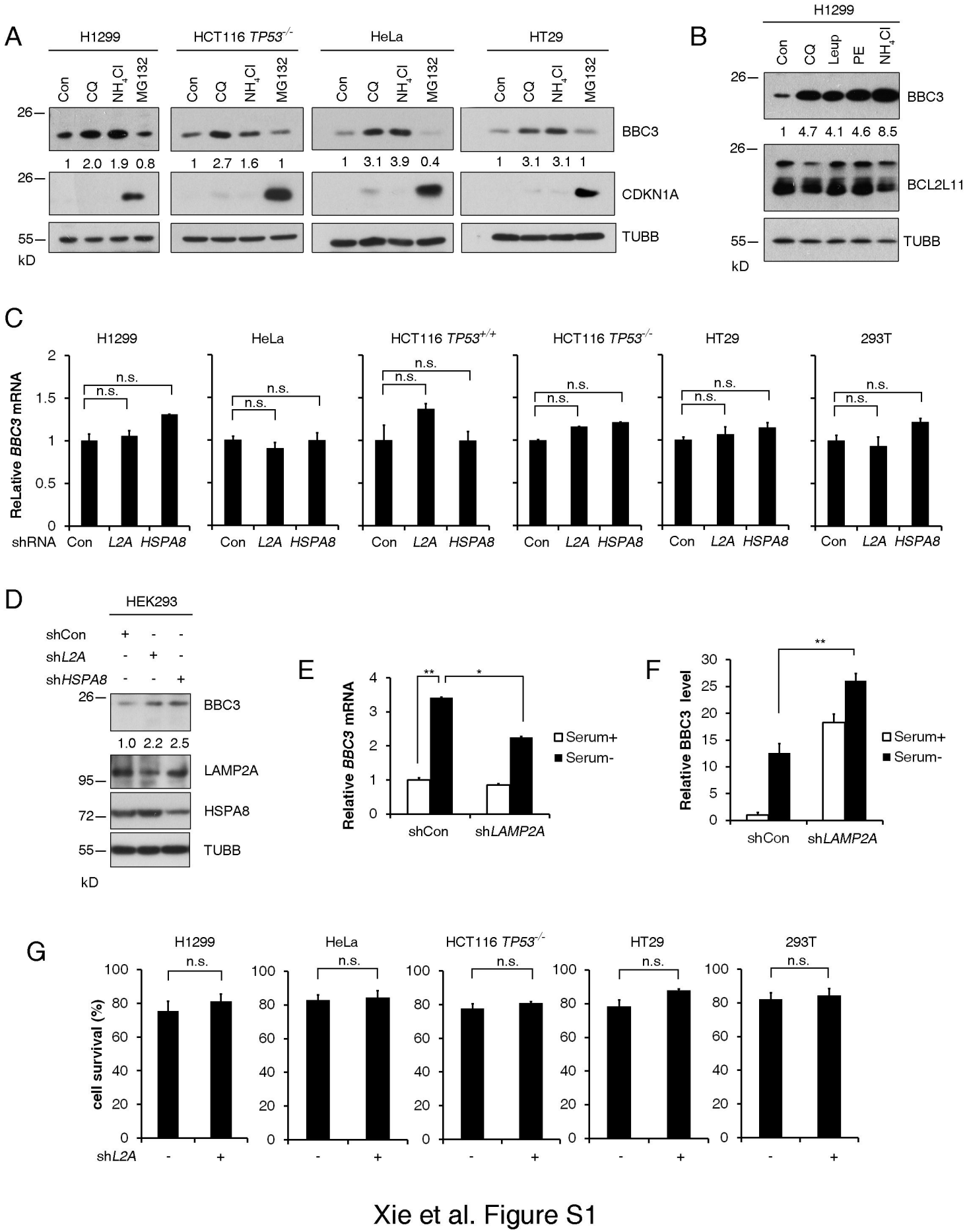
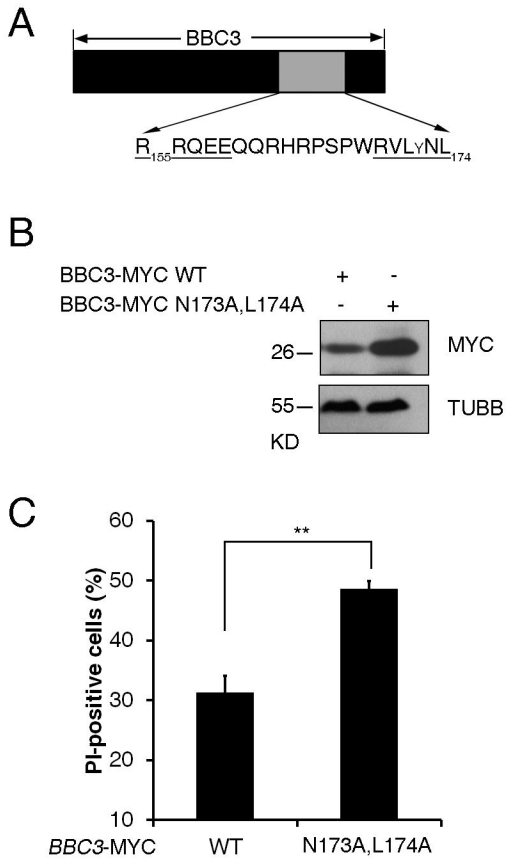
