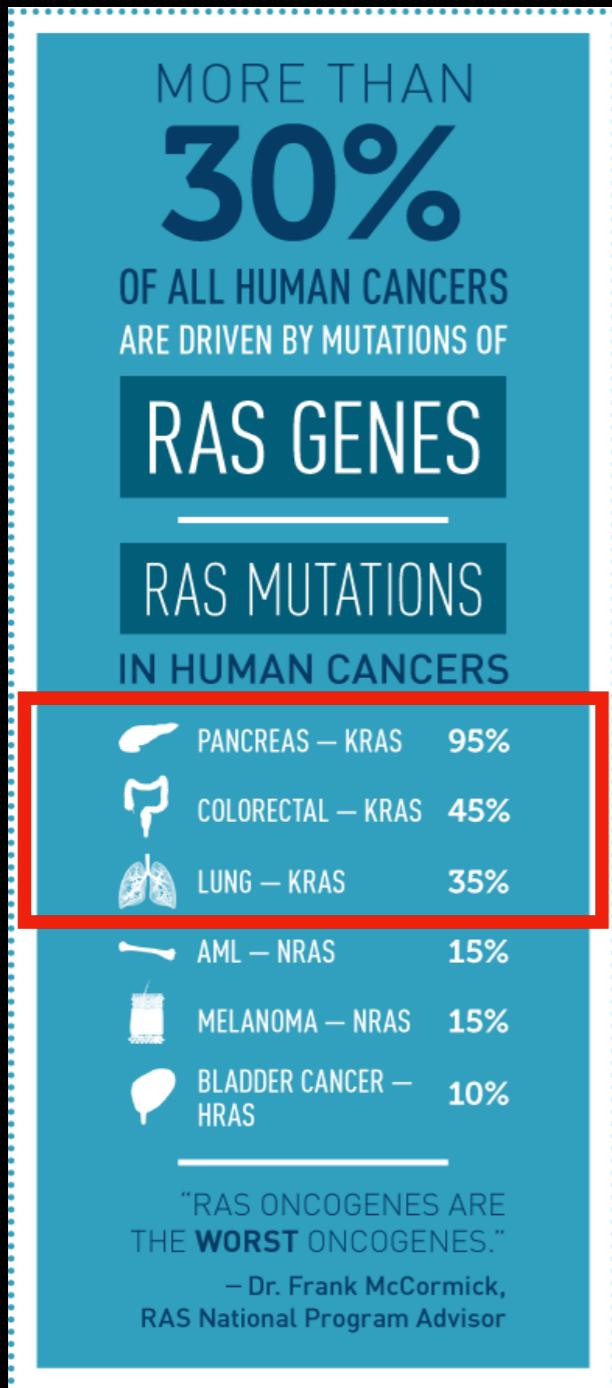


# KRAS and the non-coding Transcriptome: a Inc to Transposable Elements

Roman Reggiardo, 2nd Year BME PhD  
Daniel Kim Lab

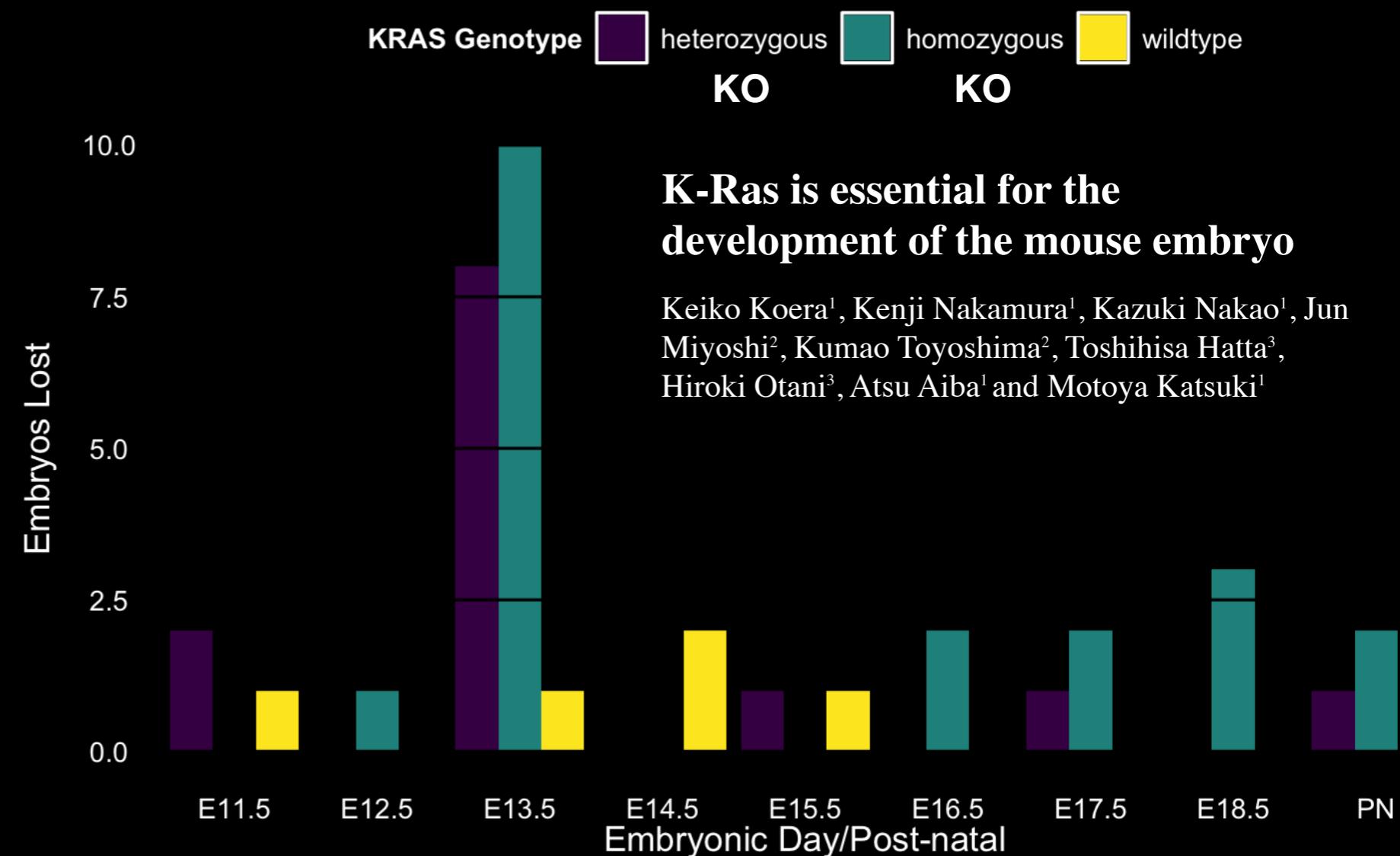
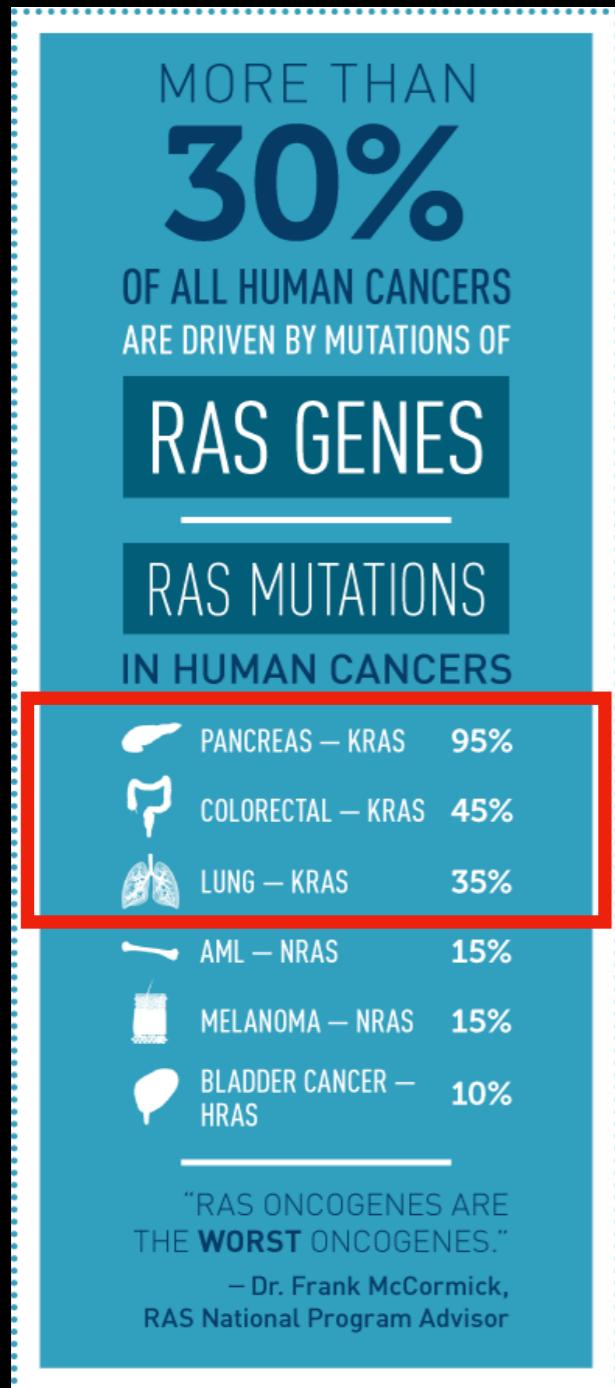
# why study KRAS?

- Prevalence in Human cancers as a potent oncogene



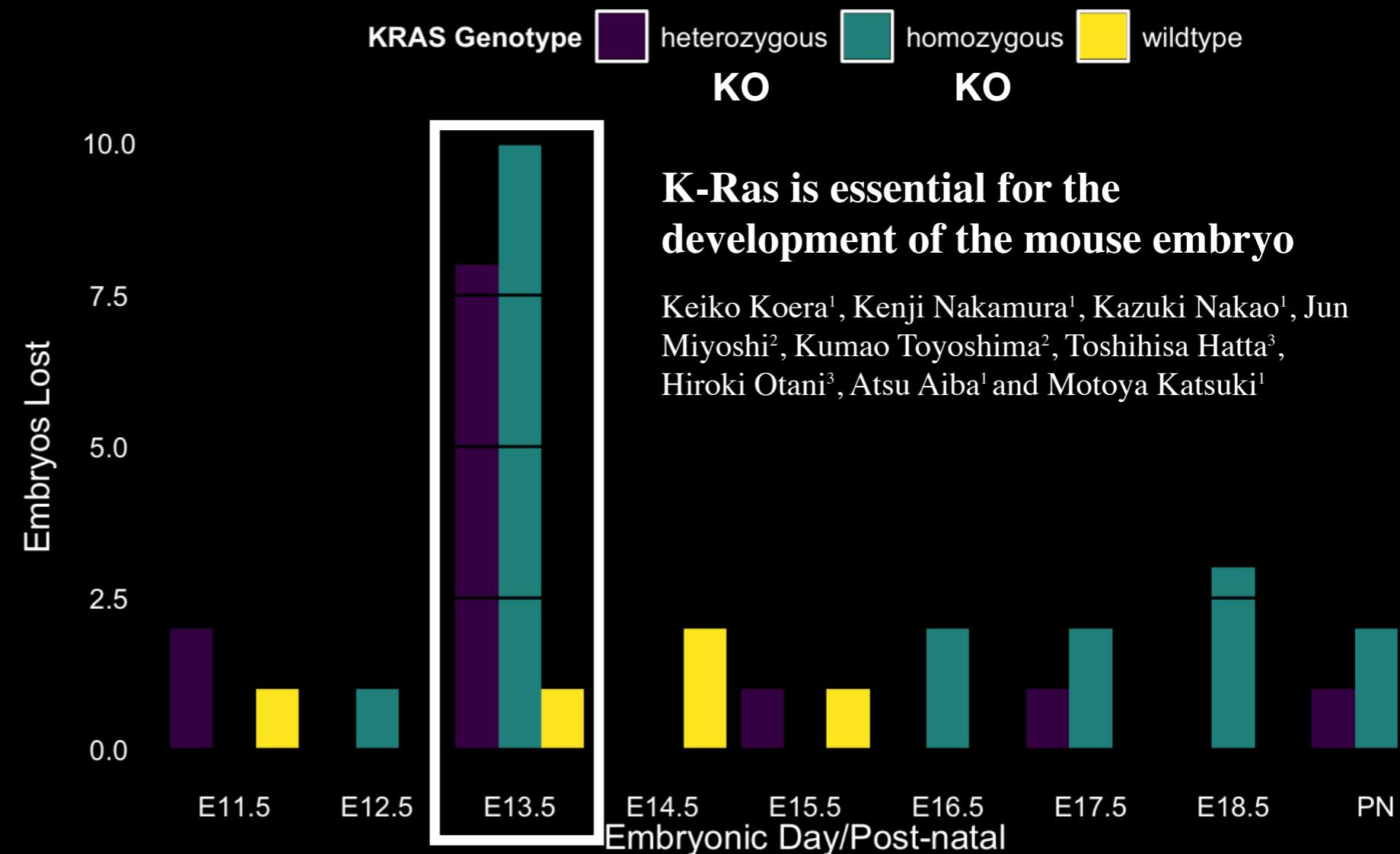
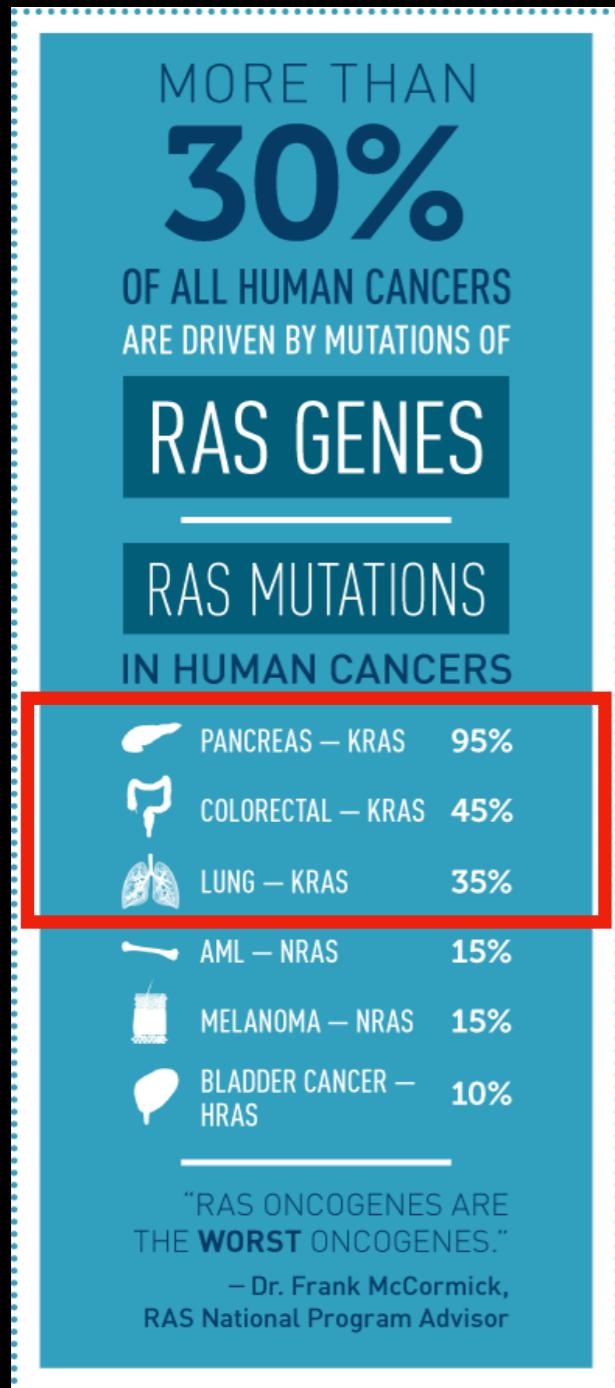
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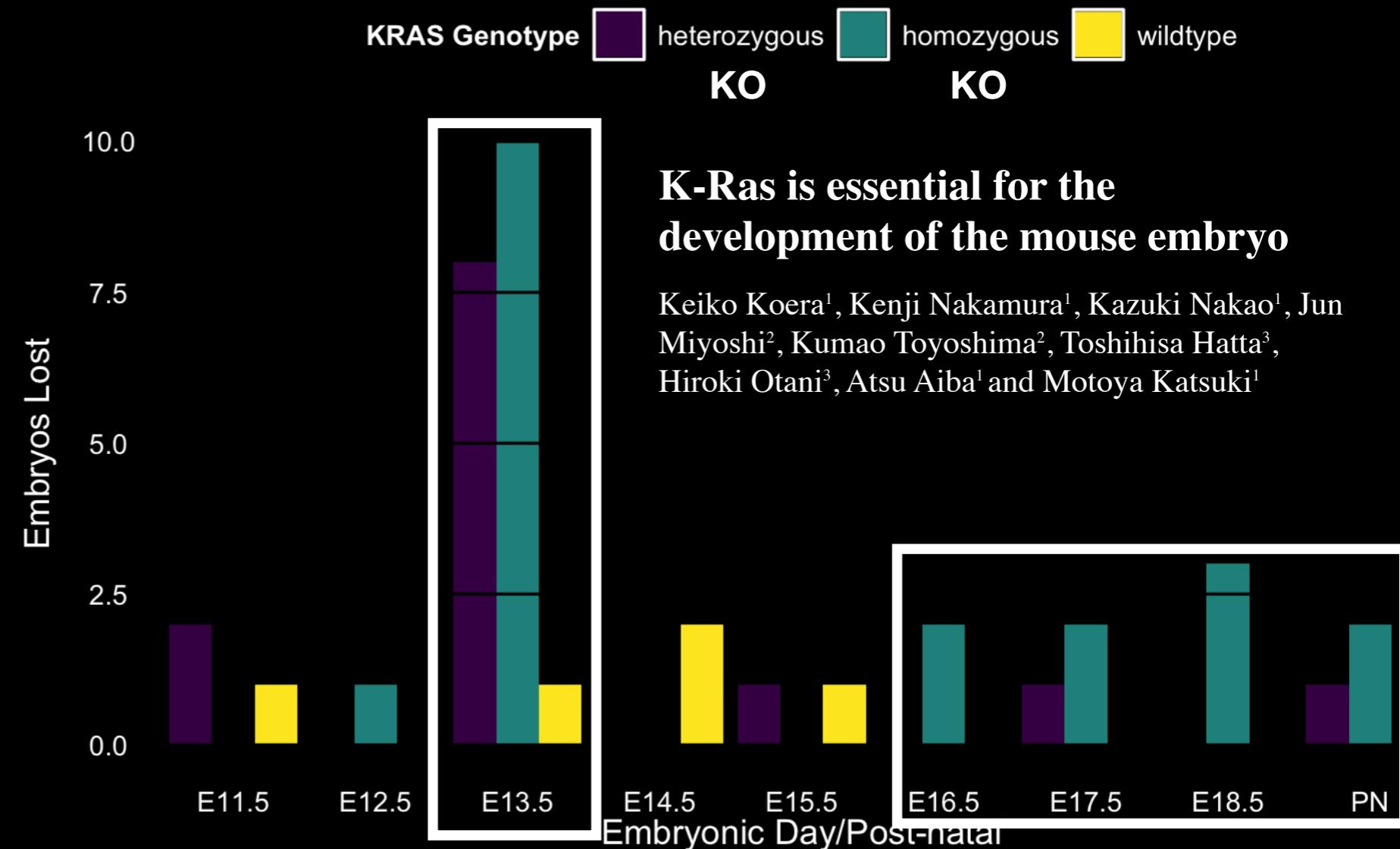
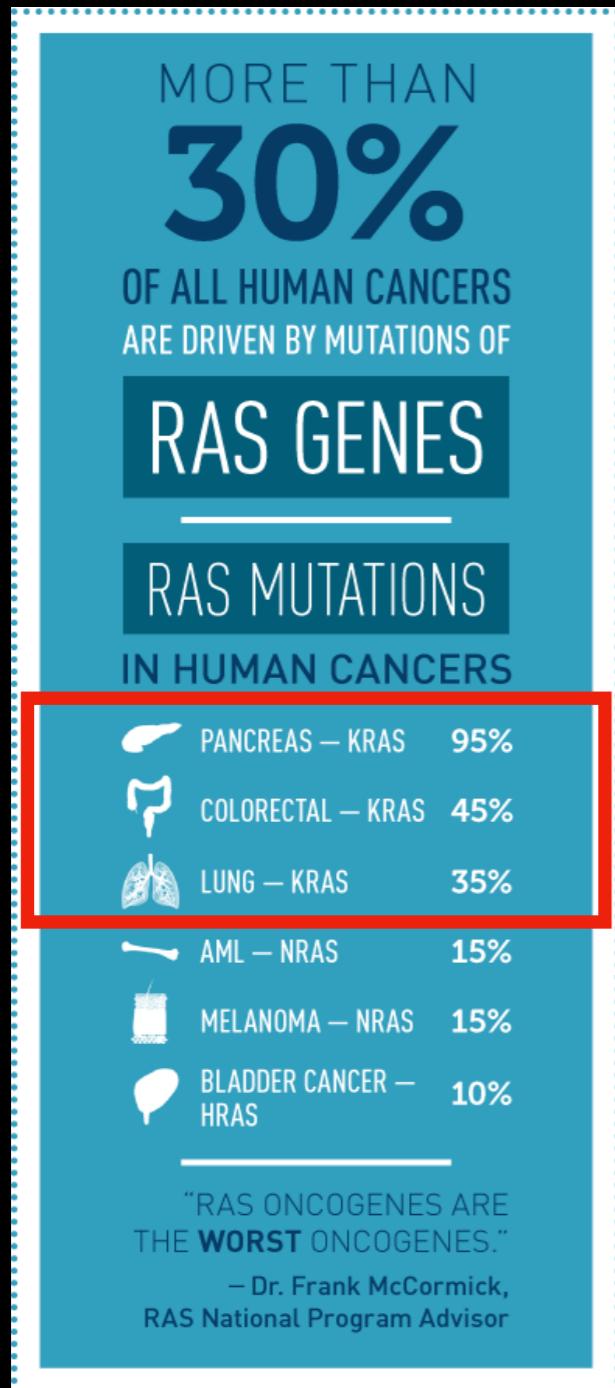
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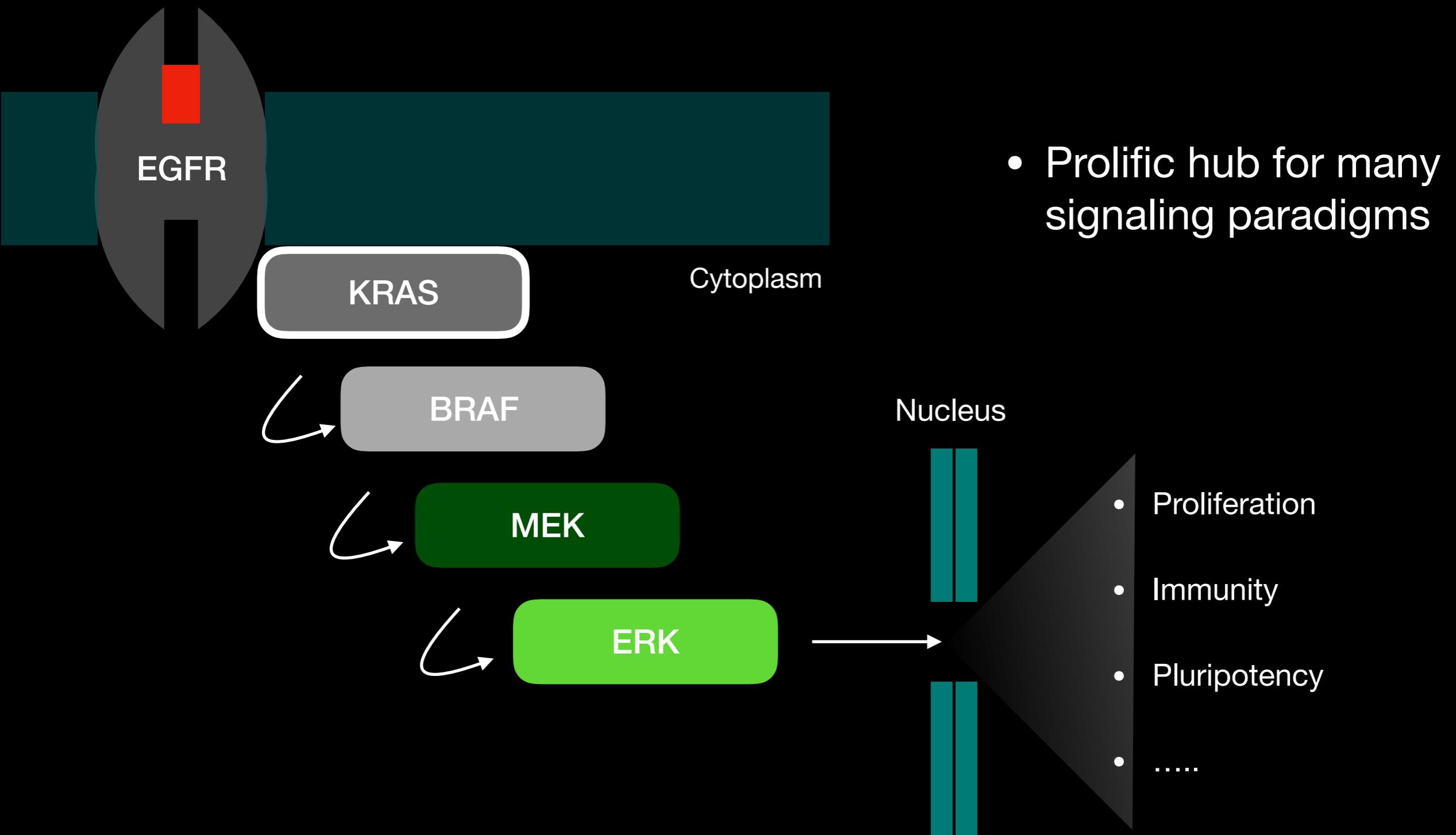
# why study KRAS?

