SUPPORTING SUPPLEMENTAL INFORMATION

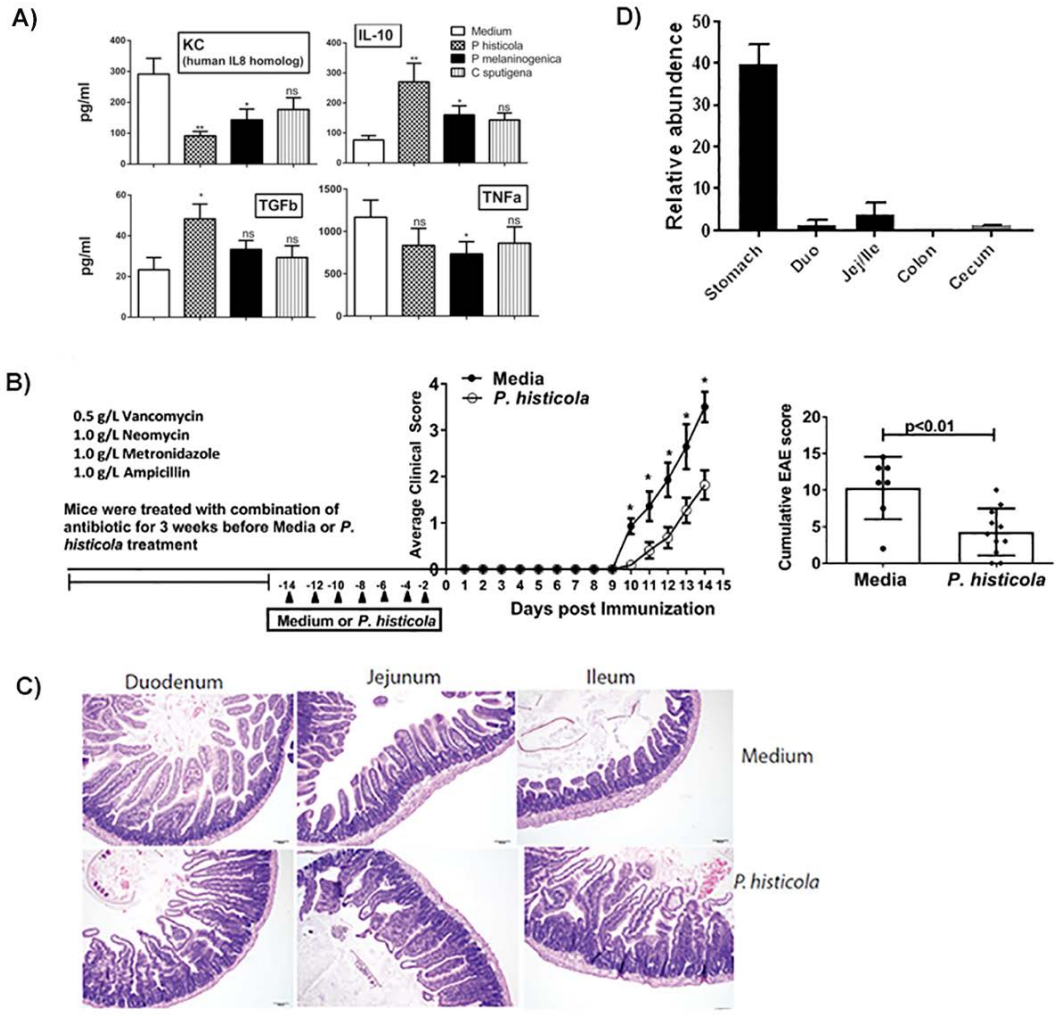

Figure S1. **(A) Human gut-derived gram negative anaerobic bacteria *P. histicola* upregulate IL-10 and TGFβ. Related to figure 1.** Animals (8-12 weeks old) were gavaged with 10^9 CFU/ml of live bacteria or medium on alternate days for a total of 7 doses and sera were collected at Day 14 (2 days after the 7^{th} dose) for cytokine analysis. *P. histicola* challenged group showed an increase in IL-10 and TGF-β together with a decrease in levels of KC. *P. melaninogenica* challenged group showed modulation in cytokines; however, the effect was less than observed in *P. histicola* challenged group. **(B) Colonization of HLA-DR3.DQ8 transgenic mice with *P. histicola* alone suppressed EAE after depletion of gut flora**. Gut flora was depleted in HLA-DR3.DQ8 transgenic mice using broad spectrum antibiotics for three week and colonized with *P. histicola* or medium. One week after the last dose, EAE was induced. The group receiving media only developed severe EAE compared to the group colonized with *P. histicola* at all the time point. The data in figures A and B represent average cumulative scores with n=7mice in the medium group and n=11 mice in *P. histicola*-challenged group. (**C) *P. histicola* challenged mice showed no adverse pathology in the upper gut.