SUPPLEMENTAL INFORMATION

Role for casein kinase 1 in the phosphorylation of Claspin on critical residues necessary for the activation of Chk1

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Evaluation of CK1γ siRNA knockdowns

(A) RT-PCR analysis of CK1γ1 mRNA levels after siRNA transfection. Cells were incubated with control siRNA (C) or siRNAs specific for CK1γ1 (#1, #2, and #3) for 48 hr. Total cellular RNA was isolated and semi-quantitative RT-PCR was performed to measure the mRNA level for the kinase. β-Actin was used as a control. Relative band intensities (indicated below the panels) were measured by using the ImageQuant program.

(B) RT-PCR analysis of CK1γ2 mRNA levels in cells treated with the indicated CK1γ2 siRNAs was carried out as described in (A).

(C) RT-PCR analysis of CK1γ3 mRNA levels in cells treated with the indicated CK1γ3 siRNAs was carried out as described in (A).

(D) Anti-human CK1γ1 antibodies were produced as described in Materials and Methods. Equal amounts (2 ng) of purified recombinant CK1γ1, CK1γ2, and CK1γ3 proteins were subjected to SDS-PAGE and immunoblotted with these anti-CK1γ1 antibodies.

(E) U2OS cells were treated with control siRNA or three different CK1γ1 siRNAs (#1, #2, and #3) for 48 hr. Whole cell lysates were immunoprecipitated with the anti-CK1γ1 antibodies and finally immunoblotted with the same antibodies.

(F) U2OS cells were treated with control siRNA or three different CK1γ2 siRNAs (#1, #2, and #3) for 48 hr and immunoblotted with commercially available anti-CK1γ2 antibodies (N-20; Santa Cruz Biotechnology).

(G) U2OS cells harboring an inducible form of a GST-tagged CKAD from *Xenopus* Claspin were incubated with doxycycline for 16 hr and then treated with APH for the indicated times.

Cell lysates were prepared and immunoblotted with antibodies against Ser864-phosphorylated Claspin and GST.

(H) Human U2OS cells were treated for 48 hr with control siRNA (lane 1), CK1γ1 siRNA #2 (lane 2), CK1γ2 siRNA #1 (lane 3), and CK1γ3 siRNA #2 (lane 4). Whole cell lysates were prepared and immunoblotted for the indicated proteins.

(I) Human U2OS cells were treated for 48 hr with no siRNA (lane 1) or the indicated siRNAs. Whole cell lysates were prepared and immunoblotted for the indicated proteins.

Supplemental Figure 2. Further characterization of the effect of CK1γ siRNA knockdowns on checkpoint responses

(A) HeLa cells were treated for 48 hr with no siRNA (lanes 1 and 2), TopBP1 siRNA (lane 3), CK1γ1 siRNA #1 (lane 4), CK1γ1 siRNA #2 (lane 5), CK1γ1 siRNA #3 (lane 6), all three CK1γ1 siRNAs (lane 7), or control siRNA (lane 8). Cells were incubated in the absence (lane 1) or presence of APH for 10 min (lanes 2-8). Total cell lysates were prepared and immunoblotted with the indicated antibodies. Band signals for phospho-Chk1 Ser345 and Chk1 were quantitated by using the ImageJ program (right).

(B) HeLa cells were treated for 48 hr with no siRNA (lanes 1 and 2), TopBP1 siRNA (lane 3), CK1γ2 siRNA #1 (lane 4), or control siRNA (lane 5). Cells were incubated in the absence or presence of APH for 10 min as indicated. Total cell lysates were prepared and immunoblotted with the indicated antibodies.

(C) HeLa cells were treated for 48 hr with no siRNA (lanes 1 and 2), TopBP1 siRNA (lane 3), CK1γ3 siRNA #2 (lane 4), or control siRNA (lane 5). Cells were incubated in the absence or presence of APH for 10 min as indicated. Total cell lysates were prepared and immunoblotted with the indicated antibodies.

