SUPPLEMENTAL MATERIAL

Supplemental 1: Methods and Materials

Animals and injections

All experiments involving animals were performed according to protocols approved by the Washington University School of Medicine Animal Studies Committee. Mice were maintained in a specified-pathogen-free barrier facility under a 12 hour light cycle. Wild type *C57BL/6, Gt(ROSA)26Sor*$^{tm1(HBEGF)Awai}$ (iDTR)[1], and B6.129(Cg)-Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J (mT/mG)[2] mice were all purchased from Jackson Laboratories. *ATP4b-Cre* mice[3] were crossed with iDTR mice, which express the inducible simian diphtheria toxin receptor under the control of the Rosa26 promoter. Littermate controls were housed together when possible to minimize differences in gastric microflora. To selectively kill parietal cells, Diphtheria Toxin (225 ng/mouse, Sigma) was injected intraperitoneally one or three times per day. Since parietal cells (PCs) die at a comparable rate to those previously published with D3 TAM, most analysis was done at D3 DT. Diphtheria Toxin was dissolved in sterile 0.9% sodium chloride saline. To induce SPEM, Tamoxifen (5 mg/20 g body weight, Toronto Research Chemicals Inc.) was injected intraperitoneally daily for three days [4] or DMP-777 (7 mg/20 g body weight, gift of DuPont-Merck Corporation) was gavaged daily for 14 days. Tamoxifen was dissolved in a vehicle of 10% ethanol and 90% sunflower oil (Sigma), and DMP-777 was suspended in 1% methylcellulose (Sigma) in distilled H2O.

Immunofluorescence

Mice were given an intraperitoneal injection containing 5-bromo-2'-deoxyuridine (BrdU, 120 mg/kg) and 5-fluoro-2'-deoxyuridine (12 mg/kg) in sterile water 90 min before sacrifice. Following sacrifice, stomachs were immediately excised and flushed with PBS, then pinned out and fixed in freshly prepared methacarn (60% methanol, 30% chloroform, 10% glacial acetic

acid) for 20 minutes and stored overnight in 70% ethanol. Tissues were arranged in 3% agar in a tissue cassette, underwent routine paraffin processing, and 5µm sections were cut and mounted on glass slides. Sections underwent a standard deparaffinization and rehydration protocol, were blocked in 1% BSA, 0.3% Triton-X100, in PBS, left overnight with primary antibodies, washed in PBS and incubated for one hour with secondary antibodies, washed, incubated 5 minutes in 1 g/ml bisbenzimide (Molecular Probes), washed, then mounted using glycerol:PBS.

Primary Antibodies used in this study: rabbit anti-human gastric intrinsic factor (1:10,000, gift of Dr. David Alpers, Washington University), goat anti-Brdu (1:20,000, gift of Dr. Jeff Gordon, Washington University), goat anti-VEGFb (1:100, Santa Cruz), goat anti-Clusterin (1:100, Santa Cruz), mouse anti-E-Cadherin (1:200, BD Biosciences), rabbit anti-GFP (1:100 Santa Cruz), mouse anti-TFF2 (1:500, Abcam), rat anti-CD44 v10-e16, ortholog of human v9 (1:200, Cosmo Bio), or 1 g/ml fluorescently labeled GSII lectin (Alexafluor488, 594, Molecular Probes). Secondary Antibodies included AlexaFluor (488, 594 or 647) conjugated donkey anti-goat, anti-rabbit, or anti-mouse (1:500, Molecular Probes).

Immunofluorescence quantification

All timepoints were quantified with at least three mice, with representatives from both genders. Stomachs were fluorescently stained with bisbenzimide and either anti-BrdU or anti-VEGFb markers along with the neck cell marker GSII lectin and zymogenic cell marker anti-GIF. Images were captured as TIFF files from a Zeiss Axiovert 200 microscope with Axiocam MRM camera and with Apotome optical sectioning filter. Each stomach had at least 5 images taken containing 10+ well-oriented gastric units each. Units were counted using the neck staining, and total quantifications of proliferating cells ($BrdU^+$) or PCs ($VEGFb^+$)[5] were averaged over total unit numbers per mouse.

