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Supplemental Information

Integrin αvβ5 Internalizes Zika Virus during Neural Stem Cells Infection and Provides a Promising Target for Antiviral Therapy

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Supplementary Figure 1 (Related to Figure 1)

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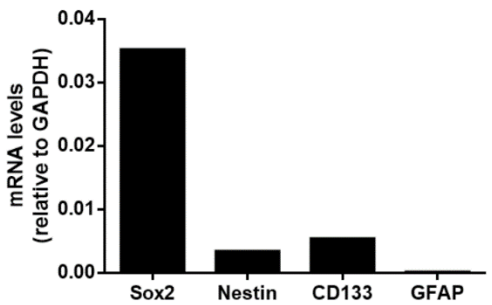

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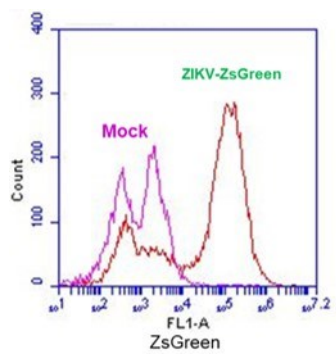

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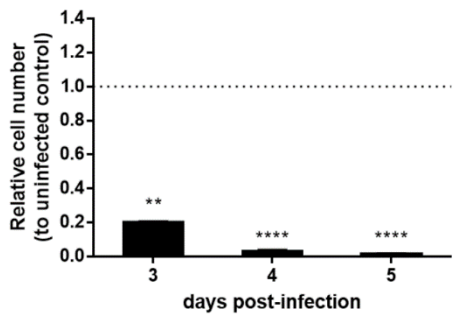

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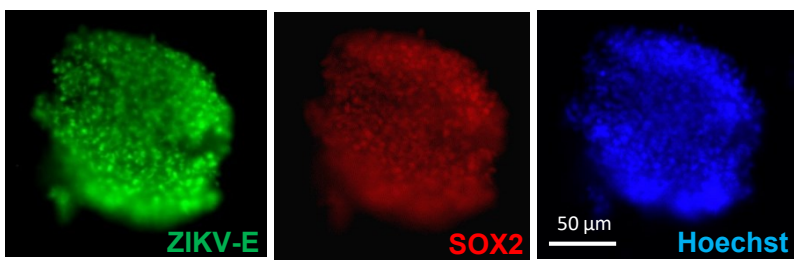

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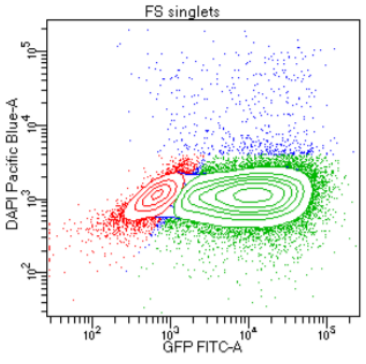
Gates for ZsGreen- populations



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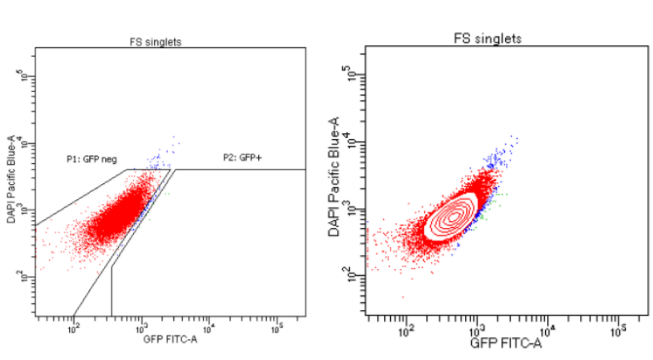
Gate for uninfected TS576



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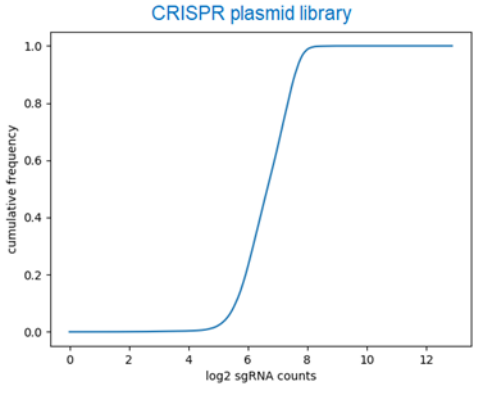

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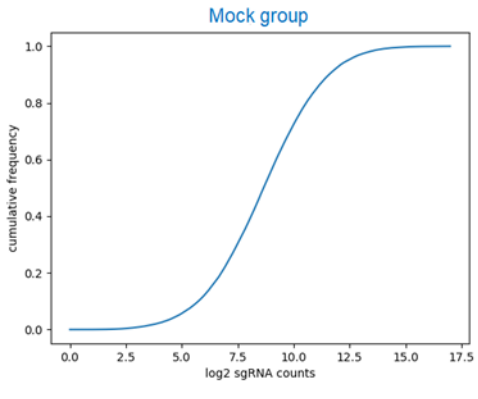

