Supplementary Material

Small RNA mediated gradual control of lipopolysaccharide biosynthesis affects antibiotics resistance in *Helicobacter pylori*

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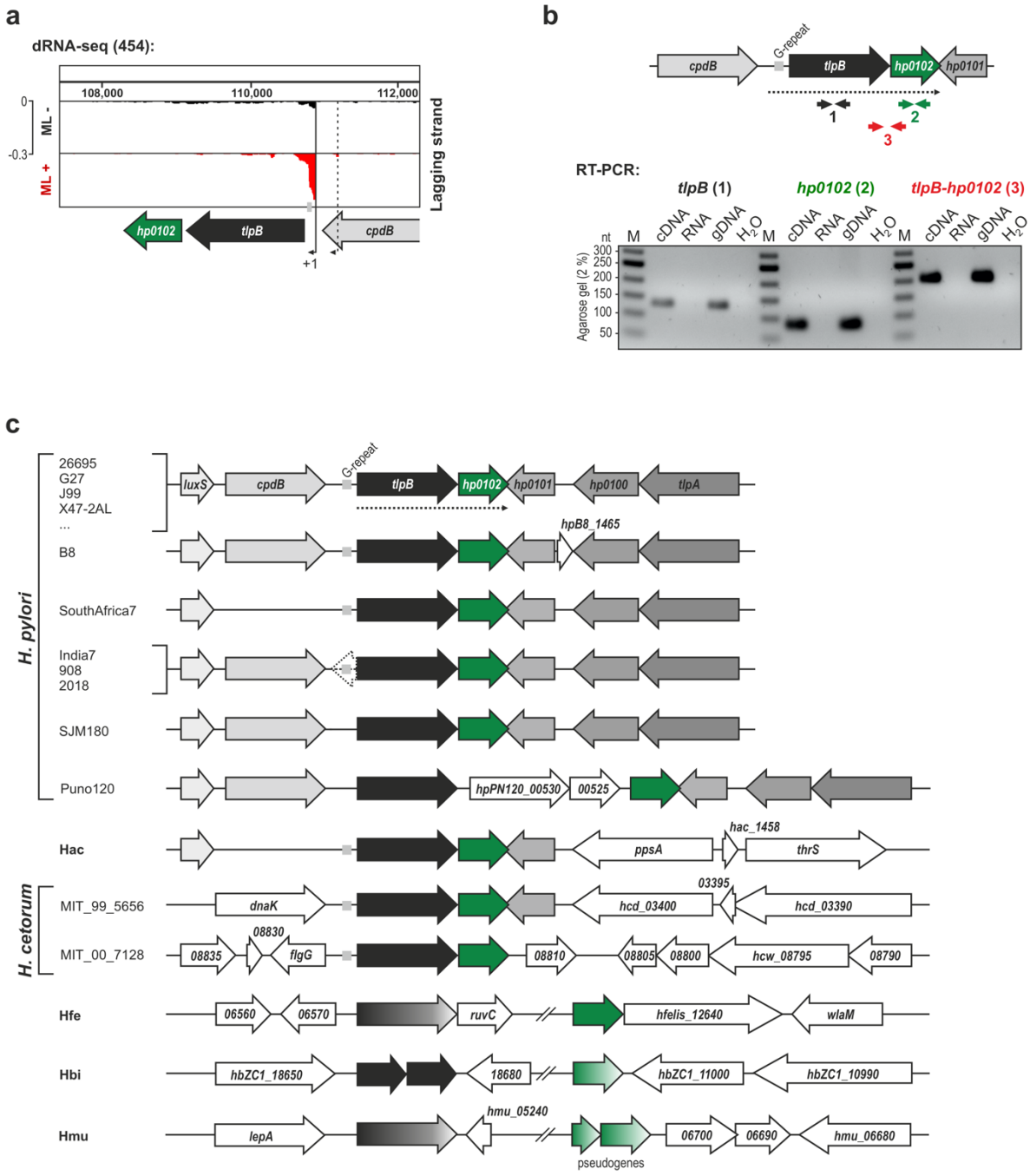
Supplementary Figures

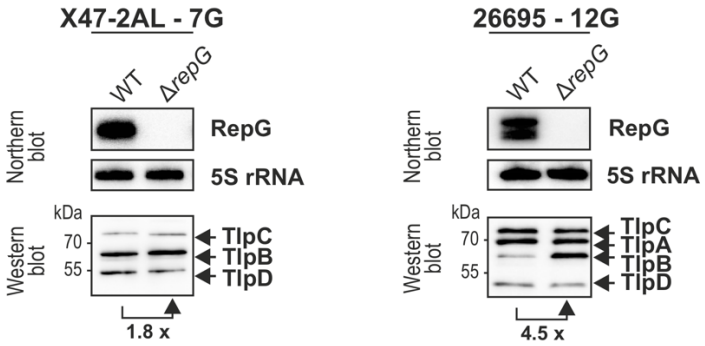
Supplementary Figure 1



Supplementary Figure 1: Conservation of the *tlpB-hp0102* operon among *Helicobacter* strains and species. (a) Screenshot of a genome browser showing cDNA read coverage at the bicistronic *tlpB-hp0102* operon based on our previous differential RNA-seq (dRNA-seq) analysis of *H. pylori* 26695[1]. RNA samples isolated from cells grown to mid-exponential growth phase (ML) were used for

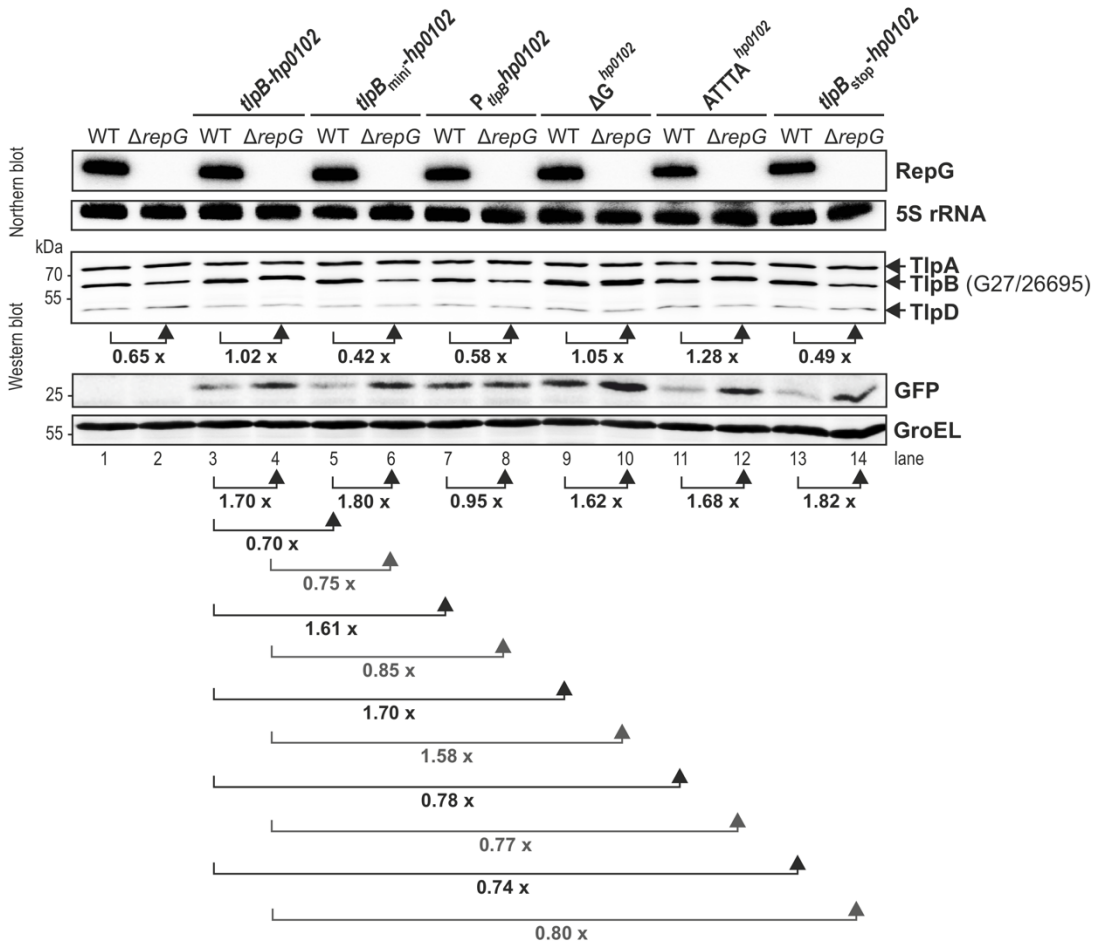
preparation of two cDNA libraries: (-) RNA left untreated, (+) RNA treated with terminator nuclease to enrich for primary transcripts. Thick arrows represent annotated open reading frames (ORFs) and thin arrows transcriptional start sites (TSS; +1; filled line - primary TSS, dotted line - secondary TSS). **(b)** *(Upper panel)* Schematic representation of the bicistronic *tlpB-hp0102* operon and RT-PCR validation strategy. Small arrows represent locations of gene-specific primers for *tlpB* (black)*, hp0102* (green), and overlapping both genes *tlpB-hp0102* (red). RT-PCR assays were performed on total RNA isolated from strain 26695. *(Lower panel)* Agarose gel of RT-PCR reactions performed in the presence (cDNA) and absence (RNA) of reverse transcriptase. Control reactions using genomic DNA (gDNA) of 26695 and water (H_2O) are indicated. Lane M contains a 50 bp DNA ladder. Underlying source data are provided as a Source Data file. **(c)** Gene synteny analysis of the *tlpB-hp0102* operon (dotted line) in different gastric *Helicobacter* strains and species. Homologs are indicated by the same colors (Hac - *H. acinonychis,* Hce - *H. cetorum,* Hfe - *H. felis*, Hbi - *H. bizzozeronii*, Hmu - *H. mustelae*), unrelated genes are indicated in white. The homopolymeric G-repeat in the 5' UTR of the *tlpB* mRNA is represented by a gray box. A potential ORF (dotted, white arrow) is annotated in the *tlpB* 5' UTR in *H. pylori* strains India7, 908, and 2018. Gradually colored arrows indicate limited sequence homology, whereas fully colored arrows represent homologs with >50 % sequence conservation. In *H. pylori* strain Puno120, two genes belonging to a type-II DNA restriction and modification system are inserted downstream of *tlpB*.

