Supplemental Data

Supplemental Figure Legends

Supplemental Fig. 1. RSK2 phosphorylates caspase-8 *in vitro*. (A) RSK2 phosphorylates caspase-8 *in vitro*. Wildtype His-caspase-8 proteins (5 μg) were purified from BL21 bacteria and subjected to an *in vitro* kinase assay with active RSK2 (100 ng) and 10 μCi of [γ-^{32}P] ATP and 50 μM unlabeled ATP. The reaction mixture was resolved by 10% SDS-PAGE and the phosphorylated caspase-8 was visualized by autoradiography. (B) Amino acid sequences for peptide synthesis. (C) Identification of the RSK2 phospho-target amino acid of caspase-8. Peptides (5 μg) synthesized in B were used for an *in vitro* kinase assay with active RSK2 (100 ng) and 10 μCi of [γ-^{32}P] ATP and 50 μM unlabeled ATP. The reaction mixture was resolved by 20% SDS-PAGE and phosphorylated caspase-8 was visualized by autoradiography. (D) Mutant caspase-8-T263A, but not mutants S119A or S256A, affects caspase-8 phosphorylation *in vitro* as determined by Western blot. An *in vitro* kinase assay was performed with caspase-8-wt or caspase-8 mutants together with active RSK2 (100 ng) and 200 μM unlabeled ATP. The phosphorylated caspase-8 was visualized by Western blot with a p-S/T antibody.

Supplemental Fig. 2. Overexpression of RSK2 induces caspase-8 degradation.
(A) RSK2 enhances caspase-8 degradation. RSK2 was transfected into HeLa cells and cells were cultured for 30 h. The cells were treated with cyclohexamide (CHX, 30 μg/ml) for the indicated time and proteins were extracted. The caspase-8 protein abundance was visualized by Western blot with a casapse-8 antibody. (B) RSK2 induces caspase-8 degradation through proteosomal-mediated caspase-8 degradation. RSK2 was transfected into HeLa cells and cells cultured for 30 h. The cells were pre-treated with MG-132 (20 μM) for 1 h and then treated with CHX (30 μg/ml). The caspase-8 protein level was visualized by Western blot using a caspase-8 antibody.

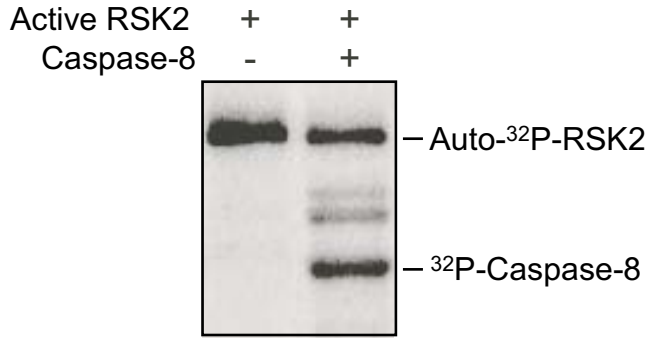
Supplemental Fig. 3. Phosphorylation of Thr263 plays an important role in caspase-8 ubiquitination. HeLa cells were co-transfected with caspase-8, caspase-8-S119A, caspase-8-S256A, or caspase-8-T263A along with HA-Ub and RSK2. Transfected cells were cultured for 30 h and then proteins were extracted. The caspase-8 proteins were immunoprecipitated with anti-V5 and the ubiquitinated caspase-8 proteins were visualized by Western blot with anti-HA.

Supplemental Fig. 4. Fas induces significant apoptosis in Jurkat A3 cells
Jurkat A3 cells were exposed to anti-Fas (200 ng/ml) for 6 h and then subjected to apoptosis analysis by Annexin V and propidium iodide (PI) staining using flow cytometry (bottom). Data are represented as means ± S.D. of the percent Annexin V positive cells

determined from triplicate experiments. The asterisk (*) indicates a significant difference ($p < 0.05$ Student's t test; bottom).

