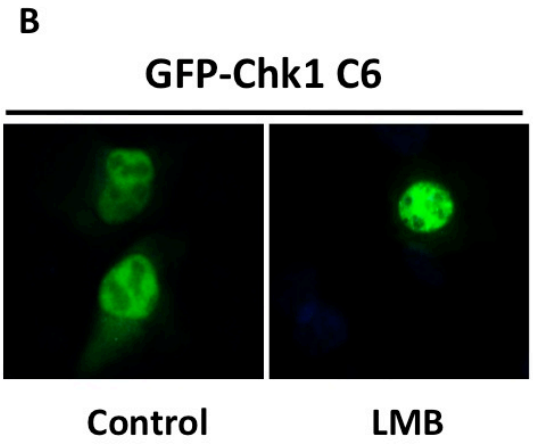
A

Chk1 Mutants	Localization
FL	N, c
N	N, C
C	N, c
Δ-C55	N, C
Δ-C108	N, c
C1	N, c
C2	N, C
C3	N, c
C4	C, n
C5	N, c
C6	N
C7	C

B



C

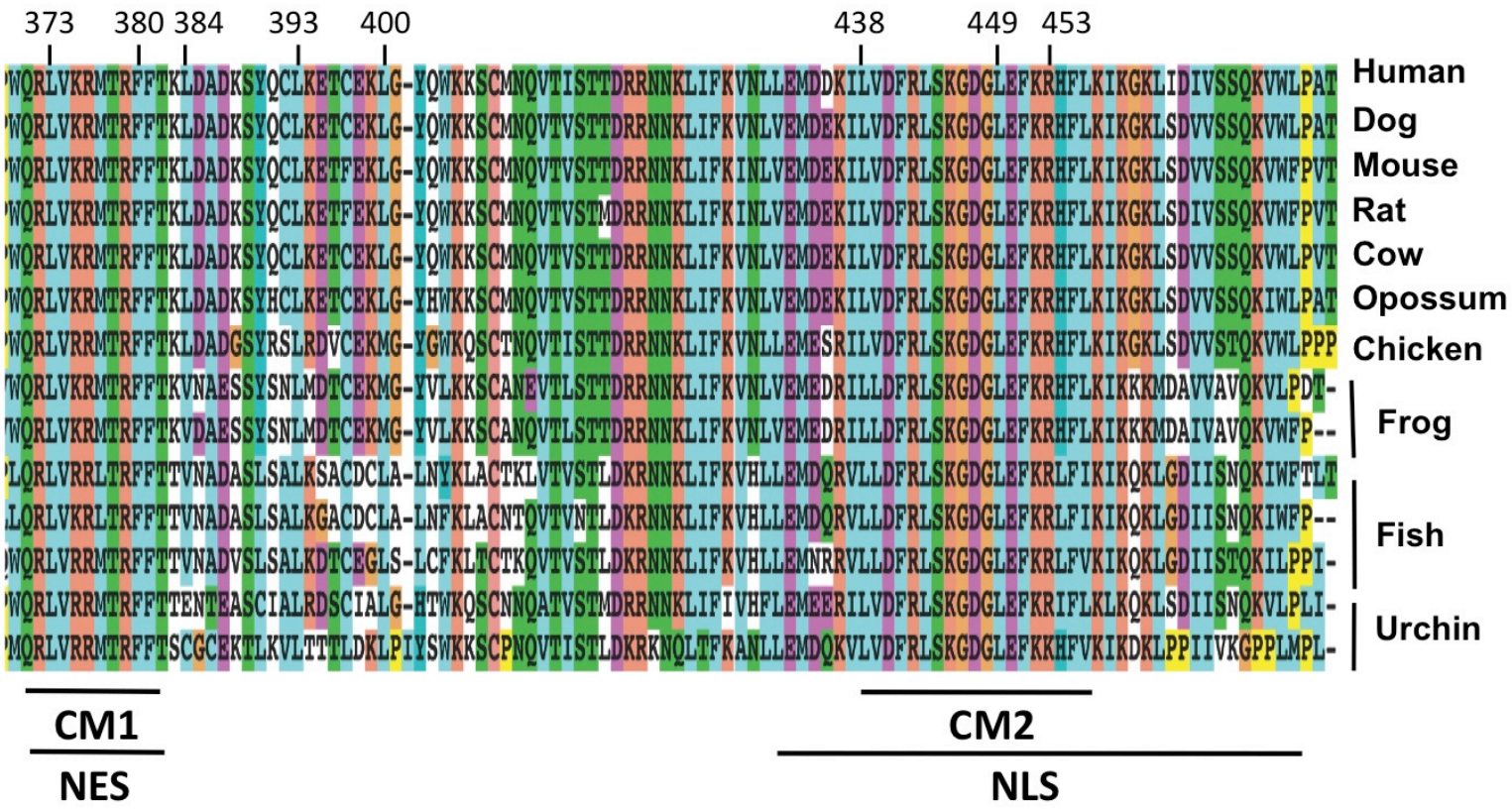


Figure S1 Chk1 protein localization. (A) Summary of protein localization of Chk1. *Left*, Chk1 fragments; *right*, cellular localization pattern. Upper and lower cases represent main and minor cellular localization, respectively. For instance, 'N, c' stands for 'mainly in the nucleus with minor cytoplasmic localization' pattern. (B) GFP-Chk1 C6 fragment was transfected into U2-OS cells grown on cover slips, treated or not with 6 ng/ml leptomycin B (LMB) for 4 h, fixed and visualized under fluorescence microscopy. (C) Alignment of the domain from CM1 to the distal C-terminus of Chk1 from different species. Conserved residues are listed. CM1 and CM2 domains function as NES and NLS, respectively.

