Supporting Information

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SI Materials and Methods

Genotyping. Genomic DNA was isolated from a mouse tissue using Proteinase K (AppliChem) digestion followed by phenol-chloroform extraction. PCR conditions: 3 min at 94 °C, 30 s at 94 °C, 30 s at 55 °C for interleukin-10 (*Il10*) and 61 °C for α2,3 sialyltransferase 4 (*St3gal4*), 45 s at 72 °C (35 cycles) and 5 min at 72 °C. Amplified DNA was separated by 1.5% agarose gel electrophoresis: 450-bp fragments for both $Il10^{-/-}$ and $St3gal4^{-/-}$, 200-bp fragment for $Il10^{+/+}$, and 260-bp fragment for $St3gal4^{+/+}$ (for primers, see Table S1).

Cross-Fostering. Cross-fostering was performed as previously described (1). Briefly, time-matched breeding was set up for mice of different genotypes and newborns were separated from their mothers and transferred to mothers of another genotype.

Flow Cytometry. Cells were stained in PBS containing 0.5% BSA (Sigma-Aldrich) and one of the following fluorescently conjugated mAbs against CD3ε (145-2C11), CD4 (GK1.5), CD8α (53-6.7), CD11b (M1/70), CD11c (HL3), CD19 (1D3), CD40 (1C10), CD45 (30-F11), CD80 (16-10A1), CD86 (P03.1), CD103 (2E7), F4/80 (CI:A3-1), Ly-6C (AL-21), Ly-6G (1A8), and MHCII (AF6-120.1) for 30 min on ice. All mAbs were purchased either from BD or eBiosciences. Cells were acquired on a FACSCanto II flow cytometer (BD) and further analyzed with FlowJo software (Tree Star).

Histology. The distal part of the colon was washed, cut longitudinally, fixed in 4% (vol/vol) formalin, and embedded in paraffin. Tissue samples were cut in 5-μm sections and stained with hematoxylin and eosin (Sigma-Aldrich). Histological evaluation was performed in a blinded fashion. Morphological changes in the colon were assessed by multiple parameters (epithelium and leukocyte infiltration) as previously described (2). Histological scores were a sum of changes ranging from score 0 = normal morphology, no inflammation through score 8 = altered morphology, severe inflammation.

Isolation of Lamina Propria Leukocytes. Lamina propria leukocytes (LPLs) were isolated from the distal part of the colon. Colon was removed, washed with PBS, cut into 0.1- to 0.5-cm pieces, and incubated 30 min at 37 °C in PBS containing 0.5% BSA, 10 mM EDTA, penicillin/streptomycin, 20 mM Hepes, and 1 mM Na-pyruvate to remove epithelial cells. Tissues were thoroughly washed with PBS and digested with 2 mg/mL of collagenase type VIII (Sigma-Aldrich) and 30 μg/mL of DNase I (Sigma-Aldrich) in complete Iscove's modified Dulbecco's medium, containing 10% FCS, for 25 min at 37 °C. Released LPLs were filtered through a 40-μm nylon cell strainer and purified using magnetic separation with anti-CD45 MicroBeads (Miltenyi Biotec) according to the manufacturer's instructions. A small aliquot of unpurified cells was taken before magnetic separation, stained with anti-CD45 mAb, and analyzed by flow cytometry. When LPLs were isolated for stimulation of DCs, collagenase type D (Roche) was used instead.

Dendritic Cell Isolation and Stimulation. Mesenteric lymph nodes (MLNs) were removed, injected with 2.5 mg/mL of collagenase type D (Roche) in RPMI 1640 containing 10% FCS, and incubated for 10 min at 37 °C. Tissues were homogenized by passing through an 18-gauge needle, incubated for 30 min at 37 °C and filtered through 30-μm preseparation filters (Miltenyi Biotec). CD11c-positive cells were isolated with anti-CD11c MicroBeads (Miltenyi Biotec) according to the manufacturer's instructions. Dendritic cells (DCs) (1×10^6 cells/mL) were stimulated in RPMI 1640 containing 10% FCS in presence of one of the sugars: 625 μM of 3SL, 6SL, or lactose and with LPS (100 ng/mL, from *Escherichia coli* EH100; Sigma-Aldrich) or water for 14 h at 37 °C, as controls. Cells were stained with fluorochrome-labeled antibodies and analyzed by flow cytometry.

Cytokine Gene Expression. Total RNA was isolated from flash frozen colon tissues using the RNeasy Protect Mini kit (Qiagen) according to the manufacturer's instructions. cDNA was obtained by reverse transcription using Omniscript RT kit (Qiagen) with oligo(dT) primers and 2 μg of RNA. Real-time PCR was performed with 100 ng of cDNA using SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich) and primers specific for TNF-α, IFN-γ, IL-4, IL-12, IL-1β, TGF-β, GAPDH (QuantiTect; Qiagen), and IL-17α, chemokine (C-C motif) ligand (CCL2), CCL5, CXCL1, and mBD1 (Microsynth; Table S1) using the following protocol: 95 °C for 10 min, 40 cycles at 95 °C for 15 s, 60–64 °C for 30 s and 72 °C for 30 s in a Mx3000P thermocycler (Stratagene). Expression levels were calculated in a relation to GAPDH expression using the $2^{-\Delta\Delta Ct}$ method (3).

