Supplemental information

CD4^{+} T-cell-derived IL-10 promotes CNS inflammation

in mice by sustaining effector T cell survival

Nir Yogev, Tanja Bedke, Yasushi Kobayashi, Leonie Brockmann, Dominika Lukas, Tommy Regen, Andrew L. Croxford, Alexei Nikolav, Nadine Hövelmeyer, Esther von Stebut, Marco Prinz, Carles Ubeda, Kevin J. Maloy, Nicola Gagliani, Richard A. Flavell, Ari Waisman, and Samuel Huber
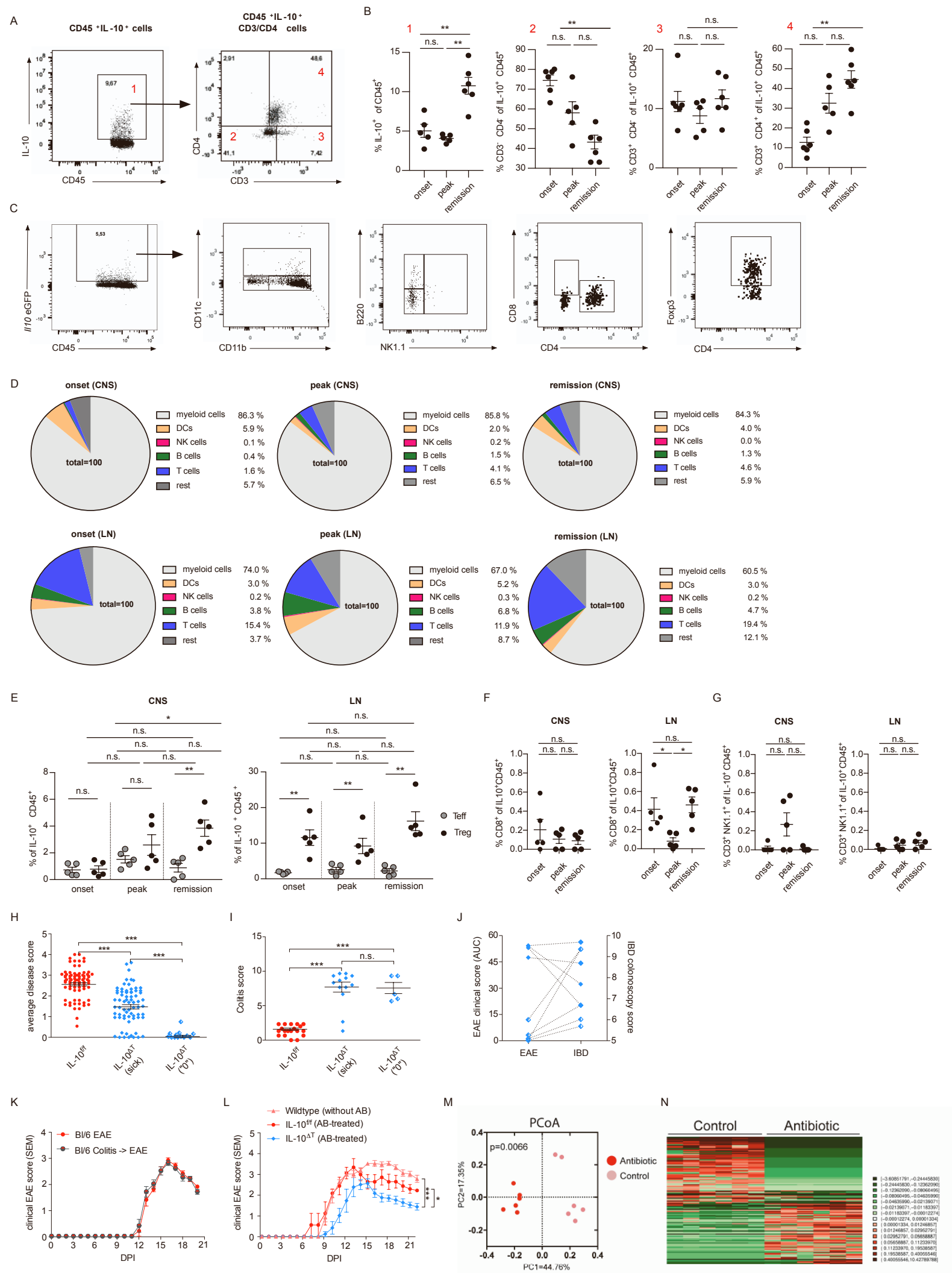
Figure S1

Figure S1 related to Figure 1: EAE mediated immune cell-derived IL-10 production and extra-intestinal inflammation.

