

Figure S1

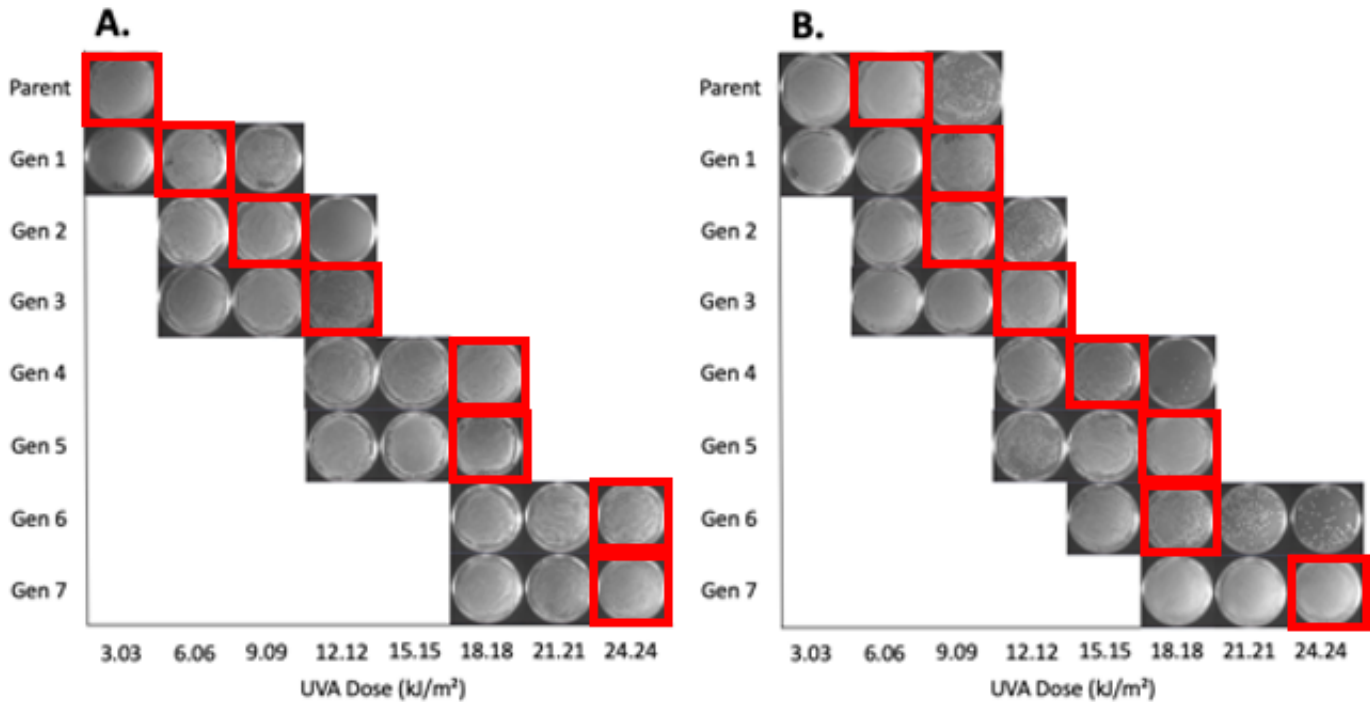


Fig. S1. Selection for the development of resistance to psoralen-UVA treatment. Two separate cultures (A and B) of strain SR108 were plated on media containing 20 $\mu\text{g/ml}$ 8-methoxypsoralen and exposed to increasing doses of UVA. The cells from the plate where viability began to become compromised were collected, grown, and the psoralen-UVA treatment/selection process was repeated. Over successive rounds, the resistance of the culture increased significantly. Images of the plates with surviving cells at each dose are shown above. A third resistant strain was subsequently isolated using this same approach.

