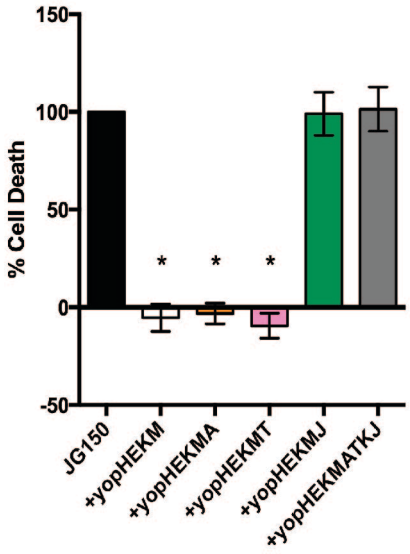

Figure S1. Cytotoxicity of *Y. pestis* T3SS mutants in immortalized macrophages. C57BL/6 immortalized macrophages (iMacs) were infected with *Y. pestis* at an MOI of 1. Loss of cell membrane integrity was monitored kinetically by assaying ethidium homodimer uptake and quantified by calculating the area under each curve (AUC), as described (see Methods of main text and (1)). Amount of cell death over 6 hours calculated by AUC was normalized to the amount of cell death caused by the T3SS-positive strain JG150 (set to 100%). Mean and standard error shown for two combined independent experiments, each with n=4. * denotes $p<0.05$ compared to JG150 (one-way ANOVA with Dunnett's correction).

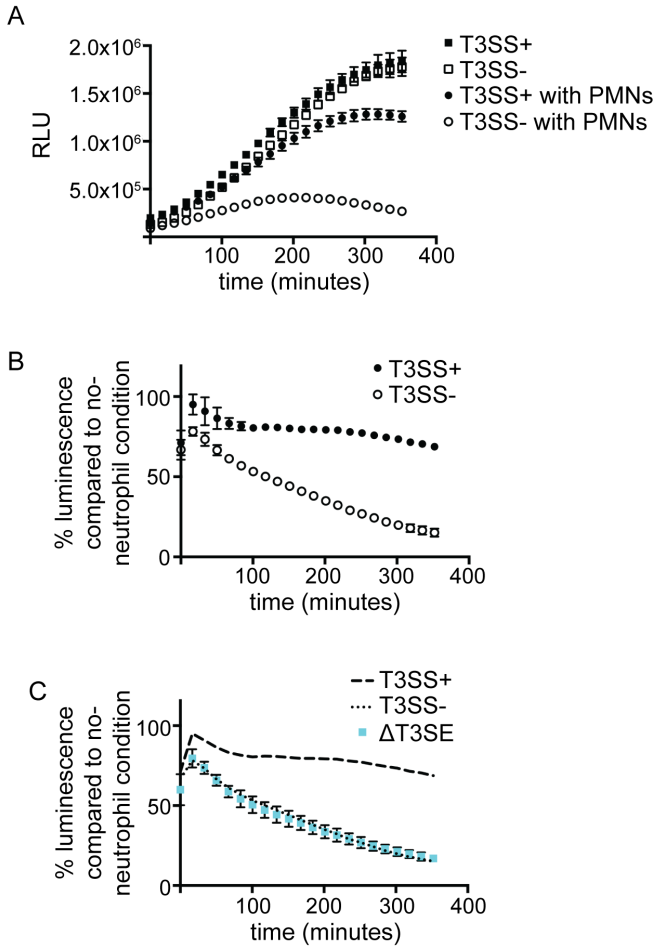

Figure S2. T3SS-dependent and effector-dependent survival of *Y. pestis* in co-culture with neutrophils. (**A**) Relative light units (RLU) of JG150L (T3SS+, shaded symbols) and JG152L (T3SS-, open symbols) in the presence (circles) and absence (squares) of primary human neutrophils. (**B**) Same data as in (A), analyzed such that the luminescence of each biological replicate in the presence of neutrophils is shown as a percentage of that replicate's luminescence in the absence of neutrophils at the same time point. (**C**) Luminescence of JG150L (T3SS+), JG152L (T3SS-), and JG150LΔT3SE (ΔT3SE) in the presence of neutrophils; data analyzed as in panel B. JG150LΔT3SE is as susceptible to neutrophil killing as JG152L. Shown: mean and standard deviation (symbols) or mean only (lines) of 3 technical replicates. Representative of 7 independent experiments.