(D) U2OS cells were treated for 48 hr with esiRNAs directed against TopBP1 (lane 2), CK1γ1 (lane 3), CK1γ2 (lane 4), and CK1γ3 (lane 5). esiRNA against firefly luciferase was used as a control (lane 1). Cells were treated with APH for 10 min and processed for immunoblotting with the indicated antibodies. esiRNAs were produced as described in Materials and Methods.
(E) U2OS cells were treated for 48 hr with no siRNA (lanes 1 and 2), TopBP1 siRNA (lane 3), CK1γ1 siRNA #2 (lane 4), CK1γ2 siRNA #1 (lane 5), CK1γ3 #2 (lane 6), or control siRNA (lane 7). Cells were incubated in the absence or presence of APH for 10 min as indicated. Cell lysates were prepared and immunoblotted with antibodies against Ser645-phosphorylated Rad17 and Rad17 protein.

Supplemental Figure 3. Properties of CK1γ1-depleted cells
(A) Cell numbers in cultures of U2OS cells were determined at the indicated times following transfection with control siRNA, CK1γ1 siRNA (#1, #2, and #3), or CK1γ2 siRNA #1.
(B) U2OS cells were treated with control siRNA, three different CK1γ1 siRNAs (#1, #2, and #3), or CK1γ2 siRNA #1 for 48 hr and then processed for indirect immunofluorescence with antibodies against γ-H2AX. Percentages of cells positive for γ-H2AX staining were determined and plotted.
(C) U2OS cells were treated for 48 hr with no siRNA (lanes 1 and 2), TopBP1 siRNA (lane 3), CK1γ1 siRNA #1 (lane 4), CK1γ1 siRNA #2 (lane 5), or control siRNA (lane 6). Cells were exposed to UV radiation (100 J/m^2) and then incubated for 2 hr. Cell lysates were prepared and immunoblotted with the indicated antibodies.

Supplemental Figure 4. Rescue of CK1γ1-depleted cells

(A) Different stable lines (clones #2 and #8) of U2OS cells harboring an inducible siRNA-resistant version of CK1γ1 isoform A were transfected with control siRNA (lanes 1, 2, 5, and 6) or CK1γ1 siRNA #2 (lanes 3, 4, 7, and 8) and either not induced or induced with 100 ng/ml doxycycline (Dox) as indicated. After 48 hr, cells were incubated in the absence or presence of APH for 10 min, and then whole cell lysates were prepared and immunoblotted.

(B) Alignment of the C-terminal regions of different CK1γ1 isoforms. Amino acid sequences of various CK1γ1 isoforms were aligned with the ClustalW2 program. Only unique regions in the C-terminal ends of the proteins are shown.

(C) Comparison of kinase activity with other members of the CK1 family. The GST-CKAD was co-expressed in U2OS cells with no kinase (lanes 1, 2, 9, and 10), isoform C of CK1γ1 (lanes 3, 4, 11, and 12), CK1α (lanes 5 and 6), CK1ε (lanes 7 and 8), or a short (lanes 13 and 14) or long form of CK1δ (lanes 15 and 16). Cells were incubated in the absence or presence of APH for 10 min as indicated. Cell lysates were prepared and immunoblotted with anti-P-Ser864, anti-GST, anti-FLAG, and anti-tubulin antibodies.

Supplemental Figure 5. Rescue of CK1γ1-depleted phenotype

(A) Asynchronous cultures of U2OS cells were treated with control siRNA, CK1γ1 siRNA #2, or treated with CK1γ1 siRNA #2 and rescued by concurrent infection with three lentiviruses encoding siRNA-resistant forms of CK1γ1 isoforms A, B, and C. Cells were stained with 7-AAD, and DNA content was analyzed by flow cytometry. Percentages of cells in each phase of the cell cycle were quantified with the FlowJo program.

