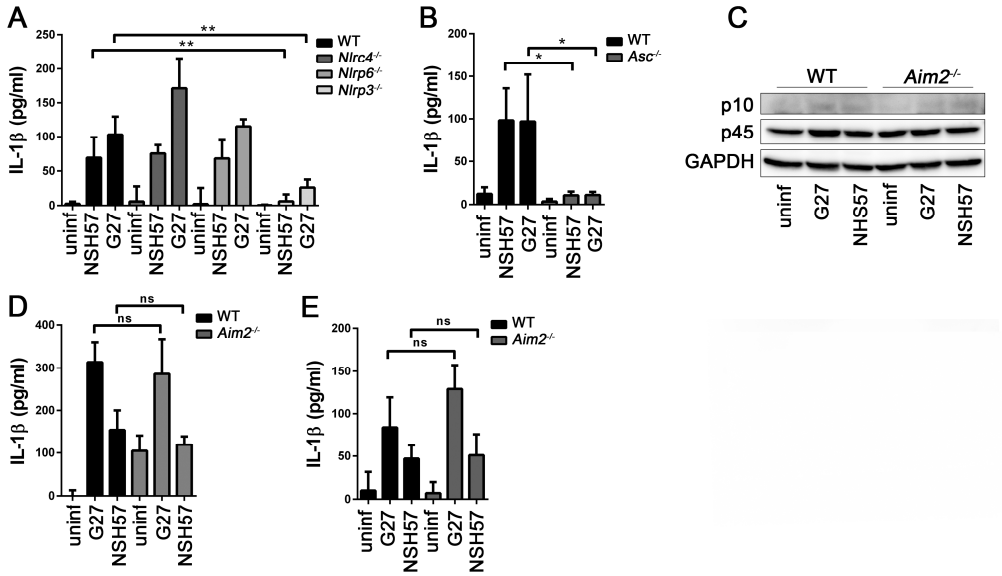
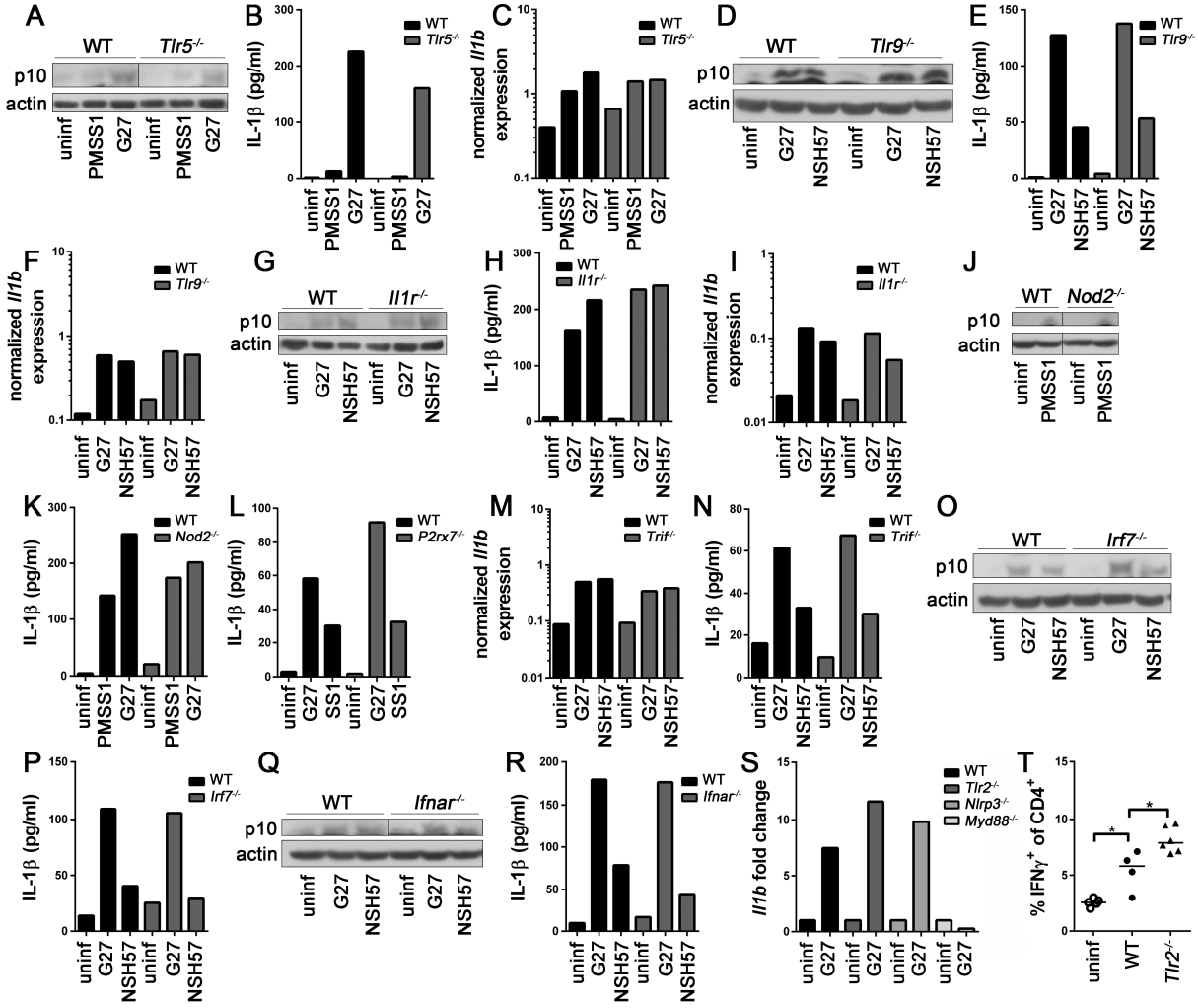
Supplemental Figures

