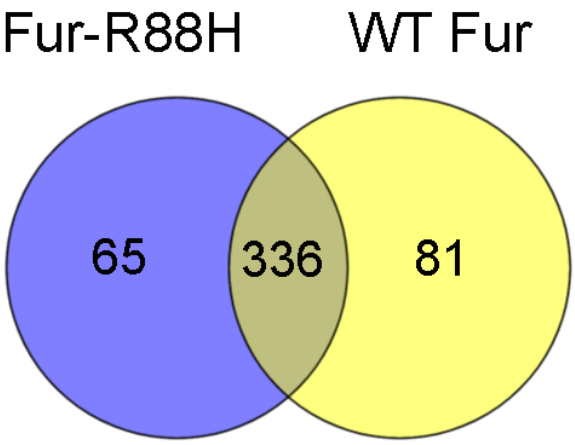
Supplementary Figure S1. Comparison of salt-responsive gene expression in *H. pylori* strains containing WT Fur or Fur-R88H. The overlap in the Venn diagrams illustrates the number of genes that are salt-responsive in both strains. A) Genes that are upregulated in response to high-salt conditions in the indicated strains (FDR<0.05, fold change >2). B) Genes that are downregulated in response to high-salt conditions in the indicated strains (FDR<0.05, fold change <0.5). A total of 336 genes are upregulated in response to high-salt conditions in both the Fur-R88H and WT FurR-containing strains (panel A), while 222 genes are downregulated in response to high-salt conditions in both the Fur-R88H and WT Fur-containing strains (panel B). The remaining numbers in the Venn diagram illustrate genes that are salt-responsive in only one of the strains.

Supplementary Figure S2. Comparison of gene expression in *H. pylori* strains containing WT Fur or Fur-R88H. *H. pylori* strains were cultured under both routine and high-salt conditions. A) Comparison of genes that were increased in expression in the Fur-R88H variant compared to the strain containing WT Fur under the indicated conditions. A total of 57 genes were increased in expression under regular salt conditions in the Fur-R88H variant compared to the strain containing WT Fur (FDR <0.05, fold >2). In contrast, only 3 genes were increased in expression under high-salt conditions in the Fur-R88H variant compared to strain containing WT Fur (FDR<0.05, fold >2). A single gene (i.e. *fecA2*, Table 1, Supplementary Table 1) was increased in expression in the Fur-R88H variant under both regular and high-salt conditions. B) Analysis of genes that were decreased in expression in the Fur-R88H variant compared to the strain containing WT Fur under the indicated conditions. A total of 53 genes were expressed at a lower level under regular salt conditions in the Fur-R88H strain compared to the strain containing WT Fur (FDR<0.05, fold <0.5). Four genes were expressed at lower levels in the Fur-R88H variant compared to the strain containing WT Fur under high-salt conditions (FDR<0.05, fold <0.5).

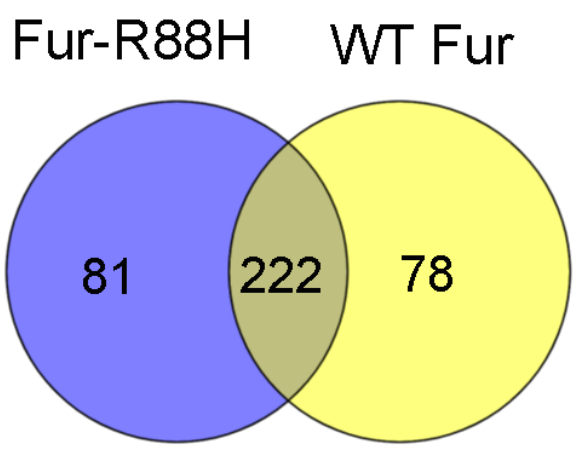
Supplementary Figure S3. Relative fitness of *fecA2* mutants in strains containing either WT Fur strain or Fur-R88H. A) Depiction of strains used for this analysis. Strain JL3B contains WT Fur and a cassette containing the chloramphenicol acetyltransferase gene and a unique DNA barcode (TAG1) inserted into the deleted *fecA2* locus. Strain JL4 is a strain that contains Fur-R88H and a *cat*-TAG2 sequence inserted into the deleted *fecA2* locus. The indicated strains were mixed 1:1 and serially passaged (once every 3.5 days) on medium containing either 0.5% (panel B) or 0.9% added NaCl (panel C). At the indicated time points, bacteria were harvested and genomic DNA was isolated. The proportion of each strain in the bacterial population was then quantified by qPCR as described in the Methods. The primers for the qPCR analyses (Supplemental Table S4) included a tag-specific primer (TSP) and a gene-specific primer (GSP). The results are from 2 independent experiments. qPCR analysis for each time point was performed in triplicate and the mean value is reported.

