Supplemental information

Species-specific deamidation of cGAS by herpes simplex virus UL37 protein facilitates viral replication

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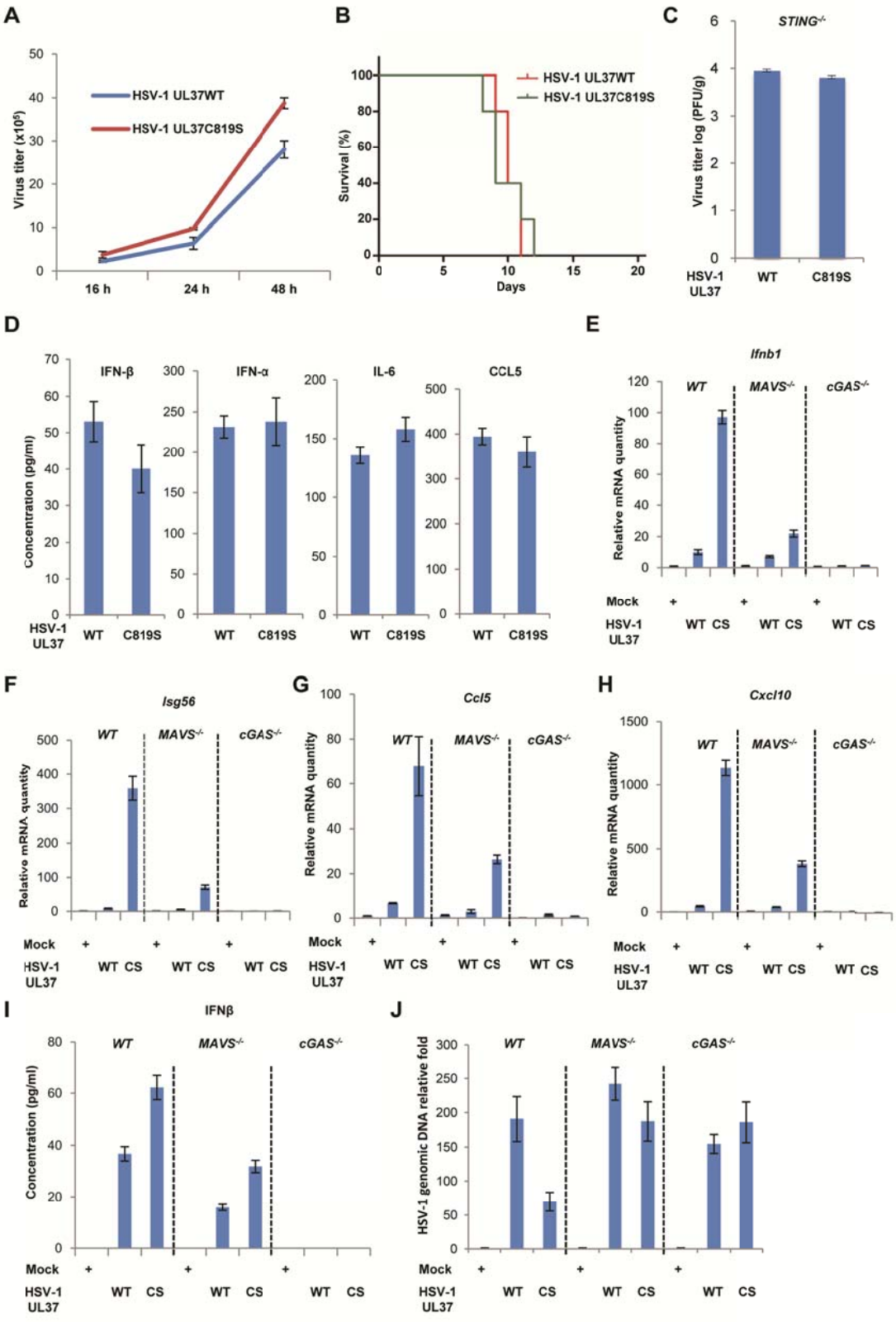

Figure S1. HSV-1 carrying the deamidase-deficient UL37C718S more robustly induces cytokine production than wild-type HSV-1 *in vivo*. Related to Figure 1.

(A) VERO cells were infected with HSV-1 UL37WT or HSV-1 UL37C819S (MOI = 0.1) and virus titer was quantified by plaque assay at indicated time points.

(B) Age- and gender-matched STING KO mice (n=10) were infected with HSV-1 UL37WT or HSV-1 UL37C819S (5×10^7 PFU). Mouse survival was recorded daily.

(C) Mice were sacrificed at 3 days post-infection and viral titer in the brain was determined by plaque assay (n=5).

(D) Age- and gender-matched STING KO mice (n=5) were infected with HSV-1 UL37WT (WT) or HSV-1 UL37C819S (C819S) (5×10^7 PFU). Blood was collected at 8 hours post-infection (hpi) and cytokines in sera were determined by ELISA.

(E-H) Primary bone marrow-derived macrophages (BMDMs) were infected with HSV-1 UL37WT or HSV-1 UL37C819S (CS) viruses (MOI = 5). Total RNA was extracted and the expression of the indicated genes was determined by reverse transcription and real-time PCR at 6 hpi.

(I and J) Primary BMDMs were infected with HSV-1 UL37WT or HSV-1 UL37C819S (CS) viruses (MOI = 5). At 20 hpi, the induction of IFN-β was determined by ELISA (I) and HSV-1 genomic DNA was quantified by real-time PCR (J).

Each value is the mean + s.d. of the results of three independent experiments (A, C-J). Statistical analysis was performed by log-rank test for (B) and unpaired *t*-test for (C and D). Data are representative of at least two independent experiments (B).