Cytokine Determination. Supernatants of stimulated cells were collected and submitted for determination of cytokine concentration by multiplex bead array to Cytolab.

Leukocyte Isolation. Blood was taken by cardiac puncture from mice anesthetized with a mixture of 0.65 mL/kg body weight of 10% ketamine (Vétoquinol), 0.5 mL/kg body weight of 2% xylazine (Streuli), and 0.07 mL/kg body weight of 10% acepromazine (Arovet). To prevent coagulation, blood was collected in PBS containing 10 mM EDTA and spun down. Erythrocytes were lysed in PharmLyse lysing buffer (BD) for 15 min in the dark. Remaining leukocytes were washed with PBS containing 0.5% BSA (Sigma-Aldrich), stained with appropriate antibodies, and analyzed by flow cytometry.

Bacterial Typization. Bacterial DNA was isolated from freshly collected fecal pellets using the QIAamp Stool Mini kit (Qiagen) according to the manufacturer's instructions. Real-time PCR was performed in a Stargene apparatus (Mx3000P). Isolated DNA was amplified using primers specific for the following bacterial lineages: Bacteroidetes, Lactobacillaceae, Enterobacteriaceae, Lachnospiraceae, and clostridial cluster IV (Table 1). Each reaction was carried out in the volume of 25 μL using PCR buffer (Sigma-Aldrich), SYBR Green I nucleic acid gel stain (Sigma-Aldrich), 0.05 units Taq polymerase (Sigma-Aldrich), reference dye ROX (Sigma-Aldrich), 0.1 mM dNTPs (Promega), 0.4-μM primers (Microsynth; Table 1), and 100 ng of DNA or water as a control. RT-PCR was performed with the following protocol: 95 °C for 10 min, 40 cycles at 95 °C for 20 s, 60–66 °C for 25 s, and 72 °C for 25 s. Expression rates were quantified using the $2^{-\Delta Ct}$ method in a relation to the amplification of the total bacterial 16S rRNA (4).

Statistics. Data were analyzed using one-way ANOVA with Bonferroni's multiple comparison post hoc tests. Error bars show mean ± SEM. P values of less than 0.05 were considered significant: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

1. Fuhrer A, et al. (2010) Milk sialyllactose influences colitis in mice through selective intestinal bacterial colonization. *J Exp Med* 207(13):2843–2854.
2. Hausmann M, et al. (2007) In vivo treatment with the herbal phenylethanoid acteoside ameliorates intestinal inflammation in dextran sulphate sodium-induced colitis. *Clin Exp Immunol* 148(2):373–381.
3. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25(4):402–408.
4. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 3(6):1101–1108.

