Expanded View Figures

Figure EV1. Schemes for gastric and pancreatic injury treatments, and hydroxychloroquine treatment in stomach tissue to inhibit paligenosis.

A Scheme of tamoxifen treatment timeline. Mice were administered high-dose tamoxifen (HDT; 5 mg/20 g body weight) by intraperitoneal injection up to two times, and stomachs were harvested at the respective/indicated timepoint as per the experimental details. Day 3 of timeline is when peak SPEM is observed. Orange bars indicate each stage of paligenosis and where they correspond in the treatment timeline.

B Scheme of cerulein (CER) treatment timeline to induce pancreatitis. Mice were administered repeated cerulein injections by intraperitoneal injection. Six hourly injections of cerulein (50 μg/kg) were given every other day for up to 7 days (peak ADM). Orange bars indicate each stage of paligenosis and where they correspond in the treatment timeline.

C Scheme of hydroxychloroquine (HCQ) treatment timeline. Mice were pretreated with 60 mg/kg HCQ (or PBS) one day prior to high-dose tamoxifen (HDT) administration alongside HCQ (60 mg/kg/day). Stomachs were harvested at day 3 (72 h) when peak SPEM is normally observed.

D Immunohistochemistry for GSII in gastric units to mark normal mucous neck cells (vehicle) and SPEM cells (72-h HDT) in mice treated with vehicle, HDT, HCQ, or HDT + HCQ. Scale bars 20 μm. Counterstained with hematoxylin. Arrows mark GSII at the base of the stomach unit indicating SPEM.

E Quantification of the fraction of gastric units exhibiting SPEM (from EV1D). Each data point = mean of counts from 40 to 50 gastric glands from an individual mouse, $n = 4$–6 mice per treatment group; black line = mean of means from each mouse ± SEM. Significance by nonparametric two-tailed *t*-test.

F Immunofluorescence of gastric units; nuclei (DAPI, blue), chief cells (GIF, red), progenitor/metaplastic cells (GSII green + red GIF), and proliferative cells (BrdU, white) after treatment with HDT or HDT + HCQ for 72 h. 72-h HDT units exhibit SPEM (GSII and GIF overlap), while 72-h HDT+HCQ units show scant SPEM and full chief cell cytoplasm packed with GIF secretory granules. Scale bars 50 μm. Stomach unit base outlined by dashed white line.

