Supplemental Figure 1. BrdU labeling characteristics of β-gal positive cells.

In panels A,D,G,J, and M, BrdU staining (red) is shown. In panels B,E,H,K and N, the signal for the FITC-labeled anti-β-gal antibody (green) has been added to the BrdU (red) signal. In panels C,F, I, L and O, the DAPI channel (blue) has been added to the BrdU (red) and anti-β-gal (green). A-C) This wild type mouse was pulse labeled with BrdU one hour prior to sacrifice. β-gal positive cells are located among the labeled cells (arrows) but the β-gal positive cells themselves are not labeled, indicating that they are not cycling in the absence of stimulation. D-I) Two examples of cells after one week of continuous BrdU labeling and one week of IFNγ treatment are shown. In D-F, the β-gal positive cell did not take up label (arrow). In G-I, the β-gal positive cell is also positive for the BrdU label (arrow). J-O) Two examples are shown of label retaining cells. These mice were given IFNγ and BrdU for one week and were sacrificed after an additional week without treatment. In both cases, only two label retaining cells are visible in the glands. In both cases, one label- retaining cell (white arrow) is positive for anti-β-gal staining while the other (yellow arrowhead) is not. In all panels, Bar = 20μm.

Supplemental Figure 2. The distribution of labelled cells is similar in two transgenic models driven by villin regulatory sequences.

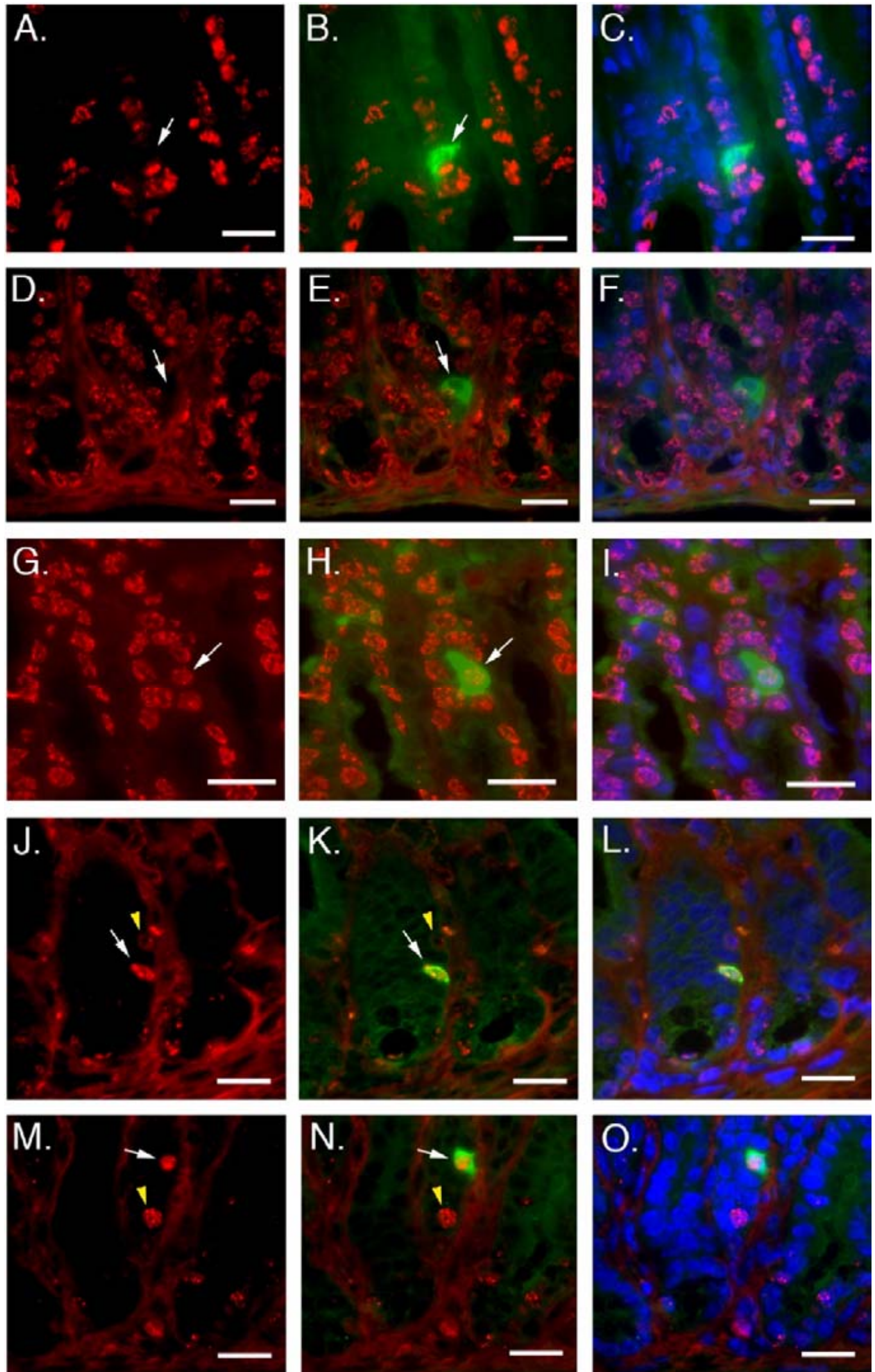
A) β-gal positive cells occupy the antrum of the 12.4KVil-LacZ mouse. Their distribution and location is similar to that seen in the Villin$^{\beta\text{-gal}/+}$ model (compare to Figure 1B). B) Two weeks of IFNγ treatment causes amplification of β-gal positive cells in the 12.4KVil-LacZ mouse antrum. C) β-gal positive cells in a 12.4KVil-LacZ mouse antrum are located deep in the gland and are triangular in shape. D) Rare labeled cells are visible

