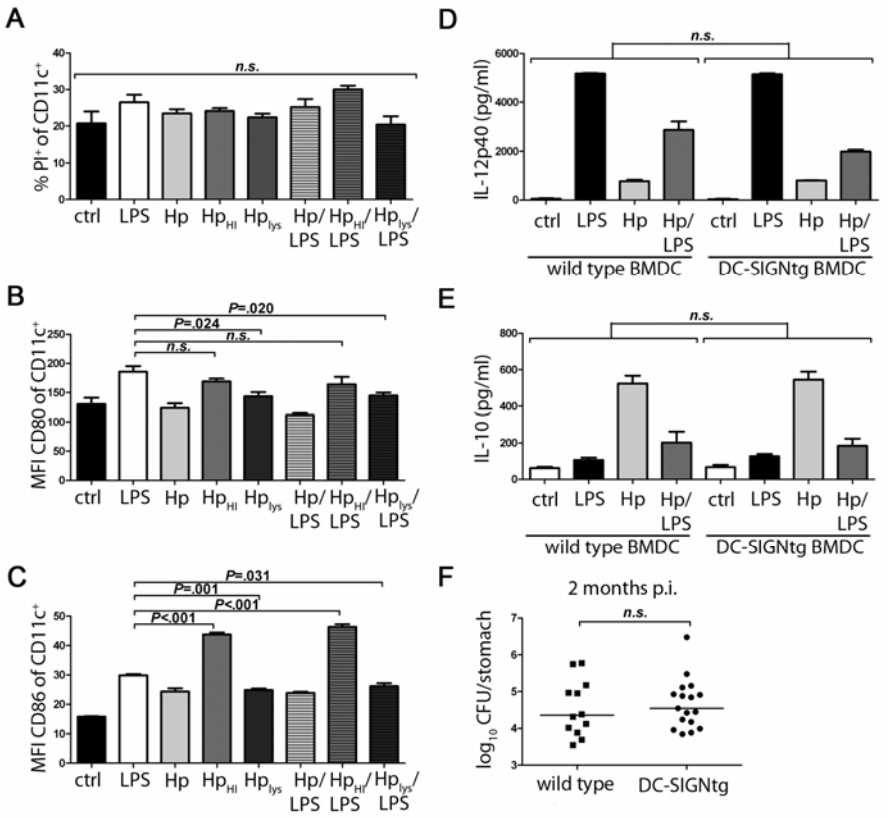
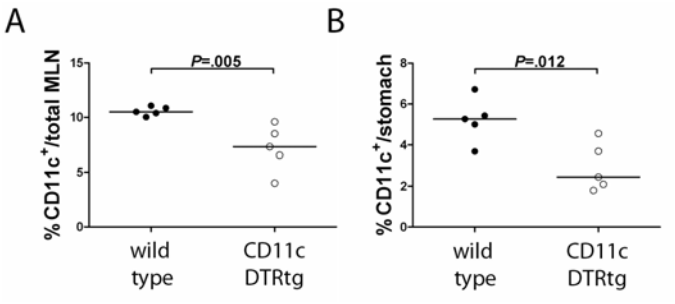
Supplemental Figures:

