

Supplementary Figure Legends

Figure S1. TRIM2 is an interferon-stimulated gene.

(A-B): A549 (A) and HEK293T (B) cells were treated with IFN- α (100 ng/mL) for 0, 4, 8, 12, 24, and 36 h. TRIM2 protein levels were assessed by western blotting. (C-D): A549 (C) and HEK293T (D) cells were treated with IFN- γ (1 ng/mL) for 0, 4, 8, 12, 24, and 36 h. TRIM2 protein levels were assessed by western blotting. (E-F): A549 (E) and HEK293T (F) cells were treated with increasing concentrations of IFN- α (0, 1, and 100 ng/mL) for 24 h. TRIM2 protein levels were assessed by western blotting. (G-H): A549 (G) and HEK293T (H) cells were treated with increasing concentrations of IFN- γ (0, 0.1, and 10 ng/mL) for 24 h. TRIM2 protein levels were assessed by western blotting. β -actin served as a loading control. Densitometric quantification from three independent experiments is shown as means $\pm$ SEM. * p < 0.05, ** p < 0.01, *** p < 0.001 compared to the control group. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test or unpaired two-tailed Student's t-test.

Figure S2. The genotyping results of WT and TRIM2 KO mice.

(A) The targeting strategy of TRIM2 KO mice. (B) The genomic DNA was extracted and purified from mouse tail samples using the DNeasy Blood and Tissue Kit (Qiagen). The PCR was performed to identify the mutant mice (524 bp) using primers 1. (C) The PCR was performed to identify the wild type mice (443 bp) using primers 2.

Figure S3. Knockdown efficiency of TRIM2-targeting siRNAs in HEK293T cells.

Serum-starved HEK293T cells were transfected with either negative control siRNAs or TRIM2-targeting siRNAs. TRIM2 protein levels were assessed by western blotting 48 h post-transfection, with β -actin serving as a loading control. The representative

results are shown.

Figure S4. TRIM2 directly interacts with MDA5 and STAT2.

(A) HEK293T cells were co-transfected with His-TRIM2 and Myc-MDA5. His pull-down assays were performed using His-binding beads, and the eluted proteins were analyzed by western blotting with anti-Myc antibody. Input lysates were analyzed as controls. (B) HEK293T cells were co-transfected with His-TRIM2 and HA-STAT2. His pull-down assays were performed as described in (A), and the eluted proteins were analyzed by western blotting with anti-HA antibody. Representative results from three independent experiments are shown.

Figure S5. Endogenous TRIM2 depletion specifically stabilizes MDA5 and STAT2.

A549 cells were transfected with TRIM2-targeting siRNA or negative control siRNA (siNC). After 48 h, cell lysates were analyzed by western blotting using the indicated antibodies. β -actin served as a loading control. Densitometric quantification from three independent experiments is shown as means $\pm$ SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to the control group. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test or unpaired two-tailed Student's t-test.

Figure S6. TRIM2 modulates poly(I:C)-induced innate immune responses independently of RIG-I.

(A) WT or RIG-I KO HEK293T cells were transfected with empty vector or TRIM2-overexpressing plasmids (20, 200, and 400 ng), along with an IFN- β luciferase reporter plasmid. After 24 h, cells were stimulated with poly(I:C) for 12 h, and luciferase activity was measured. Data are presented as means $\pm$ SEM from three independent experiments. $**p < 0.01$, $***p < 0.001$. (B) WT or RIG-I KO HEK293T

cells were transfected with empty vector or TRIM2-overexpressing plasmids (1, and 2 μ g), or with control siRNA (siNC) or TRIM2-targeting siRNA (siTRIM2). After 24 h, cells were stimulated with poly(I:C) for 12 h, and the mRNA levels of ISG56, CCL5, and IL-6 were quantified by qPCR. Data are presented as means $\pm$ SEM from three independent experiments. $**p < 0.01$, $***p < 0.001$.

Figure S7. TRIM2 specifically promotes K48-linked polyubiquitination of MDA5 and STAT2.