in an untreated 12.4KVil-EGFP mouse, here detected with an FITC conjugated anti-EGFP antibody. E) IFNγ causes amplification of the marked cells in the 12.4KVil-EGFP model. F) Labeled cells in 12.4KVil-EGFP mice are triangular and located in the bottom one third of the glands. For A-B, Bar = 50μm; In B and E, Bar = 20μm; In C and F, Bar = 10μm.

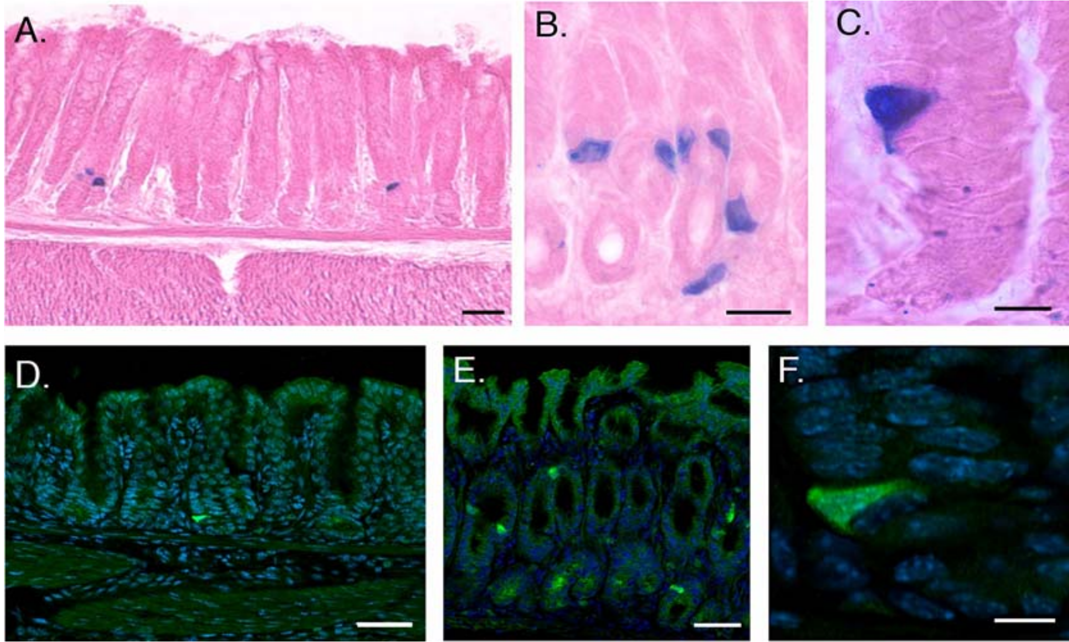
Supplemental Figure 3. A proposed life cycle of GPC-positive glands. GPC (yellow) are quiescent and reside in a niche that is located below the isthmus. B) In response to specific triggers, such as INFγ, GPC divide symmetrically along the long axis of the gland (see Figure 3J). C) Recently divided GPC migrate toward the gland base (see Figure 2B). D-F) Arrival of two GPC at the base of the gland may be a trigger for gland fission; GPC segregate to opposite sides of the gland to be separated by fission (see Figures 1C, 3G, 3H, 3J). Cycles of symmetric division and subsequent fission (yellow arrows) could explain clonal patches of GPC-containing glands seen in lineage tracing studies (see Figure 4E). G) GPC in recently divided glands migrate back up to a niche, where they can receive triggers that promote asymmetric division. H) Asymmetric division of GPC (yellow) leads to rapid gland regeneration (blue). All cells of the regenerated gland are derived from a single GPC (designated in yellow).