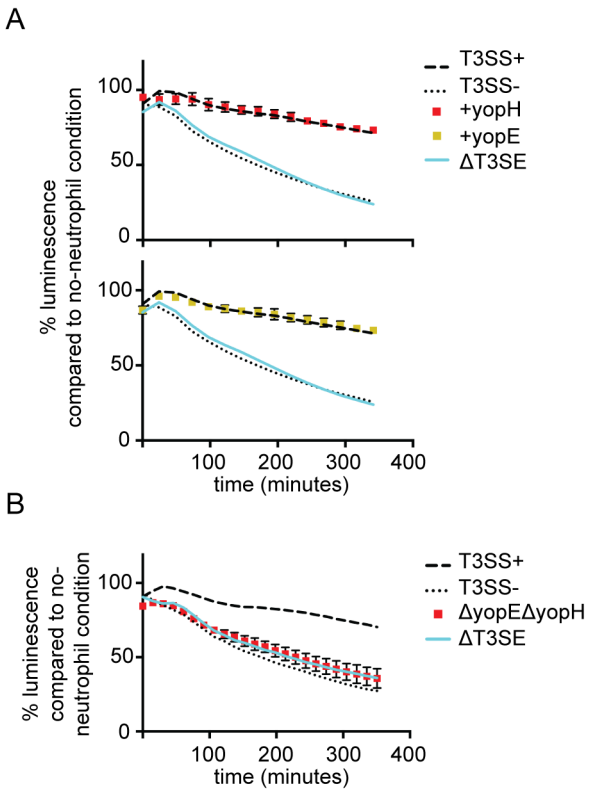

Figure S3. YopH and YopE are each sufficient to promote survival of *Y. pestis* in the presence of primary human neutrophils. (**A**) Addition of a functional *yopH* or *yopE* gene to the JG150LΔT3SE genetic background rescues bacterial survival to wild-type levels. (**B**) A strain carrying deletions of both the *yopH* and *yopE* genes from the JG150L genetic background (ΔyopEH) is as sensitive to neutrophil killing as JG150LΔT3SE (ΔT3SE). Shown: mean and standard deviation (symbols) or mean only (lines) of 3 replicates. Representative of 2 independent experiments.

