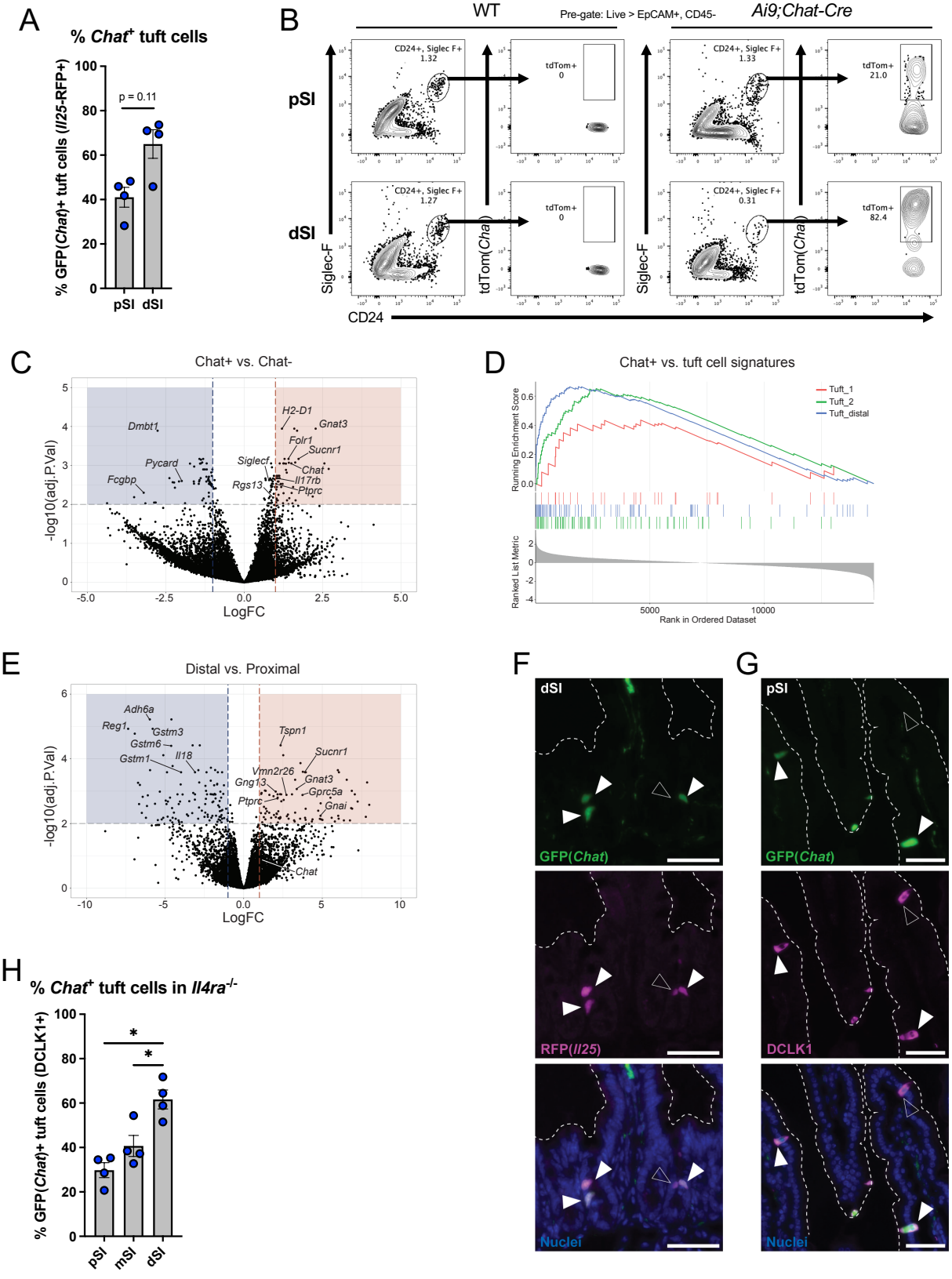
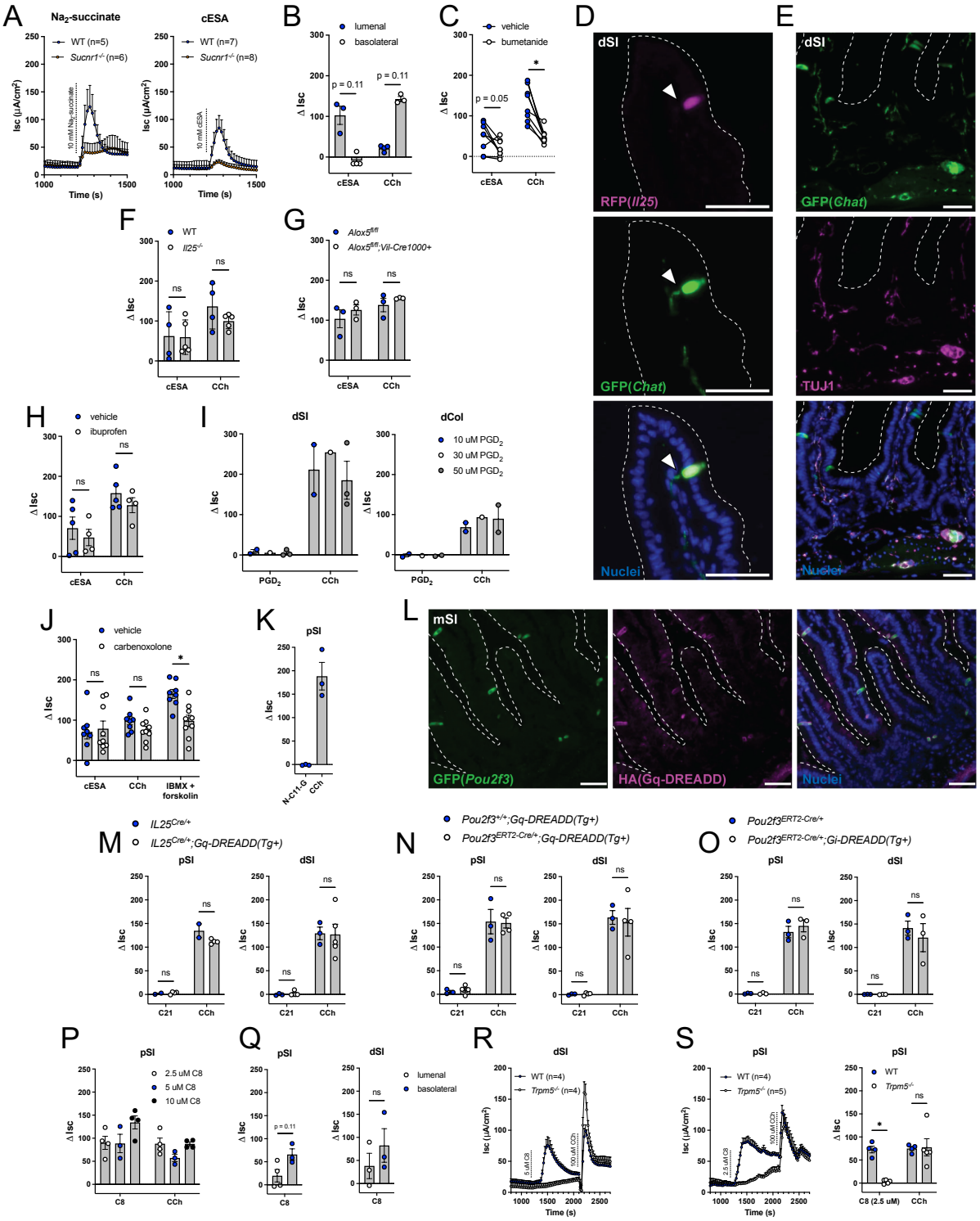