Supplementary Figure S4. Reduced fitness of a *fecA2* mutant in either the presence or absence of supplemental ferric citrate. A) Depiction of strains used for this analysis. Strain JL1 contains WT Fur and a cassette containing the chloramphenicol acetyltransferase gene and a unique DNA barcode (TAG1) inserted into the *mdaB-hydA* locus. Strain JL3A contains WT Fur and a DNA barcode (TAG2) inserted into the deleted *fecA2* locus. A 1:1 mixture of strains JL1 and JL3A were cultured on medium containing either 0.5% (panel B) or 0.9% (panel D) added NaCl. Competition experiments were also done using media supplemented with 100 μM ferric citrate (panels C, E). The bacteria from the plates were subjected to continuous passage (once every 3.5 days) for 21 days. At the indicated time points, bacteria were harvested and genomic DNA was isolated. The proportion of each strain in the bacterial population was then quantified by qPCR as described in the Methods. qPCR primer pairs involving a tag-specific primer (TSP) and gene-specific primer (GSP) are listed in Supplemental Table S4. The results are from 2 independent experiments. qPCR analysis was performed in triplicate on samples from each time point, and the mean values are reported.

A. Genes upregulated (1.0% vs 0.5% NaCl))

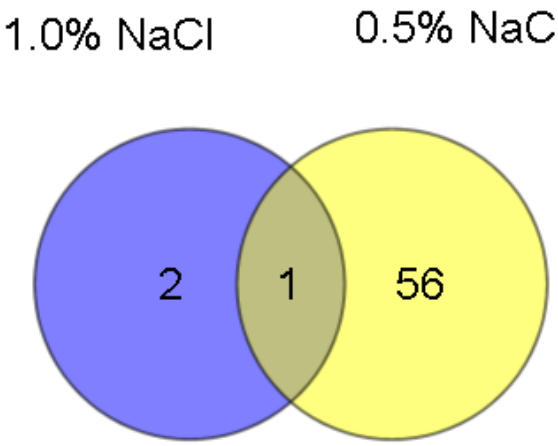


B. Genes downregulated (1.0% vs 0.5% NaCl)

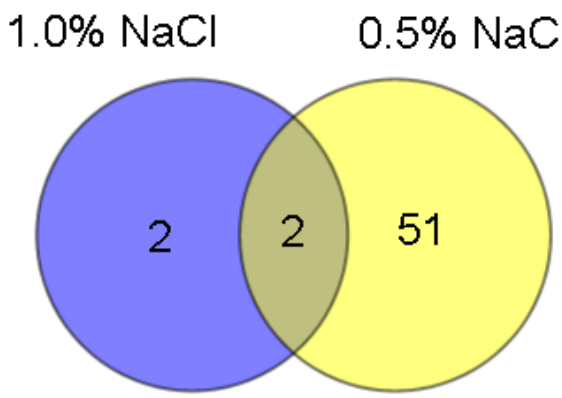


Supplementary Fig S1

A. Increased expression in Fur-R88H compared to WT Fur



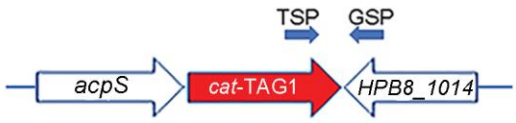
B. Decreased expression in Fur-R88H compared to WT Fur



