Supporting information for

Gain-of-function variants of FtsA form diverse oligomeric structures on lipids and enhance FtsZ protofilament bundling

Kara M. Schoenemann[1]*, Marcin Krupka[1]*, Veronica W. Rowlett[1]*, Steven L. Distelhorst[1], Bo Hu[1], and William Margolin[1†]

Department of [1]Microbiology and Molecular Genetics, McGovern Medical School, 6431 Fannin St., Houston TX 77030

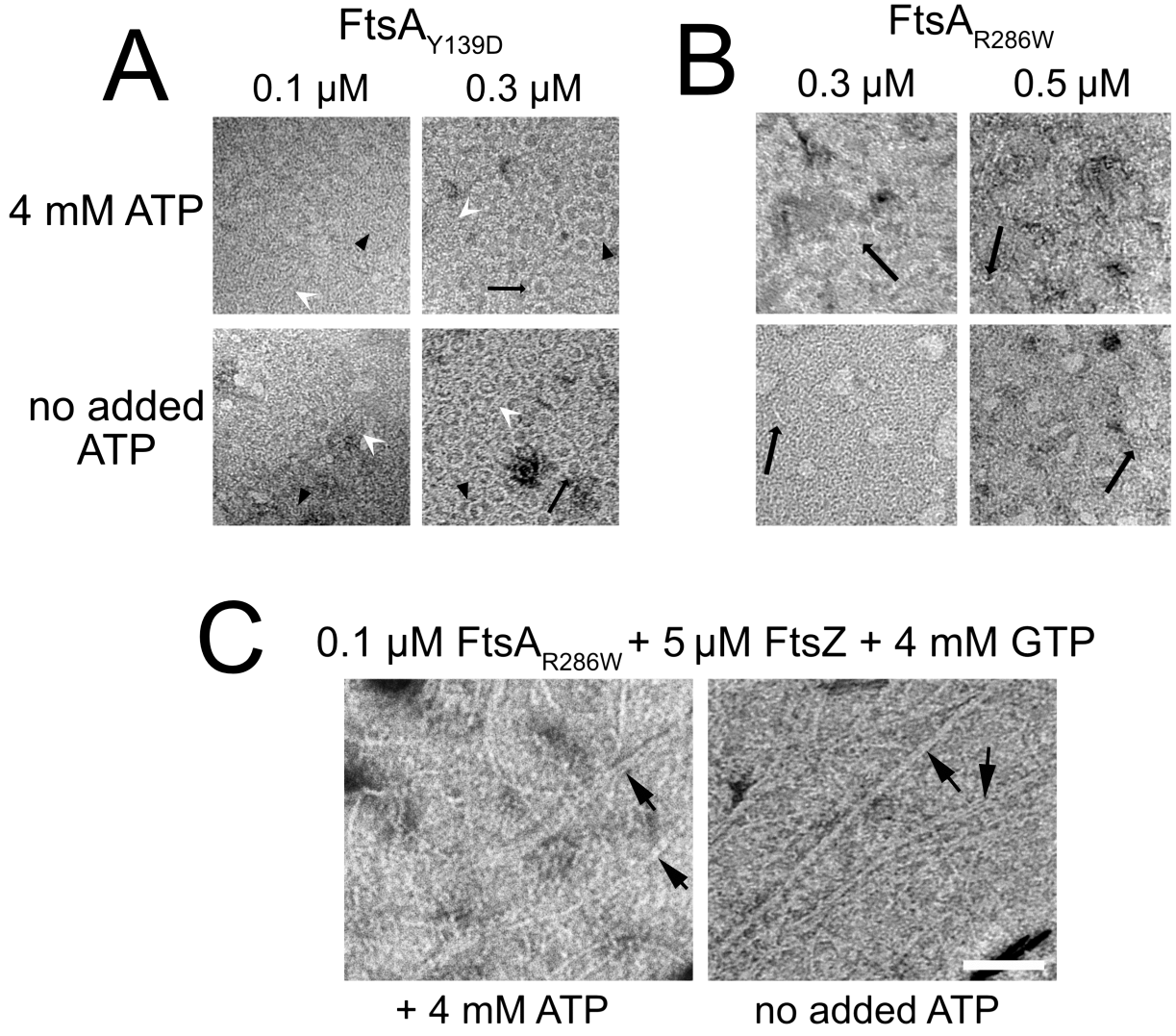

Figure S1. Examples of FtsA and FtsZ structures when added to lipid monolayers at alternative protein concentrations. Shown are negative-stain electron micrographs of lipid monolayers containing FtsA$_{Y139D}$ (A), FtsA* (B) or a mixture of low concentrations of FtsA* + FtsZ (C) at the concentrations and conditions listed, as described in Materials and Methods. In (A-B), black arrows and arrowheads highlight minirings or arcs, respectively; white arrowheads highlight straight oligomers. In (C), arrows highlight FtsZ filament bundles. Scale bar, 100 nm.

