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# Supplementary Materials for

## HDAC3 is critical in tumor development and therapeutic resistance in *Kras*-mutant non–small cell lung cancer

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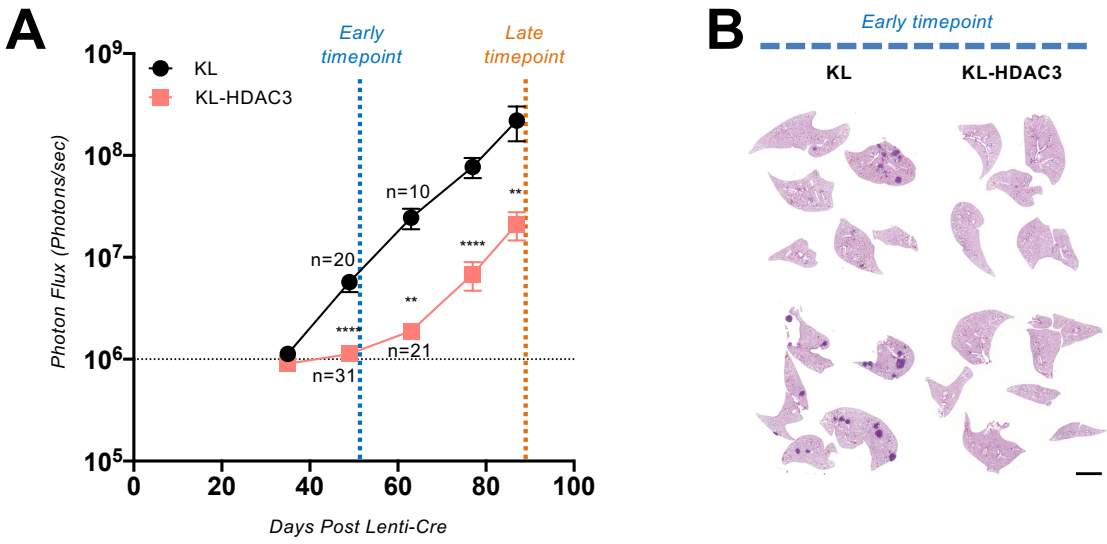
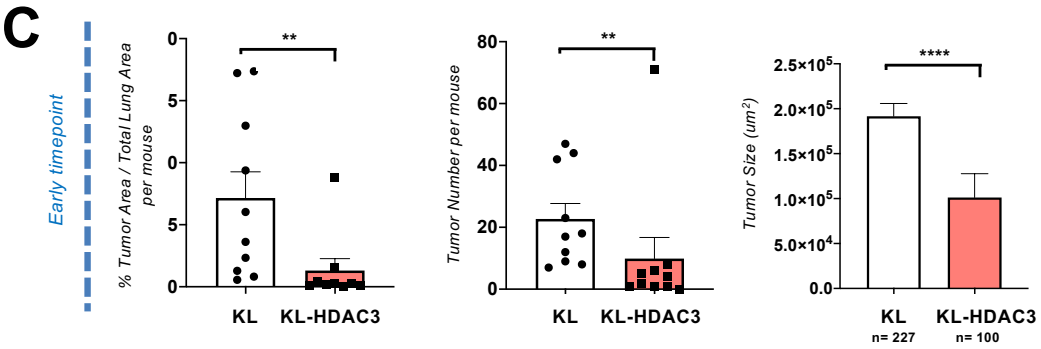
**The PDF file includes:**