Supplementary Fig S2

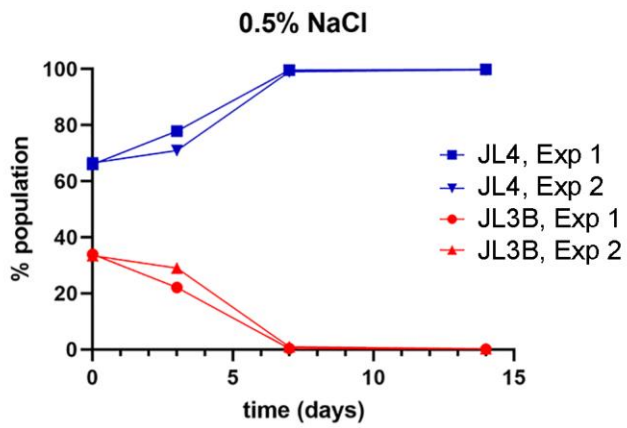
A.

Strain JL3B
WT Fur, Δ*fecA2*::*cat*-TAG1

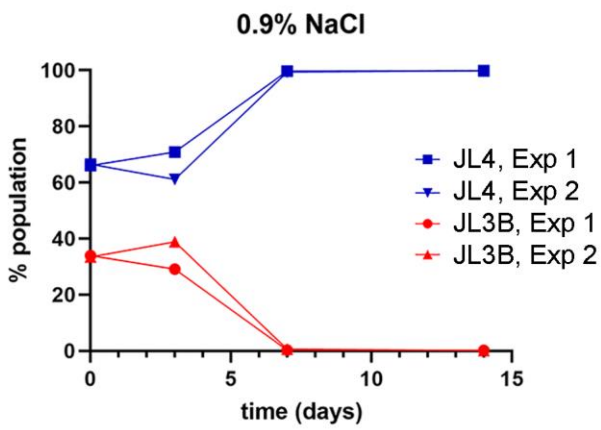


Strain JL4
Fur-R88H, Δ*fecA2*::*cat*-TAG2

B.



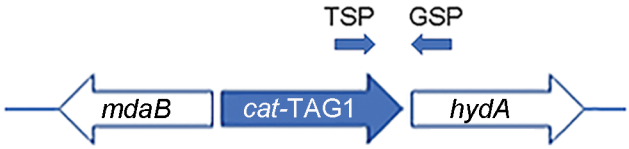
C.



Supplementary Fig S3

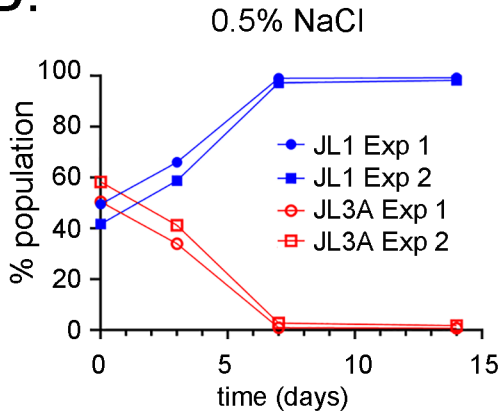
A.

