Supplemental Methods

qRT-qPCR.

RT-qPCRs were carried out using Power SYBR Green RNA-to-CT 1-Step Kit according to the specifications of the manufacturer (Applied Biosystems). This kit consists of 2x Power SYBR® Green RT-PCR Mix containing SYBR® Green I dye, AmpliTaq Gold DNA Polymerase, dNTPs, ROX (6-carboxy-X-rhodamine) passive reference dye, buffer components and 125x RT Enzyme Mix containing ArrayScript UP Reverse Transcriptase and RNase inhibitor. Reaction mixtures were scaled down to a final volume of 25 µl. Final concentrations of primers in the RT-qPCR mix were 166 nM. For each reaction, 10 ng µl of the isolated RNA (adjusted to 1 µg/µl) were added. RT-qPCR and data analysis were performed on a StepOnePlus Real-Time PCR System (Applied Biosystems). A one-step protocol combining the RT reaction and PCR reaction was performed with the following conditions: an initial cycle of 30 min at 48°C for the generation of the cDNAs; a second cycle of 10 min at 95°C to activate the hot-start Taq polymerase; and 40 cycles of denaturation at 95°C for 15 s, primer annealing at 60°C for 1 min. After 40 cycles, a melting curve with a ramp speed of 0,3% between 60°C and 95°C was determined using SYBR green fluorescence. In each reverse transcription reaction mixture, the same RNA samples were not supplemented with reverse transcriptase to detect DNA contamination. The baseline and Cq were automatically determined using the StepOne Software v2.3 (Applied Biosystems, Life Technologies). Reactions were performed in triplicates and none of the Cq values was discarded. The overall SD for the Cq variance was 0,1 calculated across all sets of data. The signal of NTC amplification plots was very late (Cq>31 for GAPDH, Cq>37 for IL-8, Cq>34 for ALPK1 and Cq>32 for TIFA) and therefore there was a high Cq value difference between the negative control and all the cDNA samples. Gene-specific amplification was confirmed by a single peak in the melting-curve analysis. The Cq values were converted into RQ via the delta-Cq method (Livak et al. 2001). Cq values of GAPDH were used as reference as the expression level of GAPDH was stable across different treatments. Finally, RQ values were transferred to GraphPad (GraphPad Prism 7.00 Software) and an unpaired two-tailed Student's t-test was used to compare two separate sets of independent samples. qRT-PCR was performed using Power SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems) according to the manufacturer´s recommendations using Tthe following primers were used:

GAPDH

5´-GGTATCGTGGAAGGACTCATGAC-3´ and 5´-ATGCCAGTGAGCTTCCCGTTCAG-3´, IL-8

5´-ACACTGCGCCAACACAGAAAT-3´ and 5´-ATTGCATCTGGCAACCCTACA-3´, TIFA

5´-TGGTAAACCGTCATCTGGAG¬-3´ and 5´-GAGTTCACTGACTCCCCAGC -3´. ALPK1

5´-CACCAAGAACACAATAGCCG-3´ 5´-ACCTGAAGGATGTGATTGGC-3´

Deletion of *RfaE* gene in *H. pylori*

Deletion of the *RfaE* gene was performed as described by Belogolova et al, 2013. Briefly, the upstream region (0.5 kb) and the downstream region (0.5 kb) of *RfaE* were amplified by using the primer combinations 0858_A/ 0858_B and 0858_C/ 0858_D respectively. Up- and downstream fragments, were gel-purified, digested with BamHI and ligated. After column purification of the ligation mixture a PCR was carried out using the ligation product as a template with the primer combinations 0858_A/ 0858_D. The 1 kb products of up- and downstream fragments were cloned into pGEM-T-easy and separated by the *KanR* gene at the BamHI restriction site. This plasmid was transformed into the dam-negative *E. coli* strain GM2199. Plasmid DNA from positive *E. coli* clones was isolated and used to transform the *H. pylori* P12 WT (strain collection no P511). Transformed *H. pylori* were selected on kanamycin-containing agar plates. *RfaE* gene knockout was confirmed by PCR amplification using primers spanning a DNA up- and downstream of sites targeted by primer 0858_A and 0858_D.

0858_A 5`-TTGGATTCGTGGGGTATCATCG-3`; 0858_B 5`- CGGGATCCATTTGATTCAAAAAGAATTTTTA-3´; 0858_C 5`- CGGGATCCCACGCTGTTCTTTAAAAATCG-3´; 0858_D 5`- TTCCAAGCTTTGCATGGACG-3´

CRISPR–Cas9 deletion of ALPK1 and TIFA in AGS SIB02 cells. Oligos encoding for ALPK1 or TIFA sgRNA were cloned into the vector lentiCRISPR version 2 (Addgene plasmid # 52961) (Sanjana et al., 2014). 293T cells (ATCC) were transfected with a mixture of the following vectors: packaging vector psPAX2 (Addgene plasmid 12260), envelope vector pMD2.G (Addgene plasmid 12259) and plentiCRISPRv-ALPK1.01, plentiCRISPRv2-

TIFA.03 or plentiCRISPRv2-empty vector using FuGENE6 (Promega) diluted in OptiMEM (Gibco). LV was harvested 2 d after transfection by filtrating (0.45 µm) and concentrating (Lenti-X Concentrator, Clontech) the supernatant. AGS SIB02 cells were transduced with 10x concentrated LV containing supernatant by adding 50 µg polyprene (Sigma) per 10-cm^2 plate. Transduced bulk cells were selected with 0,4 µg/ml puromycin (Gibco) for 1 week and subjected to single-cell cloning by limiting dilution. Single-cell clones with disrupted ALPK1 and TIFA expression were identified by p65 translocation assay upon infection with *H. pylori* and treatment with TNF-α. Clones were genotyped by sequencing of genomic DNA and qRT-PCR for ALPK1 and TIFA.

