**Virologica Sinica**

**Supplementary Data**

**Mice with type I interferon signaling deficiency are prone to** **epilepsy upon HSV-1 infection**

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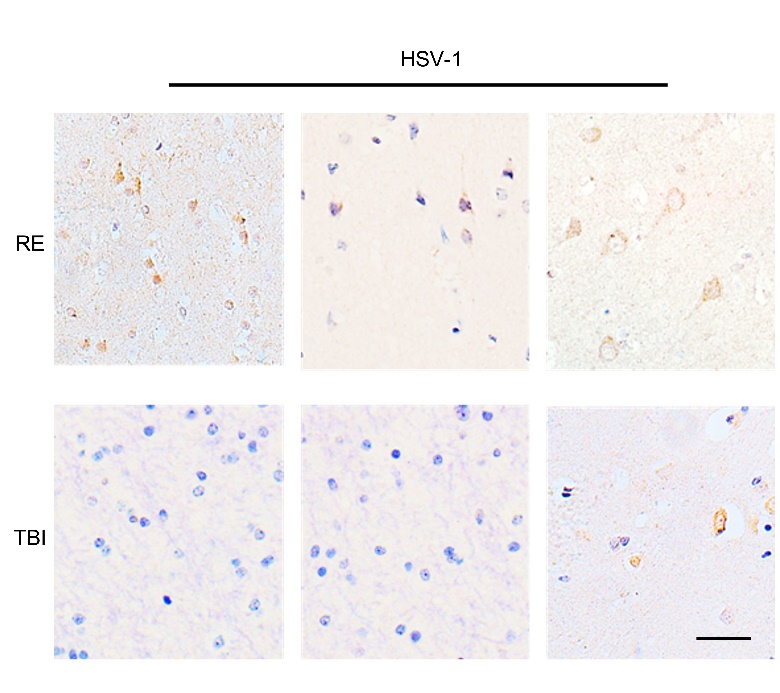
e Chinese Institute for Brain Research, Beijing, 102206, China.

\*Corresponding author:

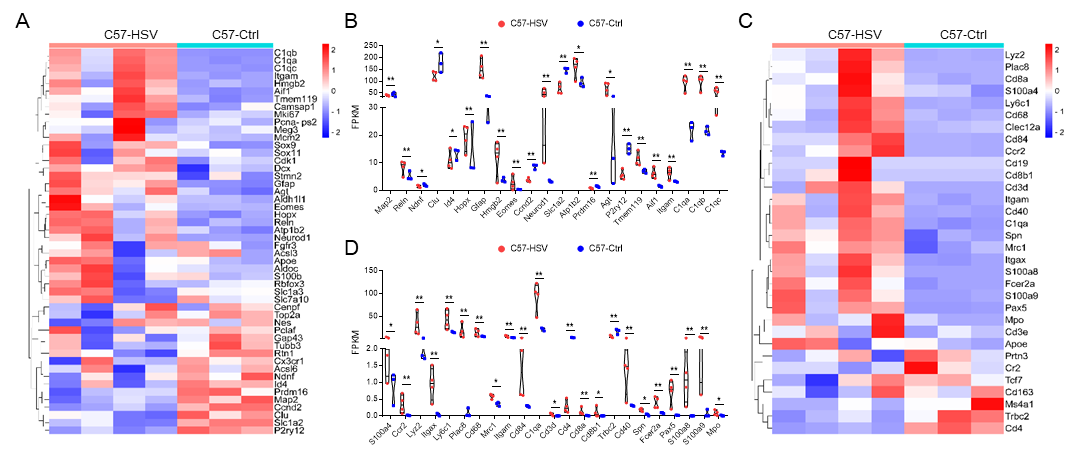
Email address: luangm@ccmu.edu.cn (G-M. Luan)

