## Supplemental materials

### Supplemental figure legends

**Figure S1. Secretion assay of strains expressing the YopD-Bla reporter.** Plasmids carrying YopD-Bla were transformed into WT and mutant *Y. pestis* strains. Secretion assays were performed to induce expression of the TTSS, and secreted proteins (S) were separated from cells (P) by centrifugation, followed by TCA precipitation and immunoblotting. Antibodies to YopK, YopE, YopM, and YopH show expressed and secreted late Yops. YscD (a structural bacterial protein) is a fractionation control. Bla and YopD antibodies recognize the YopD-Bla reporter as well as the native proteins. Note that secreted full-length Yops (➔) show degradation products (*), as the Pla protease is present.

**Figure S2. Characterizing middle Yop reporters.** *Y. pestis* strains carrying either the YopB-Bla or YopD-Bla reporter as well as arabinose inducible YopJ-GSK were used to infect CHO cells at an MOI of 10 in the presence or absence of arabinose. After 3 hours, cells were lysed and analyzed by SDS-PAGE. β-lactamase (Bla) antibody shows the expression of each reporter fusion and actin probed as a loading control. The arrowhead (➔) indicates Bla and GSK reporters while injection of the YopJ-Gsk reporter is shown by a check (✓).

**Figure S3. Secretion assay of strains expressing the YopK point mutants and YopD-Bla reporter.** WT and mutant *Y. pestis* strains expressing YopK or YopK point mutants as well as the YopD-Bla reporter were induced to secrete with low calcium and temperature shift to 37°C. Secreted proteins (S) were separated from cells (P) by centrifugation, followed by TCA precipitation and immunoblotting. Antibody raised against YopK shows expression of native YopK as well as each point mutant derivative. YscD (a structural bacterial protein) is a fractionation control. Note that secreted full-length Yops (➔) show degradation products (*), as the Pla protease is present.

**Figure S4. Secretion assay of strains expressing the YopK point mutants and YopB-Bla reporter.** WT and mutant *Y. pestis* strains expressing YopK or YopK point mutants as well as the YopB-Bla reporter were induced to secrete with low calcium and temperature shift to 37°C. Secreted proteins (S) were separated from cells (P) by centrifugation, followed by TCA precipitation and immunoblotting. Antibody raised against YopK shows expression of native YopK as well as each point mutant derivative. YscD (a structural bacterial protein) is a fractionation control. Note that secreted full-length Yops (➔) show degradation products (*), as the Pla protease is present.

**Figure S1.**

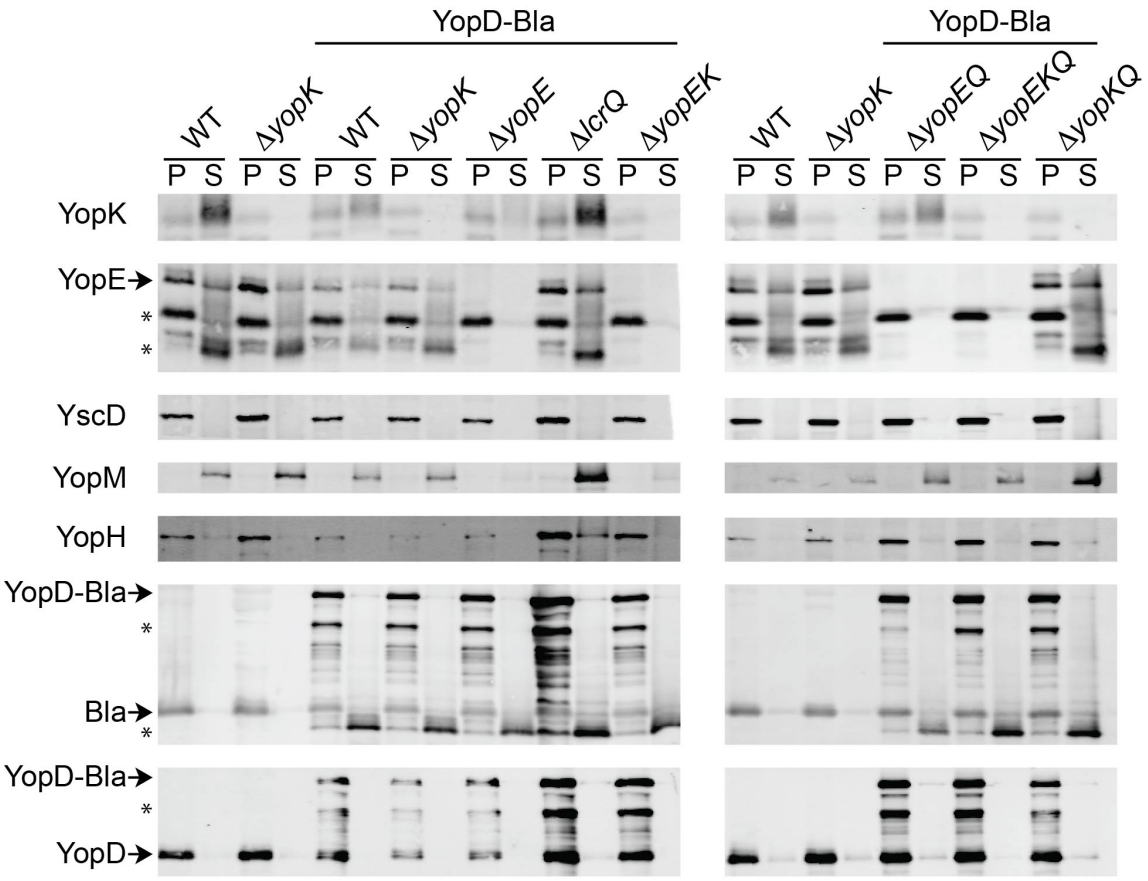
YopD-Bla
YopD-Bla
WT
ΔyopK
WT
ΔyopK
ΔyopE
ΔlcrQ
ΔyopEK
WT
ΔyopK
ΔyopEQ
ΔyopEKQ
ΔyopKQ
P S P S P S P S P S P S P S P S P S P S P S
YopK
YopE
*
*
YscD
YopM
YopH
YopD-Bla
*
Bla
*
YopD-Bla
*
YopD

