Supplemental Information

Materials and Methods

Cell lines and Viruses

HEK293T, HeLa, Vero, HCT116, BHK21, mouse embryonic fibroblasts (MEFs) and human foreskin fibroblasts (HFF) were cultured in Dulbecco's modified Eagle's medium (DMEM, Corning) supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone), penicillin (100 U/mL) and streptomycin (100 μg/mL). Wild-type and *Rig-i*$^{-/-}$ MEFs were described previously (Kato et al., 2005). Wild-type HSV-1 (KOS strain), GFP HSV-1 and HSV-1 recombinant viruses were amplified in Vero cells, with viral titers ranging from 10^7 to 10^8 pfu/ml. eGFP VSV (Dr. Sean Whelan) was amplified in BHK21 cells, with viral titer of 10^9 pfu/ml. Sendai virus was purchased from Charles River Laboratories.

Constructs

Luciferase reporter plasmids for the NF-κB, IFN-β promoter, PRDIII (ISRE) promoter, mammalian expression plasmids for RIG-I and its truncated mutants, MDA5, MAVS, IKKβ, TBK1 and IRF3-5D were described previously (Dong et al., 2010; Dong and Feng, 2011; Dong et al., 2012; He et al., 2015; Seth et al., 2005). The non-silencing (control) shRNA and shRNA against human RIG-I, human IFI16 and human STING were purchased from Thermo Scientific. HSV-1 expression library was described previously (Sen et al., 2013). Mammalian expression plasmids for truncated RIG-I and UL37, lentiviral expression plasmids for RIG-I and UL37 were generated by standard molecular biology techniques. All point mutants, including those of RIG-I and UL37, were generated by site-directed mutagenesis and confirm by sequencing. HSV-1ΔUL37 (KOS) and HSV-1(KOS) Bacmid was a gift from Dr. Thomas C. Mettenleiter.

Antibodies and reagents

Antibody against UL37 was a gift from Dr. Weiming Yuan. Antibodies against GST (Z-5), IRF3 (FL-425), TRAF6 (D10) and RIG-I (H-300) were purchased from Santa Cruz Biotechnology. Antibodies against FLAG (M2, Sigma), V5 (A190-220A, Bethyl Group), RIG-I (SS1A, Enzo Life Sciences), STING (ab92605, Abcam), dsRNA-J2 (SCICONS), Sendai virus (PD029, MBL), P-S172 TBK-1 (D52C2, Cell Signaling) and β-Actin (Ab8226, Abcam) were purchased from the indicated suppliers. The glutamine analog 6-Diazo-5-oxo-L-norleucine (DON) was purchased from Sigma. Low molecular weight Poly [I:C] (31852-29-6), ppp-dsRNA (tlrl-3prna) and control-dsRNA (tlrl-3prnac) were purchased from InvivoGen. Lipofectamine 2000 was purchased from Life Technologies.

DNA and RNA Transfection

For plasmid transfection in HEK293T cells, calcium phosphate transfection method was applied. 293T cells were plated at around 50%-60% confluence. For dsRNA and Poly [I:C] transfection in 293T cells and plasmid transfection in HeLa cells, Lipofectamine 2000 transfection reagent was used according to the manufacturer's instructions. Both cells were prepared at around 80%-90% confluence prior to transfection.

Lentivirus-mediated Stable Cell Line Construction

Lentiviruses were produced as previously described (Dong and Feng, 2011; Feng et al., 2008). Briefly, HEK293T cells were transfected with the packaging plasmids VSV-G and DR8.9 and the pCDH lentiviral expression vector or lentiviral shRNA plasmids. At 48 h post transfection, supernatant was harvested and filtered (and concentrated by centrifugation if necessary). HEK293T cells, MEFs, HeLa, HCT116 or HFF cells were infected with the supernatant in the presence of polybrene (8 μg/ml) with centrifugation at 1800 rpm for 45 minutes. Cells were selected at 48 h post infection and maintained in 10% FBS DMEM supplemented with puromycin (1~2 μg/ml).

Dual-Luciferase Reporter Assay

HEK293T cells, seeded in 24-well plates (~50% cell density), were transfected with IFN-β, PRDIII (ISRE) or NF-κB reporter plasmid cocktail (50 ng of luciferase reporter plasmid and 5 ng of pRL

Renilla luciferase control vector) and expression plasmid (empty plasmid, one or multiple plasmids depending on the experiment) by calcium phosphate precipitation. Cells were infected with SeV (100 HA/ml), HSV-1 for 15-16 h, transfected with Poly [I:C] for 16 h or directly harvested 30-36 h post transfection. Whole cell lysates were used to determine the activity of firefly luciferase and Renilla luciferase by a microplate reader (FLUOstar Omega).

Plaque Assay

HSV-1 and VSV titer were determined by plaque assay on Vero monolayer essentially as previously described (Lieber and Bailer, 2013). Briefly, 10-fold serially-diluted virus-containing supernatant was added onto Vero cells and incubated for 2 h at 37°C. Then, DMEM containing 2% FBS and 1% methylcellulose (Sigma) was added after removing the supernatant. Plaques were counted at day 3 post-infection.

Confocal Microscopy

HeLa cells were transfected with expression plasmid containing UL37 and subsequently infected with Sendai virus for 6 h (100 [HAU]/ml). Cells were fixed, permeabilized, stained with indicated primary antibody (1:100 dilution) and Alexa Fluor 488/594-congugated goat secondary antibody (1:200 dilution), and analyzed with confocal microscope (Leica). Representative images were shown for all analyses.

Protein Expression and Purification

HEK293T cells were transfected with expression vector containing Flag-tagged gene of interest. Cells were harvested and lysed with Triton X-100 buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1.5 mM $MgCl_2$, 20 mM β-glycerophosphate, 1 mM sodium orthovanadate, 10% glycerol, 0.5 mM EGTA, 0.5% Triton X-100) supplemented with a protease inhibitor cocktail (Roche). Whole cell lysates were sonicated and centrifuged at 12,000 rpm for 15 min. Supernatant was harvested, filtered, pre-cleared with protein A/G agarose beads at 4°C for 1 h and then incubated with anti-Flag agarose beads at 4°C for 4 h. The agarose beads were washed extensively and eluted with 0.2 mg/ml 3xFlag peptide. The eluted proteins were analyzed by SDS gel electrophoresis and silver staining.

For recombinant protein expression and purification, *E.coli* Bl21 (DE3) was transformed with pGEX-4T-1 or pET28 plasmid containing UL37. Recombinant GST-UL37 expression was induced by 0.1 mM IPTG at 20°C. Bacteria were harvested, lysed and incubated with glutathione sepharose 4B (GE) for 4 h at 4°C. Sepharose beads were washed extensively and GST-UL37 was eluted with 10 mM reduced glutathione. UL37 was then cleaved and purified from the fusion protein by TEV protease treatment at 4°C overnight.

