**Supplementary materials**

**Materials and methods**

**Plasmid constructs**

The cDNA encoding CKIP-1 was generated by PCR from the human 22-week fetal liver cDNA library. All deletion and point mutants of CKIP-1, c-Jun and others were generated by PCR or recombinant PCR, followed by subcloning into: (i) yeast two-hybrid plasmids pGBKT7 and pGADT7 (Clontech); (ii) pGEX-4T-2 (Pharmacia) for expression in bacteria; (iii) pCMV-Myc (Clontech), or pFlag-CMV-2 (Sigma) to express N-terminal Myc or Flag-tagged protein, and pcDNA3.1-Myc (version C) (Invitrogen) to express C-terminal Myc-tagged protein (and for stable transfection); (iv) pSP72 (Promega) for *in vitro* translation; (v) pEGFP-C1, pDsRed1-N1 (Clontech) to express GFP or RFP-fused protein.

The restriction endonuclease enzymes used in the subcloning were listed below.

(i) pGADT7-CKIP-1/C-term1/ΔC-term1, *Nde*I/*Eco*RI

(ii) pGBKT7-c-Jun/bzJun/lzJun/ΔbzJun/ΔlzJun/bzJunD/bzJunB/bzFos/bzATF2, *Nde*I/*Eco*RI

(iii) pcDNA3.1-myc-CKIP-1/CKIP-D310+345A, *Hind*III/*Kpn*I; pCMV-Myc-CKIP-1/C-term1/C-term2/ΔC-term1/ΔC-term2/c-Jun, *Eco*RI/*Kpn*I; pFlag-CMV-2-CKIP-1/c-Jun, *Eco*RI/*Kpn*I; pCMV-Myc-Bax, *Eco*RI/*Xho*I

(iv) pSP72-CKIP-1 wild-type and all mutants, *Eco*RI/*Kpn*I

(v) pGEX-4T-2-CKIP-1, *Eco*RI/*Not*I; pGEX-4T-2-PH, *Eco*RI/*Xho*I;

pGEX-4T-2-c-Jun, *Bam*HI/*Xho*I

(vi) pDsRed1-N1-CKIP-1/C-term1/ΔC-term1/ΔPH, *Eco*RI/*Kpn*I, pEGFP-C1-c-Jun, *Eco*RI/*Kpn*I

**Transfections, cell culture**

Monkey kidney COS7 cells, human embryonic kidney HEK293 cells, and mouse fibroblast NIH3T3 cells were cultured in DMEM containing 10% fetal bovine serum (FBS). SK-BR-3 and MCF7 breast cancer cells, human cervix carcinoma HeLa cells, MOLT4 leukemia cells were maintained in RPMI 1640 containing 10% FBS. SK-BR-3 and NIH3T3 cells were transfected with pcDNA3.1-Myc, pcDNA3.1-Myc-CKIP-1 or pcDNA3.1-Myc-CKIP-D310+345A plasmids by Lipofectamine 2000 (Invitrogen) or FuGene 6 reagent (Roche) according to the manufacturer's instructions. Three days later, G418 (Invitrogen) was added into the medium (800 μg/ml) for 2-3 weeks to select the stable transfectants.

**Fluorescence analysis of CKIP-1**

For localization of CKIP-1 after TNF treatment (Supplementary figure S4), HepG2 cells were transfected with RFP-CKIP-1. Twenty-four hours later, the cells were deprived of serum overnight and treated with TNF plus CHX for the indicated time. The images were visualized using a confocal microscope (Bio-Rad, Radiance 2100$^{TM}$).

