

**Supplementary Figure S1.** Purification of proteins. **(A)** The bacterially expressed and purified  $^6\text{His}$  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 ( $\alpha$ -His IB). **(B)** The Ku dimer ( $^6\text{His}$ -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.

```

Human : MSCWESYYKTEGDEE---AEEEQEENLEASGDYKYSGRDSLIFLVDASKAMFESQSEDEL-TPFDMSIQ-CIQSVYISKI : 75
Gorilla : MSCWESYYKTEGDEE---AEEEQEENLEASGDYKYSGRDSLIFLVDASKAMFESQSEDEL-TPFDMSIQ-CIQSVYISKI : 76
Pig : MSCWESYYKTEGDEE---EEEEEGRETGEDYRCYSGRDSLIFLVDASRAMFESQDEDEL-TPFDMSIQ-CIQSVYTNKI : 73
Cat : MSCWESYYKTEGDEE---EEEEEGLEAGGEYTYSGRDSLIFLVDGSKAMFESQSEVEL-TPFDMSIQ-CIQSVYTNKI : 73
Mouse : MSCWESYYKTEGDEE---EEEEESPDTGGEYTYSGRDSLIFLVDASRAMFESQDEDEL-TPFDMSIQ-CIQSVYTSKI : 73
Chicken : MADWVSYYRGDGPDEEEDGEGQEEEGPEAVADYRFSGRDSLIFLVDASKAMFEPYENEBAATPFDMTMO-CIRNVYTSKI : 79

Human : ISSDRDLLAVVFGYTEKDKNSVNFKNIYVLQELDNPGAKRILELDOFKGQQGQKRFQDLMGHGSDYSLSEVLWVCANLFS : 155
Gorilla : ISSDRDLLAVVFGYTEKDKNSVNFKNIYVLQELDNPGAKRILELDOFKGQQGQKRFQDLMGHGSDYSLSEVLWVCANLFS : 156
Pig : ISSDRDLLAVVFGYTEKDKNSVNFKNIYVLQELDNPGAKRILELDOFKGQQGQKRFQDLMGHGSDYSLSEVLWVCANLFS : 153
Cat : ISSDRDLLAVVFGYTEKDKNSVNFKNIYVLQELDNPGAKRILELDOFKGQQGQKRFQDLMGHGSDYSLSEVLWVCANLFS : 153
Mouse : ISSDRDLLAVVFGYTEKDKNSVNFKNIYVLQELDNPGAKRILELDOFKGQQGQKRFQDLMGHGSDYSLSEVLWVCANLFS : 153
Chicken : ISSDRDLLAVVFGYTEKDKNSVNFKNIYVLQELDNPGAKRILELDOFKGQQGQKRFQDLMGHGSDYSLSEVLWVCANLFS : 159

Human : DVQFKMSHKRIMLFTNEDNPHGNSAKASRARTKAGDLRDTGIFLDMHLKPKGGFDISLFYRDIISIAEDEDLRFVHFEE : 235
Gorilla : DVQFKMSHKRIMLFTNEDNPHGNSAKASRARTKAGDLRDTGIFLDMHLKPKGGFDISLFYRDIISIAEDEDLRFVHFEE : 236
Pig : DVQFKMSHKRIMLFTNEDDPPHGNSAKASRARTKAGDLRDTGIFLDMHLKPKGGFDISLFYRDIISIAEDEDLRFVHFEE : 233
