

Expanded View Figures

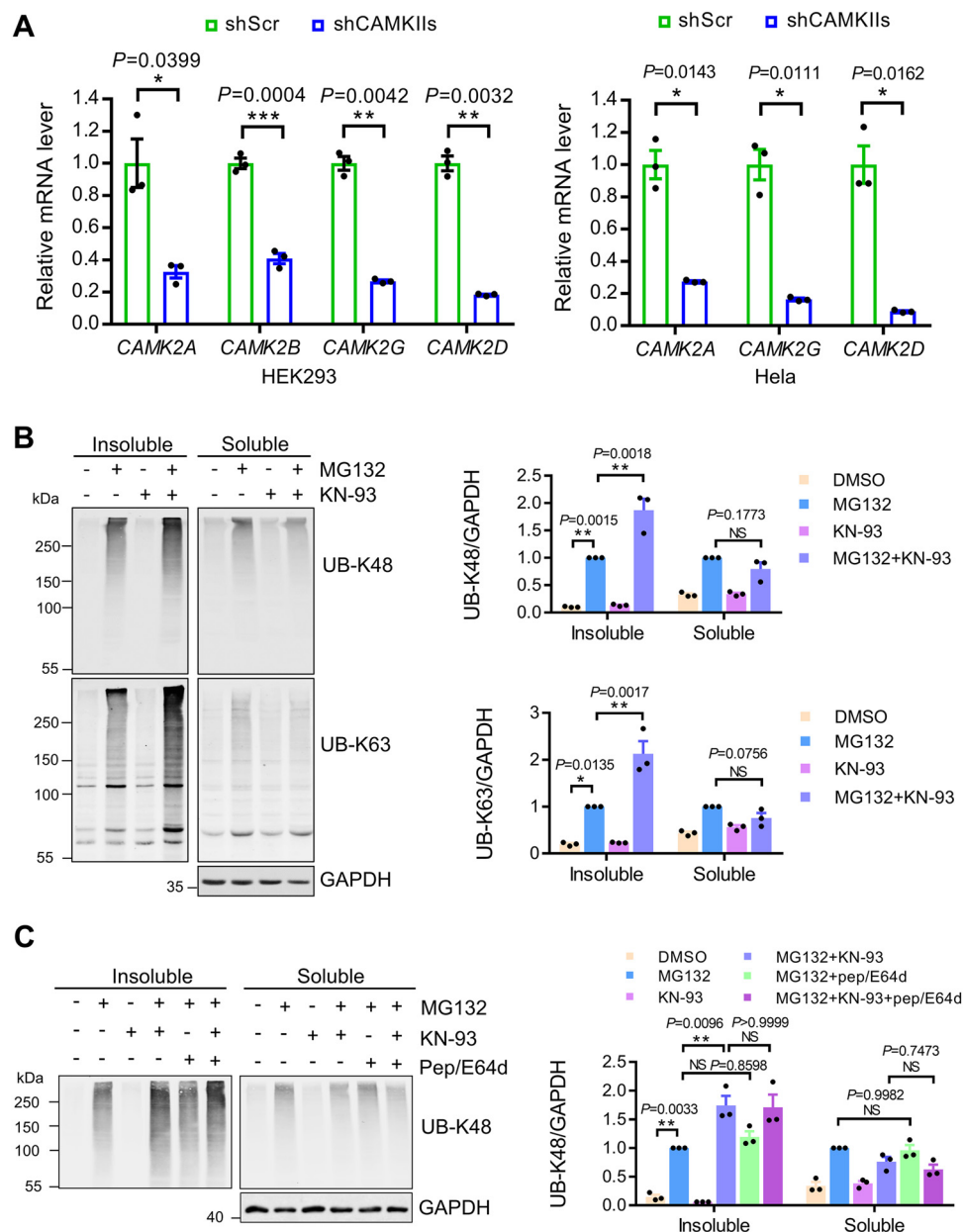


Figure EV1. CaMKII regulates the synthesis of aggregated ubiquitinated proteins in response to proteasome inhibition, related to Fig. 1.