- Prevalence in Human cancers as a potent oncogene
- Necessary in embryonic development

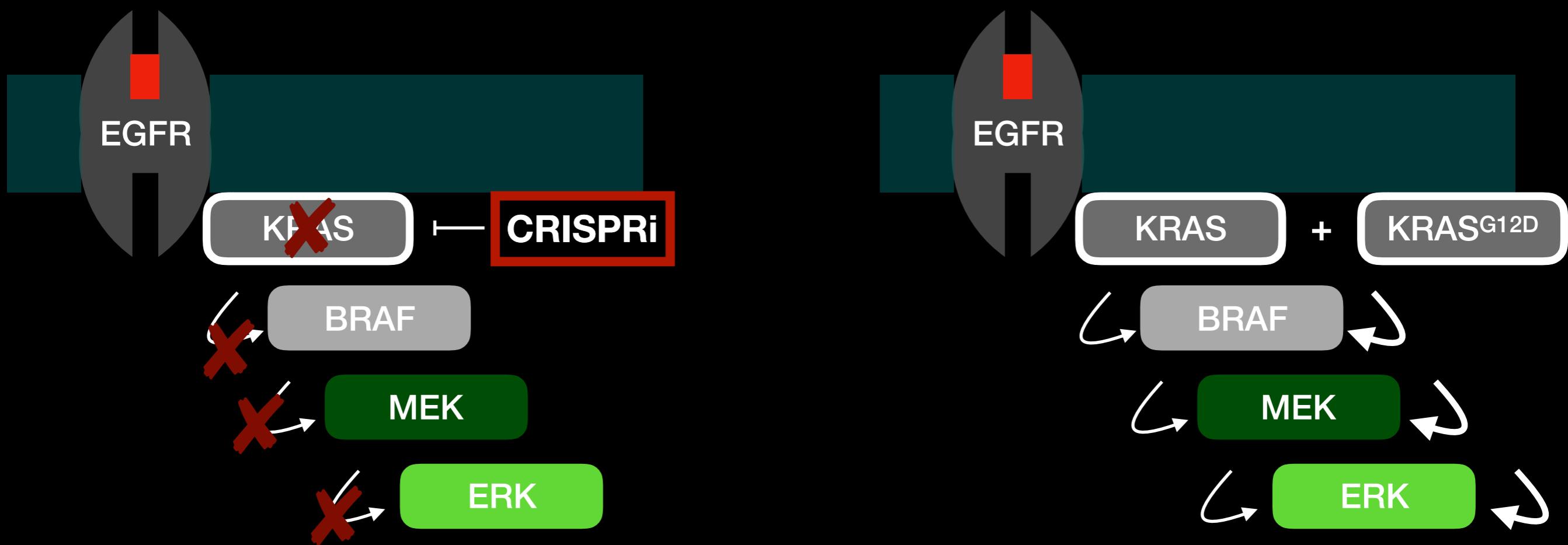


# why study KRAS?

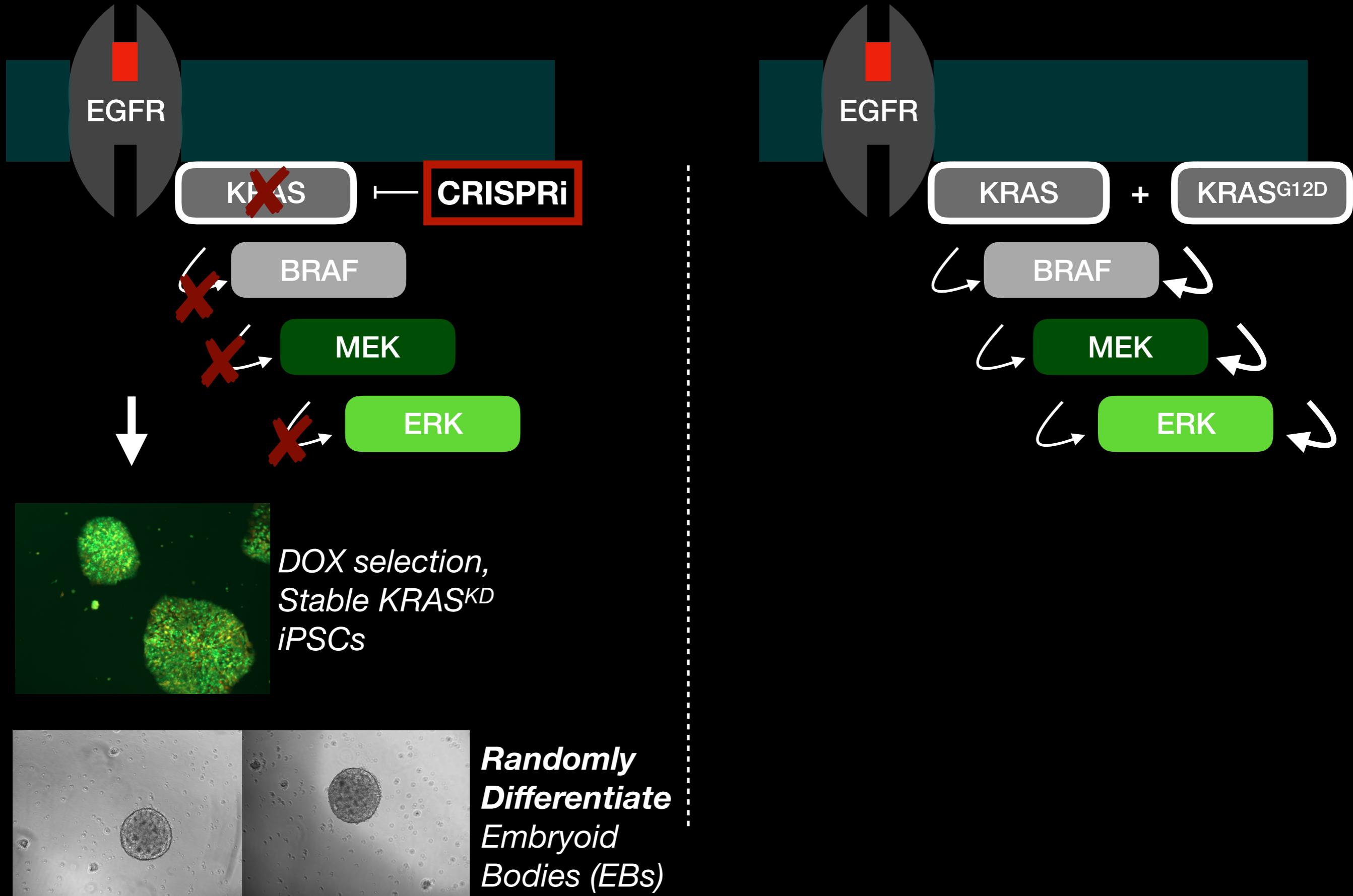
- Prevalence in Human cancers as a potent oncogene
- Necessary in Mouse and Human development



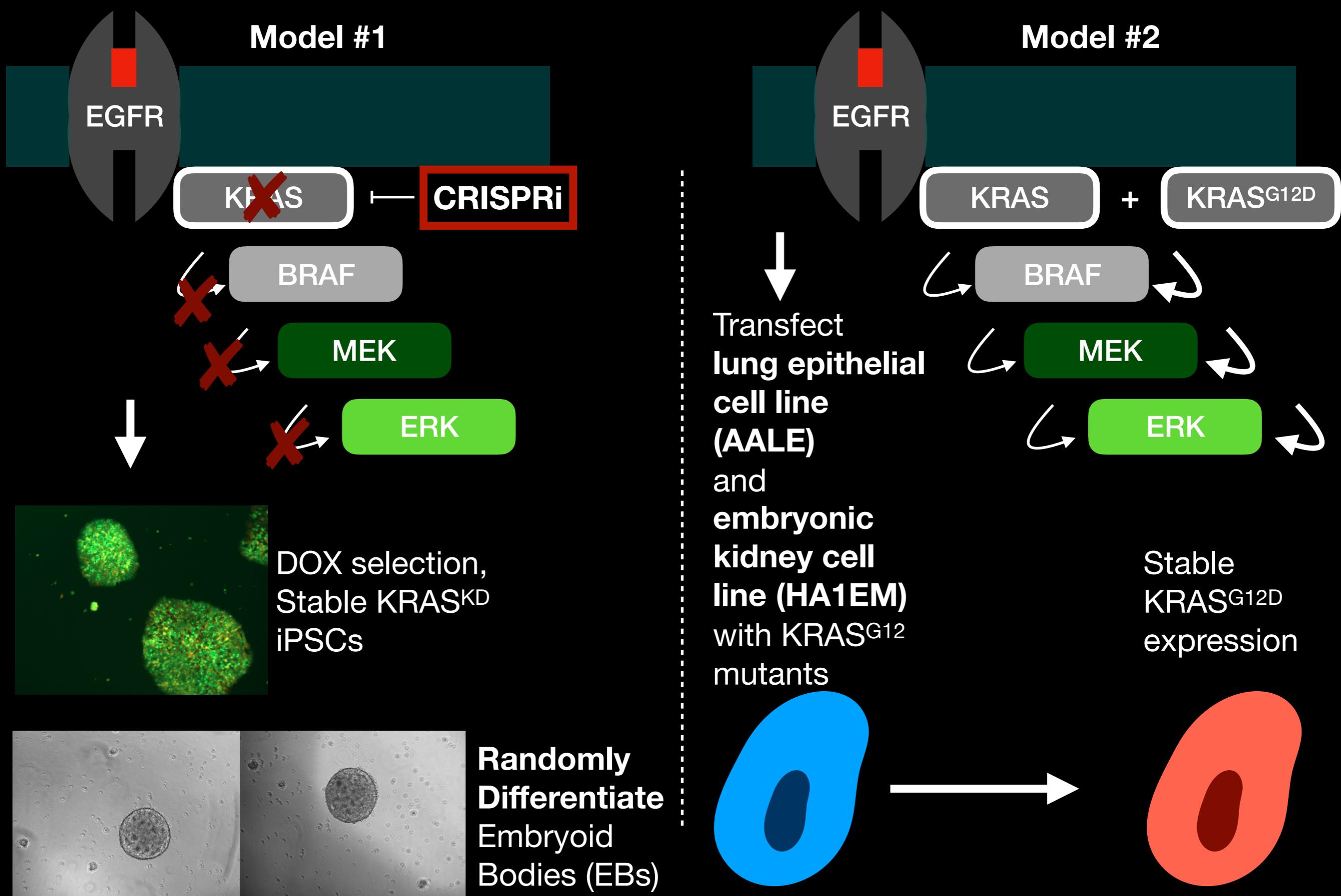
# how we model KRAS *in vitro*:



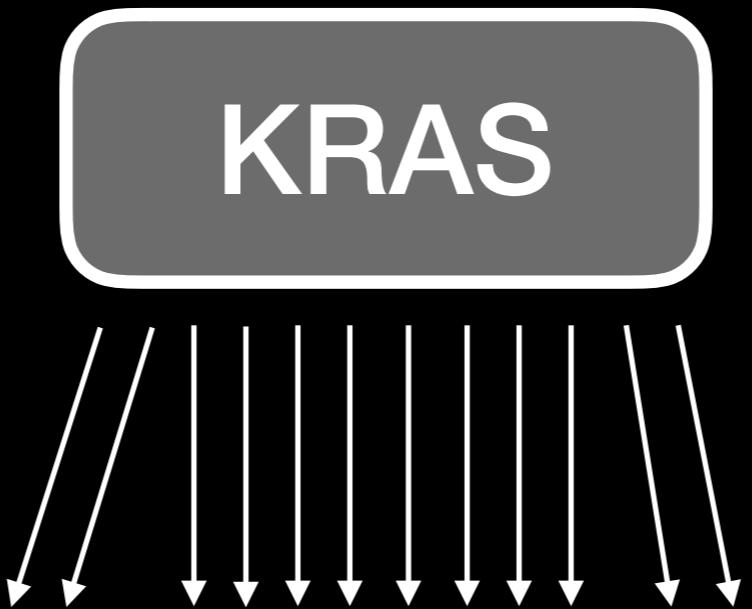
# how we model KRAS *in vitro*:



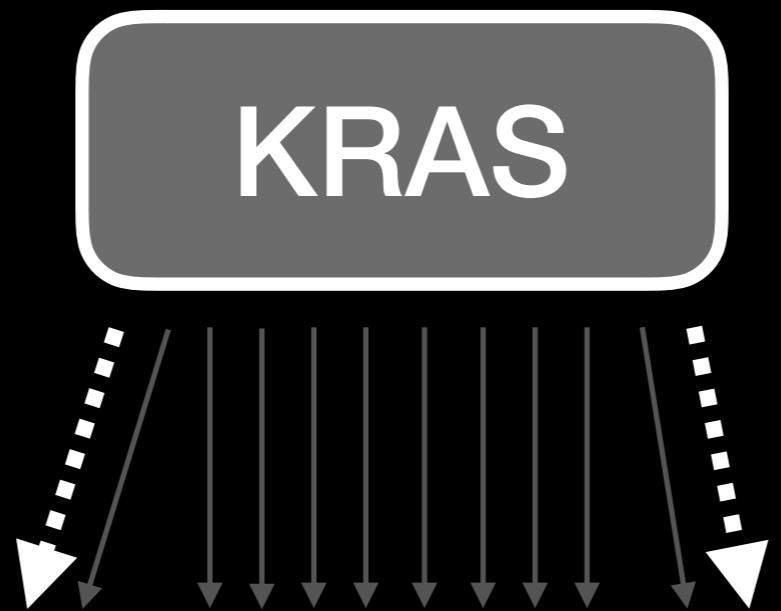
# how we model KRAS *in vitro*:



# what I'll talk about today:



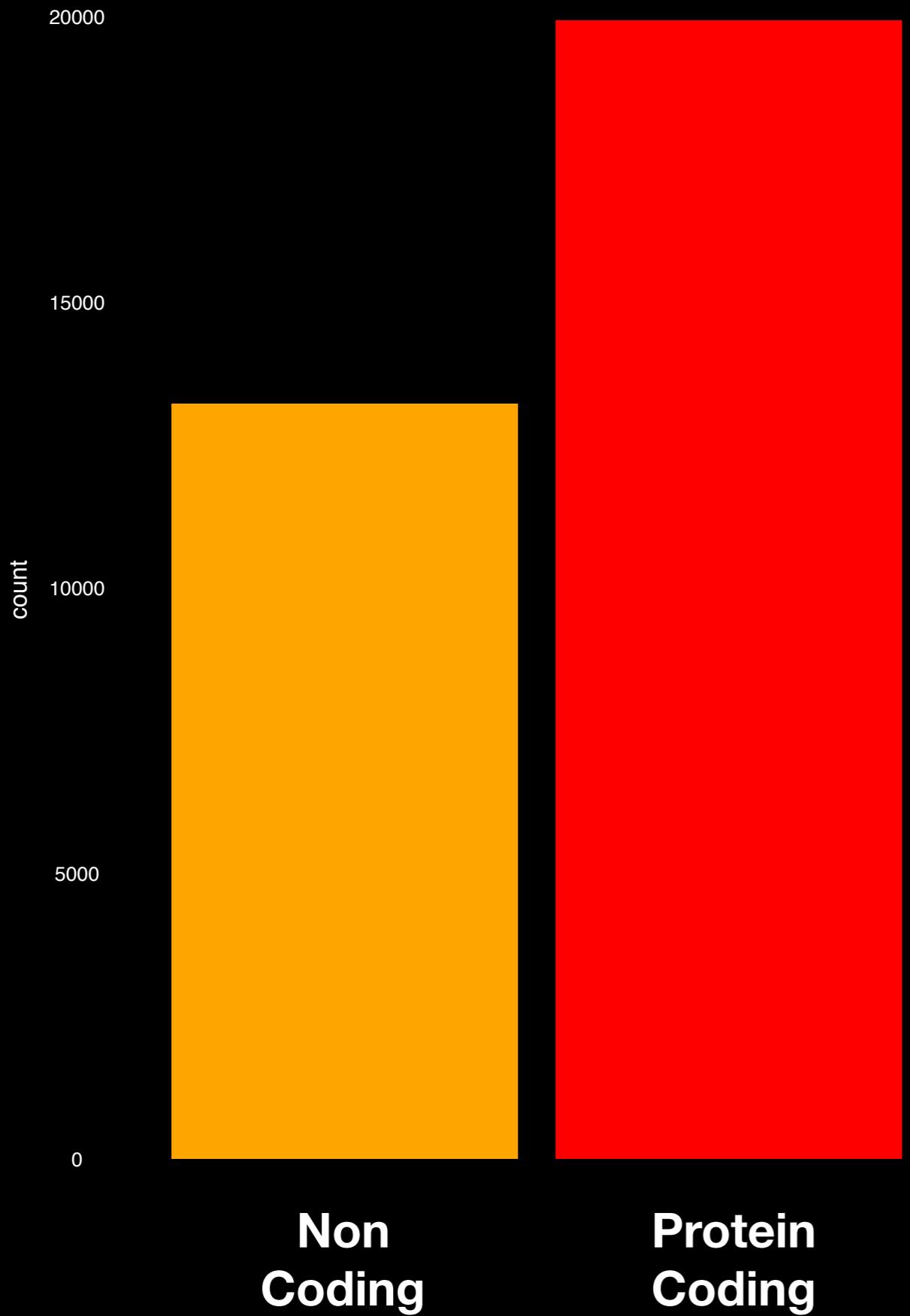
# what I'll talk about today:



long non-coding RNA ← - - - → Transposable Elements

# long non-coding RNA

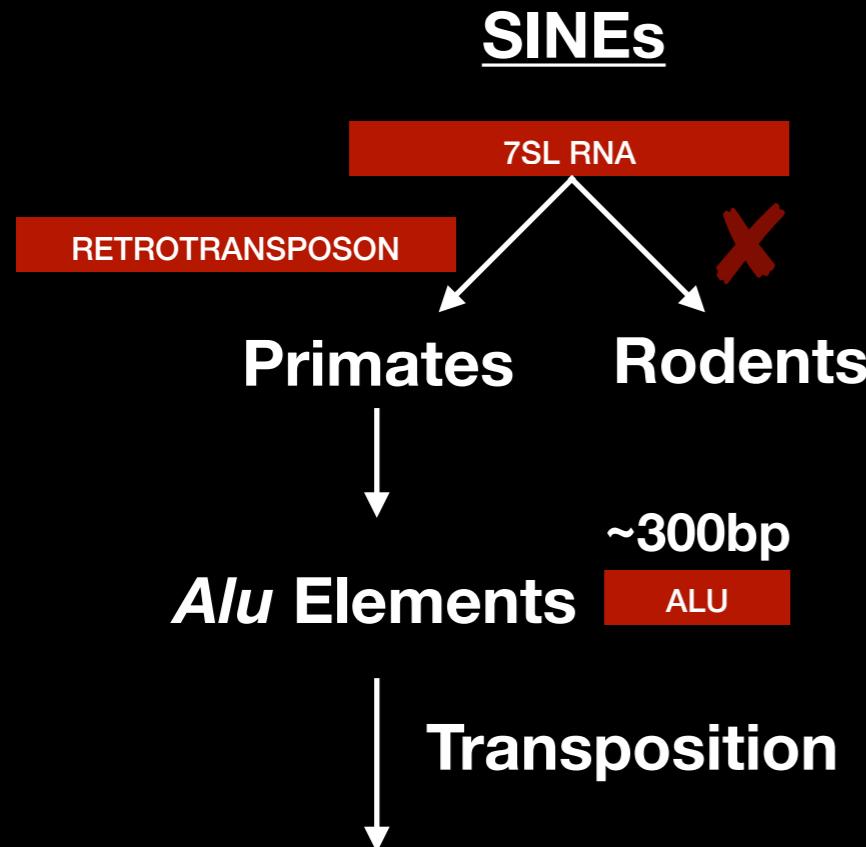
## *GENCODE* annotations



- longer than 200 Nucleotides
- not translated
- may act in *cis* or *trans* in a wide variety of functions

