

Supplementary Materials:

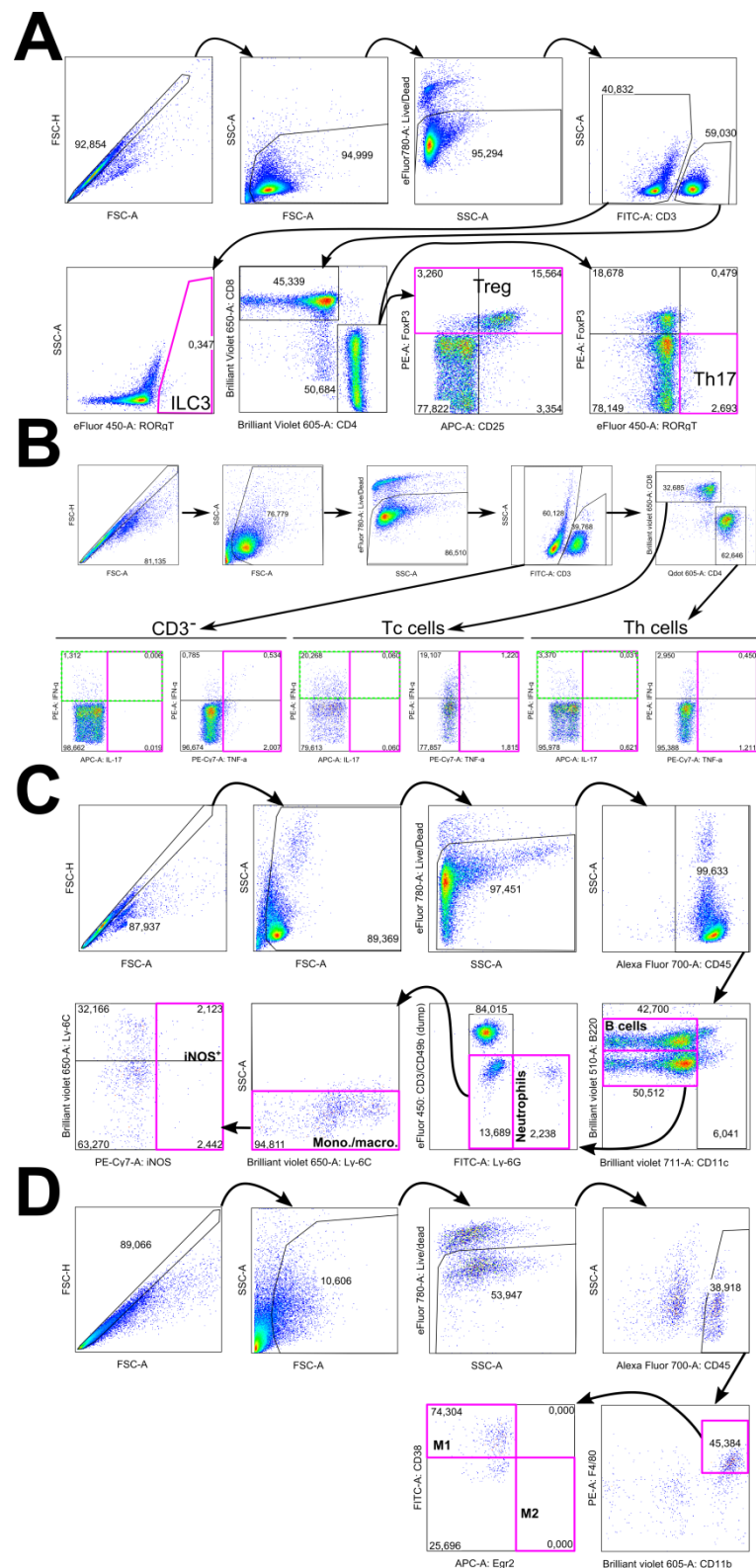


Figure 1. Gating strategy for regulatory T cells, Th17 and ILC3 (A), cytokine production by T cells (B) and monocytes, macrophages and neutrophils (C) using mesenteric lymph nodes (mLN) of placebo-treated C57BL/6J mouse suffering from EAU. Gating strategy for M1/M2 macrophages using cells isolated from ileum of healthy placebo-treated C57BL/6J mouse (D).