(A) Confirmation of *CAMK2* knockdown in HEK293 and HeLa cells. After transfected with indicated plasmids for 48 h, the mRNA levels of *CAMK2* were analyzed by qRT-PCR. Data are mean $\pm$ SEM of biological replicates ($n = 3$). The P value was determined by Student's t -test (two-sided). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(B) Immunoblotting and quantification of ubiquitin (UB-K48 and UB-K63) in NP-40-soluble and -insoluble fractions of HEK293 cells treated with indicated drugs (MG132 (1 μ M), KN-93 (10 μ M)) for 14 h. Data are mean $\pm$ SEM of biological replicates ($n = 3$). (C) Immunoblotting and quantification of UB-K48 in NP-40-soluble and -insoluble fractions of HEK293 cells treated with indicated drugs (MG132 (1 μ M), KN-93 (10 μ M), Pepstatin A (Pep, 25 μ g/ml)/E64d (25 μ g/ml)) for 14 h. Data are mean $\pm$ SEM of biological replicates ($n = 3$). For (B, C), the P value was determined by a one-way ANOVA analysis. NS not significant; * $P < 0.05$; ** $P < 0.01$.

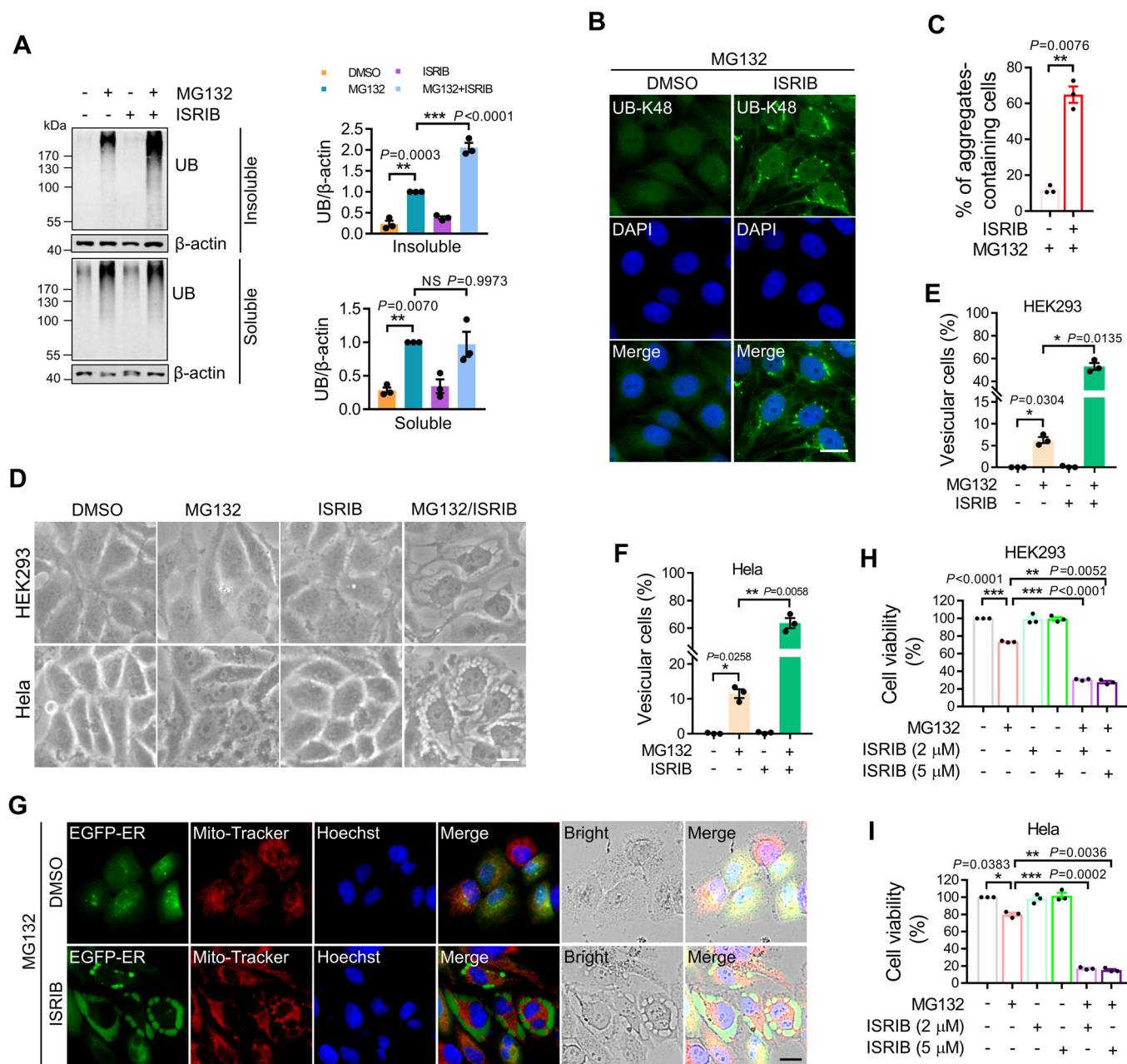


Figure EV2. Inhibiting eIF2 α phosphorylation aggravates the proteasome inhibitor-induced proteotoxicity, related to Fig. 3.

