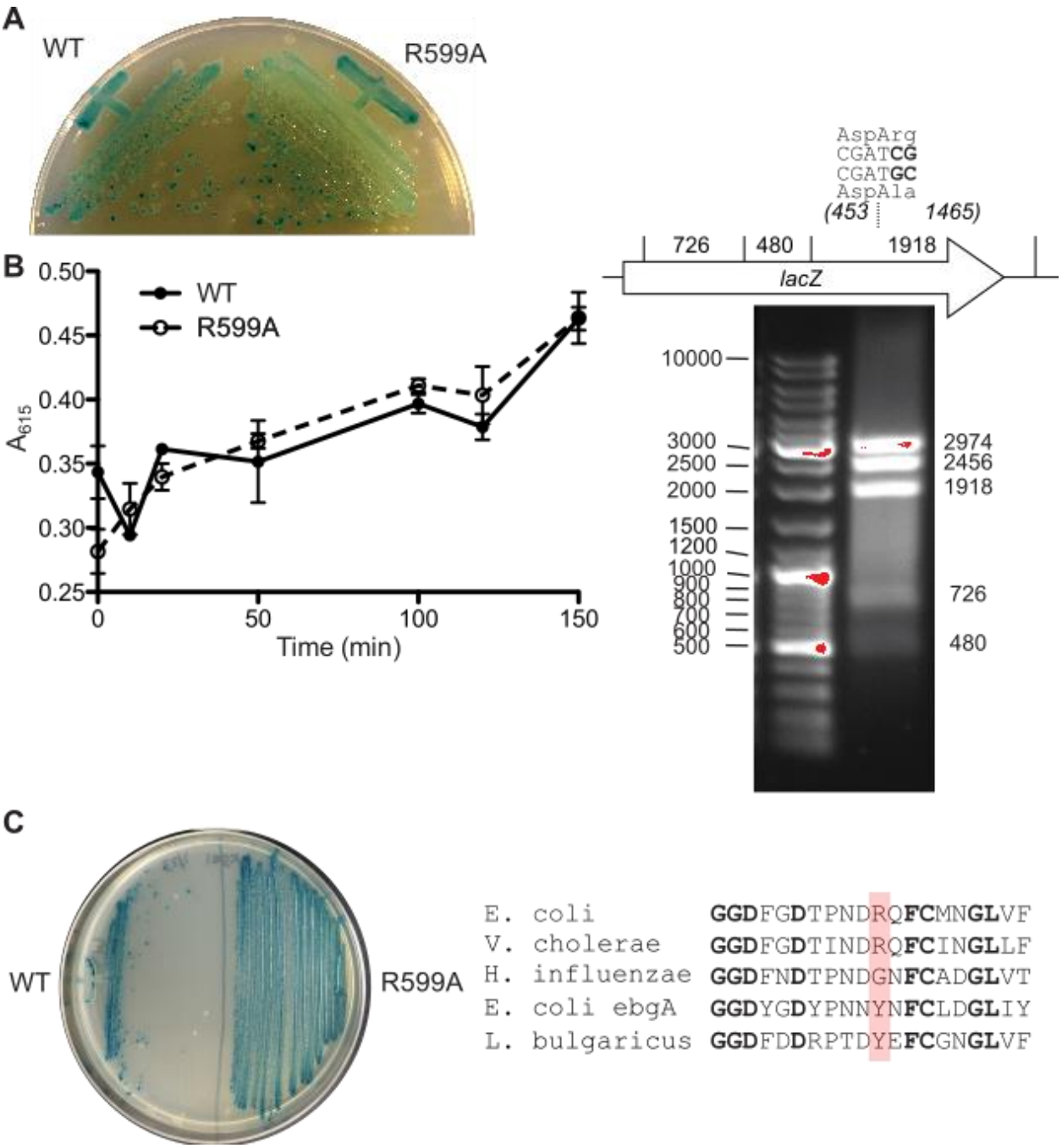

Figure S2. Arg 599 in *E. coli* β-gal is dispensable for activity *in vivo*. (A) *E. coli* cells of strain DH5α, carrying the *lacZ*Δ*M15* allele at the endogenous locus, were transformed with plasmids carrying WT *lacZ* or the R599A mutant allele under control of the yeast *ACT1* promoter and streaked on medium containing the chromogenic β-gal substrate X-gal at 32 μg/mL. Plates were incubated overnight at 37˚C prior to imaging. (B) *Left,* β-gal activity in cells as in (A) but grown in liquid culture selective for the plasmids, and then lysed at the indicated timepoints following the addition of X-gal and chloramphenicol (to halt new translation). Cleaved X-gal at each timepoint was measured in triplicate by absorbance at 615 nm ("A_{615}") after the indicated times following X-gal addition. Points show the mean, error bars are standard error of the mean. *Right,* plasmid was isolated from the remainder of the R599A culture and digested with *Pvu*I before separation by agarose gel electrophoresis. Band sizes are shown in basepairs. Red color indicates saturated pixels. The *Pvu*I recognition site overlapping with the Arg 599 codon is shown with a map (not to scale) of *Pvu*I sites in *lacZ*. DNA ladder was Thermo Scientific GeneRuler DNA Ladder Mix (#SM0331). (C) *Left,* as in (A) but with the *E. coli* strain RS302, which carries the Δ(*lacIPOZ)C29* allele at the endogenous locus, and with X-gal at 200 μg/mL. *Right*, protein sequence alignment of a portion of the indicated β-gal homologs, with *E. coli* Arg 599 highlighted in red and identical residues in bold.