Figure S1. Low levels of MG132 induce Nrf2 accumulation. (A) Representative immunoblots and (B) quantification of Nrf2 in cells treated with varying amounts of MG132. Data are presented as the mean ± SEM representative of three independent experiments and were assessed using one-way ANOVA followed by Tukey's test. **P<0.01 and ***P<0.001. Nrf2, nuclear factor erythroid 2-related factor 2.

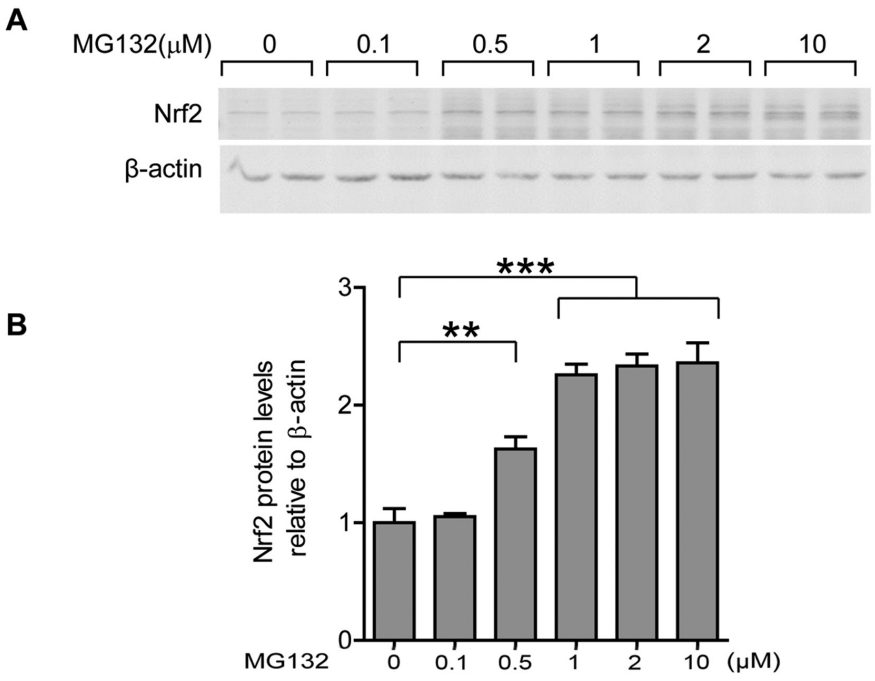

Figure S2. Generation of Nrf2 knockout cells. (A) Schematic diagram of CRISPR/Cas9 genome editing of *NEF2L2*. (B) Representative immunoblots of Nrf2 in *nrf2*$^{+/+}$ and *nrf2*$^{-/-}$ cells treated with MG132 (2 μM) for 12 h. (C) Representative images of *NEF2L2* gene fragments in *nrf2*$^{+/+}$ and *nrf2*$^{-/-}$ cells; amplified genomic DNA covered the sgRNA-targeting sequence. (D) DNA sequences of PCR fragments in created by PCR. (E) Representative images and (F) quantification of aggresomes in MG132 or MG132+NAC (1 mM) treated cells; anti-ubiquitin Lys 48 visualizes aggresomes; scale bar, 20 μm. Data are presented as the mean± SEM representative of three independent experiments and were assessed using one-way ANOVA followed by Tukey's test. ***P<0.001. Nrf2, nuclear factor erythroid 2-related factor 2; NS, not significant; sgRNA, single guide RNA; *nrf2*$^{-/-}$, Nrf2 knockout 293 cells; NAC, N-acetyl-cysteine.