Supplementary Figure 1 A-C

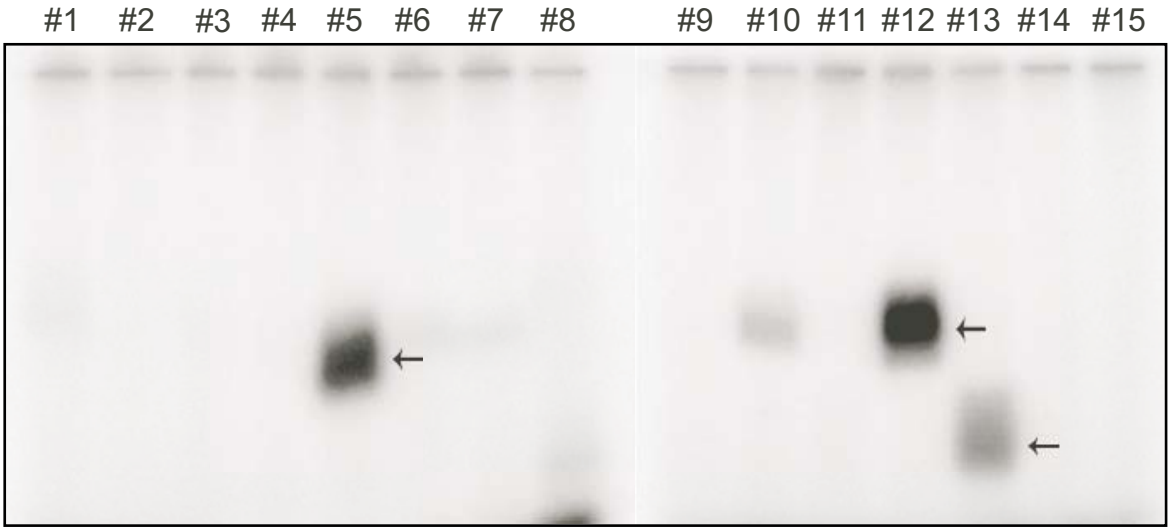
A



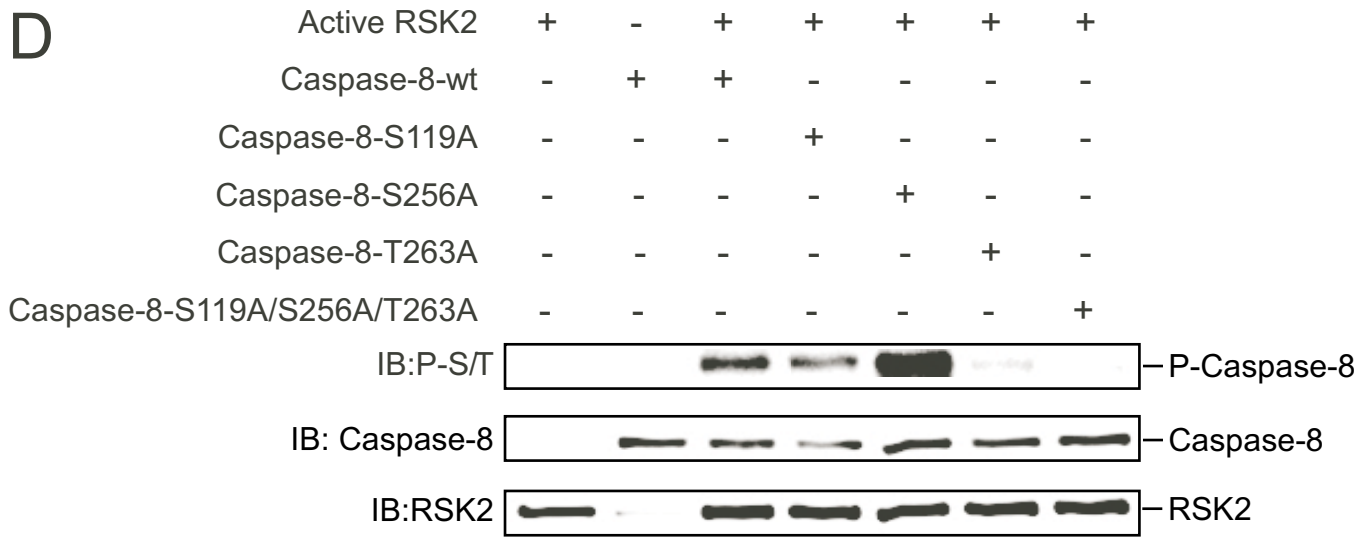
B

Peptide number	Position	Score	Mutation	Sequence
#1	S21	0.901		EDLA**S**LKFLDYIP
#2	T92	0.955		EEMERELQ**T**PGRAQI
#3	S113	0.996	S115A,S119A	EEV**S**R**A**ELR**A**FKFLL
#4	S115	0.938	S113A,S119A	EEV**A**R**S**ELR**A**FKFLL
#5	S119	0.974	S113A,S115A	EEV**A**R**A**ELR**S**FKFLL
#6	S182	0.994		LLKIINDYEEF**S**KER
#7	S187	0.997	S118A,S192A	AKERA**S**A**A**LEG**A**PDEF
#8	S188	0.997	S187A,S192A	AKERA**A**S**A**LEG**A**PDEF
#9	S192	0.99	S187A,S188A	AKERA**AA**LEG**S**PDEF
#10	S211	0.998		LCGVMAIAD**S**PREQD
#11	S217	0.982		PREQD**S**EAQALDKVY
#12	S256	0.959		REKVPKLH**S**IRDRNG
#13	T263	0.806		I**R**D**R**NG**T**HLDAGALA (RXRXXS/T)
#14	S375	0.981		GIPVEAD**S**EEQPYLE
#15	S451	0.985		EVNYEV**S**NKDDKKNM

