Table S1. Strains and Plasmids Used in this Study	
Name	**Description**
Strains	
KIM5	*Y. pestis pgm*-, (pCD1+, pMT1+, pPCP1+) (1)
CC001	KIM5 *rfaL*::CM (pKD46+)
CC003	KIM5 Δ*rfaL*
CC004	CC003 Δ*yopN*
CC006	CC003 Δ*lcrQ*
AH020	KIM5 pCD1(Δ*sycE-yopE*::*kan*, Δ*yscF*) from YP656 (2)
AH021	CC003 pCD1(Δ*sycE-yopE*::*kan*, Δ*yscF*) from YP656 (2)
MEL2	KIM5 Δ*lcrQ* (3)
MEL17	KIM5 *yscU*::CM (pMM1+)
MEL18	KIM5 *yopN*- made using pMM61
MEL20	KIM5 *yscU*::CM
KIM5-3001.P61(Δ*yscF*)	Sm^r pCD1(Δ*sycE-yopE*::*kan*, Δ*yscF*), pCP1+, pMT1+ (2)
KIM5(Δ*y1324*)(Δ*ail*)	KIM5 Δ*ail*::*kan* (pCD1+, pPCP1+, pMT1+) (4)
Plasmids	
pBSCK-YscF(D46A)	Contains YscF with glutamic acid at position 46 replaced with an alanine. (2)
pBSCK-YscF(D46C)	Contains YscF with glutamic acid at position 46 replaced with an cysteine (2)
pCP20	Flippase containing plasmid used for flipping out *cat* cassette (5)
pDONR::*rfaL*	Used to complement *rfaL* mutant (6)
pKD46	Contains Lambda-red recombinase genes (5)
pLC28	Derivative of the pEP185.2 suicide vector containing *sacB* (7)
pKOBEG-sacB	Contains the *sacB* gene and Lambda-red recombinase genes expressed under the control of the arabinose-inducible pBAD promoter (8)
pMM1	pKOBEG-sacB with *npt* cassette from Tn903 replacing the *cat* cassette.
pMM54	pLC28 + *lcrQ* containing a stop frameshift mutation inserted after the 6^{th} codon (3)

pMM61	pLC28 + *yopN* containing a stop frameshift mutation inserted after the 27th nucleotide.
pMM83	YopM with a C-terminal Bla fusion (9)
pMM85	YopE with a C-terminal Bla fusion (9)

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Table S2. Primers Used in this Study

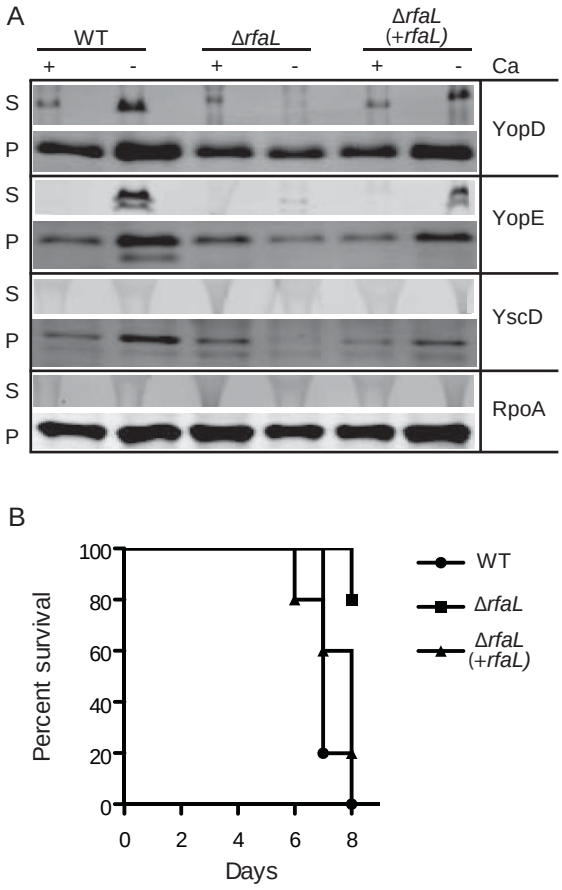
Primer name	Primer Sequence
M13 Reverse	CAGGAAACAGCTATGAC
M13(-21) Forward	TGTAAAACGACGGCCAGT
pKD13kan.P1	GTGTAGGCTGGAGCTGCTTC
pKD13kan.P4	ATTCCGGGGATCCGTCGACC
y3762-attB1	AAAAAGCAGGCTTCCTCTTTGGAAGGCTCCTTGC
y3762-attB2	AGAAAGCTGGGTCTCTGAATGTGTATTGCGTCT
y3762down	ATGATCAACATAACCCTAGT
y3762up	GCCTGAACTGGCGGTCTTAA
y3762KO-P1	TGACAACTTTATCACCGGCAATTCCGGTCAATCGCGTGTAGGCTGGAGCTGCTTC
y3762KO-P2	CGTTGGCAGCCGTTTACCTGCTATGGATTGAGCATATGAATATCCTCCTTAG
yopN.ko.Eco1	AAGAATTCGGCAATACCCCGCTGCATAATGAG
yopN.ko.Eco2	AAGAATTCATCAATAAGATAGGTTATGAAGCGTCG
yopN.ko.SalI	AAGTCGACCTCAATAGCCATATCGATCG
yopN.ko.XbaI	AATCTAGAGCAAACCGTGTTACAGAGTCC
yscU.P1	CTATAAGTTTACTGATCCCTGTTTTGGAGAAGTAATGA
yscU.P2	GTTTTAACCATATTCCTAGTTACATTGCAGCCTATTA
yscU.up	GCTTTTTCCCTGCAGTTAGT
yscU.down	GATGTTTGGTTAGAATCGGA

