```
    LVS ATGTTCGATCAATTAGGCAATAGTGGAGCATTTCTTATAAATGCTATAGTTGCAGTTGTT 60
 FSC200 ATGTTCGATCAATTAGGCAATAGTGGAGCATTTCTTATAAATGCTATAGTTGCAGTTGTT 60
  OSU18 ATGTTCGATCAATTAGGCAATAGTGGAGCATTTCTTATAAATGCTATAGTTGCAGTTGTT 60
 R13-38 ATGTTCGATCAATTAGGCAATAGTGGAGCATTTCTTATAAATGCTATAGTTGCAGTTGTT 60

    LVS ATTTTAGTTGTTGGCTTTTGGGCATCTAAGTATATAAAGTCTATTGTGTATAGCCTGTTG 120
 FSC200 ATTTTAGTTGTTGGCTTTTGGGCATCTAAGTATATAAAGTCTATTGTGTATAGCCTGTTG 120
  OSU18 ATTTTAGTTGTTGGCTTTTGGGCATCTAAGTATATAAAGTCTATTGTGTATAGCCTGTTG 120
 R13-38 ATTTTAGTTGTTGGCTTTTGGGCATCTAAGTATATAAAGTCTATTGTGTATAGCCTGTTG 120

    LVS AAAGAGTATGACAAGACAGTATCGCAATTTTTAGCTAGTCTAGTTTACACAATTTTTTA 180
 FSC200 AAAGAGTATGACAAGACAGTATCGCAATTTTTAGCTAGTCTAGTTTACACAATTTTTTA 180
  OSU18 AAAGAGTATGACAAGACAGTATCGCAATTTTTAGCTAGTCTAGTTTACACAATTTTTTA 180
 R13-38 AAAGAGTATGACAAGACAGTATCGCAATTTTTAGCTAGTCTAGTTTACACAATTTTTTA 180

    LVS GTGTTAGTGGTTGTAATTGCTTTAGCAAAGTTGGGGGTGCCGATATCTCCTGTGACAGGA 240
 FSC200 GTGTTAGTGGTTGTAATTGCTTTAGCAAAGTTGGGGGTGCCGATATCTCCTGTGACAGGA 240
  OSU18 GTGTTAGTGGTTGTAATTGCTTTAGCAAAGTTGGGGGTGCCGATATCTCCTGTGACAGGA 240
 R13-38 GTGTTAGTGGTTGTAATTGCTTTAGCAAAGTTGGGGGTGCCGATATCTCCTGTGACAGGA 240

    LVS GTTCTAACAGGTGTGGTTTTTGGTATCTCAATGTCATTAAAGACATCTTATAATACTGTT 300
 FSC200 GTTCTAACAGGTGTGGTTTTTGGTATCTCAATGTCATTAAAGACATCTTATAATACTGTT 300
  OSU18 GTTCTAACAGGTGTGGTTTTTGGTATCTCAATGTCATTAAAGACATCTTATAATACTGTT 300
 R13-38 GTTCTAACAGGTGTGGTTTTTGGTATCTCAATGTCATTAAAGACATCTTATAATACTGTT 300

    LVS GCCTGTGGTATTATGCTGGCATTTAGTAAGCCTTTTGAGGTTGGGGAAAAGGTTGATTTT 360
 FSC200 GCCTGTGGTATTATGCTGGCATTTAGTAAGCCTTTTGAGGTTGGGGAAAAGGTTGATTTT 360
  OSU18 GCCTGTGGTATTATGCTGGCATTTAGTAAGCCTTTTGAGGTTGGGGAAAAGGTTGATTTT 360
 R13-38 GCCTGTGGTATTATGCTGGCATTTAGTAAGCCTTTTGAGGTTGGGGAAAAGGTTGATTTT 360

    LVS GGCGGAATCAAAGGTACTGTTAAATCAATTGGTTTTTTGTATACTAAGCTTGATGATGAG 420
 FSC200 GGCGGAATCAAAGGTACTGTTAAATCAATTGGTTTTTTGTATACTAAGCTTGATGATGAG 420
  OSU18 GGCGGAATCAAAGGTACTGTTAAATCAATTGGTTTTTTGTATACTAAGCTTGATGATGAG 420
 R13-38 GGCGGAATCAAAGGTACTGTTAAATCAATTGGTTTTTTGTATACTAAGCTTGATGATGAG 420

    LVS GATGGTAATGAAATTGTAATCTCAAACAACCTTGTTCTATCTAAGGTAATTACTAGATTT 480
 FSC200 GATGGTAATGAAATTGTAATCTCAAACAACCTTGTTCTATCTAAGGTAATTACTAGATTT 480
  OSU18 GATGGTAATGAAATTGTAATCTCAAACAACCTTGTTCTATCTAAGGTAATTACTAGATTT 480