(A, B) Lymphocytes were isolated from the CNS of wild-type mice with an EAE score of 1.5 (onset, d11), 3 (peak, d15), and 2.5 after (remission, d23) and restimulated with PMA/Ionomycin in the presence of Monensin for 5 hours prior antibody staining for CD45, CD3, CD4, and IL-10. (A) Representative Dotplots of IL-10$^+$CD45$^+$ cells (left) and IL-10$^+$CD3$^+$CD4$^+$ (right) at disease remission. (B) Graphs summarize the percentage of CNS-infiltrating IL-10$^+$CD45$^+$ leukocytes (1) IL-10$^+$CD3$^-$CD4$^-$ (2) IL-10$^+$CD3$^+$CD4$^-$ (3), and IL-10$^+$CD3$^+$CD4$^+$ (4) cells isolated from 5 mice per time-point. (C) Analysis of *Il10*eGFP expression by CD45$^+$ leukocyte populations using the following gating strategy: CD45$^+$IL-10$^+$ cells (left) were subdivided into myeloid cells (CD11b$^+$CD11c$^-$); DCs (CD11c$^+$CD11b$^{+/-}$); B cells (NK1.1$^-$B220$^+$); NK cells (B220$^-$NK1.1$^+$); CD8 T cells (CD4$^-$CD8$^+$); CD4$^+$Foxp3$^+$ (Treg), and CD4$^+$Foxp3$^-$ (Teff). (D-G) Graphs summarize the percentage of the analyzed IL-10$^+$ cell populations isolated from the CNS and LN at the indicated time points. Data represent mean $\pm$ SEM of $n = 5$ per condition obtained from 2 independent experiments. For statistical analysis Kruskal-Wallis was used. (H) EAE disease score in both *CD4*$^{Cre.neg}$*Il10*$^{fl/fl}$ control (IL-10$^{fl/fl}$) and *CD4*$^{Cre.pos}$*Il10*$^{fl/f}$ (IL-10$^{\Delta T}$) mice. Both IL-10$^{fl/fl}$ and IL-10$^{\Delta T}$ (sick) mice developed EAE score of ≥2 that lasted for two days or longer. IL-10$^{\Delta T}$ ("0") mice on the other hand, developed EAE score of ≤1 that lasted for two days or less. (I) Colitis disease score of individual IL-10$^{fl/fl}$ and IL-10$^{\Delta T}$ animals. Representative animals were randomly selected among those analyzed for EAE development (shown in H). (J) EAE/Colitis correlative incidence analysis among representative individual IL-10$^{\Delta T}$ animals. (K) EAE disease curve of either C57BL/6 (Bl/6 EAE) or C57BL/6 mice that were previously subjected to DSS-induced colitis (Bl/6 Colitis -> EAE). (L) EAE disease curve in IL-10$^{fl/fl}$ and IL-10$^{\Delta T}$ mice that have been maintained on broad-antibiotic treatment starting from the gestational stage and throughout their entire life span as well as control mice which has not received any antibiotic treatment. (K-L) Representative disease curve of ≥2 experiments, shown as mean $\pm$ SEM of n≥8 per group. (M, N) Mice treated for three consecutive weeks with sulfadoxin-trimethoprim in the drinking water. As control, another group of mice did not receive

antibiotics. Fecal samples were collected three weeks after treatment initiation. The microbiota was analysed through 16s rRNA high-throughput sequencing. (M) Principal coordinate of analysis based on Bray-curtis distances at the level of amplicon sequence variants (ASV). The p value indicates that both groups have a significantly different microbiota composition (p<0.05, PERMANOVA test). (N) Heatmap showing the amplicon sequence variants whose abundance significantly differs between both groups of mice (ANCOM2 & FDR, q<0.05). For each ASV, the mean relative abundance was calculated. Then, for each sample, the original relative abundance was normalized as the difference respect to the mean of the corresponding ASV.

Supplementary Table 1 related to Figure 1: 16SrRNA sequence analysis of the gut microbiota.