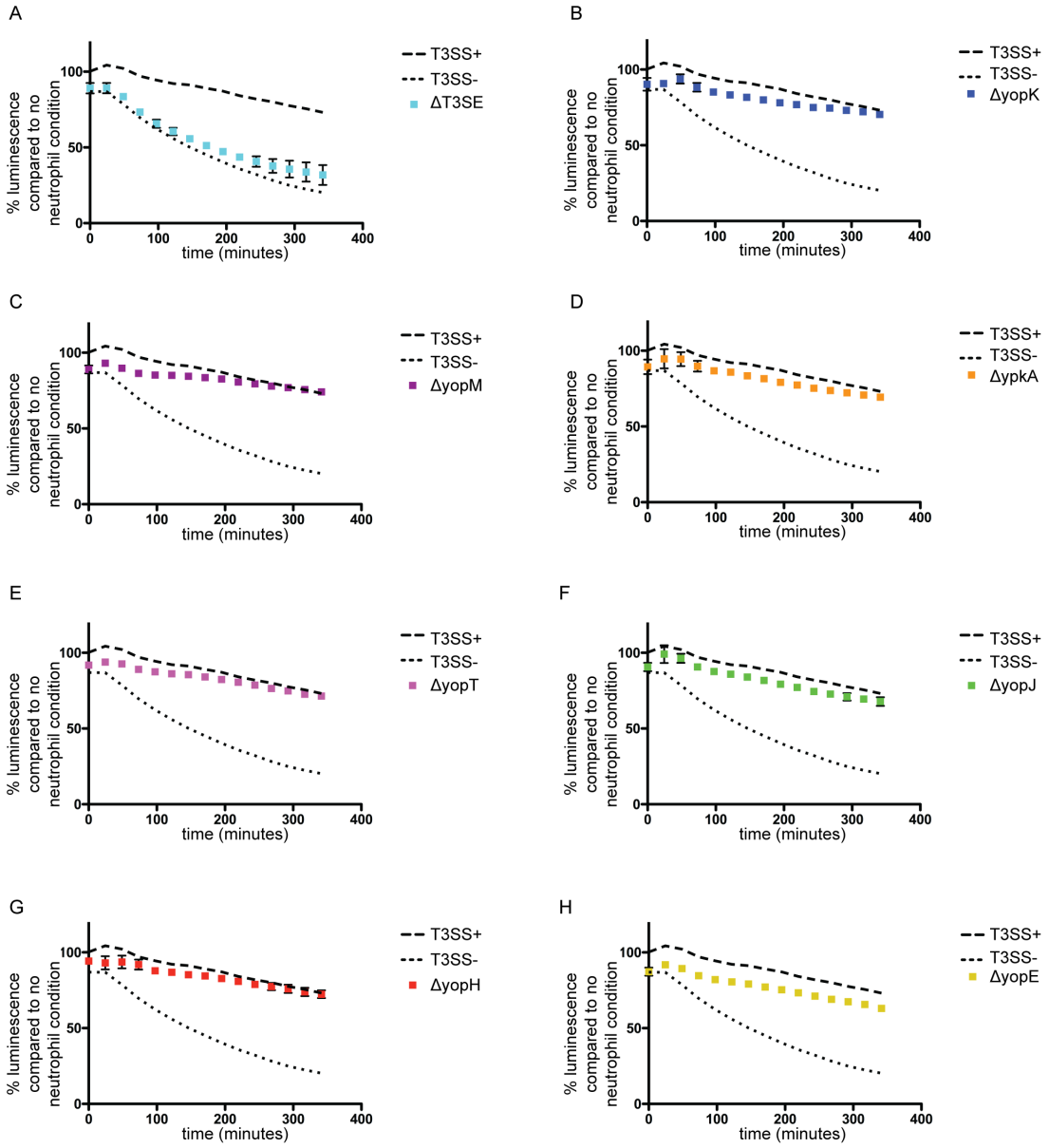

Figure S4. No single T3SS effector is required for survival of *Y. pestis* in co-culture with primary human neutrophils. Although a strain expressing no effectors is susceptible to killing by neutrophils (**A**, and see Figure S2C), deletion of any single effector from JG150L does not enhance bacterial killing (**B**, *yopK* deletion; **C**, *yopM* deletion; **D**, *ypkA* deletion; **E**, *yopT* deletion; **F**, *yopJ* deletion; **G**, *yopH* deletion; **H**, *yopE* deletion). Shown: mean and standard deviation (symbols) or mean only (lines) of 3 replicates. Representative of 2 independent experiments.

