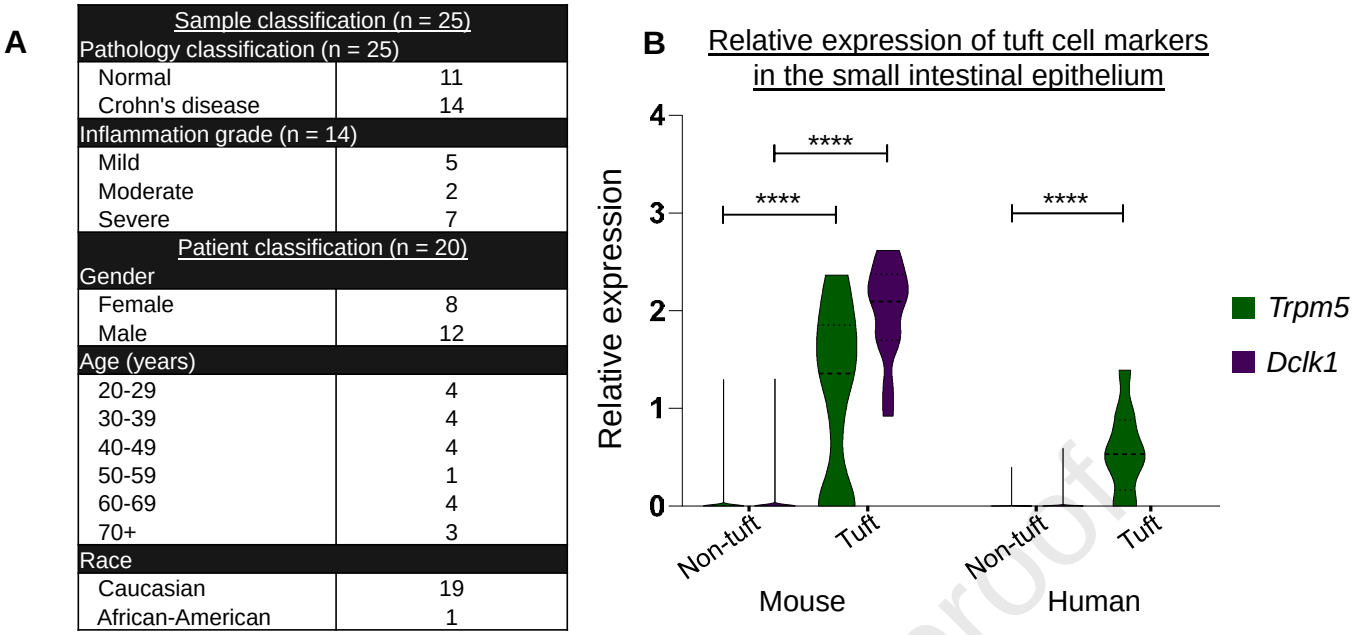
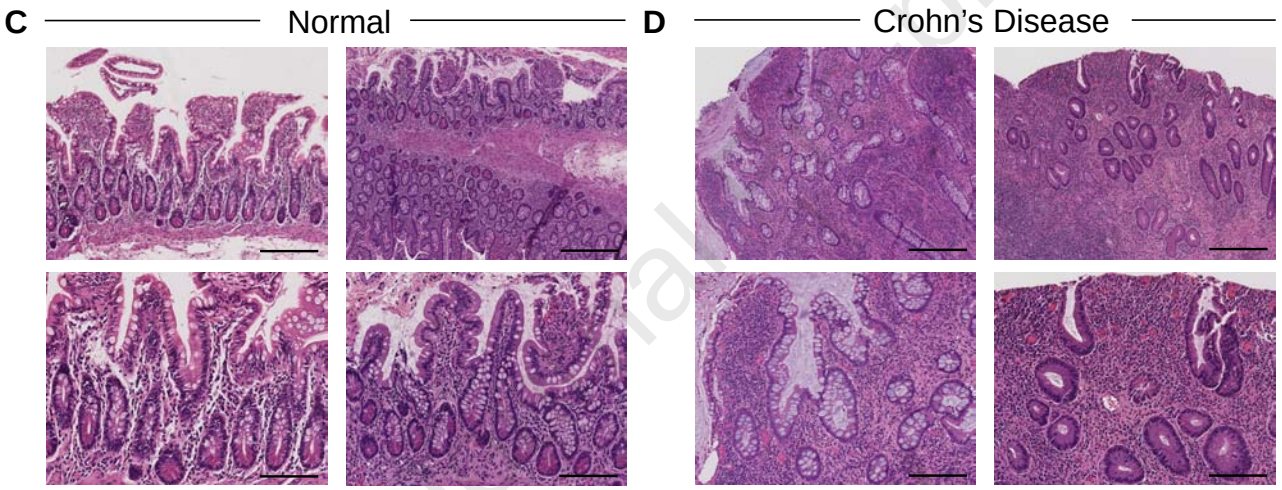
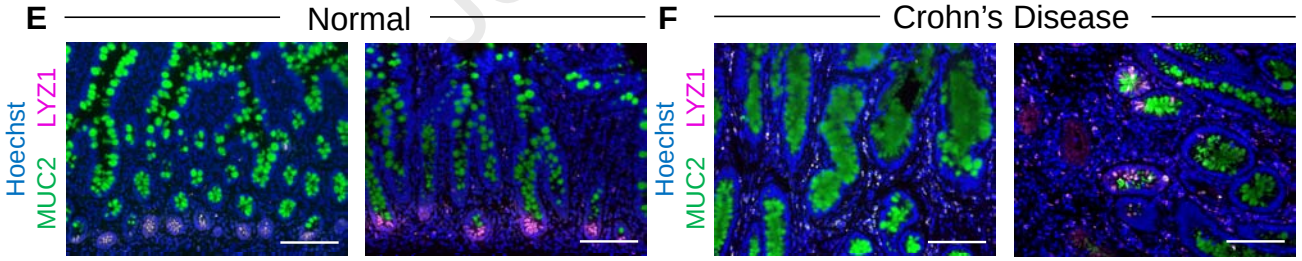
Supplementary Figure 1

A

Sample classification (n = 25)	
Pathology classification (n = 25)	
Normal	11
Crohn's disease	14
Inflammation grade (n = 14)	
Mild	5
Moderate	2
Severe	7
Patient classification (n = 20)	
Gender	
Female	8
Male	12
Age (years)	
20-29	4
30-39	4
40-49	4
50-59	1
60-69	4
70+	3
Race	
Caucasian	19
African-American	1

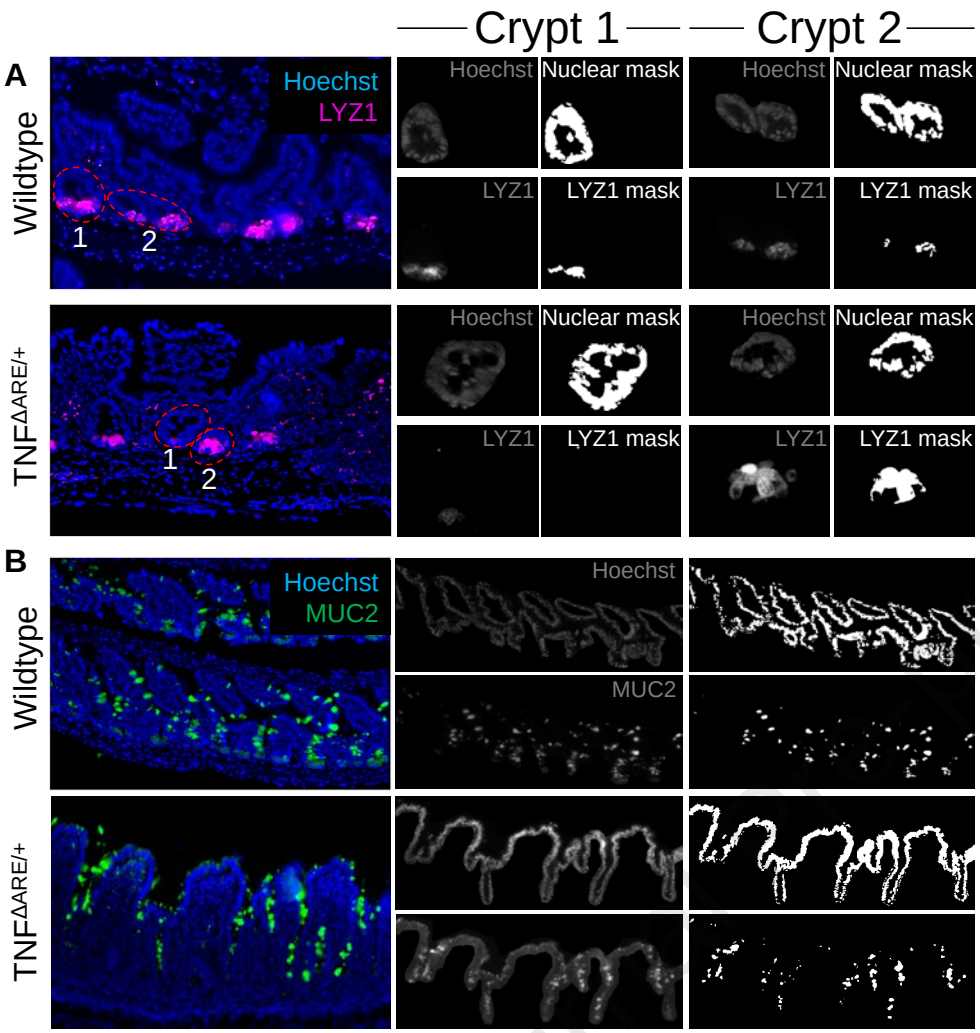






Supplementary Figure 1. (A) Sample and patient summary statistics from ileal surgical resections. **(B)** Relative gene expression of *Dclk1* (green) and *Trpm5* (magenta) in mouse and human non-tuft (n = 1166 and 1942 cells, respectively) and tuft cell (n = 13 and 9 cells, respectively) populations. ****p-value < 0.0001. **(C-D)** Representative histology of normal and CD ileum. Scale bar = 100 µm. **(E-F)** MUC2 (green) and LYZ (magenta) in normal and CD ileum. Hoechst (blue) denotes nuclei, scale bar = 100 µm.

Supplementary Figure 2



C

Category	Number of Animals Included in Quantification	Inflammation Classification	Number of Total Villi Counted per Category
Wildtype	N = 5	Normal	146
TNF$^{\Delta ARE/+}$	N = 6	Normal/Low-grade	11
		Mid-grade	125
		High-grade	62

D

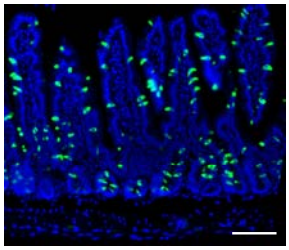
Category	Number of Animals Included in Quantification	Number of Total Villi Counted per Category	Number of Randomly Sampled Villi per Category
TNF$^{\Delta ARE/+}$ - low inflammation	N = 8	118	40
TNF$^{\Delta ARE/+}$ - high inflammation		40	40

Supplementary Figure 2. (A) Epithelial nuclear and LYZ1 masks in wildtype and TNF$^{\Delta ARE/+}$ crypts. LYZ1 expression was normalized to total nuclear area, scale bar = 100 µm. **(B)** Epithelial nuclear and MUC2 masks in wildtype and TNF$^{\Delta ARE/+}$ villi. MUC2 expression was normalized to total nuclear area, scale bar = 100 µm. **(C)** Summary of tuft cell quantification in wildtype and TNF$^{\Delta ARE/+}$ (low-, mid-, and high-grade inflammation) villi. **(D)** Summary of tuft cell quantification in MPO-low and –high TNF$^{\Delta ARE/+}$ villi. **(E)** MPO (magenta, red dots) and DCLK1 (green, white arrows) in TNF$^{\Delta ARE/+}$. Scale bar = 100 µm. **(F)** Tuft cell number per villi in MPO-low (< 35 neutrophils) and –high (≥ 35 neutrophils) TNF$^{\Delta ARE/+}$ villi. SEM for n = 40 villi per condition (n = 6 wildtype, n = 8 TNF$^{\Delta ARE/+}$ animals), **p-value < 0.01.