(B) Rescue of G2/M checkpoint. U2OS cells treated with control siRNA, CK1γ1 siRNA #2, or treated with CK1γ1 siRNA #2 and rescued by concurrent infection with three lentiviruses encoding siRNA-resistant forms of CK1γ1 isoforms A, B, and C. Cells were irradiated with UV

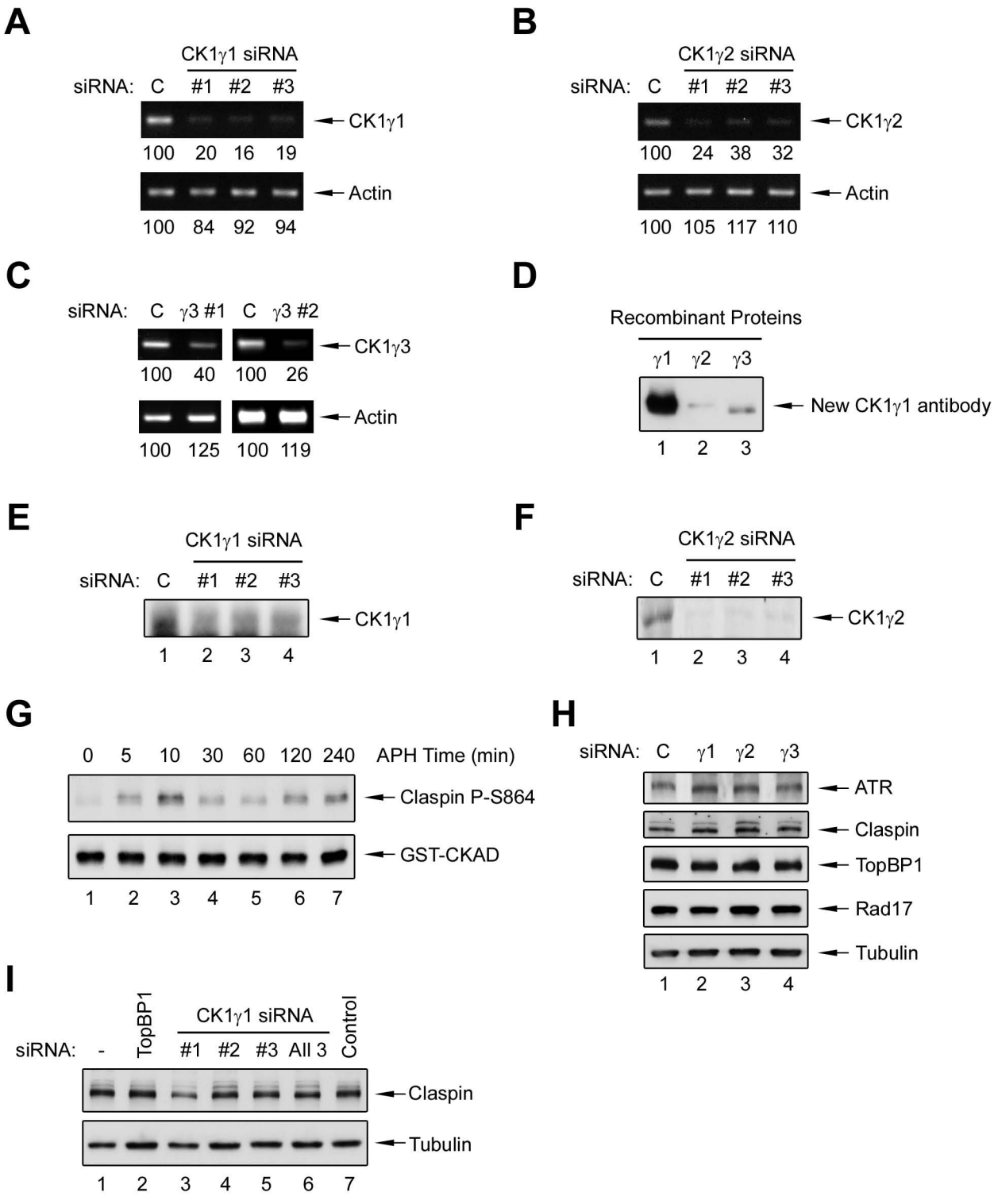
(20 J/m^2) and then incubated for 1 hr before fixation. Cells were stained with anti-phospho-histone H3 antibodies conjugated with Alexa 488 dye and with 7-AAD. Percentages of mitotic cells were determined by flow cytometry.

