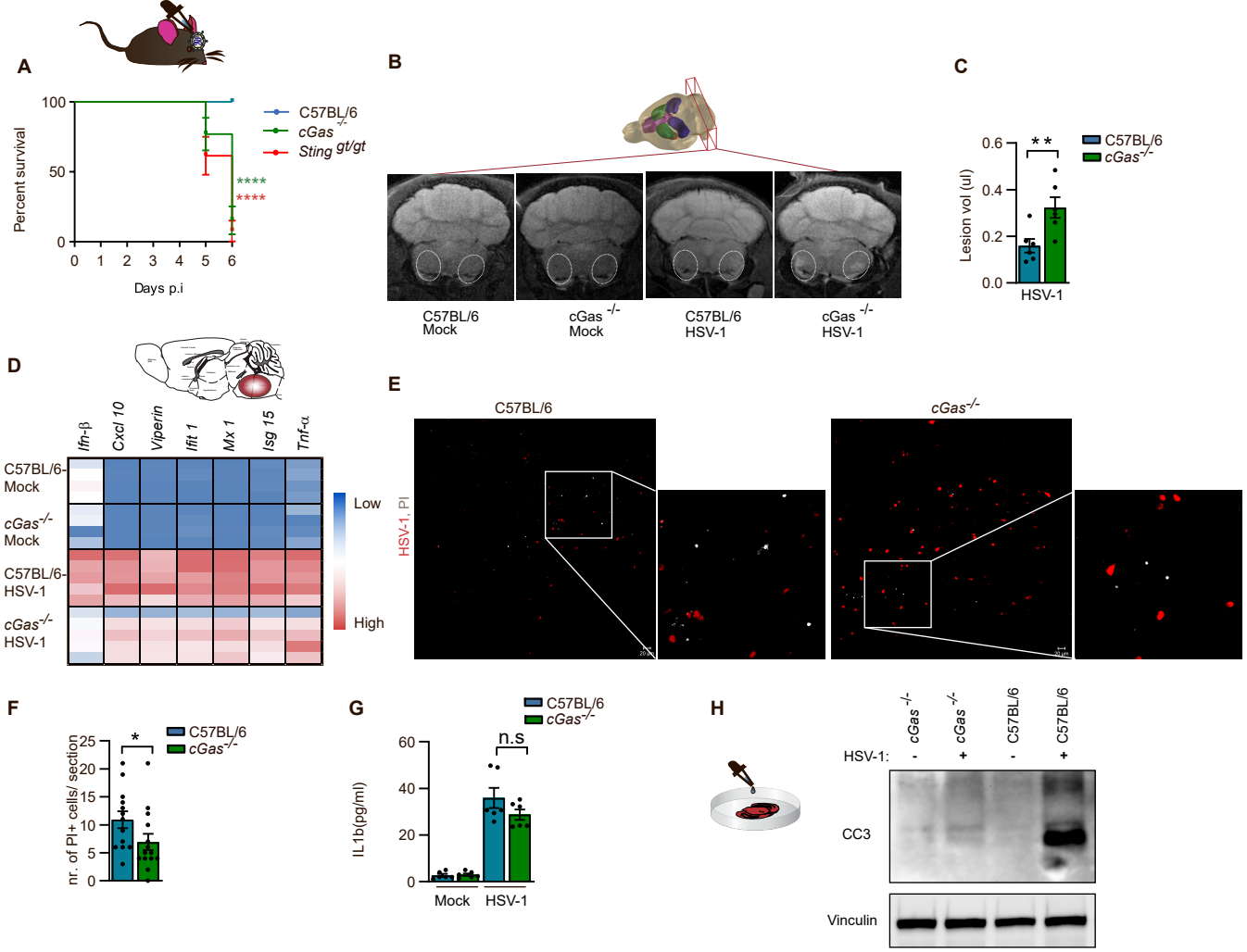
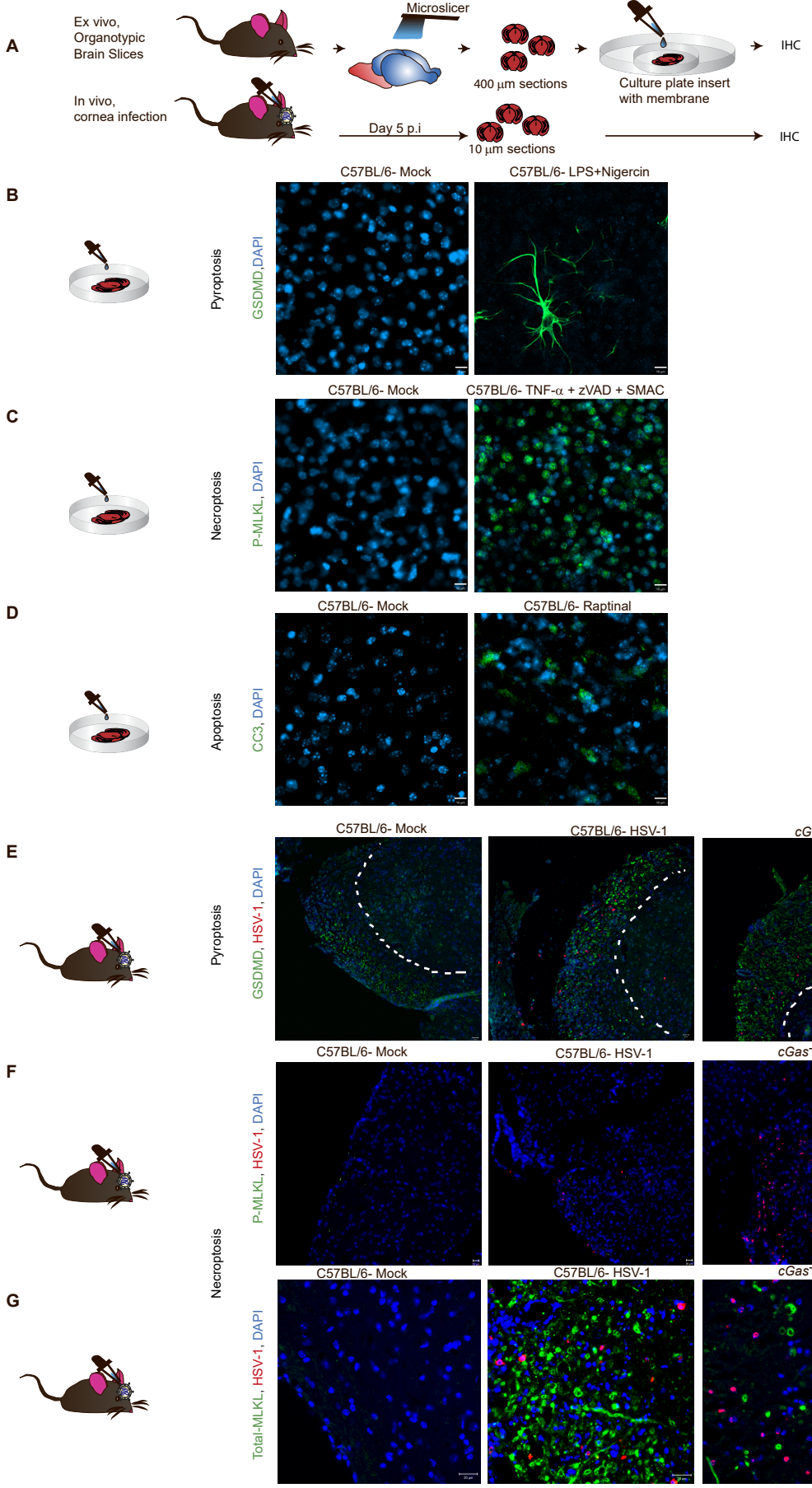
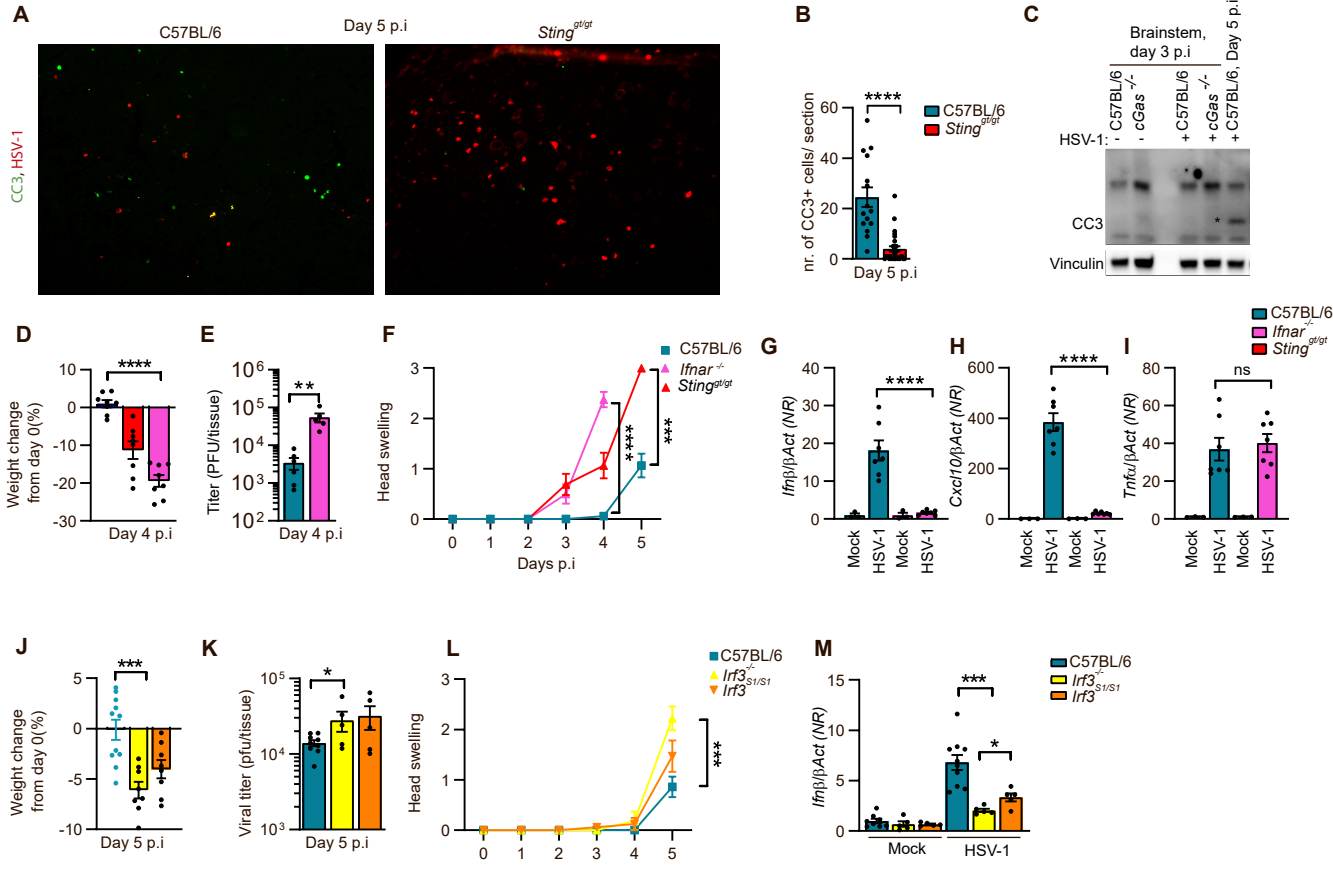