CACCGGGACCAGCGCTGCAGAGGTG (ALPK1 up-Oligo) AAACCACCTCTGCAGCGCTGGTCCC (ALPK1 down-Oligo) GTGGTAGCTGTGCTACTGCAAG (ALPK1 sequencing primer fw) ACTCATCTCTCGGTGGTCATCT (ALPK1 sequencing primer rv) CACCGGATCGTGGACAGCAGAGAGC (TIFA up-Oligo) AAACGCTCTCTGCTGTCCACGATCC (TIFA down-Oligo) AACAGAGAGAAACTCCCTTCCA (TIFA sequencing primer fw) TGATACTCTCCGAATCTGACCA (TIFA sequencing primer rv).

RNAi interference. All small interfering RNAs (siRNA) were purchased from Qiagen.

Transfections robotic system: 500 AGS SIB02 cells/well were seeded in 384 well plates with a final volume of 0.15 µl Hiperfect transfection reagent (Qiagen) and a siRNA concentration of 20 nM each per well. Transfection was performed on a BioRobot 8000 system (Qiagen). In cases two siRNAs were combined, both siRNAs were used at half concentration having the same total siRNA concentration as single siRNAs.

Manual transfections for *Protein analysis, qRT-PCR* : 50.000 cells /well were seeded in 12-well plates and were directly transfected with 1 µg/well siRNA using 6 µl Hiperfect /well. Experiments were performed 72-96 h after transfections.

siRNAs sequences:

IKK-β 5'-AAACCGAGTTTGGGATCACAT-3', IKKα 5'-AAACCGAGTTTGGGATCACAT-3'.

ALPK1 siRNA1 5'-CACCCAATTCATCGTAATCAT-3', ALPK1 siRNA2 5'- TTGGGTTTCATTGCCGGGAAA-3'.

ARD1 siRNA1 5'-CCGGGCCGCCCTGCACCTCTA-3', ARD1 siRNA2 5'-ATCAGTGAAGTGGAGCCCAAA-3',

NARG1 siRNA1 5'-AAGAAGCTACGTAATAAACAA-3', siRNA2 5'-CAAGCGGATCTTGAGGTGTTA-3',

TAK1 siRNA1 5'-AACGGACAGCCAAGACGTAGA-3', siRNA2 5'-AAAGCGTTTATTGTAGAGCTT-3',

HCF1 siRNA1 5'-CTGGTTTGTTGTATTATAGTA-3', siRNA2 5'-ACCGTTCACTATTGTAGAGTA-3',

MLL4 siRNA1 5'-CTCGAATCAGGTCAAGGTCAA-3', siRNA2 5'-CACGAGCTGGTGTTCTGTCAA-3',

Ash2L siRNA1 5'-CCCGTTTAACAAAGATGGCTA-3', siRNA2 5'-CTGTGACTTGTTATCCTACTA-3'

TRAF6 siRNA 5'-ATGGTGAAATGTCCAAATGAA-3', 5'-CAGCGCTGTGCAAACTATATA-3'

p65 siRNA 5'-AAGAGCATCATGAAGAAGAGT-3', 5'-AAGATCAATGGCTACACAGGA-3'

Control siRNA: AllStars negative control

Live fluorescence imaging. For analysis of living cells expressing fluorescent fusion proteins, cells were seeded onto µ-slide 8 well dishes (Ibidi). Cells were either transfected, infected with *H. pylori* at MOI 100 or left untreated. After treatment, cells were immediately imaged either on a Leica TCS-SP5 confocal or a Zeiss Axiovert 200M wide field microscope, both equipped with an incubator (37°C, 5% CO2). Image series were generated using LAS X (Leica) or Volocity 6.1 (Perkin Elmer for the Zeiss microscope equipped with a Hamamatsu Orca camera). Volocity was also used to prepare the videos.

Pull-down assay and MS analysis. AGS cells were seeded in 10 cm dishes (Corning) and transfected for 24 h with Myc-Flag-TIFA using FuGENE6. Cells were infected with *H. pylori* (MOI 100, 30 min) under serum-free conditions, washed with ice cold PBS, lysed (50 mM Tris HCL pH7.4, 150 mM NaCl, 1 mM EDTA, 1% (w/v) Triton X-100,complete protease inhibitor,1mM Ortho-vanadate), passed 10 times to a 23G needle and cleared from cellular

