

## Differential expression and prognostic value of long non-coding RNA in HPV-negative head and neck squamous cell carcinoma

### Authors:

Salsal-UI Haque<sup>1</sup>, Liang Niu<sup>2</sup>, Damaris Kuhnell<sup>2</sup>, Jacob Hendershot<sup>2</sup>, Jacek Biesiada<sup>2</sup>, Wen Niu<sup>2</sup>, Matthew C. Hagan<sup>3</sup>, Karl T. Kelsey<sup>4,5</sup>, Keith A. Casper<sup>6</sup>, Trisha Wise-Draper<sup>1,7</sup>, Mario Medvedovic<sup>2</sup>, Scott M. Langevin<sup>2,†</sup>

### Affiliations:

<sup>1</sup> Department of Internal Medicine, Division of Hematology/Oncology, University of Cincinnati College of Medicine, *Cincinnati, OH*

<sup>2</sup> Department of Environmental Health, University of Cincinnati College of Medicine, *Cincinnati, OH*

<sup>3</sup> Department of Pathology & Laboratory Medicine, University of Cincinnati College of Medicine, *Cincinnati, OH*

<sup>4</sup> Department of Epidemiology, Brown University, *Providence, RI*

<sup>5</sup> Department of Pathology & Laboratory Medicine, Brown University, *Providence, RI*

<sup>6</sup> Department of Otolaryngology, University of Michigan, *Ann Arbor, MI*

<sup>7</sup> Department of Cancer Biology, University of Cincinnati College of Medicine, *Cincinnati, OH*

† Correspondence should be addressed to:

Scott M. Langevin, PhD, MHA

160 Panzeca Way, ML0056

Cincinnati, OH 45267

Tel: 513-558-1066/Fax: 513-558-4397

langevst@ucmail.uc.edu

### Funding Sources:

This work was supported by the National Cancer Institute (K22CA172358 to S.M.L) and National Institute for Environmental Health Sciences through the UC Center for Environmental Genetics (2P30ES006096).

**Word Count:** 2323

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of record](#). Please cite this article as [doi:10.1002/hed.25136](https://doi.org/10.1002/hed.25136).

## ABSTRACT

**BACKGROUND:** Long non-coding RNA (lncRNA) has emerged as a new avenue of interest due to its various biological functions in cancer. Abnormal expression of lncRNA has been reported in other malignancies but has been understudied in head and neck squamous cell cancer (HNSCC).

**METHODS:** lncRNA expression was interrogated via qRT-PCR array for 19 HPV-negative HNSCC tumor-normal pairs. The Cancer Genome Atlas (TCGA) was used to validate these results. The association between differentially expressed lncRNA and survival outcomes was analyzed.

**RESULTS:** Differential expression was validated for 5 lncRNA (*SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*). There was also an inverse association between *MEG3* expression (not significantly differentially expressed in TCGA tumors but highly variable expression) and 3-year relapse-free survival (RFS).

**CONCLUSION:** We identified and validated differential expression of 5 lncRNA in HPV-negative HNSCC. Low *MEG3* expression was associated with favorable 3-year RFS, although the significance of this finding remains unclear.

Key words:

Head and neck cancer; HNSCC; lncRNA; ncRNA; TCGA; survival

Author Manuscript

## INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer type worldwide and accounts for approximately 350,000 deaths per year <sup>(1,2)</sup>. Risk factors such as tobacco, alcohol use, and more recently, human papilloma virus (HPV) have been identified as etiological factors in the occurrence of HNSCC. Despite advances in the treatment of localized HNSCC, approximately half of patients will develop recurrent disease <sup>(3)</sup>, which is a major contributor to patient mortality. In addition, HPV-positive and HPV-negative HNSCC are biologically distinct with the latter being associated with poorer prognosis. Unlike other malignancies, there are no tools in widespread use that can identify early disease and there is no systematic approach that has proven effective in monitoring for early evidence of recurrence. Hence, novel markers are needed that appropriately characterize those patients with early stage disease as well as identify and characterize the response of individual patient's treatment.

While only 1.2% of our DNA sequence encodes proteins, approximately 75% of the human genome is capable of being transcribed into RNA <sup>(4)</sup>, and it has become increasingly apparent that RNA plays a diverse and important role in genome integrity through production of both proteins as well as non-coding RNA (ncRNA), including long non-coding RNA (lncRNA). While the microRNA (miRNA) class of ncRNA have been widely studied in the context of cancer, lncRNA, which are larger (> 200 bases and ~1-2 exons in length) <sup>(5)</sup> and have a more complex secondary/tertiary structure, have recently begun to garner increased attention <sup>(6)</sup>. This is largely due to their diverse biological functions <sup>(7)</sup>, which can include inhibition of target gene transcription, initiation of alternative splicing, generation of protein scaffolding and chromatin organization, and alteration of transcription factor activity.

Even though 18,000 human long non-coding human transcripts have been catalogued in GENCODE v7<sup>(4)</sup>, the lncRNA Database (<http://www.lncrnadb.org>), a database of functionally annotated eukaryotic lncRNA, only contains information for 127 human lncRNA, highlighting the gap in our knowledge of lncRNA biology. Identification of lncRNA transcripts that are associated with human diseases and the corresponding pathobiology (e.g. aggressiveness or responsiveness to treatment) would therefore provide a welcome means for prioritizing functional studies.

Several lncRNA have recently been reported to be differentially expressed in various cancers and to play a role in cancer growth, invasion, epithelial-to-mesenchymal transition, motility, and metastatic potential<sup>(8,9)</sup>, and as such are increasingly being recognized as having a very strong potential as cancer biomarkers<sup>(10)</sup>. Several recent studies have reported that higher expression of lncRNA, such as *HOTAIR* (*HOX antisense intergenic RNA*) and *Malat-1* (*metastasis associated lung adenocarcinoma transcript 1*), is associated with invasion and metastasis in various epithelial cancers<sup>(11-17)</sup>, although the prognostic significance of lncRNA in HNSCC has been understudied to date. The aim of this study was to identify differentially expressed lncRNA in HPV-negative HNSCC and assess its impact on outcomes in these groups of patients.

## MATERIALS & METHODS

### *Tumor Samples*

Initial identification of differentially expressed lncRNA in HPV-negative HNSCC was conducted using a quantitative real-time PCR (qRT-PCR) array consisting of 84 lncRNA with reported involvement in various cancers using paired archival formalin-fixed paraffin-embedded (FFPE) tumor tissue and paired adjacent normal squamous tissue from 19 patients treated for incident HNSCC at the University of Cincinnati Cancer Institute. Raw RNA-sequencing (RNA-seq) reads for all available HPV-negative head and neck cancer tumors (n = 444) and all available adjacent normal tissue (n = 44) was downloaded from The Cancer Genome Atlas (TCGA) for independent validation of significant results.

