### Supplementary Experimental Procedures

Large Scale Isolation of Hp0579-80 Reaction Product. A 10-ml reaction mixture containing 45  $\mu$ M of Kdo<sub>2</sub>-lipid A substrate (Avanti Polar Lipids), 0.5 mg/ml HMS174 pET21a or HMS174 pHp0579-80 membranes, 50 mM Hepes pH 8.0, and 0.1% Triton TX-100 was incubated at 30 °C for 3 hours. The reaction was then converted into a two-phase Bligh-Dyer consisting of chloroform/methanol/0.01 M HCI (2:2:1.8 v/v). Phases were separated in a clinical centrifuge and the lower phase removed to a separate tube. A second extraction of the resulting upper phase was achieved by addition of fresh lower phase. The lower phases were pooled and dried under a stream of N<sub>2</sub>. Lipids were separated by DEAE anion-exchange chromatography as previously described (Tran *et al.*, 2004). Fractions containing the Kdo<sub>2</sub>-lipid A substrate and Kdo-lipid A reaction product were converted to a two-phase Bligh/Dyer, as described above, and the lipid recovered from the lower phase.

**Mild Acid Hydrolysis of Whole Cell Lysates.** Whole cell lysates were heated at 80°C for 30 minutes in 1% acetic acid as described previously (Hug *et al.*, 2010).

### Supplementary Figure Legends

Fig. S1. Genomic context of *F. tularensis* and *H. pylori* Kdo hydrolase genes. MacVector was used to generate the image.

**Fig. S2. Membrane topology prediction of** *H. pylori* **Kdo hydrolase machinery.** Predicted topology and transmembrane segments of Hp0579 (A) and Hp0580 (B) based on the TMHMM algorithm (Krogh *et al.*, 2001).

**Fig. S3. Mass spectrometry of the Kdo hydrolase reaction products.** A large scale reaction using Kdo<sub>2</sub>-lipid A substrate was performed using membranes isolated from either HMS174 pET21a or HMS174 pHp0579-80. Reaction products were purified on a DEAE column as previously described (Tran et al., 2004) and analyzed by MALDI-TOF mass spectrometry in the negative-ion mode. The HMS174 pET21a reaction produced a predominant peak at m/z 2237.5, consistent with the Kdo<sub>2</sub>-lipid A starting material **(A)**. The HMS174 pHp0579-80 reaction produced a predominant peak at m/z 2017.8, consistent with a Kdo-lipid A species **(B)**.

**Fig. S4. Mass spectrometry of the 7.13 Kdo hydrolase mutant.** Lipid A was isolated from wild type strain 7.13, 7.13 *hp0579-80::cam* and 7.13 *hp0579-80::cam*, *hp0579-80<sup>+</sup>* and analyzed by MALDI-TOF mass spectrometry in the negative-ion mode. Strain 7.13 produced a peak at m/z 1548.9 corresponding to the published wild type *H. pylori* lipid A mass (A). In addition to the wild type peak at m/z 1548.0, 7.13 *hp0579-80::cam* also displayed a peak at m/z 2091.2 (B). 2091.2 corresponds to a hexa-acylated lipid A species, with the 4'-phosphate still present. Strain 7.13 *hp0579-80<sup>+</sup>* showed complete reversion to the wild type phenotype, with a single peak present at a m/z of 1548.9 (C).

**Fig. S5. Mild acid hydrolysis of the G27 Kdo hydrolase mutant whole cell lysates.** *H. pylori* strain G27 Kdo hydrolase mutants and complements were subjected to mild acid hydrolysis treatment followed by SDS-PAGE (A) and immunoblot analysis (B, C), as described in materials and methods. U = untreated, T = mild acid hydrolysis treated.

### References

- Hug, I., M. R. Couturier, M. M. Rooker, D. E. Taylor, M. Stein & M. F. Feldman, (2010) Helicobacter pylori lipopolysaccharide is synthesized via a novel pathway with an evolutionary connection to protein N-glycosylation. *PLoS pathogens* 6: e1000819.
- Krogh, A., B. Larsson, G. von Heijne & E. L. Sonnhammer, (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol* 305: 567-580.
- Tran, A. X., M. J. Karbarz, X. Wang, C. R. Raetz, S. C. McGrath, R. J. Cotter & M. S. Trent, (2004) Periplasmic cleavage and modification of the 1-phosphate group of Helicobacter pylori lipid A. *J Biol Chem* 279: 55780-55791.

