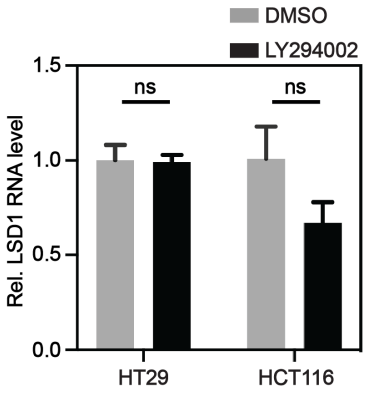
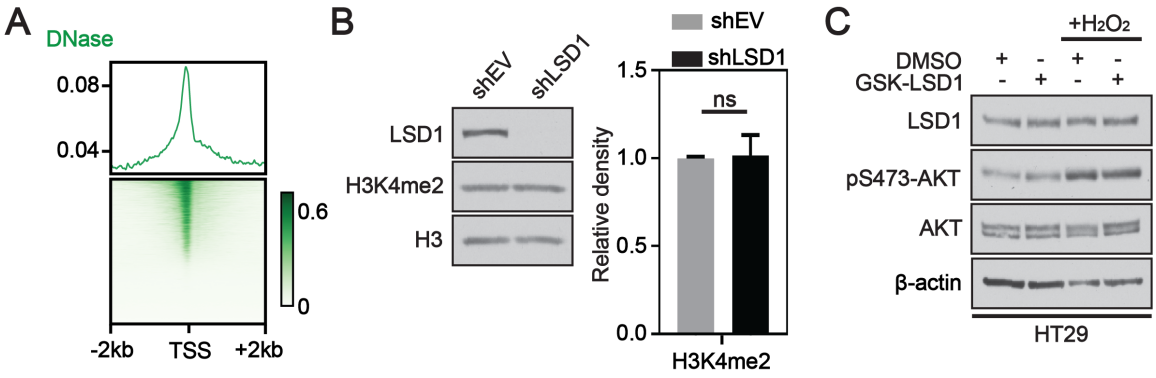
	SW480	HT29	RKO	LoVo	HCT116	AGS
Cancer Type	Colorectal Adeno-carcinoma (Primary)	Colorectal Adeno-carcinoma (Primary)	Colon Carcinoma (Primary)	Colorectal Adeno-carcinoma (Lymph Met.)	Colorectal Carcinoma (Primary)	Gastric Adeno-carcinoma (Primary)
PI3KCA	WT	P449T	H1047R E545A	WT	H1047R	E453K
KRAS	G12V	WT	WT	G13D	G13D	G12D
BRAF	WT	V600E	V600E	WT	WT	WT
p53	R273H P309S	R273H	WT	WT	WT	WT

Supplementary Table S1: Mutational status of common oncogenes in CRC and STAD cell lines.



