Supporting Information

A Two-component Kdo Hydrolase in the Inner Membrane of *Francisella novicida*

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Fig. S1. Preparation of labeled substrates used to assay the Kdo hydrolase and products generated by the hydrolase. The schematic versions for these structures are used throughout the manuscript. The ^{32}P atom is introduced by using the *E. coli* LpxK 4′-kinase. The stereochemistry of the released Kdo is not known. Color scheme: *white boxes*, Kdo; *blue*, glucosamine; *red*, phosphate groups; *green*, acyl chain.

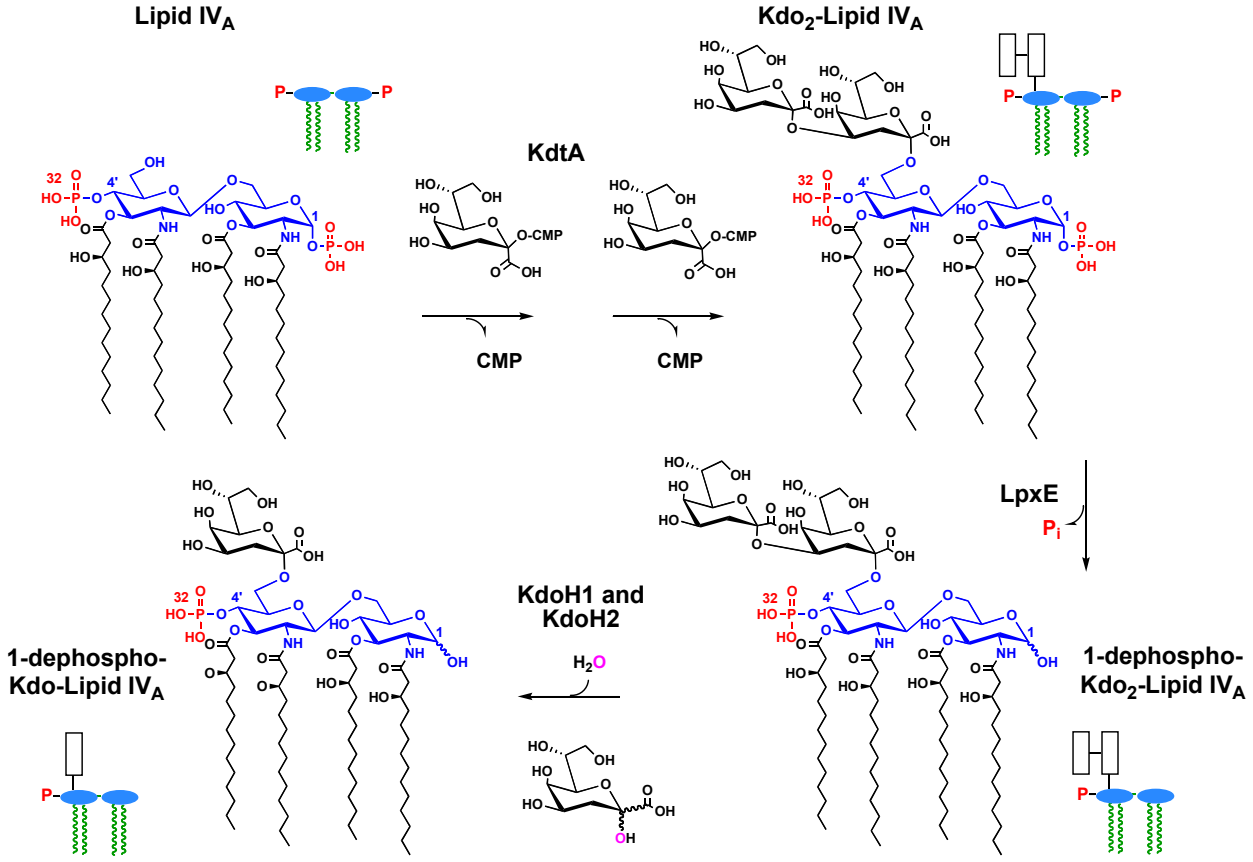

Figure S2A. Electrophoresis of LPS from *F. novicida* wild-type and mutants.
Overnight cultures of the *F. novicida* wild-type, the *kdtA* deficient strain, and the hydrolase mutant JZ-H1H2 were used to extract the LPS using a commercial kit (iNtRON Biotechnology). LPS samples were separated on 4-20% gradient SDS-PAGE gel and stained with the Pro-Q Emerald 300 LPS Gel Stain Kit (Molecular Probes). LPS from *E. coli* serotype 055:B5 was provided with the stain kit.