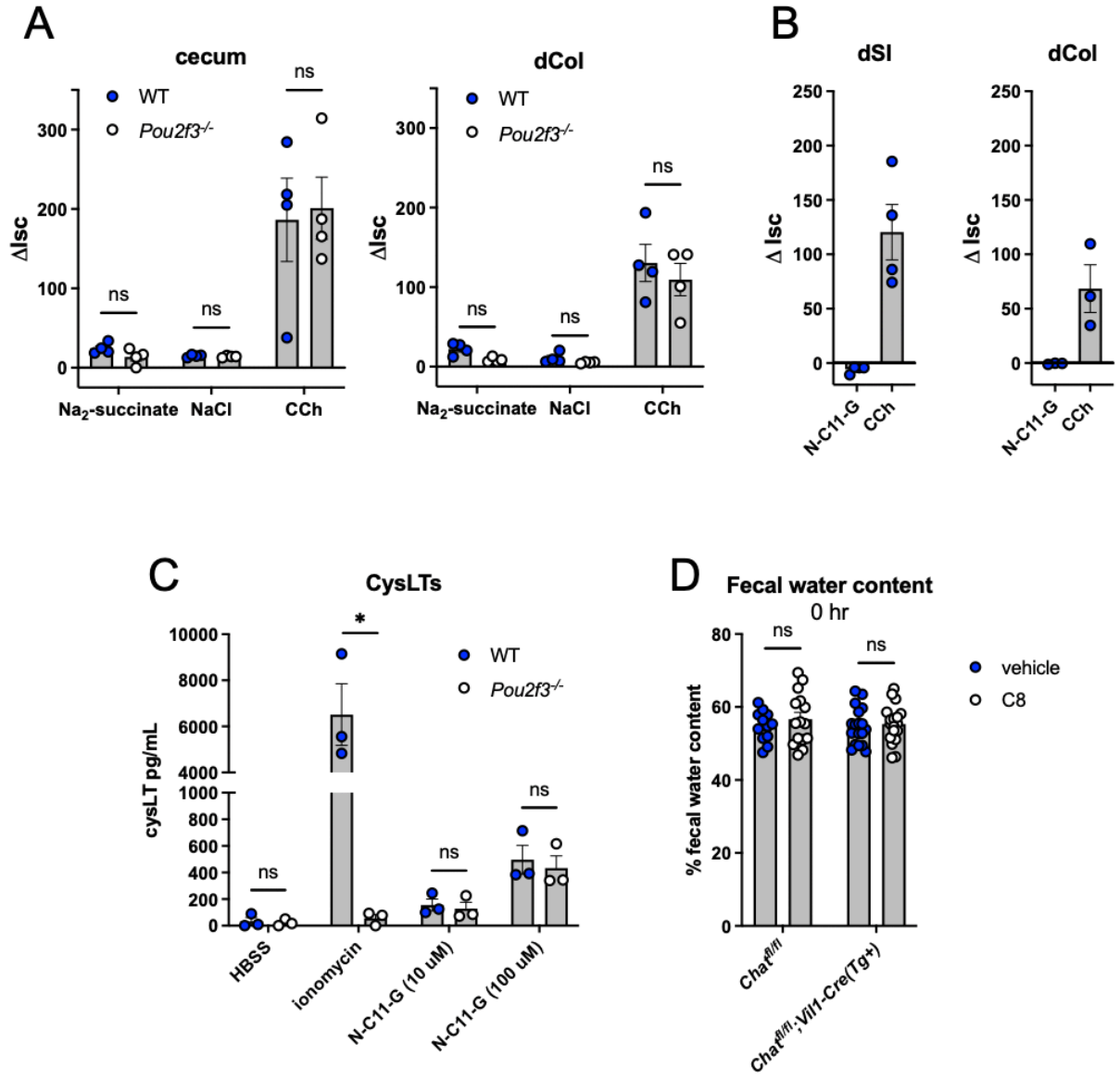


