Evidence of Y Chromosome Long Non-Coding RNAs involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells

Tayvia Brownmiller
West Virginia University, tabrownmiller@mix.wvu.edu

Follow this and additional works at: https://researchrepository.wvu.edu/etd

Part of the Cancer Biology Commons, Cell Biology Commons, Genetics Commons, Molecular Genetics Commons, and the Radiation Medicine Commons

Recommended Citation
https://researchrepository.wvu.edu/etd/7784

This Dissertation is protected by copyright and/or related rights. It has been brought to you by the The Research Repository @ WVU with permission from the rights-holder(s). You are free to use this Dissertation in any way that is permitted by the copyright and related rights legislation that applies to your use. For other uses you must obtain permission from the rights-holder(s) directly, unless additional rights are indicated by a Creative Commons license in the record and/or on the work itself. This Dissertation has been accepted for inclusion in WVU Graduate Theses, Dissertations, and Problem Reports collection by an authorized administrator of The Research Repository @ WVU. For more information, please contact researchrepository@mail.wvu.edu.
Evidence of Y Chromosome Long Non-Coding RNAs involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells

Tayvia Brownmiller

Dissertation submitted
to the West Virginia University School of Medicine
at West Virginia University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in
Cancer Cell Biology

Elena Pugacheva, PhD, Chair
Linda Vona-Davis, PhD
Heath Damron, PhD
Erik Bey, PhD
Scott Weed, PhD
Ivan Martinez, PhD, Mentor

Graduate Program in Cancer Cell Biology

Morgantown, West Virginia
2020

Keywords: Long non-coding RNA, lncRNA, non-small cell lung cancer, NSCLC, radiation, radiosensitivity, radioresistance, Y chromosome, linc-SPRY3
Copyright 2020 Tayvia Brownmiller
Evidence of Y Chromosome Long Non-Coding RNAs involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells

Tayvia Brownmiller

Non-small cell lung cancer (NSCLC) is the number one cause of cancer related mortality in the United States and worldwide. Advanced and therapeutically resistant lung tumors contribute to the high rate of mortality from NSCLC, therefore there is a need for new methods of diagnosing and treating this disease. Long non-coding RNAs (lncRNAs) have been shown to be a crucial component of human molecular biology, regulating nearly every cellular pathway from chromatin condensation to transcription and translation. Furthermore, many lncRNAs have been classified as oncogenes or tumor suppressors, highlighting the various molecular mechanisms they are involved in regarding the formation and progression of cancer and their use as prognostic and diagnostic biomarkers has been proposed in numerous cancers, including NSCLC.

Our group has discovered, for the first time, a three member family of Y chromosome lncRNAs (linc-SPRY3-2, lin-SPRY3-3, and linc-SPRY3-4) which regulate NSCLC cell response to ionizing radiation (IR). Briefly, the linc-SPRY3 family demonstrated a dose dependent induction of expression following exposure to IR in male radiosensitive NSCLC cell lines, but not in male radioresistant cell lines. This difference was revealed to be due to loss of the Y chromosome in the radioresistant cell lines. Using gain-of-function and loss-of-function experiments, we demonstrated statistically significant changes in cell viability and apoptosis in vitro. Furthermore, in vivo tumor growth delay assays showed a more radioresistant phenotype in tumors with knockdown of the linc-SPRY3 RNAs versus control tumors. We hypothesize linc-SPRY3-2/3/4 mediate their tumor suppressive effect via sequestration of the RNA binding protein IGF2BP3 demonstrated by CLIP and RNA degradation assays. Moreover, DNA FISH and bioinformatic analysis revealed a trending negative correlation in patient survival between loss of the Y chromosome and linc-SPRY3-2/3/4. These findings suggest that the linc-SPRY3 RNAs function as tumor suppressors by promoting cell death following IR through interactions with IGF2BP3, and could have potential as biomarkers in male NSCLC.
Acknowledgements

The work presented in this dissertation is the culmination of seven years of blood, sweat and many many many tears, and would not have been possible without the support of a number of individuals.

First and foremost, I need to thank my mentor, Dr. Ivan Martinez. Though there have been numerous times where I was frustrated beyond belief with both you and/or the research, this would not have been possible without your guidance. This was a learning curve for the both of us, which we have both admitted multiple times throughout this process, and I wholeheartedly believe we are both the better for it. Your unbelievable mentorship has prepared me for whatever comes next and I hope one day I get the chance to do for others what you have done for me.

I also have to thank my graduate program and my committee members. Without you, this milestone could not have been realized. Thank you all for your generosity, support and guidance. I would not be the scientist or person I am today without you.

Next, I have to thank three amazing women I had the privilege to work with. The three of you went from coworkers, to friends and now I consider you family because only family could have put up with me and my craziness for so long! Karen, Jamie, and Abby, I would not have survived this without your continual support, even after you all moved on. Karen, I will miss your birthday cakes and pies and the sage advice you always had on hand no matter what the situation. Jamie, I can’t thank you enough for showing me what true vigilance looks like. Even though most of the time you could not stand to be at the bench, you pushed through and eventually found something you love doing and its always something I will admire about you. You are both amazing women, mentors, and even more amazing friends and I will never be able to fully thank you for everything you have given me. Abby, you were my first real test as a mentor and I could not be prouder of what you have done since leaving the lab. I wasn’t sure I would be cut out for the job, but thanks to you I found a passion for mentoring and learned so much from you and about myself and for that I will be eternally grateful. Even more so, I am grateful for our friendship. From the moment I pointed out your insulin pump tubing, I knew you would become a lifelong friend and I look forward to seeing what’s next for you!

I also have to thank the many other members of the Martinez Lab, past and present. Aly, you are going to do great things and I can’t wait to see what the next chapter of your life holds for you. Thank you for allowing me to be part of your journey here at WVU. To all of the other undergraduates (there are too many to name them all) who I have the chance to work with, thank you for helping me become a better mentor and good luck with your futures! To Brandon, though your time in our lab was short, I have to say thank you for coming in and hitting the ground running like you did. I was at my wits end when you joined the lab and without your help and friendship, I don’t think I would have made it. To Emily, Michael, and Alana, in the brief time I have spent with y’all, I can see that the Martinez Lab is in good hands. You are all amazing and I wish you the best of luck in whatever that comes next.

I next would like to thank my friends, who have done so much to help keep me sane throughout all of this grad school mess. Dudley, you were my very first friend here and have always been there for me when I needed someone. I can’t tell you how much your friendship has meant to me.
One of my most treasured grad school memories will be that camping trip you organized. Even though it did not start off well or go the way we planned at all that weekend, it was still one of the best times I’ve had during my time here. Abi, from the moment we bonded over Harry Potter at our interview weekend, I knew you and I would be friends for life and I can’t imagine it any other way. You made grad school so much more fun and I couldn’t have survived it without you. I would also like to take this opportunity to formally apologize for spoiling that one episode of Buffy for you back in first year, and to tell you that I am now much more careful when discussing TV and movies with others. To Sarah, oh how I will miss our impromptu hallway conversations about all things MARVEL or obscure sci-fi, or the times we plan to hang out and binge a show on Netflix only to spend the evening ranting about school, family or whatever else is happening in our lives. You’ve truly made these last few years of grad school much more bearable. To my high school best friend Emily, though we were a thousand miles apart, you were always there when I needed someone to vent to and blindly take my side no matter what. Regardless of how much time had passed, sometimes weeks, I could always call/text and we’d pick back up right where we left off like no time had passed at all. You were one of the first people to convince me I could do this, and now years later you’re here cheering me on and the only thing I can say is thank you. You’re the best best friend anyone could ever ask for. Lastly to Evan, my rock for what were probably the hardest years of this entire journey, there are no words that can express what your support through the last couple of years has meant to me. Especially because I know I can be a handful when things get stressful and out of control. Thanks for sticking with me through all the craziness. No matter what happens next, I will always be grateful for you and what you have done for me.

I have to give a special acknowledgement to Dr. Linda Vona-Davis and her husband Ed. Twice you have opened your home to me, given me a place to stay when I had little to no other options. First when I began this journey, and now when it is coming to an end. I will never forget your kindness and generosity. Thank you for everything you have done for me! You will always hold a special place in my heart.

Finally, to my amazing family, this has been a long time coming. Mom and dad, this is just as much your accomplishment as it is mine. Thank you for always supporting me in everything, because without you I wouldn’t be where I am today. The two of you are my foundation and continue to keep me grounded every day of my life. This next chapter will be challenging, but with the two of you behind me I know I will be just as successful as I have been here. Dyllon, the best brother a girl could ever ask for, thank you for always being there, being my sounding board, and being my comic relief. Even when you were going through rough stuff of your own, I could always lean on you when I needed it. To my beautiful niece Paislee, you have been a light in a very long and dark tunnel. You are so loved and I hope maybe one day this will inspire you to pursue your own dreams as I have, no matter what they may be. And to the rest of my family, thank you all for your love and support during this journey; the cards, phone calls, care packages, and encouraging words on the rare chances we got to see each other face to face. I could not have made it to the end without any of you.
# Table of Contents

Abstract ........................................................................................................................................... ii
Acknowledgements ......................................................................................................................... iii
Table of Contents .......................................................................................................................... v
List of Main Figures ....................................................................................................................... vi

## Chapter 1: Introduction and Literature Review ........................................................................... 1

I. Non-coding RNA ......................................................................................................................... 1

II. Long Non-Coding RNAs (LncRNAs) ....................................................................................... 3
   A. Overview .................................................................................................................................. 3
   B. Characterization ....................................................................................................................... 3
      - LncRNA – Separating non-coding from coding transcripts
      - Biogenesis
      - Complexities of Classification
      - Genomic Location
      - Cellular Localization
      - Biological Functions
   C. LncRNAs in Cancer .................................................................................................................. 10
      - Tumor Suppressor LncRNAs
      - Oncogenic LncRNAs
      - Clinical Relevance

III. LncRNAs in Non-Small Cell Lung Cancer (NSCLC) .............................................................. 13
   A. Overview of NSCLC ................................................................................................................ 13
   B. Roles of LncRNAs in NSCLC .............................................................................................. 14
      - MALAT1
      - HOTAIR
      - MEG3
      - GAS5
IV. Radiation Therapy.........................................................................................................................17
   A. Overview.....................................................................................................................................17
   B. LncRNAs and Mechanisms of Cancer Cell Radiation Resistance....................................................19
   C. LncRNAs in NSCLC Radiation Response.........................................................................................20

V. The Y Chromosome in Cancer........................................................................................................22
   A. Overview.....................................................................................................................................22
   B. ChY in Cancer.............................................................................................................................23
   C. ChY LncRNAs in Cancer..............................................................................................................24

Chapter 2: Evidence of Y Chromosome LncRNAs involved in Radiation Response of Male Non-Small Cell Lung Cancer Cells ..................................................................................................39

Chapter 3: Discussion and Future Directions .......................................................................................93

Appendix ...............................................................................................................................................111

I. Figure Copyright Permissions...........................................................................................................111

II. Long non-coding RNA FAM83H-AS1 is regulated by human papillomavirus 16 E6 independently of p53 in cervical cancer cells..............................................................................153

Curriculum Vitae...................................................................................................................................223
List of Main Figures

Chapter 1: Introduction and Literature Review

Figure 1: Timeline of discoveries of RNAs in biological regulation
Figure 2: The Major classes of RNAs
Figure 3: Overview of Ribosome Profiling
Figure 4: Classes of non-coding transcripts based on genomic location relative to a coding gene
Figure 5: Four Major Groups of LncRNA Functions
Figure 6: LncRNA Molecular Mechanisms
Figure 7: LncRNAs associated with the hallmarks of cancer
Figure 8: The effects of radiation-induced DNA damage
Figure 9: Mechanisms of resistance against radiotherapy
Figure 10: LncRNAs involved in radiosensitivity of various cancers
Figure 11: Schematic diagram of the human Y chromosome

Chapter 2: Evidence of Y Chromosome LncRNAs involved in Radiation Response of Male Non-Small Cell Lung Cancer Cells

Figure 1: Microarray analysis reveals a family of Y chromosome, radiation inducible long non-coding RNAs.
Figure 2: Radiation sensitive and radiation resistant NSCLC cell lines show significant differences in the expression of the linc-SPRY3 family due to Y chromosome loss.
Figure 3: Linc-SPRY3 RNAs are important for cell survival following 8Gy irradiation
Figure 4: The linc-SPRY3 family act as molecular sponges for IGF2BP3 preventing it from stabilizing HMGA2 and c-Myc.
Figure 5: The DYZ1 region of the Y chromosome and the linc-SPRY3 family show negative correlations with patient survival.
Chapter 3: Discussion and Future Directions

Figure 1: IGF2BP3 expression is significantly upregulated in NSCLC
Figure 2: IGF2BP3 gene expression across human tissue and cancer types
Figure 3: Mechanisms of IGF2BP3
Figure 4: Major cancer types, in organs unrelated to reproductive function, with gender difference in incidence and mortality

Appendix: Long non-coding RNA FAM83H-AS1 is regulated by human papillomavirus 16 E6 independently of p53 in cervical cancer cells

Figure 1: Differential expression of host IncRNAs after expression of HPV-16 E6 in primary foreskin keratinocytes
Figure 2: Increased FAM83H-AS1 expression in primary cervical keratinocytes containing the HPV-16 genome as well as in HPV-16 positive cervical cancer and HNSCC lines
Figure 3: Regulation of FAM83H-AS1 expression by HPV-16 E6 in a p53-independent, p300-dependent manner
Figure 4: FAM83H-AS1 is localized in the nucleus in cervical pre-malignant and cancerous cell lines
Figure 5: FAM83H-AS1 knockdown altered cell proliferation, migration, and apoptosis in CaSki cells
Figure 6: FAM83H-AS1 expression is increased in human cervical cancer tissues and correlates with poor overall survival
CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1. Non-coding RNA

The accepted central dogma of biology that has been taught to us has been as follows: DNA is transcribed to RNA, which is then translated to protein, and proteins have long been considered the primary functional units of cellular and molecular biology. However, over the past several decades, we have learned that RNA plays a much larger role in the cell than previously thought (Fig. 1). There is actually an immense universe of non-coding RNA (ncRNA), RNA that do not code for a protein, which carry out regulatory roles once attributed to proteins (1). The two earliest examples of ncRNAs are transfer RNAs (tRNAs) and ribosomal RNAs, both of which are extremely important in translation. Transfer RNAs (tRNAs) are carriers for amino acids that are incorporated into a peptide. Ribosomal RNAs (rRNAs) make up the ribosome, including the ribozyme, an enzymatic RNA which is responsible for processing other RNA molecules such as the tRNA or splicing of nuclear pre-mRNA (2). While these types of RNAs have been extensively studied, they are only a small part of an expansive RNA world. Thanks to the continuing advancement of sequencing technologies and completion of The Human Genome Project followed

Figure 1: Timeline of discoveries of RNAs in biological regulation. (Reproduced with permission of Annual Reviews, Inc. from Genome Regulation by Long Noncoding RNAs, John L. Rinn and Howard Y. Chang, Volume 81, Annual Review of Biochemistry, 2012; permission conveyed through Copyright Clearance Center, Inc.)
by the Encyclopedia of DNA Elements (ENCODE), we have learned that roughly 70-80% of human genome is transcribed, but less than 2% of that RNA is translated to protein. This means that nearly 98% of the transcripts produced in a human cell are ncRNA. Originally, this 98% of RNA was considered “junk” or transcriptional noise, with no function and therefore was considered unimportant, but we now have a much better understanding of this “dark matter” of the genome (3,4).

Interestingly, they offer the connection to organism complexity that was once unknown. What we know now is that the more complex the organism, the larger the noncoding RNA content of that organism, with the human genome harboring the largest percentage of noncoding information compared to other genomes (5). We also know that ncRNAs are involved in nearly every facet of cellular biology, and are categorized in numerous ways, beginning with regulatory or housekeeping ncRNAs. Housekeeping ncRNAs are those important for translation, such as tRNAs and rRNAs. The rest are grouped into regulatory ncRNAs, a much larger group which is categorized first by size. NcRNAs larger than 200 nucleotides are classified as long non-coding RNAs (lncRNAs), which have wide ranging functions. Any ncRNAs smaller than 200 nucleotides are classified as small ncRNAs encompassing microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), small interfering RNAs (siRNAs), small nuclear RNAs (snRNAs), and PIWI-interacting RNAs (piRNAs), each of which have their own specific functions (Fig. 2) (6).

As previously mentioned, ncRNAs function in a myriad of ways which are important for every cellular process. Because they are so intricately woven into our basic biological functions, their dysregulation leads to the development of disease, including cancer (7). While the roles of
the small RNAs, especially miRNAs, have been relatively well studied in regards to cancer cell biology, lncRNAs remain poorly characterized in comparison.

II. Long Non-coding RNA

A. Overview

Long non-coding RNAs (lncRNAs) are those RNAs greater than 200 nucleotides long and lack an open reading frame, and due to their emergence over the past 15 years, there has been a fundamental shift in the landscape of molecular genetics (8). LncRNAs are comprised of intergenic transcripts, enhancer RNAs, and sense or antisense transcripts that overlap coding genes and can function in cis or trans. They also show incredible cell type and tissue specificity. Those that have been functionally characterized have revealed a myriad of roles, many of which are dictated primarily by sequence and/or cellular localization (9). As it stands, there are currently over 120,000 identified lncRNAs in the human genome (10). Unfortunately, only a handful of these have been functionally characterized leaving hundreds of thousands left to be studied.

B. Characterization

Through advancements in RNA sequencing technologies, we have learned that lncRNAs are abundant and extremely diverse. In general, a non-coding RNA (ncRNA) is classified as a lncRNA if they are larger than 200 nucleotides, but their sizes are very heterogeneous ranging from a few hundred to even larger than a few thousand nucleotides. Furthermore, they originate from nearly every part of the human genome, can be found in virtually every cellular compartment, and carry out numerous functions (8,11). Unfortunately, all of these factors combined with a limited understanding of lncRNAs has resulted in poorly defined system for classification.

LncRNA – Separating non-coding from coding transcripts

The major defining factor for any ncRNA is its lack of coding potential, or its inability to be translated into a protein, but quantitatively determining this characteristic is not an easy task. Moreover, lncRNAs have begun to emerge as a gray area in terms of coding potential, as many lncRNAs have been found to contain small open reading frames (smORF) which could produce
micropeptides. It is also important to note that some mRNAs could have non-coding functions (12).

The primary method for determining coding potential of a RNA is ribosome profiling followed by deep sequencing (RiboSeq). Ribosomes are the cornerstone of protein translation. Briefly, when ribosomes associate with an RNA during translation (protein synthesis), they also protect that piece of RNA from digestion by exonucleases. That protected RNA can be recovered and then be used for deep sequencing and the sequencing data can be aligned to a genome which determines the translational profile of a given RNA (Fig. 3) (13). LncRNAs typically have no to low enrichment of ribosome occupancy and therefore are classified as non-coding (14). However, as sequencing technologies advance and become more sensitive, we are able to better detect and identify small ORFs such as those attributed to micropeptide encoding lncRNAs (15).

There are also computational methods that can be utilized. A few of the more popular pipelines are phylogenetic analysis of codon substitution frequencies based on sequence alignment (PhyloCSF) scoring, sORF finder, coding region identification tool invoking comparative analysis (CRITICA), and micro-peptide detection pipeline (micPDP) (16–19). It is important to note that some controversy still surrounds this grey area as micropeptide potential is a very new field in the realm of lncRNAs. Furthermore, just because a lncRNA contains a small ORF and can produce a micropeptide, does not mean its primary function isn’t that of a non-coding RNA.

*Biogenesis*
In general, lncRNAs are very similar to messenger RNAs (mRNAs). Their promoters display similar epigenetic markers, they are transcribed via Pol II, undergo splicing, and can include both a 5’ cap and a Poly A tail. However, that is where the similarities end (20). Every gene is controlled first by epigenetic markers such as histone methylation and acetylation, essentially signaling if a RNA should be transcribed. Both mRNAs and lncRNAs are controlled by promoter enrichment of trimethylation of lysine 4 on histone 3 (H3K4me3), but lncRNAs typically show much higher enrichment of acetylation of lysine 27 of histone 3 (H3K27ac), with more recruitment of transcriptional regulators like Swr1, Isw2, Rsc, and Ino80 resulting in repression (21). This would explain the relatively low expression of most lncRNAs across human tissues.

As previously mentioned, lncRNAs are primarily transcribed by Pol II, and processed similarly as mRNAs by splicing and 3’ polyadenylation. However, there are some mechanisms of post-transcriptional regulation that affect lncRNAs and not mRNAs. For example, the lncRNA NEAT1 can be processed like a mRNA to produce what is known as its short form, but alternatively its long form contains a pre-tRNA like structure on its 3’ end which is recognized and cleaved by the ribonucleoprotein complex RNaseP (responsible for processing mature tRNAs). The resulting free end then forms a triple helix structure, stabilizing the lncRNA strand (22). Some other forms of alternative RNA processing that affect lncRNAs includes splicing events that result in the formation of circular RNAs (circRNAs). This includes products from canonical splicing called lariat loops, formed by a 2’,5’-phosphodiester bond, or the non-sequential splicing of introns, otherwise known as backsplicing. The difference between the two being that circRNAs formed from backsplicing are typically more stable since there are no free ends for exonucleases to recognize and degrade (20,23).

**Complexities of Classification**

Due to the ever-growing field of lncRNA biology, there is no one unified way to classify a lncRNA other than the basic attributes that they are longer than 200 nucleotides and lack an open reading frame. This cutoff of length is an arbitrary number, set to separate lncRNAs from their short ncRNA counterparts (miRNA, snoRNA, piRNA, etc.). Other than this, there are a number of subclasses of lncRNAs which divides them based on characteristics such as genomic location,
association with DNA elements, tandem repeat content, sequence or structure conservation, cellular localization, and function, just to name a few (24). This has led to some confusion in regards to lncRNA characterization, classification, and even naming. However, as the field of lncRNA biology advances one thing is constant. This species of complex RNAs is extremely important and nearly all aspects of cellular and molecular biology.

Genomic Location

The most commonly used method of classification is based on the genomic location relative to a protein coding gene (8). LncRNAs can be classified as sense or antisense, intronic, divergent (bidirectional), or intergenic (Fig. 4). Sense lncRNAs are transcribed in the same direction of a protein coding gene and contain one or more exons, and sometimes overlap or cover an entire sequence of a protein coding gene. These overlapping lncRNAs are considered non-coding transcript variants of the protein coding gene as they originate from the same promoter but lack an open reading frame. Antisense lncRNAs are transcribed in the opposite direction of a protein coding gene, usually initiate inside or downstream of a protein coding gene, and include at least one exon from the protein coding gene. Their expression typically correlates with an mRNA of the same promoter and have been shown to be very important in cancer, usually regulating the expression of tumor suppressor or oncogenic proteins (25). Intronic lncRNAs, as the name suggests, are lncRNAs that initiate from an intron, are composed entirely of introns and can be transcribed in either direction of a protein coding gene. These intronic transcripts have not been well studied, but have been implicated in regulating RNA splicing and stability (26,27). Divergent, or bidirectional, lncRNAs are transcripts that initiate in the opposite direction as its protein coding gene counterpart, typically around a few hundred bases to 1 kilobase from a neighboring protein coding gene promoter (11,28). Finally, intergenic lncRNAs (lincRNAs) are transcripts that
LncRNAs originate from their own transcriptional unit which is found between protein coding units. LncRNAs are by far the most studied among the above listed classes. They have been shown to function in both \textit{cis} and \textit{trans}, affecting transcription, translation and splicing. They have also shown high conservation between species (28,29).

\textit{Cellular Localization}

LncRNAs can also be classified by their cellular localization. The two main methods primarily used to identify a lncRNA’s primary compartment are cellular fractionation and RNA fluorescent \textit{in situ} hybridization (FISH). Cellular fractionation involves lysing cells and separating the nuclear fraction from the cytoplasmic fraction, primarily by multiple centrifugation steps, followed by methods like quantitative reverse transcriptase PCR (qRT-PCR) or even RNA-seq (30). RNA FISH is a method in which a fluorescent probe complementary to an RNA of interest is designed and incubated with cells or tissue of interest. The probe should hybridize to its target RNA and this can be imaged via fluorescent microscopy (31).

Identifying a perspective lncRNA’s compartment is a very important part of characterizing a lncRNA, as it narrows the potential functions inside a cell. Unlike mRNAs, the vast majority of lncRNAs are localized to the nucleus due to a number of factors such as inefficient splicing and polyadenylation. Many lncRNAs essentially retained to the nucleus due to their interactions with nuclear structures and proteins. Nuclear lncRNAs typically regulate epigenetic markers, chromatin structure, transcription, and post-transcriptional processing (32,33). To a much lesser extent, there are some lncRNAs which localize to the cytoplasm. Cytoplasmic lncRNAs have been shown to regulate translation, post-translational processing and interact directly with other regulators in the cytosol like microRNAs (miRNAs) and even other mRNAs. Furthermore, a few cytoplasmic lncRNAs have been shown to produce micropeptides, though this function needs more study to fully understand its role in the cell (33,34).

\textit{Biological Functions}

Unlike proteins, the extent of possible lncRNA functions has yet to be elucidated, nonetheless it has emerged as a way to classify a lncRNA. Those handful of lncRNAs that have been well characterized have revealed a number of important functions, nearly all of which are
regulatory. As mentioned before, a lncRNA’s function is highly determined by the cell compartment in which they are found. Furthermore, their functions can be categorized as cis, those that directly regulate neighboring genes, or as trans, those that translocate to another part of the cell to perform its function. The main classifications have been previously described in four major groups: signals, decoys, guides, and scaffolds (Fig. 5). However, these four groups are much more complex and a single lncRNA can have multiple functions within the cell (Fig. 6) (35–37).

**eRNA:** Enhancer lncRNAs (eRNAs) are a type of nuclear lncRNA which can enhance target gene expression, and can be considered both scaffold and signal lncRNAs (Fig. 6A). They can originate from enhancer regions of DNA and stand apart from most “traditional” lncRNAs as they can be transcribed in either direction and most do not undergo maturation such as splicing and polyadenylation. Most importantly, eRNAs function by regulating transcription by recruiting transcription factors and acting as a bridge to bring two genes close together, one of which is usually an enhancer region, primarily by intrachromosomal interactions via chromosome looping or alternatively by interchromosomal interactions by bringing two different chromosomes together (Fig. 6D) (38). An example of an eRNA is the lncRNA HOTTIP (HOXA distal transcript antisense RNA). HOTTIP functions in cis and facilitates chromosome looping which brings itself into
proximity with the HOXA gene cluster. From there, it recruits WDR5 and the MLL complex which triggers deposition of H3K4me3 to activate transcription of HOXA genes (39).

**Chromatin modifiers:** One of the better characterized lncRNA functions is their role in chromatin modification and remodeling (Fig. 6B). They can be categorized as scaffolding or guide lncRNAs, are nuclear, can act in either cis or trans, and can recruit chromatin modifiers that can either promote transcription or repress it (40). One of the most well studied examples is the lncRNA Xist (X-inactive specific transcript). Xist’s primary function is the silencing of one of the two X chromosomes in female cells (X chromosome inactivation). Xist coats the inactive X chromosome with multiple copies of itself which recruits transcriptional repressors such as the polycomb repressive complex 2 (PRC2) triggering accumulation of H3K27me3 (41).

**Transcriptional regulators:** A number of lncRNAs have been shown to directly interact with protein binding partners and thereby affect gene expression by regulating transcription (Fig. C). Their roles in transcription can happen in cis or trans, and can be activating or repressive. ANRIL is an antisense lncRNA clustered with the genes CDKN2A and CDKN2B and represses them in cis by recruiting both PRC1 and PCR2 to the gene loci (42). In contrast, the lncRNA Evf-2 (DLX6-AS1) acts as a transcriptional cofactor and forms a complex with DLX2 to activate transcription of genes located at the DLX5/6 locus (43). LncRNAs can also affect transcription by acting as decoys to sequester transcription factors which can inhibit or promote transcription of target genes (44).

**Regulation of mRNA splicing, stability, and translation:** Alongside regulating transcription, lncRNAs can also function post-transcriptionally by affecting the maturation, translation, and degradation of mRNAs (Fig. 6E-H). For example, MIAT (myocardial infarction associated transcript) sequesters splicing factor 1 (SF1) to inhibit formation of the spliceosome complex during splicing (45). Another example is the famous lncRNA MALAT1, which can bind SR splicing factor [serine-arginine (SR)-rich splicing factor] and affect its distribution, downregulating splicing (46). In terms of affecting mRNA stability, some lncRNAs have been shown to directly interact with mRNAs, either to stabilize the mRNA strand itself (e.g. BACE1-AS) or to act as a guide for proteins necessary to stabilize an mRNA (e.g. TINCR) (47,48).
Similarly but in contrast, lncRNAs can destabilize mRNA strands and downregulate protein expression by inducing degradation of a target mRNA (e.g. 1/2sbsRNAs) (49). Additionally, lncRNAs can influence (e.g. lincRNA-p21) translation of target mRNAs (50). LncRNAs can also function as competing endogenous RNAs (ceRNAs) and act as molecular sponges, not just for proteins but also for miRNAs. miRNAs regulate protein expression by binding the 3’ UTR of a target mRNA and inhibiting translation or triggering mRNA degradation. LncRNAs can “sponge” miRNAs, preventing them from targeting an mRNA, which allows that mRNA to be translated (51).

*Micropeptide encoding lncRNAs:* One of the most recent discoveries in lncRNA biology is the capacity for some lncRNAs to contain small open reading frames (smORFs) which can be translated into functional micropeptides (usually <100 amino acids). These lncRNAs could be their own transcript, or a non-coding variant transcript from a protein coding gene and typically localize to the cytoplasm of the cell (Fig. 6I). Computational analysis has predicted numerous smORFs, but studies have suggested that only around 17% of predicted smORF containing lncRNAs are translated. Moreover, these micropeptides are expressed at very low levels and are typically unstable (12,15,52). However, there are some examples of functional micropeptides derived from putative lncRNAs. DWORF is a muscle specific micropeptide (34 amino acids) which originates from the human lncRNA LOC100507537 and functions by displacing SERCA (sarco/endoplasmic reticulum Ca$^{2+}$-ATPase) membrane pump inhibitory proteins which allows increased calcium uptake and myocyte contractility (53).

Overall, it can be easily determined that lncRNAs are extremely vital to cellular and molecular biology and offer an explanation for organism complexity that scientists have sought for decades. It is important to note that, while we have made great strides in characterizing lncRNAs, we have barely scratched the surface of their importance and potential and given their broad range of functions we should look to them for answers regarding numerous pathologies, including cancer.

*C. LncRNAs and Cancer*
LncRNAs have emerged as one of the most important and diverse regulators in the human cell. Considering their widespread involvement in nearly every biological process, it is easy to assume that their dysregulation can lead to the development of disease including cancer. Cancer is a collection of diseases, but nearly all have one or more disruptions in the molecular pathways of what cancer researchers call “the hallmarks of cancer” (54). We now know that lncRNAs are just as intricately involved in these processes as proteins and act as tumor suppressors or oncogenes. However, it is important to note that a tumor suppressive or oncogenic role is highly dictated by the cancer type in which a lncRNA is dysregulated (Fig. 7) (55).

Tumor Suppressor LncRNAs

Tumor suppressor genes, as their name suggests, are those genes necessary to suppress carcinogenesis (56). There are a number of tumor suppressor proteins (p53, Rb, BRCA, etc.) and the same can be said for lncRNAs, where their dysregulation via loss or downregulation of their expression results in the promotion of a malignant phenotype. One of the most well characterized tumor suppressive lncRNAs is MEG3. Downregulated in multiple cancer types (renal, liver, lung, and brain) its primary function is regulating the tumor suppressor p53 (57). MEG3 downregulates MDM2 expression, an E3 ubiquitin ligase responsible for targeting p53 for degradation. This allows p53, a transcription factor, to function properly and regulate its target genes which are important in a number of cellular processes such as cell cycle and apoptosis (58). Another well-known example is the lncRNA GAS5 (growth arrest specific transcript 5). It has also been associated with numerous cancer types (breast, renal, prostate, and endometrial) and it is an important regulator of cell metabolism (among other mechanisms) which binds glucocorticoid receptor (GR), inhibiting the activation of GR dependent genes. This leads to metabolic stress and eventually cell death (59,60). There are even lncRNAs that are important for regulating telomeres,
the ribonucleoprotein structures at the end of chromosomes. In healthy cells, telomeres shorten every time a cell divides. Once they reach a certain size, the cell is signaled to stop dividing, either undergoing apoptosis or permanent cell growth arrest. One component of telomeres is a group of lncRNAs called telomeric repeat-containing RNAs or TERRA (61). While the complete mechanisms of TERRA have yet to be elucidated, they have been shown to interact with and inhibit telomerase, an enzyme hijacked by cancer cells to maintain telomeres and essentially mediate immortality (62). Additionally, TERRA transcripts are commonly downregulated in cancer, further supporting their role as tumor suppressors in human cells (55,63).

**Oncogenic LncRNAs**

Oncogenes are those genes that give rise to proteins or lncRNAs that promote tumorigenesis, and upregulation or activation of oncogenic factors results in phenotypes that are beneficial to cancer (56). Some well-known oncogenic proteins include members of the RAS family (KRAS, NRAS, and HRAS), Myc, and numerous receptor tyrosine kinases (RTKs, e.g. EGFR, VEGF, and RET). These proteins stimulate tumor formation via promoting proliferation, resisting apoptosis, and even reprogramming cancer cell metabolism (64). Not surprisingly, there are lncRNAs that have been shown to promote the same oncogenic phenotypes. One example of a fairly well studied oncogenic lncRNA found upregulated in multiple tumor types is HOX transcript antisense RNA (HOTAIR). It’s been associated with many different signaling pathways (proliferation, apoptosis, and invasiveness) across numerous cancers including lung, breast, pancreatic and ovarian cancer. It’s a wildly promiscuous lncRNA that can regulate the PI3K/Akt pathway by suppressing the expression of the tumor suppressor PTEN, affect cell adhesion and cancer cell epithelial-to-mesenchymal transition via inhibiting expression of proteins such as Wnt inhibitory factor-1 (WIF-1), and also promote metastasis by silencing of specific genes through PRC2 (63,65). Another example is the lncRNA PCAT1 (prostate cancer-associated transcript 1), which is named for the cancer in which it was originally identified. While it is mostly prominent in prostate cancer cell biology, we now know that it is upregulated in other cancer types and functions primarily by upregulating cell proliferation by post-transcriptionally stabilizing the oncogenic protein c-Myc. Furthermore, it essentially forms a positive feedback loop with c-Myc since c-Myc is the transcription factor that controls the expression of PCAT1 (66–68).
Clinical Relevance

Given their dysregulation in cancer and higher tissue specificity relative to their protein counterparts, lncRNAs have become attractive candidates as potential biomarkers (69). One of the best examples is PCA3, a prostate specific lncRNA that is undetectable in healthy tissue, overexpressed in >90% of prostate cancers and can be detected in the urine of patients (70). In 2012, it became the first lncRNA approved by the US Food and Drug Administration for biomarker use and is now considered more reliable than the standard prostate-specific antigen (PSA) level test since it’s use is more accurate for prostate cancer diagnosis (71). A recent search of ClinicalTrials.gov revealed 5 ongoing biomarker studies looking at lncRNAs including specific trials investigating HOTAIR for use in thyroid cancer (NCT03469544) and CCAT1 for use in colorectal cancer (NCT04269746). Though their clinical use is slowly emerging, the list of potential biomarker lncRNAs is continuously growing, and with the advancements of less invasive techniques such as the liquid biopsy, it is only a matter of time before more lncRNAs are proven as effective diagnostic and prognostic indicators.

LncRNAs have also been investigated as targets for cancer therapeutics. As mentioned before, lncRNAs are highly tissue and/or cancer specific which makes them ideal candidates as druggable targets. Current methods in development include RNAi (siRNA, miRNA), antisense oligonucleotides (ASOs), and small molecules, however none have yet to enter clinical trials. There a multiple examples of these methods effectiveness in vitro and in vivo, however the lack of an efficient systemic delivery method has proven challenging for use in humans. The alternative option to directly targeting a perspective lncRNA is identifying novel binding partners or downstream effectors and targeting them instead (69). Considering the multitude of functions attributed to lncRNAs, these strategies could one day prove useful in treating cancers.

III. LncRNAs and Non-Small Cell Lung Cancer (NSCLC)

A. Lung Cancer Overview

In the U.S., lung cancer is the number one cause of cancer related mortality, and is the second most commonly diagnosed cancer amongst both men and women. Currently, the average
overall 5 year survival rate is 18.6%, which can be attributed to the fact that more than half of lung cancer cases are diagnosed at late stages (72).

Lung cancer is typically divided into two major types, small cell lung cancer (SCLC), which makes up ~20% of cases, and non-small cell lung cancer (NSCLC), which makes up ~80% of cases. NSCLC is then further divided into three histological subtypes, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and large cell carcinoma (LCC). Of these subtypes, LUAD is the most common, followed by LUSC and then LCC. Interestingly, the subtype most found in non-smokers is LUAD, while LUSC is mostly found in those who currently smoke or have a history of smoking. While most cases can be linked to smoking, other causes also include genetic changes and carcinogenic environmental factors such as asbestos or radon gas (73). Regardless of the cause, genetic alterations such as activation or overexpression of oncogenes and/or down regulation or loss of tumor suppressors and their affected pathways are crucial in the development of lung cancer. With advancements in genomics and proteomics, there have been over two dozen alterations identified that can be classified as “driver mutations.” These are mutations which occur typically in a tumor suppressor gene or oncogene. For NSCLC, the most common are changes in TP53, KRAS, EGFR, and CDK2NA. Alterations in these genes results in drastic consequences, leading to changes in multiple cellular processes such as cell cycle regulation, DNA damage response, growth signaling and even metabolic reprogramming, leading to tumorigenesis (74,75). Additionally, like changes in protein coding genes, changes in lncRNAs can also wreak havoc in the cell.

B. Roles of LncRNAs in NSCLC

Numerous lncRNAs have been found to affect all the major hallmarks of cancer, and thanks to the continuing advancements in RNA sequencing technologies, more and more are being discovered. In NSCLC specifically, there have been a number of dysregulated lncRNAs (oncogenic and/or tumor suppressive) characterized to affect nearly all major hallmark pathways involved in carcinogenesis including epithelial-to-mesenchymal transition (EMT), apoptotic resistance, migration and metastasis, and therapy resistance (76,77).

MALAT1
Arguably one of the most studied lncRNAs associated with not only lung cancer but numerous cancers is the oncogenic Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1). As its name suggests, it was first characterized in lung adenocarcinoma as a mediator of metastasis (78), but further studies revealed it is actually one of the most abundantly expressed lncRNAs in normal human tissues. Only when is it dysregulated, usually overexpressed, does it become oncogenic (79). Since its discovery, MALAT1 has been shown to carry out multiple functions in NSCLC. The most common functions typically involve regulation of RNA splicing and are associated with pathways that promote invasion and metastasis. Moreover, studies using NSCLC cell lines have shown that depletion of MALAT1 leads to a decrease in migration, invasiveness and metastatic potential (80). Interestingly, MALAT1 can also act as a competing endogenous RNA (ceRNA) by sponging miRNA. One study describes an ERβ/MALAT1/miR-145-5p/NEDD9 axis that promotes the phenomenon vasculogenic mimicry (the ability for cancer cells to trans-differentiate into endothelial like cells) and NSCLC invasion. Briefly, estrogen receptor β (ERβ) signaling upregulates the expression of MALAT1, which in turn sequesters miR-145, allowing NEDD9 to be expressed and promote cell invasiveness (81). Another mechanism involving sponging of miR-145 promotes resistance to the chemotherapeutic agent cisplatin in NSCLC cell lines. Sequestration of miR-145 lead to an increase in Kruppel-like factor 4 (KLF4), a factor known to regulate stemness in cancer cells and promote resistance to chemotherapeutics (82). MALAT1 has also been shown to promote cisplatin resistance through STAT3 activation which upregulates MDR1 and MRP1, known mediators of drug resistance (83). Additionally, numerous studies have proposed MALAT1 as a prognostic marker for NSCLC. Overall, high expression correlates with worse overall survival and high metastatic potential in NSCLC patients (84,85).

**HOTAIR**

The lncRNA HOX Transcript Antisense RNA (HOTAIR) is another well studied and characterized oncogenic lncRNA found in numerous cancers. In lung cancer, it has been shown to be important in cell proliferation, invasion, and therapeutic response, typically when overexpressed compared to normal tissue (86). One study found that the tumor suppressor p53 negatively regulates HOTAIR expression by directly binding to and occupying its promoter. Furthermore, overexpression of HOTAIR resulted in downregulation of p53 via recruitment of
PRC2 to the p53 promoter, revealing a feedback loop between HOTAIR and p53 that causes changes in cell proliferation and invasiveness (87). Another study revealed that hypoxia is a major regulator of HOTAIR expression in NSCLC. Briefly, hypoxia turns on expression of the transcription factor HIF-1α, a major regulator of cellular hypoxia response, which binds to hypoxia response elements within the HOTAIR promoter. The upregulation in HOTAIR expression resulted in enhanced cell proliferation, migration, and invasion under hypoxic conditions (88). HOTAIR can also function as a ceRNA to influence tumorigenesis. For example, one study showed that HOTAIR can sponge the miRNA miR-217 which prevents downregulation of dachshund homolog 1 (DACH1), a cell fate determination factor favorable for tumorigenesis (89). Additionally, HOTAIR has been shown to influence cellular response to chemotherapeutics such as Crizotinib, a tyrosine kinase inhibitor, via promoting autophagy in NSCLC cell lines (90). Lastly, increased expression of HOTAIR is negatively correlated with NSCLC patient outcomes (91,92).

**MEG3**

MEG3 (maternally expressed gene 3) is one of only a handful of characterized tumor suppressor IncRNAs. It is found expressed in many normal tissues, and its loss is associated with numerous cancer types (57). Furthermore, one of the primary roles of MEG3 is activation and accumulation of the tumor suppressor protein p53. It is suggested that it stabilizes the p53 protein which promotes increased activity of the protein and proper regulation of p53 target genes which control cell cycle and apoptosis (58). In NSCLC, the loss of MEG3 is attributed to apoptotic resistance and increased proliferation. One study demonstrated that exogenous overexpression of MEG3 significantly increased the expression and activity of p53. This resulted in reduced cell proliferation and increased apoptosis *in vitro* and slower tumor growth *in vivo* (93). Another tumor suppressive mechanism attributed to MEG3 involves its regulation of Skp2, a component of the ubiquitin ligase complex responsible for degrading p27, a master cell cycle regulator. Briefly, MEG3 coordinates with miR-3163 to inhibit the translation of Skp2 which allows expression of p27 and eventually inhibits cell growth (94). Additionally, through a miRNA mediated mechanism, MEG3 has been shown to enhance the sensitivity of NSCLC cells to cisplatin. Cells with low MEG3 expression demonstrated resistance to cisplatin, which was reversed via exogenous overexpression of MEG3, mediated by a miR-21-5p/SOX7 signaling axis (95). It is
also important to note that in addition to the mechanisms outlined above, MEG3 has also been suggested as a prognostic marker in NSCLC, as low expression of the lncRNA correlates with poor survival (96).