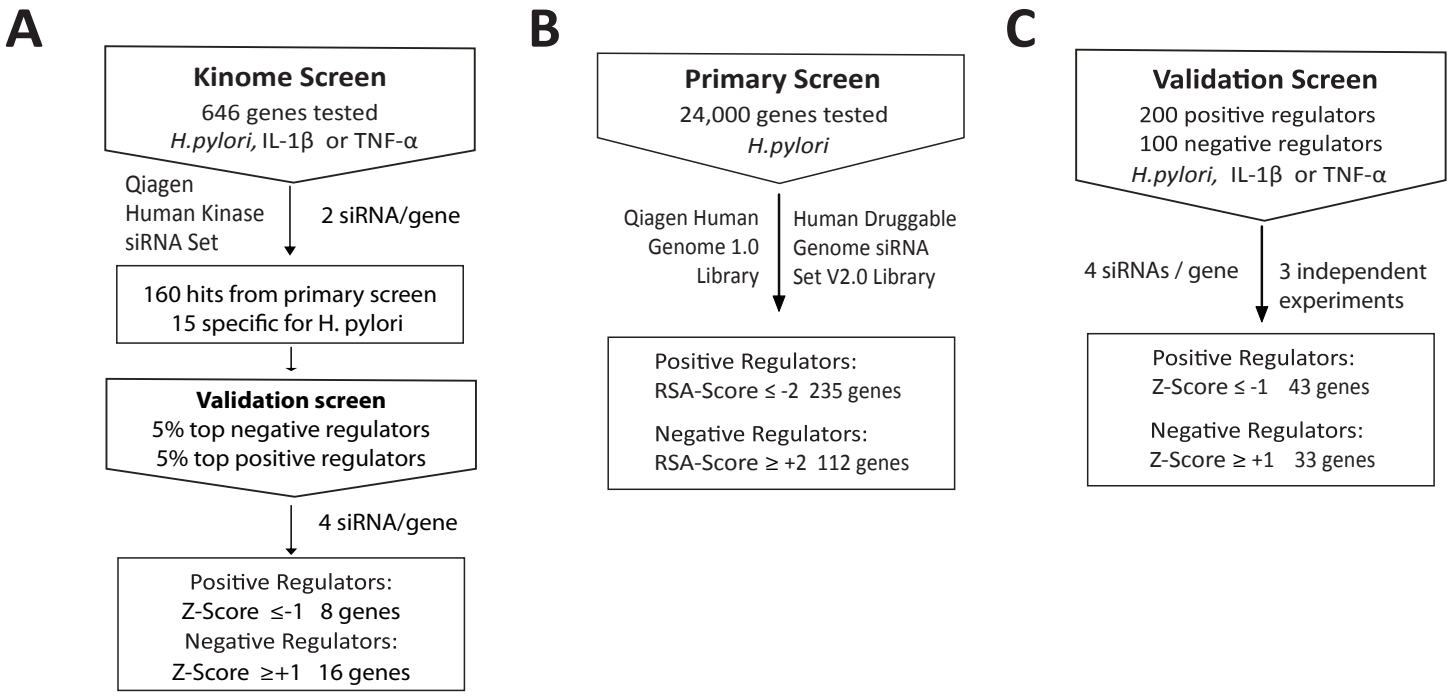
debris by centrifugation in a table top centrifuge for 10 min at 4°C at full speed. Cleared lysate was incubated with pre-cleaned anti Flag-M2 magnetic beads (Cat. No: M8823, Sigma) at 4°C for 4 h on a rotating wheel. Samples were washed with TBS (50mMTris HCL, 150mM NaCl, pH 7,4) and for Western blot analysis eluted by boiling beads for 3 min in 2x non-reducing Laemmli buffer. For MS analysis samples were eluted with acidified Glycine (0.1 M Glycine HCl, pH 3.0) and immediately neutralized with Tris HCL (0.5 M Tris HCl, pH 7.4, 1.5 M NaCl). Cysteines were reduced with Dithiothreitol (6.4 mM) at 60°C for 15 min and alkylated with iodoacetamide (12.5 mM) at room temperature for 15 min in the dark. Thereafter the proteins were digested with trypsin (0.3 µg per sample;Promega V5111) overnight at 37°C. Following digestion peptide mixtures were acidified with TFA to 0.5% (v/v), desalted using ZipTip C18 (Millipore) and then lyophilized. The peptides were analyzed using a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific) coupled on line to a Dionex UltiMate 3000 RSLC nano system (Thermo Fisher Scientific). Samples were loaded on a C18 PepMap 100 trap column (100 µm i.d.x 2cm; 5 µm particle size; Thermo Fisher Scientific) at a flow rate of 20 µl/min 2:98 (v/v) acetonitrile/water containing 0.1% TFA for pre-concentration and desalting. Separation was performed using a C18 PepMap PicoFrit (75µm i.d.x 20 cm; 3 µm particle size; uncoated Tip, 10 µm; New Objective) at a flow rate of 300 nl/min. HPLC solvent A was 0.1% (v/v) FA and peptides were eluted from the column using HPLC solvent B 80:20 (v/v) acetonitrile/water containing 0.1% FA starting from 3%, increasing to 15% in 1 minute, to 55% in 30 minutes and to 98% in 2 minutes. The peptides were analyzed in data-dependent acquisition mode that alternated between one MS scan and 10 MS/MS scans. MS scans were acquired over a mass range of m/z 350–1500 and resolution was set to 60000 with detection in the Orbitrap mass analyzer. For MS/MS scans ions were fragmented with CID in the LTQ linear ion trap. Proteins were identified using Mascot (Version 2.5; Matrix Science) searching against the Swiss-Prot human sequence database (released Sep 09, 2016, 20,178 entries), NCBI Helicobacter_P12 (released Sep 29, 2016, 3,326 entries) and an in-house sequence database containing Myc-Flag-TIFA and predicted binding partners of TIFA. Mass accuracy was set to 10 ppm for MS mode and to 0.5 Da for MS/MS mode. Searches were performed using the following parameters: max. missed cleavages 2; variable modifications carbamidomethyl (C), oxidation (M), acetylation (protein N-terminus) and pyroglutamate formation (N-term Q). The false discovery rate was set to 1% and acceptance criteria for protein identifications were at least three significant peptides.

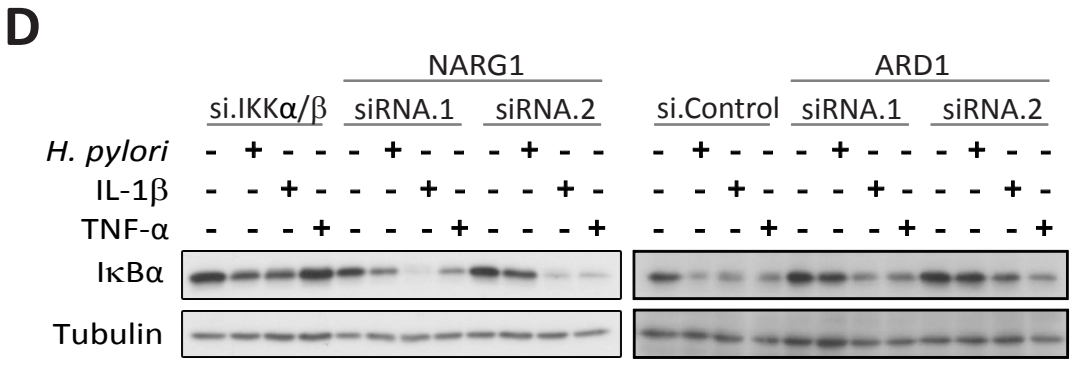
Synthesis of D-*glycero*-α,β-D-*manno*-heptose 1,7-bisphosphate and D-*glycero*-D-*manno*-heptose 7-phosphate. D-*Glycero*-D-*manno*-heptose 7-phosphate was prepared as the triethylammonium salt according to literature (Güzlek et al., 2005). The synthesis of both anomeric forms of D-*glycero*-D-*manno*-heptose 1,7-bisphosphates is described in Figure S5.