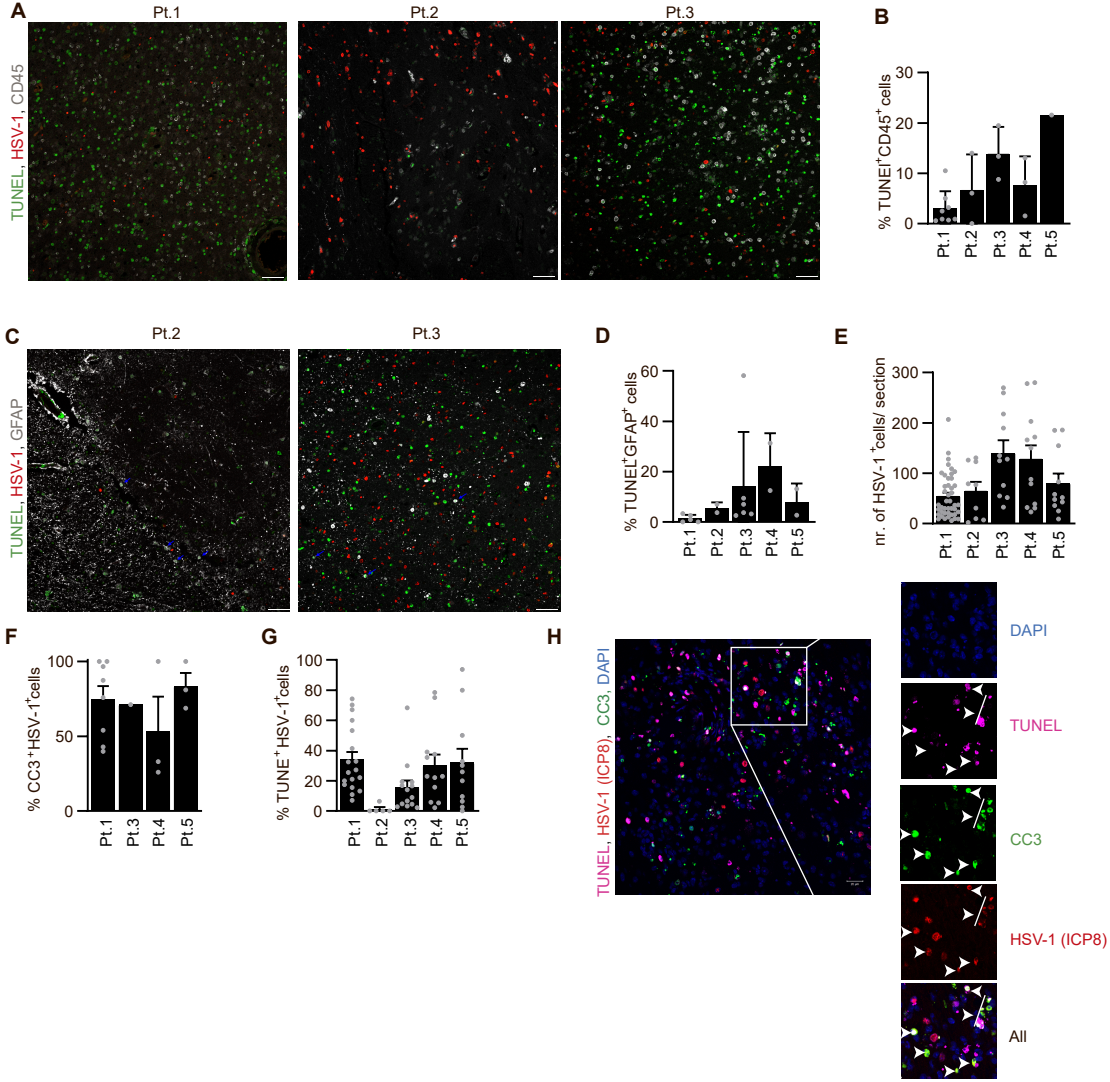
Supplemental Figure 1. cGAS or STING deficient mice are susceptible to HSE and exhibit impaired antiviral responses. Mice were infected in the cornea with 2x10^6 PFU/eye of HSV-1. **(A)** Survival of infected mice was monitored for 6 days p.i. (n=6 mice/group, ± SE), and represents at least three replicates of the experiment. **(B)** Animals were scanned using a 9.4 T preclinical MRI system. Representative MRI scans of the brainstem at day 5 p.i are shown **(C)** 3D Quantification of the edema in the affected region within the brainstem expressed as mean of volume ± SEM, n=5 mice/group, and represents at least two replicates. **(D)** IFN/ISG mRNA expression profile from brainstem of C57BL/6 (wild-type, wt) and *cGas*$^{-/-}$ mice infected for 5 days with HSV-1. mRNA expressions were normalized to *β-Actin*, each row represents mRNA from one mouse, n=4-5 mice/group. Blue and red color indicates low and high expression, respectively. **(E)** IHC of brainstem of HSV-1-infected mice (2x10^6 PFU/eye) for 5 days p.i. The sections are stained with antibody against HSV-1 (VP-5) shown in red and propidium iodide (PI) is visualized in white, scale bar is 20 μm. Areas marked by squares are magnified in the images to the right of the large images. **(F)** Quantification of PI-positive cells/sections presented as means ± SEM. Images per group (n=13-14), and animals per group (n=6-7). **(G)** Brainstem of HSV-1-infected mice (2x10^6 PFU/eye) for 5 days p.i. were homogenized and levels of interleukin-1b (IL1b) was analyzed by Elisa and presented as means ± SEM, n=6 per group and represents at least three independent experiments. **(H)** Organotypic brain slices from wt and cGAS-/- mice were cultured and infected with 1x10^4 PFU of HSV-1 for 20 h and were subject for immunobloting with CC3 and vinculin. The presented data are representative for at least three independent experiments and p-values were calculated using Log-rank (Mantel-Cox) test (A) or Wilcoxon rank-sum test (C, F-G) and assigned: p>0.05 (ns, not significant), *0.01<p<0.05, **0.001<p<0.01, ***0.0001<p<0.001 and ****p<0.0001.