Supplemental Figure 1: (**A**) Quantification of GFP+ tuft cells (RFP+) from pSI and dSI of *Chat-GFP*;*Il25*$^{RFP/+}$ mice by immunofluorescence (IF). (**B**) Representative flow cytometry of traced tdTom+ tuft cells (CD24+, Siglec-F+) from pSI and dSI of wild-type (WT) and *Ai9;Chat-Cre* mice. (**C**) Volcano plot showing log2FC of genes expressed in *Chat*+ tuft cells (n=4) versus *Chat*- tuft cells (n=3) sorted from whole SI of *Chat-GFP*;*Il25*$^{RFP/+}$ mice. (**D**) Gene set enrichment analysis comparing *Chat*+ tuft cell gene expression to Tuft-1 and Tuft-2 consensus gene signatures and the dSI tuft cell signature from (**E**) Volcano plot showing log2FC of genes expressed in tuft cells sorted from the dSI (n=4) versus tuft cells sorted from the pSI (n=4) of B6 mice. (**F**) Representative immunofluorescence image showing GFP+ (green) tuft cells (RFP+, magenta) in the SI crypt (solid white arrows), next to one GFP- tuft cell (open white arrow). Nuclei stained with DAPI (blue). Scale bars: 50 μm. (**G**) Representative immunofluorescence image showing a GFP- (green) tuft cell (DCLK1+, magenta) at the villus tip (open white arrow), past other GFP+ tuft cells (solid white arrows). Nuclei stained with DAPI (blue). Scale bars: 50 μm. (**H**) Quantification of GFP(*Chat*)+ tuft cells (DCLK1+) from denoted tissues of *Il4ra*$^{-/-}$*;Chat-GFP* mice by immunofluorescence. In the graphs, each symbol represents an individual mouse (columns represent different tissues from same mouse) from two pooled experiments. *p < 0.05, **p < 0.01, ***p < 0.001 by Mann-Whitney test (A) or one way ANOVA with Tukey's multiple comparisons test (G). mSI, medial SI. Graphs depict mean +/- SEM.

