Supporting information

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SI Text

Electron Microscopy and TUNEL assay. Pancreas were fixed with glutaraldehyde and paraformaldehyde followed by osmium tetroxide and uranyl acetate, dehydrated in ethanol, and embedded in epoxy. Sections were cut at ≈90-nm thickness, stained with lead citrate, and imaged on a FEI Techai 12 microscope using a Gatan camera and software.

Apoptotic cells were visualized by DeadEnd fluorometric TUNEL assay using the manufacturer's protocol (Promega). Bouin's fixed, paraffin sections were used for this TUNEL assay.

Real-Time RT-PCR. Whole tadpoles were killed by using ice-cold MS-222, and their abdomens were opened and immediately immersed in RNAlater (Ambion) for as long as overnight at 4°C. Total RNA and DNA were extracted with TRIzol (Invitrogen) from pools of five pancreases per replicate (three replicates for each stage) from different stages of development. The quality of RNA was determined by gel electrophoresis, and the absorbance ratio at 260/280 was determined by spectrophotometry. Real-time PCR was carried out in a DNA Engine Opticon System (MJ Research) using the QuantiTect SYBR Green (One step) RT-PCR kit (Qiagen). The primer pairs used were as follows: *amylase*, 5′-CGTTCTGGGAATGAACAACA-3′ and 5′-CCTGGATCCTGCATTGAAGT-3′; *Notch-1*, 5′-CATGCAGCAACAACATCACA-3′ and 5′-AAGGCAGAGATGCAGCAAAT-3′; *Hes-1*, 5′-TCCAACAGCATCTCCAT CAG-3′and 5′-ATTCAAGGTGCCTGAACACC-3′; *PDX1* 5′-GTTCCCTCAGCTGCTT ATCG-3′ and 5′-TACCAAGGG GTTGCTGTAGG-3′. Universal 18S primer pairs (Ambion) were used for internal standards for each of the samples. The quantity of template RNA and the annealing temperature was standardized before the actual run. The reaction was carried out in a 96-well plate according to the manufacturer's protocol (Qiagen). Each sample was run in duplicate and there were three replicates for each group. The threshold cycle value (C_t) was determined for each run as the cycle number where the threshold line intersected the amplification curve at exponential phase of the amplification. C_t value for each sample was normalized to their respective 18S ribosomal RNA internal standard. Percentage change in mRNA levels of each gene at stages NF62, 66 and adult are expressed relative to the mRNA level of that particular gene at the premetamorphic stage (considered as 100%). The quality control for real-time PCR used a no-RT reaction for each sample (for DNA contamination), melting curve analysis, and electrophoresis in a 2% agarose gel to confirm the amplicon size.

