Supplementary Information

Figure S1. Fluorescent tagged helicases retain significant activity. The ATPase activity of purified helicases was assayed in the presence of M13 ssDNA (RecG) or ϕX174 ssDNA (PriA). The activity of RecG was normalized to wild type (100%) while that of PriA was normalized to his-PriA. Coupled spectrophotometric assays were done at 37°C in a final volume of 160 µl. Reactions contained buffer (25mM Tris-OAc, pH 7.5; 10mM MgOAc and 1mM DTT), 10 µM nucleotides of ssDNA, 10 nM helicase and 1mM ATP. Assay procedures and data analysis are described in (Slocum *et al.* 2007; Abd Wahab *et al.* 2013). Results are from 2 separate assays done on the same day. The values in bars of his-RecG and his-mcherryRecG indicate the level compared to wild type and in parentheses for mcherry-RecG relative to his-RecG.

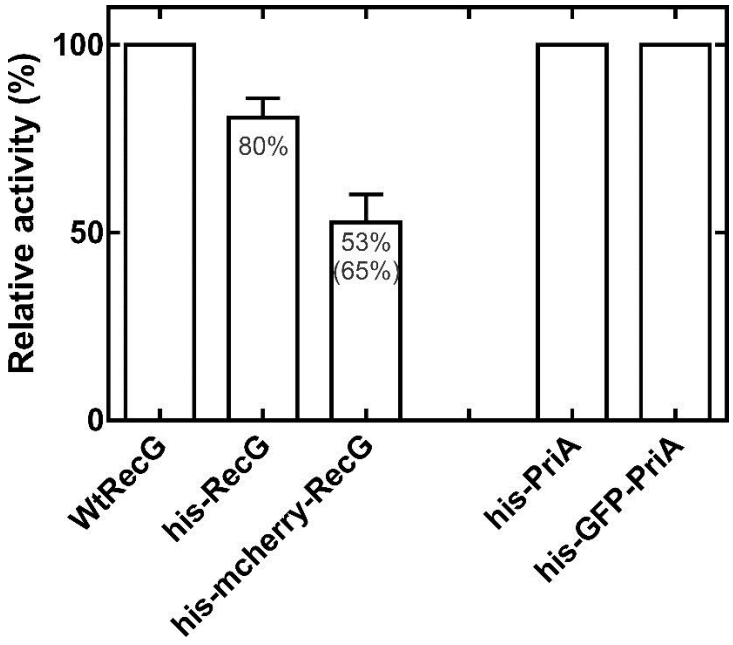


Figure S2. Fluorescent tagged helicases bind to wild type or fluorescent tagged SSB *in vitro* with moderate affinity. Panel (A), coprecipitation of SSB was done as described in the Experimental Procedures. The amount of RecG co-precipitated in each reaction was determined from the analysis of the resulting SDS-PAGE gels as described previously (Buss *et al.* 2008). The designation G indicates GFP and ¼ refers to a single SSB-GFP monomer in a tetramer; 2/4 refers to two of four subunits being SSB-GFP per tetramer. Panel (B), titration of 5 nM GFP-PriA with mCherry-SSB ¼. Data were fit with a hyperbola to obtain a K_d of 7.2±0.3 µM. Panel (C), titration of 5 nM mCherry-RecG with GFP-SSB ¼. Data were fit with a hyperbola to obtain a K_d of 5.6±0.3 µM. Error bars, standard error of the fit.