 R13-38 GATGGTAATGAAATTGTAATCTCAAACAACCTTGTTCTATCTAAGGTAATTACTAGATTT 480

    LVS ACTCAAACAGATAAATAA                                           498
 FSC200 ACTCAAACAGATAAATAA                                           498
  OSU18 ACTCAAACAGATAAATAA                                           498
 R13-38 ACTCAAACAGATAAATAA                                           498
```

Figure S1: Alignment of the first putative mechanosensitive channel in *F. tularensis* subsp. *holarctica* strains. No differences were found. The locus tags of compared loci are as follows: FTL_1753/FTL_RS08965 (LVS), FTS_RS08945 (FSC200), FTH_RS08925 (OSU18) and RB23_RS05230 (R13-38).

```
     LVS  ATGCCTTTATATAATGAAATGCTTTTCAACATAATATCAACGTTTATCAATATAATATTA 60
  FSC200  ATGCCTTTATATAATGAAATGCTTTTCAACATAATATCAACGTTTATCAATATAATATTA 60
   OSU18  ATGCCTTTATATAATGAAATGCTTTTCAACATAATATCAACGTTTATCAATATAATATTA 60
  R13-38  ATGCCTTTATATAATGAAATGCTTTTCAACATAATATCAACGTTTATCAATATAATATTA 60

     LVS  ATAACTATAGTCGCTAGCTTAGCTTTTTACTTACTAAAAAAACGCGCAACATCTACTAAA 120
  FSC200  ATAACTATAGTCGCTAGCTTAGCTTTTTACTTACTAAAAAAACGCGCAACATCTACTAAA 120
   OSU18  ATAACTATAGTCGCTAGCTTAGCTTTTTACTTACTAAAAAAACGCGCAACATCTACTAAA 120
  R13-38  ATAACTATAGTCGCTAGCTTAGCTTTTTACTTACTAAAAAAACGCGCAACATCTACTAAA 120

     LVS  CAAATCAAAAAATTAAATTGCGAGTGATTTATTTAAGTATAATTATTTTTTTCCTAGTT 180
  FSC200  CAAATCAAAAAATTAAATTGCGAGTGATTTATTTAAGTATAATTATTTTTTTCCTAGTT 180
   OSU18  CAAATCAAAAAATTAAATTGCGAGTGATTTATTTAAGTATAATTATTTTTTTCCTAGTT 180
  R13-38  CAAATCAAAAAATTAAATTGCGAGTGATTTATTTAAGTATAATTATTTTTTTCCTAGTT 180

     LVS  GTTATAAAAATTTGGCTTGGTGGAATAACTAACTTATTTACAATGTTAAGTTTAGTTGCA 240
  FSC200  GTTATAAAAATTTGGCTTGGTGGAATAACTAACTTATTTACAATGTTAAGTTTAGTTGCA 240
   OSU18  GTTATAAAAATTTGGCTTGGTGGAATAACTAACTTATTTACAATGTTAAGTTTAGTTGCA 240
  R13-38  GTTATAAAAATTTGGCTTGGTGGAATAACTAACTTATTTACAATGTTAAGTTTAGTTGCA 240

     LVS  GCTGGTCTGATAATCGTTAATAAAGAAACTGTTATGAATTTTGTTGGTTGGATTATCATC 300
  FSC200  GCTGGTCTGATAATCGTTAATAAAGAAACTGTTATGAATTTTGTTGGTTGGATTATCATC 300
   OSU18  GCTGGTCTGATAATCGTTAATAAAGAAACTGTTATGAATTTTGTTGGTTGGATTATCATC 300
  R13-38  GCTGGTCTGATAATCGTTAATAAAGAAACTGTTATGAATTTTGTTGGTTGGATTATCATC 300

     LVS  AATTGGCGCAGCCTATTTTCTGAGGGTGACTATATTGAGGTACAAAATTATCATGGCTAT 360
  FSC200  AATTGGCGCAGCCTATTTTCTGAGGGTGACTATATTGAGGTACAAAATTATCATGGCTAT 360
