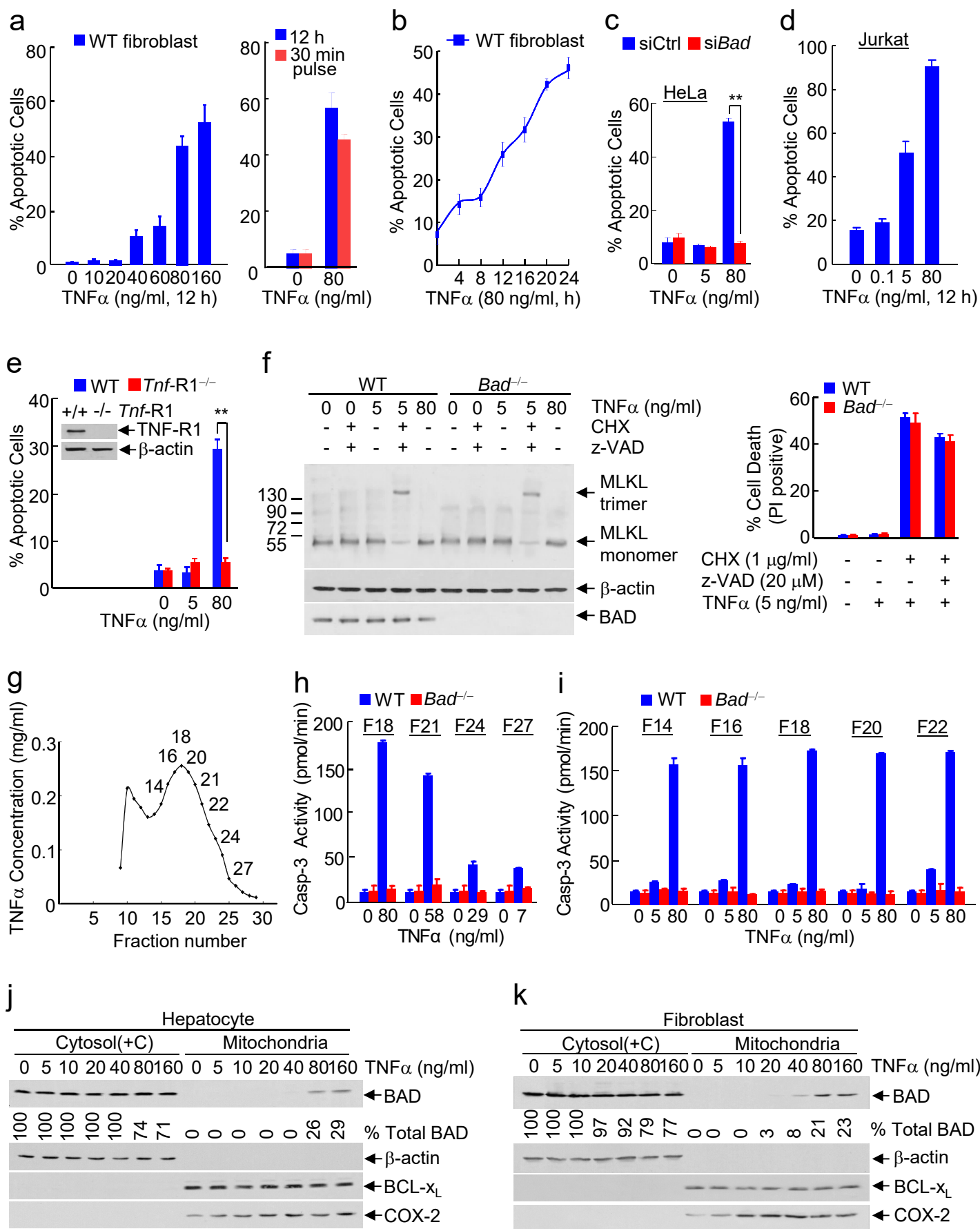
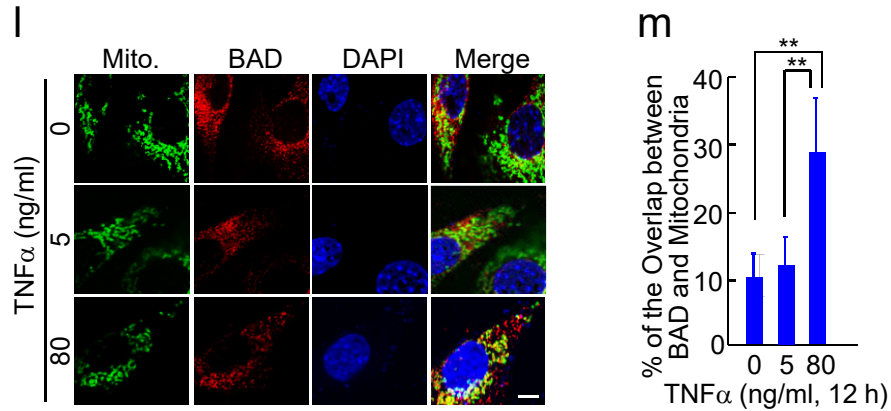
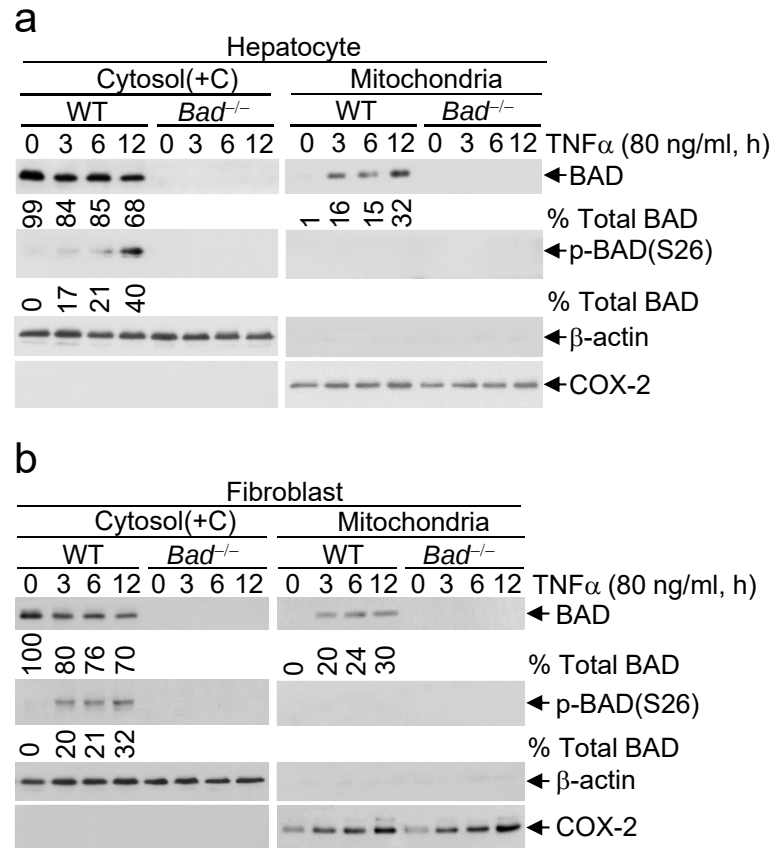