Strain JL1
WT Fur, *mdaB-hydA::cat*-TAG1

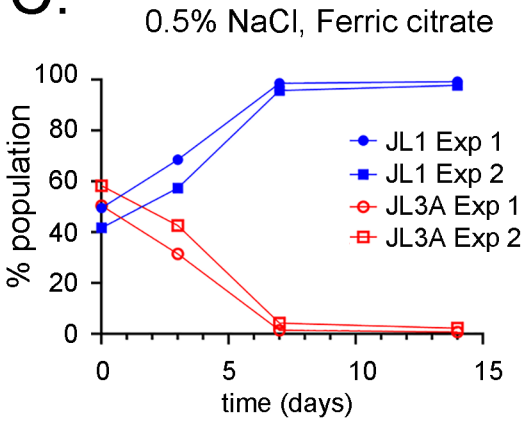


Strain JL3A
WT Fur, *ΔfecA2::cat*-TAG2

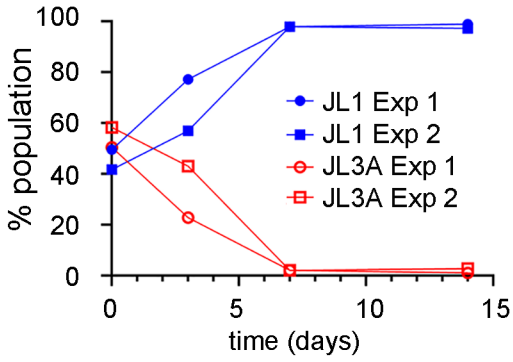
B.



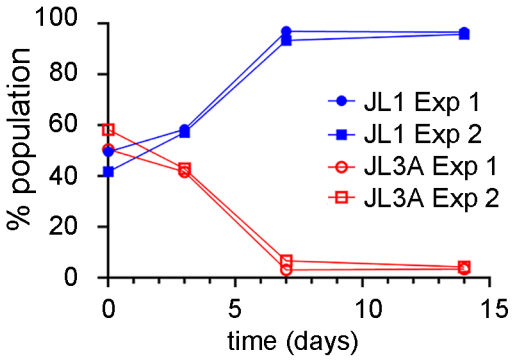
C.



D.



E.



Supplementary Fig S4

Supplemental Table S1. Genes that are differentially expressed under regular salt conditions (0.5% added NaCl) between strains expressing Fur-R88H and WT Fur[a]

B8 gene	26695/J99 gene	FDR[b]	fold[c]	Description
Increased expression in Fur-R88H				
HPB8_29	N/A	5.4E-05	2.64	putative endonuclease
HPB8_116	HP1428	2.8E-02	2.31	Ribosomal RNA large subunit methyltransferase N (*rlmN*)
HPB8_121	HP1423	2.5E-05	2.23	hypothetical protein
HPB8_123	HP1421	1.5E-02	2.12	type IV secretion system protein VirB11, conjugation
HPB8_125	HP1419	2.2E-02	20.93	flagellar biosynthetic protein (*fliQ*)
HPB8_158	HP1321	2.1E-03	2.65	hypothetical protein
HPB8_199	HP1281	9.5E-04	2.91	anthranilate synthase component II (*trpG*)
HPB8_316	HP0054	2.8E-02	2.45	conserved hypothetical protein
HPB8_380	HP1121	2.8E-04	5.81	DNA (cytosine-5-)-methyltransferase (*dcm1*)
HPB8_387	HP1116	2.4E-03	2.55	hypothetical protein
HPB8_449	HP0398	2.0E-02	2.12	hypothetical protein
HPB8_468	HP0418	3.5E-04	2.11	ferrochelatase
HPB8_490	jhp0922	3.6E-02	2.77	component of conjugal plasmid transfer system
HPB8_494	jhp1203	3.3E-02	4.93	hypothetical protein
HPB8_501	HP1003	3.0E-02	2.78	hypothetical protein
HPB8_504	HP1001	6.7E-07	2.26	hypothetical protein
HPB8_505	HP1000	1.1E-15	3.23	putative chromosome partitioning protein (*parA3*)
HPB8_512	jhp0947	3.4E-03	2.07	hypothetical protein
HPB8_521	HP0995	1.9E-03	2.83	hypothetical protein
HPB8_528	jhp0936	4.9E-02	2.31	hypothetical protein
HPB8_532	HP1000	2.5E-18	3.11	hypothetical protein
HPB8_533	jhp0934	8.1E-12	2.48	hypothetical protein
HPB8_538	HP1006	1.0E-02	2.69	type IV secretion system protein (*virD4, traG*)
HPB8_620	HP0929	1.5E-04	2.00	geranyltranstransferase (*ispA*)
HPB8_623	HP0926	4.0E-04	3.54	tRNA pseudouridine synthase D (*truD*)
HPB8_624	HP0925	1.1E-04	2.95	Recombination protein (*recR*)
HPB8_643	HP0909	2.2E-02	2.39	restriction endonuclease (*hpy8I*)
HPB8_672	HP1116	2.4E-03	2.55	hypothetical protein
HPB8_798	HP0600	4.0E-02	2.25	Phosphate import ATP-binding protein (*pstB*)
HPB8_801	HP0602	2.9E-02	2.44	Endonuclease III (*nth3*)
HPB8_855	HP0652	6.7E-15	5.15	phosphoserine phosphatase (*serB*)
HPB8_856	HP0653	8.0E-27	6.01	ferritin (*pfr*)
HPB8_877	HP0673	6.3E-06	2.57	hypothetical protein
HPB8_880	HP0677	1.8E-04	2.66	hypothetical protein
HPB8_980	HP0771	1.7E-02	2.79	hypothetical protein
HPB8_990	HP0781	1.9E-05	3.28	hypothetical protein