For quantifying units exhibiting SPEM, SPEM was defined exclusively in corpus gastric units as either 5+ cells per unit co-expressing GSII and GIF or GSII-expressing cells extending to the base of the unit.

Genotyping

Tissue was lysed with DirectPCR reagent (Viagen Biotech Inc) with added Proteinase K (New England BioLabs) at 55 ^{0}C for 11 hours, then 85 ^{0}C for 15 minutes. Genotyping PCR was run with Redtaq (Sigma). Primers: *H^+/K^+ATPase-Cre* Forward: AGGGATCGCCAGGCGTTTTC, Reverse: GTTTTCTTTTCGGATCCGCC.

Corpus gastroid culture

Gastric glands from the corpus of the stomach were isolated from *Atp4b-Cre;LSL-DTR*; ROSA$^{mT/mG}$ mice[2] according to Barker et al[6] and Stange et al[7]. Whole gastric glands were mixed with Matrigel, distributed in 48-well plates and grown in Advanced DMEM/F12 medium (Invitrogen), 50% Wnt3a conditioned medium, 10% R-Spondin1 and Noggin conditioned medium supplemented with 10mM HEPES, 1X N-2, 1X B27, 1X glutamax (Invitrogen), 2.5 mM *N*-Acetylcysteine (Sigma-Aldrich), 50 ng/mL EGF, 100 ng/mL FGF10 (Peprotech) and 10 nM gastrin (Sigma-Aldrich). 10 µM ROCK inhibitor (Y-27632, Sigma-Aldrich) was provided for the first 3 days. Three days after initial culturing, gastroids were treated with 10 ng/mL of diphtheria toxin in the absence of Wnt3a, R-Spondin 1 and Noggin. Fresh medium containing DT was added the following day. Using Cytation 3 (Biotek), all wells were microscopically scanned every 24 hours throughout the whole experiment, and the number of dead gastroids was scored.

Quantitative RT-PCR

Total RNA was extracted from corpus stomach tissue using the RNeasy Mini Kit (Qiagen). RNA was treated with DNAse I then cDNA was synthesized with Superscript III (Invitrogen) and

random primers. qRT-PCR was performed using PowerUp SYBR Green Master Mix (ThermoFisher) and gene specific primers (see table) on a QuantStudio 3 PCR System (ThermoFisher) and data analyzed using QuantStudio Design & Analysis Software. Every run was standardized to TATA Box Binding Protein (TBP) primers. All primers were exon-spanning when possible, (i.e. for genes having multiple exons of sufficient length). For full list of primers, see Supplemental Table. All graphs and statistics were completed in GraphPad Prism, using one-way ANOVA with either Dunnett's or Tukey's post-hoc multiple comparison tests to determine significance.

Supplemental Table

Gene	**Forward Primer 5'→3'**	**Reverse Primer 3'→5'**
TBP	CAAACCCAGAATTGTTCTCCTT	ATGTGGTCTTCCTGAATCCCT
GIF	GAAAAGTGGATCTGTGCTACTTGCT	AGACAATAAGGCCCCAGGATG
Mist1	GAGCGAGAGAGGCAGCGGATG	AGTAAGTATGGTGGCGGTCAG
TFF2	TGCTTTGATCTTGGATGCTG	GGAAAAGCAGCAGTTTCGAC
Clusterin	CCAGCCTTTCTTTGAGATGA	CTCCTGGCACTTTTCACACT
Wfdc2/HE4	TGCCTGCCTGTCGCCTCTG	TGTCCGCACAGTCCTTGTCCA
Mal2	GCTTTCGTCTGTCTGGAGATTG	ACACAAACATGACCCATCCTTG
Arhgap9	TGCTGCCTGACTTTCGTGATG	GCGGTCATTCGGTTCTTATCC
Casp1	GAAAGACAAGCCCAAGGTGAT	GGTGTTGAAGAGCAGAAAGCA
Ccnb2	TGAAGTCCTGGAAGTCATGC	GAGGCCAGGTCTTTGATGAT
CD14	CTCTGTCCTTAAAGCGGCTTAC	GTTGCGGAGGTTCAAGATGTT
Ceacam1	CCTCAGCACATCTCCACAAAG	TATAGCCGTAGTGTTTCCCTTG
Ceacam10	CTCCGATTTCTGTGCGATTTC	GTCCGTGGCAGATTGTGAAC
Cenpk	AATACTGGACACTCTTAACG	GGATCTTAGTTGTCAGTTCAT

