

Supplemental Figures

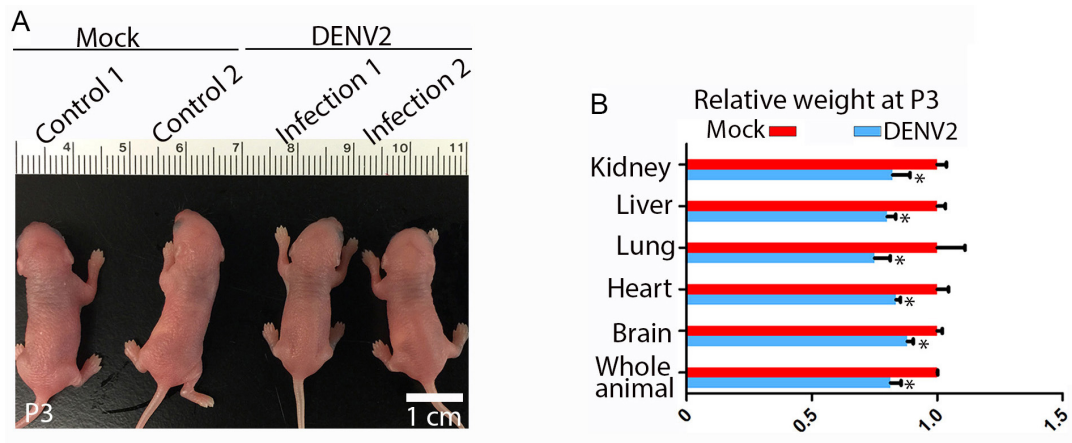


Fig.S1

Fig. S1: DENV2 is sufficient to cause growth restriction at P3. (A) Dorsal views of P3 mouse pups after mock or DENV2 intracerebral inoculation of the brain at E14.5. Scale bar: 1 cm. (B) Relative weights of different organs from control and DENV2 infected pups at P3. Error bars indicate the SEM of four independent experiments, * $p < 0.05$ (Student's t-test).

