Supplementary Materials and Methods

Tissue collection and histology

SAMP and AKR mice were euthanized by CO_2 inhalation at times required by the experimental design. The distal ilea (10 cm) and MLN were harvested. The ileum was rinsed, opened on the anti-mesenteric side and rolled into a swiss roll configuration. Samples were then fixed in Bouin's fixative, embedded in paraffin, and cut into 3-5-µm sections. The tissues were stained with H&E for histological assessment by a single experienced pathologist who was blinded to the experimental design using a standardized semiquantitative scoring system, as previously described[1]. Briefly, inflammatory indices were assessed as flattening or widening of the normal villous architecture (villous distortion index), neutrophilic infiltration to the lamina propria (active inflammatory index), lymphocyte, plasma cell and macrophage infiltration (chronic inflammatory index). Total inflammatory index was calculated as sum of the above indices.

Human mucosal tissue specimens

Colonic endoscopic biopsies from males and females, aged 18-65 years, and affected by CD or UC and non-inflamed controls, were harvested from patients undergoing flexible sigmoidoscopy or colonoscopy for diagnostic or surveillance purposes. Diagnosis of UC and CD were established by clinical, macroscopic, and histologic criteria. The control group consisted of individuals undergoing screening colonoscopy, who were included in the study only when endoscopy and pathology did not reveal any mucosal abnormalities. Specimens were maintained at 4℃ for 1–2d in RNA *later*

solution (Qiagen, Valencia, CA), followed by isolation of total RNA using the RNeasy Mini Kit (Qiagen). One microgram of total RNA was then reverse-transcribed using MultiScribe MuLV (Applied Biosystems RNA-to-cDNA kit, Life Technologies, Carlsbad, CA) in the presence of random hexamers and oligo-dT primers. Real-time quantitative PCR was performed using TaqMan probe-based gene expression assays (Life Technologies) on 1:5 diluted cDNA. All studies were approved by the Internal Review Board of Case Medical Center.

Cell isolation

MLNs were minced and digested with 1 mg/ml collagenase VIII (Sigma, St. Louis, MO) and 20 µg/ml DNase I (Sigma) for 30 min at 37 ˚C. For preparation of LP cells, terminal ilea (10 cm) were collected into 5% FBS in calcium- and magnesium-free HBSS, Peyer's patches were dissected and tissue cleaned from fat. Intestines were opened longitudinally, cut into 1 cm pieces and washed 3x 10 min in 5% HBSS buffer containing 2 mM EDTA, 100 mM N-acetyl cysteine and 10 mM HEPES to remove mucus and epithelial cells. Tissue was minced and digested with 1 mg/ml collagenase VIII (Sigma) and 20 µg/ml DNase I (Sigma) for 20 minutes at 37 ˚C. Digested material was passed through 70-µm filters, washed and resuspended in 5% FBS in HBSS. Isolated cells were counted using Vi-CELL counter (Beckman Coulter, Brea, CA).

Cell sorting and cell culture

$MHCII^{+}CD11c^{+}$ antigen presenting cells were isolated from MLN of SAMP or AKR mice. Collagenase digested MLN cells were enriched for $CD11c^{+}$ cells by positive selection

with magnetic beads (STEMCELL, Vancouver, Canada) and sorted as $MHCII^+CD11c^+$ events on FACS Aria (BD Biosciences, San Jose, CA). $CD4^+$ T cells were prepared from spleens of SAMP or AKR mice by positive selection using magnetic beads (Miltenyi, Auburn, CA) and $CD4^+CD45RB^{high}CD25^-$ naïve fraction was further FACS sorted using FACS Aria. Naïve $CD4^+$ T cells ($1x10^5$) were stimulated with 2 µg/ml anti-CD3ε (BD) and 5 ng/ml human TGFβ1 (R&D, Minneapolis, MN) in the presence of $5x10^4$ $CD11c^+MHCII^+$ cells for 5 days.

Flow cytometry

Cells ($1\text{-}3x10^6$ cells) were resuspended in staining buffer (PBS with 2% FBS, 2 mM EDTA and 0.1% sodium azide) supplemented with Fc block to reduce non-specific binding. Cells were stained with combinations of fluorescently labeled anti–mouse antibodies against TCRβ (H57-597), CD19 (1D3), CD11c (N418), CD11b (M1/70), MHCII (NIMR-4), CD103 (2E7), Foxp3 (FJK-16s) (BD Pharmingen, eBiosciences and Biolegend, San Diego, CA) and LIVE/DEAD® Aqua fixable dead cell stain (Invitrogen, Carlsbad, CA). Cells were stained for 30 minutes at 4 ℃, washed twice in staining buffer and fixed in 2% paraformaldehyde. For the Aldefluor straining cells were resuspended in Aldefluor staining buffer (STEMCELL) and incubated with 300 nM activated Aldefluor substrate for 30 min at 37 ℃. Cells were washed, stained with antibodies and analyzed within 2 hours. To block RALDH activity cells were pretreated with 100 µM 4-diethylaminobenzaldehyde (DEAB, Sigma) for 10 minutes. Flow cytometry data were acquired on a BD LSRII flow cytometer (BD Biosciences) and were analyzed with FlowJo software (Tree Star, Ashland, OR) after eliminating dead and

doublet cells as detailed in Figures 1 and 2. Frequencies of DC populations among parent gate and among all live cells were calculated in FlowJo. Absolute numbers of $CD103^+CD11b^+$ DCs were obtained as the product of flow cytometry percentage and ViCell Count-based total live cell counts.