# Transposable Elements

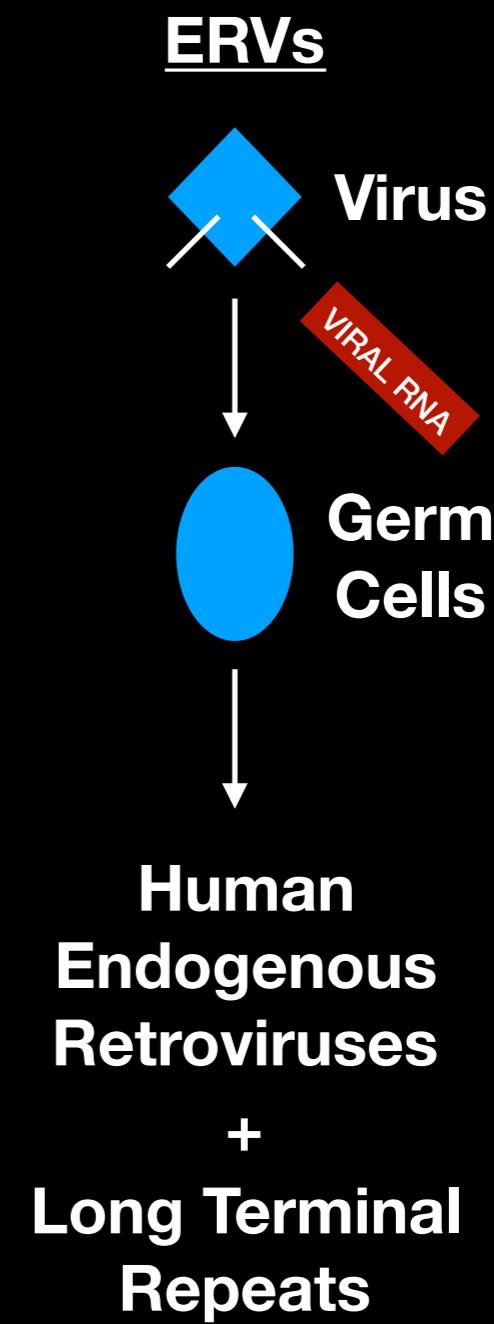
## Short INterspersed Elements



### **Alu Elements:**

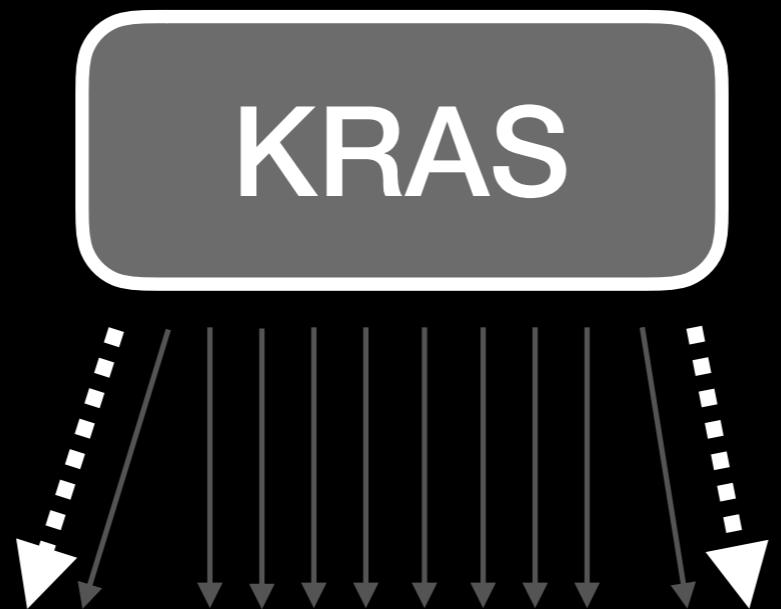
1. in new loci
2. with new variation ~ 'younger'

## Endogenous Retrovirus



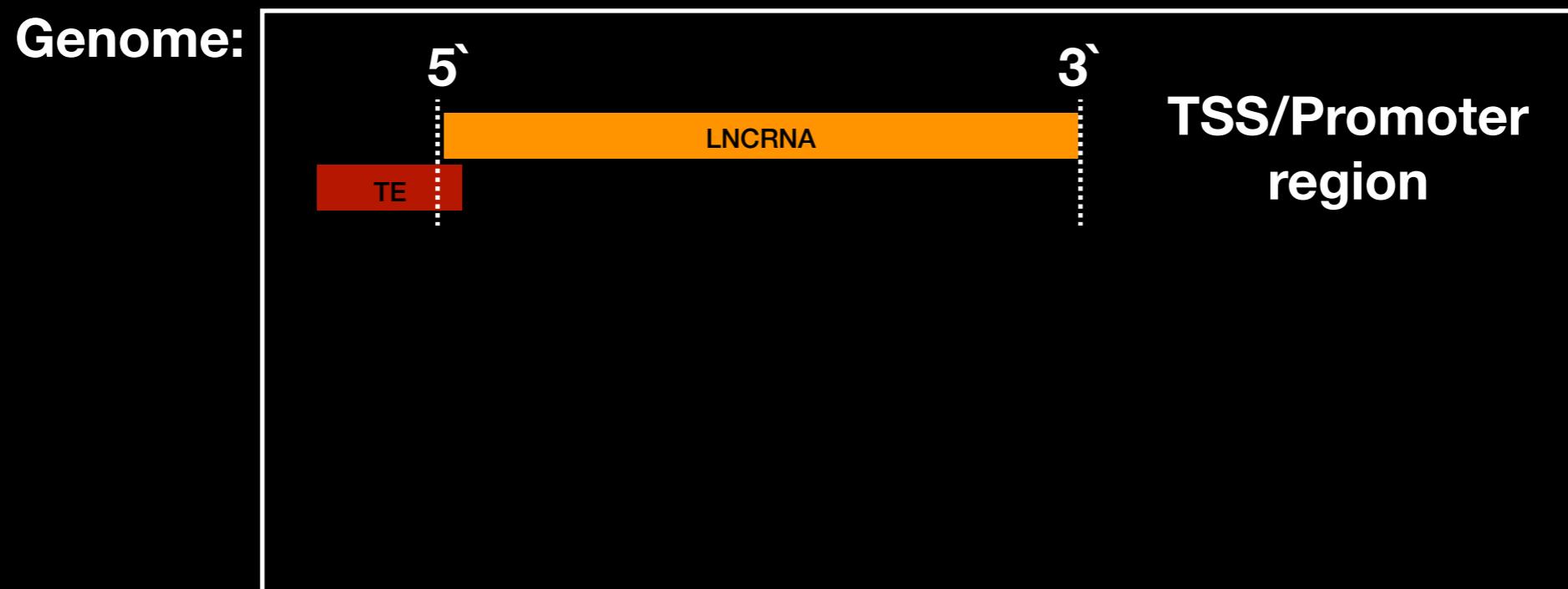
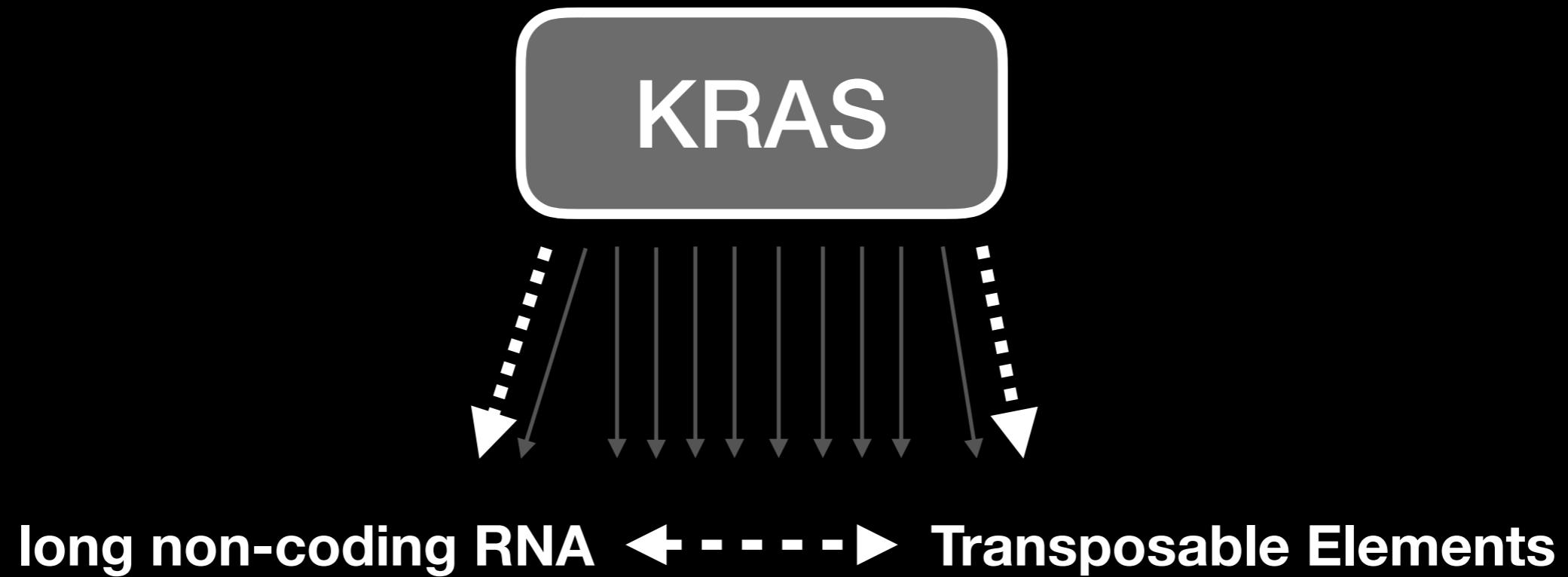
LTR | HERV | LTR

# what I'll talk about today:



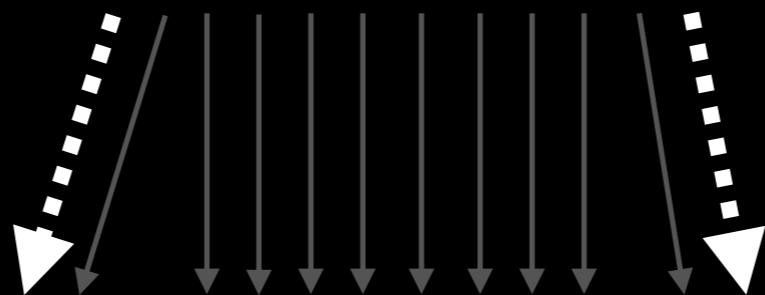
long non-coding RNA ← - - - → Transposable Elements

# what I'll talk about today:

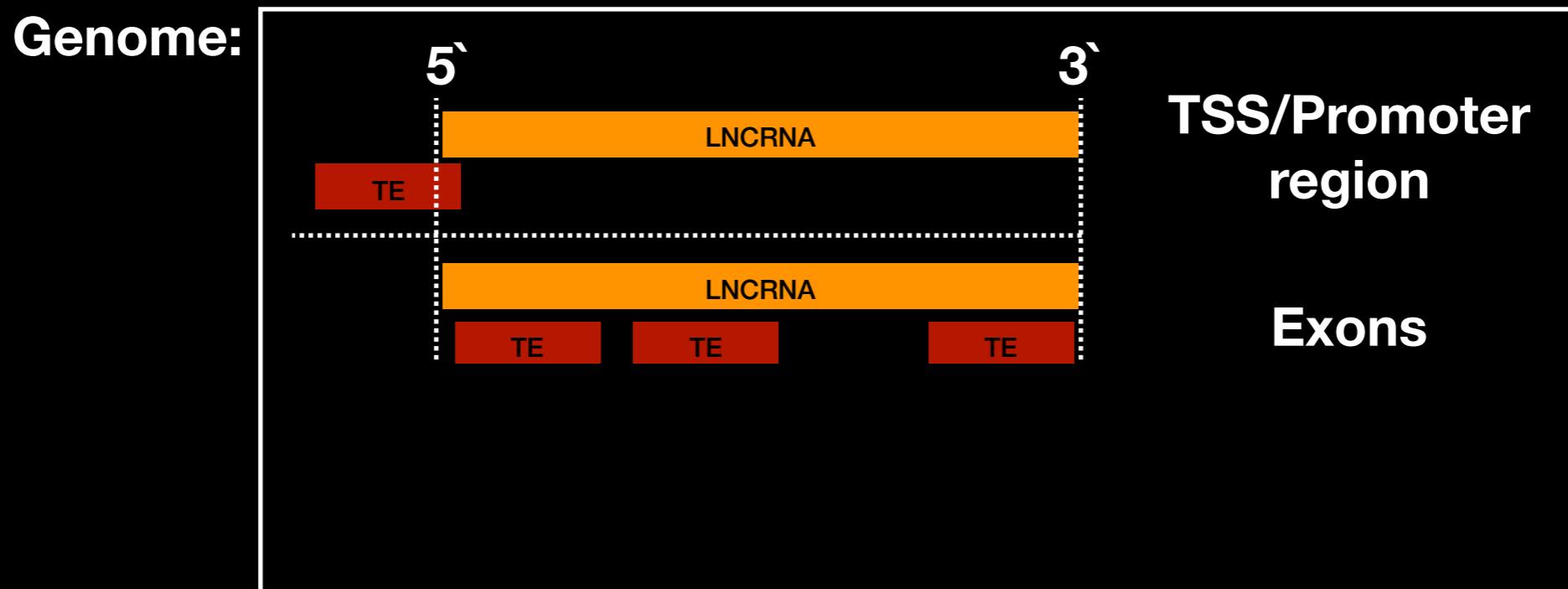


# what I'll talk about today:

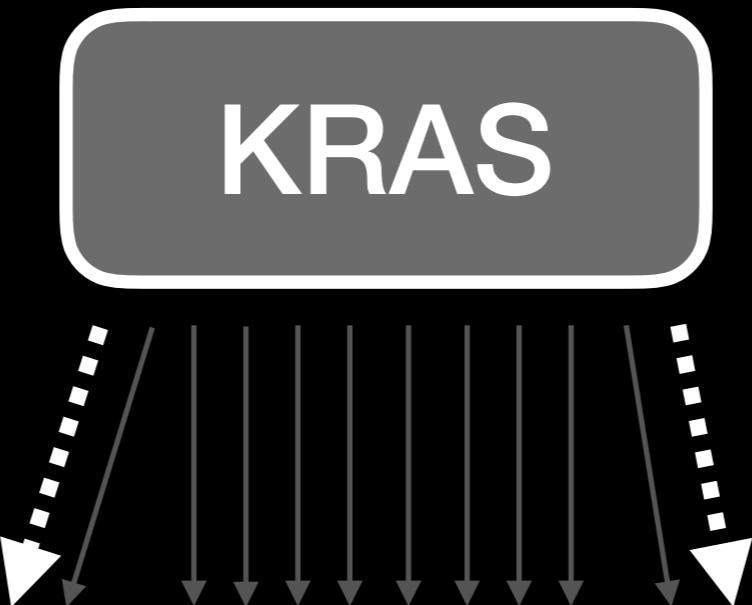
KRAS



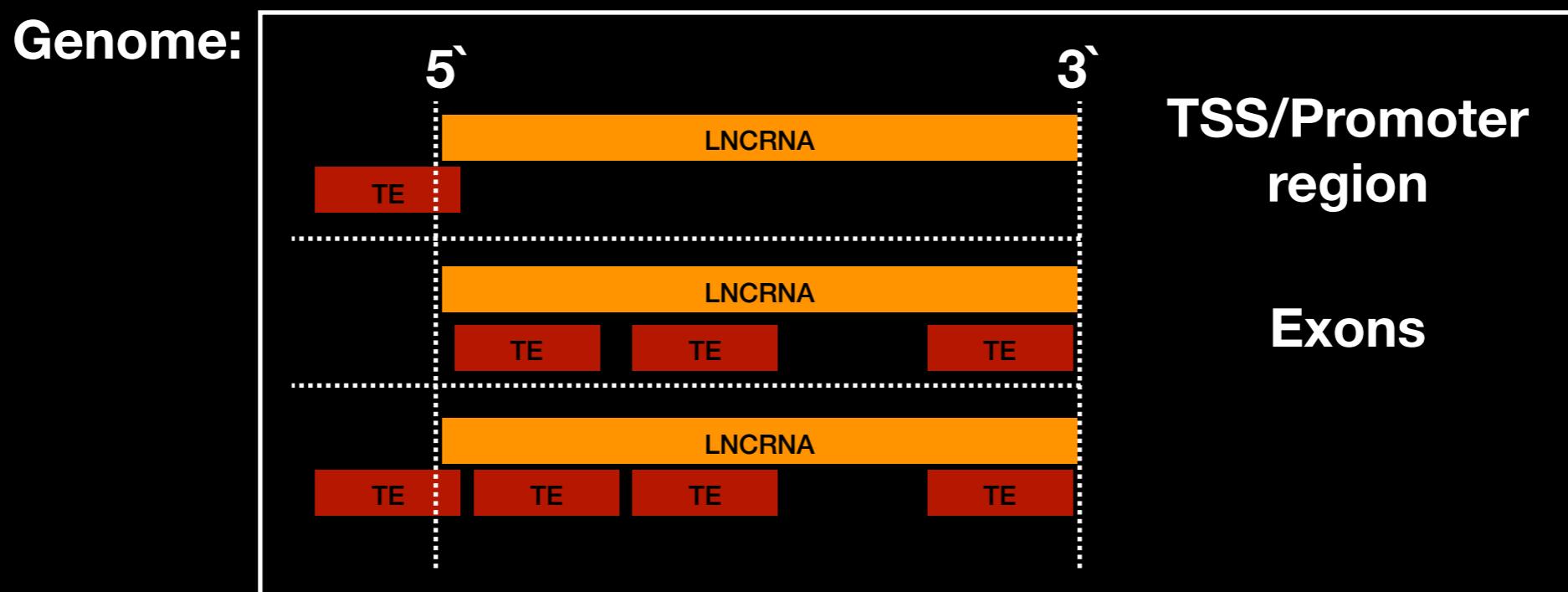
long non-coding RNA ← - - - → Transposable Elements



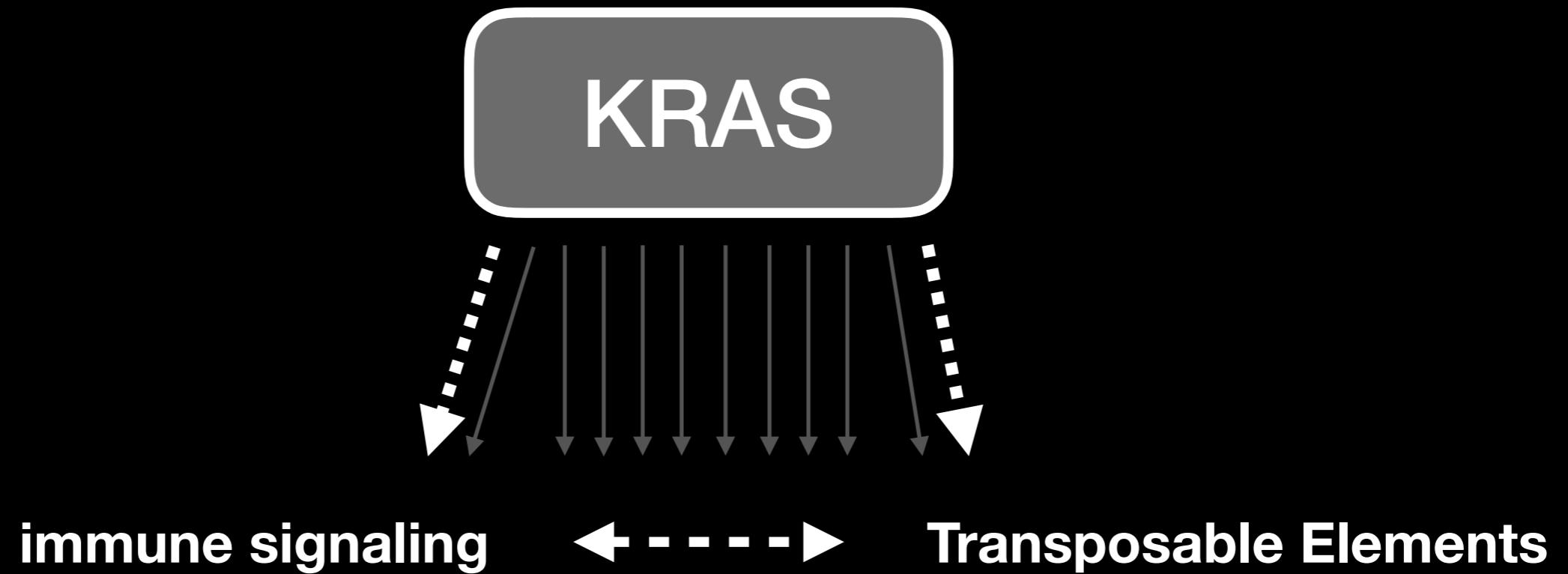
# what I'll talk about today:



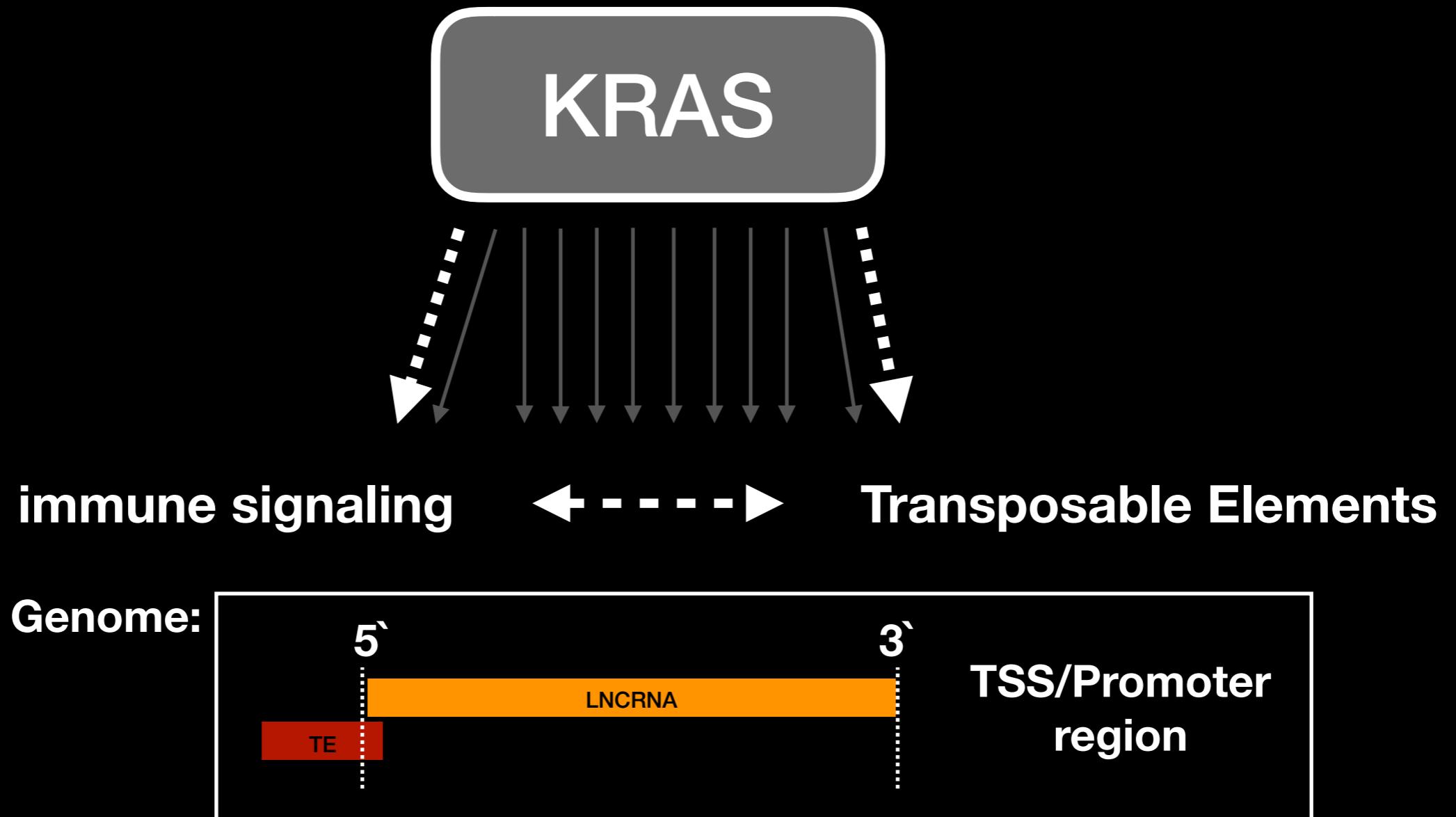
long non-coding RNA ← - - - → Transposable Elements



