

SUPPORTING INFORMATION

I. Experimental Methods:

RNAi reagents for gene silencing in fly S2 cells.

The following primers were used for PCR amplification of RNAs for gene silencing in fly S2 cells.

Tub_dsRNA_Fw: TAATACGACTCACTATAGGCCCTGCCCGCCTGT

Tub_dsRNA_Rev: TAATACGACTCACTATAGGTGATTCGCGGAATGTTAGG

Tub_dsRNA_2_Fw: TAATACGACTCACTATAGGAATGACATTTACCGGCTTGC

Tub_dsRNA_2_Rev: TAATACGACTCACTATAGGCTCGTTGTCCACTTCCATT

Pll_dsRNA_Fw: TAATACGACTCACTATAGGGGGCGTCAGCAACTCAA

Pll_dsRNA_Rev: TAATACGACTCACTATAGGCCGAGGCTGATGCTAAAC

Pll_dsRNA_2_Fw: AATACGACTCACTATAGGCCGTGTGATTGTACTGACCG

Pll_dsRNA_2_Rev: TAATACGACTCACTATAGGACAGGTTTTTCATCGAATCGC

Ire1_dsRNA_Fw: TAATACGACTCACTATAGGAACACGCCTCGTATTTCCAC

Ire1_dsRNA_Rev: TAATACGACTCACTATAGGAGGCAATCATACCCAGAACG

qRT-PCR primers.

The thermal amplification profile was as follows: Activation: 95°C for 5 minutes for 1 cycle | Amplification: 95°C for 10 sec, 60°C for 10 sec and 72°C for 10 sec for 45 cycles | Final amplification: 95°C for 5 sec, 60°C for 1 minute and 1 cycle of cooling at 40°C for 30 sec. Q-PCR results were considered valid when efficiency $E > 95\%$ and standard curve correlation $R^2 > 99\%$

were achieved. The quality of the resulting PCR products was assessed by thermal melting point determination.

The sequences of DNA primers used for qRT-PCR analysis of transcripts were as follows:

