

Supplementary Figures

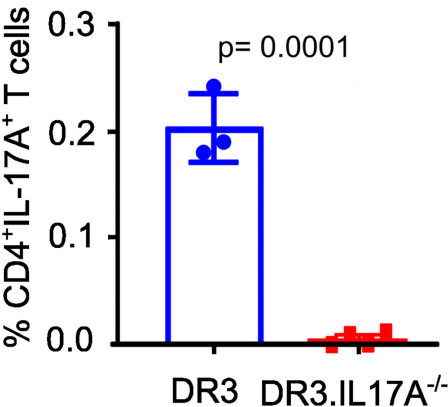


Fig. S1: DR3.IL-17A^{-/-} mice lack IL-17A-expressing CD4⁺ T cells in the spleen. 8-10-week-old DR3 and DR3.IL-17A^{-/-} transgenic mice were euthanized and splenocytes were harvested for analysis. Quantification of the frequency of CD4⁺IL17A⁺ T cells from mice in A. DR3: n=3; DR3.IL-17A^{-/-}, n = 4. *p*-value was determined using Mann-Whitney test between the experimental groups. Symbols represent individual mice, bars represent mean ± SEM.

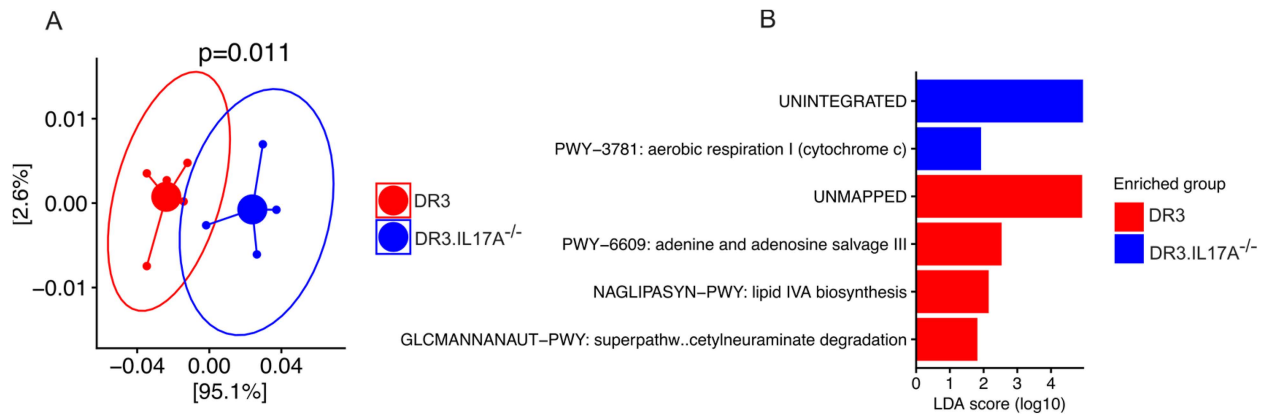


Fig. S2: DR3 and DR3.IL-17A^{-/-} transgenic mice harbor distinct functions in their gut microbiome. Fecal samples were collected from 10-week-old DR3 (n=5) and DR3.IL-17A^{-/-} (n=5) female mice. Gut microbiota composition was analyzed using shotgun metagenomic sequencing of fecal DNA. **(B)** Beta-diversity PCoA plot depicting clustering of functions between DR3 and DR3.IL-17A^{-/-} gut microbiome using Bray-Curtis dissimilarity metric. *adonis2* test was performed for A. **(B)** Differential abundance of bacteria at species level using *lefse* from *microbiomeMarker* package at *kw_cutoff*=0.01, *wilcoxon_cutoff*=0.01 and *lda_cutoff*=1 in R.

