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Supplemental Information

Microbiotas from Humans with Inflammatory Bowel Disease Alter the Balance of Gut Th17 and RORγt$^+$ Regulatory T Cells and Exacerbate Colitis in Mice

Graham J. Britton, Eduardo J. Contijoch, Ilaria Mogno, Olivia H. Vennaro, Sean R. Llewellyn, Ruby Ng, Zhihua Li, Arthur Mortha, Miriam Merad, Anuk Das, Dirk Gevers, Dermot P.B. McGovern, Namita Singh, Jonathan Braun, Jonathan P. Jacobs, Jose C. Clemente, Ari Grinspan, Bruce E. Sands, Jean-Frederic Colombel, Marla C. Dubinsky, and Jeremiah J. Faith

Table S1 (Related to Table 1). An Excel file detailing the species composition of each of the 17 arrayed culture collections derived from donor fecal microbiota.

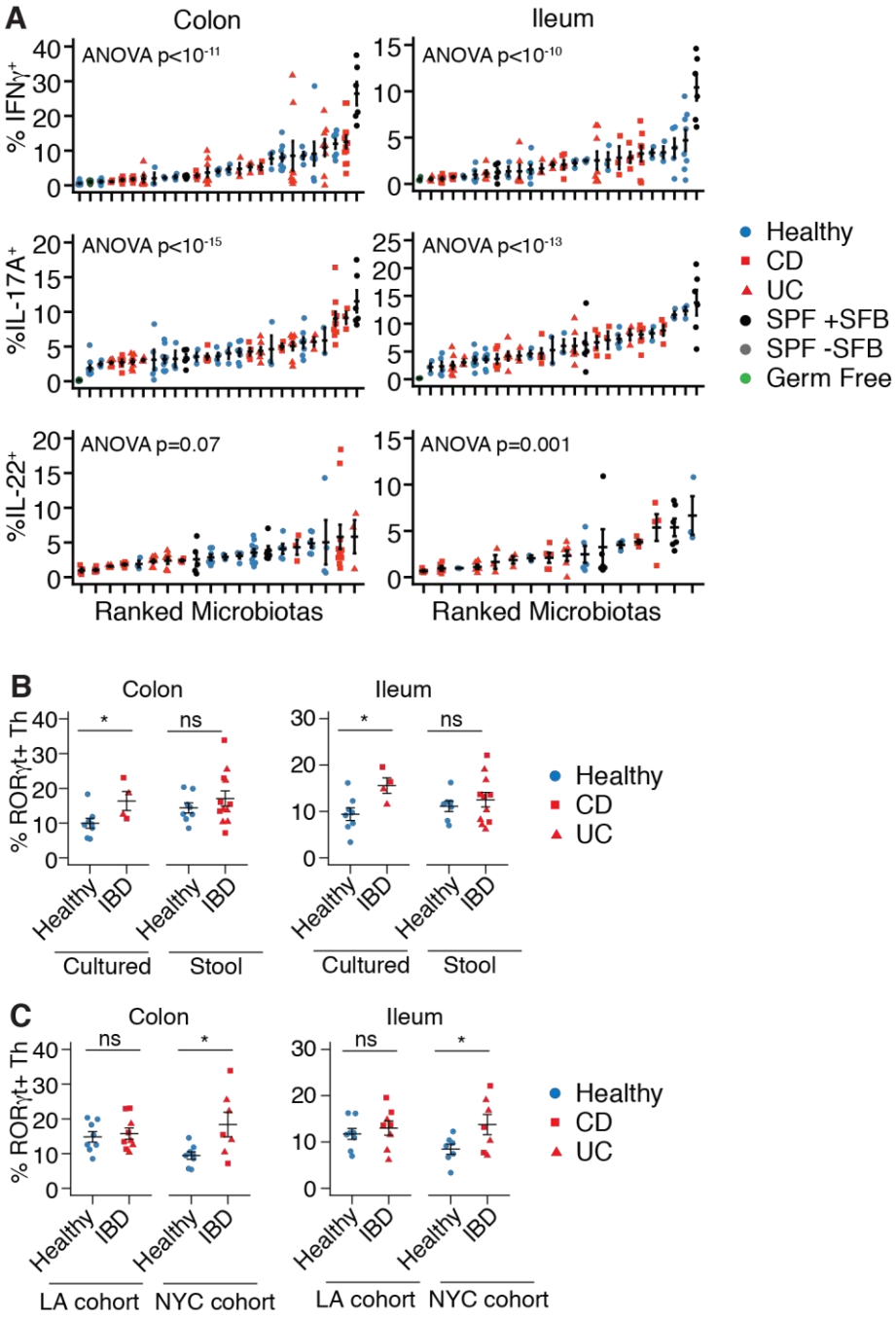

Figure S1. (Related to Figure 1). Relative frequencies of T helper cells in gnotobiotic mice colonized with IBD-associated microbiotas as compared to mice colonized with healthy donor microbiotas