(A) Immunoblotting and quantification of indicated proteins in NP-40-soluble and -insoluble fractions of HEK293 cells treated with indicated drugs (MG132 (1 μ M), ISRIB (5 μ M)) for 14 h. Data are mean $\pm$ SEM of biological replicates ($n = 3$). (B) Representative UB-K48 staining images of HeLa cells treated with indicated drugs (MG132 (0.5 μ M), ISRIB (5 μ M)) for 14 h. Scale bar: 20 μ m. (C) Quantitative analysis of results in (B). Data are mean $\pm$ SEM of biological replicates ($n = 3$). At least 50 cells were randomly selected from each group to score. (D) Cell morphology imaging of HEK293 and HeLa cells treated with indicated drugs (MG132 (1 μ M), ISRIB (5 μ M)) for 24 h. Scale bar: 10 μ m. (E, F) Quantitative analysis of results in (D). Data are mean $\pm$ SEM of biological replicates ($n = 3$). At least 50 cells were randomly selected from each group to score. (G) Representative images of HeLa cells transfected with ER-EGFP and treated with indicated drugs (MG132 (1 μ M), ISRIB (5 μ M)) for 24 h. Mitochondria and nuclei were stained with Mito-Tracker (red) and Hoechst (blue), respectively. Scale bar: 20 μ m. (H, I) Cell viability of HEK293 and HeLa cells treated with indicated drugs (MG132 (1 μ M), ISRIB (2 μ M), 5 μ M)) for 48 h. Data are mean $\pm$ SEM of biological replicates ($n = 3$). For (C), the P value was determined by Student's t test (two-sided). ** $P < 0.01$. For (A, E, F, H, I), the P value was determined by a one-way ANOVA analysis. NS not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

