Supplemental Information

aE-catenin is a positive regulator of pancreatic islet cell lineage differentiation

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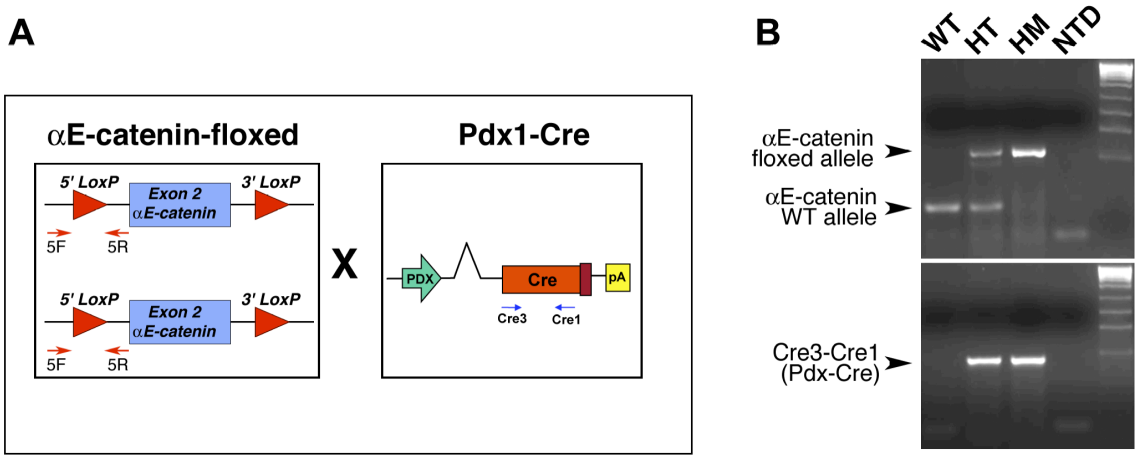

Figure S1. Breeding strategy and genotype of Pdx1-Cre;αE-catenin-KO mice (Related to Figure 1).

(A) αE-catenin$^{flox/flox}$ mice (Vasioukhin et al., 2001) were bred to Pdx1-Cre mice (Gu et al., 2002) to yield heterozygous (HT) Pdx1-Cre;αE-catenin$^{flox/-}$ mice (B). Backcrossing of these HT mice to (A) αE-catenin$^{flox/flox}$ animals yielded homozygous Pdx1-Cre;αE-catenin$^{-/-}$ mutants (B).

