Supporting Materials

TRIM34 modulates influenza virus-activated programmed cell death through targeting ZBP1 for K63-linked polyubiquitination

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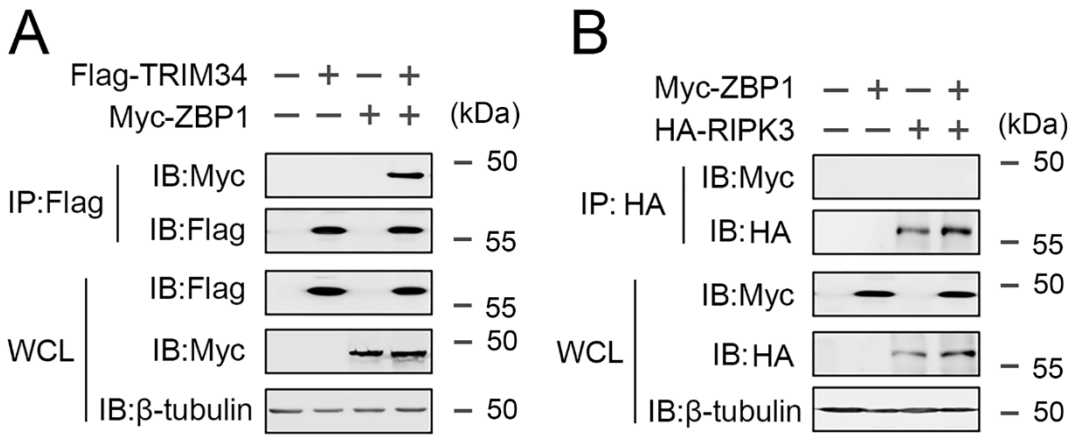

Figure S1, related to Figure 1. TRIM34 interact with ZBP1.
(A) 293T cells were transfected with vector, Myc-ZBP1 and Flag-TRIM34. Forty-eight hours post-transfection, Co-IP and immunoblot analysis were performed with the indicated antibodies. (B) Experiments were performed similar to those in (A), except indicated HA-RIPK3 were used. All experiments were repeated at least three times.

