Methods:

Animals

Wild type (WT) C57BL/6.CD45.2 animals were obtained from in-house breeding or from commercial vendors. We compared the population of $iNOS^+$ cells in small intestinal lamina propria preparations from Charles River Laboratories, from Taconic and from Jackson Laboratories and found no significant differences in the frequency of $iNOS^+$ cells (data not shown). C57BL/6.CD45.1, μMt, $Rag2^{-/-}$ and CD19-YFP were all purchased from Jackson Labs and bred in-house. $TNF\alpha^{-/-}$ and $iNOS^{-/-}$deficient animals were purchased from Taconic and crossed. $LT\beta^{-/-}$ animals were purchased from B&K Universal. $LT\beta R^{-/-}$ mice were obtained from Dr. R.D. Newberry, Washington University School of Medicine, St. Louis, USA. AID-YFP[1] were provided by Dr. R. Casellas, NIH, Bethesda, USA. $J_H^{-/-}$ mice were obtained from Dr. Simon Fillatreau, DRFZ, Berlin, Germany. All animals were housed in specific pathogen-free (SPF) conditions and all experiments were performed according to animal use protocols approved by the animal care committee of the Ontario Cancer Institute and the University of Toronto.

Germ-free mice and microbiota re-associations

C57BL/6 mice were re-derived to germ-free by two-cell embryo transfer and were maintained germ-free in flexible film isolators at the Farncombe Axenic and Germ-free Unit of Central Animal Facility, McMaster University, Canada or in the Clean Animal Facility, University of Bern, Switzerland as previously described[2]. For re-colonization experiments, C57BL/6 mouse colonies were re-associated with a low-complexity microbiota, by co-housing with gnotobiotic mice that had been associated with the

Altered Schaedler Flora (ASF, Taconic) consisting of 8 different bacteria[3], according to the protocol available at Taconic (www.taconic.com/library). These mice were used as ASF experimental mice and as sentinel colonizers for ASF re-associations of germ-free animals. Re-associated animals were maintained under barrier conditions in IVC cages in the Farncombe Axenic and Germ-free Unit or in the Clean Animal Facility, University of Bern, Switzerland, as previously described[2]. Reversible colonization experiments were carried out by gavaging GF mice four times with the *E. coli* K12 triple mutant strain HA107[2] over 14 days and subsequently rested germ-free for 14 days. All animal experiments were carried out in accordance with the McMaster University animal utilization protocols and the Canadian Council on Animal Care (CCAC) guidelines or in accordance with Swiss federal regulations.

Mixed bone marrow chimeras

$Rag2^{-/-}$ or $J_H^{-/-}$ mice were irradiated with two consecutive doses of 550 rad utilizing a MDS-Nordion Gammacell 40 irradiator and subsequently reconstituted by intravenous injection with $1x10^6$ red blood cell-depleted bone marrow cells (ratio 2:1 for all three groups - $J_H^{-/-}$:WT; $J_H^{-/-}$:TNF$\alpha^{-/-}$/iNOS$^{-/-}$ and WT: TNF$\alpha^{-/-}$/iNOS$^{-/-}$) from sex-matched donors. Mice were then provided 2 mg/ml neomycin-sulfate (Sigma-Aldrich) supplemented drinking water for 2 weeks post-irradiation. Mice were further used for experiments following 8-10 weeks of reconstitution.

Bacterial Infection

A nalidixic-acid resistant strain of *Citrobacter rodentium* DBS100 (provided by Dr. Brett Finlay) was grown overnight at 37^{o}C with moderate shaking. 1x10^{9} colony-forming units (CFU) of the overnight culture, which was washed once in PBS, were used to inoculate mice by oral gavage. The mice were fasted for three hours pre-challenge. The percentage of body weight loss of animals post-infection was monitored. At indicated time-points animals were sacrificed and organ colonization by *C. rodentium* was determined by homogenizing tissues in sterile PBS using a rotor homogenizer followed by serial dilution plating on nalidixic acid-containing LB plates. For pathological examination of tissues, large intestines were harvested, the fecal content removed and washed twice in PBS and opened longitudinally. Tissues were fixed for two days in 10% formalin, washed twice with PBS and fixed for 18 hours in 70% ethanol. Samples were embedded in paraffin, sectioned and analyzed by standard histological staining procedures using hematoxylin and eosin.

Immunofluorescence microscopy

Small intestines were cut out and mesentery and fat removed. After gently pushing out the fecal content, small intestines were washed twice in PBS and cut open longitudinally and pieces were frozen in OCT compound (Sakura Finetek). Similarly, spleens from animals were removed and frozen in OCT. Spleens and intestinal tissue sections were cut at 5 μm using a Leica CM3050 cryostat (Leica Microsystems), mounted on glass microscope slides and fixed in acetone. Sections were washed TBS followed by TBS-T (TBS plus 0.05% Tween-20 (Sigma Aldrich)), then incubated with TBS-T supplemented with 10% normal rabbit serum (Jackson Laboratories), 10% normal mouse serum