HPB8_1015	HP0807	5.8E-33	7.32	iron complex outermembrane protein (*fecA2*)
HPB8_1029	HP0820	1.3E-05	2.45	conserved hypothetical protein
HPB8_1056	HP0846	2.8E-03	2.09	type I restriction enzyme R subunit (*hsdR1*)
HPB8_1088	HP0876	3.2E-09	4.38	iron complex outermembrane r protein (*frpB1*)
HPB8_1089	HP0877	1.0E-11	6.13	crossover junction endodeoxyribonuclease (*ruvC*)
HPB8_1097	HP0484	1.3E-03	3.53	hypothetical protein
HPB8_1098	HP0051	7.0E-03	2.65	DNA (cytosine-5-)-methyltransferase (*dcm3*)
HPB8_1127	HP1011	3.2E-03	2.56	dihydroorotate oxidase (*pyrD*)
HPB8_1159	HP1041	1.4E-04	2.02	flagellar biosynthesis protein (*flhA*)
HPB8_1160	HP1042	1.3E-06	2.87	hypothetical protein
HPB8_1170	HP1051	9.1E-16	2.70	hypothetical protein
HPB8_1235	HP0329	2.2E-03	2.05	NH(3)-dependent NAD(+) synthetase (*nadE*)
HPB8_1249	HP0313	6.9E-04	2.95	hypothetical protein (*narK*)
HPB8_1282	HP0280	1.9E-04	2.02	heat shock protein B (*htrB*)
HPB8_1300	HP0262	1.8E-02	2.78	Uncharacterized protein HP_0262
HPB8_1301	HP0262	2.4E-02	2.55	Uncharacterized protein HP_0262
HPB8_1393	HP0173	1.7E-02	2.42	flagellar biosynthetic protein (*fliR*)
HPB8_1415	HP0150	1.9E-02	2.28	hypothetical protein
HPB8_1430	HP0135	1.4E-03	2.52	Uncharacterized protein jhp_0123
HPB8_1447	HP1187	2.9E-04	2.58	hypothetical protein
HPB8_1628	HP1588	1.5E-02	2.22	UPF0174 protein jhp_1494