ID (a)	p.value	q.value	mean.antibiotic	mean.control	log2FC	Taxonomy (b)
ASV0649	7,57E-05	0,000609696	0,012406567	0	3,291375309	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0637	0,002480471	0,011287939	0,015292912	0	3,565051029	p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__NA;g__NA
ASV0575	0,001003473	0,005626618	0,017464156	0	3,741349914	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NA
ASV0561	0,001889856	0,009484803	0,019237538	0	3,870898532	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Roseburia
ASV0533	0,000718979	0,004180729	0,025454047	0	4,250583315	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__UCG-005
ASV0482	0,004457614	0,015660086	0,029686752	0	4,461660538	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Marvinbryantia
ASV0461	0,004330967	0,015631305	0,031528034	0	4,54464753	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NA
ASV0429	0,004490906	0,015660086	0,037299171	0	4,777559555	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium
ASV0422	0,001988225	0,00975473	0,05112576	0	5,218170506	p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelatoclostridiaceae;g__Erysipelatoclostridium
ASV0417	0,000234494	0,001636245	0,043646455	0	4,996610791	p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae;g__DNF00809
ASV0391	0,006814125	0,021612478	0,061080381	0	5,468498889	p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__NA;g__NA
ASV0362	1,02E-06	9,66E-06	0,05686926	0	5,367849594	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0339	0,007768574	0,023541258	0,057557688	0	5,384791258	p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__NA;g__NA
ASV0310	1,63E-07	1,60E-06	0,090830032	0	6,030247566	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0294	1,44E-06	1,33E-05	0,087732912	0	5,980975394	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Eisenbergiella
ASV0253	0,00754512	0,02325489	0,111220345	0	6,318374347	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_UCG-001
ASV0164	0,003372914	0,013754479	0,215015	0	7,260637523	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0160	0,004538433	0,015660086	0,035515939	0	4,709521237	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Tannerellaceae;g__Parabacteroides
ASV0119	0,002301862	0,010845437	0,330748852	0	7,878640552	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_UCG-001
ASV0113	0,00372137	0,01442605	0,31252184	0	7,797218891	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia
ASV0011	8,37E-05	0,000654703	3,048870572	0,043093317	6,098841561	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
ASV0335	2,86E-05	0,00023612	0,069020164	0,001142272	4,785593833	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NA
ASV0342	8,55E-05	0,000654703	0,072388335	0,001313444	4,759386022	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0177	0,000452542	0,003023368	0,221150123	0,004688677	5,189240182	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
ASV0247	0,006472642	0,020738873	0,124541921	0,003264645	4,751465764	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0203	0,000342706	0,002339342	0,1857243	0,008163317	4,288640243	p__Firmicutes;c__Clostridia;o__Peptococcales;f__Peptococcaceae;g__NA
ASV0296	0,005756858	0,018829723	0,077867892	0,003829217	3,916775925	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__GCA-900066575
ASV0279	0,010566415	0,030720874	0,08908228	0,004396106	3,961833658	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Incertae_Sedis
ASV0154	0,00066571	0,004019865	0,210838067	0,011509976	4,03793259	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
ASV0246	0,011449	0,032981524	0,111757041	0,00652929	3,833068722	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0350	0,000199169	0,001421345	0,052563055	0,003330251	3,508824142	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NA
ASV0057	0,003046402	0,012926625	0,597396324	0,043473852	3,737780368	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
ASV0281	0,000613285	0,003775914	0,087703025	0,007515044	3,319506012	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK4A136_group
ASV0471	0,015873759	0,042601371	0,026713547	0,002689076	2,777996585	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0304	0,004207423	0,015542717	0,090591607	0,009517865	3,073488297	p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__NA;g__NA
ASV0260	0,001240795	0,006603554	0,096727477	0,011357192	2,942228428	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Roseburia
ASV0127	0,0016347	0,008414686	0,293225645	0,034946761	3,018588803	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0448	0,00216292	0,010448567	0,030210524	0,003935155	2,564250702	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Butyricicoccaceae;g__Butyricicoccus
ASV0104	0,006337416	0,020514933	0,30602504	0,040193751	2,88545507	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia
ASV0462	0,000708594	0,004180729	0,028509899	0,003897632	2,494659145	p__Firmicutes;c__Bacilli;o__RF39;f__NA;g__NA
ASV0142	0,007797105	0,023541258	0,239293075	0,034888042	2,729244759	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NA
ASV0151	0,004666372	0,015755278	0,21695407	0,037538951	2,48703747	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NA
ASV0244	0,001903002	0,009484803	0,097818685	0,020008981	2,211795149	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NA
ASV0123	0,015025635	0,041026516	0,244374897	0,052143188	2,198323067	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__UCG-003
ASV0303	0,002440659	0,011270103	0,064306145	0,013754917	2,115410141	p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__Anaerovorax
ASV0117	0,000873283	0,004985652	0,267487384	0,057951394	2,179432918	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NA
ASV0148	0,002987451	0,012850132	0,218434791	0,049732437	2,103862919	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0137	0,000608576	0,003775914	0,20230646	0,051443368	1,946467576	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Tannerellaceae;g__Parabacteroides
ASV0456	0,004038044	0,015094591	0,026170262	0,006681437	1,768995992	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Tannerellaceae;g__Parabacteroides
ASV0157	0,012496853	0,035351457	0,193180481	0,053519437	1,824763915	p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus
ASV0037	0,013308835	0,036982072	0,859907125	0,263941528	1,698634511	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__NA
ASV0091	0,00402165	0,015094591	0,309401988	0,10069269	1,606008282	p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio
ASV0080	0,002314154	0,010845437	0,340540412	0,112126364	1,590621063	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0068	0,010292845	0,030205172	0,413386939	0,143062099	1,521605438	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0005	0,0036528	0,01442605	6,378196003	2,27773613	1,484973343	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes
ASV0042	0,003308357	0,01366874	0,657882641	0,248133828	1,401620841	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Oscillibacter
ASV0128	0,009921734	0,029390798	0,214393442	0,081513714	1,379846572	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Colidextribacter