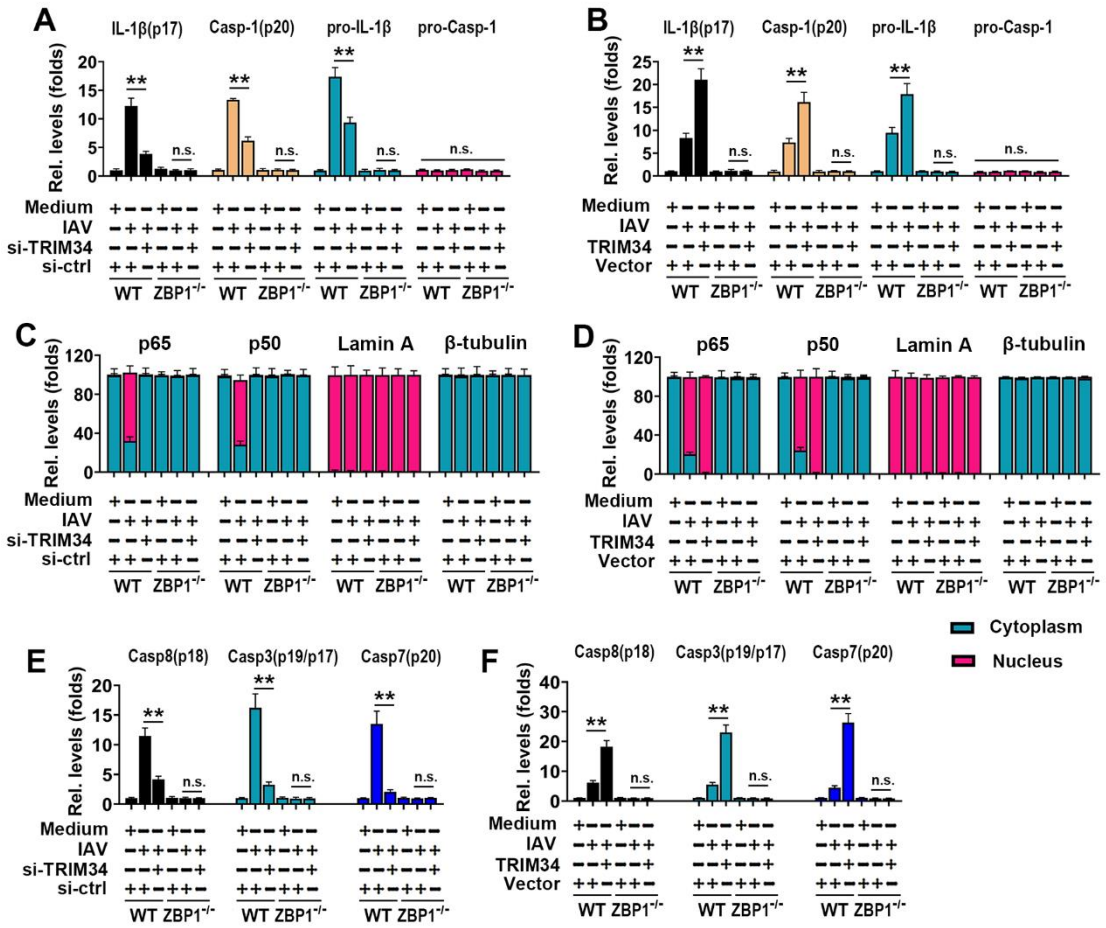

Figure S2, related to Figure 5. TRIM34 regulates IAV induced NLRP3 inflammasome and programmed cell death pathways via ZBP1 *in vitro*.

(A and B) The IL-1β (p17)/β-tubulin, Casp-1 (p20)/β-tubulin, pro-IL-1β/β-tubulin and pro-Casp-1/β-tubulin ratios were quantified as described in Fig 5B and 5D.

(C and D) Quantification of p65, p50, Lamin A and β-tubulin cytoplasmic and nuclear localization as described in Fig 5F and 5H.

(E and F) The Casp8 (p18)/Casp8, Casp3 (p19/p17)/Casp3 and Casp7 (p20)/Casp7 ratios were quantified as described in Fig 5J and 5K.

All experiments were repeated at least three times. Bar graphs present means ± SD, n = 3 (**P < 0.01; n.s., not significant).

