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Supplementary Information for

A peptide of a type I toxin-antitoxin system induces *Helicobacter pylori* morphological transformation from spiral-shape to coccoids

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Legend of movies S1, S2 and S3
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Other supplementary materials for this manuscript include the following:

Movies S1 to S3

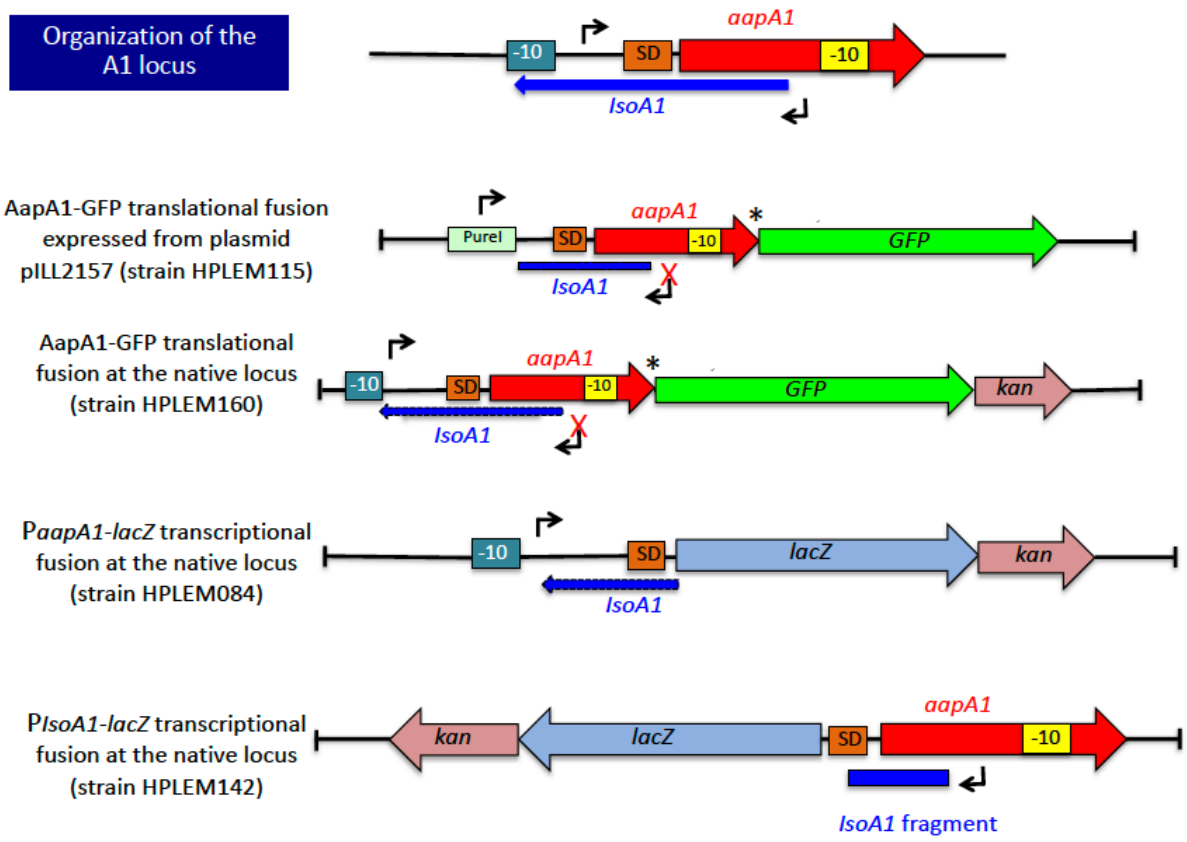


Fig. S1. Schematic representation of the native A1 locus, of the AapA1-GFP fusions expressed from plasmid pILL2157 or from the native locus and of the P*aapA1-lacZ* and P*IsoA1-lacZ* fusions expressed at their native loci from their respective native promoters.

SD indicates the Shine-Dalgarno sequence; -10, the position of the -10 box of the promoter; PureI, the promoter of the *ureI* gene from plasmid pILL2157; arrows, the direction of transcription; a red cross on an arrow, an inactivated promoter; GFP, green fluorescent protein; kan, the gene conferring kanamycin resistance; a star (*) a STOP codon that has been replaced by a codon coding for Ala. A hatched *IsoA1* arrow indicates that the corresponding RNA is not expressed. The corresponding strains are listed in Table S2, the plasmids in Table S3 and the primers used for the construction in Table S4.