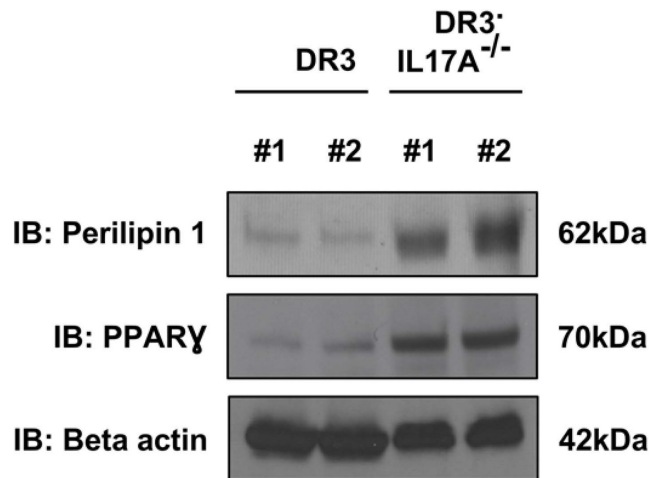


Fig S3: Plin1 and PPAR γ protein expression are increased in the colon of DR3.IL-17A^{-/-} mice compared with DR3 controls. Colon tissues from DR3 and DR3.IL-17A^{-/-} mice were lysed in RIPA buffer containing protease inhibitors, and equal amounts of protein (50 μ g) were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were probed with Anti-Perilipin 1 (1:1000), Anti-PPAR γ (1:1000), and Anti- β -actin (loading control). HRP-conjugated secondary antibodies were used (1:5000 for Perilipin 1 and PPAR γ ; 1:10,000 for β -actin), and signals were detected by chemiluminescence.

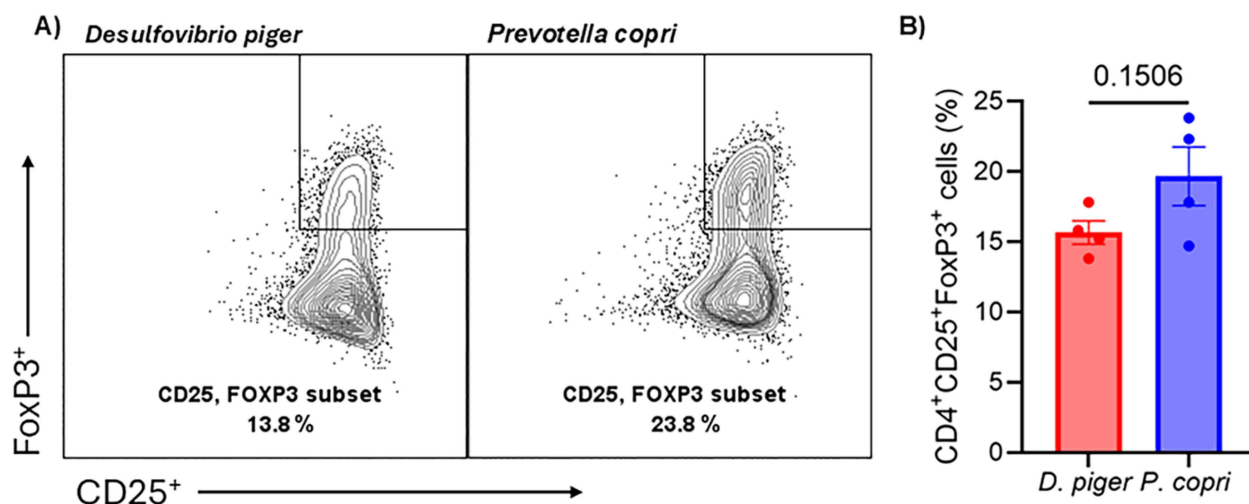


Fig S4: Dendritic cells (DCs) primed with *P. copri* show an increasing trend in CD4⁺CD25⁺FoxP3⁺ T cell differentiation compared with control bacteria primed DCs. DCs were isolated from the spleens of DR3 mice using Miltenyi CD11c⁺ selection beads and plated with plate-bound anti-CD3. Cells were stimulated overnight with *P. copri* or *D. pigr* at a multiplicity of infection (MOI) of 10. On day 2, naïve CD4⁺ T cells were isolated from DR3 spleens using a naïve CD4⁺ T cell isolation kit. The culture medium (complete RPMI with 10% FBS) was replaced, and purified naïve CD4⁺ T cells were co-cultured with DCs at a 1:1 ratio in the presence of Treg differentiation cytokines IL-2 and TGF- β . On day 5, cells were restimulated with PMA and brefeldin A (BFA) for 3 hours, followed by surface and intracellular staining for flow cytometry. (A) Representative flow cytometry plots showing CD4⁺CD25⁺FoxP3⁺ T cells in *P. copri* and *D. pigr* primed DC co-cultures. (B) Quantification showing an increasing trend in CD4⁺CD25⁺FoxP3⁺ T cell differentiation in *P. copri* primed DCs compared with *D. pigr* primed controls (n = 4). *P* values were determined using Welch's *t* test.