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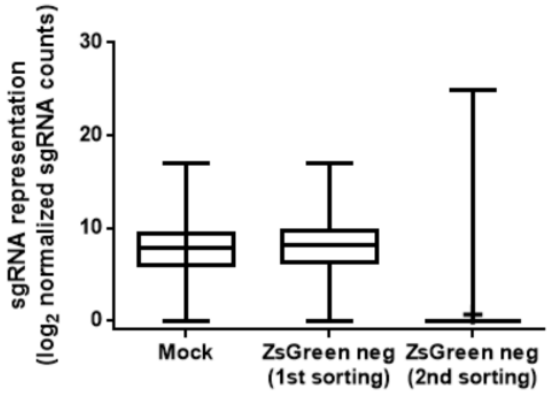


Supplemental Figure 1 (Related to Figure 1). Genome-wide CRISPR knockout screen in glioblastoma stem cells (GSCs) identifies ZIKV dependency factors.

(A) Relative maker gene expressions in glioblastoma stem cells.

(B) The ZsGreen signal in ZIKV-ZsGreen infected GSCs was quantified by flow analysis. n=3.

(C) ZIKV infection induced cytopathic effect in glioblastoma stem cell. Mean ± SD of n = 3. $^{**}p < 0.01$, $^{****}p < 0.0001$, by Student's *t* test.

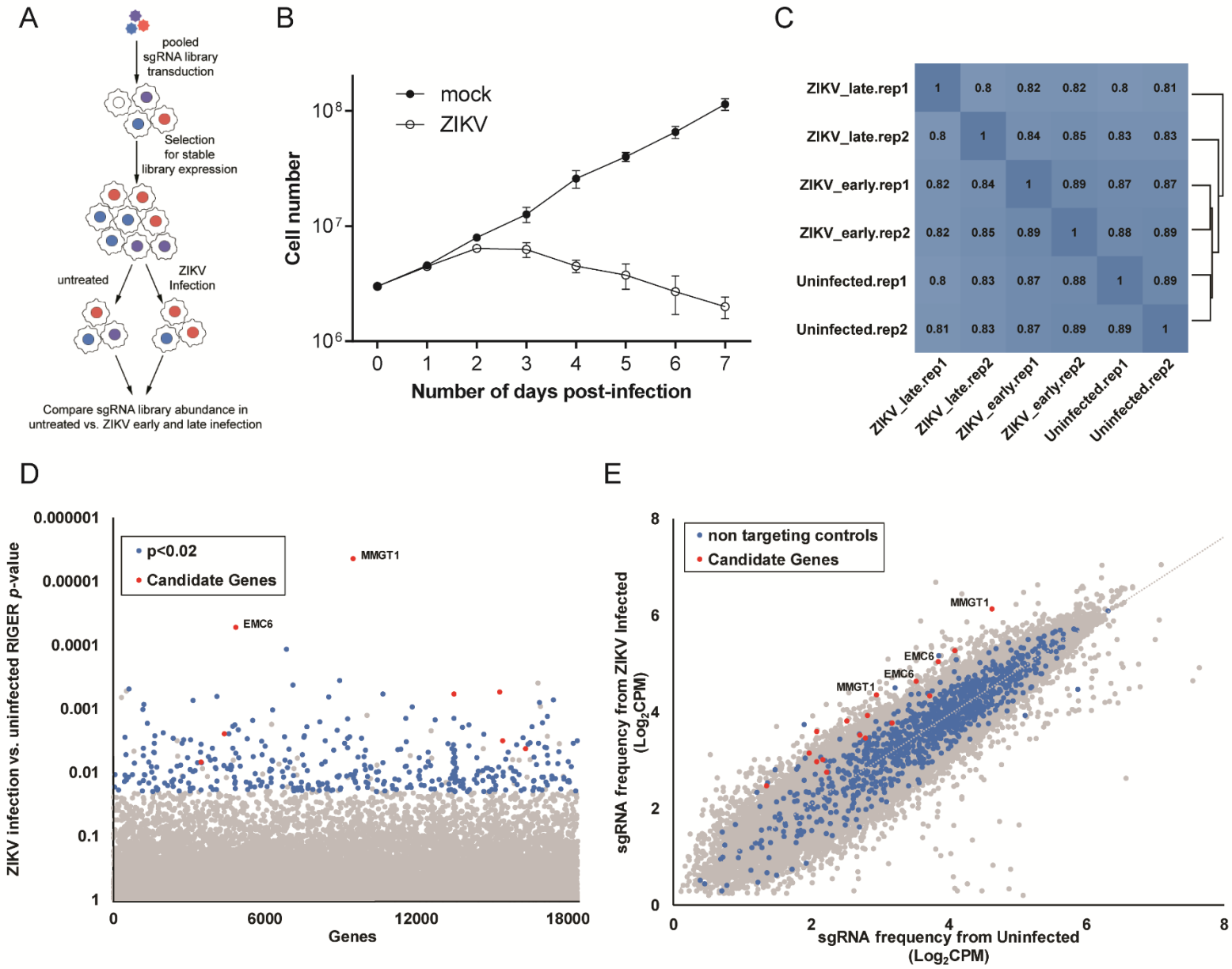
(D) Stemness of the GSCs was not affected by ZIKV infection. Stem cell marker SOX2 was detected in ZIKV-ZsGreen infected GSCs. n=3.

