Expanded View Figures

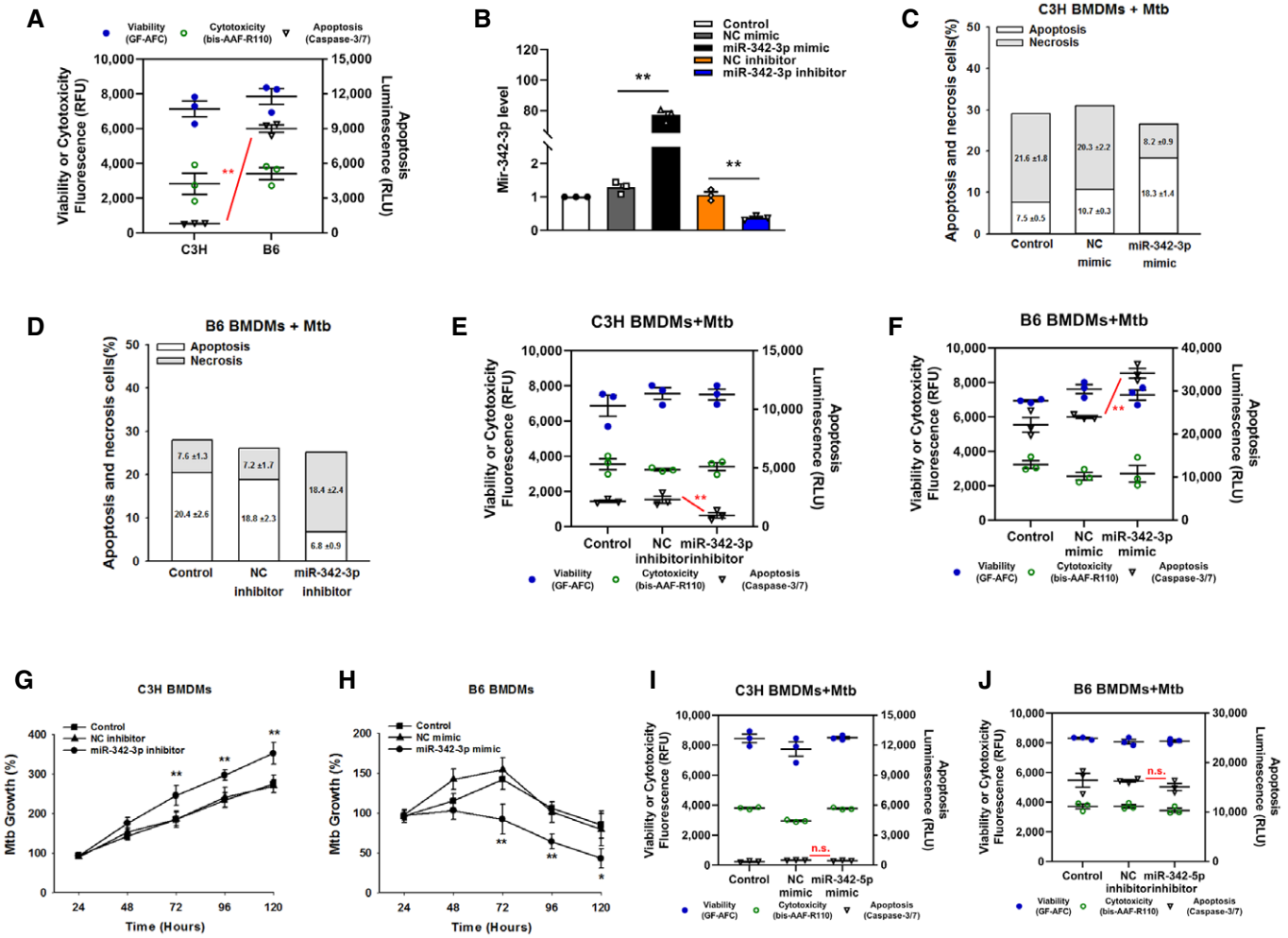

Figure EV1. MiR-342-3p is associated with TB susceptibility.

