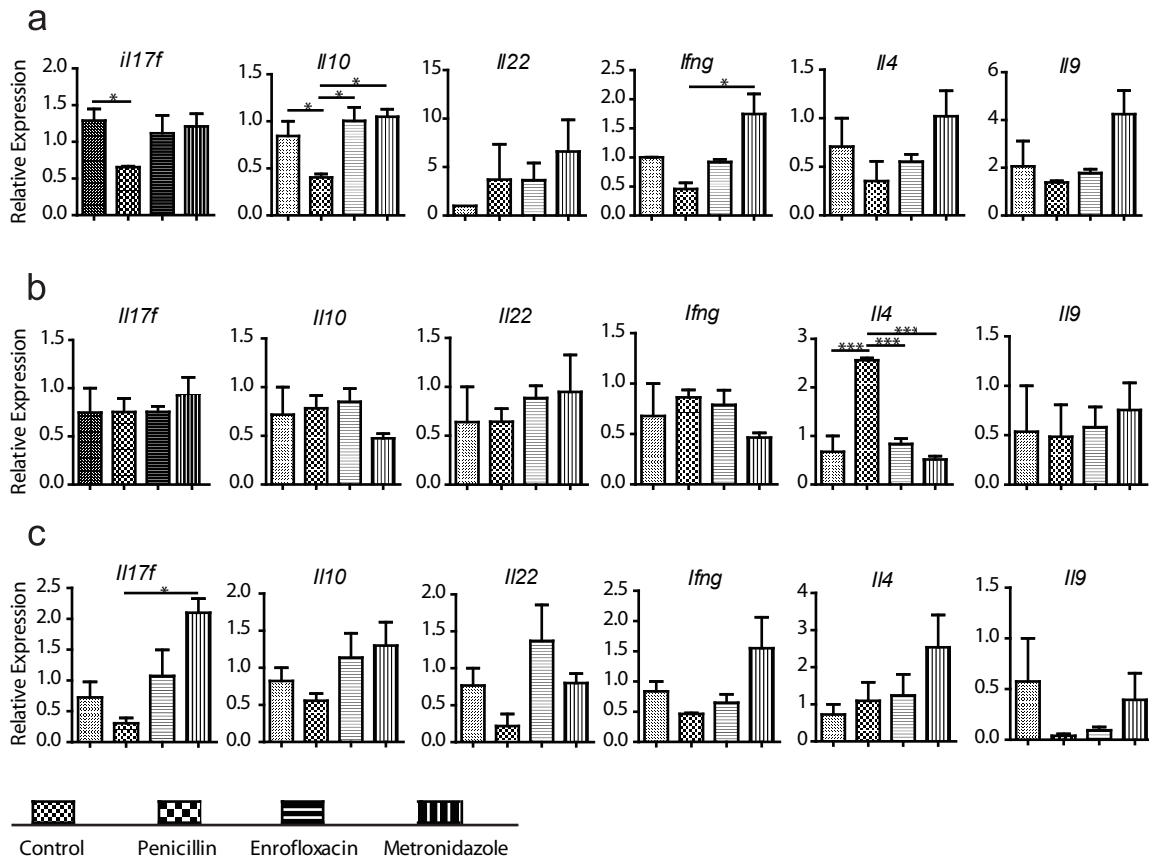


Supplementary Material

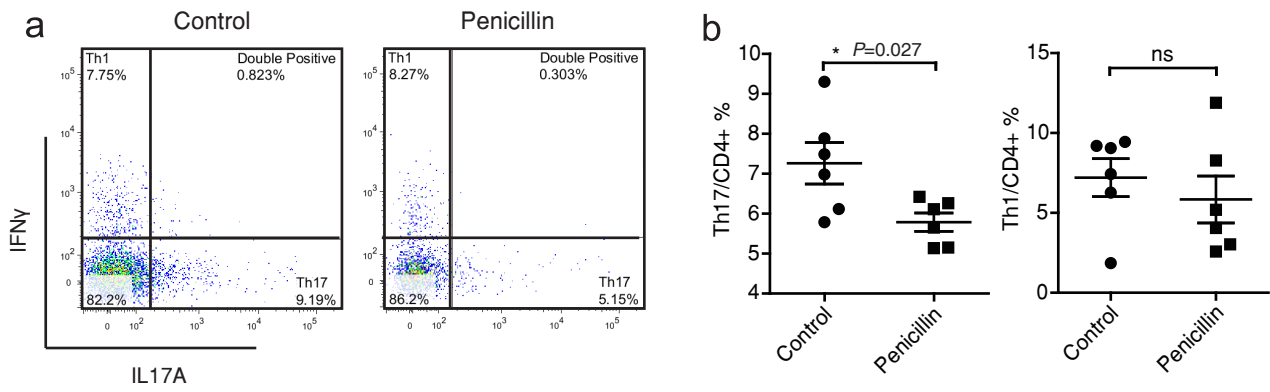
Low-dose penicillin exposure in early life decreases Th17 and the susceptibility to DSS colitis in mice through gut microbiota modification

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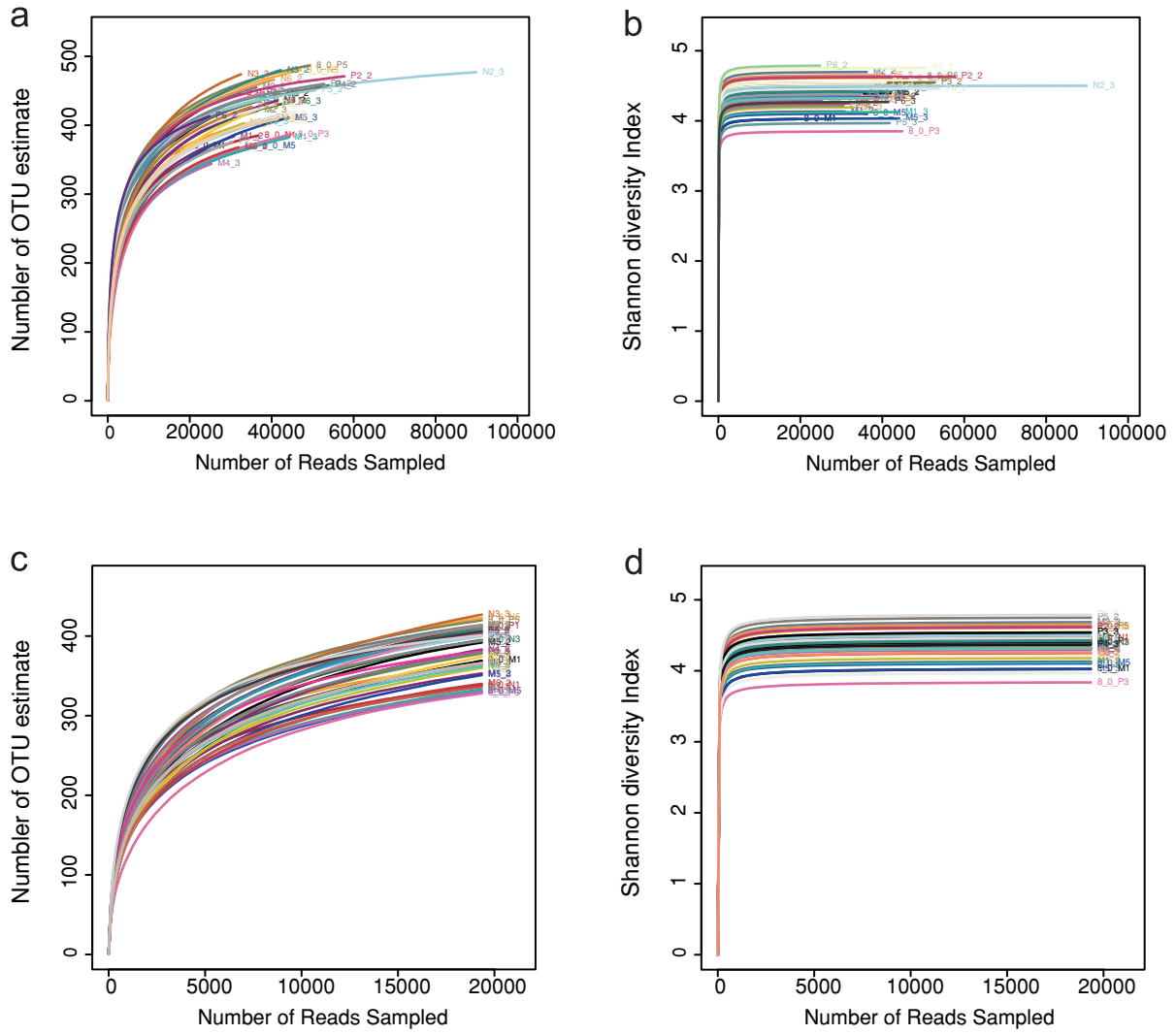
Division of Gastroenterology and Hepatology; Key Laboratory of Gastroenterology and Hepatology, Ministry of Health; Renji Hospital, School of Medicine, Shanghai Jiao-Tong University; Shanghai Institute of Digestive Disease; Shanghai Inflammatory Bowel Disease Research Center; 160 Pujian Road, Shanghai 200127, China



