Supplemental Information

Supplementary Figures and Figure Legends

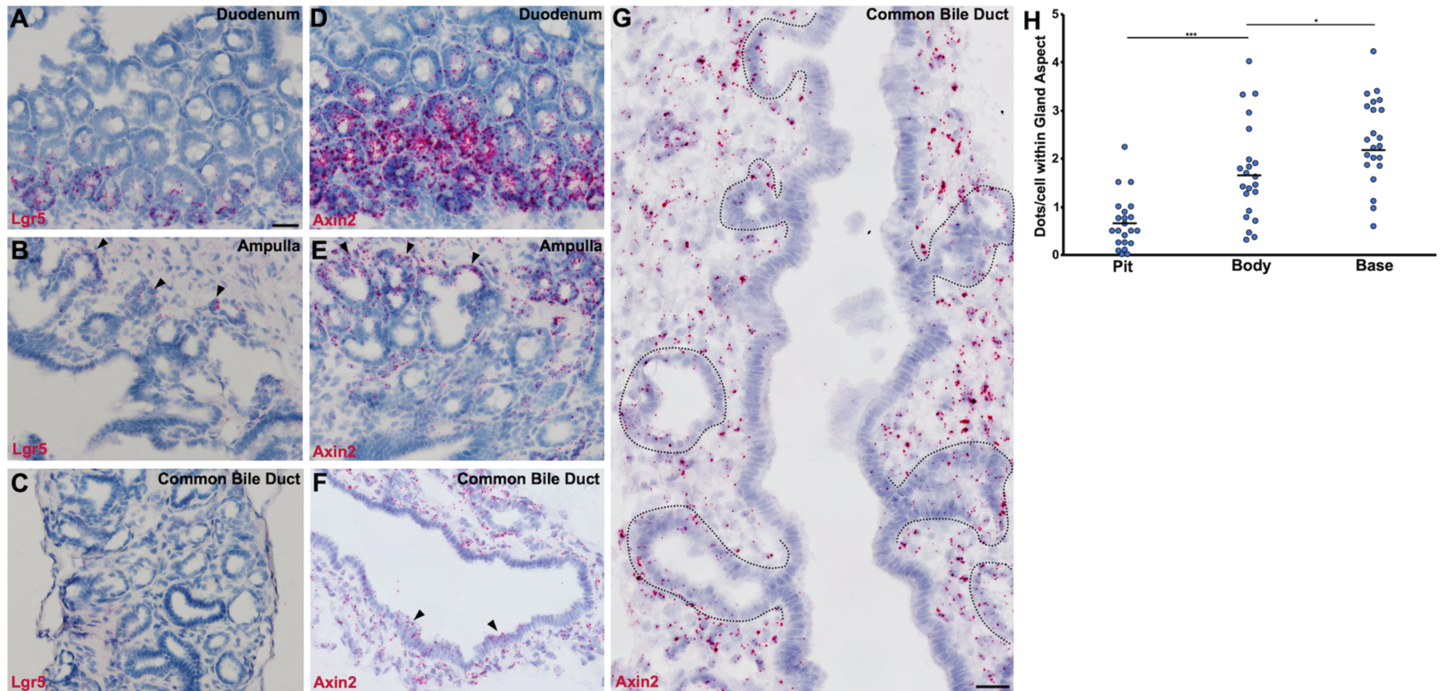


Figure S1: *Lgr5* and *Axin2 in situs* of the Duodenum, Ampulla, and Common Bile Duct, related to Figure 1.

(**A-C**) *Lgr5 in situs* of Duodenum (**A**), Ampulla, (**B**), and CBD (**C**) (n = 3). (**D-G**) *Axin2 in situs* of the Duodenum (**D**), Ampulla, (**E**), and CBD (**F** and **G**) (n = 3). (**H**) Quantification of *Axin2 in situ* signal from pit, body, and base of peribiliary glands (n = 3). Scale bars, 50 μm (**B**-**F** same scale as **A**). *** $p<0.001$, * $p<0.05$, by Student's t-test.

