# SET Contributes to the epithelial-mesenchymal transition of pancreatic cancer

## SUPPLEMENTARY MATERIALS

**Supplementary Table 1: Clinical and histological characterization of human pancreatic tumor samples**

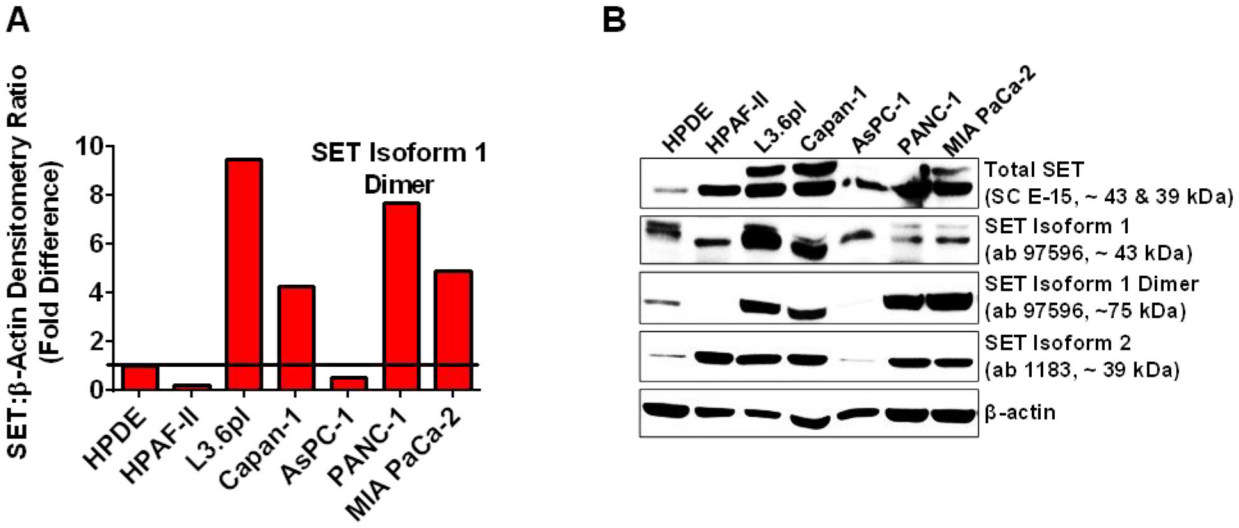
| Sample | Gender | Age | Appearance | Diagnosis | Tumor grade | Stage |
|---|---|---|---|---|---|---|
| N1 | Male | 66 | Normal | Adenocarcinoma of pancreas | AJCC G2: Moderately differentiated | Normal |
| N2 | Male | 37 | Normal | Pancreatitis, chronic | NULL | Normal |
| N3 | Male | 74 | Normal | Tumor of pancreas, intraductal, papillary, mucinous | Other Histologic Grade | Normal |
| N4 | Female | 57 | Normal | Tumor of pancreas, Islet cell | Not Reported | Normal |
| C1 | Female | 79 | Tumor | Adenocarcinoma of pancreas, ductal | AJCC G3: Poorly differentiated | IB |
| C2 | Male | 57 | Tumor | Adenocarcinoma of pancreas | AJCC G2: Moderately differentiated | IIB |
| C3 | Male | 75 | Tumor | Adenocarcinoma of pancreas, ductal | AJCC G2: Moderately differentiated | IIB |
| C4 | Female | 71 | Tumor | Adenocarcinoma of pancreas, ductal | AJCC G3: Poorly differentiated | III |
| C5 | Male | 69 | Tumor | Adenocarcinoma of pancreas, ductal | AJCC G3: Poorly differentiated | III |
| C6 | Female | 69 | Tumor | Tumor of pancreas, neuroendocrine | AJCC G1: Well differentiated | Not Reported |
| C7 | Female | 73 | Tumor | Tumor of pancreas, neuroendocrine, metastatic | AJCC G1: Well differentiated | Not Reported |
| C8 | Female | 60 | Tumor | Tumor of pancreas, neuroendocrine | Not Reported | Not Reported |
| C9 | Male | 50 | Tumor | Tumor of pancreas, Islet cell | Not Reported | Not Reported |
| C10 | Male | 66 | Tumor | Tumor of pancreas, neuroendocrine | Not Reported | Not Reported |
| C11 | Female | 64 | Tumor | Tumor of pancreas, neuroendocrine | Not Reported | Not Reported |
| C12 | Female | 60 | Tumor | Tumor of pancreas, neuroendocrine | Not Reported | Not Reported |
| C13 | Female | 60 | Tumor | Tumor of pancreas, neuroendocrine | Not Reported | Not Reported |
| C14 | Female | 61 | Tumor | Tumor of pancreas, neuroendocrine | Not Reported | Not Reported |
| C15 | Male | 51 | Tumor | Tumor of pancreas, Islet cell | Not Reported | Not Reported |
| C16 | Male | 31 | Tumor | Tumor of pancreas, Islet cell | Not Reported | Not Reported |
| C17 | Male | 76 | Tumor | Tumor of pancreas, Islet cell | Not Reported | Not Reported |
| C18 | Female | 57 | Tumor | Tumor of pancreas, neuroendocrine | Not Reported | Not Reported |