**GAS5**

Growth arrest specific transcript 5 (GAS5) is another tumor suppressor lncRNA found expressed in multiple tissues, but downregulated in a number of cancer types including NSCLC. Overall, it has been associated with a number of signaling pathways such as cell cycle, cancer cell metabolism, proliferation, and apoptosis (59). One study revealed GAS5 was downregulated in NSCLC tissues when compared to normal adjacent tissues suggesting its function as a tumor suppressor. Further investigation in the same study revealed that exogenous expression of GAS5 in NSCLC cell lines slowed cell proliferation and decreased colony formation ability as well as induced apoptosis (97). Another tumor suppressive mechanism in NSCLC attributed to GAS5 is its ability to regulate glucose signaling. In summary, GAS5 functions in a delicate feedback loop with glucose signaling. It was shown that high glucose fed NSCLC cells downregulated the expression of GAS5 which increased proliferation and migration, while exogenous overexpression of GAS5 attenuated the affect through direct interaction with TRIB3, an important mediator of glucose signaling (98). GAS5 has also been shown to interact with miRNAs to affect NSCLC cell proliferation, metastasis and radiation sensitivity. For example, GAS5 can function as a ceRNA for miR-205. In short, miR-205 suppresses the translation of the tumor suppressor protein PTEN. GAS5 therefore promotes the expression of PTEN by sponging miR-205. Expression of PTEN results in slower proliferation and decreased migration and invasion in NSCLC cells (99). Additionally, GAS5 has been shown to sponge miR-135b, inhibiting tumorigenesis and promoting radiation sensitivity both *in vitro* and *in vivo* (100). Lastly, thanks to technological advances and the development of liquid biopsy methods, low levels of circulating GAS5 has been proposed as a potential biomarker for the prognosis and diagnosis of NSCLC (101,102).

Overall, it can be concluded that lncRNAs are an extremely important factor in the tumorigenesis and treatment of NSCLC. Unfortunately, a lot more work needs to be done in regards to the clinical applicability of lncRNAs.
IV. Radiation Therapy

A. Overview

Radiation therapy (RT) is a major component of cancer therapy. Currently, there are multiple modalities (intensity modulated radiotherapy, image-guided radiotherapy, stereotactic body radiotherapy, etc.) utilized to administer radiation to tumors, typically combined with a chemotherapeutic agent which is dictated by the cancer type and location of the tumor. The clinically approved modalities use one of two kinds of radiation, photon radiation (x-rays and gamma rays) or particle radiation (electron, proton, or neutron beams). The major difference between photon radiation and particle radiation is the rate of energy emission. Overall, particle radiation has a higher linear energy transfer (LET), resulting in more DNA damage compared to photon radiation. However, photon radiation is more commonly used because the equipment needed to administer particle radiation is difficult to manufacture and much more expensive (103). Regardless of their differences, they both have proven effective in the treatment of most cancers, whether through curative or palliative care.

The component shared amongst all types of radiation therapy is the mechanisms by which it kills cancer cells. The primary mechanism is by direct DNA damage, inducing double stranded (DSB) and single stranded breaks (SSB) (Fig. 8A). The secondary mechanism is by the creation of free radicals which signals cell stress and also causes DNA damage (104). DNA damage then triggers a number of molecular signaling cascades which attempt to fix the damage [non-

Figure 8: The effects of radiation-induced DNA damage. (Reprinted/Adapted from Clinical Cancer Research, 2015, Volume 21, Issue 13, 2898-2904, Meredith A. Morgan and Theodore S. Lawrence, Molecular Pathways: Overcoming Radiation Resistance by Targeting DNA Damage Response Pathways, with permission from AACR)
homologous end joining (NHEJ), alternative end joining (alt-EJ), and homologous recombination (HR)] and slow/stop cell cycle progression, however the mass amount of stress and genomic instability typically overwhelms these repair mechanisms and results in senescence (permanent cell growth arrest), or cell death via apoptosis or necrosis (Fig. 8B and 8C) (104,105). Unfortunately, like most therapies, there are molecular mechanisms of resistance that can make treating cancers with radiation very difficult (Fig. 9).

**B. LncRNAs and Mechanisms of Cancer Cell Radiation Resistance**

While RT has proven to be an effective strategy for treating multiple types of cancers, cancer cells are notorious for adapting to circumvent cell death. These mechanisms of resistance span multiple molecular pathways (Fig. 9) and can be intrinsic or acquired, which makes overcoming them very difficult in terms of therapy and leads to poor prognosis. Some well-known examples include apoptosis resistance, hijacking of the DNA damage repair pathway, expression of stem cell like factors, and epithelial-to-mesenchymal transition (106). Additionally, as previously stated, lncRNAs play a role in nearly every molecular pathway so it is no surprise that lncRNAs have been shown to respond to and regulate RT sensitivity in multiple cancer types (Fig. 10) (107). For instance, in glioblastoma, colorectal, and gastric cancers lincRNA-p21 has strong interactions within the Wnt/β-catenin pro-survival pathway which mediates resistance to radiation with respective mechanisms within the pathway specific to each cancer type (108–110). LincRNA-ROR has been associated with radioresistance in hepatocellular carcinoma by sequestering miR-145 which allows upregulation of RAD18, a factor responsible for DNA damage repair (111). It’s also been shown to regulate the same miRNA in colorectal cancer, and promotes radioresistance via negative regulation of miR-145/p53 pathway resulting in apoptotic resistance (112). The
LncRNA NEAT1 has been shown to influence EMT in nasopharyngeal carcinoma via negative regulation of miR-204 leading to the upregulation of ZEB1, promoting EMT and enhancing radiation resistance in vitro and in vivo (113). Furthermore, the lncRNA TUG1 has also been shown to promote EMT via sponging of miR-145 and upregulating ZEB2 in bladder cancer (114). While these examples are only a small sample of the studies investigating the roles of lncRNAs in cancer radiotherapy, it is clear that lncRNAs are a potential area of therapeutic and prognostic value for multiple cancer types.

![Image: Mechanism of LncRNA in Regulation of Tumor Radiosensitivity]

Figure 10: LncRNAs involved in radiosensitivity of various cancers. (Molecular mechanisms of LncRNAs in regulating cancer cell radiosensitivity, Zhu et al, Bioscience Reports, 2019)

C. LncRNAs in NSCLC Radiation Response

While there have been great strides in understanding the mechanisms of lncRNAs in radiation response, only a handful have been characterized in non-small cell lung cancer (NSCLC). The tumor suppressive lncRNA GAS5 (Growth Arrest Specific Transcript 5) has shown the ability to sensitize NSCLC cell lines to radiation. One of these studies proposed a mechanism where
GAS5 is able to work as a competing endogenous RNA (ceRNA) and sequester or “sponge” the miRNA miR-135b. Another study showed that GAS5 functions through a miR-21/PTEN/Akt signaling axis. Interestingly, both studies showed increased ability for cells to undergo apoptosis following exogenous expression of GAS5(100,115). The lncRNA PVT1 (Plasmacytoma Variant Translocation 1) has also shown potential in regulating radioresistance in NSCLC by acting as a ceRNA for the miRNAs miR-195 and miR-424-5p. While there is no further mechanism described in regards to miR-195 other than increased apoptosis following administration of RT when PVT1 was knockdown, PVT1 sponges miR-424-5p and stabilizes the protein CARM1, promoting an anti-apoptotic phenotype. This results in upregulated Bcl-2 (anti-apoptosis protein) and matrix-metalloproteinases MMP-2 and MMP-9 (important for cell adhesion, migration and invasion), which was all attenuated upon knockdown of PVT1 or overexpression of miR-424-5p, increasing radiosensitivity (116,117).

In addition to GAS5 and PVT1, there other examples of lesser known lncRNAs which have been shown to regulate the radiosensitivity of NSCLC cells. For instance, the lncRNA CYTOR (Cytoskeleton Regulator RNA) can also sponge miR-195 similarly to PVT1. Briefly, high expression of CYTOR correlates with poor patient survival and functions as an oncogene by promoting increased proliferation, migration, invasion, and radioresistance in NSCLC cell lines by acting as a molecular sponge for miR-195. By sequestering miR-195, CYTOR allows for the upregulation of miR-195 targets such as, YAP and WNT3A which are regulators of stem-cell/developmental molecular pathways implicated in radioresistance(118). Another example is the lncRNA FAM201A (LncRNA Family with Sequence Similarity 201-member A). Along with high expression being associated with radiation resistance and poor prognosis in NSCLC patients, FAM201A functions in mediating radioresistance by sequestering miR-370, which promotes expression of EGFR and HIF-1α, both of which have been associated with pathways involved in mediating resistance to RT (119).

In conclusion, lncRNAs are clearly an important factor in regulating cancer cell radiosensitivity. However, more studies are needed to fully understand the plethora of mechanisms these complex RNAs can elicit in cancer cells. Furthermore, many have been proposed as
prognostic indicators in some cancers (120), but more clinical studies are necessary to fully classify them as relevant molecular biomarkers.

V. The Y Chromosome in Cancer

A. Overview

The human Y chromosome (ChY) has long been considered a genetic wasteland with no value beyond its roles in male development and fertility (121). This is due in large part to the miniscule number of genes found on ChY compared to other chromosomes in the human genome. The total number of annotated genes currently reported is 568, of which only 71 have been described as protein-coding, and of those 71, many of the proteins belong to the same family resulting in a minor 27 distinct protein coding genes. The rest are considered to be potential non-protein-coding genes and pseudogenes (122).

Structurally, ChY is split into three major regions: MSY, PARs and the heterochromatic block (Fig. 11). The male specific region (MSY) is comprised of euchromatin and contains the majority of the annotated coding genes along with some pseudogenes. The pseudoautosomal regions (PAR1 and PAR2), which contain a small number of coding genes, are the only portions of ChY that share homology with the X chromosome, and are the only parts of ChY which undergo meiotic recombination. The remaining region is typically referred to as the heterochromatic block, comprises roughly half of the q arm of ChY, and has been described as transcriptionally inactive (123).

Interestingly, ChY is commonly lost in aging somatic cells, otherwise referred to as mosaic loss of chromosome Y (LOY). This phenomenon has been observed for decades and was first considered a simple physiological consequence of aging in male tissues, and considering the limited number of non-essential genes, it has been largely ignored in terms of human biology (124).
However, recent evidence suggests a prognostic or pathological role for LOY. Just within the last two decades there has been an increase in research surrounding ChY. Many have found negative correlations with changes in ChY or LOY and pathologies such as cardiovascular disease, Parkinson’s, schizophrenia, age related macular degeneration, cancer and overall mortality in men (125–129). Unfortunately, more information is still needed in regards to the genetic information of the ChY in order to acquire a complete understanding of the full potential of ChY in human disease.

B. ChY in Cancer

Overall, men are more likely to develop and die from cancer (72). Many theories have been offered to explain the disparities in cancer incidence and survival between the sexes, most of which are attributed to differences in hormone regulation, immune surveillance, and external factors (e.g. tobacco use). Numerous studies have also shown associations with genome instability, aneuploidy and cancer risk and mortality primarily for somatic chromosomes, enabling the identification of strengths and weaknesses in different cancers (130,131). However, while all of these have been fairly well elucidated, it is only recently that changes in ChY, especially LOY, has emerged as an additional factor in regards to male cancer susceptibility, tumorigenesis and survival.

Studies done in prostate cancer have eluded that LOY is beneficial for tumorigenesis and could potentially be used as a biomarker. Utilizing the prostate cancer cell line PC-3, one study revealed that restoration of ChY to the cells resulted in less tumorigenic phenotypes \textit{in vitro} and \textit{in vivo} (132). Another study showed that different ChY haplotypes could potentially be used to assess prostate cancer risk (133). More recently, a group identified the ChY specific histone demethylase \textit{KDM5D} as a potential ChY linked tumor suppressor as its loss resulted in more aggressive prostate tumors (134). LOY has also been implicated in increased risk for developing testicular germ cell tumors (135). Additionally, in male breast cancer, it’s been shown that the ChY gene \textit{TMSB4Y} is a possible tumor suppressor and that loss of the gene via loss of ChY promotes a more tumorigenic phenotype (136). An additional study determined that clonal ChY in male breast cancer can begin as early as ductal carcinoma \textit{in situ} (DCIS), and is primarily associated with Estrogen Receptor (ER) and Progesterone (PR) negative breast cancer subtypes (137). In addition to prostate, testicular, and male breast cancer, LOY has been observed in other
cancers including head and neck squamous cell carcinoma, urothelial bladder cancer, clear cell renal cell carcinoma, pancreatic adenocarcinoma, and NSCLC, all of which describe LOY as beneficial for the respective tumor types (138–141). Additionally, here have been multiple studies investigating LOY in the blood as prognostic indicator for patient survival or a biomarker for cancer risk. The overall findings within these studies conclude that LOY negatively correlates with patient survival and carcinogenesis (142–144). Interestingly, it was also noted that smoking is an external factor that can accelerate LOY, which adds to the already known fact that smoking has a major role in the development of multiple types of cancers (145).

C. ChY LncRNAs in Cancer

Due to incomplete sequencing data, the non-coding regions of ChY still remain an elusive source of potential in cancer cell biology. However, there are a few studies that have sought to begin identifying and characterizing ncRNAs, specifically lncRNAs from ChY. Currently, however, there are only 2 publications describing a role for the ChY lncRNA TTTY15. Interestingly, in prostate cancer TTTY15 behaves in an oncogenic capacity and is found upregulated in patient tumor samples, but the opposite is seen in NSCLC (146,147). Since these are the only two studies available, it is obvious that more information is needed to fully elucidate the effects of ChY lncRNAs in cancer.

VI. References


63. Gutschner T, Diederichs S. The hallmarks of cancer: A long non-coding RNA point of


75. Cheung WKC, Nguyen DX. Lineage factors and differentiation states in lung cancer


a candidate tumor suppressor on the Y chromosome and is deleted in male breast cancer. Oncotarget. 2015;6.


Chapter 2

Evidence of Y Chromosome LncRNAs involved in Radiation Response of Male Non-Small Cell Lung Cancer Cells

(Accepted to Cancer Research May 2020)

Tayvia Brownmiller1, Jamie A. Juric1, Abby D. Ivey1, Brandon M. Harvey1, Emily S. Westemeier1, Michael T. Winters1, Alyson M. Stevens1, Alana N. Stanley1, Karen E. Hayes2, Samuel A. Sprowls3, Amanda G. Ammer1, Mackenzie Walker1, Xiaoliang Wu5, Zuan-Fu Lim5,6, Lin Zhu5, Erik A. Bey4, Gangqing Hu1,7, Sijin Wen8, Patrick C. Ma5, and Ivan Martinez1

1Department of Microbiology, Immunology & Cell Biology, West Virginia University Cancer Institute, School of Medicine, West Virginia University, Morgantown, West Virginia. 2Modulation Therapeutics, West Virginia University, Morgantown, West Virginia. 3Department of Pharmaceutical Sciences, School of Pharmacy, West Virginia University, Morgantown, West Virginia. 4Department of Biochemistry and Molecular Biology, School of Medicine, Indiana University, Indianapolis, Indiana. 5Penn State Cancer Institute, Penn State Health Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania. 6Cancer Cell Biology Program, West Virginia University School of Graduate Studies, West Virginia University, Morgantown, WV. 7Department of Biostatistics, School of Public Health, West Virginia University, Morgantown, West Virginia. 8Bioinformatics Core, West Virginia University, Morgantown, West Virginia.

Running Title: Radiation responsive Y chromosome LncRNAs in male NSCLC

Keywords: Non-small cell lung cancer, radiation therapy, Y chromosome, LncRNA

Corresponding Author: Ivan Martinez, West Virginia University, 1 Medical Center Dr., PO Box 9300, Morgantown, West Virginia, 26506. Email: ivmartinez@hsc.wvu.edu, Phone: 304-581-1934, Fax: 304-293-4667

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST
The authors declare no conflicts of interest.
Abstract
Numerous studies have implicated changes in the Y chromosome in male cancers, however few have investigated the biological importance of Y chromosome non-coding RNAs. Here we demonstrate a group of Y chromosome-expressed long non-coding RNAs (lincRNAs) involved in male non-small cell lung cancer (NSCLC) radiation sensitivity. Radiosensitive male NSCLC cell lines demonstrated a dose-dependent induction of linc-SPRY3-2/3/4 following irradiation, not observed in radioresistant male NSCLC cell lines. Cytogenetics revealed the loss of chromosome Y (LOY) in the radioresistant male NSCLC cell lines. Gain- and loss-of-function experiments indicated that linc-SPRY3-2/3/4 transcripts affect cell viability and apoptosis. Computational prediction of RNA binding proteins (RBPs) motifs and UV Crosslinked Immunoprecipitation (CLIP) assays identified IGF2BP3 (an RBP involved in mRNA stability) as a binding partner for the linc-SPRY3-2/3/4 RNAs. The presence of linc-SPRY3-2/3/4 reduced the half-life of known IGF2BP3 binding mRNAs, such as the anti-apoptotic HMGA2 mRNA, as well as the oncogenic c-MYC mRNA. To assess the clinical relevance of these findings, we examined the presence of the Y chromosome in NSCLC tissue microarrays and the expression of linc-SPRY3-2/3/4 in NSCLC RNAseq and microarray data. We observed a negative correlation between the loss of the Y chromosome or linc-SPRY3-2/3/4, and overall survival. Thus, linc-SPRY3-2/3/4 expression and LOY could represent an important marker of radiation therapy in NSCLC.

Graphical Abstract
Statement of Significance
This study describes previously unknown Y chromosome-expressed IncRNA regulators of radiation response in male NSCLC. Furthermore, our findings show a correlation between loss of chromosome Y and radioresistance.
I. Introduction

Lung cancer is the most commonly diagnosed cancer worldwide and is the number one cause of cancer-related mortality (1). While there have been great strides in advancing treatment modalities for NSCLC, radiation therapy (RT) resistance remains a significant challenge. Previous studies have demonstrated mechanisms of radioresistance that include alterations in proteins involved in cellular response to DNA damage, the repair of DNA, apoptosis, hypoxia, and cell cycle regulators, among others (2,3). Regulatory non-coding RNAs have also been implicated in the development of radioresistance in NSCLC (4).

Regulatory non-coding RNAs include mature microRNAs (miRNAs) of around 22 nucleotides and long non-coding RNAs (lncRNAs) of >200 nucleotides (5). Studies have shown that most LncRNAs do not contain an open reading frame and could regulate a wide variety of processes through diverse mechanisms, including acting as signals (e.g. enhancers of transcriptional regulation), guides (e.g. recruitment of chromatin modifiers enzymes), or competing endogenous RNAs (e.g. sequestering miRNAs or RNA binding proteins) (6). The dysregulation of the expression and function of lncRNAs has been implicated in numerous cancer types, suggesting their potential use as biomarkers, prognostic indicators, or therapeutic targets (7). In NSCLC, lncRNAs have been associated with nearly every facet of cancer cell biology (8). Several lncRNAs have been associated with the development of resistance to radiotherapy (9). Examples of this include MALAT1 and HOTAIR, which are responsible for inhibiting apoptosis and promoting epithelial to mesenchymal transition thereby aiding in radioresistance (10).

Non-coding RNA genes, including those coding for lncRNAs have been mapped to every human chromosome, including the X and Y chromosomes (11). LncRNAs that map to the X chromosome include XIST, which is critical for the initiation of X-inactivation. The dysregulation of XIST has been shown in multiple cancers (7,12). Other X chromosome lncRNAs include TSIX and XACT, which also function in regulating X chromosome inactivation (12). In contrast, few studies have considered the possibility that the Y chromosome contains genes encoding regulatory RNAs (13-15). Only 27 distinct protein-coding genes are present on the Y chromosome, most of which function in sex determination and male fertility (16,17). The remaining are considered
potential non-coding genes and pseudogenes (18). This suggests the Y chromosome is an untapped source of non-coding information, and could potentially provide new insights into numerous diseases - including NSCLC.

Here we report, for the first time, the importance of the lncRNAs linc-SPRY3 (also known as lnc-BPY2C): linc-SPRY3-2, linc-SPRY3-3, linc-SPRY3-4 (linc-SPRY3-2/3/4) in male NSCLC radiation response. Gain and loss of function experiments demonstrated that linc-SPRY3-2/3/4 are involved in radiation vulnerability of male NSCLC cells and tumor samples containing chromosome Y or linc-SPRY3-2/3/4. Together our findings suggest a role of male-specific lncRNAs in NSCLC radiation sensitivity.

II. Materials and Methods

Cell Culture
Non-small cell lung cancer cell lines H460, H820, H157, H1299, A549, and WVU-Ma-0005 were cultured with DMEM (Sigma-Aldrich, St. Louis, MO, USA, D7777). H1650 and Ma-ALK-0001 cells were grown in RPMI (Corning, Corning, NY, USA, 50-020-PC). DMEM and RPMI media was supplemented with 10% fetal bovine serum (Gemini Bio-products, Sacramento, CA, USA, 100-106), L-glutamine (Gibco-LifeTech, Grand Island, NY, USA, 25030-081), HEPES (GE Healthcare Life Sciences, Logan, UT, USA, SH30237.01), penicillin–streptomycin (Gibco, 15140-122), and amphotericin B (Gibco, 15290-026). Human bronchial epithelial cells (HBEC) were cultured with keratinocyte-SFM supplemented with bovine pituitary extract, human recombinant epidermal growth factor, and L-glutamine (Gibco, 10724-011). All cells were grown in a humidified incubator at 37°C in the presence of 5% CO2. Cell lines HBEC, H460, H820, H157, A549, H1299, and H1650 were kindly provided by Dr. Erik A. Bey (Indiana University) and patient derived cell lines WVU-Ma-0005 and Ma-ALK-0001 were kindly provided by Dr. Patrick C. Ma (Pennsylvania State University).

Microarray analysis
For global IncRNA expression, we used the GeneChip® Human Transcriptome Array 2.0 (ThermoFisher Scientific, Affymetrix, Santa Clara, CA, USA) that assesses 22,829 non-protein
coding human genes. Microarray processing was carried out in the West Virginia University Genomics Core Facility. Briefly, 1 μg of total RNA was labeled using the Flashtag RNA labeling kit (Genisphere, Hatfield, PA, USA) according to the manufacturer's instructions. Each sample was hybridized to the array at 48°C and 60 rpm for 16 hours then washed and stained on Fluidics Station 450 (Fluidics script FS450_0003) and finally scanned on a GeneChip® Scanner 3000 7G (Affymetrix, Santa Clara, CA, USA). Feature intensities were extracted by using GeneChip® Human Transcriptome Array 2.0 library files. Array data was deposited into the NCBI Gene Expression Omnibus (GEO) database (GEO accession #GSE147708).

**Quantitative RT-PCR**

Total RNA was extracted using Trizol Reagent (Ambion, Austin, TX, USA, 15596026) per manufacturer’s instructions, then treated with Turbo DNAfree DNase (Invitrogen, Carlsbad, CA, USA, AM1907) for 25 minutes at 37°C. RNA concentrations were determined with a Nanodrop 2000 Spectrophotometer. 0.5 – 1 μg of total RNA was converted to cDNA using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA, 170-6891), followed by qRT-PCR using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, 172-5271) and several pair of primers to specifically amplify the genes of interest in this study (Supplementary Table S4). Relative expression was calculated using the double delta CT method (relative expression = 2^(-ΔCT); where ΔCT = CT (Target RNA) – CT (endogenous control RNA)), where the endogenous control for lncRNA and mRNA was GAPDH and/or UBC.

**Virtual Northern Blot**

Total RNA (60μg) from 8Gy treated H460 cells was resolved on a 1.2% formaldehyde denaturing agarose gel overnight. The gel was imaged (to serve as ladder) and then fragmented into 25 fractions of equal size and RNA was recovered from each fraction by gel extraction. The recovered RNA was then used for qRT-PCR analysis of each individual lncRNA. The values generated by qRT-PCR were plotted as a fraction of the total RNA. Plotted values were a mean of three technical replicates per band.

**Clonogenic Cell Survival Assay**
Cells were seeded in triplicate 60mm gridded dishes containing 4 mL of media at 250 cells per dish. Cells were treated with sham or a single dose of radiation (2Gy, 4Gy, or 8Gy), and then colonies were allowed to form for 10-15 days. Colonies were fixed and stained with crystal violet in 33% methanol, and manually counted using a microscope. Surviving fraction values relative to the sham controls were plotted as a function of radiation dose.

**Viral Transduction of shRNAs**

HEK-293T cells were transfected with the lentiviral plasmid pLKO-shScramble (Addgene #1864) or pLKO.1-TRC cloning vector (Addgene #10878) containing shRNA sequences against linc-SPRY3-2, linc-SPRY3-3, or linc-SPRY3-4 and lentiviral packaging plasmids (PsPax2 Addgene #12260, and VSV-G Addgene #8454) using calcium phosphate transfection. Lentivirus was collected after 48 hours, filtered (0.45 µm) and supplemented with polybrene (1 µg/mL, MilliporeSigma, Burlington, MA, USA, TR-1003-G). WVU-Ma-0005 or H460 cells were exposed to lentivirus for 16-72 hours and allowed to recover for 48 hours post infection. Infected cells were stably selected via puromycin treatment (1.0-2.5 µg/mL) for 72 hours. Efficiency of shRNA knockdown was measured using qRT-PCR.

**Nucleofection of Y Chromosome BAC**

The bacterial artificial chromosome (BAC) RP11-88F4 clone, from the RPCI-11 Human Male BAC Library (containing a 95,171bp insert from ChrY:58,819,440-58,914,611 [GRCh37/hg19]), was purchased from the Children’s Hospital Oakland Research Institute and introduced to cells by nucleofection. A549 cells (4x10⁶ cells/cuvette) were briefly resuspended in nucleofection buffer, and 16 µg of RP11-88F4 or pBACe3.6 (control) plasmid DNA was added. The cuvette was placed on the Amaza Nucleofector II device and the appropriate program was selected (Nucleofector® Program X-001). Immediately after the Nucleofection process ended, cells were resuspended in rescue media and transferred into 6-well plates and incubated at 37ºC under 5% CO₂. Expression of linc-SPRY3-2/3/4 was measured 48 hours post transfection by qRT-PCR.

**Flow Cytometry**

Cell viability and apoptosis were evaluated via Guava Easycyte HT Flow Cytometer (Millipore). Guava Viacount (Luminex, Austin, TX, 4000-0040) reagent was used for analysis of cell viability.
and Guava Nexin (Luminex, 4500-0455) reagent for apoptosis per manufacturer’s instructions. Cells were plated at equal densities in a 6-well plate, then sham treated or treated with a single dose of radiation (8Gy). Cells were trypsinized and collected 24, 48 and 72 hours after treatment. Parameters for the flow cytometer were set using sham controls.

**Tumor Growth Delay Assay (TGD)**

shControl or shlinc-SPRY3-2 (sh1) cells were trypsinized and suspended at 5 x 10^6 cells/mL in Matrigel. 1 x 10^6 cells were injected subcutaneously into the right flanks of male nude mice (Nu/Nu-088, Charles River, Wilmington, MA, USA). Once tumors reached ±150 mm³, RT was initiated. Treatment groups (3 animals each) included sham treated control (0Gy) and radiation (20Gy, 5 fractions). Tumor volume and depth was measured using ultrasound imaging (Vevo 2100, FUJIFILM VisualSonics Inc.). Measurements were quantified using the Vevo LAB 2.2.0 software. Tumors were assessed until they reached >800mm³, day 21 after initiation of treatment, or mice showed evidence of morbidity. Relative tumor volume was determined by normalizing measured volumes to the starting volume (Day 0) of each respective animal.

**UV Crosslinking and Immunoprecipitation (CLIP)**

CLIP was performed according to the original protocols with some modifications (19,20). The detailed method is described in the Supplementary data.

**RNA Stability Assay**

Cells were treated with 3µg/mL of Actinomycin D and then collected at 0 hours, 3 hours, 6 hours and 9 hours post treatment. RNA was then extracted and used for subsequent qRT-PCR analysis. *C. elegans* RNA was added as an exogenous spike for qRT-PCR (70 ng/µL) to amplify the worm specific gene *Ama-1*. Each time point was normalized to the 0 hour control.

**siRNA Transfection**

Cells were seeded at 3.5x10^5 cells per well in a 6-well dish. siRNAs were transfected utilizing the RNAiMax Lipofectamine reagent according to the manufacturer’s standard transfection protocol. 300pmol of scramble control siRNA (Horizon ON-TARGETplus Non-targeting Control siRNA
#2, D-001810-02-05) was used. For the targeted lncRNA siRNAs, 100pmol of each linc-SPRY3 specific siRNA was used to create a pool with a total concentration of 300pmol.

**Irradiation**
Radiation protocols were carried out on an Xstrahl XenX small animal radiation research platform (Suwanee, GA, USA). The XenX was commissioned following the AAMP Task Group 61 protocol for clinical irradiators of 40-300 kV (11439485). Dosage of experimental design was confirmed using Gafchromic EBT3 film (31198412, 29923495). Mice were anesthetized via isoflurane inhalation for the duration of each treatment. Each tumor was treated to a total dose of 20Gy over 5 fractions given every other day with a 1.2 cm circular fixed collimator at a dose rate of 3.62Gy/min corrected for tumor volume per mouse. For cells, a similar confirmation of dose rate for the experimental setup was determined by Gafchromic EBT3 film. Cells were treated in single fraction doses of 2Gy, 4Gy, or 8Gy with a variable collimator at a dose rate of 1.7Gy/min.

**DNA FISH AND Tissue Array Analysis**
DNA FISH in cell lines was performed and quantified by the WVU Cytogenetics Laboratory. DNA FISH for the tissue arrays was performed as previously described with the following modifications (21). Slides were first baked at 60°C before proceeding through the protocol. Slides were deparaffinized with Skipdewax (Insitus Biotechnologies, Albuquerque, NM, USA, #T213) for 20 minutes at 80°C followed by three washes with distilled water for 2 minutes each. This process was repeated three times. The SRY probe (Cytocell Ltd., Tarrytown, NY, USA, LPU 026-A) was allowed to hybridize for 22 hours. All tissue arrays were purchased from US Biomax, Inc. (Derwood, MD, USA, Lung: OD-CT-RsLung01-009, Testis: T231a, Cervix: T103). Imaging for representative images for the tissue array FISH was performed via epifluorescent microscopy on a Nikon A1R/N SIM-E microscope. Imaging for quantification of Y chromosome in the lung tissue array was performed on an Olympus VS120 Slide Scanner microscope. Quantification of Y chromosome FISH in the lung TMA was done using IMARIS software. Kaplan-Meier method was used to estimate the survival curves and log-rank test was used to assess the difference of survival curves between groups. TMA data for each patient was evaluated by percentage of Y chromosome positive cells out of the total cells examined, where the cut-off point to define high and low was obtained from the classification and regression tree.
RNA Seq and microarray Analysis from NSCLC patient data

RNA-Seq data for patients diagnosed with NSCLC were obtained from the Gene Expression Omnibus (GEO) database (accession #GSE81089) (22). The expression of linc-SPRY3-2 was measured by TPM (transcripts per millions reads) by salmon (23). A TPM value below 0.1 was deemed as not-expressed. The threshold was determined by comparing to the values obtained from female patients, where linc-SPRY3-2 should not express. The survival period was estimated as days between surgery and vital date (i.e., day of death or latest contact per the definition from GSE81089). For the microarray data, the expression of linc-SPRY3-2 from male Early Stage Lung Squamous Cell Carcinomas patients were obtained from GEO database (accession #GSE74777) based on Affymetrix Human Transcriptome Array 2.0. The survival analysis was done by comparing the top 10% high expressing patients of linc-SPRY3-2. The survival curves were made with the survival R package, and p-value was calculated by log-rank test.

Cell Fractionation

Cells were pelleted and immediately processed for cell fractionation to extract RNA (protocol adapted from previous publication (24). Pelleted cells were disrupted using an autoclaved glass Dounce homogenizer. Homogenate was centrifuged to pellet the nuclear fraction and the cytoplasmic fraction (supernatant) was collected. Each fraction was then treated with DNase I (New England Biolabs) for 20 minutes at 37°C and then RNA was extracted using Trizol Reagent (Life Technologies) per manufacturer’s instructions. Once the RNA was extracted it was treated with Turbo DNAfree DNase (Ambion) for 20 minutes at 37°C. RNA concentrations were obtained using a Nanodrop 2000 Spectrophotometer (Thermo Scientific). qRT-PCR for cell fractionations was normalized using an exogenous spike of C. elegans RNA (70 ng/µL) and the worm specific gene Ama-1.

Statistical Analysis

Data are presented as mean ±SD, *P < 0.05; **P < 0.01; ***P < 0.001. The statistical significance between experimental groups was determined by ANOVA followed by Tukey’s multiple comparisons test. Analyses were performed using GraphPad Prism V8 and Microsoft Excel 2013.
III. Results

Identification of radiation-responsive lncRNAs expressed from the Y chromosome

To identify lncRNAs potentially involved in radiation response of NSCLC cells, we profiled the expression of over 20,000 human lncRNAs in the male NSCLC H460 cell line irradiated with 7 Gy of ionizing radiation (IR), compared to unirradiated (0 Gy) H460 cells 24 hours post-treatment. Using a change in gene expression of 1.5-fold or more, we identified 106 lncRNAs as up-regulated following exposure to radiation, while 59 lncRNAs exhibited down-regulation (Supplementary Fig. S1, Supplementary Table S1). Among the highest upregulated lncRNAs, we observed changes in transcripts annotated as linc-SPRY3-2, linc-SPRY3-3, and linc-SPRY3-4, also known as lnc-BPY2C-4, lnc-BPY2C-25, and lnc-BPY2C-2, respectively. Surprisingly, the linc-SPRY3-2/3/4 transcripts map to a region of the Y chromosome heterochromatic block known as the DYZ1 region that contains a single H3K27Ac mark suggesting transcription in this region (Fig. 1A). The DYZ1 region has been described as genetically inert since it is composed of heterochromatin and, due to its highly repetitive nature, remains poorly sequenced (16). Previously attempted sequence analyses of the human Y chromosome have revealed numerous copies of the pentanucleotide repeat motif 5’-TTCCA-3’ in the DYZ1 region (25,26). Only one previous publication has shown expression of two non-coding RNAs (AK128024.1 and AY598346.2) from the DYZ1 region in a testis-specific manner (27). The linc-SPRY3-2/3/4 sequence fragments obtained from the online database LNCipedia (28) contain several 5’-TTCCA-3’ repeat motifs (Fig. 1B) and partially overlap with AK128024.1 and AY598346.2 (Supplementary Fig. S2), further supporting that these lncRNAs are transcribed from the DYZ1 region of the Y chromosome. Using the linc-SPRY3-2/3/4 sequence fragments, we designed primers to specifically amplify each linc-SPRY3 transcript in order to perform virtual northern blot analysis. Using RNA from 8Gy treated H460 cells, we found two peaks of amplification migrating around 5Kb and 1Kb regardless of which linc-SPRY3 primers were used. This suggests linc-SPRY3-2/3/4 could be partial sequences of a longer lincRNA transcript(s). (Supplementary Fig. S3).

We next developed qRT-PCR assays of the linc-SPRY3 transcripts to assess their expression in H460 cells following exposure to a single dose of IR (8Gy) at different time points.
post-treatment versus non-irradiated controls. We observed a 5-fold induction in the expression of the linc-SPRY3-2/3/4 transcripts four hours post-exposure to radiation, that increased to almost 15-fold after eight hours and remained elevated for at least twelve hours after treatment (Fig. 1C). Furthermore, H460 cells exhibited a dose-dependent increase from 5-fold (2Gy) to 50-fold (8Gy) in the expression of linc-SPRY3-2/3/4 after 72 hours post-exposure to IR (Fig. 2B).

**Linc-SPRY3-2/3/4 expression reveals a dose responsive signature to IR**

In order to expand our findings, we examined the expression of the linc-SPRY3 transcripts in several male NSCLC cell lines: the radiosensitive cell lines H820 and H157, and three radioresistant lines: H1299, A549 and H1650 (29-33). We also included two early passage male patient-derived NSCLC cell lines: WVU-Ma-0005 and Ma-ALK-0001. Clonogenic cell survival assays were performed to determine and verify the radiation response of these NSCLC cell lines. Our clonogenic assay data confirmed the radiosensitivity of H460 and H820 as well as the radioresistance of A549 and H1299, which were used as controls for the characterization of the response of the patient-derived cell lines. Our data showed that the WVU-Ma-0005 cell line is radiosensitive and the Ma-ALK-0001 cell line is radioresistant (Supplementary Fig. S4). Next, cells were treated with a single dose of IR (2Gy, 4Gy, or 8Gy) and collected 72 hours post-treatment. Controls were established by treating the cells identically in parallel and omitting the irradiation step. We observed that human bronchial epithelial cells (HBEC), which served as a normal control, the radiosensitive cell lines H820, WVU-Ma-0005 and H157 demonstrated a dose-dependent increase in expression of the linc-SPRY3 RNAs, (Fig. 2A-D, Supplementary Fig. S5H) similar to H460 cells. In contrast, the radioresistant cell lines A549, H1299, Ma-ALK-0001 and H1650 showed minimal to no expression of these lncRNAs when compared to no retrotranscription (RT) controls (Fig. 3E-G and Supplementary Fig. S5I). To confirm that this expression profile is specific to male radiosensitive NSCLC cells, we investigated the expression of the linc-SPRY3 transcripts in two established female NSCLC cell lines H1975 (radiosensitive) and H1819 (radioresistant) (29). qRT-PCR revealed no amplification signal above background in the female cell lines, a finding consistent with the Y-specific mapping of the linc-SPRY3 genes (Supplementary Fig. S6). Finally, we assessed the coding potential and cellular location of the linc-SPRY3 RNAs in H460 and WVU-Ma-0005 cells. In both cell lines, we detected linc-SPRY3 RNA species exclusively in the nucleus (Supplementary Fig. S7A and S7B) and we found no
coding probability in any of the lncRNA transcripts (Supplementary Fig. S7C) supporting our hypothesis of their potential non-coding function.

**Loss of the linc-SPRY3 transcripts in the radioresistant cell lines is due to loss of the Y chromosome**

The differences in the expression of the linc-SPRY3 transcripts in radiosensitive and radioresistant NSCLC cell lines prompted us to hypothesize that radioresistant lines harbored genetic changes involving chromosome Y. There are reports of changes in the Y chromosome in cancers dating back several decades that include evidence for the mosaic loss of chromosome Y (LOY) (21,34-41). LOY involves the loss of the entire Y chromosome and occurs in human male tissues as a consequence of aging, however, only recent studies have begun validating a correlation between LOY and cancer biology (18,21,36-38,40-45). Using DNA-FISH, we investigated the sex chromosome status of our panel of cell lines. As expected, the immortalized HBECs showed a normal male genotype containing a single copy of the X and Y chromosomes (Fig. 2H). The radiosensitive cell lines (H460, H820, and H157) possessed multiple copies of both the X and Y chromosomes, while the patient-derived cell line WVU-Ma-0005 contained a single copy of the X and Y chromosomes (Fig. 2I-K and Supplementary Fig. S5L). Interestingly, the radioresistant cell lines A549, H1299, Ma-ALK-0001 and H1650 showed complete loss of the Y chromosome (Fig. 2L-N and Supplementary Fig. S5M). Cytogenetic analysis and quantification of LOY in all the NSCLC cell lines verified our DNA-FISH analysis, (Supplementary Table S2) suggesting that loss of the linc-SPRY3 transcripts in radioresistant cell lines is due to LOY, and that these noncoding RNAs and potentially other genes mapping to the Y chromosome could influence the response of cells to radiation.

**The DYZ1 region of the long arm of the Y chromosome restores radiosensitivity**

As described above, the repetitive nature of the sequences around the gene(s) coding for the linc-SPRY3 transcript(s) (Fig. 1B) means no complete sequence(s) for these RNAs exists hindering gene-specific gain-of-function studies. However, we were interested to determine if a bacterial artificial chromosome (BAC, RP11-88F4) encompassing the DYZ1 region of the long arm of the human Y chromosome where the linc-SPRY3 transcript(s) are located (a 95,171bp insert from ChrY:58,819,440-58,914,611 [GRCh37/hg19]) could alter the response of the Y
chromosome negative, radioresistant NSCLC cell line A549 to IR. After 72 hours, we extracted DNA from the nucleofected cells and performed PCR for the BAC plasmid (pBACe3.6) to determine efficiency of the nucleofection (Supplementary Fig. S8). Next, we detected the expression of the three linc-SPRY3 transcripts in the BAC-transfected A549 cells (A549_88F4) by RT-PCR, but not in the A549 BAC control cells (A549_Ctrl) (Fig. 3A).

We measured cell viability and apoptosis in the A549_Ctrl and the A549_88F4 cells after a single dose of 8Gy IR. Notably, the A549_88F4 cells showed significantly worse cell viability when compared to control cells in response to treatment (Fig. 3B). Additionally, A549_88F4 cells showed increased apoptosis compared to control cells after IR with this difference reaching statistical significance at 48 and 72 hours (Fig. 3C). These experiments suggest that the DYZ1 region of the long arm of the Y chromosome harbors genes that influence the response to IR, which includes the linc-SPRY3 family. To obtain further evidence to support this hypothesis, we conducted a series of loss-of-function studies using shRNAs to reduce the levels of the linc-SPRY3 transcripts in NSCLC cells.

First, we designed and validated two shRNAs; one targeting a linc-SPRY3-2 sequence (sh1) and one a linc-SPRY3-3 sequence (sh2) (Supplementary Table S3). Interestingly, we observed a significant reduction in the levels of all three linc-SPRY3 RNAs in H460 and WVU-Ma-0005 cells (Fig. 3D and 3G), consistent with our hypothesis that linc-SPRY3 transcripts may be a longer lincRNA transcript. Both H460 and WVU-Ma-0005 cells expressing the linc-SPRY3 shRNAs exhibited increased survival and reduced apoptosis after 8Gy IR in comparison to control cells (Fig. 3E-F & 3H-I); consistent with a loss of linc-SPRY3 transcripts expression resulting in resistance to radiation.

We next sought to determine if reducing the expression of the linc-SPRY3 transcripts would result in IR resistance in vivo. Briefly, 6-8 week old mice were injected with H460 or WVU-Ma-0005 cells harboring either the shCtrl or sh1 constructs. Once tumors reached a volume of 150mm³, mice were treated with fractionated IR to a total dose of 20 Gy, mimicking the clinical procedures for patients with NSCLC (Supplementary Fig. S9A). Ultrasound imaging was used to ensure accurate measurement of both tumor depth and volume, the appropriate settings for IR
administration, as well as the tracking tumor growth (Supplementary Fig. S9B-C). Untreated mice showed rapid development of tumors regardless of the type of shRNA used (Supplementary Fig. S9D-E). As expected, IR treated mice exhibited slower tumor progression, while sh1 tumors showed larger tumor volumes both during and after IR compared to shCtrl tumors. This suggests that loss of linc-SPRY3 transcripts results in tumors with higher resistance to RT (Fig. 3J-K and Supplementary Fig. S9D-E).