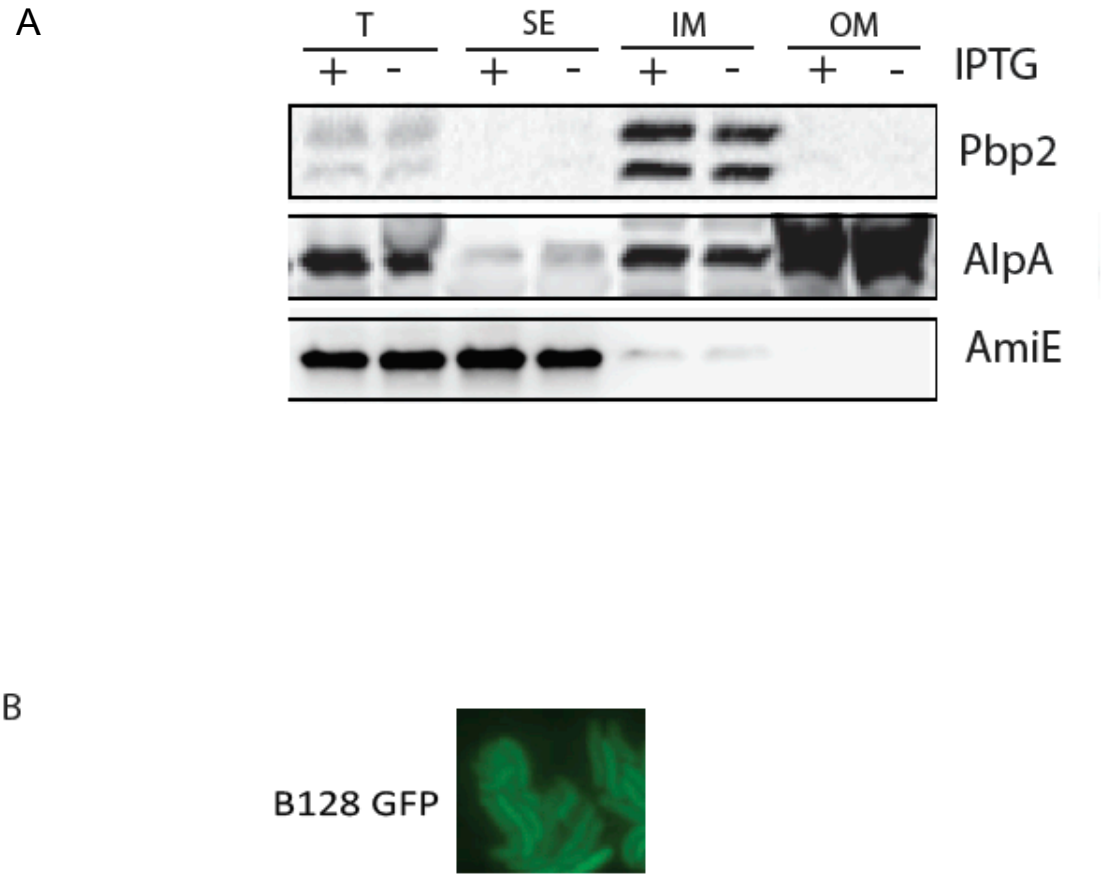


Fig. S2. Controls of the *H. pylori* fractionation procedure

A. Western blot analysis of total extract (T), soluble extract (SE), inner membrane (IM) and outer membrane (OM) fractions prepared from a control *H. pylori* B128 strain and revealed with the following control antibodies anti-Pbp2 for IM, anti-AlpA for the OM and anti-AmiE for the cytoplasmic fraction.

B. Fluorescence of a strain expressing GFP from plasmid pILL2157 is presented as a control for the data of Fig. 2B.

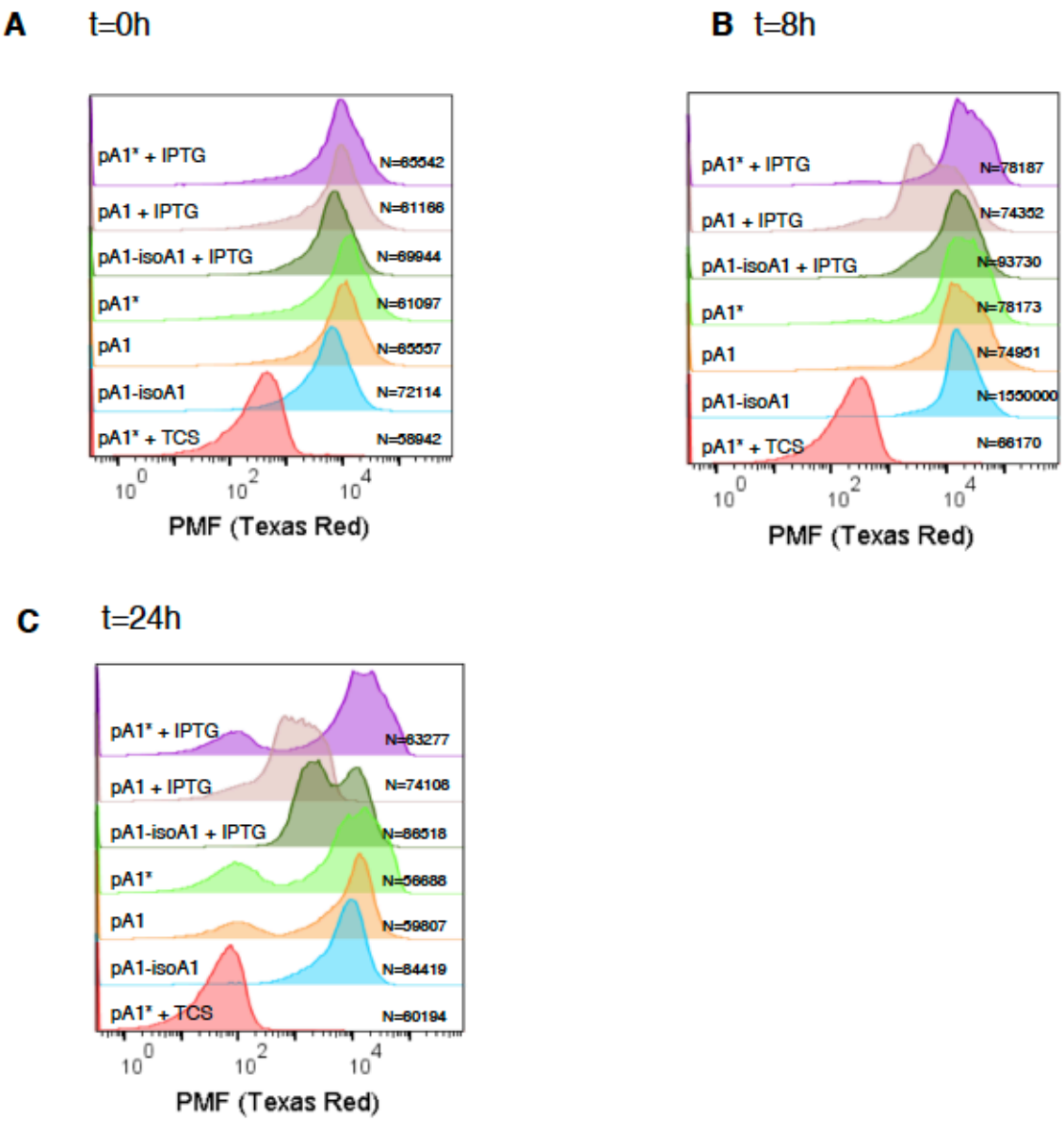


Fig. S3. Representative flow cytometry histograms of different *H. pylori* cells stained with a PMF-sensitive dye.

Histograms of fluorescence intensity of cells from strains pA1-isoA1, pA1 or pA1* stained with the PMF-sensitive MitoTracker Red CMXROS dye at 0h (A), 8h (B) or 24h (C) after 1 mM IPTG addition or not. pA1* cells treated with TCS served as a control for PMF dissipation conditions.

