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Supplemental Information

Akt kinase-mediated checkpoint of cGAS DNA sensing pathway

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SUPPLEMENTAL FIGURE LEGEND

Figure S1. Structurally and functionally conserved cGAS and OAS1 have similar Akt substrate motifs.

(A) Immunoprecipitated human cGAS-Flag was fractionated with SDS-PAGE and the expected cGAS band was digested with trypsin and peptides were identified by mass spectrometry. Each peptide shows the probability of phosphorylation (Ascore). The first phosphopeptide listed has a highly significant value (Ascore of greater than 18 is experimentally reliable).

(B) Sequence comparison of the putative cGAS phosphorylation site from the six indicated species.

(C) Akt phosphorylation target site predicted in both human and mouse cGAS and OAS1. It indicates two catalytically important sites (blue-colored) and one putative Akt phosphorylation site (red-colored). See also Figure 1.

Figure S2. Akt1 kinase phosphorylates human cGAS.

(A) Human cGAS-Flag was immunoprecipitated with anti-Flag M2 beads from lysates of 293T cells transiently transfected with vector or human cGAS-Flag and with or without HA-myr-Akt1 for 24 hours. Cells were treated for 8 h before harvesting with Akt1/Akt2 selective inhibitor VIII, as indicated. The immunoprecipitates were probed with an Akt phosphosubstrate antibody. WCLs were probed with Akt phosphosubstrate, Flag, PanAkt, and Actin (loading control) antibodies. See also Figure 1.

Figure S3. Akt negatively regulates cGAS-mediated IFN-β production.

(A) RAW 264.7 cells were electroporated with a construct of empty vector or myr-Akt1. At 48 h post-transfection, cells were treated with mock, pdAdT or HT-DNA stimulation for 9 hours , or infected with HSV-1 (moi = 5) for 12 hours. The expression of IFN-β mRNA was measured by real-time PCR.

(B) RAW 264.7 cells were treated with 5μM Akt1/2 selective inhibitor VIII or DMSO vehicle. Cells were treated with mock, pdAdT or HT-DNA stimulation for 9 hours, or infected with HSV-1 (moi = 5) for 12 hours. The expression of IFN-β mRNA was measured by real-time PCR.

(C) L929 cGAS-/- cells complemented with empty vector or mouse cGAS WT (mWT) or mouse cGAS S291A were treated with 5μM Akt1/2 selective inhibitor VIII or DMSO vehicle and stimulated with cGAMP (3μg/ml) for 10 hours. The expression of IFN-β mRNA was measured by real-time PCR.

(D) L929 cell lines used in (C) were stimulated with cGAMP (3μg/ml) for 18 hours. The production of IFN-β was measured by ELISA. * $p<0.05$. See also Figure 4.

Figure S4. Akt1 specifically regulates cGAS enzymatic activity and ultimately affects IFN-β induction.

(A) *In vitro* enzymatic assays were performed in the presence of P^{32}-α-GTP with mouse cGAS (aa141-507) WT and cGAS S291A purified from *E. coli*. Mock or immunoprecipiated active Akt1 was added to the purified cGAS WT or S291 protein in the presence of P^{32}-α-GTP. cGAMP production was analyzed by TLC and autoradiograph (top right panel). The bottom arrow shows the spotted origin and the top arrow shows the migrated cGAMP.

(B) cGAMP Bio-assay. L929 cells were stimulated with HT-DNA (2μg/ml) for 9 hours. Extracts of the cells were prepared, treated with Benzonase and heat, and incubated with permeabilized THP1- Lucia ISG cells for 18 hours. Reporter cells were added with 200 nM cGAMP as a positive control. Relative luciferase activity was measured. * $p<0.05$. See also Figure 4.

Figure S5. Akt antagonizes cGAS-mediated IFN response upon infection with DNA viruses

(A) L929 cGAS-/- cells were stably complemented with empty vector, mouse cGAS WT (mWT), or S291A. The expression of IFN-β mRNA was measured by real-time PCR after infection with MVA (moi = 5) for 10 hours.

