



Figure S1: Semi-quantitative RT-PCR of FPI genes. Reverse-transcription followed by PCR was used for verification of FPI production on transcription level. Almost all of FPI genes showed significantly decreased expression in mutant strain in contrast to WT, whereas the transcription level of rpoA remained unchanged. Taken together with iTRAQ data these results confirm role of HU protein in FPI expression. P value $<0.05^{*}, \mathrm{P}<0.01^{* *}, \mathrm{P}<0.001$ ***, $\mathrm{P}<0.0001$ ****.


Figure S2: Pro-Q Emerald staining of LPS. LPS was extracted from the membrane proteinsenriched fractions using a hot phenol-water extraction. Residual phenol was removed from the collected aqueous LPS-containing phase by acetone precipitation prior to SDS-PAGE. For LPS visualization the Pro-Q Emerald 300 Gel staining was used. Strain FSC200/wbtDEF::Cm lacking LPS was used as a negative control.


Figure S3: Western blot and immunodetection of LPS. Production of LPS was verified also by Western blot followed by immunodetection using anti-LPS antibody. F. tularensis subsp. holarctica FSC200/wbtDEF::Cm lacking LPS was used as a negative control. The typical ladder-like pattern of O-antigen was detected in the FSC200/hupB mutant but in comparison to WT strain with lower intensity in the middle part of the ladder.


Figure S4: Standard growth curve. Bacteria were grown in Chamberlain medium with appropriate antibiotics at $37^{\circ} \mathrm{C}$ for 24 hours in 96 -well plate. The growth kinetics was determined by measurement of optical density at 600 nm using microplate reader FLUOstar Optima. Mutant strain exhibits very similar growth curve like WT and complemented strains.


Figure S5: His-tagged HU protein purification. Purification of FtHU was done with Amicon Pro Affinity Concentrator and HisLink Protein Purification Resin. The samples were separated on $12 \%$ polyacrylamide gel. Samples 1-3 eluates, 4-6 eluates after imidazole removal (using PD MiniTrapG-25), 7-9 concentrated samples (using Amicon Ultra 3K). White arrow shows the position of FtHU.

TABLE S1 Bacterial strains and plasmids used in this study

| Strain | Description | Source |
| :---: | :---: | :---: |
| Francisella tularensis FSC200 | Francisella tularensis subsp. holarctica, wild type (WT), clinical isolate | Francisella Strain Collection (FSC) of the Swedish Defense Research Agency, Umeä, Sweden |
| FSC200/hupB | hup $B$ deletion mutant strain, $\triangle$ hup $B$ | This study |
| FSC200/hupB+hupB | deletion mutant strain complemented in trans | This study |
| FSC200/wbtDEF: $:$ Cm | FSC200 with inactivated O-antigen production | [65] |
| E. coli S17-1 $\lambda$ pir | Escherichia coli donor strain for conjugation TpR SmR recA, thi, pro, hsdR-M+RP4: 2-Tc:Mu: $\mathrm{Km} \operatorname{Tn} 7 \lambda$ pir | [66] |
| E. coli XL1 | Escherichia coli competent cell recAl endAl gyrA96 thi-1 hsdR17 supE44 relAl lac [ $\mathrm{F}^{\prime}$ proAB lacIq Z $\mathrm{M} 15 \mathrm{Tn} 10\left(\mathrm{Tet}^{\mathrm{R}}\right)$ ] | Stratagene |
| E. coli BL21 (DE3) | Escherichia coli strain for overproduction $\mathrm{F}^{-} \operatorname{omp} T h s d S_{B}\left(\mathrm{r}_{\mathrm{B}}{ }^{-} \mathrm{m}_{\mathrm{B}}{ }^{-}\right)$gal dcm (DE3) | Novagen |
| E. coli BL21/FtHU | Escherichia coli strain expressing recombinant $F$. tularensis subsp. holarctica FSC200 HU protein | This study |
| Plasmid | Description | Source |
| pBluescript SK+ | cloning vector, f1 ori, lacZ, ColE ori, $\mathrm{Amp}^{\mathrm{R}}$ | Invitrogen |
| pET28b | T7 expression vector, $\mathrm{Km}^{\text {R }}$ | Novagen |
| pCR4-TOPO | cloning vector, pUC ori, $\mathrm{P}_{l a c}, l a c Z, \mathrm{Kan}^{\mathrm{R}}, \mathrm{Amp}^{\mathrm{R}}$ | Invitrogen |
| pDM4 | F. tularensis suicide vector, mob $_{\mathrm{RP} 4,}$, ori $_{\mathrm{R} G \mathrm{~K}}, \operatorname{sacB}$, $\mathrm{Cm}^{\mathrm{R}}$ | [67] |
| pKK289KmGFP | E. coli/ F. tularensis shuttle vector, Ft ori, p15a ori, $\mathrm{Km}^{\mathrm{R}}$, groES promoter | [68] |

