

Supplementary Materials for

High frequency of intestinal T_H17 cells correlates with microbiota alterations and disease activity in multiple sclerosis

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- fig. S6. Relative abundance of *Prevotella* and *Streptococcus* strains in HCs and RRMS patients stratified according to immunomodulatory treatment.

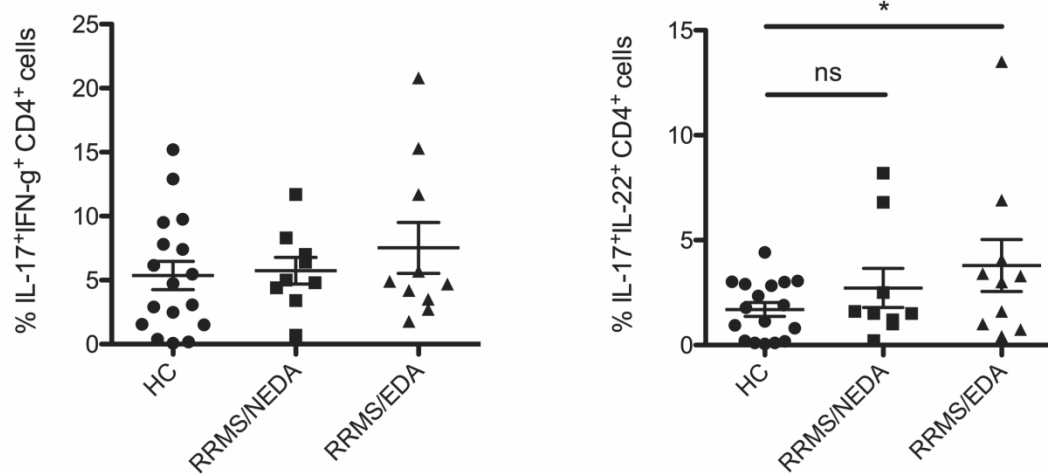


fig. S1. Percentages of double-positive IL-17⁺IFN- γ ⁺ and IL-17⁺IL-22⁺ T cells in the intestinal mucosa of HCs and RRMS patients with active disease (RRMS/EDA) or inactive disease (RRMS/NEDA). Single cell suspensions isolated from small intestinal tissues were stained with monoclonal Abs against CD3 and CD4 and for intracellular cytokines with anti-IL-17A, anti-IFN- γ and anti-IL-22 mAbs and FACS analyzed. Frequencies of double positive IL-17⁺IFN- γ ⁺ and IL-17⁺IL-22⁺ T cells among total CD3⁺CD4⁺ T cells are presented. * P < 0.05 by unpaired t -test.