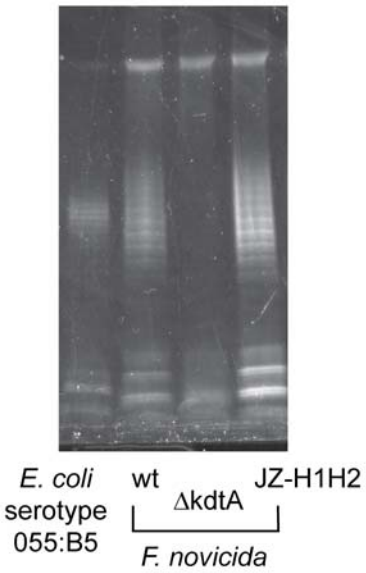

Figure S2B. TLC analysis of free lipid A from *F. novicida* wild-type and mutants. The lipids of overnight cultures of the *F. novicida* wild-type, the *kdtA* deficient strain, and the hydrolase mutant JZ-H1H2 were extracted by Bligh-Dyer method, and portions were subjected to silica TLC analysis in the solvent chloroform, methanol, pyridine, acetic acid, water (25:15:5:4:3, v/v/v/v/v). After development the plate was dried, sprayed with10 % sulfuric acid in ethanol, and charred. Species A1 (Fig. 1) and A2 are the major and minor forms of free lipid A of *F. novicida*; A2 is the same as A1 but contains an extra glucose unit at position 6′.

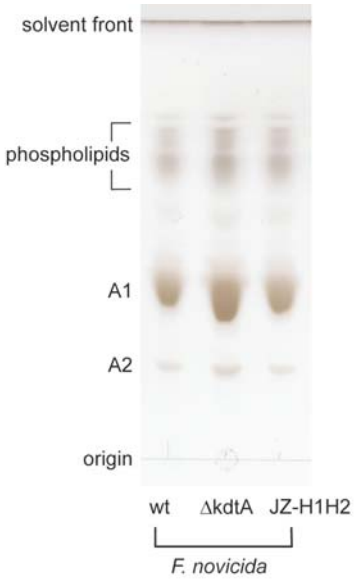

Fig. S3A. PSI-BLAST alignment of *F. novicida* KdoH1 with the crystallized *C. perfringens* NanI protein after 2 iterations with 5000 sequences. The structure of the *Clostridium perfringens* NanI sialidase was determined by Newstead et al. *J. Biol. Chem.* **283**: 9080-9088. The active site arginine residues that interact with the carboxyl group of bound sialic acid in the NanI crystal structure are highlighted in yellow. These residues appear to be conserved in KdoH1, suggesting that they may be part of the KdoH1 active site.

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Length=452
Score = 63.5 bits (153),  Expect = 4e-08, Method: Composition-based stats.
Identities = 42/198 (21%), Positives = 74/198 (37%), Gaps = 38/198 (19%)

FnKdoH1   152  TSSLNIMYSDDLGKTWSQPKTI---LSSNILNFSTLTRGAAIELDN----NRFAIPVYK-  203
               TS +N++YSDD GKTWS+P+ I   +  + + F  +  G  I++ N     R  +PVY
CpNanI    186  TSYINLVYSDDDGKTWSEPQNINFQVKKDWMKFLGIAPGRGIQIKNGEHKGRIVVPVYYT  245

FnKdoH1   204  -EFNNLNGRWFVFNKDGELIFVSEMTNDGVNLQ--------------P-----TVVPLSK  243
                E    +       +  G+   + E  ND   L+               P      VV +
CpNanI    246  NEKGKQSSAVIYSDDSGKNWTIGESPNDNRKLENGKIINSKTLSDDAPQLTECQVVEMPN  305

FnKdoH1   244  THALALYRQMHSPIKRIYTNETSDSGLSWSKV--KPTQLDNPDSGIAAIKIQ-----NGI  296
                     R +   +       + D G +W +   K T +  P   ++ I
CpNanI    306  GQLKLFMRNLSGYLN---IATSFDGGATWDETVEKDTNVLEPYCQLSVINYSQKVDGKDA  362

FnKdoH1   297  LLAYNNATDSRADLSLAF  314
               ++  N    SR++ ++
CpNanI    363  VIFSNPNARSRSNGTVRI  380
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Fig. S3B. PSI-BLAST alignment of *F. novicida* KdoH1 and *H. pylori* J99 KdoH1 after 2 iterations with 5000 sequences. The functional analysis of *H. pylori* KdoH1 and the demonstration of its Kdo trimming activity in conjunction with *H. pylori* KdoH2 is presented in the accompanying manuscript.

