

Expanded View Figures

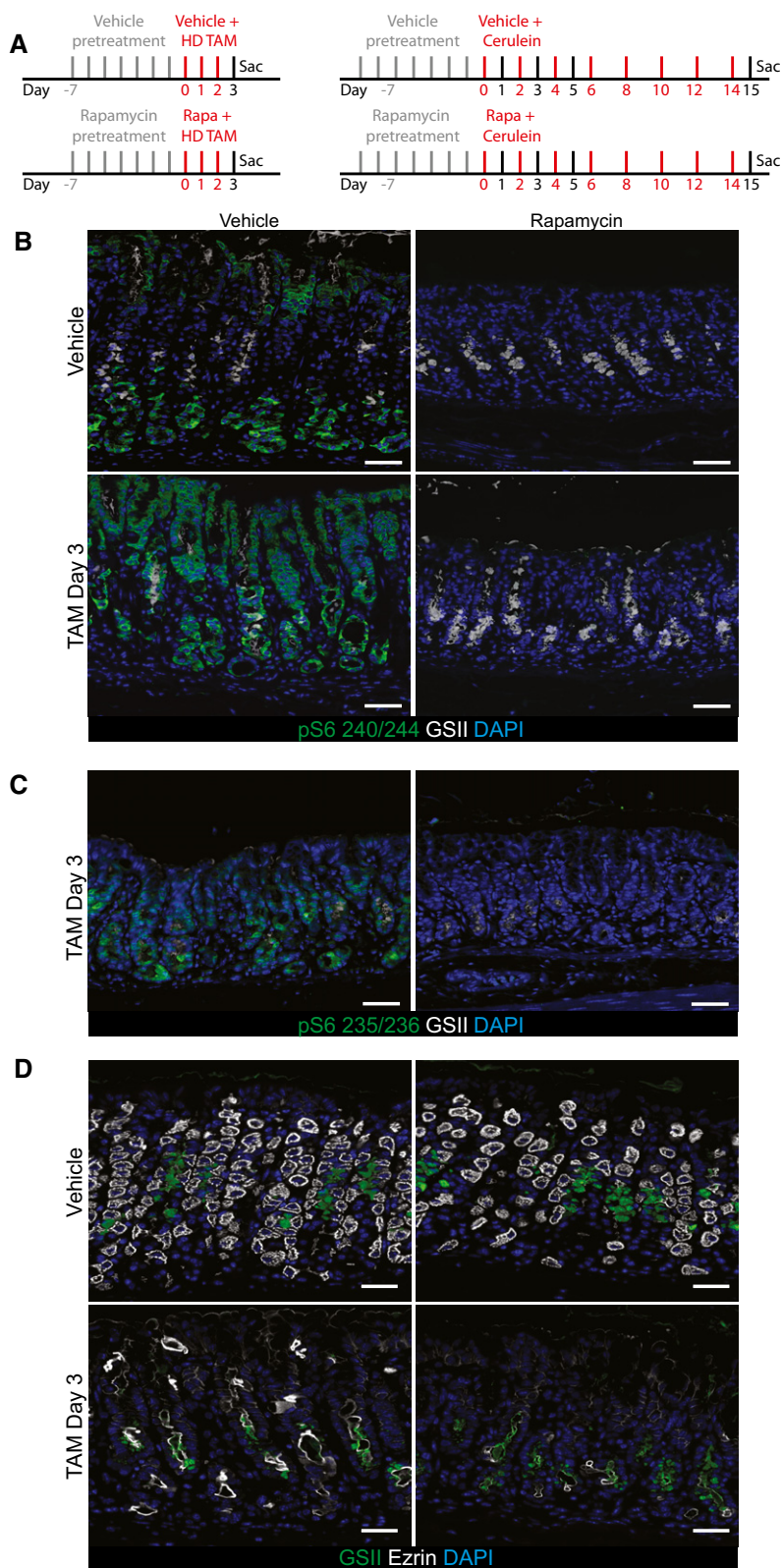


Figure EV1. pS6 is an accurate proxy for rapamycin-sensitive mTORC1 activity and shows that loss of mTORC1 does not affect parietal cell death or induction of metaplastic gene expression in reprogramming chief cells.

