Viotti et al., https://doi.org/10.1083/jcb.201705031

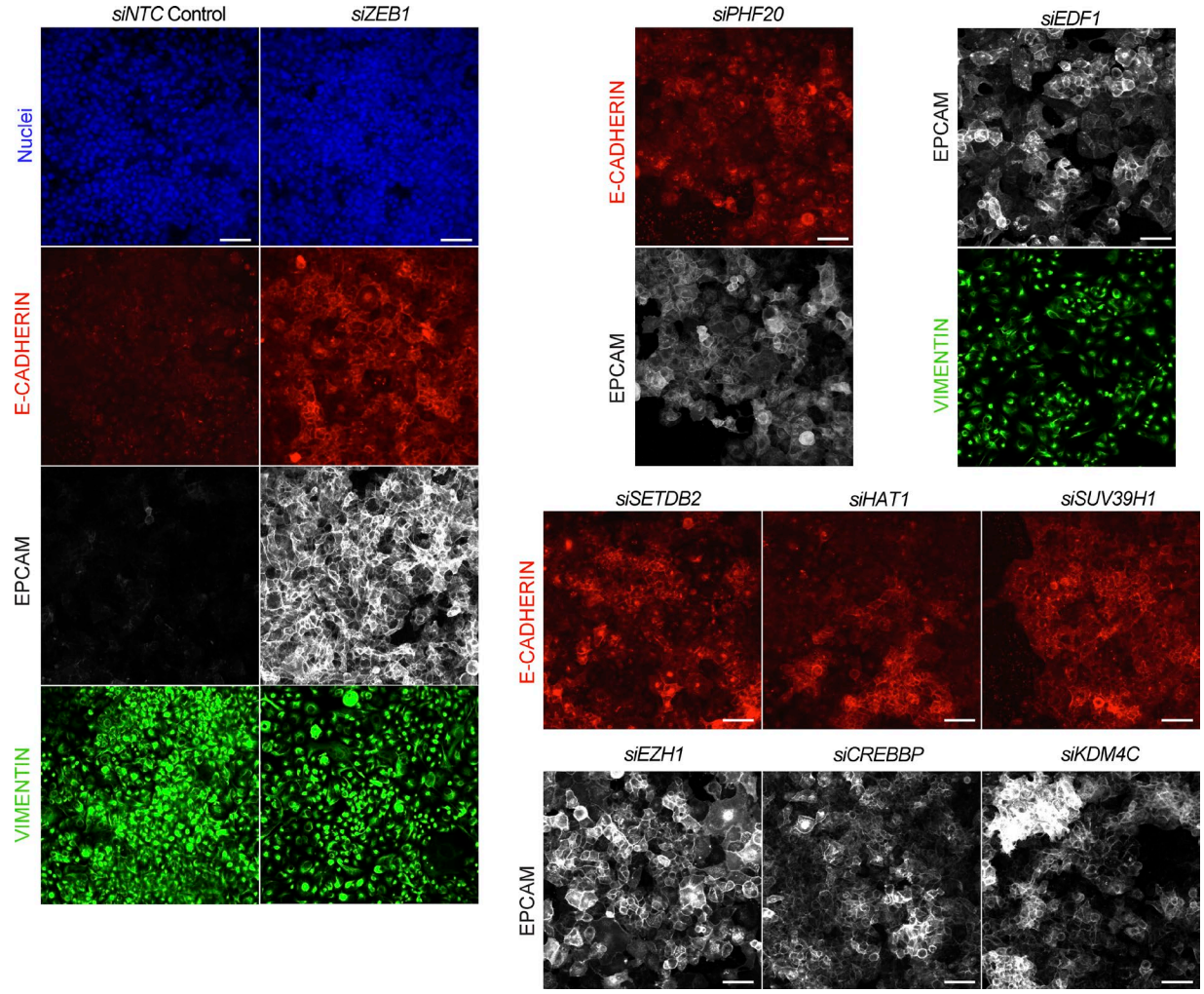

Figure S1. **Original screen images of PANC-1 cells with various epigenetic knockdown targets.** Knockdown with *NTC*, *ZEB1* positive control and several epigenetic factors that elicited significant changes in immunofluorescent intensity for E-CAD, EPCAM, and/or VIM. Bars, 50 µm.