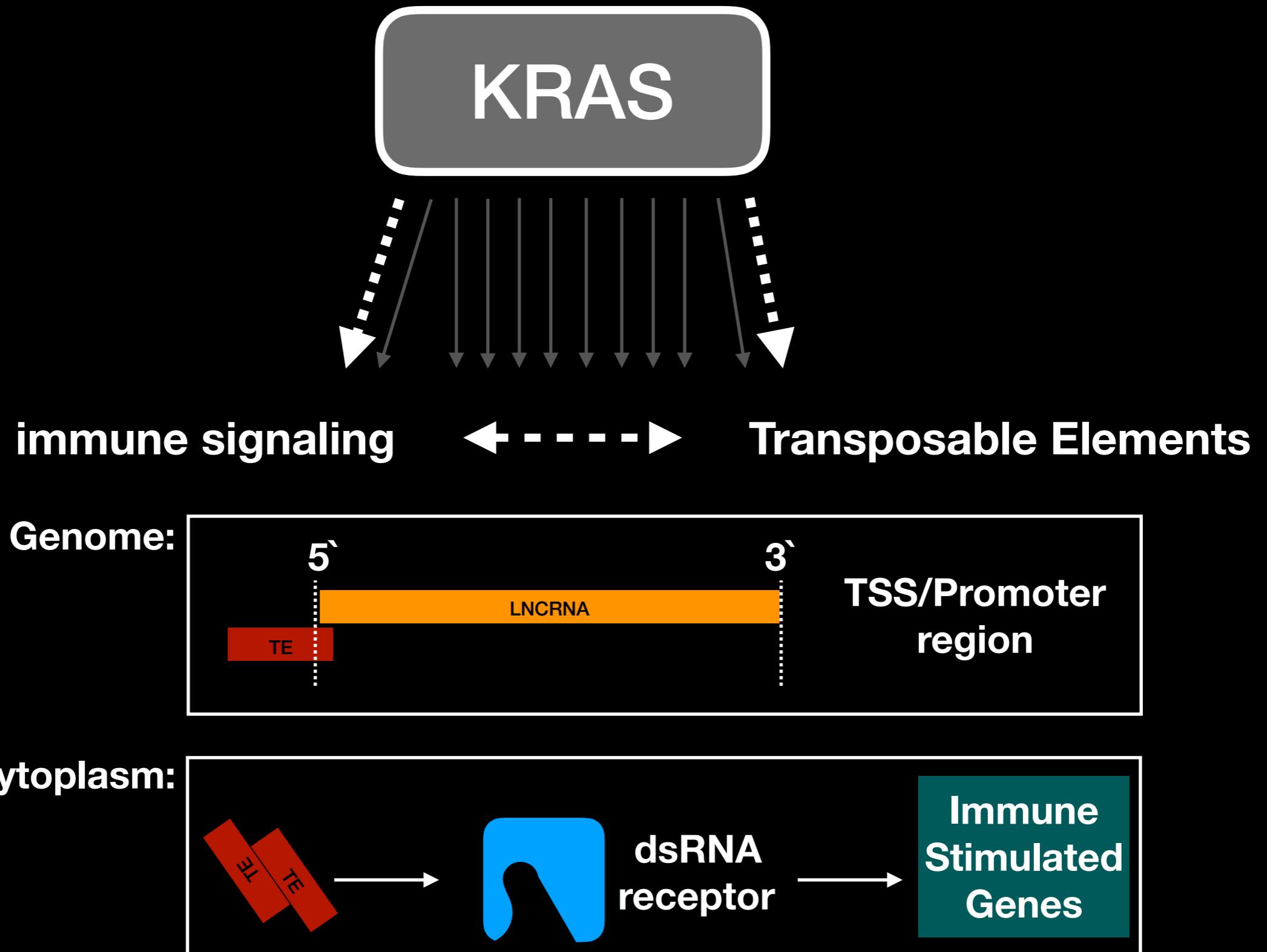
# what I'll talk about today:



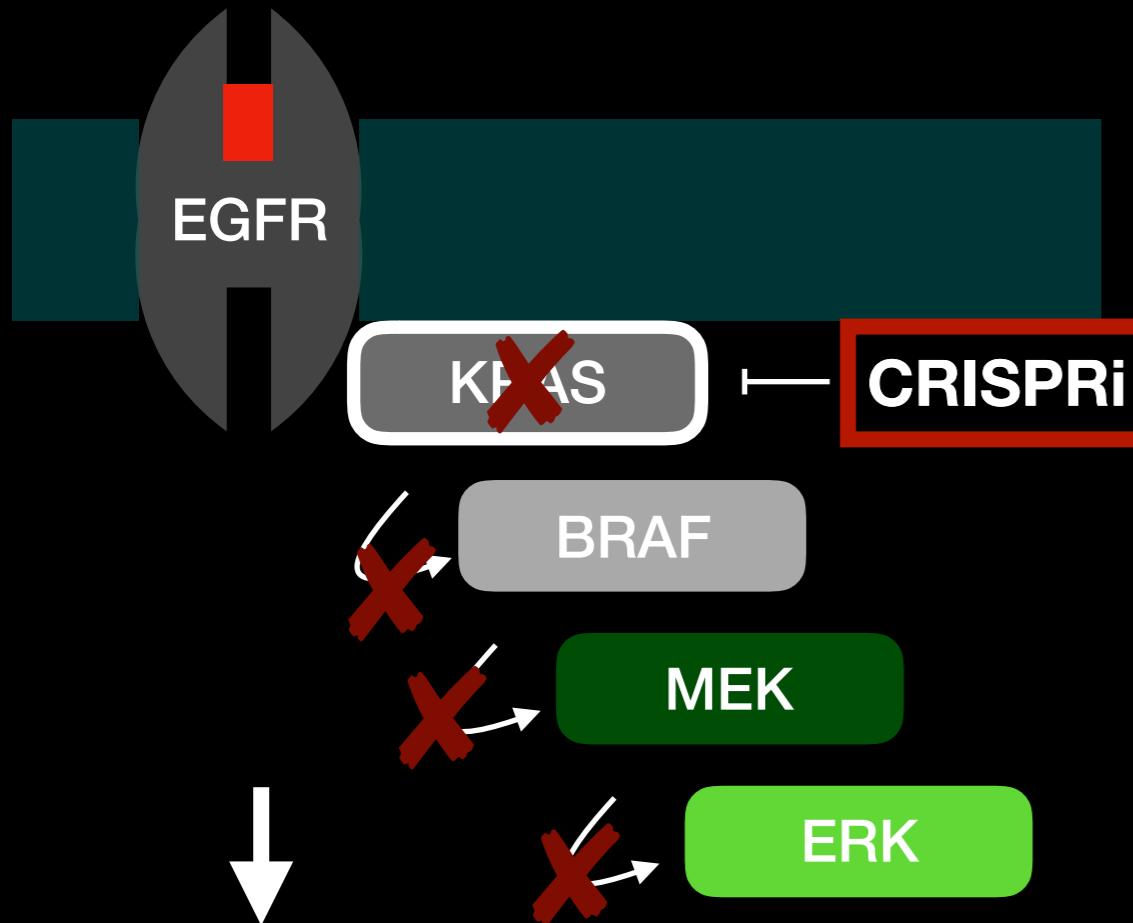
# what I'll talk about today:



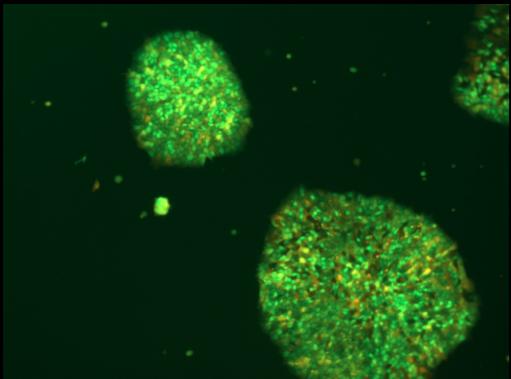
# what I'll talk about today:



# Model #1: CRISPRi induced pluripotent stem cells



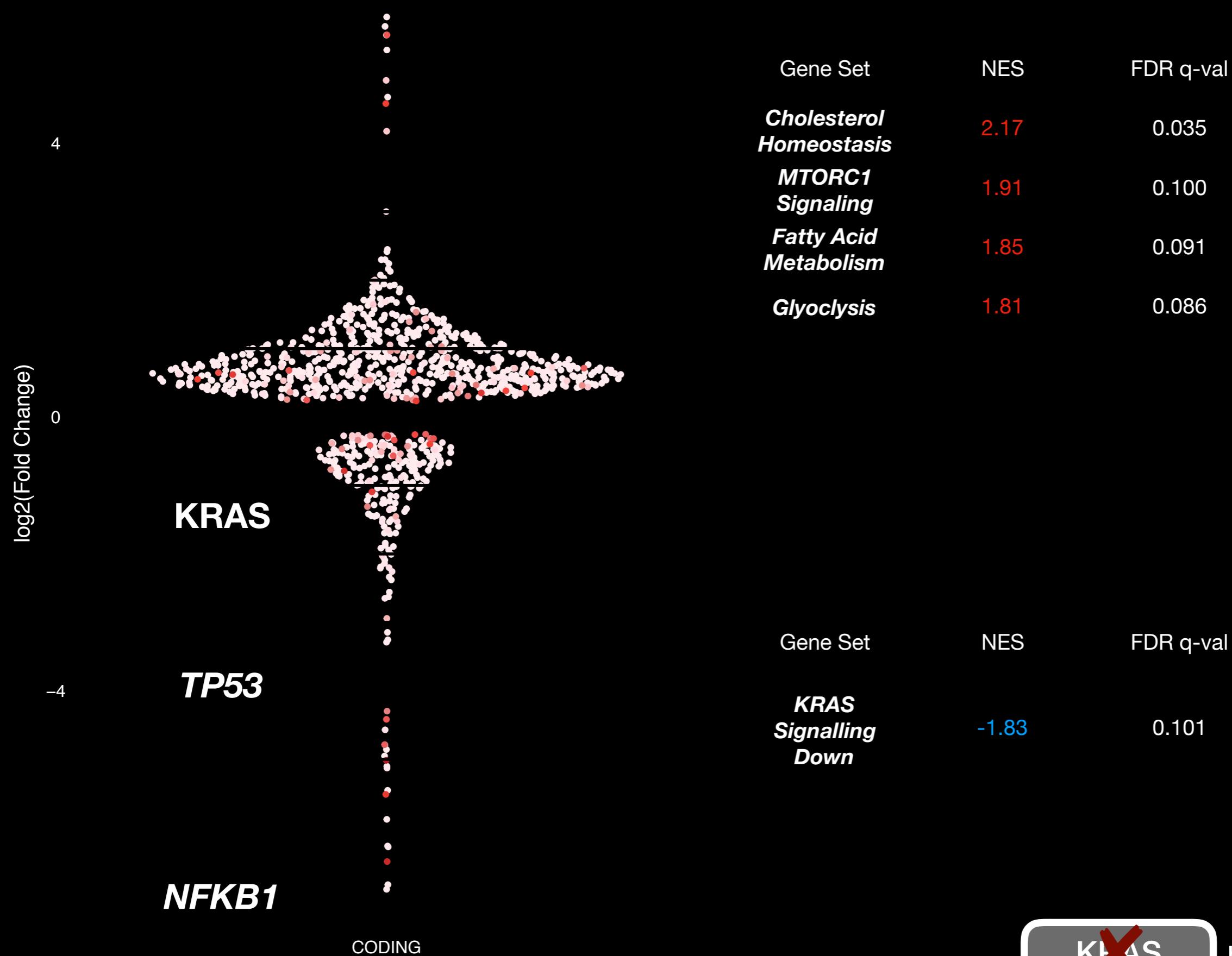
**Randomly Differentiate Embryoid Bodies (EBs)**



DOX selection,  
Stable KRAS<sup>KD</sup>  
iPSCs

- Bulk Illumina
- Single Cell 10x
- Direct RNA  
Nanopore

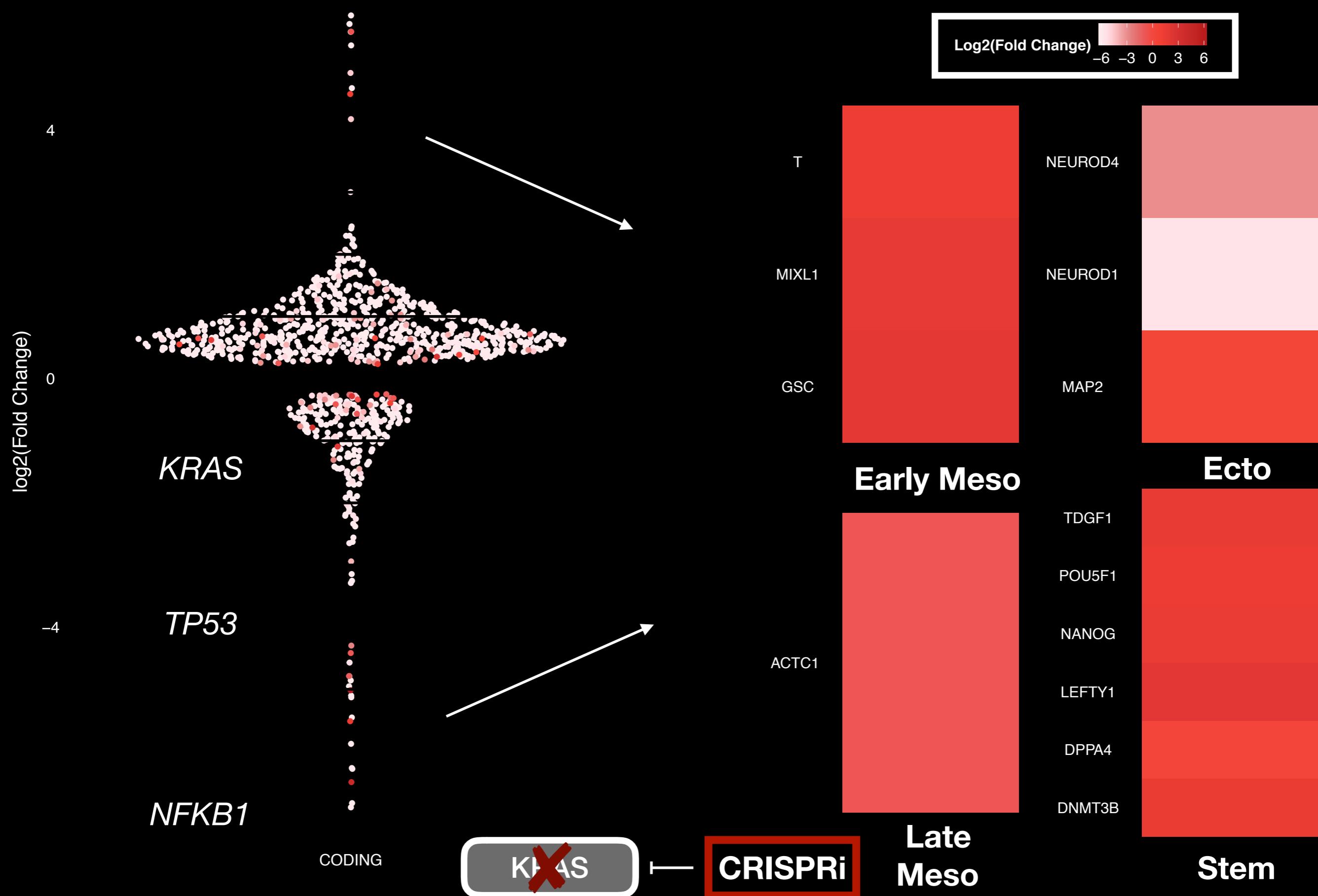
# broad transcriptional changes in KRAS<sup>KD</sup> EBs



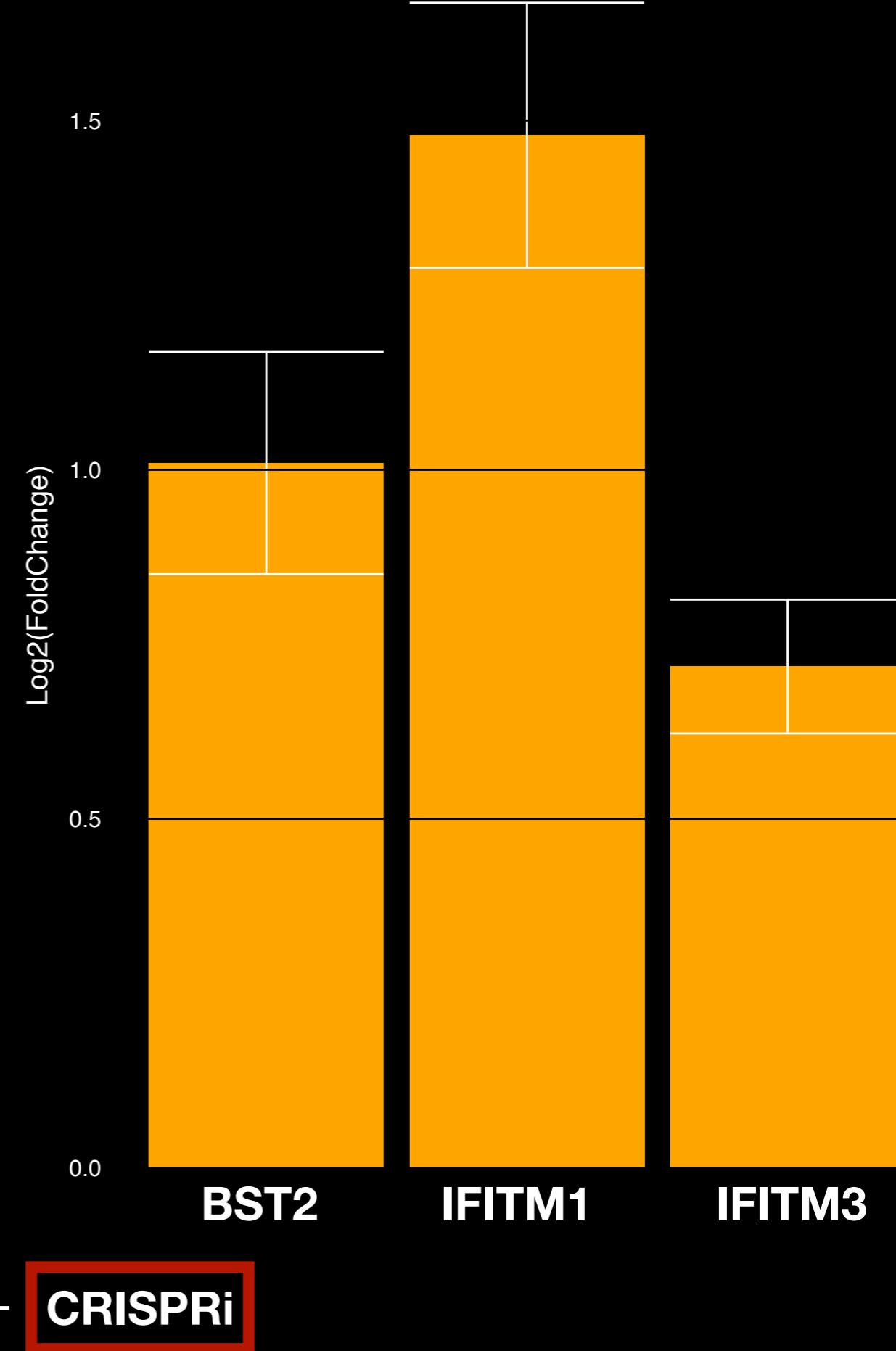
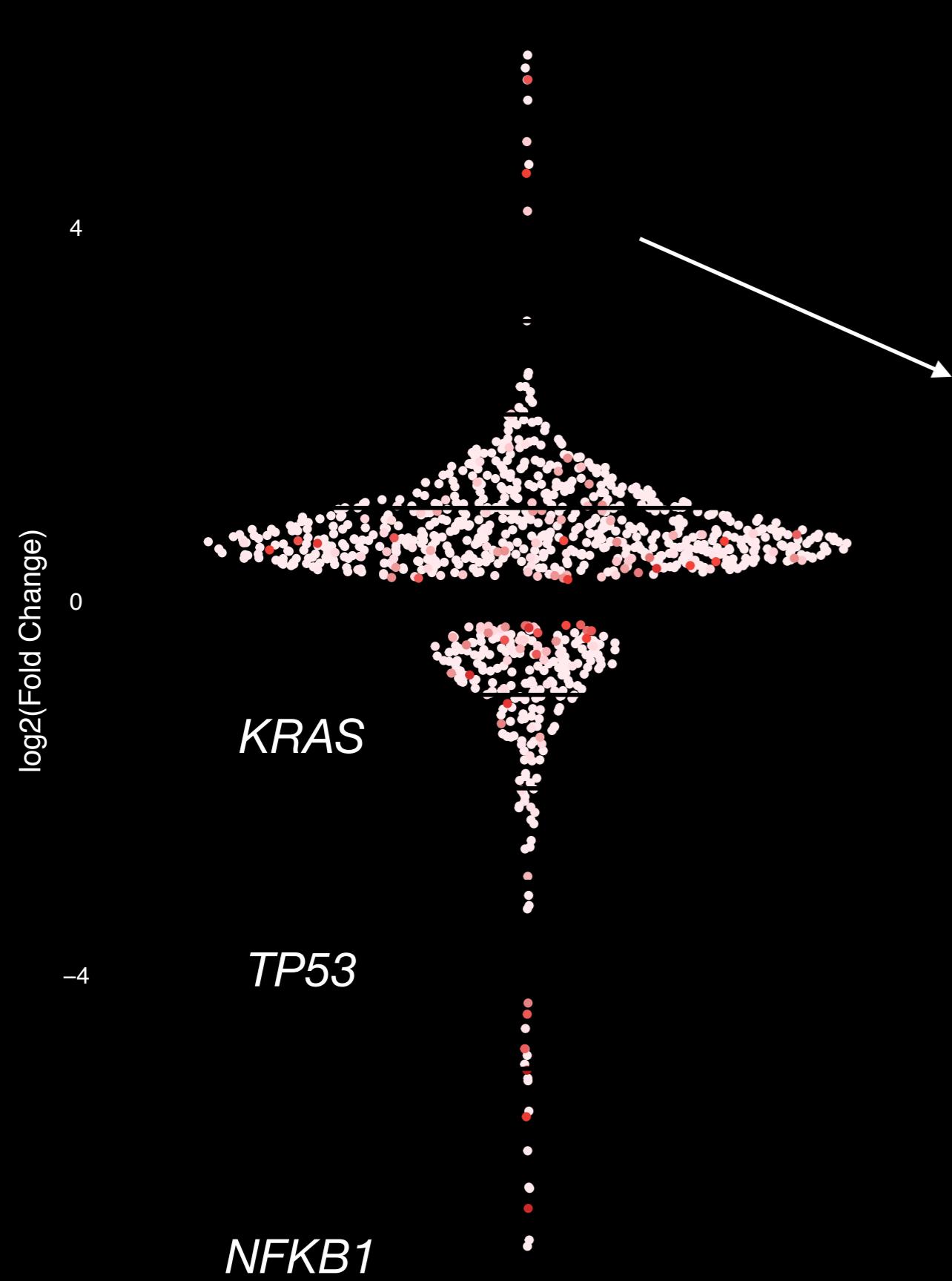
KRAS

