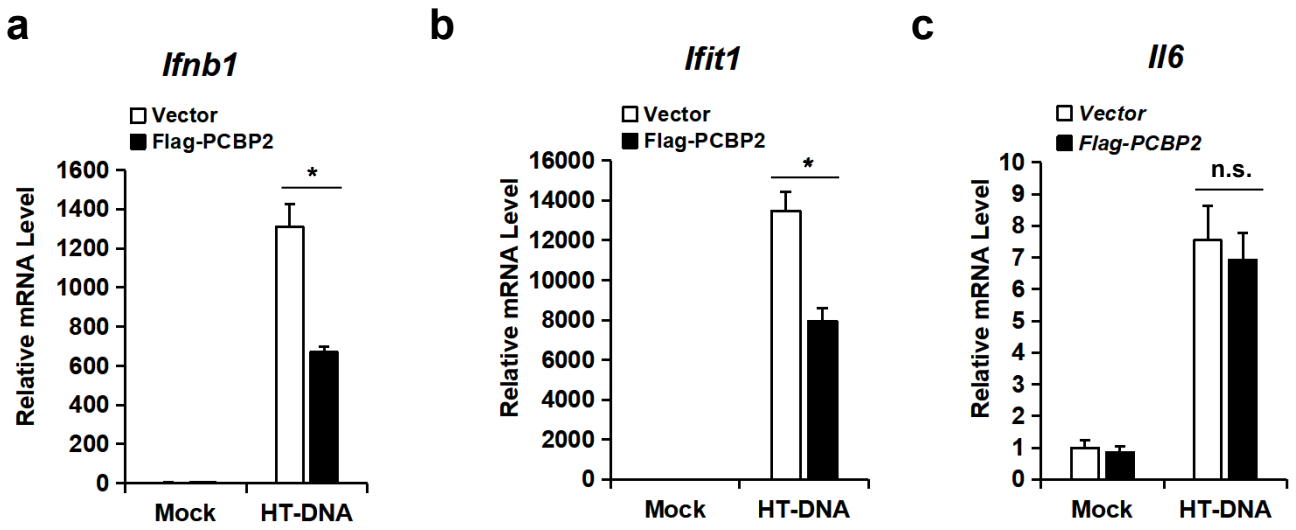
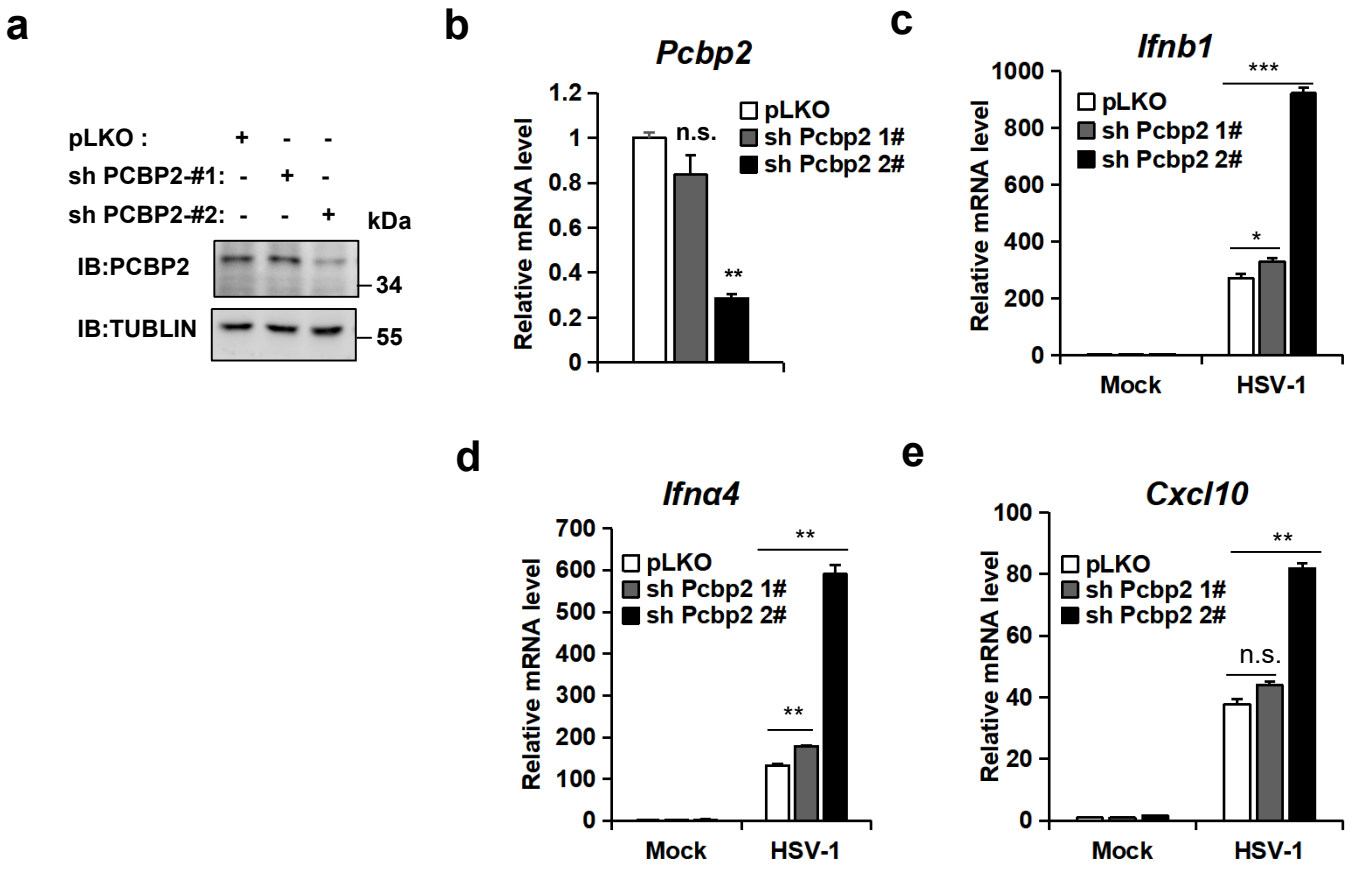
Figure S1



Supplementary Figure 1. PCBP2 overexpression inhibits innate immune response stimulated by HT-DNA in L929 cells.

(a-c) L929 cells were infected with lenti-virus expressing Flag-tagged PCBP2 or empty vector for 48 h, followed by HT-DNA (2μg/ml) transfection for 6 h. The cells were harvested to isolate RNA, transcriptional levels of *Ifnb1* (a), *Ifit1* (b), and *Il6* (c) were measured by qRT-PCR analysis.
Data shown in (a-c) are from one representative experiment of at least three independent experiments (mean ± SD of duplicate experiments).
Two-tailed Student's t-test was used to analyze statistical significance. * $P < 0.05$; n.s. not significant versus the control groups.

