1 **Restored macrophage function ameliorates disease pathophysiology in inflammatory**
2 **bowel disease**
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16 **Supplementary Information**
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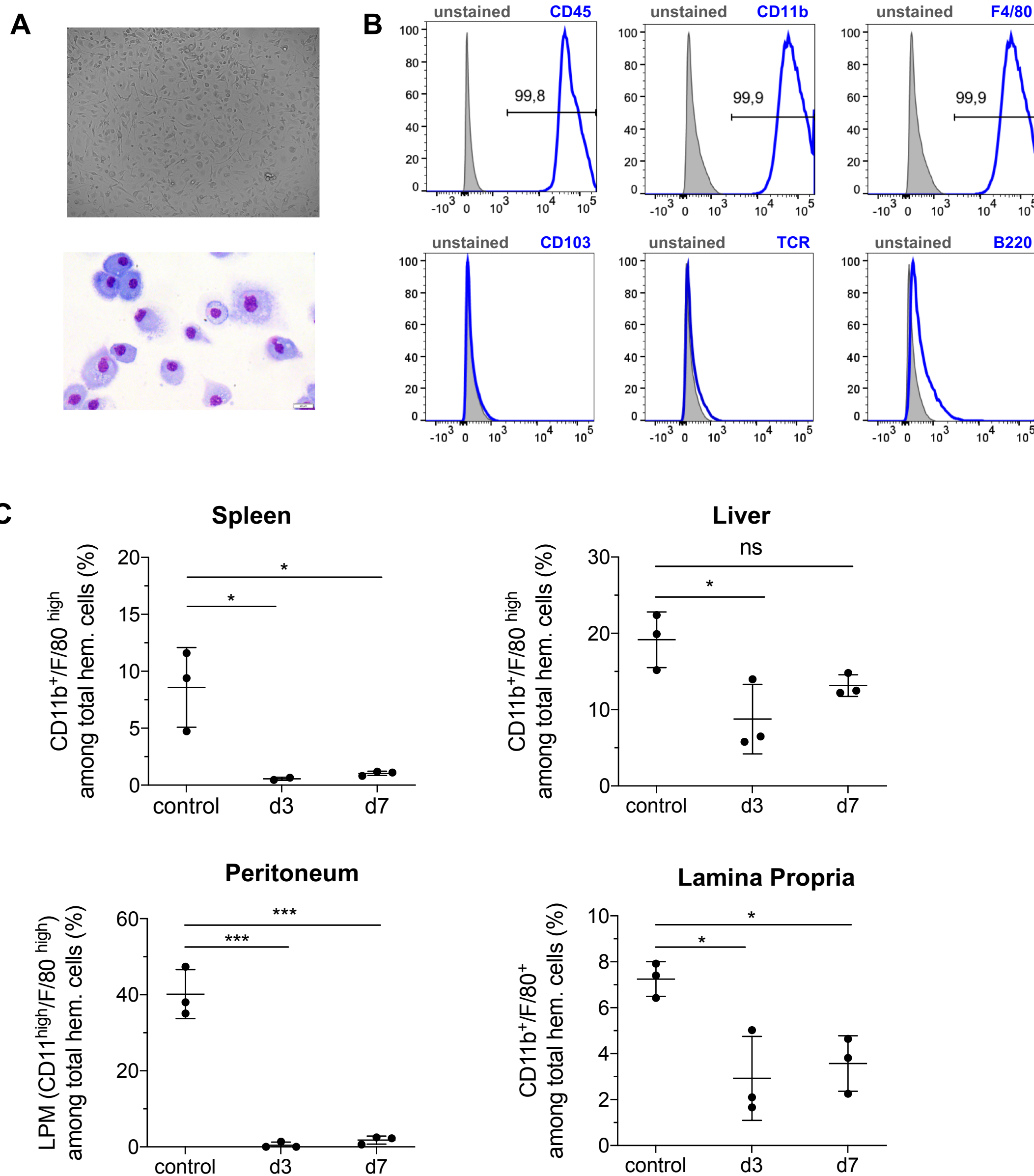
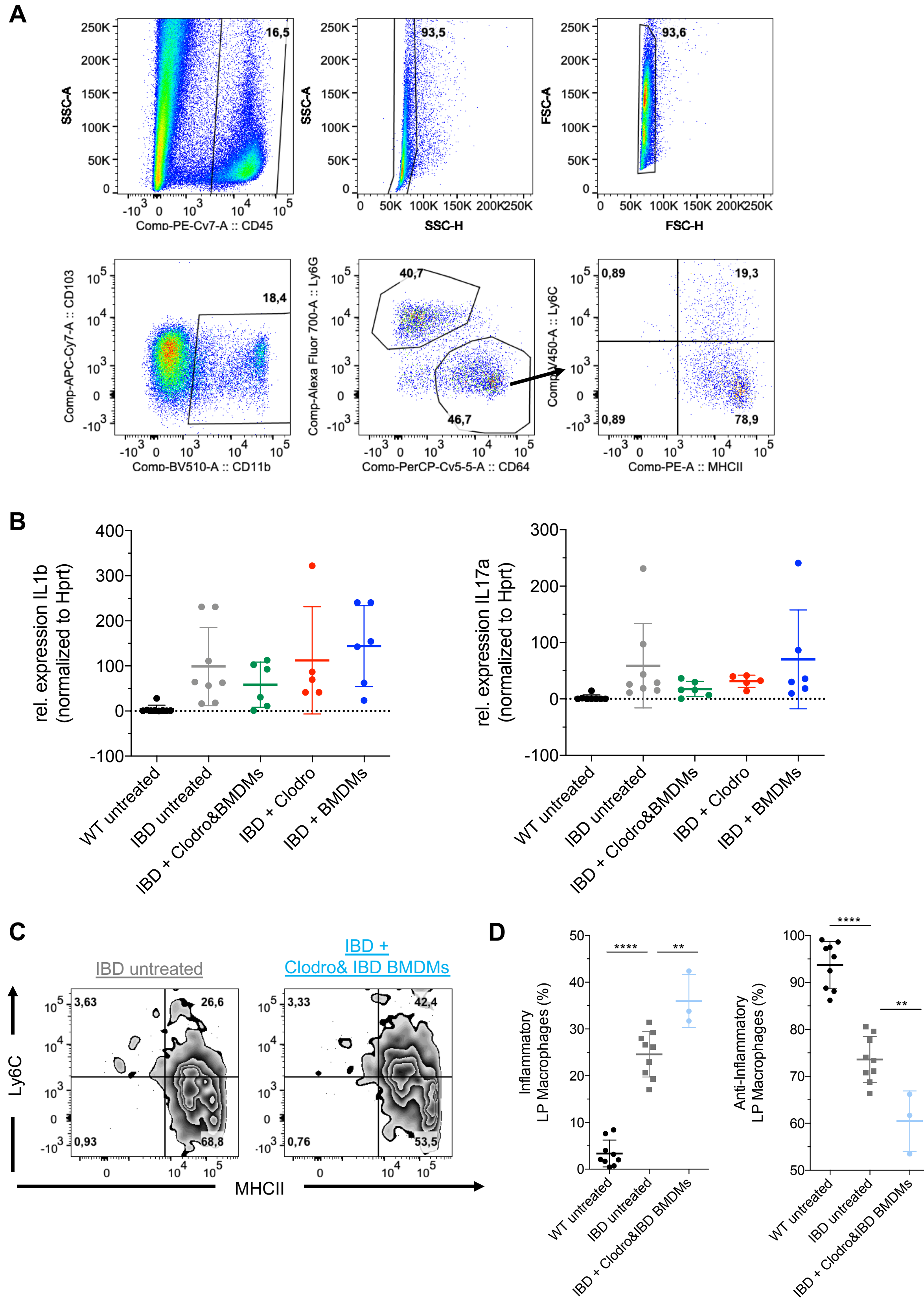
Supplementary Figures:



