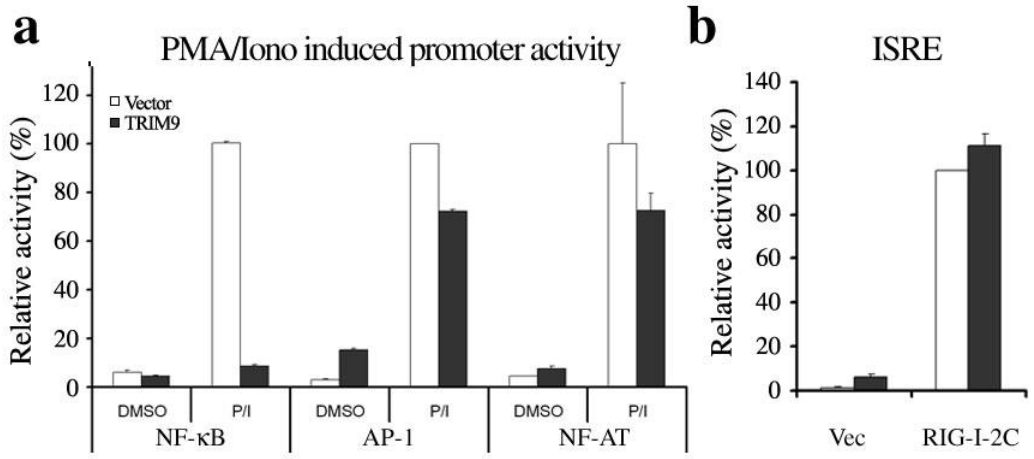
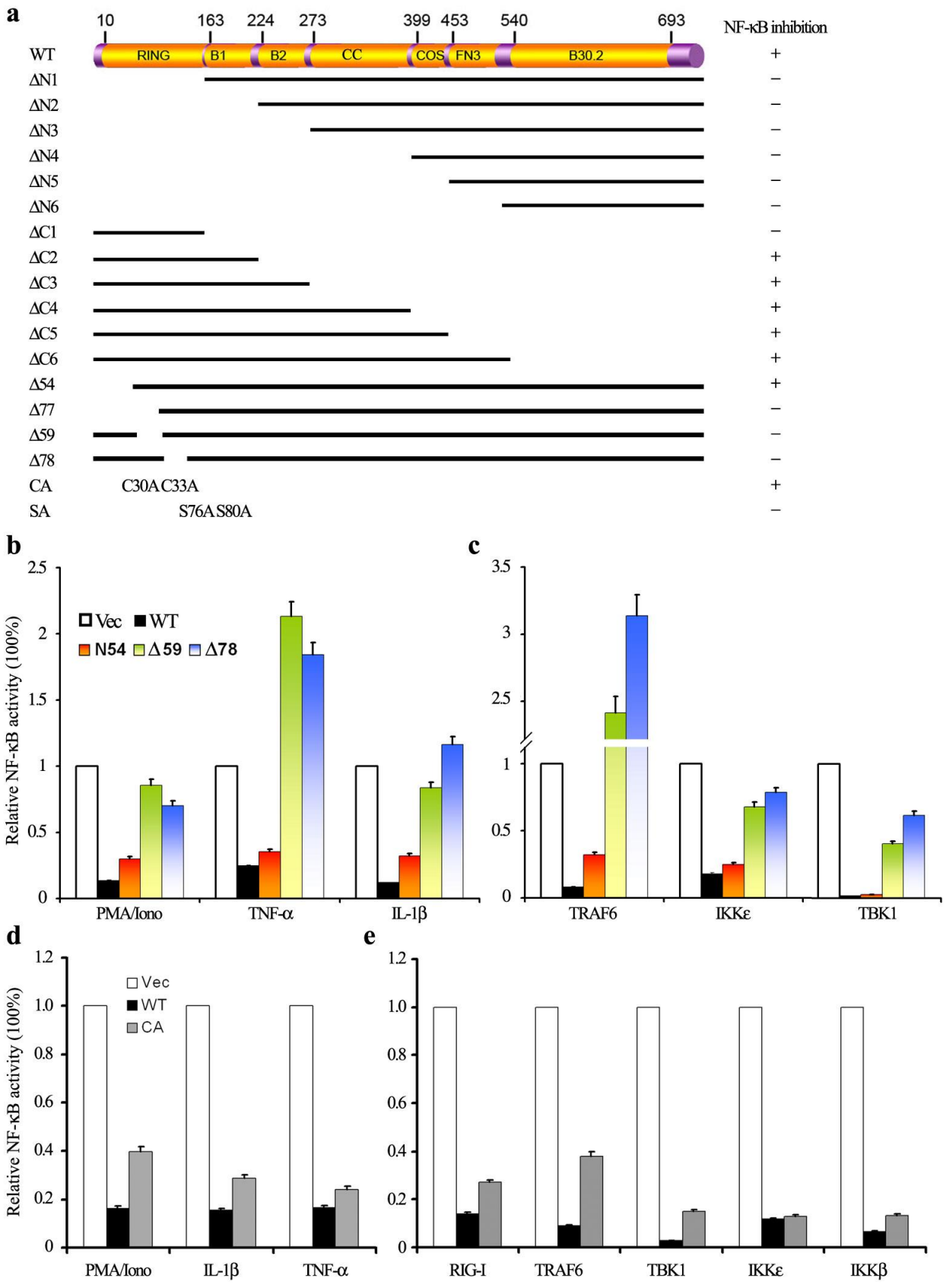
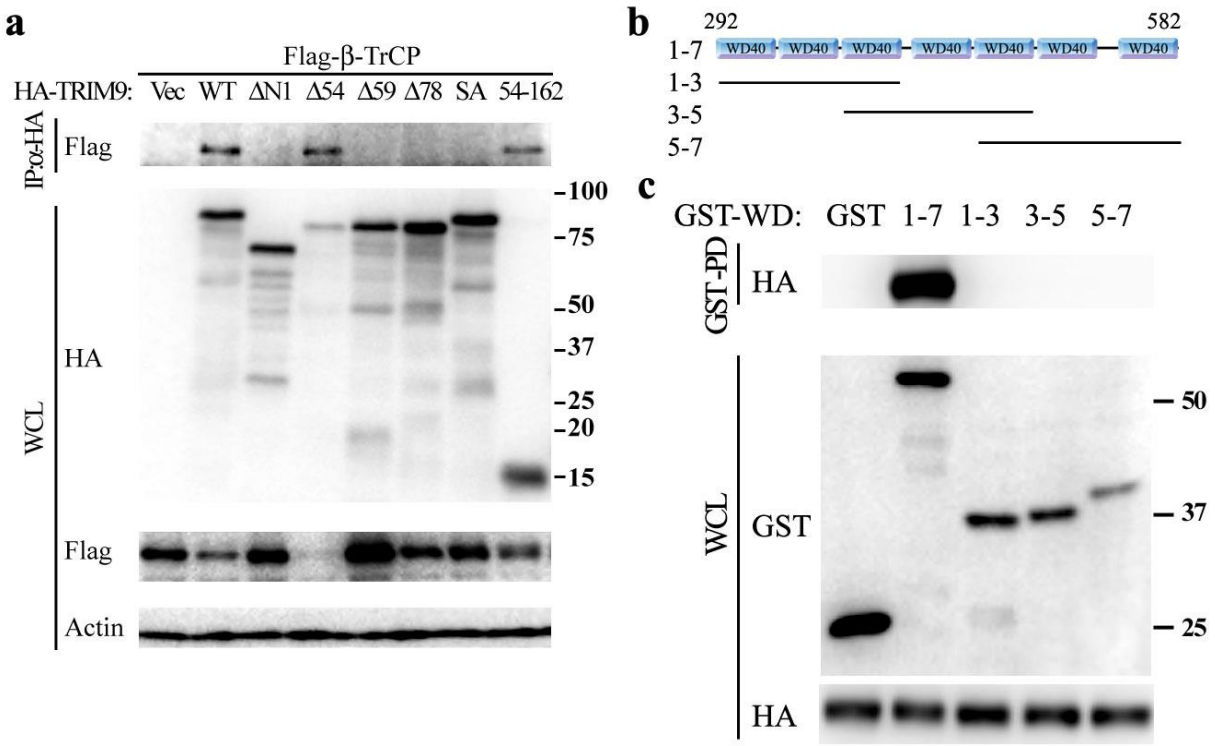