A Cell death mechanisms of C3H and B6 BMDMs after stimulation with Mtb for 36 h. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

B Relative miRNA expression was detected by qRT–PCR using miR-342-3p specific primer. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

C, D Cell death mechanisms of C3H BMDMs transfected with miR-342-3p mimic (C), or B6 BMDMs transfected with miR-342-3p inhibitor (D), followed by Mtb infection for 36 h. Representative data (from $n = 3$ biological replicates) are shown as the mean ± SEM of technical replicates.

E, F Cell death mechanisms of C3H BMDMs transfected with miR-342-3p inhibitor (E), or B6 BMDMs transfected with miR-342-3p mimic (F), followed by Mtb infection for 36 h. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

G, H Mtb growth rates of C3H BMDMs transfected with miR-342-3p inhibitor (G), or B6 BMDMs transfected with miR-342-3p mimic (H) after Mtb infection. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

I, J Cell death mechanisms of C3H BMDMs transfected with miR-342-5p mimic (I), or B6 BMDMs transfected with miR-342-5p inhibitor (J), followed by Mtb infection for 36 h. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

Data information: ANOVA followed by Bonferroni post *hoc* test was used for data analysis (A, B, E-J). $^*P < 0.05$, $^{**}P < 0.01$. Abbreviation: n.s., not significant. NC, negative control.
Source data are available online for this figure.

Figure EV2. MiR-342-3p directly targets SOCS6 to regulate anti-Mtb immunity.

A Relative expressions of miR-342-2p target genes were analyzed by qRT–PCR. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

B Cell death mechanisms of RAW264.7 cells transfected with siRNA, followed by Mtb infection for 36 h. Representative data (from $n = 3$ biological replicates) are shown as the mean ± SEM of technical replicates.

C, D MiR-342-3p mimic or inhibitor was transfected to RAW264.7 macrophages. After 48 h, cells were collected for qRT–PCR (C, data are shown as the mean ± SEM of $n = 3$ biological replicates) and Western blotting (D, representative blots from $n = 3$ biological replicates are shown) to detect the relative levels of SOCS6.

E C3H BMDMs were transfected with miR-342-3p mimic or SOCS6-overexpressing lentivirus, followed by Mtb infection. Cell death mechanisms were analyzed, respectively. Representative data (from $n = 3$ biological replicates) are shown as the mean ± SEM of technical replicates.

F B6 BMDMs were transfected with miR-342-3p inhibitor or *Socs6* siRNA, followed by Mtb infection. Cell death mechanisms were analyzed, respectively. Representative data (from $n = 3$ biological replicates) are shown as the mean ± SEM of technical replicates.

G, H SOCS6-overexpressing vector (G) or *Socs6* siRNA (H) was mixed with polyethylenimine to form a complex, which was used to infect mice by tail vein injection (*N*/P ratio=8). Lungs were collected for transfection efficiency validation. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

I Alveolar macrophages from mice treated with SOCS6-overexpressing vector or *Socs6* siRNA were collected to analyze cell death mechanisms. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

J, K Secretion of cytokines TNF-α, IL-1, IL-6, and CXCL15 in BMDMs obtained from *Mir342*$^{-/-}$ B6 and *Mir342*$^{-/-}$ B6 mice supplemented with *Socs6* siRNA (J), or from *Mir342*$^{+/+}$ C3H and *Mir342*$^{+/+}$ C3H mice supplemented with SOCS6-overexpressing vector (K), was detected by ELISA after Mtb stimulation. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

Data information: ANOVA followed by Bonferroni post *hoc* test (A, C, I-K) and two-tailed Student *t* test (G, H) were used for data analysis. *$P < 0.05$, **$P < 0.01$.
Abbreviations: n.s., not significant. NC, negative control.
Source data are available online for this figure.