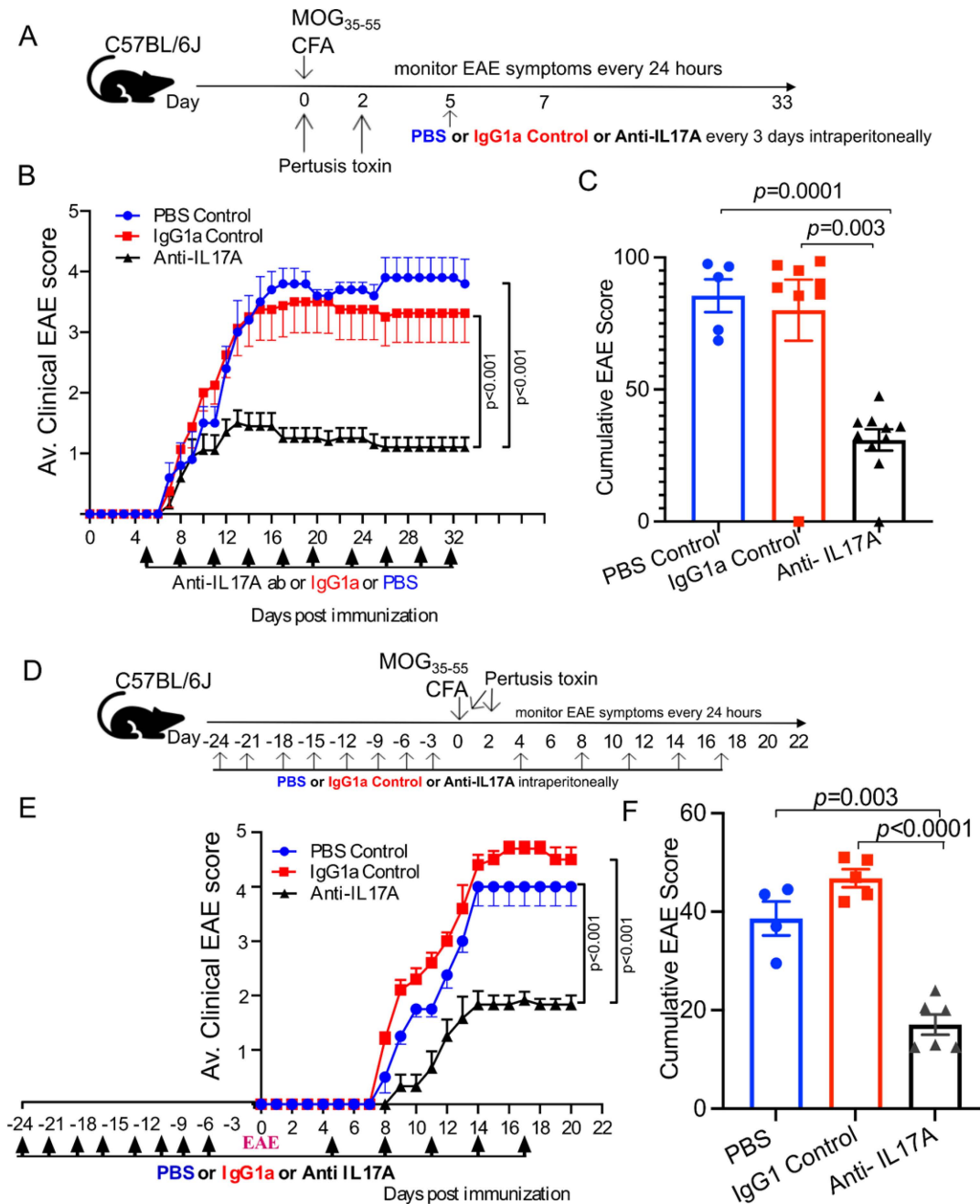


Fig. S5: Anti-IL-17A treatment lowers the severity of MOG₃₅₋₅₅ induced EAE in C57BL/6J mice. (A-B) 8-10-week-old B6 mice were immunized with MOG₃₅₋₅₅/CFA. Five days after immunization, mice received anti-mouse IL-17A monoclonal antibody, isotype control mouse IgG1, or PBS intraperitoneally every three days until the end of the experiment (day 32). (A) Average clinical EAE scores over time. Treatment days are indicated by arrowheads. (B) Average cumulative EAE scores. (C-D) 8-10-week-old B6 mice were treated every three days with anti-IL-17A, IgG1, or PBS pre- and post-immunization with MOG₃₅₋₅₅/CFA. (C) Average clinical EAE scores. Treatment days are indicated by arrowheads. (D) Average cumulative EAE scores. For plots in A, C, each bullet point represents the mean and bars represent the standard error of the mean from each experimental group. p -value was determined using two-way ANOVA following Tukey correction for EAE clinical scores (A, C) and unpaired t-test with Welch correction for cumulative EAE score (B, D). For graphs in B, D, each bar represents mean $\pm$ SEM.

