Developmental Cell, Volume *50*

Supplemental Information

Prevention and Reversion of Pancreatic Tumorigenesis through a Differentiation-Based Mechanism

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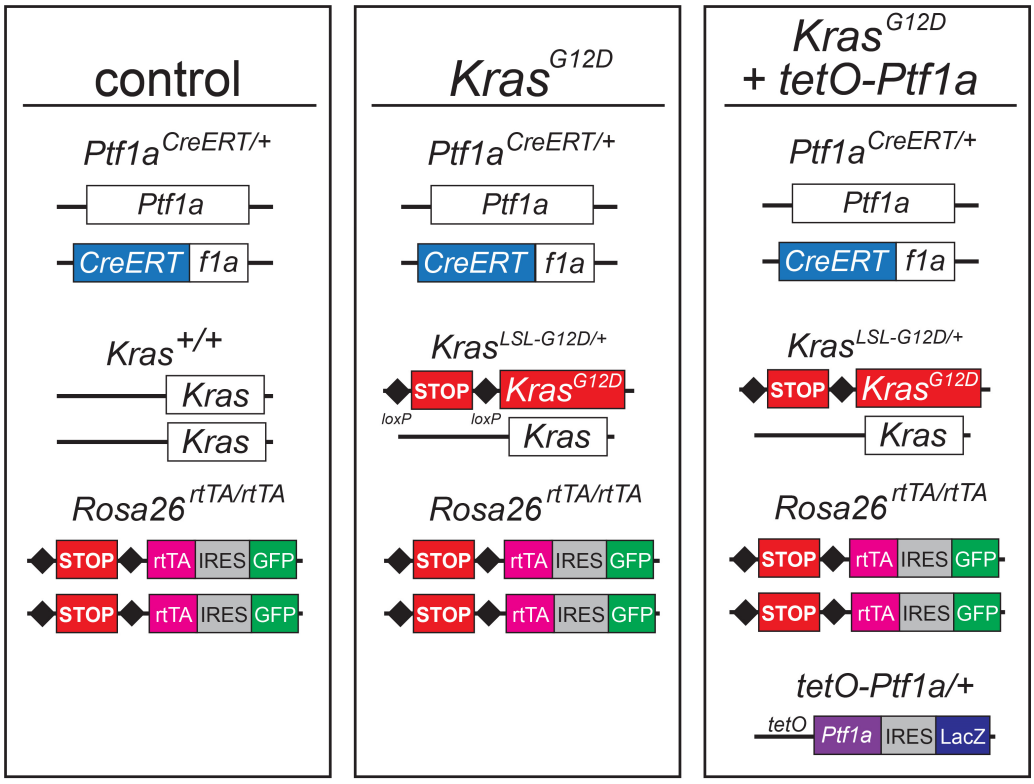

Figure S1. Related to Figure 2. Mouse alleles utilized in this study. Schematic representations of the alleles present in the genotypes referred to, in shorthand, as Control, *Kras*^{G12D}, and *Kras*^{G12D} + *tetO-Ptf1a*. Function of the system is described in Figure 1.

