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Protease Activated Receptor 1 suppresses *Helicobacter pylori* gastritis via the inhibition of macrophage cytokine secretion and Interferon Regulatory Factor 5

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ABSTRACT:*Background & Aims*

Chronic gastritis from *Helicobacter pylori* infection is a major factor in development of gastric adenocarcinoma. Factors which regulate gastritis severity are important in determining which individuals are susceptible to *H. pylori*-associated disease. While Protease-activated receptor 1 (PAR1) has been identified as one such host factor, its mechanism of action is unknown.

Methods & Results

Using chimeric mice, we demonstrated that PAR1-mediated protection against *H. pylori*-gastritis requires bone marrow-derived cells. Analyses of the gastric mucosa revealed that PAR1 suppresses cellular infiltration and both Th1 and Th17 responses to infection. Moreover, PAR1 expression was associated with reduced vaccine-mediated protection against *H. pylori*. Analyses of *H. pylori* stimulated macrophages revealed that PAR1 activation suppressed secretion of IL-12 and IL-23, key drivers of Th1 and Th17 immunity respectively. Further, PAR1 suppressed Interferon Regulatory Factor 5 (IRF5), an important transcription factor for IL-12 and IL-23, both in the infected mucosa and following bacterial stimulation.

Conclusions

PAR1 suppression of IRF5 and IL-12/23 secretion by macrophages provides a novel mechanism by which the host suppresses the mucosal Th1 and Th17 response to *H. pylori* infection. Dysregulation of this process is likely an important factor in the susceptibility of some individuals to *H. pylori*-associated disease.

INTRODUCTION

The pathological consequences of infection by *Helicobacter pylori*, including gastric adenocarcinoma and peptic ulcer disease,^{1,2} occur as a result of the chronic inflammation that develops from long term colonization by these pathogenic bacteria. This gastritis is characterized by a mixed Th1- and Th17-type immune response, and requires the presence of macrophages.³⁻⁷ Not all *H. pylori* infected persons develop disease, and factors which modify the severity of resulting inflammation appear to play a key role in determining an individual's disease susceptibility. Progression to disease is highly complex and is influenced by an interaction of environmental factors, variability in *H. pylori* virulence factors including the *cag* pathogenicity island,⁸ as well as host genetic factors^{9,10} such as cytokine polymorphisms.^{11,12}

One such host factor is the Protease Activated Receptor 1 (PAR1). PARs comprise a family of cell surface G-protein-coupled receptors that are expressed on a wide range of cell types. PAR activation occurs via a unique mechanism of activation involving cleavage of an extracellular domain by a specific serine protease, unmasking a tethered ligand that binds to the body of the PAR.¹³ This initiates a signalling cascade that can result in a variety of cell responses, including proliferation, apoptosis and/or the production of cytokines and prostanoids.¹⁴⁻¹⁶ PAR-activating proteases are derived from numerous sources including epithelial and inflammatory cells, coagulation factors and bacteria. Activation of PAR by serine proteases, released during infection and inflammation, have been shown to play essential roles in regulating the innate immune response to infection.¹⁶

We have previously shown that PAR1 is a critical suppressor of *H. pylori*-induced gastritis in an animal model, with infected PAR1-deficient mice developing a severe atrophic gastritis, in contrast to controls expressing PAR1 which only developed mild gastritis.¹⁷ The clinical relevance of this observation is supported by studies from us and others that have associated human PAR1 polymorphisms with susceptibility to gastric cancer.^{18, 19} However, since PAR1 is expressed by numerous cell types, including epithelial, endothelial, smooth muscle and immune cells, the source and precise mechanism of its protective activity is unknown.

Here we identify this mechanism, by showing that PAR1 inhibits both the Th1 and Th17 immune response to *H. pylori* in the gastric mucosa, and that suppression of these responses is mediated by PAR1 activation inhibiting the secretion of pro-inflammatory cytokines by macrophages. Further, we demonstrate that while PAR1 is protective against the pathogenic effects of *H. pylori*, it actually inhibits the ability of vaccinations to combat this infection.

RESULTS

***H. pylori* gastritis is regulated by PAR1-expressing bone marrow-derived cells**

Previously, we showed that at two months post-infection, *PAR1*^{-/-} mice are colonized by significantly less *H. pylori* than wild-type controls and develop more severe pathology,¹⁷ though the cells responsible for this effect were not identified. PAR1 is expressed by a range of cell types found in the stomach, including gastric epithelial cells and a variety of immune cells.^{15, 16} Using bone marrow chimeras we now show that mice reconstituted with *PAR1*^{-/-} bone marrow are colonized by significantly fewer *H. pylori*

than mice receiving wild-type bone marrow (Figure 1a). No significant increase in immune cell infiltration was detected in the infected gastric mucosa of mice receiving *PAR1*^{-/-} bone marrow (Figure 1b). However, mice reconstituted with *PAR1*^{-/-} bone marrow developed severe atrophic gastritis upon infection (median atrophy scores of 3), in stark contrast to mice receiving wild-type bone marrow, that only typically developed mild atrophy (median atrophy score of 1; Figure 1b-e). Notably, only mice reconstituted with *PAR1*^{-/-} bone marrow developed severe atrophic gastritis (Figure 1c-f) and this was irrespective of the PAR1 status of the recipient, indicating PAR1 regulation of *H. pylori*-induced gastritis was mediated by bone marrow-derived immune cell populations.

PAR1 suppresses the T helper response to *H. pylori* infection *in vivo*

As a key feature of *H. pylori*-induced gastritis is the induction of a mixed Th1 (i.e. IFN γ) and Th17 (i.e. IL-17A) response,³⁻⁶ we examined the effect of PAR1 on production of these cytokines in the infected mucosa. Gastritis in *PAR1*^{-/-} mice does not develop until after one month infection with *H. pylori*.¹⁷ Gastric tissues from mice with severe gastritis would be expected to contain increased levels of pro-inflammatory cytokines compared to mice with mild gastritis. In order to measure the direct regulatory effects of PAR1 on the host response to infection, without the complication of cytokine levels increasing due to gastritis, we compared cytokine levels in the stomachs of wild-type and *PAR1*^{-/-} mice at one week post-infection..

One week post-infection, both IFN γ and IL-17A were significantly elevated in *PAR1*^{-/-} mice relative to wild-type controls (Figures 2a-b). The increased cytokine levels in infected *PAR1*^{-/-} mice were not due to a failure of these mice to produce local IL-10 (Figure 2c), which is known to be an important regulatory cytokine in this infection.

Analysis of the serum antibody response revealed that *PARI*^{-/-} mice infected with *H. pylori* for two months had a significantly greater IgG2c, but not IgG1 response compared to wild-type mice (Figure 2d), supportive of an increased Th1-type immune response in *PARI*^{-/-} animals.

Flow cytometric analysis was used to quantify immune cells infiltrating into the gastric tissues of *PARI*^{-/-} mice infected with *H. pylori* for one week. This revealed a small but significant increase in the infiltration of CD4⁺ T cells, B cells, neutrophils and macrophages into the *PARI*^{-/-} stomach, relative to wild-type mice (Figure 3). The stomachs of infected *PARI*^{-/-} mice had significantly increased levels of CD4⁺ T cells, B cells and neutrophils compared to uninfected controls. There was a trend towards increased macrophage numbers, but this did not reach significance in this study (Figure 3).

In a further extension of this analysis and in order to evaluate the mucosal immune response to this infection draining the gastrointestinal tract, we also quantified cytokine levels in mesenteric lymph nodes from uninfected and infected mice. Once again, both IFN γ and IL-17A were significantly elevated in *PARI*^{-/-} mice relative to wild-type controls (Figure 4), whereas no significant increase was observed in the Th2 cytokine IL-13, the immunosuppressive cytokines IL-10 and TGF β , nor in pro-inflammatory MIP2. The absence of an increased Th2 response in *PARI*^{-/-} mice is consistent with the similarity of the IgG1 antibody response between these animals and wildtype mice.

Vaccination-mediated protection against *H. pylori* is enhanced in the absence of *PAR1*

IL-17 is potentially a key cytokine in vaccine-mediated protection against *H. pylori*.^{20,}

²¹ As our data indicated that PAR1 regulated the IL-17 response to *H. pylori in vivo* (Figure 2b), this raised the possibility that PAR1 activity may negatively impact upon vaccine effectiveness against *H. pylori*. To test this possibility, *PAR1*^{-/-} and wild-type mice were orally vaccinated prior to challenge with live bacteria. Consistent with the antibody response to infection (Figure 2d), vaccinated and challenged *PAR1*^{-/-} mice mounted a significantly greater *H. pylori*-specific serum IgG2c but not IgG1 response compared to wild-type controls (Figure 5a). In this study we also evaluated the intestinal antibody response and found IgG2c was also the only antibody class significantly increased in intestinal scrapings from vaccinated and challenged *PAR1*^{-/-} mice (Figure 5b).

Vaccination significantly reduced *H. pylori* numbers in both wild-type and *PAR1*^{-/-} mice, compared to sham-vaccinated controls (Figure 5c). However, vaccination of *PAR1*^{-/-} mice resulted in an average 93% reduction in *H. pylori* colonization, compared to only a 63% reduction in wild-type mice (Figure 5d). Hence vaccination was significantly more protective against *H. pylori* colonization in mice lacking PAR1, supporting our hypothesis that PAR1-mediated suppression of cytokines opposes vaccine efficacy.