### *RT2 lncRNA qPCR Cancer Array*

Total RNA was extracted from each sample using the RNeasy FFPE kit (Qiagen, Valencia, CA), and converted to cDNA using the RT2 First Strand kit (Qiagen), according to the manufacturer's respective suggested protocols. The RT2 Profiler lncRNA qPCR Cancer PathwayFinderArray (96-well format) was used to profile expression of 84 lncRNA transcripts that have been associated with various cancer types. The array also contains 3 reverse transcription controls, 3 positive PCR controls, a probe for human gDNA contamination, and 5 housekeeping genes for normalization: *beta actin (ACTB)*; *beta-2 microglobulin (B2M)*; *Ribosomal protein, large, P0 (RPLP0)*; *7SK small nuclear RNA (RN7SK)*; and *Small nucleolar RNA, H/ACA box 73A (SNORA73A)*. PCR mastermix was prepared with 250ng of total cDNA and dispensed in 24 $\mu$ l aliquots into each RT2 lncRNA PCR Array well. RT-qPCR was performed using an Applied Biosystems StepOnePlus™ Real-Time PCR System (ThermoFisher Scientific, Waltham, MA)

under the following conditions: 10 min at 95°C using HotStart Taq Polymerase followed by 40 cycles of 15s at 95°C and 1min at 60°C. A complete list of the 84 lncRNA included on the array can be found in **Supplemental Table S1**.

#### *Differential Expression*

Expression was normalized to the geometric mean of 5 housekeeping control genes: *beta actin (ACTB)*; *beta-2 microglobulin (B2M)*; *Ribosomal protein, large, P0 (RPLP0)*; *7SK small nuclear RNA (RN7SK)*; and *Small nucleolar RNA, H/ACA box 73A (SNORA73A)*.

Differential expression of each lncRNA was described in terms of fold-change for tumor relative to adjacent normal tissue based on a one-sample t-test, adjusted for multiple testing by false discovery rate (FDR) estimation and  $Q$  values using the methods proposed by Benjamini & Hochberg (53). Expression was considered significantly differential where  $Q \leq 0.10$ .

#### *Replication Using TCGA RNA-Sequencing Data*

RNA-sequencing (RNA-seq) data in the form of bam files of aligned reads was obtained for head and neck cancers in TCGA project where downloaded from the Cancer Genomics Hub. Reads not mapped to human genome where aligned to HPV (NC\_001526) E6 and E7 viral oncoproteins using *bowtie* aligner<sup>(18)</sup>. HPV-status was inferred by designating the sample to be from a HPV-positive tumor if more than 1,000 reads mapped to HPV oncoproteins and HPV-negative tumor otherwise. The harmonized read counts for head and neck TCGA samples aligned to lncRNA defined in the GDC.h38 GENCODE v22 GTF file were downloaded from NCI Genomic Data Commons<sup>(19)</sup> using the *TCGAbiolinks* Bioconductor package<sup>(20)</sup>.

Normalized counts (count per million) for each of the 7 lncRNA in each sample were calculated using the *cpm* function in the Bioconductor package *edgeR*<sup>(21)</sup>. Since the distributions of lncRNA expression were non-linear, differential expression was assessed non-parametrically, using the Wilcoxon rank sum test, comparing tumor expression (n = 444) to that of all available normal samples (n = 44), and was considered differential where  $p \leq 0.05$ . Median fold-change was determined for each tumor by comparing its expression to the median expression of the normal samples. A description of the distribution of expression values for the 7 lncRNA in the 44 adjacent normal samples is provided in **Supplemental Table S2**.

#### *Survival Analysis Using TCGA Samples*

To visualize 5-year overall survival (OS) and 3-year relapse-free survival (RFS), univariate Kaplan-Meier and cumulative incidence (to account for death as a competing risk<sup>(22, 23)</sup>) functions were generated, respectively, comparing curves for low, normal, and high expression levels. Discrete multivariable Cox proportional hazards and cumulative incidence models were fit to assess 5-year OS and 3-year RFS, respectively, for each of the significant lncRNA, adjusted for age, sex, race, smoking status, tumor site, and stage at diagnosis, as established *a priori*. Missing values of model covariates were imputed (m = 20) using multivariate normal regression, based on age, sex, stage, and primary tumor site. Log-log plots [i.e.  $-\log(-\log(S(t)))$  vs.  $\log(t)$ ] were generated for each model to verify that the proportional hazards assumption was met. Statistical analyses were conducted using Stata 13 (Stata Corp, College Station, TX). All statistical tests were two-sided, and significance was considered when the unadjusted  $p \leq 0.05$ .

## RESULTS

The median age for the UC cases (n = 19 tumor-normal pairs) was 64 years, 74% of which were male. UC and TCGA sets differed in terms of smoking status (p = 0.01), with fewer non-smokers in the UC set, but were comparable in terms of age, sex, race, primary tumor site, AJCC stage group, and tumor grade (**Table 1**). The majority of tumors in both sets originated in the oral cavity (63% and 66%, respectively), and presented at an advanced stage (III or IV).

Eleven of the lncRNA included on the array were not detected in any of the tumor or normal samples (**Supplemental Table S1**). Twenty lncRNA were significantly differentially expressed at a nominal p-value  $\leq 0.05$  (8 upregulated, 12 downregulated). After FDR adjustment, 7 lncRNA remained significantly differential ( $Q \leq 0.10$ : *SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*, *MEG3* and *TERC*; 4 upregulated, 3 downregulated; **Table 2**). Expression of these 7 lncRNA was also significantly differential for 5 of the 7 lncRNA in the TCGA validation set (*SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*). It should be noted, however, that although *MEG3* and *TERC* were not significantly differentially expressed in the TCGA validation set, there was wide variability in terms of expression in both directions (i.e. upregulation and downregulation) for individual tumors relative adjacent normal samples (**Figure 1B**). Interestingly, Black patients in the TCGA dataset were significantly more likely to exhibit high (> 2-fold) and low (< 0.5-fold) *MEG3* expression (**Table 3**).

The relationship between each of the 7 lncRNA and 5-year OS and 3-year RFS was assessed using the TCGA dataset. Amongst the 7 lncRNA identified, only differing levels of *Maternally Expressed 3 (MEG3)* had an impact on 3 year RFS. Patients with low *MEG3* expression (<0.5 fold change) were found to have a significantly lower 3-year RFS while higher *MEG3* expression (> 2 fold change) appeared to have better 3-year RFS, although this did not



reach statistical significance (**Figure 2 and Table 4**). We also analyzed the associations between the 7 lncRNA and clinical characteristics of HPV-negative HNSCC in the TCGA dataset and found that low *MEG3* expression was associated with locally advanced disease and low expression of *HANDS-2AS1* correlated with more locally advanced cancer, although this did not appear to have an impact on survival outcomes. Restriction of the differential expression analysis to the 44 tumor-normal pairs in the TCGA validation set yielded similar results (data not shown).

Author Manuscript

## DISCUSSION

There is increasing evidence that aberrant expression of lncRNA plays a role in the genesis and progression of HNSCC<sup>(24, 25)</sup>. Through our present study, we were able to identify and validate 5 differentially expressed lncRNA in HPV-negative HNSCC (*SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*). Further, we found that low expression of *MEG3* was associated with more favorable 3-year RFS, although the significance of this finding is unclear.

