

Supplementary materials for

A novel lncRNA linc-AhRA negatively regulates innate antiviral response in murine microglia upon neurotropic herpesvirus infection

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Supplemental Figure legends

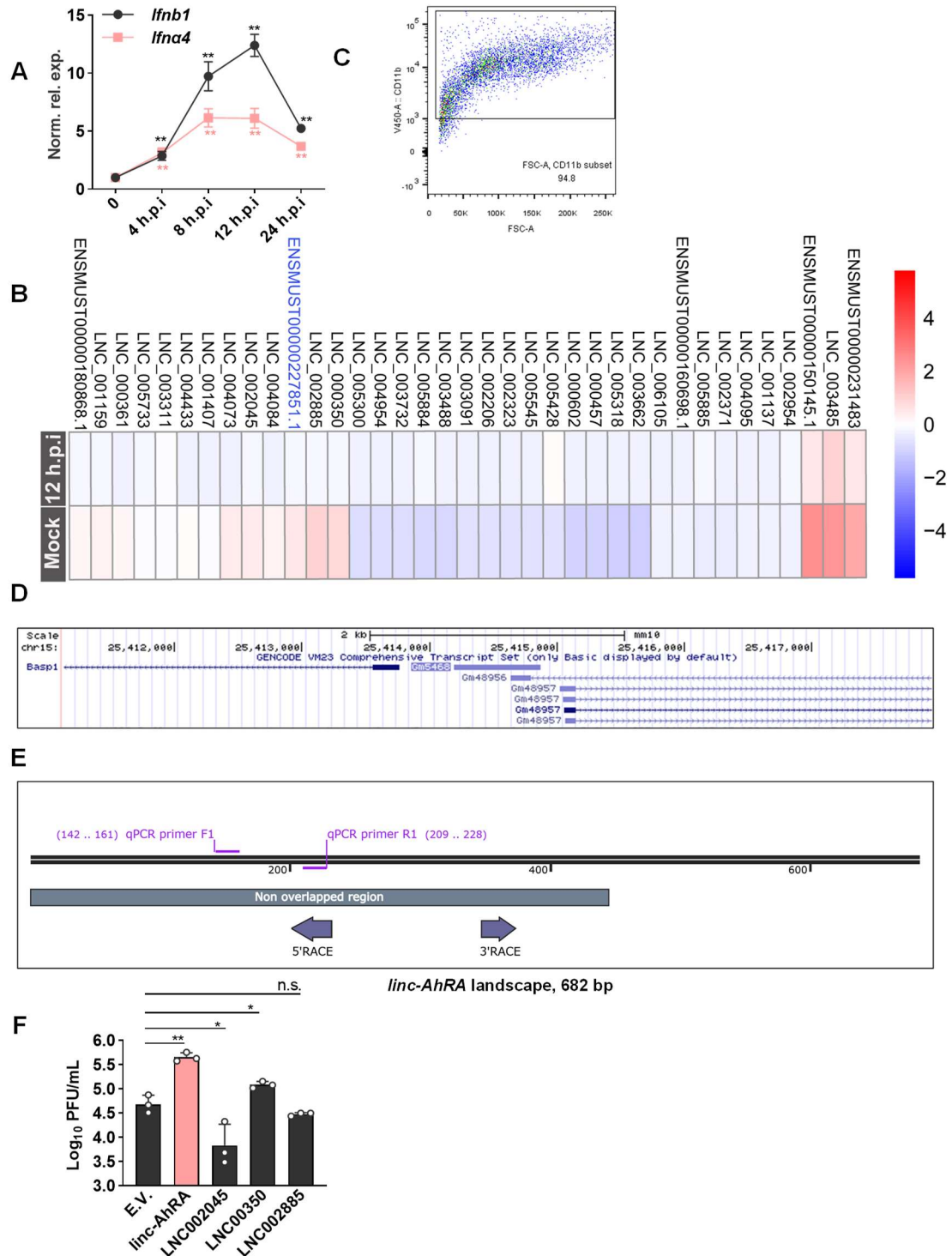


Figure S1. **A**, qPCR analysis of the expression of I-IFNs in primary microglia with HSV-1 infection (MOI 1) for the indicated hours. **B**, The cluster heat map shows upregulated DELs with expression fold change > 16 from lincRNA-sequencing data. The colors represent transcripts above (red) or below (blue) the global median scaled to the corresponding fold activation or repression respectively as shown in the scale bar. **C**, Flow cytometry analysis of microglia digested from mixed glial cultures. The ratio of the CD11b⁺ subset was presented. **D**, The genomic architecture of linc-AhRA

(GM5468) and its neighbor genes. **E**, Primers used in RACE assay (labeled with arrows) and qPCR analysis (labeled with purple stub) for linc-AhRA. The non-overlapped region with GM48956 was also labeled. **F**, Determination of HSV-1 titers in culture medium supernatants of BV2 cells transfected with plasmids expressing the indicated lincRNAs for 48 h followed by HSV-1 infection (MOI 1) for 12 h using a plaque formation assay. Data are representative of three independent experiments with $n = 2$ technical replicates (**A**) or $n = 3$ technical replicates (**F**), three independent experiments (**C**), or the average of three technical replicates as a mixture (**B**), each symbol represents a technical replicate (**F**) (shown as mean and s.d. in **A**, **F**), One-way ANOVA (and nonparametric) (**A**), Cuffdiff was used for differential expression analysis (**B**), two-tailed unpaired Student's t-test (**F**).

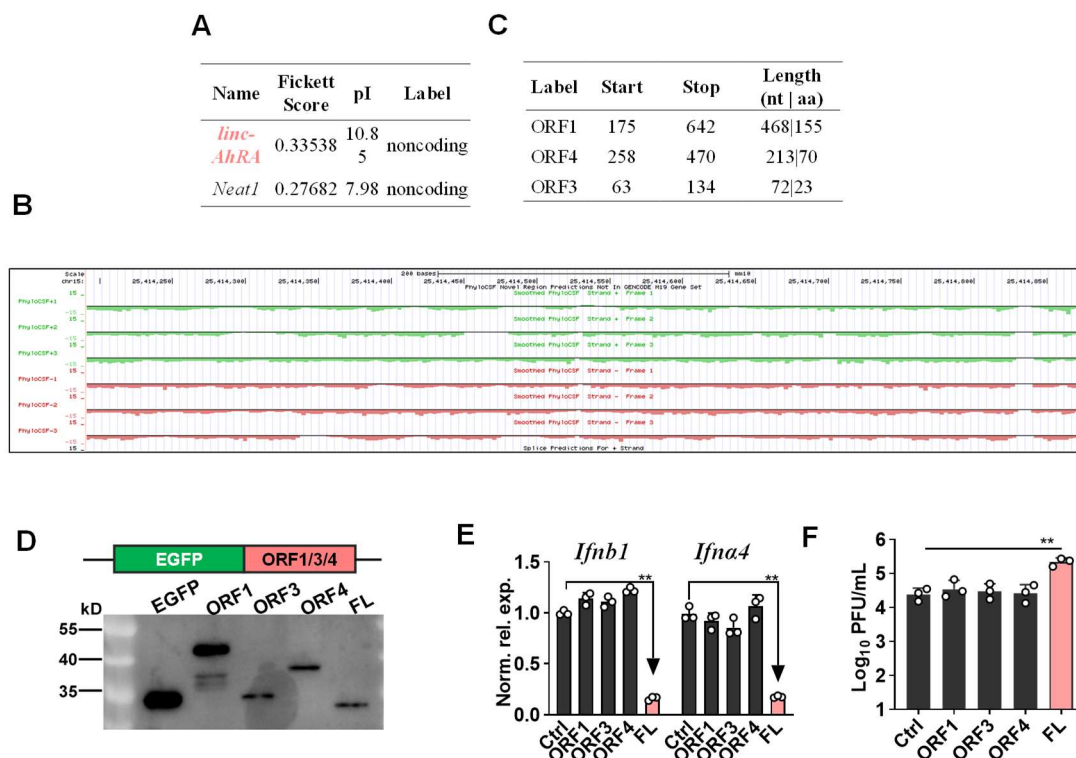


Figure S2. **A**, Coding Potential Calculator analysis of linc-AhRA and Neat1 presented according to the Fickett Score and pI, as well as the coding properties. Neat1 was present as a positive control non-coding RNA. **B**, Protein coding potential analysis of *linc-AhRA* using PhyloCSF. **C**, ORFs with a length longer than 20 amino acids within linc-AhRA. **D**, **Top**: Scheme diagram for constructing plasmids expressing EGFP-ORF. **Bottom**: Immunoblotting results for the indicated ORF-EGFP production in HEK 293T cells. **E**, qPCR analysis of the indicated RNA level following a 6 h infection with HSV-1 in BV2 cells in the presence of the indicated small peptides (10 μ g/mL) or plasmids expressing linc-AhRA with an EGFP indicator. **F**, Determination of HSV-1 titers in culture medium supernatants of BV2 cells with a 12 h HSV-1 infection following a treatment of the indicated small peptides (10 μ g/mL) for the first 6 h or a transfection of plasmids expressing linc-AhRA with an EGFP indicator. Data are representative of three independent experiments (**D**, **bottom**), three independent experiments with $n = 3$ technical replicates (**E-F**), each symbol represents an individual technical replicate (**E-F**) (shown as mean and s.d. in **E-F**), two-tailed unpaired Student's t-test (**E-F**).