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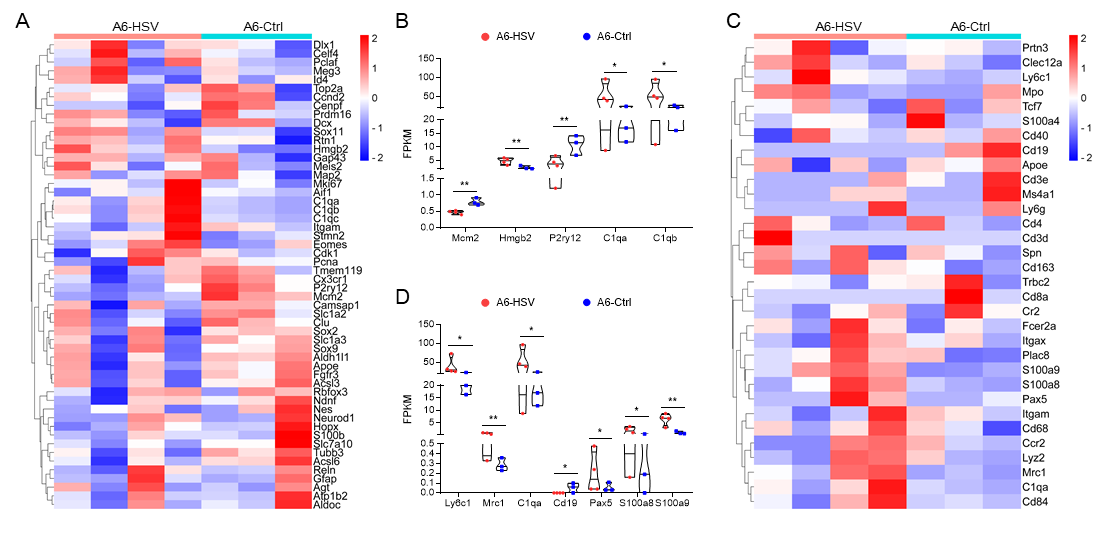
**Supplementary Materials**



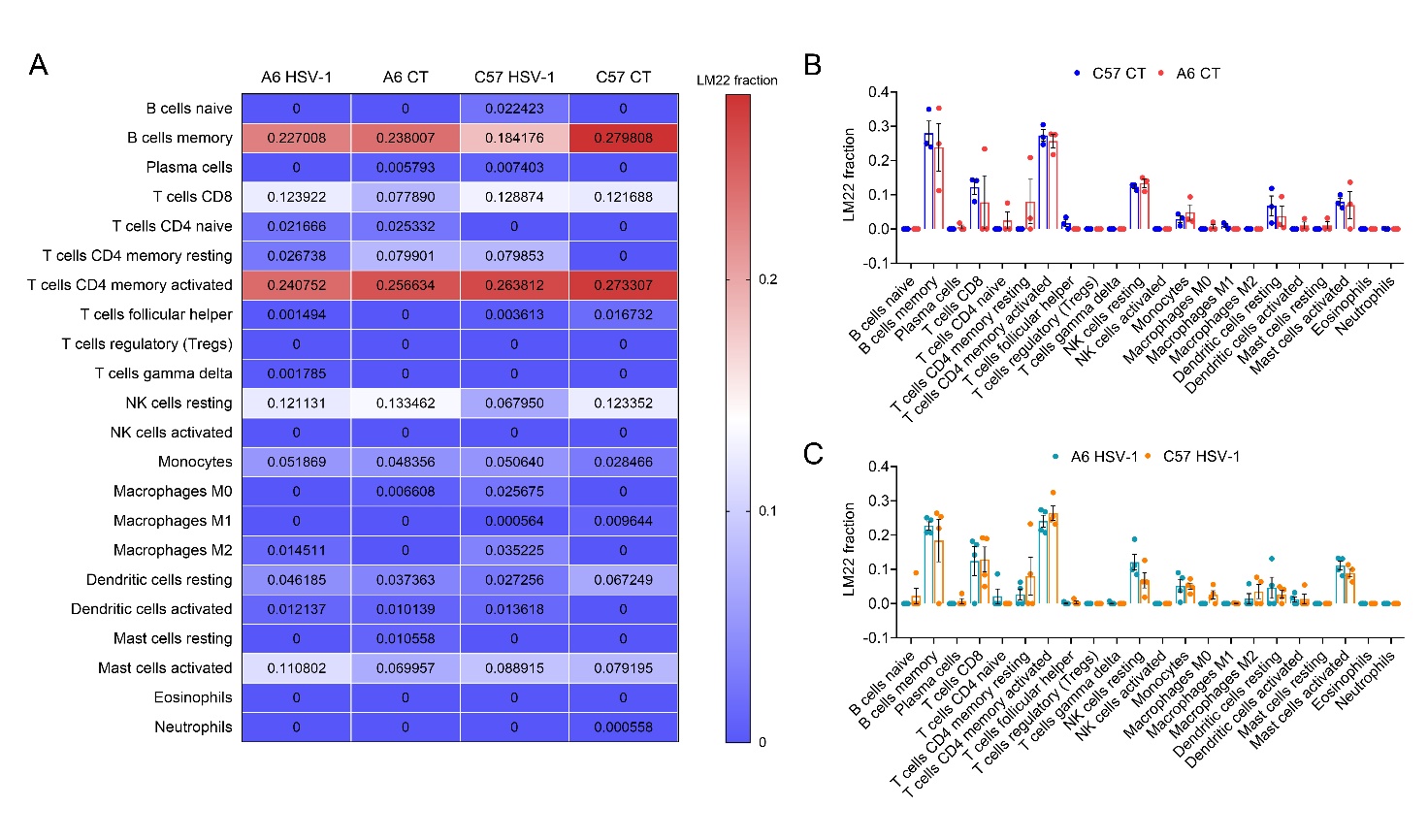
**Fig. S1** Distribution of HSV-1 antigens in RE patients. Immunohistochemistry staining of brains from RE patients. Brain tissues from traumatic brain injury (TBI) patients served as control. Scale bar, 25 μm.