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Score =  276 bits (707),  Expect = 2e-72, Method: Composition-based stats.
Identities = 80/376 (21%), Positives = 147/376 (39%), Gaps = 30/376 (7%)

FnKdoH1   2    KHKLKLVLLFAFIYLILLLVFYYSR-----IQHNYSFTISTPR---NDSITKNLDIKTIA  53
               +++LK    F  ++++L L+    +       HN +    TP      +I K  D
HpKdoH1   5    RNRLKHAAFFVGLFIVLFLIIMKHQTSPYAFTHNQALVTQTPPYFTQLTIPKPNDAL---  61

FnKdoH1   54   NLKYFKYNHASSMTTIDNK-LFITWYSSDQETAPNTKIVVAIAEKVAGKWHFNEIKPVMN  112
                      HASS+ ++ N  L   ++S  +E A + KI   + +    +W   E   ++
HpKdoH1   62   ------SAHASSLISLPNDNLLSAYFSGTKEGARDVKISANLFDSKTNRWS--EAFILLT  113

FnKdoH1   113  RQEFQSIFKKHIHHLGNPIIYSQAKRLWLVF-TSSSGGWVTSSLNIMYSDDLGKTWSQPK  171
               ++E      ++I  LGNP+++    ++ L     S GGW TS +    S      +   +
HpKdoH1   114  KEELSHHSHEYIKKLGNPLLFLHDNKILLFVVGVSMGGWATSKIYQFESALEPIHFKFAR  173

FnKdoH1   172  TILSSNILNFSTLTRGAAIELDNNRFAIPVYKEFNNLNGRWFVFNKDGELIFVSEMTNDG  231
                +  S  LN S L R   +   +  F +P+Y E          F++      +
HpKdoH1   174  KLSLSPFLNLSHLVRNKPLNTTDGGFMLPLYHELATQYPLLLKFDQQNNPRELLRPNTLN  233

FnKdoH1   232  VNLQPTVVPLSKTHALALYRQMHSPIKRIYTNETSDSGLSWSKVKPTQLDNPDSGIAAIK  291
                 LQP++ P      +A     +   K     ET  +   W K   T L N D  +  +
HpKdoH1   234  HQLQPSLTPFKDCAVMAF---RNHSFKDSLMLETCKTPTDWQKPISTNLKNLDDSLNLLN  290

FnKdoH1   292  IQNGILLAYNNATDS--RADLSLAFKADNSQQWRNIYTFPNKIKGEFSYPAFTLYQDNII  349
               +   + L +N +  S  R +L L+ K +NS  ++ +         E SYP+++L   + I
HpKdoH1   291  LNGILYLIHNPSDLSLRRKELWLS-KLENSNSFKTLKVLDK--ANEVSYPSYSLNP-HFI  346

FnKdoH1   350  LAFSDKTKGTIRIVEI  365
                    +  I+ +
HpKdoH1   347  DIVYTYNRSHIKHIRF  362
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Fig. S4. Reconstitution of Kdo hydrolase activity by mixing of *Francisella novicida* KdoH1 and KdoH2 mutant membranes in the presence of a non-ionic detergent. Membranes from the *F. novicida* U112 wild-type (lane 2), mutant JZ-H1 (lane 3), mutant JZ-H2 (lane 4), or the mixture of JZ-H1 and JZ-H2 (lane 6) were used as the enzyme source at 0.1 mg/mL under the standard Kdo hydrolase assay conditions, which include 0.1 % Triton X-100. *E. coli* W3110/pWSK29 membranes (lane 5) were used as a negative control. Lane 1 is a no enzyme control. The reactions proceeded at 30 °C for 25 hours, and then the lipids were separated by TLC in the solvent chloroform, pyridine, 88% formic acid, water (30:70:16:10, v/v/v/v) as in Fig. 6 of the main text.

