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Supplemental Information

Unanchored K48-Linked Polyubiquitin Synthesized by the E3-Ubiquitin Ligase TRIM6 Stimulates the Interferon-IKKε Kinase-Mediated Antiviral Response

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Supplementary Information

Supplementary Figure Legends

Supplementary Figure 1. LPS-mediated IFNβ and ISG54 mRNA expression is attenuated in hDCs upon TRIM6 silencing, related to Figure 1. (A) Representation of the TLR4, RIG-I and MDA5 signaling pathways investigated. **(B)** Monocyte-derived hDCs transduced with lentiviruses expressing TRIM6-specific shRNAs were stimulated with LPS. **(C-E)** Each condition was repeated in three different donors. At 2 h post treatment, cells were harvested and their TRIM6, IFNβ, ISG54, TNFα, IL-6 and IL-8 mRNA levels determined by RT-qPCR and plotted as percentage of control or fold induction over mock-induced samples, as indicated.

Supplementary Figure 2. Specific TRIM6 silencing reduces IFNβ production upon poly I:C stimulation, related to Figure 2. A549 cells were transiently tranfected with a non-targeting control or TRIM6-specific siRNAs. Forty h p.t., cells were either mock-treated or stimulated with poly I:C for 24 h. **(A)** Mock-treated cells were harvested for RT-qPCR analysis of TRIM6, TRIM1, TRIM5, TRIM13, TRIM25 mRNA to confirm specific silencing. **(C)** Suppernatants from stimulated cells were collected for IFNβ ELISA**. (B)** HEK-293T cells were transiently tranfected with a non-targeting control or TRIM6-specific targeting siRNA sequence. Silencing efficiency was confirmed by IB.

Supplementary Figure 3. IKKε interacts with TRIM6 *in vitro* and co-localize in hDCs, related to Figure 3. (A) Monocyte-derived human DCs mock-treated or stimulated with IFNβ for 45 minutes or 6 hr or infected with SeV for 6 hr were fixed and stained with anti-TRIM6 (red), anti-IKKε (green) and DAPI (nucleus, blue). Co-localization was determined by confocal microscopy. **(B)** *In vitro* binding assay. Baculovirus-produced recombinant FLAG-IKKε protein was incubated with GST-TRIM6-RBCC or GST-TRIM6-SPRY *in vitro* followed by immunoprecipitation with anti-FLAG beads.

Supplementary Figure 4. TRIM6 silencing enhances expression of IFNγ-inducible genes and IFNβ does not activate TBK1 phosphorylation, related to Figure 4.

(A) A549 cells were transiently tranfected with a non-targeting control or TRIM6-specific targeting siRNA sequence. Forty h p.t., cells were mock-treated or stimulated with IFNγ (500 U/ml). Cells were harvested at the indicated time points for RT-qPCR analysis. **(B)** hDCs transduced with lentiviruses expressing TRIM6 shRNAs were stimulated with IFNβ (100U/ml) and subjected to IB analysis.

Supplementary Figure 5. IKKε interacts with K48-linked poly-ubiquitin chains synthesized by TRIM6 in the lungs of influenza virus infected mice *in vivo*, related to Figure 5.

(A) Western blot analysis of TRIM6 silencing in MEFs using peptide-conjugated phosphorodiamidate morpholino oligomers (PPMO). **(B)** Schematic representation of *in vivo* experiments. BALB/c mice were infected i.n. with influenza A virus PR8 strain (1000 PFU). Lungs were collected at the indicated time points p.i. Half of each lung was used to determine **(C)** viral load by plaque assay and **(E)** IP of IKKε followed by IB with indicated antibodies. **(D)** The other half of the lung was used for RT-qPCR analysis for influenza A virus M1, IFNβ, TRIM6 and IFN inducible gene ADAR1 mRNA levels. **(F)** Schematic representation of TRIM6 silencing *in vivo*. BALB/c mice were treated with TRIM6-targeting PPMO, non-targeting control PPMO or vehicle only (PBS) (day -2). This treatment was repeated 24 hr later (day -1) followed by influenza PR8 infection (1000 PFU) (day 0). At indicated time points p.i. lungs were collected for RT-qPCR **(G)**. Results are from a single experiment ($n = 3$ for influenza A virus infected samples) and error bars indicate SD. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ by T-test. **(H)** Lanes from the immunoblot shown in Figure 5H for IP with the samples of the PPMO treated mice were quantified using ImageJ and normalized to the lowest value within each individual blot (arbitrarily set to 1). Results were plotted as bar graphs.

Supplementary Figure 6. IKKε interacts with K48-linked unanchored polyubiquitin chains in cell-culture, *in vivo* and *in vitro*, related to Figure 6

(A) Schematic overview of the denaturing pulldown assay. **(B)** Schematic overview of the assay for unanchored polyubiquitin chains using isopeptidase T/USP5 (IsoT), which specifically degrades unanchored Ub through recognition of its exposed C-terminal diglycine residues, while not deconjugating attached Ub chains. **(C)** WCE of HEK-293T cells, transfected with FLAG-IKKε plasmid together HA-TRIM6 were subjected to FLAG-IP. The beads which contained poly-ubiquitin chains bound to FLAG-IKKε were incubated with or without IsoT for 1 hr at 30°C and analyzed by IB. **(D)** WT or *Ifnar1-/-* mice were infected i.n. with influenza A virus PR8 strain (1000 PFU). Lungs were collected at the indicated time points, lysed, and subjected to IP with anti-IKKε or isotype control antibody. The beads containing poly-ubiquitin chains bound to IKKε from samples at day 3 p.i. were incubated with IsoT for 1hr at 30°C (last 2 lanes). Samples were analyzed by IB with indicated antibodies. **(E)** *In vitro* binding assay with ubiquitin chains. Baculovirus-produced recombinant FLAG-IKKε protein was incubated for 2 hr at 4°C with K48 or K63-linked poly-ubiquitin chains (2-7 Ub/chain), K48-linked poly-ubiquitin chains 2-16 Ub/chain, or tetra-ubiqutin with mixed linkages (K48-K643), followed by immunoprecipitation with anti-FLAG beads. **(F)** Formation of TRIM6 cytoplasmic bodies, but not colocalization with IKKε requires unanchored poly-ubiquitin chains. At 24 h p.t. with HA-TRIM6, FLAG-IKKε and His-IsoT, HeLa cells were stained with anti-His (green), anti-FLAG (red), anti-HA (violet) and DAPI (nucleus, blue). **(G)** WCE of HEK-293T cells, transfected with HA-TRIM6 or V5-TRIM25 were subjected to HA or V5 IP respectively. The beads containing HA-TRIM6 or V5-TRIM25 were incubated with or without IsoT for 1hr at 30°C and analyzed by IB.