Co-immunoprecipitation (Co-IP) and Immunoblotting

For Co-IP using exogenous protein, HEK293T cells were transfected with indicated expression plasmids for 48 h. For Co-IP using endogenous proteins, cells were directly harvested. Whole cell lysates were prepared with NP40 buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 5 mM EDTA) supplemented with 20 mM β-glycerophosphate and 1 mM sodium orthovanadate. Whole cell lysates were sonicated, centrifuged and pre-cleared with protein A/G agarose for 1 h. Pre-cleared samples were then incubated with indicated antibodies overnight and protein A/G agarose for 1 h at 4°C, or with antibody/glutathione-conjugated agarose for 4 h at 4°C. The agarose beads were washed extensively and samples were eluted by boiling at 95°C for 10 min. Precipitated proteins were analyzed by SDS gel electrophoresis and immunoblotting.

All immunoblottings were performed using the indicated primary antibodies (1:1000 dilution) and IRDye800-conjugated secondary antibodies (1:10,000 dilution, Licor). Proteins were visualized by Odyssey infrared imaging system (Licor).

Gel Filtration

Virus-infected HEK293T/Flag-RIG-I or HeLa/Flag-RIG-I stable cells were harvested and lysed in cold Triton X-100 buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1.5 mM $MgCl_2$, 20 mM β-glycerophosphate, 1 mM sodium orthovanadate, 10% glycerol, 0.5 mM EGTA, 0.5% Triton X-100, 1 mM PMSF and 10 μg/ml leupeptin). Centrifuged supernatant was filtered and subjected to

incubation with anti-Flag-conjugated agarose beads for 2 h at 4°C. Beads were then extensively washed and proteins were eluted with 3xFlag peptide at 0.2 mg/ml.

Gel filtration with superose 6 was performed as described previously (He et al., 2015). Briefly, eluted proteins (200-300 μl) were loaded to superose 6 column and subjected to gel filtration analysis with Buffer B (20 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.5% Triton X-100, 20 mM NaF, 20 mM β-glycerophosphate, 1 mM Na_3VO_4, 2.5 mM metabisulphite [sodium salt], 5 mM benzamidine). Elution was collected in 0.5 ml fractions and aliquots of fractions were analyzed by immunoblotting.

Mock- or HSV-1-infected cells (2×10^7) were harvested and lysed in 300 μl cold Triton X-100 buffer. Samples were sonicated briefly and centrifuged. Supernatant was filtered and loaded to superose 6 column and subjected to gel filtration analysis with Buffer B. Elution was collected in 0.5 ml fractions and aliquots of fractions were analyzed by immunoblotting.

Quantitative Real-time PCR (qRT-PCR)

Quantitative Real-time PCR was performed as previously described (He et al., 2015). Cells were infected or treated with viruses or agents for indicated time period. Total RNA was extracted using TRIzol reagent (Invitrogen). Complementary cDNA was synthesized from DNase I-treated total RNA using reverse transcriptase (Invitrogen). cDNA was diluted and qRT-PCR was performed using SYBR Green Master Mix (Applied Biosystems) by real-time PCR instrument (Applied Biosystems). Relative mRNA expression for each target gene was calculated by the $2^{-\Delta\Delta Ct}$ method using *β-Actin* as an internal control. The sequences of qRT-PCR primers are as follows:

Human *β-actin*	forward	5'-CTGGCACCCAGCACAATG-3'
	reverse	5'-GCCGATCCACACGGAGTACT-3'
Human *IFN-β*	forward	5'-AGGACAGGATGAACTTTGAC-3'
	reverse	5'-TGATAGACATTAGCCAGGAG-3'
Human *ISG56*	forward	5'-TCTCAGAGGAGCCTGGCTAA-3'
	reverse	5'-TGACATCTCAATTGCTCCAG-3'
Human *IL8*	forward	5'-GGCACAAACTTTCAGAGACAG-3'
	reverse	5'-ACACAGAGCTGCAGAAATCAGG-3'
Human *CXCL2*	forward	5'-GGGCAGAAAGCTTGTCTCAA-3'
	reverse	5'-GCTTCCTCCTTCCTTCTGGT-3'
Human *IFI16*	forward	5'-ACAAACCCGAGAAACAATGACC-3'
	reverse	5'-GCATCTGAGGAGTCCGAAGA-3'
Mouse *β-actin*	forward	5'- ACGGCCAGGTCATCACTATTG-3'
	reverse	5'-CAAGAAGGAAGGCTGGAAAAGA-3'
Mouse *IFN-β*	forward	5'-TCCGAGCAGAGATCTTCAGGAA-3'
	reverse	5'-TGCAACCACCACTCATTCTGAG-3'
Mouse *ISG56*	forward	5′-ACCATGGGAGAGAATGCTGAT-3′
	reverse	5′-GCCAGGAGGTTGTGC-3′

Cytokine ELISA

Commercial cytokine ELISA kits used in this study include: human IFN-β (PBL Assay Science) and human RANTES (R&D Systems). Cytokine levels in the supernatant from cultured cells were assessed according to manufacturer's instruction. Absorbance was determined with FLUOstar Omega (BMG Labtech).

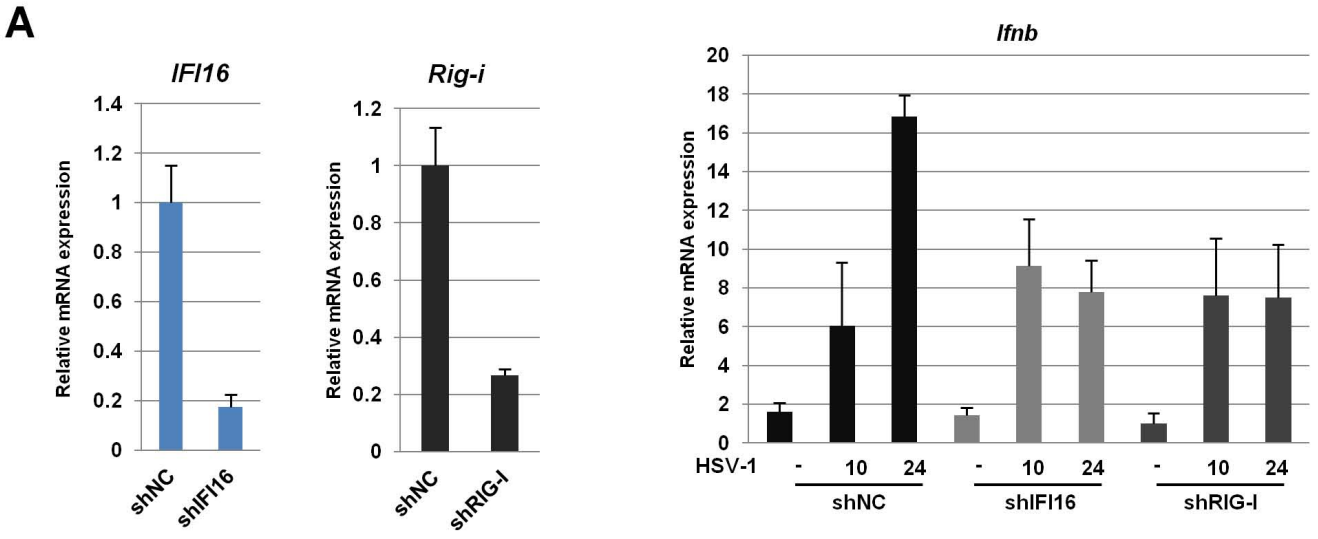
Mass Spectrometry Analysis

For identification of deamidation sites, HEK293T/Flag-RIG-I stable cell line was transfected with an expression plasmid containing UL37 or infected by HSV-1 for 10 h (MOI = 10). Flag-RIG-I was purified by anti-Flag-conjugated agarose beads for 4 h at 4°C. Beads were then extensively washed and RIG-I was eluted with 3xFlag peptide at 0.2 mg/ml. Purified RIG-I was subjected to SDS page electrophoresis and gel slices were prepared for in-gel digestion and Mass Spectrometry analysis (Harvard Taplin Mass Spectrometry Facility).