### Table S1. Oligonucleotides

Fhp05795'-GCGCGCCATATGCTTATATCTTCTTCT-3'Rhp05795'-GCGCGCCGGATCCTTAATACAACCTTTTTT-3'Fhp05805'-GCGCGCCGGATCCTCAATGGAACCTTCAAGAAAT-3'Rhp05805'-GCGCGCCGGGATCCTCAACTGAGAAGACT-3'Hp0580P15'-GCGCGCCCTGAAGCATCTCTAATGCGAC-3'Hp0580P25'-CTTAGCTCCTGAAAATCTCGGAAAGCCCCACAAAAAAGGCG-3'Hp0580P35'-TAATACCTGGAGGGAATAATGCCGCTCTCATATCAAACACA-3'Hp0580P45'-GCGCGCCTCTAGAAGCGAACTGGATAACGCTAC-3'Hp0579P35'-TAATACCTGGAGGGAATAATGCCAGTTCGCTTTTGATGCG-3'Hp0579P35'-CGCGCGCTCTAGAAGCGAACTGGATAACGCTAC-3'Hp0579P45'-GCGCGCCTCTAGAATCGTGAGCCGCCCGCCCTGCCACTC-3'F580cmp5'-GCGCGCGGAATTCTCACTGAGGAGGGATTTACGCCCCGCCCTGCCACTC-3'F580cmp5'-GCGCGCGGAATTCTCACTTGAGGAGGGATTTTAA-3'R579comp5'-GCGCGCCGAATTCTTAATACAACCTTTTTTTTAA-3'FphoA5'-GCGCGCCCTAGGCTGTTGACAACAF580phoA5'-GCGCGCCCTAGGCTGTAGGATGCCTGAGAAAT-3'F580phoA5'-GCGCGCCCTAGGCTGTTAACCCCTGTGACA-3'FphoAfus5'-GCGCGCCATATGCAGCTTCAAGAAAT-3'F580phoApet5'-GCGCGCCATATGCACTTCAAGAAAT-3'F580phoApet5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCCCCATAGGAGGCGACCTTCAAGAAAT-3'F580ghp5'-GCGCGCCAAGCTGGAACCTTCAAGAAAT-3'F580ghp5'-GCGCCCAAGCTGGAATTCA-3'F580ghp5'-GCGCCCAGTTGGAACCTTCAAGAAAT-3'F580ghp5'-GCGCCCCAGTTGGAATTCA-3'F580ghp5'-GCGCCCCAGTTGGAATTCA-3'F580gfp5'-GCGCCCCAGTTGGAATTCA-3'F580gfp5'-GCGCCCCAGTTGGAATTCA-3'F580gfp5'-GCGCCCCAGTTGGAATTCA-3'F580g	Name	Sequence
Fhp0580 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Rhp0580 5'-GCGCGCCGGATCCTCACTTGAGGAGGGATTT-3'   Hp0580P1 5'-GCGCGCCTCGAGCATGACAATCTCTATGCGAC-3'   Hp0580P2 5'-CTTAGCTCCTGAAAATCTCGGAAAGCCCCACAAAAAAGGCG-3'   Hp0580P3 5'-TAATACCTGGAGGGAATAATGCCGCTCTCATATCAAACACA-3'   Hp0580P4 5'-GCGCGCTCTAGAAGCGAACTGGATAACGCTAC-3'   Hp0580P3 5'-TAATACCTGGAGGGAATAATGCCGCTCTCATATCAAACACA-3'   Hp0570P3 5'-TAATACCTGGAGGGAATAATGTCCAGTTAGCGTAC-3'   Hp0570P4 5'-GCGCGCTCTAGAATCGTGAGCGTGTCTTCATG-3'   CamF 5'-CCGAGATTTTCAGGAGCTAAG-3'   CamR 5'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'   R580comp 5'-GCGCGCGGAATTCTCACTTGAGGAGGGATTTTAA-3'   R580comp 5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCGCCATATGCTGTTACCCCTGTGACA-3'   F580phoA 5'-GCGCGCCTAGGCTTGAGGATGGAACAT-3'   F580phoA 5'-GCGCCCTAGGCTAGGCTTGAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCCCTAGGCTAGGACTTCAAGAAAT-3'   FphoAfus 5'-GCGCCCTAGGCTAGGACCTTCAAGAAAT-3'   FphoAfus 5'-GCGCCCCTAGGAGCGACCTCAAGAAAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GC	Fhp0579	5'-GCGCGC <u>CATATG</u> CTTATATCTTCTTCT-3'