(A) HEK293T cells were co-transfected with Myc-MDA5, Flag-TRIM2, and HA-tagged ubiquitin mutants (Ub-K6, Ub-K11, Ub-K27, Ub-K29, or Ub-K33). Cell lysates were immunoprecipitated with anti-Myc antibody and immunoblotted with anti-HA antibody. Input lysates were analyzed by western blotting with the indicated antibodies. (B) HEK293T cells were co-transfected with V5-STAT2, Flag-TRIM2, and the same panel of HA-ubiquitin mutants as in (A). STAT2 ubiquitination was analyzed by Co-IP with anti-V5 antibody and immunoblotted with anti-HA antibody. Data are representative of three independent experiments.

Figure S8. MDA5 and STAT2 are required for TRIM2's proviral effect during IAV infection.

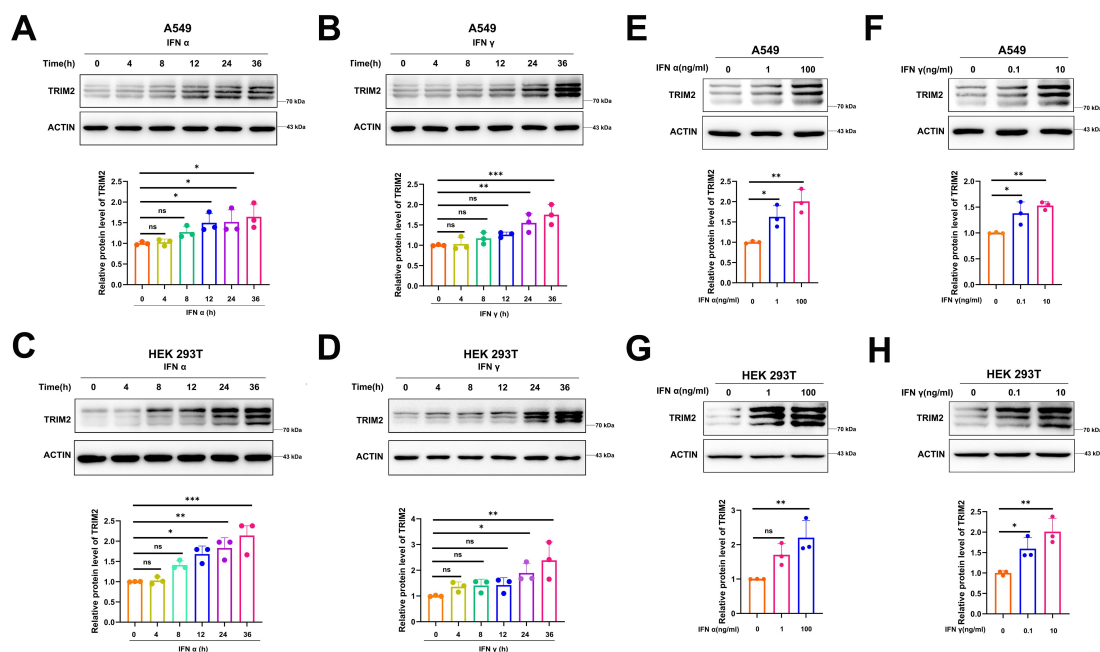
(A) PMA-differentiated THP-1 macrophage-like cells were treated with 100 ng/mL PMA for 48 h, then transfected with siNC or siTRIM2. After 24 h, cells were infected with PR8 virus (MOI = 1) for 24 h. Viral NP, MDA5, and STAT2 protein levels were assessed by western blotting. β -actin served as a loading control. (B) A549 wild-type (WT) or MDA5 knockout (KO) cells were transfected with empty vector or Flag-TRIM2. After 24 h, cells were infected with PR8 virus (MOI = 1) for 18 h. Viral NP protein levels were assessed by western blotting. (C) HEK293T cells were transfected with siNC, siTRIM2 alone, or siTRIM2 together with siSTAT2. After 48