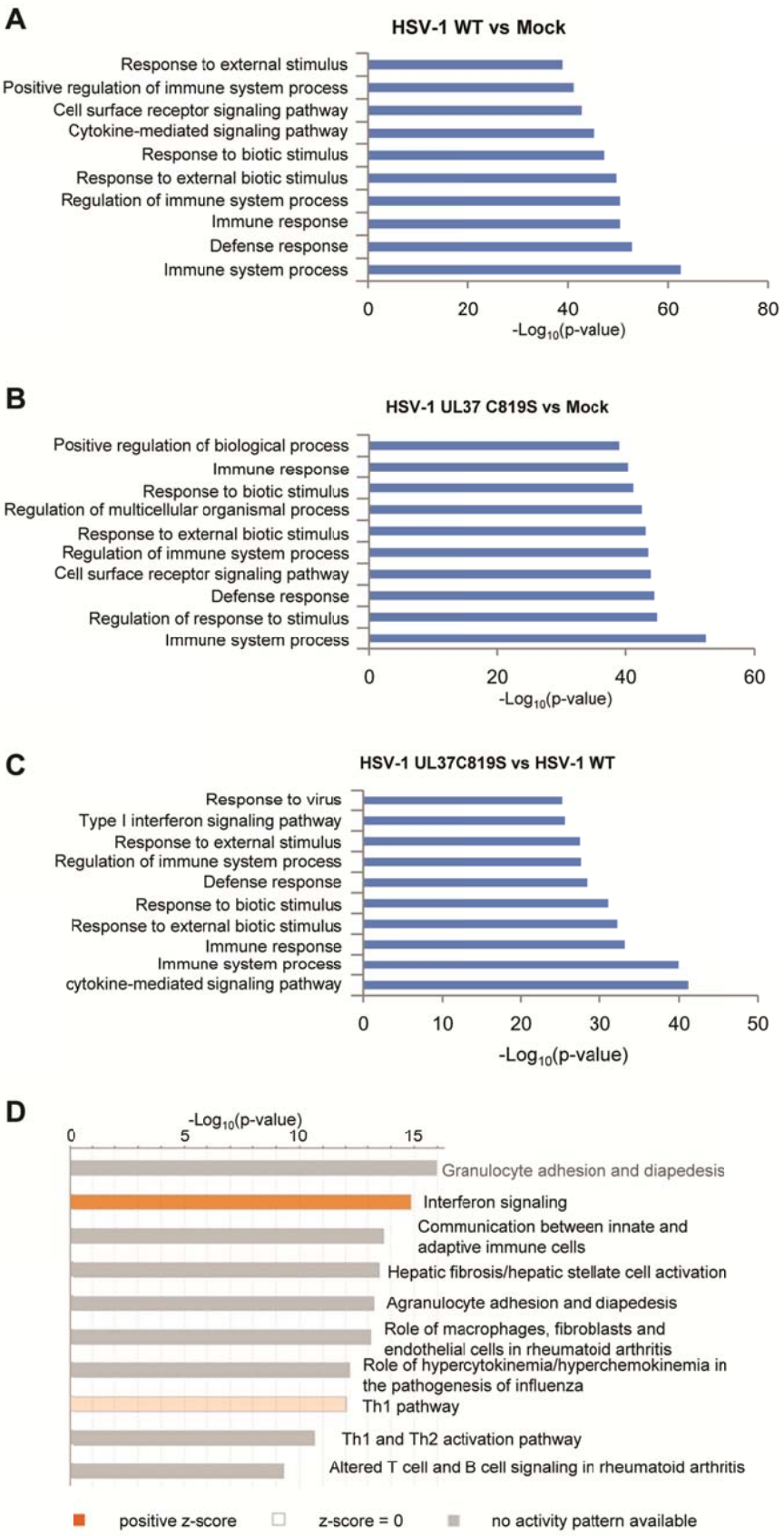
A
HSV-1 WT vs Mock
Response to external stimulus
Positive regulation of immune system process
Cell surface receptor signaling pathway
Cytokine-mediated signaling pathway
Response to biotic stimulus
Response to external biotic stimulus
Regulation of immune system process
Immune response
Defense response
Immune system process
0
20
40
60
80
-Log10(p-value)
B
HSV-1 UL37 C819S vs Mock
Positive regulation of biological process
Immune response
Response to biotic stimulus
Regulation of multicellular organismal process
Response to external biotic stimulus
Regulation of immune system process
Cell surface receptor signaling pathway
Defense response
Regulation of response to stimulus
Immune system process
0
20
40
60
-Log10(p-value)
C
HSV-1 UL37C819S vs HSV-1 WT
Response to virus
Type I interferon signaling pathway
Response to external stimulus
Regulation of immune system process
Defense response
Response to biotic stimulus
Response to external biotic stimulus
Immune response
Immune system process
cytokine-mediated signaling pathway
0
10
20
30
40
50
-Log10(p-value)
D
-Log10(p-value)
0
5
10
15
Granulocyte adhesion and diapedesis
Interferon signaling
Communication between innate and adaptive immune cells
Hepatic fibrosis/hepatic stellate cell activation
Agranulocyte adhesion and diapedesis
Role of macrophages, fibroblasts and endothelial cells in rheumatoid arthritis
Role of hypercytokinemia/hyperchemokinemia in the pathogenesis of influenza
Th1 pathway
Th1 and Th2 activation pathway
Altered T cell and B cell signaling in rheumatoid arthritis
positive z-score
z-score = 0
no activity pattern available

Figure S2. HSV-1 carrying deamidase-deficient UL37 more robustly induces innate immune responses. Related to Figure 2

(A-C) Human THP-1 monocytes were mock-infected or infected with HSV-1 UL37WT or HSV-1 UL37C819S at MOI=5 for 4 h. Total mRNA was extracted and subjected to RNA sequencing. Gene ontology (GO) analysis revealed the top 10 enriched gene sets.

(D) Ingenuity Pathway Analysis (IPA) revealed the top 10 signaling pathways that were enriched in THP-1 cells infected with HSV-1 UL37C819S vs HSV-1 WT.

Data are representative of at least two independent experiments (A-D).