References

Güzlek, H., Graziani, A., and Kosma, P. (2005). Note. Carbohydr Res 340, 2808-2811.
Sanjana, N.E., Shalem, O., and Zhang, F. (2014). Improved vectors and genome-wide libraries for CRISPR screening. Nat Meth 11, 783-784.

Fig S1





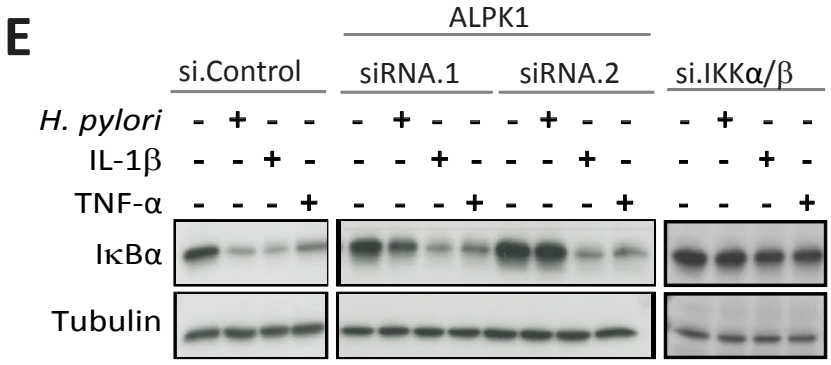


Figure S1: Screening procedures and identification of NARG1, ARD1 and ALPK1 as positive regulators for *H. pylori*-induced NF-κB activation (related to Figure 1) (A) Kinome screen: 646 genes were silenced using Qiagen Human Kinase siRNA Set. NF-κB activation was induced with *H. pylori,* IL-1β and TNF-α. 5% top hits from this screen were further validated with 4 individual siRNAs per gene (B) Primary genome-wide screen: 24,000 genes were silenced using Qiagen siRNA libraries. NF-κB activation was induced with *H. pylori*. Experiments were done in triplicates. CellHTS2 and RSA were used for data analysis. In summary, 347 hit genes were identified. Of these, 235 positively regulated and 112 negatively regulated NF-κB activation. (C) Validation screen: 300 selected candidate genes (200 positive and 100 negative regulators from the primary screen) were tested. *H. pylori* as well as IL-1β and TNF-α were used as inducers for NF-κB activation. Data analysis based on CellHTS2. (D+E) Knockdown of NARG1, ARD1 and ALPK1 blocked degradation of IκBα. Shown are representative Western blots of at least two independent experiments.

