Supplementary Materials for

Mucus Enhances Gut Homeostasis and Oral Tolerance by Delivering Immunoregulatory Signals

Meimei Shan, Maurizio Gentile, John R. Yeiser, A. Cooper Walland, Victor U. Bornstein, Kang Chen, Bing He, Linda Cassis, Anna Bigas, Montserrat Cols, Laura Comerma, Bihui Huang, J. Magarian Blander, Huabao Xiong, Lloyd Mayer, Cecilia Berin, Leonard H. Augenlicht, Anna Velcich, Andrea Cerutti*

*To whom correspondence should be addressed. E-mail: andrea.cerutti@mssm.edu or acerutti@imim.es

Published 26 September 2013 on *Science* Express
DOI: 10.1126/science.1237910

This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencemag.org/cgi/content/full/science.1237910/DC1)

Materials and Methods

Mice

6-10 weeks old WT C57/BL6, OT-II and *Lgals3*$^{-/-}$ mice were purchased from the Jackson Laboratory (Bar Harbor). *Fcgr2b*$^{-/-}$ mice were purchased from Taconic (Hudson, NY). *Clec7a*$^{-/-}$ mice were obtained from Amariliz Rivera (University of Medicine and Dentistry of New Jersey). To obtain viable mice with a selective *Ctnnb1* gene deletion in hematopoietic cells, *Ctnnb1*$^{fl/fl}$ mice from the Jackson Laboratory were crossed with transgenic mice expressing Cre recombinase under the control of the *Vav1* gene promoter as previously described (*29, 30*). All these mice were maintained under specific pathogen-free conditions in a barrier facility at Mount Sinai School of Medicine. *Muc2*$^{-/-}$ mice were maintained in a barrier facility at Albert Einstein School of Medicine. In this facility, *Muc2*$^{-/-}$ mice showed neither spontaneous colitis nor systemic invasion by gut bacteria nor intestinal tumors for up to 4 months. Only 6-10 weeks old *Muc2*$^{-/-}$ mice were used in this study. The Animal Care and Use Committee of Mount Sinai School of Medicine and Albert Einstein School of Medicine approved all the experiments performed with mice.

Cells

Mouse. SI-LP, PP, MLN and splenic mononuclear cells were obtained after processing tissue samples with type-IV collagenase for 20 minutes. Total CD11c^{+} DCs were MACSorted from mononuclear cells according to the manufacturer's instructions (Miltenyi Biotech). Intestinal and splenic CD103^{+}CD11b^{-}CX3CR1^{-}, CD103^{+}CD11b^{+} CX3CR1^{-} and CD103^{-}CD11b^{+}CX3CR1^{+} DC subsets were FACSorted from DC-enriched suspensions using Abs to the common leukocyte antigen CD45, the major

histocompatibility class-II (MHC-II) molecule I-Ab, CD11c, CD11b, CD103 and CX3CR1 (table S1). DCs were gated as $CD11c^{+}CD45^{+}MHC\text{-}II^{high}$ cells. BM-derived DCs were obtained after culturing BM mononuclear cells in complete RPMI medium supplemented with granulocyte monocyte-colony stimulating factor (GM-CSF) and 5% fetal bovine serum (FBS). Semi-adherent cells were harvested on ice on day 6 and replated immediately in fresh medium with 5% FBS. Splenic $CD4^{+}$ T cells, splenic naïve $CD4^{+}CD62L^{+}$ OT-II T cells and DC-induced $CD4^{+}CD25^{hi}$ OT-II Treg cells were MACSorted as recommended by the manufacturer (Miltenyi Biotech). IECs were isolated from the SI after microdissecting serous and muscular layers, PPs and visible isolated lymphoid follicles. Live $CD45^{-}$ IECs were FACSorted from cell suspensions obtained after processing the tissue with EDTA for 30 min., followed by centrifugation at 1000 rpm.

Human. Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats purchased from the New York Blood Center. $CD14^{+}$ monocytes were MACSorted from PBMCs as specified by the manufacturer (Miltenyi Biotech). Monocyte-derived $CD11c^{+}$ DCs (termed human DCs in the text, unless otherwise specified) were obtained by culturing $CD14^{+}$ monocytes with IL-4 and GM-CSF, whereas $CD1c^{+}$ DCs, naïve $CD4^{+}CD45RA^{+}CD45RO^{-}$ T cells and $CD4^{+}CD25^{hi}Foxp3^{+}$ Treg cells were MACSorted from PBMCs or DC-activated $CD4^{+}$ T cells as instructed by the manufacturer (Miltenyi Biotech). The phenotype of these cells was analyzed by FC using specific Abs (table S2). Caco-2 and LS174T are IEC and GC-like lines, respectively. Caco-2 generates a polarized monolayer with intercellular tight junctions as primary IECs do and thus were used in all IEC functional assays (*31*).

Cultures and reagents

Mouse. All cells were cultured in RPMI 1640 medium supplemented with 10% FBS. To generate DCs, BM-derived mononuclear cells were cultured with 1000 U/ml GM-CSF (PreproTech) for 6 days. BM-derived, SI-LP or splenic DCs (5×10^4/ml) were pulsed with 1 μg/ml OVA323-339 peptide (ISQVHAAHAEINEAGR) in the presence or absence of 50 μg/ml MUC2 purified from the mouse SI.

Human. T cells, DCs and 293 embryonic kidney cells were cultured in RPMI 1640 medium supplemented with 10% FBS. To generate DCs, monocytes were incubated with 1000 U/ml GM-CSF and 1000 U/ml IL-4 (R&D Systems) for 6 days. Monocyte-derived or primary myeloid $CD1c^+$ DCs (1×10^5/ml) were exposed to 10 ng/ml LPS (Sigma-Aldrich), 0.5 μg/ml flagellin (Invivogen) or 50 ng/ml TNF (R&D Systems) for 2 days, unless otherwise specified. These cultures were carried out in the presence or absence of MUC2 from LS174T cells, mouse SI (both generated in-house) or pig stomach (Sigma-Aldrich). MUC2 was used at 50 μg/ml unless otherwise specified. Caco-2 and LS174T cells were cultured in DMEM supplemented with 5% FBS until reaching 70-80% confluence. The gut-specific secretory mucin MUC2 was purified following a previously described protocol (*32*).

Mouse MUC2. The SI of B6 mice was freed of intraluminal contents and its surface exposed by longitudinal dissection and washed twice with 1× Dulbecco's PBS (DPBS). Mucus was gently scraped off with a microscope slide, collected into 10-mm Petri dishes together with an equal volume of ice-cold 1× DPBS containing protease inhibitors, stirred gently at 4°C for 1 hour, and centrifuged at 23,000 × *g* for 45 minutes at 4°C. The resulting pellet corresponding to gel-phase MUC2 was supplemented with six volumes of

6 M guanidinium-hydrochloride buffer, gently stirred overnight at 4°C, and centrifuged at 23,000 × *g* for 45 minutes at 4°C. Supernatants containing smaller gel-forming mucins were collected, whereas the extraction residue containing larger gel-forming mucins was resuspended in 6 M guanidinium-hydrochloride and centrifuged. After repeating this extraction step for six times, the final extraction residue was incubated for 5 h at 37°C with 6M guanidinium-hydrochloride buffer supplemented with 10 mM of the reducing agent dithiothreitol (DTT). Overnight incubation with 25 mM iodoacetamin in the dark at room temperature was followed by centrifugation at 23,000 × *g* for 45 minutes at 4°C and dialysis with double-distilled H_2O at 4°C overnight using a Slide-A-Lyser MINI dialysis cup. After removing LPS with EndoTrap red (Hyglos), pooled smaller gel-forming mucins and larger gel-forming mucins were concentrated with a Spedvac apparatus, resuspended in 1× DPBS and tested for presence of MUC2 by immunobloting after electrophoresis on a sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) with a continuous 1-4% polyacrylamide gradient containing 10 mM DTT. Both small and large mucin fractions contained MUC2 but not other secretory mucins or IgA and comparably inhibited LPS-induced IL-12 production in mouse or human DCs in a carbohydrate-dependent manner. In most binding and functional assays, larger gel-forming mucin species were used.

Human MUC2. LS174T selectively producing MUC2 were cultured in 1× DMEM supplemented with 20% FBS, 4.5 g/l glucose, 2 mM L-glutamine and 20 mM HEPES. Confluent LS174T cells were washed twice with medium and separated from the flask using a cell scraper. After adding 1 ml/cm^2 of ice-cold 6M guanidine-chloride buffer, LS174T cells were gently stirred overnight at 4°C and centrifuged at 23,000 × *g* at 4°C

for 45 minutes. Subsequent extractions steps were identical to those followed to purify MUC2 from the mouse SI. Human MUC2 exerted carbohydrate-dependent regulatory effects on LPS-activated DCs comparable to those induced by murine MUC2.

Porcine MUC2. This mucin was commercially available (Sigma-Aldrich) and contained a mixture of MUC2 glycoproteins that exerted carbohydrate-dependent regulatory effects on LPS-activated DCs comparable to those induced by murine or human MUC2.

Endotoxin measurement

The endotoxin levels in mucus preparations were determined by ELISA assays using a QCL-1000 Limulus Amebocyte Lysate kit from Lonza (Catalogue Number 50-647U) according to the manufacturer's instruction.

MUC2 binding to DCs

Native or deglycosylated MUC2 was labelled with 100 μg/ml CFSE at 4 °C and binding of 20 μg/ml labelled MUC2 to DCs was determined after pre-incubation of DCs with 0-500 μg/ml unlabeled MUC2, 30 mg/ml PTTTPISTTTVTPTPTPGTQT peptide from the glycan-binding central tandem repeat region of MUC2 (AnaSpec), 1 mg/ml mannan, 0.1 M β-lactose (Sigma-Aldrich), or 30 μg/ml Ab to Galectin-3 (R&D Systems). Free CFSE consistently stained less than 5% DCs. In some binding assays, labelled MUC2 was pre-incubated with 1× PBS, 50 μg/ml galectin-3 or 50 μg/ml control human albumin (Sigma-Aldrich) at 4 °C. MUC2 was deglycosyated using an Enzymatic Protein Deglycosylation Kit according to the manufacturer instructions (Sigma-Aldrich). A combination of *O*-glycosidase, α(2→3,6,8,9)-neuroaminidase and PNGase (Comb A) was sufficient to

abolish the binding of MUC2 to DCs. The removal of glycans was confirmed through periodic acid-Schiff staining of electrophoresed MUC2.

MUC2 binding to galectin-3

96-well plates were coated overnight with 1 μg/ml galectin-1, galectin-3 or galectin-9 in carbonate-bicarbonate buffer at 4°C. After 1-hour blocking with 1% BSA at room temperature, wells were incubated with native or deglycosylated MUC2 at 1, 0.1 and 0.01 μg/ml for 2 hours, followed by washes, incubation with biotin-conjugated Ab to MUC2 (Santa Cruz) for 1 hour, washes, and incubation with horse radish peroxidase-conjugated streptavidin for 45 minutes. After further washes, a peroxydase substrate was added to develop a colorimetric reaction, which was stopped with 2N sodium sulphate. Then, plates were read with a Synergy 2 reader (BioTek) at 450 nm.

MUC2 binding to Dectin-1 and FcγRIIB

293 cells were transfected with 1 μg empty expression plasmid (control) or expression plasmids encoding human Dectin-1 or FcγRIIB (Invivogen) using Superfect® Transfection Reagent according to the manufacter's instructions (Qiagen). After 24 hours, the transfection efficiency was analyzed by FC using specific Abs (table S2). Transfected cells were exposed to 25 μg/ml CFSE-labeled MUC2 pre-incubated with 2.5 μg/ml galectin-3 (R&D Systems) for 1 hour at 4°C. The binding of CFSE-MUC2 to transfected 293 cells was analyzed by FC.

Bacterial strains

E. coli DH5α (Invitrogen) was grown in Luria-Bertani broth (Sigma-Aldrich). mRFP-expressing *E. coli* DH5α was grown in Luria-Bertani broth supplemented with 50 μg/ml

trimethoprim, 500 μg/ml thymidine and 100 μg/ml of penicillin (Sigma-Aldrich) for 18 hr, harvested by centrifugation, and resuspended in cold PBS. *Lactobacillus plantarum* (Invitrogen) was grown at 37°C without agitation in MRS broth (Sigma-Aldrich). Bacterial concentrations were estimated by measuring the OD at 600 nm and confirmed by seeding bacterial dilutions on LB agar plates. Bacteria were collected when an OD 600 of 0.3 was reached (OD 600 of 1 = 8×10^8 CFU/ml).

Transepithelial DC sampling system

Caco-2 cells were cultured for 7-10 days in the upper chamber of 3.0-μm pore Transwell filters (Costar) positioned in a 24-well plate until a transepithelial resistance of 300 Ω/cm^2 was achieved. After turning filters upside down, DCs (0.2×10^6) were seeded on the filter facing the basolateral membrane of IECs for 4 hours to allow the attachment of DCs to the filter. Then, filters were turned upside down again and the apical surface of IECs was supplemented with native or mRFP-expressing *E. coli* (ratio of 10 bacteria to 1 DC) with or without 100 μg/ml CFSE-labeled MUC2. After 1-hr incubation, bacteria and MUC2 were washed off and replaced with RPMI 1640 medium containing 100 μg/ml of gentamycin and 10 μg/ml tetracycline. Culture supernatants and DCs were collected after 18 hours from the bottom chamber to measure the phenotype and function of DCs, including bacteria sampling. Laser confocal microscopy was performed after staining acetone-fixed filters with DAPI and Abs to CD11c and tight junction proteins, including Occludin (table S2).

Tissue immunofluorescence microscopy, confocal microscopy and FISH

Frozen tissues and cells were fixed and washed as reported (*33*) and stained with various combinations of Abs to specific mouse or human antigens (tables S1 and S2). FISH analysis was performed using the EUB338 probe for bacterial 16S ribosomal RNA as reported (*33*). Nuclear DNA was visualized with DAPI. Coverslips were applied with ProLong Gold Antifade reagent (Invitrogen). Images were acquired with a Zeiss Axioplan 2 microscope (Atto Instruments). Confocal images were generated with a Leica TCS SP5 Upright confocal microscope by acquiring up to 53 different z-planes with adequate z spacing (~ 0.25 μm). Three-dimensional views were reconstructed with Imaris 7.6.0 64 bits software and exported as avi uncompressed file.

Carnoy's fixation

A segment of mouse intestine (2 cm) containing fecal material was dissected and placed in a tube with Carnoy's solution containing 60% ethanol, 30% chloroform, and 10% glacial acetic acid for 24 hours at room temperature. Two 30-minute washes in dry methanol were followed by two 20-minute washes in absolute ethanol. Before paraffin embedding and sectioning, the fixed tissue was incubated twice in xylene for 15 minutes.

Alcian Blue tissue staining

Paraffin embedded tissue sections (5 μm) were dewaxed in xylene and rehydrated to distilled water through descending graded alcohols. After exposure to Alcian Blue 8GX at pH 2.5 for 30 minutes, sections were washed thoroughly with water, dehydrated through graded alcohols, cleared in xylene and mounted with DePeX. Cryostat tissue sections (5-8 μm) were exposed to Alcian Blue 8GX (Sigma-Aldrich) for 5 minutes, rinsed gently, and sealed with cover slip. Images were acquired with a Axio Scope.A1 microscope (Carl Zeiss Microscopy).

Flow cytometry

Cells were first incubated with an Fc-blocking reagent (Miltenyi Biotech) or blocking 2.4G2 Ab to FcγRIII/II (BD PharMingen) and then stained with various Ab combinations at 4 °C (tables S1 and S2). 7-aminoactinomycin D (7-AAD) was used to exclude dead cells from the analysis as indicated by the manufacturer (BD Pharmingen). All gates and quadrants were drawn to give ≤ 1% total positive cells in the sample stained with control Abs. Cells were acquired using a BD LSRFortessa Cell Analyzer (BD Biosciences) and data were analyzed with the FlowJo software (Tree Star).

FACSorting

Cells were stained with specific Ab cocktails (tables S1 and S2) and discrete subsets purified with a FACSAria III BSL2 cell sorter (Becton Dickinson) after exclusion of dead cells through 7-AAD staining. The purity of sorted cells was consistently above 98%.

RALDH activity

Active RALDH was detected using the ALDEFLUOR Kit as instructed by the manufacturer (Stem Cell Technologies). RALDH-induced RA was blocked using the RA antagonist LE540 as indicated by the manufacturer (Sigma-Aldrich).

Intracellular Foxp3 and cytokine staining

DC-stimulated naïve $CD4^+$ T cells or $CD4^+$ T cells from the SI-LP were restimulated for 6 h with 50 ng/ml PMA and 500 ng/ml ionomycin in the presence of GolgiPlug (BD PharMingen) and subsequently permeabilized with Cytofix/Cytoperm (BD PharMingen) and stained with Abs to CD3, CD4, CD45, Foxp3, IL-17A and/or IFN-γ (tables S1 and S2). Intracellular expression of Foxp3 and IFN-γ was analyzed in $CD4^+$ T cells exposed

to MUC2-conditioned DCs in the presence or absence of blocking Abs to IL-10 or TGF-β1 (table S3).

T cell proliferation

Human. Naïve $CD4^+$ T cells (1×10^5/ml) were labeled with 5 μM CFSE and co-cultured with irradiated allogeneic DCs (1×10^5/ml) for 5 days. CFSE dilution was measured by FC.

Mouse. Naïve OT-II cells were labeled with 5 μM CFSE and stimulated with agonistic Abs to CD3 and CD28 (table S3). CFSE dilution was measured by FC. In oral tolerance induction experiments, splenic $CD4^+$ T cells (2×10^5/well) were co-cultured with splenic DCs (2×10^4/well) for 5 days. These DCs were pulsed for 2 hours with 1 mg/ml OVA and irradiated at 3000 rad before co-culture with $CD4^+$ T cells. Bromodeoxyuridine (BrdU) was added into each well 24 h before the end of the culture. On day 5, $CD4^+$ T cells were fixed with ethanol, treated with nucleases, and supplemented with a peroxydase-conjugated Ab to BrdU and the substrate ABTS according to the manufacturer's instructions (Roche Diagnostics). The resulting colorimetric reaction was quantified by measuring the absorbance at 450nm using a Multiskan Ascent ELISA reader (Thermo Scientific).