Supplemental Figure 1



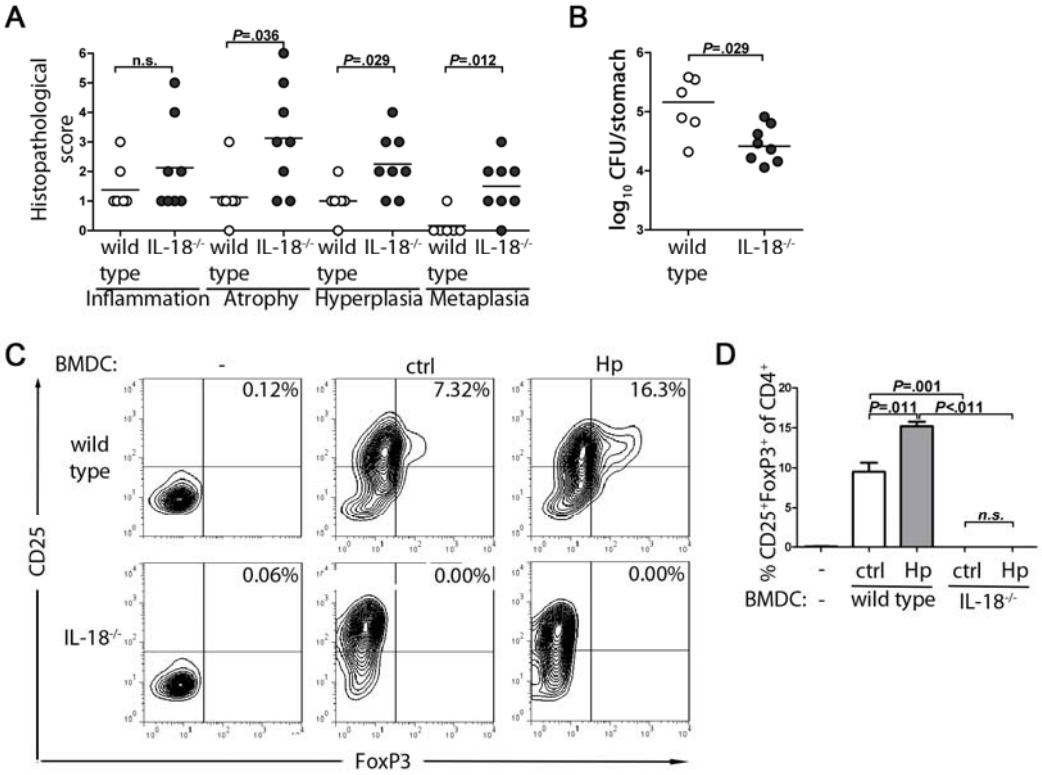
Suppl. Figure 1. BM-DC infection with *H. pylori* does not induce cytotoxicity and treatment of BM-DCs with *H. pylori* sonicate, but not heat-inactivated bacteria, phenocopies the effect of live infection on the LPS-mediated up-regulation of CD80 and CD86; DC-SIGN is not required for the inhibitory activity of *H. pylori*. (**A-C**) BM-DCs were infected with *H. pylori* strain PMSS1 at a multiplicity of infection (MOI) of 50 and/or treated with 0.5μg/ml *E. coli* LPS for 16h prior to the flow cytometric analysis of propidium iodide-positive cells **(A)**, CD80 **(B)** and CD86 expression **(C)**. Additional wells were treated with 10μg/ml *H. pylori* lysate (obtained by sonication, "lys") or heat-inactivated bacteria ("HI") corresponding to an MOI of 50 during the entire 16h exposure to *E. coli* LPS where indicated. **(D-F)** Transgenic expression of human DC-SIGN (DC-SIGNtg) under the *cd11c* promoter does not affect the inhibitory activity of *H. pylori* infection on LPS-induced DC maturation, as assessed by IL-12 and IL-10 ELISA, and *in vivo* infection experiments. Wild type and DC-SIGN-transgenic BM-DCs were infected with *H. pylori* strain PMSS1 at a multiplicity of infection (MOI) of 50 and/or treated with 0.5μg/ml *E. coli* LPS for 16h prior to the assessment of IL-12 and IL-10 production by ELISA **(D,E)**. **(F)** Wild type and DC-SIGN-transgenic mice were infected with *H. pylori* strain PMSS1 for two months and analyzed with respect to *H. pylori* colonization levels by plating and colony counting of gastric homogenate.