For Cysteine labeling experiment, bacterial purified UL37 (571-1123) was treated with N-methylacetamide (Alfa Aesar) (1 μM) at room temperature for 45 min. Samples were then blocked with Iodoacetamide (Sigma) (50 mM) at room temperature for 1 h and subjected to Mass Spectrometry analysis (Poochon Scientific).

Statistical Analysis

Statistical analysis was performed by unpaired two-tailed Student's *t*-test. A *p*-value less than 0.05 is considered statistically significant. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$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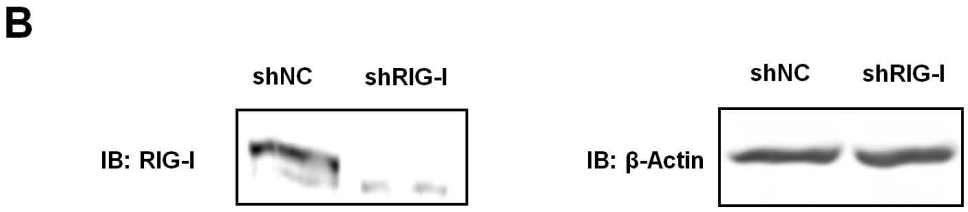
IFI16
Rig-i
Ifnb
Relative mRNA expression
shNC
shIFI16
shRIG-I
HSV-1
-
10
24


B
shNC
shRIG-I
IB: RIG-I
IB: β-Actin

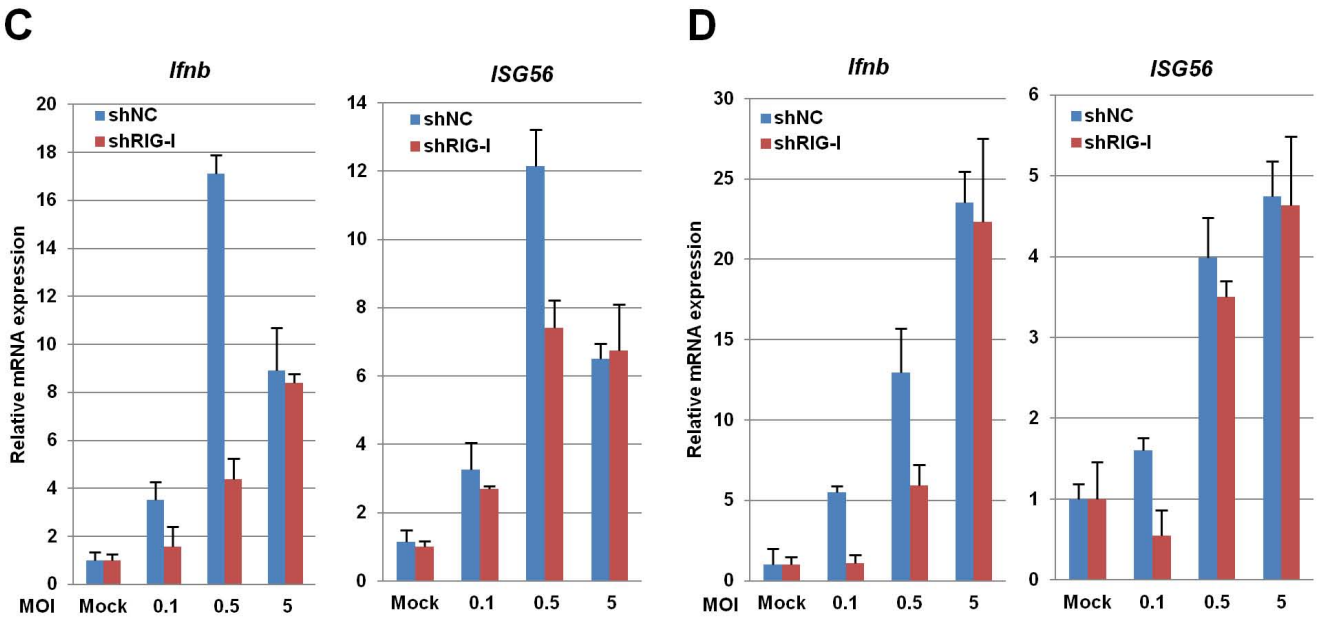

C
D
Ifnb
ISG56
shNC
shRIG-I
Relative mRNA expression
MOI
Mock
0.1
0.5
5

Figure S1. HSV-1 evades RIG-I-mediated antiviral immune responses Related to Figure 1.

(A) (Left) HFF cells stably expressing control, IFI16 or RIG-I shRNA were prepared by lentiviral transduction. RNA was extracted and cDNA was prepared to determine IFI16 and RIG-I mRNA expression by real-time PCR analysis. (Right) Stable cells were then infected with HSV-1 (MOI = 5) for the indicated hours. RNA was extracted and cDNA was prepared to determine IFN-β mRNA expression by real-time PCR analysis.

(B) 293T cells stably expressing control or RIG-I shRNAs were prepared by lentiviral transduction. WCLs were analyzed by SDS-PAGE and immunoblotting with the indicated antibodies.

(C) Control and RIG-I knockdown 293T cells were mock-infected or infected with HSV-1 of indicated MOI for 24 h. RNA was extracted and cDNA was prepared to determine IFN-β and ISG56 mRNA expression by real-time PCR analysis.

(D) Control and RIG-I knockdown HeLa cells were mock-infected or infected with HSV-1 of indicated MOI for 24 h. RNA was extracted and cDNA was prepared to determine IFN-β and ISG56 mRNA expression by real-time PCR analysis.

For Figure S1A, S1C and S1D, data are presented as mean. Error bars denote SD (n=3).