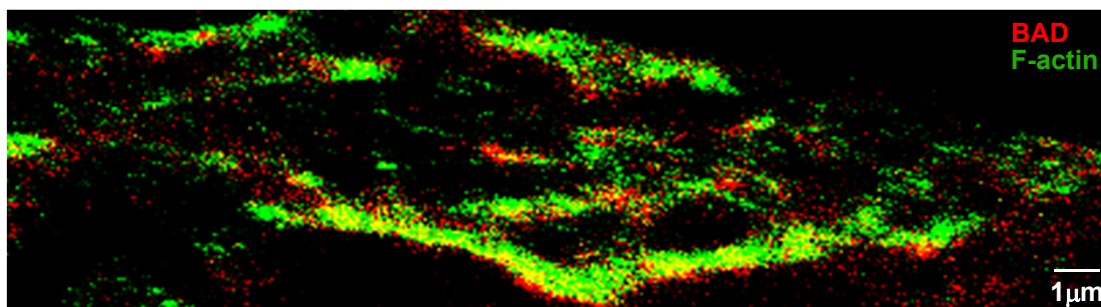


**Supplementary Information, Figure S1 TNF $\alpha$  induces apoptosis, but not necroptosis, and BAD mitochondrial translocation in a dose-dependent and time-dependent manner. (a-b)** Immortalized fibroblasts were treated without or with various doses of TNF $\alpha$  for 12 h (**a, Left panel**) or treated with cytotoxic dose TNF $\alpha$  (80 ng/ml) for 30 min and then replaced with fresh medium containing no TNF $\alpha$  for 12 h (**a, Right panel**), or treated with cytotoxic dose (80 ng/ml) TNF $\alpha$  for various times (**b**), as indicated. Apoptotic cells were determined by Annexin V/Propidium iodide (PI) staining and flow cytometric analysis. (**c-d**) HeLa cells (**c**) transfected with siBad or the control scramble siRNA, or Jurkat cells (**d**) were treated without or with various doses of TNF $\alpha$  for 12 h, as indicated. Apoptotic cell death was detected as described in (**a**). (**e**) WT and *Tnf-R1*<sup>-/-</sup> fibroblasts were treated without or with non-cytotoxic or cytotoxic dose TNF $\alpha$  for 12 h, as indicated. Apoptotic cell death was detected as described in (**a**). (**f**) WT and *Bad*<sup>-/-</sup> fibroblasts were treated without or with non-cytotoxic (5 ng/ml) in the presence of CHX (1  $\mu$ g/ml) and pan-caspase inhibitor z-VAD-FMK (20  $\mu$ M) or cytotoxic dose (80 ng/ml) TNF $\alpha$  for 6 h (MLKL Trimerization) or 12 h (Cell death assay), as indicated. MLKL trimerization was detected by non-reducing gel (SDS-PAGE without  $\beta$ -ME and DTT) and analyzed by immunoblotting of MLKL, BAD, and  $\beta$ -actin using corresponding antibodies (**Left panel**). Cell death was determined by PI staining and flow cytometric analysis (**Right panel**). (**g-i**) Different fractions (F, fraction; F14, Fraction number 14 and etc.) of the TNF $\alpha$  preparation (R&D) (**g**) were used to treat WT and *Bad*<sup>-/-</sup> immortalized fibroblasts, as indicated, and caspase-3 activity was detected (**h-i**). The concentration of TNF $\alpha$  in different fractions was shown in Table S3. (**j-k**) Primary hepatocytes (**j**) or immortalized fibroblasts (**k**) were treated without or with various doses of TNF $\alpha$  for 4 h, as indicated. BAD mitochondrial translocation was determined by cytosol [containing cytoskeleton, Cytosol(+C)] and mitochondria fractionation (see “Materials and Methods” for details), followed by immunoblotting with corresponding antibodies, as indicated. (**l-m**) WT fibroblasts were treated without or with non-cytotoxic dose or cytotoxic dose TNF $\alpha$  for 12 h and the co-localization of BAD with mitochondria was analyzed by immunofluorescence staining using anti-BAD antibody, MitoTracker and DAPI (4',6-diamidino-2-phenylindole). Images were taken with confocal microscopy. Scale bar, 5  $\mu$ m (**l**). Co-localization of BAD with mitochondria in (**l**) was quantitated by the ImageJ program in six random fields and determined by Pearson's correlation coefficient (**m**). Data in (**a-f, h-i and m**) are means  $\pm$  s.d. \*\*,  $P < 0.01$ , as analyzed by two-tailed unpaired Student's *t* test. All data represent two to three individual experiments with similar results.

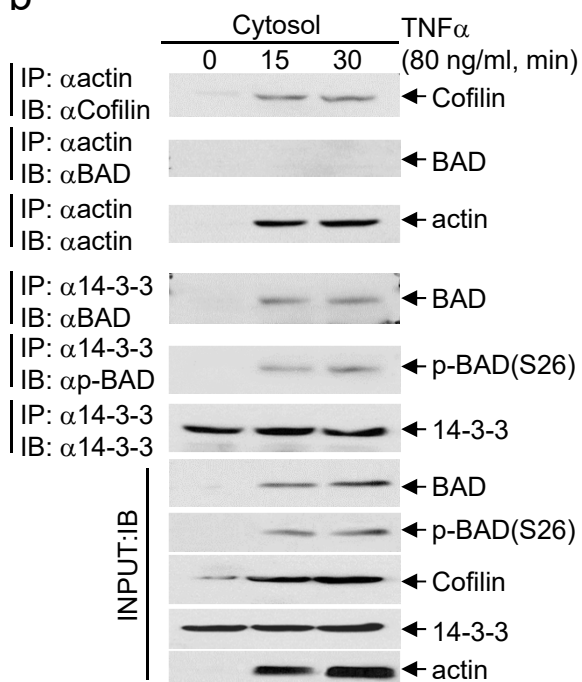


**Supplementary Information, Figure S2 Only a fraction of cytosolic BAD is phosphorylated in response to cytotoxic dose TNF $\alpha$  and the rest non-phosphorylated BAD translocates to mitochondria.** Primary hepatocytes (**a**) and immortalized fibroblasts (**b**) were treated without or with cytotoxic dose TNF $\alpha$  for various times, as indicated. BAD phosphorylation by IKK and BAD mitochondrial translocation were determined by cytosol [containing the cytoskeleton, Cytosol(+C)] and mitochondria fractionation (see "Materials and Methods" for details) and immunoblotting. The percentage of IKK-phosphorylated BAD in total cytoplasmic BAD protein was determined as described in (**Figure 1f, Supplementary information, S9t and S9u**). All data represent two to three individual experiments with similar results.