Supplemental Figure 4. β-gal positive cells are present at the squamo-columnar junction and are visible in fetal life. A) Several β-gal positive cells are located on the initial columnar region at the muco-columnar junction in this 12.4KVil-LacZ mouse. B) A 12.4KVil-EGFP mouse exhibits similar labeled cells at the forestomach junction. C)

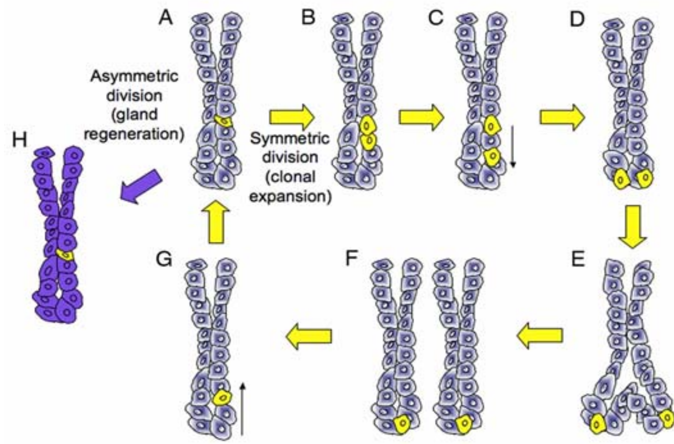
In an E16.5 mouse embryo, small triangular β-gal positive cells are located next to the basement membrane of the immature antral epithelium. For all panels, Bar = 20μm.



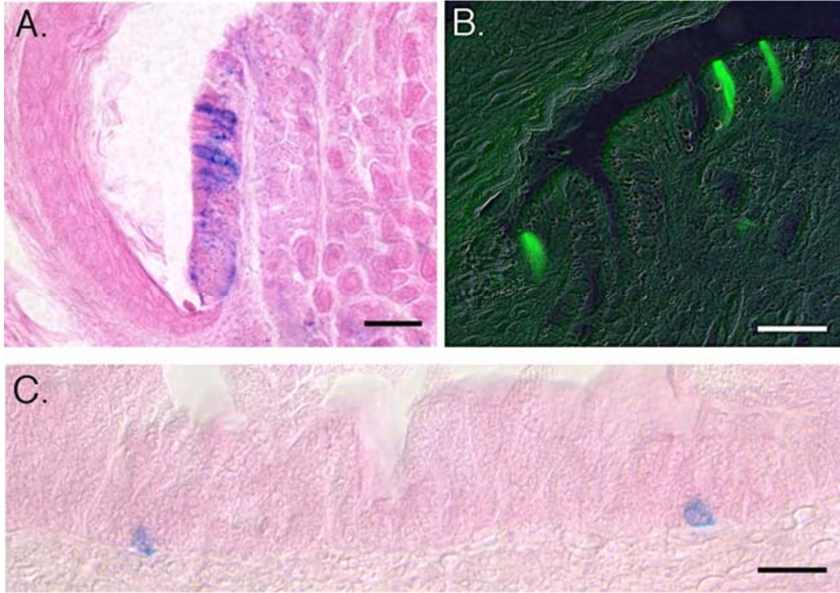
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4