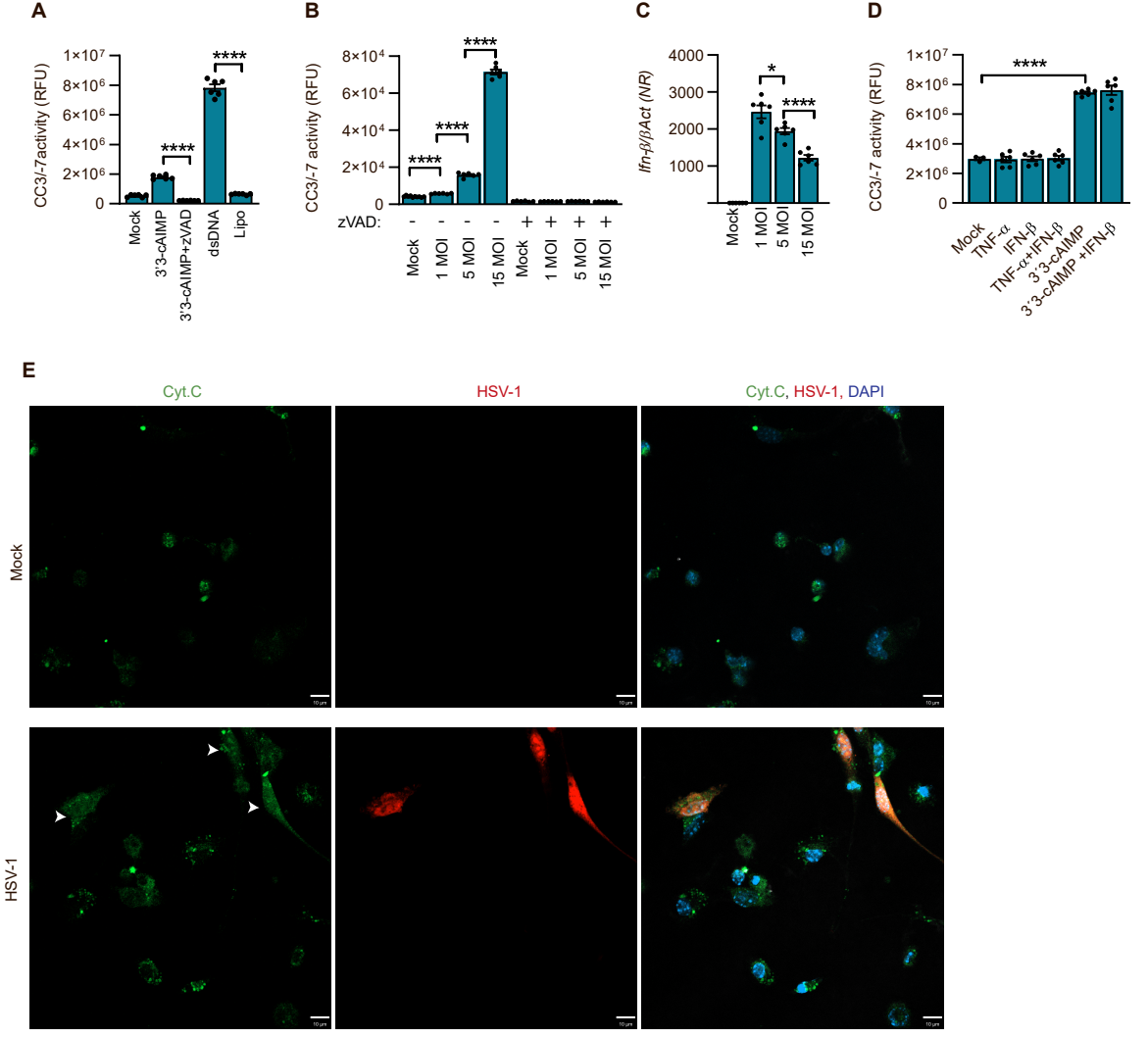
Supplemental Figure 2. Mouse brain cells do not undergo necroptosis or pyroptosis upon HSV-1 infection. (A) Overview of ex vivo and in vivo experiments. Organotypic brain slices from wt mice were sectioned and cultured before stimulation. Brainstem from mice infected with HSV-1 via the corneal route ($2x10^6$ PFU/cornea of HSV-1), were isolated 5 days p.i, fixed and sectioned before IHC staining. **(B-D)** Organotypic brain slices were treated with vehicle or the following inducers of specific PCD types **(B)** pyroptosis (LPS and Nigercin), **(C)** necroptosis (TNF-α and z-VAD), or **(D)** apoptosis (Raptinal) **(E-G)**. Tissue sections of brainstems from HSV-1-infected mice processed as above **(A)** were stained with antibody against either **(E-G)** VP-5 of HSV-1, **(B, E)** GSDMD (monoclonal) antibody, **(C, F)** P-MLKL, **(G)** total MLKL, or **(D)** cleaved caspase 3 (CC3). All PCD-related stainings are shown in green and HSV-1 (VP-5) is shown in red. Unspecific staining by GSDMD antibody of the gray matter of the brain, is located on the left side of white dotted line. All data presented originate from experiments with at least three biological replicates, n=3 mice per group, representative images are shown with scale bar of 10 µm (A-D) or 20 µm (E-G).