a



b



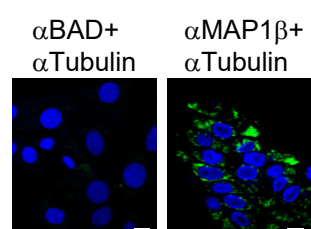
c



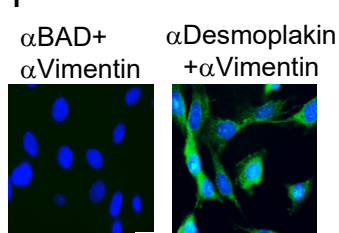
d



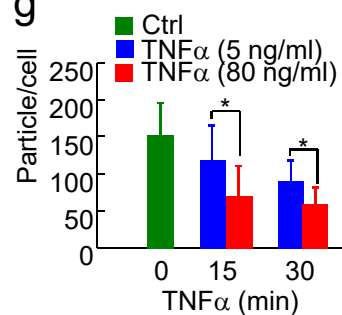
e



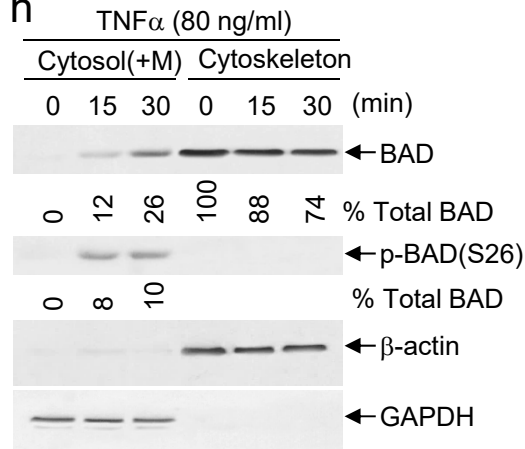
f



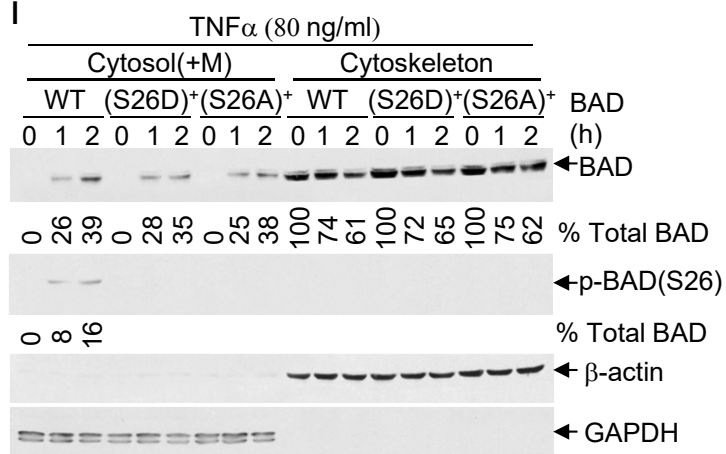
g



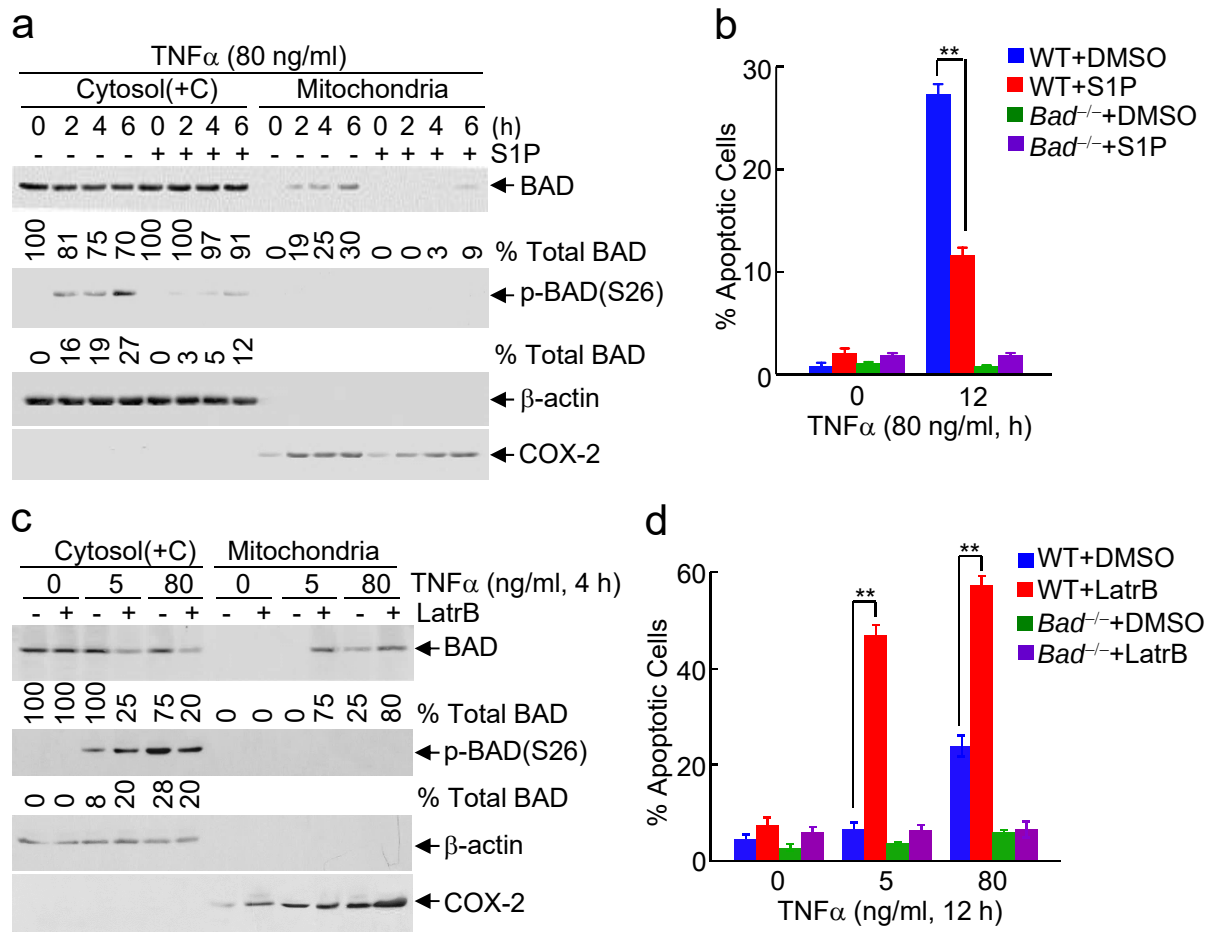
h



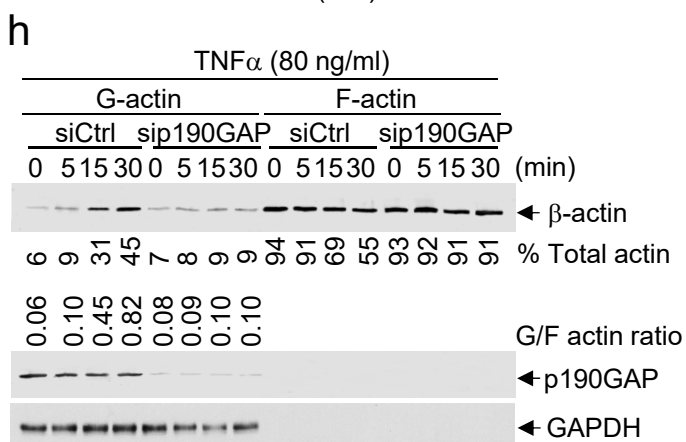
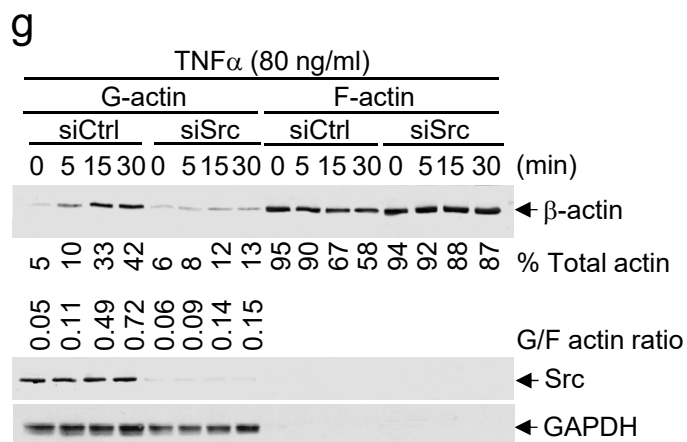
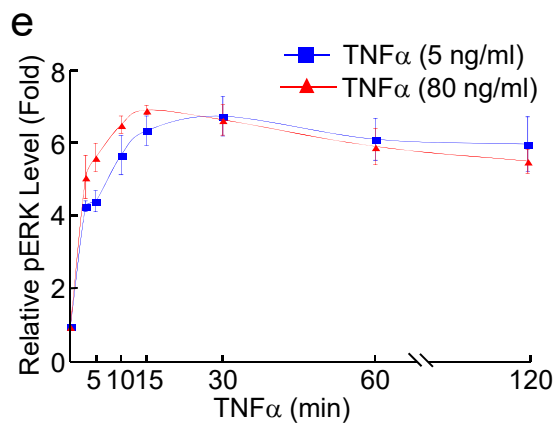
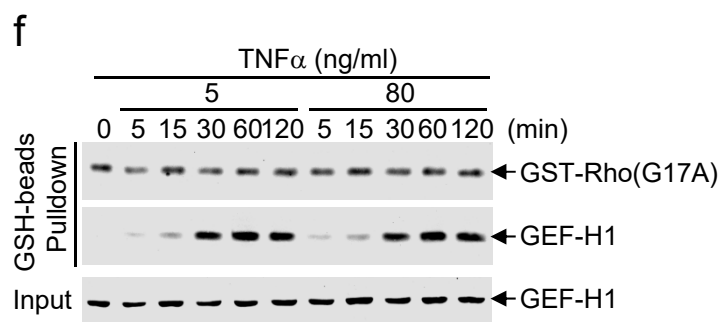
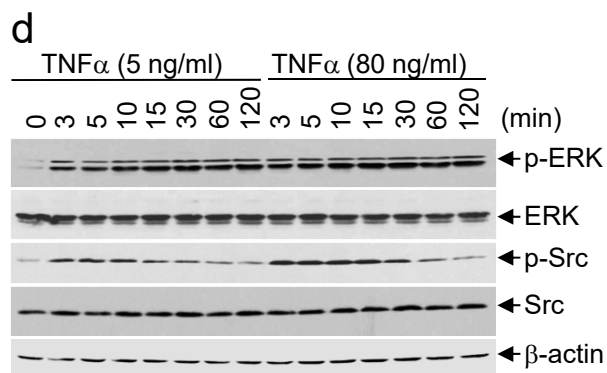
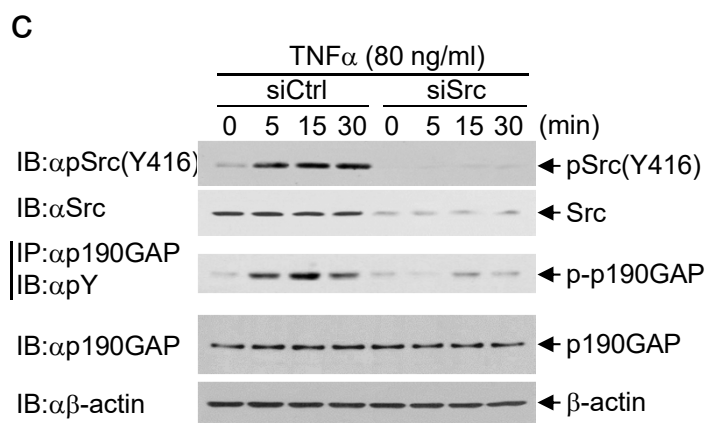
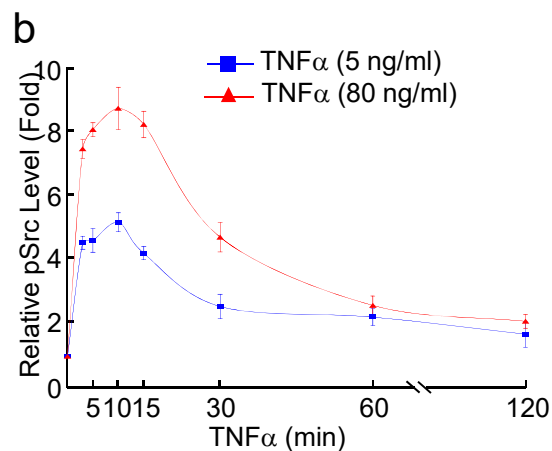
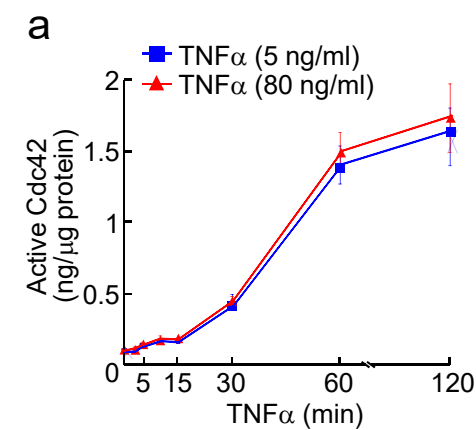
i