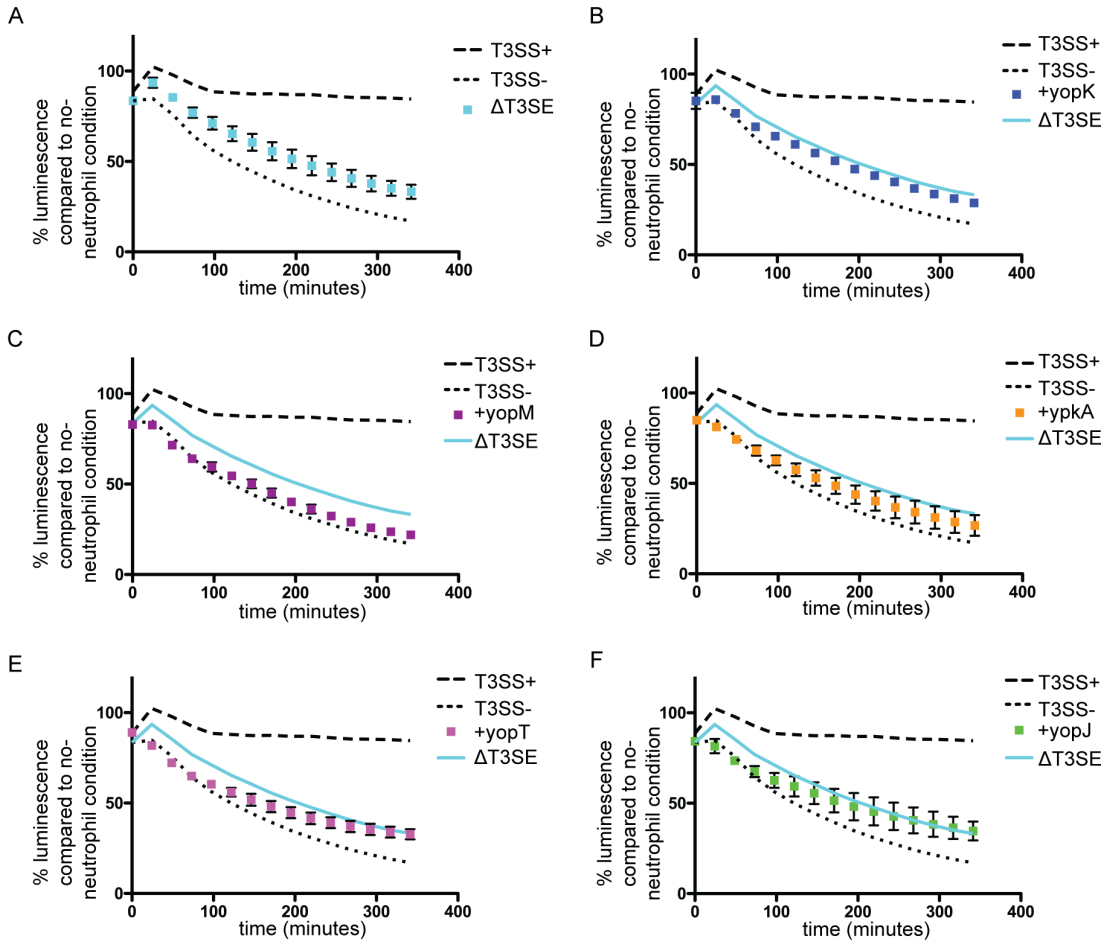

Figure S5. Unlike YopH and YopE, other T3SS effectors are not sufficient to prevent bacterial killing by primary human neutrophils. Addition of a functional *yopH* or *yopE* gene to the JG150LΔT3SE genetic background rescues bacterial survival to wild-type levels (Figure S3A). By contrast, addition of any other single effector to JG150LΔT3SE does not increase bacterial survival (**A**, no effectors; **B**, *yopK* alone; **C**, *yopM* alone; **D**, *ypkA* alone; **E**, *yopT* alone; **F**, *yopJ* alone). Shown: mean and standard deviation (symbols) or mean only (lines) of 3 replicates. Representative of 2 independent experiments.

Table S1. *Y. pestis* strains and relevant characteristics.

