Supplemental Information:

Goblet Cell Associated Antigen Passages Support the Induction and Maintenance of Oral Tolerance

Devesha H. Kulkarni[1,3], Jenny K. Gustafsson[1,3], Kathryn A. Knoop[1], Keely G. McDonald[1], Shay S. Bidani[1], Jazmyne E. Davis[1], Alexandria N. Floyd[1], Simon P. Hogan[2], Chyi-Song Hsieh[1], and Rodney D. Newberry[1, *]

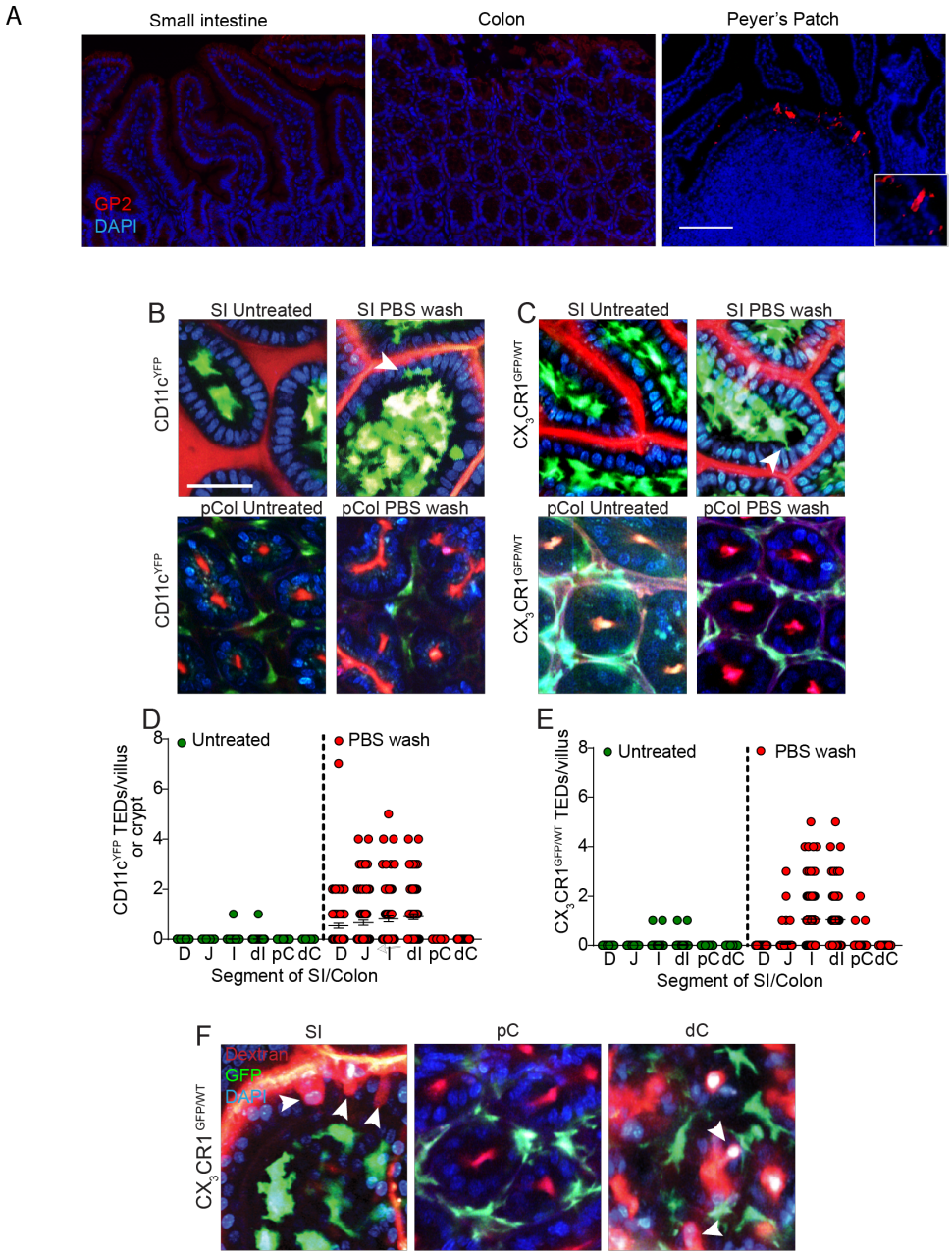
[1]Department of Internal Medicine, Washington University School of Medicine, Saint Louis, MO 63110

[2] Mary H. Weiser Food Allergy Center, University of Michigan School of Medicine, Ann Arbor, MI 48109