(B) L929 cGAS -/- cells stably transfected with cGAS-Flag were infected with 5 moi HSV-1 for indicated times. Mouse cGAS-Flag (cGAS-Flag) was then immunoprecipitated with anti-Flag M2 beads and the immunoprecipitates and whole cell lysates (WCLs) were probed with P-Akt substrate, pAKT S473, PanAkt, Actin and Flag antibodies.

(C) L929 cGAS-/- cells were stably complemented with mouse cGAS WT (mWT), or S291A. The expression of IFN-β mRNA was measured by real-time PCR after infection with HSV-1 WT (moi = 5) for indicated times.

(D) The S291A mutation enhances cGAS-mediated antiviral activity toward HSV-1 infection. L929 cGAS-/- cells stably complemented with empty vector, mouse cGAS WT (mWT) or S291A were infected with HSV-1 ICPΔ34.5 (moi = 0.1, A) for indicated times. Viral supernatants were collected at indicated times and titered using plaque assays on Vero cells. * $p<0.05$. See also Figures S5 and S6.

A

Peptide	Ascore Seq	Ascore
R.GGS#PAVTLLISEK.I	GGS#PAVTLLISEK	71.2
R.GAPM*DPT#ESPAAPEAALPK.A	GAPM*DPT#ESPAAPEAALPK	6
R.DDIS#TAAGM*VK.G	DDIS#TAAGM*VK	7.4
R.AQDTQPSDATS#APGAEGLEPPAAREPALSR.A	AQDTQPSDATS#APGAEGLEPPAAREPALSR	6.5
R.AQDTQPSDATS#APGAEGLEPPAAREPALSR.A	AQDTQPS#DATSAPGAEGLEPPAAREPALSR	0
R.AQDTQPS#DATSAPGAEGLEPPAAR.E	AQDTQPS#DATSAPGAEGLEPPAAR	7.5

B

Akt substrate motif: R/K x R/K xx S*/T*

Human cGAS: RKRGGS$_{305}$PA
Mouse cGAS: KEKPGS$_{291}$PA
Rat cGAS: EEKPGS$_{294}$PA
Dog cGAS: KKKRGS$_{294}$PA
Chicken cGAS: RKKRGS$_{268}$PA
Lizard cGAS: KKRPGS$_{383}$PA
Consensus: R/K x R/K xx S*

C

Akt substrate motif: R/K x R/K xx S*/T*

Human cGAS

VPSPGLPVSAPILVRRDAAPGASKLRAVLEKLKLSRDDISTAAGMVKGVVDHLLLRLKCDSAFRGVGLLNTGSYYEHVKISAP
NE$_{225}$FD$_{227}$VMFKLEVPRIQLEEYSNTRAYYFVKFKRNPKENPLSQFLEGEILSASKMLSKFRKIIKEEINDIKDTDVIMK
RKRGGS$_{305}$PAVTLLISE$_{319}$KISVDITLALESKSSWPASTQEGLRIQNWLSAKVRK

Mouse cGAS

RGSRKEPDKLKKVLDKLRLKRKDISEAAETVNKVVERLLRRMQKRESEFKGVEQLNTGSYYEHVKISAPNE$_{211}$FD$_{213}$VMFKL
EVPRIELQEYYETGAFYLVKFKRIPRGNPLSHFLEGEVLSATKMLSKFRKIIKEEVKEIKDIDVSVEKEKPGS$_{291}$PAVTLLIRNP
E$_{302}$EISVDIILALESKGSWPISTKEGLPIQGWLGTKVRTNLRREPFYLVPK

Human OAS1

MMDLRNTPAKSLDKFIEDYLLPDTCFRMQINHAIDIICGFLKERCFRGSSYPVCVSKVVKGGSSGKGTTLRGRSD$_{75}$AD$_{77}$LVVF
LSPLTTFQDQLNRRGEFIQERRQLEACQRERAFS$_{116}$VKFEVQAPRWGNPRALSFVLSSLQLGEGVEFD$_{148}$VLPAFDALGQLT