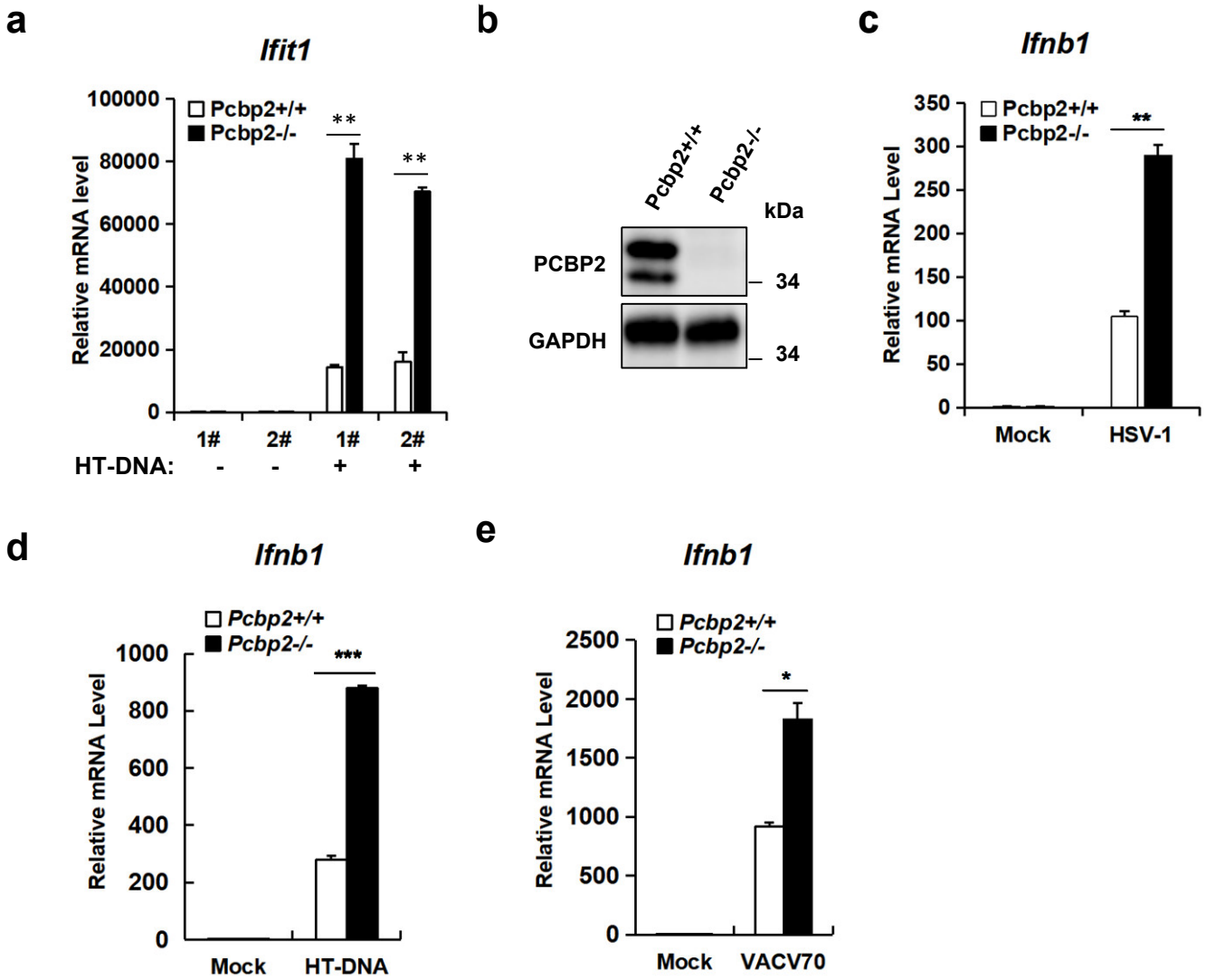
Figure S2



Supplementary Figure 2. Knockdown of PCBP2 potentiates cGAS-STING signaling

(a) THP-1 cells were infected with lentivirus-based shRNA targeting PCBP2 or empty vector for 48 h. The cells were lysed, followed by immunoblotting with the indicated antibodies.
(b-e) RAW264.7 cells were infected with lentivirus-based shRNA targeting Pcbp2 (shPcbp2) or empty vector for 48 h, then infected by HSV-1 (MOI=5) for the indicated times. Transcriptional levels of *Pcbp2* (b), *Ifnb1* (c) *Ifnα4* (d) and *Cxcl10* (e) were detected by qRT-PCR assays.
Data shown in (b-e) are from one representative experiment of at least three independent experiments (mean ± SD of duplicate experiments).
Two-tailed Student's t-test was used to analyze statistical significance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant versus the control groups.

Figure S3



Supplementary Figure 3. PCBP2 knockout increases cGAS-STING signaling

(a) Two different clones from L929 wild-type and *Pcbp2*-deficient cells were transfected with HT-DNA (2μg/ml) for 6 h. The cells were harvested for qRT-PCR analysis to measure the transcriptional levels of *Ifit1.*

(b) *Pcbp2*$^{+/+}$ and *Pcbp2*$^{-/-}$ MEFs were isolated at 13.5 dpi embryos , and lysed for immunoblotting with the indicated antibodies.

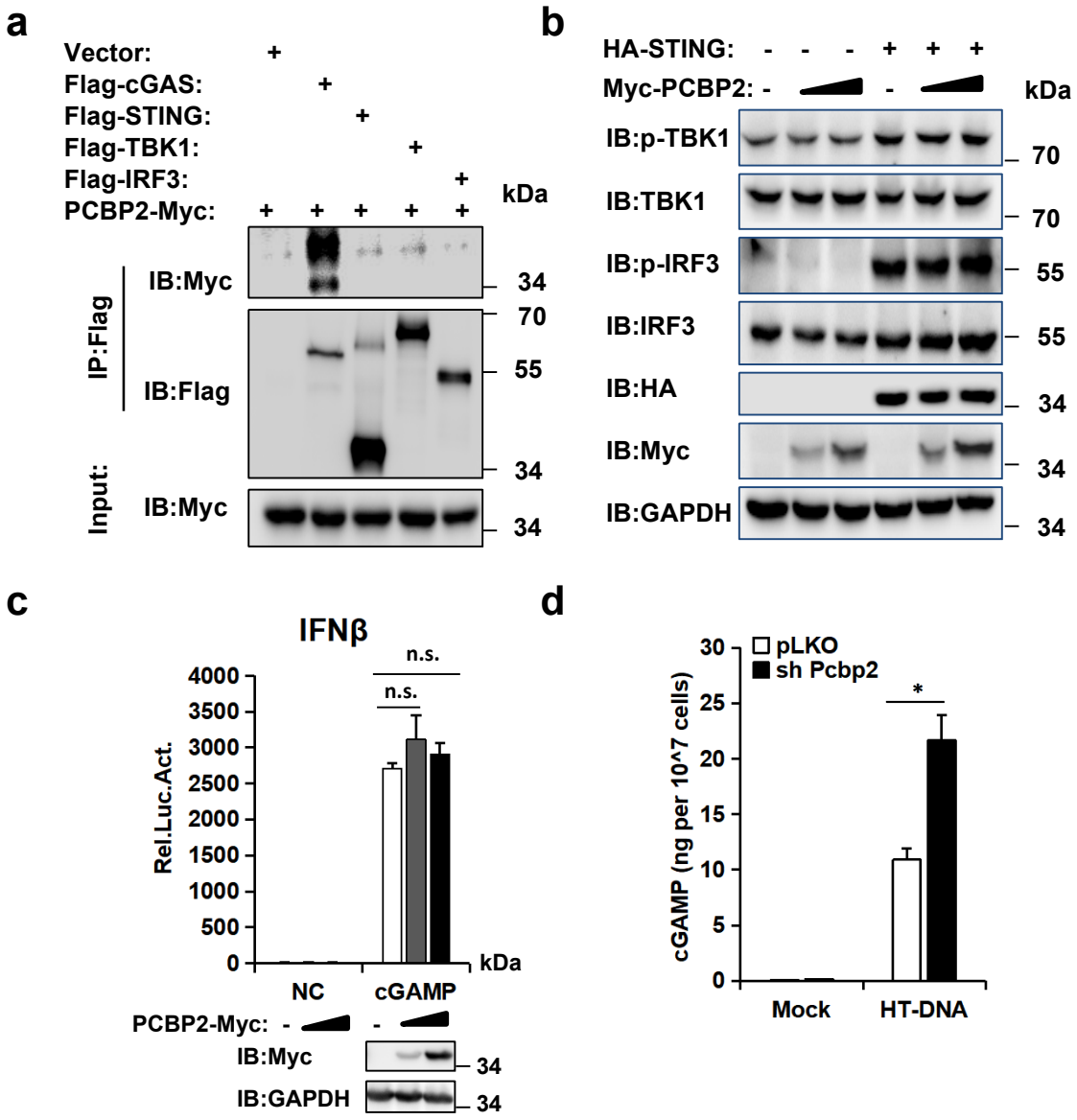
(c) *Pcbp2*$^{+/+}$ and *Pcbp2*$^{-/-}$ MEFs were infected with HSV-1（MOI=5）for 6 h, then lysed for quantification of mRNA levels of *Ifnb1.*

(d, e) *Pcbp2*$^{+/+}$ and *Pcbp2*$^{-/-}$ MEFs were transfected with HT-DNA (d) or VACV70 (e) for 6 h, then lysed for quantification of mRNA levels of *Ifnb1.*

Data shown in (a, c-e) are from one representative experiment of at least three independent experiments (mean ± SD of duplicate experiments).