The linc-SPRY3 transcripts interact with the RNA binding protein IGF2BP3 and affect RNA stability in HMGA2 and c-MYC mRNAs

In an attempt to identify a potential mechanism for the linc-SPRY3-2/3/4 RNAs we utilized A daTabase of RNA binding proteins and AssoCiated moTifs (ATtRACT) software (46) to predict RNA binding proteins (RBPs) potentially interacting with the linc-SPRY3 transcripts. The analysis revealed 13 potential RBPs interacting with the linc-SPRY3 transcripts but the largest number of predicted binding sites across all three family members was the RBP Insulin Like Growth Factor 2 mRNA Binding Protein 3 (IGF2BP3, also known as IMP3) (Fig. 4A). To confirm the predicted association of IGF2BP3 with the linc-SPRY3 RNAs, we performed UV crosslinking and immunoprecipitation (CLIP) for IGF2BP3 in irradiated H460 cells. RT-PCR of the recovered RNA revealed that IGF2BP3 does indeed directly interact with all three linc-SPRY3 family members as well as the known binding partner HMGA2 mRNA (47) but not with the control GAPDH (Fig. 4B). IGF2BP3 is a known RBP whose functions include stabilizing mRNAs. Two such targets include c-Myc and HMGA2 (48). These two targets were of specific interest to us, since previous studies have shown that downregulation of c-Myc and HMGA2 results in an increase in apoptosis in multiple cancers including lung cancer (49-54).

In order to confirm that the association of IGF2BP3 with the linc-SPRY3 family members affects its ability to target and stabilize c-Myc and HMGA2, we performed RNA stability assays using Actinomycin D (ActD) to stop global transcription in A549 cells with or without the expression of the linc-SPRY3 transcripts (A549_Ctrl and A549_88F4 cells) as well as the knockdown of the linc-SPRY3 transcripts by a pool of siRNAs (siPool). qRT-PCR analysis revealed more rapid degradation of HMGA2 and c-Myc mRNAs in the A549_88F4 siCtrl cells when compared to A549_Ctrl siCtrl cells but GAPDH mRNA was not affected (red bar compared
to black bar) (Fig. 4C). Furthermore, we observed rescue of c-Myc and HMGA2 mRNAs stabilization in the A549_88F4 siPool cells after the knockdown of the linc-SPRY3 transcripts (Fig. 4C and Supplementary Fig. S10). Overall, these data suggest that when the linc-SPRY3 RNAs are present they could potentially act as competing endogenous RNAs (ceRNAs) for IGF2BP3, thereby preventing it from stabilizing its mRNA targets c-Myc and HMGA2 affecting apoptosis response.

**Y chromosome loss and loss of the linc-SPRY3 RNAs are potential poor prognostic indicators for male NSCLC**

To assess the potential clinical relevance of the presence or absence of the Y chromosome and radiation sensitivity and resistance, we performed DNA-FISH for the Y chromosome on a lung cancer tissue microarray and quantified LOY for 30 male cores as well as a testicular cancer core (positive control) and a cervical cancer core (negative control). Representative images taken of male lung cancer cores and a testicular cancer core reveal numerous Y chromosome positive cells, while images taken of female lung cancer cores and a cervical cancer core reveal no detectable presence of the Y chromosome DYZ1 (Supplementary Fig. S11). Using images from male cores of the NSCLC tissue array, we applied a custom image analysis pipeline to determine the percentage Y chromosome positivity. The representative images demonstrate the DNA-FISH of a primary Y chromosome negative core (Fig. 5A, inset is absent of punctate dots) and a heavily Y chromosome positive core (Fig. 5C, arrows in inset indicate punctate dots). Figures 5B and 5D show the result of the image analysis in which Y chromosome negative cells are artificially colored purple and Y chromosome positive cells are artificially colored red. We next used the supplied clinical data to perform Kaplan-Meier analysis. Our analysis revealed a trend towards a negative relationship between Y chromosome loss and patient survival. Though not statistically significant, the trend revealed worse survival for those patients with < 25% Y chromosome positivity (Fig. 5E).

Finally, we accessed the publicly available microarray data (GSE74777) containing 107 Early Stage Lung Squamous Cell Carcinomas (96 male) and the NSCLC short-read RNAseq dataset (GSE81089) containing 198 samples (95 male) to assess the expression of the linc-SPRY3-2 transcript and clinical outcomes (the expression of the other two IncRNAs was only detected in
limited number of patients in the RNAseq dataset and therefore were not considered). Interestingly, male patients with high \textit{linc-SPRY3} transcripts expression (top 10\%) from the microarray data showed a better surviving outcome than the rest of the patients (\textbf{Fig. 5F}). Furthermore, in the RNA-seq study, male patients with tumors expressing low levels of \textit{linc-SPRY3}-2 exhibited a worse overall survival than those in which their tumor did express \textit{linc-SPRY3}-2 (\textbf{Supplementary Fig. S11I}). Further studies are needed to validate these initial findings (statistically not significant), but the trends observed in two independent studies suggest that the presence of the Y chromosome and the expression of \textit{linc-SPRY3} RNAs warrants further analysis for their functions in radiation response of male NSCLC tumors.

\textbf{IV. Discussion}

In this study the unbiased expression profiling of IncRNAs, following the irradiation of a NSCLC cell line, revealed the induced expression of three annotated IncRNAs that map to the highly repetitive, heterochromatic DYZ1 region of the Y chromosome: \textit{linc-SPRY3}-2, \textit{linc-SPRY3}-3, and \textit{linc-SPRY3}-4. Further investigation revealed expression of this group of IncRNAs is IR dose dependent and begins approximately 4 hours after administration of IR and maintained through 72 hours (\textbf{Fig. 1}). Interestingly, expression of these IncRNAs was only detected in radiosensitive cell lines, but not in radioresistant cell lines, regardless of their oncogenic driver mutations (\textbf{Supplementary Table S2}). Cytogenetic analysis of all cell lines showed loss of the Y chromosome in the radioresistant cell lines, while the radiosensitive cells lines retained their Y chromosome, explaining the differences seen in \textit{linc-SPRY3}-2/3/4 expression (\textbf{Fig. 2}). Gain-of-function and Loss-of-function experiments demonstrated that the \textit{linc-SPRY3} RNAs are important for cellular response to IR as statistically significant changes were seen in cell viability and apoptosis (\textbf{Fig. 3}). \textbf{In vivo} experiments further confirmed these observations as knockdown of the IncRNAs promoted radioresistance in the NSCLC cellular phenotype.

Additionally, we have provided significant evidence that these IncRNAs could act as competing endogenous RNAs (ceRNAs) for the RNA binding protein IGF2BP3 (also known as IMP3) (\textbf{Fig. 4}). In normal tissues, IGF2BP3 is primarily expressed during embryonic development, but its overexpression has been associated with numerous cancer types and as such has been
classified as an oncogenic protein (48,55). More specifically, it has been previously shown that IGF2BP3 is upregulated across all NSCLC subtypes and that its loss leads to increased apoptosis (56). As a RBP, IGF2BP3 regulates several genes primarily by binding and stabilizing target mRNAs in order to increase translation efficiency (48). Two such targets, as mentioned previously, are c-Myc and HMGA2. Interestingly, previous studies have shown that downregulation of c-Myc results in increased radiosensitivity by promoting apoptosis (49,50). Additionally, while there is little to no previous evidence of a role for HMGA2 in radiation sensitivity, there are multiple studies characterizing its relationship to apoptosis (52-54). Moreover, HMGA2 expression is controlled by the transcription factor c-Myc (57) which suggests a potential feedback loop interaction. Based on our data, we propose the hypothesis that the presence of the *linc-SPRY3-2/3/4*, following IR, sequester IGF2BP3 and inhibit the stabilization of c-Myc and HMGA2 which results in downregulation of their proteins making the cells more vulnerable to apoptosis following IR. On the other hand, we cannot discard the possibility that the *linc-SPRY3* transcripts could regulate the expression of c-MYC and HMGA2 at the transcriptional level. Lastly, DNA-FISH analysis, microarray analysis, and RNA-seq revealed a negative correlation between loss of the Y chromosome and loss of *linc-SPRY3-2* expression and overall patient survival, respectively (Fig. 5). This establishes, for the first time, a connection between the Y chromosome, the *linc-SPRY3* transcripts, and how male NSCLC cells respond to radiation.

In conclusion, we show for the first time the importance of a Y chromosome derived group of lncRNAs in regulating male NSCLC radiation response. We provide significant evidence that the *linc-SPRY3-2/3/4* transcripts are potentially tumor suppressive and their loss via LOY contributes to radioresistance in NSCLC cells. We also provide evidence that these lncRNAs directly bind IGF2BP3, preventing it from stabilizing its targets HMGA2 and c-Myc which we believe contributes to the radiosensitive phenotype. Moreover, we show a trending negative correlation between Y chromosome and *linc-SPRY3* loss and patient survival. We do, however, acknowledge the limitations of this study. More data is needed to fully validate a clinically relevant relationship of both the Y chromosome and *linc-SPRY3-2/3/4* family in NSCLC patient survival. It would also be interesting to investigate the behavior of these lncRNAs in an acquired resistance model, utilizing isogenic radioresistant cell lines generated from radiosensitive parental cells like the H460 and WVU-Ma-0005 cell lines.
Considering the dose dependent response in expression observed in the radiosensitive cell lines, we speculate that these nuclear lncRNAs could be involved in apoptosis and/or the DNA damage response and repair pathway. We also hypothesize that linc-SPRY3 transcripts and the Y chromosome could eventually be used as biomarkers for cancer radiotherapy. Overall, we believe that more attention should be given to the Y chromosome, as many of its non-coding regions remain uncharted. This has left a vast untapped resource in the realm of cancer cell biology that has yet to be explored.

V. Acknowledgements

This work was supported in part by WVU Cancer Institute, National Cancer Institute (NCI) Plan (2V882), American Cancer Society IRG Internal Pilot Funding (IRG-09-061-04), WVCTSI Award (NIH/NIGMS U54GM104942), and Tumor Microenvironment (TME) CoBRE Grant (NIH/NIGMS P20GM121322). Dr. Karen Hayes was supported in part by The Ladies Auxiliary to the VFW of the United States Cancer Research Fellowship (CK003229). Dr. Gangqing Hu was supported with Bioinformatic Core grant NIH-NIGMS U54 GM-104942. We thank Elisabeth Seftor (WVU Cancer Institute) for her assistance with flow cytometry analysis. We thank Dr. Malcolm Mattes (WVU Cancer Institute) for lending his expertise in radiation oncology. We thank Jamie Senft of the WVU Medicine Clinical Cytogenetics Laboratory for performing the DNA-FISH and providing the images and analysis for all of our cell lines. We thank Sarah Mclaughlin and the WVU Animal Models Imaging Facility (AMIF), as well as the WVU Microscope Imaging Facility (MIF) and their funding sources (NIH Grants P30GM103488, S10RR026378, U54GM104942, P20GM103434) for their assistance with animal and cell imaging. We thank Dr. Alexey Ivanov for his suggestions. We thank the staff of the WVUCI Preclinical Tumor Models core facility for their assistance with the in vivo study. We would also like to thank the WVU Bioinformatics Core and their funding source (NIGMS Grant U54 GM-104942) for their support with the RNAseq analysis. We would also like to thank Dr. Natasha Caplen of the National Cancer Institute (National Institutes of Health, Bethesda, MD) for her assistance in editing this work.
VI. References


VII. Figure Legends

Figure 1: Microarray analysis reveals a family of Y chromosome, radiation inducible long non-coding RNAs.

(A) Y chromosome schematic obtained from the UCSC Genome Browser demonstrating the genomic location of the three linc-SPRY3 transcripts in the DYZ1 region (also known as Inc-BPY2C). (B) Linc-SPRY3 transcripts sequences. Highlighted sections indicate pentanucleotide repeats. The black underline regions show the sequences target by the shRNAs and siRNAs used in this study (C) qRT-PCR analysis of the linc-SPRY3 family members validating the radiation induced expression in H460 cells. Time course expression following a single dose of 8Gy IR of linc-SPRY3-2/3/4. GAPDH mRNA was used to normalize qRT-PCR analysis. Error bars represent SD from the mean (n=3).
Figure 2: Radiation sensitive and radiation resistant NSCLC cell lines show significant difference in the expression of the linc-SPRY3 family due to Y chromosome loss.

qRT-PCR analysis of (A) HBEC and radiosensitive cell lines (B) H460, (C) H820, and (D) WVU-Ma-0005, demonstrates a dose dependent response in linc-SPRY3 expression. qRT-PCR analysis of radioresistant cell lines, (E) A549, (F) H1299, (G) Ma-ALK-0001, shows little to no change in linc-SPRY3 expression. Values are relative to untreated control (0Gy) of the same collection day. GAPDH mRNA was used to normalize qRT-PCR analysis. Error bars represent SD from the mean (n=3). (H-N) Cytogenetic analysis via DNA-FISH reveals complete loss of the Y chromosome in the radioresistant cell lines while HBECs and radiosensitive cell lines retain their Y chromosome. Images are a representative from 200 counted cells.

Figure 3: Linc-SPRY3 RNAs are important for cell survival following 8Gy irradiation

(A) RT-PCR analysis shows expression of the linc-SPRY3 family in BAC RP11-88F4 nucleofected A549 cells. (B-C) Overall cell viability and apoptosis were measured by flow cytometry. Y chromosome BAC nucleofection results in lower cell viability and increased apoptosis compared to control in radioresistant A549 cells after 8Gy IR. (D, G) qRT-PCR analysis reveals effective knockdown of the linc-SPRY3 RNAs in radiosensitive cell lines H460 and WVU-Ma-0005. (E-F, H-I) Knockdown of the linc-SPRY3 family in both H460 and WVU-Ma-0005 cells using two different shRNAs against linc-SPRY3 family (sh1 and sh2) resulted in better overall cell viability and increased resistance to apoptosis when compared with shControl (shCtrl) after 8Gy IR. Untreated controls are provided to demonstrate baseline cell viability and apoptosis. (J-K) Tumor growth delay assay (n = 3) shows higher resistance to radiation of the sh1 tumors relative to shCtrl tumors in both H460 and WVU-Ma-0005 animal experiments. Mean relative tumor volumes are plotted. Error bars represent SD from the mean (n=3) (A-I). Error bars represent SD from the mean of 3 mice in each group (J-K). Untreated tumor controls are represented in Supplementary Fig. S9. ANOVA was used to determine statistical significance.
Figure 4: The linc-SPRY3 family act as molecular sponges for IGF2BP3 preventing it from stabilizing HMGA2 and c-Myc.

(A) Predicted binding motifs (using WebLogo3) of IGF2BP3 in each linc-SPRY3 transcript. (B) RT-PCR amplification of the recovered RNA from IGF2BP3 CLIP assays of untreated (No Radiation) or treated (8 Gy) H460 cells. The lanes are as follows (from left to right): Input RNA, IgG Supernatant, IGF2BP3 Supernatant, IgG Immunoprecipitation, IGF2BP3 Immunoprecipitation. HMGA is provided as a positive control and GAPDH as a negative control for IGF2BP3 interaction. Gel images are a representative of duplicate experiments. (C) Actinomycin D (ActD) RNA stability assays show rapid degradation of HMGA2 and c-Myc mRNA when the linc-SPRY3 RNAs are present (A549_88F4 siCtrl). siRNA knockdown rescues mRNA stability of HMGA2 and c-Myc (A549_88F4 siPool). GAPDH is provided as a negative control. Error bars represent SD from the mean (n=3)

Figure 5: The DYZ1 region of the Y chromosome and the linc-SPRY3 family show negative correlations with patient survival.

(A-D) Representative images from the imaging analysis performed on 30 cores from a lung cancer tissue array to determine the percentage of Y chromosome DYZ1 positive cells. Y chromosome positive cells are red and Y chromosome negative cells are purple. (E) Kaplan-Meier analysis of the 30 cores analyzed for Y chromosome DYZ1 positivity. (F) Survival curves for NSCLC male patients (GSE74777).
VIII. Main Figures

Figure 1

A

B

Linc-SPRY3-2 (Lnc-BPY2C-4:2/Lnc-BPY2C-4:3/Lnc-BPY2C-4:4) 776/765/765 nt

Linc-SPRY3-3 (Lnc-BPY2C-25:1) 211 nt

Linc-SPRY3-4 (Lnc-BPY2C-2:2) 204 nt

C

Linc-SPRY3-2

Linc-SPRY3-3

Linc-SPRY3-4
Figure 2

A

B

C

D

E

F

G

H

I

J

K

L

M

N

Linc-SPRY3-2

Linc-SPRY3-3

Linc-SPRY3-4

Radiosensitive

Radioresistant

HBEC

H460

H820

WVU-Ma-0005

A549

H1299

Ma-ALK-0001

Relative Expression
Figure 3

A. RT-PCR

B. Cell Viability

C. Apoptosis

D. H460

E. Cell Viability

F. Apoptosis

G. WVU-Ma-0005

H. Cell Viability

I. Apoptosis

J. Relative Tumor Volume

K. Relative Tumor Volume
Figure 4

A

IGF2BP3 Sequence recognition motif:

Linc-SPRY3-2

Linc-SPRY3-3

Linc-SPRY3-4

B

<table>
<thead>
<tr>
<th>Input</th>
<th>IgG Sup.</th>
<th>IGF2BP3 Sup.</th>
<th>IgG IP</th>
<th>IGF2BP3 IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linc-SPRY3-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linc-SPRY3-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linc-SPRY3-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMGA2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No Radiation  8 Gy

C

HMG A2

Rel. values compared to CTRL at time point D

Time (hrs)

c-Myc

Rel. values compared to CTRL at time point D

Time (hrs)

GAPDH

Rel. values compared to CTRL at time point D

Time (hrs)
Figure 5

A

B

C

D

E

F

- < 25% Y-Chr positive cells (n=16)
- >= 25% Y-Chr positive cells (n=14)

Hazard Ratio = 1.33 (p = 0.59)

Overall Survival

Time (Months)

Fraction Surviving

Top 10% (n=10)
Others (n=86)  p = 0.2

Days
### IX. Supplemental Information

**Supplementary Table S1**

<table>
<thead>
<tr>
<th>LncRNA Gene</th>
<th>Transcript Cluster ID</th>
<th>Fold Change</th>
<th>ANOVA p-value</th>
<th>FDR p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF092843</td>
<td>TC12002437.hg.1</td>
<td>4.26</td>
<td>0.000022</td>
<td>0.000414</td>
</tr>
<tr>
<td>linc-OC1AD2-1</td>
<td>TC04002544.hg.1</td>
<td>4.17</td>
<td>0.000007</td>
<td>0.000159</td>
</tr>
<tr>
<td>linc-LOC100132288-2</td>
<td>TC21000805.hg.1</td>
<td>3.88</td>
<td>0.000028</td>
<td>0.000496</td>
</tr>
<tr>
<td>linc-SPRY3-4</td>
<td>TC0Y000283.hg.1</td>
<td>3.17</td>
<td>0.162867</td>
<td></td>
</tr>
<tr>
<td>linc-SPRY3-3</td>
<td>TC0Y000284.hg.1</td>
<td>3.04</td>
<td>0.09976</td>
<td></td>
</tr>
<tr>
<td>BC036485</td>
<td>TC03003223.hg.1</td>
<td>2.74</td>
<td>0.00001</td>
<td></td>
</tr>
<tr>
<td>linc-POTED-9</td>
<td>TC21000560.hg.1</td>
<td>2.73</td>
<td>0.071853</td>
<td>0.258323</td>
</tr>
<tr>
<td>linc-ZNF25-4</td>
<td>TC10002568.hg.1</td>
<td>2.55</td>
<td>0.005738</td>
<td>0.036702</td>
</tr>
<tr>
<td>linc-CDH6-5</td>
<td>TC05002316.hg.1</td>
<td>2.49</td>
<td>0.000003</td>
<td>0.000094</td>
</tr>
<tr>
<td>linc-FCGR1B-9</td>
<td>TC01005759.hg.1</td>
<td>2.43</td>
<td>0.000086</td>
<td>0.001207</td>
</tr>
<tr>
<td>BC057782</td>
<td>TC09002209.hg.1</td>
<td>2.3</td>
<td>1.12E-07</td>
<td>0.000007</td>
</tr>
<tr>
<td>AK025384</td>
<td>TC16001789.hg.1</td>
<td>2.15</td>
<td>9.63E-07</td>
<td>0.000037</td>
</tr>
<tr>
<td>linc-SPRY3-2</td>
<td>TC0Y000285.hg.1</td>
<td>2.13</td>
<td>0.187334</td>
<td></td>
</tr>
<tr>
<td>AK054573</td>
<td>TC17002305.hg.1</td>
<td>2.11</td>
<td>0.000099</td>
<td>0.001344</td>
</tr>
<tr>
<td>S81264</td>
<td>TC17002307.hg.1</td>
<td>2.11</td>
<td>1.37E-07</td>
<td>0.000002</td>
</tr>
<tr>
<td>AF085983</td>
<td>TC02004620.hg.1</td>
<td>2.07</td>
<td>6.60E-09</td>
<td>9.36E-07</td>
</tr>
<tr>
<td>NR_027299</td>
<td>TC03002817.hg.1</td>
<td>2.07</td>
<td>1.70E-07</td>
<td>0.00001</td>
</tr>
<tr>
<td>AX750576</td>
<td>TC17002329.hg.1</td>
<td>2.03</td>
<td>7.14E-07</td>
<td>0.000029</td>
</tr>
<tr>
<td>linc-NAA35-2</td>
<td>TC09002052.hg.1</td>
<td>2.01</td>
<td>0.000004</td>
<td>0.000112</td>
</tr>
<tr>
<td>NR_026866</td>
<td>TC03002367.hg.1</td>
<td>1.93</td>
<td>7.03E-07</td>
<td>0.000029</td>
</tr>
<tr>
<td>BC033874</td>
<td>TC01006039.hg.1</td>
<td>1.89</td>
<td>0.000209</td>
<td>0.002472</td>
</tr>
<tr>
<td>BC061593</td>
<td>TC05002175.hg.1</td>
<td>1.89</td>
<td>3.79E-09</td>
<td>6.30E-07</td>
</tr>
<tr>
<td>BX537506</td>
<td>TC08002539.hg.1</td>
<td>1.89</td>
<td>5.12E-09</td>
<td>7.85E-07</td>
</tr>
<tr>
<td>AY665470</td>
<td>TC12002292.hg.1</td>
<td>1.89</td>
<td>8.29E-10</td>
<td>2.12E-07</td>
</tr>
<tr>
<td>NR_044995</td>
<td>TC13001319.hg.1</td>
<td>1.85</td>
<td>2.64E-07</td>
<td>0.000013</td>
</tr>
<tr>
<td>BX647541</td>
<td>TC16001788.hg.1</td>
<td>1.85</td>
<td>2.70E-10</td>
<td>1.07E-07</td>
</tr>
<tr>
<td>AF172940</td>
<td>TC02003138.hg.1</td>
<td>1.83</td>
<td>1.90E-08</td>
<td>0.000002</td>
</tr>
<tr>
<td>M73837</td>
<td>TC10002092.hg.1</td>
<td>1.83</td>
<td>0.000549</td>
<td>0.005416</td>
</tr>
<tr>
<td>linc_luo_466</td>
<td>TC06002911.hg.1</td>
<td>1.81</td>
<td>0.313329</td>
<td>0.633221</td>
</tr>
<tr>
<td>EF210211</td>
<td>TC07002321.hg.1</td>
<td>1.81</td>
<td>2.36E-08</td>
<td>0.000002</td>
</tr>
<tr>
<td>AF086401</td>
<td>TC07002818.hg.1</td>
<td>1.81</td>
<td>0.000009</td>
<td>0.000209</td>
</tr>
<tr>
<td>NR_028034</td>
<td>TC10002196.hg.1</td>
<td>1.79</td>
<td>5.54E-07</td>
<td>0.000024</td>
</tr>
<tr>
<td>S47380</td>
<td>TC04002187.hg.1</td>
<td>1.78</td>
<td>0.003206</td>
<td>0.022936</td>
</tr>
<tr>
<td>NR_033258</td>
<td>TC02004623.hg.1</td>
<td>1.77</td>
<td>1.23E-08</td>
<td>0.000001</td>
</tr>
<tr>
<td>linc-C7orf66-2</td>
<td>TC07003112.hg.1</td>
<td>1.77</td>
<td>0.000043</td>
<td>0.000692</td>
</tr>
<tr>
<td>AK021785</td>
<td>TC11003067.hg.1</td>
<td>1.77</td>
<td>7.20E-07</td>
<td>0.000029</td>
</tr>
<tr>
<td>Gene ID</td>
<td>Accession</td>
<td>log2FC</td>
<td>P-value</td>
<td>q-value</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------</td>
<td>----------</td>
<td>-----------</td>
<td>----------</td>
</tr>
<tr>
<td>CR542200</td>
<td>TC04002080.hg.1</td>
<td>1.76</td>
<td>0.000565</td>
<td>0.005548</td>
</tr>
<tr>
<td>AF321824</td>
<td>TC08002559.hg.1</td>
<td>1.75</td>
<td>0.000075</td>
<td>0.01082</td>
</tr>
<tr>
<td>BC040985</td>
<td>TC03002416.hg.1</td>
<td>1.74</td>
<td>1.03E-08</td>
<td>0.000001</td>
</tr>
<tr>
<td>S83545</td>
<td>TC05002797.hg.1</td>
<td>1.74</td>
<td>2.33E-07</td>
<td>0.00012</td>
</tr>
<tr>
<td>NR_027140</td>
<td>TC08002257.hg.1</td>
<td>1.74</td>
<td>0.00011</td>
<td>0.00245</td>
</tr>
<tr>
<td>AY820830</td>
<td>TC06003084.hg.1</td>
<td>1.73</td>
<td>6.40E-08</td>
<td>0.000005</td>
</tr>
<tr>
<td>ENST00000537998</td>
<td>TC12002680.hg.1</td>
<td>1.73</td>
<td>0.000002</td>
<td>0.000054</td>
</tr>
<tr>
<td>linc-GRM1</td>
<td>TC06003110.hg.1</td>
<td>1.72</td>
<td>0.000749</td>
<td>0.006988</td>
</tr>
<tr>
<td>NR_036684</td>
<td>TC16001604.hg.1</td>
<td>1.71</td>
<td>5.09E-08</td>
<td>0.000004</td>
</tr>
<tr>
<td>linc-ZNF626-1</td>
<td>TC19002390.hg.1</td>
<td>1.71</td>
<td>0.017129</td>
<td>0.087898</td>
</tr>
<tr>
<td>linc-FCGR1B-6</td>
<td>TC01005756.hg.1</td>
<td>1.7</td>
<td>0.001</td>
<td>0.008555</td>
</tr>
<tr>
<td>AK057151</td>
<td>TC07002222.hg.1</td>
<td>1.7</td>
<td>0.000029</td>
<td>0.0005</td>
</tr>
<tr>
<td>NR_036431</td>
<td>TC0X002160.hg.1</td>
<td>1.69</td>
<td>0.002693</td>
<td>0.01933</td>
</tr>
<tr>
<td>linc-ELTD1-11</td>
<td>TC01005583.hg.1</td>
<td>1.67</td>
<td>0.000003</td>
<td>0.0008</td>
</tr>
<tr>
<td>AJ420500</td>
<td>TC06002615.hg.1</td>
<td>1.67</td>
<td>0.00453</td>
<td>0.004635</td>
</tr>
<tr>
<td>NR_027783</td>
<td>TC0X001624.hg.1</td>
<td>1.67</td>
<td>9.46E-08</td>
<td>0.000006</td>
</tr>
<tr>
<td>AK056630</td>
<td>TC07002487.hg.1</td>
<td>1.66</td>
<td>0.013477</td>
<td>0.073006</td>
</tr>
<tr>
<td>AF007193</td>
<td>TC07002493.hg.1</td>
<td>1.65</td>
<td>0.020372</td>
<td>0.10857</td>
</tr>
<tr>
<td>X55077</td>
<td>TC12002396.hg.1</td>
<td>1.64</td>
<td>2.21E-08</td>
<td>0.000002</td>
</tr>
<tr>
<td>AF086343</td>
<td>TC1000211.hg.1</td>
<td>1.63</td>
<td>0.000542</td>
<td>0.005365</td>
</tr>
<tr>
<td>linc-NBPFF-3</td>
<td>TC01006417.hg.1</td>
<td>1.62</td>
<td>0.000033</td>
<td>0.000559</td>
</tr>
<tr>
<td>AK026778</td>
<td>TC02004622.hg.1</td>
<td>1.61</td>
<td>0.00051</td>
<td>0.000787</td>
</tr>
<tr>
<td>D87470</td>
<td>TC11003246.hg.1</td>
<td>1.61</td>
<td>0.00013</td>
<td>0.00278</td>
</tr>
<tr>
<td>uc001gla.1</td>
<td>TC01004846.hg.1</td>
<td>1.6</td>
<td>0.00001</td>
<td>0.000044</td>
</tr>
<tr>
<td>AK123435</td>
<td>TC01005963.hg.1</td>
<td>1.6</td>
<td>0.000898</td>
<td>0.008093</td>
</tr>
<tr>
<td>linc_luo_506</td>
<td>TC07002386.hg.1</td>
<td>1.6</td>
<td>0.00063</td>
<td>0.006047</td>
</tr>
<tr>
<td>BC014926</td>
<td>TC14001766.hg.1</td>
<td>1.6</td>
<td>3.72E-07</td>
<td>0.000018</td>
</tr>
<tr>
<td>AF049885</td>
<td>TC04002887.hg.1</td>
<td>1.59</td>
<td>3.44E-11</td>
<td>3.0E-08</td>
</tr>
<tr>
<td>L12143</td>
<td>TC06002402.hg.1</td>
<td>1.59</td>
<td>1.06E-07</td>
<td>0.000007</td>
</tr>
<tr>
<td>BC006127</td>
<td>TC12002414.hg.1</td>
<td>1.59</td>
<td>0.003111</td>
<td>0.022429</td>
</tr>
<tr>
<td>NR_003136</td>
<td>TC15002630.hg.1</td>
<td>1.59</td>
<td>0.028675</td>
<td>0.131215</td>
</tr>
<tr>
<td>linc-C1QTNF1-5</td>
<td>TC17002394.hg.1</td>
<td>1.59</td>
<td>1.60E-07</td>
<td>0.000009</td>
</tr>
<tr>
<td>M34339</td>
<td>TC17002615.hg.1</td>
<td>1.59</td>
<td>0.000753</td>
<td>0.007018</td>
</tr>
<tr>
<td>L26969</td>
<td>TC05003038.hg.1</td>
<td>1.58</td>
<td>0.000017</td>
<td>0.00033</td>
</tr>
<tr>
<td>linc-PPP2R3B-2</td>
<td>TC0X01951.hg.1</td>
<td>1.58</td>
<td>0.294838</td>
<td>0.61422</td>
</tr>
<tr>
<td>linc-TMEM888-1</td>
<td>TC01004129.hg.1</td>
<td>1.57</td>
<td>0.133895</td>
<td>0.390383</td>
</tr>
<tr>
<td>AK094779</td>
<td>TC01004217.hg.1</td>
<td>1.57</td>
<td>0.000004</td>
<td>0.000117</td>
</tr>
<tr>
<td>linc-C5orf43-1</td>
<td>TC05003020.hg.1</td>
<td>1.57</td>
<td>0.000192</td>
<td>0.002313</td>
</tr>
<tr>
<td>NR_037623</td>
<td>TC15002291.hg.1</td>
<td>1.57</td>
<td>0.002041</td>
<td>0.015904</td>
</tr>
<tr>
<td>linc-FOXF1-4</td>
<td>TC16001680.hg.1</td>
<td>1.57</td>
<td>0.133609</td>
<td>0.389902</td>
</tr>
<tr>
<td>NR_036501</td>
<td>TC07002242.hg.1</td>
<td>1.56</td>
<td>0.000008</td>
<td>0.000193</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Start</td>
<td>End</td>
<td>Log2FC</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------</td>
<td>---------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>AK125888</td>
<td>TC08002503.hg.1</td>
<td>1.56</td>
<td>0.000873</td>
<td>0.007912</td>
</tr>
<tr>
<td>AK026716</td>
<td>TC0X001693.hg.1</td>
<td>1.56</td>
<td>0.003961</td>
<td>0.027195</td>
</tr>
<tr>
<td>X69207</td>
<td>TC11002905.hg.1</td>
<td>1.56</td>
<td>0.004246</td>
<td>0.028736</td>
</tr>
<tr>
<td>U80232</td>
<td>TC06002661.hg.1</td>
<td>1.55</td>
<td>0.00013</td>
<td>0.00168</td>
</tr>
<tr>
<td>AF085942</td>
<td>TC07002488.hg.1</td>
<td>1.55</td>
<td>0.048528</td>
<td>0.194753</td>
</tr>
<tr>
<td>linc-MAOA-2</td>
<td>TC0X001666.hg.1</td>
<td>1.55</td>
<td>0.005446</td>
<td>0.035176</td>
</tr>
<tr>
<td>linc-ZNF25-1</td>
<td>TC10002567.hg.1</td>
<td>1.54</td>
<td>0.017415</td>
<td>0.088988</td>
</tr>
<tr>
<td>AF029311</td>
<td>TC17002638.hg.1</td>
<td>1.54</td>
<td>0.000007</td>
<td>0.00016</td>
</tr>
<tr>
<td>linc-NBPF9-7</td>
<td>TC01004681.hg.1</td>
<td>1.53</td>
<td>0.02606</td>
<td>0.121819</td>
</tr>
<tr>
<td>AK056155</td>
<td>TC01004870.hg.1</td>
<td>1.53</td>
<td>0.000009</td>
<td>0.0002</td>
</tr>
<tr>
<td>AF086224</td>
<td>TC01006049.hg.1</td>
<td>1.53</td>
<td>0.01442</td>
<td>0.076986</td>
</tr>
<tr>
<td>AF113008</td>
<td>TC08001925.hg.1</td>
<td>1.53</td>
<td>0.185875</td>
<td>0.477108</td>
</tr>
<tr>
<td>DQ088985</td>
<td>TC15002378.hg.1</td>
<td>1.53</td>
<td>0.000001</td>
<td>0.000043</td>
</tr>
<tr>
<td>linc_luo_524</td>
<td>TC07003197.hg.1</td>
<td>1.52</td>
<td>0.000002</td>
<td>0.000402</td>
</tr>
<tr>
<td>OTTHUMP00000328528</td>
<td>TC02003287.hg.1</td>
<td>1.51</td>
<td>0.122727</td>
<td>0.369369</td>
</tr>
<tr>
<td>uc002vef.2</td>
<td>TC02003799.hg.1</td>
<td>1.51</td>
<td>2.95E-10</td>
<td>1.13E-07</td>
</tr>
<tr>
<td>AK311114</td>
<td>TC06003872.hg.1</td>
<td>1.51</td>
<td>0.000001</td>
<td>0.000043</td>
</tr>
<tr>
<td>NR_002211</td>
<td>TC17002746.hg.1</td>
<td>1.51</td>
<td>0.000001</td>
<td>0.000046</td>
</tr>
<tr>
<td>linc_luo_333</td>
<td>TC01004684.hg.1</td>
<td>1.5</td>
<td>0.04694</td>
<td>0.031159</td>
</tr>
<tr>
<td>linc_luo_1805</td>
<td>TC01005654.hg.1</td>
<td>1.5</td>
<td>0.190779</td>
<td>0.484473</td>
</tr>
<tr>
<td>NR_028308</td>
<td>TC02004133.hg.1</td>
<td>1.5</td>
<td>0.000001</td>
<td>0.000046</td>
</tr>
<tr>
<td>uc011hki.1</td>
<td>TC06002389.hg.1</td>
<td>1.5</td>
<td>0.336701</td>
<td>0.656202</td>
</tr>
<tr>
<td>AJ459885</td>
<td>TC06003566.hg.1</td>
<td>1.5</td>
<td>0.188301</td>
<td>0.480849</td>
</tr>
<tr>
<td>BC040303</td>
<td>TC06003704.hg.1</td>
<td>1.49</td>
<td>0.000003</td>
<td>0.000093</td>
</tr>
<tr>
<td>linc-ANKRD20A1-12</td>
<td>TC09001955.hg.1</td>
<td>1.49</td>
<td>0.000091</td>
<td>0.001259</td>
</tr>
<tr>
<td>AL049370</td>
<td>TC10002117.hg.1</td>
<td>1.49</td>
<td>0.013632</td>
<td>0.073681</td>
</tr>
<tr>
<td>BC033316</td>
<td>TC01004199.hg.1</td>
<td>1.49</td>
<td>-0.01105</td>
<td>0.009591</td>
</tr>
<tr>
<td>linc-CCDC80-2</td>
<td>TC03003030.hg.1</td>
<td>-1.49</td>
<td>0.29249</td>
<td>0.133067</td>
</tr>
<tr>
<td>DQ459608</td>
<td>TC04002625.hg.1</td>
<td>-1.49</td>
<td>3.08E-07</td>
<td>0.000015</td>
</tr>
<tr>
<td>linc-ZFP57-2</td>
<td>TC06003570.hg.1</td>
<td>-1.49</td>
<td>2.17E-07</td>
<td>0.000012</td>
</tr>
<tr>
<td>AL833059</td>
<td>TC09002185.hg.1</td>
<td>-1.49</td>
<td>0.003138</td>
<td>0.022584</td>
</tr>
<tr>
<td>NR_027280</td>
<td>TC19002194.hg.1</td>
<td>-1.49</td>
<td>0.000034</td>
<td>0.000579</td>
</tr>
<tr>
<td>AY211918</td>
<td>TC01004531.hg.1</td>
<td>-1.5</td>
<td>0.005576</td>
<td>0.035907</td>
</tr>
<tr>
<td>NR_027157</td>
<td>TC12003031.hg.1</td>
<td>-1.5</td>
<td>0.000005</td>
<td>0.000129</td>
</tr>
<tr>
<td>NR_036462</td>
<td>TC01004175.hg.1</td>
<td>-1.5</td>
<td>0.002087</td>
<td>0.016199</td>
</tr>
<tr>
<td>linc-PPIAL4F-5</td>
<td>TC01005804.hg.1</td>
<td>-1.5</td>
<td>0.002016</td>
<td>0.015731</td>
</tr>
<tr>
<td>linc-AADAT-2</td>
<td>TC04002818.hg.1</td>
<td>-1.5</td>
<td>0.235108</td>
<td>0.545485</td>
</tr>
<tr>
<td>D86978</td>
<td>TC07002605.hg.1</td>
<td>-1.5</td>
<td>0.000018</td>
<td>0.000342</td>
</tr>
<tr>
<td>Gene ID</td>
<td>Chromosome</td>
<td>Log2 Fold Change</td>
<td>p-value</td>
<td>Adjusted p-value</td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
<td>------------------</td>
<td>----------</td>
<td>------------------</td>
</tr>
<tr>
<td>CR601575</td>
<td>TC11003430.hg.1</td>
<td>-1.51</td>
<td>0.002021</td>
<td>0.015764</td>
</tr>
<tr>
<td>linc_luo_778</td>
<td>TC13001572.hg.1</td>
<td>-1.51</td>
<td>0.000666</td>
<td>0.006327</td>
</tr>
<tr>
<td>NR_028323</td>
<td>TC02004129.hg.1</td>
<td>-1.53</td>
<td>0.000013</td>
<td>0.000272</td>
</tr>
<tr>
<td>uc002eea.2</td>
<td>TC16001872.hg.1</td>
<td>-1.53</td>
<td>0.013494</td>
<td>0.073083</td>
</tr>
<tr>
<td>CR598627</td>
<td>TC07002576.hg.1</td>
<td>-1.54</td>
<td>0.001751</td>
<td>0.013987</td>
</tr>
<tr>
<td>linc-NBPF9-14</td>
<td>TC01004671.hg.1</td>
<td>-1.55</td>
<td>0.000047</td>
<td>0.000741</td>
</tr>
<tr>
<td>L13804</td>
<td>TC13001407.hg.1</td>
<td>-1.55</td>
<td>0.013766</td>
<td>0.074222</td>
</tr>
<tr>
<td>NR_033302</td>
<td>TC01004881.hg.1</td>
<td>-1.56</td>
<td>0.005702</td>
<td>0.036507</td>
</tr>
<tr>
<td>NR_037669</td>
<td>TC07002868.hg.1</td>
<td>-1.56</td>
<td>0.009795</td>
<td>0.056413</td>
</tr>
<tr>
<td>linc-GRAMD1B-1</td>
<td>TC11002922.hg.1</td>
<td>-1.56</td>
<td>6.54E-07</td>
<td>0.000027</td>
</tr>
<tr>
<td>uc010hrq.1</td>
<td>TC03003054.hg.1</td>
<td>-1.57</td>
<td>0.007241</td>
<td>0.044382</td>
</tr>
<tr>
<td>Z70768</td>
<td>TC09002468.hg.1</td>
<td>-1.57</td>
<td>0.000078</td>
<td>0.00112</td>
</tr>
<tr>
<td>CR614667</td>
<td>TC06003537.hg.1</td>
<td>-1.58</td>
<td>0.001279</td>
<td>0.010817</td>
</tr>
<tr>
<td>CR595160</td>
<td>TC14001578.hg.1</td>
<td>-1.58</td>
<td>0.003315</td>
<td>0.003473</td>
</tr>
<tr>
<td>NR_026778</td>
<td>TC01006247.hg.1</td>
<td>-1.59</td>
<td>0.011804</td>
<td>0.065552</td>
</tr>
<tr>
<td>U87167</td>
<td>TC0X001927.hg.1</td>
<td>-1.59</td>
<td>0.015197</td>
<td>0.012952</td>
</tr>
<tr>
<td>BC005221</td>
<td>TC15002245.hg.1</td>
<td>-1.60</td>
<td>0.020556</td>
<td>0.101559</td>
</tr>
<tr>
<td>NR_003105</td>
<td>TC01005350.hg.1</td>
<td>-1.61</td>
<td>0.000522</td>
<td>0.005197</td>
</tr>
<tr>
<td>DQ470079</td>
<td>TC04002368.hg.1</td>
<td>-1.61</td>
<td>0.000775</td>
<td>0.007175</td>
</tr>
<tr>
<td>linc-DHR57B-5</td>
<td>TC17002115.hg.1</td>
<td>-1.61</td>
<td>0.010224</td>
<td>0.058437</td>
</tr>
<tr>
<td>NR_036608</td>
<td>TC17002792.hg.1</td>
<td>-1.61</td>
<td>0.00002</td>
<td>0.000372</td>
</tr>
<tr>
<td>linc-ZNF791-1</td>
<td>TC19001991.hg.1</td>
<td>-1.62</td>
<td>0.098672</td>
<td>0.320528</td>
</tr>
<tr>
<td>linc-ZNF131-4</td>
<td>TC05002352.hg.1</td>
<td>-1.63</td>
<td>0.000329</td>
<td>0.003603</td>
</tr>
<tr>
<td>ENST00000499627</td>
<td>TC01005364.hg.1</td>
<td>-1.65</td>
<td>0.000279</td>
<td>0.003145</td>
</tr>
<tr>
<td>CR595668</td>
<td>TC0X001880.hg.1</td>
<td>-1.65</td>
<td>1.67E-08</td>
<td>0.000002</td>
</tr>
<tr>
<td>BC105606</td>
<td>TC10002226.hg.1</td>
<td>-1.65</td>
<td>0.000159</td>
<td>0.00198</td>
</tr>
<tr>
<td>AF333388</td>
<td>TC01006221.hg.1</td>
<td>-1.66</td>
<td>7.35E-07</td>
<td>0.00003</td>
</tr>
<tr>
<td>NR_034041</td>
<td>TC17002724.hg.1</td>
<td>-1.67</td>
<td>0.00003</td>
<td>0.000515</td>
</tr>
<tr>
<td>AL833658</td>
<td>TC06003604.hg.1</td>
<td>-1.68</td>
<td>0.004967</td>
<td>0.032589</td>
</tr>
<tr>
<td>S70154</td>
<td>TC06003166.hg.1</td>
<td>-1.69</td>
<td>0.000541</td>
<td>0.005356</td>
</tr>
<tr>
<td>linc-PDZD7</td>
<td>TC10002799.hg.1</td>
<td>-1.70</td>
<td>0.00004</td>
<td>0.004211</td>
</tr>
<tr>
<td>NR_026978</td>
<td>TC18000697.hg.1</td>
<td>-1.70</td>
<td>0.003329</td>
<td>0.023636</td>
</tr>
<tr>
<td>D83986</td>
<td>TC22001090.hg.1</td>
<td>-1.73</td>
<td>4.21E-08</td>
<td>0.000003</td>
</tr>
<tr>
<td>NR_024526</td>
<td>TC02003640.hg.1</td>
<td>-1.74</td>
<td>0.005067</td>
<td>0.033116</td>
</tr>
<tr>
<td>D82345</td>
<td>TC0X002162.hg.1</td>
<td>-1.74</td>
<td>0.000219</td>
<td>0.00258</td>
</tr>
<tr>
<td>AF086045</td>
<td>TC18000953.hg.1</td>
<td>-1.74</td>
<td>0.012803</td>
<td>0.070056</td>
</tr>
<tr>
<td>NR_033142</td>
<td>TC01005497.hg.1</td>
<td>-1.77</td>
<td>0.000836</td>
<td>0.007633</td>
</tr>
<tr>
<td>CR616786</td>
<td>TC06003578.hg.1</td>
<td>-1.81</td>
<td>0.00002</td>
<td>0.000374</td>
</tr>
<tr>
<td>DQ648894</td>
<td>TC02003219.hg.1</td>
<td>-1.85</td>
<td>0.011223</td>
<td>0.063019</td>
</tr>
<tr>
<td>uc010fnw.1</td>
<td>TC02003637.hg.1</td>
<td>-1.90</td>
<td>0.023072</td>
<td>0.110835</td>
</tr>
<tr>
<td>IncRNA</td>
<td>Target</td>
<td>Log2 Fold Change</td>
<td>FDR</td>
<td>Bonferroni Corrected FDR</td>
</tr>
<tr>
<td>-------------</td>
<td>---------------</td>
<td>-----------------</td>
<td>------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>uc002ufj.3</td>
<td>TC02003679.hg.1</td>
<td>-1.99</td>
<td>0.010288</td>
<td>0.058714</td>
</tr>
<tr>
<td>DQ656056</td>
<td>TC09002285.hg.1</td>
<td>-2.04</td>
<td>0.000016</td>
<td>0.000318</td>
</tr>
<tr>
<td>AK307939</td>
<td>TC10002686.hg.1</td>
<td>-2.07</td>
<td>0.000458</td>
<td>0.004673</td>
</tr>
<tr>
<td>uc002zdj.1</td>
<td>TC21000762.hg.1</td>
<td>-2.2</td>
<td>2.53E-07</td>
<td>0.000013</td>
</tr>
<tr>
<td>CR622106</td>
<td>TC12002644.hg.1</td>
<td>-2.34</td>
<td>0.000154</td>
<td>0.001929</td>
</tr>
<tr>
<td>AK021443</td>
<td>TC10002227.hg.1</td>
<td>-2.51</td>
<td>0.011029</td>
<td>0.062107</td>
</tr>
</tbody>
</table>