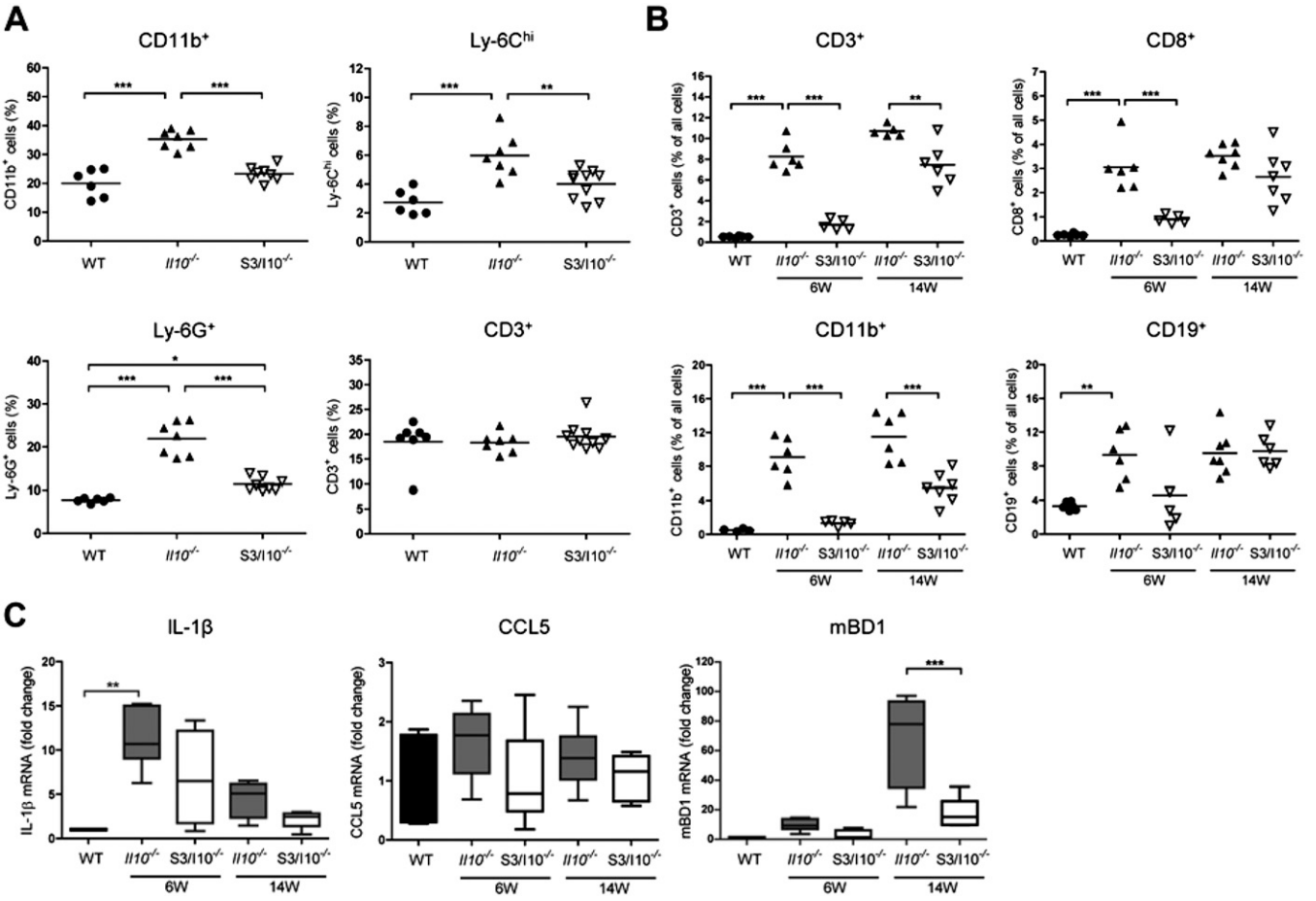

Fig. S1. Increased levels of inflammatory leukocytes in circulation of *Il10*$^{-/-}$ mice compared with S3I10$^{-/-}$ mice. (*A*) Flow cytometry analysis of peripheral blood leukocytes in C57BL/J6 (WT), *Il10*$^{-/-}$, and S3/I10$^{-/-}$ mice at 6 wk of age. Phenotype of leukocytes was determined and data are presented as percentage of CD11b^{+}, Ly6C^{hi}, Ly6G^{+}, and CD3^{+} cells of all leukocytes. (*B*) Flow cytometry analysis of lamina propria leukocytes (LPLs) isolated from the distal part of the colon of 6- and 14-wk-old *Il10*$^{-/-}$ and S3/I10$^{-/-}$ mice (n = 5–7) or 6- to 8-wk-old WT controls. Phenotype of immune cells was determined. Data are presented as percentage of CD3^{+}, CD8^{+}, CD11b^{+}, and Ly19^{+} of the total isolated cells. (*C*) Expression levels of cytokines in colons from 6- and 14-wk-old *Il10*$^{-/-}$, ST3/IL10-KO mice (n = 6–8), and WT control mice (n = 5–6) were determined by RT-PCR and normalized to GAPDH. WT, wild-type mice; S3/I10$^{-/-}$, *St3g4*$^{-/-}$*Il10*$^{-/-}$ mice; 6W, 6-wk-old mice; 14W, 14-wk-old mice. *P < 0.05; **P < 0.01; ***P < 0.001.

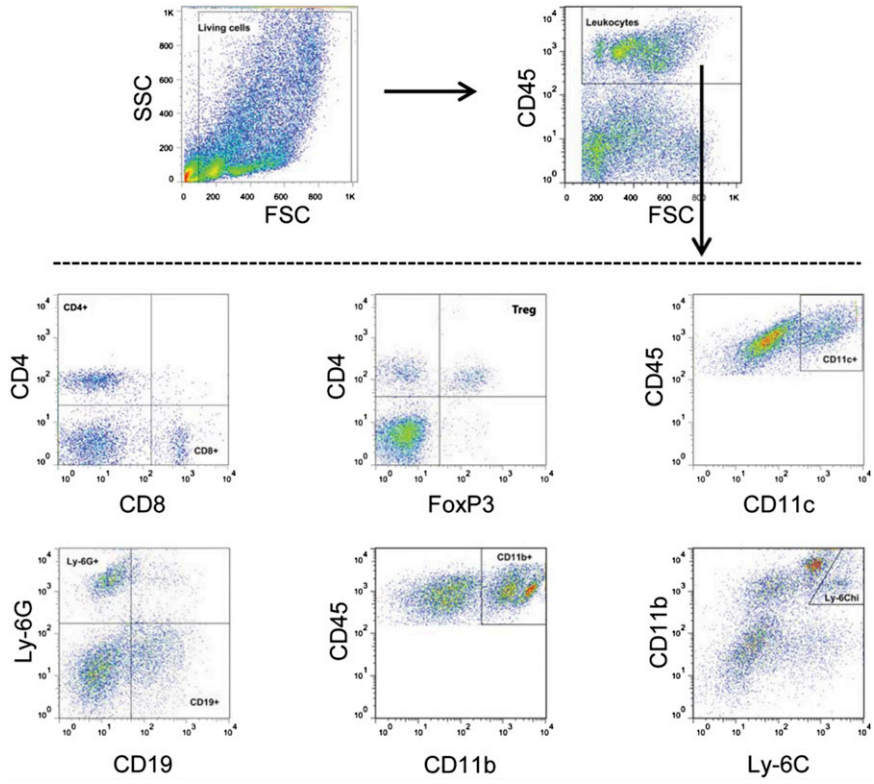

Fig. S2. Gating strategy for the analysis of lamina propria leukocytes (LPLs). Crude cell isolate from the distal colon was stained with mAbs. Living cells were first gated based on forward and side scatter. Leukocytes were gated based on CD45-APC-Cy7 staining and further analyzed with correspondent fluorescently labeled mAbs for the following markers: CD3, CD4, CD8, CD19, CD11b, CD11c, Ly-6C, Ly-6G, and FoxP3.