T cell suppression assay

Human. Naïve $CD4^+$ T cells were labeled with 5 μM CFSE and co-cultured for 5 days with DC-induced Treg cells (1×10^5/ml) in the presence of 5 μg/ml plate-bound OKT3 Ab to CD3 and 1 μg/ml soluble CD28.2 Ab to CD28 (table S3).

Mouse. Naïve OT-II cells (5×10^4) were labeled with 5 μM CFSE and co-cultured with DC-induced Treg cells (5×10^5/ml) for 5 days in the presence of 5 μg/ml plate-bound 145.2C11 Ab to CD3 and 1 μg/ml soluble 37.51 Ab to CD28 (Becton Dickinson). CFSE dilution was measured by FC.

ELISA

Commercially available kits were used to measure the concentration of human or mouse IL-6, IL-8, IL-10, IL-12p70, TNF, IFN-γ (BD Biosciences or R&D Systems), active TGF-β1 (Promega), and galectin-3 (R&D Systems) in culture fluids. OVA-specific IgM, IgG and IgA from serum, fecal pellets and SI secretions were detected using microplates coated with OVA overnight at 4ºC in carbonate-bicarbonate buffer (table S4).

Quantitative PCR analysis

RNA was extracted and cDNA synthesized as previously reported (*33*). qRT-PCRs were performed as previously described (*33*) using appropriate primer pairs (tables S5 and S6).

Ligated intestinal loop assay

WT and *Muc2*$^{-/-}$ mice were anesthetized intraperitoneally with 20 mg/ml Ketamine and 2 mg/ml Xylazine (100 μl final volume) and kept on a 37°C warming pad for the duration of the experiment. The respiratory rate and spontaneous movement during anaesthesia were monitored. The ventral abdomen from the xiphoid process to the pelvis was shaved, disinfected and draped as surgical site. A 2-4 cm midline abdominal incision was made to allow good visualization of the peritoneal cavity. The terminal ileum portion of the SI was exposed and a 3-cm long segment ligated at both extremities with surgical thread. After injecting CFSE-MUC2 and 1×10^8 CFU mRFP-*E. coli* pre-mixed in 100 μl PBS

into the ligated loop, injection sites were plugged with surgical foam gel and repositioned into the abdomen. The skin incision was closed with non-absorbable suture. Mice were euthanized 30 minutes after the injection and the ligated loop was removed. The intestinal contents were washed out thoroughly and one half of the loop was used for cell isolation, whereas the other half was embedded in OCT medium for IFA analysis.

Antibiotic treatment

WT and *Muc2*$^{-/-}$ mice were gavaged for 5 days with 0.5 mg/L ampicillin, 0.5 mg/mL gentamicin (Gemini Bio-Products), 0.5 mg/mL neomycin sulphate, 0.5 mg/mL metronidazole, and 0.25 mg/mL vancomycin (Sigma-Aldrich). The same antibiotic cocktail was added to drinking water. Fecal pellets were collected after 2 weeks of treatment and bacteria separated from food components, resuspended in sterile water and placed on Luria-Bertani agar plates at 37°C for 48 hours to determine the growth of culturable species.

Detection of bacteria-bound IgA antibodies

Fecal samples (10 mg) from WT and *Muc2*$^{-/-}$ mice were suspended in 100 μl 1× DPBS, homogenized and centrifuged at 400 × *g* for 5 minutes to remove large particles. The resulting supernatant was centrifuged at 8000 × *g* for 10 minutes to remove free antibodies and obtain a bacterial pellet that was resuspended in 1 ml 1× DPBS and bovine serum albumin (1% volume/weight). These bacteria were incubated with a $F(ab)_2$ Ab to IgA on ice for 20 minutes, washed with PBS, and further resuspended in 1× DPBS containing 4 μg/ml propidium iodide for FC analysis.

Induction and assessment of colitis

A two-cycle DSS administration protocol adapted to *Muc2*$^{-/-}$ mice was used to induce colitis. WT and *Muc2*$^{-/-}$ mice received either drinking water containing 3% PBS (control) or drinking water containing 3% DSS with an average molecular weight of 50 kDa (Cat No. BP1585-100, Fisher Scientific). DSS was administered on days 1-5 and 8-12 and mice. Mice were sacrificed on day 12 due to onset of severe colitis in the *Muc2*$^{-/-}$ group. Before DSS treatment, some *Muc2*$^{-/-}$ mice were gavaged daily with MUC2 for 7 days. To assess the severity of colitis, body weight, stool consistency and fecal blood were monitored daily. The disease activity index was recorded with the following formula: (combined score of weight loss, stool consistency, and bleeding)/3. Pathologists at Mount Sinai determined the degree of intestinal inflammation by measuring the colon length and assigning a histological score after staining colonic issue sections with hematoxyilin and eosin.

Oral tolerance induction

Oral tolerance was studied following previously described protocols (*34, 35*).

Mucosal tolerance. WT and *Muc2*$^{-/-}$ mice were tolerized by gavage for 5 consecutive days with 200 μl control phosphate buffer saline solution (PBS) with or without 1 mg/ml OVA (grade III, Sigma-Aldrich) supplemented or not with 1 mg/ml MUC2. 7, 14, and 21 days later mice were orally immunized with 1 mg OVA (grade VI, Sigma-Aldrich) and 10 μg cholera toxin. OVA-specific B and T cell responses were measured 7 days after the third oral immunization (day 28). Blood samples were collected after retro-orbital bleeding to measure serum IgE. SI contents and fecal pellets were collected by scrapping and by gentle rectal squeezing, respectively, diluted with 1 × DPBS containing 0.01% sodium azide to reach a concentration of 100 mg/ml, and homogenized by continuous

shaking for 10 min with a vortex mixer. Non-dissolved particulate debris was removed by centrifugation for 10 min at 14,000 rpm. The resulting supernatants were collected to measure IgG, IgM and IgA. DCs from PPs, SI-LP and MLNs were pulsed with OVA before incubating them with naïve OT-II cells to measure IFN-γ production.

Systemic tolerance. WT and *Muc2*$^{-/-}$ mice were gavaged with PBS, OVA or OVA plus MUC2 for 5 consecutive days. One week after the last gavage, mice were immunized with 100 μg OVA in 50 μl complete Freund adjuvant emulsion by subcutaneous injection. A week later, mice were challenged with 10 μg OVA in 20 μl PBS in the left rear footpad, while PBS with no OVA was injected into the right rear footpad for control purposes. Footpad thickness was measured prior to and 48 hours after injection with a custom-built spring-driven micrometer. OVA-specific footpad swelling was calculated as (right footpad thickness – left footpad thickness) after 24 hours – (right footpad thickness – left footpad thickness) at 0 hours. The proliferation of splenic $CD4^+$ T cells in response to OVA-pulsed DCs was analyzed through a BrdU-based assay as described earlier.

RNA interference

Gene knockdown assays were performed as previously described (*31, 36*). Pre-designed 100 nM siRNAs (Santa Cruz) targeting *CLEC7A* (sc-63276), *FcγRIIB* (sc-42774), *LGALS3* (sc-155994) and *CTTNB1* (sc-29209) or an appropriate negative control (sc-37007) were mixed in 100 μL buffer from the Amaxa Human Dendritic Cell Nucleofector Kit and nucleofected into 1 x 10^6 cells human monocyte-derived DCs according to the manufacturer's recommendations (Lonza). Nucleofected cells were seeded in complete RPMI medium for 48 hours after nucleofection. qRT-PCR and FC

showed severely impaired *CLEC7A*, *FcγRIIB*, *LGALS3* or *CTTNB1* gene expression in DCs nucleofected with specific siRNAs.

Western blotting and phospho-kinase array

Total, cytoplasmic and nuclear proteins were obtained as previously described (*37*). Equal amounts of proteins were fractionated onto a 10% SDS-PAGE and transferred onto PVDF membranes (BioRad). After blocking, membranes were probed with Abs to relevant receptors and signaling proteins (table S7), washed and incubated with appropriate secondary Abs as previously reported (*37*). Proteins were detected with an enhanced chemiluminescence detection system (Amersham). The phosphorylation of AKT, p38, ERK1/2 and CREB was analyzed using a Proteome Profiler Human Phospho-Kinase Array kit according to the manufacturer's instructions (R&D Systems). A transmission-mode scanner (Epson) and ImageJ analysis software were used to perform a comparative densitometric analysis of immunoreactions.

Immunoprecipitation assays

DCs (5×10^6) were homogenized in lysis buffer with a Dounce homogeneizer. Equal amounts of protein lysate were incubated with a control Ab with irrelevant binding activity or Abs to galectin-3 or dephospho-β-catenin (Santa Cruz) overnight at 4^oC, followed by incubation with 50 μL streptavidin-sepharose beads (GE-Healthcare) for 3 hours. Three different buffers were used to wash streptavidin-bound antigen-antibody complexes as previously reported (*37*). Denatured proteins were analyzed by SDS-PAGE and membranes immunoblotted with Abs to FcγRIIB, Dectin-1 and galectin-3 (table 7).

Electrophoretic mobility shift assays

Oligonucleotides encompassing an NF-κB-binding region (from -395 to -372) of the *IL12A* gene promoter, a CREB-binding region (from -356 to -355) of the *IL10* gene promoter (table S8) or commercially available consensus NF-κB-binding and consensus Oct1-binding DNA sequences (Santa Cruz) were labeled with [α-32P] ATP and used at approximately 50,000 c.p.m. in each reaction. Reaction samples were prepared as described and electrophoresed through a 5% non-denaturing polyacrylamide gel (*37*). The composition of DNA-bound protein complexes was determined by incubating the reaction mixture with inhibitory or supershifting Abs to p50, p65 or CREB as previously reported (*37*).

Chromatin immunoprecipitation assays

Chromatin immunoprecipitation (ChIP) assays were performed using the EZ-ChIP™ Chromatin Immunoprecipitation Kit according to the manufacturer's instructions (Millipore). Briefly, human DCs were crosslinked with 1% formaldehyde for 10 minutes. After stopping the crosslinking reaction with 0.125 mM glycine, DCs were lysed and sonicated to shear chromatin into 0.5-1 kb DNA fragments. Lysates were centrifuged and the resulting supernatants pre-cleared with protein G agarose and immunoprecipitated with 5 μg of control IgG Ab with irrelevant binding activity (Santa Cruz) or Abs to NF-κB p65 (Santa Cruz), pCREB or CBP (Cell Signaling). Immunoprecipitates were recovered with protein G agarose, extensively washed and treated with sodium chloride to dissociate DNA from proteins. Finally, DNA was purified, concentrated and analyzed by qRT-PCR using Quantifast SYBR green PCR kit (Qiagen) together with forward and reverse primers that amplified a region of the *IL12A* gene promoter spanning nucleotides -482 and -296 and a region of the *IL12B* gene promoter spanning nucleotides -102 and

+47, where - and + refer to the initiation transcription site (table S8). Soluble chromatin prior to immunoprecipitation was used as input control. Samples from three individual ChIP assays were analyzed in triplicate and results expressed as *n*-fold increase over input control.

Luciferase reporter assays

Human monocyte-derived DCs or 293 cells (20×10^6/ml) were nucleofected with 200 ng *IL12A* gene promoter (from residue -1082 to residue +61) reporter plasmid from Xiaojing Ma (Weill Cornell Medical College) or 200 ng minimal $\kappa B_{(2)}$ promoter reporter plasmid expressing firefly luciferase using an Amaxa Human Dendritic Cell Nucleofector Kit as instructed by the manufacturer (Lonza). In some experiments, 293 cells were co-transfected with 500 ng *CTTNB1* expression vector (Addgene). In all the conditions, DCs were co-transfected with 100 ng pRL-TK reporter plasmid expressing renilla luciferase under the control of the thymidine kinase promoter (Promega). After 24 hours, DCs were cultured with various stimuli. The luciferase activity was measured after 18 hours using the Dual Luciferase Assay System (Promega) and expressed as fold induction after normalizing the firefly signal to the renilla signal.

Statistical analysis

Values were expressed as mean ± standard deviation (s.d.). Statistical significance was assessed by using a one-tailed unpaired Student's *t*-test.

Supplementary references

29. C. Ruiz-Herguido, J. Guiu, T. D'Altri, J. Ingles-Esteve, E. Dzierzak, L. Espinosa, A. Bigas, Hematopoietic stem cell development requires transient Wnt/beta-catenin activity. *J. Exp. Med.* **209**, 1457-1468 (2012).

30. M. Stadtfeld, T. Graf, Assessing the role of hematopoietic plasticity for endothelial and hepatocyte development by non-invasive lineage tracing. *Development* **132**, 203-213 (2005).

31. B. He, W. Xu, P. A. Santini, A. D. Polydorides, A. Chiu, J. Estrella, M. Shan, A. Chadburn, V. Villanacci, A. Plebani, D. M. Knowles, M. Rescigno, A. Cerutti, Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity* **26**, 812-826 (2007).

32. M. A. Axelsson, N. Asker, G. C. Hansson, O-glycosylated MUC2 monomer and dimer from LS 174T cells are water-soluble, whereas larger MUC2 species formed early during biosynthesis are insoluble and contain nonreducible intermolecular bonds. *J. Biol. Chem.* **273**, 18864-18870 (1998).

33. I. Puga, M. Cols, C. M. Barra, B. He, L. Cassis, M. Gentile, L. Comerma, A. Chorny, M. Shan, W. Xu, G. Magri, D. M. Knowles, W. Tam, A. Chiu, J. B. Bussel, S. Serrano, J. A. Lorente, B. Bellosillo, J. Lloreta, N. Juanpere, F. Alameda, T. Baro, C. D. de Heredia, N. Toran, A. Catala, M. Torrebadell, C. Fortuny, V. Cusi, C. Carreras, G. A. Diaz, J. M. Blander, C. M. Farber, G. Silvestri, C. Cunningham-Rundles, M. Calvillo, C. Dufour, L. D. Notarangelo, V. Lougaris, A. Plebani, J. L. Casanova, S. C. Ganal, A. Diefenbach, J. I. Arostegui, M. Juan, J. Yague, N. Mahlaoui, J. Donadieu, K. Chen, A.

Cerutti, B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. *Nat. Immunol.* **13**, 170-180 (2012).

34. O. Alpan, G. Rudomen, P. Matzinger, The role of dendritic cells, B cells, and M cells in gut-oriented immune responses. *J Immunol* **166**, 4843-4852 (2001).

35. T. Worbs, U. Bode, S. Yan, M. W. Hoffmann, G. Hintzen, G. Bernhardt, R. Forster, O. Pabst, Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J. Exp. Med.* **203**, 519-527 (2006).

36. M. Cols, C. M. Barra, B. He, I. Puga, W. Xu, A. Chiu, W. Tam, D. M. Knowles, S. R. Dillon, J. P. Leonard, R. R. Furman, K. Chen, A. Cerutti, Stromal endothelial cells establish a bidirectional crosstalk with chronic lymphocytic leukemia cells through the TNF-related factors BAFF, APRIL, and CD40L. *J. Immunol.* **188**, 6071-6083 (2012).

37. B. He, R. Santamaria, W. Xu, M. Cols, K. Chen, I. Puga, M. Shan, H. Xiong, J. B. Bussel, A. Chiu, A. Puel, J. Reichenbach, L. Marodi, R. Doffinger, J. Vasconcelos, A. Issekutz, J. Krause, G. Davies, X. Li, B. Grimbacher, A. Plebani, E. Meffre, C. Picard, C. Cunningham-Rundles, J. L. Casanova, A. Cerutti, The transmembrane activator TACI triggers immunoglobulin class switching by activating B cells through the adaptor MyD88. *Nat. Immunol.* **11**, 836-845*Nat. Immunol.* **11**, 836-845 (2010).

Supplementary Acknowledgments

We thank Miriam Merad (Mount Sinai School of Medicine) for helpful discussions, Amariliz Rivera (University of Medicine and Dentistry of New Jersey) for *Clec7a*$^{-/-}$ mice, Xiaojing Ma (Weill Cornell Medical College) for *IL12A* plasmid, and Cristina Ruiz Herguido (Institut Hospital del Mar d'Investigacions Mèdiques) for helping with *Ctnbb1*$^{-/-}$ mice.

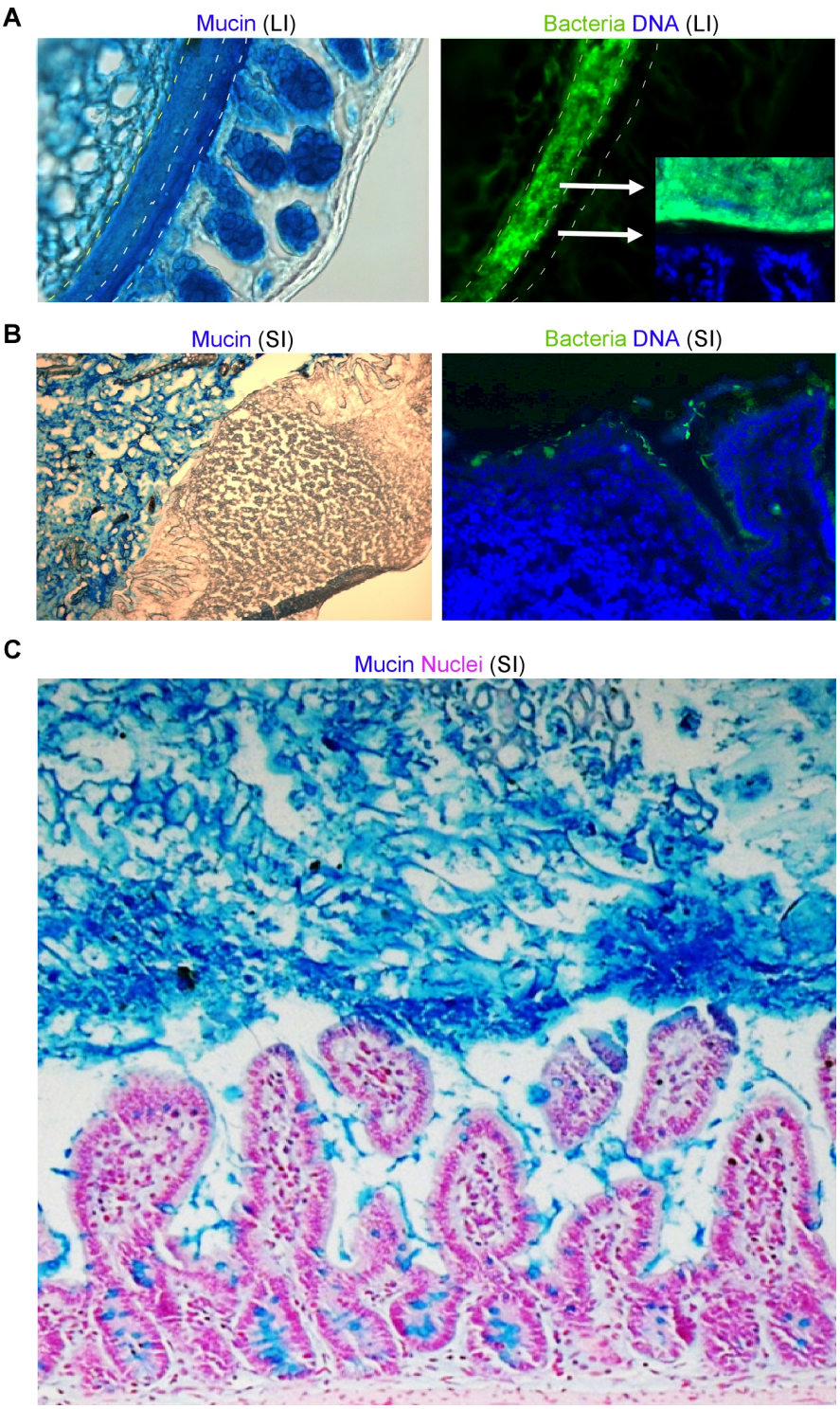

Fig. S1. **Mucus forms a porous layer pervious to bacteria in the SI**. (**A**) Light microscopy of LI tissue section stained with mucin-reactive Alcian Blue and IFA of the same tissue section after fluorescent *in situ* hybridization (FISH) of 16S ribosomal

bacterial RNA and DAPI counterstaining. Dashed lines demarcate loose (upper) and dense (bottom) mucous layers. Inset: magnification of microbiota inhabiting the loose but not dense mucous layers. (**B**) Mucin and bacterial 16S RNA from two distinct SI tissue sections visualized as in (A). (**C**) Mucin in Carnoy-fixed SI tissue section. Original magnification, ×5 (A and B) and ×20 (inset in A right panel and C). Data show one of four experiments with similar results.