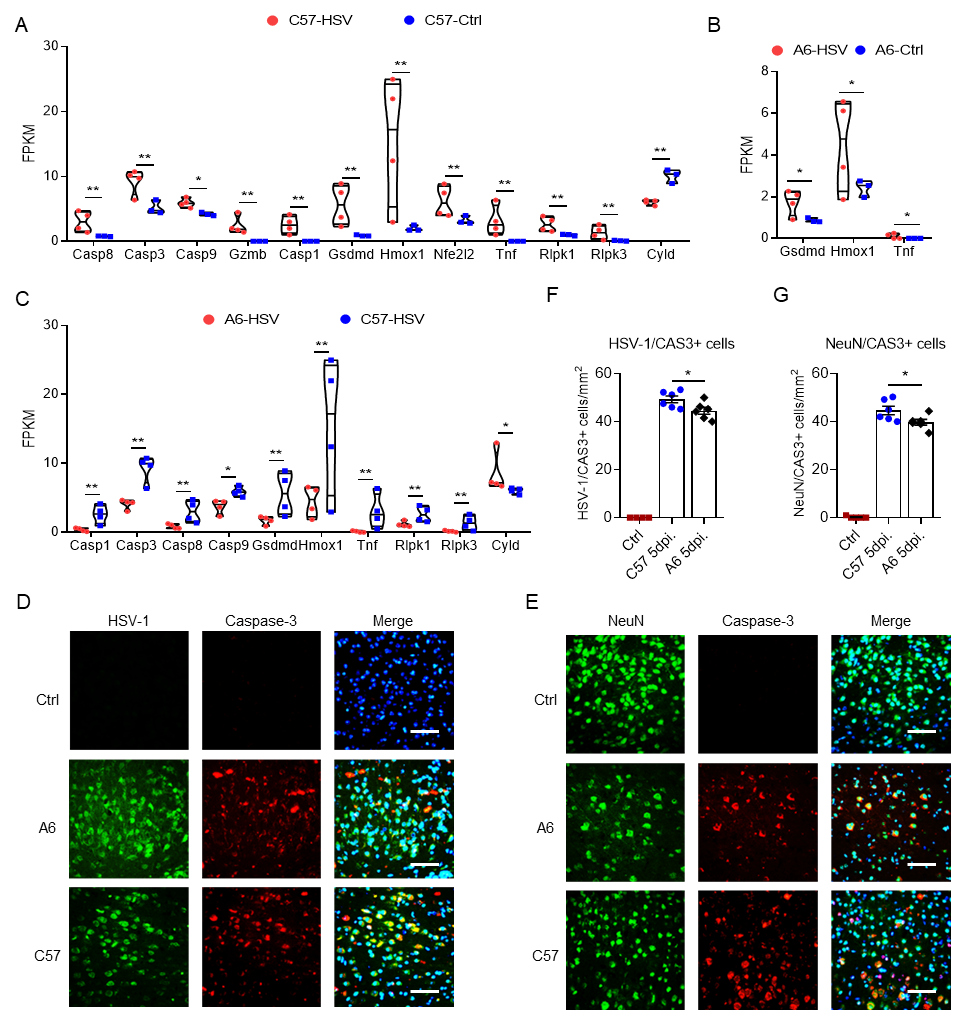
**Fig. S2** Expression of cell marker genes in HSV infected brains from WT mice. Brain tissues from HSV-1 infected infant mice at 5 dpi were subjected to RNA sequencing, PBS-injected mice served as control (n = 3–4 mice for each group). **A and B** The expression levels of markers of brain cells in brains from HSV-1 infected WT mice were shown in heatmap **(A)** and genes with significantly altered levels were shown in violin chart **(B)**. **C and D** The expression levels of markers of immune cells in brains from HSV-1 infected WT mice were shown in heatmap **(C)** and genes with significantly altered levels were shown in violin chart **(D)**. Data in this figure were expressed as FPKM value and analyzed using the two-sided Student’s *t* test. \* *P* < 0.05, \*\* *P* < 0.01.