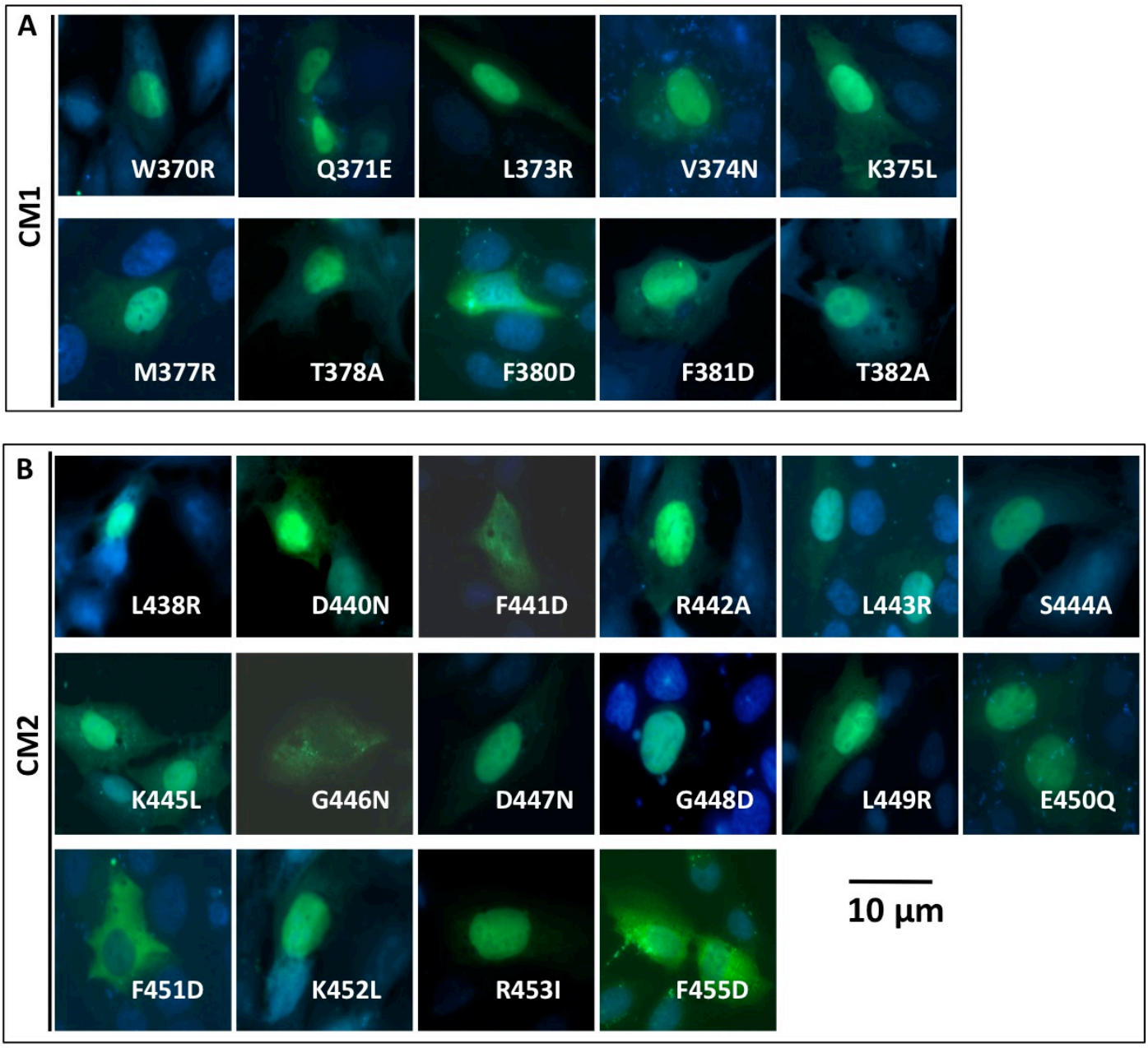


Figure S2 Representative images of protein localization of Chk1 point mutants. (A) GFP-tagged Chk1 proteins with point mutation in the CM1 domain. (B) GFP-tagged Chk1 proteins with point mutation in the CM2 domain.

	Chk1 Constructs	Localization	Phosphorylation
	WT	N, c	+
CM1	W370R	N, c	+
CM1	Q371E	N, c	+
CM1	L373R	N, c	+
CM1	V374N	N, c	+
CM1	K375L	N, c	+
CM1	M377R	N, c	+
CM1	T378A	N, c	+
CM1	F380D	C, n	-
CM1	F381D	N, c	+
CM1	T382E	N, c	+
CM2	L438R	N, c	+
CM2	D440N	N, c	+
CM2	F441D	C, n	-
CM2	R442A	N, c	+
CM2	L443R	N, c	+
CM2	S444E	N, c	+
CM2	K445L	N, c	+
CM2	G446N	C, n	-
CM2	D447N	N, c	+
CM2	G448D	N, c	+
CM2	L449R	N, c	+
CM2	E450Q	N, c	+
CM2	F451D	C, n	-
CM2	K452L	N, c	+
CM2	R453I	N, c	+
CM2	F455D	C, n	-

Figure S3 Summary of protein localization of Chk1 point mutants. Upper and lower cases represent main and minor cellular localization, respectively, as in Figure S1a. '+' and '–' indicate detection and no-detection of Chk1 phosphorylation at Ser-345, respectively.