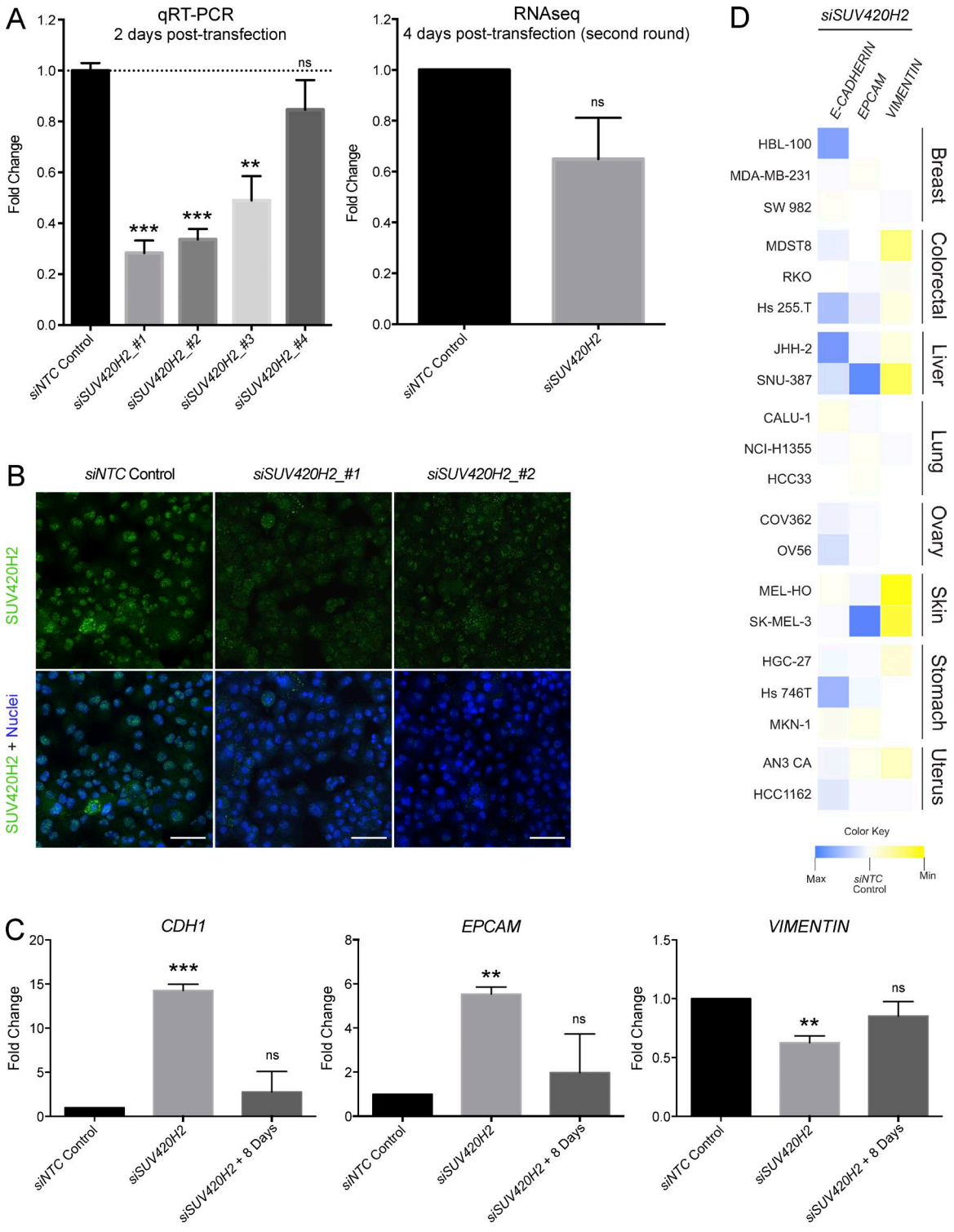

Figure S2. **Effects of *SUV420H2* knockdown in cancer cells. (A)** Knockdown efficiency of the four siRNA sequences used in the screen targeting *SUV420H2* determined by qRT-PCR (left graph). Confirmation of *SUV420H2* knockdown in RNaseq experiment by using siRNA sequence 1 analyzed 4 d after the second siRNA transfection, when siRNA knockdown efficiency is already likely to be considerably diminished (right graph). For qRT-PCR, $n = 3$ biological replicates each averaged from three technical replicates; differences were assessed by Student's *t* test compared with *siNTC* control. For RNaseq data, $n = 3$ biological replicates, and differences were assessed by using voom+limma. Bar graphs depict mean ± SD. **, $P < 0.01$; ***, $P < 0.001$. **(B)** Immunofluorescent analysis of *SUV420H2* knockdown efficiency in PANC-1 cells. Bars, 50 µm. **(C)** qRT-PCR analysis of *CDH1*, *EPCAM*, and *VIM* expression levels after 8-d recovery after *SUV420H2* knockdown. $n = 3$ biological replicates each averaged from three technical replicates; differences were assessed by Student's *t* test compared with *siNTC* control. Bar graphs depict mean ± SD. **, $P < 0.01$; ***, $P < 0.001$. **(D)** qRT-PCR expression analysis for *CHD1*, *EPCAM*, and *VIM* on *SUV420H2* knockdown in various cancer cell lines of mesenchymal identity. Data are represented as normalized to the *siNTC* control knockdown for each cell line. To facilitate comparison, the same maxima and minima are used as in Fig. 2 E. For each data point, $n = 3$ biological replicates each averaged from three technical replicates. Heat map data depicts means. ns, not significant ($P \geq 0.05$).

