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Supplementary Information for

KAT5 acetylates cGAS to promote innate immune response to DNA virus

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Supplementary Information Text

SI Materials and Methods

Mice

The *Kat5*$^{SA/SA}$ mice were kindly provided by Dr. Sheng-Cai Lin (Xiamen University) (1). Genotyping by PCR was performed using the following primers. F1, 5'-GCCCCAGCCTCGGTTTTCCC-3'; R1, 5'-ACTGCCCTACGGGCTGACCC-3'; M1, 5'-TCAAGCTGATCCCCCGCGCT-3'.

The mice were bred in specific pathogen-free facilities at Medical Research Institute of Wuhan University. Six to eight week-old mice were used in the experiments and age- and sex-matched mice were used as controls. Experiments were conducted without blinding. All animal experiments were performed in accordance with the Institutional Animal Care and Use Committee guidelines.

Reagents, antibodies, cells and viruses

2',3'-cGAMP and Lipofectamine 2000 (InvivoGen); polybrene (Millipore); SYBR Green supermix (Bio-Rad); Dual-Specific Luciferase Assay Kit (Promega); puromycin (Thermo); streptavidin agarose (Thermo); MG149 (Selleck); HDACs inhibitor (TSA) and SIRTs inhibitor (NAM) (MCE); 3xFLAG peptide , Ac-CoA and Digitonin (Sigma); and ELISA kits for murine IFN-β (PBL), IL-6 (BioLegend), CXCL10 (BOSTER), and cGAMP (Cayman) were purchased for the indicated

manufacturers.

Mouse monoclonal antibodies against HA (901515) (BioLegend); FLAG (F3165) and β-actin (A2228) (Sigma); cGAS (31659S, 15102), phospho-MITA S366 (40818) and phospho-IRF3 S396 (4947S) (Cell Signaling Technology); phospho-TBK1 S172 (ab109272), phospho-KAT5 S86 (ab73207), Ac-K (ab21623) and TBK1 (ab40676) (Abcam); KAT5 (sc-166363) and IRF3 (sc-33641) (Santa Cruz Biotechnology) were purchased from the indicated manufacturers.

The antibody that specifically recognizes acetylated 4K (K47/K56/K62/K83) of cGAS was raised by immunizing rabbits with a synthetic peptide: ALP(K-AC)AGKFGPAR(K-AC)SGSRQ(K-AC)KSAPDTQERPPVRATGARAK(K-AC)APQR. The antibodies that specifically recognize acetylated cGAS(K384/K394) and cGAS(K414) were gifts from Dr. Tao Li (National Center of Biomedical Analysis of China) (2).

HEK293 and THP-1 cells were obtained from ATCC. HFFs were provided by Dr. Min-Hua Luo (Wuhan Institute of Virology, CAS). HSV-1 and MCMV were previously described (3).

Establishment of stable KAT5-expressing or knockdown cells

To establish KAT5-overexpressing cell lines, the 293 cells were transfected with two packaging plasmids (pGAG-Pol and pVSV-G) together with pMSCV-FLAG-KAT5 retroviral plasmid or empty vector by calcium phosphate precipitation. To establish stable KAT5-knockdown cell lines, the 293 cells were transfected with two packaging

plasmids (pGAG-Pol and pVSV-G) together with control or KAT5-RNAi retroviral plasmid by calcium phosphate precipitation. Twenty-four hours after transfection, cells were incubated with new medium without antibiotics for another twenty-four hours. The recombinant virus-containing medium was collected and filtered with 0.4 μm filter (Millex) and then added into cultured THP-1 or HFF cells in the presence of polybrene (4 μg/mL). The infected cells were selected with puromycin (1 μg/mL) for 48 hours before additional experiments were performed.

Constructs

Expression plasmids for HA- or FLAG-tagged KAT5 and its truncation mutants, HA- or FLAG-tagged cGAS and its truncation mutants, FLAG-tagged KAT5 (QG/EE), cGAS(K/R) and cGAS(K/Q) mutants were constructed by standard molecular biology techniques. The ISRE reporter plasmid and expression plasmids for MITA and TBK1 were previously described (4, 5).

Preparation of primary mouse cells

The preparation of BMDMs and BMDCs was previously described (6). Briefly, for preparation of BMDMs, mouse bone marrow-derived monocytes ($5x10^6$) were cultured in 100 mm dishes in 5 mL 10% M-CSF-containing conditional medium from L929 cells for 3-5 days. For preparation of BMDCs, mouse bone marrow-derived monocytes ($5x10^6$) were cultured in medium containing murine GM-CSF (50 ng/mL) for 6-8 days.

Synthetic dsDNA

The sequences of synthetic dsDNA ISD45, HSV60, HSV120 and VAVC70 are as following.

ISD45: 5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCT ACA-3';

HSV60: 5'-TAAGACACGATGCGATAAAATCTGTTTGTAAAATTTATTAAGGGT ACAAATTGCCCTAGC-3';

VACV70: 5'-CCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATGGAAGA GAGAAAGAGATAAAACTTTTTTACGACT-3';

DNA90: 5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTAC ATACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTACA-3';

HSV120: 5'-AGACGGTATATTTTTGCGTTATCACTGTCCCGGATTGGACACGG TCTTGTGGGATAGGCATGCCCAGAAGGCATATTGGGTTAACCCCTTTTTATT TGTGGCGGGTTTTTTGGAGGACTT-3'.

Transfection and reporter assays

Transfection and reporter assays were performed as previously described (7). HEK293 cells were transfected by standard calcium phosphate precipitation method. To ensure that each transfection receives the same amount of total DNA, an empty control plasmid was added to each transfection. To normalize for transfection efficiency, pRL-TK (*Renilla* luciferase) reporter plasmid (0.01 μg) was added to each

transfection. Luciferase assays were performed using a Dual-Specific Luciferase Assay Kit. Firefly luciferase activities were normalized with *Renilla* luciferase activities.

RNAi

Double-strand oligonucleotides corresponding to the target sequences were cloned into the pSuper.retro RNAi plasmid (Oligoengine). The target sequences are as following.