Supplemental Figure 2



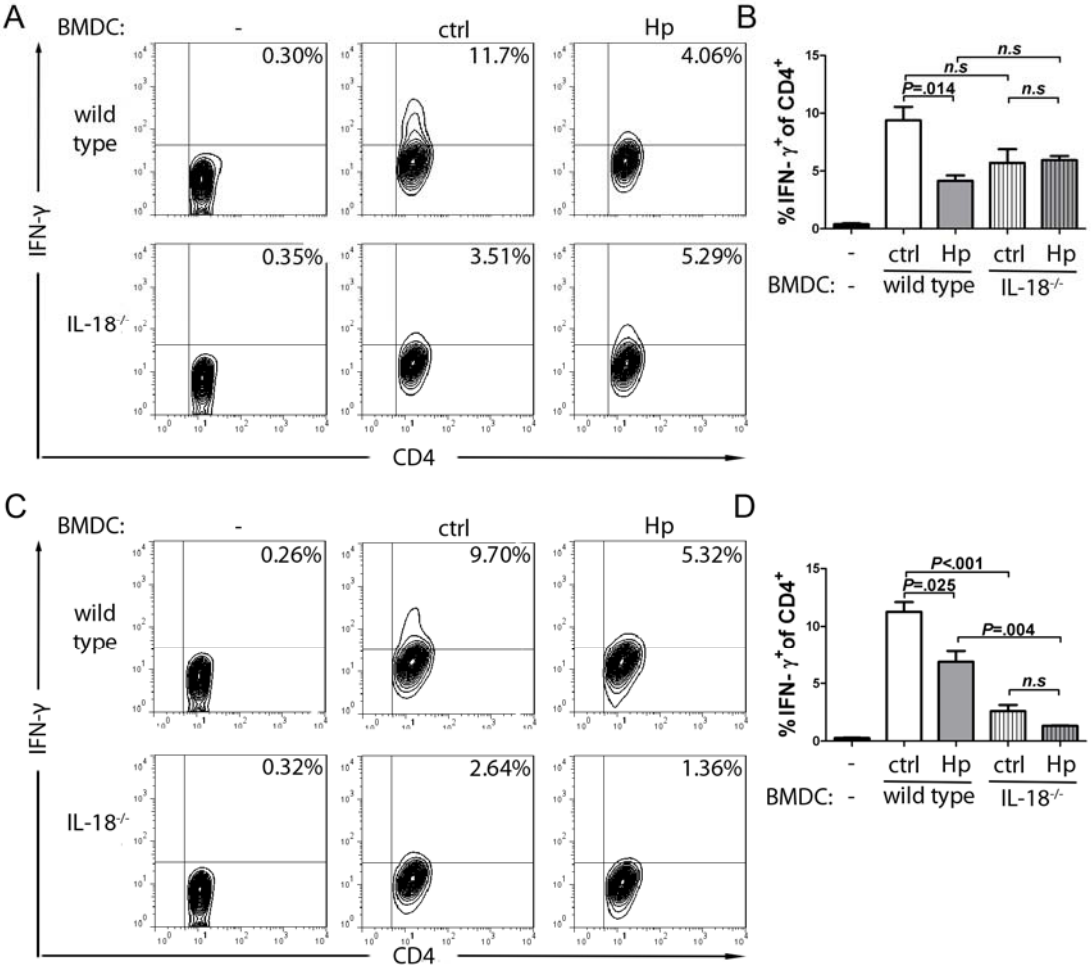
Suppl. Figure 2. DC numbers in wild type and CD11c-DTR transgenic mice after two weeks of DT administration. (**A**) % $CD11c^+$ cells in the MLN. (**B**) % $CD11c^+$ cells in the stomach.

