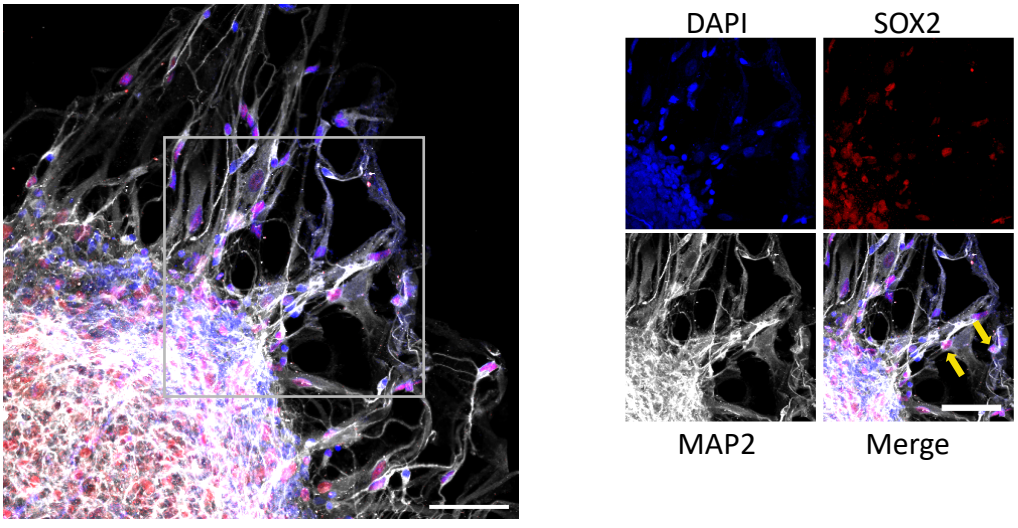
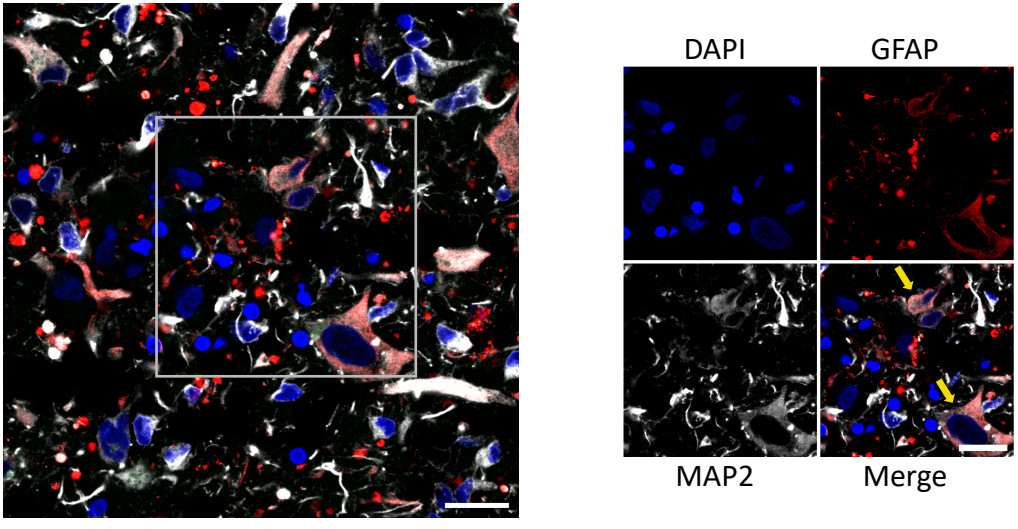
A.

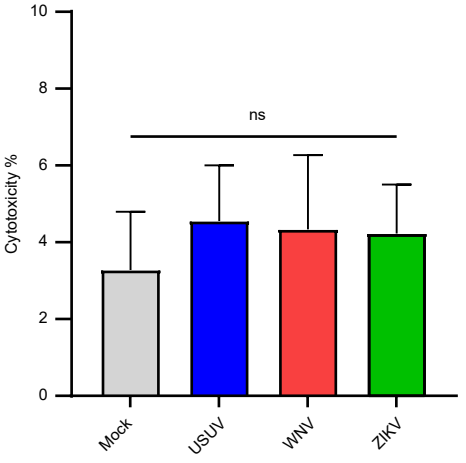


B.