Descriptive name	Genotype	Strain number	Relevant characteristics	Source
KIM1001	*pgm*$^{+}$/pMT1^{+} pCD1^{+} pPCP1^{+}		Fully virulent strain reconstructed from KIM10	(2)
JG150A	Δ*pgm*/pMT1^{+} pCD1^{+} pPCP1^{+}		Spontaneous Δ*pgm* derivative of KIM1001	(3)
JG150L	Δ*pgm*/pMT1^{+} pCD1^{+} pPCP1^{+} pML001^{+}	JG598	Luminescent version of JG150A	This work
JG152L	Δ*pgm*/pMT1^{+} pCD1^{-} pPCP1^{+} pML001^{+}	JG597	Spontaneous pCD1^{-} derivative of JG150A, luminescent	This work
KIM1001ΔT3SE	*pgm*$^{+}$/pMT1^{+} pCD1(*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG917	In-frame deletions of all seven effector proteins	This work and used in (1)
JG150LΔT3SE	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+} pML001^{+}	JG715	Δ*pgm* and luminescent derivative of JG917	This work
KIM1001ΔT3SE:: +*yopHE*	*pgm*$^{+}$/pMT1^{+} pCD1 (*yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG918	Derivative of JG917 in which the functional ORFs for *yopH* and *yopE* have been restored	This work
KIM1001ΔT3SE:: +*yopHEK*	*pgm*$^{+}$/pMT1^{+} pCD1 (*yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG919	Derivative of JG918 in which the functional ORF for *yopK* has been restored	This work
KIM1001ΔT3SE:: +*yopHEM*	*pgm*$^{+}$/pMT1^{+} pCD1(*yopK*$^{\Delta 4\text{-}181}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG920	Derivative of JG918 in which the functional ORF for *yopM* has been restored	This work

Table S1. *Y. pestis* strains and relevant characteristics (continued)

Descriptive name	Genotype	Strain number	Relevant characteristics	Source
KIM1001ΔT3SE:: +*yopHEKM*	*pgm*$^{+}$/pMT1^{+} pCD1 (*ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG921	Derivative of JG919 in which the functional ORF for *yopM* has been restored	This work
KIM1001ΔT3SE:: +*yopHEKMA*	*pgm*$^{+}$/pMT1^{+} pCD1 (*yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG922	Derivative of JG921 in which the functional ORF for *ypkA* has been restored	This work
KIM1001ΔT3SE:: +*yopHEKMT*	*pgm*$^{+}$/pMT1^{+} pCD1 (*ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$) pPCP1^{+}	JG923	Derivative of JG921 in which the functional ORF for *yopT* has been restored	This work
KIM1001ΔT3SE:: +*yopHEKMJ*	*pgm*$^{+}$/pMT1^{+} pCD1 (*ypkA*$^{\Delta 3\text{-}731}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG924	Derivative of JG921 in which the functional ORF for *yopJ* has been restored	This work
KIM1001ΔT3SE:: +*yopHEKMJT*	*pgm*$^{+}$/pMT1^{+} pCD1 (*ypkA*$^{\Delta 3\text{-}731}$) pPCP1^{+}	JG925	Derivative of JG924 in which the functional ORF for *yopT* has been restored	This work
KIM1001ΔT3SE:: +*yopHEKMAJ*	*pgm*$^{+}$/pMT1^{+} pCD1 (*yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG926	Derivative of JG922 in which the functional ORF for *yopJ* has been restored	This work
KIM1001ΔT3SE:: +*yopHEKMAT*	*pgm*$^{+}$/pMT1^{+} pCD1 (*yopJ*$^{\Delta 4\text{-}288}$) pPCP1^{+}	JG927	Derivative of JG922 in which the functional ORF for *yopT* has been restored	This work
KIM1001ΔT3SE:: +*yopHEKMAJT*	*pgm*$^{+}$/pMT1^{+} pCD1^{+} pPCP1^{+}	JG928	Derivative of JG926 in which the functional ORF for *yopT* has been restored, fully complemented strain with respect to JG917	This work
JG150ΔT3SE:: +*yopHEKM*	Δ*pgm*/pMT1^{+} pCD1 (*ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG911	Δ*pgm* derivative of JG921	This work

Table S1. *Y. pestis* strains and relevant characteristics (continued)