Immunofluorescence

For β_7 integrin staining ilea were harvested and fixed with 1% paraformaldehyde, 100 mM L-lysine (pH 7.4), 2 mg/ml $NaIO_4$ in 50 mM phosphate buffer overnight at 4℃ and then equilibrated in 30% sucrose solution in 50 mM phosphate buffer for another 24 hours[2]. Tissues were frozen in OCT and stored at -80 ℃. Cryostat sections were cut at 18 µm thickness, and air-dried for 30 minutes. Sections were rehydrated for 10 minutes in PBS and non-specific binding sites were saturated with 5% normal donkey serum and 1% mouse serum in the presence of 0.3% Triton X-100 in PBS for 1 hour. Sections were incubated overnight at 4 ℃ with purified anti-CD11b (M1/70, BD) monoclonal antibodies, washed and reacted with donkey anti-rat DyLight 568 secondary antibodies (Jackson Immunoresearch). Following washing, sections were incubated overnight at 4 ℃ with FITC-labeled anti-β_7 integrin (M293, BD) and anti-Lyve1 eFluor 660 (ALY7, eBiosciences) antibodies. Slides were washed and overlaid with Alexa Fluor 488 goat anti-FITC antibody for 1h at room temperature, washed, mounted in Prolong Gold antifade reagent (Invitrogen) with a cover slip and examined with FluoView FV10i confocal microscope (Olympus). Staining for peripheral node addressin (Meca79, eBioscences), Lyve1 (ALY7) and CCL21 (AF457, R&D) was performed with directly labeled antibodies on 10 µm cryostat sections of MLN. Sections were fixed with 4%

PFA, blocked with 5% donkey serum in the presence of 0.3% Triton X-100 in PBS for 1h and processed as described above. Imaging was performed at room temperature, using 60x/1.35 NA oil objective. Staining for CCL21 and Lyve1 was performed on whole mount ileum fixed with 4% paraformaldehyde. Blocks of tissue (2x2 mm) were permeabilized with staining buffer containing 2% FBS, 0.5% saponin, 0.09% sodium azide in PBS for at least 18h. Samples were stained for 18 hours at 22–37 ℃ with goat polyclonal antibodies to CCL21 and rabbit polyclonal or rat monoclonal antibodies to Lyve1 (ab14917, Abcam, Cambridge, MA and ALY7, eBioscience, respectively) diluted in staining buffer. Secondary antibodies conjugated to Alexa Fluor 488 and Alexa Fluor 568 were used for detection. To improve feature visibility images were processed in ImageJ software (NIH) by rolling-ball background subtraction and contrast stretching using identical procedures. Omissions of the primary antibody, isotype controls or tissues from *Itgb7*$^{-/-}$ mice were used to determine specificity of the staining.

Short-term homing

Lymph nodes were dissociated with frosted glass slides (Fisher Scientific, Waltham, MA). Equal numbers of lymphocytes from AKR mice were labeled with 5 μM CFSE and CMRA (Invitrogen) in PBS for 7 min at 37 ℃. CMRA-labelled control cells were incubated with 300 ng/ml pertussis toxin (List Biological Laboratories, Campbell, CA) for 2h. In some experiments dyes were swapped. After washing with PBS, the labeled cells were mixed and an aliquot was saved to determine the input ratio. Between 2.5 x 10^6 to 10^7 cells was retroorbitally injected into AKR or SAMP hosts. Recipient mice were sacrificed 18 hours post injection. Spleen and pooled lymph nodes were made into

single-cell suspensions and stained with mAbs to TCRβ (H57-597), CD19 (1D3), CD4 (GK1.5), CD8 (53-6.7) and LIVE/DEAD® Aqua fixable dead cell stain. Transferred CFSE- and CMRA-labeled cells were detected by flow cytometry. Lymphocyte homing to lymph nodes was also quantified using two-photon microscopy. At 18 h post cell transfer popliteal lymph nodes were harvested and fixed with 4% paraformaldehyde followed by blocking with permeabilization in 2% FBS and 0.5% saponin in PBS with 0.09% sodium azide. Lymph nodes were immobilized in a dish using tissue glue (Vetbond, 3M, St. Paul, MN), and immersed in PBS. Explants were imaged with a 20x water-dipping objective (0.95 NA; Olympus) on a Leica SP5 MP microscope using two-photon excitation by a femtosecond-pulsed Ti:Sapphire infrared laser (Chameleon Ultra II; Coherent, Santa Clara, CA) tuned to 820 nm. Emitted light was split into four channels in a non-descanned detector photomultiplier tube detection unit equipped with 495-nm, 560-nm, 620-nm dichroic long-pass mirrors and 525/50, 610/75 and 624/40 band-pass filters, so second harmonic generation (SHG, <495 nm), CFSE (500 to 550 nm), CMRA (572.5 to 620 nm) and autofluorescence (620 to 644 nm) signals could be recorded. To reconstruct a lymph node in 3D multiple stacks of images were acquired with a voxel size set to 0.892×0.892×5 µm covering up to 400 µm in Z dimension. Single volumes were stitched together with XuvTools software[3]. Since no CMRA-positive cells were detected the residual green tissue autofluorescence was extracted post acquisition from the CFSE channel and combined with yellow/orange/red autofluorescence (CMRA and autofluorescence channels) using Spectral Unmixing plugin in the ImageJ software (NIH) followed by median filtering of all channels. Cell counting was performed in the Imaris software (Bitplane, South Windsor, CT), using

Spots function set to detect ellipsoids with minor and major axes of 9 and 27 μm, respectively, with the same threshold of Quality parameter for both analyzed samples. To quantify the shortest distance between cells (spots) and the node's collagen capsule Imaris' Surface function was utilized to create an object showing the capsule based on the SHG channel. Subsequently Distance Transform function was used on the Surface, creating a new channel where intensity codes for the distance from the capsule. Distance Transform values for each spot/cell were automatically read out from Spots statistics function.