Supplementary Figure 2



Supplementary Figure 2: Analysis of RepG-mediated TlpB regulation in different *H. pylori* strains. Total RNA and protein samples of X47-2AL and 26695 WT and Δ*repG* were harvested in exponential growth phase and were analyzed by northern and western blot, respectively. RepG was detected by radioactively labeled DNA oligonucleotide CSO-0003; 5S rRNA served as loading control. The chemotaxis receptors TlpA, TlpB, TlpC, and TlpD were detected by polyclonal anti-TlpA22 serum. The results shown are representative of at least three independent experiments. Underlying source data are provided as a Source Data file.

Supplementary Figure 3

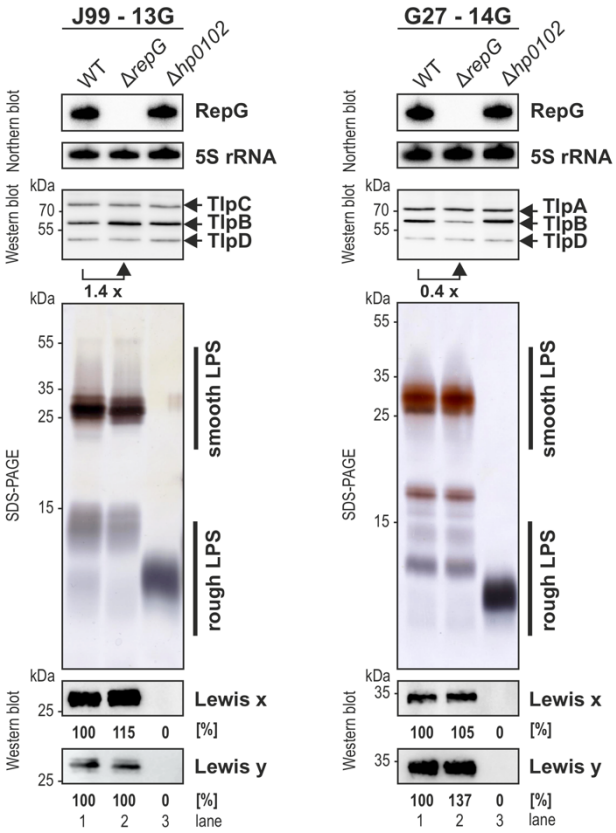


Supplementary Figure 3: Base-pairing of RepG to the homopolymeric G-repeat in the 5' UTR of the *tlpB-hp0102* mRNA results in repression of *hp0102* at the protein level. Several *hp0102*::*gfpmut3* reporter fusions (see also main Figure 2a; derived from *H. pylori* 26695) were investigated in *H. pylori* strain G27. *H. pylori* G27 wildtype, Δ*repG*, as well as WT and Δ*repG* strains carrying indicated *hp0102*::*gfpmut3* reporter fusions were grown to exponential phase, and RNA and protein samples were analyzed by northern (RepG, CSO-0003) and western blot (HP0102::GFP with anti-GFP antibody and TlpB with anti-TlpA22 antiserum), respectively. GroEL served as a loading control (anti-GroEL antibody). Depending on the G-repeat length, RepG mediates both activation of the endogenous G27 *tlpB* (14G) and repression of the ectopic *tlpB* of the 26695 (12G) reporter construct[2]. Accordingly, TlpB levels in the strains carrying the *tlpB-hp0102* (lanes 3-4), ΔG^{hp0102} (lanes 9-10) or $ATTTA^{hp0102}$ (lanes 11-12) fusions are the sum of both the endogenous G27 TlpB and ectopically expressed 26695 TlpB levels. The results shown are representative of at least three independent experiments. The joint result of all three replicates is shown in the bar diagram in Figure 2b. Source data underlying this figure is provided as a Source Data file.

Supplementary Figure 4

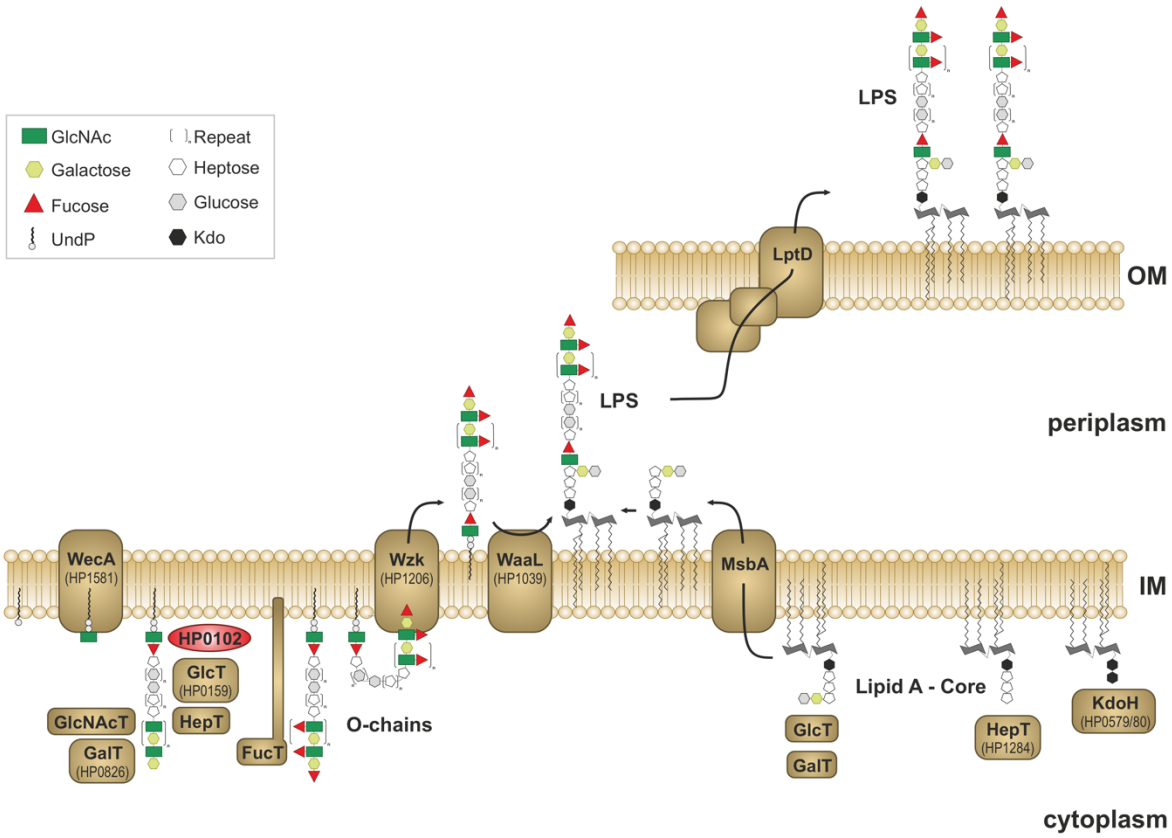
Supplementary Figure 4: Sequence alignment of the promoter, 5' UTR, and first amino acids of the *tlpB* coding region as well as the *tlpB-hp0102* intergenic region (IGR) in diverse *H. pylori* strains and *H. acinonychis* (Hac). (a-b) The *tlpB* stop codon, putative ribosome binding site (RBS), and annotated start codons of *tlpB* and *hp0102* are highlighted in light green. Potential RepG sRNA interaction sites (homopolymeric G-repeat and G^{hp0102}) are boxed in gray and orange, respectively. RepG-mediated *tlpB-hp0102* co-regulation was investigated in the strains marked in bold. **(c)** In *H. pylori* 26695, RepG was predicted to potentially base-pair to G^{hp0102} (orange) in the *tlpB-hp0102* IGR. The *tlpB* stop codon is marked in bold. Numbers indicate positions relative to the *hp0102* start codon. The previously identified *tlpB* binding site in the RepG terminator loop is shown in blue.