Fig S2

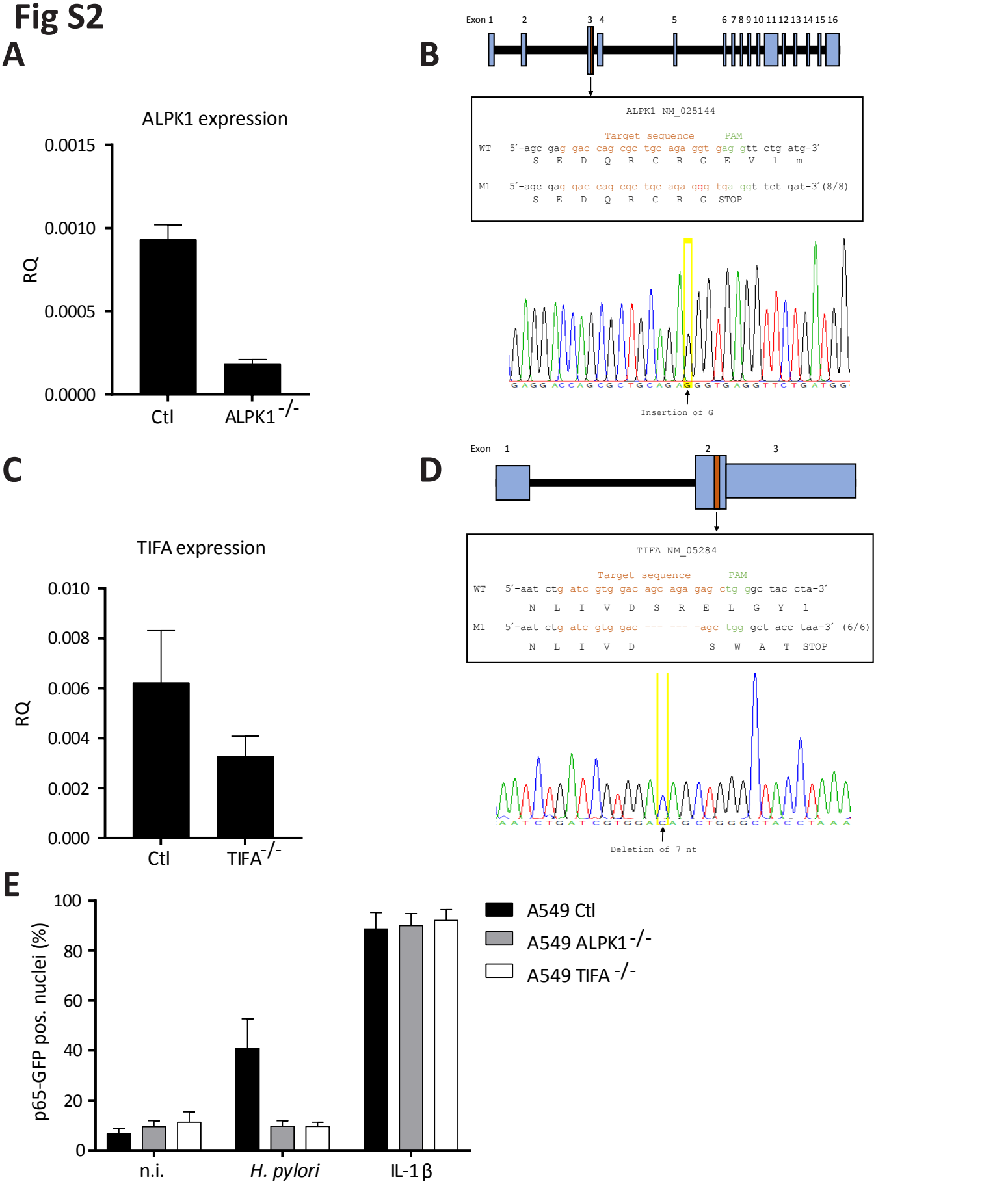


Figure S2: Generation and characterization of ALPK1 and TIFA knockout cells (related to Figure 2). (A+C) ALPK1 mRNA expression is reduced in AGS SIB02 depleted for ALPK1 by CRISPR/Cas9 and TIFA mRNA expression is reduced in AGS SIB02 depleted for TIFA by CRISPR/Cas9. The expression fold change is normalized to non-infected control cells. (B+D) Schematic representation of ALPK1 and TIFA protein. The area of the proteins corresponding to the target region for CRISPR/Cas9 based gene editing is highlighted in red. Boxes: overview of WT sequence and mutant sequence generated by CRISPR/Cas9. The number indicates how many times the sequence was detected within the same cell line. Right side: Sequence chromatograms produced by direct sequencing of PCR product from single cell lines with targeted mutations. Chromatograms produced by Clone Manager Suite 9. (E) CRISPR/Cas9-mediated silencing of ALPK1 and TIFA in A549 SIB01 NF-κB reporter cells inhibits p65 translocation after infection with *H. pylori* MOI 100 but not after stimulation with IL-1β (10 ng/mL) 30 min post infection or treatment. Shown is percentage of cells with nuclear p65 per well. Data represent mean + SEM of three independent experiments.

Fig S3

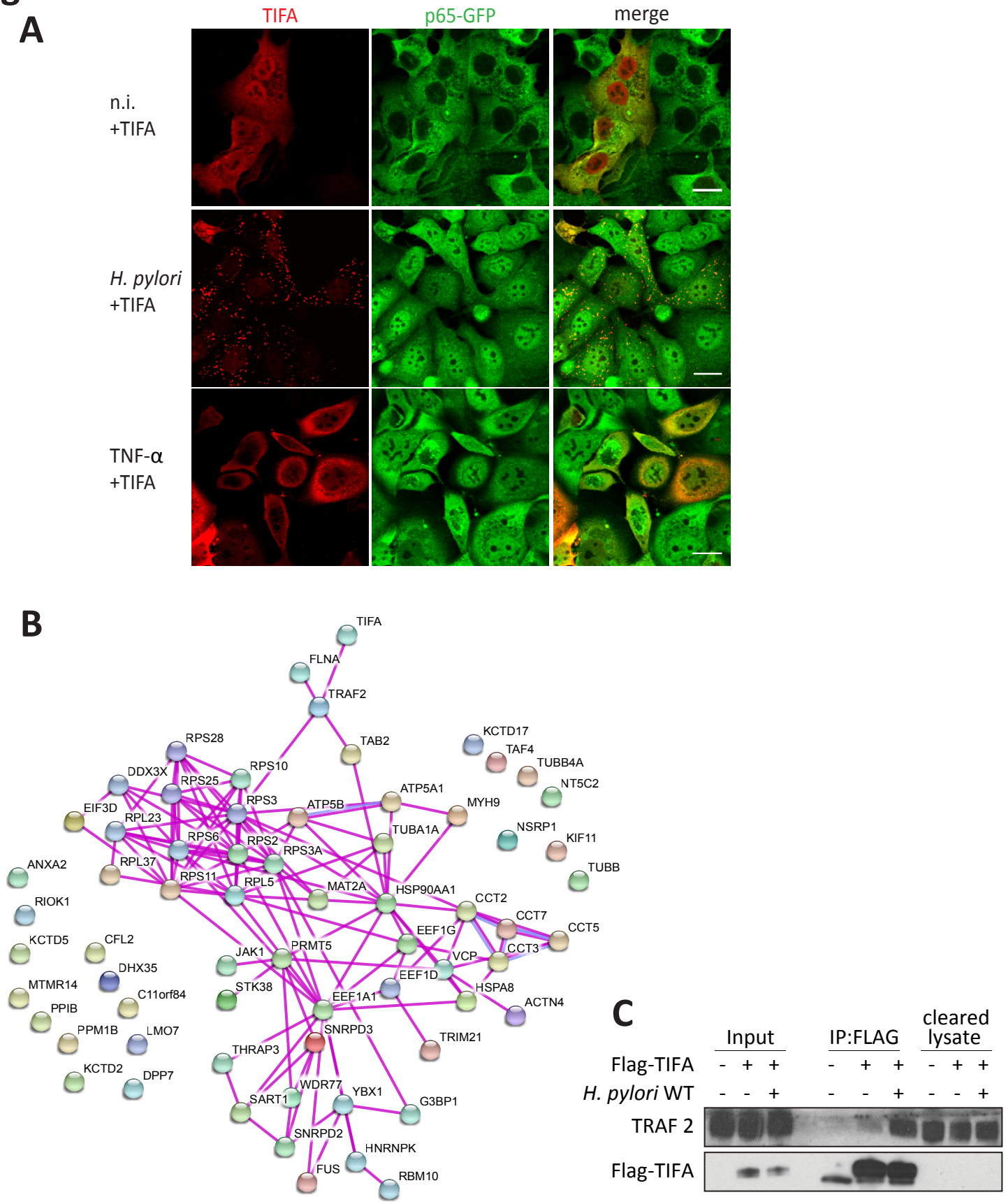


Figure S3. Characterization of TIFAsome formation and MS analysis of TIFA oligomer composition (related to Figure 2). (A) Overexpression of TIFA in AGS SIB02 wild type cells. *H. pylori* infection but not TNF-α induces TIFA oligomerization. AGS SIB02 cells were transfected for 24 h with dtTomato-TIFA (red) and then infected with *H. pylori* for (MOI 100, 30 min) or activated with TNF-α (20 ng/ml, 30 min). Scale bar*:* 20 µm. (B) Protein composition of TIFAsomes identified by mass spectrometry upon *H. pylori* infection. AGS cells were transfected for 24 h with Myc-Flag-TIFA then infected with *H. pylori* (MOI 100, 30 min). Myc-Flag-TIFA was precipitated and binding proteins were analyzed by UHPLC/MS/MS. Identified factors were depicted in a network with predicted interaction partners assembled by the STRING software (http://string.embl.de). (B) TIFA-TRAF2 interaction is induced specifically after *H. pylori* infection. Immunoprecipitation of Myc-Flag-TIFA and immunoblot for TRAF2 in AGS cells transiently transfected with Myc-Flag-TIFA and infected with *H. pylori* for 30 min at MOI 100.