CRISPRi

# lineage bias in KRAS<sup>KD</sup> EBs



# innate immune signaling in KRAS<sup>KD</sup> EBs



CODING

~~KRAS~~ — CRISPRi

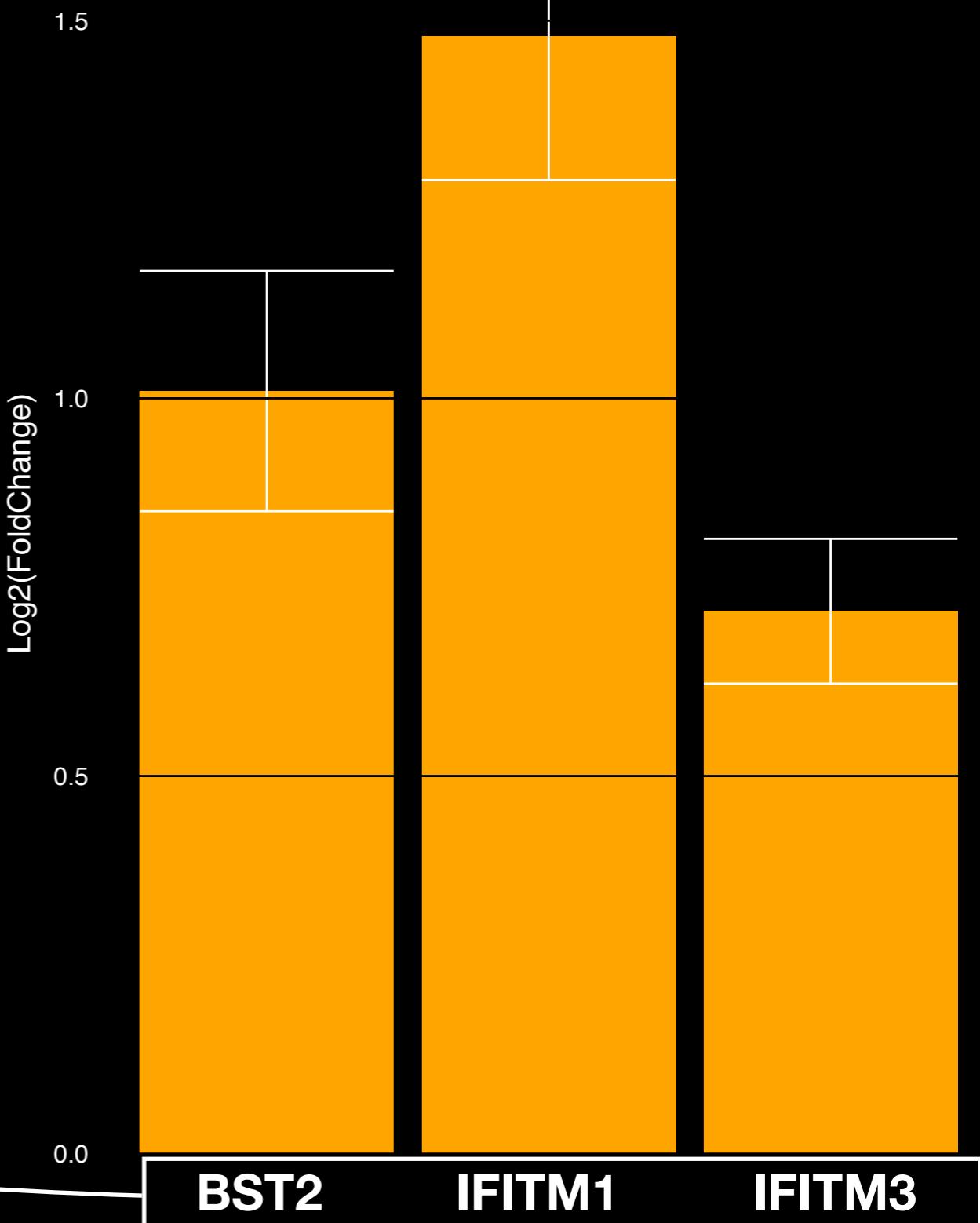
# innate immune signaling in KRAS<sup>KD</sup> EBs: pluripotent cells express a specific subset of immune stimulated genes

**IFITM:** Transmembrane Interferon induced antiviral protein, inhibits entry of virus into host cytoplasm

**BST:** Interferon induced antiviral protein, blocks release of mammalian enveloped viruses

*“Pluri/multipotent stem cells exhibit intrinsic expression of ISGs”*

Wu, X. et al. Intrinsic Immunity Shapes Viral Resistance of Stem Cells. *Cell* **172**, 423-438.e25 (2018).



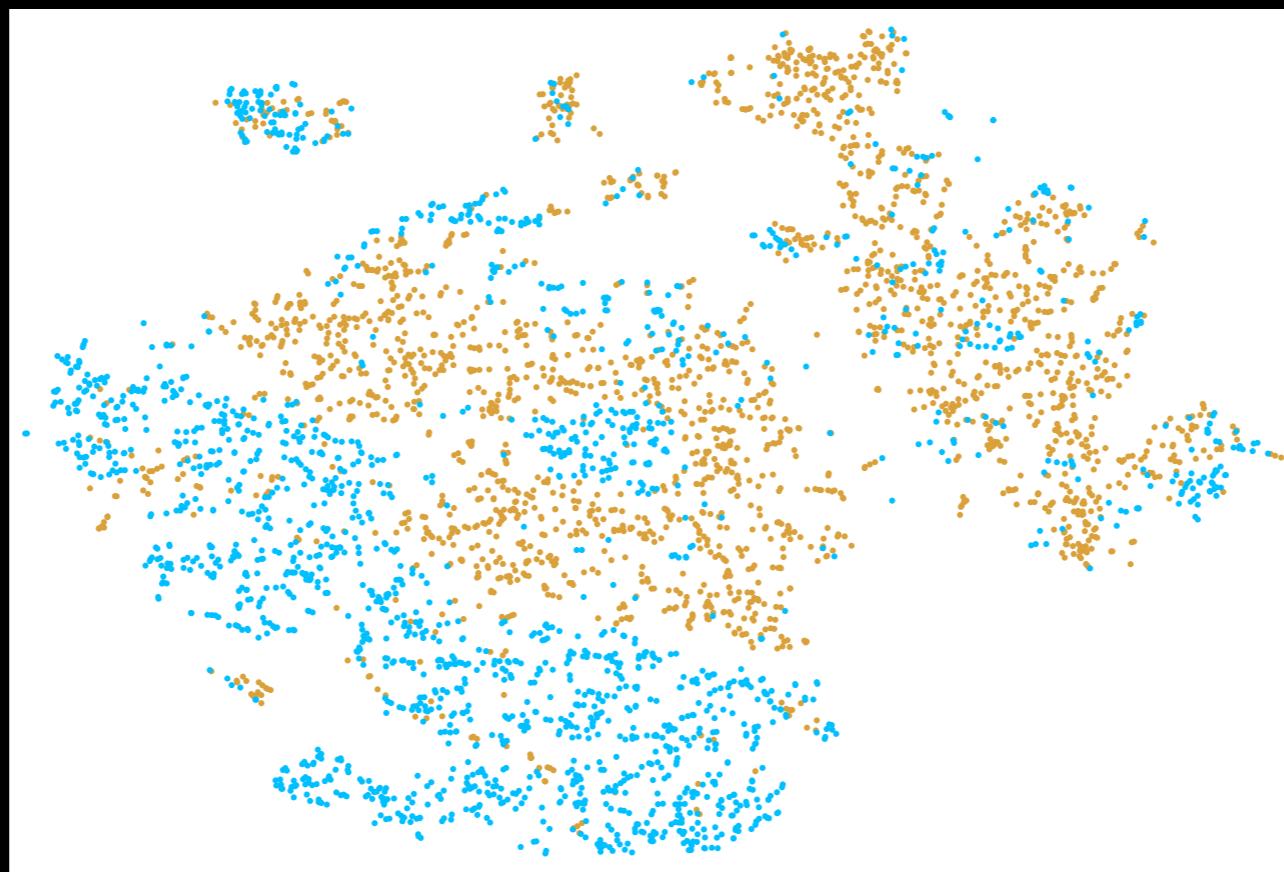
KRAS

CRISPRi

# lineage bias in KRAS<sup>KD</sup> EBs at single-cell resolution



*K-means Clusters*



*KRAS Expression*

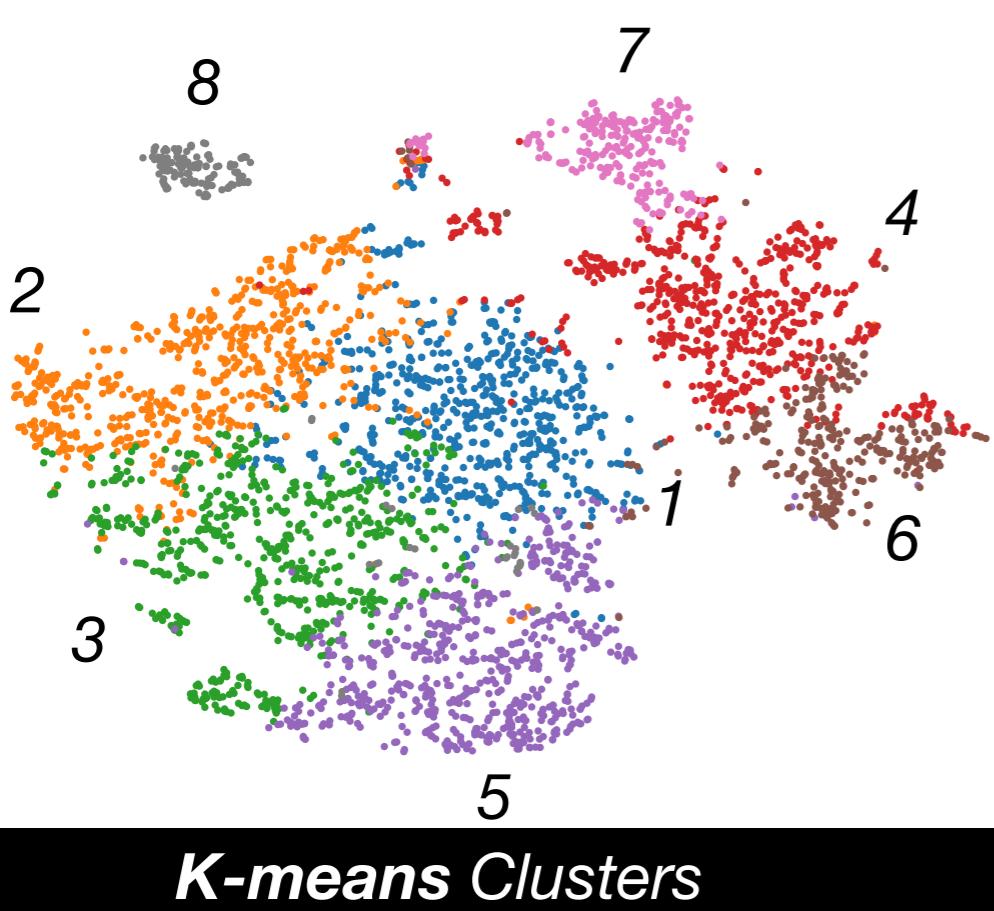
**SCRAMBLE** and **KRAS** Libraries

KRAS



CRISPRi

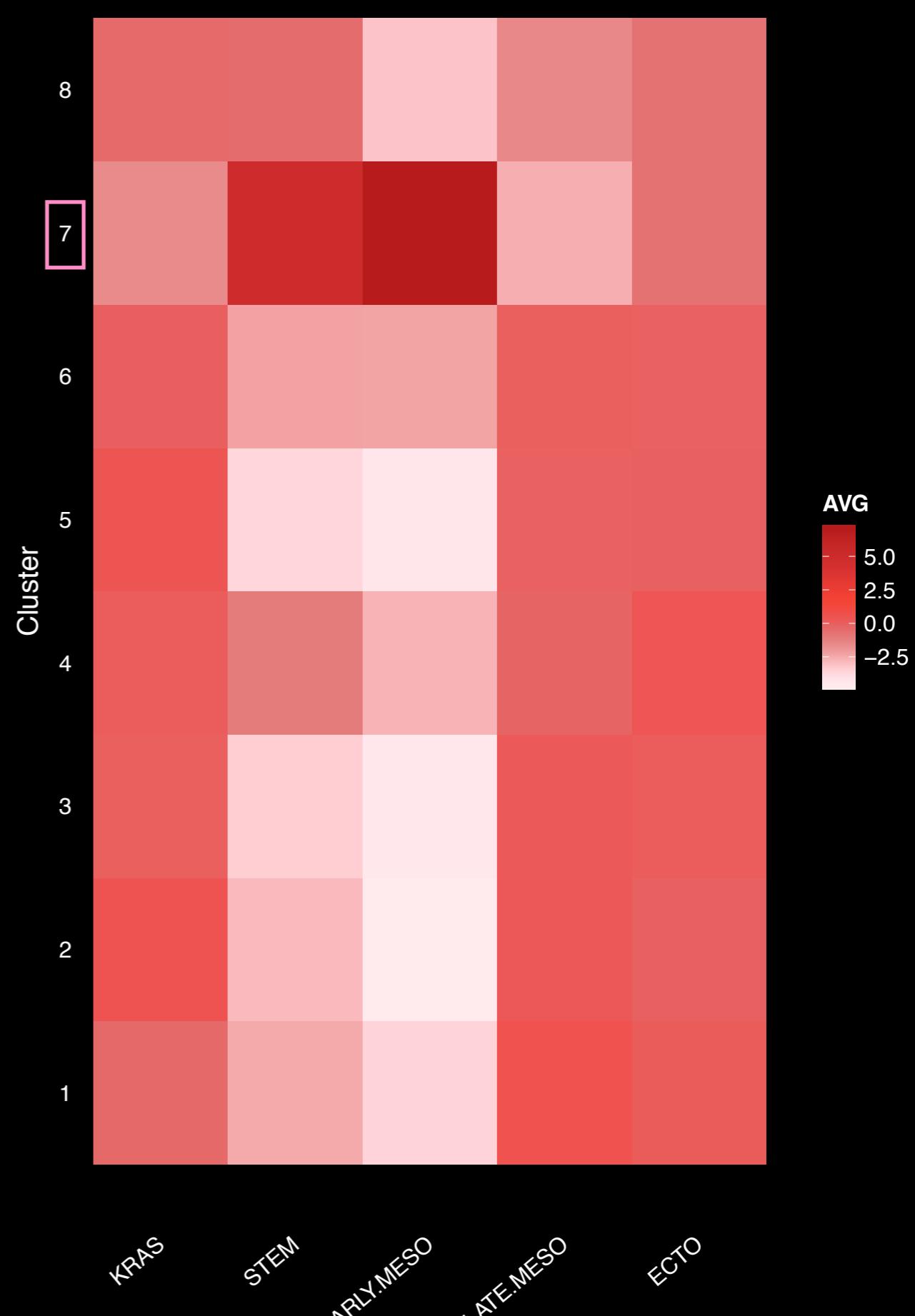
# lineage bias in KRAS<sup>KD</sup> EBs



*K-means Clusters*



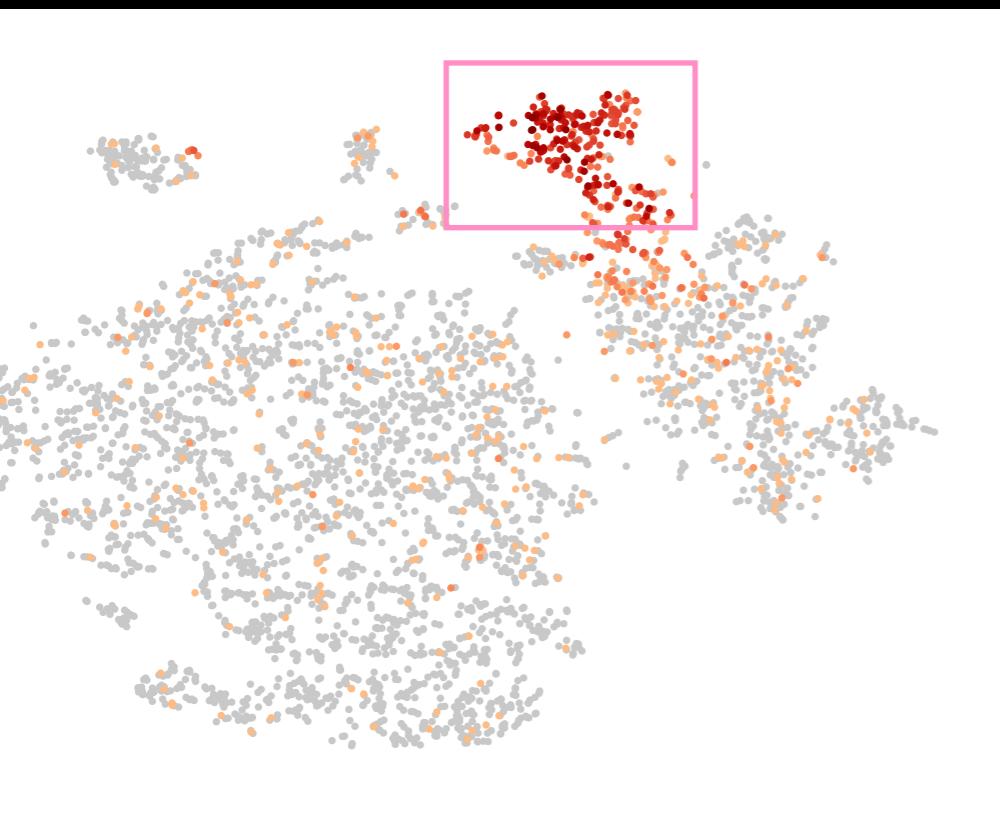
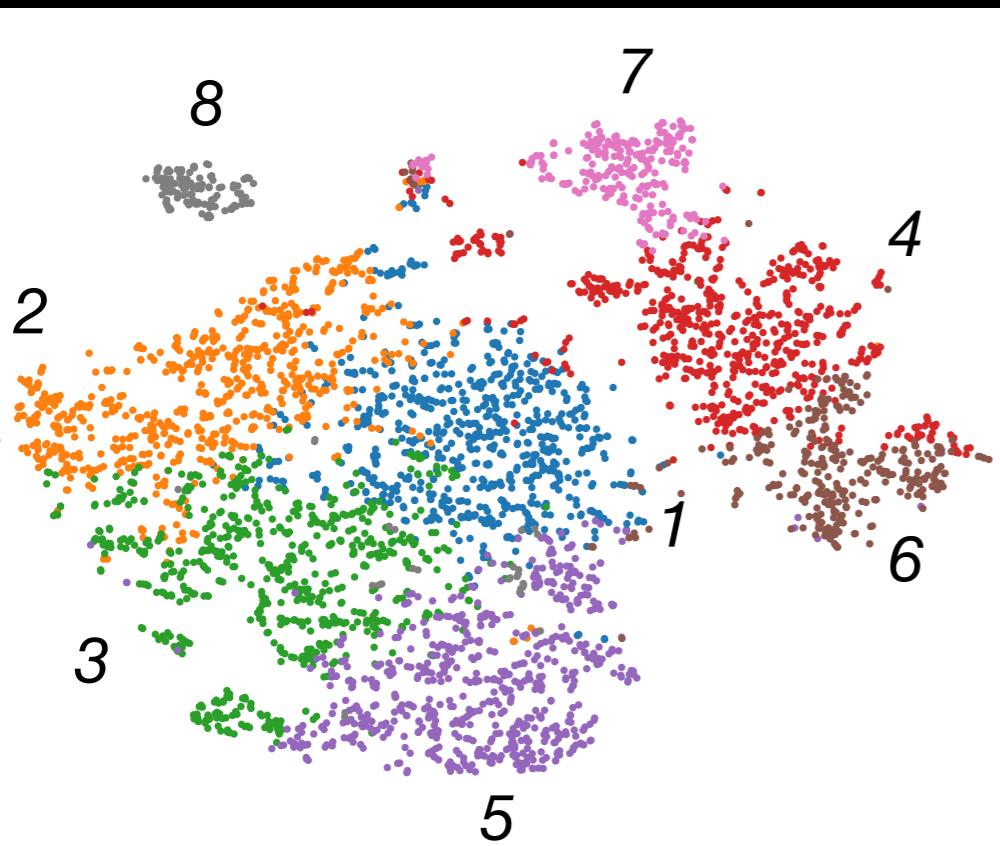
*KRAS Expression*