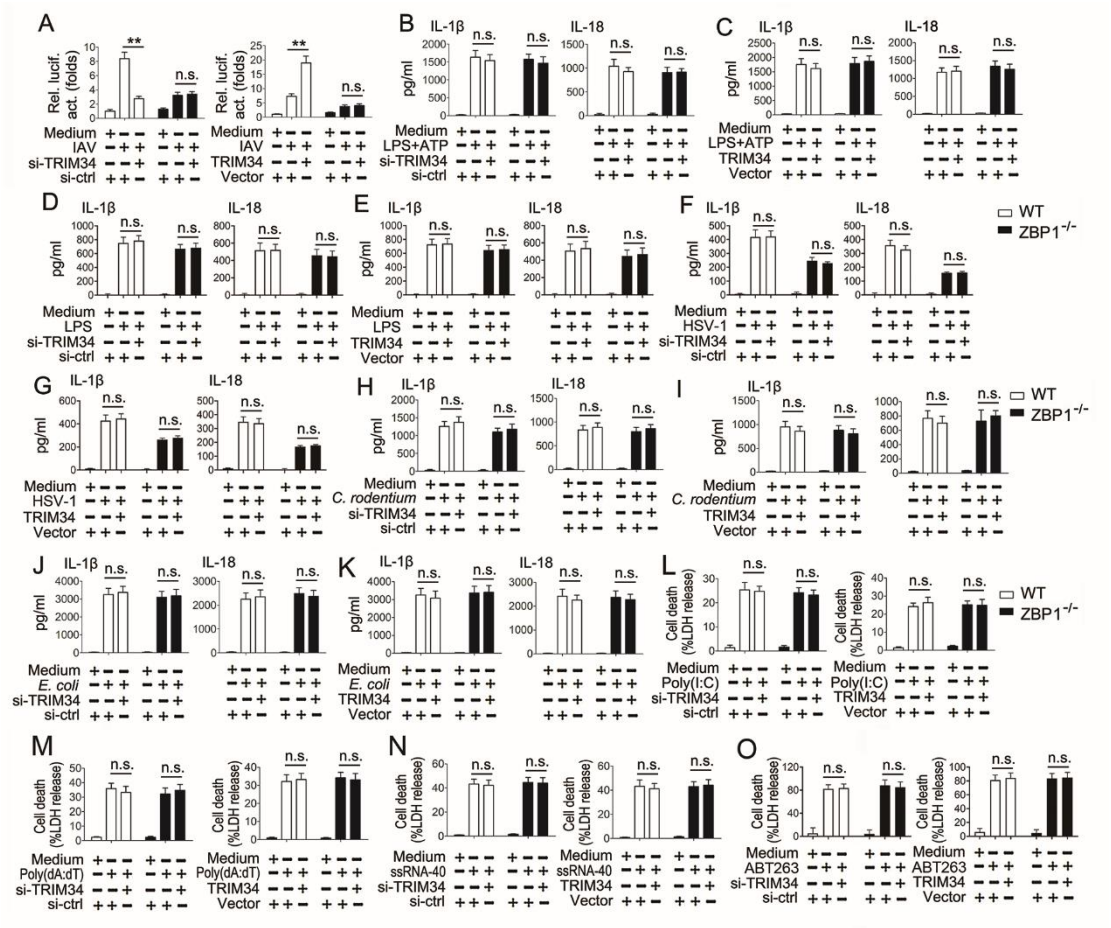

Figure S3. TRIM34 is dispensable for proinflammatory cytokine production and cell death in response to activation of canonical and non-canonical inflammasomes and transfected RNA ligands.

(A) THP-1 macrophages were transfected with indicated plasmids, si-RNAs or NF-κB dual luciferase reporter plasmid for 48 h prior to luciferase assays.

(B) THP-1 macrophages were transfected with indicated si-RNAs for 36 h. Then, cells were treated LPS (1 μg/ml for 3 h) and ATP (2.5 mM for 30 min). IL-1β and IL-18 protein levels in cell culture supernatants were determined by ELISA.

(C) Experiments were performed as described in (A), except that cells were transfected with vector control or Flag-TRIM34.

(D-K) Experiments were performed as described in (A), except that cells were treated LPS (1 μg/ml for 6 h) (D and E), infected with HSV-1 (1 MOI for 12 h) (F and G), *C. rodentium* (MOI = 20) (H and I) or *E. coli* (MOI = 20) (J and K) for 20 h.

(L) THP-1 macrophages were transfected with indicated plasmids or si-RNAs for 36 h, and transfected with poly(I:C) (1 μg/ml) for 6 h. Cell death was quantified using LDH release.

(M-O) Experiments were performed as described in (L), except that cell were transfected with poly(dA:dT) (1 μg/ml) for 6 h (M), ssRNA-40 (5 μg/ml) for 24 h (N), or ABT-263 (1 μM) for 72 h (O).

All experiments were repeated at least three times. Bar graphs present means ± SD, n = 3 (**P < 0.01; n.s., not significant).

