Morioka et al., http://www.jcb.org/cgi/content/full/jcb.201305070/DC1

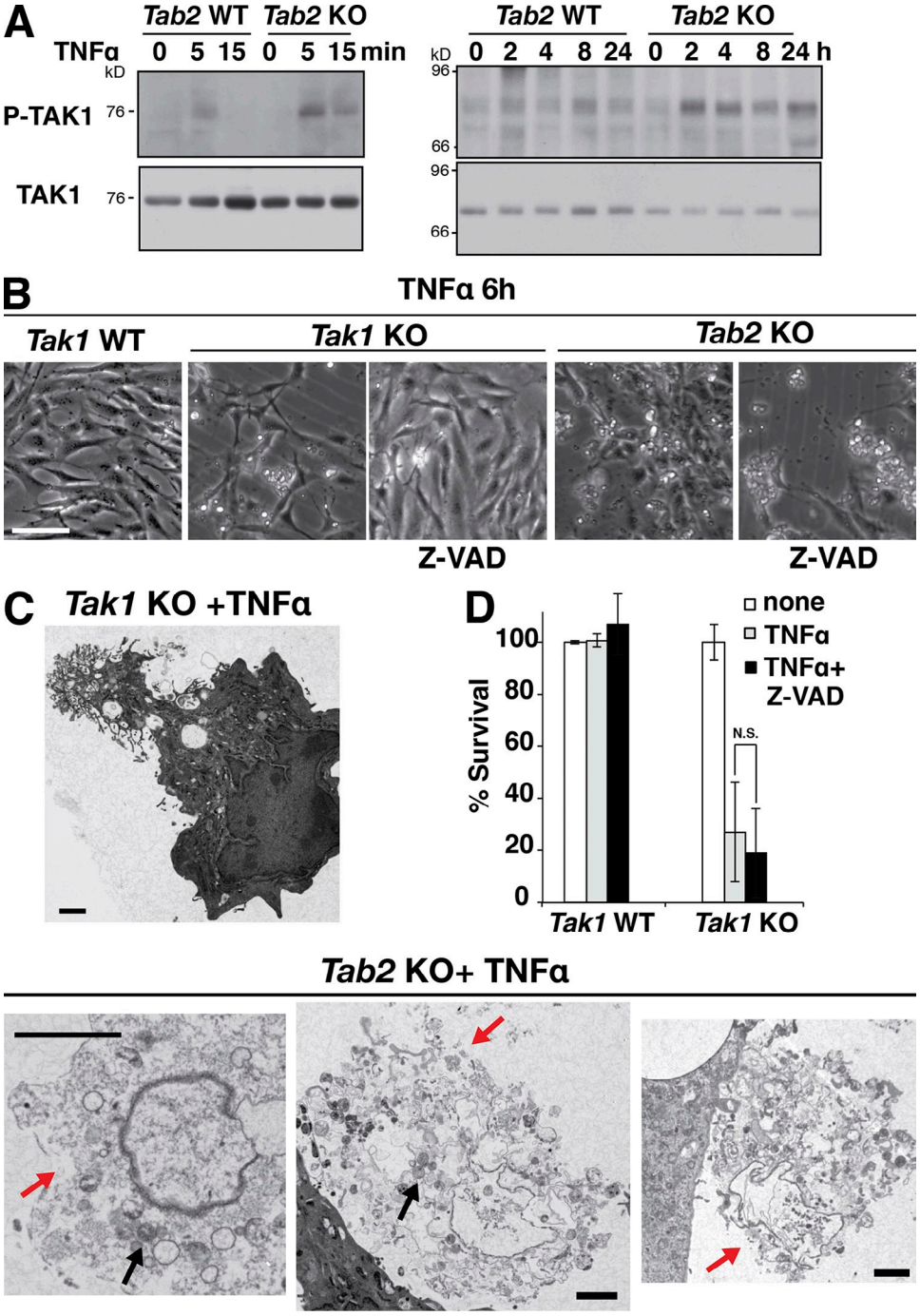

Figure S1. **TNF induces hyperactivation of TAK1 and necrotic cell death in *Tab2*-deficient fibroblasts, while apoptosis is induced in *Tak1*-deficient fibroblasts.** (A) *Tab2* WT and *Tab2* KO fibroblasts were stimulated with 20 ng/ml TNF, and cell lysates were analyzed by immunoblotting. Related to Fig. 1. (B) *Tak1*- and *Tab2*-deficient fibroblasts die within 6 h after TNF stimulation. *Tak1* WT, *Tak1* KO, or *Tab2* KO fibroblasts were stimulated with 20 ng/ml TNF, and bright-field photographs were taken at 6 h after TNF stimulation. Some cells were pre-incubated with 20 µM Z-VAD for 1 h before TNF stimulation. Bar, 100 µm. Related to Fig. 1 B. (C) Electron microscopic images. *Tak1* KO and *Tab2* KO fibroblasts were exposed to TNF (20 ng/ml for *Tak1* KO and 200 ng/ml for *Tab2* KO) for 6 h and samples were analysed using a transmission electron microscope. Black arrows, swollen mitochondria; red arrows, plasma membrane rupture. Bars, 2 µm. Related to Fig. 1 C. (D) Inhibition of caspases does not block cell death in *Tak1*-deficient dermal fibroblasts at 24 h after TNF stimulation. *Tak1* WT and *Tak1* KO were pre-treated with 20 µM vehicle (DMSO) or 20 µM Z-VAD for 1 h and stimulated with 20 ng/ml TNF. Cell viability was determined at 24 h after TNF-α stimulation by the crystal violet assay (three independent experiments, mean ± SD; N.S., not significant; P = 0.61). Related to Fig. 1B.