Human CHOP Fwd: CAGAGCTGGAACCTGAGGAG Rev: TGGATCAGTCTGGAAAAGCA

Human XBP1 total Fwd: TGACGAGGTTCCAGAGGTG Rev: TGCAGAGGTGCACATAGTCTG

Human XBP1 unspliced Fwd: CCGCAGCACTCAGACTACG Rev: ATGTTCTGGAGGGGTGACAA

Human IRAK2 Fwd: CCTCCTCTGAGGCCTGTGT Rev: TGATCTCAATTTGCCACGAA

Human IRAK 1 Fwd: GAGACCTTGGCTGGTCAGAG Rev: AGTGTGCTCTGGGTGCTTCT

Human BIP Fwd: CAACCAACTGTTACAATCAAGGTC Rev: CAAAGGTGACTTCAATCTGTGG

Human IRE1 Fwd: CCATCGAGCTGTGTGCAG Rev: TGTTGAGGGAGTGGAGGTG

Human PERK Fwd: CAGTGGGATTTGGATGTGG Rev: GGAATGATCATCTTATTCCCAA

Human ATF6 Fwd: TTGGCATTATAATACTGAACTATGGA Rev: TTTGATTTGCAGGGCTCAC

Human NQO1 Fwd: CAGCTCACCGAGAGCCTAGT Rev: GAGTGAGCCAGTACGATCAGTG

Human JUN Fwd: CCAAAGGATAGTGGATGTTT Rev: CTGTCCCTCTCCACTGCAAC

Human ATG12 Fwd: TCTTCCGCTGCAGTTTCC Rev: GTCTCCCACAGCCTTTAGCA

Mouse CHOP Fwd: GCGACAGAGCCAGAATAACA Rev: GATGCACTTCCTTCTGGAACA

Mouse IRAK2 Fwd: AGCACAGCCATCCACCAG Rev: TGATCTCAATTTTCCATGAAGTCT

Mouse XBP1 total Fwd: AGCAAGTGGTGGATTTGGAA Rev: CCGTGAGTTTTCTCCCGTAA

Mouse XBP1 unspliced Fwd: TGACGAGGTTCCAGAGGTG Rev: TGCAGAGGTGCACATAGTCTG

Mouse BIP Fwd: CAAGTTCTTGCCATTCAAGGT Rev: CTTCTGGGGCAAATGTCTTG

Mouse IRE1 Fwd: TGAACACCCCTTCTTCTGG Rev: CCTCCTTTTCTATTCCGGTCACTT

Mouse PERK Fwd: CCTTGGTTTCATCTAGCCTCA Rev: ATCCAGGGAGGGGATGAT

Mouse ATF6 Fwd: GGACGAGGTGGTGTTCAGAG Rev: GACAGCTCTTCGCTTTGGAC

For qRT-PCR analysis of fly S2 cells, the following primers were employed:

Tub _1_Fw: AGCAGAGCTCAAACAATGAC

Tub _1_Rev: CAAATGAATACATCTGGGTTG

Tub _2_Fw: ACGATGATAACGATGGTGAG

Tub _2_Rev: TGCAGCTCACTCAAGTCTG

Pll _1_Fw: CATGAATCAGTCGCAACC

Pll _1_Rev: GTCGGGATAGAGTTTTACGG

Pll _2_Fw: ACTACGTTAAGCAGCAGTGG

Pll _2_Rev: CCATCTAGTCGGTAACAAACG

XBP1_Full_Fw: TCTAACCTGGGAGGAGAAAG

XBP1_Full_Rev: GTCCAGCTTGTGGTTCTTG

XBP1_S_Fw: CAGCATCCAAAGCTGACCCTCTG

XBP1_S_Rev: ATATCTGCGAGCAGACTTTC

IRE1_Fw: TCTCCCACGAATACAATCTG

IRE1_Rev: GAATTGCAACTTCTCCACAC

rp49_Fw: ATCGTGAAGAAGCGCACCAAG

rp49_Rev: ACCAGGAACTTCTTGAATCCG

Cell Lysis

Cells were resuspended in a lysis buffer containing 20 mM HEPES (pH 7.3), 150 mM NaCl, 0.5% NP-40, 1 mM EDTA and Complete EDTA-free protease inhibitor cocktail (Roche). Lysates kept on

ice for 20 minutes were then centrifuged at 12,000g for 10 minutes to eliminate debris. Animal tissue protein lysates were prepared in a hypotonic lysis buffer containing 10 mM NaCl, 10 mM Tris-HCl pH 7.2, 1 mM EDTA, 1% Triton and Complete EDTA-free protease inhibitor cocktail (Roche). After incubation on ice for 20 min, samples were centrifuged 3 times at 12,000g for 10 minutes.

II. Supporting Figure Legends:

Figure S1: Overview of kinome screening campaign.

(A) The flow chart depicts the process used to prosecute hits from siRNA kinome library screening. See text for details. **(B)** Alva-31 and A549 cells were transfected with IRAK2, scrambled (Sc) or Caspase-8 (casp 8) siRNAs. After 2 days, cells were treated with CDDO-Im for 32 h (Alva-31) or 12 h (A549). Cell viability was measured by ATPLite assay. Non-treated controls (DMSO) were normalized to 100%. Data are mean \pm SD, n = 3.

Figure S2: Additional studies of effects of IRAK2 on UPR signaling.