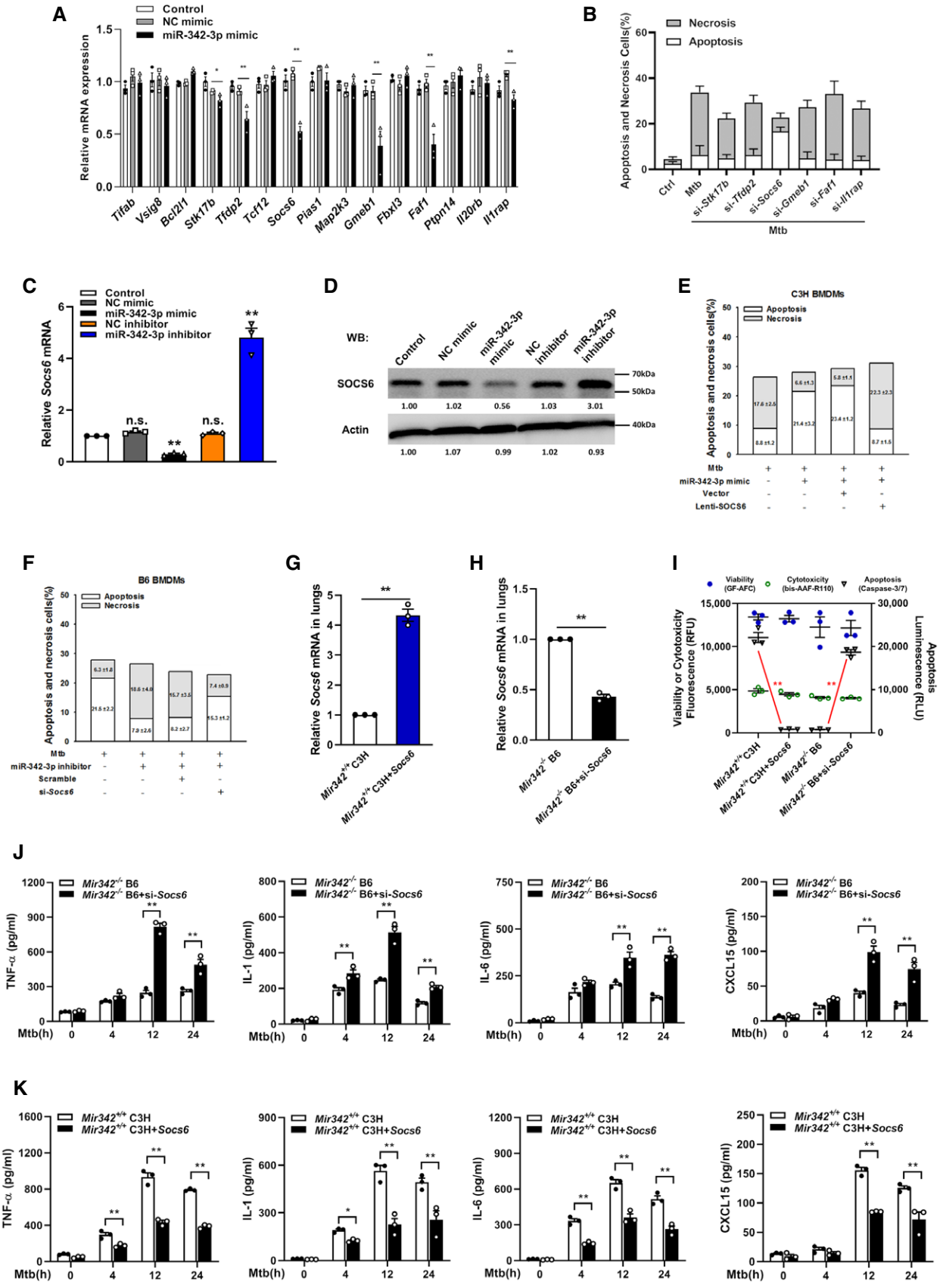

Figure EV2.

Figure EV3. SOCS6 has no effects on IFN-γ, caspase 2, and caspase 9.

A Phosphorylation states of STAT family members in response to Mtb stimulation (4 h) in *Socs6*$^{+/+}$ or *Socs6*$^{-/-}$ BMDMs were examined by Western blotting. Representative blots from $n = 3$ biological replicates are shown.

B Phosphorylation states of STAT1 and STAT3 in response to Mtb stimulation (4 h) in *Socs6*$^{+/+}$ BMDMs were examined by Western blotting. Fludarabine treatment concentration was 10 μM, and the treatment time was 24 h. Representative blots from $n = 3$ biological replicates are shown.

C Phosphorylation states of STAT1 in response to Mtb stimulation in *Socs6*$^{+/+}$ BMDMs were examined by Western blotting. Representative blots from $n = 3$ biological replicates are shown.

