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ARTICLE

Supplemental Information

“Nickel-Responsive Regulation of Two Novel *Helicobacter pylori* NikR–Targeted Genes”

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Table S1. Primers for plasmid construction and qPCR.

Promoter	**Primer name**	**Sequence (5’-3’)**
	Plasmid construction	
HPG27_1499	p1499F	CTGGGTCGACTCAGCGGAAACATAGT
	p1499R	GATTTCGGCCGAAGCGAGCGATTAG
HPG27_866	p866F	CACTCGGCCGAACGCTTGTCATTCATTC
	p866R	CATCGTCGACTGCGTGCTTTGAAAGGG
Gene	**qPCR**	
ureA	qUreAF	ACGCTGGAGAATTGGCTAAA
	qUreAR	CAGCTCTCGCTTCTTCCATAATA
HPG27_1499	q1499F	CTTGCATTTTGGCCAGTTTT
	q1499R	GCTTAGCCCTGATCTTGTGG
HPG27_866	q866F	CGCGGCTTTAAATGTCTCGCCTTT
	q866R	ATCCACACCAAACCTCCAGGCATA
16S rRNA	q16SF	CAGCGTCAGTAATGTTCCAG
	q16SR	TAGAGTGTGGGAGAGGTAGG
gyr	qgyrF	AGCGATAGAGAGGGCATTAGA
	qgyrR	CTCCATGGTGGTGAGTTTGT
	Sequencing	
	sKO866F	GCATTCTGCGTGCTTTGAAAGG
	sKO866R	GGGCTTATTACGCATGAAATCCTC
	sKO866upF	GGCTATCAACACCAGCGATC
	sKO866upR	CATAAGCGCATTGGGTGAGG
	sKO866dwnF	GTGGTCGTGTGGAGAACTTTTC
	sKO866dwnR	CCCTTGATTCGAACCGCTC

Table S2. Putative *Hp*NikR gene targets identified through ChIP-Seq.

Gene Tag[a]	Putative Description[b]	Sequence Tag[c]
HPG27_998	30S ribosomal protein S1	291
HPG27_210	3-deoxy-manno-octulosonate cytidylyltransferase	290
HPG27_663	HopE (outer membrane protein)	283
HPG27_1285	nicotinate-nucleotide adenyltransferase	262
HPG27_1419	RplY (50S ribosomal protein L25)	256
HPG27_1036	UDP-glucose 4-epimerase	233
HPG27_1429	riboflavin biosynthesis protein	231
HPG27_376	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	226
HPG27_1518	undecaprenylphosphate N-acetylglucosaminyl transferase	225
HPG27_513	23S rRNA methyltransferase	225
HPG27_242	Type II DNA modification enzyme	224
HPG27_452	neuraminyllactose-binding hemagglutinin	223
HPG27_453	phospho-N-acetylmuramoyl-pentapeptide-transferase	223
HPG27_1352	dimethyladenosine transferase	221
HPG27_768	hypothetical protein	221
HPG27_769	hypothetical protein	221
HPG27_160	peptidyl-prolylcis-transisomerase C	220
HPG27_22	HopD (outer membrane protein)	220
HPG27_903	prolipoprotein diacylglyceryl transferase	217
HPG27_1164	ABC transporter, ATP-binding protein	213
HPG27_456	hypothetical protein	211
HPG27_457	sodium-and chloride-dependent transporter	211
HPG27_81	RpoD (RNA polymerase sigma factor)	211
HPG27_1282	hypothetical protein	207
HPG27_27	hypothetical protein	206
HPG27_1286*	**NikR (nickel responsive regulator)**	205
HPG27_1287*	**ExbB (biopolymer transport protein)**	205
HPG27_1091	50S ribosomal protein L19	204
HPG27_1499	iron (III) ABC transporter, periplasmic iron-binding protein	202
HPG27_1500	alkylhydroperoxide reductase	202
HPG27_212	hypothetical protein	202
HPG27_1051	pyruvate flavodoxin oxidoreductase subunit delta	196
HPG27_1524	hypothetical protein	194
HPG27_631	HorF (outer membrane protein)	190
HPG27_866	iron-regulated outer membrane protein	190
HPG27_1153	ABC-transporter, ATP-binding domain	189
HPG27_507	CagA (cytotoxin-associated protein A)	189
HPG27_870	glyceraldehyde-3-phosphate dehydrogenase	189
HPG27_1425	HopK-3 (outer membrane protein)	186
HPG27_1426	hypothetical protein	186
HPG27_1165	undecaprenyl pyrophosphate synthase	183
HPG27_288	putative N-carbamoyl-D-amino acid amidohydrolase	183
HPG27_354	hypothetical protein	183
HPG27_361	CheY (chemotaxis protein)	180
HPG27_1229	hypothetical protein	179