Supplementary Figure 1. TRIM9 does not affect AP-1, NF-AT or ISRE activity. (a,b) At 24h post-transfection with TRIM9 or vector and indicated reporter luciferase constructs, HEK293T cells were stimulated with PMA/Ionomycin (P/I) for 16h **(a)** or transfected together with RIG-I-2CARD **(b)** and cell lysates were then used for dual-luciferase assay. Data (mean and s.e.m.) are representative of at least three independent experiments.

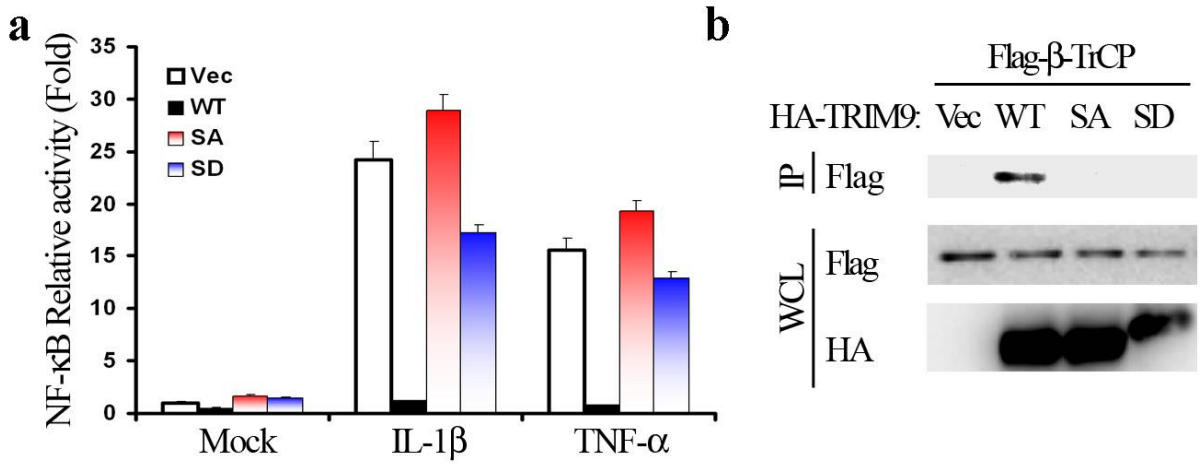

a
10
163
224
273
399
453
540
693
NF-κB inhibition
WT
RING
B1
B2
CC
COS
FN3
B30.2
+
ΔN1
ΔN2
ΔN3
ΔN4
ΔN5
ΔN6
ΔC1
ΔC2
ΔC3
ΔC4
ΔC5
ΔC6
Δ54
Δ77
Δ59
Δ78
CA
C30A C33A
SA
S76A S80A
b
Relative NF-κB activity (100%)
Vec
WT
N54
Δ59
Δ78
PMA/Iono
TNF-α
IL-1β
c
TRAF6
IKKε
TBK1
d
Vec
WT
CA
PMA/Iono
IL-1β
TNF-α
e
RIG-I
TRAF6
TBK1
IKKε
IKKβ

Supplementary Figure 2. The N-terminal of TRIM9 is critical for the NF-κB inhibition. (a) Schematic diagram of TRIM9 mutants and summary of their NF-κB inhibition ability.

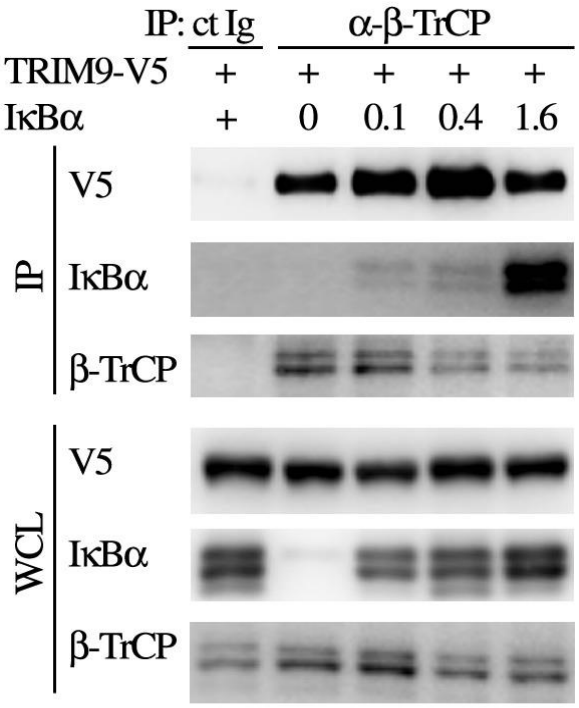
(b, c) HEK293T cells were transfected with NF-κB reporter, TK-Renilla reporter and TRIM9 WT or its mutant for 24h, followed by treatment with PMA/Ionomycin, TNF-α or IL-1β for 16h **(b)** or together with TRAF6, IKKε or TBK1 **(c)**. Cell lysates were used for dual-luciferase assay **(d, e)** HEK293T cells were transfected with NF-κB reporter, TK-Renilla reporter and TRIM9 WT or its CA (C30A C33A) mutant for 24h, followed by treatment with PMA/Ionomycin, TNF-α or IL-1β for 16h **(d)** or together with RIG-I (2 CARD), TRAF6, TBK1, IKKε or IKKβ **(e)**. Cell lysates were used for dual-luciferase assay. Data (mean and s.e.m.) are representative of at least three independent experiments.