Human KAT5: #1, 5'-GGAGAAAGAATCAACGGAA-3'; #2, 5'-GGCTGGACCTAAAGAAGAT-3'.

qPCR

Total RNAs were isolated and the reverse transcribed products were obtained for qPCR analysis to measure mRNA abundance of the indicated genes. Data shown is the relative abundance of the indicated mRNA normalized to that of GAPDH. Gene-specific primer sequences were as following. Human *IFNB1*, 5′-TTGTTGAGAACCTCCTGGCT-3′ and 5′-TGACTATGGTCCAGGCACAG-3′; human *ISG56*, 5′-TCATCAGGTCAAGGATAGTC-3' and 5′-CCACACTGTATTTGGTGTCTA-3′; human *CXCL10*, 5′-GGTGAGAAGAGATGTCTGAATCC-3′ and 5′-GTCCATCCTTGGAAGCACTGCA-3′; human *IL6*, 5′-AGACAGCCACTCACCTCTTCAG-3′ and

5′-TTCTGCCAGTGCCTCTTTGCTG-3′; human *GAPDH*,
5′-GTCTCCTCTGACTTCAACAGCG-3′ and
5′-ACCACCCTGTTGCTGTAGCCAA-3′; human *KAT5*,
5′-GGAACTCACCACATTGCCTGTC-3′ and
5′-CTCATTGCCTGGAGGATGTCGT-3′; murine *Ifnb1*,
5′-TCCTGCTGTGCTTCTCCACCACA-3′ and
5′-AAGTCCGCCCTGTAGGTGAGGTT-3′; murine *Cxcl10*,
5′-ATCATCCCTGCGAGCCTATCCT-3′ and
5′-GACCTTTTTTGGCTAAACGCTTTC-3′; murine *Isg56*,
5′-TACAGGCTGGAGTGTGCTGAGA-3′ and
5′-CTCCACTTTCAGAGCCTTCGCA-3′; murine *Il6*,
5′-TCTGCAAGAGACTTCCATCCAGTTGC-3′ and
5′-AGCCTCCGACTTGTGAAGTGGT-3′; murine *Gapdh*,
5′-ACGGCCGCATCTTCTTGTGCA-3′ and
5′-ACGGCCAAATCCGTTCACACC-3′;

Viral plaque assays

Viral plaque assays were previously described (8). Eight week-old mice were infected with HSV-1 for 5 days. The brains of mice were weighed and homogenized for 5 seconds in PBS. After homogenization, the brain suspensions were centrifuged at 1,620 g for 30 minutes, and the supernatants were used for plaque assays. Vero cells were seeded in 24-well plates, and the cells were infected by incubation for 2 hours at

152 37^{o}C with serial dilutions of the brain suspensions. After infection for 2 hours, 2%
153 methylcellulose was overlaid, and the plates were incubated for 36-48 hours. The
154 overlay was removed, and cells were fixed with 4% paraformaldehyde for 20 minutes
155 and stained with 1% crystal violet for 20 minutes before plaque counting.
156
157 **ELISA**
158 BMDMs were infected with viruses or transfected with the synthetic DNAs for 18
159 hours. The culture media were collected for measurement of IFN-β, CXCL10 and
160 IL-6. Eight week-old wild-type and *Kat5*$^{SA/SA}$ mice were infected intraperitoneally
161 with HSV-1 and MCMV for 6 hours, and then the sera of mice were collected for
162 measurement of IFN-β, CXCL10 and IL-6 by ELISA.
163
164 **Co-immunoprecipitation and immunoblotting analysis**
165 Cells were lysed in NP-40 lysis buffer (20 mM Tris-HCl pH7.4, 150 mM NaCl, 1 mM
166 EDTA, 1% NP-40, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 mM
167 phenylmethylsulfonyl fluoride). Co-immunoprecipitation and immunoblotting
168 analysis were performed as previously described (9). To detect acetylated proteins,
169 cells were treated with deacetylase inhibitors TSA and NAM for 8 hours before lysis.
170 For immunoblots, the blocking buffer contains 50 mM Tris (pH 7.5), 10% (V/V)
171 Tween-20 and 1% peptone, and incubation of the antibodies was carried out in 50 mM
172 Tris (pH7.5) and 0.1% peptone.
173

Recombinant protein purification

The cDNAs of cGAS and its mutants were cloned into the pGEX-6p-1-GST. The cDNA of KAT5 was cloned into the pET-30C. The plasmids were transformed into the BL21 *E. Coli* strain. Expression of the recombinant proteins was induced with 0.1 mM IPTG at 16^{o}C for 24 hours. The recombinant GST-fusion proteins were purified with GST resins and eluted with elution buffer (PBS, 100 mM Tris-HCl pH 8.8, 40 mM reduced glutathione). Ni^{2+}-NTA-agarose was used for purification of His-tagged KAT5 protein. To prepare FLAG-KAT5 protein, a mammal expression plasmid for FLAG-KAT5 was transfected into HEK293 cells. The cells were lysed 18 hours after transfection. FLAG antibody-conjugated beads were then used for immunoprecipitation for 4 hours at 4^{o}C. The beads were washed three times with lysis buffer. The FLAG-tagged KAT5 was eluted with 3xFLAG peptide in 250 mM Tris-HCl, pH 8.0. The FLAG-tagged KAT5 was used for acetylation assays *in vitro*.

***In vitro* acetylation**

The recombinant GST-cGAS and FLAG-KAT5 were purified, and mixed in HAT buffer (250 mM Tris-HCl, pH 8.0, 50% (vol/vol) glycerol, 0.5 mM EDTA, and 5 mM DTT) with or without 1 mM Ac-CoA at 30°C for 1.5 hours. The reaction was terminated by SDS/PAGE sample buffer and the protein samples were subjected to immunoblotting analysis with anti-Ac-K mAb.

***In vitro* pull-down assays**

In vitro pull-down assays were previously described (10). Briefly, HEK293 cells transfected with the indicated plasmids or THP-1 cells were lysed in NP-40 lysis buffer. Lysates were incubated with biotinylated-HSV120 for 1 hour at 4^{o}C, and then incubated with streptavidin beads for another 3 hours at 4^{o}C. The beads were washed three times with lysis buffer and analyzed by immunoblots with the indicated antibodies.