*MEG3* is a long non-coding RNA located at the *DLK-MEG3* locus on chromosome 14q32.3 and is reported to be a tumor suppressor gene, exerting its effect in part through interaction with tumor suppressor and master regulator p53<sup>(26, 27)</sup>. *MEG3* has been reported to be downregulated in multiple solid tumor types<sup>(28)</sup>, which is consistent with what we observed in our 19 HPV-negative HNSCC tumor-adjacent normal pairs. However, our observed association of low *MEG3* expression with better 3-year RFS is contrary to what has been reported for other solid tumor types<sup>(29)</sup> and therefore should be interpreted with caution. In particular, Jia and colleagues found that low expression of *MEG3* correlated with poorer outcomes in squamous cell carcinoma of the anterior tongue, and overexpression of *MEG3* inhibited cell proliferation and cell cycle progression in SCC-15 and Cal 27 tongue squamous cell carcinoma cell lines<sup>(30)</sup>.

While the other differentially expressed lncRNA transcripts in our study were not associated with OS or RFS in the TCGA head and neck tumors, they have been identified as potential markers for poor prognosis in other cancer types. *SPRY4-IT1*, which is located on the *SPRY4* gene, has been implicated in cell growth invasion and increased apoptosis<sup>(31)</sup>, and elevated expression of *SRPY4-IT1* has been associated with poorer outcomes in esophageal squamous cell carcinoma, which, like HPV-negative HNSCC, is also strongly associated with tobacco and alcohol<sup>(32)</sup>. Overexpression of *HEIH* has been reported in hepatocellular carcinoma,

where it is an independent predictor for recurrence and survival and interacts with the lysine methyltransferase and Polycomb Repressor Complex 2 member *Enhancer of Zeste Homolog 2* (*EZH2*)<sup>(33)</sup>. Upregulation of both *LUCAT1* and *LINC00152* have been associated with poorer outcomes for non-small cell lung cancer (NSCLC)<sup>(34, 35)</sup>, and *HAND2-ASI* has been reported to be upregulated in stage IVS neuroblastoma<sup>(36)</sup> but downregulated in metastatic hepatocellular carcinoma<sup>(37)</sup>, with the latter being more in-line with our narrative of lower expression of *HAND2-ASI* in HPV-negative HNSCC.

Strengths of our study include the comprehensive assessment of 84 cancer-associated lncRNA and our ability to access raw RNA-seq data from TCGA. This allowed the alignment of our data with annotated lncRNA sequences to validate our findings in an independent dataset and assess the impact of significantly differential lncRNA on overall and relapse-free survival, and to infer HPV-status by aligning to HPV16 E6/E7 viral mRNA transcripts. Our study also has several limitations, including the modest sample size of our initial discovery set, which may adversely impacted our power to detect smaller effect sizes, increasing the risk of false-negative results. However, our use of a stringent FDR-control and validation in an independent set of tumors using TCGA data yields high confidence in our significant results. Furthermore, since no adjustments were made for multiple comparisons in the survival analyses, we cannot rule out the spurious nature of the observed association between MEG3 and RFS. Additionally, use of archival FFPE tissue for our discovery set likely attenuated the lncRNA expression levels, which could reduce our sensitivity for detection of signal or more subtle differences in expression. Our use of the RNeasy FFPE kit, which is specifically engineered to maximize the integrity and downstream results for RNA extracted from FFPE<sup>(38)</sup>, helps mitigate this issue; it is also notable

that 5 of the 7 differentially expressed lncRNA identified with the array were replicated using RNA-seq data from fresh tissue, supporting the validity of our findings.

## CONCLUSION

Expression of lncRNA is dysregulated in HPV-negative HNSCC. Specifically, we have identified and validated 5 differentially expressed lncRNA: *SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*. Additional studies are needed to confirm the potential prognostic value of *MEG3* expression in HNSCC.

Author Manuscript

**REFERENCES**

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55(2):74-108.
2. Parkin DM, Muir CS. Cancer Incidence in Five Continents. Comparability and quality of data. *IARC Sci Publ* 1992(120):45-173.
3. Brockstein B, Haraf DJ, Rademaker AW, et al. Patterns of failure, prognostic factors and survival in locoregionally advanced head and neck cancer treated with concomitant chemoradiotherapy: a 9-year, 337-patient, multi-institutional experience. *Ann Oncol* 2004;15(8):1179-86.
4. Djebali S, Davis CA, Merkel A, et al. Landscape of transcription in human cells. *Nature* 2012;489(7414):101-8.
5. Pauli A, Rinn JL, Schier AF. Non-coding RNAs as regulators of embryogenesis. *Nature reviews Genetics* 2011;12(2):136-49.
6. Chandra Gupta S, Nandan Tripathi Y. Potential of long non-coding RNAs in cancer patients: From biomarkers to therapeutic targets. *Int J Cancer* 2016.
7. Cech TR, Steitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. *Cell* 2014;157(1):77-94.
8. Yang G, Lu X, Yuan L. LncRNA: A link between RNA and cancer. *Biochimica et biophysica acta* 2014.
9. Serviss JT, Johnsson P, Grander D. An emerging role for long non-coding RNAs in cancer metastasis. *Frontiers in genetics* 2014;5:234.
10. Qiu MT, Hu JW, Yin R, Xu L. Long noncoding RNA: an emerging paradigm of cancer research. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2013;34(2):613-20.
11. Gutschner T, Hammerle M, Eissmann M, et al. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. *Cancer research* 2013;73(3):1180-9.
12. Schmidt LH, Spieker T, Koschmieder S, et al. The long noncoding MALAT-1 RNA indicates a poor prognosis in non-small cell lung cancer and induces migration and tumor growth. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2011;6(12):1984-92.
13. Liu XH, Liu ZL, Sun M, Liu J, Wang ZX, De W. The long non-coding RNA HOTAIR indicates a poor prognosis and promotes metastasis in non-small cell lung cancer. *BMC cancer* 2013;13:464.
14. Nakagawa T, Endo H, Yokoyama M, et al. Large noncoding RNA HOTAIR enhances aggressive biological behavior and is associated with short disease-free survival in human non-small cell lung cancer. *Biochemical and biophysical research communications* 2013;436(2):319-24.
15. Zhao W, An Y, Liang Y, Xie XW. Role of HOTAIR long noncoding RNA in metastatic progression of lung cancer. *European review for medical and pharmacological sciences* 2014;18(13):1930-6.
16. Zhang J, Zhang P, Wang L, Piao HL, Ma L. Long non-coding RNA HOTAIR in carcinogenesis and metastasis. *Acta biochimica et biophysica Sinica* 2014;46(1):1-5.
17. Gutschner T, Hammerle M, Diederichs S. MALAT1 -- a paradigm for long noncoding RNA function in cancer. *Journal of molecular medicine* 2013;91(7):791-801.