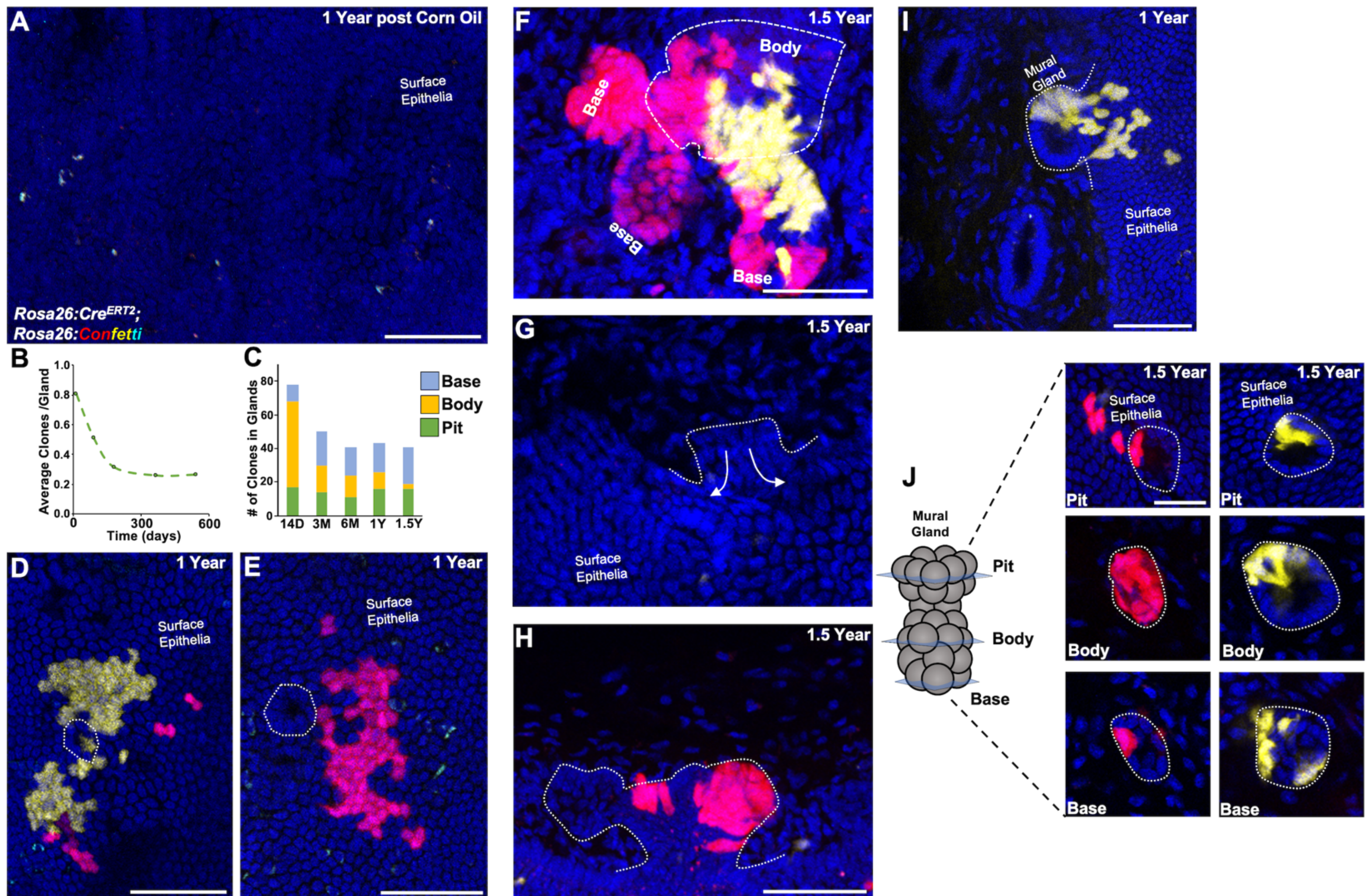


Figure S2: Clonal Analysis of Biliary Proliferation, related to Figure 2.

(**A**) 1 year after corn oil, there is no detectable recombination in *Rosa26:Cre^{ERT2}; Rosa26:Confetti* mice (n = 3). (**B**) Overtime, there is a steady decrease in the average number of clones per gland in *Rosa26:Cre^{ERT2}; Rosa26:Confetti* mice. (**C**) Total clones subdivided by compartment at indicated timepoints. (**D**) 1 year post tamoxifen, a yellow clone is seen on the surface epithelium in continuity with a peribiliary pit (dashed circle). (**E**) 1 year post tamoxifen, a red clone is seen on the surface epithelium adjacent to a peribiliary pit (dashed circle), but without labeled cells in the pit. (**F**) 1.5 years after tamoxifen, a extramural gland with 3 separate alveoli can be seen with red and yellow clones mixing in one alveolus and meeting together at the body (dashed circle) of the gland. (**G**) Pit from the gland in (**F**), showing no labeled cells going through the pit into the surface epithelium. (**H**) A two alveolar gland 1.5 years after tamoxifen showing a red clone labeling most of an alveolus coming down into the body of the gland and fragmenting. (**I**) 1 year post tamoxifen, a yellow clone in a mural gland (dashed line) can be seen going from the base to the surface epithelium. (**J**) Examples of two other mural glands at 1.5 years post tamoxifen showing base to pit labeling. Scale bars, 50 µm (**A**, **D**, **E**, **F**, **H**, **I**, **G** same scale as **F**); 25 µm (**J,** all panels same scale).