[3]These authors contributed equally to this work

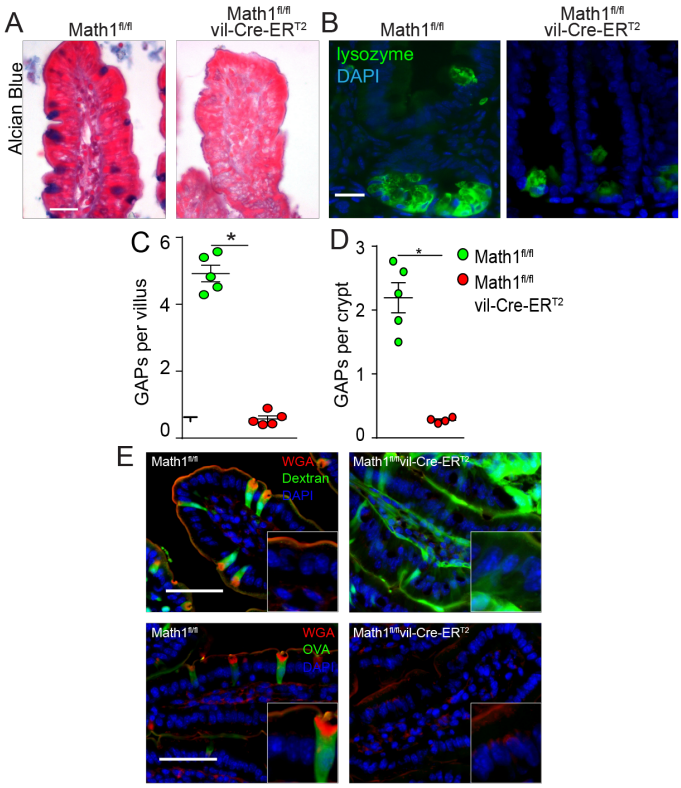
[*]Correspondence should be addressed to:
Rodney D. Newberry, M.D.
660 S. Euclid Avenue Box 8124
St. Louis, MO 63110
Ph: 314 362-2671
Fax: 314 362-2609
Email:rnewberry@wustl.edu

Supplemental Information:

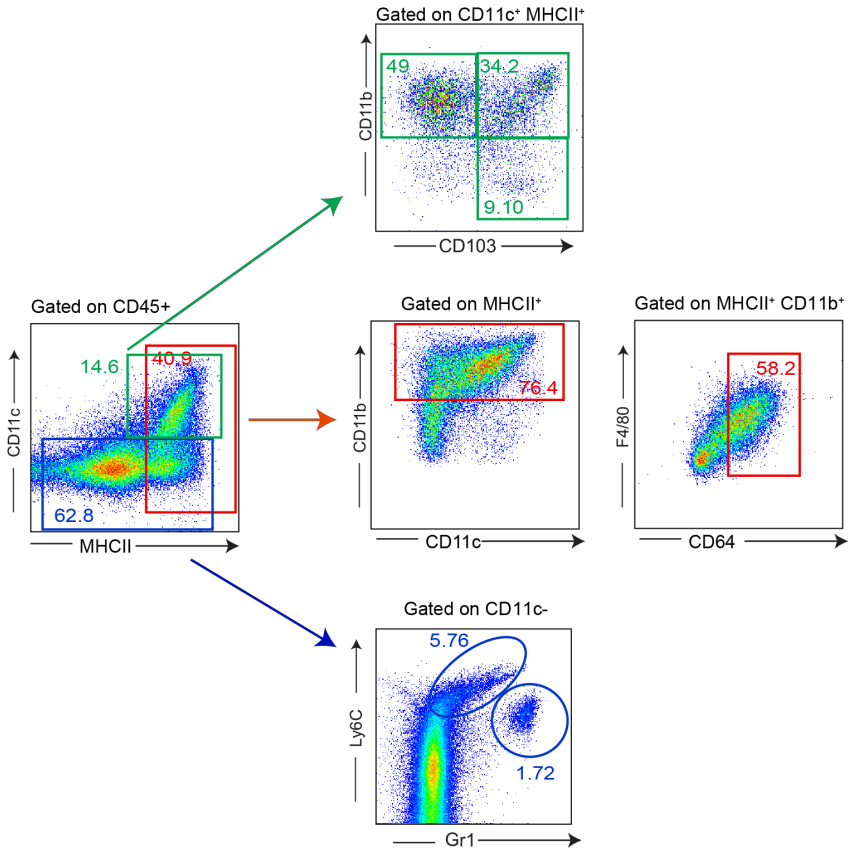


Supplemental Figure 1: *Villous M-cells and transepithelial dendrite (TED) extensions by LP-APCs are rare in the steady state, but TEDs are induced by removal of the luminal contents and mucus layer.* A) Photomicrographs of immunofluorescent staining for the M cell marker glycoprotein 2 (GP2) in the epithelium of the proximal ileum, proximal colon, and Peyer's patch. B and C) Images of SI (upper panels) and proximal colon (lower

