Supplementary Information for

β-arrestin 2 as an activator of cGAS-STING signaling and target of viral immune evasion

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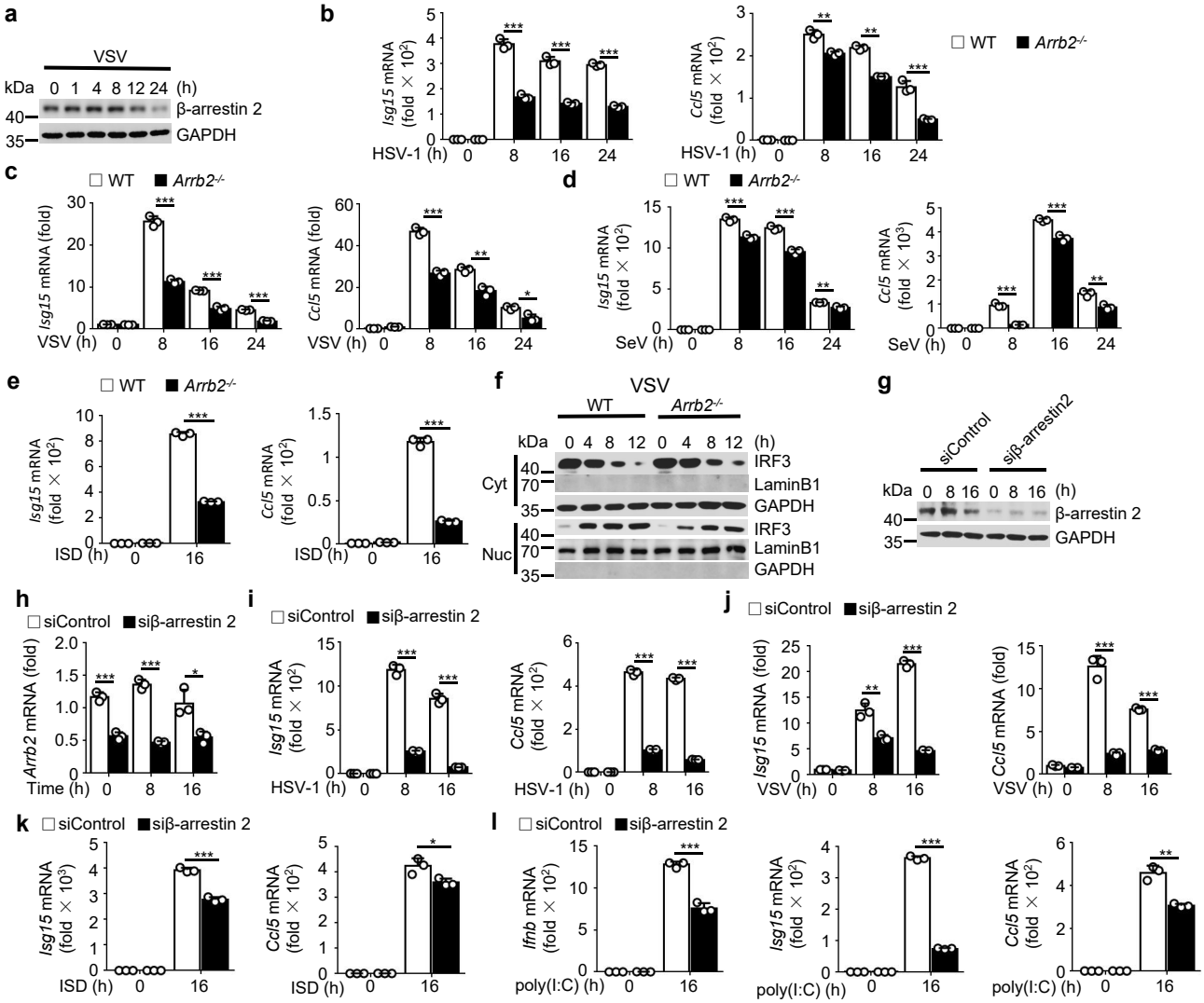
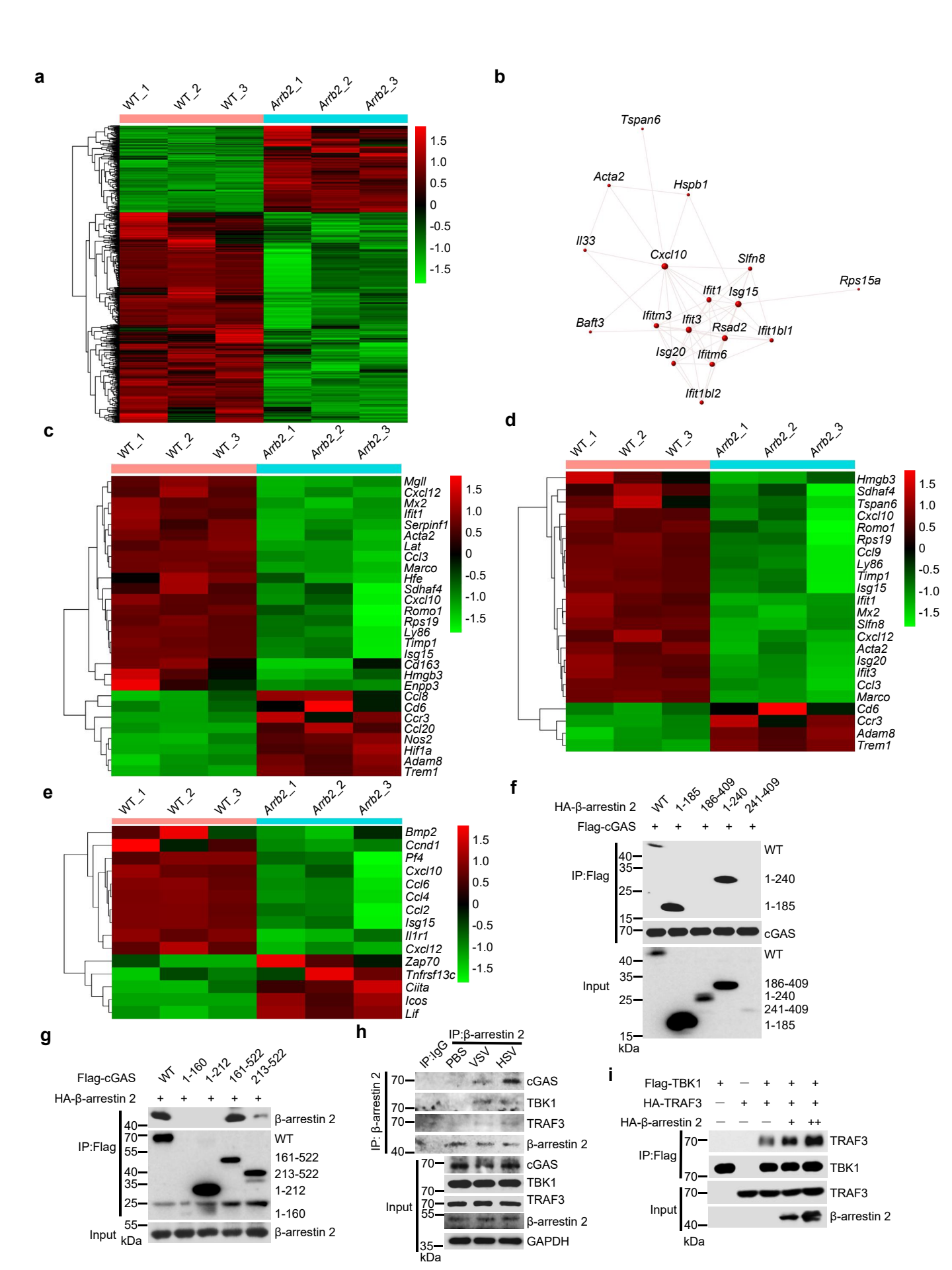
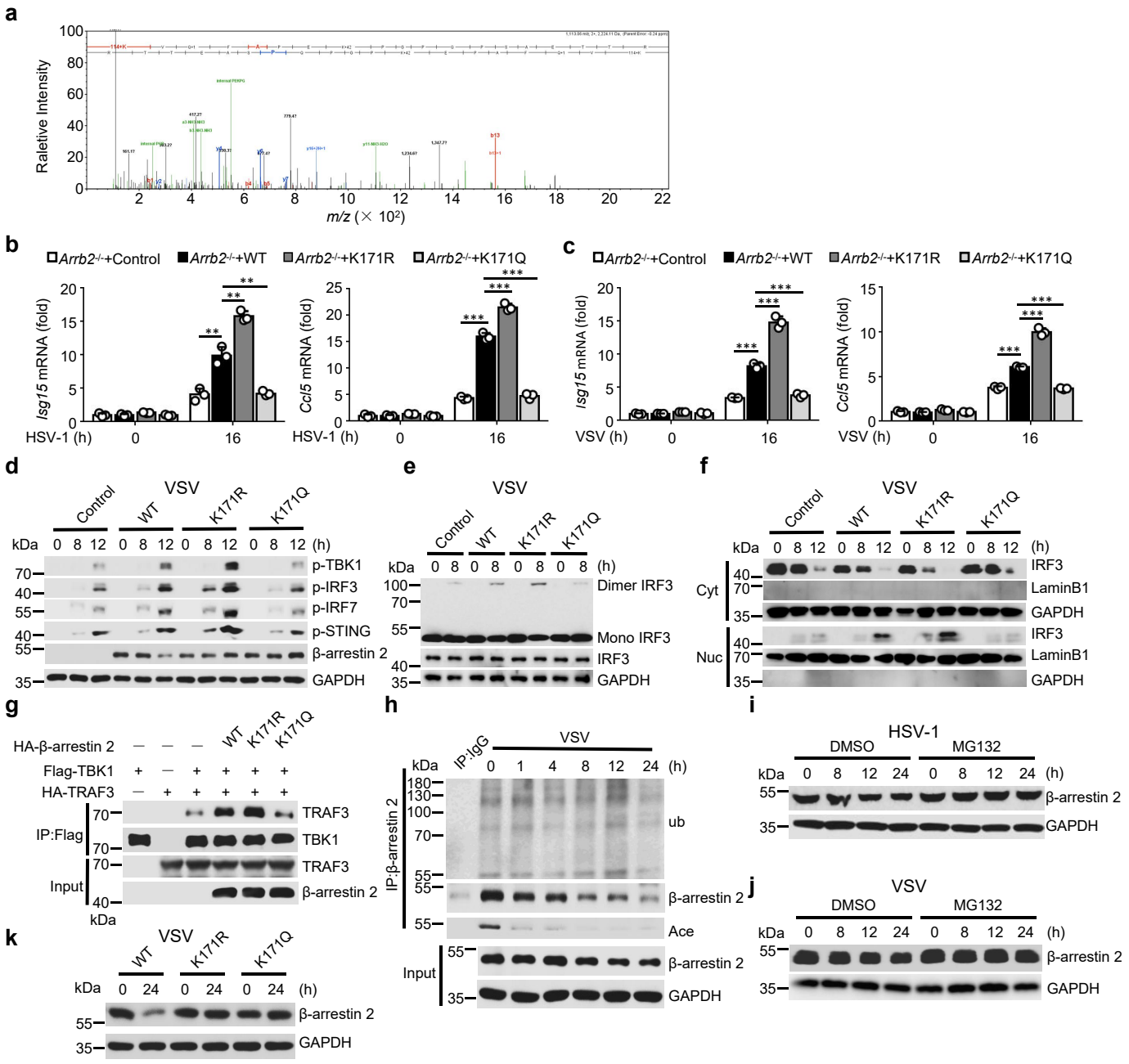


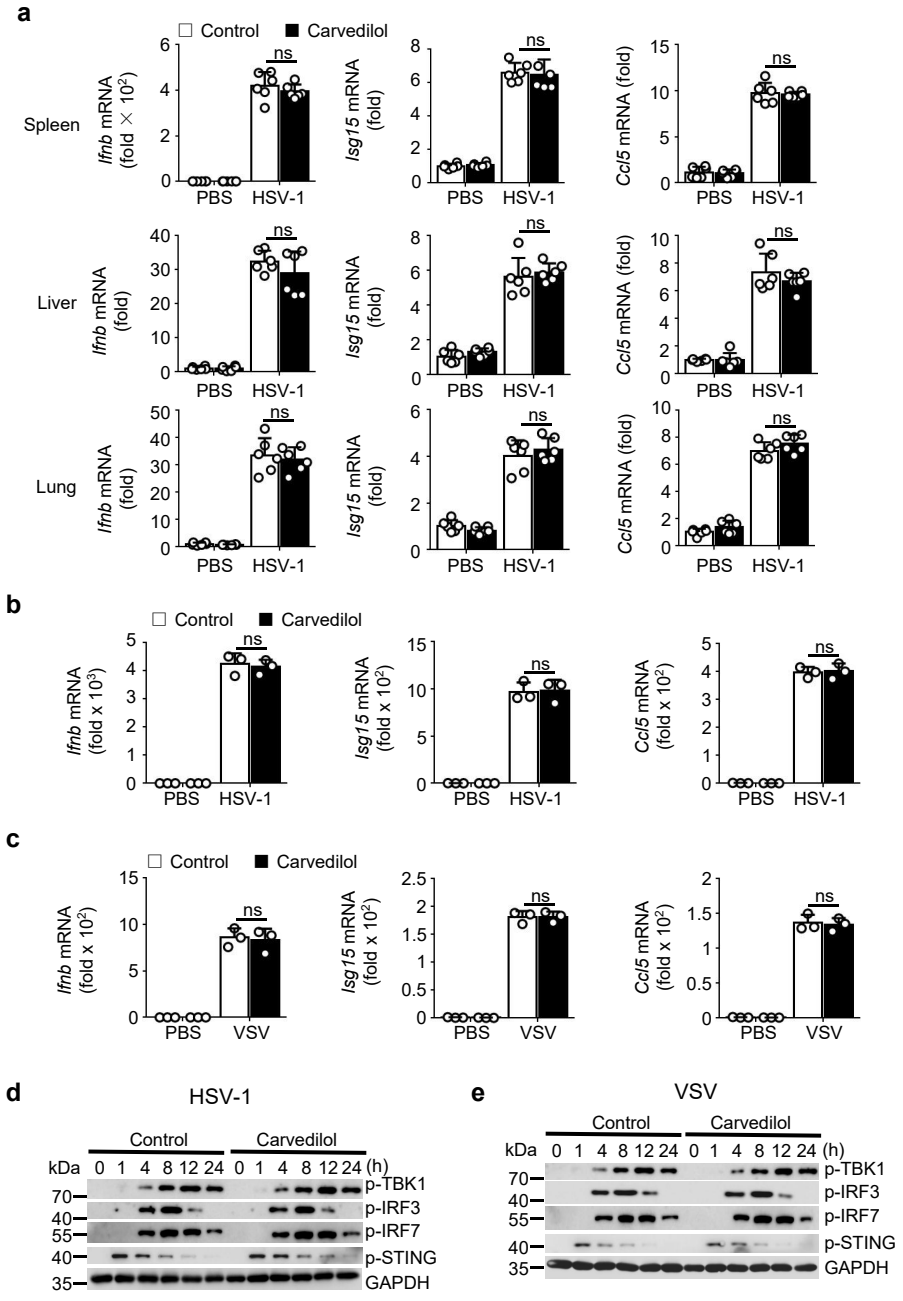
Supplementary Fig. 1: β-arrestin 2 promoted the antiviral immune response. (**a**) Immunoblot of lysates of peritoneal macrophages infected with VSV for indicated times. (**b–e**) *Isg15* and *Cxcl10* mRNA levels