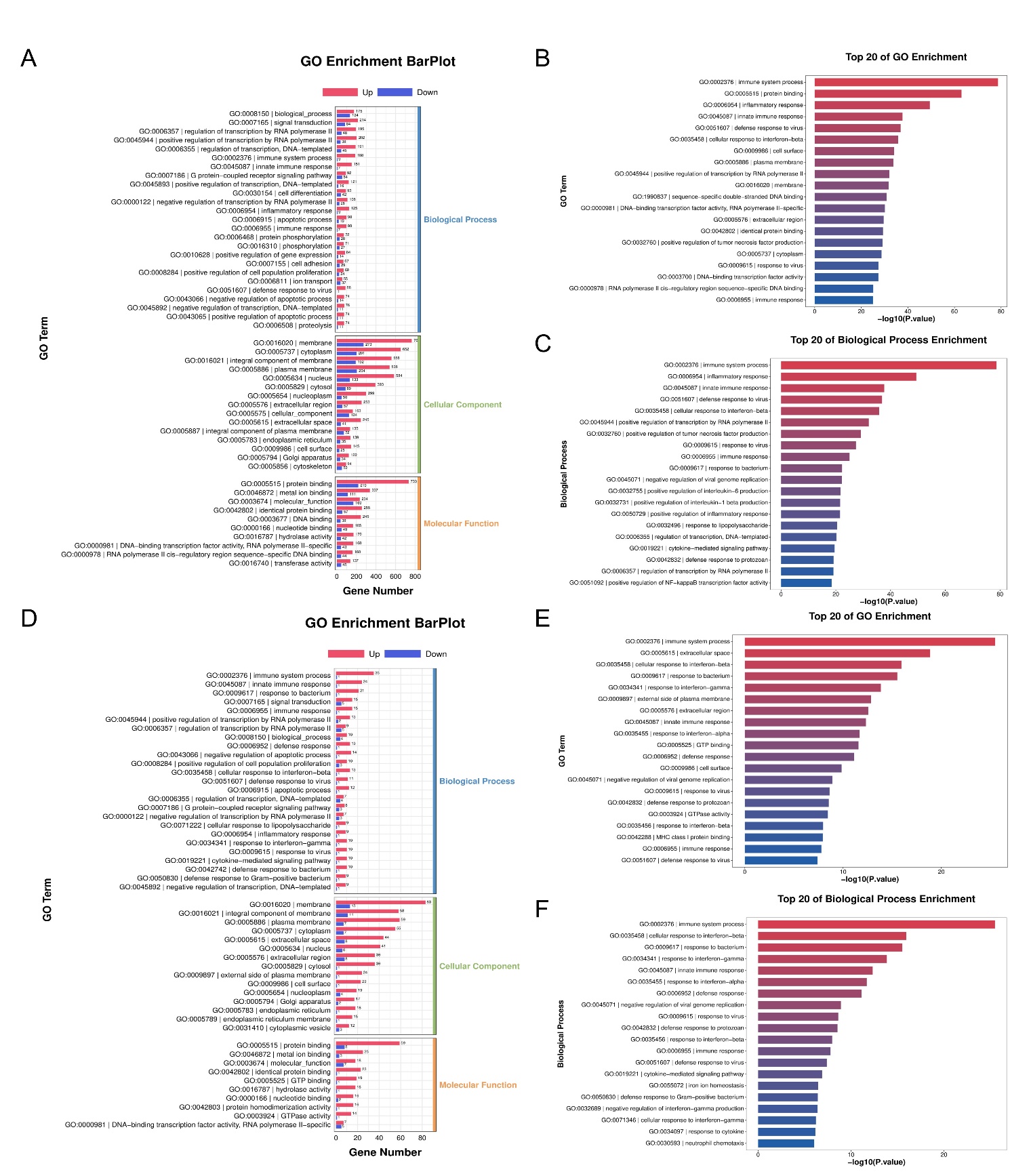
**Fig. S3** Expression of cell marker genes in HSV infected brains from A6 mice.Brain tissues from HSV-1 infected infant mice at 5 dpi were subjected to RNA sequencing, PBS-injected mice served as control (n = 3–4 mice for each group). **A and B** The expression levels of markers of brain cells in brains from HSV-1 infected A6 mice were shown in heatmap **(A)** and genes with significantly altered levels were shown in violin chart **(B)**. **C and D** The expression levels of markers of immune cells in brains from HSV-1 infected A6 mice were shown in heatmap **(C)** and genes with significantly altered levels were shown in violin chart **(D)**. Data in this figure were expressed as FPKM value and analyzed using the two-sided Student’s *t* test. \* *P* < 0.05, \*\* *P* < 0.01.