D Intracellular localization of STAT1 in Mtb-stimulated *Socs6*$^{+/+}$ and *Socs6*$^{-/-}$ BMDMs were detected by immunofluorescence. Representative images from $n = 3$ biological replicates are shown. Scale bar = 100 μm.

E, F Relative expressions of chemokines CCL5, CXCL10, ICAM1, and caspase 3, caspase 7, caspase 8 in Mtb-stimulated *Socs6*$^{+/+}$ (E) or *Socs6*$^{-/-}$ (F) BMDMs were detected by Western blotting. Representative blots from $n = 3$ biological replicates are shown.

G ELISA was performed to detect the secretion of IFN-γ in *Socs6*$^{+/+}$ and *Socs6*$^{-/-}$ BMDMs during Mtb stimulation. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

H, I caspase 2, caspase 9 in Mtb-stimulated *Socs6*$^{+/+}$ or *Socs6*$^{-/-}$ BMDMs were detected by qRT–PCR (H, data are shown as the mean ± SEM of $n = 3$ biological replicates) and Western blotting (I, representative blots from $n = 3$ biological replicates are shown).

J, K Caspase 8 in STAT1-suppressed *Socs6*$^{-/-}$ BMDMs were detected by qRT–PCR (J, data are shown as the mean ± SEM of $n = 3$ biological replicates) and Western blotting (K, representative blots from $n = 3$ biological replicates are shown). Fludarabine (10 μM) was used to treat *Socs6*$^{-/-}$ BMDMs for 24 h to specifically suppress the activation of STAT1.

Data information: ANOVA followed by Bonferroni post *hoc* test (G, H, J) was used for data analysis. $^{**}P < 0.01$. Abbreviation: n.s., not significant.
Source data are available online for this figure.