Supplementary Figure 1: *Ex vivo* generation of macrophages and *in vivo* depletion of macrophages with clodronate. Total bone marrow, lineage positive or lineage negative cells were differentiated for 7-10 days in medium containing M-CSF. **(A)** Brightfield microscopy and H/E stained cytospins of bone marrow derived macrophages (BMDMs) generated *in vitro*. **(B)** Flow cytometric analyses of *in vitro* generated BMDMs. Grey filled: unstained control, Blue line: respective surface marker. **(C)** Macrophage populations in spleen, liver, peritoneum und lamina propria (LP) were analyzed by flow cytometry 3 and 7 days post i.p. injection of clodronate liposomes in *Il10rb*$^{-/-}$ mice. Non-treated animals served as controls (n=3 animals/group, mean±SD).



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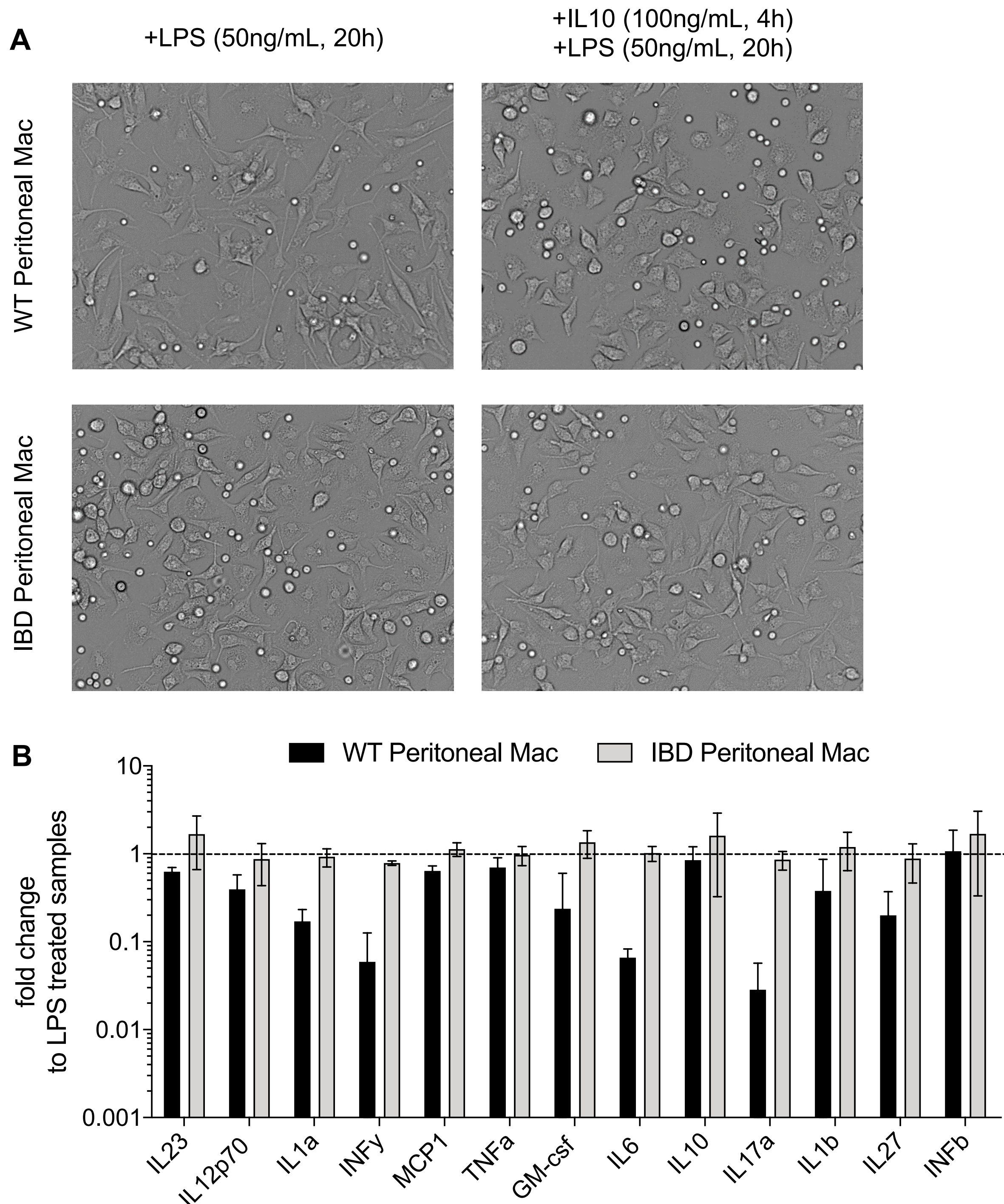
2 **Supplementary Figure 2: Therapeutic effect of a macrophage-based therapy on colitis**

3 **in *Il10rb*$^{-/-}$ mice. (A)** Representative gating strategy for the analysis of lamina propria