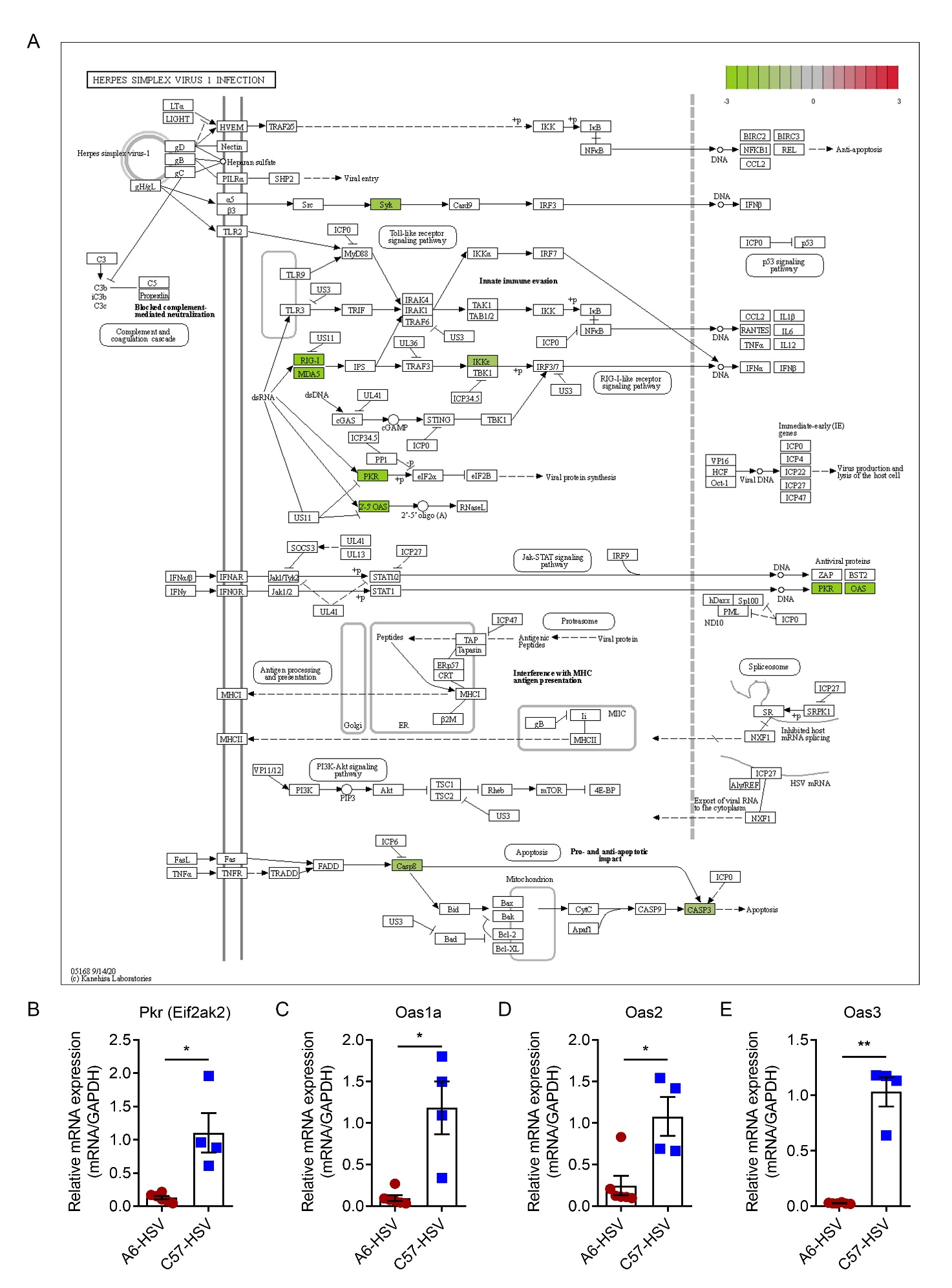
**Fig. S4** Immune landscape analysis of HSV-infected brain tissues. The proportion of infiltrated immune cells in brain tissues from WT and A6 infant mice were calculated by CIBERSORT algorithm. **A** The differences in immune cells of HSV-infected brain tissues from WT and A6 infant mice were shown by heatmap. **B and C** The differences in immune cells of brain tissues from control **(B)** and HSV-1 infected mice **(C)** (n = 3–4 mice for each group).