CFTR	CTGGACCACACCAATTTTGAGG	GCGTGGATAAGCTGGGGAT
Chek2	TCGGCTATGGGCTCTTCA	CGTCCTTCTCAACAGTGGTC
Ctss	TCTATGACGACCCCTCCTG	TTGCCATCCGAATGTATCCTT
Cxcl17	AGGTGGCTCTTGGAAGGTG	CTCTGGAGGGTCTTTGCGA
Dmbt1	ACCTCCTCACGGTGCTACAG	GCTTCTTCACATCCTCCACTG
ETV5	GCTCTTGGTGCTAAGTAGGA	TCTGATGGGTGGGTGACA
Fignl1	TTATATTCCCCTCCCAGAAGC	GCCAGAAAACCCATCAGACT
Glipr1	CCAGCTTCGGTCAAAAGTGAG	TGGGTGTATCCGTGAATGCAG
Gpx2	CAGGGCTGTGCTGATTGAG	CGGACATACTTGAGGCTGTTC
Ly6a	GACTTCTTGCCCATCAATTACC	TTAGTACCCAGGATCTCCATAC
Lyz2	GCCAGAACTCTGAAAAGGAATG	CTTTGGTCTCCACGGTTGTAG
Mad2l1	TGCTTACAACTACTGACCCCG	ACTGCCATCTTTCAAGGACTTC
Mmp12	CATGAAGCGTGAGGATGTAGAC	CTAGTGTACCACCTTTGCCA
Ms4a6b	TCCCTCCAATCTACACTTTACC	GACTTTGTCTCCGTGACGATG
Ms4a6c	AAAAGACGAGTCCCAGCCTAC	ATGGGACAGGAGGAACAGATG
Muc4	GCTGCCTGTATTCTTGCCT	ATGTTCTGGTGCTGCTGGA
Pigr	GATTTGGGAGGCAATGACAAC	GCTTTCTTGGATTCTTCTGGC
Prom1	TGGATAACACAGGAAGGAAGAG	CAGGGTAGAGGCAAATGTCAG
Slfn9	TCCTTAGTGGTGAAACGGTCT	TCAGGTTGCTCACTCTGGTTG
Tmem48	GCTGCTACAAATGGGAGGAT	CACGGAAGGCGTCTGACTA
Top2a	CGAAATGGCTATGGAGCTAA	TATCTTTGTCCAGGCTTTGC
Traf4	CAGGTGTTAGGCTTGGCTATC	CGATTAGGGCAGGGGACTA
Tyrobp	GGTGTTGACTCTGCTGATTGC	AAGCTCCTGATAAGGCGACTC
Ube2c	CAACATCTGCCTGGACATC	CCTGCTTTGAATAGGTTTCTTGC
Vil1	TCAAAGGCTCTCTCAACATCAC	GGTGCTGGAAGGAACAGG

Supplemental References

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2. Muzumdar MD, et al. Genesis 2007; 45: 593-605.
3. Syder AJ, et al. Proc Natl Acad Sci U S A 2004; 101: 4471-4476.
4. Saenz JB, et al. Methods Mol Biol 2016; 1422: 329-339.
5. Mills JC, et al. J Biol Chem 2003; 278: 46138-46145.
6. Barker N, et al. Cell stem cell 2010; 6: 25-36.
7. Stange DE, et al. Cell 2013; 155: 357-368.

Supplemental Figure 1: *Atp4b-Cre;LSL-DTR* drives iDTR specifically in parietal cells

Top) Natural fluorescence in *Atp4b-Cre;LSL-DTR;ROSA*^{mTmG} mice. Cells with Cre driver express GFP (green), and all cells without Cre driver express mTomato (red). *Bottom*) IF staining for GFP (green), driven by Cre driver, and the parietal cell marker VEGFB (red).