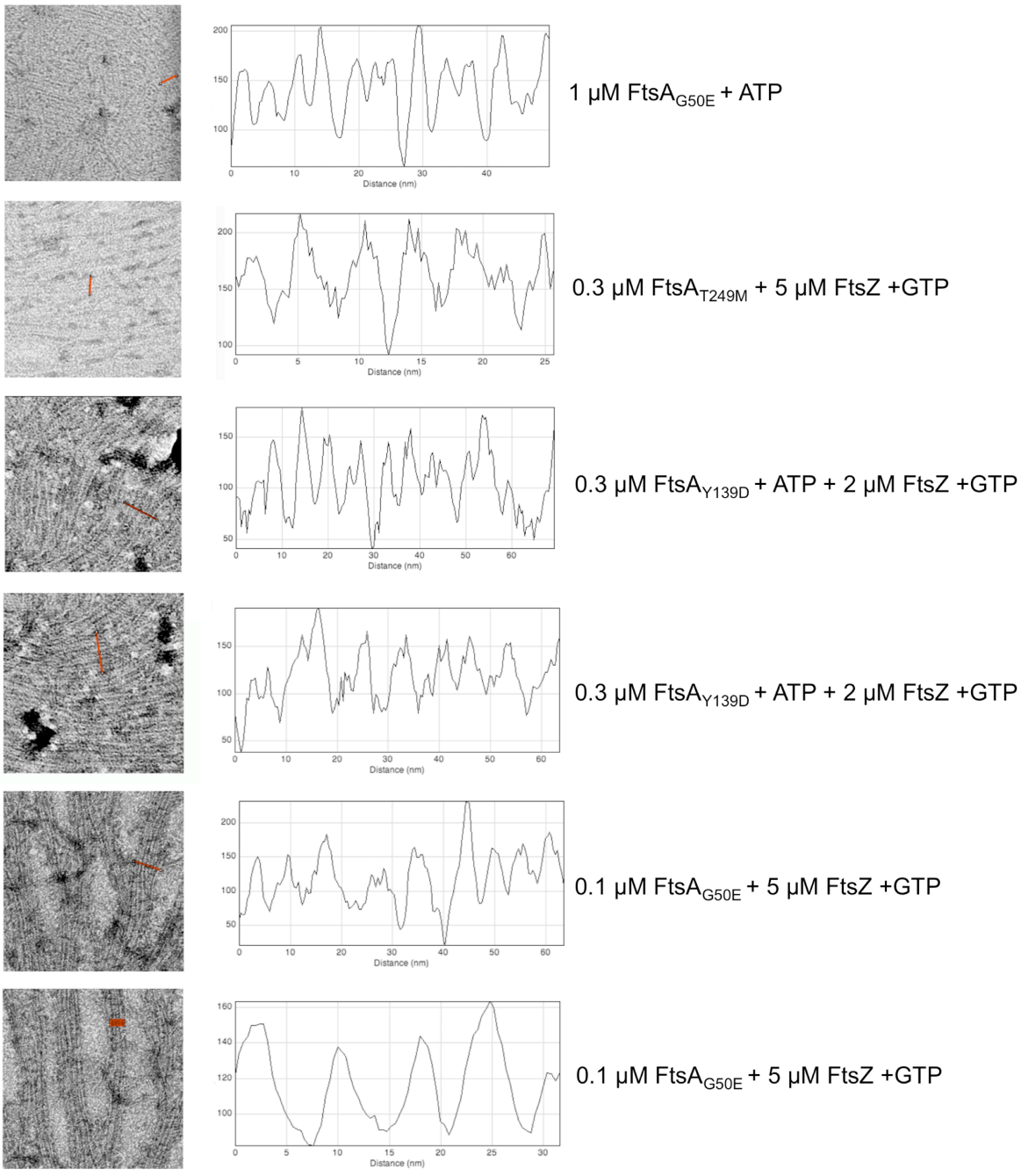

Figure S2. Examples of distances between FtsA or FtsZ filaments. Shown are electron micrographs of the conditions (described at the right). The area in red was subjected to the Plot Profile analysis in ImageJ, and the number of peaks was divided by the distance to obtain a rough average of inter-filament distances that were reported in the text.