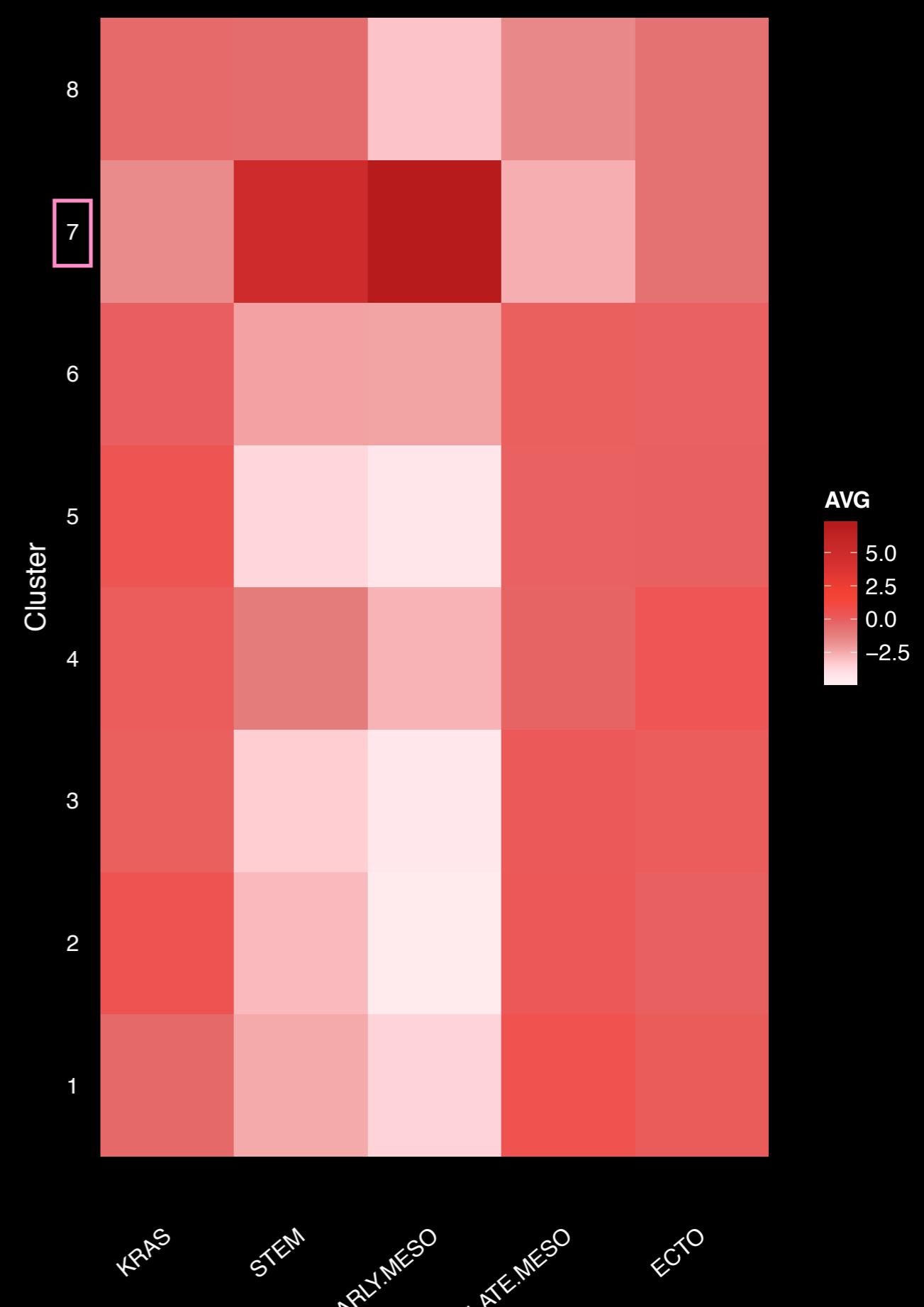
KRAS

CRISPRi

# lineage bias in KRAS<sup>KD</sup> EBs



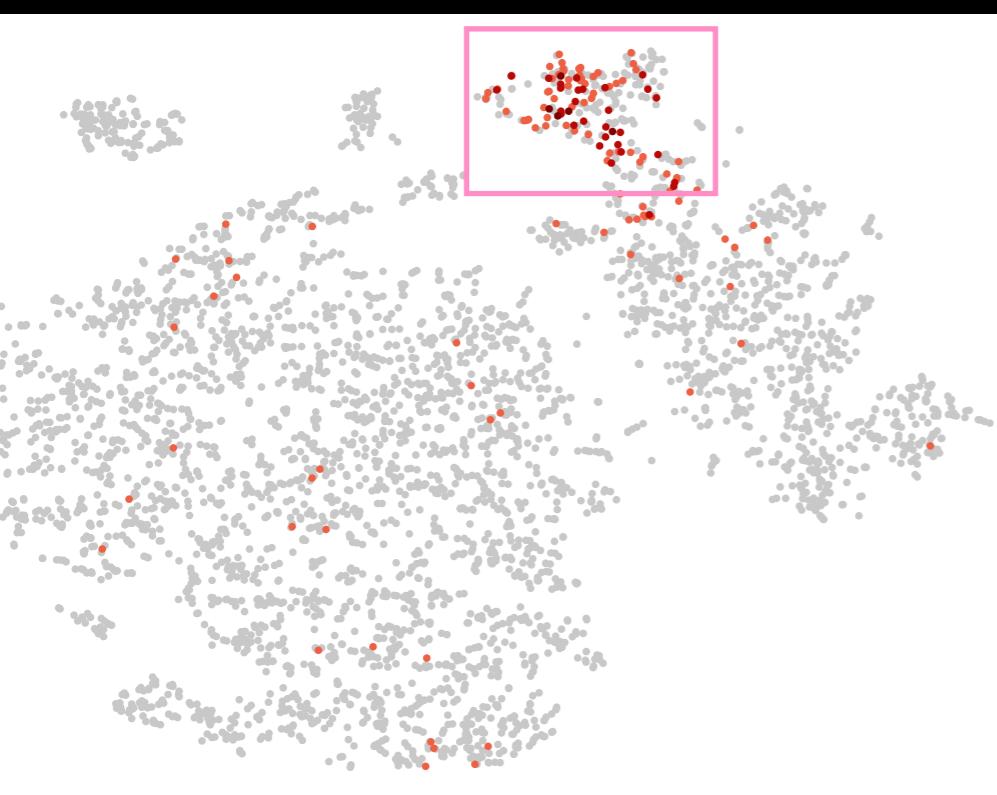
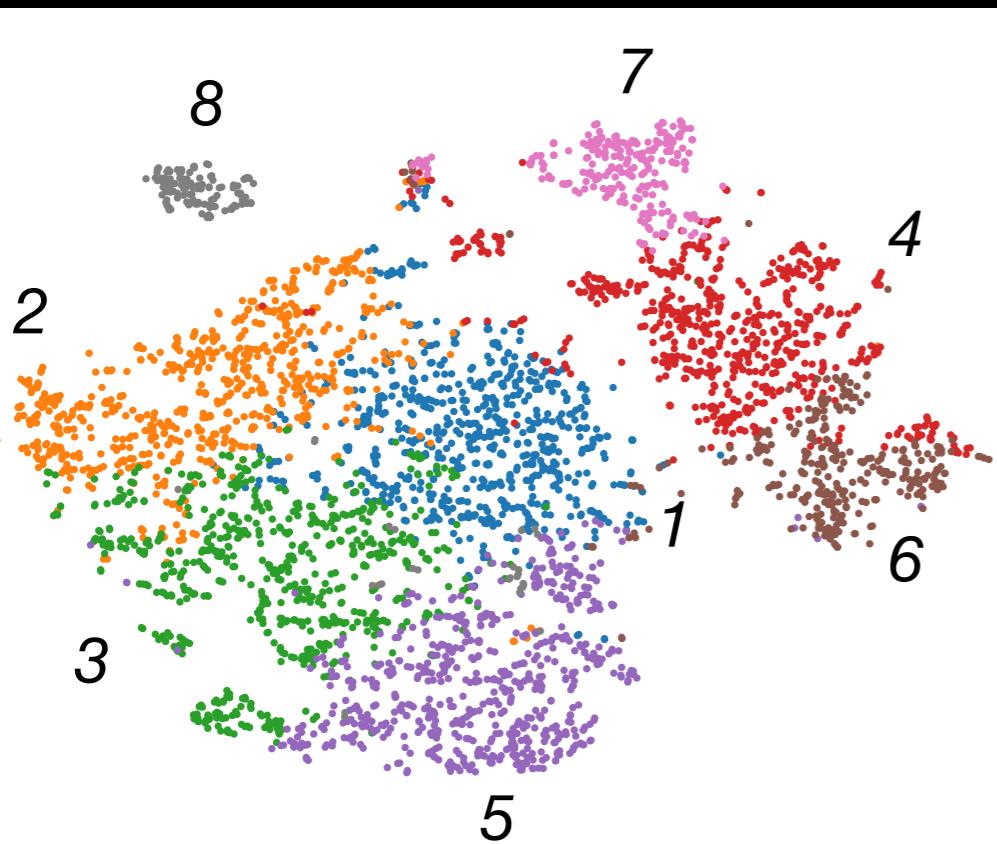
*OCT4 Expression*



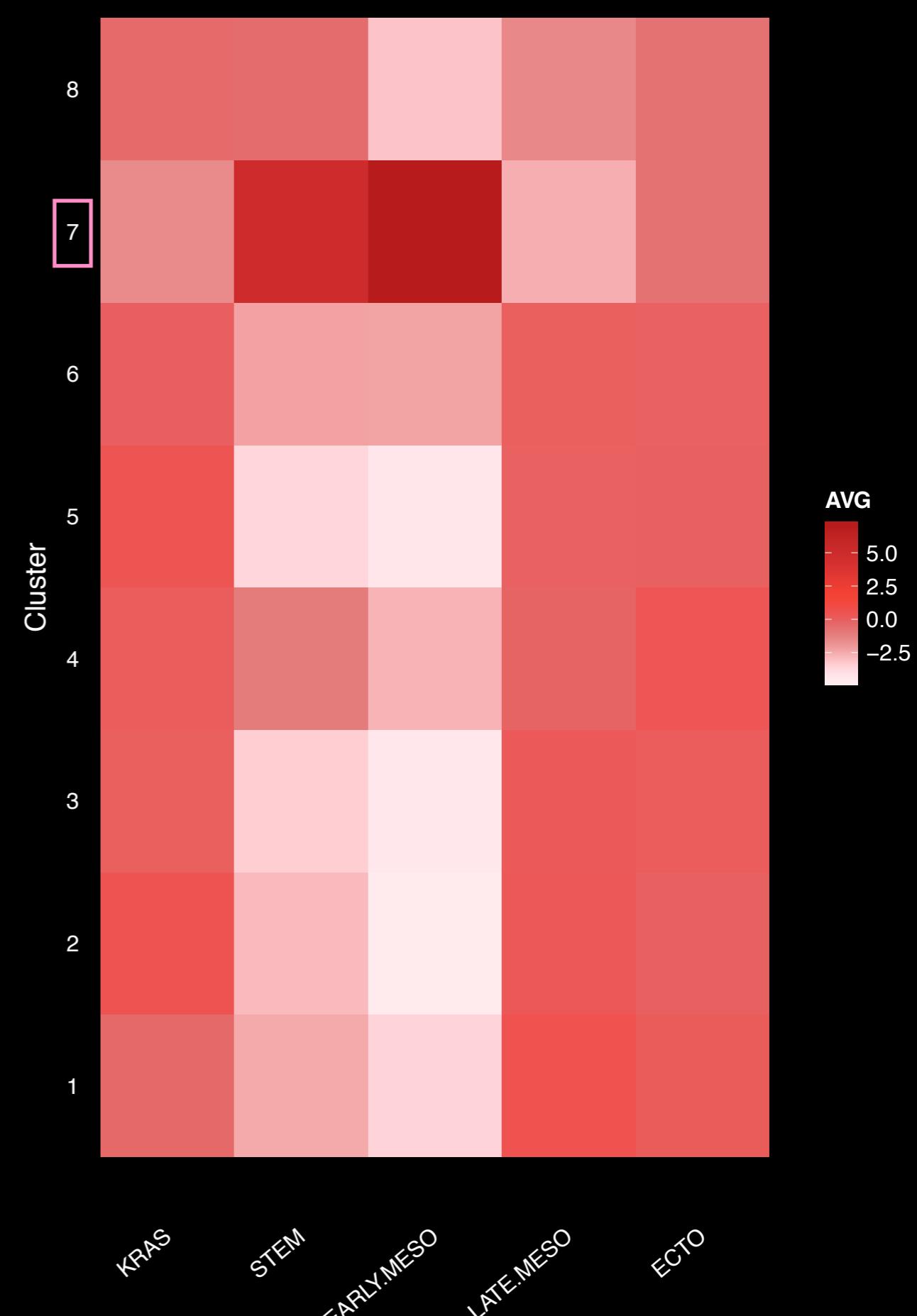
KRAS

CRISPRi

# lineage bias in KRAS<sup>KD</sup> EBs



*NANOG Expression*



KRAS

CRISPRi

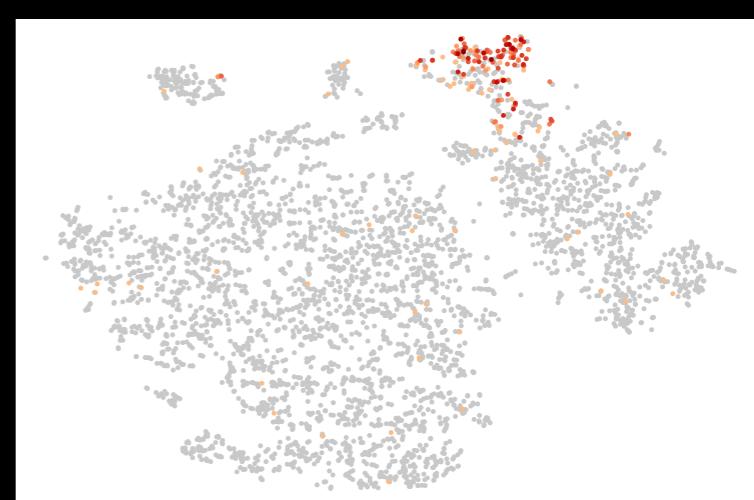
# this cluster also has significant enrichment of uncharacterized lncRNAs

## Top 10 Significant Genes

Gene	Log2(Fold Change)
LINC01356	7.10649714743636
GAL	6.95359492089621
TDGF1	7.58294672215199
<b>RP11-1144P22.</b>	<b>6.13612908550736</b>
MIXL1	7.0137424728284
POU5F1	5.37568016978003
CDA	5.96881660194689
NODAL	5.80414732595607
<b>LINC00458</b>	<b>6.9510762449523</b>
FOXH1	5.25916426320356



KRAS Expression



KRAS



CRISPRi

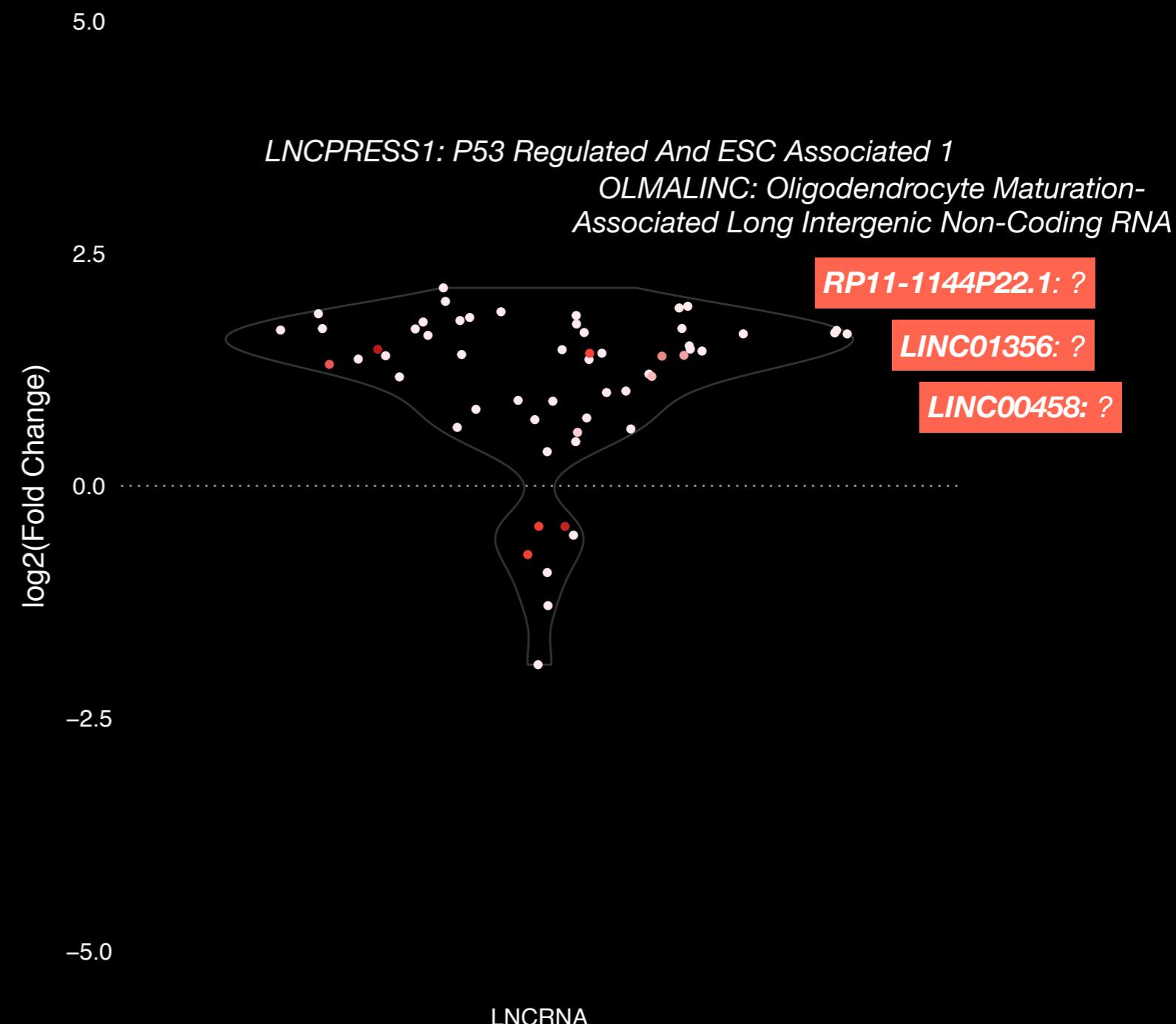
# uncharacterized lncRNAs are upregulated in both cluster 7 and the bulk RNA-seq data

## Single Cell, Cluster 7

### Top 10 Significant Genes

Gene	Log2(Fold Change)
LINC01356	7.10649714743636
GAL	6.95359492089621
TDGF1	7.58294672215199
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CDA	5.96881660194689
NODAL	5.80414732595607
LINC00458	6.9510762449523
FOXH1	5.25916426320356