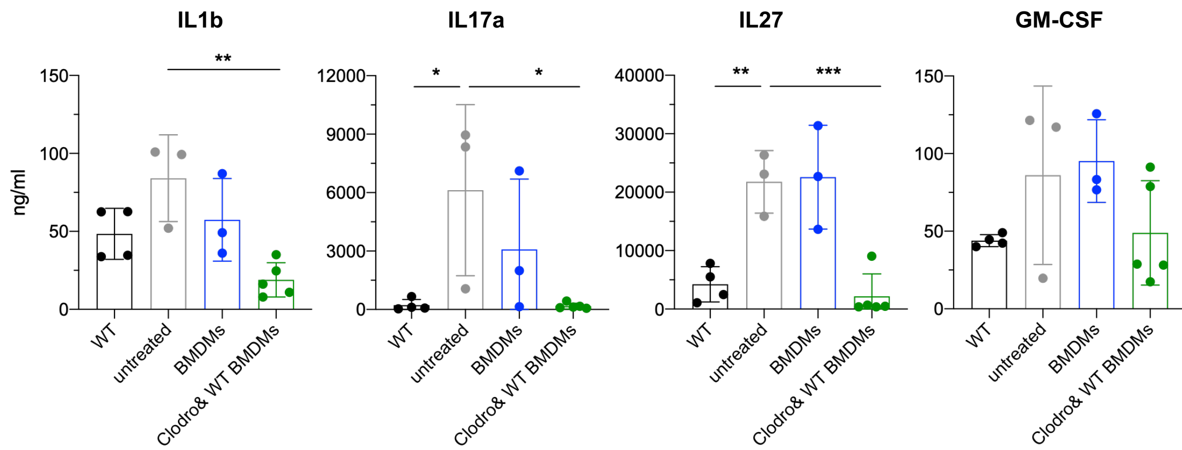
4 macrophages and granulocytes by flow cytometry. Pre-gating on total CD45^{+} hematopoietic

cells was followed by two single-cell gating steps. Myeloid, non-dendritic cell populations were defined as CD11b$^+$/CD103$^-$ cells. Subsequently, granulocytes and monocyte/macrophages were separated by expression of Ly6G and CD64 as CD45$^+$/CD11b$^+$/CD103$^-$/Ly6G$^+$/CD64$^-$ granulocytes and CD45$^+$/CD11b$^+$/CD103$^-$/Ly6G$^-$/CD64$^+$ monocyte/macrophages. Pro- and anti-inflammatory macrophages were identified by analyzing expression of Ly6C and MHCII. **(B)** Relative expression of IL1β (left) and IL17α (right) in total lamina propria (LP) cell extracts analyzed by qRT-PCR (data represent three independent experiments, individual values with mean±SD, n=5-8). **(C)** Representative flow cytometry plots showing the frequency of Ly6C$^+$/MHCII$^+$ inflammatory and Ly6C$^-$/MHCII$^+$ anti-inflammatory LP monocyte/macrophages in IBD animals treated with clodronate liposomes and IBD macrophages as well as untreated animals. **(D)** Quantification of n=3 animals. (individual values with mean±SD, n=3-9).

(Significances calculated by one-way ANOVA with Dunnett's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****<0.0001, ns not significant)



Supplementary Figure 3: Functional consequences of IL10Rb deficiency in peritoneal macrophages. (A) Brightfield microscopy of WT and *Il10rb*$^{-/-}$ IBD macrophages derived from the peritoneal lavage fluid after stimulation with LPS alone or LPS in combination with IL10. **(B)** Legendplex-based cytokine secretion analysis of WT and *Il10rb*$^{-/-}$ IBD peritoneal cavity derived macrophages after 24 hours of stimulation. Values are given as fold change for IL10 and LPS vs. LPS only treated samples (n=3, mean±SD, dashed line represents 1).



Supplementary Figure 4: Cytokine profiling in the Bl6 *Il10rb*$^{-/-}$ model. Secretion of IL1β, IL17α, IL27 and GM-CSF by macrophages derived from the peritoneal lavage fluid of different experimental groups (WT untreated, Bl6 *Il10rb*$^{-/-}$ IBD untreated, IBD+BMDMs and IBD+Clodro/BMDMs) after LPS stimulation (Data represent one experiment, individual values with mean$\pm$SD, n=4-5 per group, cells isolated 6 days post transplantation). Significances are calculated by one-way ANOVA with Dunnett's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****<0.0001, ns not significant.