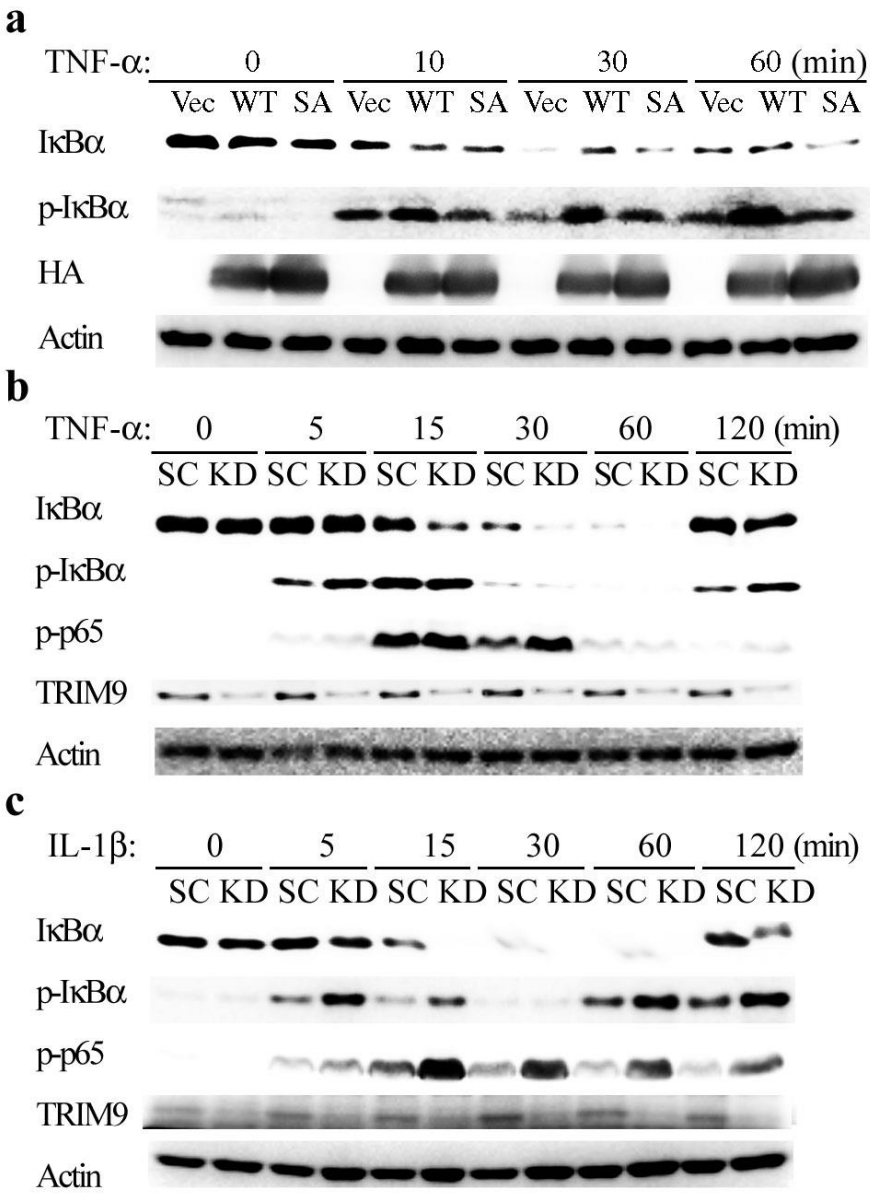
Supplementary Figure 3. The C-terminal 7 WD40 motifs of TrCP are required for TRIM9 binding. (a) At 48h posttransfection with Flag-β-TrCP together with HA-TRIM9 WT or its mutants, HEK293T cells were used for IP and IB with the indicated antibodies. **(b)** Schematic diagram of the GST-β-TrCP WD40 (1-7) repeats and deletion mutants. **(c)** At 48h post-transfection with mammalian GST-TrCP C-terminal fusion construct and HA-TRIM9, HEK293T cell lysates were subjected to GST-Pull-down (PD) and IB analysis. The data are representative of three independent experiments.