Fig. S1

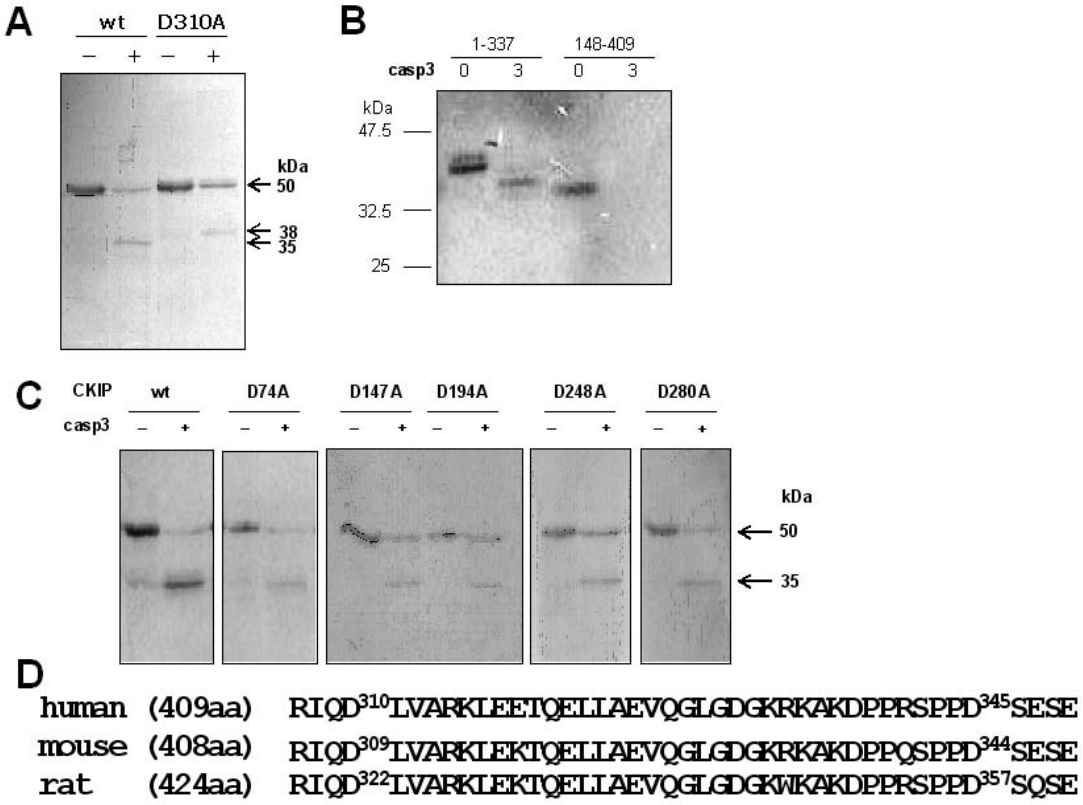


**Figure S1.** Analysis of cleavage of CKIP-1 by caspase-3 *in vitro*.

**(A)** Wild-type CKIP-1 and the D310A mutant were incubated with purified caspase-3 *in vitro*. Cleavage of wt-CKIP-1 generated a 35 kDa fragment, whereas D310A generated a 38 kDa fragment, suggesting that D310 is one cleavage site. The other one is located closely C-terminal of D310. **(B)** CKIP-1 truncates 1-337 and 148-409, in which the C-term1 and the N-terminal PH domain was deleted respectively, were subjected to cleavage *in vitro.* The former was cleaved into a slightly smaller fragment, confirming that a cleavage site exists close to residue 337. The band of the latter disappeared after incubation with caspase-3, possibly due to the fact that the cleaved products contain only 2 Met residues and therefore the signal is weak. **(C)** A series of point-mutants of CKIP-1 were cleaved by caspase-3 *in vitro*. The sizes of cleaved fragments were the same as that of wild-type CKIP-1, excluding the sites D74, D147, D194, D248 and D280 as cleavage sites, although the DLSD74 motif conforms to the caspase-3 consensus sequence DXXD. **(D)** Alignment of human, mouse and rat CKIP-1 shows conserved caspase-3 cleavage sites (D310 and D345 in human) and conserved GSK3 and CK2 phosphorylation sites (S342 and S346 in human). Genbank accession numbers: human CKIP-1, AAK28027; mouse CKIP-1, NP_075809; rat CKIP-1, XP_227451.