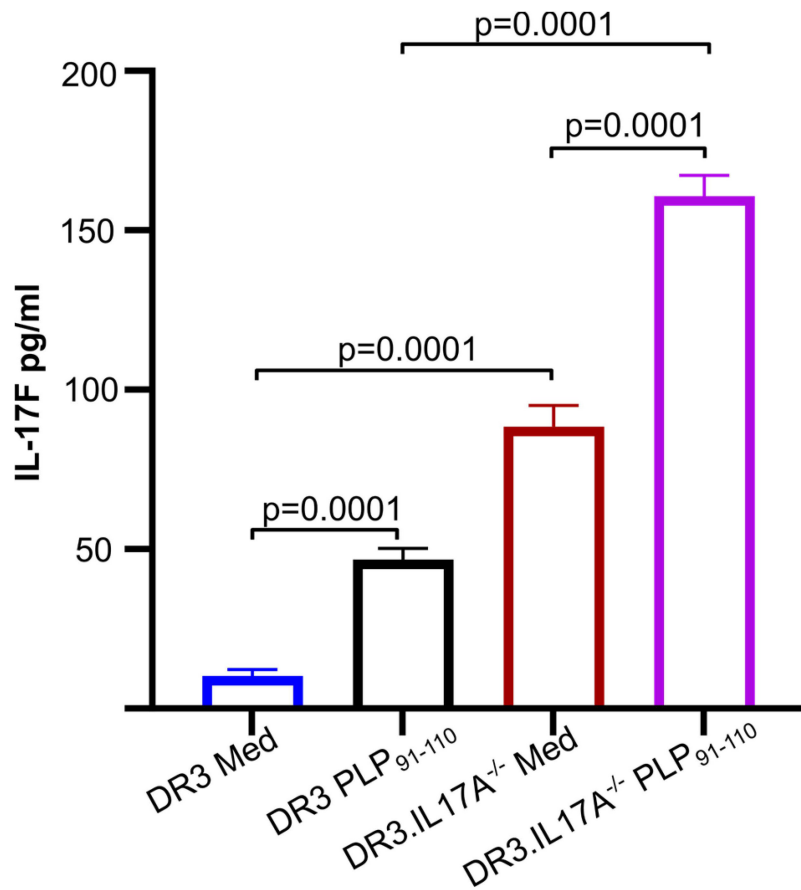


Fig. S6: Levels of inflammatory cytokines IL-17F in DR3 and DR3.IL-17A^{-/-} mice. 8-10-week-old mice were immunized with PLP₉₁₋₁₁₀/CFA to induce EAE. After 20 days, mice were euthanized, spleens were harvested, and splenic cells were cultured in the presence of PLP₉₁₋₁₁₀ antigen for five days following stimulation with Phorbol myristate acetate (PMA) and ionomycin in the presence of Brefeldin A (BFA). The supernatant was collected and levels of IL17F were measured using ELISA. Cytokine values from DR3 mice were compared to those of DR3.IL-17A^{-/-} mice in either media (Med) or after stimulation with PLP₉₁₋₁₁₀. *p*-value was determined using unpaired t-test with Welch correction. Bars represent mean±S.E.M.