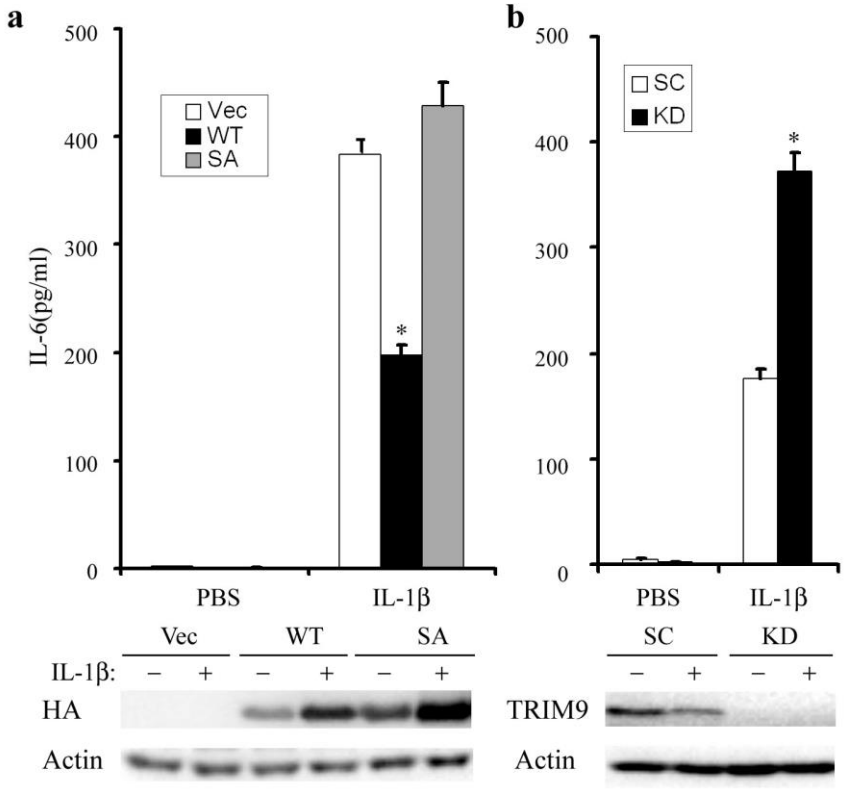
Supplementary Figure 4. **Both SA, and SD mutant fail to bind β-TrCP and to inhibit NF-κB activity**. **(a)** HEK293T cells were transfected with NF-κB reporter, TK-Renilla reporter and TRIM9 WT SA or SD mutant for 24h, followed by stimulation with mock, IL-1β or TNF-α. Cell lysates were used for dual-luciferase assay. **(b)** At 48h post transfection with Flag-β-TrCP and HA-TRIM9 WT, SA or SD mutant, HEK293T cell lysates were subjected to IP and IB analysis. Data (mean and s.e.m.) are representative of at least three independent experiments.



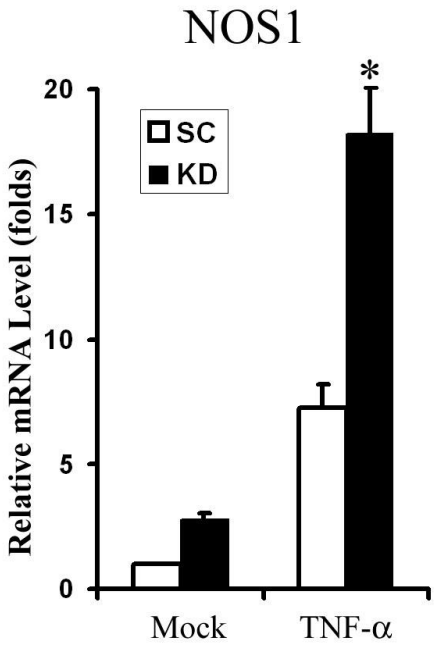
Supplementary Figure 5. **IκBα does not complete with TRIM9 for β-TrCP binding**. At 48h post transfection with Flag-β-TrCP and V5-TRIM9 and increasing amounts of IκBα (0-1.6 μg), HEK293T cell lysates were used for IP with anti-β-TrCP, followed by IB with anti-IκBα, anti-β-TrCP or anti-V5 antibody. The data are representative of three independent experiments.



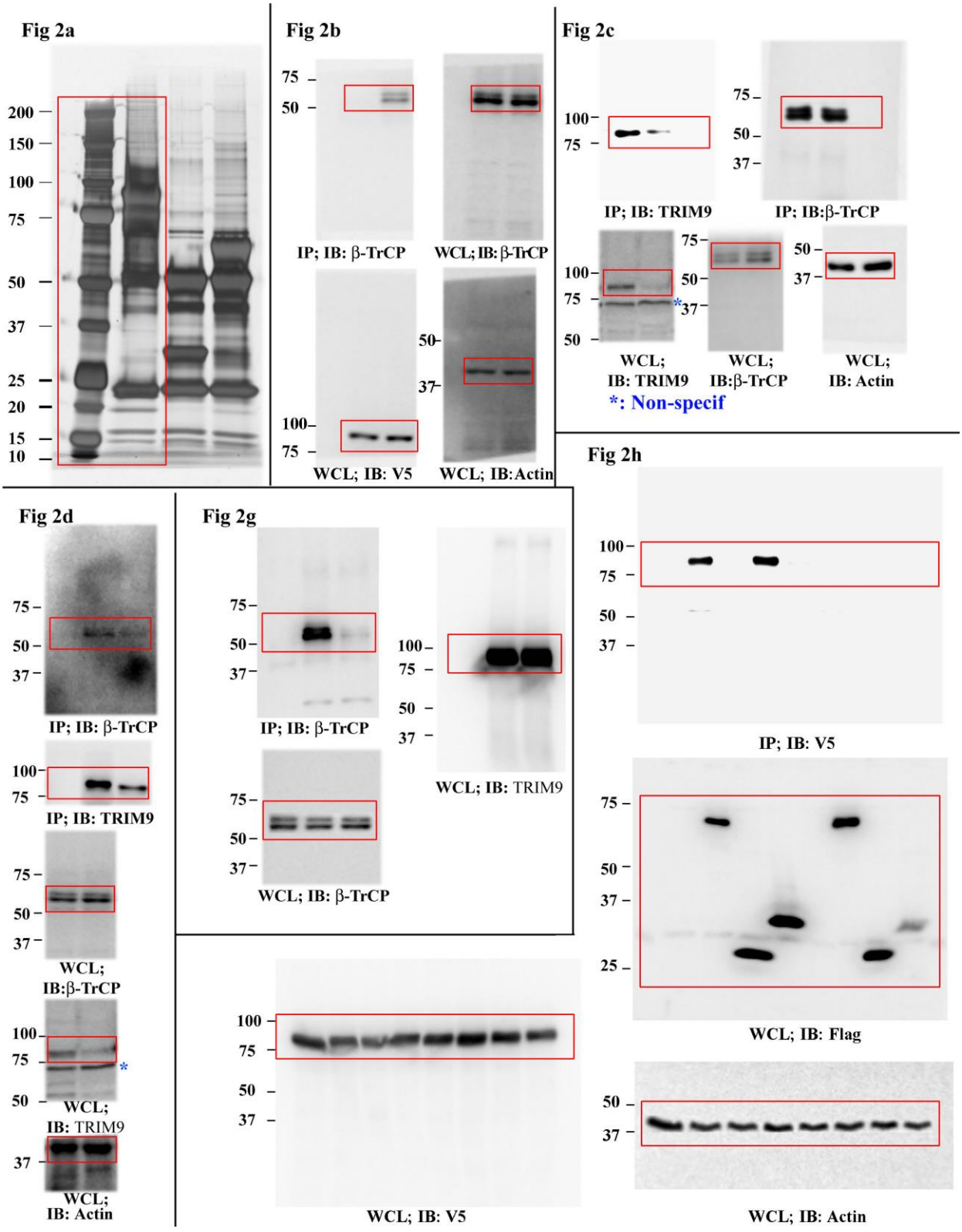
Supplementary Figure 6. TRIM9 suppresses IκBα degradation and p65 phosphorylation. (a) At 24 h posttransfection with TRIM9 or its SA mutant, HEK293T cells were stimulated with TNF-α for indicated times (min) and cell lysates were used for IB with the indicated antibodies. **(b** and **c)** A549 cells carrying lentivirus containing scrambled shRNA (SC) or TRIM9-specific shRNA (KD) were stimulated with TNF-α or IL-1β for indicated times and cell lysates were then used for IB with the indicated antibodies. The data are representative of three independent experiments.



