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Supplementary Materials for

SERINC proteins potentiate antiviral type I IFN production and proinflammatory signaling pathways

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This PDF file includes:

Figure S1

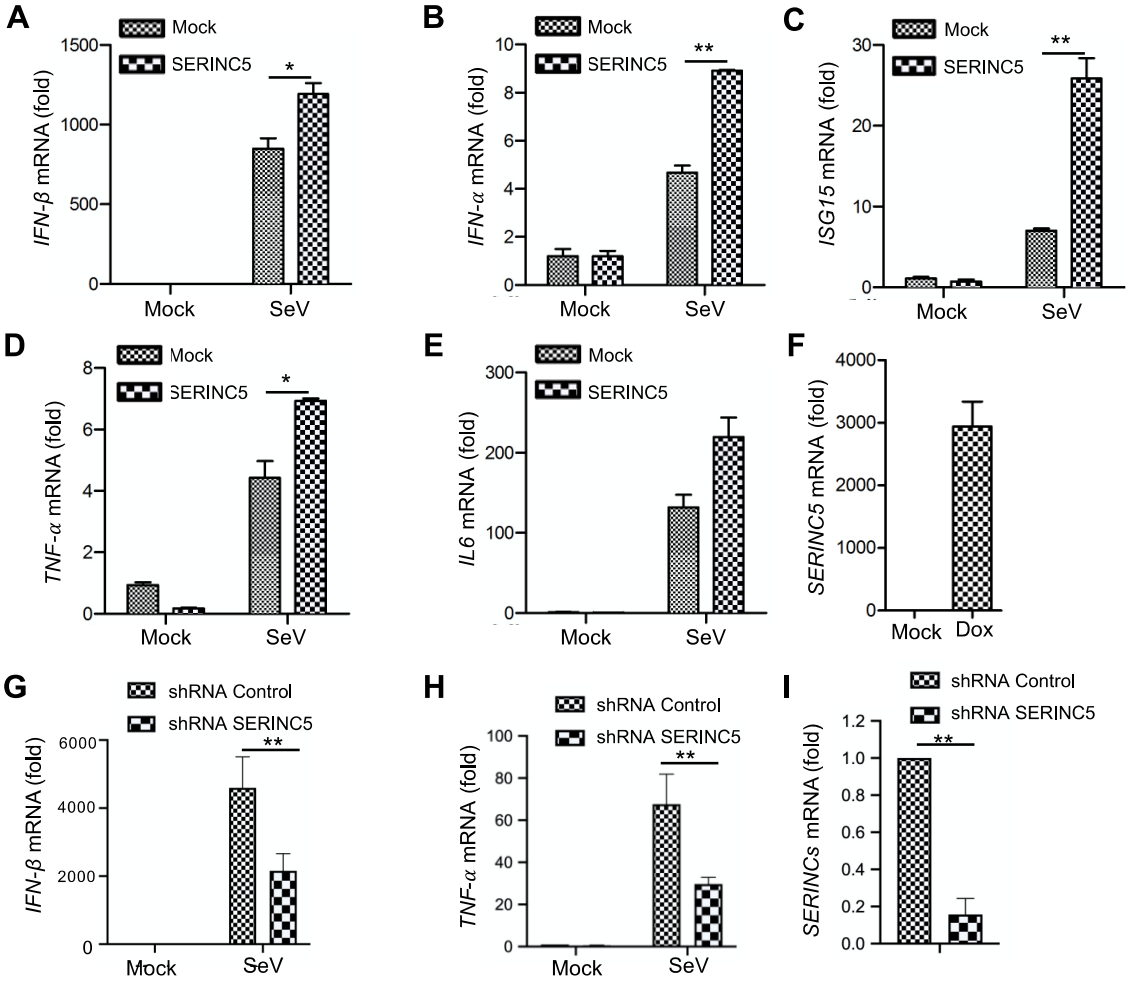

Fig. S1. SERINC5 enhances the type I IFN production and NF-κB signaling pathways in PMA-stimulated THP-1 cells. (**A** to **F**) THP-1 cells stably expressing lentiviral vector Tet-on-SERINC5 were treated with doxycycline (500 ng/ml) and PMA (10 ng/ml) before the cells were left uninfected (Mock) or were incubated with SeV. The relative amounts of mRNAs for IFN-α, IFN-β, ISG15, TNF-α, IL-6, and SERINC5 were then determined by qPCR analysis. The values of THP-1 control cells at time zero were set to zero for comparisons. Data are from at least three independent experiments. (**G** to **I**) THP-1 cells were treated with PMA (10 ng/ml) and then transduced with lentivirus pseudotypes expressing SERINC5-specific shRNA or scrambled control shRNA. The cells were left uninfected (Mock) or were incubated with SeV for 8 hours and the relative amounts of mRNAs for IFN-β and TNF-α were measured by qPCR analysis. (I) Measurement of the efficiency of SERINC5 knockdown. **P* < 0.05; ***P* < 0.01.

