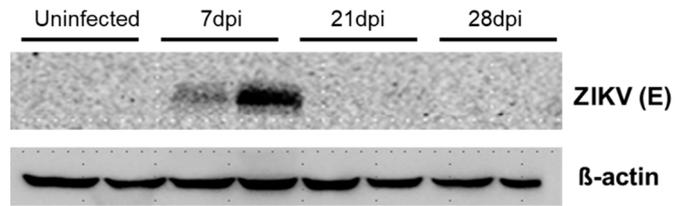


## **Supplemental Information**

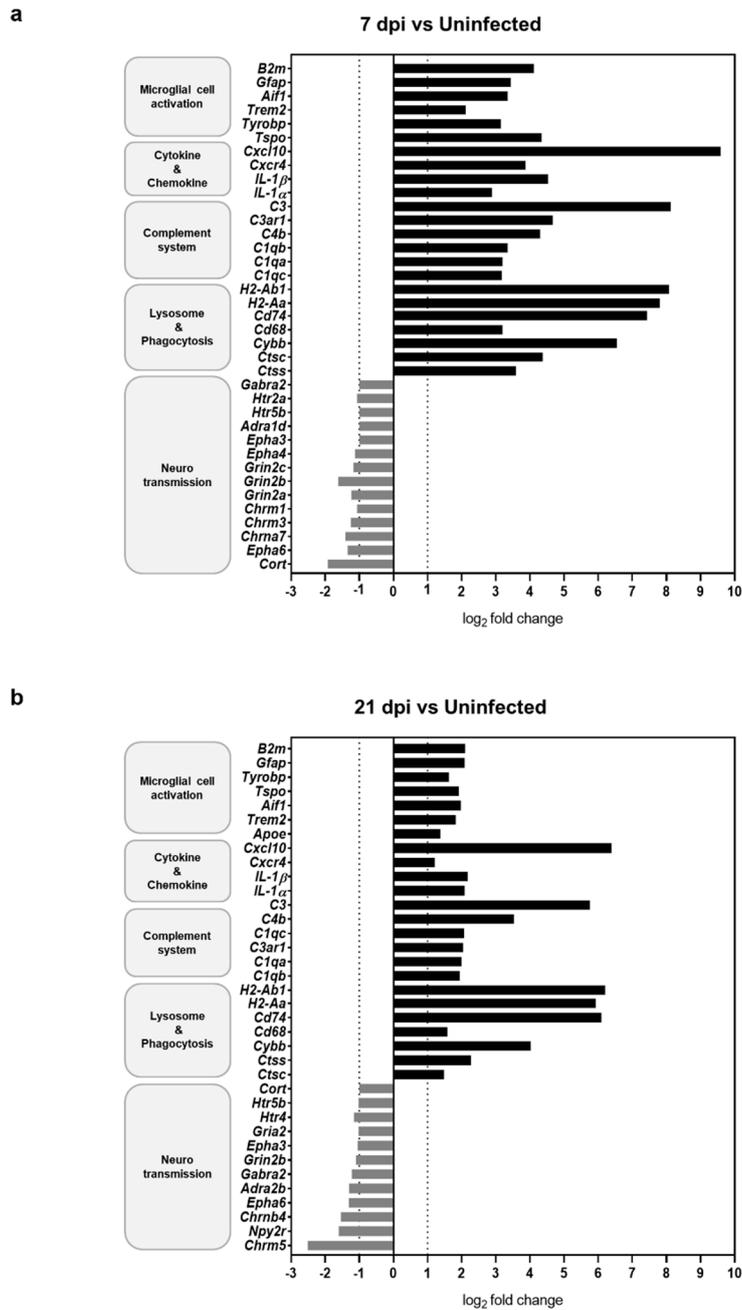
### **Sustained Microglial Activation Promotes Synaptic Loss and Neuronal Dysfunction after Recovery from ZIKV Infection**

