Supplementary information, Figure S1

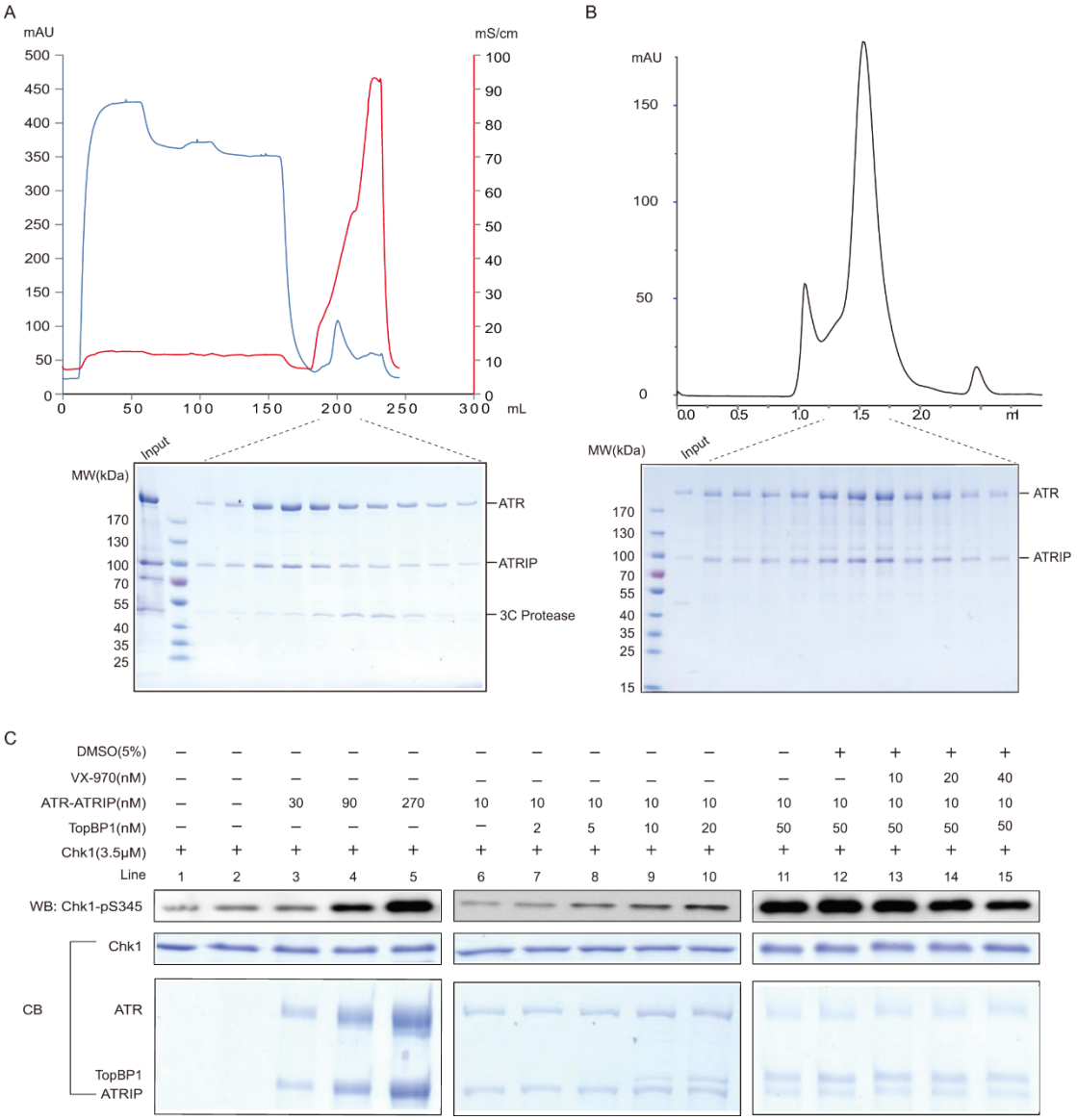

Figure S1 Complex purification and characterization of ATR-ATRIP. (A, B) Cation exchange (A) and size exclusion chromatogram (B) of the human ATR-ATRIP complex. The cation exchange was performed using Mono S column (5/50 GL, GE Healthcare) and Gel filtration using a Superose 6 increase column (5/150 GL, GE Healthcare). The peak fractions were subjected to SDS-PAGE for Coomassie blue staining. (C) *In vitro* kinase assays. Phosphorylation of purified Chk1 by increasing

amount of ATR-ATRIP in the presence or the absence of VX-970, the inhibitor of ATR. The concentration of the protein and VX-970 used in the reactions are indicated above. The activities of ATR-ATRIP were detected by immunoblotting with antibodies targeting phospho-Ser-345 of Chk1. The reaction products were subjected to SDS-PAGE followed by immunoblotting (5 μl reaction products) and Coomassie blue staining (15 μl reaction products). The phosphorylation of $S345^{Chk1}$ increased in the presence of increasing amount of ATR-ATRIP and decreased in the presence of VX-970. The basal phosphorylation of full-length Chk1 (D130A, the kinase dead mutant) may be occurred during expression in 293F cells.