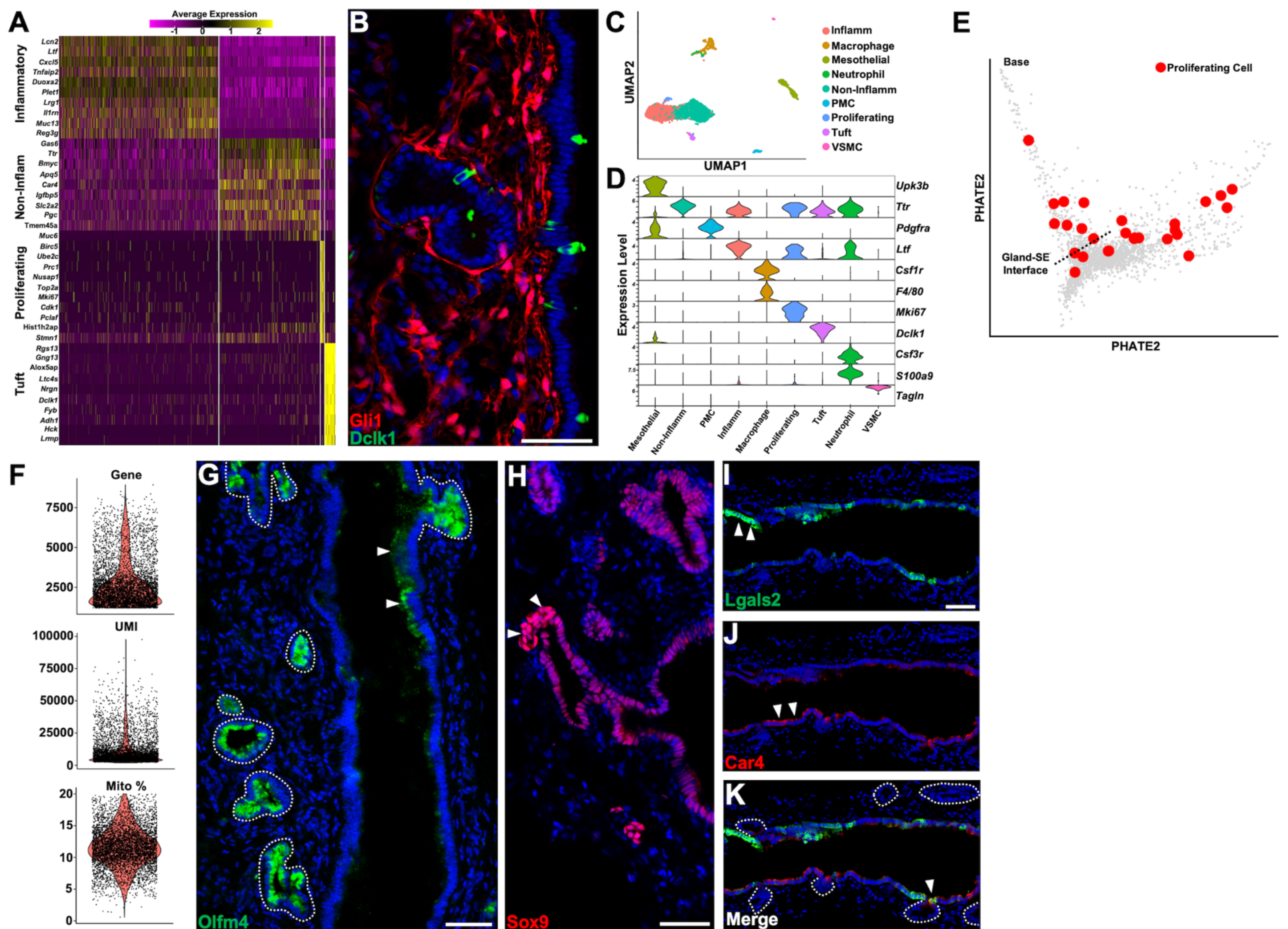


Figure S3: Analysis of Different Populations of Cells within the Common Bile Duct, related to Figure 3

(**A**) Marker genes for low resolution clustering of epithelia from the CBD. (**B**) Staining for *Dclk1* in the CBD showing tuft cells in a *Gli1:Cre*^{ERT2}*; Rosa26:lox-STOP-lox-tdTomato* animal in which tamoxifen mediated recombination has labeled the adjacent mesenchyme (n = 3). (**C**) UMAP reduction of epithelial and non-epithelial cell populations from the CBD. (**D**) Violin plot of marker genes for non-epithelial cell populations. (**E**) PHATE trajectory analysis with proliferating cells highlighted in red. (**F**) Gene, UMI, and % mitochondrial counts per cell from (**C**). (**G**) *Olfm4* staining showing predominant expression within the glands (dashed circles) but with some expression within the surface epithelium (arrowheads) (n = 3). (**H**) *Sox9* staining showing higher expression (arrowheads) within the bases of glands and lower expression towards the lumen (n = 3). (**I-K**) *Car4* and *Lgals2* double staining showing areas of *Lgals2* high, *Car4* low epithelium (arrowheads, **I**), *Lgals2* low, *Car4* high epithelium (arrowhead, **J**), and double positive epithelium (arrowhead, **K** with varying degrees of expression of both proteins) (n = 3). Scale bars, 50 μm (**J** and **K** same scale as **I**).