(A) HeLa cells were transiently transfected with 2 independent IRAK2 siRNAs or scrambled controls (SC). After 2 days, cells were cultured with 5 μ M TG or 100 μ M Etoposide (Eto) for 3 hrs then total RNA was extracted. Levels of endogenous CHOP and IRAK2 mRNAs were measured by qRT-PCR and normalized relative to housekeeping gene, cyclophilin (mean \pm SD, n = 3). **(B,C)** PPC-1 cells stably expressing scrambled or IRAK2 shRNAs were cultured with 5 μ M Thapsigargin (TG) for 3 hrs or 100 ng/mL TNF- α for 0-60 min. Lysates were prepared, normalized for total protein content, and analyzed by immunoblotting using antibodies specific for phosphor-JNK1, total JNK1, cleaved ATF6, or actin. Data representative of 3 or more experiments. **(D)** qRT-PCR for CHOP

mRNA was performed using samples from Fig. 4a and displayed as ratios relative to housekeeping gene, cyclophilin. Data are mean±SD, n = 3.

Figure S3: IRAK2 mRNA expression is selectively induced by ER stress.

(A) The levels of NQ01, JUN and ATF6 mRNAs were analyzed by qRT-PCR using samples from Fig. 4c and normalized relative to housekeeping gene, cyclophilin (mean±SD, n = 3). **(B)** PPC-1 cells were cultured with 5 μM TG, 5 μM CDDO-Im, or DMSO for various times, then total RNA was extracted and levels of the indicated mRNAs were analyzed by qRT-PCR with normalization relative to housekeeping gene, cyclophilin (mean±SD, n = 3).

Figure S4: Confirmation of target knockdown by RNA silencing.

(A) Lysates were prepared from *Ire1^{-/-}*, *Perk^{-/-}* and *Atf6^{-/-}* mouse embryonic fibroblasts, normalized for total protein content, and analyzed by immunoblotting using the indicated antibodies. **(B)** The levels of mRNAs for ASK1, CHOP, and XBP1 were measured in qRT-PCR in PPC-1 transfected with various siRNAs as indicated, using 2 independent siRNAs for each target. Data are mean±SD, n = 3.

Figure S5: Proposed mechanism of IRAK2 regulation of ER stress signaling.

IRE1 activation by ER stress stimulates IRAK2 gene expression via a XBP1-dependent mechanism. Reciprocally, IRAK2 promotes ER stress-induced expression of the IRE1 gene. The IRE1 pathway stimulates stress kinase activation, which is known to promote apoptosis.