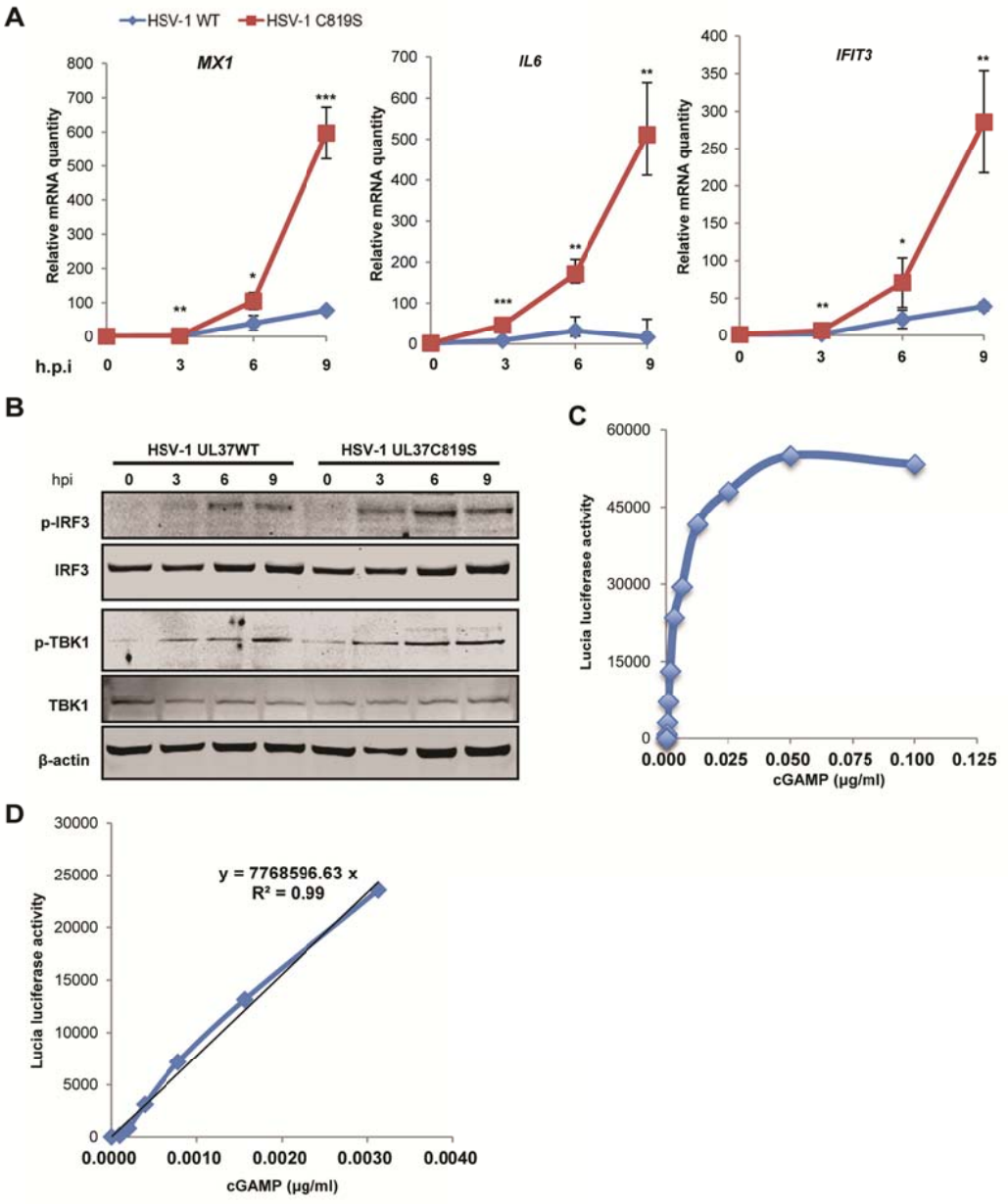

Figure S3. HSV-1 carrying deamidase-deficient UL37 more robustly induces innate immune responses. Related to Figure 2

(A) THP-1 cells were infected with HSV-1 UL37WT or HSV-1 UL37C819S at MOI=5. Total RNA was extracted and the induction of inflammatory cytokines was determined by real-time PCR at indicated time points.

(B) Whole cell lysates (WCLs) were analyzed by immunoblotting with indicated antibodies.

(C, D) cGAMP was delivered to digitonin-permeabilized THP1-Lucia ISG cells for 30 min at 37°C. Cells were cultured for 20 h and Lucia reporter activity was measured. Pure cGAMP was diluted and used to establish a standard for the assay.

Each value is the mean + s.d. of the results of three independent experiments (A). Statistical analysis was performed by unpaired *t*-test for (A); $*P<0.05$, $**P<0.01$, $***P<0.005$. Data are representative of at least two independent experiments (B-D).