**Supplementary Information, Figure S3 BAD directly interacts with F-actin in actin stress fibers at the cytoskeleton.** (a) WT fibroblasts were analyzed by double immunofluorescence staining with anti-BAD antibody (red) and phalloidin (actin filament marker; green), and visualized with super-resolution microscopy based on Ground State Depletion program. Scale bar, 1  $\mu$ m. (b) WT fibroblasts were treated without or with cytotoxic dose TNF $\alpha$  for various times, as indicated. The cytosol fractions were separated from the cytoskeleton fractions by ultracentrifugation (see “Materials and Methods” for details). Potential protein-protein association between G-actin and BAD in the cytosol was analyzed by immunoprecipitation with anti-actin antibody, followed by immunoblotting with anti-BAD antibody. The associations between G-actin and Cofilin and 14-3-3 with phosphorylated BAD were used as positive controls. IKK-phosphorylation of BAD, expression levels of BAD, Cofilin, G-actin and 14-3-3 were determined by immunoblotting with corresponding antibodies (Input). (c) Purified G-actin monomers were incubated with purified GST, GST-Cofilin or GST-BAD protein separately *in vitro*. An aliquot of the mixture was subjected to ultracentrifugation to detect the interaction between GST-fusion proteins and F-actin in actin filaments. In parallel, another aliquot of the mixture was used in GST pulldown assay to detect the interaction between GST-fusion proteins and G-actin monomers. GST-fusion proteins and actin were detected by immunoblotting with anti-GST and anti-actin antibodies, respectively. A third aliquot of the mixture was used as Input. (d-f) Negative controls (d) and positive controls (e-f) for proximity ligation assay (Figure 3b). WT fibroblasts were stained with anti-BAD antibody only, anti- $\beta$ -actin antibody only, and no primary antibodies (d), or anti-MAP1 $\beta$  and anti-Tubulin (e), anti-Desmoplakin and anti-Vimentin (f) instead, as indicated. Nuclei were detected by DAPI. Scale bar, 5  $\mu$ m. (g) Quantitation of co-localization of BAD with actin by proximity ligation assay (Figure 3b) with the ImageJ program in eight different fields. Data are means  $\pm$  s.d. \*,  $P < 0.05$ , as analyzed by two-tailed unpaired Student's *t* test. (h) WT fibroblasts were treated without or with cytotoxic dose TNF $\alpha$  for various times, as indicated, followed by the cytoskeleton fractionation (see “Materials and Methods” for details). The percentage of IKK-phosphorylated BAD in total cytoplasmic BAD protein was determined as described (Figure 1f) (Supplementary information, Figure S9v). (i) Bad-deficient fibroblasts were transfected with WT Bad, Bad(S26D) or Bad(S26A) mutant, followed by treatment without or with 80 ng/ml TNF $\alpha$  for various times, as indicated. Subcellular localizations of BAD and IKK-phosphorylated BAD were analyzed and quantitated as described in (h) (Supplementary information, Figure S9w). All data represent two to three individual experiments with similar results.

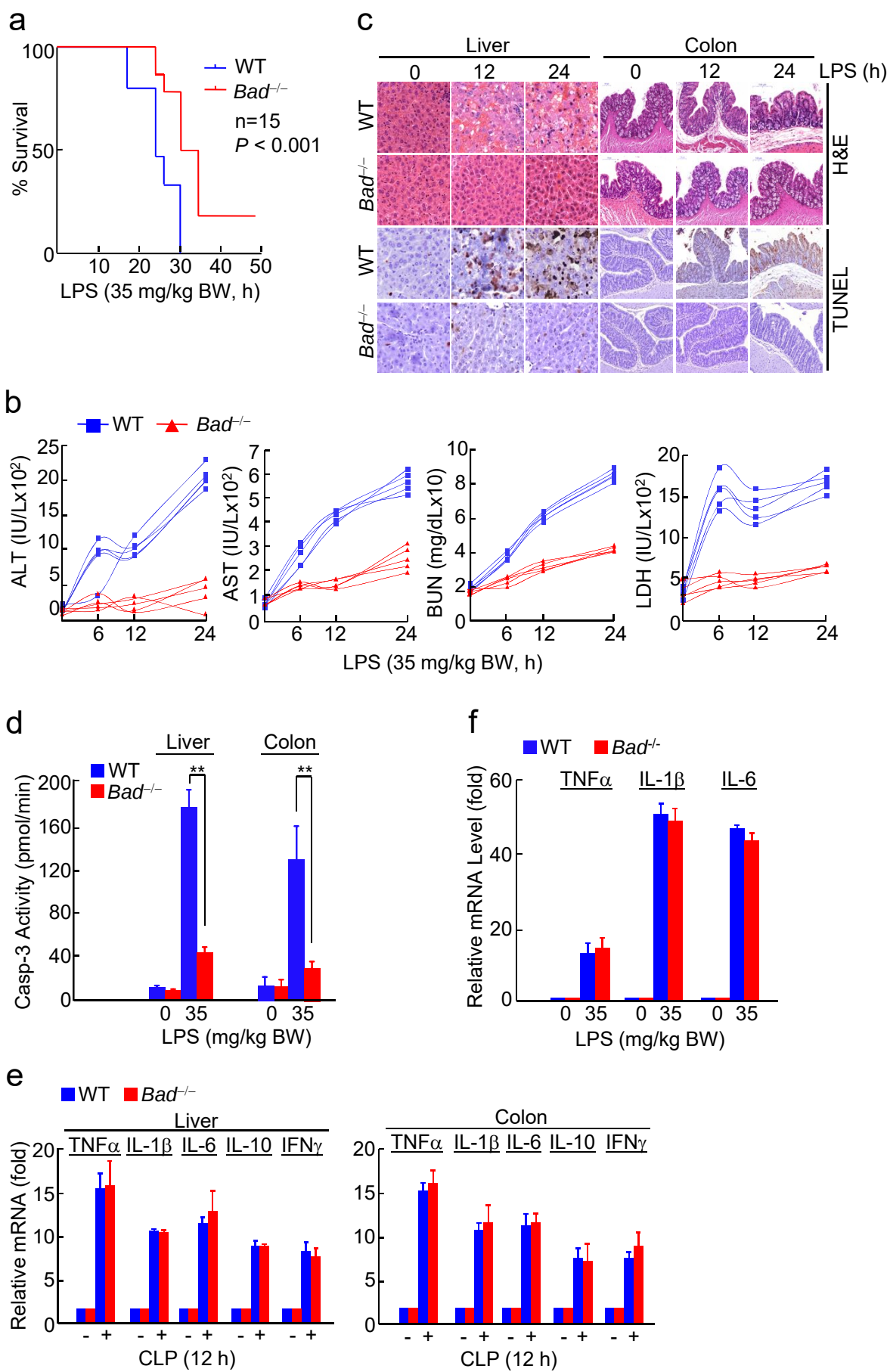


**Supplementary Information, Figure S4 The ability of TNF $\alpha$  to induce apoptosis is regulated by actin stress fiber polymerization.** WT and *Bad*<sup>-/-</sup> fibroblasts were pretreated without or with 5  $\mu$ M Sphingosine-1-phosphate (S1P) (**a-b**), 2.5  $\mu$ g/ml Latrunculin B (**c-d**) or DMSO (control) for 1 h, followed by stimulation without or with non-cytotoxic or cytotoxic dose TNF $\alpha$  for various times, as indicated. BAD phosphorylation and BAD mitochondrial translocation were determined by cytosol [containing cytoskeleton, Cytoso(+C)] and mitochondria fractionation (see “Materials and Methods” for details) and immunoblotting (**a**, **c**). The percentage of IKK-phosphorylated BAD in total cytoplasmic BAD protein (**a**, **c**) was determined, as described in (**Figure 1f and Supplementary Information, Figure S9x-S9y**). Apoptotic cells were determined as described in (**Figure 1a**) and present as means  $\pm$  s.d. \*\*,  $P < 0.01$ , as analyzed by two-tailed unpaired Student’s  $t$  test (**b**, **d**). All data represent two to three individual experiments with similar results.

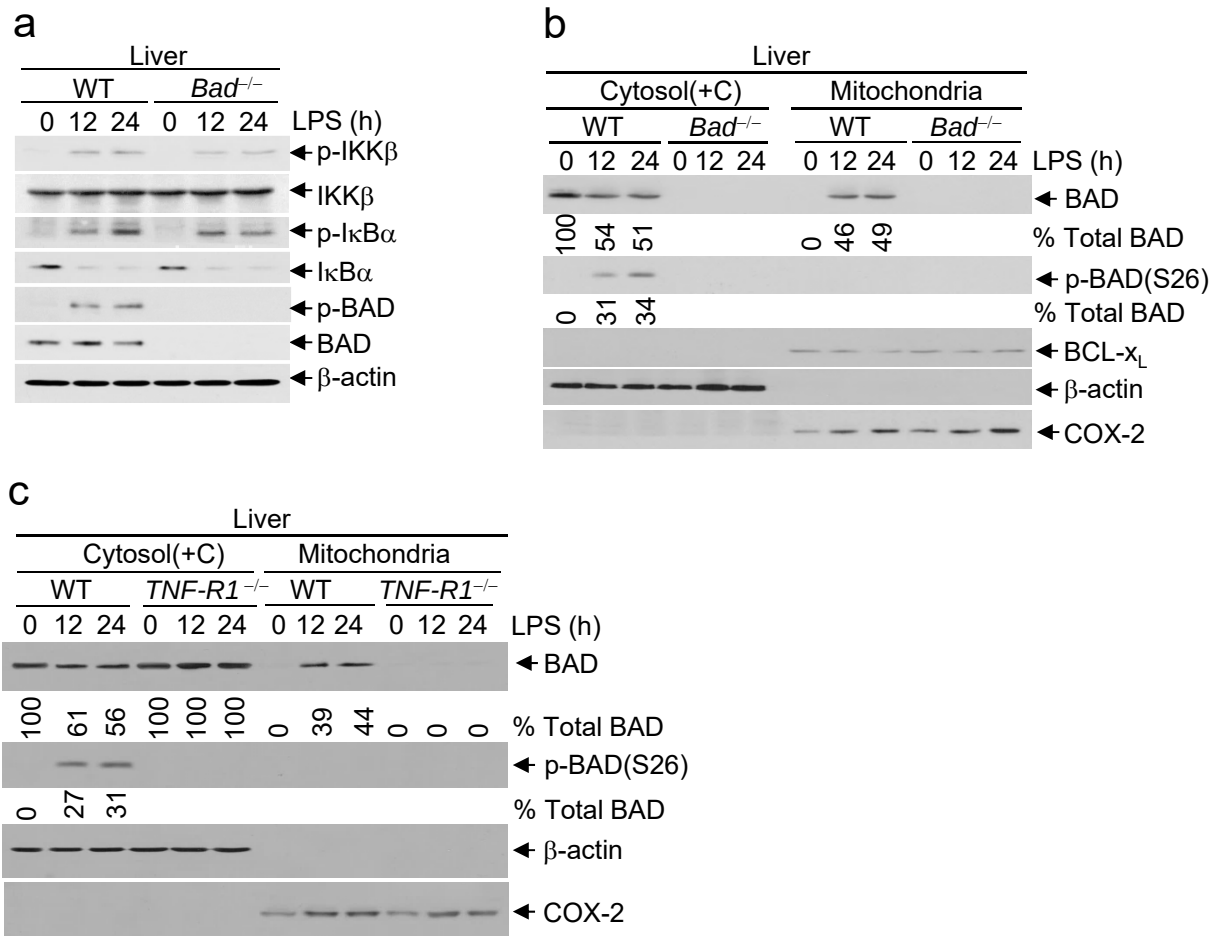


**Supplementary Information, Figure 5 Cytotoxic dose TNF $\alpha$  induces massive depolymerization of actin stress fibers through Src-p190GAP-mediated inactivation of RhoA.**