** HLA-DR3.DQ8 transgenic mice were with PLP_{91-110} immunized mice were either challenged with *P. histicola* or medium staring at 7 days post-immunization for a total of 7 doses and small intestinal tissue were stained with hematoxylin and eosin. **(D) *P. histicola* colonized stomach and jejunum/ileum.** Naïve HLA-DR3.DQ8 transgenic mice (8-12 weeks old) were gavaged with either medium or *P. histicola* (7 doses on alternate days) and sacrificed at day 14 to quantify the levels of *P. histicola* by qPCR (Sybr Green) using *Prevotella histicola* specific primers (Forward- CACGTGTGATTGTTTGCAGGT and Reverse- TCCAGCCTACGCTCCCTTTA).

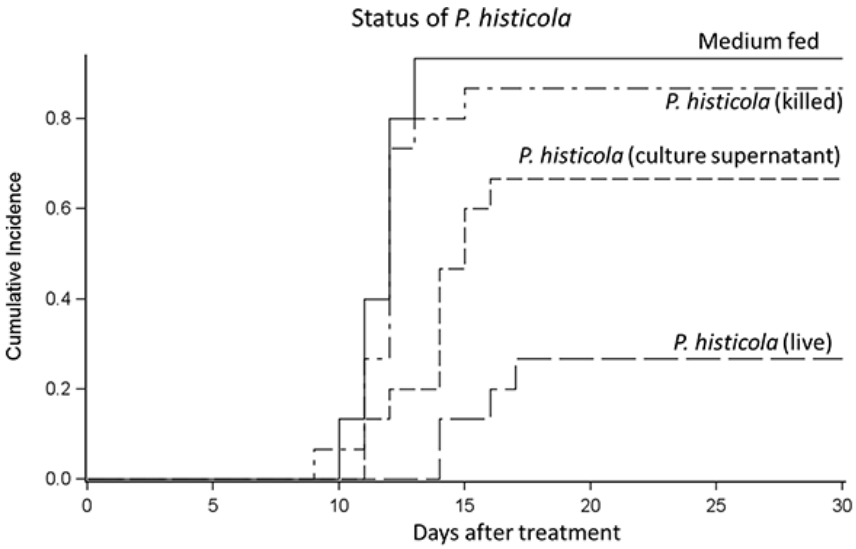

Figure S2. Effect of *P. histicola* on the cumulative incidence of EAE in HLA-DR3.DQ8 transgenic mice. Related to Figure 1. Live *P. histicola* culture showed maximum disease suppressive activity indicating a requirement of active bacteria for suppressive effect. HLA-DR3.DQ8 transgenic mice were immunized with PLP_{91-110} peptide emulsified in CFA and pertussis toxin was given at day 0 and 2 post-immunization. Immunized mice were either challenged bacteria or medium staring at 7 days post-immunization and then every other day for total of 7 doses and weighed daily (as stated in material and methods), and the daily mean weight for each group was plotted. The Kaplan-Meier method was used to estimate the probability of cumulative incidence.