Supplementary Figure 7. TRIM9 inhibited IL-6 production in SK-N-AS cells. (a,b) SK-N-AS cells stably expressing vector, TRIM9 wt or SA mutant (a) or carrying lentivirus containing scrambled shRNA (SC) or TRIM9-specific shRNA (KD) (b) were stimulated with PBS or IL-1β for 16 hrs and their supernatants were used for IL-6 ELISA. TRIM9 expressions were analyzed by IB (lower panel). The mean±s.d. is shown: *$p<0.05$. The data are representative of three independent experiments.

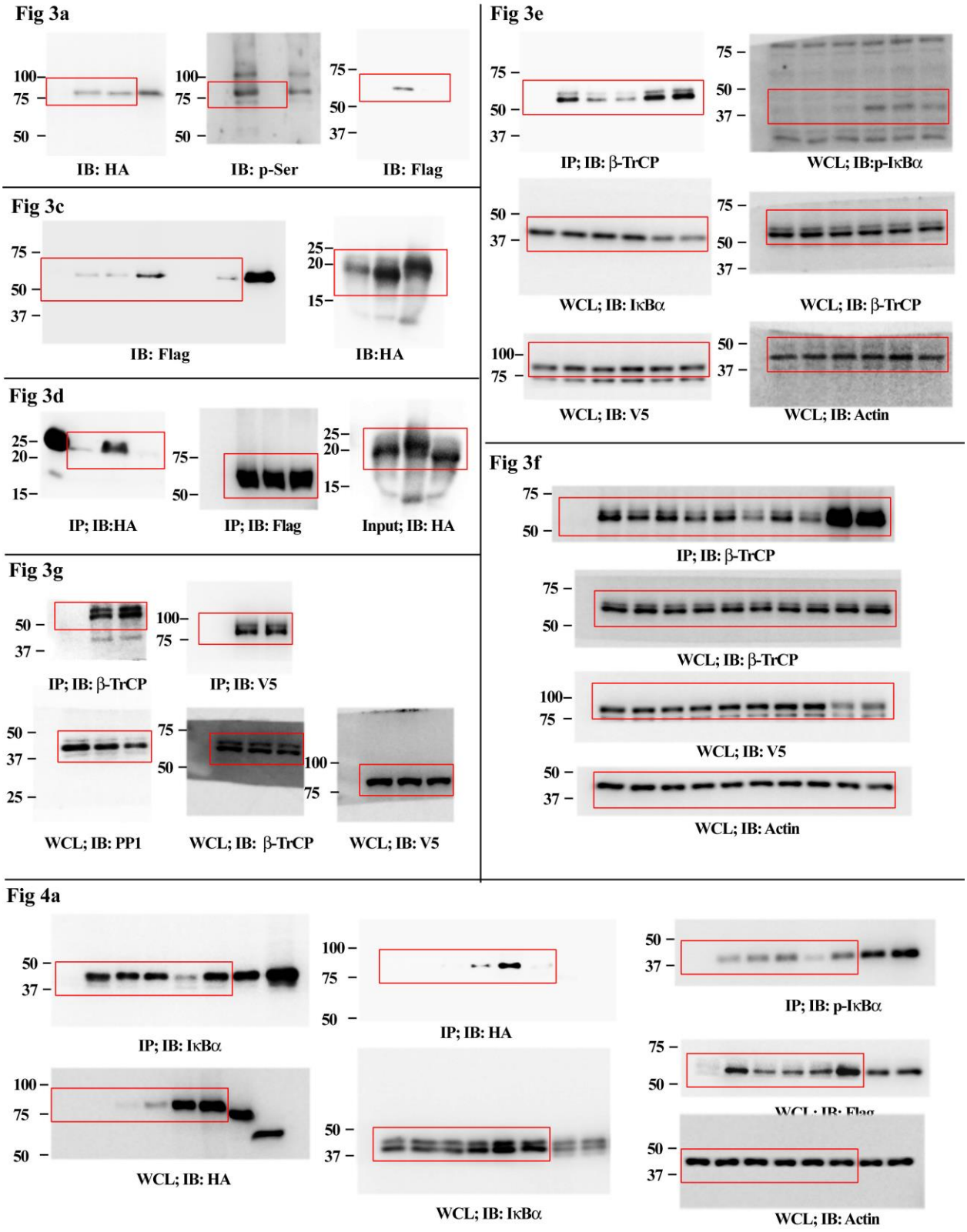


Supplementary Figure 8. Q-PCR analysis for NOS1 mRNA levels of primary neuron cells. At 48h post infection with scramble (SC) lentivirus or TRIM9-specific shRNA lentivirus, primary rat neuron cells were stimulated with TNF-α for 2h, followed by Q-PCR analysis for NOS1 mRNA levels. The mean±s.d. is shown: *$p<0.05$, The data are representative of three independent experiments. .