in WT or *Arrb2*$^{-/-}$ mouse peritoneal macrophages stimulated with HSV-1 (**b**) (***P < 0.0001, < 0.0001, < 0.0001 in sequence, left panel; **P = 0.0025, ***P < 0.0001, **P = 0.0007, right panel), VSV (**c**) (***P < 0.0001, = 0.0001, < 0.0001 in sequence, left panel; ***P < 0.0001, **P = 0.0020, *P = 0.0125, right panel), SeV (**d**) (***P = 0.000, 0.0002 in sequence, **P = 0.0024, left panel; ***P < 0.0001, =0.0009 in sequence, **P = 0.0035, right panel), or ISD (**e**) (***P < 0.0001 in all panels) for indicated times. (**f**) Immunoblot analysis of nuclear and cytoplasmic fractions as in WT or *Arrb2*$^{-/-}$ mouse peritoneal macrophages infected with VSV for indicated times. (**g**) Immunoblot of lysates of RAW264.7 cells treated with control siRNA or siβ-arrestin 2 and infected with HSV-1 for indicated times. (**h**) *Arrb2*$^{-/-}$ mRNA levels in RAW264.7 cells treated with control siRNA or siβ-arrestin 2 and infected with HSV-1 for indicated times (***P = 0.0003, ***P < 0.0001, *P = 0.0164). (**i–l**) *Ifnb*, *Isg15*, and *Cxcl10* mRNA levels in RAW264.7 cells transfected with control siRNA or β-arrestin 2 siRNA and then stimulated with HSV-1 (**i**) (***P < 0.0001 in all panels), VSV (**j**) (**P = 0.0035, ***P < 0.0001 left panel; ***P = 0.0001, ***P < 0.0001, right panel), ISD (**k**) (***P = 0.0001, left panel; **P = 0.0306, right panel), or poly(I:C) (**l**) (***P = 0.0002, left panel; ***P < 0.0001, middle panel; **P = 0.0014, right panel) for indicated times. Data are representative of at least three independent experiments (mean ± SEM in **b–e, h–l,** n = 3). Two-tailed unpaired Student's t-test.