Supplemental Figure 2: DT specifically kills PCs in gastroids.

Top) Gastroids from DTR mice with the *Atp4b*$^{+}$ parietal cell lineage fluorescing green (*Atp4b-Cre;LSL-DTR;ROSA*^{mTmG} mice) and all other lineages in red. The same gastroids were monitored over 3 days of control or DT treatment. Note DT treatment does not affect gastroid survival, but PCs are specifically extruded into lumen of gastroids by Day 1 (arrowheads in *inset*) and then are largely gone by Day 3. PC extrusion – which is consistent with cell death in these cultures – does not occur in controls. *Bottom*) Immunofluorescence co-staining with anti-GFP (green) and anti-PC-marker H^{+}/K^{+}ATPase (red) antibodies.

Supplemental Figure 3: Changes in protein localization for markers of SPEM and chief cell differentiation following TAM and DT.

A) Base of D14 DT treated unit with anti-E-Cadherin (green) and anti-GIF (red). (B-D) Immunofluorescence of stomachs following three days of vehicle, DT, or TAM injections. B) Red CD44v, Green: GSII, magenta: BrdU. C) Red: GIF, Green: Tff2. D) Red: GIF, Green: Clusterin.

Supplemental Figure 4: Quantitative real-time PCR of selected transcripts implicated in SPEM.

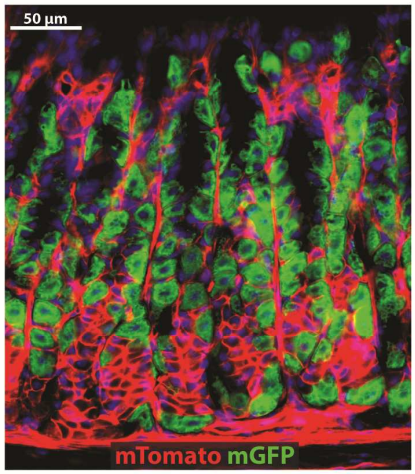
Transcripts were analyzed from RNA isolated from the whole gastric corpus of mice treated with vehicle, DT or TAM for three days. 12 transcripts with significant changes in experimental groups compared to control (* indicates $p \leq 0.05$, ** indicates $p \leq 0.01$) are shown.

Supplemental Figure 5: DMP-777 control showing deletion of PCs.

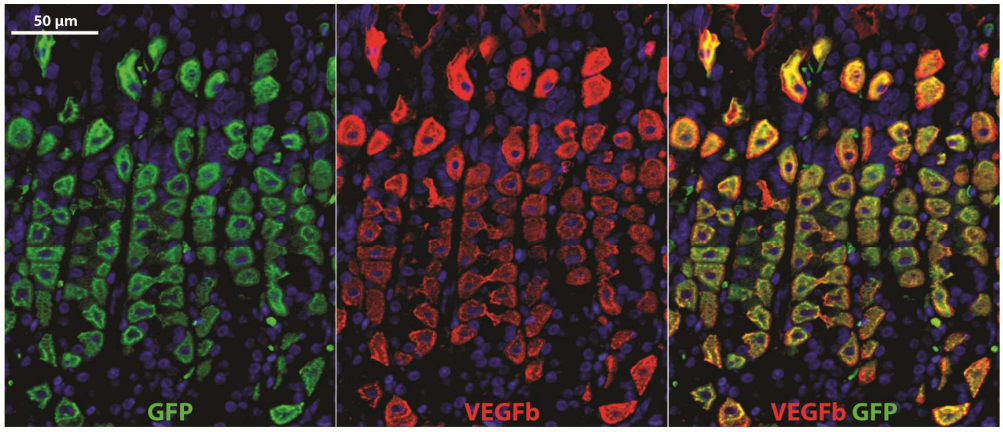
IF of stomachs following 16 days control, DT, or DT then DMP-777 for the PC marker VEGFB (red).