<u>Decreased expression in Fur-R88H</u>

HPB8_45	HP1485	3.1E-02	0.44	hypothetical protein
HPB8_78	HP1456	3.1E-19	0.42	membrane associated lipoprotein (*lpp20*)
HPB8_79	HP1455	1.9E-22	0.29	hypothetical protein
HPB8_80	HP1454	6.5E-30	0.21	hypothetical protein
HPB8_95	HP1441	9.5E-04	0.44	peptidyl-prolyl cis-trans isomerase B (*ppiA*)
HPB8_105	HP1436	3.5E-02	0.41	hypothetical protein
HPB8_141	HP1338	7.1E-04	0.45	nickel-responsive regulator (*nikR*)
HPB8_142	HP1337	4.5E-03	0.47	nicotinate-nucleotide adenylyltransferase (*nadD*)
HPB8_154	HP1325	3.5E-06	0.25	fumarate hydratase (*fumC*)
HPB8_155	HP1324	2.3E-05	0.30	hypothetical protein
HPB8_162	HP1317	7.1E-03	0.37	ribosomal protein L23 (*rplW*)
HPB8_224	HP1256	1.7E-02	0.25	ribosome recycling factor (*frr*)
HPB8_285	HP1205	2.8E-06	0.39	elongation factor EF-Tu
HPB8_286	HP1204	3.9E-06	0.41	ribosomal protein L33 (*rpmG*)
HPB8_304	HP1186	1.4E-08	0.22	carbonic anhydrase (*cah*)
HPB8_377	HP1123	3.8E-05	0.45	peptidyl-prolyl cis-trans isomerase (*slyD*)
HPB8_381	HP1120	5.3E-03	0.10	hypothetical protein
HPB8_466	HP0416	3.5E-06	0.38	cyclopropane-fatty-acyl-phospholipid synthase (*cfa*)
HPB8_580	HP0966	1.3E-04	0.44	hypothetical protein

HPB8_652	HP0900	5.4E-05	0.28	hydrogenase nickel incorporation protein (*hypB*)
HPB8_653	HP0899	1.7E-03	0.40	hydrogenase formation protein (*hypC*)
HPB8_697	HP0546	7.0E-04	0.49	*cagC*
HPB8_731	HP0515	1.5E-04	0.49	heat shock protein (*hslV*)
HPB8_745	HP0551	5.5E-05	0.40	50S ribosomal protein L31 (*rpmE*)
HPB8_763	HP0567	3.8E-06	0.27	hypothetical membrane protein
HPB8_787	HP1589	5.2E-07	0.40	2-oxoglutarate ferredoxin oxidoreductase (*oorA*)
HPB8_788	HP1590	5.2E-07	0.39	2-oxoglutarate ferredoxin oxidoreductase (*oorB*)
HPB8_803	HP0604	8.8E-06	0.43	uroporphyrinogen decarboxylase (*hemE*)
HPB8_925	HP0719	2.0E-06	0.27	hypothetical protein
HPB8_926	HP0721	8.7E-05	0.31	hypothetical protein
HPB8_985	HP0776	2.9E-02	0.50	DNA-directed RNA polymerase subunit (*rpoZ*)
HPB8_986	HP0777	6.3E-06	0.50	uridylate kinase (*pyrH*)
HPB8_1020	HP0812	4.5E-02	0.43	hypothetical protein
HPB8_1033	HP0824	4.5E-08	0.23	thioredoxin 1 (*trxA*)
HPB8_1034	HP0825	3.6E-08	0.40	thioredoxin reductase (NADPH) (*trxB3*)
HPB8_1061	HP0851	1.1E-03	0.37	hypothetical protein
HPB8_1198	HP1079	1.0E-04	0.39	hypothetical protein
HPB8_1247	HP0318	3.1E-08	0.30	hypothetical protein
HPB8_1264	HP0298	8.6E-06	0.45	dipeptide ABC transporter (*dppA*)
HPB8_1285	HP0277	2.2E-05	0.41	ferredoxin (*fer*)
HPB8_1370	HP0197	2.5E-06	0.48	S-adenosylmethionine synthetase (*metK*)
HPB8_1425	HP0140	9.3E-09	0.45	lactate transporter%2C LctP family (*lctP3*)
HPB8_1455	HP0110	2.9E-06	0.47	molecular chaperone (*grpE*)
HPB8_1456	HP0106	1.3E-08	0.38	molecular chaperone (*dnaK*)
HPB8_1480	jhp0078	5.9E-03	0.14	Uncharacterized protein jhp_0078
HPB8_1481	HP0084	3.1E-07	0.42	ribosomal protein L13 (*rplM*)
HPB8_1502	HP0065	4.4E-02	0.45	hypothetical protein
HPB8_1503	HP0064	6.7E-03	0.28	hypothetical protein
HPB8_1511	HP0057	3.8E-05	0.37	hypothetical protein
HPB8_1564	HP1410	2.3E-02	0.50	hypothetical protein
HPB8_1596	HP0028	3.4E-03	0.50	hypothetical protein
HPB8_1597	HP0027	6.1E-06	0.42	isocitrate dehydrogenase (*icd*)
HPB8_1620	HP0005	4.5E-04	0.48	orotidine-5'-phosphate decarboxylase (*pyrF*)