Descriptive name	Genotype	Strain number	Relevant characteristics	Source
JG150ΔT3SE::+*yopHEKMA*	Δ*pgm*/pMT1^{+} pCD1 (*yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG912	Δ*pgm* derivative of JG922	This work
JG150ΔT3SE::+*yopHEKMT*	Δ*pgm*/pMT1^{+} pCD1 (*ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$) pPCP1^{+}	JG913	Δ*pgm* derivative of JG923	This work
JG150ΔT3SE::+*yopHEKMJ*	Δ*pgm*/pMT1^{+} pCD1 (*ypkA*$^{\Delta 3\text{-}731}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG914	Δ*pgm* derivative of JG924	This work
JG150ΔT3SE::+*yopHEKMAJT*	Δ*pgm*/pMT1^{+} pCD1^{+} pPCP1^{+}	JG915	Δ*pgm* derivative of JG928	This work
JG150LΔ*yopH*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$) pPCP1^{+} pML001^{+}	JG673	In-frame deletion of *yopH*; luminescent	This work
JG150LΔ*yopE*	Δ*pgm*/pMT1^{+} pCD1 (*yopE*$^{\Delta 40\text{-}197}$) pPCP1^{+} pML001^{+}	JG674	In-frame deletion of *yopE*; luminescent	This work
JG150LΔ*yopK*	Δ*pgm*/pMT1^{+} pCD1 (*yopK*$^{\Delta 4\text{-}181}$) pPCP1^{+} pML001^{+}	JG675	In-frame deletion of *yopK*; luminescent	This work
JG150LΔ*yopM*	Δ*pgm*/pMT1^{+} pCD1 (*yopM*$^{\Delta 3\text{-}408}$) pPCP1^{+} pML001^{+}	JG679	In-frame deletion of *yopM*; luminescent	This work
JG150LΔ*ypkA*	Δ*pgm*/pMT1^{+} pCD1 (*ypkA*$^{\Delta 3\text{-}731}$) pPCP1^{+} pML001^{+}	JG677	In-frame deletion of *ypkA*; luminescent	This work
JG150LΔ*yopT*	Δ*pgm*/pMT1^{+} pCD1 (*yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+} pML001^{+}	JG676	In-frame deletion of *yopT*; luminescent	This work
JG150LΔ*yopJ*	Δ*pgm*/pMT1^{+} pCD1 (*yopJ*$^{\Delta 4\text{-}288}$) pPCP1^{+} pML001^{+}	JG678	In-frame deletion of *yopJ*; luminescent	This work
JG150ΔT3SE::+*yopH*	Δ*pgm*/pMT1^{+} pCD1 (*yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG734	Derivative of JG150ΔT3SE in which the functional ORF for *yopH* has been restored	This work
JG150LΔT3SE::+*yopH*	Δ*pgm*/pMT1^{+} pCD1 (*yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+} pML001^{+}	JG680	Luminescent version of JG734	This work

Table S1. *Y. pestis* strains and relevant characteristics (continued)

Descriptive name	Genotype	Strain number	Relevant characteristics	Source
JG150ΔT3SE::+*yopE*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG733	Derivative of JG150ΔT3SE in which the functional ORF for *yopE* has been restored	This work and used in (1, 4)
JG150LΔT3SE::+*yopE*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+} pML001^{+}	JG681	Luminescent version of JG733	This work
JG150ΔT3SE::+*yopK*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG736	Derivative of JG150ΔT3SE in which the functional ORF for *yopK* has been restored	This work and used in (1)
JG150LΔT3SE::+*yopK*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+} pML001^{+}	JG682	Luminescent version of JG736	This work
JG150ΔT3SE::+*yopM*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG732	Derivative of JG150ΔT3SE in which the functional ORF for *yopM* has been restored	This work and used in (4)
JG150LΔT3SE::+*yopM*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+} pML001^{+}	JG683	Luminescent version of JG732	This work
JG150ΔT3SE::+*ypkA*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG730	Derivative of JG150ΔT3SE in which the functional ORF for *ypkA* has been restored	This work
JG150LΔT3SE::+*ypkA*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *yopJ*$^{\Delta 4\text{-}288}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+} pML001^{+}	JG684	Luminescent version of JG730	This work
JG150ΔT3SE::+*yopT*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$) pPCP1^{+}	JG708	Carries in-frame deletions of all effectors except for *yopT*	This work