Supplementary Figure 5



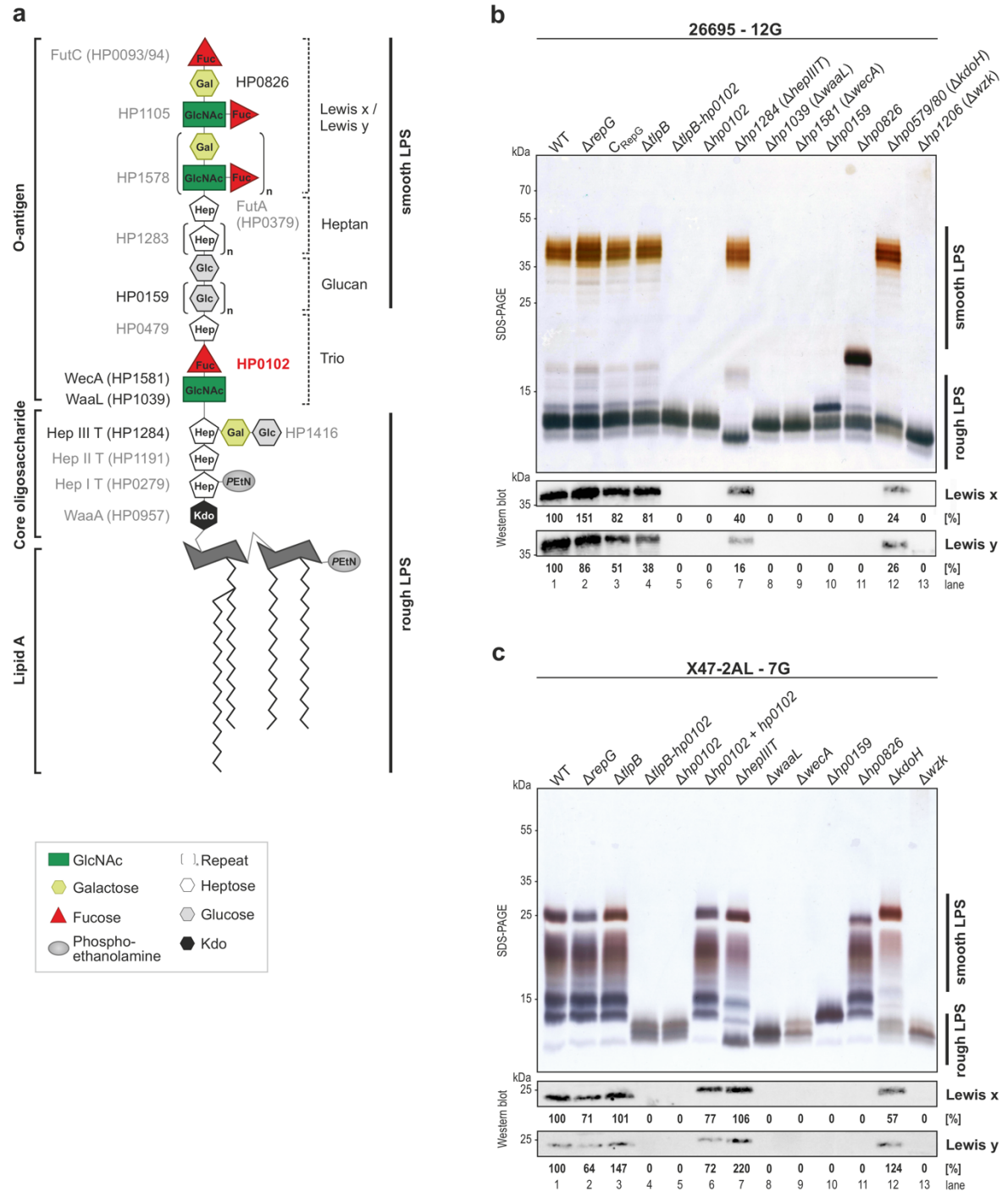
Supplementary Figure 5: HP0102 is involved in smooth LPS production in various *H. pylori* strains. Wildtype, Δ*repG*, and Δ*hp0102* deletion mutants of *H. pylori* strains J99 and G27 were grown to exponential growth phase. Total RNA and protein samples were analyzed by northern and western blot, respectively. RepG and the chemotaxis receptors TlpA, TlpB, TlpC, and TlpD were detected using a polyclonal anti-TlpA22 antiserum. *H. pylori* strains J99 and G27 each express only three chemotaxis receptors. LPS patterns and Lewis x/y antigen expression were analyzed by silver staining and western blot using a Lewis x/y antigen-specific antibody, respectively. The results shown are representative of at least three independent experiments. Source data underlying this figure is provided as a Source Data file.

Supplementary Figure 6



Schematic of the LPS biosynthesis pathway in *H. pylori*. Summary of the LPS biosynthesis pathway in *H. pylori* based on literature[3,4] and results from this study. The lipid A-core (right) and O-chains (left) are assembled independently from each other in the cytoplasm. Lipid A-core: Heptose and glucose units are added to the lipid A by multiple heptosyltransferases (HepTs) and glucosyltransferases (GlcTs). O-chains: The O-chains are assembled onto a polyisoprenoid membrane anchor (UndP). The initiating glycosyltransferase WecA transfers an N-acetylglucosamine unit from a nucleotide-activated donor (uridine diphosphate (UDP)-GlcNAc) to the lipid carrier, providing the platform for the O-chain synthesis. The linear O-chain backbone is assembled by processive galactosyltransferases (GalTs) and N-acetylglucosaminyltransferases (GlcNAcTs). Fucosyltransferases (FucTs) attach fucose units to the O-chain backbone, generating Lewis antigens. O-chains and the lipid A-core moiety are translocated through the inner membrane (IM) by the flippase Wzk and transmembrane protein MsbA, respectively, and are fused together by the O-chain ligase WaaL. The LPS molecule is then transported to the outer leaflet of the outer membrane (OM). Our results together with recent observations by Li et al. [4] indicate that HP0102 is the fucosyltransferase involved in the biosynthesis of the conserved trisaccharide of the O-antigen. See also Figure S7.

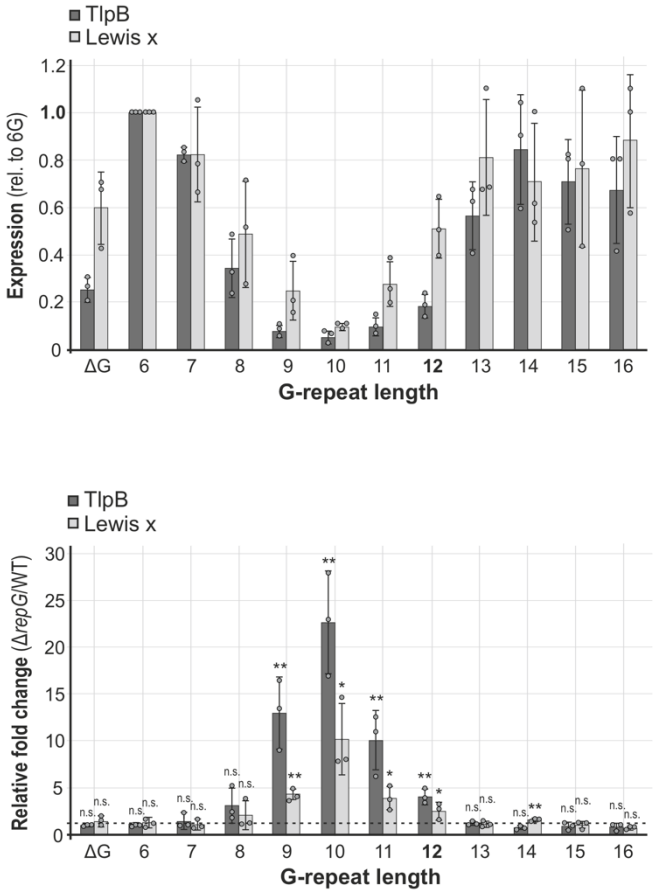
Supplementary Figure 7



Supplementary Figure 7: Comparison of LPS profiles elicited by deletion *hp0102* and additional genes involved in LPS biosynthesis in *H. pylori* strains 26695 and X47-2AL. (a) Schematic representation of the *H. pylori* 26695 LPS structure and genes involved in its synthesis according to and illustration inspired by Li et al. (2019)[4]. LPS samples harvested from WT and indicated mutant strains of *H. pylori* strains 26695 **(b)** and X47-2AL **(c)** during exponential growth were separated on 15 % SDS-PAGE gels and either directly visualized by silver staining, or electro-blotted to PVDF membranes and

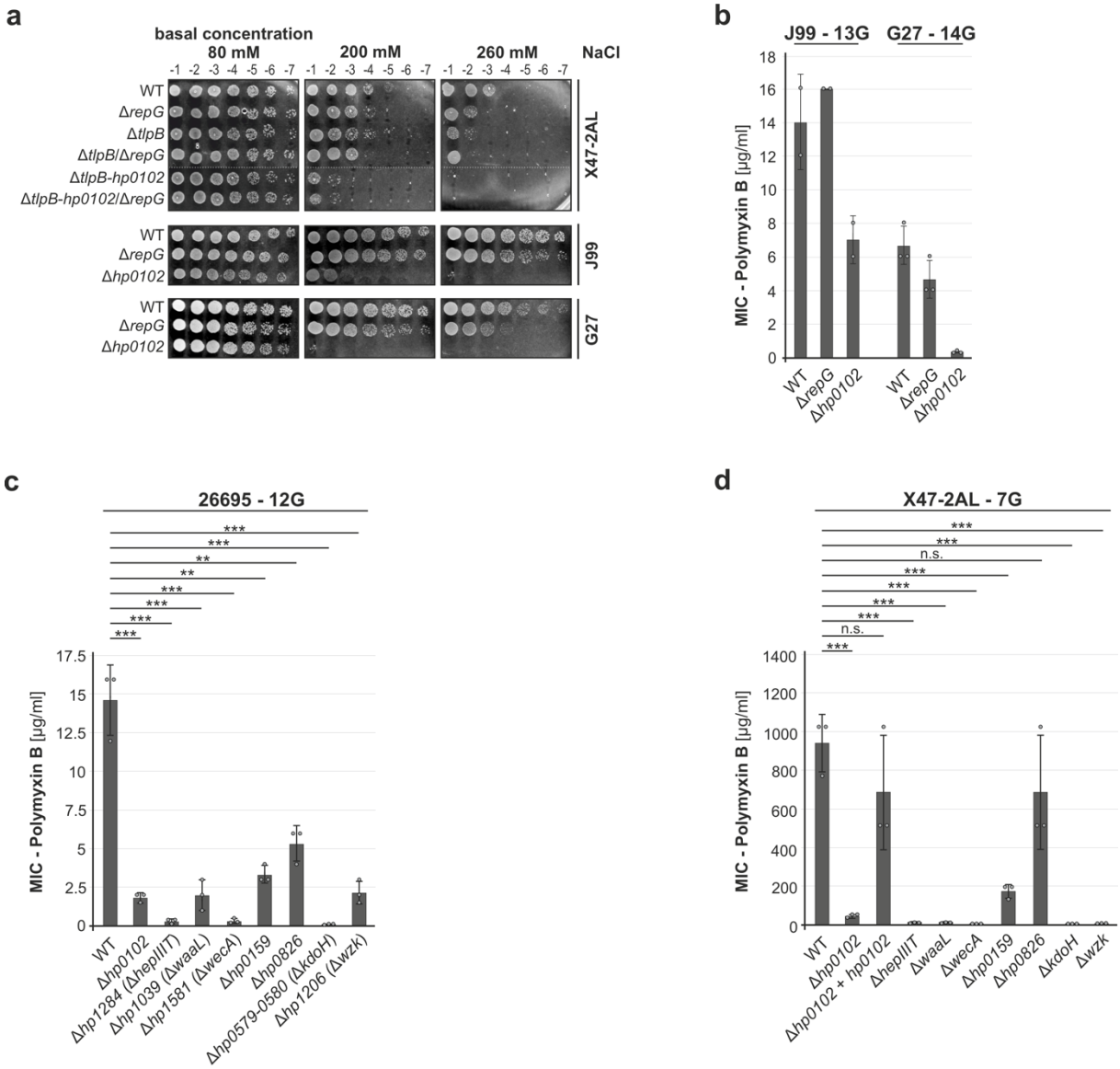
probed with Lewis x/y antigen-specific antibodies. The results shown are representative of at least three independent experiments. Source data underlying b and c are provided as a Source Data file.