**Figure S2.**

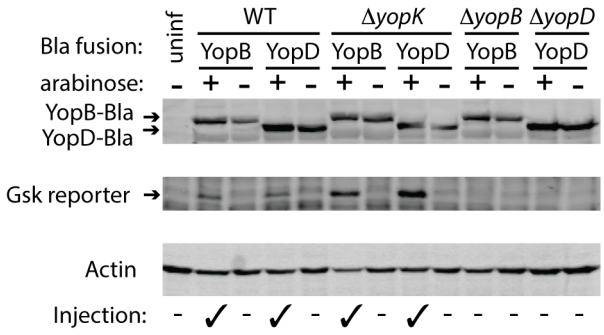
uninf
WT
ΔyopK
ΔyopB
ΔyopD
Bla fusion:
YopB
YopD
YopB
YopD
YopB
YopD
arabinose:
- + - + - + - + - + - +
YopB-Bla
YopD-Bla
Gsk reporter
Actin
Injection:
- ✓ - ✓ - ✓ - ✓ - - - - -

**Figure S3.**

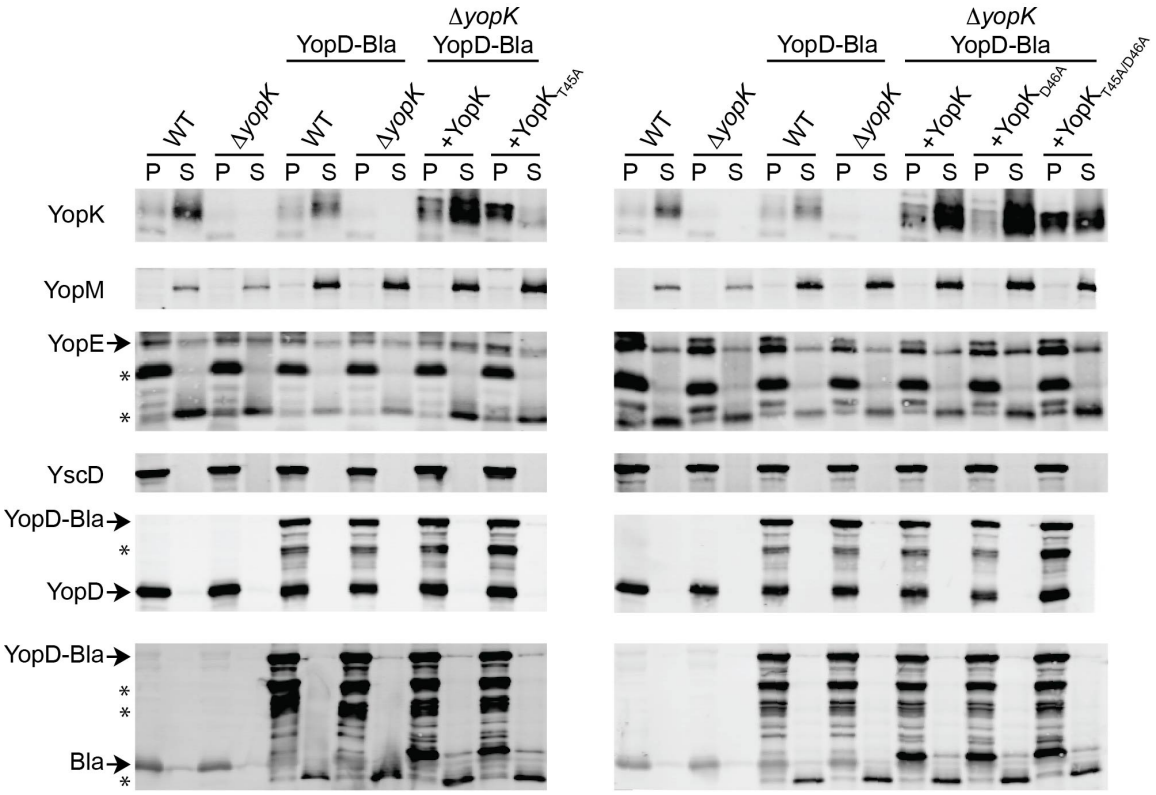
YopD-Bla
ΔyopK YopD-Bla
WT
ΔyopK
+YopK