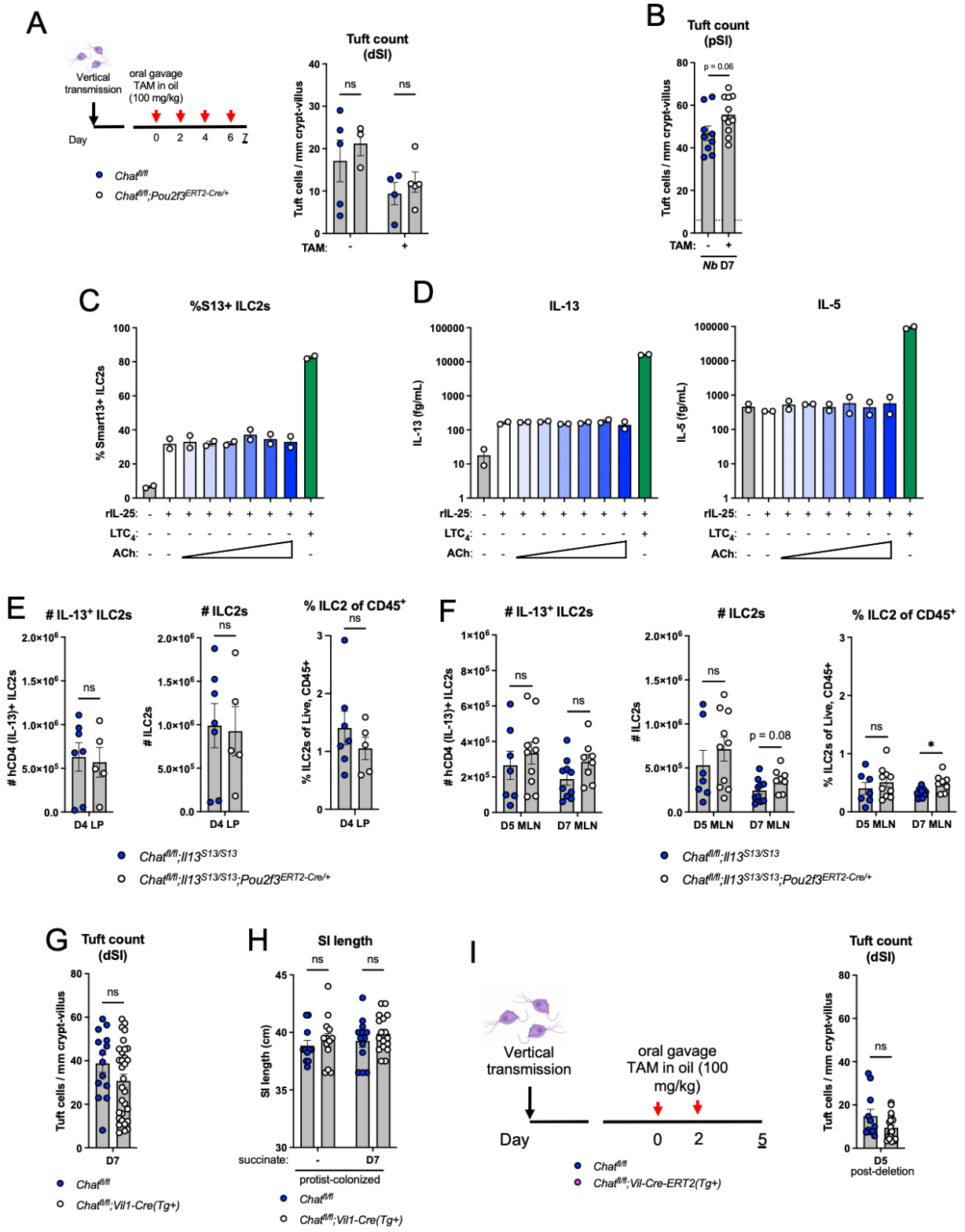


Supplemental Figure 2: (**A**) Average Isc traces of dSI from WT or *Sucnr1*$^{-/-}$ mice stimulated as indicate (Na_2-succinate and cESA, lumenal). (**B**) ΔIsc values of WT dSI stimulated as indicated. (**C**) ΔIsc values of WT dSI pretreated 15 min with vehicle or bumetanide (100 μM, basolateral), stimulated as indicated (10 mM cESA lumenal; 100 μM CCh, basolateral). (**D**) Representative immunofluorescence image of a GFP+ (green) neuronal process (indicated by solid white arrow) approaching a GFP+/RFP+ (magenta) tuft cell from the dSI of *Chat-GFP*;*Il25*$^{RFP/+}$ mice. Nuclei stained with DAPI (blue). Scale bars: 50 μm. (**E**) Representative immunofluorescence image of GFP+ (green) neurons co-stained for BIII tubulin (TUJ1, magenta) in the dSI. Nuclei stained with DAPI (blue). Scale bars: 50 μm. (**F**, **G**, and **H**) (F and G) ΔIsc values of dSI from indicated genotypes or (H) WT dSI compared to dSI pretreated 15 min with ibuprofen (10 μM, bilateral), stimulated as indicated. (**I**) ΔIsc values of WT tissues stimulated as indicated (PGD_2, basolateral). (**J**) ΔIsc values for WT dSI compared to dSI pretreated 15 min with carbenoxolone (1 mM, lumenal), stimulated as indicated (100 μM IBMX + 10 μM forskolin, bilateral). (**K**) ΔIsc values of WT pSI stimulated as indicated (100 μM N-C11-G, lumenal). (**L**) Representative immunofluorescence image of GFP+ (green) tuft cells expressing HA+ Gq-DREADDs (magenta) in the crypts and villi of the medial SI (mSI). Nuclei stained with DAPI (blue). Scale bars: 50 μm. (**M**, **N**, and **O**) (M) ΔIsc values of indicated tissues from unmanipulated mice or (N and O) indicated tissues from mice 7 days after start of tamoxifen chow, stimulated as indicated (1 μM C21, bilateral). (**P**) ΔIsc values of WT pSI stimulated as indicated (C8, bilateral). (**Q**) ΔIsc values of WT tissues from pSI and dSI stimulated as indicated. (**R**) Average Isc traces of dSI stimulated as indicated (5 μM C8, basolateral). (**S**) Average Isc traces and ΔIsc values of pSI stimulated as indicated (2.5 μM C8, basolateral). In the graphs, each symbol represents an individual mouse (one tissue or average of two) pooled from two or more experiments. Groups represent sequential stimulations of the same tissue. In (C) paired vehicle and bumetanide-treated tissues are from the same mouse. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.001$ by multiple Mann-Whitney tests with Holm Sídák's multiple comparisons test (B, F-H, J, M-O, S), Wilcoxon matched-pairs signed rank test with Holm Sídák's multiple comparisons test (C), or Mann-Whitney test (Q). ns, not significant. Graphs depict mean +/- SEM.