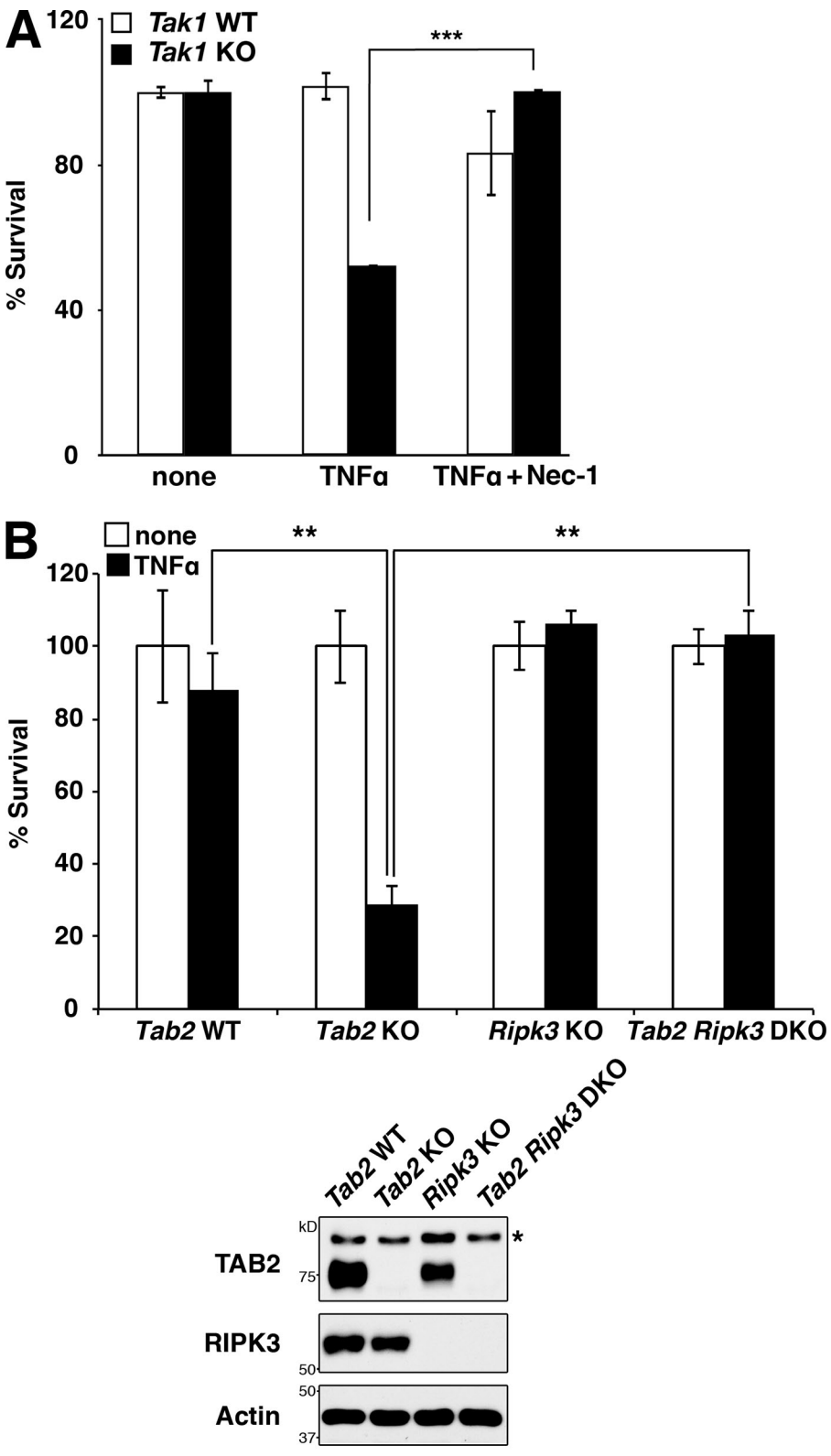

Figure S2. **Nec-1 blocks TNF-induced cell death in *Tak1*-deficient fibroblasts, and *Ripk3* deletion rescues TNF-induced cell death in *Tab2*-deficient fibroblasts.** (A) *Tak1* WT and *Tab1* KO fibroblasts were pre-treated with 30 µM vehicle (DMSO) or 30 µM Nec-1 for 1 h, and then stimulated with 20 ng/ml TNF-α for 24 h. Cell survival was determined by the crystal violet assay (three independent experiments, mean ± SD; ***, P = 0.00023). Related to Fig. 2. (B) Ripk3 deficiency completely rescued TNF-induced cell death in *Tab2*-deficient fibroblasts. *Tab2* WT, *Ripk3* KO, *Tab2* KO, and *Tab2 Ripk3* DKO fibroblasts were treated with 200 ng/ml TNF for 24 h. Cell survival was determined by the crystal violet assay (three independent experiments, mean ± SD; **, P < 0.001, P = 0.0093, and P = 0.0082 from the left). Proteins of TAB2, RIPK3, and actin were analyzed by immunoblotting. Asterisk indicates a non-specific band. Related to Fig. 3.