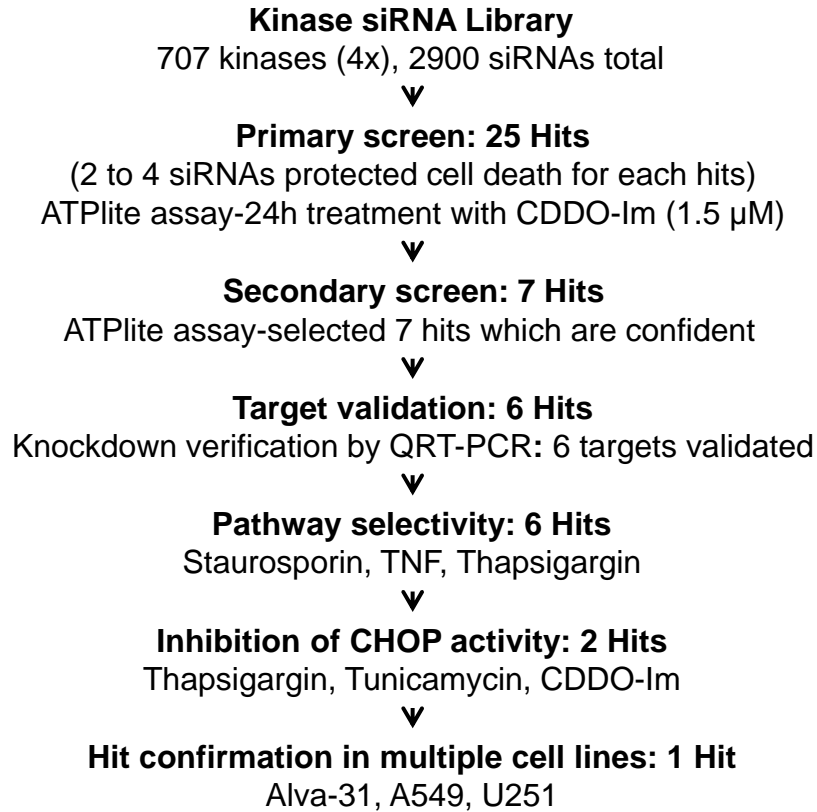
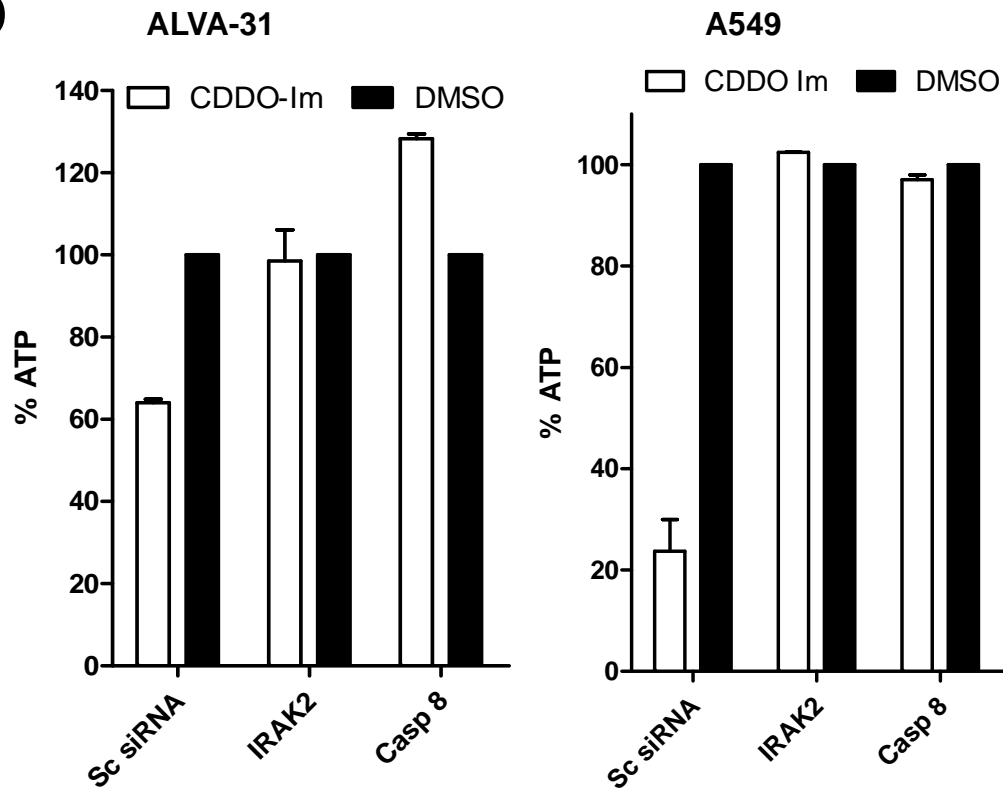
a**b**