**Nahyun Kim, Hanul Choi, Uijin Kim, Suyeon Kim, Young Bong Kim\* and Ha Youn Shin\***

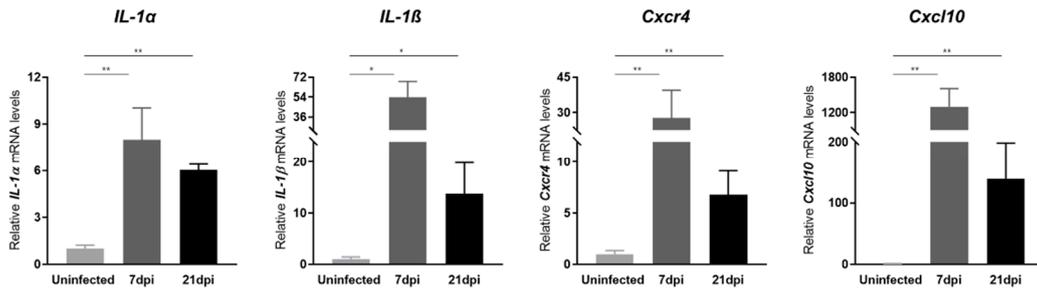
\*Correspondence: [kimera@konkuk.ac.kr](mailto:kimera@konkuk.ac.kr); [hayounshin@konkuk.ac.kr](mailto:hayounshin@konkuk.ac.kr)



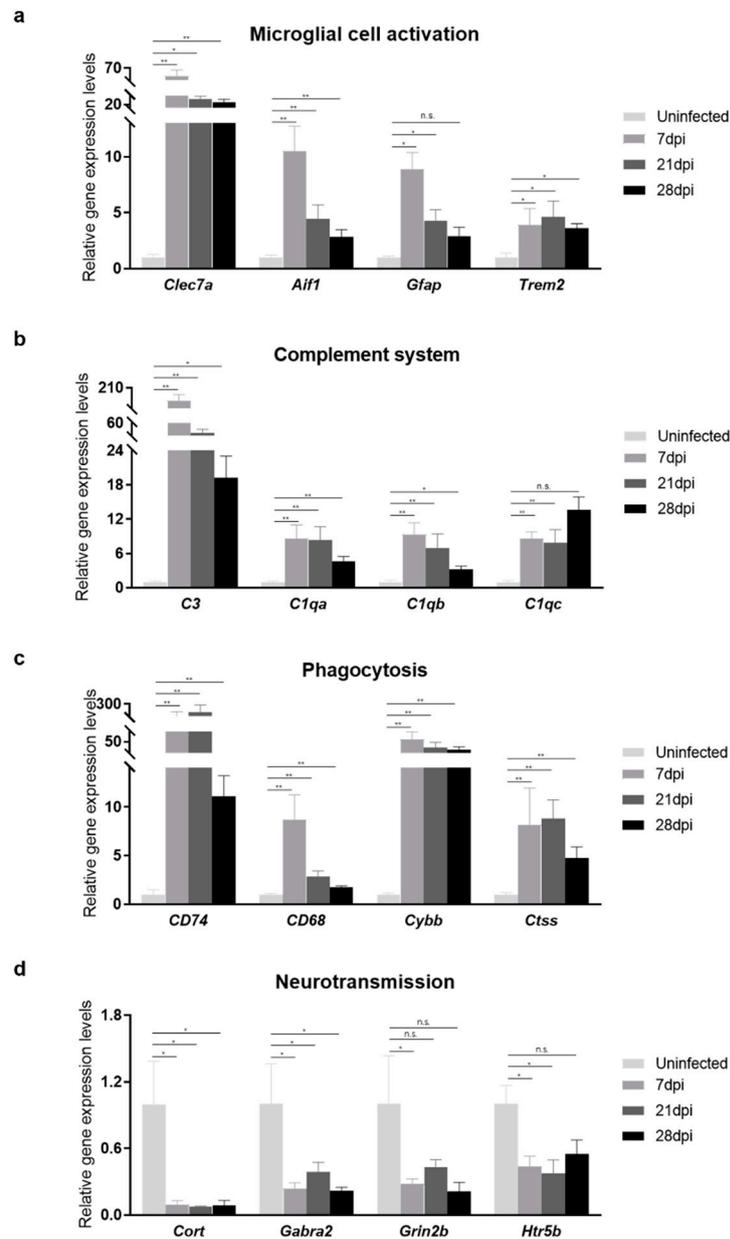
**Figure S1. Expression levels of ZIKV E protein in ZIKV-infected brain tissues at different time points.** Western blot analyses were performed on brain tissues obtained at 7, 21, and 28 days post-infection (dpi), with two samples per time point ( $n = 2$  per group). The expression levels of ZIKV E protein were assessed, with  $\beta$ -actin used as the loading control.



