Supplementary Materials

Target	Forward primer (5'->3')	Reverse primer (5'->3')
EcN	AACTGTGAAGCGATGAACCC	GGACTGTTCAGAGAGCTATC
EcO	CTAATGCGGGCAGAGAAATAAAGT	ATAAAGACGGCAGGGTAACACAC

Table S1. List of used primers.

References**:** (Blum-Oehler et al., 2003; Hejnova et al., 2005)**.**

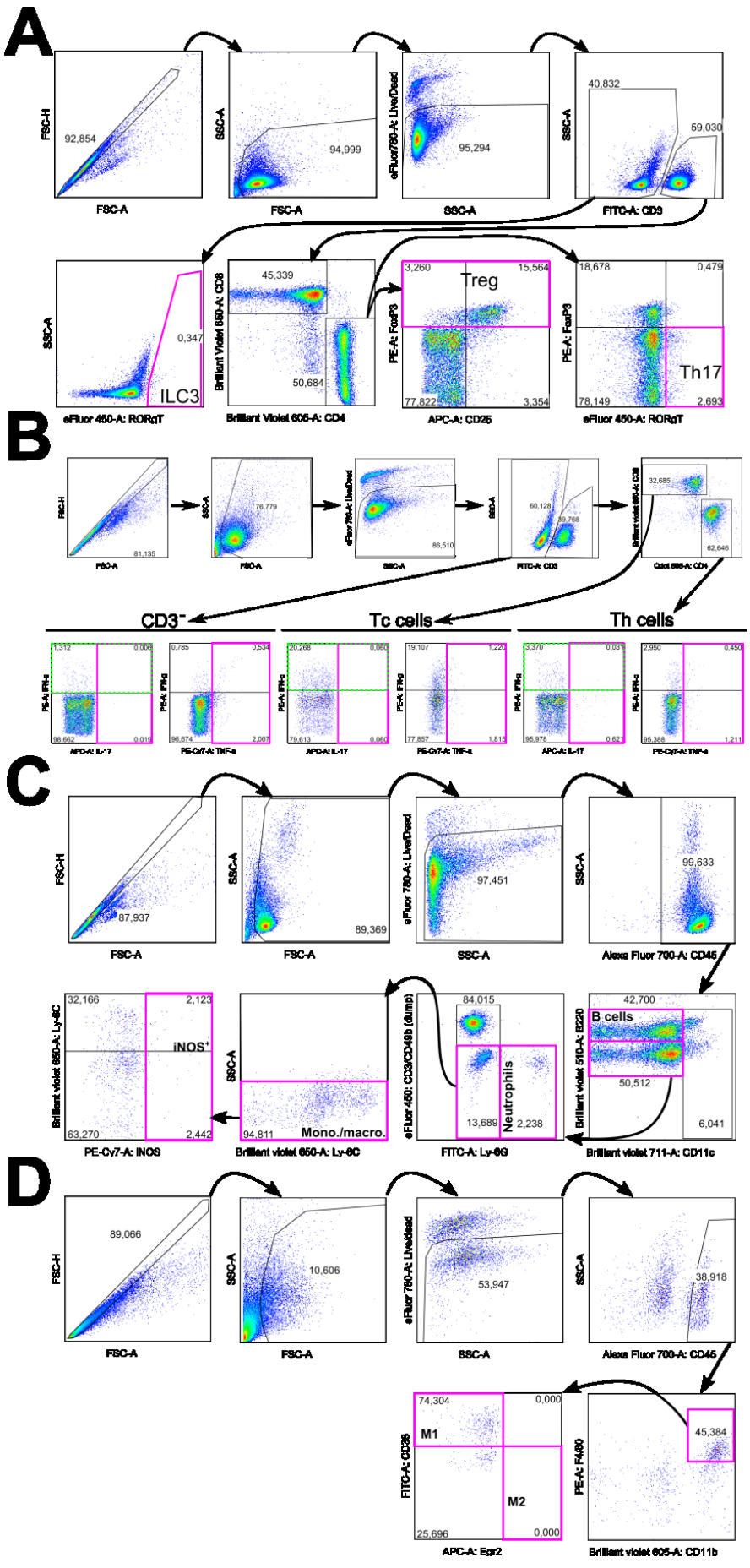
Epitope — Fluorochrome	Clone	Manufacturer	Cat#
CD3ε (purified)	145-2C11	BioLegend	100359
CD28 (purified)	37.51	BioLegend	102121
CD16/32 (purified)	93	BioLegend	101302
Fixable Viability Dye eFluor 780[1, 2, 3, 4,6]	-	Thermo Fisher Scientific	65-0865-14
Fixable Viability Dye eFluor 450[5]	-	Thermo Fisher Scientific	65-0863
CD3 — eFluor 450[3]	17A2	Thermo Fisher Scientific	48-0032-82
CD3ε — FITC[1, 2]	145-2C11	BioLegend	100306
CD4 — Brilliant violet 605[1, 2]	GK1.5	BioLegend	100451
CD8 — Brilliant violet 650[1, 2]	53-6.7	BioLegend	100741
CD11b — Brilliant violet 605[4,6]	M1/70	BioLegend	1106285
CD11c — Brilliant violet 711[3,6]	N418	BioLegend	117349
CD14 - APC[5]	Sa2-8	Thermo Fisher Scientific	17-0141-81