Figure S2

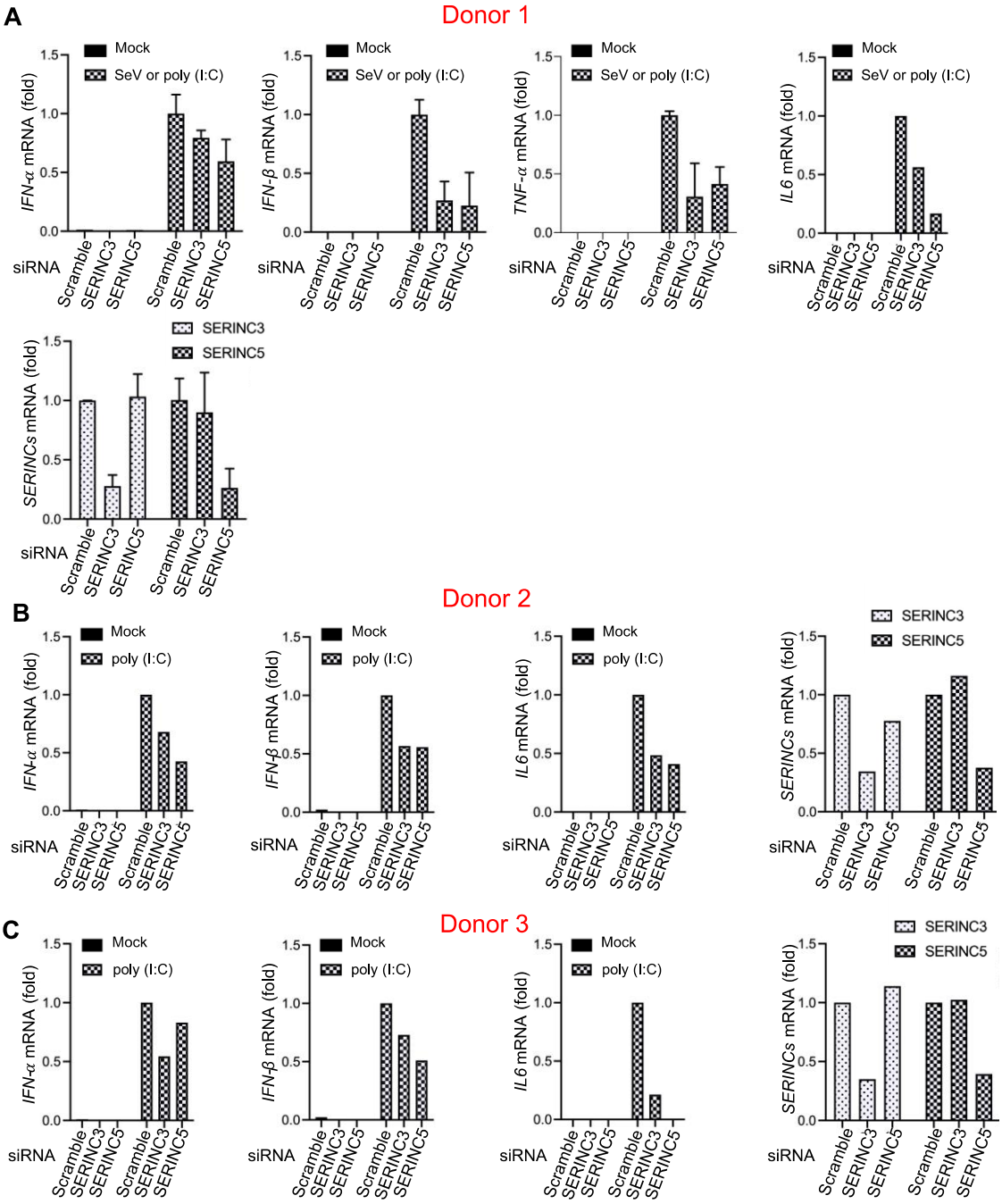

Fig. S2. Knockdown of SERINC3 or SERINC5 in human MDMs reduces type I IFN production and inflammatory cytokines in the presence of SeV or poly(I:C). (**A** to **C**) Experiments were performed as described for Fig. 1, D to H. Human MDMs from three different donors were transduced with lentiviruses expressing shRNAs targeting SERINC3 or SERINC5 or expressing a scrambled control shRNA. The cells were incubated with SeV or poly(I:C), and the relative amounts of mRNAs for IFN-α, IFN-β, IL-6, and TNF-α were measured by qPCR analysis. Analysis of the relative knockdown efficiencies of SERINC3 and SERINC5 is shown in the last graph for each donor.