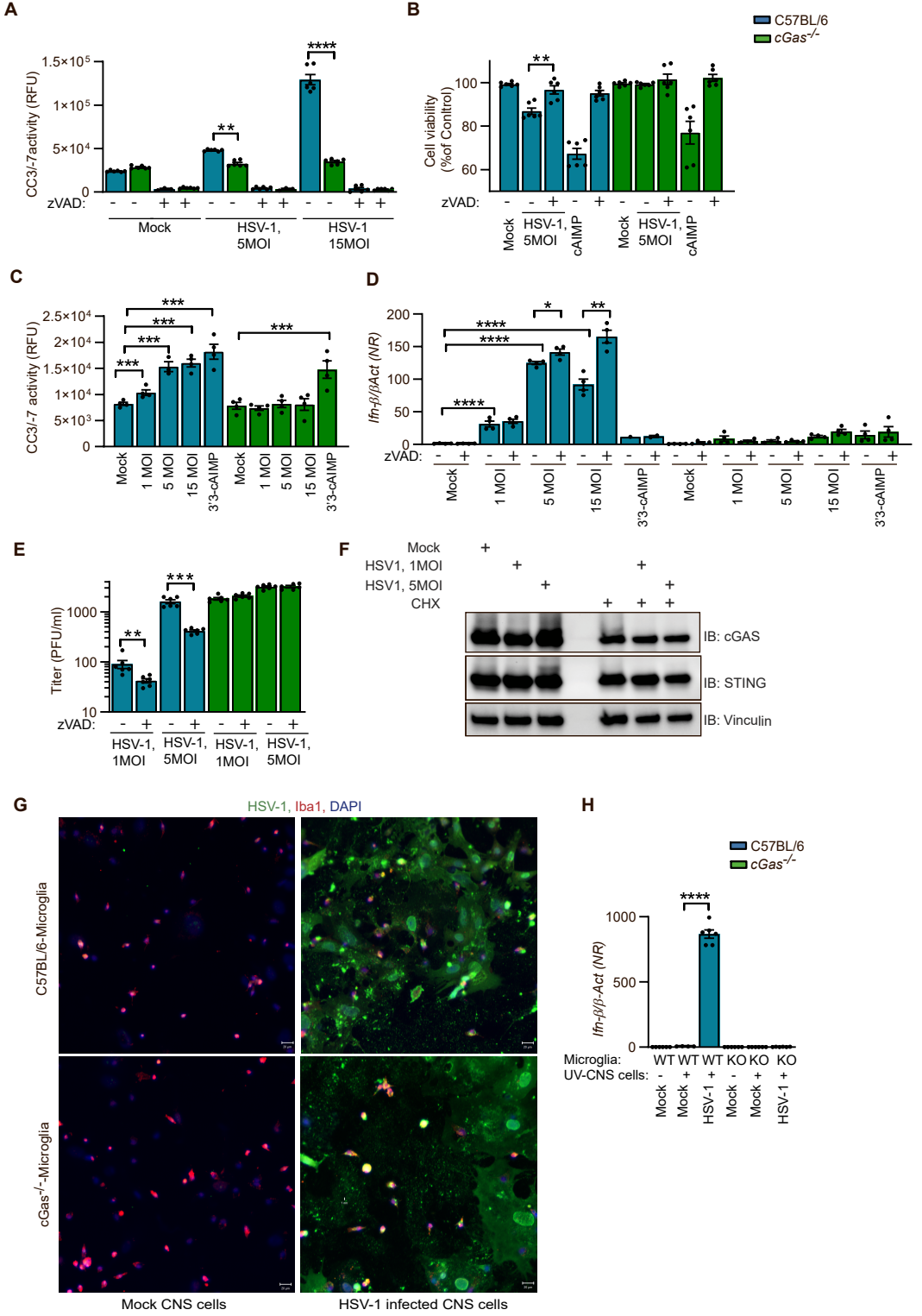
Supplemental Figure 3. STING, IFNAR and IRF3 are important for IFN-I-mediated antiviral response. The wt and indicated knock-out mice were infected with $2x10^6$ PFU/cornea of HSV-1 and the brainstems isolated after either day 5 p.i **(A, B, J-M)**, day 3 p.i **(C)**, or day 4 p.i. (D-I). **(A-B)** IHC of brainstem sections stained with antibodies against HSV-1 (VP-5) (red) and cleaved caspase 3 (CC3) (green) and representative images are shown in (A), the original magnifications are 20x. $CC3^{pos}$ cells and HSV-1-infected cells per section were quantified and presented as means ± SEM. n=15-35 images per group, and 5-7 animals per group. **(C)** Immunoblotting was use for detection of cleaved caspase 1 (P20)* in brainstem lysates isolated at day 3 p.i or day 5 p.i. **(D, F, J, L)** Weight changes and head swelling of wt , *Ifnar* $^{-/-}$, *Sting* $^{-/-}$, *Irf3* $^{-/-}$, *Irf3* $^{s1/s1}$ mice were compared to day 0. **(E, G-L, K, M)** Brainstems from infected animals were homogenized at day 5 p.i., and viral titer or the expression of *Ifnb, Cxcl10* and *Tnfa* determined by plaque assay and RT-qPCR, respectively. Values were normalized to β-*Actin* transcript levels and presented as normalized ratio compared to wt mock ($2^{-\Delta\Delta CT}$means ± SEM) (n=4-9 mice per group). This figure represents at least three independent experiments and p values were calculated by two-tailed Student's t-test (B, D, E, G-K), one-way ANOVA with Tukey's multiple comparison test (M), or 2-way repeated-measures ANOVA with Sidak's multiple comparison test (F, L) and assigned: $p>0.05$ (ns, not significant), * $0.01<p<0.05$, ** $0.001<p<0.01$,*** $0.0001<p<0.001$; **** $p<0,0001$.