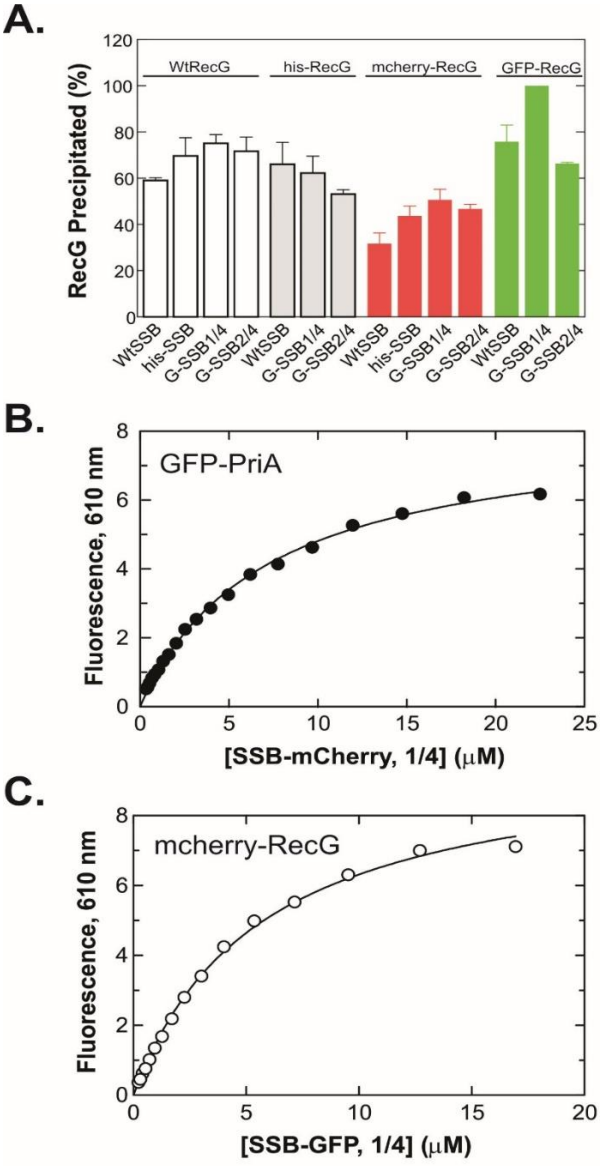


Figure S3. Efficient co-localization of RecG and PriA requires the C-terminus of SSB. Fluorescence microscopy images and analysis of cells where GFP-PriA, mcherry-RecG and SSBΔC8 were co-expressed. Mutant SSB was expressed from pET28 (high copy number vector) while the helicases were expressed from low copy number vectors. To determine localization, overnight cultures from a single colony were diluted 1:100 into 5mL of fresh LB containing antibiotics and grown for 4 hours in the absence of IPTG. Cells were harvested by centrifugation, resuspended into 10 mM $MgSO_4$, attached to poly-L-lysine coated coverslips and imaged as described in Experimental Procedures. Representative microscopy images are presented. The magnification in each microscope image is 400x. (A), DIC image; (B), fluorescence image captured with the GFP filter to image PriA; (C), fluorescence image of mcherry-RecG and (D), colour composite image. SSBΔC8.

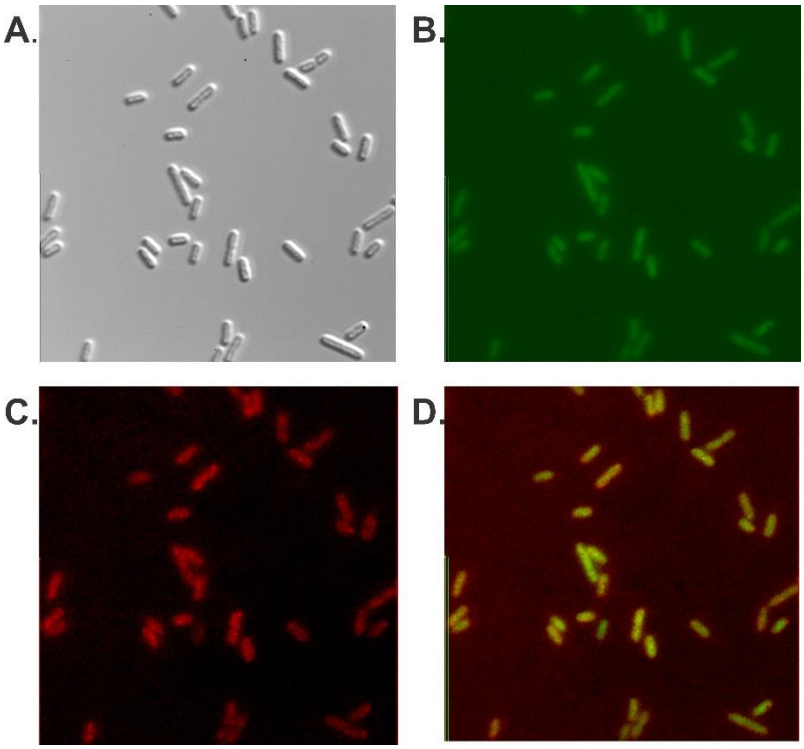


Figure S4. Elution profiles from nickel columns. (A), his-SSB and wtRecG. (B), wtSSB and his-RecG. (C), his-SSB and wtPriA. (D), wtSSB and his-PriA. Proteins were eluted using an imidazole gradient from 30 mM to 500 mM. The red curve indicates the absorbance at 280 nm; the blue line indicates the imidazole concentration.