   OSU18  AATTGGCGCAGCCTATTTTCTGAGGGTGACTATATTGAGGTACAAAATTATCATGGCTAT 360
  R13-38  AATTGGCGCAGCCTATTTTCTGAGGGTGACTATATTGAGGTACAAAATTATCATGGCTAT 360

     LVS  GTTAGTGAGATAAAAGTATTTTATTTTAGGATGTATGAAACTATTGAGCATGGTGATAAA 420
  FSC200  GTTAGTGAGATAAAAGTATTTTATTTTAGGATGTATGAAACTATTGAGCATGGTGATAAA 420
   OSU18  GTTAGTGAGATAAAAGTATTTTATTTTAGGATGTATGAAACTATTGAGCATGGTGATAAA 420
  R13-38  GTTAGTGAGATAAAAGTATTTTATTTTAGGATGTATGAAACTATTGAGCATGGTGATAAA 420

     LVS  AGAACAACTGGGAAACTCTTAAATAGTAATTACAAGTATTATTAA               465
  FSC200  AGAACAACTGGGAAACTCTTAAATAGTAATTACAAGTATTATTAA               465
   OSU18  AGAACAACTGGGAAACTCTTAAATAGTAATTACAAGTATTATTAA               465
  R13-38  AGAACAACTGGGAAACTCTTAAATAGTAATTACAAGTATTATTAA               465
```

Figure S2: Alignment of the second putative mechanosensitive channel in *F. tularensis* subsp. *holarctica* strains. No differences were found. The locus tags of compared loci are as follows: FTL_0945/FTL_RS04830 (LVS), FTS_RS04840 (FSC200), FTH_RS04840 (OSU18), RB23_RS01900 (R13-38).

```
    LVS  TTGGCTACTGAGAAACTATTTTTGTGCACAGAACCTAGTTTATTTAGGTTTCTATATAAA  60
 FSC200  TTGGCTACTGAGAAACTATTTTTGTGCACAGAACCTAGTTTATTTAGGTTTCTATATAAA  60
  OSU18  --------------------TTGTGCACAGAACCTAGTTTATTTAGGTTTCTATATAAA  39

    LVS  AGTAAAAATATTGCTATCGAAAAGAAAAAAGCTGAAACAAAAGATGGTTATGATGATTTT  120
 FSC200  AGTAAAAATATTGCTATCGAAAAGAAAAAAGCTGAAACAAAAGATGGTTATGATGATTTT  120
  OSU18  AGTAAAAATATTGCTATCGAAAAGAAAAAAGCTGAAACAAAAGATGGTTATGATGATTTT  99

    LVS  AGAGATATCAATGCTATATTTAAAGCTTTAGAGCTTGGAGCTATAGTAGTTTCAGTTATA  180
 FSC200  AGAGATATCAATGCTATATTTAAAGCTTTAGAGCTTGGAGCTATAGTAGTTTCAGTTATA  180
  OSU18  AGAGATATCAATGCTATATTTAAAGCTTTAGAGCTTGGAGCTATAGTAGTTTCAGTTATA  159

    LVS  TTGATTTTGGCAGCTTTTCGTGTTCCTCTTACTGCTTTAGGAGCTTTCAGTGGTGTTGCC  240
 FSC200  TTGATTTTGGCAGCTTTTCGTGTTCCTCTTACTGCTTTAGGAGCTTTCAGTGGTGTTGCC  240
  OSU18  TTGATTTTGGCAGCTTTTCGTGTTCCTCTTACTGCTTTAGGAGCTTTCAGTGGTGTTGCC  219

    LVS  TTGGCTGGTTTAACTCTTTCGCAAAGTACTCTGTTGACAAATCTATTTGGAGGCTTGTTT  300
 FSC200  TTGGCTGGTTTAACTCTTTCGCAAAGTACTCTGTTGACAAATCTATTTGGAGGCTTGTTT  300
  OSU18  TTGGCTGGTTTAACTCTTTCGCAAAGTACTCTGTTGACAAATCTATTTGGAGGCTTGTTT  279

    LVS  GTAGTATTTAATCGTAAATATTCTGAGGGCGATATTATTTCTTCAGAGATAAATTCGACA  360