Supplemental Figure 1



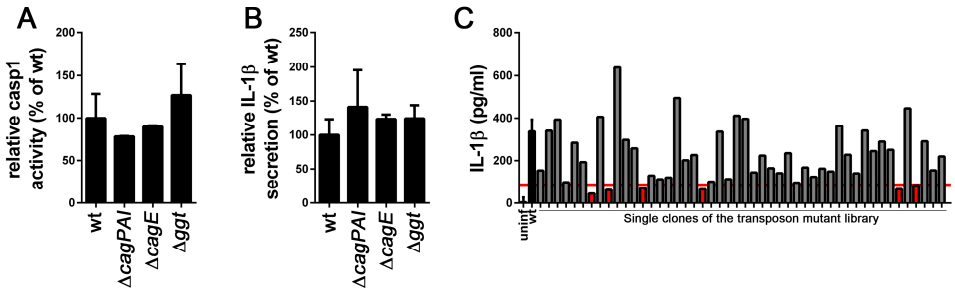
Suppl. Figure 1: IL-1β secretion by *H. pylori*-infected DCs depends on NLRP3 and ASC, but is independent of NLRC4, NLRP6 and AIM2. (**A-E)** BMDCs generated from mice of the indicated genotypes were infected overnight with *H. pylori* NSH57 and G27 at an MOI of 50, with or without prior *E. coli* LPS stimulation. **(A and B)** IL-1β ELISA of samples generated as shown in main Figure panels 1B and 1D, but without prior *E. coli* LPS stimulation. Pooled data of 3 independent experiments are shown (n=3). **(C)** WB analysis of caspase-1 activation (p10) in the cell supernatant compared to full length caspase-1 (p45) and GAPDH in the cell extract. One representative experiment of 3 is shown (n=3). **(D and E)** IL-1β ELISA of samples stimulated with 5ng/ml *E. coli* LPS for 3h prior to infection **(D)** or w/o prestimulation **(E)**. Pooled data of 3 independent experiments (n=3) are shown. Data represent mean ± SD; statistics: Mann-Whitney *U* test.

Supplemental Figure 2



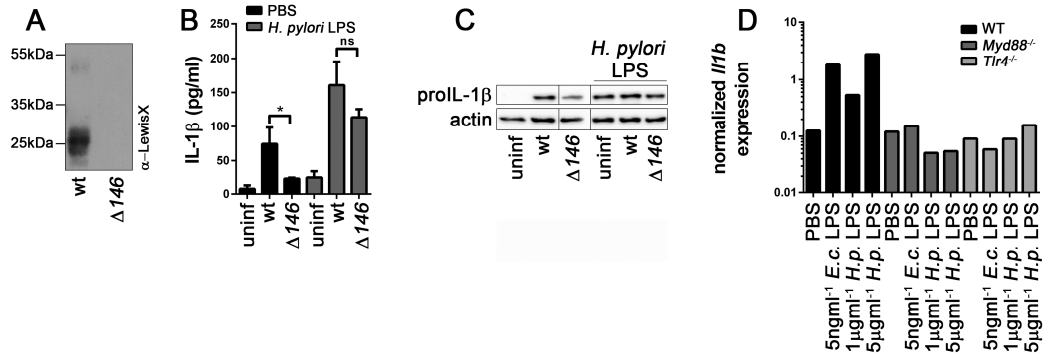
Suppl. Figure 2: Caspase-1 activation and IL-1β secretion by *H. pylori*-infected DCs is independent of TLR5 and TLR9, IL-1R, Nod2, P2X7R and the TRIF-IRF7-IFNaR axis. (**A-R)** BMDCs generated from mice of the indicated genotypes were infected overnight at an MOI of 50 with *H. pylori* NSH57, G27, SS1 and/or PMSS1. **(A, D, G, J, O and Q)** WB analysis of caspase-1 activation (p10) in the cell supernatant compared to actin expression in the cell extract. Lanes in the p10 WB in panels A, J and Q were run on the same gel, but were noncontiguous. **(B, E, H, K, L, N, P and R)** IL-1β secretion was analyzed by IL-1β ELISA of cell supernatants. **(C, F, I and M)** *Il1b* transcription was measured by qRT-PCR (normalized to *Gapdh*). **(S)** *Il1b* transcription in spleen DCs was measured by qRT-PCR and normalized to *Gapdh* and to uninfected controls. **(T)** MLN single cell suspensions derived from individual mice were re-stimulated with PMA/ionomycin and stained for IFNγ and CD4. Representative experiments are shown throughout (A-I, L, O, P and T: n=2); J and K: n=4; M, N, and Q-S: n=3). Each symbol represents one mouse. Horizontal lines indicate the median; statistics: Mann-Whitney *U* test.