Fig S4

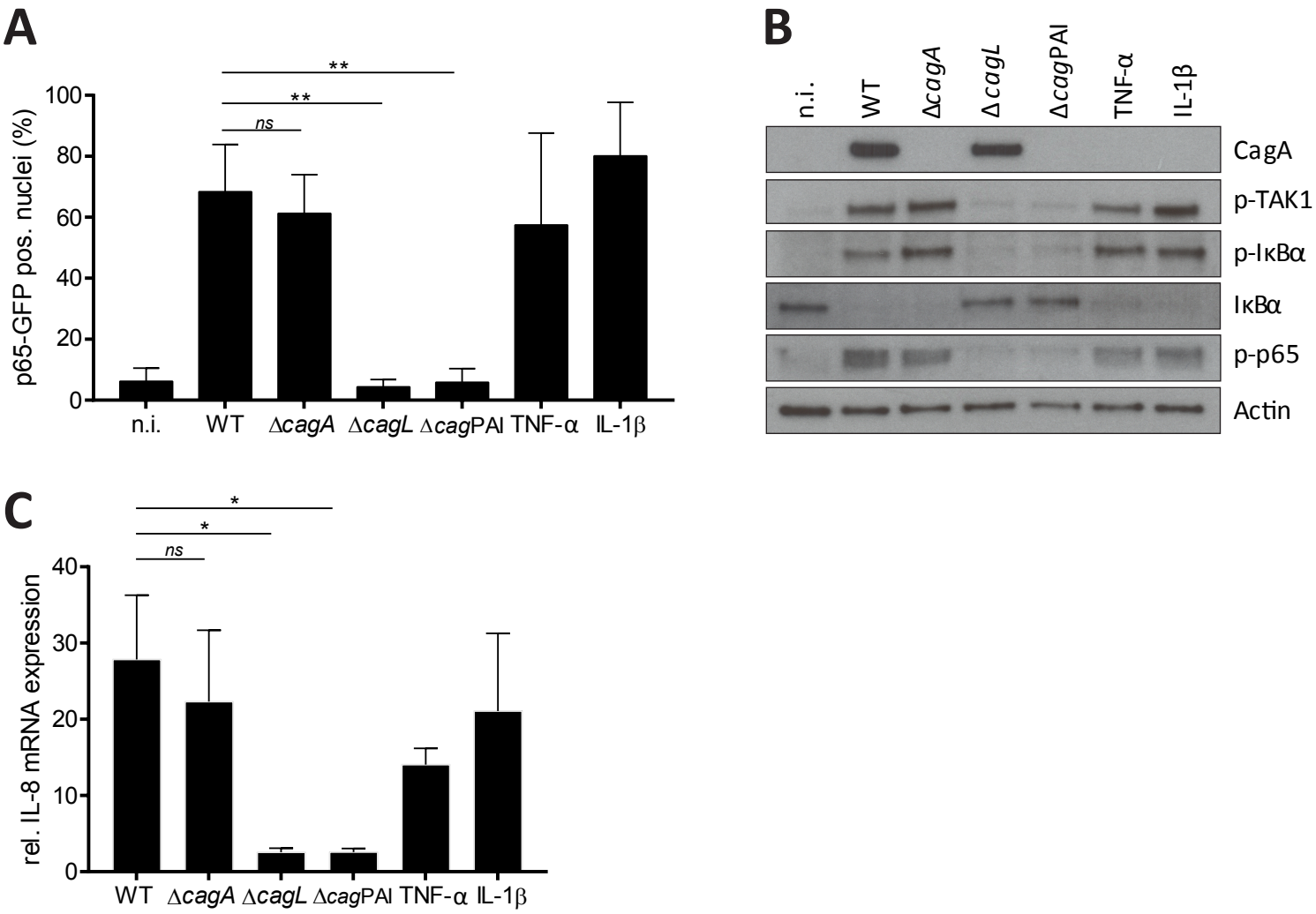
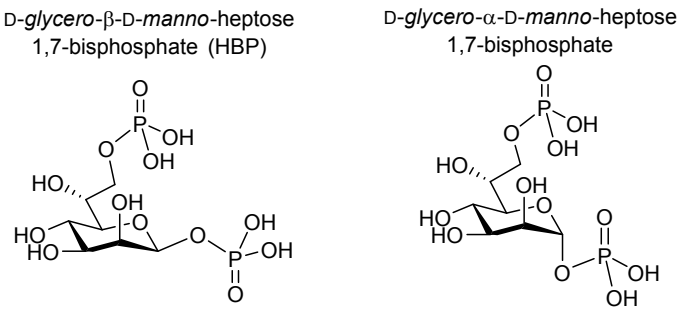