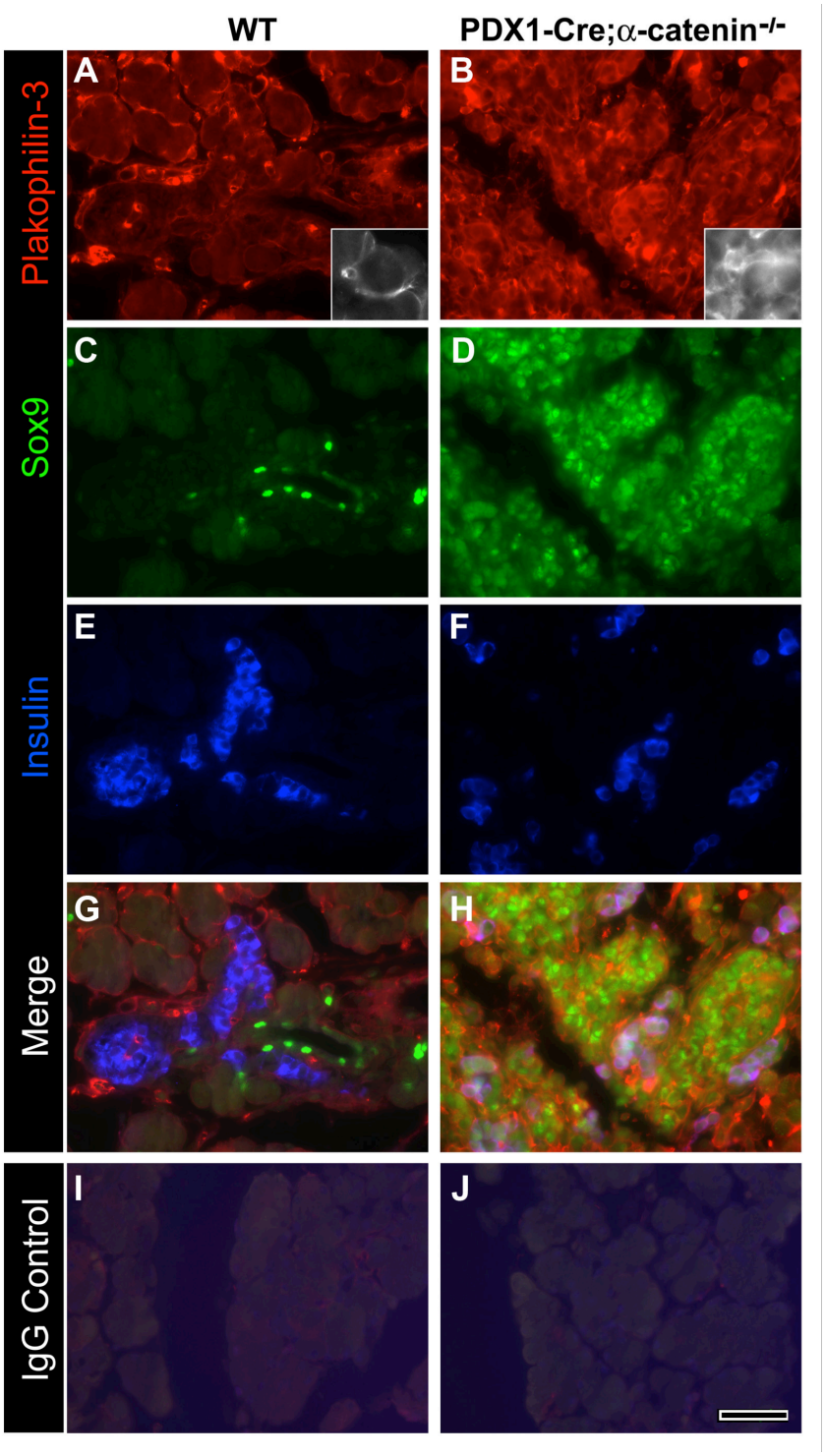

Figure S2. Expression pattern of Plakophilin-3 in Pdx1-Cre;αE-catenin-KO pancreata (Related to Figures 2 & 3). In the pancreas of WT mice (left panels) Plakophilin-3-specific immunoreactivity is primarily detected at the cell surface of all epithelial cells (A, G), albeit most prominently in ductal cells. This pattern is altered in Pdx1-Cre;αE-catenin-KO pancreata (right panels) where significant immunoreactivity is also detected in the cytoplasm (B, H). Insets in A and B provide monochromatic examples of immunoreactivity for Plakophilin-3. Note the accumulation of Sox9^{+} cells in mutant pancreata (D), compared to WT (C), and the rare insulin^{+} cells (F *vs* E). (G, H) three-color merging of individual immunoreactivities. (I and J) Negative control staining performed using irrelevant species-matched control IgGs as primary antibodies. Reference bar= 50 μm.