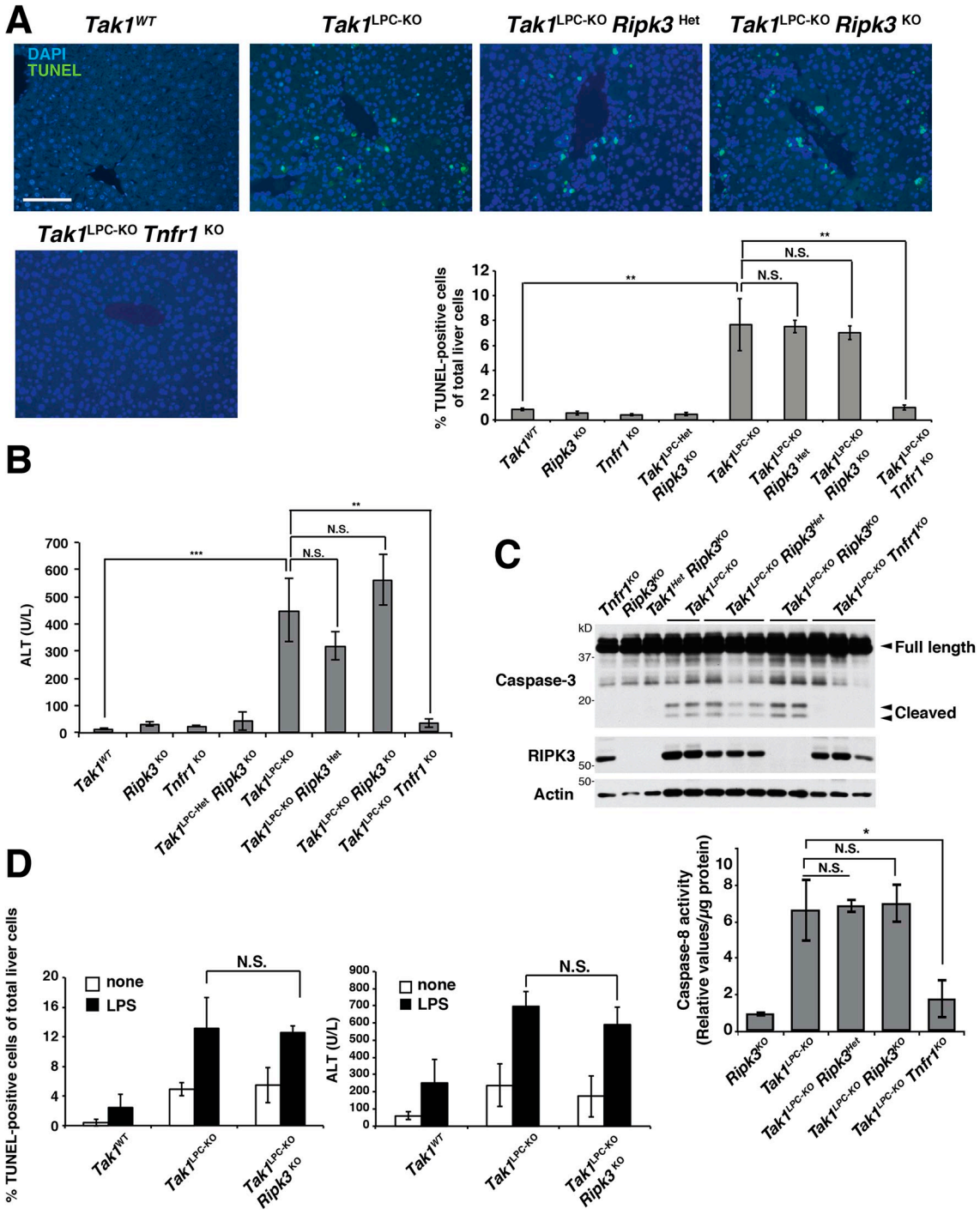

Figure S3. ***Ripk3* deletion does not rescue liver injury in *Tak1*-deficient liver.** (A) Livers from Tak1$^{flox/flox}$ (Tak1 WT), Ripk3$^{-/-}$ (Ripk3^{KO}), Tnfr1$^{-/-}$ (Tnfr1^{KO}), Tak1$^{flox/+}$ Alb.Cre Ripk3$^{-/-}$ (Tak1$^{LPC\text{-}Het}$ Ripk3^{KO}), Alb.Cre Tak1$^{flox/flox}$ (Tak1$^{LPC\text{-}KO}$), Alb.Cre Tak1$^{flox/flox}$ Ripk3$^{-/-}$ (Tak1$^{LCP\text{-}KO}$ Ripk3^{KO}), Alb.Cre Tak1$^{flox/+}$ Ripk3$^{-/-}$ (Tak1$^{LPC\text{-}KO}$ Ripk3^{Het}), and Alb.Cre Tak1$^{flox/flox}$ Tnfr1$^{-/-}$ (Tak1$^{LCP\text{-}KO}$ Tnfr1^{KO}; all *n* = 4) mice at 1–2 months of age were analyzed by TUNEL staining. Percentages of TUNEL-positive cells in total liver cells (DAPI-stained cells) are shown (mean ± SD; **, P < 0.01; N.S., not significant; P = 0.0048, P = 0.95, P = 0.64, and P = 0.0054 from the left). (B) Sera from the mice described in A were analyzed by an ALT assay kit (*n* = 4 per genotype; ***, P < 0.001; **, P < 0.01; N.S., not significant; P = 0.0006, P = 0.15, P = 0.18, and P = 0.0036 from the left). (C) Caspase-3 activity was determined by immunoblotting using protein extracts from the mice described in A. Caspase-8 activity was also determined (bottom graph; *n* = 4 per genotype; *, P < 0.05; N.S., not significant; P = 0.83, P = 0.77, and P = 0.015 from the left). (D and E) *Tak1 WT*, *Tak1*$^{LPC\text{-}KO}$, and *Tak1*$^{LCP\text{-}KO}$ *Ripk3*^{KO} mice at 4–7 months old were intraperitoneally injected with LPS at 10 mg/ kg for 24 h. Sera from the mice were analyzed by an ALT assay kit (D; *n* = 3 per genotype; N.S., not significant; P = 0.073). Percentages of TUNEL-positive cells in total liver cells (DAPI-stained cells) are shown (E; *n* = 3 per genotype; N.S., not significant; P = 0.83). Related to Fig. 4.

