**Supplemental Data**

# Murine protein serine/threonine kinase 38 activates apoptosis signal-regulating kinase 1 via Thr$^{838}$ phosphorylation

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## Figure S1

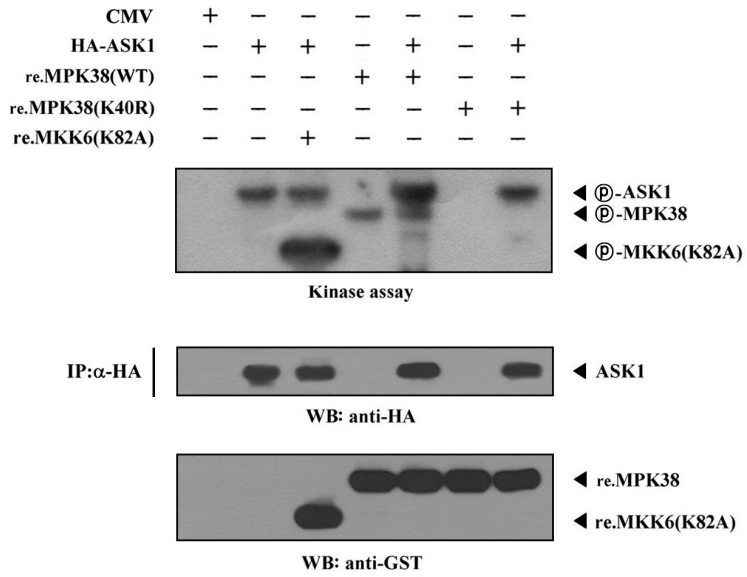
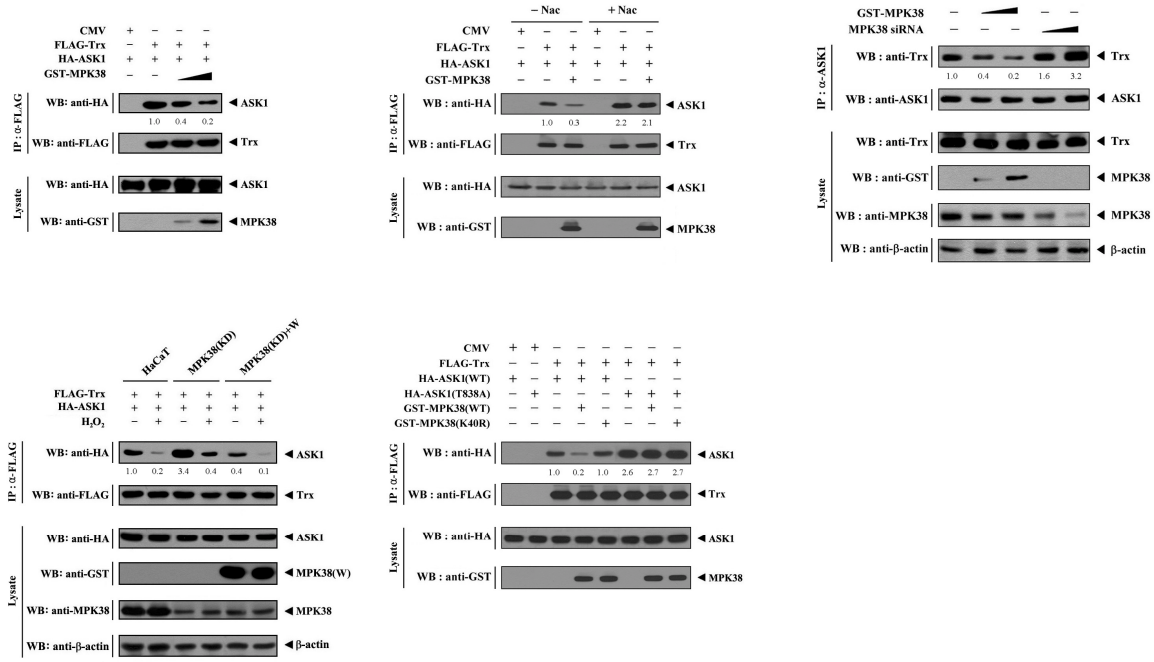



**Fig. S1.** MPK38 is not phosphorylated by ASK1. Approximately 3-4 μg of recombinant wild-type MPK38, MPK38(K40R), and MKK6(K82A) were mixed with 10 μM ATP, 5 μCi of [γ-$^{32}$P]-ATP, and 10 mM $MgCl_2$ in 20 μl of kinase buffer and incubated with the immunoprecipitated ASK1 for 15 min at 37$^{o}$C. The circled P-MPK38, P-ASK1, and MKK6(K82A) indicate the phosphorylated wild-type MPK38, autophosphorylated ASK1, and phosphorylated MKK6(K82A), respectively.