(E and F) Gating protocol for ZsGreen positive and negative cells in sorting.

(G and H) The distribution of the sgRNA was shown in our plasmid (G) and mock (H) group.

(I) sgRNA representation in the CRISPR screen.

Supplementary Figure 2 (Related to Figure 2)



Supplementary Figure 2 (Related to Figure 2). CRISPR knockout screen in 293FT cells identifies cell specific ZIKV dependency factors.
(A) Schematic representation of the genome-wide CRISPR/Cas9 screen. Briefly, 293FT cells were transduced with Gecko sgRNA pooled library and selected in puromycin. Transduced cells were then mock-infected or ZIKV-infected and genomic DNA harvested at early (Day 4 post infection) or late infection (Day 7 post infection) time points. sgRNA sequencing libraries were prepared from genomic DNA by nested PCR and quantified on Illumina NextSeq.
(B) Growth curves of 293T cells mock or ZIKV-infected (MOI = 10) over a time period of 7 days. Error bars represent ± s.e.m, n=3.
(C) Pearson Correlation of normalized sgRNA read counts between treatment conditions and biological replicates.
(D) Identification of sgRNAs consistently enriched in ZIKV infected vs uninfected cells by RIGER *p* value analysis. Genes with *p*-value < 0.02 are highlighted in blue.
(E) Scatterplot of log normalized read counts between ZIKV infected and uninfected cells. Enrichment of specific sgRNAs identified by RIGER analysis and chosen for subsequent validation are shown in red as well as the set of non-targeting controls in blue.
(F) *MMGT1* and *EMC6* are part of the ER membrane protein complex. STRING-db protein-protein network is shown.
(G) Pathway analysis with STRING-db revealed enrichment for membrane vesicle and endocytosis pathway with *COPB2* and *DNM2*.
(H) sgRNA validation (2 different sgRNAs per gene) of candidate genes reveals inhibition of ZIKV replication. Student's *t* test ** $p < 0.005$, *** $p < 0.0005$, and error bars indicate ± s.e.m, n=3.

Supplementary Figure 3 (Related to Figure 2)

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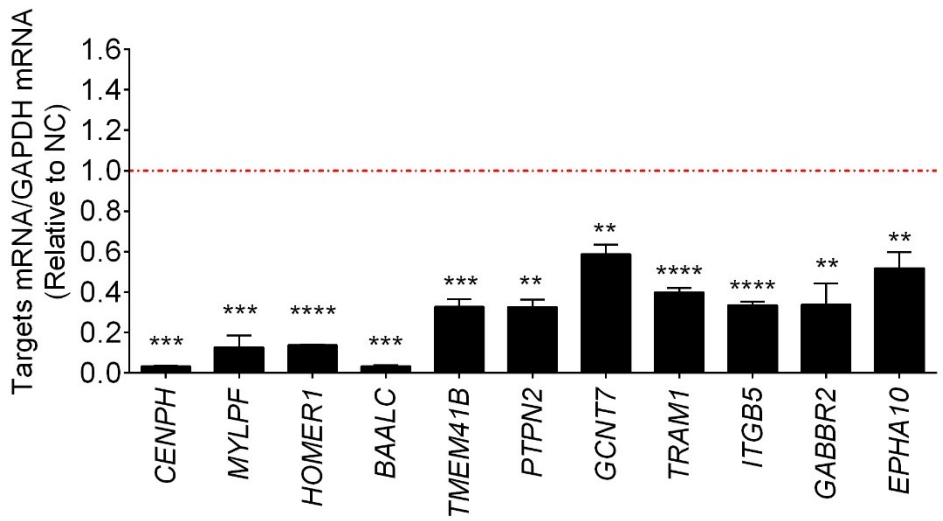