Two-tailed Student's t-test was used to analyze statistical significance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus control groups.

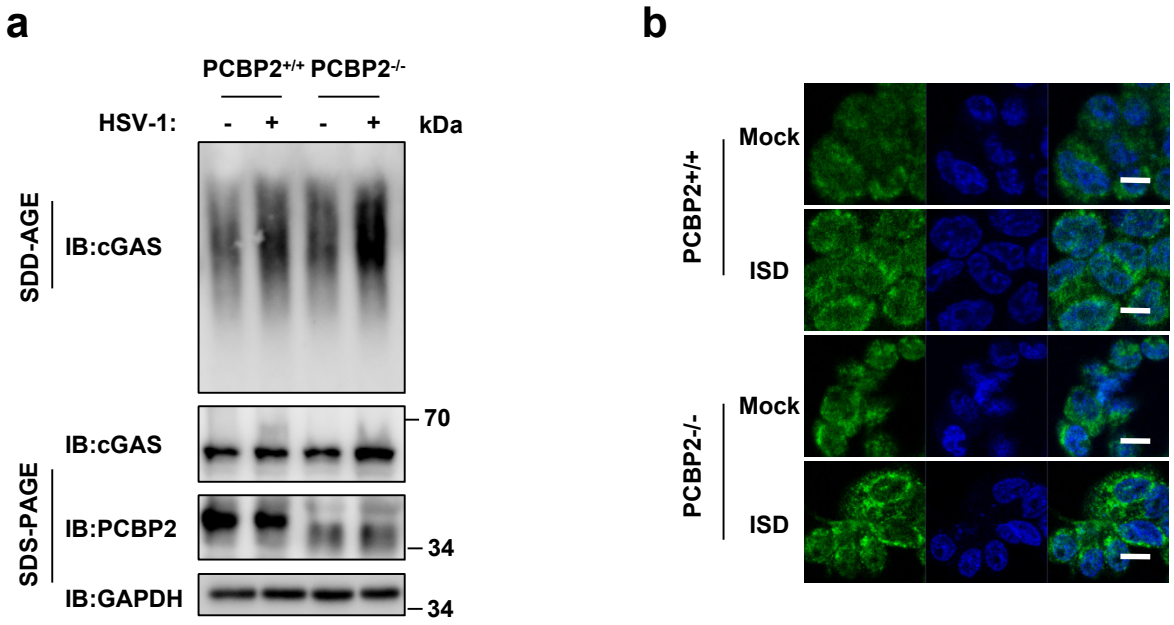
Figure S4



Supplementary Figure 4. PCBP2 specifically targets cGAS to inhibit its activation

(a) HEK293T cells were transfected with the indicated plasmids. Thirty hours after transfection, the cells were lysed and immunoprecipitated with Flag beads, followed by immunoblotting with the indicated antibodies.
(b) HEK293T cells were transfected with the indicated plasmids. Twenty-four hours after transfection, the cells were lysed and followed by immunoblotting with the indicated antibodies.
(c) HEK293T cells were transfected with the indicated vector together with IFNβ-Luc and Renilla which served as an internal control. Twenty-four hours after transfection, the cells were treated with cGAMP for 12 h, then lysed for luciferase reporter assays (upper panel) and immunoblotting assays (lower panels).
(d) RAW264.7 stable PCBP2-knockdown cells and control cells were transfected with HT-DNA for 6 h, and harvested for cGAMP extraction. The abundance of cGAMP was quantitated by cGAMP ELISA kit.
Data shown in (c,d) are from one representative experiment of at least three independent experiments (mean ± SD of duplicate experiments).
Two-tailed Student's t-test was used to analyze statistical significance. * $P < 0.05$; n.s. not significant versus the control groups