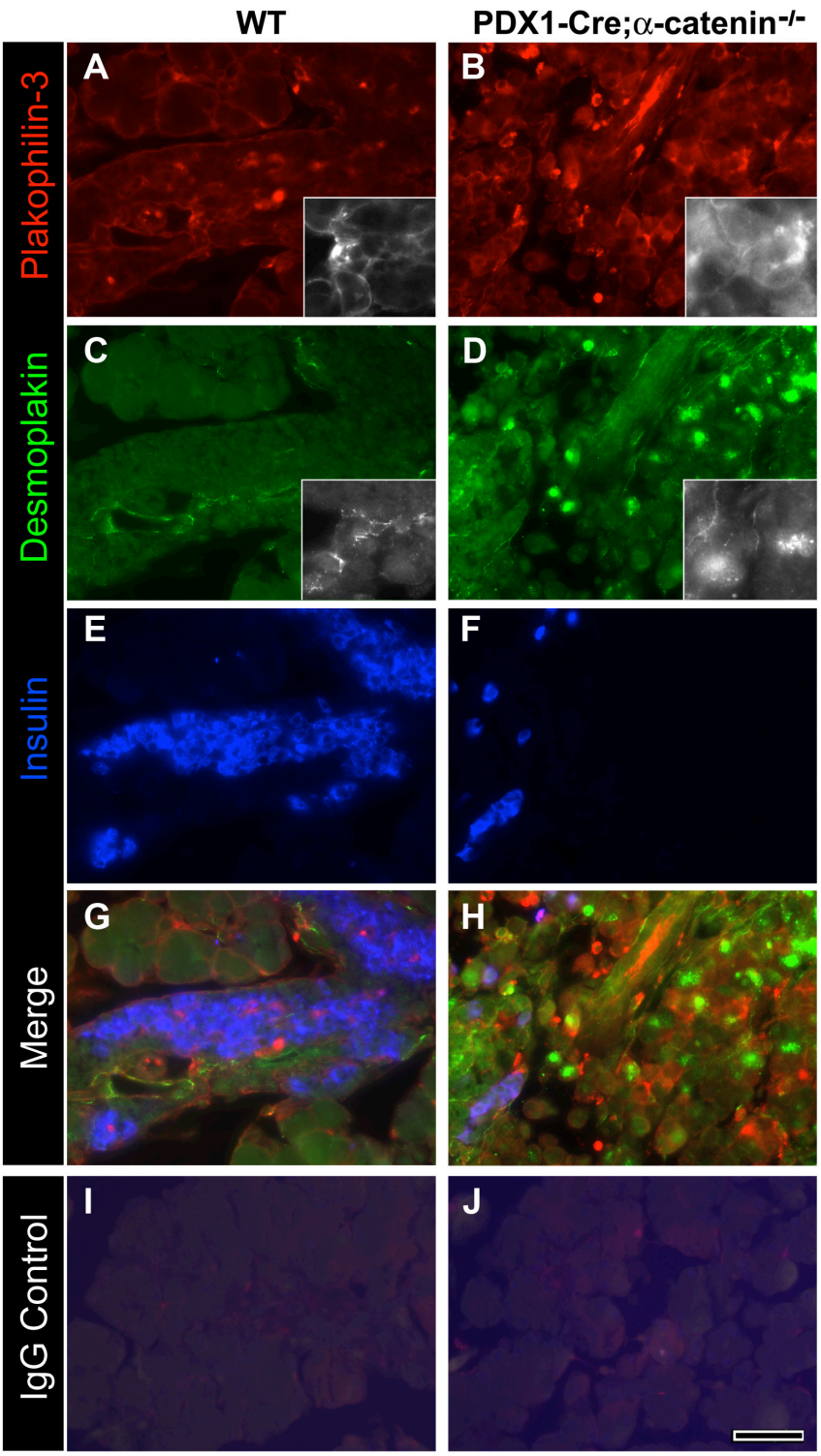

Figure S3. Expression pattern of Desmoplakin in Pdx1-Cre;αE-catenin-KO pancreata (Related to Figures 2 & 3). In the pancreas of WT mice (left panels), Desmoplakin-specific immunoreactivity is primarily detected at the cell surface of ductal and exocrine cells (C, and G). This pattern is altered in Pdx1-Cre;αE-catenin-KO pancreata (right panels) where significant immunoreactivity is also detected in the cytoplasm (D and H). Black and white insets show monochromatic examples of immunoreactivity for Plakophilin-3 (A and B), and for Desmoplakin (C and D). (I and J) Negative control staining performed using irrelevant species-matched control IgGs as primary antibodies. Reference bar= 50 μm.