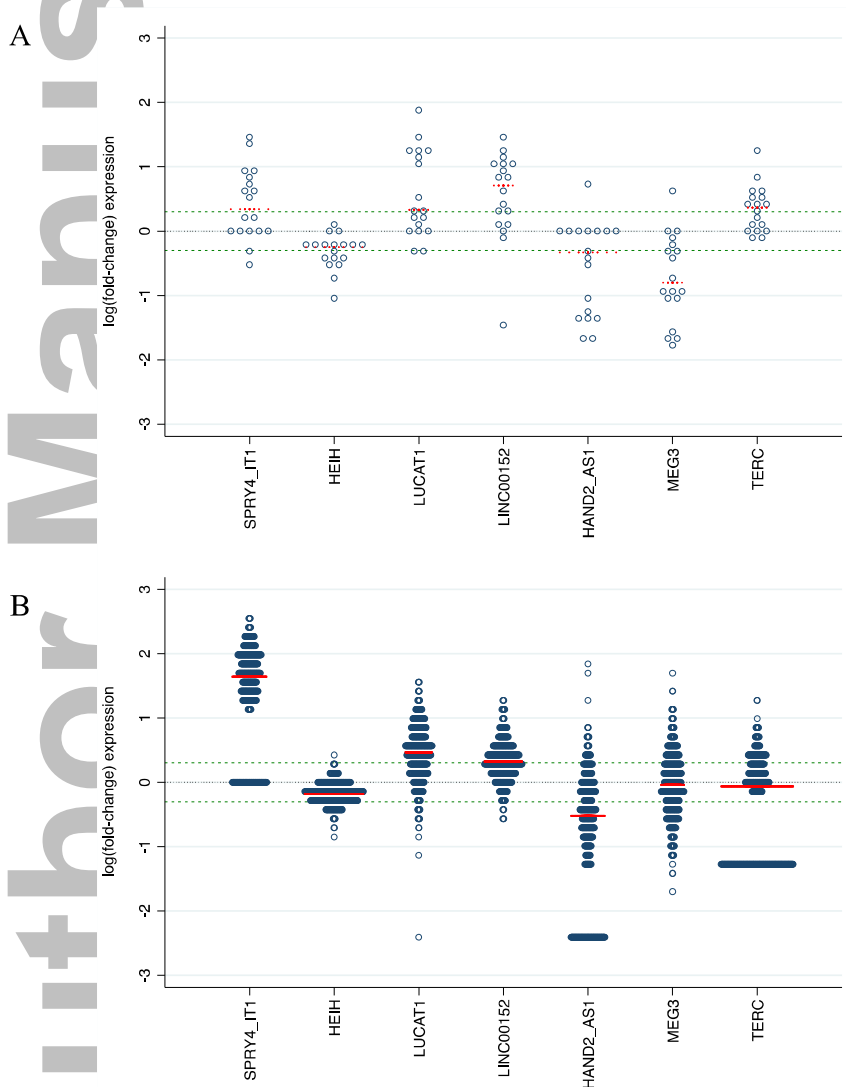
18. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* 2009;10(3):1-10.
19. Grossman RL, Heath AP, Ferretti V, et al. Toward a Shared Vision for Cancer Genomic Data. *New England Journal of Medicine* 2016;375(12):1109-1112.
20. Colaprico A, Silva TC, Olsen C, et al. TCGAAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res* 2016;44(8):e71.
21. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 2010;26(1):139-40.
22. Kim HT. Cumulative incidence in competing risks data and competing risks regression analysis. *Clin Cancer Res* 2007;13(2 Pt 1):559-65.
23. Satagopan JM, Ben-Porat L, Berwick M, Robson M, Kutler D, Auerbach AD. A note on competing risks in survival data analysis. *Br J Cancer* 2004;91(7):1229-35.
24. Nohata N, Abba MC, Gutkind JS. Unraveling the oral cancer lncRNAome: Identification of novel lncRNAs associated with malignant progression and HPV infection. *Oral Oncol* 2016;59:58-66.
25. Zou AE, Zheng H, Saad MA, et al. The non-coding landscape of head and neck squamous cell carcinoma. *Oncotarget* 2016;7(32):51211-51222.
26. Tang W, Dong K, Li K, Dong R, Zheng S. MEG3, HCN3 and linc01105 influence the proliferation and apoptosis of neuroblastoma cells via the HIF-1alpha and p53 pathways. *Sci Rep* 2016;6:36268.
27. Zhou Y, Zhong Y, Wang Y, et al. Activation of p53 by MEG3 non-coding RNA. *J Biol Chem* 2007;282(34):24731-42.
28. Zhou Y, Zhang X, Klibanski A. MEG3 noncoding RNA: a tumor suppressor. *J Mol Endocrinol* 2012;48(3):R45-53.
29. Cui X, Jing X, Long C, Tian J, Zhu J. Long noncoding RNA MEG3, a potential novel biomarker to predict the clinical outcome of cancer patients: a meta-analysis. *Oncotarget* 2017.
30. Jia LF, Wei SB, Gan YH, et al. Expression, regulation and roles of miR-26a and MEG3 in tongue squamous cell carcinoma. *Int J Cancer* 2014;135(10):2282-93.
31. Zou Y, Jiang Z, Yu X, et al. Upregulation of long noncoding RNA SPRY4-IT1 modulates proliferation, migration, apoptosis, and network formation in trophoblast cells HTR-8SV/neo. *PLoS One* 2013;8(11):e79598.
32. Zhang CY, Li RK, Qi Y, et al. Upregulation of long noncoding RNA SPRY4-IT1 promotes metastasis of esophageal squamous cell carcinoma via induction of epithelial-mesenchymal transition. *Cell Biol Toxicol* 2016;32(5):391-401.
33. Yang F, Zhang L, Huo XS, et al. Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. *Hepatology* 2011;54(5):1679-89.
34. Renhua G, Yue S, Shidai J, Jing F, Xiyi L. 165P: Long noncoding RNA LUCAT1 is associated with poor prognosis in human non-small cell lung cancer and affects cell proliferation via regulating p21 and p57 expression. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2016;11(4 Suppl):S129.

35. Chen QN, Chen X, Chen ZY, et al. Long intergenic non-coding RNA 00152 promotes lung adenocarcinoma proliferation via interacting with EZH2 and repressing IL24 expression. *Mol Cancer* 2017;16(1):17.
36. Voth H, Oberthuer A, Simon T, Kahlert Y, Berthold F, Fischer M. Identification of DEIN, a novel gene with high expression levels in stage IVS neuroblastoma. *Mol Cancer Res* 2007;5(12):1276-84.
37. Yang Y, Chen L, Gu J, et al. Recurrently deregulated lncRNAs in hepatocellular carcinoma. *Nat Commun* 2017;8:14421.
38. Belder N, Coskun O, Doganay Erdogan B, et al. From RNA isolation to microarray analysis: Comparison of methods in FFPE tissues. *Pathol Res Pract* 2016;212(8):678-85.