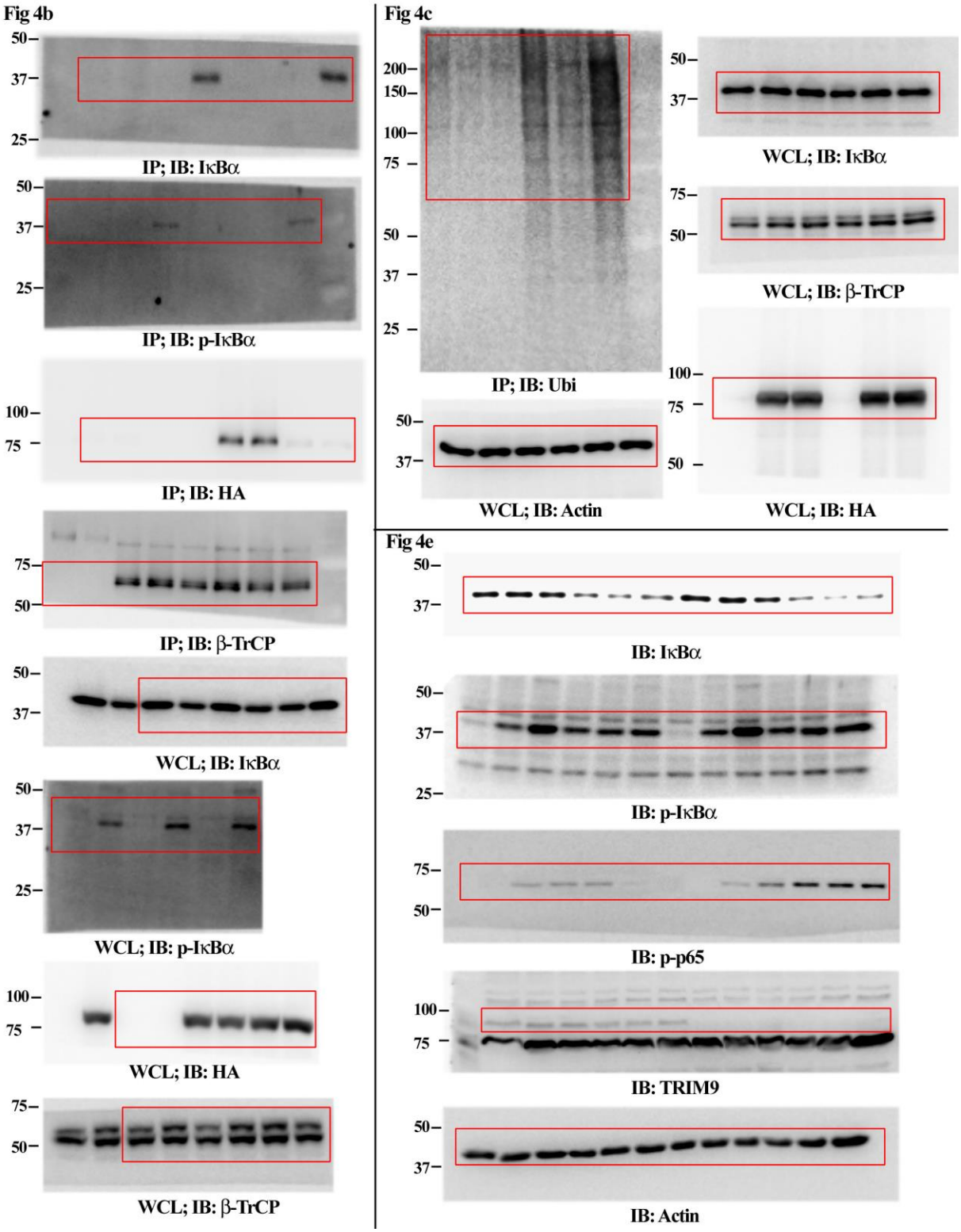
Supplementary Figure 9.

Full scan image of the Western blots used in the manuscript for Fig 2.



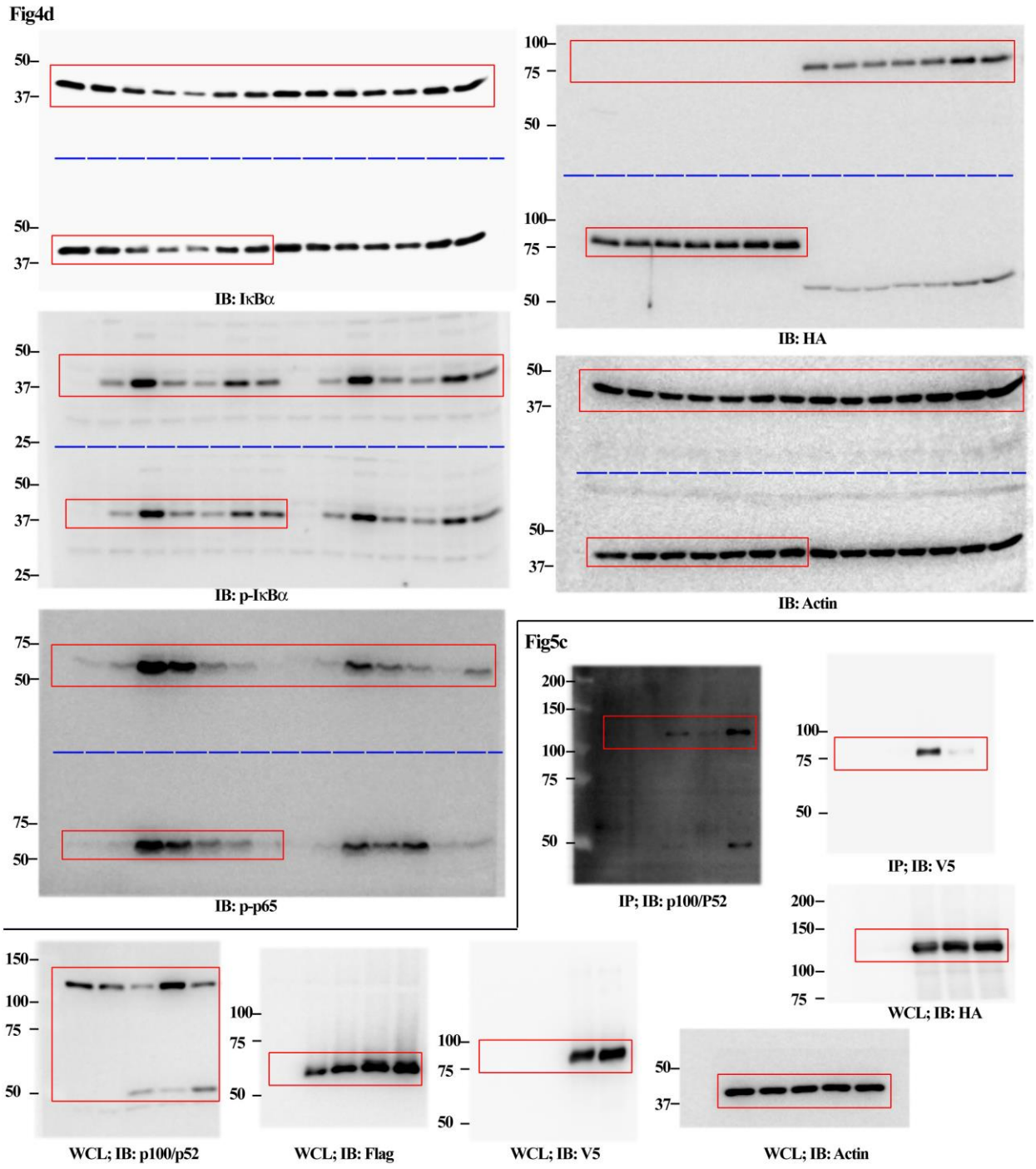
Supplementary Figure 9 (Continued).