Supplementary Figure 7. K48-linked poly-ubiqitin chains interact with the kinase domain of IKKε, promoting its oligomerization and activation. related to Figure 7.

(A) Purified FLAG-IKKε or a kinase-dead FLAG-IKKε mutant (K38A) were incubated with recombinant E1, UbE2K, and TRIM6 with mono-ubiquitin WT, K48R mutant or K48only mutant, in the presence of ATP. After 1 h incubation at 30°C, the reaction mixture was subjected to IB with the indicated antibodies. **(B)** Endogenous unanchored poly-Ub chains induce IKKε-mediated STAT1 phosphorylation. Schematic representation of the assay. **(C)** Schematic representations of the FLAG-IKKε deletion mutants used for CoIP. **(D)** WCE of HEK-293T cells transfected with FLAG-IKKε WT or FLAG-IKKε truncations or a FLAG-IKKε S172A mutant (transactivation loop) together with HA-TRIM6, were subjected to IP with anti-FLAG beads, followed by IB. **(E-F)** HEK-293T cells were transfected with ISG54 luciferase reporter plasmid together with empty vector, and increasing concentrations of FLAG-IKKε WT or FLAG-IKKε S172A **(E)**, and in the presence of TRIM6 **(F)**. At 24 h p.i., cells were lysed and subjected to luciferase assay. **(G)** IKKε oligomerization assay. Purified FLAG- IKKε protein was incubated with increasing amounts of K48-linked ubiquitin chains (2-7) in the absence of ATP (left panel) or with K48-linked ubiquitin chains (2-7 and 2-16 ub/chain) in presence of ATP (right panel) at 37°C for 15 minutes to allow formation of complexes, followed by native PAGE and IB using anti-FLAG antibody. RIG-I-YFP, which runs at 130 Kd, was used as a control of molecular weight. **(H)** TRIM6 interacts with JAK1 kinase. WCE of HEK-293T cells, that had been transfected with FLAG-JAK1 together with empty vector or HA-TRIM6 were subjected to IP with anti-FLAG beads (left panel) or anti-HA beads (right panel) and analysed by IB with indicated antibodies. Potential TRIM6 phorsphorylated bands oberved in the presence of JAK1 are indicated with arrows in the right panel. **(I)** WCE of HEK-293T cells transfected with FLAG-JAK1 or HA-TRIM6 were subjected to IP with anti-HA beads and analysed by IB with an anti-phospho-tyrosine or the indicated antibodies. **(J)** Proposed model of IKKε activation: TRIM6 and UbE2K cooperate in the synthesis of K48-linked unanchored poly-Ub chains, which activate IKKε for STAT1 phosphorylation and induction of a subset ISGs required for the antiviral response.

Supplemental Experimental procedures

Cell culture and Transfection

HEK-293T, HeLa and A549 were purchased from ATCC. *Ikbke*$^{+/+}$ and *Ikbke*$^{-/-}$ (IKKε knockout) MEFs were previously described (Tenoever et al., 2007). All cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin-streptomycin (Gibco-BRL). Transient transfections were performed with TransIT-LT1 (Mirus) or Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Plasmids

Reporter plasmids expressing firefly luciferase under the control of the ISG54-ISRE, NF-κB, and the IFNβ promoter were described previously (Kochs et al., 2007; Yoneyama et al., 1998). FLAG-tagged RIG-I(2CARD) was previously described (Nistal-Villan et al., 2010). The reporter plasmid carrying the *Renilla* luciferase gene (REN-Luc/pRL-TK) was purchased from Promega. The HA-TRIM6 plasmid was kindly provided by Andrea Ballabio (Reymond et al., 2001). TRIM6 mutants were generated by mutagenesis PCR and cloned in-frame with the HA-tag sequence into the pCAGGS-HA expression plasmid using KpnI and XhoI restriction sites. All sequences were confirmed by sequencing analysis (Genewiz, NJ).

Viruses

Sendai virus (SeV; Cantell strain) was obtained from Charles River Laboratories. Influenza A/Puerto Rico/8/1934 virus (PR8) virus titers were determined by plaque assay using MDCK cells. The PR8-GFP virus was described previously (Manicassamy et al., 2010).

IFNβ, NF-kB and ISG54 Luciferase Reporter Assay

HEK-293T cells were transfected in 24-well plates (Falcon, Becton Dickinson, NJ) with 50 ng of IFNβ, NF-kB or ISG54 reporter plasmid together with 20 ng of Renilla luciferase, 0.2-1 ng of stimulating plasmid (RIG-I[2CARD], IKKε, TBK-1) and 1-20ng of TRIM6 plasmid. Empty pCAGGs plasmid was used to bring the total amount of plasmid to 100ng/well. Plasmids were transfected using TransIT-LT1 (Mirus) at a ratio of 1:3. 30 h p.t., cells were lysed and a dual-luciferase assay was performed according to the manufacturer's instructions (Promega). Values were normalized to Renilla, and the fold induction was calculated as the ratio of stimulated samples, or samples transfected with inducing plasmid versus samples transfected with empty plasmid (no stimulation). WT and *Ikbke* $^{-/-}$ MEFs were transfected using Lipofectamine 2000 at a ratio 1:2.