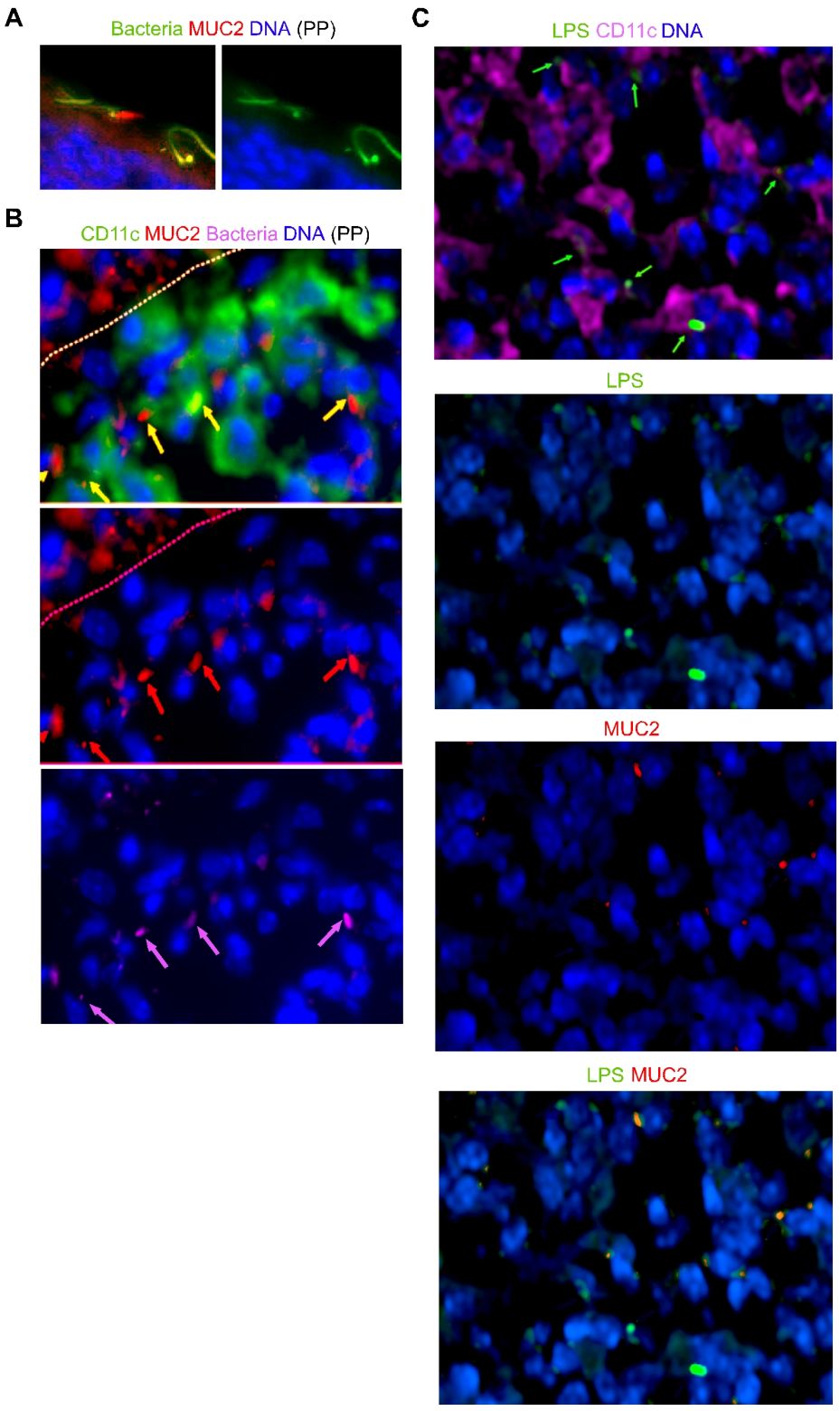

Fig. S2. **Some DCs from PPs contain MUC2-coated bacteria**. (**A** and **B**) FISH of bacterial 16S ribosomal RNA (bacteria) and IFA of CD11c and MUC2 in SI tissue

sections from WT mice counterstained with DAPI. Dashed line: border between PP epithelium and subepithelial dome. Arrows: MUC2-coated bacteria in DCs. (**C**) IFA of LPS, CD11c, MUC2 and DAPI in PP tissue section from a WT mouse. Green arrows: LPS-expressing MUC-positive and MUC2-negative bacteria in DCs. Original magnification ×5 (upper left panel) and ×63 (bottom left panel and right panels). Data show one of four experiments with similar results.

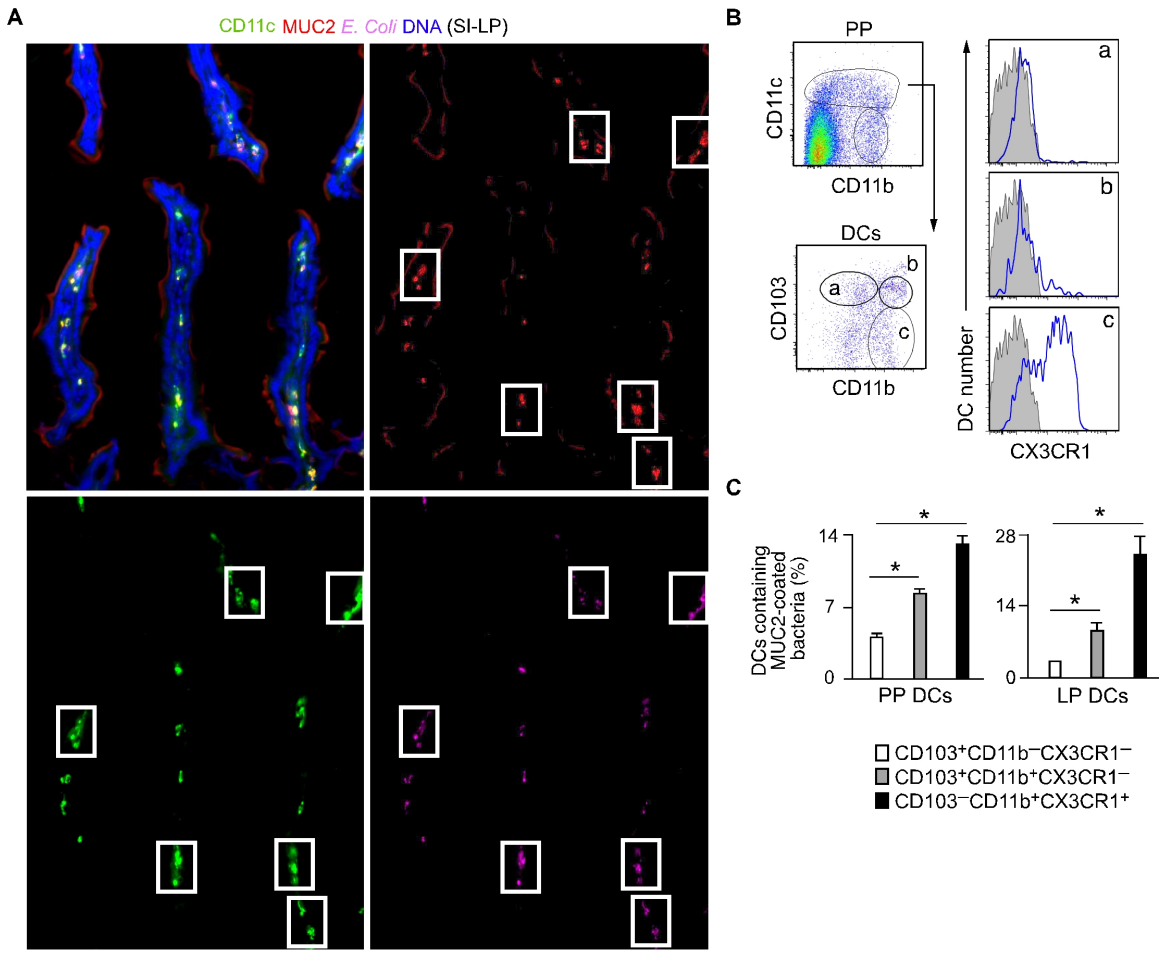

Fig. S3. **DCs from the SI capture MUC2-coated bacteria**. (**A**) IFA of CD11c, CFSE-MUC2 (digitally converted in red), monomeric red fluorescent protein (mRFP)-*E. coli* (digitally converted in purple) and DAPI in SI-LP tissue section from a WT mouse 30 minutes after injection of MUC2 and bacteria into a ligated intestinal loop. Boxes: DCs containing MUC2-coated bacteria. Original magnification, ×20. (**B**) Left panels: FC of CD11c, CD11b and CD103 on PP DCs. Right panels: FC of CX3CR1 on $CD11b^{-}$ $CD103^{+}$ (gate a) $CD11b^{+}CD103^{+}$ (gate b) and $CD11b^{+}CD103^{-}$ (gate c) DCs. (**C**) FC of CFSE-MUC2 in PP and SI-LP $CD103^{+}CD11b^{-}CX3CR1^{-}$, $CD103^{+}CD11b^{+}CX3CR1^{-}$ and $CD103^{-}CD11b^{+}CX3CR1^{+}$ DCs from (B). Data summarize three experiments (error bars, s.d.; unpaired *t* test, $*P < 0.05$) or show one of four experiments with similar results.

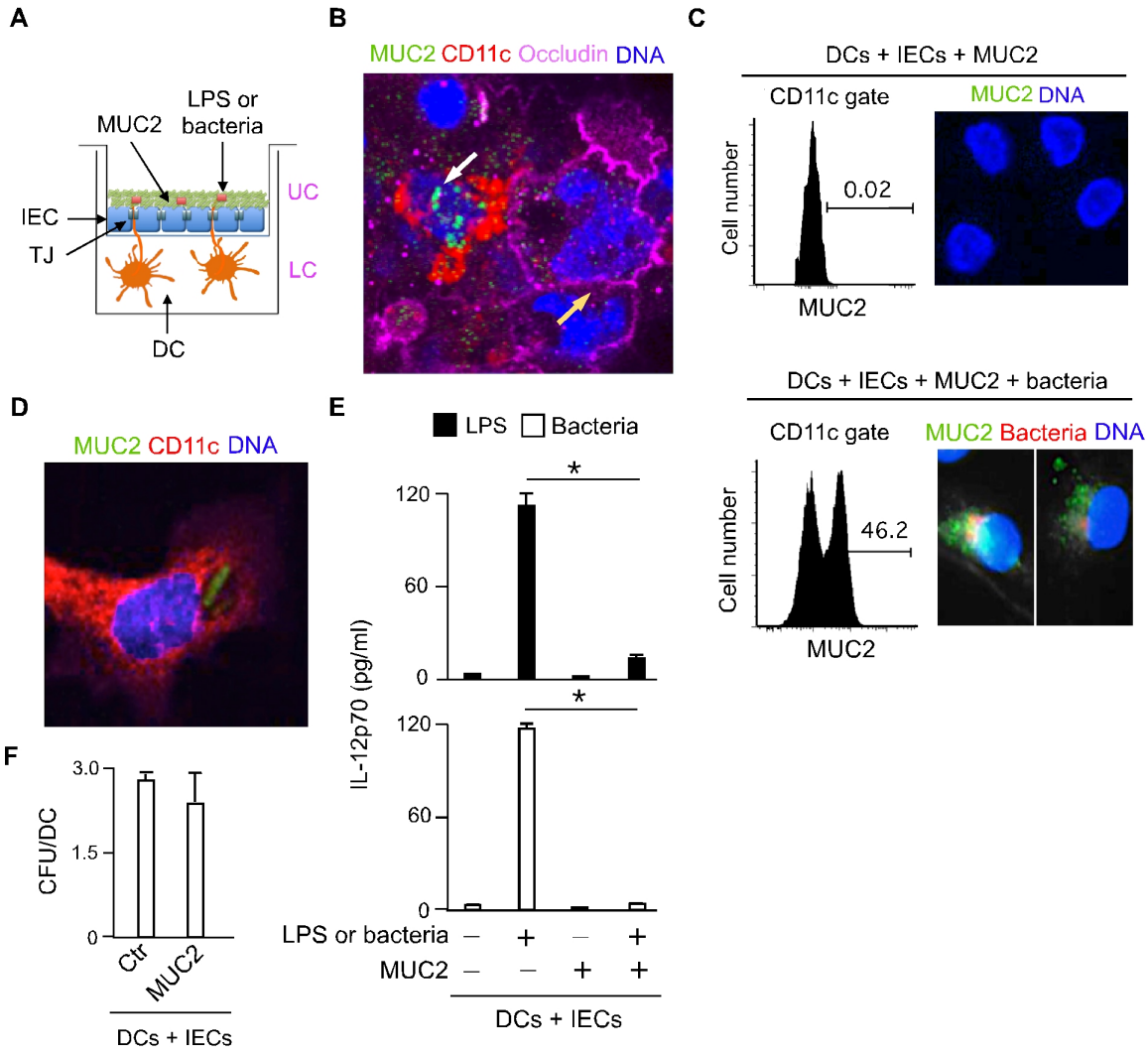

Fig. S4. **DCs capture MUC2-coated bacteria across IECs**. (**A**) Transwell system for human DC sampling of LPS or *E. coli* (native or RFP) across a polarized monolayer of IECs supplemented with CFSE-MUC2. TJ, tight junction; UC, upper chamber; LC, lower chamber. (**B**) Confocal microscopy of MUC2, CD11c, Occludin (digitally converted in purple) and DAPI in the filter of a transwell system supplemented 12 hours earlier with CFSE-MUC2 and *E. coli* from the apical surface of IECs. White arrow, MUC2-coated bacteria; yellow arrow, TJ. Original magnification, ×63. (**C**) FC of MUC2 in CD11c-gated DCs and IFA of MUC2, bacteria and DAPI in CD11c-MACSorted DCs from the

LC of a transwell system challenged with CFSE-MUC2 and mRFP-*E. coli* for 12 hours. % of DCs containing bacteria or MUC2. Original magnification, ×20. (**D**) Confocal microscopy of MUC2, CD11c and DAPI in a DC from the LC of a transwell system used as in (B). Original magnification, ×63. (**E**) ELISA of IL-12p70 from the LC supernatant of a transwell system supplemented 2 days earlier with LPS or *E. coli* from the apical surface of IECs with or without MUC2. (**F**) Colony-forming unit (CFU) from DCs in the LC of a transwell system used as in (E). Data summarize three experiments (error bars, s.d.; unpaired *t* test, $*P < 0.05$) or show one of four experiments with similar results.

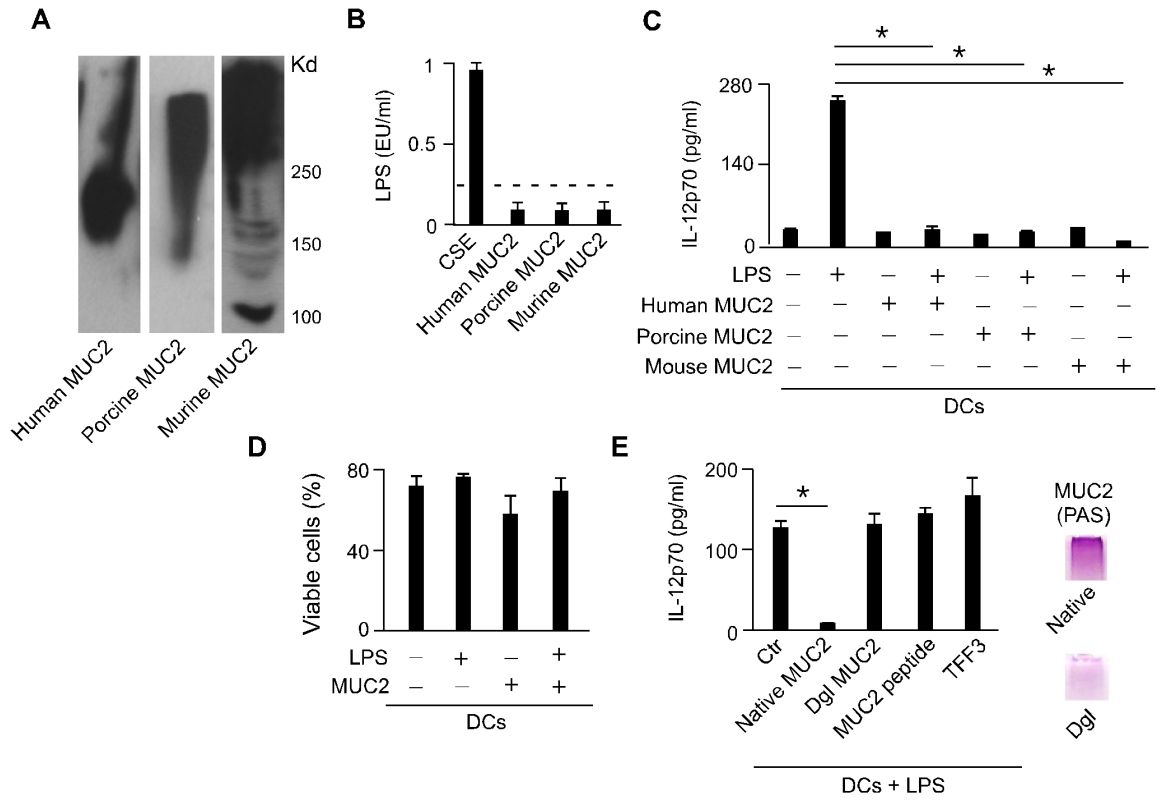

Fig. S5. **DCs attenuate LPS-induced IL-12 production in response to MUC2**. (**A**) WB of reduced MUC2 isolated from human GCs and porcine or murine SI. kD, kilodalton. (**B**) Photometric measurement of LPS in MUC2 preparations. CSE, control standard endotoxin; EU, endotoxin unit. Dashed line: upper limit of virtually endotoxin-free conditions. (**C**) ELISA of IL-12p70 from human monocyte-derived DCs cultured with or without LPS and/or human, porcine or murine MUC2 for 2 days. (**D**) FC of viable propidium iodide$^-$annexin-V$^-$ human monocyte-derived DCs cultured with or without LPS and/or MUC2 for 2 days. (**E**) ELISA of IL-12p70 from human monocyte-derived LPS-stimulated DCs exposed to PBS (ctr), fully glycosylated MUC2, deglycosylated (dgl) MUC2, MUC2 peptide or trefoil factor 3 (TFF3). Rightmost gels show control Periodic Acid Schiff (PAS) staining of native or dgl MUC2. Data summarize three

experiments (error bars, s.d.; unpaired *t* test, **P* <0.05) or show one of four experiments with similar results.

