**Virologica Sinica**

**Supplementary Data**

**Ferroptosis contributes to JEV-induced neuronal damage and neuroinflammation**

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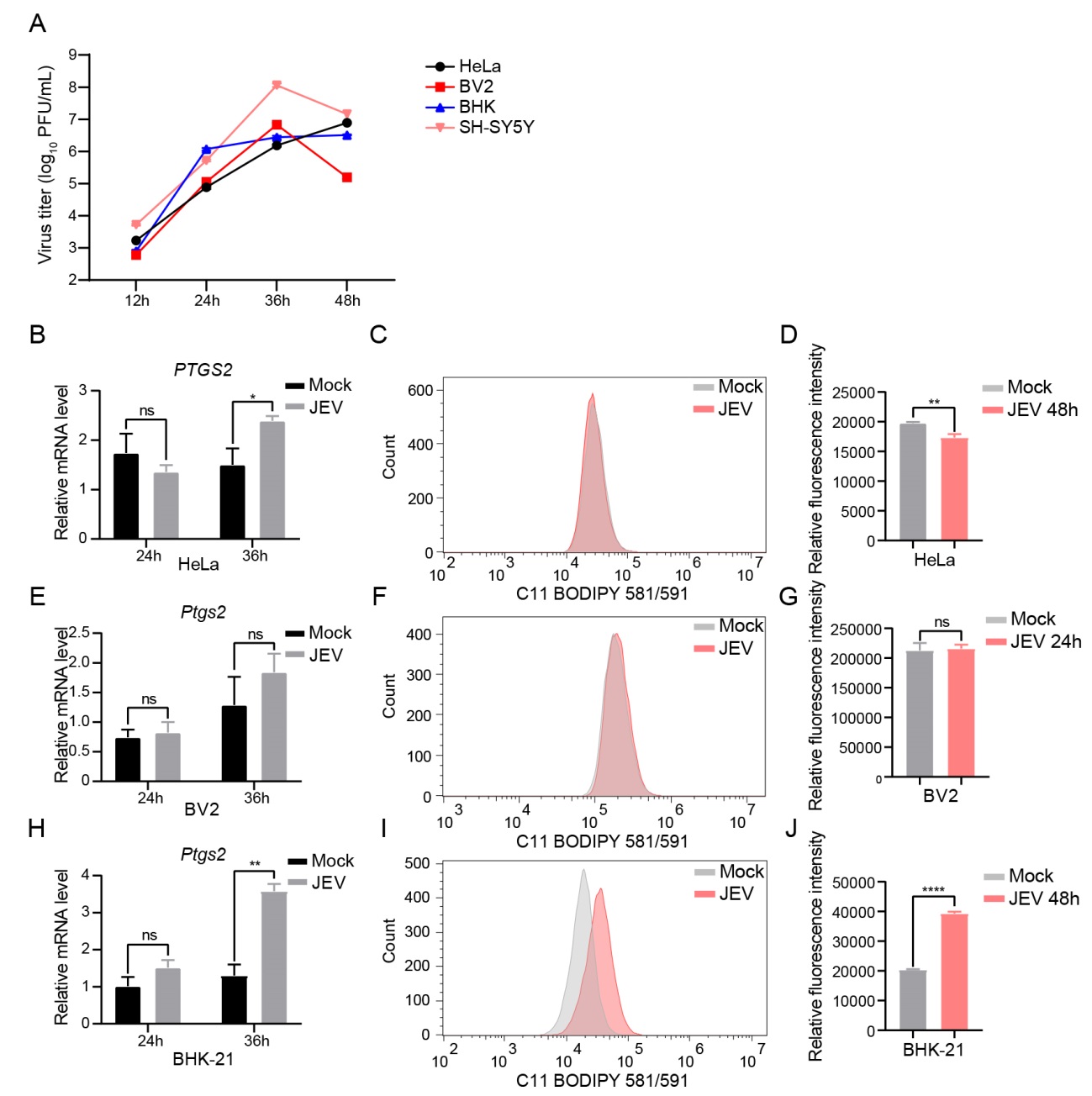
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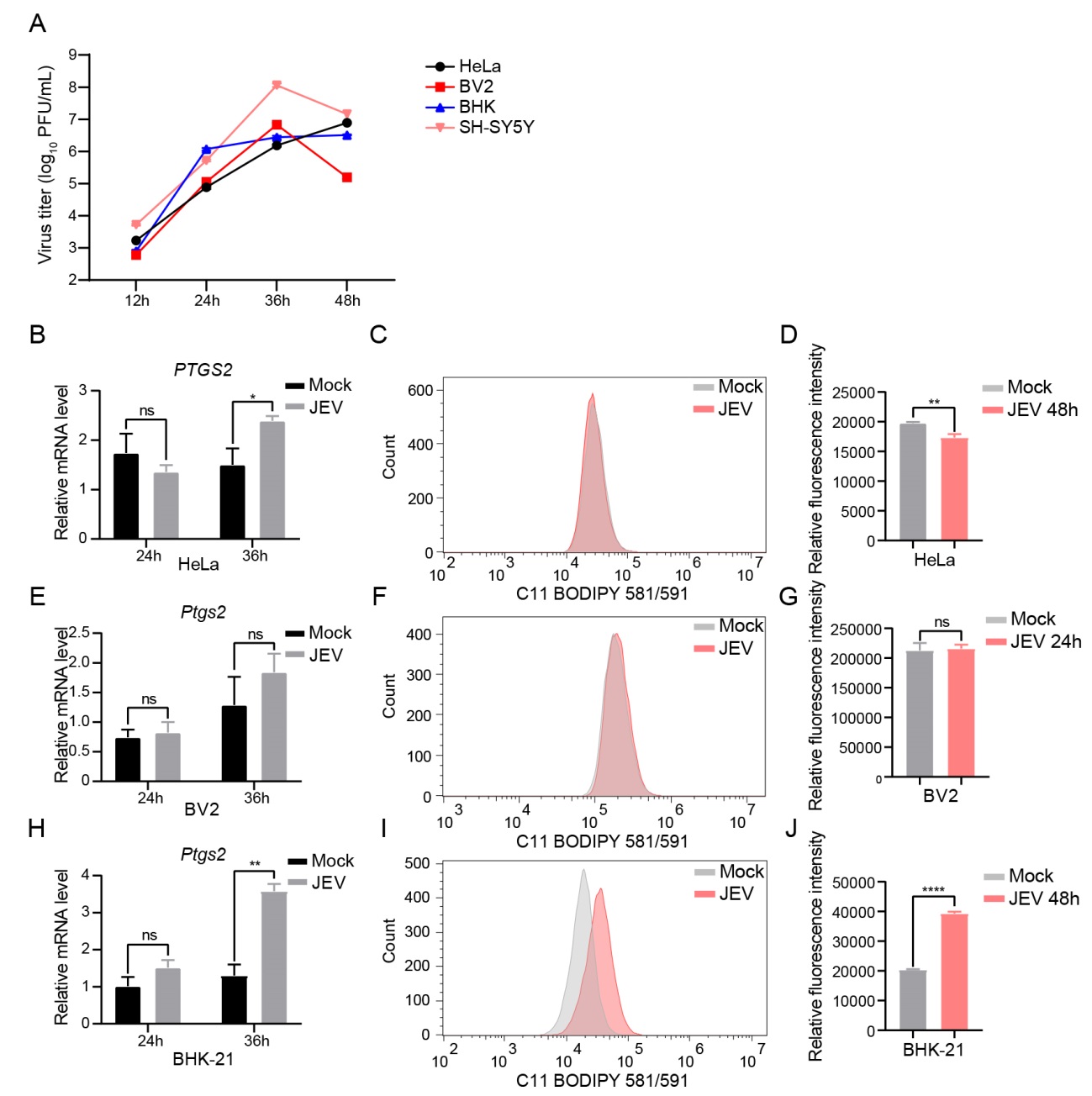
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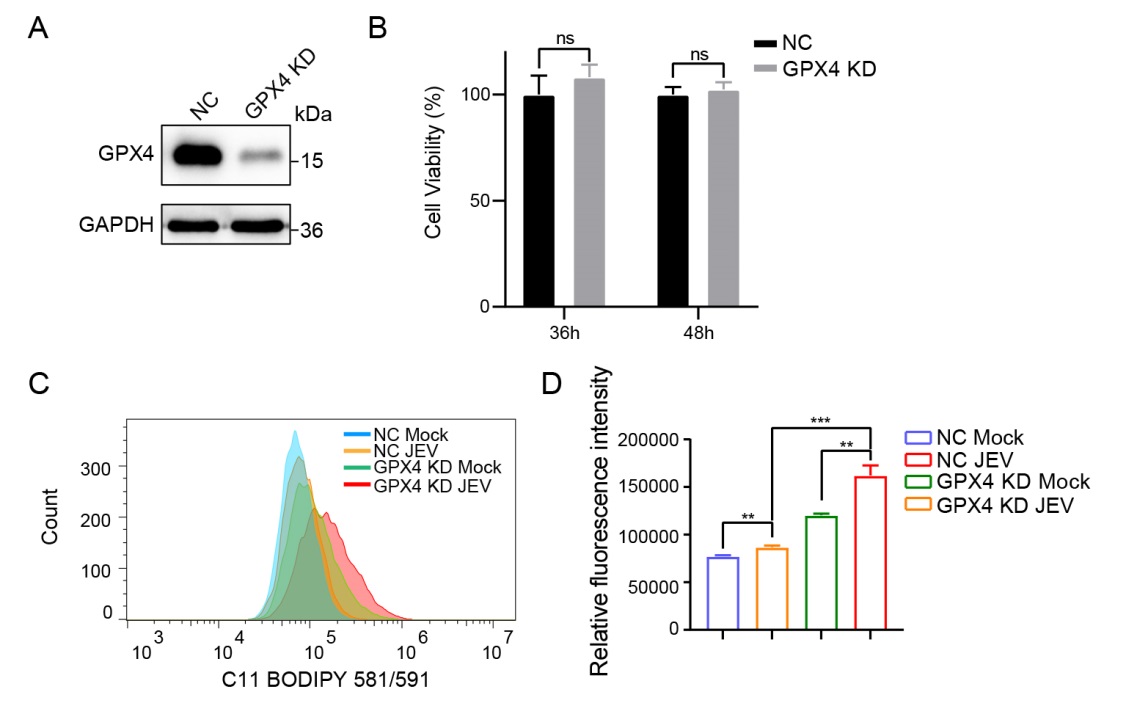
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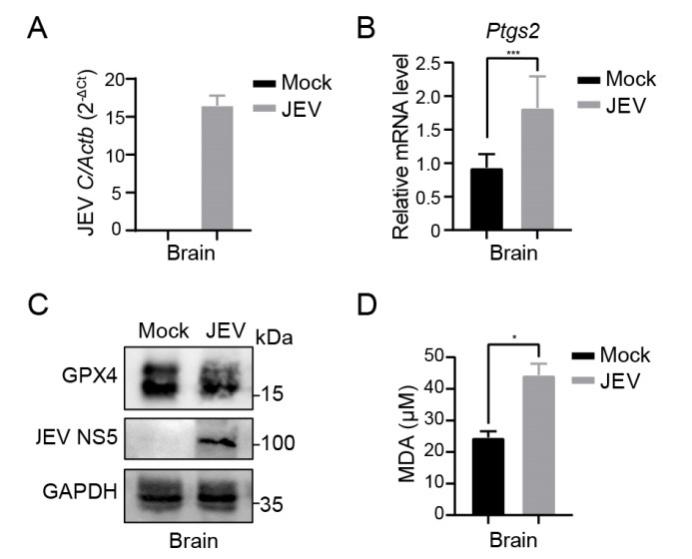
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**Figure S1.** Erastin treatment upregulated the expression of *PTGS2* at mRNA levels in SH-SY5Y cells and mouse primary neurons. **A** and **B** SH-SY5Y cells or mouse primary neurons were infected or mock-infected with JEV at an MOI of 1. At indicated time post-infection, cells were subjected to RT-qPCR to detect the mRNA level of JEV *C*. **C** to **F** SH-SY5Y cells were treated with Erastin (10 µmol/L) or the vehicle (DMSO) for 12 h, cells were subjected to RT-qPCR to detect the mRNA level of *PTGS2* (**C**) and the level of lipid peroxidation through flow cytometry (**E**). Mouse primary neurons were treated with Erastin (20 µmol/L) or vehicle (DMSO) for 48 h, cells were subjected to RT-qPCR to detect the mRNA level of *Ptgs2* (**D**) and the level of lipid peroxidation through flow cytometry (**F**). Data are representative of three independent experiments with three biological replicates. Significance was analyzed using a Student’s two-tailed unpaired *t*-test (n = 3 in each group), \**P*<0.05, \*\**P*<0.01, \*\*\*\**P*<0.0001.