Screen for E2 ubiquitin ligases

HA-TRIM6 was immuno-purified from HEK-293T cells using HA-beads and eluted with HA peptide. To determine the E2 ubiquitin ligase that cooperates with TRIM6 for synthesis of ubiquitin chains, E2 SCAN kit was used according to the manufacturer's instructions (Ubiquigent, Cambridge, USA). In brief, E1 enzyme UbE1, ubiquitination buffer, ubiquitin and 0.5 µl immuno-purified HA-TRIM6 protein were mixed and added to a 96-well plate containing each of the 29 E2 enzymes as described by the manufacturer. The reaction was initiated with the addition of ATP and incubated for 1h at 30°C. The reaction was stopped by addition of Laemmli sample buffer (BioRad) containing β-Mercaproethanol, and boiled for 5 min. The poly-ubiquitin was detected by western blot using anti-ubiquitin antibody.

Isolation and analysis of endogenous poly-ubiquitin chains

We followed a previously described protocol to isolate chains from RIG-I(2CARD) used by Zeng *et.al.* (Zeng et al., 2010). In brief, HEK-293T cells were transfected in 10cm dishes with 5μg of FLAG-IKKε plasmid alone or together with TRIM6. Cells were lysed in IP buffer, supplemented with 10mM N-ethylmaleimide (NEM) (Sigma). After centrifugation at 15,000xg, 5mM DTT was added to quench the NEM. FLAG-IKKε was isolated using FLAG M2 affinity gel (Sigma). The beads containing IKKε were incubated at 75°C/5 min. After centrifugation, the supernatant containing the poly-ubiquitin chains was used for *in vitro* kinase assay. For deubiquitinating enzyme treatment, the supernatants containing the dissociated poly-ubiquitin were incubated with 200nM of Isopeptidase T/USP5 (IsoT) (Boston Biochem) for 1h/30°C. These treated supernatants were used for *in vitro* kinase assay.

Confocal Microscopy

HeLa cells or hDCs were seeded into Lab-Tek II 8-well chamber slides (CC2 Glass slide, Nunc). After 12-16 h, plasmids harboring HA or GST-tagged TRIM6, FLAG-IKKε, and ubiquitin (His, FLAG or HA tagged) plasmids were transfected with Lipofectamine 2000 (Invitrogen) at a ratio 1:1. Six h p.t. the medium was changed. Twenty-four h later, cells were washed with PBS, fixed with methanol-acetone (1:1), permeabilized with 0.5% NP-40 (v/v) in PBS, and blocked with 0.5% BSA and 0.2% fish gelatin in PBS. For triple stains, cells were stained with directly conjugated mouse monoclonals anti-HA (Alexa Fluor 488 or 647, Cell Signaling), anti-FLAG (Alexa Fluor 555, Cell Signaling), anti-His (Alexa Fluor 488, Millipore), or anti-GST (Alexa Fluor 647, Cell Signaling) as well as DAPI (Invitrogen). For double stains, secondary anti-mouse or anti-rabbit antibodies conjugated to Alexa-fluor 488 and Alexa-fluor 555 (Invitrogen), respectively, were used to visualize the proteins. Images were taken on a Leica SP5 DM confocal

microscope (Leica Microsystems) at a magnification of 63x. Confocal laser scanning microscopy was performed at the MSSM-Microscopy Shared Resource Facility.

Electrophoretic mobility shift assay (EMSA)

Binding assays were performed with whole cell extracts in NP-40 lysis buffer (1% Nonidet P40, 50mM Tris pH 7.4, 5mM ETDA, 30mM NaF, 10% glycerol, supplemented with 1mM PMSF and protease inhibitor cocktail (Roche)). 50µg of protein extract were normalized in 10mM HEPES pH7.9, 2% glycerol, 40mM KCl, 1mM EDTA, 0.2mM $MgCl_2$, and 1mM DTT in a total volume of less than 15µl. 1µg of poly(dI:dC) (Sigma) was added to each sample to eliminate non-specific binding and the reaction was incubated on ice for 10min. 100000-300000 CPU of labeled probe was added and incubated at room temperature for 20min. Samples were resolved on a 6% native acrylamide gel. Gels were dried and exposed by autoradiogram. Probe sequences:

MxA: CCGCGCCGCGAAGAAATGAAACTCACA

OAS1: CCTTCTGAGGAAACGAAACCAACAGCA

ISG15: CTCGGGAAAGGGAAACCGAAACTGAAG

***In vitro* STAT1 kinase assay**

GST-tagged murine STAT1 (aminoacids 609-749) was purified from XL1blue bacteria and FLAG-tagged IKKε was isolated from HEK-293T with anti-FLAG beads. 0.1µl of purified Flag-tagged IKKε was incubated for 1h at 4˚C in 20mM Hepes pH7.5 and 10% glycerol with increasing amounts (14, 100, or 700 ng/µl) of commercial recombinant ubiquitin chains (Boston Biochem or K48-linked 2-16 from Millipore) or endogenous chains isolated as described above.

Subsequently, 5µg of GST-STAT1 and 1µl of [gamma ^{32}P]-ATP (3000Ci/mmol) were added and the kinase reaction was incubated for 30min at 30˚C in kinase buffer (25 mM Tris HCl, pH 7.5, 10mM $MgCl_2$, 5mM glycerophosphate, 2mM DTT, 1mM Na3VO4). The reaction was analysed by SDS-PAGE and visualized by Coomassie staining or auto-radiography. Baculovirus produced recombinant IKKε was purified as previously described (Tenoever et al., 2007).