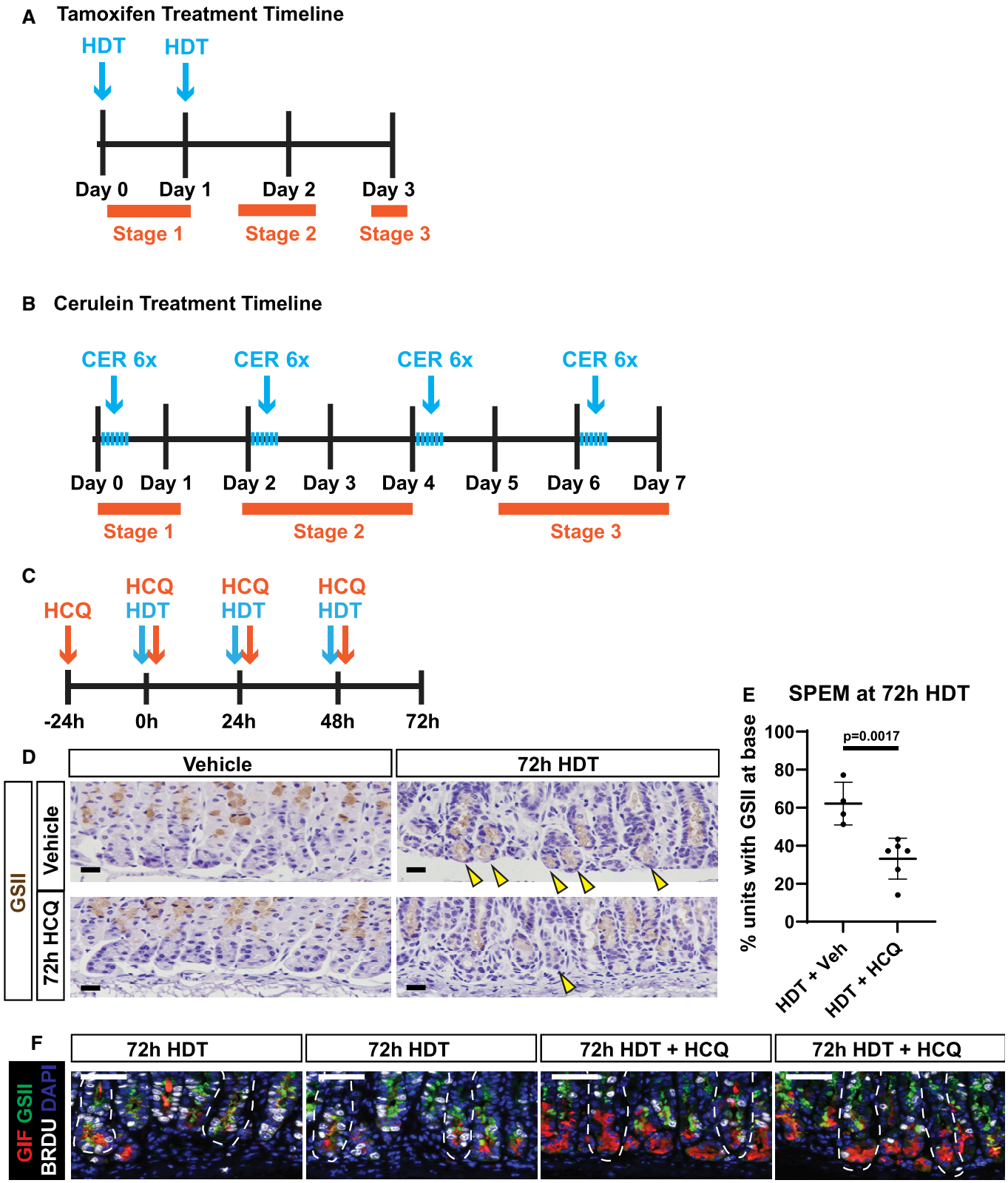

Figure EV1.

