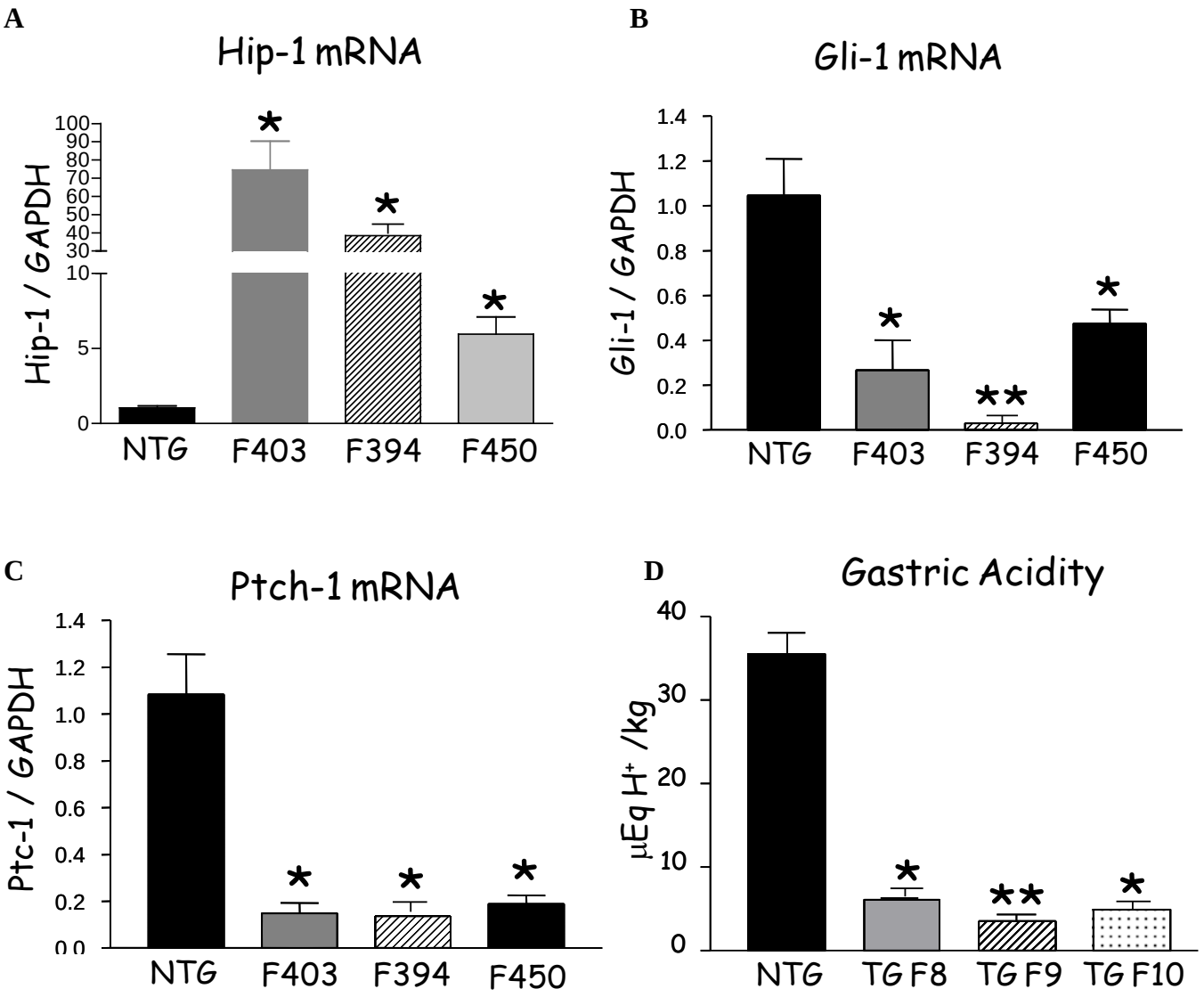
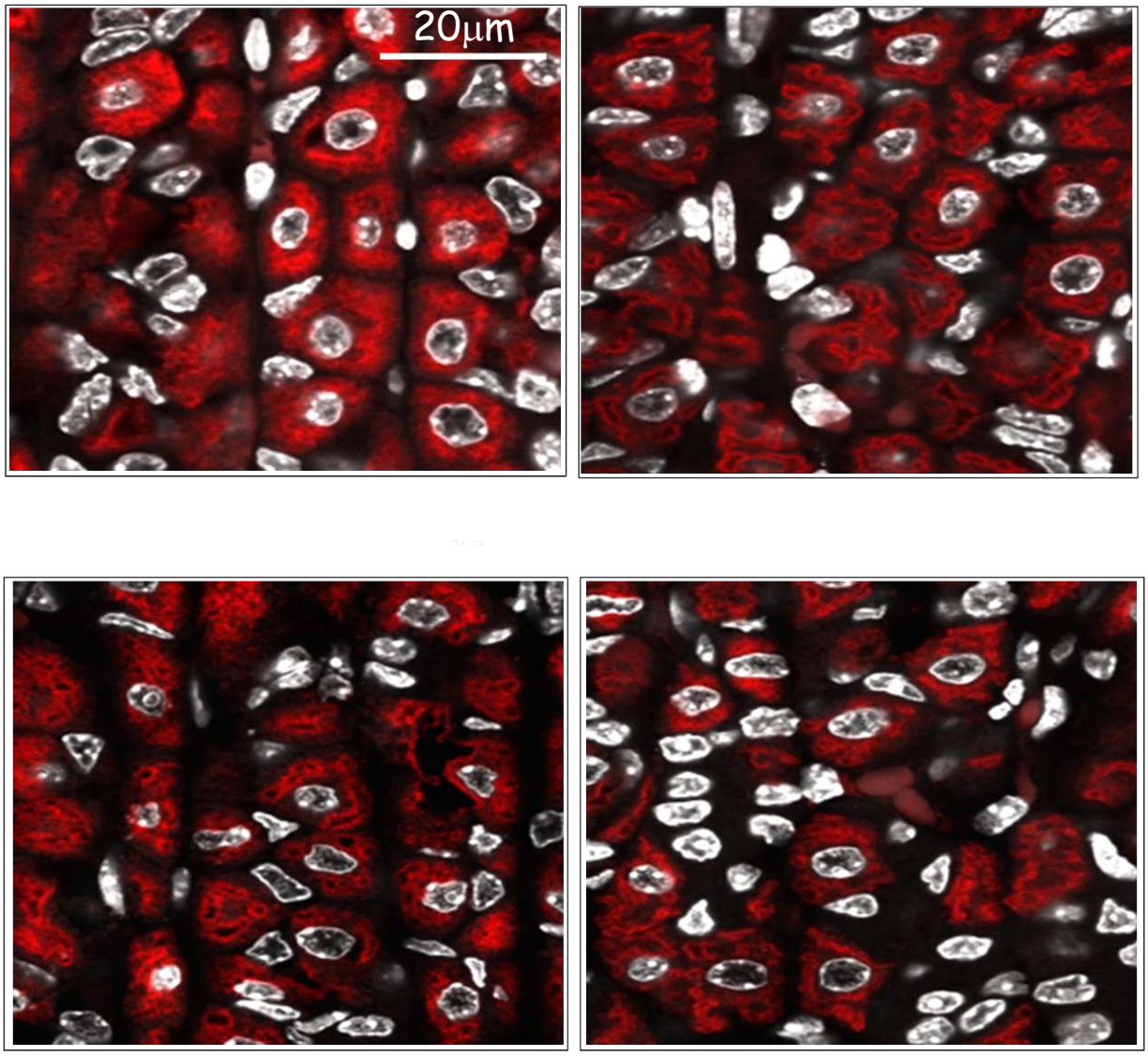
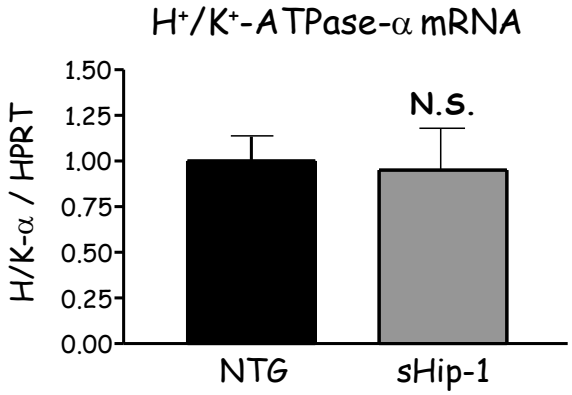


