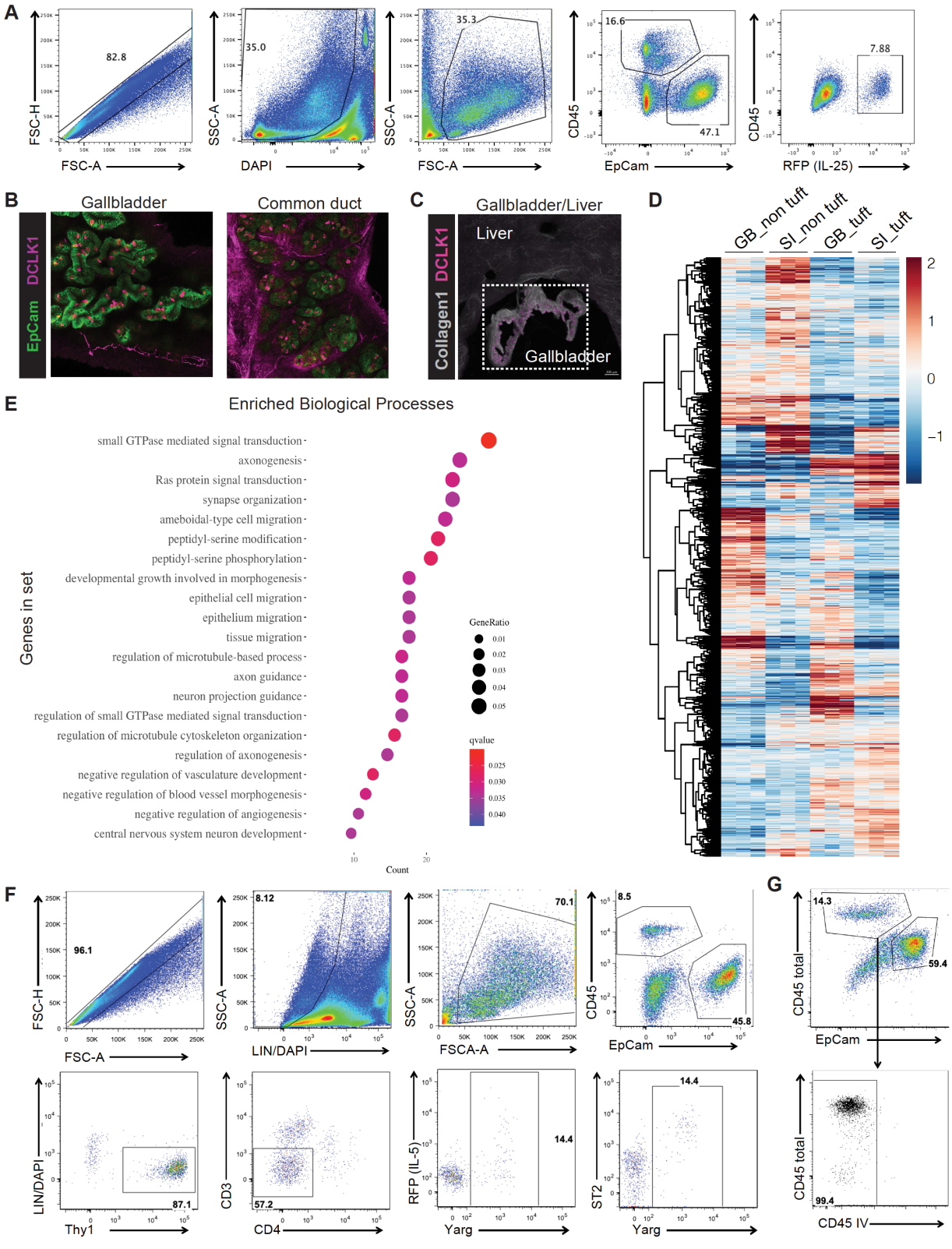

A
FSC-H
FSC-A
82.8
SSC-A
DAPI
35.0
SSC-A
FSC-A
35.3
CD45
EpCam
16.6
47.1
CD45
RFP (IL-25)
7.88
B
Gallbladder
Common duct
EpCam
DCLK1
C
Gallbladder/Liver
Liver
Collagen1
DCLK1
Gallbladder
D
GB_non tuft
SI_non tuft
GB_tuft
SI_tuft
2
1
0
−1
E
Enriched Biological Processes
small GTPase mediated signal transduction
axonogenesis
Ras protein signal transduction
synapse organization
ameboidal-type cell migration
peptidyl-serine modification
peptidyl-serine phosphorylation
developmental growth involved in morphogenesis
epithelial cell migration
epithelium migration
tissue migration
regulation of microtubule-based process
axon guidance
neuron projection guidance
regulation of small GTPase mediated signal transduction
regulation of microtubule cytoskeleton organization
regulation of axonogenesis
negative regulation of vasculature development
negative regulation of blood vessel morphogenesis
negative regulation of angiogenesis
central nervous system neuron development
Genes in set
Count
GeneRatio
0.01
0.02
0.03
0.04
0.05
qvalue
0.025
0.030
0.035
0.040
F
FSC-H
FSC-A
96.1
SSC-A
LIN/DAPI
8.12
SSC-A
FSCA-A
70.1
CD45
EpCam
8.5
45.8
LIN/DAPI
Thy1
87.1
CD3
CD4
57.2
RFP (IL-5)
Yarg
14.4
14.4
ST2
Yarg
14.4
G
CD45 total
EpCam
14.3
59.4
CD45 total
CD45 IV
99.4