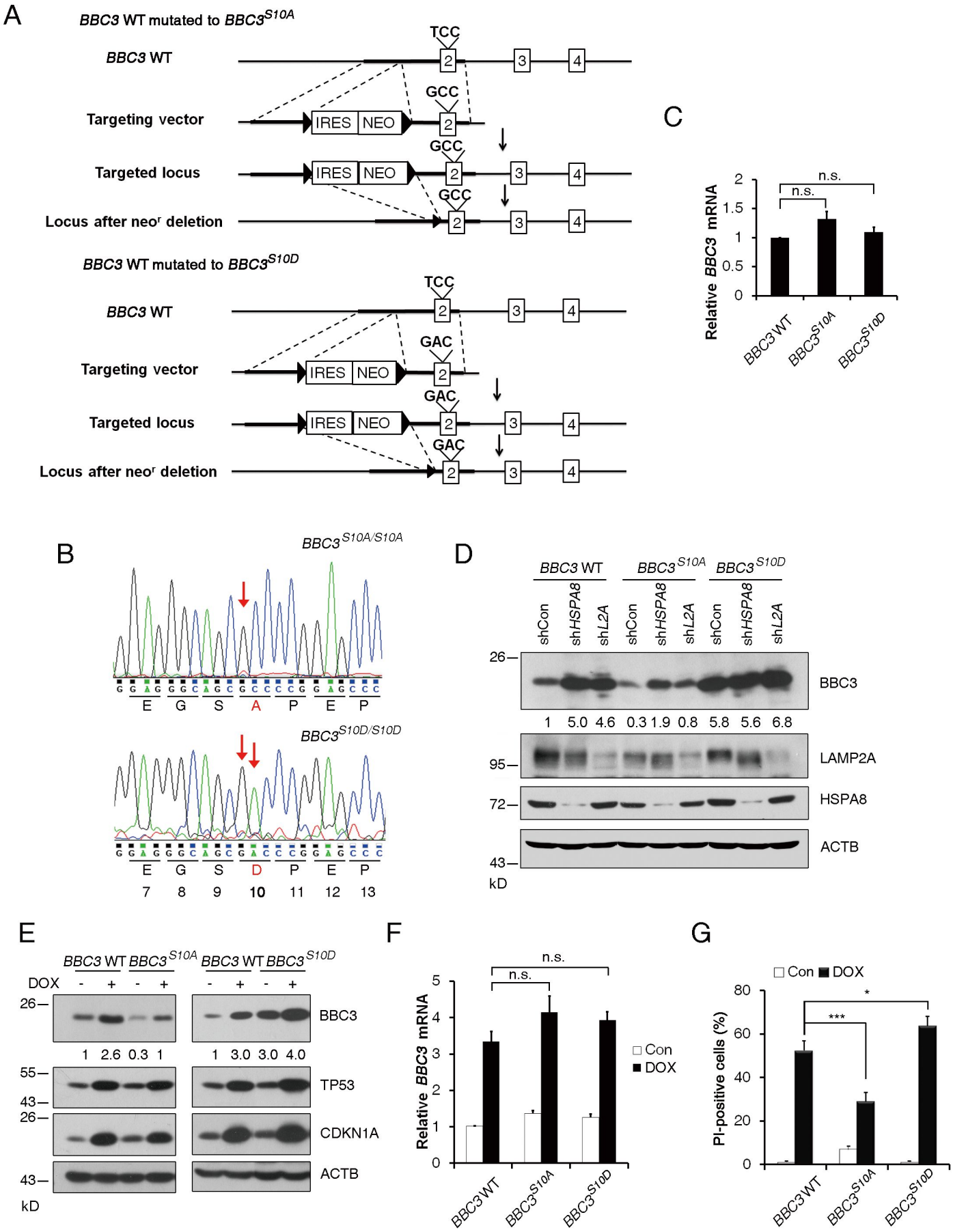