**Supplementary Table 2: Genes profiled for RT-PCR profiler array**

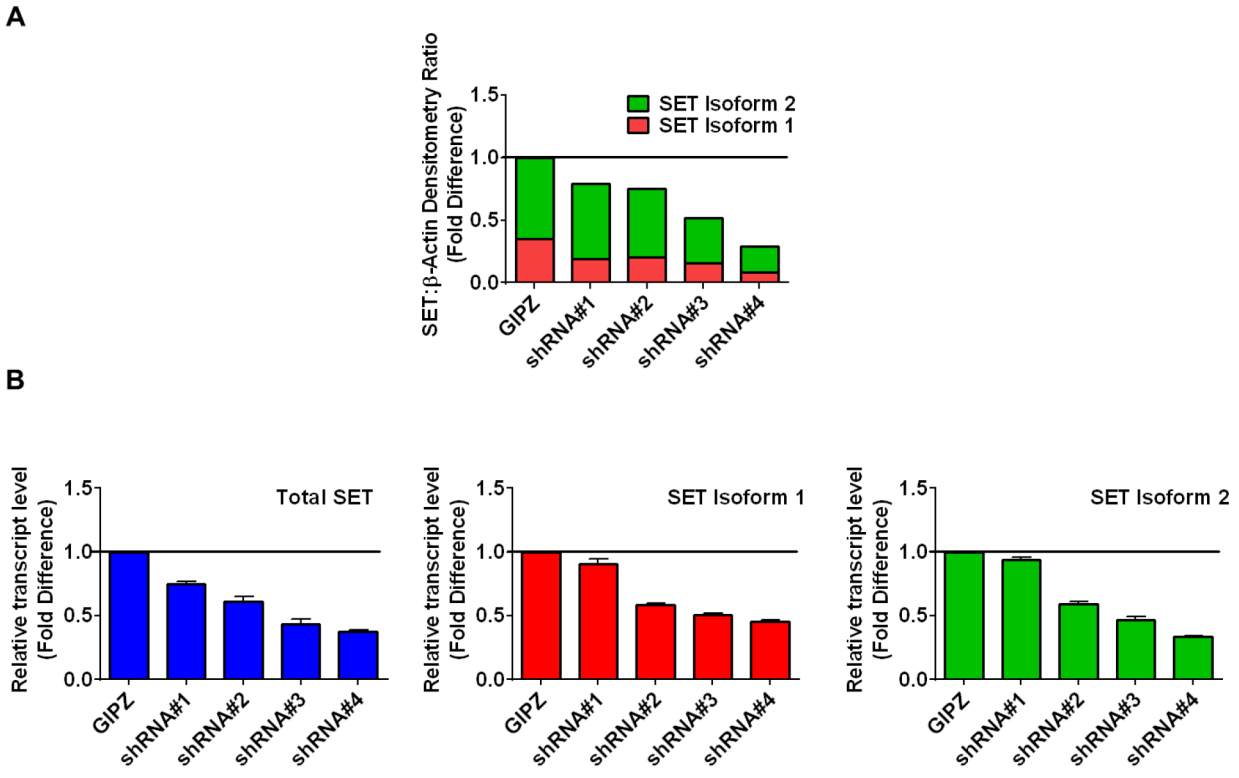
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AHNAK | AKT1 | BMP1 | BMP2 | BMP7 | CALD1 | CAMK2N1 | CAV2 | CDH1 | CDH2 | COL1A2 | COL3A1 |
| COL5A2 | CTNNB1 | DSC2 | DSP | EGFR | ERBB3 | ESR1 | F11R | FGFBP1 | FN1 | FOXC2 | FZD7 |
| GNG11 | GSC | GSK3B | IGFBP4 | IL1RN | ILK | ITGA5 | ITGAV | ITGB1 | JAG1 | KRT14 | KRT19 |
| KRT7 | MAP1B | MMP2 | MMP3 | MMP9 | MSN | MST1R | NODAL | NOTCH1 | NUDT13 | OCLN | PDGFRB |
| PLEK2 | PPPDE2 | PTK2 | PTP4A1 | RAC1 | RGS2 | SERPINE1 | SIP1 | SMAD2 | SNAI1 | SNAI2 | SNAI3 |
| SOX10 | SPARC | SPP1 | STAT3 | STEAP1 | TCF3 | TCF4 | TFPI2 | TGFB1 | TGFB2 | TGFB3 | TIMP1 |
| TMEFF1 | TMEM132A | TSPAN13 | TWIST1 | VCAN | VIM | VPS13A | WNT11 | WNT5A | WNT5B | ZEB1 | ZEB2 |
| ACTB | B2M | GAPDH | HPRT1 | RPLP0 | HGDC | RTC | RTC | RTC | PPC | PPC | PPC |