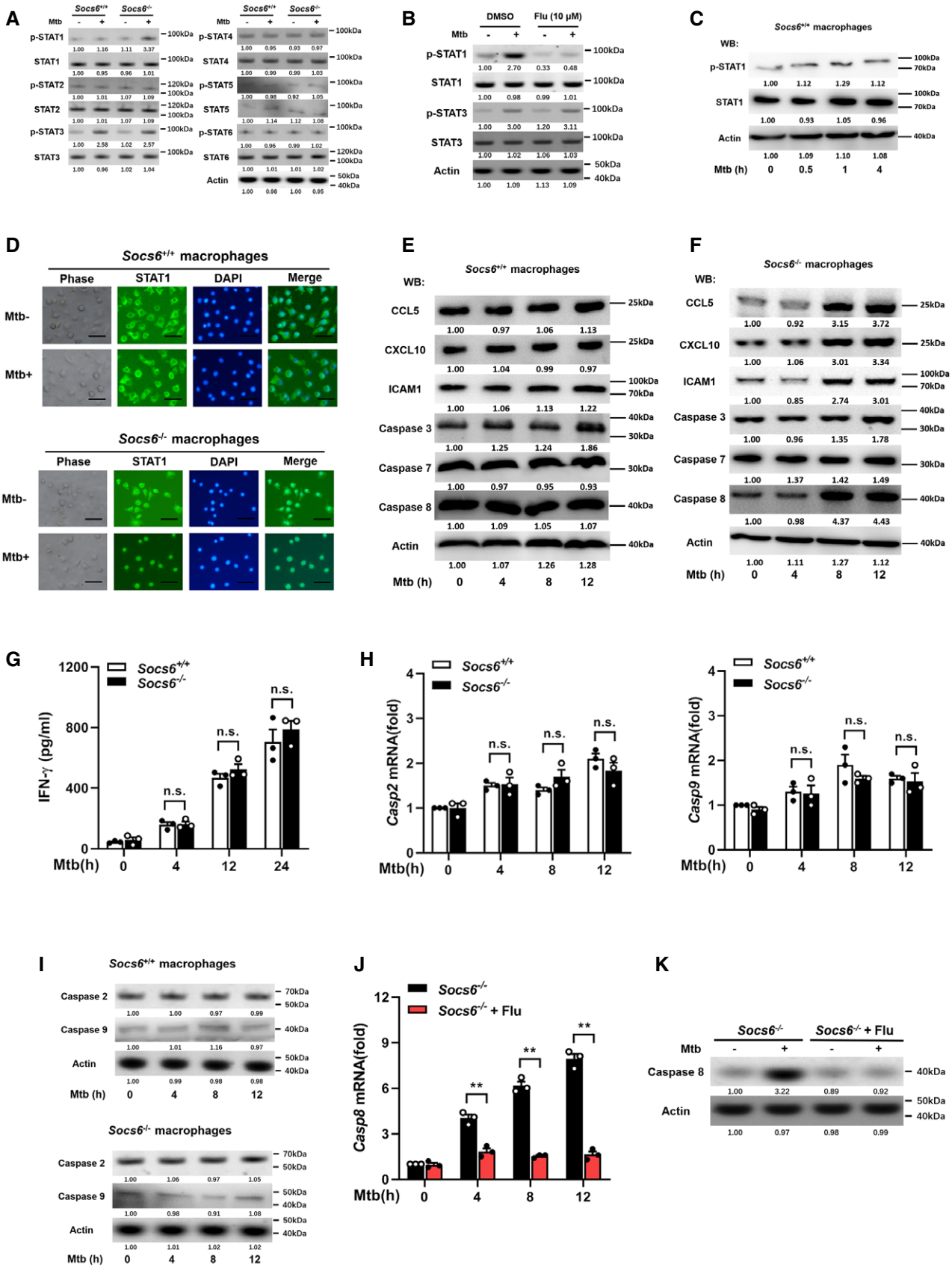

Figure EV3.

Figure EV4. RIPK3 is critical for SOCS6-regulated cell death mechanisms.

A Relative expressions of RIPK1 and RIPK3 were analyzed by Western blotting in *Socs6*$^{+/+}$ BMDMs stimulated with Mtb for 0–24 h. Representative blots from $n = 3$ biological replicates are shown.

B *Socs6*$^{+/+}$ BMDMs were stimulated with Mtb for 0–24 h, and cell lysates were collected and immunoprecipitated using an anti-RIPK1 antibody. The recruitment of caspase 8, RIPK3, FADD, and MLKL was analyzed by immunoblots. The lower panel represents the immunoblot analysis of whole cell lysates. Representative blots from $n = 3$ biological replicates are shown.

C Cell death mechanisms of Mtb-infected *Socs6*$^{-/-}$ BMDMs that were transfected with plasmids expressing Myc-RIPK3 or Myc-RIPK3 K51A mutant as indicated. Representative data (from $n = 3$ biological replicates) are shown as the mean ± SEM of technical replicates.

D *Socs6*$^{+/+}$ BMDMs were transfected with *Ripk3* siRNA or *Mlkl* siRNA for 24 h. Afterward, transfected cells were stimulated with Mtb for 12 h, and cell lysates were collected and immunoprecipitated using an anti-RIPK1 antibody. The recruitment of caspase 8, RIPK3, FADD, and MLKL was analyzed by immunoblot. The lower panel represents the immunoblot analysis of whole cell lysates. Representative blots from $n = 3$ biological replicates are shown.

E–H Cell viabilities (E, data are shown as the mean ± SEM of $n = 3$ biological replicates), cell death mechanisms [F, data are shown as the mean ± SEM of $n = 3$ biological replicates. G, representative data (from $n = 3$ biological replicates) are shown as the mean ± SEM of technical replicates], or Mtb growth rates (H, data are shown as the mean ± SEM of $n = 3$ biological replicates) of Mtb-infected *Socs6*$^{+/+}$ BMDMs that were transfected with *Ripk3* siRNA or *Mlkl* siRNA as indicated. Z-VAD treatment concentration was 20 μM, and the treatment time was 24 h.

I Cell death mechanisms of *Socs6*$^{-/-}$ BMDMs transfected with plasmids expressing Myc-MLKL and stimulated with Mtb for 36 h. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

J Cell viabilities of *Socs6*$^{-/-}$ BMDMs transfected with plasmids expressing Myc-MLKL for 24 h and stimulated with Mtb or z-VAD for 24 h. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

K Mtb growth rates of *Socs6*$^{-/-}$ BMDMs transfected with plasmids expressing Myc-MLKL and stimulated with Mtb for 0–120 h. Data are shown as the mean ± SEM of $n = 3$ biological replicates.