Supplemental Figure 3



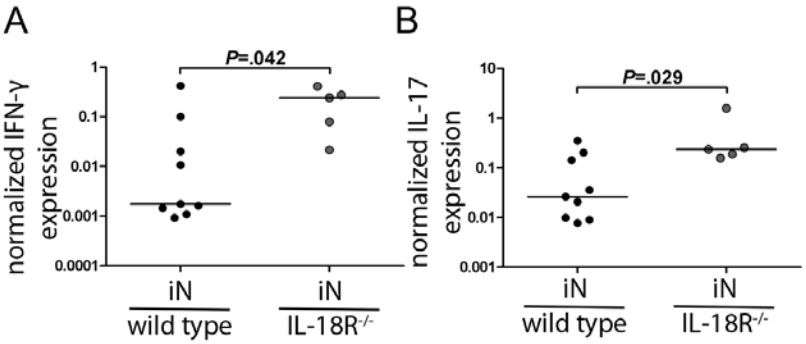
Suppl. Figure 3. IL-18 is required for the restriction of gastric *H. pylori* infection-induced immunopathology and for ovalbumin-specific Treg induction by *H. pylori*-exposed DCs. (A) Histopathology scores of wild type and IL-18$^{-/-}$ mice infected at six weeks of age with *H. pylori* PMSS1 for 1 month; horizontal lines indicate the means. Scores on a scale from 0-6 were assigned independently for the parameters gastric inflammation, atrophy, epithelial hyperplasia and intestinal metaplasia, as described in detail previously (1, 2) **(B)** *H. pylori* PMSS1 colonization levels as assessed by colony count assay; medians are represented by horizontal lines. (**C,D**) Wild type and IL-18$^{-/-}$ BM-DCs treated as described in Figure 7 were loaded with 20μg/ml ovalbumin prior to co-culturing with OTII T-cells in the presence of rTGF-β and rIL-2. CD25 and FoxP3 staining of the CD4^{+} gate are shown for representative mice (**D**) along with the quantification for all mice (**D**).

Supplemental Figure 4



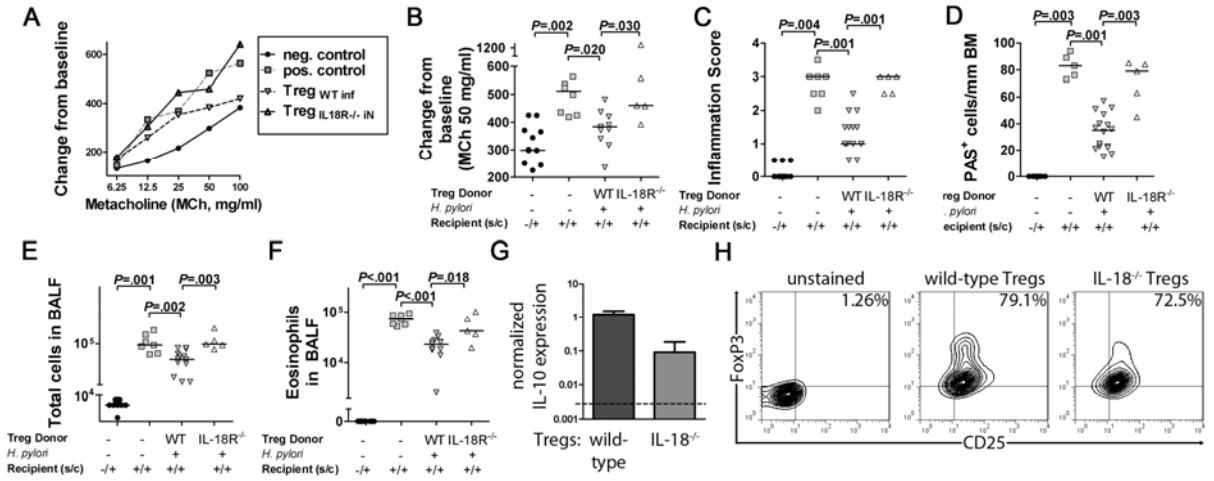
Suppl. Figure 4. IL-18$^{-/-}$ BM-DCs fail to induce IFN-γ expression in co-cultured T-cells. (**A,B**) Wild type and IL-18$^{-/-}$ BM-DCs were infected as described in Figure 2A prior to co-culturing with immunomagnetically isolated, splenic OTII CD4^{+}CD25^{-} T-cells for 3 days in the presence of 10ng/ml rIL-2 and 1μg/ml anti-CD3ε mAb. (**C,D**) The same cells were alternatively loaded with 20μg/ml ovalbumin during the 16h *H. pylori* infection; under these circumstances, anti-CD3ε mAb was not included in the BM-DC/T-cell co-cultures. IFN-γ-producing CD4^{+} T-cells were quantified by intracellular cytokine staining; representative FACS plots and averages of triplicate measurements +/- SEM are shown in A and C, and B and D, respectively. T-cells cultured without DCs served as controls (-).