Cat : DVQFKMSHKRIMLFTNEDDPPHGNSAKASRARTKAGDLRDTGIFLDMHLKPKGGFDISLFYRDIISIAEDEDLRFVHFEE : 233
Mouse : DVQFKMSHKRIMLFTNEDDPPHGNSAKASRARTKAGDLRDTGIFLDMHLKPKGGFDVSVFYRDIISIAEDEDLRFVHFEE : 233
Chicken : DVQFKMSHKRIMLFTNEDNPHGNSAKAKLARTRAGDLRDTGIFLDMHLKPKGGFDISLFYRDIISIAEDEDLRFVHFEE : 239

Human : SSKLEDLLRKVRAKETRKRALSRLKLNKDIIVISVGIYNLVQKAKKPPPIKLYRETNEPVKTKTRIFNTSTGCLLLPSD : 315
Gorilla : SSKLEDLLRKVRAKETRKRALSRLKLNKDIIVISVGIYNLVQKAKKPPPIKLYRETNEPVKTKTRIFNTSTGCLLLPSD : 316
Pig : SSKLEDLLRKVRAKETRKRALSRLKLNKDIIVISVGIYNLVQKAKKPPPIKLYRETNEPVKTKTRIFNVNTGCLLLPSD : 313
Cat : SSKLEDLLRKVRAKETRKRALSRLKLNKDIIVISVGIYNLVQKAKKPPPIKLYRETNEPVKTKTRIFNVNTGCLLLPSD : 313
Mouse : SSKLEDLLRKVRAKETRKRALSRLKLNKDIIVISVGIYNLVQKAKKPPPIKLYRETNEPVKTKTRIFNVNTGCLLLPSD : 313
Chicken : SSKLEDLLRKVRAKETRKRALSRLKLNKDIIVISVGIYNLVQKAKKPPPIKLYRETNEPVKTKTRIFNVNTGCLLLPSD : 319

Human : TKRSQIYGSRQIILEKEETEELKRFDDPGLMLMGFKPLVLLKKHHYLRPSLFVYPEESLVVIGSSTLFSALLIKCLEKEVA : 395
Gorilla : TKRSQIYGSRQIILEKEETEELKRFDDPGLMLMGFKPLVLLKKHHYLRPSLFVYPEESLVVIGSSTLFSALLIKCLEKEVA : 396
Pig : TKRSQIYGNRQIVLEKEETEELKRFDEPGLMLMGFKPLVLLKKHHYLRPSLFVYPEESLVVIGSSTLFSALLIKCLEKEVM : 392
Cat : TKRSQIYGNRQIVLEKEETEELKRFDEPGLMLMGFKPLVLLKKHHYLRPSLFVYPEESLVVIGSSTLFSALLIKCLEKEVM : 393
Mouse : TKRSQIYGNRQIVLEKEETEELKRFDEPGLMLMGFKPLVLLKKHHYLRPSLFVYPEESLVVIGSSTLFSALLIKCLEKEVM : 393
Chicken : TKRSQIYGNRQIVLEKEETEELKRFDEPGLMLMGFKPLVLLKKHHYLRPSLFVYPEESLVVIGSSTLFSALLIKCLEKEVM : 399

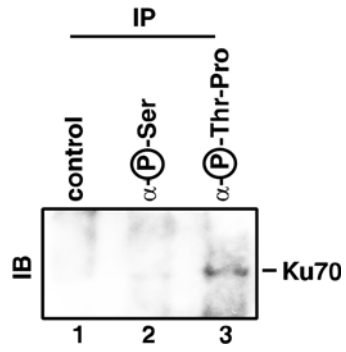
Human : ALCRY-----TPRRNIPPYFVALVPQEEELDDQKIQVTPPGFQLVFLPFADDKRVKMPFTEKIMATPEQVQKMKKAI VEKLR : 470
Gorilla : ALCRY-----TPRRNIPPYFVALVPQEEELDDQKIQVTPPGFQLVFLPFADDKRVKMPFTEKIMATPEQVQKMKKAI VDKLR : 471
Pig : AVCRY-----TPRKNLPPYFVALVPQEEELDDQKVQVTPPGFQLVFLPYADDKRVKMPFTEKIMANPEQVDKMKKAI VQKLR : 467