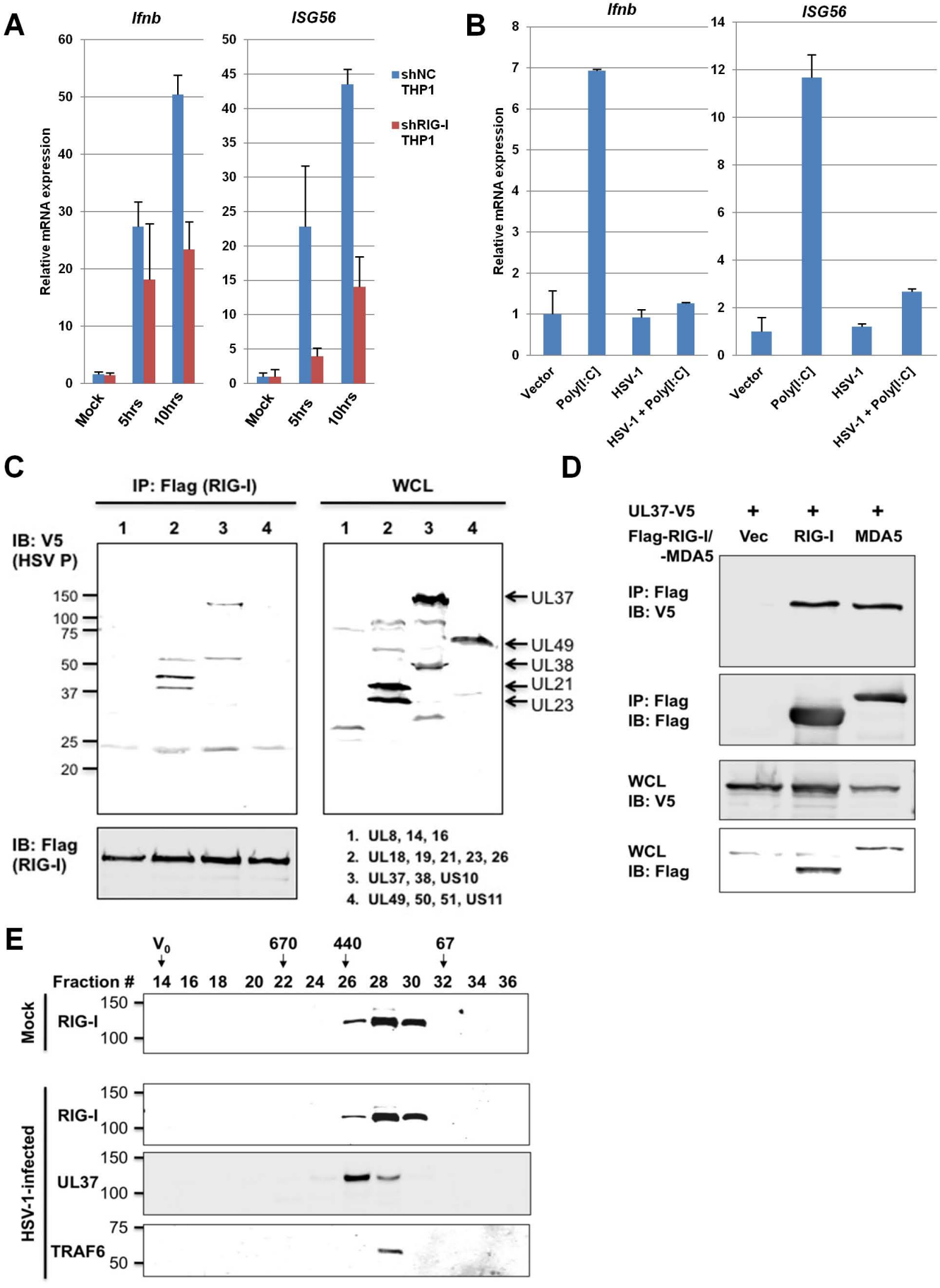

A
Ifnb
ISG56
Relative mRNA expression
shNC THP1
shRIG-I THP1
Mock
5hrs
10hrs
B
Ifnb
ISG56
Relative mRNA expression
Vector
Poly[I:C]
HSV-1
HSV-1 + Poly[I:C]
C
IP: Flag (RIG-I)
WCL
IB: V5 (HSV P)
IB: Flag (RIG-I)
UL37
UL49
UL38
UL21
UL23
1. UL8, 14, 16
2. UL18, 19, 21, 23, 26
3. UL37, 38, US10
4. UL49, 50, 51, US11
D
UL37-V5
Flag-RIG-I/ -MDA5
Vec
RIG-I
MDA5
IP: Flag IB: V5
IP: Flag IB: Flag
WCL IB: V5
WCL IB: Flag
E
Fraction #
Mock
HSV-1-infected
RIG-I
UL37
TRAF6

Figure S2. HSV-1 evades RIG-I-mediated antiviral immune responses Related to Figure 1.

(A) THP1 cells stably expressing control or RIG-I shRNA were prepared by lentiviral transduction. Cells were then infected with HSV-1 (MOI = 5) for the indicated hours. RNA was extracted and cDNA was prepared to determine IFN-β and ISG56 mRNA expression by real-time PCR analysis.

(B) 293T cells were infected with HSV-1 (MOI = 5) for 2 h and then transfected with LMV Poly [I:C] for 8 h. RNA was extracted and cDNA was prepared to determine IFN-β and ISG56 mRNA expression by real-time PCR analysis.

(C) 293T cells were transfected with a plasmids containing RIG-I and those containing indicated ORFs of HSV-1. Whole cell lysates (WCLs) were harvested and RIG-I was immunoprecipitated, followed by SDS-PAGE analysis and immunoblotting with the indicated antibodies.

(D) 293T cells were transfected with plasmids containing UL37 and RIG-I/MDA5. RIG-I/MDA5 was immunoprecipitated. Precipitated proteins and WCL were analyzed by immunoblotting with indicated antibodies.

(E) 293T cells were infected by HSV-1 (MOI = 5) for 15 h. WCLs were analyzed by gel filtration and immunoblotting with indicated antibodies.

For Figure S2A and S2B, data are presented as mean. Error bars denote SD (n=3).