TABLE S2 Primers used in this study ${ }^{a}$

| Primer | Sequence | reverse/forward | Application |
| :---: | :---: | :---: | :---: |
| A | 5'GCATGTCTCGAGTATGTGCGTATGGCTTT3' | F |  |
| B | $\begin{aligned} & \text { 5'ACTTTTTATTATTTTTCACTCTTGTTCATGTT } \\ & \text { TTTAAA3' } \end{aligned}$ | R | deletion |
| C | 5'ACAAGAGTGAAAAATAATAAAAAGTTACA AAAAAGTAA3' | F | construct |
| D | ```5'GCATGTGAGCTCTCTTATCTATCTTCTTTCC GCT3'``` | R |  |
| 1F | 5'TGGGGTAAGAGGGCAAAAGT3' | F |  |
| 2R | 5'CTACTAGAGTTACGCTATCAC3' | R |  |
| F1 | 5'AATGACAGGTGAGGTGACAC3' | F | PCR screening of mutant strain |
| R1 | 5'CTCAAGTTTATCCATTCCACC3' | R |  |
| pKK_0886_F | 5'AAACATATGAACAAGAGTGAATTAGTAAG3 | F |  |
| pKK_0886_R | $\begin{aligned} & \text { 5'AACGAGCTCTTATTTTACAGCGTCTTTAAG } \\ & \text { AC3' } \end{aligned}$ | R | complementatio n in trans |
| pET_rHuB_F | 5'CCATGGCTAACAAGAGTGAATTAG3' | F |  |
| pET_rHuB_R | 5'CTCGAGTTTTACAGCGTCTTTAAGACC3' | R | recombinant protein |

${ }^{a}$ The restriction sites are underlined.

TABLE S3 iTRAQ tags mixtures

| iTRAQ | set 1 | set 2 |
| :--- | :--- | :--- |
| 114 | FSC200 (1) | FSC200 (3) |
| 115 | $200 / h u p B(1)$ | $200 / h u p B(3)$ |
| 116 | FSC200 (2) | FSC200 (4) |
| 117 | $200 / h u p B(2)$ | $200 / h u p B(4)$ |

TABLE S5 Primers used in RT-PCR

| Primer | Sequence | reverse/forward |
| :---: | :---: | :---: |
| iglA_F | 5'CCGCGGAGCAAAAAATAAAATCCCAAATTCA3' | F |
| iglA_R | 5'CTCGAGCTTACCATCTACTTGTTGATTA3' | R |
| iglB_F | 5'ACAATAAATAAATTAAGTCTCACT3' | F |
| iglB_R | 5'GTTATTATTTGTACCGAATAATTC3' | R |
| iglC_F | 5'CCGCGGAAGTGAGATGATAACAAGACAAC3' | F |
| iglC_R | 5'CTCGAGTGCAGCTGCAATATATCCTATT3' | R |
| iglD_F | 5'CTCTTAATCATTATTATTTAGGTGAT3' | F |
| iglD_R | 5'AGAAAAGGCTATAAAGAAATCAA3' | R |
| iglE_F | 5'TACAATAAATTATTGAAAAATCTTTG3' | F |
| iglE_R | 5'ATCTTTTTCTATGCTACTATCATT3' | R |
| iglF_F | 5'AATAATAATATTGATAAATGGTTTGA3' | F |
| iglF_R | 5'TCAGTACAATCTAAGAGGTTATC3' | R |
| iglG_F | 5'TTAAATATTATAAATGACTCCTTAAA3' | F |
| iglG_R | 5'AGATGTTTTTACATTTATTTGTCC3' | R |
| iglH_F | 5'GATGAAAAAAGAAAAGATTTAAGTA3' | F |
| iglH_R | 5'TATAGAGTTATTTAAAACAATCTTTT3' | R |
| iglI_F | 5'CCGCGGAAGTCAGATAATATCTACACTAAAT3' | F |
| iglI_R | 5'CTCGAGTATGTCAAAAAGATCTTCAAAATA3' | R |
| iglJ_F | 5'AAGACTATTTTGAAGATCTTTTTG3' | F |
| iglJ_R | 5'TAAATTAAAATAACTTAGGTATATCT3' | R |
| pdpA_F | 5'TTTGGACTAAGCACAAACCAT3' | F |
| pdpA_R | 5'GTCATTATTAACATTTTCTCCAAT3' | R |


| pdpB_F | 5'GCTATCTATAAAAGCTCTATAAA3' | F |
| :---: | :---: | :---: |
| pdpB_R | 5'CAGTTTTATTATAAAAAAGTAGTG3' | R |
| pdpC_F | 5'TATCTAAAGATATTATAAAATCATATA3' | F |
| pdpC_R | 5'AAGCGTCAGCATATTTTTGTAA3' | R |
| pdpE_F | 5'CCGCGGAAGTAAAAAAATATTTAAATTATTATCAA3' | F |
| pdpE_R | 5'CTCGAGTATTATAGTAATTTTCTTTTCATAAT3' | R |
| vgrG_F | 5'TCAAAAGCAGACCATATTTTCAA3' | F |
| vgrG_R | 5'TCCAACCATTGTTGCTGTAGA3' | R |
| dotU_F | 5'AAAGACTTTAAAGAGATAGAAATTA3' | F |
| dotU_R | 5'CCAGCTTAATAAAATTAGTAAGC3' | R |
| pigR_F | 5'ATGGCGAATCAATATTCTGGAA3' | F |
| pigR_R | 5'CAGTCAAGATTTAGCTTTGATTA3' | R |
| rpoA_F | 5'GTGAGTAATAATAATTCAAAACTG3' | F |
| rpoA_R | 5'TTATTTTCCTTCAACTAGCTCTC3' | R |