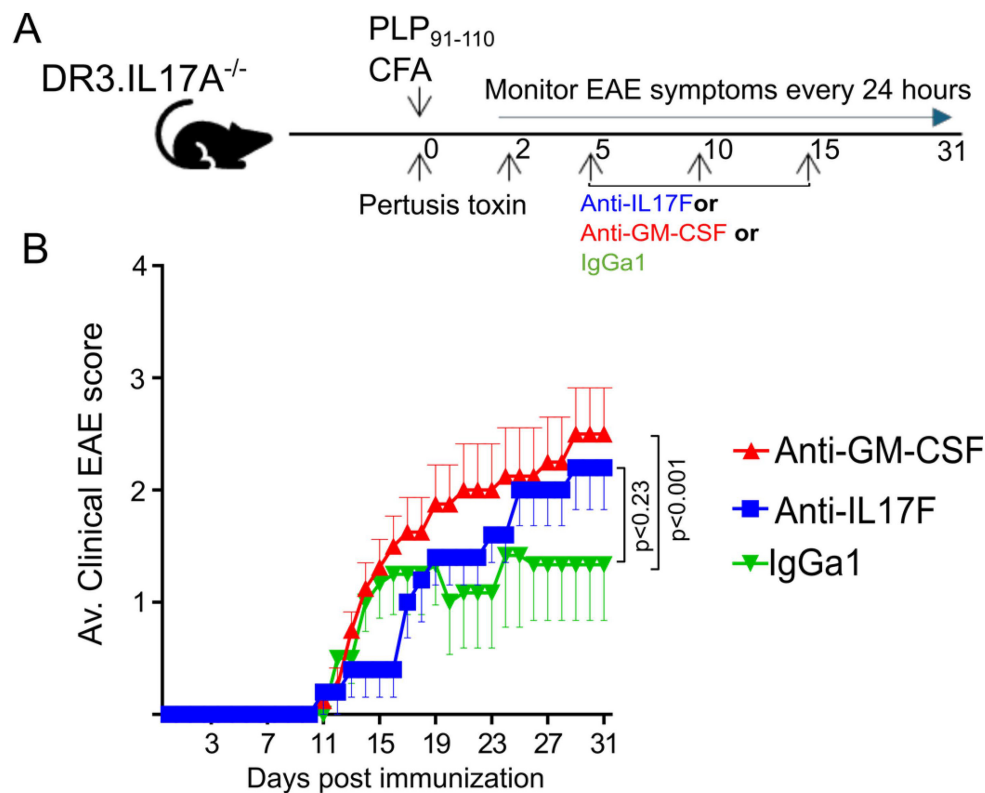


Fig. S7: IL-17F and GM-CSF have no role in disease severity in (A) Schematic diagram of the experimental workflow. EAE was induced in 8-10-week-old in DR3.IL-17A^{-/-} transgenic mice. At days 5, 10, and 14 post-EAE induction, mice received intraperitoneal injections of either: 1) anti-GM-CSF monoclonal antibody, 2) anti-IL-17F monoclonal antibody, or 3) IgGa1 isotype control and clinical EAE scores were monitored for 31 days. (B) Average clinical EAE score of mice in each group over time. Bullet points represent the mean and bars represent the standard error of the mean of each experimental group from two independent experiments (n ≥ 3 mice per group). *p*-value was determined by a two-way ANOVA following Tukey correction for average clinical EAE score (B).