Full scan image of the Western blots used in the manuscript for Fig 3 and Fig 4a.



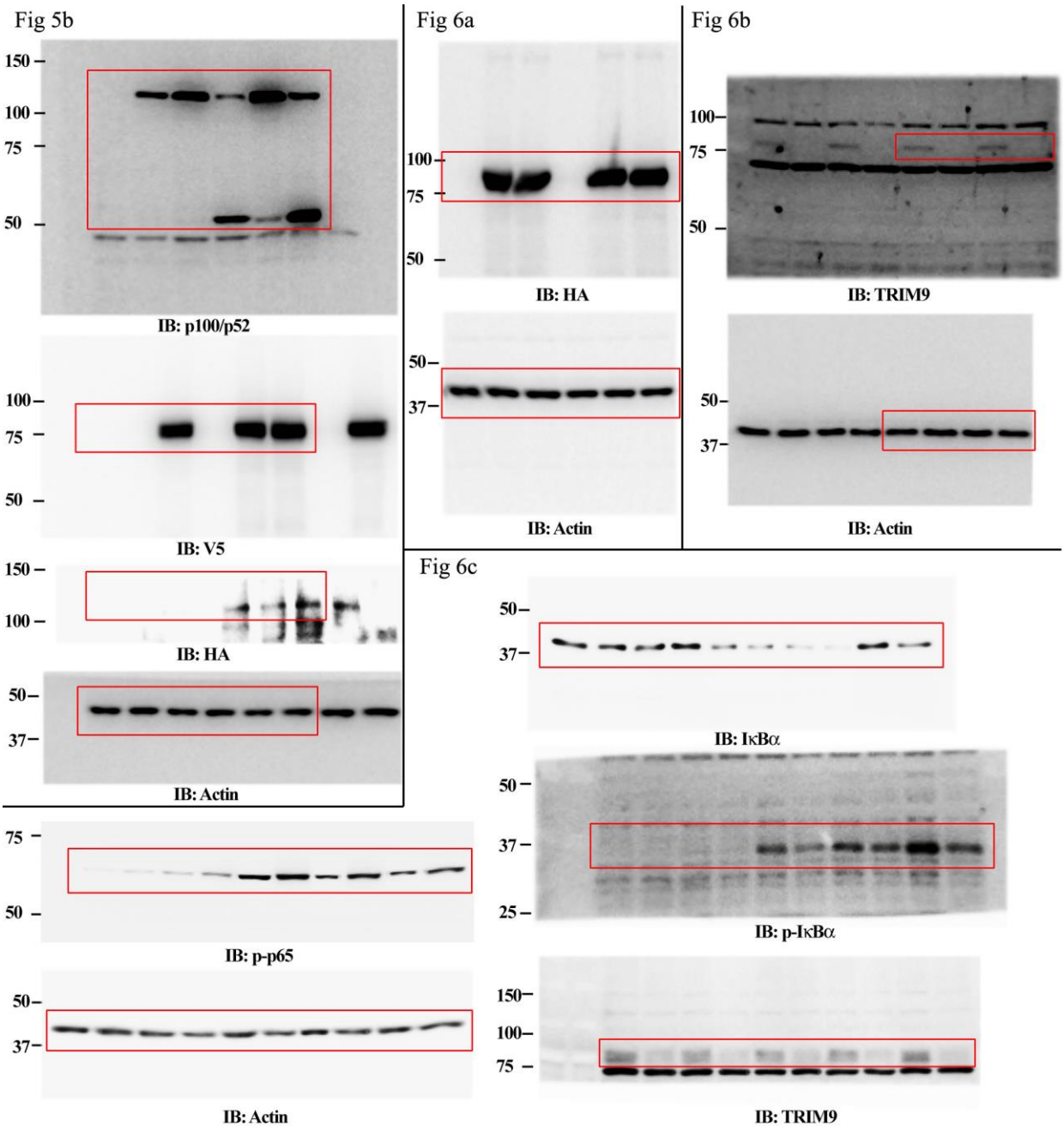
Supplementary Figure 9 (Continued).

Full scan image of the Western blots used in the manuscript for Fig 4b, c and e.