# Figure S2

## A


CMV
FLAG-Trx
HA-ASK1
GST-MPK38
IP : α-FLAG
WB: anti-HA
ASK1
WB: anti-FLAG
Trx
Lysate
WB: anti-GST
MPK38
– Nac
+ Nac
GST-MPK38
MPK38 siRNA
IP : α-ASK1
WB : anti-Trx
WB : anti-ASK1
WB : anti-GST
WB : anti-MPK38
WB : anti-β-actin
β-actin
HaCaT
MPK38(KD)
MPK38(KD)+W
H2O2
HA-ASK1(WT)
HA-ASK1(T838A)
GST-MPK38(WT)
GST-MPK38(K40R)
MPK38(W)

## B

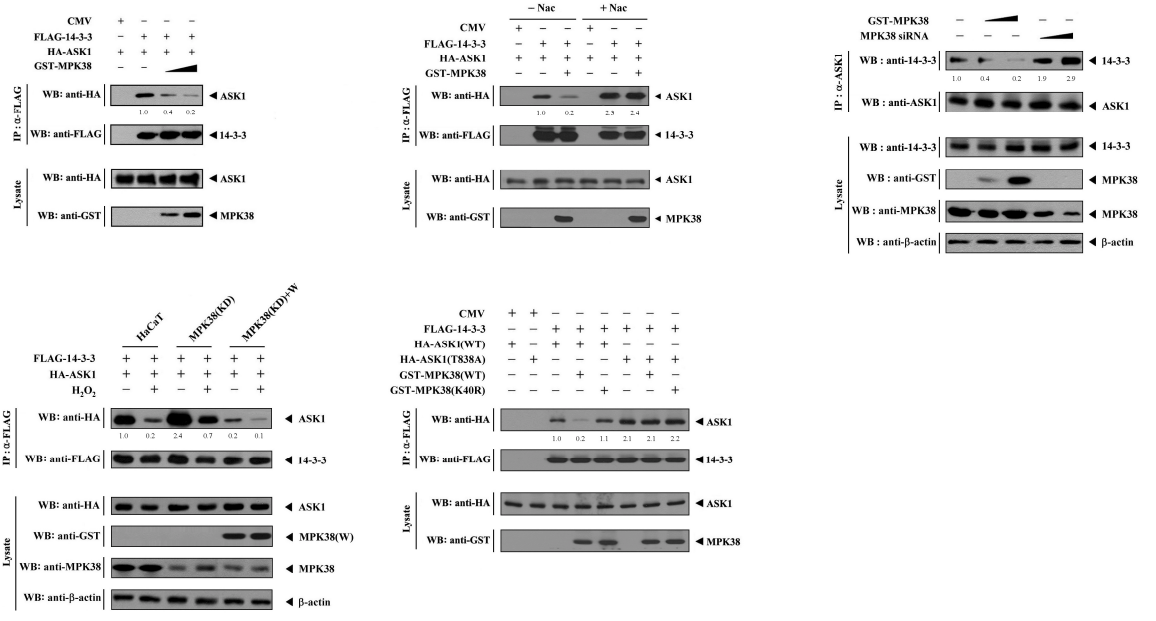

CMV
FLAG-14-3-3
HA-ASK1
GST-MPK38
IP : α-FLAG
WB: anti-HA
ASK1
WB: anti-FLAG
14-3-3
Lysate
WB: anti-GST
MPK38
– Nac
+ Nac
GST-MPK38
MPK38 siRNA
IP : α-ASK1
WB : anti-14-3-3
WB : anti-ASK1
WB : anti-GST
WB : anti-MPK38
WB : anti-β-actin
β-actin
HaCaT
MPK38(KD)
MPK38(KD)+W
H2O2
HA-ASK1(WT)
HA-ASK1(T838A)
GST-MPK38(WT)
GST-MPK38(K40R)
MPK38(W)