CD25 — APC[1]	PC61.5	Thermo Fisher Scientific	17-0251-82
CD38 — FITC[4,5]	90	BioLegend	102705
CD40 — PE[3,5,6]	1C10	Thermo Fisher Scientific	12-0401-82
CD45 — Alexa Fluor 700[3, 4]	30-F11	BioLegend	103128
CD80 - PerCP/Cyanine5.5[6]	16-10A1	BioLegend	104722
CD80 - Brilliant violet 711[5]	16-10A1	BD Biosciences	740698
CD86 - Brilliant violet 605[5]	GL1	BioLegend	105037
CD86 - FITC[6]	GL1	Thermo Fisher Scientific	11-0862-81
B220 (CD45R) — Brilliant violet 510[3]	RA3-6B2	BioLegend	103247
CD49b — eFluor 450[3]	DX5	Thermo Fisher Scientific	48-5971-82
I-A/I-E - Alexa Fluor 700[5,6]	M5/114.15.2	Thermo Fisher Scientific	56-5321-82
CD103 - eFluor 450[6]	2E7	Thermo Fisher Scientific	48-1031-82
Egr2 — APC[4]	erongr2	Thermo Fisher Scientific	17-6691-82
F4/80 — PE[4]	BM8	BioLegend	123110
F4/80 - APC[6]	BM8	Thermo Fisher Scientific	17-4801-82
Foxp3 — PE[1]	FJK-16s	Thermo Fisher Scientific	12-5773-82
IFN-γ — PE[1, 2]	XMG1.2	Thermo Fisher Scientific	12-7311-81
IL-17A — APC[1, 2]	eBio17B7	Thermo Fisher Scientific	17-7177-81