## Bulk, lncRNAs

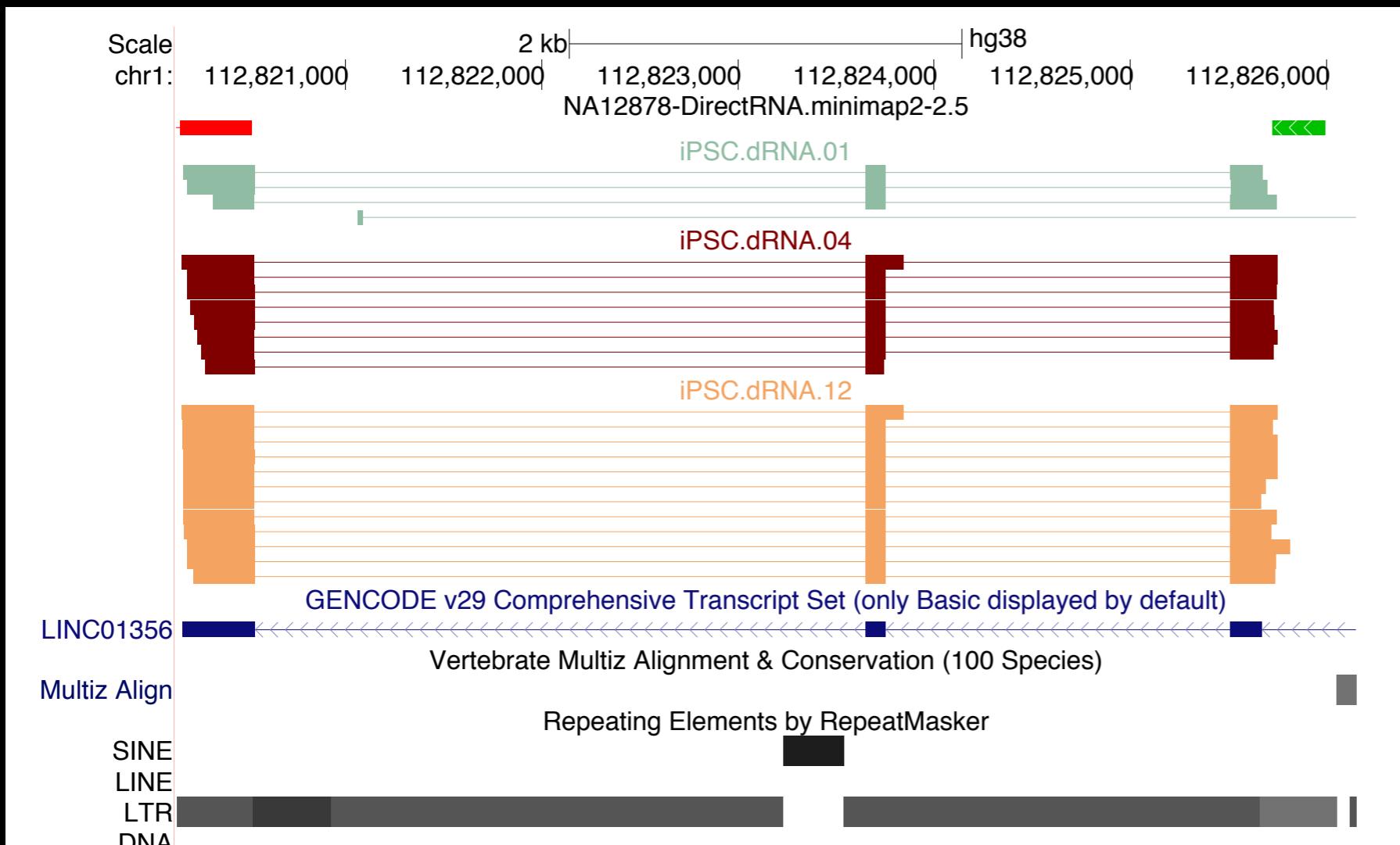


KH4S

CRISPRi

# long non-coding RNA upregulated in KRAS<sup>KD</sup> EBs are closely associated with LTR7

## LINC01356



HERVH ← LTR7

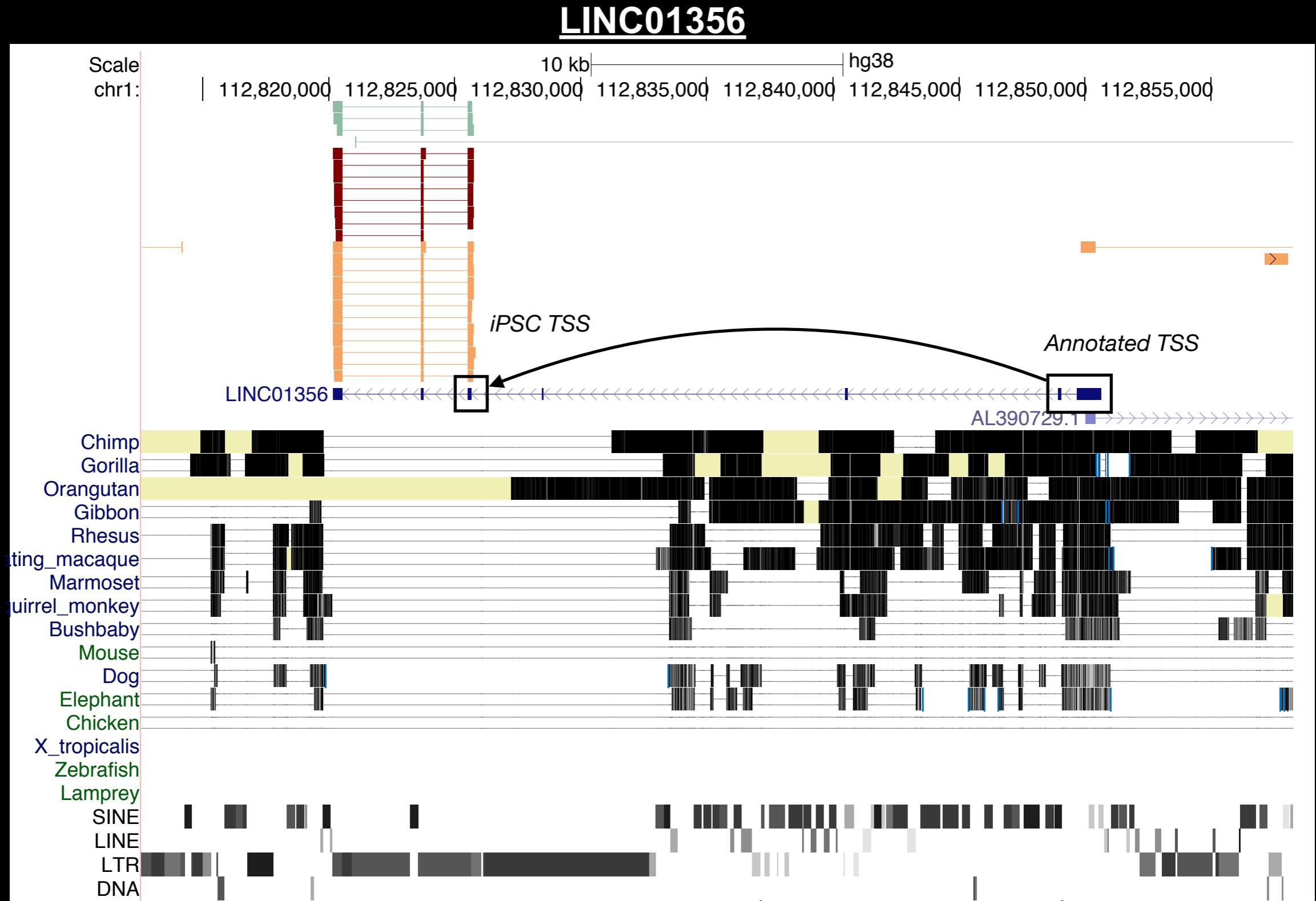


*LINC01356 Expression*

KRAS

CRISPRi

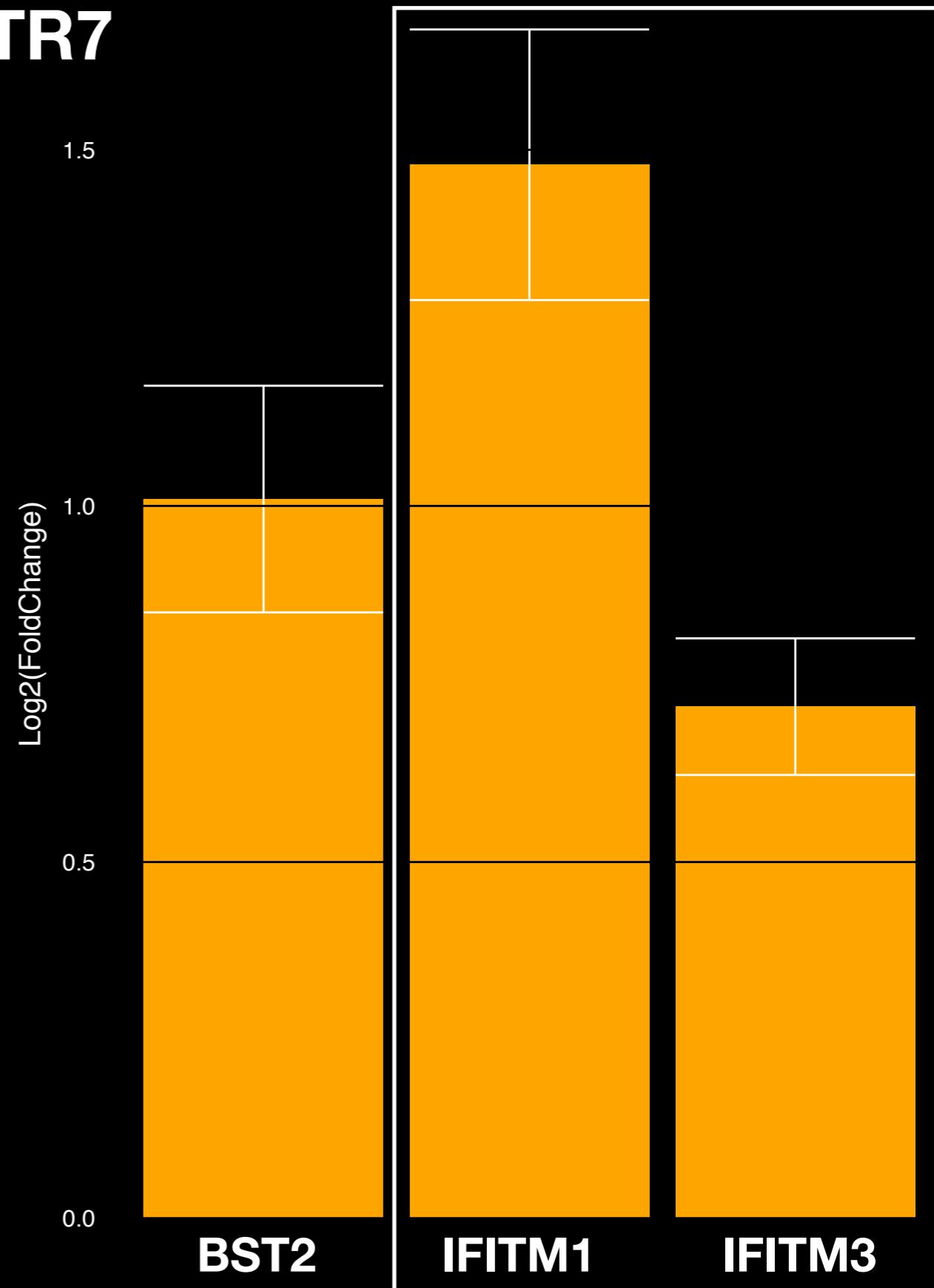
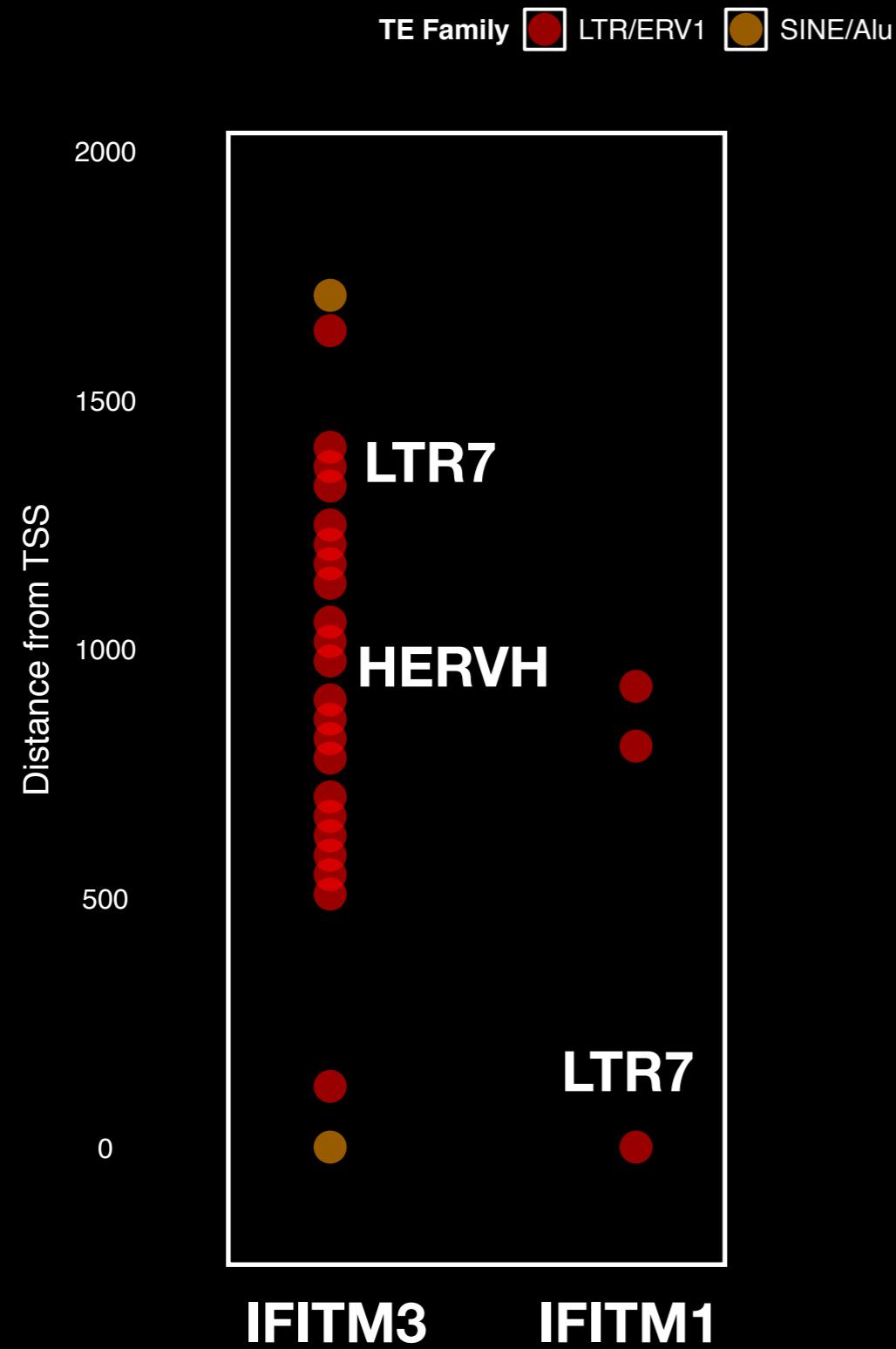
# LTR7 can serve as an alternative TSS in KRAS<sup>KD</sup> EBs



KRAS

CRISPRi

# Innate immune signaling in KRAS<sup>KD</sup> EBs is also associated with LTR7



# Take Aways:

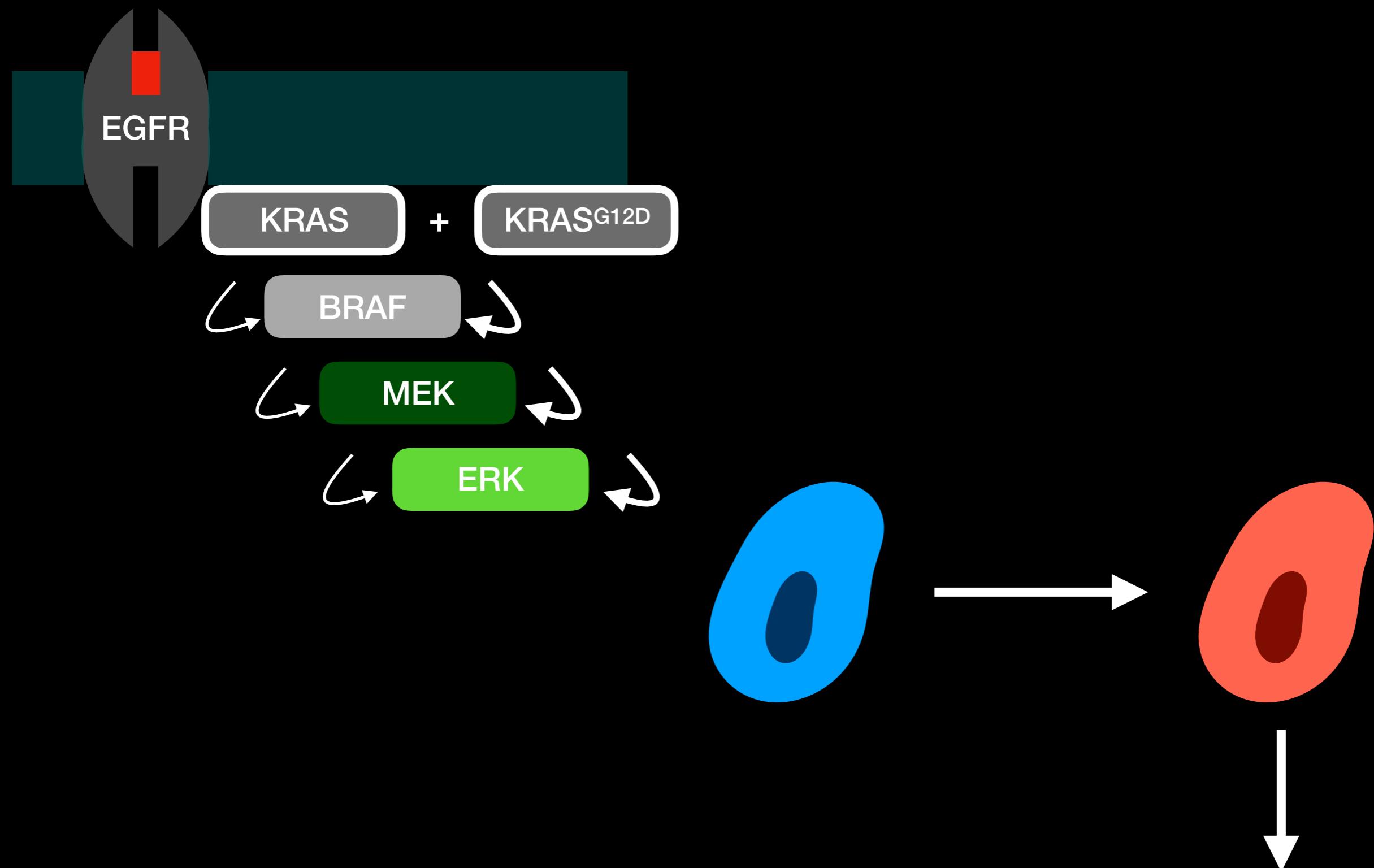
## iPSC/EB Model



### KRAS is required for:

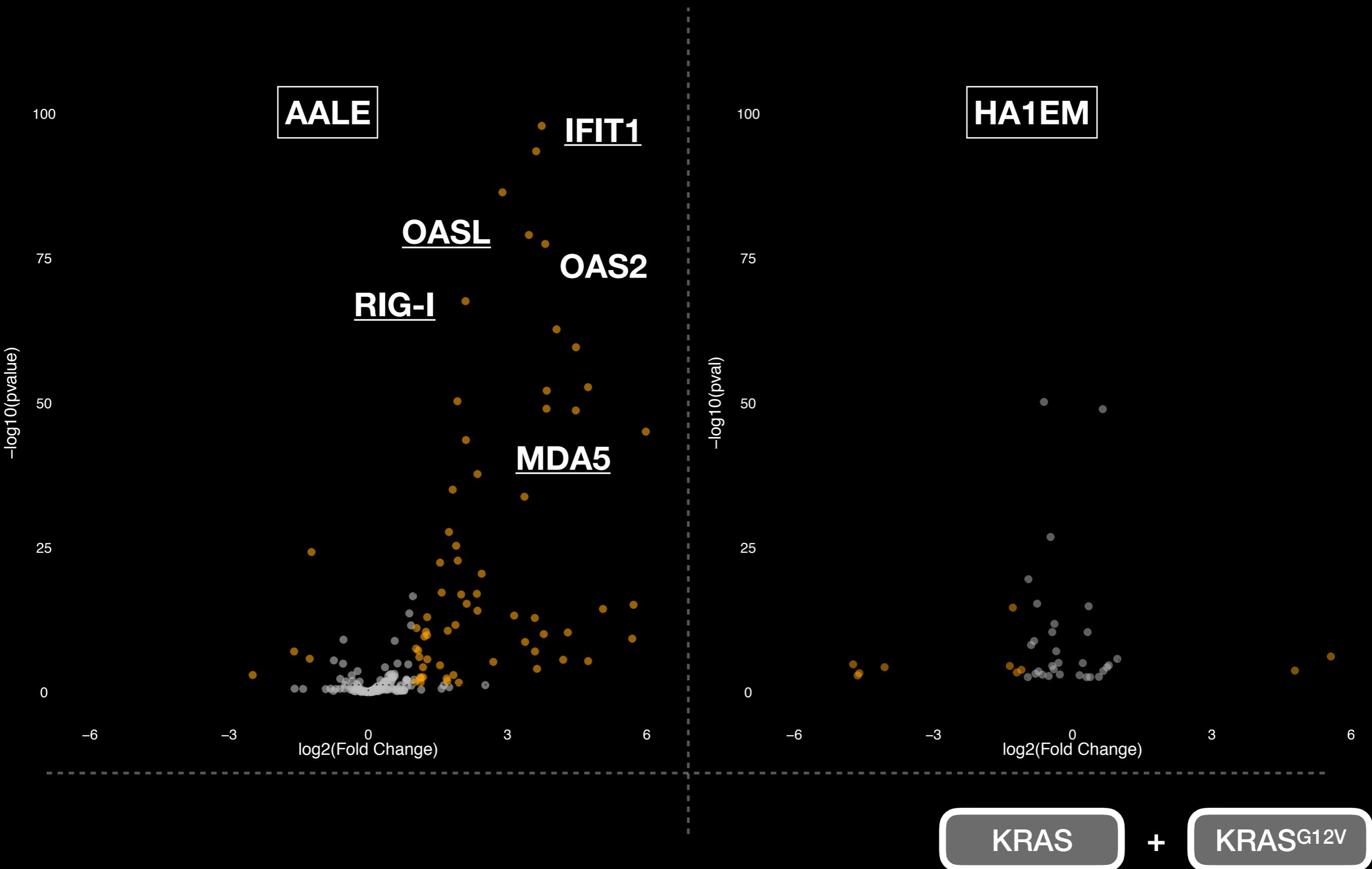
- Proper regulation of LTR/LTR-derived lncRNA expression
- Proper stem cell differentiation, particularly ectoderm
- Proper exit from pluripotent state

# Model #2: lung and kidney cells expressing mutant KRAS

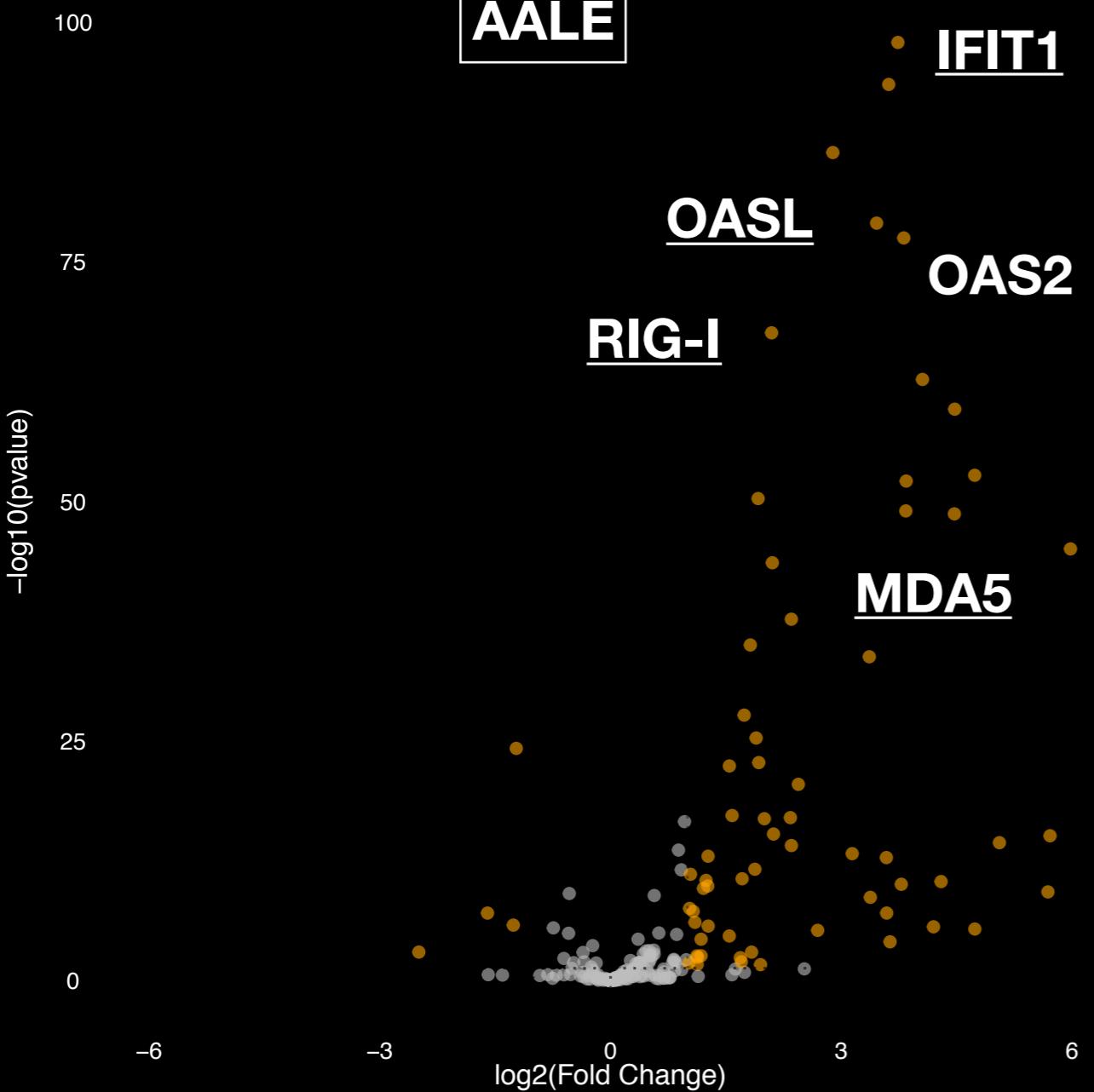


- Bulk Illumina
- Single Cell 10x

# mutant KRAS upregulates immune signaling gene expression in lung in the absence of interferon



# mutant KRAS upregulates immune signaling gene expression in lung in the absence of interferon



IFIT1 - ssRNA binding

RIG-I

MDA5

OASL

OAS2

**OAS2 - activated by upstream dsRNA binding proteins, activates immune response**

Cytoplasm:

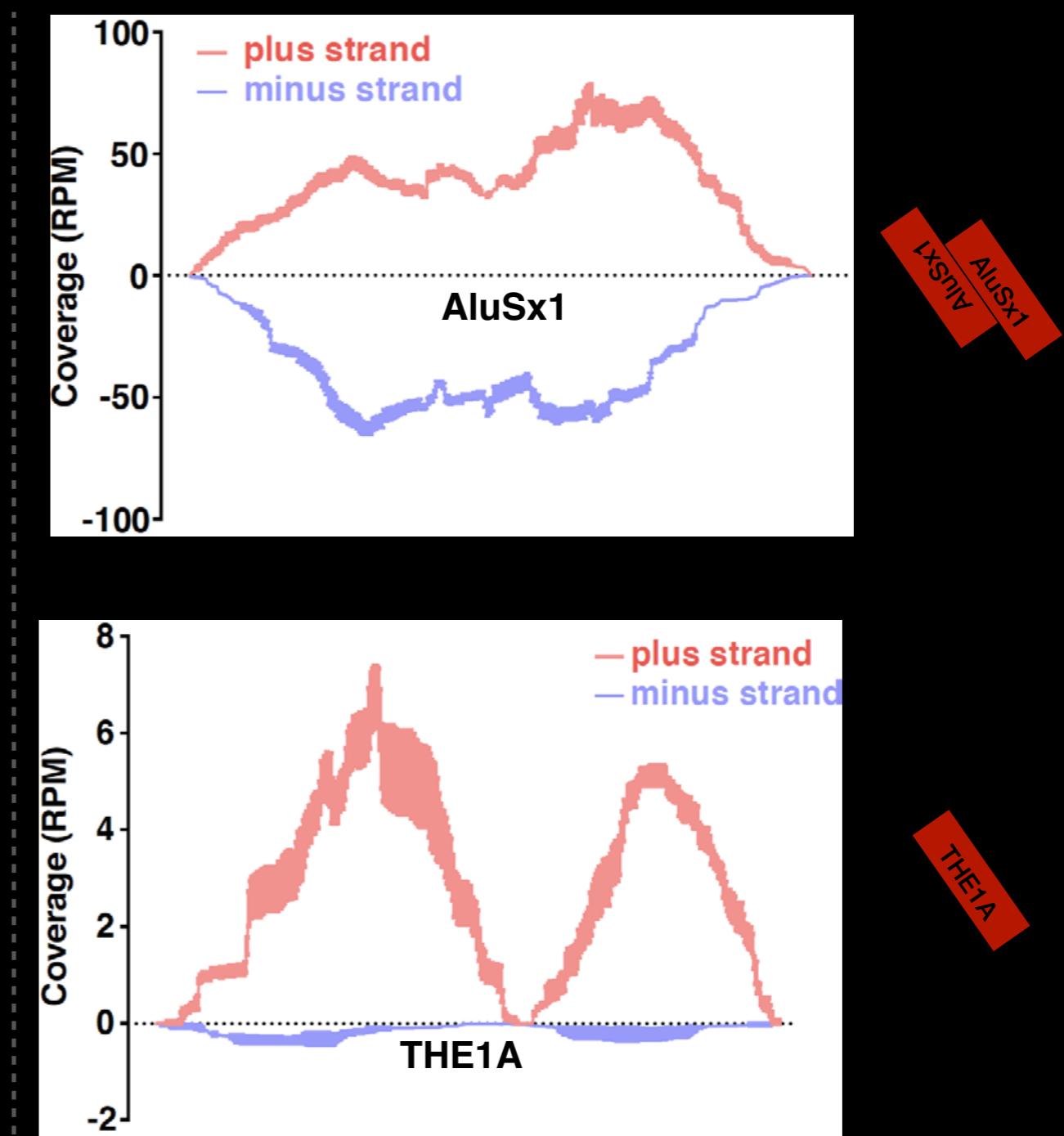
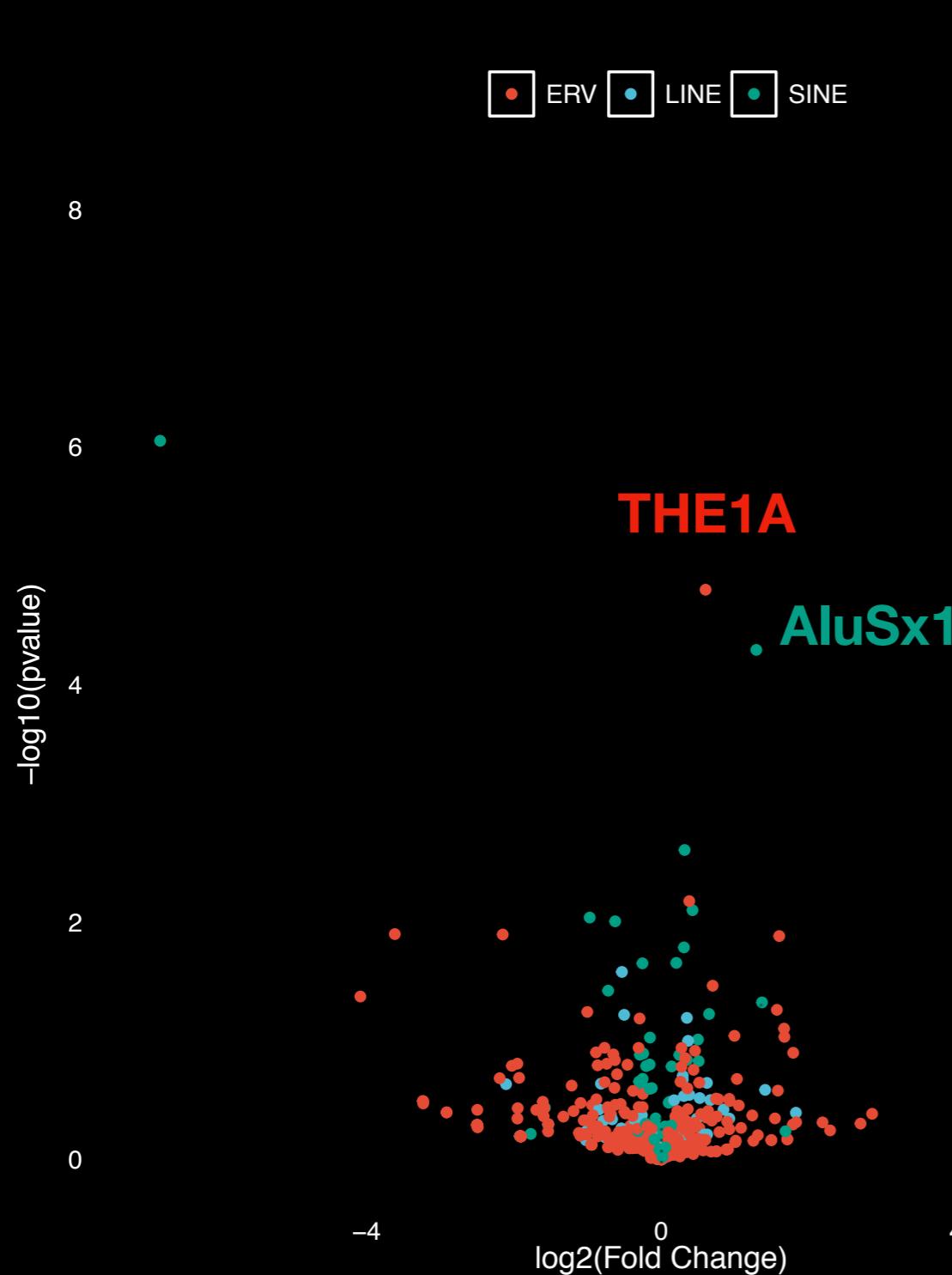