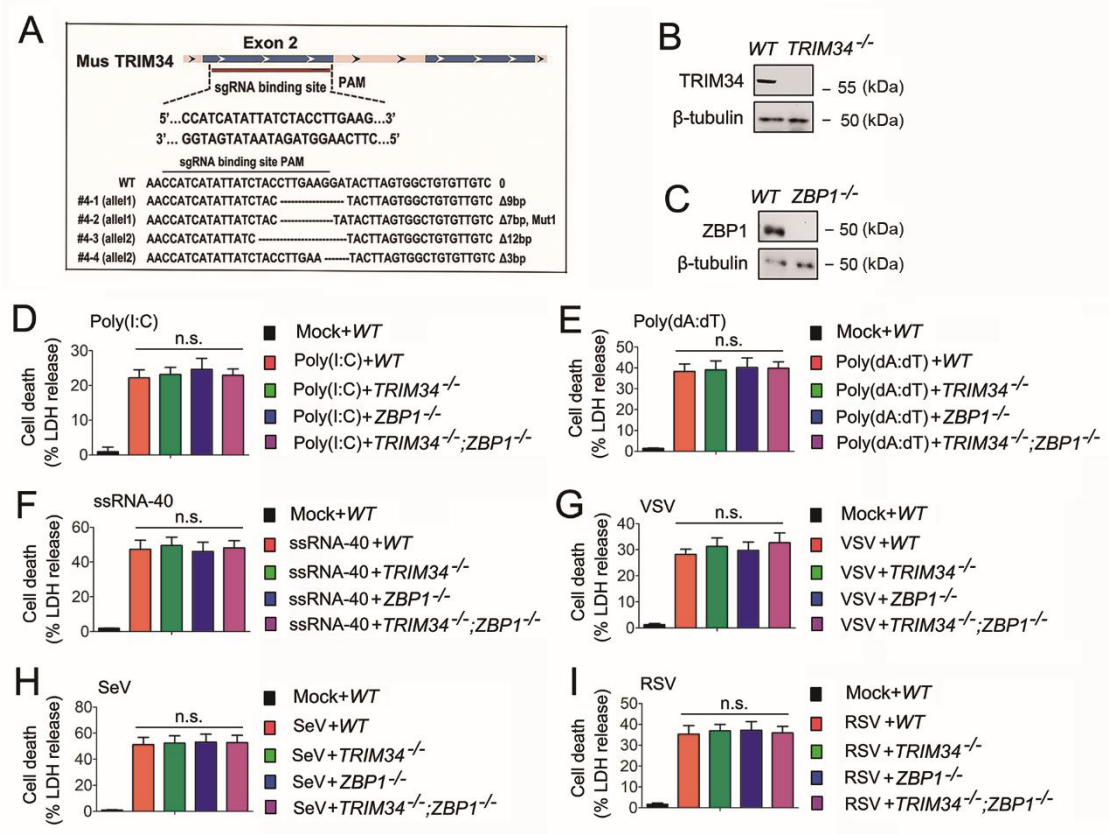

Figure S4. TRIM34 is dispensable for cell death in response to transfected RNA ligands and RNA viruses belonging to Paramyxoviridae and Rhabdoviridae families.

(A) schematic showing the single guide RNA (sgRNA)-binding site in the mouse *Trim34* gene (top). Sequence of the genomic single guide RNA-binding site (bottom).

(B) *Trim34* expression in the lung of WT and *Trim34*$^{-/-}$ mice was detected using Western blot analyses.

(C) *ZBP1* expression in the lung of WT and *ZBP1*$^{-/-}$ mice was detected using Western blot analyses.

(D) BMDMs were isolated from indicated mice and transfected with poly(I:C) (1 μg/ml) for 6 h. Cell death was quantified using LDH release.

(E and F) Experiments were performed as described in (D), except that cell were transfected with poly(dA:dT) (1 μg/ml) for 6 h (E), or ssRNA-40 (5 μg/ml) for 24 h (F).

(G) BMDMs were isolated from indicated mice and infected with VSV (MOI=5) for 24 h. Cell death was quantified using LDH release.

(H and I) Experiments were performed as described in (G), except that cell were infected with SeV (MOI = 5) (H), and RSV (MOI = 10) (I) for 24 h.

All experiments were repeated at least three times with consistent results. Bar graphs represent the means ± SEM, n = 3 (**P < 0.01; *P < 0.05).

Table S1 Antibodies used in this study

Antibodies	Source	Dilution
TRIM34	Abcam (ab180130)	1:1000
ZBP1	Abcam (ab180130)	1:2000
HA	Sigma (H6908)	1:2000
Flag	Sigma (M2)	1:2000
Myc	Abcam (ab32)	1:2000
Caspase 3	Abcam (ab13847)	1:5000
Caspase 7	Abcam (ab 69540)	1:5000
Caspase 8	Abcam (ab32397)	1:5000
Caspase 9	Abcam (ab202068)	1:5000
Ubiquitin	Abcam (ab7780)	1:5000
Ubiquitin(k48)	Abcam (ab140601)	1:5000
Ubiquitin(k63)	Abcam (ab179434)	1:1000
Caspase-1	Abcam (ab138483)	1:5000
IL-1β	Abcam (ab200478)	1:500
p65	Cell Signaling (8242)	1:2000
p50	Cell Signaling (3035)	1:2000
β-actin	Abcam (ab179467)	1:5000
β-tubulin	Abcam (ab210797)	1:5000
Lamin A	Abcam (ab26300)	1:2000

Table S2 Primers used in qRT-PCR

Gene name	5'primer	3'primer
ZBP1	5'- CAGGAGTAGATGACAGGGAGTTGC -3'	5'- CAGGTAGCCATGATGGAAGGTAA -3'
IL-6	5'- ACTCACCTCTTCAGAACGAATTG -3'	5'-AGCCATCTTTGGAAGGTTCAG-3'
IL-1β	5'- TGGCAATGAGGATGACTTGTTC -3'	5'- GCTGTAGTGGTGGTCGGAGATT -3'
TNF-α	5'- CCCTGGTATGAGCCCATCTATC -3'	5'- CTTAGTGGTTGCCAGCACTTCA -3'
β-actin	5'- GGACTTCGAGCAAGAGATGG-3'	5'-AGGAAGGAAGGCTGGAAGAG-3'