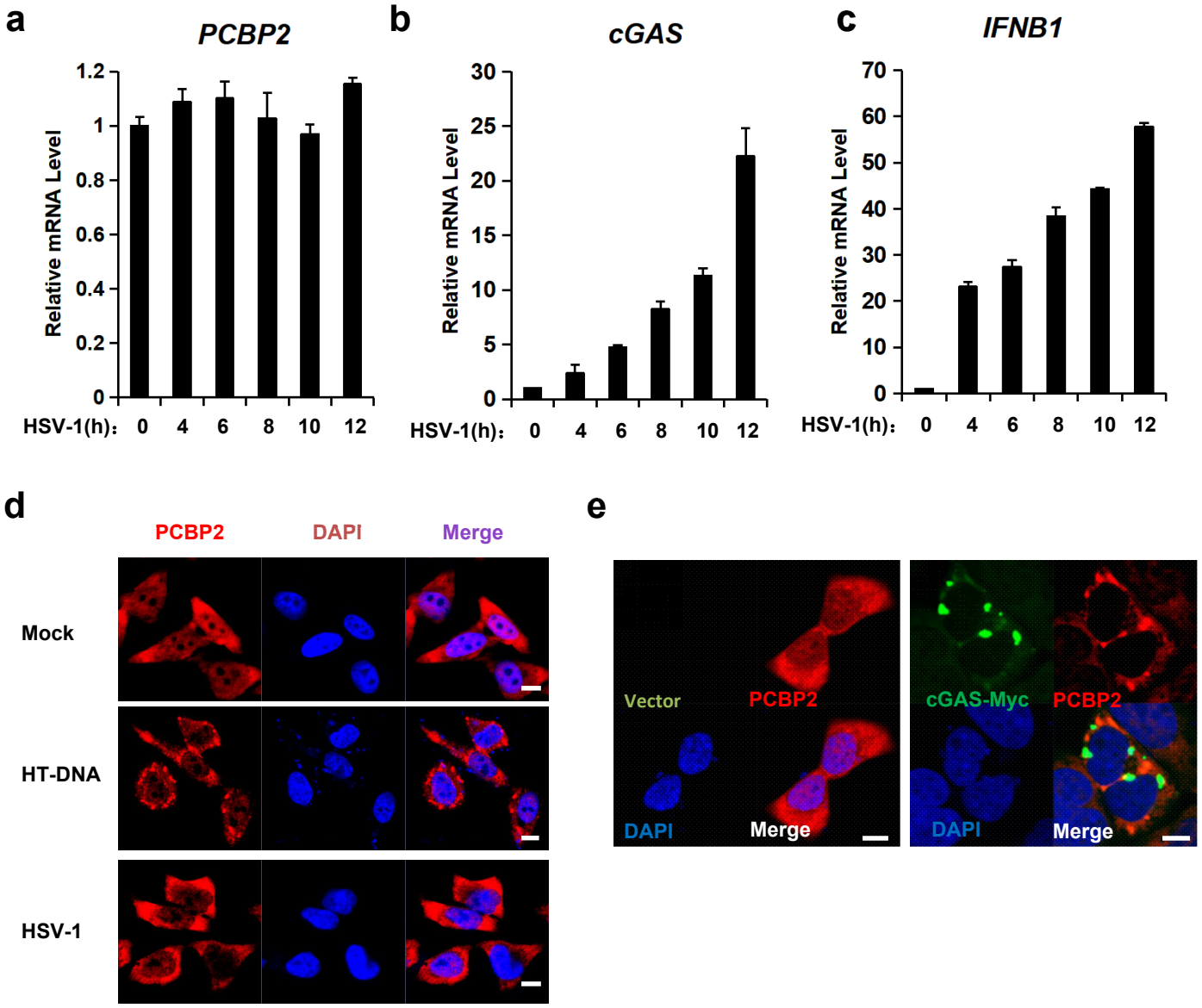
Figure S5



Supplementary Figure 5. PCBP2 attenuates cGAS oligomerization

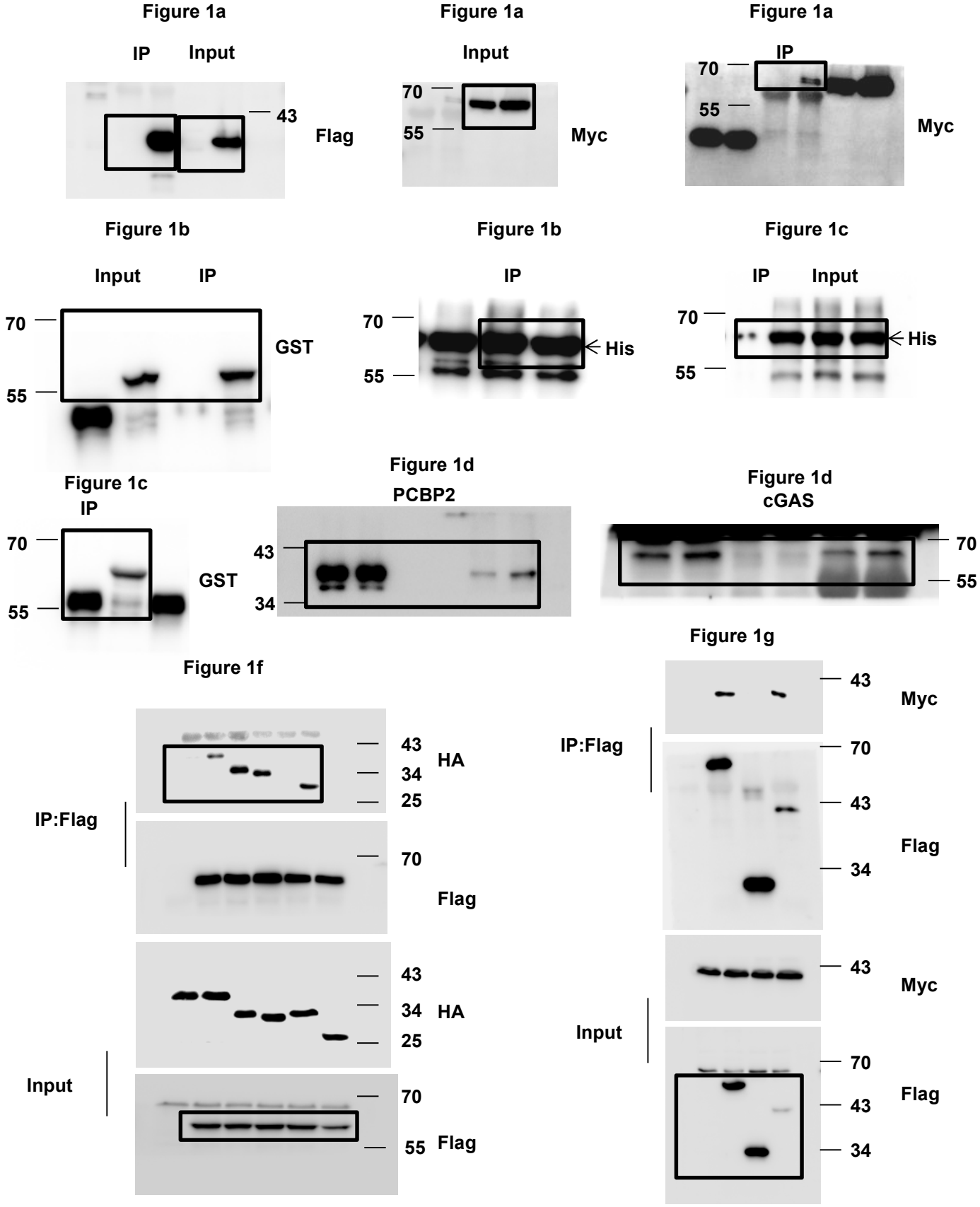
(a) THP-1 *PCBP2+/+* and *PCBP2-/-* cells were infected with HSV-1(MOI=5) for 12 h, cell lysates were prepared for SDD-AGE (upper panel) and SDS-PAGE assays (lower panels), followed by immunoblotting with the indicated antibodies.
(b) THP-1 *PCBP2+/+* and *PCBP2-/-* cells were transfected with or without ISD (2μg/ml) for 6 h. The cells were fixed, stained with cGAS antibody (Cell Signaling Technology, green) and DAPI (blue), and observed by confocal microscopy. Scale bars, 10 μm.

Figure S6



Supplementary Figure 6. PCBP2 mRNA expression is not induced by HSV-1 infection and PCBP2 can translocate to the cytoplasm under different stimuli

(a-c) THP-1 cells were infected with HSV-1(MOI=5) for the indicated times, the cells were then lysed for qRT-PCR analysis to measure the transcript levels of *PCBP2* (a) *cGAS* (b) and *IFNB1* (c) mRNA.
(d) Hela cells were left untreated(upper panels), transfected with HT-DNA (2μg/ml) for 6 h (middle panels) or infected with HSV-1(MOI=2) for 9 h (lower panels). Cells were then fixed, stained with PCBP2(red) antibody and DAPI (blue), and observed by confocal microscopy. Scale bars, 10 μm.
(e) HEK293A cells were transfected with empty vector or C-terminal Myc-tagged cGAS. Twenty-four hours after transfection, the cells were fixed, stained with anti-Myc (green), anti-PCBP2 (red) antibody and DAPI (blue), and observed by confocal microscopy. Scale bars, 10 μm.



Supplementary Figure 7. Original western blots in main and supplementary figures. Panels corresponding to the figures are indicated.

