Supplementary Information

TRAF2 phosphorylation promotes NF-κB-dependent gene expression and inhibits oxidative stress-induced cell death

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Supplementary Data

A

```
                    Ser-11
                      ↓
          1           15
hTRAF2: MAAASVTPPGSLELL
mTRAF2: MAAASVTSPGSLELL
        ******* *******
                                  Ser-55
                                    ↓
        32                                                  76
hTRAF2: YLCSACRNVLRRPFQAQCGHRYCSFCLASILSSGPQNCAACVHEG
mTRAF2: YLCSACKNILRRPFQAQCGHRYCSFCLTSILSSGPQNCAACVYEG
        ****** * ********************************** **
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                              TRAF2 RING domain
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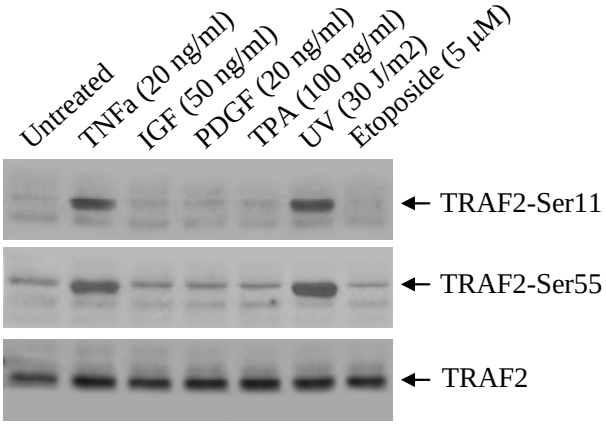
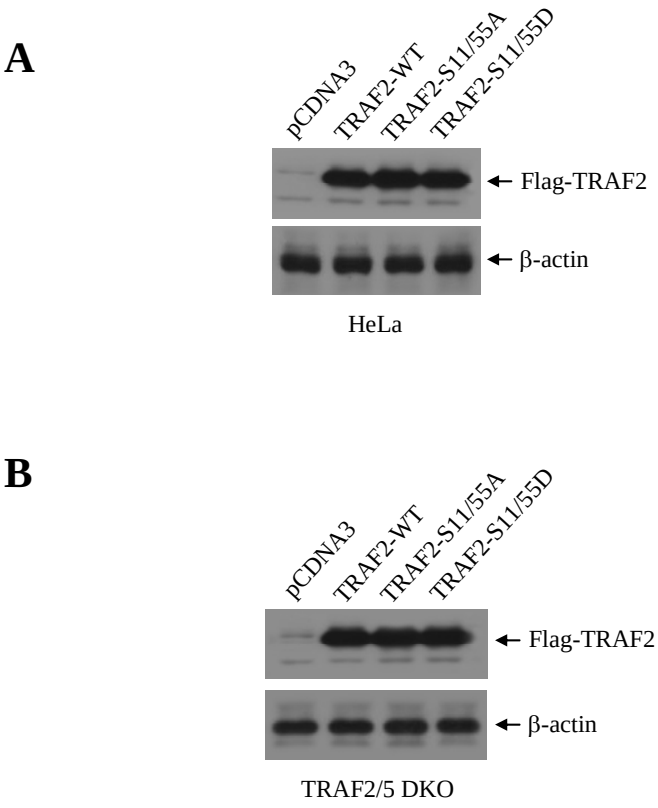
B



HeLa

Supplementary Figure 1. (A) Sequence comparison between human and mouse TRAF2, with phosphorylation sites and amino acid sequence of the RING domain indicated. (B) UV and TNFα induce TRAF2 phosphorylation at Ser-11 and Ser-55. HeLa cells were treated as indicated for 30 min, after which TRAF2 phosphorylation was detected by Western blotting using TRAF2 phosphoantibodies, TRAF2-Ser11 and TRAF2-Ser55.



Supplementary Figure 2. Western blot analysis of wild-type and phosphomutant TRAF2 expression in HeLa and TRAF2/5 DKO cells.