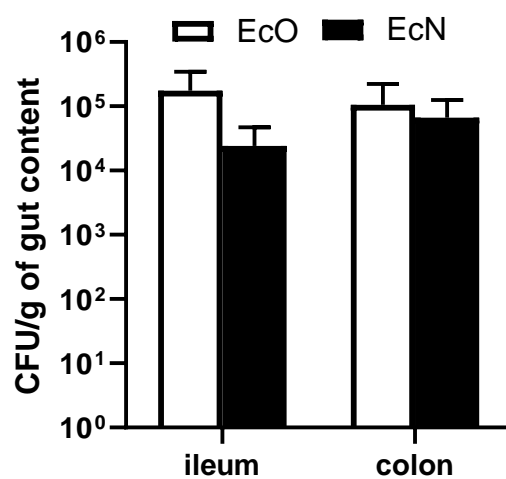


Figure 2. Probiotic EcO and EcN colonize mice used in our experiments for at least 48 h after a single dose. The results were calculated by comparing threshold cycle with a standard curve constructed from known number of CFU for each bacterium and then related to the sample weight.

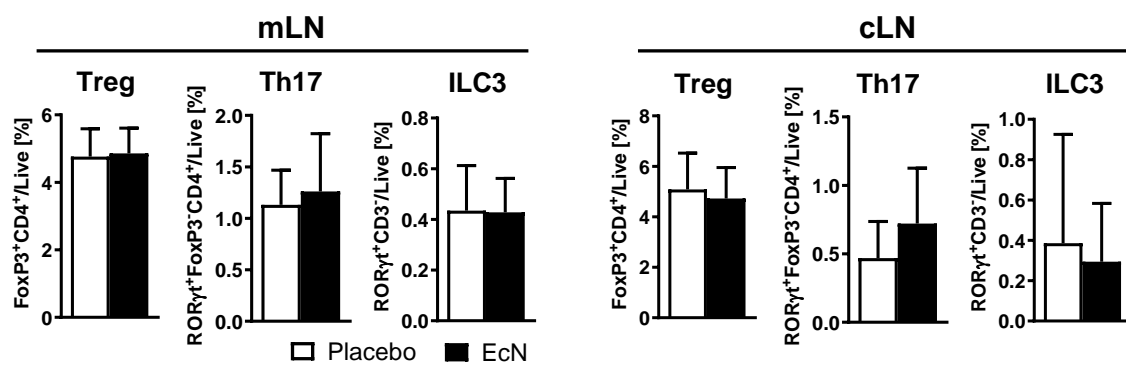


Figure 3. Live probiotic EcN does not change proportion of regulatory T cells, Th17 cells or innate lymphoid cells (ILC) 3 in mLN or cLN at day 28 post-induction. (n = 22 (placebo), 23 (EcN) in total).

