



**Figure S4. Deletion of DN756\_21815 and DN756\_21820, identified gene targets of IscR, results in a small but significant decrease in type III secretion. (A)** Read coverage of IscR-binding peaks proximal to DN756\_21815\_DN756\_21820. Counts per million reads (CPM) are plotted versus the genomic position. Dashed lines correspond to the predicted 30 bp IscR type II motif. Arrows indicate transcriptional start sites (50). **(B)** Expression of DN756\_21815, and DN756\_21820 genes under varying iron conditions as measured by RNA-seq. Reads are represented by Trimmed Mean of M-values (TMM) of WT (black) and  $\Delta$ iscR strains (grey) grown in M9 minimal media containing FeSO<sub>4</sub> (non-iron starved, NIS), iron starved in chelex-treated M9 minimal media with no iron source added back (Chelex), supplemented with 5 mM hemin (+Heme), or supplemented with FeSO<sub>4</sub> (+FeSO<sub>4</sub>). \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$  (EdgeR with a corrected FDR post-hoc test). **(C)** *Yersinia* strains were grown under rich media, T3SS inducing conditions. The secretome of these cultures was visualized with Coomassie blue. The effector protein, YopE, was quantified by densitometry relative to the WT control to measure the relative efficiency of the Ysc T3SS. The average of 5 biological replicates  $\pm$  standard deviation is shown. \*\*\*\* $p < 0.0001$ ; \* $p < 0.05$  (one way ANOVA with Dunnett's post-hoc test).