Production of lentivirus VLPs for TRIM6 knockdowns

SIV VLPs were produced by Fugene6 (Roche) mediated co-transfection of HEK-293T cells with minigenome plasmid pSIV3+ and VSV-G envelope vector pHCMV-VSVG in a ratio of 5:1. Lentiviruses expressing anti-TRIM6 shRNAs were produced by transfecting HEK-293T cells with HIV gag-pol plasmid pCRV1-gag-pol, mini-genome plasmids harboring TRIM6-specific shRNA sequences (Santa Cruz biotechnology) and VSV-G envelope plasmid vector pHCMV-VSVG in a ratio of 5:5:1. At 48-72 h p.t. supernatants were collected, cleared by low-speed centrifugation and purified over a 20% sucrose cushion as previously described. Virus stocks were titered by exo-RT activity (EnzCheck, Invitrogen). A GFP expressing control virus was routinely produced, which was in addition tittered by TCID50 on 293T cells and hDCs in the presence of SIV VLPs. The lowest amount of SIV and GFP lentivirus required to transduce >95% of hDCs by FACS was used for transduction with anti-TRIM shRNA expressing lentiviruses produced together with the GFP control virus.

Isolation and differentiation of human DCs

Peripheral blood mononuclear cells were isolated from buffy coats of healthy human donors by Ficoll density gradient centrifugation. Buffy coats were obtained from the Mount Sinai Blood Donor Center and New York Blood Center. In brief, CD14+ cells were purified using anti-human

CD14 antibody-labeled magnetic beads and iron-based MiniMACS LS columns (Miltenyi Biotech). After elution from the columns, 2 x 10^5 cells were plated in 96-well plates in DC medium (RPMI medium [Invitrogen], 10% fetal calf serum [HyClone, Thermo Scientific], 2 mM L-glutamine, 1 mM sodium pyruvate, 50 µg/ml gentamycin, 100 U/ml penicillin, and 100 µg/ml streptomycin [Gibco, Invitrogen]) supplemented with 500 U/ml human granulocyte-macrophage colony-stimulating factor (hGM-CSF; Peprotech), and 1,000 U/ml human interleukin-4 (hIL-4; Peprotech).

TRIM6 knockdown in human DCs with shRNA lenti-viral vectors

Freshly isolated monocytes were transduced with VSV-G pseudo-typed SIV VLPs and shRNA plasmid containing VLPs for 3 h by spinoculation in the presence of 2 µg/mL polybrene (Sigma). Equal amounts of each virus were added, just sufficient to transduce >95% of the cells. Each condition was represented by two (donor 3 and 4) or four (donor 1 and 2) replicate wells. Subsequently, cells were washed, resuspended in DC medium supplemented with hGM-CSF and hIL-4, and incubated for 5 days at 37°C until stimulation. At 5 d post-transduction, hDCs were either induced with a final concentration of 0.4 ng/mL LPS (Alexis Biochemicals; 581-008-L002) or IFNβ 1000 U/ml.

siRNA-mediated knockdown

Transient knockdown of endogenous TRIM6 in A549 or HEK-293T cells, seeded in 24-well plates (30,000 cell/well), was achieved by transfection of 10 picomol of non-targeting (scramble) control or siRNA specific for TRIM6 (Invitrogen/Life technologies, sleath RNAi TRIM6-specific sequence targeting the 5'-UTR region of transcript variant 2; sense: GCUGCUUCAAGUCCUUGGCUCUGAU and antisense: AUCAGAGCCAAGGACUUGAAGCAGC). The RNAi was transfected with RNAiMAX (Invitrogen) according to the manufacturer's instructions. After 24 h, cells were transfected again

with the same siRNA amounts. After 6-8 h medium was replaced with fresh medium and cells were left for additional 12 h. TRIM6 knockdown efficiency was determined by qPCR using specific primers, and by IB with a specific TRIM6 antibody (Ab1, Sigma). For viral infections, cells were infected with SeV (10 HAU/ml) or PR8 influenza (MOI=2) viruses for the indicated time points. Cells were then harvested for qPCR or IB.

GST pull-down assay, immunoprecipitation, and immunoblot analysis

HEK-293T and human DCs were lysed in IP buffer (50 mM TRIS, pH8.0, 280 mM NaCl, 0.5% [v/v] NP40, Glycerol 10%, protease inhibitor cocktail [Roche], 10mM N-ethylmaleimide (NEM). GST pull-down and immunoprecipitations were performed using Glutathione Sepharose 4B (GE Healthcare), anti-FLAG M2 affinity gel (Sigma), mouse monoclonal anti-human IKKε (Abcam) or mouse monoclonal anti-mouse IKKε (Imgenex). Its important to note that for the ubiquitination assays in which IKKε and TRIM6 were co-expressed together, a very low amount of IKKε was used (and the amount of TRIM6 was carefully determined in titration experiments). Normally, about 400,000 HEK-293T cells were trasfected using TransIT-LT1 (Mirus) with 4ng of Flag-IKKε together with 50-200ng of HA-TRIM6 (or GST-TRIM6 as indicated) in 6 well plates.

For immunoblotting, proteins were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane (Immobilon-P, Millipore). The following primary antibodies were used: anti-FLAG (Rabbit,Sigma), anti-HA (Rabbit, Sigma), anti-GST (Rabbit) (Sigma), anti-β-actin (Abcam), anti-human IKKε (Rabbit, Abcam), anti-phosphoIKKε T500 Rabbit, Novus Biologicals), anti-TRIM6 (Rabbit, Ab1, Sigma), anti-phosphoIRF3 (S386) (rabbit, Epitomics), anti-ubiquitin K48-specific (Rabbit, Apu2, millipore), anti-ubiquitin K63-specific (mouse, HWA4C4, millipore), anti-ubiquitin (mouse, Enzo), anti-pSTAT1(Y701) (Rabbit, Cell Signaling), anti-pSTAT2(Y689)(rabbit, Upstate), anti-STAT2 (Rabbit, Santa Cruz).