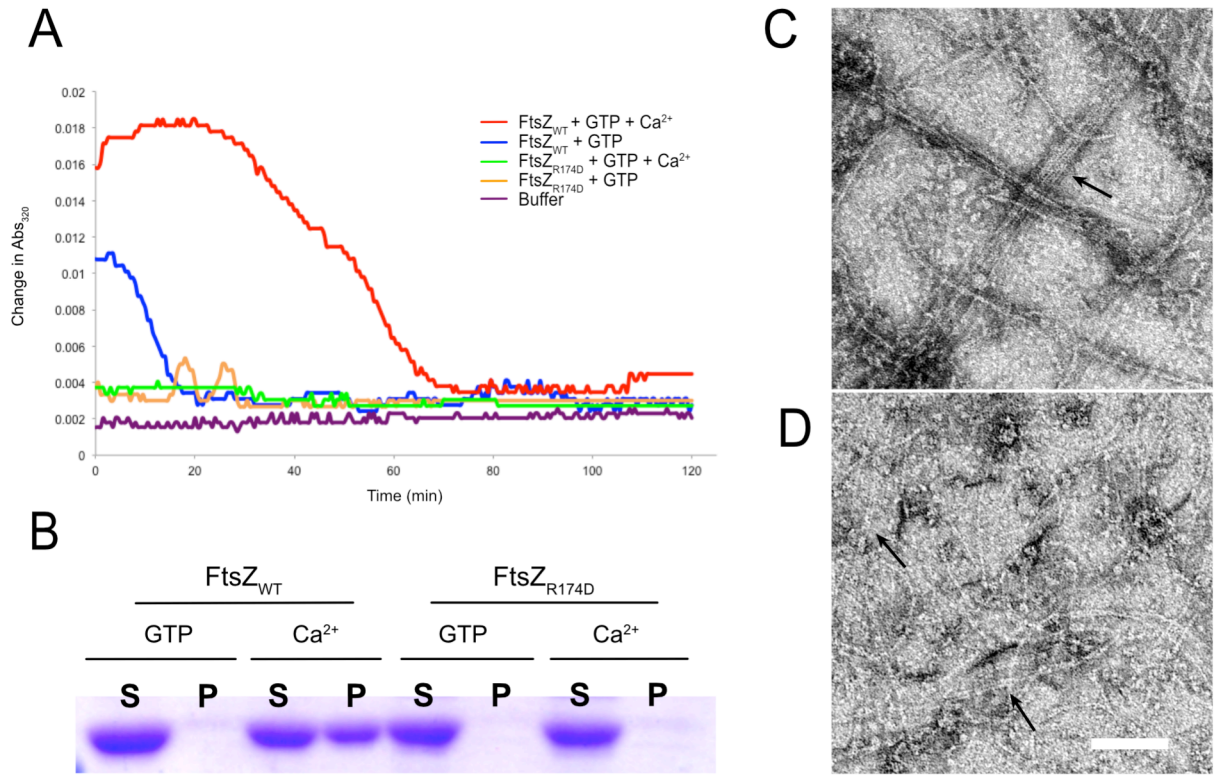

Figure S3. Evidence that $FtsZ_{R174D}$ can assemble into protofilaments but is defective in lateral interactions between protofilaments. (A) Microplate monitoring of changes in FtsZ and $FtsZ_{R174D}$ polymerization by light scattering. Purified proteins (15 μM) were incubated either in the presence or absence of 10 mM $CaCl_2$ at 25˚C for 10 minutes. The data plotted show the change in absorbance following addition of GTP at time point 0. Absorbance was measured for 3 minutes prior to GTP addition, and the average from 3 replicate reactions was subtracted from the measurements taken after GTP addition. (B) Coomassie-stained SDS-PAGE of the supernatant (S) and pellet (P) fractions from sedimentation reactions containing 10 μM of purified FtsZ or $FtsZ_{R174D}$ in the presence or absence of 10 mM $CaCl_2$ after incubation for 10 min at 25˚C. (C-D) Transmission EM images of 15 μM purified FtsZ (C) and $FtsZ_{R174D}$ (D) incubated at 25˚C in the presence of 1 mM GTP and 10 mM $CaCl_2$ and negatively stained with uranyl acetate. Most FtsZ filaments associated into bundles, some consisting of 6-7 filaments (arrow), whereas $FtsZ_{R174D}$ filaments were mostly single and only occasionally associated as doublets (arrows). Scale bar, 100 nm.