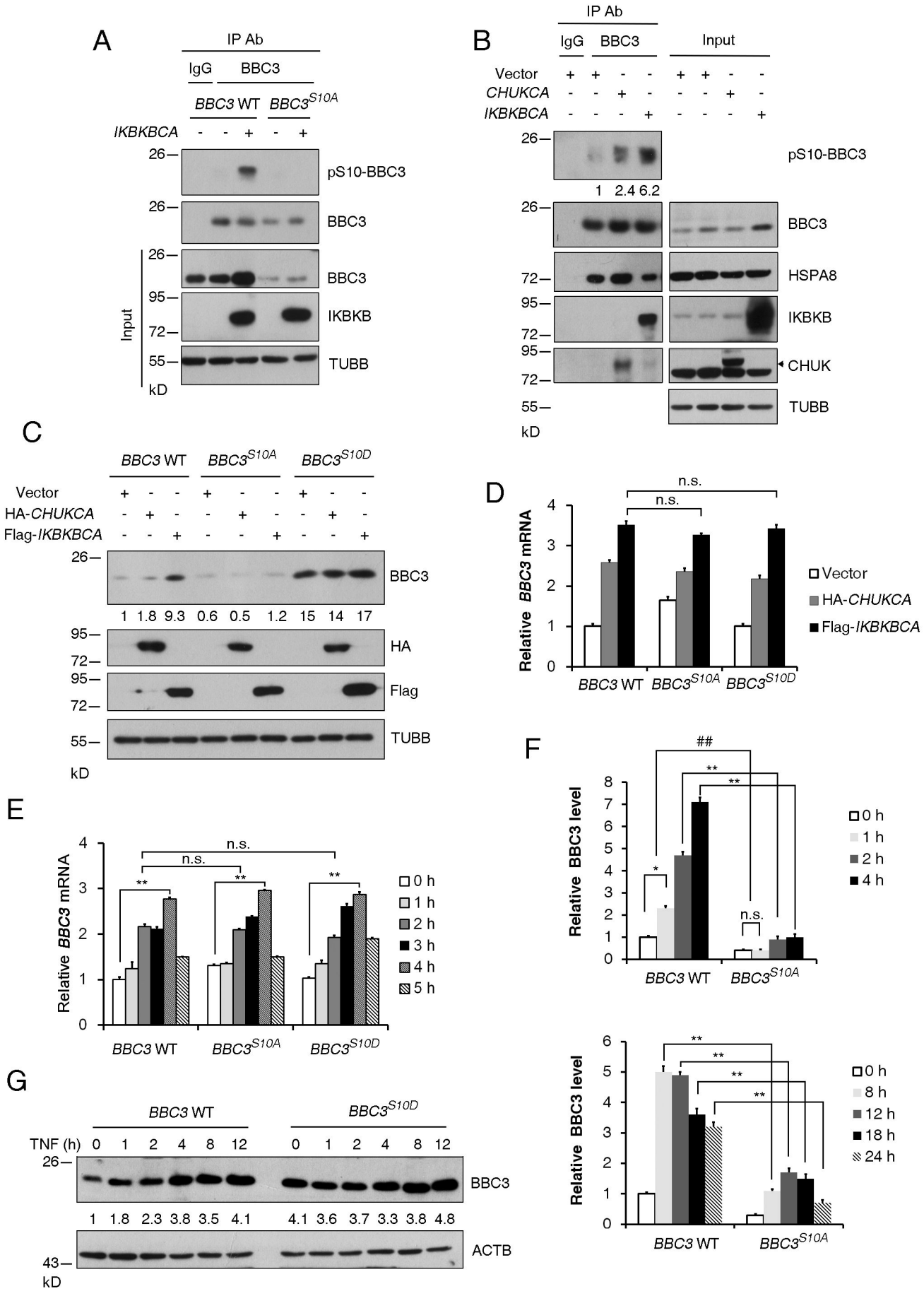
Xie et al. Figure S1



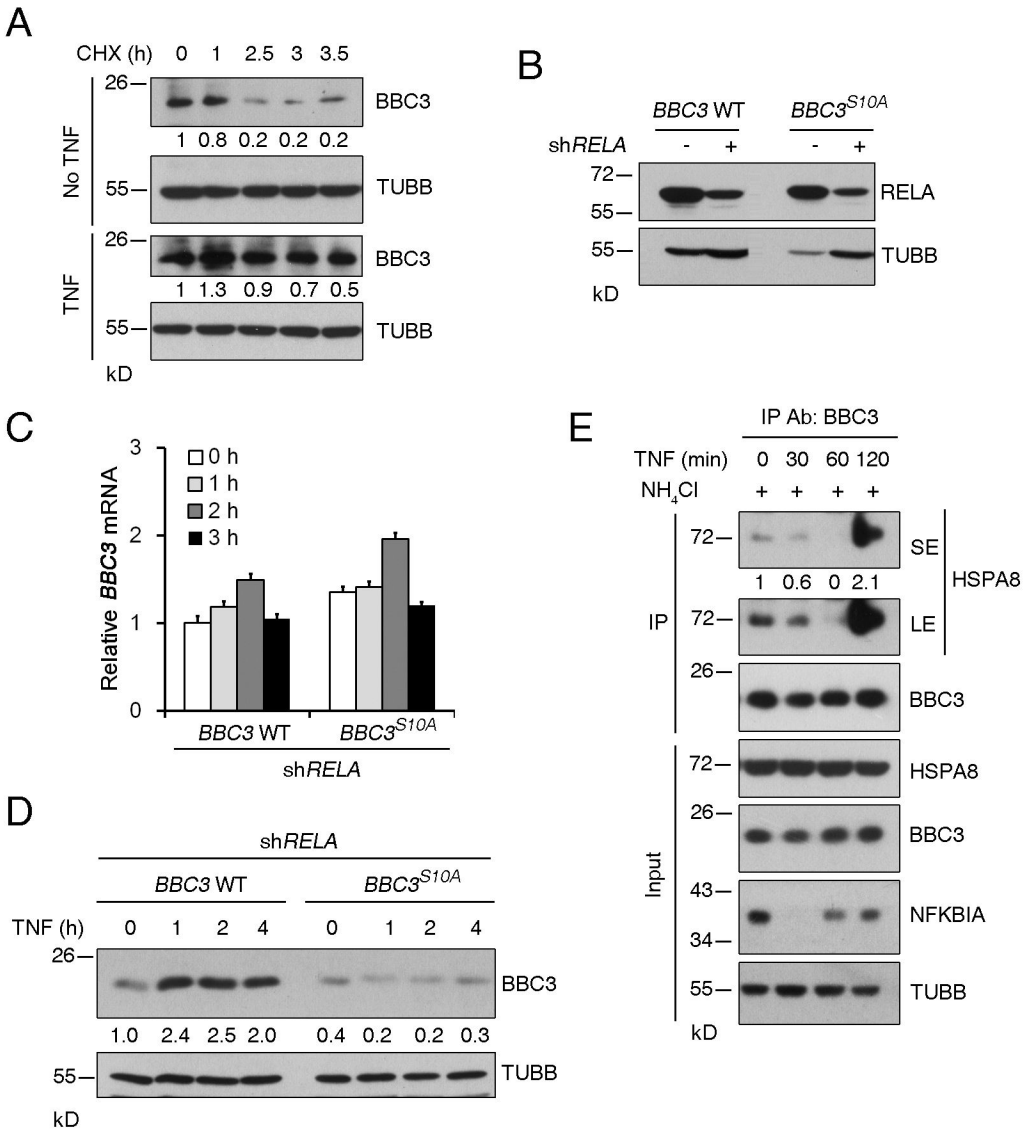
Xie et al. Figure S2



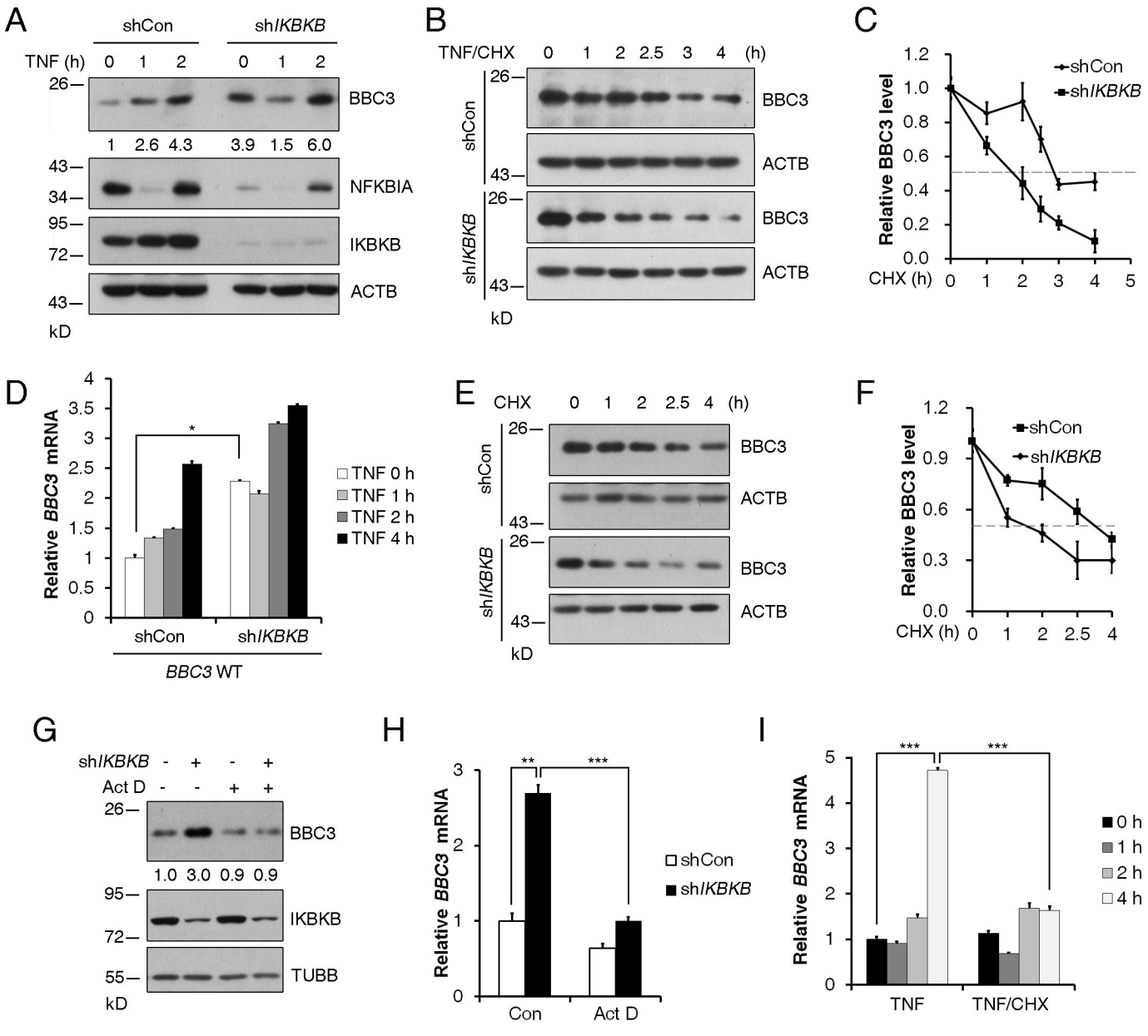
Xie et al. Figure S3



Xie et al. Figure S4



Xie et al. Figure S5



Xie et al. Figure S6

Supplemental Figure Legends

Figure S1. Inhibition of CMA leads to BBC3 induction. (**A**) Representative immunoblots and densitometric data (n=3 or 4) of human cancer cell lines incubated with 25 μM CQ, 20 mM NH_4Cl or 20 μM MG132 for 6 h showing upregulation of BBC3 in response to lysosomal inhibitors. The BBC3 protein levels were quantified relative to TUBULIN. (**B**) Representative western blots with densitometric analysis (n=3) of H1299 cells incubated with the indicated inhibitors (25 μM CQ, 100 μM leupeptin, 10 μg/ml pepstatin A/E64D or 20 mM