Immunoblots were developed with the following secondary antibodies: Anti-rabbit IgG-HRP conjugated antibody and anti-mouse IgG-HRP from sheep (GE Healthcare; England). The proteins were visualized by an enhanced chemiluminescence reagent (Pierce).

TRIM6 knockdown *in vivo* using PPMOs

The peptide-conjugated phosphorodiamidate morpholino oligomers (PPMO) were produced by previously published methods (Abes et al., 2006). The TRIM6-targeting PMO sequence was: AAGCTTAGGACCGACCTGGTACTCC and was designed to specifically target the exon2-intron 2 junction of TRIM6 pre-mRNA, in an effort to interfere with splicing of pre-mRNA. The non-targeting PMO control sequence (NC-705) was: CCTCTTACCTCAGTTACAATTTATA, which has no perfect homology to mouse or influenza viral sequences. TRIM6 PPMO is designed to remove exon 2, and PPMO efficacy was assessed by RT-qPCR using primers designed to amplify the exon2 and exon3 region (primer sequences located in table in supplementary information). TRIM6-silencing by PPMO was also assessed by immunoblots. Immunoblots from the experiments with PPMO treated mice were quantified using ImageJ (National Institutes of Health). In brief, the entire surface from each blot presented in the figures was scanned, with lanes (indicated) based on the automatic detection of the software, and the signal then quantified within each set lane. The quantified values were normalized to the lowest value within each individual blot (arbitrarily set to 1).

RT-qPCR analysis

Total RNA was isolated using RNeasy kit (Qiagen) and subjected to DNAse digestion with Turbo DNase (Ambion). Reverse transcription was performed using the high capacity cDNA reverse transcription kit (Applied Biosystems). Real-time qPCR was performed in 384-well plates in triplicate using SYBR green I master mix (Roche) in a Roche LightCycler 480. Relative mRNA

values were calculated using the ΔΔCt method (Livak and Schmittgen, 2001) using 18S rRNA as internal control for human cells or β-actin for mouse cells, and plotted as relative values or fold change by normalizing to mock-control samples.

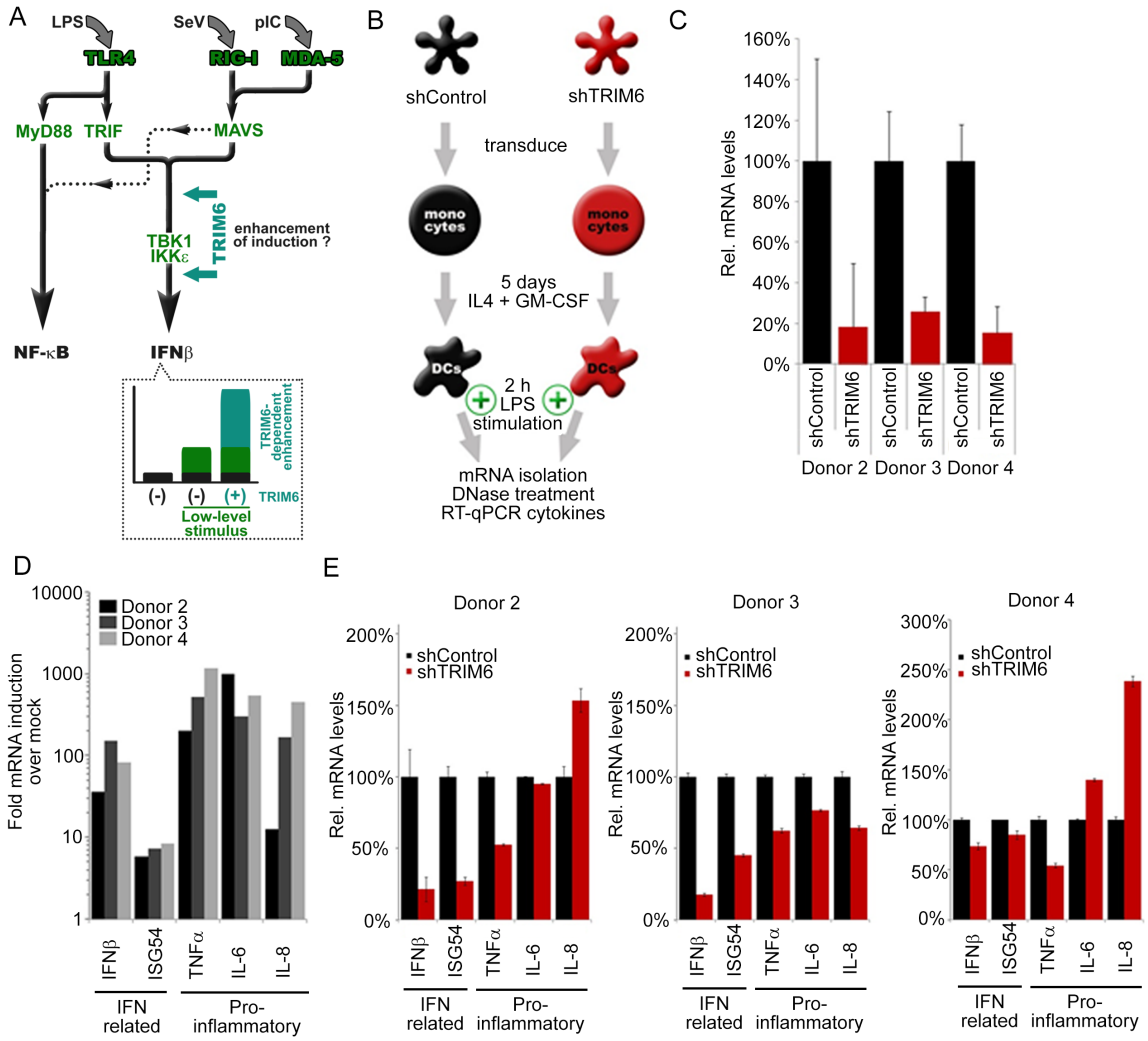
The PCR primers used were:

RT-qPCR primers

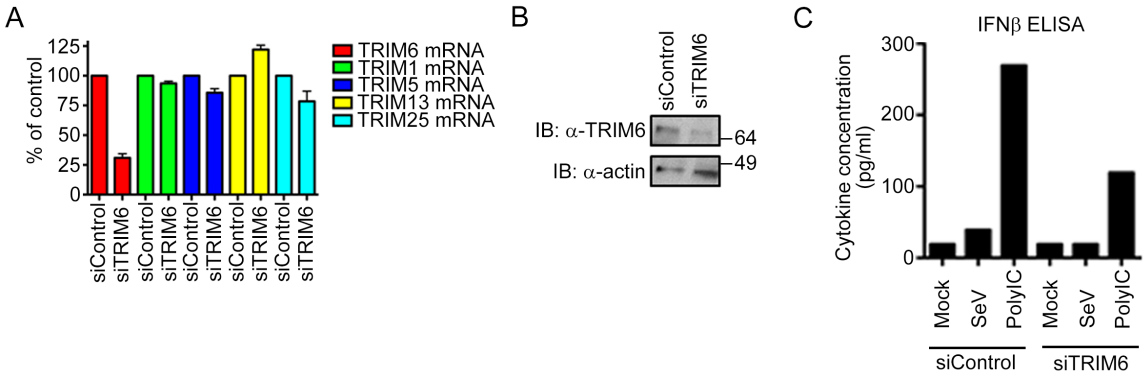
Target	Primer sequence
hTRIM6	Fwd: 5'-GGGGTATGCAGCAATTCACT-3' Rev: 5'-ACCCAATCACCCAGTATCCA-3'
hTRIM1	Fwd: 5'-CCACACCCCAGAGAGGTTTA-3' Rev: 5'-TATTTCCTGCTGCCCCATAG-3'
hTRIM5α	Fwd: 5'-AGCAATGAGCTGCAAAACCT-3' Rev: 5'-GCTGCACCATCTCAGTTTCA-3'
hTRIM25	Fwd: 5'-CGGATGACTGCAAACAGAAA-3' Rev: 5'-TCCTTGTCGAGGTGGTCTCT-3'
hTRIM13	Fwd: 5'-CAACGGATGGCCTTTAACAT-3' Rev: 5'-GGCTTGCAGGCAAATTAGAG-3'
Influenza-M1	Fwd: 5'-AGATGAGTCTTCTAACCGAGTCG-3' Rev: 5'-TGCAAAAACATCTTCAAGTCTCT-3'
hRIG-I	Fwd: 5'- GGCATGTTACACAGCTGACG-3' Rev: 5'- TGCAATATCCTCCACCACAA-3'
hMxA	Fwd: 5'- GTGGCTGAGAACAACCTGTG -3' Rev: 5'- GGCATCTGGTCACGATCCC -3'
hOAS1	Fwd: 5'- GATCTCAGAAATACCCCAGCCA-3' Rev: 5'- AGCTACCTCGGAAGCACCTT-3'
hSTAT1	Fwd: 5'- ACAGCAGAGCGCCTGTATTG-3' Rev: 5'- CAGCTGATCCAAGCAAGCAT-3'
18S rRNA	Fwd: 5'-GTAACCCGTTGAACCCCATT-3' Rev: 5'-CCATCCAATCGGTAGTAGCG-3'
hIFNβ	Fwd: 5'-TCTGGCACAACAGGTAGTAGGC-3' Rev: 5'-GAGAAGCACAACAGGAGAGCAA-3'
hTNFα	Fwd: 5'-AGTGAAGTGCTGGCAACCAC-3' Rev: 5'-GAGGAAGGCCTAAGGTCCAC-3'
hISG15	Fwd: 5'-TCCTGGTGAGGAATAACAAGGG-3' Rev: 5'-GTCAGCCAGAACAGGTCGTC-3'
hISG54	Fwd: 5'-ATGTGCAACCTACTGGCCTAT-3' Rev: 5'-TGAGAGTCGGCCCATGTGATA-3'
mActin	Fwd: 5'-CGGTTCCGATGCCCTGAGGCTCTT-3' Rev: 5'-CGTCACACTTCATGATGGAATTGA-3'

mIFNβ	Fwd: 5'-CAGCTCCAAGAAAGGACGAAC-3' Rev: 5'-GGCAGTGTAACTCTTCTGCAT-3'
mADAR1	Fwd: 5'-GGAAAGGCAAGGCGATGTCTA-3' Rev: 5'-GCTTCTTGTCCGTCAAGTACC-3'
mTRIM6	Fwd: 5'-CTCTTTTGCAAGGAGGATGG-3' Rev: 5'-CAGGCTCCACCTGACTCTTC-3'