Supplementary Methods:

Mice: *Il10rb*$^{-/-}$ (Il10rb^{tm1Agt}) (RRID: MGI:3603437) 129SvEv mice, B6.129S2-*Il10rb*^{tm1Agt}/J and their matched controls were housed in a specific pathogen-free animal facility at Boston Children's Hospital. Mice had access to food and water ad libitum. Both male and female mice were used throughout this study. The animals were 3-5 month of age at the beginning of treatment. All experimental groups were randomly allocated to co-housed littermates. All experiments were conducted following approval from the Animal Resources at Children's Hospital following the guidelines of the Institutional Animal Care and Use Committees (IACUC protocols 17-05-3410R and 17-01-3364R).

Isolation of BM& lineage depletion: Iliac crest, femur and tibias were isolated from euthanized animals and bone marrow isolated by flushing the bones with a syringe or crushing the bones with mortar and pestle. Isolated total bone marrow cells were either used directly for differentiation or subjected to lineage depletion using a MACS-based sorting strategy by using a lineage cell depletion kit (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instructions and subsequently differentiated to macrophages.

Generation and preparation of lentiviral vectors

The 3rd generation lentiviral vector PGK-*mIL10rb* (pCCL-hPGK-*coIL10rb*-T2A-mVenus-wPRE) was generated by replacing GFP in the pCCL-PGK-GFP-WPRE with a codon optimized (GeneArt, Invitrogen, Carlsbad, USA) variant of the murine *Il10rb* cDNA-T2A-mVenus fragment via BamH1 – Sal1 restriction cut sites.[1] Vector production was performed by transient transfection of HEK293T cells following standard procedures.[2]

Transduction of lineage negative cells

Lineage negative bone marrow cells were prestimulated at 0.5-1*10^6 cells/ml for 24h in CellGro SCGM media supplemented with mSCF, hTPO and hFLT-3L (each 100ng/ml,

Peprotech, Rocky Hill, NJ, USA). Viral supernatants, titered previously under identical conditions on lineage negative cells, were added at a multiplicity of infection (MOI) of 1-2 overnight.

STAT3 phosphorylation:

Cells were stimulated for 20min with 50ng/ml IL10, then fixed for 10min at 37C (BD Cytofix #554655). After washing the cells were permeabilized using ice cold Methanol buffer (BD PermBuffer III #558050) and stained for 45min with anti P-STAT3 antibody (pY705, BD #612569).

Hematopoietic stem cell transplantation

Il10rb-/- 129SvEv mice were irradiated using a 7Gy+4.5Gy split dose gamma irradiation spaced 24h apart. Transduced lineage negative cells were washed twice in PBS and administered via retroorbital injection. Mice received Baytril™ supplemented drinking water (1.9ml/250ml) for two weeks post irradiation.

Macrophage Differentiation: Total bone marrow, lineage negative or lineage positive cells were differentiated into macrophages by 7-9 days of cultivation in DMEM medium supplemented with 10% FCS, 1 mM penicillin/streptomycin, and 30% macrophage colony-stimulating factor (M-CSF) conditioned supernatant produced by L929 cells as described previously.[3] Cells were cultured on non-tissue culture treated plate and non-adherent cells were removed by media change after 3-4 days.

Treatment with Clodronate Liposomes: Clodronate liposomes were purchased from Liposoma (Amsterdam, The Netherlands) and 100-200ul (approx. 50 mg/kg body weight) were injected intraperitoneally (i.p.) to the animals under isoflurane anesthesia.