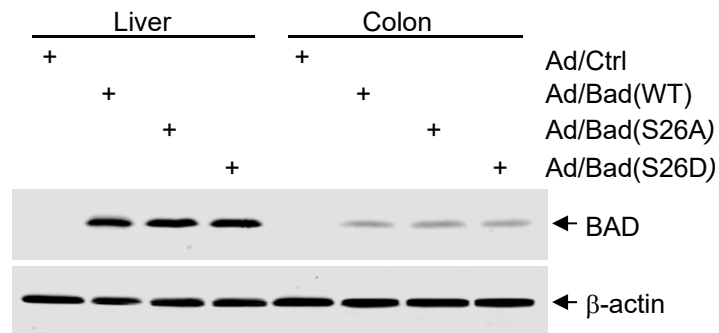
**(a)** Cytotoxic and non-cytotoxic dose TNF $\alpha$  induced similar level of Cdc 42 activation, as measured by the G-LISA Cdc42 activation assay. Data are means  $\pm$  s.d. **(b-e)** WT fibroblasts were treated without or with non-cytotoxic or cytotoxic dose TNF $\alpha$  for various times as indicated. Phospho-Src, phospho-ERK. Expression levels of Src, ERK and  $\beta$ -actin were determined by immunoblotting **(c-d)** and the fold of phosphorylation of Src and ERK were quantitatively analyzed by ImageJ program and presented as means  $\pm$  s.d. **(b, e)**. Phosphorylated p190GAP was detected by immunoprecipitation with anti-p190GAP antibody and immunoblotting with anti-phosphor-Tyrosine ( $\alpha$ pY) antibody **(c)**. **(f)** Active GEF-H1 was detected (see “Materials and Methods” for details). Expression level of GEF-H1 and the input GST-Rho(G17A) were detected with immunoblotting. **(g-h)** WT fibroblasts were transfected with scramble siRNA (siCtrl) **(g-h)**, siSrc **(g)** or sip190GAP **(h)**, and treated without or with cytotoxic dose TNF $\alpha$  for various times, as indicated. G-actin and F-actin were detected by fractionation and immunoblotting, and quantitated by the ImageJ program. Expressing level of Src **(g)** and p190GAP **(h)** was detected with corresponding antibodies, respectively. All data represent two to three individual experiments with similar results.



**Supplementary Information, Figure S6 Loss of Bad protects mice from septic shock-induced tissue damage of multiorgans and mortality.** (a) WT littermates and *Bad*<sup>-/-</sup> mice were intraperitoneally (i.p.) injected with lethal dose LPS (35 mg/kg body weight) and the mortality rate was determined.  $P < 0.001$ ;  $n = 15$ , as analyzed by log rank (Mantel-Cox) test. (b-d) WT littermates and *Bad*<sup>-/-</sup> mice were intraperitoneally (i.p.) injected with lethal dose LPS (35 mg/kg body weight), respectively. Blood samples from the tail vein were collected at various times as indicated and the serum was used for ALT, AST, BUN and LDH determination (b). Liver and colon tissues from pre-removed or at indicated times after injections were analyzed by H&E staining and TUNEL staining (c). Relative Caspase 3 activity in liver and colon samples were analyzed. \*\*,  $P < 0.01$ , as determined by two-tailed unpaired Student's *t* test (d). (e) WT littermates and *Bad*<sup>-/-</sup> mice were subjected to high-grade CLP surgery. Expressions of TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-10 and IFN $\gamma$  in liver and colon were quantitated by real-time PCR (see "Materials and Methods" for details). (f) *Bad*<sup>-/-</sup> mice and WT littermate intraperitoneally (i.p.) injected with lethal dose LPS (35 mg/kg body weight). Expressions of TNF $\alpha$ , IL-1 $\beta$  and IL-6 in liver were quantitated by real-time PCR. Data are means  $\pm$  s.d. and represent three individual experiments. All results represent three individual experiments with similar results.

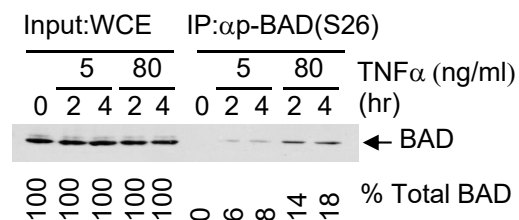


**Supplementary Information, Figure S7 LPS utilizes the same mechanism as cytotoxic dose TNFα to stimulate BAD pro-apoptotic activity.** *Bad*<sup>-/-</sup> mice and WT littermate were i.p injected with lethal dose LPS (35 mg/kg body weight). Liver tissues were extracted at different time as indicated to either analyze IKK and NF-κB activation (**a**), or further separated into mitochondrial and cytosol [contain cytoskeleton, Cytosol(+C)] fractions to analyze BAD phosphorylation and mitochondrial translocation (see “Materials and Methods” for details) (**b**) (**Supplementary information, Figure S9z**). (**c**) *Tnf-R1*<sup>-/-</sup> mice and WT littermate were i.p injected with lethal dose LPS (35 mg/kg body weight). Liver tissues were extracted at different time as indicated and separated into the mitochondrial and cytosol [contain cytoskeleton, Cytosol(+C)] fractions (see “Materials and Methods” for details) to analyze BAD phosphorylation and mitochondrial translocation (**Supplementary information, Figure S9aa**). All results represent three individual experiments with similar results.

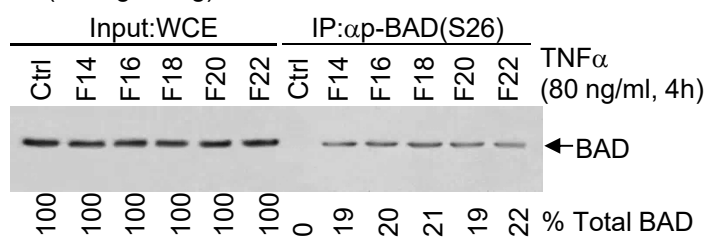


**Supplementary Information, Figure S8** Expression of adenoviral vector encoding various BAD proteins (WT, S26A or S26D mutant). *Bad*<sup>-/-</sup> mice were intravenously injected with Ad/WT, Ad/S26A, Ad/S26D BAD, or Ad/Ctrl(LacZ) respectively for 48 h. Expression level of various BAD proteins in liver and colon were detected by immunoblotting using anti-BAD antibody.

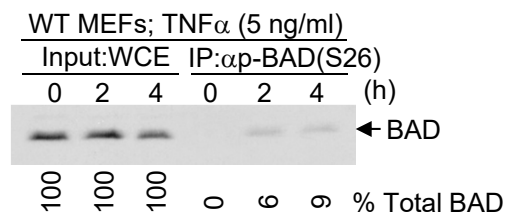
**a** (for Figure 1f)



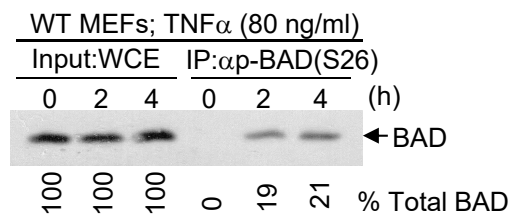
**b** (for Figure 1g)



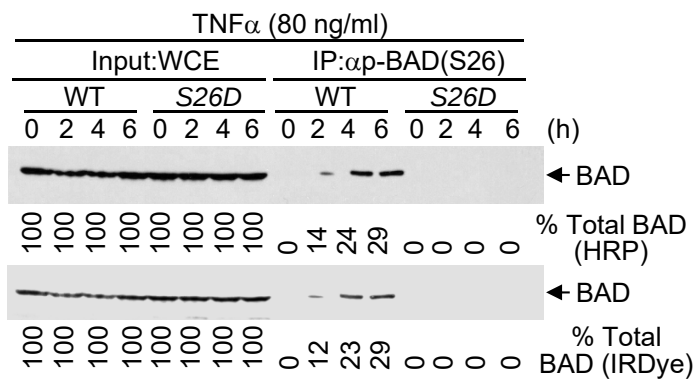
**c** (for Figure 2a)