Figs. S1 to S5

**Other Supplementary Material for this manuscript includes the following:**

Table S1



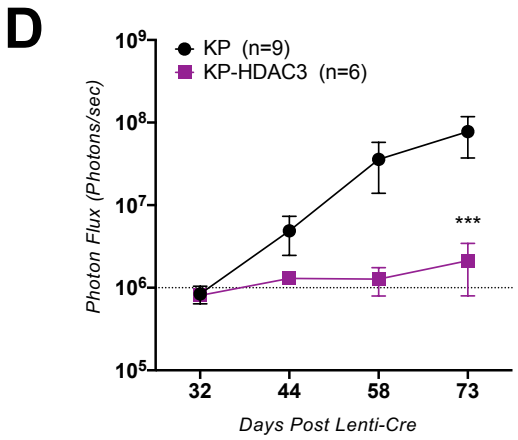




**Figure S1. HDAC3 deletion *in vivo* impairs tumor growth in *KL* and *KP* GEMM models of NSCLC**
**(A)** Average longitudinal BLI data from the *KL-HDAC3* experiment.
**(B)** Representative H&E-stained sections from the *KL-HDAC3* early timepoint. Scale bar 1000um.
**(C)** Quantitation from H&E-stained sections from the early timepoint cohort: Tumor area as a percentage of total lung area per mouse (n=10), tumor number per mouse (n=10), and average tumor size (n=227 or 100 as indicated).
**(D)** Average longitudinal BLI data from the *KP-HDAC3* experiment.
*Values are expressed as mean ± s.e.m. ** p-value < 0.01, *** p-value < 0.001, **** p-value < 0.0001 determined by two-tailed Mann-Whitney test.*

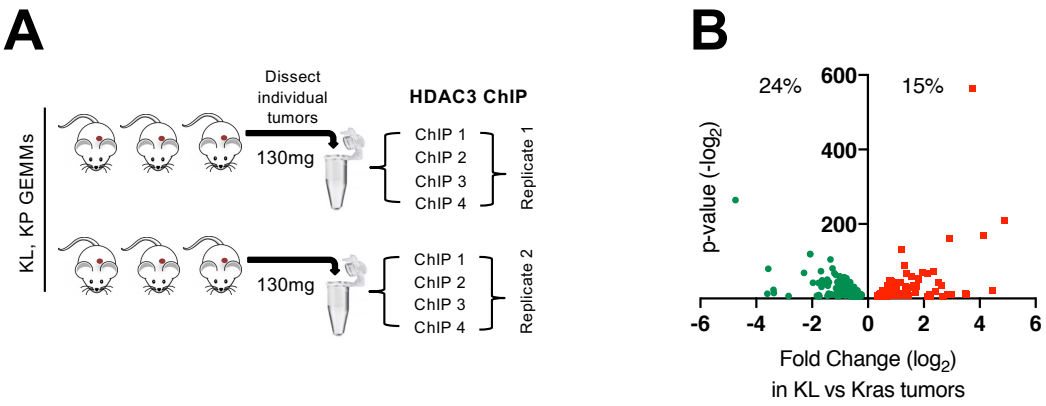



**Figure S2. HDAC3 genome occupancy in primary tumors.**
**(A)** Schematic of HDAC3 ChIP-seq experimental design in primary *KL* and *KP* tumors.
**(B)** Plot of RNA-seq differential expression between *KL* versus *Kras* primary tumors for the HDAC3 target genes in Figure 2A.