- A Injection schemes for injury experiments with rapamycin in stomach (*left*) and pancreas (*right*).
- B Representative epifluorescence images of the distribution of pS6 in the normal and injured stomach \pm rapamycin treatment. pS6 is restricted to the chief cell zone (base) and pit zone of the normal corpus unit. At peak (HD-Tam day 3) SPEM stages, it is located at high level throughout the unit. Upon rapamycin treatment, all pS6 staining is lost throughout the normal and injured corpus unit. The characteristic induction of GSII staining in reprogramming chief cells at the base of gastric units (indicating SPEM) occurs at least as markedly in the presence of rapamycin, indicating mTORC1 is not required for metaplastic gene induction. Green, pS6; white, GSII; blue, DAPI. Scale bars: 50 μ m.
- C At peak metaplasia stages, pS6 235/6 is upregulated in the stomach epithelium and rapamycin treatment at this stage abolishes all staining. Scale bars: 50 μ m.
- D Representative epifluorescence images of the loss parietal cells (marked by ezrin) upon injury and rapamycin treatment. Treatment with HD-Tam caused the loss of the vast majority of parietal cells throughout the corpus. Rapamycin does not rescue that injury. Green, GSII; white, ezrin; blue, DAPI. Scale bars: 50 μ m.

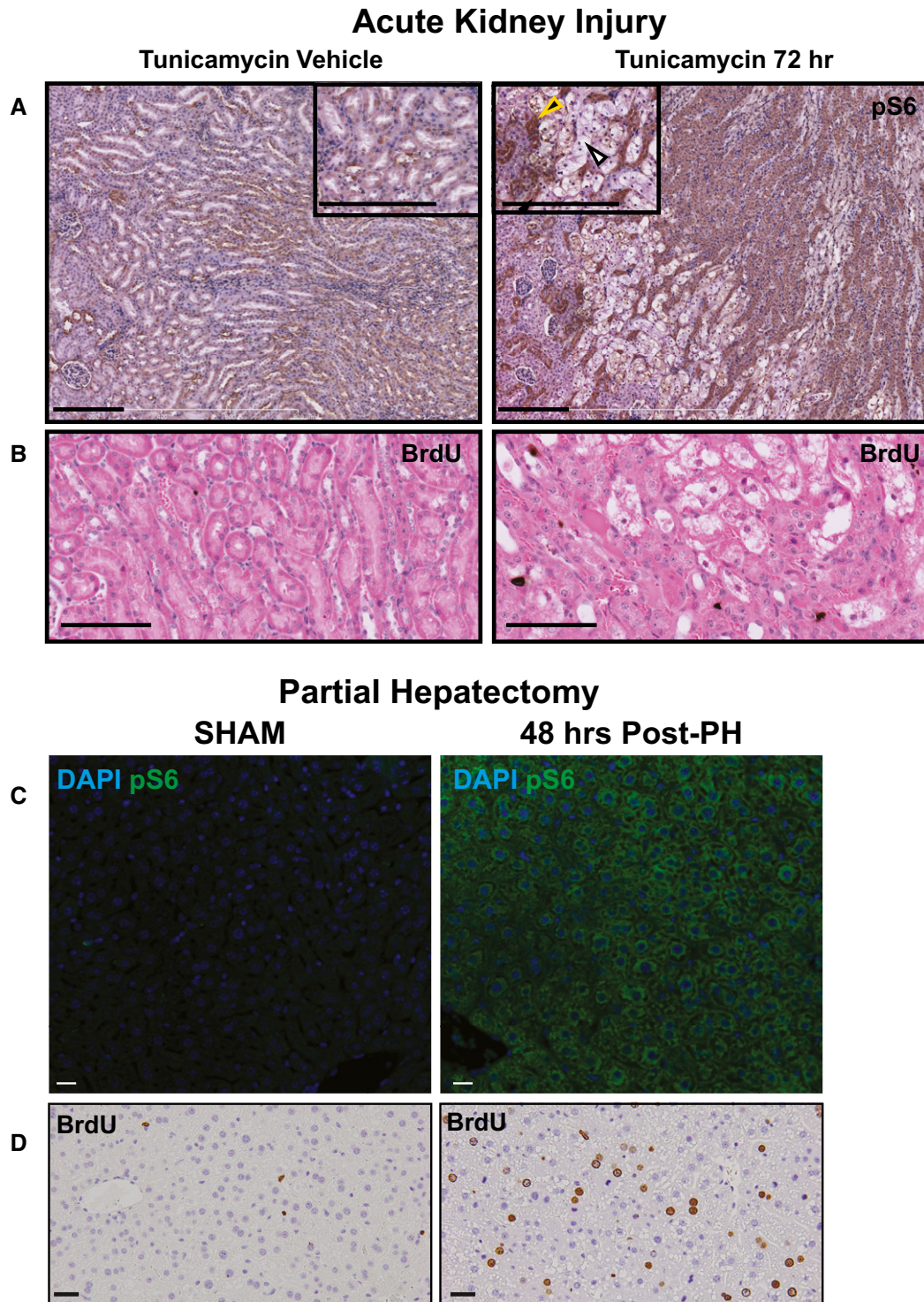


Figure EV2. Acute kidney injury and partial hepatectomy both cause upregulation of mTORC1 activity during proliferative phases.

- A Upon injury with tunicamycin, tubule cells in the kidney are damaged (white arrowhead) and surviving tubule cells (yellow arrowhead) upregulate pS6. Scale bars: 100 μ m.
- B Upregulation of the pS6 is associated with increased proliferation in this injury model as seen by BrdU⁺ nuclei. Scale bars: 100 μ m.
- C Two-thirds partial hepatectomy causes a pronounced upregulation of pS6 in the remaining hepatocyte mass. Scale bars: 20 μ m.
- D The pS6⁺ hepatocytes are highly proliferative at this stage. Scale bars: 20 μ m.