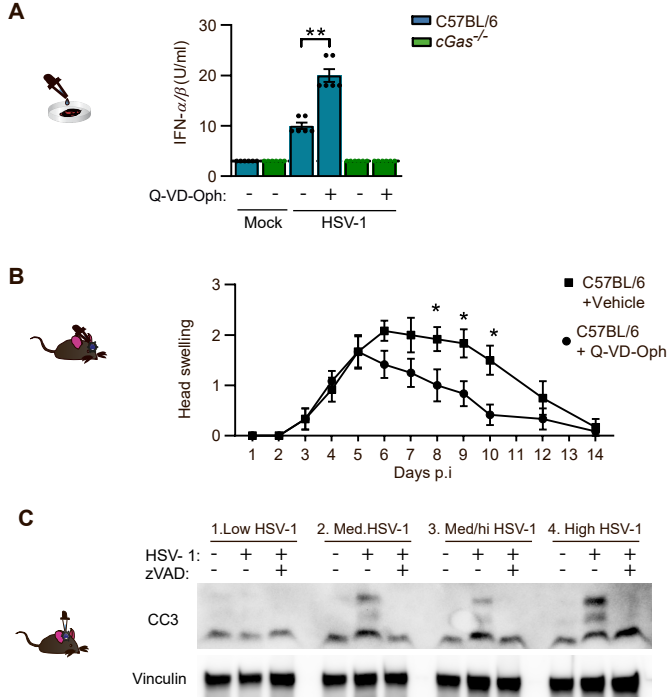
Supplemental Figure 4. Microglia and other immune cells undergo apoptosis in brain tissue of human HSE patients. Representative brain sections from five HSE patients were stained for HSV-1 (ICP8) (red), TUNEL (green), and either **(A-B)** the human leukocyte/microglia marker, CD45 (white) or **(C-D)** astrocyte marker, GFAP (white), scale bar is 50 μm. The percentages of TUNEL^{pos} cells also showing positive staining for either **(B)** CD45 or **(D)** GFAP, were quantified and shown mean ± SE (n=1-7 sections per patient). **(E-G)** Number of HSV-1^{pos} cells, and percentages of CC3^{pos} or TUNEL^{pos} cells among the total HSV-1^{pos} cells in the sections analyzed. Data are shown as means ± SE (n=1-43 sections stained per patient). **(H)** Wt mice were infected with HSV-1 (2x10^6 PFU/cornea) and the brainstems dissected at day 5 p.i. Representative images of the tissue sections stained for TUNEL, CC3 and HSV-1(ICP8) are shown, scale bar is 20μm. The area marked by the white square is magnified in the images to the right and arrows indicate cells positive for both TUNEL, CC3 and HSV-1(ICP8).