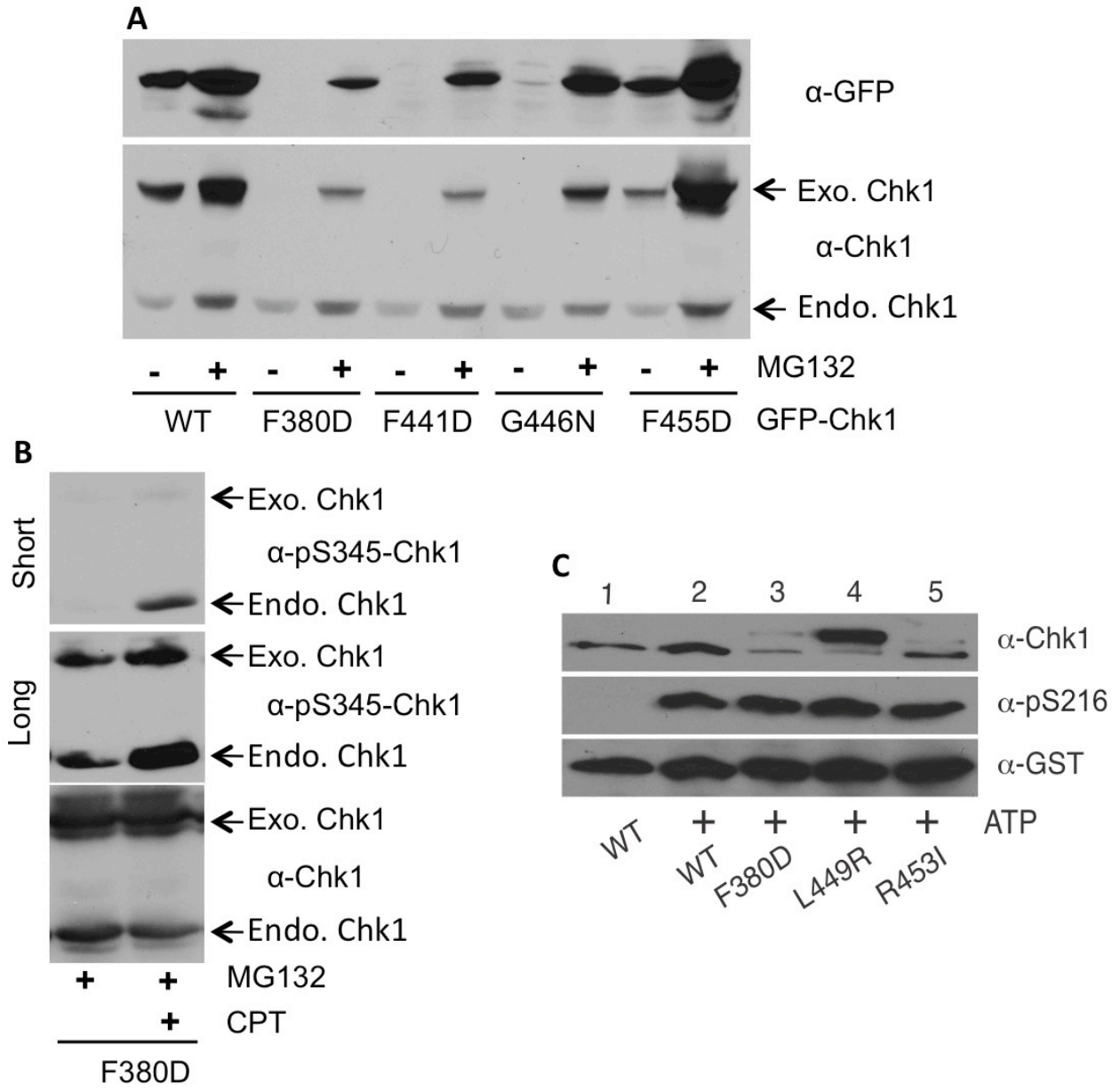


Figure S4 Function of cytoplasmic Chk1 mutants. (A) HEK293T cells were transfected with GFP-tagged Chk1 WT or mutants for 48 h, treated or not with 2 μM MG132 for additional 12 h, and immunoblotted with indicated antibodies. Exogenous (Exo) and endogenous (Endo) Chk1 proteins are shown by arrows. (B) HEK293T cells were transfected with GFP-Chk1 F380D for 48 h, treated or not with 2 μM MG132 for additional 12 h with 500 nM CPT added during the last 2 h, and immunoblotted with indicated antibodies. Exogenous (Exo) and endogenous (Endo) Chk1 proteins are shown by arrows. (C) In vitro kinase. GFP-tagged Chk1 proteins (WT or mutants) were expressed and immunoprecipitated from HEK293T cells and used to perform the *in vitro* kinase assay. The GST-Cdc25C fragment (residues 200-256) was used as the substrate. This Cdc25C fragment contains a known Chk1 phosphorylation site, Ser-216; thus, it is a surrogate for assessing the catalytic activity of Chk1 .