**Fig. S2.** MPK38-mediated modulation of the association between ASK1 and Trx or 14-3-3. HEK293 cells were transfected with the indicated combinations of expression plasmids. Cell lysates were then subjected to immunoprecipitation with an anti-FLAG antibody, and the resulting immunoprecipitates were analyzed by immunoblot analysis with an anti-HA antibody to determine the association of Trx (***A***) and 14-3-3 (***B***) with ASK1 in the presence or absence of 15 mM Nac for 30 min. The expression levels of ASK1 and MPK38 in total cell lysates were analyzed by immunoblot using anti-HA and anti-GST antibodies. The effect of MPK38 knockdown on the association between ASK1 and Trx or 14-3-3 was determined by immunoblotting with the indicated antibodies using HEK293 cells transiently transfected with MPK38 siRNA #1 (***A*** and ***B***, *upper right*) or HaCaT cells [*MPK38(KD)*] stably expressing MPK38 shRNA (***A*** and ***B***, *lower left*). MPK38(KD) cells were transfected with 4 μg of plasmid [MPK38(W)] containing a wobble mutant cDNA encoding MPK38 with three synonymous point mutations within the siRNA target sequence (***A*** and ***B***, *lower left*). The relative level of ASK1-Trx or ASK1-14-3-3 complex formation was quantitated by densitometric analyses and fold increase relative to control expressing ASK1 and Trx or MKK3 in the absence of MPK38 was calculated.