Figure S3

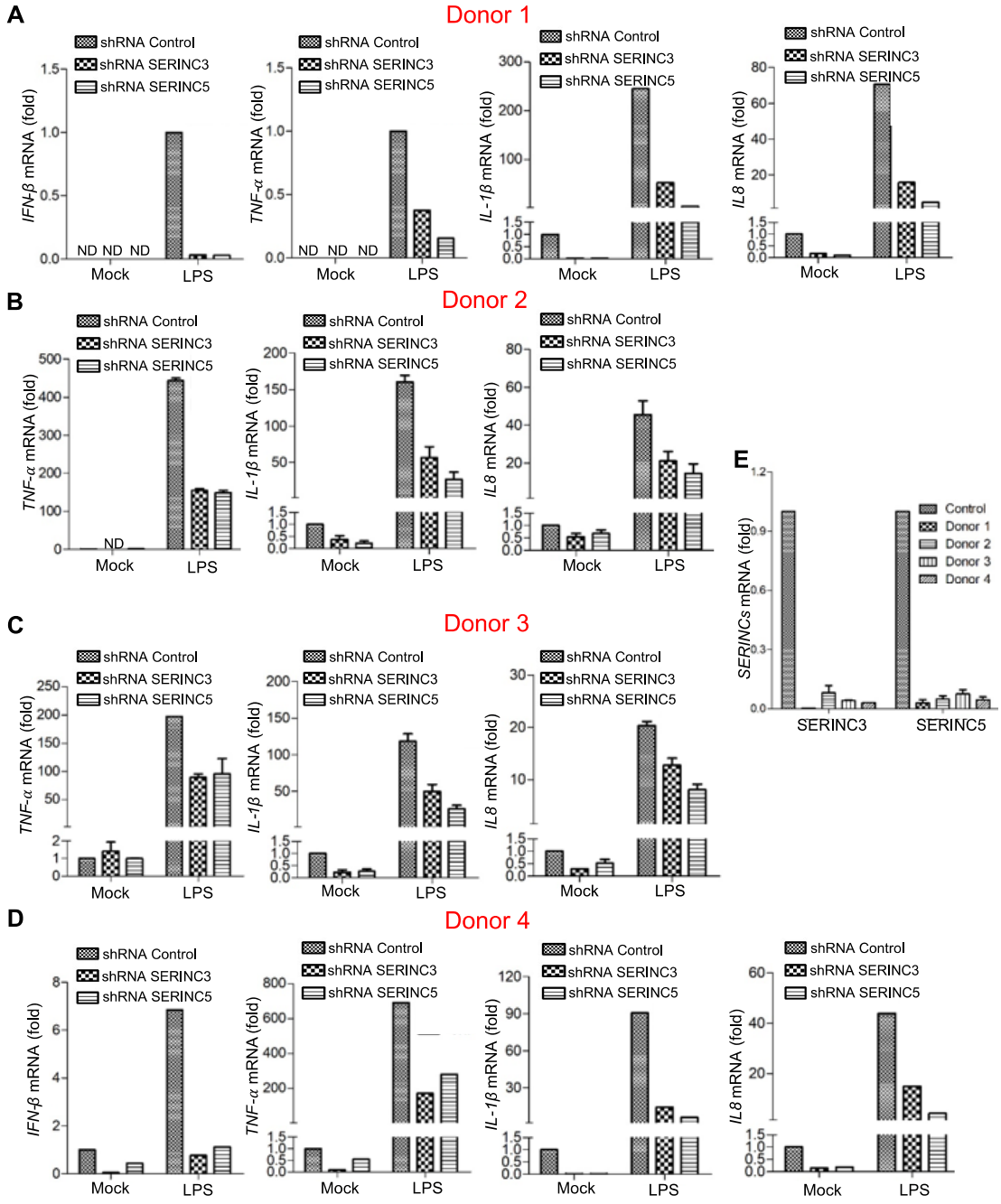

Fig. S3. Knockdown of SERINC3 or SERINC5 in human MDMs reduces type I IFN production and inflammatory cytokines in the presence of LPS. (**A** to **E**) Experiments were performed as described in Fig. 1, I to M. Human MDMs from four different donors were transduced with lentiviruses expressing shRNAs targeting SERINC3 or SERINC5 or expressing a scrambled control shRNA. The cells were stimulated with LPS (100 ng/ml) for 8 hours and then the relative amounts of mRNAs for IFN-β, TNF-α, IL-1β, and IL-8 were measured by qPCR analysis. (E) Analysis of the relative knockdown efficiencies of SERINC3 and SERINC5 in all four donors. Note that samples from donors 2 and 3 were examined twice; bars represent the SD.