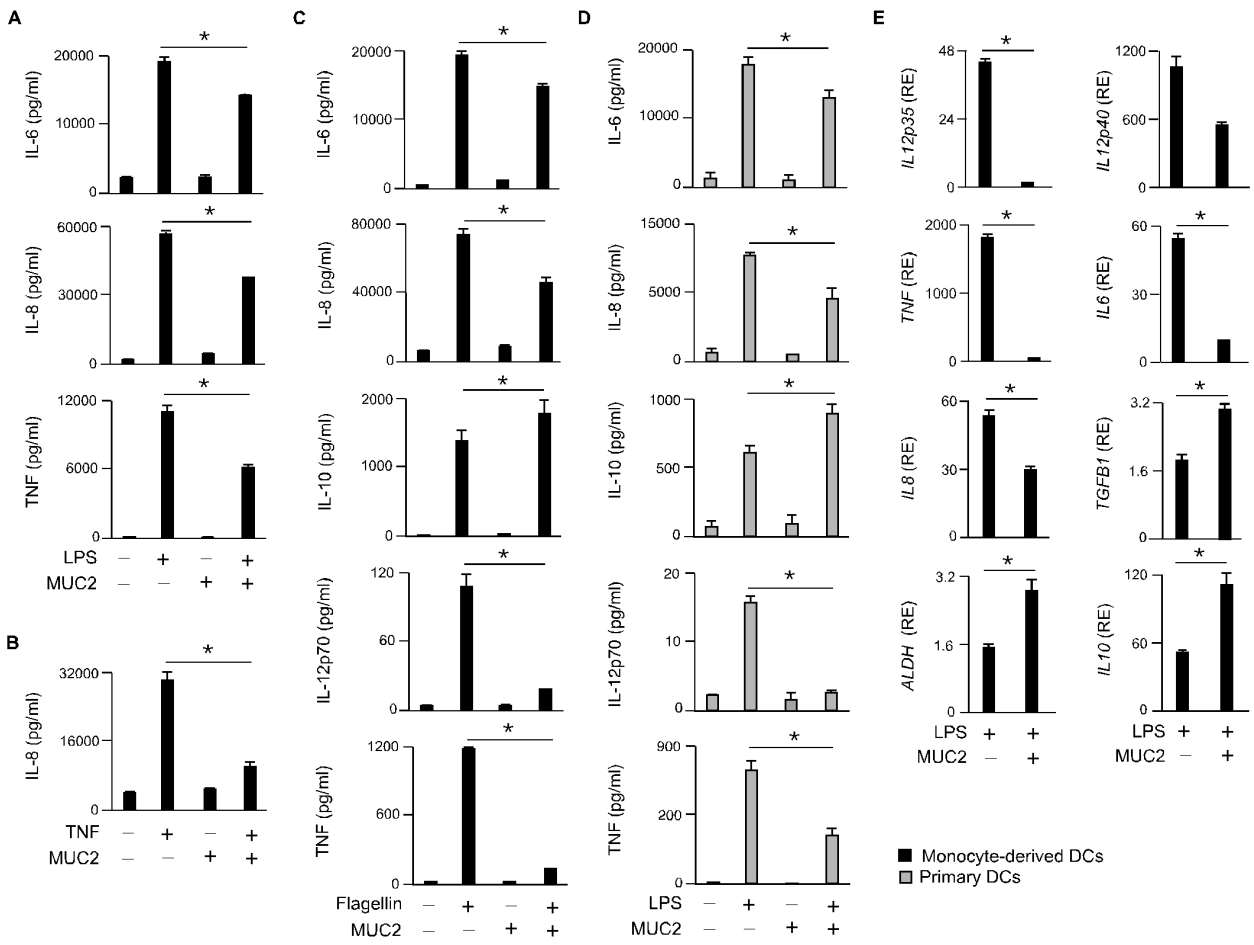

Fig. S6. **DCs attenuate pro-inflammatory but not anti-inflammatory cytokine production when exposed to LPS, flagellin or TNF in the presence MUC2**. (**A-D**) ELISA of IL-6, IL-8, IL-10, IL-12p70 and TNF from human monocyte-derived DCs (black bars) or circulating myeloid $CD1c^+$ DCs (gray bars) cultured with or without LPS, flagellin, TNF and/or MUC2 for 2 days. (**E**) qRT-PCR of mRNA for IL-6, IL-8, IL-10, IL-12p35 (*IL12A*), IL-12p40 (*IL12B*), TNF, TGF-β1 and RALDH (*ALDH*) in human DCs cultured with LPS with or without MUC2 for 12 hours. RE, relative expression compared to DCs exposed to medium alone. Data summarize three experiments (error bars, s.d.; unpaired *t* test, $*P < 0.05$).

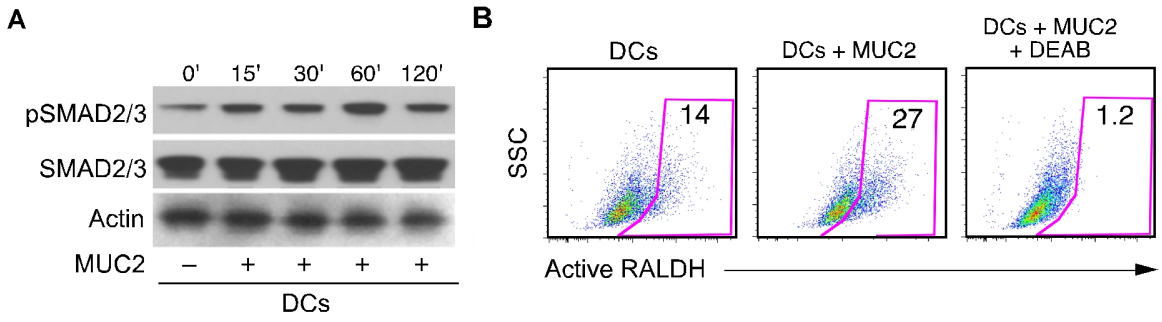

Fig. S7. **DCs induce TGF-β1 signaling and RALDH activity in response to MUC2**. (**A**) Immunoblotting of phospho (p) SMAD2/3 (a TGF-β1-induced transcription factor induced by ongoing TGF-β1 signaling), total SMAD2/3 and actin (loading control) in human monocyte-derived DCs cultured with MUC2. (**B**) FC of active RALDH in human DCs exposed to medium alone or MUC2 with or without the RALDH inhibitor DEAB for 12 hours. Numbers: % of positive cells. Data show one of four experiments with similar results

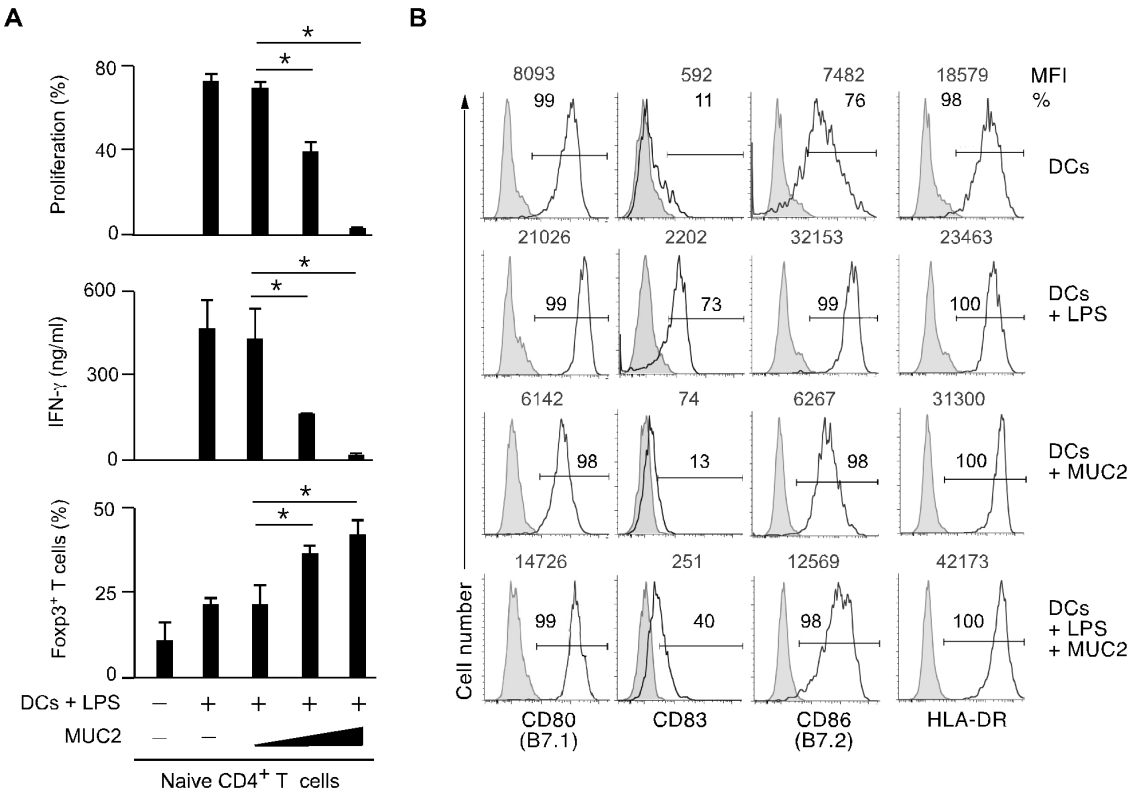

Fig. S8. **DCs acquire tolerogenic properties when exposed to LPS in the presence of MUC2**. (**A**) FC of proliferation-induced $CFSE^{low}$, FC of Foxp3 and ELISA of IFN-γ from human naïve $CD4^+$ T cells exposed for 5 days to allogeneic monocyte-derived DCs primed with LPS in the presence of absence of 10, 50 and 100 μg/ml of MUC2 for 2 days. (**B**) FC of CD80 (B7.1), CD83, CD86 (B7.2) and HLA-DR on human DCs cultured with or without LPS and/or MUC2 for 2 days. Upper numbers: mean fluorescence intensity (MFI); bottom numbers: % of positive DCs. Data summarize three experiments (error bars, s.d.; unpaired *t* test, **P* <0.05) or show one of four experiments with similar results.

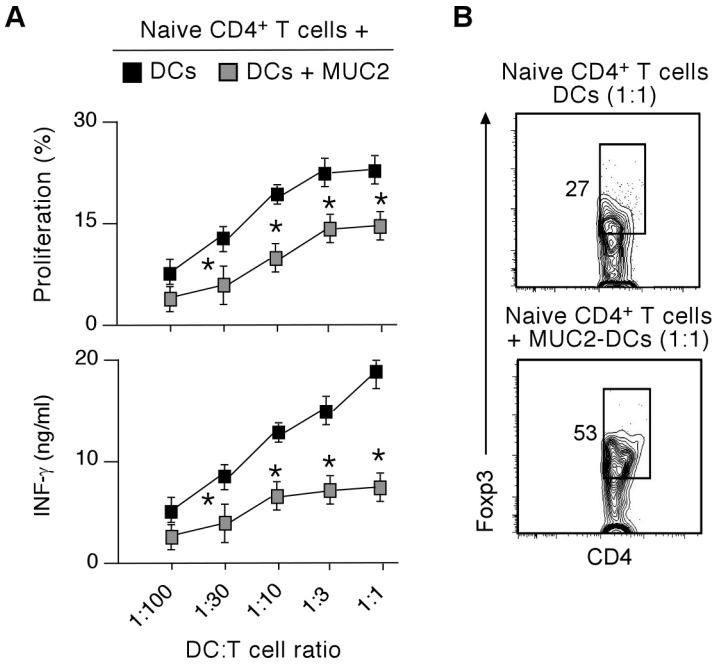

Fig. S9. **DCs acquire tolerogenic properties when exposed to MUC2 alone**. (**A**) FC of proliferation-induced $CFSE^{low}$ and ELISA of IFN-γ from human naïve $CD4^+$ T cells exposed for 5 days to allogeneic monocyte-derived DCs primed with MUC2 for 2 days. (**B**) FC of CD4 and Foxp3 from human naïve $CD4^+$ T cells cultured as in (A). Numbers: % of Treg cells. Data summarize three experiments (error bars, s.d.; unpaired *t* test, **P* <0.05) or show one of four experiments with similar results.

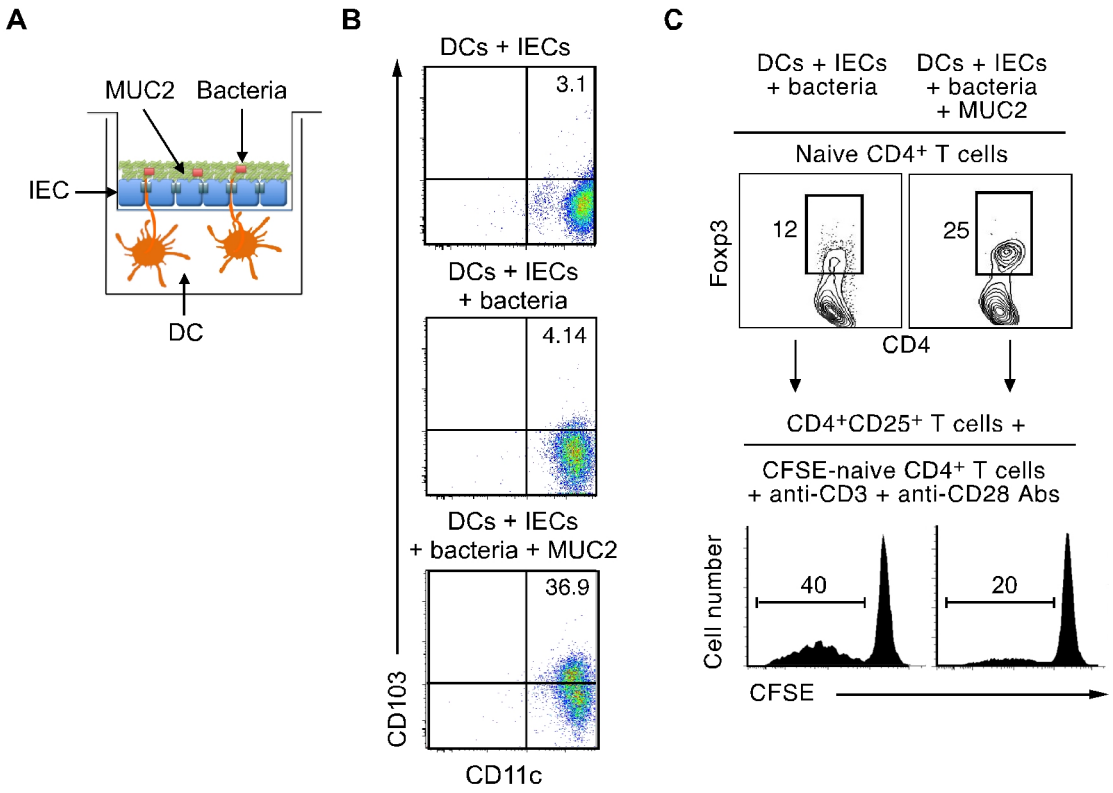

Fig. S10. **DCs acquire tolerogenic properties after sampling MUC2-coated bacteria across IECs**. (**A**) Transwell system for human subepithelial DC sampling of bacteria across a polarized monolayer of IECs topped by MUC2. (**B**) FC of CD103 on human monocyte-derived DCs from the bottom chamber of the transwell system shown in (A) before and after a 12-hour challenge with bacteria with or without MUC2. (**C**) FC of CD4 and Foxp3 from human naïve $CD4^+$ T cells exposed to isogenic DCs cultured for 2 days with IECs and bacteria in the presence or absence of MUC2 as in (B). $CD4^+CD25^+$ Treg cells from these cultures were sorted and incubated with CFSE-labeled naïve $CD4^+$ T cells in the presence of agonistic Abs to CD3 and CD28 for 5 days. Numbers: % of Treg cells (top) and divided $CFSE^{low}$ $CD4^+$ T cells (bottom). Data show one of four experiments with similar results.