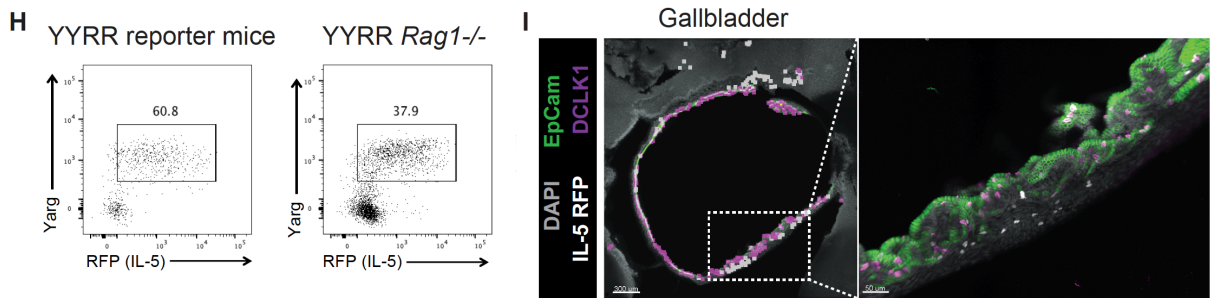


Fig. S1. Biliary tuft cells express tuft cell- and tissue-specific gene signature and are not dependent on type 2 cytokines. A) Gating scheme for analysis of tuft cells. The same gating was used for total GB/EHBD digests and epithelial prep. **B**) Whole-mount confocal imaging of DCLK1-stained GB/bile duct. **C**) Thick section imaging of gallbladder and liver for collagen 1 and DCLK1. **D**) Tuft (IL-25+) and non-tuft (IL-25-) epithelial cells were sorted from duodenum or GB of Flare25 mice and analyzed by RNAsequencing. Heatmap shows expression and hierarchical clustering of top 1000 gene by variance from biliary tuft (GB_tuft), biliary non-tuft (GB_non tuft), small intestinal tuft (SI_tuft), and small intestinal non-tuft (SI_non tuft) cells, as defined by FDR > 0.05 in any pair-wise comparison. **E**) Gene ontology biological processes enrichment for genes upregulated in biliary tuft cells compared to small intestinal tuft cell and non-tuft biliary epithelium. **F**) Identification of ILC2s from Arginase-1 (Yarg)/IL-5 (Red5) reporter mice. **G**) Representative flow plot. Anesthetized mice were injected retro-orbitally with anti-CD45 antibody 3 minutes before euthanasia. CD45+ cells in total GB/EHBD digests were stained with an alternate fluorophore; circulating cells were identified by the presence of the I.V. injected fluorophore. **H**) Representative plots. Analysis of ILC2s from total GB/EHBD digests in *Rag1*+/+ and *Rag1*-/- Yarg/Red5 reporter mice. Previously gated on live singlets, FSC-A x SSC-A, CD45+lineage-Thy1+. **I**) Thick-section imaging of GB/liver from Red5;R26$^{Ai14\text{-}RFP}$ mice

stained for EpCam (green), DCLK1 (magenta), and DAPI (gray). ILC2s detected by native fluorescence. ILC2s and DCLK1+ cells shown as surfaces in low magnification image.