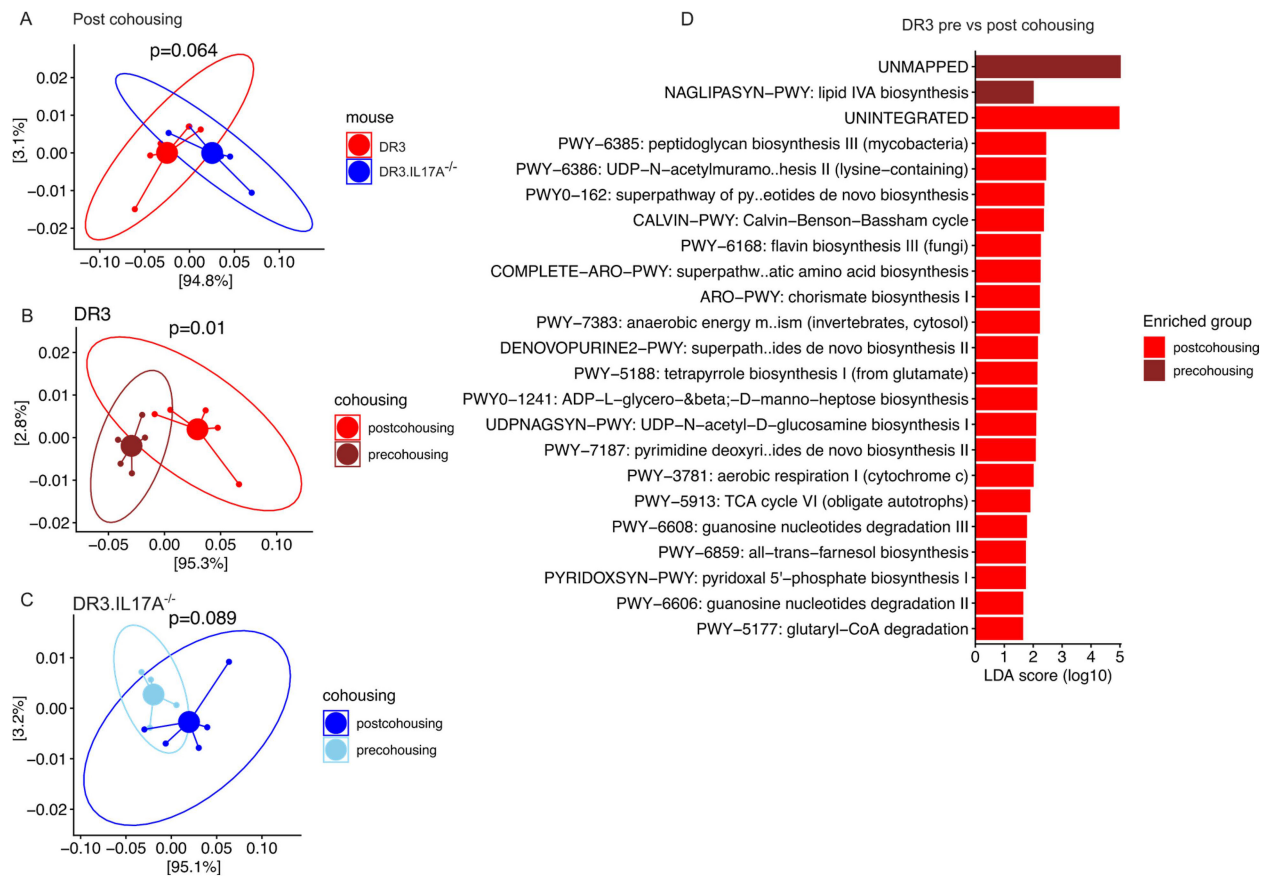


Fig. S8: DR3.IL-17A^{-/-} transfer functional pathways to DR3 transgenic mice. Eight to ten-week-old DR3 (n = 5) and DR3.IL-17A^{-/-} (n= 5) mice fecal samples were collected. Fecal from DR3 mice was transplanted to DR3.IL-17A^{-/-} mice, and conversely, fecal materials from DR3.IL-17A^{-/-} mice was transplanted into DR3 mice via oral gavage. Five days after oral gavage, DR3 mice were cohoused with DR3.IL-17A^{-/-} mice. Fecal samples were collected before and after co-housing/fecal transplant for shotgun metagenomic sequencing. Humann3 was used with default parameters to profile the pathway abundances in the clean reads using MetaCyc databases. (A) Bray-Curtis dissimilarity metric-based PCoA plots showing microbiome functional composition between DR3 and DR3.IL-17A^{-/-} post cohousing microbiome. (B) DR3 pre- and post-cohousing functions of the microbiome (C) DR3.IL-17A^{-/-} pre- and post-cohousing functions of the microbiome. (D) Differential abundance of bacterial species using *lefse* from *microbiomeMarker* package at *kw_cutoff*=0.01, *wilcoxon_cutoff*=0.01 and *lda_cutoff*=1 in R for DR3 mice pre- and post-cohousing. *adonis2* test was performed for A, B and C.

Supplemental Table:

Table S1: Differentially expressed gene list in the colon of DR3.IL-17A^{-/-} compared to DR3 transgenic mice calculated using *limma* package within *edgeR* in R. All 123 differentially expressed genes with *LogFC*>1.0 and *adj.p.val*<0.05 are shown.

SN	Genes	logFC	AveExpr	t	P.Value	adj.P.Val
1	Eno1b	-12.154	1.443	-19.576	0.000	0.000
2	Hoxb13	-11.448	-1.890	-19.897	0.000	0.000
3	Hoxd13	-9.589	-1.902	-10.871	0.000	0.000
4	Casp14	-8.793	-1.658	-13.128	0.000	0.000
5	Glo1-ps	-8.601	-0.491	-9.588	0.000	0.000
6	2310079G19Rik	-8.148	-2.842	-8.433	0.000	0.000
7	Rpl15-ps6	-7.886	0.116	-6.560	0.000	0.002
8	Gm13237	-7.823	-0.170	-8.445	0.000	0.000
9	Ly6g	-7.548	-2.237	-11.113	0.000	0.000
10	Tgm3	-6.671	3.560	-31.635	0.000	0.000
11	Vtcn1	-6.393	-3.095	-8.575	0.000	0.000
12	Mettl7a2	-6.307	-3.303	-8.664	0.000	0.000
13	Igkv8-18	-6.293	-0.666	-8.186	0.000	0.000
14	Fxyd4	-6.059	-1.910	-4.809	0.000	0.025
15	5033404E19Rik	-5.855	-2.774	-5.566	0.000	0.008
16	Gm7334	-5.659	1.342	-5.672	0.000	0.007
17	Sval1	-5.631	3.704	-19.493	0.000	0.000
18	Evx2	-5.587	-2.795	-6.518	0.000	0.002
19	Gm8540	-5.532	-3.496	-7.667	0.000	0.000
20	Atp12a	-5.477	0.033	-18.752	0.000	0.000
21	Chit1	-4.989	-3.144	-5.998	0.000	0.004
22	Trpv6	-4.912	-0.557	-8.873	0.000	0.000
23	Igkv2-112	-4.725	1.275	-9.585	0.000	0.000
24	Fut9	-4.701	-1.951	-6.631	0.000	0.002
25	Ighv7-1	-4.595	0.648	-6.919	0.000	0.001
26	Ninj2	-4.593	-1.906	-5.754	0.000	0.006
27	St8sia5	-4.551	-1.819	-5.220	0.000	0.013
28	Ighv1-61	-4.532	1.365	-7.272	0.000	0.001
29	Tmprss13	-4.269	-0.183	-8.813	0.000	0.000
30	Gjb5	-4.261	-1.541	-4.778	0.000	0.025
31	Hoxd12	-4.259	-3.247	-4.345	0.000	0.049
32	Vsig1	-4.255	-1.212	-6.516	0.000	0.002
33	Ighv10-3	-4.253	3.640	-11.518	0.000	0.000
34	B3gnt7	-4.195	5.973	-34.648	0.000	0.000