ASV0009	0,018101709	0,047764172	3,086078087	1,726080655	0,83775516	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0102	0,004844681	0,016183295	0,119942673	0,249296331	-1,046784144	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Incertae_Sedis
ASV0035	0,004607727	0,015726373	0,346577473	0,807778858	-1,217437848	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0050	0,012625037	0,035395193	0,191387621	0,564855014	-1,554383397	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__Odoribacter
ASV0192	0,01156511	0,033013132	0,041457496	0,135378653	-1,673960872	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0044	0,001473394	0,00771076	0,178654404	0,634645363	-1,82063147	p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Turicibacter
ASV0010	0,008463878	0,025311026	0,843027998	3,017416554	-1,837922782	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotellaceae_UCG-001
ASV0322	0,016872291	0,044897451	0,014718203	0,056514376	-1,844496609	p__Cyanobacteria;c__Vampirivibrionia;o__Gastranaerophilales;f__NA;g__NA
ASV0132	0,003195773	0,013379635	0,049675498	0,233042767	-2,198281376	p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus
ASV0141	0,003914722	0,014990521	0,041506977	0,20614122	-2,273810548	p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus
ASV0287	0,015593196	0,042209168	0,004107804	0,076756673	-3,824055034	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium
ASV0282	0,013672699	0,037659889	0,001680465	0,075727335	-4,640920193	p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__NA;g__NA
ASV0677	0,000561041	0,003595241	0	0,010309926	-3,053965046	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1
ASV0650	0,002623888	0,01177001	0	0,012512342	-3,302376904	p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__NA
ASV0640	0,002668954	0,011803544	0	0,0121331	-3,262537001	p__Firmicutes;c__Clostridia;o__Clostridia_vadinBB60_group;f__NA;g__NA
ASV0576	0,001148821	0,006219477	0	0,01684567	-3,693285945	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0566	0,002906146	0,012674026	0	0,018081169	-3,787755058	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0554	0,007086522	0,02225168	0	0,020122565	-3,931445062	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides
ASV0505	0,004308264	0,015631305	0	0,023667947	-4,151331138	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnoclostridium
ASV0493	3,85E-09	4,64E-08	0	0,024987079	-4,225286242	p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae;g__NA
ASV0467	0,003694768	0,01442605	0	0,029275768	-4,442467202	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__A2
ASV0453	0,003430602	0,013810371	0	0,030101097	-4,480755791	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0385	9,51E-05	0,000711013	0	0,042781413	-4,968643907	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0272	5,72E-11	1,38E-09	0	0,092893997	-6,062173013	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0270	0,000158915	0,001160452	0	0,092719804	-6,059505718	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0269	2,75E-14	4,32E-12	0	0,094002049	-6,079025304	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0267	0,007554136	0,02325489	0	0,097875992	-6,136443219	p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__NA
ASV0232	4,77E-09	5,54E-08	0	0,118716311	-6,411329854	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0216	4,19E-12	1,88E-10	0	0,13749952	-6,620920937	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides
ASV0205	1,75E-09	2,29E-08	0	0,146218593	-6,70874679	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0202	0,001120937	0,006174984	0	0,1612099	-6,848277197	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0201	2,73E-12	1,43E-10	0	0,157006559	-6,810496855	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0189	5,92E-10	9,78E-09	0	0,16423091	-6,874832201	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0187	2,20E-08	2,30E-07	0	0,177603955	-6,986844332	p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__Parasutterella
ASV0178	6,74E-10	1,06E-08	0	0,182803516	-7,028150899	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0176	5,25E-10	9,16E-09	0	0,191678679	-7,096035051	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0174	2,51E-06	2,25E-05	0	0,105404758	-6,241890712	p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__Parasutterella
ASV0173	1,67E-10	3,08E-09	0	0,192428103	-7,101623618	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0167	0,00054269	0,003550095	0	0,215681766	-7,265075348	p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__NA;g__NA
ASV0161	1,08E-09	1,55E-08	0	0,204765428	-7,190643225	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0159	1,41E-08	1,53E-07	0	0,215499172	-7,263861404	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0150	2,07E-05	0,000175632	0	0,233153449	-7,376747881	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0139	7,92E-11	1,78E-09	0	0,256657881	-7,51451949	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0131	5,99E-13	3,76E-11	0	0,273800035	-7,607301492	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0122	1,07E-09	1,55E-08	0	0,304195962	-7,758440394	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0114	0,004508365	0,015660086	0	0,316863559	-7,817034757	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Oscillibacter
ASV0111	2,04E-09	2,56E-08	0	0,323391487	-7,846325559	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Oscillibacter
ASV0109	0,005101813	0,016862833	0	0,337362074	-7,907082023	p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae;g__Oscillibacter
ASV0093	6,46E-09	7,24E-08	0	0,386171084	-8,101264499	p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__Parasutterella
ASV0077	1,50E-05	0,000131102	0	0,456511709	-8,341866515	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0076	1,99E-15	6,26E-13	0	0,440751262	-8,291338344	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0074	8,95E-11	1,87E-09	0	0,488916573	-8,440508277	p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium
ASV0071	1,22E-10	2,40E-09	0	0,498799119	-8,469296547	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0065	2,58E-11	6,74E-10	0	0,561597784	-8,639919617	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes
ASV0063	1,34E-09	1,83E-08	0	0,593431168	-8,719269059	p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae;g__Parasutterella
ASV0061	1,13E-11	3,91E-10	0	0,617025275	-8,775387047	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0051	1,25E-11	3,91E-10	0	0,701737133	-8,960590654	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0030	1,40E-11	3,99E-10	0	1,345957485	-9,898834192	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0028	1,21E-13	1,26E-11	0	1,502948188	-10,05783876	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0023	2,34E-13	1,84E-11	0	1,555678983	-10,107542	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0015	5,73E-12	2,25E-10	0	2,294691593	-10,66787667	p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__NA
ASV0006	3,59E-08	3,64E-07	0	7,21703583	-12,32037786	p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae;g__Akkermansia