Germ free B6 mice were colonized with fecal microbiotas from human donors with and without IBD. Effector CD4 T cells from the colon and ileum of these mice were analyzed by flow cytometry.

(**A**) The proportions of colon and ileum IFNγ^+, IL-17A$^+$ and IL-22$^+$ CD4$^+$ T cells. Each symbol represents data from one mouse. The numbers of cytokine$^+$ cells are presented as a proportion of live, CD45$^+$.CD4$^+$ cells.

(**B**) The proportion of RORγt$^+$Th induced by donor microbiotas in colon and ileum of B6 gnotobiotic mice colonized with either cultured collections of microbes or total stool microbiota. Each point represents the mean value of a group of 2-12 mice colonized with a single microbiota. Data is also shown in Figure 1C, but here is segregated according to the type of microbiota inoculum. The numbers of RORγt$^+$ cells are presented as a proportion of live, CD45$^+$, CD4$^+$, FoxP3$^-$ cells.

(**C**) The proportion of RORγt$^+$Th induced by donor microbiotas in colon and ileum of B6 gnotobiotic mice colonized with microbiotas from two independent cohorts of donors. Each point represents the mean value of a group of 2-12 mice colonized with a single microbiota. Data is also shown in Figure 1C, but here is segregated according to donor cohort. The numbers of RORγt$^+$ cells are presented as a proportion of live, CD45$^+$, CD4$^+$, FoxP3$^-$ cells.

(**A**) n=11 healthy, 6 UC and 7 CD microbiotas (IFNγ and IL-17A), n=8 healthy, 4 UC and 6 CD microbiotas (IL-22),; each point represents data from one mouse. (**B** and **C**) each point represents the mean value of a group of 2-12 mice colonized with a single microbiota. ns – not significant, *$p<0.05$, Students t-test; horizontal lines indicate mean ± SEM.