(Jackson Laboratories), 5% BSA (Sigma) and 2 mg/ml of a rat anti-mouse CD16/CD32 antibody ("Fc-block", clone: 2.4G2) for 30 minutes to block non-specific staining. Fluorochrome labeled antibodies were applied for 45 minutes in the dark. The following antibodies were used: rat anti-mouse IgA-FITC (11-44-2, Southern Biotech), rabbit anti-mouse iNOS-PE (N-20, Santa Cruz Biotechnology), rat anti-mouse CD8α-PE (53-6.7) rat anti-mouse EpCAM-APC (G8.8), rat anti-mouse TNFα-APC (MP6-XT22), rabbit anti-mouse TNFα (610325, R&D) followed by biotinylated anti-Rabbit (Molecular probes) and Streptavidin-PE, rat anti-mouse CD21/CD35-biotin (8D9) followed by Streptavidin-ALEXA488 (InVitrogen). Except otherwise mentioned, all antibodies were purchased from eBioscience. After removal of the staining solution slides were washed three times with TBS-T, once with TBS and once with PBS. Finally, slides were stained with DAPI nucleic acid stain (InVitrogen) for 30 seconds and washed three times with PBS before being mounted with Gel/Mount (Biomeda Corp.). Images were acquired with a Leica DMRA2 microscope (Leica Microsystems) equipped with a Retiga EXi digital camera (Q Imaging) using OpenLab software (Improvision) and Adobe Photoshop. Quantification of IgA and $CD8\alpha^+$ cells was performed using Image J.

Isolation of intestinal lamina propria cells

Small and large intestines were cut out and mesentery and fat removed, flushed, Peyer's patches were removed and the intestine was cut open longitudinally and into pieces of ~5 mm. The caecum was opened, the content removed and the tissue cut in pieces. Tissue pieces were washed twice by gentle vortexing for a few seconds in ice-cold buffer (HBSS (Gibco) supplemented with 2% FBS (PAA) and 15 mM HEPES pH 7.4). The epithelial

cells and intestinal epithelial lymphocytes were then removed by transferring the gut pieces to an EDTA-containing buffer (HBSS (Gibco) supplemented with 10% FBS (PAA), 5 mM EDTA, 15 mM HEPES, buffered with NaOH at pH 7.4) and shaken vigorously at room temperature for 10 minutes, vortexed gently for a few seconds, before decanting the supernatant. This wash step was repeated three times. Gut pieces were then washed three times in cold HBBS buffer (Gibco) to remove residual EDTA before transfer into RPMI 1640 supplemented with 10% FBS (PAA), 15 mM HEPES pH7.4, Collagenase type IV (5 mg/ml, Sigma) and DNase I (0.5 mg/ml, Roche) for digestion of tissue for approximately 1-2 hours at 37°C with occasional vortexing. The resulting suspension was filtered through a 70 μm nylon cell strainer to obtain a single cell suspension. Finally cells were washed and resuspended in ice-cold FACS buffer containing PBS supplemented with 2% FBS (PAA) and analyzed by flow cytometry or used for cytospin preparations.

Isolation of splenocytes

Spleens were harvested and fat was removed. Tissue was gently homogenized with glass slides in digestion buffer (HBSS (Gibco) supplemented with 10mM HEPES, 150mM NaCl, 5mM KCl, 1mM $MgCl_2$, 1.8mM $CaCl_2$, 1 mg/ml Collagenase D (Roche) and 0.2 mg/ml DNase I (Roche) and incubated for 30 minutes at 37°C. The cell suspension was homogenized by gentle pipetting before being incubated for an additional 15 minutes at 37°C prior to the addition of EDTA (final concentration 1 mM) and incubation for 10 minutes at room temperature. Cells were pelleted and resuspended in red blood cell lysis buffer for 1 minute on ice (150 mM NH_4Cl, 100 mM $NaHCO_3$, 1 mM EDTA pH 8.0).

Cells were then resuspended in FACS buffer (PBS supplemented with 2% FBS (PAA)) and pelleted by centrifugation. Finally, cells were filtered through a 70 µm nylon cell strainer and pelleted by centrifugation, resuspended in FACS buffer (PBS supplemented with 2% FBS (PAA)) and analyzed by flow cytometry.

Flow cytometry

Cells were washed with ice-cold PBS containing 2% FBS (PAA) and prior to antibody staining a live/dead stain was applied utilizing the fixable aqua dead cell stain kit (InVitrogen). After that, cells were incubated with 1 mg/ml of a rat anti-mouse CD16/CD32 antibody ("Fc-block", clone: 2.4G2) to block unspecific staining for 15 minutes at 4°C. Predetermined concentrations of fluorochrome labeled antibodies were added in a total volume of 100 µl, thoroughly mixed with the cells and incubated for 15 minutes at 4°C. The following antibodies were used: murine anti-mouse CD45.1-Pacific Blue (A20), murine anti mouse CD45.2-Pacific blue (104, Biolegend), rat anti-mouse CD11b-FITC (M1/70), rat anti-mouse Ly6C-PerCP-Cy5.5 (HK1.4), rat anti-mouse CD11b-APC (M1/70), hamster anti-mouse CD11c-APC (N418), rat anti-mouse CD11b-PE-Cy7 (M1/70), hamster anti-mouse CD11c-PE-Cy7 (N418), rat anti-mouse CD19-FITC (1D3), rat anti-mouse CD4-PE (GK1.5), rat anti-mouse CD8α-PerCP-Cy5.5 (53-6.7), rat anti-mouse CD45R (B220)-eFluor450 (RA3-6B2), rat anti-mouse Ly6G-PE (1A8, BD Biosciences), and rat anti-mouse CD138-APC (281-2, BD Biosciences). After washing with FACS buffer, cells were fixed and permeabilized using a cytofix/cytoperm kit from BD Biosciences according to the manufacturer's protocol. Intracellular staining was then performed for 30 min at 4°C using the following antibodies: rat anti-mouse

IgA-FITC (11-44-2, Southern Biotech), IgA-PE (11-44-2, Southern Biotech), rat anti-mouse IgA-biotin (11-44-2) followed by Streptavidin-APC-Cy7 or Streptavidin PerCp-Cy5.5, murine anti-mouse iNOS-FITC (6, BD Biosciences), mouse anti-mouse IgG_1 iNOS-ALEXA647 (C-11, Santa Cruz Biotechnology used fresh at 1/25 and compared with an ALEX647-conjugated mouse anti-mouse IgG_1 isotype control), rat anti-mouse TNFα-PE (MP6-XT22, BD), rat anti-mouse TNFα-APC (MP6-XT22, BD). Except otherwise mentioned, all antibodies have been purchased from eBioscience. Cells were then washed twice with Perm/Wash buffer and resuspended in FACS buffer prior analysis by flow cytometry using either a FACS Cailbur or a LSR-II (BD Biosciences). Acquired data was analyzed and processed using FlowJo (Tree Star Inc.).