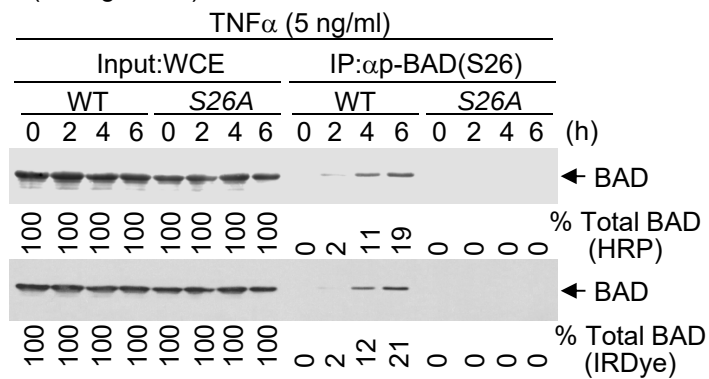
**d** (for Figure 2b)



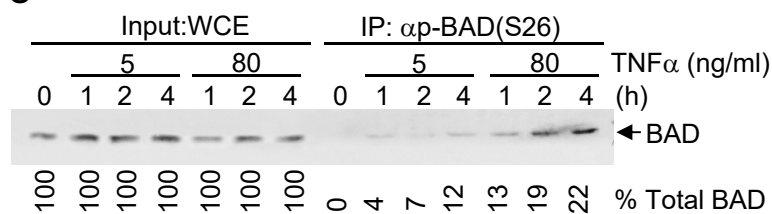
**e** (for Figure 2c)



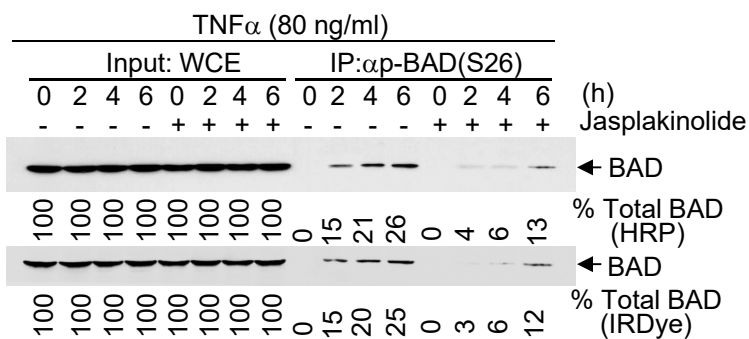
**f** (for Figure 2d)



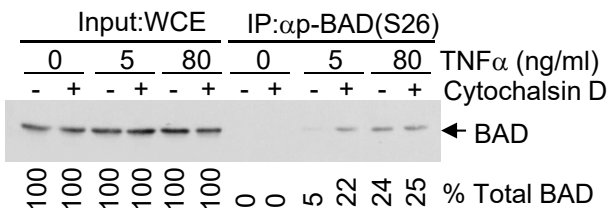
**g** (for Figure 3c)

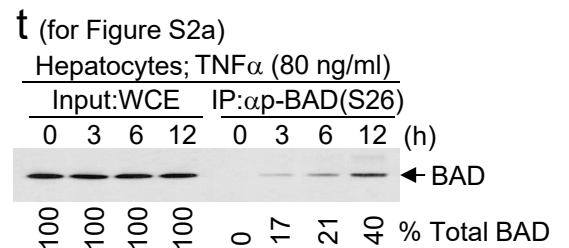
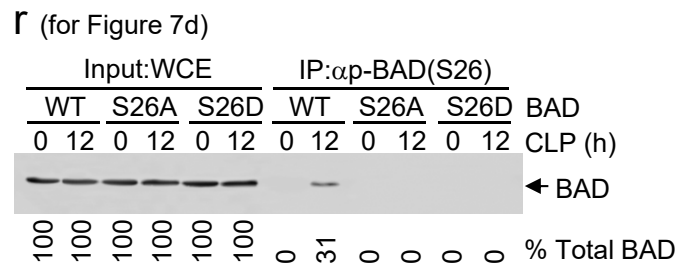
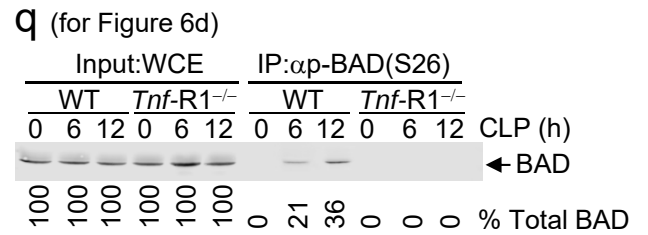
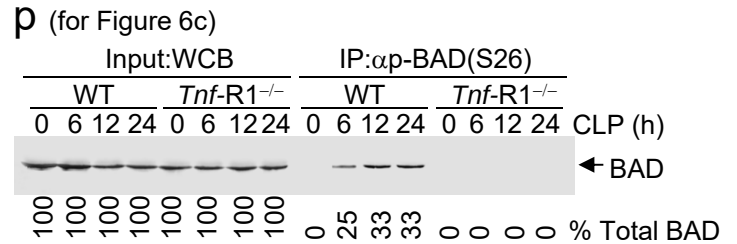
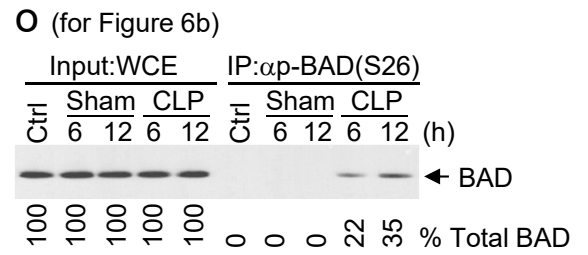
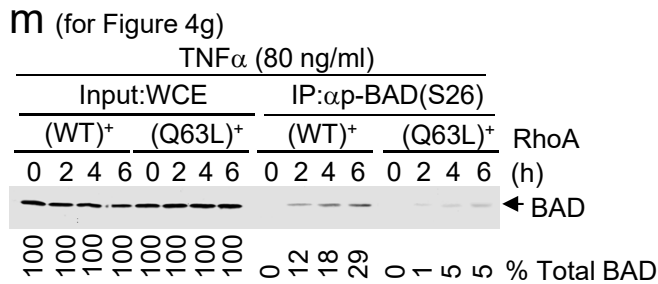
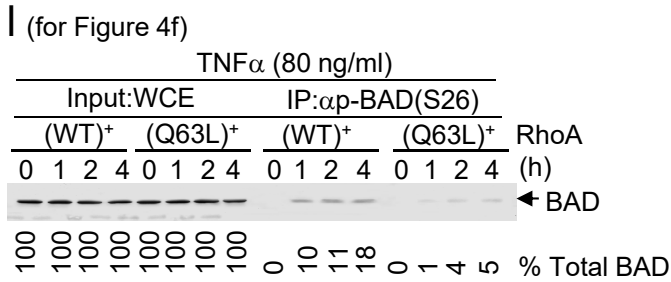
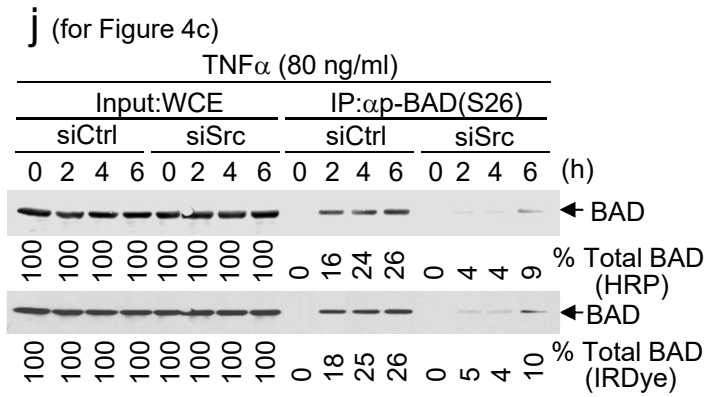


**h** (for Figure 3f)

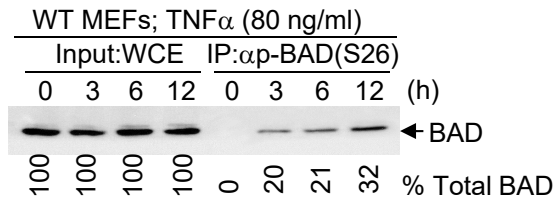


**i** (for Figure 3h)

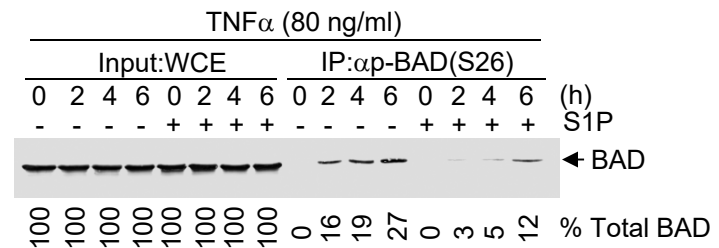




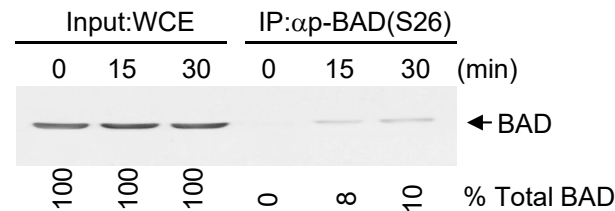
**U** (for Figure S2b)