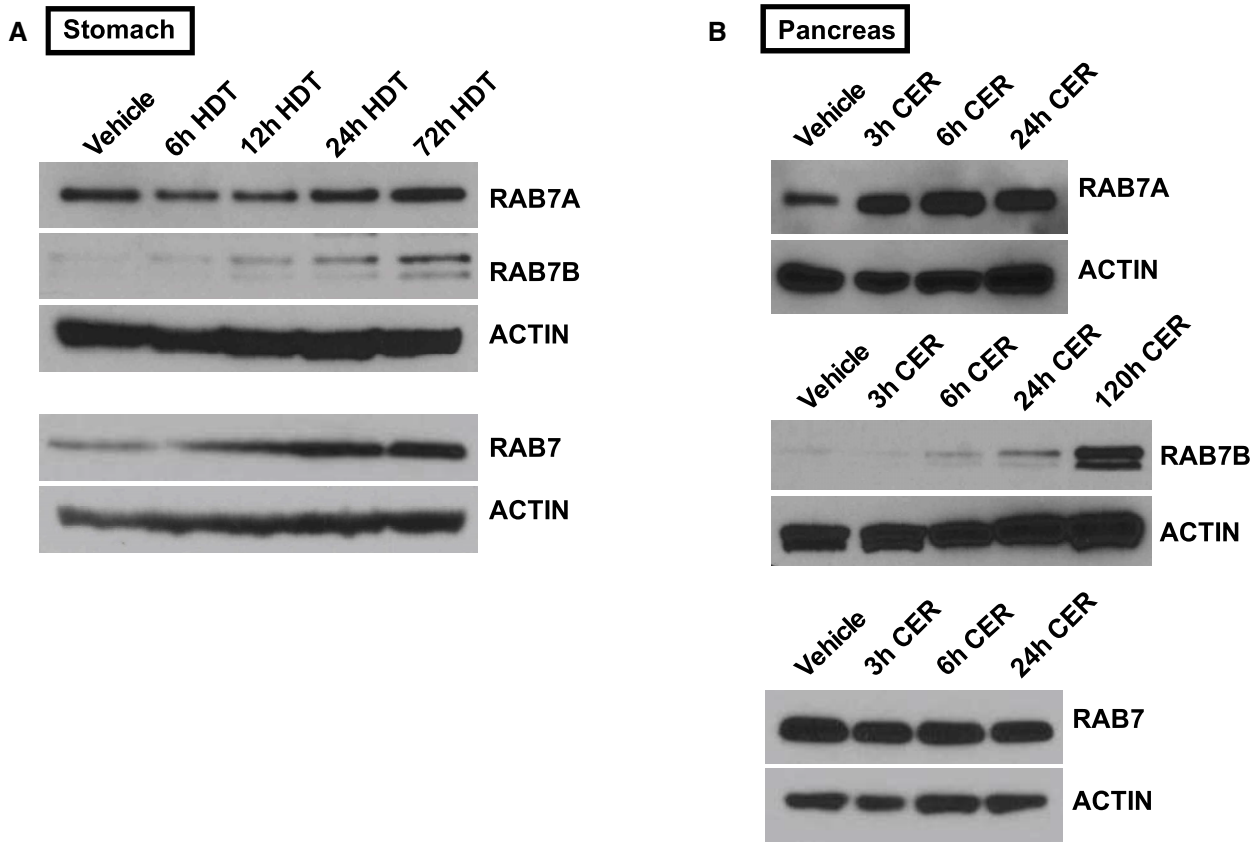

Figure EV2. Protein expression of RABs in stomach and pancreas.

A RAB7A, RAB7B, RAB7 expression by Western blot in stomachs treated with high-dose tamoxifen (HDT) compared to ACTIN loading control.

B RAB7A, RAB7B, RAB7 expression by Western blot in pancreata treated with cerulein (CER) compared to ACTIN loading control.