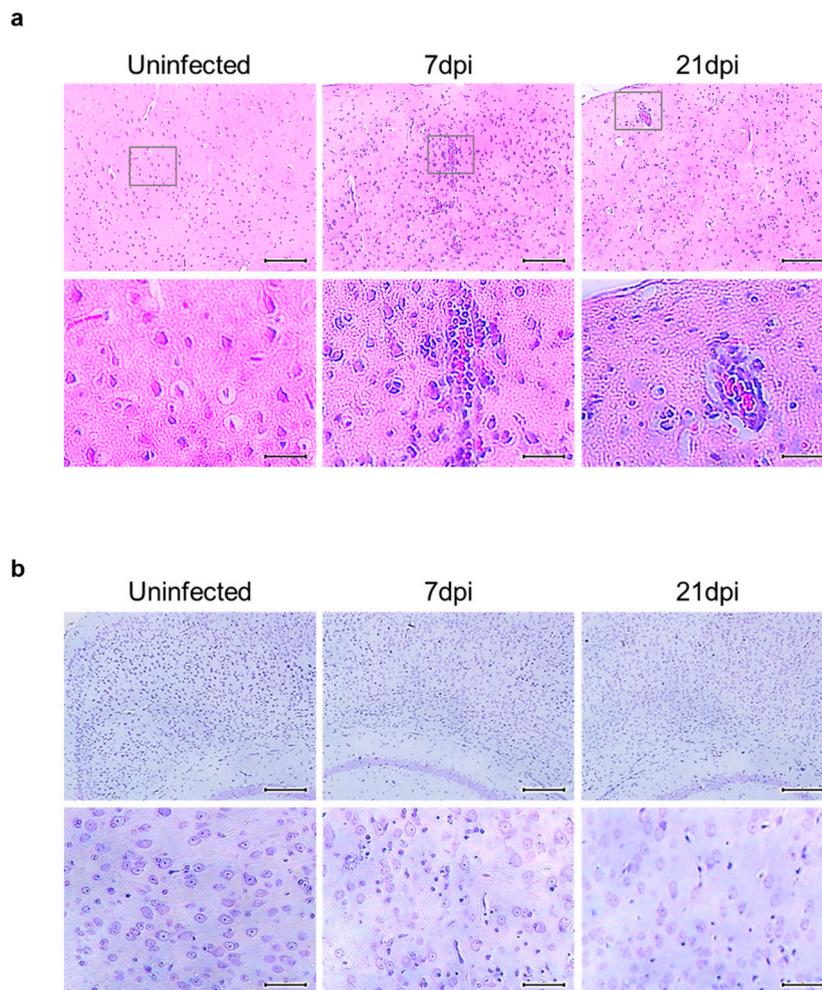
**Figure S2. Transcriptional analysis of upregulated and downregulated genes in the mouse brain following ZIKV infection.** (a) Log<sub>2</sub>-fold changes of significantly regulated genes ( $p < 0.05$ ) in the cerebral cortex of ZIKV-infected mice obtained at 7 dpi. (b) Log<sub>2</sub>-fold changes of significantly regulated genes ( $p < 0.05$ ) in the cerebral cortex of ZIKV-infected mice obtained at 21 dpi.



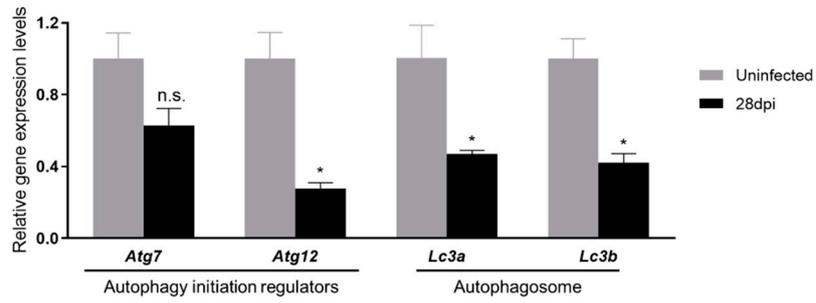
**Figure S3. RT-qPCR analysis of cytokine genes.** Relative mRNA levels of the cytokine genes, *IL-1α*, *IL-1β*, *Cxcr4* and *Cxcl10*, at 7 and 21 dpi in the cerebral cortex of mice infected with ZIKV-FLR strain. mRNA levels were normalized to *Gapdh* mRNA levels. Data represent the means  $\pm$  SEM of at least four independent replicates (\*p < 0.05; \*\*p < 0.01).