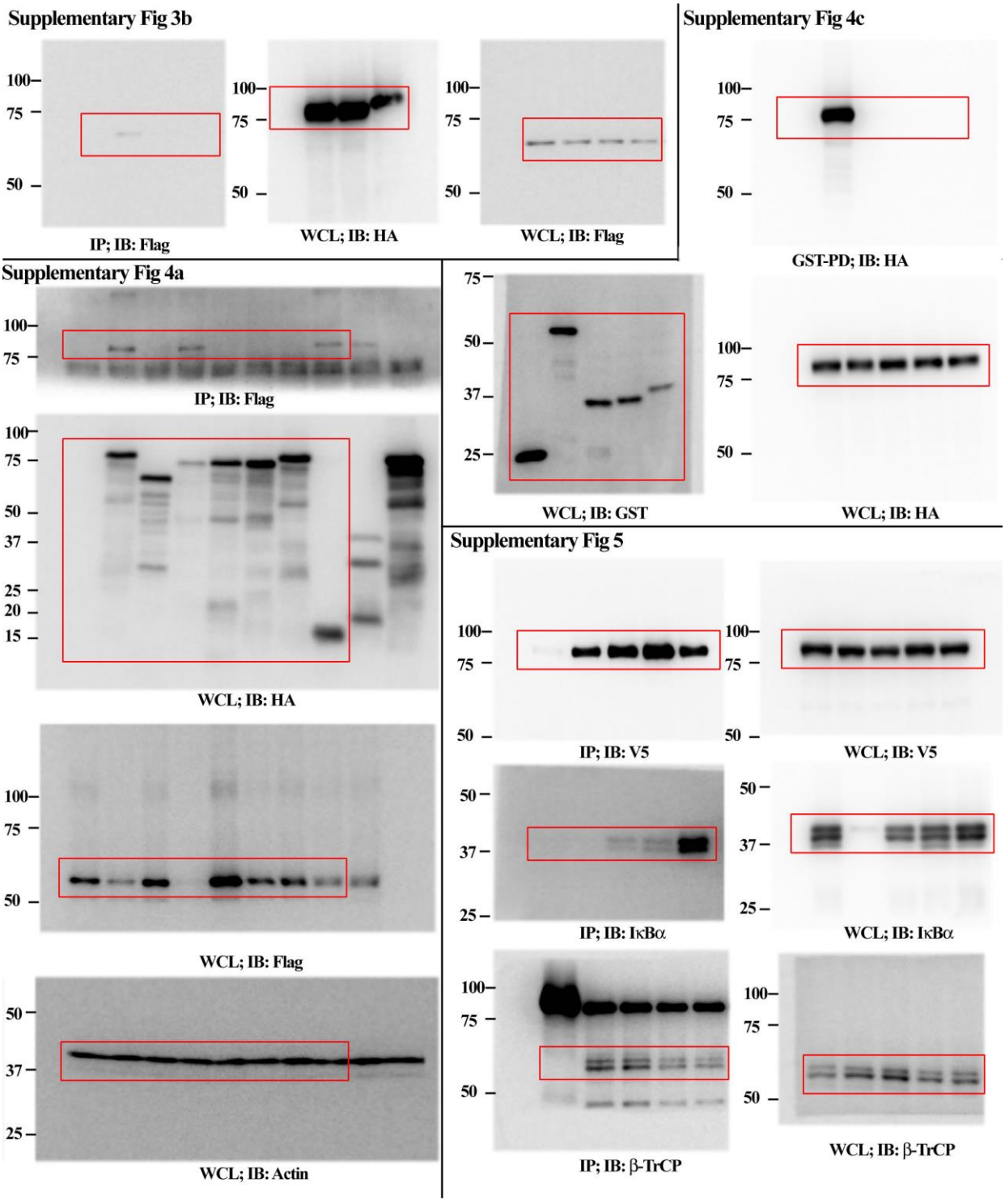
Supplementary Figure 9 (Continued).

Full scan image of the Western blots used in the manuscript for Fig 4d and Fig 5c.



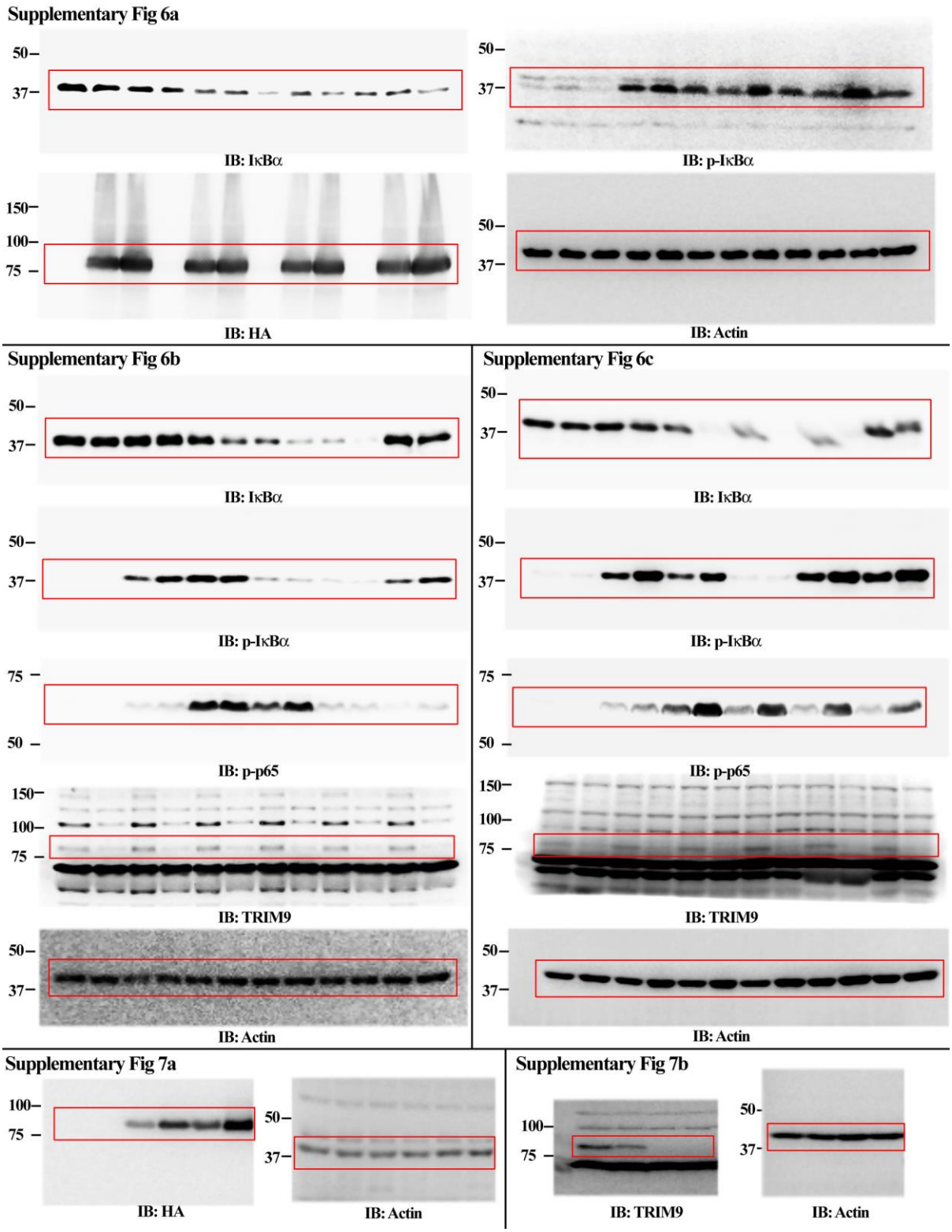
Supplementary Figure 9 (Continued).

Full scan image of the Western blots used in the manuscript for Fig 5b and Fig 6.



Supplementary Figure 9 (Continued).

Full scan image of the Western blots used in the manuscript for Supplementary Fig 3b, 4 and 5.



Supplementary Figure 9 (Continued).

Full scan image of the Western blots used in the manuscript for Supplementary Fig 6 and 7.