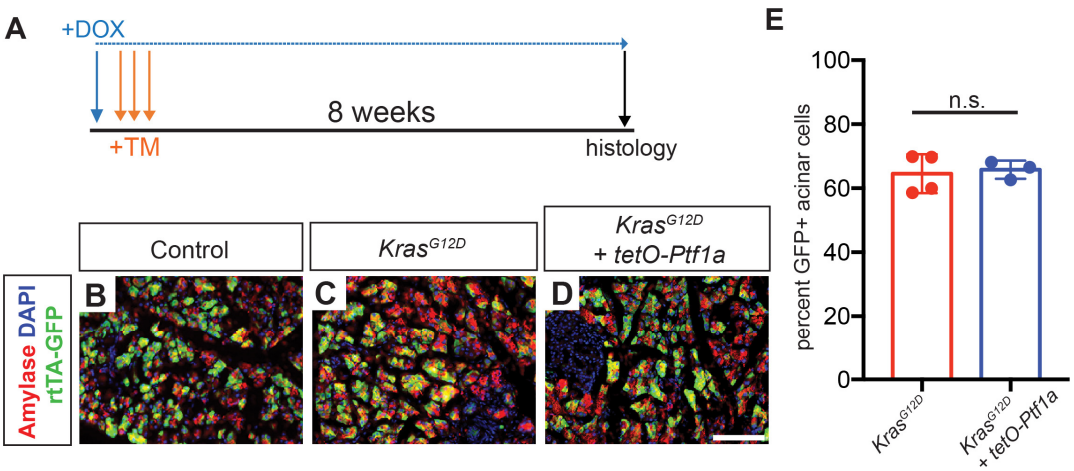

Figure S2. Related to Figure 2. *Ptf1a*^{CreERT} recombination efficiency following tamoxifen treatment. (A) 6-8-week-old mice were administered tamoxifen on three consecutive days (3 x 0.25 mg/g mouse) while DOX was present in the drinking water (1 mg/ml), and pancreata were harvested after an 8-week chase. **(B-D)** Immunofluorescence for amylase (red) and GFP (green), reporting recombination of *R26*^{rtTA}, on pancreata of indicated genotypes (20x, scale bar is 100μm). For all mice, only histologically normal areas were imaged in an effort to provide an accurate quantification of Cre-mediated recombination. **(E)** The proportion of GFP expression among amylase+ acinar cells were compared between *Kras*^{G12D}, and *Kras*^{G12D} + *tetO-Ptf1a* genotypes, with no significant difference found.

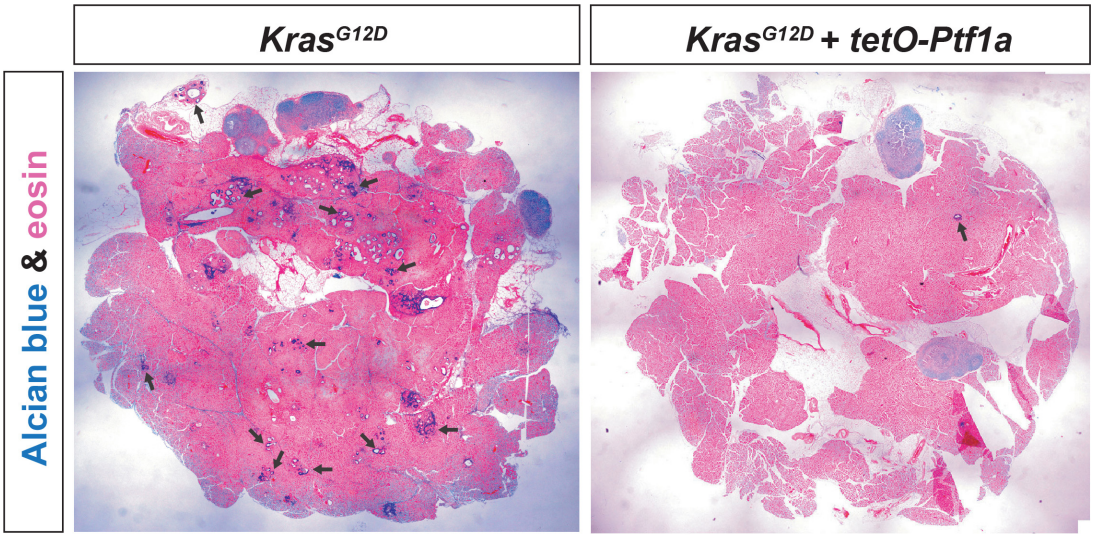

Figure S3. Related to Figure 2. Alcian blue staining, highlighting PanIN formation in mice of the indicated genotypes 8 weeks after TM administration. Each image is a Photomerge of 16 4x images, showing a section through the entire pancreas. Three images are quantified per mouse to yield a count of PanIN burden throughout the organ. Black arrows indicate examples of pancreatic lobules containing Alcian blue+ PanINs.