 FSC200  GTAGTATTTAATCGTAAATATTCTGAGGGCGATATTATTTCTTCAGAGATAAATTCGACA  360
  OSU18  GTAGTATTTAATCGTAAATATTCTGAGGGCGATATTATTTCTTCAGAGATAAATTCGACA  339

    LVS  ATTAAATTTAGTGGTACTATCAAAAAAATTGGTACCTTAACTACTAGAGTTGATAATTCT  420
 FSC200  ATTAAATTTAGTGGTACTATCAAAAAAATTGGTACCTTAACTACTAGAGTTGATAATTCT  420
  OSU18  ATTAAATTTAGTGGTACTATCAAAAAAATTGGTACCTTAACTACTAGAGTTGATAATTCT  399

    LVS  GAAACAGCACCAATGCATATTCCAAATTCGGTGTTTCTTAATACTTGTATAACAACAACA  480
 FSC200  GAAACAGCACCAATGCATATTCCAAATTCGGTGTTTCTTAATACTTGTATAACAACAACA  480
  OSU18  GAAACAGCACCAATGCATATTCCAAATTCGGTGTTTCTTAATACTTGTATAACAACAACA  459

    LVS  TCACGTCGAACTCATAGGCGCATAGTACAGTTTATAACTATTGATTATAAACATATTGAT  540
 FSC200  TCACGTCGAACTCATAGGCGCATAGTACAGTTTATAACTATTGATTATAAACATATTGAT  540
  OSU18  TCACGTCGAACTCATAGGCGCATAGTACAGTTTATAACTATTGATTATAAACATATTGAT  519

    LVS  AAAATCCCTGTTATTAAACAAAAGCTTTTAGAGATACTTAAATCACATCCAAATATTGAC  600
 FSC200  AAAATCCCTGTTATTAAACAAAAGCTTTTAGAGATACTTAAATCACATCCAAATATTGAC  600
  OSU18  AAAATCCCTGTTATTAAACAAAAGCTTTTAGAGATACTTAAATCACATCCAAATATTGAC  579

    LVS  CAGAATAAAACTTTAGCTGTCTCATTAGCATCAGGAGGTACCAATATTTCTGGTAAGCTA  660
 FSC200  CAGAATAAAACTTTAGCTGTCTCATTAGCATCAGGAGGTACCAATATTTCTGGTAAGCTA  660
  OSU18  CAGAATAAAACTTTAGCTGTCTCATTAGCATCAGGAGGTACCAATATTTCTGGTAAGCTA  639

    LVS  GAGGGTAGTTTTGGTAGTAGTGGTATTAATATACAAATTTATGCGATGGTTAATAAAGTT  720
 FSC200  GAGGGTAGTTTTGGTAGTAGTGGTATTAATATACAAATTTATGCGATGGTTAATAAAGTT  720
  OSU18  GAGGGTAGTTTTGGTAGTAGTGGTATTAATATACAAATTTATGCGATGGTTAATAAAGTT  699

    LVS  TTCTTTAGTGACTTTATCAATGTTCAGGATGAAATTTTTATAAATATTGCCAAAGAATTA  780
 FSC200  TTCTTTAGTGACTTTATCAATGTTCAGGATGAAATTTTTATAAATATTGCCAAAGAATTA  780
  OSU18  TTCTTTAGTGACTTTATCAATGTTCAGGATGAAATTTTTATAAATATTGCCAAAGAATTA  759

    LVS  AATGATTTAGATATAGAGTTTGCGATAAATCCAGTTACGTTACATAAATATTAG       834
 FSC200  AATGATTTAGATATAGAGTTTGCGATAAATCCAGTTACGTTACATAAATATTAG       834
  OSU18  AATGATTTAGATATAGAGTTTGCGATAAATCCAGTTACGTTACATAAATATTAG       813
```

Figure S3: Alignment of the third putative mechanosensitive channel in *F. tularensis* subsp. *holarctica* strains. Differences are highlighted in black. The locus tags of compared loci are as follows: FTL_1588 /FTL_RS08095 (LVS), FTS_RS08075 (FSC200) and FTH_RS08065(OSU18). A gap in the R13-38 WGS assembly precluded this strain from comparison.