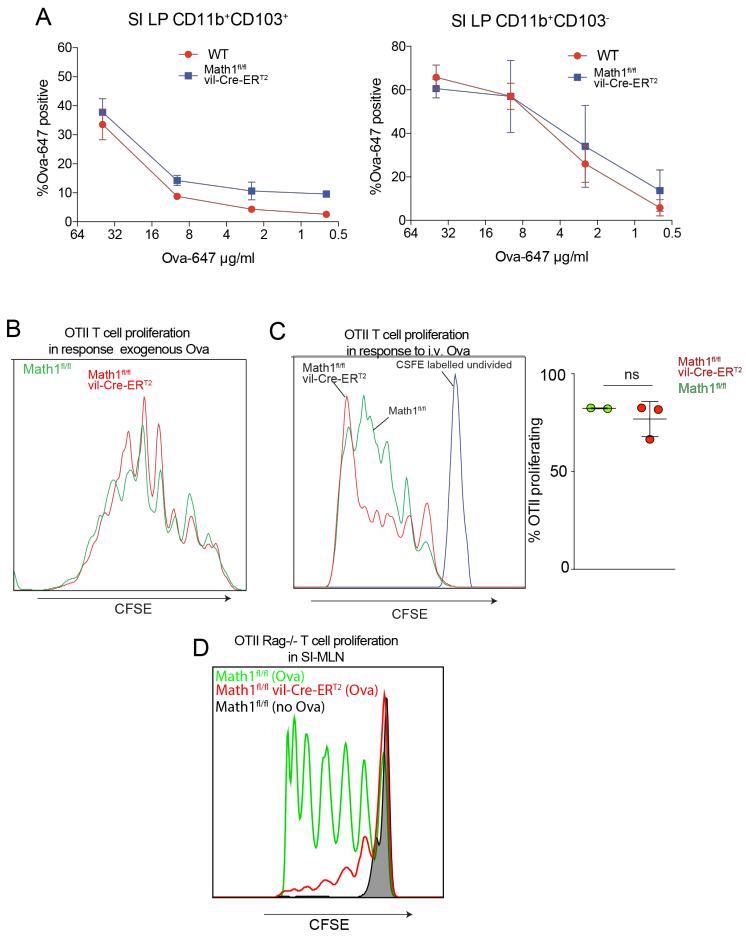
panels) obtained during *in vivo* two photon imaging of B) $CD11c^{YFP}$ reporter mice and C) $CX_3CR1^{GFP/WT}$ reporter mice demonstrate that TEDs are rare in the steady state and induced following the removal of the luminal contents and mucus layer by rinsing with PBS. Arrowheads denote LP-APC TED extension. D and E) Quantification of TED extension in regions of the GI tract in D) $CD11c^{YFP}$ reporter mice and E) $CX_3CR1^{GFP/WT}$ reporter mice that were untreated or treated with PBS to remove the luminal contents and mucus layer. F) Images from *in vivo* two photon imaging in SI and colon in $CD11c^{YFP}$ reporter mice, arrowheads denote GAPs. Panel A contains representative images from 4 mice in which immunohistochemistry for GP2 was performed on multiple sections of duodenum, jejunum, ileum, proximal colon, and distal colon. D=Duodenum, J=jejunum, I = proximal ileum, dI = distal ileum, pC = proximal colon, dC = distal colon. Scale bars = 50μm. Panels B, C and F are representative images, each dot in panels D and E represents a single villus or crypt imaged from surface to base. n = 8 $CD11c^{YFP}$ reporter mice and 8 $CX_3CR1^{GFP/WT}$ reporter mice. Scale bar: panel A 100 μm.



Supplemental Figure 2*: Inducible deletion of Math1 in intestinal epithelial cells results in goblet cell deletion, reduction of GAPs and increased leak of low molecular weight substances from the gut lumen.* A) Photomicrographs of Alcian blue staining and B) immunofluorescent staining for lysozyme on SI sections from Math1$^{f/f}$ mice and Math1$^{f/f}$vil-Cre-ER^{T2} mice treated with tamoxifen. C) Quantification of GAPs per SI villus in Math1$^{f/f}$ mice and Math1$^{f/f}$vil-Cre-ER^{T2} mice treated with tamoxifen. D) GAP density in the distal colon of Math1$^{f/f}$ mice and Math1$^{f/f}$vil-Cre-ER^{T2} mice treated with tamoxifen. E) Fluorescent microscopy of fixed tissue section of SI from Math1$^{f/f}$ mice and Math1$^{f/f}$vil-Cre-ER^{T2} mice treated with tamoxifen and given 3kD lysine fixable FITC dextran or FITC Ova in the gut lumen demonstrating leak of low molecular weight dextran but not Ova when goblet cells are deleted. Each data point represents an individual mouse, images are representative of two or more mice for each treatment group.