Figure 2a,b,c

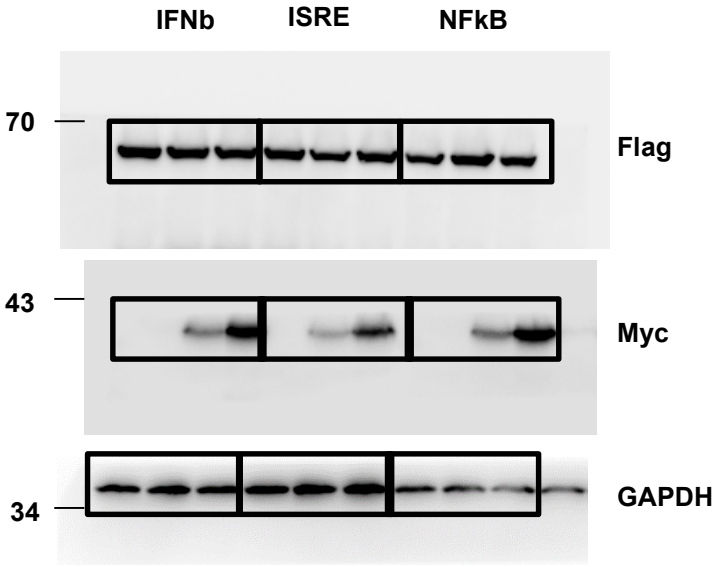


Figure 2g

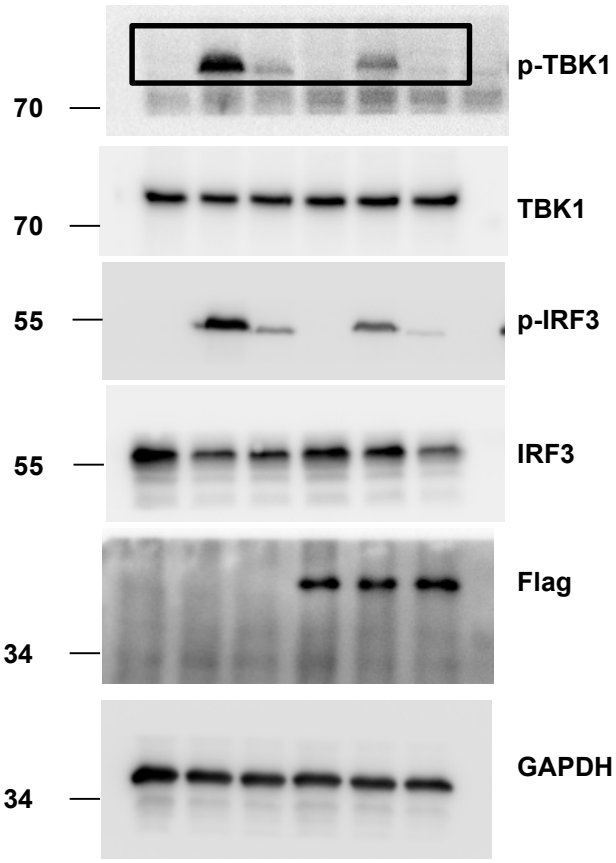
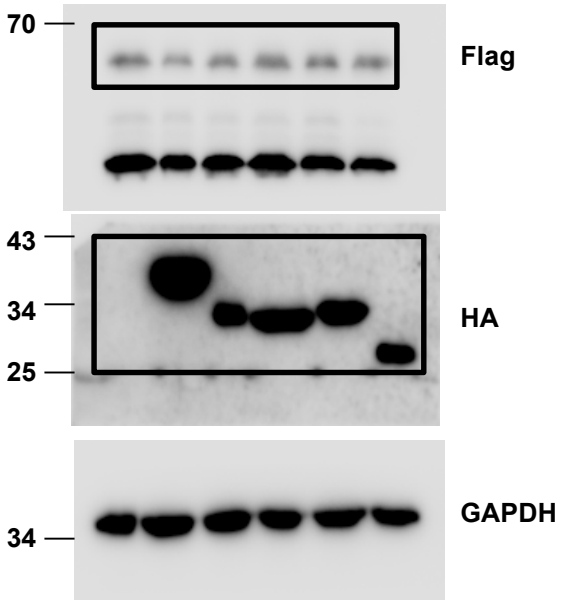


Figure 2h



Supplementary Figure 7 continued.

Figure 3e

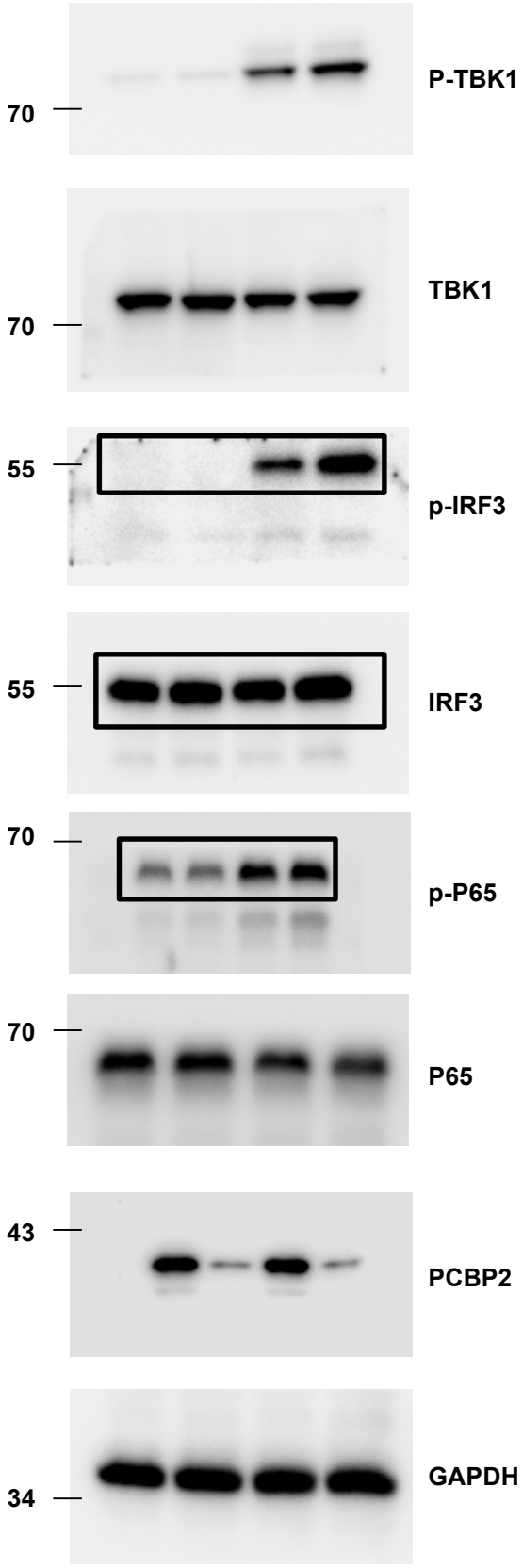
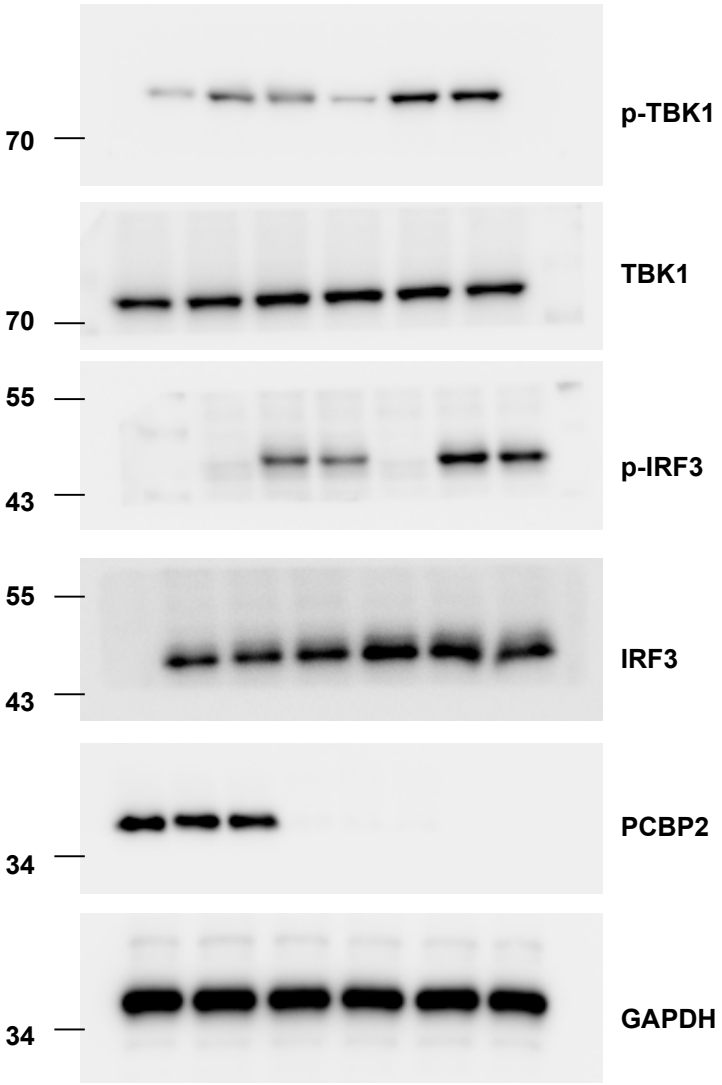


Figure 4b



Supplementary Figure 7 continued.