Analysis of VDJ recombination

Intestinal lamina propria cells (LPC) and splenocytes were isolated and stained with the antibodies as described above. LPC and splenocytes were sorted for $CD3\varepsilon^+$ or $CD19^+$ populations. In addition LPC were stained for CD3ε and B220 and the $CD3\varepsilon^-B220^-$ population was then sorted for $CD11c^{lo}iNOS^+$ and $CD11c^{hi}iNOS^+$ populations using a FACSAria (BD Biosciences) and placed in trizol (Sigma-Aldrich). Genomic DNA from 10^5-10^6 sorted cells of the shown phenotype were then subject to PCR to amplify all heavy chain V(D)J-rearranged products, however this PCR will preferentially amplify V-D-JH_4 products as this product is the smallest amongst PCR products[4]. Briefly, the VH_{all} primer and JH_4-C intronic primer were used to amplify joints. 35 cycles (94°C for 30 seconds, 54°C for 30 seconds, 72°C for 2 minutes) were carried out. PCR products were

separated on 0.9% agarose gels, southern blotted and probed with an internal (coding region) JH4-specific probe.

Analyses of serum Ig

Sera from animals were prepared and analyzed by sandwich ELISA on serially diluted samples by coating the primary antibody (goat anti-mouse Ig (H+L), Southern Biotechnologies) to 96-well plates (MaxiSorp; Nalgene Nunc, Roskilde, Denmark). IgA (eBioscience) and IgG_1 (15H6, Southern Biotechnologies) isotypes were used as standards. Standard and bound antibodies of plated sera samples were then detected by sequential incubation with biotinylated secondary antibodies (goat anti-mouse IgA-biotin and goat anti-mouse IgG_1-biotin, both from Southern Biotechnologies), horseradish peroxidase-conjugated streptavidin (R&D Systems), and TMB (Sigma-Aldrich). Reaction was stopped by acidification with 0.5 M H_2SO_4 (Sigma-Aldrich) and absorbance was read at 450 nm.

Quantitative real-time PCR amplification of 16S rRNA

DNA was extracted from the scraped epithelial content ("Small Intestinal Surface Scrape"), as well as from the remaining tissue ("Small Intestinal Tissue") of the terminal ileum using a QIAamp DNA Stool mini kit (Qiagen). Quantitative real time PCR (qRT-PCR) analysis was conducted using an AB 7300 system (Applied Biosystems, Foster City, CA) and sequence detection software (version 1.3.1, Applied Biosystems, Foster City, CA). The qRT-PCR program began with an initial step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min, and a final dissociation stage

of 95°C for 15 s, 60°C for 30 s and 95°C for 15 s. Data was acquired in the final step at 60°C. Each reaction mixture was run in duplicate in a total volume of 10 µl in 96-well optical reaction plates (Applied Biosystems, Foster City, CA) sealed with optical adhesive film (Applied Biosystems, Foster City, CA). Amplification reactions consisted of 5 µl Power SYBR green PCR master mix (Applied Biosystems, Foster City, CA) mixed with 1 µl of forward and reverse primers (0.5 µM) and 4 µl of genomic DNA (diluted to approximately 10 ng/µl). Primer pairs were as follows: Other Eubacteria, UniF340 (5'-ACTCCTACGGGAGGCAGCAGT) and UniR514 (5'-ATTACCGCGGCTGCTGGC)[5]; Bacteroides, AllBac296f (5'-GAGAGGAAGGTCCCCCAC) and AllBac412r (5'-CGCTACTTGGCTGGTTCAG)[6]; Bacillus, (5'-GCGGCGTGCCTAATACATGC) and (5'-CTTCATCACTCACGCGGCGT)[6]; *Clostidium coccoides* (Clostridium cluster IV), UniF338 (5'-ACTCCTACGGGAGGCAGC) and C.cocR491 (5'-GCTTCTTAGTCAGGTACCGTCAT)[5]; *Clostridium leptum* (Clostridium cluster XIVa), FClept09 (5'-CCTTCCGTGCCGSAGTTA) and RClept08 (5'-GAATTAAACCACATACTCCACTGCTT)[7]; Segmented Filamentous Bacteria, SFB736F (5'-ACGCTGAGGCATGAGAGCAT) and SFB844R (5'-GACGGCACGGATTGTTATTCA)[5]. The relative quantity of 16S rDNA for each bacterial group was calculated by the ΔCt method and normalized to the amount of total Eubacteria in the sample.