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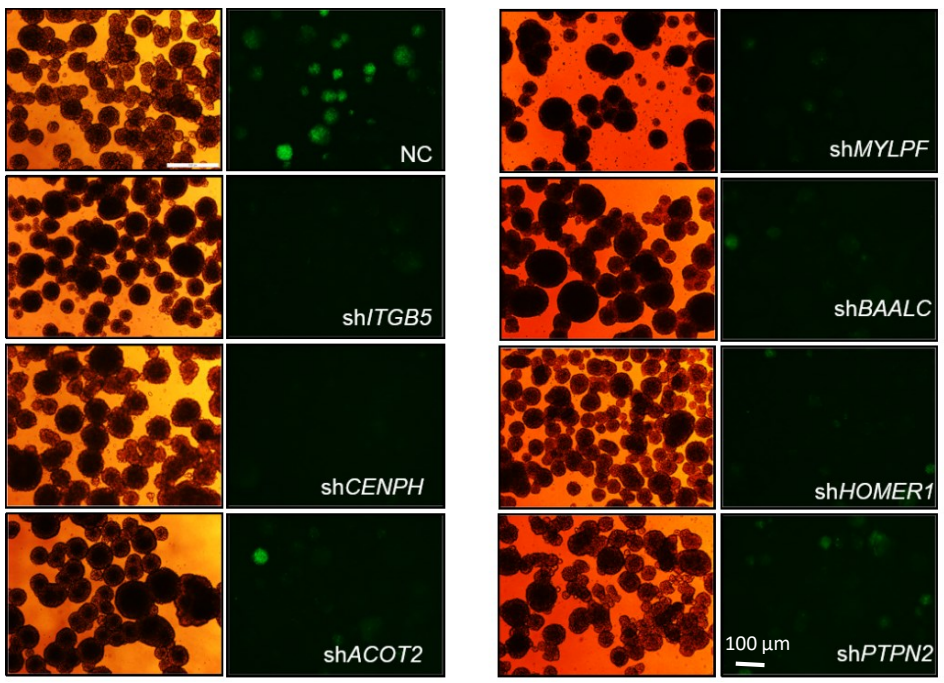

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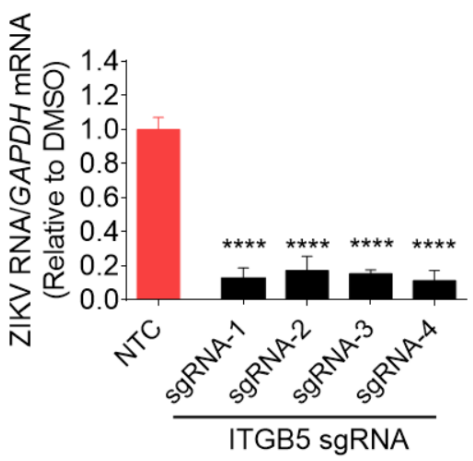

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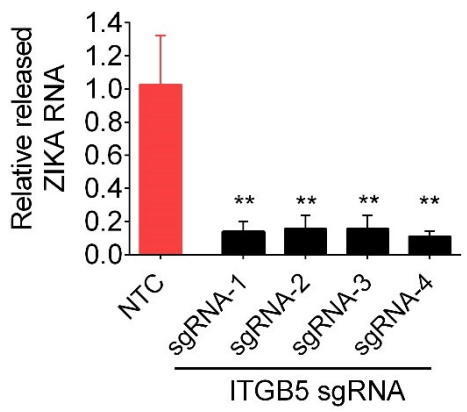

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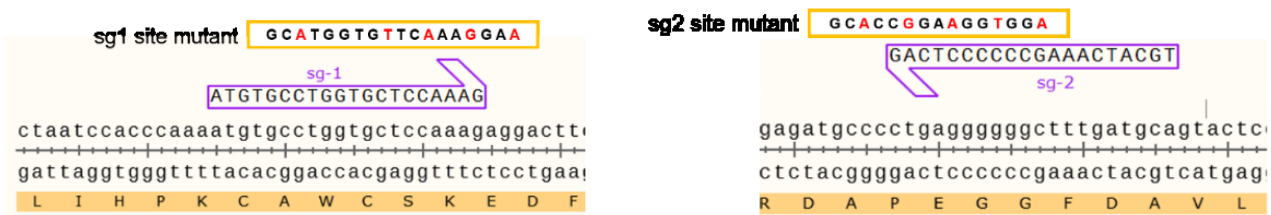


Supplemental Figure 3 (related to Figure 2). *ITGB5* is a dependency factor for ZIKV replication.