# Fig. S2

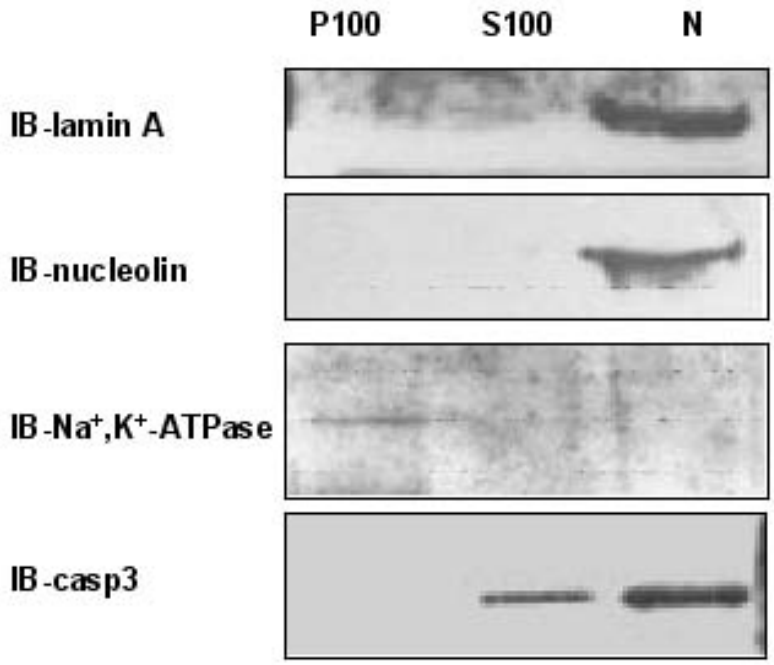


**Figure S2. Efficiency of the cellular fractionation.**

P100, S100 and N fractions of SK-BR-3 cells were prepared as described (Safi *et al.*, 2004). To determine the efficiency of the cellular fractionation, the expression of Lamin A, nucleolin, $Na^+/K^+$-ATPase α1 and caspase-3 was detected by Western blotting analysis. Lamin A and nucleolin were used as nuclear marker proteins, while $Na^+/K^+$-ATPase α1 was used as the plasma membrane-localized protein. Caspase-3 was distributed in the cytoplasm and nucleus.