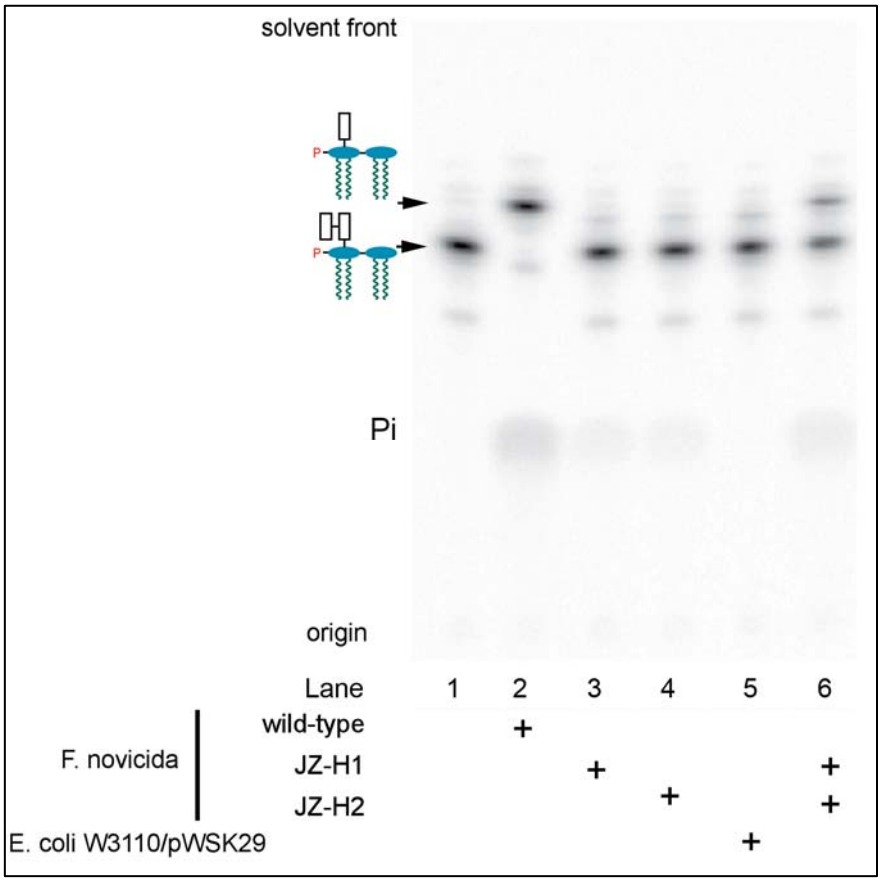

Table S1. PCR primers.

Primer name	Description
nKdtA_for	5′-CCGGGGAGCTCAGGAGGTTAAAGA*GTG*GAACATTTAAAA-3′ Forward primer for cloning *FnkdtA* into pBAD33 vector: a clamp region, a SacI restriction site (underlined), a ribosome binding site with a spacer, and the original *kdtA* coding sequence starting with the GTG (italic and underlined)
nKdtA_rev	5′-CCCGGCTGCAGGTCGACTTTTATTATATTTTCACTAC-3′ Reverse primer for cloning *FnkdtA* into pBAD33 vector: a clamp region, a PstI restriction site (underlined), and matching anti-coding strand downstream of *FnkdtA*.
nMpH1_for	5′-GCGCGCACGCGTTATAGCTTTTTGAGAATATTCAACTTGTTG TGTC-3′ Forward primer for cloning *FnkdoH1* or *FnkdoH1H2* into pMP529 vector: a clamp region, a MluI restriction site (underlined), and a matching sequence about 300 base pairs upstream of *kdoH1*.
nMpH1_rev	5′-GCGCGCACGCGT*TTA*AACATTAGAATTTTCTCCTTTT-3′ Reverse primer for cloning *FnkdoH1* into pMP529 vector: a clamp region, a MluI restriction site (underlined), and a matching anti-coding strand segment at the end of *kdoH1*, including the stop codon (italic).
nMpH2_rev	5’-GCGCGCACGCGT*TTA*GTAATATATAATCTTATCTTTATGC TGTTTATTTTTTATTGTT-3’ Reverse primer for cloning *FnkdoH1H2* or *FnkdoH2* into pMP529 vector: a clamp region, a MluI restriction site (underlined), and a matching anti-coding strand segment at the end of *kdoH2*, including the stop codon (italic)
nPromH2_rev	5′-GAGGTTTGTTGTCAAAAGGTTATAATTATTAAA*CAT*AGTT TTTAAAATTAAGTAAGTATATTGTGCTATTGTAATTTAATT-3′ Primer for cloning *FnkdoH2* into pMP529 vector: a match to anti-coding strand of the beginning of *kdoH2* (underlined), the start codon (italic and underlined), and the anti-coding strand of sequence immediately upstream of *kdoH1*.