Figure S1

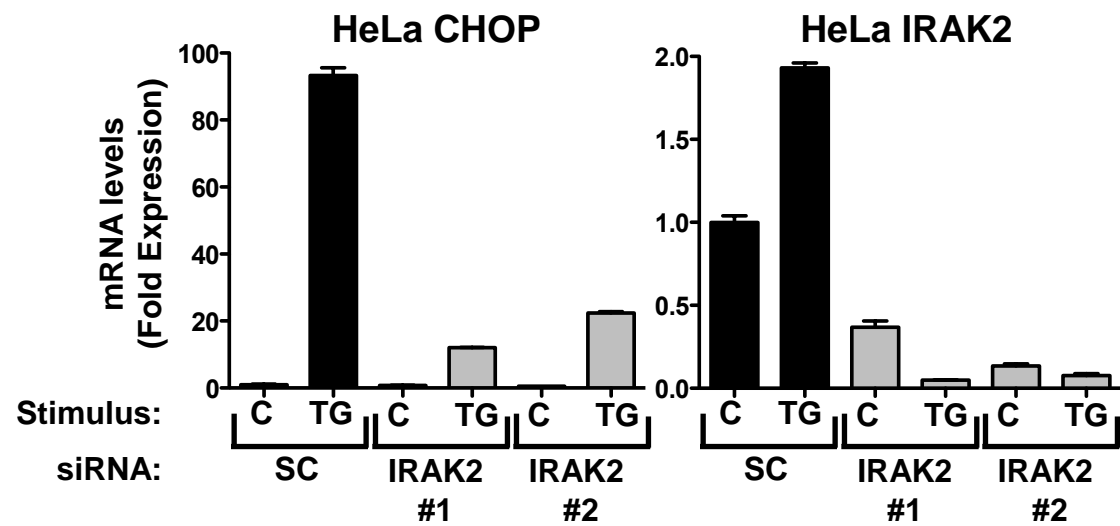
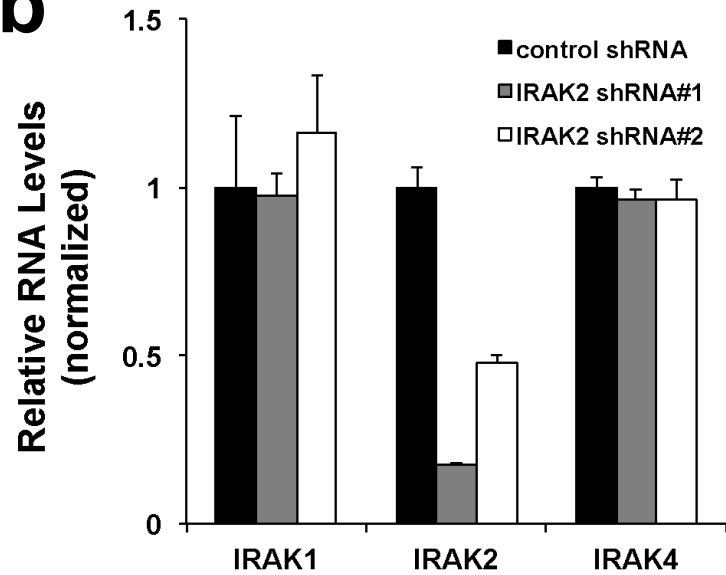
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Figure S2

