Supplemental Figure legends

Suppl. Figure 1: *H. pylori PMSS1, but not its derivative SS1, injects CagA into gastric epithelial cells and induces gastric cancer precursor lesions in C57BL/6 mice.* (A) Groups of 6 week old C57BL/6 mice were infected with PMSS1 (squares) or SS1 (triangles) for 2 weeks, 1 month and 2 months. Gastric colonization was determined by plating and colony counting. (B) To demonstrate CagA delivery, AGS gastric adenocarcinoma cells were co-cultured with both strains for 8h. Phase contrast micrographs show elongation and scattering (the "hummingbird phenotype") of cells co-cultured with PMSS1, but not SS1. (C) Representative micrographs of Giemsa-stained paraffin sections of mice infected with PMSS1 or SS1 for 2 months. (D) Pathology scores for the indicated parameters (chronic inflammation, atrophy, epithelial hyperplasia, intestinal metaplasia) as assigned to all mice shown in A.

Suppl. Figure 2: *The CagA protein of H. pylori PMSS1 harbours three predicted C-terminal EPIYA phosphorylation motifs.* Upper panel: DNA and amino acid sequence of PMSS1 CagA; predicted C-terminal EPIYA phosphorylation motifs are marked in color. Lower panel: Schematic of the C-terminus of the PMSS1 CagA protein.

Suppl. Figure 3: *Neonatally infected mice fail to produce gastric pro-inflammatory cytokines in response to H. pylori infection.* The gastric production of the proinflammatory cytokines IFN-γ, IP-10 and Mip-2 was evaluated by RT-PCR for the same mice as shown in Figure 2. GAPDH levels are shown for comparison. UreaseB-specific PCR was performed as an additional measure of colonization (uppermost panels).

Suppl. Figure 4: *The antrum histology does not differ between uninfected and infected mice of both age groups.* The same mice as shown in Figure 2 were examined with respect to antral histopathology. No obvious differences were detected between uninfected and infected groups or between neonatally and adult-infected groups. Representative Alcian blue- and Periodic Acid Schiff-stained micrographs of the 4 month time point are shown.

Suppl. Figure 5: *Differences in pathology based on age at the time of infection are independent of the type IV secretion system and can also be observed in H. felis infected mice*. C57BL6 mice were infected at 7 days (iN) or 5 weeks (iA) of age in groups of 4-7 animals with *H. felis* (A,B), or PMSS1ΔCagE (C,D). Mice were sacrificed after 1 and 3 months and analyzed with respect to colonization (A,C) and gastric histopathology (B,D). Despite similar levels of colonization, adult-infected and neonatally infected mice differed substantially with respect to gastric histopathology at the three month time point: whereas adult infected mice had developed gastritis as well as preneoplastic epithelial lesions, no such lesions were detected in neonatally infected mice (B,D). Histopathological parameters were scored as described in the figure legend to Figure 1. The colonization of *H. felis* (A) was assessed by qPCR using flagellin B (*flaB*) specific primers, as *H. felis* does not form single colonies and thus cannot be assessed by colony counting. *H. felis* genome copies per stomach were calculated based on the premise that 2 fg of *H. felis* chromosomal DNA is equivalent to 1 copy of the genome. Each sample was analyzed in triplicate; averages are plotted and medians are indicated for all groups.

Suppl. Figure 6: *Depletion of regulatory T-cells breaks tolerance of neonatally infected mice.* (A-C) Mice were infected with PMSS1 at 7 days of age, and depleted of Treg by regular weekly i.p. injections of anti-CD25 antibody, starting on the day of infection (clone PC61; 1. dose: 100µg; 2.-4. dose: 50µg, 6 animals). A control group of 5 mice remained untreated. All mice were sacrificed four weeks p.i. and analyzed with respect to *H. pylori* colonization as assessed by colony counting (A) and gastric histopathology (B,C). Pathology scores for the four indicated parameters are shown in B, and representative micrographs are shown in C.

Suppl. Figure 7: *Natural Treg are generated at normal levels, but the induction of Treg in vitro is impaired in CD4-dnTßRII mice.* (A) $CD4^+FoxP3^+$ cells were quantified as a fraction of all $CD4^+$ T-cells in the MLN and spleens of five wild type and five CD4-dnTßRII mice. Means and standard deviations are shown per group. (B) Bone marrow-derived dendritic cells (DC) were generated by differentiation of freshly isolated wild type bone marrow cells in 20ng/ml GM-CSF for 7 days. 200'000 $CD4^+CD25^-$ T-cells immunomagnetically isolated from a wild type (top panels) or a CD4-dnTßRII (bottom panels) donor spleen were added to 100'000 DC and stimulated with 1µg/ml anti-CD3ε antibody (clone 145-2C11, Pharmingen), 10ng/ml recombinant IL-2 and 10ng/ml TGF-β as indicated. The $FoxP3^+$ fraction of the $CD4^+$ population was determined by flow cytometry.