Supplementary Figure 8



Supplementary Figure 8: The length of the homopolymeric G-repeat in the *tlpB* mRNA leader impacts RepG-mediated TlpB and HP0102 regulation, and in turn Lewis x antigen expression. (*Upper panel*) Quantification of relative TlpB protein and Lewis x antigen levels in the different *tlpB* leader mutants of *H. pylori* strain 26695 in the wild-type background grown to exponential growth phase. TlpB protein and Lewis x antigen levels in the *tlpB* 6G-leader variant were used as reference and set to 1. (*Lower panel*) Relative fold change of TlpB protein and Lewis x antigen levels upon *repG* deletion in *tlpB* leader mutants when compared to the respective wild-type backgrounds. Bars represent the mean determinations of triplicate measurements (n = 3) ± standard deviation (s.d.) derived from western blot quantification (see main Figure 5b). ** - significant, p-value < 0.01; * - significant, p-value < 0.05; n.s., not significant; Student's t-test, two-tailed. Source data underlying this figure is provided as a Source Data file.

Supplementary Figure 9



Supplementary Figure 9: *H. pylori* Δ*hp0102* mutant strains show increased susceptibility to high salt-induced membrane stress and to polymyxin B. (a) Growth of wild-type and indicated mutant strains of *H. pylori* X47-2AL, J99, and G27 on GC-agar plates without (80 mM, basal concentration) or with increased NaCl concentrations (200 mM, 260 mM). Ten-fold dilutions of the bacterial suspensions were spotted on indicated GC-agar plates and incubated for 3 to 5 days at 37 °C under microaerobic conditions. The results shown are representative of at least two biological replicates. **(b-d)** Antibiotic sensitivity testing using E-tests (bioMérieux, Inc.) for *H. pylori* strains J99 and G27 (b), X47-2AL (c), and 26695 (d). *H. pylori* cells corresponding to an OD_{600nm} of 0.01 were plated on GC-agar plates and E-test stripes (0.064-1024 µg/ml polymyxin B) were placed on top. MICs were determined after 3 days of incubation under microaerobic conditions. Bars represent the mean determinations of triplicate measurements of MICs (n = 3, exception: n = 2 for J99) ± standard deviations (s.d.). *** - highly significant, p-value < 0.001; ** - very significant, p-value < 0.01; n.s., not significant; Student's t-test, two-tailed. Source data underlying b, c, and d are provided as a Source Data file.

Supplemental Tables

Supplementary Table 1: Minimal inhibitory concentrations (MICs) of *H. pylori* 26695, X47-2AL, J99, and G27 wild-type and mutant strains against polymyxin B. MICs against polymyxin B (PxB) were determined using Epsilometer (E)-tests (bioMérieux) in three biological replicates and the averages are given in µg/ml with the corresponding standard deviations (s.d.). Fold changes of *H. pylori* mutant strains were calculated relative to their respective parental wildtype. Positive (+) fold changes indicate an increased resistance to polymyxin B compared to WT, negative (-) fold changes represent decreased resistance (-xx-fold more sensitive compared to WT; +xx-fold less sensitive compared to WT).

Strains	**Role in LPS biosynthesis**	**PxB MIC** [µg/ml]	**Fold change** (relative to WT)
***H. pylori* strain 26695**			
WT	full-length LPS	14.7 ± 2.3	
Δ*repG*		29.3 ± 4.6	+2.0
C_{RepG}		14.7 ± 2.3	–
Δ*tlpB*		21.3 ± 4.6	+1.5
Δ*tlpB-hp0102*		2.0 ± 0.0	-7.2
Δ*hp0102*	Trio fucosyltransferase	1.8 ± 0.3	-7.8
Δ*hp1284* (Hep III T)	Hep III transferase	0.3 ± 0.1	-48.7
Δ*hp1039* (WaaL)	O-antigen ligase	2.0 ± 1.0	-7.0
Δ*hp1581* (WecA)	O-antigen initiating ligase	0.3 ± 0.2	-45.9
Δ*hp0159*	Glycosyltransferase	3.3 ± 0.6	-4.3
Δ*hp0826*	Galactosyltransferase	5.3 ± 1.2	-2.7
Δ*hp0579-580*	KDO hydrolase	0.1 ± 0.035	-137.3
Δ*hp1206* (Wzk)	O-antigen flippase	2.2 ± 0.8	-6.6
***H. pylori* strain X47-2AL**			
WT	full-length LPS	938.7 ± 147.8	
Δ*repG*		682.7 ± 147.8	-1.4
Δ*tlpB*		768.0 ± 256.0	-1.2
Δ*tlpB-hp0102*		32.0 ± 0.0	-29.3
Δ*hp0102*	Trio fucosyltransferase	42.7 ± 9.2	-22.0
Δ*hp0102* + *hp0102*		682.7 ± 295.6	-1.4
Δ*hp1284* (Hep III T)	Hep III transferase	6.0 ± 2.0	-156.4
Δ*hp1039* (WaaL)	O-antigen ligase	6.0 ± 2.0	-156.4
Δ*hp1581* (WecA)	O-antigen initiating ligase	0.4 ± 0.2	-2,631.8
Δ*hp0159*	Glycosyltransferase	170.7 ± 37.0	-5.5
Δ*hp0826*	Galactosyltransferase	682.7 ± 295.6	-1.4
Δ*hp0579-580*	KDO hydrolase	0.1 ± 0.043	-16,822.0
Δ*hp1206* (Wzk)	O-antigen flippase	2.5 ± 0.9	-375.5
***H. pylori* strain J99**			
WT	full-length LPS	14.0 ± 2.0	
Δ*repG*		14.0 ± 2.0	–
Δ*hp0102*	Trio fucosyltransferase	7.3 ± 1.5	-1.9
***H. pylori* strain G27**			
WT	full-length LPS	7.0 ± 1.4	
Δ*repG*		5.0 ± 1.4	-1.4
Δ*hp0102*	Trio fucosyltransferase	0.3 ± 0.09	-23.3

Supplementary Table 2: SSRs associated with genes involved in LPS biosynthesis in *H. pylori* strain 26695

***H. pylori* 26695 – homopolymeric repeats**					
Promoter		**5' UTR**		**Coding sequence**	
ID (SSR)	**Function**	**ID (SSR)**	**Function**	**ID (SSR)**	**Function**
hp0651 (A7)	*futB*, fucosyltransferase	*hp0208* (A11[b])	*rfaJ2*, glucosyltransferase	*hp0093-94* (C14)	*fucC*, fucosyltransferase
		***hp0102* (G12[a])**	**fucosyltransferase**	*hp0208* (AG11)	*rfaJ2*, glycosyltranferase
		hp1105 (A15)	N-acetyl-glycosamyltransferase	*hp0217* (G12)	N-acetylgalactosaminyltransferase
				hp0379 (C13)	*futA,* fucosyltransferase
				hp0580 (C8)	Kdo-hydrolase
				hp0619 (C13)	*lex2B*, glycosyltransferase
				hp0651 (C13)	*futB*, fucosyltransferase
				hp1417 (AG9)	hypothetical protein[c]

[a]*hp0102* is located in an operon with *tlpB*, G12 in the 5' UTR of the *tlpB* mRNA

[b]*hp0208* does not have a defined TSS, but RNA-seq indicated a potential 110-nt long 5' UTR (Pernitzsch and Sharma, unpublished)

[c]*hp1417* is potentially involved in LPS core assembly[3]

Supplementary Table 3: G-repeat length in the *tlpB* 5' UTR of sequential *H. pylori* isolates from human or after re-isolation from animals. Lines frame *H. pylori* isolates that were obtained from the same patient or that were re-isolated after mice/gerbil infection studies. The lengths of the G-repeats in the *tlpB* 5' UTRs of isolates from Narino, Colombia, from the study of Kennemann *et al*. (2011)[5] were re-sequenced by Sanger sequencing.

Strain	NCBI Acc. No	Time scale	G-repeat length	G-repeat variation	Origin/Comment	Reference
908	NC_017357		17G-TGGTTTT-17G		West African duodenal ulcer disease patient in France	Ref[6]
2017	NC_017374	10 yrs	15G	yes	Re-isolate from antrum	Ref[7]
2018	NC_017381	10 yrs	13G	yes	Re-isolate from corpus	
NQ367	NZ_CADL00000000		15/16G°		Isolates from Narino, Colombia	Ref[5]
NQ1671	NZ_CADM00000000	3 yrs	13G	yes		
NQ4191	NZ_CADN00000000	16 yrs	13G	yes		
NQ392	NZ_CADI00000000		13G°		Isolates from Narino, Colombia	Ref[5]
NQ1707	NZ_CADJ00000000	3 yrs	14G	yes		
NQ4060	NZ_CADK00000000	16 yrs	15/16G°	yes		
NQ315	NZ_CADE00000000		12G		Isolates from Narino, Colombia	Ref[5]
NQ1712	NZ_CADF00000000	3 yrs	13G	yes		
NQ352	NZ_CADG00000000		12G°		Isolates from Narino, Colombia	Ref[5]
NQ1701	NZ_CADH00000000	3 yrs	14G°	no		
BM012A	NZ_CP006888.1		12G		Isolate from asymptomatic human	Ref[8]
BM012B	NZ_CP007605.1	44 d	11G	yes	Isolate after re-infection of cured patient with strain BM012A	
BM012S	NZ_CP006889.1	5 mo	10G	yes	Spouse of patient with BM012A, natural transmission between spouses (timepoint of transmission unclear)	
BM013A	NZ_CP007604.1		12G		Isolate from asymptomatic human	Ref[8]
BM013B	NZ_CP007606.1	20 d	12G	no	Isolate after re-infection of cured patient with strain BM013A	

BCM-300	NZ_LT837687.1		14G		*cagA*-positive *H. pylori* strain	Ref[9]
HE136/09	NZ_LT635473.1	12 wk	14G	no	Re-isolates from five patients infected with BCM-300, placebo group	
HE141/09	NZ_LT635471.1	12 wk	15G	yes		
HE143/09	NZ_LT635458.1	12 wk	14G	no		
HE147/09	NZ_LT635477.1	12 wk	14G	no		
HE170/09	NZ_LT635472.1	12 wk	14G	no		
HE93/10	NZ_LT838273.1	12 wk	13G	yes	Re-isolates from seven patients infected with BCM-300, patients were previously challenged with prophylactic vaccine candidate	
HE101/09	NZ_LT635456.1	12 wk	14G	no		
HE132/09	NZ_LT635459.1	12 wk	15G	yes		
HE134/09	NZ_LT635476.1	12 wk	14G	no		
HE142/09	NZ_LT635478.1	12 wk	14G	no		
HE171/09	NZ_LT635474.1	12 wk	14G	no		
HE178/09	NZ_LT635460.1	12 wk	13G	yes		
Hp141			12G-TGC[#]		Woman with gastritis in Poitiers, France	Ref[10]
HP141*		150 d	10G-C[#]	yes	Re-isolate from femal C57BL/6 inbred mice	
HP145			10G		Woman with prepyloric ulcer in Poitiers, France	Ref[10]
HP145*		150 d	10G	no	Re-isolate from femal C57BL/6 inbred mice	
HP87			13G		Original human isolate	Ref[11]
HP87 P7*			16/17/18G	yes	Gerbil adapted strain	
HP87 P7 *tlpD*			16/17/18G		*tlpD* mutant of gerbil adapted strain	Ref[11]
HP87 P7 *tlpD* RI		6 wk	16/17/18G		Re-isolate from gerbil antrum	

° The G-repeat length determined by Sanger sequencing differed from the genome sequence determined by 454- sequencing.