Figure S2

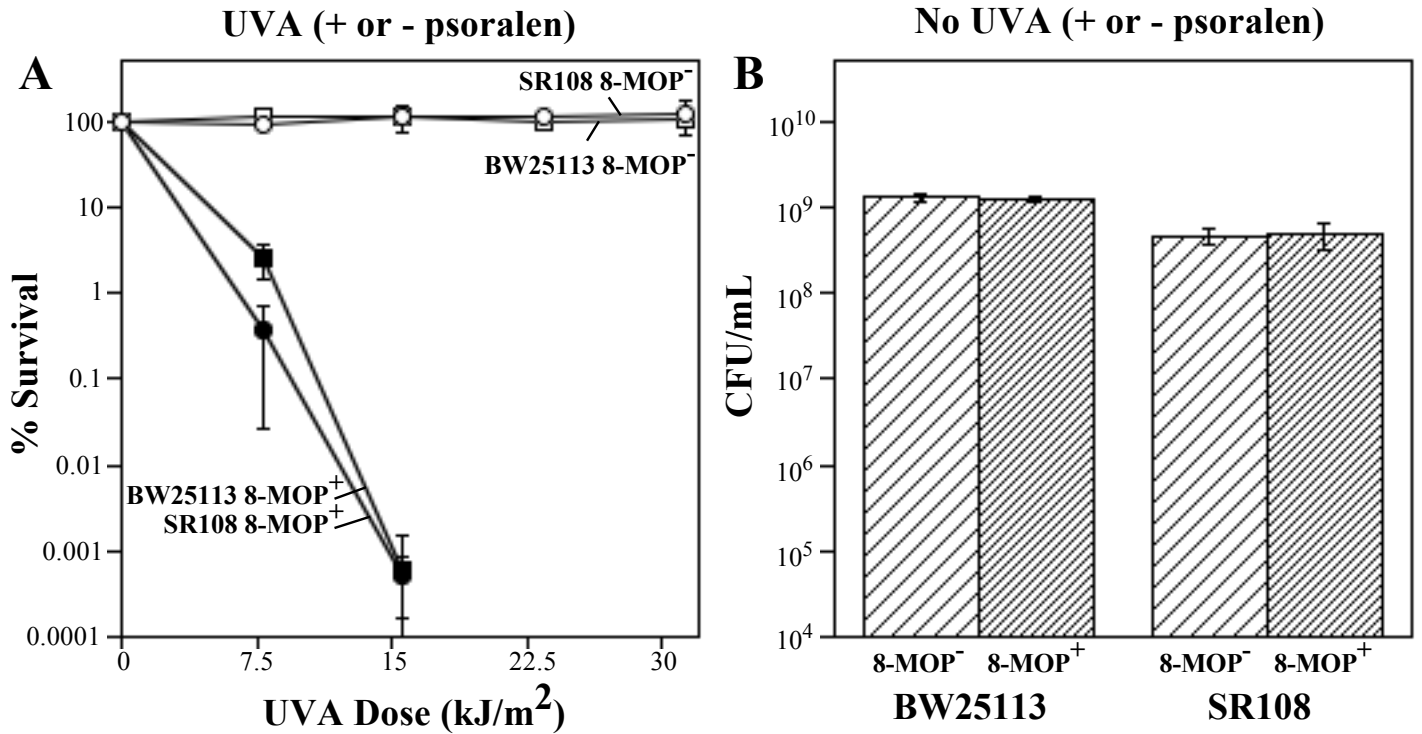


Fig. S2. SR108 and BW25113 do not significantly differ in sensitivity to psoralen-UVA. UVA or psoralen alone do not contribute to lethality. (A) Survival of BW25113 and SR108 in the presence of psoralen (filled squares and filled circles respectively) and BW25113 and SR108 without psoralen (empty squares and empty circles respectively) at the indicated UVA doses is plotted. (B) Number of colony forming units for BW25113 (left) and SR108 (right) with and without psoralen and no exposure to UVA irradiation are plotted. Plots represent the average of at least two independent experiments. Error bars represent the standard error of the mean.