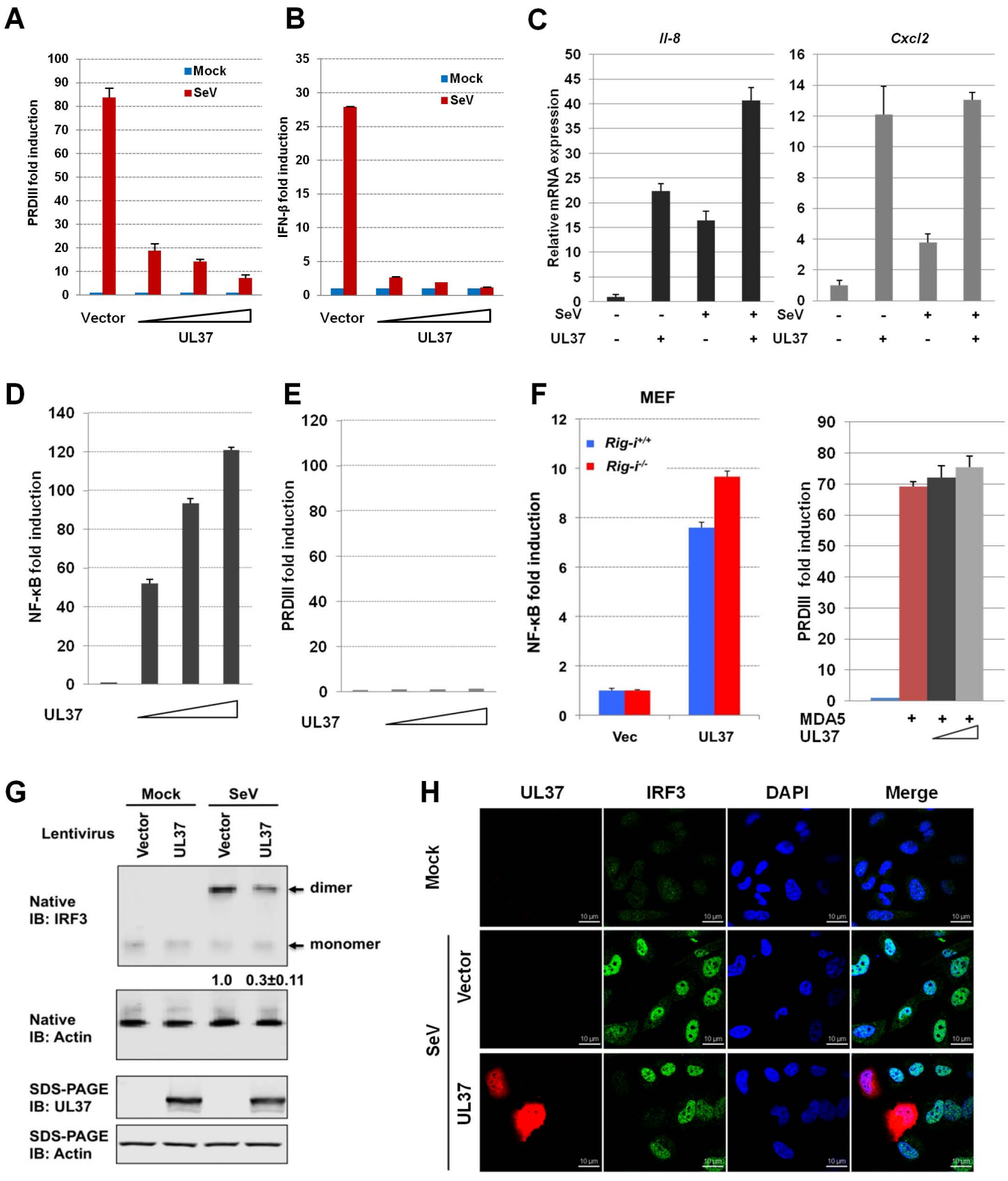

A
PRDIII fold induction
Mock
SeV
Vector
UL37
B
IFN-β fold induction
Mock
SeV
Vector
UL37
C
Il-8
Cxcl2
Relative mRNA expression
SeV
UL37
D
NF-κB fold induction
UL37
E
PRDIII fold induction
UL37
F
MEF
Rig-i+/+
Rig-i-/-
NF-κB fold induction
Vec
UL37
PRDIII fold induction
MDA5
UL37
G
Mock
SeV
Lentivirus
Vector
UL37
Vector
UL37
Native
IB: IRF3
dimer
monomer
1.0
0.3±0.11
Native
IB: Actin
SDS-PAGE
IB: UL37
SDS-PAGE
IB: Actin
H
UL37
IRF3
DAPI
Merge
Mock
SeV
Vector
UL37

Figure S3. UL37 inhibits RIG-I-mediated antiviral immune responses Related to Figure 2.

(A) 293T cells were transfected with a PRDIII (ISRE) reporter cocktail and increasing amounts of a plasmid containing UL37. Transfected cells were subsequently infected with Sendai virus (SeV, 100 [HAU]/ml) for 15 h. Fold induction of the PRDIII reporter was determined by luciferase assay.

(B) 293T cells were transfected with an IFN-β reporter cocktail and increasing amounts of a plasmid containing UL37. Transfected cells were subsequently infected with SeV (100 [HAU]/ml) for 15 h. Fold induction of the IFN-β reporter was determined by luciferase assay.

(C) Control 293T and 293T cells stably expressing UL37 were infected with SeV (100 [HAU]/ml) for 10 h. RNA was extracted and cDNA was prepared to determine IL8 and CXCL2 mRNA expression by real-time PCR analysis.

(D) 293T cells were transfected with an NF-κB reporter cocktail and increasing amounts of a plasmid containing UL37. Fold induction of the NF-κB reporter was determined by luciferase assay.

(E) 293T cells were transfected with PRDIII reporter cocktail and increasing amounts of a plasmid containing UL37. Fold induction of the PRDIII reporter was determined by luciferase assay.

(F) (Left) *Rig-i*$^{+/+}$ and *Rig-i*$^{-/-}$ MEF cells were transfected with an NF-κB reporter cocktail and a plasmid containing UL37 via NEON transfection system. Fold induction of the NF-κB reporter was determined by luciferase assay. (Right) 293T cells were transfected with a PRDIII reporter cocktail and a plasmid containing MDA5, with increasing amounts of a plasmid containing UL37. Fold induction of the PRDIII reporter was determined by luciferase assay.

(G) Control 293T and 293T cells stably expressing UL37 were infected with SeV (100 [HAU]/ml) for 8 h. WCLs were analyzed by native PAGE and immunoblotting with anti-IRF3 antibody.

(H) HeLa cells were transfected with a control plasmid or a plasmid containing UL37, and then infected with SeV (100 [HAU]/ml) for 6 h. IRF3 nuclear translocation was analyzed by immunofluorescence microscopy with anti-IRF3 antibody.

For Figure S3A-S3F, data are presented as mean. Error bars denote SD (n=3).