Real-Time Quantitative PCR

Mouse tissue samples were collected in RNAlater and frozen in -80℃. Tissues were homogenized in TRIzol (Invitrogen) and RNA was extracted using RNeasy columns (Qiagen). cDNA was synthesized using the Omniscript RT Kit (Qiagen). QPCR was performed using a Roche Lightcycler 480 with CXCL13, CCL19, and CCL21 primer assays (SABiosciences/Qiagen) that amplify both CCL21a and CCL21b isoforms. SYBR Green QPCR Mastermix was purchased from Roche. Each reaction was measured in triplicate and data were normalized to the expression levels of the house-keeping gene Rpl13a.

Supplementary references:

1. Burns RC, Rivera-Nieves J, Moskaluk CA, et al. Antibody blockade of ICAM-1 and VCAM-1 ameliorates inflammation in the SAMP-1/Yit adoptive transfer model of Crohn's disease in mice. Gastroenterology 2001;121:1428-36.

2. Gerner MY, Kastenmuller W, Ifrim I, et al. Histo-cytometry: a method for highly multiplex quantitative tissue imaging analysis applied to dendritic cell subset microanatomy in lymph nodes. Immunity 2012;37:364-76.
3. Emmenlauer M, Ronneberger O, Ponti A, et al. XuvTools: free, fast and reliable stitching of large 3D datasets. J Microsc 2009;233:42-60.

Supplementary Figure 1. Defect in migratory DCs is similar in CCR7-deficient and SAMP mice. MLN DC populations were analyzed by FACS in CCR7-deficient (CCR7 KO) and age-matched wild type (CCR7 WT) C57BL/6J mice. (A) MLN cells were gated on $MHCII^{hi}$ and $MHCII^{int}$ DCs. Subpopulations of $MHCII^{hi}$ cells were gated on CD103 and CD11b. Percentages of parent gate (mean ± SEM) are indicated on the plots, # - denotes statistically significant difference between CCR7 KO and WT populations. (B) Frequency of $MHCII^{hi}$ and $MHCII^{int}$ and (C) $MHCII^{hi}$ subpopulations among all live MLN cells was calculated. N=4 in CCR7 WT and n=3 in CCR7 KO groups, shown is mean±SEM of data polled from two experiments.

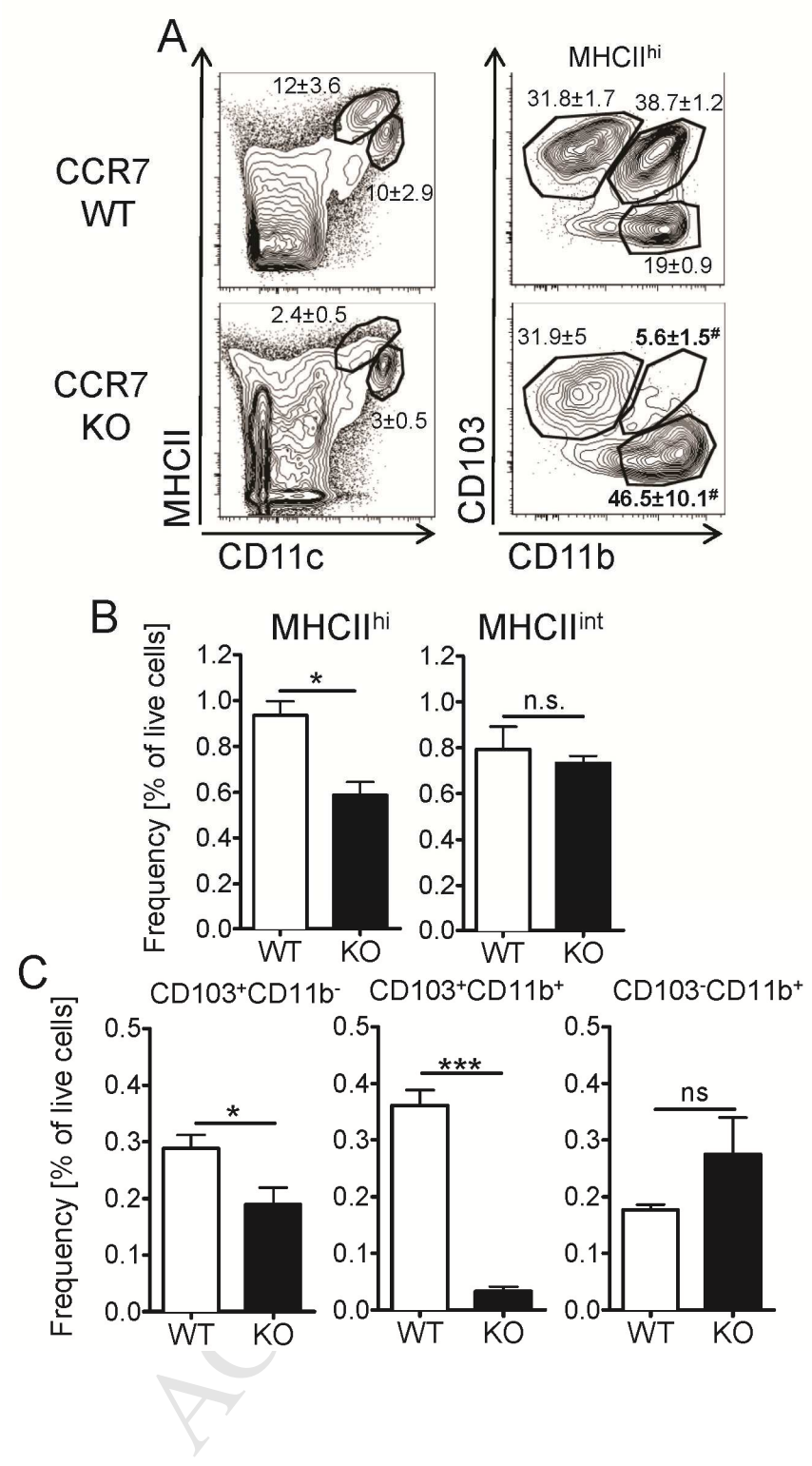
Supplementary figure 2. Short term homing of lymphocytes is impaired in SAMP lymph nodes. CSFE- or CMRA-labelled AKR lymphocytes were injected intravenously into AKR and SAMP mice and homing of transferred CD4 and CD8 T cells to lymph nodes and spleen was determined by flow cytometry. CMRA-labelled control cells were incubated with 300 ng/ml pertussis toxin for 2 hours. Representative FACS plots showing homing of labeled CD4 cells to spleen (A) and LNs (B) of AKR and SAMP hosts. Homing to the spleen, which is not dependent on CCR7[53], was affected only minimally. Control cells pretreated with pertussis toxin were not found in the lymph nodes but were present in the spleen. (C) Frequency of adoptively transferred CD4 or CD8 T cells homing to lymph nodes. Shown is mean±SEM of data polled form two experiments, n=6 mice/strain. (D) CCL21 immunofluorescence (*red*) on PFA-fixed frozen sections of spleen from AKR and SAMP mice. Sections were counterstained with DAPI (blue). Bar=20 µm