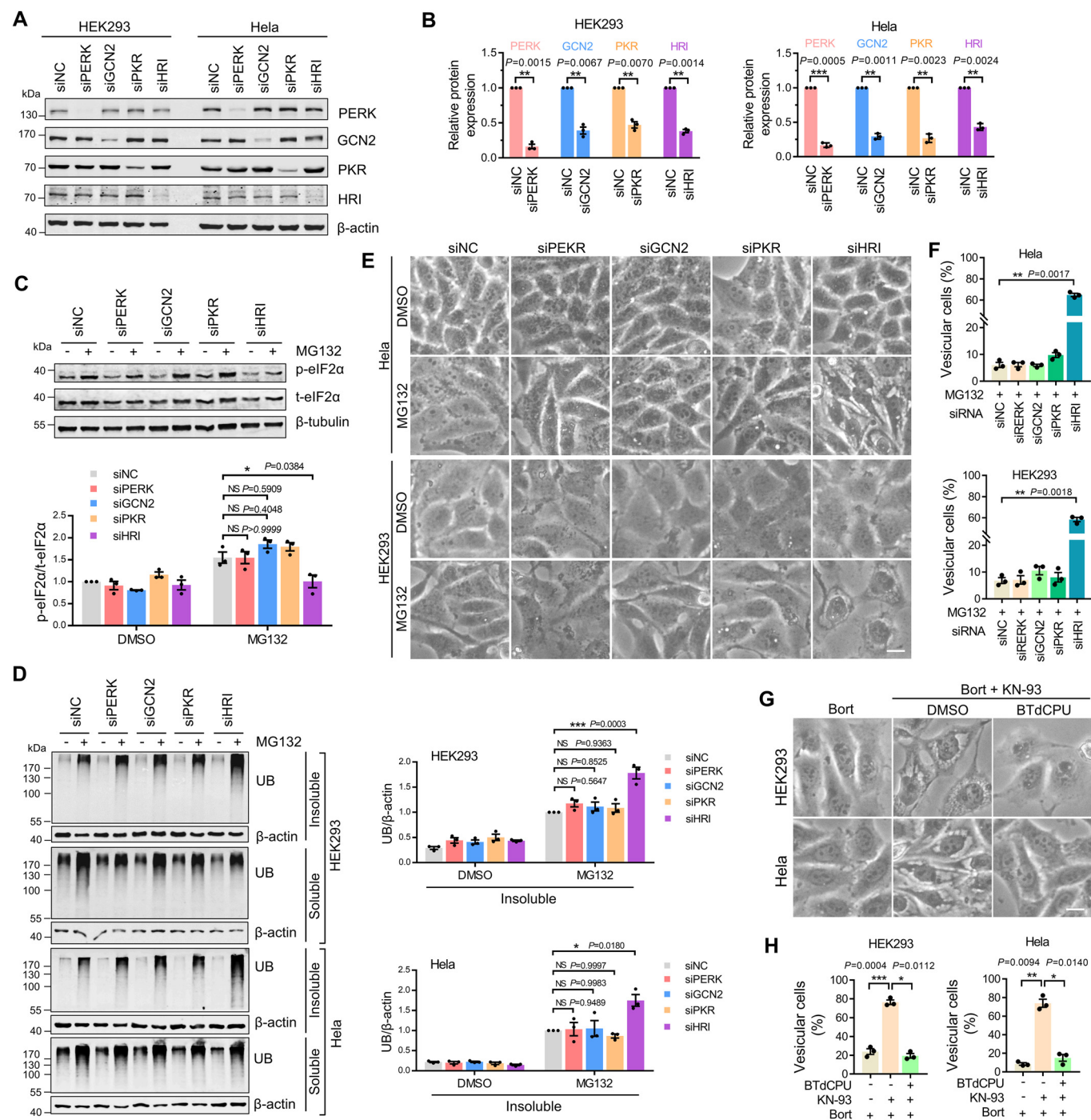
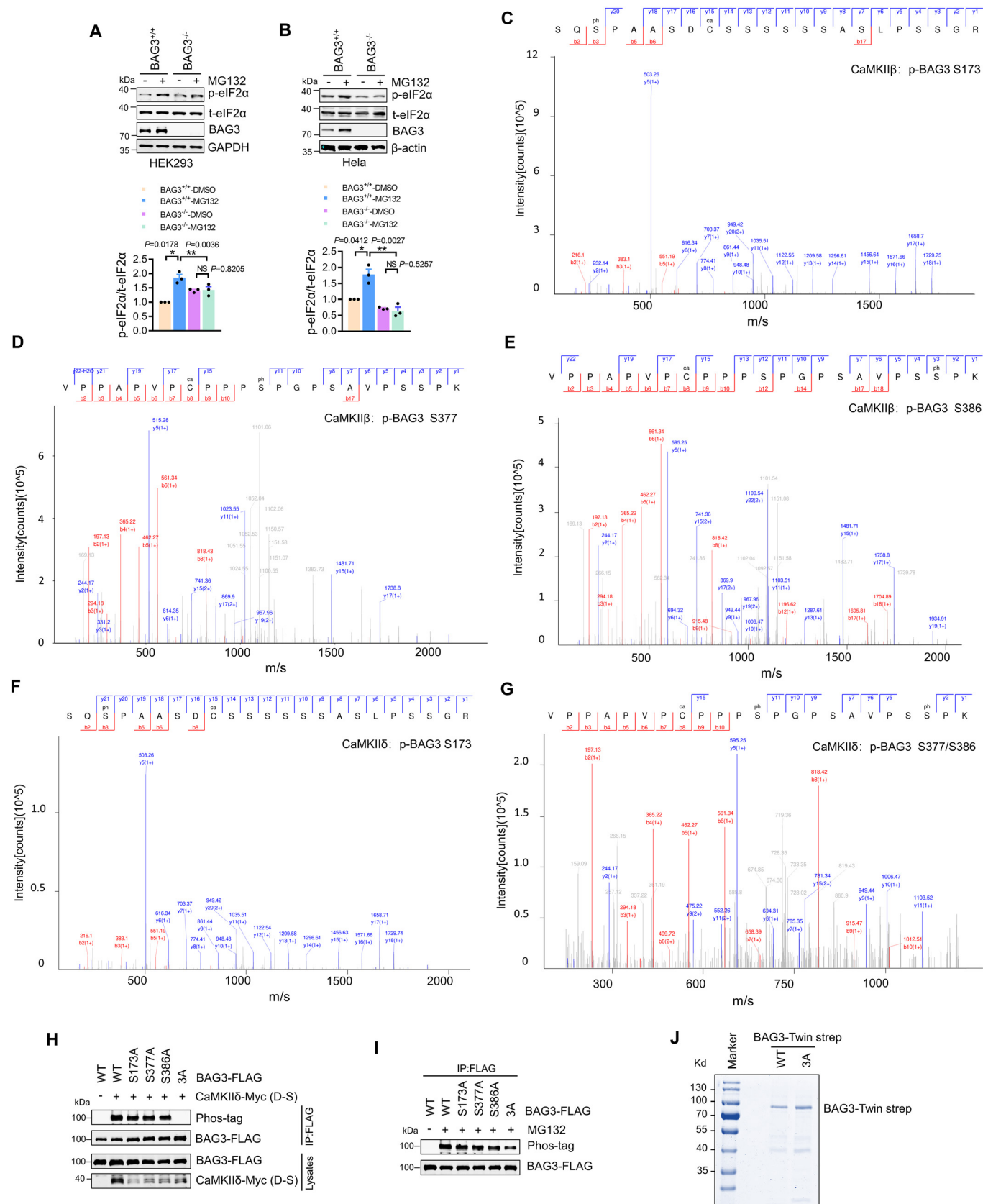