(a) ASVs have the same order as in the heatmap in the supplementary figure: from lower to higher fold change comparing the antibiotic-treate group to the control group

(b) p=phylum;c=class;o=order;f=family;g=genus

Supplementary Table 1 related to Figure 1: 16S rRNA sequence analysis of the gut microbiota.

Taxonomy and q values obtained for each amplicon sequence variants (ASV). For each ASV, the mean relative abundance was calculated. Then, for each sample, the original relative abundance was normalized as the difference respect to the mean of the corresponding ASV. The obtained value represented with a colour as depicted. The taxonomy and q values obtained for each ASV are shown.
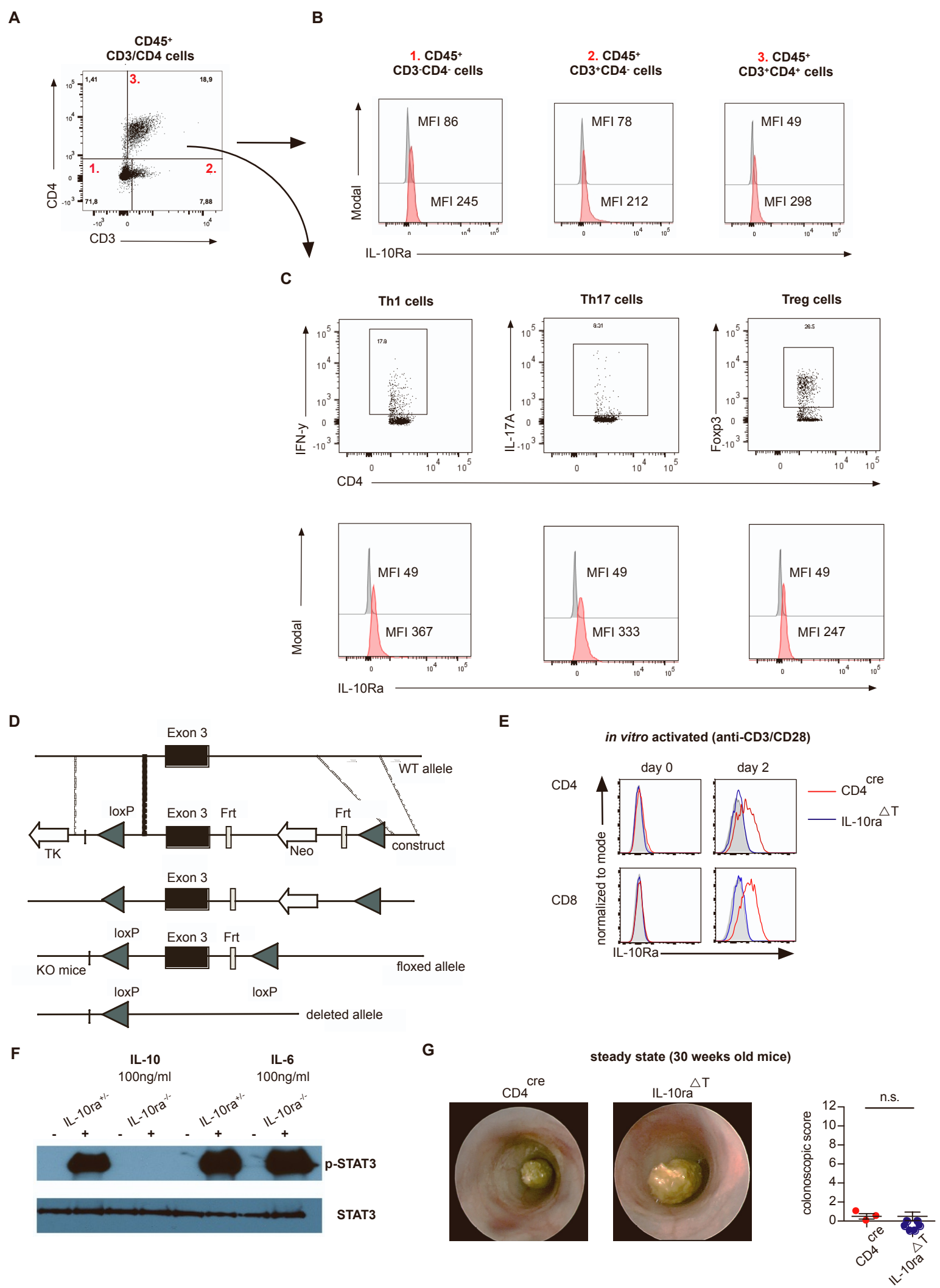
A

CD45+
CD3/CD4 cells

1,41 3. 18,9

CD4

1. 2.

71,8 7,88

CD3

B

1. CD45+
CD3-CD4- cells

2. CD45+
CD3+CD4- cells

3. CD45+
CD3+CD4+ cells

Modal

MFI 86
MFI 245

MFI 78
MFI 212

MFI 49
MFI 298

IL-10Ra

C

Th1 cells | Th17 cells | Treg cells

IFN-γ 17,9

IL-17A 8,31

Foxp3 26,5

CD4

Modal

MFI 49
MFI 367

MFI 49
MFI 333

MFI 49
MFI 247

IL-10Ra

D

Exon 3

WT allele

loxP Exon 3 Frt Frt

TK Neo construct

Exon 3 Frt

loxP Exon 3 Frt

KO mice floxed allele

loxP loxP

deleted allele

E

in vitro activated (anti-CD3/CD28)

day 0 day 2

CD4

CD8

normalized to mode

IL-10Ra

CD4cre

IL-10raΔT

F

IL-10
100ng/ml

IL-6
100ng/ml

IL-10ra+/- IL-10ra-/- IL-10ra+/- IL-10ra-/-

- + - + - + - +

p-STAT3

STAT3

G

steady state (30 weeks old mice)

CD4cre IL-10raΔT

colonoscopic score

12
10
8
6
4
2
0

n.s.