Mouse OAS1

MEQDLRSIPASKLDKFIENHLPDTSFCADLREVIDALCALLKDRFFRGPVRRMRASKGVKGKCTALKGRSD$_{71}$AD$_{73}$LVVFLNN
LTYFEDQLNQQGVLIKEIKKQLYEVQHERRFGVKFEVQSLRSPNSRALS$_{129}$FKLSAPDLLKEVKFD$_{144}$VLPAYDLLDHLNILKKP
NQQFYANLISGRTPLGKEGKLLTCFMGLRKYFLNCRPTKLKRLIHLVTHWYQLCKEK

Figure S1

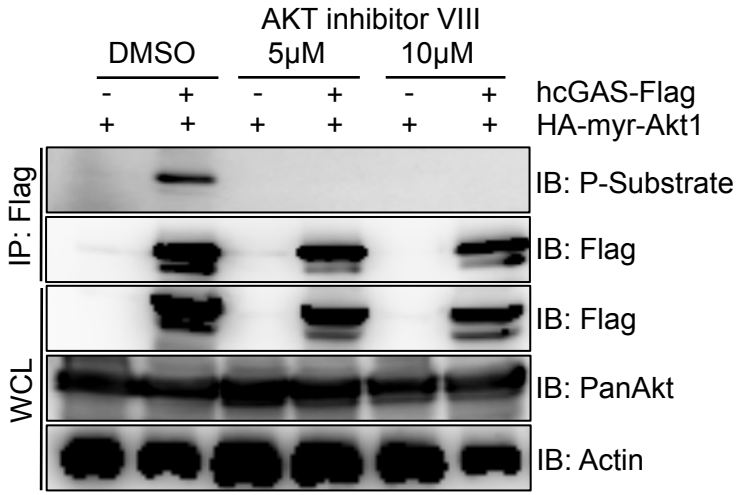
AKT inhibitor VIII
DMSO
5μM
10μM
- + - + - +
hcGAS-Flag
+ + + + + +
HA-myr-Akt1
IP: Flag
IB: P-Substrate
IB: Flag
WCL
IB: Flag
IB: PanAkt
IB: Actin