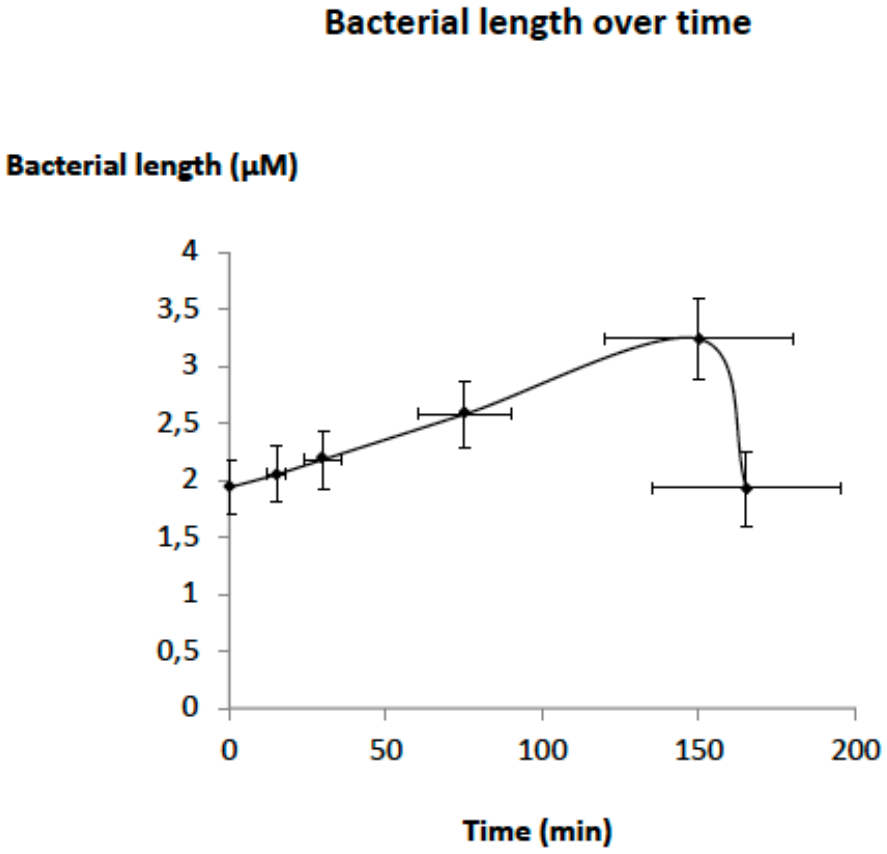


Fig. S4. Measurement of *H. pylori* cell length during growth over time.

Mean curve obtained from the analysis of the growth of 61 individual *H. pylori* bacteria (strain HPLEM213 without IPTG, Table S2). Bacteria were analyzed by live microscopy, and their size and time of division were measured from their separation following a division to the next division. The curves were analyzed and normalized. Mean division time is 165 min, initial length mean is 1.9 µm, mean length when division occurs is 3.2 µm.

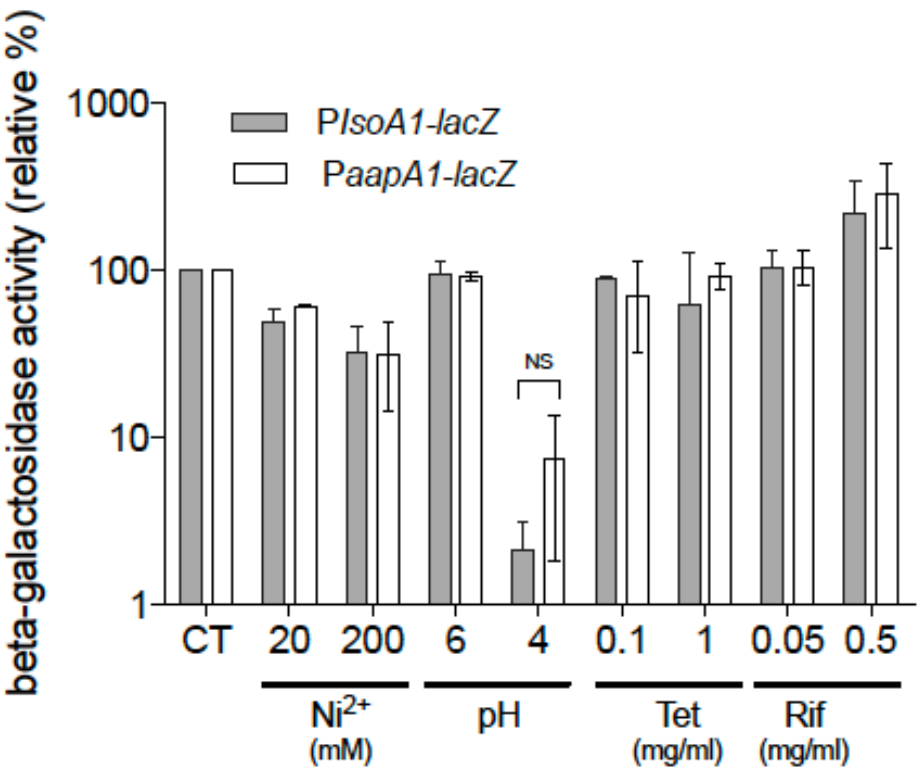


Fig. S5. Response of the *aapA1* and *IsoA1* promoters to different stresses. β-galactosidase activities expressed by strains expressing the P*aapA1-lacZ* and P*IsoA1-lacZ* fusions from the native locus were measured after 6h treatment with different stresses, $NiCl_2$ (20 and 200 mM), pH 4, Tetracycline (0.1 and 1 mg/ml) or Rifampicin (0.05 and 0.5 mg/ml). β-galactosidase activities are presented as ratio (expressed in %) of activities measured with stress versus activities of untreated samples. Results from 3 independent experiments performed in duplicates are shown. Error bars represent the standard deviation, NS corresponds to non-significant, (P >0.05).

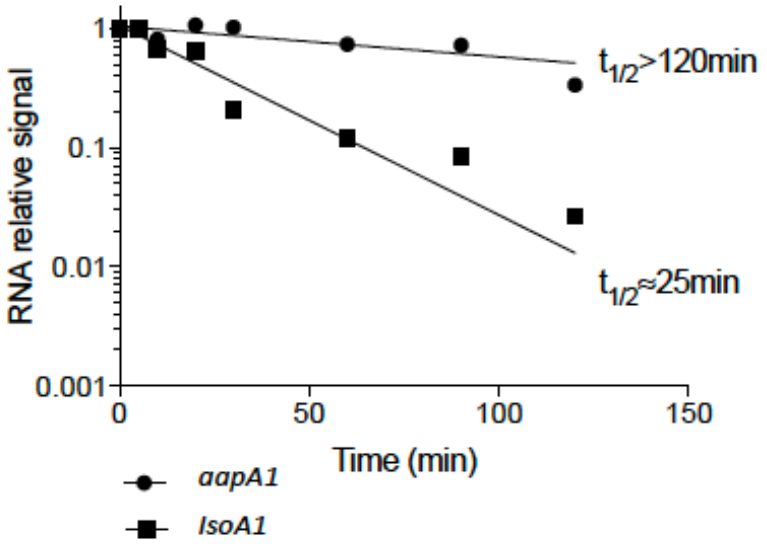


Fig. S6. Half-life of the *aapA1* and *IsoA1* RNAs

RNA decay was determined by plotting normalized intensities (RNA signal relative to time 0) of bands corresponding to full length *aapA1* and *IsoA1* transcripts as a function of time after rifampicin addition. Approximate half-lives (min) measurements from three independent experiments are indicated for each transcript.