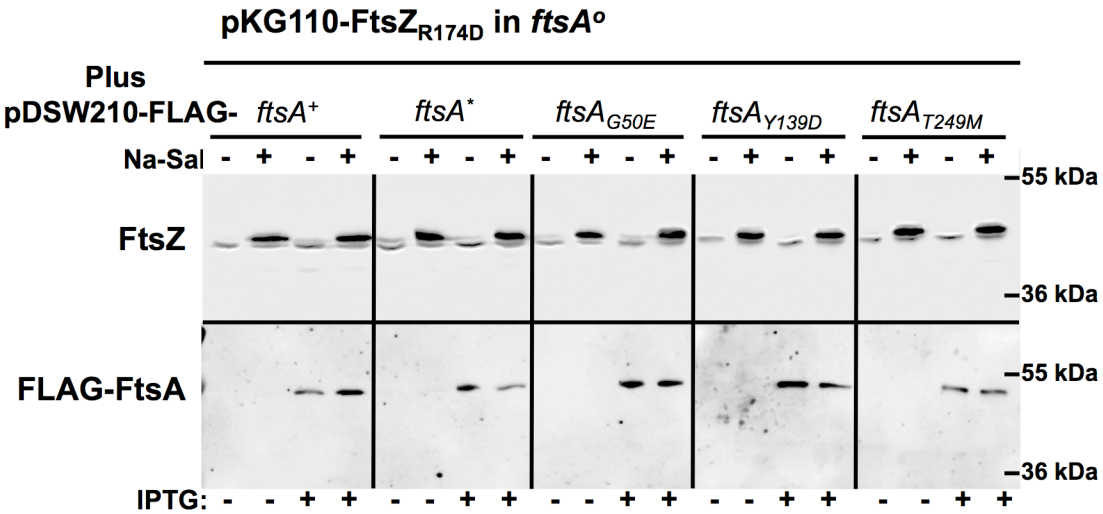

Figure S4. Levels of endogenous FtsZ and overproduced $FtsZ_{R174D}$ or FLAG-FtsA under different FtsA expression conditions. The native *ftsA* allele was inactivated in strains carrying pKG110-$FtsZ_{R174D}$ and each of the FLAG-FtsA variants (noted at the top) expressed from IPTG-inducible pDSW210F. Cells were then grown to early log phase and either induced or not induced with 2.5 μM sodium salicylate (Na-Sal) to express $FtsZ_{R174D}$ from pKG110-$FtsZ_{R174D}$, or IPTG to express the pDSW210F-FtsA derivative, and harvested proteins were separated by SDS-PAGE. Western blots of these gels were then probed with either anti-FtsZ (top) or anti-FLAG (bottom). Molecular weight markers are shown at the right. Note that whereas anti-FLAG only detects the FLAG-FtsA, anti-FtsZ detects both native FtsZ, visible as a faint lower band, and the $FtsZ_{R174D}$ produced from the plasmid (upper band, due to 5 extra residues at the N terminus).