Mass spectrometry

HEK293 cells were transfected with FLAG-cGAS and HA-KAT5 plasmids for 20 hours. The cells were then treated with the deacetylase inhibitors TSA and NAM for 8 hours before lysis. The lysates were subjected to immunoprecipitation with anti-FLAG agarose beads at 4°C for 3 hours. The bound proteins were analyzed by MS, which was performed by SpecAlly (Wuhan) Life Science and Technology Company.

Immunofluorescent microscopy

HeLa or HT1080 cells were seeded on coverslips in 24-well plates. After transfection with HT-DNA for 5 hours or with the indicated plasmids for 20 hours, the cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.1% Triton X-100 for 10 minutes and blocked in 1% BSA for 1 hour. Cells were then incubated with a primary antibody for 2 hours, washed three times with washing buffer, and then incubated with Alexa Fluor 488-conjugated secondary antibody for 1 hour before

images were acquired using a ZEISS LSM confocal microscope.

Microscale thermophoresis technology (MST)

MST analysis was performed using a NanoTemper Monolith NT.115 instrument (NanoTemper Technologies GmbH). For detecting affinity, the protein samples were mixed with 20 nM Cy5-labeled HSV60. The samples were loaded into Premium Coated Capillaries and MST measurements were performed using 20% MST power and 40% LED power at 25°C. Laser-on and -off times were 30 and 5 seconds respectively. NanoTemper Analysis 1.2.20 software was used to fit the data and to determine the apparent Kd values.

cGAMP quantification

BMDMs (1×10^7) were mock-infected or infected with HSV-1 for 2 hours. Cells were then homogenized by dunce homogenizer in hypotonic buffer (10 mM Tris-HCl [pH 7.4], 10 mM KCl, and 1.5 mM $MgCl_2$). After centrifugation at 13,000 rpm for 20 min, the supernatant was heated at 95°C for 10 min and centrifuged at 13,000 rpm for 10 min to remove denatured proteins. cGAMP in the heat-resistant supernatants were measured by ELISA kit.

The indicated THP-1 cells (1×10^8) were left un-infected or infected with HSV-1 (MOI=2) for 3 hours. Cells were then homogenized by dunce homogenizer in hypotonic buffer (10 mM Tris-HCl, pH 7.4, 10 mM KCl, 1.5 mM $MgCl_2$). After centrifugation at 13,000 rpm for 20 minutes, the supernatant was heated at 95°C for

10 minutes and centrifuged at 13,000 rpm for another 10 minutes to remove denatured proteins. The heat-resistant supernatants containing cGAMP were delivered for cGAMP measurements with an Ultimate 3000 UHPLC Dionex (Sunnyvale, CA) coupled with a TSQ Quantiva (Thermo Fisher, Waltham, MA). The chromatography separation was performed on a Waters C18 column at 40˚C. Selective reaction monitoring (SRM) and the appropriate product ions were chosen to quantify cGAMP. The ration is based on the standard.

Digitonin permeabilization

cGAMP (40 nM) was delivered to THP-1 cells pretreated with digitonin permeabilization solution (50 mM HEPES pH 7.0, 100 mM KCl, 3 mM $MgCl_2$, 0.1 mM DTT, 85 mM Sucrose, 0.2% BSA, 1 mM ATP, 0.1 mM GTP and 2 μg/mL digitonin) at 37^{o}C for 20 minutes (11). Four hours later, the THP-1 cells were collected for qPCR analysis.

Statistics

Unpaired Student's *t* test was used for statistical analysis of most experimental results in this study. For the mouse survival study, Kaplan-Meier survival curves were generated and analyzed by Log-Rank test. $P < 0.05$ was considered significant.

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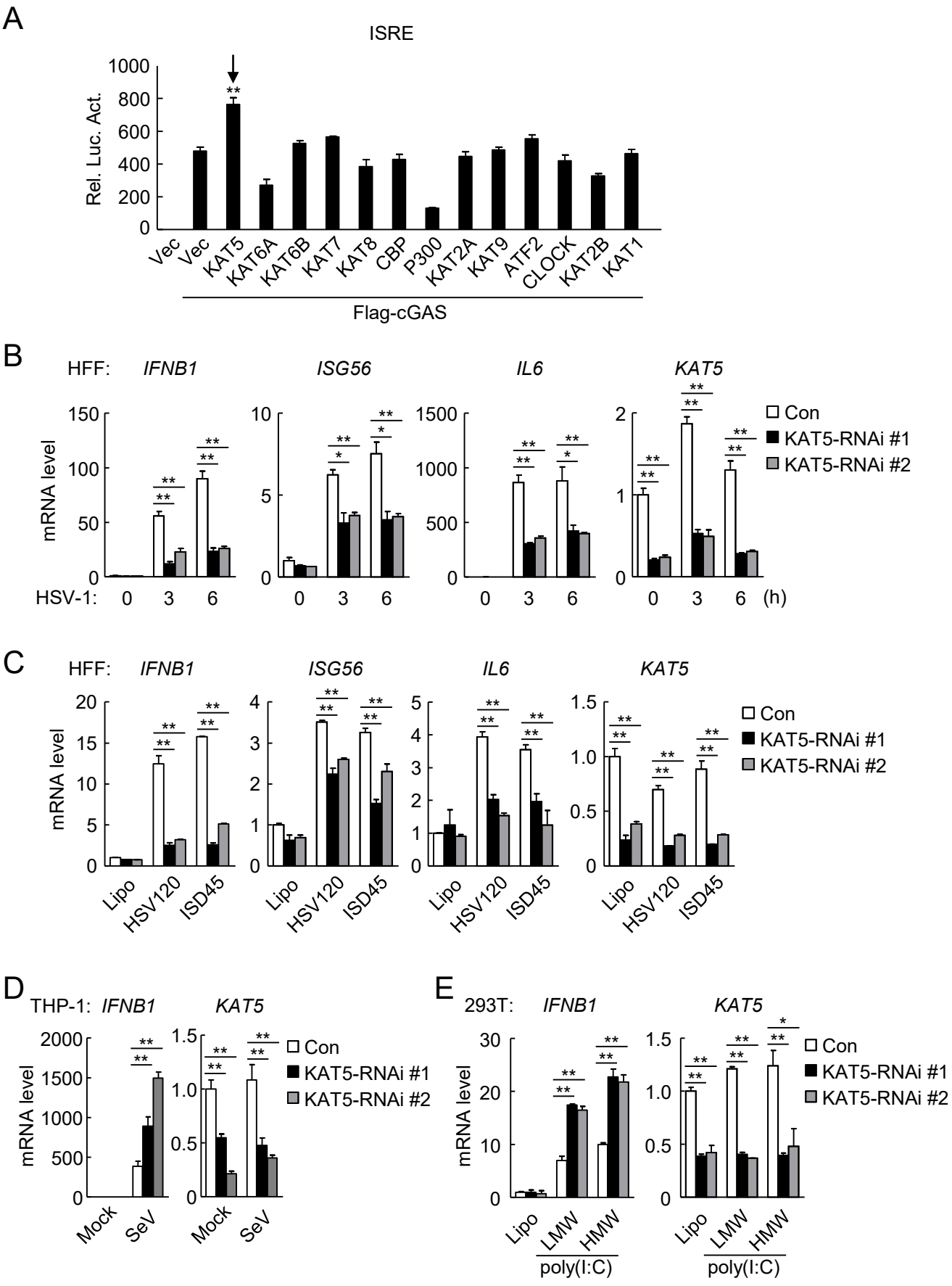
307
308

