

Table S1. Oligonucleotides

YscE-P1	5'-ACCTCGATGACACAATTAGAGGAGCAACTGCATAACGTGTGTGTAGGCTGGAGCTGCTTC-3'
YscE-P2	5'-TTTTAGGTCTCCTGCTACATAATGAATAATGGCTATAGCCATATGAATATCCTCCTTAGT-3'
YscE-CTL-F	5'-ATGCGGTGATTGTGAGTAAAGGAA-3'
YscE-CTL-R	5'-GTCTGCTGGCTTCTTGAGCGTTG-3'
YscI-SmaI-F	5'-TTTCCCGGGAAGAGGCGGTTCAATTGATTCGC-3'
YscI-R	5'-TATGTTGCGCATTTATGTATCCAT-3'
YscI-F	5'-ATCGCCAAGACTGCCGGCGAATG-3'
YscI-SmaI-R	5'-TTTCCCGGGAACGACAACAAGCCAATAAGATGC-3'
YscI-CTL-F	5'-GTTGGATTACCTTATTTATCAGAG-3'
YscI-CTL-R	5'-TGGCTCTTTGTCTGCGGAAAGGCC-3'
YscF-XbaI-F	5'-TTTTTCTAGACAGTTTGGCAGAGAGAATCTAAA-3'
YscF-HindIII-R	5'-TTTTAAGCTTTTATGGGAACCTCTGTAGGATGCC-3'
YscF-86-HindIII-R	5'-TTTTAAGCTTTCAGAACTTCTGTAGGATGCCTTGCAT-3'
YscF-85-HindIII-R	5'-TTTTAAGCTTTCACCTTCTGTAGGATGCCTTGCATTAA-3'
YscF-80-HindIII-R	5'-TTTTAAGCTTTCATTGCATTAAGTCTTTCATGCTACG-3'
YscF-1-R	5'-CATTATTTTAGGTCTCCTGTAC-3'
YscF-6-F	5'-GGATTTACGAAAGGAACCGATATC-3'
YscF-11-F	5'-ACCGATATCGCAGACTTAGATGCG-3'
YscF-16-F	5'-TTAGATGCGGTGGCTCAAACGCTC-3'
YscF-21-F	5'-CAAACGCTCAAGAAGCCAGCAGAC-3'
YscG-HindIII-F	5'-TTTTAAGCTTAAATATAAACTCAACGTACTGTTA-3'
YscG-R	5'-GAGCCGTATGTTTTTAGTTGCTCCCT-3'
YscE-F	5'-AATGGAGGTAAACCTCGATGACAC-3'
YscE-6xHis-BglII-R	5'-TTTAGATCTTCAATGATGATGATGATGATGAGAACCCCTTTTAGGTCTCCTGCTACATA-3'
YscE-6xHis-KpnI	5'-TTTTGGTACCGGATCCTAGAATACAGACATTTGT-3'
YscE-6xHis-XbaI-R	5'-TTTTTCTAGAGACTATCTATCTAGATCTTCAATG-3'
YscE-234-F	5'-CCGCAGAGGAGCAACTGCATAACGTG-3'
YscE-234-R	5'-CCGCCATCGAGGTTTACCTCCATTGAG-3'
YscE-8910-F	5'-CCGCAGTGGAGACAGTGCCTCTATC-3'
YscE-8910-R	5'-CCGCTTGCTCCTCTAATTGTGTCAT-3'
YscE-1112-F	5'-GCTACAGTGCCTCTATCACCATG-3'
YscE-1112-R	5'-TGCGTTATGCAGTTGCTCCTCTAA -3'