Figure EV3. HRI is critical for eIF2α activation and proteotoxicity reduction during proteasome dysfunction, related to Fig. 3.

(A) Confirmation of EIF2AKs knockdown in HEK293 and HeLa cells. After transfected with indicated siRNAs, the whole cell lysates were subjected to western blot analysis with indicated antibodies. (B) Quantitative analysis of results in (A). Data are mean $\pm$ SEM of biological replicates ($n = 3$). (C) Immunoblotting and quantification of p-eIF2α (S51) in HeLa cells transfected with indicated siRNAs and treated with MG132. Data are mean $\pm$ SEM of biological replicates ($n = 3$). (D) Immunoblotting and quantification of UB in NP-40-soluble and -insoluble fractions of HEK293 and HeLa cells transfected with indicated siRNAs and treated with MG132. Data are mean $\pm$ SEM of biological replicates ($n = 3$). (E) Cell morphology imaging of HEK293 and HeLa cells transfected with indicated siRNAs and treated with MG132. Scale bar: 10 μ m. (F) Quantitative analysis of results in (E). Data are mean $\pm$ SEM of biological replicates ($n = 3$). At least 50 cells were randomly selected from each group to score. (G) Cell morphology imaging of HEK293 and HeLa cells treated with indicated drugs (Bortezomib (1 μ M), KN-93 (10 μ M), BTdCPU (0.5 μ M)) for 24 h. Scale bar: 10 μ m. (H) Quantitative analysis of results in (G). Data are mean $\pm$ SEM of biological replicates ($n = 3$). At least 50 cells were randomly selected from each group to score. For (B), the P value was determined by Student's t test (two-sided). ** $P < 0.01$; *** $P < 0.001$. For (C, D, F, H), the P value was determined by a one-way ANOVA analysis. NS not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.



**Figure EV4. CaMKII phosphorylates BAG3, related to Fig. 4.**

(A, B) Immunoblotting and quantification of p-eIF2 α (S51) in BAG3 knockout cells treated with 1 μ M MG132 for 14 h. Data are mean $\pm$ SEM of biological replicates ($n = 3$). The P value was determined by Student's t test (two-sided). NS not significant; * $P < 0.05$; ** $P < 0.01$. (C–G) Mass spectrum of the phosphopeptide containing the S173/S377/S386 phosphorylated residue of BAG3. (H, I) Phosphorylation level of re-expressed BAG3 (WT or mutants) in BAG3 $^{-/-}$ HEK293 cells treated with MG132 (I) or not (H). (J) Confirmation of purified BAG3-Twin Strep (WT or 3A) by coomassie blue staining.