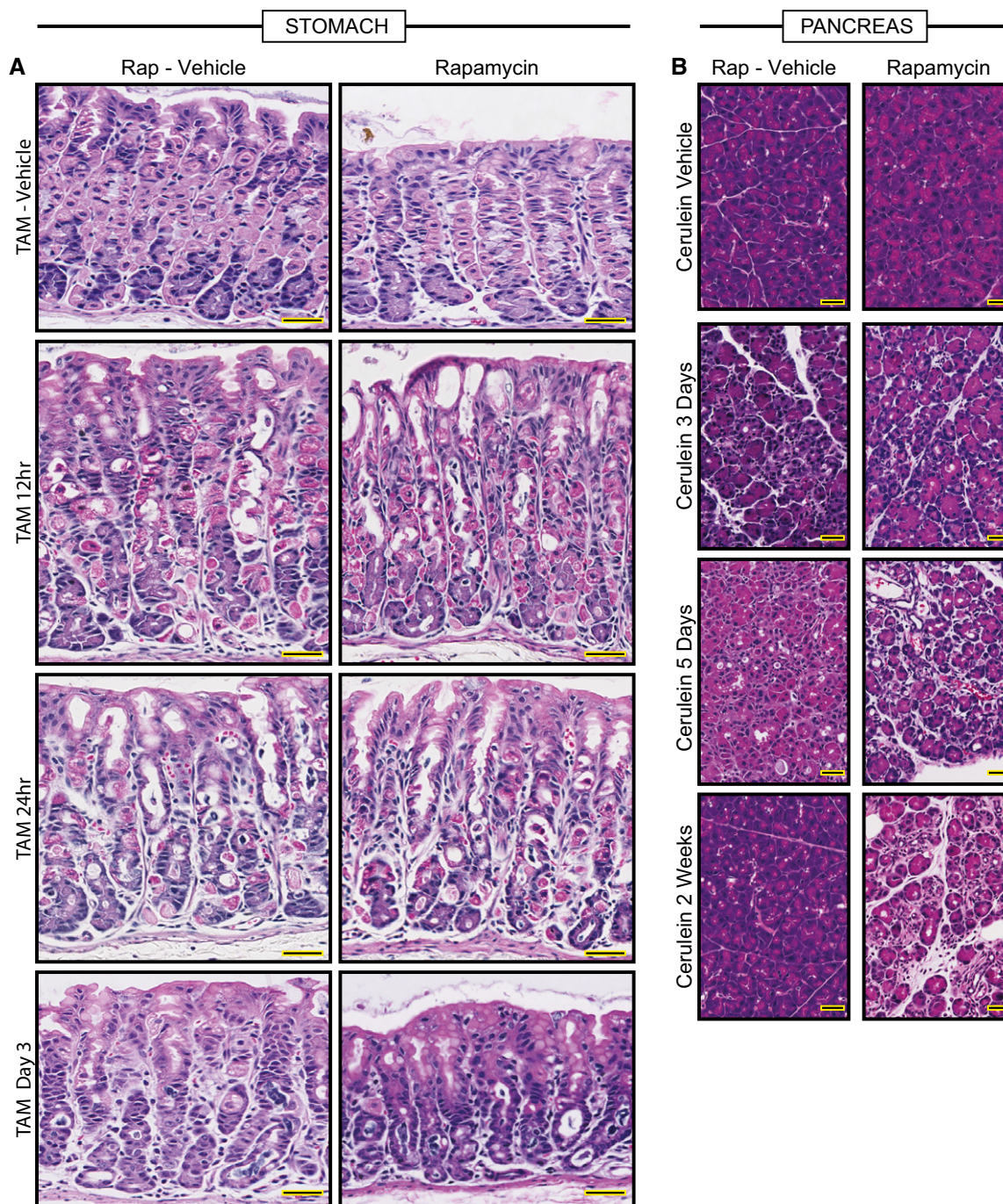


Figure EV3. Histological changes in the injured stomach and pancreas with and without rapamycin treatment.

- A Representative hematoxylin and eosin counterstained images of HD-TAM stomach tissue \pm rapamycin. Treatment with tamoxifen causes acute loss of parietal cells (large eosinophilic cells) by 12–24 h post-injury. By 3 days, chief cells have reprogrammed into SPEM cells. The general pattern of loss of parietal cells and conversion of chief cells to metaplastic cells is not affected by rapamycin (*right panels*). Scale bars, 50 μ m.
- B Representative hematoxylin and eosin counterstained images of pancreas tissue injured with cerulein at various stages \pm rapamycin. Cerulein injury causes mosaic, asynchronous conversion of acinar cells into proliferative, acinar-ductal metaplastic cells with maximal features of the process at day 5 in our protocol. By 2 weeks, the pancreas has compensated for the continuous injury and recovers a relatively normal morphology. Dual treatment with rapamycin and cerulein does not rescue the metaplastic response by day 5 and impedes normal tissue compensation by 2 weeks injury, with most of the tissue continuing to show abundant metaplastic forms. Scale bars, 50 μ m.