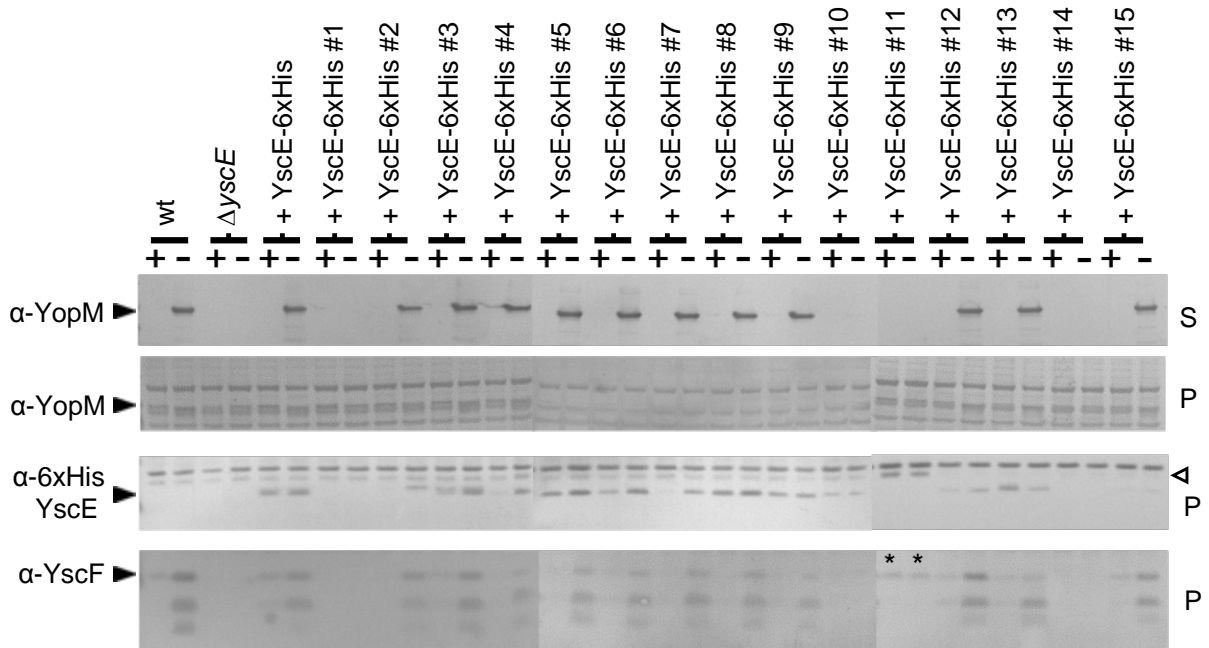
YscE-1314-F 5'-GCTCGCTCTATCACCATGCAACTA-3'
YscE-1314-R 5'-TGCCTCCACGTTATGCAGTTGCTC-3'
YscE-1516-F 5'-GCTATCACCATGCAACTAGAAATG-3'
YscE-1516-R 5'-TGCCACTGTCTCCACGTTATGCAG-3'
YscE-1920-F 5'-GCTCTAGAAATGGCACTAACTAAG-3'
YscE-1920-R 5'-TGCGGTGATAGAGCGCACTGTCTC-3'
YscE-2223-F 5'-GCTGCACTAACTAAGCTCAAAAAA-3'
YscE-2223-R 5'-TGCTAGTTGCATGGTATAGAGCG-3'
YscE-2627-F 5'-GCTCTCAAAAAAGATATGATGCGCGG-3'
YscE-2627-R 5'-TGCTAGTGCCATTTCTAGTTGCAT-3'
YscE-3031-F 5'-GCTATGATGCGCGGTGGTATGCC-3'
YscE-3031-R 5'-TGCTTTGAGCTTAGTTAGTGCCAT-3'
YscE-323334-F 5'-CCGCAGGTGGTATGCCAAGCAGTAT-3'
YscE-323334-R 5'-CCGCATCTTTTTGAGCTTAGTTAG-3'
YscE-353637-F 5'-ACAAGGCCAAGCAGTATCAGGTTTGG-3'
YscE-353637-R 5'-GCTTGCGCATCATATCTTTTTGAGC-3'
YscE-3940-F 5'-GCTTATCAGGTTTGGCAGAGAGAA-3'
YscE-3940-R 5'-TGCGGCATCACCACCGCGCATCAT-3'
YscE-4243-F 5'-GCTTGGCAGAGAGAATCTAAAGCT-3'
YscE-4243-R 5'-TGCATACTGCTTGGCATCACCACC-3'
YscE-50515354-F 5'-CGACGCATAGCCATTATTCATTATGTAGCAG-3'
YscE-50515354-R 5'-TTCCTTCTTTTTAGATTCTCTCTGCCAAAC-3'
YscE-6465-F 5'-GCTAAAGGGGGTTCTCATCATCAT-3'
YscE-6465-R 5'-TGCTCCTGCTACATAATGAATAAT-3'
SSSSSS-F 5'-AGTTCGAGTAAAGGAACCGATATCGCAGACTTA-3'
SSSSSS-R 5'-CGAACTCGAACTCATTTATTTTAGGTCTCCTGC-3'
ISSISI-F 5'-ATTAGTATCAAAGGAACCGATATCGCAGACTTA-3'
ISSISI-R 5'-CGAACTAATACTCATTTATTTTAGGTCTCCTGC-3'
IIIIII-F 5'-ATCATTATCAAAGGAACCGATATCGCAGACTTA-3'
IIIIII-R 5'-AATGATAATACTCATTTATTTTAGGTCTCCTGC-3'
SIISSI-F 5'-AGTTCGATTAAAGGAACCGATATCGCAGACTTA-3'
SIISSI-R 5'-AATGATCGAACTCATTTATTTTAGGTCTCCTGC-3'
YscF-KpnI-F 5'-TTTGGTACCCAGGTTTGGCAGAGAGAATCTAAAG-3'
YscF-1-R 5'-TTTGAATTCCATTTATTTTAGGTCTCCTGCTAC-3'

