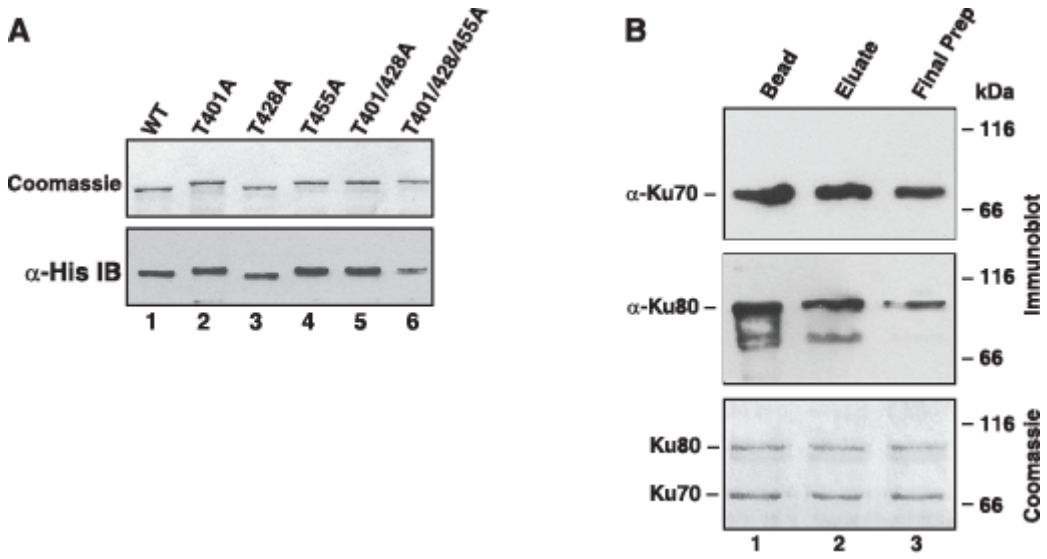
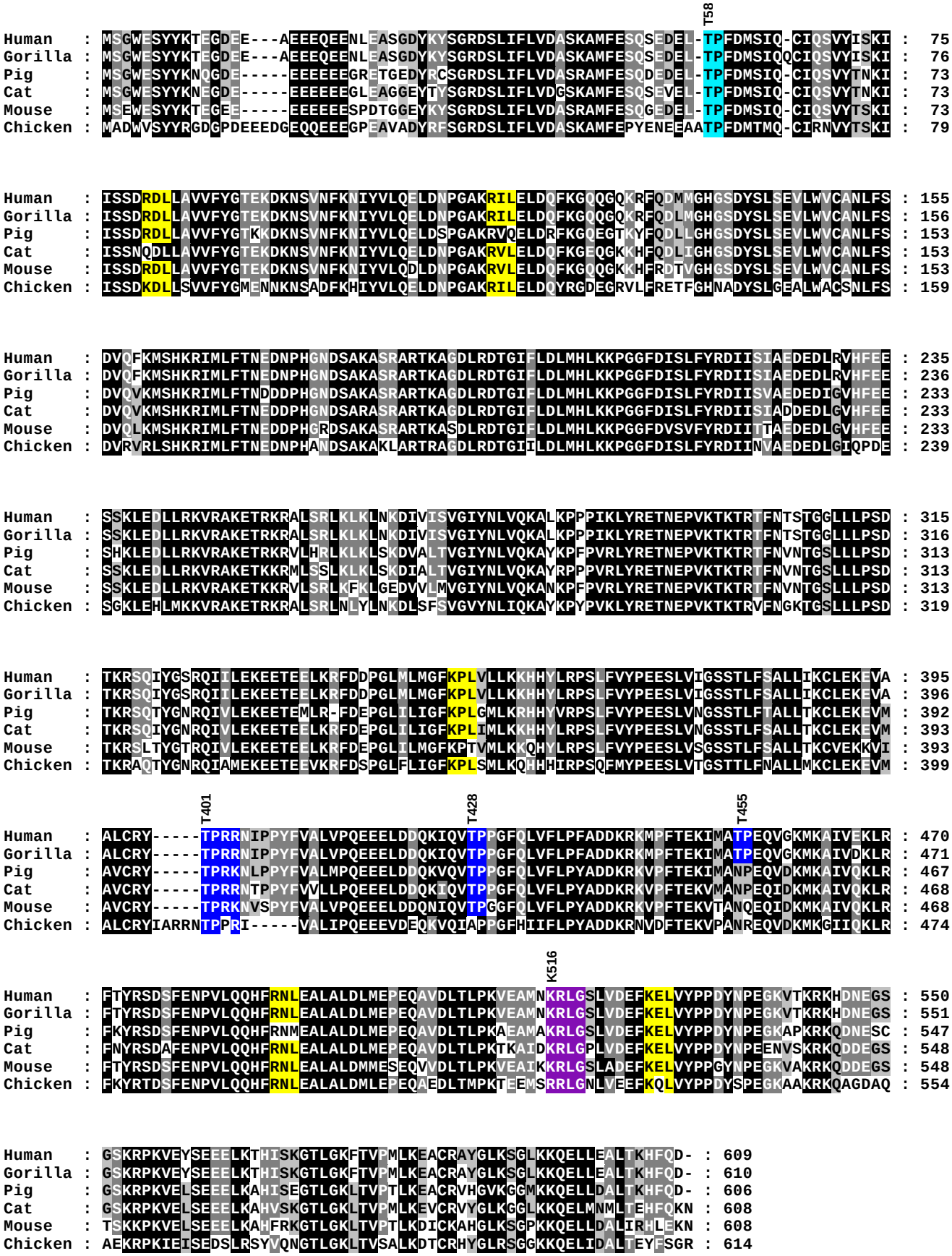
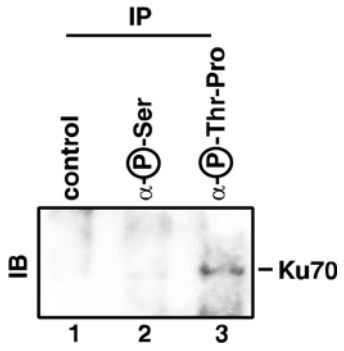


