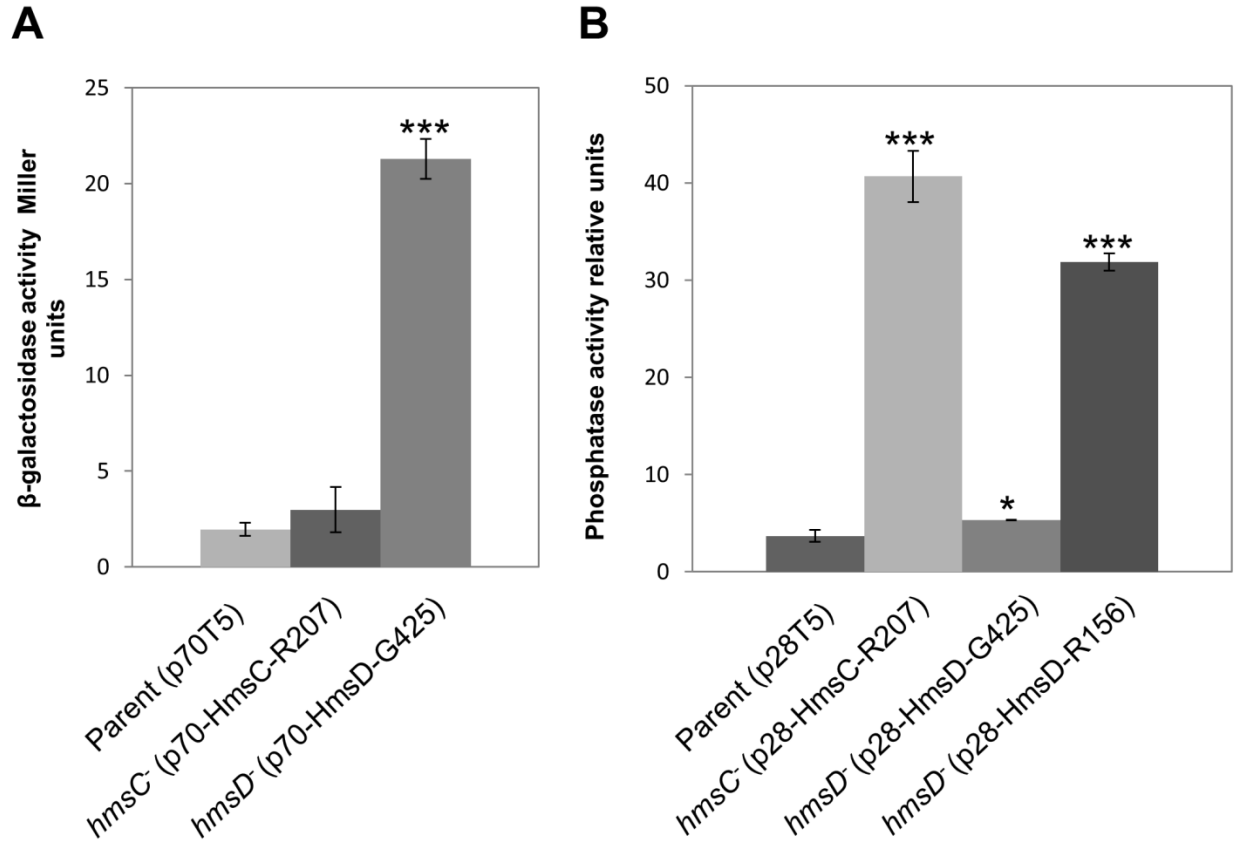


**The *Yersinia pestis* HmsCDE regulatory system is essential for blockage of the oriental rat flea (*Xenopsylla cheopis*), a classic plague vector**

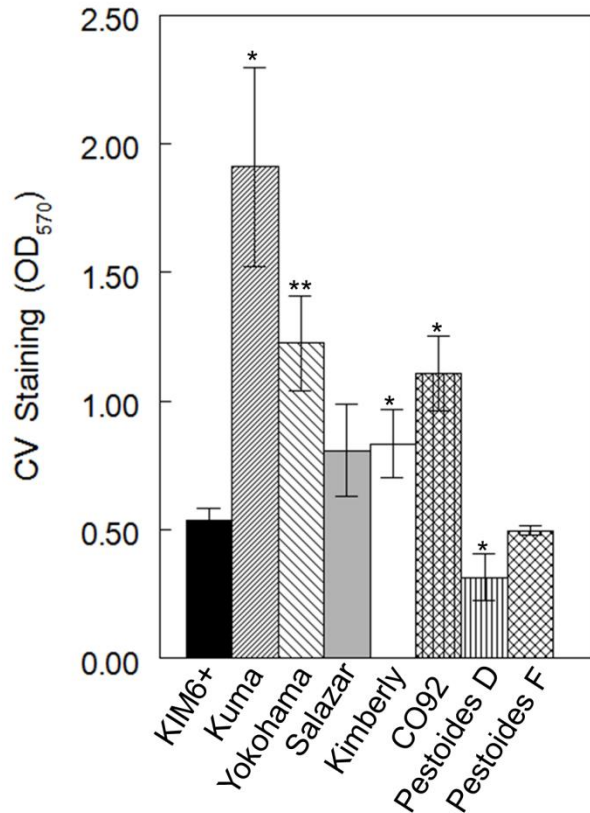
Alexander G. Bobrov, Olga Kirillina Viveka Vadyvaloo, Benjamin J. Koestler, Angela K. Hinz, Dietrich Mack, Christopher M. Waters, and Robert D. Perry

**SUPPORTING INFORMATION**

**SUPPORTING FIGURES**



**Fig. S1. Enzymatic activities of Hms-β-gal and Hms-PhoA fusion proteins.** β-Gal (A) and PhoA (B) enzyme assays were performed with permeabilized cells of *Y. pestis* strains KIM6+ (parent), KIM6-2173.1+ (*hmsC*<sup>-</sup>) and KIM6-2159.1+ (*hmsD*<sup>-</sup>) carrying the indicated plasmids. HmsC and HmsD fusions were expressed from a T5 promoter. Vector plasmids for *lacZ* and *phoA* gene fusions are p70T5 and p28T5, respectively. Fusion sites are indicated by the residue number of the relevant protein (HmsC or HmsD). Results are averages of duplicate assays from two independent cultures. Error bars indicate standard deviations. The asterisks indicate statistically significant differences by the Student's T-test (\*P<0.05, \*\*\* P<0.001) between the parent strains carrying vector plasmids and mutants carrying HmsC or HmsD fusions.



**Fig. S2. *In vitro* biofilm formation in epidemic and endemic strains of *Y. pestis*.**

A CV staining assay was used to assess Hms-dependent biofilm formation in different strains of *Y. pestis*. Biovar Mediaevalis strain – KIM6+; Biovar Orientalis strains – CO92, Salazar, Kimberly; Biovar Antiqua strains– Kuma, Yokohama; Endemic strains – Pestoides D, Pestoides F. Endemic strains are a subspecies that cause plague in most rodents (except guinea pigs) but are avirulent in humans. Results are averages of duplicate assays from two independent cultures. Error bars indicate standard deviations. The asterisks indicate statistically significant differences between *Y. pestis* KIM6+ and the other strains by the Student's T-test (\*P<0.05, \*\*P<0.005).