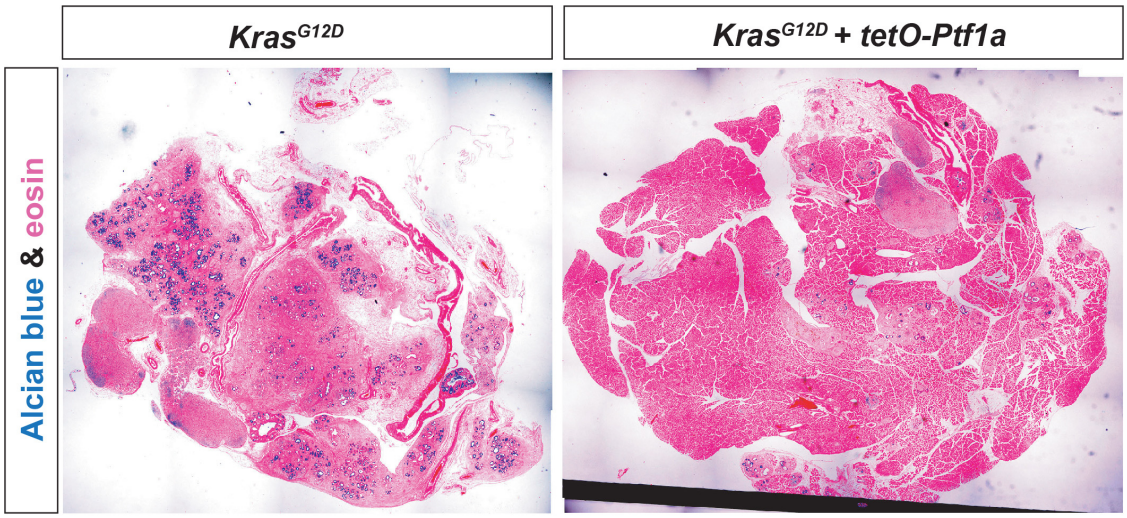

Figure S4. Related to Figure 3. Alcian blue staining, highlighting PanIN formation in mice of the indicated genotypes 3 weeks after caerulein treatment administration (see Figure 3 for the entire experimental schematic). Each image is a Photomerge of ~16 4x images. Three images are quantified per mouse to yield a count of PanIN burden throughout the organ.