Supplemental Table 1. siRNA sequences

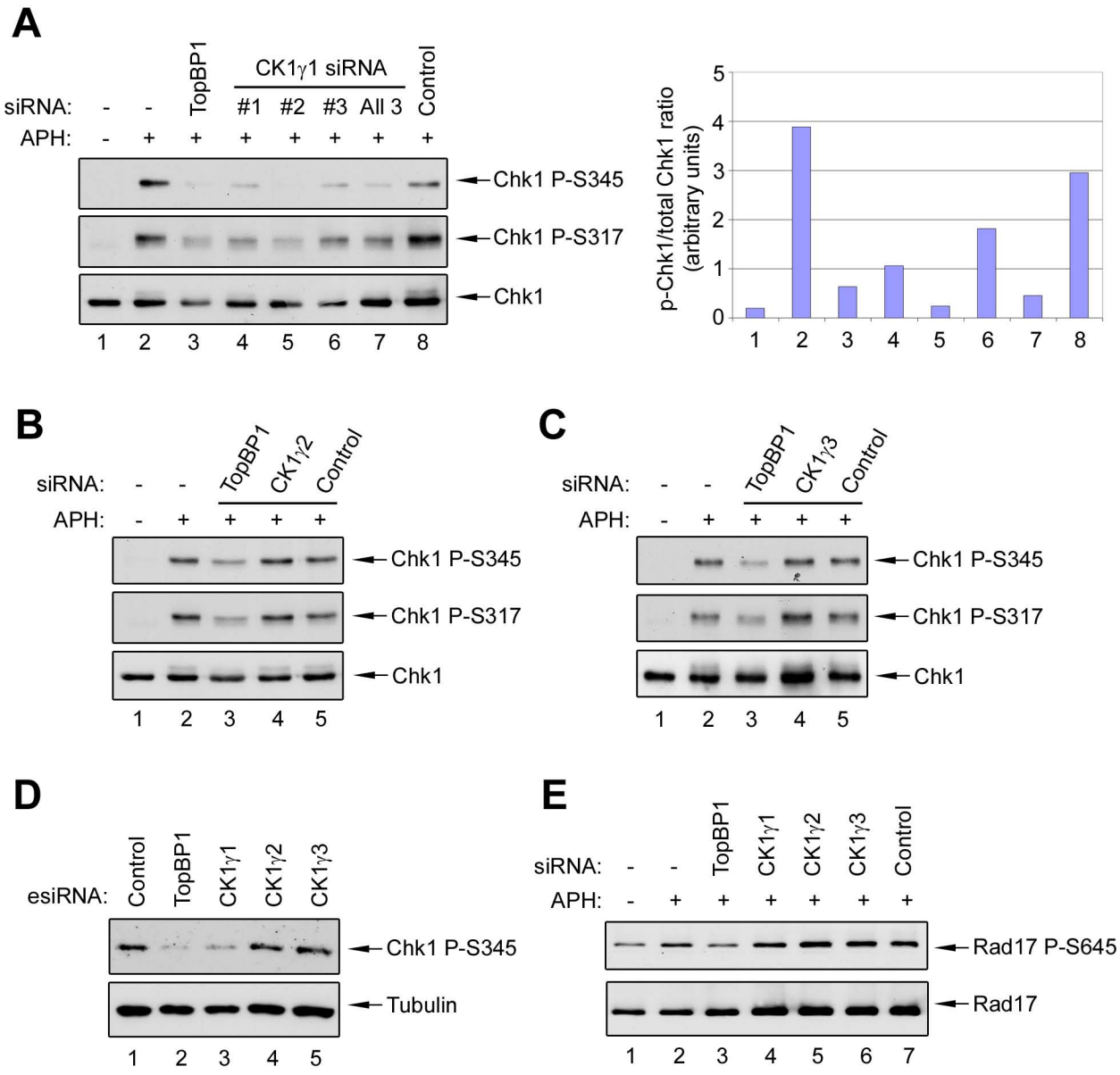
Name	**Sense Strand Sequence (5′-3′)**
CK1γ1 #1	ACA AGG CAA UAA GAA AGA GCA UGU U
CK1γ1 #2	CCA CAG CUU CAU UUA GAG UAC AGA U
CK1γ1 #3	UGA AGA CGG UGU UAA UGA UAG CCA U
CK1γ2 #1	GCC UAG GAA AGA AUC UCU AUA CAA A
CK1γ2 #2	ACG UGG CUA UCA AAU UGG AGC CGA U
CK1γ2 #3	CAG CUG CAC CUG GAG UAC CGG UUC U
CK1γ3 #1	UCU UCG UUA UGU AAG AAG GCU AGA U
CK1γ3 #2	GGC UGA CAC AUU AAA GGA GAG GUA U
Isoform C #71	UAA ACA GGA AUA AUG UGC UGG AAA U
Isoform C #156	CAG AUG ACA GGA GUG AGA GCC UUC A
Chk1	GCG UGC CGU AGA CUG UCC A dT dT