Author Manuscript

## FIGURES

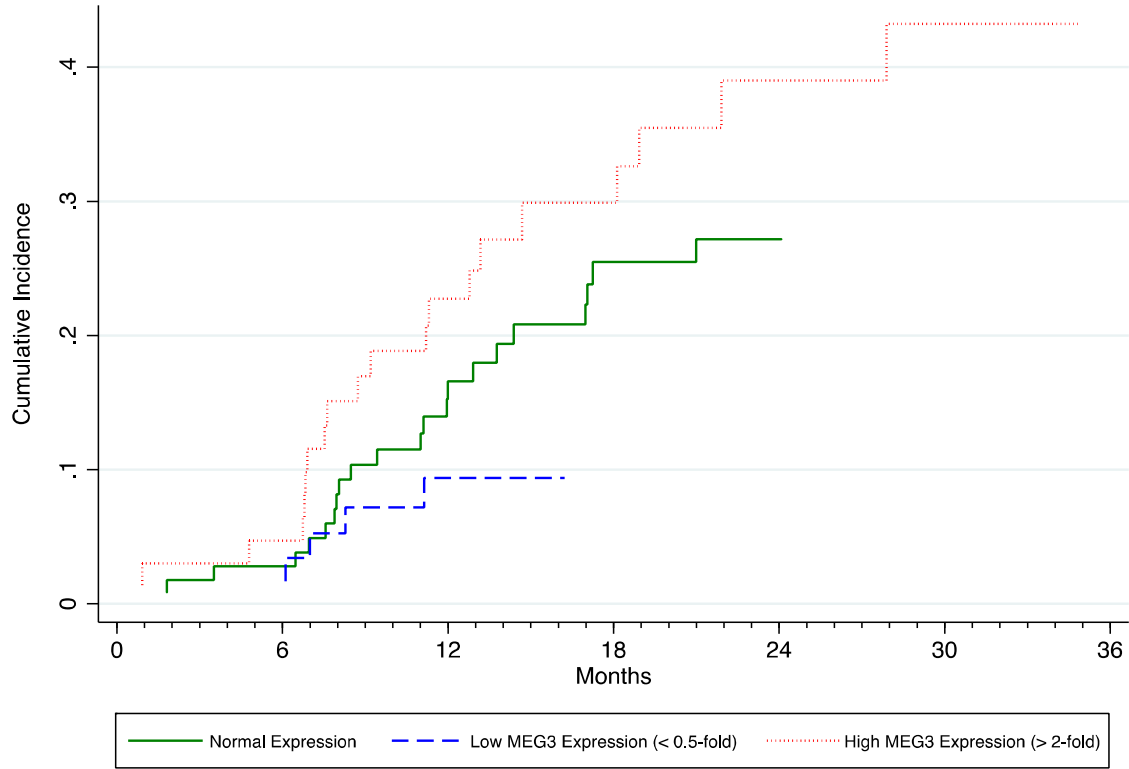
**Figure 1.** Distribution of log(fold-change) in HPV-negative head and neck squamous cell carcinoma (HNSCC) tumors relative to adjacent non-malignant squamous tissue for (A) University of Cincinnati patients (n = 19 tumor-normal pairs) and (B) HPV-negative tumors (n = 444) and adjacent normal tissue (n = 44) with RNA-sequencing data through The Cancer Genome Atlas (TCGA). The black dashed center line at 0 corresponds to neutral expression and blue dashed lines above and below the center line correspond to respective fold changes of 2.0 and 0.5 respectively; mean log(fold-change) for each lncRNA is denoted by a red dashed line.

Footnote:

Fold-change for the 19 tumors samples in panel A was calculated by comparing the expression of each tumor to its paired adjacent normal sample; fold-change for the 444 TCGA tumors in panel B was calculated by comparing the expression to the median expression of the 44 adjacent normal TCGA samples.



**Figure 2.** Cumulative incidence function for 3-year relapse-free survival of HPV-negative TCGA head and neck squamous cell carcinoma (HNSCC) tumors according to *MEG3* expression level.



Author

## TABLES

**Table 1.** Clinicodemographic characteristics of HPV-negative head and neck squamous cell carcinoma (HNSCC) cases from the University of Cincinnati and TCGA validation set.

	University of Cincinnati (n = 19)	TCGA Validation Set (n = 444)	P <sub>difference</sub>
Age			0.28 <sup>a</sup>
Years, median (range)	64 (51-86)	61.5 (19-90)	
Sex			0.83 <sup>b</sup>
Male	14 (74%)	317 (71%)	
Female	5 (26%)	127 (29%)	
Race			0.44 <sup>b</sup>
White	16 (84%)	355 (83%)	
Black	3 (16%)	44 (10%)	
Other	0 (0%)	30 (7%)	
Smoking Status			0.01 <sup>b</sup>
Never	1 (5%)	93 (21%)	
Former	15 (79%)	181 (42%)	
Current	3 (16%)	160 (37%)	
Primary Tumor Site			0.75 <sup>b</sup>
Oral cavity	12 (63%)	294 (66%)	
Pharynx	1 (5%)	41 (9%)	
Larynx	6 (32%)	109 (25%)	
AJCC Stage Group			0.68 <sup>b</sup>
I	0 (0%)	23 (5%)	
II	2 (11%)	74 (17%)	
III	5 (28%)	82 (18%)	
IV	11 (61%)	265 (60%)	
Tumor Grade			0.25 <sup>b</sup>
Well differentiated	6 (32%)	59 (13%)	
Moderately differentiated	9 (47%)	276 (63%)	
Poorly differentiated	4 (21%)	96 (22%)	
Undifferentiated	0 (0%)	2 (<1%)	
Undetermined	0 (0%)	8 (2%)	

<sup>a</sup> Wilcoxon Rank Sum test<sup>b</sup> Fisher's Exact testAbbreviations:

TCGA = The Cancer Genome Atlas; AJCC = American Joint Committee on Cancer  
 hrHPV = high-risk human papillomavirus

**Table 2.** Differentially expressed lncRNA in HPV-negative head and neck squamous cell carcinoma (HNSCC).

lncRNA	Ensembl ID	Human lncRNA qPCR Cancer Array (n <sub>T-N pairs</sub> = 19)			TCGA RNA-seq Replication Set (n = 444)	
		Median Fold- Change	p-value	Q-value	Median Fold-Change	p-value
<i>SPRY4-IT1</i>	ENSG00000281881	2.19	0.006	<b>0.07</b>	3.52	< 0.0001
<i>HEIH</i>	ENSG00000278970	0.56	0.0001	<b>0.01</b>	0.64	< 0.0001
<i>LUCAT1</i>	ENSG00000248323	2.14	0.002	<b>0.04</b>	1.76	< 0.0001
<i>LINC00152</i>	ENSG00000222041	5.11	0.003	<b>0.06</b>	1.56	< 0.0001
<i>HAND2-AS1</i>	ENSG00000237125	0.47	0.005	<b>0.06</b>	0.13	< 0.0001
<i>MEG3</i>	ENSG00000214548	0.16	0.0003	<b>0.01</b>	0.33	0.50
<i>TERC</i>	ENSG00000270141	2.32	0.0007	<b>0.02</b>	0.64	0.12

**Table 3.** Association between expression of significantly differential lncRNA and patients characteristics and clinical features in the TCGA HPV-negative head and neck squamous cell carcinoma (HNSCC) samples (n = 444).