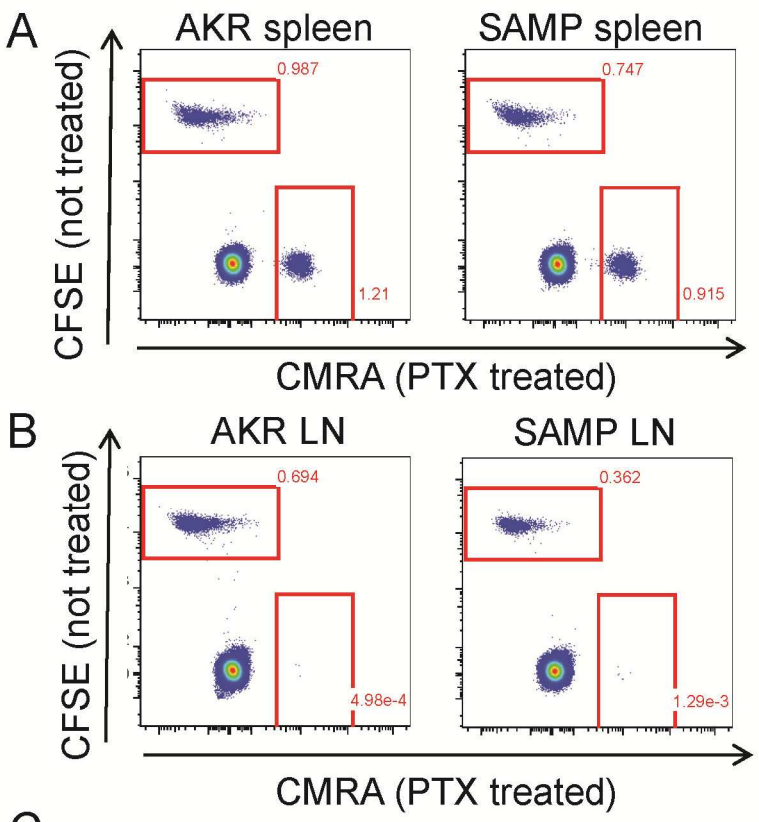
Supplementary figure 3. Expression of CCL21, CCL19, CXCL13 and CCR7 in SAMP mice following R848 treatment.

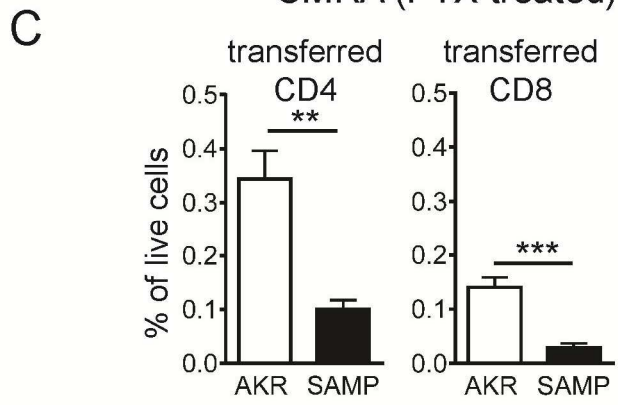
(A) Quantitative RT-PCR for CCL21, CCL19 and CXCL13 in the intestine and MLN, and (B) FACS analysis of CCR7 expression on $CD11c^{+}MHCII^{hi}$ DCs isolated from MLNs of AKR and SAMP mice following 18 hours of treatment with R848. Control mice received sterile water; mean±SEM for n=4 mice/group. Ribosomal protein L13A (Rpl13a) mRNA was used as a reference.