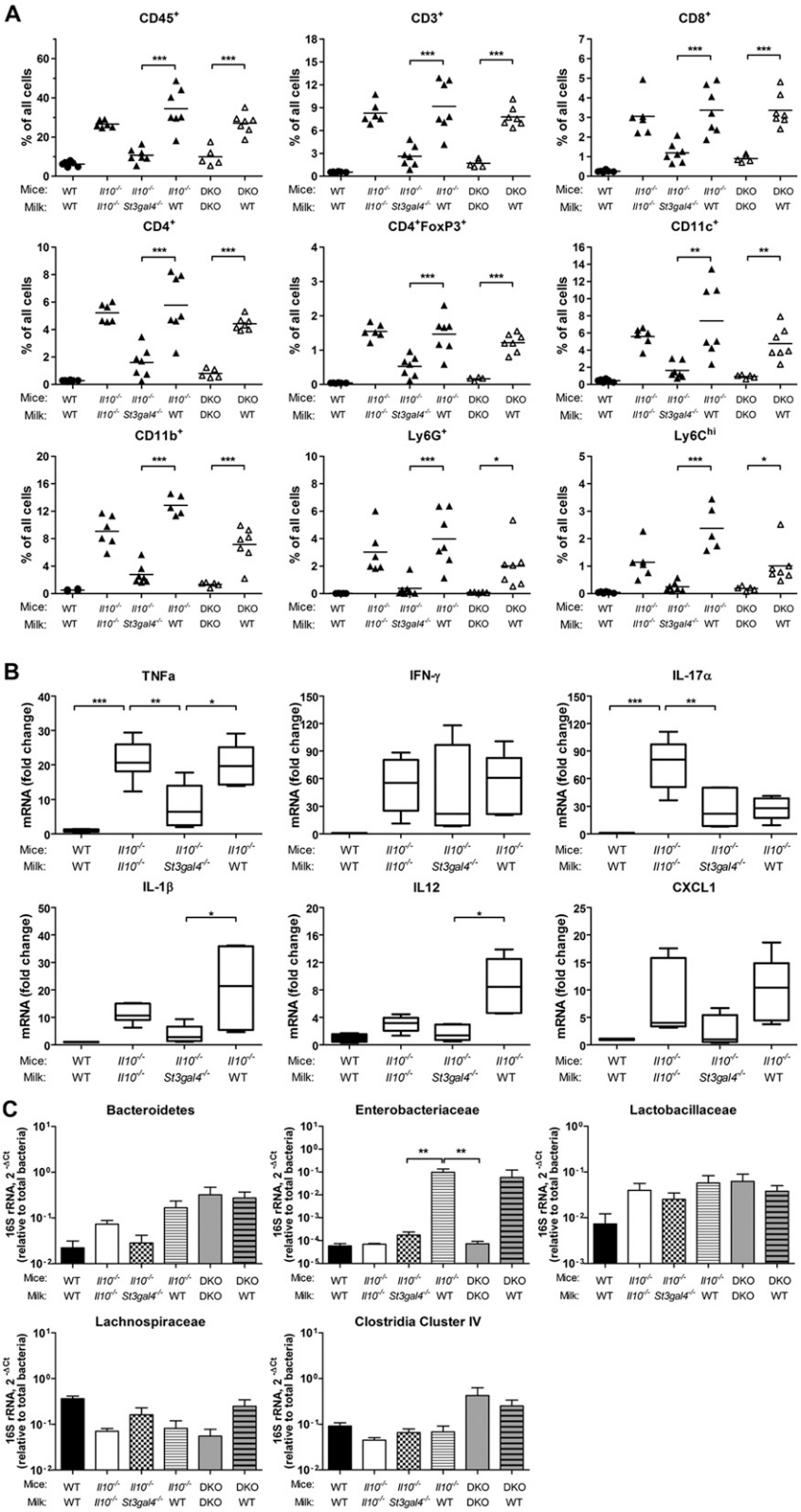

Fig. S3. Milk oligosaccharide sialyl(α2,3)lactose (3SL) increases susceptibility to spontaneous intestinal inflammation in *Il10*$^{-/-}$ mice. Newborn *Il10*$^{-/-}$ mice were cross-fostered to WT mothers (3SL-rich milk) or to *St3g4*$^{-/-}$ mothers (3SL-poor milk). *Il10*$^{-/-}$ and WT mice without cross-fostering were used as controls. Double *St3gal4*$^{-/-}$; *Il10*$^{-/-}$ mice (DKO) were cross-fostered to DKO (3SL-poor milk) or to WT mothers (3SL-rich milk). All mice were analyzed at the age of 6–7 wk. (*A*) Flow cytometry analysis of LPLs isolated from the distal part of a colon. Phenotype of immune cells was determined with corresponding mAbs. Data are presented as percentage of CD45$^+$, CD3$^+$, CD8$^+$, CD4$^+$, CD4$^+$FoxP3$^+$ (Treg), CD11c$^+$, CD11b$^+$, Ly6C^{hi+}, and Ly6G$^+$ cells of all isolated cells. (*B*) Expression levels of cytokines in colon samples were determined by RT-PCR and normalized to GAPDH. (*C*) Analysis of gastrointestinal microbiota. Bacterial DNA was isolated from feces of 6-wk-old *Il10*$^{-/-}$ and S3/I10$^{-/-}$ mice either cross-fostered or kept with mothers (n = 5–6). Bacterial DNA from 6-wk-old WT was used as a control. Bacterial strains were determined by RT-PCR; expression of different phylogenic groups of bacteria was calculated using the $2^{-\Delta ct}$ method in relation to total bacterial 16S rRNA. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