309 **Table S1 MS analysis of acetylation sites at the N-terminus of cGAS**

Protein Accession	Peptide	Acetylation site	m/z	PTM
Q8N884|CGAS_HUMAN	R.Q(-17.03)K(+42.01)KSAPDTQERPPVR.A	62	587.9814	Pyro-glu (Q); Acetylation (K)
Q8N884|CGAS_HUMAN	R.AK(+42.01)KAPQRAQDTQPSDATSAPGAEGLEPPAAR.E	82	1053.523	Acetylation (K)
Q8N884|CGAS_HUMAN	A.PEAALPKAGK(+42.01)FGPAR.K	50	776.4293	Acetylation (K)
Q8N884|CGAS_HUMAN	E.SPAAPEAALPKAGK(+42.01)FGPAR.K	50	939.5099	Acetylation (K)
Q8N884|CGAS_HUMAN	R.GAP(+31.99)MDPTESPAAPEAALPK(+42.01).A	47	641.972	Dihydroxy (P); Acetylation (K)
Q8N884|CGAS_HUMAN	R.G(+43.01)APMDPTESPAAPEAALPK(+42.01).A	47	967.9744	Carbamylation (G); Acetylation (K)
Q8N884|CGAS_HUMAN	R.ASEAGATAPK(+42.01)ASAR.N	21	665.3377	Acetylation (K)
Q8N884|CGAS_HUMAN	R.ASEAGATAPK(+42.01)ASAR(+14.02).N	21	672.3414	Acetylation (K); Methylation(KR)
Q8N884|CGAS_HUMAN	R.Q(-17.03)KK(+42.01)SAPDTQERPPVR.A	63	587.9794	Pyro-glu (Q); Acetylation (K)
Q8N884|CGAS_HUMAN	K.K(+43.01)(+42.01)SAPDTQERPPVR.A	63	783.3968	Carbamylation (K); Acetylation (K)
Q8N884|CGAS_HUMAN	K.K(+42.01)(+14.02)APQ(+.98)RAQDTQPSDATSAPGAEGLEPPAAR.E	83	992.1501	Acetylation (K); Methylation(KR); Deamidation (NQ)