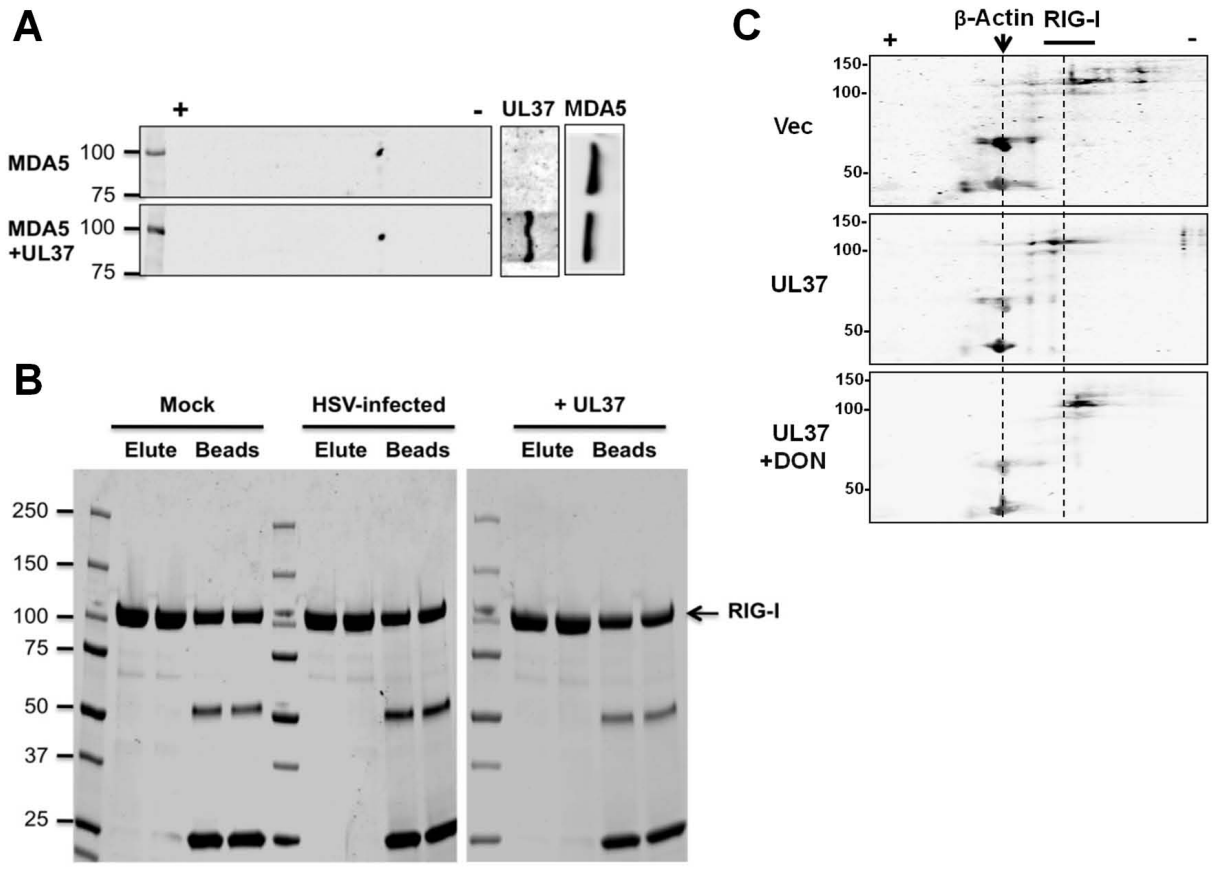

A
+
-
UL37
MDA5
MDA5
100
75
MDA5
+UL37
100
75
B
Mock
HSV-infected
+ UL37
Elute
Beads
Elute
Beads
Elute
Beads
250
150
100
75
50
37
25
RIG-I
C
β-Actin
RIG-I
+
-
Vec
UL37
UL37
+DON
150
100
50

Figure S4. UL37 deamidates RIG-I
Related to Figure 3.

(A) 293T cells were transfected with a plasmid containing MDA5 and either a vector or a plasmid containing UL37. WCLs were analyzed by 2-dimensional gel electrophoresis (2DGE) and immunoblotting with indicated antibodies.

(B) 293T/RIG-I cells were mock-infected or infected with HSV-1 (MOI = 5) or transfected with a plasmid containing UL37. RIG-I was purified and analyzed by SDS-PAGE and Coomassie staining.

(C) 293T/RIG-I cells were transfected with a plasmid containing UL37 and subsequently treated with or without DON (10 μM). WCLs were analyzed by 2DGE and immunoblotting with indicated antibodies. β-actin served as an internal control.

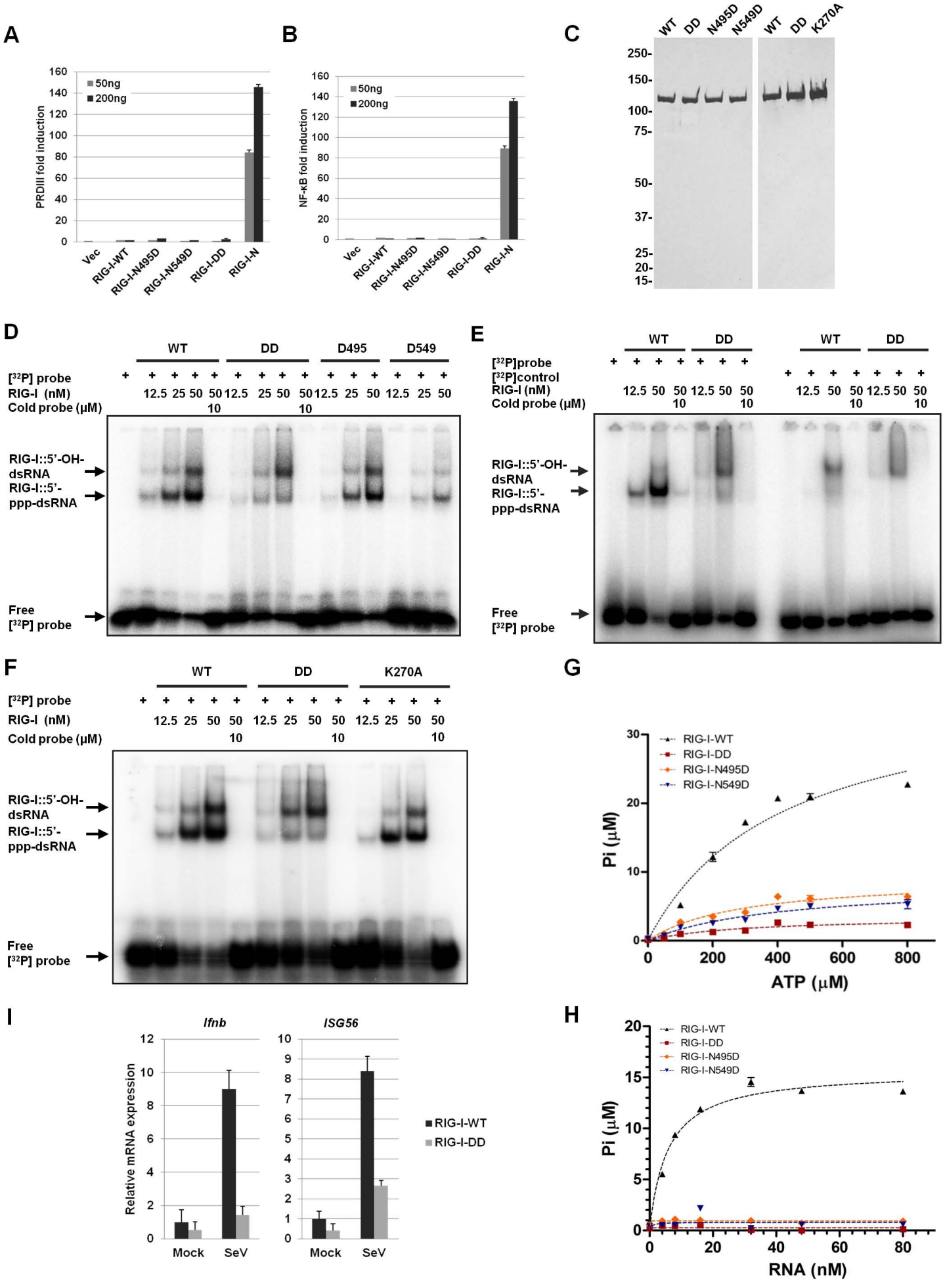

A
PRDIII fold induction
50ng
200ng
Vec
RIG-I-WT
RIG-I-N495D
RIG-I-N549D
RIG-I-DD
RIG-I-N
B
NF-κB fold induction
C
WT
DD
N495D
N549D
K270A
250-
150-
100-
75-
50-
37-
25-
20-
15-
D
WT
DD
D495
D549
[32P] probe
RIG-I (nM)
Cold probe (μM)
RIG-I::5'-OH-dsRNA
RIG-I::5'-ppp-dsRNA
Free [32P] probe
E
[32P]probe
[32P]control
F
K270A
G
RIG-I-WT
RIG-I-DD
RIG-I-N495D
RIG-I-N549D
Pi (μM)
ATP (μM)
H
RNA (nM)
I
Ifnb
ISG56
Relative mRNA expression
Mock
SeV

Figure S5. Deamidation inactivates RIG-I to sense dsRNA
Related to Figure 4.

(A) 293T cells were transfected with a PRDIII reporter cocktail and plasmids containing RIG-I, RIG-I-N495D, RIG-I-N549D, RIG-I-DD and RIG-I-N (1-200), respectively. Fold induction of the PRDIII reporter was determined by luciferase assay.

(B) 293T cells were transfected with an NF-κB reporter cocktail and plasmids containing RIG-I, RIG-I-N495D, RIG-I-N549D, RIG-I-DD and RIG-I-N (1-200), respectively. Fold induction of the NF-κB reporter was determined by luciferase assay.