NH_4Cl) for 6 h showing that BH3-only protein BCL2L11 remains unchanged upon lysosomal inhibition. The BBC3 protein levels were quantified relative to TUBULIN. (**C**) QRT-PCR analysis of *BBC3* mRNA normalized against *TBP* in the indicated cancer cell lines infected with vectors containing control, *LAMP2A* or *HSPA8* shRNA showing that the *BBC3* mRNA level remains unchanged under CMA ablation. (**D**) Representative western blots and densitometric data (n=3 or 4) showing that CMA ablation leads to BBC3 upregulation in HEK293 cells. HEK293 cells were infected with vectors containing control, *LAMP2A*, or *HSPA8* shRNA and then lysed for immunoblotting analysis. (**E**) QRT-PCR analysis of *BBC3* mRNA normalized against *TBP* in H1299 cells serum deprived for 48 h following transduction with control or *LAMP2A* lentiviruses showing transcriptional induction of *BBC3*. (**F**) LAMP2A depletion further promotes BBC3 induction upon serum withdrawal. Quantitative analysis of western blot results is presented as mean ±

SEM of 3 independent experiments. (**G**) FACS analysis of cell survival by ANXA5 and PI staining in the indicated human cancer cell lines infected with *LAMP2A* shRNA. CMA inhibition had no effect on cell survival in the absence of TP53. Statistics are depicted as mean ± SEM; n.s., not significant; *, $P < 0.05$; and **, $P < 0.01$; n=3, t test.

Figure S2. BBC3 interacts with CMA components. (**A**) Schematic representation of KFERQ-like motif in the C terminus of BBC3. (**B**) Representative BBC3-myc immunoblots (n=3) of HCT116 cells transfected with wild-type or the $BBC3^{N173A,L174A}$ mutant. (**C**) FACS analysis of cell death by PI staining in cells shown in (**B**) showing that ectopic expresion of the $BBC3^{N173A,L174A}$ mutant leads to enhanced cell death. Data are represented as mean ± SEM of 3 independent experiments. **, $P < 0.01$, t test.

Figure S3. Ser10 is crucial for protecting BBC3 from CMA-mediated degradation. (**A**) Targeting strategy used to generate $BBC3^{S10A/S10A}$ and $BBC3^{S10D/S10D}$ HCT116 cells. (**B**) Sequence analysis of genomic DNA isolated from $BBC3^{S10A/S10A}$ and $BBC3^{S10D/S10D}$ HCT116 cells. Chromatograms demonstrating the successful targeting of the *BBC3* locus and translated amino acids are shown below the codons. Red letters and bold numbers denote the targeted amino acids (S10A and S10D). Arrows point to targeted nucleotides. (**C**) QRT-PCR analysis of