Supp Fig 1: *Atp4b-Cre;LSL-DTR* drives iDTR specifically in parietal cells

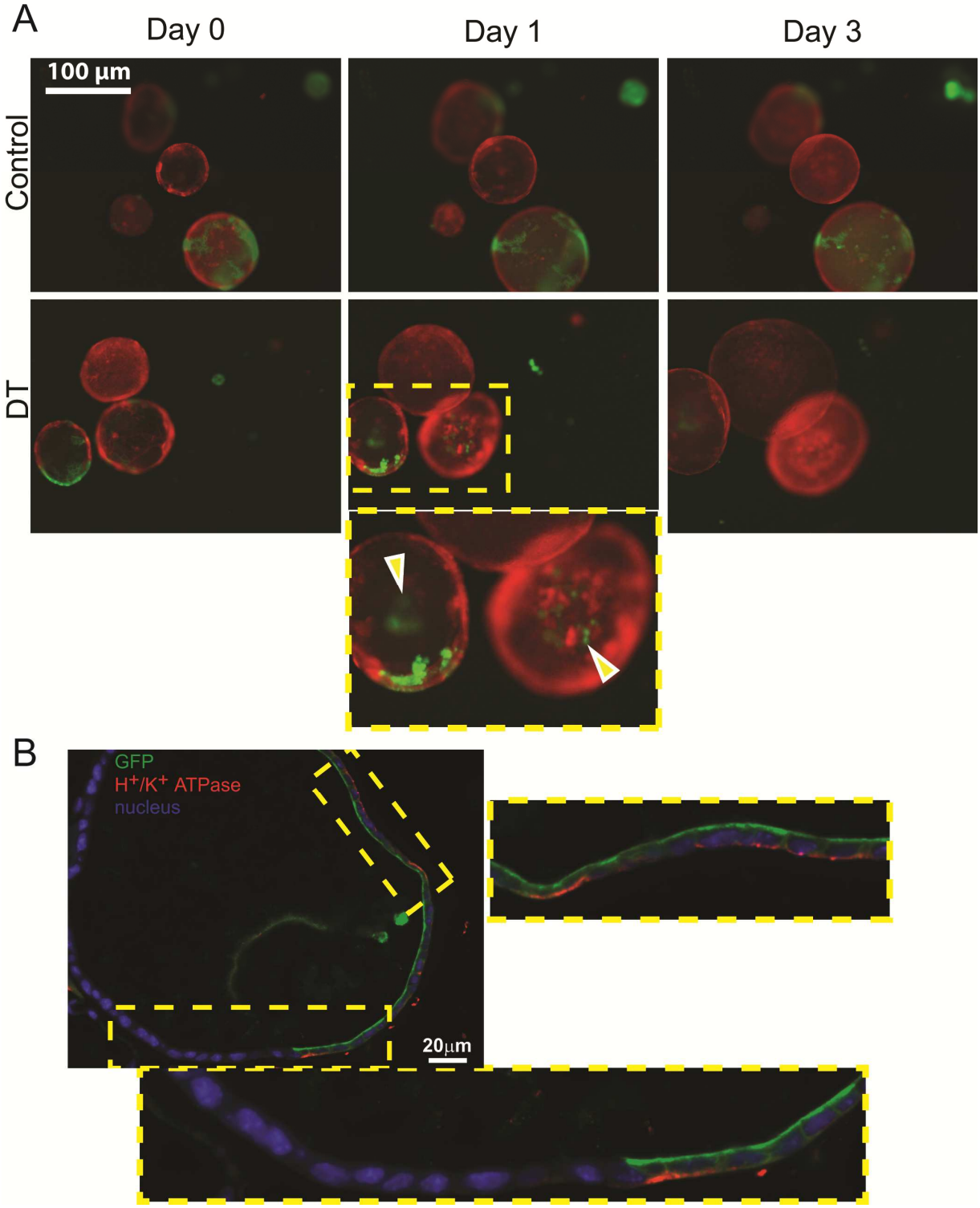
Natural Fluorescence



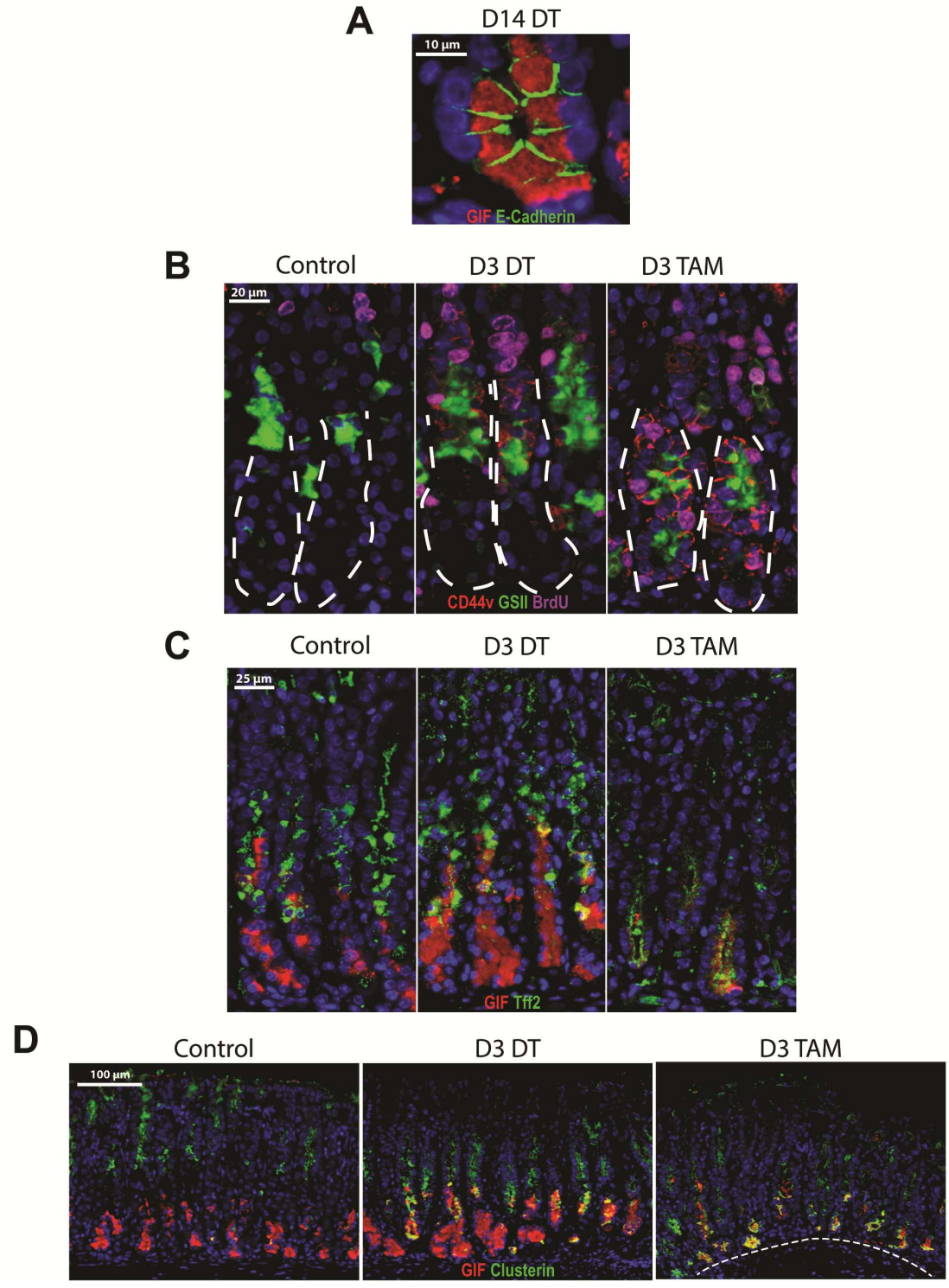
Immunofluorescence