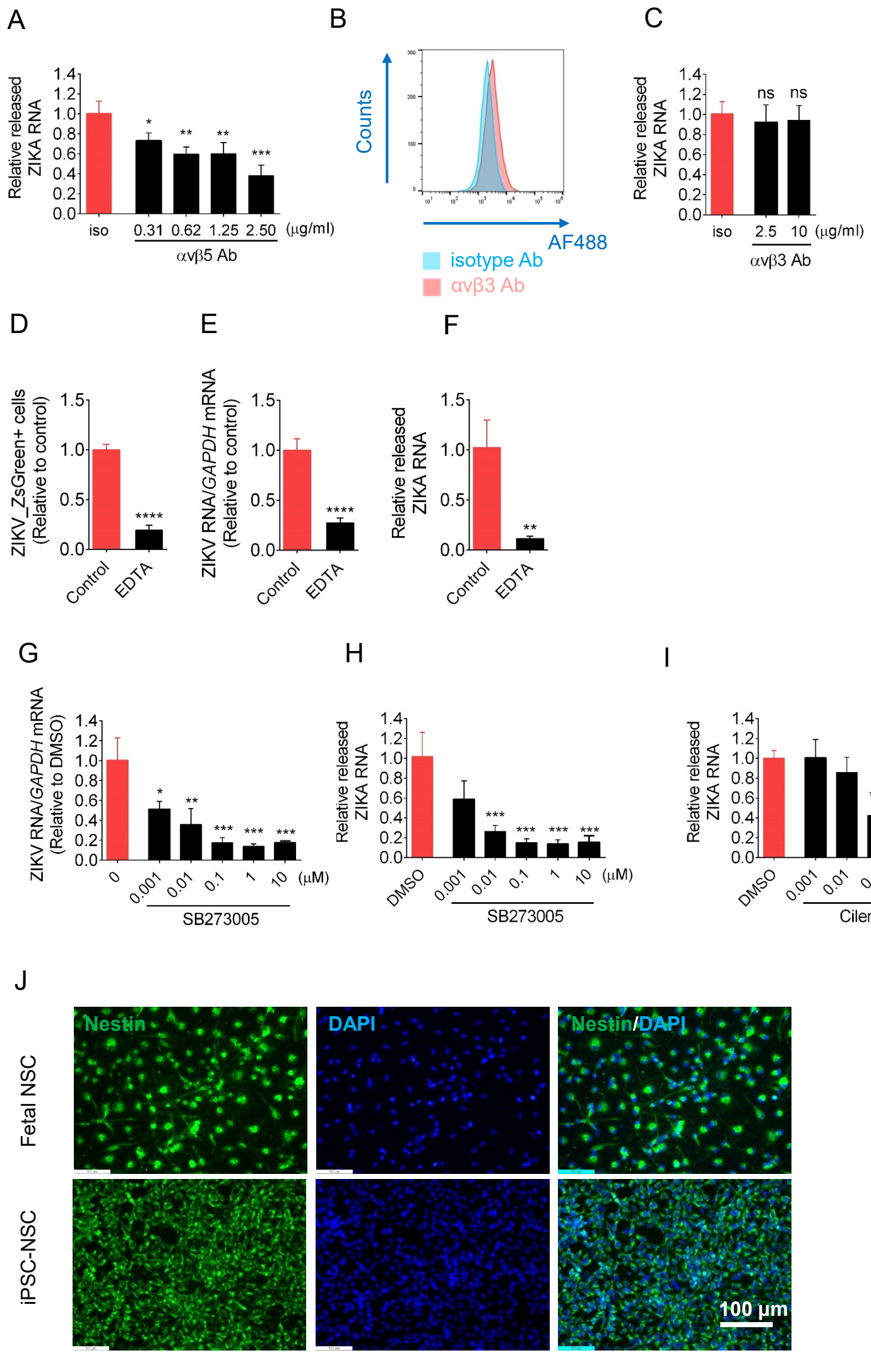
(A) Knock-down efficiency of the eleven genes in Figure 2B is shown. GSCs were transduced with lentiviruses expressing non-targeting control (NC) shRNA or pooled shRNAs for indicated genes. After 2 days, cells were infected with ZIKA ZsGreen at an MOI of 0.01 for 3 days. Cells were harvested to extract the RNA and RT-qPCR was performed to quantify the expression of target genes relative to GAPDH. Mean ± SD of n = 3. ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001, by Student's *t* test.

(B) ZIKV infection was performed in knock-down cells as described above in Figure 2B. Bright field and green fluorescence field is shown to illustrate cells and ZIKV infection. One representative of three independent experiments is shown.

(C and D) Cells were treated as in Figure 2D, and cellular (C) and released ZIKV RNA (D) levels were quantified to indicate ZIKV infection and release. Mean ± SD of n = 3. **p < 0.01, ****p < 0.0001, by Student's t test.

(E) Illustration for the design of mutant vectors. Purple arrow indicated the sequences of the sgRNA. Yellow box contains the mutant sequences. Red nucleotide is the mutated sites.

Supplementary Figure 4 (Related to Figure 3)



Supplemental Figure 4 (related to Figure 3). αvβ5 is essential for ZIKV replication.

(A) Viral titer in the supernatant was quantified using RT-PCR. Cells were treated as in Figure 3A. Mean ± SD of n = 3. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by Student's *t* test.

(B) αvβ3 was expressed on TS576 cells. TS576 cells were stained with αvβ3 antibody or isotype antibody, and flow cytometry was used to identify the expression αvβ3. Blue shading represents cells staining with isotype antibody and the red shading represents TS576 staining with αvβ3 antibody. n=3.