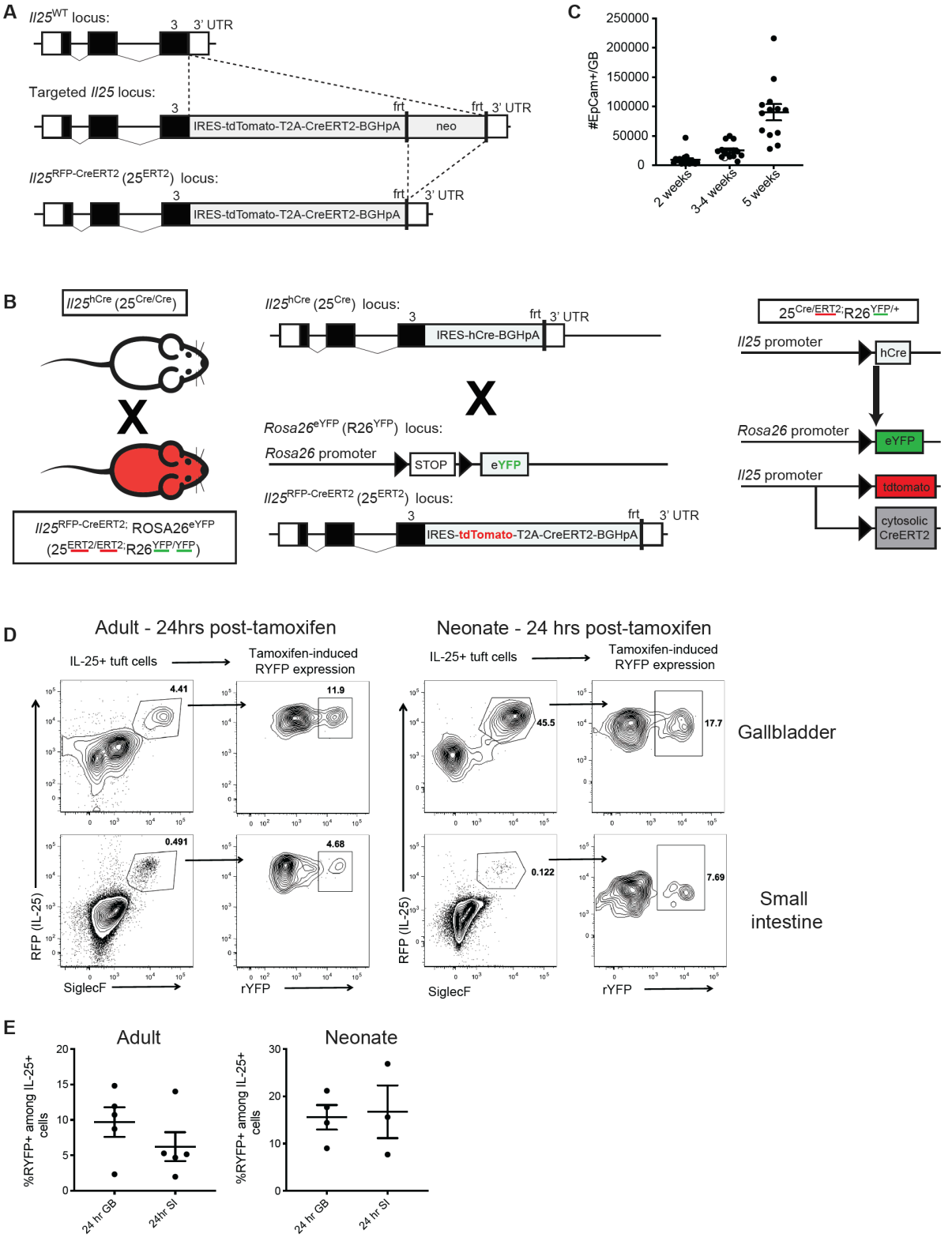


Fig. S2. Biliary tuft cells exhibit developmental regulation. A) Targeting strategy for IL-25 driven CreERT2/tdTomato expression. **B**) Number of epithelial cells from per total GB/EHBD digests isolated from wildtype mice of the indicated ages, as quantified by flow cytometry. **C**) Schematic showing intercross of $25^{Cre/Cre}$ mouse with $25^{ERT2/ERT2}$;$R26^{YFP/YFP}$ mouse to generate $25^{Cre/ERT2}$;$R26^{YFP/+}$ fate mapping mouse in which active IL-25 is reported by tdTomato from the 25^{ERT2} allele while YFP is expressed in any current or former IL-25+ cell downstream of 25^{Cre} activity. **D**) Representative flow plots showing tuft cell gating and tamoxifen inducible YFP labelling in $25^{ERT2/ERT2}$;$R26^{YFP/YFP}$ adult or neonatal (p10-p12) mice injected twice with tamoxifen and analyzed by flow cytometry on epithelial prep one day after the last injection (24 hr). **E**) Flow cytometry quantification of YFP+ tuft cells among small intestinal or biliary epithelial cells from mice treated as in (**C**).