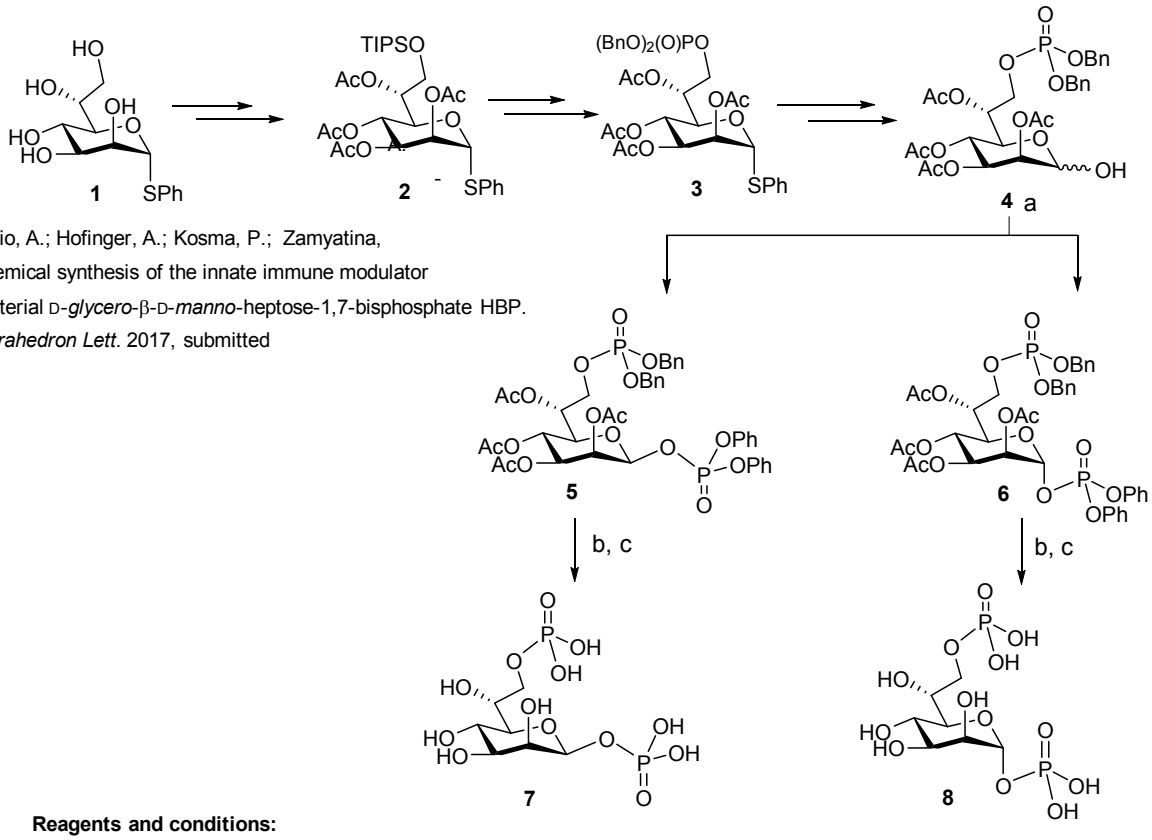
Figure S4: *H. pylori*-induced NF-κB activation is dependent on a functional type IV secretion system (related to Figure 4). (A) Transient NF-κB activation by *H. pylori* depends on the T4SS. AGS SIB02 cells were infected with wild type P12, Δ*cag*A, Δ*cag*L, and ΔPAI *H. pylori* (MOI 100, 30 min) or activated with TNF-α (20 ng/ml) or IL1-β (10 ng/ml, both 30 min). Cells were fixed, stained with Hoechst 33342 and then analyzed with automated microscopy for mean percentage of cells with nuclear GFP-p65 per well. Data shown as mean ± SEM of three independent experiments (B) *H. pylori*-induced NF-κB activation through activation of TAK1. AGS cells were infected with *H. pylori* or activated with TNF-α or IL1-β as in (A) then levels of total CagA, TAK1 phosphorylation at Thr-184/187, p65 phosphorylation at Ser536, as well as IκBα phosphorylation at Ser32, and IκBα degradation were analyzed by Western blot. Blot is representative of at least two independent experiments. (C) Impact of *H. pylori* infection on IL-8 mRNA expression. qRT-PCR analysis of IL-8 expression in AGS cells infected with wild type P12, Δ*cag*A, Δ*cag*L, and ΔPAI *H. pylori* 3 h p.i. at MOI 100. The expression fold change is normalized to non-infected control cells. Data represent mean + SEM of two independent experiments. Statistical analysis was performed using Student´s *t*-test (* $p \leq 0.05$; ** $p \leq 0.001$; *ns* = not significant)

Fig S5

A



B



Borio, A.; Hofinger, A.; Kosma, P.; Zamyatina, Chemical synthesis of the innate immune modulator bacterial D-*glycero*-β-D-*manno*-heptose-1,7-bisphosphate HBP. *Tetrahedron Lett.* 2017, submitted

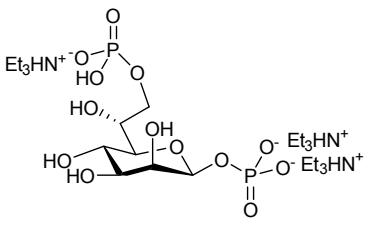
Reagents and conditions:

a) DMAP, DCM, $ClP(O)(OPh)_2$ slow addition of a DCM solution over 6 h, 0.5 equiv/h, rt;

b) H_2, PtO_2, MeOH, rt, 16 h;

c) MeOH/H_2O/Et_3N, 7:3:1 (v/v/v), pH=12, rt, 48 h

C



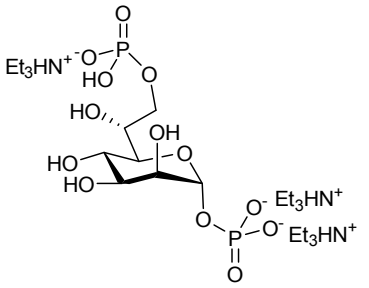
D-*glycero*-β-D-*manno*-heptopyranosyl-1,7-diphosphate (triethylammonium salt)

$[\alpha]_D^{20}$ = -4 (c =0.5, D_2O); ^{1}H-NMR (600 MHz, D_2O): 5.05 (d, 1H, H-1, $^3J_{1,P}$ = 8.5 Hz); 4.11 (dt, 1H, H-6, $^3J_{7a,6}$ = 7.4 Hz, $^3J_{5,6}$ = $^3J_{7b,6}$ = 3.5 Hz); 4.02 (ddd, 1H, H-7a, $^2J_{7a,7b}$ = 11.1 Hz, $^3J_{7a,P}$ = 6.6 Hz); 3.92 (m, 1H, H-7b); 3.91 (m, 1H, H-2); 3.67 (dd, 1H, H-4); 3.60 (dd, 1H, H-3, $^3J_{2,3}$ = 3.2 Hz, $^3J_{3,4}$ = 9.8 Hz); 3.44 (dd, 1H, H-5 $^3J_{5,6}$ = 3.1 Hz); 3.13 (q, 17H, 2.8xEt_3N, $J_{CH2,CH3}$ = 7.2 Hz); 1.21 (t, 23H, 2.8xEt_3N).