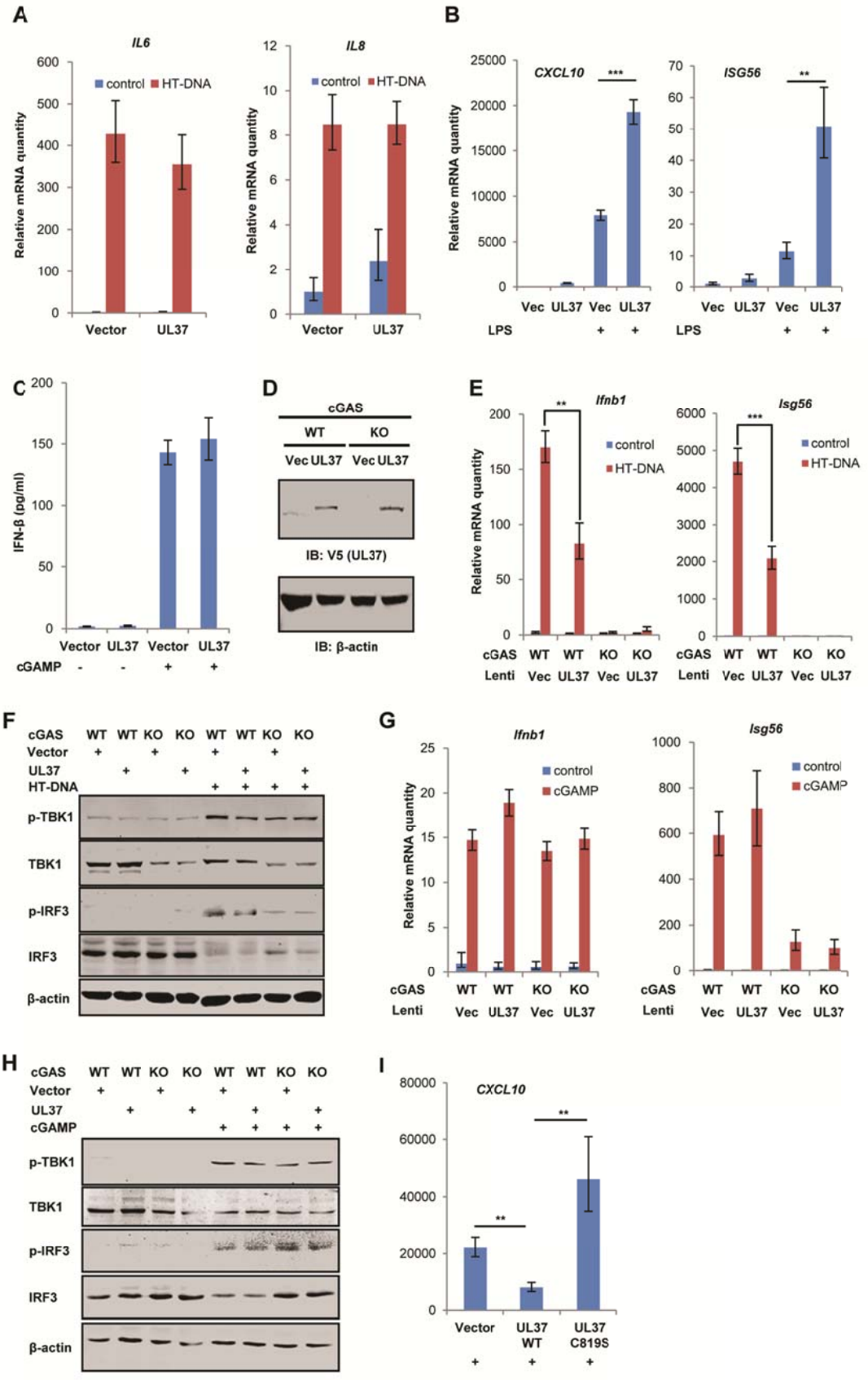

Figure S4. UL37 targets cGAS to inhibit innate immune activation. Related to Figure 3.

(A) THP-1 stable cell lines were transfected with HT-DNA (2 μg/ml) and cells were harvested at 6 hours post-transfection. The expression of *IL6* and *IL8* was analyzed by real-time PCR.

(B) THP-1 stable cell lines were stimulated with LPS (2 μg/ml) for 6 h and the expression of *CXCL10* and *ISG56* was analyzed by real-time PCR.

(C) THP-1 stable cell lines were transfected with cGAMP (2 μg/ml). IFN-β in the supernatant was determined by ELISA at 16 hours post-transfection.

(D) Wild-type (WT) or cGAS KO L929 cells were infected with control (Vec) or UL37-expressing lentivirus to establish stable cell lines and WCLs were analyzed by immunoblotting with the indicated antibodies.

(E) WT or cGAS KO L929 stable cell lines as describe in (D) were transfected with HT-DNA (2 μg/ml) and the induction of *Ifnb1* and *Isg56* was analyzed at 6 h post-transfection.

(F, H) WT or cGAS KO stable L929 cells were transfected with HT-DNA (F) or cGAMP (H). Cells were harvested at 6 h post-transfection and WCLs were analyzed by immunoblotting with indicated antibodies.

(G) L929 stable cell lines were transfected with cGAMP (2 μg/ml). The expression of *Ifnb1* and *Isg56* was analyzed by real-time PCR at 6 hours post-transfection.

(I) THP-1 stable cell lines were transfected with HT-DNA. Cells were harvested at 6 h post-transfection. The relative mRNA quantity of *CXCL10* was determined by real-time PCR.

Each value is the mean + s.d. of the results of three independent experiments (A-C, E, G,

I). Statistical analysis was performed by unpaired *t*-test for (A-C, E, I); ***P*<0.01, ****P*<0.005. Data are representative of at least two independent experiments (D, F, H).