Gene Tag[a]	Putative Description[b]	Sequence Tag[c]
HPG27_573	ABC transporter. ATP-binding protein	179
HPG27_89	hypothetical protein	178
HPG27_1459*	**FecA3 (putative nickel transport protein)**	177
HPG27_257	guanosine pentaphosphate phosphohydrolase	177
HPG27_895	hypothetical protein	177
HPG27_134	cytochrome c oxidase (diheme subunit)	176
HPG27_751	Tig (trigger factor)	176
HPG27_1032	ribonucleotide-diphosphate reductase subunit beta	174
HPG27_1289*	**TonB (siderophore-mediated iron transport protein)**	174
HPG27_876	HtpX (heat shock protein)	174
HPG27_223	NapA (neutrophil activating protein, bacterioferritin)	173
HPG27_890	alanine racemase	173
HPG27_518	3-oxoacyl-(acyl carrier protein) synthase II	172
HPG27_988	GMP synthase	172
HPG27_1414	phosphate permease	171
HPG27_1415	conserved hypothetical nifU-like protein	171
HPG27_1120	HopQ (outer membrane protein)	170
HPG27_1158	F0F1 ATP synthase subunit C	170
HPG27_1398	phosphopantetheine adenylyltransferase	169
HPG27_775	hypothetical protein	169
HPG27_1421	hypothetical protein	168
HPG27_795	hypothetical protein	168
HPG27_841	FecE (iron (III) dicitrate ABC transporter)	168
HPG27_1064	FlgK (flagellar hook-associated protein)	167
HPG27_1440	hypothetical protein	166
HPG27_736	bifunctional aconitate hydratase 2/2-methylisocitrate dehydratase	165
HPG27_1435*	**FrpB4 (putative nickel transport protein)**	164
HPG27_300	arginyl-tRNA synthetase	164
HPG27_418	polyphosphate kinase	164
HPG27_571	ABC transporter, permease	164
HPG27_531	adenine phosphoribosyltransferase	163
HPG27_1522	hypothetical protein	161
HPG27_1523	FlgG (flagellar basal body rod protein)	161
HPG27_287	hypothetical protein	161
HPG27_1474	putative type II methylase protein	159
HPG27_65	UreE (urease accessory protein)	158
HPG27_1155	type II adenine specific DNA methyltransferase	157
HPG27_116	HorB (outer membrane protein)	156
HPG27_1170	coproporphyrinogen III oxidase	155
HPG27_1171	cytochrome c553	155
HPG27_1313	chemotaxis response regulator	154
HPG27_145	hypothetical protein	154
HPG27_703	hypothetical protein	154
HPG27_325	single-stranded-DNA specific exonuclease	152
HPG27_1199	biotin synthesis protein	150
HPG27_1391	branched-chain amino acid aminotransferase	150
HPG27_1025	deoxycytidine triphosphate deaminase	149