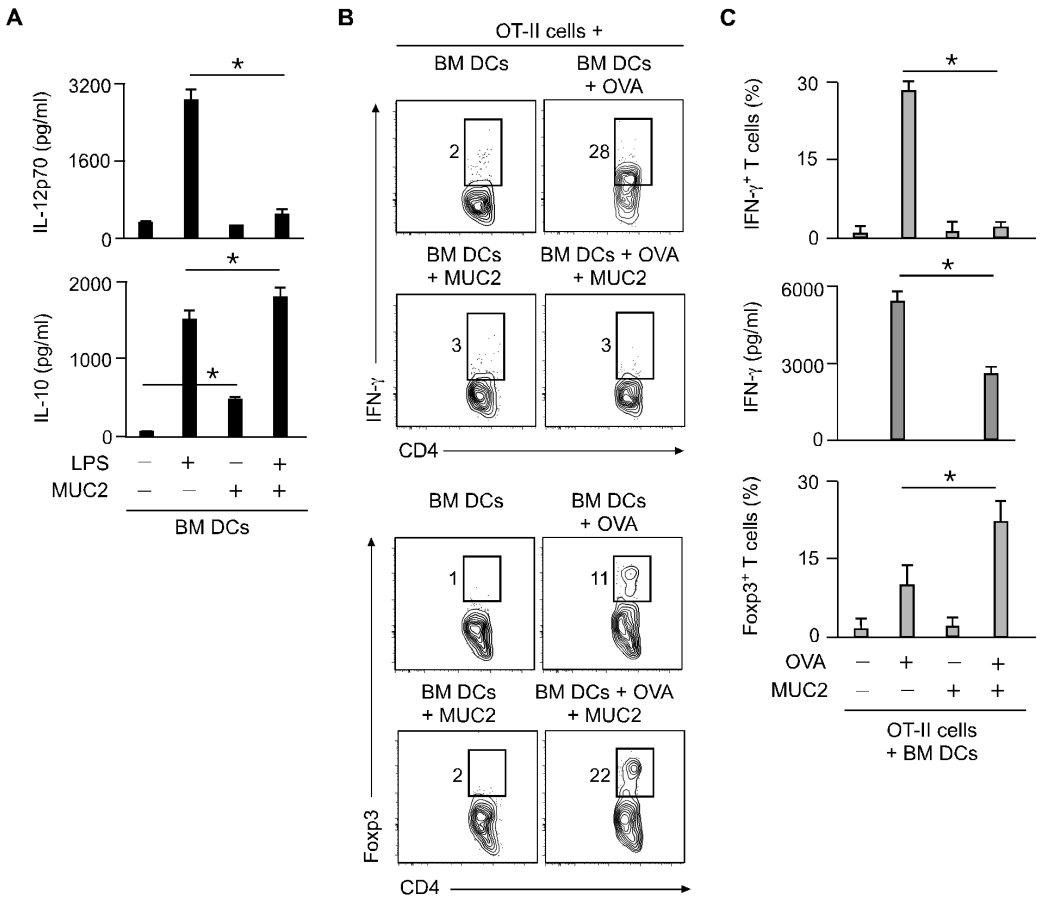

Fig. S11. **DCs confer tolerogenic properties to OVA-specific T cells in response to MUC2**. (**A**) ELISA of IL-12p70 and IL-10 from mouse bone marrow (BM)-derived DCs cultured with or without LPS and/or MUC2 for 2 days. (**B** and **C**) FC of CD4, IFN-γ and Foxp3 and ELISA of IFN-γ from mouse OT-II cells cultured for 5 days with isogenic BM-derived DCs pulsed with OVA in the presence or absence of MUC2. Data summarize results from 3 mice for each experimental condition (error bars, s.d.; unpaired *t* test, **P* <0.05)

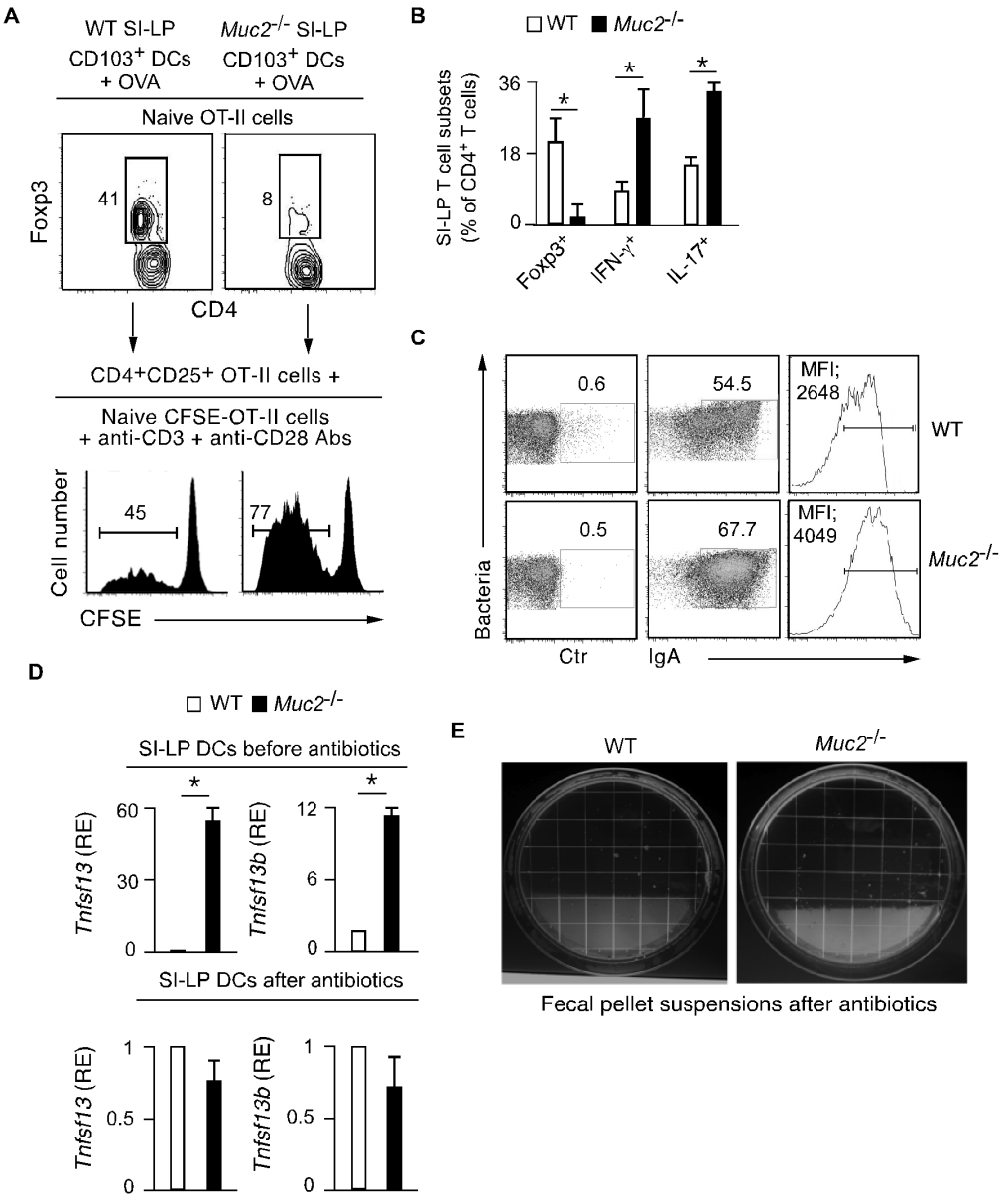

Fig. S12. **Intestinal DCs and T cells express more pro-inflammatory but less tolerogenic properties in the absence of MUC2**. (**A**) FC of Foxp3 and CD4 from mouse naïve OT-II cells cultured for 2 days with SI-LP CD103$^+$ DCs from WT or *Muc2*$^{-/-}$ mice orally immunized with OVA. CD4$^+$CD25$^+$ OT-II cells from these cultures were incubated with CFSE-labeled naïve OT-II cells and agonistic Abs to CD3 and CD28 for 5 days and divided CFSE^{low} cells were quantified by FC. Numbers: % of Treg cells (top) and divided

$CD4^+$ T cells (bottom). (**B**) FC of Foxp3, IFN-γ and IL-17 in mouse SI-LP $CD4^+$ T cells from WT or *Muc2*$^{-/-}$ mice. (**C**) FC of IgA bound on intestinal bacteria from fecal pellets of WT and *Muc2*$^{-/-}$ mice. Ctr, control antibody with irrelevant binding activity. (**D**) qRT-PCR of mRNAs for APRIL (*Tnfsf13*) and BAFF in mouse SI-LP DCs from WT and *Muc2*$^{-/-}$ mice before and after treatment with oral antibiotics. RE, relative expression compared to DCs from WT mice. (**E**) Agar plates seeded for 48 hours with suspensions of fecal pellets from WT and *Muc2*$^{-/-}$ mice after oral antibiotics. Data summarize results from 3 mice for each experimental condition (error bars, s.d.; unpaired *t* test, $*P < 0.05$) or show one of four experiments with similar results.

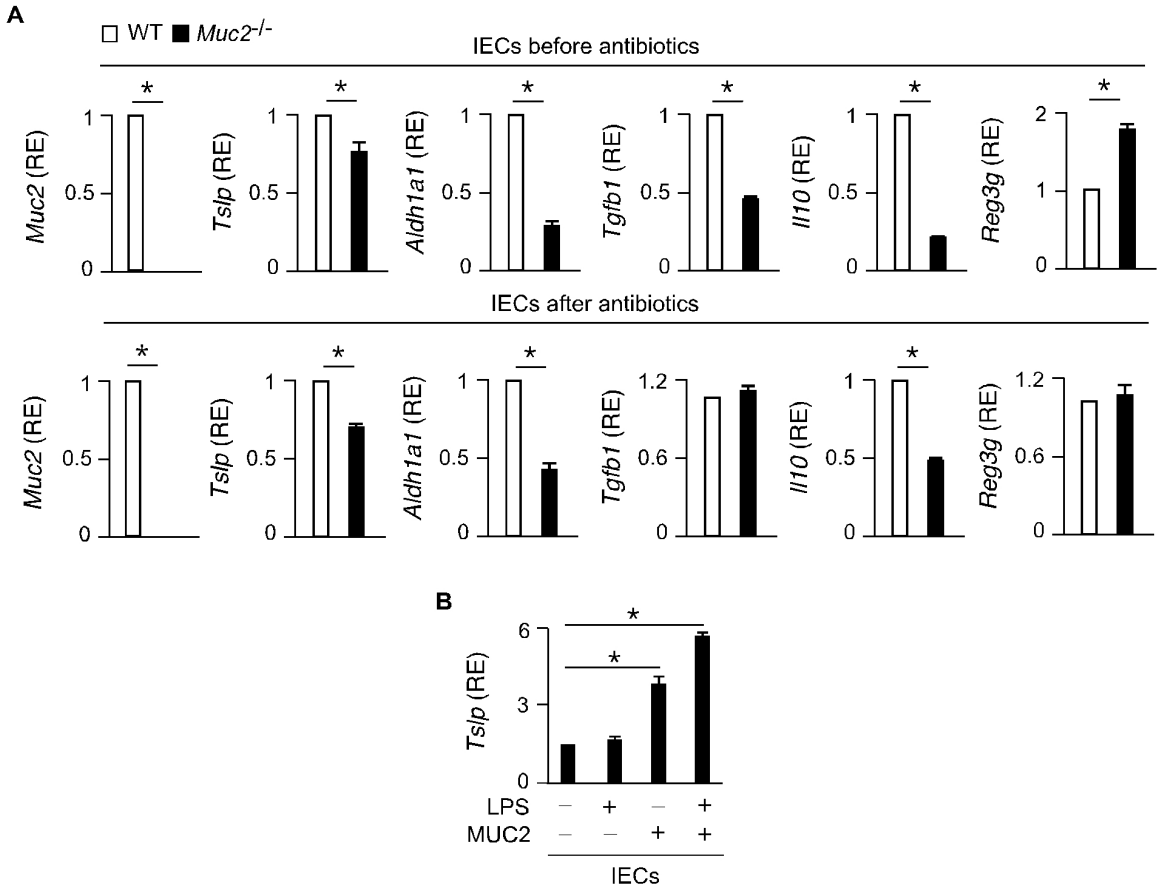

Fig. S13. **IECs express less tolerogenic properties in the absence of MUC2**. (**A**) qRT-PCR of mRNAs for MUC2, TSLP, RALDH1 (*Aldh1a1*) TGF-β1, IL-10 and RegIIIγ in mouse SI-LP IECs from WT and *Muc2*$^{-/-}$ mice before and after oral antibiotics. RE, relative expression compared to WT mice. (**B**) qRT-PCR of TSLP from human IECs (Caco-2 line) cultured for 12 hours with or without LPS and/or MUC2. RE, relative expression compared to unstimulated IECs. Data summarize results from 3 mice for each experimental condition (error bars, s.d.; unpaired *t* test, **P* <0.05) or summarize three experiments with Caco-2.

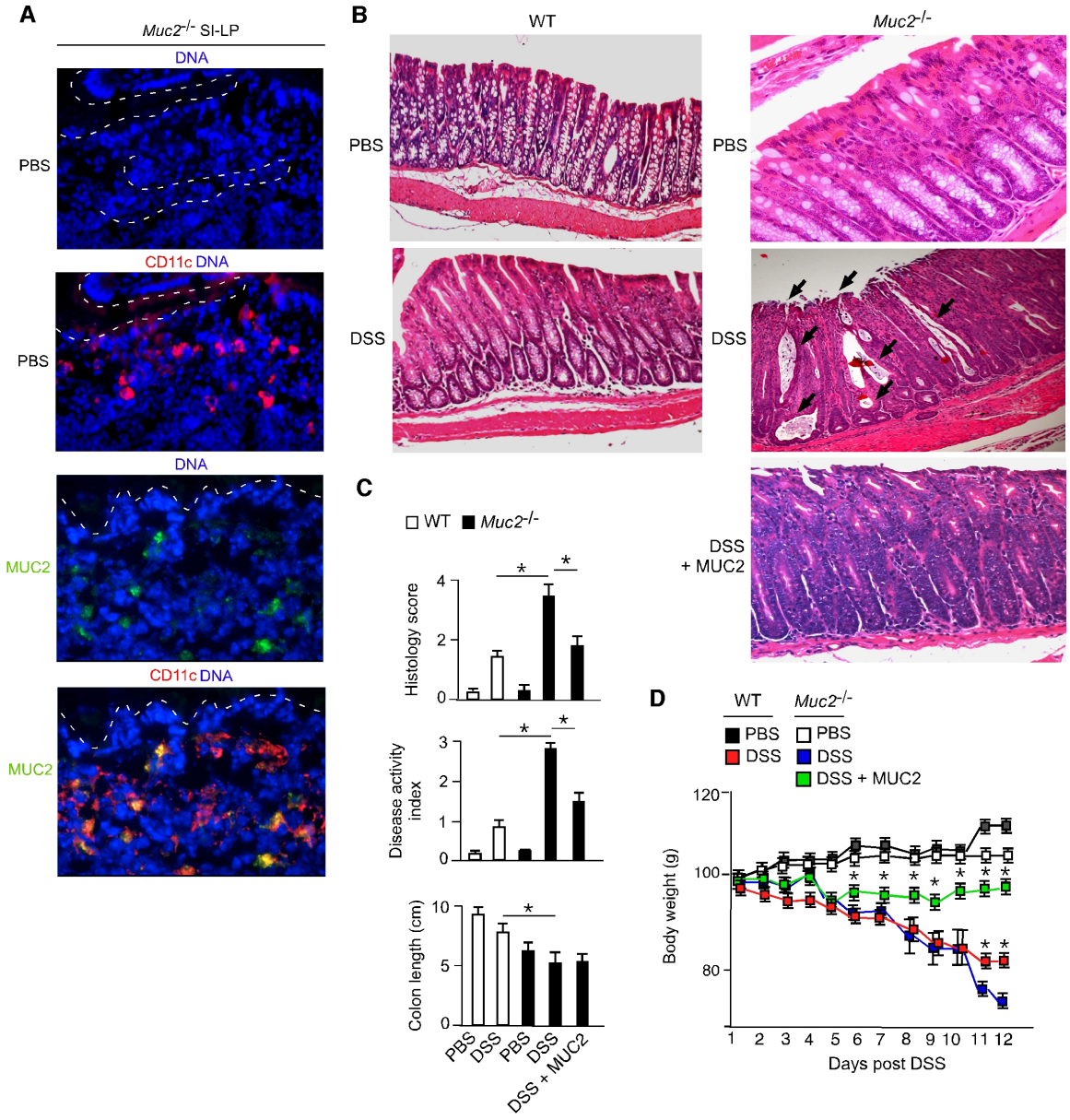

Fig. S14. **MUC2 enhances intestinal resistance to DSS-induced inflammation**. (**A**) IFA of CD11c and DAPI in LI sections from WT and *Muc2*$^{-/-}$ mice gavaged with PBS or CFSE-MUC2 for 5 days. Dashed lines: epithelial border. Original magnification, ×10. (**B**) Conventional microscopy of hematoxylin and eosin-stained LI tissue section from WT and *Muc2*$^{-/-}$ mice challenged with PBS or DSS for 5 days with or without 7-day pre-

treatment with intragastric MUC2. Histology was assessed 12 days after the end of DSS treatment. Original magnification, ×5. (**C** and **D**) Histology score, disease activity index, colon length and body weight in WT and *Muc2*$^{-/-}$ mice treated as in (B). Arrows point to superficial erosions and abscesses. Data summarize results from 4 mice for each experimental condition (error bars, s.d.; unpaired *t* test, **P* <0.05) or show one of four experiments with similar results.