Figure S3

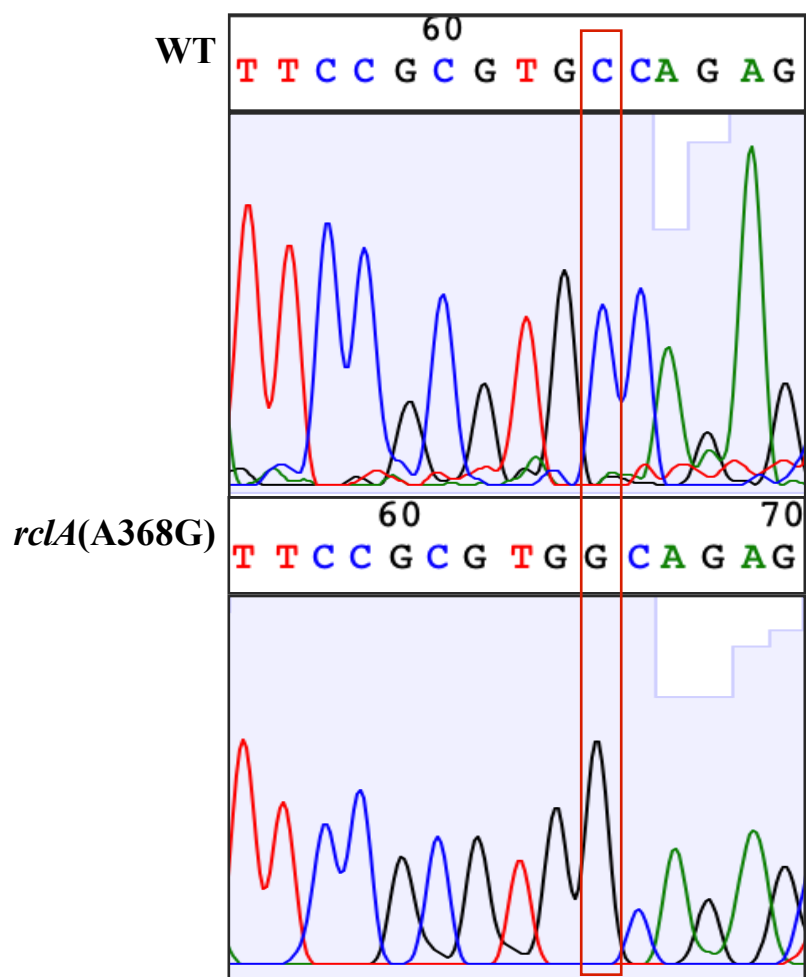


Fig. S3. Sequencing of *rclA* supports successful transduction of *rclA*(A368G) mutation into BW25113. (Top) Wild type *rclA*. (Bottom) *rclA*(A368G) mutation in CL5233. Red box indicates location of point mutation.

Figure S4

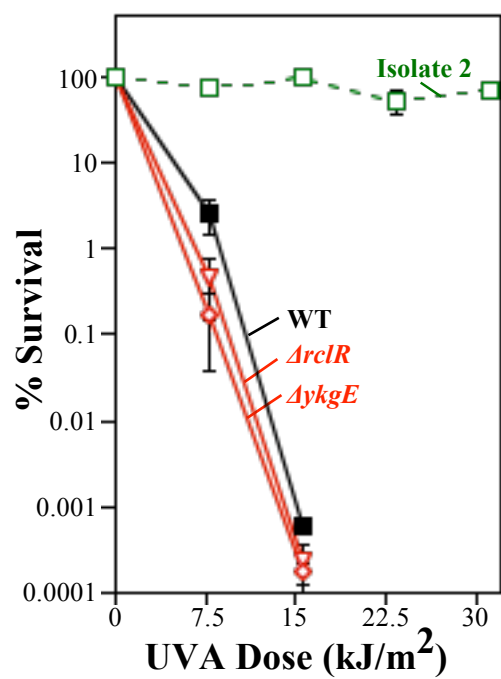


Fig. S4. $\Delta rclR$ and $\Delta ykgE$ show wild type sensitivity to psoralen-UVA. Survival of BW25113 (black, filled square), $\Delta rclR$ (red, open inverted triangle), $\Delta ykgE$ (red, open diamond) in the presence of 20 $\mu\text{g/ml}$ 8-methoxypsoralen at the indicated UVA doses is plotted. Plots represent the average of at least three independent experiments. Error bars represent the standard error of the mean.

