Supporting Information

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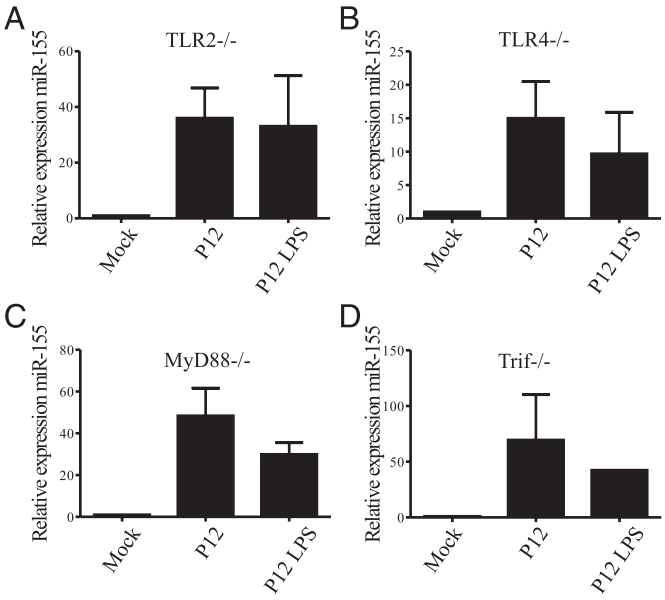

Fig. S1. Up-regulation of microRNA-155 (miR-155) is similar in Toll-like receptor 2 (TLR2$^{-/-}$), Toll-like receptor 4 (TLR4$^{-/-}$), MyD88$^{-/-}$, and Trif$^{-/-}$ bone marrow-derived macrophages (BMMs). (*A* and *B*) *H. pylori* P12 [multiplicity of infection (MOI) of 50] and P12 LPS (1.5 μg/mL, 2,000 endotoxin unit/mL) led to up-regulation of miR-155 in TLR2$^{-/-}$ (*A*) and TLR4$^{-/-}$ BMMs (*B*). (*C* and *D*) BMMs lacking the adaptor protein MyD88 (*C*) show an up-regulation of miR-155 in response to P12 and P12 LPS similar to that seen in BMMs lacking the adaptor protein Trif (*D*). Experiments were performed with at least two biologically independent experiments and are presented as mean + SE.

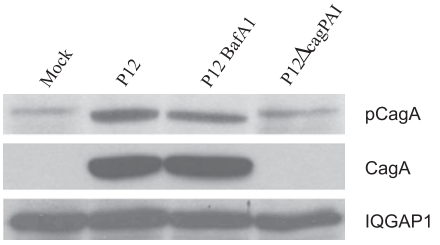

Fig. S2. Functional type IV secretion system in bafilomycin A1 (BafA1)-treated BMMs. A representative Western blot (of three total) shows phosphorylated CagA in P12-infected WT BMMs (3 h) as well as in P12-infected WT BMMs treated with 100 nM BafA1.

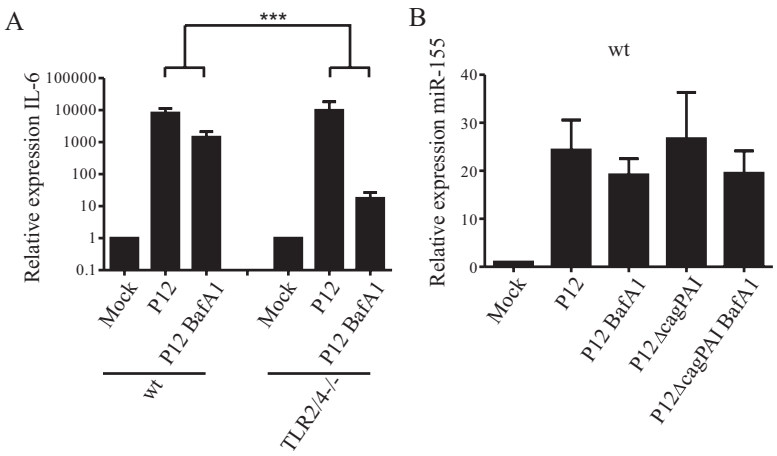

Fig. S3. Control experiments for the BafA1 effects. (*A*) BafA1 treatment (100 nM 1 h before infection) almost completely blocks IL-6 mRNA expression response to P12 infection (MOI of 50; 3 h) in TLR2/4$^{-/-}$ BMMs but not in WT BMMs. (*B*) WT BMMs were pretreated with 100 nM BafA1 or were left untreated and were infected with P12 (MOI of 50; 3 h). Experiments were performed with at least three biologically independent replicates and are presented as mean + SE. To determine the statistical significance, Student's *t* test was performed between the ratio of BafA1-treated to untreated BMMs; ***$P < 0.001$.