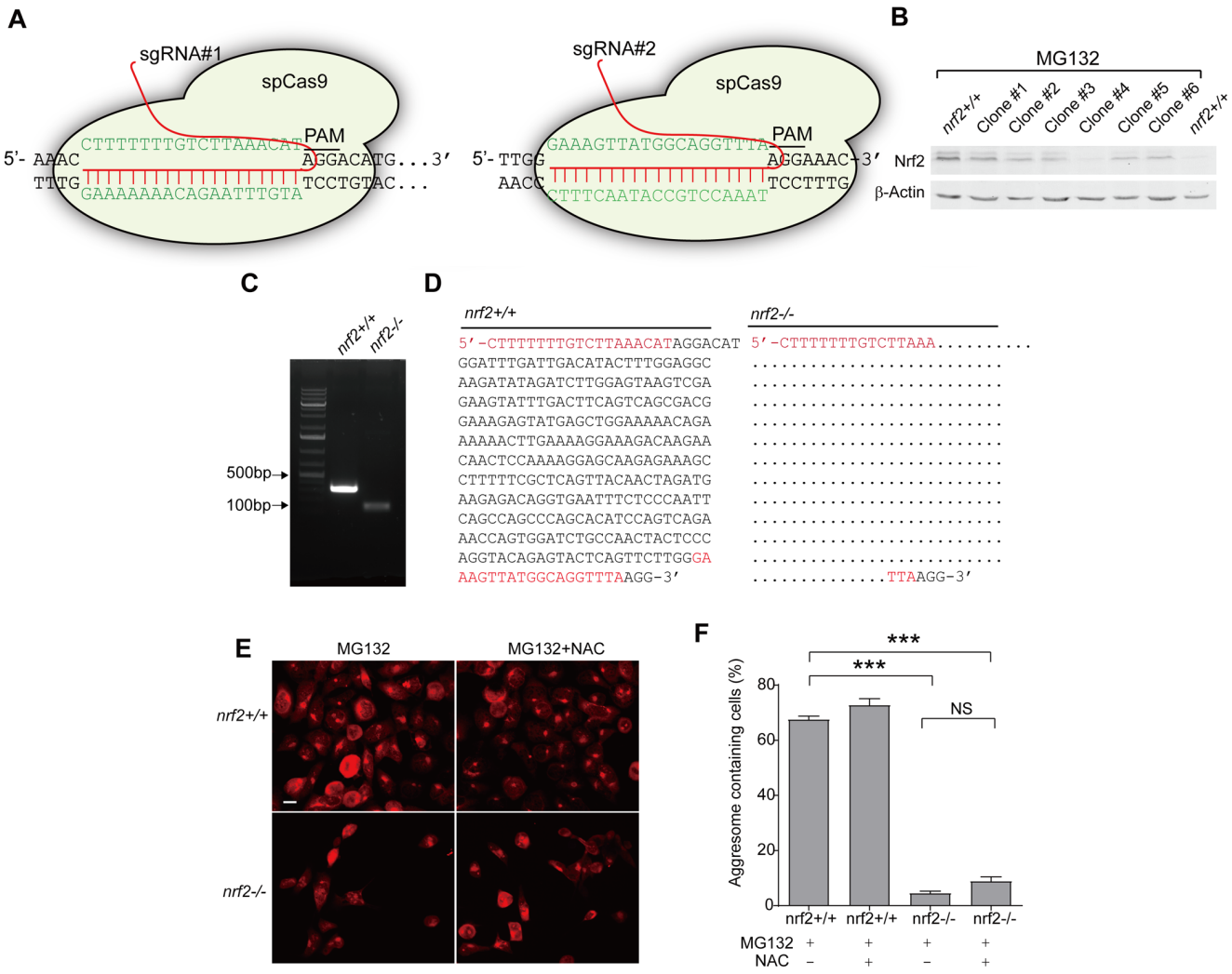

Figure S3. MG132 induces cell death in 293 cells. Representative quantification of viable 293 cells stained with Annexin V and PI at different time points after MG132 (2 μM) treatment; live cells, Annexin V$^-$PI$^-$. Data are presented as the mean ± SEM representative of three independent experiments and were assessed using one-way ANOVA followed by Tukey's test. *P<0.05, ***P<0.001 and ****P<0.0001. Nrf2, nuclear factor erythroid 2-related factor 2; PI, propidium iodide.

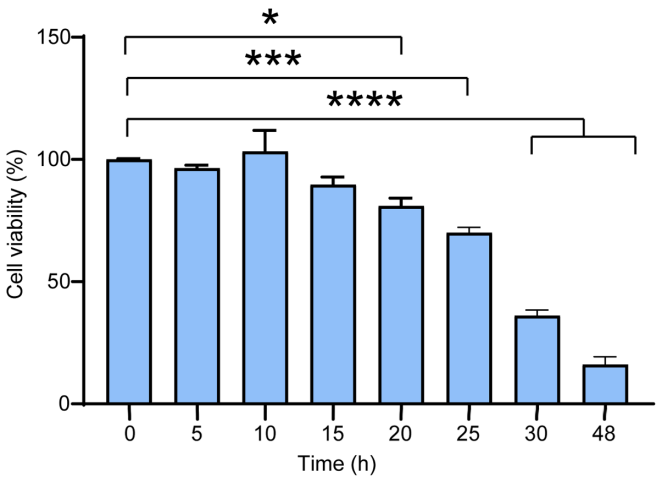

Figure S4. Loss of Nrf2 reduces HO1 expression without affecting other stress-induced genes during proteasomal inhibition. (A) Representative immunoblots and (B) quantification of HO1 in cells treated with DMSO or MG132 (2 μM) for 12 h. (C) Representative immunoblots and (D) quantification of HSP70 in cells treated with DMSO or MG132 (2 μM) for 12 h. Data are presented as the mean ± SEM representative of three independent experiments and were assessed using one-way ANOVA followed by Tukey's test. **P<0.01. Nrf2, nuclear factor erythroid 2-related factor 2; *nrf2*$^{-/-}$, Nrf2 knockout 293 cells; HO1, heme oxygenase-1; HSP70, 70-kDa heat shock protein.