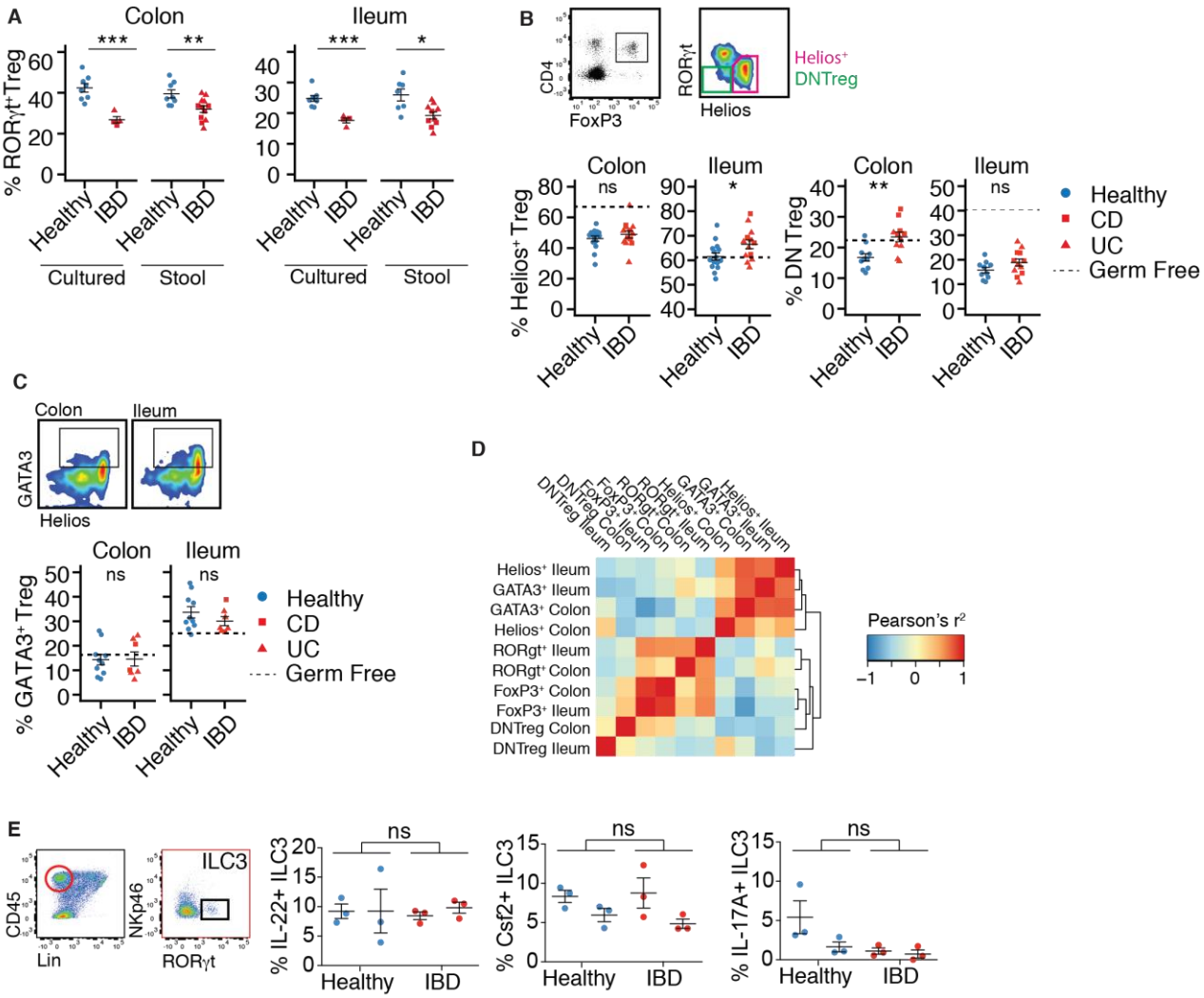

Figure S2. (Related to Figure 3). Transfer of microbiotas from healthy donors specifically increases numbers of RORγt⁺ Treg cells.

Germ free B6 mice were colonized with fecal microbiotas from human donors with and without IBD. Regulatory T cell subsets in the colon and ileum of these mice were analyzed by flow cytometry.

(**A**) The proportion of RORγt⁺Treg induced by donor microbiotas in colon and ileum of B6 gnotobiotic mice colonized with either cultured collections of microbes or total stool microbiota. Each point represents the mean value of a group of 2-12 mice colonized with a single microbiota. Data is also shown in Figure 3C, but here is segregated according to the type of microbiota inoculum. The numbers of RORγt⁺ cells are presented as a proportion of live, CD45⁺, CD4⁺, FoxP3⁺ cells.

(**B**) The mean proportion of Helios$^+$Treg and DNTreg (RORγt- Helios-) cells in colon and ileum of mice colonized with healthy or IBD donor microbiotas. Shown is the mean proportion of Helios$^+$ cells RORγt-Helios- cells as a percentage of CD4$^+$FoxP3$^+$ cells.
(**C**) The mean proportion of GATA3$^+$Treg cells in colon and ileum of mice colonized with healthy or IBD donor microbiotas. Shown is the mean proportion of GATA3$^+$ cells as a percentage of CD4$^+$FoxP3$^+$ cells.
(**D**) Correlations between the proportions of colon and ileum FoxP3$^+$Treg, GATA3$^+$Treg, Helios$^+$Treg, DNTreg and RORγt$^+$Treg cells induced by different human microbiotas.
(**E**) Induction of colon IL-22$^+$, Csf2$^+$ and IL-17A$^+$ ILC3 by four different human microbiotas. Shown is the proportion of cytokine positive cells as a percentage of CD45$^+$, CD3/CD19$^-$, NKp46$^-$, RORγt$^+$ cells. Each point represents data from one mouse and each group shows data from a different microbiota. Statistical comparisons are made using data from all mice colonized with healthy or IBD microbiotas.

(**A-C**) n=15 healthy, 8 UC and 7 CD microbiotas; data are the mean value of a group of 2-12 mice colonized with a single microbiota. ns – not significant, *p<0.05, ** p<0.01, ***p<0.0001, Students t-test (**A**-**C** and **E**); solid horizontal lines indicate mean ± SEM, dashed horizontal lines indicate the mean proportion of cells from a group of germ free mice.