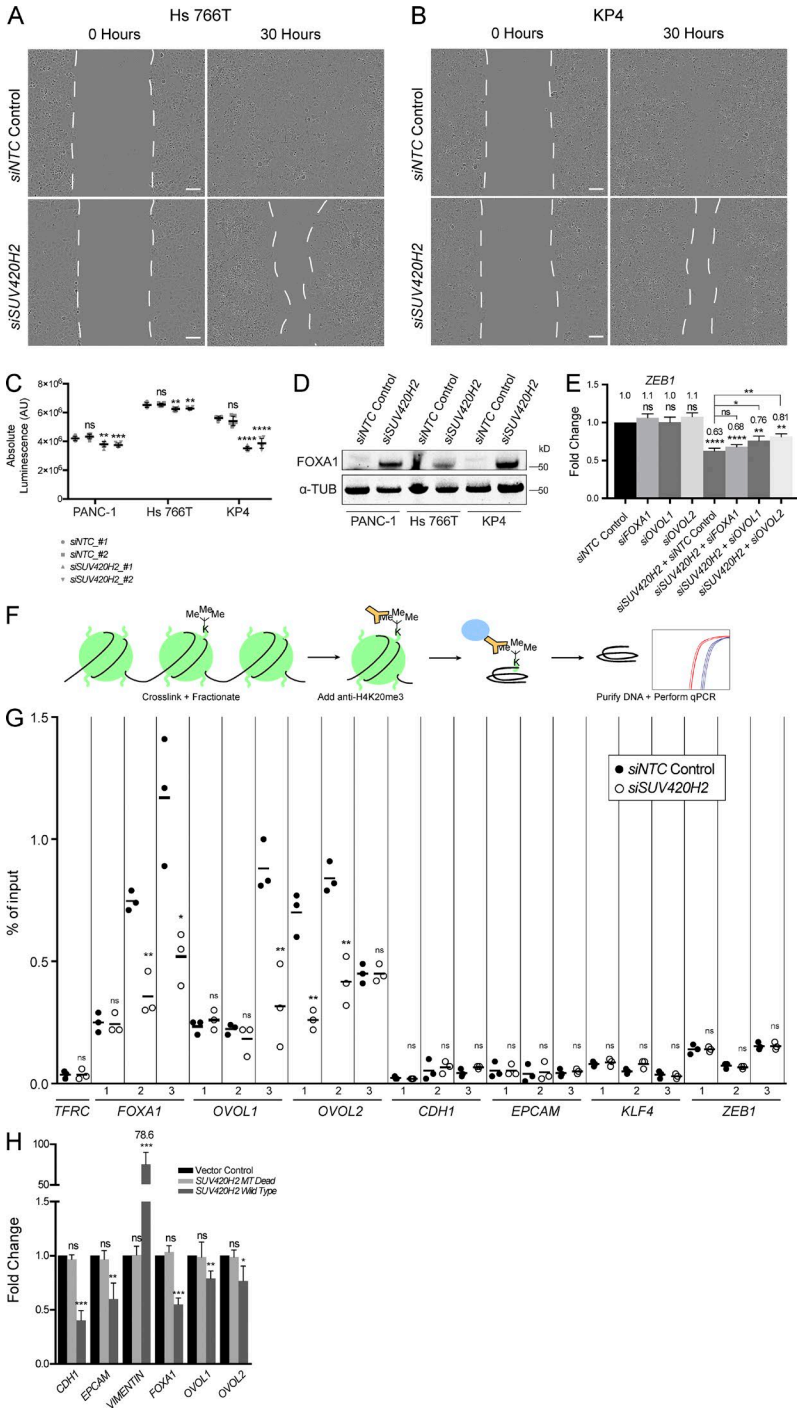

Figure S3. **Analysis of SUV420H2 function and mechanism in pancreatic cancer cells. (A)** Migration assay representative images of Hs 766T cells. Bars, 50 μm. **(B)** Migration assay representative images of KP4 cells. Bars, 50 μm. **(C)** Absolute quantitation of PANC-1, Hs 766T, and KP4 cells with NTC control and *SUV420H2* knockdown with no drug exposure, related to Fig. 4 D. *n* = 6 biological replicates. Lines represent means ± SD. Differences assessed by Student's *t* test compared with *siNTC_#1* control. **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. **(D)** Western blot for FOXA1 in mesenchymal pancreatic cancer cells with NTC control or *SUV420H2* knockdown. Note a nonspecific smear for *siNTC* control lane in Hs 766T cells. The loading control is α-TUB ULIN. **(E)** qRT-PCR expression analysis of *ZEB-1* in PANC-1 cells in double-knockdown rescue experiments. Data are normalized to *siNTC* control; bar graphs indicate mean ± SD. *n* = 3 biological replicates each averaged from three technical replicates; differences were assessed by Student's *t* test compared with *siNTC* control, unless otherwise indicated. *, P < 0.05; **, P < 0.01; ****, P < 0.0001. **(F)** Assay diagram of ChIP-qPCR experiment to quantify levels of the H4K20me3 mark. **(G)** ChIP-qPCR results quantifying levels of H4K20me3 on different genes in PANC-1 cells, with control or *SUV420H2* knockdown. Three regions per genes were probed (indicated by 1, 2, and 3), except for TFRC control (single region). Each dot represents a value for one biological replicate, averaged from three technical replicates. Black lines indicate means. Differences were assessed by Student's *t* test compared with *siNTC* control for each probe. *, P < 0.05; **, P < 0.01. **(H)** qRT-PCR analysis of expression of epithelial/mesenchymal factors in Panc 04.03 cells transfected with vector control, MT Dead, and wild-type *SUV420H2* overexpression plasmid. Data normalized to empty vector transfection level for each gene assayed. Bar graphs indicate mean ± SD. *n* = 3 biological replicates each averaged from three technical replicates; differences were assessed by Student's *t* test compared with cells transfected with empty vector. *, P < 0.05; **, P < 0.01; ***, P < 0.001. ns, not significant (P ≥ 0.05).