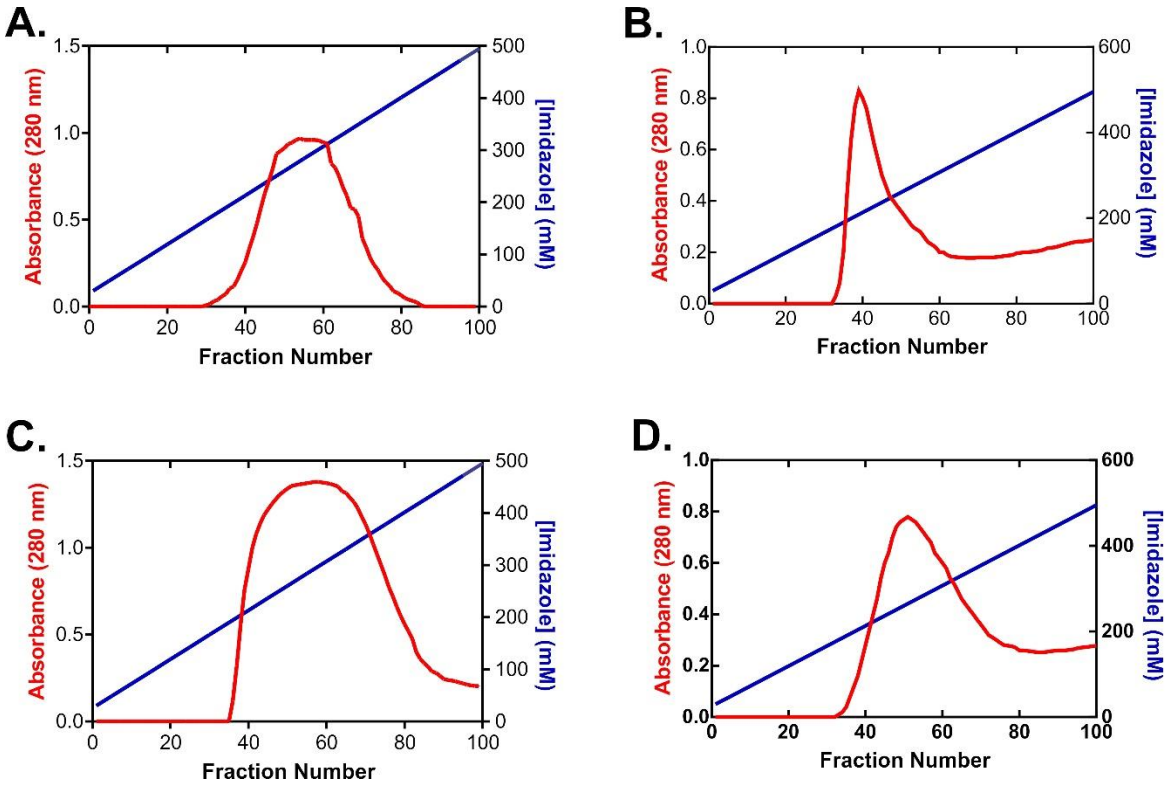


Figure S5. SSB forms complexes with RecG and PriA in the absence of IPTG. 100mL cultures of Tuner cells containing high copy number vectors expressing his –SSB and RecG and separately, his-SSB and PriA were grown to stationary phase in the absence of IPTG. SDS-PAGE gels showing eluted fractions from separate 1mL columns of the his-SSB-RecG (left) and his-SSB-PriA lysate (right) are shown. WCL, whole cell lysate; CCL, cleared cell lysate, FT, flow through. Numbers indicate fractions eluted from the column.

