S10 Fig.

S10 Fig. **AcrIIA22 nicks supercoiled plasmids.** (**A**) A Coomassie stain of an N-terminally His6-tagged AcrIIA22 construct shows no co-purifying proteins. (**B**) The nicking activity for this protein preparation (bottom) correlates with the intensity of the Coomassie-stained protein band across purification fractions (top). In each lane, supercoiled (SC) plasmid DNA represents the un-nicked fraction whereas open circle (OC) and linear DNA have been nicked at least once. (**C**) This panel is a quantification of the experiment depicted in panel B across all 13 fractions collected. (**D**) His6-AcrIIA22 nicks supercoiled plasmids in a time and concentration dependent manner. A decrease in the proportion of supercoiled plasmid DNA indicates nicking activity, as depicted in Fig 5B. (**E**) A silver stain of a C-terminally twin-strep-tagged AcrIIA22 construct shows no co-purifying proteins. Equal volumes of each protein fraction were loaded in each lane, for all samples. Fraction 4 was concentrated and used for all *in vitro* experiments. (**F**) A C-terminal, but not N-terminal twin-strep tag is compatible with AcrIIA22's ability to protect a target plasmid from SpyCas9 elimination *in vivo*. Statistically significant differences in plasmid retention between SpyCas9-inducing and non-inducing conditions were determined via a Student's t-test (n=3); '**' indicates $p \leq 0.001$. All p-values were adjusted for multiple hypotheses using the Bonferroni correction. (**G**) The D14A mutation in AcrIIA22 impairs nicking activity. Over time, the wild-type AcrIIA22-twin-strep construct consistently converts a higher fraction of plasmid DNA from its supercoiled (SC) form to an open-circle (OC) conformation than a D14A mutant. Control plasmids include a miniprepped sample and sample pre-treated with the commercial nickase, Nb.BssSI. Reaction times are indicated to the right of each image. (**H**) AcrIIA22a (Fig 3B) is impaired for nicking activity relative to AcrIIA22. As in panel G, both constructs were purified via C-terminal twin-strep tags. The individual numerical values and original images for the data presented in this figure may be found in S1_Data and S1_raw_images, respectively.