Supplemental Figure 5. Murine microglia cell line (BV2) induced apoptosis in response to the STING agonists, dsDNA or HSV-1, correlates inversely with *Ifnb* transcript levels. The microglia cell line (BV2) cells were either mock- or HSV-1 infected at increasing multiplicity of infection (MOI), or stimulated with STING agonist 3'3-cAIMP (0.2 µg/µl), TNF-α (150 ng/ml), IFNb (25U/ml), transfected Lipofectamine, or dsDNA (1 µg) for 12 h in presence of vehicle or zVAD-FMK (1,5 µg/ml). These samples were analyzed for **(A-B, D)** Caspase-3/7 activity or **(C)** expression of *Ifnb* gene normalized to β-*Actin* by RT-qPCR. Values presents (means ± SEM), (n=6 per group). Data presented represent (A-D) at least 6 biological replicates per experiment, and are representative for at least three independent experiments and p values were calculated by one-way ANOVA with Tukey's multiple comparison test and assigned: * 0.01<p<0.05, **** p<0,0001. **(E)**The primary wt microglia cell were treated with either HSV-1 (15MOI) for 8 h. Cells were fixed and stained with DAPI (blue) and antibodies against and Cyt.C and HSV-1(polyclonal), and representative images are shown in (E) with scale bar of 10 µm. The cyt.C released from into the cytoplasm (including above/below the nucleus) is marked by white arrowheads. The images are representative for at least 2 biological replicate standings per experiment and three independent experiments.