+YopK T145A
+YopK D46A
+YopK T145A/D46A
P S
YopK
YopM
YopE
YscD
YopD-Bla
YopD
Bla
*

**Figure S4.**

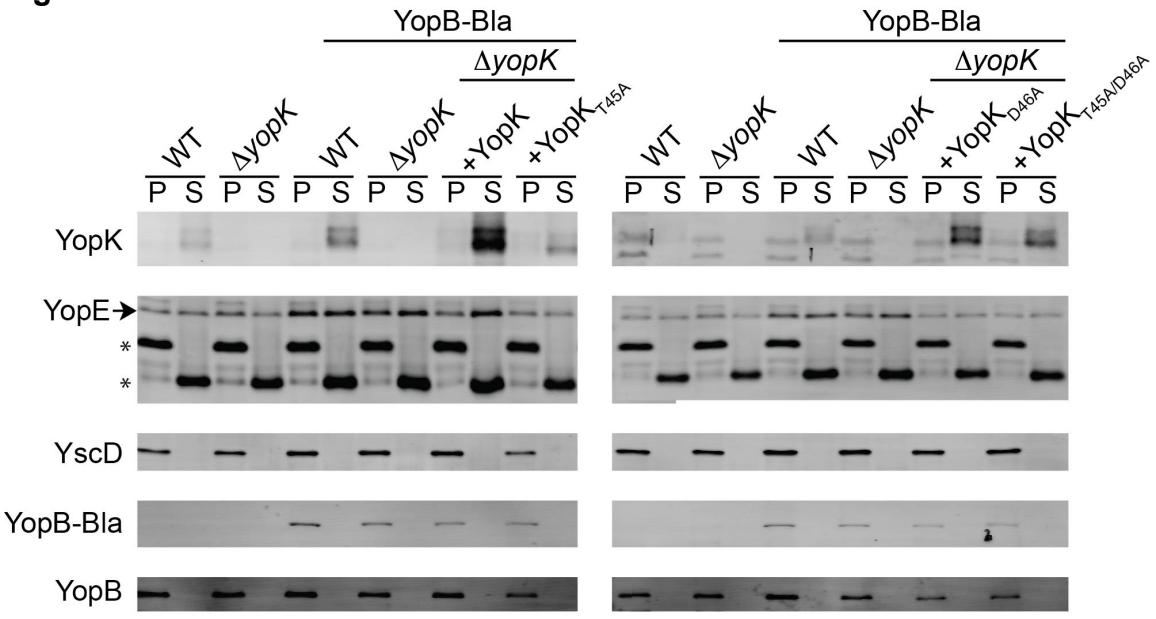
YopB-Bla
ΔyopK
YopB-Bla
ΔyopK
WT
ΔyopK
WT
ΔyopK
+YopK
+YopK T45A
WT
ΔyopK
WT
ΔyopK
+YopK D46A
+YopK T45A/D46A
P S P S P S P S P S P S
P S P S P S P S P S P S
YopK
YopE
*
*
YscD
YopB-Bla
YopB