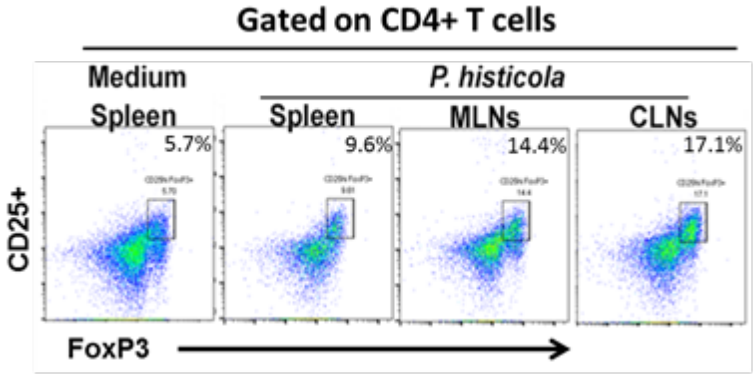

Figure S3. ***P. histicola*** **induces Treg population in naïve mice. Related to Figure 3.** Naïve HLA-DR3.DQ8 transgenic mice (8-12 weeks old) mice were challenged with either medium or *P. histicola* on alternate days for a total of 7 doses. Animals were sacrificed at day 14 (one week after the 7th dose) to collect spleen, MLNs, and CLNs for flow cytometry analysis. Cells were analyzed by flow cytometry using specific antibodies for the expression levels of $CD4^+$, $CD25^+$, and $FoxP3^+$ T cells. We observed that *P. histicola* challenged mice showed higher levels of $CD4^+CD25^+FoxP3^+$ Tregs in spleen, MLNs and CLNs compared to the medium only group. Thus *P. histicola* can induce Treg population in naïve mice.

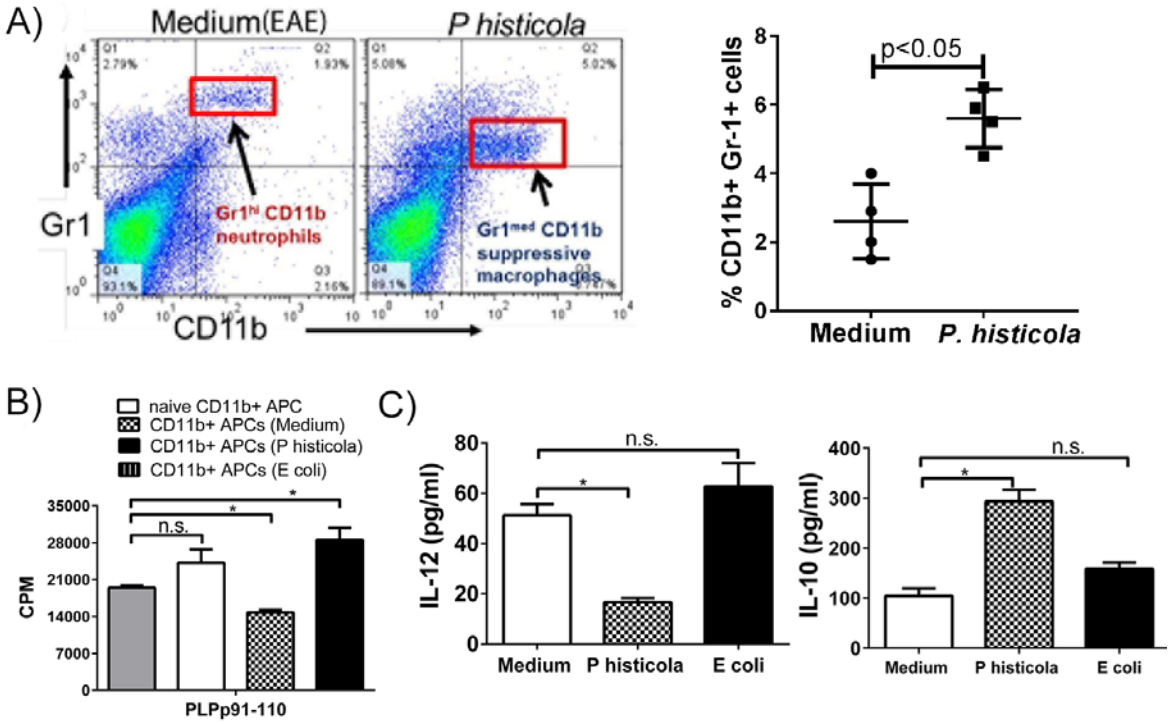

Figure S4. *P. histicola* challenge increased the frequency and activity of suppressive macrophages. Related to Figure 3. **A)** The *P. histicola* −challenged group showed increased frequency of CD11b^{+} GR-1^{med} suppressive macrophages compared with the medium challenged group, which showed increased frequency of CD11b^{+}GR-1^{hi} neutrophils. Numbers in scatter plots indicate the percentage of positive cells. **B)** Co-culture of CD11b^{+} cells from the *P. histicola* −challenged group with PLP specific CD4 T cells resulted in reduced T cell proliferation compared to medium treated or naïve group. The data are presented as the mean±SD CPM and are the average of 2 independent experiments with n=3 mice per group. **C)** CD11b^{+} macrophages from the *P. histicola* −challenged group produce high IL-10 and low IL-12 compared with the medium challenged group. The data presented are the average of 2 independent experiments with n=3 mice per group. A single asterisk indicates p≤0.05 and 'n.s.' indicates not significant, when compared to naïve CD11b^{+} group (B), or medium challenged group (C).