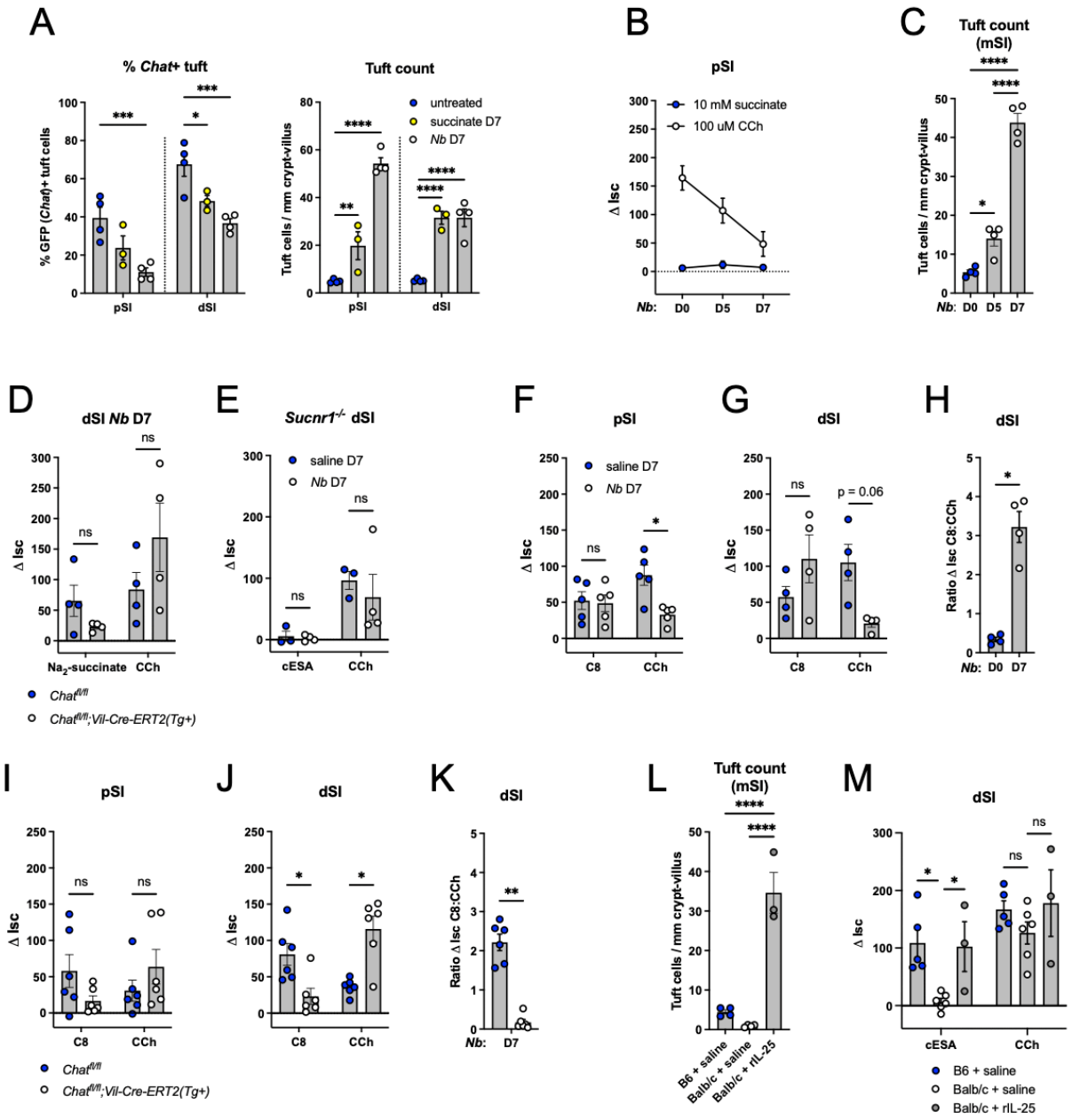
Supplemental Figure 3: (**A**) ΔIsc values of WT and *Pou2f3*$^{-/-}$ tissues stimulated as indicated (10 mM Na_2-succinate and 20 mM NaCl, lumenal; 100 μM CCh, basolateral). (**B**) ΔIsc values of WT tissues stimulated as indicated (100 μM N-C11-G, lumenal). (**C**) Cysteinyl leukotriene (CysLTs) production from WT and *Pou2f3*$^{-/-}$ SI epithelial monolayers stimulated as indicated. (**D**) Quantification of water content of fecal pellets collected from indicated mice immediately after oral treatment with C8 (30 mg/kg). In the graphs, each symbol represents an individual mouse pooled from two or more experiments. In (A-B) groups

represent sequential stimulations of the same tissue and in (C) groups represent individual monolayers. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001 by multiple Mann-Whitney tests with Holm Sídák's multiple comparisons test (A, D), or multiple unpaired t tests with Holm Sídák's multiple comparisons test (C). ns, not significant. Graphs depict mean +/- SEM.