310

Figure S1


A
ISRE
Rel. Luc. Act.
1000
800
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400
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Vec
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CBP
P300
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KAT9
ATF2
CLOCK
KAT2B
KAT1
Flag-cGAS
B
HFF:
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ISG56
IL6
KAT5
mRNA level
HSV-1:
0
3
6
(h)
Con
KAT5-RNAi #1
KAT5-RNAi #2
C
HFF:
IFNB1
ISG56
IL6
KAT5
mRNA level
Lipo
HSV120
ISD45
Con
KAT5-RNAi #1
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D
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SeV
Con
KAT5-RNAi #1
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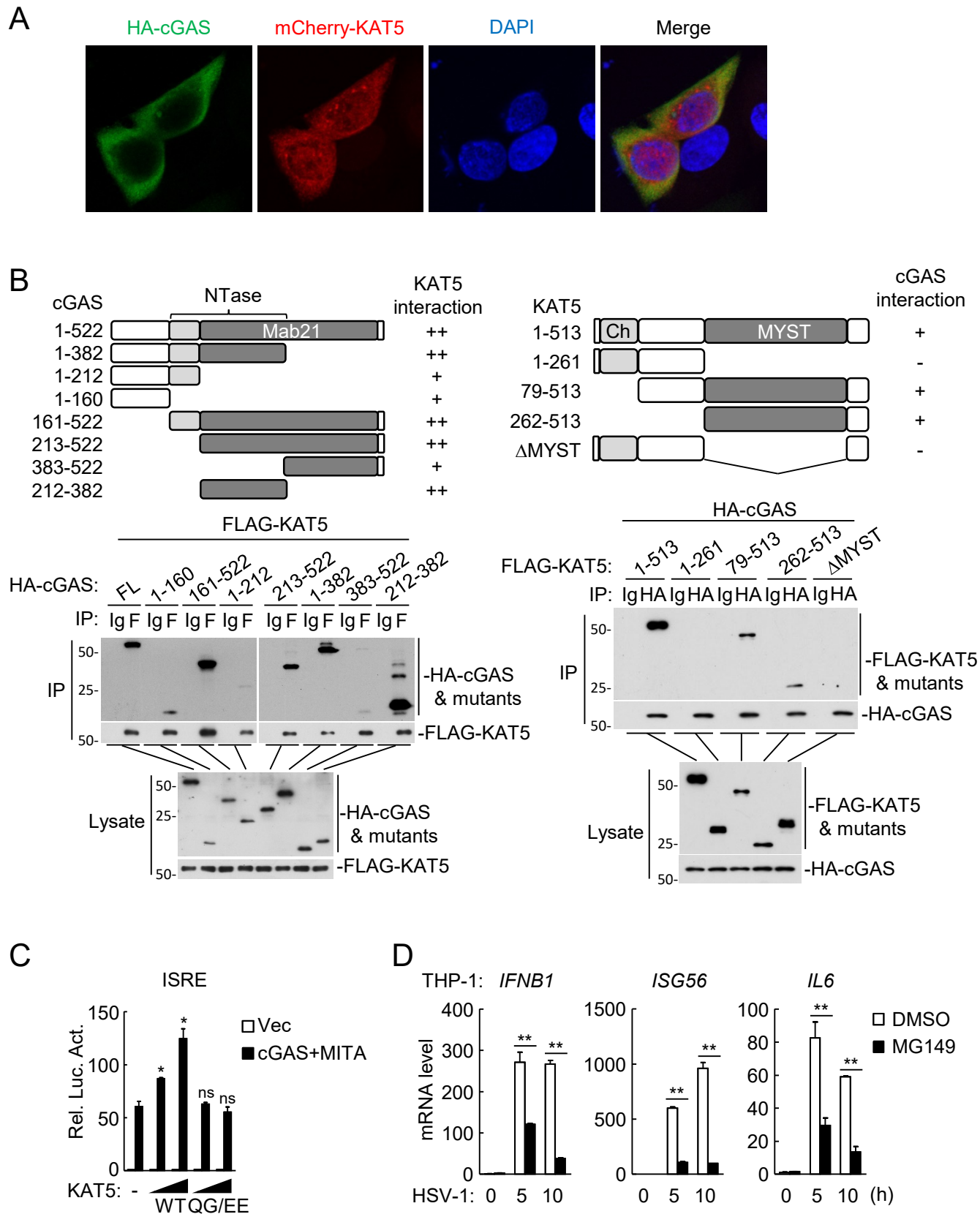
E
293T:
IFNB1
KAT5
mRNA level
Lipo
LMW
HMW
poly(I:C)
Con
KAT5-RNAi #1
KAT5-RNAi #2

Figure S1. KAT5 positively regulates HSV-1-induced transcription of downstream genes.

A. Screens of the acetyltransferase family members for their ability to regulate cGAS-mediated ISRE activation. HEK293 cells stably-expressing MITA were transfected with the indicated plasmids together with ISRE reporter plasmid for 20 hours before luciferase assays.
B. Effects of KAT5 knockdown on HSV-1-induced transcription of downstream genes in HFFs. The control and KAT5-knockdown HFFs were infected with HSV-1 for the indicated times before qPCR analysis of the indicated genes.
C. Effects of KAT5 knockdown on dsDNA-induced transcription of downstream genes in HFFs. The control and KAT5-knockdown HFFs were transfected with dsDNA for the indicated times before qPCR analysis of the indicated genes.
D. Effects of KAT5 knockdown on SeV-induced transcription of *IFNB1* gene. The control and KAT5-knockdown THP-1 cells were infected with SeV for 8 hours before qPCR analysis.
E. Effects of KAT5 knockdown of on transfected poly(I:C)-induced transcription of *IFNB1* gene. The control and KAT5-knockdown HEK293 cells were transfected with poly(I:C) for 8 hours before qPCR analysis. LMW, low molecular weight; HMV, high molecular weight.

* $P < 0.05$, ** $P < 0.01$ (unpaired *t* test). Data shown are mean ± SD from one representative experiments performed in triplicate.

Figure S2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HA-cGAS
mCherry-KAT5
DAPI
Merge
B
cGAS
NTase
Mab21
KAT5 interaction
1-522 ++
1-382 ++
1-212 +
1-160 +
161-522 ++
213-522 ++
383-522 +
212-382 ++
KAT5
Ch
MYST
cGAS interaction
1-513 +
1-261 -
79-513 +
262-513 +
ΔMYST -
FLAG-KAT5
HA-cGAS:
FL 1-160 161-522 1-212 213-522 1-382 383-522 212-382
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IP
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25-
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-HA-cGAS & mutants
-FLAG-KAT5
Lysate
50-
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-HA-cGAS
Lysate
50-
25-
50-
-FLAG-KAT5 & mutants
-HA-cGAS
C
ISRE
Rel. Luc. Act.
150
100
50
0
Vec
cGAS+MITA
* * ns ns
KAT5: - WT QG/EE
D
THP-1: IFNB1 ISG56 IL6
mRNA level
400
300
200
100
0
1500
1000
500
0
100
80
60
40
20
0
** ** ** ** ** **
DMSO
MG149
HSV-1: 0 5 10 0 5 10 0 5 10 (h)

Figure S2. KAT5 plays a role in innate immune response to DNA virus by targeting cGAS.

A. cGAS and KAT5 partially colocalized in the cytoplasm. HeLa cells were transfected with the indicated plasmids for 20 hours before immunofluorescent analysis.
B. Domain mapping of the interaction between KAT5 and cGAS. HEK293 cells were transfected with the indicated plasmids for 20 hours before coimmunoprecipitation and immunoblotting analysis with the indicated antibodies.
C. Effects of KAT5 and KAT5(QG/EE) on cGAS-mediated ISRE activation. HEK293 cells were transfected with the indicated plasmids for 20 hours before luciferase assays were performed. ns, no significance. * $P < 0.05$ (unpaired *t* test). Data shown are mean ± SD from one representative experiments performed in triplicate.
D. Effects of KAT5 acetyltransferase inhibitor MG149 on HSV-1-induced transcription of downstream genes. THP1 cells were treated with MG149, then were left un-infected or infected with HSV-1 (MOI=1) for the indicated times before qPCR analysis of the indicated genes. ** $P < 0.01$ (unpaired *t* test). Data shown are mean ± SD from one representative experiments performed in triplicate.