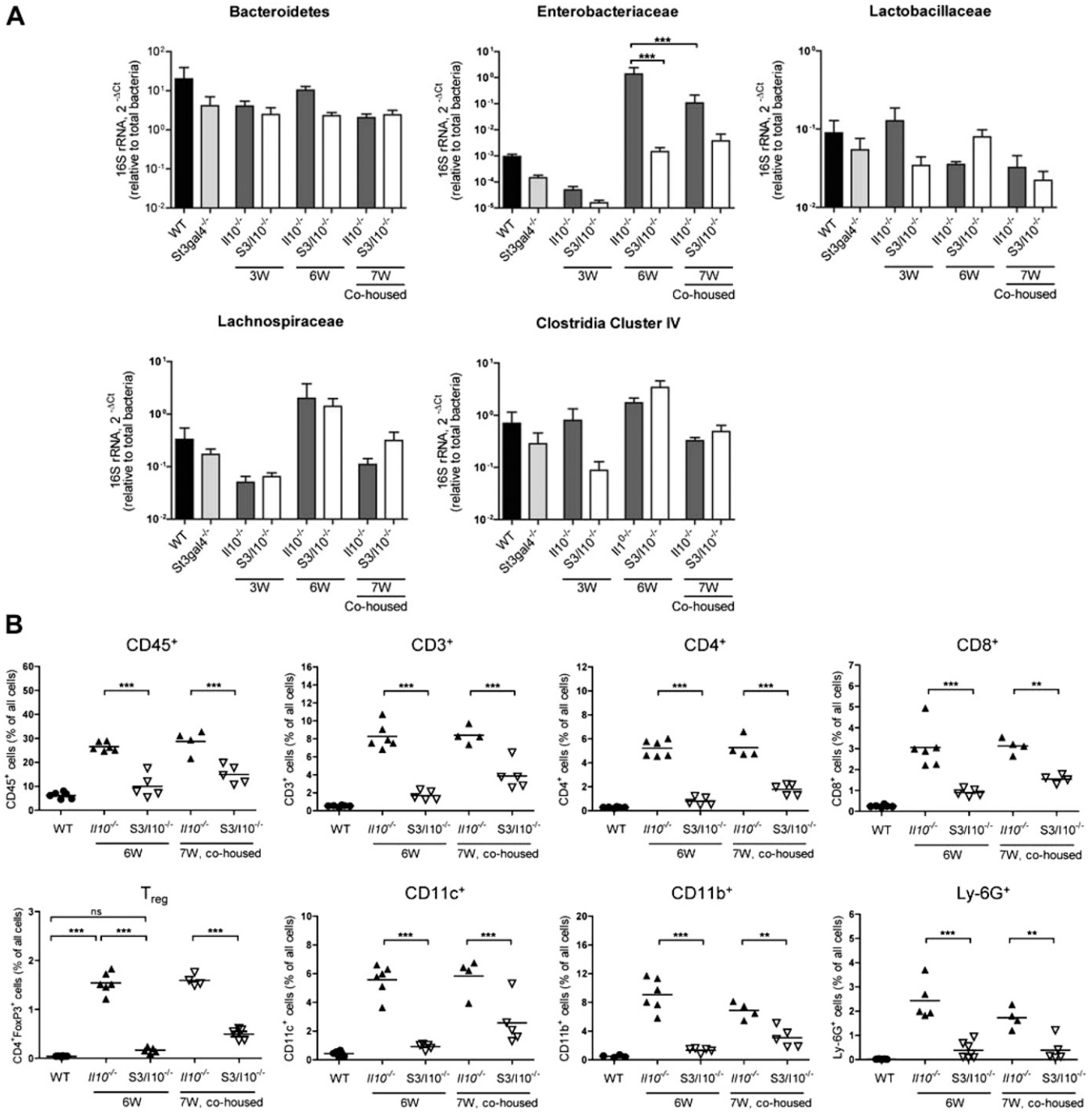

Fig. S4. Changes in gastrointestinal microbiota do not alter the onset of inflammation in *Il10*$^{-/-}$ and ST3/IL10-KO mice. *Il10*$^{-/-}$ and S3/I10$^{-/-}$ mice were co-housed for 4 wk after weaning or housed conventionally. (*A*) Analysis of gastrointestinal microbiota. Bacterial DNA was isolated from feces of 3- and 7-wk-old *Il10*$^{-/-}$ and S3/I10$^{-/-}$ mice (n = 5–6). Bacterial DNA from 7-wk-old WT and *St3g4*$^{-/-}$ mice was used as a control. Bacterial strains were determined by RT-PCR; expression of different phylogenic groups of bacteria was calculated using the $2^{-\Delta ct}$ method in relation to total bacterial 16S rRNA. (*B*) FACS analysis of LPLs isolated from the distal part of the colon of 7-wk-old mice. Phenotype of immune cells was determined with corresponding mAbs. Data are presented as percentage of CD45$^+$, CD3$^+$, CD4$^+$, CD8$^+$, CD4$^+$FoxP3$^+$ (Treg), CD11c$^+$, CD11b$^+$, and Ly6G$^+$ cells of all isolated cells. WT, wild-type mice; S3/I10$^{-/-}$, *St3g4*$^{-/-}$*Il10*$^{-/-}$ mice; 3W, 3-wk-old mice; 6W, 6-wk-old mice; 7W, 7-wk-old mice. $^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant.