Supplementary Figure 1. Comparison of different sHip-1 founder lines. RT-qPCR of Hip-1 (A), Gli-1 (B), and Ptch-1 (C) in the fundus of nontransgenic versus 3 different sHip-1 founder lines. D) Gastric acidity by base titration is shown for 3 different sHip-1 founder lines versus non-transgenic controls (NTG). Error bars represent SEM. * $P < 0.05$ and ** $P < 0.01$.



Supplementary Figure 2. H^+/K^+-ATPase-β immunofluorescence in sHip-1 mice. H^+/K^+-ATPase staining (red) in 3 different sHip-1 founder lines (F403, F394, F450) versus a non-transgenic control (NTG). The fold increase in Hip-1 mRNA is indicated in parentheses. Staining was visualized by confocal microscopy. The nuclei are counterstained with DAPI (pseudocolored in grey).

A



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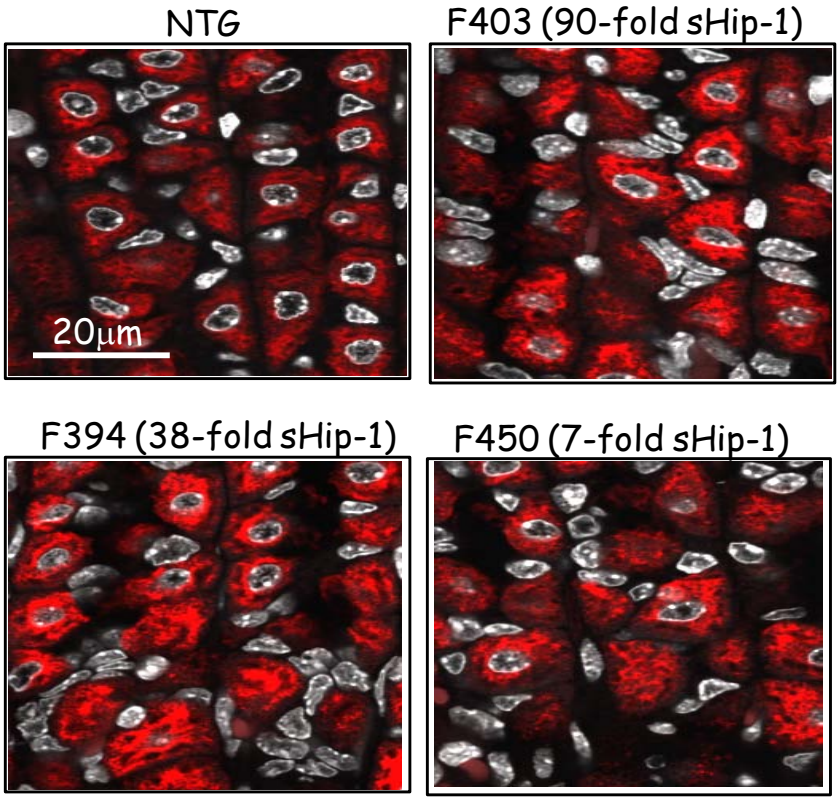
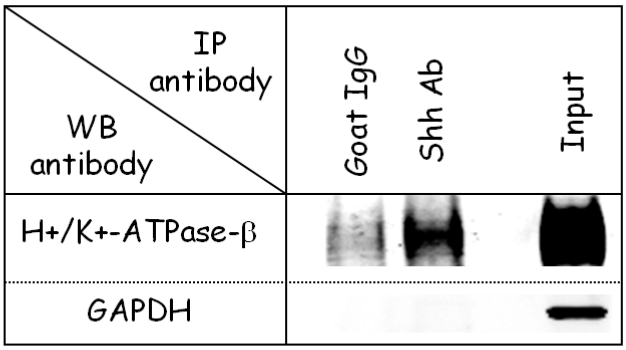


Supplementary Figure 3. H^+/K^+-ATPase-α RT-qPCR and immunofluorescence in sHip-1 mice. A) RT-qPCR of H^+/K^+-ATPase-α subunit mRNA expression in sHip-1 versus non-transgenic littermate. B) H^+/K^+-ATPase-α staining (red) for 3 different sHip-1 founder mouse lines (F403, F394, F450) versus a non-transgenic control (NTG). The fold increase in Hip-1 mRNA over NTG levels is shown. Staining was visualized by confocal microscopy. The nuclei are counterstained with DAPI (pseudocolored in grey).