Supplementary Figure 3

AtohKO

A



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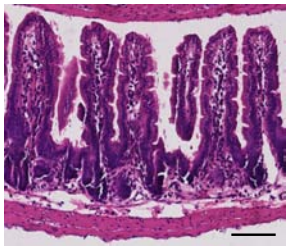
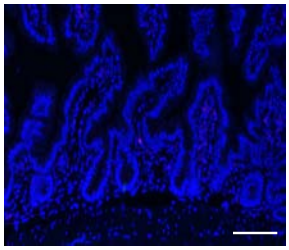


C



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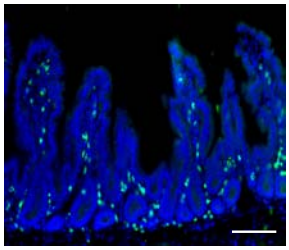

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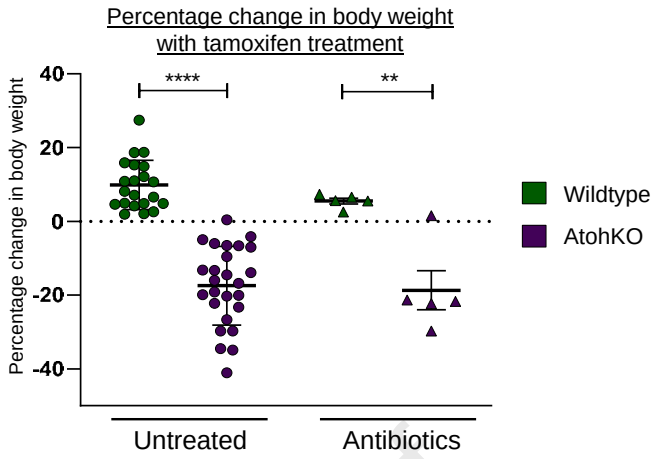
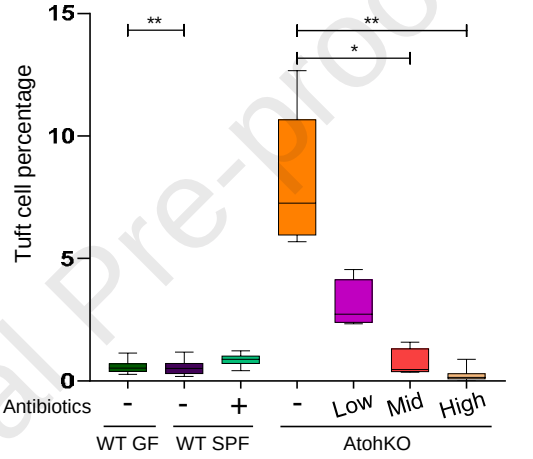


F

Colony Testing (n = 5)

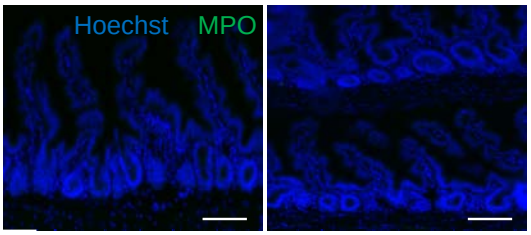
Pathogen	Result
Aspiculuris tetraptera	Negative
Helicobacter bilis	Negative
Helicobacter ganmani	Negative
Helicobacter hepaticus	Negative
Helicobacter mastomyrinus	Negative
Helicobacter rodentium	Negative
Helicobacter spp.	Negative
Helicobacter typhinoius	Negative
Murine Norovirus	Negative
Mycoptes	Negative
Radfordia/Myobia	Negative
Syphacia muris	Negative
Syphacia obvelata	Negative
Tritrichomonas muris	Negative
Parasite	**Result**
Helminth ova	Negative
Coccidial oocysts	Negative

G



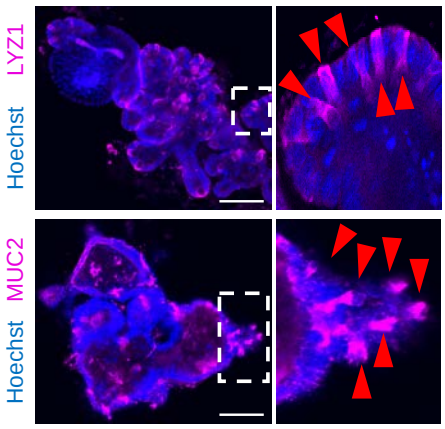
H

AtohKO + Antibiotics



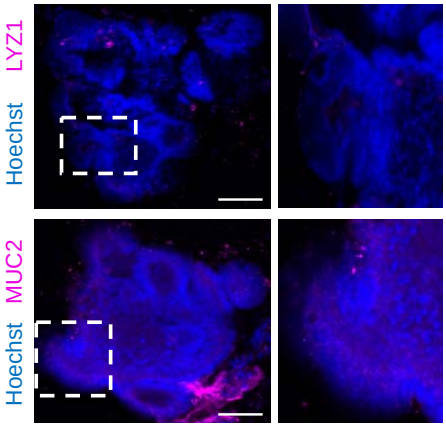
I

Vehicle

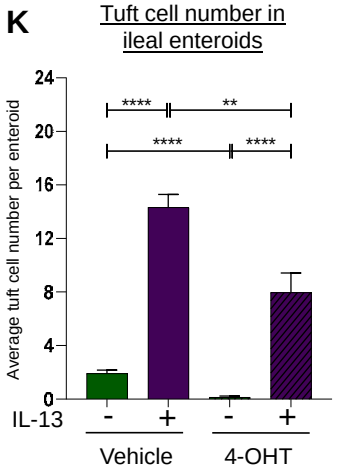


J

4-OHT

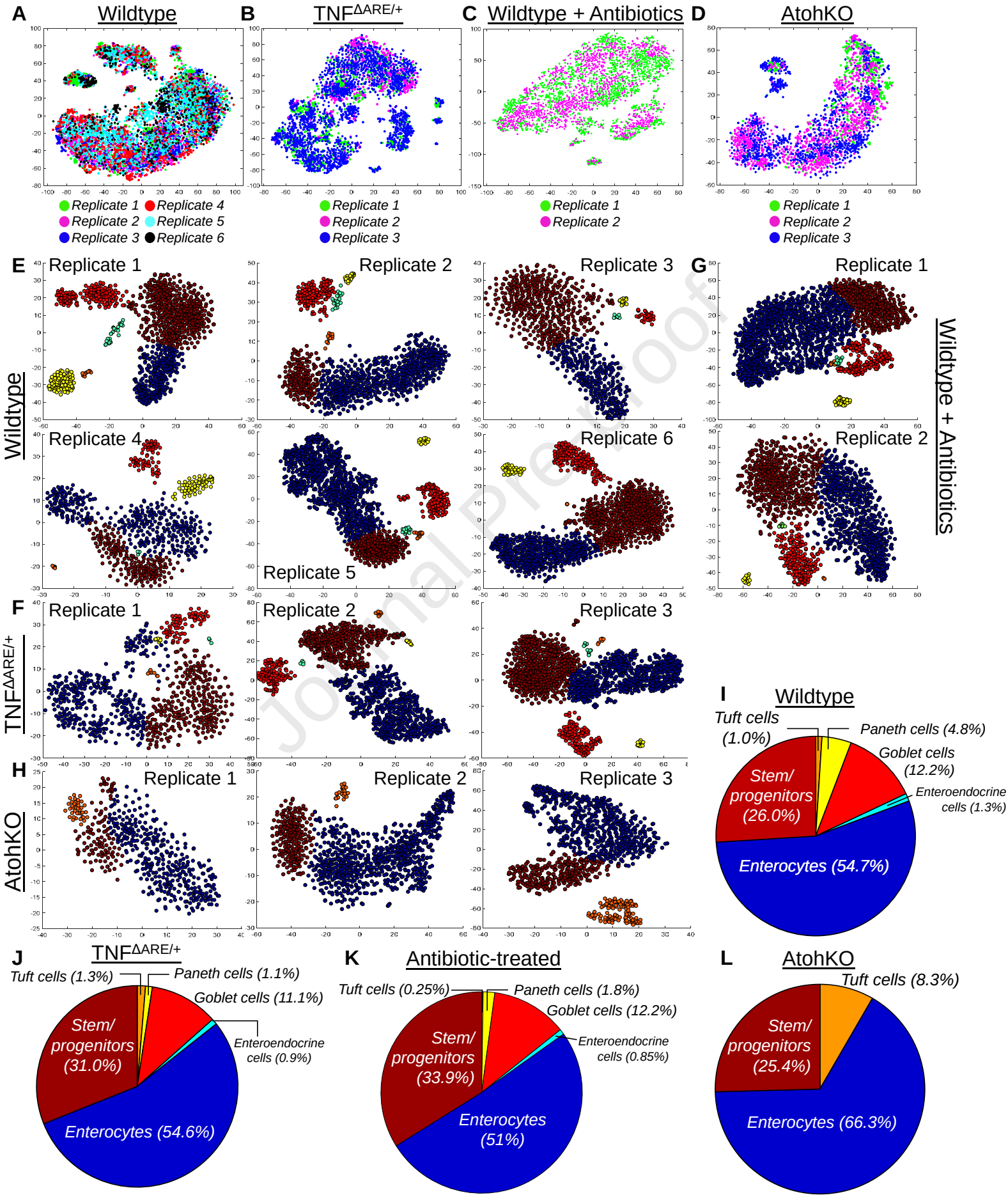


K



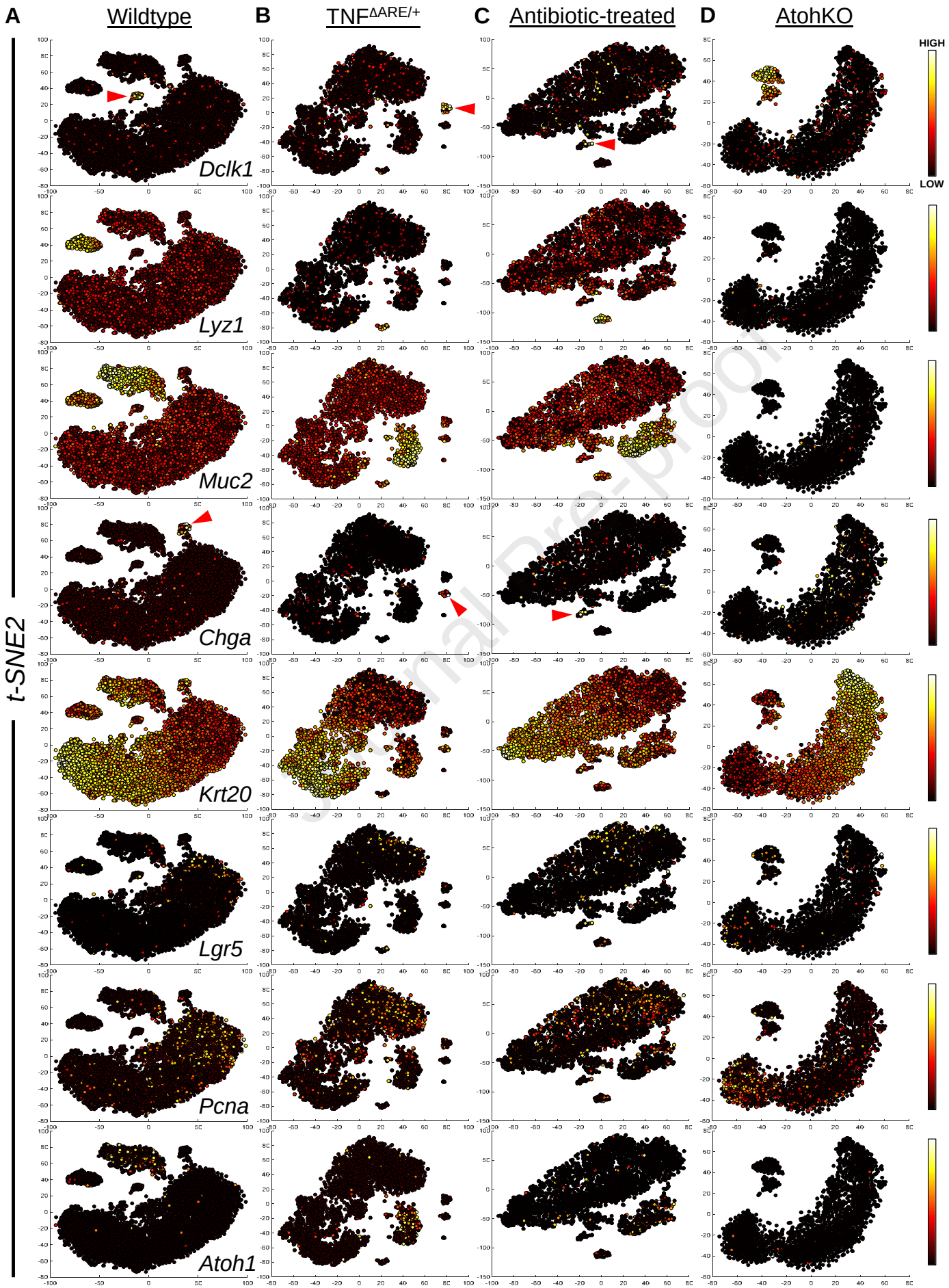
Supplementary Figure 3. (A-D) Representative images of DCLK1 (green), histology, MUC2 (green) and LYZ1 (magenta), and MPO (green) in the AtohKO ileum. Hoechst (blue) and scale bar = 100 µm. **(E)** Percentage body weight change in tamoxifen and antibiotic treatment. Symbols represent individuals animals and error bars represent SEM calculated from untreated wildtype (n = 21), untreated AtohKO (n = 26), antibiotic-treated wildtype (n = 5), and antibiotic-treated AtohKO (n = 5) animals, ****p-value < 0.0001, **p-value < 0.01 by t-test. **(F)** Summary of parasite testing (n = 5 mice). **(G)** Normalized tuft cell count in germ-free (n = 4), untreated (n = 3) and antibiotic-treated (n = 4) wildtype, untreated AtohKO (n = 4) and low/mid (n = 2), and high (n = 3) antibiotic-treated AtohKO ileum, *p-value < 0.05, **p-value < 0.01. **(H)** Representative IF of MPO (green) in antibiotic-treated AtohKO ileum, scale bar = 100 µm. **(I-J)** Representative IF of LYZ1+ (magenta) and MUC2+ (magenta) in vehicle- and 4-OHT-treated enteroids. Hoechst (blue) denotes nuclei, scale bar = 50 µm. **(K)** Average tuft cell number in vehicle-treated control (green) and IL-13 (magenta) and 4-OHT-treated control (green, striped) and IL-13-treated (magenta, striped) enteroids. ****p-value < 0.0001, **p-value < 0.01.