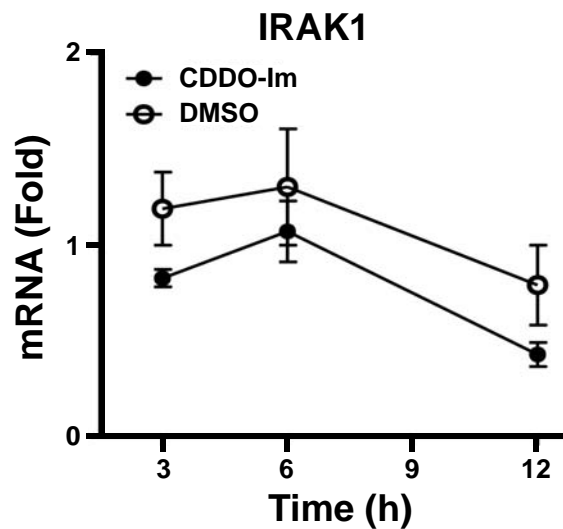
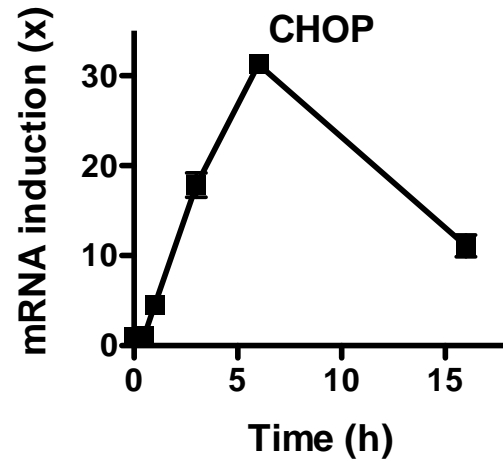
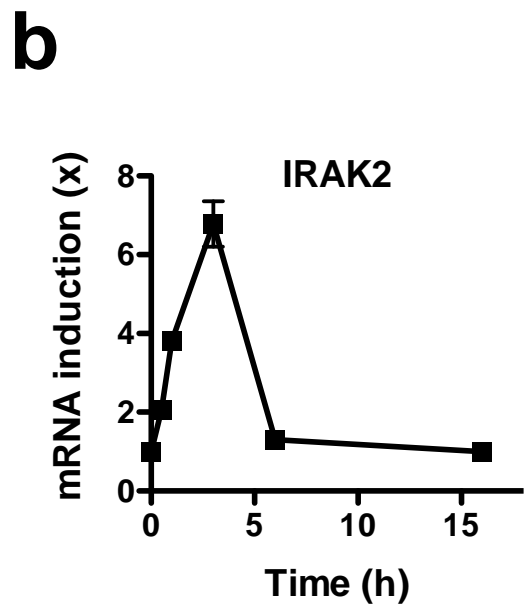
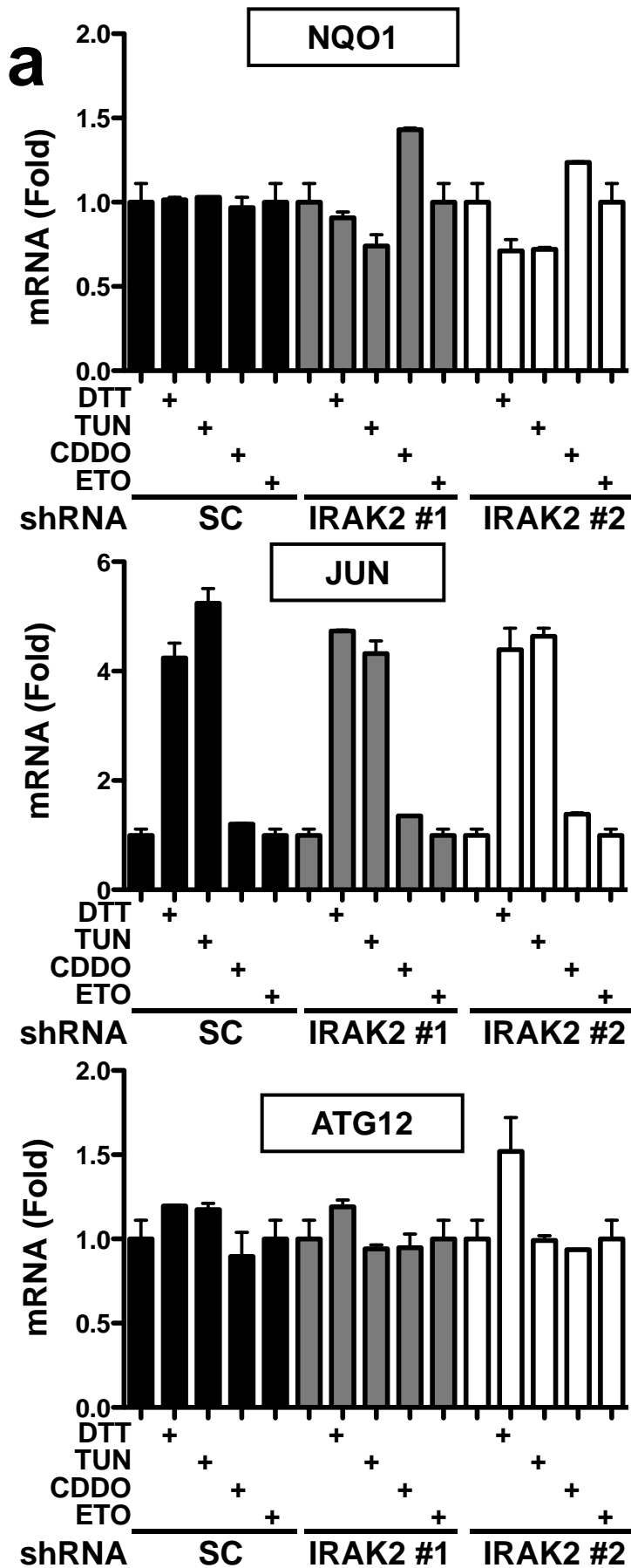