(C) RIG-I WT and the indicated mutants were purified from transfected 293T cells and analyzed by SDS-PAGE and silver staining.

(D) Purified RIG-I, RIG-I-N495D, RIG-I-N549D and RIG-I-DD were incubated with ^{32}P-labeled 5′-triphosphate dsRNA (100 nM), with and without a 100-fold excess of cold 5′-triphosphate dsRNA. RNA-RIG-I complex was analyzed by PAGE and autoradiography.

(E) Purified RIG-I and RIG-I-DD were incubated with ^{32}P-labeled 5′-triphosphate dsRNA (100 nM) or ^{32}P-labeled control dsRNA, with and without a 100-fold excess of cold dsRNA. RNA-RIG-I complex was analyzed by PAGE and autoradiography.

(F) Purified RIG-I, RIG-I-DD and RIG-I-K270A were incubated with ^{32}P-labeled 5′-triphosphate dsRNA (100 nM), with and without a 100-fold excess of cold 5′-triphosphate dsRNA. RNA-RIG-I complex was analyzed by PAGE and autoradiography.

(G) Purified RIG-I, RIG-I-N495D, RIG-I-N549D and RIG-I-DD (20 nM) were incubated with increasing concentrations of ATP in the presence of 5′-triphosphate dsRNA (80 nM) and analyzed by ATP hydrolysis assay.

(H) Purified RIG-I, RIG-I-N495D, RIG-I-N549D and RIG-I-DD (20 nM) were incubated with increasing concentrations of dsRNA in the presence of ATP (500 μM) and analyzed by ATP hydrolysis assay.

(I) “Reconstituted” MEFs as shown in Figure 4(G) were infected with Sendai virus (100 [HAU]/ml) for 10 h. RNA was extracted and cDNA was prepared to determine IFN-β and ISG56 mRNA expression by real-time PCR analysis.

For Figure S5A, S5B, S5G-S5I, data are presented as mean. Error bars denote SD (n=3).

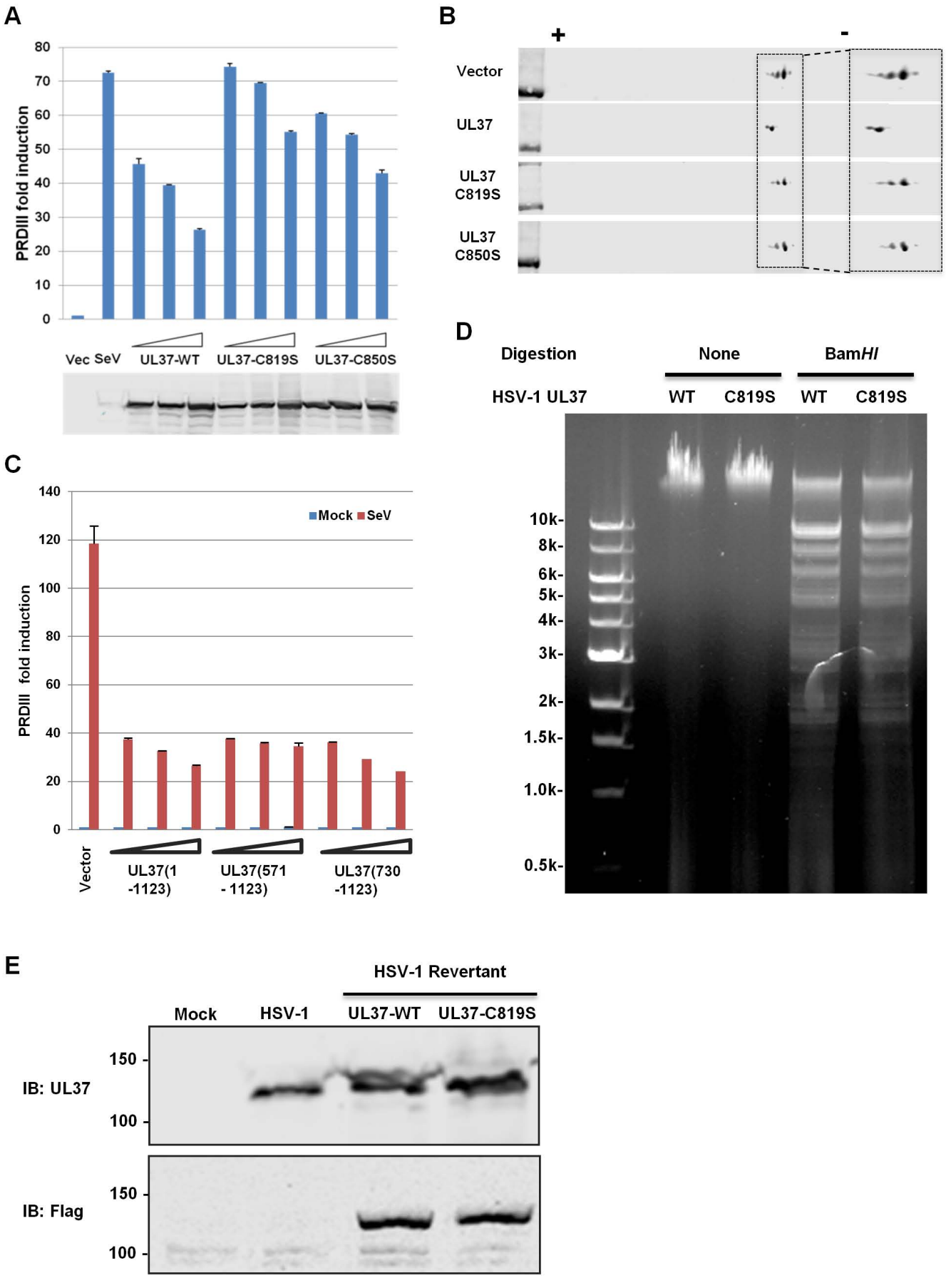

A
PRDIII fold induction
80
70
60
50
40
30
20
10
0
Vec SeV
UL37-WT
UL37-C819S
UL37-C850S
B
+
-
Vector
UL37
UL37 C819S
UL37 C850S
C
PRDIII fold induction
140
120
100
80
60
40
20
0
Mock
SeV
Vector
UL37(1 -1123)
UL37(571 - 1123)
UL37(730 -1123)
D
Digestion
None
BamHI
HSV-1 UL37
WT
C819S
WT
C819S
10k-
8k-
6k-
5k-
4k-
3k-
2k-
1.5k-
1.0k-
0.5k-
E
HSV-1 Revertant
Mock
HSV-1
UL37-WT
UL37-C819S
IB: UL37
150
100
IB: Flag
150
100

Figure S6. The carboxyl terminal half of UL37 contains a deamidase domain Related to Figure 6.

(A) 293T cells were transfected with a PRDIII reporter cocktail and increasing amount of a plasmid containing UL37-WT, UL37-C819S or UL37-C850S. Transfected cells were subsequently infected with Sendai virus (SeV, 100 [HAU]/ml) for 15 h. Fold induction of the PRDIII reporter was determined by luciferase assay.

(B) 293TRex/RIG-I cells were transfected with a plasmid containing UL37-WT, UL37-C819S or UL37-C850S. WCLs were analyzed by 2-dimensional gel electrophoresis and immunoblotting with indicated antibodies.