Supplementary Figure 4



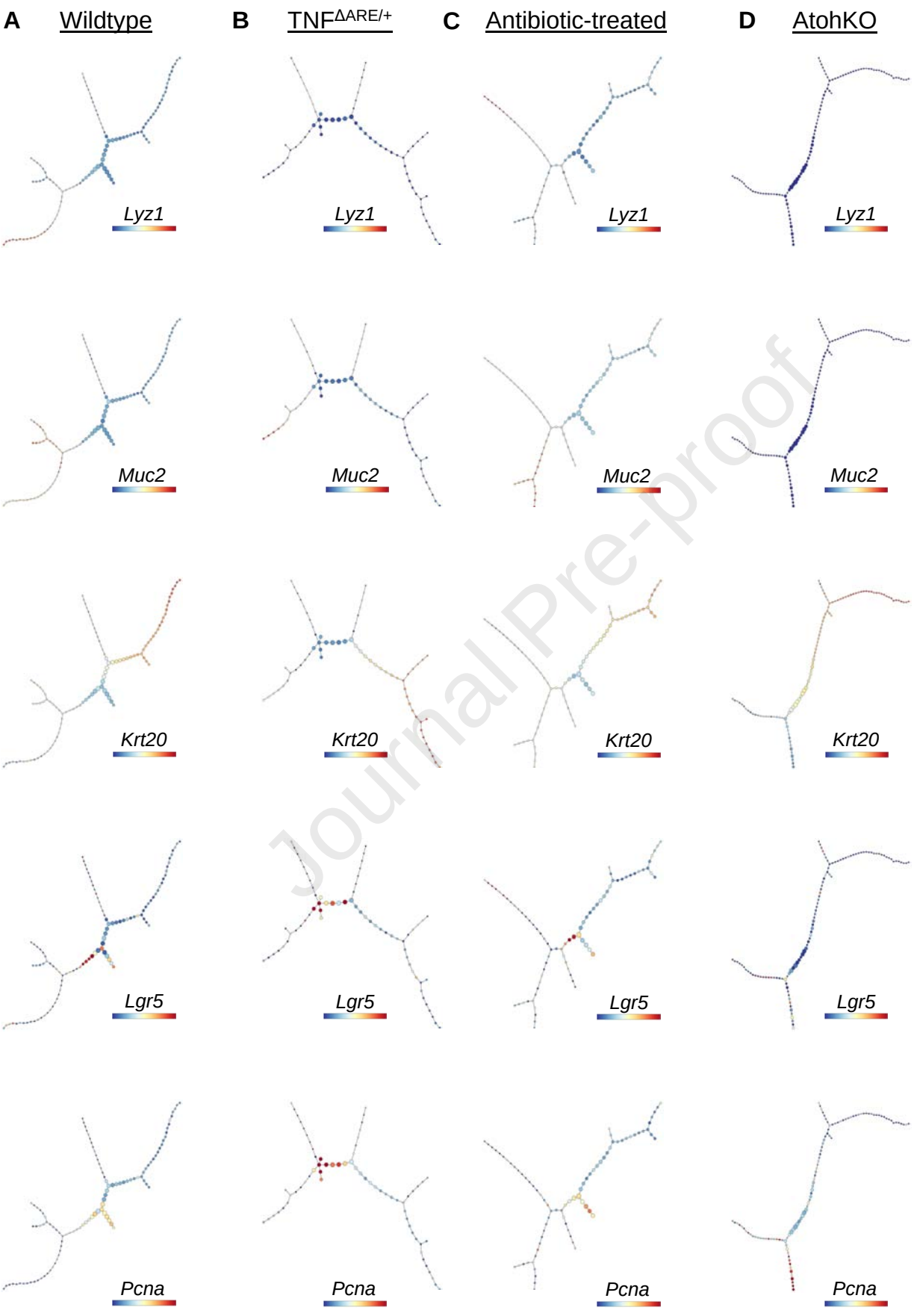
Supplementary Figure 4. (A-D) t-SNE and cell clustering of wildtype, TNF$^{\Delta ARE/+}$, antibiotic-treated wildtype, and AtohKO datasets across multiple replicates. **(E-H)** Cell population annotation in wildtype, TNF$^{\Delta ARE/+}$, antibiotic-treated wildtype, and AtohKO across biological replicates. **(I-L)** Cell population distribution in wildtype, TNF$^{\Delta ARE/+}$, antibiotic-treated wildtype, and AtohKO datasets.

Supplementary Figure 5



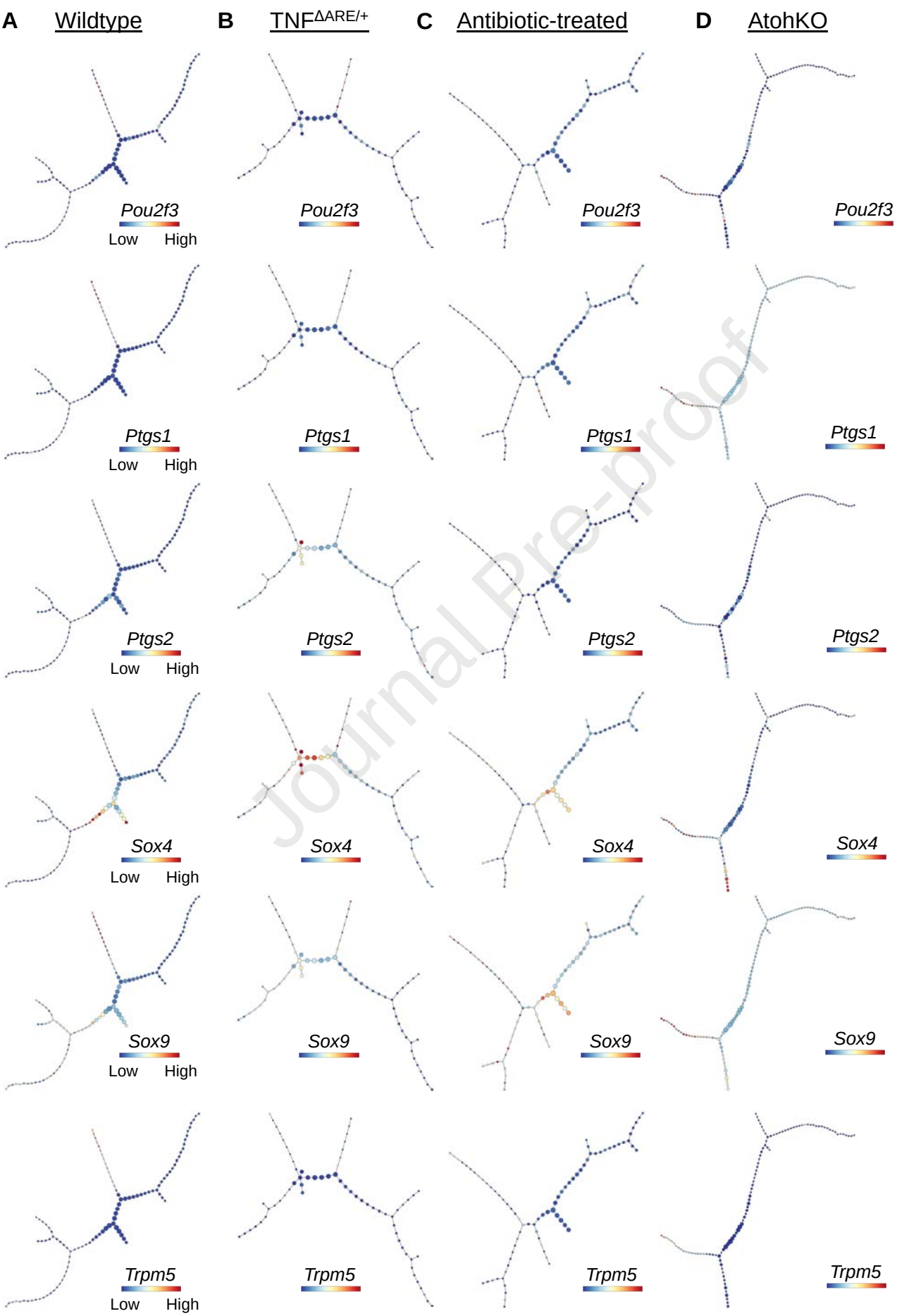
Supplementary Figure 5. (A-D) ArcSinh-scaled data overlay of cell type markers onto t-SNE plots.

Supplementary Figure 6



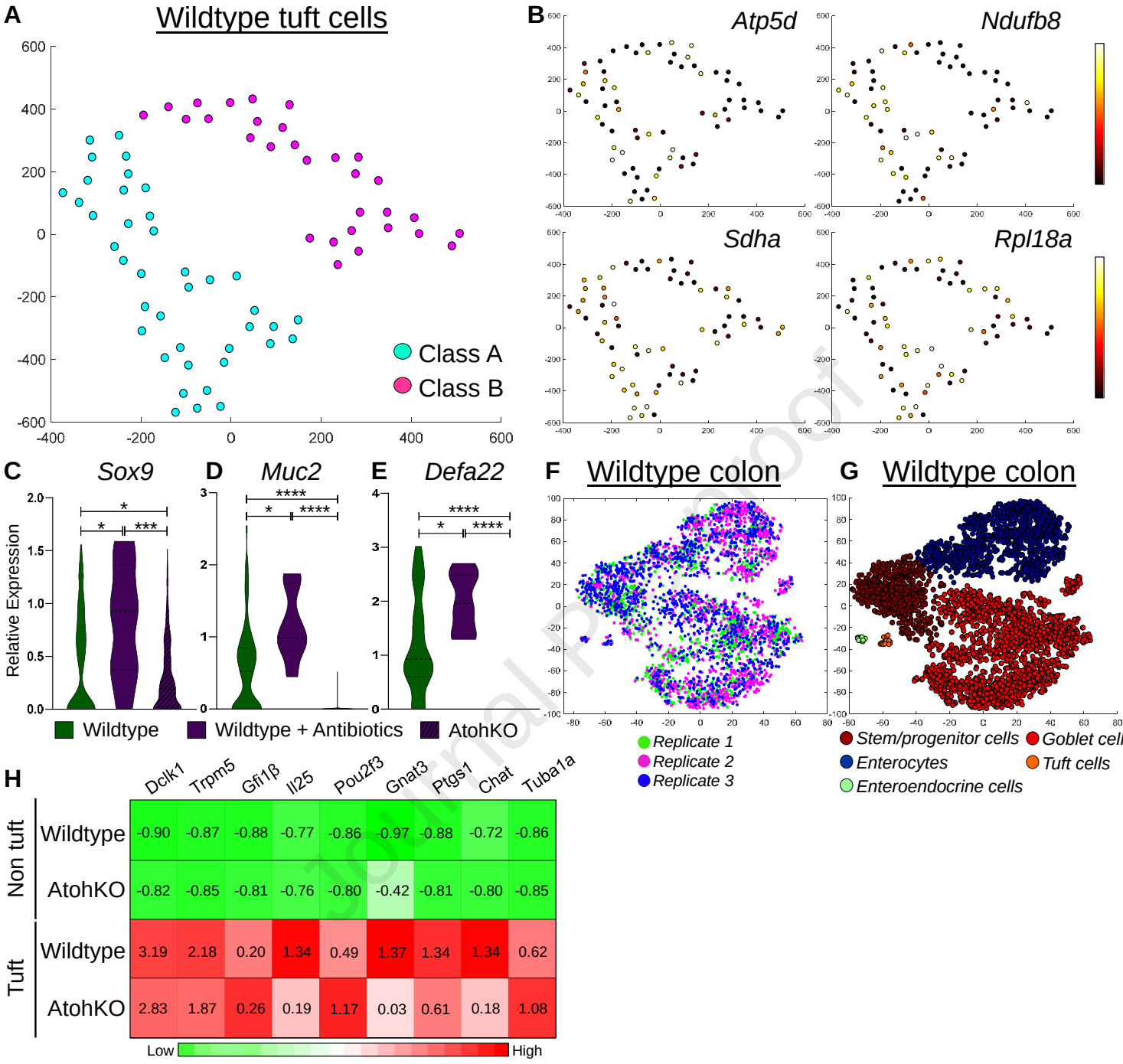
Supplementary Figure 6. (A-D) ArcSinh-scaled data overlay of cell identity genes onto p-Creode topologies.