change of *BBC3* mRNA levels in *BBC3* WT, *BBC3*^{S10A} and *BBC3*^{S10D} HCT116 cells normalized to *TBP*. (**D**) Densitometric analyses and representative western blots (n=4) of *BBC3* WT, *BBC3*^{S10A} and *BBC3*^{S10D} HCT116 cells infected with the indicated shRNAs showing that CMA ablation differentially affects the stability of wild-type and mutant BBC3. The ratio of BBC3 protein normalized to ACTIN relative to control (marked as 1) is indicated below each lane. (**E**) Representative immunoblots and densitometric quantification (n=4) of *BBC3* WT, *BBC3*^{S10A} and *BBC3*^{S10D} HCT116 cells incubated with 0.5 μM Dox for 16 h. Quantification of BBC3 protein levels was done relative to ACTIN. (**F**) QRT-PCR analysis of *BBC3* mRNA levels normalized to *TBP* in response to treatment shown in (**E**). (**G**) FACS analysis of cell death in *BBC3* WT, *BBC3*^{S10A} and *BBC3*^{S10D} HCT116 cells incubated with 0.5 μM Dox for 72 h showing that BBC3^{S10A} protects while BBC3^{S10D} potentiates DNA damage-induced apoptosis. Statistics are depicted as mean ± SEM; n.s., not significant; *, $P < 0.05$; and ***, $P < 0.001$;, n=3, t test.

Figure S4. IKBKB-dependent phosphorylation of BBC3 stabilizes BBC3. (**A**) Representative western blots following immunoprecipitation (n=4) using IgG or anti-BBC3 antibody showing that IKBKBCA overexpression induces BBC3 Ser10 phosphorylation only in wild-type cells but not in *BBC3*^{S10A} cells. Of note, BBC3 levels in the coIP experiment do not change in this case in the *IKBKBCA* lane, owing to saturation of the anti-BBC3 antibody. (**B**) Representative protein gel blots

following immunoprecipitation (n=3) with IgG or anti-BBC3 antibody in HCT116 cells transfected with vector, HA-*CHUKCA* or Flag-*IKBKBCA* showing that IKBKB overexpression phosphorylates BBC3 at Ser10, which attenuates its binding to HSPA8. CHUK is indicated with an arrowhead. Of note, BBC3 levels in the IP experiment do not change in this case in the IKBKBCA lane, owing to saturation of the anti-BBC3 antibody. The pSer10-BBC3 protein levels were quantified relative to BBC3 protein levels in the IP experiment. (**C**) Representative immunoblots (n≥4) and associated densitometric analysis of HCT116 cells transfected with vector, HA-*CHUKCA*, or Flag-*IKBKBCA* showing that Ser10 is essential for BBC3 stabilization in response to active form of IKBKB. The ratio of BBC3 protein normalized to TUBULIN relative to control (marked as 1) is indicated below each lane. (**D**) Total RNA of cells in (**C**) was extracted and subjected to QRT-PCR analysis for detection of *BBC3* mRNA levels. (**E**) QRT-PCR analysis of the change of *BBC3* mRNA normalized to *TBP* in *BBC3* WT, $BBC3^{S10A}$ and $BBC3^{S10D}$ HCT116 cells showing time-dependent induction of *BBC3* mRNA upon 10 ng/ml TNF exposure. (**F**) Serine to alanine mutation results in compromised BBC3 accumulation upon TNF exposure. Quantitative analysis of western blot results is presented as Mean ± SEM of 5 independent experiments. (**G**) Representative western blots and densitometric data (n=4) of *BBC3* WT and $BBC3^{S10D}$ HCT116 cells treated with 10 ng/ml TNF showing that $BBC3^{S10D}$ confers resistance to TNF-mediated protein induction. Quantification of BBC3 protein levels was done

relative to ACTIN. Statistics are depicted as mean ± SEM. n.s., not significant; *, $P < 0.5$; **, 0.01; For Fig. S4D, n=3, t test., For Fig. S4E and S4F, one-way ANOVA for indicated comparison; $^{\#\#}$, $P < 0.01$, two-way ANOVA for comparison of magnitude of changes between different groups.