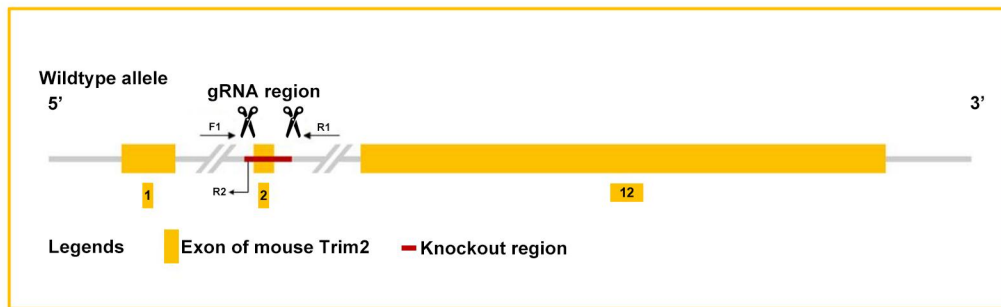
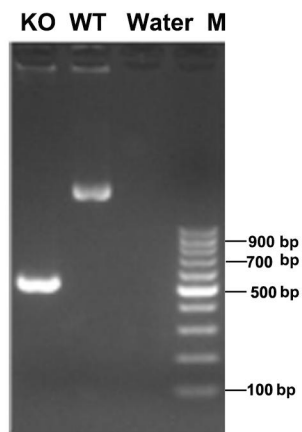
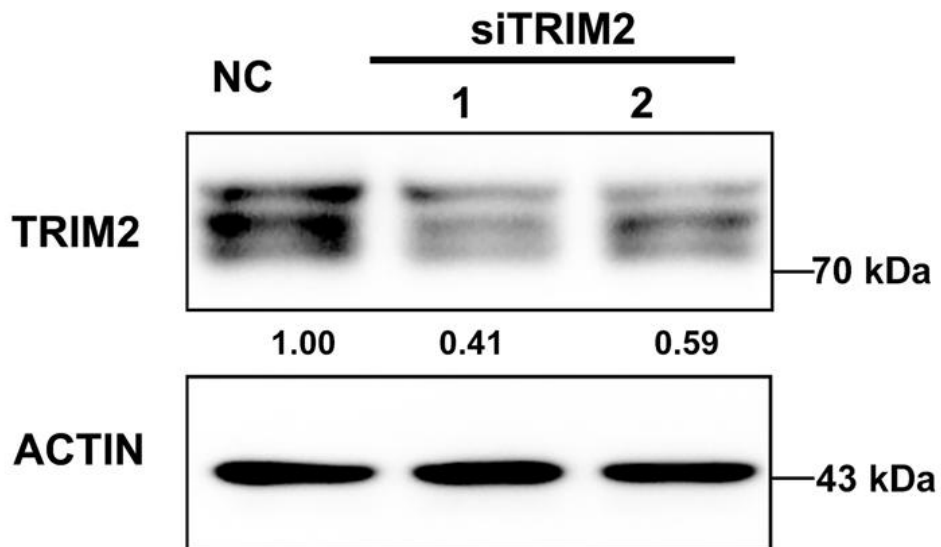
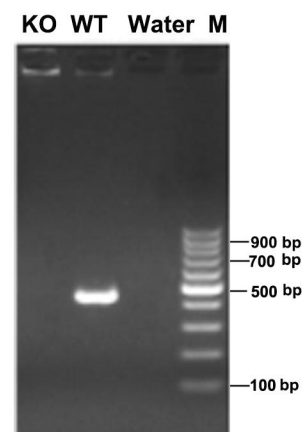
h, cells were infected with PR8 virus (MOI = 1) for 18 h. Viral NP protein levels were assessed by western blotting. β -actin served as a loading control. Densitometric quantification from three independent experiments is shown as means $\pm$ SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to the control group. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test or unpaired two-tailed Student's t-test.