Figure S5

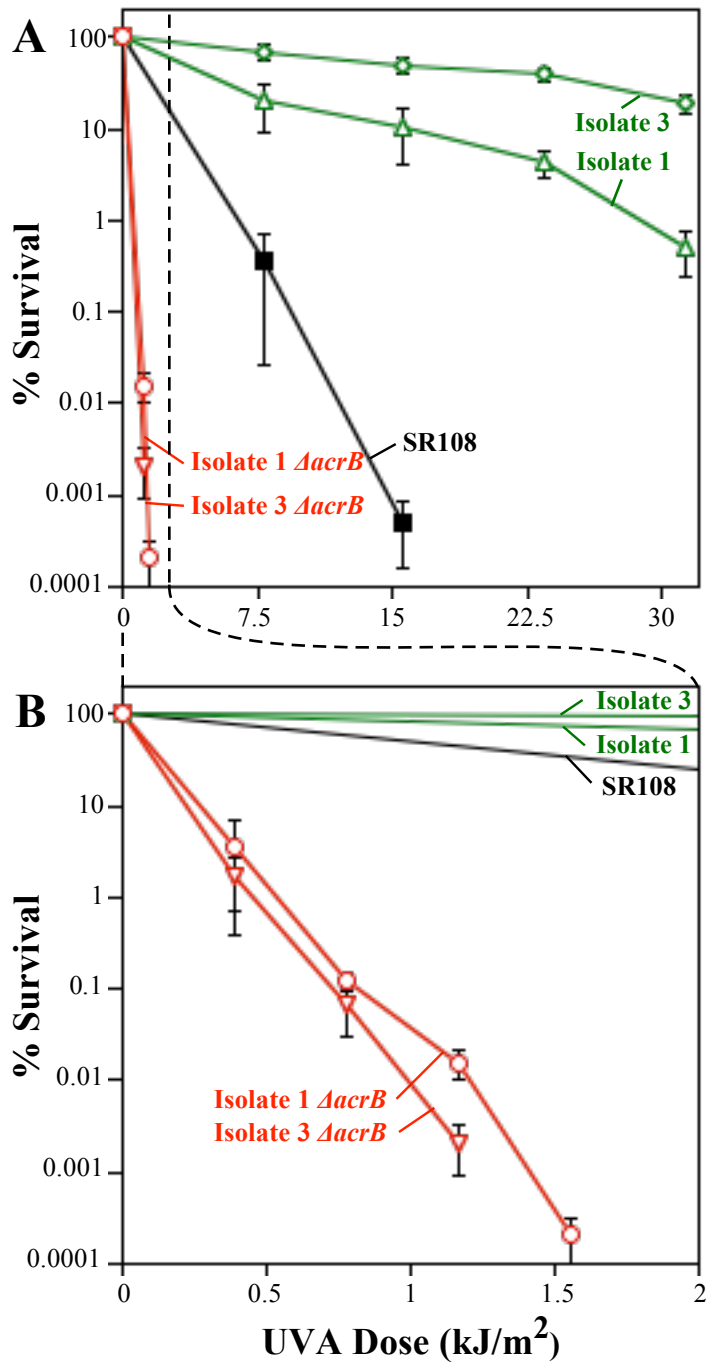


Fig. S5. Deletion of *acrB* in resistant isolates 1 and 3 reversed resistance to PUVA. Survival of BW25113 (black, filled square), *acrB* deletion in resistant isolate 1 background (red, open triangle), and *acrB* deletion in resistant isolate 3 background (red, open diamond) in the presence of 20 $\mu\text{g/ml}$ 8-methoxypsoralen at the indicated UVA doses is plotted. Plots represent the average of at least three independent experiments. Resistant isolates 1 and 3 were derived in SR108 background (indicated by dotted line). Error bars represent the standard error of the mean.

Figure S6

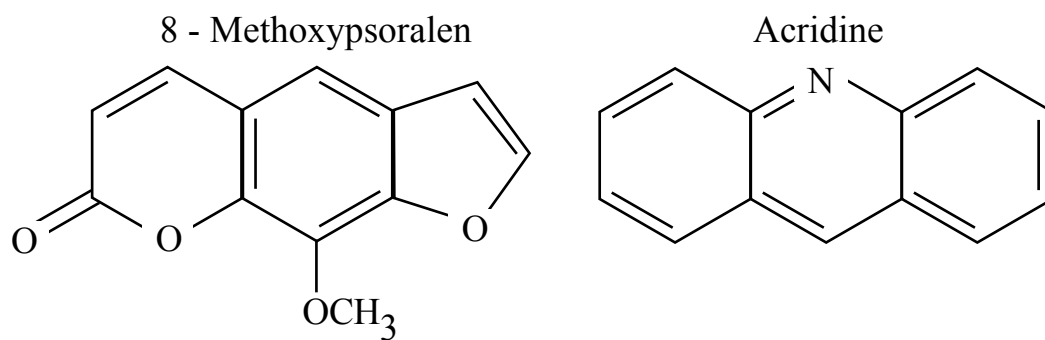


Fig. S6. Structures of 8 – methoxypsoralen and acridine are similar. (Left) 8 – methoxypsoralen. (Right) acridine.