a
WT_1
WT_2
WT_3
Arrb2_1
Arrb2_2
Arrb2_3
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
b
Tspan6
Acta2
Hspb1
Il33
Cxcl10
Slfn8
Rps15a
Ifit1
Isg15
Baft3
Ifitm3
Ifit3
Rsad2
Ifit1bl1
Isg20
Ifitm6
Ifit1bl2
c
Mgll
Cxcl12
Mx2
Ifit1
Serpinf1
Acta2
Lat
Ccl3
Marco
Hfe
Sdhaf4
Cxcl10
Romo1
Rps19
Ly86
Timp1
Isg15
Cd163
Hmgb3
Enpp3
Ccl8
Cd6
Ccr3
Ccl20
Nos2
Hif1a
Adam8
Trem1
d
Hmgb3
Sdhaf4
Tspan6
Cxcl10
Romo1
Rps19
Ccl9
Ly86
Timp1
Isg15
Ifit1
Mx2
Slfn8
Cxcl12
Acta2
Isg20
Ifit3
Ccl3
Marco
Cd6
Ccr3
Adam8
Trem1
e
Bmp2
Ccnd1
Pf4
Cxcl10
Ccl6
Ccl4
Ccl2
Isg15
Il1r1
Cxcl12
Zap70
Tnfrsf13c
Ciita
Icos
Lif
f
HA-β-arrestin 2
WT
1-185
186-409
1-240
241-409
Flag-cGAS
IP:Flag
Input
cGAS
kDa
g
Flag-cGAS
HA-β-arrestin 2
WT
1-160
1-212
161-522
213-522
IP:Flag
Input
β-arrestin 2
kDa
h
IP:β-arrestin 2
IP:IgG
PBS
VSV
HSV
IP: β-arrestin 2
Input
cGAS
TBK1
TRAF3
β-arrestin 2
GAPDH
kDa
i
Flag-TBK1
HA-TRAF3
HA-β-arrestin 2
IP:Flag
Input
TRAF3
TBK1
β-arrestin 2
kDa

