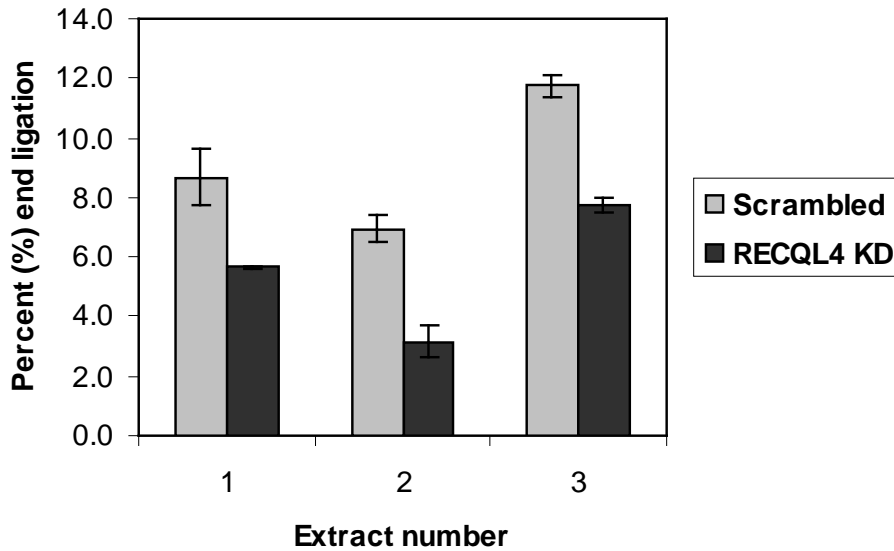
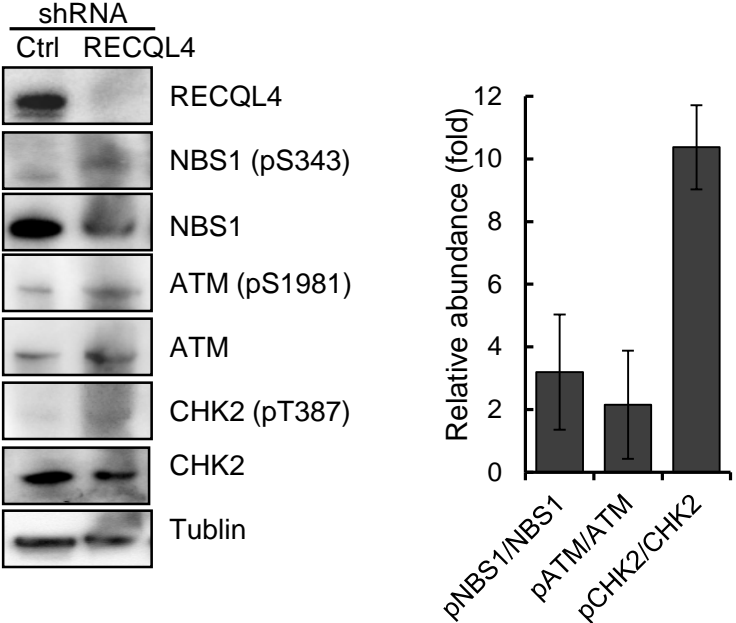