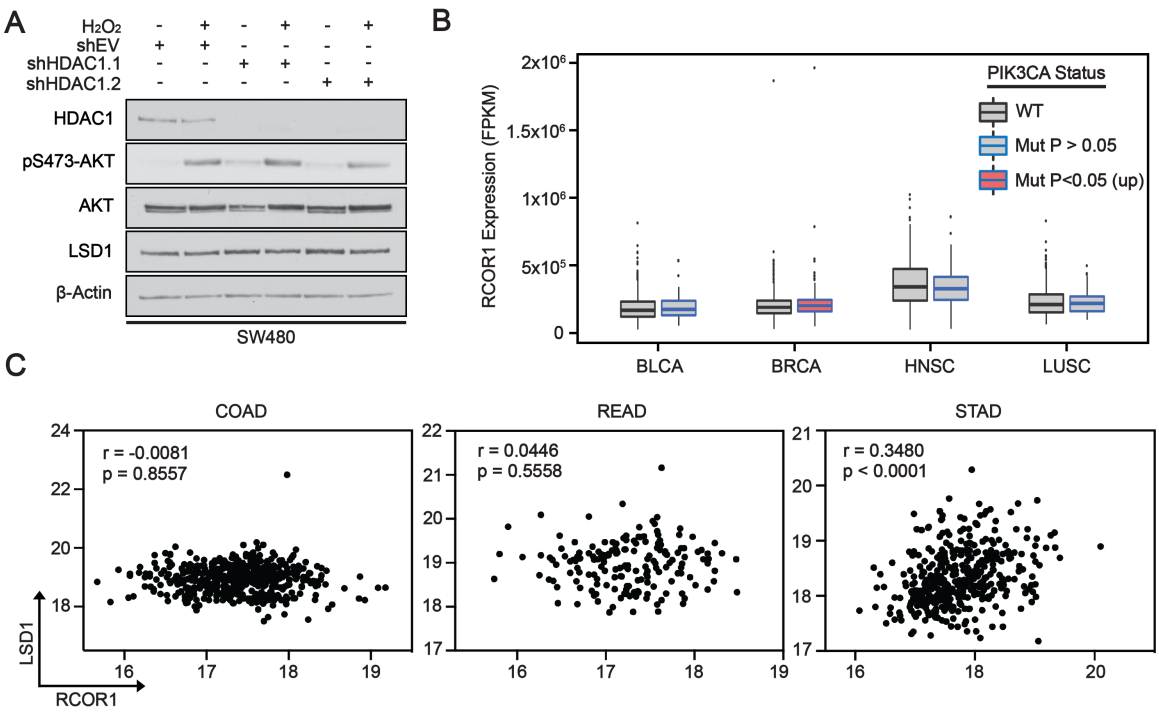
Supplementary Figure S1: Mutant PIK3CA does not regulate LSD1 expression.

Real-time PCR analysis of *LSD1* RNA expression levels after 48H DMSO or 20 μM LY294002 treatment in HT29 and HCT116 cells. Expression was normalized to *Gapdh* and DMSO. Results are represented as mean ± SD. Significance determined by Welch's t-test. ns – not significant.



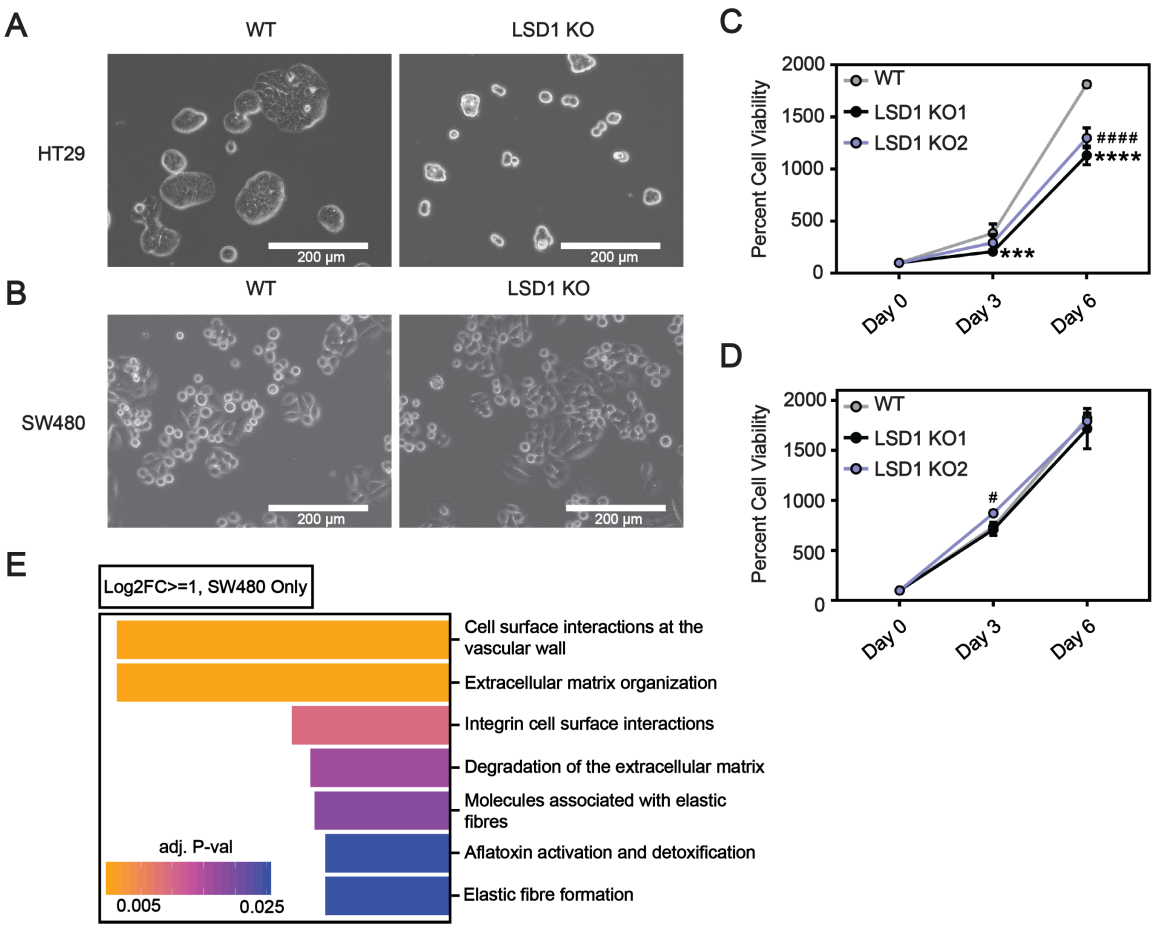
Supplementary Figure S2: DNase signal is not generally depleted at TSS's, and LSD1 does not reduce global H3K4me2.