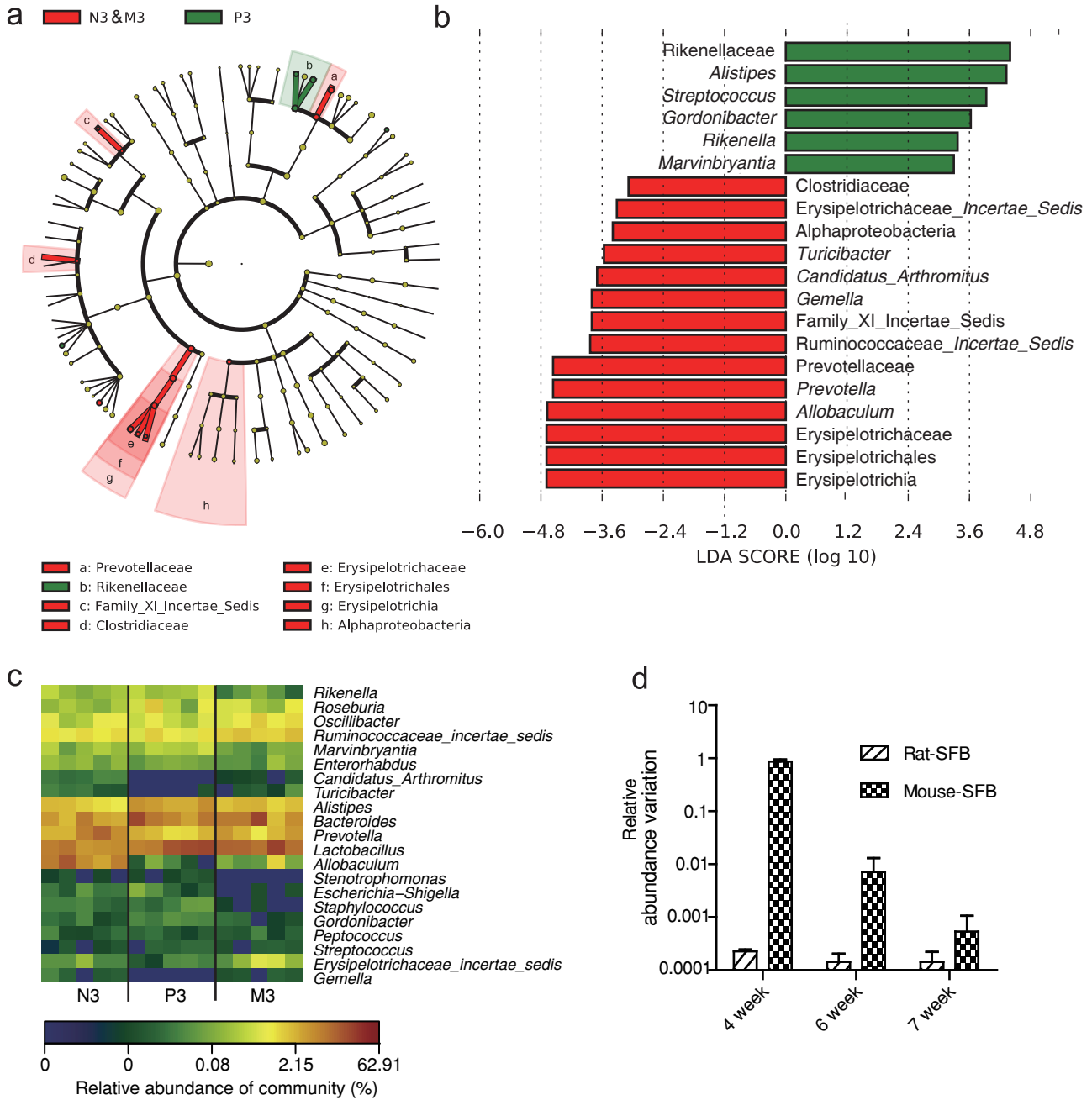
Supplementary figure S1: Low-dose antibiotics' impact on cytokine expression quantified by qPCR (n=3/group). Relative expression of indicated cytokine genes after 2-week treatment was measured in terminal ileum tissue (a), mesenteric lymph nodes (b) and Peyer's patches (c). Data are presented as mean \pm SEM. *P<0.05, ** P<0.01, ***P<0.001, one-way ANOVA.