**Supplementary Table 3: List of EMT-related genes (including house-keeping genes) from a RT-PCR profiler array with fold changes on SET overexpression (SET-HA) compared with control cells (pLNCX2) in PANC-1.**

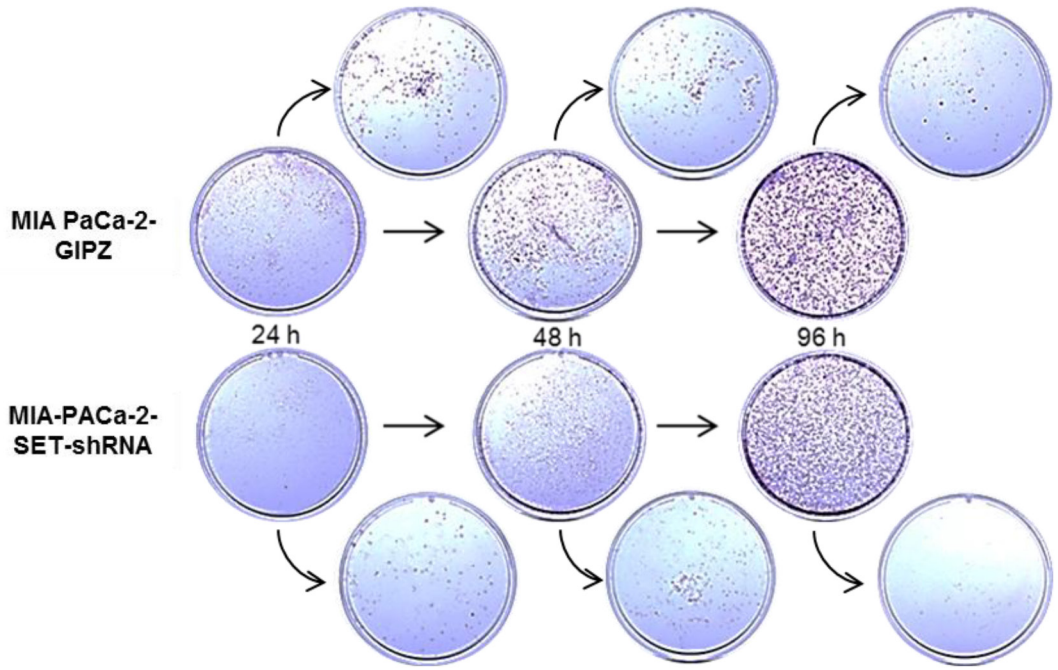
**See Supplementary File 1**



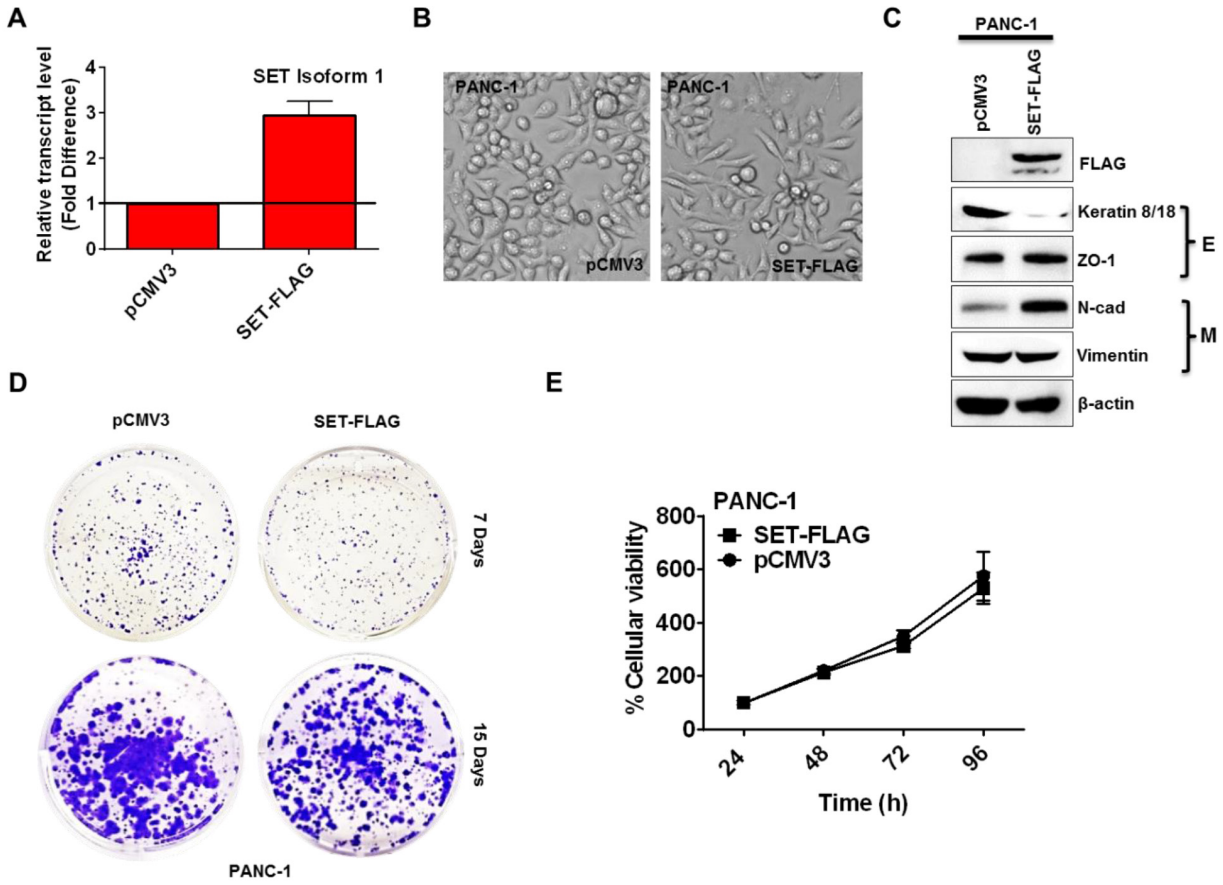

**Supplementary Figure 1: (A)** The bands of SET Isoform 1 dimer from Fig. 1B were quantified by densitometry and SET:β-actin ratios plotted. **(B)** Protein levels of Total SET, SET isoform 1, and SET