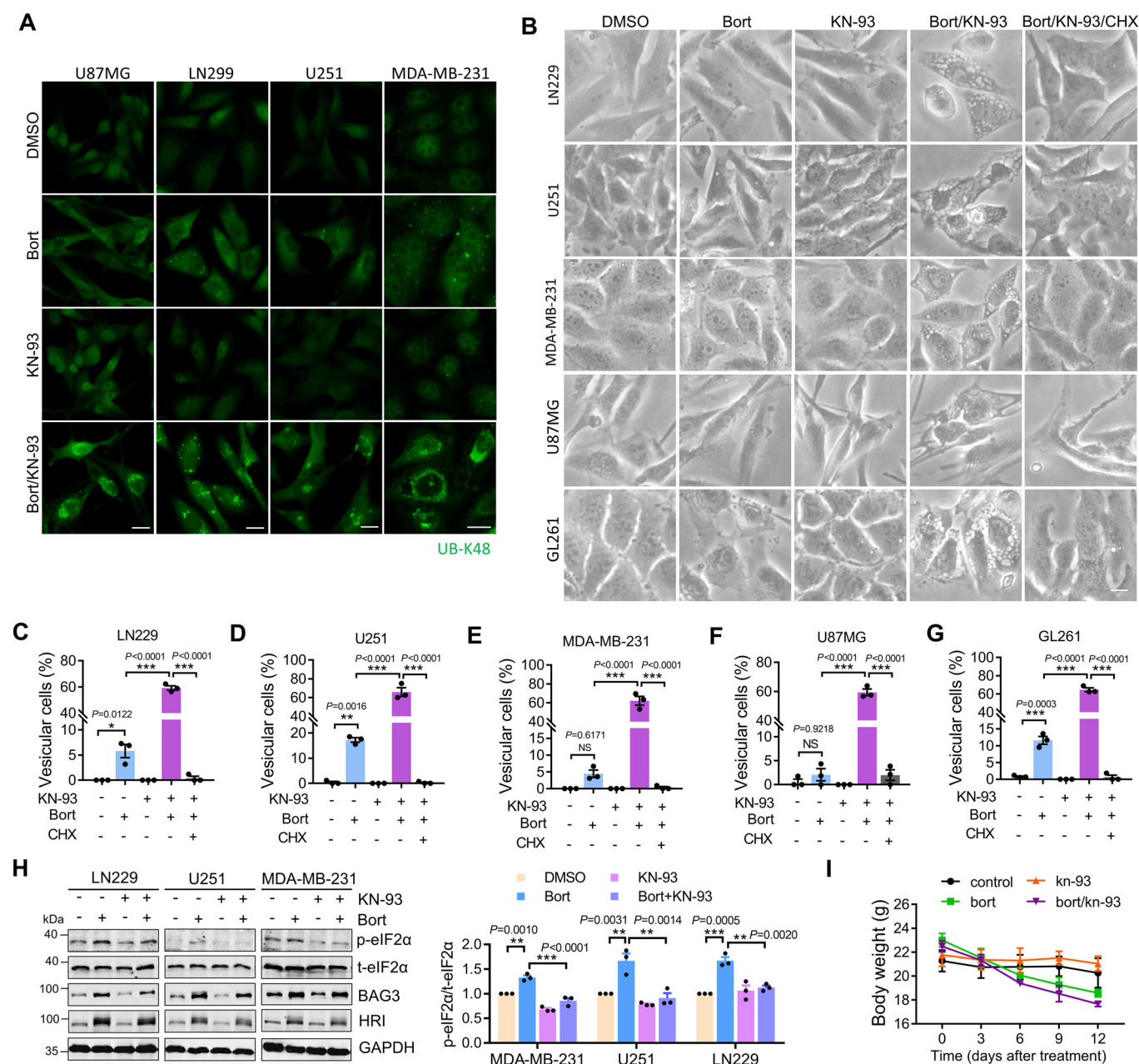


Figure EV5. Inhibition of CaMKII enhances the anti-tumor activity of proteasome inhibitor, related Fig. 7.

(A) Representative UB-K48 staining images of tumor cells treated with indicated drugs. Scale bar: 20 μ m. (B) Cell morphology imaging of tumor cells treated with indicated drugs (Bortezomib (10 nM), KN-93 (10 μ M), CHX (25 μ g/ml)) for 24 h. Scale bar: 10 μ m. (C–G) Quantitative analysis of results in (B). Data are mean $\pm$ SEM of biological replicates ($n = 3$). At least 50 cells were randomly selected from each group to score. (H) Immunoblotting and quantification of indicated proteins in tumor cells treated with indicated drugs. Data are mean $\pm$ SEM of biological replicates ($n = 3$). (I) The body weight of each group was calculated once time every three days. Data are mean $\pm$ SEM of 5 mice. For (C–H), the P value was determined by a one-way ANOVA analysis. NS not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.