PAR1 inhibits Th1 and Th17 cytokine secretion by suppressing macrophage production of IL-12 and IL-23

To determine whether concurrent PAR1 activation was required for Th1 and Th17 inhibition, we evaluated the *in vitro* cytokine response of murine splenocytes stimulated

with *H. pylori*. Addition of a PAR1-activating, but not control peptide significantly suppressed the IFN γ and IL-17A response of splenocytes stimulated with *H. pylori* for 24 hours (Figure 6a). However this effect was preceded by PAR1-mediated suppression of both IL-12 (a key inducer of IFN γ) and IL-23 (a key inducer of IL-17) at 18 hours post-*H. pylori* stimulation (Figure 6b).

These data indicated that PAR1 activation might not directly suppress T cells, but rather act upstream by inhibiting the secretion of IL-12 and IL-23. The main cell types that secrete IL-12 and IL-23 are macrophages and dendritic cells (DCs). Despite repeated attempts (six experiments) we found no evidence that PAR1 activation inhibits the secretion of IL-12 or IL-23 by DCs (Figure 7a). However, PAR1 activation reproducibly suppressed cytokine secretion by macrophages (Figure 7b). Importantly, we showed that *H. pylori*-induced secretion of IFN γ (a cytokine produced by T cells and innate immune cells, i.e. NK cells) by splenocytes was macrophage dependent, as depletion of macrophages blocked release of this cytokine (Figure 7c). Moreover, the suppressive effects of PAR1 peptide on IFN γ secretion could be reversed by the addition of rIL-12 (Figure 7c).

PAR1 suppression of IRF5 expression

Bacterial-induced secretion of IL-12 and IL-23 by macrophages is promoted by the transcription factor Interferon Regulatory Factor 5 (IRF5).²² We thus hypothesized that PAR1 may regulate *H. pylori*-induced gastritis via IRF5. Analysis of complete gastric tissues revealed no significant difference in *IRF5* mRNA levels between uninfected wild-type and *PAR1*^{-/-} mice. When infected with *H. pylori* however, *PAR1*^{-/-} gastric tissues expressed significantly elevated *IRF5*, compared to wild-type mice (Figure 8a). A direct

comparison of immune cells revealed that *PAR1*^{-/-} macrophages (Figure 8b) and splenocytes (Figure 8c) have elevated *IRF5* even when unstimulated, demonstrating a fundamental role for PAR1 in *IRF5* regulation. When wild-type splenocytes were stimulated with *H. pylori in vitro*, *IRF5* expression was upregulated, but importantly, upregulation of *IRF5* was completely blocked by the addition of a PAR1-activating, but not a control peptide (Figure 8d) proving that *H. pylori* induces *IRF5* upregulation in immune cells via a process that is inhibited by PAR1 activation.

In order to relate these *in vitro* observations back to the gastric mucosa, *IRF5* levels were quantified in macrophages isolated from the stomachs of uninfected and infected mice. *IRF5* levels were higher in *PAR1*^{-/-} gastric macrophages from both uninfected and infected mice; due to small group sizes, this only reached significance when the groups were pooled (Figure 8).

Finally, we confirmed that regulation of *IRF5* mRNA levels in immune cells translated to modified expression of *IRF5* protein. Western blot analysis showed that *PAR1*^{-/-} splenocytes and macrophages both express increased levels of *IRF5* protein compared to wildtype cells (Figure 9).

DISCUSSION

Host factors that regulate the severity of *H. pylori*-induced gastritis likely play a critical role in determining which individuals succumb to associated disease, including gastric adenocarcinoma. In this study we have demonstrated that one such host factor, PAR1, suppresses both Th1- and Th17-immune responses in the gastric mucosa of *H. pylori*-infected mice, via a process involving bone marrow-derived cells. The latter observation

was surprising as we had previously shown that PAR1 inhibits the production of the inflammatory cytokine MIP-2 by primary gastric epithelial cells.¹⁷ These findings indicate that while PAR1 plays a role in regulating epithelial cytokine production *in vitro*, during gastric *H. pylori* infection it is PAR1 expressing immune cells that have the dominant effect. In support of this, *in vitro* analyses of immune cells revealed that PAR1 suppresses Th1 and Th17 responses by inhibiting the secretion of both IL-12 (a key Th1-promoting cytokine) and IL-23 (a key Th17-promoting cytokine) by macrophages.

These data, combined with our previous demonstration of a greatly exacerbated atrophic gastritis in *PAR1*^{-/-} but not wild-type mice,¹⁷ clearly indicate that in the case of *H. pylori* infection, PAR1 acts to suppress inflammation. However this is not always the role of PAR1. While the literature consistently demonstrates an important role for PAR1 in the regulation of inflammation, the nature of that role varies. For example, PAR1 is anti-inflammatory in oxazolone-induced colitis,²³ and *H. pylori* infection,¹⁷ but pro-inflammatory in viral (influenza) infection of mice.²⁵ These findings suggest a highly complex role for PAR1 in regulating inflammation and, in particular, indicate that PAR1 can have either pro- or anti-inflammatory activity, depending on the nature of the stimulus, and/or the immune cells involved. The latter point is supported by observations that PAR1 is pro-inflammatory for dendritic cells (DCs) in a mouse model of endotoxic shock,²⁶ while our data presented here clearly show anti-inflammatory activity for PAR1 in macrophages.

Related to this is the effect of PAR1 on vaccination. The mechanism by which vaccines reduce *H. pylori* colonisation in mice is unknown, but is believed to be antibody independent,^{27, 28} be T-cell mediated and has been linked to Th17 cells and IL-17,^{20, 21, 27}

although not all evidence supports this.²⁹ Hence while PAR1 protects against the pathological consequences of *H. pylori* infection by suppressing the production of pro-inflammatory cytokines, the same property appears to also reduce the efficacy of vaccine-mediated protection. The increased vaccine efficacy seen in *PAR1*^{-/-} mice, which when infected have elevated levels of gastric IL-17, is consistent with protection being mediated via this cytokine. However the effects of PAR1-suppression of other cytokines on vaccine-mediated protection cannot be ruled out.

In support of this possibility, while PAR1 modifies vaccine-mediated protection, there is no evidence that it influences colonisation during natural infection in the absence of severe gastritis. We have shown that wild-type and *PAR1*^{-/-} mice exhibit no difference in *H. pylori* colonisation levels from one day to one month post-infection,¹⁷ and this occurs despite elevated IL-17 levels in the gastric mucosa of infected mice (Figure 2). Hence increased gastric IL-17 levels alone were not sufficient to reduce *H. pylori* colonisation, and vaccination must therefore induce an additional, currently unidentified, response. Colonisation does drop in *PAR1*^{-/-} mice relative to wild-type controls, but only after 2 months infection and coincident with development of severe gastritis,¹⁷ which is well known to impact upon *H. pylori* numbers in the gastric mucosa.⁵ Interestingly, while macrophage PAR1 appears to inhibit *H. pylori* vaccine efficacy, dendritic cell PAR2 appears to promote vaccine-mediated protection,^{30, 31} indicating a complex interaction of these two PAR family members exerting their effects via different immune cells.

The most important discovery relating to the precise mechanism by which PAR1 regulates *H. pylori* pathogenesis is the finding that this receptor not only suppresses expression of the important transcription factor IRF5 in the infected gastric mucosa, but

that it also regulates IRF5 expression by immune cells. It is notable that *PAR1*^{-/-} macrophages express extremely high levels of IRF5 compared to other cells (Figure 8b/c), and that *PAR1*^{-/-} macrophages isolated from the gastric mucosa express more *IRF5* than wildtype macrophages (Figure 8e). This observation, combined with our demonstration of significantly higher macrophage numbers in the gastric mucosa of *H. pylori* infected *PAR1*^{-/-} compared to wildtype mice (Figure 3) suggests the elevated levels of *IRF5* observed in the infected *PAR1*^{-/-} stomach (Figure 8a) is the result of gastric macrophages.

As IRF5 is a key promoter of IL-12 and IL-23 secretion and thus Th1 and Th17 immunity,²² our finding that PAR1 is an important regulator of IRF5 provides a mechanism for the suppression of inflammation by this receptor. Based on our data, we propose a model by which *H. pylori* infection activates IRF5 (as shown in Figure 8d), which then drive macrophages to secrete IL-12 and IL-23 (Figures 7b/c); these cytokines then promote the development of a mixed Th1 and Th17 immune response (Figures 2 and 6). *H. pylori* infection has been shown to increase thrombin levels in the gastric mucosa.³² Thrombin (considered the most important PAR1 activating serine protease)¹⁴ would then activate PAR1 on macrophages, causing reduced IRF5 expression, and Th1 and Th17 responses, resulting in a suppressed gastritis.

In conclusion, PAR1 suppression of IRF5 and IL-12/23 secretion by macrophages provides a novel mechanism by which the host can suppress the Th1 and Th17 response to *H. pylori* and thereby protect itself against the pathological consequences of this infection. Dysregulation of this process may be an important factor in the susceptibility of some individuals to *H. pylori* associated disease, and may explain the association of a

PAR1 polymorphism (*PAR1* IVSn -14 A/T; known to affect PAR1 activity), with susceptibility to *H. pylori* associated gastric cancer.¹⁹

METHODS

Bacterial culture

For infection of mice and cell stimulation assays, *H. pylori* strain SS1,³³ was cultivated in brain heart infusion broth (BHI; Oxoid, Basingstoke, UK) containing 5% horse serum (JRH Biosciences, USA), 0.02% amphotericin B and Skirrow's Selective Supplements, under microaerophilic conditions for 24 hours at 37°C. For formalin-fixation for vaccination,³⁴ bacteria were suspended in 0.01 M formaldehyde in PBS and adjusted to an OD₆₀₀ of 1.5. After 2 hours gentle shaking at 37°C, the bacteria were shaken overnight at room temperature, washed 3 times in PBS and resuspended at 10⁸ bacteria/mL.