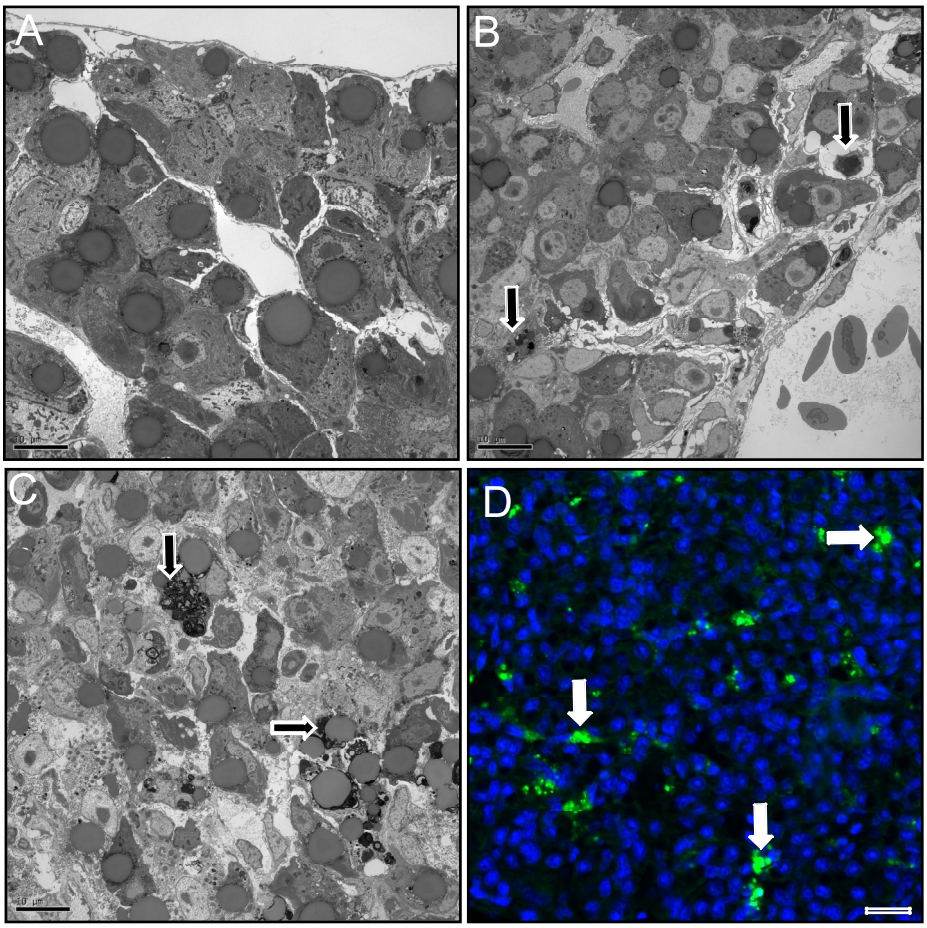

Fig. S1. Electron micrographs of the *X. laevis* pancreas at different developmental stages. (*A*) Acinar cells in premetamorphic (NF55) pancreas are large in shape with abundant cytoplasm and polarized nuclei. (*B*) At metamorphic climax (NF63) acinar cells have lost most of their cytoplasm and their nuclei are centrally located. (*C*) Pancreas from a premetamorphic tadpole that has been exposed to 10 nM T_3 for 4 days. (*D*) TUNEL assay confirms the presence of significant number apoptotic cells (green; nucleus stained with DAPI, blue) at climax (NF62) of metamorphosis. Dying and apoptotic cells and cell debris are marked with arrows. (Scale bar: *A*–*C*, 10 μm; *D*, 20 μm.)

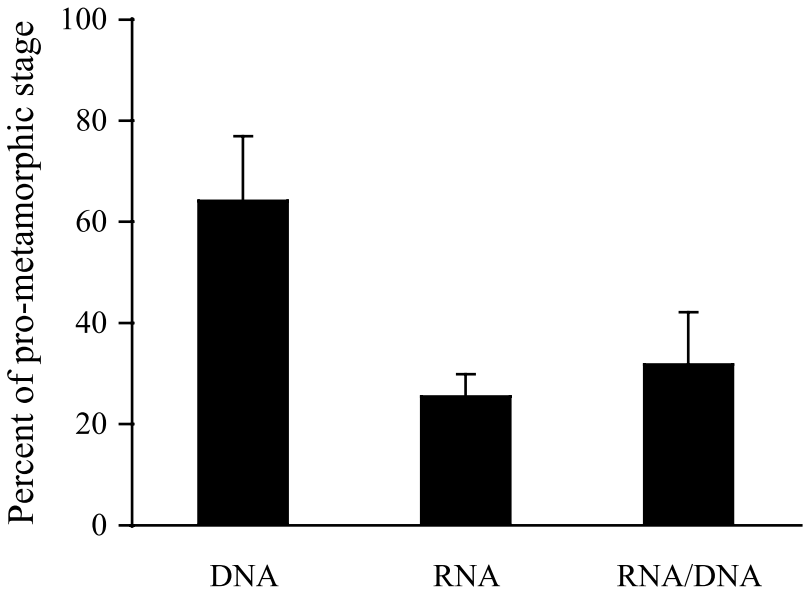

Fig. S2. Total DNA, RNA, and RNA/DNA were measured from pancreases at prometamorphosis (NF58) and climax (NF62) stages of *X. laevis*. Pancreases from five tadpoles was pooled for each replicate. There were three replicates for each stage of development. The amount of total pancreatic RNA and DNA and the RNA/DNA ratio at climax is represented as the percent of the same measurement at prometamorphosis (prometamorphosis = 100%). Metamorphic climax is characterized by loss of DNA (by 40%) and RNA (by 70%). Each bar gives the mean value ($n = 3$) ± SEM.