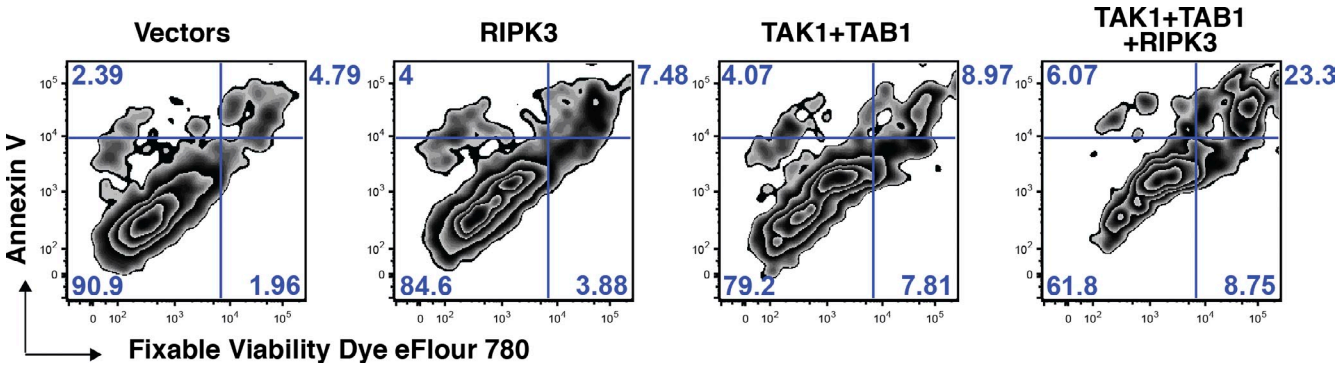

Figure S4. **Hyperactivation of TAK1 promotes RIPK3-dependent cell death in fibroblasts.** Dermal fibroblasts were transfected with 1 μg of Flag-TAB1 (TAB1), DsRedMT7-TAK1 (TAK1), HA-RIPK3 (RIPK3), or their control vectors for the total amount of 3 μg at 48 h after transfection. DsRed-positive transfected cells were gated, and cell death was analyzed by annexin V and fixable viability dye eFluor 780 staining. Related to Fig. 5.

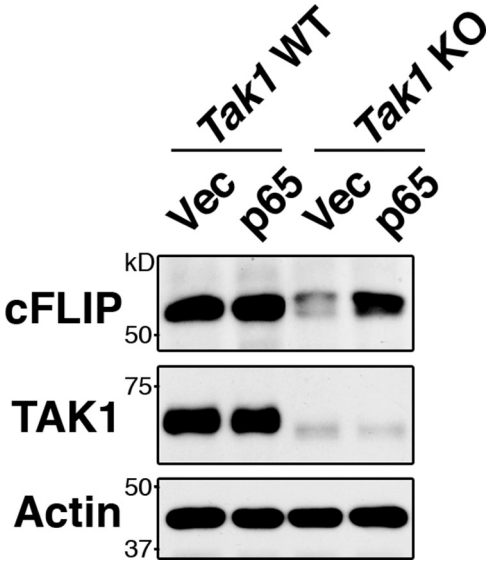

Figure S5. ***Tak1* deficiency reduces the expression of cFLIP, which is rescued by p65 overexpression.** *Tak1* WT and *Tak1* KO fibroblasts were stably transfected with p65. Immunoblots of cFLIP, TAK1, and β-actin are shown. Related to Fig. 6 D.