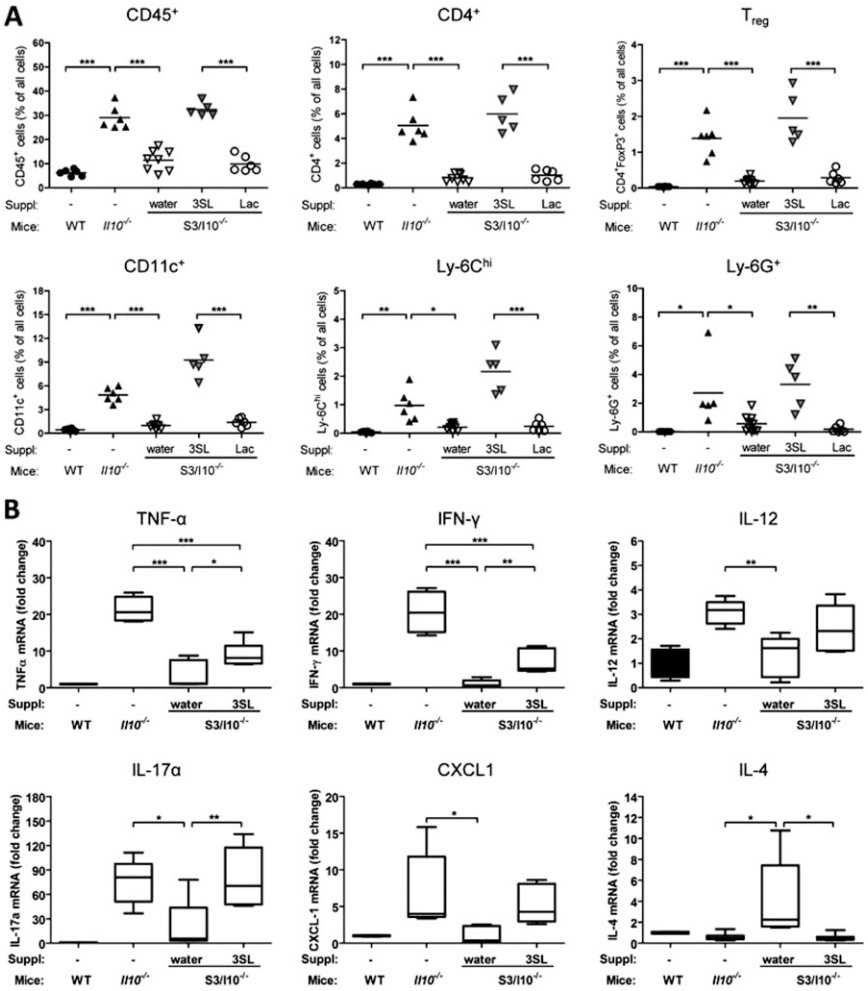

Fig. S5. Sialyl(α2,3)lactose (3SL) supplementation accelerates colonic inflammation. S3/I10$^{-/-}$ mice were fed daily from birth until weaning (21 d) with 25 mM 3SL or 25 mM lactose; control mice were fed with water. Mice were analyzed at the age of 6 wk (day 48 after birth). (*A*) Flow cytometry analysis of LPLs isolated from the distal part of the colon. Phenotypic characterization of LPLs was performed. Data are presented as percentage of CD45^{+}, CD4^{+}, CD4^{+}FoxP3^{+} (Treg), CD11c^{+}, Ly6C^{hi+}, and Ly6G^{+} of all isolated cells. (*B*) Expression levels of cytokines in colon samples from 6-wk-old WT, S3/I10$^{-/-}$ mice, either 3SL supplemented or water controls (n = 6–8) were measured by RT-PCR and normalized to GAPDH. WT, wild-type mice; S3/I10$^{-/-}$, *St3g4*$^{-/-}$/*Il10*$^{-/-}$ mice; 3SL, sialyl(α2,3)lactose. *P < 0.05; **P < 0.01; ***P < 0.001.