iNOS — PE-Cyanine7[3]	CXNFT	Thermo Fisher Scientific	25-5920-80
Ly-6C — Brilliant violet 605[3]	HK1.4	BioLegend	128036
Ly-6G — FITC[3]	1A8	BioLegend	127605
Rorγt — Brilliant violet 421[1]	Q31-378	BD Biosciences	562894
TNF-α— PE-Cyanine7[1, 2]	TN3-19.12	Thermo Fisher Scientific	25-7423-82

Table S2. List of used antibodies. Used to analyze: [1] regulatory T cells, Th17 and ILC3, [2] cytokine production by T cells, [3] monocytes and macrophages, [4] M1/M2 monocytes and macrophages, [5] bone marrow derived macrophages or [6] bone marrow derived dendritic cells.

Task
Assistance with experiment termination (tissue processing, cell counting etc.)
Derivation of leukocytes from colon samples
FACS preparation
Colonization assessment (sample collection, DNA isolation, RT-PCR, analysis)
Stimulation of BMDMs (stimuli preparation, cell derivation, cultivation, ELISA)

Table S3. Contributions to the research: (Dusek et al., 2020)**.**


A
FSC-H
FSC-A
92,854
SSC-A
FSC-A
94,999
eFluor780-A: Live/Dead
SSC-A
95,294
SSC-A
FITC-A: CD3
40,832
59,030
SSC-A
eFluor 450-A: RORgT
0,347
ILC3
Brilliant Violet 650-A: CD3
Brilliant Violet 605-A: CD4
45,339
50,684
PE-A: FoxP3
APC-A: CD25
3,260
15,564
Treg
77,822
3,354
PE-A: FoxP3
eFluor 450-A: RORgT
18,678
0,479
Th17
78,149
2,693
B
FSC-H
FSC-A
81,135
SSC-A
FSC-A
76,779
eFluor 780-A: Live/Dead
SSC-A
86,510
SSC-A
FITC-A: CD3
60,128
59,768
Brilliant violet 650-A: CD3
Qdot 605-A: CD4
32,685
62,646
CD3-
Tc cells
Th cells
PE-A: IFN-g
APC-A: IL-17
PE-Cy7-A: TNF-a
1,312
0,006
98,662
0,019
0,785
0,534
96,674
2,007
20,268
0,060
79,613
0,060
19,107
1,220
77,857
1,815
3,370
0,031
95,978
0,621
2,950
0,450
95,388
1,211
C
FSC-H
FSC-A
87,937
SSC-A
FSC-A
89,369
eFluor 780-A: Live/Dead
SSC-A
97,451
SSC-A
Alexa Fluor 700-A: CD45
99,633
Brilliant violet 650-A: Ly-6C
PE-Cy7-A: iNOS
32,166
2,123
iNOS+
63,270
2,442
SSC-A
Brilliant violet 650-A: Ly-6C
94,811
Mono./macro.
eFluor 450: CD3/CD49b (dump)
FITC-A: Ly-6G
84,015
13,689
Neutrophils
2,238
Brilliant violet 510-A: B220
Brilliant violet 711-A: CD11c
42,700
B cells
50,512
6,041
D
FSC-H
FSC-A
89,066
SSC-A
FSC-A
10,606
eFluor 780-A: Live/dead
SSC-A
53,947
SSC-A
Alexa Fluor 700-A: CD45
38,918
FITC-A: CD206
APC-A: Egr2
74,304
0,000
M1
M2
25,696
0,000
PE-A: F4/80
Brilliant violet 605-A: CD11b
45,384