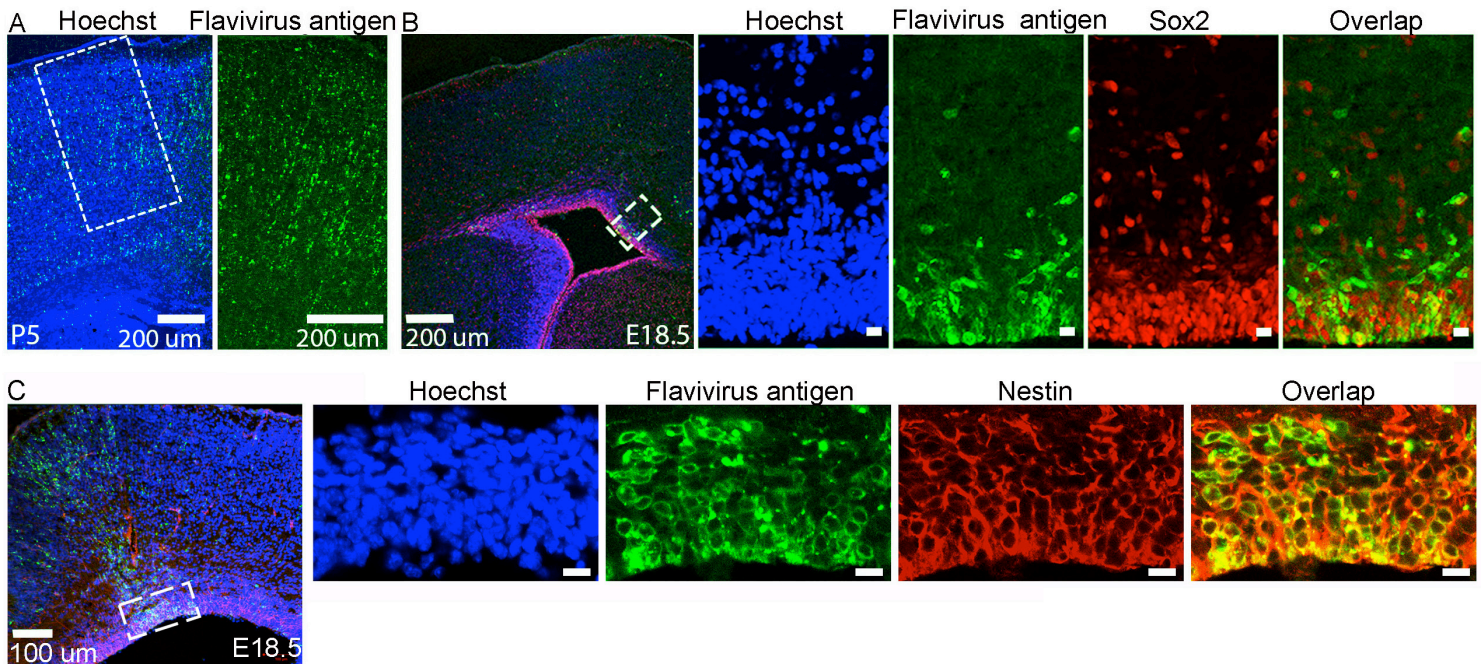


Fig. S2: DENV2 infection has minimal impact on NPC proliferation. (A) Confocal imaging of E18.5 cerebral cortex stained with antibodies against CldU (green) after 2hr CldU pulse. Hoechst stains nuclei (blue). Scale bar: 100 μm. (B) Quantification of the percentage of CldU-positive cells out of total cells in DENV2-infected cortices normalized to controls. Error bars indicate SEM of 9 sections from three independent experiments. Student's t-test revealed no significant difference between mock and DENV2-infected groups. (C) Confocal imaging of E18.5 cerebral cortex stained with antibodies against Cyclin D1 (red). Hoechst stains nuclei (blue). Scale bar: 100 μm. (D) Quantification of the percentage of Cyclin D1-positive cells out of total cells in DENV2-infected cortices normalized to controls. Error bars indicate SEM of 9 sections from three independent experiments. Student's t-test revealed no significant difference between mock and DENV2-infected groups.