Isolation of bone marrow-derived B220^{+} progenitors

B220^{+} progenitors were freshly isolated from bones of C57BL/6.CD45.2, C57BL/6.CD45.1 or AID-cre x YFP mice. Briefly, a single cell suspension of bone marrow was prepared by gentle grinding of femurs and tibiae using a mortar and pestle. The cells were then flushed with MACS buffer (PBS$^{-Ca2+, -Mg2+}$, supplemented with 1 mM EDTA, 0.25% BSA (Sigma)). B cell progenitors were positively selected using anti-B220-beads (clone : RA3-6B2, Miltenyi) and a VarioMACS magnet with an LS adaptor (Miltenyi). Cells were washed three times with MACS buffer and eluted in OptiMEM (Life Technologies). Typically about 8-14 x 10^{6} B220^{+} cells were recovered from the bone marrow of one mouse (two femurs and two tibiae). In culture, IL-7 promotes the development of B cells from bone marrow-derived B220^{+} precursors[8]. Bone-marrow derived differentiated B cells are ideal for these experiments since the secondary lymphoid organs contain a mixed population of innate, adaptive and antigen-experienced B cells.

Co-Culture of stromal cells and bone marrow cells

For co-culture experiments, either 200 bone marrow (BM)-derived S17 cells, or 80,000 (live) BM-derived stromal cells, or 80,000 (live) lamina propria-derived stromal cells were plated together with 300,000 BM-derived B220^{+} cells. For the lamina propria-derived stromal cells, cells were isolated in the same manner as described above for lamina propria-derived cells, washed 2 times in Opti-MEM and plated in 24-well plates. After 2 days, a slowly growing adherent monolayer is obtained. By day 7 of culture, the monolayer reaches confluence. In two cases, the stromal cell monolayer appeared less

viable at day 7 when the $B220^+$ bone marrow derived cells expanded more rapidly than expected, and those experiments were discarded.

Cultures were performed in OptiMEM (Life Technologies) supplemented with 10% non-heat inactivated FBS (Gibco), β-mercaptoethanol (50 μM), $NaHCO_3$ (2.4 g/l), penicillin-streptomycin (100 μg/ml), IL-21 (30 ng/ml, R&D Systems) TGF-β (2.5ng/ml, R&D Systems), IL-7 (0.5 ng/ml), and 2 μg of rat IgG2a anti-mouse CD40 (clone : 3/23; BD Biosciences) or rat IgG2a (clone : HB9419) as isotype control. The supernatant from the stably transfected J558 line was used as a source of IL-7 (supplied by Dr. Ana Cumano, Institut Pasteur, Paris). The co-culture was performed in 24 well plates in a total volume of 1 ml/well. On day 4, 0.5 ml of the above indicated culture mixture was added in addition, and on day 7 cells were harvested and analyzed by flow cytometry or were transferred to a second stromal cell culture by gently removing non-adherent cells and washing 3 times in PBS + 3%FBS to remove any cytokines/anti-CD40 Ab. Cells were then re-plated onto fresh stroma as indicated and co-cultured for an additional 2 days.

Statistical Analyses

Statistical analyses were performed using Graphpad Prism software (GraphPad Software Inc.). Student's T-test were used for data with a normal distribution. Mann-Whitney was used for non-gaussian distributed data and 2-way Anova for weight loss over time.

Supplementary Figure Legends

Supplementary Figure 1

Characterization of intestinal lamina propria-derived iNOS^{+}TNFα^{+} cells in wild-type, Rag2$^{-/-}$ and μMt mice.

a, Intestinal lamina propria cells (LPC) of wild-type (WT), Rag2$^{-/-}$and μMt mice were isolated and analyzed by flow cytometry. Representative flow cytometry plots of LPC stained for CD11c and iNOS are shown. A significant reduction in iNOS^{+} cells was observed in the small intestinal lamina propria (n = 4 per group). NB: The residual appearance of some iNOS-producing cells in μMt mice may be associated with the small numbers of immature IgA^{+} B cells in their intestinal LP[9]. **b,** Splenocytes and intestinal lamina propria cells were stained and sorted by flow cytometry. Genomic DNAs from sorted cells were amplified by PCR for heavy chain V(D)J-rearranged products using the $V_H$all primer and the J_H4-C intronic primer to amplify joints, separated on an agarose gel, southern blotted and probed with an internal (coding region) J_H4-specific probe.

Supplementary Figure 2

Characterization of iNOS expression in small intestinal lamina propria-derived cells by cytospin and immune fluorescence microscopy.

Small intestinal lamina propria cells (LPC) of wild-type mixed chimeric mice were isolated, cytospins prepared and stained and analyzed by fluorescence microscopy. **a**, DAPI nucleic acid stain (blue) and IgA (green), **b**, DAPI (blue) and iNOS (red), **c**, DAPI (blue), IgA (green) and iNOS (red). As depicted by the arrows, the expression of iNOS is

restricted to IgA^+ plasma cells. Cytoplasmic localization of IgA appears to positively correlate with high iNOS expression, declining upon cell surface localization of IgA. Embedded in the pictures are magnifications of single cells. Representative pictures are shown following image acquisition under oil at 630x.

Supplementary Figure 3

Characterization of iNOS and TNFα expression in small intestinal lamina propria-derived cells by cytospin and immune fluorescence microscopy.

Small intestinal lamina propria cells (LPC) of wild-type mixed chimeric mice were isolated, cytospins prepared and stained and analyzed by fluorescence microscopy. **a**, DAPI nucleic acid stain (blue) and IgA (green), **b**, DAPI (blue) and TNFα (red), **c**, DAPI (blue), IgA (green) and TNFα (red), **d**, iNOS (blue), **e**, iNOS (blue) and TNFα (red), **f**, iNOS (blue), IgA (green) and TNFα (red). On top of the pictures magnifications of single cells are shown. As depicted by the arrows, the expression of iNOS and/or TNFα is restricted to IgA^+ plasma cells, indicating the presence of $IgA^+iNOS^+TNF\alpha^+$, $IgA^+iNOS^-TNF\alpha^+$ as well as $IgA^+iNOS^+TNF\alpha^-$ plasma cells. Representative pictures are shown following image acquisition under oil at 630x.