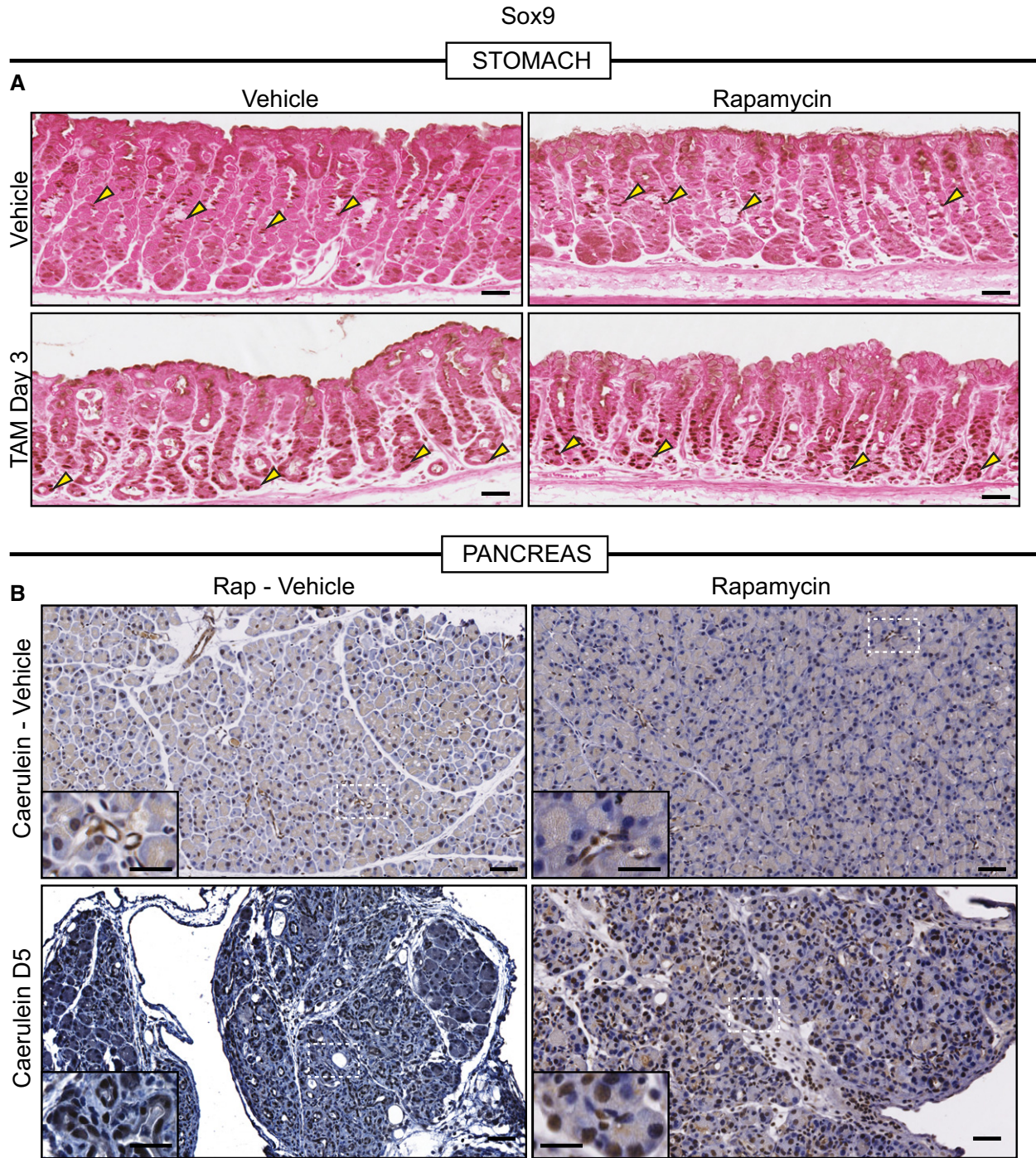


Figure EV4. mTORC1 is not required for increased SOX9 during metaplasia.

A Representative eosin counterstained IHC images of normal or metaplastic gastric tissue stained for SOX9. SOX9, in control tissue, stains the isthmal and mucous neck cells, which are proliferative progenitors (yellow arrowheads), of the corpus units and is generally excluded from the base of units. Upon injury with HD-TAM, SOX9 expression is induced in the base of units (yellow arrowheads). Treatment with rapamycin does not alter either the normal or metaplasia distribution of SOX9 (yellow arrowheads). Scale bars, 50 μ m.

B Representative hematoxylin counterstained IHC images of normal or metaplastic pancreatic tissue stained for SOX9. SOX9 expression in normal pancreatic tissue is restricted to the duct (see inset in top left panel which is a high magnification view of the boxed area). At peak metaplasia stages, SOX9 becomes expressed in dedifferentiating acinar cells (see bottom left inset). Treatment with rapamycin in normal (see top right inset) or injured (see bottom right inset) does not alter SOX9 expression. Scale bars 50 μ m; inset 25 μ m.