[a]*H. pylori* strains expressing either Fur-R88H or WT Fur were grown in Brucella broth containing 0.5% NaCl for 15 h. RNA-seq was performed as described in Materials and Methods. Genes considered differentially expressed demonstrated fold change values (Fur-R88H vs WT Fur) either >2 or <0.5, with a false-discovery rate <0.05.
[b]FDR, false-discovery rate.
[c]"Fold" indicates fold change values. The fold change values are a ratio of RNA-seq reads from cultures expressing Fur-R88H to RNA-seq reads from cultures expressing WT Fur.

Supplemental Table S2. Genes that are differentially expressed in strains containing Fur-R88H or WT Fur, cultured under high-salt conditions [a]

B8 gene	26695 gene	[b]FDR	fold	Description
increased expression in Fur-R88H				
HPB8_545	HP0040	2.25E-02	3.30	type IV secretion system protein (*virB9*)
HPB8_1015	HP0807	1.45E-05	2.23	outer membrane protein (*fecA2*)
HPB8_1322	HP0241	5.82E-03	2.27	conserved hypothetical protein
decreased expression in Fur-R88H				
HPB8_80	HP1454	1.50E-06	0.41	conserved hypothetical protein
HPB8_389	HP0489/HP1115	7.08E-04	0.03	conserved hypothetical protein
HPB8_580	HP0966	1.24E-03	0.48	conserved hypothetical protein
HPB8_581	HP0965	1.45E-05	0.39	probable tRNA modification GTPase (*trmE*)

[a]*H. pylori* strains containing either Fur-R88H or WT Fur were grown in BB-FBS broth containing 1.0% added NaCl for 15 h. RNA-seq was performed as described in Materials and Methods. Genes considered differentially expressed demonstrated fold change values (Fur-R88H vs WT Fur) either >2 or <0.5, with a false-discovery rate <0.05.

[b]FDR, false-discovery rate.

[c]"Fold" indicates fold change values. The fold change values are a ratio of RNA-seq reads from the strain containing Fur-R88H to RNA-seq reads from the strain containing WT Fur.

