Supplementary Material:

Methods:

Media, cytokines, and neutralizing antibodies. Complete medium (CM) consisted of RPMI-1640 with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Two recombinant cytokines (R&D Systems, Minneapolis, MN) were diluted in CM: mouse granulocyte/macrophage colony-stimulating factor (GM-CSF, 10 ng/mL) and mouse IL-4 (10 ng/mL). Anti–TGF-β and anti–IL-10 antibodies and isotype control were purchased from R&D Systems. The antibodies used to deplete $CD25^+$ cells were purified using protein G columns (GE Healthcare, Piscataway, NJ) from the supernatant of the PC61 hybridoma cell line (American Type Culture Collection, Manassas, VA).

Mice. Specific pathogen–free female C57BL/6 mice aged 8–10 wk were purchased from Jackson Laboratory (Bar Harbor, ME) and housed in the Animal Maintenance Facility at the University of Michigan Health System. Experiments were conducted on mice aged 10–14 wk. All experiments were approved by the University of Michigan Animal Care and Use Committee. The CD11c-YFP mouse[1] is a gift of Michel Nussenzweig of the Rockefeller University.

Bacterial strains and culture conditions. *H pylori* (SS1, 26695 wild type, 26695 VacA and CagA mutant strains) organisms were grown on *Campylobacter*-selective agar (BD Biosciences, Bedford, MA) supplemented with 5% sterile horse blood, trimethoprim (5 μg/mL), vancomycin (10 μg/mL), and nystatin (10 μg/mL)[2] for 2 days at 37°C in a

humidified microaerophilic chamber (BBL Gas System, with CampyPak Plus packs, BD Biosciences). The bacterial concentrations were calculated by using a spectrophotometer (O.D. 600 nm) and determined using 1 x 10^9 CFU/mL per O.D. measured.

Comparison of DC density in uninfected versus infected mice by two-photon microscopy. The stomach was harvested and attached to a plastic cover slip using a thin layer of Vetbond tissue adhesive. The stomach was bisected in a plane parallel with the cover slip using fine tipped surgical scissors and the overlying half removed to expose the luminal surface. Gastric contents were gently removed from the tissue by swirling in a dish of medium. The tissue was placed in an imaging chamber containing CO_2 independent medium (Gibco). The corpus was identified by bright field microscopy and then imaged by two-photon microscopy at room temperature. Each z series consisted of 71, 2.5 μm steps to capture a sample volume of 225x250x177.5 μm.

Localization of DCs in the stomach by confocal microscopy. To visualize DCs in gastric units, stomachs were excised immediately after sacrifice, flushed with PBS, inflated with Tissue-Tek O.C.T. compound (Sakura Finetek, Torrance, CA) via the duodenal stub, and frozen in Cytocool II (Thermo Scientific, Kalamazoo, MI). 7-μm sections were cut and placed on Superfrost slides (Fisher HealthCare, Houston TX), fixed in 70% ethanol for 5 s, rehydrated briefly in deionized water, and incubated in 10 μg/mL Alexa Fluor 594–tagged *Griffonia simplicifolia* (lectin GS-II; Invitrogen, Carlsbad, CA) in PBS for 1 h at room temperature. Lectin GS-II specifically labels mucous neck cells, which are located below the isthmal stem cell zone.[3] After labeling for 5 min in 1 μg/mL bis-benzimide (Invitrogen) in PBS, slides were rinsed in PBS and cover-slipped in a 1:1 solution of PBS and glycerol.

Images seen with the Axiovert 200 microscope (Carl Zeiss Light Microscopy, Göttingen, Germany) were captured with an AxioCam MRm camera (Zeiss).

DC isolation procedure for mouse stomach. Stomachs were removed, washed in Hanks' Balanced Salt Solution (HBSS), cut into small pieces. Tissue pieces were incubated while being shaken in complete medium containing 5% FBS, L-glutamine, 1% antibiotic/antimyotic (Gibco-Invitrogen,) with 1% (v/v) liberase blendzymes 3 (Roche, Indianapolis, IN), 5U/ml Deoxyribonuclease I (Sigma-Aldrich, St. Louis, MO), and 1 mM dithiotheitol in RPMI 1640 at 37°C for 90 min. Digested fragments were filtered though a 100μm cells strainer, and cell suspensions washed twice in were HBSS. Cells isolated by Optiprep™ (Sigma-Aldrich, St. Louis, MO) density gradient centrifugation (600 *g* for 20 min). The low density cells at the interface were harvested, washed twice, and used as a gastric DC-enriched fraction.

Generation of bone marrow–derived DCs. Erythocyte-depleted murine bone marrow cells were cultured in CM with GM-CSF (10 ng/mL) and IL-4 (10 ng/mL) at 1×10^6 cells/mL.[4] On day 6, nonadherent bone marrow–derived DCs were harvested by vigorous pipetting and enriched by gradient centrifugation using Optiprep density solution (Sigma-Aldrich, St. Louis, MO). The low-density interface containing DCs was collected by gentle aspiration. The recovered bone marrow–derived DCs were washed twice with RPMI-1640 and cultured in CM with GM-CSF (10 ng/mL).