***** *H. pylori* isolates that were re-isolated from C57BL/6 inbred mice or gerbils.

[#] Additional nucleotide variations that were identified in the flanking region of the homopolymeric G-repeat.

Supplementary Table 4: Bacterial strains

Name	Description	Strain number	*H. pylori*	Resistance
WT / 26695	Wildtype (NCBI Acc-no. NC_000915), kindly provided by T. F. Meyer (MPI-IB, Berlin, Germany); Ref[12]	CSS-0004	26695	
[a]**Δ*repG***	*repG*::*aphA*-3	JVS-7014	26695	Kan^R
[a]**C_{RepG}**	*repG*::*aphA*-3, *rdxA*::*repG*:*catGC*	CSS-0046	26695	Kan^R Cm^R
[a]**SL 2**	*repG*::*aphA*-3, *rdxA*::*repG*-SL 2:*catGC*	CSS-0747	26695	Kan^R Cm^R
[a]**ΔCU**	*repG*::*aphA*-3, *rdxA*::*repG*-ΔCU:*catGC*	CSS-0157	26695	Kan^R Cm^R
[a]**Δ*tlpB***	*tlpB*::*rpsL-erm*	CSS-0163	26695	Erm^R
Δ*hp0102*	*hp0102*::*rpsL-erm*	CSS-1000	26695	Erm^R
Δ*tlpB* (Kan)	*tlpB*::*aphA*-3	CSS-5924	26695	Kan^R
Δ*hp0102* (Kan)	*hp0102*::*aphA*-3	CSS-5942	26695	Kan^R
Δ*tlpB-hp0102*	*tlpB-hp0102*::*aphA*-3	CSS-5926	26695	Kan^R
Δ*hp1284*	*hp1284*::*aphA*-3	CSS-5928	26695	Kan^R
Δ*hp1039*	*hp1039*::*aphA*-3	CSS-5930	26695	Kan^R
Δ*hp1581*	*hp1581*::*aphA*-3	CSS-5932	26695	Kan^R
Δ*hp0159*	*hp0159*::*aphA*-3	CSS-5934	26695	Kan^R
Δ*hp0826*	*hp0826*::*aphA*-3	CSS-5936	26695	Kan^R
Δ*hp0579-0580*	*hp0579-0580*::*aphA*-3	CSS-5938	26695	Kan^R
Δ*hp1206*	*hp1206*::*aphA*-3	CSS-5940	26695	Kan^R
[a]***tlpB*::3xFLAG*/ *tlpB* ΔG**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* ΔG::*tlpB*:*rpsL-erm*	CSS-0471	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* 6G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 6G::*tlpB*:*rpsL-erm*	CSS-0472	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* 7G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 7G::*tlpB*:*rpsL-erm*	CSS-0473	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* 8G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 8G::*tlpB*:*rpsL-erm*	CSS-0474	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* 9G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 9G::*tlpB*:*rpsL-erm*	CSS-0475	26695 Str^R	Str^R Erm^R
[a]***tlpB*-3xFLAG*/ *tlpB* 10G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 10G::*tlpB*:*rpsL-erm*	CSS-0476	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* 11G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 11G::*tlpB*:*rpsL-erm*	CSS-0477	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* WT (12G)**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 12G::*tlpB*:*rpsL-erm*	CSS-0470	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* 13G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 13G::*tlpB*:*rpsL-erm*	CSS-0478	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* 14G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 14G::*tlpB*:*rpsL-erm*	CSS-0479	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* 15G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 15G::*tlpB*:*rpsL-erm*	CSS-0480	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ *tlpB* 16G**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *tlpB* 16G::*tlpB*:*rpsL-erm*	CSS-0481	26695 Str^R	Str^R Erm^R
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* ΔG**	*rpsL-str*^{R}, *tlpB*::*tlpB*-3xFLAG*, *repG*::*aphA*-3, *tlpB* ΔG::*tlpB*:*rpsL-erm*	CSS-0483	26695 Str^R	Str^R Erm^R Kan^R

[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 6G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 6G*::tlpB:rpsL-erm*	CSS-0484	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 7G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 7G*::tlpB:rpsL-erm*	CSS-0485	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 8G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 8G*::tlpB:rpsL-erm*	CSS-0486	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 9G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 9G*::tlpB:rpsL-erm*	CSS-0487	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 10G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 10G*::tlpB:rpsL-erm*	CSS-0488	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 11G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 11G*::tlpB:rpsL-erm*	CSS-0489	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* WT (12G)**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 12G*::tlpB:rpsL-erm*	CSS-0482	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 13G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 13G*::tlpB:rpsL-erm*	CSS-0490	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 14G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 14G*::tlpB:rpsL-erm*	CSS-0491	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 15G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 15G*::tlpB:rpsL-erm*	CSS-0492	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
[a]***tlpB*::3xFLAG*/ Δ*repG* / *tlpB* 16G**	*rpsL-str*^{R}*, tlpB::tlpB*-3xFLAG*, *repG::aphA*-3, *tlpB* 16G*::tlpB:rpsL-erm*	CSS-0493	26695 Str^{R}	Str^{R} Erm^{R} Kan^{R}
tlpB-hp0102* 10th::*gfpmut3	*rdxA::tlpB-hp0102* 10th*-gfpmut3:catGC*	CSS-2104	G27	Cm^{R}
tlpB-hp0102* 10th::*gfpmut3* / Δ*repG	*repG::aphA*-3, *rdxA::tlpB-hp0102* 10th*-gfpmut3:catGC*	CSS-2107	G27	Cm^{R} Kan^{R}
tlpB*$_{mini}$-*hp0102* 10th::*gfpmut3	*rdxA::tlpB*$_{mini}$*-hp0102* 10th*-gfpmut3:catGC*	CSS-2116	G27	Cm^{R}
tlpB*$_{mini}$-*hp0102* 10th::*gfpmut3* / Δ*repG	*repG::aphA*-3, *rdxA::tlpB*$_{mini}$*-hp0102* 10th*-gfpmut3:catGC*	CSS-2119	G27	Cm^{R} Kan^{R}
P$_{tlpB}$*hp0102* 10th::*gfpmut3*	*rdxA::*P$_{tlpB}$*hp0102* 10th*-gfpmut3:catGC*	CSS-2138	G27	Cm^{R}
P$_{tlpB}$*hp0102* 10th::*gfpmut3* / Δ*repG*	*repG::aphA*-3, *rdxA::*P$_{tlpB}$*hp0102* 10th*-gfpmut3:catGC*	CSS-2141	G27	Cm^{R} Kan^{R}
***tlpB-hp0102* 10th::*gfpmut3* ΔG^{hp0102}**	*rdxA::tlpB-hp0102* 10th*-gfpmut3* ΔG^{hp0102}*:catGC*	CSS-2150	G27	Cm^{R}
tlpB-hp0102* 10th::*gfpmut3* ΔG^{hp0102} / Δ*repG	*repG::aphA*-3, *rdxA::tlpB-hp0102* 10th*-gfpmut3* ΔG^{hp0102}*:catGC*	CSS-2153	G27	Cm^{R} Kan^{R}
***tlpB-hp0102* 10th::*gfpmut3* ATTTA^{hp0102}**	*rdxA::tlpB-hp0102* 10th*-gfpmut3* ATTTA^{hp0102}*:catGC*	CSS-2156	G27	Cm^{R}
tlpB-hp0102* 10th::*gfpmut3* ATTTA^{hp0102} / Δ*repG	*repG::aphA*-3, *rdxA::tlpB-hp0102* 10th*-gfpmut3* ATTTA^{hp0102}*:catGC*	CSS-2159	G27	Cm^{R} Kan^{R}
tlpB*$_{stop}$-*hp0102* 10th::*gfpmut3	*rdxA::tlpB*$_{stop}$*-hp0102* 10th*-gfpmut3:catGC*	CSS-3241	G27	Cm^{R}
tlpB*$_{stop}$-*hp0102* 10th::*gfpmut3*/ Δ*repG	*repG::aphA*-3, *rdxA::tlpB*$_{stop}$*-hp0102* 10th*-gfpmut3:catGC*	CSS-3244	G27	Cm^{R} Kan^{R}
X47-2AL	Wildtype (DDBJ/EMBL/GenBank Acc-no. AWNG00000000, AWNG01000000); Ref[13]	CSS-0996	X47-2AL	
X47-2AL Δ*repG*	*repG::aphA*-3	CSS-0997	X47-2AL	Kan^{R}
X47-2AL Δ*tlpB*	*tlpB::aac*(3)-IV	CSS-1123	X47-2AL	Gen^{R}
X47-2AL Δ*tlpB* / Δ*repG*	*tlpB::aac*(3)-IV, *repG::aphA*-3	CSS-1769	X47-2AL	Gen^{R} Kan^{R}
X47-2AL Δ*tlpB-hp0102*	*tlpB-hp0102::aac*(3)-IV	CSS-1743	X47-2AL	Gen^{R}
X47-2AL Δ*tlpB-hp0102* / Δ*repG*	*tlpB-hp0102::aac*(3)-IV, *repG::aphA*-3	CSS-1773	X47-2AL	Gen^{R} Kan^{R}
X47-2AL Δ*tlpB-hp0102* + *tlpB-hp0102*	*tlpB-hp0102::aac*(3)-IV, *rdxA::tlpB-hp0102:aphA*-3	CSS-2046	X47-2AL	Gen^{R} Kan^{R}
X47-2AL Δ*tlpB-hp0102* + *hp0102*	*tlpB-hp0102::aac*(3)-IV, *rdxA::hp0102:aphA*-3	CSS-2080	X47-2AL	Gen^{R} Kan^{R}
X47-2AL Δ*hp0102*	*hp0102::aac*(3)-IV	CSS-2019	X47-2AL	Gen^{R}
X47-2AL Δ*hp0102* / Δ*repG*	*hp0102::aac*(3)-IV, *repG::aphA*-3	CSS-2022	X47-2AL	Gen^{R} Kan^{R}
X47-2AL Δ*hp0102* + *hp0102*	*hp0102::aac*(3)-IV, *rdxA:: hp0102:aphA*-3	CSS-2087	X47-2AL	Gen^{R} Kan^{R}