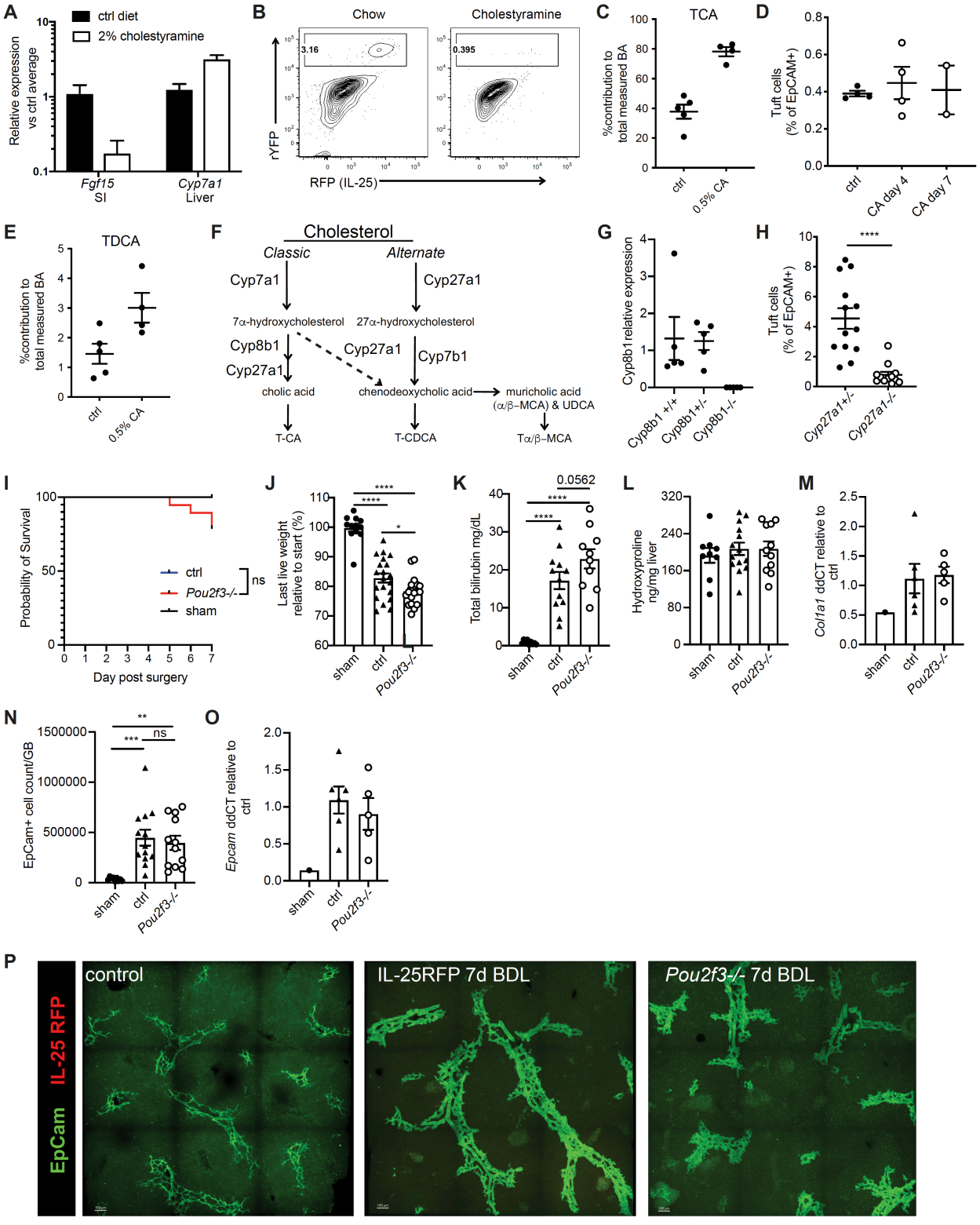

A
ctrl diet
2% cholestyramine
Relative expression vs ctrl average
Fgf15 SI
Cyp7a1 Liver
B
Chow
Cholestyramine
3.16
0.395
rYFP
RFP (IL-25)
C
TCA
%contribution to total measured BA
ctrl
0.5% CA
D
Tuft cells (% of EpCAM+)
ctrl
CA day 4
CA day 7
E
TDCA
%contribution to total measured BA
ctrl
0.5% CA
F
Cholesterol
Classic
Alternate
Cyp7a1
Cyp27a1
7α-hydroxycholesterol
27α-hydroxycholesterol
Cyp8b1
Cyp27a1
Cyp27a1
Cyp7b1
cholic acid
chenodeoxycholic acid
muricholic acid (α/β–MCA) & UDCA
T-CA
T-CDCA
Tα/β–MCA
G
Cyp8b1 relative expression
Cyp8b1 +/+
Cyp8b1+/-
Cyp8b1-/-
H
****
Tuft cells (% of EpCAM+)
Cyp27a1+/-
Cyp27a1-/-
I
Probability of Survival
ctrl
Pou2f3-/-
sham
ns
Day post surgery
J
****
****
*
Last live weight relative to start (%)
sham
ctrl
Pou2f3-/-
K
0.0562
****
****
Total bilirubin mg/dL
sham
ctrl
Pou2f3-/-
L
Hydroxyproline ng/mg liver
sham
ctrl
Pou2f3-/-
M
Col1a1 ddCT relative to ctrl
sham
ctrl
Pou2f3-/-
N
**
***
ns
EpCam+ cell count/GB
sham
ctrl
Pou2f3-/-
O
Epcam ddCT relative to ctrl
sham
ctrl
Pou2f3-/-
P
EpCam
IL-25 RFP
control
IL-25RFP 7d BDL
Pou2f3-/- 7d BDL

Fig. S3. Biliary tuft cell abundance is modulated by bile acids. A) Terminal ileum and liver from mice fed control (AIN93G, ctrl) or 2% cholestyramine diet for two weeks were analyzed by qPCR for expression of *Fgf15* (SI) and *Cyp7a1* (liver) relative to the housekeeping gene, *Rps17.* Five mice per condition. **B**) $25^{Cre/ERT2}$;$R26^{YFP/+}$ mice fed control diet (chow) or 2% cholestyramine diet for two weeks were analyzed by flow cytometry for presence of fate-mapped (YFP+) cells that no longer expressed IL-25 (RFP). Representative flow plots from epithelial prep previously gated on live singlets, EpCam+CD45- cells. **C**). Concentration of tauro-cholic acid (TCA) was analyzed by GC-MS from gallbladder of mice fed control diet (ctrl) or 0.5% cholic acid (CA). **D**) Tuft cell frequency among proximal small intestinal epithelial cells was quantified by flow cytometry in mice fed 0.5% CA for indicated periods. **E**) Concentration of tauro-deoxycholic acid (TDCA) was analyzed by GC-MS from gallbladder of mice fed control diet (ctrl) or 0.5% cholic acid (CA). **F**) Schematic of bile acid synthesis in hepatocytes. **G**) qPCR for *Cyp8b1* gene expression in liver from *Cyp8b1*+/+, *Cypb8b1*+/- and *Cypb8b1-/-* mice. **H**) The frequency of DCLK1+ tuft cells was examined by flow cytometry on GB/EHBD of *Cyp27a1*+/+ and *Cyp27a1-/-* mice. **I**) Relative survival of *Pou2f3-/-* and littermate control mice following BDL surgery: 18/22 *Pou2f3-/-,* 23/24 control mice. **J**) Weight of *Pou2f3-/-* and littermate mice seven days after BDL, or last live weight, relative to starting body weight. **K**) Total serum bilirubin of *Pou2f3-/-* and littermate mice seven days after BDL. **L**) Liver hydroxyproline from *Pou2f3-/-* and littermate mice seven days after BDL. **M**) Relative expression of *Col1a1* from liver as assessed by qPCR relative to sham operated controls. **N**) Total EpCam+ cell count per GB/EHBD digest from *Pou2f3-/-* and littermate mice analyzed by flow cytometry seven days after BDL. **O**) Relative expression of *Epcam* from liver as assessed by qPCR relative to sham operated controls. **P**) Representative thick section liver imaging of Flare25 *Pou2f3-/-* and control

littermate mice seven days after BDL or sham surgery stained for EpCam (green) and anti-RFP (red). No RFP+ cells were observed. P value calculated by one way ANOVA in **F,G,J**, *p<.05, **p<.01, ***p<.001, **** p<.0001.