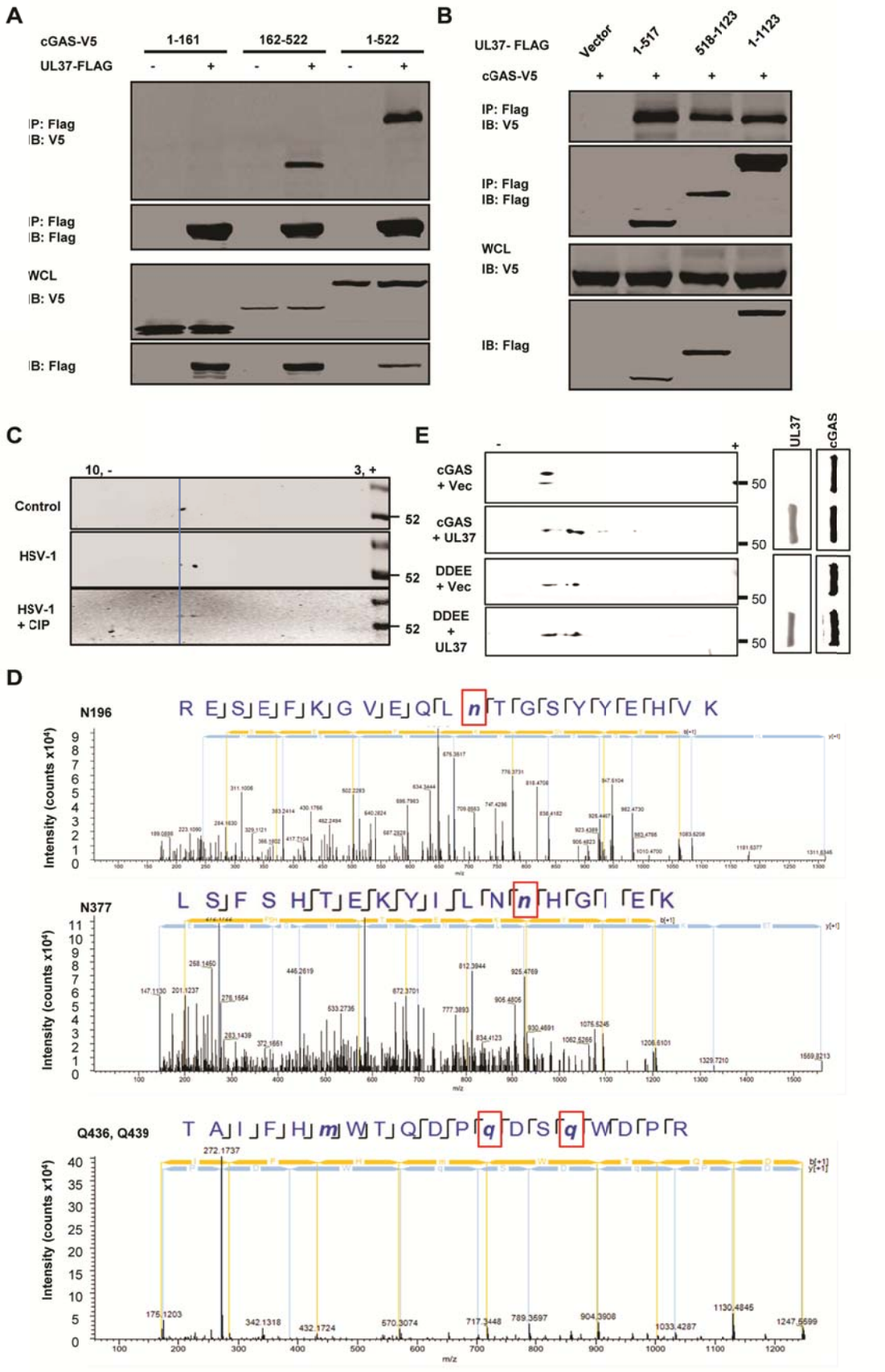

Figure S5. UL37 deamidates cGAS. Related to Figure 4

(A) 293T cells were transfected with a plasmid encoding cGAS(1-161)-V5, cGAS(162-522)-V5 or cGAS-V5 and an empty vector or UL37-FLAG plasmid. Centrifuged whole cell lysates (WCLs) were incubated with anti-FLAG agarose. Precipitated proteins and WCLs were analyzed by immunoblotting with indicated antibodies.

(B) 293T cells were co-transfected with a plasmid containing cGAS-V5 and plasmids containing UL37(1-517)-FLAG, UL37(518-1123)-FLAG, UL37 or empty vector in 293T cells. Immunoprecipitation and immunoblotting were performed as described in (A).

(C) cGAS-expressing 293T cells were mock-infected or infected with HSV-1 (MOI=10). At 4 hpi, cells were harvested and FLAG-cGAS was immunoprecipitated and treated with alkaline phosphatase (CIP) (20 U) for 20 min at 37°C. The purified proteins were analyzed by 2-DGE and immunoblotting with cGAS antibody.

(D) mcGAS deamidated by UL37 *in vitro* was analyzed by tandem mass spectrometry. The m/z spectrum of the deamidated peptide containing N196, N377 or Q436 and Q439 (homologous to hcGAS N210, N389, Q451 and Q454) was shown.

(E) 293T cells stably expressing wild-type cGAS or the deamidated cGAS-DDEE mutant were transfected with vector or that containing UL37. WCLs were prepared, and analyzed by two-dimensional gel electrophoresis and immunoblotting.

Data are representative of at least two independent experiments (A-E).