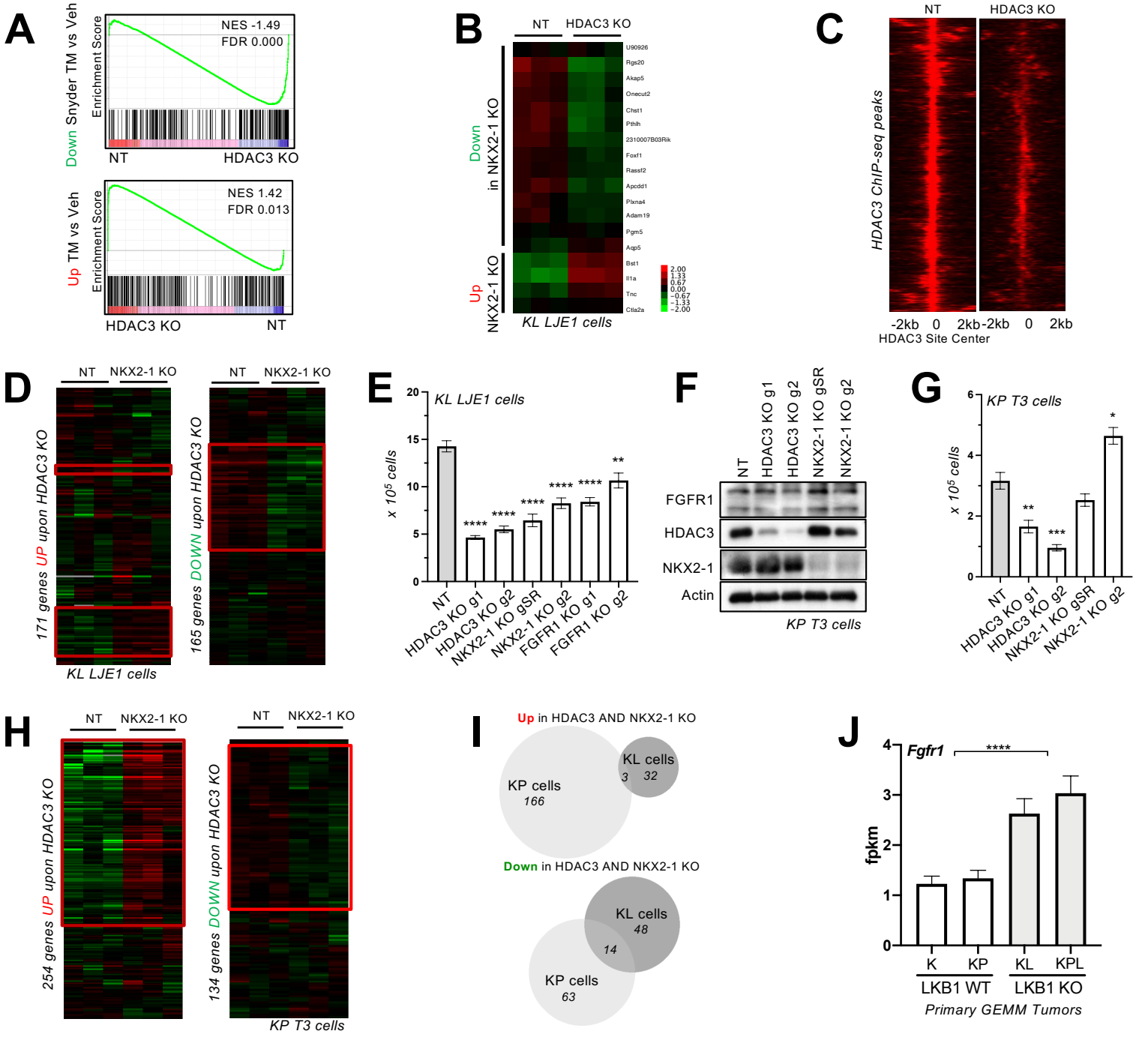



**Figure S3. HDAC3 and NKX2-1 regulate the expression of a common set of target genes.**

**(A)** GSEA plots for genes deregulated (downregulated, top plot; upregulated, bottom plot) upon tamoxifen (TM)-mediated *in vivo* deletion of *NKX2-1* in *Kras* tumors (Snyder et al. *Mol Cell*, 2013 (*44*)) queried across *HDAC3* KO RNA-seq data from KL LJE1 cells. NT, Non-Targeting control.

**(B)** Heatmap of RNA-seq data showing FPKM read counts from NT or *HDAC3* KO cells for genes deregulated upon *NKX2-1* KO (adj. p-value <0.05, fold +/-0.5) in KL LJE1 cells.

**(C)** 3,728 HDAC3 ChIP-seq peaks identified in KL LJE1 NT cells. Peaks were called using Input from NT cells, and HDAC3 ChIP-seq from HDAC3 KO cells as background.

**(D)** Heatmap of RNA-seq data showing FPKM read counts from NT or *NKX2-1* KO KL LJE1 cells for the genes deregulated upon *HDAC3* KO (adj. p-value <0.05, fold +/-0.5).

**(E)** Proliferation assessment of KL LJE1 cells after 5 days (n=6).

**(F)** Western blot analysis from KP T3 cells.

**(G)** Proliferation assessment of KP T3 cells after 5 days (n=6).

**(H)** Heatmap of RNA-seq data showing FPKM read counts from NT or *NKX2-1* KO cells for the genes deregulated upon *HDAC3* KO (adj. p-value <0.05, fold +/-0.5) in KP T3 cells. 159/254 genes were strongly upregulated upon *HDAC3* KO or *NKX2-1* KO (red box, *left*), whereas 68/134 genes were mildly downregulated upon *HDAC3* KO or *NKX2-1* KO (red box, *right*).