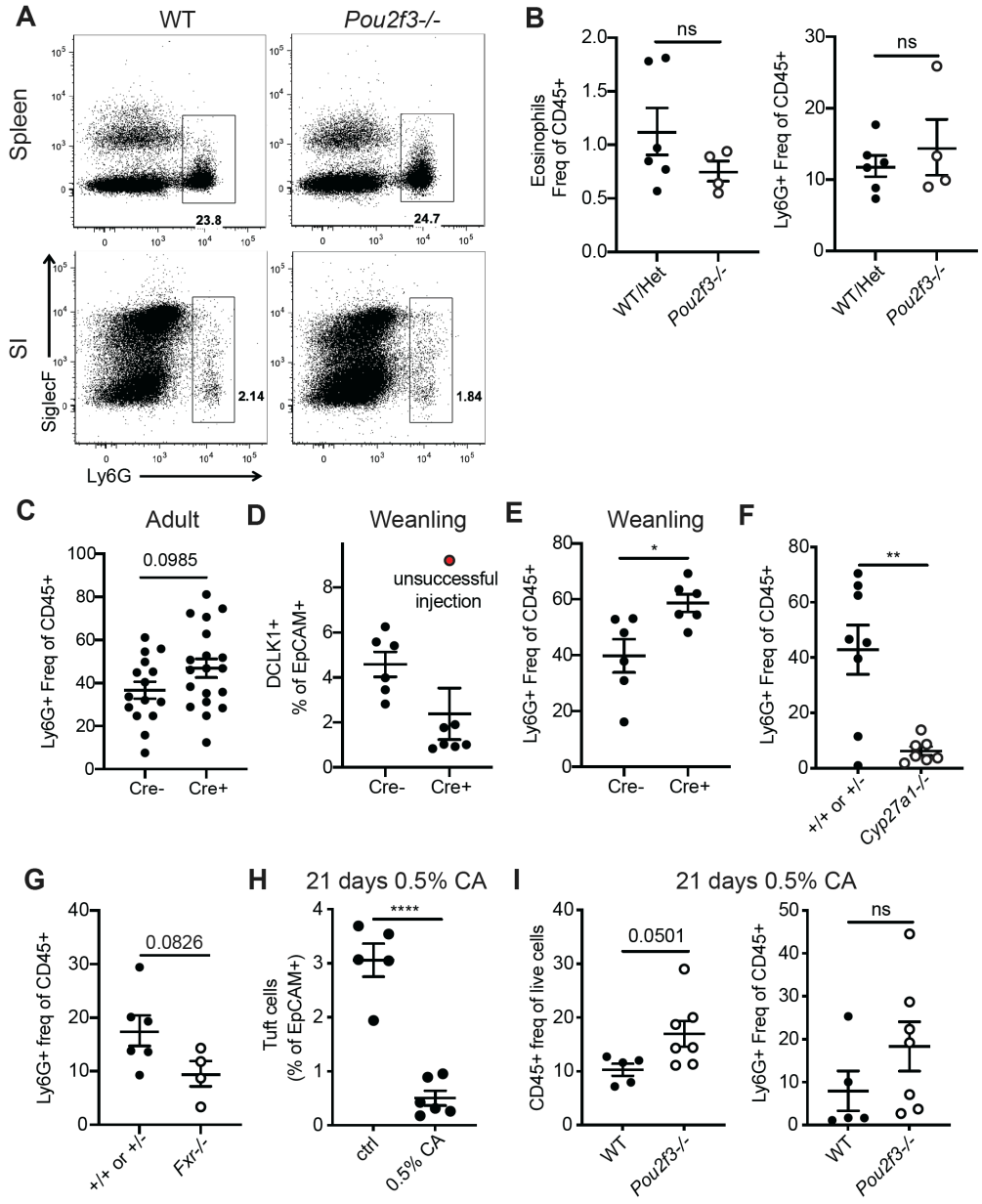
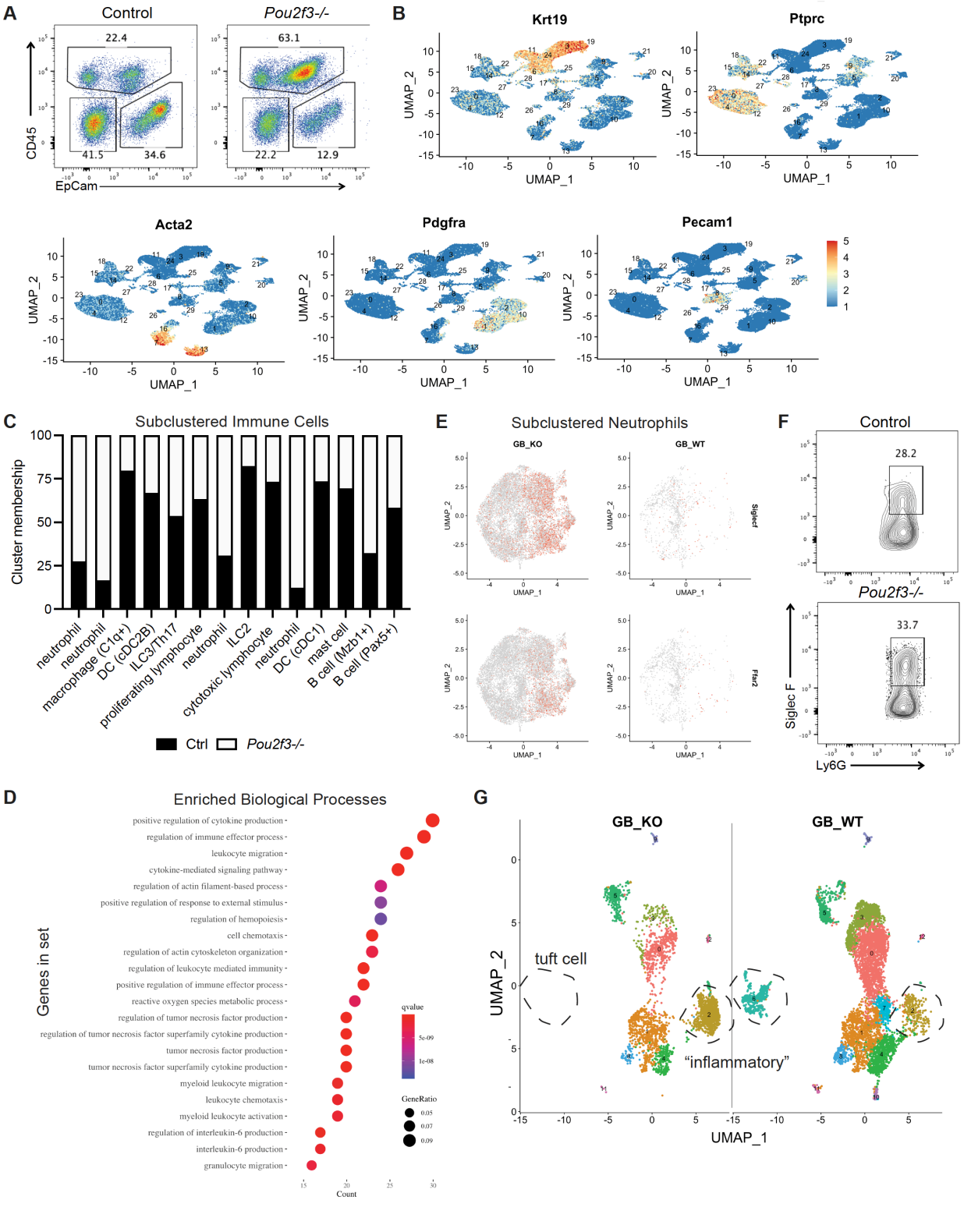