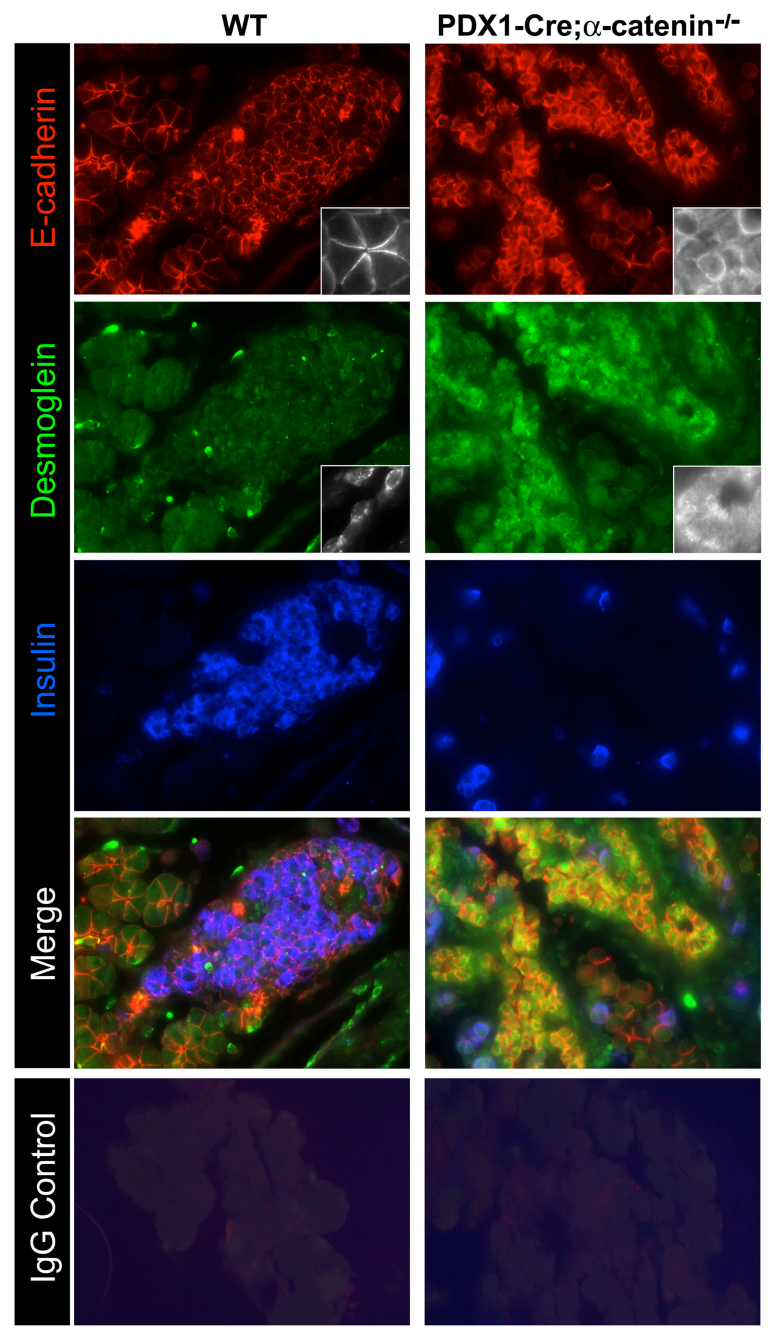

Figure S4. Expression pattern of Desmoglein in Pdx1-Cre;αE-catenin-KO pancreata (Related to Figures 2 & 3). In the pancreas of WT mice (left panels) Desmoglein-specific immunoreactivity (C) is detected at the cell surface of both epithelial and non-epithelial (E-cadherin-negative) cells. This pattern is altered in Pdx1-Cre;αE-catenin-KO pancreata (right panels) where significant immunoreactivity is detected in cords of epithelial E-cadherin^{+} cells and within the cell cytoplasm (D, and H). Black and white insets show monochromatic examples of immunoreactivity for E-cadherin (A and B), and for Desmoglein (C and D). (I and J) Negative control staining performed using irrelevant species-matched control IgGs as primary antibodies. Reference bar= 50 μm.