(C) Viral titer in the supernatant was quantified using RT-qPCR. Cells were treated as in Figure 3B. Mean ± SD of n = 3. ns, no significance, by Student's *t* test.

(D-F) EDTA treatment inhibited ZIKV infection. We pretreated cells with 5mM EDTA and infected with ZIKV. After 1 h incubation, virus and EDTA were removed. Virus infection was detected using flow cytometry (D), cellular (E) and released ZIKV RNA (F) was quantified by RT-PCR. Mean ± SD of n = 3. **$p < 0.01$, ****$p < 0.0001$, by Student's *t* test.

(G) Cellular RNA was quantified using RT-PCR. Cells were treated as Figure 3E. Mean ± SD of n = 3. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by Student's *t* test.

(H-I) Viral titer in the supernatant was quantified using RT-qPCR. Cells were treated as Figure 3E and 3F. Mean ± SD of n = 3. ***$p < 0.001$, ****$p < 0.0001$, by Student's *t* test.

(J) Characterization of human neural stem cells (fetal NSCs or iPSC-derived) showing expression of neural stem cells (NSC) marker nestin (Green) co-labelled with nuclear marker DAPI (Blue). One representative of three independent experiments is shown.

Supplementary Figure 5 (Related to Figure 5)

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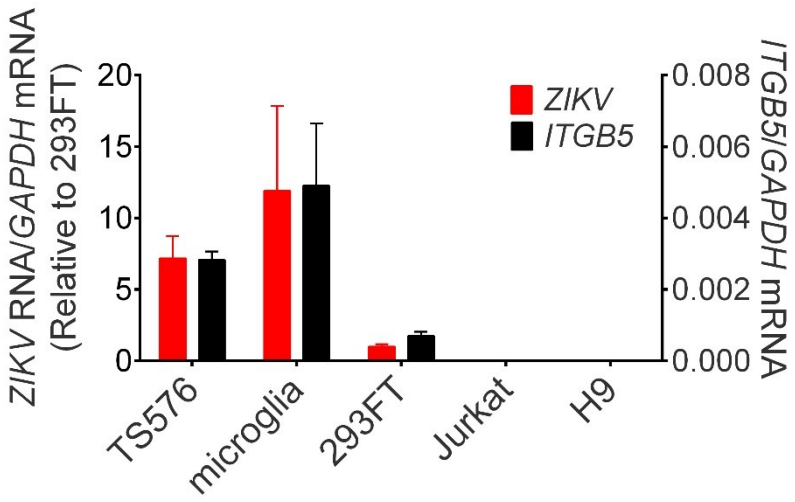

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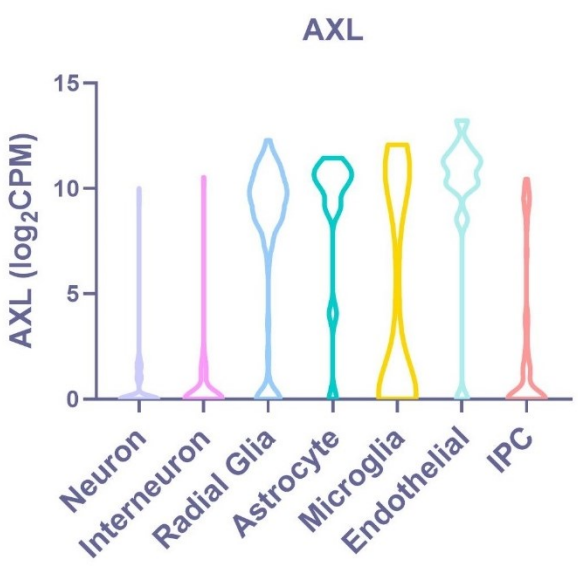

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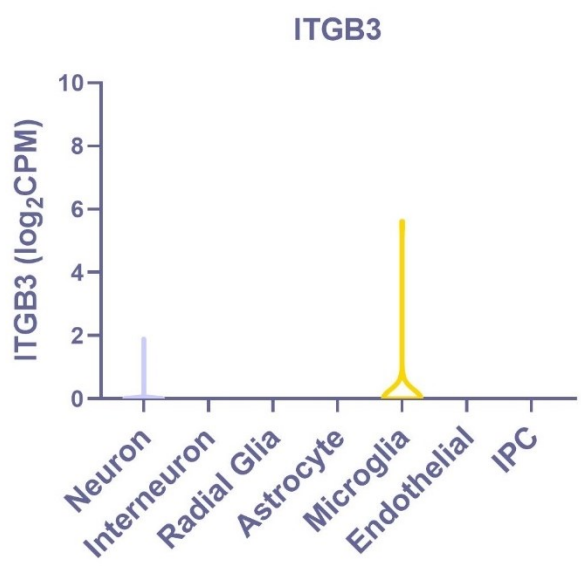