Supplementary Figure 7



Supplementary Figure 7. (A-D) ArcSinh-scaled data overlay of tuft cell genes onto p-Creode topologies.

Supplementary Figure 8



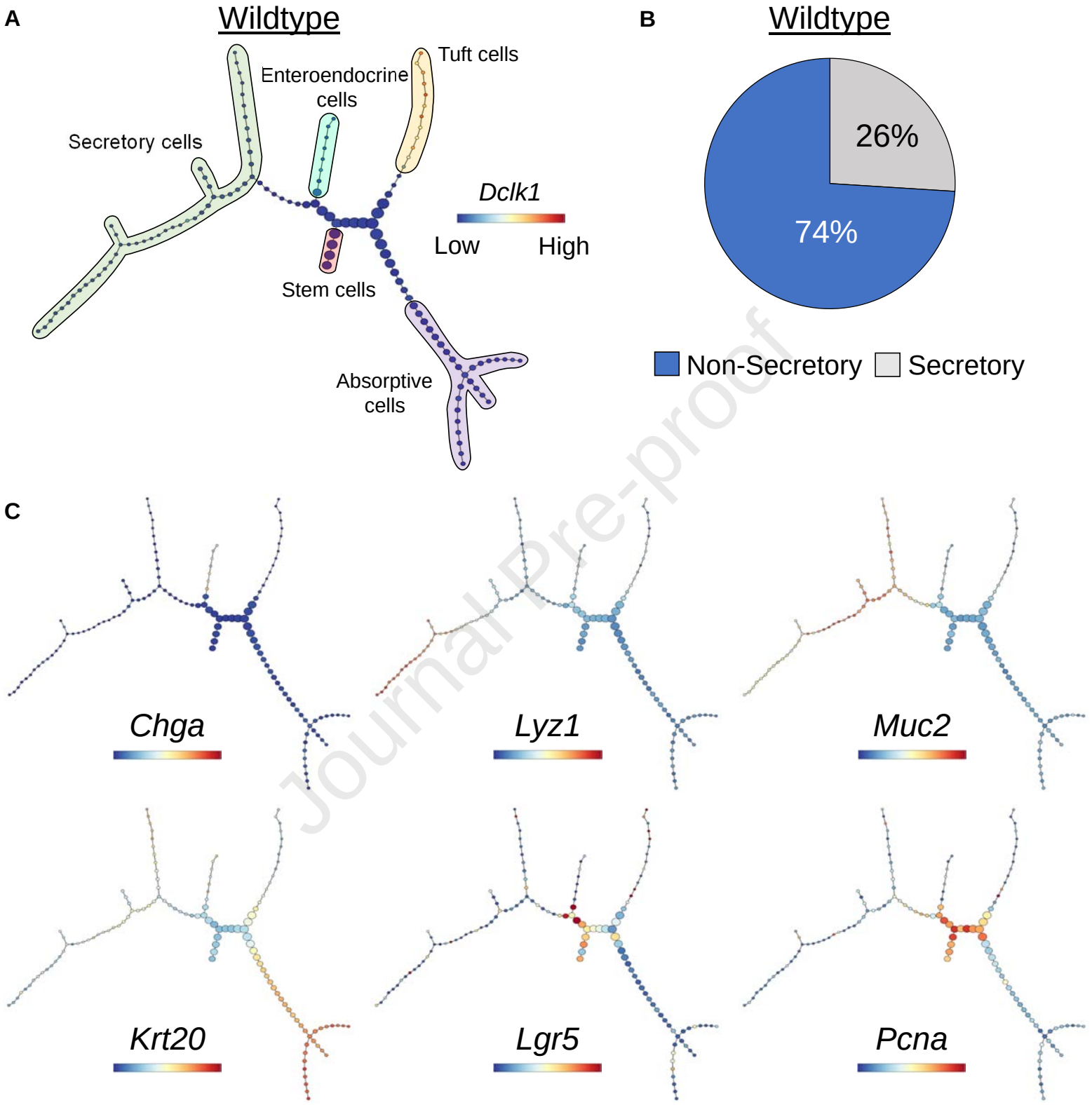
H

		Dclk1	Trpm5	Gfi1β	Il25	Pou2f3	Gnat3	Ptgs1	Chat	Tuba1a
Non tuft	Wildtype	-0.90	-0.87	-0.88	-0.77	-0.86	-0.97	-0.88	-0.72	-0.86
	AtohKO	-0.82	-0.85	-0.81	-0.76	-0.80	-0.42	-0.81	-0.80	-0.85
Tuft	Wildtype	3.19	2.18	0.20	1.34	0.49	1.37	1.34	1.34	0.62
	AtohKO	2.83	1.87	0.26	0.19	1.17	0.03	0.61	0.18	1.08

Low High

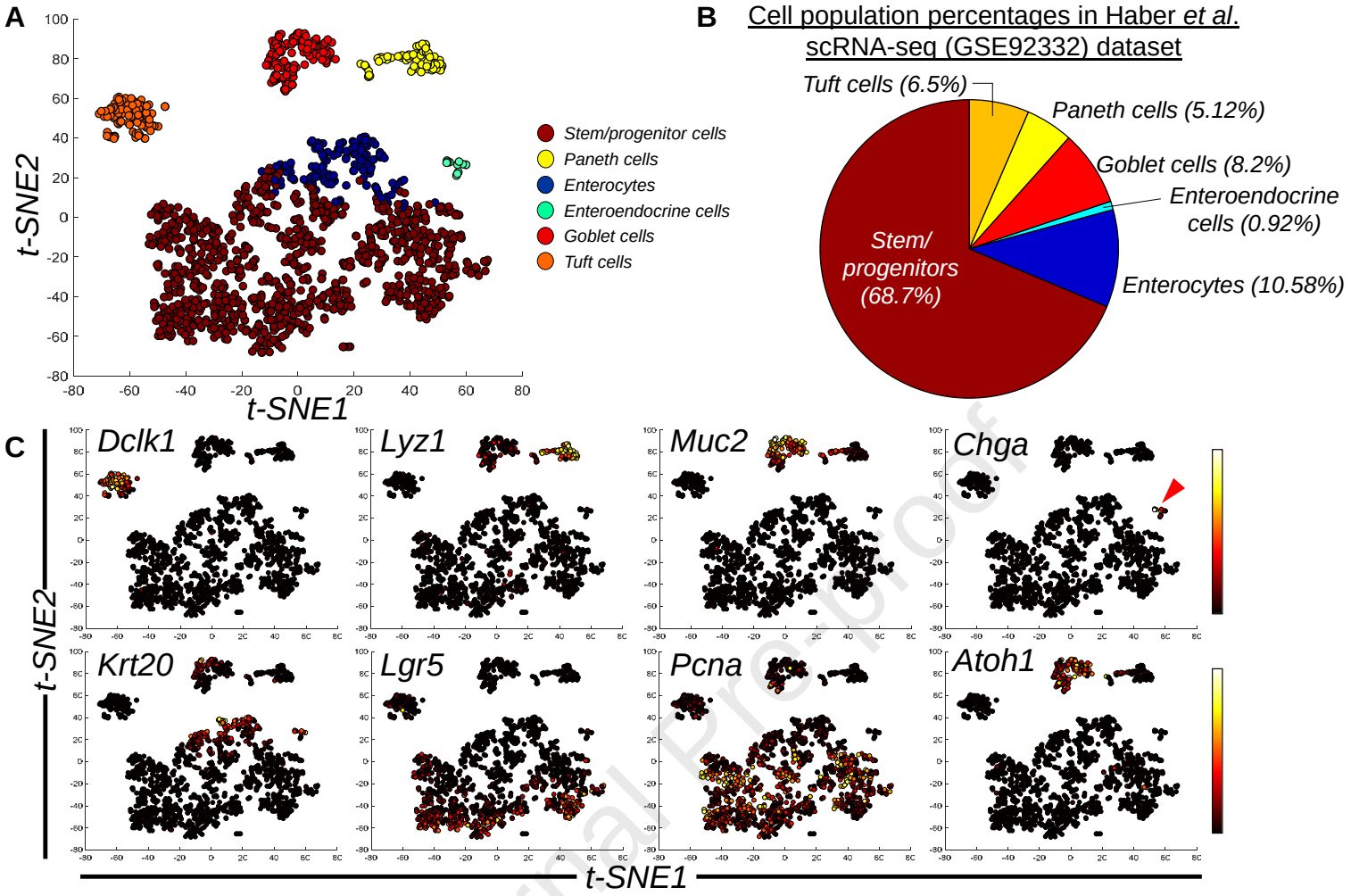
Supplementary Figure 8. (A) t-SNE and cell clustering of wildtype (non-antibiotic-treated) tuft cells with class A (teal) and B (magenta) subpopulations. **(B)** Overlay of ArcSinh-scaled gene expression of Class A-enriched genes onto t-SNE plot of wildtype tuft cells. **(C)** *Sox9,* **(D)** *Muc2,* and **(E)** *Defa22* relative expression in untreated wildtype (green), antibiotic-treated wildtype (magenta), and AtohKO (magenta, striped) tuft cells, *p-value < 0.05, ***p-value < 0.001, ****p-value < 0.0001 by t-test. **(F)** Wildtype colon scRNA-seq replicates (n = 3) and **(G)** annotation of cell identity clusters onto t-SNE plot. **(H)** Heatmap of z-score normalized tuft cell signature expression in wildtype and AtohKO tuft and non-tuft cell populations. All values are statistically significant between tuft and non-tuft cells, ***p-value < 0.001.

Supplementary Figure 9



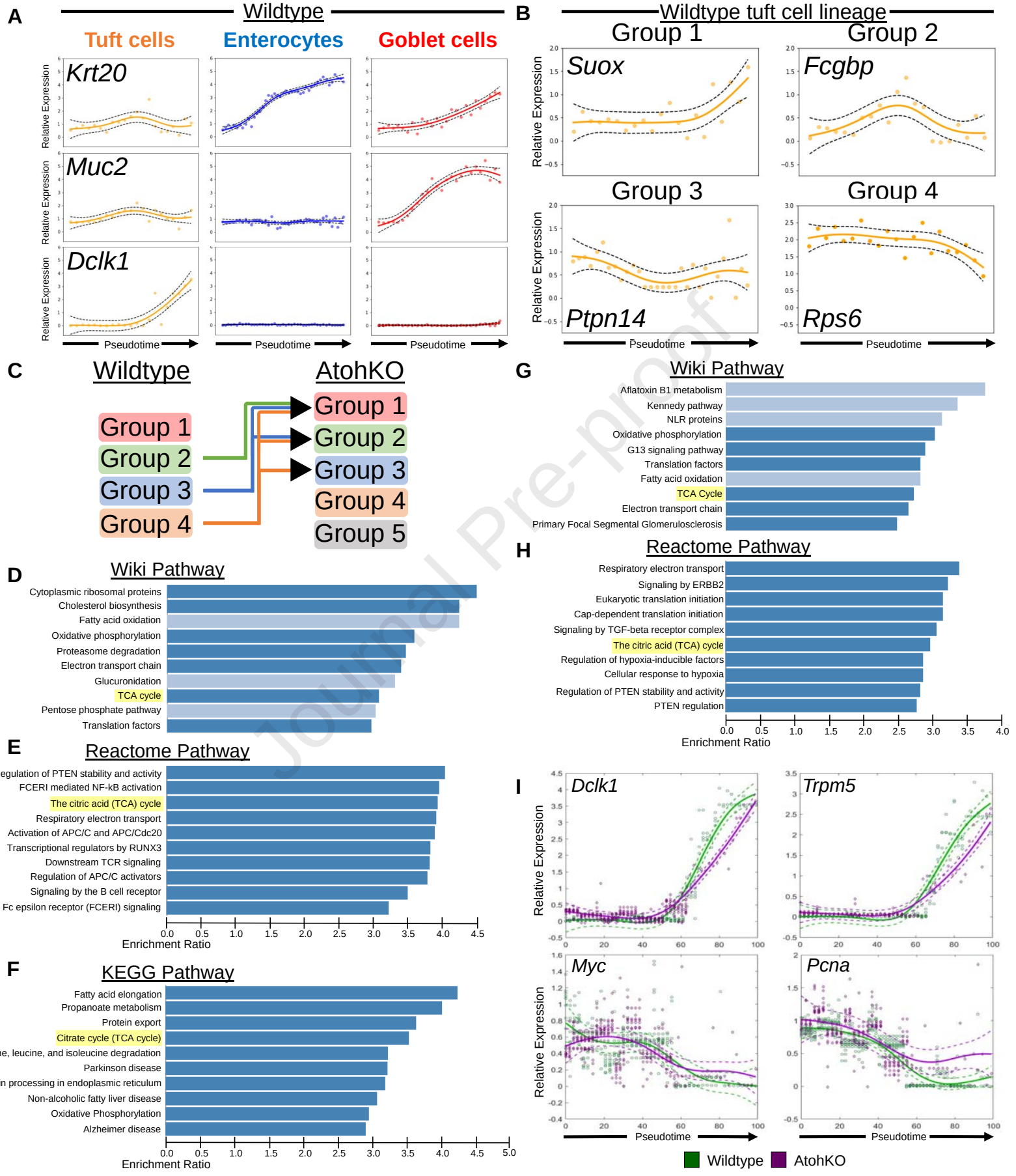
Supplementary Figure 9. (A) Representative p-Creode topology of wildtype scRNA-seq dataset, including enteroendocrine cells. Graph overlay depicts ArcSinh-scaled *Dclk1* gene expression and annotation of cell lineages. Node size represents cell state density and edges represent possible transitions. **(B)** Quantification of non-secretory and secretory tuft cell placement in wildtype topologies. **(C)** Overlay of ArcSinh-scaled gene expression onto the topology.