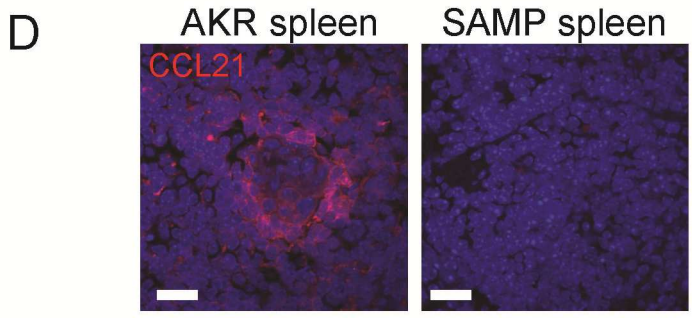
Supplementary figure 4. Expression of CCL19 and CCL21 in uninvolved control colon samples, involved ulcerative colitis or involved Crohn's disease patients. Chemokine expression was measured by TaqMan probe-based gene expression assays. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as a reference.

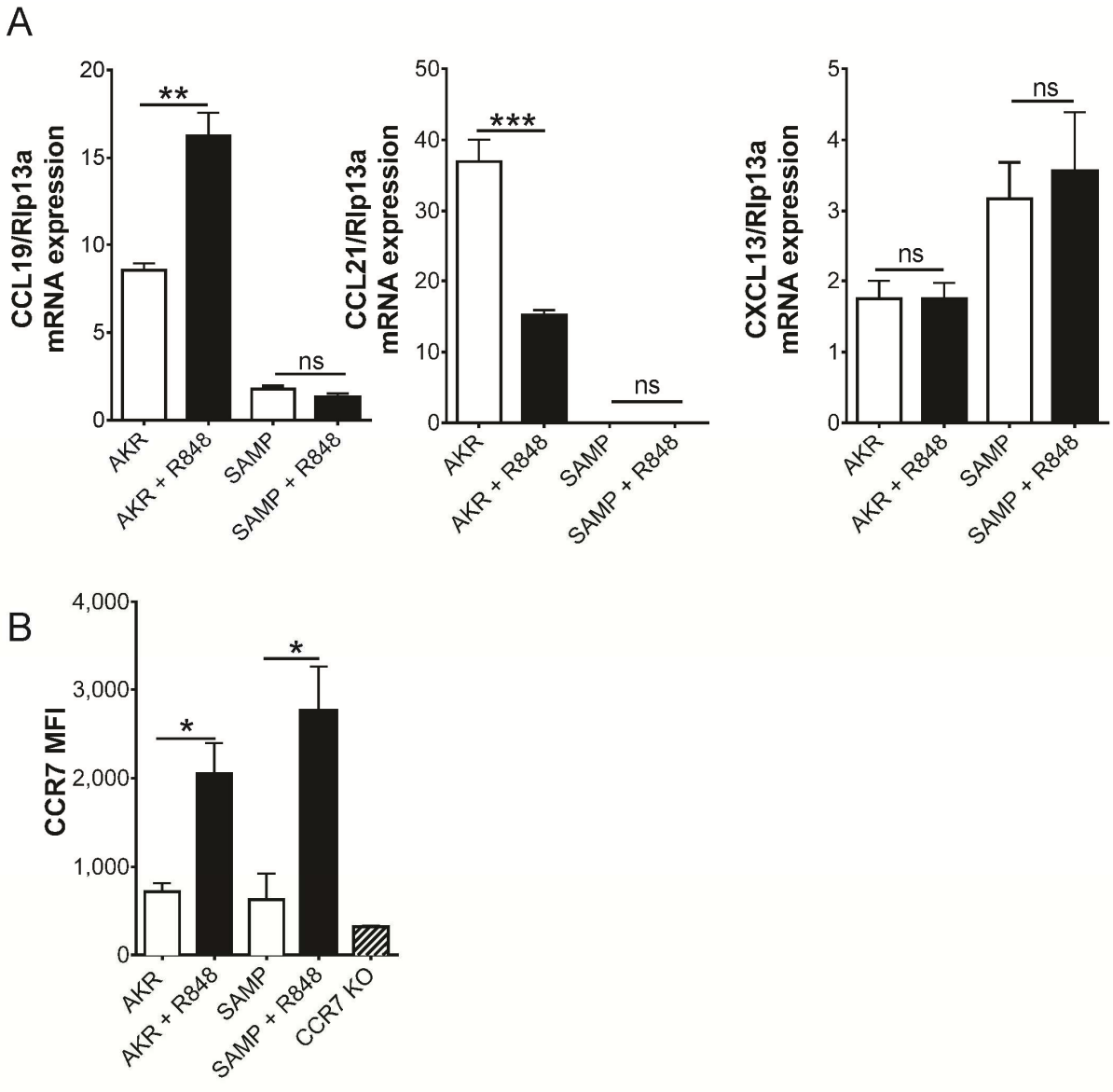
Supplementary Movie 1. Analysis of lymphocyte homing into AKR and SAMP popliteal lymph nodes by two-photon microscopy. CFSE-labeled T cells are shown in *green*, collagen-rich lymph node capsule (*red*) was visualized by second harmonic generation. Autofluorescence signals appear as *blue*. Movie shows series of consecutive optical sections of representative lymph nodes reconstructed from several stitched volumes followed by their rendering in Imaris software. Lymphocytes are represented as ellipsoid spots and their distance from the capsule (*red surface*) is color-coded.