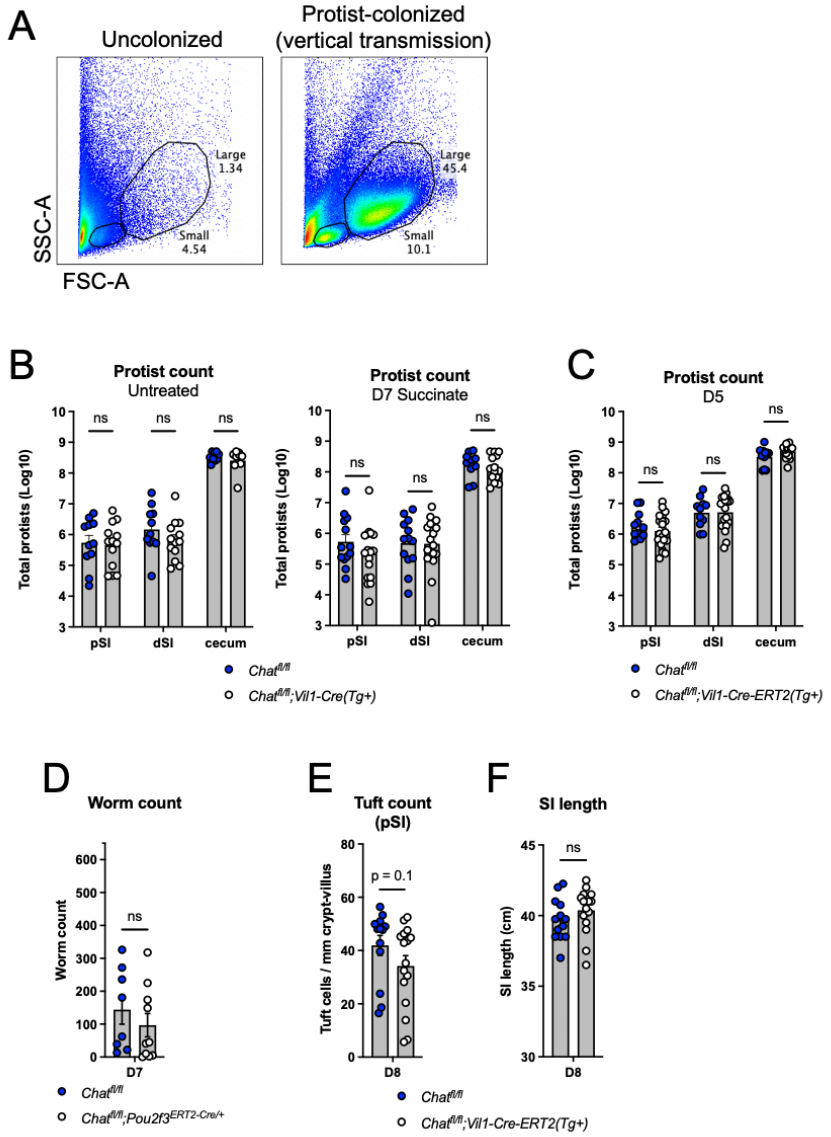
Supplemental Figure 4: (**A**) Schematic of tamoxifen (TAM) treatment of protist-colonized *Chat-fl; Pou2f3*$^{ERT2\text{-}Cre/+}$ mice and quantification of dSI tuft counts by immunofluorescence at D7 after start of treatment. (**B**) Quantification of pSI tuft counts by immunofluorescence of WT mice treated with or without

458 tamoxifen and infected with *Nb* for 7 days. (**C** and **D**) (C) Flow cytometric quantification of percent hCD4+
459 (IL-13+) SILP ILC2s and (D) IL-13 and IL-5 concentration in their supernatant after 6 hr *in vitro* culture
460 with the indicated conditions (0.1 ng/mL rIL-25, serial 10-fold dilutions of ACh from 10 mM to 0.1 μM, 1
461 nM LTC_4). (**E** and **F**) Quantification of number of hCD4+ (IL-13+) ILC2s, total ILC2s, and percent ILC2s at
462 the indicated timepoints, tissues, and genotypes. (**G**) Quantification of dSI tuft counts by
463 immunofluorescence from indicated mice treated with 150 mM succinate drinking water for 7 days. (**H**) SI
464 length from indicated mice vertically-colonized with *T. rainier* protists with or without 7 days of additional
465 150 mM succinate drinking water treatment. (**I**) Schematic of acute deletion of *Chat* from vertically *T.*
466 *musculis* (*Tm*) -colonized mice and quantification of dSI tuft counts by immunofluorescence 5 days after
467 start of treatment. In the (A-B, E-I), each symbol represents an individual mouse from two or more pooled
468 experiments. In (C and D) each symbol represents a technical replicate of cells sorted from pooled mice.
469 *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001 by multiple Mann-Whitney tests with Holm Sídák's
470 multiple comparisons test (A, F, H) or Mann-Whitney test (B, E, G, I). ns, not significant. Graphs depict
471 mean +/- SEM.