Supplemental Figure 3



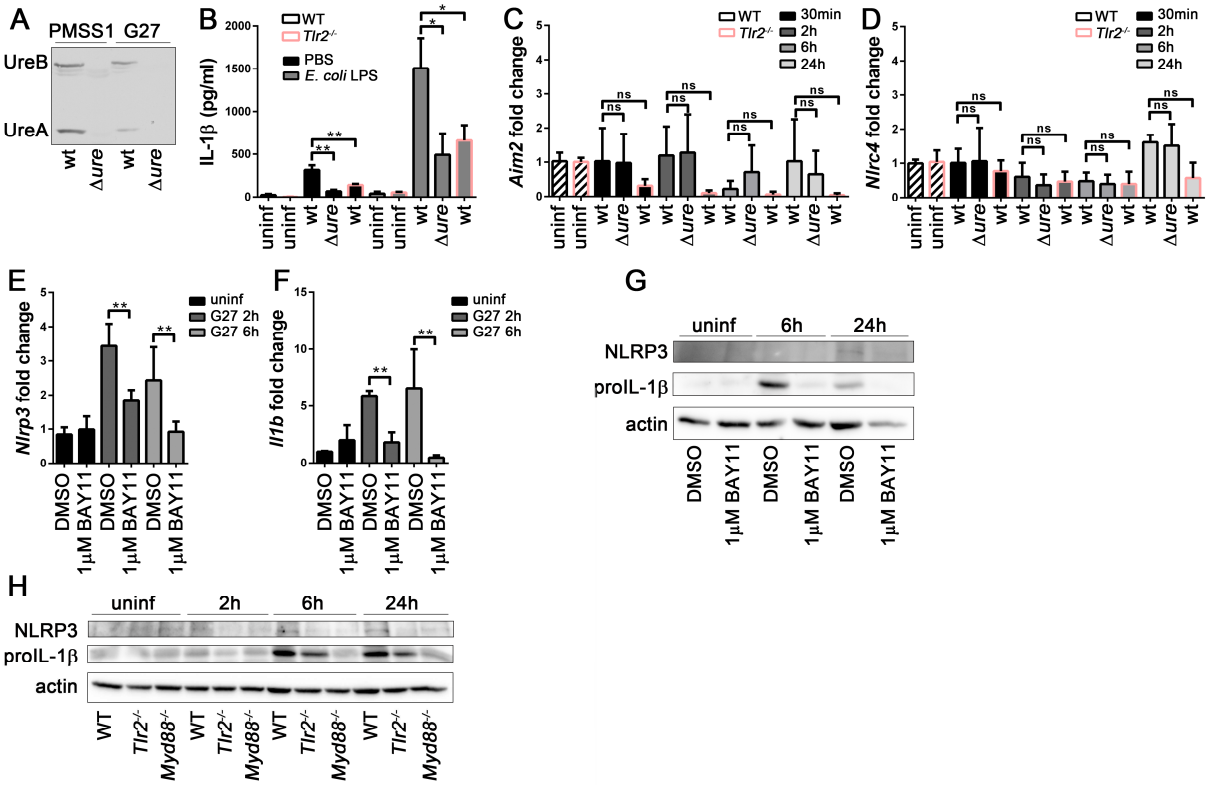
Suppl. Figure 3: IL-1β secretion by *H. pylori*-infected DCs is independent of known *H. pylori* virulence factors. (**A and B)** BMDCs generated from wild type mice were infected overnight with *H. pylori* G27 wild type and the indicated mutants and assessed with respect to caspase-1 activation (by quantification of Western blot signals for the p10 subunit, **(A)**) and IL-1β secretion (by ELISA, **(B)**). At least two and up to four experiment are pooled in A and B. Data represent mean ± SD. **(C)** Representative 96 well plate IL-1β ELISA result of the transposon mutant library screen, where all clones (in red) exhibiting IL-1β expression levels under the 75% reduction cut-off (indicated by the horizontal line) were selected for a second round of analysis.