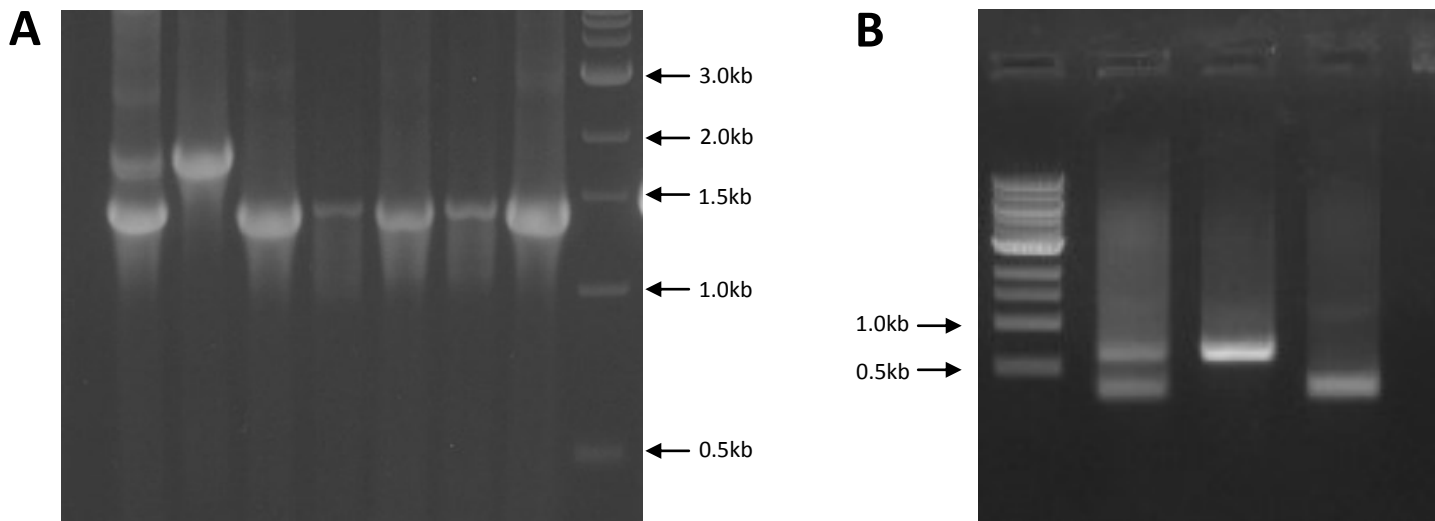
Figure S4: pictures of gels demonstrating deletion of FTL_1753 and FTL_0945. Genomic DNA was isolated from wild-type LVS, primary recombinants and secondary recombinants and amplified with the primers indicated. **A)** FTL_1753 (*Ft*MscS) deletion. DNA was amplified with the 1753_UP_F & 1753_DOWN_R primers, yielding a WT band of ~2000 BP and a deletion band of ~1500 BP. Lane 1: primary recombinant with both bands. Lane 2: WT DNA template. Lanes 3-7: secondary recombinants with the deletion. **B)** FTL_0945 deletion. DNA was amplified with the 0945_SCREEN_F and 0945_SCREEN_R primers, yielding a WT band of ~670 BP and a deletion band of ~330 BP. Lane 1: primary recombinant with both bands. Lane 2: WT DNA template. Lane 3: secondary recombinant with the deletion.