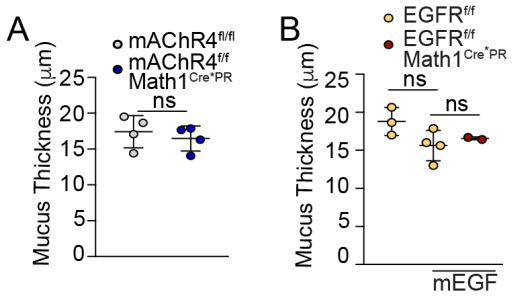


Supplemental Figure 3: Flow cytometric gating strategy to identify LP-APC subsets. LP populations were gated on forward scatter, side scatter, and CD45+ (not shown). Populations were subsequently gated as CD11c+ MHCII+ (green) and analyzed for CD11b and CD103 expression to identify CD103+ CD11b-, CD103+ CD11b+, and CD103- CD11b+ LP-APCs, gated as MCHII+ (red) and analyzed for CD11b+ F4/80+ CD64+ macrophages, or gated as MHCII- (blue) and analyzed as Ly6C (hi) monocytes or Gr1+ Ly6C (int) neutrophils.

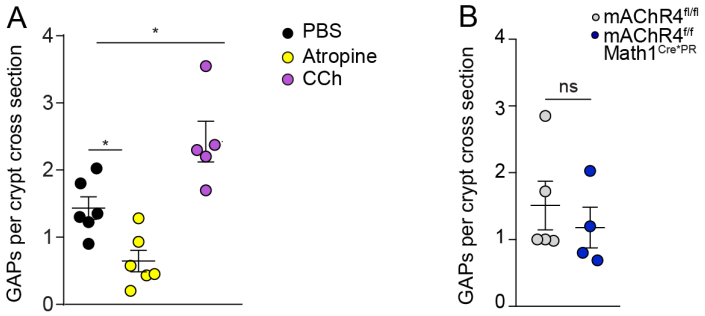


Supplemental Figure 4: Goblet cell deficient mice have functional *LP-APCs and lower levels of dietary Ova specific T cell proliferation in the MLN.* A) Quantification of fluorescent Ova uptake in culture by LP-APCs isolated from mice lacking goblet cells (Math1$^{f/f}$ vil-Cre-ER^{T2} mice) and controls as assessed by flow cytometry. B) Histograms demonstrating CFSE dilution by OTII T cells cultured with Ova and LP-APCs isolated from mice lacking goblet cells and their littermate controls. C) Histograms of CFSE dilution and quantification of proliferation by OTII T cells in the MLN that were adoptively transferred into mice lacking goblet cell and their littermate control following i.v. (systemic) Ova administration. D) Histograms of CFSE dilution OTII Rag-/- T cells adoptively transferred into mice lacking goblet cells or their littermate controls demonstrating residual CFSE dilution following goblet cell deletion. ns = not significant. Data is presented as the mean

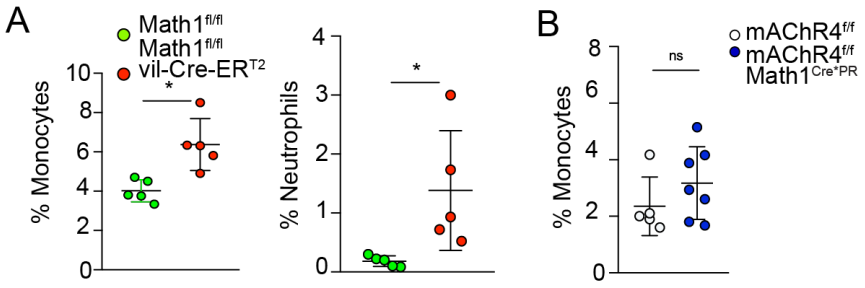
+/- SEM. Panel A represents data obtained from pooling APCs from two or more mice from each genotype. Histograms in panels B-D are representative of two or more mice for each condition. Each data point represents an individual mouse in panel C.



Supplemental Figure 5: Inhibition of GAPs does not alter the thickness of the mucus layer. Thickness of the mucus layer on Carnoy's fixed SI sections from A) mice with inducible deletion of mAChR4 in goblet cells and their littermate controls or B) mice with inducible deletion of EGFR in goblet cells or their littermate controls treated with mEGF. ns = not significant. Each data point represents an individual mouse.



Supplemental Figure 6: *GAPs in the distal colon are not dependent upon mAChR4 signaling.* A) Quantification of GAPs in the distal colon of mice given PBS, the pan acetylcholine muscarinic receptor antagonist atropine, or the cholinergic agonist carbamycholine (CCh) or B) in mice lacking mAChR4 in goblet cells ($mAChR4^{f/f}$ $Math1^{Cre*PR}$ mice) and their littermate controls * = $P < 0.05$, ns = not significant. Data is presented as the mean +/- SEM. Each data point represents an individual mouse.



Supplemental Figure 7: Deletion of goblet cells, but not inhibition of GAPs results in the influx of inflammatory cells. A) Monocytes and neutrophils in the SI lamina propria of inducible goblet cell deficient mice or their littermate controls. B) Monocytes in the SI lamina propria of mice with inducible deletion of mAChR4, to inhibit GAPs, or their littermate controls. * = P <0.05, ns = not significant. Each data point represents an individual mouse.