Fig. S4. *Pou2f3-/-* mice have increased biliary neutrophil infiltration under homeostatic conditions. A) Spleen and small intestinal lamina propria (SI) were analyzed for presence of neutrophils in *Pou2f3-/-* and littermate controls by flow cytometry. Representative plots,

previously gated on live singlets, CD45+EpCam-, CD11b+Thy1-. B) Frequency of eosinophils (CD11b+Siglec F+) and neutrophils (CD11b+ Ly6G+CD64-) among CD45+ cells in peripheral blood from *Pou2f3-/-* and littermate controls was quantified by flow cytometry. **C-E**) $25^{cre/+}$;$R26^{iDTR}$ or $25^{+/+}$;$R26^{iDTR}$ littermate mice received two retroorbital injections of diptheria toxin and frequency of tuft cells (**D**) and neutrophils (**C,E**) in total GB/EHBD digests were determined by flow cytometry. **B**) Adult mice were injected at 8 weeks of age and chased 1-6 months. Data pooled from four experiments. Non-normalized flow data from **Fig. 4G**. **D,E**) Mice were injected at weaning and analyzed 4-6 weeks later, data pooled from two experiments. **D**) One failed injection ($25^{cre/+}$;$R26^{iDTR}$ mouse) was removed from further analysis. **E**) Non-normalized flow data from **Fig. 4I**. **F,G**) Frequency of neutrophils in total GB/EHBD digests from *Cyp27a1-/-* (**F**) or *Fxr-/-* (**G**) relative to littermate controls was determined by flow cytometry. **H**) Tuft cell frequency in WT mice fed 0.5% CA for 21 days was determined by flow cytometry on total GB/EHBD digests. **I**) *Pou2f3-/-* and littermate controls were fed 0.5% CA diet for 21 days. Total CD45+ cells and frequency of neutrophils in total GB/EHBD digests were determined by flow cytometry. Statistical significance determined by unpaired student's T-test (**B**,**C**,**E-I**). *p<.05, **p<.01, ****p<.0001. All data shown with +/- SEM.


A
Control
Pou2f3-/-
22.4
63.1
41.5
34.6
22.2
12.9
CD45
EpCam
B
Krt19
Ptprc
Acta2
Pdgfra
Pecam1
UMAP_1
UMAP_2
C
Subclustered Immune Cells
Cluster membership
neutrophil
neutrophil
macrophage (C1q+)
DC (cDC2B)
ILC3/Th17
proliferating lymphocyte
neutrophil
ILC2
cytotoxic lymphocyte
neutrophil
DC (cDC1)
mast cell
B cell (Mzb1+)
B cell (Pax5+)
Ctrl
Pou2f3-/-
D
Enriched Biological Processes
positive regulation of cytokine production
regulation of immune effector process
leukocyte migration
cytokine-mediated signaling pathway
regulation of actin filament-based process
positive regulation of response to external stimulus
regulation of hemopoiesis
cell chemotaxis
regulation of actin cytoskeleton organization
regulation of leukocyte mediated immunity
positive regulation of immune effector process
reactive oxygen species metabolic process
regulation of tumor necrosis factor production
regulation of tumor necrosis factor superfamily cytokine production
tumor necrosis factor production
tumor necrosis factor superfamily cytokine production
myeloid leukocyte migration
leukocyte chemotaxis
myeloid leukocyte activation
regulation of interleukin-6 production
interleukin-6 production
granulocyte migration
Genes in set
Count
qvalue
GeneRatio
E
Subclustered Neutrophils
GB_KO
GB_WT
Siglecf
Ffar2
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GB_KO
GB_WT
tuft cell
"inflammatory"