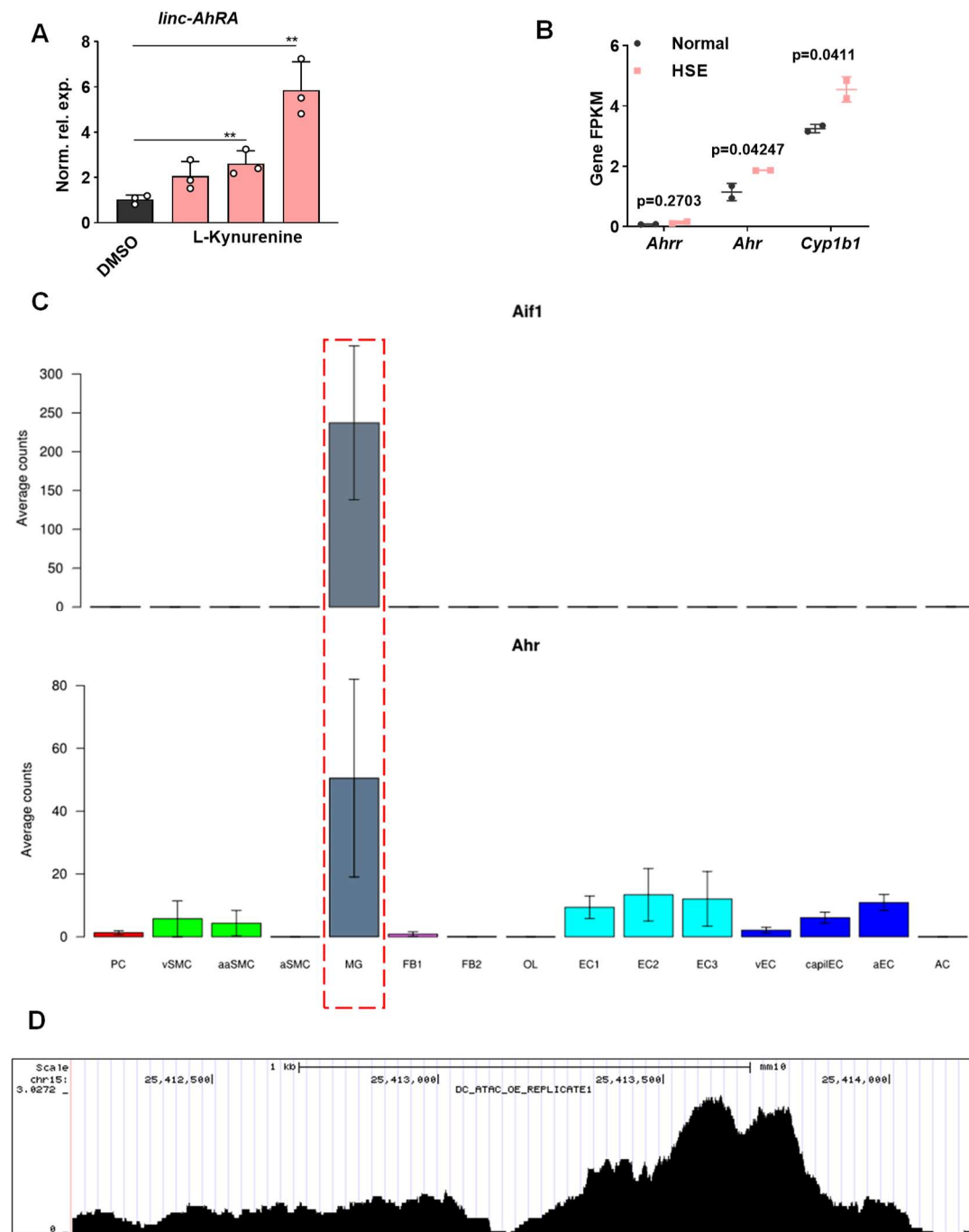


Figure S3. **A**, qPCR analysis of *linc-AhRA* expression in BV2 cells with a stimulation of L-Kynurenine (100, 200, and 400 μ M) for 6 h. Data are normalized relative to DMSO-treated BV2 cells. **B**, The level of AhR-activated genes reflected by the average FPKM value in RNA-sequencing results for olfactory bulb isolated from mice with an infection of 2.0×10^6 PFU for 9 days, $n = 2$ mice per group. **C**, The level of AhR in mouse microglia using the published single-cell RNA-sequencing data for the main CNS cell population (<http://betsholtzlab.org/VascularSingleCells/database.html>). Abbreviations: PC, Pericytes; SMC, Smooth muscle cells; MG, Microglia; FB, Vascular fibroblast-like cells; OL, Oligodendrocytes; EC, Endothelial cells; AC, Astrocytes; v, venous; capil, capillary; a, arterial; aa, arteriolar; 1,2,3, subtypes. **D**, Binding landscape of AhR within *linc-AhRA* promoter in published ChIP data obtained from mouse macrophages with LPS (100 ng/mL) stimulation for 2

h using Cistrome Data Browser analysis (<http://cistrome.org/db/#/>). Data are representative of three independent experiments with $n = 3$ technical replicates (**A**) or the average of one experiment with $n = 2$ mice (**B**), each symbol represents an individual technical replicate or one mouse (**A-B**) (shown as mean and s.d. in **A-B**), One-way ANOVA (and nonparametric) (**A**), Cuffdiff differential expression analysis (**B**).

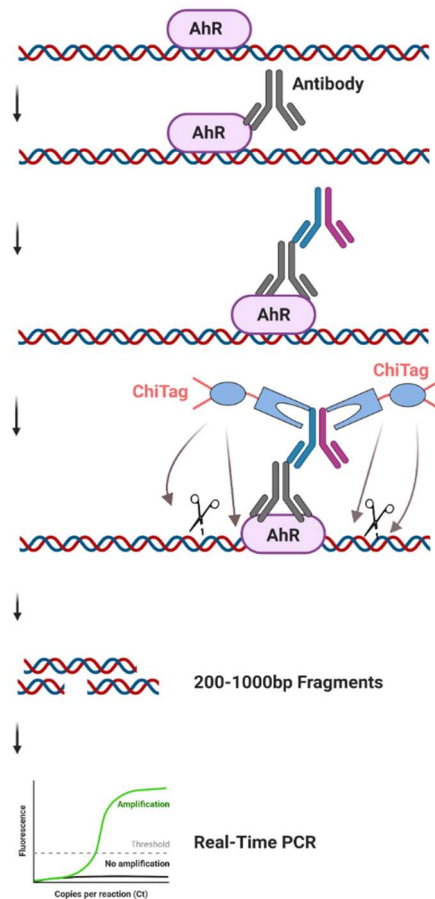


Figure S4. Workflow for detecting the enrichment of AhR within linc-AhRA promoter using AhR CUT&Tag assay.

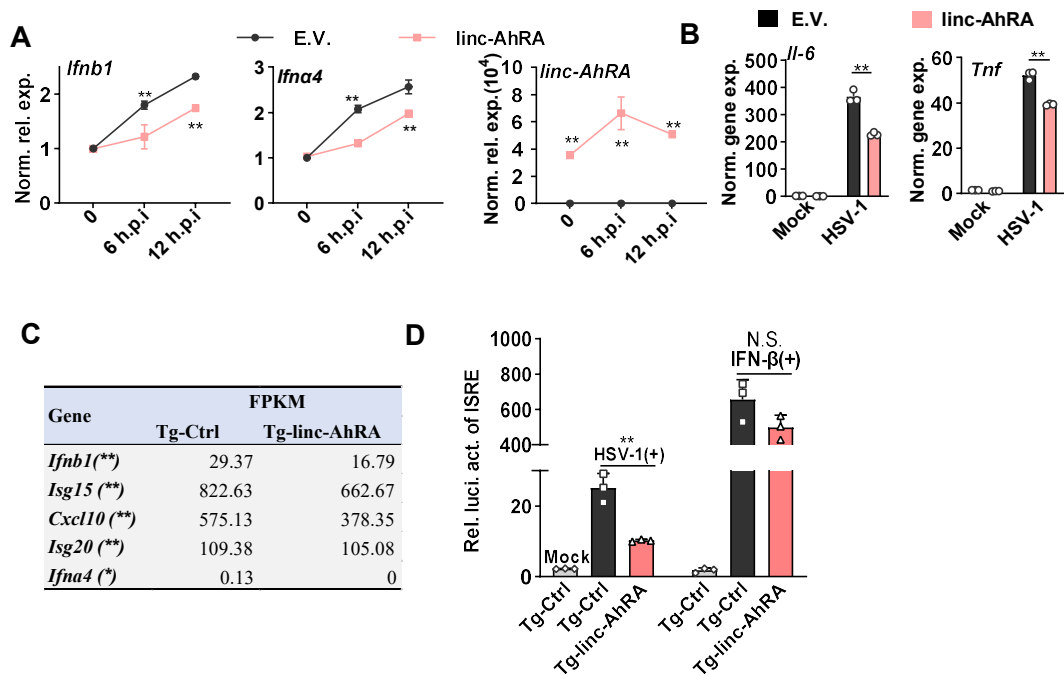


Figure S5. **A**, qPCR analysis of the expression of the indicated genes in primary microglia with the transfection of linc-AhRA-expressing and E.V. plasmids for 48 h and subsequent HSV-1 infection (MOI 1) for the indicated hours. **B**, qPCR analysis of the expression of the inflammatory factors in BV2 cells transfected with linc-AhRA-expressing and E.V. plasmids, followed 48 h later by HSV-1 infection (MOI 1) for 12 h. **C**, Determination of the average FPKM of I-IFNs and ISGs in BV2 cells stably expressing linc-AhRA with HSV-1 infection (MOI 1) for 6 h using RNA-sequencing. **D**, Dual luciferase analysis of ISRE activity in linc-AhRA-expressing stable BV2 cells 24 h after co-transfecting a firefly luciferase reporter (ISRE-Luc) and TK-renilla plasmids, followed by HSV-1 (MOI 1) or stimulation with IFN- β (100 pg/mL) for another 12 h. Data are three independent experiments with $n = 3$ technical replicates (**A-B, D**), or the average of three technical replicates as a mixture (**C**), each symbol represents an individual technical replicate (**B, D**) (shown as mean and s.d. in **A-B, D**), two-way ANOVA (**A**), two-tailed unpaired Student's t-test (**B, D**), Cuffdiff was used for differential expression analysis (**C**).

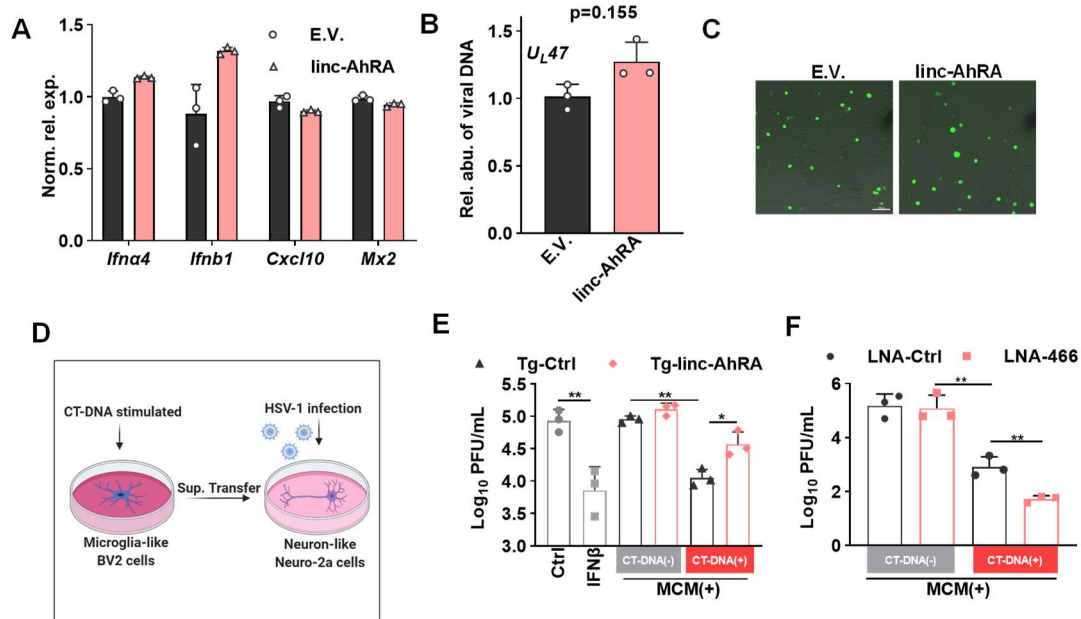


Figure S6. **A**, qPCR analysis of the expression of the indicated genes in Neuro-2a cells with the transfection of linc-AhRA-expressing and E.V. plasmids for 48 h and subsequent HSV-1 infection (MOI 1) for 6 h. **B**, Determination of viral DNA abundance in Neuro-2a cells with the transfection of linc-AhRA-expressing and E.V. plasmids for 48 h and subsequent HSV-1 infection (MOI 1) for 24 h. **C**, Fluorescence microscopy images of viral replication (green) in Neuro-2a cells with EGFP-HSV-1 infection (MOI 1) for 24 h, following the transfection of plasmids expressing linc-AhRA for 48 h, scale bar, 100 μm . **D**, Schematic illustration of a cellular model used to study intercellular communication between microglia and neuron. Supernatant from microglia stably expressing linc-AhRA (**E**) or microglia with linc-AhRA knockdown (**F**) treated for 24 h with jetPRIME buffer or CT-DNA (1 $\mu\text{g}/\text{mL}$) was transferred to neuron-like Neuro-2a cells, followed by HSV-1 infection (MOI 1) for 36 h. Supernatants were harvested and the HSV-1 titers was measured using a plaque formation assay. The medium and IFN- β (100 pg/mL) were used as the negative and positive controls, respectively. MCM, microglia conditioned medium. Data are representative of three independent experiments with $n = 3$ technical replicates (**A-B**, **E-F**), three independent experiments (**C**), each symbol represents an individual technical replicate (**A-B**, **E-F**) (shown as mean and s.d. in **A-B**, **E-F**), two-tailed unpaired Student's t-test (**A-B**, **E-F**).