Figure S4

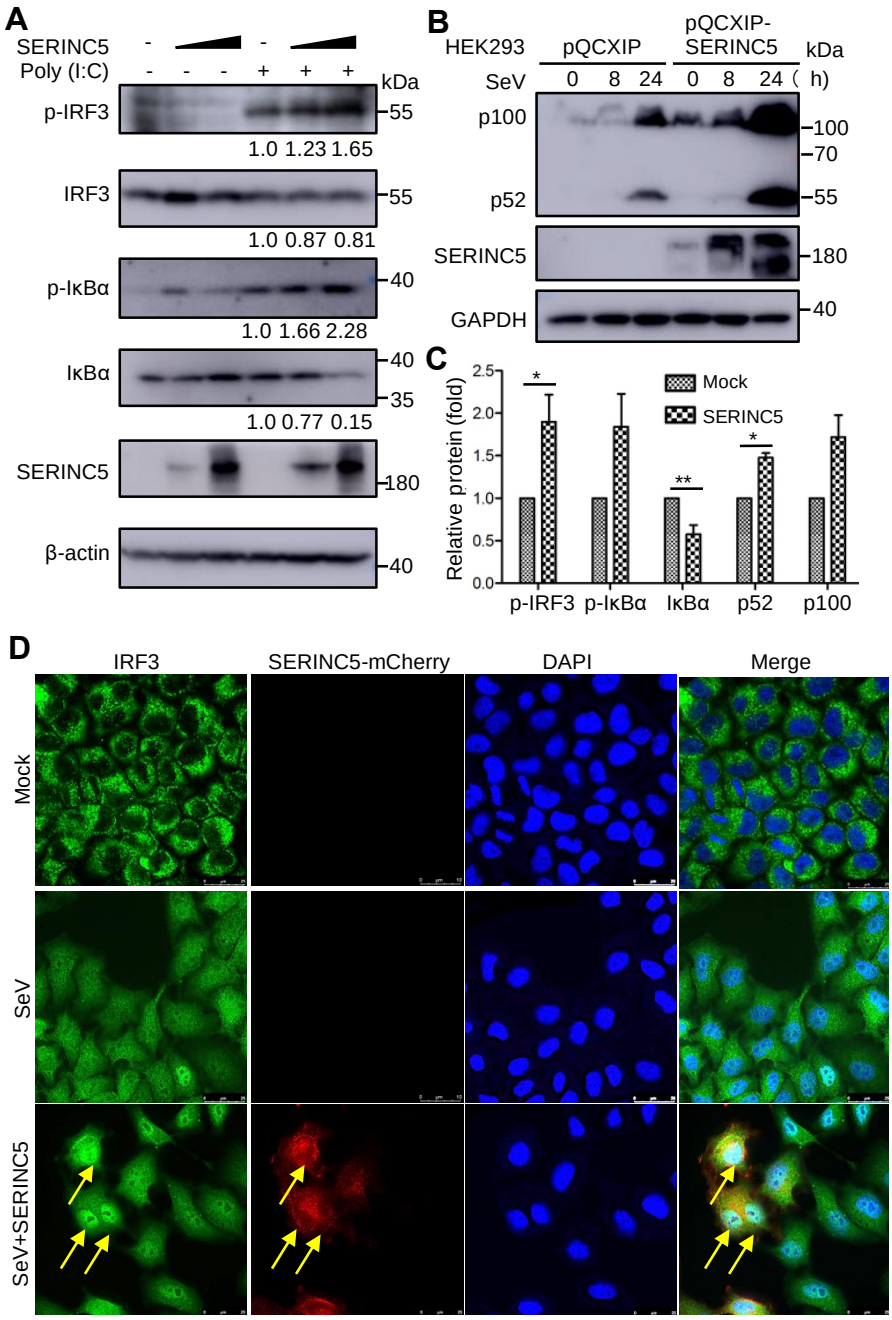

Fig. S4. SERINC5 promotes the phosphorylation of IRF3 and IκBα and enhances the nuclear translocation of IRF3. (**A**) 293T cells were transfected with increasing amounts of pBJ5-SERINC5-HA plasmids in the presence or absence of poly(I:C). Thirty-six hours after transfection, immunoblotting analysis of cell lysates was performed to detect the indicated proteins. The intensities of the bands were quantified with NIH ImageJ software (numbers below blots). (**B**) HEK293 cells stably transfected with a FLAG-tagged SERINC5 expression vector or an empty vector as a control were incubated with SeV (at an MOI of 4) for the indicated times. Immunoblotting of cell lysates was then performed to detect the indicated proteins. The intensities of the bands were quantified with ImageJ software. Note that SERINC5 sometimes

forms high-molecular weight species (>180 kD), despite treatment of the cell lysate samples at 55°C rather than under boiling denaturing conditions. (**C**) The relative band intensities corresponding to p-IRF3 (n = 7 experiments), p-IκBa (n = 4), IκBa (n = 4), p52 (n = 4), and p100 (n = 4) were determined from the experiments shown in (A) and (B). (**D**) HeLa cells were first left untransfected (Mock) or were transfected with pBJ5-SERINC5-mCherry. Twenty-four hours after transfection, the cells were infected with SeV (at an MOI of 4) for 8 hours. The subcellular localizations of IRF3 and SERINC5 were visualized by immunofluorescence staining with anti-IRF3 antibody (which detects both phosphorylated and unphosphorylated IRF3) and 3D deconvolution imaging. Arrows indicate cells positive for SERINC5-mCherry. Data are representative of three independent experiments.