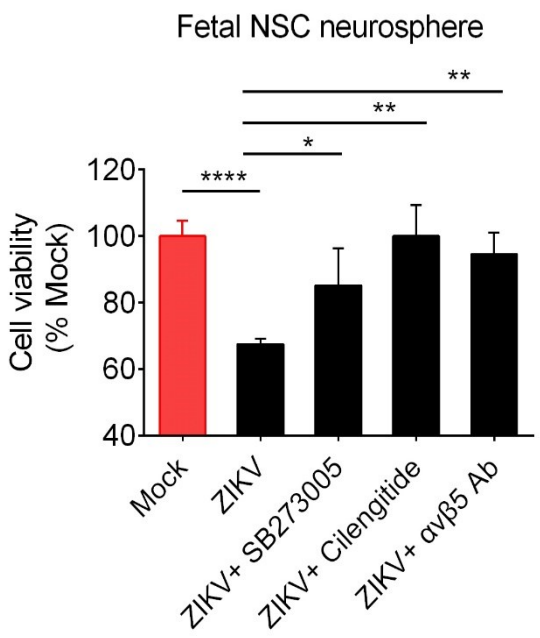
Supplemental Figure 5 (related to Figure 5). Integrin β5 (*ITGB5*) expression in various cell types.

(A) *ITGB5* expression and ZIKV infection were quantified by RT-qPCR in cDNA reverse-transcribed of RNA isolated from various infected cell lines. Mean ± SD of n = 3.

(B and C) Single-cell transcriptomics from developing human cerebral cortex were extracted from Nowakowski et.al (Nowakowski et al., 2016). Violin plots showing distribution of expression levels of *AXL* (B) and *ITGB3* (C) across single cells of each respective cell type including neuron, interneuron, radial glia, astrocyte, microglia, endothelial and IPC.

Supplementary Figure 6 (Related to Figure 6)

A



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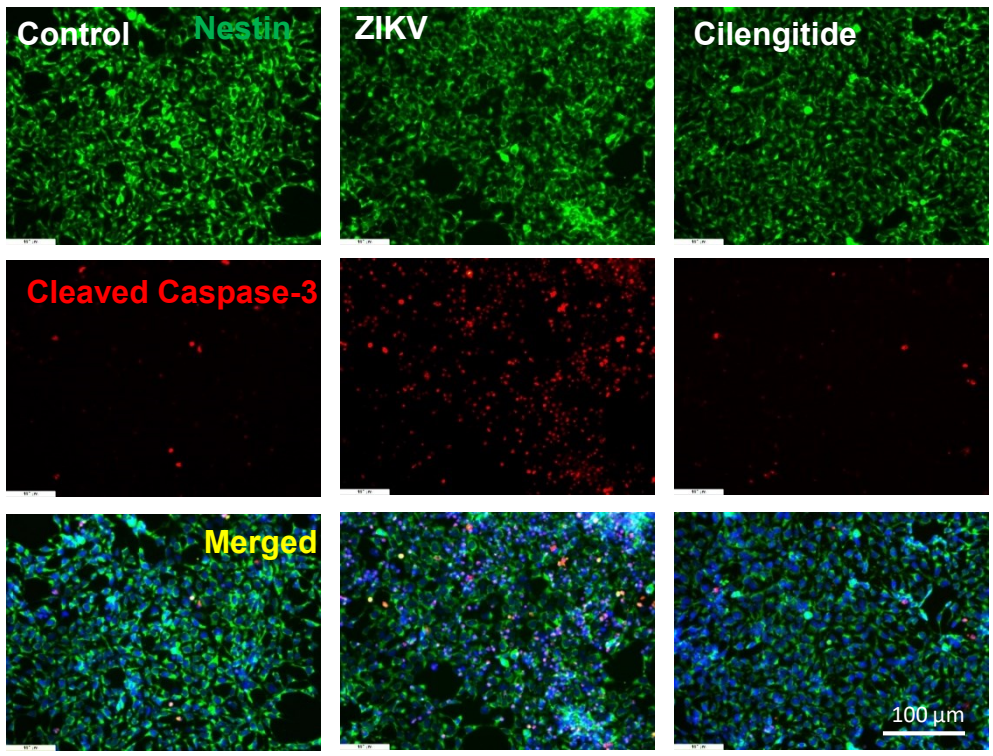