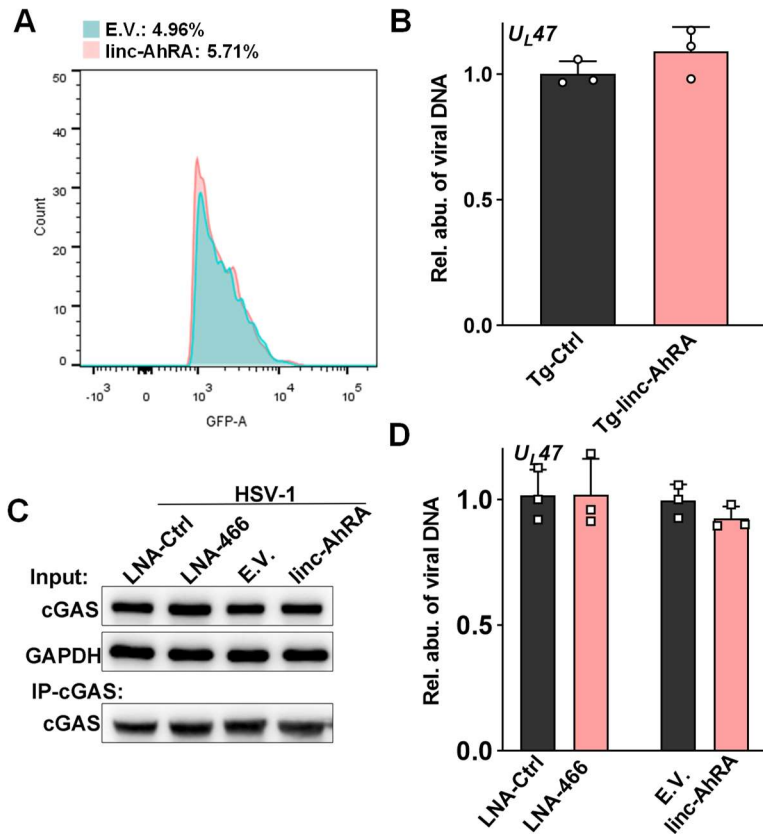


Figure S7. **A**, Flow cytometry analysis of EGFP in BV2 cell with EGFP-HSV-1 (MOI 3) infection for 90 min in the context of linc-AhRA overexpression after removing the free viral particle. **B**, qPCR-based determination of the amount of viral DNA in BV2 cells stably expressing linc-AhRA with HSV-1 infection (MOI 3) for 90 min and subsequent removal of the free virus. **C**, BV-2 cells with indicated expression of linc-AhRA were infected with HSV-1(MOI 3) for 2 h. Lysates were immunoprecipitated with anti-cGAS and the eluate was analyzed by immunoblotting to determine the efficiency of the precipitation (**C**) or qPCR to detect viral DNA after isolating DNA (**D**) (data are shown as relative abundance). Data are representative of three independent experiments (**A**, **C**), three independent experiments with $n = 3$ technical replicates (**B**, **D**), each symbol represents an individual technical replicate (**B**, **D**) (shown as mean and s.d. in **B**, **D**), two-tailed unpaired student's t-test (**B**, **D**).

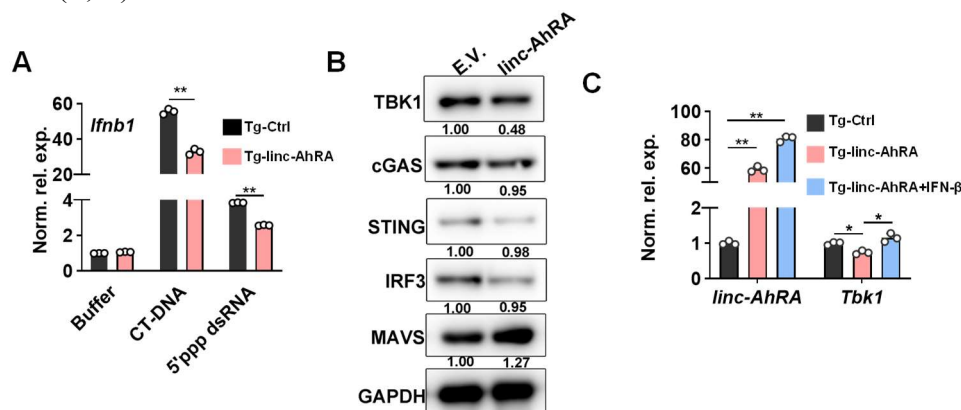


Figure S8. **A**, qPCR analysis of the expression of the indicated genes in BV2 cells stably expressing

linc-AhRA, followed by the transfection of CT-DNA (1 $\mu\text{g}/\text{mL}$) or 5'ppp-dsRNA (1 $\mu\text{g}/\text{mL}$) for 6 h. Data are normalized relative to buffer-stimulated control BV2 cells. **B**, Immunoblot analysis of the indicated factors in primary microglia following the transfection of linc-AhRA expression plasmids for 48 h. **C**, qPCR analysis of the expression of the indicated genes in BV2 cells stably expressing linc-AhRA, followed by IFN- β stimulation (100 pg/mL) for 6 hours. Data are representative of three independent experiments with $n = 3$ technical replicates (**A**, **C**), three independent experiments (**B**), each symbol represents an individual technical replicate (**A**, **C**) (shown as mean and s.d. in **A**, **C**), two-tailed unpaired Student's t-test (**A**, **C**).

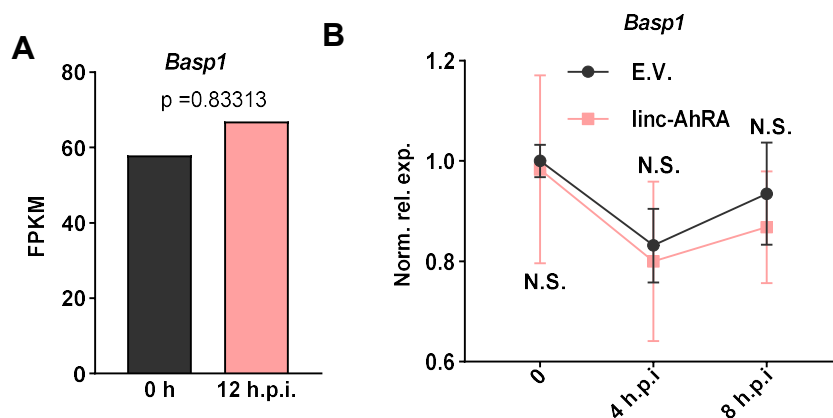


Figure S9. A, The average FPKM of *Basp1* determined by analyzing the RNA-sequencing results for samples as illustrated in **Figure 1 (A)**. **B**, qPCR analysis of *Basp1* expression in BV2 cells with the transfection of linc-AhRA-expressing and E.V. plasmids for 48 h and subsequent HSV-1 infection (MOI 1) for the indicated hours. Data are representative of three independent experiments with $n = 3$ technical replicates (**A**) (shown as mean and s.d. in **B**). Data are the average of three technical replicates as a mixture (**A**). Cuffdiff was used for differential expression analysis (**A**), two-way ANOVA (**B**).

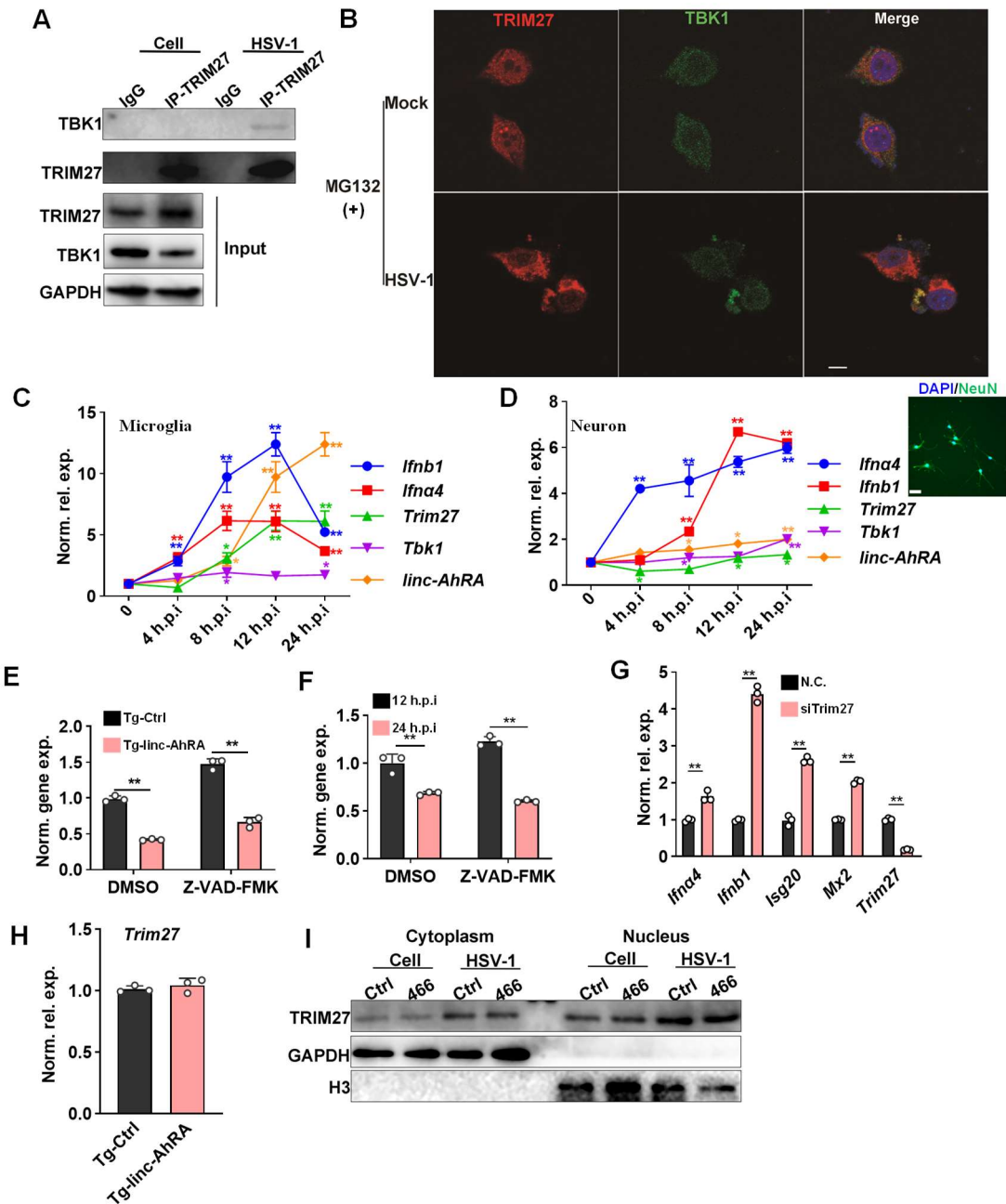


Figure S10. A, Co-immunoprecipitation (Co-IP) analysis of the TBK1-TRIM27 interaction in BV2 cells with HSV-1 infection (MOI 1) for 24 h. **B**, Confocal microscope-based fluorescence analysis of the co-localization of endogenous TBK1 (green) and TRIM27 (red) in BV2 cells with HSV-1 infection (MOI 1) for 18 h and subsequent treatment with MG-132 (10 μ M) for 6 h. Nuclei were labeled with DAPI (blue). Scale bars, 10 μ m. **C**, qPCR analysis of the expression of indicated genes in primary microglia with HSV-1 infection (MOI 1) for the indicated hours as in **Figure S1A**. **D**, qPCR analysis of the expression of indicated genes in primary neuron with HSV-1 infection (MOI 1) for the indicated hours. **E**, qPCR analysis of the expression of *Ifnb1* following a 24 h infection with HSV-1 (MOI 1) in BV2 cells stably expressing linc-AhRA with an incubation of Z-VAD-FMK (50 μ M) for final 6 h. **F**, qPCR analysis of the expression of *Ifnb1* following a indicated hours infection with HSV-1 (MOI 1) in BV2 cells with an incubation of Z-VAD-FMK (50 μ M) for final 6 h. **G**, qPCR analysis of the expression of the indicated genes in BV2 cells 6 h after HSV-1 infection