Supplemental Table 2. RT-PCR primer sequences

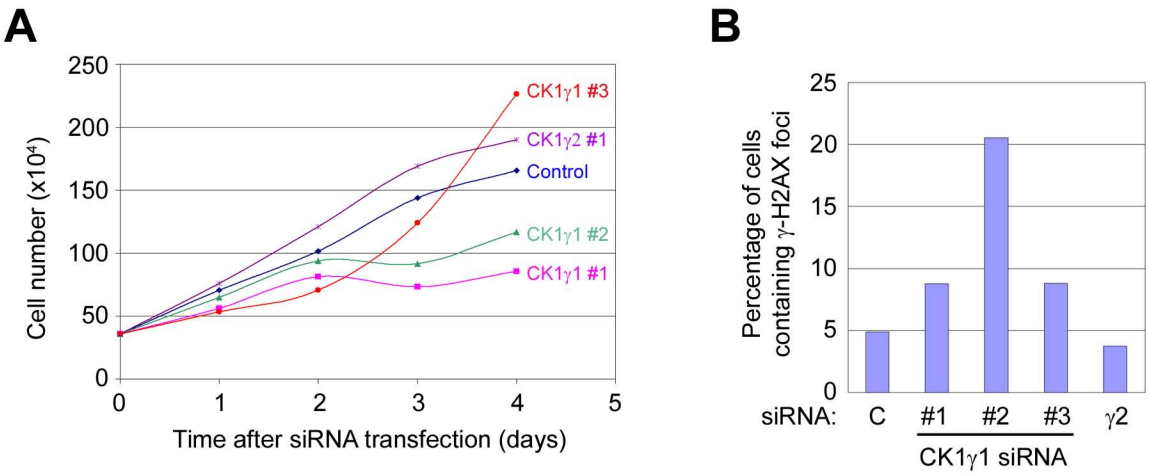
Name	**Sense Strand Sequence (5′-3′)**
CK1γ1 5′	GGA GTG CAC ACT GCT CTC GAC C
CK1γ1 3′	ACC TGT GGG AGA CCT TCA CCT GC
CK1γ2 5′	CAG AGG GCG TCC CTC AGG TCT AC
CK1γ2 3′	GTG GAT GGC ATG CTG CCG CTT G
CK1γ3 5′	GGT CGA TCG GGA CAC AAC ACT CG
CK1γ3 3′	ACC ACA AGG GCC GAA ATA GTA AAC TTG
β-actin 5′	GCT CGT CGT CGA CAA CGG CTC
β-actin 3′	CAA ACA TGA TCT GGG TCA TCT TCT C