Supplementary Figure 4

Characterization and localization of intestinal IgA^+ plasma cells in AID^+YFP^+ animals.

Longitudinal and transverse sections of small intestines of AID^+YFP^+ animals were stained with specific fluorochrome-tagged antibodies for EpCAM (blue) which stains the

epithelial layer, and IgA (red), and analyzed by fluorescence microscopy. The arrows highlight AID^+IgA^+ plasma cells, which appear in yellow. Representative pictures are shown from a total of 2 separate experiments, n=3 mice per experiment.

Supplementary Figure 5

***In vitro* generation of iNOS-producing IgA^+ plasma cells.**

a, $B220^+$ bone marrow (BM) cells derived from AID-YFP mice were co-cultured for up to 7 days with $CD45.1^+$ intestinal lamina propria cells ("Gut stroma") or the S17 bone marrow stromal cell line, in the presence of IL-7, TGFβ, IL-21 and αCD40 antibody. The expression of IgA, iNOS and YFP was analyzed by flow cytometry. IgA^+iNOS^+ cells = green rectangle, IgA^+iNOS^- cells = blue rectangle and IgA^-iNOS^- cells = red rectangle. Data are representative of at least three independent experiments. Representative flow cytometry plots are shown. **b**, $B220^+$ bone marrow (BM) cells from $CD45.2^+$ wild type mice were co-cultured for 7 days in the presence of IL-7, TGFβ, IL-21 and αCD40 antibody with: the BM-derived stromal cell line S17, $CD45.1^+$ BM-derived cells ("BM stroma"), $CD45.1^+$ intestinal lamina propria cells ("Gut stroma"), $CD45.1^+$ intestinal lamina propria cells ("Gut stroma") from $LT\beta R^{-/-}$ animals (NB, $LT\beta R^{-/-}$ mice have been back-crossed to the CD45.1 congenic background). To ensure selective analysis of BM-derived precursors, cells were pre-gated on the $CD45.1^-$ population. Representative flow cytometry plots of cells analyzed for iNOS and IgA. IgA^+iNOS^+ cells are depicted by the red rectangle. Data are representative of at least three independent experiments.

Supplementary Figure 6

Generation of mixed bone marrow chimeric mice

Schematic representation of mixed bone marrow chimera generation. $Rag2^{-/-}$ or $J_H^{-/-}$ mice were irradiated to deplete radio-sensitive cells and animals were reconstituted with a mixture of 1) bone marrow from wild-type (WT) + $J_H^{-/-}$ animals (yielding mice where all B cells are $TNF\alpha^+iNOS^+$), 2) bone marrow from $TNF\alpha^-iNOS^-$ double-deficient + WT animals (yielding reconstituted mice where most B cells and other radio-sensitive cells are $TNF\alpha^+iNOS^+$) and 3) bone marrow from $TNF\alpha^-iNOS^-$ double-deficient + $J_H^{-/-}$ animals (yielding reconstituted mixed chimeras where all B cells are $TNF\alpha^-iNOS^-$).

Supplementary Figure 7

Analysis of splenic architecture of mixed chimeric mice.

Sections of spleens from WT and dKO reconstituted mixed bone marrow chimeric mice were stained with specific fluorochrome-tagged antibodies for **a,** CD35 (green), B220 (red) and CD3ε (blue) **b,** CD3 ε (green), MadCAM1 (red) and B220 (blue), and analyzed by fluorescence microscopy. High levels of CD35 are expressed by Follicular Dendritic Cells (FDC), and MadCAM1 is expressed in the marginal zone (MZ) sinus. In mice that lack TNFα in B cells, FDC networks and the MZ are disrupted[10]. Data are representative of at least four independent mixed chimeric animals analyzed. Representative pictures are shown. **c,** Spleens from WT and dKO reconstituted mixed chimeric mice were isolated, single cell suspensions prepared and the total counts of the indicated cell subsets were determined by flow cytometry. Absolute cell subset counts (logarithmic scale) in spleen are not different between WT and dKO mice (black bars versus grey bars respectively) in non-infected mice.

Supplementary Figure 8

iNOS/TNFα double-deficient mixed chimeras are more susceptible to infection with *Citrobacter rodentium*.

a, Large intestines (LI) from WT and dKO mixed chimeras mice were harvested 11 days post-infection and the pathological scores were analyzed by standard histological staining procedures using hematoxylin and eosin (H&E). LI's from dKO animals show more severe pathology. H&E staining of two representative dKO mice and WT mice are shown. Note the infiltrates in the dKO mice (arrows) and the shortened crypt length (scale bars). The panel shows the original magnification of 10x and scale bars represent 250 μm. **b,** The percentage of body weight loss in WT + dKO → $J_H^{-/-}$ versus $J_H^{-/-}$ + dKO → $J_H^{-/-}$ mixed chimeras after *C. rodentium* infection over time is depicted. Significantly higher body weight loss in $J_H^{-/-}$ + dKO → $J_H^{-/-}$ mice was observed from day 7 to day 11 post-infection (n = 7 per group). NB: One $J_H^{-/-}$ + dKO → $J_H^{-/-}$ mouse was found dead at day 10 post-infection. Also note that in this experiment, *C. rodentium* was somewhat attenuated compared to prior experiments, thus resulting in later onset of weight loss and larger variability in weight loss. **c,** The colonization by *C. rodentium* was determined 11 days after infection by homogenizing spleens, livers and caecums followed by serial dilution plating on nalidixic acid-containing LB plates and counting of colonies. *C. rodentium* colonization of all 3 organs is significantly enhanced in dKO mice at day 11 post-infection. * $p < 0.05$, ** $p < 0.01$.