A



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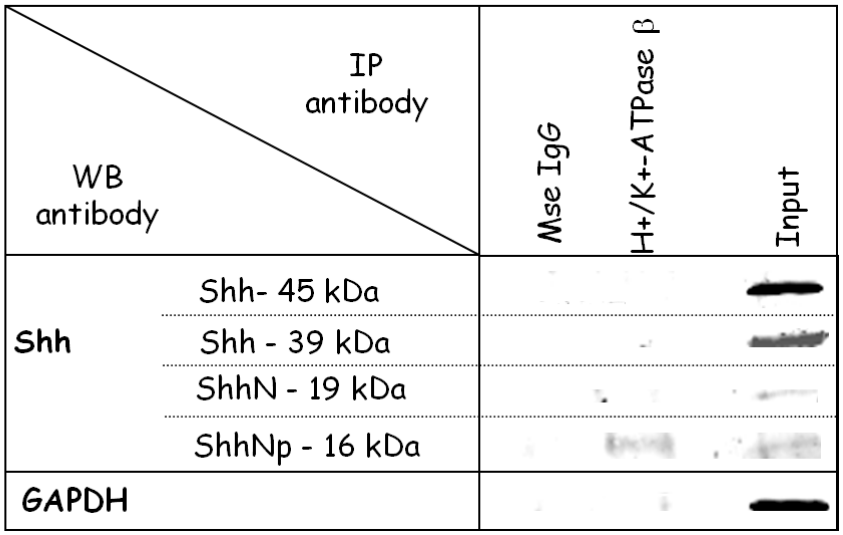
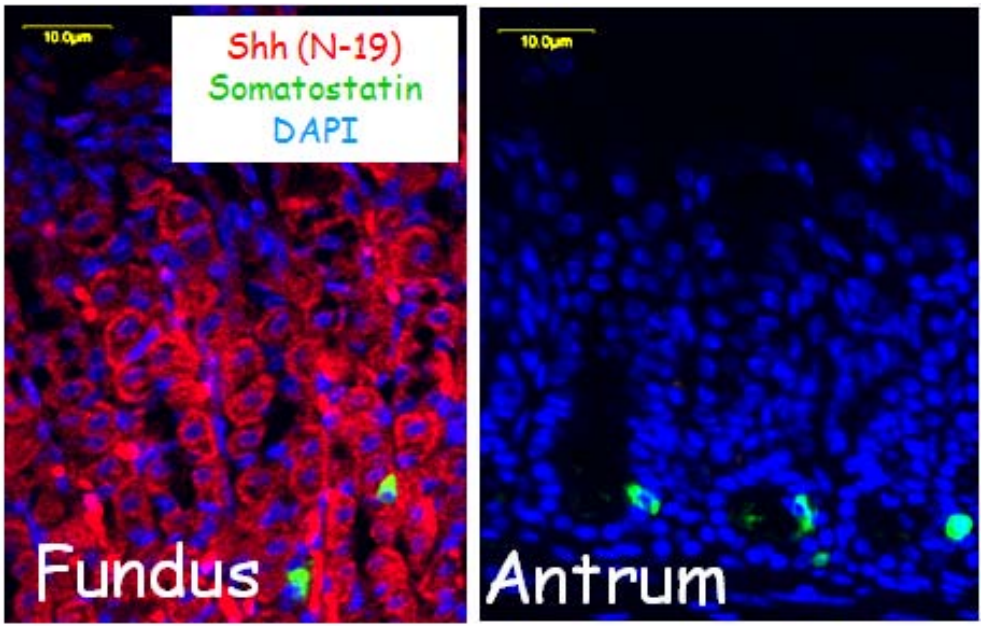