**Supplementary Table S1: Differentially expressed IncRNAs after IR in H460 cells**
Supplementary Figure S1: Microarray analysis of differentially expressed lncRNAs after IR in NSCLC cell line H460. Heat map of lncRNAs microarray data comparing H460 no radiated (Control) against H460 irradiated (7Gy) after 24 hrs. Only lncRNAs differentially expressed are shown.
Supplementary Figure S2

>AK128024.1 Homo sapiens cDNA FLJ46143 fis, clone TESTI2053561 (1986bp)
(with AY598347.3 fragment is called AK47)

CCATTCCTTCTGACTCCCTCCTATTCCTTTTCCAGTCATTTGCATTTGAACTTCTATTTCTATACATTCGTTCCATTTCACTCCAGTTC
CCATTCCTTCTGACTCCTTCCTATTTCTATACATTCGTTCCATTTCACTCCAGTTC

Lnc-SPRY3-2

Lnc-SPRY3-4

AY598347.3 sequence (591-816)

Splicing region

>AY598346.2 Homo sapiens heterochromatic block map Yq12 transcribed DYZ1 sequence mRNA, partial sequence (1441)
GGTTCCGTACGATTCATTCATTTGGAGTTGCAATTCATTTGCATTTCACTTCCAGACATTTCCATTTCTTATCGAGTCCATTCCATTGTCCATTTCTGCTGGTGCATTCCATTTTGTTCTATGATTCGCCATTACATTCCTTGCTTATGACGATTCCGTTTGCATTTGATTCTATTCTCTTCTACTG

CAACCAATTTCCACTGTTTCCATTTTGATTCATTTCCATTTCACTTTGTTTCTATGATTC GCCATTACATTCCTTGCTTATGACGATTCCGTTTGCATTTGATTCTATTCTCTTCTACTG

Lnc-SPRY3-3

Splicing region

Supplementary Figure S2: Overlapping sequences between linc-SPRY3-2/3/4 and clones AY598347.3 and AY598346.2
Supplementary Figure S3: Virtual northern blot analysis of linc-SPRY3-2/3/4 in H460 cells demonstrates potential transcript size. (A) Schematic outlining the virtual northern blot method. (B) Virtual northern blot reveals two large peaks, one at ~5kb and one at ~1kb. (C) These two peaks align when the individual graphs are merged. Similar peaks are seen when a second set of primers for linc-SPRY3-2 and linc-SPRY3-3 are used. GAPDH was used as a control.
Supplementary Figure S4: Clonogenic cell survival assay shows the radiation response of a panel of male NSCLC cell lines. Clonogenic cell survival assay shows the relative radiation response of a panel of male NSCLC cell lines. Surviving fractions are plotted as a function of dose. Error bars represent SD from the mean of triplicate measurements from a single experiment. Shown here is a representative of two independent experiments with similar result.
Supplementary Table S2

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Driver Mutation</th>
<th>Observed Radiation Response</th>
<th>% Y Chromosome (+) Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>H460</td>
<td>KRAS</td>
<td>Sensitive</td>
<td>98%</td>
</tr>
<tr>
<td>H820</td>
<td>EGFR</td>
<td>Sensitive</td>
<td>100%</td>
</tr>
<tr>
<td>H1299</td>
<td>NRAS</td>
<td>Resistant</td>
<td>0%</td>
</tr>
<tr>
<td>A549</td>
<td>KRAS</td>
<td>Resistant</td>
<td>0%</td>
</tr>
<tr>
<td>WVU-Ma-0005</td>
<td>Unknown</td>
<td>Sensitive</td>
<td>100%</td>
</tr>
<tr>
<td>Ma-ALK-0001</td>
<td>ALK Fusion</td>
<td>Resistant</td>
<td>0%</td>
</tr>
</tbody>
</table>

Supplementary Table S2: Cell lines used in this study.
Outline of the observed radiation response of each cell line used in this study color coded to correspond with Supplementary Fig. 2. Cytogenetic analysis and quantification were performed by the WVU Cytogenetics Laboratory in the Department of Pathology, Anatomy and Laboratory Medicine. The percentages shown are a result of the quantification of a population of 200 cells.
Supplementary Figure S5: Radiation response of cell line panel normalized to the housekeeping gene UBC and two additional NSCLC cell lines. (A-G) qRT-PCR analysis of the linc-SPRY3 family normalized to UBC. (H-I) qRT-PCR analysis of the linc-SPRY3 family in the additional radiosensitive cell line H157 and radioresistant cell line H1650 normalized to GAPDH. (J-K) qRT-PCR analysis of the linc-SPRY3 family in the additional radiosensitive cell line H157 and radioresistant cell line H1650 normalized to UBC. (L-M) Y chromosome DNA FISH of H157 and H1650 cells.
Supplementary Figure S6: Female NSCLC cell lines show no expression of the linc-SPRY3 family. qRT-PCR analysis of the linc-SPRY3 family in female NSCLC cell lines (A) H1975 and (B) H1819. Cells were plated at equal density and treated with one dose of radiation (2Gy, 4Gy, or 8Gy) and collected 72 hours after treatment. Values are relative to untreated control of the same collection day (0Gy). GAPDH mRNA was used to normalize qRT-PCR analysis. Error bars represent SD from the mean of triplicate experiments.
Supplementary Figure S7

Supplementary Figure S7: Cell fractionation of radiosensitive cell lines reveals nuclear localization of the linc-SPRY3 family. qRT-PCR analysis of the linc-SPRY3 family in fractionated (A) H460 and (B) WVU-Ma-0005 cell lines. The dotted line represents the total relative RNA of each experiment. Mature β-Actin mRNA was used as a cytoplasmic control and U6 small nucleolar RNA was used as a nuclear control. Error bars represent SD from the mean of triplicate measurements from a single experiment. Shown here is a representative of three independent experiments with similar result. Normalization was done using C. elegans total RNA as an exogenous spike for the amplification of worm specific ama-1 gene. (C) Coding probability (CP) scores of the linc-SPRY3-2/3/4 showing no coding probabilities for these lncRNAs (a CP of 0.364 and above is considered a coding RNA). Coding-Potential Assessment Tool (CPAT) was used for these calculations.
Supplementary Figure S8: PCR of the pBACe3.6 plasmid backbone in A549 cells
Lane 1: A549 Parental cells, Lane2: A549 cells nucleofected with the empty vector pBACe3.6, Lane 3: A549 cells nucleofected with the BAC clone RP11-88F4. GAPDH is provided as a loading control. DNA was extracted using the Thermo Scientific GeneJET Genomic DNA Purification Kit (#K0722) following the manufacturers protocol for adherent cells. PCR was run using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, 172-5271). PCR products were resolved on a 1% agarose gel with ethidium bromide.
Supplementary Figure S9: In vivo study scheme and untreated controls for the tumor growth delay assays. (A) Schematic detailing the tumor growth delay assay. (B and C) Representative images of ultrasound measurements utilized in determining tumor volume and depth respectively. (D) Tumor growth delay assay with untreated controls for H460 shCtrl and sh1 cells. (E) Tumor growth delay assay with untreated controls for WVU-Ma-005 shCtrl and sh1 cells.
Supplementary Figure S10: siRNA knockdown of linc-SPRY3 RNAs in A549 cells nucleofected with the BAC clone RP11-88F4. Representative replicate from triplicate experiments. Error bars represent SD from the mean of 3 technical replicates.
Supplementary Figure S11: Representative images of DYZ1 fluorescent in situ hybridization in tissue microarrays. (A-C) Images taken of male cores from the lung cancer tissue microarray. (D-F) Images taken of female cores from the lung cancer tissue microarray. (G) Image taken of a core from the testis tissue microarray as a positive control. (H) Image taken of a core from a cervical cancer tissue microarray as a negative control. (I) Survival curves for NSCLC male patients (GEO GSE81089) which express and do not express linc-SPRY3-2. P-value by log rank test.
### Supplementary Table S3

<table>
<thead>
<tr>
<th>ID</th>
<th>Target Sequence</th>
<th>Hairpin oligo sequences</th>
<th>For cloning:</th>
<th>siRNA sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>sh1</td>
<td>GAAUCCAUUCCA UUAGAGGAC</td>
<td>FWD</td>
<td>AgeI/EcoRI</td>
<td>5'-GTCCCTCTAATGGAAATGGATTC-3'</td>
</tr>
<tr>
<td>linc-SPRY3-2</td>
<td></td>
<td>REV</td>
<td></td>
<td>5'-TCATAGAATGTAATGGAAATGC-3'</td>
</tr>
<tr>
<td>sh2</td>
<td>GCAUCCAUUA CAUUCUAUGA</td>
<td>FWD</td>
<td></td>
<td>5'-GGAGTGTATGGGAACGGACTC-3'</td>
</tr>
<tr>
<td>linc-SPRY3-3</td>
<td></td>
<td>REV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Supplementary Table S4**

**qRT-PCR/PCR Primer Sequences**

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>linc-SPRY3-2 FWD</td>
<td>CATCCAAATCCCATCTACTCCAT</td>
</tr>
<tr>
<td>linc-SPRY3-2 REV</td>
<td>TAGAATGGAACGAAATTTCACG</td>
</tr>
<tr>
<td>linc-SPRY3-3 FWD</td>
<td>TCCAGTCAATGATTTTGAGTTC</td>
</tr>
<tr>
<td>linc-SPRY3-3 REV</td>
<td>GGAATAAAGTGGAATGCTACGG</td>
</tr>
<tr>
<td>linc-SPRY3-4 FWD</td>
<td>GTCCACTCCACCCATTCTA</td>
</tr>
<tr>
<td>linc-SPRY3-4 REV</td>
<td>CGAACGGGAATGGAATAAAAAT</td>
</tr>
<tr>
<td>linc-SPRY3-2 FWD (2\textsuperscript{nd} set)</td>
<td>TCCAGTCCATTTCACTCCAG</td>
</tr>
<tr>
<td>linc-SPRY3-2 REV (2\textsuperscript{nd} set)</td>
<td>TGCAATAAAATCGACTCAGATAGA</td>
</tr>
<tr>
<td>linc-SPRY3-3 FWD (2\textsuperscript{nd} set)</td>
<td>CCGTTTCATTGCATTCCA</td>
</tr>
<tr>
<td>linc-SPRY3-3 REV (2\textsuperscript{nd} set)</td>
<td>GCAAGCGAAAGGAAAGGA</td>
</tr>
<tr>
<td>GAPDH FWD</td>
<td>CCACTCCTCCACCTTTGAC</td>
</tr>
<tr>
<td>GAPDH REV</td>
<td>ACCTGTGGCTGTGCA</td>
</tr>
<tr>
<td>UBC FWD</td>
<td>GATTTGGGTGCGCAGTCTTCTG</td>
</tr>
<tr>
<td>UBC REV</td>
<td>CCTTATCTTGGATCTTGGC</td>
</tr>
<tr>
<td>Beta-Actin FWD</td>
<td>AGCACAGAGCCTCGCCTTTT</td>
</tr>
<tr>
<td>Beta-Actin REV</td>
<td>CCACGATGGAGGGGAAGAC</td>
</tr>
<tr>
<td>U6 FWD</td>
<td>GTGCTCGCTTCCGGCAGCACATAT</td>
</tr>
<tr>
<td>U6 REV</td>
<td>AAAAATATGGGAACGCTTCCACGAA</td>
</tr>
<tr>
<td><em>C. elegans</em> ama-1 FWD</td>
<td>GGAGCTCGAGTGGATCTTTCG</td>
</tr>
<tr>
<td><em>C. elegans</em> ama-1 REV</td>
<td>GCGCAGAGATCTCCTGGAC</td>
</tr>
<tr>
<td>pBACe3.6 FWD</td>
<td>TTGAGTCTGCAAGAAGGACTTGA</td>
</tr>
<tr>
<td>pBACe3.6 REV</td>
<td>GATGGATGTGTCATCAGGCCTT</td>
</tr>
<tr>
<td>HMGA2 FWD</td>
<td>GCCCCAGGAAGCAGCAA</td>
</tr>
<tr>
<td>HMGA2 REV</td>
<td>TCGAACGTTGGCGCCCCTA</td>
</tr>
<tr>
<td>C-MYC FWD</td>
<td></td>
</tr>
<tr>
<td>C-MYC REV</td>
<td></td>
</tr>
</tbody>
</table>
Ultra-Violet Radiation Crosslinking and Immunoprecipitation (CLIP)

Reagents

**NET-2 Buffer (Nuclease-free) stored at 4C**
(150 mM NaCl; 0.05% NP-40; 50 mM Tris-HCl, pH7.4) 500 mL
2 M Sodium Chloride (NaCl) 37.5 mL
Surfact-Amps NP-40 (10% Nonidet P-40) 2.5 mL
1 M Tris, pH 7.4 (tris(hydroxymethyl)aminomethane) 25 mL
Bring volume up to 500 mL with Nuclease-free water, autoclave
Add Protease inhibitor and RNasin just before using

**Proteinase K Buffer (Nuclease-free)**
(50 mM Tris-Cl, pH 7.5, 5 mM EDTA, 1.5% SDS, 300 mM NaCl) 100mL
1 M Tris-Cl (Tris(hydroxymethyl)aminomethane hydrochloride), pH 7.5 5 mL
0.5 M EDTA (Ethylene-diaminetetraacetic acid) 1 mL
10% SDS (sodium dodecyl sulfate) 15 mL
2 M NaCl (Sodium Chloride) 15 mL
Mix and bring volume up to 100 mL with Nuclease-free water; autoclave.

**BSA Solution (Nuclease-free) stored at 4**
(2 mg/mL) 100 mL
BSA (Bovine serum albumin) 200 mg
Mix and bring volume up to 100 mL with Nuclease-free water; autoclave.

**1X PXL (wash buffer made fresh)**
1x PBS (tissue culture grade; no Mg++, no Ca++) 9.89 mL
0.1% SDS (0.01 ml of 10% SDS in 0.99 ml water) 0.01 mL
0.5% sodium deoxycholate (5 mg in 1 ml water) 0.05 mL
0.5% NP-40 (4.7 ul in 1 ml water) 0.05 mL

Protocol

1. **72 hours before Day 1 (if using treated cells)**
   - Treat cell lines with 8Gy radiation (6cm dishes)
   - Let incubate for 72 hours before beginning.

2. **Day 1 – UV X-linking, BCA assay and Bead Prep (complete this for all samples)**
   - Aspirate media from cells. Rinse with tissue culture grade PBS, remove, and replace PBS.
   - UV irradiate using Stratalinker (On → energy → value → start) with the dish lid off. Can irradiate 2 6cm dishes at a time. MUST be on ice. Irradiate one time for 400 mJ/cm², rotate the plate 90° and irradiate an additional 200 mJ/cm².
• Collect suspension (scrape) for all dishes, pellet cells at 2500 rpm for 5 min at 4°C.
• Resuspend pellet in (~3x dry volume) of tissue culture grade PBS; quick spin at 4°C, remove supernatant and freeze pellets at -80°C until use.
• Resuspend each tube of cross-linked lysate using 700 µl of 1X PXL + 300 µl of HALT protease.
• Add 15 µL RNAsin and let sit on ice for 10 min.
• Add 30 µl of RQ1 DNase or DNAse 1 (Promega, M6101 or NEB) to each tube; incubate at 37°C for 5 min, 1000 rpm.
• Supernatant was collected after 10 min centrifugation at 16,000Xg at 4°C
• Quantitate protein with BCA assay…100 ug of each lysate was used for IP

*Clip end of pipette tip before pipette beads

• 600 µL Protein G-Sepharose beads in 1.5mL tube; wash beads (spin at 4°C for 3 min at 1000 rpm, then remove supernatant) 2 times with about 500 µL PXL buffer each wash (protein G beads are stored at 4°C).
• Block Protein G Sepharose beads with 900 uL of 2 mg/mL BSA for 1hr or overnight at 4°C (BSA stored at 4°C).

3. Day 2 - Immunoprecipitation
• Wash beads 2 times with 500uL PXL Buffer each time (Did not add RNasin/PI during the washes to save reagents). After last wash, add 600 µL of PXL, 6.0 µl HALT, and 15 µl RNasin.
• Measure out the volume of lysate (100 ug) determined from BCA assay. Divide supernatants into 2 1.5mL tubes (2 antibodies – IGF2BP3, Abcam #177942 and IgG, Abcam #172730).
• Preclear lysates by adding 90uL of blocked/washed beads to each lysate; incubate for 1hr at 4°C on rotator.
• Spin down precleared beads/lysate at 4°C 1,000 rpm for 3 min (low speed when working with beads).
• Transfer supernatant into new tubes and discard beads used for preclearing; bring up to equal volume (500 µl).
• Add desired antibody to remaining lysates (8 ug antibody/lysate). Incubate for 3 hrs at 4°C on rotator.
• Add 50uL of blocked/washed beads to each lysate/antibody tube; Incubate for 1hr at 4°C on rotator.
• Spin down lysate/antibody/beads at 4°C 1,000 rpm for 3 min (low speed when working with beads).
• Divide supernatant into 2 tubes (IP supernatant for RNA and protein).
• Wash pelleted beads 5 times with about 200 uL each wash with NET2-Buffer (Spin down beads at 4°C 1,000 rpm for 3 min. add the following volumes of RNasin to each wash:
  o  Wash 1: 14 uL RNAsin
  o  Wash 2: 14 uL RNasin
  o  Wash 3: 6 uL RNasin
- Wash 4: 3 uL RNasin
- Wash 5: 3 uL RNasin
- Take 25% (125uL) of beads for Western Blot analysis -> Spin down beads at 4°C 1,000 rpm for 3 min.
  - Spin remove supernatant resuspend in 50 µL PXL buffer
  - Add 10 µL 5X LSB and boil at 100°C for 5 min to remove protein from beads
- Spin down remaining 75% (375uL) of beads at 4°C 1,000 rpm for 3 min. Incubate beads with Proteinase K Buffer (150ul) (w/ 300 mM NaCl) and 1.5mg/mL proteinase K (11.25uL of 20mg/mL) for 30 min at 50°C.
- Then incubate beads for 60 min at 70°C (Add 0.5 uL RNasin/sample).
- RNAs (from beads and supernatant) were recovered with phenol/chloroform extraction
  - Add same volume of Phenol-chloroform-isoamyl alcohol/tube.
  - Vortex 20 seconds (10 up and 10 angled).
  - Centrifuge 10 minutes at 11,400 rpm at RT.
  - Transfer aqueous phase to another centrifuge tube w/ 50 µL of 3M Sodium Acetate (NaOAc), at pH 5.2.
  - Add 1 mL of 100% Ethanol (precooled to -20˚ C), fill to top of tube, add 1 µL of GlycoBlue (15 µg/µL) Store at -20˚C overnight or for a few days to increase yield.

4. **Day 3 – Finish RNA extraction**
   - Spin down RNA for 15 minutes at 11,400 rpm in micro-centrifuge at 4°C.
   - Aspirate Ethanol (leave a little).
   - Wash with 500 uL of 75% Ethanol (precooled to -20°C); don’t shake just add.
   - Spin down for 1 minute at 11,400 rpm.
   - Aspirate Ethanol, remove any remaining with pipette.
   - Re-suspend RNA pellet in 20 µL of Nuclease-Free water (Ambion).
   - DNase treat samples.
   - Collect supernatant.
   - Quantify samples.
   - Store RNA in -80 until use.
CHAPTER 3
DISCUSSION AND FUTURE DIRECTIONS

For decades, biomedical research has focused on the central dogma of molecular biology; DNA is the blueprint, RNA is the messenger, and proteins are the functional mediators. However, thanks to the Encyclopedia of DNA Elements (ENCODE), which determined that <3% of the human genome gives rise to functional proteins, this dogma has started to be challenged (1,2). The emergence of functional non-coding RNAs (ncRNAs) has revealed that the realm of human biology is much more complex than previously thought, and researchers have only begun to scratch the surface of their impact in molecular biology and disease. We now know that a single ncRNA can function in numerous ways, in numerous pathways, which makes their study imperative to understanding human disease (3,4).

Long non-coding RNAs (lncRNAs) are ncRNAs >200 nucleotides long, lack a functional open reading frame, and have emerged as vital regulators of many important genes and signaling pathways in human cells. Furthermore, their dysregulation has been implicated in multiple cancer types, and current studies are focused around identifying mechanisms of carcinogenesis and their potential as biomarkers for cancer diagnosis and/or prognosis (5). Lung cancer is the number one cause of cancer related mortality and in late stages is notoriously resistant to therapy (6,7). Our group’s overall goal is to elucidate the roles of ncRNAs in regulating radiation therapy responsive genes and signaling pathways in an attempt to identify potential therapeutic targets or biomarker candidates. Therefore, we set out to investigate potential ncRNAs, specifically lncRNAs, involved in lung cancer radiation therapy response.

Not only have previous studies showed radioresponsive lncRNAs, but many lncRNAs have been implicated in the radioreistance of cancers (8,9). A few of the mechanisms identified include regulation of anti-apoptotic pathways, DNA damage repair pathways, and promotion of epithelial-to-mesenchymal transition (EMT). Therefore, in the initial stages of the project presented in Chapter 2, we began with a microarray analysis utilizing RNA from a well-known non-small cell lung cancer (NSCLC) cell line (NCI-H460) treated with ionizing radiation to identify candidates
for study. Not surprisingly, we found hundreds of differentially expressed lncRNAs and among
the highest upregulated genes we discovered a three-member family; linc-SPRY3-2, linc-SPRY3-
3, and linc-SPRY3-4 (also known as lnc-BPY2C-4, lnc-BPY2C-25, and lnc-BPY2C-2, respectively) (Chapter 2, Fig. S1 and Table S1). Unexpectedly, further investigation into the linc-
SPRY3 transcripts on LNCipedia revealed that they were transcribed from the Y chromosome
(ChY), and to our knowledge, were the first examples of Y chromosome linked lncRNAs (Chapter 2, Fig. 1A).

It was determined that the linc-SPRY3 family maps specifically to the DYZ1 region of the
heterochromatic block of the q arm of ChY. Interestingly, this region has been traditionally
described as genetically inert, and is characterized by a 3.4 kilobase pentanucleotide tandem repeat
sequence which can vary in the number of copies from 2000-4000 within the heterochromatic
block (10). Beyond structural characterizations, investigations into its use in forensics, gender
verification in sports, or as a tool for monitoring genomic instability in males, there is little known
about the DYZ1 region (11–14). This is not surprising considering its suggested lack of
transcriptional potential. Our study challenges this notion and proposes that more attention should
be paid not only to this region but to ChY in general.

We demonstrate that the linc-SPRY3 transcripts are upregulated after exposure to radiation
in cells that have retained ChY (Chapter 2, Fig. 1C, Fig. 2A-D, and Fig. S5A-D, H and I). It is
well-known that radiation induces a signaling cascade to trigger cell death. Amongst these signals
is changes in chromatin structure to turn specific genes on and off in order to promote the necessary
gene expression for cellular responses (15). It the context of the linc-SPRY3 RNAs, it would be
interesting to tease out changes in the chromatin marks of the DYZ1 region in response to
radiation. As there is currently little to no sequencing data available for the q arm of ChY, using
tools such as the UCSC Genome Browser has proven difficult and unsuccessful. Therefore,
identifying chromatin changes in the DYZ1 region would allow for the identification of potential
promoter sites within the region. Furthermore, specifically identifying the promoter(s) of the linc-
SPRY3-2/3/4 transcripts would allow for investigation into upstream regulators of their
expression.
One of the major limitations to the study presented in Chapter 2 is the lack of full sequences for linc-SPRY3 transcripts. The sequence fragments we were able to mine from LNCipedia.org gave us enough information to design primers for quantitative reverse transcriptase PCR (qRT-PCR), but without the full sequences we were unable to perform methods such as, northern blotting, RNA fluorescent in situ hybridization (FISH), and cloning to exogenously express the IncRNAs. This also prevents us from determining if the linc-SPRY3 RNAs are a family of IncRNAs or one large transcript. An attractive option for attempting to sequence the linc-SPRY3 family is the Oxford Nanopore minION platform. This technology is one of few that can perform direct RNA long read sequencing. Based on the data we acquired from virtual northern blot analysis, we predict that the SPRY3 IncRNAs are roughly 5 kilobases long, and the near perfect alignment of peaks between all three family members further supports the hypothesis that these three family members could be one large transcript, originally classified as 3 transcripts due to realignment errors because of the highly repetitive nature of their sequences (Chapter 2, Fig. S3).

Long read sequencing has shown much higher accuracy at reading highly repetitive sequences, and the ability to perform direct RNA sequencing is much more ideal as it removes the need to generate a cDNA library, a step which usually involves fragmenting the DNA which is then read and aligned to a reference genome (16–18). If successful, minION sequencing would provide the full sequence(s) of the linc-SPRY3 RNAs. This would then allow us to perform more rigorous studies on their structure and function and ultimately better understand their roles and potential clinical relevance in NSCLC which are further explained in the below paragraphs.

An extremely useful tool to studying RNA is RNA fluorescent in situ hybridization (FISH), and it has become the gold standard for identifying transcript localization (19). Unfortunately, successful RNA FISH relies on sequence specificity in order for the fluorescent probes to hybridize to a transcript of interest. This has made it a challenge to visualize the compartmentalization of linc-SPRY3-2/3/4 using fluorescent microscopy. We did attempt to utilize the sequence fragments that we currently have to design Stellaris RNA FISH probes, but were not successful. Therefore, it is important that we define the complete sequence(s) in order to be able to move forward with this method. However, we have been able to identify that these IncRNAs are nuclear via cellular fractionation (Chapter 2, Fig. S7). An even more important use for this method is its applications...
in determining clinical relevance. Successful RNA FISH would enable us to use patient tissue samples such as a tumor microarrays to better elucidate if the linc-SPRY3-2/3/4 correlate with patient therapeutic response and/or survival. Considering the tumor suppressive characteristics we have observed regarding the linc-SPRY3-2/3/4 *in vitro* and *in vivo*, and the survival trends seen using microarray and RNA-seq data (Chapter 2), we predict that a more in depth analysis with a large patient cohort would demonstrate a negative correlation between loss of the linc-SPRY3 RNAs and patient outcomes.

Another important method used for characterizing novel RNAs is northern blotting. Briefly, northern blotting is a method used to visualize RNA expression and determine transcript size, somewhat similar to the way western blots are used to look at protein expression. Northern blots traditionally use radiolabeled (e.g. $^{32}$P) oligonucleotide probes (cDNA or RNA) which are complementary to a transcript of interest. In order to design a probe, the sequence of a target RNA is necessary, therefore identifying the full sequence of the linc-SPRY3 RNAs is needed in order to perform this method. Like RNA FISH, we attempted to synthesize northern blot probes based on the sequence fragments mined from LNCipedia, but were unsuccessful at detecting the linc-SPRY3 transcripts. qRT-PCR has been essential in assaying the expression of these IncRNAs, however, how large these transcripts are or if they are a family or a single large transcript is still uncertain. The ability to perform traditional northern blots would complement the data from the previously mentioned virtual northern blots and allow us to better ascertain the potential size(s) of the linc-SPRY3 transcript(s).

Identifying the full sequence(s) of linc-SPRY3-2/3/4 would also be imperative for designing expression vectors (retroviral or lentiviral vectors) to better characterize the effect and molecular functions of these RNAs. A standard in most molecular biology based projects is the ability to knockdown and overexpress genes of interest. While we were very successful at suppressing the expression of the SPRY3 IncRNAs via short-hairpin RNA (shRNA) lentiviral constructs, the opposite proved to be more difficult. However, we were able to circumvent the traditional methods of exogenous expression such as cloning the IncRNAs into a viral vector by utilizing an old tool in a new way: transient expression of the linc-SPRY3 RNAs by transfecting a
bacterial artificial chromosome containing the human Y-chromosome fragment where these lncRNAs are expressed.

Bacterial artificial chromosomes (BACs), bacterial plasmids which contain large fragments of DNA, are tools that were vital for the completion of the Human Genome Project and the completion of sequencing numerous other genomes (20,21), but we were able to take advantage of this technology in a different way. Since we could not clone linc-SPRY3-2/3/4 into an expression vector, we acquired two ChY BAC clones from bacpacresources.org, RP11-80F8 and RP11-88F4. These BACs were chosen because they both contain large fragments of the q arm from the human ChY which includes the DYZ1 region. Fortunately, we were able to introduce these BACs to the radioresistant NSCLC cell line A549 via nucleofection and subsequently detect robust expression of the SPRY3 lncRNAs by qRT-PCR. Additionally, we determined that restoration of linc-SPRY3-2/3/4 expression via BAC nucleofection resulted in a more radiosensitive phenotype in vitro (Chapter 2, Fig. 3A-C). While these data were very exciting, there are some drawbacks to the method. This system is highly transient, meaning lengthy studies such as in vivo tumor growth delay assays are not possible. Also, there is the potential of introducing unknown ChY factors that might be present in the ChY fragment within the BACs which could influence cellular response to radiation. Therefore, fully sequencing linc-SPRY3-2/3/4 would enable us to generate a more stable and pure expression system and allow us to investigate their functions more specifically and more in depth.

Another future route in regards to studying the linc-SPRY3 family, is fully vetting the molecular mechanism(s) by which they regulate NSCLC cell response to radiation. Our group has previously shown that cell lines that express the SPRY3 lncRNAs are more sensitive to ionizing radiation, thus we sought to determine a potential mechanism by which they mediate this effect (Chapter 2, Fig. 2, Fig. S4, Fig. S5, Table S2). Through in silico analysis followed by UV-crosslinked immunoprecipitation (CLIP) we identified the RNA binding protein (RBP) insulin growth factor 2 binding protein 3 (IGF2BP3; also known as IMP3) as a direct binding partner of linc-SPRY3-2/3/4 (Chapter 2, Fig. 4A and 4B). IGF2BP3 presented as an interesting target because previous studies have demonstrated that it is consistently upregulated in numerous tumor types when compared to normal adjacent tissue controls, including lung adenocarcinoma (ADC,
LUAD) and lung squamous cell carcinoma (SCC, LUSC) (Fig.1 and Fig. 2 black box), as well as mediates multiple oncogenic mechanisms in cancer including radioresistance (22–24).

While CLIP is a good start at demonstrating the interaction between linc-SPRY3-2/3/4 and IGF2BP3, additional experiments to confirm the interaction are still needed. In addition to confirming the CLIP experiments in other NSCLC cell lines, it would be complementary to perform multiplexed RNA FISH for linc-SPRY3-2/3/4 and IF for IGF2BP3 to visualize co-localization of the RNA with this RBP. Furthermore, as linc-SPRY3-2/3/4 expression is induced upon treatment with radiation, RNA FISH/IF could reveal clues towards confirming our ceRNA hypothesis as we predict there would be higher enrichment.
of IGF2BP3 in the nucleus versus cytoplasm in treated cells as linc-SPRY3-2/3/4 would sequester the RBP to the nucleus.

According to previous studies, IGF2BP3 has multiple targets, and among them is HMGA2 and the oncogene c-Myc, both of which have ties to regulating cell viability and apoptosis (25,26). IGF2BP3 regulates these targets by stabilizing their mRNA which allows for their translation (27–29). We hypothesized that when the linc-SPRY3 RNAs are expressed, they act as ceRNAs to sponge IGF2BP3. This in turn prevents stabilization of its targets, c-Myc and HMGA2, resulting in a shorter half-life of their mRNA and subsequent downregulation in expression. To confirm this, we performed actinomycin-D RNA degradation assays which revealed that expression of the SPRY3 lncRNAs resulted in faster degradation of the mRNAs of HMGA2 and c-Myc (Chapter 2, Fig. 4C). The RNA degradation assay data was obtained from manipulated A549 cells without radiation, and while these data provided valuable results, they only revealed the tip of the iceberg of a potential mechanism for the SPRY3 lncRNAs. Future experiments should include the same RNA degradation assay in other cell lines and confirm downregulation in expression of HMGA2 and c-Myc at the protein level (Western blots). Furthermore, since we provide evidence that increased mRNA degradation of HMGA2 and c-Myc is as a result of IGF2BP3 being sequestered by the SPRY3 lncRNAs, which are only upregulated in radiosensitive cell lines following radiation, it is imperative that we perform knockdown experiments of IGF2BP3 and look at mRNA degradation of HMGA2 and c-Myc as well as cellular response (e.g. viability and apoptosis) to radiation. If our hypothesis is correct, and the linc-SPRY3 transcripts do sequester IGF2BP3 as its primary function, then exogenous overexpression of IGF2BP3 should promote a more radioresistant phenotype in radiosensitive cell lines similar to what was seen upon knockdown of linc-SPRY3-2/3/4, and knockdown of IGF2BP3 in radioresistant cell lines should lead to increased sensitivity to radiation.

It is also important to consider the other functions of IGF2BP3 which could be regulated by the linc-SPRY3 RNAs. We provide significant evidence of a downstream effect on the ability of IGF2BP3 to stabilize mRNA targets such as c-Myc and HMGA2, two targets that were chosen because of their proven roles in regulating cell viability and apoptosis in cancer cells. However, IGF2BP3 is a prolific RBP with thousands of predicted targets and multiple functions aside from
mRNA stability which include miRNA biogenesis, RNA degradation, and RNA localization (Fig. 3) (30). These pathways would provide alternative avenues of study regarding the potential molecular functions mediated downstream of the linc-SPRY3/IGF2BP3 interactions.

Numerous studies investigating lncRNAs have revealed that many of them have multiple functions, and moreover interact with more than one binding partner which can be DNA, RNA, or proteins (31). We have provided evidence of a direct interaction between linc-SPRY3-2/3/4 and the RBP IGF2BP3, but we cannot discount other potential interactions of linc-SPRY3 RNAs. In order to investigate other potential interactions, we could employ the method of chromatin isolation by RNA purification (ChIRP). Briefly, biotinylated probes are designed complementary to a transcript of interest (in our case the linc-SPRY3 RNAs) and incubated with lysates from crosslinked cells. Next, magnetic streptavidin beads are used to recover hybridized RNA and anything potentially bound to that RNA due to crosslinking. The recovered material can then be

Figure 3: Mechanisms of IGF2BP3 (IGF2BP3 From Physiology to Cancer: Novel Discoveries, Unsolved Issues, and Future Perspectives, Front. Cell Dev. Biol., Caterina Mancarella and Katia Scotlandi, 2020)
subjected to sequencing for DNA elements or RNA binding partners, or mass spectrometry for protein binding partners. As mentioned previously regarding other suggested methods, the linc-SPRY3-2/3/4 sequence(s) will need to be determined first so effective probes can be designed to pull down the lncRNAs. In our hands it would be interesting to perform this using lysates from cells either treated or untreated with radiation and then compare the recovered DNA/RNA/protein to identify primary interactions in response to radiation.

An additional aspect of the project presented in Chapter 2 that should be investigated in more depth is the use of ChY as a predictive marker for patient response to radiation therapy as well as overall survival. Previous studies have implicated loss of ChY (LOY) as a marker for poor prognosis/survival in multiple cancer types (32–37). Furthermore, data we collected utilizing ChY DNA FISH from a NSCLC tumor microarray (TMA) revealed a trending negative correlation between LOY and overall survival in males, though the sample size was too small to provide statistical significance (Chapter 2, Fig. 5). We intend on increasing the sample size by adding more TMAs in an attempt to reach statistical significance. Additionally, in an attempt to build on this, we initiated a retrospective study in collaboration with Dr. Malcolm Mattes of Rutgers Cancer Institute of New Jersey (formerly WVU Cancer Institute) to examine LOY using ChY DNA FISH in treatment naïve NSCLC tumor samples. The goal of this study is to determine if LOY could be used as a predictive biomarker for patient response to radiation therapy. Our current hypothesis is that patients whose tumors exhibit high LOY will respond worse to radiation than those whose tumors have managed to retain ChY. If correct, this could eventually lead to a clinical trial to validate LOY as a predictive biomarker for radiation therapy use in male NSCLC patients.

While our group has primarily focused on ChY and linc-SPRY3-2/3/4 in NSCLC, we also have an interest in pursuing similar studies outlined above and in Chapter 2 in other cancer types. It is well documented that disparities between the sexes, independent of race, exist in both incidence and survival for the vast majority of cancer types (Fig. 4). Many mechanisms have been proposed to explain these disparities, though most are primarily attributed to differences in sex hormone function and regulation (38,39). It is only recently that the sex chromosomes and their resident genes have gained significant attention in regards to their roles in regulating carcinogenesis, though the X chromosome has received far more attention than ChY. A recent
PubMed search using the keywords X chromosome and cancer resulted in nearly 4,000 publications, versus Y chromosome and cancer which yielded less than half that at ~1,700 publications.

Overall, we believe that ChY as a whole and its genes, discovered and undiscovered, deserve a lot more attention. Additional studies into their relationship to cancer could provide a better understanding of the rampant sex disparities seen in incidence and mortality across multiple cancers leading to the development of precision medicine not just informed by diagnostic standards such as cancer type and driver mutations but by inherent sexual differences.
Project Summary

1. Identification of Candidate LncRNAs
   - Treat cells with radiation and extract RNA
   - Send for microarray analysis
   - Identify significantly up and downregulated genes

2. Characterization of Linc-SPRY3-2/3/4
   - UCSC Genome Browser reveals the linc-SPRY3 RNAs originate from the Y chromosome
   - qRT-PCR Analysis reveals a dose dependent increase in expression of linc-SPRY3-2/3/4 in radiosensitive male NSCLC cell lines but not in radioresistant cell lines
   - DNA FISH Reveals Y chromosome loss in radioresistant male NSCLC cell lines

3. Manipulation of linc-SPRY3-2/3/4 expression changes NSCLC cell line radiosensitivity
   - Exogenous expression of linc-SPRY3-2/3/4 via Y chromosome BAC results in increased radiosensitivity in A549 cells
3. Manipulation of linc-SPRY3-2/3/4 expression changes NSCLC cell line radiosensitivity (cont.)

- Knockdown of linc-SPRY3-2/3/4 via shRNAs results increased radioresistance in vitro and in vivo in H460 and WVU-Ma-0005 cells

4. Evidence suggests the linc-SPRY3 RNAs function through sequestration of the RBP IGF2BP3

5. The Y chromosome and linc-SPRY3 RNAs have potential clinical relevance by showing trending negative correlations with male NSCLC patient survival.
Future Directions

- Sequence the linc-SPRY3 RNAs via minION nanopore sequencing
- Fully vet the linc-SPRY3/IGF2BP3 interaction and proposed mechanism
- Perform ChIRP to identify potential binding partners, which will allow us to further investigate the Linc-SPRY3 molecular functions
- Use DNA/RNA FISH to correlate Y chromosome presence and Linc-SPRY3 expression with patient survival and validate their potential use as a biomarker for male NSCLC
- Determine if these IncRNAs have a role in other cancers

References


Figure Copyright Permissions

(Copyright permissions were obtained for figures reused from journals which required requested permission. This section contains the permission licenses for the necessary figures. All others were obtained from open access journals.)
# Chapter 1: Figure 1

## Annual Reviews, Inc. - License Terms and Conditions

This is a License Agreement between Tayvia Brownmiller ("You") and Annual Reviews, Inc. ("Publisher") provided by Copyright Clearance Center ("CCC"). The license consists of your order details; the terms and conditions provided by Annual Reviews, Inc., and the CCC terms and conditions.

All payments must be made in full to CCC.

