

## Supplementary information

### **Engineering stem cell-derived 3D brain organoids in a perfusable organ-on-a-chip system**

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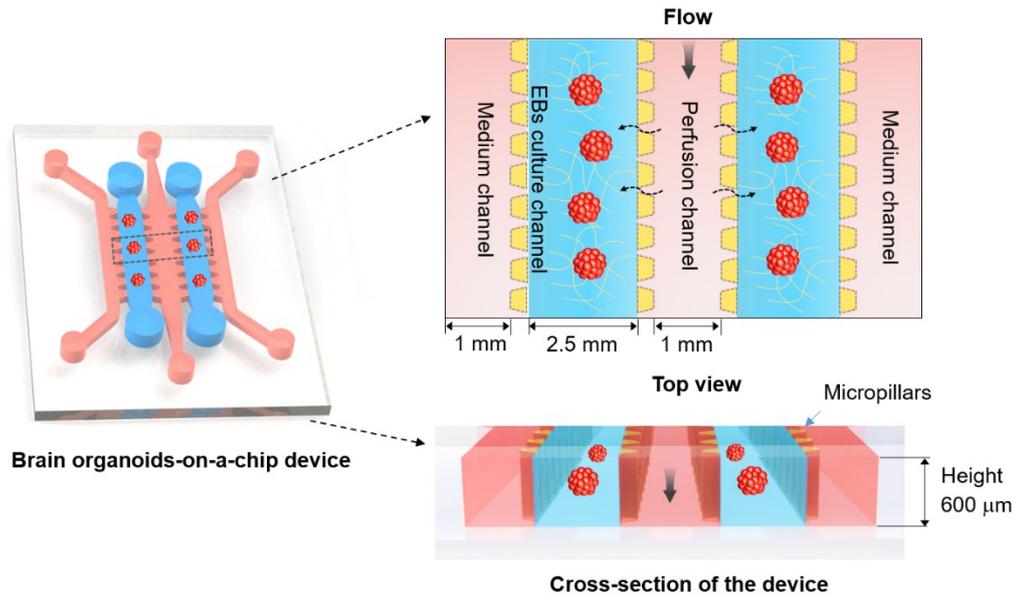
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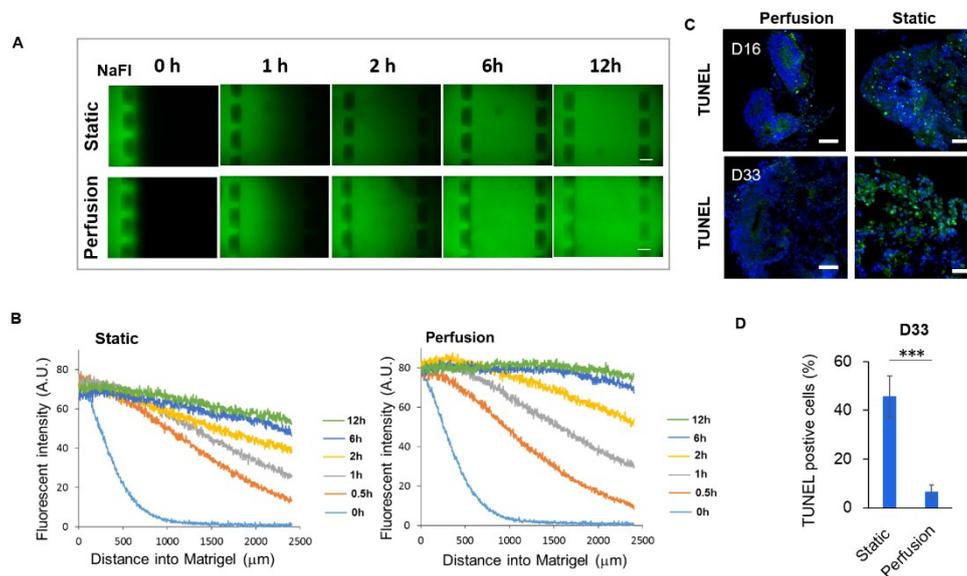
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The authors have no conflicts of interest to declare.