**Table S1. Plasmids used in this study.**

| | | |
|---|---|---|
| pRD206 | *yopK* under native promoter on pUC19 backbone | (Dewoody *et al.*, 2011) |
| pRD12 | pMM206 with T45A/D46A mutations to YopK | This work |
| pRD13 | pMM206 with T45A mutation to YopK | This work |
| pRD15 | pMM206 with D46A mutation to YopK | This work |
| pMM115 | YopB-Bla | This work |
| pMM117 | YopD-Bla | This work |
| pMM91 | GST with C-terminal Bla fusion expressed from *yopM* promoter on pHSG576 backbone | (Marketon *et al.*, 2005) |
| pMM83 | YopM with C-terminal Bla fusion expressed from *yopM* promoter on pHSG576 backbone | (Marketon et al., 2005) |
| pMM84 | YopK-Bla with YopK promoter on pHSG576 backbone | This work |
| pRD17 | pMM84 with T45A/D46A mutations to YopK | This work |
| pRD22 | pMM84 with T45A mutations to YopK | This work |
| pRD24 | pMM84 with D46A mutations to YopK | This work |
| phmKeima-Red-MClinker | humanized monomeric Keima-Red fluorescent protein expression vector | MBL International Corp |
| pRD1 | *yopK* ORF inserted as a C-terminal fusion to *keima* | (Dewoody et al., 2011) |
| pUC19 | Bacterial cloning vector, high copy number | Fermentas Life Sciences |
| pHSG576 | Low-copy-number bacterial expression vector | (Takeshita *et al.*, 1987) |
| pRD16 | pRD1 with T45A and D46A mutations | This work |
| pRD19 | pRD1 with D46A mutation | This work |
| pRD21 | pRD1 with T45A mutation | This work |
| pMM112 | YopJ-Bla fusion expressed from yopN promoter on pHSG576 backbone | (Dewoody et al., 2011) |
| YopJ-GSK | $YopJ_{1-288}$-GSK on the pBAD30 expression vector | (Garcia *et al.*, 2006) |

**Table S2. Strains and cell lines used in this study.**

| | | |
|---|---|---|
| KIM5 | Wild-type parent strain, attenuated *Y. pestis* mediaevalis strain lacking the *pgm* locus | (Brubaker, 1969) |
| MEL27 | Δ*yopK* | (Dewoody et al., 2011) |
| MEL19 | Δ*yopE* | (Dewoody et al., 2011) |
| MEL2 | Δ*lcrQ* | (Sorg *et al.*, 2005) |
| DEW1 | Δ*yopEK* | (Dewoody et al., 2011) |
| PMY1 | Δ*yopE*, Δ*lcrQ* | This work |
| PMY2 | Δ*yopEK,* Δ*lcrQ* | This work |
| PMY3 | Δ*yopK*, Δ*lcrQ* | This work |
| PMY4 | Δ*yopB* | This work |
| MEL18 | Δ*yopN* | (Lee *et al.*, 1998) |
| PMY12 | Δ*yopD* | This work |
| | | |
| CMV-*bla* CHO-K1 | CHO cells constitutively expressing β-lactamase | Invitrogen |
| CHO-K1 | Chinese hamster ovary eukaryotic cell line | ATCC |

Citations:


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Garcia, J. T., F. Ferracci, M. W. Jackson, S. S. Joseph, I. Pattis, L. R. W. Plano, W. Fischer & G. V. Plano, (2006) Measurement of effector protein injection by type III and type IV secretion systems by using a 13-residue phosphorylatable glycogen synthase kinase tag. *Infect Immun* **74**: 5645-5657.

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Marketon, M. M., R. W. DePaolo, K. L. DeBord, B. Jabri & O. Schneewind, (2005) Plague bacteria target immune cells during infecion. *Science* **309**: 1739-1741.

Sorg, J. A., N. C. Miller, M. M. Marketon & O. Schneewind, (2005) Rejection of impassable substrates by *Yersinia* type III secretion machines. *Journal of Bacteriology* **187**: 7090-7102.

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