Rhp0580 5'-GCGCGCGGATCCTCACTTGAGGAGGGATTT-3'   Hp0580P1 5'-GCGCGCCTCGAGCATGACAATCTCTATGCGAC-3'   Hp0580P2 5'-CTTAGCTCCTGAAAATCTCGGAAAGCCCCACAAAAAAGGCG-3'   Hp0580P3 5'-TAATACCTGGAGGGAATAATGCCGCTCTCATATCAAACACAC-3'   Hp0580P4 5'-GCGCGCTCTAGAAGCCGAACTGGATAACGCTAC-3'   Hp0580P4 5'-GCGCGCTCTAGAAGCGAACTGGATAACGCTAC-3'   Hp0579P3 5'-TAATACCTGGAGGGAATAATGTCCAGTTCGCTTTGATGCG-3'   Hp0579P4 5'-GCGCGCCTCTAGAATCGTGAGCGTGTCTTCATG-3'   CamF 5'-CCATTATTCCCTCCAGGTATTACGCCCGCCCTGCCACTC-3'   F580comp 5'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'   R580comp 5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'   R579comp 5'-GCGCGCGAATTGCTATGACACCTTTTATAAC-3'   F580cphoA 5'-GCGCGCCTTAGATTGCACTTGAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCGCCTAGGCTGTTGACACGTTGAGAAAT-3'   F580phoA 5'-GCGCGCCTAGGCTGTTGACCCTGTGACA-3'   FphoAfus 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   F580phoA 5'-GCGCGCCATAGGCTGTTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fs80phoApet 5'-GCGCGCCATAGGAGGTCGACTCTAAGAAAT-3'   Fgfp 5'-GCGCGCCATAGCAGGTCGACTCTAAGAGAAT-3'	Rhp0579	5'-GCGCGC <u>GGATCC</u> TTAATACAACCTTTTTT-3'
Hp0580P1 5'-GCGGC <u>CTCGAG</u> CATGACAATCTCTATGCGAC-3'   Hp0580P2 5'-CTTAGCTCCTGAAAATCTCGGAAAGCCCCACAAAAAAGGCG-3'   Hp0580P3 5'-TAATACCTGGAGGGAATAATGCCGCTCTCATATCAAACACA-3'   Hp0580P4 5'-GCGCGCTCTAGAAGCGAACTGGATAACGCTAC-3'   Hp0579P3 5'-TAATACCTGGAGGGAATAATGTCCAGTTCGCTTTTGATGCG-3'   Hp0579P4 5'-GCGCGCTCTAGAACGGAACTGGAGCGTGTCTTCATG-3'   CamF 5'-CCGAGATTTTCAGGAGCTAAG-3'   CamR 5'-GCGCGCGGATCCCAGTAAAAAAGCGCCTTGCCACTC-3'   F580comp 5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'   R580comp 5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCGCCAATGCTGTTTACCCCTGTGACA-3'   F580phoA 5'-GCGCGCCTAGGCTGTTGACCAGGAAAT-3'   F580phoA 5'-GCGCGCCTAGGCTATGCCAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCGCCTAGGCTGAGGCTGTGAGAAAT-3'   FphoAfus 5'-GCGCGCCTAGGCTGTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   FphoAfus 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCGCCATATG	Fhp0580	5'-GCGCGC <u>CATATG</u> GAACCTTCAAGAAAT-3'
Hp0580P2 5'-CTTAGCTCCTGAAAATCTCGGAAAGCCCCACAAAAAAGGCG-3'   Hp0580P3 5'-TAATACCTGGAGGGAATAATGCCGCTCTCATATCAAACACA-3'   Hp0580P4 5'-GCGCGCCTCTAGAAGCGAACTGGATAACGCTAC-3'   Hp0579P3 5'-TAATACCTGGAGGGAATAATGTCCAGTTCGCTTTGATGCG-3'   Hp0579P4 5'-GCGCGCTCTAGAACGTGAGCGTGTCTTCATG-3'   CamF 5'-CCGAGATTTTCAGGAGCTAAG-3'   CamR 5'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'   F580comp 5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'   R580comp 5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'   R579comp 5'-GCGCGCCATATGCTGTTACCCCTGTGACA-3'   FphoA 5'-GCGCGCCTTAGGATTGGCATTCAAGAAAT-3'   R580phoA 5'-GCGCGCCTAGGCTTAGGCTTGAGGAGGGATTTTAA-3'   FphoAfus 5'-GCGCCCTAGGCTGTTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCGCCTAGGCTTAGGCTTGAGGAGGGATTTTAA-3'   FphoAfus 5'-GCGCCCTAGGCTGTTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCGCCTAGGCTGTTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fpfp 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCGCCCATATGGAACCTTCAAGAGAAT-3'   Fgfp 5'-GCGCCCATATGG	Rhp0580	5'-GCGCGC <u>GGATCC</u> TCACTTGAGGAGGGATTT-3'