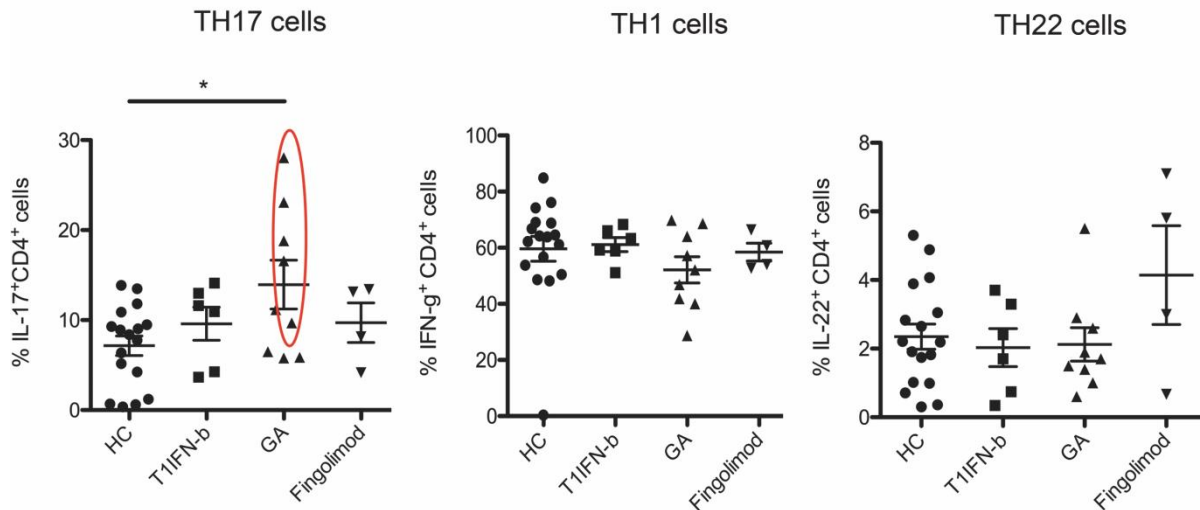


fig. S2. Percentages of intestinal T_H cell subsets in RRMS patients stratified according to immunomodulatory treatment. At the time of EGD and intestinal tissue sampling all RRMS patients enrolled in this study were undergoing treatment with immune modulatory drugs as Beta-interferon (type 1 interferon beta-T1IFN-b), Glatiramer Acetate (GA) and Fingolimod. Percentages of intestinal TH17, TH1 and TH22 cells out of total CD4⁺ T cells in the three cohorts of RRMS patients and healthy controls (HC) are shown. **P* < 0.05 by unpaired *t*-test.

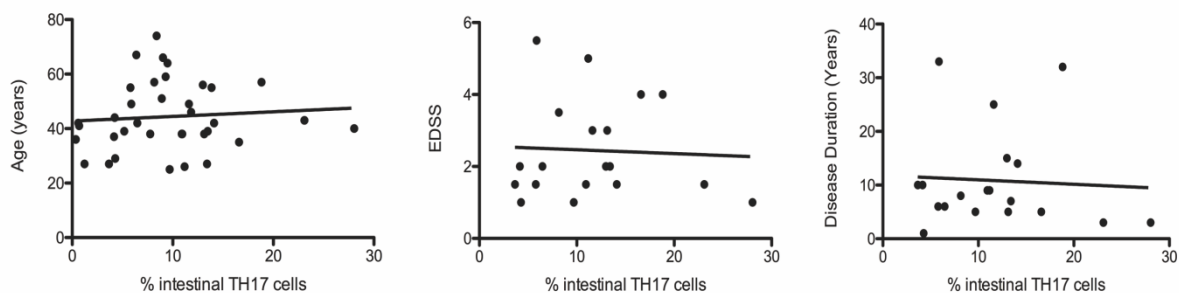


fig. S3. Correlative statistical analysis between percentages of intestinal T_H17 cells and age, EDSS score, and disease duration. Linear regression analysis showed no correlation between age (years) and intestinal TH17 cell percentages in HC and RRMS patients ($r=0.006$). The percentages of intestinal TH17 cells did not correlate also with the disability (EDSS score) and disease duration at the time of intestinal immunological profiling (EGD and sample collection) in RRMS patients ($r=-0.04$ for EDSS and $r=-0.003$ for disease duration).

	TATCGTTAAWGTCAACGACGAAATCGAAATCGTTGGTATCAAAGAAGAACTCAAAAAGCAGTTGTTACTGGTGTGAAATGTTCCGTAAACAACCTTGACGAAGGTCTTGCCGGAGATAACGTAGGTGTCTTCTTCGTGGTGTCAACGTGATGAAATCGAACGTG
RRMS6	TGAGCACATCCTTCTTTCACGTCAGGTTGGTGTAAACACCTTATCGTCTTCATGAACAAAGTTGACTTGGTTGACGACGAAGAATTGCTTGAATTGGTTGAAATGAAATCCGTGACCTATTGTCAGAATACGACTTCCCAGGTGACGATCTCCAGTTATCCAAGGTTGAGTCTTAAAGCCCTTGAAGGTGACACTAAATACGAAGAYATCGTTATGGAATTGATGAACACAGTTGATGAGTACATCCAGAACCCAGAACGTGACACTGACAAACCATTGCTTCTCCAGTGAAGACGTATTCTCAATCACTGGWCGTGGTACAGTTGCTTCAGGACGTATCGACCGTGTATCGTTAAAGTCAACGACGAAATCGAAATCGTTGGTATCAAAGAAGAACTCAAAAAGCAGTTGTTACTGGTGTGAAATGTTCCGTAAACAACCTTGACGAAGGTCTTGCCGGAGATAACGTAGGTGTCTTCTTCGTGGTGTCAACGTGATGAAATCSAACGTG
RRMS7	TGAGCACATCCTTCTTTCACGTCAGGTTGGTGTAAACACCTTATCGTCTTCATGAACAAAGTTGACTTGGTTGACGACGAAGAATTGCTTGAATTGGTTGAAATGAAATCCGTGACCTTCTTTCAGAATACGACTTCCCAGGTGACGATCTCCAGTTATCCAAGGTTGAGTCTTAAAGCTCTTGAAGGTGACTCTAAATACGAAGACATCATATGGAATTGATGAACACTGTTGATGAGTACATYCCAGAACCCAGAACGTGACTGACAAACCATTGCTTCTCCAGTGAAGACGTATTCTCAATCACTGGACGTGGTACAGTTGCTTCAGGACGTATCGACCGTGGTAYGTTCGTGTCAACGACGAAATCGAAATCGTTGGTATCAAAGAAGAAAYCAAAAAGCAGTTGTTACTGGTGTGAAATGTTCCGTAAACAACCTTGACGAAGGTCTTGCCGGAGATAACGTAGGTGTCTTCTTCGTGGTGTCAACGTGATGAAATCSAACGTG