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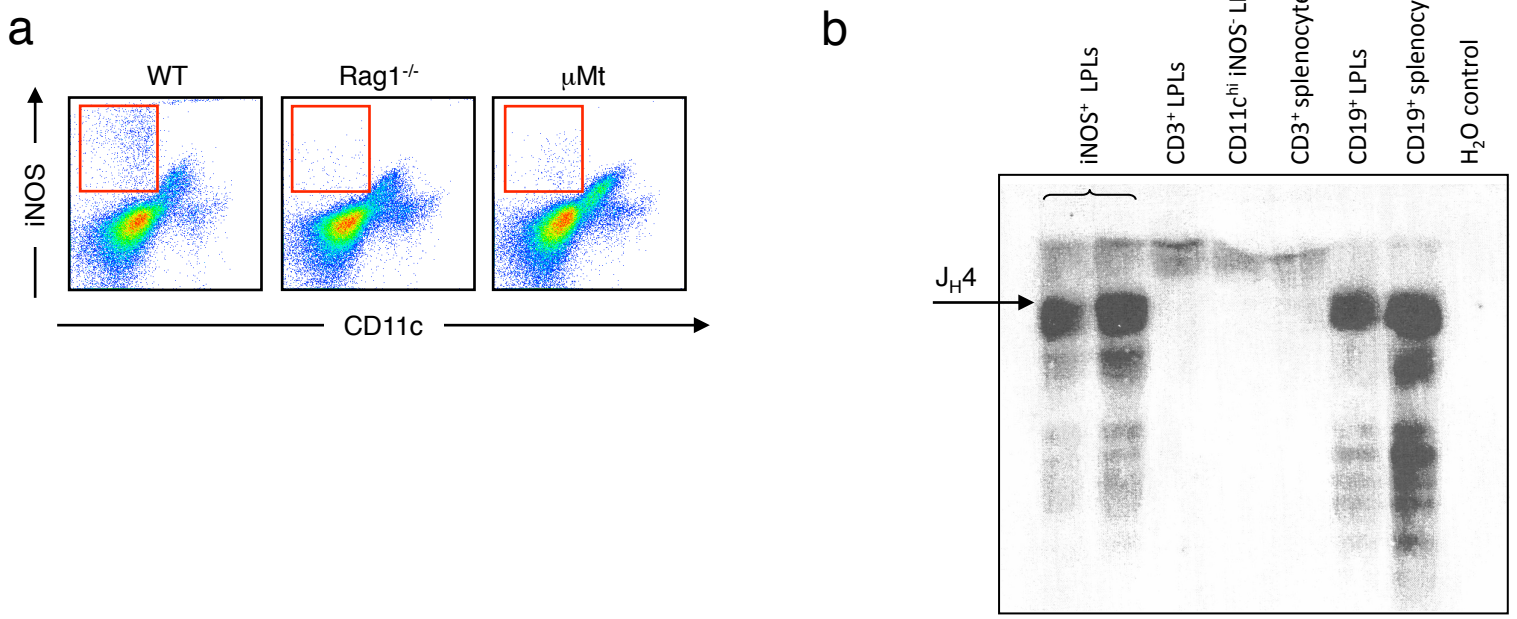
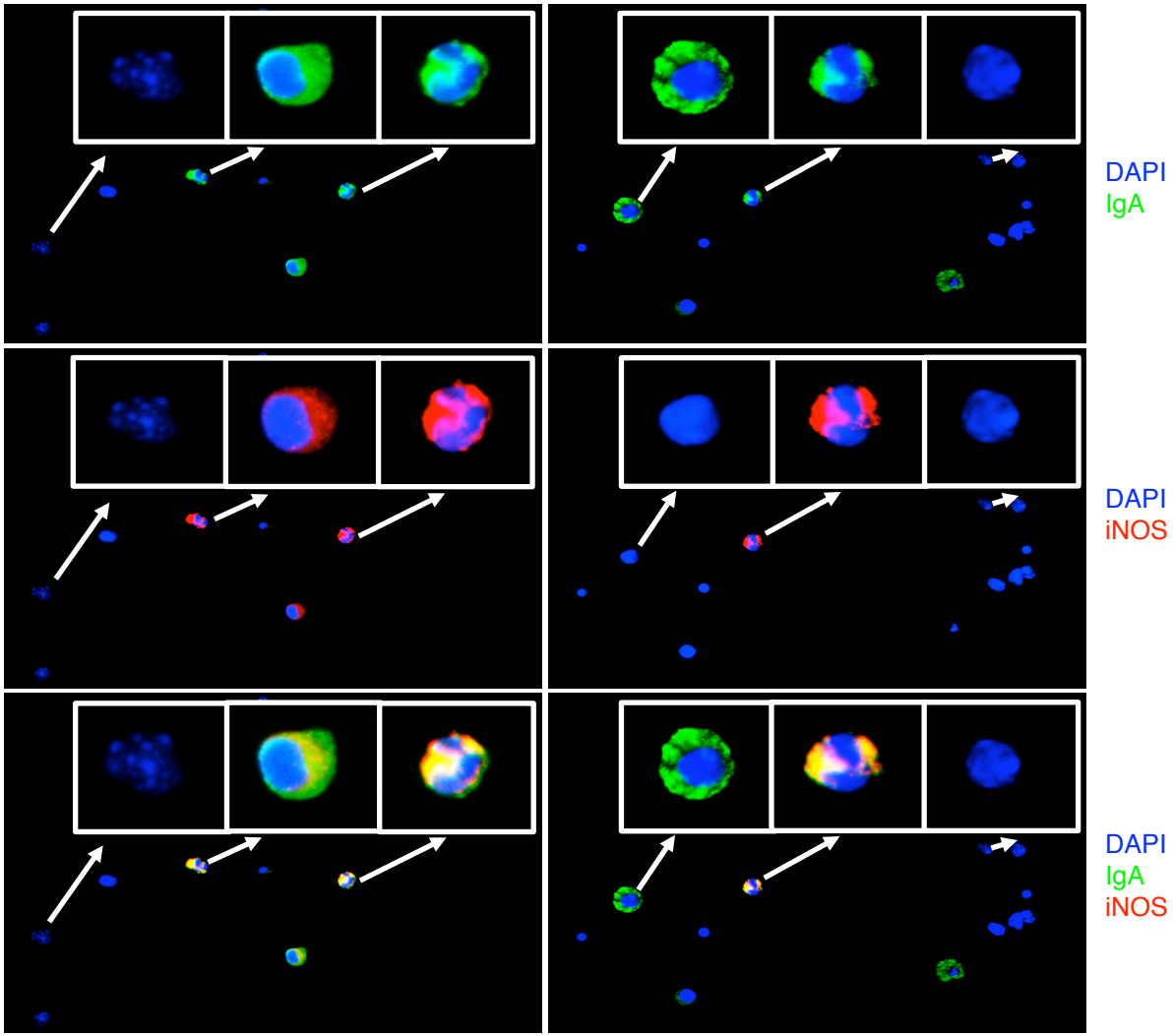