X47-2AL Δ*hp1284*	*hp1284*::*aac*(3)-IV	CSS-5910	X47-2AL	Gen^R
X47-2AL Δ*hp1039*	*hp1039*::*aac*(3)-IV	CSS-5912	X47-2AL	Gen^R
X47-2AL Δ*hp1581*	*hp1581*::*aac*(3)-IV	CSS-5914	X47-2AL	Gen^R
X47-2AL Δ*hp0159*	*hp0159*::*aac*(3)-IV	CSS-5916	X47-2AL	Gen^R
X47-2AL Δ*hp0826*	*hp0826*::*aac*(3)-IV	CSS-5918	X47-2AL	Gen^R
X47-2AL Δ*hp0579-580*	*hp0579-580*::*aac*(3)-IV	CSS-5920	X47-2AL	Gen^R
X47-2AL Δ*hp1206*	*hp1206*::*aac*(3)-IV	CSS-5922	X47-2AL	Gen^R
G27	Wildtype (NCBI Acc-no. NC_011333), kindly provided by T. F. Meyer; Ref[14]	CSS-0010	G27	
[a]G27 Δ*repG*	*repG*::*aphA*-3	CSS-0169	G27	Kan^R
G27 Δ*hp0102*	*hp0102*::*rpsL-erm*	CSS-1007	G27	Erm^R
J99	Wildtype (NCBI Acc-no. NC_000921), kindly provided by T. F. Meyer, MPI-IB, Berlin, Germany; Ref[15]	CSS-0001	J99	
[a]J99 Δ*repG*	*repG*::*aphA*-3	CSS-0732	J99	Kan^R
J99 Δ*hp0102*	*hp0102*::*rpsL-erm*	CSS-1019	J99	Erm^R
TOP 10	mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74recA1araD139 Δ(ara-leu)7697galUgalK λ⁻rpsLendA1nupG from Invitrogen	CSO-0296	*E. coli*	

Str^R: streptomycin resistant; Kan^R: kanamycin resistant; Cm^R: chloramphenicol resistant; Erm^R: erythromycin resistant; Gen^R: gentamicin resistant

[a] Mutant strains were constructed as described in Ref[2]

Supplementary Table 5: DNA oligonucleotides. Sequences are given in 5' –> 3' direction; P~ denotes a 5' monophosphate.

Name	Sequence 5' -> 3'	Description
CSO-0003	GAAAGGAGGGGGAGGT	Northern blot probe for RepG
CSO-0017	gttttttCTAGAGATCAGCCTGCCTTTAGG	RepG cloning
CSO-0018	gttttttCTCGAGCTTAGCGCTTAATGAAACGC	RepG cloning
CSO-0039	gttttttCTCGAGTCTCAAAATCCGCTGAAATCT	Cloning of *tlpB*
CSO-0040	gttttttCTAGATCAGTTGCAACCAGGAGATT	Cloning of *tlpB*
CSO-0051	TGTCACTTATATTTACAAGTTCGCT	Verification of *tlpB* deletion
CSO-0086	gttttttATGCATAGGACTTTGAATGTCAAATCCATT	Verification/Sequencing of *tlpB-hp0102*, *hp0102*
CSO-0146	gttttttATCGATGTATGCTCTTTAAGACCCAGC	Cloning GFP fusion, *tlpB-hp0102*
CSO-0147	gttttttCATATGCTCGAATTCAGATCCACGTT	Cloning GFP fusion, *tlpB-hp0102*
CSO-0205	AATTACAACAGTACTGCGATGAGT	Verification/Sequencing of GFP fusions
CSO-0206	AATCTCACGCCAAGCATTT	Verification/Sequencing of *tlpB-hp0102*, *hp0102*
CSO-0207	AGTTCTGATTTCATGCCCTT	Verification/Sequencing of *tlpB-hp0102*, *hp0102*
CSO-0263	GACTGATGTCATCAGCGGT	Cloning of *tlpB*
CSO-0293	gttttttGAATTcTCCAACGTCATCTCGTTCT	Cloning of *tlpB*, *tlpB-hp0102*
CSO-0309	gttttttGAATTCTTACTTATTAAATAATTTATAGCTATTGAAAAGA	Cloning of *tlpB, hp0102*
CSO-0581	gttttttATCGATTTATTATTTTATCTTTAAGCCTAACTTAA	Cloning of GFP fusion
CSO-0683	gttttttTGCTAGCAGTAAAGGAGAAGAACTTTTCACTGGA	Cloning of GFP fusion
CSO-0867	AGCCATTTATGAACGCTACGGC	Quantitative RT-PCR (*hp0102*)
CSO-0868	CACCACAAACAAGCGGATGAT	Quantitative RT-PCR (*hp0102*)
CSO-0869	gttttttGGATCCAACCCTTTTTTAATAAACTCC	Cloning *hp0102*
CSO-0870	gttttttCTCGAGTTATCAATGATATTGCCGATCAAAC	Cloning *hp0102*
CSO-0871	gttttttGAATTCCATGCTTTTAAAATACCCTAG	Cloning *hp0102*, *tlpB-hp0102*
CSO-0872	gttttttTCTAGATTTGGATACAACTATTCGCAAGC	Cloning *hp0102*
CSO-0873	gttttttTCTAGAGGCATCAAATAAAACGAAA	Cloning *hp0102*
CSO-0874	gttttttCTCGAGGTGAAGACGAAAGG	Cloning *hp0102*, *tlpB-hp0102*
CSO-0940	gttttttGGATCCCAGCGATTTCTTCTAATTCTGT	Cloning *tlpB-hp0102*
CSO-0941	gttttttGGATCCACAAGCTGAAATTATAGAACACC	Cloning *tlpB-hp0102*
CSO-0942	gttttttGAATTCTCAGGGGGAGTTTATTAAAAAAG	Cloning of *tlpB*
CSO-1173	GGTAGTGGTTTTTGTGTGATGG	Quantitative RT-PCR (6S RNA)
CSO-1174	CCAGATGACCGCTACTTTTACA	Quantitative RT-PCR (6S RNA)
CSO-1359	gttttttCTCGAGTTTGGATACAACTATTCGCAAGC	Cloning *hp0102*, *tlpB-hp0102*

CSO-1737	gttttttTCTAGATTATCAATGATATTGCCGATCAAAC	Cloning *hp0102*
CSO-1738	TGTCAAAGAGAGCAAGGATGC	Verification of *hp0102* deletion
CSO-1739	gttttttGGATCCAACACGCCGTGATCACAGAA	Cloning *hp0102*
CSO-1740	gttttttCATATGTAAGATTTTTTATTTTATTTTTAAGCCTAAC	Cloning *tlpB-hp0102*
CSO-1741	gttttttATCGATAGTTAGGGATTGAAATACCCTTA	Cloning *tlpB-hp0102*
CSO-1742	AGCTAAAATTATAGAACACCCTTTT	Cloning *hp0102*
CSO-1743	P~TCAGGGGGAGTTTATCAAAAAAG	Cloning *hp0102*
CSO-1745	CATTTATCTCCTATAAATCATTTTTAAGT	Cloning of *tlpB*
CSO-1803	gttttttGCTAGCACACGCCGTGATCACAGAAA	Cloning of GFP fusion
CSO-1813	gttttttGGATCCTTTTATGGATAATTTTTAAAATCATTTG	Cloning *tlpB-hp0102*
CSO-1814	gttttttGCTAGCTATTCCCTCCAGGTACTAAAACA	Cloning *tlpB-hp0102*
CSO-1984	GGCTAACACGACCAGCATGA	Cloning of GFP fusion
CSO-1985	P~TAATCAGGGGGAGTTTATTAAAAAAG	Cloning of GFP fusion
CSO-2052	P~AGTTTATTAAAAAAGGGTTGGATTG	Cloning of GFP fusion
CSO-2053	TGATTAAGTTTTAAACAAATTCACTTGT	Cloning of GFP fusion
CSO-2055	P~TCAGGGGGAGTTTATTAAAAAAGG	Cloning of GFP fusion
CSO-2056	AGCTGAAATTATAGAACACCCTTTTT	Cloning of GFP fusion
CSO-2061	P~ATTTAAGTTTATTAAAAAAGGGTTGGATTG	Cloning of GFP fusion
CSO-2274	TTGAAGAAAATTACTATTATCTCCTATAAATCATTTTTAAGTCAAA	Cloning of GFP fusion
CSO-2275	TAGGAGATAATAGTAATTTTCTTCAATGTTTGCTTCGTTG	Cloning of GFP fusion
CSO-3195	gttttttGGATCCGACTGATGTCATCAGCGGT	Cloning *hp0102*
CSO-SP001	TCGCGGCTATAACTTTGATATG	Cloning of *tlpB* (Kan) in 26695
CSO-SP004	CCATAAAGCAAGGTTTTAGGGT	Cloning of *tlpB* (Kan) in 26695
CSO-SP008	TAGAGATCCGCCATATTGTGTT	Amplification of *aac*(3)-IV cassette
CSO-SP009	TCCAACGTCATCTCGTTCTC	Amplification of *aac*(3)-IV cassette
CSO-SP010	ATGGATTTTATAGGGTTTGAAGATTT	Cloning of *hp1284* in 26695 and X47
CSO-SP013	AATCAAACCCATATCGTTTGAGC	Cloning of *hp1284* in 26695 and X47
CSO-SP014	GCCCATAAAAGCATGGCAGA	Verification of *hp1284* deletion in 26695 and X47
CSO-SP015	ATGGCTCACTCTGCTTTTTTGG	Cloning of *hp1039* in 26695 and X47
CSO-SP018	ATCATTCCTACAAAAAAACTTCCC	Cloning of *hp1039* in 26695 and X47
CSO-SP019	CTTTAACTCAAGTCTTGTTGATTG	Verification of *hp1039* deletion in 26695 and X47
CSO-SP020	ATGCTTTTTGTGGATAACGCTAAT	Cloning of *hp1581* in 26695 and X47
CSO-SP023	CATCGCCAATCTCTTCTAAAATG	Cloning of *hp1581* in 26695 and X47
CSO-SP024	GCTATAATAGTGAAAATACTAAATCCA	Verification of *hp1581* deletion in 26695 and X47
CSO-SP025	TTGCGTGTTTTTGCCATTTCTTTAA	Cloning of *hp0826* in 26695 and X47