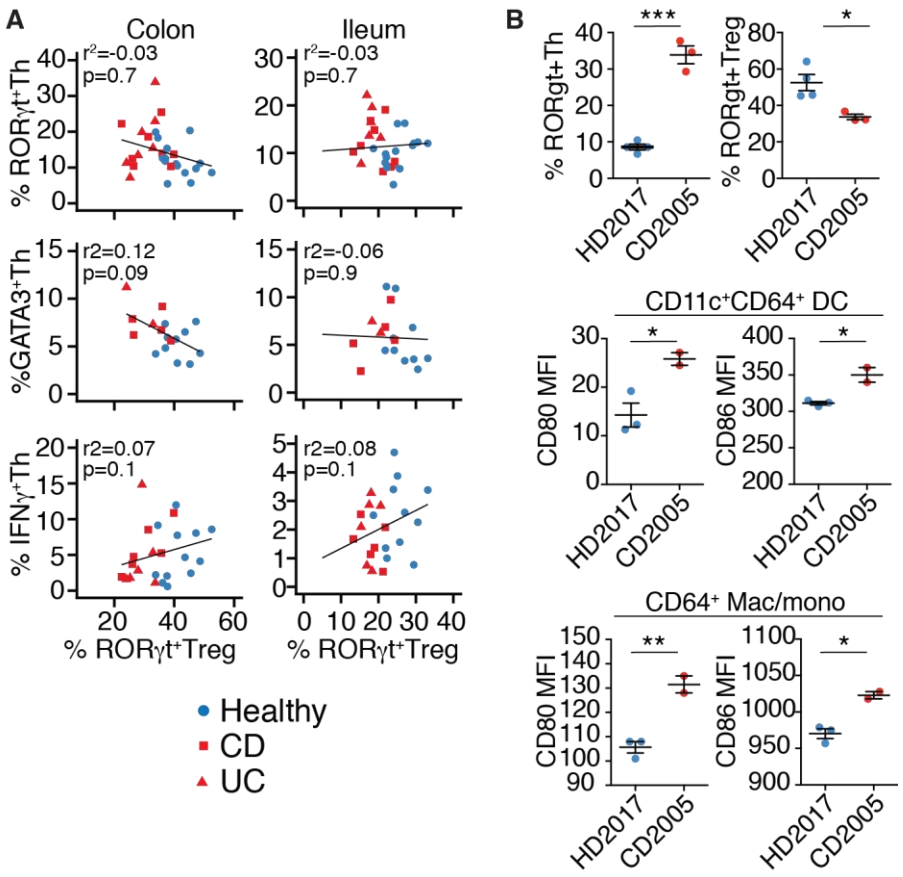

Figure S3 (related to Figure 3) Correlation between the number of RORγt⁺ Treg and antigen presenting cell activation

Germ free B6 mice were colonized with fecal microbiotas from human donors with and without IBD. The proportions of T cell subsets in the colon and ileum of these mice and the activation of antigen presenting cells in the colon was analyzed by flow cytometry.

(A) Correlations between the proportions of colon and ileum RORγt⁺Treg with RORγt⁺Th, GATA3⁺Th and IFNγ⁺Th induced by different human microbiotas. Each point represents the mean data from a group of 2-12 mice.

(B) Expression of CD80 and CD86 on dendritic cells (DC) and macrophages/monocytes (mac/mono) from the colon mice colonized with representative healthy (high RORγt⁺Treg, low RORγt⁺Th) and IBD (low RORγt⁺Treg, high RORγt⁺Th) microbiotas. DC were gated as MHCII⁺, CD64⁻, CD11c⁺ cells. Macrophages/monocytes were gated as MHCII⁺, CD64⁺ cells. Each point represents data from an individual mouse.