Figure 4g

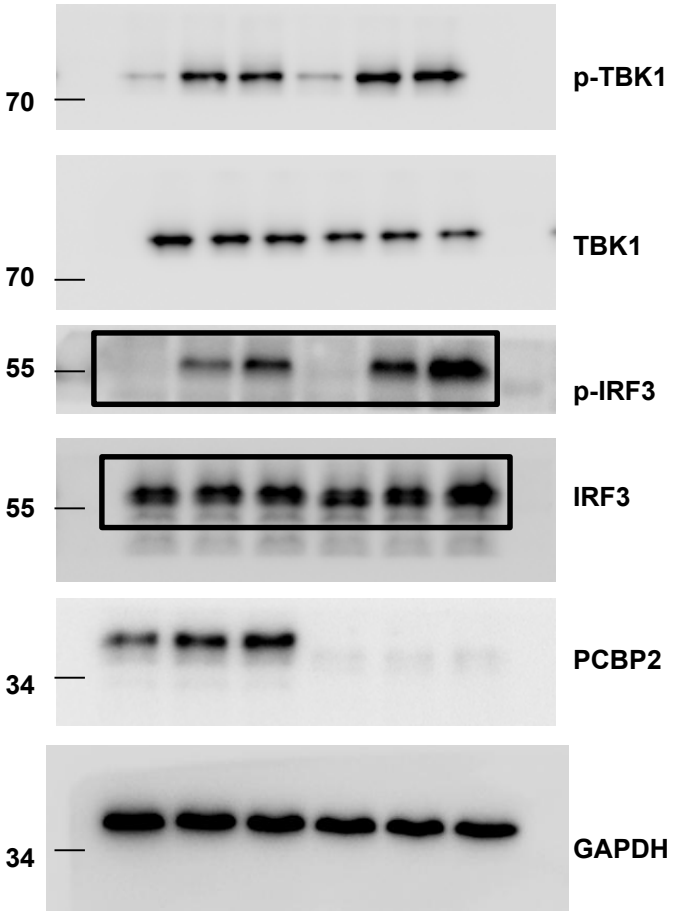
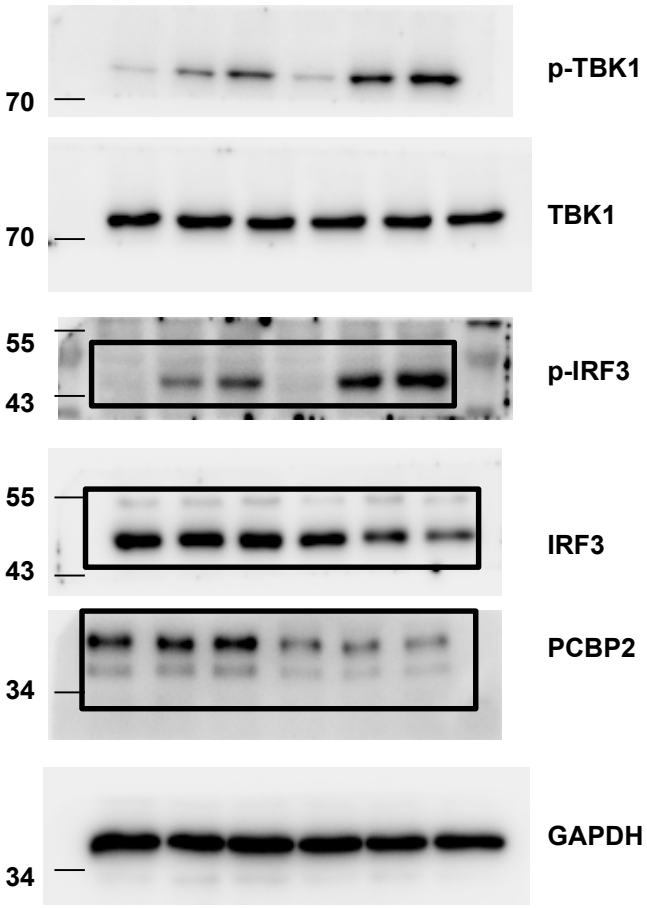


Figure 4k



Supplementary Figure 7 continued.

Figure 5a

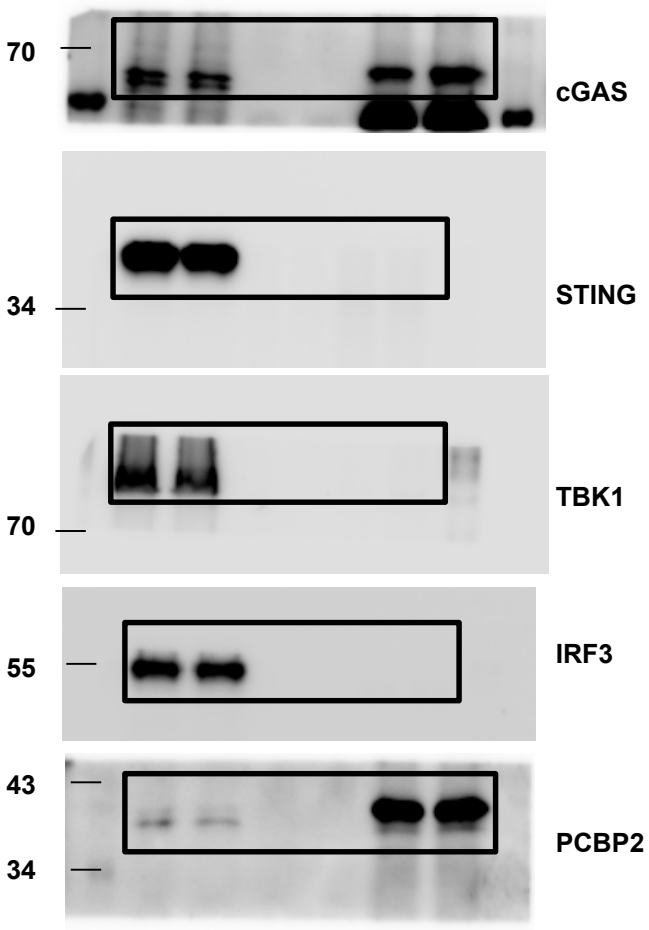


Figure 5b,c,d,e

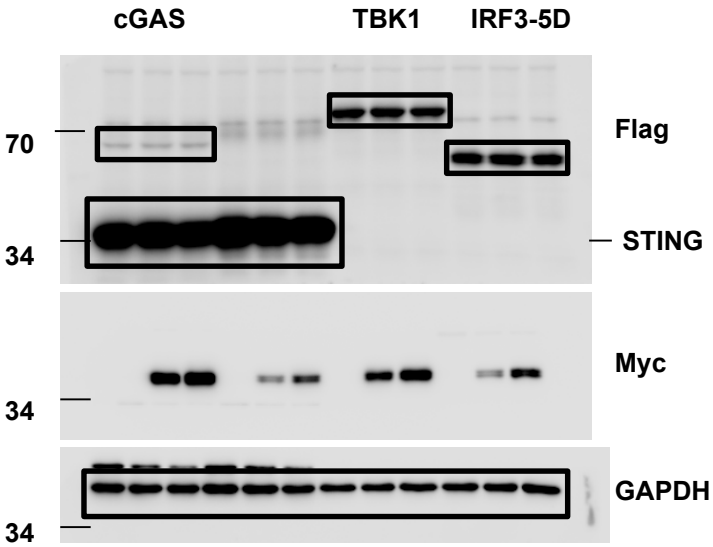
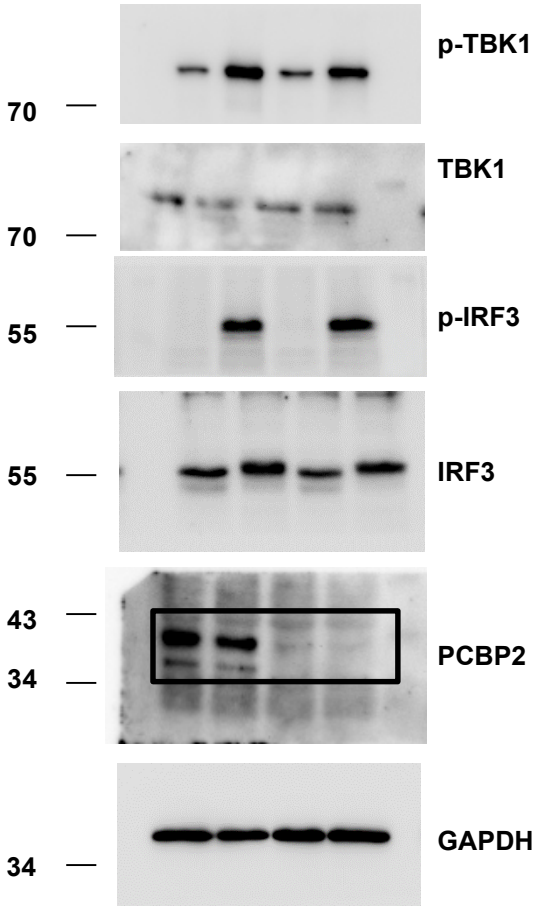


Figure 5f



Supplementary Figure 7 continued.