Hp0580P3 5'-TAATACCTGGAGGGAATAATGCCGCTCTCATATCAAACACA-3'   Hp0580P4 5'-GCGCGCTCTAGAAGCGGAACTGGATAACGCTAC-3'   Hp0579P3 5'-TAATACCTGGAGGGAATAATGTCCAGTTCGCTTTTGATGCG-3'   Hp0579P4 5'-GCGCGCTCTAGAATCGTGAGCGTGTCTCATG-3'   CamF 5'-CCGAGATTTTCAGGAGCGTAAG-3'   CamR 5'-CCGCGCGGATCCCAGGTATTACGCCCGCCCTGCCACTC-3'   F580comp 5'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'   R580comp 5'-GCGCGCGAATTCTCACATGAGGAGGGATTTTAA-3'   R579comp 5'-GCGCGCGAATTCGTATACAACCTTTTTTAAC-3'   F580phoA 5'-GCGCGCCATATGCTGTGACAA-3'   F580phoA 5'-GCGCGCCTAGGCTGTGAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCCCTAGGCTGTAGACCTTCAAGAAAT-3'   FphoAfus 5'-GCGCCCTAGGCTGTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   FphoAfus 5'-GCGCCCATAGGCTGTTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fg80phoApet 5'-GCGCCCAGTAGCAGCTGCAACTTCAAGAAAT-3'	Hp0580P1	5'-GCGCGC <u>CTCGAG</u> CATGACAATCTCTATGCGAC-3'
Hp0580P4 5'-GCGCGCTCTAGAAGCGAACTGGATAACGCTAC-3'   Hp0579P3 5'-TAATACCTGGAGGGAATAATGTCCAGTTCGCTTTTGATGCG-3'   Hp0579P4 5'-GCGCGCTCTAGAATCGTGAGCGTGTCTTCATG-3'   CamF 5'-CCGAGATTTCAGGAGCTAAG-3'   CamR 5'-GCGCGCGGATCCCAGGTATTACGCCCGCCCTGCCACTC-3'   F580comp 5'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'   R580comp 5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'   R579comp 5'-GCGCGCGAATTCGTCATGACACCTTTTTTAAC-3'   F580phoA 5'-GCGCGCCATATGCTGTTACCCCTGTGACA-3'   F580phoA 5'-GCGCGCCTGCAGCTGAGCTTGAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCCCTAGGCTGTTGCCCAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCCCTGCAGCCTAGGCTTGAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   F580phoA 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   F580phoApet 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   F580phoAppet 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   F580phoApet 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   F580phoApet 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   F360phoApet 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   F360phoApet 5'-GCGCCCAAGCTGCAACTTCAAGAAAT-3'   F360phoApet 5'	Hp0580P2	5'- <b>CTTAGCTCCTGAAAATCTCGG</b> AAAGCCCCACAAAAAAGGCG-3'