Supplemental Figure 4



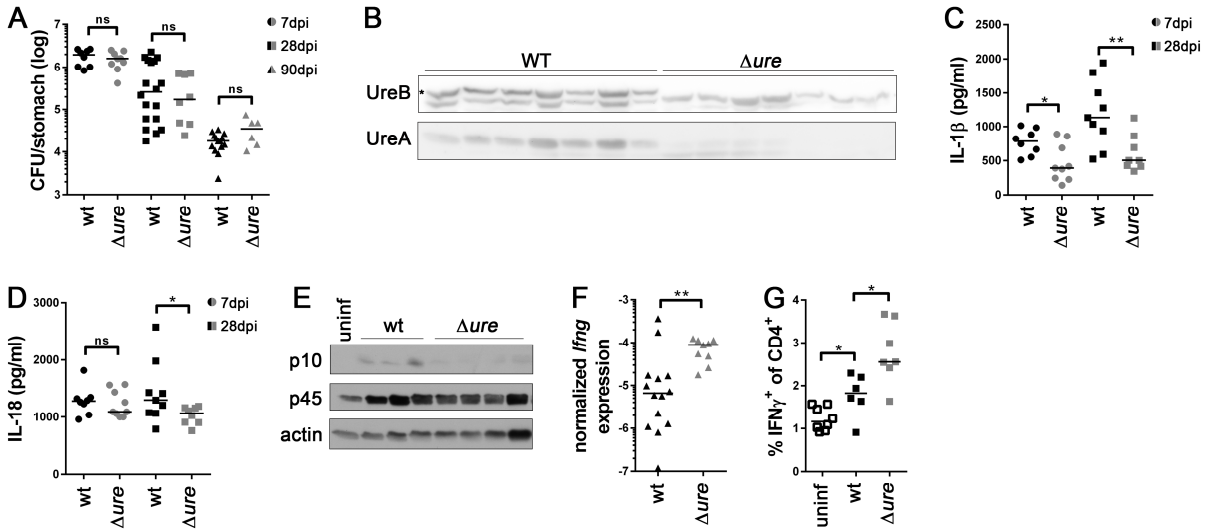
Suppl. Figure 4: *H. pylori* LPS induces the transcription of *Il1b* via TLR4 and MyD88. (A) Western blot of Lewis antigen expression of wild type G27 and the Δ*146* mutant strain. One representative blot of 3 is shown (n=3). **(B)** Wild type BMDCs were infected overnight with *H. pylori* G27 wild type and the Δ*146* mutant in the absence or presence of 1μg/ml purified *H. pylori* LPS, and assessed with respect to IL-1β secretion by ELISA. Pooled data of 3 independent experiments are shown (n=3). Data represent mean ± SD; statistics: Mann-Whitney *U* test. **(C)** BMDCs were treated as described in B and analyzed for proIL-1β expression by Western blotting of cell extract (lanes were run on the same gel, but are not contiguous). One representative experiment of 2 is shown (n=2). **(D)** Wild type, *Myd88*$^{-/-}$ and *Tlr4*$^{-/-}$ BMDCs were treated overnight with either purified *E. coli* or *H. pylori* LPS at the indicated concentrations and assessed with respect to *Il1b* expression by qRT-PCR (samples were normalized to *Gapdh*). One representative experiment of 2 is shown (n=2).

Supplemental Figure 5



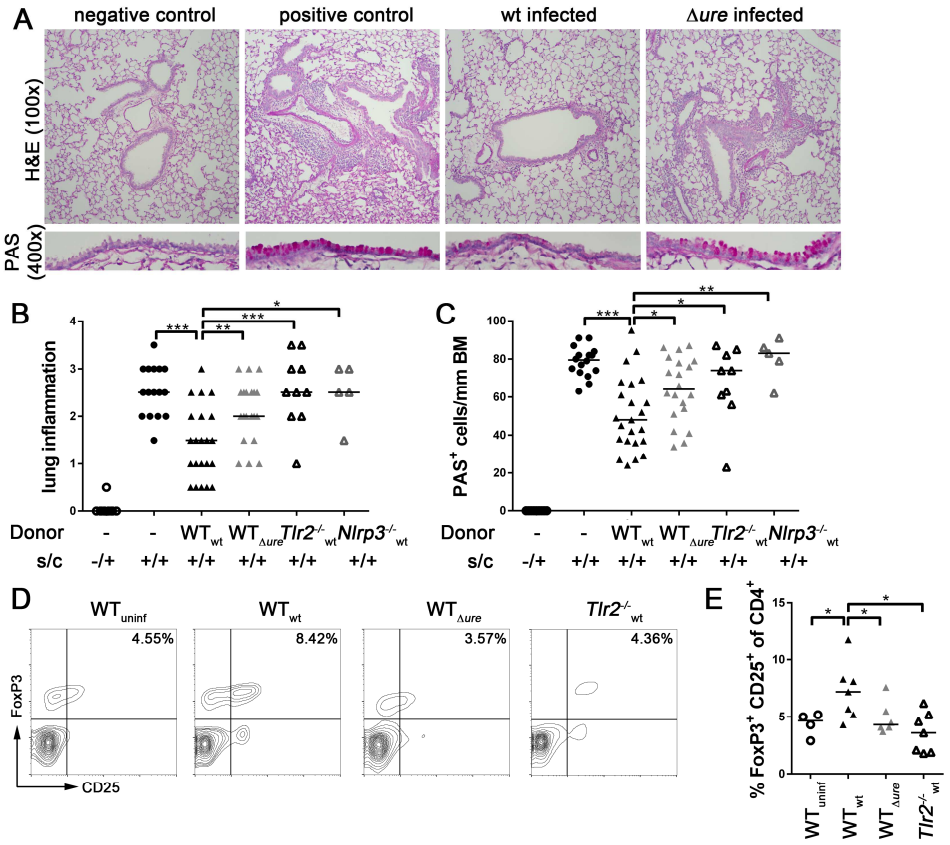
Suppl. Figure 5: Urease-proficient *H. pylori* induces the transcription of *Nlrp3*, but not of *Aim2* and *Nlrc4*, via TLR2, MyD88 and NF-κB. (A) UreA- and UreB-specific Western blot of extracts generated from PMSS1 and G27 wild type and Δ*ure* strains. One representative blot of 3 is shown (n=3). **(B-H)** BMDCs of the indicated genotypes were infected overnight with *H. pylori* G27 wild type and the Δ*ure* mutant as indicated and assessed with respect to IL-1β secretion by ELISA, and proIL-1β, AIM2, NLRC4 and NLRP3 expression by Western blotting and/or qRT-PCR. **(B)** IL-1β ELISA of the 24h-infected samples shown in Figure 2I, along with parallel samples that were additionally pre-stimulated with *E. coli* LPS for 3h. Pooled data of 3 independent experiments are shown (n=3). **(C and D)** qRT-PCR for *Aim2* **(C)** and *Nlrc4* **(D)** of the samples shown in Figure 2I. Pooled data of 4 independent experiments are shown (n=4). **(E-G)** Wild type BMDCs were treated with DMSO or the NF-κB inhibitor BAY11 for 1h prior to infection with wild type G27. Transcription of *Nlrp3* **(E)** and *Il1b* **(F)** was analyzed at the indicated time points using qRT-PCR (normalized to *Gapdh* and to completely untreated, uninfected controls). Pooled data of 3 independent experiments are shown in E and F (n=3). **(G)** NLRP3 and proIL-1β expression as analyzed by Western blotting of cell extracts relative to actin as loading control. One representative experiment of 3 is shown (n=3). **(H)** WT, *Tlr2*$^{-/-}$ and *Myd88*$^{-/-}$ BMDCs were infected for the indicated time points with wild type G27; NLRP3 and proIL-1β expression was analyzed by Western blotting of cell extracts relative to actin as loading control. One representative experiment of 3 is shown (n=3). Data in B-F represent mean ± SD; statistics: Mann-Whitney *U* test.