***H pylori*–specific in vitro stimulation.** Erythocyte-depleted splenic $CD4^+$ T cells isolated using MACS MicroBeads were stimulated for 7 days with syngeneic bone marrow–derived

DCs (1×10^6 cells/mL) and *H pylori* SS1 sonicate (5 μg/mL). $CD4^+$ T cells stimulated with either DCs or *H pylori* SS1 sonicate served as controls.

FACS. The percentage of $CD4^+CD25^+Foxp3^+$ Treg was measured using a mouse Treg staining kit (eBioscience, San Diego, CA). Dot plots and histograms were obtained Flowjo version 4.2 (Tree Star, Inc., Ashland, OR).

Animal studies. Naïve C57BL/6 mice were given an intraperitoneal (IP) injection of *H pylori* SS1–stimulated DCs (10^6 cells per injection) on days 0 and 14. Unstimulated DCs treated with phosphate-buffered saline (PBS) served as controls. Beginning on day 21, all mice were infected with *H pylori* SS1; an oral gavage of 10^8 colony forming units (CFU) per mL live organisms administered three times over 1 wk. Mice were sacrificed on week 19 and the spleens and stomachs were removed. Stomachs were cut along the greater curvature into 2-mm strips containing fundic and antral tissue. Paraffin sections were prepared for H&E. Spleens were flushed with RPMI-1640 and $CD4^+$ T cells were isolated using MACS MicroBeads (Miltenyi Biotec, Auburn, CA). For in vivo Treg depletion, C57BL/6 mice (n = 10 per group) were given an IP injection of either PBS or a single 1-mg dose of anti-CD25 monoclonal antibody (PC61). After 30 days, the success of Treg depletion was determined by fluorescence-activated cell sorting (FACS) analysis of isolated splenocytes.

Quantification of *Helicobacter* colonization. A standard curve was generated by extracting total RNA using TRIzol reagent (Invitrogen) from *H pylori* SS1 cultures with densities ranging from 1×10^3–1×10^9 total bacteria. Total RNA also was isolated from stomach tissue using TRIzol reagent. Primer pairs C97 and C98 were used to amplify the 16S rRNA

species specific for *Helicobacter*, and an amplicon of ~400 base pairs was generated.[5] Reverse transcriptase–polymerase chain reaction (RT-PCR) amplifications were performed in a total volume of 25 μL, containing 10× PCR buffer with $MgCl_2$, 10 nM dNTPs, 200 nM primers, 5 μL cDNA, 100 nM Taq polymerase GOLD, and 2.5 μL SYBR Green (Invitrogen). Each PCR amplification was performed in duplicate wells using the iCycler iQ detection system (Bio-Rad Laboratories, Hercules, CA) at 94°C for 10 min, followed by 35 two-temperature cycles at 94°C for 1 min and 55°C for 1 min.

Statistical analysis. Statistical significance was determined by unpaired Student *t* test. Correlation was quantified by Pearson correlation using commercially available software (Prism, GraphPad Software, La Jolla, CA). **P* < .05, ** *P* < .01, and *** *P* < .05 were considered significant.

Supplementary Data:

Supplementary Figure 1. **Treg skewed response of HP-DC is independent of *H pylori* VacA or CagA.** Bone marrow–derived DCs were pulsed with wild-type *H pylori* 26695 (26695 WT), VacA mutant (VacA KO), CagA mutant (CagA KO), or *E coli* (EC) for 18 h. Bacteria-pulsed DCs were cocultured with syngeneic naïve splenocytes for 72 h. (*A*) Induction of $CD4^+CD25^+Foxp3^+$ Tregs was measured by FACS and (*B*) IL-17 production was measured by ELISA. VacA or CagA deletion did not affect Treg-skewing of HP-DC. Data shown are from three separate experiments (**P* < .05).

Supplementary Figure 2. *H pylori* colonization density and gastritis scores of PBS-DC and HP-DC transferred mice. Please refer to **Figure 5*A*** for details of experimental groups.

(*A*) Total stomach RNA was isolated from PBS-DC and HP-DC mice (n = 15). Quantitative RT-PCR was performed to quantify *H pylori* colonization. (*B*) Histological and polymorphic neutrophil scores (gastritis scores[6]) of stomach tissue from PBS-DC and HP-DC mice were similar. (*C*) IL-17 and (*D*) IFN-γ gastric mRNA expressions were determined by quantitative RT-PCR. The mRNA expressions were similar for IL-17, but higher in HP-DC mice for IFN-γ. (*E*) *H pylori* specific IFN-γ^+ CD4 T cells in the spleen were measured by ELISpot per manufacturer's instruction (BD Biosciences). Results shown are from three independent experiments.

Supplementary Figure 3. IL-17, not IFN-γ, production is correlated inversely with Treg induction. *H pylori*–specific IL-17 and IFN-γ production were plotted against the percentage of $CD4^+CD25^+Foxp3^+$ Tregs. Correlation was compared ($P < .05$).

References:

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