fig. S4. Characterization of *Streptococcus* species by amplification and sequencing. The *Streptococci tuf* gene was amplified starting from 500 ng of extracted DNA using the FastStart High Fidelity PCR System (Roche, Basel, Switzerland) with a double hemi-nested PCR protocol. The outer amplification was performed with the following primers: Tuf_FW_IN-OUT CCA ATG CCA CAA ACT CGT and Tuf_Rev_Outer ACG TTC GAT TTC ATC ACG TTG; and the following cycling conditions: 94°C for 3 min, 30 cycles of 94°C/30", 55°C/1', 72°C/90", 72°C 10 min and then stored at 4°C until usage. A second amplification step was performed to amplify two fragments within the outer region using the following primers: Tuf_FW_IN-OUT CCA ATG CCA CAA ACT CGT and Tuf_Rev_Inner CCT GAA CCA ACA GTA CGT for the first fragment; Tuf_FW_Inner GTA CAG TTG CTT CAG GAC GTA TC and Tuf_Rev_Outer ACG TTC GAT TTC ATC ACG TTG for the second fragment. The following amplification protocol was used: 94°C for 3 min, 35 cycles of 94°C/30", 55°C/1', 72°C/90", 72°C 10 min and then stored at 4°C until usage. The amplification products were sequenced using the AB 3130 XL Genetic Analyzer (Applied Biosystems) and the sequences were analyzed using SeqScape software (Thermo Fisher Scientific). After electropherogram manual editing and ambiguous base permutation, species identification was performed by using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