Suppl. Figure 8*: Interleukin-10 produced by $CD4^+$ T-cells is required for stable tolerance to H. pylori infection.* Wild type BL6, IL-$10^{-/-}$ and IL-$10^{fl/fl}$CD4-Cre mice were infected at 7 days (iN, A,B,E) or 5 weeks (iA, C,D,E) of age with *H. pylori* PMSS1, and groups of 5-7 animals were assessed with respect to colonization (A,C) and pathology (B,D,E) 1 and 2 months later, as indicated. (A) Neonatally infected mice of both IL-$10^{-/-}$ genotypes remain tolerant after one

month p.i., but have cleared or greatly reduced the infection at 2 months p.i. (C) Adult infected mice of both strains are also less densely colonized than wild type after one month (not shown) and two months p.i. (B,D,E) The gastric histopathology is inversely correlated with colonization: mice that are colonized at high levels show no histopathological lesions, whereas those that clear the infection develop severe gastritis accompanied by atrophy, hyperplasia and metaplasia. (E) Representative micrographs of the two month infection time point.

Suppl. Figure 9: *Neonatal and adult mice differ with respect to their H. pylori colonization levels and their CD4$^+$ FoxP3$^+$ T-cell compartment.* (A) Seven day old (iN) and five week old (iA) C57BL6 mice were infected with 10^7 *H. pylori* PMSS1 and sacrificed at 1, 2, 3, 4, 9, 13, 16, 21 and 28 days p.i. The median colonization of at least 2 and up to 6 mice per time point is shown for both age groups. (B, C) Four seven day old (N) and three six week old (A) C57BL6 mice were sacrificed and their CD4$^+$ (B) and CD4$^+$ FoxP3$^+$ (C) T-cell compartments in the spleens and MLN were analyzed flow cytometrically and results were confirmed in two independent experiments. Means and standard deviations are shown per group.

Supplemental Methods

Bacterial strains and culture conditions

The *H. pylori* strain used in this study for *in vivo* experimentation, PMSS1, is a clinical isolate of a duodenal ulcer patient and the parental strain of the mouse-derivative Sydney strain 1 (SS1).[1] The PMSS1ΔCagE isogenic mutant was constructed by replacement of the entire *cagE* gene with the chloramphenicol resistance cassette of *C. coli* (NCBI accession number M35190; for primer sequences, see suppl. Table 1). *H. felis* CS1 was purchased from ATCC (49179). *H. pylori* was grown on horse blood agar plates and in liquid culture as described previously.[2] Cultures were routinely assessed by light microscopy for contamination, morphology, and motility.

Cell culture and determination of CagA translocation *in vitro*

AGS cells (ATCC CRL 1739; human gastric adenocarcinoma epithelial cell line) were cultured in DMEM (Gibco/BRL) supplemented with 10% heat-inactivated FBS (Gibco/BRL). For assessment of CagA translocation into cultured epithelial cells, AGS cells were infected with *H. pylori* for 16 hours; extracts were subjected to immunoblotting using either CagA- or phospho-CagA-specific antibodies[2] or stained for 3D-confocal microscopy using previously described reagents and procedures.[2] For visual quantitative assessment of the "hummingbird phenotype" as a measure of CagA translocation and phosphorylation, the percentage of elongated cells was determined after 8 hours by microscopical examination and scored on a scale of 0-4. The scores indicate the following: 0, no elongated cells; 1, <10% of cells elongated; 2, 10-30% of cells elongated; 3, 30-50% elongated; 4, 50-100% elongated. All results were verified by another examination after 16 hours.