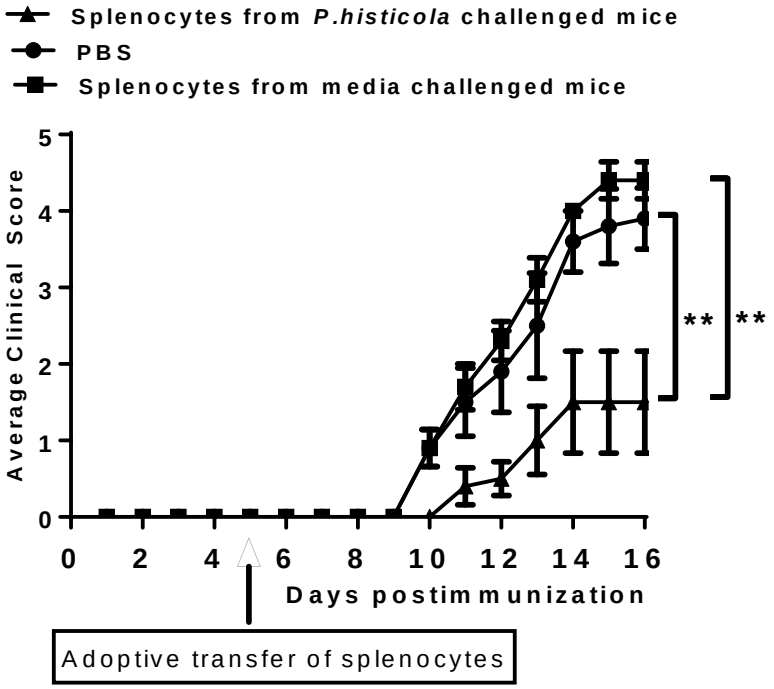

Figure S5. Adoptive transfer of splenocytes from *P. histicola* challenged mice can suppress EAE. Related to Figure 3. To analyze whether disease protection observed in *P. histicola* challenged mice is transferrable, EAE was induced in HLA-DR3.DQ8 transgenic mice and five days postimmunization, splenocytes ($1X10^7$ cells/mouse) from *P. histicola* challenged mice or media treated mice were transferred i.v.. The group receiving splenocytes from *P. histicola* challenged mice showed lower disease severity compared to PBS treated or mice receiving splenocytes from media treated group. The data in figures average clinical scores with n=5 mice in each group. These data suggests that *P. histicola* induced splenocytes can transfer its protective effect.

Treatment	*Disease incidence (%)*	*Disease free, Mean ± SE, days*[A]	*Hazard ratio (95% CI)*	*p-value*
Medium fed	14/15 (93%)	11.7 ± 0.3	1 (ref)	
P. histicola (live)	4/15 (26.7%)	16.5 ± 0.3	0.09 (0.03-0.3)	<0.0001
P. histicola (culture supernatant)	10/15 (66.7%)	14.4 ± 0.5	0.3 (0.1-0.7)	0.005
P. histicola (heat killed)	13/15 (86.7%)	12.3 ± 0.5	0.7 (0.3 -1.5)	0.34

Table S1. Disease suppressive activity of *P. histicola* require active bacteria, related to Figure 1

A- Estimated by the log-rank test

Treatment	*Disease incidence (%)*	*Mean onset of disease±SD*	*Number of mice with maximum severity score*					
			0	1	2	3	4	5
Medium	09/10 (90%)	11.5 ± 1	1	-	-	4	3	2
P. histicola (10^7 CFU)	7/10 (70%)	13.5 ± 2.0	3	-	2	3	1	-
P. histicola (10^8 CFU)	3/10 (30%)	15.1 ± 1.0	7	-	1	2	-	-
P. histicola (10^9 CFU)	4/10 (40%)	14.5 ± 2.0	6	-	-	3	1	-

Table S2. Dose titration of *P. histicola.* Related to Figure 1

Supplemental methods
Isolation and culture of bacteria

The identification and characterization of *P. histicola, P. melaninogenica, and C. sputigena* were performed by the Clinical Microbiology Laboratory. Briefly, pigmented bacteria isolated on laked kanamycin-vancomycin blood agar plates were sub-cultured and frozen in skim milk. *Prevotella* species were grown in trypticase soy broth. A murine strain of *Escherichia coli (E. coli)* was used as a control bacterium.