Supplemental Figure 5



Suppl. Figure 5. Treg differentiation and the development of *H. pylori*-specific tolerance requires IL-18 signaling *in vivo*. (**A,B**) Gastric mucosal IFN-γ and IL-17 expression of the C57BL/6 wild type and BL/6.IL-18R$^{-/-}$ mice neonatally infected with *H. pylori* shown in Figure 8C-F, as determined by qPCR and normalized to GAPDH expression.

Supplemental Figure 6



Suppl. Figure 6. IL-18 signaling is required for the generation of Tregs with suppressive activity and for T-regulatory IL-10 production (**A-F**) Wild type C57BL/6 mice were sensitized with two i.p. doses of alum-adjuvanted ovalbumin prior to challenge with aerosolized ovalbumin 2 weeks after the last sensitization. Two groups of sensitized recipients received 250,000 immunomagnetically isolated $CD4^+CD25^+$ T-cells isolated from the MLNs of either neonatally infected wild type or IL-$18R^{-/-}$ donors one day before the first challenge. Negative controls were challenged without prior sensitization. (**A,B**) Airway hyper-responsiveness as assessed by challenge with increasing doses of metacholine (**A**) and the 50 mg/ml dose (**B**), respectively. (**C,D**) Tissue inflammation and goblet cell metaplasia as assessed and scored on H&E and PAS-stained tissue sections. (**E**) Total cells contained in 1ml of BALF. (**F**) Eosinophils in 1ml of BALF. Horizontal lines indicate medians; "s/c" stands for "sensitized/challenged". (**G**) $CD4^+CD25^+$ T-cells were immunomagnetically isolated from single cell MLN suspensions of neonatally infected (PMSS1, four weeks of infection) C57BL/6 wild type or BL/6.IL-$18^{-/-}$ mice (five per group) and subjected to RNA isolation and IL-10-specific real time RT-PCR. IL-10 transcript levels were normalized to GAPDH expression. Data represent means +/- SEM. The dashed line indicates the average IL-10 expression of Tregs from three uninfected wild type and three uninfected IL-$18^{-/-}$ controls. (**H**) Representative FACS plots of FoxP3 and CD25 staining demonstrating that $CD4^+CD25^+$ T-cells from IL-$18^{-/-}$ donors do not differ from wild type $CD4^+CD25^+$ T-cells in terms of FoxP3 expression.

Supplemental References:

1. Arnold, I.C., Lee, J.Y., Amieva, M.R., Roers, A., Flavell, R.A., Sparwasser, T., and Muller, A. 2011. Tolerance rather than immunity protects from Helicobacter pylori-induced gastric preneoplasia. *Gastroenterology* 140:199-209.
2. Sayi, A., Kohler, E., Hitzler, I., Arnold, I., Schwendener, R., Rehrauer, H., and Muller, A. 2009. The CD4+ T cell-mediated IFN-gamma response to Helicobacter infection is essential for clearance and determines gastric cancer risk. *J Immunol* 182:7085-7101.

Supplemental Methods:

Real time RT-PCR

For real-time RT-PCR, total RNA was isolated from one-sixth of every stomach (antrum and corpus) using NucleoSpin RNA II kits (Macherey-Nagel). The corresponding cDNA served as a template for real-time PCR performed using the LightCycler 480 SYBR Green I master kit (Roche). Absolute values of IFN-γ, IL-17 and IL-10 expression were normalized to GAPDH expression (conditions: Tm 55°C, 50 cycles; primers: GAPDH fw GAC ATT GTT GCC ATC AAC GAC C; GAPDH rv CCC GTT GAT GAC CAG CTT CC; IFN-γ fw CAT GGC TGT TTC TGG CTG TTA CTG; IFN-γ rv GTT GCT GAT GGC CTG ATT GTC TTT; IL-17 fw GCT CCA GAA GGC CCT CAG A; IL-17 rv AGC TTT CCC TCC GCA TTG A; IL-10 fw CTA GAG CTG CGG ACT GCC TTC A; IL-10 rv CCT GCT CCA CTG CCT TGC TCT TAT).