Supp Fig 2: DT specifically kills parietal cells in gastroids



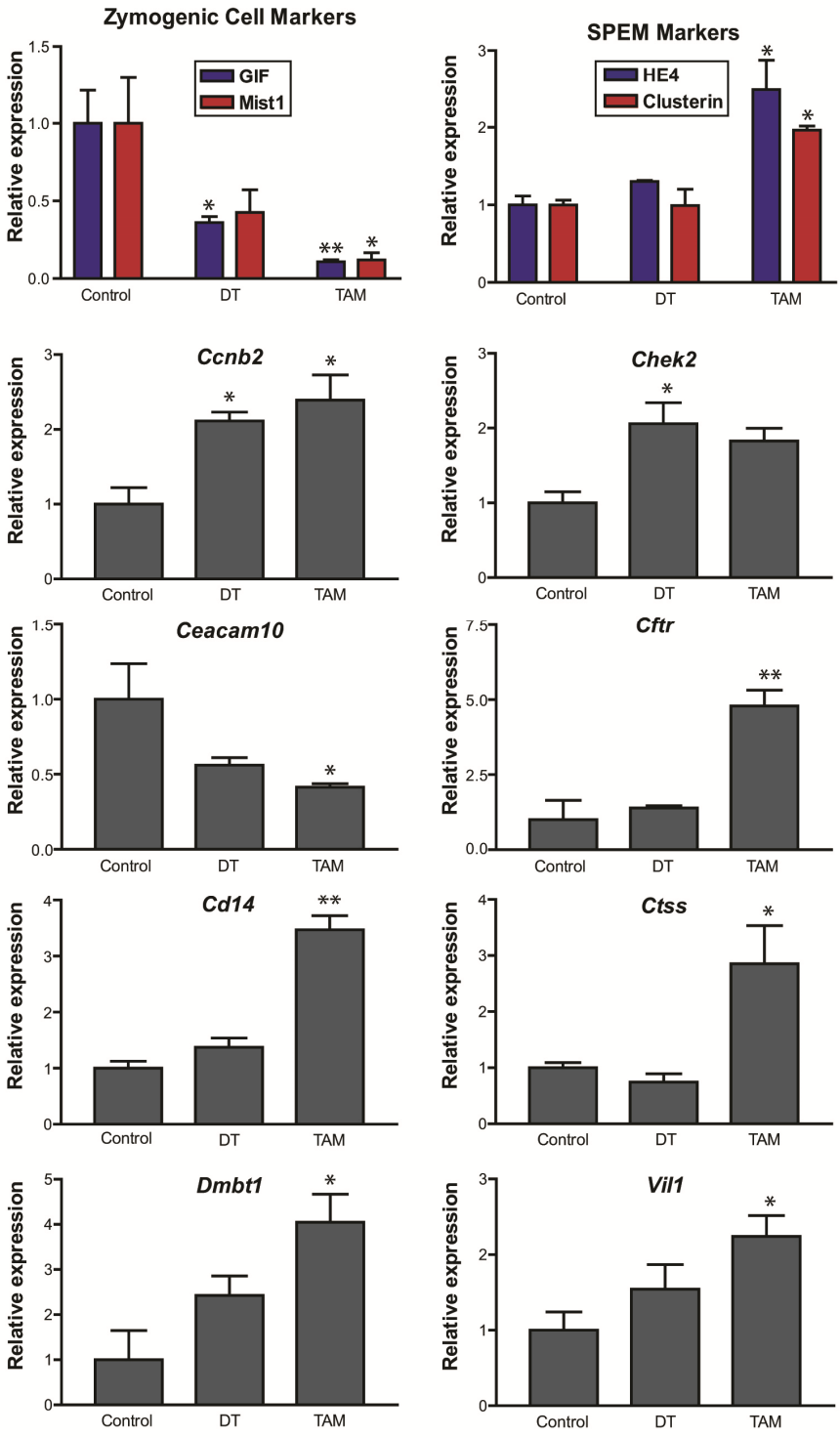
Supp Fig 3: Changes in protein localization following TAM and DT



Supp Fig 4: Quantitative Real-Time PCR of selected transcripts implicated in SPEM

No significant change

Arhgap9
Casp1
Ceacam1
Cenpk
Cxcl17
ETV5
Fignl1
Glipr1
Gpx2
Ly6a
Lyz2
Mad2l1
Mal2
Mmp12
Ms4a6b
Ms4a6c
Muc4
Pigr
Prom1
Slfn9
Tff2
Tmem48
Top2a
Traf4
Tyrobp
Ube2c



Supp Fig 5: DMP-777 control showing deletion of parietal cells