Hp0579P3 S'-TAATACCTGGAGGGAATAATGTCCAGTTCGCTTTTGATGCG-3'   Hp0579P4 S'-GCGCGC <u>TCTAGA</u> ATCGTGAGCGTGTCTTCATG-3'   CamF S'-CCGAGATTTTCAGGAGCTAAG-3'   CamR S'-CATTATTCCCTCCAGGTATTACGCCCCGCCCTGCCACTC-3'   F580comp S'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'   R580comp S'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'   R579comp S'-GCGCGCCAATTGCTGTAAAAAAGCCTTTTTTAAC-3'   FphoA S'-GCGCGCCATATGCTGTTACCCCTGTGACA-3'   FphoA S'-GCGCGCCTAGGCTGTGGAACCTTCAAGAAAT-3'   F580phoA S'-GCGCGCCTAGGCTGTTTACCCCTGTGACA-3'   FphoAfus S'-GCGCGCCTAGGCTGTTTACCCCTGTGACA-3'   FphoAfus S'-GCGCGCCATAGGCTGTTTACCCCTGTGACA-3'   FphoAfus S'-GCGCGCCATAGGCTGTTTACCCCTGTGACA-3'   FphoAfus S'-GCGCGCCATAGGCTGTTTACGCCCAGAGGAGATTTTAA-3'   FphoAfus S'-GCGCGCCATAGGACCTTCAAGAAAT-3'   Fgfp S'-GCGCGCCATAGGACCTTCAAGAAAT-3'   Fgfp S'-GCGCGCCATAGGAGGCGACTCTAGAGAGAT-3'   Fgfp S'-GCGCGCCATAGGAGCGACCTTCAAGAGAAT-3'   Fgfp S'-GCGCGCCATAGCAGGTCGACTCTAGAGAGAT-3'   Fgfp S'-GCGCGCCATAGCAGTGGACCTTCAAGAGAAT-3'   Fgfp S'-GCGCCCAGTTGGAATTCA-3'	Hp0580P3	5'- <b>TAATACCTGGAGGGAATAATG</b> CCGCTCTCATATCAAACACA-3'
Hp0579P4 5'-GCGCGCTCTAGAATCGTGAGCGTGTCTTCATG-3'   CamF 5'-CCGAGATTTTCAGGAGCTAAG-3'   CamR 5'-CATTATTCCCTCCAGGTATTACGCCCCGCCCTGCCACTC-3'   F580comp 5'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'   R580comp 5'-GCGCCGCGAATTCTCACTGAGGAGGGATTTTAA-3'   R579comp 5'-GCGCGCGAATTCTTAATACAACCTTTTTTAAC-3'   FphoA 5'-GCGCCGCATATGCTGTTACCCCTGTGACA-3'   FphoA 5'-GCGCCGCTAGGCTTAGGCTTGAGGAGGGATTTTAA-3'   F580phoA 5'-GCGCCCTAGGCTGTAGGCTTGAGGAGGGATTTTAA-3'   FphoAfus 5'-GCGCCCTAGGCTGTTGACGAGGGAGTTTTAA-3'   FphoAfus 5'-GCGCCCTAGGCTGTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCCCTAGGCTGTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCCCTAGGCTGTTTACCCCTGTGACA-3'   FphoAfus 5'-GCGCCCTAGGCTGTTACCCCTGTGACA-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCCCTAGCAGGTCGACTCTAGAGGAT-3'   Fgfp 5'-GCGCCCATATGGAATTCA-3'   Fgfp 5'-GCGCCCATAGGAGTCGACTTCAAGAGAT-3'   Fgfp 5'-GCGCCCATAGCAGTGCGACTTCAAGAGAAT-3'   Fgfp 5'-GCGCCCAGTAGCAGTCGACTTCAAGAGAAT-3'   F	Hp0580P4	5'-GCGCGC <u>TCTAGA</u> AGCGAACTGGATAACGCTAC-3'
CamF 5'-CCGAGATTTTCAGGAGCTAAG-3'   CamR 5'-CATTATTCCCTCCAGGTATTACGCCCCGCCCTGCCACTC-3'   F580comp 5'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'   R580comp 5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'   R579comp 5'-GCGCGCGAATTCTTAATACAACCTTTTTTAAC-3'   FphoA 5'-GCGCGCCATATGCTGTTACCCCTGTGACA-3'   FphoA 5'-GCGCGCCTAGGTTGGAACCTTCAAGAAAT-3'   F580phoA 5'-GCGCGCCTAGGCTAGGCTTGAGGAGGGATTTTAA-3'   FphoAfus 5'-GCGCGCCTAGGCTTGAGGCTGGACA-3'   FphoAfus 5'-GCGCGCCTAGGCTTGAGGCTGGACA-3'   F580phoAph 5'-GCGCGCCATATGGAACCTTCAAGAAGGGATTTTAA-3'   FphoAfus 5'-GCGCGCCAAGCTTTTATTCCACCCCAGAGC-3'   FphoAfus 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAAGGAT-3'   Fgfp 5'-GCGCCCATATGGAACCTTCAAGAGAGAT-3'   Fgfp 5'-GCGCCCATATGGAATTCA-3'   Fgfp 5'-GCGCCCAGGTGGAATTCA-3'   Fgfp 5'-GCGCCCAGGTGGAATTCA-3'   Fj-GCGCCCAGGTGGAATTCA-3'	Hp0579P3	5'- <b>TAATACCTGGAGGGAATAATG</b> TCCAGTTCGCTTTTGATGCG-3'