	MIC (µg/mL)		
	WT	**ΔFTL_1753**	**ΔFTL_0945**
gentamicin	4	4	4
kanamycin	8	16	8
streptomycin	16	16	16
tetracycline	0.5	0.5	0.5
erythromycin	>16	>16	>16
chloramphenicol	2	2	2
trimethoprim	>16	>16	>16
polymyxin B	>16	>16	>16
ampicillin	>16	>16	>16

Table S1: Minimum Inhibitory Concentrations (MICs) of WT LVS and the ΔFTL_1753 and ΔFTL_0945 strains to indicated antibiotics.

```
holarctica   MFDQLGNSGAFLINAIVAVVILVVGFWASKYIKSIVYSLL   40
tularensis   MFDQLGNSGAFLINAIVAVVILVVGFWASKYIKSLVYSLL   40
  novicida   MFDQLGNSGAFLINAIVAVVILVVGFWASKYIKSLVYSLL   40

holarctica   KEYDKTVSQFLASLVYTIFLVLVVVIALAKLGVPISPVTG   80
tularensis   KEYDKTVSQFLASLVYTIFLVLVVVIALAKLGVPISPVTG   80
  novicida   KEYDKTVSQFLASLVYTIFLVLVVVIALAKLGVPISPVTG   80

holarctica   VLTGVVFGISMSLKTSYNTVACGIMLAFSKPFEVGEKVDF   120
tularensis   VLTGVVFGISMSLKTSYNTVACGIMLAFSKPFEVGEKVDF   120
  novicida   VLTGVVFGISMSLKTSYNTVACGIMLAFSKPFEVGERVDF   120

holarctica   GGIKGTVKSIGFLYTKLDDEDGNEIVISNNLVLSKVITRF   160
tularensis   GGIKGTVKSIGFLYTKLDDEDGNEIVISNNLVLSKVITRF   160
  novicida   GGIKGTVKSIGFLYTKLDDEDGNEIVISNNLVLSKVITRF   160

holarctica   TQTDK                                      165
tularensis   TQTDK                                      165
  novicida   TQTDK                                      165
```

Figure S5: Protein-level alignment of FTL_1753 (*Ft*MscS) to equivalent genes in subsp. *tularensis* SchuS4 and subsp. *novicida* U112. Substitutions involving similar amino acids are highlighted gray, while substitutions involving dissimilar amino acids or gaps are shaded black using default parameters in Multiple Align Show (http://www.bioinformatics.org/sms/multi_align.html). The locus tags/protein IDs used are as follows: subsp. *holarctica*: FTL_1753/FTL_RS08965/WP_003017262.1; subsp. *tularensis in silico* translated from FTT_0135; subsp *novicida* FTN_1581/FTN_RS08090/WP_003041103.1

```
holarctica   MPLYNEMLFNIISTFINIILITIVASLAFYLLKKRATSTK   40
tularensis   MPLYNEMLFNIISTFINIVLITIVASLAFYLLKKRATSTK   40
  novicida   MPLYNEMLFNIISTFINIVLITIVASLAFYLLKKRATSTK   40

holarctica   QIKKIKLRVIYLSIIIFFLVVIKIWLGGITNLFTMLSLVA   80
tularensis   QIKKIKSRVIYLSIIIFFLVVIKIWLGGITNLFTMLSLVA   80
  novicida   QIKKIKSRVIYLSIIIFFLAVIKIWLGGITNLFTMLSLVA   80

holarctica   AGLIIVNKETVMNFVGWIIINWRSLFSEGDYIEVQNYHGY  120
tularensis   AGLIIVNKETVMNFVGWIIINWRSLFSEGDYIEVQNYHGY  120
  novicida   AGLIIVNKETVMNFVGWIIINWRSLFSEGDYIEVQNYHGY  120

holarctica   VSEIKVFYFRMYETIEHGDKRTTGKLLNSNYKYY------  154
tularensis   VSKIKVFYFRMYETIEHGDKRTTGKLLNSNYKYY------  154
  novicida   VSEIKVFYFRMYETIEHGDKRTTGKLLKLPNSIVITSIIK  160

holarctica   ----------------------------------------  154
tularensis   ----------------------------------------  154
  novicida   TFSSDENIALHKVPWLISIEKDPLEVAQKLEQLIKRIIAD  200