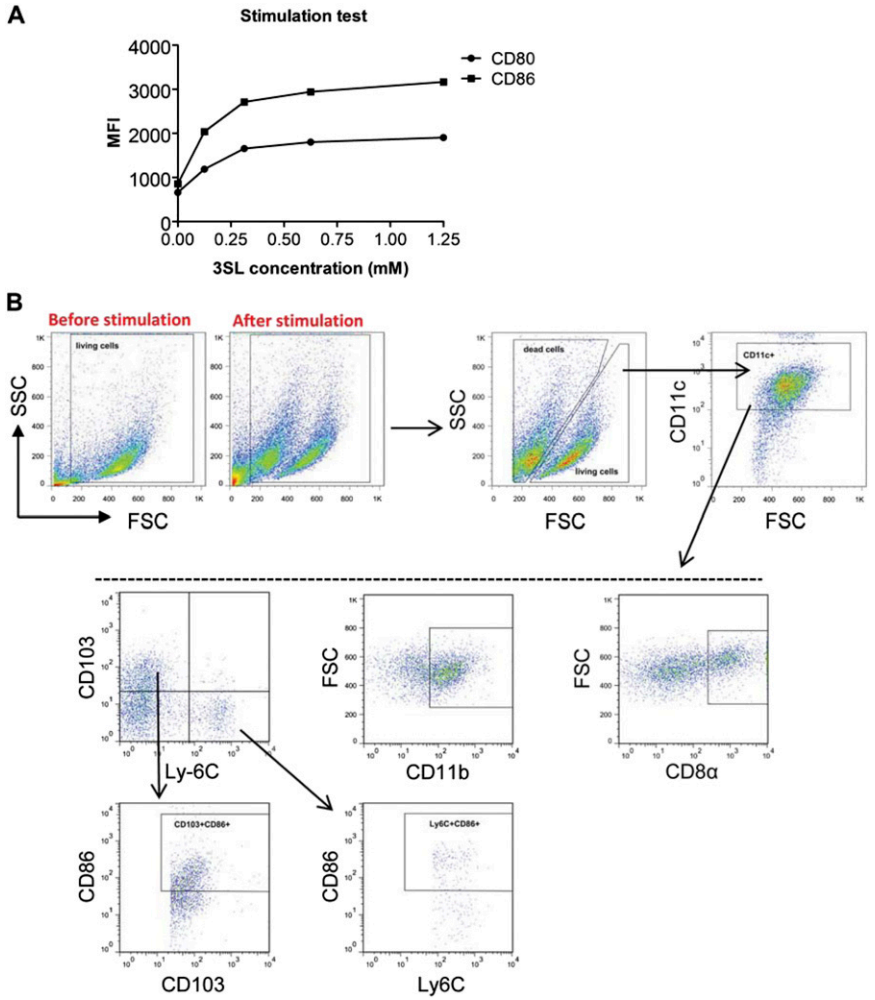

Fig. S6. (*A*) 3SL directly stimulates dendritic cells (DCs). DCs were isolated from mesenteric lymph nodes of 6-wk-old WT mice and purified with CD11c MicroBeads. CD11c^{+}/MHCII^{+} cells were stimulated with 3SL at increasing concentrations and expression of CD80 and CD86 was determined. (*B*) Gating strategy for the analysis of dendritic cells. Cells were isolated from mesenteric lymph nodes and analyzed by flow cytometry using corresponding mAbs. Living cells were gated based on the forward and side scatter. Before stimulation, cells represented a homogenous population but after the stimulation, two separate cell populations (smaller and larger cells) were observed. The smaller cells were positive for propidium iodide staining. The population of the living cells was gated for CD11c/MHCII double positive cells. Further analysis using combination of mAbs against CD8α, CD11b, CD103, Ly-6C, and CD86 was performed. Ly-6C/CD86 or CD103/CD86 double positive cells were gated based on the intensity of CD86 expression.

