



**Fig. S3.** Interactions of wild-type ParF and ParF<sup>H</sup> with ParG in sedimentation assays and in segrosome assembly. **(A)** Co-sedimentation assays of ParF proteins and ParG in which equimolar protein concentrations (4-8  $\mu$ M) were incubated in the absence (-) or presence (2 mM) of ATP $\gamma$ S for 10 minutes at 30°C, and the reactions were then centrifuged. In all, 100% and 33%, respectively, of the pellet (P) and supernatant (S) fractions were resolved on 2% SDS gels and stained with Coomassie blue. The percentages of ParF (filled bars) ParG (open bars) protein detected in the pellet fractions are shown. Data are representative examples of experiments performed at least in duplicate with standard deviations  $\pm$  10%. **(B)** Supershifting of the ParG:*parH* complex in the presence of wild-type or mutated ParF. A 320-bp biotinylated PCR product encompassing *parH* (28) was incubated simultaneously with ParG (0.5  $\mu$ M) and increasing concentrations of the indicated ParF proteins and analyzed by gel retardation assays. ParF concentrations ( $\mu$ M monomer, left to right): 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 16 and 20. Open, single filled and double filled arrows indicate unbound DNA, ParG-*parH* complexes, and ParG-ParG-*parH* complexes, respectively. The band above free *parH* in some reactions is likely to be the same fragment with an atypical secondary structure that is commonly observed in DNA preparations of the centromere site (27, 28). Data are representative examples of experiments performed at least in duplicate.