holarctica   ----------------------------------------  154
tularensis   ----------------------------------------  154
  novicida   KYFFSSDFSKSSVINKSKLKNYGINDFTPYIEIKTFNDKE  240

holarctica   ------------------------------------     154
tularensis   ------------------------------------     154
  novicida   NVINIQANFYCYVEHRKEIEQAYTRELINIINSEAFS    277
```

Figure S6: Protein-level alignment of FTL_0945 to equivalent loci in subsp. *tularensis* SchuS4 and subsp. *novicida* U112. Substitutions involving similar amino acids are highlighted gray, while substitutions involving dissimilar amino acids or gaps are shaded black using default parameters in Multiple Align Show (http://www.bioinformatics.org/sms/multi_align.html). The locus tags/protein IDs used are as follows: subsp. *holarctica*: FTL_0945/FTL_RS04830/WP_003015729.1; subsp. *tularensis* FTT_0670c/YP_169687.1; subsp *novicida* FTN_1012/FTN_RS05215/WP_003039467.1

```
holarctica  MA----------------------------------------  2
tularensis  MNLLNNILLRVENYSTTYILLIDFVICLVLSVIISLLGSL  40
  novicida  MNLLNNILLRVENYSTTYILLIDFVICLVLSVIISLLGSL  40

holarctica  ----------------------------------------  2
tularensis  LVIKHKDTIRYSFVKSFKLTLYLVIWSYFIKTCVDLPVIV  80
  novicida  LVIKHKDTIRYSFVKSFKLTLYLVIWSYFIKTCVDLPVIV  80

holarctica  ------------TEKLF-----LCTEPSLFRFLYKSKNIA  25
tularensis  HLPDYKEVIVSYSDKIFDFCIYLAIIISLFRFLYKSKNIA  120
  novicida  HLPDYKEVIVSYSDKIFDFCIYLAIIISLFRFLYKSKNIA  120

holarctica  IEKKKAETKDGYDDFRDINAIFKALELGAIVVSVILILAA  65
tularensis  IEKKKAETKDGYDDFRDINAIFKALELGAIVVSVILILAA  160
  novicida  IEKKKAETKDGYDDFRDINAIFKALELGAIVVSVILILAA  160

holarctica  FRVPLTALGAFSGVALAGLTLSQSTLLTNLFGGLFVVFNR  105
tularensis  FRAPLTALGAFSGVALAGLTLSQSTLLTNLFGGLFVVFNR  200
  novicida  FRVPLTALGAFSGVALAGLTLSQSTLLTNLFGGLFVVFNR  200

holarctica  KYSEGDIISSEINSTIKFSGTIKKIGTLTTRVDNSETAPM  145
tularensis  KYSEGDIISSEINSTIKFSGTIKKIGTLTTRVDNSETAPM  240
  novicida  KYSEGDIISSEINSTIKFSGTIKKIGTLTTRVDNSETAPM  240

holarctica  HIPNSVFLNTCITTTSRRTHRRIVQFITIDYKHIDKIPVI  185
tularensis  HIPNSVFLNTCITTTSRRTHRRIVQFITIDYKHIDKIPVI  280
  novicida  HIPNSVFLNTCITTTSRRTHRRIVQFITIDYKHIDKIPVI  280

holarctica  KQKLLEILKSHPNIDQNKTLAVSLASGGTNISGKLEGSFG  225
tularensis  KQKLLEILKSHPNIDQNKTLAVSLASGGTNISGKLEGSFG  320
  novicida  KQKLLEILKSHPNIDQNKTLAVSLASGGTNISGKLEGSFG  320

holarctica  SSGINIQIYAMVNKVFFSDFINVQDEIFINIAKELNDLDI  265
tularensis  SSGINIQIYAMVNKVFFSDFINVQDEIFINIAKELNDLDI  360
  novicida  SSGINIQIYAMVNKVFFSDFINVQDEIFINIAKELNDLDI  360

holarctica  EFAINPVTLHKY                              277
tularensis  EFAINPVTLHKY                              372
  novicida  EFAINPVTLHKY                              372
```