Supplemental movie 1: *LP-APCs extend TEDs after washing with PBS to remove the luminal contents and the mucus layer.* Two photon in vivo imaging of $CD11c^{YFP}$ reporter mice following the administration of luminal dextran (red) and luminal DAPI (blue) to stain epithelial cell nuclei. (left side) With the luminal contents and mucus layer undisturbed, LP-APCs probe the epithelium, remain compact and do not extend dendrites into the lumen denoted by red luminal dextran. (right side) Approximately 10 minutes following the removal of the luminal contents and mucus layer by washing with PBS, LP-APCs extend more dendrites within the LP, extend dendrites into the lumen, with some LP-APCs migrating into the epithelium (white arrow).

Supplemental Table I: Antibodies and staining reagents

Reagent	Clone	Manufacturer
Dextran Tetramethylrhodamine		Invitrogen
DAPI (4',6-Diamidino-2-phenylindole, Dihydrochloride)		Invitrogen
Fluorescein Ulex Europaeus Agglutinin I (UEA1)		Vector Laboratories
Fluorescein Wheat Germ Agglutinin (WGA)		Vector Laboratories
Ovalbumin Texas Red		Invitrogen
BSA		Invitorgen
PNA		Vector Laboratories
ABL		Vector Laboratories
LPS		Sigma
Ovalbumin Alexa Fluor 647		Invitrogen
Dextran FITC 4,000 MW		Invitrogen
Aldefluor		Stemcell Technologies
CSFE		eBioscience
anti-lysozyme	PA5-16668	Invitrogen
anti-chromogranin A	PA5-16685	Invitrogen
anti-GP2	2F-11-C3	MBL
anti-CD45	30-F11	eBioscience
anti-CD45.1	A20	eBioscience
anti-MHCII	NIMR-4	eBioscience
anti-CD11c	N418	eBioscience
anti-CD103	M290	BD Pharmingen
anti-Vβ5	MR9-4	eBioscience
anti-Vα2	B20.1	eBioscience
anti-CD62	MEL-14	BD Pharmingen
anti-CD11b	M1/70	eBioscience
anti-F4/80	BM8	eBioscience
anti-IL-10	JES5-16E3	eBioscience
anti-Foxp3	FJK-16s	eBioscience
anti- Helios	22F6	eBioscience
anti-RoRγt	B2D	eBioscience
anti-CD4	GK 1.5	eBioscience
anti-α4β7	DATK32	Southern Biotech
anti- CCR9	CW-1.2	eBioscience
anti-IRF4	3E4	eBioscience
anti- CD64	X54—5/7.1	Biolegend
anti-Ly6G	HK1.4	Invitrogen
anti-Gr1	RB6-8C5	eBioscience

Materials and Methods

Mice: All mice were 10 or more generations on the C57BL/6 background, with the exception of the mAChR4$^{fl/fl}$ mice, which were 6-7 generations on the C57BL/6 background at the time of these studies. C57BL/6 mice, congenic CD45.1 B6SJL mice, OTII T-cell receptor transgenic mice[1], CD11c^{YFP} transgenic mice[2], CX_3CR1^{GFP} mice[3], Math1$^{fl/fl}$ mice[4], FoxP3^{GFP} mice[5], were purchased from The Jackson Laboratory (Bar Harbor, ME) or The National Cancer Institute (Frederick, MD). Transgenic mice in which a tamoxifen-dependent Cre recombinase is expressed under the control of the villin promoter (vil-Cre-ERT2) mice[6] were a gift from Sylvie Robine (Institut Curie, Paris, France). Math1$^{fl/fl}$ mice were bred to vil-Cre-ER^{T2} mice to generate mice with inducible depletion of goblet cells following deletion of Math1 in villin expressing cells. Math1$^{fl/fl}$vil-Cre-ER^{T2} mice and the injection protocol to induce goblet cell deletion have been previously described[7]. EGFR$^{fl/fl}$ mice[8] were a gift from Dr. David Threadgill, University of North Carolina. mAChR4$^{fl/fl}$ mice [9] were a kind gift from Jurgen Wess (National Institute Health, Bethesda, MD). EGFR$^{fl/fl}$ mice and mAChR4$^{fl/fl}$ mice were bred to Math1^{Cre*PR} mice [10] to generate mice with an inducible deletion of EGFR or mAChR4 in goblet cells. These mice and Cre negative littermate controls were injected i.p. with mifepristone (10mg/kg) every day starting four days prior to use in experiments. Mice were bred in house and cohoused littermates were used as experimental controls. Mice were housed in a specific-pathogen-free facility and fed routine chow diet. Mice of both sexes were used in this study. Animal procedures and protocols were performed in accordance with the IACUC at Washington University School of Medicine.