1 Transplantation of BMDMs: Bone marrow derived macrophages (BMDMs) were resuspended
2 in PBS and 4-12x10^6 cells in 200ul volume were injected i.p. to the animals under isoflurane
3 anesthesia.
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5 Isolation of lamina propria cells:
6 Lamina propria immune cells were prepared as we reported recently.[4] Briefly, colons were cut
7 in pieces and stripped of epithelial cells by performing agitation in 10 mM EDTA containing
8 buffer for 40 min at 37°C. Subsequently, tissues were further minced with a scalpel and
9 digested using collagenase VIII (Sigma-Aldrich, St. Louis, MI, USA) for 30–45 min at 37°C.
10 Single cell suspensions were filtered and stained for flow cytometry.
11
12 Cytospins: Cytospins were generated utilizing a Shandon cytocentrifuge (Thermo Scientific,
13 Waltham, MA, USA). Slides were stained for 5 min in May-Grünwald stain and 10 min in 5%
14 of Giemsa Azur-Eosin-Methyleneblue solution and washed extensively in aqua dest.
15
16 Histology and scoring: Colon sections were fixed in 4% PFA overnight. Paraffin embedding
17 and H/E staining were performed at the Beth-Israel Diaconess Medical Center Histology Core
18 following standard procedures. Blinded scoring was performed following adapted guidelines
19 from Erben et. al..[5] In brief parameters for colitis were: Inflammatory infiltrates (severity 0-4
20 and extent 0-3), Hyperplasia (0-4), Goblet cell loss (0-4) and Erosion (0-4), (1: minimal; 2: mild;
21 3: moderate; 4: severe.
22
23 Flow cytometry: PBS supplemented with 2 mM EDTA and 5% FCS (FACS buffer) was used
24 during the staining process. Fc blocking reagent was used to prevent unspecific binding. Cells
25 were rinsed with FACS buffer, analyzed on a LSRII machine (BD, Franklin Lakes, NJ, USA)
26 and raw data were analyzed using FlowJo software (TreeStar, Ashland, OR, USA). Used
27 antibodies:
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Antigen	Fluorochrome	Catalog number	Provider
CD45	PE -Cy7	103113	Biolegend
CD103	APC-Cy7	121431	Biolegend
CD11b	BV510	101245	Biolegend
CD11b	PE	101207	Biolegend
Ly6G	AlexaFluor700	127622	Biolegend
CD64	PerCp-Cy5.5	139308	Biolegend
Ly6C	eFluor 450	48-5932-82	Invitrogen
MHCII	PE	107607	Biolegend
B220	Pacific Blue	558108	BD Pharmingen
B220	AlexaFluor 488	103228	Biolegend
CD3	APC	17-0032-80	Invitrogen
TCRb	eFluor 450	109207	Biolegend
Gr1	PE	108407	Biolegend
Gr1	APC-Cy7	552985	BD Biosciences
CD45	FITC	103107	Biolegend
CD45.1	PE-Cy7	4334853	Invitrogen

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3 RNA Isolation and qRT-PCR: Total RNA was extracted from whole colonic tissue using
4 TRIzol® Reagent (Ambion® by life technologies, Carlsbad, CA, USA) according to
5 manufacturer's instructions. Complementary DNA (cDNA) was generated from 1-2 μg RNA
6 using iScriptTM Select cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA).
7 Transcipt analysis was performed using SsoAdvanced™ Universal SYBR® Green Supermixes
8 on a CFX96 Real-Time System (Bio-Rad). The following primers were used: *Il17a*-2F 5' GGC
9 CCT CAG ACT ACC TCA AC 3', *Il17a*-2R 5' TCT CGA CCC TGA AAG TGA AGG 3';

Il1b-F 5’ GCA ACT GTT CCT GAA CTC AAC 3’, *Il1b*-R 5’ ATC TTT TGG GGT CCG TCA ACT 3’; *Hprt*-F 5’ GTT GGA TAC AGG CCA GAC TTT GTT G 3’, *Hprt*-R 5’ GAG GGT AGG CTG GCC TAT AGG CT 3’. Gene expression was normalized against hypoxanthine-guanine phosphoribosyl transferase (*Hprt*). Relative expression was quantified using the $2^{-\Delta Ct}$ method.

PCR: The *Il10rb* and *Gtcd1* genes were amplified from gDNA using Promega GoTaq Polymerase (Cat# M3005) using the following conditions: 95C 2:00min, (95C 30s, 60C 30s, 70C 60s) x35, 72C 5min. Primer sequences: *Il10rb*-F 5’ GGAGACAAGACTTTGGAGGGG 3’, *Il10rb*-R 5’ CACCTGGCACCAGAAGGAAG 3’. *Gtcd1*-F 5’ GAAGTTCAGGTTAATTAGCTGCTG 3’, *Gtcd1*-R 5’ GGCACCTTAACATTTGGTTCTG 3’.

Legendplex: For analysis of cytokine secretion macrophages were starved in X-VIVO 15 medium (Lonza, Basel, Switzerland) over night and stimulated with 50ng/ml LPS (sigma) and 100ng/ml IL10 (Peprotec, Hamburg, Germany) for 24 hours. Supernatants were analyzed using the Legendplex Mouse Inflammation Panel (13-Plex, Biolegend, San Diego, CA, USA) following the manufacturer’s instructions.

Statistics:

GraphPad Prism 7 or 8 Software (San Diego, CA, USAI were applied to perform analysis of variance (ANOVA). Asterisks indicated: * P< .05; ** P< .01; ***P< .001

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