# Fig. S3

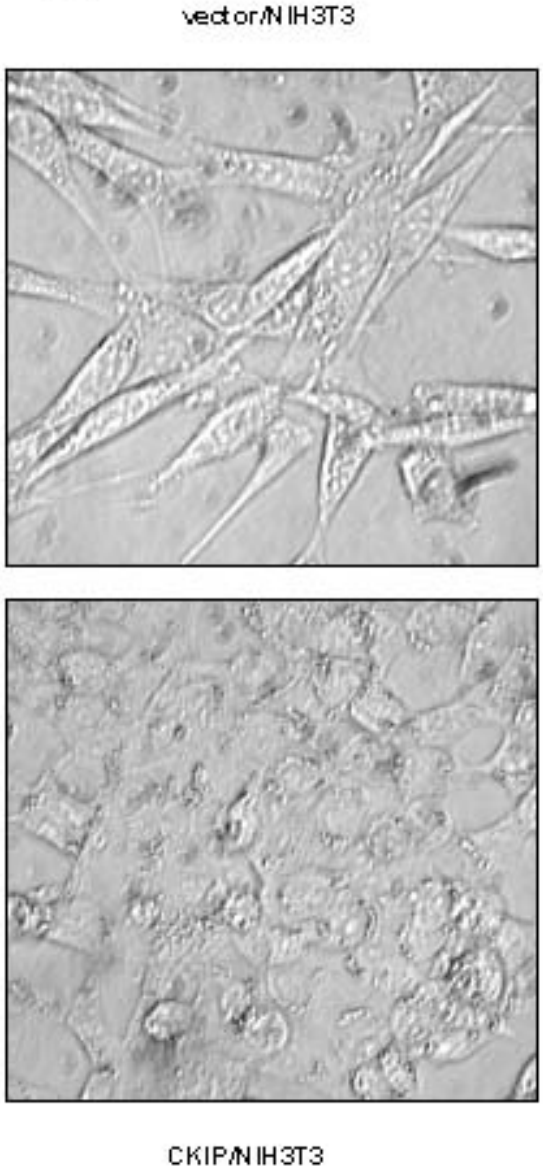


**Figure S3.** Stable expression of CKIP-1 in NIH3T3 cells clearly altered the cellular morphology.

# Fig. S4

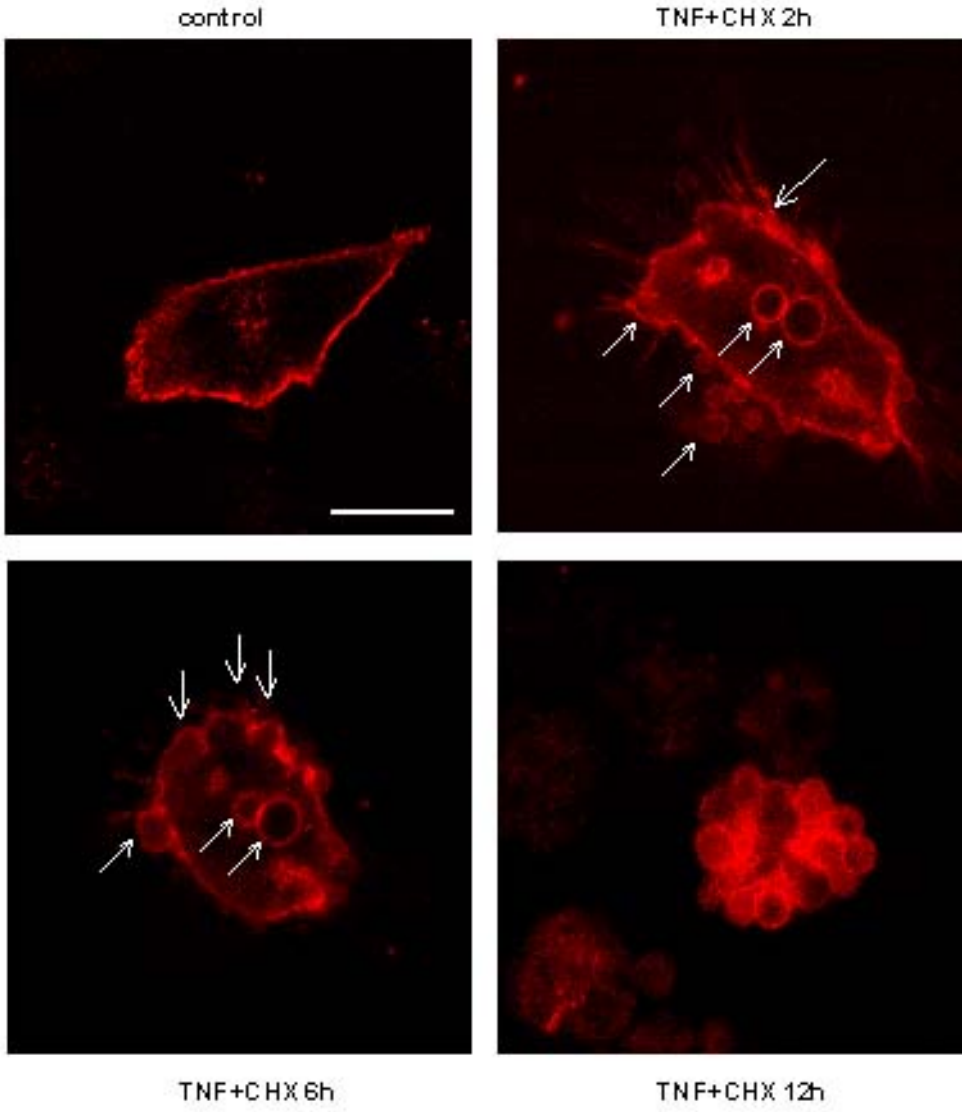


**Figure S4.** CKIP-1 causes membrane ruffling and vesicle formation in response to TNF. RFP-CKIP-1 was overexpressed in HepG2 cells and images were visualized after treatment of TNF (20 ng/ml) plus CHX (10 μg/ml) for the indicated time. The images were visualized using a confocal microscope. The arrows indicate membrane ruffling and pinocytic vesicle formation both outside and inside the cells. *Scale bar*, 10 μm.

Fig. S5

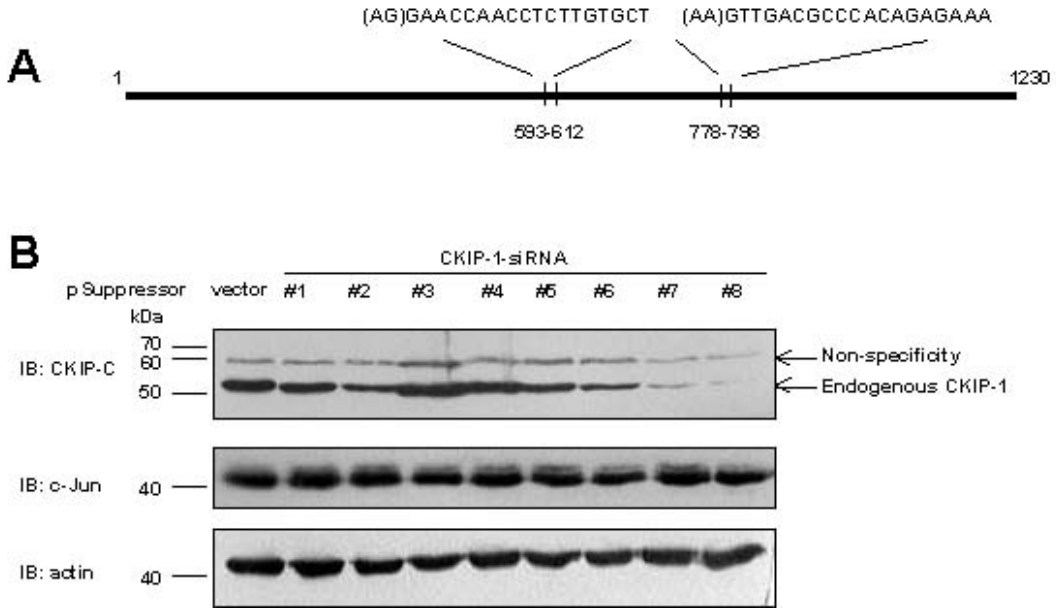


**Figure S5. Establishment of cells with diminished CKIP-1 expression by RNAi.**

**(A)** The sequence and relative position of the efficient CKIP-1 siRNAs. **(B)** HEK293 cells were transfected with pSuppressor-CKIP-1-siRNA or pSuppressor vector (1 μg) for 48 hr, lysed and subjected to immunoblot analysis for CKIP-1, c-Jun and actin proteins.