**(I)** Overlap of genes significantly upregulated or downregulated upon both *HDAC3* KO and *NKX2-1* KO in KP T3 *(KP)* versus KL LJE1 *(KL)* cells.

**(J)** *Fgfr1* mRNA levels (FPKM) from RNA-seq data across *Kras (K)* (n=9), *KP* (n=8), *KL* (n=9), and *KPL* (n=15) primary tumor (n=8-15).

*Values are expressed as mean ± s.e.m. * p-value < 0.05, ** < 0.01, *** < 0.001, **** p-value < 0.0001 determined by two-tailed student's t-test.*

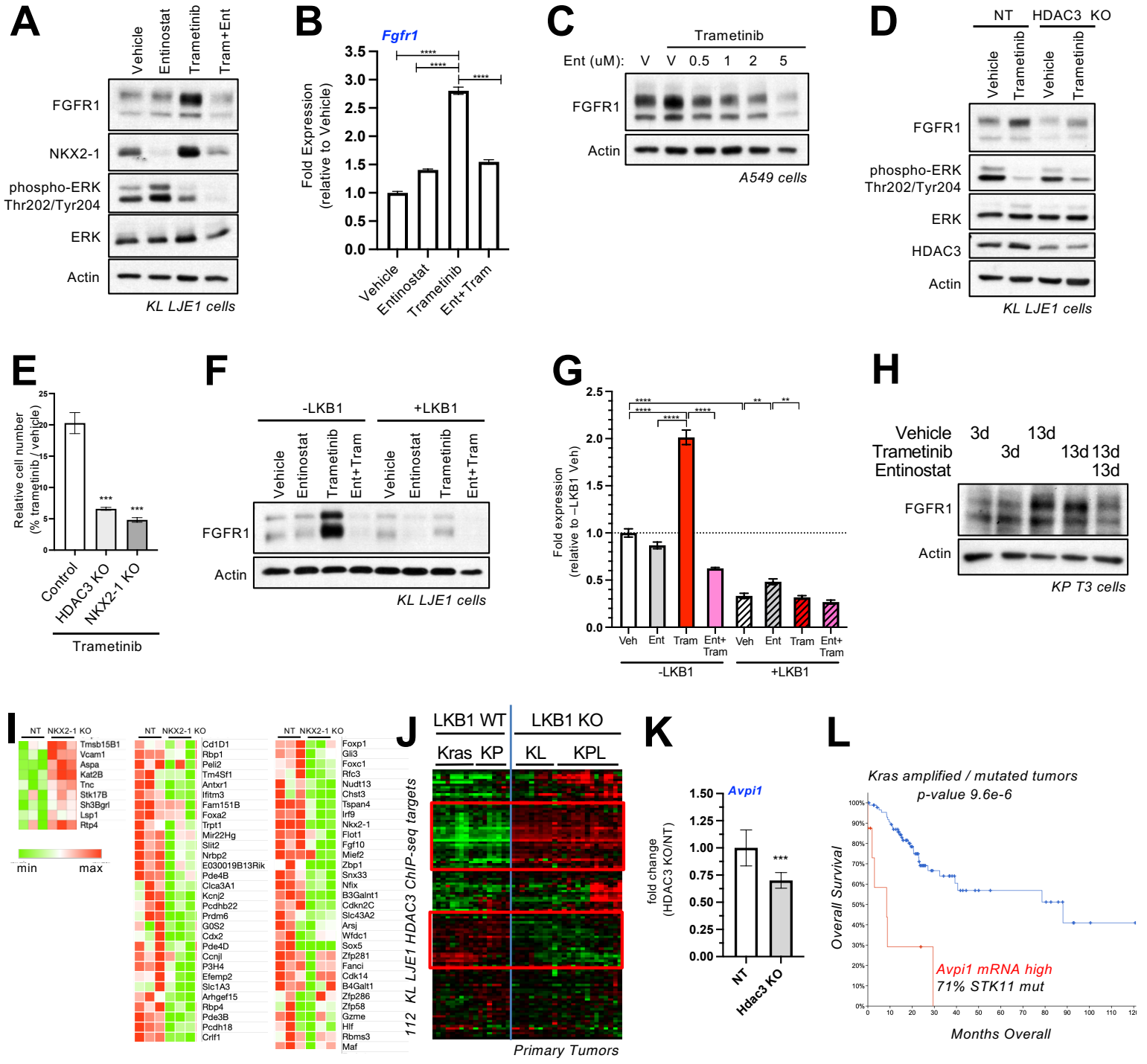



**Figure S4. HDAC3 and NKX2-1 common target genes are aberrantly engaged upon trametinib resistance**

**(A)** Western blot analysis from KL LJE1 cells treated with vehicle, 10nM trametinib, or 1uM entinostat for 13 days.

**(B)** qRT-PCR for *Fgfr1* mRNA expression in KL LJE1 cells treated as in (A) (n=3).

**(C)** Western blot analysis from human A549 cells treated for 9 days with vehicle (V) or 10nM trametinib, and during the last 6 days co-treated with V or entinostat (Ent) at the doses indicated.

**(D)** Western blot analysis of NT or *HDAC3* KO KL LJE1 cells treated with vehicle or 10nM trametinib for 10 days.

**(E)** Proliferation assessment after 6 days of treatment with vehicle or 10nM trametinib in NT, *HDAC3* KO, or *NKX2-1* KO KL LJE1 cells (n=6).

**(F-G)** Western blot (F) and qRT-PCR (G) analysis from cells with (+) or without (-) LKB1 re-expression treated for 13 days; 10nM trametinib (Tram), 1uM entinostat (Ent).