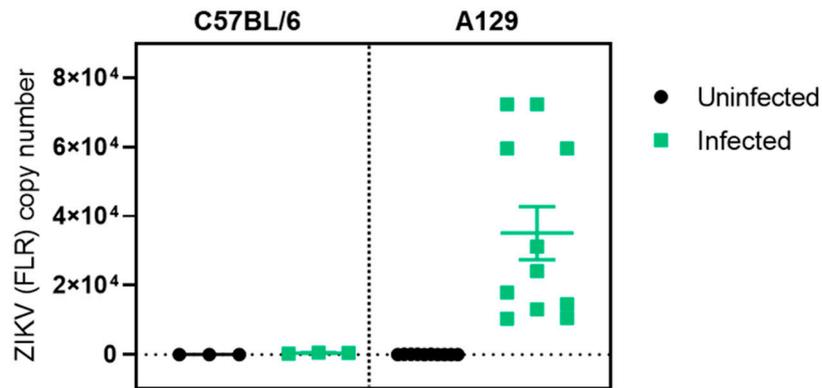
**Figure S4. Transcriptional analysis of genes related to microglial activation, complement system, phagocytosis, and neurotransmission in the brains of ZIKV-infected and recovered mice.** RT-qPCR analyses were performed to assess genes associated with (a) microglial activation, (b) the complement system, (c) phagocytosis, and (d) neurotransmission. mRNA levels were normalized to *Gapdh* and presented relative to uninfected controls. Data are shown as means  $\pm$  SEM from at least four independent replicates (\* $p < 0.05$ ; \*\* $p < 0.01$ ; n.s., not significant).



**Figure S5. Histopathological analysis of ZIKV-infected mouse brain cortices.** (a) Representative images of hematoxylin & eosin (H&E) staining in the cortex, with boxes highlighting areas of immune cell infiltration and nuclear shrinkage compared to uninfected controls (n = 3 per group; magnification: 40x and 400x; scale bar: 20  $\mu$ m). (b) Representative images of Nissl staining in the cortex. (n = 3 per group, magnification: 40x and 200x, Scale bar, 20  $\mu$ m).



**Figure S6. RT-qPCR analysis of autophagy-related genes in the cerebral cortex of ZIKV-infected mice.** Relative mRNA levels of *Atg7*, *Atg12*, *Lc3a*, and *Lc3b* were measured at 28 dpi in mice infected with the ZIKV-FLR strain. mRNA levels were normalized to *Gapdh*. Data are presented as means  $\pm$  SEM of four independent replicates (\* $p < 0.05$ ; *n.s.*, not significant).



**Figure S7. Comparison of ZIKV replication in the brain cortices of immunocompetent C57BL/6 and immunocompromised A129 mice.** ZIKV (FLR) RNA levels in mouse brain tissues were quantified using RT-qPCR analysis. Relative ZIKV RNA levels are shown in the brains of both immunocompetent and immunocompromised mice at 7 dpi. Data are presented as means  $\pm$  SEM from at least three mice per group.