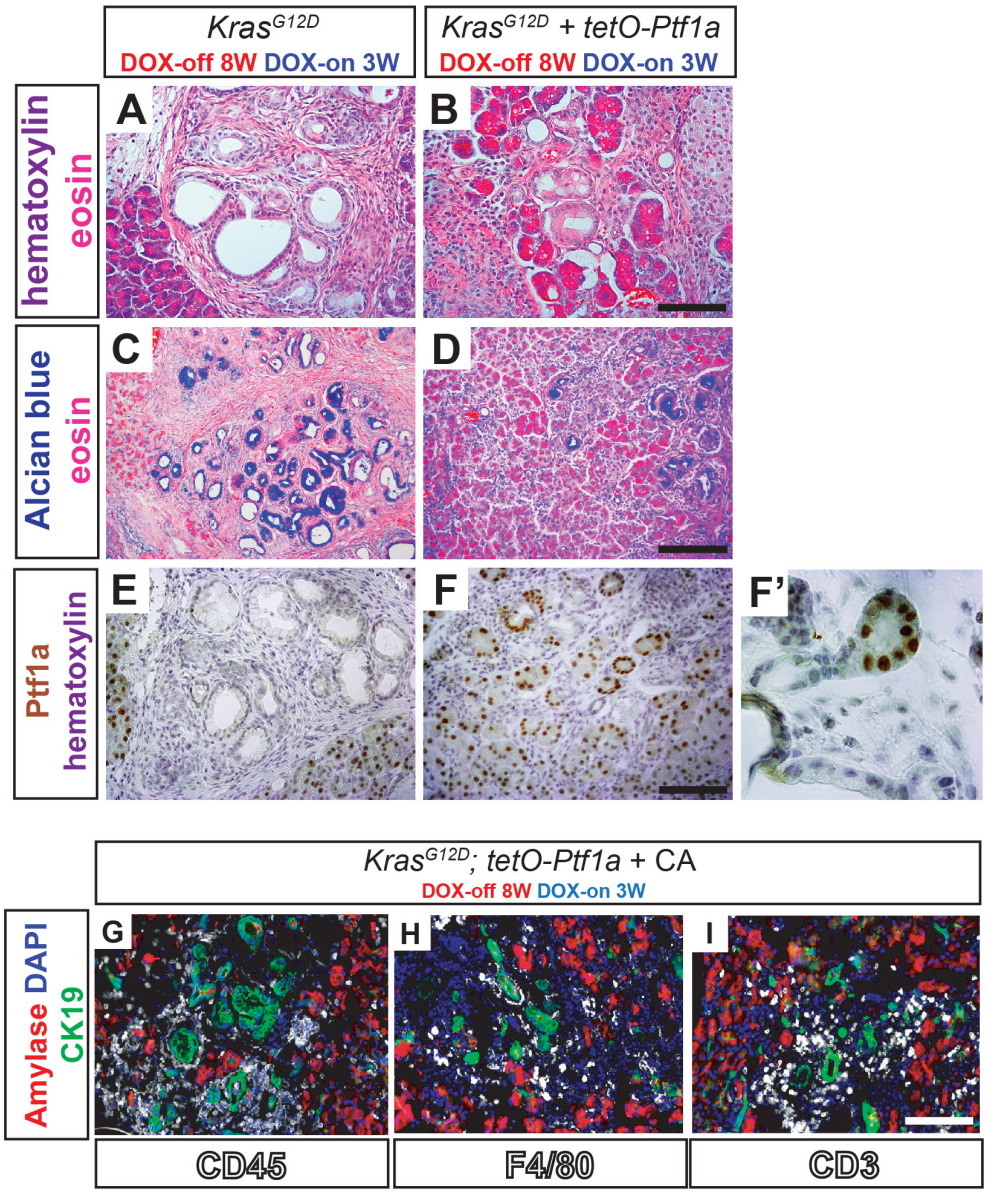

Figure S5. Related to Figure 4. *Ptf1a* re-expression in PanINs leads to the emergence of lesion-localized acinar cells in *Kras^{G12D}* + *tetO-Ptf1a* pancreata. (A-B) H&E (40x, scale bar is 100μm) and **(C-D)** Alcian blue (20x, scale bar is 200μm) staining of pancreata from mice of indicated genotypes and treatments. Eosinophilic primitive acinar cells are observed trapped within PanIN lesions of *Ptf1a* re-expressing mice. **(E-F)** Immunohistochemistry for Ptf1a, showing absence of Ptf1a in PanINs from *Kras^{G12D}* pancreata and wide Ptf1a expression in duct-like structures in *Kras^{G12D}* + *tetO-Ptf1a* pancreata (40x, scale bar is 100μm). **(F')** Ptf1a+ acinar-like cluster emerging from a ductal structure. **(G-I)** Immunofluorescence for Amylase, CK19, DAPI and CD45, F4/80, or CD3 in resolving areas of *Kras^{G12D}* + *tetO-Ptf1a* pancreata (20x, scale bare is 100μm).