CamR5'-CATTATTCCCTCCAGGTATTACGCCCCGCCCTGCCACTC-3'F580comp5'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'R580comp5'-GCGCGCGGAATTCTCACTTGAGGAGGGATTTTAA-3'R579comp5'-GCGCGCGCAATTCTTAATACAACCTTTTTTAAC-3'FphoA5'-GCGCGCCCATATGCTGTTTACCCCTGTGACA-3'F580phoA5'-GCGCGCCTCTAGATTGGAACCTTCAAGAAAT-3'R580phoA5'-GCGCGCCCTAGGCTGTAGGCTGTGAGGAGGGATTTTAA-3'FphoAfus5'-GCGCGCCCTAGGCTGTTACCCCTGTGACA-3'FphoAfus5'-GCGCGCCCTAGGCTGTTTACCCCTGTGACA-3'FphoAfus5'-GCGCGCCCTAGGCTGTTTACCCCTGTGACA-3'FphoAfus5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCGCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCCGCGCTAGCAGGTCGACTCTAGAGGAT-3'Fgfp5'-GCGCCCCAGTTGGAATTCA-3'Fgfp5'-GCGCCCAGTTGGAATTCA-3'F580gfp5'-GCGCCCAAGCTTGCTAGCTTGGAACCTTCAAGAAAT-3'	Hp0579P4	5'-GCGCGC <u>TCTAGA</u> ATCGTGAGCGTGTCTTCATG-3'
F580comp5'-GCGCGCGGATCCCAGTAAAAAAGCGCGTTTGTC-3'R580comp5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'R579comp5'-GCGCGCGAATTCTTAATACAACCTTTTTTAAC-3'FphoA5'-GCGCGCCATATGCTGTTACCCCTGTGACA-3'F580phoA5'-GCGCGCCTAGGATTGGAACCTTCAAGAAAT-3'FphoAfus5'-GCGCGCCTAGGCTGTTACCCCTGTGACA-3'FphoAfus5'-GCGCGCCATATGCAGCTTCAAGAAAT-3'F580phoApet5'-GCGCGCCTAGGCTGTTACCCCTGTGACA-3'Fgfp5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCCAGCAGGTCGACTCTAGAGGAT-3'Fgfp5'-GCGCGCAAGCTTGGAATCA-3'Fgfp5'-GCGCCCAGGCAGGTCGACTCTAGAGGAT-3'Fgfp5'-GCGCCAAGCTTGGAATTCA-3'Fgfp5'-GCGCCAAGCTTGGAATTCA-3'Fgfp5'-GCGCCAAGCTTGGAATTCA-3'Fgfp5'-GCGCCAAGCTTGGAATTCA-3'Fgfp5'-GCGCCAAGCTTGGAATTCA-3'Fgfp5'-GCGCCAAGCTTGGAATTCA-3'F580gfp5'-GCGCCAAGCTTGCTAGCGCTTGGAACCTTCAAGAAAT-3'	CamF	5'-CCGAGATTTTCAGGAGCTAAG-3'
R580comp5'-GCGCGCGAATTCTCACTTGAGGAGGGATTTTAA-3'R579comp5'-GCGCGCGAATTCTTAATACAACCTTTTTTAAC-3'FphoA5'-GCGCGCCATATGCTGTTACCCCTGTGACA-3'F580phoA5'-GCGCGCTCTAGATTGGAACCTTCAAGAAAT-3'R580phoA5'-GCGCGCCTGCAGCCTAGGCTTGAGGAGGGATTTTAA-3'FphoAfus5'-GCGCGCCTAGGCTGTTACCCCTGTGACA-3'FphoAfus5'-GCGCGCCAAGCTTTTATTTCAGCCCCAGAGC-3'F580phoApet5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCCATATGGAATTCA-3'Fgfp5'-GCGCGCCATATGGAATTCA-3'Fgfp5'-GCGCCCATATGGAATTCA-3'	CamR	5'-CATTATTCCCTCCAGGTATTACGCCCCGCCCTGCCACTC-3'
R579comp5'-GCGCGCGAATTCTTAATACAACCTTTTTTAAC-3'FphoA5'-GCGCGCCATATGCTGTTACCCCTGTGACA-3'F580phoA5'-GCGCGCCTAGATTGGAACCTTCAAGAAAT-3'R580phoA5'-GCGCGCCTGCAGCCTAGGCTGGAGGGGATTTTAA-3'FphoAfus5'-GCGCGCCCTAGGCTGTTACCCCTGTGACA-3'RphoAfus5'-GCGCGCCAAGCTTTATTCAGCCCCAGAGC-3'F580phoApet5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCCAAGCTGAGGACCTTCAAGAAAT-3'Fgfp5'-GCGCCGCAAGCTGACCTTCAAGAAAT-3'Fgfp5'-GCGCCGCAAGCTTGAACACTTCAAGAAAT-3'Fgfp5'-GCGCCCAAGCTTGGAATTCA-3'F580gfp5'-GCGCCCAAGCTTGCTAGCTTGGAACCTTCAAGAAAT-3'	F580comp	5'-GCGCGC <u>GGATCC</u> CAGTAAAAAGCGCGTTTGTC-3'