Supplementary Figure 10



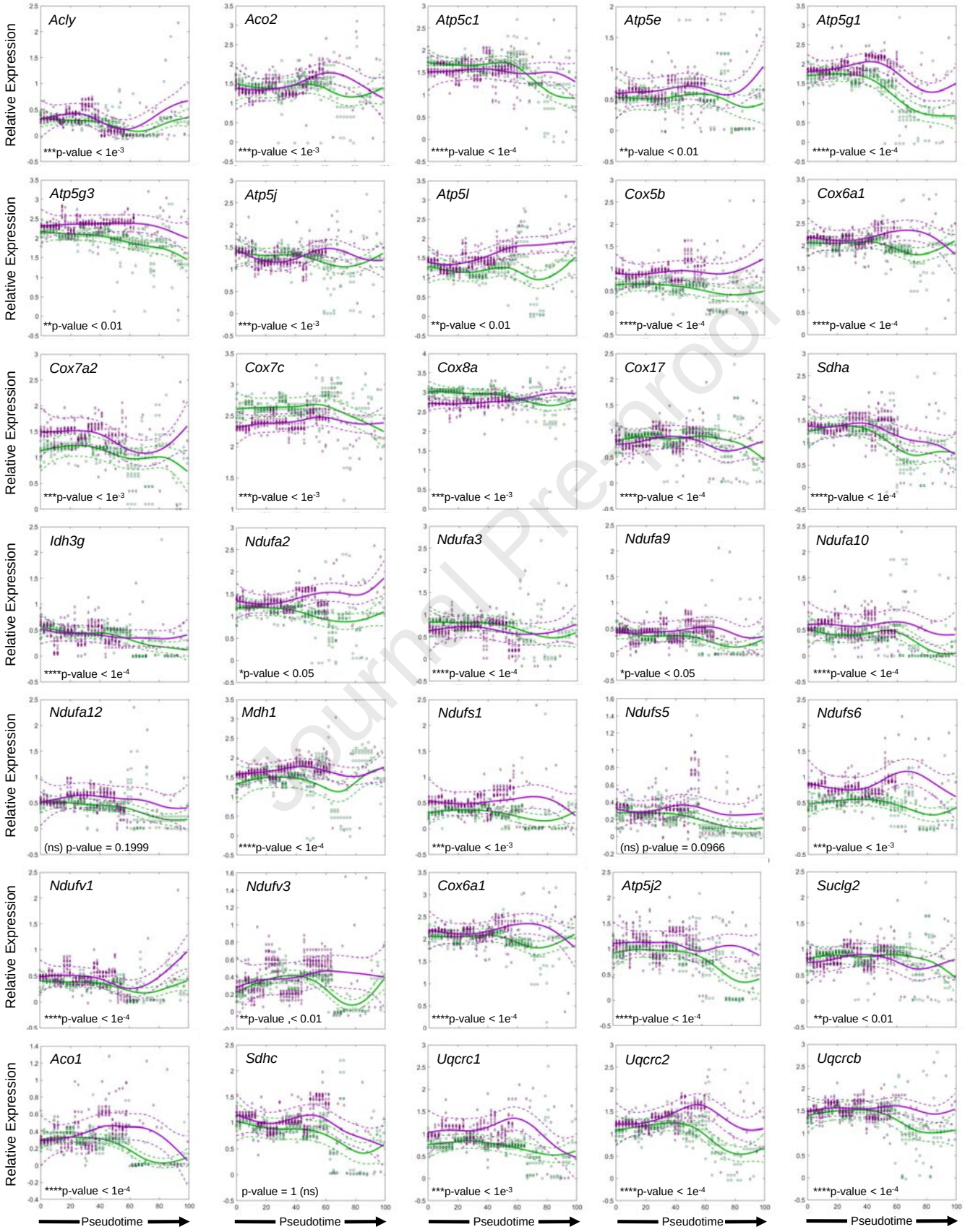
Supplementary Figure 10. (A-B) Cell population annotation and distribution in the 1,522 cell-dataset GSE92332 scRNA-seq dataset. **(C)** Overlay of *Dclk1*, *Lyz1*, *Muc2*, *Chga*, *Krt20*, *Lgr5*, *Pcna*, and *Atoh1* ArcSinh-scaled data. **(D)** Overlay of *Dclk1* ArcSinh-scaled expression on GSE92332 p-Creode topology and annotation of cell lineages. **(E)** Non-secretory and secretory tuft cell placement in p-Creode maps. **(F)** Overlay of ArcSinh-scaled gene expression onto the topology.

Supplementary Figure 11



Supplementary Figure 11. (A) ArcSinh-scaled gene expression of cell identity genes along the wildtype tuft cell, enterocyte, and goblet cell lineages. **(B)** Representative gene trends (solid orange line) across pseudotime from Groups 1-4. Solid circles depict raw data from single representative p-Creode graph and dashed black line represents the confidence interval for gene expression. **(C)** 1,755 genes class-switched between wildtype groups 2-4 to AtohKO groups 1-3. **(D-E)** Wiki and Reactome Pathways over-representation analysis of 5-trend dynamics. **(F-H)** KEGG, Wiki Pathway, and Reactome Pathways over-representation analysis of 3-trend dynamics. **(I)** Representative tuft cell-specific (*Dclk1* and *Trpm5*) and stem cell-specific (*Myc* and *Pcna*) gene trends over the wildtype (green) and AtohKO (magenta) tuft cell lineages. Solid lines represent mean expression trends and dashed lines represent confidence intervals fitted to raw data from 10 top-scoring p-Creode topologies. Datapoints are scaled expression data. Consensus alignment and significance testing was performed between conditions, *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, and ****p-value < 0.0001.

Supplementary Figure 12



Supplementary Figure 12. Representative TCA cycle-related genes over the wildtype (green) and AtohKO (magenta) tuft cell lineage pseudotime. Solid lines represent mean expression trends and dashed lines represent confidence intervals fitted to raw data from 10 top-scoring p-Creode topologies. Datapoints are scaled expression data. Consensus alignment and significance testing was performed between conditions, *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, and ****p-value < 0.0001.

Supplementary Figure 12

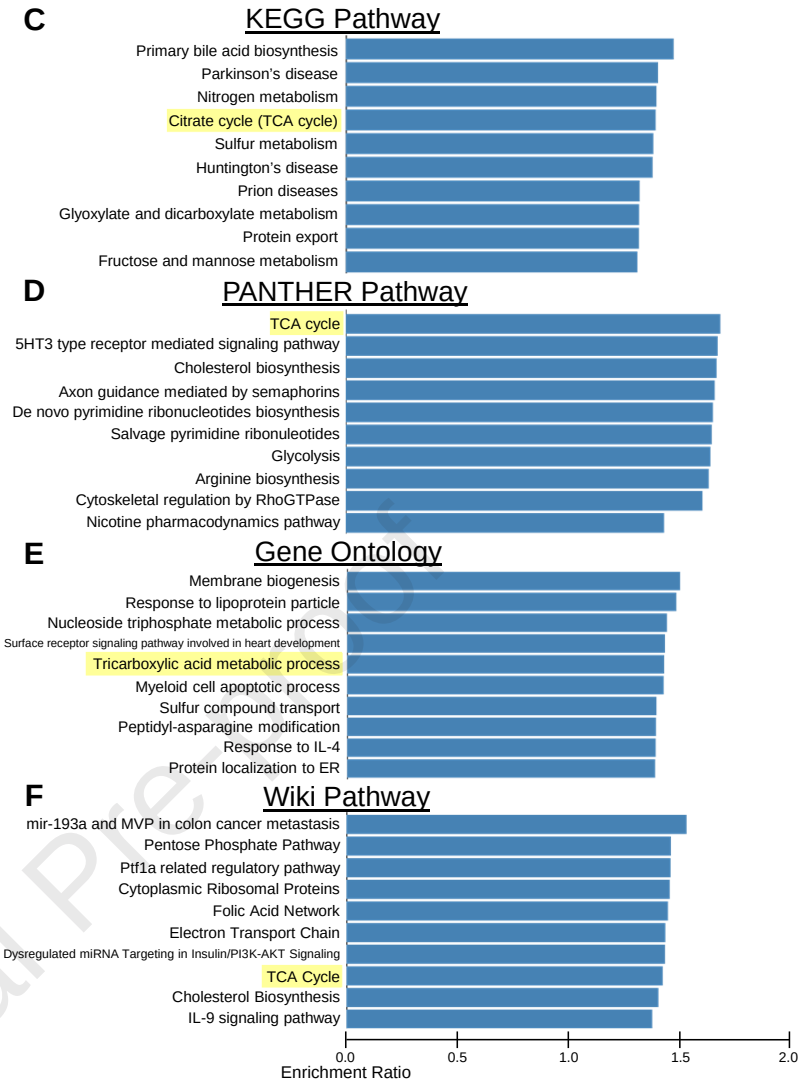
A

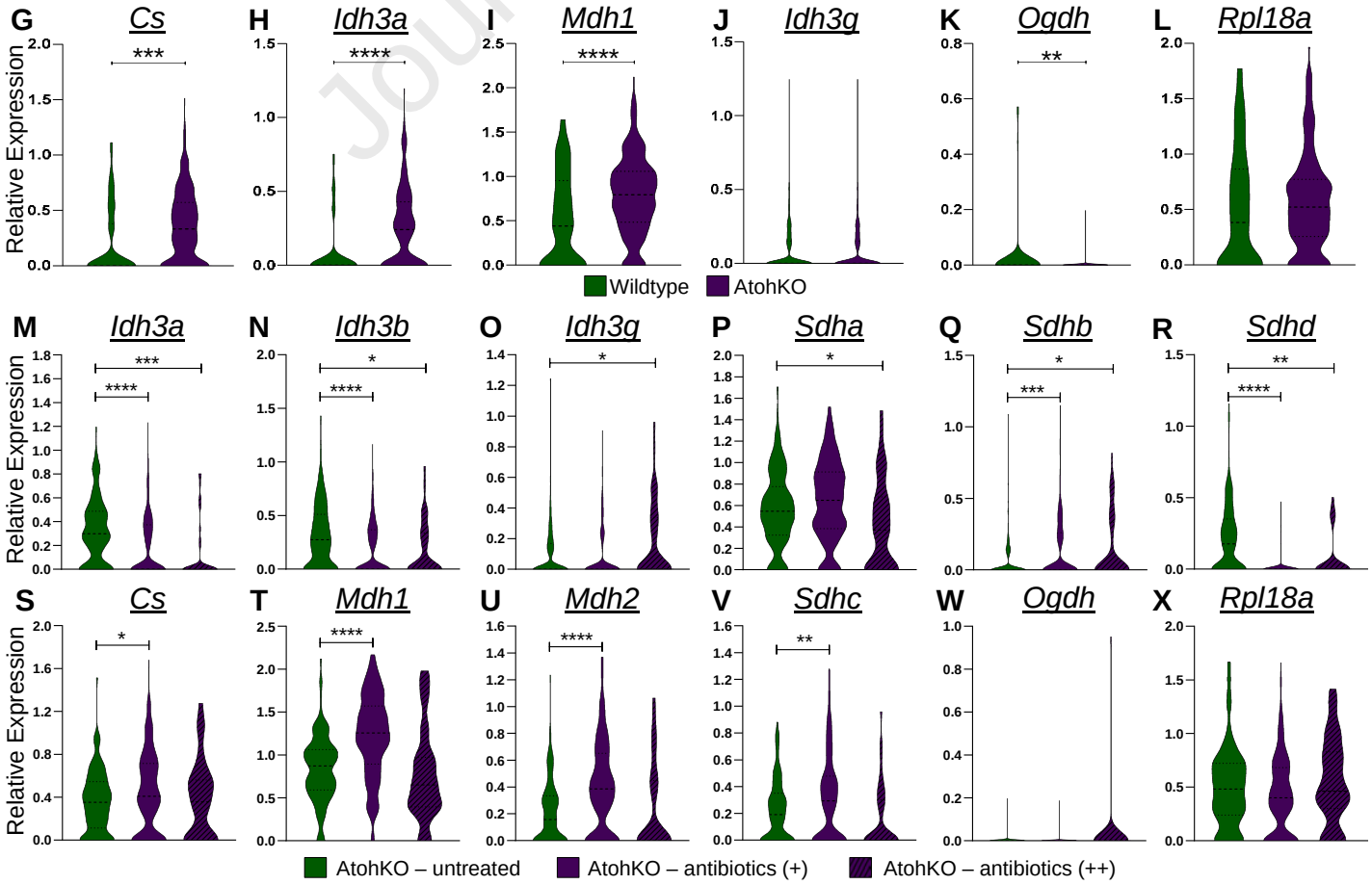
NAME	NES	NOM p-val
BARRIER_COLON_CANCER_RECURRENCE_DN	1.54	0.008
REACTOME_PEPTIDE_CHAIN_ELONGATION	1.54	0
SA_TRKA_RECEPTOR	1.54	0.039
BANDRES_RESPONSE_TO_CARMUSTIN_MGMT_24HR_DN	1.53	0.013
HAHTOLA_CTCL_CUTANEOUS	1.53	0.012
MOOTHA_TCA	1.52	0.011
HE_PTEN_TARGETS_UP	1.52	0.03
VERRECCHIA_RESPONSE_TO_TGFB1_C5	1.52	0.019
KEGG_RIBOSOME	1.52	0
REACTOME_NONSENSE_MEDIATED_DECAY_ENHANCED_BY_THE_EXON_JUNCTION_COMPLEX	1.52	0
WANG_BARRETTS_ESOPHAGUS_DN	1.52	0.018
REACTOME_INFLUENZA_LIFE_CYCLE	1.52	0
MOOTHA_GLYCOLYSIS	1.51	0.007
BUCKANOVICH_T_LYMPHOCYTE_HOMING_ON_TUMOR_DN	1.51	0.011
REACTOME_OXYGEN_DEPENDENT_PROLINE_HYDROXYLATION_OF_HYPOXIA_INDUCIBLE_FACTOR_ALPHA	1.51	0.023
CHEN_LUNG_CANCER_SURVIVAL	1.51	0.016
REACTOME_REGULATION_OF_HYPOXIA_INDUCIBLE_FACTOR_HIF_BY_OXYGEN	1.5	0.026
REACTOME_INTERACTION_BETWEEN_L1_AND_ANKYRINS	1.5	0.035
BIOCARTA_TFF_PATHWAY	1.5	0.009
REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE	1.5	0