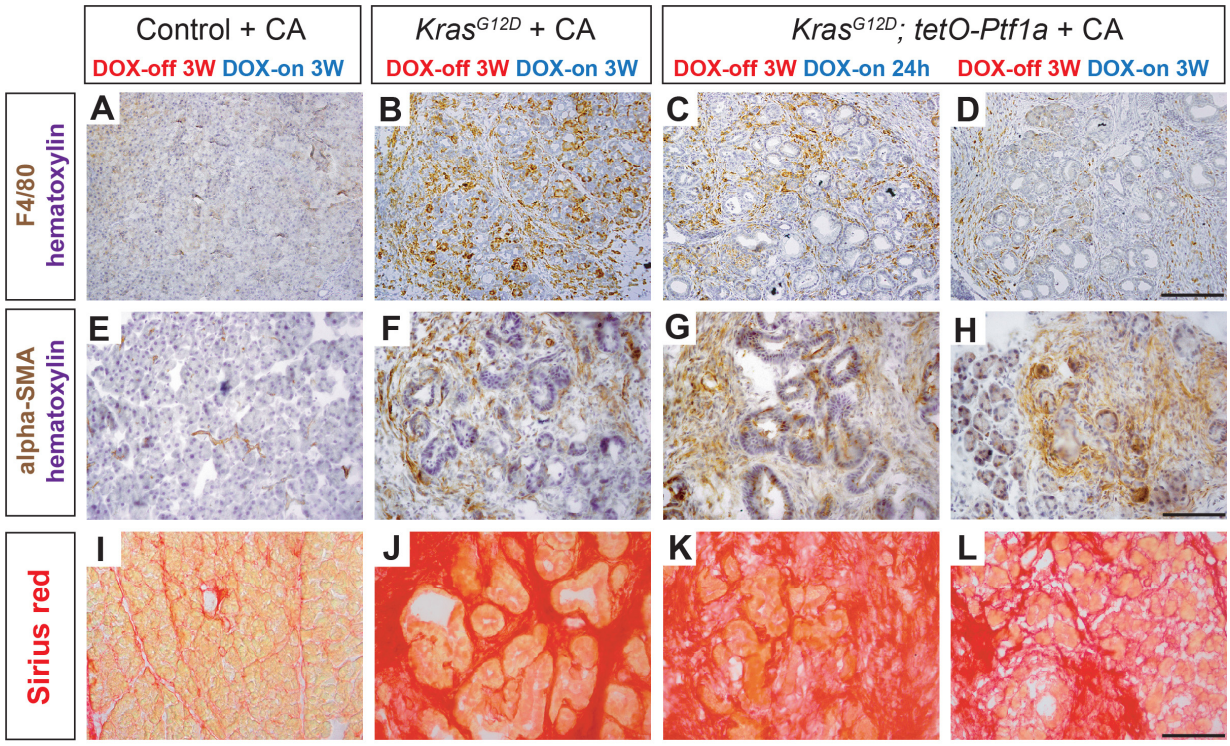

Figure S6. Related to Figure 6. Microenvironmental changes associated with PanIN-to-acinar re-differentiation. (A-D) Immunohistochemistry for F4/80, showing macrophage infiltration in *Kras*^{G12D} and *Kras*^{G12D} + *tetO-Ptf1a* pancreata (20x, scale bar is 200μm). Black arrow shows a representative area of resolving *Kras*^{G12D} + *tetO-Ptf1a* pancreata 3W after DOX treatment that has few F4/80+ cells. **(E-H)** Immunohistochemistry for αSMA, showing fibroblast activation in *Kras*^{G12D} and *Kras*^{G12D} + *tetO-Ptf1a* pancreata (40x, scale bar is 100μm). The arrows in H highlight an area of resolved pancreas that has few αSMA-positive cells. (I-L) Sirius red staining, highlighting areas of fibrosis in *Kras*^{G12D} and *Kras*^{G12D} + *tetO-Ptf1a* pancreata. Re-differentiating acini are present in areas of low Sirius red staining (L, 40x, scale bar is 100μm).

Table S1. Related to Figures 2 and 7. PCR primer sequences.

Target	Strand	Sequence	Amplicon size (bp)
		genotyping	
Ptf1a	top	TCCAGCAAGCGGGTACTATC	807 (wt),
	bottom (wt-specific)	AGGCGCTTTTCGTAGGGTAG	560 (CreERT)
	bottom (CreERT-specific)	TAAGCAATCCCCAGAAATGC	
Kras$^{LSL-G12D}$	top	AGCTAGCCACCATGGCTTGAGTAAGTCTGCA	597
	bottom	CCTTTACAAGCGGCAGACTGTAGA	
R26R^{rtTA}	top	AAAGTCGCTCTGAGTTGTTAT	317 (wt),
	bottom (wt-specific)	GAAAGACCGCGAAGAGTTTG	235 (rtTA)
	bottom (rtTA-specific)	TAAGCCTGCCCAGAAGACTC	
tetO-Ptf1a	top	TTGACCTCCATAGAAGACACCGG	257
	bottom	CGCGGTAGCAGTATTCGTGTAGC	
		RT-PCR	
PPIA	top	CCCACCGTGTTCTTCGACATT	275
(cyclophilin A)	bottom	GGACCCGTATGCTTTAGGATGA	
CPA1	top	CGCTCCACCGACACTTTTAAC	139
	bottom	AAATGGGACGCCCTTCATAGG	
PRSS1	top	AGCCAGGCTAAGTGTGAAGC	100
	bottom	AATCACCCTGACATGAATCCTTG	
AMY2A	top	AATACACAACAAGGACGGACATC	102
	bottom	TCCAAATCCCTTCGGAGCTAAA	
CELA2A	top	ACCCCACTTACCCACCTTATG	227
	bottom	CTCCGCAACGTAGAGGTTGT	