**Figure S3**

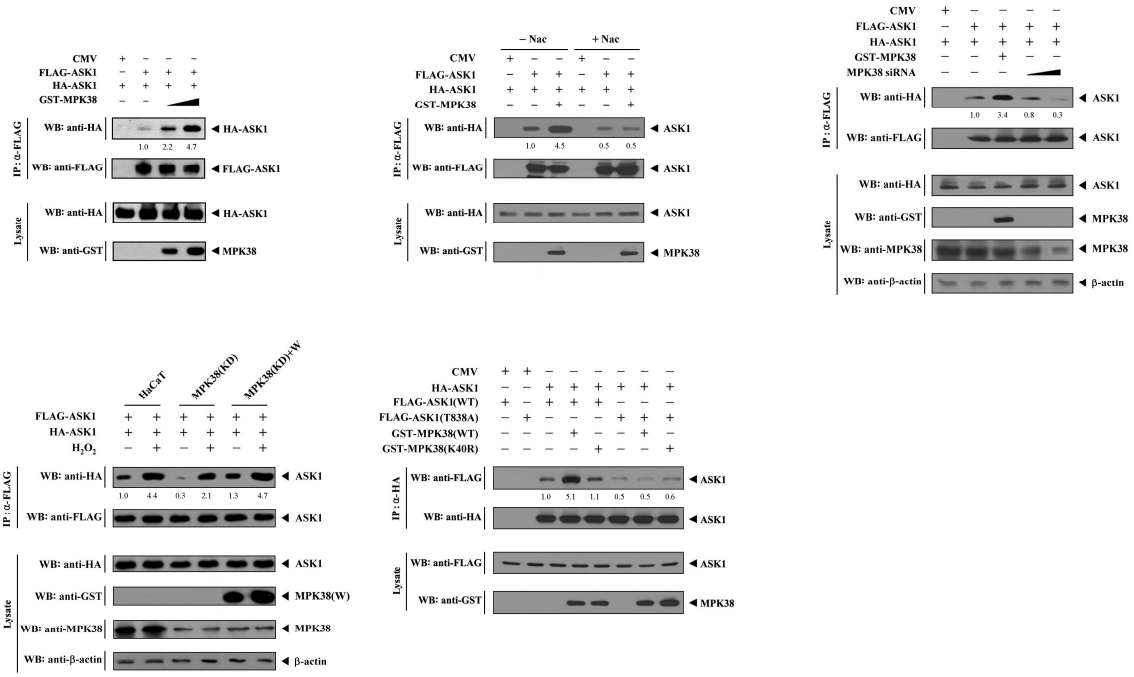



**Fig. S3.** MPK38-mediated modulation of ASK1 homo-oligomerization. HEK293 cells were transfected with the indicated combinations of FLAG-ASK1, FLAG-ASK1(T838A), HA-ASK1, GST-MPK38, and GST-MPK38(K40R). Cell lysates were then subjected to immunoprecipitation with anti-FLAG or anti-HA antibodies, and the resulting immunoprecipitates were analyzed by immunoblot analysis with anti-HA or anti-FLAG antibodies to determine the ASK1 homo-oligomerization in the presence or absence of 15 mM Nac for 30 min. The expression levels of ASK1 and MPK38 in total cell lysates were analyzed by immunoblot using anti-HA, anti-FLAG, and anti-GST antibodies. The effect of MPK38 knockdown on the ASK1 homo-oligomerization was determined by immunoblotting with the indicated antibodies using HEK293 cells transiently transfected with MPK38 siRNA #1 (*upper right*) or HaCaT cells [*MPK38(KD)*] stably expressing MPK38 shRNA (*lower left*). MPK38(KD) cells were transfected with a plasmid (*W*) containing a wobble mutant cDNA encoding MPK38 with three synonymous point mutations within the siRNA target sequence (*lower left*).

**Figure S4**

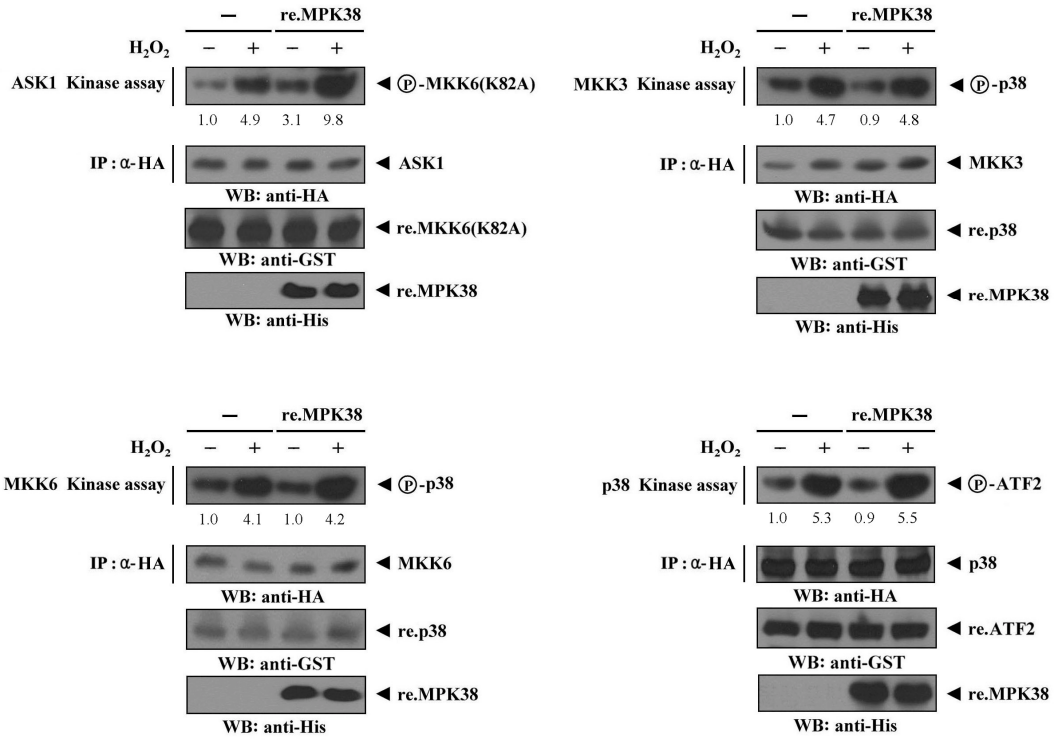
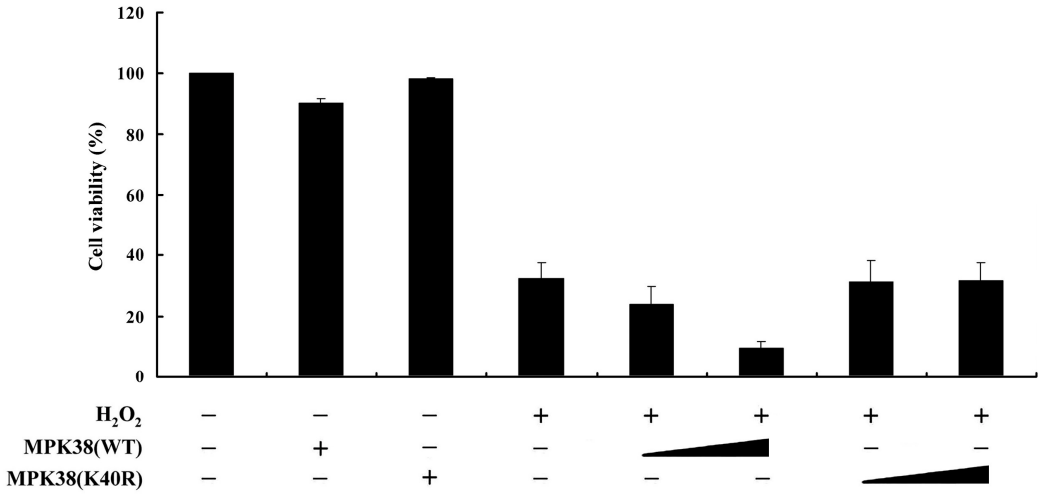