<table>
<thead>
<tr>
<th>Order Date</th>
<th>30-May-2020</th>
<th>Type of Use</th>
<th>Republish in a thesis/dissertation ANNUAL REVIEWS Image/photo/illustration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order license ID</td>
<td>1038456-1</td>
<td>Publisher</td>
<td></td>
</tr>
<tr>
<td>ISSN</td>
<td>1545-4509</td>
<td>Portion</td>
<td></td>
</tr>
</tbody>
</table>

### LICENSED CONTENT

- **Publication Title**: Annual review of biochemistry
- **Date**: 01/01/1932
- **Language**: English
- **Country**: United States of America
- **Rightsholder**: Annual Reviews, Inc.
- **Publication Type**: e-Journal
- **URL**: http://arjournals.annualreviews.org/loi/biochem

### REQUEST DETAILS

- **Portion Type**: Image/photo/illustration
- **Format (select all that apply)**: Print, Electronic
- **Who will republish the content?**: Academic institution
- **Duration of Use**: Life of current edition
- **Lifetime Unit Quantity**: More than 2,000,000
- **Rights Requested**: Main product
- **Distribution**: Worldwide
- **Translation**: Original language of publication
- **Copies for the disabled?**: No
- **Minor editing privileges?**: Yes
- **Incidental promotional use?**: No
- **Currency**: USD

### NEW WORK DETAILS

- **Title**: Evidence of Y Chromosome Long Non-Coding RNAs involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells
- **Instructor name**: Tayvia Brownmiller
- **Institution name**: West Virginia University
- **Expected presentation date**: 2020-06-01

### ADDITIONAL DETAILS

- **Order reference number**: N/A
CCC Republication Terms and Conditions

1. Description of Service: Defined Terms. This Republication License enables the User to obtain licenses for republication of one or more copyrighted works as described in detail on the relevant Order Confirmation (the "Work(s)"). Copyright Clearance Center, Inc. ("CCC") grants licenses through the Service on behalf of the rightsholder identified on the Order Confirmation (the "Rightsholder"). "Republication", as used herein, generally means the inclusion of a Work, in whole or in part, in a new work or works, also as described on the Order Confirmation. "User", as used herein, means the person or entity making such republication.

2. The terms set forth in the relevant Order Confirmation, and any terms set by the Rightsholder with respect to a particular Work, govern the terms of use of Works in connection with the Service. By using the Service, the person transacting for a republication license on behalf of the User represents and warrants that he/she/it (a) has been duly authorized by the User to accept, and hereby does accept, all such terms and conditions on behalf of User, and (b) shall inform User of all such terms and conditions. In the event such person is a "freelancer" or other third party independent of User and CCC, such party shall be deemed jointly a "User" for purposes of these terms and conditions. In any event, User shall be deemed to have accepted and agreed to all such terms and conditions if User republishes the Work in any fashion.

3. Scope of License; Limitations and Obligations.

3.1. All Works and all rights therein, including copyright rights, remain the sole and exclusive property of the Rightsholder. The license created by the exchange of an Order Confirmation (and/or any invoice) and payment by User of the full amount set forth on that document includes only those rights expressly set forth in the Order Confirmation and in these terms and conditions, and conveys no other rights in the Work(s) to User. All rights not expressly granted are hereby reserved.

3.2. General Payment Terms: You may pay by credit card or through an account with us payable at the end of the month. If you and we agree that you may establish a standing account with CCC, then the following terms apply: Remit Payment to: Copyright Clearance Center, 29118 Network Place, Chicago, IL 60673-1291. Payments Due: Invoices are payable upon their delivery to you (or upon our notice to you that they are available to you for downloading). After 30 days, outstanding amounts will be subject to a service charge of 1-1/2% per month or, if less, the maximum rate allowed by applicable law. Unless otherwise specifically set forth in the Order Confirmation or in a separate written agreement signed by CCC, invoices are due and payable on "net 30" terms. While User may exercise the rights licensed immediately upon issuance of the Order Confirmation, the license is automatically revoked and is null and void, as if it had never been issued, if complete payment for the license is not received on a timely basis either from User directly or through a payment agent, such as a credit card company.

3.3. Unless otherwise provided in the Order Confirmation, any grant of rights to User (i) is "one-time" (including
the editions and product family specified in the license), (ii) is non-exclusive and non-transferable and (iii) is subject to any and all limitations and restrictions (such as, but not limited to, limitations on duration of use or circulation) included in the Order Confirmation or invoice and/or in these terms and conditions. Upon completion of the licensed use, User shall either secure a new permission for further use of the Work(s) or immediately cease any new use of the Work(s) and shall render inaccessible (such as by deleting or by removing or severing links or other locators) any further copies of the Work (except for copies printed on paper in accordance with this license and still in User’s stock at the end of such period).

3.4. In the event that the material for which a republication license is sought includes third party materials (such as photographs, illustrations, graphs, inserts and similar materials) which are identified in such material as having been used by permission, User is responsible for identifying, and seeking separate licenses (under this Service or otherwise) for, any such third party materials; without a separate license, such third party materials may not be used.

3.5. Use of proper copyright notice for a Work is required as a condition of any license granted under the Service. Unless otherwise provided in the Order Confirmation, a proper copyright notice will read substantially as follows: "Republished with permission of [Rightsholder's name], from [Work's title, author, volume, edition number and year of copyright]; permission conveyed through Copyright Clearance Center, Inc." Such notice must be provided in a reasonably legible font size and must be placed either immediately adjacent to the Work as used (for example, as part of a by-line or footnote but not as a separate electronic link) or in the place where substantially all other credits or notices for the new work containing the republicated Work are located. Failure to include the required notice results in loss to the Rightsholder and CCC, and the User shall be liable to pay liquidated damages for each such failure equal to twice the use fee specified in the Order Confirmation, in addition to the use fee itself and any other fees and charges specified.

3.6. User may only make alterations to the Work if and as expressly set forth in the Order Confirmation. No Work may be used in any way that is defamatory, violates the rights of third parties (including such third parties’ rights of copyright, privacy, publicity, or other tangible or intangible property), or is otherwise illegal, sexually explicit or obscene. In addition, User may not conjoin a Work with any other material that may result in damage to the reputation of the Rightsholder. User agrees to inform CCC if it becomes aware of any infringement of any rights in a Work and to cooperate with any reasonable request of CCC or the Rightsholder in connection therewith.

4. Indemnity. User hereby indemnifies and agrees to defend the Rightsholder and CCC, and their respective employees and directors, against all claims, liability, damages, costs and expenses, arising out of any use of a Work beyond the scope of the rights granted herein, or any use of a Work which has been altered in any unauthorized way by User, including claims of defamation or infringement of rights of copyright, publicity, privacy or other tangible or intangible property.

5. Limitation of Liability. UNDER NO CIRCUMSTANCES WILL CCC OR THE RIGHTSHOLDER BE LIABLE FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL OR INCIDENTAL DAMAGES (INCLUDING WITHOUT LIMITATION DAMAGES FOR LOSS OF BUSINESS PROFITS OR INFORMATION, OR FOR BUSINESS INTERRUPTION) ARISING OUT OF THE USE OR INABILITY TO USE A WORK, EVEN IF ONE OF THEM HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. In any event the total liability of the Rightsholder and CCC (including their respective employees and directors) shall not exceed the total amount actually paid by User for this license. User assumes full liability for the actions and omissions of its principals, employees, agents, affiliates, successors and assigns.

6. Limited Warranties. THE WORK(S) AND RIGHT(S) ARE PROVIDED "AS IS". CCC HAS THE RIGHT TO GRANT TO USER THE RIGHTS GRANTED IN THE ORDER CONFIRMATION DOCUMENT. CCC AND THE RIGHTSHOLDER DISCLAIM ALL OTHER WARRANTIES RELATING TO THE WORK(S) AND RIGHT(S), EITHER EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. ADDITIONAL RIGHTS MAY BE REQUIRED TO USE ILLUSTRATIONS, GRAPHS, PHOTOGRAPHS, ABSTRACTS, INSERTS OR OTHER PORTIONS OF THE WORK (AS OPPOSED TO THE ENTIRE WORK) IN A MANNER CONTEMPLATED BY USER USER UNDERSTANDS AND AGREES THAT NEITHER CCC NOR THE RIGHTSHOLDER MAY HAVE SUCH ADDITIONAL RIGHTS TO GRANT.
7. Effect of Breach. Any failure by User to pay any amount when due, or any use by User of a Work beyond the scope of the license set forth in the Order Confirmation and/or these terms and conditions, shall be a material breach of the license created by the Order Confirmation and these terms and conditions. Any breach not cured within 30 days of written notice thereof shall result in immediate termination of such license without further notice. Any unauthorized (but licensable) use of a Work that is terminated immediately upon notice thereof may be liquidated by payment of the Rightsholder’s ordinary license price therefor; any unauthorized (and unlicensable) use that is not terminated immediately for any reason (including, for example, because materials containing the Work cannot reasonably be recalled) will be subject to all remedies available at law or in equity, but in no event to a payment of less than three times the Rightsholder’s ordinary license price for the most closely analogous licensable use plus Rightsholder’s and/or CCC’s costs and expenses incurred in collecting such payment.

8. Miscellaneous.

8.1. User acknowledges that CCC may, from time to time, make changes or additions to the Service or to these terms and conditions, and CCC reserves the right to send notice to the User by electronic mail or otherwise for the purposes of notifying User of such changes or additions; provided that any such changes or additions shall not apply to permissions already secured and paid for.

8.2. Use of User-related information collected through the Service is governed by CCC’s privacy policy, available online here: https://marketplace.copyright.com/rs-ui-web/mp/privacy-policy

8.3. The licensing transaction described in the Order Confirmation is personal to User. Therefore, User may not assign or transfer to any other person (whether a natural person or an organization of any kind) the license created by the Order Confirmation and these terms and conditions or any rights granted hereunder; provided, however, that User may assign such license in its entirety on written notice to CCC in the event of a transfer of all or substantially all of User’s rights in the new material which includes the Work(s) licensed under this Service.

8.4. No amendment or waiver of any terms is binding unless set forth in writing and signed by the parties. The Rightsholder and CCC hereby object to any terms contained in any writing prepared by the User or its principals, employees, agents or affiliates and purporting to govern or otherwise relate to the licensing transaction described in the Order Confirmation, which terms are in any way inconsistent with any terms set forth in the Order Confirmation and/or in these terms and conditions or CCC’s standard operating procedures, whether such writing is prepared prior to, simultaneously with or subsequent to the Order Confirmation, and whether such writing appears on a copy of the Order Confirmation or in a separate instrument.

8.5. The licensing transaction described in the Order Confirmation document shall be governed by and construed under the law of the State of New York, USA, without regard to the principles thereof of conflicts of law. Any case, controversy, suit, action, or proceeding arising out of, in connection with, or related to such licensing transaction shall be brought, at CCC’s sole discretion, in any federal or state court located in the County of New York, State of New York, USA, or in any federal or state court whose geographical jurisdiction covers the location of the Rightsholder set forth in the Order Confirmation. The parties expressly submit to the personal jurisdiction and venue of each such federal or state court. If you have any comments or questions about the Service or Copyright Clearance Center, please contact us at 978-750-8400 or send an e-mail to support@copyright.com.

v 1.1
**Chapter 1: Figure 3**

**SPRINGER NATURE LICENSE TERMS AND CONDITIONS**

May 30, 2020

This Agreement between Tayvia Brownmiller ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

<table>
<thead>
<tr>
<th>License Number</th>
<th>4839041147275</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td>May 30, 2020</td>
</tr>
<tr>
<td>Licensed Content Publisher</td>
<td>Springer Nature</td>
</tr>
<tr>
<td>Licensed Content Publication</td>
<td>Nature Reviews Molecular Cell Biology</td>
</tr>
<tr>
<td>Licensed Content Title</td>
<td>Ribosome profiling reveals the what, when, where and how of protein synthesis</td>
</tr>
<tr>
<td>Licensed Content Author</td>
<td>Gloria A. Brar et al</td>
</tr>
<tr>
<td>Licensed Content Date</td>
<td>Oct 14, 2015</td>
</tr>
<tr>
<td>Type of Use</td>
<td>Thesis/Dissertation</td>
</tr>
<tr>
<td>Requestor type</td>
<td>academic/university or research institute</td>
</tr>
<tr>
<td>Format</td>
<td>print and electronic</td>
</tr>
<tr>
<td>Portion</td>
<td>figures/tables/illustrations</td>
</tr>
<tr>
<td>Number of figures/tables/illustrations</td>
<td>1</td>
</tr>
</tbody>
</table>
Title: Evidence of Y Chromosome Long Non-Coding RNAs involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells

Institution name: West Virginia University

Expected presentation date: Jun 2020

Portions: Figure 1a

Requestor Location: PO Box 9300
MORGANTOWN, WV 26505
United States
Attn: Tayvia Brownmiller

Total: 0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the Licence) between you and Springer Nature Customer Service Centre GmbH (the Licensor). By clicking 'accept' and completing the transaction for the material (Licensed Material), you also confirm your acceptance of these terms and conditions.

1. Grant of License
1. The Licensor grants you a personal, non-exclusive, non-transferable, worldwide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1.2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1.3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2.1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2.2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2.3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2.4. Where permission has been granted free of charge for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2.5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3.1. A licence for is valid from the date of purchase (‘Licence Date’) at the end of the relevant period in the below table:

<table>
<thead>
<tr>
<th>Scope of Licence</th>
<th>Duration of Licence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post on a website</td>
<td>12 months</td>
</tr>
<tr>
<td>Presentations</td>
<td>12 months</td>
</tr>
<tr>
<td>Books and journals</td>
<td>Lifetime of the edition in the language purchased</td>
</tr>
</tbody>
</table>
4. Acknowledgement

4.1. The Licensor's permission must be acknowledged next to the Licensed Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5.1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5.2. You must not use any Licensed Material as part of any design or trademark.

5.3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6.1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations
8.1. **BOOKS ONLY**: Where 'reuse in a dissertation/thesis' has been selected the following terms apply: Print rights of the final author’s accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9.1. Licences will expire after the period shown in Clause 3 (above).

9.2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION] (Article name, Author(s) Name), [COPYRIGHT] (year of publication)

For Advance Online Publication papers:
Reprinted by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION] (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION] (Article name, Author(s) Name), [COPYRIGHT] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:
Reprinted/adapted/translated by permission from [the Licensor]: on behalf of Cancer Research UK: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION] (Article name, Author(s) Name), [COPYRIGHT] (year of publication)

For Advance Online Publication papers:
Reprinted by permission from The [the Licensor] on behalf of Cancer Research UK: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION] (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [the Licensor]: [Book Publisher (e.g. Palgrave Macmillan, Springer etc)] [Book Title] by [Book author(s)] [COPYRIGHT] (year of publication)
Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.
This Agreement between Tayvia Brownmiller ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

<table>
<thead>
<tr>
<th>License Number</th>
<th>4839050188348</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td>May 30, 2020</td>
</tr>
<tr>
<td>Licensed Content Publisher</td>
<td>Springer Nature</td>
</tr>
<tr>
<td>Licensed Content Publication</td>
<td>Springer eBook</td>
</tr>
<tr>
<td>Licensed Content Title</td>
<td>History, Discovery, and Classification of IncRNAs</td>
</tr>
<tr>
<td>Licensed Content Author</td>
<td>Julien Jarroux, Antonin Morillon, Marina Pinskaya</td>
</tr>
<tr>
<td>Licensed Content Date</td>
<td>Jan 1, 2017</td>
</tr>
<tr>
<td>Type of Use</td>
<td>Thesis/Dissertation</td>
</tr>
<tr>
<td>Requestor type</td>
<td>academic/university or research institute</td>
</tr>
<tr>
<td>Format</td>
<td>print and electronic</td>
</tr>
<tr>
<td>Portion</td>
<td>figures/tables/illustrations</td>
</tr>
<tr>
<td>Number of figures/tables/illustrations</td>
<td>1</td>
</tr>
</tbody>
</table>
Will you be translating?  no  
Circulation/distribution  50000 or greater  
Author of this Springer Nature content  no  
Title  Evidence of Y Chromosome Long Non-Coding RNAs involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells  
Institution name  West Virginia University  
Expected presentation date  Jun 2020  
Portions  Figure 1.4  
Requestor Location  Tayvia Brownmiller  1 Medical Center Dr  PO Box 9300  Room 1815  MORGANTOWN, WV 26505  United States  Attn: Tayvia Brownmiller  
Total  0.00 USD  

Terms and Conditions

Springer Nature Customer Service Centre GmbH  
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the Licence) between you and Springer Nature Customer Service Centre GmbH (the Licensor). By clicking 'accept' and completing the transaction for the material (Licensed Material), you also confirm your acceptance of these terms and conditions.

1. Grant of License

   1.1. The Licensor grants you a personal, non-exclusive, non-transferable, worldwide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and
for no other use, subject to the conditions below.

1.2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1.3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2.1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2.2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2.3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2.4. Where permission has been granted free of charge for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2.5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3.1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

<table>
<thead>
<tr>
<th>Scope of Licence</th>
<th>Duration of Licence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post on a website</td>
<td>12 months</td>
</tr>
<tr>
<td>Presentations</td>
<td>12 months</td>
</tr>
<tr>
<td>Books and journals</td>
<td>Lifetime of the edition in the language purchased</td>
</tr>
</tbody>
</table>

4. Acknowledgement
4. The Licensor’s permission must be acknowledged next to the Licensed Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book’s homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5.1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5.2. You must not use any Licensed Material as part of any design or trademark.

5.3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6.1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8.1. BOOKS ONLY Where ‘reuse in a dissertation/thesis’ has been selected the following terms apply: Print rights of the final author’s accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use
only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)

For Advance Online Publication papers:
Reprinted by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM]).]

For Adaptations/Translations:
Adapted/Translated by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [the Licensor]: on behalf of Cancer Research UK: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)

For Advance Online Publication papers:
Reprinted by permission from The [the Licensor]: on behalf of Cancer Research UK: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi 10.1038/sj. [JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [the Licensor]: [Book Publisher (e.g. Palgrave Macmillan, Springer etc) [Book Title] by [Book author(s)] [COPYRIGHT] (year of publication)

Other Conditions:
Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.
**Chapter 1: Figure 5**

**ELSEVIER LICENSE TERMS AND CONDITIONS**

May 30, 2020

---

This Agreement between Tayvia Brownmiller ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

<table>
<thead>
<tr>
<th>License Number</th>
<th>4839050395708</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td>May 30, 2020</td>
</tr>
<tr>
<td>Licensed Content</td>
<td>Elsevier</td>
</tr>
<tr>
<td>Publisher</td>
<td></td>
</tr>
<tr>
<td>Licensed Content</td>
<td>Molecular Cell</td>
</tr>
<tr>
<td>Publication</td>
<td></td>
</tr>
<tr>
<td>Licensed Content</td>
<td>Molecular Mechanisms of Long Noncoding RNAs</td>
</tr>
<tr>
<td>Title</td>
<td></td>
</tr>
<tr>
<td>Licensed Content</td>
<td>Kevin C. Wang, Howard Y. Chang</td>
</tr>
<tr>
<td>Author</td>
<td></td>
</tr>
<tr>
<td>License Date</td>
<td>Sep 16, 2011</td>
</tr>
<tr>
<td>Licensed Content</td>
<td>43</td>
</tr>
<tr>
<td>Volume</td>
<td></td>
</tr>
<tr>
<td>Licensed Content</td>
<td>6</td>
</tr>
<tr>
<td>Issue</td>
<td></td>
</tr>
<tr>
<td>Content Pages</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Start Page</td>
<td>904</td>
</tr>
<tr>
<td>End Page</td>
<td>914</td>
</tr>
</tbody>
</table>
INTRODUCTION
1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at permissions@elsevier.com). No modifications can be made to any Lancet figures/tables and they must be reproduced in full.

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials
beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. Translation: This permission is granted for non-exclusive world English rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article.

16. Posting licensed content on any Website: The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the
Elsvier homepage for books at http://www.elsevier.com; Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at http://www.elsevier.com. All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.

17. For journal authors: the following clauses are applicable in addition to the above:

Preprints:

A preprint is an author's own write-up of research results and analysis, it has not been peer-reviewed, nor has it had any other value added to it by a publisher (such as formatting, copyright, technical enhancement etc.).

Authors can share their preprints anywhere at any time. Preprints should not be added to or enhanced in any way in order to appear more like, or to substitute for, the final versions of articles however authors can update their preprints on arXiv or RePEc with their Accepted Author Manuscript (see below).

If accepted for publication, we encourage authors to link from the preprint to their formal publication via its DOI. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help users to find, access, cite and use the best available version. Please note that Cell Press, The Lancet and some society-owned have different preprint policies. Information on these policies is available on the journal homepage.

Accepted Author Manuscripts: An accepted author manuscript is the manuscript of an article that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and editor-author communications.

Authors can share their accepted author manuscript:

- immediately
  - via their non-commercial person homepage or blog
  - by updating a preprint in arXiv or RePEc with the accepted manuscript
  - via their research institute or institutional repository for internal institutional uses or as part of an invitation-only research collaboration work-group
  - directly by providing copies to their students or to research collaborators for their personal use
  - for private scholarly sharing as part of an invitation-only work group on commercial sites with which Elsevier has an agreement
- After the embargo period
via non-commercial hosting platforms such as their institutional repository
via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license - this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article.

Published journal article (JPA): A published journal article (PJA) is the definitive final record of published research that appears or will appear in the journal and embodies all value-adding publishing activities including peer review co-ordination, copy-editing, formatting, (if relevant) pagination and online enrichment.

Policies for sharing publishing journal articles differ for subscription and gold open access articles:

Subscription Articles: If you are an author, please share a link to your article rather than the full-text. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help your users to find, access, cite, and use the best available version.

Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

If you are affiliated with a library that subscribes to ScienceDirect you have additional private sharing rights for others' research accessed under that agreement. This includes use for classroom teaching and internal training at the institution (including use in course packs and courseware programs), and inclusion of the article for grant funding purposes.

Gold Open Access Articles: May be shared according to the author-selected end-user license and should contain a CrossMark logo, the end user license, and a DOI link to the formal publication on ScienceDirect.

Please refer to Elsevier’s posting policy for further information.

18. For book authors the following clauses are applicable in addition to the above:
Authors are permitted to place a brief summary of their work online only. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. Posting to a repository: Authors are permitted to post a summary of their chapter only in their institution's repository.

19. Thesis/Dissertation: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for Proquest/UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission. Theses and
dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

Elsevier Open Access Terms and Conditions

You can publish open access with Elsevier in hundreds of open access journals or in nearly 2000 established subscription journals that support open access publishing. Permitted third party re-use of these open access articles is defined by the author’s choice of Creative Commons user license. See our open access license policy for more information.

Terms & Conditions applicable to all Open Access articles published with Elsevier:

Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author’s honour or reputation. If any changes have been made, such changes must be clearly indicated.

The author(s) must be appropriately credited and we ask that you include the end user license and a DOI link to the formal publication on ScienceDirect.

If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.

Additional Terms & Conditions applicable to each Creative Commons user license:

CC BY: The CC-BY license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article and to make commercial use of the Article (including reuse and/or resale of the Article by commercial entities), provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by/4.0.

CC BY NC SA: The CC BY-NC-SA license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article, provided this is not done for commercial purposes, and that the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. Further, any new works must be made available on the same conditions. The full details of the license are available at http://creativecommons.org/licenses/by-nc-sa/4.0.

CC BY NC ND: The CC BY-NC-ND license allows users to copy and distribute the Article, provided this is not done for commercial purposes and further does not permit distribution of the Article if it is changed or edited in any way, and provided the user gives appropriate credit (with a link to the formal publication through the
relevant DOI), provides a link to the license, and that the licensor is not represented as endorsing the use made of the work. The full details of the license are available at [http://creativecommons.org/licenses/by-nc-nd/4.0](http://creativecommons.org/licenses/by-nc-nd/4.0). Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.

Commercial reuse includes:

- Associating advertising with the full text of the Article
- Charging fees for document delivery or access
- Article aggregation
- Systematic distribution via e-mail lists or share buttons

Posting or linking by commercial companies for use by customers of those companies.
Chapter 1: Figure 7

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

May 30, 2020

This Agreement between Tayvia Brownmiller ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

<table>
<thead>
<tr>
<th>License Number</th>
<th>4839050600821</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td>May 30, 2020</td>
</tr>
<tr>
<td>Licensed Content Publisher</td>
<td>John Wiley and Sons</td>
</tr>
<tr>
<td>Licensed Content Publication</td>
<td>Journal of Gene Medicine</td>
</tr>
<tr>
<td>Licensed Content Title</td>
<td>Long non-coding RNAs in cancer: Another layer of complexity</td>
</tr>
<tr>
<td>Licensed Content Author</td>
<td>Jaqueline Carvalho Oliveira, Luana Caroline Oliveira, Carolina Mathias, et al</td>
</tr>
<tr>
<td>Licensed Content Date</td>
<td>Jan 8, 2019</td>
</tr>
<tr>
<td>Licensed Content Volume</td>
<td>21</td>
</tr>
<tr>
<td>Licensed Content Issue</td>
<td>1</td>
</tr>
</tbody>
</table>
Licensed Content Pages

Type of use  Dissertation/Thesis

Requestor type  University/Academic

Format  Print and electronic

Portion  Figure/table

Number of figures/tables  1

Will you be translating?  No

Title  Evidence of Y Chromosome Long Non-Coding RNAs involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells

Institution name  West Virginia University

Expected presentation date  Jun 2020

Portions  Figure 1

Requestor Location
Tayvia Brownmiller  
1 Medical Center Dr
PO Box 9300  
Room 1815  
MORGANTOWN, WV 26505  
United States  
Attn: Tayvia Brownmiller

Publisher Tax ID  EU826007151
Total 0.00 USD

Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a "Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.

- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, and any CONTENT (PDF or image file) purchased as part of your order, is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner. For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts, You may not alter, remove or suppress in any manner any copyright,
trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto.

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect.
as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY’s prior written consent.

- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

- These terms and conditions together with CCC’s Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties’ successors, legal representatives, and authorized assigns.

- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC’s Billing and Payment terms and conditions, these terms and conditions shall prevail.

- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC’s Billing and Payment terms and conditions.

- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state’s conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

**WILEY OPEN ACCESS TERMS AND CONDITIONS**
Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

**The Creative Commons Attribution License**

The [Creative Commons Attribution License (CC-BY)](https://creativecommons.org/licenses/by/4.0/) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

**Creative Commons Attribution Non-Commercial License**

The [Creative Commons Attribution Non-Commercial (CC-BY-NC) License](https://creativecommons.org/licenses/by-nc/4.0/) permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes. (see below)

**Creative Commons Attribution-Non-Commercial-NoDerivs License**

The [Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND)](https://creativecommons.org/licenses/by-nc-nd/4.0/) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

**Use by commercial "for-profit" organizations**

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Further details can be found on Wiley Online Library [http://onelibrary.wiley.com/WileyCDA/Section/id-410895.html](http://onelibrary.wiley.com/WileyCDA/Section/id-410895.html)

**Other Terms and Conditions:**

v1.10 Last updated September 2015

Questions? [customercare@copyright.com](mailto:customercare@copyright.com) or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.
Chapter 1: Figure 8

AMERICAN ASSOCIATION FOR CANCER RESEARCH LICENSE TERMS AND CONDITIONS

May 30, 2020

This Agreement between Tayvia Brownmiller ("You") and American Association for Cancer Research ("American Association for Cancer Research") consists of your license details and the terms and conditions provided by American Association for Cancer Research and Copyright Clearance Center.

License Number 4839050908719

License date May 30, 2020

Licensed Content Publisher American Association for Cancer Research

Licensed Content Publication Clinical Cancer Research

Licensed Content Title Molecular Pathways: Overcoming Radiation Resistance by Targeting DNA Damage Response Pathways

Licensed Content Author Meredith A. Morgan, Theodore S. Lawrence

Licensed Content Date Jul 1, 2015

Licensed Content Volume 21

Licensed Content Issue 13

Type of Use Thesis/Dissertation

Requestor type academic/educational
Format: print and electronic

Portion: figures/tables/illustrations

Number of figures/tables/illustrations: 1

Will you be translating? no

Circulation: 999999

Territory of distribution: Worldwide

Title: Evidence of Y Chromosome Long Non-Coding RNAs involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells

Institution name: West Virginia University

Expected presentation date: Jun 2020

Portions: Figure 1

Requestor Location: Tayvia Brownmiller
1 Medical Center Dr
PO Box 9300
Room 1815
MORGANTOWN, WV 26505
United States
Attn: Tayvia Brownmiller

Total: 0.00 USD

Terms and Conditions

American Association for Cancer Research (AACR) Terms and Conditions

INTRODUCTION
The Publisher for this copyright material is the American Association for Cancer
Research (AACR). By clicking "accept" in connection with completing this licensing transaction, you agree to the following terms and conditions applying to this transaction. You also agree to the Billing and Payment terms and conditions established by Copyright Clearance Center (CCC) at the time you opened your Rightslink account.

LIMITED LICENSE
The AACR grants exclusively to you, the User, for onetime, non-exclusive use of this material for the purpose stated in your request and used only with a maximum distribution equal to the number you identified in the permission process. Any form of republication must be completed within one year although copies made before then may be distributed thereafter and any electronic posting is limited to a period of one year. Reproduction of this material is confined to the purpose and/or media for which permission is granted. Altering or modifying this material is not permitted. However, figures and illustrations may be minimally altered or modified to serve the new work.

GEOGRAPHIC SCOPE
Licenses may be exercised as noted in the permission process

RESERVATION OF RIGHTS
The AACR reserves all rights not specifically granted in the combination of 1) the license details provided by you and accepted in the course of this licensing transaction, 2) these terms and conditions, and 3) CCC's Billing and Payment terms and conditions.

DISCLAIMER
You may obtain permission via Rightslink to use material owned by AACR. When you are requesting permission to reuse a portion for an AACR publication, it is your responsibility to examine each portion of content as published to determine whether a credit to, or copyright notice of a third party owner is published next to the item. You must obtain permission from the third party to use any material which has been reprinted with permission from the said third party. If you have not obtained permission from the third party, AACR disclaims any responsibility for the use you make of items owned by them.

LICENSE CONTINGENT ON PAYMENT
While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you, either by the publisher or by the CCC, as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions, or any of the CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unretracted license, may constitute copyright infringement and the publisher reserves the right to take any and all action to protect its copyright in the materials.

COPYRIGHT NOTICE
You must include the following credit line in connection with your reproduction of the
TRANSLATION
This permission is granted for non-exclusive world English rights only.

WARRANTIES
Publisher makes no representations or warranties with respect to the licensed material.

INDEMNIFICATION
You hereby indemnify and agree to hold harmless the publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

REVOCATION
The AACR reserves the right to revoke a license for any reason, including but not limited to advertising and promotional uses of AACR content, third party usage and incorrect figure source attribution.

NO TRANSFER OF LICENSE
This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

NO AMENDMENT EXCEPT IN WRITING
This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

OBJECTION TO CONTRARY TERMS
Publishers hereby objects to any terms contained in any purchase order, acknowledgement, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC’s Billing and Payment terms and conditions. These terms and conditions together with CCC’s Billing and Payment terms and conditions (which are incorporated herein) comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions, and those established by CCC’s Billing and Payment terms and conditions, these terms and conditions shall control.

THESIS/DISSERTATION TERMS
If your request is to reuse an article authored by you and published by the AACR in your dissertation/thesis, your thesis may be submitted to your institution in either in print or electronic form. Should your thesis be published commercially, please reapply.

ELECTRONIC RESERVE
If this license is made in connection with a course, and the Licensed Material or any portion thereof is to be posted to a website, the website is to be password protected and made available only to the students registered for the relevant course. The permission is granted for the duration of the course. All content posted to the website must maintain the copyright information notice.

JURISDICTION
This license transaction shall be governed by and construed in accordance with the
laws of Pennsylvania. You hereby agree to submit to the jurisdiction of the federal and state courts located in Pennsylvania for purposes of resolving any disputes that may arise in connection with this licensing transaction.

Other Terms and Conditions:

v1.0

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.
Chapter 3: Figure 4

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

May 30, 2020

This Agreement between Tayvia Brownmiller ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number 4839051330343
License date May 30, 2020
Licensed Content Publisher Springer Nature
Licensed Content Publication Nature Reviews Cancer
Licensed Content Title Sexual dimorphism in cancer
Licensed Content Author Andrea Clocchiatti et al
Licensed Content Date Apr 15, 2016
Type of Use Thesis/Dissertation
Requestor type academic/university or research institute
Format print and electronic
Portion figures/tables/illustrations
Number of figures/tables/illustrations 1
Title: Evidence of Y Chromosome Long Non-Coding RNAs involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells

Institution name: West Virginia University

Expected presentation date: Jun 2020

Portions: Figure 1

Requestor Location: Tayvia Brownmiller
1 Medical Center Dr
PO Box 9300
Room 1815
MORGANTOWN, WV 26505
United States
Attn: Tayvia Brownmiller

Total: 0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the Licence) between you and Springer Nature Customer Service Centre GmbH (the Licensor). By clicking 'accept' and completing the transaction for the material (Licensed Material), you also confirm your acceptance of these terms and conditions.

1. Grant of License
1. The Licensor grants you a personal, non-exclusive, non-transferable, worldwide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1.2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1.3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2.1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2.2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2.3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2.4. Where permission has been granted free of charge for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2.5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3.1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

<table>
<thead>
<tr>
<th>Scope of Licence</th>
<th>Duration of Licence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post on a website</td>
<td>12 months</td>
</tr>
<tr>
<td>Presentations</td>
<td>12 months</td>
</tr>
<tr>
<td>Books and journals</td>
<td>Lifetime of the edition in the language purchased</td>
</tr>
</tbody>
</table>
4. Acknowledgement

4. 1. The Licensor’s permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book’s homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations
8. **BOOKS ONLY**: Where ‘reuse in a dissertation/thesis’ has been selected the following terms apply: Print rights of the final author’s accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [the Licenser]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)]

For Advance Online Publication papers:
Reprinted by permission from [the Licenser]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM]).]

For Adaptations/Translations:
Adapted/Translated by permission from [the Licenser]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)]

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [the Licenser]: on behalf of Cancer Research UK: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)]

For Advance Online Publication papers:
Reprinted by permission from The [the Licenser]: on behalf of Cancer Research UK: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM]).]

For Book content:
Reprinted/adapted by permission from [the Licenser]: [Book Publisher (e.g. Palgrave Macmillan, Springer etc) [Book Title] by [Book author(s)] [COPYRIGHT] (year of publication)
Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.
Long non-coding RNA FAM83H-AS1 is regulated by human papillomavirus 16 E6 independently of p53 in cervical cancer cells

Jamie A. Barr¹, Karen E. Hayes¹, Tayvia Brownmiller¹, Abby D. Harold¹, Rajaganapathi Jagannathan², Paul R. Lockman², Saleem Khan³, and Ivan Martinez¹,*

¹Department of Microbiology, West Virginia University Cancer Institute, School of Medicine, West Virginia University, Morgantown, West Virginia 26506; ²Department of Basic Pharmaceutical Sciences, Health Sciences Center, School of Pharmacy, West Virginia University, Morgantown, West Virginia 26506; ³Department of Microbiology and Molecular Genetics, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15219

Key Words: Long non-coding RNA, Human papillomavirus, FAM83H-AS1, Cervical cancer

*Correspondence: Ivan Martinez, (ivmartinez@hsc.wvu.edu)

*The authors disclose no potential conflicts of interest.

Published in: Scientific Reports
I. Abstract

High-risk human papillomavirus (HPV) infection is one of the first events in the process of carcinogenesis in cervical and head and neck cancers. The expression of the viral oncoproteins E6 and E7 are essential in this process by inactivating the tumor suppressor proteins p53 and Rb, respectively, in addition to their interactions with other host proteins. Non-coding RNAs, such as long non-coding RNAs (lncRNAs) have been found to be dysregulated in several cancers, suggesting an important role in tumorigenesis. In order to identify host lncRNAs affected by HPV infection, we expressed the high-risk HPV-16 E6 oncoprotein in primary human keratinocytes and measured the global lncRNA expression profile by high-throughput sequencing (RNA-seq). We found several host lncRNAs differentially expressed by E6 including GAS5, H19, and FAM83H-AS1. Interestingly, FAM83H-AS1 was found overexpressed in HPV-16 positive cervical cancer cell lines in an HPV-16 E6-dependent manner but independently of p53 regulation. Furthermore, FAM83H-AS1 was found to be regulated through E6-p300 pathway. Knockdown of FAM83H-AS1 by siRNAs decreased cellular proliferation, migration and increased apoptosis. FAM83H-AS1 was also found to be altered in human cervical cancer tissues and high expression of this lncRNA was associated with worse overall survival, suggesting an important role in cervical carcinogenesis.

II. Introduction

High-risk HPV infection (e.g. HPV-16) is one of the most common causes of cervical cancer (1-3), as well as a subset of head and neck squamous cell carcinoma (HNSCC) (1). The HPV oncoproteins E6 and E7 have been shown to contribute to carcinogenesis by modulating the degradation of human proteins, such as the tumor suppressors p53 (4) and Rb (5) as well as a plethora of other cellular proteins (2,3,6-8). The HPV-16 E6 protein can abrogate p53 function by proteasomal degradation as it forms a complex with E6-associated protein (E6AP) (9), or by targeting the p53 coactivator CBP-p300 (8,10). Upon transmission, HPV infects the undifferentiated keratinocytes at the basal layer of the stratified epithelia and its genome remains episomal maintaining low copy numbers. During the course of cancer development, the viral genome frequently becomes integrated into the host cell DNA (11).
The recent discovery of different classes of non-coding RNAs (ncRNAs) expressed in human cells has opened a new chapter in the understanding of cellular processes, such as chromatin remodeling, transcriptional control, and post-transcriptional regulation. One of these classes of ncRNAs called long non-coding RNAs (lncRNAs) are defined as RNAs larger than 200 nucleotides that are not translated into proteins. Recent findings indicate that lncRNAs are involved in gene regulation at the transcriptional level by functioning as signal, guide, decoy, or scaffold RNAs (12-14). Dysregulation of lncRNAs occurs in a variety of cancers, suggesting a potential use of these ncRNAs as biomarkers for diagnosis, prognosis, stage of cancer, and response to therapy (15-18). LncRNAs have been shown to be altered in cervical cancer (19-21), however, only a few publications have studied lncRNAs that are specifically regulated by the HPV E6 oncoprotein, such as MALAT1 and CCEPR (22,23). These lncRNAs were found altered in cervical cancer but it is unknown if these alterations are part of the early events in cervical carcinogenesis. A few studies have looked at aberrant expression of lncRNAs in progression from pre-malignant cervical intraepithelial neoplasia (CIN) to cervical cancer (24,25). For these reasons, it is important to understand if certain lncRNAs are important in the first stages of immortalization and transformation caused by HPV infections.

In this study, we demonstrated that the lncRNA FAM83H-AS1 (also known as onco-lncRNA-3) is up-regulated in primary keratinocytes expressing HPV-16 oncogene E6 as well as HPV-16 positive human cervical cancer cell lines and cervical tumor samples. We show that FAM83H-AS1 is regulated by HPV-16 E6 through the presence of p300 instead of the tumor suppressor p53. Finally, we show that FAM83H-AS1 is involved with cellular proliferation, migration, and apoptosis and is associated with worse overall survival in cervical cancer patients.

III. Results

A. High-risk HPV-16 E6 oncoprotein alters host long non-coding RNAs in primary keratinocytes.

As an initial screen to identify host lncRNAs that are regulated specifically by HPV-16 E6, we developed a system to look specifically at the effect of E6 expression alone in primary human
foreskin keratinocytes (HEKα). HEKα were infected with a retroviral vector expressing HPV-16 E6 oncogene or GFP as a control. After puromycin selection and stable expression of HPV-16 E6 was confirmed by RT-PCR (Fig. S1), RNA was extracted, and samples were analyzed by RNA high-throughput sequencing (RNA-seq) to determine gene expression alterations in long non-coding RNAs (lncRNAs). Following bioinformatics analysis, we found 151 up- and 100 down-regulated host lncRNAs altered greater than 1.5-fold change when HPV-16 E6 was expressed in HEKα cells compared to GFP control (Fig. 1A, Table S1). From these host lncRNAs, we randomly chose 8 up- and 8 down-regulated host lncRNAs to validate our RNA-seq data by qRT-PCR. The expression of all the lncRNAs selected for validation followed the same trend of up- or down-regulation found by RNA-seq (Fig. 1B). In order to determine the importance of these 251 lncRNAs in cervical carcinogenesis, a variety of filtering methods were utilized to reduce our scope (see Methods section). After filtering, we performed preliminary experiments with many lncRNAs, however, some of our top altered lncRNAs from our RNAseq analysis of foreskin keratinocytes (HEKα) were not altered in cervical cells (HCK) with HPV-16 E6 expression (e.g. SNHG15). This is not surprising, as lncRNAs are known to typically be tissue specific (26). We also found differences in the expression of some lncRNAs between pre-malignant and cancerous cervical cells CaSki and W12/201402 to HCK (e.g. miR205HG). One of the up-regulated lncRNAs in our RNA-seq dataset, FAM83H-AS1 was intriguing. Its expression recently was found to correlate with poor overall survival in a variety of human cancers (27-32) but had not previously been shown in the context of cervical cancer. In addition, it was recently shown to be involved in regulating cellular processes associated with the hallmarks of cancer (e.g. proliferation and migration) (28,31,32). To ensure that expression of FAM83H-AS1 is comparable to lncRNAs with well-known functions, we utilized our RNAseq dataset to compare their RPKM values (Table S2).

B. FAM83H-AS1 expression is higher in HPV-16+ pre-malignant and cancerous samples.

Because many lncRNAs are known to be tissue specific (26) and our RNA-seq screening was performed with foreskin keratinocytes expressing HPV-16 E6 oncogene, it was critical to confirm the expression changes of FAM83H-AS1 in epithelial keratinocytes of the cervix where HPV naturally infects (11). Additionally, we considered that the E6 and E7 oncogenes can be
synergistic with each other (33), so we needed to develop a model that closely mimics HPV infection by using the entire HPV-16 genome. Therefore, primary human cervical keratinocytes (HCK) were transfected with the entire HPV-16 genome (by releasing the viral genome from a plasmid construct and circularizing it by ligation before transfection), then passaged several times (around 10-15 divisions) for growth selection of HPV positive immortalized cells. HPV-16 E6 and E7 expression was confirmed by RT-PCR (Fig. S2A), as well as p53 degradation through the HPV-16 E6 pathway by Western Blot (Fig. S2B). After confirmation of HPV-16 oncogene expression in these cells, we named them JAMM-16. It was confirmed by qRT-PCR that FAM83H-AS1 is also up-regulated in JAMM-16 cervical keratinocytes expressing the entire HPV-16 genome in comparison to the parental cervical keratinocytes (Fig. 2A). We then used HPV-16 positive low-grade cervical (W12/20863 [episomal HPV-16], W12/201402 [integrated HPV-16]) and carcinoma (CaSki [integrated HPV-16]) cell lines to investigate the expression of FAM83H-AS1. As shown in Figure 2B, we found higher expression of FAM83H-AS1 in all the HPV-16 positive cell lines in comparison to HCK cells. Interestingly, FAM83H-AS1 was expressed at lower levels in HPV-negative cervical cancer C33A cells compared to HCK as well as HPV-16 positive cervical cells (Fig. 2B). Furthermore, HPV-16 positive and HPV-negative head and neck squamous cell carcinoma (HNSCC) cell lines were compared, and higher expression of FAM83H-AS1 was observed in the HPV-16 positive versus the HPV-negative HNSCC (Fig. 2C). Overall, we conclude that presence of HPV-16 correlates with elevated levels of FAM83H-AS1 expression in early stages of cervical carcinogenesis (newly immortalized JAMM-16 cells and cervical low-grade pre-malignant cell lines) as well as cervical cancer and HNSCC cell lines.