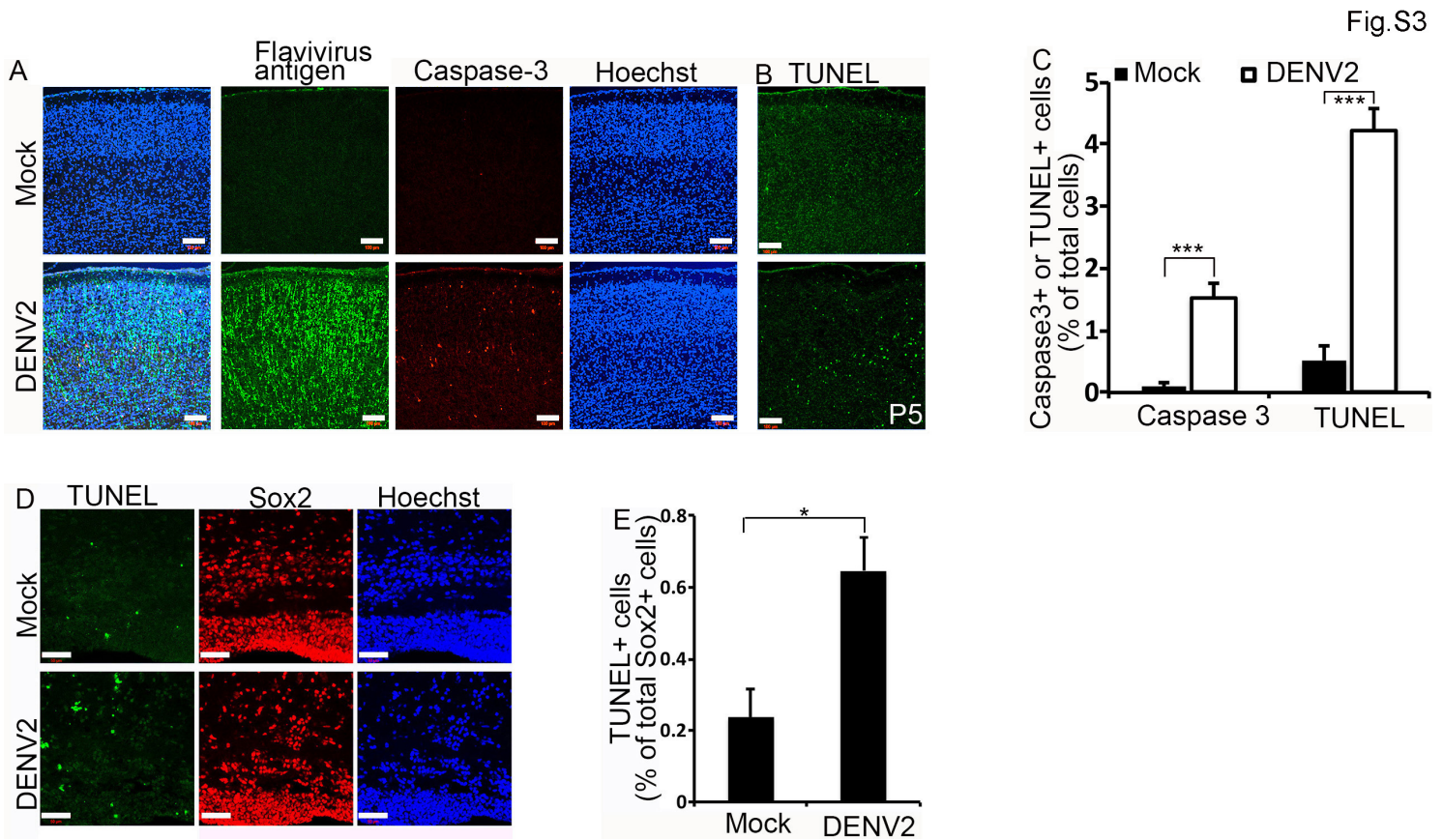


Fig. S3: DENV2 infection leads to increased cell death in neurons and NPCs. (A) Confocal imaging of P5 cerebral cortex stained with antibodies against Flavivirus group antigen (labeling DENV2, green) and cleaved Caspase 3 (red). Hoechst stains nuclei (blue). Scale bar: 100 μ m. (B) TUNEL staining (green) reveals an increase in cell death in DENV2-infected cortices compared to controls. Hoechst stains nuclei (blue). Scale bar: 100 μ m. (C) Quantification of the percentage of Caspase-3 or TUNEL-positive cells out of total cells in DENV2-infected cortices normalized to controls. Error bars indicate SEM of 9 sections from three independent experiments, *** p < 0.001 (Student's t-test). (D) TUNEL staining (green) reveals apoptotic cell death in Sox2-positive neural progenitor cells (NPCs) (red). Hoechst stains nuclei (blue). Scale bar: 50 μ m. (E) Quantification of the percentage of TUNEL- and Sox2-double positive cells out of total Sox2-positive cells in D. Error bars indicate SEM of 9 sections from three independent experiments, * p < 0.05 (Student's t-test).