Fig. S1. Confirmation of *rfaL* mutant phenotypes. A) Secretion assays were performed with wild type, Δ*rfaL*, and the Δ*rfaL* (+*rfaL*) strains. Cultures were centrifuged to separate secreted proteins (S) from the cell pellet (P). Antibodies to the α subunit of the RNA polymerase (RpoA) and to YscD, a structural component of the T3SS injectisome, were used as controls for proper fractionation. **B)** 6-8 week old C57BL/6 mice were infected retro-orbitally with each strain at a dose of ~10^2 CFU. Mice were monitored until they became moribund or recovered. Moribund mice were euthanized. Survival trends were compared using the Log-rank test.

Fig. S2. Mutations in *yscF* cannot bypass the secretion defect of a Δ*rfaL* mutant. Wild type, wild type expressing YscF(D46A) or YscF(D46C), Δ*rfaL*, and Δ*rfaL* strains expressing YscF(D46A) or YscF(D46C) were grown in DMEM at 37°C with or without EGTA (-Ca or +Ca). Following the incubation the cultures were centrifuged to separate secreted proteins (S) from cells (P). The proteins in both fractions were TCA precipitated and immunoblotting was performed using antibodies against YscD, YopD, YopH, YopM, and RpoA. The < indicates full length YopH and * represents YopH degradation products.

Fig. S3. Effects of RfaL on Pla abundance and plasminogen activation. A-C) Plasminogen activation assays were performed on wild type, Δ*rfaL*, and Δ*rfaL*+*rfaL* strains cultured at 26ºC or 37ºC in the presence or absence of 2.5 mM $CaCl_2$. The assay was performed twice with samples analyzed in duplicate, and one representative trial is shown. **D)** Pla protein levels were assessed in the same strains by immunoblot analysis. Decreased abundance of autoprocessed Pla (arrow) in the Δ*rfaL* strain compared to wild-type or complement is observed. RpoA is shown as a loading control.