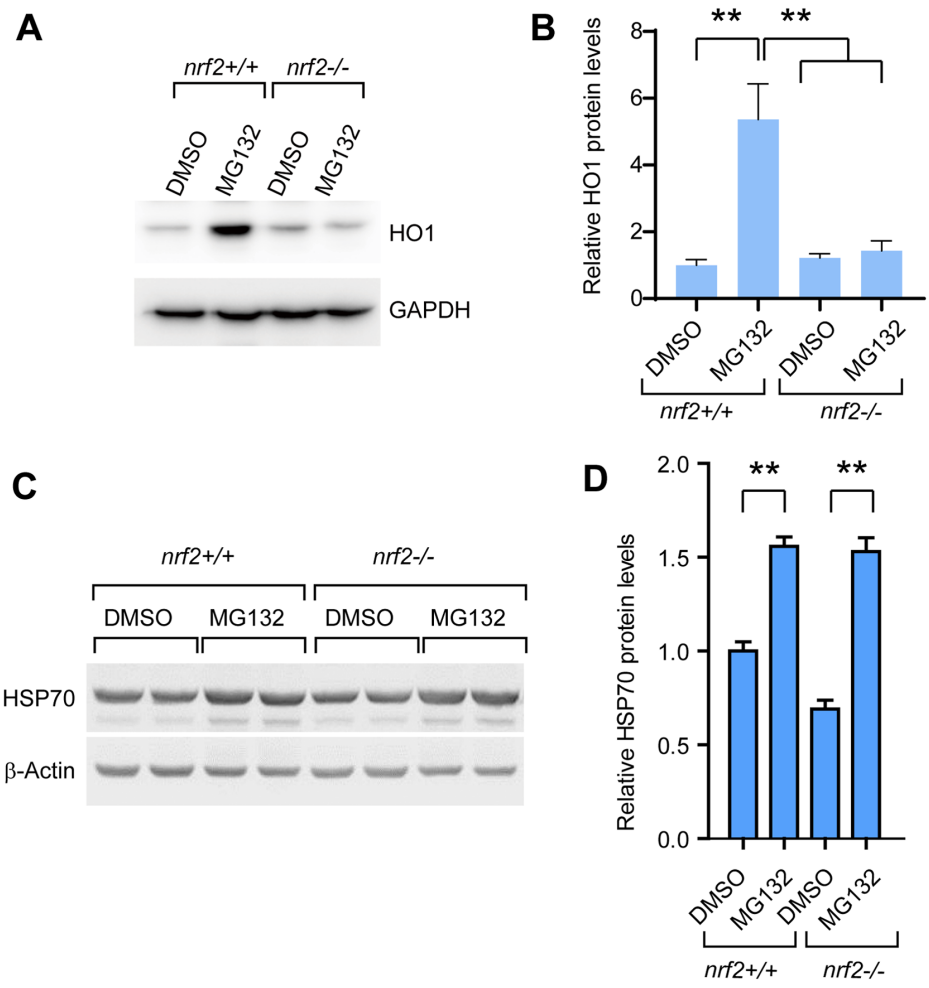

Figure S5. Generation of *nrf2*$^{-/-}$[Flag-p62] and *nrf2*$^{-/-}$[Flag-Nrf2] cells. (A) Nrf2 mRNA levels in *nrf2*$^{+/+}$, *nrf2*$^{-/-}$, *nrf2*$^{-/-}$[Flag-Nrf2], *nrf2*$^{+/+}$[Vector] and *nrf2*$^{-/-}$[Vector] cells. (B) p62 mRNA levels in *nrf2*$^{+/+}$, *nrf2*$^{-/-}$, *nrf2*$^{-/-}$[Flag-p62], *nrf2*$^{+/+}$[Vector] and *nrf2*$^{-/-}$[Vector] cells. Data are presented as the mean ± SEM representative of three independent experiments and were assessed using one-way ANOVA followed by Tukey's test. ***P<0.001, ****P<0.0001. Nrf2, nuclear factor erythroid 2-related factor 2; *nrf2*$^{-/-}$, Nrf2 knockout 293 cells; [], transfection construct.