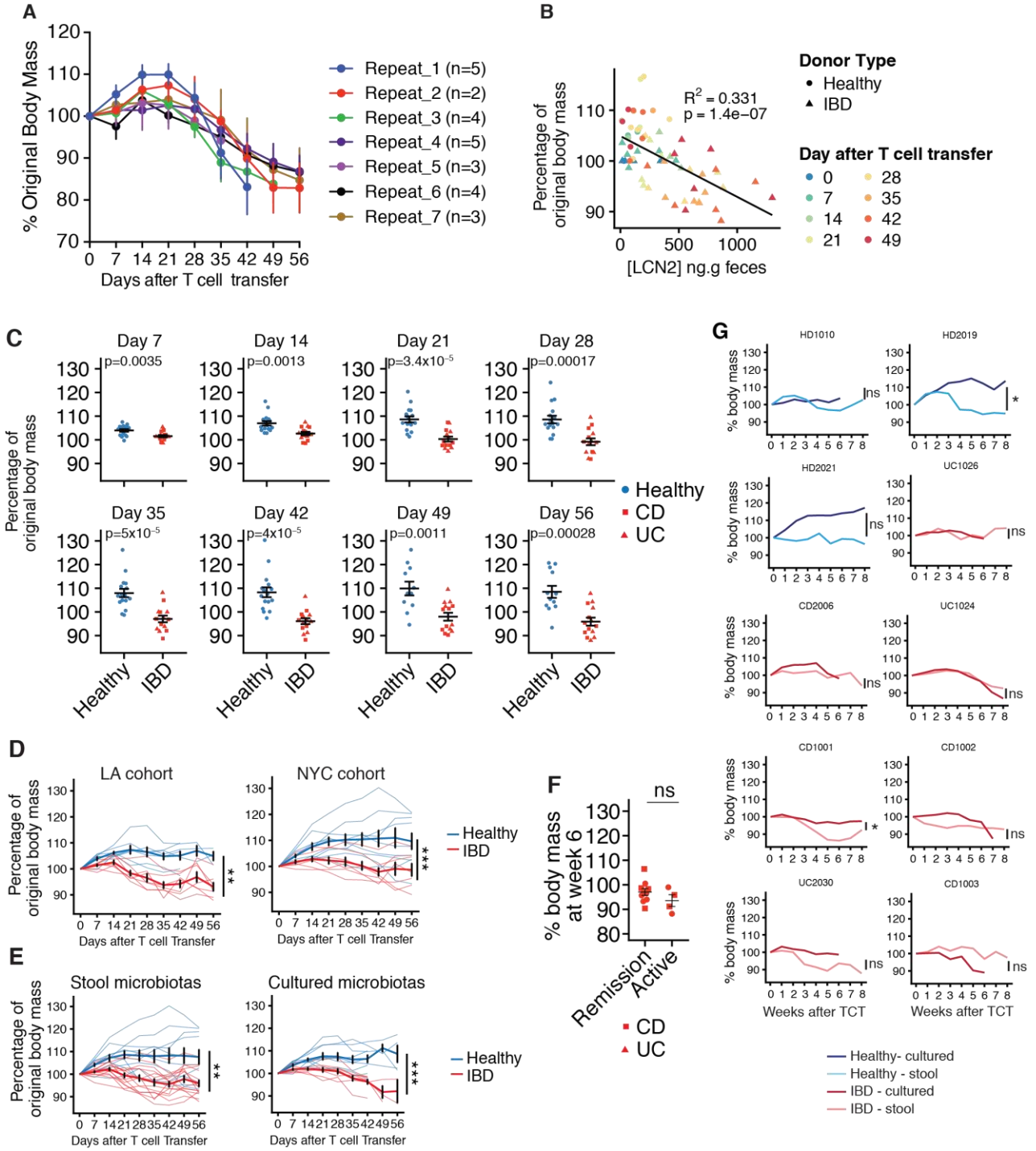

Figure S4. (Related to Figure 4.) IBD-associated microbiotas transmit enhanced colitis severity to susceptible mice.

Colitis was induced by transferring naïve CD4 T cells into Rag1-deficient mice colonized with healthy or IBD donor microbiotas. Colitis severity was assessed by monitoring by changes in body mass and lipocalin 2 in feces.

(**A**) A group of mice colonized with donor microbiota UC1024 was included in all T cell transfer colitis experiments to assess the efficacy and consistency of colitis induction. Shown are weight loss curves for 7 groups of mice, which served as the control group for RagTCT experiments performed at intervals over approximately 24 months.
(**B**) Following induction of T cell transfer colitis, loss in body mass is significantly correlated with an elevation of fecal lipocalin2.
(**C**) Exacerbated colitis in mice colonized with IBD microbiotas can be detected early after T cell transfer.
(**D**) IBD-associated microbiotas from two donor cohorts transmit more severe colitis to mice than healthy donor microbiotas.
(**E**) Complete fecal microbiotas and cultured collections of bacteria from IBD donors both transmit more severe colitis to mice than equivalent microbiotas derived from healthy donors.
(**F**) Colitis severity in RagTCT mice colonized with IBD microbiotas from donors who had active disease or were in remission at the time of fecal microbiota sample colleciton.
(**G**) Comparison of ten donor microbiotas that were assessed for colitogenicity in mice as both stool and cultured collection.