Supplemental Figure 5: (**A**) Quantification of percent GFP(*Chat*)+ tuft cells and RFP(*Il25*)+ tuft cells from the pSI and dSI of WT mice untreated, treated with 150 mM Na_2-succinate drinking water (succinate), or infected with *N. brasiliensis* (*Nb*) for the duration indicated. (**B**) ΔIsc values of pSI from WT mice infected with *Nb* for the indicated number of days and stimulated as indicated (10 mM succinate, lumenal; 100 μM CCh, basolateral). (**C**) Quantification of tuft cells (DCLK1+) by immunofluorescence from medial SI (mSI) of mice in (B). (**D**) ΔIsc values of dSI from mice of indicated genotypes infected with *Nb* for 7 days and stimulated as indicated. (**E**) ΔIsc values of *Sucnr1*$^{-/-}$ dSI with or without 7 day *Nb* infection, stimulated as indicated. (**F-G**) ΔIsc values of (F) pSI and (G) dSI from WT mice with or without 7 day *Nb* infection, stimulated as indicated. (**H**) Ratio of C8 ΔIsc values to CCh ΔIsc values in (G). (**I** and **J**) ΔIsc values of (I)

pSI and (J) dSI from mice of indicated genotype infected with *Nb* for 7 days, stimulated as indicated. (**K**) Ratio of C8 ΔIsc values to CCh ΔIsc values from (J). (**L**) Quantification of tuft cells (DCLK1+) by immunofluorescence in the mSI of mice of indicated genotypes treated as indicated. (**M**) ΔIsc values of dSI from mice in (L) stimulated as indicated. In the graphs, each symbol represents an individual mouse (one tissue or average of two) from two or more pooled experiments. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001 by two way ANOVA with Dunnett's multiple comparisons test (A) two way ANOVA with Tukey's multiple comparisons test (M), Mann-Whitney test (B, H, K), one way ANOVA with Tukey's multiple comparisons test (C, L), or multiple Mann-Whitney tests with Holm Sídák's multiple comparisons test (D-G, I-J). ns, not significant. Graphs depict mean +/- SEM.



Supplemental Figure 6: (**A**) Representative flow cytometry of cecal contents from uncolonized and protist-colonized mice showing gating of protists by size. The "Large" gate contains *Tritrichomonas sp.* protists. (**B**) Quantification of total protists by flow cytometry in indicated tissues of vertically-colonized mice of indicated genotypes left untreated or given 150 mM Na_2-succinate in drinking water for 7 days. (**C**) Quantification of total protists by flow cytometry in indicated tissues of vertically-colonized mice of indicated genotypes administered tamoxifen 5 days prior to analysis. (**D**) Quantification of total SI *Nb* in mice of indicated genotype infected with *Nb* for 7 days without tamoxifen administration. (**E** and **F**) (E) Quantification of tuft cells (DCLK1+) by immunofluorescence and (F) total SI length 8 days post *Nb* infection of mice of indicated genotype given a single dose of tamoxifen (125 mg/kg) on day 5. In the graphs, each symbol represents an individual mouse (one tissue or average of two) from two or more pooled experiments. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001 by multiple Mann-Whitney tests with

632 Holm Sídák's multiple comparisons test (B-C) or Mann-Whitney test (D-F). ns, not significant. Graphs
633 depict mean +/- SEM.

