

Figure Legends for Supplemental Figures

Fig. S1. Translocation of Bla reporters is increased in the *yopK* mutant.

Plasmids carrying YopN-Bla, YopJ-Bla, YopM-Bla, or YopH-Bla were introduced into WT, $\Delta yopK$, and $\Delta yscU$ *Y. pestis*. Each strain was then used to infect CHO cells at an MOI=10 for 3 hours, followed by CCF2-AM staining and flow cytometry. White bars: green (uninjected) cells, gray bars: aqua (low-level injection) cells, black bars: blue (high-level injection) cells.

Fig. S2. Gst-YopK is expressed but not secreted by *Y. pestis*. Plasmids carrying Gst-YopK or YopK were transformed into WT and $\Delta yopK$ *Y. pestis* carrying either YopM-Bla or Gst-Bla. 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 detect native YopK as well as Gst-YopK. The YopM antibody recognized native YopM as well as YopM-Bla. $\alpha YscD$ (a component of the TTSS machinery) was used as a fractionation control.

Fig. S3. Secretion profile of *Y. pestis* mutant strains. WT or mutant *Y. pestis* strains carrying the YopM-Bla reporter were induced to secrete Yops (S) that were separated by centrifugation from cells (P), TCA precipitated and immunoblotted. $\alpha YopH$, $\alpha YopK$ and $\alpha YopE$ were used to confirm deletions while $\alpha YopM$ recognized native YopM and the YopM-Bla reporter. $\alpha YscD$ was utilized as a fractionation control.

Fig. S4. Complementation of $\Delta yopE$. *Y. pestis* strains carrying the YopM-Bla reporter were transformed with plasmids expressing either native YopE or a GAP deficient YopE (YopE_{R144A}) and induced to secrete Yops (S) that were separated by centrifugation from cells (P). Samples were TCA precipitated and immunoblotted with $\alpha YopE$ to show complementation while $\alpha YopM$ recognized native YopM and the YopM-Bla reporter and $\alpha YscD$ was used as a fractionation control. * denotes degradation products of YopE due to Pla protease.

Fig. S5. Complementation of *Y. pestis* mutant strains with YopK or YopE. *Y. pestis* strains carrying the YopM-Bla reporter were transformed with plasmids expressing either native YopK or YopE. Strains were induced to secrete Yops (S) that were separated by centrifugation from cells (P), TCA precipitated and immunoblotted. Antibodies raised against YopK and YopE were used to confirm complementation while $\alpha YopM$ recognized native YopM and the YopM-Bla reporter. $\alpha YscD$ was used as a fractionation control. * denotes degradation products of YopE due to Pla protease.