(A) Lines represent the mean ± SEM of groups of mice colonized with donor UC1024. (B) Each point shows data from one mouse at one timepoint from the time of T cell transfer. (C) Each point represents the mean weight loss of a group of 5-15 mice colonized with a microbiota at the indicated time after T cell transfer. Lines represent the mean ± SEM of all healthy or IBD microbiotas. (D and E) Each thin line represents the mean data from a group of 5-15 mice colonized with a single microbiota. Bold lines represent the mean ± SEM of all groups of mice colonized with either a healthy donor or IBD donor microbiotas. (F) Each point represents the mean weight loss of a group of 5-15 mice colonized with a microbiota 6 weeks after T cell transfer. (G) The mean weight change of groups of 5-15 mice colonized with either the complete stool microbiota or the cultured collection microbiota from the same donor. Statistical comparisons were made by f-test (B) or Students t-test (all other panels) *$p<0.05$.

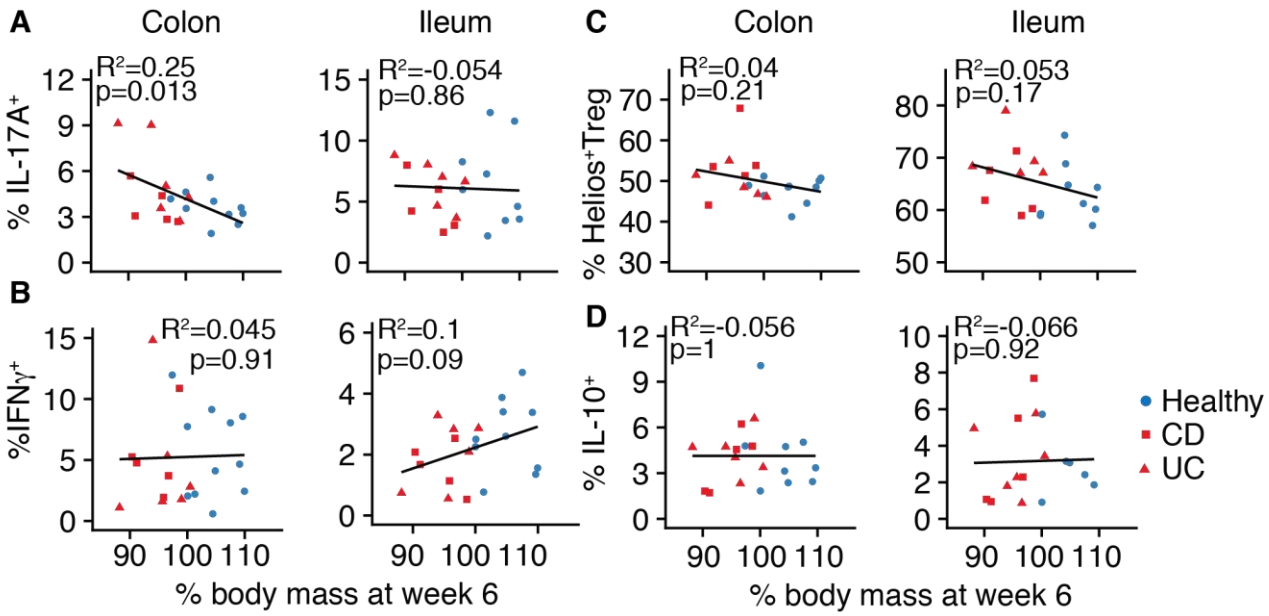

Figure S5. (Related to Figure 5). The relationship between T cell populations in B6 mice and colitis severity in RagTCT mice

(A-D) Relationships between the proportions of T cell populations in the colon and ileum of B6 mice and colitis severity (as measured by weight loss) of RagTCT mice colonized with the same microbiotas. Each symbol represents the mean value from a group of 3-12 mice colonized with a single microbiota. p values calculated by f-test.