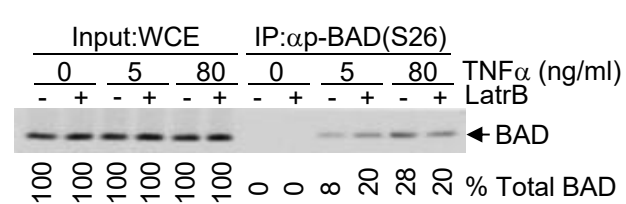
**X** (for Figure S4a)



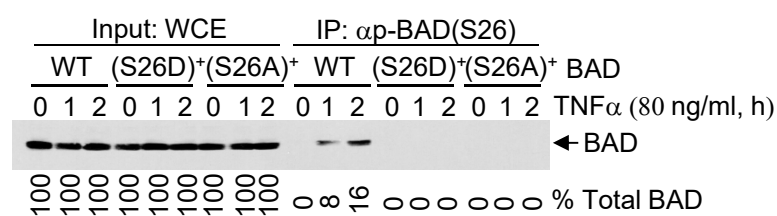
**V** (for Figure S3h)



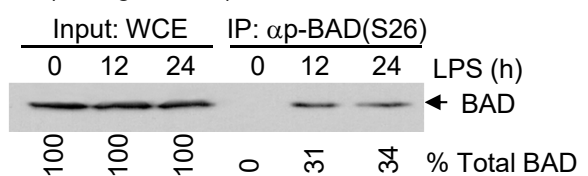
**Y** (for Figure S4c)



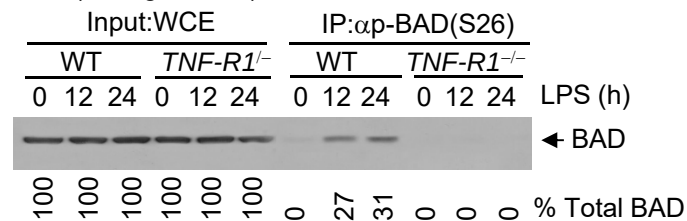
**W** (for Figure S3i)



**Z** (for Figure S7b)



**aa** (for Figure S7c)



### Supplementary Information, Figure S9 Quantitation of phosphorylated BAD proteins.

**(a-aa)** Cells were treated the same way as indicated in their corresponding figures. The percentage of IKK-phosphorylated BAD in total cytoplasmic BAD was determined by immunoprecipitation of Ser26-phosphorylated BAD with anti-Ser26 antibody in combination with immunoblotting with anti-BAD antibody, and quantitated by the ImageJ program and/or IRDye fluorescence analyzed by Odyssey Imager. BAD in the total cell extracts were calculated as 100%.

## Supplementary Information, Table S1. MS/MS analysis of the TNF $\alpha$ preparation

Matched peptides shown in red. TNF $\alpha$  protein sequence coverage: 55%

1 MSTESMIRDV ELAEEALPQK MGGFQNSRRC LCLSLFSLL VAGATTLFCL  
 51 LNFGVIGPQR DEKFPNGLPL ISSMAQTLTL **RSSSQNSSDK PVAHVVANHQ**  
 101 **VEEQLEWLSQ RANALLANGM DLKDNQLVVP ADGLYLVYSQ VLFKGQGPCD**  
 151 **YVLLTHTVSR FAISYQEKVN LLSAVKSPCP KDTPEGAELK PWYEPIYLG**  
 201 **VFQLEKGDQL SAEVNLPKYL DFAESGQVYF GVIAL**

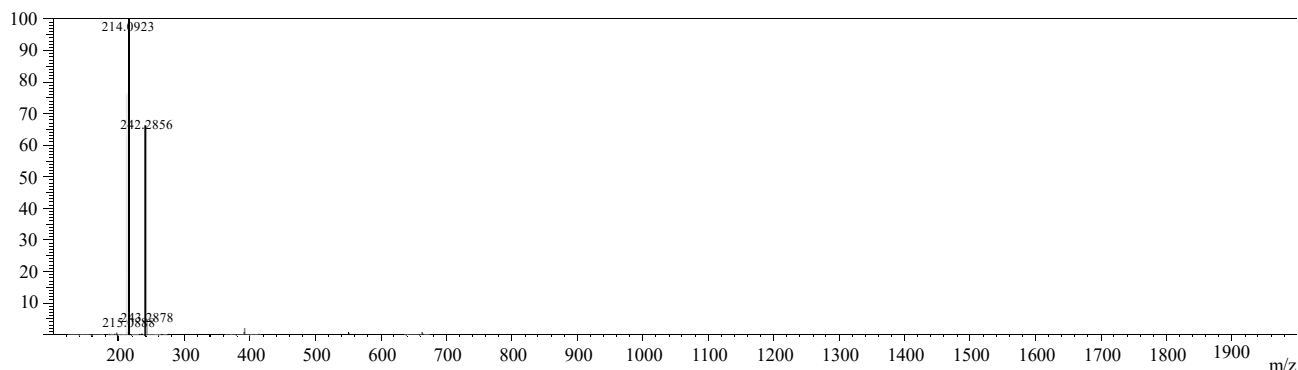
| Start |   | End | Observed | Mr(expt) | Mr(calc)  | ppm    | score | Peptide                                                       |
|-------|---|-----|----------|----------|-----------|--------|-------|---------------------------------------------------------------|
| 82    | – | 111 | 848.1648 | 3388.63  | 3388.6396 | -2.8   | 48    | R.SSSQNSSDKPVAHVVANHQV<br>EEQLEWLSQR.A                        |
| 82    | – | 111 | 678.7339 | 3388.633 | 3388.6396 | -1.91  | 12    | R.SSSQNSSDKPVAHVVANHQV<br>EEQLEWLSQR.A                        |
| 82    | – | 111 | 848.1667 | 3388.638 | 3388.6396 | - 0.56 | 51    | R.SSSQNSSDKPVAHVVANHQV<br>EEQLEWLSQR.A                        |
| 82    | – | 111 | 1130.885 | 3389.633 | 3389.6236 | 2.74   | 55    | R.SSSQNSSDKPVAHVVANHQV<br>EEQLEWLSQR.A +<br>Deamidated (NQ)   |
| 82    | – | 111 | 848.6676 | 3390.641 | 3390.6076 | 9.94   | 47    | R.SSSQNSSDKPVAHVVANHQV<br>EEQLEWLSQR.A + 2<br>Deamidated (NQ) |
| 112   | – | 123 | 615.8267 | 1229.639 | 1229.6438 | -4     | 49    | R.ANALLANGMDLK.D                                              |
| 124   | – | 144 | 596.075  | 2380.271 | 2380.2679 | 1.3    | 33    | DNQLVVPADGLYLVYSQVLFK                                         |
| 145   | – | 160 | 601.6303 | 1801.869 | 1801.8781 | -4.9   | 40    | GQGCPDYVLLTHTVSR                                              |
| 169   | – | 181 | 471.9361 | 1412.787 | 1412.7697 | 11.9   | 33    | K.VNLLSAVKSPCPK.D +<br>Deamidated (NQ)                        |
| 182   | – | 206 | 960.4846 | 2878.432 | 2878.4429 | - 3.79 | 4     | K.DTPEGAELKPWYEPIYLG<br>VFQLEK.G                              |
| 207   | – | 218 | 635.8337 | 1269.653 | 1269.6565 | - 2.85 | 38    | K.GDQLSAEVNLPK.Y                                              |
| 207   | – | 218 | 636.3326 | 1270.651 | 1270.6405 | 8.01   | 31    | K.GDQLSAEVNLPK.Y +<br>Deamidated (NQ)                         |

## Supplementary Information, Table S2. IT-TOP accurate mass report of small chemicals in the TNF $\alpha$ preparation

#1 Ret.Time:Averaged 1.723-49.987(Scan#:70-5561) BG

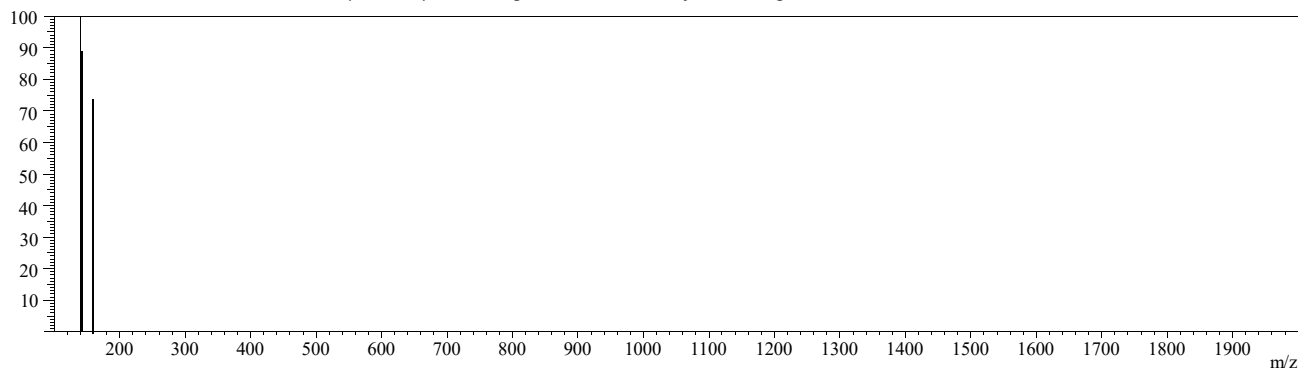
Mode:None

Mass Peaks:122 Base Peak:214.09(106631) MS Stage:MS Polarity:Pos Segment1 - Event1 Precursor:----- Cutoff: Ionization Mode:ESI