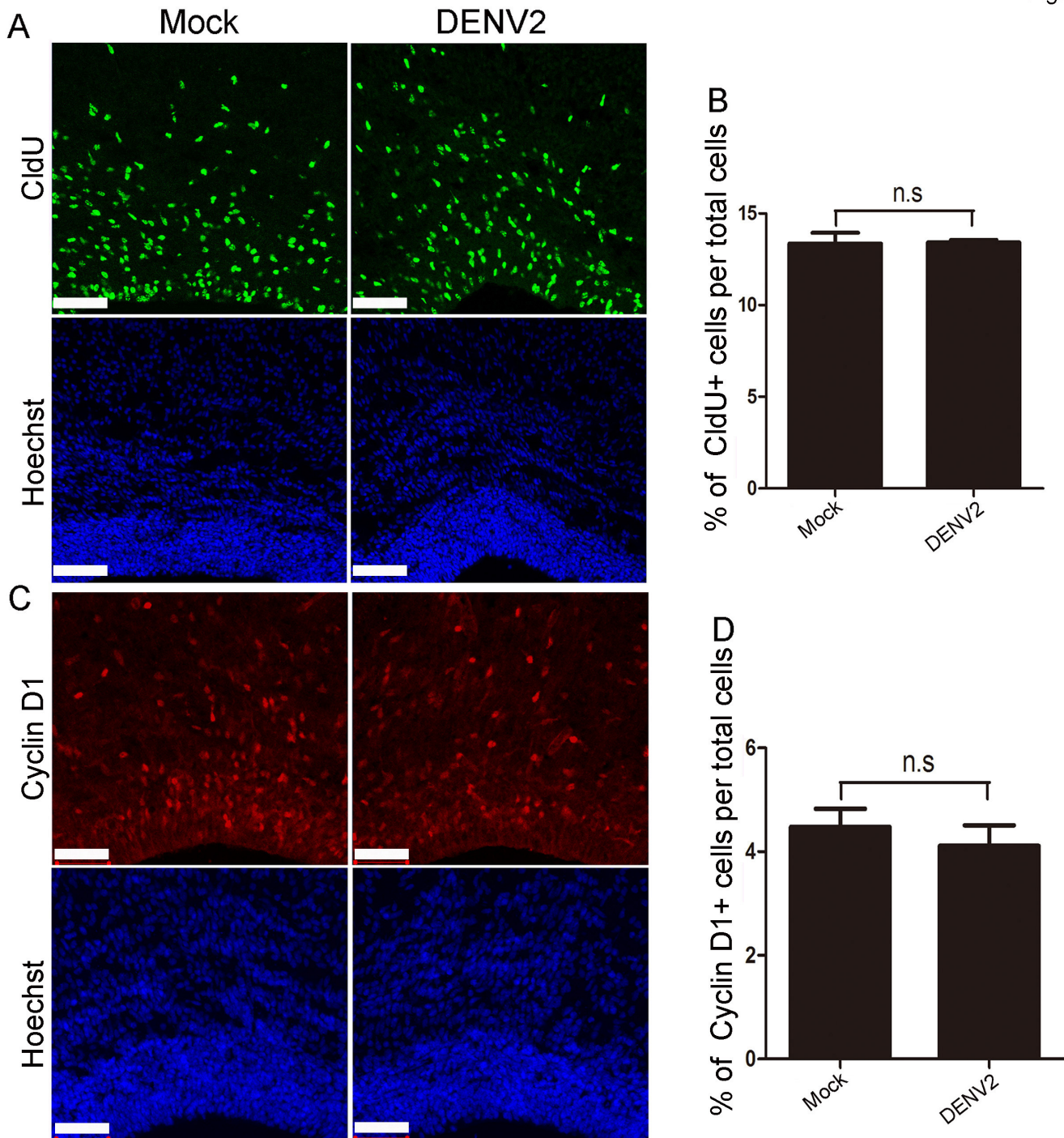


Fig. S4: MEX1-44, but not DENV2, infection causes NPC cell cycle arrest.

(A) Confocal imaging of E18.5 cerebral cortex stained with antibodies against Ki67 (labeling proliferating cells, far red) and Flavivirus group antigen (labeling DENV2 and MEX1-44, green). Hoechst stains nuclei (blue). Scale bar: 100 μ m. (B) Quantification of the percentage of Ki67-positive cells out of total cells. Error bars indicate SEM of 9 sections from three independent experiments. Two-way ANOVA revealed a significant difference between MEX1-44 and mock, and between MEX1-44 and DENV2, but no significant difference between mock and DENV2, $*p < 0.05$. (C) Confocal imaging of E18.5 cerebral cortex stained with antibodies against p-Histone3 (labeling mitotic cells, red). Hoechst stains nuclei (blue). Scale bar: 100 μ m. (D) Quantification of the percentage of p-Histone3-positive cells out of total cells in DENV2/MEX1-44 infected cortexes normalized to mock. Error bars indicate SEM of 9 sections from three independent experiments, Two-way ANOVA revealed a significant difference between MEX1-44 and mock, and between MEX1-44 and DENV2, but no significant difference between mock and DENV2, $*p < 0.05$.