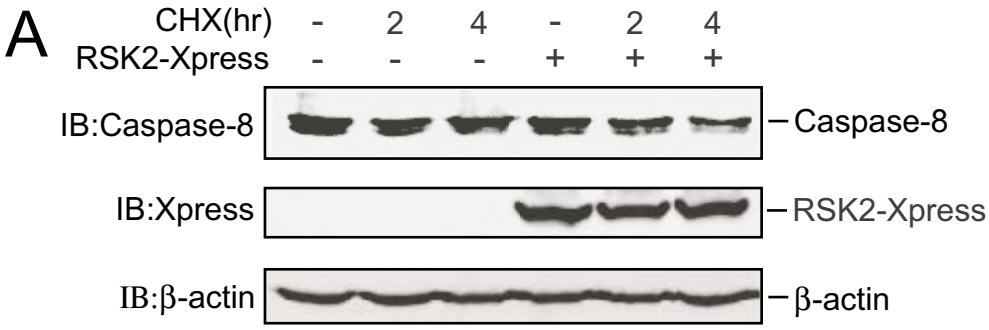
C

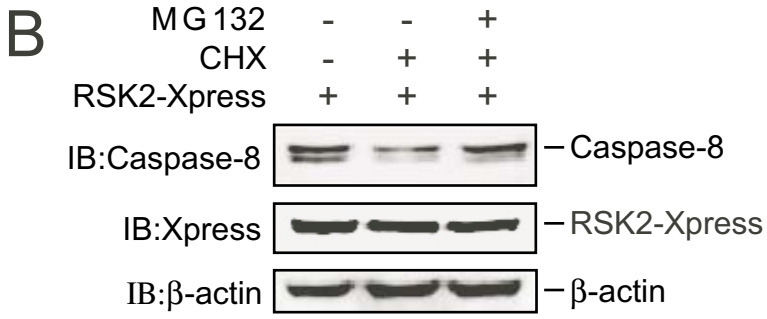


Supplementary Figure 1D

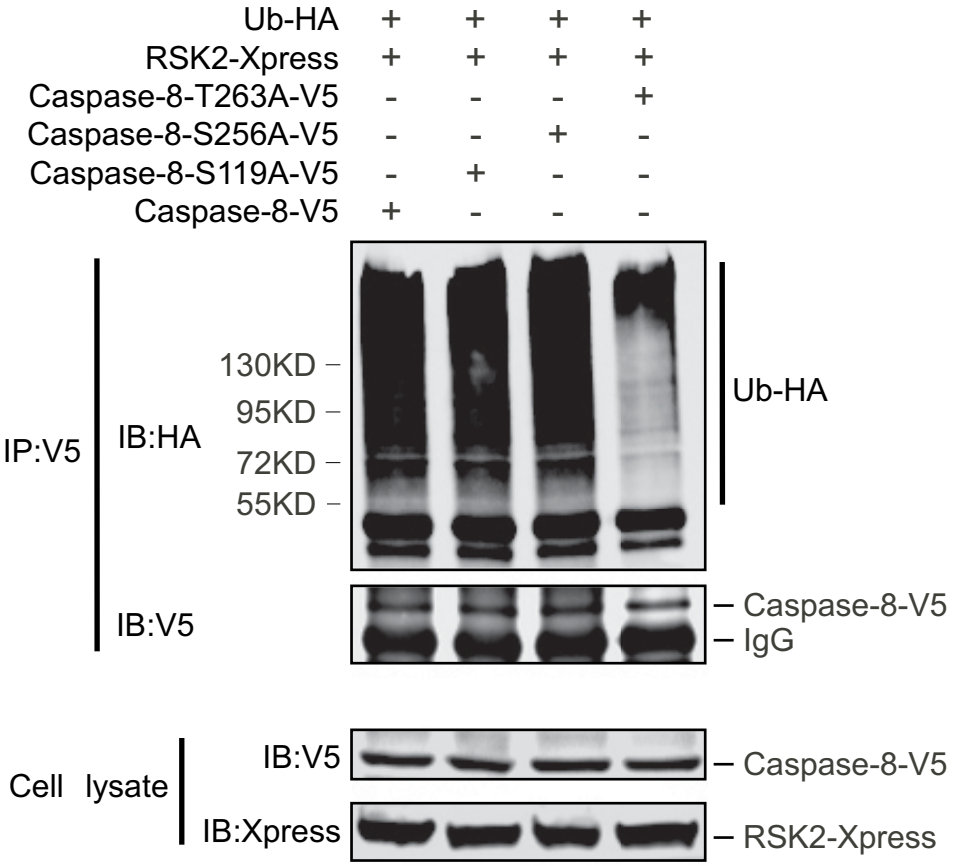


Supplementary Figure 2 A-B





Supplementary Figure 3



Supplementary Figure 4