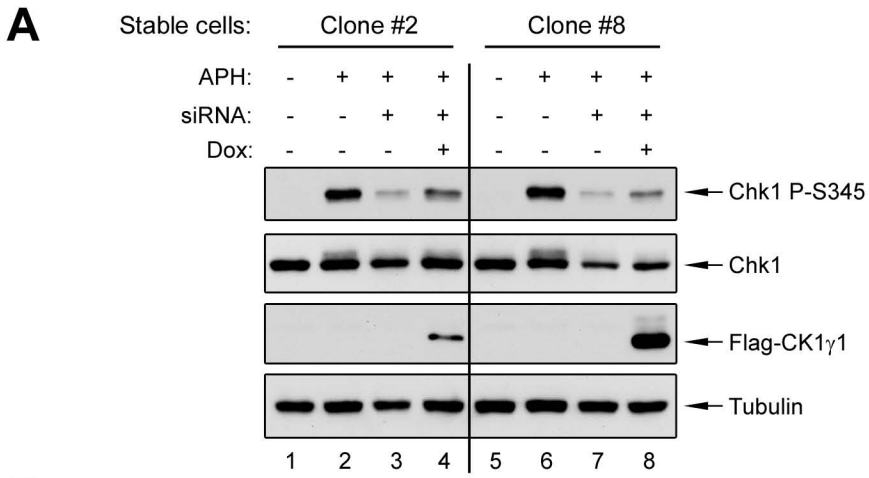
Supplemental Figure 1

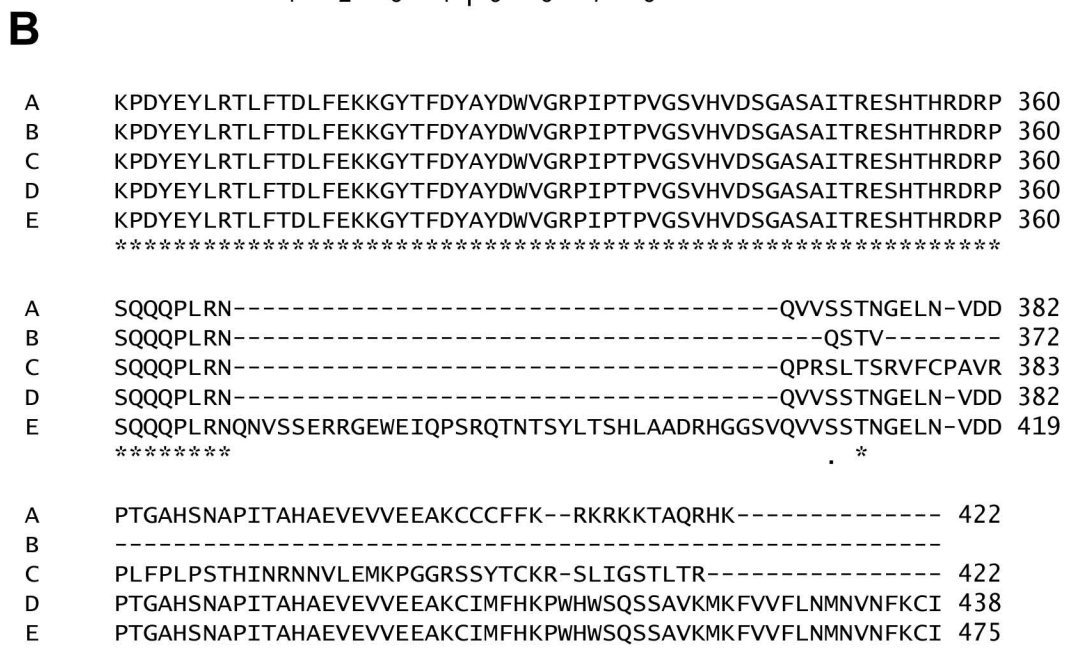


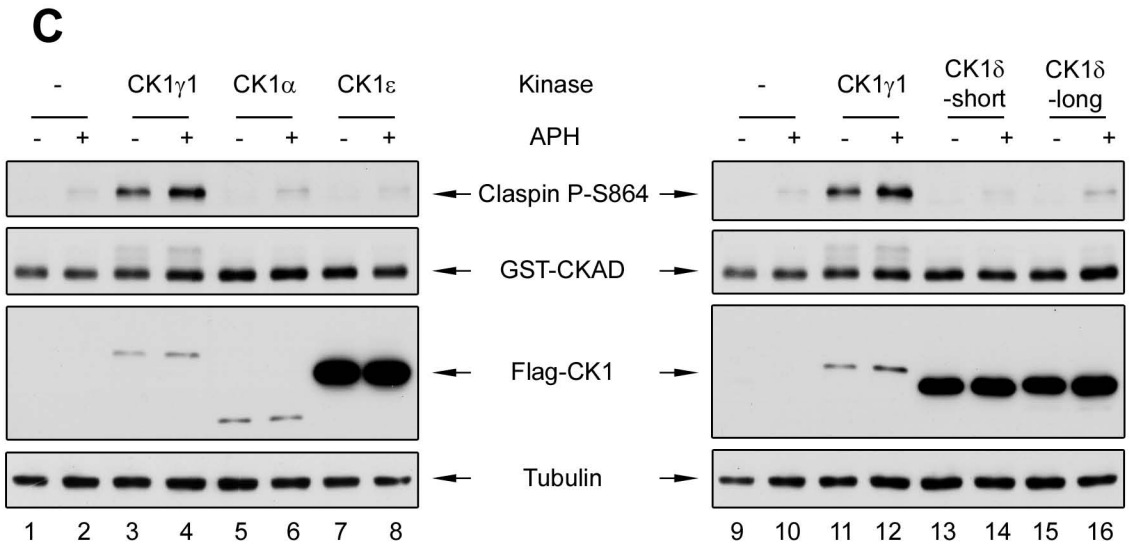
Supplemental Figure 2



Supplemental Figure 3

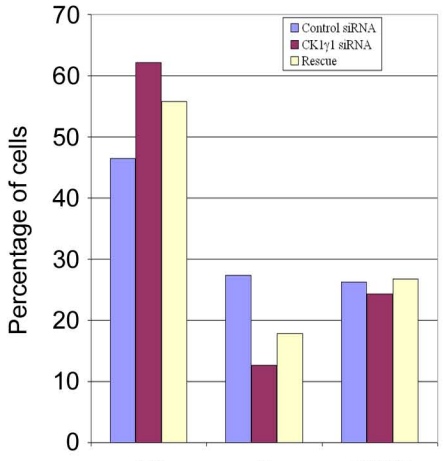