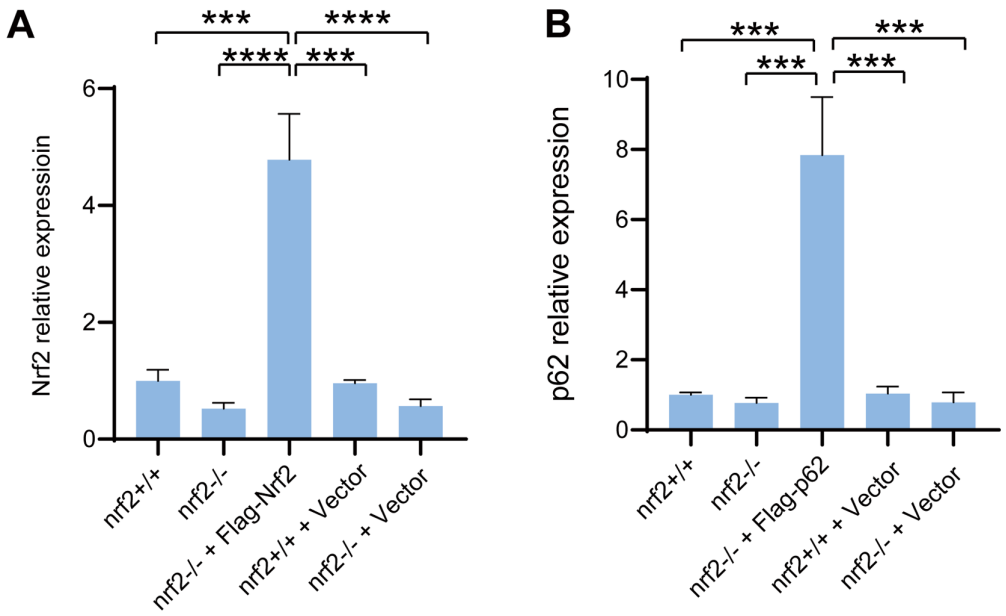

Table SI. Primers used in this study.

Primer	Sequence (5'-3')	Purpose
p62 *Eco*RI	GATCGAATTCATGGCGTCGCTCACCGTG	SQSTM1 from cDNA
p62 *Xba*I	GATCTCTAGATTACAACGGCGGGGGATG	SQSTM1 from cDNA
Nrf2 *Bam*HI	GATCGGATCCATGATGGACTTGGAGCTG	Nrf2 from cDNA
Nrf2 *Xba*I	GATCTCTAGAGTTTTTCTTAACATC	Nrf2 from cDNA
SQSTM Pro-F	GATCGATCGGATCCGGATGATGCCCACACCTG	p62 promoter region
SQSTM Pro-R	GATCGATCGAATTCGGAGCGGGCCCGGCGG	p62 promoter region
EGFP-F	GATCGAATTCATGG TGAGCAAGGGCGAGGAG	EGFP introduction
EGFP-R	GATCTCTAGATTACTTGTACAGCTCGTCCATGCC	EGFP introduction
Nrf2 gRNA1-F	CACCGCTTTTTTTGTCTTAAACAT	Annealing
Nrf2 gRNA1-R	AAACATGTTTAAGACAAAAAAA	Annealing
NrfF2 gRNA2-F	CACCGGAAAGTTATGGCAGGTTTA	Annealing
Nrf2 gRNA2-R	AAACTAAACCTGCCATAACTTTC	Annealing
Primer-F	AACAGTGGCATAATGTGAATTA	CRISPR/Cas9 validation
Primer-R	GGTTAGGTACTGAACTCATC	CRISPR/Cas9 validation
Nrf2 RT-F	CAGCGACGGAAAGAGTATGA	RT-qPCR
Nrf2 RT-R	TGGGCAACCTGGGAGTAG	RT-qPCR
p62 RT-F	TGAGGAACAGATGGAGTCGG	RT-qPCR
p62 RT-R	GAGATGTGGGTACAAGGCAG	RT-qPCR
GAPDH-F	CCCACTCCTCCACCTTTG	RT-qPCR
GAPDH-R	CACCACCCTGTTGCTGTAG	RT-qPCR

Nrf2, nuclear factor erythroid 2-related factor 2; F, forward; R, reverse; RT, reverse transcription; q, quantitative; gRNA, guide RNA; EGFP, enhanced GFP; SQSTM1, sequestosome-1.