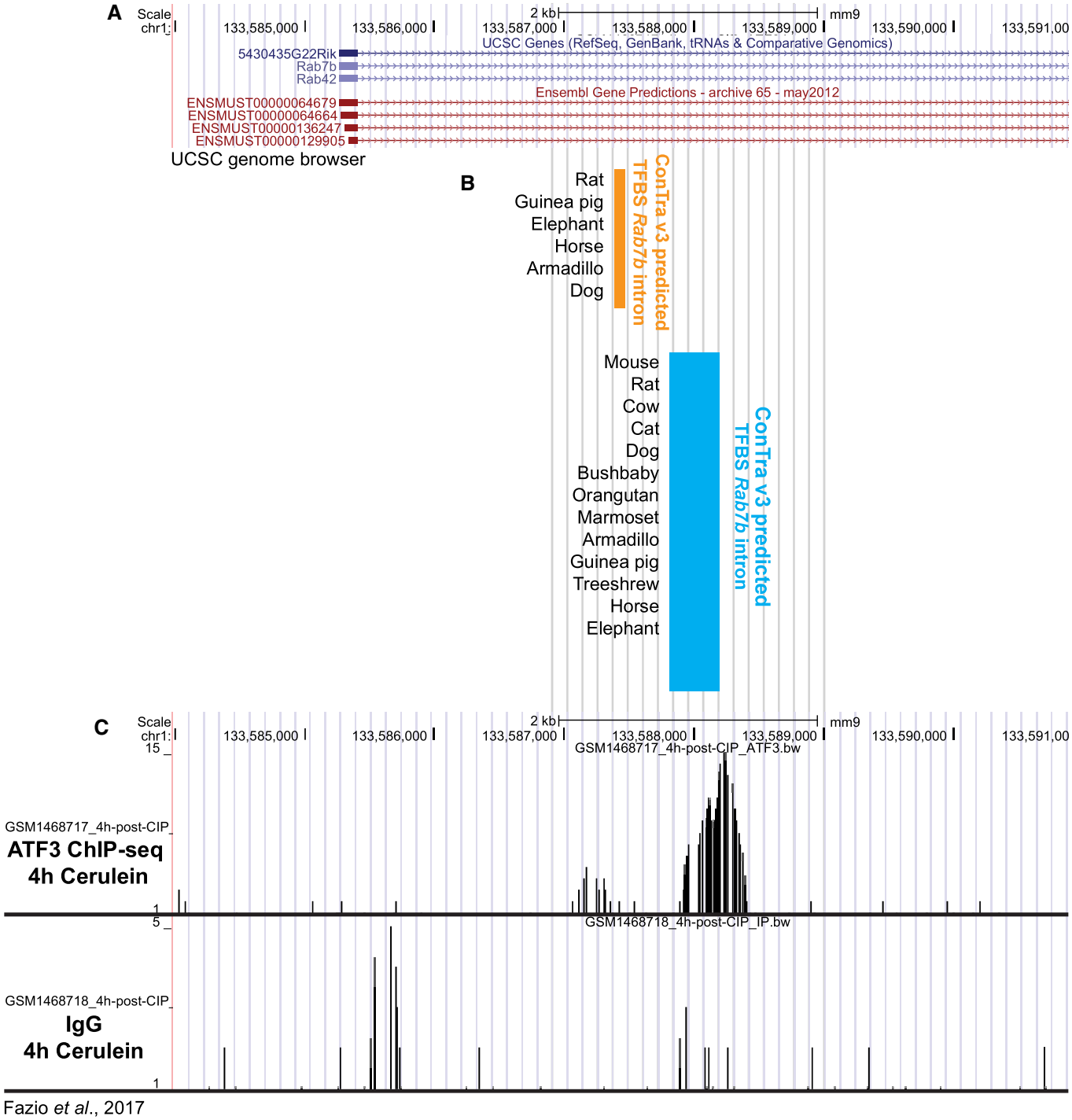

Figure EV3. Analysis of ATF3-binding sites in the mouse *Rab7b* first intron.

A Mouse *Rab7b* gene alignment using UCSC genome browser (NCBI37/mm9).

B Regions of predicted conserved ATF3 binding derived from ConTra v3. Orange indicates conserved ATF3 sites in one region of *Rab7b* and blue indicates a second region of conserved ATF3 sites downstream, but still in the first intron. Highlighted regions are aligned to the gene in (A). TFBS = transcription factor binding sites.

C Alignment of ATF3 ChIP-seq analysis from 4-h Cerulein-treated mouse pancreas published in Fazio *et al*, 2017, GEO accession GSE60250. ChIP-seq peaks aligned to gene in (A).

Figure EV4. Markers of Spasmolytic Polypeptide-Expressing Metaplasia, cell proliferation, and cell death in the stomach following injury.

A Representative immunofluorescence of gastric units stained for DAPI (blue), gastric intrinsic factor (GIF, cyan), and CD44v (marker of spasmolytic polypeptide-expressing metaplasia [SPEM] when expressed in cells at the unit base, red). WT (top) and *Atf3*$^{-/-}$ (bottom) mice were treated with 72-h high-dose tamoxifen (HDT). Arrowhead indicates base dropout in *Atf3*$^{-/-}$stomach. Scale bars 20 μm. Stomach units outlined by dashed yellow line.

B Representative immunohistochemistry of GSII in gastric units to mark normal mucous neck cells (vehicle) and SPEM cells (72-h HDT) in WT (top) and *Atf3*$^{-/-}$ (bottom) mice. Scale bars 50 μm. Counterstained with hematoxylin. Stomach units outlined by dashed black line.

C Quantification of the percent of gastric units exhibiting SPEM as counted by the number of units with GSII at the unit base over the number of unit total. Each data point = mean of counts from 40 to 50 gastric glands from an individual mouse, $n = 5$ mice per treatment group. Significance by nonparametric two-tailed *t*-test. Error bars denote standard deviation.

D Representative immunohistochemistry of BrdU in WT (top) and *Atf3*KO (bottom) gastric units with vehicle or 72-h high-dose tamoxifen (HDT) treatment. BrdU administered 90 minutes prior to sacrifice to capture cells in S phase. Eosin counterstain. Scale bars 50 μm. Stomach units outlined by dashed black line.

E Representative immunohistochemistry of cleaved-caspase 3 in gastric units of WT (top) and *Atf3*KO (bottom) mice when treated with vehicle or 72-h high-dose tamoxifen (HDT). Counterstained with eosin. Arrowheads point to ZCs with high expression of cleaved-CASP3. Scale bars 50 μm. Stomach units outlined by dashed black line.

F Graph of the apoptosis rate following 72-h HDT injury as counted by the number of cleaved-caspase 3+ cells from a 40× field. Each data point = mean of counts from 40 to 50 gastric glands from an individual mouse, $n = 4$ mice per treatment group. Significance by nonparametric two-tailed *t*-test. Error bars denote standard deviation.