Supplemental Figure 6



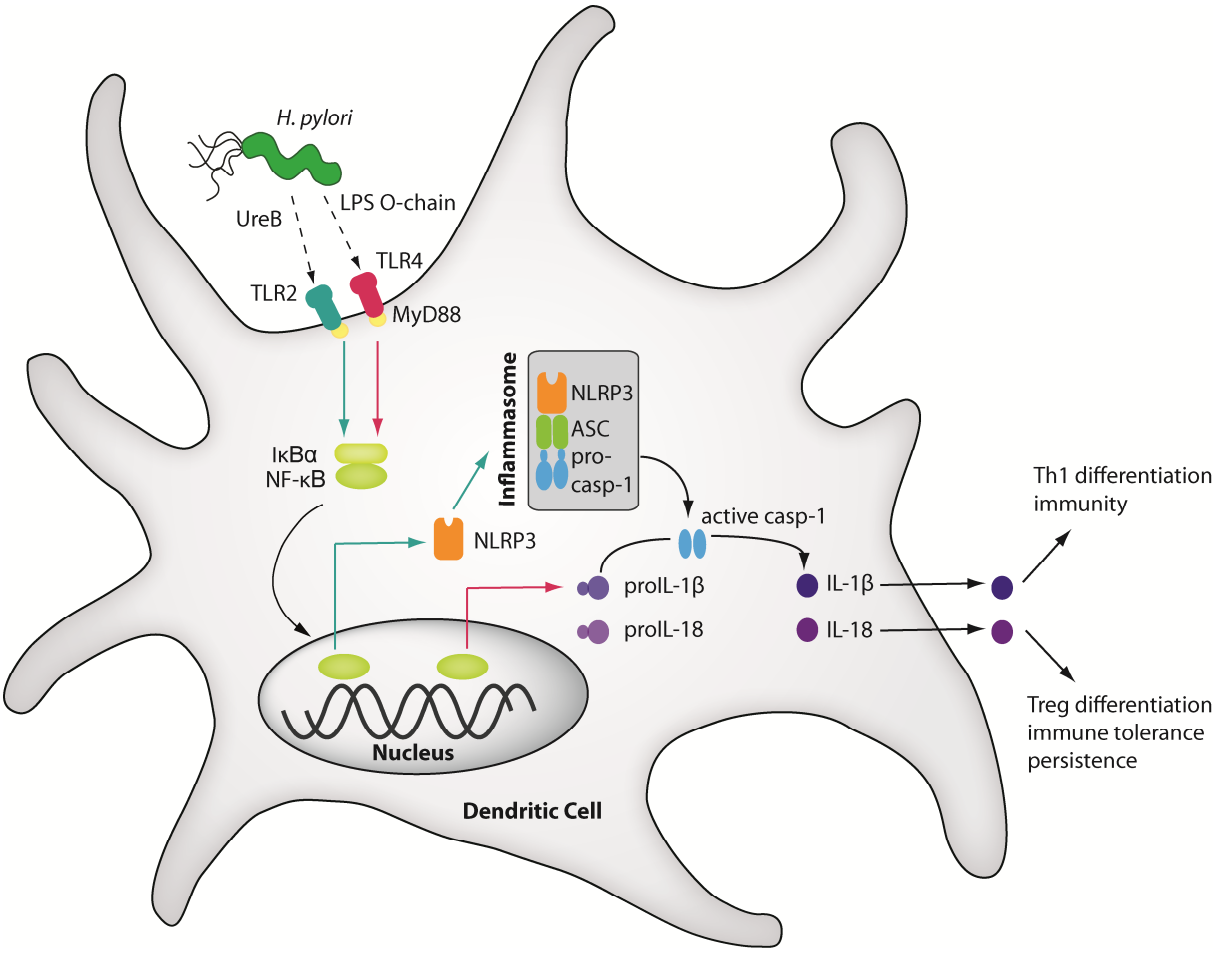
Suppl. Figure 6: *H. pylori* urease is required for inflammasome activation in adult-infected mice. **(A-G)** Mice were infected at 6 weeks of age with *H. pylori* PMSS1 wild type or the Δ*ure* mutant. **(A)** Gastric *H. pylori* colonization at the indicated time points post infection, as determined by plating and colony counting. **(B)** UreA- and UreB-specific Western blot of *H. pylori* re-isolates recovered after 28 days of infection. Each lane represents pooled re-isolates from one animal. **(C and D)** IL-1β **(C)** and IL-18 **(D)** ELISA of gastric mucosal homogenates obtained at the indicated time points post infection. Each symbol represents one animal. **(E)** Western blotting analysis of activated caspase-1 p10, full-length caspase-1 p45 and actin as loading control of gastric mucosal homogenates after 28 days of infection. Each lane represents one animal. **(F)** *Ifng* expression in the gastric mucosa after 90 days of infection, as measured by qRT-PCR and normalized to *Gapdh*. **(G)** MLN single cell suspensions derived from individual mice were re-stimulated with PMA/ionomycin and stained for IFNγ and CD4. Each symbol represents one mouse. Horizontal lines indicate the median; statistics: Mann-Whitney *U* test. Pooled data from 2 independent experiments are shown throughout (n=2); note that for technical reasons not every parameter could be analyzed for each mouse.

