



**Figure S1. Experimental strategy to determine genes essential for fitness in competitive, infection-relevant contexts.** The transposon mutant pool (a) was first cultured in nutrient broth (b) to generate a reproducible inoculum for subsequent experiments. To identify genes contributing to fitness in infection, bacteria were inoculated in duplicate *ex vivo* into aqueous fluid, vitreous fluid and whole blood (d). Additionally, genes contributing to persistence and proliferation in an abscess *in vivo* (e) were identified. Each output was sequenced and compared to nutrient broth outgrowth control (c).