isoform 2 in a panel of pancreatic cancer cells. Whole cell lysates (50 µg) prepared with HEPES Lysis Buffer were subjected to Western blotting analysis. β-actin, the internal loading control, is shown with a representative blot.



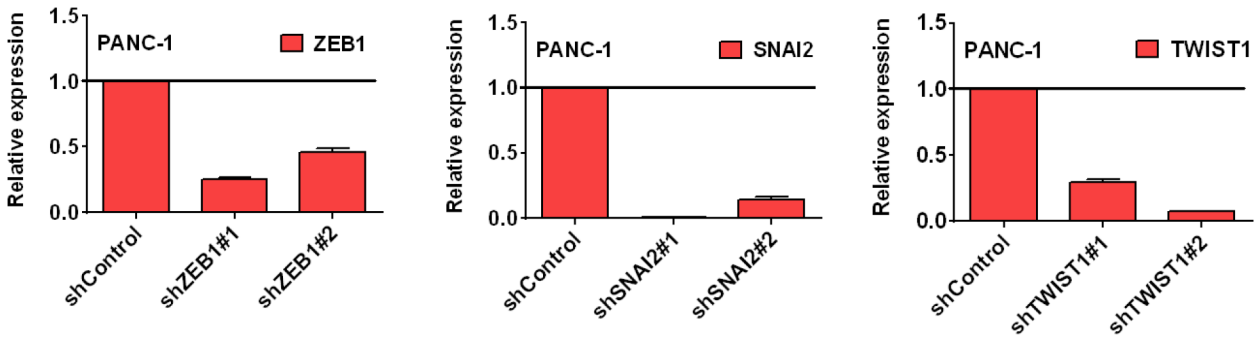

**Supplementary Figure 2: Stable knockdown of SET (SET-shRNA) in MIA PaCa-2. (A)** Protein levels of SET Isoform 1 and Isoform 2 in SET shRNA (From Fig 3C) transduced MIA PaCa-2 cells were quantified by densitometry and SET:β-actin ratios plotted. **(B)** Expression