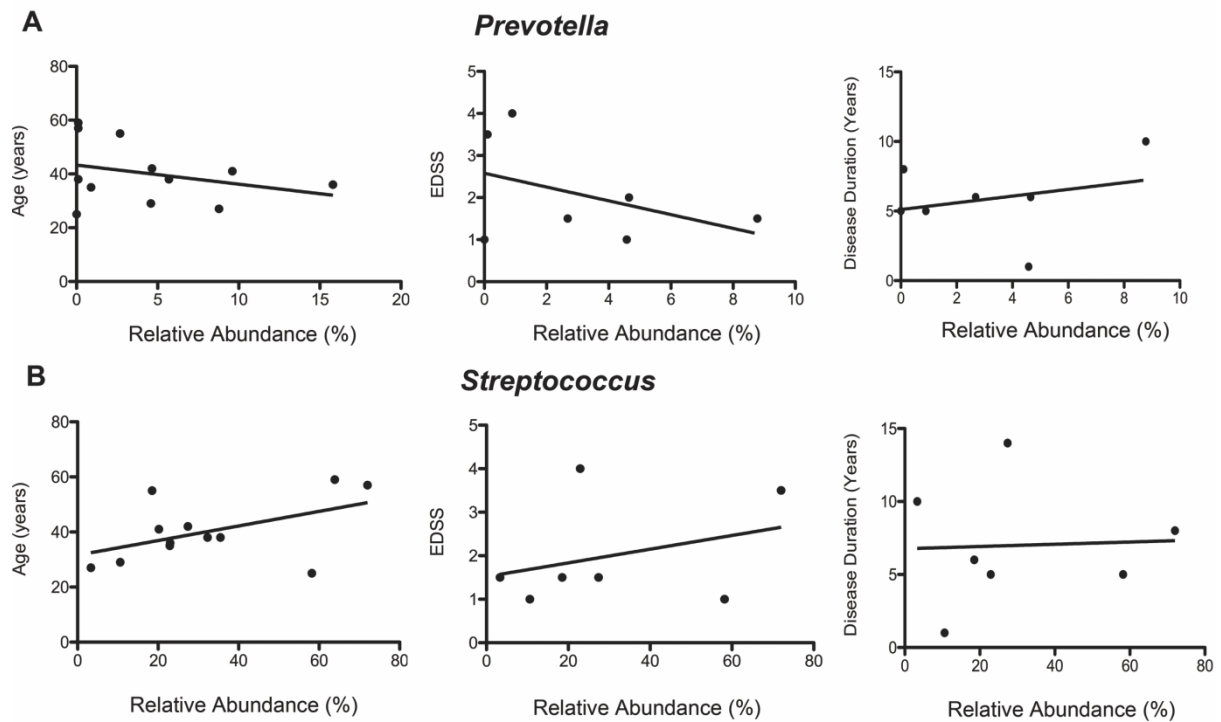


fig. S5. Correlative analysis of relative abundance of *Prevotella* and *Streptococcus* strains in mucosa-associated microbiota and age, EDSS score, and disease duration. Microbiota profiles were assessed by 16S Amplicon Sequencing of DNA purified from human small intestinal tissue of HC and RRMS. Linear regression analysis showed no statistically significant correlation between age (years) and relative abundance of *Prevotella* ($r=0.09$) and *Streptococcus* ($r=0.09$) strains in HC and RRMS patients. We also found no statistically significant correlation between the relative abundance of *Prevotella* and *Streptococcus* strains and disability (EDSS score) or disease duration at the time of intestinal tissue collection in RRMS patients ($r=-0.3$ and $r=0.4$ for EDSS and $r=-0.4$ and 0.01 for disease duration).

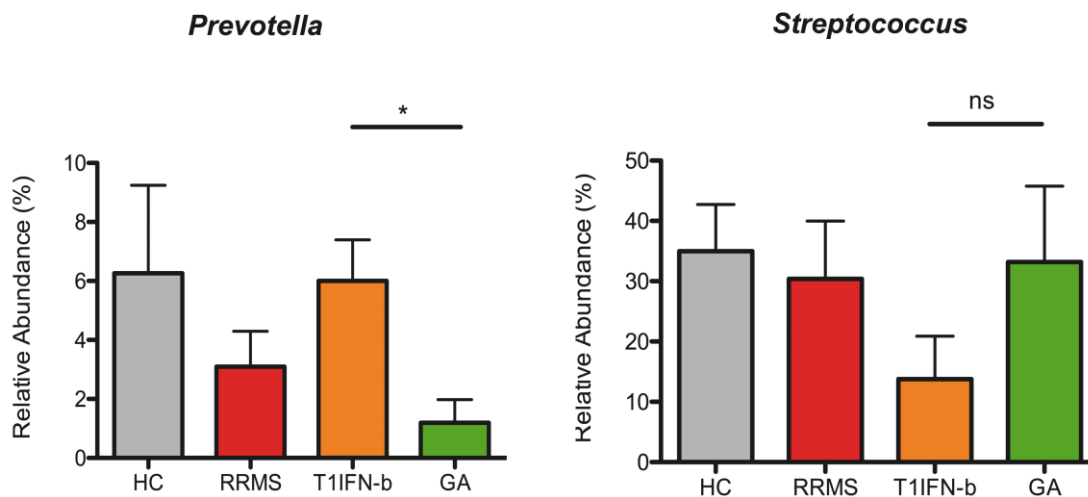


fig. S6. Relative abundance of *Prevotella* and *Streptococcus* strains in HCs and RRMS patients stratified according to immunomodulatory treatment. Gut microbiota profiles were analysed by 16S Amplicon Sequencing of DNA purified from human small intestinal tissue of HC (n=5) and RRMS patients stratified according to the type of immune modulatory treatment that they were receiving at the time of tissue sample collection: Beta-interferon (type 1 interferon beta-T1IFN-b, n=3) or Glatiramer Acetate (n=3). Data are presented as mean \pm s.e.m. of the relative abundance of different phyla. ** $P \leq 0.01$ by unpaired *t*-test.