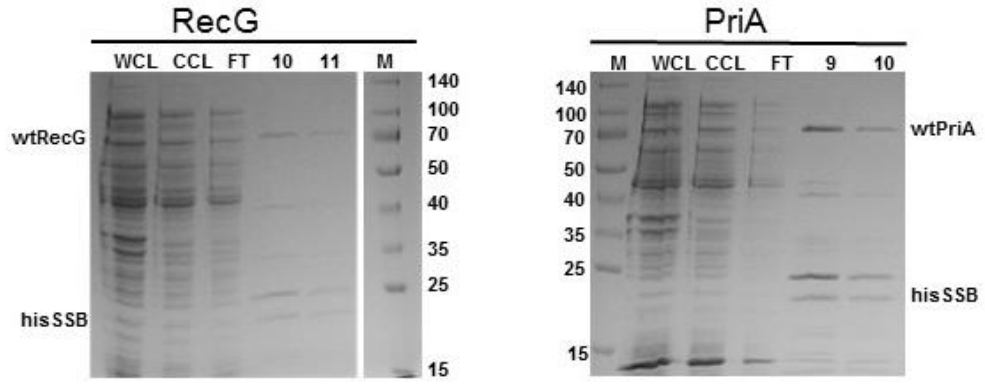


Figure S6. SSB and RecG do not form a complex following cell lysis. SDS-PAGE gels showing eluted fractions of an SSB-RecG lysate mixture. Separate 1L cultures expressing his-SSB or RecG were grown, harvested and lysed. The resulting cleared cell lysates were immediately mixed and split into two equal fractions. (A). Mixed lysate loaded immediately onto the column; (B) Lysates mixed and stirred for 30 minutes prior to loading. W, whole cell lysate; C, cleared cell lysate; L, load mixture; F, flow through and numbers indicate column fractions. In panel A, the W and C are from the RecG culture while in panel B, they are from the SSB culture.

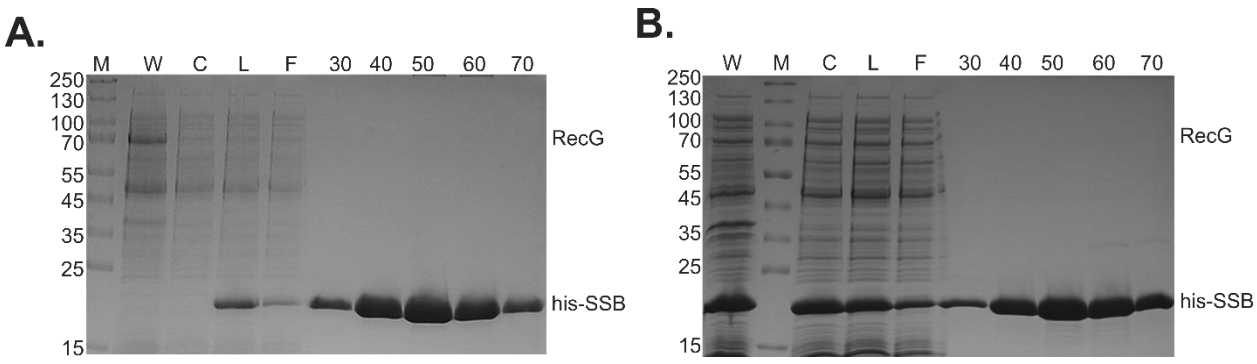


Figure S7. Components of the resolvasome do not bind to SSB. Cleared cell lysates of his-tagged SSB and (A) RuvA, (B) RuvB, or (C), RuvC were subjected to 1 ml nickel column chromatography as described in the Experimental Procedures. The 12% SDS-PAGE gels show various stages during the purification. M, molecular weight marker; WCL, whole cell lysate; CCL, clear cell lysate; FT, column flow through; Wash, extensive wash with binding buffer and binding buffer with 0.2% NP40. The numbers at the top of the lanes indicate the five, 1ml elution fractions.