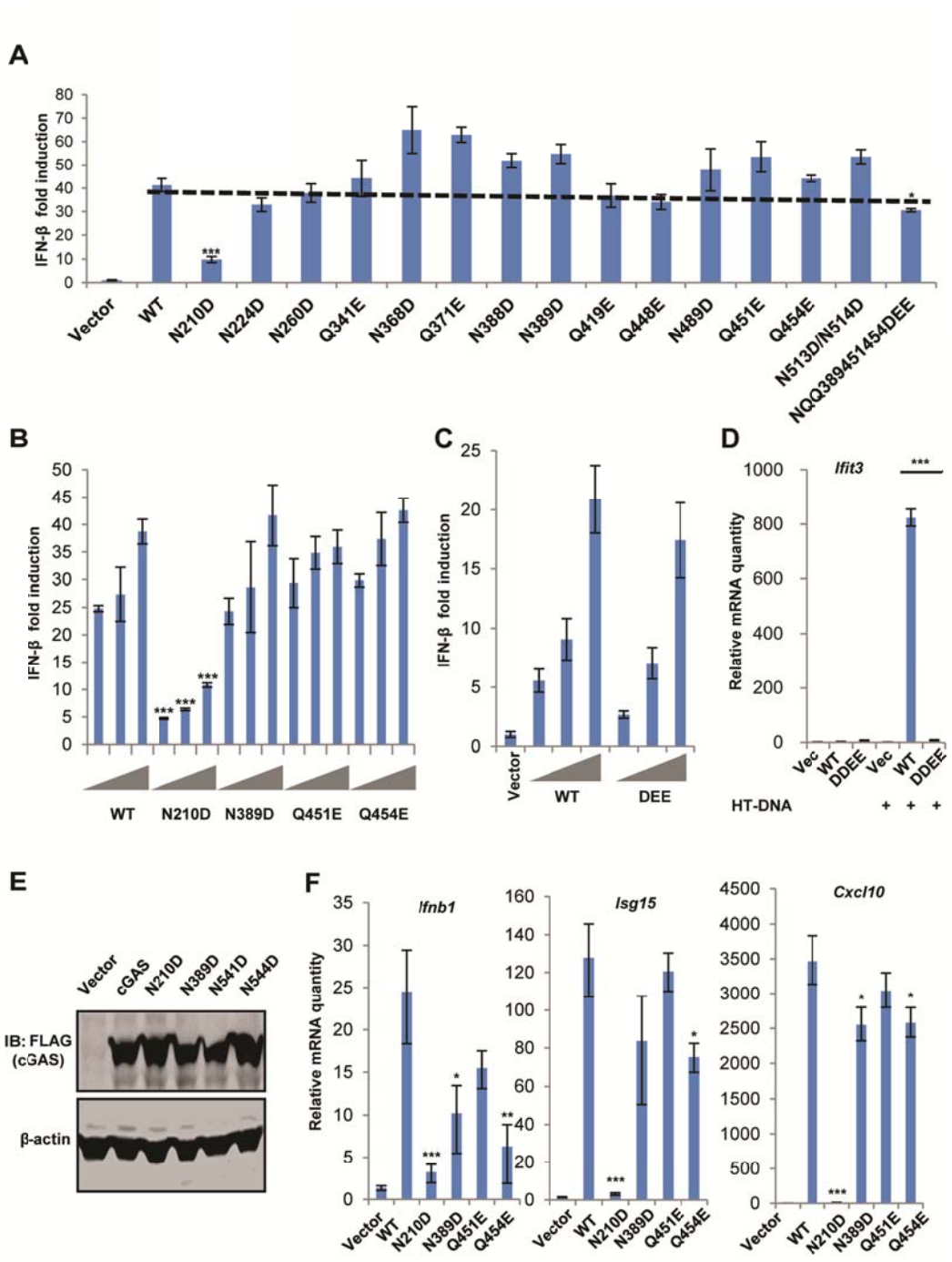

Figure S6. Deamidated cGAS fails to respond to DNA stimulation. Related to Figure 5

(A-C) 293T cells were transfected with the IFN-β reporter plasmid cocktail with plasmids containing cGAS wild-type or mutants. At 30 h post-transfection, IFN-β activation was determined by luciferase assay.

(D) cGAS KO L929 cells “reconstituted” with control lentivirus (Vec), or lentivirus containing cGAS or cGAS-DDEE were transfected with HT-DNA, and *Ifit3* expression was analyzed by real-time PCR at 6 h post-transfection.

(E) cGAS KO L929 cells were “reconstituted” with control lentivirus (vector), or lentivirus containing cGAS, cGAS-N210D, cGAS-N389D, cGAS-Q451E and cGAS-Q454E. WCLs of these stable cell lines were analyzed by immunoblotting with indicated antibodies.

(F) The “Reconstituted” cells as described in (E) were transfected with HT-DNA (2 μg/ml) and the expression of the indicated genes was analyzed by real-time PCR at 6 h post-transfection.

Each value is the mean + s.d. of the results of three independent experiments (A-D, F). Statistical analysis was performed by unpaired *t*-test for (A-D, F); **P*<0.05, ***P*<0.01, ****P*<0.005. Data are representative of two independent experiments (E).

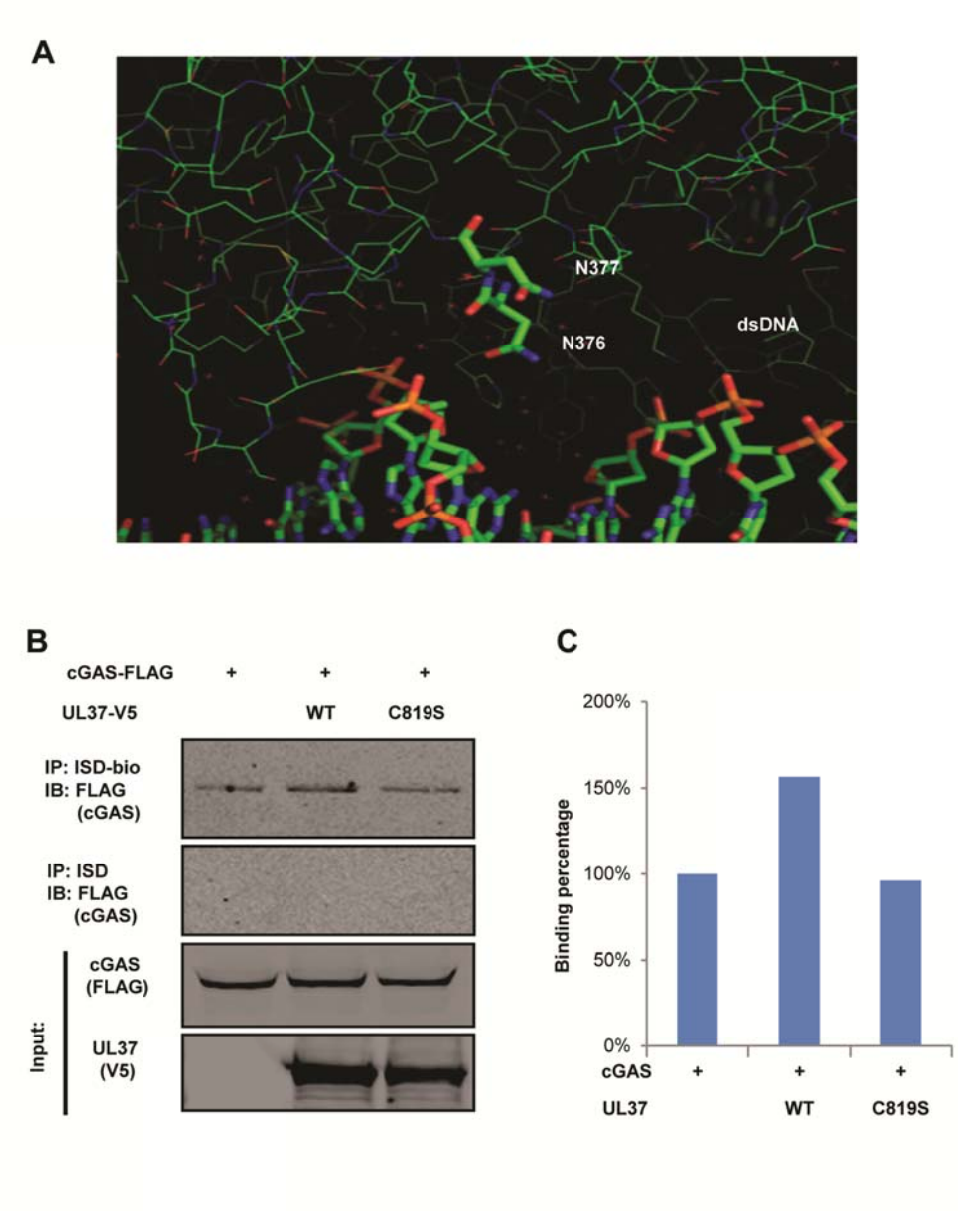

Figure S7. cGAS deamidation doesn't impair its DNA-binding activity. Related to Figure 6

(A) The structure of cGAS with N376 and N377 extending into the minor groove of dsDNA that bound to cGAS was shown (PDB: 4K9B).