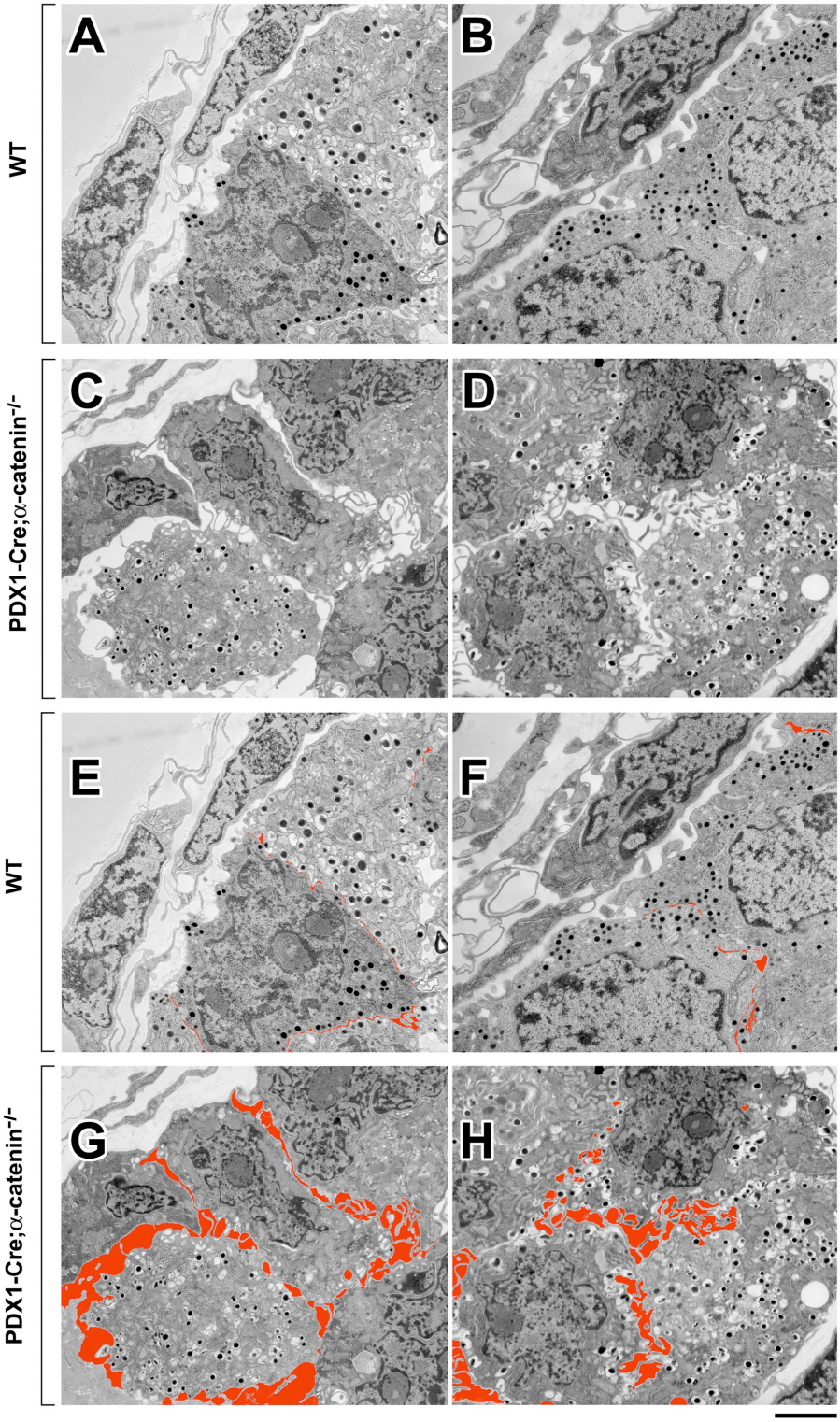

Figure S5. Transmission Electron Microscopy of pancreatic endocrine cells from WT and Pdx1-Cre;αE-catenin-KO mice (Related to Figure 3). Representative images of pancreatic endocrine cells from WT (A and B) and Pdx1-Cre;αE-catenin-KO mice (C and D). Digital tracing of inter-cellular spaces (E-H, red) highlights the significant loss of close cell-cell interactions in the tissue from Pdx1-Cre;αE-catenin-KO mice (G & H) as compared to wild type controls (E & F). Reference bar= 1 μm.