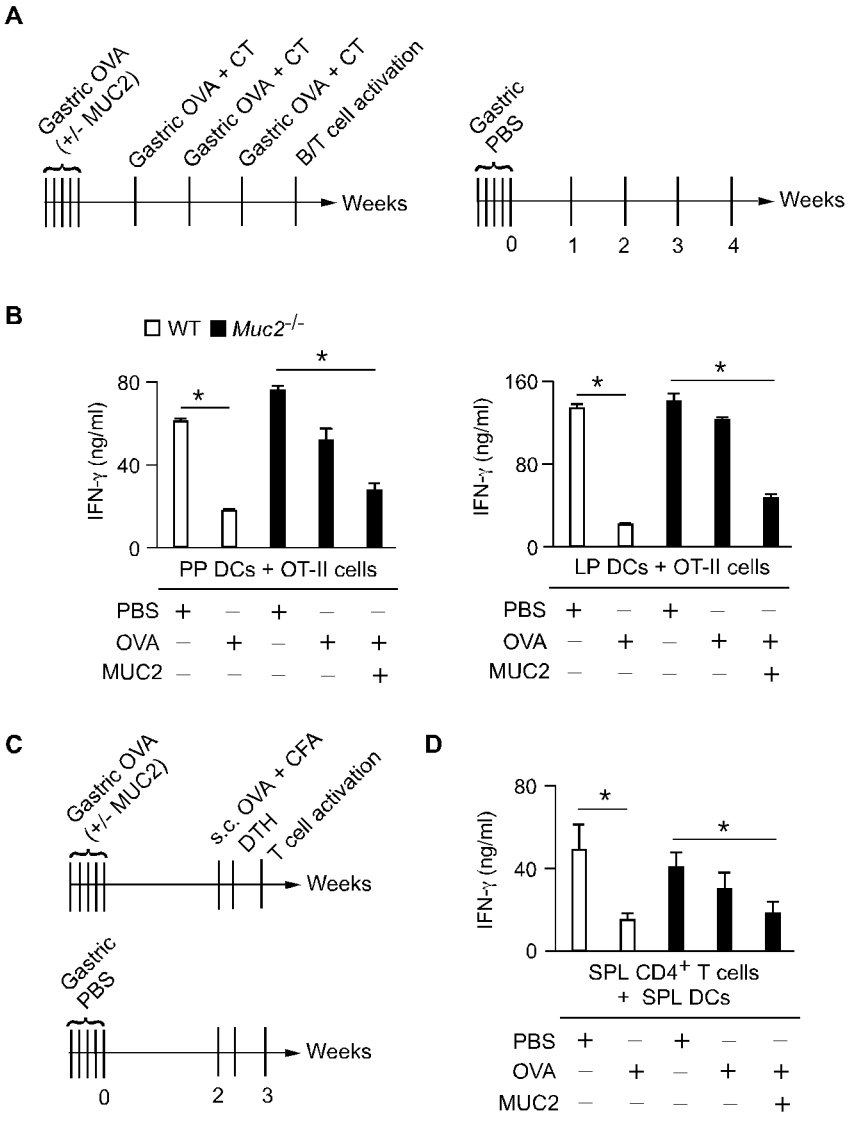

Fig. S15. **MUC2 enhances intestinal and systemic tolerance to OVA**. (**A**) Protocol for the induction of intestinal tolerance to OVA. CT, cholera toxin. (**B**) ELISA of OVA-induced IFN-γ from OT-II cells incubated for 2 days with PP or SI-LP DCs from WT or *Muc2*$^{-/-}$ mice tolerized and immunized as in (A). (**C**) Protocol for the induction of systemic tolerance to OVA. CFA, complete Freund adjuvant; DTH, delayed-type hypersensitivity; s.c., subcutaneous. (**D**) ELISA of IFN-γ secreted by SPL CD4^{+} T cells

from WT or *Muc2*$^{-/-}$ mice tolerized and immunized as in (C). CD4^{+} T cells were activated with OVA-pulsed SPL DCs for 5 days. Data summarize results from 4 mice for each experimental condition (error bars, s.d.; unpaired *t* test, **P* <0.05) or show one of four experiments with similar results.

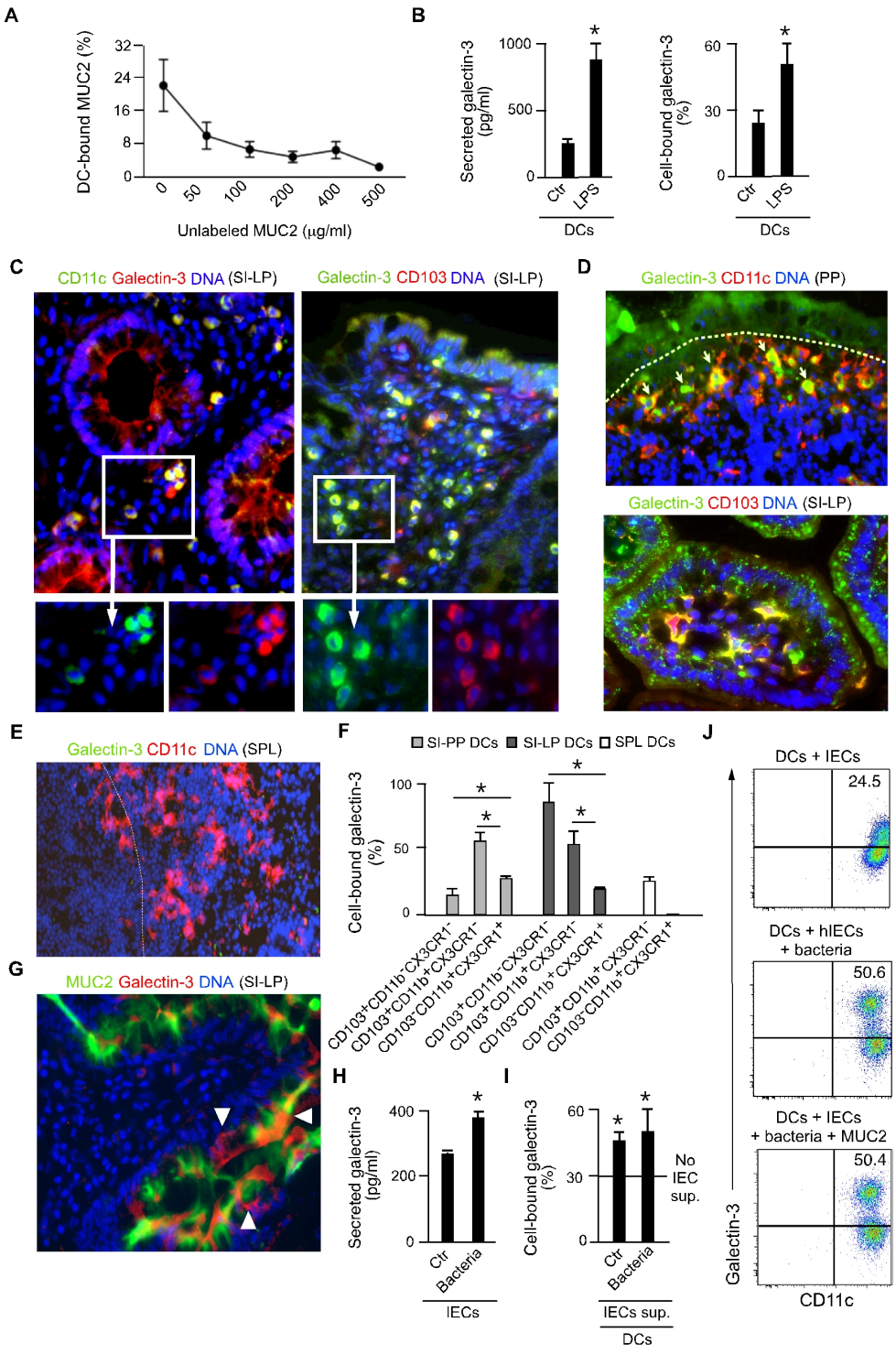

Fig. S16. **Intestinal DCs contain abundant galectin-3 and acquire surface galectin-3 from IECs**. (**A**) FC of CFSE-MUC2 on human monocyte-derived DCs pre-incubated with unlabeled MUC2. (**B**) ELISA of soluble galectin-3 (left) and FC of membrane-

bound galectin-3 (right) from human DCs incubated with medium alone (ctr) or LPS for 2 days. (**C-E**) IFA of human SI-LP (C), mouse PP and SI-LP (D), and mouse SPL (E) tissue sections stained for CD11c, galectin-3, CD103 and/or DAPI. Boxes: galectin-3^{+} DCs magnified in bottom panels. Dotted line: basolateral border of follicle-associated epithelium in PP. Original magnification, ×10 (C) or ×20 (D and E). (**F**) FC of galectin-3 on mouse PP, SI-LP and SPL DC subsets. (**G**) IFA of GCs from the human SI-LP stained for MUC2, galectin-3 and DAPI. Original magnification, ×20. (**H**) ELISA of galectin-3 from human IEC monolayer incubated with or without bacteria for 2 days. (**I**) FC of galectin-3 on human DCs incubated with culture supernatant from IECs treated as in (H). Dotted line: mean % of DCs with surface galectin-3 under basal conditions. (**J**) FC of CD11c and galectin-3 on human DCs undergoing trans-IEC sampling of bacteria in the presence or absence of MUC2. Data summarize three experiments (error bars, s.d.; unpaired *t* test, **P* <0.05) or show one of four experiments with similar results.

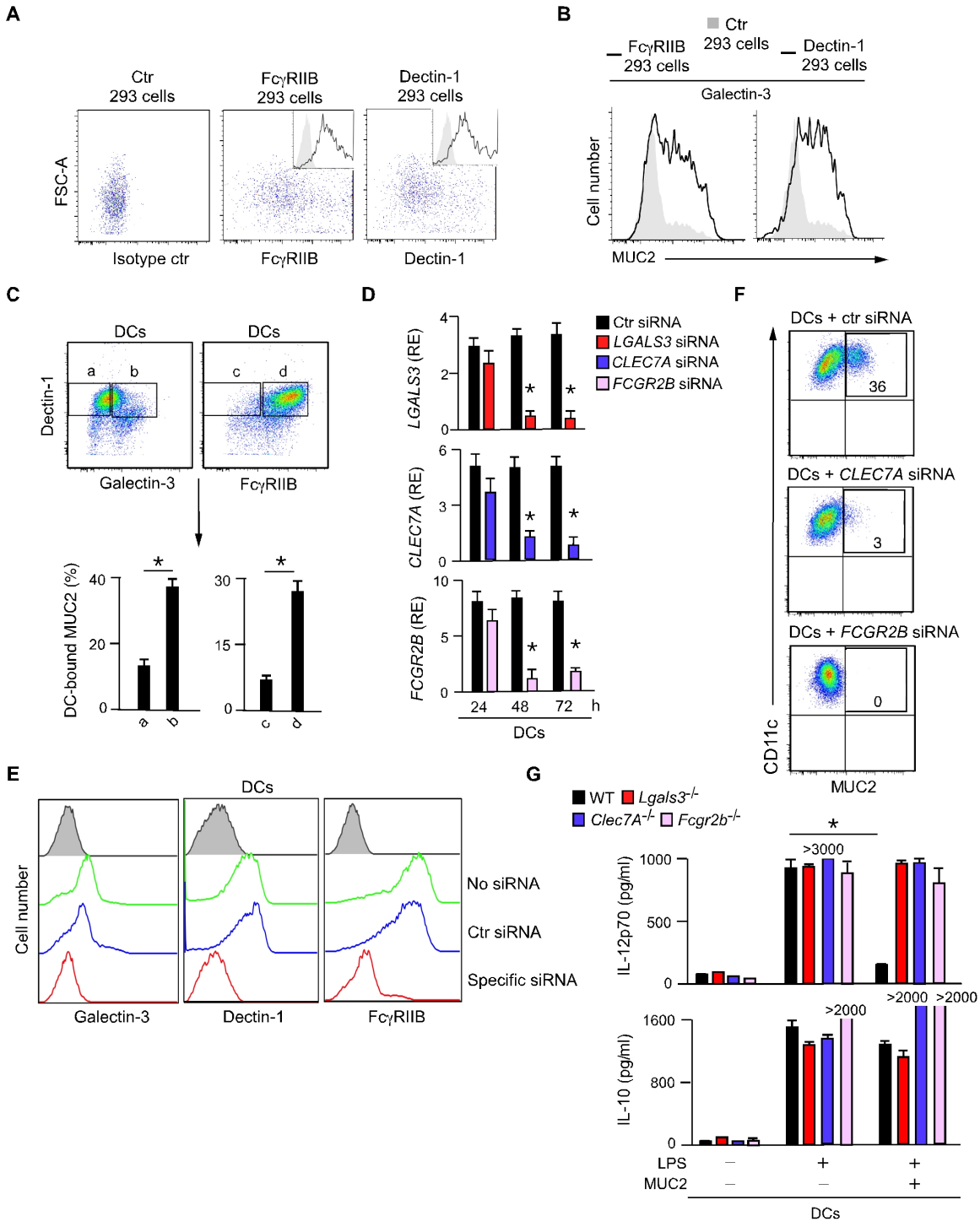

Fig. S17. MUC2 binds galectin-3, FcγRIIB and Dectin-1 on DCs. (**A**) FC of isotype ctr Ab in untransfected 293 cells and FC of FcγRIIb and Dectin-1 in transfected FcγRIIb-

293 cells or Dectin-1-293 cells, respectively. Insets: ctr Ab (solid profile) and FcγRIIb or Dectin-1 (open profiles) in FcγRIIb-293 or Dectin-1-293 cells, respectively. (**B**) FC of CFSE-MUC2 on untransfected (solid profile) and transfected (open profile) 293 cells expressing FcγRIIb or Dectin-1. (**C**) FC of galectin-3, FcγRIIB, Dectin-1 and CFSE-MUC2 on human monocyte-derived galectin-3^{lo} (a), galectin-3^{hi} (b), FcγRIIB^{lo} (c) and FcγRIIB^{hi} (d) DCs. (**D**) qRT-PCR of *LGALS3*$^{-/-}$, *CLEC7A*$^{-/-}$ or *FCGR2B*$^{-/-}$ in human DCs exposed to scrambled (ctr) siRNA or siRNA to galectin-3, Dectin-1 or FcγRIIB. RE, relative expression compared to DCs incubated with no siRNA. (**E**) FC of galectin-3, Dectin-1 or FcγRIIB on human DCs treated as in (D) for two days. Gray profile: DC staining by an irrelevant Ab. (**F**) FC of CD11c and CFSE-MUC2 on human DCs treated with scrambled (ctr), *FCGR2B* or *CLEC7A* siRNAs. Numbers: % of positive cells. (**G**) ELISA of IL-12p70 and IL-10 from BM-derived WT, *Lgals3*$^{-/-}$, *Clec7a*$^{-/-}$ or *Fcgr2b*$^{-/-}$ DCs cultured with or without LPS and/or MUC2 for 48 hours. Data summarize three experiments (error bars, s.d.; unpaired *t* test, $*P < 0.05$) or show one of four experiments with similar results.

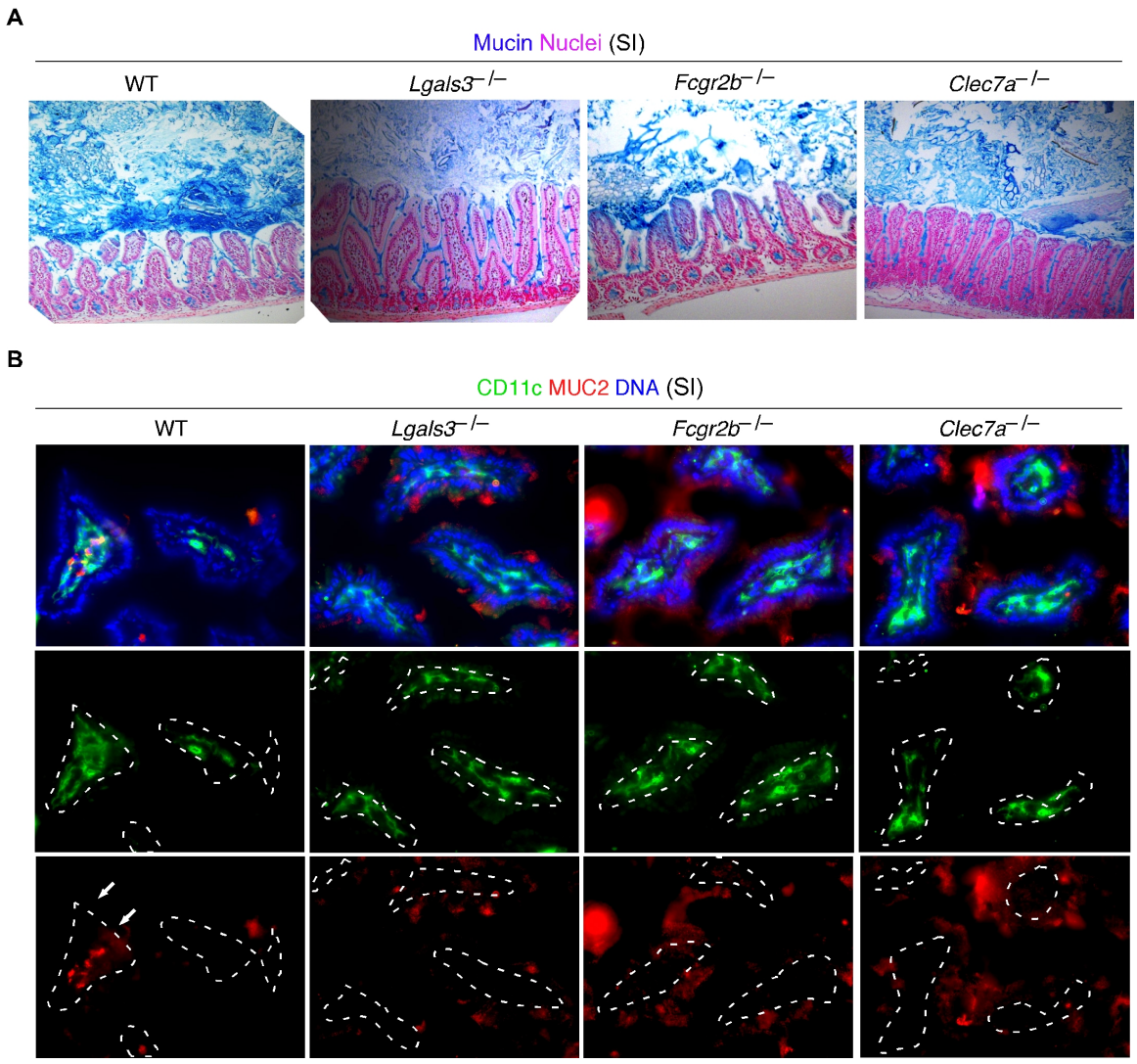

Fig. S18. **Lack of galectin-3, FcγRIIB or Dectin-1 decreases MUC2 internalization by intestinal DCs**. (**A**) Light microscopy of mucin in Carnoy-fixed SI tissues sections from WT, *Lgals3*$^{-/-}$, *Fcgr2b*$^{-/-}$and *Clec7A*$^{-/-}$ mice. Original magnification, ×5. (**B**) IFA of CD11c, MUC2 and DAPI in SI-LP tissue sections from WT, *Lgals3*$^{-/-}$, *Fcgr2b*$^{-/-}$and *Clec7A*$^{-/-}$ mice. Dashed lines: epithelial border. Arrows: MUC2-containing SI-LP DCs. Original magnification, ×20. Data show one of 10-12 stainings from each mouse strain that yielded similar results.