Fig. S4. Complement Sensitivity Assay. Wild type, Δ*rfaL,* and Δ*ail* strains were grown overnight in HIB at 26 °C. Cells were then incubated at 37 °C for 1 hour in normal human serum (NHS), heat inactivated serum (HIS), or saline (PBS). Where indicated EGTA was added to the overnight cultures as well as the serum assays. After the incubation period, cells were serially diluted and plated in triplicate on HIA. Colonies were counted and the average Log (10) CFU/mL was determined. Data shown is representative of two independent trials.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ΔrfaL (+rfaL)
+
-
+
-
+
-
Ca
S
P
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YscD
RpoA
B
Percent survival
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2
4
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Days
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ΔrfaL
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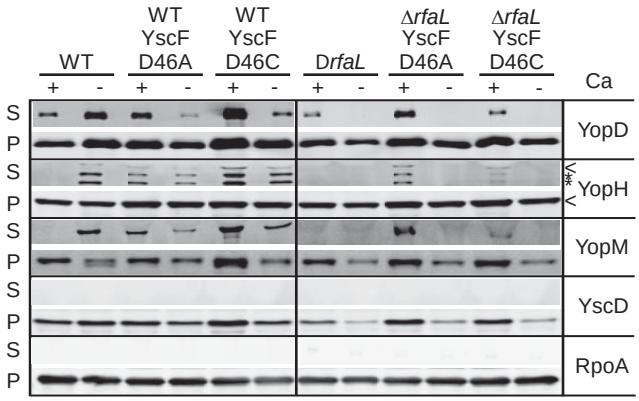