Figure S5. Phosphorylation of BBC3 at Ser10 enables maximum BBC3 induction upon TNF exposure. (**A**) Densitometric analyses and representative protein gel blots (n=3) of HCT116 cells treated with 25 μg/ml CHX or cotreated with 10 ng/ml TNF and 25 μg/ml CHX for the indicated time periods showing that TNF treatment increases the protein half-life of BBC3. Quantification of BBC3 protein levels was done relative to TUBULIN (loading control). (**B**) Western blots of BBC3 WT and *BBC3*^{S10A} HCT116 cells infected with lentivirus encoding control or *RELA* shRNA. (**C**) QRT-PCR analysis of *BBC3* mRNA levels normalized to *TBP* in *BBC3* WT and *BBC3*^{S10A} HCT116 cells infected with lentivirus encoding *RELA* shRNA followed by TNF treatment for the indicated time periods. Statistics are depicted as mean ± SEM; n.s. = not significant, $P < 0.05$*, n=3, t test. (**D**) Representative immunoblots and densitometric data (n=3) of cells in (**C**) under TNF treatment for the indicated time periods showing that Ser10 phosphorylation is crucial for transient BBC3 induction upon TNF exposure. Quantification of BBC3 protein levels was done relative to TUBULIN (loading control). (**E**) Representative western blots with densitometric analysis following immunoprecipitation (n=3) in

HCT116 cells incubated with TNF for the indicated times after pretreatment with 20 mM NH_4Cl for 1 h showing that TNF treatment weakens the interaction between endogenous BBC3 and HSPA8. SE, short exposure; LE, long exposure. The HSPA8 protein levels were quantified relative to BBC3 protein levels in the IP experiment.

Figure S6. IKBKB-dependent phosphorylation of BBC3 at Ser10 enables maximum BBC3 induction upon TNF exposure. (**A**) Representative immunoblots and densitometric quantification (n=3) of HCT116 cells treated with 10 ng/ml TNF for indicated time periods following infection with vectors containing control or *IKBKB* shRNA showing that depletion of IKBKB profoundly impairs TNF-induced upregulation of BBC3. The ratio of BBC3 protein normalized to ACTIN relative to control (marked as 1) is indicated below each lane. (**B**) Representative western blots (n=4) of HCT116 cells cotreated with 25 μg/ml CHX and 10 ng/ml TNF following infection with control or *IKBKB* shRNA showing that silencing IKBKB results increased turnover rate of the BBC3 protein under TNF treatment. (**C**) BBC3 protein levels of cells in (**B**) were quantified relative to ACTIN. (**D**) QRT-PCR analysis of *BBC3* mRNA level in *BBC3* WT HCT116 cells treated with 10 ng/ml TNF for the indicated hours following control or *IKBKB* shRNA infection showing that knockdown IKBKB leads to induction of *BBC3* mRNA. (**E**) Representative protein gel blots (n=3) of *BBC3* WT HCT116 cells exposed to 25

μg/ml CHX following infection with control or *IKBKB* shRNA showing that silencing IKBKB results in increased turnover rate of the BBC3 protein under basal conditions. (**F**) Quantification of the BBC3 protein levels in (**E**) was done relative to ACTIN. (**G**) Representative immunoblots (n=4) and associated densitometric analysis of *BBC3* WT HCT116 cells treated with 0.2 μg/ml actinomycin D for 16 h following infection with vectors containing control or *IKBKB* shRNA. Quantification of BBC3 protein levels was done relative to TUBULIN. (**H**) QRT-PCR analysis of *BBC3* mRNA level in response to the treatment shown in (**G**) showing that IKBKB depletion induces BBC3 via transcriptional machinery. (**I**) QRT-PCR analysis of *BBC3* mRNA level in *BBC3* WT HCT116 cells treated with 10 ng/ml TNF or cotreated with 10 ng/ml TNF and 2.5 μg/ml CHX for the indicated times showing that TNF-mediated transcriptional induction of *BBC3* was compromised upon CHX treatment. Statistics are depicted as mean ± SEM; *, $P < 0.05$; **, 0.01; and ***, 0.001, n=3, t test.