Data information: ANOVA followed by Bonferroni post *hoc* test (E, F, H-K) was used for data analysis. *$P < 0.05$, **$P < 0.01$. Abbreviation: n.s., not significant.
Source data are available online for this figure.

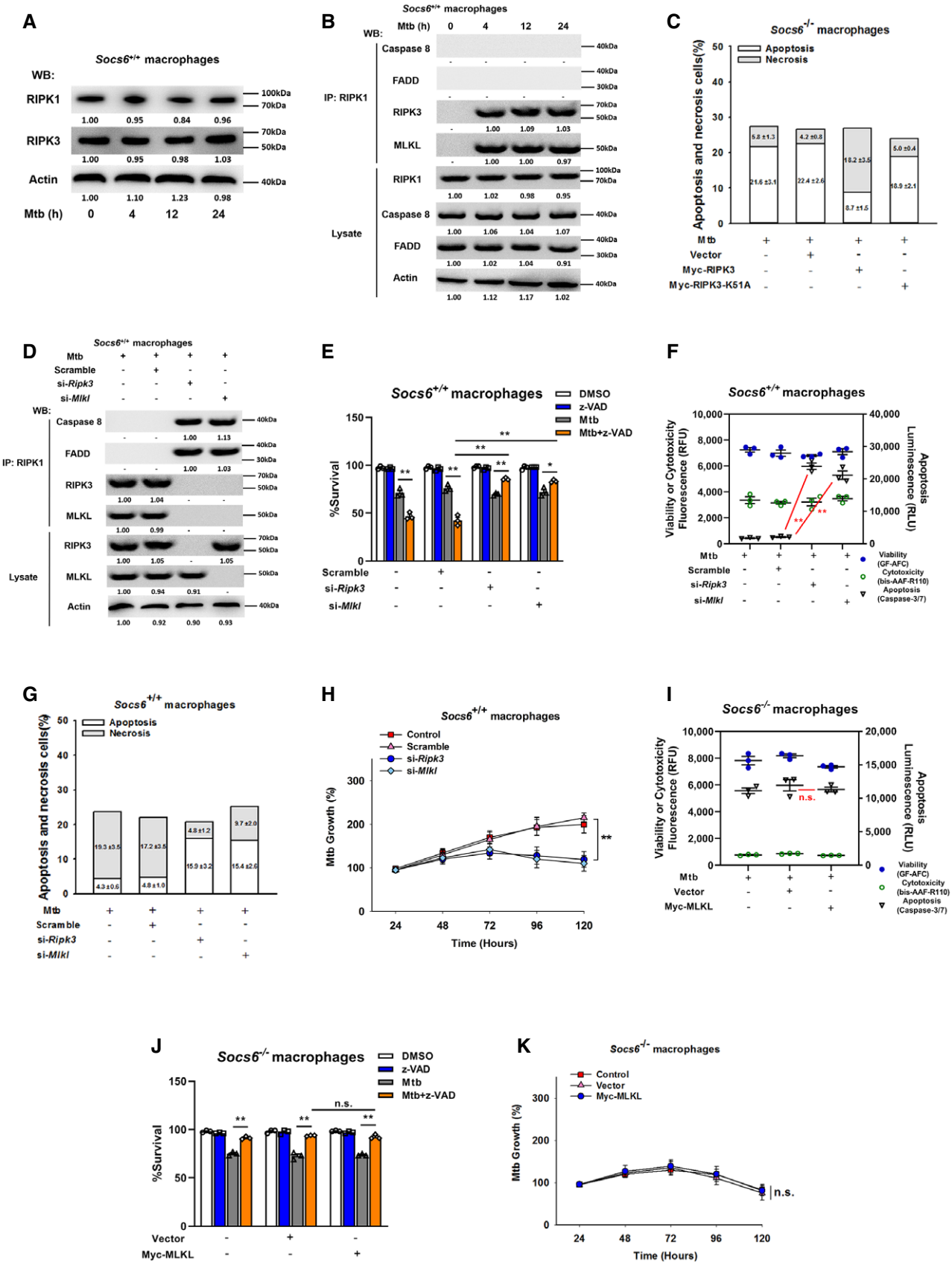

Figure EV4.