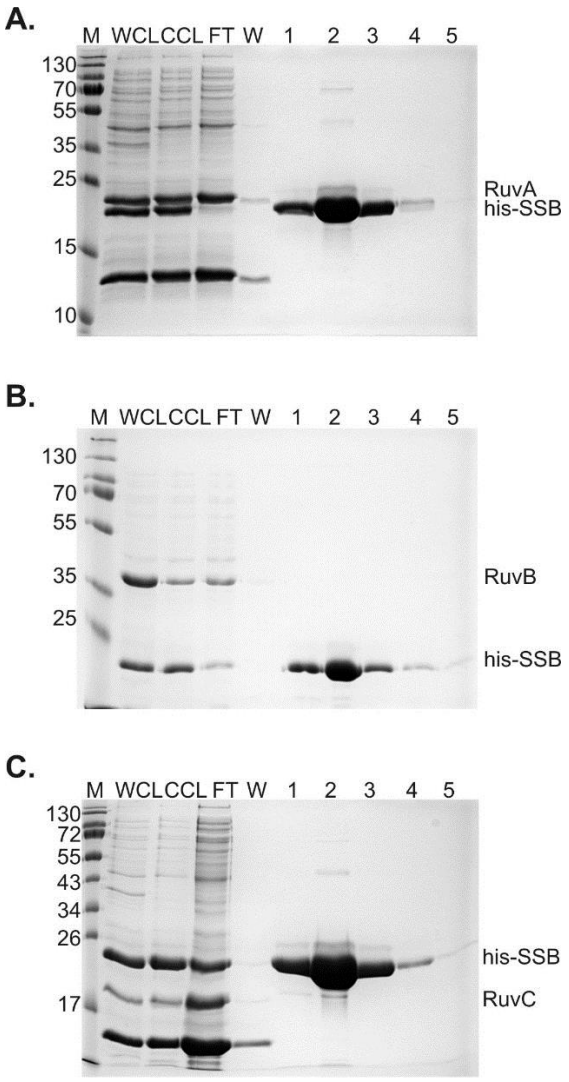


Figure S8. The purified his-SSB-RecG-PriA complex exists in two forms. The fractions eluted from a nickel column containing his-SSB, RecG and PriA were pooled, concentrated using an Amicon spin column and applied to a Superose-6 column equilibrated in the same running buffer used to generate the standard curve as described in the Experimental Procedures. Fractions were analyzed by SDS-PAGE to determine which proteins were present in each fraction. The molecular weight of fractions eluting at 14 and 15 ml were determined from the K_{av} values generated from the standard curve.

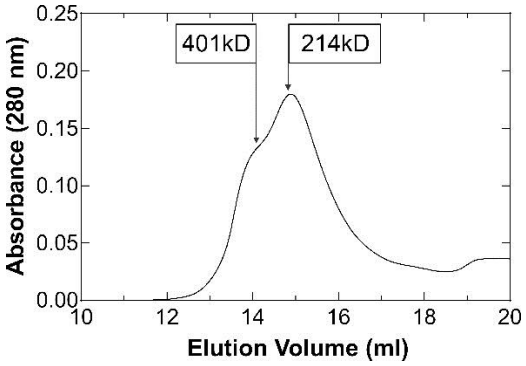
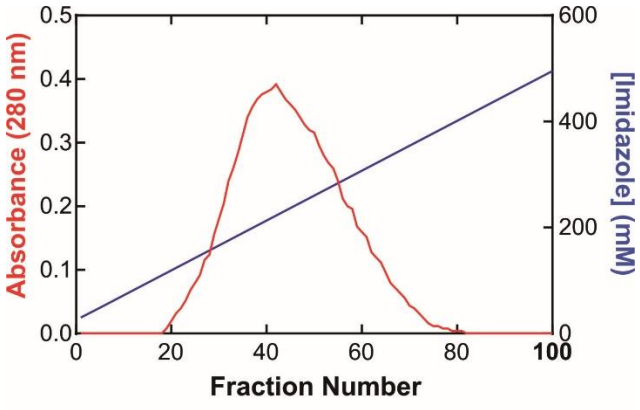


Figure S9. The PriA-RecG complex elutes as a single peak. The elution profile from a nickel column is shown. The profile corresponds to the gel shown in Figure 7D. Proteins were eluted using an imidazole gradient from 30 mM to 500 mM. The red curve indicates the absorbance at 280 nm; the blue line indicates the imidazole concentration.



References for Supplementary Figures

Abd Wahab, S., Choi, M. & Bianco, P.R. (2013) Characterization of the ATPase activity of RecG and RuvAB proteins on model fork structures reveals insight into stalled DNA replication fork repair. *J Biol Chem* **288**, 26397-26409.

Buss, J.A., Kimura, Y. & Bianco, P.R. (2008) RecG interacts directly with SSB: implications for stalled replication fork regression. *Nucleic Acids Res* **36**, 7029-7042.

Slocum, S.L., Buss, J.A., Kimura, Y. & Bianco, P.R. (2007) Characterization of the ATPase activity of the Escherichia coli RecG protein reveals that the preferred cofactor is negatively supercoiled DNA. *J Mol Biol* **367**, 647-664.