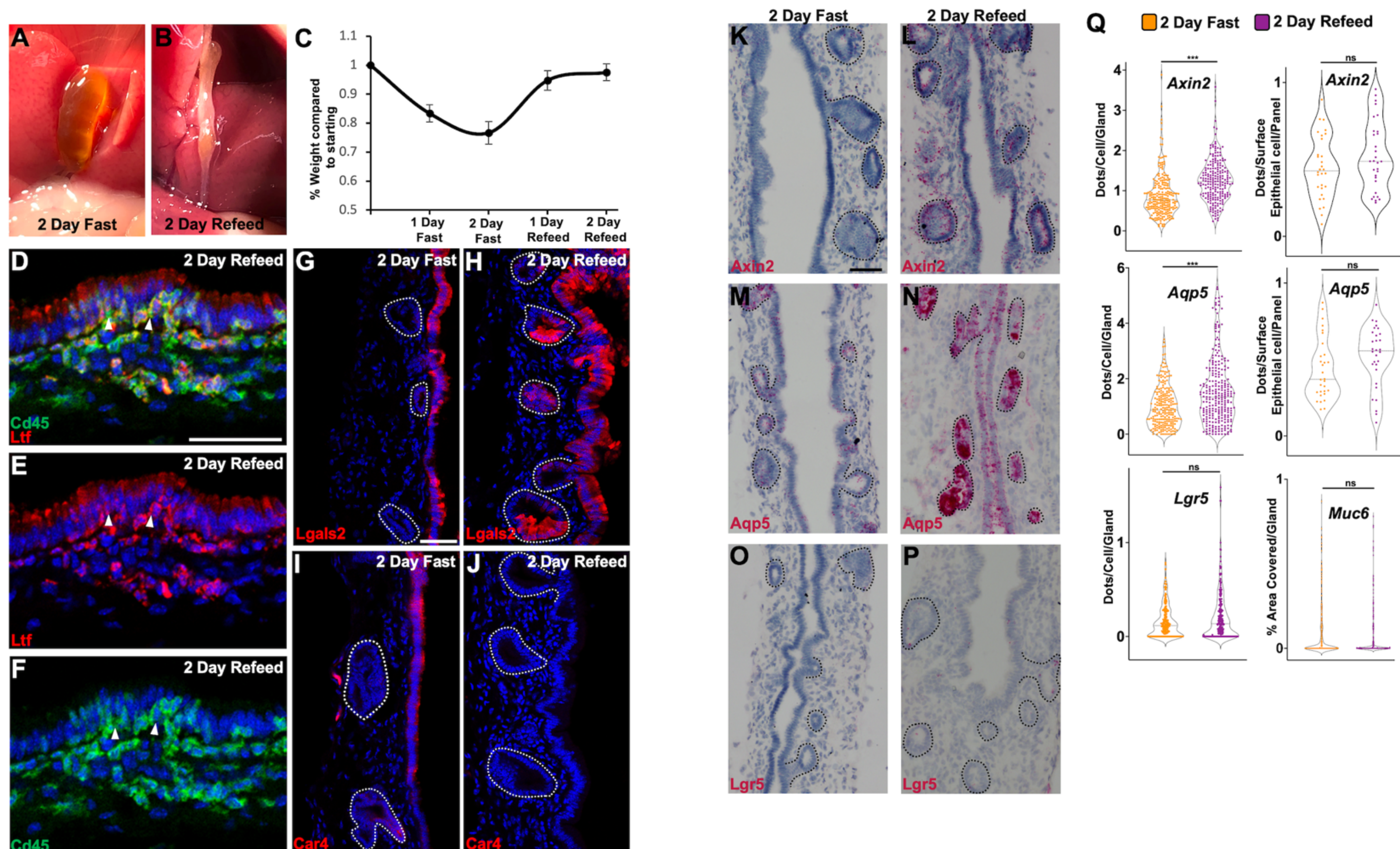


Figure S4: Changes in Glandular Marker Gene Expression after Fasting/Refeeding, related to Figure 4.

(**A**) Fasting for two days causes the gallbladder to swell with bile. (**B**) Two days after refeeding, the gallbladder is collapsed. (**C**) Weights of animals during fasting and refeeding (n = 22 for each timepoint, error bars show SEM). (**D-F**) *Ltf* and *Cd45* staining showing infiltrating intraepithelial *Ltf*$^+$*Cd45*$^+$ (arrowheads) neutrophils 2 days after refeeding (n = 6). (**G** and **H**) *Lgals2* staining in fasting (**G**) and refeed (**H**) states with glands in dashed circles (n = 6). (**I** and **J**) *Car4* staining in fasting (**I**) and refeed (**J**) states with glands in dashed circles (n = 6). (**K** and **L**) *Axin2 in situ* in 2 day fast (**K**) and 2 day refeed states (**L**). (**M** and **N**) *Aqp5 in situ* in 2 day fast (**M**) and 2 day refeed states (**N**). (**O** and **P**) *Lgr5 in situ* in 2 day fast (**O**) and 2 day refeed states (**P**). (**Q**) Quantification of *in situs* of indicated genes (n = 6 animals per group). Scale bars, 50 μm (**E** and **F** same scale as **D**, **H-J** same scale as **G**, **L-P** same scale as **K**). *** p<0.001, ns = not significant, by Student's t-test.