Cat : AVCRY-----TPRRNIPPYFVALVPQEEELDDQKIQVTPPGFQLVFLPYADDKRVKMPFTEKIMANPEQVDKMKKAI VQKLR : 468
Mouse : AVCRY-----TPRKNLPPYFVALVPQEEELDDQKIQVTPPGFQLVFLPYADDKRVKMPFTEKIMANPEQVDKMKKAI VQKLR : 468
Chicken : ALCRYIARRNTPPRI-----VALIQEEVDEQKQVQIAPPGFHIIIFLPYADDKRVKMPFTEKIMANPEQVDKMKKAI VQKLR : 474

Human : FTYRSDSFENPVLQQHFERNLEALALDLMPEQAVDLTLPKVEAMNKRRLGSLVDEFKELVYPPDYNPEGKVTKRKHEDNEGS : 550
Gorilla : FTYRSDSFENPVLQQHFERNLEALALDLMPEQAVDLTLPKVEAMNKRRLGSLVDEFKELVYPPDYNPEGKVTKRKHEDNEGS : 551
Pig : FKYRSDSFENPVLQQHFERNLEALALDLMPEQAVDLTLPKVEAMNKRRLGSLVDEFKELVYPPDYNPEGKAPKRKQDNESC : 547
Cat : FNYRSDSFENPVLQQHFERNLEALALDLMPEQAVDLTLPKVEAMNKRRLGSLVDEFKELVYPPDYNPEGKVTKRKHEDNEGS : 548
Mouse : FTYRSDSFENPVLQQHFERNLEALALDLMPEQAVDLTLPKVEAMNKRRLGSLVDEFKELVYPPDYNPEGKVTKRKHEDNEGS : 548
Chicken : FKYRSDSFENPVLQQHFERNLEALALDLMPEQAVDLTLPKVEAMNKRRLGSLVDEFKELVYPPDYNPEGKAPKRKQAGDAQ : 554

Human : GSKRPKVEYSEELKTHISKGTLGKFTVPMLEACRAYGLKSGLKKQELLEALTKHFOD- : 609
Gorilla : GSKRPKVEYSEELKTHISKGTLGKFTVPMLEACRAYGLKSGLKKQELLEALTKHFOD- : 610
Pig : GSKRPKVELSEELKTHISEGTLGKLTVPMLKEACRVHGVKGGMKQELLDALTKHFOD- : 606
Cat : GSKRPKVELSEELKTHISKGTLGKLTVPMLKEACRVHGVKGGMKQELLDALTKHFOD- : 608
Mouse : TSKRPKVELSEELKTHISEGTLGKLTVPMLKEACRVHGVKGGMKQELLDALTKHFOD- : 608
Chicken : ASKRPKVELSEELKTHISEGTLGKLTVPMLKEACRVHGVKGGMKQELLDALTKHFOD- : 614

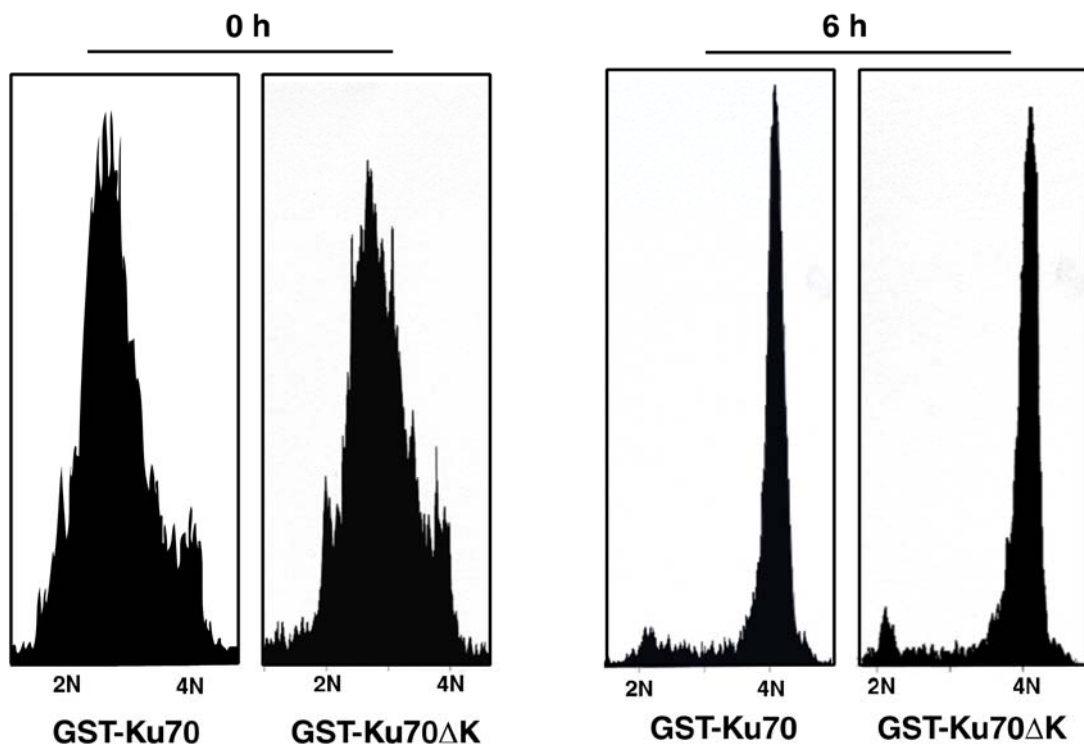
```

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  $\alpha$ -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 phospho-Thr-Pro moieties and the presence of Ku70 in the precipitates was examined by  $\alpha$ -Ku70 immunoblotting (IB).

After double thymidine block



**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.