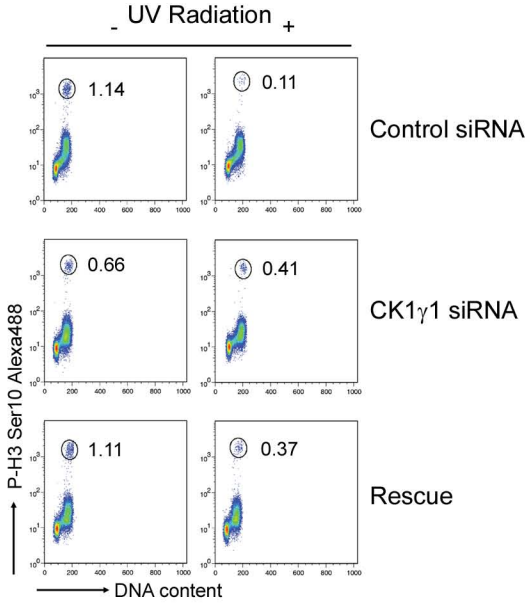
Supplemental Figure 4

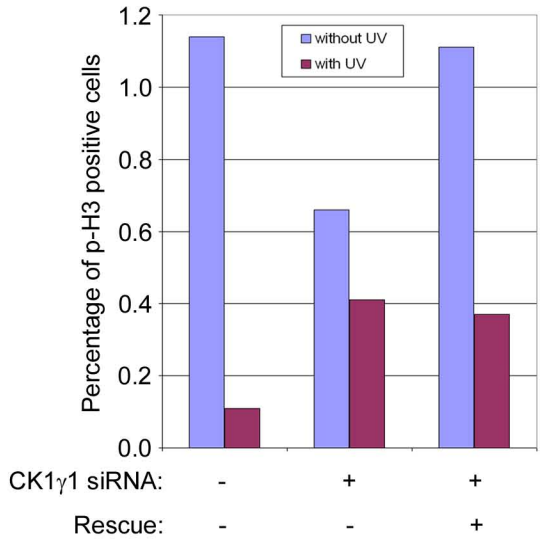
A



	G1	S	G2/M
Control siRNA:	46.5	27.4	26.3
CK1γ1 siRNA:	62.2	12.6	24.3
Rescue:	55.8	17.8	26.8

B





Supplemental Figure 5