**Fig. S4.** Direct stimulation of ASK1 kinase activity by MPK38. HEK293 cells were transiently transfected with HA-tagged ASK1, MKK3, MKK6, or p38, and incubated in the presence or absence of 2 mM $H_2O_2$ for 30 min. HA immunoprecipitates were assayed for ASK1, MKK3, MKK6, or p38 activities by *in vitro* kinase assays using the indicated substrates (each 5 μg) in the presence or absence of purified recombinant MPK38 (10 μg). These experiments were performed independently at least three times with similar results. re., recombinant.

**Figure S5**

**A**


Cell viability (%)
120
100
80
60
40
20
0
H2O2 – – – + + + + +
MPK38(WT) – + – – – –
MPK38(K40R) – – + – – –

**B**

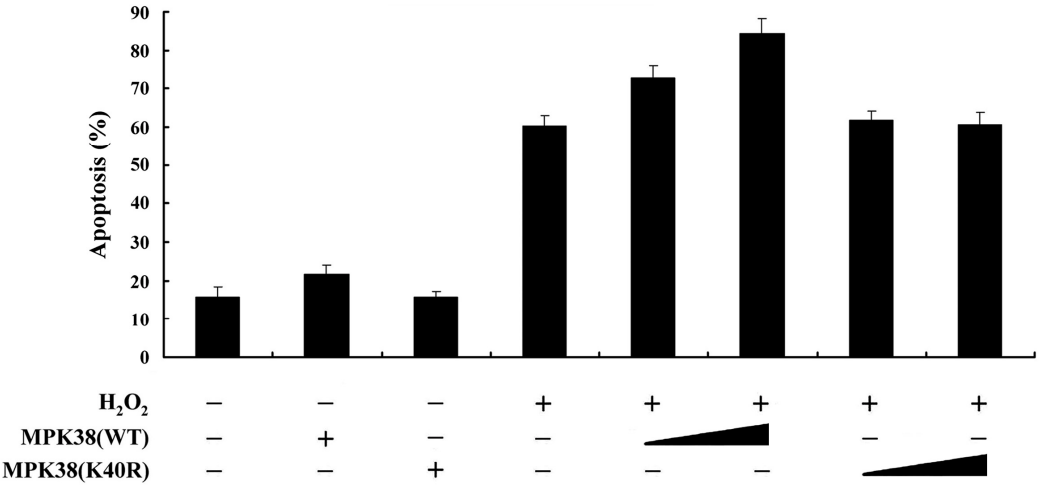
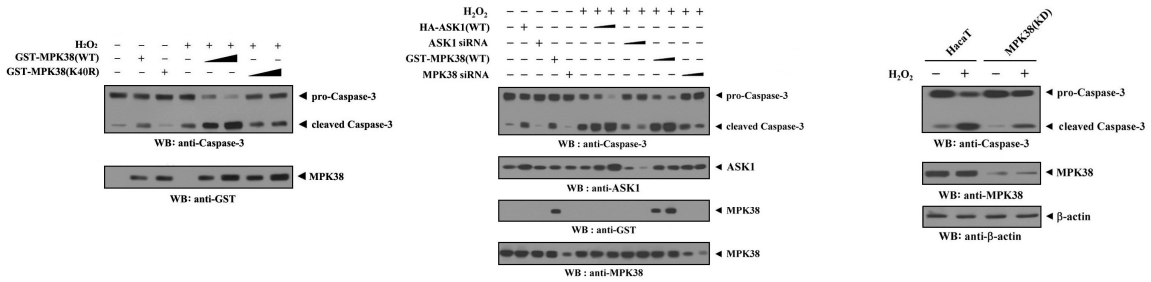