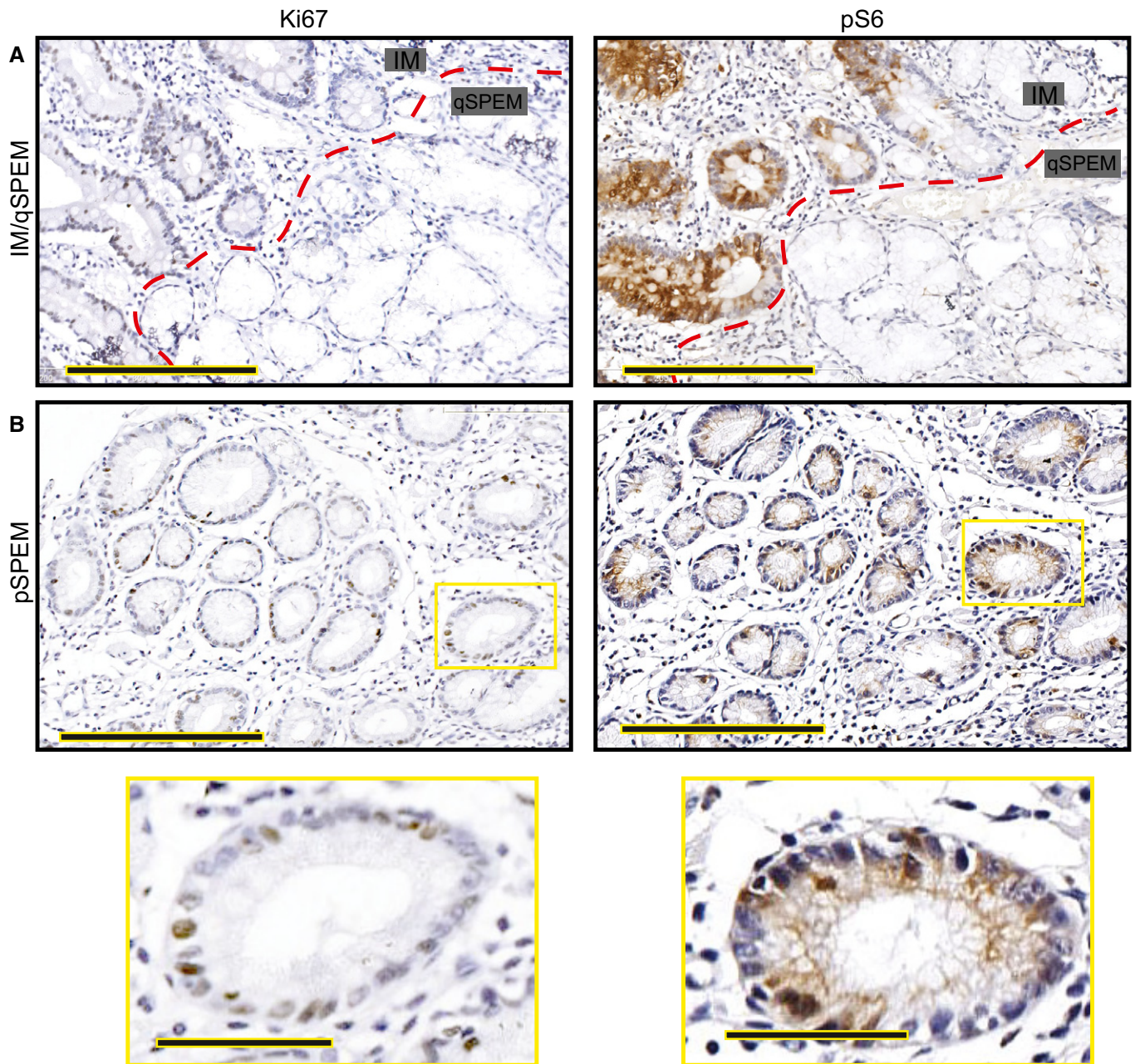


Figure EV5. Representative IHC images from human tissue microarray.

A Intestinal metaplasia ("IM" indicating the glands to upper left of red dashed line) is generally proliferative as evinced by frequent Ki-67⁺ cells (*left*) and is strongly pS6 positive. Most SPEM has a quiescent phenotype (glands labeled on "qSPEM" side of panels) characterized by cells with abundant mucus, flattened basal nuclei, and a lack of both Ki-67 and pS6 staining Scale bar, 200 μ m.

B Rare SPEM lesions show cells with cuboidal columnar morphology. These lesions show Ki-67 positivity usually associated with pS6 positivity. Boxed regions are shown at higher magnification below. Scale bar, 200 μ m; pullout, 50 μ m.