Intravital two-photon (2P) microscopy: Mice were anesthetized using nebulized isofluorane in 95% O_2/5% CO_2. Intravital preparation of the intestine was performed as previously described[11]. To detect GAPs, lysine fixable fluorescently-labeled dextran

10,000 MW (10 mg/mL) and diamidino-2-phenylindole (DAPI; 10 mg/mL) were injected intraluminally 20 minutes prior to imaging, and imaging was performed for up to one hour. To detect the extension of trans-epithelial dendrites by APCs, $CD11c^{YFP}$ or CX^3CR1^{GFP} mice were imaged as above using fluorescent dextran to outline the epithelial surface. In some experiments the luminal contents and mucus layer were removed prior to imaging by gently flushing the lumen 3 times with 1ml of 37°C tissue culture grade PBS. Tissues were excited using a Ti:sapphire laser tuned to 890nm (Chameleon XR, Coherent, Santa Clara, CA). Time-lapse imaging was performed with a custom-built 2P microscope running ImageWarp acquisition software (A&B Software, New London, CT). Epithelial integrity was assessed by dextran and DAPI staining as described[11]. Following imaging, tissues were placed in 10% formalin buffered phosphate solution (Fisher Scientific, Fair Lawn, NJ) to fix dextran in place to confirm 2P findings and for further analysis by immunofluorescence microscopy.

Evaluation of luminal antigen uptake by epithelial cells: Tetramethylrhodamine-labeled 10 kD dextran or Texas Red labeled ovalbumin was administered in the SI, proximal and distal colon of anesthetized mice. After 1 hour, mice were sacrificed, and tissues thoroughly washed with cold PBS before fixing in 10% formalin buffered solution. Tissues were embedded in optimal cutting temperature compound (Fisher Scientific, Pittsburgh, PA) and 6 μm sections prepared. For studies in Figure 1, sections were stained with wheat germ agglutinin (WGA), *Ulex europaeus* agglutinin I (UEA I), anti-lysozyme antibodies, or anti-chromogranin A antibodies to identify goblet cells in the SI, goblet cells in the colon, Paneth cells, and enteroendocrine cells respectively. Sections were then stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, St Louis, MO) and imaged using an

Axioskop 2 microscope with a Plan-Neofluar 20x/0.5 objective (Carl Zeiss Microscopy, Thornwood, NY).

Enumeration of GAPs: In two photon imaging, GAPs were identified as dextran-filled columns measuring approximately 20μm (height) x 5μm (diameter) traversing the epithelium and containing a nucleus. SI GAPs were enumerated as GAPs per villus. GAPs in the distal colon were predominantly within the crypts and were enumerated as GAPs per crypt. The number of GAPs per villus and GAPs per crypt seen by 2P imaging were confirmed by immunofluorescence microscopy of tissues that were fixed following 2P imaging. In Figure 1 GAPs were identified as UEA I or WGA stained epithelial cells containing fluorescent ovalbumin. For other studies using fixed tissue sections, GAPs were identified as fluorescent dextran or fluorescent ovalbumin filled epithelial cells with goblet cell morphology measuring approximately 20 μm (height) x 5 μm (diameter) traversing the epithelium and containing a nucleus, and were enumerated as GAPs/villus cross section in the SI or GAPs/crypt cross section in the colon. In some studies goblet cells were identified by alcian blue staining (Sigma Aldrich, St Louis, MO) performed per the manufacturer's recommendations.

Intestinal Permeability Assay: Mice were removed from food for 4hr with ad libitum water supply. Mice were gavaged with 400mg/kg body weight 4kD FITC dextran (Sigma Aldrich, St. Louis, MO) or 40kD FITC-dextran (Thermo Fisher). After 4hr incubation serum was collected. Twenty μl of serum were aliquoted into a black 96-well plate and mixed with 80 μl PBS. A standard curve was generated from the 4 kD or 40kD FITC dextran with known concentrations. The intensity of fluorescence in sera was then measured using excitation 485/20 and emission 528/20. To visualize leak, 400mg/kg 3kD lysine fixable FITC dextran

(Thermo Fisher) was injected into the small intestine lumen of anesthetized mice and tissues harvested two hours later and placed in 10% formalin buffered solution.

Isolation of cellular populations and flow cytometry: SIs or colons were harvested, rinsed with PBS, and Peyer's patches were removed from the SI tissue. Epithelial cellular populations were released by incubating for 15 min three times in a 37°C rotating incubator in HBSS media (BioWhittaker, Walkersville, MD) containing 5 mM EDTA. Splenic and LP cellular populations were isolated as described previously[12]. Antibodies and other staining reagents used are listed in supplemental information table S1. SI and colonic APCs were identified as $CD45^+$, $CD11c^+$, $MHCII^+$. $CD11b^+$, and $CD103^+$ or $CD103^-$ for flow cytometry assisted cell sorting. For intracellular antigens and cytokines, cells were stimulated with PMA and ionomycin in the presence of brefeldin A, fixed, permeabilized overnight, and stained per the manufacturer's recommendations (eBioscience, San Diego, CA). Flow cytometry was performed with a FACScan cytometer (BD Biosciences, San Jose, CA) retrofitted with additional lasers or a four laser Attune NxT cytometer (Invitrogen, Carlsbad, CA). Flow cytometry assisted cell sorting was performed using a Sony iCyt Synergy (San Jose, CA). Data acquisition and analysis was performed using FlowJo (Tree Star, Ashland, OR) or Attune NxT (Invitrogen) software.