35	Rpl15-ps2	-3.829	-0.635	-4.963	0.000	0.019
36	Itih2	-3.817	-1.521	-4.471	0.000	0.041
37	Mettl7a3	-3.571	-1.774	-4.445	0.000	0.042
38	Pla2g4f	-3.458	0.531	-5.797	0.000	0.006
39	Trpv3	-3.403	3.252	-12.943	0.000	0.000
40	Thbs4	-3.334	3.057	-21.508	0.000	0.000
41	Hoxa13	-3.280	-1.700	-8.765	0.000	0.000
42	Itln1	-3.187	6.879	-10.731	0.000	0.000
43	Sycn	-3.151	2.937	-24.593	0.000	0.000
44	Nxpe4	-3.057	3.610	-15.040	0.000	0.000
45	Insl5	-2.941	-1.314	-6.488	0.000	0.002
46	Ighv2-5	-2.897	2.075	-4.897	0.000	0.021
47	Adgrf1	-2.818	0.607	-9.433	0.000	0.000
48	Igkv3-4	-2.711	3.280	-8.454	0.000	0.000
49	Igkv4-57	-2.655	4.588	-12.494	0.000	0.000
50	Slc28a3	-2.572	2.546	-4.625	0.000	0.032
51	Igkv4-68	-2.565	4.316	-8.356	0.000	0.000
52	Pla2g2f	-2.494	3.879	-7.097	0.000	0.001
53	Ighv3-3	-2.458	1.456	-8.997	0.000	0.000
54	Cyp2d12	-2.407	1.294	-9.324	0.000	0.000
55	Best2	-2.363	0.866	-10.732	0.000	0.000
56	Gm47283	-2.290	5.085	-10.117	0.000	0.000
57	Adh1	-2.274	5.559	-21.681	0.000	0.000
58	Ighv1-39	-2.271	3.886	-4.873	0.000	0.022
59	Cyp2c68	-2.248	4.529	-8.006	0.000	0.000
60	B3gnt5	-2.203	3.517	-6.569	0.000	0.002
61	Ighv5-4	-2.151	2.640	-5.001	0.000	0.018
62	Ggh	-2.100	5.137	-16.144	0.000	0.000
63	Cyp2d9	-2.078	0.066	-4.490	0.000	0.040
64	Zfp791	-2.041	2.587	-4.591	0.000	0.034
65	Slc15a2	-2.026	2.158	-4.924	0.000	0.020
66	Nox1	-2.008	3.528	-7.361	0.000	0.001
67	Nt5e	-1.973	5.284	-10.629	0.000	0.000
68	Duoxa2	-1.952	6.303	-10.058	0.000	0.000
69	Ang4	-1.951	7.834	-9.720	0.000	0.000
70	Ighv2-9-1	-1.949	3.374	-4.962	0.000	0.019
71	Slc15a1	-1.948	6.682	-8.009	0.000	0.000
72	Igkv3-5	-1.942	3.424	-5.541	0.000	0.008
73	Cldn8	-1.909	4.312	-9.203	0.000	0.000

