*All sub-sections below are associated with R scripts hosted on* [*GitHub*](https://github.com/rreggiar/KRAS-AALE-Paper/blob/master/KRAS-AALE-CODE/) *and organized in the “aale.2019.analysis.notebook.Rmd”*

RNA-seq psuedoalignment and quantification

All *fastq* files were trimmed with *Trimmomatic 2 (0.38)* [] using the Illumina NextSeq PE adapters. The resulting trimmed files were assessed with *FastQC* [] and then passed through the following analytical pipeline:

Salmon (0.14.1): pseudoalignment of RNA-seq reads performed with *Salmon* [] using the following arguments:

--validateMappings --rangeFactorizationBins 4 --gcBias --numBootstraps 10

using an index created from the *GENCODE* version 29 transcriptome fasta file using standard arguments.

Sleuth (0.30.0): transcript differential expression was performed using *Sleuth* [] and *Wasabi* (1.0.1) to convert the *Salmon* output into the proper format. Upon completion, the transcripts with q-values below 0.05 in the likelihood-ratio test were used to filter salmon output from which log2fc was manually calculated and paired to the sleuth output.

DESeq2 (1.24.0): *Salmon* output was imported into a DESeq object using *tximport* [] and differential expression analysis was performed with standard arguments.

Transposable Element Content Analysis

Exon and 5’/3’ UTR Overlap: a whole genome *.gtf* file was downloaded from the UCSC genome browser Table browser utility. This file was parsed and merged with the GENCODE v.29 reference transcriptome. This modified *.gtf* (now a *.bed* file) was passed to *bedtools* [] where the overlap function was used with the following arguments:

-a modified.gtf.bed -b all.ucsc.rmsk.genes.bed -wao -s > retained.overlap.bed

alongside a whole genome *.gtf* retrieved as described above except generated from the repeat-masked browser track. The resulting overlapped bed file was processed and visualized using custom *R* scripts.

Differential Expression: Differential transcript abundance was determined using the *Salmon* and *Sleuth* procedures described above provided with a custom index comprising both the *GENCODE* version 29 transcripts and all transcripts extracted from the Hammel lab GTF file as described in the single cell procedures. *Sleuth* output was filtered and visualized using *R* and *Tidyverse*.

Zinc Finger Protein Analysis

ChIP-exo data and supplementary information were extracted from supplementary data provided by *Imbeault et al* []. ZNF genes were cross referenced with *DESeq2* and *RepeatMasker* outputs to extract relevant differential expression data of ZNF proteins and Transposable Element transcripts using *R*. *RepeatMasker* output from promoter analyses was cross referenced with ChIP-exo target data to identify potential regulatory targets of differentially expressed KZNFs. Only KZNF targets with ‘score’ [see Imbeault *et al*] >= 75 were kept for analysis. Analysis of all data was performed and visualized in *R* using custom scripts.

Gene Set Enrichment Analysis

Genes determined to be significantly differentially expressed in *DESeq2* output were first ‘pre-ranked’ in *R* by the following metric:

Score metric = sin(log2FoldChange) \* -log10(p-value)

The resulting ranked files objects were processed using the *R* package *fgsea* [] alongside gene set files downloaded from msigdb [] using the *R* package *msigdbr* []. Additional code was written for select vizualizations.

Gene Ontology Analysis

Upregulated gene names were extracted from *DESeq2* output using bash command line tools. Name lists were pasted into the *Gene Ontology Consortium*’s *Enrichment Analysis* tool powered by *PANTHER*. Output data was exported as *.txt* files and parsed using bash command line tools. Parsed data was visualized using custom *R* scripts.

Single Cell Analysis:

*All single cell analyses are associated with R scripts hosted on* [*GitHub*](https://github.com/rreggiar/KRAS-AALE-Paper/blob/master/KRAS-AALE-CODE/) *and organized in the “aale.2019.single.cell.analysis.Rmd”*

10x Processing: Single cell output data was processed using 10x pipeline *CellRanger* [The *mkfastq* functionality was used to generate *fastq* files for further downstream analysis. Output was also aggregated and quantified using the *aggr* and *count* functionalities, respectively. This output was visualized using the 10x Loupe browser.

Downstream Analysis: *fastq* files generated above were passed to Salmn *alevin* [] with the following arguments:

--libtype A –chromium –dumpCsvCounts -p 16.

*alevin* was used to psuedoalign the libraries to both the GENCODE v.29 reference transcriptome as well as a composite transcriptome reference built by combining the GENCODE v.29 reference with one built from the GRCh38\_rmsk\_TE.gtf hosted by the Hammel lab at <http://labshare.cshl.edu/shares/mhammelllab/www-data/TEToolkit/TE_GTF/> . A salmon index was built from this reference with standard arguments. These alevin output matrices were imported into R using *tximport*. GSEA/ cluster correlations were calculated using the *R corr()* function. Normalization and clustering were performed with *Seurat* [] and additional code was written to handle select visualizations.

TCGA ZNF analysis:

TCGA-LUAD and GTEX lung phenotype and normalized count data were downloaded from the UCSC Xena browser TOIL data repository (<https://xenabrowser.net/datapages/?cohort=TCGA%20TARGET%20GTEx&addHub=https%3A%2F%2Fxena.treehouse.gi.ucsc.edu&removeHub=https%3A%2F%2Fxena.treehouse.gi.ucsc.edu%3A443>). The files were combined and patients were grouped by their KRAS mutation status and identity. These data were compared to and visualized alongside of data generated from our analysis using custom *R* code. Significance was determined with a one-way t test implemented in the *R* *t.test()* function.

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