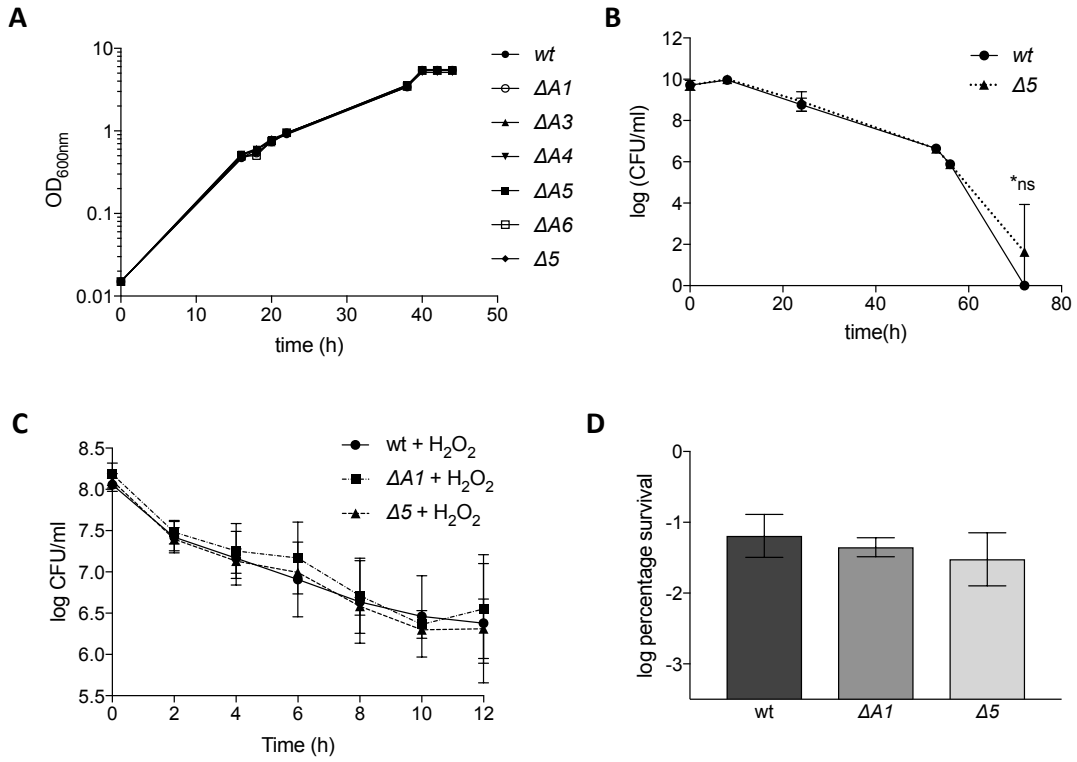


Fig. S7: Growth and viability of B128 WT strain and multiple isogenic class A TA systems deletion mutants under normal conditions or upon exposure to hydrogen peroxide

A) Growth of the B128 WT strain, of six isogenic mutants carrying *AapA1-IsoA1* deletions (Δ*A1, ΔA2, ΔA3, ΔA4-2+A4-2, ΔA5 or ΔA6*) and of a multiple mutant strain carrying deletions of every functional class A TA system (Δ*5*) was followed under normal conditions during 44h. The growth curve of the mutants was similar to that of the parental WT strain.

B) Viability of the B128 WT strain and of the Δ5 multiple TA mutant was measured during 72h by determining colony forming units (CFU) by plating on blood agar medium. No significant difference was observed in the kinetics of loss of viability between these strains.

C) Exponentially growing B128 WT strain, Δ*A1* and Δ*5* isogenic mutants were exposed to 1% hydrogen peroxide. Their viability was measured during 12h by counting the colony forming units (CFU/mL) on blood agar plates.

D) Exponentially growing B128 WT strain, Δ*A1* and Δ*5* isogenic mutants were exposed to 1% hydrogen peroxide during 8 h. The percentage of survival was calculated by dividing the number of CFU/mL in the culture after 8h with hydrogen peroxide by the number of CFU/mL after 8 h of incubation without stress.

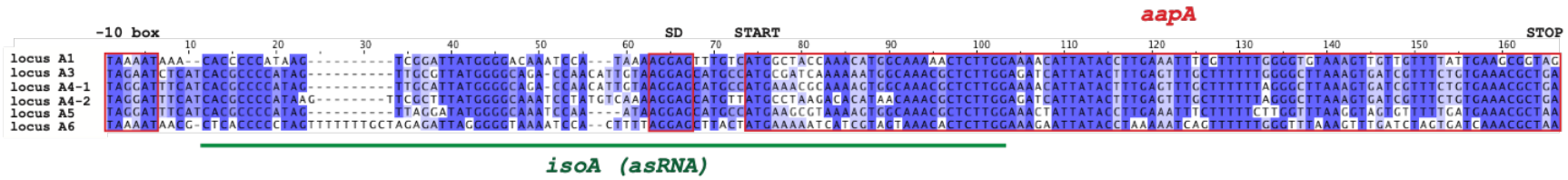


Fig. S8. Sequence alignment of the six functional class A TA modules present on the *H. pylori* B128 genome.

Sequences alignment of the six functional class A TA module colored according to the percentage identity with their consensus sequence. The -10 box, Shine-Dalgarno (SD) and AapA toxin coding sequence are framed in red and the start and stop codons are indicated above the sequences. *IsoA* antisense RNA sequence is represented by a green bar under the sequence alignment. Note that in B128, two consecutive TA modules are found at the locus A4, here referred as locus A4-1 and locus A4-2. A locus corresponding to the position of the A2 locus in other *H. pylori* strains was identified but its corresponding A2 ORF was inactivated in B128 strain.

Table S1. Summary of the muropeptide composition of peptidoglycan extracted from B128 WT strain during exponential phase (24h), early stationary (36h) and late stationary phase (72h culture) and from the B128 Δ*aapA1-IsoA1* + pA1 strain, 8 and 24 hours after toxin induction by IPTG addition and, as a negative control, from B128 Δ*aapA1-IsoA1* + pA1* 8h and 24 h after IPTG addition. Panel A**:** Summary of the muropeptide composition of peptidoglycan extracted from *H. pylori* B128 WT strain during exponential phase (24h, first column); stationary phase (36h, second column); after 72h culture (“aging” coccoids, third column); of toxin-induced coccoids of B128 Δ*aapA1-IsoA1* + pA1 strain after 8h of induction (equivalent to 24h of culture, fourth column) or 24 h of induction (equivalent to 36h of culture, fifth column); and as a control, of strain B128 Δ*aapA1-IsoA1* + pA1* after 8h of induction (sixth column) and 24 h of induction (seventh column). Each condition was analyzed in triplicates. The relative abundance of muropeptides in each sample was calculated according to Glauner *et al.* (1). Arrows show major statistically significant changes measured in the muropeptide composition when comparing exponential phase grown bacteria with aging coccoids and with A1 toxin induced coccoids (cultures at equivalent timepoints). These data show that both "aging coccoids" and toxin-induced coccoids present similar changes namely significant GM2 increase and GM3 reduction, which for the toxin-induced condition is already visible at 8h post-induction and is accentuated at 24h post-induction. In contrast, for the control condition, pA1*, no such changes are measured.