Supplementary Figure 1



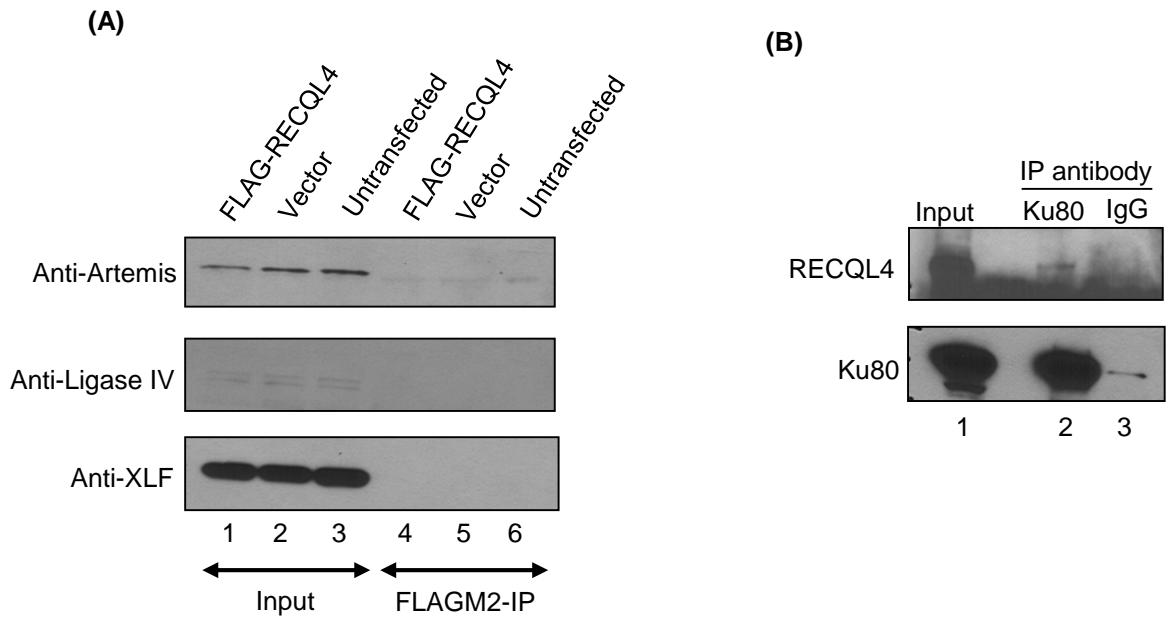
Supplementary Figure S1: RECQL4-deficient U2OS cells show end joining defect *in vitro*. *In vitro* NHEJ assay on cohesive-end containing DNA substrate with cellular extracts prepared from scrambled, RECQL4 KD U2OS cells. The end-joining was performed with EcoR1-linearized pKS plasmid DNA substrate. The products were resolved by agarose gel electrophoresis and quantified with the ImageJ software. Bar graph represents the % end ligation activities of scrambled and RECQL4 KD U2OS cell extracts. The experiments were done 3 times with the three independently prepared extracts. Error bars represents the standard deviation (+/-) of 3 independent experiments.