CD4cre IL-10raΔT

Figure S2

Figure S2 related to Figure 2: Gating strategy for the identification of IL10Ra expressing immune cells and characterization of *CD4^Cre^Il10ra^fl/fl^* mice.

(A) Gating strategy of IL10 receptor expression: CD45$^+$ cells (not shown) were first subdivided into CD3$^-$CD4$^-$, CD3$^+$CD4$^-$, and CD3$^+$CD4$^+$ cell subsets. (B) Histograms show FMO for IL-10R staining (grey) of the indicated subset overlayed with IL-10R staining (red) of the same subset.

(C) Gating of *Ifng* FP635$^+$ Th1 cells, *Il17a* eGFP$^+$ Th17 cells and *Foxp3* RFP$^+$ Treg cells from CD3$^+$CD4$^+$ (as shown in A). lower Panel: Histograms show FMO for IL-10R staining (grey) of CD4$^+$ T cells overlayed with IL-10R staining (red) of the indicated subset. (D) *Il10ra* construct used for the generation of *CD4^Cre^Il10ra^fl/fl^* mice. (E) IL-10Rα expression on CD4$^+$ T cells and CD8$^+$ T cells directly after isolation from spleens of untreated *CD4^Cre^Il10ra^wt/wt^* (CD4^{Cre}) and *CD4^Cre^Il10ra^fl/fl^* (IL-10r$\alpha^{\Delta T}$) mice and after 2 days of *in vitro* activation in the presence of 1µg/ml soluble anti-CD3 and anti-CD28 mAb. (F) Western blot of STAT3 phosphorylation of CD4$^+$ T cells isolated from heterozygeous *CD4^Cre^Il10ra^fl/wt^* and *CD4^Cre^Il10ra^fl/fl^* (IL-10r$\alpha^{\Delta T}$) mice after *in vitro* stimulation with 100 ng/ml IL-10 or 100 ng/ml IL-6 as a positive control, respectively. (G) Endoscopy of 30 weeks old untreated CD4^{Cre} and IL-10r$\alpha^{\Delta T}$ mice as described in material and methods.
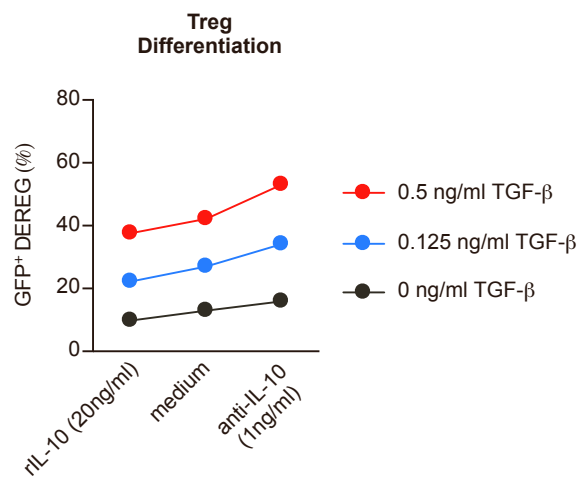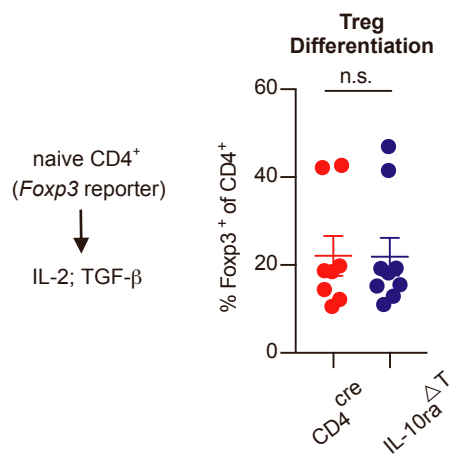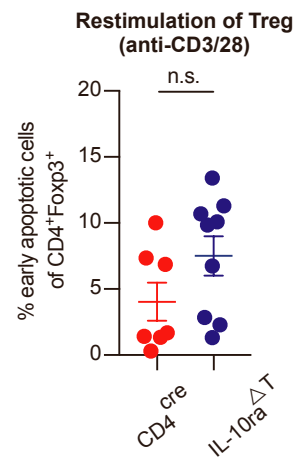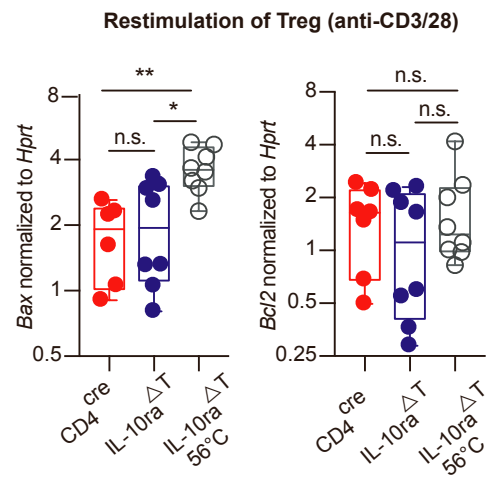
A

Treg Differentiation

GFP⁺ DEREG (%) vs rIL-10 (20ng/ml), medium, anti-IL-10 (1ng/ml)