nMpH2_for	5′-ATGTTTAATAATTATAACCTTTTGACAACAAACCTC-3′ Forward primer for cloning *FnkdoH2* into pMP529 vector: a match to the beginning of the *kdoH2* coding sequence, including the start codon (underlined).
nWskH1_for	5′-GCGCGCTCTAGAAAGGAGAAAAACT*GTG*AAACATAAAC TAAAGCTAGTTTTGC-3′ Forward primer for cloning *FnkdoH1* into pWSK29 vector: a clamp region, a XbaI restriction site (underlined), a ribosome binding site and a match to the coding strand, including the starting codon (italic and underlined)
nWskH1_rev	5′-GCGCGGGATCCTTGTCAAAAGGTTATAATTA*TTA*AACAT TAG-3′ Reverse primer for cloning *FnkdoH1* into pWSK29 vector: a clamp region, a BamHI restriction site (underlined), and a match to anti-coding strand including the stop codon (italic and underlined).
nWskH2_for	5′-GCGCGCTCTAGAAAGGAGAAAATTCTA*ATG*TTTAATAAT TATAACCTTTTGACAACAA-3′ Forward primer for cloning *FnkdoH2* into pWSK29 vector: a clamp region, a XbaI restriction site (underlined), a ribosome binding site and a match to the coding strand, including the starting codon (italic and underlined).
nWskH2_rev	5′-GCGCGCGGATCCTTTCTGACACTGCTGATGGTAATGCA CCCG-3′ Reverse primer for cloning *FnkdoH2* into pWSK29 vector: a clamp region, a BamHI restriction site, and a match to the anti-coding strand of DNA located about 70 bases downstream of *kdoH2*.
nKan_for	5′-ATGAGCCATATTCAACGG-3′ Forward primer for amplifying *kan* cassette from pET28b: a match to the beginning of the *kan* cassette including the start codon (underlined)
nKan_rev	5′-TTAGAAAAACTCATCGAGCA-3′ Reverse primer for amplifying *kan* cassette from pET28b: a match to the anti-coding sequence at the end of *kan* cassette including the stop codon (underlined).

nHmut_for	5′-TATTCTTTTTAAAAGAATTTGAATA-3′ Common forward primer for construction of *FnkdoH* mutants: a match to the coding strand located about 2 kb upstream of *kdoH1*.
nHmut_rev	5′-AAGATTGAAGAAATTGAGATCA-3′ Common reverse primer for construction of *FnkdoH* mutants: a match to the anti-coding strand about 2 kb downstream of *kdoH2*.
nH1Kan_rev	5′-CCGTTGAATATGGCT*CAT*AGTTTTTAAAATTAAGTAAGTAT ATTGT-3′ Reverse primer for construction of *FnkdoH1* mutant: a match to the anti-coding sequence of the beginning of kanamycin cassette from the pET28b vector (underlined) including the start codon (italic), and a match to the anti-coding sequence upstream of *kdoH1*.
nH1Kan_for	5′-TGCTCGATGAGTTTTTC*TAA*AGAATTGTTGAGATAAAA GGAG-3′ Forward primer for construction of *FnkdoH1* mutant: a match to the end of the kanamycin cassette (underlined) including the stop codon, and a match to a portion of the *kdoH1* sequence before the stop codon.
nH2Kan_rev	5′-TTTAAATTCTCCTTC*GCTTA*TTAAACATTAGAATTTTCT CCTT-3′ Reverse primer for construction of *FnkdoH2* mutant: a spacer sequence for the downstream kanamycin cassette, a ribosome binding site derived from pET28b (underlined) and a spacer sequence (italic) followed by a match to the anti-coding sequence of the end of *kdoH1*.
nH2Kan_for	5′-TAAGCGAAGGAGAATTTAAA*ATG*AGCCATATTCAACGG-3′ Forward primer for construction of *FnkdoH2* mutant: a spacer sequence for the upstream *kdoH1*, a ribosome binding site (underlined) and a spacer sequence followed by a match to the coding sequence of the beginning of kanamycin cassette including the starting codon (italic).