levels of total SET, isoform 1, and isoform 2 transcripts in MIA PaCa-2 SET-shRNAs analyzed by qRT-PCR with taqman probe. GUSB was used as an internal control. Bars, SD.



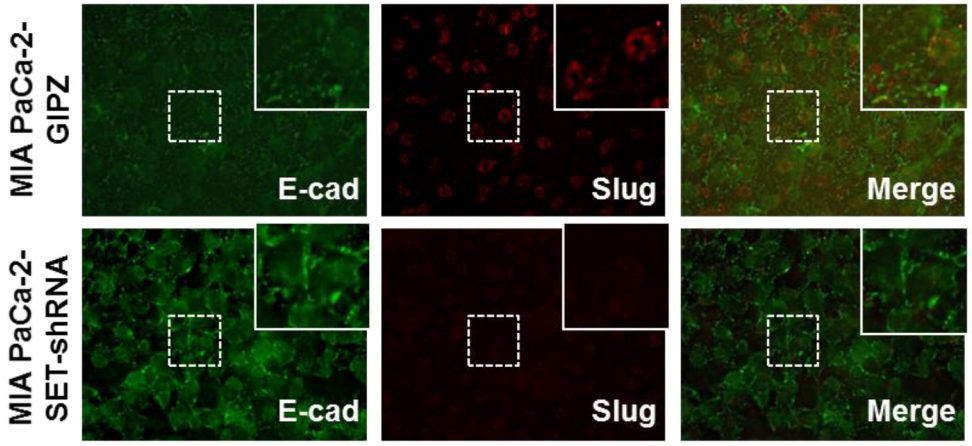

**Supplementary Figure 3: Supernatants were collected from cells cultured in primary dishes for the indicated time periods and reseeded and cultured in secondary dishes (curved arrows).** After 14 days, colonies developed in secondary dishes were fixed and stained with methylene blue for visualization.