Table S1. *Y. pestis* strains and relevant characteristics (continued)

Descriptive name	Genotype	Strain number	Relevant characteristics	Source
JG150LΔT3SE::+*yopT*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopJ*$^{\Delta 4\text{-}288}$) pPCP1^{+} pML001^{+}	JG685	Luminescent version of JG708	This work
JG150ΔT3SE::+*yopJ*	Δ*pgm*/pMT1^{+} pCD1 (*yopH*$^{\Delta 3\text{-}467}$ *yopE*$^{\Delta 40\text{-}197}$ *yopK*$^{\Delta 4\text{-}181}$ *yopM*$^{\Delta 3\text{-}408}$ *ypkA*$^{\Delta 3\text{-}731}$ *yopT*$^{\Delta 3\text{-}320}$) pPCP1^{+}	JG735	Derivative of JG150ΔT3SE in which the functional ORF for *yopJ* has been restored	This work

Table S2. Primers used to construct and complement *Y. pestis* T3SS mutants

Primer Name	Sequence 5' to 3'
yopM-A	ATAGAGCTCTTCAAAAGGGGTACTGGATAC
yopM-B	GAACATATTGAATGCCTTTCT
yopM-C	AGAAAGGCATTCAATATGTTCGAGTAGTACGCAAGAGCGTTC
yopM-D	GGGTCTAGATTTACCAATTTTTTGATGGGG
yopJ-A	ATAGAGCTCCACTACTGATTCAACTTGGACG
yopJ-B	ACGGCAAATGCAGAGCAGTCCGATCATTTATTTATCCTTATTCA
yopJ-C	CTGCTCTGCATTTGCCGTTAATGTATTTTGGAAATCTTGCT
yopJ-D	GGGTCTAGACTGATGTCGTTTATTTCTGGGTAT
yopE-A	ATAGAGCTCAGCATTACACACTCCACAGTTGGGT
yopE-B	ACGCAGGCAGCAAATGAGATCAAA
yopE-C	CTCATTTGCTGCCTGCGTATATTGATCACTTGTTTG
yopE-D	ATATCTAGATATCCAGGCTGTTCAATGGTTGTCGAT
yopH-A	ATAGAGCTCAACTGCATCCGTCCGGTG
yopH-B	GTTCATGCTTCCCTCCTT
yopH-C	AAGGAGGGAAGCATGAACAGCTAATGTAAATATTTATTCCTATGA
yopH-D	GGGTCTAGACGGTGGTACTAAAAATAGGAGGGA
ypkA-A	ATAGAGCTCGGGACGGGTTTAATCAATGAT
ypkA-B	TTTCATGCTTTACTCATCCCC
ypkA-C	GGGGATGAGTAAAGCATGAAAATGTGACAAGTGCCCCCTAAG
ypkA-D	GGGTCTAGAGATCCATCCGATATATCAGTTTCC
yopK-A	GGGGAGCTCTGTTAGCCATTATTTTGCTATAC
yopK-B	ACGGCAAATGCAGAGCAGAATAAACATAGTTACTACTCCCAAA
yopK-C	CTGCTCTGCATTTGCCGTGGATGAAGCTATATTAAAGAGTT
yopK-D	ATATCTAGACATTTAAAACAGGGCATGG
yopT-A	ATAGAGCTCTAACTTTGTATGGTACCGCGT
yopT-B	GTTTAATGCAGACAACCTTCAC
yopT-C	GTGAAGGTTGTCTGCATTAAACGTTCATCTGTATAACCTATTTATGTTAGC
yopT-D	AAATCTAGACTCAATGAGCTTCCCAT

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