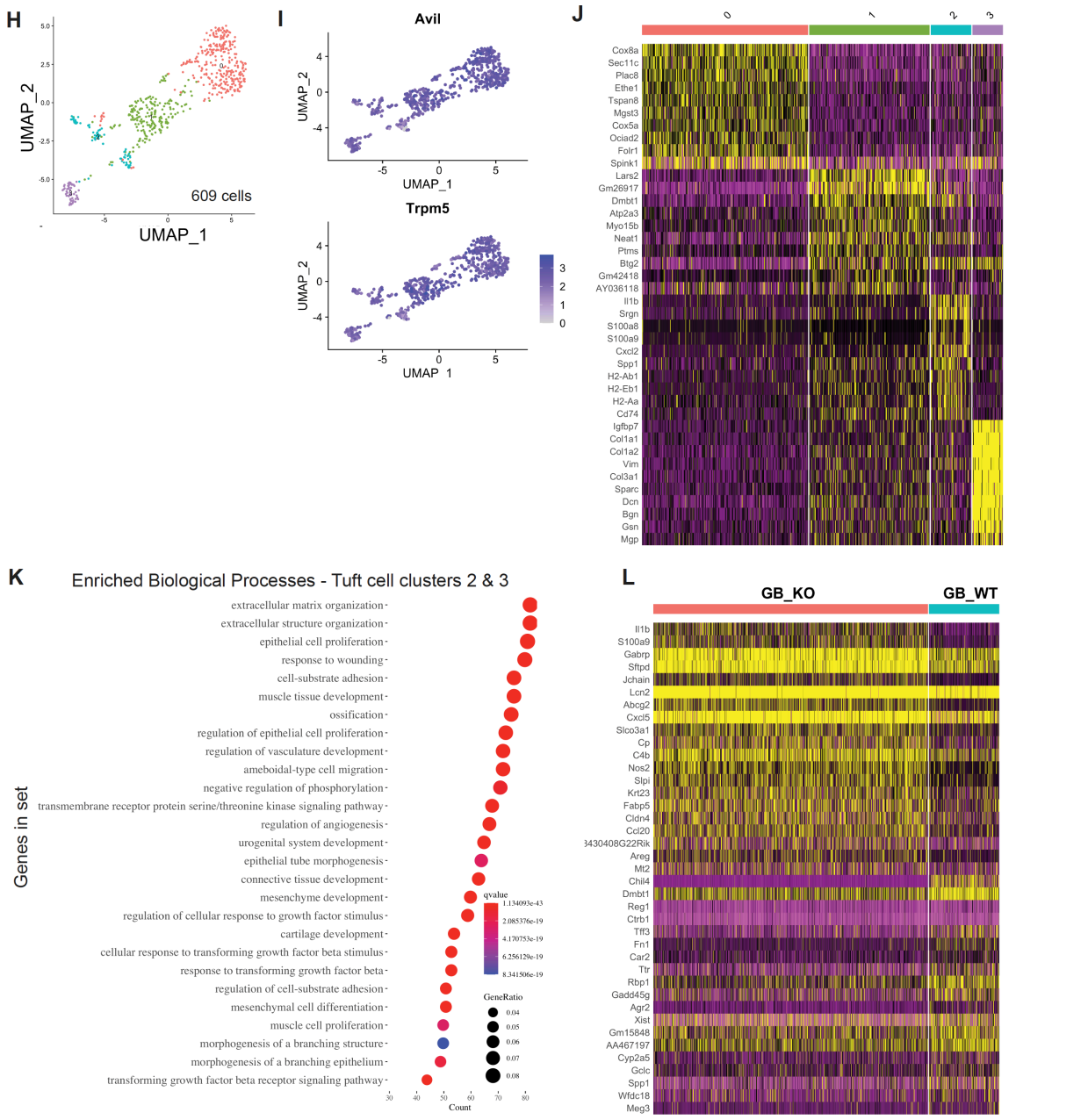

Fig. S5. Total biliary scRNA-seq reveals activated state of biliary neutrophils in the absence of tuft cells. A) Live cells were sorted from total GB/EHBD digests from *Pou2f3-/-* and littermate controls. Input to 10X analysis was analyzed by flow cytometry for relative frequency of CD45+ cells, epithelial cells (EpCam+) and stromal cells (EpCam-CD45-). **B**) Featureplots showing cell type-defining gene expression. **C**) After subclustering CD45+ cells, relative cluster

membership was determined per genotype, normalized to the total number of CD45+ cells sequenced per sample. **D**) Gene ontology biological processes enrichment analysis on genes upregulated in neutrophils from *Pou2f3-/-* mice compared to controls. **E**) Featureplots showing expression of *Ffar2* and *Siglecf* on subclustered neutrophils, split by samples. **F**) Representative flow plot. Total GB/EHBD digests from littermate *Pou2f3-/-* and control mice were analyzed by flow cytometry for expression of Siglec F on neutrophils. Previously gated on: live cells, singlets, FSC-A x SSC-A, EpCam-CD45+, Thy1- CD11b+, CD64-Ly6G+). **G**) UMAP of subclustered epithelial cells, split by sample. **H**) UMAP of subclustered tuft cells (control sample only). **I**) Featureplots indicated high expression of tuft cell marker genes, *Avil* and *Trpm5*, among all tuft cell clusters. **J**) Top 10 most differentially expressed genes defining tuft cell subclusters. **K**) Gene ontology biological processes enrichment analysis on genes upregulated in tuft cells from cluster 2/3 compared to tuft cells in clusters 0/1. **L**). Top 20 most differentially expressed genes among cluster 2 “inflammatory” epithelial cells from *Pou2f3-/-* or control sample.