Figure S2

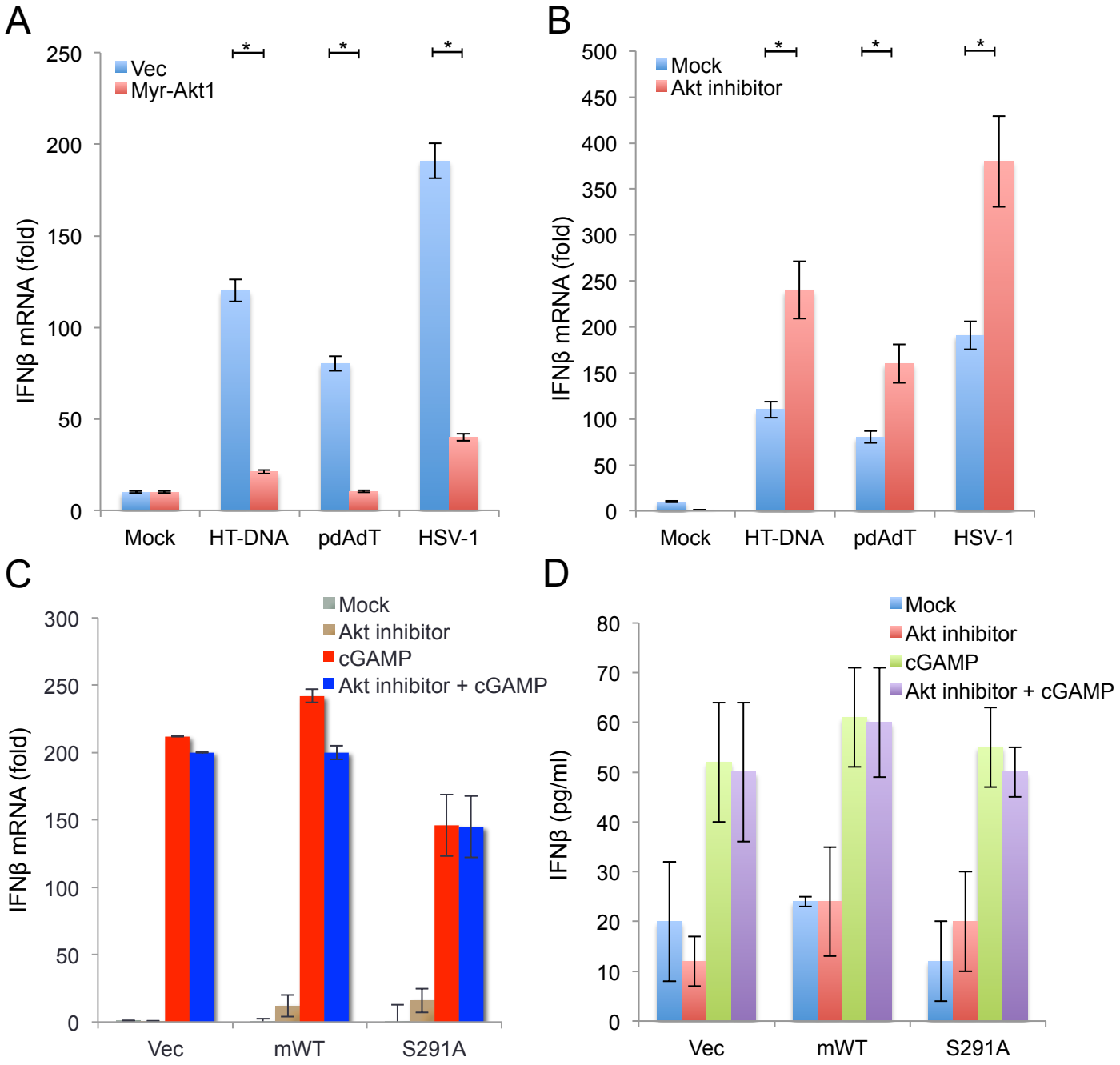
A
IFNβ mRNA (fold)
250
200
150
100
50
0
Vec
Myr-Akt1
*
*
*
Mock
HT-DNA
pdAdT
HSV-1
B
IFNβ mRNA (fold)
500
450
400
350
300
250
200
150
100
50
0
Mock
Akt inhibitor
*
*
*
Mock
HT-DNA
pdAdT
HSV-1
C
IFNβ mRNA (fold)
300
250
200
150
100
50
0
Mock
Akt inhibitor
cGAMP
Akt inhibitor + cGAMP
Vec
mWT
S291A
D
IFNβ (pg/ml)
80
70
60
50
40
30
20
10
0
Mock
Akt inhibitor
cGAMP
Akt inhibitor + cGAMP
Vec
mWT
S291A