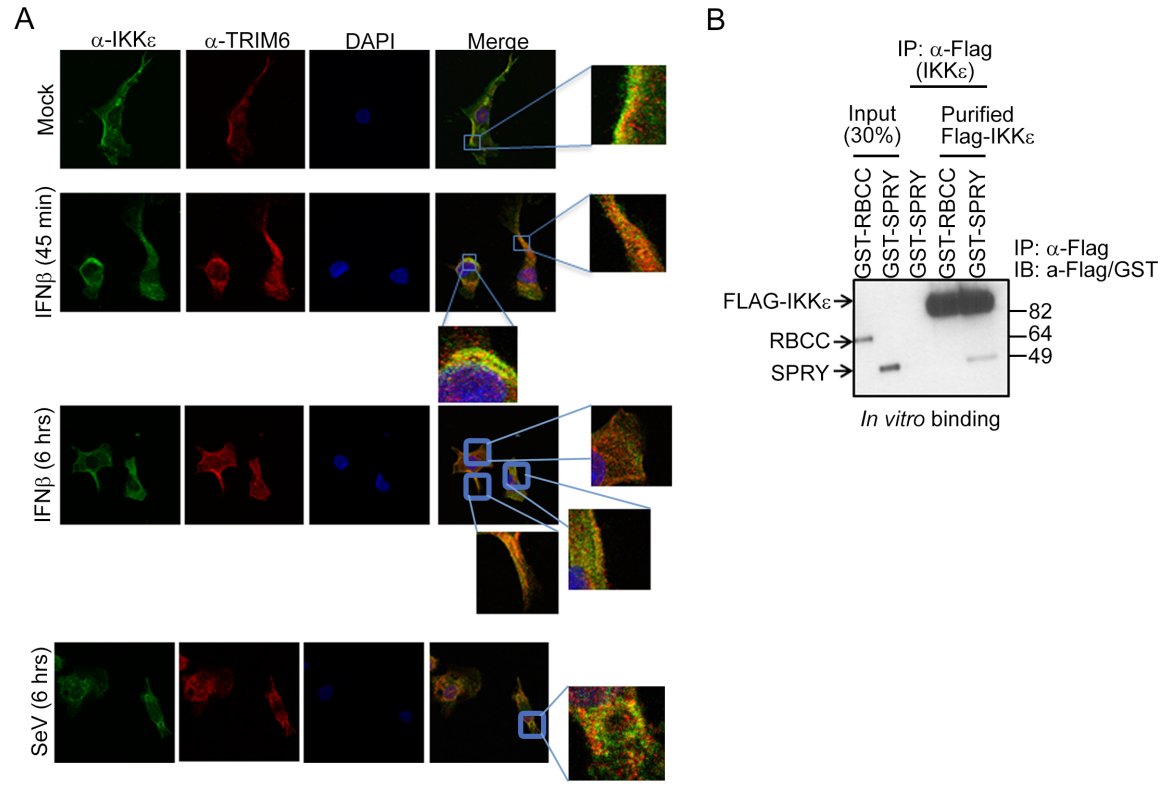
Supplementary Figure 1



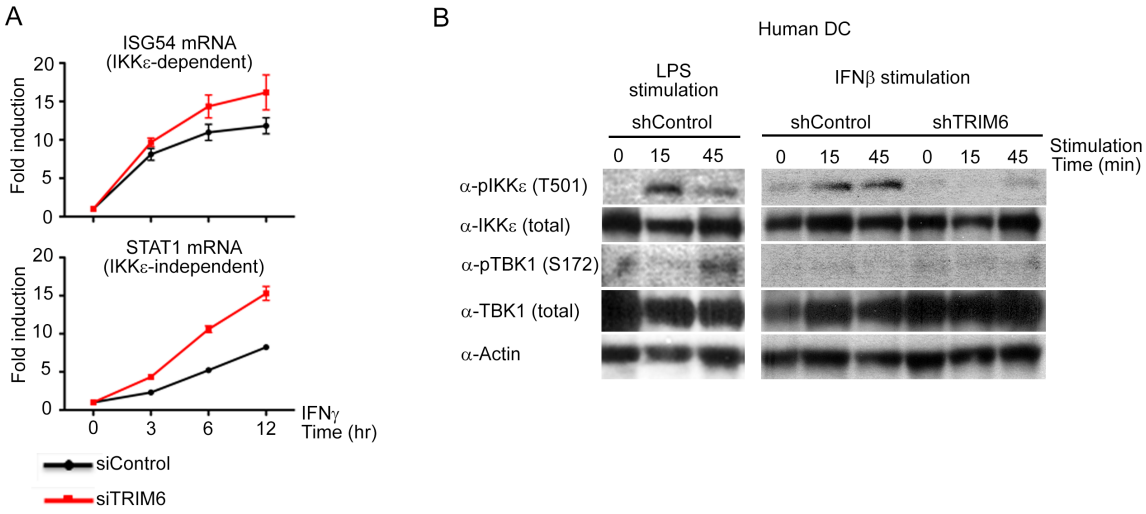
Supplementary Figure 2