Supplementary Figure 4. Co-immunoprecipitation of H^+/K^+-ATPase and Shh from gastric lysates. A) Immunoprecipitation of Shh peptide from gastric lysates using goat polyclonal Shh antibody (N-19) versus concentration-matched goat IgG. The immunoprecipitate was probed with H^+/K^+-ATPase-β subunit antibody on a western blot. B) Immunoprecipitation of H^+/K^+-ATPase-β protein using H^+/K^+-ATPase-β antibody versus concentration-matched mouse IgG. The Shh forms were detected by western blot using Shh antibody (N-19). Shh-45kDa is the full-length protein; Shh-39kDa is the protein minus the signal peptide; ShhN-19kDa and ShhNp-16kDa are the N-terminally processed and presumed lipid-modified forms.

A



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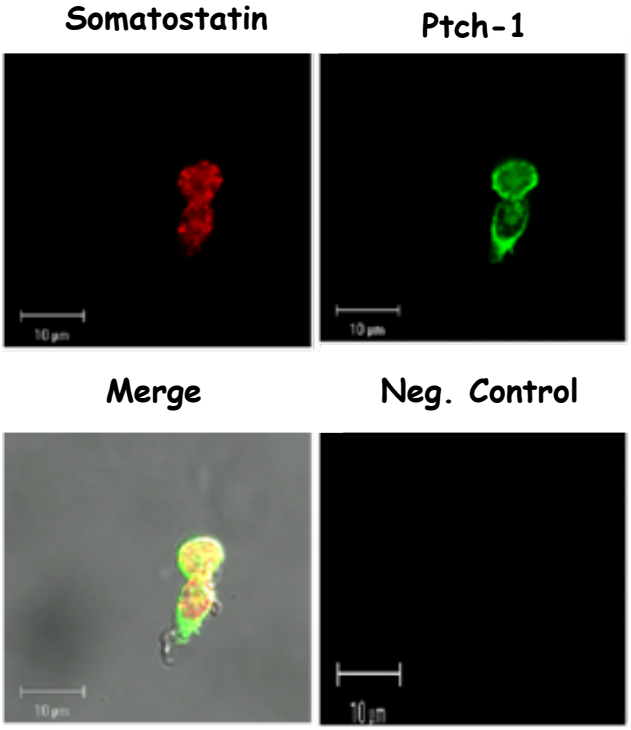
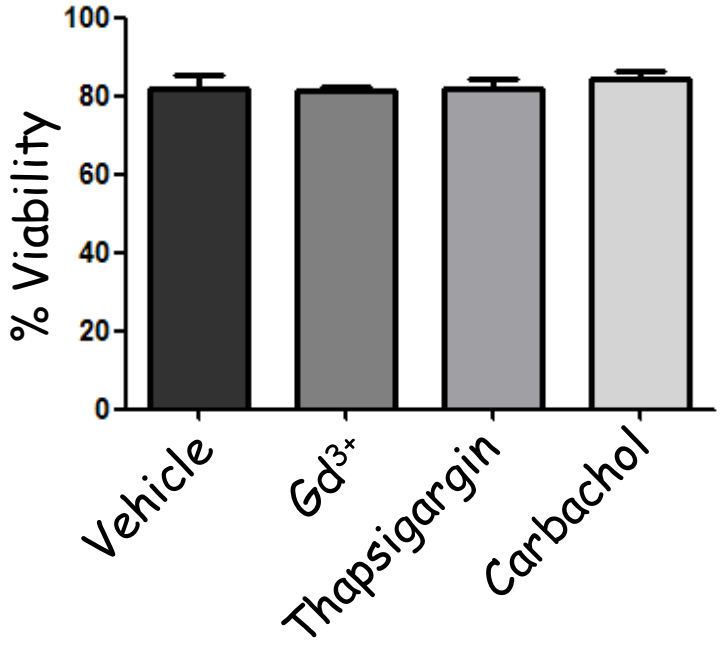
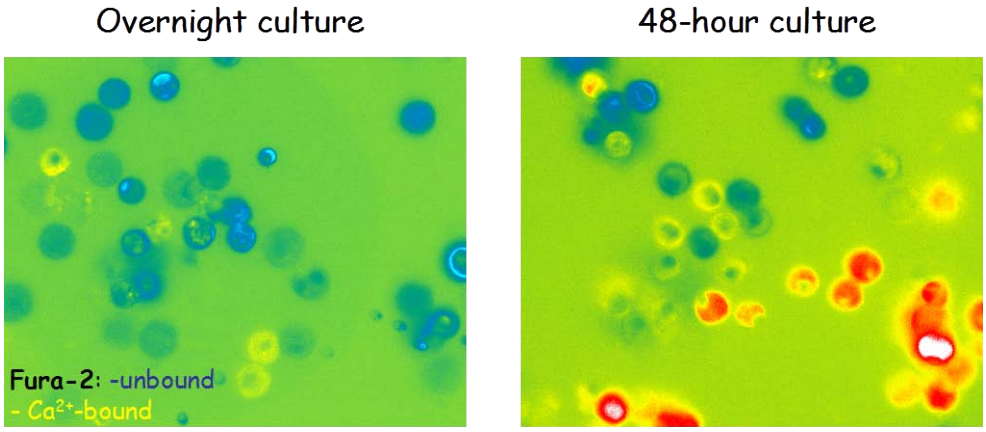