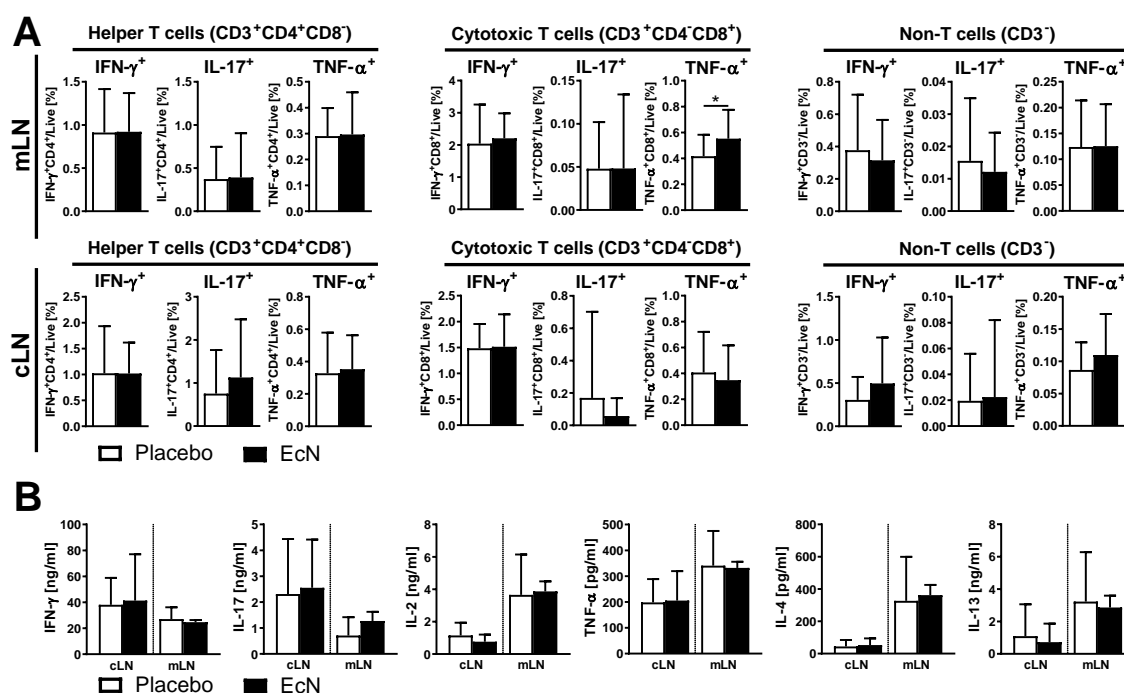


Figure 4. Oral EcN has no effect on expression of pro-inflammatory intracellular cytokine of anti-CD3/CD28 activated helper or cytotoxic T cells in mLN or cLN, as measured by FACS (A). In addition, there is no accumulation of T cell cytokines in supernatants of activated T cells isolated from mLN and cLN, as measured by ELISA (B). Differences were quantified by unpaired Mann-Whitney test; * $p < 0.05$. Data are pools from 5 independent experiments ($n = 24$ per group in total). Data are from day 28 post-induction.

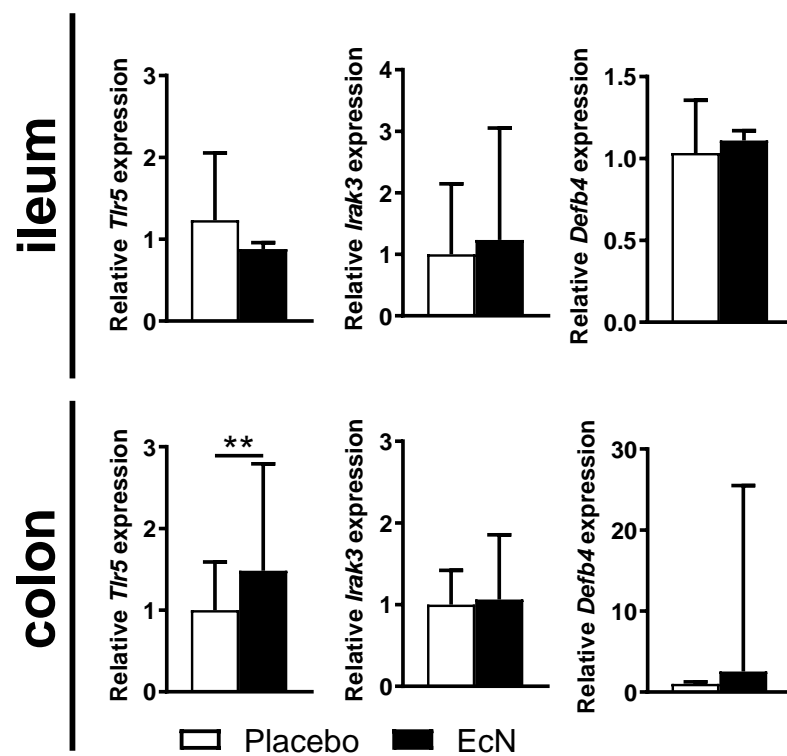


Figure 5. Live EcN increases the expression of *tlr5* in the colon at day 28 post-induction. Data are pool from 5 independent experiments ($n = 3-8/\text{group}$) and differences were quantified by unpaired Mann-Whitney test; * $p < 0.05$ ** $p < 0.01$.

