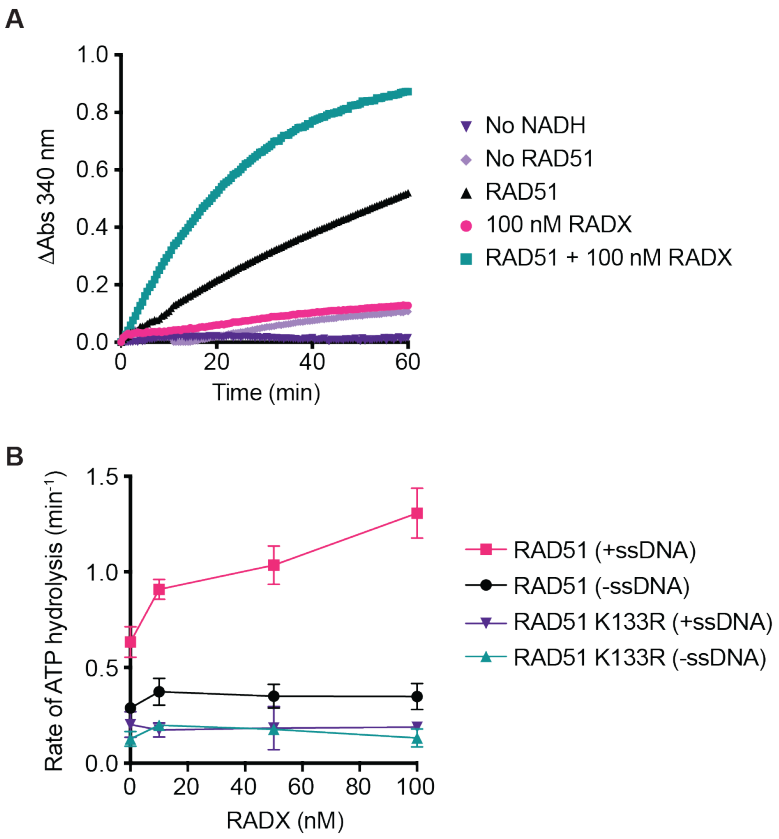

Figure S1. Example of ATPase assay (related to Figure 2). Representative data of the raw change in absorbance of NADH measured at 340 nm used to calculate rate of ATP hydrolysis in Figure 2. The 'No NADH' control represents the background absorbance used to normalize the experimental measurements. (B) ATPase assay with RAD51 or RAD51 K133R and increasing concentrations of RADX added to the reaction after the addition of RAD51. The reactions were performed in the presence or absence of ssDNA as indicated in the legend. (n=2, +/- SD)