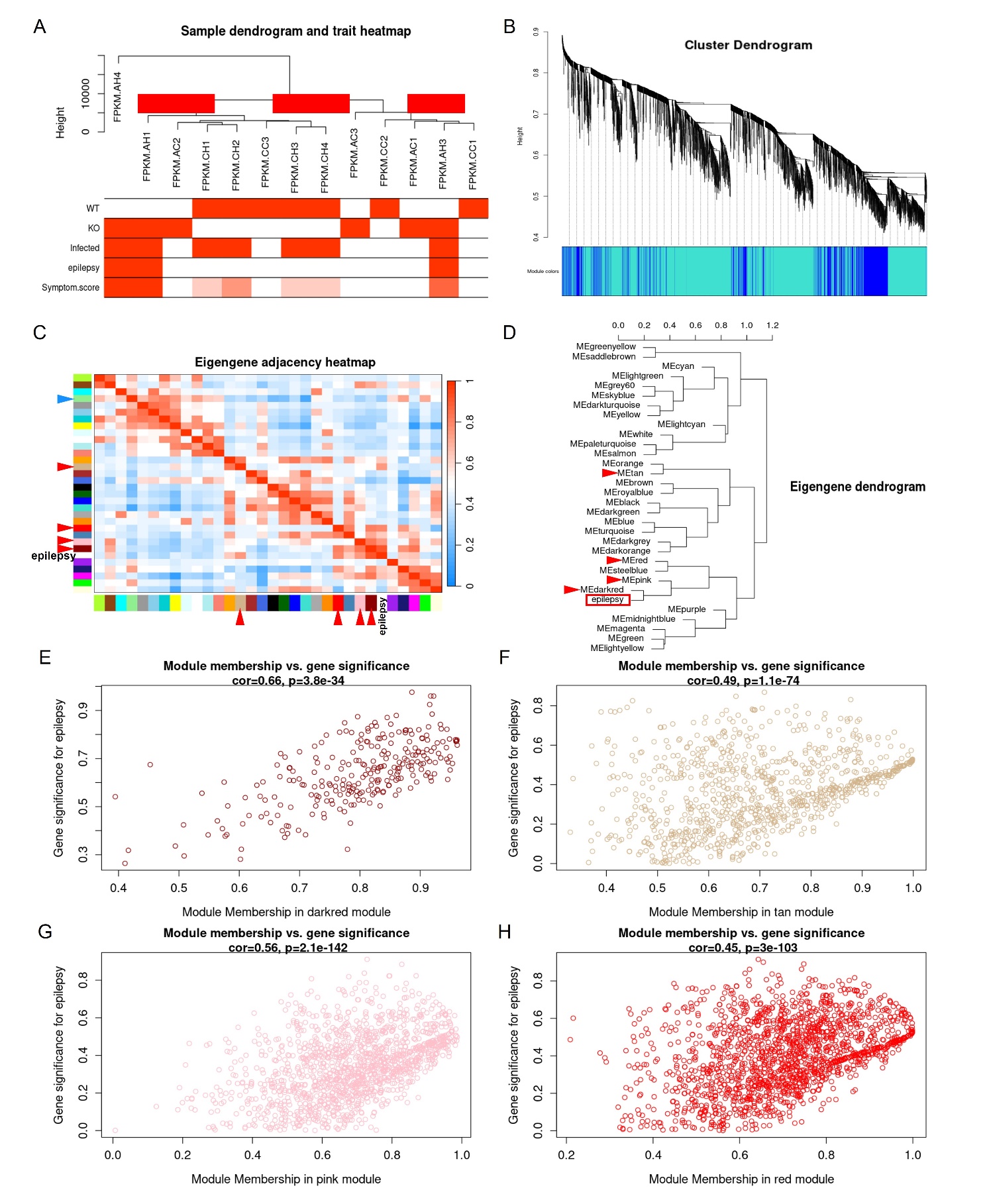
**Fig. S5** Expression of cell death-related genes in HSV infected brains.Brain tissues from HSV-1 infected infant mice at 5 dpi were subjected to RNA sequencing, PBS-injected mice served as control (n= 3-4 mice for each group). **A** The expression levels of cell death-related genes in brains from HSV-1 infected and control WT mice with significantly altered levels were shown in violin chart. **B** The expression levels of cell death-related genes in brains from HSV-1 infected and control A6 mice with significantly altered levels were shown in violin chart. **(C)** The expression levels of cell death-related genes in brains from HSV-1 infected A6 compared with that of WT mice with significantly altered levels were shown in violin chart. **D**–**G** Brain tissues from HSV-1 infected WT and A6 mice at 5 dpi (n = 4–6 mice for each group) were subjected to co-immunofluorescence staining with anti-Caspase-3 antibody and anti-HSV-1 antibody **(D)**, or anti-NeuN antibody **(E)**. Nuclei were shown with DAPI. Scale bar, 50 μm. The number of HSV-1+ Caspase-3+ cells **(F)** and NeuN+ Caspase-3+ cells **(G)** were calculated by Image J. Scale bar, 25 μm. Results were shown as means ± SEM and analyzed using the two-sided Student’s *t* test. \* *P* < 0.05, \*\* *P* < 0.01. Data in this figure expressed as FPKM value were analyzed using the two-sided Student’s *t* test. \* *P* < 0.05, \*\* *P* < 0.01.

