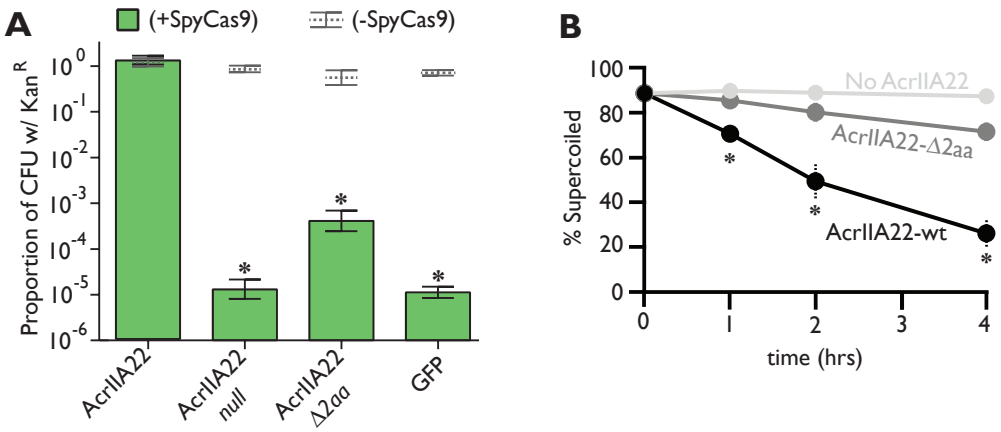

S9 Fig. A 2-aa truncation mutant of AcrIIA22 is impaired for SpyCas9 inhibition and nicking activity. (**A**) An *in vivo* plasmid protection assay. Asterisks depict statistically significant differences in plasmid retention under SpyCas9-inducing conditions with either wild-type AcrIIA22, a null mutant with an early stop codon, a 2-aa truncation, or a negative control *gfp* gene (adj. $p < 0.005$, Student's t-test, n=3). The truncation mutant retains mild but severely impaired activity, as it protects a plasmid from SpyCas9 more effectively than a null mutant ($p = 0.012$) or GFP control ($p = 0.015$). All p-values were corrected for multiple hypotheses using Bonferroni's method. (**B**) The 2-aa truncation mutant is impaired for nicking *in vitro*, relative to wild-type AcrIIA22. In both cases, 25μM of protein was used following NiNTA-based purification of an N-terminal, His6-tagged construct. An asterisk (*) denotes significant differences between AcrIIA22-treated and untreated substrates (Student's t-test, $p < 0.05$, n=3). Standard deviations are indicated by dashed lines (in most cases, the data points obscure these error bars). The individual numerical values that underlie the summary data in this figure may be found in S1_Data.