Analysis of luminal fluorescent antigen uptake by LP-APCs: Mice were anesthetized and 200 µg of Alexa Fluor 647 labeled ovalbumin (Ova-A647), dissolved in phosphate buffered saline (PBS), or PBS alone (controls), was injected into the SI lumen, or given via enema using a 16G plastic cannula inserted 3cm transanal into the colon. In some experiments, anesthetized mice were treated intraluminally with 10 µg murine EGF (Shenandoah Biotechnology, Warwick, PA) dissolved in PBS, or PBS alone 20 minutes prior to Ova-A647 administration. Two hours later cellular populations were isolated from the non-

Peyer's patch bearing SI or distal colon as described previously[12]. The distal colon segment represents the last two cm of the colon. Isolated LP cells were stained for APC markers and evaluated for Ova-A647 positive staining by flow cytometry.

Analysis of luminal antigen delivery to LP-APCs and induction of T cell proliferation in vitro: Mice were anesthetized and 2mg of ovalbumin (Ova) dissolved in phosphate buffered saline (PBS), or PBS alone (controls), was injected intraluminally into the SI. For delivery of Ova by enema or a 16g plastic cannula was inserted 3 cm transanal into the colon. In some experiments, anesthetized mice were intraluminally treated with 10μg murine EGF (Shenandoah Biotechnology, Warwick, PA) 20 minutes prior to Ova administration. Two hours later cellular populations were isolated from the non-Peyer's patch bearing SI LP. APC populations and Ova specific CD4+ OTII T cells were isolated with flow cytometric cell sorting and cultured at a ratio of 1:10 APCs ($1x10^4$) to T-cells ($1x10^5$). As a positive control, 10μg Ova was added to cultures of APC populations isolated from mice receiving luminal PBS. After 3 days, cultures were evaluated for the number of T-cells by flow cytometry and cell counting.

Adoptive T cell transfer and analysis of in vivo antigen specific T cell responses to luminal Ova: To evaluate the role of goblet cells and GAPs on delivery of luminal antigen and antigen specific T cell proliferation in the draining lymph nodes, single cell suspensions of Ova-specific T cells were prepared from spleens and MLNs of $CD45.1^+$ OTII T cell receptor transgenic mice, and CD4 T cell enrichment was performed using magnetic beads (Stemcell Technology, Vancouver, BC). Enriched $CD4^+$ T cells were labeled with 2μM CFSE (Invitrogen, Carlsbad, CA) and $2×10^6$ CFSE-labeled cells were *i.v*. transferred into sex matched recipient mice. Twenty-four hours after transfer, mice were orally gavaged with 15 mg Ova (Sigma-Aldrich, St. Louis, MO) or in some experiments

mice were administered 25 mg of ovalbumin via enema using a 16G plastic cannula as above. $EGFR^{fl/fl}$ or $EGFR^{fl/fl}Math1^{Cre*PR}$ mice, were administered with 10μg of murine EGF 20 minutes prior to receiving 15 mg ovalbumin in saline orally. Two days later SI draining MLNs or distal colon draining caudal and iliac LNs were removed and single-cell suspensions were prepared and analyzed by flow cytometry for CD45.1, CD3, Vβ5, Vα2 and CSFE. To evaluate the effect of systemic antigen administration on transferred T cells, 24 hours post adoptive transfer 200 μg of Ova was administered *i.v.* and transferred T cells evaluated on the same schedule as described above.

pTreg generation in vivo and in vitro: To evaluate *de novo* induction of pTreg cells, single cell suspensions from spleen and MLNs from Ova-specific $CD45.1^{+}$ $Foxp3^{GFP}$ OTII T cell receptor transgenic mice were flow cytometrically sorted for GFP^{-}, $V\beta5^{+}$, $V\alpha2^{+}$, $CD45.1^{+}$, $CD62^{hi}$ cells. 5×10^{5} cells were *i.v.* administered into recipient $Math1^{fl/fl}$vil-Cre-ER^{T2} or $Math1^{fl/fl}$ mice 7 days after start of tamoxifen treatment. Recipient mice were gavaged with 15 mg Ova, and SI draining MLNs were evaluated five days later for $Foxp3^{GFP+}$ cells among the transferred cells. To evaluate the *de novo* generation of pTregs *in vitro* naive $Foxp3^{GFP-}$ CD45.1 OTII T cells were isolated as above and cultured with flow cytometrically sorted LP-APCs at a ratio of 10:1 with 40μg of exogenous Ova. Five days later cultures were harvested and evaluated for $Foxp3^{GFP+}$ expression by T cells.