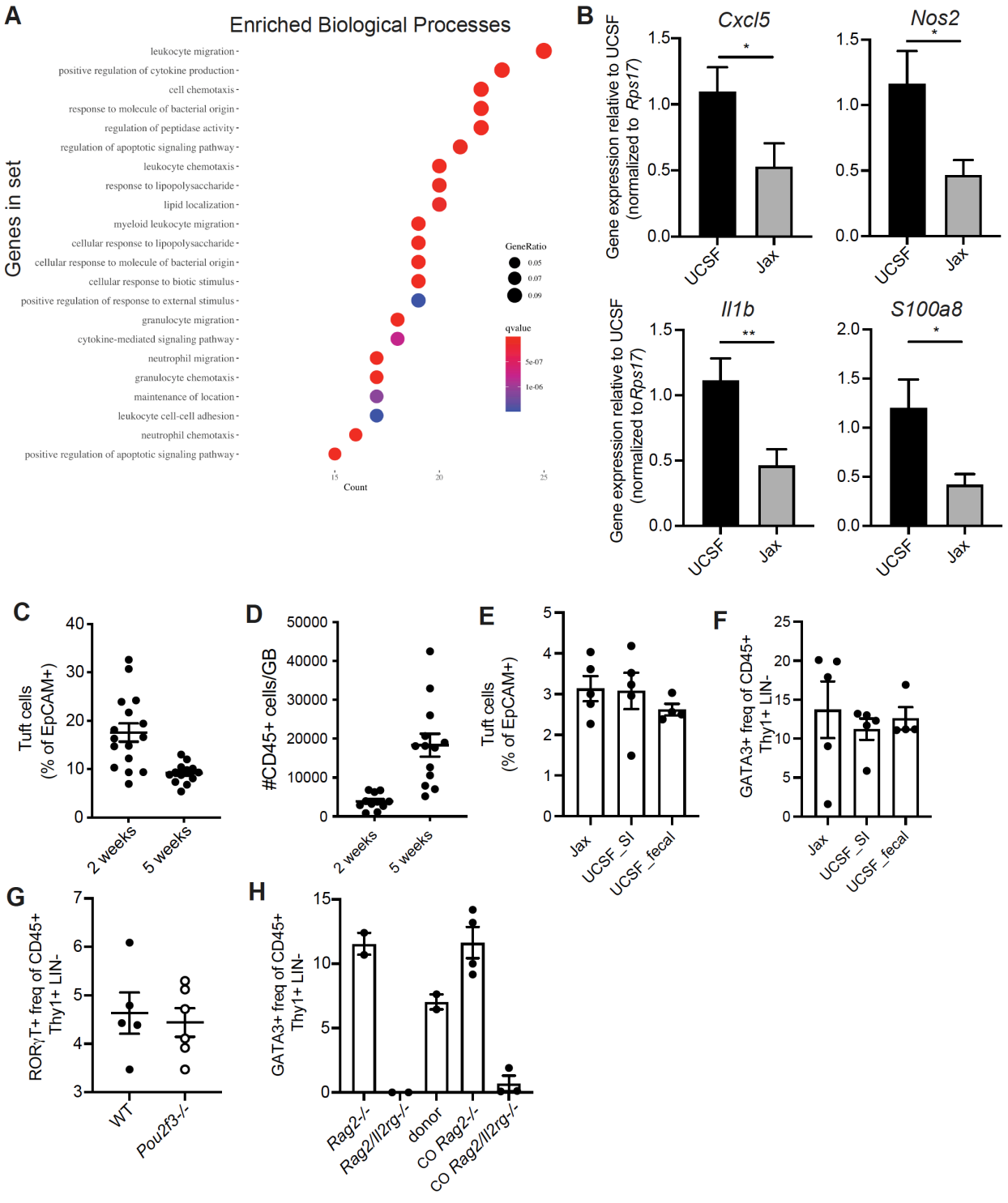

Fig. S6. The microbiome induces biliary neutrophil influx. A) Gene ontology biological processes enrichment analysis on genes upregulated in cluster 2 "inflammatory" epithelial cells from *Pou2f3-/-* mice compared to controls, from single cell sequencing analysis. **B**) Total

GB/EHBD from UCSF-raised WT mice (n=10) and Jax mice (9) was analyzed by qPCR for the indicated target genes relative to the housekeeping gene Rps17. Jax RQ normalized to average of WT mice. Statistical significance determined by unpaired student's T-test; *p<.05, **p<.01. **C,D**) Flare25 mice were analyzed at 2 wks of age and 5 wks of age for tuft cell frequency (**C**) and CD45+ cell count (**D**) by flow cytometry on total GB/EHBD digests. Mice were taken from three litters, with littermates analyzed across the two time-points. **E,F**) Jax mice received fecal or small intestinal contents from UCSF donor mice by oral gavage and were analyzed 6 wks later by flow cytometry on total GB/EHBDs for tuft cells (**E**) or ILC2s (**F**) as identified by intracellular staining. **G**) Innate lymphocytes from non-littermate WT and *Pou2f3-/-* were examined by flow cytometry on total GB/EHBDs for presence of RORγT by intracellular staining. **H**) Rag2-/- or Rag2/Il2rg-/- mice from Taconic were cohoused with UCSF donor mice for 6 wks. ILC2s were quantified by flow cytometry on total GB/EHBDs subjected to intracellular staining. All data shown with +/- SEM.