(C) 293T cells were transfected with the PRDIII reporter plasmid cocktail and increasing amounts of a plasmid containing UL37 (1-1123), UL37 (571-1123) or UL37 (730-1123). At 30 hours later, cells were infected with SeV (100 [HAU]/mL) for 16 hours. Fold induction of the PRDIII promoter was determined by luciferase assay.

(D) Recombinant HSV-1 carrying flag-tagged wild-type UL37 or C819S UL37 was generated by homologous recombination. Viral DNA was extracted from infected Vero cells and digested by BamHI. DNA fragments were analyzed by agarose gel electrophoresis.

(E) 293T cells were infected with HSV-1 (KOS), HSV-1 UL37-WT or HSV-1 UL37-C819S (MOI = 5) for 20 h, respectively. WCLs were analyzed by immunoblotting with indicated antibodies.

For Figure S6A and S6C, data are presented as mean. Error bars denote SD (n=3).

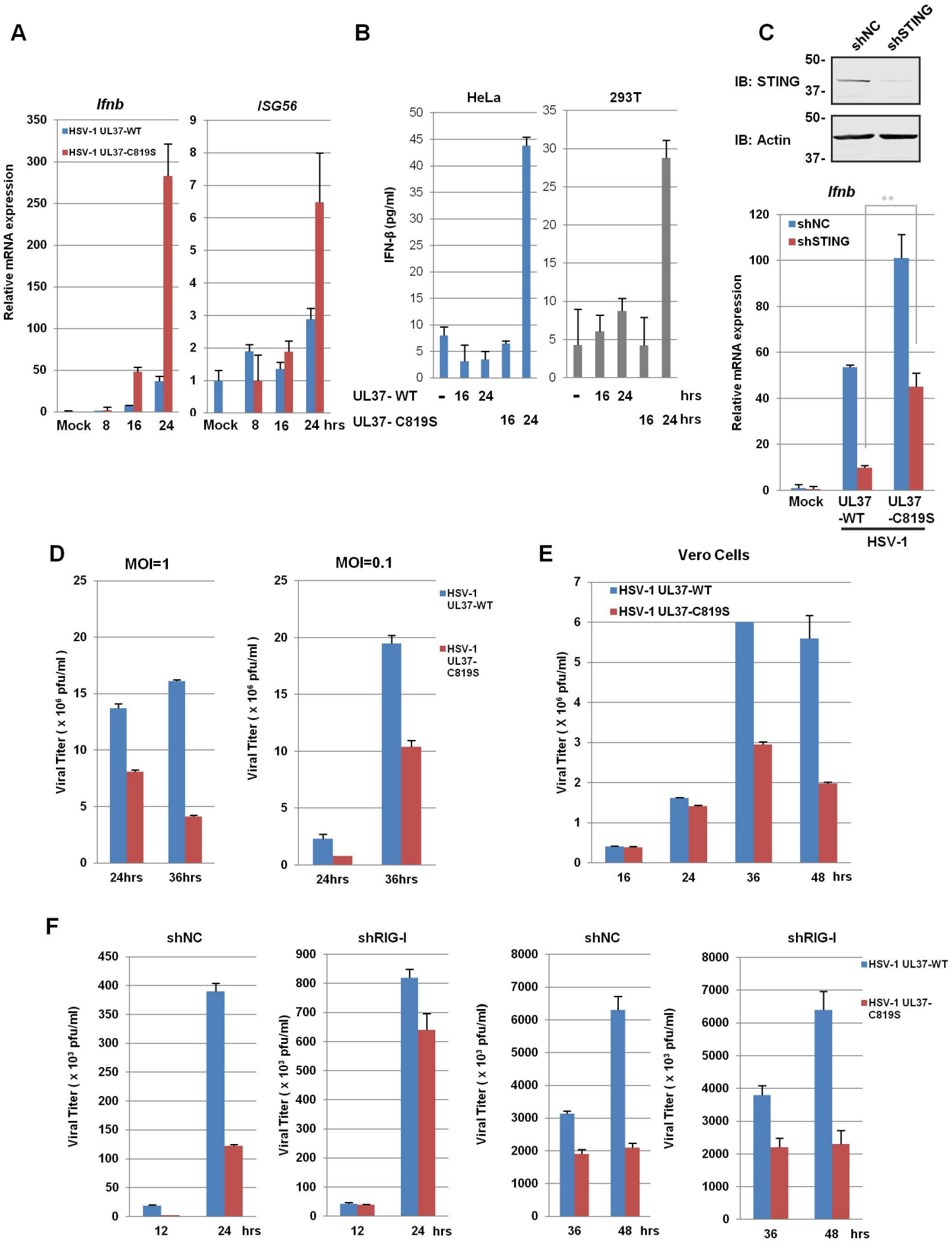

A
Ifnb
ISG56
HSV-1 UL37-WT
HSV-1 UL37-C819S
Relative mRNA expression
Mock 8 16 24 hrs
B
HeLa
293T
IFN-β (pg/ml)
UL37- WT
UL37- C819S
hrs
C
shNC
shSTING
IB: STING
IB: Actin
Ifnb
Relative mRNA expression
Mock
UL37 -WT
UL37 -C819S
HSV-1
D
MOI=1
MOI=0.1
Viral Titer (x 10⁶ pfu/ml)
24hrs
36hrs
HSV-1 UL37-WT
HSV-1 UL37-C819S
E
Vero Cells
Viral Titer (X 10⁶ pfu/ml)
16 24 36 48 hrs
F
shNC
shRIG-I
Viral Titer (x 10³ pfu/ml)
12 24 hrs
36 48 hrs
HSV-1 UL37-WT
HSV-1 UL37-C819S

Figure S7. The carboxyl terminal half of UL37 contains a deamidase domain Related to Figure 6.

(A) HeLa cells were infected with recombinant HSV-1 UL37-WT or HSV-1 UL37-C819S (MOI = 5) for the indicated hours. RNA was extracted and cDNA was prepared to determine IFN-β and ISG56 mRNA expression by real-time PCR analysis.

(B) HeLa and 293T cells were infected with recombinant HSV-1 as in (A). Supernatant was harvested and IFN-β was quantified by ELISA.

(C) (Top) HFF cells stably expressing control or STING shRNA were prepared by lentiviral transduction. WCLs were analyzed by immunoblotting with the indicated antibodies. (Bottom) HFF stable cells were infected with recombinant HSV-1 (MOI = 5) as in (A). RNA was extracted and cDNA was prepared to determine IFN-β mRNA expression by real-time PCR analysis.

(D) HeLa cells were infected with recombinant HSV-1 (MOI = 0.1/1) as in (A) for the indicated hours. Supernatant was harvested and HSV-1 viral titer was measured by plaque assay.

(E) Vero cells were infected with recombinant HSV-1 (MOI = 0.1) as in (A) for the indicated hours. Supernatant was harvested and HSV-1 viral titer was measured by plaque assay.

(F) HFF cells stably expressing control or RIG-I shRNA were prepared by lentiviral transduction as in FigS1A. Cells were then infected with recombinant HSV-1 (MOI = 0.1) as in (A) for the indicated hours. Supernatant was harvested and HSV-1 viral titer was measured by plaque assay.

For Figure S7A-S7F, data are presented as mean. Error bars denote SD (n=3). $^{**}p<0.01$

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