Supplemental Figure 6. cGAS is important for activation of apoptosis in Microglia. (A) Primary microglia obtained from wt or *cGas*$^{-/-}$ mice were infected in vitro with HSV-1 at the indicated MOI in presence of vehicle or zVAD-FMK 1.5 µg/ml for 12 h, at which point caspase-3/7 activity was measured. **(B)** In a similar experiment as in (A), including stimulation with STING agonist, 3'3-cAIMP (0.2 µg/µl), cell viability was measured by flow cytometry as Annexin V^{Neg} and PI^{Neg} staining. **(C-D)** Primary spleen cells (90% CD45^{pos}cells) were treated as in (A) including 3'3-cAIMP (0.2 µg/µl) and the caspase-3/7 activity and *Ifnb* expression levels were measured (n=4) **(E)** Cells were treated as in (A), followed by washing of cells 3-times after 1 h incubation to remove extracellular virus. Fresh medium containing vehicle or zVAD was added, and cells were incubated for 48 h. Conditioned medium was collected and the viral titer determined by conventional plaque assay. **(F)** The microglia cell line (BV2) cells were pretreated either with vehicle or CHX (20 µg/ml) for 1 h, and then infected with mock or HSV-1 for 6 h. The cell lysate was subject to western blotting with antibodies against STING, cGas or Vinculin. **(G)** Primary neurons and astrocytes were mixed and infected with HSV-1-GFP (10 MOI) for 24 h. Subsequently, microglia was added for 24 h and cells were fixed and stained with DAPI (blue), and antibodies against GFP (green) or Iba-1 (red) scale bar is 20 µm. **(H)** primary microglia obtained from wt or *cGas*$^{-/-}$ mice were incubated with UV-treated HSV-1-infected mixed neuron/astrocyte cultures. After 6 h incubation, *Ifnb* expression was measured by RT-qPCR. Values in this figure presents means ± SEM, (n=4-6 per group) and represents at least three independent experiments. The p values were calculated by one-way ANOVA with Tukey's multiple comparison test (A, C-E, F) and two-tailed Student's t-test (B, H) and assigned: * 0.01<p<0.05, ** 0.001<p<0.01,*** 0.0001<p<0.001; **** p<0,0001.