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WT YscF D46A
WT YscF D46C
DrfaL
ΔrfaL YscF D46A
ΔrfaL YscF D46C
Ca
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S
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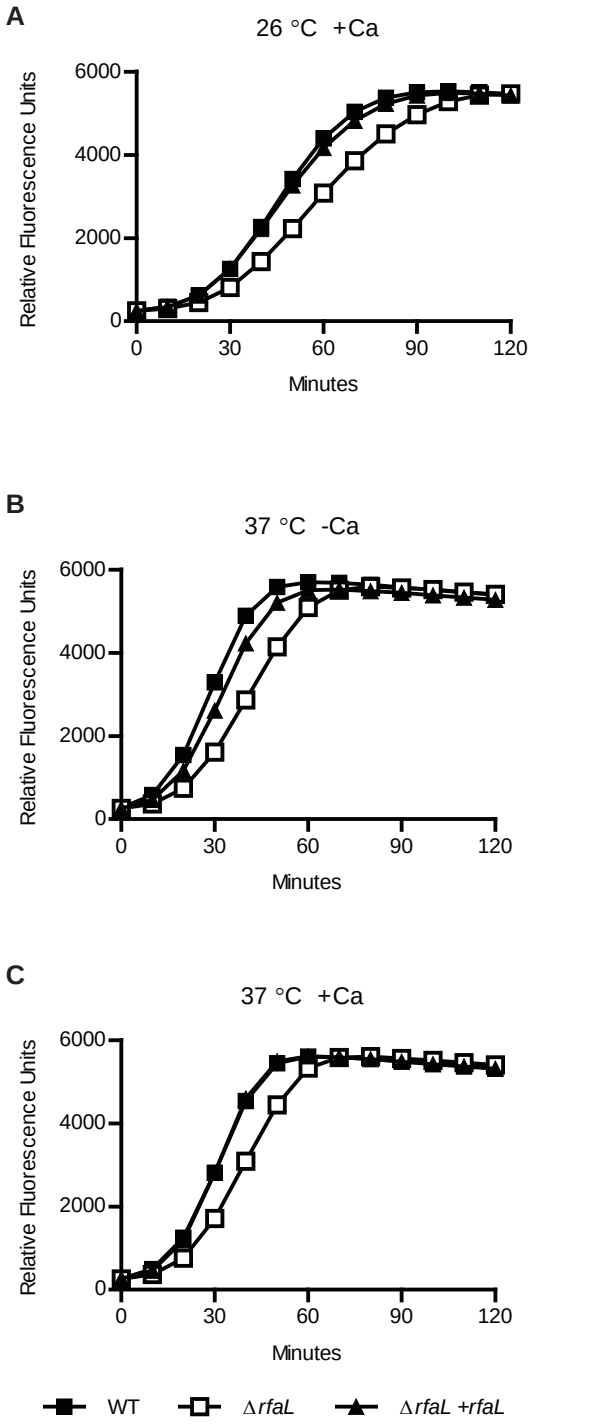
YscD
RpoA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26 °C +Ca
Relative Fluorescence Units
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2000
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0
30
60
90
120
Minutes
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37 °C -Ca
Relative Fluorescence Units
6000
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2000
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C
37 °C +Ca
Relative Fluorescence Units
6000
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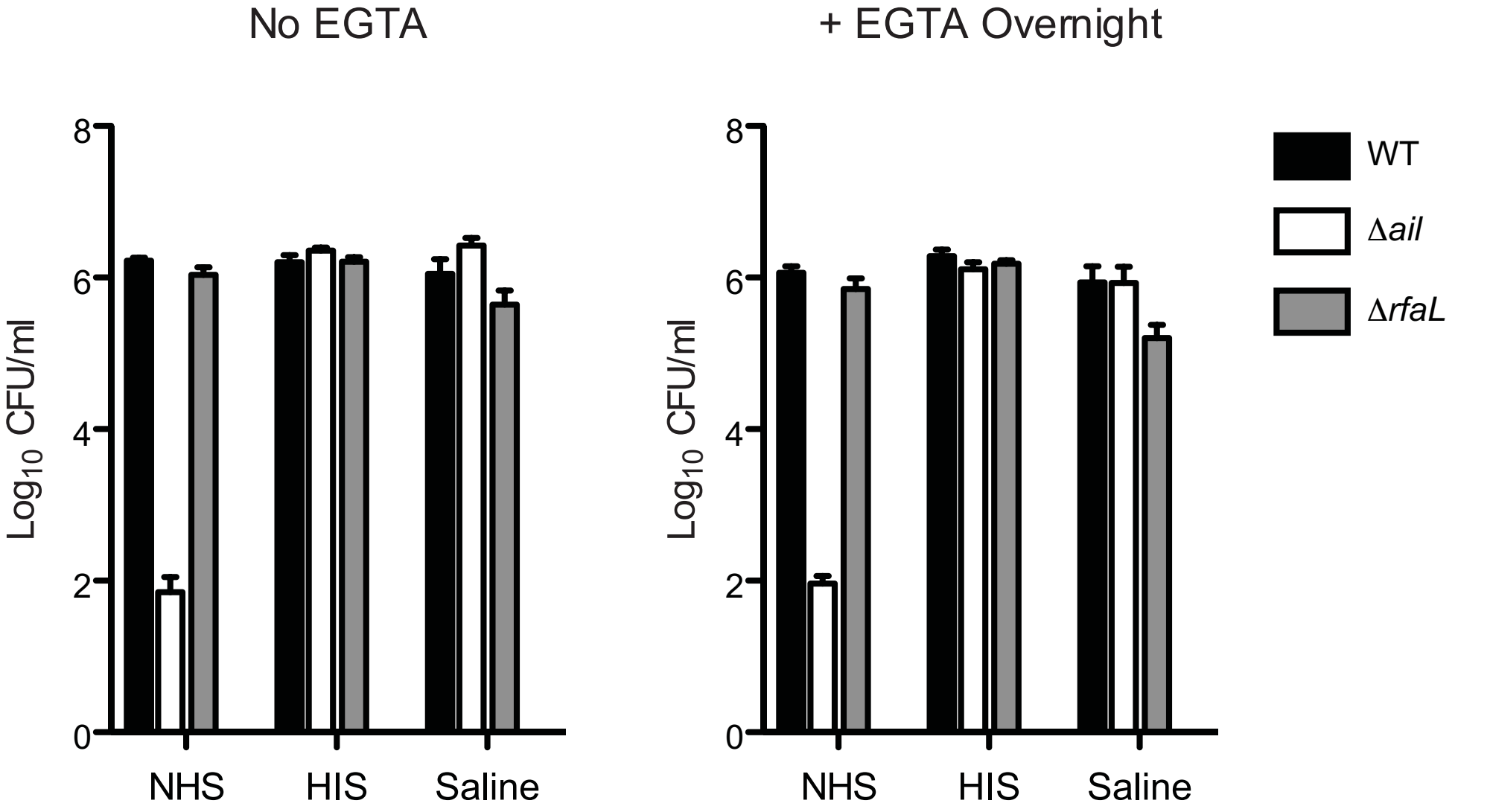
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+ EGTA Overnight
Log10 CFU/ml
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6
4
2
0
NHS
HIS
Saline
WT
Δail
ΔrfaL