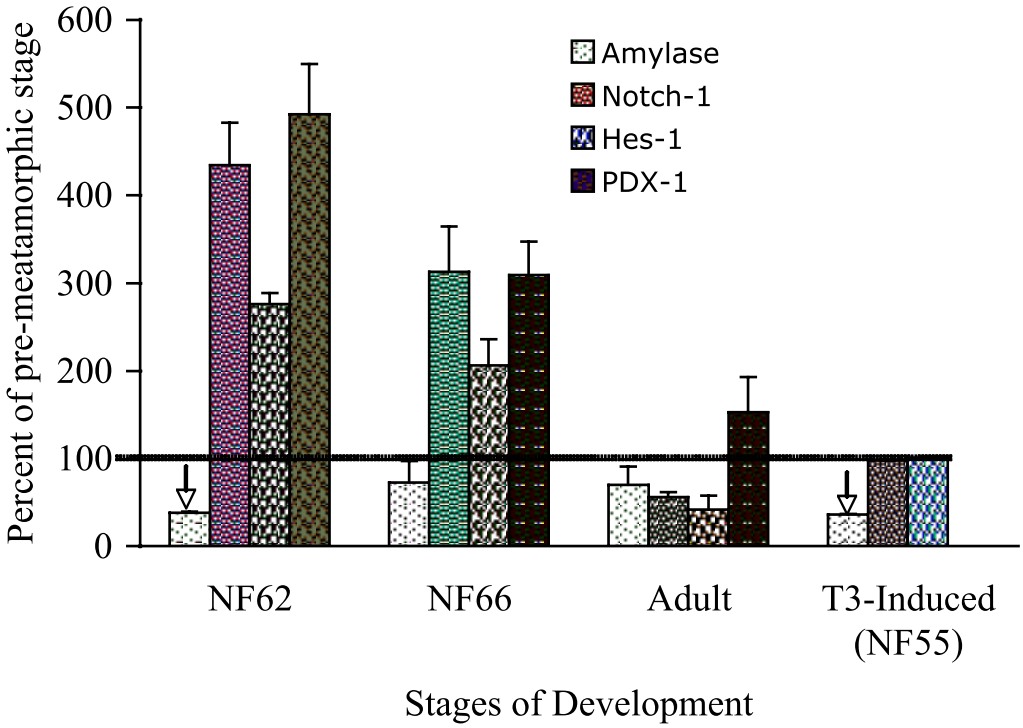

Fig. S3. Changes in gene expression profile during spontaneous and T_3-induced metamorphosis. RT-PCR was used to quantify the mRNA levels of *amylase*, *PDX-1*, *Notch-1*, and *Hes-1*. Down-regulation of amylase and up-regulation of PDX-1, Notch-1, and Hes-1 were observed at climax stage (NF62) and froglet (NF66), the transition of dedifferentiation and redifferentiation process. The change in gene expression at climax (NF62), froglet (NF66), and adult has been expressed as the percent of the value in premetamorphosis stage tadpoles (premetamorphosis = 100%). Induction by 10 nM T_3 of premetamorphic tadpoles for 4 days caused down-regulation of the exocrine gene, amylase but failed to up-regulate Notch-1 and Hes-1 mRNA. Each bar represents mean (n = 3) ± SEM.