Infection of mice

PAR1^{-/-},³⁵ and wild-type (WT) specific-pathogen free C57BL/6 mice were bred within the Veterinary Science animal house, University of Melbourne, Parkville. All experiments involved age-matched female mice and were performed under University of Melbourne Animal Ethics Committee approval. Mice were infected intragastrically once with 10⁷ *H. pylori* suspended in 0.1 mL BHI.

Generation of radiation chimeras

Recipient mice were irradiated with 2 doses of 550 rads, three hours apart, using a Co⁶⁰ source (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia). The next day, bone marrow cells were harvested from the femurs and tibias of donor mice in PBS with 1% FCS (HyClone, Logan, UT, USA). Cells were washed once then

2×10^6 cells injected into recipients intravenously in PBS. Mice were maintained on drinking water containing 2 mg/mL neomycin (Gibco, Basel, Switzerland) for two weeks. Mice were left for two months prior to infection to allow reconstitution.

Quantification of *H. pylori* colonization by colony-forming assay

H. pylori infection levels within mouse gastric tissues were quantified by colony-forming assay. Briefly, stomachs were opened along the inner curvature and divided into two halves. One half was placed in BHI and homogenized (GmbH Polytron[®] homogenizer, Kinematica, Switzerland). Ten-fold serial dilutions were prepared in BHI broth and aliquots spread over GSSA selective agar plates (Blood Agar Base No. 2 with 5% horse blood, vancomycin hydrochloride (12 $\mu\text{g}/\text{mL}$), polymyxin B (0.4 $\mu\text{g}/\text{mL}$), bacitracin (24 $\mu\text{g}/\text{mL}$), nalidixic acid (1.3 $\mu\text{g}/\text{mL}$) and amphotericin B (5 mg/mL)). After 5 days culture as above, colonies were counted and the number of colony forming units calculated per stomach.³⁶

Histological assessment of gastritis

Gastritis was assessed histologically as described previously.³⁷ Briefly, stomach halves were fixed in 10% neutral buffered formalin, embedded in paraffin and 4 μm thick sections stained with H&E and scored blinded under light microscopy. Inflammation was assessed as: (i) Cellular infiltration (lymphocytes and neutrophils) into the lamina propria, graded from 0-6 (0=none; 1=mild multifocal; 2=mild widespread or moderate multifocal; 3=mild widespread and moderate multifocal, or severe multifocal; 4=moderate widespread; 5=moderate widespread and severe multifocal; 6=severe widespread); (ii) Atrophy (loss of parietal cells), graded from 0-3 (absent, mild, moderate, severe).

Flow cytometry

Gastric cellular infiltrate to be analysed by flow cytometry were isolated by perfusion using a technique modified from Alderuccio et al.³⁸ Stomachs were collected into HBSS (Gibco), perfused with HBSS containing 5% FCS (Gibco), 5 mM EDTA and 1 mM dithiothreitol (perfusion solution) and incubated at 37°C for 15 min then cut into small pieces and poured through a 70 µM cell strainer. After a further 15 min in 10 mL of perfusion solution, pieces were vortexed and again poured through a 70 µM cell strainer. Cells in both filtrates were pooled and collected by centrifugation at 600 g for 5 min. The cell pellet was resuspended in 4 mL of 20% Percoll (Sigma), then overlaid on a 70%/40% Percoll discontinuous gradient and centrifuged at 600 g for 10 min. The interphase was collected, washed with 2% FCS in PBS and blocked in 100 µL of 20% mouse serum (collected in house) and 1 µg/mL anti-FcγII/FcγIII (2.4G2, Walter and Eliza Hall Institute) on ice for 20 min. Cells were stained with anti-MHCII-FITC (M5/114.15.2; Caltag Laboratories, Burlingame, CA, USA), anti-CD45-PE.Cy5.5 (30-F11; BioLegend, San Diego, CA, USA), anti-CD11b-Brilliant Violet 421 (M1/70; BioLegend), anti-CD19-Brilliant Violet 510 (6D5; BioLegend), anti-CD4-Brilliant Violet 650 (RM4-5; BioLegend) and anti-Ly6G-APC.Cy7 (1A8; BioLegend). Cells were washed twice, resuspended in 0.25 mg/mL propidium iodide (ImmunoChemistry Technologies, Bloomington, MN, USA) to exclude dead cells and acquired on a BD Influx cell sorter (BD Biosciences, San Jose, CA, USA). Cell counts were normalised using AccuCount Fluorescent Particles (Spherotech, Lake Forest, IL, USA). Data was analysed using FCS Express (De Novo Software, Los Angeles, CA, USA).

Cell stimulation assays

Peritoneal macrophages and bone marrow-derived dendritic cells were prepared as described previously.¹⁷ Briefly, non-induced peritoneal cells obtained by sterile lavage with ice cold PBS supplemented with 1% (v/v) FCS were washed, and resuspended in RPMI 1640 with 10% FCS (Gibco, complete RPMI). Peritoneal macrophages were enriched to >95% purity by adherence to tissue culture plastic for 1 hour, with non-adherent cells removed by washing. Red cell-depleted splenocytes, macrophages and BMDC were cultured at 10^6 cells/mL in complete RPMI with 100 μ M TFLLR PAR1 agonist peptide or FTLLR control peptide (Auspep, Victoria, Australia) for 10 minutes prior to addition of *H. pylori* (MOI 10). Macrophages were depleted from splenocytes as described above for peritoneal macrophage enrichment. Recombinant IL-12 was purchased from eBioscience (San Diego, CA, USA).

Quantification of cytokine levels by ELISA

Half stomachs and whole mesenteric lymph nodes were homogenized in PBS. Culture supernatants and homogenates were centrifuged to remove debris prior to ELISA. 96-well Maxisorp plates (Nunc, Roskilde, Denmark) were coated overnight with anti-mouse IFN γ (0.1 μ g/well) or IL-10 (0.05 μ g/well) (BD Biosciences San Jose, CA, USA); IL-17A (0.5 μ g/well), IL-23 (0.1 μ g/well), TGF β (0.1 μ g/well) or IL-13 (0.5 μ g/well) (eBioscience) or MIP-2 (0.1 μ g/well; R&D Systems, Minneapolis, MN, USA) in bicarbonate coating buffer, pH 9.6. Plates were blocked with 1% BSA (Sigma) in PBS (blocker) for one hour prior to addition of samples in duplicate for three hours at room temperature or 4°C overnight. Captured cytokines were then labelled with biotinylated anti-mouse IFN γ (0.05 μ g/well) or IL-10 (0.05 μ g/well; BD Biosciences); IL-17A (0.025

µg/well), IL-23 (0.05 µg/well), TGFβ (0.05 µg/well) or IL-13 (0.5 µg/well) (eBioscience); MIP-2 (0.1 µg/well) (R&D Systems) in blocker for one hour prior to the addition of 50 µL horseradish peroxidase conjugated streptavidin (Pierce, Rockford, IL, USA) 1/5000 in blocker for 30 min. Colour was developed with 100 µL of TMB solution prepared as 0.1% of 10 mg/mL TMB (Sigma) in DMSO and 0.006% hydrogen peroxide in phosphate-citrate buffer, pH 5.0, the reaction stopped with equal volume 2M sulphuric acid prior to reading absorbance at 450 nm. Sample concentration was determined against a standard curve of respective recombinant cytokines (same manufacturers as antibodies).. IL-12p70 was quantified by ELISA kit (R&D Systems) as per manufacturer's instructions.

Quantification of antibody response to infection

Sera were collected by cardiac puncture. Intestinal mucus scrapings were collected from the lower 10 cm of the longitudinally opened small intestine using a scalpel blade, weighed, then mixed with an equal volume of PBS containing complete mini-EDTA-free proteinase inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Anti-*Helicobacter* antibody levels were determined by direct ELISA. Maxisorp immunoplates (Nunc, Denmark) were coated overnight with 50 µL of *H. pylori* lysate (100 µg/mL) in bicarbonate buffer, then blocked as above for 45 min at RT. Samples were serially diluted 1/10 in blocker and 50 µL added to duplicate wells, before incubation at RT for 1 hour. After washing, 50 µL of either horseradish peroxidase conjugated goat-anti-mouse IgG1 (Southern Biotech, Birmingham, AL, USA; diluted 1/6,000), IgG2c (Immunology Consultants Laboratory, Newberg, OR, USA; diluted 1/5,000), or IgA (Southern Biotech; diluted 1/5,000) in blocker were added per well and incubated at RT for 45 min. Colour

was developed by addition of TMB solution, and the reaction stopped by adding sulphuric acid as above. Absorbance was read at 450 nm and end point titres calculated.

Vaccination against *Helicobacter pylori*

Mice (n=8) were dosed orogastrically with 200 μ L of either (i) PBS, or (ii) 10^7 formalin-fixed *H. pylori* SS1 mixed with 20 μ g UEA-I.³⁹ Mice received two vaccinations 3 weeks apart. Four weeks after the second vaccination, all mice were challenged with a single oro-gastric dose of 10^7 *H. pylori* SS1 in 100 μ L of BHI broth. Four weeks post-challenge, *H. pylori* infection levels within mouse gastric tissues were quantified by colony-forming assay as above.