Area - % for each muropeptide[a]								
		B128			B128 Δ*aapA1-IsoA1* + pA1		B128 Δ*aapA1-IsoA1* + pA1*	
		Exponential phase (24h)	Stationary phase (36h)	"Aging" coccoids (72h)	8h post-induction (24h culture)	24h post-induction (36h culture)	8h post-induction (24h culture)	24h post-induction (36h culture)
Peak n°	*Monomers*	69.11 ± 1.00	73.11 ± 1.17	73.38 ± 0.23	59.83 ± 0.41	61.23 ± 0.58	58.75 ± 1.07	59.98 ± 1.04
3	GM2	9.04 ± 0.57	24.67 ± 1.24 ↗	27.27 ± 0.54 ↗	15.62 ± 1.24 ↗	23.72 ± 1.21 ↗	6.55 ± 0.7	13.19 ± 3.51
1	GM3	9.42 ± 0.94	7.49 ± 0.71	4.67 ± 2.26 ↘	5.00 ± 1.3 ↘	1.12 ± 0.19 ↘	8.14 ± 0.72	5.51 ± 1.23
4	GM4	16.91 ± 0.34	10.8 ± 0.48	14.52 ± 2.77	9.43 ± 0.76	9.02 ± 0.23	11.97 ± 0.77	10.33 ± 1.39
5	GM5	28.06 ± 0.8	23.92 ± 0.69	20.53 ± 0.54	25.99 ± 1.95	23.30 ± 0.6	28.16 ± 2.09	26.69 ± 0.23
2	GM4+gly[b]	5.69 ± 0.26	6.24 ± 0.44	6.41 ± 0.74	3.79 ± 0.35	4.07 ± 0.19	3.94 ± 0.13	4.25 ± 0.27
	Dimers	16.13 ± 0.2	13.63 ± 0.04	11.66 ± 1.32	15.41 ± 1.07	13.11 ± 0.71	15.40 ± 0.24	14.7 ± 1.94
7	GM3 + GM4	2.21 ± 0.16	1.66 ± 0.3	1.41	2.43 ± 0.08	1.96 ± 0.11	2.52 ± 0.24	1.94 ± 0.41
8	GM4 + GM4+gly	0.93 ± 0.08	1.02 ± 0.01	1.01 ± 0.13	0.77 ± 0.04	0.69 ± 0.07	0.77 ± 0.06	0.81 ± 0.02
9	GM4 + GM4	7.1 ± 0.19	5.84 ± 0.09	5.66 ± 0.76	5.73 ± 0.12	5.23 ± 0.17	6.11 ± 0.52	5.94 ± 0.15
10	GM5 + GM4	5.89 ± 0.13	5.12 ± 0.37	4.29 ± 0.3	6.49 ± 0.97	5.22 ± 0.55	6.00 ± 0.38	6.01 ± 0.16
	Trimers							
	GM4 + GM3 + GM4	0.06 ± 0.00	0.06 ± 0.01	0.12 ± 0.1	0.1 ± 0.01	0.09 ± 0.00	0.11 ± 0.01	0.10 ± 0.01
	Anhydromuropeptides	14.28 ± 0.88	12.8 ± 1.09	14.42 ± 1.19	22.63 ± 0.57	22.46 ± 0.18	23.77 ± 0.94	23.51 ± 0.63
12	G(anhM)2	0.23 ± 0.02	0.54 ± 0.01	0.88 ± 0.01	0.7 ± 0.03	1.09 ± 0.06	0.65 ± 0.04	0.97 ± 0.29

6	G(anhM)3	0.62 ± 0.04	0.54 ± 0.08	0.62 ± 0.16	1.01 ± 0.07	0.46 ± 0.03	1.06 ± 0.08	0.94 ± 0.1
11	G(anhM)4	1.56 ± 0.13	0.84 ± 0.13	1.61 ± 0.07	1.59 ± 0.17	1.06 ± 0.02	2.29 ± 0.05	1.77 ± 0.46
13	G(anhM)5	2.29 ± 0.2	2.06 ± 0.12	1.89 ± 0.37	2.49 ± 0.22	1.9 ± 0.04	3.00 ± 0.25	2.87 ± 0.11
14	G(anhM)4 + GM3	1.23 ± 0.11	1.06 ± 0.21	1.11 ± 0.1	2.52 ± 0.25	2.66 ± 0.15	2.38 ± 0.2	2.21 ± 0.3
15	G(anhM)4+GM4+gly	0.29 ± 0.03	0.41 ± 0.01	0.47 ± 0.01	0.48 ± 0.03	0.55 ± 0.02	0.44 ± 0.03	0.52 ± 0.2
16	G(anhM)4 + GM4	3.63 ± 0.22	3.24 ± 0.33	3.61 ± 0.08	6.02 ± 0.03	6.47 ± 0.07	6.33 ± 0.47	6.36 ± 0.2
17	G(anhM)4 + GM5	1.5 ± 0.08	1.57 ± 0.22	1.63 ± 0.13	2.3 ± 0.08	2.41 ± 0.06	2.15 ± 0.09	2.3 ± 0.1
18	G(anhM)5 + GM4	1.94 ± 0.1	1.61 ± 0.08	1.49 ± 0.3	3.09 ± 0.04	3.07 ± 0.06	3.18 ± 0.08	3.12 ± 0.07
19	G(anhM)3+ G(anhM)4	0.15 ± 0.02	0.15 ± 0.00	0.17 ± 0.03	0.46 ± 0.05	0.5 ± 0.05	0.41 ± 0.05	0.42 ± 0.06
20	G(anhM)4+ G(anhM)4	0.41 ± 0.03	0.36 ± 0.04	0.47 ± 0.02	1.05 ± 0.07	1.22 ± 0.04	1.05 ± 0.08	1.15 ± 0.11
21	G(anhM)5+ G(anhM)4	0.42 ± 0.04	0.45 ± 0.06	0.49 ± 0.06	0.93 ± 0.08	1.07 ± 0.02	0.84 ± 0.05	0.90 ± 0.05
	Additional muropeptides	0.42 ± 0.07	0.44 ± 0.03	0.44 ± 0.01	2.03 ± 0.14	3.11 ± 0.02	1.97 ± 0.23	1.71 ± 0.03
22	GM4 + AmDap [c]	0.15 ± 0.03	0.14 ± 0.01	0.14 ± 0.00	0.83 ± 0.07	1.29 ± 0.03	0.89 ± 0.10	0.73 ± 0.03
23	GM4 + AmDap [d]	0.06 ± 0.01	0.07 ± 0.00	0.07 ± 0.01	0.19 ± 0.01	0.28 ± 0.02	0.14 ± 0.05	0.16 ± 0.02
24	GM4 + AAmDap [e]	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.64 ± 0.06	0.93 ± 0.01	0.64 ± 0.07	0.53 ± 0.02
25	GM5 + AmDap [f]	0.09 ± 0.02	0.12 ± 0.01	0.11 ± 0.01	0.37 ± 0.03	0.61 ± 0.02	0.30 ± 0.03	0.28 ± 0.01

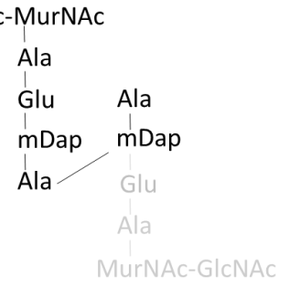
GM2: GlcNAc-Mur*N*Ac-dipeptide; GM3: GlcNAc-Mur*N*Ac-tripeptide; GM4: GlcNAc-Mur*N*Ac-tetrapeptide; GM5: GlcNAc-Mur*N*Ac-pentapeptide; AnhM: *N*-acetyl-anhydromuramic acid.