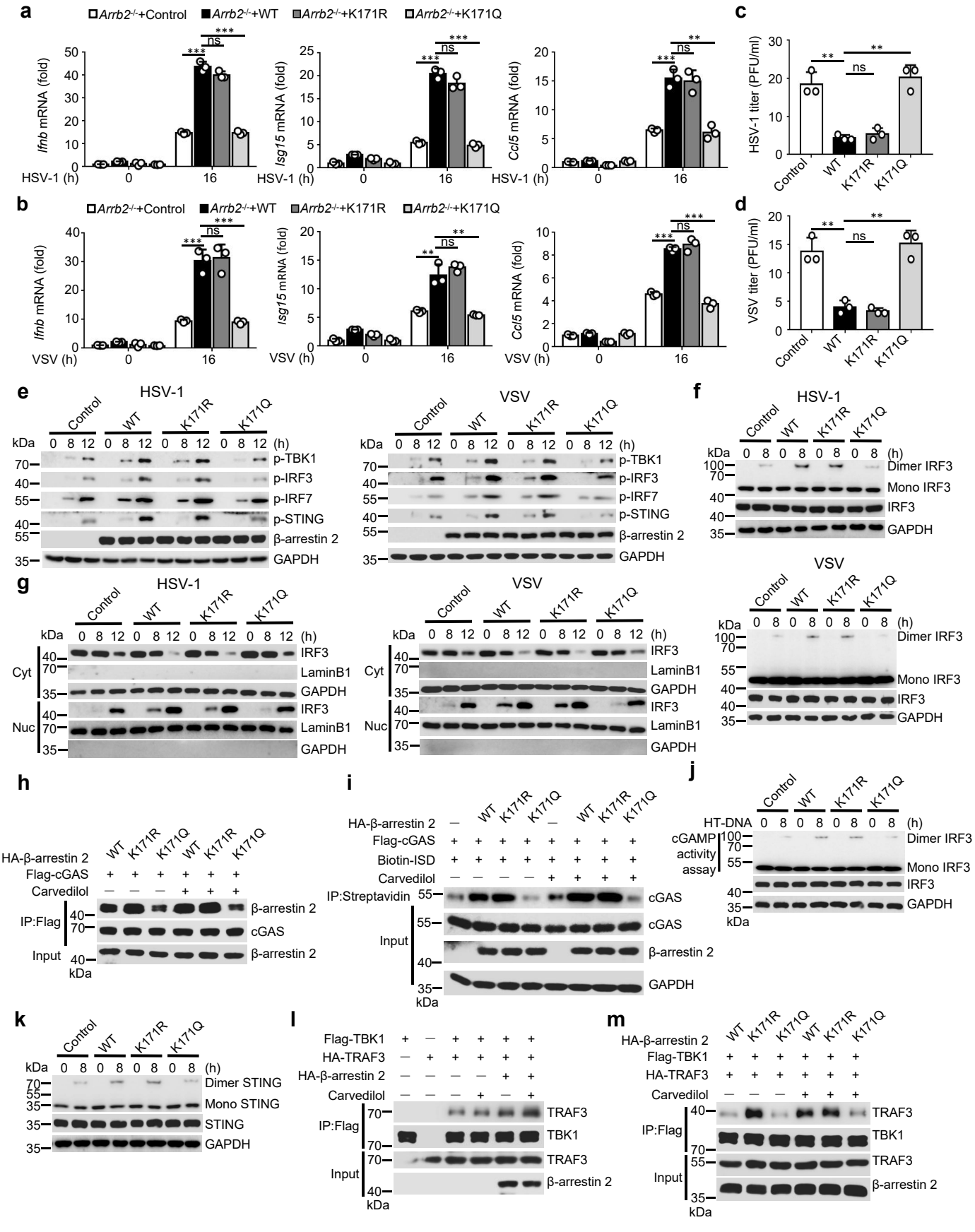
Supplementary Fig. 2: β-arrestin 2 mediated the modulation of host antiviral transcriptional program during HSV-1 infection. (**a**) Heatmap shown the differentially expressed genes (fold change > 2 and p-value < 0.05). (**b**) Network model based on differential expressed genes. (**c**) Heatmap of the differential expressed genes involved in antiviral signaling pathway. (**d**) Heatmap of differential genes involved in the terms in red as in Figure 4**b**. (**e**) Heatmap of differential genes involved in the terms in red as in Figure 4**c**. (**f**) Co-immunoassay of lysates of HEK293T cells expressing β-arrestin 2 trunctions and cGAS. (**g**) Co-immunoassay of HEK293T cells expressing cGAS trunctions and β-arrestin 2. (**h**) Immunoassay of lysates of peritoneal macrophages infected with HSV-1 or VSV. (**i**) Immunoassay of HEK293T cells expressing various vectors. Data are representative of at least three independent experiments.