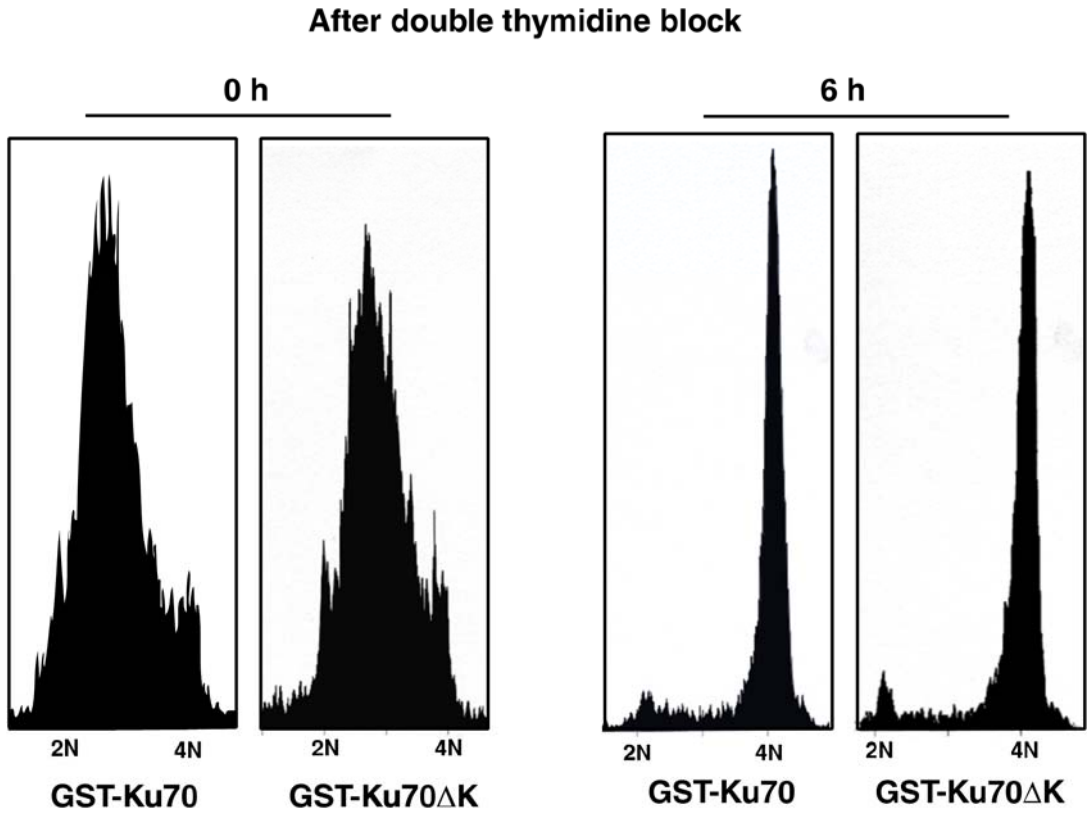
Supplementary Figure S1. Purification of proteins. **(A)** The bacterially expressed and purified ^{6}His tagged wild type and mutant Ku70 proteins were analysed by SDS-PAGE followed by Coomassie blue staining (Coomassie). The identities of the proteins were confirmed by immunoblotting with antibodies against His-tag (α-His IB). **(B)** The Ku dimer (^{6}His-Ku70/SBP-Ku80) was expressed in insect cells and purified by Ni-agarose beads. The purified proteins were analysed by SDS-PAGE followed by Coomassie blue staining and immunoblotting as indicated.



Supplementary Figure S2. The amino acid sequences of Ku70 from the indicated organisms were aligned by ClustalW and shading was done in Genedoc. The canonical Cdk phosphorylation target sites and the adjacent minimal sites are marked with blue shade. A well-conserved minimal Cdk target site on the N-terminal portion is indicated by cyan shading. The most conserved Cy-motif is shaded with purple colour and the other putative R/KxL Cy-motifs are marked with yellow shading. The accession numbers/IDs of Ku70 protein sequences: Human, AAH18259.1; Gorilla, ENSGGOP00000015496; Pig, ENSSSCP00000000065; Cat, ENSFCAT00000018079 ; Mouse, BAA28874.1; Chicken, BAA32018.1.



Supplementary Figure S3. Presence of Ku70 specifically in the α-phospho-Thr-Pro immunoprecipitate from HeLa cell extract. Immunoprecipitation was carried out from HeLa cell extract using either a control IgG or antibodies against phospho-Ser or phosphor-Thr-Pro moieties and the presence of Ku70 in the precipitates was examined by α-Ku70 immunoblotting (IB).



Supplementary Figure S4. Flow cytometer profiles of HeLa cells expressing GST-Ku70 and GST-Ku70ΔK proteins collected as per the workflow depicted in Fig. 5C.