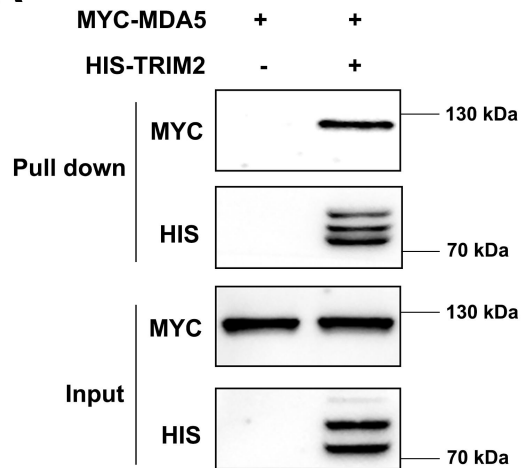
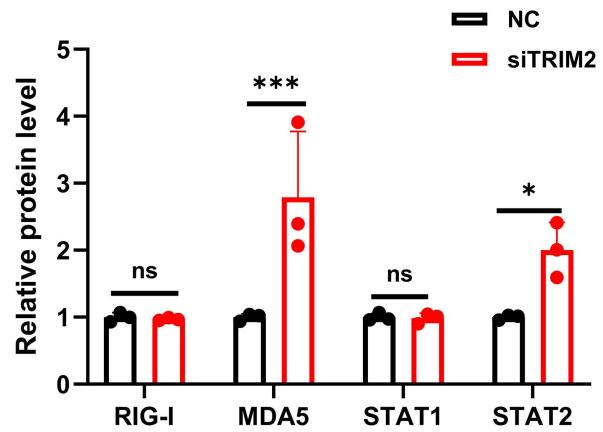
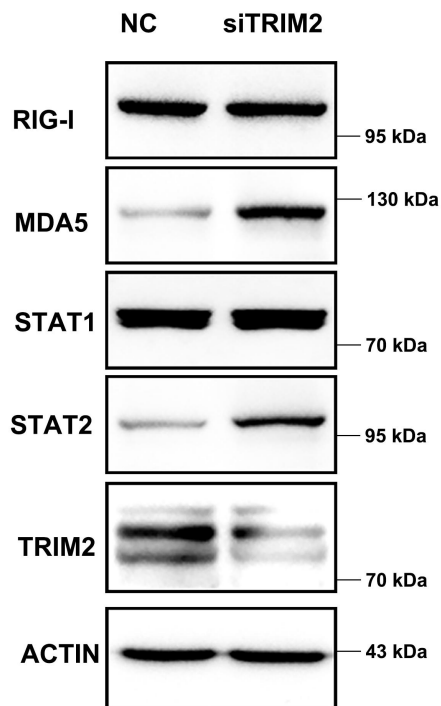
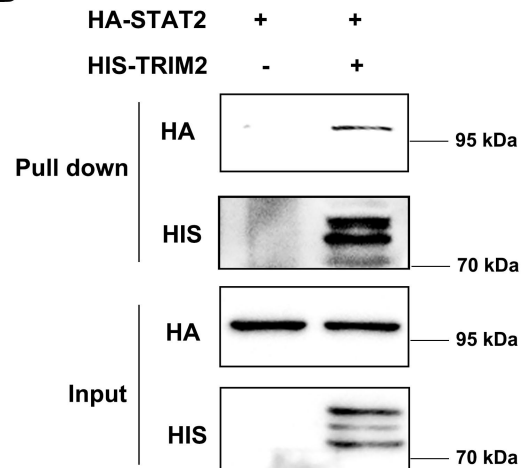
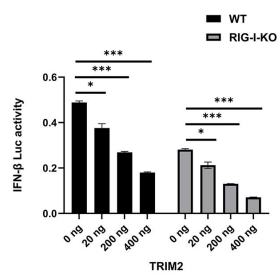
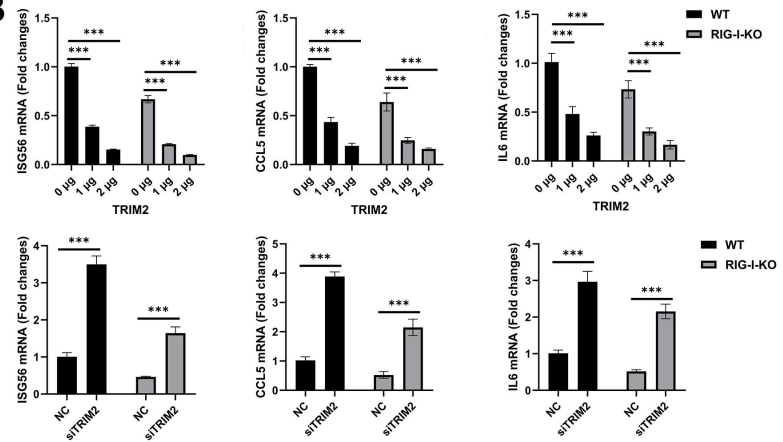
Figure S9. TRIM2 knockdown suppresses VSV replication.