(B) 293T-cGAS-FLAG stable cells were transfected with empty vector, plasmids encoding UL37-V5 or UL37C819S-V5, and WCLs were precipitated with ISD-biotin. Precipitated proteins and WCLs were analyzed by immunoblotting with indicated antibodies.

(C) The relative intensity of precipitated cGAS was determined by densitometry analysis.

Data are representative of three independent experiments (B, C).

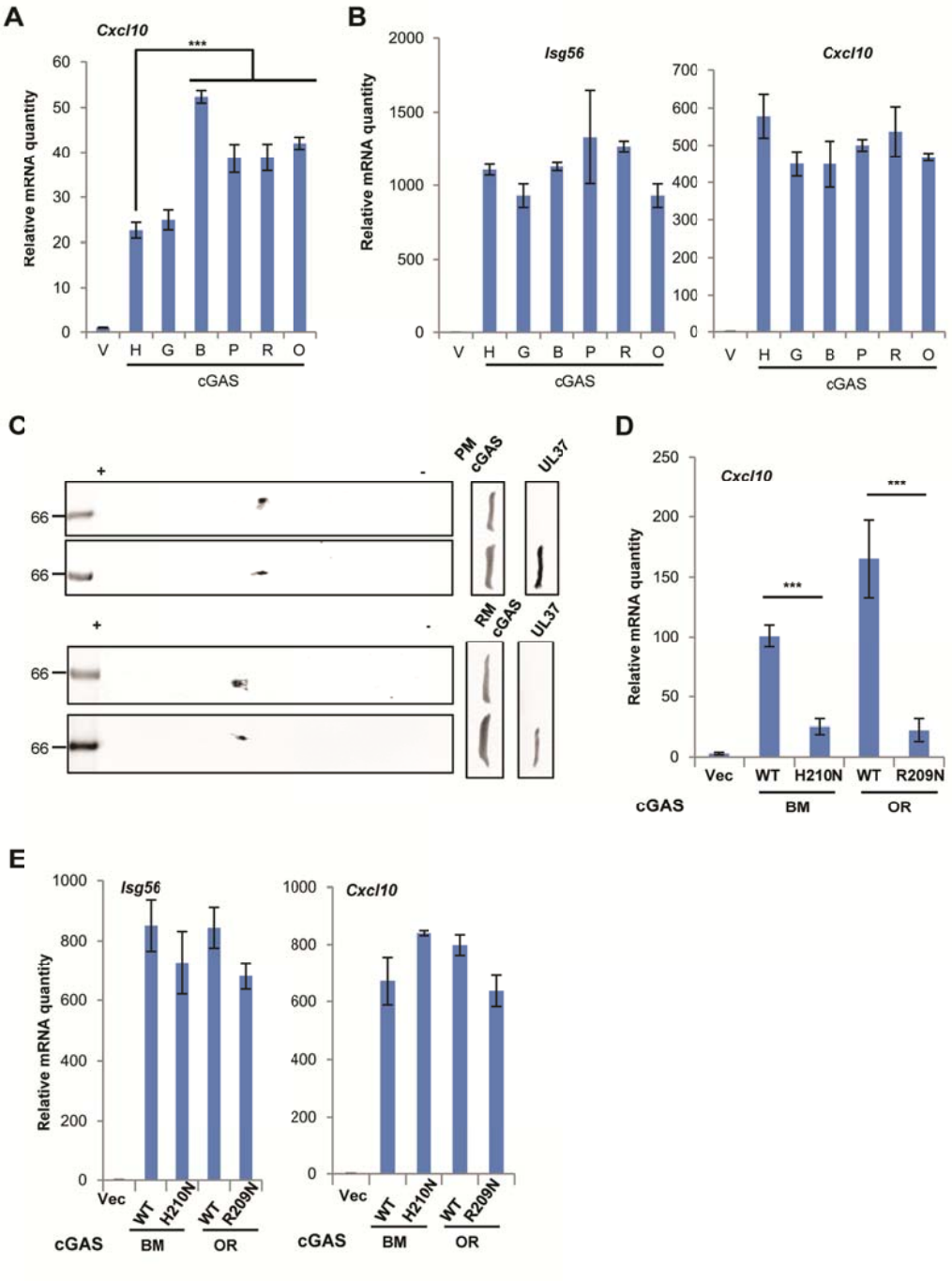

Figure S8. cGAS of nonhuman primates induces stronger innate immune response against HSV-1. Related to Figure 7

(A) cGAS KO L929 cells were “reconstituted” with control lentivirus (V), or lentivirus

containing cGAS of human (H), gorilla (G), black mangabey (B), pastas monkey (P), rhesus macaque (R), or orangutan (O). Stable cell lines were infected with HSV-1 UL37WT at MOI=5 and cells were harvested at 6 h post-infection. The relative mRNA quantity of *cxcl10* was determined by real-time PCR.

(B) “Reconstituted” cells as described in (A) were transfected with HT-DNA (2 μg/ml) and harvested at 6 h post-transfection, and the expression of the indicated genes was analyzed by real-time PCR.

(C) 293T cells were transfected with plasmids containing pastas monkey (PM) cGAS or rhesus macaque (RM) cGAS, with vector or that containing UL37 in 293T cells. WCLs were prepared, and analyzed by two-dimensional gel electrophoresis and immunoblotting.