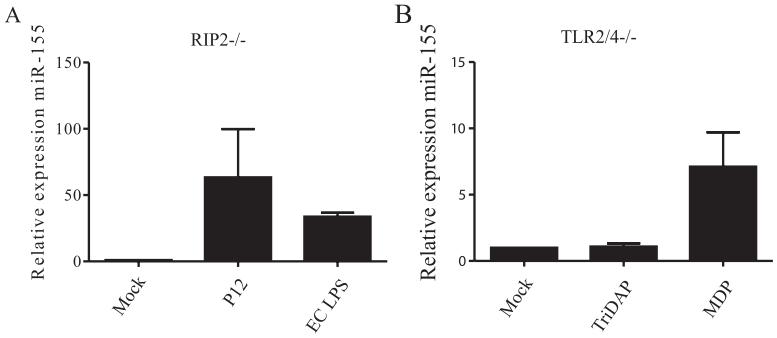

Fig. S4. Experiments with NOD1/2 ligands. (*A*) BMMs lacking the NOD adaptor molecule RIP2 were infected with *H. pylori* P12 (MOI of 50), *Escherichia coli* LPS (EC LPS, 100 ng/mL) for 3 h. (*B*) BMMs lacking TLR2/4 were incubated with the NOD1 ligand TriDAP (2.5 μg/mL) and the NOD2 ligand MDP (100 μg/mL) for 3 h. Experiments were performed with at least two biologically independent replicates and are presented as mean + SE.

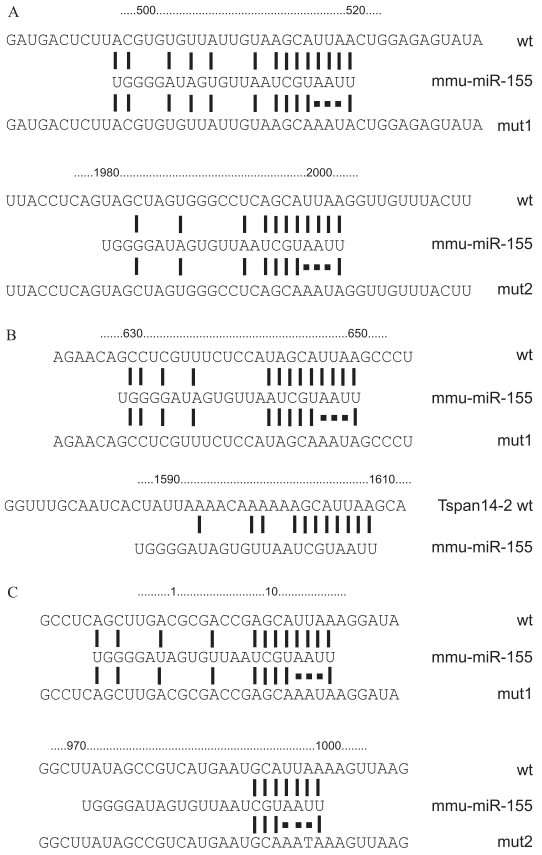

Fig. S5. Mutation of the miR-155 targets *Pmaip1*, *Tspan14*, and *Lpin1*. Sequences of the putative binding sites for Mus musculus (mmu) mmu-miR-155 (mouse) and their mutated versions *Pmaip1*-3′ UTR (*A*), *Tspan14*-3′ UTR (*B*), and *Lpin1*-3′ UTR (*C*). All putative targets here possess two binding sites for mmu-miR-155. In Lpin1 and Pmaip1 constructs, both binding sites were mutated; the double mutation showed the stronger effect. In case of Tspan14, a full-length construct (Tspan14-2), and a truncated version (Tspan14-1) were used; only the truncated version was mutated.

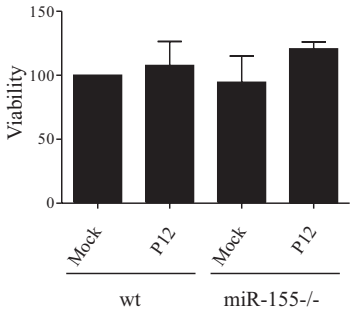

Fig. S6. Control of viability of BMMs before cisplatin treatment. To detect any differences in survival of the infected cells, water-soluble tetrazolium salts (WST-8) assays were performed. WT and miR-155$^{-/-}$ BMMs were infected with *H. pylori* P12 for 30 h (MOI of 50) or were mock infected. The results were normalized to mock-infected WT BMMs at 100%. The WST-8 assays did not reveal any significant differences among any of the cells. Data are presented as percentage (viability) and represent mean + SE of three biologically independent experiments.

Table S1. Microarray analysis shows known and unknown targets of miR-155 during infection with *H. pylori*