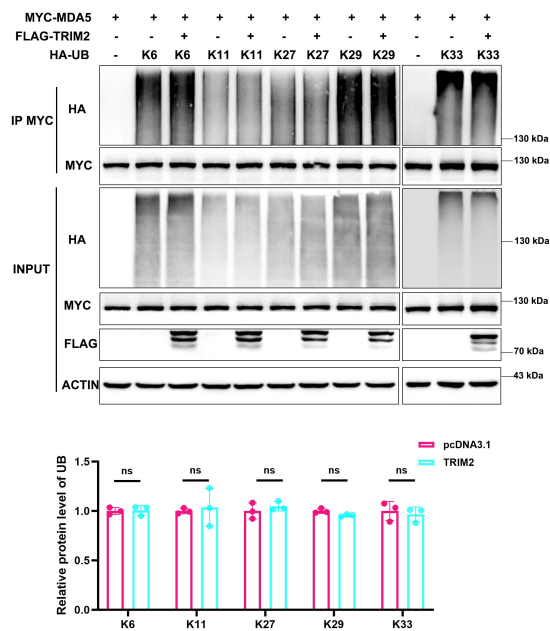
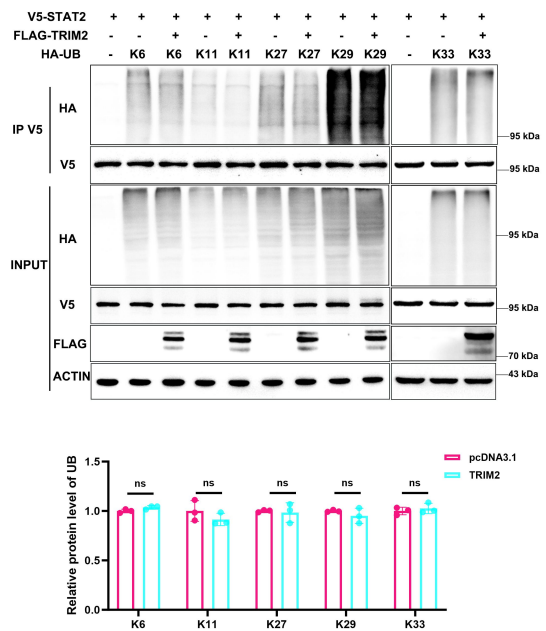
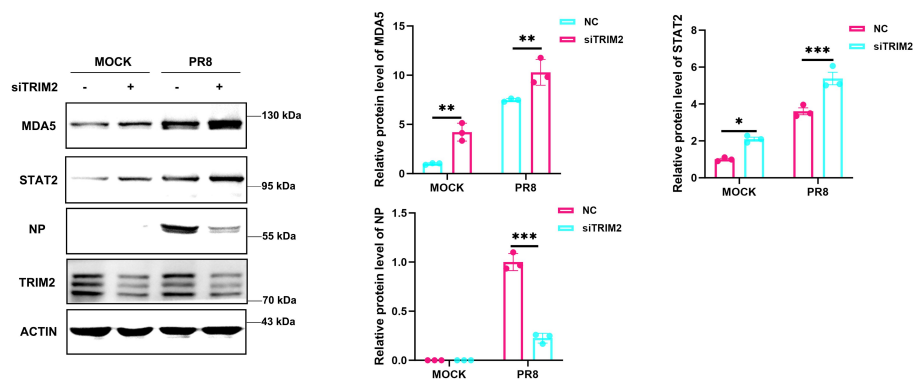
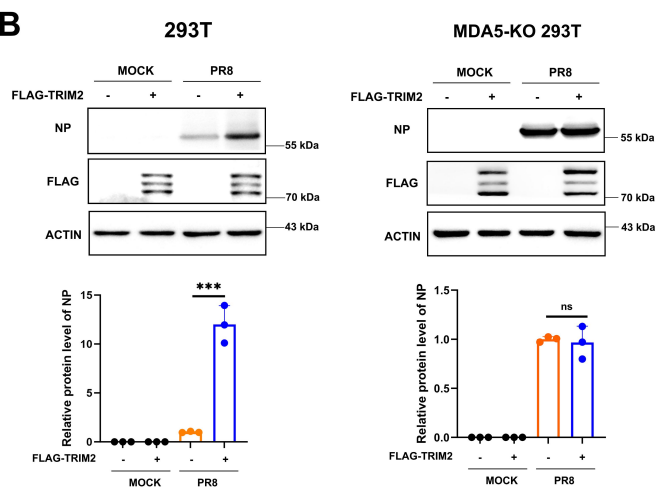
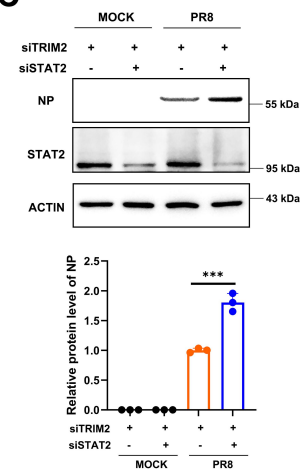
(A) Control (siNC) or TRIM2-knockdown (siTRIM2) A549 cells were infected with VSV (MOI = 0.1) for 24 h. VSV N protein mRNA levels were quantified by qPCR and normalized to GAPDH. Data are presented as means $\pm$ SEM from three independent experiments. $*p < 0.05$, $**p < 0.01$. (B) Control (siNC) or TRIM2-knockdown (siTRIM2) A549 cells were infected with VSV (MOI = 0.1) for 24 h. VSV G protein levels were assessed by western blotting. β -actin served as a loading control. Densitometric quantification of VSV G protein levels from three independent experiments is shown as means $\pm$ SEM. $*p < 0.05$, $**p < 0.01$.