YscF-3-R 5'-TTTGAATTCGTTACTCATTATTTTAGGTCTCC-3'
YscF-6-R 5'-TTTGAATTCCTCCAGAGAAGTTACTCATTATTTTAG-3'
YscF-9-R 5'-TTTGAATTCCTTCGTAAATCCAGAGAAGTTACTC-3'
YscF-12-R 5'-TTTGAATTCATCGGTTCTTCGTAAATCCAGAG-3'
YscF-15-R 5'-TTTGAATTCGTCTGCGATATCGGTTCTTCGT-3'
YscF-20-R 5'-TTTGAATTCAGCCACCGCATCTAAGTCTGCGAT-3'
YscF-30-R 5'-TTTGAATTCGCATCGTCTGCTGGCTTCTTGAG-3'
YscF-40-R 5'-TTTGAATTCGTCTGCTATCGAGTCATTAACCGC -3'
YscF-50-R 5'-TTTGAATCTAGCGCCGGTTGTCAGGCTTATC -3'
YopM-30-F 5'-GAAAATGTTAAATCTAAGACTGAA-3'
YopM-STOP-HindIII-R 5'-TTTAAGCTTCTACTCAAATACATCATCTTCAAG -3'

Table S2. YscE-6xHis mutants

<u>YscE-6xHis mutant #</u>	<u>YscE-6xHis mutant</u>
1	YscE-6xHis (T2A Q3A L4A)
2	YscE-6xHis (E5A E6A K7A)
3	YscE-6xHis (V11A E12A)
4	YscE-6xHis (T13A V14A)
5	YscE-6xHis (R15A S16A)
6	YscE-6xHis (M19A Q20A)
7	YscE-6xHis (E22A M23A)
8	YscE-6xHis (T26A K27A)
9	YscE-6xHis (K30A D31A)
10	YscE-6xHis (M32A M33A R34A)
11	YscE-6xHis (G35K G36H D37K)
12	YscE-6xHis (K39A, Q40A)
13	YscE-6xHis (Q42A V43A)
14	YscE-6xHis (A50K L51K S53R A54R)
15	YscE-6xHis (D64A L65A)

Fig. S1. Functional analysis of YscE-6xHis point mutants.



Y. pestis KIM5-3001, an isogenic $\Delta yscE$ mutant and the $\Delta yscE$ mutant carrying plasmid pYscE-6xHis or derivatives of this plasmid expressing YscE-6xHis point mutants (Table S2) were grown in the presence (+) or absence (-) of 2.5 mM $CaCl_2$ and cell pellets and culture supernatants were analyzed by SDS-PAGE and immunoblot analysis with antibodies specific for YopM YscF and penta-His (YscE) (closed arrowheads). The YscF stabilized by YscE-6xHis (G35K G36H D37K) is marked by asterisks. The location of the YscE-6xHis (G35K G36H D37K) protein is marked by an open arrowhead.