[a] Percentages were calculated as in Glauner *et al.* (1).

[b] GM4+gly: GlcNAc-Mur*N*Ac-Tetrapeptide with an additional glycine moiety.

The structures of GM4 + AmDap [c], GM4 + AmDap [d], GM4 + AAmDap [e] and GM5 + AmDap [f] are represented below

(c)


GlcNAc-MurNAc
Ala
Glu
mDap
Ala
Ala
mDap
Glu
Ala
MurNAc-GlcNAc

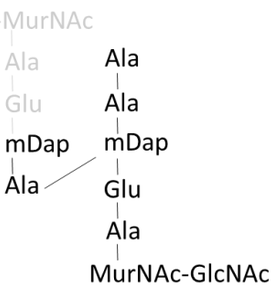
(d)


GlcNAc-MurNAc
Ala
Glu
mDap
Ala
Ala
mDap
Glu
Ala
MurNAc-GlcNAc

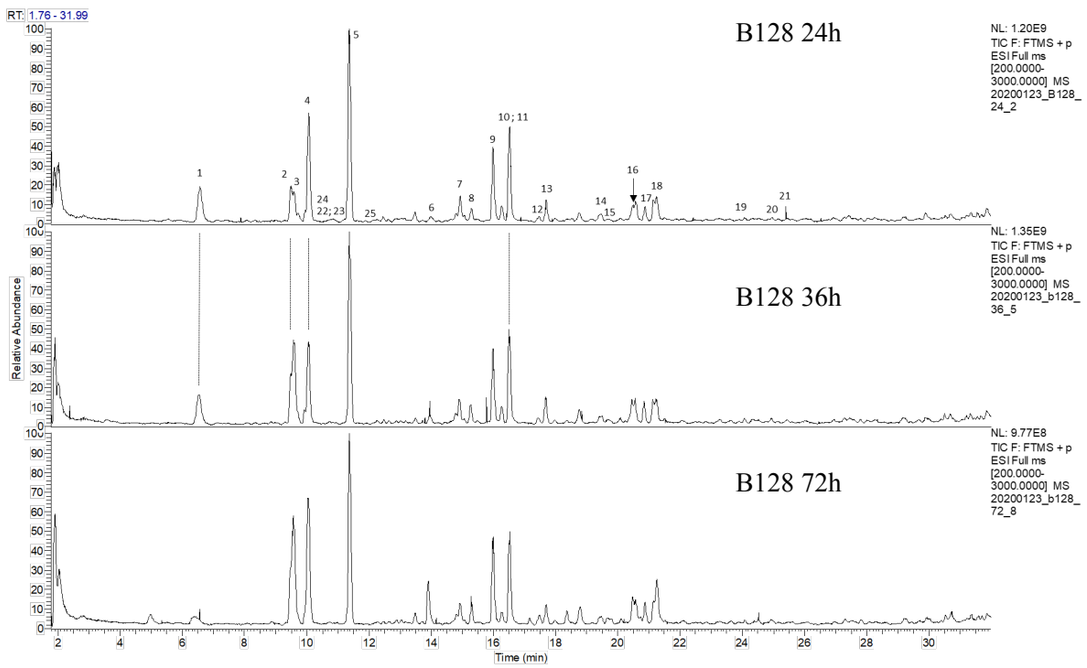
(e)


GlcNAc-MurNAc
Ala
Glu
mDap
Ala
Ala
Ala
mDap
Glu
Ala
MurNAc-GlcNAc

(f)


GlcNAc-MurNAc
Ala
Glu
mDap
Ala
Ala
Ala
mDap
Glu
Ala
MurNAc-GlcNAc

Panel B: Total ion current chromatograms of the HPLC/MS profiles of muropeptides of the seven samples indicated above (only one replicate is represented). Peak numbers correspond to those of Panel A and are identical in every graph.



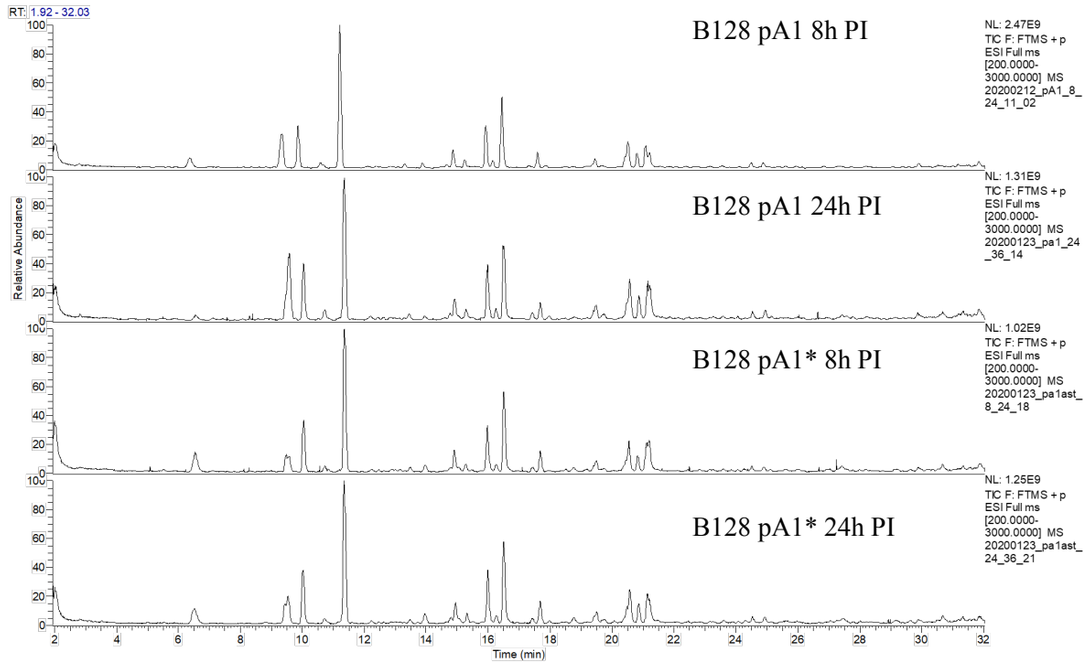


Table S2. List of strains used in this study.