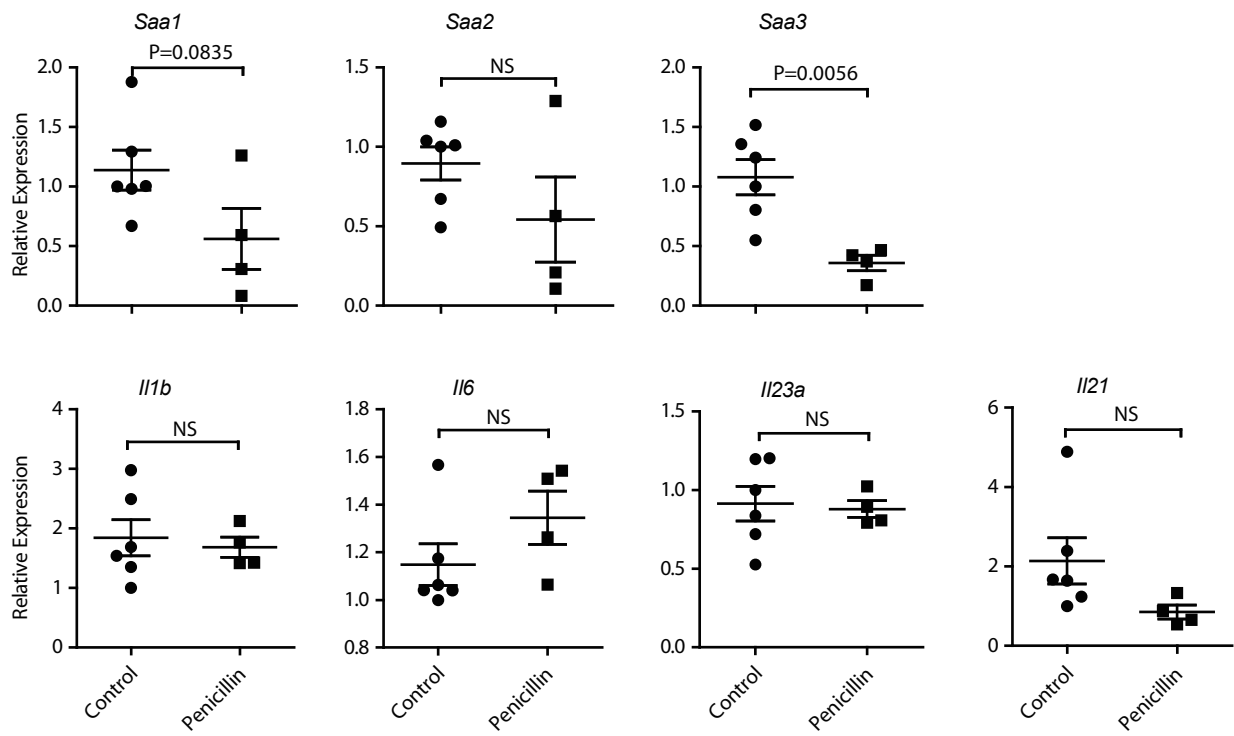
Supplementary figure S2: Two weeks' treatment of low-dose penicillin suppressed Th17 differentiation in SI-LP. (a) Intracellular staining of Small intestinal lamina propria (SI-LP) mononuclear cells for IL-17A and IFN γ after stimulation by PMA and ionomycin. Plots were gated on CD4⁺ cells. (b) Statistics of a. *: $P < 0.05$, Student's *t*-test.