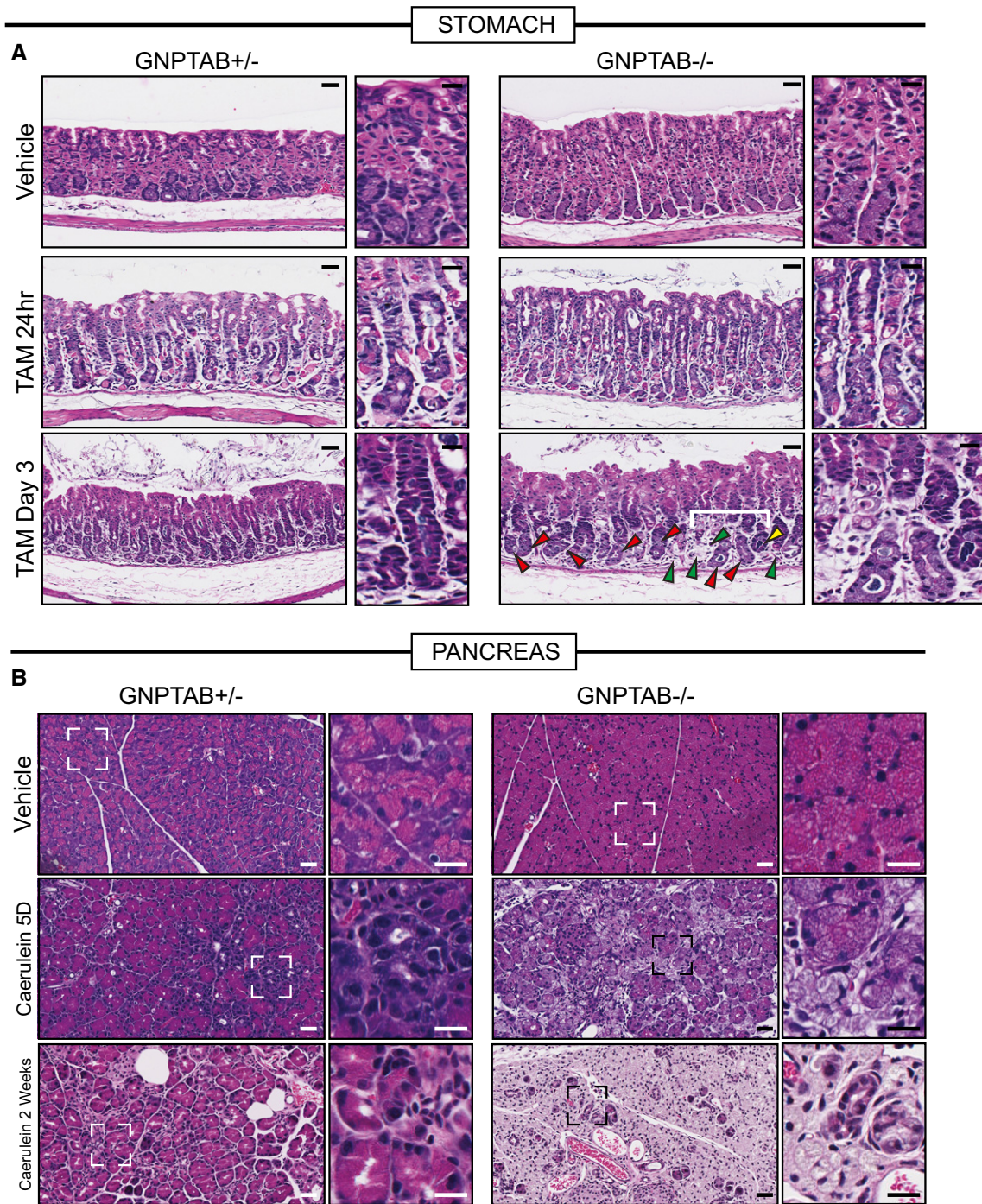


Figure EV6. Histological appearance of *Gnptab*^{-/-} stomach and pancreas tissue at injury time points.

A Representative hematoxylin and eosin counterstained images of *Gnptab*^{+/-} and *Gnptab*^{-/-} stomach tissue. *Gnptab*^{-/-} chief cell cytoplasm has a hypertrophic, frothy appearance compared to control zymogenic cells. Loss of parietal cells (fried-egg appearing eosinophilic cells) following HD-Tam is not affected by loss of GNPTAB; however, the base zones in *Gnptab*^{-/-} mice at day 3 HD-Tam are usually resistant to dedifferentiation (red arrowheads) with large, frothy chief cells remaining largely non-reprogrammed. Another, less common phenotype is that all chief cells are lost such that most of the base of the unit disappears (green arrowheads). Rare units partially undergo morphological metaplastic changes, though usually those are also associated with loss of basal cells (yellow arrowheads). Higher magnification views are to the right of each panel, with white bracket delineating particular region of interest in *Gnptab*^{-/-} stomach. Scale bar 50 μ m; pullout, 25 μ m.