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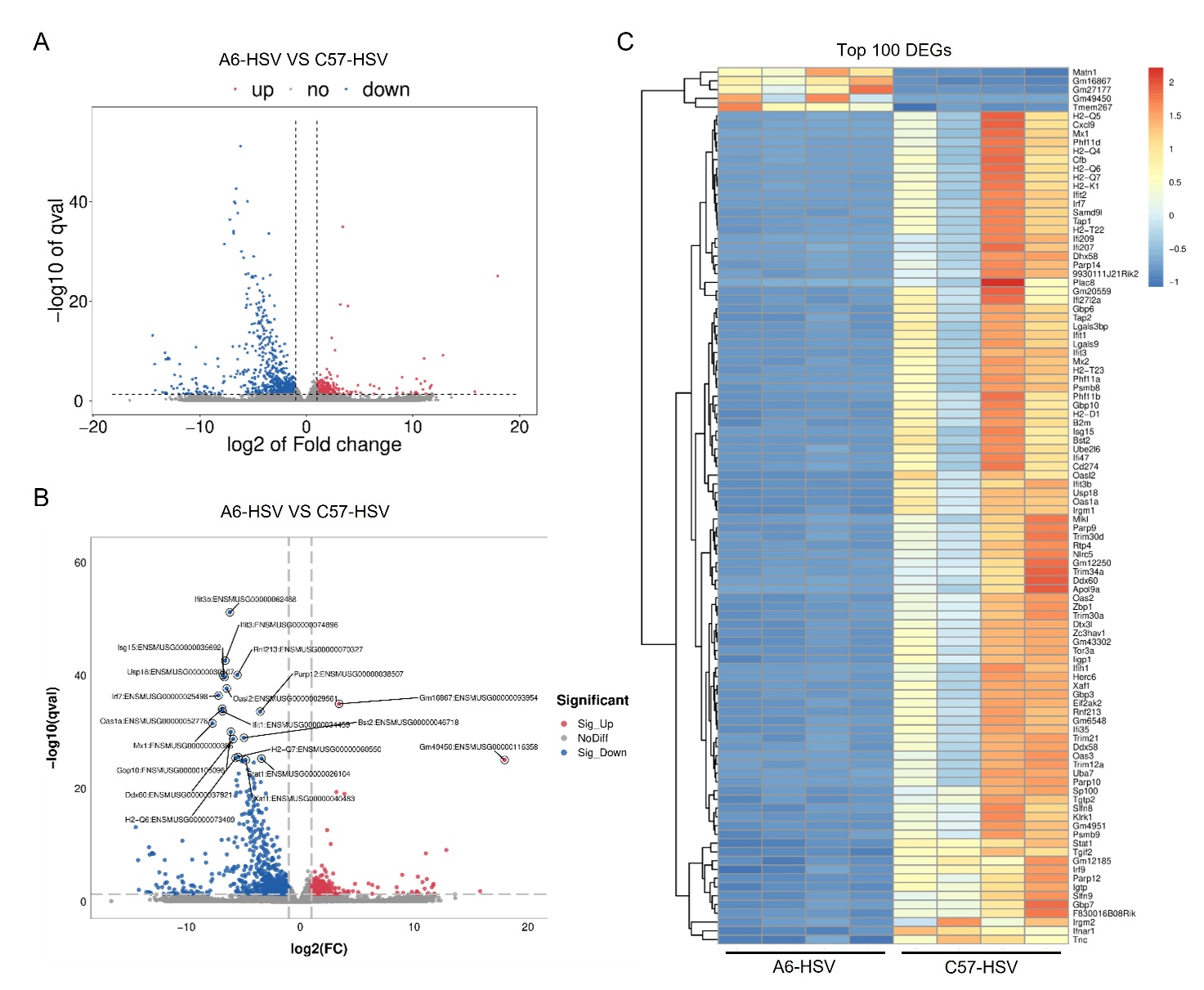
**Fig. S6** GO analysis of DEGs of HSV-infected brains. Brain tissues from HSV-1 infected infant mice at 5 dpi (n= 3–4 mice for each group) were subjected to RNA sequencing, PBS-injected mice served as control. **A**–**C** The DEGs between brains from HSV-1 infected and control WT mice were subjected to GO analysis. The top 10 most significantly enriched GO terms in BP, MF and CC **(A)**, and the top 20 most significantly enriched GO terms **(B)** and the top 20 most significantly enriched GO terms in biological process **(C)** were shown. **D**–**F** The DEGs between brains from HSV-1 infected and control A6 mice were subjected to GO analysis. The top 10 most significantly enriched GO terms in BP, MF and CC **(D)**, and the top 20 most significantly enriched GO terms **(E)** and the top 20 most significantly enriched GO terms in biological process **(F)** were shown.