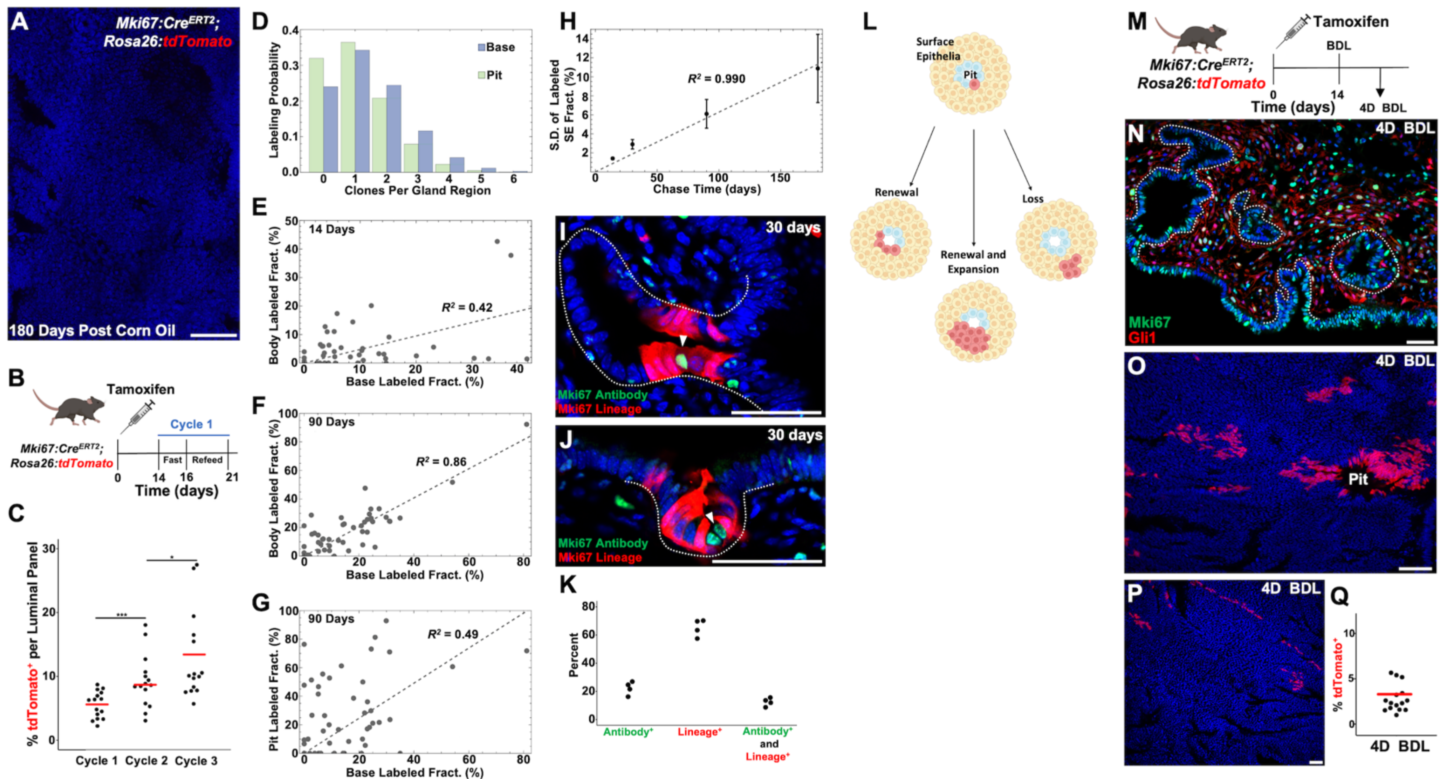


Figure S5: Lineage Tracing with *Mki67*, related to Figure 5.