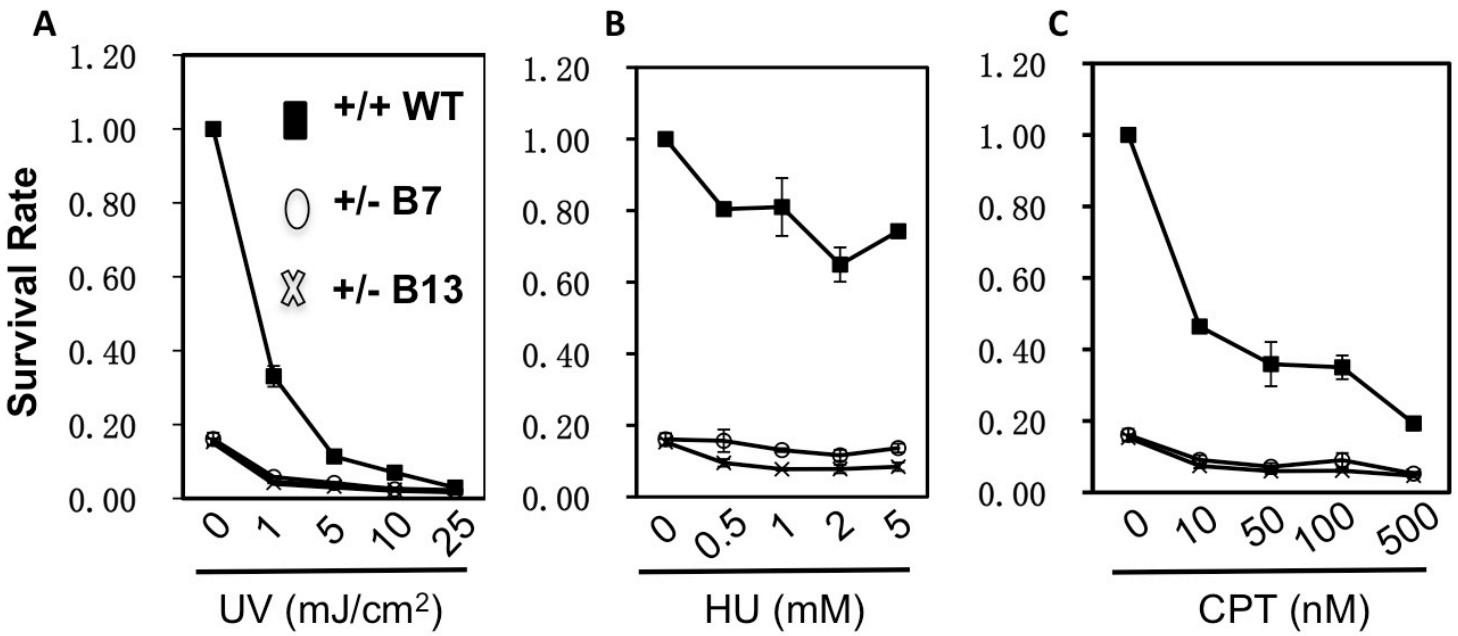


Figure S5 (A)-(C) Clonogenic assay of HCT116 WT or heterozygous cells after treatment with UV, HU and CPT, respectively. Data shown here are the same as those in Fig. 7B-D. However, different ways of normalization were used. The value of the WT non-treated group is set to as 1 for both WT and heterozygous cells here; whereas in Fig. 7B-D, the non-treated group for WT or the heterozygous was each set to as '1'. Thus, there is a significant reduction in colony formation in heterozygous cells compared to the WT even in the absence of any damage as shown here, but not in Fig. 7B-D. Data represent mean and standard deviation from three experiments.