Figure S5

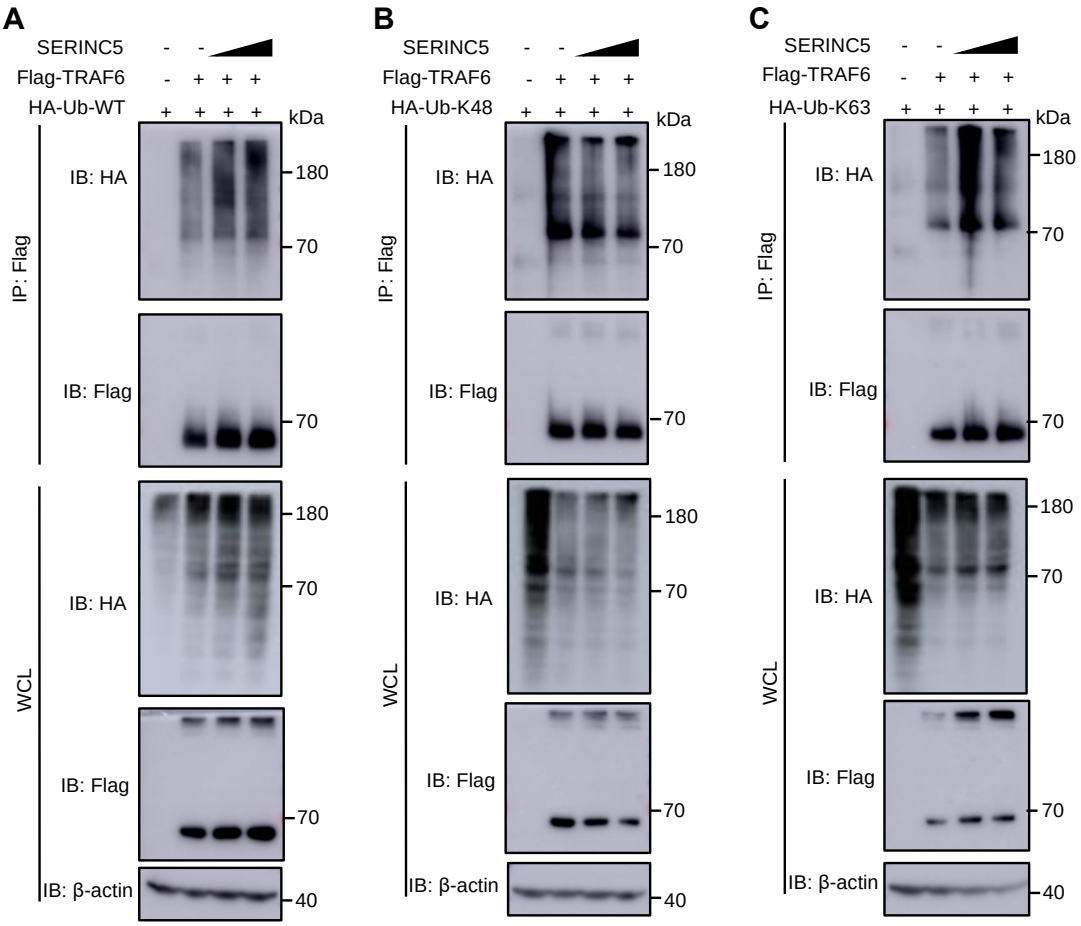

Fig. S5. SERINC5 expression leads to decreased K48-linked but increased K63-linked ubiquitylation of TRAF6. (**A** to **C**) HEK293T cells were transfected with plasmids encoding Flag-tagged TRAF6 and WT SERINC5 (untagged), together with plasmid encoding HA-tagged WT (A), K48-only (B), or K63-only (C) ubiquitin. Whole-cell lysates (WCLs) were subjected to immunoprecipitation with an anti-Flag antibody, which was followed by immunoblotting analysis with anti-HA and anti-Flag antibodies. The less strict correlation between SERINC5 abundance and K63-linked TRAF6 ubiquitylation in (C) is due to uneven loading. Blots are representative of three experiments.