ALDH activity: To evaluate the expression of ALDH in DCs, intestinal LP cells were stained using ALDEFLUOR (StemCell Technologies, Vancouver, BC, Canada) per the manufacturer's recommendations. Briefly, 5 x 106 cells were resuspended in 200ul of assay buffer containing 10ul Aldefluor reagent without or with 5ul of the ALDH inhibitor diethylaminobenzaldehyde (DEAB). Samples were incubated for 30 minutes in the dark, following which surface staining for DC markers was performed using assay buffer.

ALDH+ Cells were identified as cells with brighter fluorescent intensity than a DEAB-inhibited sample run in parallel from corresponding tissue.

Analysis of CCR9 and α4β7 induction by T cell in vitro: Cellular populations were isolated from the non-Peyer's patch bearing SI LP of mice lacking goblet cells and littermate controls. CD11c+MHCII+CD103+CD11b+ populations and Ova specific CD4+ OTII T cells were isolated with flow cytometric cell sorting. Cell were cultured at a ratio of 1:10 APCs ($1x10^4$) to T-cells ($1x10^5$) and 2μg Ova was added each well. After 3 days, cultures were evaluated for the expression of CCR9 and α4β7 on T-cells by flow cytometry.

Measurement of mucus thickness: To determine the thickness of mucus layer, SI tissue containing luminal matter were fixed in Carnoy's fixative overnight. Subsequently, tissues were passed reducing concentration of methanol, before being embedded in OCT. Tissue sections were cut to a thickness of 6μm and slides were dried to room temperature before staining with Alcian Blue for mucus.

Oral tolerance and Delayed Type Hypersensitivity Responses: Mice were given Ova 20g/L in drinking water, or drinking water alone for two weeks, or alternatively were gavaged with 20mg Ova daily for two weeks concurrent with gavage of 10μg murine EGF or given 25 mg Ova via enema. Two weeks and four weeks following dietary Ova exposure mice were immunized subcutaneously with 100μg Ova in incomplete Freund's Adjuvant (Sigma Aldrich). Two weeks after the last immunization mice were challenged with 20μg Ova in the footpad and the change in footpad thickness evaluated using measurements taken with micrometer calipers before and 24 hours after challenge. Blood was collected 24

hours after footpad challenge and serum levels of IFNγ were measured using Mouse IFNγ ELISA kit (eBioscience, San Diego, CA), according to manufacturer's protocol.

Statistical Analysis: Data analysis using a two sided student's *t* test for studies involving two groups or one way ANOVA with a Dunnett's or Tukey's posttest with correction for multiple comparisons for studies involving 3 or more groups was performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA). A cut off of $p<0.05$ was used for significance.

References:

1 Barnden, M. J., Allison, J., Heath, W. R. & Carbone, F. R. Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. *Immunol Cell Biol* **76**, 34-40. (1998).

2 Lindquist, R. L. *et al.* Visualizing dendritic cell networks in vivo. *Nat Immunol* **5**, 1243-1250, doi:10.1038/ni1139 (2004).

3 Jung, S. *et al.* Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol* **20**, 4106-4114 (2000).

4 Shroyer, N. F. *et al.* Intestine-specific ablation of mouse atonal homolog 1 (Math1) reveals a role in cellular homeostasis. *Gastroenterology* **132**, 2478-2488, doi:10.1053/j.gastro.2007.03.047 (2007).

5 Kim, J. M., Rasmussen, J. P. & Rudensky, A. Y. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat Immunol* **8**, 191-197, doi:10.1038/ni1428 (2007).

6 el Marjou, F. *et al.* Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genesis* **39**, 186-193, doi:10.1002/gene.20042 (2004).

7 Knoop, K. A., McDonald, K. G., McCrate, S., McDole, J. R. & Newberry, R. D. Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. *Mucosal Immunology* **8**, 198-210, doi:10.1038/mi.2014.58 (2015).

8 Lee, T. C. & Threadgill, D. W. Generation and validation of mice carrying a conditional allele of the epidermal growth factor receptor. *Genesis* **47**, 85-92, doi:10.1002/dvg.20464 (2009).

9 Jeon, J. *et al.* A subpopulation of neuronal M4 muscarinic acetylcholine receptors plays a critical role in modulating dopamine-dependent behaviors. *J Neurosci* **30**, 2396-2405, doi:10.1523/JNEUROSCI.3843-09.2010 (2010).

10 Rose, M. F., Ahmad, K. A., Thaller, C. & Zoghbi, H. Y. Excitatory neurons of the proprioceptive, interoceptive, and arousal hindbrain networks share a developmental requirement for Math1. *Proc Natl Acad Sci U S A* **106**, 22462-22467, doi:10.1073/pnas.0911579106 (2009).

11 McDole, J. R. *et al.* Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature* **483**, 345-349, doi:10.1038/nature10863 (2012).

12 McDonald, K. G. *et al.* Epithelial expression of the cytosolic retinoid chaperone cellular retinol binding protein II is essential for in vivo imprinting of local gut dendritic

cells by lumenal retinoids. *Am J Pathol* **180**, 984-997, doi:10.1016/j.ajpath.2011.11.009 (2012).