- 0.5 ng/ml TGF-β
- 0.125 ng/ml TGF-β
- 0 ng/ml TGF-β

B

naive CD4⁺ (*Foxp3* reporter) → IL-2; TGF-β

Treg Differentiation

% Foxp3⁺ of CD4⁺ — n.s. — CD4cre, IL-10raΔT

C

Restimulation of Treg (anti-CD3/28)

% early apoptotic cells of CD4⁺Foxp3⁺ — n.s. — CD4cre, IL-10raΔT

D

Restimulation of Treg (anti-CD3/28)

Bax normalized to *Hprt* — ** , * , n.s. — CD4cre, IL-10raΔT, IL-10raΔT 56°C

Bcl2 normalized to *Hprt* — n.s., n.s., n.s. — CD4cre, IL-10raΔT, IL-10raΔT 56°C

Figure S3

Figure S3 related to Figure 3: IL-10 signaling in Treg is dispensable for their survival *in vitro*.

(A) FACSorted naïve CD4 T cells, isolated from the FoxP3-GFP reporter mice were plated on 96 well plate ($2x10^5$ /well, triplicates) in serum free/TGFβ-free medium. Cells were stimulated with anti CD3/CD28, TGFβ at the indicated concentrations, and treated with either rIL-10 (20ng/ml), anti-IL-10 (1µg/ml) or without farther stimuli. 72hrs post stimulation, the triplicates were pooled, stained and analyzed by FACS. Data shown as percentages of GFP$^+$ iTreg of total viable CD4$^+$ T cells. One representative experiment of two. (B) Flow cytometric analysis of the frequencies of induced *Foxp3* RFP$^+$ Treg after 5 days of *in vitro* differentiation from naïve IL10R$\alpha^{\Delta T}$ T cells (blue; n=9) and CD4^{Cre} CD4$^+$ T cells (red; n=8). (C) FACS analysis of early apoptotic Annexin$^+$ Pacific Orange$^-$ iTreg (left) and (D) mRNA expression levels of the pro-apoptotic gene *Bax* and anti-apoptotic gene *Bcl2* in sorted IL-10R$\alpha^{\Delta T}$ and CD4^{Cre} iTreg after 16hr (for FACS analysis) and 12 hrs (for qPcR analysis) of restimulation with plate-bound 1 µg/ml anti-CD3 and soluble anti-CD28 mAb. Grey bars show gene expression after heat induced apoptosis induction as positive control.
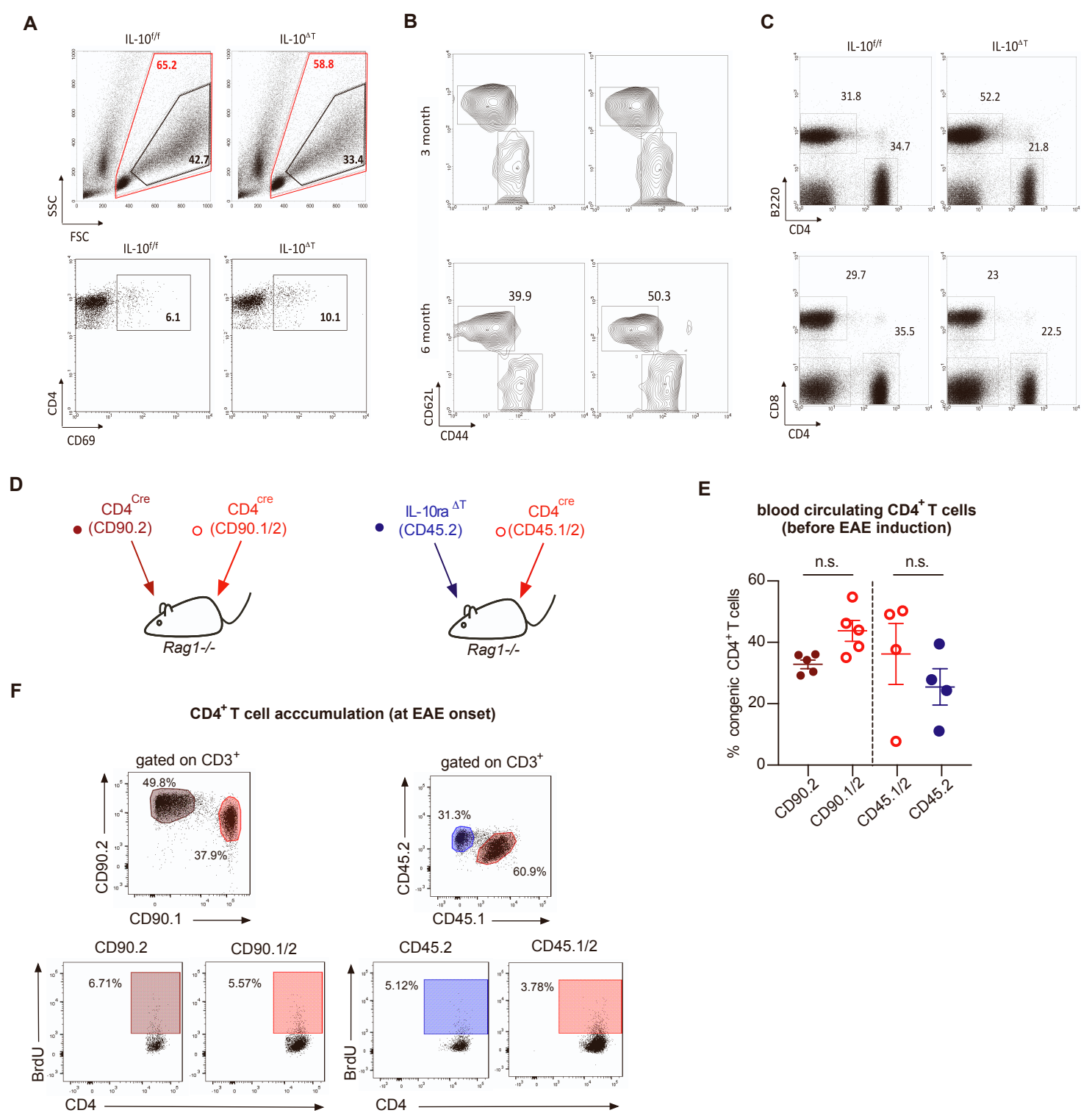
A