C. FAM83H-AS1 expression is regulated by HPV-16 E6 in a p53-independent, p300-dependent manner.

As shown in Figure 1, foreskin keratinocytes (HEKα) expressing HPV-16 E6 up-regulated FAM83H-AS1 expression. To further confirm HPV-16 E6 regulation of FAM83H-AS1 in cervical cells, cervical keratinocytes (HCK) stably expressing HPV-16 E6 were developed (Fig. S3). To measure if FAM83H-AS1 regulation could be affected by HPV-16 E7 oncogene in synergistic or antagonistic manner, we generated HCK stable cell lines expressing HPV-16 E7 or co-expressing HPV-16 E6 and E7 (Fig. S3). FAM83H-AS1 was up-regulated when cells expressed HPV-16 E6
or HPV-16 E6 and E7, but not when they expressed HPV-16E7 alone (Fig. 3A) suggesting a specific regulation by E6. To further confirm these findings, HPV-16 E6 was knocked down in CaSki (Fig. 3B, Fig. S4A) and W12/201402 (Fig. 3C, Fig. S4B) cell lines by two different siRNAs against HPV-16 E6-E7. FAM83H-AS1 expression was down-regulated after the reduction of HPV-16 E6 expression confirming the regulation of this lncRNA by HPV-16 E6. It is well known that one of the major HPV E6 targets is the tumor suppressor p53 (34), which is involved in the regulation of cell proliferation, DNA repair, and apoptosis (35). Interestingly, when we knocked down p53 in HCK by using two different siRNAs, we observed that FAM83H-AS1 expression did not change (Fig. 3D, Fig. S4C), suggesting a regulation by HPV-16 E6 in a p53-independent manner. It is also known that HPV-16 E6 is able to regulate the expression of other important genes in carcinogenesis such as hTERT through the regulation of transcriptional coactivators such as p300 (36). By using the UCSC Genome Browser (https://genome.ucsc.edu/) to investigate potential p300 binding site in the promoter region of FAM83H-AS1, we found three predicted p300 binding sites (Fig. 3E). In order to measure the potential regulation of FAM83H-AS1 by p300, we used two siRNAs against p300 and quantified FAM83H-AS1 expression. When p300 was knocked down in primary cervical keratinocytes, FAM83H-AS1 expression was also reduced (Fig. 3F, Fig. S4D) suggesting direct and/or indirect p300 regulation of FAM83H-AS1. Previous publications have shown a greater affinity of HPV-16 E6 to interact with p300 in comparison with other high-risk HPV E6 such as HPV-18 E6(37). Interestingly, FAM83H-AS1 expression was found to be up-regulated in HPV-16 positive cell lines (Fig. 2), but down-regulated in HPV-18 positive HeLa cells and HPV-31b positive CIN-612 cells (Fig. S4E). Altogether, we found that FAM83H-AS1 is regulated by HPV-16 E6 independently of p53 but influenced by the presence of p300.

D. FAM83H-AS1 is localized to the nucleus and does not regulate transcription of nearby FAM83H.

The cellular localization of lncRNAs can provide information on their potential function. Nuclear lncRNAs can regulate at the transcriptional level by interacting with critical epigenetic regulators and enhancing chromatin looping, as well as interact with splicing factors to regulate splicing (38). Meanwhile cytoplasmic lncRNAs have been found to function at the post-
transcriptional level as competing endogenous RNAs by acting as microRNA sponges and binding to mRNAs leading to the recruitment of RNA binding proteins that promote decay, suppress translation, or factors that initiate translation (39). For this reason, we investigated the cellular localization of FAM83H-AS1 in two HPV-16 positive cervical cell lines by cellular fractionation. We used U6 as a nuclear RNA control and β-actin mature mRNA as a cytoplasmic RNA control. We found significantly higher amplification of FAM83H-AS1 in the nuclear fractions in comparison to the cytoplasmic fractions by qRT-PCR in CaSki (Fig. 4A) and W12/201402 (Fig. 4B) cells, suggesting that FAM83H-AS1 is a nuclear lncRNA. Because many nuclear lncRNAs can act in cis (38), we hypothesized that FAM83H-AS1 could regulate its nearby protein coding gene FAM83H. The lncRNA FAM83H-AS1 and protein coding gene FAM83H share a promoter region but are transcribed in opposite directions (27) (Fig. 3E, Fig. S5). FAM83H is required for the organization of the keratin cytoskeleton in epithelial cells (40) and has been shown over-expressed in different tumor samples compared to their matching normal tissues (41). Interestingly, we found increased expression of FAM83H in HCK expressing HPV-16 E6 in comparison to parental HCK cells (Fig. S5B), but when we transfected an siRNA against FAM83H-AS1 in HPV-16 positive CaSki cells, we were unable to detect changes in FAM83H expression (Fig. S5C), suggesting that FAM83H-AS1 is not involved in regulation of FAM83H expression.

**E. FAM83H-AS1 knockdown in cervical cancer cells causes reduced cellular proliferation and migration, as well as induction of apoptosis.**

To understand the significance of FAM83H-AS1 in cervical cancer cells, we analyzed the effects on cellular proliferation when FAM83H-AS1 was knocked down by siRNA in CaSki and W12/201402 cervical cells. First, knockdown efficiency of a pool of 4 individual siRNAs (SMARTpool), as well as each of the individual siRNAs, was evaluated by qRT-PCR. Knockdown of FAM83H-AS1 in CaSki with the siRNA SMARTpool was maintained over a time-course from 24 to 120 hours, which was sufficient for all functional assays conducted (Fig. S6). All of the individual siRNAs, as well as the SMARTpool showed greater than 51% knockdown of FAM83H-AS1 in CaSki (Fig. 5A) and greater than 49% in W12/201402 cells (Fig. S7A). Therefore, we decided to randomly choose two of the individual siRNAs and SMARTpool to knockdown
FAM83H-AS1 and monitor cellular functional changes. Two siRNAs against FAM83H-AS1 were transfected independently into CaSki and W12/201402 cells, cultured for 48 hours, and replated to measure cell proliferation by cell counting. In both CaSki and W12/201402 cells, we observed a decrease (≥48%) in cell number with knockdown of FAM83H-AS1 compared to control (Fig. 5B, Fig. S7B). Cellular proliferation assay (CCK-8) showed that there was a decrease in cellular proliferation when FAM83H-AS1 is knocked down in CaSki cells and W12/201402 as monitored from 48 hours to 96 hours after replating. We found significantly less proliferation in CaSki (64% decrease) and W12/201402 (73% decrease), in the siRNA FAM83H-AS1 knockdown compared to siRNA control cells at the 96-hour time point (Fig. 5C, Fig. S7C). We observed similar functional changes between the two individual siRNAs and the SMARTpool so for future functional assays we only used the siRNA SMARTpool. In order to identify changes in cell cycle that could explain the differences found in cellular proliferation after FAM83H-AS1 knockdown, we performed cell cycle flow cytometry analysis. CaSki and W12/201402 cells had a significant reduction (43% and 56%, respectively) of cells in S-phase when FAM83H-AS1 was knocked down in comparison to control suggesting that FAM83H-AS1 is important in the G2/S-phase transition (Fig. 5D, Fig. S7D). Other important hallmarks of cancer such as migration and resistance to apoptosis were measured after FAM83H-AS1 knockdown. We found that cellular migration was significantly decreased in CaSki and W12/201402 after siRNA knockdown of FAM83H-AS1 compared to siRNA control (Fig. 5E, Fig. S7E). Also, we measured a significant increase in early and late apoptosis in CaSki and W12/201402 cells with knockdown of FAM83H-AS1 compared to control cells by using Annexin V/PI staining and flow cytometry (Fig. 5F, Fig. S7F and S7G-H). W12/201402 cells with knockdown of FAM83H-AS1 showed a significant increase in necrosis (Fig. S7F), while only a slight increase in necrosis was observed in CaSki cells (Fig. 5F, Fig. S7H). Altogether, we observed significant alterations in cellular proliferation, cell cycle, migration, and apoptosis by the absence of FAM83H-AS1, suggesting an important role in cervical carcinogenesis.

**F. FAM83H-AS1 expression is increased in cervical cancer human tissues & is associated with worse overall survival.**
In order to extrapolate our findings into a more clinically relevant setting, we extracted RNA from pre-malignant and cervical cancer patient samples and analyzed the expression of FAM83H-AS1 by qRT-PCR. We found high expression of FAM83H-AS1 in the pre-malignant sample (CIN3) as well as the cervical cancer (CaCx) samples in comparison to normal cervix tissue (Fig. 6A). These findings corroborate our in vitro data suggesting an importance of FAM83H-AS1 in clinical tumor samples at different stages of carcinogenesis. We also took advantage of the cervical cancer samples deposit in the TCGA database to compare the expression of FAM83H-AS1 between normal cervix and cervical cancer samples obtained from different cancer stages (120 Stage I, 35 Stage II, 30 Stage III, 7 Stage IV, 4 Stage unavailable). The TCGA data showed elevated expression of FAM83H-AS1 (RPKM values) in cervical cancer patients compared to normal cervix control (Fig. 6B). This coincides with our previous observations of FAM83H-AS1 expression being higher in cervical cancer cells lines (Fig. 2B). Finally, we used the TCGA data set from the TANRIC database to divide cervical cancer patients into high versus low expression groups and measured overall survival based on FAM83H-AS1 expression. Interestingly, we found that patients with higher FAM83H-AS1 expression yielded a worse overall survival than patients with lower FAM83H-AS1 expression suggesting a biological importance of this IncRNA in cervical cancer reflected in patients' clinical outcomes (Fig. 6C). Overall, we conclude that FAM83H-AS1 expression is elevated in cervical cancer patients and high expression correlates with overall poor survival.

IV. Discussion

4.5% of all cancers worldwide are attributable to HPV infection. Almost all cervical cancers and a substantial amount of other anogenital and oropharyngeal cancers have been found to be infected by high-risk HPVs. HPV-16 and -18 contribute to 73% of HPV-associated cancers (42), implying a higher ability to induce tumorigenesis in comparison to other types of HPVs. Although the prevalence of HPV-associated cancers has decreased due to development of the preventative vaccine and early detection screening methods (2), there is still a great need for prognostic and therapeutic options specially to people already infected with HPV as well as those affected in less developed countries where the access to the vaccine is limited.
Long noncoding RNAs (lncRNAs) have been shown to regulate a variety of critical cellular processes, including transcription and chromosome remodeling (12-14). Dysregulation of lncRNAs has been shown to be associated with the development and progression of many cancers (15-17), and interestingly they are commonly tissue specific (26) and only altered in one cancer type (32). Therefore, lncRNAs are currently being studied in the context of biomarkers for diagnosis and prognosis of cancer, as well as therapeutic targets (15-18).

Previous studies have shown that the high-risk HPV E6 protein expressed is clearly involved in the progression of carcinogenesis. HPV E6-regulation of non-coding RNAs such as microRNAs has been well studied (43), however long non-coding RNA regulation by high-risk HPV E6 needs to be studied further; there are only a couple lncRNAs shown to be specifically HPV-16 E6 regulated, including MALAT1 and CCEPR (22,23). To add to this field of study, we sought out to identify an HPV-16 E6 regulated gene that was altered from the early stages of HPV infection until carcinogenesis and therefore we considered to be important in both the development and progression of carcinogenesis. Thus, we developed a new HPV-16 positive cell line referred to as JAMM-16 to represent early infection, but also analyzed expression in established HPV-16 positive cervical cell lines such as CaSki and W12 cells as well as pre-malignant and malignant cervical tumor samples. We found FAM83H-AS1 overexpression in W12/20863 and W12/201402 (which came from a CIN2 tumor) similar to CaSki cells (cervical carcinoma). Additionally, in Figure 6A, the CIN3 (considered Stage 0 cervical carcinoma) patient sample shows similar expression to later stage cervical carcinoma (CaCx) samples. For this reason, we believe that FAM83H-AS1 up-regulation in pre-malignant cervical samples could be linked to the expression of viral oncoproteins in early HPV infection.

From the host lncRNAs altered by HPV-16 E6 in our RNA-seq analysis (Fig. 1), we found several of these lncRNAs previously described to be altered in cervical cancers, confirming that our data aligned with former studies. For example, it was previously found that decreased expression of GAS5 is associated with poor prognosis of cervical cancer patients (44,45) as well as is tumor suppressive in other types of cancer such as breast cancer (46,47) and prostate cancer (47). Furthermore, GAS5 expression was also found altered in vitro in HPV-16 positive CaSki cells (44) and HPV-18 positive HeLa cells (45). Another lncRNAs affected in our study was H19.
It was shown previously that DNA methylation alterations at the IGF2/H19 imprinted domain may mediate the association between HPV and invasive cervical cancer (48) and high H19 expression has also been shown to be predictive of poor prognosis in cervical cancer (49) as well as in a variety of other human cancers, including HNSCC (50) and breast cancer (51).

A class of lncRNAs known as onco-lncRNAs were also interesting to us because, as the majority of lncRNAs are tissue specific (26), this group of lncRNAs exhibit differential expression across multiple cancers and are hypothesized to have conserved oncogenic or tumor suppressive functions. One such onco-lncRNA which was found in our RNA sequencing analysis of an E6-regulated lncRNA is onco-lncRNA-3, referred to as FAM83H-AS1. This lncRNA is transcribed from chromosome 8 and its function in normal cells is unknown. It was first characterized in 2015 (32), and as of now multiple publications have shown increased expression of FAM83H-AS1 in breast (29,32), lung (31,32), colorectal (28,30,32), kidney (32), bladder (32), and pancreatic cancers (27) and increased expression correlates with worse overall survival in most of these cancers (27,29-31). Our findings show for the first time that FAM83H-AS1 is overexpressed in human cervical cancer (CESC) tissues and high expression in patients correlates with poor overall survival (Fig. 6).

According to previous studies, FAM83H-AS1 is an epithelial lncRNA (27) supporting our data obtained from foreskin and cervical keratinocytes. Determining the localization of a lncRNA can predict functionality of the lncRNA; our findings that FAM83H-AS1 is localized in the nucleus of cervical cancer cells (Fig. 4) is consistent with previous findings of its nuclear localization in lung cancer cells (14). Functionally, it has been found to be co-expressed with protein coding genes that were enriched for cell cycle-related genes (32), and knockdown of FAM83H-AS1 altered cell cycle (31,32), proliferation (28,31), migration (28,31), invasion (31), and apoptosis (28) in certain cancers. Our group shows here that in the context of cervical cancer, FAM83H-AS1 is involved in cell cycle, proliferation, migration, and apoptosis (Fig. 5). It is unknown if FAM83H-AS1 elicits its functions in cis or trans, but our findings suggest that FAM83H-AS1 does not elicit cis regulation on the nearby protein coding gene FAM83H (Fig. S5), which is up-regulated in a variety of human cancers. For this reason, it will be interesting in future
studies to identify the protein, RNA, and/or DNA interactions of FAM83H-AS1 in cervical cancers.

Previously, it was shown that FAM83H-AS1 regulates MET/EGFR signaling in lung cancer cells (31) and that when FAM83H-AS1 was downregulated it exhibited an anti-proliferative role by suppressing the Notch signaling pathway in colorectal cancer (28). To elucidate additional downstream targets of FAM83H-AS1, a group recently conducted RNA-seq on a pancreatic cancer cell line with siRNA knockdown of FAM83H-AS1 compared to control and identified gene alterations (78 activated and 68 inhibited targets) (27). Our group plans to determine if these downstream regulators are also involved in FAM83H-AS1 mediated functional changes observed in cervical cancer cells.

HPV-16 E6 and -18 E6 are well known to contribute to the degradation of p53, however, it is important to note that HPV-16 and HPV-18 vary in their interactions with other proteins to regulate carcinogenesis. For example, previous studies have shown that HPV-16 E6 directly interacts with CBP/p300 (8,10,37), but HPV-18 E6 appears to be unable to interact with p300 (37). This could be a possible explanation for variation in FAM83H-AS1 expression between HPV-16 and -18 positive cancers observed in our study (Fig. 2, Fig. S4E). Supporting our data, another publication previously showed low expression of FAM83H-AS1 in HeLa (HPV-18 positive) cells (27). This information led us to elucidate the mechanism of FAM83H-AS1 up-regulation by E6 in a p53-independent and p300-dependent manner (Fig. 3, Fig. S4). This regulation is interesting because the majority of E6 regulation of several coding and non-coding genes is primarily through the p53 pathway. Interestingly, a previous study showed that overexpression of cyclooxygenase (COX-2) gene was the result of the recruitment of p300 to its promoter region via the overexpression of HPV-16 E6 protein in CaSki cells as well as the exogenous expression of HPV-16 E6 in HPV-negative C-33A cells (52). Recently, p300 inhibitors such as C646 have been shown to be good candidates as anti-cervical cancer drugs, demonstrating the importance of p300 not only in the regulation of host genes but also of HPV viral genes (53).

In summary, the identification of FAM83H-AS1 up-regulation in the early stages of cervical carcinogenesis, correlation with overall survival in cervical cancer, and involvement in
different hallmarks of cancer contributes further evidence of the importance of this IncRNA in cancer. Further studies on this IncRNA could enhance the use of FAM83H-AS1 as a potential biomarker or therapeutic target in multiple cancers.

V. Methods

Detailed experimental protocols are described in the Supplementary Methods section. All experiments were performed in compliance with the Institutional Biosafety Committee at West Virginia University, number 15-03-03.

A. Cells

The following cell lines were used: human primary foreskin keratinocytes (HEKa) (Invitrogen, C-005-5C); human primary cervical keratinocytes (HCK) and J2-3T3 murine fibroblast feeder cells (obtained from Dr. Alison McBride's laboratory, NIH, Bethesda, MD); 3T3M murine fibroblast feeder cells, as well as CaSki (HPV-16 positive), HeLa (HPV-18 positive), and C-33A (HPV negative) cervical carcinoma cells (obtained from Dr. Daniel DiMaio's laboratory, Yale University, New Haven, CT); W12/201402 (HPV-16 positive) and W12/20863 (HPV-16 positive) pre-malignant cervical cells (obtained from Paul F. Lambert, University of Wisconsin-Madison, Madison, WI); CIN-612 (HPV-31b positive) (obtained from Dr. Laimonis A. Laimins' laboratory, Northwestern University, Chicago, IL); UMSCC-1 (HPV negative), UMSCC-47 (HPV-16 positive), and UMSCC-104 (HPV-16 positive) head and neck squamous cell carcinoma (HNSCC) cell lines (obtained from Dr. Scott A. Weed's laboratory, West Virginia University, Morgantown, WV). Further details are in the Supplementary Methods section.

B. High-throughput RNA sequencing

Three replicates each of human foreskin keratinocytes (HEKa) stably expressing HPV-16 E6 or GFP were sent for RNA high-throughput sequencing (Illumina). FASTQ files were subsequently imported into Strand NGS suites for analysis. Reads were aligned to the human hg19 reference genomes using the Bowtie algorithm. These were then quantified against Ensemble
transcript and including small and lncRNA annotations. Any lncRNA that were detected in human subjects were used for further analysis. Raw lncRNA counts were then normalized to the total number of lncRNA reads per sample and expression values calculated against the control samples. Further Mapping rate visualization done using Strand NGS software. In order to determine the importance of the lncRNAs obtained from the RNA-seq analysis, we used the following filtering strategy: First, only lncRNAs with reasonable expression (RPKM greater than 1) were analyzed further. Then, we used The Atlas of Noncoding RNAs in Cancer (TANRIC, MD Anderson Cancer Center) (54), which contains 297 sequenced human cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) patient data from The Cancer Genome Atlas (TCGA), to analyze the expression and clinical outcomes of these lncRNAs in patient samples. Finally, we searched previous publications to identify lncRNAs altered in other types of cancer and/or involved in hallmarks of cancer. To increase our novelty, we eliminated lncRNAs that were previously shown to be involved specifically in cervical cancer (e.g. H19).

C. Functional Analysis

For cell counting experiments, CaSki cells were transiently transfected with Lincode Human FAM83H-AS1 siRNA SMARTpool (Dharmacon, R-188909-00-000), Individual: Lincode FAM83H-AS1 siRNA (N-188909-02-0002 and N-188909-04-0002), or Lincode Non-targeting siRNA #1 (Dharmacon, D-001320-01-05) using Lipofectamine® RNAiMAX according to manufacturer's instructions (Invitrogen).

CaSki cells were cultured with siRNA-containing media for 48 hours, re-plated in equal cell numbers (200,000 cells/well of 6-well), cultured for another 48 hours, and attached cells were re-counted with a hemocytometer.

For all other functional assays (CCK-8 cell proliferation, FACS cell cycle, transwell migration, annexin V-FITC/PI apoptosis), CaSki cells were transiently transfected with Lincode Human FAM83H-AS1 siRNA SMARTpool or Lincode Non-targeting siRNA #1 (Dharmacon, D-001320-01-05) using Lipofectamine® RNAiMAX according to manufacturer's instructions (Invitrogen). Cells were incubated with siRNA-containing media for 24 hours then re-plated in
equal cell numbers for to initiate experiments described below. To monitor cell proliferation, transfected cells were plated in 96-well plates and after 24, 48, 72, and 96 hours analyzed with CCK-8 kit (Sigma-Aldrich) according to manufacturer’s protocol. Alterations in cell cycle were determined by flow cytometry propidium iodide DNA staining. Transfected cells were plated in media containing 10% fetal bovine serum (FBS). Cells were allowed to attach and then were serum-starved for 24 hours. Samples were then fixed with ethanol, stained with propidium iodide, and analyzed by flow cytometry (Fortessa S10). For transwell migration assay, transfected cells were seeded onto upper chambers of transwell inserts (8µm pore size) with 20% FBS chemoattractant in the lower chamber of 24-well plate. After 48 hours, migrated cells located on the underside of the transwell insert were stained with 0.5% crystal violet in methanol. Migrated cells were quantified using ImageJ software. To monitor apoptosis, transfected cells were plated and incubated 24, 48, and 72 hours. At desired time point, attached and floating cells were pelleted and co-stained with annexin V-FITC and propidium iodine and immediately analyzed by flow cytometry (Fortessa S10).

VI. Data Availability

The RNA-seq raw data generated during and/or analyzed during the current study are available in the Gene Expression Omnibus (GEO-NCBI) repository, accession number: GSE115334.

VII. References


VIII. Acknowledgements

This work was supported in part by WVU Cancer Institute, National Cancer Institute (NCI) Plan (2V882), American Cancer Society IRG Internal Pilot Funding (IRG-09-061-04), WVCTSI Award (NIH/NIGMS Award Number U54GM104942), Tumor Microenvironment (TME) CoBRE Grant (NIH/NIGMS P20GM121322). Karen Hayes was supported in part by The Ladies Auxiliary to the VFW of the United States Cancer Research Fellowship (CK003229). Neil Infante (WVU Bioinformatics Core) assisted with the high-throughput RNA
sequencing data analysis. We acknowledge Molly Lovern, a West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) undergraduate student, and WV-INBRE funding source [NIH (NIGMS) Grant #P20GM103434] assisted in developing the JAMM-16 cell line. Sijin Wen (WVU Biostatistics Core) assisted with biostatistics on the TANRIC/TCGA data analysis. We thank Kathy Brundage in the WVU Flow Cytometry Core for assistance with FACS analysis, along with funding sources for the Fortessa S10 (OD016165) and overall core support (MBRCC CoBRE grant: GM103488). We thank Alison McBride at the NIH for kindly providing primary human cervical keratinocytes.

**IX. Author Contributions:**

J.A.B., K.E.H. and I.M. designed research; S.K. provided initial analysis and collected cervical tissue samples; J.A.B, K.E.H., T.B., A.D.H, and I.M. performed research; J.A.B, K.E.H., T.B., A.D.H, R.J. P.R.L., and I.M. analyzed data; J.A.B. and I.M. wrote the paper.

**X. Additional Information:**

The authors declare no competing interests.
XI. Figure Legends:

**Figure 1:** Differential expression of host IncRNAs after expression of HPV-16 E6 in primary foreskin keratinocytes. (A) Waterfall plot of host IncRNAs altered 1.5-fold or greater in primary foreskin keratinocytes (HEKa) expressing HPV-16 E6 compared to uninfected HEKa by high-throughput RNA sequencing (RNA-seq) analysis. Triplicates for each sample were sent for RNA-seq. (B) qRT-PCR validation of representative differentially expressed host IncRNAs found by RNA-seq analysis. Red bars represent the IncRNAs up-regulated with HPV-16 E6 expression, and green bars represent the IncRNAs down-regulated with HPV-16 E6 expression. GAPDH mRNA was used to normalize the qRT-PCR analyses, which are shown relative to uninfected HEKa (grey bars).

**Figure 2:** Increased FAM83H-AS1 expression in primary cervical keratinocytes containing the HPV-16 genome as well as in HPV-16 positive cervical cancer and head and neck squamous cell carcinoma cell lines. (A) qRT-PCR analysis showing the increase of FAM83H-AS1 expression in newly immortalized cervical keratinocytes expressing entire HPV-16 genome (JAMM-16) compared to uninfected primary cervical keratinocytes (HCK). (B) qRT-PCR analysis showing the increase of FAM83H-AS1 expression in HPV-16 positive cervical cell lines (CaSki, W12/20863, W12/201402) and decrease of FAM83H-AS1 in HPV negative cervical cancer cell line (C-33A) compared to uninfected cervical keratinocytes (HCK). (C) qRT-PCR analysis showing the increase of FAM83H-AS1 expression in HPV-16 positive head and neck squamous cell carcinoma (HNSCC) cell lines (UMSCC-47 and UMSCC-104) compared to HPV negative HNSCC cell line (UMSCC-1). All graphs in the figure show the average of two individual experiments. Similar results were obtained in at least three independent experiments. GAPDH mRNA was used to normalize the qRT-PCR analyses. Two-tailed t test results are indicted as **p ≤ 0.01.

**Figure 3:** Regulation of FAM83H-AS1 expression by HPV-16 E6 in a p53-independent, p300-dependent manner. (A) FAM83H-AS1 expression by qRT-PCR analysis in cervical keratinocytes (HCK) stably individually expressing HPV-16 E6 or E7, or co-expressing E6/E7 compared to GFP control. (B-C) qRT-PCR analysis of HPV-16 E6 and FAM83H-AS1 expression in HPV-16
positive CaSki (B) and W12/201402 (C) cervical cell lines transfected with an siRNA against HPV-16E6 compared to siRNA control. (D) qRT-PCR analysis of p53 and FAM83H-AS1 expression in HCK transfected with an siRNA against p53 compared to siRNA control. (E) Genome representative image showing location of FAM83H, FAM83H-AS1, and three predictive p300 binding sites in FAM83H-AS1 promoter region. (F) p300 and FAM83H-AS1 expression in HCK transfected with an siRNA against p300 compared to siRNA control. All graphs in the figure show the average of two individual experiments. Similar results were obtained in at least three independent experiments. GAPDH mRNA was used to normalize the qRT-PCR analyses. Two-tailed t test results are indicted as * p ≤ 0.05 and ** p ≤ 0.01. CTRL, control.

**Figure 4:** FAM83H-AS1 is localized in the nucleus in cervical pre-malignant and cancerous cell lines. (A) qRT-PCR of FAM83H-AS1 expression in fractionated HPV-16 positive cervical cancer CaSki (A) and pre-malignant W12/201402 (B) cell lines. U6 small nuclear RNA (snRNA) was used as a nuclear control RNA and mature Beta Actin was used as a cytoplasmic control RNA. Representative images; similar results were obtained in at least three independent experiments. Normalization was done using C. Elegans total RNA as an exogenous spike for the amplification of worm-specific ama-1 gene. Two-tailed t test results are indicted as ** p ≤ 0.01.

**Figure 5:** FAM83H-AS1 knockdown altered cell proliferation, migration, and apoptosis in CaSki cells. (A) Knockdown efficiency of individual and SMARTpool siRNA against FAM83H-AS1 in HPV-16 positive CaSki cell line, measured by qRT-PCR analysis. Because of variations in the expression of GAPDH after the knockdown of FAM83H-AS1, we used UBC mRNA to normalize the qRT-PCR analyses. The graph shows average of two individual experiments. (B) CaSki cells were transfected with individual siRNAs against FAM83H-AS1, siRNA SMARTpool against FAM83H-AS1, or siRNA control for 48 hours. Cells were then re-plated in equal numbers (200,000 cells/well, represented by dashed line in graph) and cultured another 48 hours prior to re-counting attached cells. Data were obtained in triplicate, and the graph shows the average of two individual experiments. (C) CaSki cells were transfected with individual siRNAs against FAM83H-AS1, siRNA SMARTpool against FAM83H-AS1, or siRNA control for 24 hours then plated in equal numbers. Transfected cells were analyzed for cellular proliferation assessment by CCK-8 assay at 48, 72, and 96 hours post-plating. The graph shows the average of two individual
experiments; similar results were obtained in three independent experiments. (D-F) CaSki cells were transfected with siRNA SMARTpool against FAM83H-AS1 or siRNA control for 24 hours then plated in equal numbers for experiments. (D) Transfected cells were analyzed for cell cycle alterations by FACS analysis. CaSki cells with knockdown of FAM83H-AS1 exhibit less cells in S-phase of cell cycle compared to control cells. The graph shows the average of two individual experiments; similar results were obtained in three independent experiments. (E) Transwell migration of transfected cells was analyzed 48 hours post-plating in upper chamber with chemoattractant in lower chamber. The graph shows the average of three individual experiments. (F) Transfected CaSki cells were collected at 1, 2, and 3 days post-plating, stained with Annexin V/PI, and analyzed by flow cytometry to show alterations in apoptosis compared to siRNA control. The graph shows the average of three individual experiments. Two-tailed t test results are indicated as * p ≤ 0.05 and ** p ≤ 0.01.

**Figure 6:** FAM83H-AS1 expression is increased in human cervical cancer tissues and correlates with poor overall survival. (A) Increased FAM83H-AS1 expression in human cervical cancer and cervical intraepithelial neoplasia (CIN) stage 3 patient samples compared to non-cancerous cervical tissue as measured by qRT-PCR analysis. (B) The Cancer Genome Atlas (TCGA) analysis of FAM83H-AS1 RPKM values in cervical cancer tissues (n=196) compared to non-cancerous tissues (n=3). (C) Survival plot of cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) patient data mined from TCGA with low (n=22) vs. high (n=174) expression of FAM83H-AS1. High expression of FAM83H-AS1 expression correlates with worse overall survival in CESC patients.
Figure 1:

A

Fold Change
(HEKα+HPV-16 E6 vs. HEKα+GFP)

151 up-regulated IncRNAs

100 down-regulated IncRNAs

B

Relative Expression

H19
HIRC205HG
HOXC-AS5
GASS
SNHG15
MAEG-AS1
FAM83H-AS1
RP6-65023.13
GS1-60012.8
RP3-649022.8
LP1-301057
FAM32A1
AC00710.7

Figure 2:

A

Primary Cervical Cells

FAM83H-AS1

Relative Expression

HCK
JAMM-16

**

B

Cervical Cells

FAM83H-AS1

Relative Expression

HCK
CASKI
W127/20863
W127/201402
C-S3A

**

C

HNSCC Cells

FAM83H-AS1

Relative Expression

UMSCC-1
UMSCC-47
UMSCC-104

**
Figure 4:

A

CaSki

B

W12/201402

Relative Expression (normalized to spike)

U6  Beta Actin  FAM83H-AS1

Total Cell  Cytoplasmic  Nuclear

Figure 5:

A

CaSki

B

CaSki

C

CaSki

D

CaSki

E

CaSki

F

Early Apoptosis  Late Apoptosis  Necrosis

% Cells

siRNA  CTRL  #1  #2  #3  #4  Pool

Absorbance (OD)/nm

siRNA  CTRL  si-2  si-4  si-pool

Cell Cycle Distribution (%)

si-CTRL  si-FAM83H-AS1

siRNA  CTRL  FAM83H-AS1

24h  48h  72h

24h  48h  72h

24h  48h  72h
Figure 6:

A

B

C

180
Figure S1: Validation of stable HPV-16 E6 expression in primary foreskin keratinocytes. RT-PCR was used to confirm stable HPV-16 E6 expression in primary foreskin keratinocytes (HEKa) compared to HEKa expressing GFP control and HPV-16 positive CaSki cell line.
Figure S2: Validation of HPV-16 expression in primary cervical keratinocytes. RT-PCR was used to confirm HPV-16 E6 and E7 expression in cervical keratinocytes transfected with entire HPV-16 genome (JAMM-16) compared to uninfected primary cervical keratinocytes (HCK) and HPV-16 positive CaSki and W12/201402 cell lines. GAPDH was used as loading control. (B) Western blot analysis was used to confirm p53 degradation in HPV-16 positive JAMM-16, CaSki, W12/20863, W12/201402 cell lines compared to HCK and HPV negative, p53-mutated C-33A cell line. Note: N/A represents a cervical cell line that was not used in this study.
Supplementary Figure S3

<table>
<thead>
<tr>
<th></th>
<th>GFP</th>
<th>HPV-16 E6</th>
<th>HPV-16 E7</th>
<th>HPV-16 E6/E7</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16 E6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-16 E7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure S3: Validation of stable HPV-16 E6 and E7 expression in primary cervical keratinocytes. RT-PCR was used to confirm stable HPV-16 E6 and E7 expression, as desired, in primary cervical keratinocytes (HCK) compared to HCK expressing GFP control.
Supplementary Figure S4

A

![Graph for CaSki](image)

B

![Graph for W12/201402](image)

C

![Graph for HCK](image)
Figure S4: FAM83H-AS1 expression is regulated by HPV-16 E6 in a p53-independent, p300-dependent manner. (A-B) qRT-PCR analysis of HPV-16 E6 and FAM83H-AS1 expression in HPV-16 positive Caski (A) and W12/201402 (B) cell lines transfected with a second siRNA (siRNA #2) against HPV-16 E6 compared to siRNA control. (C) qRT-PCR analysis of p53 and FAM83H-AS1 expression in primary cervical keratinocytes (HCK) transfected with a different siRNA (siRNA #2) against p53 compared to siRNA control. (D) p300 and FAM83H-AS1 expression in HCK transfected with a different siRNA (siRNA #2) against p300 compared to siRNA control. (E) qRT-PCR analysis of FAM83H-AS1 expression in HPV-18 positive HeLa cells and HPV-31b positive CIN-612 cervical cell lines compared to primary cervical keratinocytes (HCK). (A-D) The graphs show the average of two independent experiments. Similar results were obtained in at least three independent experiments. GAPDH mRNA was used to normalize the qRT-PCR analyses. Two-tailed t test results are indicated as ** p ≤ 0.01. CTRL, control.
Supplementary Figure S5

**Figure S5: FAM83H expression is not regulated by FAM83H-AS1.** (A) Schematic diagram depicting possible cis-regulation of protein coding gene FAM83H by IncRNA FAM83H-AS1. (B) qRT-PCR analysis of FAM83H protein coding gene expression in primary cervical keratinocytes (HCK) stably expressing HPV-16 E6 compared to GFP control. (C) qRT-PCR analysis of FAM83H and FAM83H-AS1 expression in CaSki transfected with siRNA against FAM83H-AS1. The graphs shows the average of two independent experiments. Similar results were obtained in at least three independent experiments. GAPDH mRNA was used to normalize the qRT-PCR analyses. Two-tailed t test results are indicated as ** p ≤ 0.01. CTRL, control.
Figure S6: FAM83H-AS1 expression at 24, 48, 72, 96, and 120 hours post-transfection with FAM83H-AS1 siRNA SMARTpool by qRT-PCR analysis.
Supplementary Figure S7

A

W12/201402

Relative Expression

siRNA
CTRL #1 #2 #3 #4 Pool

B

W12/201402

Number of Cells (K,1000 cells)

siRNA
CTRL #2 #4 Pool

C

W12/201402

Absorbance (OD,600)

siRNA
si-CTRL si-#2 si-#4 si-pool

48h 72h 96h

D

W12/201402

Cell Cycle Distribution (%)

si-CTRL si-FAM83H-AS1

E

W12/201402

si-CTRL

si-FAM83H-AS1

Feeders Alone

% Relative Migration

siRNA
CTRL Pool

188
Figure S7: FAM83H-AS1 knockdown altered cell proliferation, migration, and apoptosis in W12/201402 cells. (A) Knockdown efficiency of individual and SMARTpool siRNA against FAM83H-AS1 in HPV-16 positive W12/201402, measured by qRT-PCR analysis. Because of variations in the expression of GAPDH after the knockdown of FAM83H-AS1, we used GUSB mRNA to normalize the qRT-PCR analyses. The graph shows average of two individual experiments. (B) W12/201402 cells were transfected with individual siRNAs against FAM83H-AS1, siRNA SMARTpool against FAM83H-AS1, or siRNA control for 48 hours. Cells were then re-plated in equal numbers (200,000 cells/well, represented by dashed line in graph) and cultured another 48 hours prior to re-counting attached cells. The graph shows the average of two individual experiments. (C) W12/201402 cells were transfected with individual siRNAs against FAM83H-AS1, siRNA SMARTpool against FAM83H-AS1, or siRNA control for 48 hours then plated in equal numbers. Transfected cells were analyzed for cellular proliferation assessment by CCK-8 assay at 48, 72, and 96 hours post-plating. The graph shows the average of two individual experiments. (D-G) W12/201402 cells were transfected with siRNA SMARTpool against FAM83H-AS1 or siRNA control for 24 hours then plated in equal numbers for experiments. (D) Transfected cells were analyzed for cell cycle alterations by FACS analysis. W12/201402 cells with knockdown of FAM83H-AS1 exhibit less cells in S-phase of cell cycle compared to control cells. The graph shows the average of two individual experiments. (E) Transwell migration of transfected cells was analyzed 48 hours post-plating in upper chamber with chemoattractant in lower chamber. The graph shows the average of two individual experiments. (F-H) Transfected W12/201402 or CaSki cells were collected at 1, 2, and 3 days post-plating, stained with Annexin V/PI, and analyzed by flow cytometry to show alterations in apoptosis compared to siRNA control. (F) Quantitative analysis of Annexin V/PI flow cytometry data in W12/201402 cells. The graph shows the average of two individual experiments. (G) Dot plot analysis of Annexin V/PI flow cytometry data in W12/201402 cells. (H) Dot plot analysis of Annexin V/PI flow cytometry data in CaSki cells. Representative images shown for siRNA FAM83H-AS1 and siRNA control at each time point. Two-tailed t test results are indicted as * p ≤ 0.05 and ** p ≤ 0.01.
Table S1: Host lncRNAs altered after expression of HPV-16 E6 in primary foreskin keratinocytes.