FphoA5'-GCGCGCCATATGCTGTTACCCCTGTGACA-3'F580phoA5'-GCGCGCTCTAGATTGGAACCTTCAAGAAAT-3'R580phoA5'-GCGCGCCTGCAGCCTAGGCTTGAGGAGGGATTTTAA-3'FphoAfus5'-GCGCGCCCTAGGCTGTTACCCCTGTGACA-3'RphoAfus5'-GCGCGCAAGCTTTATTTCAGCCCCAGAGC-3'F580phoApet5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCGCAGGCTAGGATCGACTCTAGAGGAT-3'Rgfp5'-GCGCCCAAGCTTGCAAGCTTCAAGAAAT-3'F580gfp5'-GCGCCCAAGCTTGCTAGCTTGGAACCTTCAAGAAAT-3'	R580comp	5'-GCGCGC <u>GAATTC</u> TCACTTGAGGAGGGATTTTAA-3'
F580phoA5'-GCGCGCTCTAGATTGGAACCTTCAAGAAAT-3'R580phoA5'-GCGCGCCTGCAGCCTAGGCTTGAGGAGGGATTTTAA-3'FphoAfus5'-GCGCGCCCTAGGCTGTTTACCCCTGTGACA-3'RphoAfus5'-GCGCGCAAGCTTTTATTTCAGCCCCAGAGC-3'F580phoApet5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCGCAGGTCGACTCTAGAGGAT-3'Rgfp5'-GCGCTCAGTTGGAATTCA-3'F580gfp5'-GCGCCAAGCTTGCTAGCTTGGAACCTTCAAGAAAT-3'	R579comp	5'-GCGCGC <u>GAATTC</u> TTAATACAACCTTTTTTTAAC-3'
R580phoA5'-GCGCGC <u>CTGCAGCCTAGG</u> CTTGAGGAGGGATTTTAA-3'FphoAfus5'-GCGCGC <u>CCTAGG</u> CTGTTTACCCCTGTGACA-3'RphoAfus5'-GCGCGC <u>AAGCTT</u> TTATTTCAGCCCCAGAGC-3'F580phoApet5'-GCGCGC <u>CATATG</u> GAACCTTCAAGAAAT-3'Fgfp5'-GCGCGC <u>GCTAGC</u> AGGTCGACTCTAGAGGAT-3'Rgfp5'-GCGCCCCATGTGGAATTCA-3'F580gfp5'-GCGCGC <u>AAGCTTGCTAGC</u> TTGGAACCTTCAAGAAAT-3'	FphoA	5'-GCGCGC <u>CATATG</u> CTGTTTACCCCTGTGACA-3'
FphoAfus5'-GCGCGC <u>CCTAGG</u> CTGTTTACCCCTGTGACA-3'RphoAfus5'-GCGCGC <u>AAGCTT</u> TTATTTCAGCCCCAGAGC-3'F580phoApet5'-GCGCGC <u>CATATG</u> GAACCTTCAAGAAAT-3'Fgfp5'-GCGCGC <u>GCTAGC</u> AGGTCGACTCTAGAGGAT-3'Rgfp5'-GCGCTCAGTTGGAATTCA-3'F580gfp5'-GCGCGC <u>AAGCTTGCTAGC</u> TTGGAACCTTCAAGAAAT-3'	F580phoA	5'-GCGCGC <u>TCTAGA</u> TTGGAACCTTCAAGAAAT-3'
RphoAfus5'-GCGCGCAAGCTTTTATTTCAGCCCCAGAGC-3'F580phoApet5'-GCGCGCCATATGGAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCGCTAGCAGGTCGACTCTAGAGGAT-3'Rgfp5'-GCGCTCAGTTGGAATTCA-3'F580gfp5'-GCGCGCAAGCTTGCTAGCACCTTCAAGAAAT-3'	R580phoA	5'-GCGCGC <u>CTGCAGCCTAGG</u> CTTGAGGAGGGATTTTAA-3'
F580phoApet5'-GCGCGC <u>CATATG</u> GAACCTTCAAGAAAT-3'Fgfp5'-GCGCGCGC <u>GCTAGC</u> AGGTCGACTCTAGAGGAT-3'Rgfp5'-GCGCTCAGTTGGAATTCA-3'F580gfp5'-GCGCGC <u>AAGCTTGCTAGC</u> TTGGAACCTTCAAGAAAT-3'	FphoAfus	5'-GCGCGC <u>CCTAGG</u> CTGTTTACCCCTGTGACA-3'
Fgfp5'-GCGCGCGCGCTAGCAGGTCGACTCTAGAGGAT-3'Rgfp5'-GCGCTCAGTTGGAATTCA-3'F580gfp5'-GCGCGCAAGCTTGCTAGCTTGGAACCTTCAAGAAAT-3'	RphoAfus	5'-GCGCGC <u>AAGCTT</u> TTATTTCAGCCCCAGAGC-3'
Rgfp5'-GCGCTCAGTTGGAATTCA-3'F580gfp5'-GCGCGCAAGCTTGCTAGCF580gfp5'-GCGCGCAAGCTTGCTAGC	F580phoApet	5'-GCGCGC <u>CATATG</u> GAACCTTCAAGAAAT-3'
F580gfp 5'-GCGCGC <u>AAGCTTGCTAGC</u> TTGGAACCTTCAAGAAAT-3'	Fgfp	5'-GCGCGC <u>GCTAGC</u> AGGTCGACTCTAGAGGAT-3'
	Rgfp	5'-GCGCTCAGTTGGAATTCA-3'
R580gfp 5'-GCGCGC <u>TCTAGA</u> CTTGAGGAGGGATTTTAA-3'	F580gfp	5'-GCGCGC <u>AAGCTTGCTAGC</u> TTGGAACCTTCAAGAAAT-3'
	R580gfp	5'-GCGCGC <u>TCTAGA</u> CTTGAGGAGGGATTTTAA-3'