Apoptosis (%)
90
80
70
60
50
40
30
20
10
0
H2O2 – – – + + + + +
MPK38(WT) – + – – – –
MPK38(K40R) – – + – – –

**Fig. S5.** Effect of MPK38 on $H_2O_2$-mediated apoptosis. ***A,*** HEK293 cells were transiently transfected with increasing amounts of wild-type (WT) and kinase-dead (K40R) forms of MPK38 (0.8 and 1.6 μg) as indicated in the presence or absence of $H_2O_2$. Cells were washed with PBS and then incubated for 40 h in DMEM supplemented with 1% FBS. Cell viability was determined by trypan blue exclusion assay. Mock-transfected cells were used as 100% viability. ***B,*** HEK293 cells were transiently transfected with increasing amounts of wild-type (WT) and kinase-dead (K40R) forms of MPK38 (0.8 and 1.6 μg) as indicated in the presence or absence of $H_2O_2$, and apoptotic cell death was assessed by GFP system. Data shown are means (± SE) of three independent experiments. $p < 0.05$ relative to control; significance calculated by Student's t-test.

**Figure S6**

**A**




**B**

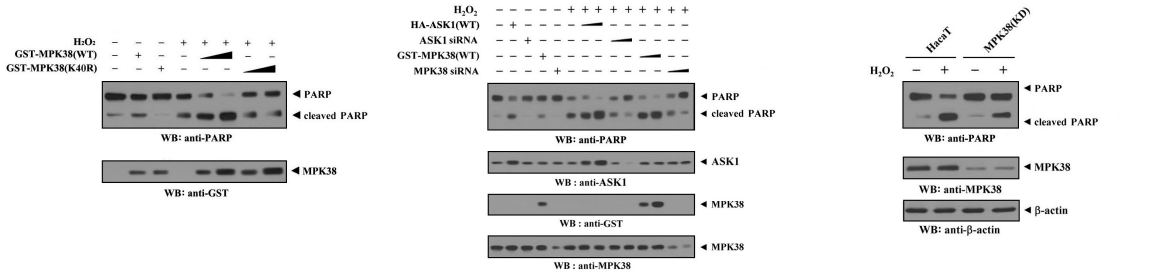



**Fig. S6.** Effect of MPK38 on the degradation of caspase-3 and PARP. ***A,*** Caspase-3 activation by MPK38. HEK293 cells were transiently transfected with increasing amounts of wild-type (WT) and kinase-dead (K40R) forms of MPK38 (0.8 and 1.6 μg), wild-type ASK1 (0.5 and 1 μg), or MPK38 and ASK1 siRNAs (100 and 200 nM) in the presence or absence of $H_2O_2$. Cell lysates were then analyzed by immunoblot analysis with an anti-caspase-3 antibody to determine the degradation of caspase-3, and the expression levels of ASK1 and MPK38 were determined by anti-ASK1 and anti-GST immunoblotting, respectively. HaCaT cells stably expressing MPK38 shRNA [*MPK38(KD)*] were incubated with 2 mM $H_2O_2$ for 30 min. Cells were lysed and then subjected to immunoblot analysis using the indicated antibodies (*right*). ***B,*** Stimulation of PARP degradation by MPK38. HEK293 cells transfected with the indicated expression vectors or siRNAs for MPK38 and ASK1 were lysed and subjected to immunoblot analysis using the indicated antibodies (*left* and *middle*). PARP degradation was also measured

by immunoblotting with an anti-PARP antibody in HaCaT cells stably expressing MPK38 shRNA [*MPK38(KD)*] treated with or without $H_2O_2$ (right). The data are representative of at least three independent experiments.