CSO-SP028	GAAGCTAATCCTAAAAAGTCTTAGT	Cloning of *hp0826* in 26695 and X47
CSO-SP029	GTTTTTAGTTGGATATTTAATCATCAAATT	Verification of *hp0826* deletion in 26695 and X47
CSO-SP030	ATGAGTATTATTATTCCTATTGTCATC	Cloning of *hp0159* in 26695 and X47
CSO-SP033	CGTTTGAAAATCCTATTCAATCTAAA	Cloning of *hp0159* in 26695 and X47
CSO-SP034	ACCCCAATCTAATTGGTTATTATAG	Verification of *hp0159* deletion in 26695 and X47
CSO-SP035	ATCTTGGAACCTTCAAGAAATCG	Cloning of *hp0579-580* in 26695 and X47
CSO-SP038	ACAACCTTTTTTTAACAAGAGATAAAG	Cloning of *hp0579-580* in 26695 and X47
CSO-SP039	TGCCCAGTAAAAAAGCGCGTT	Verification of *hp0579-580* deletion in 26695 and X47
CSO-SP040	CTACTTTAAAATACTTTTTGCGCTC	Cloning of *hp1206* in 26695 and X47
CSO-SP043	TTAGCCGAGATTGTCTTTGTGTT	Cloning of *hp1206* in 26695 and X47
CSO-SP044	TAGCTATAATCTAGCTCAATTTGG	Verification of *hp1206* deletion in 26695 and X47
CSO-SP047	AACACAATATGGCGGATCTCTAGTTAGGGAGGTTGTGATCTTTA	Cloning of *hp1284* in X47
CSO-SP048	GAGAACGAGATGACGTTGGAAATCGCATGGAAACTTAAAGACAA	Cloning of *hp1284* in X47
CSO-SP049	AACACAATATGGCGGATCTCTACCAATGGTTTGAGCGATTTTTTG	Cloning of *hp1039* in X47
CSO-SP050	GAGAACGAGATGACGTTGGAGTTATATGGTGGTCGTGAGTC	Cloning of *hp1039* in X47
CSO-SP051	AACACAATATGGCGGATCTCTACCTAAAAAAATCTTTCCTAAAGGGA	Cloning of *hp1581* in X47
CSO-SP052	GAGAACGAGATGACGTTGGATATTTTTTGGGTTTGGTGTGCG	Cloning of *hp1581* in X47
CSO-SP053	AACACAATATGGCGGATCTCTATATCTCGTGGTTTTTATGGCTC	Cloning of *hp0826* in X47
CSO-SP054	GAGAACGAGATGACGTTGGACAAGAGCGTGTGGGGATCA	Cloning of *hp0826* in X47
CSO-SP055	AACACAATATGGCGGATCTCTAGCCGCTCCAAAATAATAGCC	Cloning of *hp0159* in X47
CSO-SP056	GAGAACGAGATGACGTTGGATCCCTTTATGAGCATTACCTTAAT	Cloning of *hp0159* in X47
CSO-SP057	AACACAATATGGCGGATCTCTACAAACTTAAAACGAATCGGCTCT	Cloning of *hp0579-580* in X47
CSO-SP058	GAGAACGAGATGACGTTGGATCAAAAATAAGCCATTGGGTTTGA	Cloning of *hp0579-580* in X47
CSO-SP059	AACACAATATGGCGGATCTCTAGGAGTAGAAAAAAACGATCACAG	Cloning of *hp1206* in X47
CSO-SP060	GAGAACGAGATGACGTTGGAAGCGGATATTATTATGGGGCTTA	Cloning of *hp1206* in X47
CSO-SP061	ATTCTTCAGATATTTTAAACATCGCTAC	RT-PCR (*tlpB-hp0102*)
CSO-SP062	TATAAGTTTGATGAAGCACGGAAAGA	RT-PCR (*tlpB-hp0102*)
CSO-SP063	TCCTAGTTAGTCACCCGGGTAAAACAAGCTGAAATTATAGAACACC	Cloning of *tlpB-hp0102* in 26695
CSO-SP064	ATTGTTTTAGTACCTGGAGGGAATATGGAACGACCATGAATTTAGAC	Cloning of *tlpB-hp0102* in 26695
CSO-SP065	ATTGTTTTAGTACCTGGAGGGAATAATGAGTGAAAAAGATGAGGGCAT	Cloning of *hp0102* in 26695
CSO-SP066	TCCTAGTTAGTCACCCGGGTAGTTAGGGAGGTTGTGATCTTTA	Cloning of *hp1284* in 26695
CSO-SP067	ATTGTTTTAGTACCTGGAGGGAATAAATCGCATGGAAACTTAAAGACAA	Cloning of *hp1284* in 26695
CSO-SP068	TCCTAGTTAGTCACCCGGGTACCAATGGTTTGAGCGATTTTTTG	Cloning of *hp1039* in 26695
CSO-SP069	ATTGTTTTAGTACCTGGAGGGAATAGTTATATGGTGGTCGTGAGTC	Cloning of *hp1039* in 26695
CSO-SP070	TCCTAGTTAGTCACCCGGGTACCTAAAAAAATCTTTCCTAAAGGGA	Cloning of *hp1581* in 26695

CSO-SP071	ATTGTTTTAGTACCTGGAGGGAATATATTTTTTGGGTTTGGTGTGCG	Cloning of *hp1581* in 26695
CSO-SP072	TCCTAGTTAGTCACCCGGGTATATCTCGTGGTTTTTATGGCTC	Cloning of *hp0826* in 26695
CSO-SP073	ATTGTTTTAGTACCTGGAGGGAATACAAGAGCGTGTGGGGATCA	Cloning of *hp0826* in 26695
CSO-SP074	TCCTAGTTAGTCACCCGGGTAGCCGCTCCAAAATAATAGCC	Cloning of *hp0159* in 26695
CSO-SP075	ATTGTTTTAGTACCTGGAGGGAATATCCCTTTATGAGCATTACCTTAAT	Cloning of *hp0159* in 26695
CSO-SP076	TCCTAGTTAGTCACCCGGGTACAAACTTAAAACGAATCGGCTCT	Cloning of *hp0579-580* in 26695
CSO-SP077	ATTGTTTTAGTACCTGGAGGGAATATCAAAAATAAGCCATTGGGTTTGA	Cloning of *hp0579-580* in 26695
CSO-SP078	TCCTAGTTAGTCACCCGGGTAGGAGTAGAAAAAAACGATCACAG	Cloning of *hp1206* in 26695
CSO-SP079	ATTGTTTTAGTACCTGGAGGGAATAAGCGGATATTATTATGGGGCTTA	Cloning of *hp1206* in 26695
CSONIH-0033	TCAAAGCCACTAGTAAGTCTTACTT	Verification oligo for insertion of *rpsL-erm* cassette
JVO-0155	CCGTATGTAGCATCACCTTC	Cloning of GFP fusion [1616]
JVO-0485	TCGGAATGGTTAACTGGGTAGTTCCT	Northern blot probe for *Helicobacter pylori* 5S rRNA
JVO-2134	AAACCATAAGGAATGGTTGGAT	Northern blot probe for RepG
JVO-5069	CTTCACGCCCCTTGTAAATA	Verification of *repG* deletion mutant
JVO-5070	GATAAGGTTTAGCGATGTAATCGT	RepG cloning
JVO-5072	CGTTTCTTGACACGCTTAATT	RepG cloning
JVO-5267	ACGGGGTGGTATTGTTTGAT	Quantitative RT-PCR (*tlpB*)
JVO-5268	AAGTGTAGCCTCCCCCTTTT	Quantitative RT-PCR (*tlpB*)
JVO-5257	TATAGGTTTTCATTTTCTCCCAC	Verification of *repG* deletion mutant
pZE-A	GTGCCACCTGACGTCTAAGA	Colony PCR and sequencing of pZE12-*luc* derived plasmids
pZE-*XbaI*	TCGTTTTATTTGATGCCTCTAGA	Colony PCR and sequencing of pZE derived plasmids
HPK-1	GTACCCGGGTGACTAACTAGG	Amplification of *aphA-3* cassette
HPK-2	TATTCCCTCCAGGTACTAAAACA	Amplification of *aphA-3* cassette