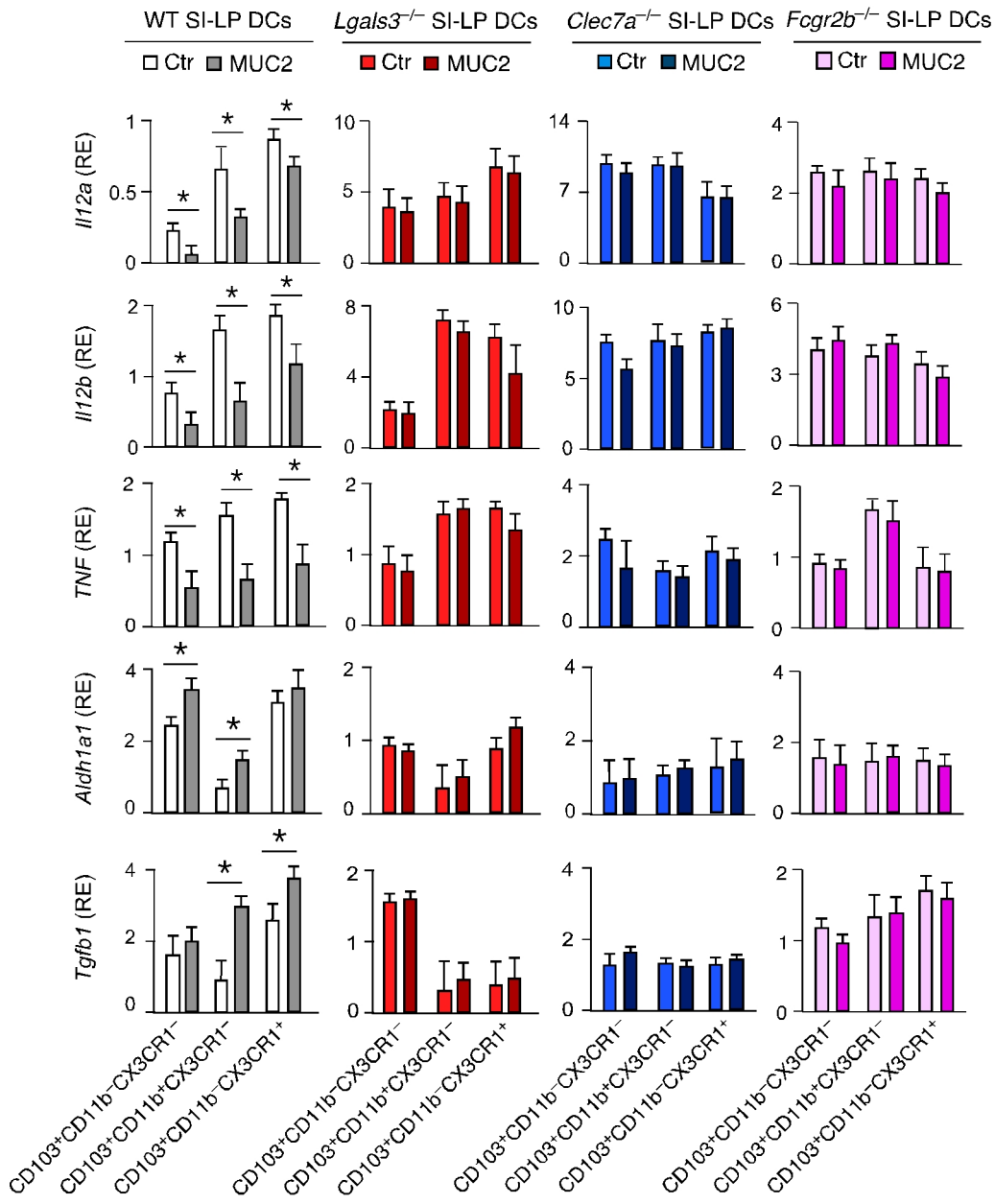

Fig. S19. **Lack of galectin-3, FcγRIIB or Dectin-1 decreases the tolerogenic response of intestinal DC subsets to MUC2**. qRT-PCR of *Il12a*, *Il12b*, *Tnf*, *Aldh1a1* and *Tgfb1* in SI-LP DC subsets from WT, *Lgals3*$^{-/-}$, *Clec7A*$^{-/-}$ or *Fcgr2b*$^{-/-}$ mice incubated with PBS (ctr) or MUC2 for 2 days. RE, relative expression compared to *Gapdh*. Data summarize results from 4 mice for each experimental condition (error bars, s.d.; unpaired *t* test, **P* <0.05).

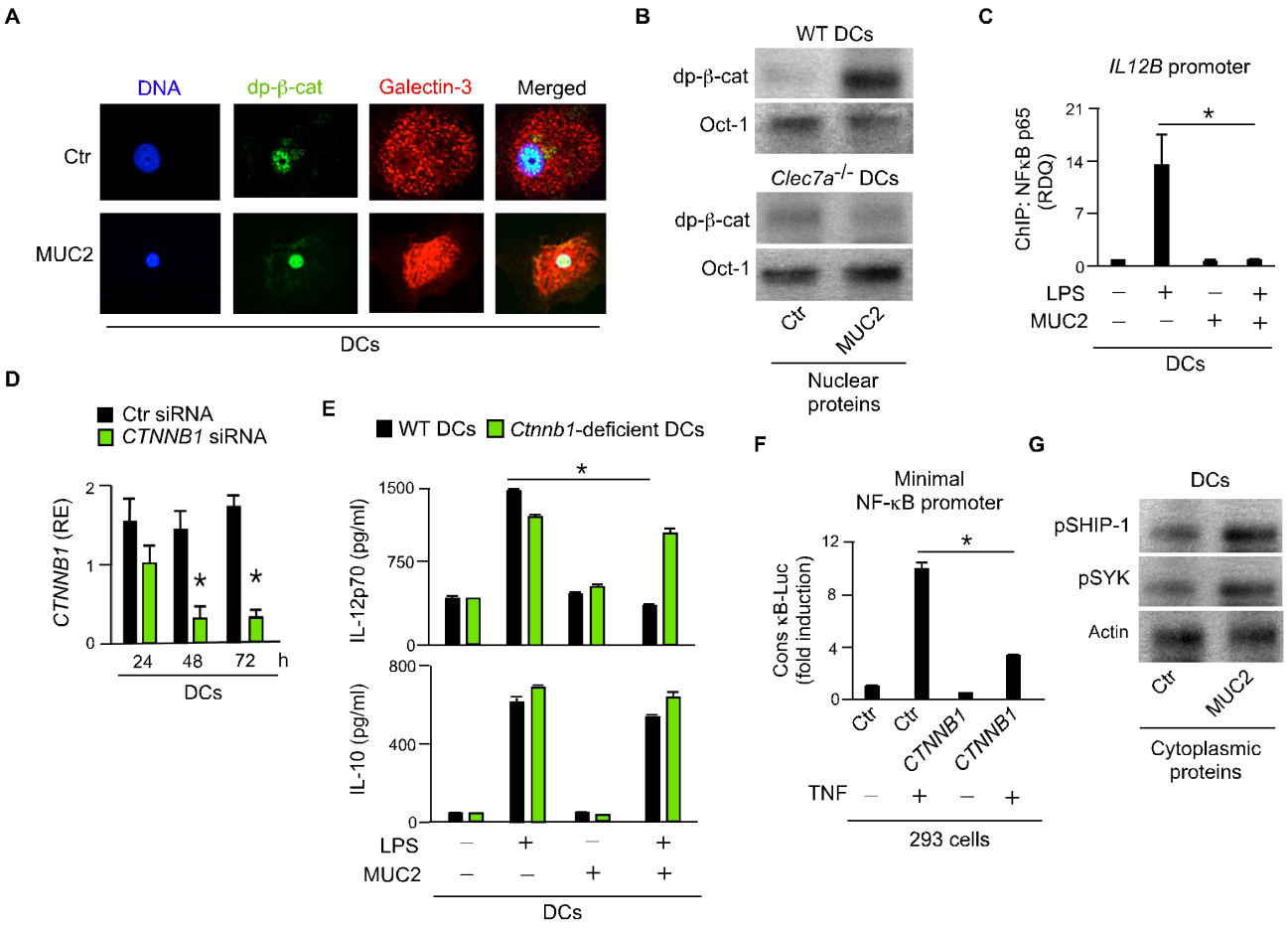

Fig. S20. **MUC2 inhibits DC production of IL-12 by inhibiting NF-κB activation via Dectin-1-induced β-catenin**. (**A**) IFA of dp-β-cat, galectin-3 and DAPI in human monocyte-derived DCs exposed to medium alone (ctr) or MUC2 for 15 minutes. (**B**) WB of nuclear dp-β-cat and Oct-1 (loading control) with protein lysates from gut DCs of WT or *Clec7a*$^{-/-}$ mice exposed to medium alone (ctr) or MUC2 for 15 minutes. (**C**) ChIP assay of *IL12B*-bound NF-κB p65 in human DCs cultured with or without LPS and/or MUC2 for 3 hours. RDQ, relative DNA quantity measured by qRT-PCR. (**D**) qRT-PCR of *CTNBB1* in human DCs exposed to scrambled (ctr) or β-catenin-specific siRNAs. RE, relative expression compared to DCs incubated with no siRNA. (**E**) ELISA of IL-12p70 and IL-10 produced by bone marrow-derived DCs from WT or *Ctnnb*$^{-/-}$ mice and

cultured as in (C) for 2 days. (**F**) Luciferase reporter activity of a minimal NF-κB promoter in human 293 cells transfected with empty (ctr) or *CTNNB1* expression vectors and stimulated with or without TNF for 48 hours. (**G**) WB of pSHIP-1, pSYK and actin (loading control) in human DCs exposed to medium alone (ctr) or MUC2 for 15 minutes. Data summarize three experiments (error bars, s.d.; unpaired *t* test, $*P < 0.05$) or show one of four experiments with similar results.

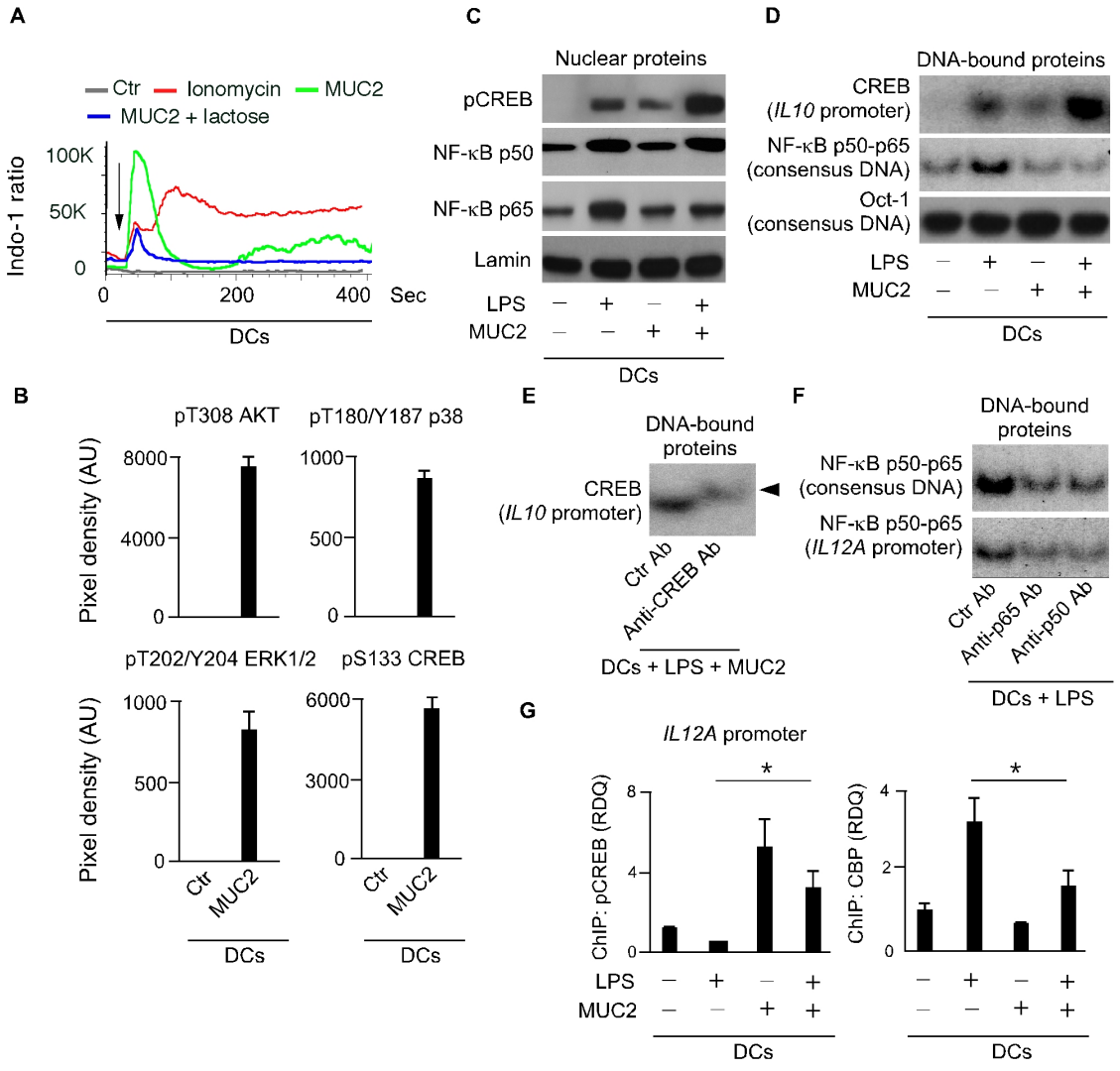

Fig. S21. **MUC2 enhances CREB but reduces CBP binding to the *IL12* gene promoter in DCs**. (**A**) FC of calcium flux in human DCs incubated with medium alone (ctr), ionomycin, MUC2 alone or MUC2 plus lactose (a competitor of galectin-3). Arrow indicates time of stimulation. (**B**) Densitometric analysis of pAKT, pp38, pERK-1/2 and pCREB from phospho-kinase arrays performed with proteins from human DCs cultured with medium alone (ctr) or MUC2 for 10 minutes. T, threonine; Y, tyrosine; S, serine. (**C**) WB of nuclear pCREB, NF-κB p65, NF-κB p50 and lamin (loading control) from human monocyte-derived DCs cultured with or without LPS and/or MUC2 for 3 hours.

(**D**) Electrophoretic mobility gel shift assay (EMSA) of nuclear CREB bound to a CREB-binding element of the *IL10* promoter and of NF-κB p65-p50 bound to a consensus DNA sequence in DCs cultured as in (C) for 3 hours. Oct-1 binding to a consensus DNA sequence is a loading control. (**E**) Identification of DNA-bound CREB by a super-shifting Ab to CREB in DCs exposed to LPS and MUC2. Arrow points to the supershifted complex. (**F**) Identification of DNA-bound NF-κB p50-p65 by inhibitory Abs to p65 and p50 in DCs exposed to LPS. (**G**) ChIP assay of *IL12A*-bound pCREB and CBP in DCs cultured as in (C) for 3 hours. RDQ, relative DNA quantity measured by qRT-PCR. Data summarize three experiments (error bars, s.d.; unpaired *t* test, $*P < 0.05$) or show one of four experiments with similar results.

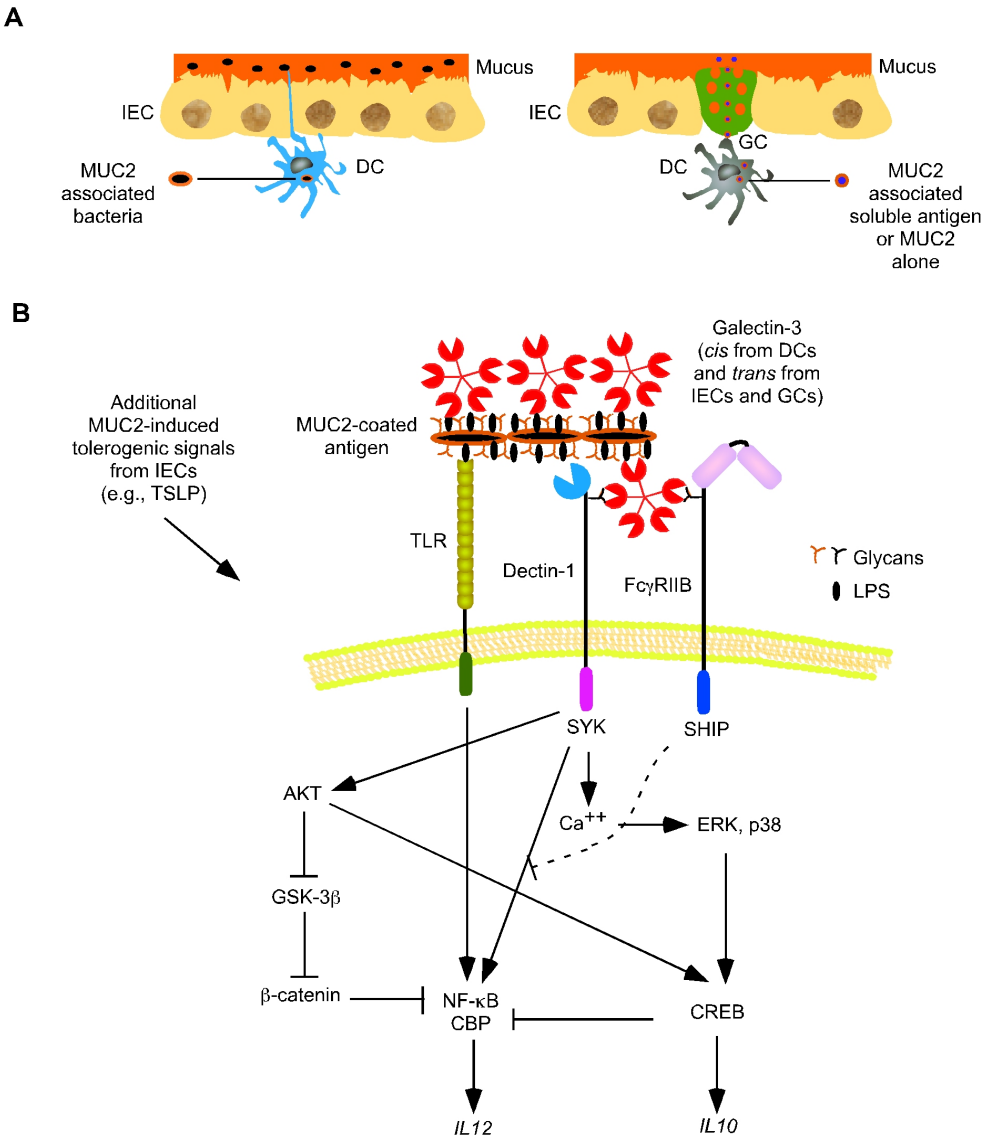

Fig. S22. **Proposed mechanism underlying the homeostatic and tolerogenic functions of intestinal mucus**. Gut CX3CR1^{+} and CD103^{+} DCs sample MUC2-associated antigen across IECs or via GC-associated passages. MUC2 triggers galectin-3 oligomerization on antigen-sampling DCs, thereby facilitating the formation of a galectin-3-Dectin-1-FcγRIIB receptor complex. By inactivating GSK-3β via AKT, Dectin-1 inhibits GSK-3β-mediated degradation of β-catenin, thus allowing β-catenin translocation to the nucleus. Nuclear β-catenin interacts with NF-κB induced by bacterial TLR ligands (e.g., LPS) or

cytokines (e.g., TNF), thus inhibiting antigen-induced activation of NF-κB-dependent proinflammatory genes (e.g., *IL12*). Concomitantly, Dectin-1 induces CREB via calcium, p38, ERK and AKT signals, thus sustaining antigen-induced activation of CREB-dependent anti-inflammatory genes (e.g., *IL10*). CREB also removes CBP from DNA-bound NF-κB, which further amplifies the inhibition of NF-κB-dependent proinflammatory genes. Finally, FcγRIIB activates SHIP-1 to constrain SYK-induced proinflammatory NF-κB signals but not tolerogenic CREB signals emanating from Dectin-1.