Figure S3

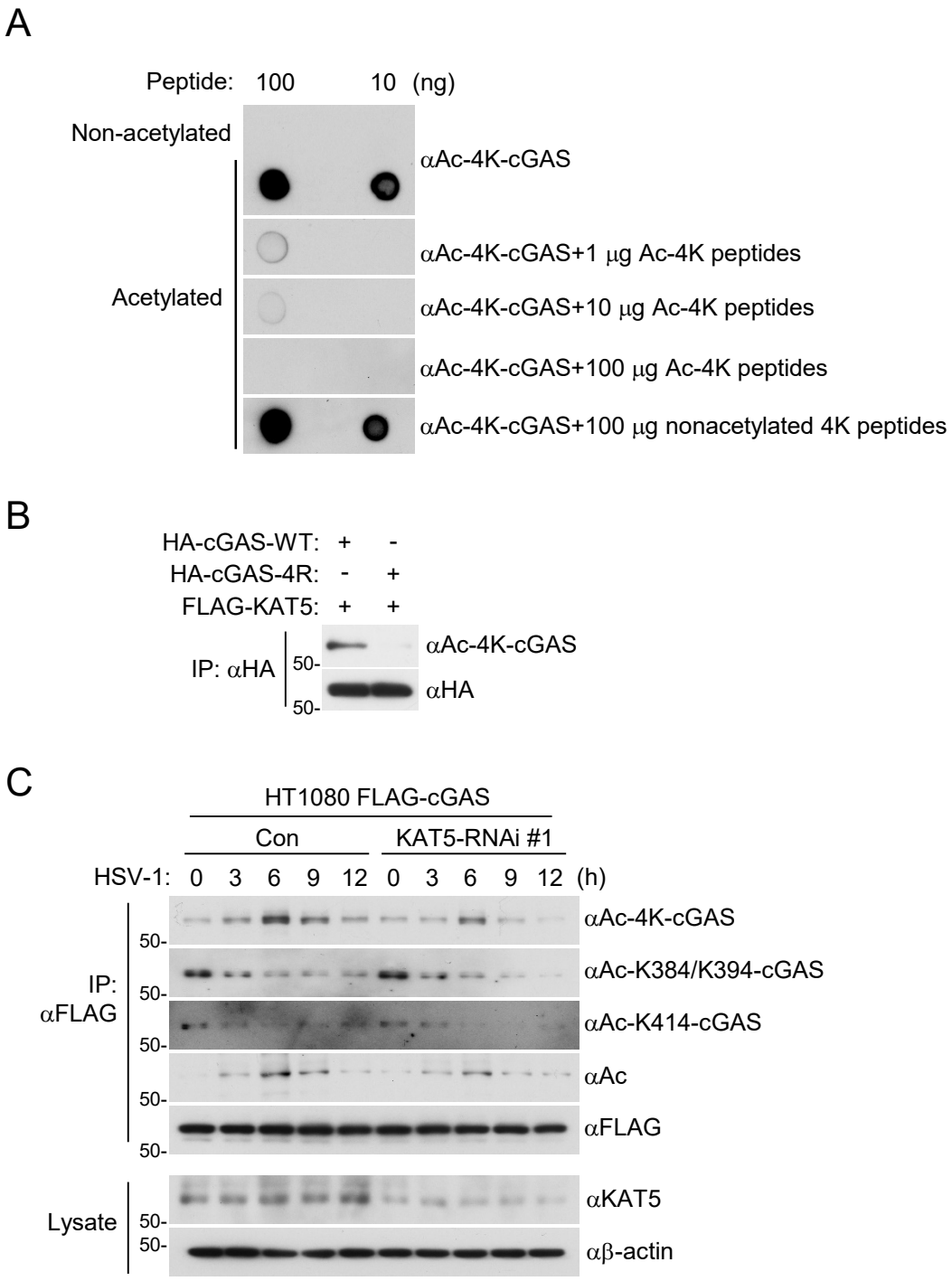


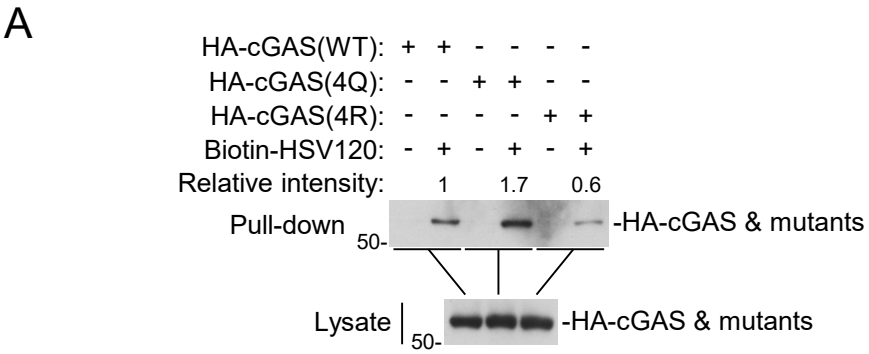
Figure S3. KAT5 catalyzes cGAS acetylation.

A. Dot blotting analysis of acetylated and non-acetylated 4K peptides with anti-Ac-4K-cGAS antibody without or with the indicated peptide competitors.

B. HEK293 cells were transfected with the indicated plasmids for 20 hours before coimmunoprecipitation and immunoblotting analysis with the indicated antibodies.

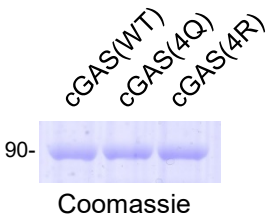
C. Effects of KAT5 knockdown on HSV-1-induced acetylation of cGAS. The KAT5-knockdown and control HT1080-FLAG-cGAS cell lines were infected with HSV-1 (MOI=2) for the indicated times before coimmunoprecipitation and immunoblotting analysis with the indicated antibodies.