**Fig. S7** Herpes simplex virus 1 infection pathway visualization with mapped data of DEGs between HSV-infected brains from A6 and WT infant mice. **(A)** DEGs between HSV-infected brains from A6 and WT infant mice were subjected to “Herpes simplex virus 1 infection pathway” for visualization by Pathview. **(B**–**E)** The expression levels of antiviral gene *Pkr (Eif2ak2)* **(B)**, *Oas1a* **(C)**, *Oas2* **(D)** and *Oas3* **(E)** in brains from HSV-1 infected A6 compared with that of WT mice were analyzed by RT-qPCR (n = 4–6 mice for each group). Results were shown as means ± SEM and analyzed using the two-sided Student’s *t* test. \* *P* < 0.05, \*\* *P* < 0.01.



**Fig. S8** WGCNA analysis to select epilepsy-correlated gene modules. **A** Clustering of module genes. **B** Cluster dendrogram for selecting gene modules. **C and D** Association between the gene modules and HSV-induced epilepsy. **E**–**H** Module membership vs. gene significance for epilepsy in darkred module **(E)**, tan module **(F)**, pink module **(G)** and red module **(H)**.



**Fig. S9** DEGs between HSV-infected brains from A6 and WT infant mice. **A** Volcano plot of all detected transcripts between HSV-1 infected brains from A6 and WT infant mice. The threshold of screening DEGs is set at |logFC| ≥ 1.0 and p (p.adjust) < 0.05. Points are colored according to expression status: non-significant genes, grey, significant up-regulated genes, red; and down-regulated genes, blue. **B** Volcano plot of top 20 DEGs between HSV-infected brains from A6 and WT infant mice. **C** Heatmap of top 100 DEGs between HSV-infected brains from A6 and WT infant mice.

**Supplementary Table S1.** DEGs between uninfected brains from A6 and WT infant mice.

**Supplementary Table S2.** Marker genes of brain cells.

**Supplementary Table S3.** Marker genes of immune cells.

**Supplementary Table S4.** Table of cell death-related genes.

**Supplementary Table S5.** Marker genes of epilepsy.

**Supplementary Table S6.** Top 10 hub genes of overlapping genes calculated by cytoHubba using Degree scoring method.

**Supplementary Table S7.** Expression of hub genes in HSV-infected brain tissues from A6 and WT infant mice at transcriptome level.