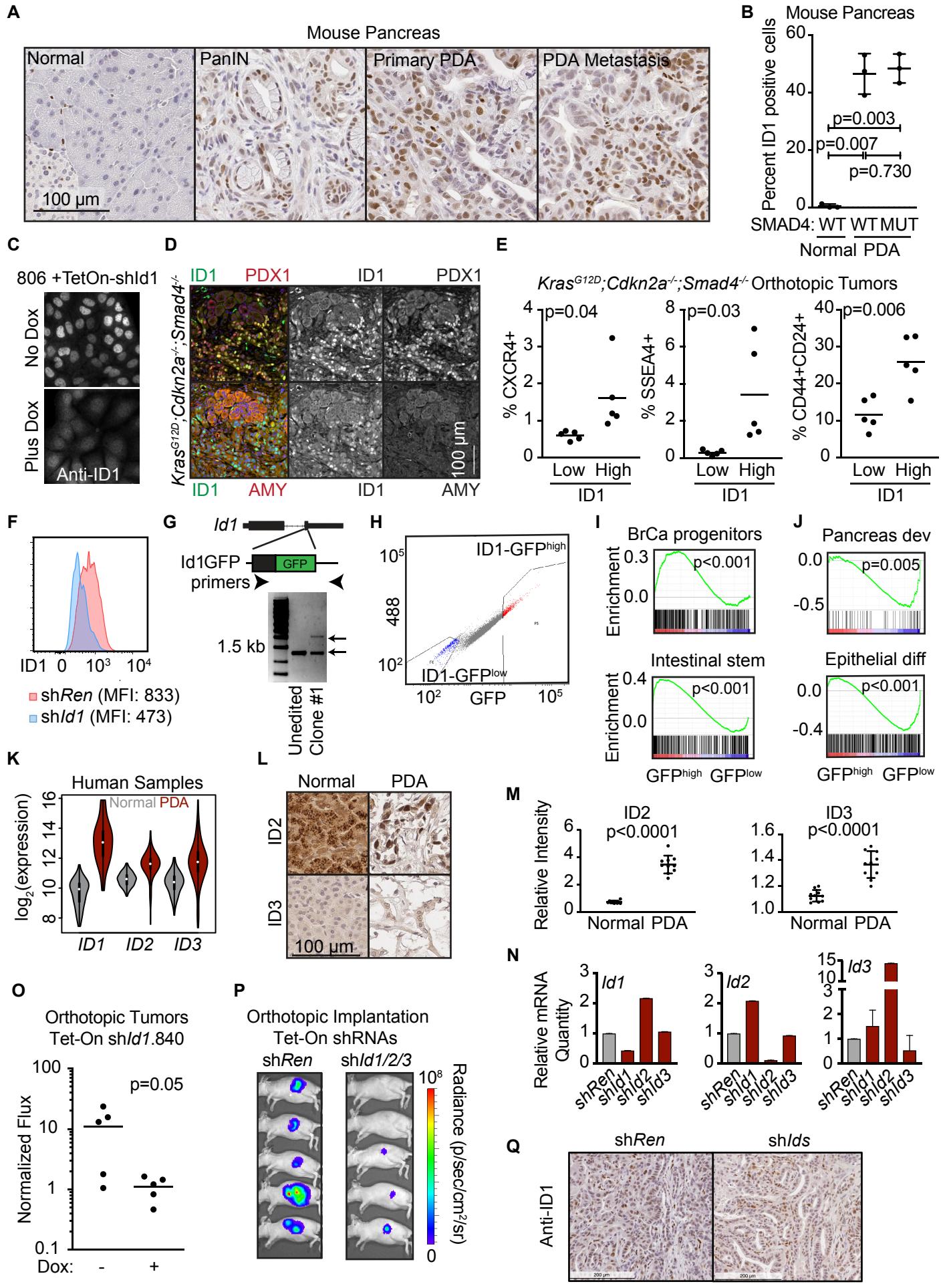
Supplementary Fig. S2



Supplementary Fig. S2: ID1 expression and effects in PDA progenitors

A) Representative samples of mouse normal pancreas, PanIN, primary PDA, and PDA metastasis subjected to ID1 IHC analysis.

B) Quantification of ID1+ epithelial cells in mouse PDA tissues as determined by IHC. n=3 per group, two-sided unpaired t-tests.

C) *Kras^{G12D};Cdkn2a$^{-/-}$;Smad4$^{-/-}$* mouse PDA cells with a doxycycline-inducible shRNA targeting *Id1* were subjected to anti-ID1 immunofluorescence staining.

D) Immunofluorescence analysis of ID1 and amylase (AMY) in autochthonous *Kras^{G12D};Cdkn2a$^{-/-}$;Smad4$^{-/-}$* mouse PDA tissue.

E) Orthotopically-implanted *Kras^{G12D};Cdkn2a$^{-/-}$;Smad4$^{-/-}$* mouse tumors were dissociated and analyzed by flow cytometry. Cytokeratin 19 (CK19) was used to distinguish PDA cells from stromal cells. The ID1-low and ID1-high components of CK19+ population were analyzed for the indicated markers. n=5 mice.

F) Flow cytometry analysis of anti-ID1 staining control of *Kras^{G12D};Cdkn2a$^{-/-}$;Smad4$^{-/-}$* mouse PDA cells engineered with a Tet-ON-*shId1* construct. MFI, mean fluorescence intensity.

G) An ID1-GFP reporter was constructed using CRISPR/Cas9 to insert *green fluorescent protein* fused to the 3' end of *Id1* coding region in the endogenous locus in *Kras^{G12D};Cdkn2a$^{-/-}$;Smad4$^{-/-}$* mouse PDA cells, as verified by PCR analysis.

H) Flow cytometry sorting for ID1-GFP^{high} and ID1-GFP^{low} cells. The overlapping spectra of Alexa 488 and GFP were used for better graphic separation of the cell populations.

I,J) ID1-GFP^{high} and ID1-GFP^{low} cells were collected for RNA-seq. Differentially expressed genes were determined by DESeq2 and ranked by fold change in expression for GSEA. n=2 per group.

K) Analysis of RNA-seq data from the human pancreas samples described in Figure 1E.

L) IHC of the human PDA samples characterized in Figure 2B.

M) Quantitative scoring of ID1 and ID3 IHC staining in human PDA samples characterized in Figure 2A.

N) qRT-PCR analysis of *Id1-3* transcripts in *Kras^{G12D};Cdkn2a$^{-/-}$;Smad4$^{-/-}$* mouse PDA cells engineered with Tet-ON shRNAs targeting individual *Id* genes. Mean ±range, representative of 2 independent experiments.

O) Mice were treated with doxycycline from 3 days after orthotopic implantation of *Kras^{G12D};Cdkn2a$^{-/-}$;Smad4$^{-/-}$* PDA cells engineered with a Tet-ON-sh*Id1* construct. BLI measurements were done 2 weeks post-implantation. Two-tailed unpaired t-test, n=5 per group.

P) Bioluminescent measurements of orthotopically implanted *Kras^{G12D};Cdkn2a$^{-/-}$;Smad4$^{-/-}$* PDA cells at 4 weeks post-implantation. Quantified in Figure 2H.

Q) IHC for ID1 in tumors collected from orthotopic implantation assays at endpoint.