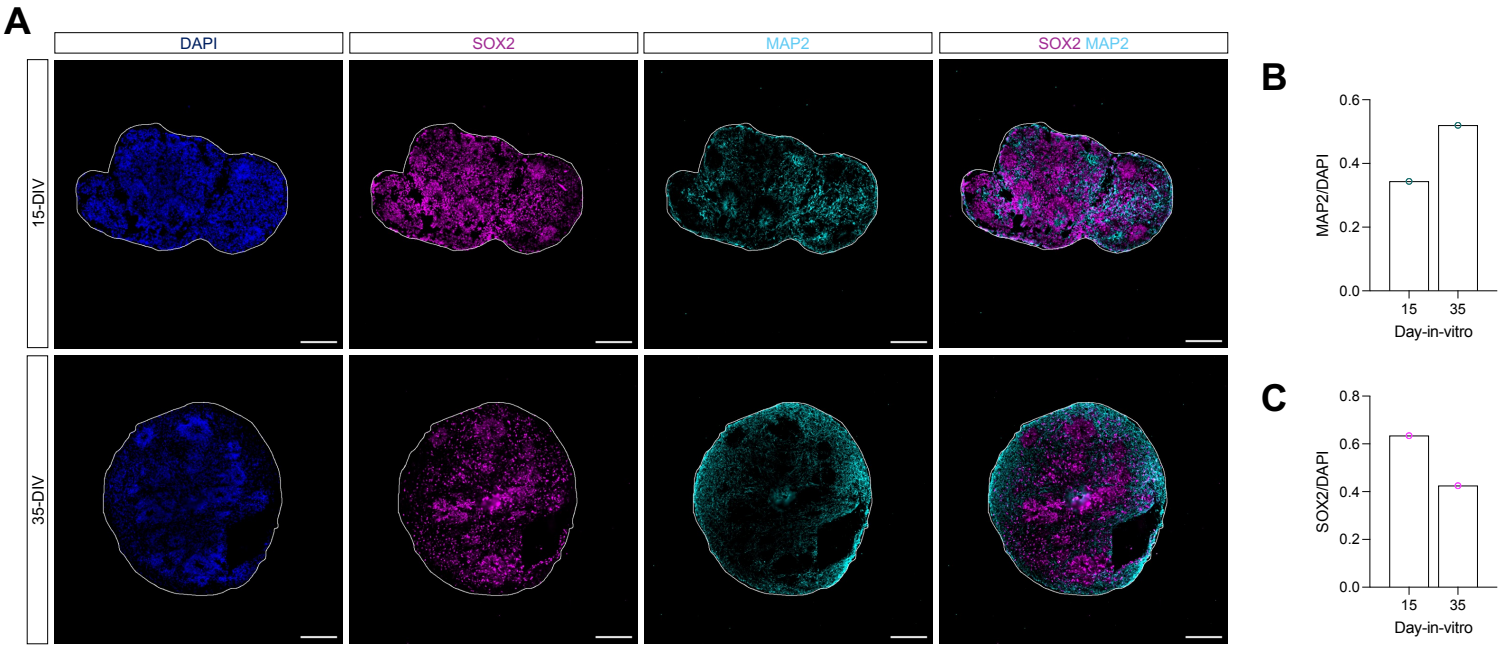
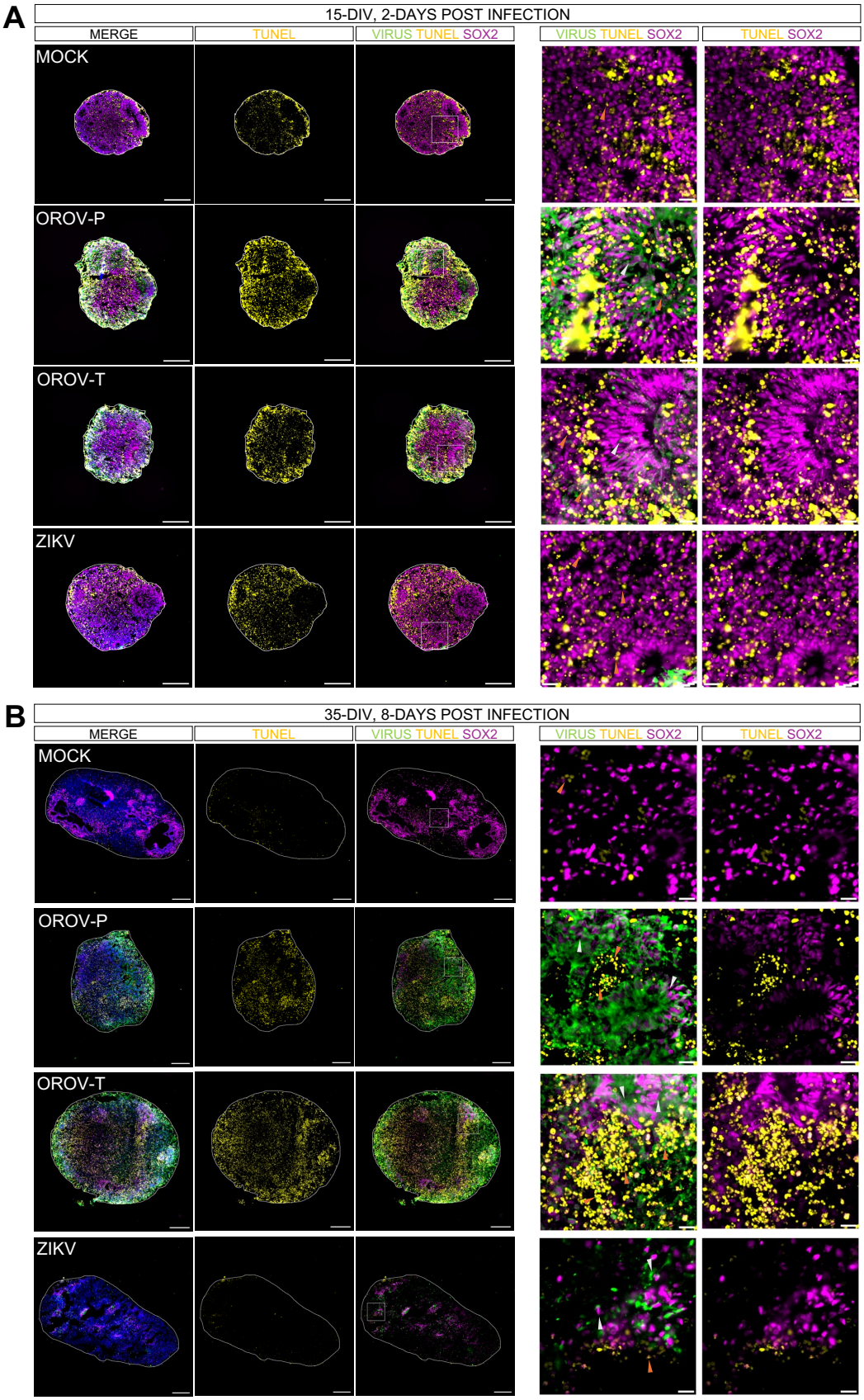
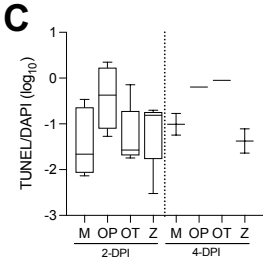
Supplementary Figure 1: Neuronal cell types in 15- and 35-DIV forebrain organoids