Supplementary Figure 2



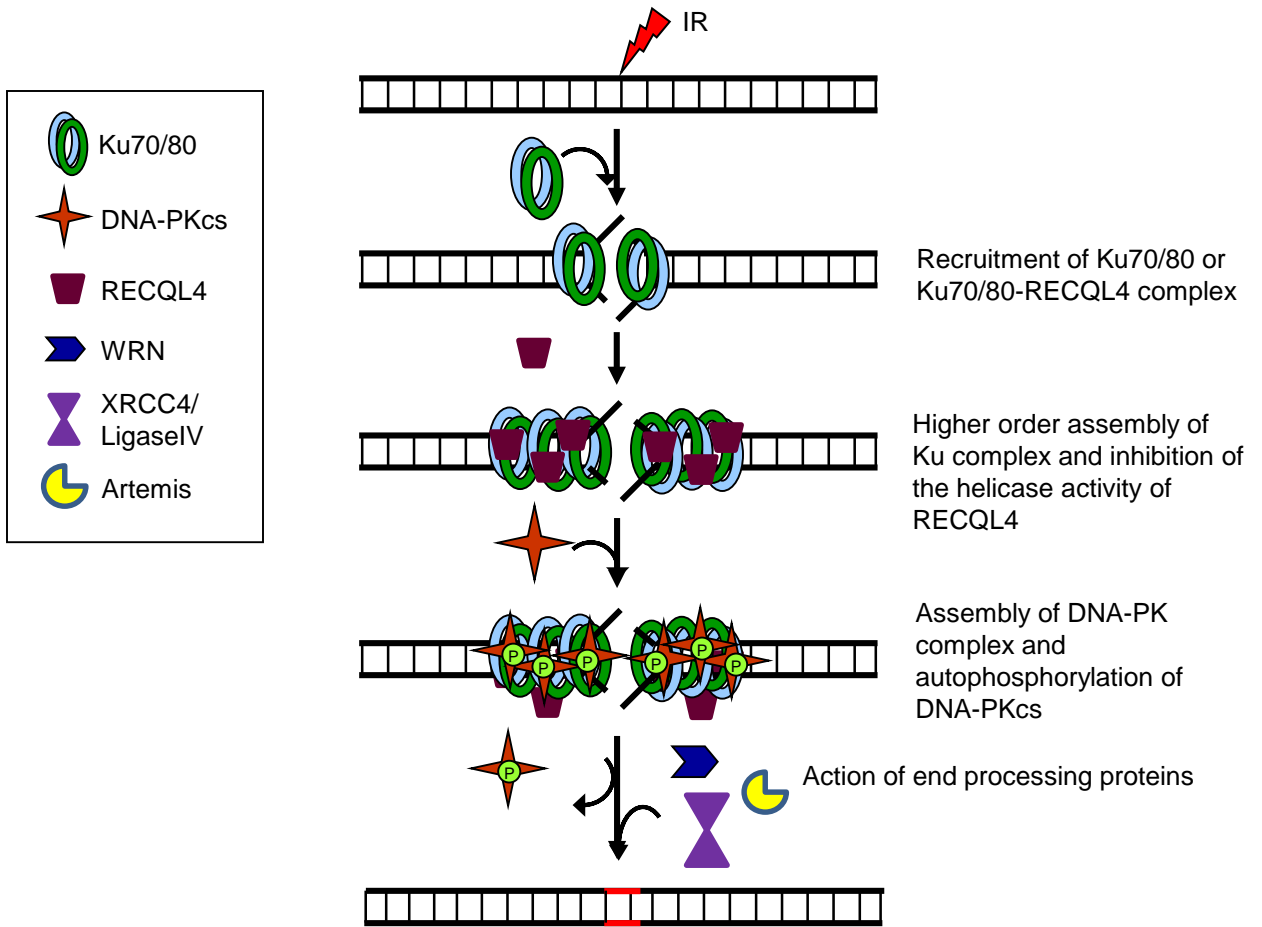
Supplementary Figure 2. Immunoblot analysis of NBS1, ATM and CHK2 in RECQL4 knockdown cells. Bargraph shows the ratios of phosphorylated to total amount of NBS1, ATM and CHK2 normalized to tubulin.

Supplementary Figure 3



Supplementary Figure 3. (A) FLAG tagged RECQL4 does not interact with NHEJ factors Artemis, XLF or Ligase IV. The HeLa cells were transfected either with FLAG-RECQL4, empty vector or untransfected cells. 24 hrs post transfection, the cells were lysed and immunoprecipitated with FLAG-M2 beads. The blot was probed with Artemis, Ligase IV or XLF antibodies. The inputs are shown in lanes 1, 2 and 3. The FLAG-M2 immunoprecipitated products are shown in lanes 4, 5 and 6 as indicated for each lanes. (B) RECQL4 co-immunoprecipitates with Ku80. Immunoprecipitation was performed with Ku80 (lane 2) and normal IgG (lane 3) antibodies and HeLa cell extracts. Immunoprecipitates were probed for Ku80 and RECQL4 by western blotting.

Supplementary Figure 4



Supplementary Figure 4. Model for the RECQL4 functions in NHEJ. Ionizing radiation-induced DSB are recognized by the Ku70/Ku80 heterodimer. RECQL4 interacts with Ku70/Ku80, and in the close vicinity of broken DNA ends, the interaction enhances higher order binding and stabilization of the Ku70/Ku80 complex with the DNA on DSB. Following the stabilization of Ku-DNA complex, DNA-PKcs associates with DNA-bound Ku forming active DNA-PK complex. DNA-PK undergoes autophosphorylation and mediates the NHEJ pathway by activating the downstream proteins, including nucleases (Artemis, WRN), polymerases and ligases (XRCC4/LigaseIV). Following the repair of the damaged DNA, the NHEJ proteins are cleared from the DSB.