Supplemental Figure 7



Suppl. Figure 7: TLR2, NLRP3 and *H. pylori* urease are required for the generation of asthma-suppressing Tregs. (A) Representative images of H&E- (upper panel, magnification 100x) and PAS- (lower panel, magnification 400x) stained sections of the lungs described and scored in main Figure 3H and 3I. **(B and C)** Neonatal wild type, *Tlr2*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice were infected with either wild type PMSS1 or its Δ*ure* mutant for 28 days. CD4^{+}CD25^{+} Tregs were immunomagnetically isolated from Peyer's patches and MLNs of these donor mice and adoptively transferred i.v. to ovalbumin-sensitized wild type recipients one day before ovalbumin challenge (s/c, sensitized/challenged). Lung inflammation **(B)** and goblet cell metaplasia **(C)** was quantified on H&E- and PAS-stained tissue sections, respectively (BM, basement membrane). Pooled data of 3 experiments are shown (n=3). **(D and E)** Neonatal wild type and *Tlr2*$^{-/-}$ mice were infected with either wild type PMSS1 or the Δ*ure* mutant for 28 days. MLNs were isolated and stained for CD4, CD25 and FoxP3. Representative FACS plots of the CD4^{+} gate **(D)** are shown along with quantitative data for all animals **(E)**. Horizontal lines indicate the median; statistics: Mann-Whitney *U* test. Data in D and E are representative of 2 independent experiments (n=2).

Supplemental Figure 8



Suppl. Figure 8: Model of *H. pylori*-induced inflammasome activation. *H. pylori* LPS and the urease B subunit (UreB) collaborate to promote NLRP3 inflammasome and caspase-1 activation as well as IL-1β and IL-18 processing and secretion. *H. pylori* LPS signals via TLR4, MyD88 and NF-κB to activate *Il1b* transcription (indicated by red arrows), whereas UreB signals via TLR2, MyD88 and NF-κB to activate *Nlrp3* transcription (green arrows). The assembly of NLRP3, ASC and pro-caspase-1 is triggered through an as yet unknown mechanism leading to caspase-1 activation, and to the processing of proIL-1β and proIL-18. The mature cytokines are released and promote Th1 differentiation and *H. pylori* clearance in the case of IL-1β, and Treg differentiation, immune tolerance and persistence in the case of IL-18. Note that the pro-form of IL-18 is constitutively expressed in DCs. While shown representatively for a dendritic cell, other immune cells and gastric epithelial cells may activate the NLRP3 inflammasome in a similar manner upon exposure to *H. pylori* and may contribute as much or more mature IL-1β and IL-18 to the overall cytokine levels in the infected gastric mucosa.

Supplemental Table 1

gene #	gene name	# of hits
LPS synthesis		
HPG27_38	mannose-6-phosphate isomerase	3
HPG27_39	GDP-D-mannosedehydratase	4
HPG27_104	L-fuculose 1-phosphate aldolase	1
HPG27_146	lipopolysaccharide1,2-glycosyltransferase	5
HPG27_437	DD-heptosyl transferase	1
Urease gene cluster		
HPG27_67	ureaseB	3
HPG27_68	ureaseA	5
Restriction-modification system		
HPG27_436	type II methyltransferase	1
HPG27_746	putative type I R-M enzyme	1
HPG27_806	type I restriction enzyme M protein	1
HPG27_945	phage/colicin/tellurite resistance cluster Y protein	1
HPG27_1316	type III restriction enzyme R protein	1
HPG27_1328	putative type III restriction enzyme R	2
HPG27_1444	type III R-M system modification enzyme	1
Chemotaxis		
HPG27_95	methyl-accepting chemotaxis protein	8
Outer membrane proteins		
HPG27_739	outer membrane protein	1
HPG27_1501	putative outer membrane lipoprotein	1
Hypothetical proteins		
HPG27_773	hypothetical protein	1
HPG27_936	hypothetical protein	1
HPG27_1131	hypothetical protein	1
HPG27_1133	hypothetical protein	1
HPG27_1275	hypothetical protein	1
HPG27_1282	hypothetical protein	1
HPG27_1358	hypothetical protein	1
Others		
HPG27_487	cag pathogenicity island protein X	1
HPG27_603	putative 3-hydroxy acid dehydrogenase	1
HPG27_772	flagellar rotor protein	1
HPG27_1129	alpha carbonic anhydrase	1
HPG27_1138	aldo-keto reductase	1
	plasmid	1
	Different non coding regions	9
	No sequences due to growth deficit	2