**(H)** Western blot analysis of protein lysates from KP T3 cells treated with vehicle, 10nM trametinib, or 1uM entinostat for 13 days.

**(I)** Heatmap of RNA-seq data showing FPKM read counts for TIER genes differentially expressed between NT or *NKX2-1* KO KL LJE1 cells.

**(J)** Heatmap of RNA-seq data showing FPKM read counts from *Kras* (n=9), *KP* (n=8), *KL* (n=9), and *KPL* (n=15) primary tumors for the 112 TIER genes which are HDAC3 ChIP-seq target genes (Figure 4E).

**(K)** qRT-PCR for *Avpi1* mRNA expression in KL LJE1 cells (n=3).

**(L)** Overall survival data from patients with *Kras* amplified or *G12*-mutant tumors, comparing tumors with (n=8) or without (n=92) high *AVPI1* mRNA expression in the Firehose Legacy LUAD TCGA dataset.

*Values are expressed as mean ± s.e.m. ** < 0.01. *** < 0.001. **** p-value < 0.0001 determined by two-tailed student's t-test.*

**A**

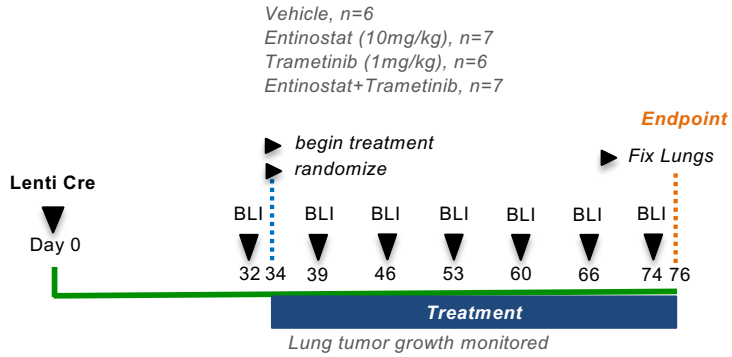



**Figure S5. *in vivo* trametinib plus entinostat combination treatment in *KL* NSCLC GEMM.**
**(A)** Schematic of experimental design. Lung tumors were initiated in *Kras*$^{G12D/+}$, *LKB1*$^{L/L}$ *(KL)* mice by Lenti-Cre administration and mice were imaged weekly (BLI) starting 4 weeks post-Lenti-Cre. Treatment was initiated 34 days post-Lenti-Cre. Mice were treated for 42 days with vehicle, entinostat (Ent, 10mg/kg), trametinib (Tram, 1mg/kg), or entinostat plus trametinib (Ent+Tram) administered by oral gavage.