Quantification of IRF5

RNA from gastric tissues and cultured cells was extracted using Tri Reagent (Ambion, Austin, TX, USA) according to manufacturer's instructions. RNA from FACS-sorted cells was extracted by disrupting in buffer RLT by passing through a 27G syringe, adding 640 μ g of carrier RNA (Qiagen, Venlo, Netherlands), then using the RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. cDNA was transcribed using the Quantitect Reverse Transcription Kit (Qiagen) according to manufacturer's instructions.

IRF5 mRNA expression levels in gastric tissues and cells were quantified by polymerase chain reaction (qPCR). Primers for murine *Actb* and *IRF5* were as described.^{40, 41} The reaction was performed in duplicate in 25 μ L containing 2 μ L cDNA, 0.2 μ M primers and 12.5 μ L 2x SYBR Green PCR Mastermix (Promega, Madison, WI, USA) using an Mx3005P cycler, (Stratagene, La Jolla, CA, USA). Cycling conditions: 1 cycle at 94°C for 5 min, 40 cycles at 94°C for 30 s, 45 cycles at 60°C for 30 s and 40

cycles at 72°C for 30 s. Expression was determined by the REST formula,⁴² relative to β -actin.

For western blot, macrophages were lysed in radioimmunoprecipitation buffer (150 mmol/L NaCl, 1% IGEPAL, 0.5% deoxycholic acid, 0.1% SDS, 50 mmol/L Tris, pH 7.5) containing protease inhibitor cocktail (Roche). Protein samples were resolved on a denaturing 10% SDS-PAGE gel and transferred to nitrocellulose membrane (Amersham Biosciences). The membrane was blocked with PBS containing 5% skim milk and 0.05% Tween 20 (Sigma) then probed with anti-IRF5 monoclonal antibody (1:500, Abcam, Cambridge, UK) and anti-GAPDH (1:1000, Cell Signaling Technology, Danvers, MA, USA). The primary antibodies were detected with HRP-conjugated anti-rabbit antibody (1:1000, Dako, Glostrup, Denmark). Blots were developed with ECL Prime Detection Reagent (Amersham Biosciences, Amersham, UK) and visualized using an ImageQuant LAS 4000 (GE Healthcare, Fairfield, CT, USA). Densitometry was analysed using ImageJ.

Statistics

Statistical analyses were performed using SPSS software, version 21.0. Histological grading scores were compared by non-parametric Mann-Whitney analysis. All other data were log-transformed and compared by one-way ANOVA with Dunnett's post-hoc analysis where required.

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DISCLOSURES

The authors have no conflict of interest to disclose.

REFERENCES

1. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M *et al.* *Helicobacter pylori* infection and the development of gastric cancer. *The New England journal of medicine* 2001; **345**(11): 784-789.
2. Yuan Y, Padol IT, Hunt RH. Peptic ulcer disease today. *Nature clinical practice Gastroenterology & hepatology* 2006; **3**(2): 80-89.
3. Shiomi S, Toriie A, Imamura S, Konishi H, Mitsufuji S, Iwakura Y *et al.* IL-17 is involved in *Helicobacter pylori*-induced gastric inflammatory responses in a mouse model. *Helicobacter* 2008; **13**(6): 518-524.
4. Bamford KB, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK *et al.* Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. *Gastroenterology* 1998; **114**(3): 482-492.
5. Eaton KA, Mefford M, Thevenot T. The role of T cell subsets and cytokines in the pathogenesis of *Helicobacter pylori* gastritis in mice. *J Immunol* 2001; **166**(12): 7456-7461.
6. Kimang'a A, Revathi G, Kariuki S, Sayed S, Devani S, Vivienne M *et al.* IL-17A and IL-17F gene expression is strongly induced in the mucosa of *H. pylori*-infected subjects from Kenya and Germany. *Scandinavian journal of immunology* 2010; **72**(6): 522-528.
7. Kaparakis M, Walduck AK, Price JD, Pedersen JS, van Rooijen N, Pearse MJ *et al.* Macrophages are mediators of gastritis in acute *Helicobacter pylori* infection in C57BL/6 mice. *Infection and immunity* 2008; **76**(5): 2235-2239.
8. Tegtmeyer N, Wessler S, Backert S. Role of the cag-pathogenicity island encoded type IV secretion system in *Helicobacter pylori* pathogenesis. *The FEBS journal* 2011; **278**(8): 1190-1202.

9. Vinall LE, King M, Novelli M, Green CA, Daniels G, Hilkens J *et al.* Altered expression and allelic association of the hypervariable membrane mucin MUC1 in *Helicobacter pylori* gastritis. *Gastroenterology* 2002; **123**(1): 41-49.
10. McGuckin MA, Every AL, Skene CD, Linden SK, Chionh YT, Swierczak A *et al.* Muc1 mucin limits both *Helicobacter pylori* colonization of the murine gastric mucosa and associated gastritis. *Gastroenterology* 2007; **133**(4): 1210-1218.
11. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA *et al.* Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**(6776): 398-402.
12. Sugimoto M, Furuta T, Shirai N, Nakamura A, Xiao F, Kajimura M *et al.* Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. *Journal of gastroenterology and hepatology* 2007; **22**(1): 51-59.
13. Dery O, Corvera CU, Steinhoff M, Bunnett NW. Proteinase-activated receptors: novel mechanisms of signaling by serine proteases. *Am J Physiol* 1998; **274**(6 Pt 1): C1429-1452.
14. Soh UJ, Dores MR, Chen B, Trejo J. Signal transduction by protease-activated receptors. *British journal of pharmacology* 2010; **160**(2): 191-203.
15. Sekiguchi F, Takaoka K, Kawabata A. Proteinase-activated receptors in the gastrointestinal system: a functional linkage to prostanoids. *Inflammopharmacology* 2007; **15**(6): 246-251.
16. Shpacovitch V, Feld M, Bunnett NW, Steinhoff M. Protease-activated receptors: novel PARTners in innate immunity. *Trends in immunology* 2007; **28**(12): 541-550.
17. Wee JL, Chionh YT, Ng GZ, Harbour SN, Allison C, Pagel CN *et al.* Protease-activated receptor-1 down-regulates the murine inflammatory and humoral response to *Helicobacter pylori*. *Gastroenterology* 2010; **138**(2): 573-582.
18. Lurje G, Husain H, Power DG, Yang D, Groshen S, Pohl A *et al.* Genetic variations in angiogenesis pathway genes associated with clinical outcome in localized gastric adenocarcinoma. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2010; **21**(1): 78-86.
19. Castano-Rodriguez N, Kaakoush NO, Goh KL, Fock KM, Chionh YT, Sutton P *et al.* PAR-1 polymorphisms and risk of *Helicobacter pylori*-related gastric cancer in a Chinese population. *Anticancer research* 2012; **32**(9): 3715-3721.

20. DeLyria ES, Redline RW, Blanchard TG. Vaccination of mice against *H pylori* induces a strong Th-17 response and immunity that is neutrophil dependent. *Gastroenterology* 2009; **136**(1): 247-256.
21. Velin D, Favre L, Bernasconi E, Bachmann D, Pythoud C, Saiji E *et al.* Interleukin-17 is a critical mediator of vaccine-induced reduction of *Helicobacter* infection in the mouse model. *Gastroenterology* 2009; **136**(7): 2237-2246 e2231.
22. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N *et al.* IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nature immunology* 2011; **12**(3): 231-238.
23. Cenac N, Cellars L, Steinhoff M, Andrade-Gordon P, Hollenberg MD, Wallace JL *et al.* Proteinase-activated receptor-1 is an anti-inflammatory signal for colitis mediated by a type 2 immune response. *Inflammatory bowel diseases* 2005; **11**(9): 792-798.
- 24.
25. Khoufache K, Berri F, Nacken W, Vogel AB, Delenne M, Camerer E *et al.* PAR1 contributes to influenza A virus pathogenicity in mice. *The Journal of clinical investigation* 2013; **123**(1): 206-214.
26. Niessen F, Schaffner F, Furlan-Freguia C, Pawlinski R, Bhattacharjee G, Chun J *et al.* Dendritic cell PAR1-S1P3 signalling couples coagulation and inflammation. *Nature* 2008; **452**(7187): 654-658.
27. Ermak TH, Giannasca PJ, Nichols R, Myers GA, Nedrud J, Weltzin R *et al.* Immunization of mice with urease vaccine affords protection against *Helicobacter pylori* infection in the absence of antibodies and is mediated by MHC class II-restricted responses. *J Exp Med* 1998; **188**(12): 2277-2288.
28. Sutton P, Wilson J, Kosaka T, Wolowczuk I, Lee A. Therapeutic immunization against *Helicobacter pylori* infection in the absence of antibodies. *Immunology and cell biology* 2000; **78**(1): 28-30.
29. Delyria ES, Nedrud JG, Ernst PB, Alam MS, Redline RW, Ding H *et al.* Vaccine-induced immunity against *Helicobacter pylori* in the absence of IL-17A. *Helicobacter* 2011; **16**(3): 169-178.
30. Velin D, Narayan S, Bernasconi E, Busso N, Ramelli G, Maillard MH *et al.* PAR2 promotes vaccine-induced protection against *Helicobacter* infection in mice. *Gastroenterology* 2011; **141**(4): 1273-1282, 1282 e1271.
31. Sutton P. Vaccinating against *Helicobacter pylori*: dissecting the mechanism. *Gastroenterology* 2011; **141**(4): 1149-1151.