	Strain designation	Genotype	Plasmid	Antibiotic resistance markers [a]	Reference or source
B128	HPLEM001	Parental wild type strain	-	-	(2, 3)
B128 Δ*rpsL::rpsL1*	HPLEM015	Δ*rpsL::rpsL1*	-	Str	(4)
B128 Δ *TA A1::Kan* + *pA1-isoA1*	HPLEM011	Δ*AapA1-isoA1::aphA-3*	pA1-isoA1	Kan, Cm	(5)
B128 Δ *TA A1::Kan* + *pA1*	HPLEM012	Δ*AapA1-isoA1::aphA-3*	pA1	Kan, Cm	(5)
B128 Δ *TA A1::Kan* + *pA1**	HPLEM086	Δ*AapA1-isoA1::aphA-3*	pA1*	Kan, Cm	(5)
B128 Δ*rpsL::rpsL1* Δ*A1 PureA-GFP* Δ*flaA::Apra* + *pA1*	HPLEM213	Δ*rpsL::rpsL1* Δ*A1* Δ*flaA::Apra* Δ*ureA::GFP-mut2*	pA1	Str, Kan, Apr, Cm	This study
B128 Δ*rpsL::rpsL1* Δ*TA A1* + *pA1-GFP*	HPLEM155	Δ*rpsL::rpsL1* Δ*A1*	pA1-GFP	Str, Cm	This study
B128 Δ*TA A1-isoA1:: AapA1-222nt-GFP-253nt*	HPLEM160	Δ*A1-isoA1:: AapA1-222nt-gfp-mut2-253nt*	-	Kan	This study
B128 Δ *TA A1 AapA1-isoA1::PaapA1-lacZ-Kan*	HPLEM084	Δ *AapA1-isoA1::PaapA1-lacZ-Kan*	-	Kan	This study
B128 Δ *TA A1 AapA1-isoA1::PisoA1-lacZ-Kan*	HPLEM142	Δ *AapA1-isoA1::PisoA1-lacZ-Kan*	-	Kan	This study
B128 Δ*rpsL::rpsL1* Δ*TA* Δ*A5* Δ*A3* Δ*A1* Δ*A6* Δ*A4::Kan*	HPLEM159	Δ*rpsL::rpsL1* Δ*TA* Δ*A5* Δ*A3* Δ*A1* Δ*A6* Δ*A4::Kan*	-	Str, Kan	This study
B128 Δ*rpsL::L1* Δ*TA A1*	HPLEM214	Δ*rpsL::L1* Δ*TA A1*	-	Str	This study
B128 Δ*rpsL::L1* Δ*TA A3*	HPLEM080	Δ*rpsL::L1* Δ*TA A3*	-	Str	This study
B128 Δ*rpsL::L1* Δ*TA A4::Kan*	HPLEM157	Δ*rps::L1* Δ*TA A4::Kan*	-	Str, Kan	This study
B128 Δ*rpsL::L1* Δ*TA A5*	HPLEM067	Δ*rpsL::L1* Δ*TA A5*	-	Str	This study
B128 Δ*rps::L1* Δ*TA A6*	HPLEM216	Δ*rps::L1* Δ*TA A6*	-	Str	This study
B128 Δ*rpsL::rpsL1* Δ*TA* Δ*A5* Δ*A3* Δ*A1* Δ*A6* Δ*A4::Kan*	HPLEM159	Δ*rpsL::rpsL1* Δ*TA* Δ*A5* Δ*A3* Δ*A1* Δ*A6* Δ*A4::Kan*	-	Str, Kan	This study

Table S3. List of plasmids used in this study.

Name	Description	Resistance (*)	Reference or source
pILL2157	Derivative of the pHeL-2 *E. coli-H. pylori* shuttle vector, carries *lacZ* under the control of p*ureI* with 2 LacI-binding sites	Cm	(6)
pILL2157bis	pILL2157 without *lacZ* (pILL2157bis)	Cm	This study
pDifWT-RC	*rpsL-cat* cassette flanked by *difH*		(7)
pA1-isoA1	*AapA1-isoA1* locus cloned into pILL2157bis	Cm	(5)
pA1	*AapA1-isoA1* locus cloned into pILL2157bis with *isoA1* promoter inactivated	Cm	(5)
pA1*	*AapA1-isoA1* locus cloned into pILL2157bis with isoA1 promoter inactivated and AapA1 start codon mutated to ATT	Cm	(5)
pGEM ΔTA A1	Suicide plasmid with *difH-rpsL-cat-difH* flanked by upstream and downstream region *A1* for markerless deletion of A1 locus.	Amp/Cm	This study
pGEM ΔTA A3	Suicide plasmid with *difH-rpsL-cat-difH* flanked by upstream and downstream region *A3* for markerless deletion of A3 locus.	Amp/Cm	This study
pGEM ΔTA A5	Suicide plasmid with *difH-rpsL-cat-difH* flanked by upstream and downstream region *A5* for markerless deletion of A5 locus.	Amp/Cm	This study
pGEM ΔTA A6	Suicide plasmid with *difH-rpsL-cat-difH* flanked by upstream and downstream region *A6* for markerless deletion of A6 locus.	Amp/Cm	This study
pJET-pureA-GFP-mut2-Kan-ureA	Suicide plasmid with *gfp-mut2* under the control of *ureA* promoter for chromosomal integration at the *ureA* locus	Amp/Kan	(8)
pA1-GFP	*gfp-mut2* cloned in translational fusion between AapA1 sequence 222 nt (from 0 to the 222 nt aapA1) and 253 nt (from 223 to the 253 nt) into vector pILL2157bis. AapA1-222nt-GFP-253nt is under the control of the *pureI* promoter.	Cm	This study

Table S4. List of primers used in this study.

Name	Sequence 5'→3'	Description
oLEM001	GTAAGCATTGCCGACAAACAC	***rpsl::rpsl1*** Forward primer to amplify upstream region of *rpsL.*
oLEM002	CCAATTGATTTATGGTAGGCACTATTTTTCCTTATTC	***rpsl::rpsl1*** Reverse primer to amplify upstream region of *rpsL.* Primer contains a homolougous region to *rpsL1.*
oLEM003	GTGCCTACCATAAATCAATTGG	***rpsl::rpsl1*** Forward primer to amplify *rpsL1* from pDifWT-RC.
oLEM004	CTAACGGATTTGTCTGTATG	***rpsl::rpsl1*** Reverse primer to amplify *rpsL1* from pDifWT-RC.
oLEM005	CATACAGACAAATCCGTTAGAGGAAAACAAAAACATGAGAAG	***rpsl::rpsl1*** Forward primer to amplify downstream region of rpsL. Primer contains a homologous region to *rpsL1.*
oLEM006	CCATTCTAACTCCAATTACCAG	***rpsl::rpsl1*** Reverse primer to amplify downstream region of *rpsL.*
oLEM192	GGATGTATAGACCGTTATGG	***flaA::apr*** Forward primer to amplify upstream region of *flaA.*
oLEM193	cactccCTAgTTAgTCACcatTGTTGTAACTCCTTG	***flaA::apr*** Reverse primer to amplify upstream region of *flaA*. Primer contains a homolougous region to *apr* resistance cassette with a stop codon and an RBS.
oLEM120	TGAcTAAcTAGgagtgcaATGtcgtgcaa	***flaA::apr*** Forward primer to amplify *apr* resistance cassette with stop codon upstream of the RBS.
oLEM066	cgatccgctccacgtgttgcc	***flaA::apr***