(MOI 1) following the transfection of siRNA targeting *Trim27* for 42 h. **H**, qPCR analysis of *Trim27* expression in BV2 cells stably expressing linc-AhRA. **I**, Immunoblotting analysis of TRIM27 in subcellular-fraction of BV2 cells 24 h after LNA transfection followed by HSV-1 infection (MOI 1) for another 24 h. Data are representative of three independent experiments (**A-B**, **I**), three independent experiments with n = 2 technical replicates (**C-D**) or with n = 3 technical replicates (**E-H**), each symbol represents an individual technical replicate (**E-H**) (shown as mean and s.d. in **C-H**). One-way ANOVA (and nonparametric analysis) (**C-D**), two-tailed unpaired Student's t-test (**E-H**).

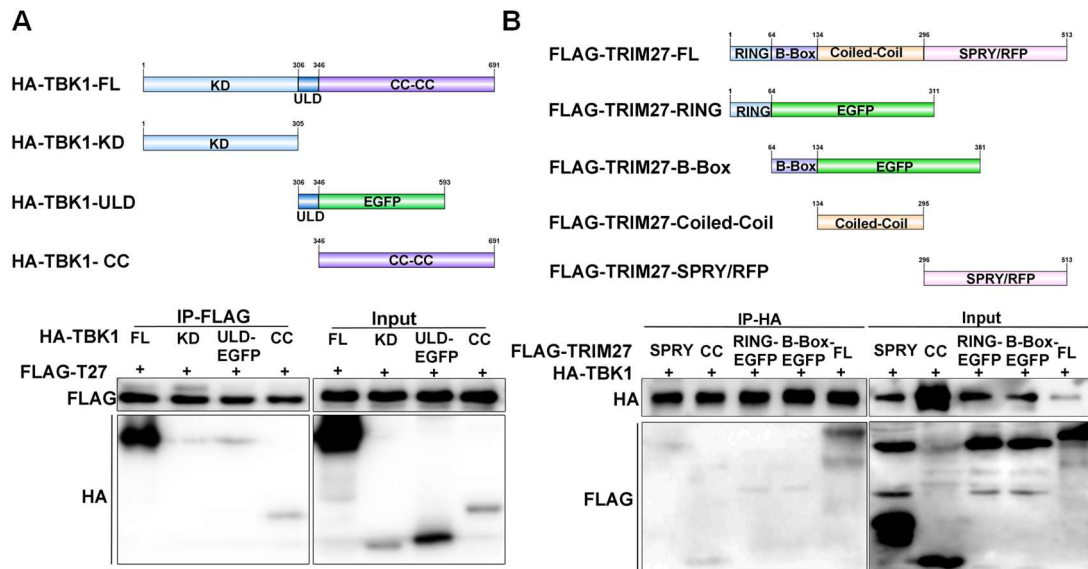


Figure S11. A, Top: Schematic illustration of HA-tagged indicated domain of TBK1. FL, full length; KD, kinase domain; ULD, ubiquitin-like domain; EGFP, enhanced Green Fluorescent Protein; CC, coiled-coil domain. Numbers above indicate the positions of amino acids; **bottom:** Co-IP and immunoblot analysis of HEK 293T cells transfected with the indicated domain of TBK1 along with FLAG-TRIM27. **B, Top:** Schematic illustration of FLAG-tagged indicated domain of TRIM27. SPRY, SPlA/Ryanodine receptor. Numbers above indicate the positions of amino acids; **bottom:** Co-IP and immunoblot analysis of HEK 293T cells transfected with the indicated domain of FLAG-TRIM27 along with HA-TBK1. Data are representative of three independent experiments (**A-B**[**bottom**]).

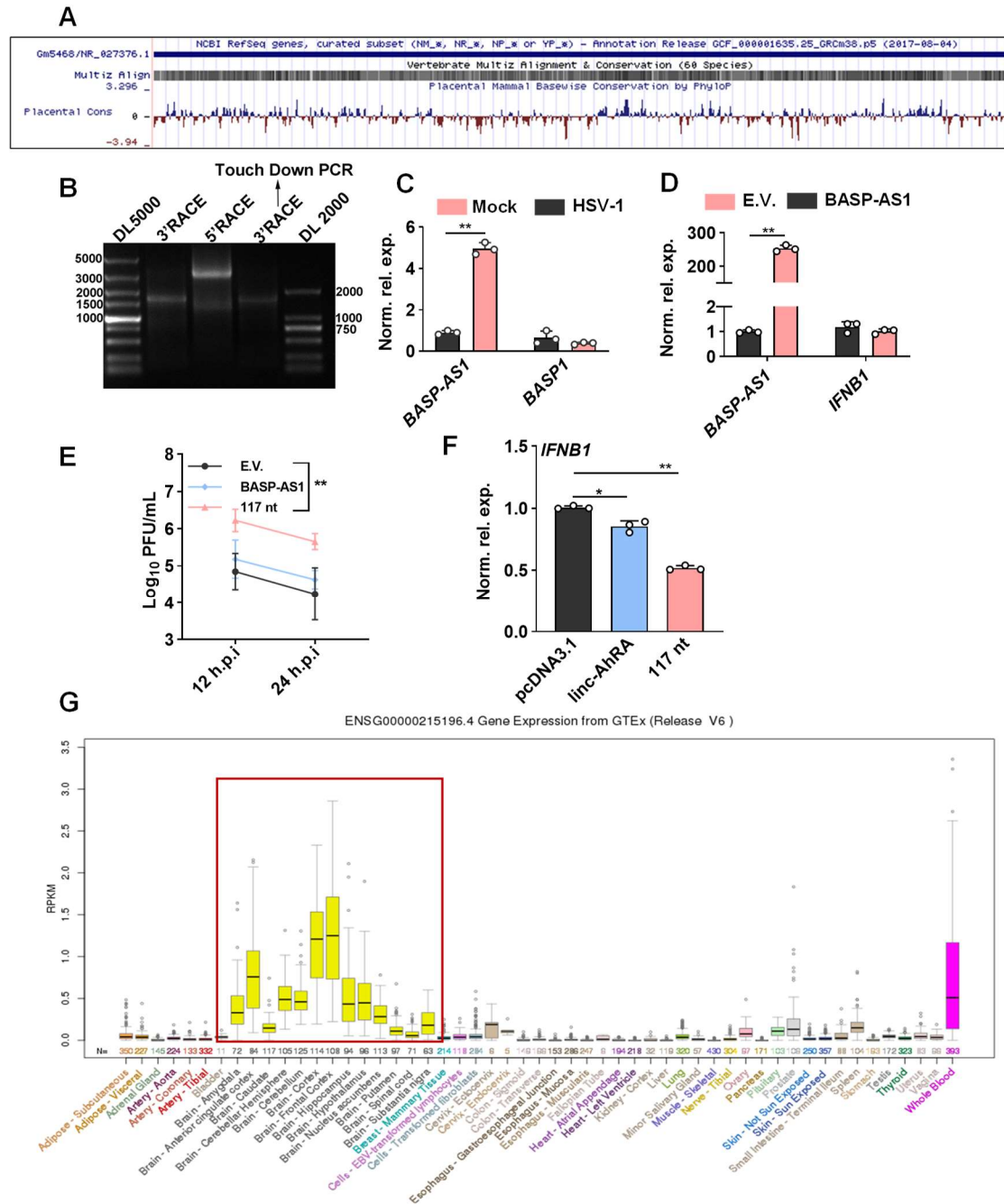


Figure S12. A, The vertebrate multiz alignment and conservation (60 species)-based analysis of linc-AhRA conservation and PhyloP-based placental mammal basewise conservation. **B**, 5'RACE and 3'RACE results for RNA from HMC3 cells with HSV-1 infection for 12 h to obtain the 5' and 3' end sequences for BASP-AS1. **C**, qPCR analysis of *BASP-AS1* and *BASP1* expression in HMC3 cells following a 12-h infection with HSV-1 (MOI 1). **D**, qPCR analysis of *IFNB1* and *BASP-AS1* levels in BASP-AS1-expressing and E.V. plasmids-transfected BV2 cells with HSV-1 infection (MOI 1) for 6 h; **E**, Determination of HSV-1 titers in culture medium supernatants from HMC3 cells transfected with linc-AhRA- or conserved 117nt-expressing plasmids followed 48 h later by HSV-1 infection (MOI 1) for the indicated duration using a plaque formation assay. **F**, qPCR analysis of *IFNB1* levels in HMC3 cells transfected with linc-AhRA or conserved 117nt-expressing plasmids followed 48 h later by HSV-1 infection (MOI 1) for 12 h. **G**, The expression of *BASP-AS1* in human

tissues as presented in GTEx (**version 6.0**). Data are representative of three independent experiments (**B**) or three independent experiments with $n = 3$ technical replicates (**C-F**), each symbol represents an individual technical replicate (**C-D, F**) (shown as mean and s.d. in **C-F**), two-tailed unpaired Student's t-test (**C-D, F**), two-way ANOVA (**E**).

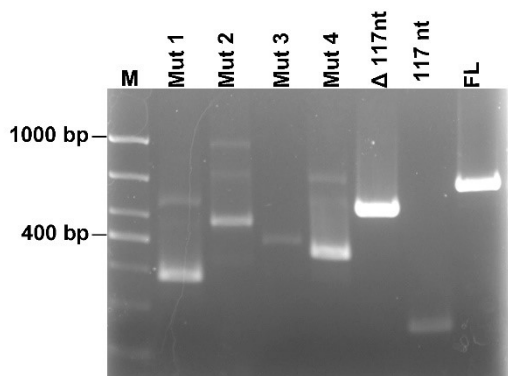


Figure S13. PCR analysis of indicated linc-AhRA deletion mutants amplified from corresponding plasmids. Data are representative of three independent experiments.