Figure S7

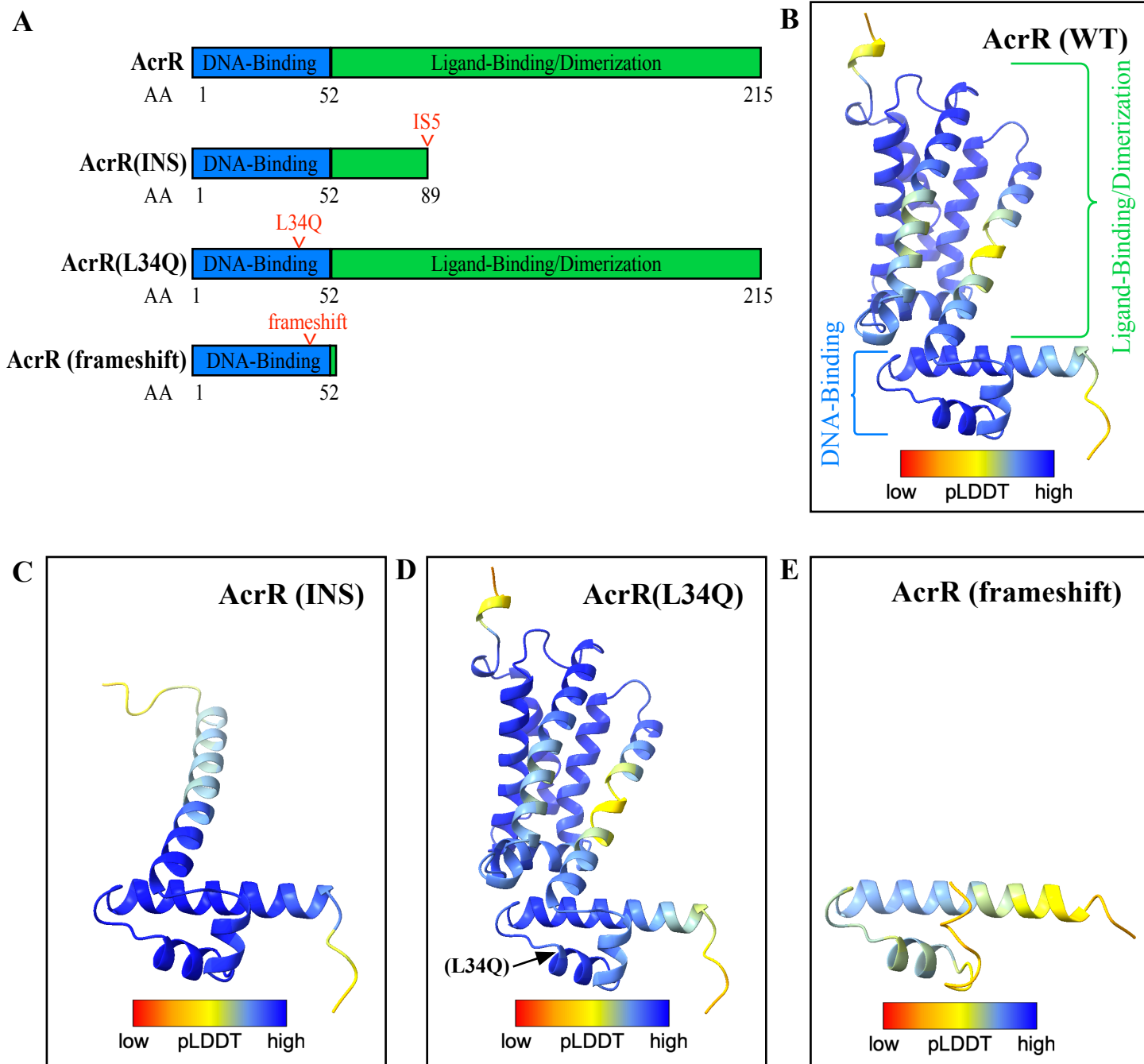


Fig. S7. DNA-binding domain is retained in *acrR* mutants. (A) Locations of mutations in *acrR* are shown relative to DNA-binding domain (blue) and ligand-binding/dimerization domain (green). AlphaFold predicted structures of (B) AcrR (WT); (C) AcrR (INS) from resistant isolate 1; (D) AcrR (L34Q) from resistant isolate 2; (E) AcrR (frameshift) from resistant isolate 3 are shown using ChimeraX. Colors on models correspond to pLDDT confidence score (Mariani et al 2013) from AlphaFold predictions (see included color key).