(**A**) 6 months after corn oil injection, there is no labeling in *Mki67:Cre*^{ERT2}; *Rosa26:lox-STOP-lox-tdTomato* animals (n = 3). (**B**) Cartoon describing strategy for cyclic fasting and refeeding experiments in *Mki67:Cre*^{ERT2}; *Rosa26:lox-STOP-lox-tdTomato* animals. (**C**) Surface epithelial labeling in cyclical fasting and refeeding experiment (n = 3 animals per group). (**D**) Predicted number of clones per gland in the base and pit region from the *Mki67* labeling assay inferred from the frequency of unlabeled glands at the 14 day time point. (**E**) Correlation between the body and base labeled cell fraction in individual glands at day 14 of the *Mki67* lineage tracing assay. Each dot represents an individual gland. A linear fit to the data shows a poor correlation (R^2=0.42) with a slope of 0.5 pointing to a bias towards base labeling at the time of induction. (**F**) Correlation between the body and base labeled cell fraction in individual glands at 90 days post-induction. Each dot represents an individual gland. Note that the fraction of labeled cells in the base and body region of individual glands is now more highly correlated than at 14 days post-labeling (R^2=0.86) with a slope of 1.02, consistent with the base region maintaining the body. (**G**) Correlation between the pit and base labeled cell fraction in individual glands at 90 days post-induction. Each dot represents an individual gland. Note that the fraction of labeled cells in the base and pit regions are poorly correlated (R^2=0.49) with a slope of 1.23, consistent with the two regions being largely compartmentalized. (**H**) Standard deviation of the labeled cell fraction on the surface epithelium as a function of chase time. Note that the standard deviation grows approximately linearly with time, as predicted by a model based on the neutral competition of surface epithelial cells which is constantly fed by migrating cells from the pit. Points show data (error bars denotes standard error). Line

shows a linear fit with a slope 0.063 per day. (**I**) *Mki67:Cre*^{ERT2}; *Rosa26:lox-STOP-lox-tdTomato* gland (dashed outline) 30 days post tamoxifen where lineage labeling in the pit region has one cell that is also positive for *Mki67* antibody staining (arrowhead). (**J**) Like (**I**) but where the pit of this gland has lineage labeling but also *Mki67* antibody positive cells that are lineage negative (arrowhead). (**K**) Quantification showing relative amounts of *Mki67* antibody positive cells, *Mki67:Cre*^{ERT2} lineage positive cells, and double positive cells (n = 4). (**L**) Cartoon showing possible outcomes of labeled pit cells. (**M**) Cartoon describing experimental design. (**N**) *Mki67* staining 4 days after BDL in *Gli1* lineage labeled animals, dashed lines indicate glands. (**O**) 4 days after BDL in *Mki67:Cre*^{ERT2}; *Rosa26:lox-STOP-lox-tdTomato* animals, positive labeling could be seen emanating from a peribiliary gland. (**P**) The majority of the surface epithelium 4 days after BDL was unlabeled. (**Q**) % labeling of surface epithelial panels 4 days after BDL (n = 3, 5 low power panels per animal). Scale bars, 50 µm. *** $p<0.001$, * $p<0.05$, ns = not significant, by Student's t-test.