32. Suzuki Y, Gabazza EC, Imoto I, James RF, Hirayama T, Wada A *et al.* Vacuolating cytotoxin A is associated with increased thrombin generation in gastric mucosa. *Helicobacter* 2005; **10**(4): 323-331.
33. 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**(4): 1386-1397.
34. Raghavan S, Hjulstrom M, Holmgren J, Svennerholm AM. Protection against experimental *Helicobacter pylori* infection after immunization with inactivated *H. pylori* whole-cell vaccines. *Infection and immunity* 2002; **70**(11): 6383-6388.
35. Connolly AJ, Ishihara H, Kahn ML, Farese RV, Jr., Coughlin SR. Role of the thrombin receptor in development and evidence for a second receptor. *Nature* 1996; **381**(6582): 516-519.
36. Sutton P. Considering increased mouse stomach mass, when calculating prophylactic vaccine efficacy against *Helicobacter pylori*. *Helicobacter* 2007; **12**: 210-212.
37. Sutton P, Danon SJ, Walker M, Thompson LJ, Wilson J, Kosaka T *et al.* Post-immunisation gastritis and *Helicobacter* infection in the mouse: a long term study. *Gut* 2001; **49**(4): 467-473.
38. Alderuccio F, Toh BH, Gleeson PA, van Driel IR. A novel method for isolating mononuclear cells from the stomachs of mice with experimental autoimmune gastritis. *Autoimmunity* 1995; **21**(3): 215-221.
39. Chionh YT, Wee JL, Every AL, Ng GZ, Sutton P. M-cell targeting of whole killed bacteria induces protective immunity against gastrointestinal pathogens. *Infection and immunity* 2009; **77**(7): 2962-2970.
40. Rad R, Brenner L, Bauer S, Schwendy S, Layland L, da Costa CP *et al.* CD25⁺/Foxp3⁺ T cells regulate gastric inflammation and *Helicobacter pylori* colonization in vivo. *Gastroenterology* 2006; **131**(2): 525-537.
41. Yanai H, Chen HM, Inuzuka T, Kondo S, Mak TW, Takaoka A *et al.* Role of IFN regulatory factor 5 transcription factor in antiviral immunity and tumor suppression. *Proceedings of the National Academy of Sciences of the United States of America* 2007; **104**(9): 3402-3407.
42. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001; **29**(9): e45.

FIGURE LEGENDS**Figure 1: *H. pylori* induced atrophic gastritis is associated with PAR1 expression on bone marrow-derived cells**

Irradiated wild-type (WT) or *PAR1*^{-/-} mice (n=5-7) were reconstituted with WT or *PAR1*^{-/-} bone marrow for 2 months prior to infection with *H. pylori*. (a) Two months post-infection, stomachs were removed and *H. pylori* colonization determined by colony-forming assay. Mice reconstituted with *PAR1*^{-/-} bone marrow had significantly less *H. pylori* colonization than WT controls reconstituted with WT bone marrow (*ANOVA). (b-d) Severity of inflammation was scored histologically for cell infiltrate (0-6) and atrophy (0-3). (b) and (c) show cell infiltrate and atrophy scores for separate groups, while (d) presents scores with all mice grouped according to the genotype of their bone marrow donor. Mice reconstituted with *PAR1*^{-/-} bone marrow developed more severe atrophic gastritis than those receiving WT bone marrow (*Mann-Whitney). Boxplots present the median (horizontal bar), interquartile range (box) and 10th and 90th percentiles (bars). Representative pictures of histology are shown of typical infected mice transplanted with (e) wildtype bone marrow and (f) *PAR1*^{-/-} bone marrow. Bars represent 200 μ M.

Figure 2: PAR1 suppression of T helper cytokines in gastric tissues from mice with acute *H. pylori* infection

(a-c) Cytokine levels in the gastric mucosa of WT or *PAR1*^{-/-} mice, either uninfected (n=3) or infected (n=4) with *H. pylori* for 1 week, were quantified by ELISA. (d) Sera collected from WT and *PAR1*^{-/-} mice infected with *H. pylori* for 2 months (n=8) were analysed by ELISA for IgG subclass antibody response. Uninfected control levels were subtracted. Box-plots present the median (horizontal bar), interquartile range (box) and 10th and 90th percentiles (bars). *H. pylori* infected *PAR1*^{-/-} mice had significantly greater levels of gastric IFN γ and IL-17A, and serum IgG2c compared to infected WT controls (*ANOVA). IL-10 levels were not significantly different (n.s.).

Figure 3: Immune cell infiltration in *H. pylori* infected *PAR1*^{-/-} mice

Gastric cells perfused from the stomachs of uninfected (n=4) and 1 week *H. pylori* infected (n=5/6) wildtype and *PAR1*^{-/-} mice were analysed by flow cytometry.

a) Identification of immune cell subsets was as illustrated: Th cells were CD45⁺CD4⁺CD19⁻, B cells were CD45⁺CD4⁻CD19⁺, neutrophils were CD45⁺Ly6G⁺CD11b⁺, macrophages were CD45⁺Ly6G⁻CD11b⁺MHC-II⁺CD19⁻ (filled histogram = anti-CD19. Empty histogram = control cells without anti-CD19 antibody.

b) Increased cell infiltration in infected *PAR1*^{-/-} mice (ANOVA).

Figure 4: Increased Th1 and Th17 cytokines in lymphoid tissue draining the mucosal gastrointestinal tract of *H. pylori* infected *PARI*^{-/-} mice

Cytokine levels in mesenteric lymph nodes (MLN) of WT or *PARI*^{-/-} mice, either uninfected (n=4) or infected (n=8) with *H. pylori* for 1 month, were quantified by ELISA. Box-plots present the median (horizontal bar), interquartile range (box) and 10th and 90th percentiles (bars). *H. pylori* infected *PARI*^{-/-} mice had significantly greater levels of lymphoid IFN γ and IL-17A compared to infected WT controls (*ANOVA), but not other cytokines tested.

Figure 5: Increased efficacy of vaccinations against *H. pylori* in *PARI*^{-/-} mice

WT or *PARI*^{-/-} mice (n=8) were orally vaccinated against *H. pylori*. Unvaccinated controls were sham-treated with PBS. Four weeks post-vaccination, mice were challenged with live *H. pylori* and samples collected a further four weeks later. Box-plots present the median (horizontal bar), interquartile range (box) and 10th and 90th percentiles (bars).

(a) serum and (b) intestinal anti-*H. pylori* antibody responses were quantified by ELISA. Vaccinated/challenged *PARI*^{-/-} mice had a significantly greater serum and intestinal IgG2c response than WT controls (*ANOVA).

(c) Efficacy of vaccination was determined by colony-forming assay. Vaccination of both WT and *PARI*^{-/-} mice resulted in a significant reduction in *H. pylori* colonization, as compared to unvaccinated controls (*ANOVA). The percentage of protective immunity induced by vaccination of individual WT and *PARI*^{-/-} mice was calculated using the

following formula: $[100 - (\text{CFU of vaccinated mouse} / \text{Median of unvaccinated control group})] \times 100$. The percentage reduction of *H. pylori* colonization in vaccinated *PAR1*^{-/-} mice was significantly greater than that resulting from vaccination of WT mice (*Mann-Whitney).

Figure 6: PAR1 mediated suppression of Th1 and Th17 cytokine production is preceded by inhibition of IL-12 and IL-23 secretion

Splenocytes from WT mice (n=7) were stimulated with *H. pylori* SS1. After 18 and 24 hours, cytokines secreted into supernatants were quantified by ELISA, with background levels from unstimulated cultures subtracted. Addition of a PAR1 activating, but not a control scrambled peptide, significantly reduced cytokine secretion (*ANOVA). Data presented are from one of three similar experiments. Box-plots present the median (horizontal bar), interquartile range (box) and 10th and 90th percentiles (bars).

Figure 7: PAR1 activation suppresses macrophage secretion of IL-12 and IL-23

(a) Bone marrow-derived DCs and (b) peritoneal macrophages from WT mice (n=4) were stimulated with *H. pylori* SS1 for 12 or 18 hours, then secreted cytokines quantified by ELISA. Addition of a PAR1 activating (TF), but not control scrambled (FT) peptide, significantly reduced cytokine secretion by macrophages (*p<0.05, **p<0.01; ANOVA) but not DC. Data shown are representative of a) six and b) two experiments performed.

(c) Splenocytes from WT mice (n=4) were stimulated with *H. pylori* SS1 for 24 hours, with or without PAR1 activating peptide and recombinant IL-12 (rIL-12) at 5 or 10 µg/mL. The inhibitory effects of PAR1 activation on *H. pylori* induced secretion of IFN γ (*ANOVA) was reversed by addition of rIL-12 (#p<0.008 cf *H. pylori* + PAR1 peptide; ANOVA). In parallel cultures within the same experiment, depletion of macrophages significantly reduced secretion of *H. pylori*-induced IFN γ (^ANOVA). Box-plots present the median (horizontal bar), interquartile range (box) and 10th and 90th percentiles (bars).