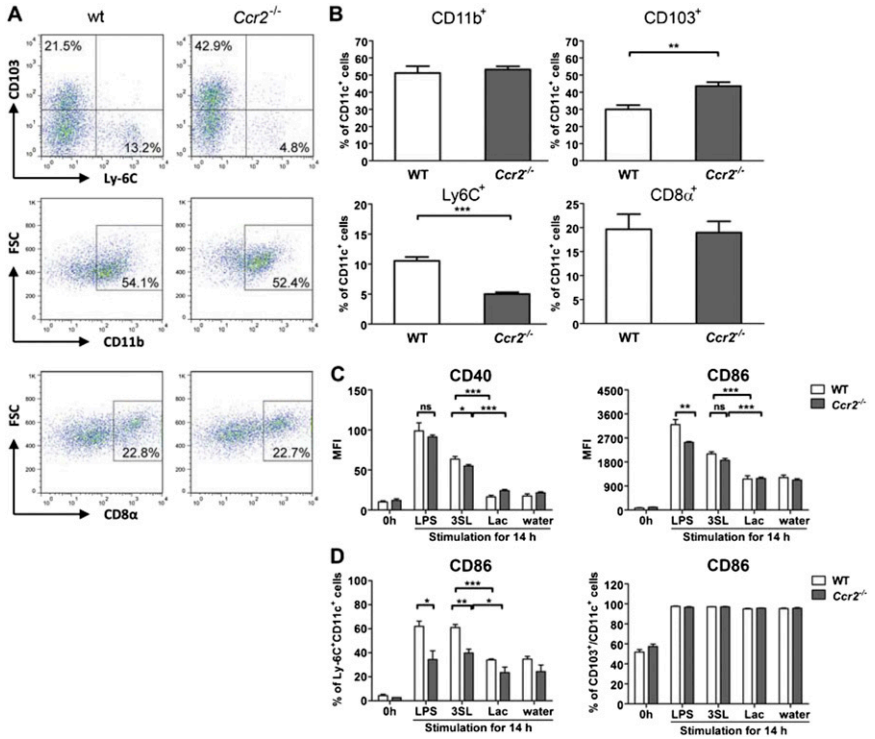

Fig. S7. Ly-6C^{hi}–derived CD11c^{+} cells respond to 3SL stimulation. DCs were isolated from MLNs of 6-wk-old WT and *Ccr2*$^{-/-}$ mice. (*A*) Representative plots of CD11c^{+} cells from WT and *Ccr2*$^{-/-}$ mice stained with anti-CD103, anti–Ly-6C, anti-CD11b, and anti-CD8α mAbs. (*B*) Quantification of DC subpopulations from WT and *Ccr2*$^{-/-}$ mice (three independent experiments). (*C*) CD11c^{+} cells from WT and *Ccr2*$^{-/-}$ mice were stimulated with 625 μM 3SL or lactose for 14 h. Stimulation with LPS (500 ng/mL) or water was used as controls. (*D*) Cell surface expression of CD86 on Ly-6C^{+} or CD103^{+} cells was analyzed by flow cytometry and quantified (three independent experiments). Lac, lactose; MFI, mean fluorescence intensity; 3SL, sialyl(α2,3)lactose. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table S1. List of primers used in real-time PCR

Names	Primers	Sequences 5′–3′	Fragment length, bp	Source
St3Gal4	Fw	TCCAGGGTGAGGCAGAGAGA	260	1
	Rv	GTCCCAAAGGGCAGCTCATA		
IL10	Fw	GCCTTCAGTATAAAAGGGGGACC	200	This study
	Rv	GTGGGTGCAGTTATTGTCTTCCCG		
CCL2	mCCL2 fw	TTAAAAACCTGGATCGGAACCAA	121	1
	mCCL2 rv	GCATTAGCTTCAGATTTACGGGT		
CCL5	mCCL5 fw	TGCTGCTTGCCTACCTC	93	2
	mCCL5 rv	ACACTTGGCGGTTCCTTC		
CXCL1	mCXCL1 fw	CTGGGATTCACCTCAAGAACATC	117	1
	mCXCL1 rv	CAGGGTCAAGGCAAGCCTC		
IL-17α	mIL17a fw	TGTGAAGGTCAACCTCAAAGTC	131	3
	mIL17a rv	AGGGATATCTATCAGGGTCTTCATT		
mBD1	mBD1 fw	GGCTGCCACCACTATGAAAACTC	148	4
	mBD1 rv	GAGACAGAATCCTCCATGTTGAA		
Total bacteria	515F	GTGCCAGCMGCCGCGGTAA	300	5
	805R	GACTACCAGGGTATCTAAT		
Bacteroidetes	Bac303R	CCAATGTGGGGGACCTTC	267	5
	Bac32F	AACGCTAGCTACAGGCTT		
Enterobacteriaceae	Eco1457F	CATTGACGTTACCCGCAGAAGAAGC	195	1
	Eco1652R	CTCTACGAGACTCAAGCTTGC		
Lachnospiraceae	Ccocc1F	CGGTACCTGACTAAGAAGC	429	1
	Ccocc1R	AGTTTYATTCTTGCGAACG		
Clostridial cluster IV	Clep866mF	TTAACACAATAAGTWATCCACCTGG	314	1
	Clept1240mR	ACCTTCCTCCGTTTTGTCAAC		
Lactobacillaceae	F_Lacto 05	AGCAGTAGGGAATCTTCC	383	1
	AR_Lacto 04	CGCCACTGGTGTTCYTCCATATA		

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2. Läubli H, Spanaus K-S, Borsig L (2009) Selectin-mediated activation of endothelial cells induces expression of CCL5 and promotes metastasis through recruitment of monocytes. *Blood* 114(20):4583–4591.
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4. Bailey MT, et al. (2010) Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium. *Infect Immun* 78(4): 1509–1519.
5. Frank DN, et al. (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 104(34):13780–13785.