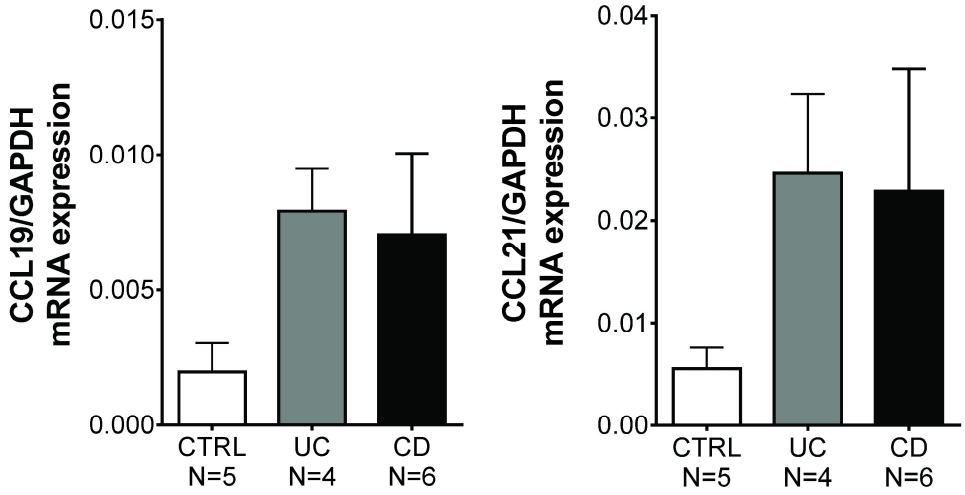

A
CCR7 WT
CCR7 KO
MHCII
CD11c
12±3.6
10±2.9
2.4±0.5
3±0.5
MHCIIhi
31.8±1.7
38.7±1.2
19±0.9
31.9±5
5.6±1.5#
46.5±10.1#
CD103
CD11b
B
MHCIIhi
MHCIIint
Frequency [% of live cells]
*
n.s.
WT
KO
C
CD103+CD11b-
CD103+CD11b+
CD103-CD11b+
***
ns

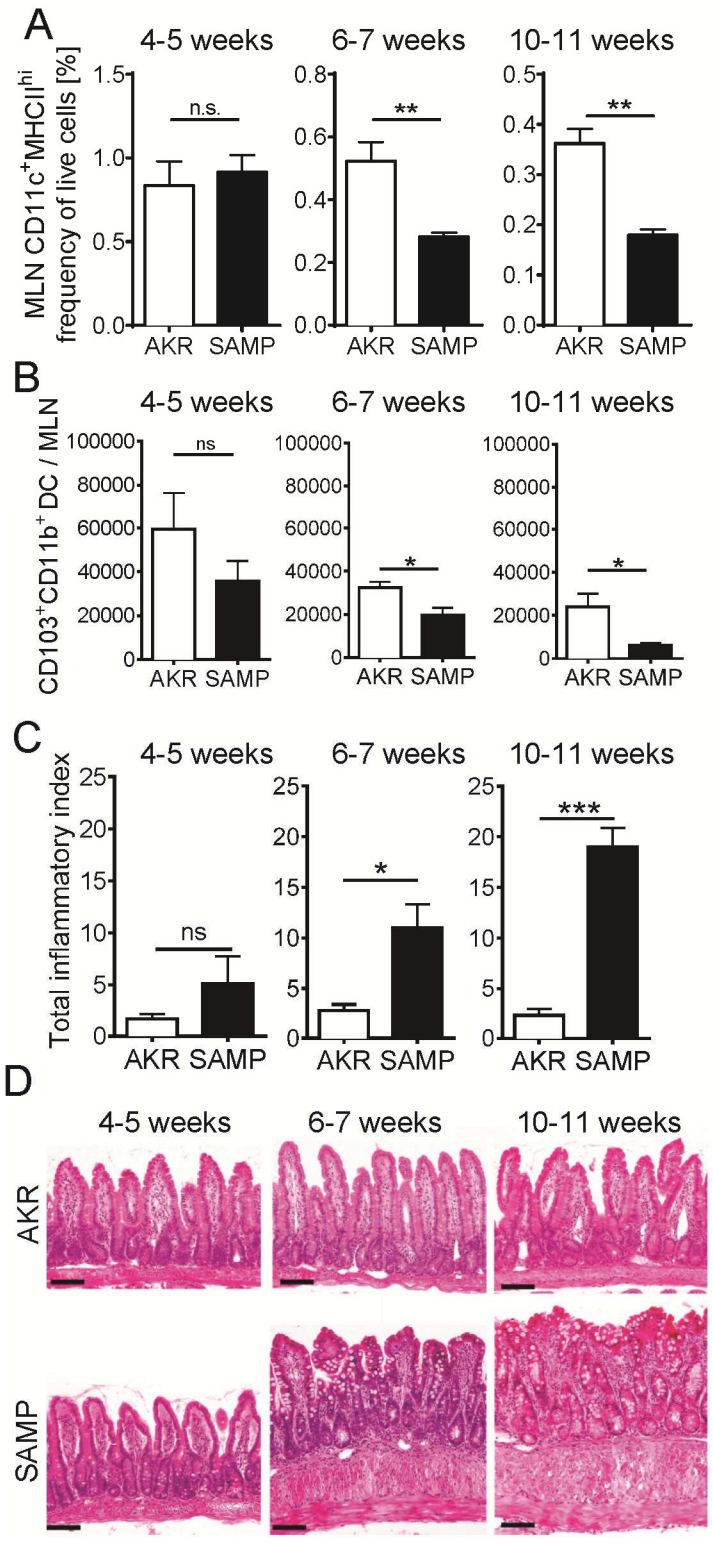

A
AKR spleen
SAMP spleen
0.987
1.21
0.747
0.915
CFSE (not treated)
CMRA (PTX treated)
B
AKR LN
SAMP LN
0.694
4.98e-4
0.362
1.29e-3
CFSE (not treated)
CMRA (PTX treated)