Supplementary figure S3: Rarefaction of samples. Rarefaction curves (a, c) and Shannon-Wiener curves (b, d) before rarefying of data (a, b) and after rarefying the sequence depth to 19345 reads per sample (c, d).



Supplementary figure S4: Key bacterial alterations after antibiotic cessation. (a) Cladogram generated by LefSe analysis showing enriched taxa in control/metronidazol super-group (red) and penicillin group (green) 3-week feces. (b) LDA scores of enriched taxa from a. (c) Heatmap showing relative abundance of key genera generated by LefSe analysis. (d) Rat-SFB and Mouse-SFB relative abundance quantified by qPCR in indicated age. Data in d are expressed as relative fold difference to one of the samples.



Supplementary figure S5: Relative expression of Th17-differentiation-related proteins in terminal ileum tissue. Data are represented as mean ± SEM. NS: not significant, Student's *t*-test.

Primers used in the experiment:

Gene	Forward Primer	Reverse Primer
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
<i>Saa3</i>	TGCCATCATTC TTTGCATCTTGA	CCGTGAACTTCTGAACAGCCT
<i>Saa2</i>	TGGCTGGAAAGATGGAGACAA	AAAGCTCTCTCTTGCATCACTG
<i>Saa1</i>	GACAGAGGATGAAGCTACTCACC	CCTTGGAAGCCTCGTGAAC
<i>Il1b</i>	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
<i>Il6</i>	CCTACCCCAATTTCCAATGCTCTCC	GCATAACGCACTAGGTTTGCCGA
<i>Il23a</i>	CAGCAGCTCTCTCGGAATCTC	TGGATACGGGGCACATTATTTTT
<i>Il21</i>	GGACCCTTGCTGTCTGGTAG	TGTGGAGCTGATAGAAGTTCAGG
<i>Il9</i>	TTGTGTCTCTCCGTCCCAAC	TGGTCAGCTGTAACAGTCCC
<i>Il22</i>	CAGCTCAGCTCCTGTCACATC	AGTTCCCAATCGCCTTGAT
<i>Il4</i>	TCGGCATTTTGAACGAGGTC	TGGTGTCTTCGTTGCTGTGA
<i>Ifng</i>	AGACAATCAGGCCATCAGCAA	TGTGGGTGTTGACCTCAAAC
<i>Il17a</i>	ACCTCACACGAGGCACAAGT	GCAACAGCATCAGAGACACAGA
<i>Il17f</i>	AATCCTGGTCCTTCGGAGGG	GCCAACTTTTAGGAGCATCTTCT
<i>Il10</i>	CAGGACTTTAAGGGTTACTTGGGT	GCCTGGGGCATCACTTCTAC

Supplementary Table S1: Primers used in qPCR, related to Fig. 2, Supplementary Fig. S1 and Supplementary Fig. S5.