Figure 6a

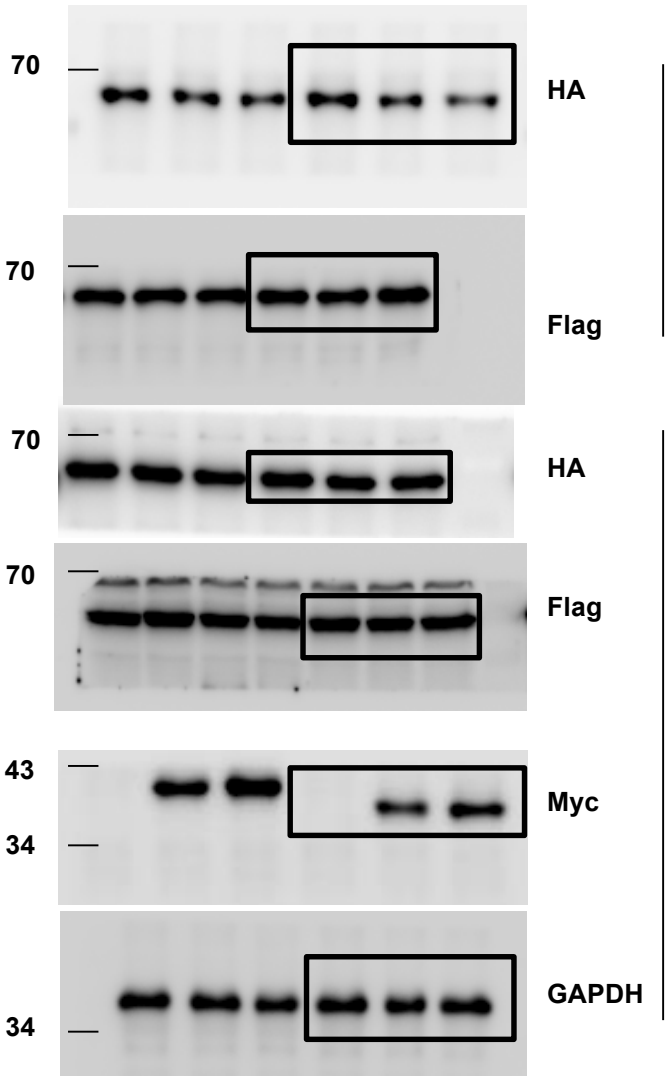
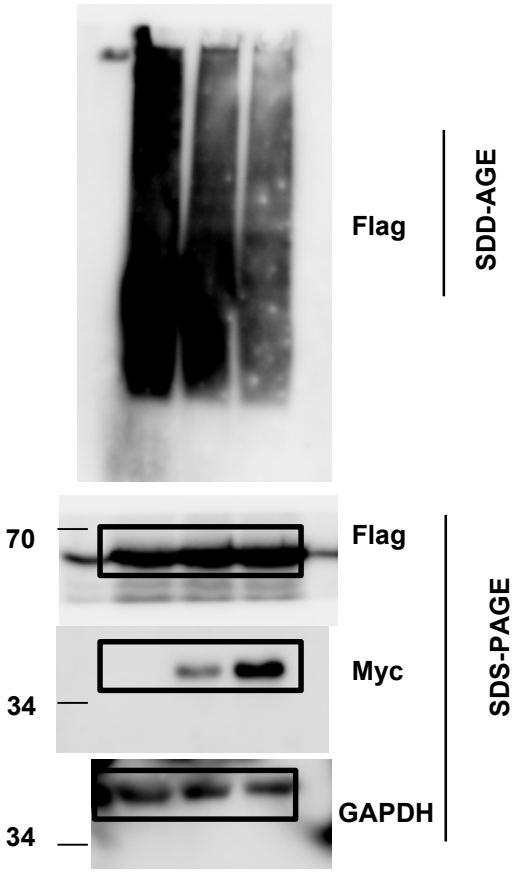


Figure 6b



Supplementary Figure 7 continued.

Figure 6c SDD-AGE

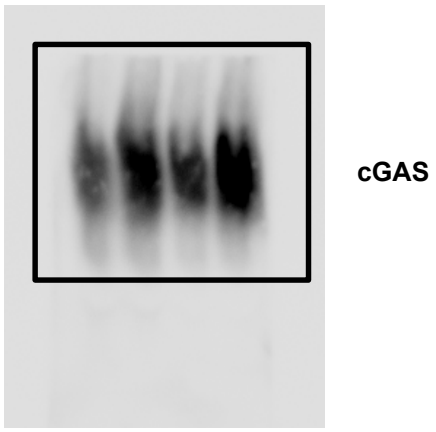


Figure 6c SDS-PAGE

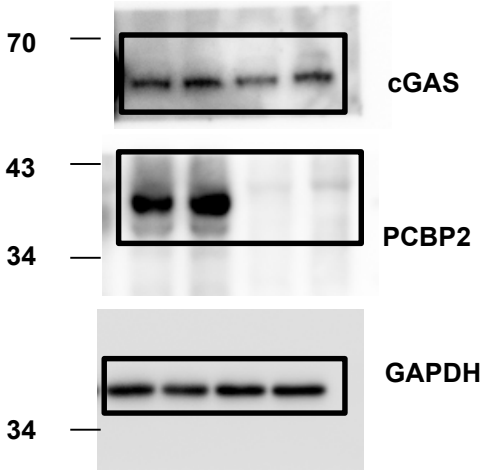
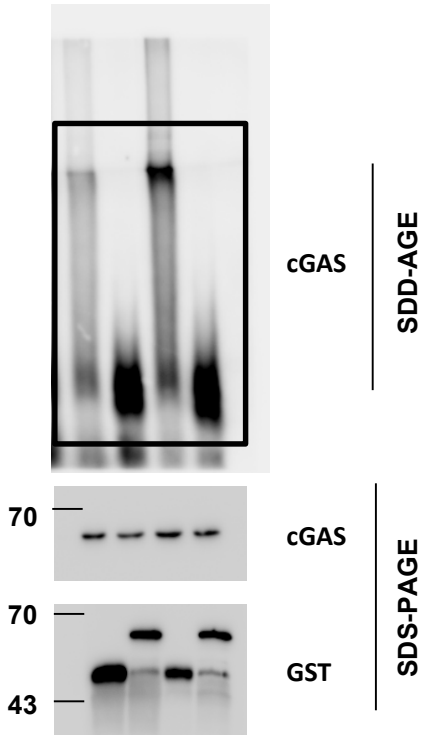
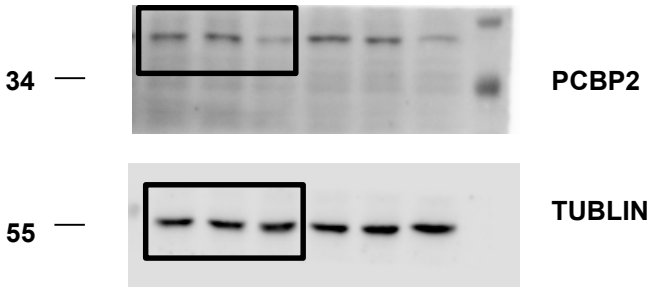


Figure 6d

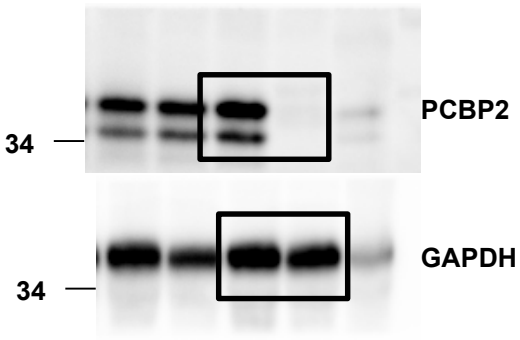


Supplementary Figure 7 continued.