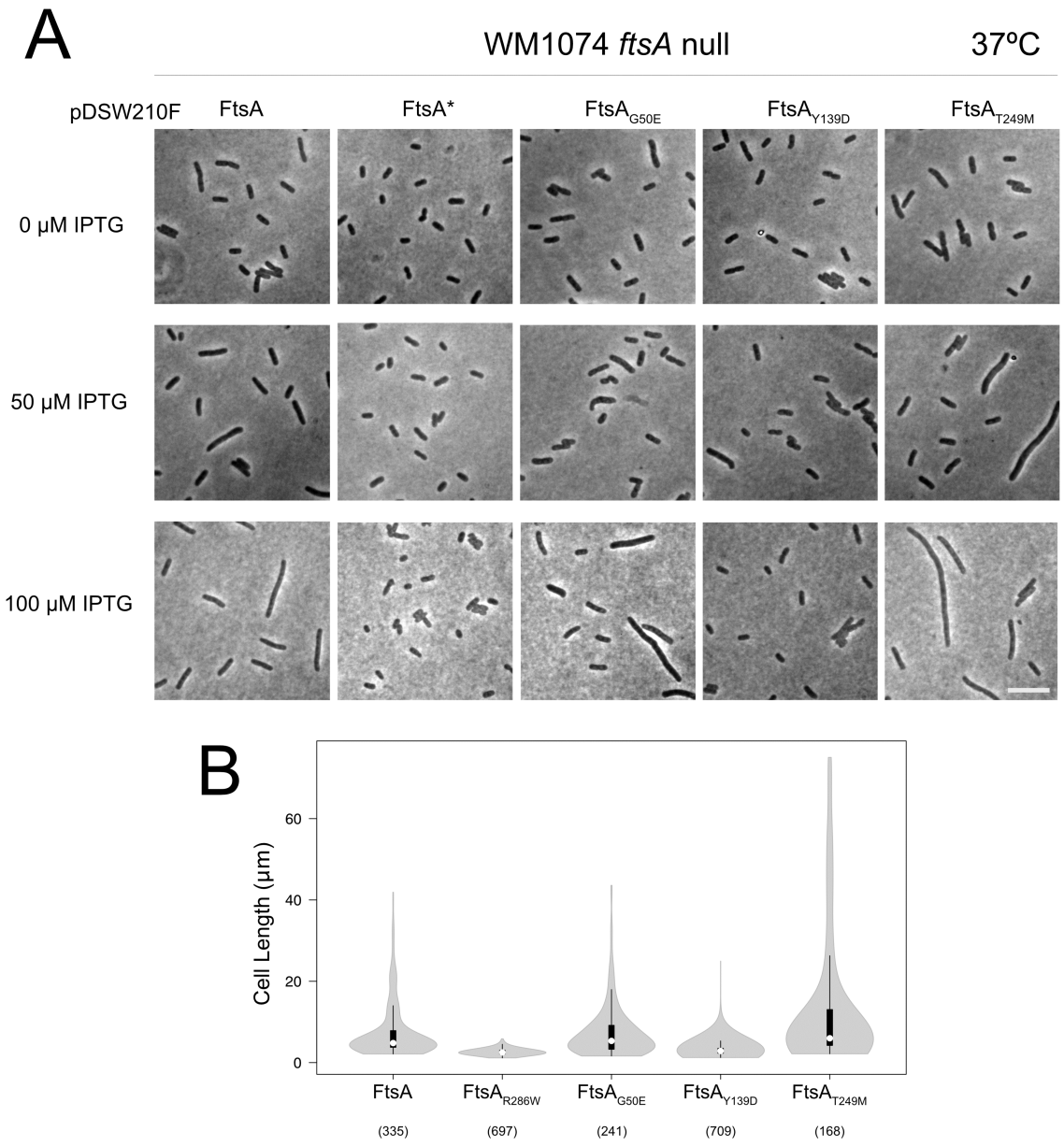

Figure S5. Differences in the effects of expression of FtsA*-like alleles on cell length. (A) Phase contrast micrographs of WM1074 *ftsA null (ftsA^{o})* cells containing pDSW210-FtsA variants following induction at 0, 50 or 100 µM IPTG at 37°C. Scale bar, 10 µm. (B) Violin plot of measured cell lengths in µm at 100 µM IPTG using the BoxPlotR web tool (http://shiny.chemgrid.org/boxplot/r). White dots show the medians; black bar limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25 