Flow cytometry

Expression of HLA-DR and HLA-DQ molecules on peripheral blood leukocytes (PBLs), lymph node cells (LNCs), and splenocytes were analyzed by flow cytometry using monoclonal antibodies (mAbs). L227 and IVD12 clones specific for HLA-DR and HLA-DQ (1) respectively, were used to analyze expression of human HLA class II molecules as described previously (2). Surface expression of CD4 (GK1.5), CD8 (53.6.72), B cells (RA3-6B2), CD11c+ DCs (HL3), CD11b+ monocytes/macrophages (M1/70), NK1.1 cells (PK136), CD25 (PC61), Ly6G/Gr-1 (RB6-8C5), CD103 (M290) were analyzed using fluorescent-conjugated mAb from BD Biosciences (San Jose, CA). FoxP3+ (FJK-16s) regulatory CD4 T cells were analyzed using an Anti-Mouse/Rat FoxP3 staining kit from eBiosciences™ (San Diego, CA).

Peptide

The peptide PLP_{91-110} (YTTGAVRQIFGDYKTTICGK) was synthesized at the peptide core facility of Mayo Clinic, Rochester, MN.

Disease induction and scoring

For disease induction, 8-12 weeks old transgenic mice were immunized subcutaneously in both flanks with 100 μg of PLP_{91-110} emulsified in CFA containing *Mycobacterium tuberculosis* H37Ra (400 μg/mice). Pertussis toxin (Sigma Chemicals, St. Louis, MO; 100ng) was injected i.v. at day 0 and 2, post immunization. The disease severity was scored using standard EAE score criteria (3), which were as follows: 0, normal; 1, loss of tail tone; 2, hind limb weakness; 3, hind limb paralysis; 4, hind limb paralysis and forelimb paralysis or weakness; 5, morbidity/death. Mice were observed daily for clinical symptoms.

T cell proliferation and cytokine assay

Splenocytes and lymph nodes were collected from immunized mice and challenged *in vitro* with antigen (4). After 30 h, [$3H^1$] thymidine was added and 18 h later, the cells were harvested, and thymidine uptake was measured using a β-scintillation counter. The results were presented as stimulation indices (CPM of test sample/CPM of the control). For *in vitro* inhibition experiments, mAbs specific for CD4 (GK1.5), CD8 (TIB 105), HLA-DQ (IVD12), and HLA-DR (L227) were added to LNCs challenged *in vitro* with human PLP_{91-110} (20 μg/ml). All of the neutralizing antibodies were generated in the Mayo Clinic antibody core facility.

Splenocytes were collected 3 week post-immunization and stimulated with the PLP_{91-110} peptide as mentioned before in the T cell proliferation section. Supernatants were collected from culture 48 h after peptide stimulation, and the concentration of cytokines (IL-4, IL-10, IL-12, IL-17, IL-23, IFN-γ, and TNF-α) in the supernatant were measured by sandwich ELISA using pairs of relevant anti-cytokine monoclonal antibodies (Pharmingen, San Diego, CA).

Isolation of Tregs, dendritic cells, and macrophages

Regulatory T cells were isolated from splenocytes using the $CD4^+CD25^+$ Regulatory T cell isolation kit (Miltenyi Biotec Inc., San Diego, CA, USA). DCs were isolated from total splenocytes using CD11c Microbeads whereas macrophages were isolated using CD11b Microbeads (both from (Miltenyi Biotec Inc., San Diego, CA, USA). The purity of specific cell populations was analyzed by flow cytometry; all were more than 90% pure.