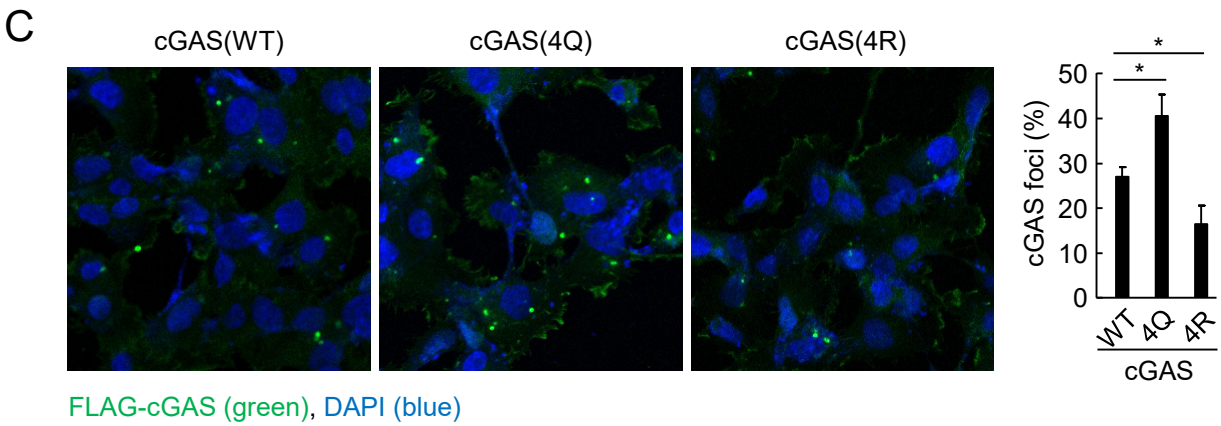
Figure S4



B

Target	Ligand	Kd (nM)
Cy5-HSV60	1. cGAS(WT)	714.01±17.52
	2. cGAS(4Q)	118.24±10.47
	3. cGAS(4R)	721.41±46.25





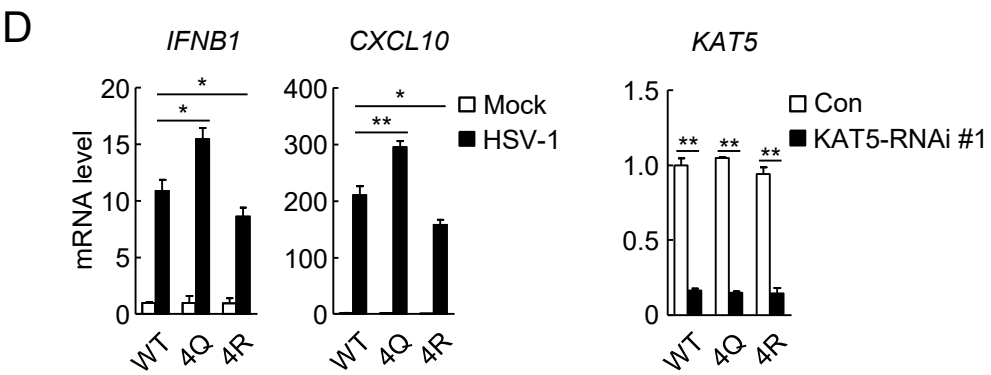


Figure S4. Acetylation of cGAS by KAT5 increases its activity.

A. Binding of dsDNA by wild-type cGAS, cGAS(4Q) and cGAS(4R). HEK293 cells were transfected with the indicated plasmids for 20 hours. The cell lysates were then incubated with biotinylated-HSV120 and streptavidin-sepharose beads. The bead-bound proteins were analyzed by immunoblots with the indicated antibodies.

B. Binding affinities of wild-type cGAS, cGAS(4Q) and cGAS(4R) to dsDNA. Recombinant GST-cGAS, GST-cGAS(4Q) and GST-cGAS(4R) were purified *from E. Coli*., and then mixed with Cy5-labeled HSV60 before MST measurements. The purified recombinant proteins were stained with Coomassie blue (right gel).

C. Foci formation of wild-type cGAS, cGAS(4Q) and cGAS(4R). The HT1080 cells expressing wild-type cGAS, cGAS(4Q) or cGAS(4R) were transfected with HT-DNA for 5 hours before immunofluorescent staining (left panels). Cells with or without foci in three viewing fields were counted (right histograph). * $P < 0.05$ (unpaired *t* test).

D. Effects of cGAS and its mutants on HSV-1-induced transcription of downstream genes in KAT5-RNAi knockdown cells. The HT1080 cells expressing wild-type cGAS, cGAS(4Q) or cGAS(4R) were transduced with KAT5-RNAi retroviruses. The cells were infected with HSV-1 for 9 hours or left un-infected before qPCR analysis. * $P < 0.05$, ** $P < 0.01$ (unpaired *t* test). Data shown are mean ± SD from one representative experiment performed in triplicates.