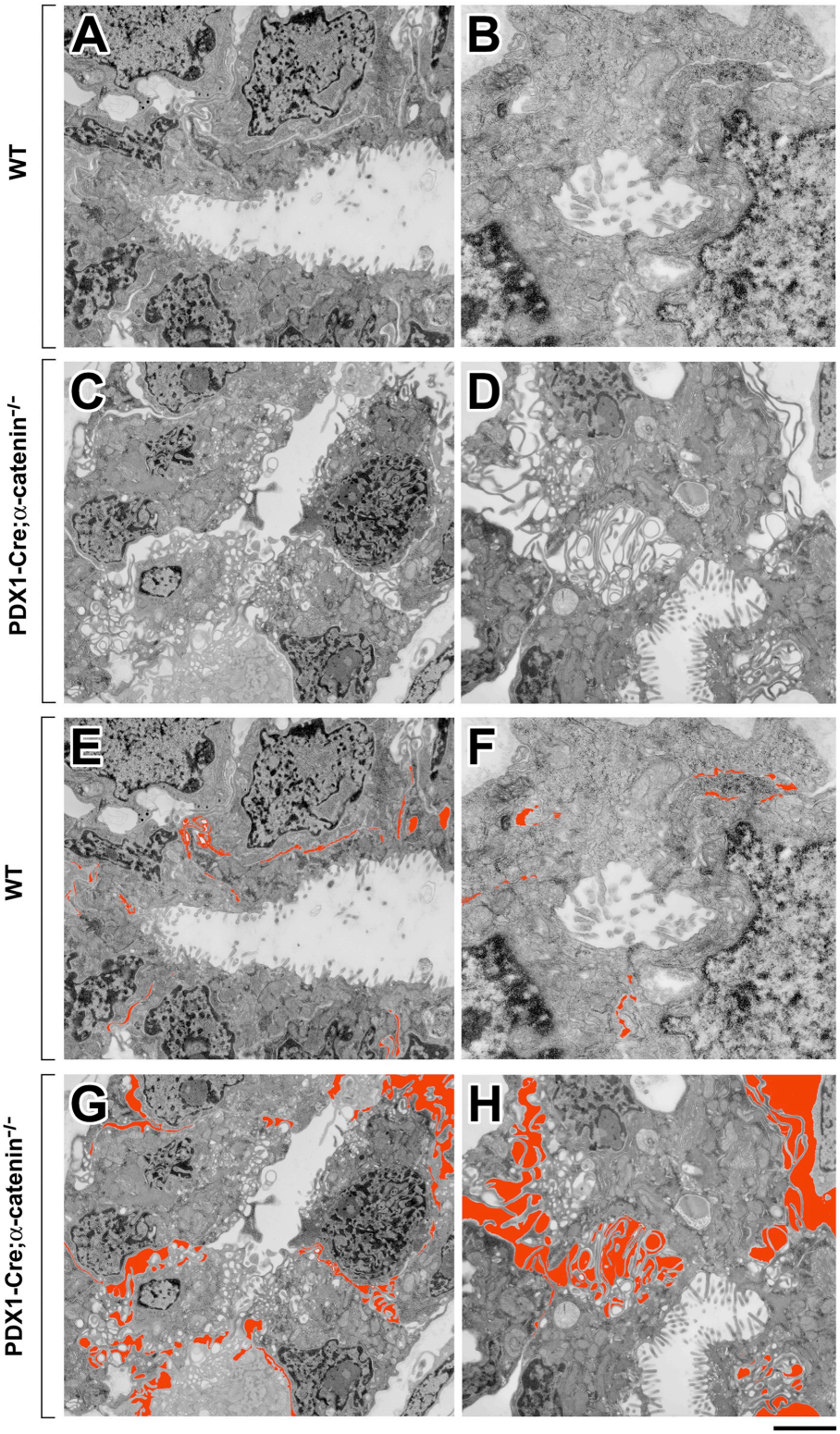

Figure S6. Transmitted electron microscopy of pancreatic ductal cells from WT and Pdx1-Cre;αE-catenin-KO mice (Related to Figure 3). Representative images of pancreatic ductal cells from WT (A & B) and Pdx1-Cre;αE-catenin-KO mice (C & D). Digital tracing of inter-cellular spaces (E-H, red) shows increased inter-cellular spaces between these pancreatic cell types in Pdx1-Cre; α E-catenin-KO mice (G & H) as compared to WT pancreata (E & F). Reference bar= 1 μm.