Figure S7: Protein-level alignment of FTL_1588 to equivalent loci in subsp. *tularensis* SchuS4 and subsp. *novicida* U112. Substitutions involving similar amino acids are highlighted gray, while substitutions involving dissimilar amino acids or gaps are shaded black using default parameters in Multiple Align Show (http://www.bioinformatics.org/sms/multi_align.html). The locus tags/protein IDs used are as follows: subsp. *holarctica*: FTL_1588/FTL_RS08095/WP_080502286.1; subsp. *tularensis* FTT_0475/YP_169513.1; subsp *novicida* FTN_0566/FTN_RS02930/WP_003033340.1

```
holarctica  MSLLKKYYFDIINNHGFSVALSCLTIILIVLLLSFIINRL  40
tularensis  MSLLKKYYFDIINNHGFSVALSCLTIILIVLLLSFIINRL  40
  novicida  MSLLKKYYFDIINNHGFSVALSCLTIILIVLLLSFIINRL  40

holarctica  ASKYIVLIAKKFFDKSNSMLGKYLLEYKFFVKLSHIIPGI  80
tularensis  SSKYIVLIAKKFFDKSNSMLGKYLLEYKFFVKLSHIIPGI  80
  novicida  ASKYIVLIAKKFFDKSNSMLGKYLLEYKFFVKLSHIIPGI  80

holarctica  FAYVIIGFATDNDYLWTQHLVTGLQLLLQIYITVTIISFL  120
tularensis  FAYVIIGFATDNDYLWTQHLVTGLQLLLQIYITVTIISFL  120
  novicida  FAYVIIGFSTDNDYLWTQHLVTGLQLLLQIYITVTIISFL  120

holarctica  VCFVDVFLTTLR----------------ILLILKIIHYVA  144
tularensis  VCFVDALFDYLENLAYFKNHSLRSYAQVIKIILYIIGFIL  160
  novicida  VCFVDALFDYLENLAYFKNHSLRSYAQVIKIILYIIGFIL  160

holarctica  MLK-------------------------------------  147
tularensis  VVSLLLNKSPIAFLTGLGALSAVLMLVFKDTILGFVTNVQ  200
  novicida  VVSLLLNKSPIAFLTGLGALSAVLMLVFKDTILGFVTNVQ  200

holarctica  ----------------------------------------  147
tularensis  VAALDLVRVGDWITIPSANVDGTVMDVSINTVKIRNFDKT  240
  novicida  VAALDLVRVGDWITIPSANVDGTVMDVSINTVKIRNFDKT  240

holarctica  ----------------------------------------  147
tularensis  ISMIPTYTLTTHSVQNWRGMVETGGRRIKRSINIDIDTIK  280
  novicida  ISMIPTYTLTTHSVQNWRGMVETGGRRIKRSINIDIDTIK  280

holarctica  ----------------------------------------  147
tularensis  FCDQELLERLGKETLLADFIKAKANEKLTNISLFRAYIEN  320
  novicida  FCDQELLERLGKETLLADFIKAKANEKLTNISLFRAYIEN  320

holarctica  ----------------------------------------  147
tularensis  YLRNQPKIHLGLTFLIRELQPTQFGLPVELYIFTNDTNWV  360
  novicida  YLRNHPKIHLGLTFLIRELQPTQFGLPVELYIFTNDTNWV  360

holarctica  ----------------------------------  147
tularensis  NYEKIQADIFDYVFASLHMFDLKAFQAITGRINNQF  396
  novicida  NYEKIQAEIFDYVFASLHMFDLKAFQAITGRINNQF  396
```

Figure S8: Protein-level alignment of FTL_1209 to equivalent loci in subsp. *tularensis* SchuS4 and subsp. *novicida* U112. Substitutions involving similar amino acids are highlighted gray, while substitutions involving dissimilar amino acids or gaps are shaded black using default parameters in Multiple Align Show (http://www.bioinformatics.org/sms/multi_align.html). The locus tags/protein IDs used are as follows: subsp. *holarctica*: in silico translated from FTL_1209/FTL_RS06185; subsp. *tularensis* FTT_0992/YP_169980.1; subsp *novicida* FTN_0872/FTN_RS04490/WP_011733656.1