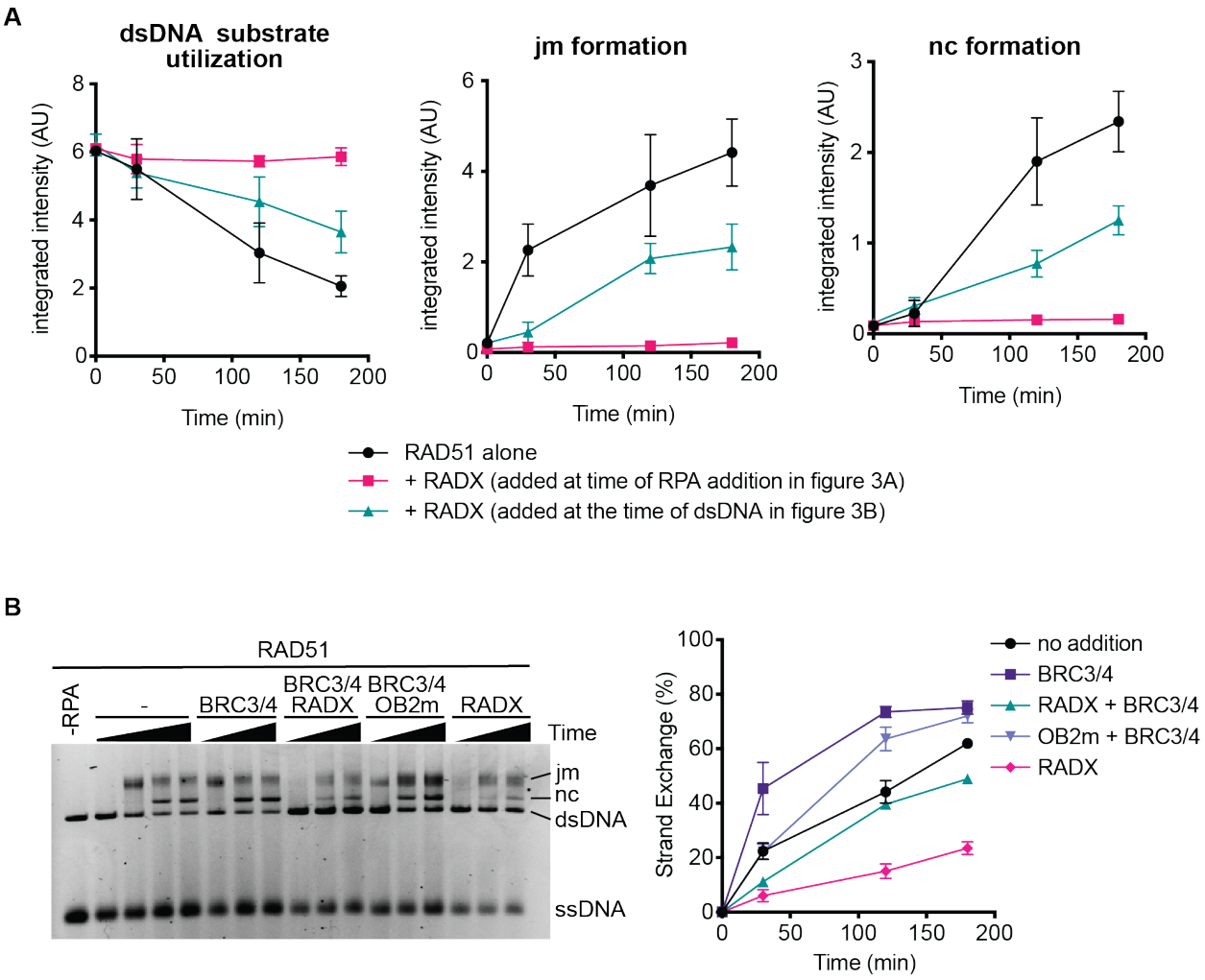

Figure S2. BRC3/4 motifs neutralize the inhibition of RAD51 strand exchange by RADX (related to Figure 3). (A) Quantification of dsDNA utilization and product formation of joint molecules (jm) and nicked circles (nc) from the strand exchange assays in Figure 3. (B) RAD51 mediated strand exchange assay in which RADX was added at the same time as the linear dsDNA to initiate the reaction. A representative gel and quantitation from n=3 experiments is shown.