KRAS

+

$\text{KRAS}^{\text{G12V}}$

# mutant KRAS induces up regulation of Alu transcripts, particularly AluSx1

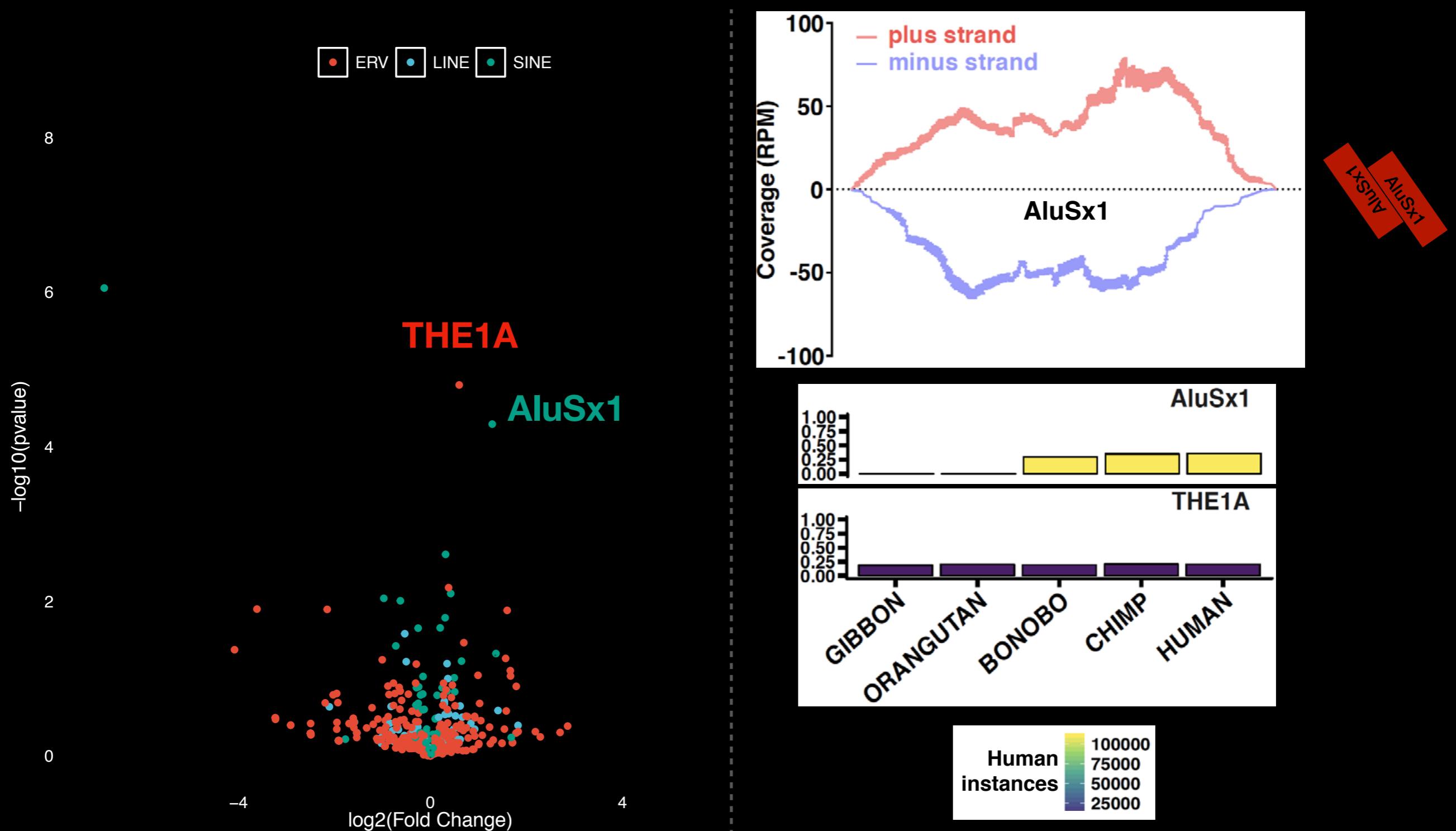


KRAS

+

KRAS<sup>G12V</sup>

# AluSx1 is detected from both strands, is highly abundant in the genome, and a more recent insertion than THE1A

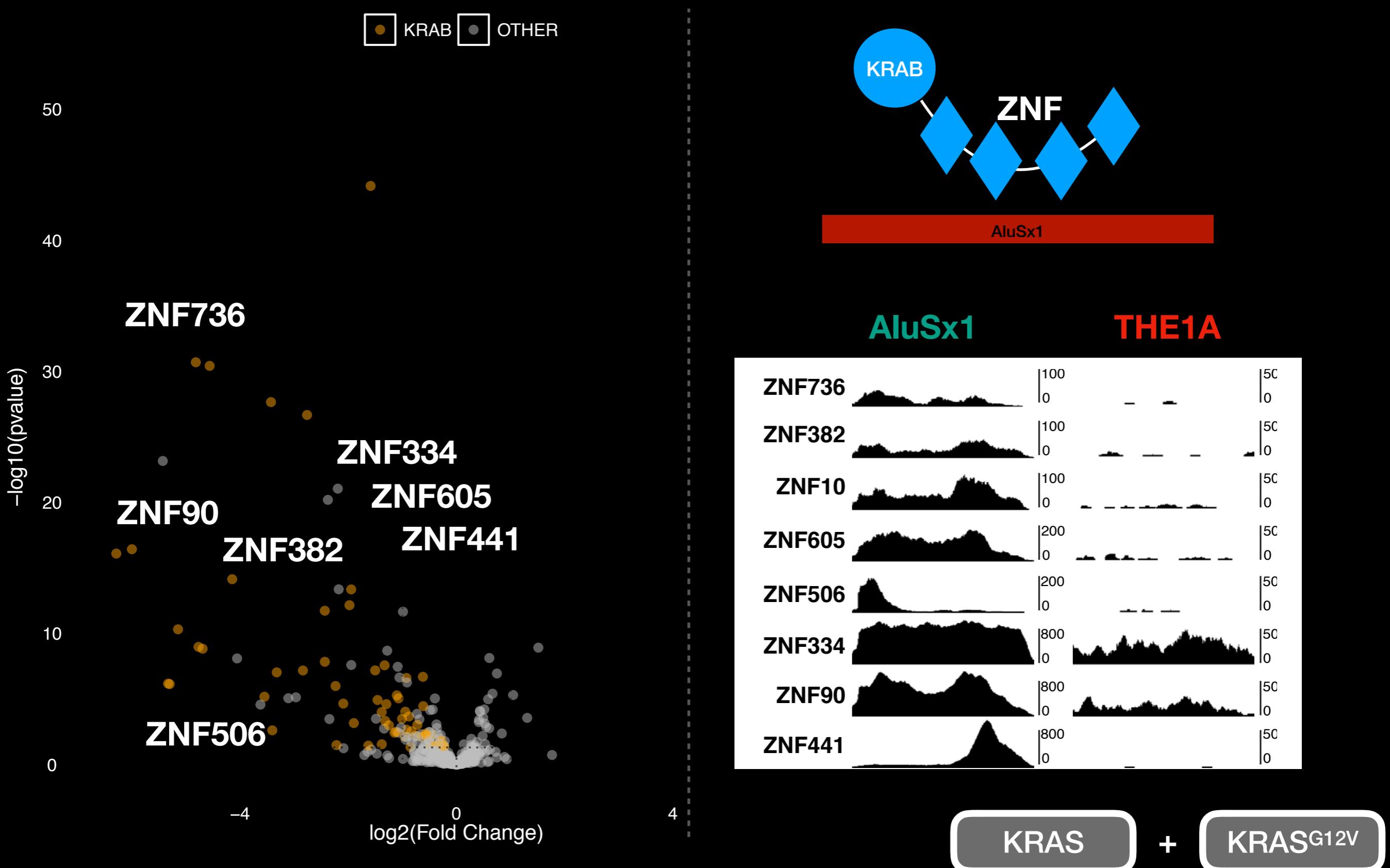


KRAS

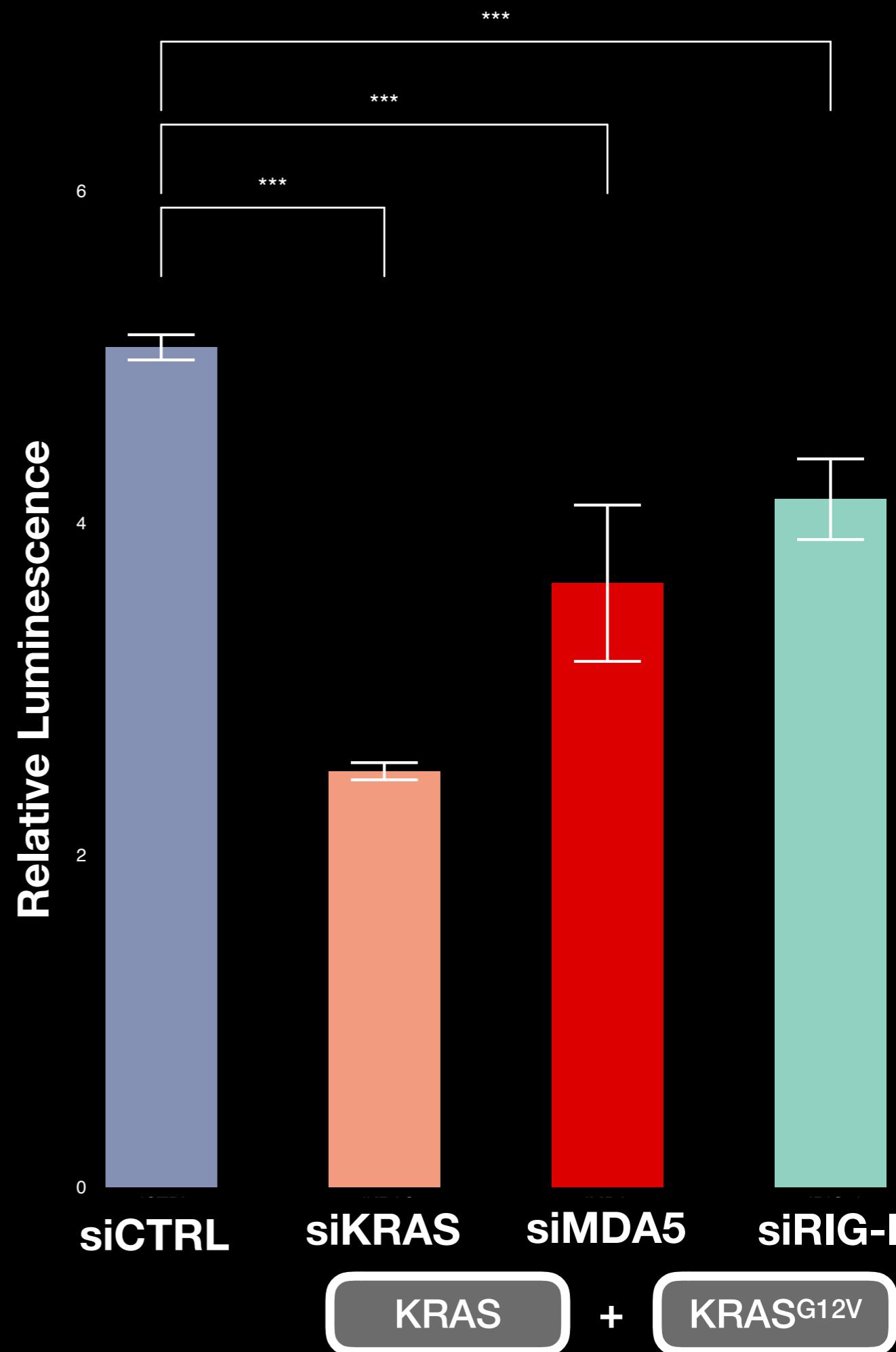
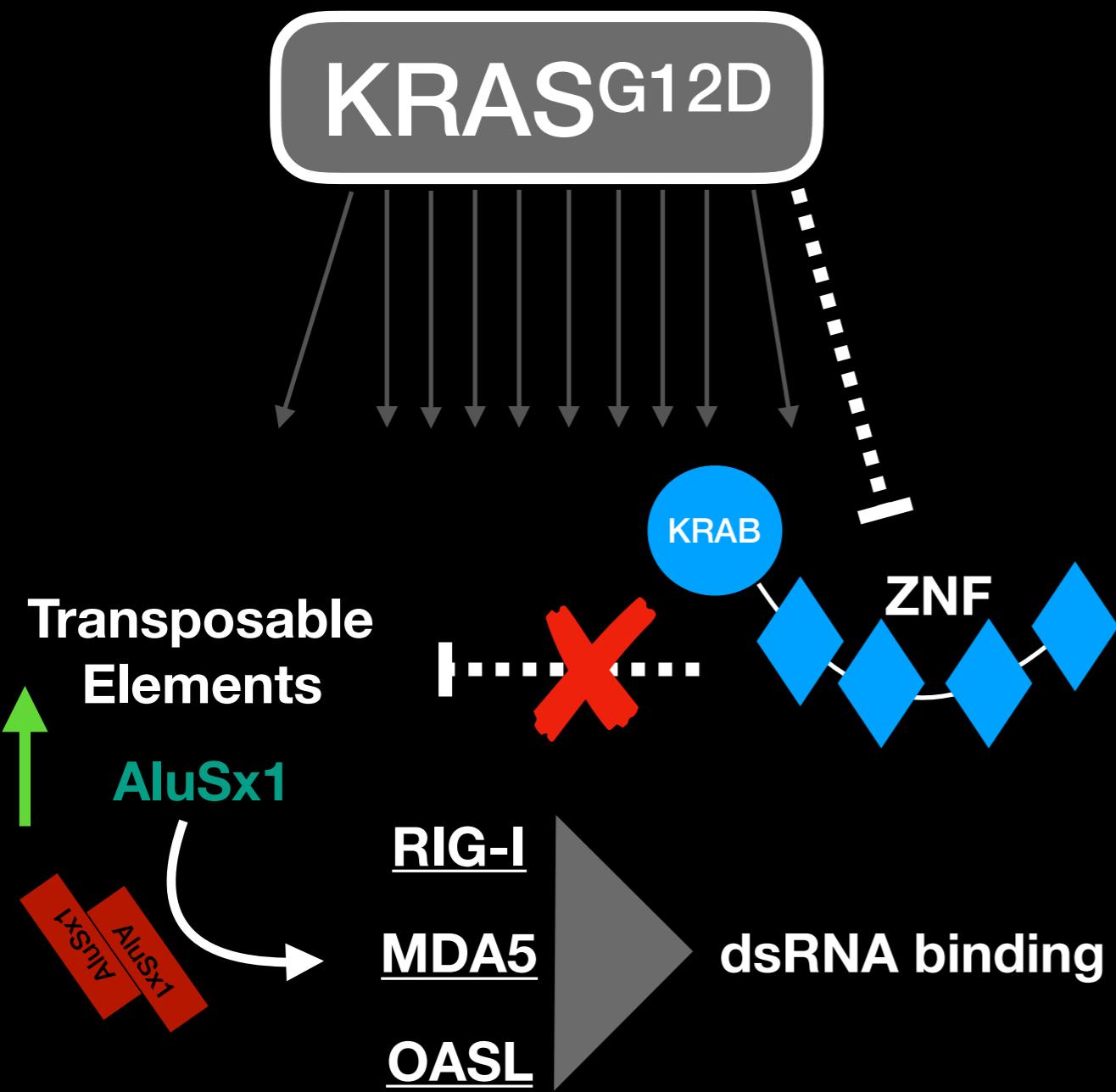
+

KRAS<sup>G12V</sup>

# mutant KRAS induces large scale down regulation of KRAB ZNFs



# Mutant KRAS induces a beneficial immune response

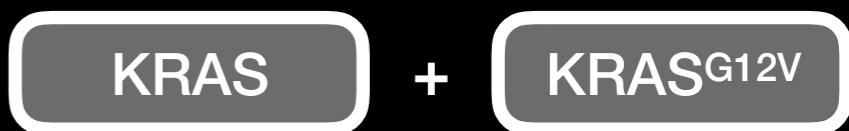


# Take Aways:

## iPSC/EB Model



## AALE/HA1EM Model



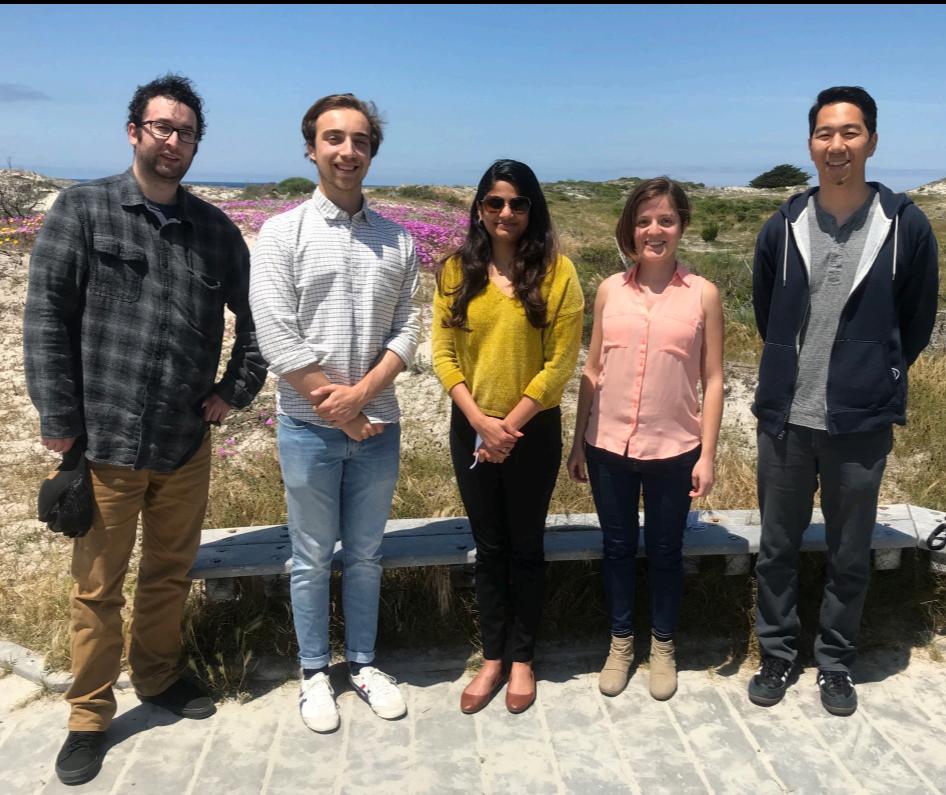
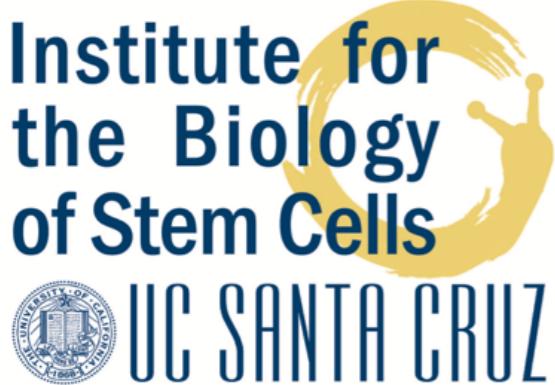
### KRAS is required for:

- Proper regulation of LTR/LTR-derived lncRNA expression
- Proper stem cell differentiation, particularly ectoderm
- Proper exit from pluripotent state

### Mutant KRAS induces:

- Tissue-specific changes to the transcriptome
- Lung-specific loss of ZNF expression
- Lung-specific up regulation of AluSx1 and a concordant ISG response

# Thank You!



- Daniel Kim
  - Sreelakshmi Velandi Maroli
  - Haley Halasz
  - David Carrillo
  - Erin LaMontagne
  - Paula Esquetini
- KRAS CRISPRi
- KRAS + KRAS<sup>G12V</sup>