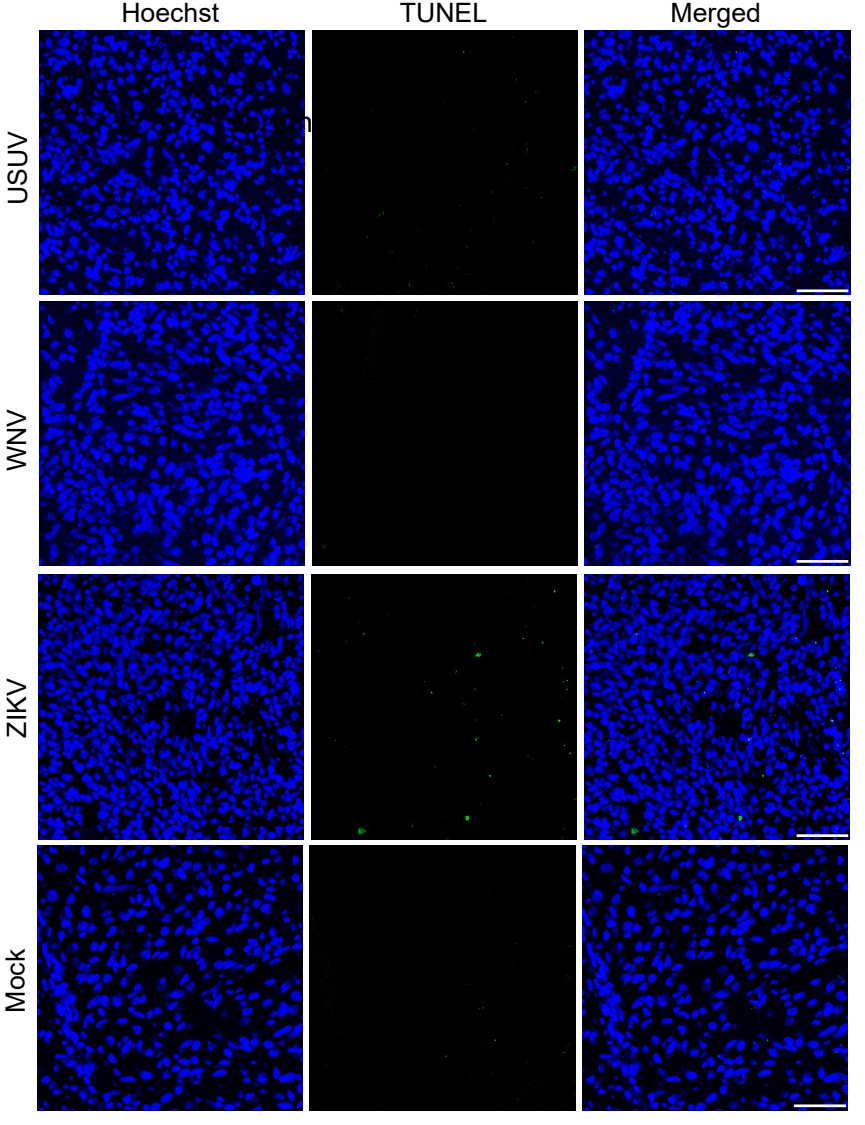


Supplementary figure 1. Co-staining of cell markers: A. Immunofluorescent staining of 3D tissue-cleared hfOBSCs for SOX2 (stem cell marker) and MAP2 (neuronal marker). Scale bars represent 50µm. **B.** Immunofluorescent staining of cryosectioned hfOBSCs for GFAP (astrocytic marker) and MAP2. Scale bars represent 20µm. Yellow arrows indicate examples of co-staining.

A.


10
8
6
4
2
0
Cytotoxicity %
ns
Mock
USUV
WNV
ZIKV

B.


Hoechst
TUNEL
Merged
USUV
WNV
ZIKV
Mock

Supplementary figure 2. USUV, WNV and ZIKV do not induce cell death in the hfOBSCs: A. Cytotoxicity induced at 72 hours post-infection, as quantified via detection of lactate-dehydrogenase (LDH) in the supernatants from hfOBSC infected with 10^6 TCID50 of USUV, WNV or ZIKV. Data was normalised to positive control hfOBSC that were lysed for maximal LDH release. Error bars represent SD. Data represents the mean of 3 independent donors, with 3 replicates per donor**.** ns = non-significant. One-way ANOVA. **B.** Immunofluorescent staining for terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) of USUV-, WNV- and ZIKV-infected brain slices. Images are representative of 3 independent donors. Scale bars represent 50μm.