Figure 8: PAR1 regulates IRF5 expression in both the infected gastric mucosa and in immune cells

IRF5 mRNA in *PAR1*^{-/-} and WT mice and cells was quantified by qPCR in (a) the stomachs of uninfected and *H. pylori* infected mice (n=8); (b) unstimulated peritoneal macrophages (n=4) and (c) unstimulated splenocytes (n=7). Data presented are from one of two similar experiments. PAR1 deficient cells contained significantly increased *IRF5* levels, even without stimulation (ANOVA).

(d) Stimulation of splenocytes from WT mice (n=4) with live *H. pylori* induced a significant increase in *IRF5* (*ANOVA cf unstimulated controls). The addition of PAR1 activating peptide, but not a control peptide, specifically suppressed *H. pylori* induction of *IRF5* to background levels.

(e) Gastric macrophages were isolated from the stomach of uninfected (n=3) and 1 week infected (n=5) wildtype and *PAR1*^{-/-} mice by flow cytometry using the parameters described in Figure 3 and *IRF5* mRNA levels assessed by qPCR. *PAR1*^{-/-} gastric macrophages had significantly higher levels of *IRF5* (ANOVA).

Figure 9: PAR1 suppresses IRF5 protein expression in immune cells.

Basal *IRF5* protein levels relative to GAPDH were assessed by western blotting in (a) splenocytes and (b) peritoneal macrophages. Densitometry values are shown (individual mice and median). *PAR1*^{-/-} cells express significantly higher levels of *IRF5* protein than WT cells (ANOVA).