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Restriction enzyme sequences are <u>underlined</u> Cam cassette complimentary sequence is in **bold** RBS and start codon sequences are in *italics* 

## Francisella tularensis

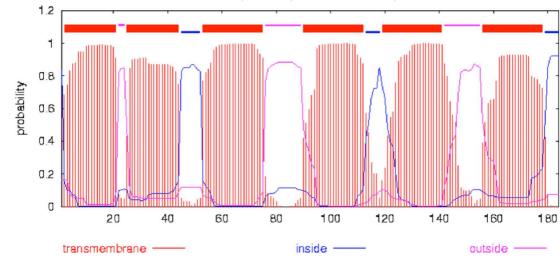


# Helicobacter pylori



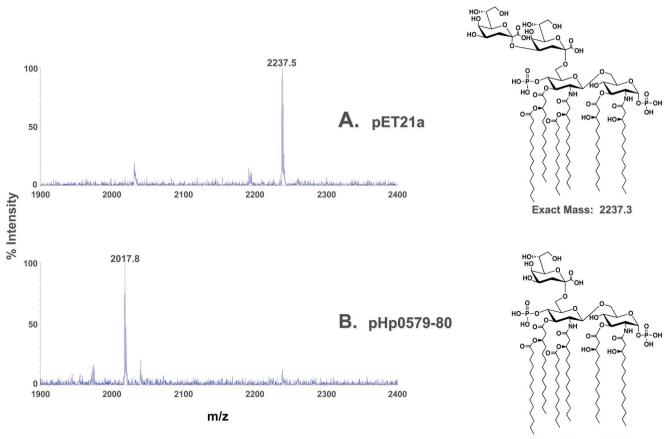


### TMHMM posterior probabilities for Sequence

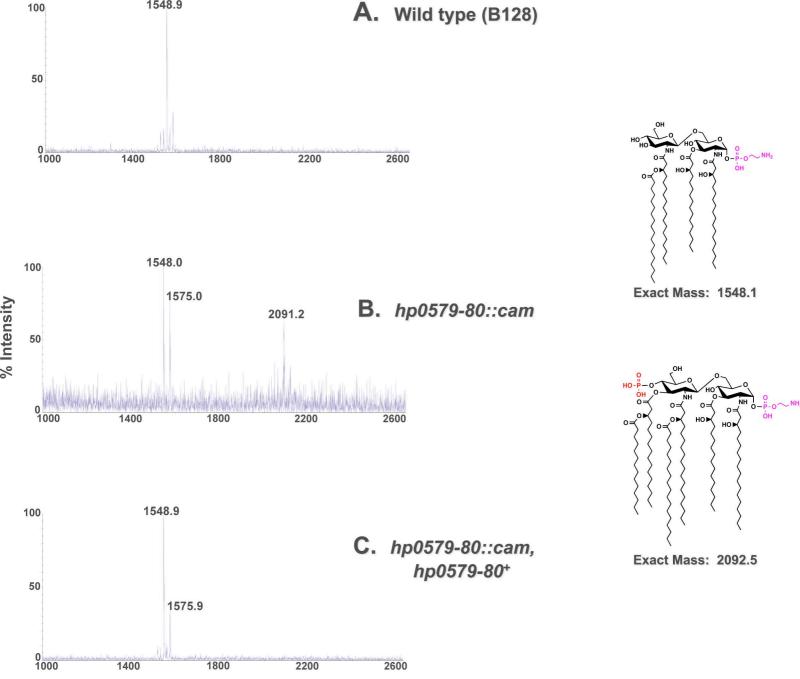




#### TMHMM posterior probabilities for Sequence 1.2 1 0.8 probability 0.6 0.4 0.2 ٥ 50 100 150 200 250 300 350 inside · transmembrane outside



Exact Mass: 2017.3



m/z