		Reverse primer to amplify *apr* resistance cassette.
oLEM194	ggcaacacgtggagcggatcgCAAGCCAATACCGTTCAAC	***flaA::apr*** Forward primer to amplify downstream region of *flaA*. Primer contains a homologous region to *apr* resistance cassette.
oLEM195	CATAGCATAAAATCGCATCC	***flaA::apr*** Reverse primer to amplify downstream region of *flaA*.
oLEM015	CTCCCACCGCAATTGATTG	**Δ*A1* with marker less system** Forward primer to amplify upstream region of *A1* Toxin antitoxin locus.
oLEM035	CATACTCGAGGCTTGATTGAGTGCATCAAAAC	**Δ*A1* with marker less system** Reverse primer to amplify upstream region of *A1* Toxin antitoxin locus. Primer contains a *Xho*I restriction site.
oLEM036	CATAGGATCCCGAAGTTTCTGTAAAACGATAG	**Δ*A1* with marker less system** Forward primer to amplify downstream region of A1 Toxin antitoxin locus. Primer contains a *Bam*HI restriction site.
oLEM018	CTCAATGCGTTTAGGATTAATC	**Δ*A1* with marker less system** Reverse primer to amplify downstream region of *A1* Toxin antitoxin locus.
oLEM019	CATTCAAAGATGTTGGTAG	**Δ*A3* with marker less system** Forward primer to amplify upstream region of *A3* Toxin antitoxin locus.
oLEM037	CATACTCGAGCTAGATCGCATCCAATACG	**Δ*A3* with marker less system** Reverse primer to amplify upstream region of *A3* Toxin antitoxin locus. Primer

		contains a *Xho*I restriction site.
oLEM038	CATAGGATCCCAAGAGCGTTCCCTTAAGC	**Δ*A3* with marker less system** Forward primer to amplify downstream region of *A3* Toxin antitoxin locus. Primer contains a *Bam*HI restriction site.
oLEM022	CTTGAAAGGCTTCAATCAAG	**Δ*A3* with marker less system** Reverse primer to amplify downstream region of *A3* Toxin antitoxin locus.
oLEM027	CATGCTTGTCAAACCACAG	**Δ*A5* with marker less system** Forward primer to amplify upstream region of *A5* Toxin antitoxin locus.
oLEM041	CATACTCGAGGCTCTTAAATGCAACCAC	**Δ*A5* with marker less system** Reverse primer to amplify upstream region of *A5* Toxin antitoxin locus. Primer contains a *Xho*I restriction site.
oLEM042	CATAGGATCCCTAAGAGCGTTCCCCTAAG	**Δ*A5* with marker less system** Forward primer to amplify downstream region of *A5* Toxin antitoxin locus. Primer contains a *Bam*HI restriction site.
oLEM030	CTCAGTATGTGAATTTAGCG	**Δ*A5* with marker less system** Reverse primer to amplify downstream region of *A5* Toxin antitoxin locus.
oLEM031	GCCAAGCACCATCTTCTTTATG	**Δ*A6* with marker less system** Forward primer to amplify upstream region of *A6* Toxin antitoxin locus
oLEM043	CATACTCGAGGCTGCAAACCACTCATTTAAAG	**Δ*A6* with marker less system** Reverse primer to amplify upstream region of *A6* Toxin

		antitoxin locus. Primer contains a *Xho*I restriction site.
oLEM044	CATAGGATCCGGGTTATCCTTAAGTGGA	**Δ*A6* with marker less system** Forward primer to amplify downstream region of *A6* Toxin antitoxin locus. Primer contains a *Bam*HI restriction site
oLEM034	CTCATTACGACACTATTGC	**Δ*A6* with marker less system** Reverse primer to amplify downstream region of *A6* Toxin antitoxin locus.
oLEM045	CATACTCGAGatttaaaagtttgaaaagtgcag	**marker less system** Forward primer to amplify *rpsl-cat* cassette from pDifWT-RC. Primer contains a *Xho*I restriction site.
oLEM046	CATAGGATCCtcatttagttatgaaaactgcac	**marker less system** Reverse primer to amplify *rpsl-cat* cassette from pDifWT-RC. Primer contains a *Bam*HI restriction site.
oLEM023	GAGGCTGTAAGGATAAGG	**Δ*A4::Kan*** Forward primer to amplify upstream region of *A4* Toxin antitoxin locus.
oLEM107	gTTAgTCAcccgggtaccCAAACGCTAAAACGAGGCAC	**Δ*A4::Kan*** Reverse primer to amplify upstream region of *A4* Toxin antitoxin locus. Primer contains a homologous region to the Kanamycin resistance cassette.
oLEM009	GGTACCCGGGTGACTAAC	**Δ*A4::Kan*** Forward primer to amplify the Kanamycin resistance cassette.
oLEM010	CATTATTCCCTCCAGGTAC	**Δ*A4::Kan*** Reverse primer to amplify the Kanamycin resistance cassette
oLEM108	gtacctggagggaataATGGTTGGTCATTTTGGTATAAAAC	**Δ*A4::Kan*** Forward primer to amplify downstream region of *A4* Toxin

		antitoxin locus. Primer contains a homologous region to the Kanamycin resistance cassette.
oLEM026	CCCTAATAGTAGAAAATGGAG	***∆A4::Kan*** Reverse primer to amplify downstream region of *A4* Toxin antitoxin locus.
oLEM073	GATCATTAAAGGCTCCTTTTG	***pA1-GFP*** Forward primer to amplify upstream region of purel in pILL2157bis.
oLEM080	CAAAATGCCCGCTTCAATAAAAC	***pA1-GFP*** Reverse primer to amplify *aapA1* where stop codon of the A1 toxin has been replaced by Ala codon .

Movie S1. Movie of *H. pylori* morphological transformation.

Representative movie of the transformation of *H. pylori* upon expression of AapA1 toxin. Snapshots of the cells were taken at intervals of 10min.

Movie S2. Movie of *H. pylori* morphological transformation.

Representative movie of the transformation of *H. pylori* upon expression of AapA1 toxin. Snapshots of the cells were taken at intervals of 10min.

Movie S3. Large view movie of *H. pylori* morphological transformation.

Representative movie of the transformation of *H. pylori* upon expression of AapA1 toxin. Snapshots of the cells were taken at intervals of 10min.

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