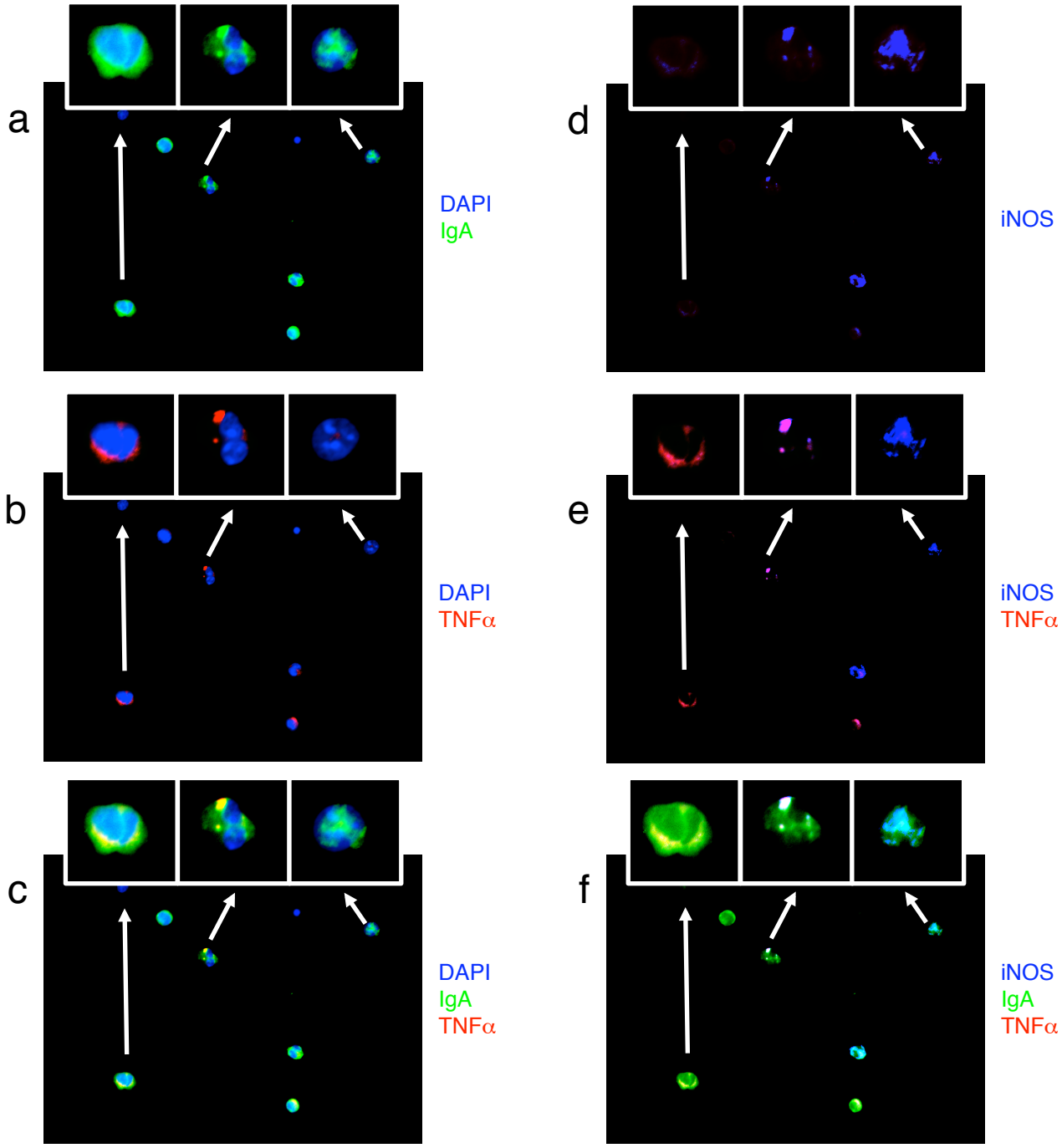
Characterization of intestinal lamina propria-derived iNOS$^+$TNFα$^+$ cells in wild-type, Rag2$^{-/-}$and μMt mice.
a, Intestinal lamina propria cells (LPC) of wild-type (WT), Rag2$^{-/-}$and μMt mice were isolated and analyzed by flow cytometry. Representative flow cytometry plots of LPC stained for CD11c and iNOS are shown. A significant reduction in iNOS$^+$ cells was observed in the small intestinal lamina propria (n = 4 per group). NB: The residual appearance of some iNOS-producing cells in μMt mice may be associated with the small numbers of immature IgA$^+$ B cells in their intestinal LP[9]. **b**, Splenocytes and intestinal lamina propria cells were stained and sorted by flow cytometry. Genomic DNAs from sorted cells were amplified by PCR for heavy chain V(D)J-rearranged products using the $V_H$all primer and the J_H4-C intronic primer to amplify joints, separated on an agarose gel, southern blotted and probed with an internal (coding region) J_H4-specific probe.