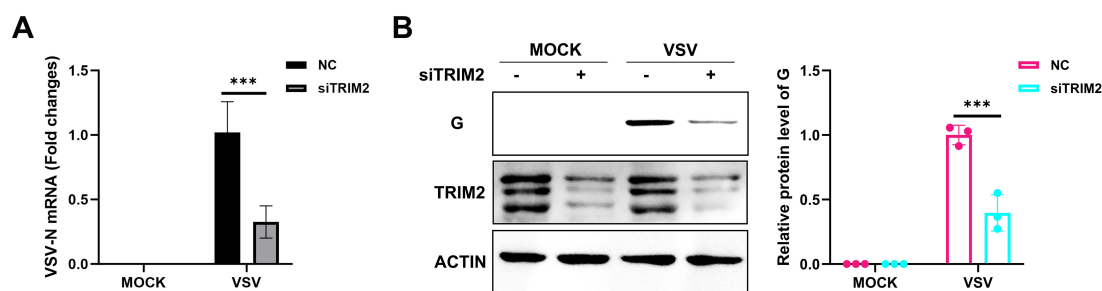
Supplementary Figures



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Supplementary Tables

Supplementary Table 1. Potential ubiquitination sites within helicase domain of MDA5.

Potential ubiquitinated lysine residues within helicase domain of MDA5 were identified using the dbPTM, PhosphoSitePlus®, and PTMGPT2 models, and were cross-referenced with published literature.

: Helicase

GMENNSSSESSFADSSVVSSESDTSLAEGSVSCLDESLGHNSNMGSDSGTMGSDSDSEENVAAARASPEPELQLRPYQMEVAQPALEG
 KNIIICLP TGSGKTRVAVYIAKDHLDDKKKASEPGKVIVLVNKKVLLVEQLFRKEFQPFLLKKWYRVIGLSGDTQLKISFPEVVKSCDIIISTA
 QILENSLLNLENGEDAGVQLSDFSLIIDECHHTNKEAVYNNIMRHYLMQ^{K465}LKNRLKKENKPVIPQLQILGTASPGVGGATKQAK
 AEEHILKLCANLDAFTI^{K519}TVKENLDQL^{K529}NQIQEPCK^{K538}FAIADATREDPFKEKLEIMTRIQTQCMSPMSDFGTQPYEQWAI
 QMEKKAKEGNRKERVCAEHLR^{K606}YNEALQINDTIRMIDAYTHLETIFYNEEKDKKFAVIEDDSDEGGDDEYCDGDEDEDLKKPL
 KLDETDRFLMTLFENNKMLKRLAENPEYENE^{K700}LTKLRNTIMEQYTRTEESARGIIFTKTRQSAYALSQWITENE^{K743}FAEVGVKA
 HHLIGAGHSSEF^{K764}PMTQNEQKEVIS^{K777}FRTGKINLLIATTVAEEGLDIKECNIVIRYGLVTNEIAMVQARGRARADESTYVLVAHS
 GSGVIEHETVNDFREKMMYKAIHCVQNMKPEEYAHKILELQMQSIMEKKMKT

Residue	Partial amino acid sequence near the mutation site	Source (predictor of protein ubiquitination sites or publications)
465	HYLMQ ^K LKNNR	Phosphosite;dbPTM
519	DAFTI ^K TVKEN	Phosphosite;dbPTM
529	NLDQL ^K NQIQE	Phosphosite;dbPTM
538	QEPCK ^K FAIAD	Phosphosite;dbPTM
606	AEHLR ^K YNEAL	Phosphosite;dbPTM
700	EYENE ^K LTKLR	Phosphosite;dbPTM
743	ITENE ^K FAEVG	Phosphosite;dbPTM;
764	HSSEF ^K PMTQN	Phosphosite;dbPTM;PTMGPT2
777	KEVIS ^K FRTGK	Phosphosite;dbPTM

Supplementary Table 2. The qPCR primers sequence used in this study.

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH/Human	AAC TTTGGCATTGTGGAAGG	ACACATTGGGGGTAGGAACA
GAPDH/Mouse	ACAAC TTTGGCATTGTGGAA	GATGCAGGGATGATGTTCTG
β-actin/Human	GCCGCCAGCTCACCAT	TCGATGGGGTACTTCAGGGT
IFN-β/Human	CAACAAGTGTCTCCTCCAAAT	TCTCCTCAGGGATGTCAAAG
IFN-β/Mouse	TGAATGGAAAGATCAACCTCACCTA	CTCTTCTGCATCTTCTCCGTCA
ISG56/Human	GCCTTGCTGAAGTGTGGAGGAA	ATCCAGGCGATAGGCAGAGATC
CCL5/Human	CCTGCTGCTTTGCCTACATTGC	ACACACTTGGCGGTTCTTTTCGG
IL-6/Human	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
IRF1/Human	GAGGAGGTGAAAGACCAGAGCA	TAGCATCTCGGCTGGACTTCGA
SOCS1/Human	TTTTTCGCCCTTAGCGTGA	AGCAGCTCGAAGAGGCAGTC
VSV-G	TGAATGTGCCTCGTTCAGATA	CCAAAGTCGATCAAATAAGGC
PR8-NP	GGCCGTCATGGTGGCGAAT	CTCAATATGAGTGCAGACCGTGCT