B

Table 2: Top Genes from GSEA: Reactome_Citric_Acid_Cycle_TCA_Cycle

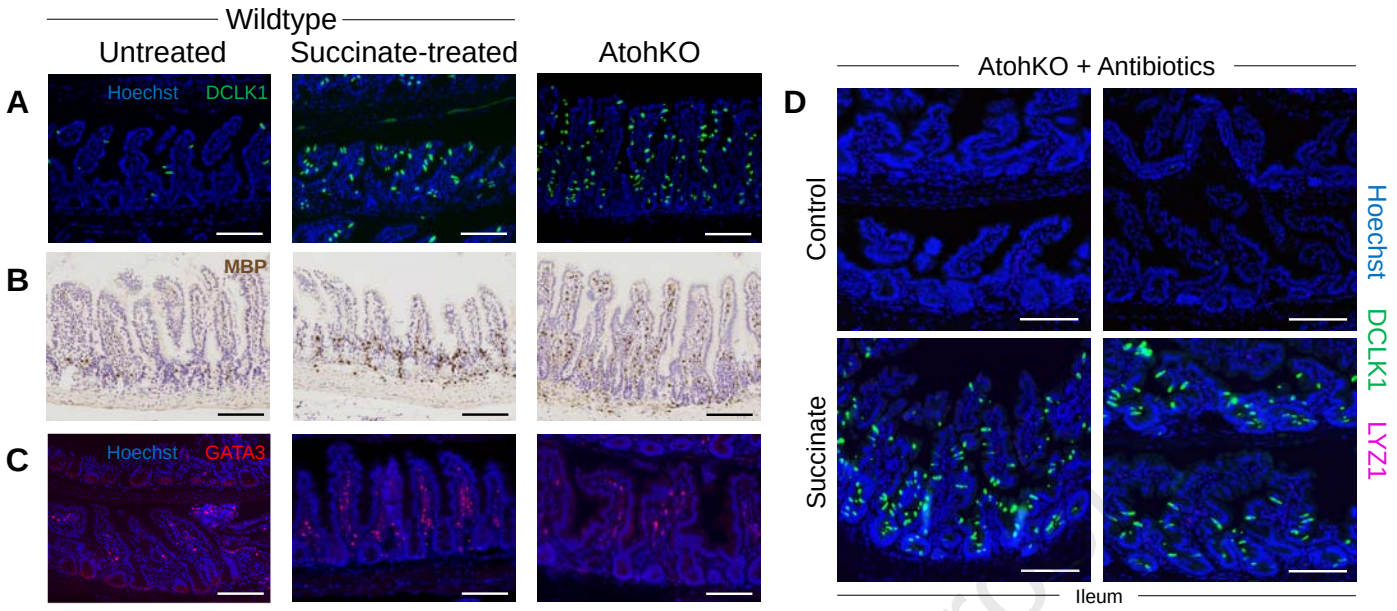
Rank in Gene List	Gene Name	Running ES	Core Enrichment
28	*Mdh2*	0.23	Yes
88	*Sdha*	0.42	Yes
164	*CS*	0.58	Yes
242	*Sdhc*	0.72	Yes
257	*Ogdh*	0.85	Yes
539	*Idh3b*	0.86	Yes
571	*Idh3g*	0.89	Yes
576	*Sdhd*	0.91	Yes
614	*Idh3a*	0.93	Yes





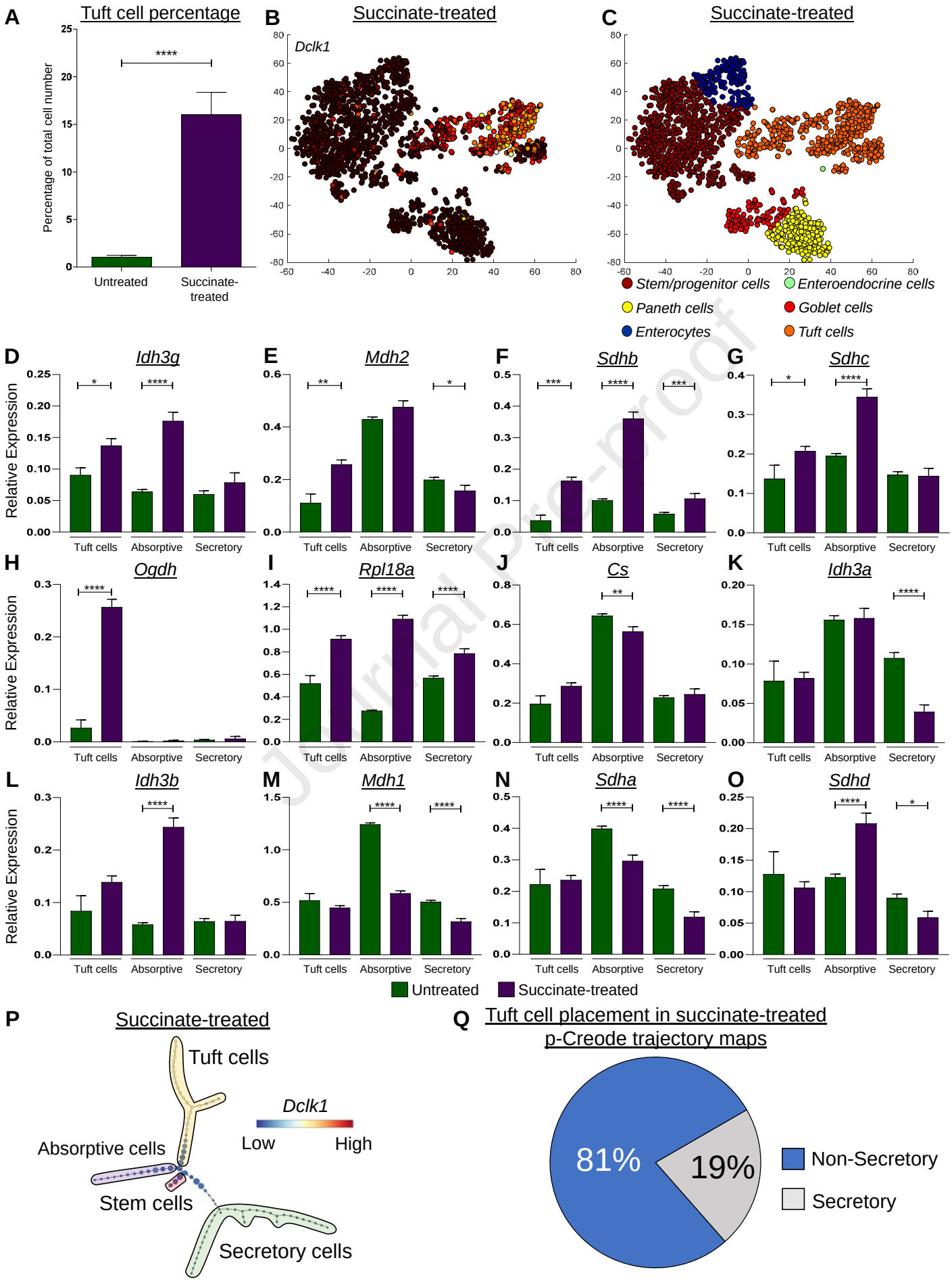
Supplementary Figure 13. (A) Top 20 GSEA gene sets comparing wildtype and AtohKO median tuft cell gene expression. Highlighted gene sets are related to the TCA cycle and metabolism pathways. **(B)** NES-ranked gene list from the metabolism-related gene list "Reactome Citric Acid Cycle TCA Cycle." Genes contributing to the running enrichment score (ES) are labeled "Yes" for Core Enrichment. **(C-F)** KEGG, PANTHER pathway, Gene Ontology, and Wiki Pathway analysis of wildtype and AtohKO tuft cell gene expression. **(G-L)** Scaled TCA cycle gene expression in wildtype (green) and AtohKO (magenta) tuft cells. **p-value < 0.01, ***p-value < 0.001, and ****p-value < 0.0001 by t-test. **(M-X)** Scaled TCA cycle gene expression in untreated (green), low-dose antibiotic- (magenta, solid), and high-dose antibiotic-treated (magenta, dashed) AtohKO tuft cells. *p-value < 0.05, **p-value < 0.01, and ****p-value < 0.0001 by t-test.

Supplementary Figure 14



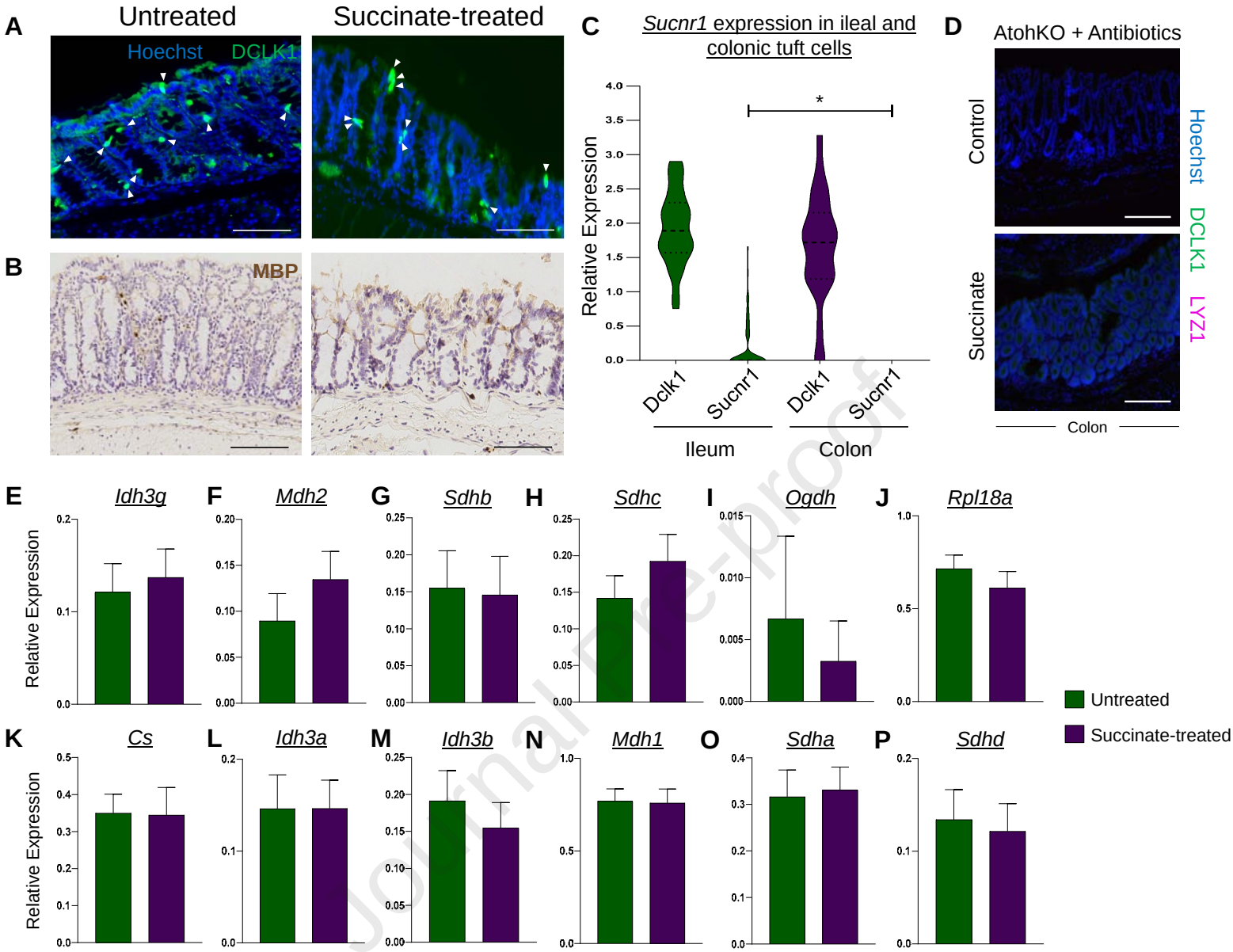
Supplementary Figure 14. (A-C) Representative imaging of DCLK1 (green), Major basic protein (brown), and GATA3 (red) in wildtype untreated, wildtype succinate-treated, and AtohKO small intestine. Scale bar = 100 μm. **(D)** Representative IF of DCLK1 (green) and LYZ1 (magenta) in antibiotic-treated AtohKO ileum with and without exogenous succinate administration. Hoechst denotes nuclei. Scale bar = 100 μm.