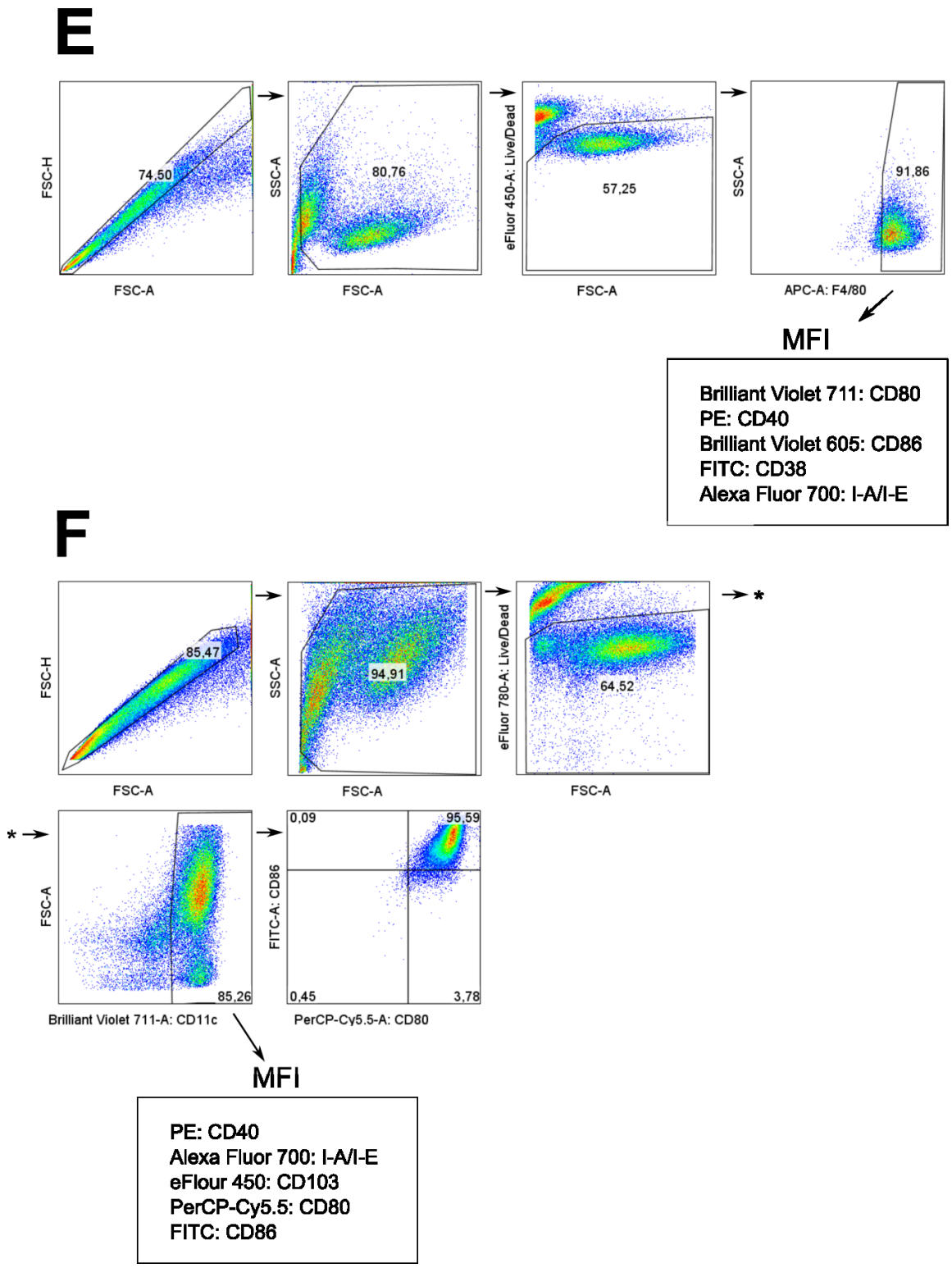


Figure S1. Gating strategies for regulatory T cells, Th17 and ILC3 **(A)**, cytokine production by T cells **(B)** and monocytes, macrophages and neutrophils **(C)** using mesenteric lymph nodes (mLN) of placebo-treated C57BL/6J mouse suffering from EAU. Gating strategy for M1/M2 macrophages using cells isolated from ileum of healthy placebo-treated C57BL/6J mouse **(D)**. Gating strategies for BMDMs **(E)** and BMDDCs **(F)**.