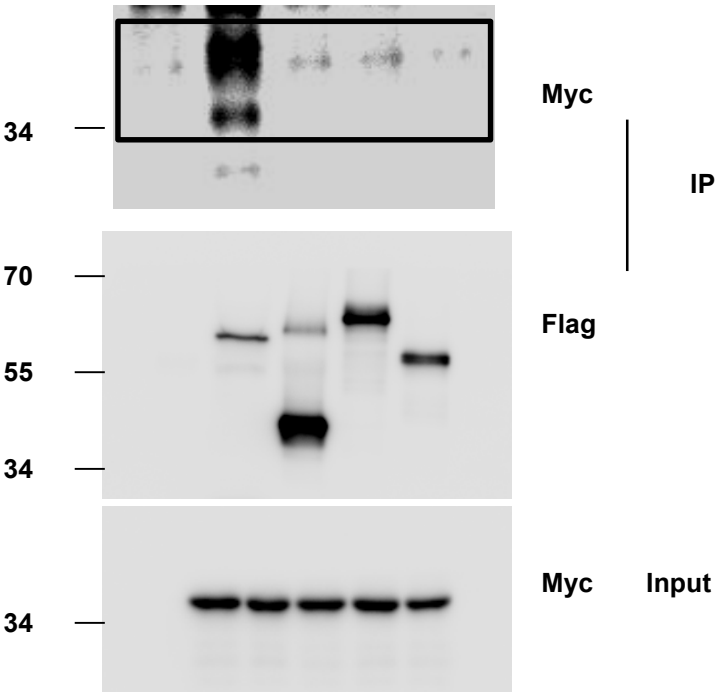
Supplementary Figure 2a



Supplementary Figure 3b



Supplementary Figure 4a



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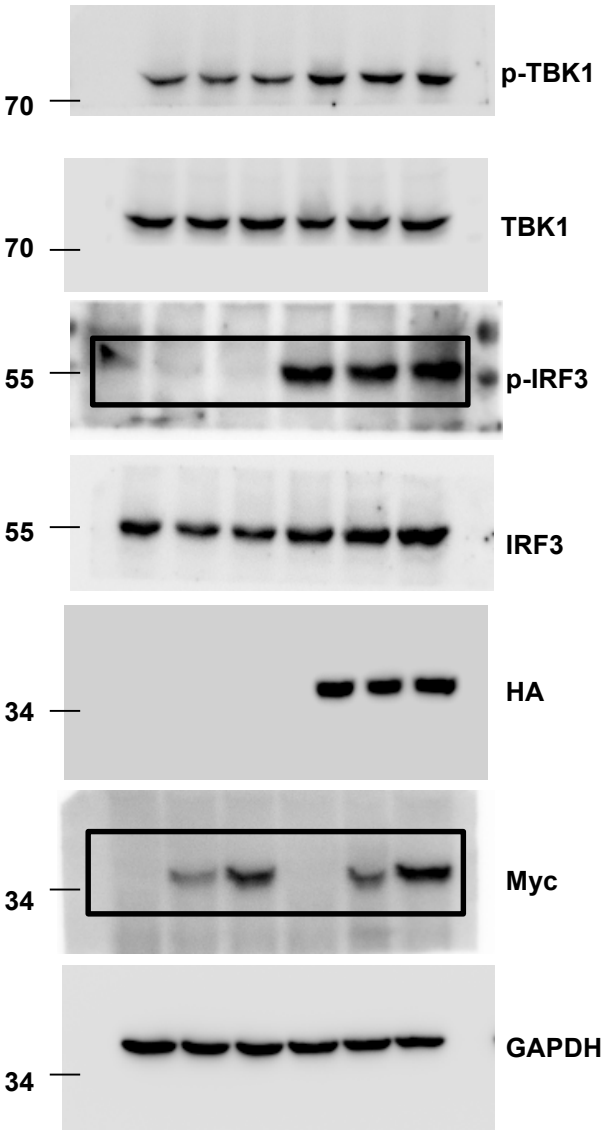
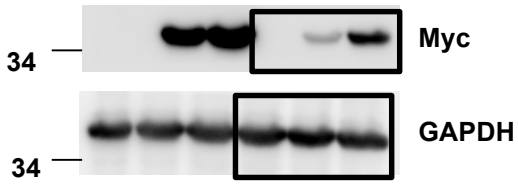
Supplementary Figure 4b

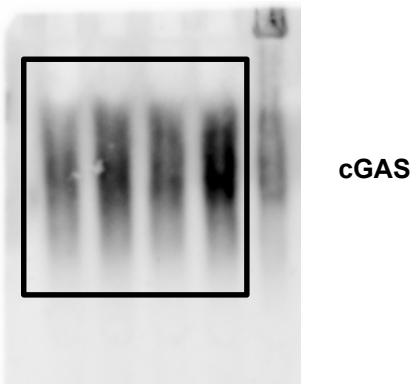


Supplementary Figure 4c



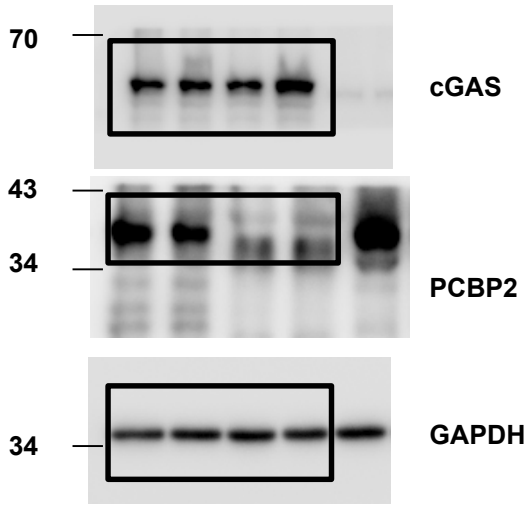
Supplementary Figure 6a

SDD-AGE



Supplementary Figure 6a

SDS-PAGE



Supplementary Figure 7 continued.

Supplementary Table 1. Sequences of primers used in qRT-PCR experiments

Gene name	Forward primers	Reverse primers
Human *GAPDH*	ATGACATCAAGAAGGTGGTG	CATACCAGGAAATGAGCTTG
Human *IFNB1*	AGGACAGGATGAACTTTGAC	TGATAGACATTAGCCAGGAG
Human *IFIT1*	GATCTGGAAAGCTTGAGCCT	GGGTGCCTAAGGACCTTG
Human *CXCL10*	GTGGCATTCAAGGAGTACCTC	TGATGGCCTTCGATTCTGGATT
Human *IL6*	AAATTCGGTACATCCTCGACGG	GGAAGGTTCAGGTTGTTTTCTGC
Human *cGAS*	TATAACCCTGGCTTTGGA	GCTTTAGTCGTAGTTGCTTC
Human *PCBP2*	ACTCTCACCATCCGGCTACTT	TGTTGATACGTGCACCACTCT
Mouse *β-Actin*	TCCAGCCTTCCTTCTTGGGT	GCACTGTGTTGGCATAGAGGT
Mouse *Ifnb1*	ATGGTGGTCCGAGCAGAGAT	CCACCACTCATTCTGAGGCA
Mouse *Ifit1*	CTGAGATGTCACTTCACATGGAA	GTGCATCCCCAATGGGTTCT
Mouse *Cxcl10*	GAATCCGGAATCTAAGACCATCAA	GTGCGTGGCTTCACTCCAGT
Mouse *Ifna4*	AGCCTGTGTGATGCAGGAACC	CAGCAAGTTGGTTGAGGAAGAG
Mouse *Il-6*	TCCATCCAGTTGCCTTCTTG	GGTCTGTTGGGAGTGGTATC
Mouse *Pcbp2*	ACTCTCACCATCAGGCTACTT	TGTTGATACGTGCACCACTCT