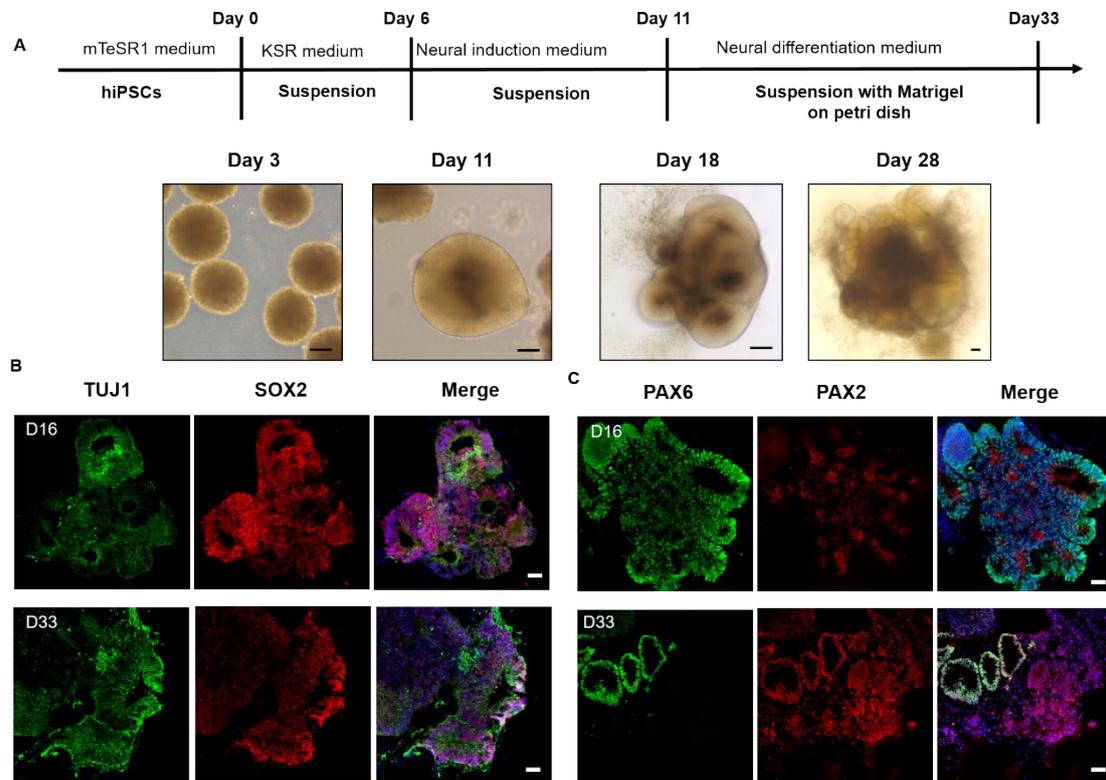


**Fig. S1 Schematic diagram of the top view and cross-section of the brain organoids-on-a-chip device.** The upper right graph is the top view of the device. Two parallel culture chambers (width: 2.5 mm) were separated by a perfusion channel (width: 1 mm). The cross-section view of the device shows the height of the channels (600  $\mu\text{m}$ ).



**Fig. S2 Evaluation of sodium fluorescein distribution across the gel channel of the device.** (A) Time-lapse fluorescent micrographs of sodium fluorescein tracer (NaFI, MW= 376 Da, 250  $\mu\text{M}$ ) across the gel channel under static and perfused culture conditions. Green, NaFI. Bars, 500  $\mu\text{m}$ . (B) Quantitative graphs showing the

distribution profiles of NaFI across the gel in the culture channel under different conditions. The horizontal axis shows the distance into Matrigel ( $\mu\text{m}$ ), and the vertical axis represents fluorescent intensity (A.U.). (C) TUNEL staining for dead cells (green) within brain organoids at day 16 and day 33 under perfusion and static culture conditions on chip. Scale bars = 50  $\mu\text{m}$ . (D) Percentage of TUNEL positive cells in 33-day organoids displayed from five different tissues. \*\*\* $P < 0.001$  in two-tailed Student's *t*-test.



**Fig. S3 Characterization of brain organoid growth under the conventional petri dish cultures.** (A) Flow chart and light microscopic images of the brain organoid culture and differentiation on dish. Scale bars = 100  $\mu\text{m}$ . (B-C) Identification of neurogenesis and specific brain regions in brain organoids. Immunohistochemical staining for SOX2, TUJ1 (B), PAX6 and PAX2 (C) markers in brain organoids at 16 and 33 days. Scale bars = 50  $\mu\text{m}$ .