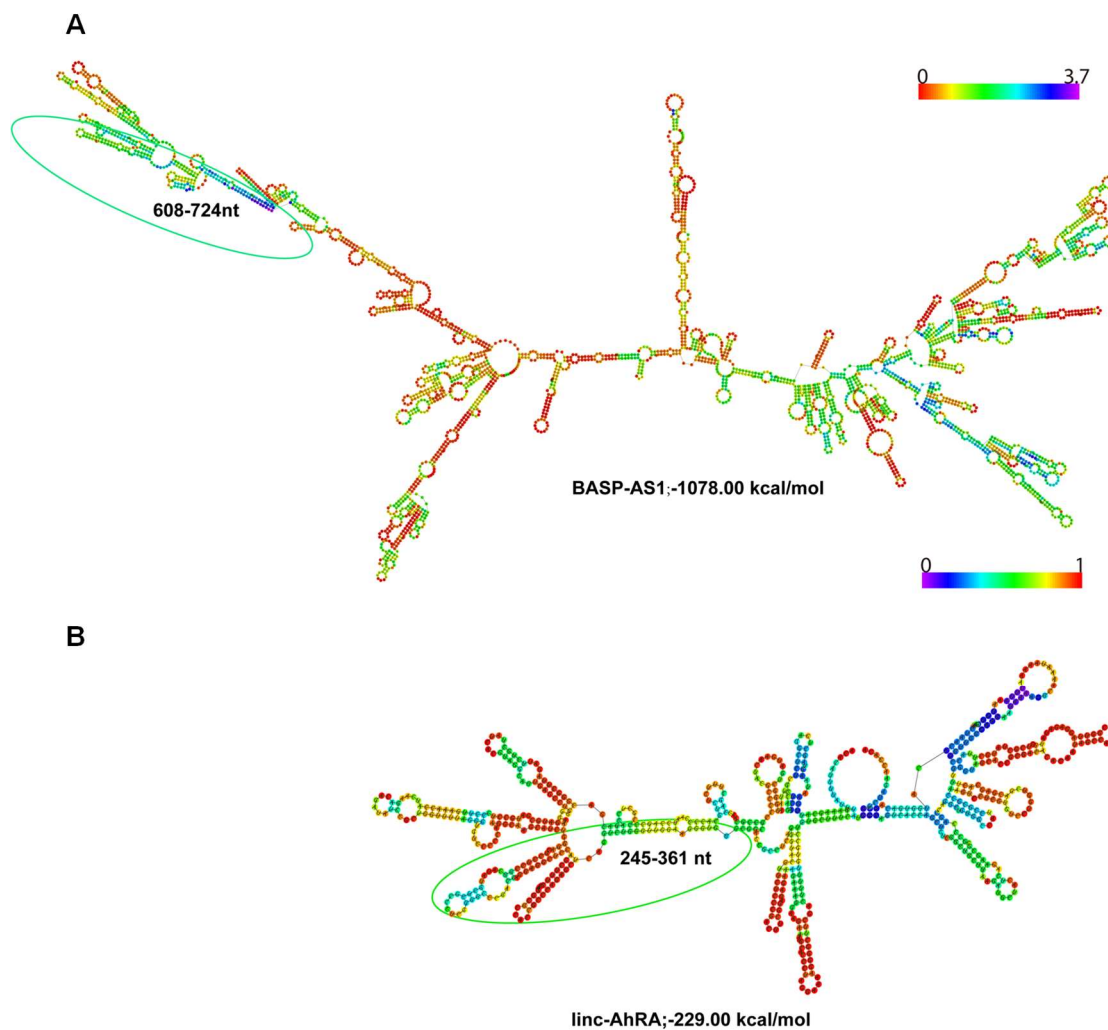


Figure S14. Secondary structure of *BASP-AS1* (**A**) and linc-AhRA (**B**) with minimum free energy predicted by the Vienna RNA web server. The ensemble free energy for them is also labeled.

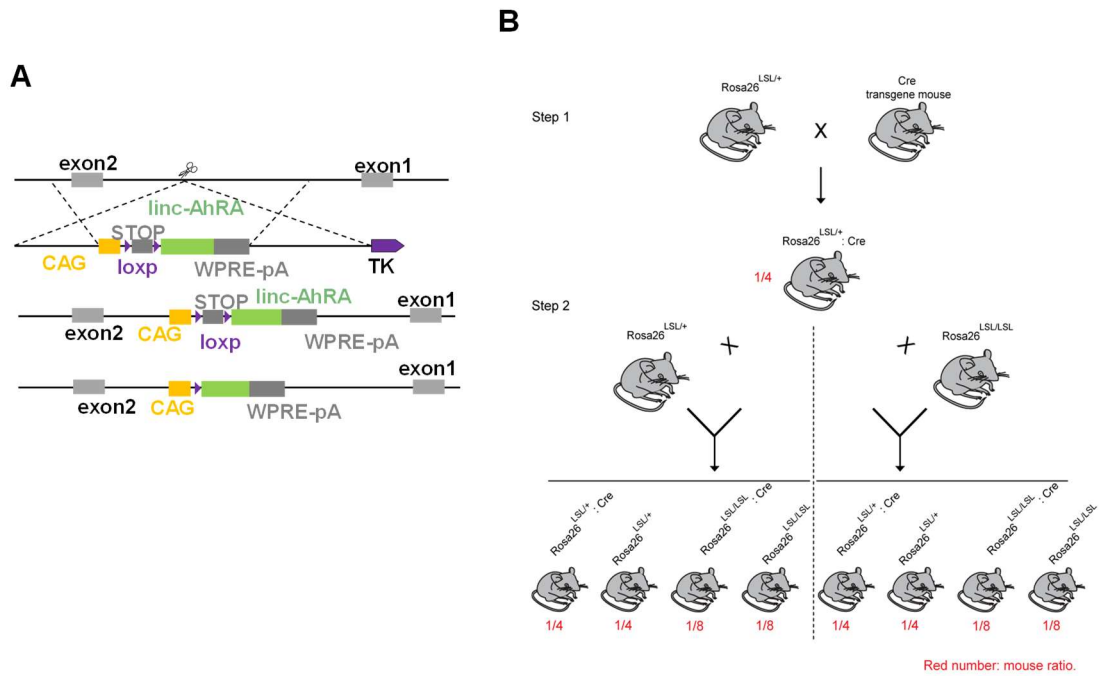


Figure S15. A, Strategy for establishing Rosa26-LSL-*linc-AhRA* mice. CAG, CAG promoter; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element; pA, PolyA. **B**, Breeding scheme for generating control and inducible microglial *linc-AhRA* KI mice.

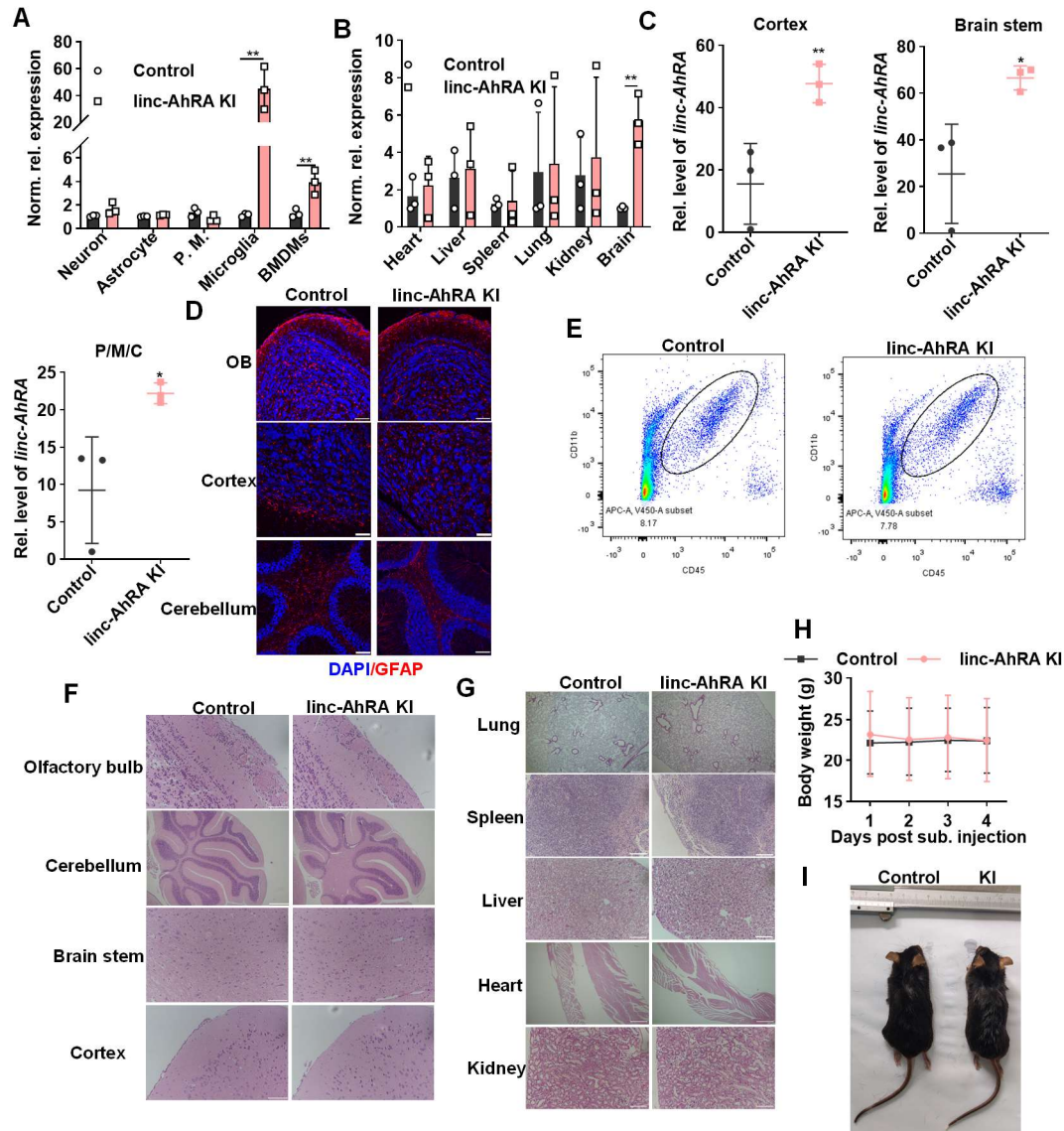


Figure S16. **A**, qPCR analysis of the expression of linc-AhRA in the indicated primary cells isolated from control and microglial linc-AhRA KI mice. All linc-AhRA KI mice described in this figure represented those mice have been received a treatment of TAM. P.M., peritoneal macrophages. BMDMs, bone marrow derived macrophages. $n = 3$ mice per group. **B**, qPCR analysis of the expression of linc-AhRA in the indicated tissues of control and microglial linc-AhRA KI mice. $n = 3$ mice per group. **C**, qPCR analysis of the expression of linc-AhRA in the indicated brain sections of control and microglial linc-AhRA KI mice. $n = 3$ mice per group. **D**, Brain histology of several brain regions that were subjected to immunofluorescence for GFAP to detect astrocytes. Scale bars represent $100 \mu\text{m}$. $n = 3$ mice per group. **E**, Flow cytometry analysis of microglia in single-cell suspension generated with the digestion of brain tissue from control and microglial linc-AhRA KI mice. The ratio of the $\text{CD11b}^+\text{CD45}^{\text{low}}$ subset is also labeled. $n = 3$ mice per group. **F**, Brain histology for several brain regions that were stained with H&E. Scale bars represent $100 \mu\text{m}$. $n = 3$ mice per group. **G**, The H&E stain-based histology of the indicated tissues isolated from WT and microglial linc-AhRA KI mice, $n = 3$ mice per group. Scale bars represent $500 \mu\text{m}$ (lung) and $100 \mu\text{m}$ (spleen, liver, heart, and kidney). **H**, The weight changes in control and microglial linc-AhRA KI mice ($n = 4-6$ mice per group, 8-9 weeks old) over a period of 4 days. **I**, Photograph of control

and linc-AhRA KI mice aged 8-9 weeks to record body size. Data are representative of three independent experiments (A-G) and each symbol represents an individual mouse (A-C). Two-tailed unpaired Student's t-test for (A-C), two-way ANOVA for H.