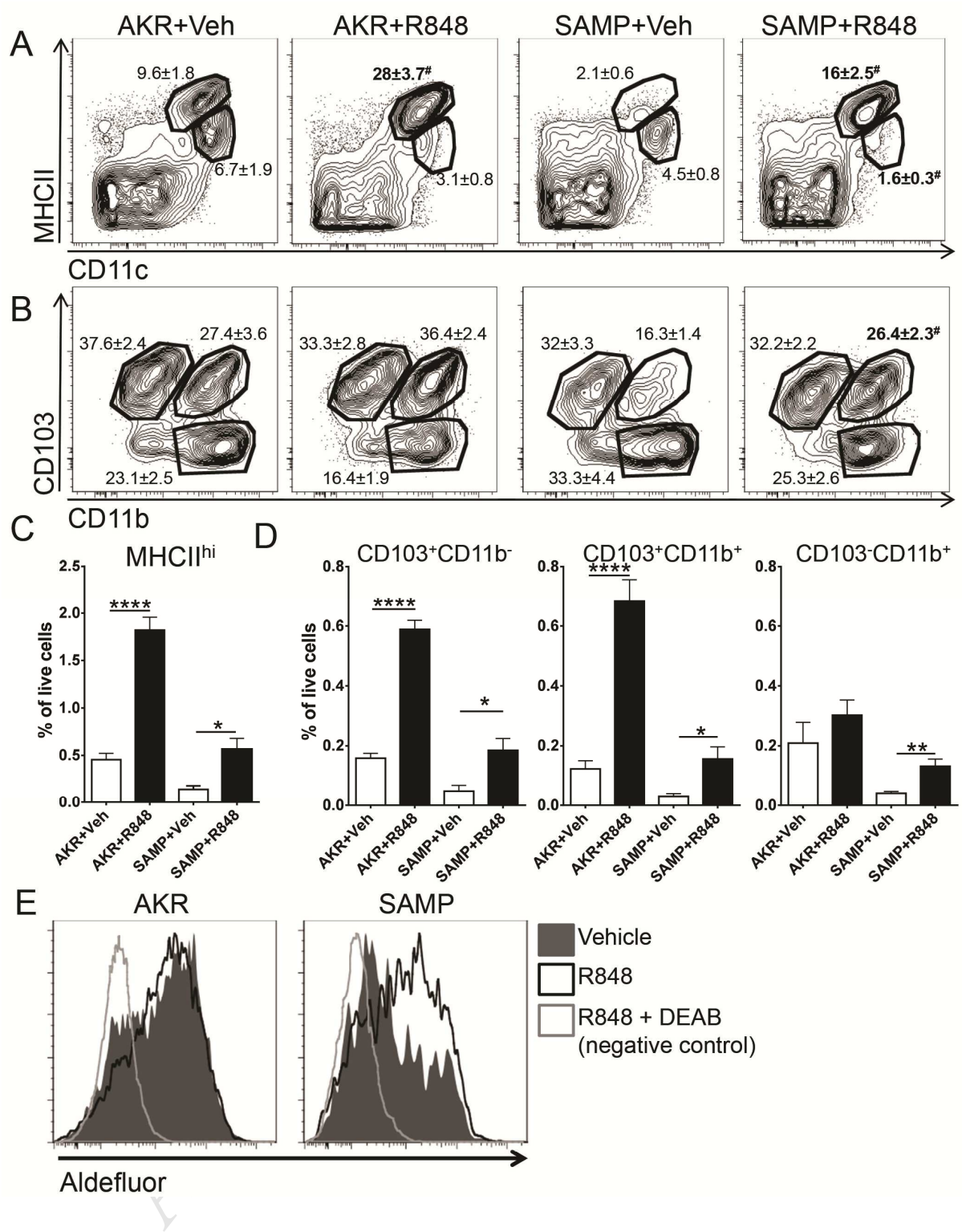

C
transferred CD4
transferred CD8
% of live cells
0.5
0.4
0.3
0.2
0.1
0.0
**
***
AKR
SAMP