IL-10^{f/f}   IL-10^{ΔT}

65.2   58.8
42.7   33.4

SSC / FSC

IL-10^{f/f}   IL-10^{ΔT}

6.1   10.1

CD4 / CD69

B

3 month

6 month

39.9   50.3

CD62L / CD44

C

IL-10^{f/f}   IL-10^{ΔT}

31.8   52.2
34.7   21.8

B220 / CD4

29.7   23
35.5   22.5

CD8 / CD4

D

CD4^{Cre} (CD90.2)   CD4^{cre} (CD90.1/2)

Rag1-/-

IL-10ra^{ΔT} (CD45.2)   CD4^{cre} (CD45.1/2)

Rag1-/-

E

blood circulating CD4+ T cells (before EAE induction)

% congenic CD4+ T cells

n.s.   n.s.

CD90.2   CD90.1/2   CD45.1/2   CD45.2

F

CD4+ T cell acccumulation (at EAE onset)

gated on CD3+

49.8%
37.9%

CD90.2 / CD90.1

gated on CD3+

31.3%
60.9%

CD45.2 / CD45.1

CD90.2   CD90.1/2

6.71%   5.57%

BrdU / CD4

CD45.2   CD45.1/2

5.12%   3.78%

BrdU / CD4

Figure S4

Figure S4 related to Figure 4: Loss of effector *Il10*-deficient T cells *in vivo* and reconstitution efficiency after co-transfer of *Il10ra*-proficient and -deficient T cells into Rag1$^{-/-}$ mice.

(A) MACS-enriched CD4$^+$ T cells isolated from ing.LN of EAE mice (day 10-post immunization) and co-cultured with MOG$_{35-55}$-pulsed BMDCs, IL-2, IL-12 and IL-23 for 5 days. (Upper panel) percentage of total viable cells (red) or cell-blasts (black). (Lower panel) percentage of activated (CD69$^+$) CD4$^+$ T cells. (B) Percentage of naïve CD4$^+$ T cells found in LN of healthy, non-challenged, young and old mice (3 vs 6 month-old, respectively). (C) B cell (B220$^+$) to CD4$^+$ T cell ratio (upper panel), and CD8$^+$ to CD4$^+$ T cell ratio (lower panel), as seen in healthy, non-challenged, young mice. (D) Experimental layout of CD4$^+$ T cell co-transfer into *Rag1$^{-/-}$* mice: Co-transfer of CD4$^+$ T cells derived from littermate control mice (*CD4^{Cre}Il10ra$^{wt/wt}$*, short CD4^{Cre}), expressing either CD90.2 or CD90.1/2 congenic marker (dark red, full circles red vs red, empty circles, respectively). (right) Co-transfer of CD4$^+$ T cells derived from either CD4^{Cre} control and IL-10rα^{ΔT} mice or littermate CD4^{Cre} control mice, expressing either CD45.2 or CD45.1/2 congenic marker (blue, full circle vs red, empty circle, respectively). (E) To determine the reconstitution efficiency of congenic CD4^{Cre} wild-type and IL-10rα^{ΔT} CD4$^+$ T cells peripheral blood was sampled 4 to 5 weeks after reconstitution and stained for CD4, CD90.1 and CD90.2 or CD4, CD45.1 and CD45.2, respectively. The Diagram shows the ratio of congenic CD4$^+$ T cells after transfer of a mixture of equal numbers of CD4^{Cre}:CD4^{Cre} control cells (left) and after transfer of a mixture of equal numbers of IL-10rα^{ΔT}:CD4^{Cre} control cells (right). (F) After 4 to 5 weeks of reconstitution, the mice were immunized with MOG$_{35-55}$ as described in the Material and Methods section, and gating strategy of FACS analyses of T cell subpopulations were performed at the indicated time points, as shown here for BrdU staining. Co-transfer of CD4$^+$ T cells derived from littermate control mice (*CD4$^{Cre.pos}$Il10ra$^{wt/wt}$*; short CD4^{Cre}), expressing either CD90.2 (brown) or CD90.1/2 (red) congenic marker (also labeled as full vs empty circles, respectively). (right) Co-transfer of CD4$^+$ T cells derived from IL-10rα^{ΔT} and CD4^{Cre} control mice, expressing either CD45.2 (blue) or CD45.1/2 (red) congenic marker (also labeled as full vs empty circle, respectively).