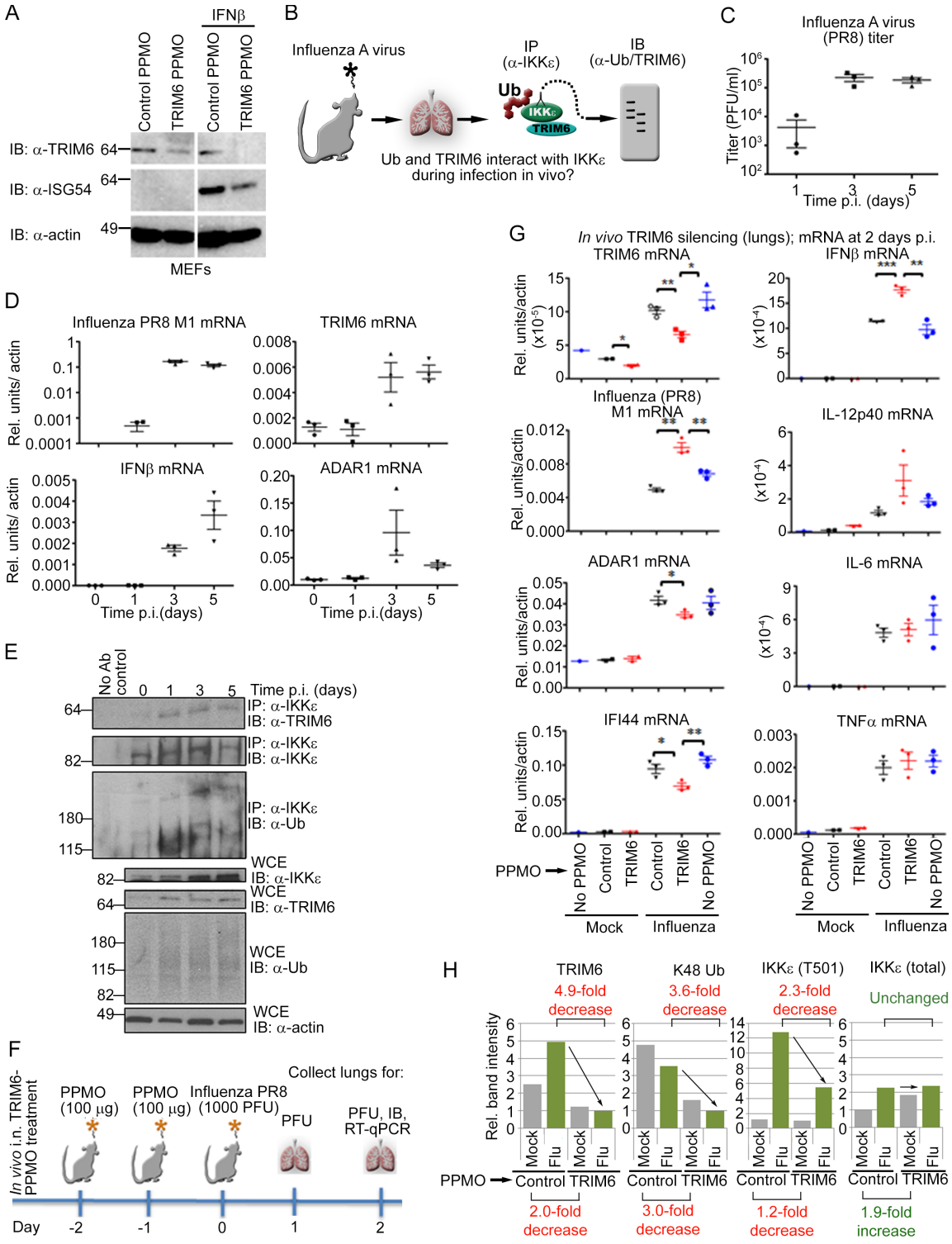
Supplementary Figure 3



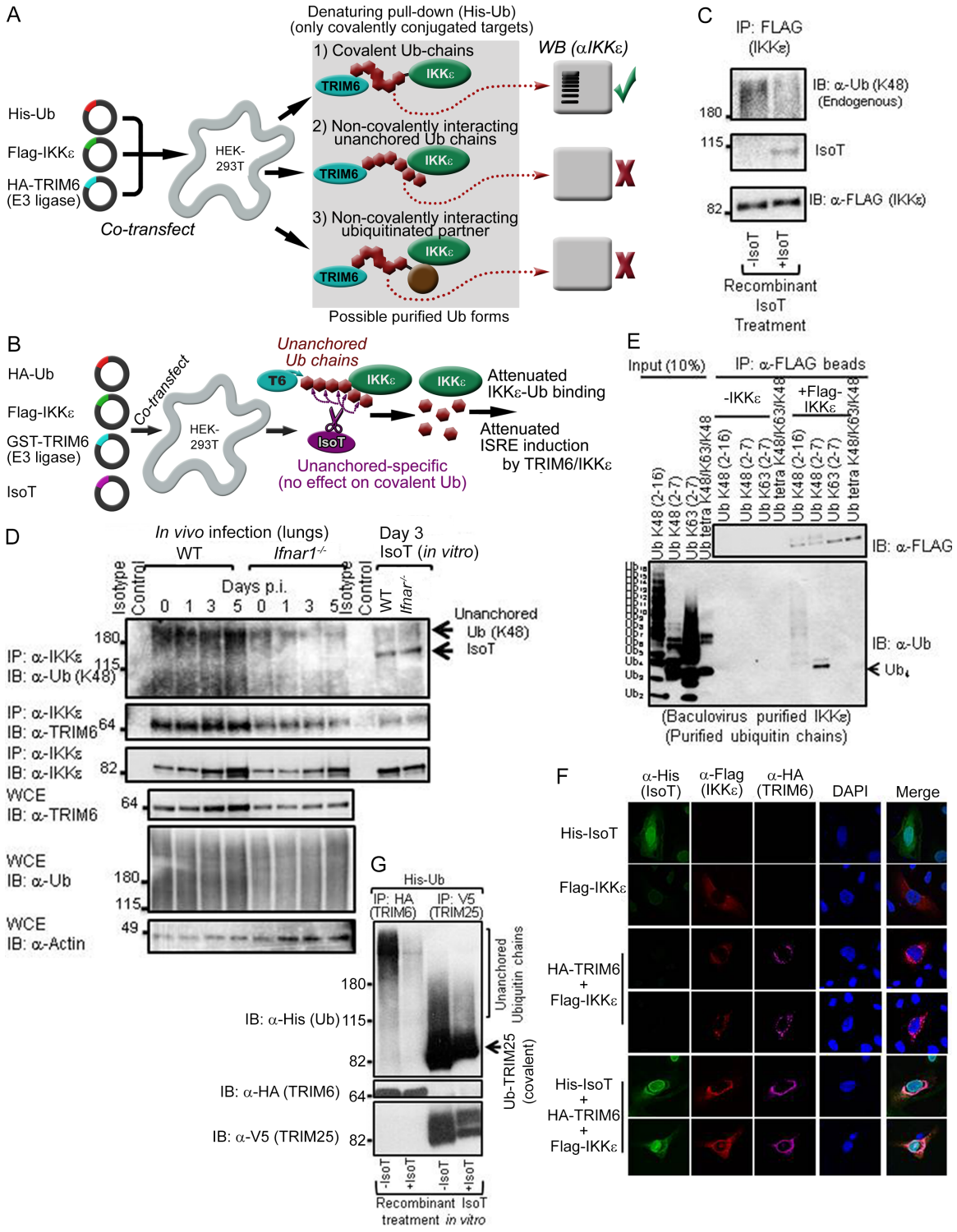
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7