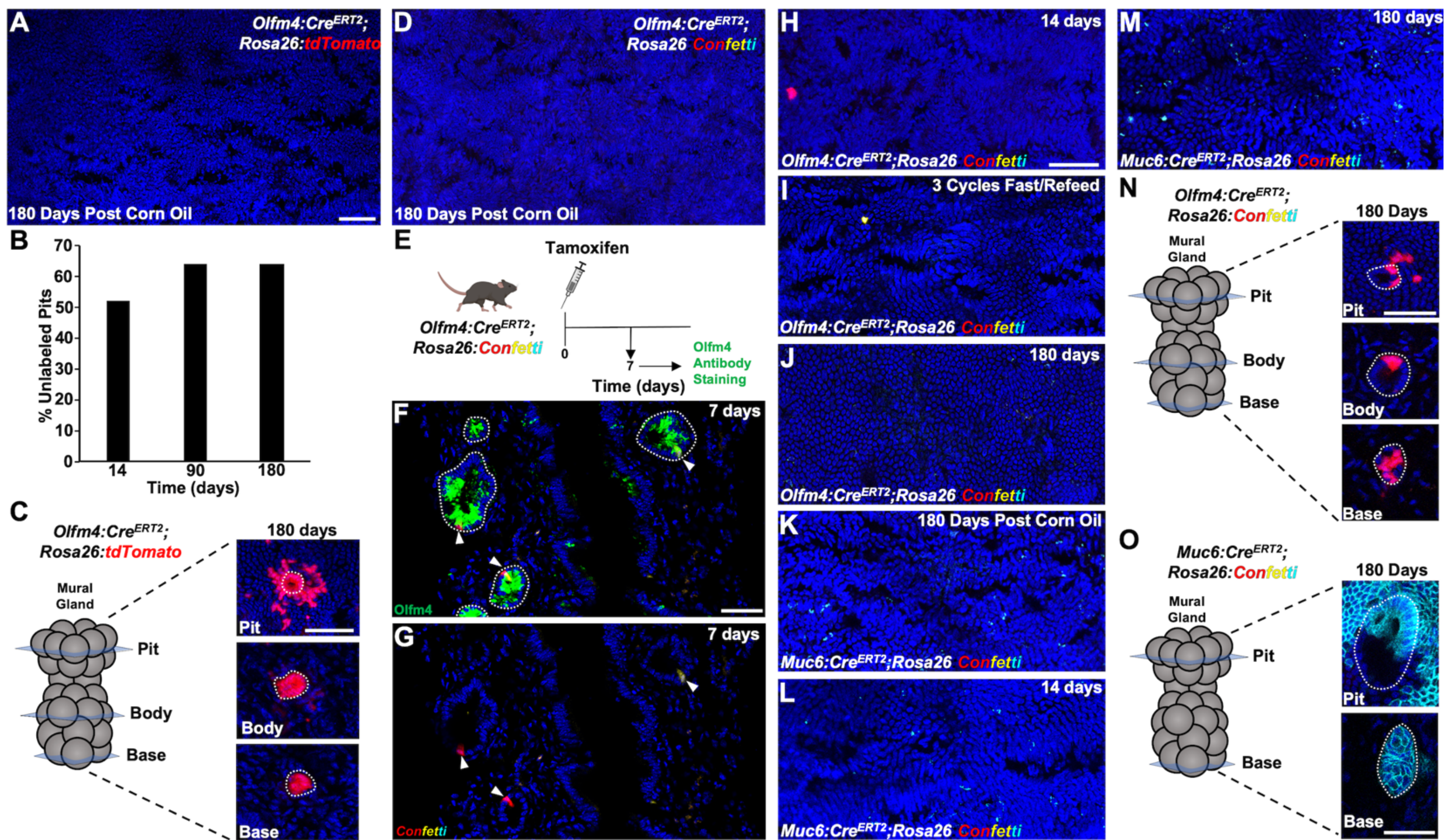
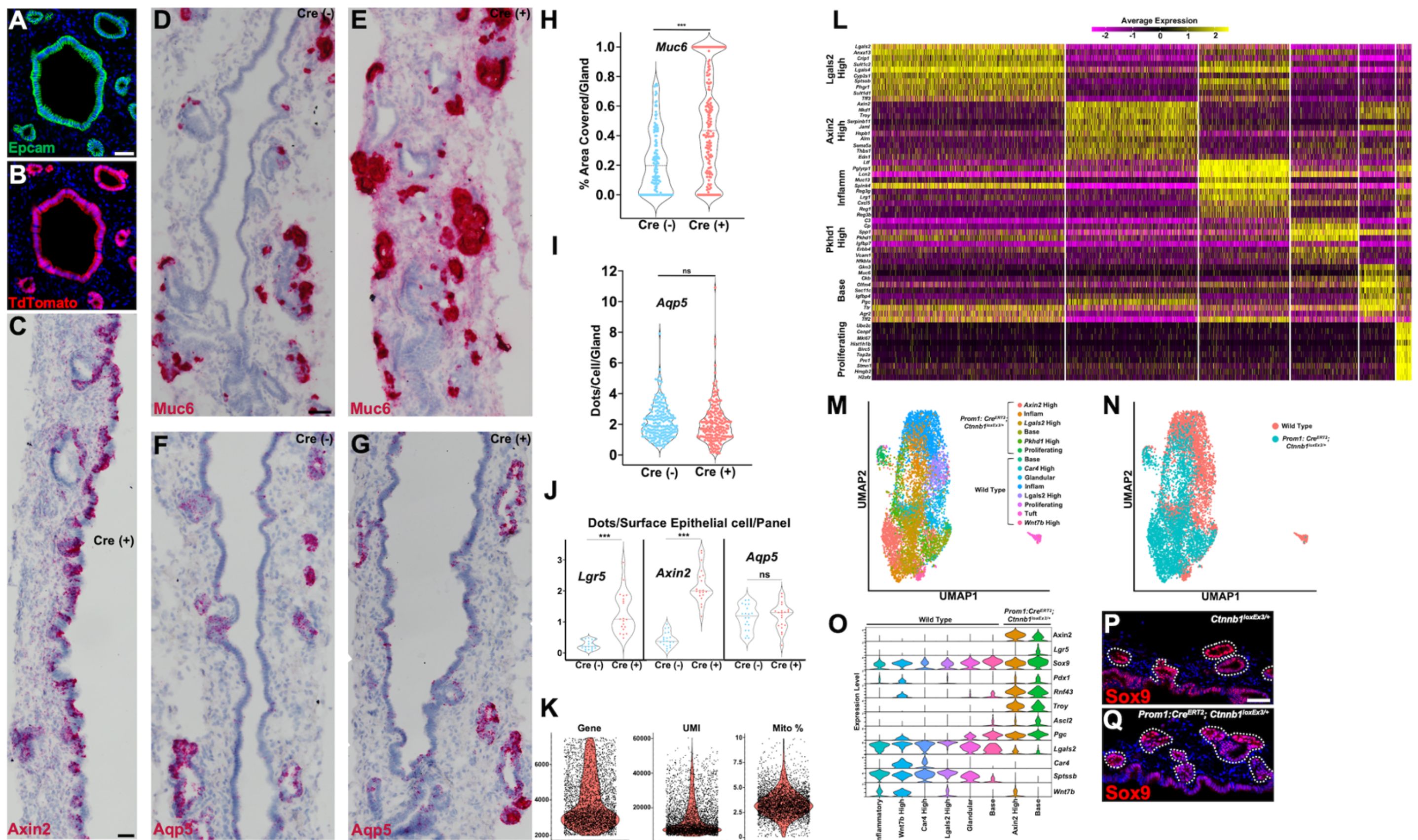


Figure S6: Lineage Tracing with *Olfm4* and *Muc6*, related to Figure 6.