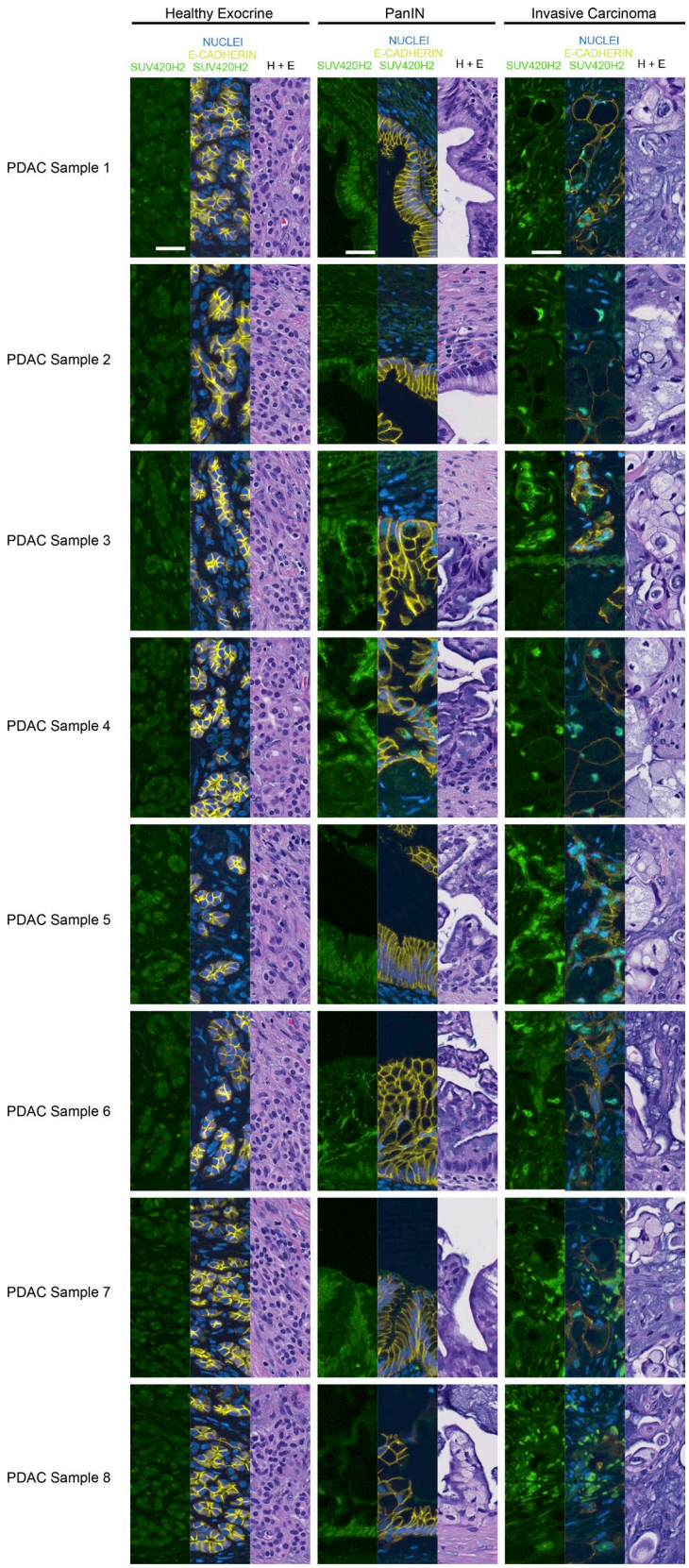

Figure S4. **SUV420H2 and E-CAD in progressive stages of PDAC.** Immunofluorescence for SUV420H2, E-CAD, nuclear stain, and H+E stain in eight human PDAC samples. For each sample, a region of healthy exocrine, PanIN, and a region of invasive carcinoma are shown. Bars, 50 µm.