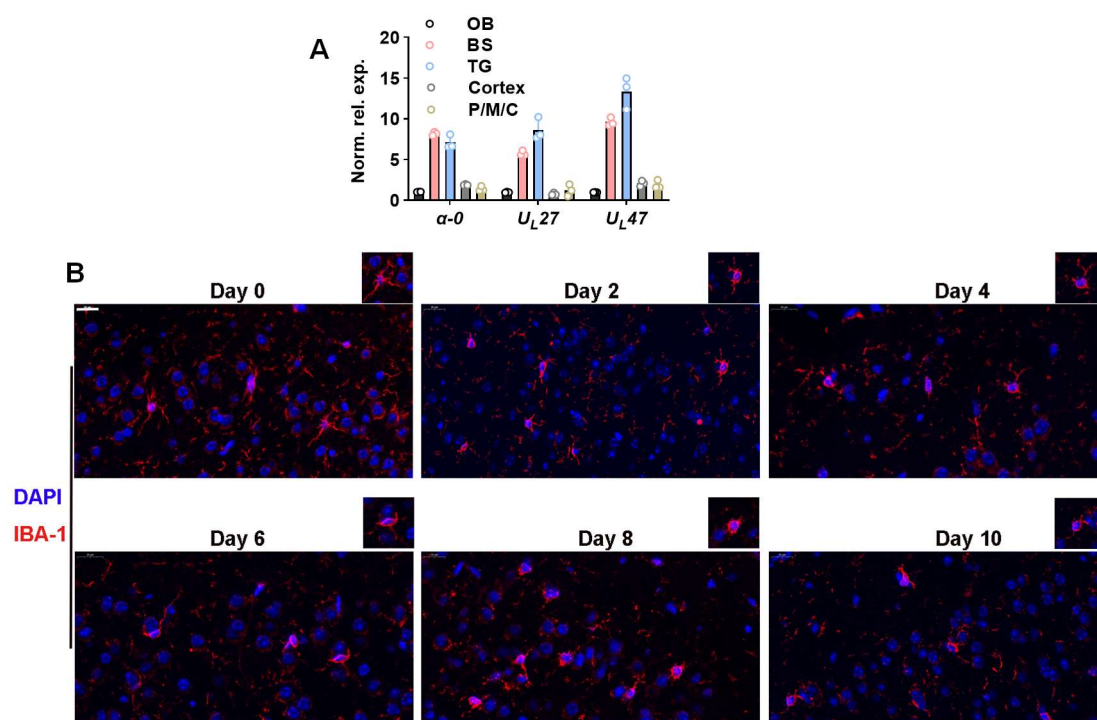


Figure S17. A, Determination of viral DNA copies in homogenized tissues of several brain sections from WT mice at 9 days after intranasal inoculation with HSV-1 (1×10^7 PFU/mouse) using qPCR, $n = 3$ mice, each symbol represents an individual technical replicate. **B**, Tissue sections of BS from mice infected intranasally with HSV-1 (1×10^7 PFU/mouse) for the indicated days were stained with an antibody against IBA-1, $n = 3$ mice per group. Scale bars represent 20 μ m. Data are representative of three independent experiments with $n = 3$ technical replicates (A), three independent experiments (B) (shown as mean and s.d. in A).

Supplemental tables

Table S1. Top15 DELs ranked by basic FPKM and FPKM_{HSV-1}/FPKM_{Cell}.

Top15 DELs ranked by FPKM _{HSV-1} /FPKM _{Cells}		Top15 DELs ranked by basic FPKM	
Transcript ID	Log2(fold change)	Transcript ID	Cell FPKM
LNC_000361	8.842804802	LNC_003485	1.549412
LNC_004084	8.03634865	ENSMUST00000150145.1	0.896818
LNC_003732	7.992301174	ENSMUST00000231483	0.816353
LNC_004073	7.426886355	LNC_005428	0.291923
LNC_004433	7.330950785	LNC_003311	0.145579
LNC_000350	7.272670066	LNC_002885	0.144511
LNC_001407	7.245165248	ENSMUST00000180868.1	0.129002
LNC_005318	7.206946765	LNC_001159	0.125979

linc-AhRA	6.945560733	LNC_005733	0.099695
LNC_000602	6.812333042	LNC_002954	0.091674
ENSMUST00000160698.1	6.781017341	LNC_000350	0.07221
LNC_002045	6.707534069	linc-AhRA	0.065611
LNC_003091	6.637491442	LNC_002045	0.064985
LNC_002206	6.447464883	LNC_005885	0.05853
LNC_002885	6.404661921	LNC_005884	0.054507

Table S2. Full-length sequence of linc-AhRA as determined by RACE

ACACATTTTATTTGAAAGCATTGAATAAGTAGCTCAGGCTCTCGCTCAGACTTAAGG
GGCTGATGAACTACGCCCCGCCGCAACCAGCTTTCTCTGCACCCCAATCCAGCCCC
GCTCCAGACGACGGGCTTAGATTCTGCTATCACGTAGGCAGTGAGCCTGGGGAAGA
TGCGATGCCTCCCTTCAAACCATCAAGACAGATATCCGCTGGTTTTAGGGTGTCCCC
CAAGGCAAGCATCTGGGACGCCTCCGGCATATGCTCGCCCTCGGGGCCCGACCAGG
AGGCTCCAGCTTTGCAAGGCGCCACCGCCCAGAGCTCAGGTCTGGGTGGAAGGGT
GGGCGCACCGCCTCAGGGTACGTTCTCCCGACTTCGGAGAGAAAGAAAGGAAGA
AATGCACCCTAGGAAAAGGGGCTTCGACTTTTTGTGCCTGTGCTTTCATGGAAGTCA
CCAGCTCTCGGCTGCTTAAGATGCTACTCAGAAGCGCGCCCAGCCAGGCTGTGGCC
ATCCGCGCCAAGTCCTCGTGTCTGGTTCGACGAGCACTTTGGGTAGCACCGCGACA
CACAATAGCCACCACGGTTAGGAGATATGCTCGCTATGCTTACCTGTTGGCAAAAATA
AAATGCAAAAATAAAAATTTAAATTTGGAACTAACACATGGTGACATCTTCTAGAAC
CACA

Table S3. Potential transcription factors (TFs) regulating the expression of linc-AhRA

Name	Score	Start	End	Sequence	Mutant
Ahr::Arnt	8.06995	314	319	agcgtg	atcata
Ahr::Arnt	8.06995	1494	1499	agcgtg	atcata
Arid3b	7.60394	496	506	cttttaatggt	
Ar	7.14583	809	825	ccgaacctcgtgttcct	
Arid3a	6.85157	708	713	ataaaa	
Arid3a	6.85157	713	718	ataaaa	
Arid3b	6.15987	201	211	gttttattcta	
Ahr::Arnt	6.15805	451	456	tgtgtg	tatatac
Arid3a	6.02799	421	426	atgaaa	
Arid3b	6.01764	776	786	gctttaatctg	

Table S4. Full-length sequence of BASP-AS1 as determined by RACE

CTGCTCCCTCCCTCCTCCGGCGCAGACCCTCCCTCTCCCTCCAGCCTGGACAC
GCCCGCTCCCTTGACTCCCCCAGCTCTGGGCCCCACCTCCCTCCCTCCAGC
ACAGTCACCCCATTTCTCTCCTATCCGCCATCCTGGTTCCTCCCTTCCCCCACCTC
CCAACTCTGTGCCCGCCAACGTTTCCTAAATGCCCTCTATTCAGATCCCCCTCCGC
CTCCCTCTCCTCTCCTCCATTCTGCGTCCCCCTTCCCCGCCGCGCCTGGGCT
GTCCGTGGACTTCTCCCACTCTCTCACTCTCTCACTCACTCTCTCTCTCTCTCT
CTCTCACTCTCTCTCTCTCTCTCCCCCTATTTATTTGGAACCGTTGGATAAGAAGTG
CTCGGGCTCTCGCTCAGACTTAGGGAGCTGCCTCGAGGTGATGAATGACACCCCT
GGCACCAGCTACCCTTCTCAGACCCAGTCCAGCCCGCTCCCGACGTCGACTACGAT
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GGCTGCGCCGCTGCAAACGAGTTGCGCACCTTGGGCGGCTCCGCACCTGCACCCGC
ACCCGCGGGGCTCAGCCCCGAAGGCTGCAGCTTCGGGGGAGGCGCGGTCGCCGAG
GTCCAGCTGGTGGGGCGAGAGACGTCGCCCTCGGAGGATGCTCTCGGAACTTGGG
AGAGGAAGGAGGAAGAGAAGAGGGGAAAGGGGCCGTCGATGTTTTTGATGTCTG
TGCTTTAATGGAGGCCACCAATATTGAGAAGACGGGGTTGGCCGAGGCAGCCCGCA
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TGCCCTAAGGGGGCGGACACTTGGTTTAGCACCGGGACACAGAATAGCCACCGGGG
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GCCTGCCTCCCTGTTACCTTCCACCATGATTGTAAGCTTCCTGAGGCCTCCCAGCCA
TGCCTGCTGTATAGCTGCAGAACAGTTTCAGTCGATTTGATTATAAAGCAAAGGTTT
GGGAAGAAAGACTCATATTCCTCCTGAGATGCAACCTCAAGTCTTAGGAAAAGAAA
GTTTTGAACTCAGGTTCCGGGAATGTGGAAGAGGAAGCTCTTAAAGGGCAAGTAGA
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TCCCCTAACTCCACCTAAGCCACTCCATAAAGTTAGGTCCCATTCTCTATTTCCTTCA
CTACTAATGACAGCTAATAGAGCTAAACAGAATACCACAATAAGCCAGAAGCTGCCT
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GTTTGGCAAAGCCTGTGCAAATGAAACCAAGTACCTATTTTTTGGCTCATCATAAAC
CAAAAGTTTTCTAGAGAACTAACTGAAAGAGATTTTCACCAAATCTTTTTATTTTTT
AATCTAGAGAATAAATTGAGAACCAAATCAATAAATATCCTCAACTGTTACCTTTGT
TATAAGGGAACCTACGATGAACCGTGCTTGCCCCACATTTACCCTAGCAGCAACTATG
CTTTTTCTATCTCTGGCCTTACCCTGCCTTCTGCCTCCAGAGTCTGAGATGGAGAAA
GGCAAAGTCAGATGGAGGATAGAGCTGGGCAGGGAGTTGCTGCCAGCAACAATTG
GAGTTGCTGGTTTGTCTTCAACACTGACCCCACTTTATTGGCCATGAGTTAAGGCAG
TCAAATGGGCATCTAGGAAACATGACCAAGATCTGCATTAGGGAAGCAAAGCAGAT
TAAAAGGCACAATTGCTGGCCAGGCATGGTGGCTCACACCTCTAATCCTAGTGAGAG
GTGAAGCCAGTTGGACTTCTGGGTCGAGTGGGGTGGGGTCTTGAGAAATTTTCT
ATCTAGCTAGAGGATTATAAATGCACCAATCAGCTCTGTGTCTAGCTAAAGTTTGTA
AACGCACCAATCAGCACTCTGTAAAACGCACCAATCAATGCTCTGTGTCTAGCTGA
AGTTTTGTAAATGCACCAATCAGCACTCCGTAAAACGGACTGATCAGTGCTTTGTAA
AATGGACCAATCAGCAGGATGTAGGCAGGGCCAAATAAGGGAAAAAAAGCTGGCA
CCCAAGCCAACAGGGGCAACCTGCTTGAGTCCCTTTCCACATTGTGGAAGCTTTGTT
CCTTCACTCTTCATGATAAATCTTGCTGCTGCTCACTCTTTGGGTCCGCACTACCTTT
ATGAGCTGTAACACTCACCACGAGGGTCTGTGGCTTCATTCTTAAGTTAGCAAGAC
CACGAACCCACAGGGAGGAACAAACAACCTCCGGGCACACCACCTTTAAGAGCTATA
ACACTCACTGCGAAGGTCTGTGGCTTCACTCCTGTAGTCAGCAAACCACAAACCC
ACCGGAAGGAAGAACTCCAGACACATCTAAACATCTGAAGGAACAAACTCTGGA
CACACCATCTTTAAGAAGTGTAACTCACCGTGAGGGTCCGTGGCTTCACTTTGA
AGTCAGGGAGACCAAGAACCACCGGAAGGAACAAACTCCGGACACACTAGCACT
TACAGGAGGCCAGGTGGGAAGATCACTTGAGGCCAGGAGTTTGAGACCAGCCTG
GGCAACGTAGTCAGACCCCATCTTACAAAAAATTTAAAAAATTAGCCGAGCATGG
TGGCAAGTGCCAGTAATCCCAGCTACTCTTGAGGGCTGAGGCAGGAAGAGCCCTTG
AGCCCAGGAGCTGGAGGCTGCAGTGAACCTATGATCAAACCACTGTAGTCCAGCCT
GAGTGACAGAGTGAGACCCTGCCTCTAAAAAATAAAAAA