B Representative hematoxylin and eosin counterstained images of *Gnptab*^{+/-} and *Gnptab*^{-/-} pancreas. Similar to the stomach zymogenic cells, pancreatic acinar cells also have a hypertrophic, frothy appearance. Whereas control samples treated with cerulein show diffuse, asynchronous acinar-to-ductal metaplasia, *Gnptab*^{-/-} mice have acinar cells that simply become less eosinophilic and foamy over time without undergoing ADM. By 2 weeks, wild-type pancreas has largely adapted to cerulein, whereas *Gnptab*^{-/-} pancreas parenchyma comprises only lobules of excessively pale (hyaline), frothy acinar cells and scattered reactive ducts. Scale bar 50 μ m; pullout, 25 μ m.

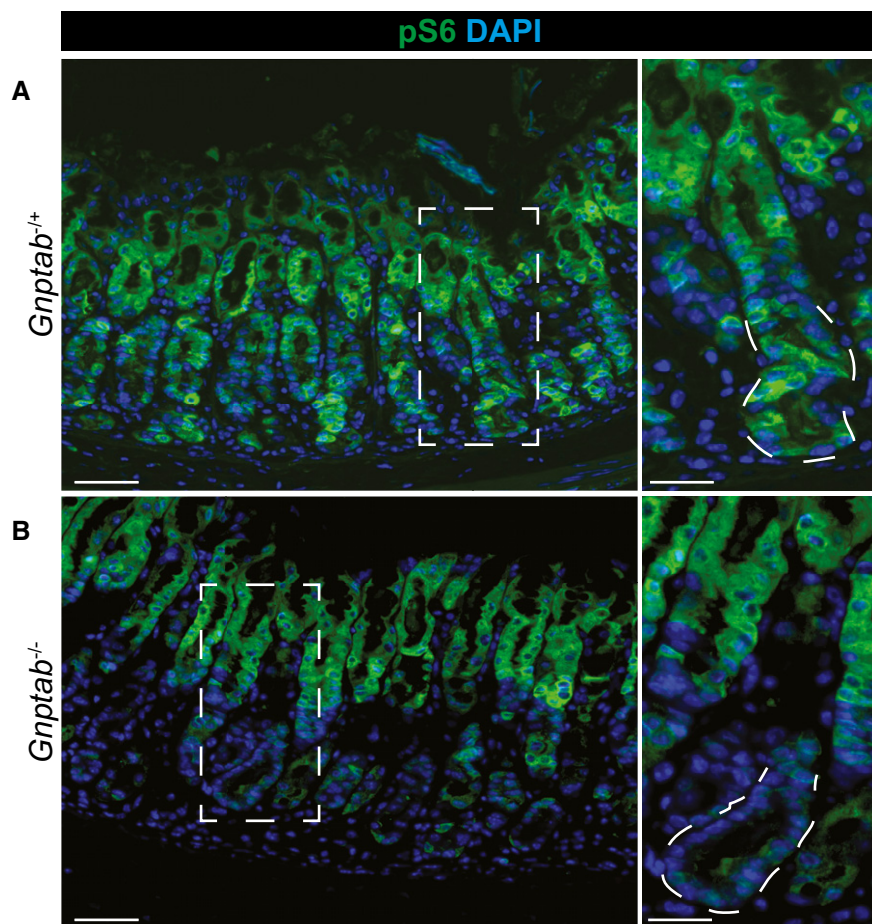


Figure EV7. Lysosomal activity is required to re-activate mTORC1 following HD tamoxifen injury.

- A At peak metaplasia stages in *Gnptab*^{-/-} tissue, pS6 is re-expressed throughout the stomach epithelium, including intense staining within the pit and metaplastic base. Scale bars: 50 μm; pullout, 25 μm.
- B In *Gnptab*^{-/-} tissue, pS6 is not reactivated in the base, indicating lysosomal activity is required for mTORC1 re-activation at later stages following injury. Boxed regions are shown at higher magnification at right with a representative base (in which pS6 remains inactive without lysosomal activity) outlined by dotted line. Lysosomal activity appears dispensable for pit cells (at top of gastric unit) mTORC1 activity. Scale bars: 50 μm; pullout, 25 μm.