Figure S3

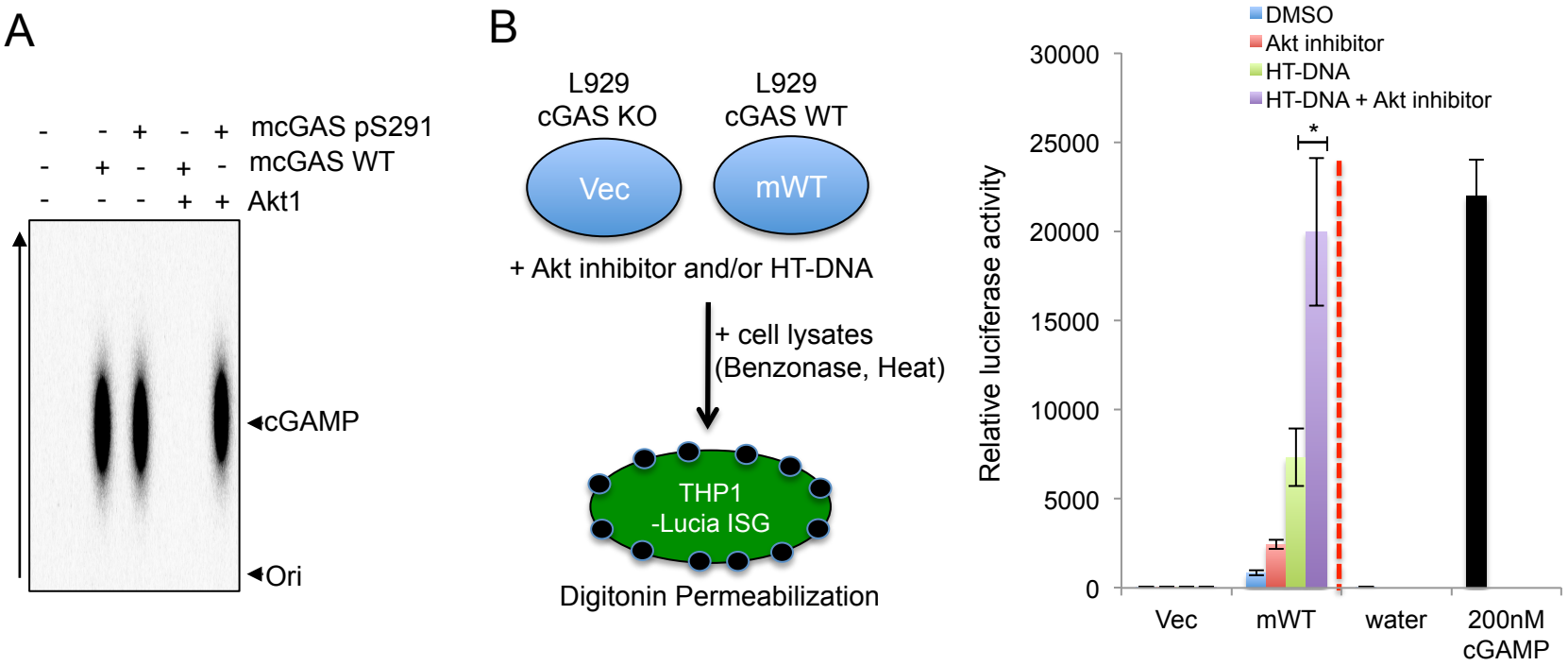
A
- - + - + mcGAS pS291
- + - + - mcGAS WT
- - - + + Akt1
cGAMP
Ori
B
L929
cGAS KO
L929
cGAS WT
Vec
mWT
+ Akt inhibitor and/or HT-DNA
+ cell lysates
(Benzonase, Heat)
THP1
-Lucia ISG
Digitonin Permeabilization
DMSO
Akt inhibitor
HT-DNA
HT-DNA + Akt inhibitor
Relative luciferase activity
30000
25000
20000
15000
10000
5000
0
*
Vec
mWT
water
200nM
cGAMP