Supplemental Figure 7. Apoptosis inhibits hyper activation of host antiviral activity. **(A)** Organotypic brain slices from wt mice were cultured and infected with HSV-1 ($1x10^4$) for 5 days in presence or absence of caspase inhibitor Q-VD-Oph (100 μM). The IFN-I protein secreted in to the media for the last 3 dys of infection was mesured by IFN-I bioassay and presented as means ± SEM, n=6 per group and represents at least three independent experiments. **(B)** Mice were HSV-1 infected ($2x10^7$ PFU/cornea) and treated on day 5, 6 and 9 p.i with Q-VD-Oph (20 mg/kg). Head swelling were monitored. Data are presented as means ± SEM, n=6 per group. **(C)** Wild-type mice were infected intracranially with HSV-1 expressing GFP ($1x10^7$ PFU) and treated with the caspase inhibitor (zVAD), or vehicle as control (n=4-6 mice/group). At 48 h post-infection, animals were sacrificed and GFP-expressing brain biopsies (indicative for HSV-1 infection) dissected. Representative images of immunoblotting for CC3 and Vinculin are shown for the 4 subgroups defined in (Figure 7G). The p values were calculated by two-tailed Student's t-test (A) or 2-way repeated-measures ANOVA with Sidak's multiple comparison test (B) and assigned: * $0.01<p<0.05$, ** $0.001<p<0.01$.

Supplemental Information

Brain immune cells undergo cGAS-STING-dependent apoptosis during herpes simplex virus type 1 infection to limit type I interferon production

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Supplemental Materials and Methods

IFN-α/β bioassay

IFN-α/β bioactivity was measured by use of a L929 cell–based bioassay. Samples and murine IFN-α/β standard was added to 96-well plates in successive 1.5-fold dilutions. Prior to use, HSV-1 in samples was inactivated by UV light treatment for 6 min. L929 cells (2 × 104 cells/well) were added to each well, and the plates were incubated overnight at 37 °C. The next day vesicular stomatitis virus (VSV/V10) was added to the wells, and the plates were further incubated for 2–3 d. The dilution mediating 50% protection was defined as 1 U of IFN-α/β per ml. The bioassay had a detection limit of 3 U/ml.

Interleukin-1β (IL1β) ELISA

Tissues were homogenized with steel beads (Qiagen) in a Tissuelyser (II) (Qiagen) in 0.5ml RIPA buffer and the protein content in samples were normalized by standard Bradford Protein Assays (Bio-Rad) to 5mg/ml of proteins. Samples and standard curve were immediately used for IL1b ELISA according to the manufacturers protocol (mouse IL-1b DuoSet ELISA, DY401, R&D).

Western blotting

Western blotting was performed as described previously (25) using the following antibodies: mouse polyclonal anti-caspase-1 (p20)(1:500; Casper-1, AdipoGen), rabbit polyclonal anti-cleaved caspase-3 (1:500; Asp175, Cell signaling), rabbit polyclonal anti-Phospho-RIPK3 (1:1000; Thr231/Ser232, Cell Signaling), monoclonal anti-RIPK3 (1:1000; R8J3L, Cell Signaling), rabbit polyclonal anti-MLKL (1:1000, C-terminal, ab172868, Abcam), rabbit monoclonal anti-phospho-MLKL (1:1000, pS345, EPR9515(2), Abcam), rabbit monoclonal anti-GSDMD (1:500; EPR19828, Abcam), rabbit polyclonal anti-GSDMD (1:250; 126-138, Sigma-Aldrich), monoclonal mouse anti-cytochrome C oxidase complex IV (1 µg/mL; 673803, R&D), mouse anti-cytochrome C (1:200; ab65311, Abcam), monoclonal rabbit anti-STING (1:1000; D1V5L, Cell Signaling) or rabbit monoclonal anti-cGAS (1:1000; D1D3G, Cell Signaling).

Virus plaque assay

The brainstems or organotypic brain slice cultures were isolated, immediately put on dry ice, and stored at –80°C until future use. Organs were thawed and homogenized in DMEM and pelleted by centrifugation at 1,600 g for 30 min. Supernatants were used for plaque assay as described (25).