Supplemental methods

H. pylori *strains, infection of DCs and transposon library screening*

The following *H. pylori* strains were used for *in vitro* infection of BMDCs. Strains G27, SS1 and PMSS1 have been described previously (1-3). NSH57 is a mouse-adapted derivative of strain G27 that was generated by three 3-week passages in FVB mice (4). To create the transposon library used here (generously donated by Nina R. Salama, Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, USA), genomic DNA prepared from the original 10,000-clone G27 library (5) was transformed into NSH57 by natural transformation (4). For the analysis of individual clones with respect to their induction of IL-1β secretion by BMDCs, bacteria were cultured from frozen stocks on horse blood agar supplemented with 25 μg/ml of chloramphenicol at 37°C for 2 days under microaerophilic conditions. Colonies were picked and expanded individually o/n on fresh horse blood plates prior to inoculation of 100μl liquid cultures (Brucella broth (Difco) containing 10% FBS (Life Technologies)) in 96 well format. Liquid cultures were grown for 6h with shaking at 37°C and used for BMDC infection in 96 well flat bottom plates and for cryopreservation of individual clones. The supernatants of infected BMDCs were subjected to IL-1β ELISA after 16h of co-culture. For sequencing of the transposon flanking regions, the following primer was used: 5'-CAG TTC CCA ACT ATT TTG TCC-3'. The screen was saturated after examination of ~2500 mutants. All *H. pylori* liquid cultures were routinely assessed by light microscopy for contamination, morphology, and motility prior to use in infections.

Generation and infection of BMDC, spleen DCs and human blood-derived DCs

For the generation of BMDCs, single cell suspensions were prepared from hind leg bone marrow and seeded at 10^5 cells per well in 96 well plates for ELISA or at $2x10^6$ cells per well in 6 well plates for Western blotting in RPMI/10% FCS and 4ng/ml GM-CSF and cultured for 6 days. BMDCs were infected for 16h with *H. pylori* at a multiplicity of infection of ~50. BMDCs were pretreated for 3h with 5ng/ml LPS (Sigma Aldrich) to stimulate *Il1b* expression where indicated. BAY11-7082 (Sigma Aldrich) was added at 1μM final concentration 1h prior to infection of BMDCs to inhibit NF-κB signaling. For the isolation of splenic DCs, spleen cell suspensions were prepared by digestion using 1mg/ml CollagenaseD and 0.1% DNaseI (both from Roche) in serum-free RPMI for 1h at 37°C. DCs were immunomagnetically isolated using CD11c MicroBeads (Miltenyi Biotec) according to the manufacturer's instructions and seeded at $2x10^5$ cells per well in 96 well round bottom plates. For the generation of human DCs, peripheral blood mononuclear cells (PBMC) were isolated from buffy coats of healthy volunteer blood donors obtained from the blood donation center Zürich (ZHBSD) using Ficoll-Paque™ (GE Healthcare). $CD14^+$ monocytes were isolated from PBMCs using CD14 MicroBeads (Miltenyi Biotec) according to the manufacturer's instructions and cultured in CellGro DC medium (Cellgenix) supplemented with 1000U/ml GM-CSF and 500IU/ml IL-4 (both R&D Systems) for 6 days.

Induction and quantification of allergic asthma

Ovalbumin model: Mice were sensitized by i.p. injection of 20µg ovalbumin (Sigma-Aldrich) emulsified in 2.25mg aluminum hydroxide (Alum Imject; Pierce) at 6 and 8 weeks of age. Sensitized mice were challenged with 1% aerosolized ovalbumin using an ultrasonic nebulizer (NE-U17; Omron) for 20 min daily on days 31, 32 and 33 post initial sensitization. For Treg transfer experiments, neonatally infected donor mice were sacrificed at the age of 5 weeks and Tregs were immunomagnetically isolated from MLN and Peyer's Patches single cell suspensions ($CD4^+CD25^+$ mouse regulatory T cell isolation kit; R&D). $1x10^6$ Tregs were injected per animal into sensitized wild type recipient mice one day before the first allergen challenge. House dust mite (HDM) model: Mice were sensitized by intranasal administration of 1µg HDM (Greer) at 6 weeks of age. Mice were challenged with 10µg HDM per animal on five consecutive days, starting on day 8 after sensitization. Anti-IL-18 antibody (YIGIF74-1G7, BioXCell) was applied weekly (50µg per dose) starting at 7 days of age. After sacrifice, lungs were lavaged via the trachea with 1ml PBS. Bronchoalveolar lavage fluid (BALF) cells were counted using trypan blue dye exclusion. Differential eosinophil counts were performed on cytocentrifuged preparations stained with the Microscopy Hemacolor Set (Merck). Lungs were fixed by inflation and immersion in 10% formalin and embedded in paraffin. Tissue sections were stained with H&E and periodic acid-Schiff and examined in blinded fashion on a BX40 Olympus microscope. Peribronchial inflammation was scored on a scale from 0 to 4. PAS-positive goblet cells were quantified per 1mm of basement membrane. For lung re-stimulation, single cell suspensions were prepared by collagenase 1A (Sigma Aldrich) digestion for 45min at 37°C. Cells were seeded at $5x10^5$ cells per well and stimulated with 250µg/ml ovalbumin for 3 days. Supernatants were analyzed for secretion of IL-5 by ELISA (eBioscience) according to the manufacturer's instructions. For detection of HDM-specific IgE, plates were coated with 25µg/ml HDM antigen; HDM-bound IgE was detected using α-mouse IgE-HRP (GTX77227, GeneTex).