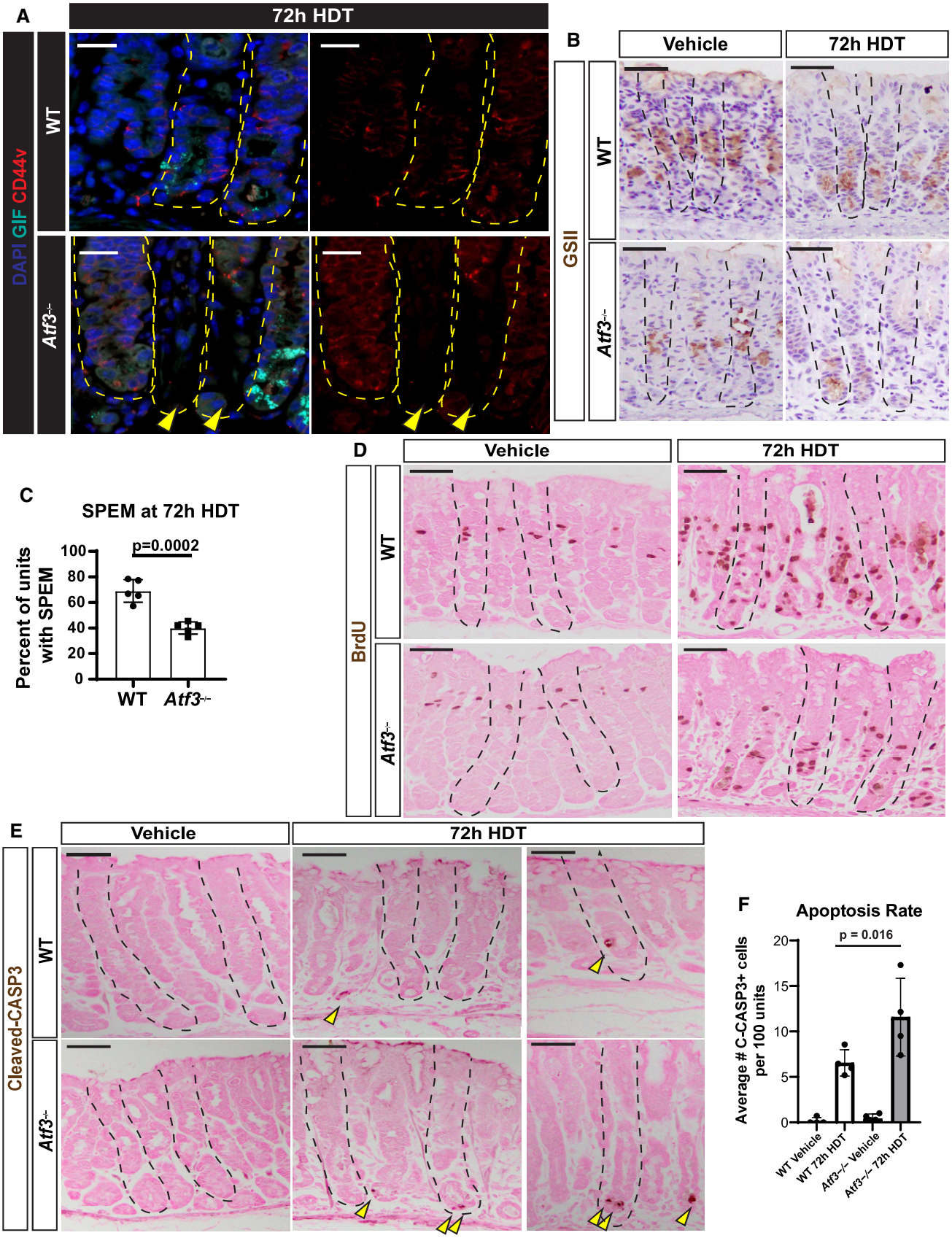

Figure EV4.