Supplementary Figure 15



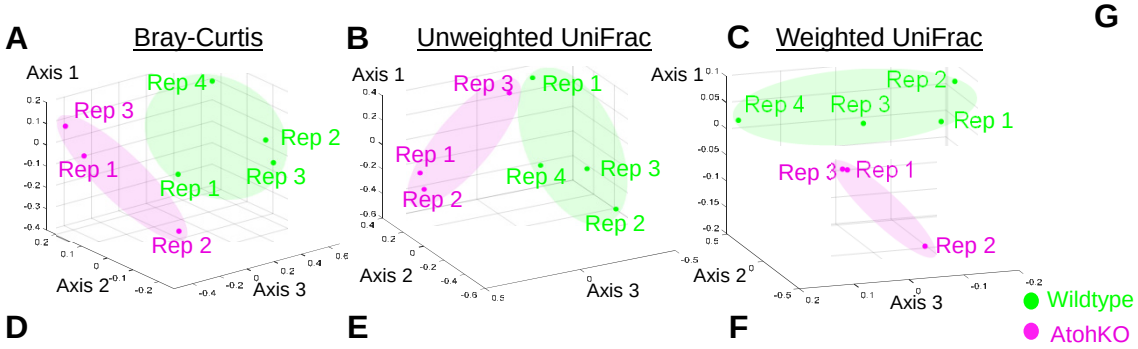
Supplementary Figure 15. (A) Tuft cell percentage in untreated and succinate-treated wildtype scRNA-seq datasets. ****p-value < 0.0001 by t-test. **(B)** ArcSinh-scaled *Dclk1* expression and **(C)** cell population annotation of succinate-treated ileal data. **(D-O)** Lineage-specific TCA cycle gene expression. *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, and ****p-value < 0.0001 by t-test. **(P)** p-Creode topology of succinate-treated ileum. Graph depicts *Dclk1* overlay and annotation of cell lineages based on ArcSinh-scaled gene expression data. **(Q)** Quantification of non-secretory (blue) and secretory (grey) tuft cell placement in n = 100 succinate-treated wildtype p-Creode topologies.

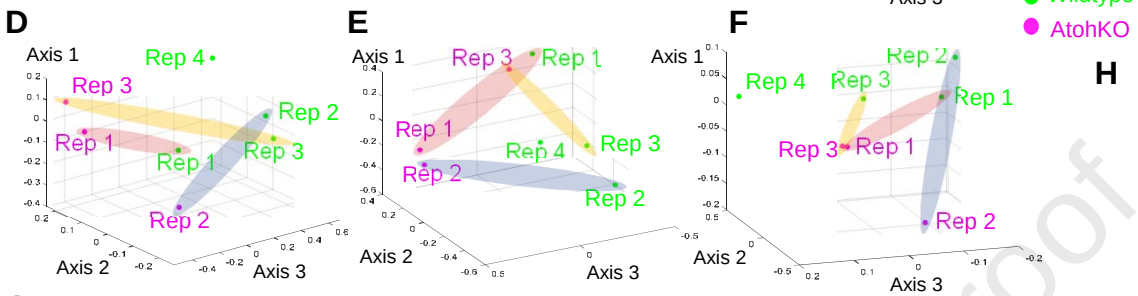
Supplementary Figure 16

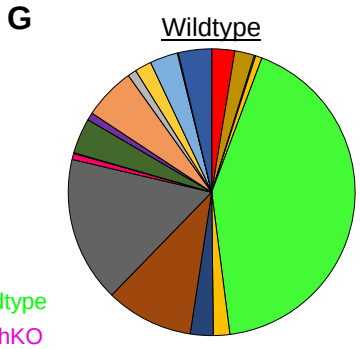


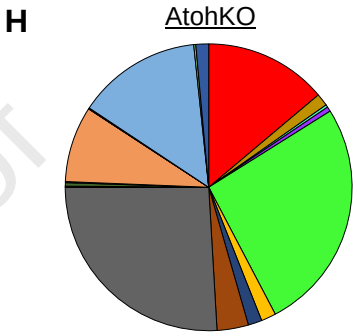
Supplementary Figure 16. (A) DCLK1 (green) and **(B)** MBP (brown) in untreated and succinate-treated colon. Hoechst (blue) denotes nuclei and white arrows identify tuft cells. Scale bar = 100 µm. **(C)** *Dclk1* and *Sucnr1* expression in untreated wildtype ileal (green) and colonic (magenta) tuft cells. **(D)** DCLK1 (green) and LYZ1 (magenta) staining in antibiotic-treated AtohKO colon absent and subsequent to exogenous succinate administration. Hoechst denotes nuclei. Scale bar = 100 µm. **(E-P)** TCA cycle gene expression in untreated (green) and succinate-treated (magenta) colonic tuft cells.

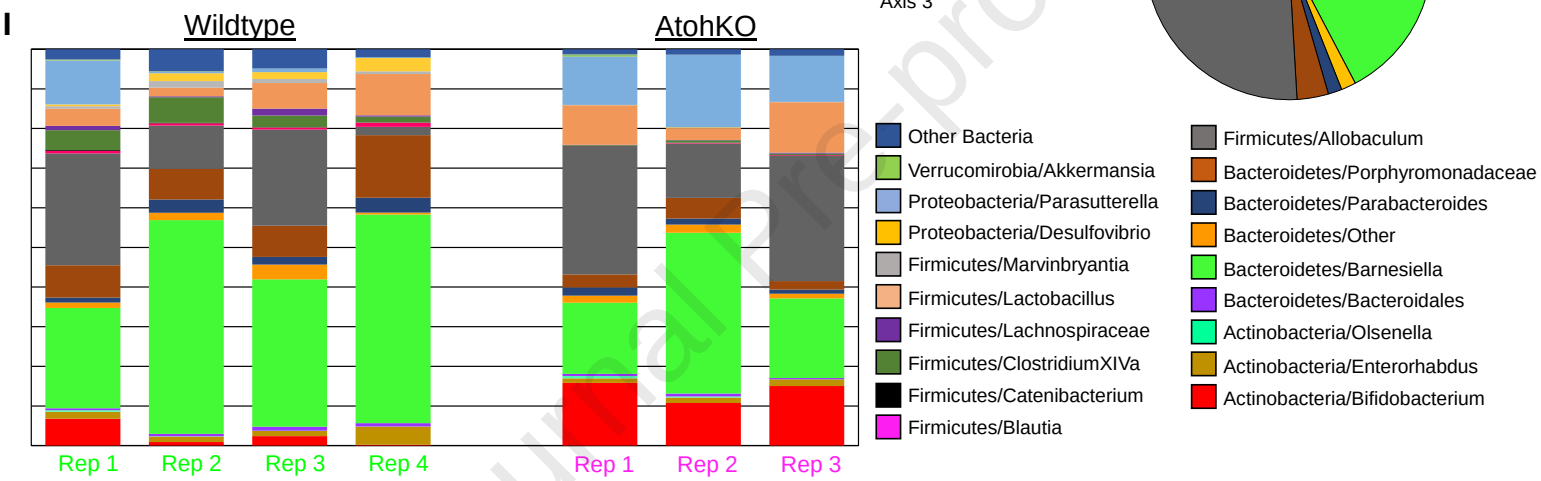
Supplementary Figure 17











J

Chlorocyclohexane and chlorobenzene degradation pathway (KEGG k00361) [Simplified]

γ-Hexachlorocyclohexane

↓

1-2-4-Trichlorobenzene

↓

3,4,6-Trichlorocatechol

↓

2-Chloro-3-oxoadipate

↓

Succinate

↓

Chloroacetate

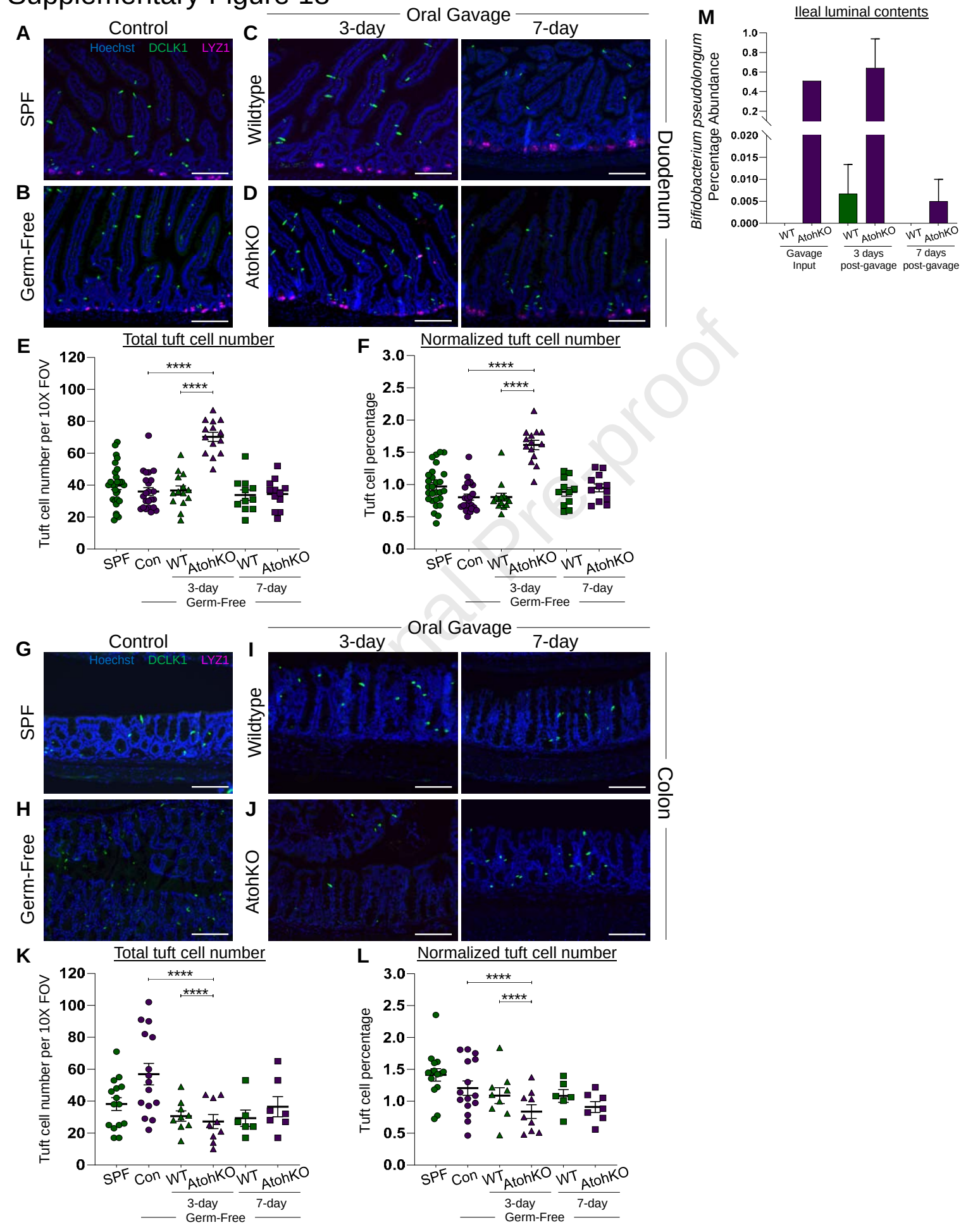
K

	Wildtype	AtohKO
Bifidobacterium pseudolongum (pval < 1e-07)	0.023	0.128
Parabacteroides chinchilla (pval < 0.001)	0.088	0.025
Parasutterella excrementihominis (pval < 0.001)	0.028	0.133
otu1712:Barnesiella intestinihominis (pval < 0.05)	0.056	0.022
otu623:Parabacteroides distasonis (pval < 0.05)	0.004	0.006
otu5745:Barnesiella viscericola (pval – ns)	0.022	0.014
Enterorhabdus mucosicola (pval – ns)	0.019	0.013
otu5516:Barnesiella intestinihominis (pval – ns)	0.128	0.071
otu5773:Barnesiella viscericola (pval – ns)	0.006	0.010
otu2975:Barnesiella viscericola (pval – ns)	0.013	0.019
otu5142:Barnesiella viscericola (pval – ns)	0.014	0.009
otu4006:Parabacteroides distasonis (pval – ns)	0.004	0.006
Lactobacillus taiwanensis (pval – ns)	0.006	0.025
otu5351:Barnesiella intestinihominis (pval – ns)	0.029	0.013
otu156:Paraprevotella clara (pval – ns)	0.004	0.005
otu568:Pontibacter_ramchander (pval – ns)	0.019	0.017
otu1791:Barnesiella_intestinihominis (pval – ns)	0.114	0.094
Lactobacillus_murinus (pval – ns)	0.043	0.048
otu2294:Tannerella_forsythia (pval – ns)	0.010	0.011