Supplementary Figure 1a,b



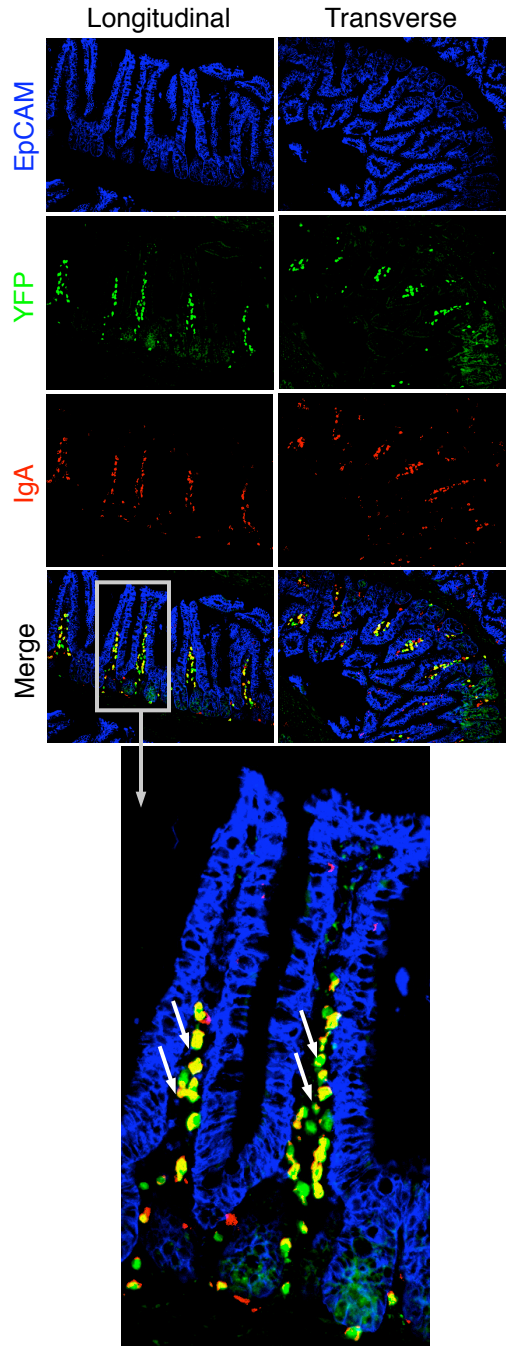
Characterization of small intestinal lamina propria-derived cells by cytospin and immune fluorescence microscopy.
Characterization of iNOS expression in small intestinal lamina propria-derived cells by cytospin and immune fluorescence microscopy.
Small intestinal lamina propria cells (LPC) of wild-type mixed chimeric mice were isolated, cytospins prepared and stained and analyzed by fluorescence microscopy. **a**, DAPI nucleic acid stain (blue) and IgA (green), **b**, DAPI (blue) and iNOS (red), **c**, DAPI (blue), IgA (green) and iNOS (red). As depicted by the arrows, the expression of iNOS is restricted to IgA^+ plasma cells. Cytoplasmic localization of IgA appears to positively correlate with high iNOS expression, declining upon cell surface localization of IgA. Embedded in the pictures are magnifications of single cells. Representative pictures are shown following image acquisition under oil at 630x.

Supplementary Figure 2a-c



Characterization of iNOS and TNFα expression in small intestinal lamina propria-derived cells by cytospin and immune fluorescence microscopy. Small intestinal lamina propria cells (LPC) of wild-type mixed chimeric mice were isolated, cytospins prepared and stained and analyzed by fluorescence microscopy. **a**, DAPI nucleic acid stain (blue) and IgA (green), **b**, DAPI (blue) and TNFα (red), **c**, DAPI (blue), IgA (green) and TNFα (red), **d**, iNOS (blue), **e**, iNOS (blue) and TNFα (red), **f**, iNOS (blue), IgA (green) and TNFα (red). On top of the pictures magnifications of single cells are shown. As depicted by the arrows, the expression of iNOS and/or TNFa is restricted to IgA^+ plasma cells, indicating the presence of $IgA^+iNOS^+TNF\alpha^+$, $IgA^+iNOS^-TNF\alpha^+$ as well as $IgA^+iNOS^+TNF\alpha^-$ plasma cells. Representative pictures are shown following image acquisition under oil at 630x.

Supplementary Figure 3a-f



Characterization and localization of intestinal IgA⁺ plasma cells in AID⁺YFP⁺ animals.
Longitudinal and transverse sections of small intestines of AID⁺YFP⁺ animals were stained with specific fluorochrome-tagged antibodies for EpCAM (blue) which stains the epithelial layer, and IgA (red), and analyzed by fluorescence microscopy. The arrows highlight AID⁺IgA⁺ plasma cells, which appear in yellow. Representative pictures are shown.

Supplementary Figure 4