74	Eno3	-1.762	3.750	-6.374	0.000	0.002
75	Clic6	-1.762	3.833	-5.762	0.000	0.006
76	Cd177	-1.749	6.807	-16.983	0.000	0.000
77	Adamts15	-1.691	4.728	-5.747	0.000	0.006
78	Ccl28	-1.628	7.068	-12.416	0.000	0.000
79	Igkv9-120	-1.622	4.390	-4.733	0.000	0.027
80	Hoxa11	-1.621	0.018	-5.452	0.000	0.009
81	Ceacam10	-1.593	5.986	-8.932	0.000	0.000
82	Aqp4	-1.573	6.556	-14.118	0.000	0.000
83	Saa1	-1.550	7.353	-7.683	0.000	0.000
84	Ano1	-1.550	3.699	-6.163	0.000	0.003
85	Tcf7l1	-1.529	2.331	-4.795	0.000	0.025
86	Cela1	-1.499	4.613	-7.343	0.000	0.001
87	Gm15401	-1.437	3.493	-4.550	0.000	0.036
88	Prdx6	-1.317	8.165	-8.030	0.000	0.000
89	Retnlb	-1.259	5.150	-4.445	0.000	0.042
90	Scd2	-1.133	9.600	-5.131	0.000	0.015
91	Plin4	1.287	6.266	4.615	0.000	0.033
92	Cyp4b1	1.379	4.923	4.760	0.000	0.026
93	Slc13a1	1.393	7.159	5.688	0.000	0.006
94	Hbb-bs	1.412	5.567	5.082	0.000	0.016
95	Naaladl1	1.451	8.878	5.901	0.000	0.005
96	Oas2	1.473	3.640	4.420	0.000	0.044
97	Tef	1.533	4.008	5.337	0.000	0.011
98	Oasl2	1.568	5.620	8.209	0.000	0.000
99	Fabp4	1.636	8.267	8.666	0.000	0.000
100	Ifit1	1.636	6.835	13.024	0.000	0.000
101	Rpl3	1.638	7.213	9.483	0.000	0.000
102	Thrsp	1.641	5.122	4.949	0.000	0.020
103	Ddx60	1.649	4.067	6.229	0.000	0.003
104	Ifit3	1.670	4.893	6.902	0.000	0.001
105	Retn	1.716	4.432	5.022	0.000	0.018
106	Sis	1.727	7.589	5.489	0.000	0.009
107	Igkv12-98	1.757	2.715	4.414	0.000	0.044
108	Oas3	1.774	4.742	8.831	0.000	0.000
109	Dbp	1.855	4.802	9.977	0.000	0.000
110	Rpl15	1.893	7.014	8.315	0.000	0.000
111	Igkv14-100	1.910	3.744	5.128	0.000	0.015
112	Car3	2.300	7.529	24.744	0.000	0.000

113	Cfd	2.334	7.125	24.230	0.000	0.000
114	Adipoq	2.474	4.756	11.111	0.000	0.000
115	Plin1	2.574	4.541	7.430	0.000	0.001
116	Lgals2	2.688	5.834	6.063	0.000	0.004
117	Tcaim	2.984	2.326	6.378	0.000	0.002
118	Trgc2	2.984	1.211	4.446	0.000	0.042
119	Cyp2e1	3.009	5.145	15.014	0.000	0.000
120	Lym7	3.114	0.716	4.632	0.000	0.032
121	Gm16011	4.232	-0.321	4.370	0.000	0.047
122	Igkv14-126	5.013	0.842	7.976	0.000	0.000
123	Cyle2	8.644	-1.325	9.999	0.000	0.000

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Table S2. Effect of *Prevotella copri* (*P. copri*) DSM 18205 supplementation on PPAR pathways genes, FoxP3, and IL10 gene expression in the colon of DR3 mice

	DR3						DR3IL17A				Unpaired T-test with Welch correction
Plin1	0.001	0.004	0.007	0.002		0.011	0.016	0.011	0.007		p=0.0123
Plin4	0.067	0.061	0.071	0.053		0.103	0.103	0.093	0.111		p=0.0003
Adipoq	0.407	1.879	4.558	0.287		4.552	7.031	7.363	0.347		p=0.17
FABP4	0.638	1.919	2.078	0.411		3.040	3.758	3.026	1.772		p=0.033
	Media						P. copri				
IL10	0.200	3.900	0.600	0.800	2.700	5.000		0.500	19.100	23.500	p=0.15
FoxP3	3.800	0.100	3.700	0.900	0.800	0.200	0.100	9.100	6.100	11.700	p=0.2
FABP4	2.127	0.923	0.992	1.340	0.384	2.105	2.456	1.093	1.921	2.762	p=0.053
Plin1	1.448	1.008	0.685	3.279	0.305	2.790	6.372	1.096	1.494	2.237	p=0.22
Plin4	1.050	0.700	2.548	0.441	1.211	0.738	1.734	1.239	0.691	0.949	p=0.78
Adipoq	3.238	1.341	1.204	1.526	0.125	1.849	3.462	1.862	1.881	4.438	p=0.13