^{13}C-NMR (150.9 MHz, D_2O): 95.54 (C-1, $^2J_{1,P}$ = 4.4 Hz); 76.73 (C-5); 72.84 (C-3); 70.89 (C-6, $^3J_{6,P}$ = 6.6 Hz); 70.57 (H-2, $^3J_{2,P}$ = 7.7 Hz); 67.06 (C-4); 65.41 (C-7, $^2J_{7,P}$ = 5.5 Hz); 46.73 (Et_3N); 8.26 (Et_3N).

^{31}P NMR (243.0 MHz, D_2O): 0.68 (P-7) and -1.22 (P-1).

HRMS (ESI-TOF) m/z: calc. for $[M+H]^+ = C_7H_{16}O_{13}P_2$+H$^+$: m/z = 371,0139; found: m/z = 371,0139



D-*glycero*-α-D-*manno*-heptopyranosyl-1,7-diphosphate (triethylammonium salt)

$[\alpha]_D^{20}$ = +21 (c =0.5, D_2O); ^{1}H NMR (600 MHz, D_2O): 5.36 (dd, 1H, H-1, $^3J_{1,2}$ = 1.9 Hz, $^3J_{1,P}$ = 8.3 Hz); 4.14 (dt, 1H, H-6, $^3J_{7a,6}$ = 7.6 Hz, $^3J_{5,6}$ = $^3J_{7b,6}$ = 3.3 Hz); 4.05 (ddd, 1H, H-7a, $^2J_{7a,7b}$ = 10.9 Hz, $^3J_{7a,P}$ = 6.5 Hz); 3.93 (m, 2H, H-7b and H-2); 3.88 (dd, 1H, H-5 $^3J_{5,6}$ = 2.9 Hz, $^3J_{4,5}$ = 9.8 Hz); 3.85 (dd, 1H, H-3, $^3J_{2,3}$ = 3.3 Hz, $^3J_{3,4}$ = 9.5 Hz); 3.79 (dd, 1H, H-4); 3.17 (q, 14H, 2.4x Et_3N, $J_{CH2,CH3}$ = 7.2 Hz); 1.25 (t, 23H, 2.4x Et_3N).

^{13}C NMR (150.9 MHz, D_2O): 96.36 (C-1, $^2J_{1,P}$ = 5.2 Hz); 74.00 (C-5); 71.45 (C-6, $^3J_{6,P}$ = 7.7 Hz); 71.02 (H-2, $^3J_{2,P}$ = 8.8 Hz); 70.79 (C-3); 67.62 (C-4); 66.25 (C-7, $^2J_{7,P}$ = 4.4 Hz); 47.30 (Et_3N); 8.83 (Et_3N).

^{31}P NMR (243.0 MHz, D_2O): 0.73 (P-7) and -1.81 (P-1).

HRMS (ESI-TOF) m/z: calc. for $[M+H]^+ = C_7H_{16}O_{13}P_2$+H+: m/z = 371,0139; found: m/z = 371,0139

Figure S5: Schemes and synthesis of D-*glycero* -D-*manno*-heptose 1,7-bisphosphate (related to Figure 5). (A) Chemical structure of D-*glycero*-β-D-*manno*-heptose 1,7-bisphosphate and D-*glycero*-α-D-*manno*-heptose 1,7-bisphosphate. (B) Chemical synthesis of D-glycero-β-D-*manno*-heptose 1,7-bisphosphate and D-glycero-α-D-manno-heptose 1,7-bisphosphate. (C) Structural characterisation of D-glycero-β-D-*manno*-heptopyranosyl-1,7-diphosphate (βHBP) and D-glycero-α-D-*manno*-heptopyranosyl-1,7-diphosphate (αHBP).

Fig S6

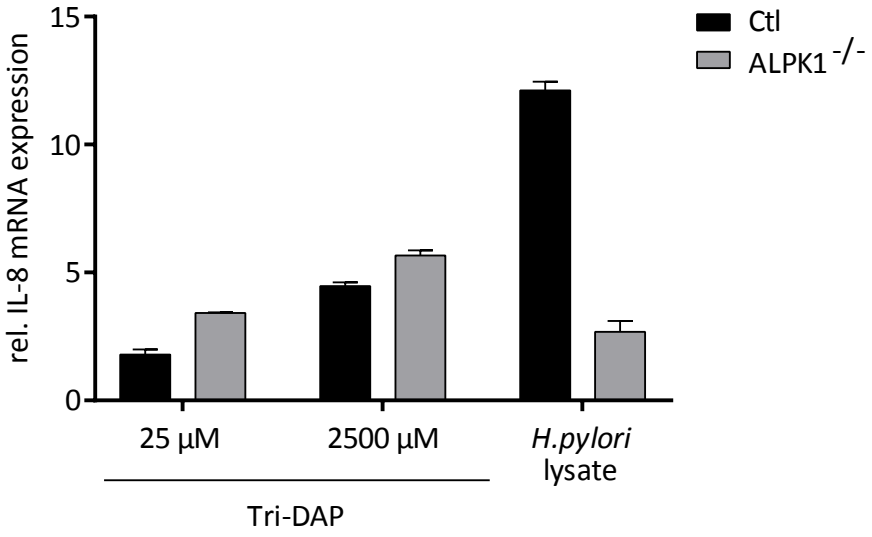


Figure S6: The NOD1 agonist Tri-Dap is not sufficient to induce NF-κB activation (related to Figure 5). Permeabilized AGS SIB02 control and ALPK1 deficient cells were treated with L-Ala-γ-D-Glu-Dap (Tri-Dap) for 3 h, as described for bacterial lysates. Levels of IL-8 mRNA were measured using RT-qPCR, normalized to mock treated control cells. Data is representative of two independent experiments. Data represent mean + SEM of experiment performed in triplicates.