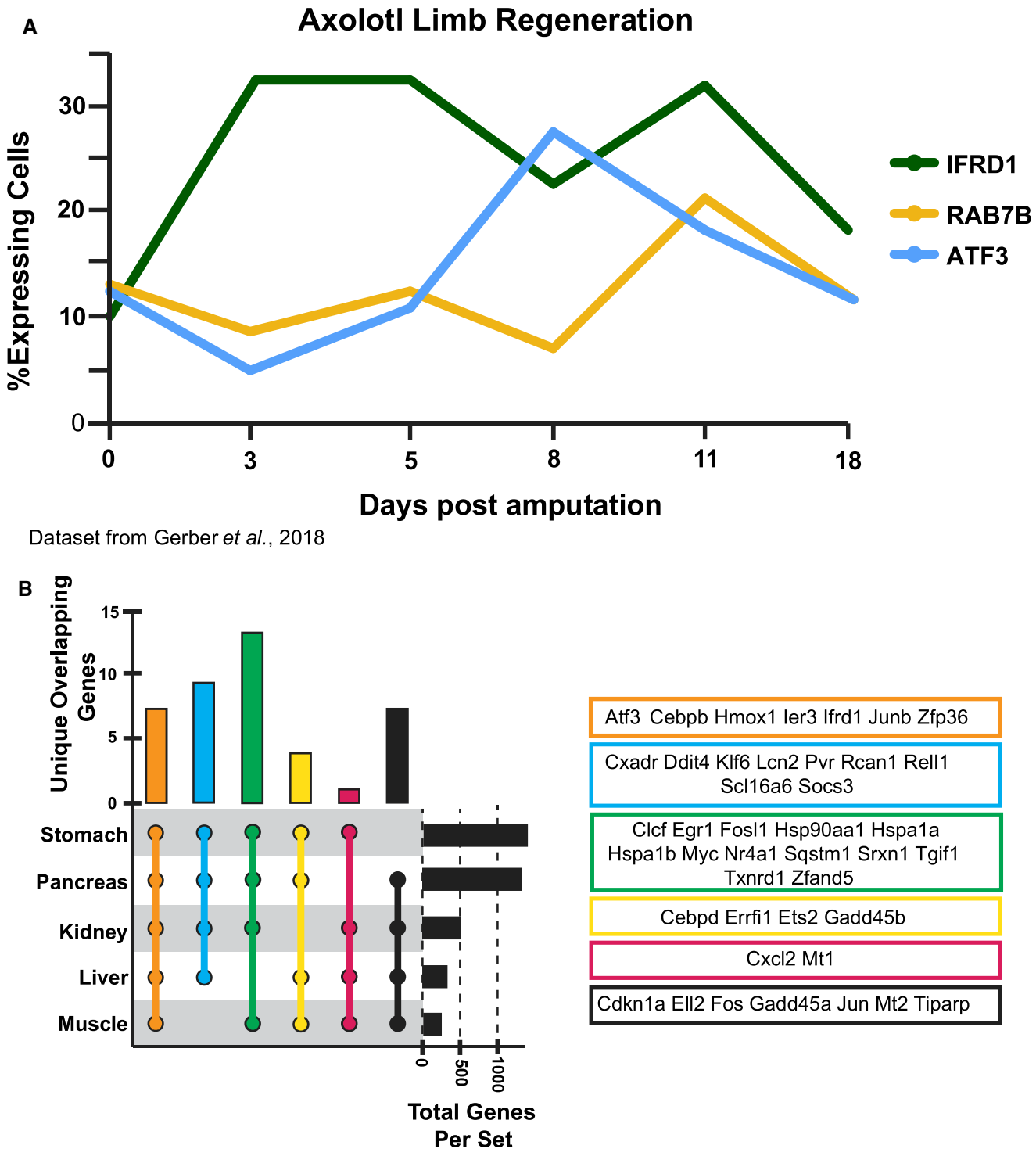

Figure EV5. Conservation of *Atf3* expression across tissues after injury.

A Fraction of cells expressing ATF3, RAB7B, and IFRD1 in previously published single-cell RNA-sequencing of regenerating cells after axolotl limb amputation. IFRD1 is a previously published paligenosis gene known to be activated during Stage 1.

B Intersection of transcripts upregulated by paligenotic injury in 5 tissues. Only intersections of gene sets ≥ 4 and transcripts unique for that intersection are depicted. Unique genes from each intersection are listed above the UpSet plot depicting the intersection combination, the number of overlapping genes, and the total number of genes in the dataset.