



Fig. S5. Transfection of type III secreted molecules into primary macrophages does not induce TNF- α or IFN β .

(A,D) MyD88^{-/-}/Trif^{-/-} macrophages were infected with varying amounts of live *Y. pseudotuberculosis* for two hours. Filtrates from cultures of *Y. pseudotuberculosis* grown under type III secretion-inducing conditions (B,E) or HKYP (C,F) were transfected into MyD88^{-/-}/Trif^{-/-} macrophages and incubated for two hours. Total RNA was isolated and *tnfa* (A-C) and *ifnb* (D-F) mRNA levels (normalized to 18s rRNA) were quantified. Data shown is the average \pm sem from one independent, representative experiment and each experiment was repeated for a total of two replicates. * Statistically significant increase ($p < 0.0001$) in cytokine mRNA levels according to the student t-Test compared to uninfected (A) or lipofectamine only (C,F) controls. ** $p = 0.0001$. *** $p < 0.0005$. *Y. pseudotuberculosis* strains lacking the regulatory protein YopN hypersecrete Yops [86] and the $\Delta 6/\Delta yopN$ strain was included to test whether T3SS cargo from a hypersecreting *Y. pseudotuberculosis* strain could trigger IL-8 production.