(A) Averageplot and heatmap depicting SW480 DNase-seq reads mapped over transcription start sites (TSSs) genome wide. Values are derived from CPM (counts per million) normalized reads. (B) Empty vector (shEV) or LSD1 KD (shLSD1) SW480 cells were analyzed by western blot. Western blot was quantified by densitometric analysis and normalized to Histone H3 loading control and shEV. Graph represents mean ± SD. ns – not significant. Significance determined by two-tailed Welch's t-test. (C) HT29 cells pretreated with DMSO or 40 nM GSK-LSD1 for 48H then treated with 250 μM H_2O_2 for 1H.



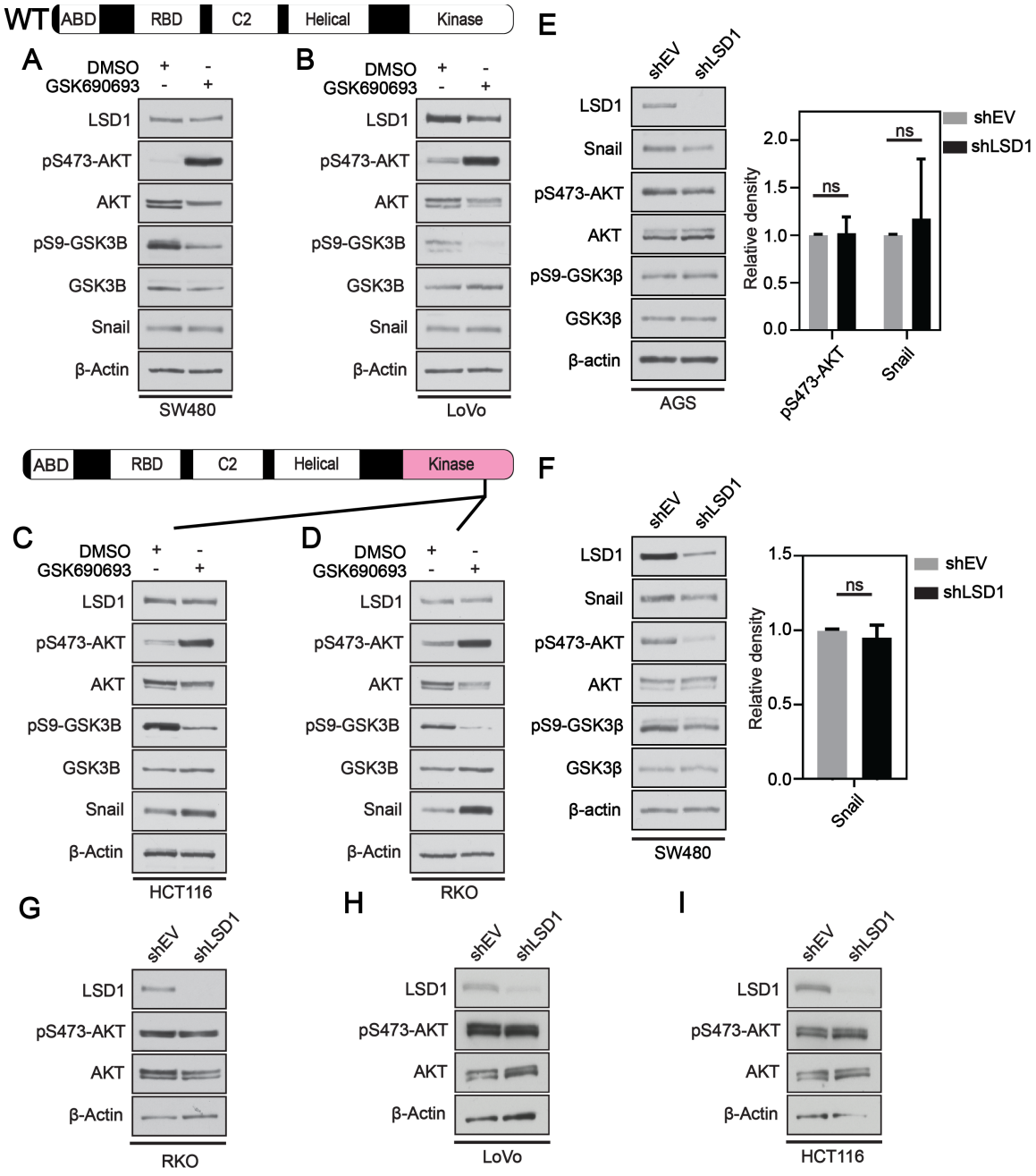
Supplementary Figure S3: *RCOR1* is generally not upregulated in *PIK3CA* mutant versus WT in cancers arising from non-gastrointestinal tissues, and generally does not correlate with LSD1 expression.

(A) shEV or shHDAC1 SW480 cells treated with 250 μM H_2O_2 for 1H, and analyzed by western blot. (B) Box and whisker plot of fragments per kilobase of transcript per million mapped reads (FPKM) expression values for *RCOR1* across different TCGA datasets. Box limits are set at the third and first quartile range with central line at the median, with whiskers depicting 1.5 times the interquartile range. Data points outside this range are represented at outliers (black dots). Black and blue outline indicates data for WT and *PIK3CA* mutant tumors, respectively. Red fill represent significant increase in *LSD1* expression, respectively, between *PIK3CA* mutant and *PIK3CA* WT tumors. (C) Spearmans correlation was calculated between LSD1 and RCOR1 normalized expression from TCGA datasets.



Supplementary Figure S4: LSD1 KO reduces cell viability over time in HT29 but not SW480 cells.