FIGURE 1

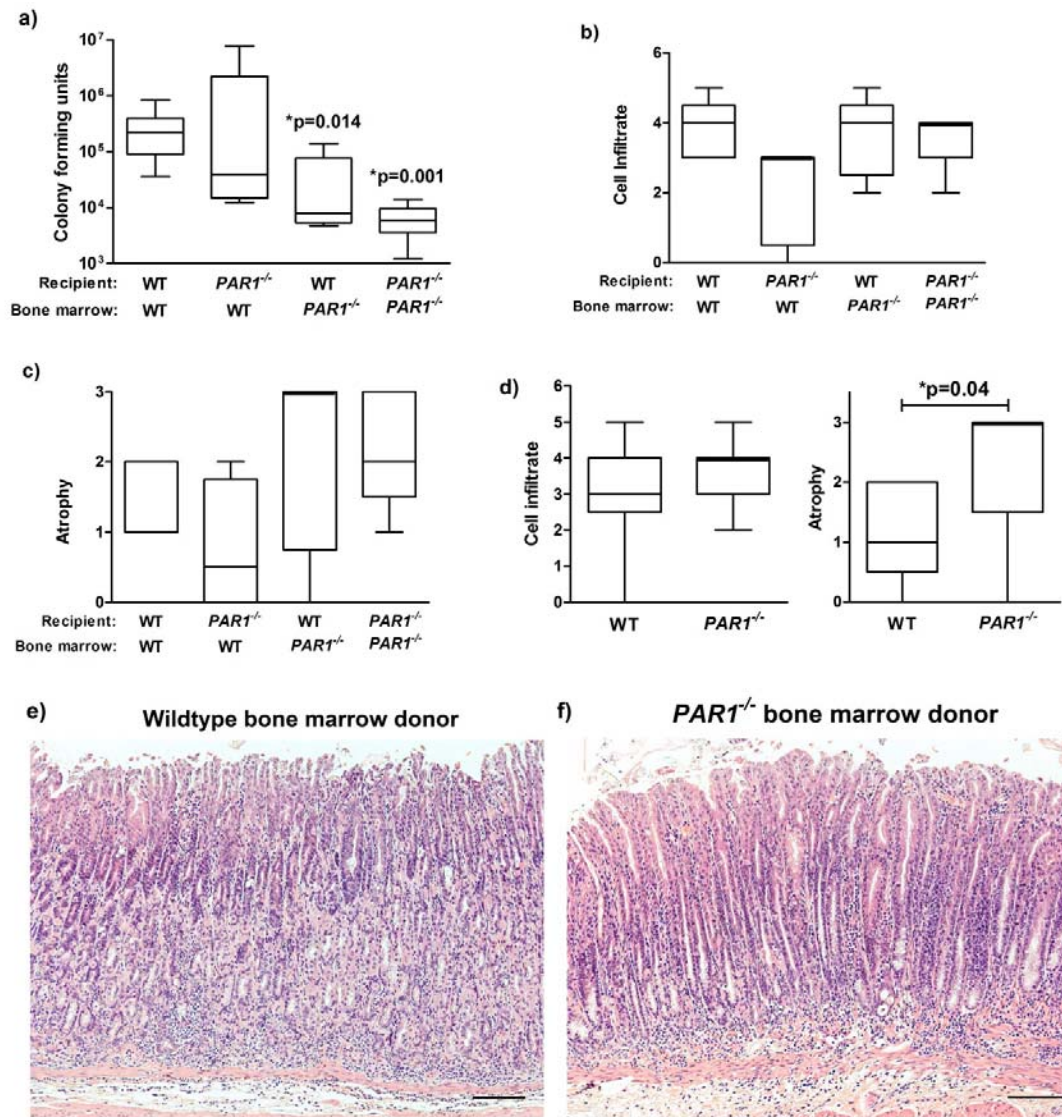


FIGURE 2

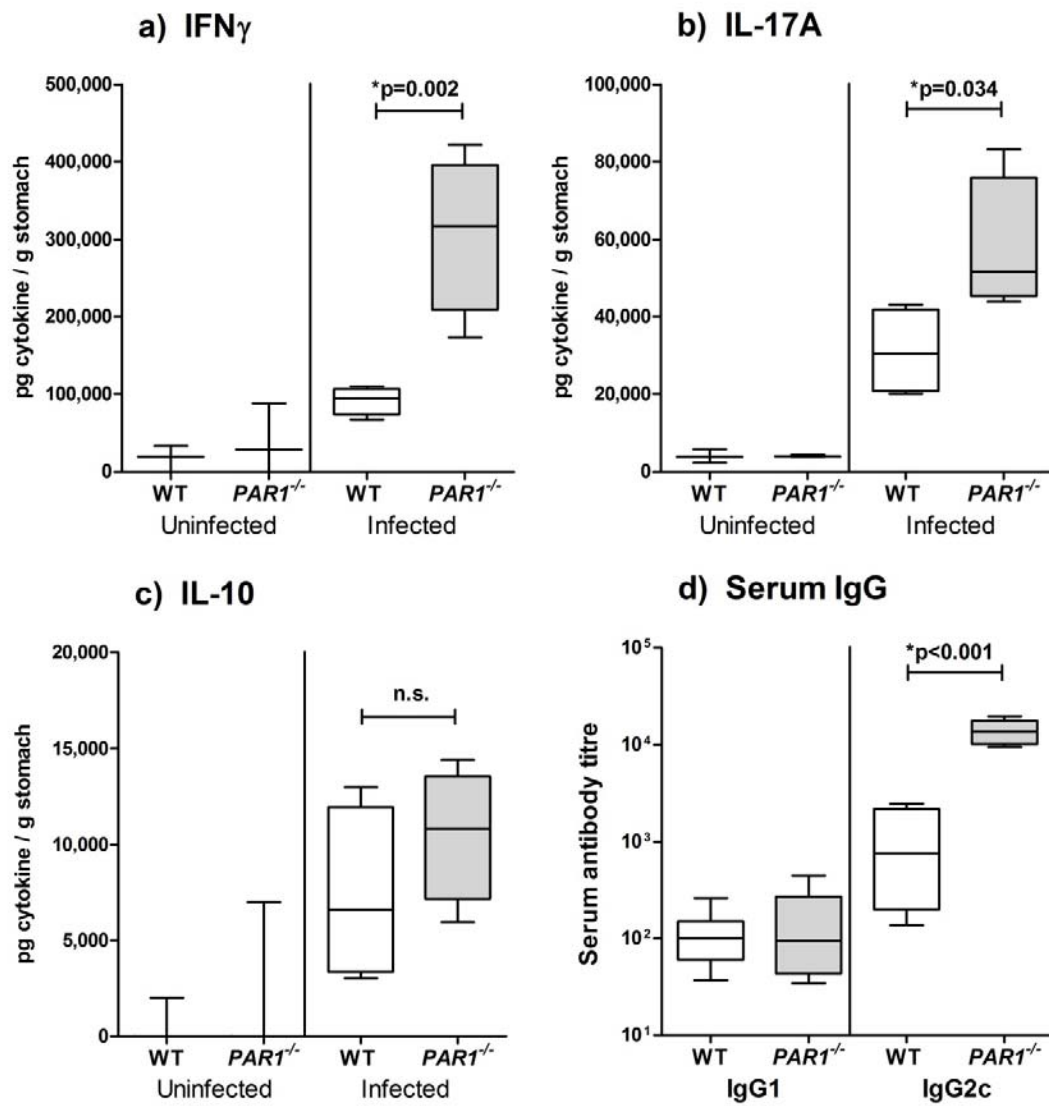


FIGURE 3

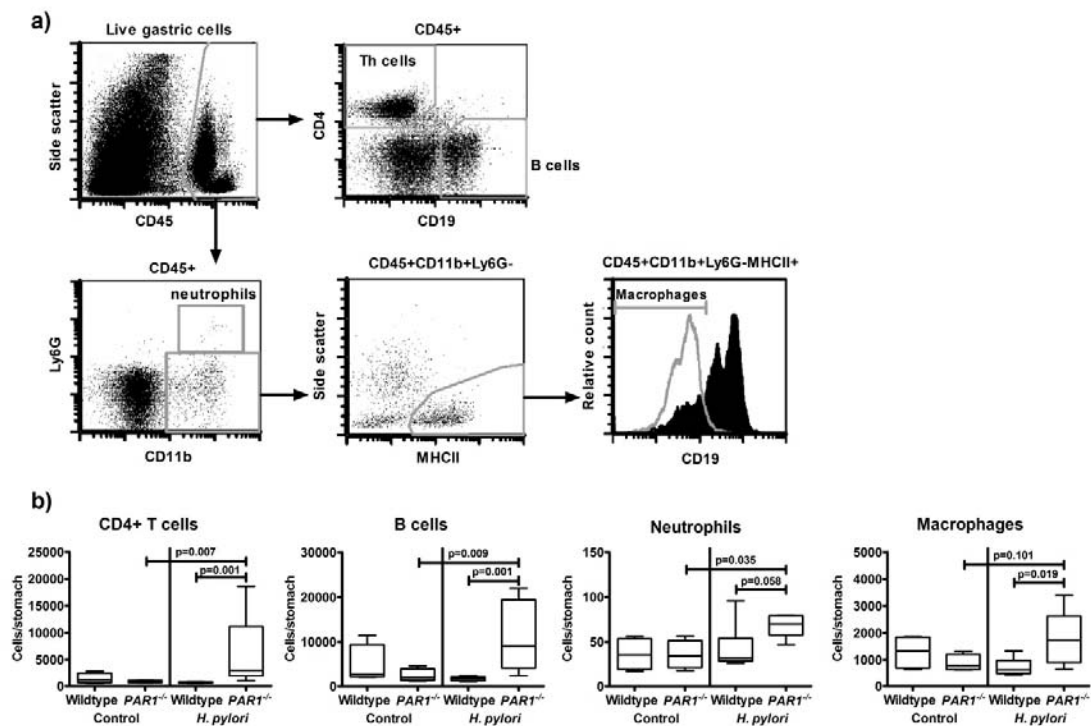


FIGURE 4

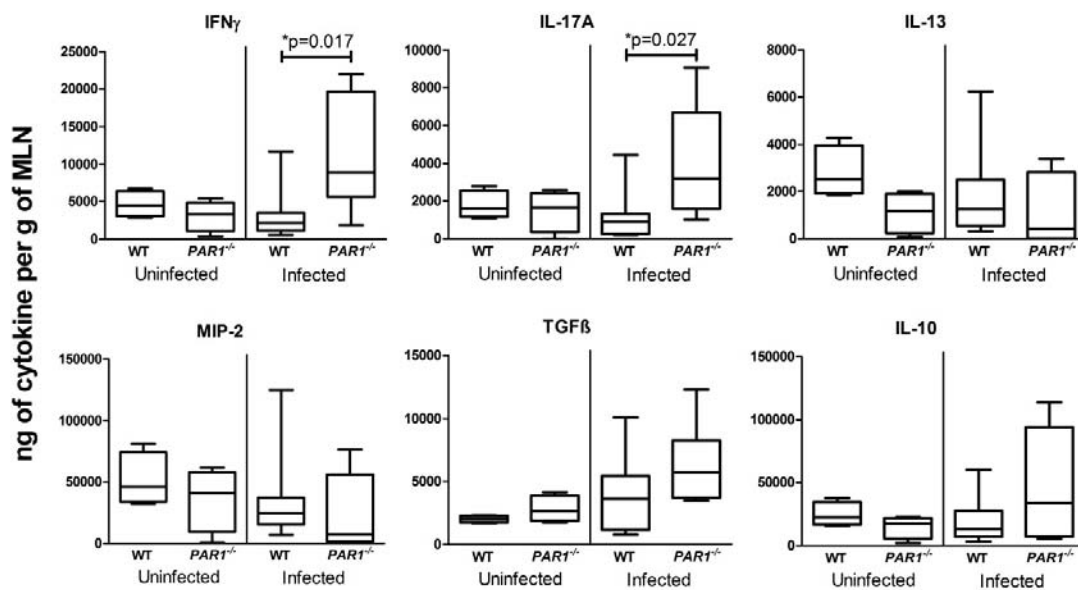
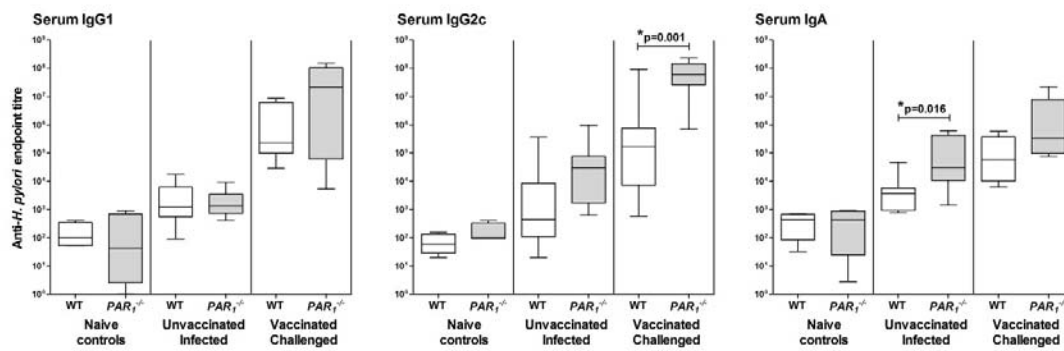
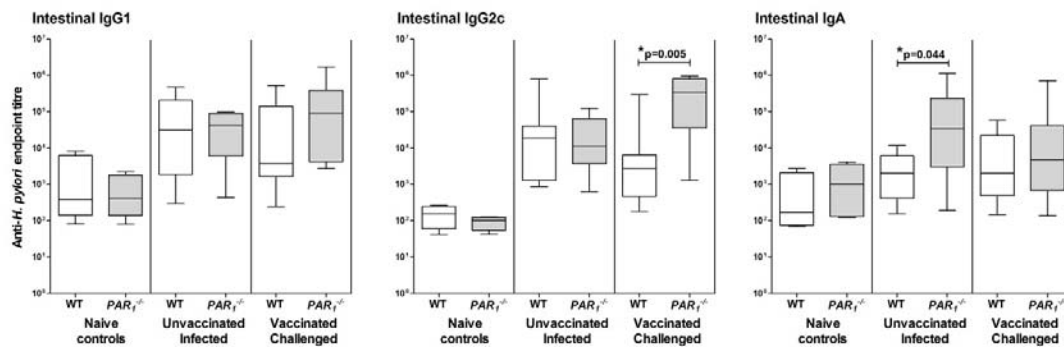