Array 1		Array 2			
Fold change	*P* value	Fold change	*P* value	Accession no.	Primary sequence name
−7.45	5.35E-30	−2.16	6.69E-16	NM_178165	Fcrl1
−5.95	0	−2.10	1.02E-30	NM_021897	Trp53inp1
−5.64	2.42E-32	−1.57	5.51E-12	NM_008043	Frat1
−4.83	0	−3.27	5.55E-39	NM_021451	Pmaip1
−4.69	1.50E-27	−1.80	1.16E-20	NM_175398	6530418L21Rik
−4.57	0	−1.89	2.86E-22	NM_015763	Lpin1
−3.96	0	−1.68	1.01E-18	NM_177733	E2f2
−3.74	4.28E-12	−1.88	1.84E-06	NM_010559	Il6ra
−3.74	3.08E-40	−1.62	2.54E-06	NM_008873	Plau
−3.62	1.11E-36	−1.70	3.24E-06	NM_013753	X99384
−3.32	2.16E-13	−1.60	1.41E-06	NM_001012450	Ankrd6
−3.08	2.97E-12	−1.74	3.82E-08	NM_007520	Bach1
−3.05	0	−1.54	1.92E-14	NM_028808	P2ry13
−2.94	1.52E-13	−1.90	1.39E-12	NM_133218	Zfp704
−2.83	6.42E-14	−1.52	1.09E-06	NM_019464	Sh3glb1
−2.79	2.13E-42	−1.65	2.16E-10	NM_024191	Arl2bp
−2.76	0	−1.86	7.21E-13	NM_008245	Hhex
−2.44	0	−1.89	1.24E-10	NM_145928	Tspan14
−2.20	3.67E-36	−2.48	3.20E-37	NM_133738	Antxr2
−2.12	1.14E-12	−2.18	4.10E-18	NM_024173	Atp6v1g1
−2.12	8.66E-18	−1.54	6.14E-09	NM_172595	Arl15
−2.09	1.28E-20	−1.67	5.70E-07	NM_011890	Sgcb
−2.07	5.30E-14	−1.87	1.47E-07	NM_145959	D15Ertd621e
−2.07	1.47E-07	−1.81	9.15E-13	NM_029019	Stard6
−2.00	3.94E-12	−2.03	1.55E-12	NM_008541	Smad5
−1.97	4.75E-07	−2.15	1.64E-06	NM_178592	Bat5
−1.91	1.15E-09	−1.72	5.01E-07	NM_134002	Csnk1g2
−1.85	2.40E-13	−1.73	2.75E-21	NM_175127	Fbxo28
−1.84	2.78E-19	−1.63	1.46E-06	NM_172742	BB128963
−1.82	1.22E-24	−1.65	3.58E-18	NM_138667	Map3k7ip2
−1.80	1.79E-17	−1.86	8.32E-09	NM_007929	Emp2
−1.60	3.92E-06	−1.72	1.14E-07	NM_146032	Srp68
−1.55	8.88E-12	−1.51	2.63E-10	NM_145958	Kbtbd2
−1.55	1.64E-15	−1.58	4.41E-09	NM_199307	Ece1
−1.54	3.15E-07	−1.87	2.96E-07	NM_173432	Pskh1

Data presented here show the overlap of down-regulated hits of array 1 and array 2 that are predicted by TargetScan5.1 possessing a 7-mer or 8-mer seed of mmu-miR-155.

Table S2. Primer sequences

Primer name	5′–3′ forward	5′–3′ reverse
Primers for quantitative RT-PCR (SYBR-green method)		
β-Actin	CGTGAAAAGATGACCCAGATCA	CACAGCCTGGATGGCTACGT
IL-6	ACAAGTCGGAGGCTTAATTACACAT	TTGCCATTGCACAACTCTTTTC
Pmaip1/Noxa	CCGCCGGTTGATGGAA	AGCGTTTCTCTCATCACATCACA
Bach1	CCGCAGCATCCATTTCAAC	TCCGCATAGGAAGGGCTCTT
Lpin1	GAGCATGCCAAGACCAACATC	CAATGGGAAGACGTGATCGA
Tspan14	GGCGAGGACCCTGATCTCA	TGCTGCATCTGCTTCTCAGAA
Primers for cloning		
Lpin1 3′ UTR	CCGCTCGAGCGCGACCGAGCATTAAAGG	ATAAGAATGCGGCCGCGGCTGCAGAGGGCATTTG
Pmaip1 3′ UTR	CCGCTCGAGGTTCTTCCAAAGCTTTTGCAAG	ATAAGAATGCGGCCGCGCATTTTTCAATAGTTAC
Tspan14-1 3′ UTR	CCGCTCGAGGAAGCAGATGCAGCAAATG	ATAAGAATGCGGCCGCGCATGCCTCGGAGTATCCAG
Tspan14-2 3′ UTR	CCGCTCGAGGAAGCAGATGCAGCAAATG	ATAAGAATGCGGCCGCGCTTAATGCTTTTTTGTTTTAATAG
Primers for mutagenesis		
Lpin1 mut1 3′ UTR	GAGCGCGACCGAGCAAATAAGGATAG	CCCCACAACCTATCCTTATTTGCTC
Lpin1 mut1/mut2 3′ UTR	GCCGTCATGAATGCAAATAAAG	CACACTTAACTTTATTTGCATTC
Pmaip1 mut1 3′ UTR	GTGTGTTATTGTAAGCAAATACTGG	CTTTATACTCTCCAGTATTTGCTTAC
Pmaip1 mut1/mut2 3′ UTR	GCTAGTGGGCCTCAGCAAATAGG	GTAAACAACCTATTTGCTGAGG
Tspan14-1 mut1 3′ UTR	CTCGTTTCTCCATAGCAAATAGCCC	CCCTCAGTAGGGCTATTTGCTATG