Figure S5

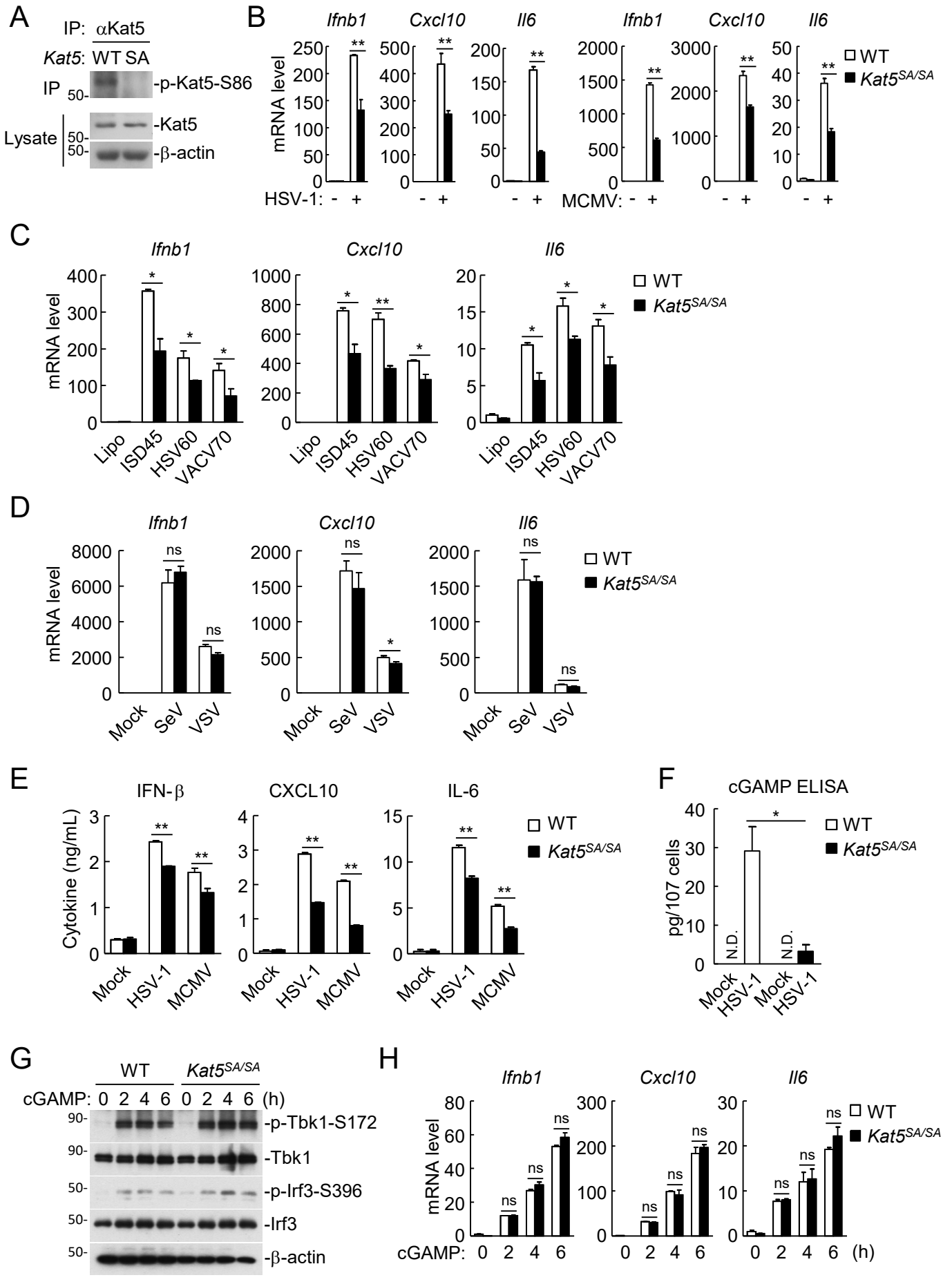

A
IP: αKat5
Kat5: WT SA
IP
-p-Kat5-S86
Lysate
-Kat5
-β-actin
B
Ifnb1
Cxcl10
Il6
mRNA level
HSV-1:
MCMV:
WT
Kat5SA/SA
C
Lipo
ISD45
HSV60
VACV70
D
Mock
SeV
VSV
ns
E
IFN-β
CXCL10
IL-6
Cytokine (ng/mL)
HSV-1
MCMV
F
cGAMP ELISA
pg/107 cells
N.D.
G
cGAMP: 0 2 4 6 0 2 4 6 (h)
-p-Tbk1-S172
-Tbk1
-p-Irf3-S396
-Irf3
-β-actin
H
cGAMP: 0 2 4 6 0 2 4 6 (h)

Figure S5. Inhibition of DNA-triggered innate immune response in *Kat5*$^{SA/SA}$ mice.

A. Kat5-S86 was not phosphorylated in *Kat5*$^{SA/SA}$ BMDCs. Cell lysates from wild-type or *Kat5*$^{SA/SA}$ BMDCs were immunoprecipitated with anti-KAT5, and the immunoprecipitates and lysates were analyzed by immunoblots with the indicated antibodies.
B. Inhibition of DNA virus-induced transcription of downstream antiviral genes in *Kat5*$^{SA/SA}$ BMDCs. Wild-type and *Kat5*$^{SA/SA}$ BMDCs were left uninfected or infected with HSV-1 or MCMV for 6 hours before qPCR analysis.
C. Inhibition of transfected DNA-induced transcription of downstream antiviral genes in *Kat5*$^{SA/SA}$ BMDCs. Wild-type and *Kat5*$^{SA/SA}$ BMDCs were transfected with the indicated dsDNA for 4 hours before qPCR analysis.
D. RNA virus-induced transcription of downstream antiviral genes was comparable in wild-type and *Kat5*$^{SA/SA}$ BMDMs. Wild-type and *Kat5*$^{SA/SA}$ BMDMs were left uninfected or infected with SeV or VSV for 6 hours before qPCR analysis.
E. Inhibition of DNA virus-induced production of IFN-β, CXCL10 and IL-6 in *Kat5*$^{SA/SA}$ BMDMs. Wild-type and *Kat5*$^{SA/SA}$ BMDMs were left un-infected or infected with HSV-1 or MCMV for 18 hours before ELISA analysis.
F. Inhibition of HSV-1-induced cGAMP production in *Kat5*$^{SA/SA}$ BMDMs. Wild-type and *Kat5*$^{SA/SA}$ BMDMs were left un-infected or infected with HSV-1 for 2 hours before ELISA analysis. N.D., not detectable.
G. cGAMP-induced phosphorylation of Tbk1-S172 and Irf3-S396 were comparable in wild-type and *Kat5*$^{SA/SA}$ BMDMs. BMDMs were left untreated or treated with 2'3'-cGAMP (40 nM) for the indicated times before immunoblotting analysis with the indicated antibodies.
H. cGAMP-induced transcription of downstream antiviral genes were comparable in Wild-type and *Kat5*$^{SA/SA}$ BMDMs. BMDMs were left untreated or treated with 2'3'-cGAMP (40 nM) for the indicated times before qPCR analysis.

Data shown are mean ± SD from one representative experiments performed in duplicate (F) or triplicate (B-E, H). ns, no significance. * $P < 0.05$, ** $P < 0.01$ (unpaired *t* test).