In vitro functional analysis of Tregs, DCs and macrophages

Suppressive abilities of $CD4^+CD25^+$ regulatory T cells isolated from *P. histicola* or medium challenged HLA-DR3.DQ8 transgenic mice were analyzed by co-culturing of Tregs ($5X10^4$ cells/well) with PLP_{91-110} specific $CD4^+$ T cells ($5X10^4$ cells/well) in the presence of irradiated APCs loaded with antigen. T cell proliferation was measured using a standard $^3H1$ Thymidine incorporation assay. To study the antigen presentation function of DCs and macrophages, $CD4^+$ T cells were plated at $1X10^5$ cells/well in presence or absence of 20μg/ml of PLP_{91-110}. Magnetically sorted DCs or macrophages from *P. histicola* or medium treated HLA-DR3.DQ8 transgenic mice were added at 0.4 X 10^5 cells/well to $CD4^+$ T cells culture in 96 well plates and $^3H1$ thymidine incorporation was measured as described previously. To analyze the ability of DCs and macrophages to produce cytokines, cells were stimulated with LPS for 16-24 h and cell free supernatant was analyzed for levels of IL-10, IL-12 and IL-23 using commercially available ELISA kits (BD Biosciences).

Pathology

Brain and spinal cord pathology were analyzed as described previously (5). Mice were perfused via intracardiac puncture with 50 ml Trump's fixative (4% paraformaldehyde + 0.5% glutaraldehyde). The spinal cords and brains were removed and postfixed for 24–48 h in Trump's fixative in preparation for morphologic analysis. All grading were performed without knowledge of the experimental group.

Spinal cords were cut into 1-mm coronal blocks and every third block postfixed in osmium and embedded in glycol methacrylate. Two-micrometer sections were stained with a modified eriochrome/cresyl violet stain. Morphologic analysis was performed on 12–15 sections per spinal cord. In brief, each quadrant from every coronal section of each spinal cord was graded for the presence or absence of inflammation and demyelination. The score was expressed as the percentage of pathologic abnormality in the spinal cord quadrants examined. A maximum score of 100 indicated a particular pathologic abnormality in each quadrant of each spinal cord section. Brain pathology was assessed after perfusion. Two coronal cuts in the intact brain (one section through the optic chiasm and a second section through the infundibulum) resulted in three paraffin-embedded blocks. The resulting slides were stained with H&E and analyzed for pathology in the cortex, corpus callosum, hippocampus, brainstem, striatum, and cerebellum. Each area of brain was graded on a four-point scale: 0 = no pathology; 1 = no tissue destruction but minimal inflammation; 2 = early tissue destruction, demyelination, and moderate inflammation; 3 = moderate tissue destruction (neuronal loss, demyelination, parenchymal damage, cell death, neurophagia, neuronal vacuolation); 4 = necrosis (complete loss of all tissue elements with associated cellular debris). Meningeal inflammation was graded as follows: 0 = no inflammation; 1 = one cell layer of inflammation; 2 = two cell layers of inflammation; 3 = three cell layers of inflammation; 4 = four or more cell layers of inflammation. The area with maximum tissue damage was used to assess each brain region.

Supplemental References

1. Lampson LA & Levy R (1980) Two populations of Ia-like molecules on a human B cell line. *Journal Of Immunology (Baltimore, Md.: 1950)* 125(1):293-299.
2. Bradley DS*, et al.* (1997) HLA-DQB1 polymorphism determines incidence, onset, and severity of collagen-induced arthritis in transgenic mice. Implications in human rheumatoid arthritis. *J Clin Invest* 100(9):2227-2234.
3. Mangalam A*, et al.* (2009) HLA-DQ8 (DQB1*0302)-restricted Th17 cells exacerbate experimental autoimmune encephalomyelitis in HLA-DR3-transgenic mice. *J Immunol* 182(8):5131-5139.
4. Das P*, et al.* (2000) Complementation between specific HLA-DR and HLA-DQ genes in transgenic mice determines susceptibility to experimental autoimmune encephalomyelitis. *Hum Immunol* 61(3):279-289.

5. Mangalam AK, *et al.* (2014) Absence of IFN-gamma increases brain pathology in experimental autoimmune encephalomyelitis-susceptible DRB1*0301.DQ8 HLA transgenic mice through secretion of proinflammatory cytokine IL-17 and induction of pathogenic monocytes/microglia into the central nervous system. *J Immunol* 193(10):4859-4870.