Supporting Information

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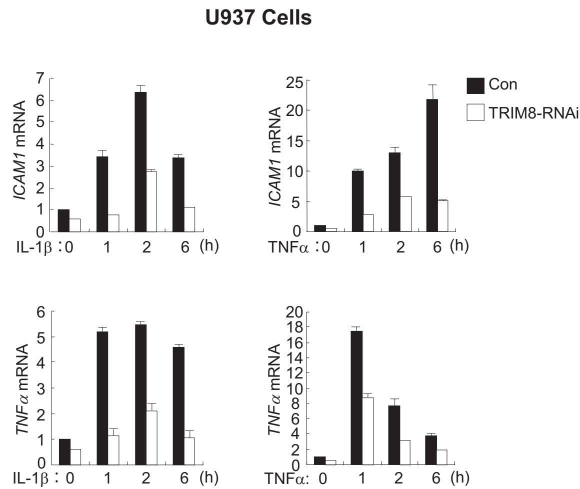

Fig. S1. Effects of TRIM8 RNAi on TNFα- or IL-1β–induced transcription of endogenous *ICAM1* and *TNFα* genes in U937 cells.U937 cells stably tranduced with GFP control or TRIM8 RNAi were either untreated or treated with TNFα (10 ng/mL) or IL-1β (10 ng/mL) for the indicated time before RT-PCR experiments were performed.

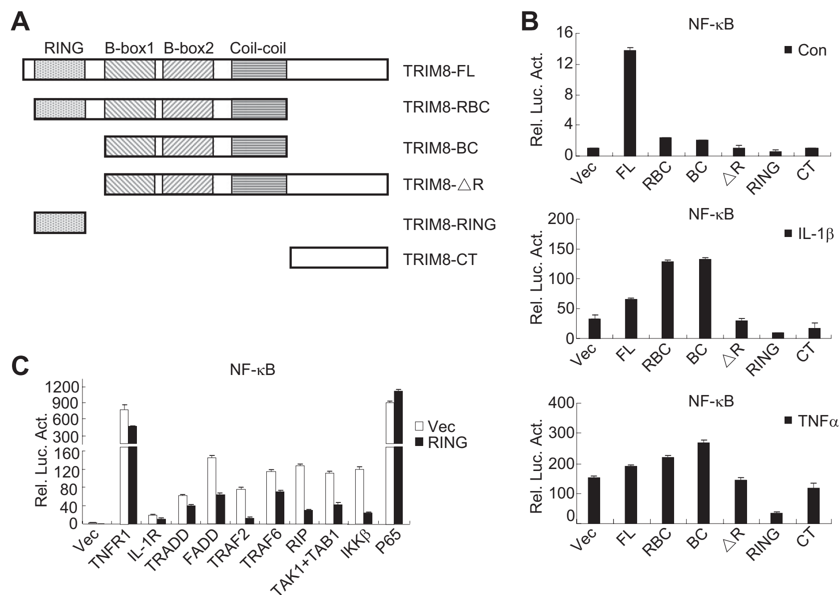

Fig. S2. TRIM8 mutant inhibits TAK1- but not p65-mediated NF-κB activation. (*A*) A schematic representation of wild-type TRIM8 and its deletion mutants. (*B*) Ring finger domain of TRIM8 (TRIM8-RING) acts as a dominant negative mutant. A total of 293 cells (1×10^5) were transfected with NF-κB reporter (0.01 μg) and the indicated expression plasmids (0.2 μg each). Twenty hours after transfection, cells were treated with buffer, TNFα, or IL-1β for 10 h before luciferase assays were performed. (*C*) The effects of TRIM8-RING on NF-κB activation mediated by various signaling components. A total of 293 cells (1×10^5) were transfected with NF-κB reporter (0.01 μg) and the indicated expression plasmids (0.1 μg) for 20 h before luciferase assays were performed.

Fig. S3. TRIM8 interacts with TAK1. (*A*) TRIM8 interacts with TAK1 in the mammalian overexpression system. A total of 293 cells (2×10^6) were transfected with the indicated plasmids (5 μg). Coimmunoprecipitation and immunoblot analysis were performed with the indicated antibodies (*Upper* panels). Expression of the transfected proteins was analyzed by immunoblots with anti-Flag and anti-HA (*Lower*). (*B*) Endogenous TRIM8 is associated with TAK1 following TNFα or IL-1β stimulation. A total of 293 cells (2×10^7) were left untreated or stimulated with TNFα or IL-1β for the indicated times. Immunoprecipitation and immunoblot analysis were performed with the indicated antibodies.

Fig. S4. TAK1(K158R) inhibits TNFα- and IL-1β–induced NF-κB activation. A total of 293 cells (1×10^5) were transfected with NF-κB reporter (0.01 μg) and the indicated expression plasmids. Twenty hours after transfection, cells were left untreated or stimulated with TNFα (10 ng/mL) or IL-1β (10 ng/mL) for 10 h before luciferase assays were performed. $^*P < 0.05$; $^{**}P < 0.01$.

Fig. S5. Knockdown of TRIM8 reduces TNFα- and IL-1β–induced IκBα degradation. A total of 293 cells stably transduced with GFP control or TRIM8 RNAi were treated with buffer, TNFα (10 ng/mL), or IL-1β (10 ng/mL) for the indicated times. Cell lysates were then analyzed by immunoblots with the indicated antibodies.

Fig. S6. TRIM8 is involved in TNFα- and IL-1β–induced JNK-AP1 but not in ELK1 and CHOP activation. (*A*) TRIM8 mediates TNFα- and IL-1β–induced JNK-AP1 activation. (Left) TRIM8 activates AP1 in reporter assays. A total of 293 cells (1×10^5) were transfected with AP1 reporter (0.1 μg) and an empty control or TRIM8 plasmid. Twenty hours after transfection, cells were left untreated or stimulated with TNFα (10 ng/mL) or IL-1β (10 ng/mL) for 10 h before luciferase assays were performed. (*Right*) TRIM8 mediates TNFα- and IL-1β–induced JNK activation. U937 or THP-1 cells stably transduced with GFP control or TRIM8 RNAi were either untreated or treated with TNFα (10 ng/mL) or IL-1β (10 ng/mL) for the indicated times. Cell lysates were then analyzed by immunoblots with the indicated antibodies. (*B*) TRIM8 is not involved in TNFα- and IL-1β–induced activation of ELK1 and CHOP. A total of 293 cells (1×10^5) were transfected with pFB (0.2 μg), pFA2-ELK1 or pFA2-CHOP (0.02 μg), and GFP control or TRIM8 RNAi plasmid (0.5 μg). Thirty-six hours after transfection, cells were left untreated or treated with TNFα (10 ng/mL) or IL-1β (10 ng/mL) for 10 h before luciferase assays were performed.