Gene Tag[a]	Putative Description[b]	Sequence Tag[c]
HPG27_1026	biotin carboxyl carrier protein	149
HPG27_1066	type II DNA modification enzyme	149
HPG27_1510	DNAse	148
HPG27_41	HypE (hydrogenase expression/formation protein)	148
HPG27_975	hypothetical protein	148
HPG27_1214	NADH dehydrogenase subunit J	147
HPG27_1497	FlgB (flagellar basal body rod protein)	147
HPG27_1498	FtsW (cell division protein)	147
HPG27_495	CagM (cag pathogenicity island protein)	137
HPG27_190	lipopolysaccharide 1,2-glycosyltransferase	26
HPG27_191	outer membrane protein	26

[a] Known targets of direct *Hp*NikR regulation are marked with an asterisk

[b] As annotated in the NCBI Gene Database

[c] As determined by SISSR

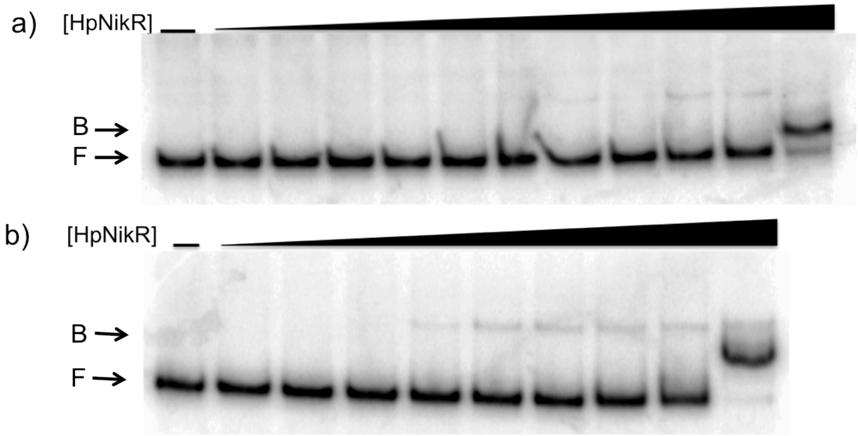
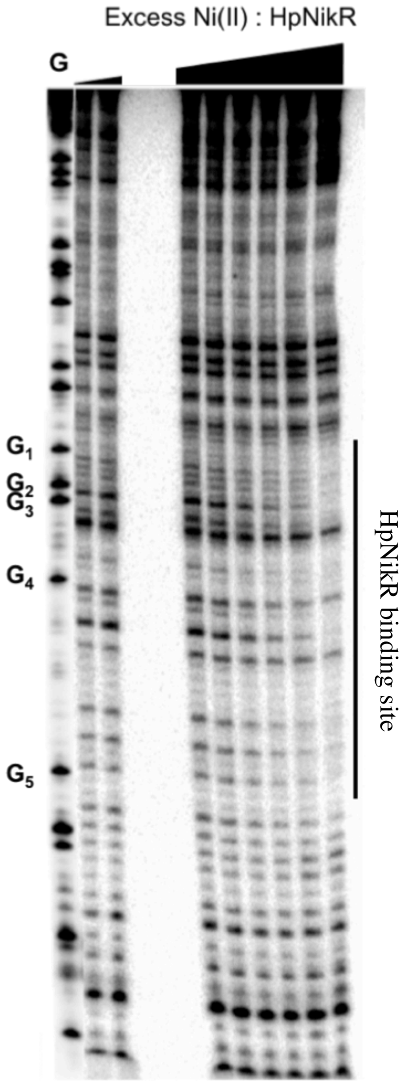


Figure S1. DNA-binding activity of apo-*Hp*NikR.