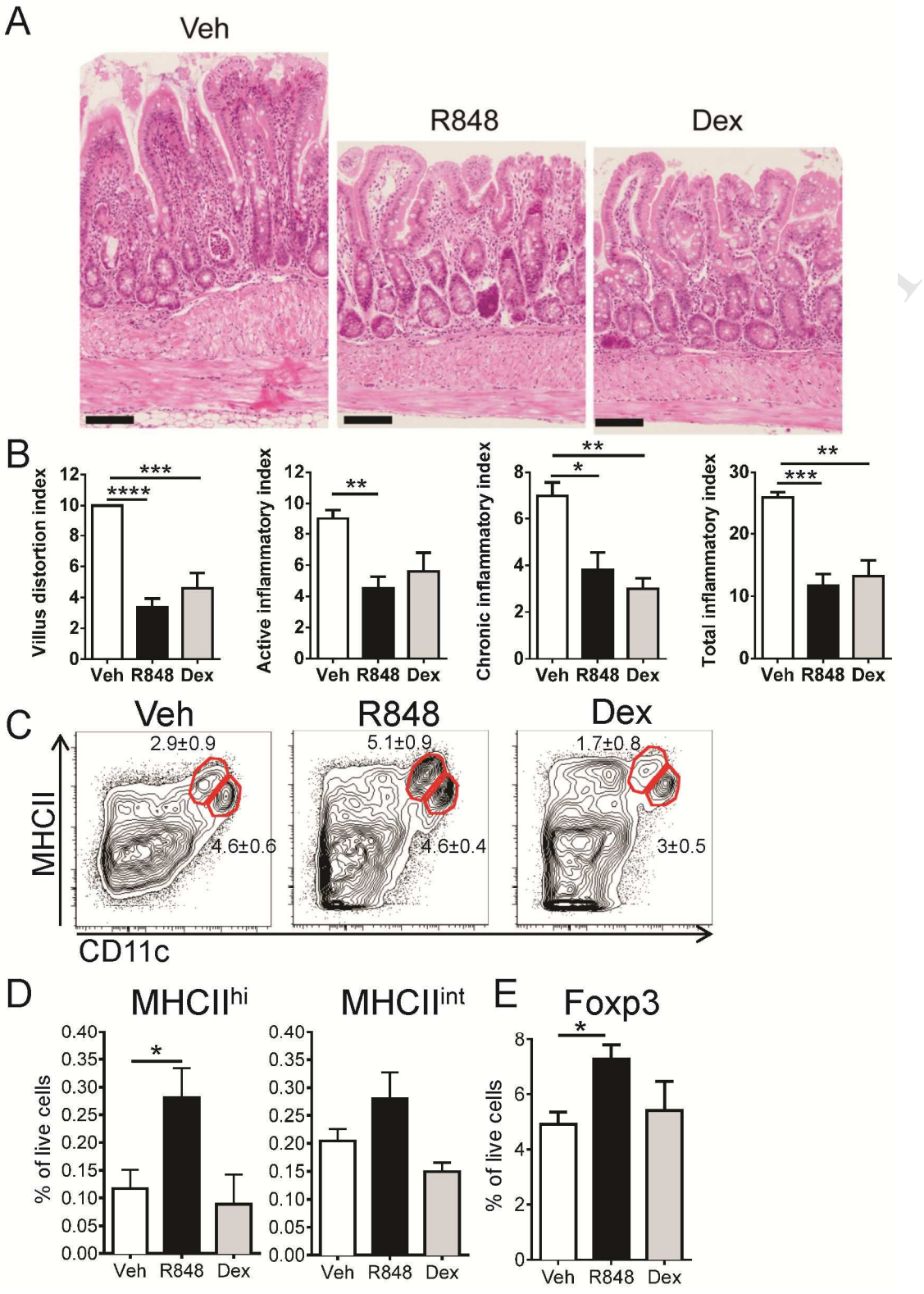

D
AKR spleen
SAMP spleen
CCL21


A
CCL19/Rlp13a mRNA expression
20
15
10
5
0
**
ns
AKR
AKR + R848
SAMP
SAMP + R848
CCL21/Rlp13a mRNA expression
50
40
30
20
10
0
***
ns
AKR
AKR + R848
SAMP
SAMP + R848
CXCL13/Rlp13a mRNA expression
5
4
3
2
1
0
ns
ns
AKR
AKR + R848
SAMP
SAMP + R848
B
CCR7 MFI
4,000
3,000
2,000
1,000
0
*
*
AKR
AKR + R848
SAMP
SAMP + R848
CCR7 KO




A
4-5 weeks
6-7 weeks
10-11 weeks
MLN CD11c+MHCIIhi frequency of live cells [%]
n.s.
**
**
AKR SAMP
B
4-5 weeks
6-7 weeks
10-11 weeks
CD103+CD11b+ DC / MLN
ns
*
*
AKR SAMP
C
4-5 weeks
6-7 weeks
10-11 weeks
Total inflammatory index
ns
*
***
AKR SAMP
D
4-5 weeks
6-7 weeks
10-11 weeks
AKR
SAMP


A
AKR+Veh
AKR+R848
SAMP+Veh
SAMP+R848
9.6±1.8
6.7±1.9
28±3.7#
3.1±0.8
2.1±0.6
4.5±0.8
16±2.5#
1.6±0.3#
MHCII
CD11c
B
37.6±2.4
27.4±3.6
23.1±2.5
33.3±2.8
36.4±2.4
16.4±1.9
32±3.3
16.3±1.4
33.3±4.4
32.2±2.2
26.4±2.3#
25.3±2.6
CD103
CD11b
C
MHCIIhi
% of live cells
****
*
D
CD103+CD11b-
CD103+CD11b+
CD103-CD11b+
**
E
AKR
SAMP
Vehicle
R848
R848 + DEAB
(negative control)
Aldefluor


A
Veh
R848
Dex
B
Villus distortion index
Active inflammatory index
Chronic inflammatory index
Total inflammatory index
Veh R848 Dex
C
Veh
R848
Dex
2.9±0.9
4.6±0.6
5.1±0.9
4.6±0.4
1.7±0.8
3±0.5
MHCII
CD11c
D
MHCIIhi
MHCIIint
% of live cells
E
Foxp3
% of live cells