

## Legends Figure Supplementary

**A:** Phase contrast images of the progenitor cells (Ntera/C12D1, up), the minibrain cells: NT2-N/A (middle) and CHME (down).

**B:** YFV-FNV and YFV-17D have the same growth and virus production in Vero cells. Student's t-test two-tailed.

**C:** Immunofluorescence images of a non-infected human neuron (right) and a Nf200 negative cell (Left) [NEFH in green, nucleus in blue].

**D:** Graphical abstract.

The FNV strain was used here as a prototype of neuroinvasive/neurovirulent YF viruses, while 17D was used as a prototype of non-[neuroinvasive/neurovirulent] virus. We compared the capacity of FNV and 17D viruses to cross the BBB, in the BBB-Minibrain model, we assessed their ability to infect the Minibrain, multiply in it, and perturbate its homeostasis. Our results demonstrate that it is feasible to detect neurovirulent variants, reliably and timely *in cellulo* with the BBB-minibrain. This system represents a promising development, and could ultimately enable the replacement of animals for neurovirulence testing of vaccine from which live virions can cross the BBB.