Table S5. The interaction propensity between linc-AhRA and TBK1

#	Protein region	RNA region	Interaction Propensity	Discriminative Power	Normalized Score
1	54-105	301-352	13.92	37	2.92
2	476-527	301-352	13.81	37	2.89
3	651-702	301-352	13.22	37	2.76
4	54-105	307-358	12.55	35	2.6
5	476-527	307-358	12.43	35	2.58
6	654-705	301-352	12.36	35	2.56
7	604-655	301-352	12.04	35	2.49
8	154-205	301-352	11.93	33	2.46
9	651-702	307-358	11.67	33	2.41

10326-377	301-352	11.25	33	2.31
11301-352	301-352	11.03	33	2.26
12401-452	301-352	11.02	33	2.26
13654-705	307-358	10.81	32	2.21
14651-702	326-377	10.81	32	2.21
15554-605	301-352	10.68	32	2.18
16604-655	307-358	10.65	32	2.17
17154-205	307-358	10.63	32	2.17
18576-627	301-352	10.59	32	2.16
19104-155	301-352	10.56	32	2.15
2051-102	301-352	10.45	32	2.13

Table S6. The interaction propensity between linc-AhRA and TRIM27

#	Protein region	RNA region	Interaction Propensity	Discriminative Power	Normalized Score
1	188-239	301-352	19.28	52	4.03
2	188-239	307-358	17.46	47	3.63
3	188-239	326-377	15.52	42	3.21
4	438-489	301-352	14.3	40	2.94
5	188-239	226-277	13.9	37	2.85
6	126-177	301-352	13.55	37	2.77
7	188-239	282-333	13.39	37	2.74
8	438-489	326-377	13.14	37	2.68
9	188-239	232-283	13.01	37	2.65
10	438-489	307-358	12.94	35	2.64
11	88-139	301-352	12.92	35	2.63
12	188-239	251-302	12.75	35	2.59
13	288-339	301-352	12.66	35	2.58
14	188-239	207-258	12.64	35	2.57
15	326-377	301-352	12.58	35	2.56
16	226-277	301-352	12.18	35	2.47
17	126-177	307-358	12.1	35	2.45
18	88-139	307-358	11.57	33	2.33
19	288-339	307-358	11.37	33	2.29
20	326-377	307-358	11.33	33	2.28

Table S7

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Cell lines (All cell lines were negative for mycoplasma contamination)		
BV2	National Infrastructure of Cell Line Resource	3111C0001CCC000063

HMC3		American Type Culture Collection	CRL-3304
L929		American Type Culture Collection	N/A
HEK 293T		American Type Culture Collection	N/A
Neuro-2a		American Type Culture Collection	CCL-131
Vero		American Type Culture Collection	CCL81
Virus Strains			
HSV-1 F strain		Prior study (1)	N/A
HSV-1 EGFP reporter strain		Prior study (1)	N/A
Influenza A virus PR8(strain A/Puerto Rico/8/1934 H1N1)		Prior study (2)	N/A
CVB3		Prior study (3)	N/A
Antibodies			
cGAS (For Human)		Cell Signaling Technology	Cat#15102S
cGAS (Mouse specific)		Cell Signaling Technology	Cat#31659S
STING		Cell Signaling Technology	Cat#13647S
TBK1		Cell Signaling Technology	Cat# 3504S
Phospho-TBK1 (Ser172)		Cell Signaling Technology	Cat# 5483S
IRF3		Cell Signaling Technology	Cat#4302S
Phospho-IRF3(Ser396)		Cell Signaling Technology	Cat#4947S
MAVS		Santa Cruz	Cat#SC-166583
RIG-1		Cell Signaling Technology	Cat#4200S
DDX19A		Sigma-Aldrich	Cat#HPA045252
DTX4		Proteintech	Cat#25222-1-AP
TRAIP		Proteintech	Cat#10332-1-AP
STAT1		Santa Cruz	Cat#sc-464
Phospho-STAT1		Santa Cruz	Cat#sc-8394
Phospho-JAK1		Cell Signaling Technology	Cat#3331S
GAPDH		Cell Signaling Technology	Cat #2118S
AhR		GeneTex	Cat#GTX22770
TRIM27		Proteintech	Cat#12205-1-AP
Ubiquitin		Cell Signaling Technology	Cat#3936
NeuN		Proteintech	Cat#26975-1-AP
GFAP		Proteintech	Cat#16825-1-AP
FLAG		Beyotime	Cat # AF5051
HA		Cell Signaling Technology	Cat #3724
IBA1		Proteintech	Cat #10904-1-AP
Alexa Fluor Antibodies	Secondary	Thermo Scientific	Cat #A11001; A21206; A21203; A21442; Z25308
APC	anti-mouse	BioLegend	Cat #101211
CD11b(M1/70)			
APC/Cyanine7	anti-mouse	BioLegend	Cat #103116
CD45			
Mouse	IgG	Cell Signaling Technology	Cat #3420S

(Sepharose® Bead Conjugate)			
Rabbit	IgG	Cell Signaling Technology	Cat #3423S
(Sepharose® Bead Conjugate)			
Mouse HRP-conjugated		Cell Signaling Technology	Cat #7076S
Rabbit HRP-conjugated		Cell Signaling Technology	Cat #7074S
Chemicals and Recombinant proteins			
5'ppp-dsRNA		InvivoGen	Cat #ttrl-3prna
3'3'-cGAMP		InvivoGen	Cat #ttrl-nacga
2'3'-c-di-AM(PS)2 (Rp,Rp)		InvivoGen	Cat #ttrl-nacda2r-01
Indirubin		TargetMol	Cat #T6169
Darolutamide (ODM-201)		TargetMol	Cat #T6915
CH 223191		TargetMol	Cat #T2448
MG132		TargetMol	Cat #T2154
Z-VAD-FMK		Beyotime	Cat #C1202
Cycloheximide		4A Biotech	Cat #FXP130
L-Kynurenine		Selleck	Cat #s5839
β-mercaptoethanol		Macklin	Cat #M6230
Tert-Pentyl Alcohol		Macklin	Cat #A800282
Mouse IFNβ		R&D Systems	Cat #8234-MB-010
3-MA		Sigma-Aldrich	Cat #M9281
CT-DNA		Solarbio	Cat #D8020
Poly(I:C)		Sigma-Aldrich	Cat #P1530-
Restricted enzymes		Takara	N/A
Tribromoethanol		Meryer Chemical Technology Company	Cat #M33110
Tamoxifen		MedChemExpress	Cat #HY-13757A
Corn oil		YuanyeBio	Cat #S50856
DMSO		Sigma-Aldrich	Cat #D2650
Main kits			
EndoFree Maxi Plasmid Kit		TIANGEN	Cat #DP120
Mouse IFN-β ELISA Kit		4A Biotech	Cat #CME0116
RNAprep Pure Micro Kit		TIANGEN	Cat #DP420
RIP Kit		Millipore	Cat #17-701
RNA pull-down Kit		Pierce	Cat #20164
Silver stain Kit		Beyotime	Cat #P0017S
SMARTer RACE 5'/3'kit		Takara	Cat #634858
CUT & Tag kit		Novoprotein	Cat #N259-YH0
EasyPure® Viral DNA/RNA Kit		TransGen Biotech	Cat #ER201
BCA Protein Assay Ki		Beyotime	Cat #P0011
Mouse Tail Direct PCR Kit-UNG		FORGENE	Cat #TP-01341

Software		
Prism version 8.0	GraphPad Software	N/A
Coding Potential Calculator	Peking University	http://cpc.cbi.pku.edu.cn/programs/run_cpc.jsp
Multiple Alignments of 60 vertebrates	UCSC Genome Browser	http://genome.ucsc.edu/
Adobe illustrator CC 2015	Adobe Systems Incorporated	N/A
FlowJo™	Becton, Dickinson and Company	N/A
Image J	National Institutes of Health	https://imagej.nih.gov/ij/
catRAPID	RNA Systems Biology - Italian Institute of Technology (IIT)	http://service.tartaglialab.com/page/catrapid_group
UbiBrowser	Beijing Institute of Radiation Medicine	http://ubibrowser.ncpsb.org/ubibrowser/
RNAfold web server	University of Basel	http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi
Illustrator for Biological Sequences	Sun Yat-sen University	http://ibs.biocuckoo.org/
ZEN 2 (blue editon) for Microscope	ZEISS	N/A
NIS viewer (version 4.5) for Microscope	Nikon	N/A

Table S8. Primers for qPCR analysis in this study

Genes	Forward (5'-3')	Reverse (5'-3')
<i>mIfnb1</i>	ATGAGTGGTGGTTGCAGGC	TGACCTTTCAAATGCAGTAGATTCA
<i>mTrim27</i>	CTCACGCTACCTTCTGTGGG	CCCAACATGGCCAGAAAACC
<i>mCxcl10</i>	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTCAA
<i>mIfna4</i>	TGATGAGCTACTACTGGTCAGC	GATCTCTTAGCACAAAGGATGGC
<i>mIsg15</i>	GATTGCCCAGAAGATTGGTG	TCTGCGTCAGAAAGACCTCA
<i>linc-AhRA</i>	TATCACGTAGGCAGTGAGCC	GGGGGACACCCTAAAACCAG
<i>mIl-6</i>	CACAGAGGATACTCCCAACA	TCCACGATTTCAGAGAACA
<i>mTnf</i>	CATCTTCTCAAATTCGAGTGACA A	CCAGCTGCTCCTCCACTTG
<i>mMx2</i>	GAGGCTCTTCAGAATGAGCAA	CTCTGCGGTCAGTCTCTCT
<i>mGapdh</i>	TGTGTCCGTCGTGGATCTGA	CCTGCTTACCACCTTCTTGA
<i>mCgas</i>	CTGCGCAGAATGCAGAAACG	CTTGTAAGCTCAATCCTGGGGA
<i>mSting</i>	GCTGCTGATGCCATACTCCA	TGGATCCTTTGCCACCCAAA
<i>mTbk1</i>	ATCAAGAAGGCACGCATCCA	GGCTCATTGCTTTTGTGGCA
<i>mIrf3</i>	AGGCTTGTGATGGTCAAGGT	AATAACCACCAGCCTAGACGC
<i>mU6</i>	GTGCTCGCTTCGGCAGCATAT	AAAATATGGAACGCTTCACGAA