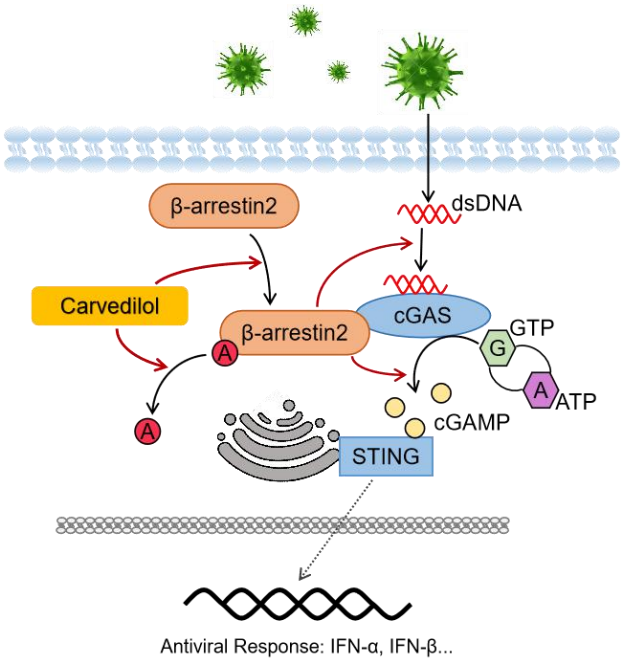
Supplementary Fig. 3: Lys171 was the pivotal regulation residue of β-arrestin 2. (**a**) Mass spectrometry analysis of a β-arrestin 2 peptide modified with acetylation. (**b, c**) *Ifnb*, *Isg15*, and *Il6* mRNA levels in *Arrb2*$^{-/-}$ MEFs transfected with control vector, β-arrestin 2, or β-arrestin 2 mutants and infected with HSV-1 (**b**) (**P = 0.0030, 0.0025, 0.0018 in sequence, left panel; ***P < 0.0001, = 0.0006, < 0.0001 in sequence, right panel) or VSV (**c**) (***P < 0.0001, = 0.0003, < 0.0001 in sequence, left panel; ***P < 0.0001, < 0.0001, < 0.0001 in sequence, right panel) for 16 h. (**d**) Immunoassay of lysates of *Arrb2*$^{-/-}$ MEFs transfected as in (**c**) and infected with VSV for indicated times. (**e**) Immunoblot analysis of monomeric and dimeric IRF3 as in (**d**). (**f**) Immunoblot analysis of nuclear and cytoplasmic fractions as in (**d**). (**g**) Immunoassay of HEK293T cells expressing various vectors. (**h**) Immunoassay of lysates of peritoneal macrophages infected with VSV for indicated times. (**i,j**) Immunoassay of lysates of peritoneal macrophages infected with HSV-1 (**i**) and VSV (**j**) for indicated times and treated with MG132 before lysed. (**k**)Immunoassay of lysates as in (**d**) for 24 h. Data are representative of at least three independent experiments (mean ± SEM in **b, c,** n = 3). Two-tailed unpaired Student's t-test.



Supplementary Fig. 4: Carvedilol has no effect on *Arrb2*$^{-/-}$ mice and *Arrb2*$^{-/-}$ cells. (**a**) *Ifnb*, *Isg15*, and *Ccl5* mRNA levels in the spleens, livers, and lungs of *Arrb2*$^{-/-}$ 6-week-old-male mice (n = 6 per group) intraperitoneally injected with PBS or HSV-1 (2×10^7 PFU per mouse) for 24 h and then treated with PBS or carvedilol for another 24 h. (**b, c**) *fnb*, *ISG15*, and *Ccl5* mRNA in *Arrb2*$^{-/-}$ peritoneal macrophages infected for 16 h with HSV-1 (**b**) or VSV (**c**) and then treated with PBS or carvedilol, n = 3. (**d**, **e**) Immunoblot of lysates of peritoneal macrophages from *Arrb2*$^{-/-}$ mice infected with HSV-1 (**d**) or VSV (**e**) for indicated times and treated with PBS or carvedilol. Data are representative of at least three independent experiments (mean ± SEM in **a–c**). ns > 0.05, two-tailed unpaired Student's t-test.