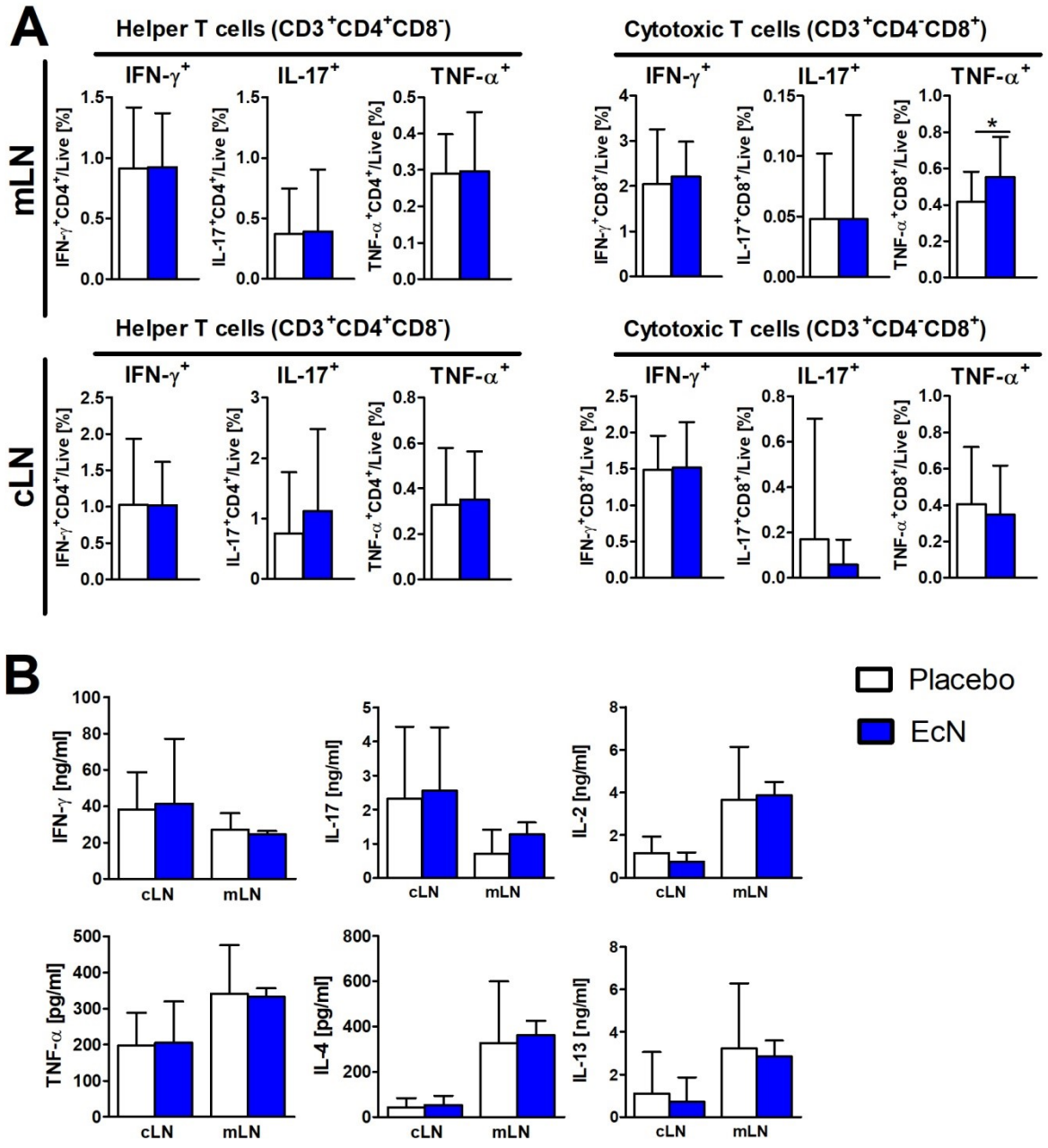


Figure S2. Responsiveness of T cells to anti-CD3/anti-CD28 stimulus remains unchanged by EcN treatment. Response was evaluated by intracellular expression of proinflammatory cytokines of T cells derived from cLNs and mLNs assessed by FACS (**A**) and production of cytokines by activated T cells derived from cLN and mLN assesed by ELISA (**B**). Data are pools from 5 independent experiments (n = 24 per group in total). Differences were quantified by unpaired Mann–Whitney test; *p<0.05. Data are from day 28 post-induction.