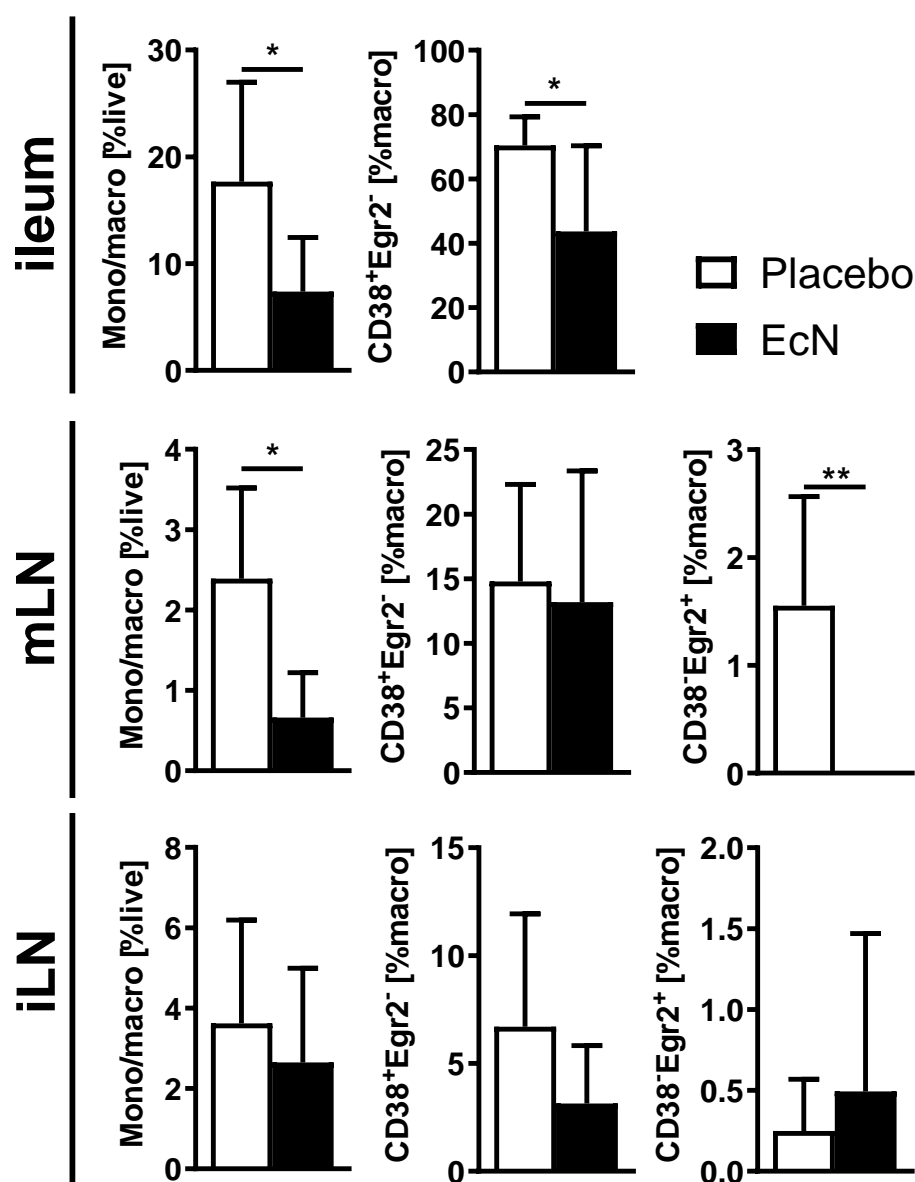


Figure 6. Live EcN decreases proportion of macrophages and shift their M1 at day 7 post-induction. This effect is clearly apparent in the ileum (A). The trend is still visible in mesenteric lymph nodes (B) and in inguinal lymph nodes (C). Differences were quantified by unpaired Mann-Whitney test ($n = 9$ (placebo) or 7 (EcN)); $*p < 0.05$, $**p < 0.01$. The macrophages were defined as live CD45⁺F4/80⁺CD11b⁺ cells and M1 (CD38⁺Egr2⁻) and M2 (CD38⁻Egr2⁺) markers were selected according to previously published data [1] Ileum did not contained clear M2 population.