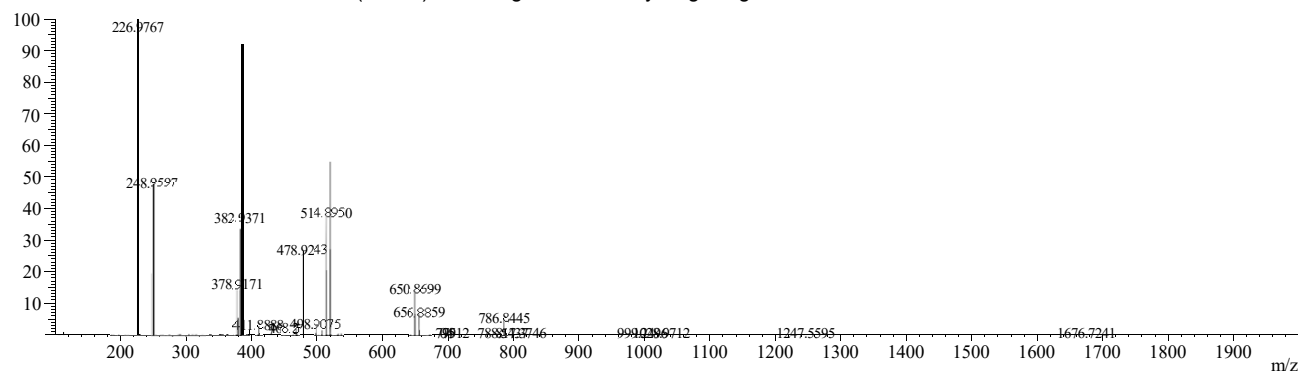
#2 Ret.Time:Averaged 1.723-1.723(Scan#:71-71) BG Mode:None

Mass Peaks:2 Base Peak:141.00(117529) MS Stage:MS/MS Polarity:Pos Segment1 - Event2 Precursor:214.09 Cutoff:59 Ionization Model:ESI



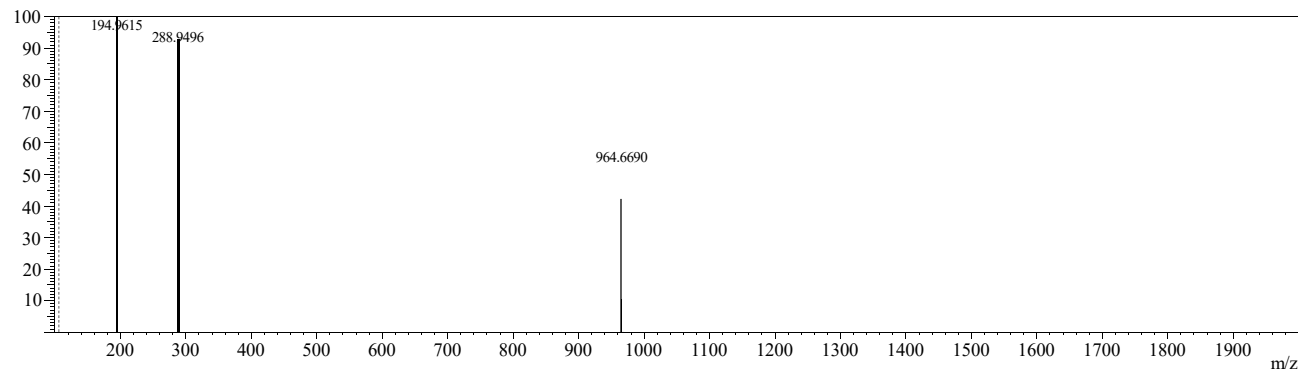
#3 Ret.Time:Averaged 1.723-49.987(Scan#:72-5562) BG Mode:None

Mass Peaks:94 Base Peak:226.97(60912) MS Stage:MS Polarity:Neg Segment1 - Event3 Precursor:----- Cutoff: Ionization Mode:ESI



#4 Ret.Time:Averaged 1.750-1.750(Scan#:75-75) BG Mode:None

Mass Peaks:3 Base Peak:194.96(47488) MS Stage:MS/MS Polarity:Neg Segment1 - Event4 Precursor:382.94 Cutoff:105 Ionization Mode:ESI



### Supplementary Information, Table S3. Concentration of different fractions of the TNF $\alpha$ preparation

| TNF fraction | PLN     | Concentration (mg/mL) | Volume (mL) | Volume (mL) w/ 1% OF | Mass (mg) | 100 mg/mL BSA to Add (mL) | 10 mg/mL BSA to Add (mL) | Total Volume (mL) | Final Concentration (mg/mL) |
|--------------|---------|-----------------------|-------------|----------------------|-----------|---------------------------|--------------------------|-------------------|-----------------------------|
| 9            | 1478329 | 0.0700                | 0.3000      | 0.3030               | 0.0210    | 0.0105                    | N/A                      | 0.3135            | 0.0670                      |
| 10           | 1478330 | 0.2420                | 0.3000      | 0.3030               | 0.0726    | 0.0363                    | N/A                      | 0.3393            | 0.2140                      |
| 11           | 1478331 | 0.2180                | 0.3000      | 0.3030               | 0.0654    | 0.0327                    | N/A                      | 0.3357            | 0.1948                      |
| 12           | 1478332 | 0.1980                | 0.3000      | 0.3030               | 0.0594    | 0.0297                    | N/A                      | 0.3327            | 0.1785                      |
| 13           | 1478333 | 0.1770                | 0.3000      | 0.3030               | 0.0531    | 0.0266                    | N/A                      | 0.3296            | 0.1611                      |
| 14           | 1478334 | 0.1830                | 0.3000      | 0.3030               | 0.0549    | 0.0275                    | N/A                      | 0.3305            | 0.1661                      |
| 15           | 1478335 | 0.2060                | 0.3000      | 0.3030               | 0.0618    | 0.0309                    | N/A                      | 0.3339            | 0.1851                      |
| 16           | 1478336 | 0.2520                | 0.3000      | 0.3030               | 0.0756    | 0.0378                    | N/A                      | 0.3408            | 0.2218                      |
| 17           | 1478337 | 0.2810                | 0.3000      | 0.3030               | 0.0843    | 0.0422                    | N/A                      | 0.3452            | 0.2442                      |
| 18           | 1478338 | 0.2960                | 0.3000      | 0.3030               | 0.0888    | 0.0444                    | N/A                      | 0.3474            | 0.2556                      |
| 19           | 1478339 | 0.2810                | 0.3000      | 0.3030               | 0.0843    | 0.0422                    | N/A                      | 0.3452            | 0.2442                      |
| 20           | 1478340 | 0.2510                | 0.3000      | 0.3030               | 0.0753    | 0.0377                    | N/A                      | 0.3407            | 0.2210                      |
| 21           | 1478341 | 0.2060                | 0.3000      | 0.3030               | 0.0618    | 0.0309                    | N/A                      | 0.3339            | 0.1851                      |
| 22           | 1478342 | 0.1600                | 0.3000      | 0.3030               | 0.0480    | 0.0240                    | N/A                      | 0.3270            | 0.1468                      |
| 23           | 1478343 | 0.1300                | 0.3000      | 0.3030               | 0.0390    | 0.0195                    | N/A                      | 0.3225            | 0.1209                      |
| 24           | 1478344 | 0.0960                | 0.3000      | 0.3030               | 0.0288    | 0.0144                    | N/A                      | 0.3174            | 0.0907                      |
| 25           | 1478346 | 0.0620                | 0.3000      | 0.3030               | 0.0186    | 0.0093                    | 0.0930                   | 0.3960            | 0.0470                      |
| 26           | 1478347 | 0.0410                | 0.3000      | 0.3030               | 0.0123    | 0.0062                    | 0.0615                   | 0.3645            | 0.0337                      |
| 27           | 1478348 | 0.0250                | 0.3000      | 0.3030               | 0.0075    | 0.0038                    | 0.0375                   | 0.3405            | 0.0220                      |
| 28           | 1478349 | 0.0170                | 0.3000      | 0.3030               | 0.0051    | 0.0026                    | 0.0255                   | 0.3285            | 0.0155                      |
| 29           | 1478350 | 0.0130                | 0.3000      | 0.3030               | 0.0039    | 0.0020                    | 0.0195                   | 0.3225            | 0.0121                      |

### Supplementary Information, Table S4. Correlation between the dose and biological activities of TNF $\alpha$ from different commercial sources (based on the manufacturer's information)

| Brand      | Ctrl  | Low Dose    | High Dose         | Extra High Dose |
|------------|-------|-------------|-------------------|-----------------|
| R&D        | 0 [0] | 5 [100-650] | 80 [1,600-10,400] |                 |
| GeneScript | 0 [0] | 5 [>100]    | 80 [>1,600]       | 520 [>10,400]   |
| PeproTech  | 0 [0] | 5 [>50]     | 80 [>800]         | 1040 [>10,400]  |

Dose (ng/ml) [Total biological activity (Units/ml)]

### **Supplementary Information, Movie S1&S2**

WT MEFs were treated with or without non-cytotoxic (5 ng/ml) or cytotoxic dose TNF $\alpha$  (80 ng/ml) for various durations, as indicated, and then analyzed by immunofluorescence staining with anti-BAD antibody for BAD protein, phalloidin for actin filament, and DAPI for nuclei, and visualized under confocal microscope with 3D program.