High
Mid
Low

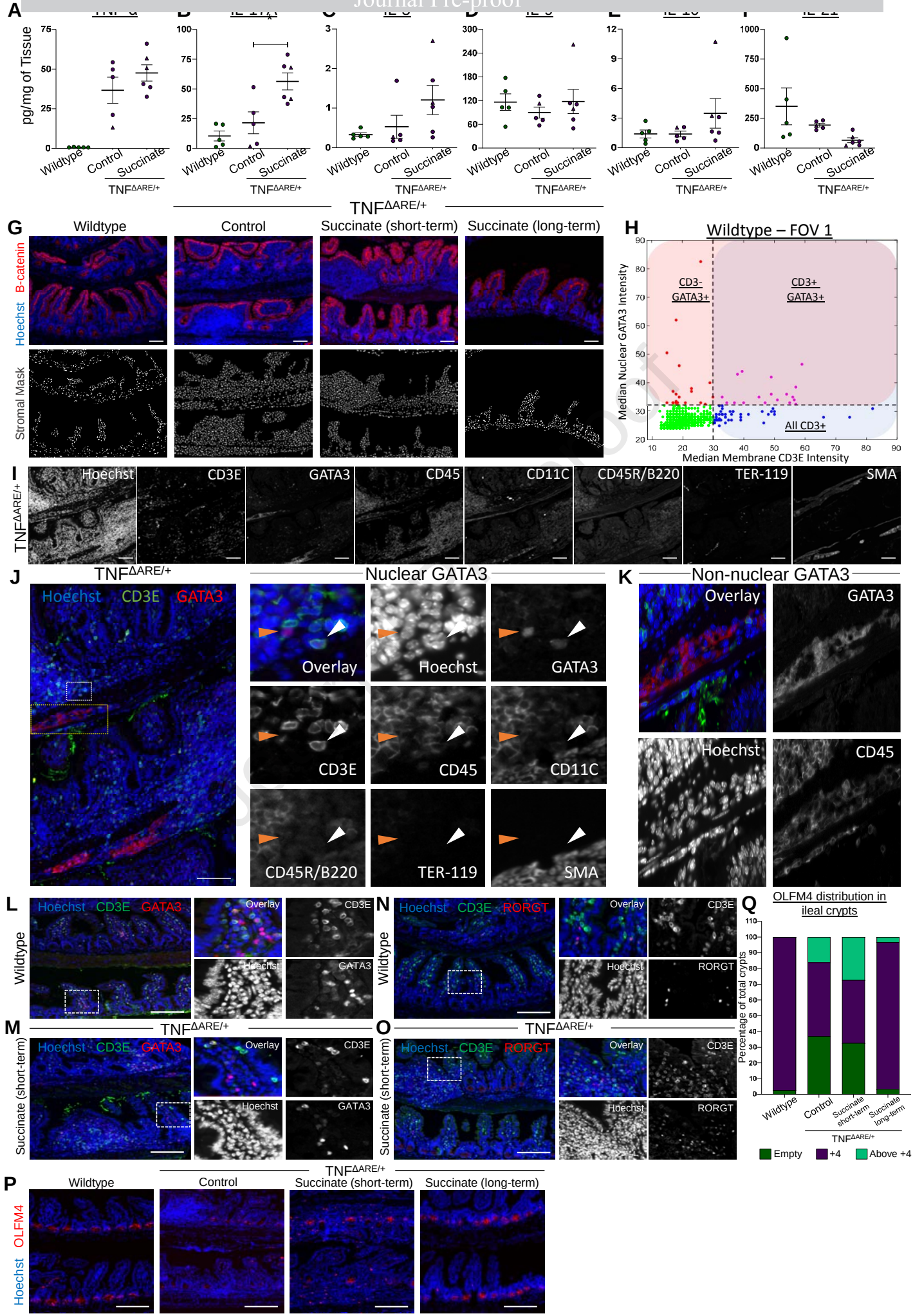
Supplementary Figure 17. (A-C) PCA plots of wildtype (n = 4, green) and AtohKO (n = 3, magenta) replicates analyzed by Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac. **(G-I)** Genus representation in wildtype (n = 4) and AtohKO (n = 3) replicates. **(J)** Simplified diagram of KEGG “Chlorocyclohexane and chlorobenzene degradation” pathway. **(K)** Heatmap of relative abundance at the operational taxonomic unit (OTU) level in wildtype and AtohKO replicates.

Supplementary Figure 18



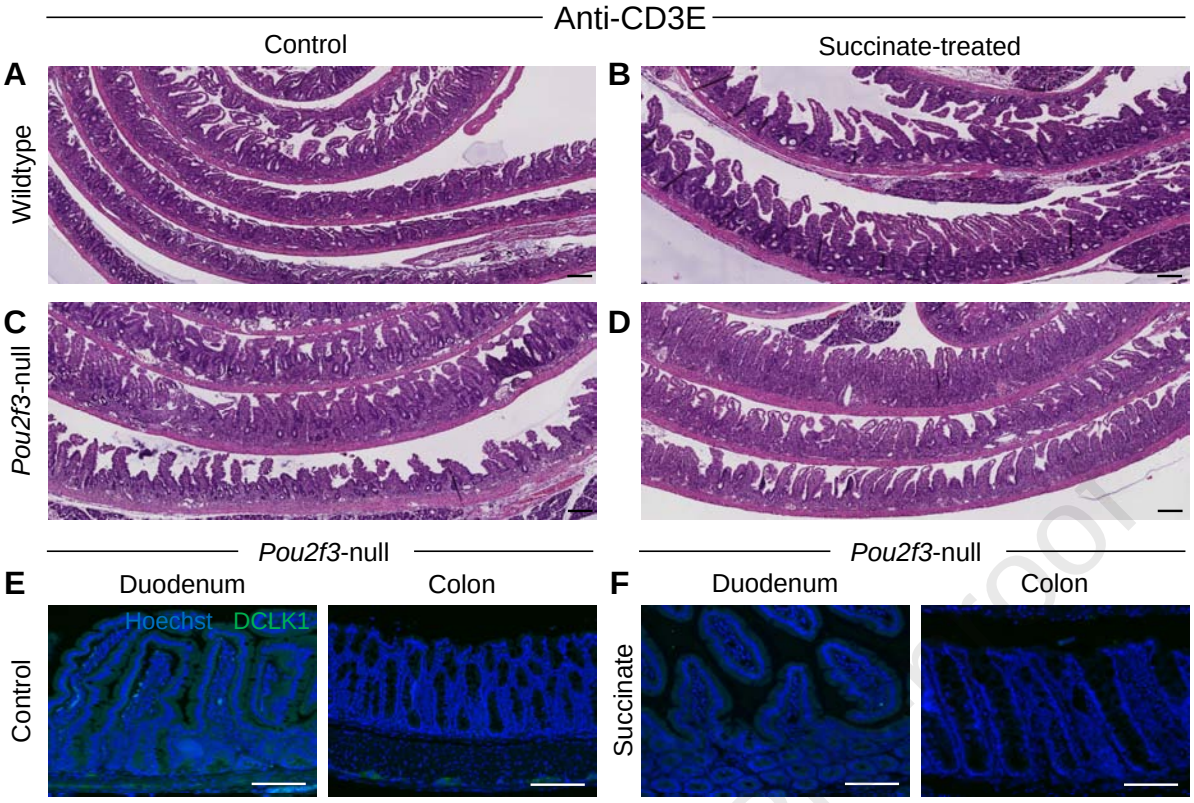
Supplementary Figure 18. (A-D) Representative IF of DCLK1 (green) and LYZ1 (magenta) in the untreated SPF, untreated germ-free, wildtype-gavaged and AtohKO-gavaged duodenum, 3 and 7 days post-gavage. **(E-F)** Total and normalized tuft cell number in untreated and oral-gavaged animals. Each symbol represents single FOVs and SEM is calculated across multiple biological replicates. ****p-value < 0.0001 by t-test. **(G-J)** Representative IF of DCLK1 (green) and LYZ1 (magenta) imaging in the untreated SPF, untreated germ-free, wildtype-gavaged, and AtohKO-gavaged colon, 3 and 7 days post-gavage. **(K)** Total tuft cell number and **(L)** Normalized tuft cell number in untreated and oral-gavaged animals. Each symbol represents single FOVs and SEM is calculated across multiple biological replicates. ****p-value < 0.0001 by t-test. **(M)** Relative abundance of *Bifidobacterium pseudolongum* in wildtype and AtohKO gavage inoculum and in ileal luminal contents of GF animals 3 or 7 days post-gavage with wildtype or AtohKO inoculum. SEM calculated across multiple biological replicates.

Supplementary Figure 19



Supplementary Figure 19. (A-F) Luminex cytokine measurements from wildtype, control $TNF^{\Delta ARE/+}$, and short-term succinate-treated $TNF^{\Delta ARE/+}$ ileal tissues. Error bars represent SEM calculated across multiple biological replicates (circles: males, triangles: females). *p-value < 0.05 by t-test. **(G)** Epithelial β-catenin (red) demarcates epithelial lining. Hoechst (blue) denotes nuclei and grey elements are evaluated stromal cells. Scale bar = 100 µm. **(H)** Scatter plot of CD3E and GATA3 median intensity per FOV. Circles represent single cells, dashed lines represent intensity thresholds, and shaded squares demarcate respective populations. **(I)** Multiplexed imaging of Hoechst, immune cell markers (CD3E, GATA3, CD45, CD11C, CD45R/B220, TER-119), and SMA in a representative FOV. **(J)** Co-localization of CD3E (green) and GATA3 (red) in representative FOV. White dashed box demarcates analysis of nuclear GATA3 expression and exclusion of other analytes. Orange arrows denote CD3E- GATA3+ ILC2s and white arrows denote CD3E+ GATA3+ Th2s. Hoechst (blue) and scale bar = 100 µm. **(K)** Yellow dashed box demarcates analysis of non-nuclear GATA3. **(L-M)** Representative IF of CD3E (green) and GATA3 (red) in wildtype and short-term succinate-treated $TNF^{\Delta ARE/+}$ ileum. Magnified inset shows individual expression of markers. Hoechst denotes nuclei and scale bar = 100 µm. **(N-O)** Representative IF of CD3E (green) and RORGT (red) in wildtype and short-term succinate-treated $TNF^{\Delta ARE/+}$ ileum. Magnified inset shows individual expression of markers. Hoechst denotes nuclei and scale bar = 100 µm. **(P)** Representative IF of OLFM4 (red) in wildtype, untreated $TNF^{\Delta ARE/+}$, short- and long-term succinate-treated $TNF^{\Delta ARE/+}$ ileum. Scale bar = 100 µm. **(Q)** IF quantification of OLFM4 (empty: green, +4: magenta, above +4: teal) in wildtype, untreated $TNF^{\Delta ARE/+}$, short- and long-term succinate-treated $TNF^{\Delta ARE/+}$ conditions.

Supplementary Figure 20



Supplementary Figure 20. (A-D) Representative histology of anti-CD3E control and succinate-treated wildtype and *Pou2f3*-null small intestine. Scale bar = 100 µm. **(E-F)** Representative IF of DCLK1 (green) in control and succinate-treated *Pou2f3*-null small intestine and colon. Hoechst (blue) and scale bar = 100 µm.