(A) HeLa cells cultured in 6-well plates were co-transfected with NF-κB-Luc (0.2 μg), pRL-TK (0.01 μg) and pCDNA3, Flag-TRAF2-WT, -S11/55A or -S11/55D (0.2 μg), using Lipofectamine 2000 reagents. 36 hrs after transfection, the cells were harvested and expression of TRAF2-WT, -S11/55A and -S11/55D was monitored by Western blotting using an anti-Flag antibody.

(B) TRAF2/5 DKO MEFs cultured in 6-well plates were co-transfected with NF-κB-Luc (0.2 μg), pRL-TK (0.01 μg) and pCDNA3, Flag-TRAF2-WT, -S11/55A or -S11/55D (0.2 μg), using Lipofectamine 2000 reagents. 36 hrs after transfection, the cells were harvested and expression of TRAF2-WT, -S11/55A and -S11/55D was monitored by Western blotting using an anti-Flag antibody.

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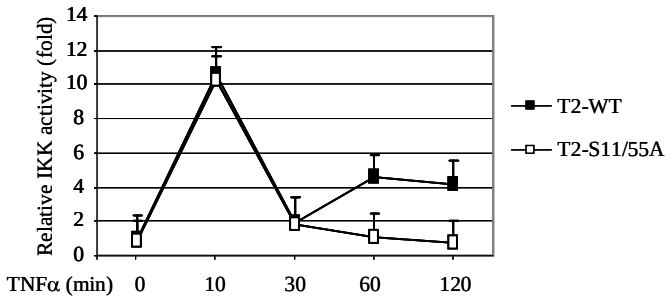

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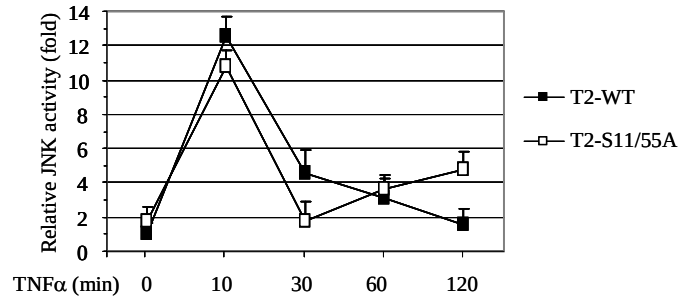
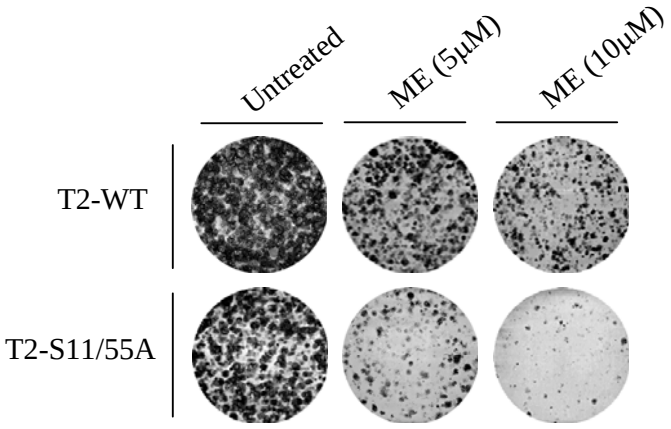


Supplementary Figure 3. TRAF2 phosphorylation regulates prolonged phases of IKK and JNK activation in response to TNFα stimulation.

(A) TRAF2/5 DKO cells reconstituted with TRAF2-WT (T2-WT) or -S11/55A (T2-S11/55A) were treated with mTNFα (10ng/ml) for the indicated times. The IKK complex was immunoprecipitated with an anti-IKKγ antibody, and then subjected to *in vitro* kinase assays using GST-IκBα$^{1-55}$ as substrate. Reaction mixtures were separated by SDS-PAGE, transferred onto a nitrocellulose membrane and exposed to X-ray film for 6 hrs. The average IKK activity from three independent kinase assays was then summarized.