Table shows details for each host lncRNA that was altered 1.5-fold or greater in primary foreskin keratinocytes (HEKα) expressing HPV-16 E6 compared to uninfected HEKα. Triplicates for each sample were sent for high-throughput RNA sequencing analysis.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Fold Change ([HPV-16 E6] vs [Control])</th>
<th>Gene Symbol</th>
<th>Chro mosome</th>
<th>Start</th>
<th>End</th>
<th>Strand</th>
<th>RPKM GFP Control -1</th>
<th>RPKM GFP Control -2</th>
<th>RPKM HPV-16 E6-1</th>
<th>RPKM HPV-16 E6-2</th>
<th>RPKM HPV-16 E6-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENSG00000130600</td>
<td>4.99</td>
<td>H19</td>
<td>chr11</td>
<td>2016406</td>
<td>2022700</td>
<td>-</td>
<td>2.12904</td>
<td>2.1426</td>
<td>5.6363</td>
<td>993</td>
<td>19.22</td>
</tr>
<tr>
<td>ENSG00000254236</td>
<td>3.54</td>
<td>KB-1639H6.2</td>
<td>chr8</td>
<td>104032415</td>
<td>104033656</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0.2837</td>
<td>624</td>
<td>3.250</td>
</tr>
<tr>
<td>ENSG00000244528</td>
<td>3.31</td>
<td>AC134873.1</td>
<td>chr2</td>
<td>243064438</td>
<td>243064620</td>
<td>+</td>
<td>1.27679</td>
<td>34</td>
<td>2.8750</td>
<td>565</td>
<td>2.7582</td>
</tr>
<tr>
<td>ENSG00000273413</td>
<td>3.07</td>
<td>RP11-96C23.15</td>
<td>chr10</td>
<td>88729994</td>
<td>88730583</td>
<td>-</td>
<td>8.39354</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>4.790</td>
</tr>
<tr>
<td>ENSG00000228626</td>
<td>3.06</td>
<td>RP11-495P10.9</td>
<td>chr1</td>
<td>147760107</td>
<td>147761057</td>
<td>-</td>
<td>2.02880</td>
<td>84</td>
<td>0.8839</td>
<td>2633</td>
<td>2.2444</td>
</tr>
<tr>
<td>ENSG00000230937</td>
<td>2.94</td>
<td>MIR205HG</td>
<td>chr1</td>
<td>209602165</td>
<td>209606183</td>
<td>+</td>
<td>61.5482</td>
<td>64</td>
<td>49.323</td>
<td>284</td>
<td>62.656</td>
</tr>
<tr>
<td>ENSG00000254860</td>
<td>2.73</td>
<td>TMEM9B-AS1</td>
<td>chr11</td>
<td>8986222</td>
<td>8999074</td>
<td>+</td>
<td>0.80623</td>
<td>74</td>
<td>1.7021</td>
<td>289</td>
<td>0.9305</td>
</tr>
<tr>
<td>ENSG00000249641</td>
<td>2.69</td>
<td>HOXC-AS5</td>
<td>chr12</td>
<td>54329112</td>
<td>54333427</td>
<td>-</td>
<td>0.95938</td>
<td>82</td>
<td>1.8041</td>
<td>867</td>
<td>0.9305</td>
</tr>
<tr>
<td>ENSG00000270168</td>
<td>2.56</td>
<td>LA16c-380H5.4</td>
<td>chr16</td>
<td>3051301</td>
<td>3052017</td>
<td>+</td>
<td>6.47689</td>
<td>87</td>
<td>0.8551</td>
<td>222</td>
<td>4.0893</td>
</tr>
<tr>
<td>ENSG00000235314</td>
<td>2.53</td>
<td>LINCO0957</td>
<td>chr7</td>
<td>44078770</td>
<td>44081905</td>
<td>+</td>
<td>0.35596</td>
<td>678</td>
<td>0.7332</td>
<td>8674</td>
<td>0.7004</td>
</tr>
<tr>
<td>ENSG00000230409</td>
<td>2.50</td>
<td>TCEA1P2</td>
<td>chr3</td>
<td>37317087</td>
<td>37318089</td>
<td>+</td>
<td>7.46950</td>
<td>8</td>
<td>9.5921</td>
<td>29</td>
<td>10.218</td>
</tr>
<tr>
<td>ENSG00000233223</td>
<td>2.45</td>
<td>AC113189.5</td>
<td>chr17</td>
<td>7485282</td>
<td>7487390</td>
<td>-</td>
<td>1.45960</td>
<td>63</td>
<td>2.9885</td>
<td>232</td>
<td>2.0333</td>
</tr>
<tr>
<td>ENSG00000254192</td>
<td>2.36</td>
<td>CTC-55802.2</td>
<td>chr5</td>
<td>168081518</td>
<td>168094766</td>
<td>+</td>
<td>1.23549</td>
<td>65</td>
<td>0.9035</td>
<td>5885</td>
<td>1.6458</td>
</tr>
<tr>
<td>ENSG00000251580</td>
<td>2.35</td>
<td>RP11-939L10.3</td>
<td>chr4</td>
<td>6672452</td>
<td>6675557</td>
<td>-</td>
<td>0.83309</td>
<td>1336</td>
<td>1.3336</td>
<td>157</td>
<td>1.0557</td>
</tr>
<tr>
<td>ID</td>
<td>Description</td>
<td>Chromosome</td>
<td>Start</td>
<td>End</td>
<td>Gene 1</td>
<td>Gene 2</td>
<td>Log2 Fold Change</td>
<td>P-Value 1</td>
<td>P-Value 2</td>
<td>False Discovery Rate</td>
<td>False Discovery Rate Offset</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------</td>
<td>------------</td>
<td>-------</td>
<td>-----</td>
<td>--------</td>
<td>--------</td>
<td>------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>ENSG00000220804</td>
<td>2.29</td>
<td>chr2</td>
<td>243030784</td>
<td>243102304</td>
<td>+</td>
<td>1.02285 7</td>
<td>1.6583 912</td>
<td>2.8178 344</td>
<td>4.230 8583</td>
<td>2.589 8807</td>
<td>5.207 1075</td>
</tr>
<tr>
<td>ENSG00000201428</td>
<td>2.28</td>
<td>chr12</td>
<td>112704881</td>
<td>112705198</td>
<td>+</td>
<td>0.05682 099</td>
<td>0.2640 6604</td>
<td>0.3352 5583</td>
<td>0.033 64112 6</td>
<td>0.464 84584</td>
<td>11.79 032</td>
</tr>
<tr>
<td>ENSG00000238107</td>
<td>2.27</td>
<td>chr1</td>
<td>147767287</td>
<td>147769663</td>
<td>+</td>
<td>1.19644 03</td>
<td>0.5992 599</td>
<td>0.7781 85</td>
<td>3.452 5478</td>
<td>1.896 4818</td>
<td>2.127 1327</td>
</tr>
<tr>
<td>ENSG00000228463</td>
<td>2.25</td>
<td>chr1</td>
<td>227615</td>
<td>267253</td>
<td>-</td>
<td>0.92179 984</td>
<td>1.8147 042</td>
<td>2.4698 875</td>
<td>3.840 908</td>
<td>3.520 3686</td>
<td>3.766 3238</td>
</tr>
<tr>
<td>ENSG00000263934</td>
<td>2.22</td>
<td>chr17</td>
<td>19091329</td>
<td>19092027</td>
<td>+</td>
<td>56.5595 66</td>
<td>96.541 93</td>
<td>87.400 215</td>
<td>115.8 12065</td>
<td>101.6 3637</td>
<td>442.5 1657</td>
</tr>
<tr>
<td>ENSG00000267984</td>
<td>2.21</td>
<td>chr19</td>
<td>51848423</td>
<td>51856547</td>
<td>+</td>
<td>0.03900 8502</td>
<td>0</td>
<td>0</td>
<td>0.093 65486</td>
<td>0</td>
<td>10.77 701</td>
</tr>
<tr>
<td>ENSG00000200488</td>
<td>2.18</td>
<td>chr2</td>
<td>76672205</td>
<td>76672536</td>
<td>-</td>
<td>0.39458 07</td>
<td>2.4766 135</td>
<td>0.8919 96</td>
<td>1.181 4926</td>
<td>0.604 2596</td>
<td>21.76 185</td>
</tr>
<tr>
<td>ENSG00000255717</td>
<td>2.13</td>
<td>chr11</td>
<td>62619460</td>
<td>62623386</td>
<td>-</td>
<td>7.09001 87</td>
<td>3.8827 178</td>
<td>5.6702 17</td>
<td>13.07 7036</td>
<td>11.90 0302</td>
<td>9.669 952</td>
</tr>
<tr>
<td>ENSG00000259001</td>
<td>2.12</td>
<td>chr14</td>
<td>20811207</td>
<td>20811844</td>
<td>-</td>
<td>15.0019 23</td>
<td>52.592 74</td>
<td>31.222 376</td>
<td>27.47 0306</td>
<td>36.77 239</td>
<td>231.4 2776</td>
</tr>
<tr>
<td>ENSG00000234741</td>
<td>2.10</td>
<td>chr1</td>
<td>173833038</td>
<td>173838020</td>
<td>-</td>
<td>75.5498 6</td>
<td>34.682 1</td>
<td>63.601 818</td>
<td>141.4 7461</td>
<td>116.2 6347</td>
<td>93.72 997</td>
</tr>
<tr>
<td>ENSG00000215039</td>
<td>2.08</td>
<td>chr12</td>
<td>6548167</td>
<td>6560733</td>
<td>-</td>
<td>0.66829 19</td>
<td>0.8538 55</td>
<td>0.8496 1987</td>
<td>3.074 375</td>
<td>1.768 162</td>
<td>1.665 7759</td>
</tr>
<tr>
<td>ENSG00000259970</td>
<td>2.06</td>
<td>chr3</td>
<td>49721913</td>
<td>49722416</td>
<td>-</td>
<td>0.57804 507</td>
<td>1.3075 932</td>
<td>0.8779 387</td>
<td>2.843 681</td>
<td>2.354 5742</td>
<td>1.696 4713</td>
</tr>
<tr>
<td>ENSG00000254578</td>
<td>2.04</td>
<td>chr8</td>
<td>145689200</td>
<td>145690484</td>
<td>+</td>
<td>2.56431 48</td>
<td>1.6997 473</td>
<td>2.1004 875</td>
<td>4.821 358</td>
<td>4.543 132</td>
<td>3.554 0457</td>
</tr>
<tr>
<td>ENSG00000202198</td>
<td>2.04</td>
<td>chr6</td>
<td>52860418</td>
<td>52860748</td>
<td>+</td>
<td>77.7898 2</td>
<td>202.92 421</td>
<td>175.02 835</td>
<td>174.8 5051</td>
<td>183.0 6963</td>
<td>733.0 992</td>
</tr>
<tr>
<td>ENSG00000255831</td>
<td>2.04</td>
<td>chr13</td>
<td>111291555</td>
<td>111292340</td>
<td>+</td>
<td>0</td>
<td>2.5623 991</td>
<td>0</td>
<td>0</td>
<td>0.016 7576</td>
<td>4.200 331</td>
</tr>
<tr>
<td>ENSG00000271992</td>
<td>2.02</td>
<td>chr1</td>
<td>70910754</td>
<td>70911219</td>
<td>+</td>
<td>0.34897 357</td>
<td>0.0900 998</td>
<td>0.8007 2904</td>
<td>2.295 6822</td>
<td>1.722 0101</td>
<td>2.083 4737</td>
</tr>
<tr>
<td>ENSG00000227195</td>
<td>2.01</td>
<td>chr20</td>
<td>26167556</td>
<td>26232162</td>
<td>-</td>
<td>0.89503 71</td>
<td>1.0255 735</td>
<td>1.4251 992</td>
<td>1.545 9784</td>
<td>1.247 556</td>
<td>6.125 4115</td>
</tr>
<tr>
<td>ENSG00000239002</td>
<td>1.98</td>
<td>chr12</td>
<td>6619388</td>
<td>6619717</td>
<td>+</td>
<td>0.54754 776</td>
<td>1.9084 775</td>
<td>1.3999 47</td>
<td>1.167 0413</td>
<td>2.879 6296</td>
<td>6.134 6283</td>
</tr>
<tr>
<td>ENSG00000263917</td>
<td>1.97</td>
<td>chr18</td>
<td>29598792</td>
<td>29691742</td>
<td>+</td>
<td>1.69259 02</td>
<td>0.7714 8676</td>
<td>1.5012 345</td>
<td>1.865 241</td>
<td>3.775 4347</td>
<td>2.767 804</td>
</tr>
<tr>
<td>ENSG00000266929</td>
<td>1.96</td>
<td>chr17</td>
<td>40688528</td>
<td>40714080</td>
<td>+</td>
<td>2.64025 28</td>
<td>2.2248 166</td>
<td>2.0022 075</td>
<td>4.118 1316</td>
<td>4.623 1112</td>
<td>4.685 91</td>
</tr>
<tr>
<td>ENSG00000223891</td>
<td>1.96</td>
<td>chr20</td>
<td>42839600</td>
<td>42854667</td>
<td>+</td>
<td>0.42706 716</td>
<td>1.3540 938</td>
<td>1.7394 654</td>
<td>2.074 9772</td>
<td>3.062 2957</td>
<td>2.806 612</td>
</tr>
<tr>
<td>Genes</td>
<td>Gene</td>
<td>Chromosome</td>
<td>start</td>
<td>end</td>
<td>size (bp)</td>
<td>TSS/FUTS</td>
<td>const</td>
<td>start</td>
<td>end</td>
<td>size (bp)</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>-------</td>
<td>--------</td>
<td>-----------</td>
<td>----------</td>
<td>-----------</td>
<td>-------</td>
<td>--------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>RP11-301H24.4</td>
<td>chr4</td>
<td>146435767</td>
<td>146438270</td>
<td>-</td>
<td>6.98976</td>
<td>66</td>
<td>3.0939</td>
<td>543</td>
<td>5.3921</td>
<td>43</td>
<td>10.33</td>
</tr>
<tr>
<td>RP11-815J21.2</td>
<td>chr15</td>
<td>86122277</td>
<td>86123409</td>
<td>-</td>
<td>1.83370</td>
<td>24</td>
<td>1.1494</td>
<td>807</td>
<td>1.1902</td>
<td>504</td>
<td>1.597</td>
</tr>
<tr>
<td>SEMA3B</td>
<td>chr3</td>
<td>50304990</td>
<td>50314977</td>
<td>+</td>
<td>0.94935</td>
<td>43</td>
<td>0.3429</td>
<td>1044</td>
<td>1.0639</td>
<td>977</td>
<td>2.247</td>
</tr>
<tr>
<td>PAXIP1-AS1</td>
<td>chr7</td>
<td>154795158</td>
<td>154797413</td>
<td>+</td>
<td>1.07325</td>
<td>18</td>
<td>2.6334</td>
<td>62</td>
<td>2.4573</td>
<td>538</td>
<td>3.006</td>
</tr>
<tr>
<td>AC005062.2</td>
<td>chr7</td>
<td>19958604</td>
<td>20180076</td>
<td>-</td>
<td>4.87558</td>
<td>13</td>
<td>7.2985</td>
<td>44</td>
<td>3.9218</td>
<td>314</td>
<td>6.931</td>
</tr>
<tr>
<td>SNHG17</td>
<td>chr20</td>
<td>37049235</td>
<td>37063996</td>
<td>-</td>
<td>2.54244</td>
<td>57</td>
<td>1.8823</td>
<td>376</td>
<td>4.6329</td>
<td>513</td>
<td>4.370</td>
</tr>
<tr>
<td>RP11-533E19.7</td>
<td>chr1</td>
<td>179850742</td>
<td>179851730</td>
<td>-</td>
<td>0.29894</td>
<td>31</td>
<td>1.2788</td>
<td>76</td>
<td>1.0368</td>
<td>878</td>
<td>1.577</td>
</tr>
<tr>
<td>DYS1C1-CCPG1</td>
<td>chr15</td>
<td>55647446</td>
<td>55790558</td>
<td>-</td>
<td>2.09689</td>
<td>38</td>
<td>2.6955</td>
<td>016</td>
<td>2.8982</td>
<td>72</td>
<td>5.290</td>
</tr>
<tr>
<td>LINCO0963</td>
<td>chr9</td>
<td>132245730</td>
<td>132275947</td>
<td>+</td>
<td>1.01223</td>
<td>13</td>
<td>0.8181</td>
<td>0194</td>
<td>0.7509</td>
<td>8056</td>
<td>2.690</td>
</tr>
<tr>
<td>SNHG8</td>
<td>chr4</td>
<td>119199864</td>
<td>119200978</td>
<td>+</td>
<td>95.6830</td>
<td>75</td>
<td>85.640</td>
<td>51</td>
<td>110.78</td>
<td>4874</td>
<td>193.3</td>
</tr>
<tr>
<td>RMRP</td>
<td>chr9</td>
<td>35657748</td>
<td>35658015</td>
<td>-</td>
<td>61.7190</td>
<td>86</td>
<td>117.54</td>
<td>486</td>
<td>105.27</td>
<td>629</td>
<td>99.84</td>
</tr>
<tr>
<td>RN7SL5P</td>
<td>chr9</td>
<td>9442060</td>
<td>9442347</td>
<td>+</td>
<td>89.3426</td>
<td>7</td>
<td>171.01</td>
<td>642</td>
<td>175.36</td>
<td>769</td>
<td>146.4</td>
</tr>
<tr>
<td>AC018638.1</td>
<td>chr7</td>
<td>128293740</td>
<td>128293989</td>
<td>-</td>
<td>2.08112</td>
<td>76</td>
<td>0.8533</td>
<td>8426</td>
<td>0.6139</td>
<td>542</td>
<td>2.203</td>
</tr>
<tr>
<td>MRPS31P4</td>
<td>chr13</td>
<td>53191693</td>
<td>53211581</td>
<td>+</td>
<td>0.99767</td>
<td>58</td>
<td>0.6966</td>
<td>341</td>
<td>0.8349</td>
<td>367</td>
<td>2.199</td>
</tr>
<tr>
<td>RP11-31F15.1</td>
<td>chr1</td>
<td>113499037</td>
<td>113542118</td>
<td>+</td>
<td>3.05203</td>
<td>82</td>
<td>2.3166</td>
<td>275</td>
<td>2.4479</td>
<td>396</td>
<td>5.745</td>
</tr>
<tr>
<td>LINCO0847</td>
<td>chr5</td>
<td>180257957</td>
<td>180262726</td>
<td>+</td>
<td>2.51798</td>
<td>3</td>
<td>1.9512</td>
<td>683</td>
<td>1.9726</td>
<td>743</td>
<td>3.667</td>
</tr>
<tr>
<td>LEPREL2</td>
<td>chr12</td>
<td>693752</td>
<td>6949018</td>
<td>+</td>
<td>1.43181</td>
<td>74</td>
<td>1.6433</td>
<td>642</td>
<td>1.4975</td>
<td>213</td>
<td>3.065</td>
</tr>
<tr>
<td>RP11-96D1.11</td>
<td>chr16</td>
<td>68259872</td>
<td>68263048</td>
<td>-</td>
<td>1.01642</td>
<td>31</td>
<td>1.7172</td>
<td>104</td>
<td>1.3368</td>
<td>715</td>
<td>2.896</td>
</tr>
<tr>
<td>PKD1P6</td>
<td>chr16</td>
<td>15219099</td>
<td>15248421</td>
<td>-</td>
<td>4.10675</td>
<td>76</td>
<td>2.8266</td>
<td>087</td>
<td>4.4332</td>
<td>04</td>
<td>4.759</td>
</tr>
<tr>
<td>AL353662.2</td>
<td>chr9</td>
<td>34195642</td>
<td>34195883</td>
<td>+</td>
<td>1.73161</td>
<td>97</td>
<td>0.8091</td>
<td>1475</td>
<td>1.1475</td>
<td>528</td>
<td>3.164</td>
</tr>
<tr>
<td>ENSG00000257086</td>
<td>1.72</td>
<td>RP11-783K16.13</td>
<td>chr11</td>
<td>64014411</td>
<td>64016966</td>
<td>-</td>
<td>1.58257</td>
<td>52</td>
<td>1.1539</td>
<td>651</td>
<td>1.8851</td>
</tr>
<tr>
<td>ENSG00000230795</td>
<td>1.72</td>
<td>HLA-K</td>
<td>chr6</td>
<td>29894236</td>
<td>29897009</td>
<td>+</td>
<td>0.61804</td>
<td>247</td>
<td>1.1913</td>
<td>046</td>
<td>1.5108</td>
</tr>
<tr>
<td>ENSG000000180672</td>
<td>1.71</td>
<td>AC007362.1</td>
<td>chr2</td>
<td>206642540</td>
<td>206644433</td>
<td>+</td>
<td>1.37003</td>
<td>06</td>
<td>0.6789</td>
<td>9704</td>
<td>1.1776</td>
</tr>
<tr>
<td>ENSG00000254682</td>
<td>1.71</td>
<td>RP11-60L16.2</td>
<td>chr11</td>
<td>71159720</td>
<td>71163203</td>
<td>+</td>
<td>1.14260</td>
<td>32</td>
<td>0.7100</td>
<td>659</td>
<td>0.4964</td>
</tr>
<tr>
<td>ENSG00000267023</td>
<td>1.71</td>
<td>LRRC37A16P</td>
<td>chr17</td>
<td>66121918</td>
<td>66148609</td>
<td>-</td>
<td>1.45405</td>
<td>14</td>
<td>1.6921</td>
<td>423</td>
<td>1.4601</td>
</tr>
<tr>
<td>ENSG00000227081</td>
<td>1.71</td>
<td>RP11-543P15.1</td>
<td>chr12</td>
<td>3320775</td>
<td>3321096</td>
<td>-</td>
<td>7.24820</td>
<td>5</td>
<td>1.5755</td>
<td>806</td>
<td>5.4170</td>
</tr>
<tr>
<td>ENSG00000223745</td>
<td>1.71</td>
<td>RP4-71I23.3</td>
<td>chr1</td>
<td>93727743</td>
<td>93811582</td>
<td>-</td>
<td>0.95840</td>
<td>377</td>
<td>1.4749</td>
<td>708</td>
<td>1.4199</td>
</tr>
<tr>
<td>ENSG00000226752</td>
<td>1.70</td>
<td>PSMD5-AS1</td>
<td>chr9</td>
<td>123587106</td>
<td>123616651</td>
<td>+</td>
<td>0.90399</td>
<td>02</td>
<td>0.6311</td>
<td>066</td>
<td>1.0164</td>
</tr>
<tr>
<td>ENSG00000227632</td>
<td>1.70</td>
<td>AC018804.6</td>
<td>chr2</td>
<td>130959885</td>
<td>130970201</td>
<td>+</td>
<td>1.50575</td>
<td>63</td>
<td>2.2844</td>
<td>598</td>
<td>2.4223</td>
</tr>
<tr>
<td>ENSG00000228327</td>
<td>1.69</td>
<td>RP11-206L10.2</td>
<td>chr1</td>
<td>700237</td>
<td>714006</td>
<td>-</td>
<td>3.12584</td>
<td>38</td>
<td>1.0653</td>
<td>372</td>
<td>3.5199</td>
</tr>
<tr>
<td>ENSG00000228638</td>
<td>1.69</td>
<td>FCP1P2</td>
<td>chr3</td>
<td>48332283</td>
<td>48332865</td>
<td>-</td>
<td>0.71284</td>
<td>515</td>
<td>0.7201</td>
<td>8015</td>
<td>0.2133</td>
</tr>
<tr>
<td>ENSG00000258186</td>
<td>1.69</td>
<td>SLC7A5P2</td>
<td>chr16</td>
<td>21531151</td>
<td>21531686</td>
<td>-</td>
<td>8.26003</td>
<td>46758</td>
<td>14.699</td>
<td>861</td>
<td>13.65</td>
</tr>
<tr>
<td>ENSG00000225648</td>
<td>1.68</td>
<td>SBDSP1</td>
<td>chr7</td>
<td>72300004</td>
<td>72307909</td>
<td>+</td>
<td>10.7959</td>
<td>62</td>
<td>11.587</td>
<td>327</td>
<td>13.133</td>
</tr>
<tr>
<td>ENSG00000272711</td>
<td>1.68</td>
<td>RP11-259N19.1</td>
<td>chr2</td>
<td>75059782</td>
<td>75061114</td>
<td>-</td>
<td>1.45040</td>
<td>58</td>
<td>1.1969</td>
<td>146</td>
<td>1.6662</td>
</tr>
<tr>
<td>ENSG00000256462</td>
<td>1.67</td>
<td>RP11-116G8.5</td>
<td>chr10</td>
<td>5566939</td>
<td>5567705</td>
<td>-</td>
<td>3.94108</td>
<td>49</td>
<td>0</td>
<td>0</td>
<td>3.097</td>
</tr>
<tr>
<td>ENSG00000236439</td>
<td>1.67</td>
<td>RP11-175B9.3</td>
<td>chr1</td>
<td>202440937</td>
<td>202441258</td>
<td>-</td>
<td>6.70856</td>
<td>76</td>
<td>3.0561</td>
<td>595</td>
<td>4.4500</td>
</tr>
<tr>
<td>ENSG00000233016</td>
<td>1.67</td>
<td>SNHG7</td>
<td>chr9</td>
<td>13961581</td>
<td>139622636</td>
<td>-</td>
<td>3.80191</td>
<td>66</td>
<td>2.5988</td>
<td>64</td>
<td>3.9963</td>
</tr>
<tr>
<td>ENSG00000234975</td>
<td>1.67</td>
<td>FTH1P2</td>
<td>chr1</td>
<td>228823162</td>
<td>228823574</td>
<td>+</td>
<td>2.36462</td>
<td>16</td>
<td>8.7332</td>
<td>3</td>
<td>5.5768</td>
</tr>
<tr>
<td>ENSG000000228998</td>
<td>1.66</td>
<td>RP11-697E2.7</td>
<td>chr15</td>
<td>90818266</td>
<td>90820841</td>
<td>+</td>
<td>1.66241</td>
<td>1</td>
<td>1.0105</td>
<td>448</td>
<td>1.4692</td>
</tr>
<tr>
<td>ENSG000000251022</td>
<td>1.66</td>
<td>THAP9-AS1</td>
<td>chr4</td>
<td>83814162</td>
<td>83822113</td>
<td>-</td>
<td>2.55584</td>
<td>17</td>
<td>2.0884</td>
<td>433</td>
<td>4.3881</td>
</tr>
<tr>
<td>ENSG000000256633</td>
<td>1.64</td>
<td>RP11-169D0.2</td>
<td>chr11</td>
<td>72295616</td>
<td>72299023</td>
<td>+</td>
<td>0.36736</td>
<td>2219</td>
<td>0.2809</td>
<td>585</td>
<td>0.585</td>
</tr>
<tr>
<td>Gene</td>
<td>chr</td>
<td>Start</td>
<td>End</td>
<td>Length</td>
<td>Status</td>
<td>Description</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-----</td>
<td>---------</td>
<td>---------</td>
<td>--------</td>
<td>--------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000271581</td>
<td>chr6</td>
<td>31324424</td>
<td>31325414</td>
<td>+</td>
<td>10.9642 39</td>
<td>24.6846 31324424</td>
<td>0.99347 425</td>
<td>0.8375 4015</td>
<td>1.0657 19</td>
<td>1.652 3628</td>
<td>1.481 0578</td>
</tr>
<tr>
<td>ENSG00000234694</td>
<td>chr1</td>
<td>43820355</td>
<td>43824329</td>
<td>-</td>
<td>1.44880 64</td>
<td>2.5090 265</td>
<td>1.8279 302</td>
<td>0.653 2786</td>
<td>1.049 3617</td>
<td>1.6349 619</td>
<td>2.750 0532</td>
</tr>
<tr>
<td>ENSG00000231970</td>
<td>chr10</td>
<td>99160872</td>
<td>99179281</td>
<td>+</td>
<td>0.84985 04</td>
<td>1.4388 658</td>
<td>1.8384 619</td>
<td>2.750 0532</td>
<td>2.498 7977</td>
<td>1.538 8129</td>
<td></td>
</tr>
<tr>
<td>ENSG00000258824</td>
<td>chr14</td>
<td>64889653</td>
<td>64915275</td>
<td>-</td>
<td>0.54871 875</td>
<td>0.3577 88</td>
<td>0.8857 678</td>
<td>2.461 6747</td>
<td>1.143 732</td>
<td>1.395 1521</td>
<td></td>
</tr>
<tr>
<td>ENSG00000224886</td>
<td>chr10</td>
<td>81444250</td>
<td>81444739</td>
<td>-</td>
<td>0.14750 265</td>
<td>1.242 577</td>
<td>0.5076 731</td>
<td>1.309 9444</td>
<td>0.603 35094</td>
<td>3.709 9042</td>
<td></td>
</tr>
<tr>
<td>ENSG00000259623</td>
<td>chr17</td>
<td>40004770</td>
<td>40007699</td>
<td>-</td>
<td>1.19021 55</td>
<td>0.7809 776</td>
<td>1.3159 648</td>
<td>2.417 0635</td>
<td>1.441 4529</td>
<td>1.755 5282</td>
<td></td>
</tr>
<tr>
<td>ENSG00000214184</td>
<td>chr2</td>
<td>109123971</td>
<td>109150652</td>
<td>-</td>
<td>1.13318 16</td>
<td>1.5004 897</td>
<td>1.6879 808</td>
<td>2.188 2014</td>
<td>1.909 4998</td>
<td>2.645 182</td>
<td></td>
</tr>
<tr>
<td>ENSG00000231864</td>
<td>chr9</td>
<td>139957987</td>
<td>139959033</td>
<td>+</td>
<td>6.8776 64</td>
<td>0.3192 18</td>
<td>1.6303 605</td>
<td>3.468 3156</td>
<td>2.224 2556</td>
<td>1.746 7672</td>
<td></td>
</tr>
<tr>
<td>ENSG00000259948</td>
<td>chr15</td>
<td>89744322</td>
<td>89744999</td>
<td>-</td>
<td>0.14750 265</td>
<td>1.242 577</td>
<td>0.5076 731</td>
<td>1.309 9444</td>
<td>0.603 35094</td>
<td>3.709 9042</td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>Symbol</td>
<td>Chromosome</td>
<td>Start</td>
<td>End</td>
<td>Length</td>
<td>Strandedness</td>
<td>Distance</td>
<td>Enrichment</td>
<td>Direction</td>
<td>Orientation</td>
<td>Coverage</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>------------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>--------------</td>
<td>----------</td>
<td>------------</td>
<td>-----------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td>ENSG00000203875</td>
<td>SNHG5</td>
<td>chr6</td>
<td>86370710</td>
<td>86388451</td>
<td>102081</td>
<td>-</td>
<td>635</td>
<td>49.028</td>
<td>72.932</td>
<td>136.5</td>
<td>115.0</td>
</tr>
<tr>
<td>ENSG00000223361</td>
<td>FTH1P10</td>
<td>chr5</td>
<td>17353804</td>
<td>17354733</td>
<td>-</td>
<td>1.1208</td>
<td>73</td>
<td>1.9096</td>
<td>3.1168</td>
<td>3.494</td>
<td>3.568</td>
</tr>
<tr>
<td>ENSG00000234797</td>
<td>RPS3AP6</td>
<td>chr15</td>
<td>60060543</td>
<td>60061347</td>
<td>+</td>
<td>8.03755</td>
<td>8</td>
<td>4.6485</td>
<td>6.0571</td>
<td>10.87</td>
<td>9.480</td>
</tr>
<tr>
<td>ENSG00000186831</td>
<td>KRT1P2</td>
<td>chr17</td>
<td>18330175</td>
<td>18335162</td>
<td>+</td>
<td>2.67693</td>
<td>57</td>
<td>5.9414</td>
<td>8.2313</td>
<td>8.073</td>
<td>7.960</td>
</tr>
<tr>
<td>ENSG00000235298</td>
<td>RP11-575L7.8</td>
<td>chr9</td>
<td>86587148</td>
<td>86590692</td>
<td>+</td>
<td>0.99603</td>
<td>415</td>
<td>0.1577</td>
<td>1.2684</td>
<td>1.084</td>
<td>1.106</td>
</tr>
<tr>
<td>ENSG00000244398</td>
<td>RP11-466H18.1</td>
<td>chr11</td>
<td>16996240</td>
<td>16996560</td>
<td>-</td>
<td>210.415</td>
<td>99</td>
<td>118.90</td>
<td>159.80</td>
<td>323.5</td>
<td>266.5</td>
</tr>
<tr>
<td>ENSG00000232956</td>
<td>SNHG15</td>
<td>chr7</td>
<td>45022622</td>
<td>45026560</td>
<td>-</td>
<td>4.96357</td>
<td>63</td>
<td>4.2213</td>
<td>6.0738</td>
<td>7.899</td>
<td>7.568</td>
</tr>
<tr>
<td>ENSG00000265688</td>
<td>MAFG-AS1</td>
<td>chr17</td>
<td>79885705</td>
<td>7988629</td>
<td>+</td>
<td>2.63169</td>
<td>65</td>
<td>1.0524</td>
<td>1.6549</td>
<td>2.523</td>
<td>3.253</td>
</tr>
<tr>
<td>ENSG00000205746</td>
<td>RP11-1212A22.1</td>
<td>chr16</td>
<td>18428257</td>
<td>18483936</td>
<td>-</td>
<td>2.70093</td>
<td>97</td>
<td>1.9503</td>
<td>2.9362</td>
<td>3.497</td>
<td>3.365</td>
</tr>
<tr>
<td>ENSG00000205763</td>
<td>RP9P</td>
<td>chr7</td>
<td>32956427</td>
<td>32982788</td>
<td>-</td>
<td>1.58876</td>
<td>7</td>
<td>2.6736</td>
<td>4.2468</td>
<td>3.486</td>
<td>3.794</td>
</tr>
<tr>
<td>ENSG00000260107</td>
<td>AC005606.15</td>
<td>chr16</td>
<td>2047655</td>
<td>2048375</td>
<td>+</td>
<td>9.51927</td>
<td>2</td>
<td>5.6106</td>
<td>7.2765</td>
<td>8.348</td>
<td>12.49</td>
</tr>
<tr>
<td>ENSG00000204194</td>
<td>RPL12P1</td>
<td>chr6</td>
<td>33367836</td>
<td>33368333</td>
<td>-</td>
<td>8.16444</td>
<td>3</td>
<td>4.4001</td>
<td>10.382</td>
<td>8.702</td>
<td>12.67</td>
</tr>
<tr>
<td>ENSG00000254473</td>
<td>RP11-522I20.3</td>
<td>chr9</td>
<td>86322509</td>
<td>86328293</td>
<td>+</td>
<td>0.62335</td>
<td>21</td>
<td>0.6170</td>
<td>0.9024</td>
<td>2.064</td>
<td>1.439</td>
</tr>
<tr>
<td>ENSG00000261971</td>
<td>RP11-473M20.7</td>
<td>chr16</td>
<td>3101992</td>
<td>3109371</td>
<td>-</td>
<td>0.38817</td>
<td>36</td>
<td>0.9558</td>
<td>1.3753</td>
<td>1.559</td>
<td>1.193</td>
</tr>
<tr>
<td>ENSG00000235725</td>
<td>AC007389.3</td>
<td>chr2</td>
<td>65816700</td>
<td>65867311</td>
<td>-</td>
<td>0.91346</td>
<td>866</td>
<td>0.7893</td>
<td>1.0001</td>
<td>1.792</td>
<td>1.434</td>
</tr>
<tr>
<td>ENSG00000229212</td>
<td>RP11-561C5.4</td>
<td>chr15</td>
<td>85747141</td>
<td>85778026</td>
<td>-</td>
<td>1.54617</td>
<td>49</td>
<td>2.1187</td>
<td>2.2953</td>
<td>2.896</td>
<td>2.832</td>
</tr>
<tr>
<td>ENSG00000259328</td>
<td>RP11-152F13.7</td>
<td>chr15</td>
<td>82944773</td>
<td>82974312</td>
<td>+</td>
<td>1.00161</td>
<td>61</td>
<td>1.3405</td>
<td>1.1754</td>
<td>1.238</td>
<td>1.825</td>
</tr>
<tr>
<td>ENSG00000223396</td>
<td>RPS10P7</td>
<td>chr1</td>
<td>20148731</td>
<td>201499602</td>
<td>+</td>
<td>3.12924</td>
<td>72</td>
<td>1.9355</td>
<td>2.9419</td>
<td>4.337</td>
<td>4.088</td>
</tr>
<tr>
<td>ENSG00000269032</td>
<td>AC016629.7</td>
<td>chr19</td>
<td>59097525</td>
<td>59097723</td>
<td>+</td>
<td>0.32712</td>
<td>79</td>
<td>0.8532</td>
<td>0.6416</td>
<td>0</td>
<td>2.120</td>
</tr>
<tr>
<td>ENSG00000203394</td>
<td>RPS-930J4.4</td>
<td>chr1</td>
<td>21069480</td>
<td>21070455</td>
<td>+</td>
<td>4.33596</td>
<td>2.2852</td>
<td>2.7163</td>
<td>5.946</td>
<td>4.018</td>
<td>3.844</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>Chromosome</td>
<td>Position (Start)</td>
<td>Position (End)</td>
<td>Type</td>
<td>Description</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000225151</td>
<td>chr15</td>
<td>84867600</td>
<td>84898888</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000244257</td>
<td>chr16</td>
<td>16404198</td>
<td>16428047</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000267580</td>
<td>chr19</td>
<td>33790840</td>
<td>33792074</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000267151</td>
<td>chr17</td>
<td>41522075</td>
<td>41528568</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000270005</td>
<td>chr15</td>
<td>30780166</td>
<td>30782516</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000228989</td>
<td>chr2</td>
<td>242629829</td>
<td>242633704</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000262791</td>
<td>chr17</td>
<td>1629042</td>
<td>1641879</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000248092</td>
<td>chr5</td>
<td>43571696</td>
<td>43603332</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000270232</td>
<td>chr5</td>
<td>99381632</td>
<td>99382078</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000255125</td>
<td>chr11</td>
<td>10804860</td>
<td>10823172</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000265415</td>
<td>chr17</td>
<td>57280038</td>
<td>57281190</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000232358</td>
<td>chr20</td>
<td>49615907</td>
<td>49626556</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000231298</td>
<td>chr10</td>
<td>4692377</td>
<td>4720346</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000269834</td>
<td>chr19</td>
<td>52892095</td>
<td>52901019</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000228649</td>
<td>chr7</td>
<td>22893797</td>
<td>22901021</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000250234</td>
<td>chr5</td>
<td>34656517</td>
<td>34657355</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000260549</td>
<td>chr16</td>
<td>56651388</td>
<td>56652730</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000269983</td>
<td>chr5</td>
<td>69745463</td>
<td>69746130</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000232656</td>
<td>chr10</td>
<td>1068606</td>
<td>1090138</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000273132</td>
<td>chr6</td>
<td>150173598</td>
<td>150174328</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000262413</td>
<td>chr17</td>
<td>79825597</td>
<td>79826428</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genes</td>
<td>Mapped Genes</td>
<td>Strain</td>
<td>Genomic Start</td>
<td>Genomic Stop</td>
<td>Strand</td>
<td>Expression (RPKM)</td>
<td>Log2 Fold Change</td>
<td>p-Value (BH)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td>---------------</td>
<td>--------------</td>
<td>--------</td>
<td>------------------</td>
<td>-----------------</td>
<td>--------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000233144</td>
<td>-1.55</td>
<td>RP11-537A6.9</td>
<td>chr10</td>
<td>75141191</td>
<td></td>
<td>1.73480</td>
<td>1.7642</td>
<td>0.579</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>75143254</td>
<td></td>
<td>1.02477</td>
<td>1.0846</td>
<td>1.376</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.02477</td>
<td></td>
<td>1.0579</td>
<td>1.0693</td>
<td>1.206</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000226963</td>
<td>-1.55</td>
<td>AC078883.4</td>
<td>chr2</td>
<td>173292502</td>
<td>-</td>
<td>3.65149</td>
<td>3.6949</td>
<td>0.984</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.6295</td>
<td>-</td>
<td>2.4602</td>
<td>3.7637</td>
<td>1.833</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.4602</td>
<td>-</td>
<td>1.8333</td>
<td>1.5695</td>
<td>2.079</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000234327</td>
<td>-1.55</td>
<td>AC012146.7</td>
<td>chr17</td>
<td>5014763</td>
<td>+</td>
<td>1.95390</td>
<td>2.1499</td>
<td>1.5286</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.5286</td>
<td>+</td>
<td>0.9844</td>
<td>0.9946</td>
<td>1.408</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000259153</td>
<td>-1.56</td>
<td>RP6-65G23.3</td>
<td>chr14</td>
<td>71276922</td>
<td>+</td>
<td>1.99365</td>
<td>2.7267</td>
<td>1.2656</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.7267</td>
<td>+</td>
<td>1.0455</td>
<td>1.0579</td>
<td>0.530</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000258791</td>
<td>-1.58</td>
<td>LINCO00520</td>
<td>chr14</td>
<td>56247864</td>
<td>-</td>
<td>0.64874</td>
<td>2.9121</td>
<td>2.3695</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.9121</td>
<td>-</td>
<td>0.3227</td>
<td>0.3298</td>
<td>1.232</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000267777</td>
<td>-1.58</td>
<td>AC006116.24</td>
<td>chr19</td>
<td>56888073</td>
<td>-</td>
<td>2.15047</td>
<td>3.7224</td>
<td>2.8212</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.7224</td>
<td>-</td>
<td>1.8324</td>
<td>1.8324</td>
<td>1.608</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000257181</td>
<td>-1.58</td>
<td>RP11-61102.5</td>
<td>chr12</td>
<td>69235068</td>
<td>-</td>
<td>5.82705</td>
<td>7.4925</td>
<td>6.0171</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.4925</td>
<td>-</td>
<td>4.1696</td>
<td>4.1696</td>
<td>3.725</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000267774</td>
<td>-1.58</td>
<td>RP11-2N1.2</td>
<td>chr18</td>
<td>57363691</td>
<td>+</td>
<td>4.9585</td>
<td>2.7897</td>
<td>5.4442</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.7897</td>
<td>+</td>
<td>1.4676</td>
<td>1.4676</td>
<td>3.439</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000241749</td>
<td>-1.59</td>
<td>RPSAP52</td>
<td>chr12</td>
<td>66151800</td>
<td>-</td>
<td>3.20631</td>
<td>1.0826</td>
<td>1.4755</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0826</td>
<td>-</td>
<td>0.9031</td>
<td>0.9031</td>
<td>0.572</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000268854</td>
<td>-1.59</td>
<td>CTD-2545M3.2</td>
<td>chr19</td>
<td>50983376</td>
<td>-</td>
<td>4.72710</td>
<td>2.3693</td>
<td>1.3470</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3693</td>
<td>-</td>
<td>3.4677</td>
<td>3.4677</td>
<td>1.066</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000224018</td>
<td>-1.59</td>
<td>RP11-134G8.8</td>
<td>chr1</td>
<td>20143511</td>
<td>-</td>
<td>3.14244</td>
<td>7.4775</td>
<td>3.8924</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.4775</td>
<td>-</td>
<td>2.8257</td>
<td>2.8257</td>
<td>2.930</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000232815</td>
<td>-1.59</td>
<td>LINCO00537</td>
<td>chr9</td>
<td>68413482</td>
<td>-</td>
<td>3.58854</td>
<td>3.3276</td>
<td>2.7501</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.3276</td>
<td>-</td>
<td>2.0340</td>
<td>2.0340</td>
<td>2.185</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000240449</td>
<td>-1.61</td>
<td>RP11-796G6.1</td>
<td>chr14</td>
<td>102144280</td>
<td>+</td>
<td>3.58287</td>
<td>1.8213</td>
<td>2.3123</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.8213</td>
<td>+</td>
<td>1.8448</td>
<td>1.8448</td>
<td>1.181</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000258017</td>
<td>-1.62</td>
<td>RP11-386G11.10</td>
<td>chr12</td>
<td>49521565</td>
<td>+</td>
<td>27.8705</td>
<td>18.402</td>
<td>17.180</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18.402</td>
<td>+</td>
<td>12.053</td>
<td>12.053</td>
<td>12.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000034063</td>
<td>-1.62</td>
<td>UHRF1</td>
<td>chr19</td>
<td>4903092</td>
<td>+</td>
<td>6.74429</td>
<td>1.2855</td>
<td>2.7702</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.2855</td>
<td>+</td>
<td>2.0752</td>
<td>2.0752</td>
<td>1.767</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000254258</td>
<td>-1.62</td>
<td>RP11-398H6.1</td>
<td>chr8</td>
<td>140472305</td>
<td>-</td>
<td>4.54776</td>
<td>7.7839</td>
<td>6.3626</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.7839</td>
<td>-</td>
<td>3.9253</td>
<td>3.9253</td>
<td>3.584</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000213412</td>
<td>-1.64</td>
<td>HNRNPA1P33</td>
<td>chr10</td>
<td>47133338</td>
<td>-</td>
<td>14.4520</td>
<td>8.3262</td>
<td>6.1332</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.3262</td>
<td>-</td>
<td>8.4667</td>
<td>8.4667</td>
<td>4.197</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000126005</td>
<td>-1.64</td>
<td>MMP24-AS1</td>
<td>chr20</td>
<td>33804265</td>
<td>-</td>
<td>9.15239</td>
<td>7.7007</td>
<td>6.1065</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.7007</td>
<td>-</td>
<td>5.4591</td>
<td>5.4591</td>
<td>5.335</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000172965</td>
<td>-1.65</td>
<td>MIR4435-1HG</td>
<td>chr2</td>
<td>111953927</td>
<td>-</td>
<td>3.55057</td>
<td>3.3823</td>
<td>3.6901</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.3823</td>
<td>-</td>
<td>1.7802</td>
<td>1.7802</td>
<td>2.279</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000253161</td>
<td>-1.65</td>
<td>RP11-150K12.1</td>
<td>chr8</td>
<td>37278859</td>
<td>-</td>
<td>11.0290</td>
<td>12.441</td>
<td>9.9816</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.441</td>
<td>-</td>
<td>6.3110</td>
<td>6.3110</td>
<td>8.075</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG000000261068</td>
<td>-1.65</td>
<td>RP11-7K24.3</td>
<td>chr6</td>
<td>42059976</td>
<td>-</td>
<td>1.63533</td>
<td>8.6070</td>
<td>3.6644</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.6070</td>
<td>-</td>
<td>1.8943</td>
<td>1.8943</td>
<td>2.506</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The table represents gene expression data with columns for gene ID, expression value, and fold change. The data is normalized by strain and genomic coordinates.
<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Ensembl</th>
<th>Chr</th>
<th>Start</th>
<th>Stop</th>
<th>Orientation</th>
<th>Distance</th>
<th>K</th>
<th>Freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENSG000000235847</td>
<td>-1.89</td>
<td>chr2</td>
<td>85004383</td>
<td>85005347</td>
<td>-</td>
<td>2.74156</td>
<td>7</td>
<td>2.0730</td>
</tr>
<tr>
<td>ENSG00000273038</td>
<td>-1.90</td>
<td>chr10</td>
<td>33176189</td>
<td>33178239</td>
<td>-</td>
<td>2.26414</td>
<td>06</td>
<td>2.7482</td>
</tr>
<tr>
<td>ENSG000000231991</td>
<td>-1.91</td>
<td>chr9</td>
<td>33624223</td>
<td>33625532</td>
<td>+</td>
<td>134.588</td>
<td>53</td>
<td>110.10</td>
</tr>
<tr>
<td>ENSG000000256929</td>
<td>-1.91</td>
<td>chr17</td>
<td>40673736</td>
<td>40674065</td>
<td>+</td>
<td>3.25574</td>
<td>8</td>
<td>8.0733</td>
</tr>
<tr>
<td>ENSG000000255959</td>
<td>-1.92</td>
<td>chr11</td>
<td>60603469</td>
<td>60610438</td>
<td>-</td>
<td>5.55156</td>
<td>56</td>
<td>2.3799</td>
</tr>
<tr>
<td>ENSG000000251292</td>
<td>-1.93</td>
<td>chr4</td>
<td>23724885</td>
<td>23735202</td>
<td>-</td>
<td>38.6029</td>
<td>8</td>
<td>68.663</td>
</tr>
<tr>
<td>ENSG000000262410</td>
<td>-1.96</td>
<td>chr17</td>
<td>80702944</td>
<td>80703585</td>
<td>+</td>
<td>2.04051</td>
<td>07</td>
<td>3.6950</td>
</tr>
<tr>
<td>ENSG000000266088</td>
<td>-1.97</td>
<td>chr17</td>
<td>38673278</td>
<td>38683254</td>
<td>+</td>
<td>4.31365</td>
<td>35</td>
<td>1.3317</td>
</tr>
<tr>
<td>ENSG000000255234</td>
<td>-1.98</td>
<td>chr11</td>
<td>82997171</td>
<td>83134559</td>
<td>+</td>
<td>74.2644</td>
<td>35</td>
<td>131.67</td>
</tr>
<tr>
<td>ENSG000000231826</td>
<td>-1.98</td>
<td>chr2</td>
<td>43254992</td>
<td>43266668</td>
<td>-</td>
<td>16.923</td>
<td>4</td>
<td>4.0733</td>
</tr>
<tr>
<td>ENSG000000235823</td>
<td>-2.00</td>
<td>chr10</td>
<td>10213332</td>
<td>10214312</td>
<td>+</td>
<td>2.90408</td>
<td>1</td>
<td>3.2670</td>
</tr>
<tr>
<td>ENSG000000265992</td>
<td>-2.00</td>
<td>chr3</td>
<td>54666149</td>
<td>54673884</td>
<td>-</td>
<td>233.581</td>
<td>45</td>
<td>439.66</td>
</tr>
<tr>
<td>ENSG000000260834</td>
<td>-2.02</td>
<td>chr16</td>
<td>65224876</td>
<td>65268817</td>
<td>-</td>
<td>56.8772</td>
<td>7</td>
<td>96.663</td>
</tr>
<tr>
<td>ENSG000000273387</td>
<td>-2.07</td>
<td>chr22</td>
<td>31478142</td>
<td>31479551</td>
<td>-</td>
<td>3.50702</td>
<td>29</td>
<td>1.8962</td>
</tr>
<tr>
<td>ENSG000000255021</td>
<td>-2.08</td>
<td>chr3</td>
<td>14313873</td>
<td>14345345</td>
<td>-</td>
<td>14.2297</td>
<td>64</td>
<td>26.088</td>
</tr>
<tr>
<td>ENSG000000100181</td>
<td>-2.08</td>
<td>chr22</td>
<td>17082777</td>
<td>17179632</td>
<td>+</td>
<td>20.2850</td>
<td>84</td>
<td>40.960</td>
</tr>
<tr>
<td>ENSG000000236824</td>
<td>-2.12</td>
<td>chr2</td>
<td>47558199</td>
<td>47571656</td>
<td>+</td>
<td>12.5237</td>
<td>26</td>
<td>24.176</td>
</tr>
<tr>
<td>ENSG000000224081</td>
<td>-2.12</td>
<td>chr1</td>
<td>95104017</td>
<td>95285837</td>
<td>-</td>
<td>59.7651</td>
<td>8</td>
<td>112.70</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>Chromosome</td>
<td>Start Position</td>
<td>End Position</td>
<td>Expression Level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
<td>----------------</td>
<td>--------------</td>
<td>-----------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000225383</td>
<td>chr10</td>
<td>10826400</td>
<td>10836943</td>
<td>33.8832</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2.15</td>
<td>chr10</td>
<td>124869633</td>
<td>124904345</td>
<td>5.0390</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-285420.2</td>
<td>chr10</td>
<td>142688379</td>
<td>142688925</td>
<td>1.71771</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNX18P16</td>
<td>chr10</td>
<td>231658134</td>
<td>231664302</td>
<td>4.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-147C22.8</td>
<td>chr10</td>
<td>76653619</td>
<td>76698911</td>
<td>3.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-54A9.1</td>
<td>chr10</td>
<td>94218480</td>
<td>94241000</td>
<td>8.6134</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDHA4</td>
<td>chr10</td>
<td>14921335</td>
<td>14922332</td>
<td>7.4155</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-879F14.2</td>
<td>chr10</td>
<td>59252979</td>
<td>59274149</td>
<td>4.6269</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-334E6.3</td>
<td>chr11</td>
<td>119243416</td>
<td>119252323</td>
<td>7.4735</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC006262.5</td>
<td>chr11</td>
<td>46692423</td>
<td>46706340</td>
<td>2.6412</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-96C23.12</td>
<td>chr11</td>
<td>88786941</td>
<td>88872324</td>
<td>1.6392</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-993823.3</td>
<td>chr12</td>
<td>28111450</td>
<td>28122746</td>
<td>2.6412</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNHG9</td>
<td>chr16</td>
<td>2014960</td>
<td>2015510</td>
<td>5.08295</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTD-2354A18.1</td>
<td>chr18</td>
<td>70992176</td>
<td>71017113</td>
<td>7.45266</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-394J1.2</td>
<td>chr12</td>
<td>96616575</td>
<td>96617751</td>
<td>11.3985</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC005618.1</td>
<td>chr5</td>
<td>140699661</td>
<td>140700339</td>
<td>3.83701</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LINCO00707</td>
<td>chr10</td>
<td>6821560</td>
<td>6884868</td>
<td>3.58438</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMT25AH</td>
<td>chr10</td>
<td>47740330</td>
<td>47747200</td>
<td>15.1145</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-527N22.2</td>
<td>chr8</td>
<td>37262957</td>
<td>37264242</td>
<td>3.75136</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP11-277P12.10</td>
<td>chr12</td>
<td>10485460</td>
<td>10490891</td>
<td>6.18079</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP13-</td>
<td>chr10</td>
<td>5636954</td>
<td>5638081</td>
<td>18.6134</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table S2: Comparison of FAM83H-AS1 RPKM (Reads Per Kilobase Million) with other previously published functional LncRNAs