Western Blotting and ELISA of cell culture supernatants and extracts, and of gastric mucosal homogenates

To detect proteins in cell culture supernatants, 500µl of supernatants were TCA-precipitated and subjected to Western blotting. Cell extracts and extracts of scraped and shock frozen murine gastric mucosa were prepared using RIPA-Buffer supplemented with protease inhibitors (complete Mini, Roche). For lysis of bacteria, 200µg/ml lysozyme (Sigma Aldrich) was added to the lysis buffer. For Lewis antigen detection, bacterial lysates were treated with 125ng/ml Proteinase K (Macherey-Nagel) o/n. The following antibodies were used: α-Casp1 p10/p45 (sc514, Santa Cruz), α-NLRP3 (ab91525, abcam), α-IL-1β (AF-401-NA, R&D Systems), α-actin (sc1616, Santa Cruz), α-GAPDH (G9545, Sigma Aldrich), α-*H. pylori* Urease (ab51954, abcam), α-LewisX (ab3358, abcam). Cytokines in gastric mucosal extracts and the supernatants of infected BMDCs or splenic DCs were analyzed by ELISA (human IL-

1β and mouse IL-1β, both BD Biosciences; IL-18, eBioscience) according to the manufacturers' instructions.

Real time qRT-PCR of cytokines in cell extracts and gastric mucosal homogenates

For real-time RT-PCR of shock frozen stomach mucosa or BMDC cell pellets, total RNA was isolated using NucleoSpin RNA II kits (Macherey-Nagel). For isolation of RNA from splenic DCs, the RNeasy microkit (Qiagen) was used. RNA was reversely transcribed into cDNA using superscript III (Invitrogen). The corresponding cDNA served as a template for real-time PCR performed using the LightCycler 480 SYBR Green I master kit (Roche). Samples were normalized to GAPDH expression (conditions: Tm 60°C, 50 cycles; primers: GAPDH fw: 5'-GAC ATT GTT GCC ATC AAC GAC C-3' / GAPDH rv: 5'-CCC GTT GAT GAC CAG CTT CC-3', pro-IL-1β fw: 5'-TTG ACG GAC CCC AAA AGA TG-3' / pro-IL-1β rv: 5'-TGG ACA GCC CAG GTC AAA-3', NLRP3 fw: 5'-CCC TTG GAG ACA CAG GAC TC-3' / NLRP3 rv: 5'-GGT GAG GCT GCA GTT GTC TA-3', IFNγ fw: 5'-ATC TGG AGG AAC TGG CAA AA-3' / IFNγ rv: 5'-TTC AAG ACT TCA AAG AGT CTG AGG TA-3', AIM2 fw: 5'-CAG GCA ATT GCA TCT GAG AG-3' / AIM2 rv: 5'-CGC CTC ACA AAG ATT TTC ACT-3', NLRC4 fw: 5'-GAA GAA TCC TGT GAT CTC CAA GAG-3' / NLRC4 rv: 5'-GAT CAA ATT GTG AAG ATT CTG TGC-3').

Single cell preparations of MLN and Peyer's patches and FACS staining

Single cell suspensions of MLNs and Peyer's patches were prepared by collagenaseIV (Sigma Aldrich) digestion for 25min at 37°C. For detection of intracellular cytokines, cells were seeded at $2x10^5$ cells per well and treated with 100nM PMA (Sigma Aldrich), 1μg/ml Ionomycin (Sigma Aldrich) and 2μg/ml Monensin (Enzo LifeScience) for 4h. Fixation and permeabilization was performed with the Cytofix/Cytoperm^{TM} Kit (BD Bioscience). The following antibodies were used for staining: CD4-FITC (RM4-5, Biolegend), CD25-Biotin (MAGM208, PartNo 860126, R&D), Streptavidin-eFluor450 (48-4317-82, eBioscience), IFNγ-PECy7 (XMG1.2, BD Biosciences), IL-17-APC (TC11-18H10.1, Biolegend) and FoxP3-APC (FJK-16s, eBioscience).

Cloning, expression and purification of recombinant proteins

UreaseA and UreaseB gene were amplified from bacterial genomic DNA using pfu-DNA polymerase (ThermoScientific) with the following primers: UreA fw: 5'-ATA TGA ATT CTT AAT TCT CCT TAA TTG TTT TT-3', UreA rv: 5'- ATA TGG ATC CAA ACT CAC CCC AAA AGA-3', UreB fw: 5'- ATA TGT CGA CCG AAC ACA TGG TAA GTT T-3', UreB rv: 5'- ATA TGA ATT CAA AAA GAT TAG CAG AAA AGA-3'. Amplified fragments were inserted into the pGEX-4T-1 plasmid (GE Healthcare) using BamHI and EcoRI restriction sites for UreaseA and SalI and EcoRI restriction sites for UreaseB (all restriction enzymes obtained from New England Biolabs). After amplification and sequencing

of the resulting plasmids, positive clones were transformed into *E. coli* BL21. Induction of overexpression of the GST-tagged proteins was done by the addition of 1mM IPTG. Bacterial cells were homogenized by sonication and precleared lysates were applied to GST GraviTrap columns (GE Healthcare). Tagged proteins were isolated according to manufacturer's instructions.

Supplemental references

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