1031 **Table S3**

Reagent or Resource	Source	Identifier
B6.Cg-Tg(RP23-268L19-EGFP)2Mik/J (Chat-GFP)	Jackson Laboratory	JAX #007902
B6.*Il25*$^{Flare25/Flare25}$ (Il25-RFP)	R. Locksley (PMID: 26675736)	NA
B6.*Il13*$^{Smart13/Smart13}$ (Smart13)	R. Locksley (PMID: 22138715)	NA
C57BL/6N-Pou2f3<tm1(KOMP)Vlcg>/Tcp (*Pou2f3*$^{-/-}$)	Canadian Mouse Mutant Repository	CMMR #ABDF
B6.129P2-Trpm5^{tm1Dgen}/J (Trpm5$^{-/-}$)	Jackson Laboratory	JAX #005848
B6.*Sucnr1*$^{-/-}$	In-house (PMID: 30021144)	NA
B6.*Il25*$^{-/-}$	A. McKenzie (PMID: 16606668)	NA
B6;129-Chat^{tm1Jrs}/J (*Chat*$^{fl/fl}$)	Jackson Laboratory	JAX #016920
B6.Cg-Tg(Vil1-cre)997Gum/J	Jackson Laboratory	JAX #004586
B6.Cg-Tg(Vil1-cre)1000Gum/J (Vil1-Cre1000)	Jackson Laboratory	JAX #021504
B6.Cg-Tg(Vil1-cre/ERT2)23Syr/J (*Vil1-Cre-Ert2*)	Jackson Laboratory	JAX #020282
B6(129S4)-*Pou2f3*$^{tm1.1(cre/ERT2)Imt}$/J (Pou2f3-Cre-Ert2)	Jackson Laboratory	JAX #037511
B6.129S-Chattm1(cre)Lowl/MwarJ (*Chat-Cre*)	Jackson Laboratory	JAX #031661
B6.*Il25-Cre*	R. Locksley (PMID: 35245089)	NA
B6.Cg-*Gt(ROSA)26Sor*$^{tm9(CAG-tdTomato)Hze}$/J (Ai9)	Jackson Laboratory	JAX #007909
B6N;129-Tg(CAG-CHRM3*,-mCitrine)1Ute/J (Gq-DREADD)	Jackson Laboratory	JAX #026220
B6.129-*Gt(ROSA)26Sor*$^{tm1(CAG-CHRM4*,-mCitrine)Ute}$/J (Gi-DREADD)	Jackson Laboratory	JAX #026219
B6.*Alox5*$^{fl/fl}$	In-house (PMID: 32160525)	NA
B6.*Il4ra*$^{fl/fl}$	F. Brombacher (PMID: 15142530)	NA

1032

1033 **Table S4**

Reagent or Resource	Dilution Factor	Source	Identifier
Rabbit α-DCLK1	1:1000	Abcam	Cat#ab31704
Rabbit α-TUJ1 (Beta-III tubulin)	1:500	Abcam	Cat#ab18207
Goat α-GFP	1:500	Novus Bio	Cat#NB100-1770
Rabbit α-dsRed	1:500	Clontech	Cat#632496
Rabbit α-HA, clone 16B12	1:1000	Biolegend	Cat#901516
WGA-488	1:150	Thermo	Cat#W11261
Donkey α-Rabbit IgG AF594	1:1000	Thermo	Cat#A-21207
Donkey α-Goat IgG AF488	1:500	Thermo	Cat#A-11055
CD16 / CD32, clone 2.4G2	1:1000	Tonbo	Cat# 70-0161-M001
CD3 PerCP-Cy5.5, clone 145-2C11	1:100	Biolegend	Cat#100328
CD3 BV421, clone 145-2C11	1:400	Biolegend	Cat#100335
CD4 BV711, clone RM4-5	1:250	Biolegend	Cat#100549
CD4 eF450, clone RM4-5	1:200	eBioscience	Cat# 48-0042-80
hCD4 PE, clone RPA-T4	1:50	Biolegend	Cat#300508
CD5 PerCP-Cy5.5, clone 53-7.3	1:500	Biolegend	Cat#100624
CD5 eF450, clone 53-7.3	1:400	Biolegend	Cat#100607
CD8 PerCP-Cy5.5, clone 53-6.7	1:200	Biolegend	Cat#100724
CD8 BV421, clone 53-6.7	1:400	Biolegend	Cat#100737
CD11b AF700, clone M1/70	1:250	Biolegend	Cat#101222
CD11b BV421, clone M1/70	1:400	Biolegend	Cat# 101235
CD19 PerCP-Cy5.5, clone 6D5	1:250	Biolegend	Cat#115533
CD19 BV421, clone 6D5	1:400	Biolegend	Cat# 115537

CD24 PE, clone M1/69	1:300	Biolegend	Cat#101807
CD24 PerCP-Cy5.5, clone M1/69	1:300	Biolegend	Cat#101824
CD45 BV605, clone 30F11	1:300	Biolegend	Cat#103155
CD45 BV650, clone 30F11	1:500	Biolegend	Cat#103151
EpCAM PE-Dazzle, clone G8.8	1:300	Biolegend	Cat#118235
EpCAM AF488, clone G8.8	1:300	Biolegend	Cat#118210
EpCAM PE-Cy7, clone G8.8	1:300	Biolegend	Cat#118215
FcER1 BV421, clone Mar-1	1:400	Biolegend	Cat#334623
IL17RB APC, clone 9B10	1:100	Biolegend	Cat#146307
KLRG1 PE-Cy7, clone 2F1	1:250	Biolegend	Cat#138416
NK1.1 PerCP-Cy5.5, clone PK136	1:100	Biolegend	Cat#108728
NK1.1 BV421, clone PK136	1:200	Biolegend	Cat#108731
Siglec-F APC-Cy7, clone E50-2440	1:100	BD	Cat#565527
Siglec-F AF647, clone E50-2440	1:100	BD	Cat# 562680
Thy1.2 (CD90.2) BV605, clone 53-2.1	1:500	Biolegend	Cat#140318