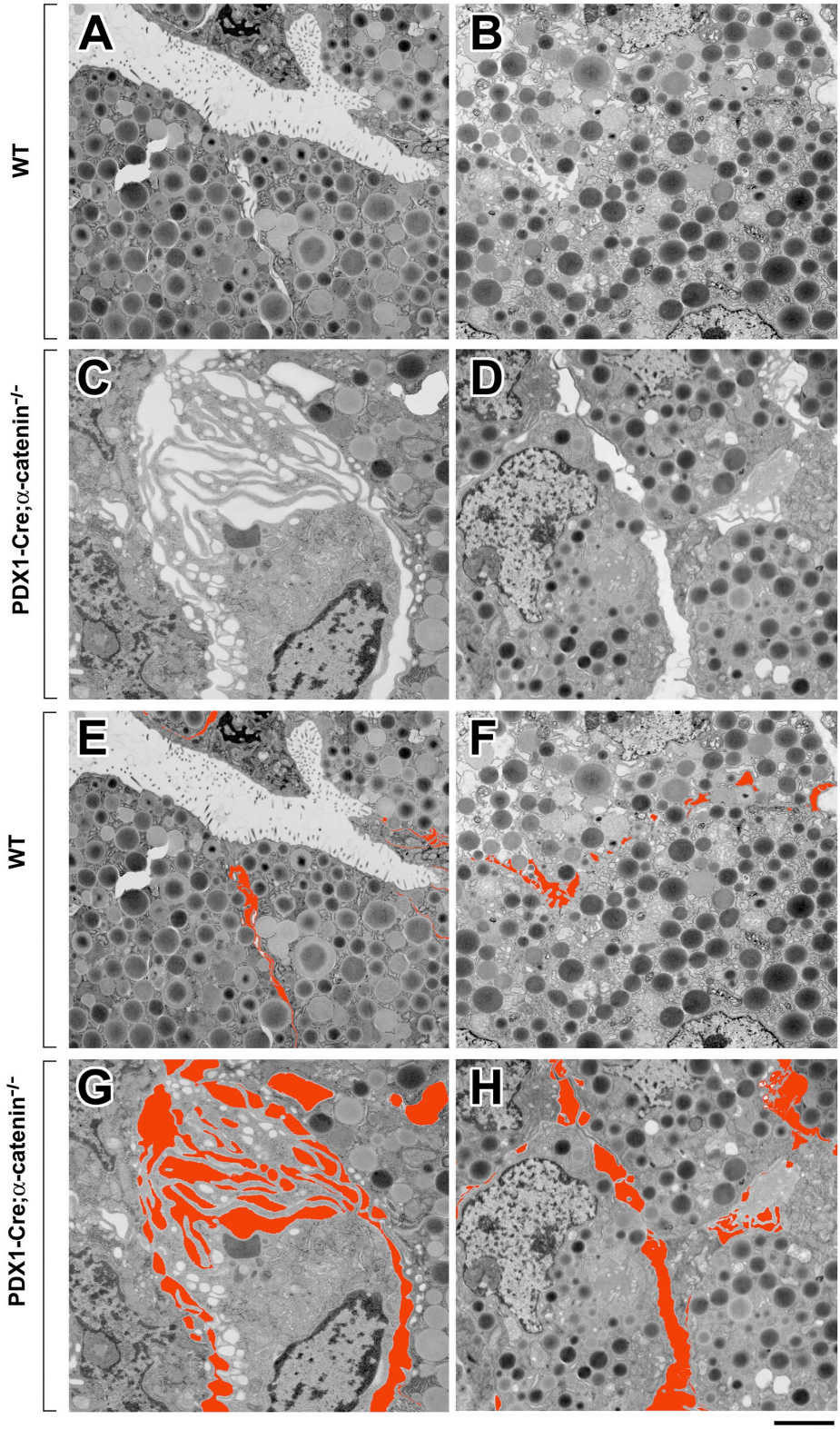

Figure S7. Transmitted electron microscopy of pancreatic acinar cells from WT and Pdx1-Cre;αE-catenin-KO mice (Related to Figure 3). Representative examples of pancreatic acinar cells from WT (A & B) and Pdx1-Cre; α E-catenin-KO mice (C & D). Inter-cellular spaces between acinar cells are significantly enlarged in the pancreas of Pdx1-Cre; α E-catenin-KO mice (G & H), compared to WT pancreata (E & F). Reference bar= 1 μm.

Table S1. Antibodies used for immunohistochemistry

Antigen	Species	Source	Dilution
Sox9	Rabbit	Chemicon	1:800
Insulin	Guinea Pig	Dako	1:400
Glucose	Mouse	Sigma	1:400
Glucose	Rabbit	Dako	1:400
Somatostatin	Rabbit	Dako	1:100
Pancreatic polypeptide	Rabbit	Dako	1:100
Ghrelin	Goat	Santa Cruz	1:800
Alpha-catenin	Mouse	BD Transduction Lab	1:200
Beta-catenin	Mouse	BD Transduction Lab	1:200
E-Cadherin	Mouse	BD Transduction Lab	1:200
Amylase	Rabbit	Sigma	1:400
CPA-1	Rabbit	Proteintech Group	1:400
Mucin-1	Rabbit	Thermo Scientific	1:400
Claudin 3	Rabbit	Invitrogen	1:100
Claudin 7	Rabbit	Invitrogen	1:50
Pan-Cytokeratin	Mouse	Sigma	1:400