<table>
<thead>
<tr>
<th>LncRNA</th>
<th>ENSEMBL ID</th>
<th>Avg. RPKM GFP</th>
<th>Avg. RPKM 16E6</th>
<th>Known function</th>
</tr>
</thead>
<tbody>
<tr>
<td>XIST</td>
<td>ENSG00000229807</td>
<td>8.137879</td>
<td>12.21193233</td>
<td>miRNA sponge in cervical cancer (Zhu et al 2018)</td>
</tr>
<tr>
<td>HOTAIR</td>
<td>ENSG00000228630</td>
<td>1.817852513</td>
<td>1.5785181</td>
<td>miRNA sponge in cervical cancer (Liu et al 2018)</td>
</tr>
<tr>
<td>NEAT1</td>
<td>ENSG00000245532</td>
<td>34.23374033</td>
<td>27.70111733</td>
<td>miRNA sponge in cervical cancer (Han et al 2018)</td>
</tr>
<tr>
<td>TINCR</td>
<td>ENSG00000223573</td>
<td>16.26229083</td>
<td>16.07113433</td>
<td>miRNA sponge in gastric cancer (Chen et al 2017) and breast cancer (Liu et al 2018)</td>
</tr>
<tr>
<td>H19</td>
<td>ENSG00000130600</td>
<td>3.3026914</td>
<td>15.14256633</td>
<td>miRNA sponge in cervical cancer (Ou et al 2018)</td>
</tr>
</tbody>
</table>
Table S3: Primers used in this study for qRT-PCR.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM83H-AS1 FWD</td>
<td>TCCCAATAAACAGGGCAGAC</td>
</tr>
<tr>
<td>FAM83H-AS1 REV</td>
<td>CAAGATCACCACACCCCTCT</td>
</tr>
<tr>
<td>Gapdh FWD</td>
<td>CCACTCCTCCACCTTTTGAC</td>
</tr>
<tr>
<td>Gapdh REV</td>
<td>ACCCTGTGTGCTGAGCCA</td>
</tr>
<tr>
<td>UBC FWD</td>
<td>GATTTGGTGCGAGGCTTCTTG</td>
</tr>
<tr>
<td>UBC REV</td>
<td>CCTTATCTGCGATCTTGGCTT</td>
</tr>
<tr>
<td>Beta-Actin FWD</td>
<td>AGCACAGACGCCTGCCTTT</td>
</tr>
<tr>
<td>Beta-Actin REV</td>
<td>CCACGATGGAGGGGAAGAC</td>
</tr>
<tr>
<td>U6 FWD</td>
<td>GTGCTCGCTTCGCGACACATAT</td>
</tr>
<tr>
<td>U6 REV</td>
<td>AAAAATAGGAAAGCCTTACGAA</td>
</tr>
<tr>
<td>C. Elegans ama-1 FWD</td>
<td>GGAGCTCGAGTGAGATCTTGC</td>
</tr>
<tr>
<td>C. Elegans ama-1 REV</td>
<td>GCGCAGAGAGTATCCTGGA</td>
</tr>
<tr>
<td>HPV-16E6 FWD</td>
<td>AATGTTCAGGACCCACAGG</td>
</tr>
<tr>
<td>HPV-16E6 REV</td>
<td>ACTGTTGCTTCAGTACACACA</td>
</tr>
<tr>
<td>HPV-16E7 FWD</td>
<td>ACAAGCAGAAACCAGGACAGAG</td>
</tr>
<tr>
<td>HPV-16E7 REV</td>
<td>GCCCATTAACAGGTCTTCCA</td>
</tr>
<tr>
<td>p53 FWD</td>
<td>TTTGGTCTTTCGAACTTCTTG</td>
</tr>
<tr>
<td>p53 REV</td>
<td>CCACAAAACACACCAGTGC</td>
</tr>
<tr>
<td>p300 FWD</td>
<td>AGCGGCTAAAATCTCTACCTC</td>
</tr>
<tr>
<td>p300 REV</td>
<td>CACCATTGGTGTTCACACTC</td>
</tr>
<tr>
<td>FAM83H FWD</td>
<td>CGACAAGTGGCGGTCAACC</td>
</tr>
<tr>
<td>FAM83H REV</td>
<td>ACTTCCCCAGTGGCAGTAG</td>
</tr>
<tr>
<td>H19 FWD</td>
<td>AGTGGAAGGAGTGGACCTGTAT</td>
</tr>
<tr>
<td>H19 REV</td>
<td>CTCCGGAAAGCTTACACTCC</td>
</tr>
<tr>
<td>MIR205HG FWD</td>
<td>TCATTAAAGAGAGAAATACTATTCA</td>
</tr>
<tr>
<td>MIR205HG REV</td>
<td>TAAAGCACCAGTGGCAG</td>
</tr>
<tr>
<td>Gene</td>
<td>FWD Sequence</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>HOXC-AS5</td>
<td>CTCCCTGGAGCAGTACACCTG</td>
</tr>
<tr>
<td>GAS5</td>
<td>CTTCTGGGCTCAAAGTGATCCT</td>
</tr>
<tr>
<td>LINC00963</td>
<td>AACTGCCCTTGGGAAGCAAGTAG</td>
</tr>
<tr>
<td>SNHG15</td>
<td>GCTGAGGTGACGGTCTCAA</td>
</tr>
<tr>
<td>MAFG-AS1</td>
<td>AGGACTCGGGAGGAAGATAAC</td>
</tr>
<tr>
<td>RP6-65G23.3</td>
<td>GAGATAGGAGGCCAATAAGTTC</td>
</tr>
<tr>
<td>GS1-600G8.5</td>
<td>ACTGTCCGGTTACTGAGGG</td>
</tr>
<tr>
<td>RP3-510D11.2</td>
<td>CCAGACCGACGGGACAGCG</td>
</tr>
<tr>
<td>RP11-479G22.8</td>
<td>TGGGAAACCTAAAAAACCTTAAAGC</td>
</tr>
<tr>
<td>LINC01057</td>
<td>GTGAATCTCTTGGAAGATGAGG</td>
</tr>
<tr>
<td>SFTA1P</td>
<td>CAGCATTCAGGTGGGCTTT</td>
</tr>
<tr>
<td>RP13-463N16.6</td>
<td>TTGGAAATCACCCTTTTCCACTT</td>
</tr>
<tr>
<td>AC007879.7</td>
<td>ACAGGGAGCCAGGACACC</td>
</tr>
</tbody>
</table>
XIII. Supplementary Methods

A. Cell Culture

Primary human foreskin keratinocytes (HEKa) were cultured as described by supplier (EpiLife® Medium supplemented with Human Keratinocyte Growth Supplement (HKGS)). Primary human cervical keratinocytes (HCK) and J2-3T3 mouse fibroblast feeders were cultured as described by Alison McBride's laboratory (NIH, Bethesda, MD) (55). HCK were maintained in F-media [3:1 F12:DMEM with 5% Fetal Bovine Serum (FBS), 0.4µg/mL Hydrocortisone, 5µg/mL Insulin, 8.4ng/mL Cholera Toxin, 10ng/mL Epidermal Growth Factor, 24µg/mL Adenine, 10U/mL Penicillin, 10ug/mL Streptomycin, 2mM L-Glutamine, Amphotericin B] containing 10µM Y-27632 ROCK Inhibitor (Tocris). HCK cells were co-cultured with J2-3T3 feeder cells rendered mitotically inactive in 8ug/mL Mitomycin C for 3 hours; these growth-arrested feeder cells were replenished every 3-4 days. J2-3T3 stock cells were cultured in Feeder media [Dulbecco's Modified Eagles Medium (DMEM), high glucose, 10% Newborn Calf Serum, 2mM L-Glutamine, 10U/mL Penicillin, 10ug/mL Streptomycin, Amphotericin B]. J2-3T3 feeder cells are sensitive to trypsin; HCK, J2-3T3 stock, and growth-arrested J2-3T3 cells were centrifuged to remove the trypsin when passaged. HPV-16 positive CaSki cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% FBS, Streptomycin-Penicilllin, HEPES Buffer, Amphotericin B. HPV-16 positive W12/20863 and W12/201402 and HPV-31b positive cervical (CIN-612) were co-cultured in E-media (1:1 DMEM:Ham's Nutrient Mix F12 medium supplemented with 5% FBS, 10mM HEPES Buffer, Penicillin-Streptomycin, Amphotericin B, 0.02µM Triiodothyronine, 0.4µg/mL Hydrocortisone, 0.1µg/mL Cholera Toxin, 5µg/mL Transferrin, 180µM Adenine, 5µg/mL Insulin ) with murine 3T3 fibroblast cells (3T3M) that were rendered mitotically inactive with 4ug/mL Mitomycin C for at least 2 hours. HPV-negative cervical C33A cells, HPV-18 positive cervical (HeLa), HPV-16 positive HNSCC cell lines UMSCC-47 and -104, HPV negative HNSCC cell line UMSCC-1, HEK293T, and 3T3M cells were all cultured in DMEM supplemented with 10% FBS, Penicillin-Streptomycin, HEPES Buffer, and Amphotericin B. To passage stock cells, 0.25% trypsin was utilized. Growth-arrested J2-3T3 and 3T3M fibroblasts were removed with Versene solution and gently pipetting, prior to
pelleting and when otherwise desired. Cells were cultured at 37°C in a humidified 5% CO2 cell culture incubator.

B. Generation of stable cell lines

Plasmids MSCV-N-GFP (Plasmid #37855), MSCV-N-16E6 (Plasmid #37875), and MSCV-N-16E7 (Plasmid #37881) were purchased from Addgene. For retroviral production, these plasmids were co-transfected with packaging (pCL-ECO) and envelope (pVSV-GF) plasmids into HEK293T cells using calcium phosphate co-precipitation. 12-16 hours later, HEK293T media was replaced with fresh target cell media. After 48-hour incubation, retrovirus-containing target cell media was harvested, filtered (0.45µm), and mixed with polybrene (4µg/mL final concentration) to increase infection efficiency. 24 hours before infection, co-cultured J2-3T3 fibroblasts were removed with Versene solution and gentle pipetting from HCK cells. HEKa and HCK cells were infected with recombinant retroviruses for 8 hours, virus was removed and replaced with fresh target cell media. Growth arrested J2-3T3 were added to infected HCK and replenished every 3 days. Cells were allowed to recover for 24 hours prior to 72-hour puromycin selection (HEKa-2.5µg/mL; HCK-3µg/mL). Cells were allowed to recover and expression of exogenous HPV-16 E6 and E7, as desired, was confirmed by RT-PCR (Fig. S1, Fig. S3).

C. Generation of HPV-16 positive cervical JAMM-16 cell line

According to Buck et al. protocol described previously (56), the HPV-16 insert was cut out of pBR322 HPV-16 plasmid by performing a restriction digestion of two separate 25ug plasmid reactions in 225uL each with the restriction enzyme BamHI (NEB) according to manufacturer's recommended conditions (37°C for 2 hours). Digested samples were then PCR purified by QIAquick® PCR Purification Kit (Qiagen) resuspended in 200uL Buffer TE. Purified samples were then ligated under dilute conditions (9mL total volume) by adding 1X Ligase Buffer and 6uL of high concentration (2m U/mL) T4 DNA Ligase (NEB) and incubated at 16°C overnight. <25ug in 9mL ligation reactions were conducted to avoid concatemer formation. The ligated samples were then treated with 4.5mL of 7.5M Ammonium acetate and mixed. 35mL of 95% ethanol was added to each, tubes were mixed, and incubated at 4°C overnight. The next day, the samples were
brought back to room temperature and centrifuged at ~5,000 x g at room temperature for 1 hour. Pellets were washed with 70% ethanol, spun briefly, and washed again with 70% ethanol. The pellets were spun one last time to remove any residual ethanol, then air dried for several minutes. Pellets were resuspended in 100uL Buffer TE each, pellets were combined, and Nanodrop was used to calculate concentration retrieved. The entire product was run in 1% agarose gel for 3 hours. Desired band [expect to see supercoiled and relaxed circular bacterial backbone (~1.8 and ~3.2 kb) and supercoiled and relaxed circular (nicked) HPV genome (~6.2 and >16 kb)] was cut out and gel extracted with QIAquick® Gel Extraction Kit (Qiagen). Before transfection of the HPV-16 genomes, growth-arrested J2-3T3 fibroblasts were removed by Versene solution and pipetting from co-cultured primary normal cervical keratinocytes (HCK). HCK were plated in a 6-well plate on day prior to transfecting according to manufacturer’s protocol for Lipofectamine LTX (Invitrogen). On day of transfection, HCK were at about 70% confluency. Media was replenished on each well. For each well, 2.8ug of re-ligated HPV-16 insert was diluted in 150uL Opti-MEM I Reduced Serum Media with 2.5uL PLUS Reagent, 15uL Lipofectamine LTX was diluted in Opti-MEM I Reduced Serum Media. Diluted DNA and diluted Lipofectamine LTX/Plus complex were combined and incubated for 5 minutes at room temperature prior to adding to well. The transfected cells were passaged at least 10-15 times so HPV-16 immortalized cells could overgrowth the untransfected HCK cells prior to experimentation.

D. RNA Extraction

Total RNA of cultured cell lines and human tissues was purified with TRIzol reagent (Invitrogen) and treated with Turbo DNA-free DNase (20 minutes at 37°C, Ambion) according to manufacturers’ protocols. RNA concentration was determined with a Nanodrop 2000 Spectrophotometer (Thermo Scientific).

E. cDNA synthesis, qualitative or quantitative RT-PCR

RNA was reverse transcribed to complementary DNA (cDNA) under standard iScript cDNA synthesis kit (Bio-Rad) instructions in T100™ Thermal Cycler (Bio-Rad). Exogenous spike of C. Elegans (70ng) was added for normalization of results if GAPDH mRNA levels were altered
during processing (cellular fractionation). RT-PCR or qRT-PCR using SsoAdvanced™ Universal SYBR® Green enzyme was performed according to manufacturer's protocol (Bio-Rad) in CFX Connect™ Real-Time System (Bio-Rad). The oligonucleotide primer sequences can be found in Supplementary Table S3. GAPDH was primarily used as housekeeping gene, however Ubiquitin C (UBC) or Glucuronidase Beta (GUSB) were used if GAPDH mRNA levels were altered with significant apoptosis (siRNA FAM83H-AS1 vs. siRNA-CTRL) and ama-1 was used for cellular fractionation studies. To validate cell fractionation efficiency, U6 snRNA was used as nuclear control and mature mRNA beta-actin as cytoplasmic control. Qualitative RT-PCR product was resolved by agarose gel electrophoresis to visualize or quantitative RT-PCR (qRT-PCR) relative expression was calculated using the double delta CT method (relative expression = 2-ΔCT; where ΔCT=CT (Target RNA) - CT (mRNA endogenous GAPDH/UBC control or C. Elegans mRNA Ama-1 was exogenous spike control). Fold changes were calculated relative to control siRNA or the mean value of normal samples.

**F. Western Blot analysis**

Cell lysates were boiled in sample buffer, separated by SDS-PAGE, and transferred onto Immobilon-Fl polyvinylidene difluoride membranes. After blocking for 1 hour with 5% milk-TBST buffer (5% non-fat dry milk, 25mM Tris-HCl pH 8.0, 125mM NaCl, 0.5% Tween-20), the membranes were incubated with primary antibodies against human p53 (Cell Signaling, #9282) or Actin (C-11) (Santa Cruz, sc-1615) overnight at 4°C. The membranes were then washed with TBST, incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies [anti-goat IgG (Santa Cruz, sc-2020); anti-mouse IgG (Thermo Scientific)] for 1 hour at room temperature, washed with TBST, and the proteins were detected on the membrane using Pierce SuperSignal West Pico or Femto Maximum Sensitivity Substrate (Thermo Scientific) chemiluminescence.

**G. Transient transfection**

The following siRNAs were used to knock down FAM83H-AS1: Lincode Human FAM83H-AS1 siRNA - Set of 4 (Dharmacon, RU-188909-00-0002), Lincode Human FAM83H-
AS1 siRNA SMARTpool (Dharmacon, R-188909-00-000), Lincode Non-targeting siRNA #1 (Dharmacon, D-001320-01-05). Two different siRNA against HPV-16E6: 5’-GAGGUAUAUGACUUUGCUUTT-3’ (Dharmacon) and 5’-UCCAUAUGCUGUAUGUGAUTC-3’ (siRNA 209, Dharmacon), two different siRNA against p53 (NEB, #2011S; Thermo Scientific Dharmacon®, J-003329-16), two different ONTARGETplus Human siRNA against p300 (Dharmacon, J-003486-11-0002; Dharmacon, J-003486-12-0002), and CTLsiRNA (Ambion, AM16104) were used.

HCK, CaSki, and W12/201402 cells were transiently transfected using standard Lipofectamine® RNAiMAX protocol (siRNA FAM83H-AS1 and siRNA HPV-16E6 #1). W12/201402 and CaSki cells were transfected with reverse transfection protocol (siRNA HPV-16 E6 #2 to improve transfection efficiency. For standard transfection, cells were plated at appropriate confluence to be about 70% confluent for transfection 24 hours post-plating. Growth-arrested J2-3T3 fibroblasts were co-cultured with HCK, and on day of transfection removed with Versene solution and gently pipetting. On day of CaSki, W12/201402, and HCK standard transfection, media was replenished on each well. For each well, Opti-MEM I Reduced Serum Media was used to dilute Lipofectamine RNAiMAX (9uL per well of 6-well), as well as siRNA (40pmol per well of 6-well). Diluted RNAiMAX was combined with diluted siRNA, incubated 5 minutes at room temperature and added to each well. Cells were incubated 48 hours in media containing siRNA prior to harvesting, unless otherwise stated.

H. Cellular fractionation

Growth arrested 3T3M cells were removed by Versene solution and pipetting the day prior to fractionation for the W12/201402. Cellular fractionation protocol was modified from previous publication (57). On ice, CaSki (~70% confluent) were washed two times with cold PBS, scraped, pelleted, and resuspended in RSB Buffer (10-1.25mM Tris, pH 7.4; 10-1.25mM NaCl; 3-0.38mM MgCl2), incubated on ice for 5 minutes, centrifuged (1500rpm for 4 minutes at 4°C). RSB Buffer was removed and swollen pellets were resuspended in RSB-G40 Buffer [2.5-0.63mM Tris, pH 7.4; 2.5-0.63mM NaCl; 0.75-0.19mM MgCl2; 2.5-0.63% glycerol; 0.125-0.03% NP-40; 0.125-0.03mM DTT; 100U/mL RNasin Plus RNase Inhibitor (Promega)] in ≥ 4 times volume of pellet.
Cells were disrupted with Dounce homogenizer; homogenate was centrifuged (4000 rpm, 4 minutes) to pellet nuclear fraction. The cytoplasmic fraction (supernatant) was collected. The pelleted nuclear fraction was resuspended in RSB-G40 Buffer, 3.3% of sodium deoxycholate and 6.6% of Tween20 were added to the volume of RSB-G40 (10% of final volume was sodium deoxycholate and 10% was Tween 20), samples were tapped to mix, incubated on ice for 5 minutes, and pelleted by centrifugation (7000rpm, 3 minutes). The supernatant was collected and combined with first cytoplasmic fraction, and nuclear pellet was washed two times with RSB-G40 Buffer, and then resuspended in RSB-G40 Buffer. Fractionated cells were Turbo DNase I-treated, according to manufacturer's instructions (NEB), prior to RNA extraction to remove bound DNA and improve recovery of RNA. Total cell (second pellet), cytoplasmic, and nuclear RNA was extracted using TRIzol Reagent (Life Technologies) per manufacturer's instructions (Glycoblue was added during isopropanol precipitation step due to small amounts of RNA recovered from nuclear fraction) and treated with Turbo DNAfree DNase (Ambion) for 20 minutes at 37°C. qRT-PCR analysis was performed using cDNA generated using equal concentration of RNA with exogenous C. elegans RNA (70ng) added for normalization.

I. Cell proliferation assay

Cell proliferation was monitored using Cell Counting Kit - 8 (CCK-8) (Sigma-Aldrich). 24 hours (CaSki) or 48 hours (201402) after transfection with FAM83H-AS1 siRNA, CaSki cells were detached with 0.05% trypsin and W12/201402 cells were detached with 0.25% trypsin. After cell detachment, trypsin was inactivated with trypsin neutralizer (CaSki) or E-media containing serum (201402), pelleted, resuspended in appropriate media containing serum, and plated at desired concentration (3E3 cells per well and of four separate 96-well plates; technical triplicates). In equal density per well, 3T3M fibroblasts treated with mitomycin C were added to the W12/201402 cells and fibroblast alone control wells were plated. At desired time (48, 72, or 96h post-plating), CCK-8 dye (100uL/well) was added to each well, incubated for 2 hours (CaSki) or 3 hours (201402). Then, absorbance was read with a spectrophotometer (BioTek Synergy H1 Hybrid Reader) (OD$_{450}$) according to manufacturers' protocols. Absorbances of feeders alone were subtracted from W12/201402 co-cultured with feeders. Biological replicate was conducted.
**J. Flow-cytometric analysis of cell cycle.**

3T3M fibroblasts were stained with CellTrace™ Far Red Cell Proliferation Kit (Invitrogen) the day before plating transfected 201402 cells. 24 hours (CaSki) or 48 hours (201402) after transfection with FAM83H-AS1 siRNA, cells were detached with 0.05% trypsin (CaSki) or 0.25% trypsin (201402). After cell detachment, trypsin was inactivated with trypsin neutralizer (CaSki) or media containing serum (201402), and cells were plated at desired concentration (4E6 for CaSki and 2.3E6 for 201402) in 100mm dishes in media containing fetal bovine serum (FBS). Mitomycin C treated, far red stained 3T3M cells were added to the 201402 cells. Cells were allowed to attach (about 5 hours) then were washed 3 times with 1X D-PBS to remove residual FBS. Cells were then serum-starved for 24 hours. After incubation, cells were trypsinized with 0.25% trypsin, suspended in serum-free media (to avoid stimulation by serum), washed with 1X PBS, pelleted, and resuspended in PBS (200µL). Harvested cells were then fixed by adding 70% cold ethanol (1.8mL) while vortexing the cells and stored at 4°C until processing. Cells in ethanol were pelleted, washed with PBS, centrifuged, and resuspended in room temperature 0.2% Tween 20 in PBS (300-1,000µL, depending on pellet size). 100µL of each sample was placed in a U-bottom 96-well plate (3 wells/technical triplicates) and incubated 15 minutes at 37°C. PBS (100µL) was added to each well, cells were pelleted, resuspended in 10uL of RNase A-PBS (180µg/mL stock), tapped gently to mix, and incubated at room temperature for 15 minutes. After incubation, 20uL of PI-PBS (final concentration of 50µg/mL) was added to each well, pipetted to mix, and incubated for 15 minutes at room temperature. After incubation, the volume was brought up to 300uL and analyzed in the dark by flow cytometry (Fortessa S10). Gates were placed around 201402 population. Biological replicate was conducted.

**K. Transwell migration assay**

24 hours (CaSki) or 48 hours (201402) after transfection with FAM83H-AS1 siRNA, cells were detached with 0.05% trypsin. After cell detachment, trypsin was inactivated with trypsin neutralizer, pelleted, resuspended in serum-free media, and cells were plated [1E5 (CaSki) or 0.75E5 (201402), 200uL well, technical duplicates] in upper chambers of 24-well transwell (8mm pore size) with 800uL serum-free media in lower chamber. In equal density per well, 3T3M
fibroblasts treated with mitomycin C were added to the W12/201402 cells and fibroblast alone control wells were plated. Cells were then allowed to attach for ~3 hours prior to adding fetal bovine serum (final concentration of 20% for CaSki and 5% for W12/201402) to lower chamber. After 24 hours, media in upper chamber was replenished with appropriate fresh serum free media and lower chamber with appropriate fresh media containing with FBS chemoattractant. 48 hours after cells were plated, lower chamber/underside of transwell and upper chamber of transwell were washed with 25% D-PBS. D-PBS was removed and cells from the top of the upper chamber transwell membrane (non-migrated cells) were wiped away using a cotton swab. The lower chamber/underside of the transwell and upper chamber of transwell were washed again with 25% D-PBS and then the underside of the transwell (migrated cells) was fixed with 4% formaldehyde in 25% D-PBS in the lower chamber for 5 minutes at room temperature. The formaldehyde solution was removed and the lower chamber/underside of transwell and upper chamber of transwell were washed with 25% D-PBS. The cells on the underside of the transwell were then stained using 0.5% crystal violet in 20% ethanol in water. The stain was added to the lower chamber, submerging the transwell for 20 minutes at room temperature. The stain was removed and the lower chamber/underside of the transwell and upper chamber of transwell washed 3 times with 25% D-PBS. The top of the upper chamber transwell membrane was then cleaned with a cotton swab. The transwells were allowed to dry overnight and then the underside of the transwell imaged with an Olympus MVX10 microscope. Migration was quantitated with ImageJ. Feeders alone were subtracted from W12/201402 co-cultured with feeders. Biological replicate was conducted.

L. Flow-cytometric analysis of apoptosis

3T3M fibroblasts were stained with CellTrace™ Far Red Cell Proliferation Kit (Invitrogen) the day before plating transfected 201402 cells. 24 hours (CaSki) or 48 hours (201402) after transfection with FAM83H-AS1 siRNA, cells were detached with 0.05% trypsin (CaSki) or 0.25% trypsin (201402). After cell detachment, trypsin was inactivated with trypsin neutralizer (CaSki) or media containing serum (201402), pelleted, resuspended in appropriate media containing serum, and cells were plated in 100mm dishes at desired concentration (1.75E6 for CaSki and 1.1E6 for 201402). At 24, 48, and 72h later post-plating, attached and floating cells were collected. On day of harvest, media containing dead cells was collected, PBS was added to
the plates, collected, and added to the media. Attached cells were trypsinized with 0.25% trypsin, cells were pelleted (2 to 5E6) at 4°C, resuspended in PBS, and 100uL of each sample was aliquoted into 3 wells of a 96 well plate (technical triplicates). Cells were pelleted, PBS was removed, Annexin V Binding Buffer (100uL; diluted with autoclaved milliQ water) was added to each tube, Annexin V-FITC (5uL) was added to each tube, and incubated in the dark at room temperature for 10 minutes. After incubation, cold Binding buffer (200uL) was added to each tube, cells were pelleted, washed with cold Binding buffer two times, incubated on ice for 5 minutes in propidium iodine in Binding buffer (2ug/mL final concentration, 300uL), and analyzed by flow cytometry (Fortessa S10) immediately. Gates were placed around 201402 population. Biological replicate was conducted.

**M. Human cervical tissue specimen**

10 human specimens were obtained from patients under the tissue collection protocol [Prognostic Marker (IRB0406147)] at the University of Pittsburgh. Patient samples were categorized as cervical intraepithelial neoplasia stage 3 (CIN3), invasive cervical cancer (CaCx), or non-cancerous.

**N. TCGA Analysis**

The Cancer Genome Atlas (TCGA) (https://cancergenome.nih.gov/) contains patient survival and RNA sequencing data from 196 cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) human patient samples. TCGA-CESC reads per kilobase million (RPKM) data was extracted, and average RPKM for all patients combined was calculated. TCGA also contains RPKM data from 3 individual non-cancerous cervical samples that were averaged together to compare to CESC RPKM values. The Atlas of non-coding RNA in Cancer (TANRIC) (MD Anderson Cancer Center) (54) that utilizes TCGA-CESC dataset to characterize the expression profiles of long non-coding RNAs (lncRNAs) was used to extract FAM83H-AS1 expression in each of the TCGA-CESC patient samples. Classification and Regression Trees (CART) analysis was used to statistically define high/low lncRNA expression groups. The cut-off z score value was 3 to discern between high and low expression groups. Survival curves were
estimated by the Kaplan-Meier method. The log-rank test was used to estimate statistical differences between survival curves. GraphPad software was used to make the survival plots.

O. Statistics

Student's t-test was utilized to determine mean values differences between groups examined and significance was determined at p ≤ 0.05 (*) and p ≤ 0.01 (**). Scale bars represent Standard Deviations (SD).

XIV. Supplemental References


11. Moody C. Mechanisms by which HPV Induces a Replication Competent Environment in Differentiating Keratinocytes. Viruses 2017;9


22. Sharma S, Munger K. Expression of the cervical carcinoma expressed PCNA regulatory (CCEPR) long noncoding RNA is driven by the human papillomavirus E6 protein and modulates cell proliferation independent of PCNA. Virology 2018;518:8-13


28. Lu S, Dong W, Zhao P, Liu Z. IncRNA FAM83H-AS1 is associated with the prognosis of colorectal carcinoma and promotes cell proliferation by targeting the Notch signaling pathway. Oncology letters 2018;15:1861-8


42. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. International journal of cancer 2017;141:664-70


Tayvia B. Brownmiller
PhD Candidate, West Virginia University School of Medicine
Office: 1 Medical Center Dr. (304) 581-1901
PO Box 9300
Morgantown, WV 26505
tabrownmiller@mix.wvu.edu

Home: 3092 Abbey Cove (870) 866-3123
Benton, AR 72015
(permanent)

Education
West Virginia University School of Medicine, Morgantown, WV 08/2013 – 05/2020
PhD in Biomedical Sciences – Cancer Cell Biology
Current GPA: 3.73
PhD conferral: 05/2020

Henderson State University, Arkadelphia, AR 08/2009 – 05/2013
Bachelor of Science in Biology
Final GPA: 3.44

Research Experience
West Virginia University School of Medicine 08/2013 – 05/2020
Principal Investigator: Ivan Martinez, PhD
Project: The Importance of the Lnc-SPRY3 Family in Non-Small Cell Lung Cancer Therapeutic Response

Henderson State University Spring 2012 – Spring 2013
Mentor: Martin Campbell, PhD
Project: Nicotinic Acid Ester-Based Ionic Liquids

Baylor College of Medicine Summer 2012
Summer Medical and Research Training (SMART) Program
Mentor: Hoang Nguyen, PhD
Project: Sox9 and Sox11 Inhibit Differentiation of Skin Epithelial Cells

Relevant Skills
Laboratory-based:
RNA isolation and purification
Mammalian cell culture
RT-PCR/qRT-PCR analysis
Northern Blotting
Western Blot analysis
Immunoprecipitation
Cloning

Bacterial Cell Culture/plasmidpurification
Xstrahl Xenex cell irradiation
RNAi techniques
CRISPR
Retro/Lentiviral transduction
Guava easycyte flow cytometry
Cellular Fractionation
Mouse Based:
- Cell line derived xenografts
- Xstrahl Xenex small animal irradiation
- Tumor ultrasound imaging via Vevo 2100

Other:
- Microsoft Office
- ImageJ and GraphPad Prism
- Scientific writing/presentation
- Mentoring undergraduate/fellow graduate students

Publications


Hayes, K.E., Barr, J.A., Harold, A., Brownmiller, T., Wilusz, J.E., Martinez, I. Discovery of Novel Non-Coding Circular RNAs Generated by High-Risk Human Papillomaviruses. (Manuscript in preparation)

Honors/Awards
Awards:
- Graduate:
  o First Place – Poster: Basic Science, 2019 WVU Cancer Institute Annual Meeting
  o WVU Biomedical Sciences PhD Programs – Cancer Cell Biology Graduate Student of the Year Award 2019
  o 2019 WVU Three Minute Thesis Competition Finalist
  o WVU Office of Research and Graduate Education, Robert C. Byrd Health Sciences Center Doctoral Student Travel Award – Spring 2019
  o Second Place – Poster: Clinical Science, 2018 WVU Cancer Institute Annual Meeting
  o WVU Office of Research and Graduate Education, Robert C. Byrd Health Sciences Center Doctoral Student Travel Award – Spring 2018
  o Outstanding Graduate Student Award – 2016 Team Award, Mad Scientists ACS Relay for Life Team
  o First Place – Poster: Basic Science 2013-Group 2, 2015 Van Liere Memorial Convocation & HSC Research Day
- Undergraduate:
  o Arkansas Academy of Science Undergraduate Research Award
  o Arkansas Space Grant Consortium Undergraduate Research Grant
  o Poster – American Chemical Society Excellence in Undergraduate Polymer Research
Scholarships:
- Henderson State University Presidential Scholarship: Fall 2009 – Spring 2013
- Arkansas Academic Challenge Scholarship: Fall 2010 – Spring 2013
- Parkers Chapel Scholarship: Fall 2009
- Henderson State University Match Fall: 2009
- Hartsell Pest Control Scholarship: Fall 2009
- Academic Honor Roll: Fall 2009 – Spring 2013

Memberships
National:
- RNA Society 2019 - Present
- American Association of University Women (AAUW) 2018 - Present
- American Association for the Advancement of Science (AAAS) 2015 – Present
- American Association for Cancer Research (AACR) 2015 – Present
- AACR Women in Cancer Research 2015 – Present

West Virginia University:
Graduate Student Organization Fall 2013 – Present
- President: Summer 2015 – Summer 2016
- Vice President: Fall 2014 – Summer 2015
Cell Biology Training Program Fall 2014 – Present
First Year Student Mentorship Program Fall 2014 – Present

Henderson State University:
Biology Club Spring 2010 – Spring 2013
- President: Fall 2012- Spring 2013
Chemistry Club Spring 2011 – Spring 2013
Tri Beta Biological Honor Society Spring 2011 – Spring 2013
- President: Fall 2012 – Spring 2013
Gamma Beta Phi Academic Honor Society Spring 2011 – Spring 2013
American Chemical Society Fall 2012 – Spring 2013

Professional Development
Meetings/Conferences and Abstracts:
October 2019 - WVU Cancer Institute Annual Scientific Meeting, Morgantown, WV. Poster presented: “A Family of Y Chromosome LncRNAs is Involved in the Radiation Response of Male Non-Small Cell Lung Cancer Cells”


March 2019 – Van Liere Research Day: West Virginia University’s yearly celebration of the biomedical research being conducted at its Health Sciences Center campus. Flash Talk and
Poster presented: “The Importance of the Lnc-SPRY3 Family in Non-small Cell Lung Cancer Therapeutic Response.”


October 2018 – WVU Cancer Institute Annual Scientific Meeting, Morgantown, WV. Poster presented: “The Importance of the Lnc-SPRY3 Family in Non-small Cell Lung Cancer Therapeutic Response.”


March 2017 – Van Liere Research Day: West Virginia University’s yearly celebration of the biomedical research being conducted at its Health Sciences Center campus. Poster presented: “The Importance of the Lnc-SPRY3 Family in Non-small Cell Lung Cancer Therapeutic Response.”


March 2016 – Van Liere Research Day: West Virginia University’s yearly celebration of the biomedical research being conducted at its Health Sciences Center campus. Poster presented: “The Importance of the PRYP4 Long Non-coding RNA family in Non-small Cell Lung Cancer Radiation Response.”


October 2015 – “RNA and the New Genetics” Symposium at University of Toronto, Toronto, ON.

March 2015 - NIH Symposium “RNA Biology 2015” at NCI, Bethesda, MD.

February 2015 - Van Liere Research Day: West Virginia University’s yearly celebration of the biomedical research being conducted at its Health Sciences Center campus. Poster presented entitled “The Importance of miRNAs in the Cancer Resistant Animal Model: The Naked Mole-rat”

Other:
September 2019 – Workshop on Long-read Sequencing, The Jackson Laboratory for Genomic Medicine – JAX Genomic Medicine, Farmington, CT.

Spring 2019 – WVU Three Minute Thesis Competition, West Virginia University, Morgantown, WV.

Fall 2018 – Guest lecturer for first year graduate students Cellular Methods course: “In Vitro Methods of Genetic Manipulation”

Fall 2017 – Spring 2018: West Virginia University, Student representative to the Cancer Cell Biology Graduate Program faculty meetings

Fall 2015 – Spring 2016: West Virginia University PhD in Biomedical Sciences admissions committee member

June 2014 – “Setting the PACE” Writing Workshop by Laura Brady, Nathalie Singh-Corcoran, Andrea Bebell, and James Holsinger at West Virginia University Health Sciences Center, Morgantown, WV.

References
Ivan Martinez, PhD
Pl/Mentor
Associate Professor, Microbiology, Immunology & Cell Biology/WVUCI
West Virginia University School of Medicine
Phone: 304-581-1934 Email: ivmartinez@hsc.wvu.edu

Elena Pugacheva, PhD
Dissertation Committee Chair
Associate Professor, Biochemistry/WVUCI
West Virginia University School of Medicine
Phone: 304-293-5295 Email: epugacheva@hsc.wvu.edu