<i>mI8S</i>	CAGCCACCCGAGATTGAGCA	TAGTAGCGACGGGCGGGTGT
<i>mNeat1</i>	TTGGGACAGTGGACGTGTGG	TCAAGTGCCAGCAGACAGCA
<i>mBasp1</i>	GAGAGAGAGAGCCTTTGCTGAG	CTTGCCTCCCATCTTGAGTT
<i>mCyp1b1</i>	ACATCCCCAAGAATACGGTC	TAGACAGTTCCTCACCGATG
<i>mTiparp</i>	GCCAGACTGTGTAGTACAGCC	GGGTTCCAGTTCCCAATCTTTT
<i>hGAPDH</i>	CACCATCTTCCAGGAGCGAG	AGAGGGGGCAGAGATGATGA
<i>hBasp1</i>	TTCAGACTCAAACCCGGCA	CCTTGGGTGTGGAAGTAGG
<i>hBasp-as1</i>	CGAGTCAAAGCTAGGGCCAA	GAACGCATCTTCTACCCCG
<i>hIfnb1</i>	TCTCCTGTTGTGCTTCTCCAC	GCCTCCATTCAATTGCCAC
<i>U_L47</i>	ACGATGATGATGAGGTTCCC	CAGCTCCTCTAGGAACAGCG
<i>α-0</i>	CCCCTATCAGGTACACCAGCTT	CTGCGCTGCGACACCTT
HSV-1-120	AGACGGTATATTTTTGCGTTATCA CTGTCCCCG	AAGTCCTCCAAAAAACCCGCCACAA ATAAAAAGG

Note: m-prefix means mouse, while h-prefix means human.

Table S9. Primers for constructing mammalian expression plasmids

Genes	Accession	Forward (5'-3')	Reverse (5'-3')
lincAhRA (antisense)	N/A	cttggtaccgagctcgatccTGTGG TTCTAGAAGATGTCACCAT G	tgctggatatctgcagaattcACACATTTTATTT GAAAGCATTGAATAA
m-cGAS(FL)	NM_17338 6.5	ggccatggaggcccgaattcGGATG GAAGATCCGCGTAGAAGG	gcgcccgcggtacctcgagaTCAAAGCTTGTC AAAAATTGGAAA
m-cGAS (C terminal)	XM_00651 1006.3	ggccatggaggcccgaattcGGATG GAAGATCCGCGTAGAAGG	gcgcccgcggtacctcgagaTCACAAGATAG AAAGCACCTGTTTC
m-STING	XM_01731 7994.1	ggccatggaggcccgaattcGGATG CCATACTCCAACCTGCATC	gcgcccgcggtacctcgagaTCAGATGAGGTC AGTGCGGAG
m-TBK1	NM_01978 6.4	ggccatggaggcccgaattcGGATG GCACCTCCAACCATC	cgcggtacctcgagaCTAAAGACAGTCCAC ATTGCGA
m-IRF3	NM_01684 9.4	ggccatggaggcccgaattcGGATG CCCCGAAACCGCGGA	cgcggtacctcgagaTCAGATATTTCCAGTG GCCTGG
m-IRF3 (D)	N/A	aaaaccgtggacttgcacatcgacAAC AGCCAGCCTATCTCCCTT	gtgcaagtccacggtttccaggtcAGAGGCTCCC CCTTCCCG
m-IκKα	NM_00770 0.2	ggccatggaggcccgaattcGGATG GGCCCCCGGGGCTGC	cgcggtacctcgagaTCATTCTGCTAACCA ACTCAA
m-AhR	NM_00131 4027.1	ggccatggaggcccgaattcGGATG CCATACTCCAACCTGCATC	gcgcccgcggtacctcgagaTCAGATGAGGTC AGTGCGGAG
mTRIM27	NM_00905	ttgcggccgcaattcatcgATGGCC	ctctagagtcgactgtaccTCACGGAGAGGT

4.3	TCCGGGAGCGTG	CTCCATGG
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Note: linc-AhRA(antisense) was cloned into pcDNA3.1(+) plasmids, TRIM27 was cloned into p3 ×FLAG-CMV-10 plasmids. Other plasmids were cloned into pCMV-HA plasmids.

Table S10. The sequence of siRNA used in this study

Genes	Sense(5'-3')	Antisense(5'-3')
Trim27-1	CCACCUAAGAAGAGUGAAAGA	UCUUUCACUCUUCUUAGGUGG
Trim27-2	GCUCAGUUAUACUCAGUUGAU	AUCAACUGAGUAUAACUGAGC
Arid3a-1	CGGGAGCAACAGUAUUAGCAU	AUGC UAAUACUGUUGCUC CCGG
Arid3a-2	UGAGGGAGAUAGGCAUUUGAU	AUCAA AUGCCUAUCUCCCUCA
Arid3b-1	CCACAGGGACAACAAACUAAA	UUUAGUUUGUUGUCCUGUGG
Arid3b-2	GCAUCAUAUUGUCUGUGGAUA	UAUCCACAGACAUAUUGAUGC
Ahr-1	UUUAUGCAUCGGCAACAAUUG	CAAUUGUUGCCGAUGCAUAAA
Ahr-2	GAGAUGCACAAGUACAGUUAU	AUAACUGUACUUGUGCAUCUC
Ar-1	UGGAUGGGACUGAUGGUUUU	AAAUACCAUCAGUCCCAUCCA
Ar-2	ACGAUUGUACCAUUGAUAAA	AUUUAUCA AUGGUACA AU CGU
N.C.	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Table S11. The primers for constructing plasmids used in dual luciferase assay

Plasmids backbone	Name	Forward (5'-3')	Reverse (5'-3')
pGL4.11-(luc2p)	<i>linc-AhRA promoter-luc.</i>	ggatcttccagagatctcgagATGATG	gccgttcgacgatagatctGCGAGACAG
		GGGAGATCAGATGGG	AGAGAGGAGAGAAAT
pGL4.11-(luc2p)	<i>mIrfb1 promoter-luc.</i>	CTGTCTCGAG	CTGTAGATCT
		TTTCTCTTATAGTACACT	GAGCTGCTTATAGTTGAT
pGL4.11-(luc2p)	<i>linc-AhRA promoter-luciferase (AhR BSs mutant)</i>	BS1-F: GCTCCTTTGAACatcataAAAATCACTGGGGCAGAGGC	
		BS1-R: tatgatGTTCAAAGGAGCACAGCGCA	
		BS2-F: CGCATtatatcCCATCCGATCCAGGGATTCT	
		BS2-R: CGGATGGgatataATGCGTGGCTGGGGACCG	
		BS3-F: CGGatcataGAGCGAGTTGGAGCGCTGC	
		BS3-R: AACTCGCTCtatgatCCGGGCTCCGCCGTCGAG	
Beyotime	ISRE-luc	Cat# D2179	

Table S12. Primers for RACE assay

Gene	5'RACE	3'RACE
linc-AhRA	GATTACGCCAAGCTTCCTTGGG GGACACCCTAAAACCAGCGGA TAT	GATTACGCCAAGCTTCCGCCTCAGGGTA CGTTCTCCCGACTT

BASP-AS1	GATTACGCCAAGCTTgggtgcggtg gcaggtgcgagc	GATTACGCCAAGCTTgcggtccgcacctgcacc gcacc
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Table S13. Primers for constructing plasmids for tRSA RNA pull-down assay

Name	Forward (5'-3')	Reverse (5'-3')
tRSA-linc-AhRA	aaaaaaagaattcggatccACACATTTTA TTTGAAAGCATTGAATAA	gtggatatctgcagaattcTGTGGTTCTAGAAGAT GTCACCATG
Mut 1	ttgtaccgagctcggatccACACATTTTAT TTGAAAGCATTGAATAA	gtggatatctgcagaattcCCCAGATGCTTGCCTT GGG
Mut 2	ttgtaccgagctcggatccACGCCTCCGG CATATGCT	gtggatatctgcagaattcTGTGGTTCTAGAAGAT GTCACCATG
Mut 3	ttgtaccgagctcggatccACACATTTTAT TTGAAAGCATTGAATAA	gtggatatctgcagaattcGTACCCTGAGGCGGTG CC
Mut 4	ttgtaccgagctcggatccGTTCTCCCGA CTTCGGAGAGA	gtggatatctgcagaattcTGTGGTTCTAGAAGAT GTCACCATG
Δ117nt	ccccaaggaagcatctgggGTTCTCCCG ACTTCGGAGAGA	gtggatatctgcagaattcTGTGGTTCTAGAAGAT GTCACCATG
117nt	ccaagctggctagtaagcttACGCCTCCG GCATATGCT	tgctggatatctgcagaattcGTACCCTGAGGCGGT GCC
pcDNA3.1 (+)-tRSA	Synthesized from Tsingke (Beijing, China)	

Table S14. Primers for CUT-&Tag qPCR analysis

Genes	Forward (5'-3')	Reverse (5'-3')
CUT&Tag-set 1	CAAAATCCTGAAATCGCACCCAT GT	GTTTCCGCCTTGGAGCGAACTCGGT
CUT&Tag-set 2	AAGCGTTCGATGAATATTCATGAA AAGAATGCGGT	AACCAGAATCCCTGGATCGGATGGC

Table S15. Targeted sites of LNA against linc-AhRA

#	Cat.no.	Targeted sites
LNA-N.C.339515LG0000001-DDA		G*C*T*C*C*C*T*T*C*A*A*T*C*C*A*A
LNA-466	339511LG00240466-DDA	A*G*A*A*A*G*C*T*G*G*T*T*G*C*G*G
LNA-467	339511LG00240467-DDA	A*T*C*A*G*C*C*C*C*T*T*A*A*G*T*C

Table S16. Primers for linc-AhRA genotyping of mice

Primer	Sequence (5'→3')	Primer type
P1	TCAGATTCTTTTATAGGGGACACA	Forward
P2	TAAAGGCCACTCAATGCTCACTAA	Reverse
P3	CTCGGCTGCTTAAGATGCTACTC	Forward
P4	GACGATGATTTCCCCGACAAC	Reverse

Table S17. Primers for Cre genotyping of mice

Primer	Sequence (5'→3')	Primer type
P1	AAGACTCACGTGGACCTGCT	Common Forward
P2	CGGTTATTCAACTTGCACCA	Mutant Reverse
P3	AGG ATGTTGACTTCCGAGTTG	Wild type Reverse

Reference

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2. Ge H, Liu G, Xiang YF, Wang Y, Guo CW, Chen NH, Zhang YJ, Wang YF, Kitazato K, Xu J. 2014. The mechanism of poly-galloyl-glucoses preventing Influenza A virus entry into host cells. *PLoS One* **9**:e94392.
3. Wang X, Wang Y, Ren Z, Qian C, Li Y, Wang Q, Zhang Y, Zheng L, Jiang J, Yang C, Wang D, Zhang Y, Fan J, Wang Y. 2012. Protective effects of 20(s)-protopanaxtriol on viral myocarditis infected by coxsackievirus B3. *Pathobiology* **79**:285-289.