Cytokeratin 19	Mouse	Sigma	1:25
PCNA	Mouse	Dako	1:100
Histone 3	Rabbit	Upstate	1:100
Pax6	Rabbit	Chemicon	1:1000
Nkx6.1	Mouse	M. Sander, UCSD, CA, USA	1:500
Nkx6.1	Mouse	Beta Cell Biology Consortium	1:1000
PDX1	Goat	M. Sander, UCSD, CA, USA	1:1000
Ngn3	Guinea Pig	M. Sander, UCSD, CA, USA	1:800
Ngn3	Mouse	Beta Cell Biology Consortium	1:2000
Synaptophysin	Rabbit	Dako	1:100
HNF-3beta	Goat	R&D Systems	1:100
Desmoplakin	Rabbit	Abcam	1:100
Desmoglein	Rabbit	Abcam	1:100
Plakiphilin-3	Mouse	Santa Cruz	1:100

Table S2. Sequence of primers used for quantitative PCR.

Gene	Forward	Reverse
Gli 1	CCAAGCCAACTTTATGTCAGGG	AGCCCGCTTCTTTGTTAATTGA
Gli 2	CAACGCCTACTCTCCCAGAC	GAGCCTTGATGTACTGTACCAC
Smo	GAGCGTAGCTTCCGGGACTA	CTGGGCCGATTCTTGATCTCA
Ptch 1	GATCCTCATGTGCCTTTT	CACGTCCTGTAGCTCTAT
Cyclin E	CCAGCAGTAAGAAGCAGAG	TCCACACCACTGTCTTTG
Fgf 15	TACTCGGAGGAAGACTGTA	ATGAAGATGATATGGAGATGGT
E-Cadherin	ACTGGACTGTCTGTGTTAG	AAAGGCACAGTTTATATCTCAG
PDX1	ACATCTCCCCATACGAAGTGCC	CCAGGCTCGGTTCCATTCGG
Nkx6.1	AGTATCCTGGCTGCTCGC	CCCGCCACAATTTCTAGGTTAA
Neurogenin 3	CCGGATGACGCCAAACTTA	CATAGAAGCTGTGGTCCGCTATG