Figure S6

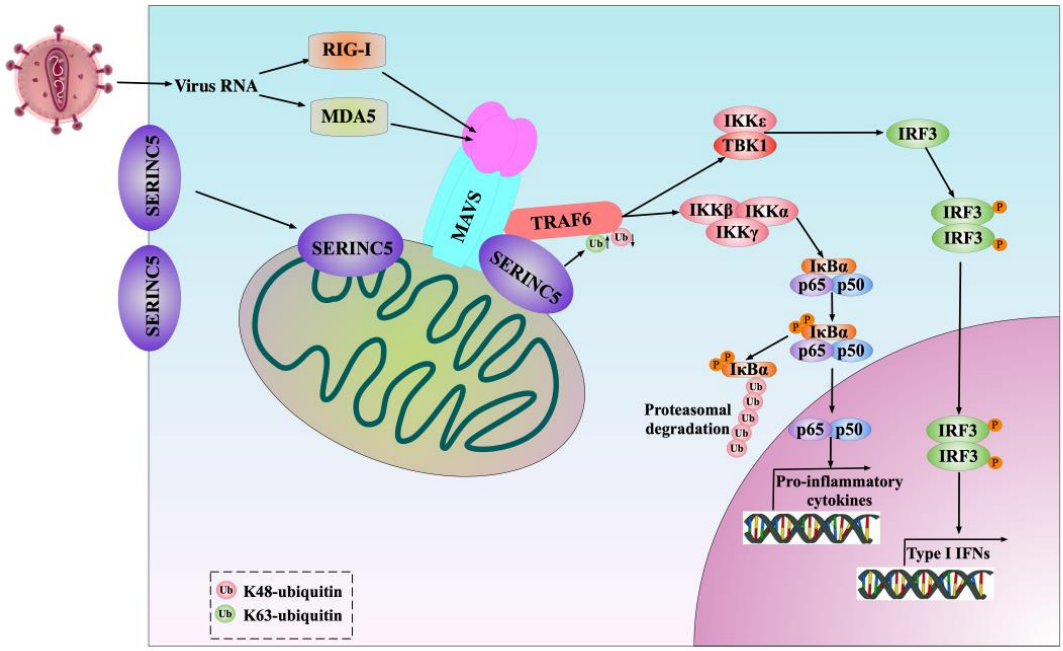

Fig. S6. Working model of the SERINC-mediated enhancement of type I IFN production and inflammatory signaling. SERINC proteins are normally localized at the plasma membrane of the cell. Upon viral infection or stimulation by poly(I:C) or LPS, SERINCs become internalized and translocate to the mitochondrial membrane, where they associate with MAVS and TRAF6. This results in the aggregation of MAVS, which is complexed with TRAF6 likely through its K63-mediated ubiquitylation, the phosphorylation of IRF3 and IκBα, leading to the activation of NF-κB and the expression of genes encoding type I IFNs.

Table S1. Primers used for qPCR analysis.

GAPDH-Forward	5'-ATGACATCAAGAAGGTGGTG-3'
GAPDH-Reverse	5'-CATACCAGGAAATGAGCTTG-3'
IFN-α-Forward	5'-GTACTGCAGAATCTCTCCTTTCTCCT-3'
IFN-α-Reverse	5'-GTGTCTAGATCTGACAACCTCCCAGG-3'
IFN-β-Forward	5'-AGGACAGGATGAACTTTGAC-3'
IFN-β-Reverse	5'-TGATAGACATTAGCCAGGAG-3'
IL-6-Forward	5'-ACTCACCTCTTCAGAACGAATTG-3'
IL-6-Reverse	5'-CCATCTTTGGAAGGTTCAGGTTG-3'
mSerinc3-Forward	5'-AATGAACCTGAGCGGTCCTG-3'
mSerinc3-Reverse	5'-GCATAGGCAGGAGCAAGAGT-3'
mSerinc5-Forward	5'-AAAAGTTGCCGGGCCTACAT-3'
mSerinc5-Reverse	5'-CCCACATAGCGCCAGACTTT-3'
mStat1-Forward	5'-TGTCATCCCGCAGAGAGAAC-3'
mStat1-Reverse	5'-AGCAGAGCTGAAACGACCTA-3'
m-β-actin-Forward	5'-GGCTGTATTCCCCTCCATCG-3'
m-β-actin-Reverse	5'-CCAGTTGGTAACAATGCCATGT-3'
SERINC3-Forward	5'-AATTCAGGAACACCAGCCTC-3'
SERINC3-Reverse	5'-GGTTGGGATTGCAGGAACGA-3'
SERINC5-Forward	5'-ATCGAGTTGTGACGCTCTGC-3'
SERINC5-Reverse	5'-GCTCTTCAGTGTCCTCTCCAC-3'
TNF-α-Forward	5'-GCCGCATCGCCGTCTCCTAC-3'
TNF-α-Reverse	5'-CCTCAGCCCCCTCTGGGGTC-3'