**Table S1. qPCR primers used in the study**

| <b>Gene</b>          | <b>Forward primer</b>         | <b>Reverse primer</b>         |
|----------------------|-------------------------------|-------------------------------|
| <b><i>Clec7a</i></b> | 5'-CCAGCTAGGTGCTCATCTACTG-3'  | 5'-CCTTCACTCTGATTGCGGGAAAG-3' |
| <b><i>Aif1</i></b>   | 5'-TCTGCCGTCCAAACTTGAAGCC-3'  | 5'-CTCTTCAGCTCTAGGTGGGTCT-3'  |
| <b><i>Gfap</i></b>   | 5'-CACCTACAGGAAATTGCTGGAGG-3' | 5'-CCACGATGTTCTCTTGAGGTG-3'   |
| <b><i>Trem2</i></b>  | 5'-CTACCAGTGTCAGAGTCTCCGA-3'  | 5'-CCTCGAAACTCGATGACTCCTC-3'  |
| <b><i>C3</i></b>     | 5'-AGCAGGTCATCAAGTCAGGC-3'    | 5'-GATGTAGCTGGTGTGGGCT-3'     |
| <b><i>C1qa</i></b>   | 5'-GTGGCTGAAGATGTCTGCCGAG-3'  | 5'-TTAAACCTCGGATACCAGTCCG-3'  |
| <b><i>C1qb</i></b>   | 5'-CAACCAGGCACTCCAGGGATAA-3'  | 5'-CCAACCTTGCCTGGAGTCCCAG-3'  |
| <b><i>C1qc</i></b>   | 5'-AAGGACGGGCATGATGGACTCC-3'  | 5'-TTTCCCACGGTGGCCAGGCAT-3'   |
| <b><i>CD74</i></b>   | 5'-GCTGGATGAAGCAGTGGCTCTT-3'  | 5'-GATGTGGCTGACTTCTTCCTGG-3'  |
| <b><i>CD68</i></b>   | 5'-GGCGGTGGAATACAATGTGTCC-3'  | 5'-AGCAGGTCAAGGTGAACAGCTG-3'  |
| <b><i>Cybb</i></b>   | 5'-TGCGATCTCAGCAAAAGGTGG-3'   | 5'-GTACTGTCCCACCTCCATCTTG-3'  |
| <b><i>Ctss</i></b>   | 5'-GCATAGAGGCAGACGCTTCCTA-3'  | 5'-CCACTGCTTCTTTCAGGGCATC-3'  |
| <b><i>Cort</i></b>   | 5'-GGTCGCAGCCTCCGCCCTTC-3'    | 5'-TTGGGAAGCCCACTCGTGCCA-3'   |
| <b><i>Grin2b</i></b> | 5'-TTCTATCCCCGGCATCCAGCG-3'   | 5'-CGTGGAGCGTGGTCATTCCCA-3'   |
| <b><i>Gabra2</i></b> | 5'-CTCTCCCAAGTGTCATTCTGGC-3'  | 5'-CGAGCACTGATGCTCAAGGTTG-3'  |
| <b><i>Htr5b</i></b>  | 5'-GTGGTGCTCTTCGTCTACTGGA-3'  | 5'-GGCTGTGAACACCATCTCAGAC-3'  |
| <b><i>Atg7</i></b>   | 5'-CCTGTGAGCTTGGATCAAAGGC-3'  | 5'-GAGCAAGGAGACCAGAACAGTG-3'  |
| <b><i>Atg12</i></b>  | 5'-GAAGGCTGTAGGAGACACTCCT-3'  | 5'-GGAAGGGGCAAAGGACTGATTC-3'  |
| <b><i>Lc3a</i></b>   | 5'-CTGCCTGTCCTGGATAAGACA-3'   | 5'-CTGGTTGACCAGCAGGAAGAAG-3'  |
| <b><i>Lc3b</i></b>   | 5'-GTCCTGGACAAGACCAAGTTCC-3'  | 5'-CCATTCACCAGGAGGAAGAAGG-3'  |
| <b><i>Gapdh</i></b>  | 5'-TTGCTGTTGAAGTCGCAGGAG-3'   | 5'-TGTGTCCGTCGTGGATCTGA-3'    |

**Table S2. Antibodies used in the study**

| <b>Antibody</b>                                    | <b>Source</b>             | <b>Identifier</b> |
|--|---------------------------|-------------------|
| Phospho-Tau (Ser396) (PHF13) mouse mAb             | Cell Signaling Technology | Cat# 9632         |
| MAP2 rabbit pAb                                    | Abcam                     | Cat# ab32454      |
| Alexa Fluor 488 goat anti-mouse antibody           | Invitrogen                | Cat# R37120       |
| Alexa Fluor 594 goat anti-rabbit antibody          | Invitrogen                | Cat# R37117       |
| Anti-Zika Envelope mAb                             | Bio front technology      | Cat# BF-1176-56   |
| Anti-beta actin                                    | Abcam                     | Cat# ab8277       |
| Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP  | Invitrogen                | Cat# 31430        |
| Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP | Invitrogen                | Cat# 31460        |