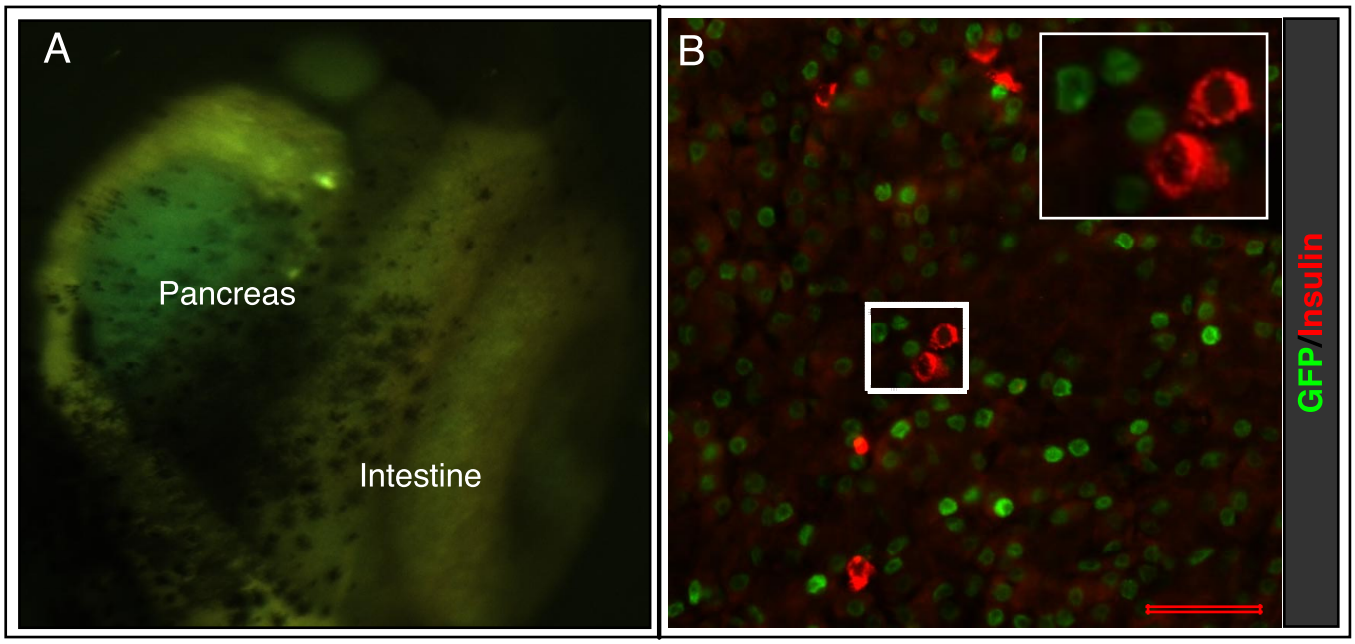

Fig. S4. The pElastase-TRDN-GFP transgene is not expressed in (endocrine) β-cells in an NF54 tadpole. (*A*) Ventral view of the fluorescent pancreas. (*B*) Section of the pancreas immunostained with antibodies for both GFP (green) and insulin (red). The GFP protein is localized in the nucleus. (Scale bar = 40 μm.)