**Figure S2.** JEV is able to induce ferroptosis in BHK-21 cells but not in HeLa cells or BV2 cells. HeLa, BV2, BHK-21 and SH-SY5Y cells were infected with JEV at an MOI of 1, the supernatants of JEV-infected cells were harvested at 12-48 h post-infection for plaque assay, and the titration was performed on BHK-21 cells (**A**). HeLa, BV2 and BHK-21 cells were infected or mock-infected with JEV at an MOI of 1. At indicated time post-infection, cells were subjected to RT-qPCR to detect the mRNA level of *PTGS2* (**B, E** and **H**) and the level of lipid peroxidation by flow cytometry (**C, D, F, G, I** and **J**). Data are representative of three independent experiments with three biological replicates. Significance was analyzed using a Student’s two-tailed unpaired *t*-test (n = 3 in each group), \**P*< 0.05, \*\**P*< 0.01, \*\*\*\**P*< 0.0001. ns, not significant.



**Figure S3.** Knockdown of GPX4 promotes ferroptosis induced by JEV infection. **A** and **B** GPX4 KD cells were subjected to Western blotting analysis of the expression of GPX4 (**A**) and detect the cell viability at different time (**B**)**. C** and **D** NC and GPX4 KD cells were infected or mock-infected with JEV at an MOI of 1. At 24 h post-infection, the cells were subjected to detect the level of lipid peroxidation. Data are representative of three independent experiments with three biological replicates. \*\**P*< 0.01, \*\*\**P*< 0.001. ns, not significant.



**Figure S4.** JEV infection induces ferroptosis in mouse brains. C57BL/6 mice were infected with JEV as described in Fig 5A. The mice were sacrificed at 7 dpi, and brains were collected and homogenized. **A** and **B** The mRNA levels of JEV *C* and *Ptgs2* in brain lysates were measured by RT-qPCR. **C** and **D** Detection of expression level of GPX4 and MDA in brain lysates. Data are representative of three independent experiments with three biological replicates. \**P* < 0.05, \*\*\**P* < 0.001. ns, not significant.

**Supplementary Table S1. Primers used for qRT-PCR**

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| Name | Sequence |
| *ACTB(H) F* | 5′- AGCGGGAAATCGTGCGTGAC -3′ |
| *ACTB(H) R* | 5′- GGAAGGAAGGCTGGAAGAGTG -3′ |
| *PTGS2(H) F* | 5′- CGGTGAAACTCTGGCTAGACAG -3′ |
| *PTGS2(H) R* | 5′- GCAAACCGTAGATGCTCAGGGA -3 |
| *ACSL4(H) F* | 5′- TCTGCTTCTGCTGCCCAATT -3′ |
| *ACSL4(H) R* | 5′- CGCCTTCTTGCCAGTCTTTT -3′ |
| *YAP1(H) F* | 5′- TCTTACACCGTGCTGCCATT -3′ |
| *YAP1(H) R* | 5′- AGCACCTGTCCAGGTATCAC -3′ |
| *JEV-C F* | 5′- GGCTCTTATCACGTTCTTCAAGTTT -3′ |
| *JEV-C R* | 5′- TGCTTTCCATCGGCCTAAAA -3′ |
| *Actb(M) F* | 5′- CACTGCCGCATCCTCTTCCTCCC -3′ |
| *Actb(M) R* | 5′- CAATAGTGATGACCTGGCCGT -3′ |
| *Ptgs2(M) F* | 5′- CGGACTGGATTCTATGGTGAAA -3′ |
| *Ptgs2(M) R* | 5′- CTTGAAGTGGGTCAGGATGTAG -3′ |
| *Tnf-α(M) F* | 5′- TGTCTCAGCCTCTTCTCATTCC -3′ |
| *Tnf-α(M) R* | 5′- TTAGCCCACTTCTTTCCCTCAC -3′ |
| *Il-6(M) F* | 5′- CATGTTCTCTGGGAAATCGTG -3′ |
| *Il-6(M) R* | 5′- TCCAGTTTGGTAGCATCCATC -3′ |
| *Ccl-2(M) F* | 5′- CGGCGAGATCAGAACCTACAAC -3′ |
| *Ccl-2(M) R* | 5′- GGCACTGTCACACTGGTCACTC -3′ |
| *Ccl-5(M) F* | 5′- TGCCCACGTCAAGGAGTATTTC -3′ |
| *Ccl-5(M) R* | 5′- AACCCACTTCTTCTCTGGGTTG -3′ |

(M: mouse; H: human)