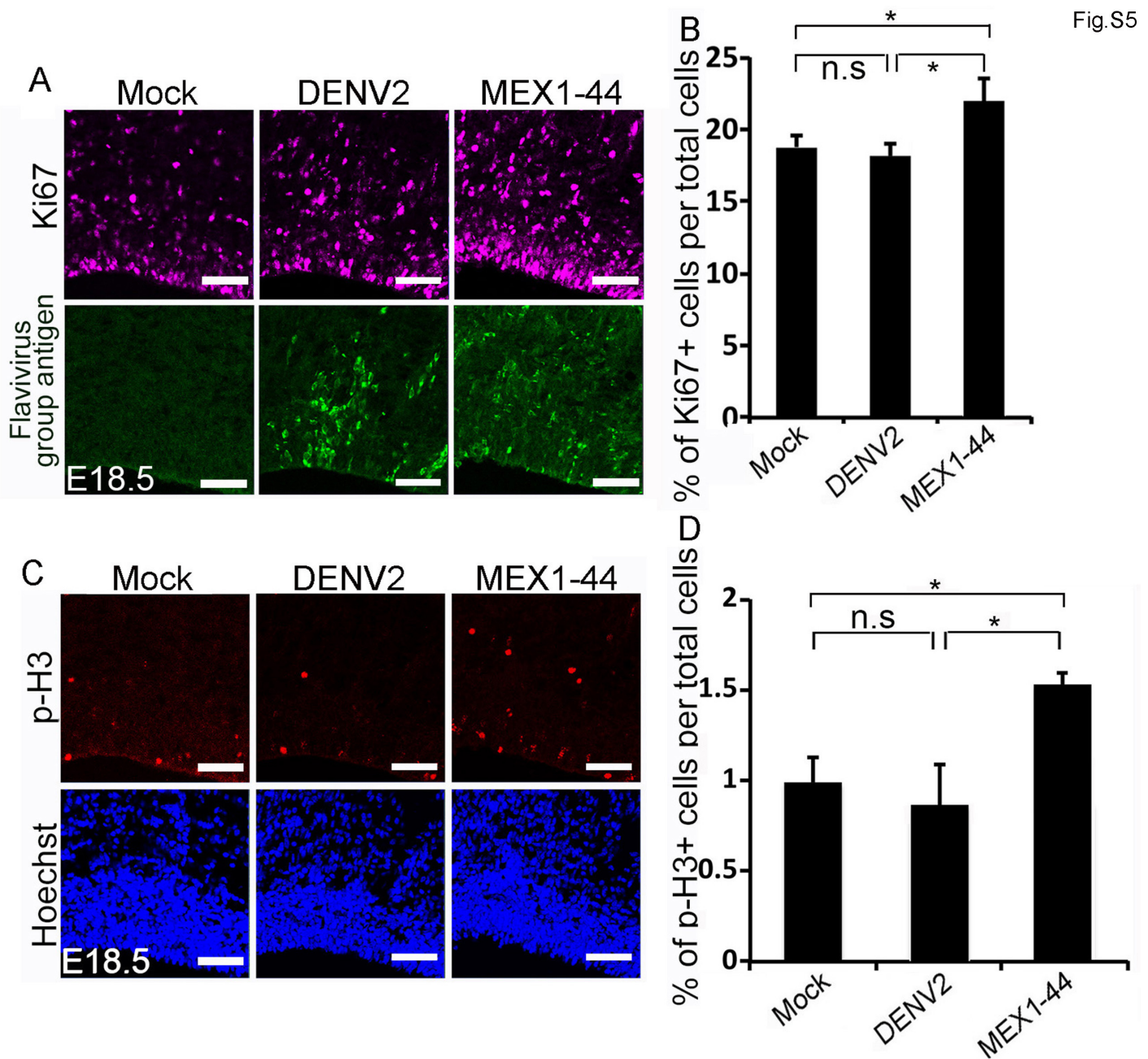


Fig. S5: DENV2 infects NPCs and is detected in the cortical plate. (A) Confocal imaging of E18.5 cortex after DENV2 inoculation of E14.5 brains stained with antibodies against Sox2 (red), and Flavivirus group antigen (green). Hoechst stains nuclei (blue). Scale bar: 200 μ m (left panel), 10 μ m (right panels). (B) Confocal imaging of P5 cortex after DENV2 inoculation of E14.5 brains stained with antibodies against Flavivirus group antigen (green). Hoechst stains nuclei (blue). Scale bar: 200 μ m. (C) Confocal imaging of E18.5 cortex after DENV2 inoculation of E14.5 brains stained with antibodies against Nestin (red), and Flavivirus group antigen (green). Hoechst stains nuclei (blue). Scale bars: 100 μ m (left panel), 10 μ m (right panels).

Table S1. List of antibodies used in the study

Antigen	Cat#	Supplier	IgG type	Dilution	Note
Flavivirus Group Antigen	MAB10216	Millipore	IgG2a	1:400(IHC)	
Tbr1	Ab31940	Abcam	Rabbit IgG	1:200(IHC)	Layer V-VI
Ctip2	Ab18465	Abcam	Rat IgG	1:200(IHC)	Layer V-VI
Cux1	Sc-13024	Santa Cruz	Rabbit IgG	1:200(IHC)	Layer II-IV
NeuN	MAB377	Millipore	IgG1	1:500(IHC)	
Caspase 3	559565	BD Bioscience	Rabbit	1:200(IHC)	
Iba1	019-19741	Wako	Rabbit	1:200(IHC)	
GFAP	Ab7260	Abcam	Rabbit	1:200(IHC)	
Sox2	14-9811	eBioscience	Rat	1:200(IHC)	
Nestin	Ab6142	Abcam	Mouse IgG1	1:200(IHC)	
Cldu	ab6326	Abcam	Alexa594	1:200(IHC)	Antigen retrieval
Ki67	M7249	Dako	Rat	1:200(IHC)	
p-Histon3(ser10)	9701	Cell signaling	Rabbit IgG	1:200(IHC)	
Cyclin D1	RM-2113-S0	Fisher	Rabbit IgG	1:200(IHC)	

Abbreviation: IHC, Immunohistochemical staining.