(**A**) 180 days after corn oil, there was no detectable recombination in *Olfm4:Cre^{ERT2}*; *Rosa26:lox-STOP-lox-tdTomato* animals (n = 3). (**B**) Percent of unlabeled pits at indicated times from *Olfm4:Cre^{ERT2}*; *Rosa26:lox-STOP-lox-tdTomato* chase experiment. (**C**) Example of a mural gland 180 days post tamoxifen from a *Olfm4:Cre^{ERT2}*; *Rosa26:lox-STOP-lox-tdTomato* animal in which the gland is fully labeled and expanding in all directions on the surface epithelium. (**D**) 180 days after corn oil, there was no detectable recombination in *Olfm4:Cre^{ERT2}*; *Rosa26:Confetti* animals (n = 3). (**E**) Cartoon describing experimental design. (**F** and **G**) 7 days after tamoxifen in *Olfm4:Cre^{ERT2}*; *Rosa26:Confetti* animals, clones (arrowheads, **G**) can be seen induced and colocalizing with *Olfm4* antibody (**F**) stained peribiliary glands. (**H-J**) Little to no labeling of the surface epithelium was seen in *Olfm4:Cre^{ERT2}*; *Rosa26:Confetti* animals at the indicated timepoints. (**K**) 180 days after corn oil, there was no detectable recombination seen in *Muc6:Cre^{ERT2}*; *Rosa26:Confetti* animals (n = 3). (**L** and **M**) No labeling of the surface epithelium was seen in *Muc6:Cre^{ERT2}*; *Rosa26:Confetti* animals at the indicated timepoints. (**N**) Example of a mural gland 180 days post tamoxifen from a *Olfm4:Cre^{ERT2}*; *Rosa26:Confetti* animal in which a red clone is seen extending from the base down to the surface epithelium. (**O**) Example of a mural gland 180 days post tamoxifen from a *Muc6:Cre^{ERT2}*; *Rosa26:Confetti* animal in which a cyan clone is seen extending from the base and spreading onto the surface epithelium. Scale bars, 50 μm (**D** same scale as **A**, **G** same scale as **F**, **I-M** same scale as **H**).



<u>Figure S7: Expansion of *Muc6*$^{+}$ Cells after *β-catenin* Gain of Function, related to Figure 7.</u>

(**A** and **B**) 7 days after tamoxifen in *Prom1:Cre*^{ERT2}; *Rosa26:lox-STOP-lox-tdTomato* animals, recombination (**B**) is seen in virtually all epithelia when compared to *Epcam* staining (**A**) (n = 5). (**C**) Low power image of *Axin2 in situ* from a *Prom1:Cre*^{ERT2}; *Ctnnb1*$^{loxEx3/+}$ animal 21 days post tamoxifen, where areas of confluent *in situ* signal represent areas of *β-catenin* gain of function. (**D** and **E**) *Muc6 in situs* in Cre (-) (*Ctnnb1*$^{loxEx3/+}$) and Cre (+) (*Prom1:Cre*^{ERT2}; *Ctnnb1*$^{loxEx3/+}$) animals. (**F** and **G**) *Aqp5 in situs* in Cre (-) and Cre (+) animals. (**H**) Quantification of glandular area covered from *Muc6 in situ* (n = 4 for each group). (**I**) Quantification of *in situ* signal from *Aqp5* (n = 4 for each group). (**J**) Quantification of surface epithelial *in situ* signal of indicated genes (n = 4 group for each group, 5 panels for each animal). (**K**) Gene, UMI, and % mitochondrial counts per cell from *Prom1:Cre*^{ERT2}; *Ctnnb1*$^{loxEx3/+}$ single cell sequencing. (**L**) Cluster markers for epithelia from *Prom1:Cre*^{ERT2}; *Ctnnb1*$^{loxEx3/+}$ animals. (**M**) Integrated UMAP of wild type and *Prom1:Cre*^{ERT2}; *Ctnnb1*$^{loxEx3/+}$ single cell sequencing data. (**N**) Same as (**M**) but colored by experiment. (**O**) Violin plots of indicated genes comparing non-proliferative, non-tuft cholangiocyte clusters from wild type dataset to *Axin2* high and base clusters from *Prom1:Cre*^{ERT2}; *Ctnnb1*$^{loxEx3/+}$. (**P** and **Q**) *Sox9* staining from Cre (-) (**P**) and Cre (+) (**Q**) animals with glands in dashed outlined. Scale bars, 50 μm (**B** same scale as **A, E-G** same scale as **D, Q** same scale as **P**). *** $p<0.001$, ns = not significant, by Student's t-test