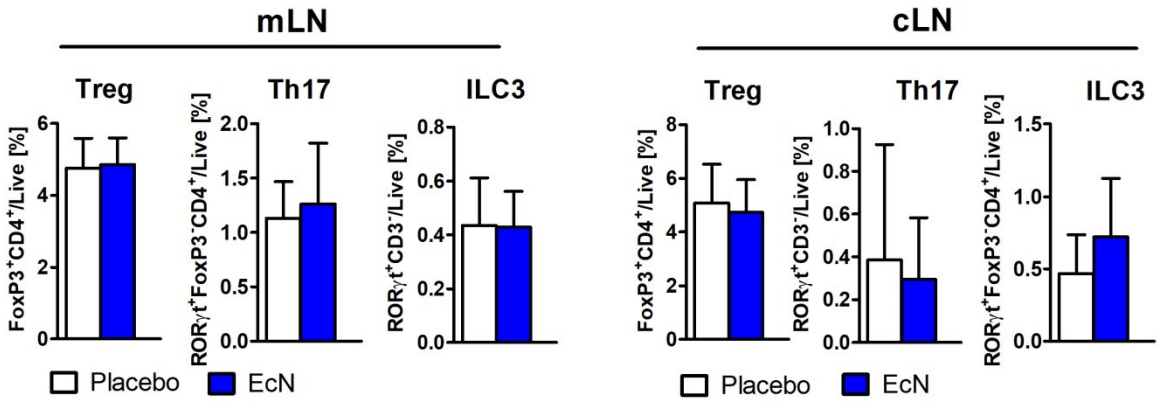


Figure S3. Treatment of EAU with EcN does not affect proportions of Treg, Th17 and ILC3 populations in mLNs or cLNs. Proportions of cells in mesenteric and cervical lymph nodes were evaluated by FACS at day 28 post-induction (n = 22 (placebo), 23 (EcN) in total).

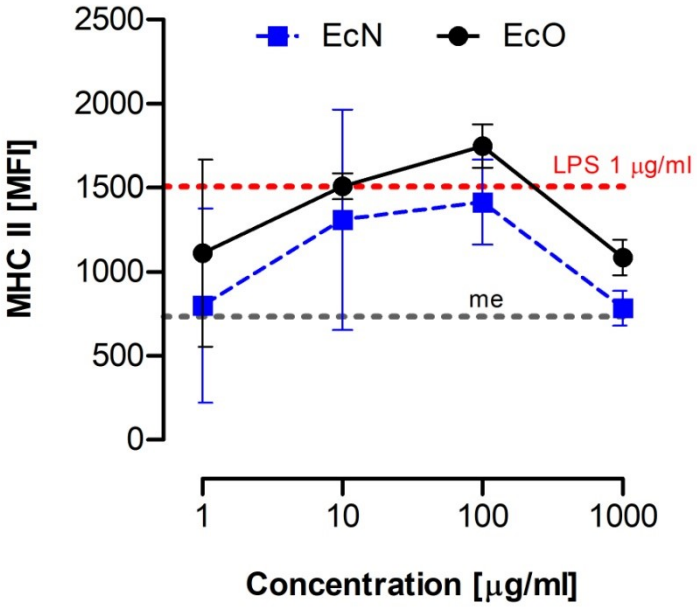


Figure S4. There are no significant differences in the expression of MHC II by BMDM in reaction to the two lysates. Data were obtained by FACS, gated to singlet, live, F4/80 positive cells and adjusted to mean fluorescence intensity (MFI) of each sample. X axis shows concentration of the stimulus—the bacterial lysate. Red dotted line represents positive control — 1 μg/ml of LPS, black dotted line represents negative control—culture medium only. Figure shows representative graph from 1 out of 3 independent experiments. Differences were quantified by One-way ANOVA with Tukey´s multiple comparison test; * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

Supplementary references

Blum-Oehler, G., Oswald, S., Eiteljörge, K., Sonnenborn, U., Schulze, J., Kruis, W., & Hacker, J. (2003). Development of strain-specific PCR reactions for the detection of the probiotic Escherichia coli strain Nissle 1917 in fecal samples. *Research in Microbiology*, *154*(1), 59–66. https://doi.org/10.1016/s0923-2508(02)00007-4

Dusek, O., Fajstova, A., Klimova, A., Svozilkova, P., Hrncir, T., Kverka, M., … Heissigerova, J. (2020). Severity of Experimental Autoimmune Uveitis Is Reduced by Pretreatment with Live Probiotic Escherichia coli Nissle 1917. *Cells*, *10*(1). https://doi.org/10.3390/cells10010023

Hejnova, J., Dobrindt, U., Nemcova, R., Rusniok, C., Bomba, A., Frangeul, L., … Buchrieser, C. (2005). Characterization of the flexible genome complement of the commensal Escherichia coli strain A0 34/86 (O83 : K24 : H31). *Microbiology (Reading, England)*, *151*(Pt 2), 385–398. https://doi.org/10.1099/mic.0.27469-0