th and 75 th percentiles. The gray areas extend vertically to include all outliers; the widths of the gray areas represent probability densities. The number of cells counted for sample is shown below in parentheses.

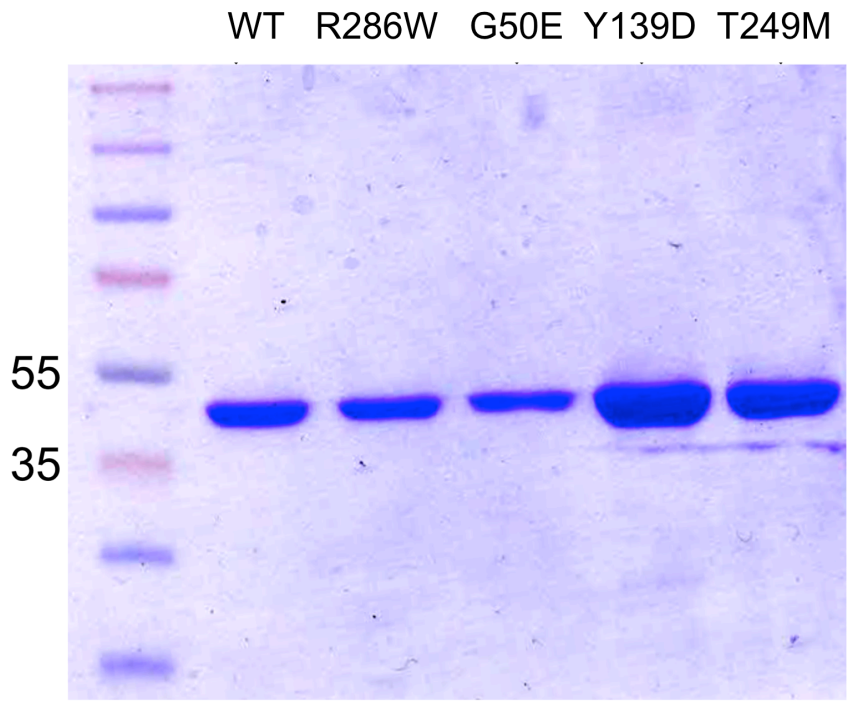

Figure S6. Purified FtsA derivatives. Shown is a Coomassie-stained SDS-PAGE of purified FtsA proteins used for this study. Alleles are shown at the top, and relevant molecular weight markers in kDa are shown at the left.