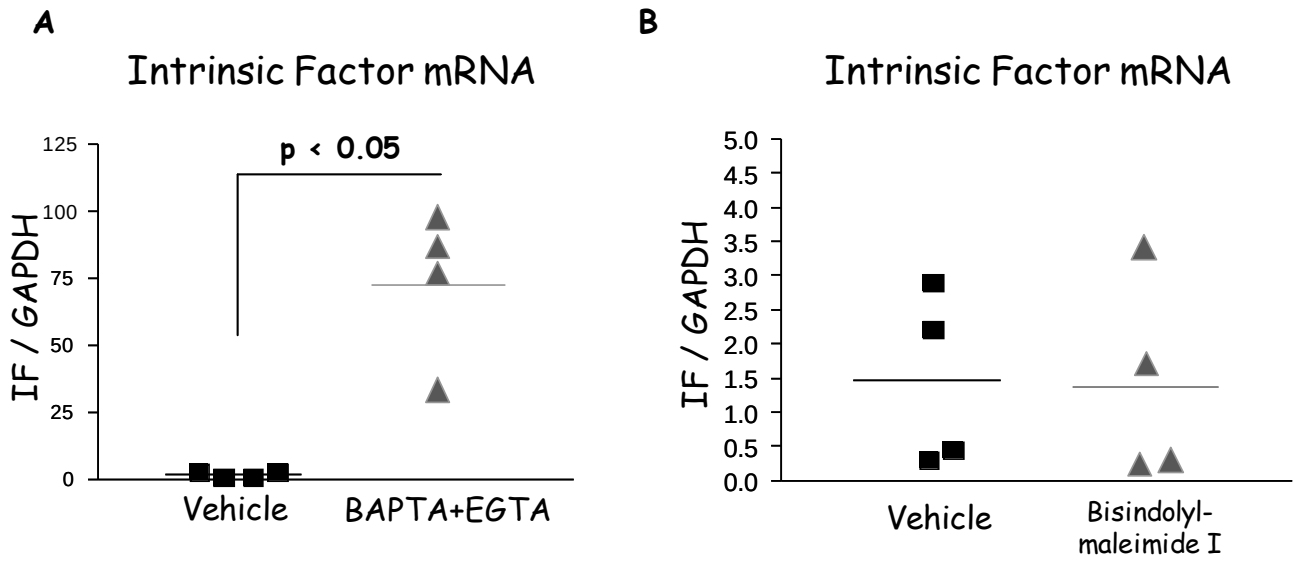
Supplementary Figure 5. D-cells express Ptch-1 receptor but not Shh. A) Immunofluorescence for somatostatin (green) and Shh (red) in the gastric fundus and antrum. B) Immunofluorescence for somatostatin (red) and Ptch-1 (green) in an enriched culture of canine D-cells.