(B) T2-WT and -S11/55A cells were treated with mTNFα (10ng/ml) for the indicated times. The JNK1 was immunoprecipitated with an anti-JNK1 antibody, and subjected to *in vitro* kinase assays using GST-jun$^{1-87}$ as substrate. Reaction mixtures were separated by SDS-PAGE, transferred onto a nitrocellulose membrane and exposed to X-ray film for 6 hrs. The average JNK1 activity from three independent kinase assays was then summarized.



Supplementary Figure 4. TRAF2 phosphorylation inhibits menadione-induced apoptosis. TRAF2/5 DKO cells reconstituted with TRAF2-WT (T2-WT) or -S11/55A (T2-S11/55A) were plated in 6-well plates at a density of 500 cells/well. The next day, cells were left untreated or treated with menadione (ME; 5 and 10 μM). Every other day, the medium was replaced with fresh medium containing ME. 14 days later, colonies were visualized by crystal violet staining.

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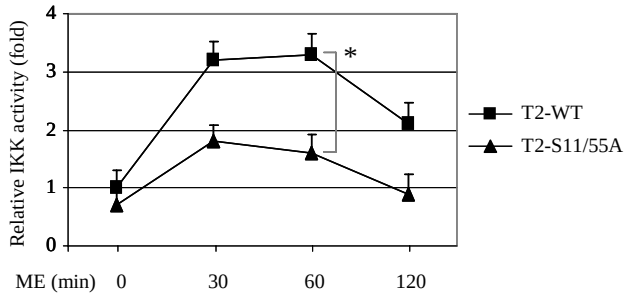

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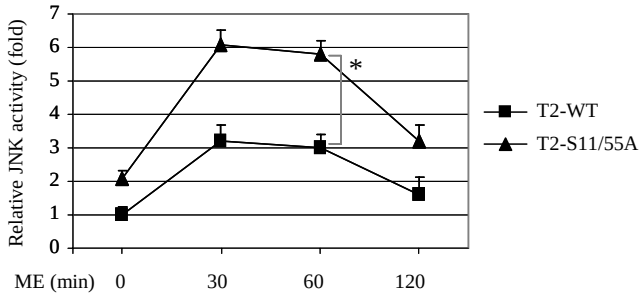

Supplementary Figure 5. Menadione induces strong and prolonged JNK activation in TRAF2-S11/55A-expressing cells.

(A, B) TRAF2/5 DKO cells reconstituted with TRAF2-WT (T2-WT) or -S11/55A (T2-S11/55A) were treated with menadione (ME; 20 μM) as indicated, and IKK (A) and JNK (B) activities were then assessed by kinase assays as in Supplementary Figure 3. Data represent the average IKK and JNK activities from three independent kinase assays. "*" indicates $p<0.05$.

Supplementary Table-1. Primers used for real-time RT-PCR

Gene	5' Primer	3' Primer	PCR amplicon size	Flanking intron?
GAPDH	CAGCAAGGACACTGAGCAAG	GGGTGCAGCGAACTTTATTG	157	Yes
IP-10	TCATCCTGCTGGGTCTGAGT	CCTATGGCCCTCATTCTCAC	106	Yes
RANTES	GTGCCCACGTCAAGGAGTAT	CCACTTCTTCTCTGGGTTGG	110	Yes
ICAM-1	TGGAGACGCAGAGGACCTTA	GTGGGCTTCACACTTCACAG	110	Yes
IκBα	AGACCTGGCCTTCCTCAACT	GCTTTCAGAAGTGCCTCAGC	102	Yes
cFLIP	AGAGCAAGCCCCTAGGAATC	ATGATAGCCCAGGAAGTGA	92	Yes
cIAP2	TCCCCAGAAGATGAGAATGC	TCCAAGGCTTTAACCAC		