a
Arrb2-/-+Control
Arrb2-/-+WT
Arrb2-/-+K171R
Arrb2-/-+K171Q
Ifnb mRNA (fold)
Isg15 mRNA (fold)
Ccl5 mRNA (fold)
HSV-1 (h)
0
16
ns
***
**
b
VSV (h)
c
HSV-1 titer (PFU/ml)
Control
WT
K171R
K171Q
d
VSV titer (PFU/ml)
e
HSV-1
VSV
kDa
0 8 12 0 8 12 0 8 12 0 8 12 (h)
p-TBK1
p-IRF3
p-IRF7
p-STING
β-arrestin 2
GAPDH
f
Dimer IRF3
Mono IRF3
IRF3
g
Cyt
Nuc
LaminB1
h
HA-β-arrestin 2
Flag-cGAS
Carvedilol
IP:Flag
Input
β-arrestin 2
cGAS
i
IP:Streptavidin
Biotin-ISD
j
HT-DNA
cGAMP activity assay
k
Dimer STING
Mono STING
STING
l
Flag-TBK1
HA-TRAF3
TRAF3
TBK1
m

Supplementary Fig. 5: Carvedilol activated β-arrestin 2 through Lys171. (**a, b**) *Ifnb*, *Isg15*, and *Ccl5* mRNA levels in *Arrb2*$^{-/-}$ MEFs transfected with control vector, β-arrestin 2, or β-arrestin 2 mutants, infected with HSV-1 (**a**) (***P < 0.0001, < 0.0001 in sequence, left panel; ***P < 0.0001, < 0.0001 in sequence, middle panel; ***P < 0.0001, **P = 0.0012, right panel) or VSV (**b**) (***P = 0.0009, 0.0008 in sequence, left panel; **P = 0.0051, 0.0035 in sequence, middle panel; ***P < 0.0001, < 0.0001 in sequence, right panel) for 16 h and treated with PBS or carvedilol. (**c, d**) The virus titers as in (**a**) and (**b**) for 72 h (**P = 0.0018, 0.0011 in sequence in **c**, **P = 0.0031, 0.0018 in sequence in **d**). (**e**) Immunoassay of lysates of *Arrb2*$^{-/-}$ MEFs transfected as in (**a**), infected with HSV-1 (left) and VSV (right) for indicated times and treated with PBS or carvedilol. (**f**) Immunoblot analysis of monomeric and dimeric IRF3 as in (**a**) and (**b**). (**g**) Immunoblot analysis of nuclear and cytoplasmic fractions as in (**a**) and (**b**). (**h**) Immunoassay of lysates of HEK293T cells expressing various vectors. (**i**) Immunoassay of cell lysates and streptavidin-precipitated proteins from HEK293T cells transfected with various vectors and stimulated with biotin-ISD. (**j**) cGAMP activity measured by extracts from HT-DNA-stimulated (6 h) WT and *Arrb2*$^{-/-}$ peritoneal macrophages. (**k**) Immunoblot analysis of monomeric and dimeric STING in *Arrb2*$^{-/-}$ MEFs transfected as in (**a**) infected with HSV-1 for indicated times and treated with PBS or carvedilol. (**l**) Immunoassay of lysates of HEK293T cells expressing various vectors. (**m**) Immunoassay of lysates of HEK293T cells expressing various vectors. Data are representative of at least three independent experiments (mean ± SEM in **a-d,** n = 3). Two-tailed unpaired Student's t-test.



Supplementary Fig. 6:
Diagram depicting the Carvedilol–β-arrestin 2–cGAS–STING–TBK1–IRF3–Type I IFN axis.

Supplementary Table 1: Primer sequences used to amplify human gene and mouse gene in real-time quantitative PCR.

Human *Ifnb*	forward	TCTGGCACAACAGGTAGTAGGC
	reverse	GAGAAGCACAACAGGAG
Human *Cxcl10*	forward	GGAACCTCCAGTCTCAGCACCA
	reverse	AGACATCTCTTCTCACCCTTC
Human *Isg15*	forward	TTTGCCAGTACAGGAGCTTGTG
	reverse	GGGTGATCTGCGCCTTCA
Human *GAPDH*	forward	GCAAATTCCATGGCACCGT
	reverse	GCCCCACTTGATTTTGGAGG
mouse *Ifnb*	forward	AGTTACACTGCCTTTGCC
	reverse	GTTGAGGACATCTCCCAC
mouse *Cxcl10*	forward	CCAAGTGCTGCCGTCATTTT
	reverse	GATAGGCTCGCAGGGATGAT
mouse *Isg15*	forward	GGTGTCCGTGACTAACTCCAT
	reverse	TGGAAAGGGTAAGACCGTCCT
mouse *Gapdh*	forward	CCCACTAACATCAAATGGGG
	reverse	CCTTCCACAATGCCAAAGTT