	<i>SPRY4-IT1</i>		<i>HEIH</i>		<i>LUCAT1</i>		<i>LINC00152</i>		<i>HAND2-AS1</i>		<i>MEG3</i>		<i>TERC</i>	
	Low (n = 89)	High (n = 0)	Low (n = 92)	High (n = 2)	Low (n = 19)	High (n = 291)	Low (n = 6)	High (n = 291)	Low (n = 275)	High (n = 43)	Low (n = 134)	High (n = 107)	Low (n = 189)	High (n = 99)
N = 444														
Age, per decade	1.08 (0.87-1.34)	---	<b>0.75 (0.60-0.94)</b>	---	1.11 (0.65-1.88)	0.99 (0.82-1.21)	---	1.09 (0.83-1.43)	0.87 (0.70-1.08)	<b>0.67 (0.48-0.92)</b>	0.84 (0.68-1.04)	0.93 (0.74-1.16)	1.05 (0.86-1.28)	<b>1.40 (1.08-1.81)</b>
Sex														
Male	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Female	0.72 (0.41-1.26)	---	0.56 (0.30-1.06)	---	0.60 (0.15-2.44)	1.07 (0.64-1.78)	---	0.90 (0.46-1.77)	0.60 (0.35-1.01)	0.62 (0.26-1.47)	1.45 (0.84-2.50)	1.00 (0.55-1.81)	1.36 (0.81-2.30)	0.65 (0.33-1.28)
Race														
White	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Black	1.26 (0.49-3.23)	---	1.15 (0.51-2.58)	---	0.49 (0.06-4.40)	0.74 (0.36-1.50)	---	0.94 (0.35-2.54)	1.16 (0.51-2.64)	1.57 (0.47-5.30)	<b>3.34 (1.43-7.80)</b>	<b>2.85 (1.18-6.90)</b>	0.90 (0.41-1.99)	1.05 (0.43-2.57)
Other	0.97 (0.37-2.58)	---	<b>3.50 (1.47-8.35)</b>	---	0.88 (0.09-8.95)	1.41 (0.54-3.68)	---	0.81 (0.28-2.38)	0.90 (0.37-2.23)	---	0.74 (0.29-1.86)	0.41 (0.13-1.30)	0.85 (0.35-2.09)	1.00 (0.34-2.98)
Smoking status														
Never	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Former	1.27 (0.65-2.45)	---	1.12 (0.53-2.38)	---	0.73 (0.19-2.83)	0.67 (0.36-1.26)	---	0.60 (0.25-1.43)	0.59 (0.45-1.72)	0.54 (0.20-1.47)	1.64 (0.84-3.22)	0.68 (0.35-1.34)	1.57 (0.84-2.90)	1.59 (0.73-3.50)
Current	1.59 (0.77-3.30)	---	1.37 (0.65-2.91)	---	0.39 (0.07-2.20)	0.79 (0.40-1.54)	---	0.78 (0.30-2.00)	0.59 (0.29-1.20)	0.45 (0.16-1.24)	0.90 (0.44-1.85)	0.53 (0.26-1.08)	1.48 (0.77-2.84)	1.30 (0.56-3.03)
Primary tumor site														
Oral cavity	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Pharynx	1.34 (0.52-3.48)	---	0.53 (0.19-1.47)	---	3.20 (0.83-12.40)	0.61 (0.28-1.29)	---	<b>0.36 (0.14-0.92)</b>	1.08 (0.49-2.40)	0.52 (0.13-2.09)	1.29 (0.58-2.90)	0.92 (0.36-2.32)	1.15 (0.50-2.64)	1.73 (0.67-4.49)
Larynx	0.82 (0.43-1.54)	---	0.93 (0.50-1.74)	---	1.25 (0.31-5.08)	0.96 (0.55-1.66)	---	0.53 (0.26-1.06)	1.33 (0.74-2.41)	0.48 (0.16-1.47)	0.71 (0.39-1.30)	0.86 (0.46-1.62)	0.56 (0.31-1.01)	1.49 (0.77-2.88)
AJCC Stage Group														
Early (I or II)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Late (III or IV)	1.09 (0.52-2.28)	---	0.62 (0.28-1.37)	---	2.06 (0.42-10.13)	1.48 (0.78-2.82)	---	1.49 (0.65-3.42)	<b>2.86 (0.74-2.41)</b>	0.75 (0.20-2.81)	1.11 (0.56-2.18)	1.01 (0.48-2.14)	1.84 (0.94-3.60)	<b>0.39 (0.17-0.91)</b>
N classification														
N0	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
N1-3	<b>0.88 (0.47-1.65)</b>	---	1.65 (0.86-3.19)	---	0.47 (0.13-1.71)	0.82 (0.47-1.44)	---	1.25 (0.59-2.63)	<b>0.28 (0.15-0.54)</b>	1.31 (0.39-4.44)	<b>0.49 (0.28-0.88)</b>	0.72 (0.38-1.34)	0.97 (0.56-1.69)	<b>2.68 (1.26-5.69)</b>
Tumor grade														
Low	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Moderate	1.39 (0.66-2.92)	---	1.22 (0.56-2.64)	---	1.87 (0.20-17.04)	1.38 (0.73-2.58)	---	1.34 (0.58-3.13)	1.07 (0.52-2.19)	0.74 (0.25-2.19)	0.83 (0.41-1.66)	1.01 (0.48-2.19)	0.94 (0.48-1.85)	1.17 (0.49-2.81)
High/undifferentiated	0.64 (0.28-1.46)	---	0.67 (0.26-1.72)	---	7013 (0.78-64.74)	1.22 (0.58-2.58)	---	1.21 (0.45-3.28)	0.87 (0.38-1.96)	0.94 (0.28-3.21)	0.65 (0.29-1.47)	1.33 (0.31-1.85)	0.62 (0.28-1.35)	0.71 (0.26-1.90)

**Table 4.** Crude and adjusted hazard ratios (HR) and subhazard ratios (SHR) for the association between expression of differentially expressed lncRNA and 5-year overall survival (OS) and 3-year relapse-free survival (RFS), respectively, in patients with HPV-negative head and neck squamous cell carcinoma (HNSCC).