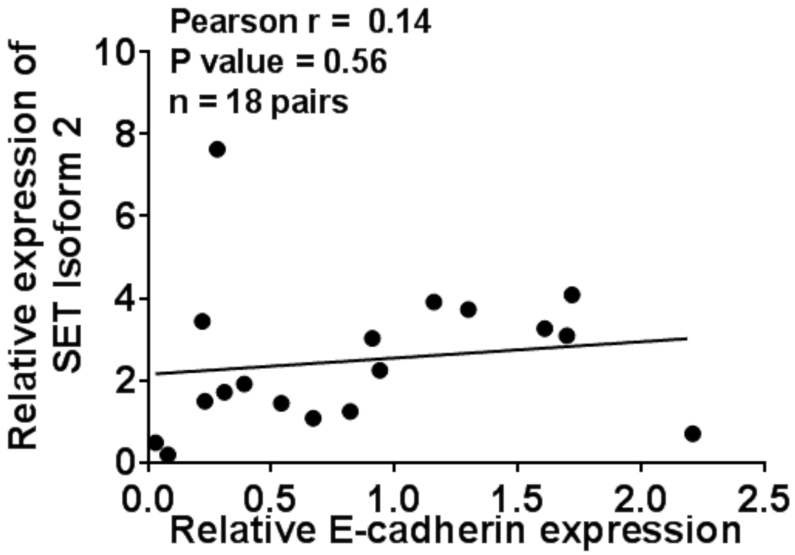

**Supplementary Figure 4: SET isoform 1 induces EMT-like characteristics but has no effect on growth of pancreatic cancer cells. (A)** Stable overexpression of SET Isoform 1 (SET-FLAG) in PANC-1. Expression levels of SET Isoform 1 transcripts in PANC-1 stably overexpressing SET Isoform 1 analyzed by qRT-PCR with taqman probe. GUSB was used as an internal control. **(B-C)** SET isoform 1 (SET-FLAG) overexpression induces EMT like characteristics in PANC-1. **(B)** Cells with overexpression of SET Isoform 1 have a more mesenchymal morphology as compared with control cells (pCMV3). **(C)** Overexpression of SET isoform 1 in PANC-1 increases N-cadherin and decreases Keratin 8/18 protein levels. Whole cell lysates (50 µg) were subjected to Western blotting analysis. β-actin, the internal loading control, is shown with a representative blot. **(D and E)** Expression of SET isoform 1 does not significantly affect the colony formation abilities (D) or the cellular proliferation (E) of PANC-1 unlike SET Isoform 2. For colony formation assays, cells were fixed and stained with crystal violet at indicated time points (D). For cellular viability assays, cells were grown for 96 h and % cellular viability was measured with MTT assay (E). Bars, SD.




**Supplementary Figure 5: Knockdown of EMT-regulating transcription factors (ZEB1, SNAI2, and TWIST1) in PANC-1.** Stable knockdown of ZEB1, SNAI2 (Slug), and TWIST1 using respective shRNAs#1-2 in PANC-1. Transcript levels of individual genes analyzed with qRT-PCR and compared against control cells. GUSB was used as an internal control. Bars, SD.




**Supplementary Figure 6: E-cadherin and Slug expressions were oppositely correlated in MIA PaCa-2 (GIPZ & SET-shRNA).** Slug (red) is expressed in the cytoplasm, while E-cadherin (green) is expressed on the cell surface. E-cadherin images in the left panels (MIA PaCa-2 GIPZ and MIA PaCa-2 SET-shRNA) are also shown in Fig. 4E.




**Supplementary Figure 7: E-cadherin transcripts showed no correlation with SET Isoform 2 expression in pancreatic cancer tissues (n = 18).**