Figure S3

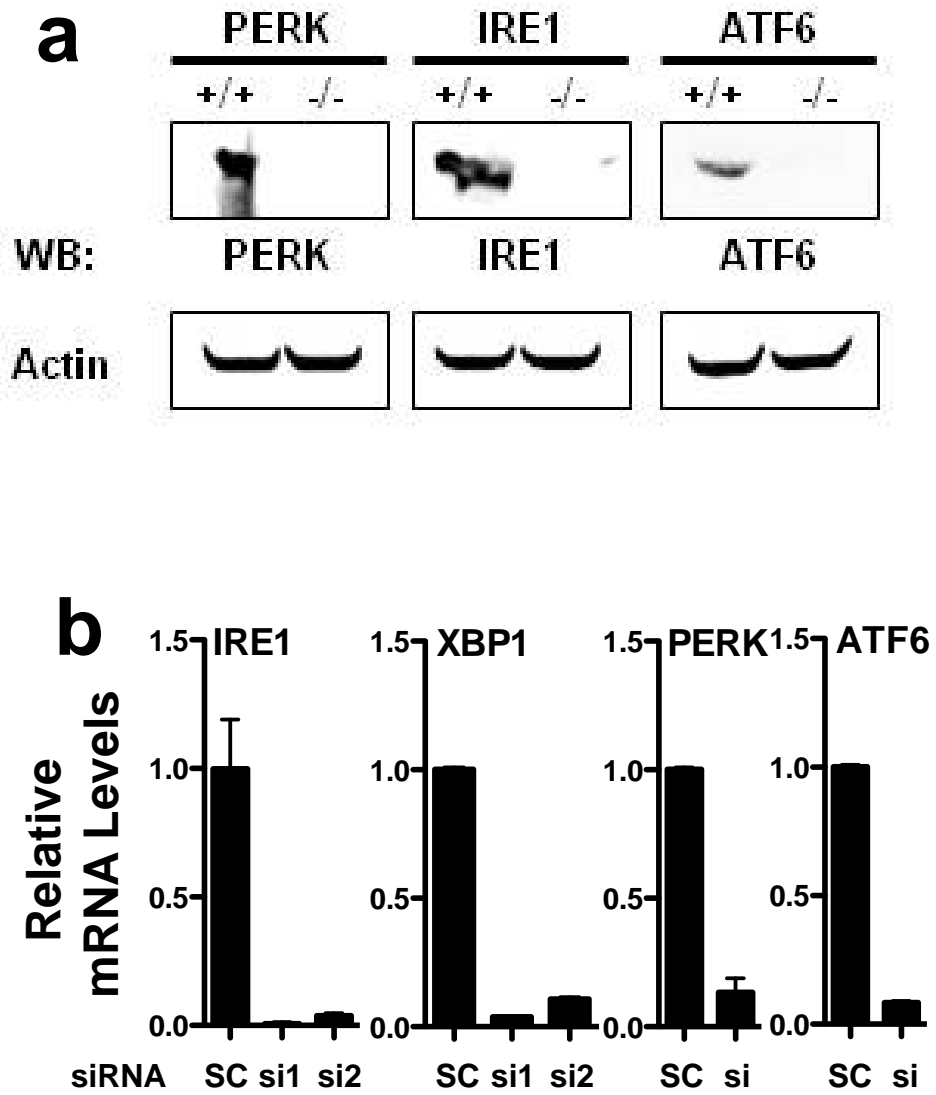