lncRNA expression	5-year Overall Survival (OS) (N = 439)			3-year Relapse-Free Survival (RFS) (N = 272)		
	n	Crude HR	Adjusted HR <sup>a</sup>	n	Crude SHR	Adjusted SHR <sup>a</sup>
<i>SPRY4-IT1</i>						
Low (<0.5-fold)	0	---	---	0	---	---
Normal <sup>b</sup>	87	1.00 (reference)	1.00 (reference)	54	1.00 (reference)	1.00 (reference)
High (>2-fold)	352	0.93 (0.62-1.38)	0.89 (0.60-1.34)	218	1.40 (0.63-3.12)	1.30 (0.54-3.13)
<i>HEIH</i>						
Low (<0.5-fold)	92	0.82 (0.54-1.28)	0.88 (0.56-1.36)	59	0.91 (0.45-1.84)	1.14 (0.54-2.40)
Normal <sup>b</sup>	345	1.00 (reference)	1.00 (reference)	212	1.00 (reference)	1.00 (reference)
High (>2-fold)	2	---	---	1	---	---
<i>LUCAT1</i>						
Low (<0.5-fold)	18	1.49 (0.67-3.32)	1.61 (0.71-3.68)	12	3.07 (0.84-11.19)	2.58 (0.71-9.33)
Normal <sup>b</sup>	131	1.00 (reference)	1.00 (reference)	80	1.00 (reference)	1.00 (reference)
High (>2-fold)	290	0.92 (0.64-1.31)	0.85 (0.60-1.23)	180	1.50 (0.74-3.02)	1.54 (0.70-3.41)
<i>LINC00152</i>						
Low (<0.5-fold)	8	---	---	7	---	---
Normal <sup>b</sup>	68	1.00 (reference)	1.00 (reference)	41	1.00 (reference)	1.00 (reference)
High (>2-fold)	363	0.84 (0.55-1.28)	0.74 (0.48-1.14)	224	1.32 (0.52-3.34)	1.11 (0.41-3.04)
<i>HAND2-AS1</i>						
Low (<0.5-fold)	271	0.92 (0.64-1.31)	0.96 (0.66-1.39)	167	0.87 (0.47-1.64)	0.83 (0.43-1.60)
Normal <sup>b</sup>	126	1.00 (reference)	1.00 (reference)	80	1.00 (reference)	1.00 (reference)
High (>2-fold)	42	0.63 (0.30-1.34)	0.71 (0.33-1.51)	25	0.94 (0.31-2.83)	0.82 (0.25-2.65)
<i>MEG3</i>						
Low (<0.5-fold)	133	1.09 (0.75-1.57)	1.11 (0.76-1.62)	71	<b>0.38 (0.14-1.00)</b>	<b>0.28 (0.10-0.78)</b>
Normal <sup>b</sup>	202	1.00 (reference)	1.00 (reference)	127	1.00 (reference)	1.00 (reference)
High (>2-fold)	104	0.88 (0.56-1.40)	0.84 (0.53-1.34)	74	1.63 (0.89-2.98)	1.43 (0.75-2.74)
<i>TERC</i>						
Low (<0.5-fold)	187	1.01 (0.69-1.47)	0.90 (0.61-1.33)	115	1.25 (0.62-2.51)	1.30 (0.61-2.79)
Normal <sup>b</sup>	155	1.00 (reference)	1.00 (reference)	97	1.00 (reference)	1.00 (reference)
High (>2-fold)	97	0.98 (0.62-1.54)	0.97 (0.60-1.55)	60	1.75 (0.82-3.72)	2.24 (0.95-5.25)

<sup>a</sup> Adjusted for age, sex, race/ethnicity, smoking status, primary tumor site, and AJCC stage group

<sup>b</sup> Tumor expression >0.5-fold and < 2-fold was considered to be within normal range