Table S1. Antibodies to mouse antigens for immunostainings.

Antigen	Label	Isotype	Clone	Manufacturer	Use
CD103	Pacific Blue	Hamster IgG	2E7	Biolegend	FC[#]
CD103	PE	Rat IgG2a	M290	BD Biosciences	FC, IFA
CD103	Biotin	Hamster IgG	2E7	Biolegend	FC, IFA
CD103	AF647	Hamster IgG	2E7	Biolegend	FC, IFA
CD11b	PE	Rat IgG2b	M1/70	BD Biosciences	FC, IFA[¶]
CD11b	PE	Rat IgG2b	M1/70	BD Biosciences	FC, IFA
CD11c	Biotin	Hamster IgG1	N418	BD Biosciences	FC, IFA
CD11c	APC-Cy7	Hamster IgG	N418	Biolegend	FC, IFA
CD19	PE-Cy7	Rat IgG2a	1D3	BD Biosciences	FC
CD19	FITC	Rat IgG2a	1D3	BD Biosciences	FC
CD25	APC-Cy7	Rat IgG1	PC61	BD Biosciences	FC
CD25	APC	Rat IgG1	PC61	BD Biosciences	FC
CD32b	FITC	Rat IgG2b	2.4G2	Santa Cruz	FC
CD4	PE-Cy7	Rat IgG2b	GK1.5	eBioscience	FC
CD40	FITC	Hamster IgM	HM40-3	BD Biosciences	FC
CD45	PE-Cy7	Rat IgG2b	30-F11	eBioscience	FC
CD80	Pacific Blue	Hamster IgG	16-10A1	Biolegend	FC
CD86	APC-Cy7	Rat IgG2a	GL-1	Biolegend	FC
CD8a	PerCP-Cy5.5	Rat IgG2a	53-6.7	Biolegend	FC
CX3CR1	Mouse/Human	Rabbit IgG	Bs-1728R	Bioss	FC, IFA
dp-β-catenin		Rabbit IgG	9561	Cell Signaling	IFA
F4/80	Biotin	Rat IgG2a	BM8	Biolegend	FC, IFA
Foxp3	V450	Rat IgG2b	MF23	BD Biosciences	FC
Galectin-3	PE	Ms IgG1	Gal397	Biolegend	FC, IFA
IFN-γ	FITC	Rat IgG1	XMG1.2	BD Biosciences	FC
IgA	Biotin	Rat IgG1	C10-1	BD Biosciences	FC
IgA	FITC	Rat IgG1	11-44-2	Southern Biotech	FC
IgD	PE	Rat IgG2a	11-26c.2a	BD Biosciences	FC
IgD	APC	Rat IgG2a	11-26c.2a	BD Biosciences	FC
IgM	APC	Rat IgG2a	II/41	BD Biosciences	FC
IL-10	V450	Rat IgG2b	JES5-16E3	BD Biosciences	FC
MHC II	Biotin	Rat IgG2B	M5/114.15.2	eBiosciences	FC
MUC2		IgG1	996/1	Abcam	IFA
pAKT (S473)	Mouse/Human	Rabbit IgG	D9E	Cell Signaling	IFA
pGSK-3β (Ser21/9)	Mouse/Human	Rabbit IgG	37F11	Cell Signaling	IFA
β-catenin		Rabbit IgG	9561	Cell Signaling	IFA
IL-17A	Mouse	Rat IgG1		TC11-18H10	FC

[#]FC, flow cytometry
[¶]IFA, immunofluorescence analysis

Table S2. Antibodies to human antigens for immunostainings.

Antigen	Label	Isotype	Clone or catalogue number	Manufacturer	Use
dp-β-catenin (Ser33/37/Thr41)		Rabbit IgG	D13A1	Cell Signaling	¶IFA
CD103	FITC	Mouse IgG1	B-Ly7	eBioscience	#FC, IFA
CD103	PE	Mouse IgG2a	MCA708	Serotec	FC, IFA
CD11b	PB	Mouse IgG2a	ICRF44	Biolegend	FC
CD11c	PE	Mouse IgG1	3.9	iCyt	FC, IFA
CD11c	PE-Cy7	Mouse IgG1	3.9	iCyt	FC
CD11c		Mouse IgG2a	5D11	Santa Cruz	IFA
CD14	Biotin	Mouse IgG2a	UCHM-1	Southern Biotec	Sorting
CD14	APC-Cy7	Mouse IgG1	HCD14	Biolegend	FC
CD16	Biotin	Mouse IgG1	3G8	BD Biosciences	FC
CD19	PE-Cy7	Mouse IgG1	HIB19	Biolegend	FC
CD25	PE-Cy7	Mouse IgG1	BC96	eBioscience	FC
CD3	PE	Mouse IgG1	SK7	BD Biosciences	FC
CD32b	PE	Rat IgG2b	2.4G2	Santa Cruz	FC
CD4	APC-Cy7	Mouse IgG1	RPA-T4	BD Biosciences	FC
CD45RA	PE-Cy7	Mouse IgG2b	HI100	eBiosciences	FC
CD45RO	eFluor 650NC	Mouse IgG2a	UCHL1	eBiosciences	FC
CD80	PE	Mouse IgG1	B7-1	BD Biosciences	FC
CD83	PE-Cy7	Mouse IgG1	HB15e	Ancell	FC
CD86	PE	Mouse IgG2b	IT2.2 BD	Biosciences	FC
CX3CR1		Rabbit IgG1	2093	ProSci Inc.	FC, IFA
CX3CR1	PE	Rat IgG2b	2A9-1	Biolegend	FC, IFA
Dectin-1	APC	Mouse IgG2b	259931	R&D Systems	FC
FOXP3	FITC	Mouse IgG1	259D/C7	BD Biosciences	FC
Galectin-3	PE	Mouse IgG1	Gal397	Biolegend	FC
Galectin-3		Mouse IgG1	Gal397	Biolegend	FC
Galectin-3		Rabbit IgG	Ab31707	abcam	$IP
IFN-γ	PE	Mouse IgG1	4S.B3	eBioscience	FC
IL-10	PE	Mouse IgG1	JES3-19E1	Biolegend	FC
IL-17A	PE	Mouse IgG1	eBio64DEC17	eBioscience	FC
MHC-II	PE	Mouse IgG1	TDR31.3	Ancell	FC
MUC2		Rabbit IgG	Ab11197	Abcam	IFA
MUC2		Mouse IgG1	CCP58	BD Biosciences	IFA
MUC2		Rabbit IgG	H-300	Santa Cruz	IFA, FC
Occludin		Rabbit IgG	71-1500	Invitrogen	IFA

Table S3. Antibodies for functional assays.

Antigen	Isotype	Clone or catalogue number	Manufacturer	Use
Human IL-10	Rat IgG1	JES3-9D7	Biolegend	Blocking
Human TGF-β1	Mouse IgG1	2E6	Abcam	Blocking
Mouse CD3e	Hamster IgG1	145-2C11	BD Biosciences	Stimulation
Mouse CD28	Hamster IgG2	37.51	BD Biosciences	Stimulation
Human CD3	Mouse IgG2a	HIT3a	BD Biosciences	Stimulation
Human CD3	Mouse IgG1	UCHT1	BD Biosciences	Stimulation
Human CD28	Mouse IgG1	CD28.2	BD Biosciences	Stimulation
Irrelevant	Rat IgG1	RTK2071	Biolegend	Control
Irrelevant	Rat IgG2a	RTK2758	Biolegend	Control
Irrelevant	Mouse IgG1	11711	R&D Systems	Control
Irrelevant	Goat IgG	AB-108-C	R&D Systems	Control
Irrelevant	Hamster IgG2	Ha4/8	BD Biosciences	Control
Irrelevant	Mouse IgG2a	G155-178	BD Biosciences	Control

Table S4. Coating strategy for ELISA.

Purified antigen	Source	Company	Concentration or dilution
OVA	*Chicken egg white*	Sigma	0.5 μg/ml
Human galectin-3	*E. Coli*	R&D Systems	0.1 μg/ml
Mouse galectin-3	*E. Coli*	R&D Systems	0.1 μg/ml
Human galectin-1	*E. Coli*	R&D Systems	0.1 μg/ml
Mouse galectin-1	*E. Coli*	R&D Systems	0.1 μg/ml
Human galectin-9	*E. Coli*	R&D Systems	0.1 μg/ml
Mouse galectin-9	*E. Coli*	R&D Systems	0.1 μg/ml
Mouse IgA	Rat (IgG1, 11-44-2)	Southern Biotec	1:1000
Mouse IgM	Rat (IgG1, 1B4B1)	Southern Biotec	1:1000
Mouse IgG	Goat	Southern Biotec	1:1000

Table S5. Primers used to amplify human gene products.

Target gene		Primer sequence
ACTB	Sense	GGATGCAGAAGGAGATCACT
	Antisense	CGATCCACACGGAGTACTTG
ALDH	Sense	ACCGTACTCTCCCAGTTCTCTTC
	Antisense	AATTGCTATGGCGTGGTAAGTG
IL6	Sense	GGTACATCCTCGACGGCATCT
	Antisense	GTGCCTCTTTGCTGCTTTCAC
IL8	Sense	CCAAACCTTTCCACCC
	Antisense	ACTTCTCCACAACCCT
IL10	Sense	ACCTGCCTAACATGCTTCGAG
	Antisense	TGTCCAGCTGATCCTTCATTTG
IL12A	Sense	TTCACCACTCCCAAAACCTGC
	Antisense	GAGGCCAGGCAACTCCCATTAG
IL12B	Sense	GGGAACTGAAGAAAGATGTTTATG
	Antisense	CTCTGGTCCAAGGTCC
TNFA	Sense	CCCAGGGACCTCTCTCTAATCA
	Antisense	GCTACAGGCTTGTCACTCGG
TGFB1	Sense	AAGGACCTCGGCTGGAAGTGG
	Antisense	CCGGGTTATGCTGGTTGTA
TGFB1	Sense	AAGGACCTCGGCTGGAAGTGG
	Antisense	CCGGGTTATGCTGGTTGTA
TSLP	Sense	TAGCAATCGGCCACATTGCC
	Antisense	CTGAGTTTCCGAATAGCCTG

Table S6. Primers used to amplify mouse gene products.

Target gene		Primer sequence
Actin	Sense	GGATGCAGAAGGAGATCACT
	Antisense	CGATCCACACGGAGTACTTG
Aldh1a1	Sense	GGGAAAGAGCCCTTGCATTGTGTT
	Antisense	GCGACACAACATTGGCCTTGATGA
Aldh1a2	Sense	ACCGTGTTCTCCAACGTCACTGAT
	Antisense	TGCATTGCGGAGGATACCATGAGA
Gapdh	Sense	CCTGTTGCTGTAGCCGTATTCA
	Antisense	CCAGGTTGTCTCCTGCGACTT
Il10	Sense	GGTGATGCCCCAAGCTGA
	Antisense	TCCCCCAGGGAGTTCACA
Il12a	Sense	CACCCTTGCCCTCCTAAACC
	Antisense	CACCTGGCAGGTCCAGAGA
Il12b	Sense	ACAGCACCAGCTTCTTCATCAG
	Antisense	TCTTCAAAGGCTTCATCTGCAA
Lgals3	Sense	TTGAAGCTGACCACTTCAAGGTT
	Antisense	AGGTTCTTCATCCGATGGTTGT
Tgfb1	Sense	AAGGACCTCGGCTGGAAGTGG
	Antisense	CCGGGTTATGCTGGTTGTA
Tnfsf13	Sense	GTGATGTGGCAACCAGCTCTT
	Antisense	CCCTTGGTGTAAATGGAAGAC
Tnfsf13b	Sense	ACCGCGGGACTGAAAATCT
	Antisense	CACGCTTATTTCTGCTGTTCTGA
Tslp	Sense	AGGCTACCCTGAAACTGAG
	Antisense	GGAGATTGCATGAAGGAATACC
RegIIIγ	Sense	TTCCTGTCCTCCATGATCAA
	Antisense	CATCCACCTCTGTTGGGTTC

Table S7. Antibodies for immunoblotting and immunoprecipitation.

Antigen	Species	Isotype	Clone or catalogue number	Manufacturer	Use
β-catenin	Mouse/Human	Rabbit IgG	9561	Cell Signaling	WB
dp-β-catenin	Mouse/Human	Rabbit IgG	D13A1	Cell Signaling	*WB
AKT	Mouse/Human	Rabbit IgG	11E7	Cell Signaling	WB
β-catenin	Mouse/Human	Rabbit IgG	6B3	Cell Signaling	WB
CBP	Mouse/Human	Rabbit IgG	D6C5	Cell Signaling	&ChIP
CREB	Mouse/Human	Rabbit IgG	48H2	Cell Signaling	WB
Dectin-1	Human	Rabbit IgG	9051	Cell Signaing	WB
Dectin-1	Mouse	Rat IgG2b	2A11	AbD Serotec	WB
FcγRIIB	Human	Rabbit IgG	EP888Y	Abcam	WB
FcγRIIB	Mouse	Mouse IgG2a	190907	R&D Systems	WB
Galectin-3	Mouse/Human	Mouse IgG1	Gal397	Biolegend	WB
Galectin-3	Human	Rabbit IgG	Ab31707	Abcam	$IP
GSK-3β	Mouse/Human	Rabbit IgG	27C10	Cell Signaling	WB
MUC2	Mouse/Human	Rabbit IgG	Ab76774	Abcam	WB
MUC2	Human	Mouse IgG1	CCP58	Abcam	WB
MUC2	Mouse/Human	Rabbit IgG	H-300	Santa Cruz	WB
OCT-1	Human	Rabbit IgG	4428	Cell Signaling	WB
β-catenin	Mouse/Human	Rabbit IgG	9561	Cell Signaling	WB, IFA
pAKT (S473)	Mouse/Human	Rabbit IgG	D9E	Cell Signaling	WB
pCREB (S133)	Mouse/Human	Rabbit IgG	D1G6	Cell Signaling	ChIP, WB
pGSK-3β (Ser21/9)	Mouse/Human	Rabbit IgG	37F11	Cell Signaling	WB
p50	Mouse/Human	Rabbit IgG	H-119	Santa Cruz	WB
p65	Mouse/Human	Rabbit IgG	C-20	Santa Cruz	WB
pSHIP (Tyr1020)	Mouse/Human	Rabbit IgG	3941	Cell Signalling	WB
pSMAD2(Ser464/467)/ pSMAD3(Ser423/425)	Mouse/Human	Rat IgG	D27F4	Cell Signaling	WB
pSYK (Tyr525/526)	Mouse/Human	Rabbit IgG	C87C1	Cell Signalling	WB
SHIP	Mouse/Human	Rabbit IgG	D1163	Cell Signalling	WB
SMAD2/SMAD3	Mouse/Human	Rabbit IgG	5678	Cell Signaling	WB
SYK	Mouse/Human	Rabbit IgG	2712	Cell Signalling	WB

*WB, Western blotting
$IP, immunoprecipatation
&ChIP, chromatin immunoprecipatation

Table S8. Primers used for EMSA* and ChIP.

Target gene		Primer sequence	Region	Use
IL10	Sense	TTGTCCACGTCACTGTGACCTAG	From -356 to -335	EMSA
	Antisense	CTAGGTCACAGTGACGGGACAA		
IL12A	Sense	TTCTCTAGGTCTTTCCTCCCAGG	From -395 to -372	EMSA
	Antisense	CCTGGGAGGAAAGACCTAGAGAA		
IL12A	Sense	CGGTCTAATGCCTGCTTGTT	From -482 to -296	ChIP^
	Antisense	GGATGAGAGCTGGCTGACTC		
IL12B	Sense	CTGACTTGGGAAGAACCAGGATT	From -39 to -16	EMSA
	Antisense	AATCCTGGTCTTCCCAAGTCAG		
IL12B	Sense	TCCTGATTACACCTCCTTCC	From -102 to +47	ChIP
	Antisense	TCAGACGGGAGGCTGAGTTC		

*EMSA, electrophoretic mobility gel shift assay
^ChIP, chromatin immunoprecipitation

Supplementary Movie Legends

Movie S1.

Gut DC interacts with MUC2. Confocal microscopy and three-dimensional animation of human gut tissue section stained for CD11c (green) and MUC2 (red) and counterstained with DAPI (blue). The movie was generated by acquiring up to 53 x,y planes with 0.25 μm z spacing, three-dimensional animation and volume enhancement of the green and red channels. Original magnification, ×63; digital magnification ×5.5. One of three experiments yielding similar results.

Movie S2.

Gut DCs contain MUC2. Confocal microscopy and three-dimensional animation of a human gut tissue section section stained for CD11c (green) and MUC2 (red) and counterstained with DAPI (blue). The movie was generated by acquiring up to 47 x,y planes with 0.25 μm z spacing, three-dimensional animation and volume enhancement of the green and red channels in selected areas. Original magnification, ×63; digital magnification ×2.9. One of three experiments yielding similar results.