Figure S4

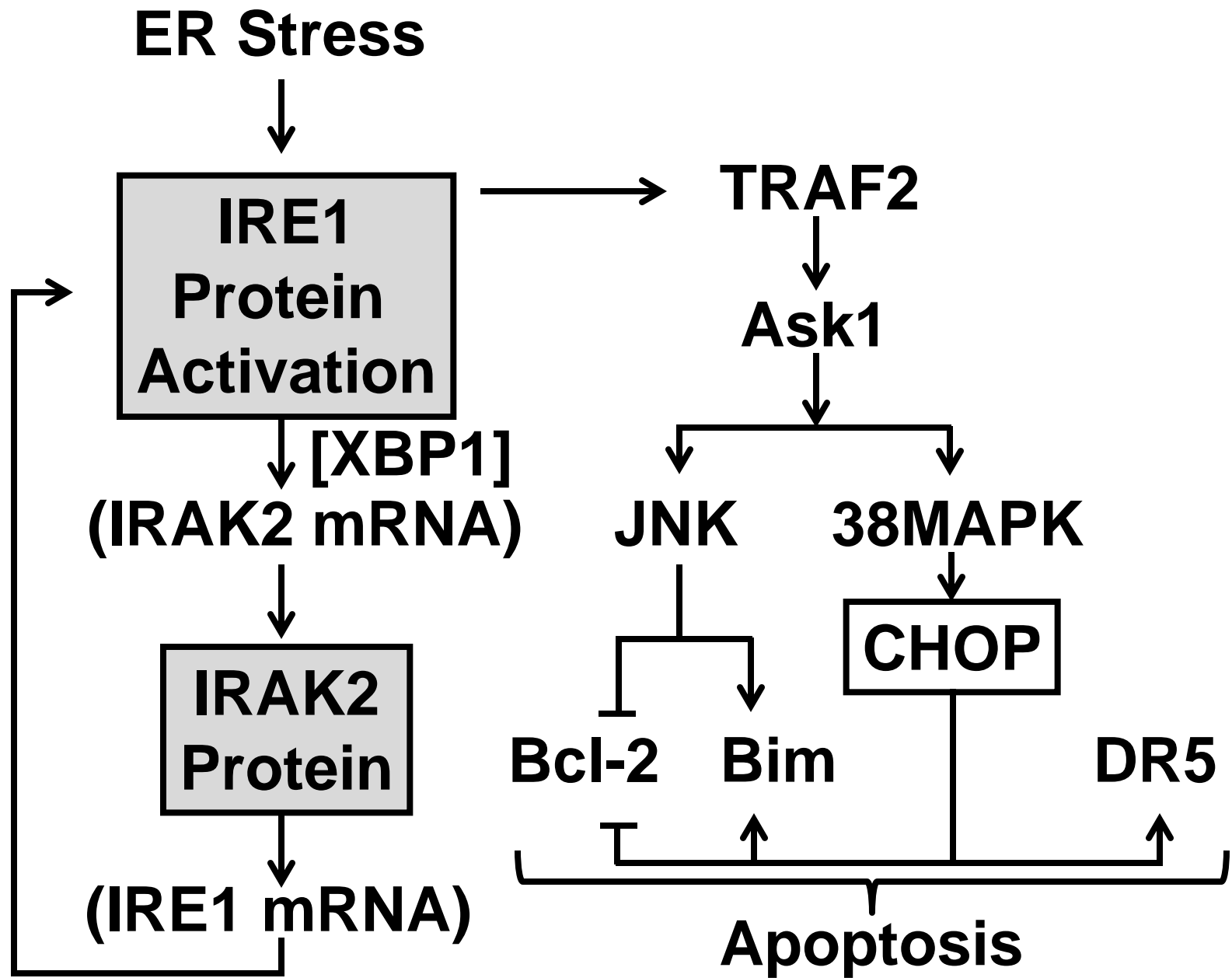


Figure S5