Supplementary Table 6: Plasmids

Name	Description/Generation	Origin / marker	Reference
pBA1-1	Intermediary plasmid for construction of pBA7-4	p15A/ Amp^R Erm^R	This study
pBA4-2	Intermediary plasmid for construction of pSP189-4 (pSP190-1)	p15A/ Amp^R Gen^R	This study
pBA5-4	Intermediary plasmid for construction of pBA13-5	p15A/ Amp^R	This study
pBA7-4	Plasmid for deletion of *hp0102* in diverse *H. pylori* strains	p15A/ Amp^R Erm^R	This study
pBA13-5	Plasmid for deletion of *tlpB* in *H. pylori* strain X47-2AL	p15A/ Amp^R Gen^R	This study
pJV752-1	Cloning vector, pZE12-*luc* with modified p15A origin	p15A/ Amp^R	Ref[17]
pMA5-2	Plasmid for translational fusion of the *cagA*-5' UTR including the 28th amino acid to *gfpmut3*	p15A/ Amp^R Cm^R	Ref[2]
pSP39-3	Plasmid for complementation of *repG* deletion with RepG in *H. pylori* strain 26695	p15A/ Amp^R Cm^R	Ref[2]
pSP60-2	Backbone plasmid for deletion or nucleotide exchange in G-stretch in 5' UTR of *tlpB*	p15A/ Amp^R Erm^R	Ref[2]
pSP127-3	Plasmid for deletion of the *tlpB-hp0102* operon in *H. pylori* strain X47-2AL	p15A/ Amp^R Gen^R	This study
pSP186-2	Plasmid for deletion of *hp0102* in *H. pylori* strain X47-2AL	p15A/ Amp^R Gen^R	This study
pSP189-4	Intermediary plasmid for construction of pSP190-1	p15A/ Amp^R Gen^R	This study
pSP190-1	Plasmid for complementation of the *H. pylori* X47-2AL Δ*tlpB-hp0102* mutant with the *tlpB-hp0102* operon	p15A/ Amp^R Kan^R	This study
pSP192-1	Plasmid for complementation of the *H. pylori* X47-2AL Δ*tlpB-hp0102* and Δ*hp0102* mutants with *hp0102* alone	p15A/ Amp^R Kan^R	This study
pSP195-6	Plasmid for construction of *tlpB-hp0102* 10th::*gfpmut3* reporter fusion	p15A/ Amp^R Cm^R	This study
pSP197-3	Plasmid for construction of *tlpB*$_{mini}$-*hp0102* 10th::*gfpmut3* reporter fusion	p15A/ Amp^R Cm^R	This study
pSP198-4	Plasmid for construction of P$_{tlpB}$*hp0102* 10th::*gfpmut3* reporter fusion	p15A/ Amp^R Cm^R	This study
pSP200-2	Plasmid for construction of *tlpB-hp0102* 10th::*gfpmut3* ΔG^{hp0102} reporter fusion	p15A/ Amp^R Cm^R	This study
pSP201-1	Plasmid for construction of *tlpB-hp0102* 10th::*gfpmut3* ATTTA^{hp0102} reporter fusion	p15A/ Amp^R Cm^R	This study
pSP205-17	Plasmid for construction of *tlpB*$_{stop}$-*hp0102* 10th::*gfpmut3* reporter fusion	p15A/ Amp^R Cm^R	This study
pUC1813apra	Plasmid carrying apramycin/gentamicin resistance cassette *aac*(3)-IV	pMB1/ Amp^R Gen^R	Ref[18]

Kan^R: kanamycin resistant; Cm^R: chloramphenicol resistant; Erm^R: erythromycin resistant; Gen^R: gentamicin resistant

Supplementary Table 7: Sequences of *tlpB-hp0102*::*gfpmut3* reporter fusions used in this study. The homopolymeric G-repeat in the 5' UTR of *tlpB* mRNA and the G^{hp0102}-repeat in the *tlpB-hp0102* IGR are underlined. The RBSs as well as start codons and ORFs are shown in light green and gray letters, respectively. Mutations within the G^{hp0102}-repeat are shown in red. Alteration of the *tlpB* start codon is shown in blue (two stop codons). The *NheI* restriction site is marked in yellow, the *gfpmut3* coding sequence is highlighted in green.

Name	Sequence 5' → 3'
tlpB-hp0102* 10th-*gfpmut3	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGA CTTAAAAATGATTTATAGGAGATAAATGATGTTTTCTTCAATGTTTGCTTCGTTGGGGACTCGTATCATGCTGGTCGTGTTAGCCGCTCTTTTAGGTTTAGGGGGGCTTTTTAT TGGTTTTGTAAAGGTTATGCAAAAAGATGTGTTAGCGCAACTCATGGAGCATTTAGAAACCGGGCAATACAAAAAGCGTGAAAAAACGCTCGCTTACATGACAAAAATTATTGA ACAGGGCATTCATGAGTATTACAAAAATTTTGACAATGCTACTGCAAGAAAAATGGCGTTAGATTATTTCAAACGCATCAACGACGATAAGGGCATGATTTATATGGTGGTGGT GGATAAAAACGGGGTGGTATTGTTTGATCCGGTCAATCCTAAAACCGTAGGCCAATCAGGGCTTGACGCTCAGAGCGTTGATGGGGTGTATTATGTTAGGGGGTATTTGGAGGC GGCCAAAAAAGGGGGAGGCTACACTTATTATAAAATGCCTAAATACGATGGAGGCGTACCGGAGAAAAAATTCGCCTACTCGCATTATGATGAAGTTTCTCAAATGGTGATCGC AACGACTTCCTATTACACTGACATTAACACAGAAAATAAAGCGATCAAAGAAGGCGTGAATAAGGTTTTTGATGAAAACACCACGAAATTATTCCTTTGGATACTGACAGCGAC GATAGCGCTAGTGGTTTTGACGCTCATATACGCTAAATTAAGGATCGTGAAACGCATTGATGAACTGGTCCTTAAAATCAACGCTTTTAGCCGTGGGGATAAGGATTTGAGAGC CAAAATTGATGTGGGTGATCGCAACGATGAAATCTCGCAAGTGGGCCGTGGGATCAATTTGTTTGTGGAAAACGCCCGCTTGATTATGGAAGAGATTAAAGGGATTTCCACCCT CAATAAAACTTCAATGGATAAATTAGTCCAAATCACGCAAGAAACCCAAAAGAGCATGAAAGATTCCTCAACCACCCTAAATTCCGTGAAAAATAAAGCCACTGATATAGCGAG CATGATGAATGCTTCCATAGAGCAATCTCAAGGGTTAAGGAAGCGTTTGATTGAAACGCAAGGGCTGGTCAAAGAGAGCAAGGATGCGATCGGGGATTTATTTTCTCAAATCAC AGAGAGCGCGCACACTGAAGAGGAACTCTCTAGCAAAGTGGAGCAGCTAAGCCGTAACGCTGATGATGTCAAATCCATTCTGGATATTATCAATGATATTGCCGATCAAACGAA TTTATTAGCCCTAAACGCTGCTATTGAAGCCGCAAGGGCTGGCGAGCATGGCAGAGGCTTTGCGGTGGTGGCTGATGAAGTTAGGAATTTAGCCGGGCGCACTCAAAAGTCTTT AGCCGAAATCAATTCCACTATCATGGTGATTGTCCAAGAAATCAATGCCGTGAGTTCGCAAATGAATCTCAATTCGCAAAAAATGGAGCGTTTGAGCGATATGAGTAAAAGCGT GCAAGAAACTTACGAAAAAATGAGTTCTAATTTAAGCTCAGTCGTGTCAGACAGCAATCAAAGCATGGACGATTACGCCAAATCCGGACACCAAATTGAAGTTATGGTAAGCGA TTTTGCAGAGGTGGAAAAAGTGGCTTCTAAGACTTTAGCGGATTCTTCAGATATTTTAAACATCGCTACGCATGTGAGTGGAACGACCATGAATTTAGACAAACAAGTGAATTT GTTTAAAACTTAATCAGGGGGGAGTTTATTAAAAAAGGGTTGGATTGTTAAAAGTTTCTGTGATCACGGCGTGTGCTAGCAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCC AATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGG AAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCC CGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGG TATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTT CAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCAC ACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAA
tlpB*$_{mini}$-*hp0102* 10th-*gfpmut3	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGA CTTAAAAATGATTTATAGGAGATAAATGATGTTTTCTTCAATGTTTGCTTCGTTGGGGACTCGTATCATGCTGGTCGTGTTAGCCTAATCAGGGGGAGTTTATTAAAAAAGGGT TGGATTGTTAAAAGTTTCTGTGATCACGGCGTGTGCTAGCAGT-GFPMUT3-TAA
P*$_{tlpB}$*hp0102* 10th-*gfpmut3	TCAGGGGGAGTTTATTAAAAAAGGGTTGGATTGTTAAAAGTTTCTGTGATCACGGCGTGTGCTAGCAGT-GFPMUT3-TAA
***tlpB-hp0102* 10th-*gfpmut3* ΔG^{hp0102}**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGA CTTAAAAATGATTTATAGGAGATAAATGATG-TLPB CODING REGION-TAATCAAGTTTATTAAAAAAGGGTTGGATTGTTAAA AGTTTCTGTGATCACGGCGTGTGCTAGCAGT-GFPMUT3-TAA

tlpB-hp0102* 10th-*gfpmut3* ATTTA*hp0102	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGA CTTAAAAATGATTTATAGGAGATAAATGATG-TLPB CODING REGION-TAATCAATTTAAGTTTATTAAAAAAGGGTTGGATTG TTAAAAGTTTCTGTGATCACGGCGTGTGCTAGCAGT-GFPMUT3-TAA
tlpB*stop-*hp0102* 10th-*gfpmut3	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGA CTTAAAAATGATTTATAGGAGATAATAGTAATTTTCTTCAATGTTTGCTTCGTTGGGGACTCGTATCATGCTGGTCGTGTTAGCCTAATCAGGGGGAGTTTATTAAAAAAGGGT TGGATTGTTAAAAGTTTCTGTGATCACGGCGTGTGCTAGCAGT-GFPMUT3-TAA

Supplementary Table 8: Sequences of *tlpB* leader mutants of *H. pylori* strain 26695. The length-variable homopolymeric G-repeat in the 5' UTR of the *tlpB* mRNA is shown in red, and the RBS as well as start codon are marked in light green.

Name	Sequence 5' → 3'
***tlpB* WT**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* ΔG**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 6G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 7G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 8G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 9G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 10G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 11G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 13G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 14G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 15G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG
***tlpB* 16G**	TGTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCATTTTTCGGGGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAGGAGATAAATG

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