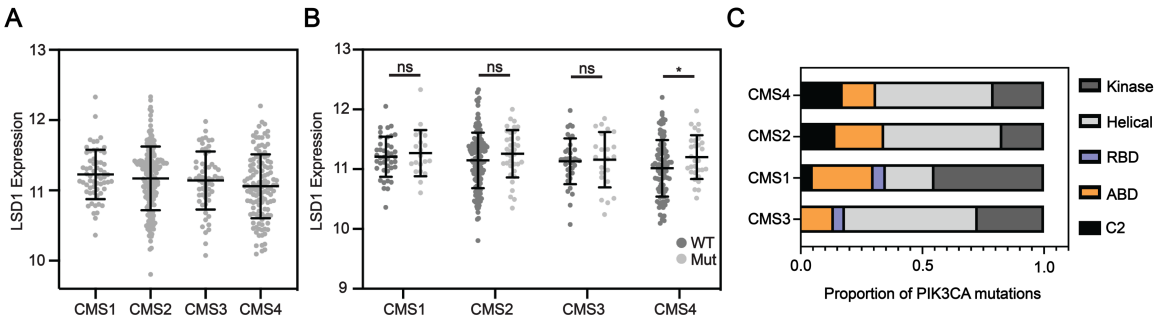
20x phase contrast images of WT and LSD1 KO (A) HT29 or (B) SW480 cells. Cell-Titer Glo assay readout of WT, LSD1 KO clone 1, and LSD1 KO clone 2 in (C) HT29 (n=4) or (D) SW480 (n=5) cells over a 6 day time course. Statistical analyses are performed using 2way ANOVA and Dunnett's multiple comparisons test. Results are represented as mean ± SD (adj. p-value; * <.05, ***<.001, ****<.0001) (WT vs. LSD1 KO1 = * , WT vs. LSD1 KO2 = #). All statistically significant comparisons are shown. (E) GO analysis was performed on genes uniquely upregulated in SW480 cells (Log2FC>=1).



Supplementary Figure S5: Inhibiting AKT does not reduce Snail protein level in *PIK3CA* WT or kinase domain mutant cells, and the LSD1-AKT-GSK3β-Snail axis is context dependent.

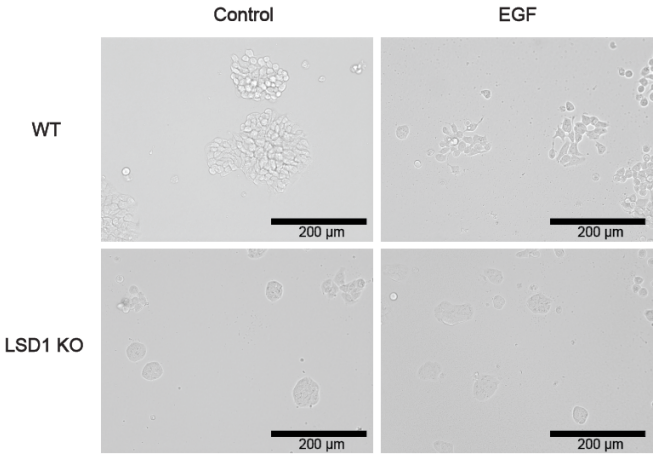
(A) SW480, (B) LoVo, (C) HCT116 and (D) RKO cells were treated with DMSO or 10 μM GSK690693 for 48H and analyzed by western blot. Empty vector (shEV) or LSD1 KD (shLSD1) (E) AGS and (F) SW480 cells were analyzed by western blot. Western blot

was quantified by densitometric analysis and normalized to β-actin loading control and shEV. Graph represents mean ± SD. ns – not significant. Significance determined by two-tailed Student's t-test. shEV and shLSD1 analyzed by western blot in (G) RKO (H) LoVo and (I) HCT116 cells.



Supplementary Figure S6: LSD1 is upregulated in *PIK3CA* mutants classified as CRC subtype CMS4, where C2 domain mutations are also most common.

(A) Normalized LSD1 expression from COADREAD TCGA data and (B) separated based on *PIK3CA* mutational status (WT = wild type, Mut = mutant). Graphs represent mean ± SD. ns – not significant, p-value; * < 0.05. Significance was determine by Welch's t-test. (C) Proportion of total *PIK3CA* mutations occurring in the different domains indicated across various CMS classifications.



Supplementary Figure S7: EGF treatment causes morphological changes in HT29 WT but not LSD1 KO cells.

20x phase contrast images of WT or LSD1 KO HT29 cells treated with 100 ng/ml EGF for 48H.