Supplemental Table S3. List of plasmids and strains used in this study

Plasmids

Name	Relevant Characteristics	Reference
p630cat-TAG1	pUC57, *cat*-TAG1 sequence inserted into *mdaB-hydA* intergenic region	45
p630cat-TAG2	pUC57, *cat*-TAG1 sequence inserted into *mdaB-hydA* intergenic region	45
pΔfecA2-catTAG1	pUC57 containing 500 bp upstream and downstream of *fecA2. fecA2* replaced with *cat*-TAG1 nucleotide sequences	This study
pΔfecA2-catTAG2	pUC57 containing 500 bp upstream and downstream of *fecA2. fecA2* replaced with *cat*-TAG2 nucleotide sequences	This study
p177::*aacC4-rpsL*	pGEMT, *aacC4-rpsL* cassette inserted into intergenic region between HPB8_1388 (*neuB*) and HPB8_1389 (*efp*)	This study
pfecA2	pGEMT, *fecA2* inserted into *neuB-efp* intergenic region	This study
<u>*H. pylori* strains</u>		
7.13 WTFur-1	7.13, *rpsL-K43R*, Sm^R, WT Fur	14
7.13 Fur-R88H-1	7.13, *rpsL-K43R*, Sm^R, Fur-R88H	14
JL1	7.13 WTFur-1, *cat*-TAG1 insertion into *mdaB-hydA* intergenic region, Sm^R, Cm^R	This study
JL2	7.13 Fur-R88H-1, *cat*-TAG2 insertion into *mdaB-hydA* intergenic region, Sm^R, Cm^R	This study
JL3A	7.13 WTFur-1, replacement of *fecA2* with *cat*-TAG2, Sm^R, Cm^R	This study
JL3B	7.13 WTFur-1, replacement of *fecA2* with *cat*-TAG1, Sm^R, Cm^R	This study
JL4	7.13 Fur-R88H-1, replacement of *fecA2* with *cat*-TAG2, Sm^R, Cm^R	This study
JL5	JL4, *aacC4-rpsL* inserted in *neuB-efp* intergenic region, $Gent^R$, Cm^R	This study
JL6	JL5, *neuB-efp* intergenic region restored, Sm^R, Cm^R	This study
JL7	JL5, *fecA2* inserted in *neuB-efp* intergenic region, Sm^R, Cm^R	This study

Supplemental Table S4. Primers used in this study

RT-qPCR primers

Gene	Forward primer	Reverse primer
fecA1	5′-ATGGTATGCGAACTACCGCC-3′	5′-TAGCGTTGCCCCACTTCAAT-3′
fecA2	5′-TCTCGCACGGTGATTTCCAA-3′	5′-GCGCACCGAAATTTTAGGCA-3′
fecA3	5′-ATGTGGGTATCCAAGCGCAA-3′	5′-TCTTGCTCGCTGAGTGATCC-3′
frpB1	5′-TATTGCACCCCAAGCTTTTC-3′	5′-AAGGCTGTCTGTGGCTTCAT-3′
gyrB	5-CGTGGATAACGCTGTAGATGAGAGC-3′	5′-GGGATTTTTTCCGTGGGGTG-3′
16S rRNA	5′-GGAGTACGGTCGCAAGATTAAA-3′	5′-CTAGCGGATTCTCTCAATGTCAA-3′
HPB8_80	5'-ATGCCTTATGGCTTTTGTGG- 3'	5'-CCACGCTAATAGACGCCACT-3'
HPB8_389	5'-ACAGCCCGAAGTGAAAGAAA-3'	5'-TTCGCTTTCAAGCTCTCTCC-3'
HPB8_545	5'-CCTTGCAAATTGGCGTAGAT-3'	5'-TGGGTGCTATGAGTGGGTTT-3'
HPB8_580	5'-AGGCAGTAGCGTTTTGCCTA-3'	5'-TACTTCGGCGCATTCTCTTT-3'
HPB8_581	5'- CTTTGAATGACAGCGTTGGA-3'	5'-CCGTCTTTGCTCCTATCTCG-3'
HPB8_1322	5'-GCTTATCCGCTATGGCTTGA-3'	5'-AGCGTCCAATAGAGCGTGAT-3'

Competition Assays

Strain	Forward primer (TAG specific, TSP)	Reverse primer (Gene specific, GSP)
JL1	5′-TTCCGACGTACAGTTGTACAG-3′	5′-TTTGGCTCCATTAATGATGAGT-3′
JL2	5′-CTACGTTCCCGAGGCCGTACA-3′	5′-TTTGGCTCCATTAATGATGAGT-3′
JL3B	5′-TTCCGACGTACAGTTGTACAG-3′	5′-CGATCCACAAAAACCATTCC-3′
JL3A, JL4, JL5, JL6, JL7	5′-CTACGTTCCCGAGGCCGTACA-3′	5′-CGATCCACAAAAACCATTCC-3′