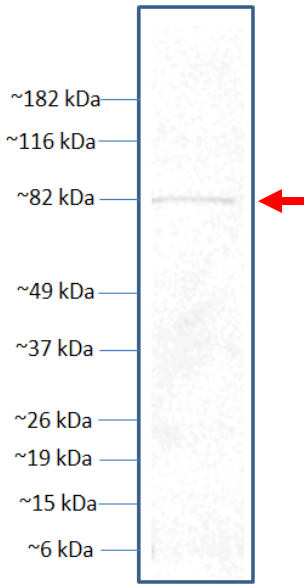
Supplementary Figure 6. Trypan blue viability assay. Trypan blue viability assay of mouse primary gastric cultures treated with vehicle, Gd^{3+}, thapsigargin or Carbachol for 6 h. Bar graphs represent the percentage of trypan blue-negative cells over the total cell number. Three experiments were performed in duplicate. Shown is the mean +/- SEM.



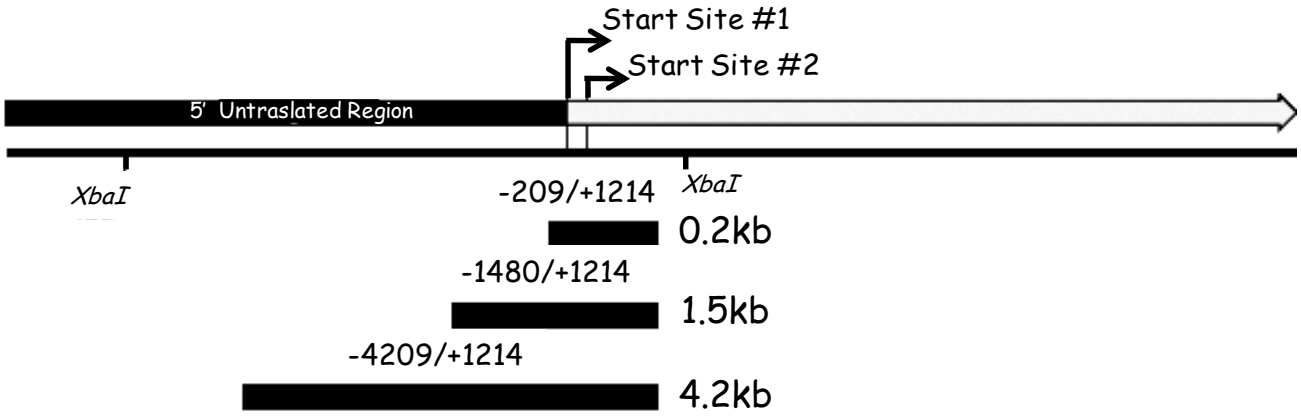
Supplementary Figure 7. $Ca^{2+}_{(i)}$ levels in parietal cells at 24 and 48 hours post-culture. Fura-2 imaging of canine parietal cells cultured for 24 h (left panel) and 48 h (right panel). The blue and yellow/red signals indicate low and high intracellular calcium-binding to Fura-2 respectively.



Supplementary Figure 8. Intrinsic factor gene expression after $Ca2^{+}_{(i)}$ chelation or PKC inhibition. Intrinsic factor expression in response to EGTA plus BAPTA-AM ($p < 0.05$) (A) and Bisindolylmaleimide I (P-value not significant) (B) in mouse fundic organ cultures determined by RT-qPCR. Each datapoint corresponds to one mouse.



Supplementary Figure 9. Activated PKCα/β protein expression in isolated canine parietal cells (~80kDa). The antibody detects PKCα at phosphorylation site threonine-638 and PKCβ_{II} at phosphorylation site threonine-641. Canine parietal cell extract was resolved on a 4-20% Tris-Glycine gradient gel, blotted on a PVDF membrane, and probed with phospho-PKCα/β_{II} (Thr638/641) antibody (#9375, Cell Signaling, Boston, MA).



Supplementary Figure 10. Diagram illustrating the Shh sequences inserted into the pGL3-basic luciferase-reporter vector. The promoter sequence for Shh has been previously described and is reported to have two transcriptional start sites[1]. The 3 bars indicate the positions of the 0.2kb, 1.5kb, and 4.2kb DNA promoter segments used in the luciferase reporter constructs.

References

1. Kitazawa S, Kitazawa R, Tamada H, Maeda S. Promoter structure of human sonic hedgehog gene. Biochim Biophys Acta 1998;1443:358-63.