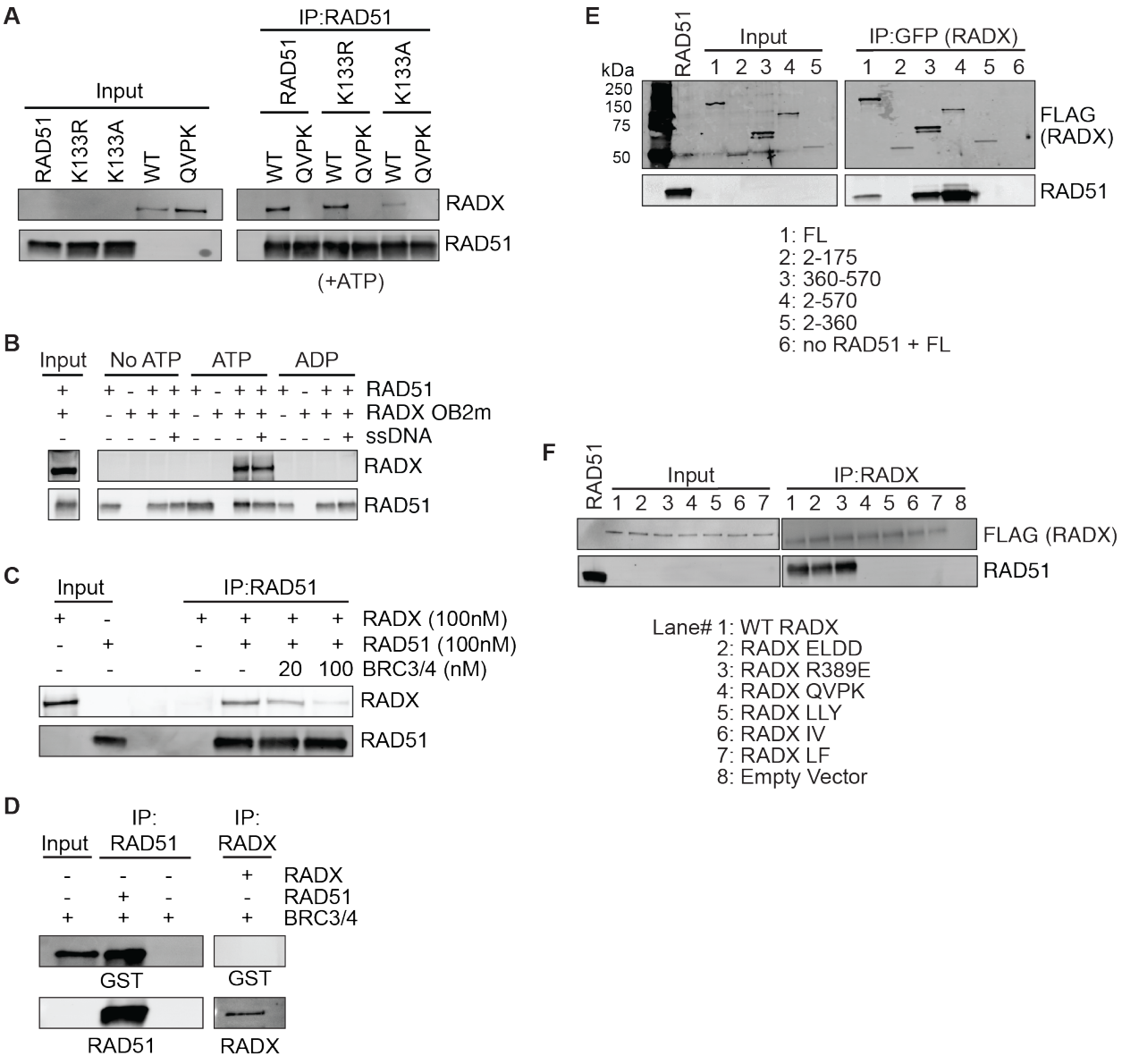

Figure S3. RADX and RAD51 interact directly (related to Figure 4). (A) Direct interaction of RADX wild-type (WT) or RADX QVPK with RAD51, RAD51 K133R, and K133A mutants was assessed using RAD51 antibody conjugated to Protein G beads. Binding reactions contained the indicated proteins and ATP. After washing, the beads were boiled in SDS loading buffer, and eluted proteins detected by immunoblotting. (B) Direct interaction of RADX OB2m and RAD51 was assessed using RAD51 antibody conjugated to Protein G beads. Binding reactions contained the indicated proteins, nucleotide, and ssDNA. (C) RAD51 and increasing

concentrations of GST-BRC3/4 were preincubated before the addition of RADX to the binding reactions to assess competition between BRC3/4 and RADX. (D) RAD51 co-immunoprecipitates with GST-BRC3/4 but RADX does not. (E and F) Interactions of purified RAD51 and FLAG-GFP-RADX fragments and mutants were assessed with GFP or RADX antibodies conjugated to Protein A beads in the presence of ATP. FL, full length.