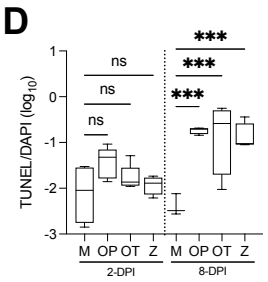


Supplementary Figure 1: Neuronal cell types in 15- and 35-DIV forebrain organoids. (A) Representative immunofluorescent images of DIV15 and DIV35 forebrain organoids. Sectioned organoids were outlined using DAPI (*blue*) and stained for SOX2 *(magenta*) and MAP2 (*cyan*). Scale bars of whole organoids = 200 µm. Quantification of **(A)** MAP2+ or **(C)** SOX2+ organoid area relative to DAPI+ region. Data presented as means ± SEM (n = 1 organoid, N = 1 organoid).

Supplementary Figure 2: OROV infection induces cell death in organoids

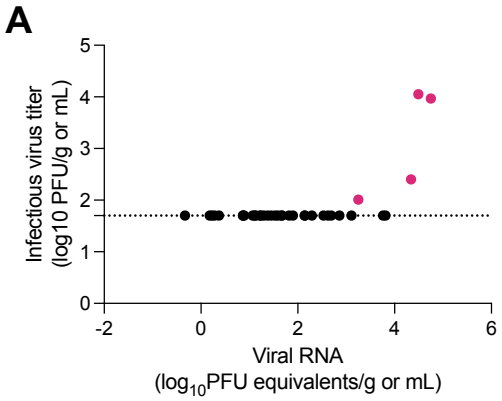






Supplementary Figure 2: OROV infection induces cell death in organoids. (A) 15-DIV or **(B)** 35-DIV iPSC-derived human forebrain organoids were mock-infected (MOCK or M) or infected with Zika virus (ZIKV or Z), the historical Oropouche prototypical strain (OROV-P or OP), or a recent traveler isolate of Oropouche (OROV-T or OT) at $5x10^4$ PFU for 24 hours. Representative immunofluorescent images of organoids at **(A)** 2-dpi or **(B)** 8-dpi. Organoid sections were outlined using DAPI (*blue*) and stained for TUNEL (*yellow*), OROV or ZIKV antigen (*green*), and SOX2 neural progenitor cell marker *(magenta)*. White box indicates inset. Gray arrowheads indicate live virus-infected SOX2+ cells. Orange arrowheads indicate apoptotic cells. Scale bars of whole organoids = 200 µm and inset = 20 µm. **(C, D)** Quantification of TUNEL+ organoid area relative to DAPI+ area in immunofluorescent images at **(C)** 2- and 4-dpi or **(D)** 2- and 8-dpi. Data presented as box and whisker plot; whiskers indicate minimum and maximum (n ≥ 2 organoids, N ≥ 1 independent experiments). Statistical analysis performed with one-way ANOVA followed by Sidak's multiple comparisons test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Figure 3: High levels of OROV RNA are needed to detect infectious virus



Supplementary Figure 3: High levels of OROV RNA are needed to detect infectious virus. (A) Viral RNA and viral titers, quantified via RT-qPCR and plaque assay respectively, from fetal heads and placentas were represented in a correlation plot. Pink dots indicate samples with detectable viral plaques (n>14 samples, N=4 independent experiments).