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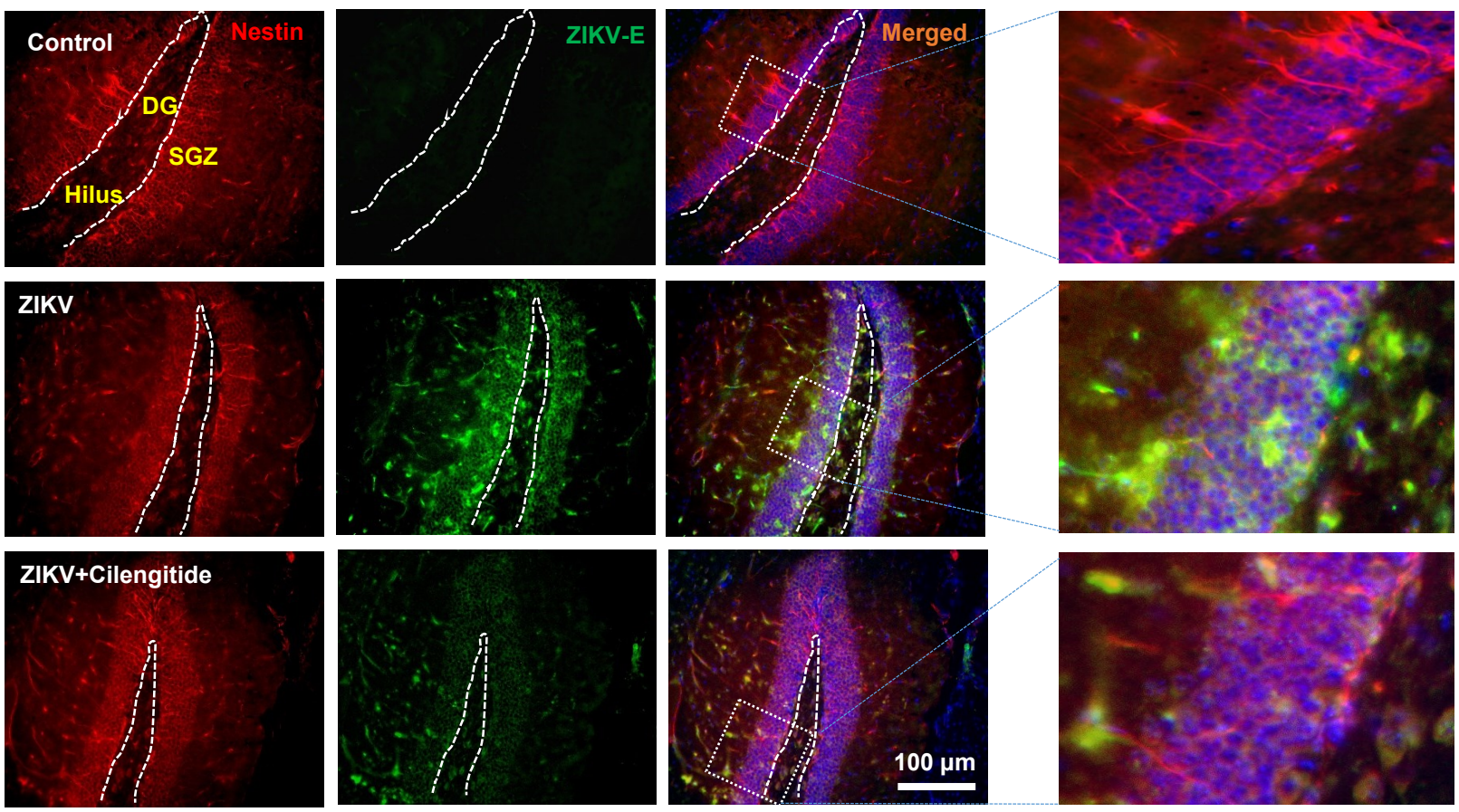

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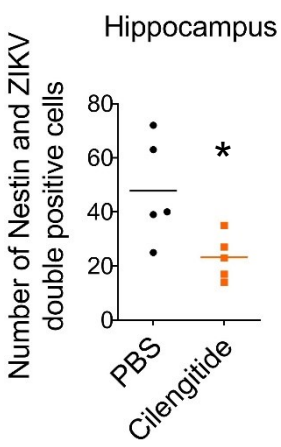

Supplemental Figure 6 (related to Figure 6). Cilengitide inhibits the ZIKV infection in hNSCs and in mouse brain

(A and B) αvβ5 inhibitors and blocking antibody decreases ZIKV-induced cytopathic effect in NSCs. Cell viability quantification was performed in hfNSC as in Figure 6C (A). Cleaved caspase-3 staining in ZIKV infected cell mock treated or pre-incubated with 2.5 μM cilengitide is shown (B). Mean ± SD of n = 4. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, by Student's *t* test.

(C-D) Mice were treated as in Figures 6D and E. (C) Images showing immunostaining of neural stem cell marker (Nestin) (Red) with ZIKV envelope flavivirus group antigen antibody (ZIKV-E) cells (Green) in the dentate gyrus region of the hippocampus in PBS-treated or cilengitide treated mice after 6 days post-infection. (D) Nestin/ZIKV-E double positive cells were quantified. DG = dentate gyrus, SGZ = sub-granular zone. *$p < 0.05$, by Student's *t* test.