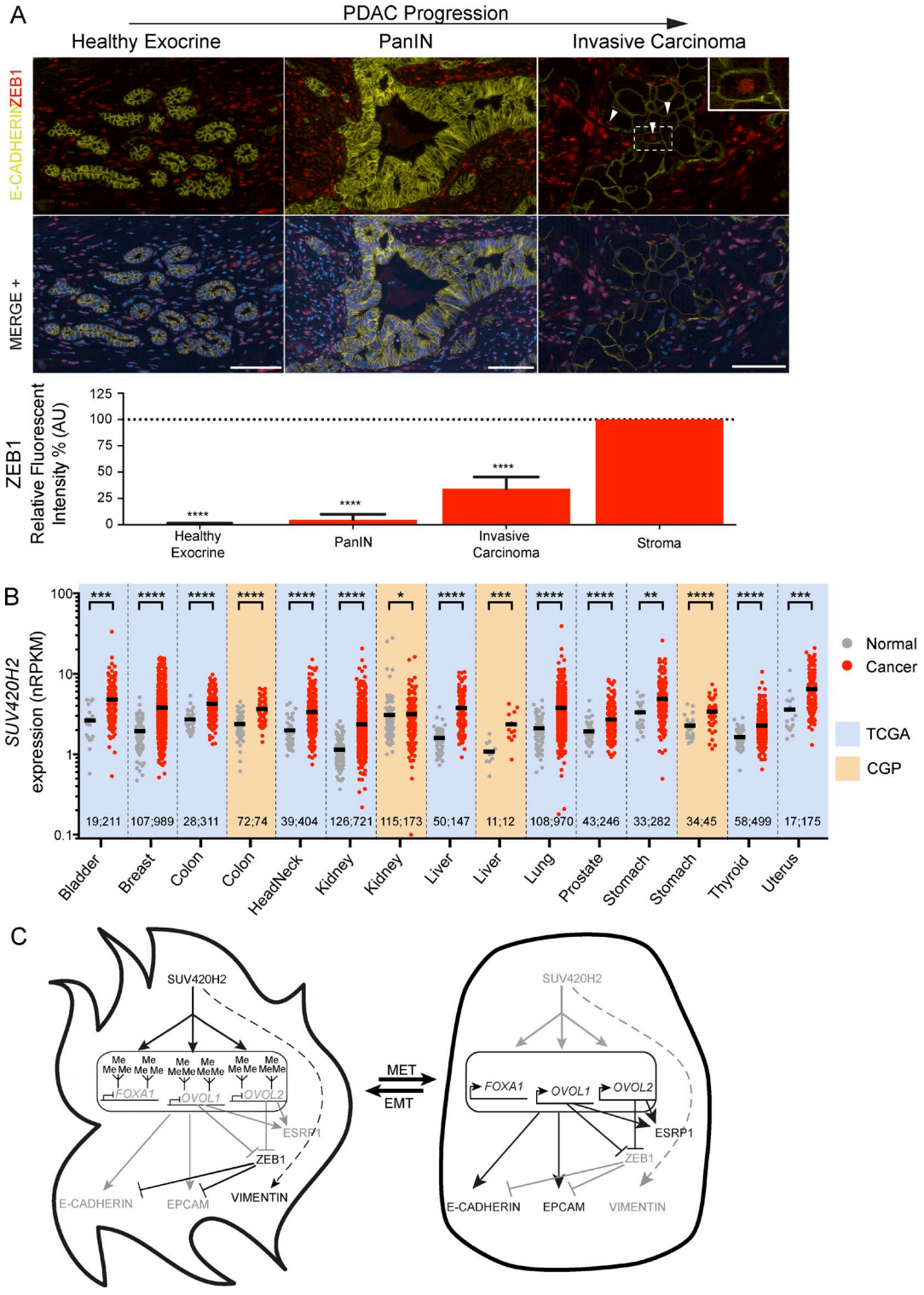

Figure S5. **ZEB1 in pancreatic cancer progression, expression of *SUV420H2* in human cancers, and working model of SUV420H2. (A)** Immunofluorescence for ZEB1 and E-CAD in sections of human pancreatic tissue with healthy exocrine epithelium, early pancreatic cancer lesion (PanIN), or invasive cancer. The inset in top right panel depicts high magnification of the dashed box. White arrowheads point out invasive cells with nuclear ZEB1 stain. Histogram depicts quantitation of fluorescent signal during cancer progression. *n* = 8 PDAC samples from separate patients, analysis of one healthy exocrine region, one PanIN, and one invasive carcinoma region in each, signal quantified in 16 cells per region for a total of 128 measurements per stage. Measurements for 128 stromal cells in total collected evenly from all images. Data are normalized to levels in stroma. Bars depict mean ± SD. Differences were assessed by Student's *t* test compared with stroma. ****, P < 0.0001. **(B)** Summary of *SUV420H2* gene expression data retrieved from TCGA (blue) and Genentech's Cancer Genome Project (orange) in human cancers. Each data point represents the normalized RPKM value for an individual tissue sample; black lines indicate means. Significance was determined with voom+limma analysis. Numbers above the x axis indicate the number for normal and cancer samples (*n*) for each tissue type. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. **(C)** Working model of SUV420H2 controlling epithelial/mesenchymal cell states in pancreatic cancer.

Provided online are four tables in Excel. Table S1 lists siRNAs used in the study. Table S2 shows RNaseq results for highly and significantly up-regulated genes on *SUV420H2* knockdown in PANC-1 cells, listed by protein coding genes (tab one) and other genes (tab two); tab three shows full results of RNAseq. Tables S3 and S4 show gene set enrichment analysis results with MsigDB Hallmark gene sets and with the MsigDB C2 curated signature collection, respectively. Table S5 lists clinical information for PDAC samples used in this study.