Assessment of anti-*H. pylori* serum titers, gastric cytokine responses and bacterial colonization

For the evaluation of *Helicobacter*-specific antibody responses, blood was harvested post mortem by cardiac puncture. The serum was diluted 1/10.000 and assessed by ELISA on 96-well plates (Nunc, Roskilde, Denmark) pre-coated with 5µg of PMSS1 sonicate per well. For conventional RT-PCR of IFN-γ, IP-10, MIP-2, and GAPDH, total gastric RNA was isolated using RNeasy Mini columns (Qiagen, Hilden, Germany). 1.5µg of total RNA was used for first strand cDNA synthesis with Superscript reverse transcriptase III (Invitrogen, Switzerland) and served as a template for PCR reactions (for conditions and primer sequences, see suppl. Table 1). IFN-γ-specific real time PCR (LightCycler; Roche, Basel, CH) was performed with the LightCycler 480 SYBR Green I master kit (Roche, Basel, CH). Absolute values of IFN-γ expression were normalized to GAPDH expression. For the PCR-based assessment of *H. pylori* colonization, whole stomach genomic DNA was subjected to amplification of the urease B gene (for conditions and primer sequences, see suppl. Table 1).

Adoptive transfer of $CD4^+CD25^-$ T-cell populations and flow cytometry

$CD4^+CD25^-$ T-cells were purified from single cell suspensions of freshly isolated spleens. Immunomagnetic sorting was performed using a $CD4^+CD25^+$ T-cell purification kit (R&D Systems, Minneapolis, USA), according to the manufacturer's instructions. 300.000 cells were adoptively transferred into immunodeficient (TCR-$\beta^{-/-}$ BL6) hosts by i.v. injection in 200 µL volume. The $FoxP3^+$ fraction of the $CD4^+$ population was determined by flow cytometry. Single cell suspensions of spleen, stomach or mesenteric lymph nodes were stained for CD4 (clone RM4-5, eBioscience, San Diego, USA) prior to fixation, permabilization and $FoxP3^+$ staining

(clone FJK-16s, eBioscience) or IFN-γ staining (clone XMG1.2, BD Biosciences). FACS analyses were performed on a CyanADP instrument (Dako, Glostrup, Denmark).

Statistical analysis

All p-values were calculated using Graph Pad prism 5.0 or R software. The significance of categorical differences in histopathology scores was calculated by Mann-Whitney or unpaired Wilcox test and the significance of numerical differences (e.g. colony counts, serum antibody titers) was calculated by Student's t-test. In all graphs showing colonization data and histopathology scores (scatter plots), the medians are indicated by horizontal bars. In column bar graphs, standard deviations are indicated by vertical bars. n.a. denotes 'not applicable', and n.s. stands for 'not significant'.

Supplemental References

1. Lee A, O'Rourke J, De Ungria MC, Robertson B, Daskalopoulos G, Dixon MF. A standardized mouse model of Helicobacter pylori infection: introducing the Sydney strain. Gastroenterology 1997;112:1386-97.
2. Tan S, Tompkins LS, Amieva MR. Helicobacter pylori usurps cell polarity to turn the cell surface into a replicative niche. PLoS Pathog 2009;5:e1000407.

Supplemental Table 1: Primer sequences, annealing temperatures and cycle number of PCR reactions performed for the generation of *H. pylori* PMSS1ΔCagE, *H. pylori* quantification and reverse transcription PCR of cytokine genes.

Gene	Nucleotide Sequence (5′- 3′)	Tm (°C)	PCR cycles
CagE N-ter	Fw: CGTATGGGTCAGCAAATGACGA Rv: ATCCACTTTTCAATCTATATCCCCTCTCTTTATAGATATACC	52	30
CagE C-ter	Fw: CCCAGTTTGTCGCACTGATAAAATGAAACAAAGTTTGCGCGA Rv: GCATGTCCTCGCTTATGTTGTT	52	30
Cp^r (ΔCagE)	Fw:GATATAGATTGAAAAGTGGAT Rv: TTATCAGTGCGACAAACTGGG	52	30
ureB (PMSS1)	Fw: CGTCCGGCAATAGCTGCCATAGT Rv: GTAGGTCCTGCTACTGAAGCCTTA	55	34
flaB (*H.felis*)	Fw: TTCGATTGGTCCTACAGGCTCAGA Rv: TTCTTGTTGATGACATTGACCAACGCA	58	30
IFN-γ	Fw: GGTGACATGAAAATCCTGCAGAGC Rv: TCAGCAGCGACTCCTTTTCCGCTT	58	35
IP10	Fw: CCTATCCTGCCCACGTGTTGAG Rv: CGCACCTCCACATAGCTTACA	55	33
MIP2	Fw: AGTTTGCCTTGACCCTGAAGCC Rv: GGAACTAGCTACATCCCACCCA	55	35
GAPDH	Fw: GACATTGTTGCCATCAACGACC Rv: CCCGTTGATGACCAGCTTCC	55	32