FIGURE 5

a) Serum antibody response



b) Intestinal antibody response



c) Vaccine-induced protection

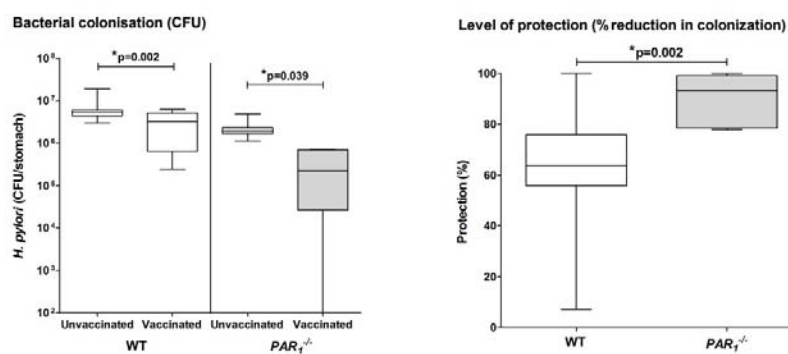


FIGURE 6

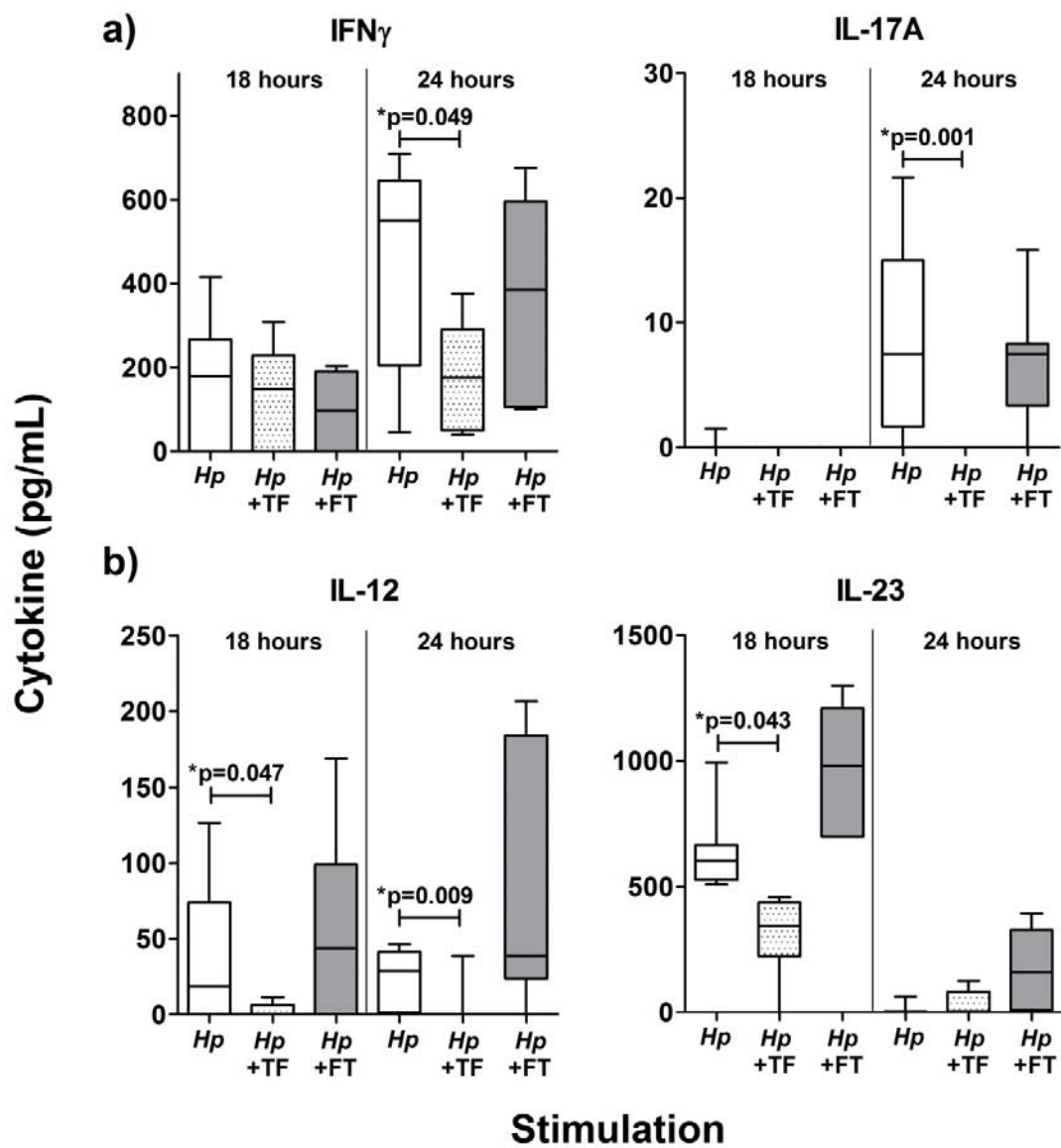
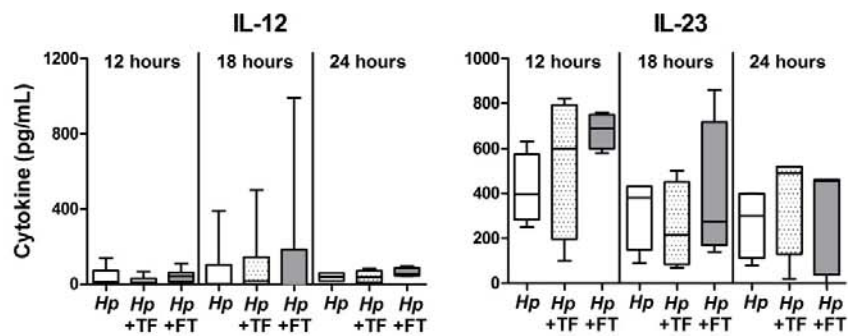
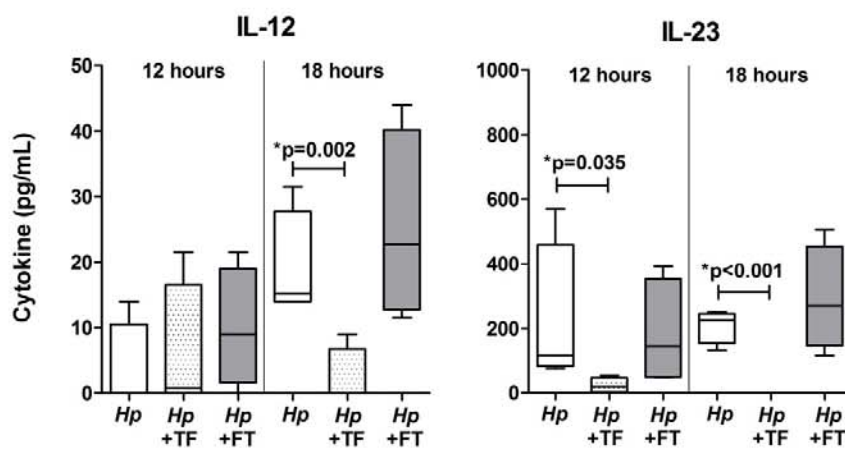


FIGURE 7

a) Bone marrow-derived dendritic cells



b) Peritoneal macrophages



c) Splenocytes

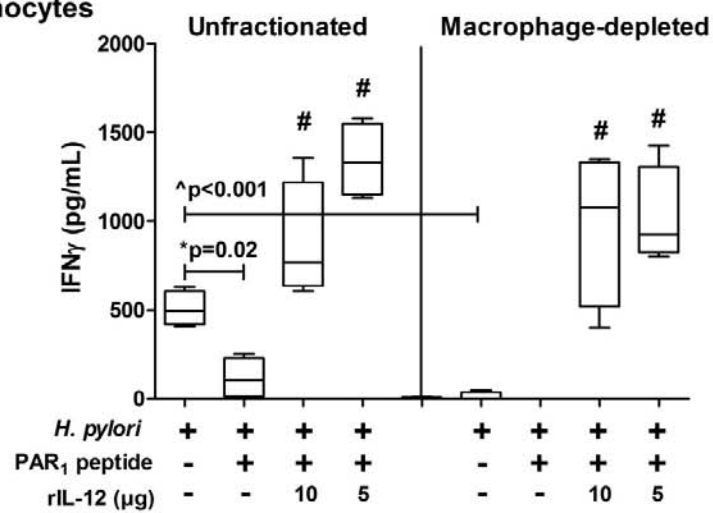


FIGURE 8

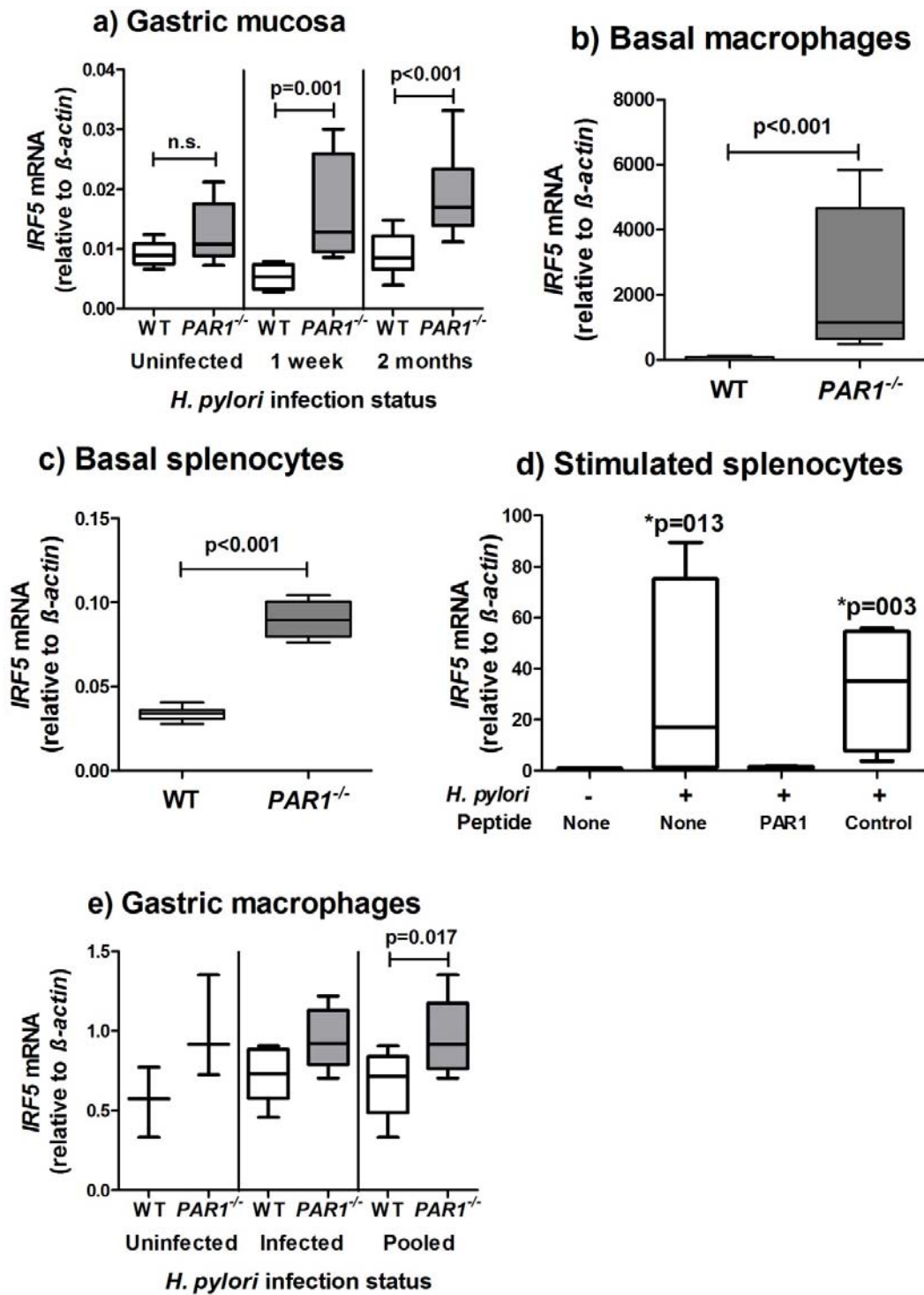


FIGURE 9