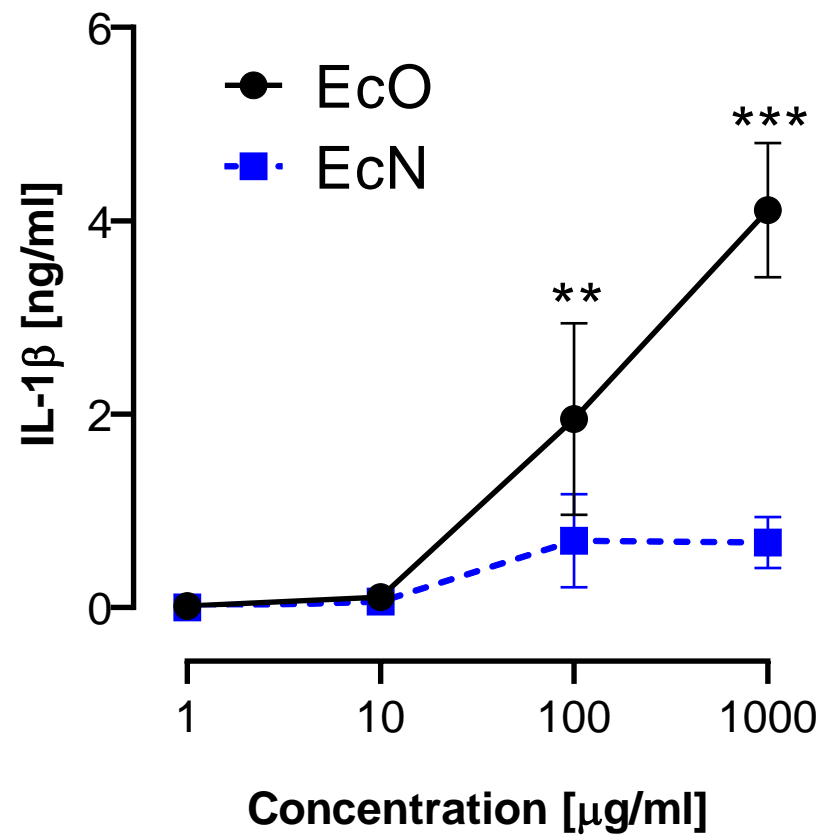


Figure 7. EcO lysate induces higher IL-1 β production in BMDM compared to EcN lysate. The differences between EcO (n = 4) and EcN (n = 4) were analyzed by 2-way ANOVA with Bonferroni's multiple comparisons test (n = 4); **p<0.01, ***p<0.001

Table 1. List of antibodies.

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}	-	Thermo Fisher Scientific	65-0865-14
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	M1/70	BioLegend	1106285
CD11c – Brilliant violet 711 ³	N418	BioLegend	117349
CD25 – APC ¹	PC61.5	Thermo Fisher Scientific	17-0251-82
CD38 – FITC ⁴	90	BioLegend	102705
CD40 – PE ³	1C10	Thermo Fisher Scientific	12-0401-82
CD45 – Alexa Fluor 700 ^{3, 4}	30-F11	BioLegend	103128
B220 (CD45R) – Brilliant violet 510 ³	RA3-6B2	BioLegend	103247
CD49b – eFluor 450 ³	DX5	Thermo Fisher Scientific	48-5971-82
Egr2 – APC ⁴	erongr2	Thermo Fisher Scientific	17-6691-82
F4/80 – PE ⁴	BM8	BioLegend	123110
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
Roryt – Brilliant violet 421 ¹	Q31-378	BD Biosciences	562894
TNF-α – PE-Cyanine7 ^{1, 2}	TN3-19.12	Thermo Fisher Scientific	25-7423-82

Used to analyze: ¹regulatory T cells, Th17 and ILC3, ²cytokine production by T cells, ³monocytes and macrophages or ⁴M1/M2 monocytes and macrophages.

Table 2. Primers used in qPCR and RT-PCR.

Target	Forward primer (5'→3')	Reverse primer (5'→3')	Reference
EcN	AACTGTGAAGCGATGAACCC	GGACTGTTTCAGAGAGCTATC	[2]
EcO	CTAATGCGGGCAGAGAAATAAAGT	ATAAAGACGGCAGGGTAACACAC	[3]
Defb4	ATCTCACCAGGCTTCAGT	TCTTCATGGAGGAAATTC	this study
Tlr5	CAGGATGTTGGCTGGTTTCT	CGGATAAAGCGTGGATT	[4]
Irak3	TTGGTCCTGGGCACAGAAA	AATAGCTCGACGATGTCCCAT	[5]
pan-Defensin α	GGTGATCATCAGACCCCAGCATCAGT	AAGAGACTAAAAGTGAAGGAGCAGC	[6]
Defa5	TCCTGCTCAACAATTCTCCA	GACACAGCCTGGTCCTCTTC	[7]
S100a8	AAATCACCATGCCCTCTACAAG	CCCACCTTTTATCCCATCGCAA	[8]
S100a9	GGCCAACAAAGCACCTTCTCA	CTTGCTCAGGGTGTCAAGG	this study
Reg3β	CTGCCTTAGACCGTGCTTTC	CCCTTGTCATGATGCTCTT	[9]
Reg3γ	TTCCTGTCCTCCATGATCAAAA	CATCCACCTCTGTTGGGTTC	[10]
Muc13	GAGGAGAAACAGGAGCATAG	GGACATCAAGGGAGAAG	this study
Tjp1 (ZO-1)	CCACCTCTGTCCAGCTCTTC	CACCGGAGTGATGGTTTTCT	[11]
Eef2	CTGACACTCGCAAGGATGAG	CCCGGTCCATCTTGTTTCATC	[12]

Supplementary References:

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