Protein	Symbol	HM0096	HM0126	HM0527	HM0888	HM0909	HM0732	HM0793	HM0859	HM0868	HM0888	HM1003	HM1028	HM1068	HM1129	HM1140	HM1155	HM1295	HM1332	avg(ncs/CI) avg(FC)	p-value				
E02	MEI1	-0.895960	0.6199582	-0.323381	1.460145	-1.360673	0.4019759	0	0.972228	-1.018872	-0.901083	-0.507986	1.623641	-0.163493	-0.113554	0.913074	0	-1.828137	-0.703777	-0.770161	0.017860	0.0026201			
E03	HEI1	-0.178421	-0.412056	-0.257119	-0.750704	-0.897212	1.0497575	-0.222588	0.0305669	-0.805977	-0.207706	-0.623881	0.1068203	-0.163486	-0.294038	-0.518767	-0.208730	-0.381345	-0.137659	-0.615426	-0.0062621	0.0016749			
G04	TEAC	-0.606863	0.6048789	0.5973259	0.4370497	0.2381967	0.52107287	0.4884649	0.1227027	0.2132525	0.8301784	-0.080558	0.526322	-0.0176007	-0.0479511	1.2362393	0.6730288	0.0186789	0.0679315	0.543671	2.2662261	0.0016749			
D12	LKCAT1	1.007801	1.024860	1.2629751	1.2662209	1.2448191	0.7120722	0.1309744	-0.593377	-0.3182164	0	0.018227	0.0206162	0.2305001	0.0221517	1.6431199	0.9598887	0.4068403	0.5735257	1.748888	0.0238436	0.0016749			
D03	LINC0152	1.045184	-1.310463	0.6025914	0.2019475	0.2701164	0.8146165	1.1084863	0.6017855	1.020221	0	0.8917895	1.6164341	0.10027474	1.08835	-1.434127	0.8132004	0.1081585	1.2664919	0.8466928	3.528815	0.0026201			
E07	SNY11	1.514168	0	0	0.8976441	0.4857338	0.8871446	0.8876568	-0.133941	-0.664654	0	1.484274	0.505478	0.6881369	0.1629541	0	0.8464945	0	0.1781148	0.1498767	0.8464945	0.0016749			
NI2	HAND2L1	1.1957739	0	0	0	-1.3477517	-1.6397781	-0.401283	-0.266051	-1.084297	-1.2646011	-0.566823	0	0	0	0.7749356	1.348846	-1.062839	-0.563071	0.2717059	0.0016749				
E05	MIR378	1.8476681	0	-0.9779175	1.8964887	1.1788184	1.008168	-1.252451	1.2069654	0.9877174	0.0271489	0.0881849	-0.105563	0.1667882	0.1678411	2.0432376	1.159312	0.0713276	1.6076588	0.5231378	3.5665997	0.0016749			
C11	IMP1	0.0775851	0.5423723	0.8577007	-0.2022764	-1.8666411	-0.1730883	-1.394556	-0.3960169	-1.79588	1.1641608	0.6088967	-0.489455	0	-0.912736	-0.6968319	-1.2026484	0.3697245	-0.579921	-0.1024837	0.0124837	0.0016749			
G03	BMO	-0.196802	0.3060735	0.06139166	-0.092391	0.00800878	1.0427111	0	0.8224185	1.002792	-1.268411	0.7946108	-0.05328	0.3607699	0.9737284	0.02081916	1.2779258	0.1299977	1.1007546	0.3662105	1.1882504	0.0124837			
NI3	ILK1L2	-0.547662	0.0484731	-0.4777807	-0.882339	-0.4831905	0.177366	0.3782922	0.2660221	-0.862091	-0.449031	-0.1758487	-0.181923	-0.186177	0.1277183	0.3072205	-0.6699862	0.318462	0.6044647	0.0250647	0.0016749				
G01	SNHG8	-0.12776	0.840721	0.4325387	0.9393066	0.2062389	0.6070192	0.2035606	0.0954973	0.0416072	0.4770941	-0.0727419	-0.016031	-0.025252	-0.107606	-0.0793976	0.0363499	0.0089117	0.0062839	0.1221601	1.3247749	0.0216126			
NI2	PCSK5	0.8418016	0.6165219	0	0	0.0778642	0.9508494	1.3748681	0	0.8817860	0	0	0	0	0	0	0	0	0	0	0.2660238	1.9108766	0.0236409		
A08	CAM1	0	-1.209115	-0.003168	0	0	0	0	-0.47144	-1.3899169	0	0	0	0	0	0	0	0	0	0	0.6168771	0.3657209	0.0238886		
G12	ZC3H11	1.2829511	0.1272002	0.2614664	0.1451578	0.0566953	0.3222018	0.2566155	-0.109231	0.2071256	0.4081317	0.5111816	0.3721216	-0.002788	0.0076828	0.1226761	0.8371756	0.0795181	0.0693526	0.10761	1.2605105	0.0236409			
G07	TUFI1	0.2181786	0.411785	0.2984889	0.4624739	0.0133093	-0.2091521	0.6839074	0.3637499	-0.203076	0.7623694	0.2287799	0.3494912	-0.103846	-0.335818	0.1038477	0.04018704	0.0008677	-0.141223	0.1507061	1.4148424	0.0202473			
D05	LINC0141	-0.732828	-0.3706045	-0.109797	0.1877808	-0.9338452	-0.3538005	-0.1032013	0.2071013	0.0330166	0.0207925	-0.733317	0.5170228	-0.513022	1.2715662	0.842326	-0.4464824	-0.700787	-0.280905	0.1884604	0.0100127	0.0016749			
A12	CENSD18A	0	0	0	0.2271882	0.4140018	0.5410414	0	0	0.6029267	0	0	0	0	0	0	0	0	0	0	-0.6027478	0.1646056	0.0016749		
F09	PVT1	0.4163913	0.6054602	0.4247184	0.5942887	-0.260314	1.848333	0.0018276	-0.560214	0.6385117	0	-0.916859	1.7881583	0.163486	0.00381057	0.1774266	0.207078	0.1972263	0	0.2411802	1.743482	0.0417059			
B06	PTC	0.039951	0.4731656	-0.020086	-0.450424	-0.7009511	0.0164304	0	-0.2531596	-0.6654662	0.5479166	-0.3948182	-1.266095	-0.0090756	-1.083739	1.016248	0.432064	0.2702275	-0.7632109	-0.262323	0.9606364	0.012265			
E07	MIR213-AS1	-0.809447	0.1999119	0	-0.6959788	-0.277775	0.1324147	0	-0.0115748	-0.482678	0	0	0.0449861	0	0.7349191	0	0.7424252	0	-0.26071	0.6317895	0.5647399	0.0016749			
G09	LKAT1	-0.189792	0.4273892	0.5400106	-0.102323	0.4606664	1.681597	0.9654923	-0.8604903	0.5088464	1.4543238	1.0561802	1.7560909	0.1228264	0.0923284	0.0714613	1.666679	0.3326472	-0.378121	1.2067488	2.2502629	0.0124837			
NI1	H19	-0.462470	1.0064645	-1.779252	1.1159728	-1.3391245	-1.1845249	-0.1417623	0.2710126	-0.289441	-0.789318	1.6730704	0.3500929	-0.020935	-1.05443	-0.312297	-0.966393	-1.707749	-0.331124	-0.470494	0.1831784	0.0886013			
D10	HPIA-AS2	0.2992872	-0.2022974	1.9814628	0.228311	0	-1.1229416	-0.174392	-0.091222	0.8746089	0.9166098	0.9129185	0.041962	0	0	0	0	0	0	0.1862107	0.9891147	0.0177057			
F04	POLS1F75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1160495	1.3003308	0.0236409		
F06	PTC31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0016749			
C02	LOT1B	0	0.2232084	-0.2047458	0	1.00999184	0.2534839	0	0.3489045	-0.3500901	0	0.8904115	-1.118205	0.859402	0	-0.059932	0.0382006	-0.6174427	0.2997969	0.3306351	0.2121431	0			
D10	LINC01234	0	0	1.1920796	0	0.1941296	0	0.64880967	0	0	1.0929293	0	0	0	0	0	0	0	0	0	0	0.1234878	1.3288802		
NI2	PAN3A1	0	0.6913713	0	0	0	0	0	0	0	0	0	0.9790493	0	0	0	0	0	0	0	0	0.0468907	1.1017182	0.0236409	
E01	MAAT1	0.2279782	-0.226269	-0.0570499	-0.4761137	0.1718282	0.1433315	-0.0084195	-0.2268721	-0.707343	-0.938475	-0.125263	-0.023776	0.5282228	-0.226661	0.2999441	0.8830516	-0.1011303	-0.4048446	-1.862766	0.6121249	1.3472177	0.0016749		
C02	H19-AS1	0	1.1520864	0	0	0.7817271	-0.1817178	0	0	0.3080441	0.3071219	0.3447866	0.0028889	0	-0.4405741	0	0.8780905	0.3082886	0	-0.1729095	0.1287452	1.3162209	1.3476703		
E06	LINC0041	0	-1.191141	0.3623165	0.0354619	-0.4048316	0	1.0552508	0.5752364	0.617539	0	0.3388511	-0.8138916	-0.2870979	-0.219005	0	0.3889928	0.8172148	0.0564405	0.1518857	1.1608399	0.1417381			
D04	LINC0041	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0042751	1.3331545	0.0236409			
F01	PCAJ	-0.292515	0	-0.082621	0	0	0	0	0	-1.2979505	-0.7464519	0	0.8381197	0.0011386	0	0.9838828	0	-1.254402	0	-0.222483	0.5848978	0.1574448			
F02	PCAT1	0	0	0.8986236	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0468391	1.0890928	0.0174021	
A04	ARX	0	0.0114124	0	0	1.1855878	0	0	0	0	-0.105961	0	0	0	0	0	0	0	-0.984212	1.0187744	0	0.1819252	1.3101517	0.1891462	
F03	PCMY1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.315551	1.0006792	0.1660561	
E03	MIR153HG	0	0	0	0	0.7287818	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0064879	1.0977193	0.1610372	
A05	BANX1	0.6207201	0	0	0.2163844	0	0.38909755	0	0	0	0	1.8845984	0	0	0	0	0	0	0	0	0	0	0.1032028	1.2882343	0.1849548
A13	ADAP1-AS1	0	0	0.8316885	0.8912842	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0076732	1.1883601	0.1735222
C04	HNF1A-AS1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0016749		
E10	NDA1	-0.063566	0.1158996	-0.2640819	-0.3186083	0.1279664	0.8548026	0.0563718	-0.1681442	-0.3491655	-0.103762	0.1526268	0.0649545	-0.2709162	-0.272161	0.1342488	-0.624428	-0.266213	-0.129278	0.7418697	0.2781817	0.0016749			
D08	LINC0089	-0.186616	0	0.1700578	0.2951308	-0.491051	1.524128	0	-0.875191	0.8498916	0.3676969	-0.672076	-0.364143	1.0025764	-0.0										

lncRNA	Average Normalized Count	Standard Deviation	Median Normalized Count	Inter-Quartile Range	Inter-Quartile Range	P <sub>skewness-kurtosis</sub>
SPRY4_IT1	0.11	0.018	0	0.00, 0.06	0, 0.018	0.0009
HEIH	20.36	8.47	19.59	8.56, 42.91	14.26, 23.49	0.007
LUCAT1	0.39	0.44	0.24	0.02, 1.92	0.08, 0.57	0.0001
LINC00152	7.07	7.52	5.26	0.90, 38.66	2.80, 7.89	< 0.0001
HAND2_AS1	0.67	0.95	0.28	0.00, 4.25	0.13, 0.73	< 0.0001
MEG3	15.71	23.84	5.67	0.49, 116.51	2.29, 19.23	< 0.0001
TERC	0.02	0.03	0.02	0.00, 0.12	0.00, 0.04	0.0007

230x101mm (300 x 300 DPI)

Author Mar