Apo-*Hp*NikR (50 nM – 100 μM) was incubated with DNA containing the HPG27_1499 promoter sequence (a) or the HPG27_866 sequence (b) then resolved on 6% native polyacrylamide gels with 3 mM $MgSO_4$. Binding was observed only at the maximum *Hp*NikR concentrations used.

a)



b)

5’
TCGACTCAGCGGAAACATAGTGGAGTATATTCTTAAAAAGTTAATGAAAAGATATTTCCTTTTAAATTTT
AAGATTTTGTGAAAAAAAGTTTCATTTTCACTGCTTGTATTTCCTT**G**$_1$AATA**G**$_2$T**G**$_3$TTATAATC**G**$_4$CTTCAT
AAATCATAC**G**$_5$AAAAGGAATTGTCATGCTAATCGCTCGCTTCGGCCG 3’

Figure S2. DNA binding by *Hp*NikR in excess nickel on the 1499 promoter.

a) *Hp*NikR (50 nM, 100 nM, 200 nM, 250 nM, 300 nM, 500 nM, 1 μM, 5 μM) was incubated with a ^{32}P-labeled 184-bp DNA probe in the presence of 35 μM NiCl$_2$ for 30 minutes at room temperature prior to the addition of DNase I. The reactions were resolved on an 8% denaturing polyacrylamide gel and the area of protection is indicated. Lane G was loaded with a Maxam-Gilbert G reaction. The apparent DNA-binding affinity was determined to be 330 ± 40 nM with a Hill coefficient of 2.02 ± 0.04, calculated as an average of two independent experiments. The DNA strand was labeled on the 3’ end with α^{32}P and Klenow fragment. **b)** DNA sequence of 1499 promoter (reverse complement of sequence in Figure 3b). The binding region is underlined and the guanines within in the footprint are bolded and noted on the footprint gel.

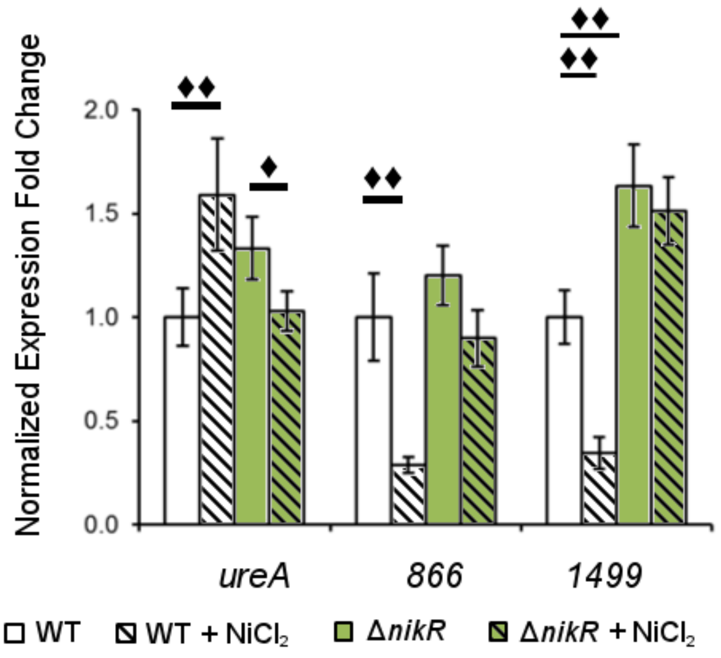


Figure S3. Expression of *H. pylori* genes upon transient nickel exposure.

Quantitative PCR was performed for *H. pylori* G27 and its isogenic *ΔnikR* mutant grown to mid-log phase and exposed to 100 µM $NiCl_2$ for 15 minutes. Fold expression is relative to *16S* and *gyr* expression under the same conditions and normalized to WT without nickel. Statistically significant differences between expression levels are marked with diamonds (♦ = $P < 0.05$, ♦♦ = $P < 0.01$, Student's t-test). The data represent the average of at least three independent biological and technical replicates, and the error bars indicate one standard deviation.

Figure S4. Alignment of the HPG27_866/*frpB2* promoters from *H. pylori* strains G27 and 26695.

The *Hp*NikR recognition consensus sequence proposed by Delany et al[28] is circled by a dashed line and the consensus sequence proposed by Stoof et al[29] is highlighted in blue. Both promoter sequences are the same distance upstream of the start codon.