Figure S4

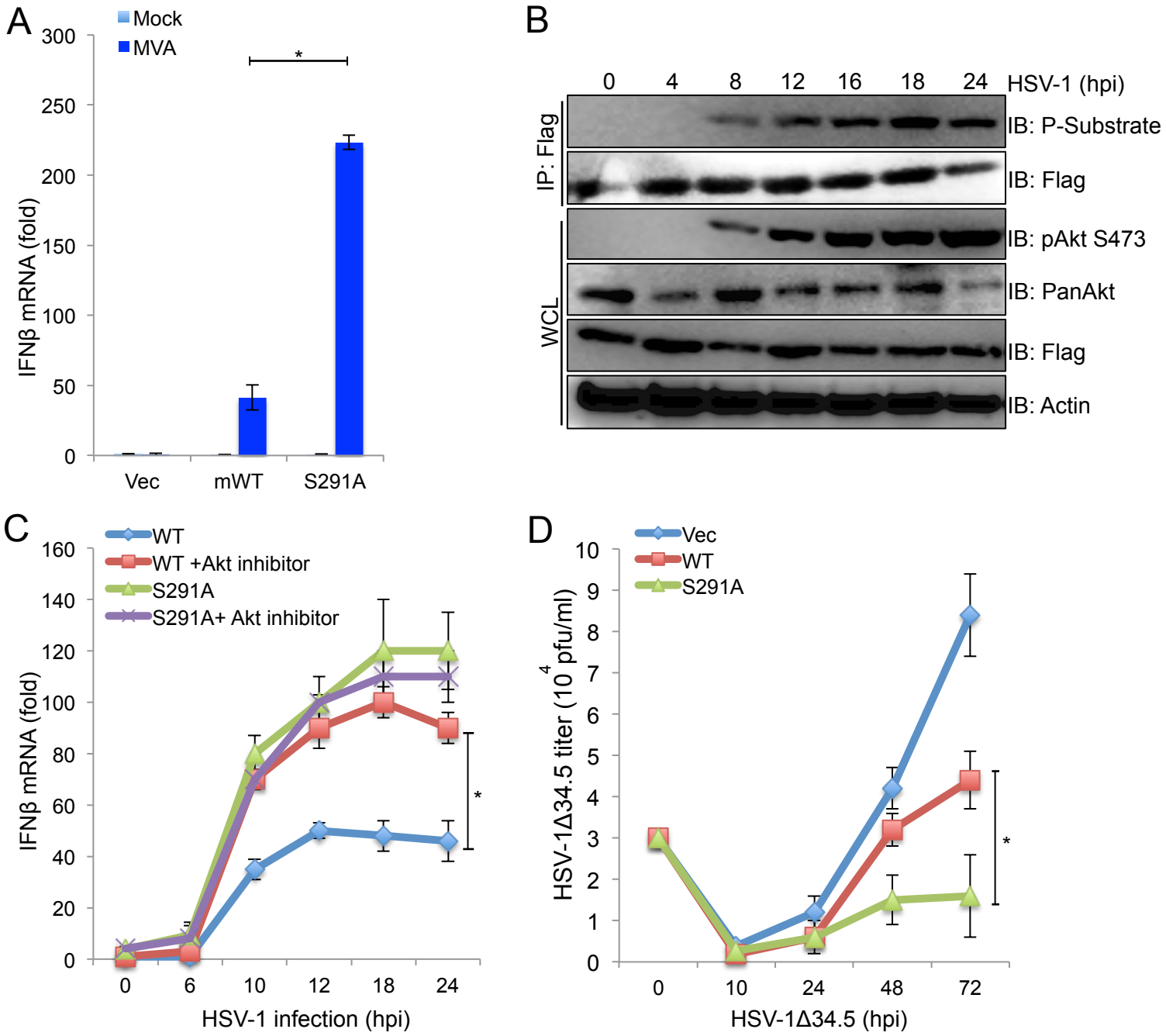
A
Mock
MVA
IFNβ mRNA (fold)
300
250
200
150
100
50
0
*
Vec
mWT
S291A
B
0
4
8
12
16
18
24
HSV-1 (hpi)
IP: Flag
IB: P-Substrate
IB: Flag
WCL
IB: pAkt S473
IB: PanAkt
IB: Flag
IB: Actin
C
WT
WT +Akt inhibitor
S291A
S291A+ Akt inhibitor
IFNβ mRNA (fold)
160
140
120
100
80
60
40
20
0
*
0
6
10
12
18
24
HSV-1 infection (hpi)
D
Vec
WT
S291A
HSV-1Δ34.5 titer (10^4 pfu/ml)
10
9
8
7
6
5
4
3
2
1
0
*
0
10
24
48
72
HSV-1Δ34.5 (hpi)

Figure S5