(D, E) cGAS KO L929 cells “reconstituted” with control lentivirus (Vec), or lentivirus containing black mangabey (BM) cGAS, orangutan (OR) cGAS or their mutants were infected with HSV-1 (D) or transfected with HT-DNA (2 μg/ml) (E), and the expression of the indicated genes was analyzed by real-time PCR at 6 h post-infection (D) or post-transfection (E).

Each value is the mean + s.d. of the results of three independent experiments (A, B, D, E). Statistical analysis was performed by unpaired *t*-test for (A, D); ***$P<0.005$. Data are representative of at least two independent experiments (C).

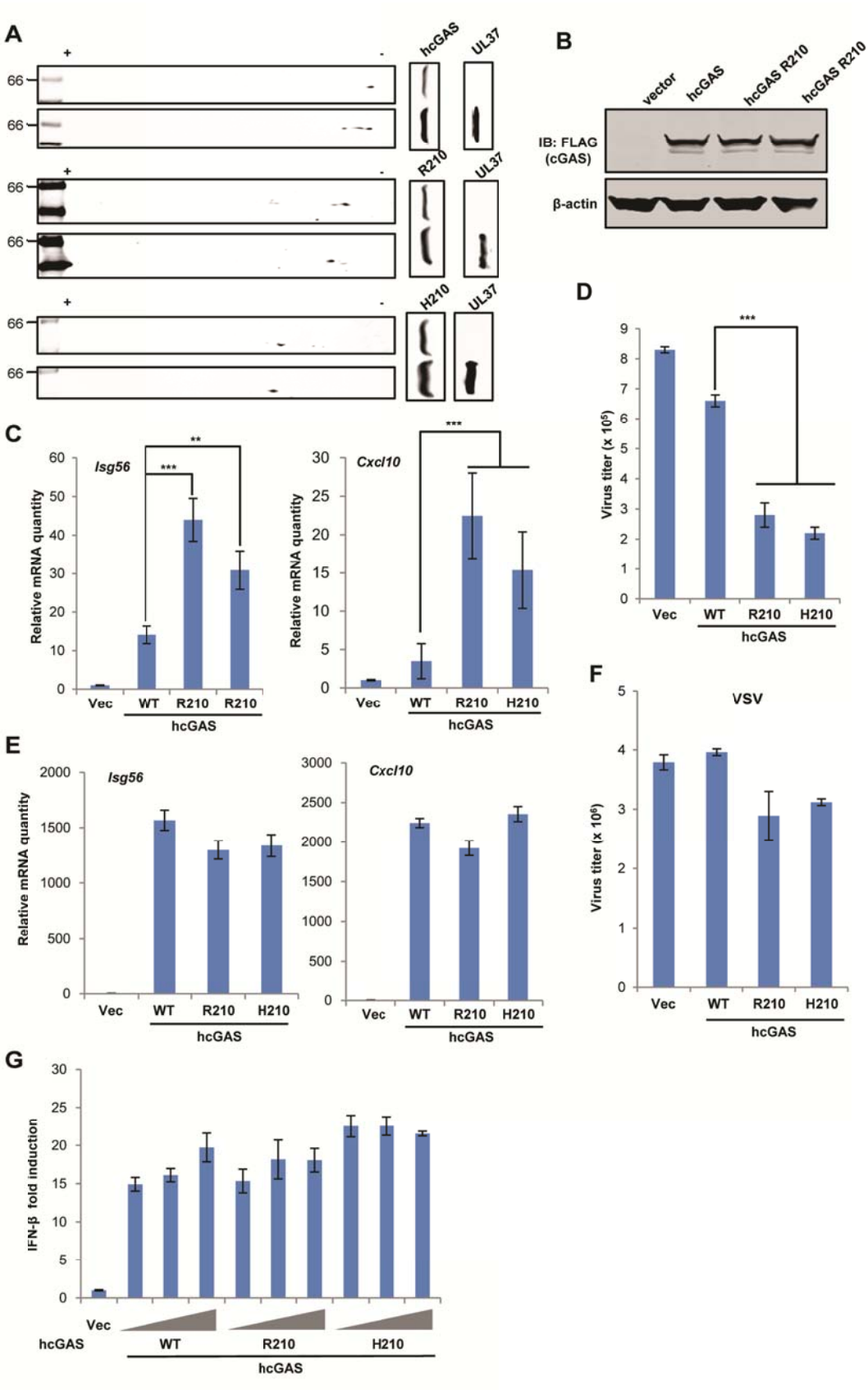

Figure S9. cGAS of nonhuman primates induces stronger innate immune response against HSV-1. Related to Figure 7

(A) 293T cells were transfected with plasmids containing human cGAS wild-type or mutants, with vector or that containing UL37 in 293T cells. WCLs were prepared, and analyzed by two-dimensional gel electrophoresis and immunoblotting.

(B, C) cGAS KO L929 cells were “reconstituted” with control lentivirus (Vector or Vec), or lentivirus containing human cGAS wild-type or mutants (B). Stable cell lines were infected with HSV-1 at MOI=5 and the expression of the indicated genes was analyzed by real-time PCR at 6 h post-infection (C).

(D) Stable cell lines from (B) were infected with HSV-1 (MOI=0.01), Viral titer was measured by plaque assay at 16 h post-infection.

(E) cGAS KO L929 cells “reconstituted” with control lentivirus (Vec), lentivirus containing human cGAS wild-type or mutants were transfected with HT-DNA (2 μg/ml). Cells were harvested 6 h post-transfection and the expression of the indicated genes was analyzed by real-time PCR.

(F) “Reconstituted” cells as described in (B) were infected with VSV (MOI=0.01). Viral titer in the supernatant was measured by plaque assay at 16 h post-infection.

(G) 293T cells were transfected with the IFN-β reporter plasmid cocktail with plasmids containing human cGAS wild-type or indicated mutants. At 30 h post-transfection, IFN-β activation was determined by luciferase assay.

Each value is the mean + s.d. of the results of three independent experiments (C-G). Statistical analysis was performed by unpaired *t*-test for (C, D); $^{**}P<0.01$, $^{***}P<0.005$. Data are representative of at least two independent experiments (A, B).