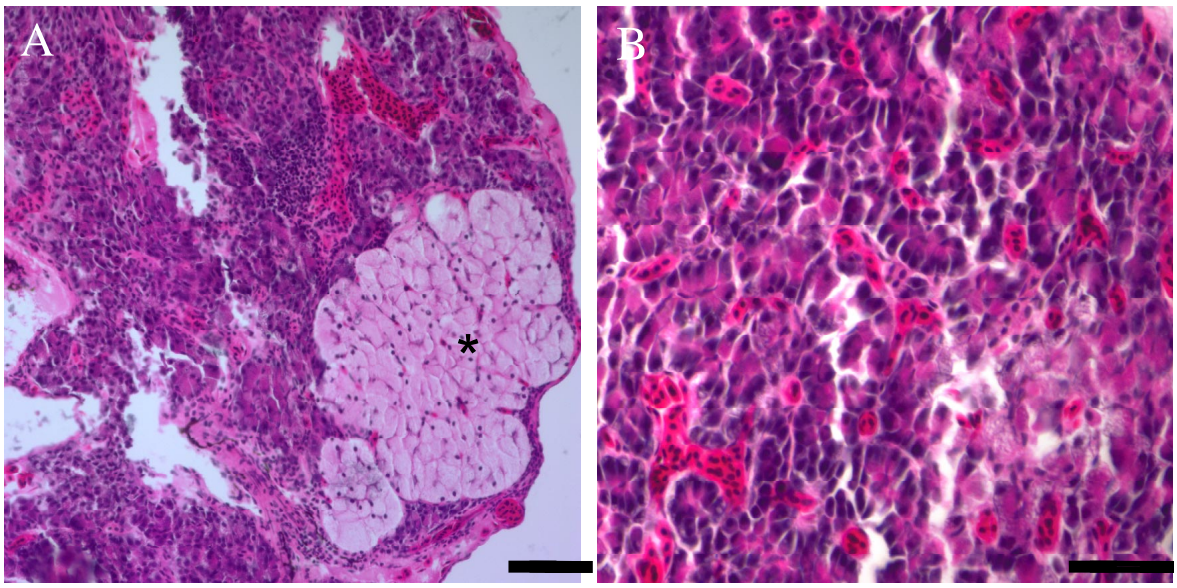

Fig. S5. Sections of a 2-month-old TRDN transgenic frog pancreas. (*A*) Metaplastic regions without acinar cells have fat bodies (*) and other unidentified nonpancreatic cell types. (*B*) A disorganized region with acinar cells. (Scale bar = 40 μm.)

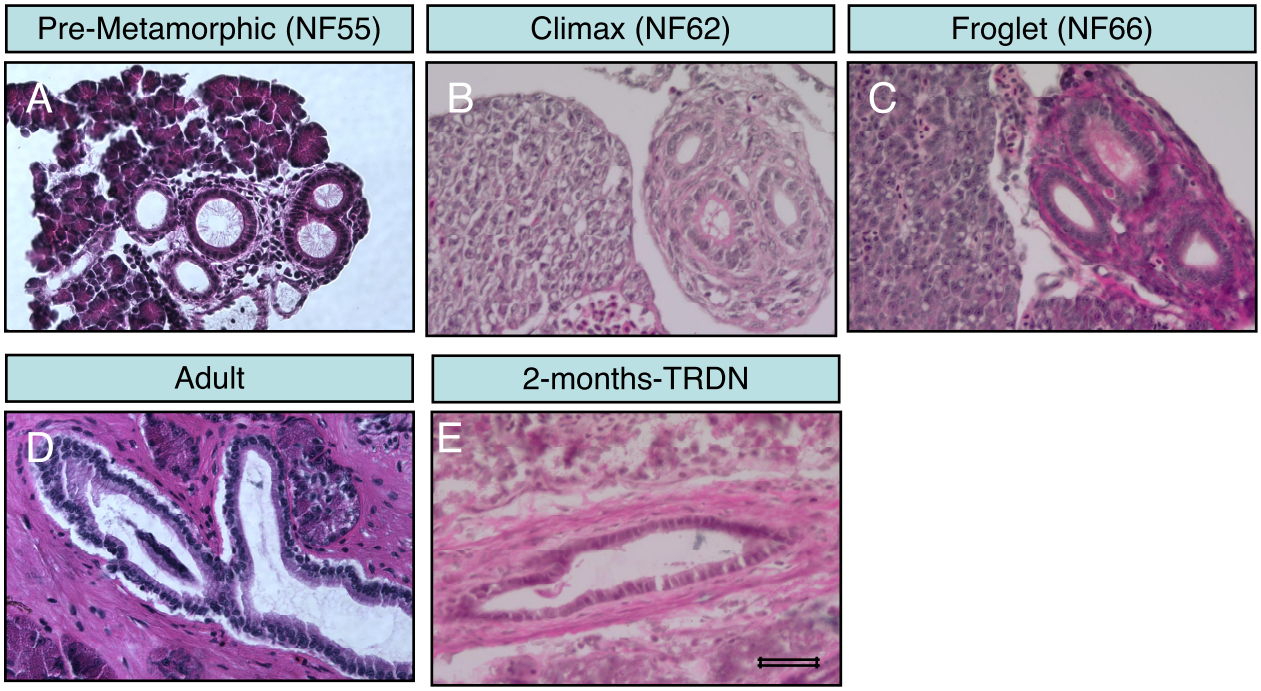

Fig. S6. Micrograph of extrapancreatic ducts at varying developmental stages. (*A*) In a premetamorphic tadpole the ducts have a single cell layer of epithelial cells without surrounding matrix. (*B*) Climax, NF62. (*C*) NF66 froglet, some extracellular matrix has begun to form. (*D*) Adult pancreas showing thick collagen matrix surrounding the duct. (*E*) A 2-month-old TRDN transgenic frog has normal maturation of the extra pancreatic duct. (Scale bar = 40 μm.)