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Supplemental information

A microengineered Brain-Chip

to model neuroinflammation in humans

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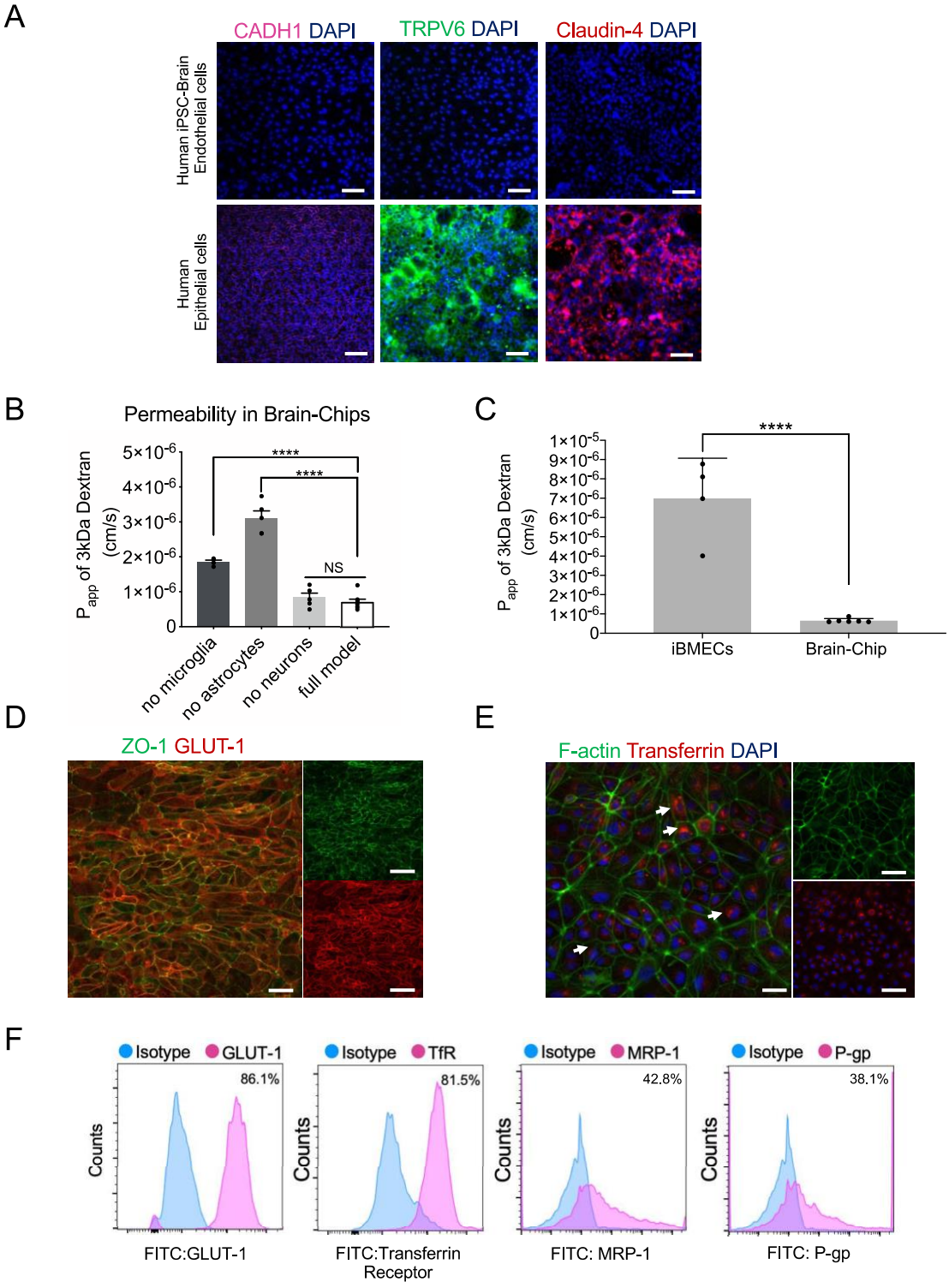
A
CADH1 DAPI
TRPV6 DAPI
Claudin-4 DAPI
Human iPSC-Brain Endothelial cells
Human Epithelial cells
B
Permeability in Brain-Chips
P app of 3kDa Dextran (cm/s)
5×10-6
4×10-6
3×10-6
2×10-6
1×10-6
0
****
****
NS
no microglia
no astrocytes
no neurons
full model
C
P app of 3kDa Dextran (cm/s)
1×10-5
9×10-6
8×10-6
7×10-6
6×10-6
5×10-6
4×10-6
3×10-6
2×10-6
1×10-6
0
****
iBMECs
Brain-Chip
D
ZO-1 GLUT-1
E
F-actin Transferrin DAPI
F
Isotype GLUT-1
86.1%
Counts
FITC:GLUT-1
Isotype TfR
81.5%
Counts
FITC:Transferrin Receptor
Isotype MRP-1
42.8%
Counts
FITC: MRP-1
Isotype P-gp
38.1%
Counts
FITC: P-gp

Figure S1, related to Figure 2. Characterization of endothelial cells and barrier properties

(**A**) Top: Representative merged confocal image of the brain endothelial-like cells on chip, stained with epithelial markers CADH1 (magenta), TRPV6, Claudin-4 and DAPI (blue) compared to bottom: Human epithelial cells stained positive for the same markers (bar,100 µm).

(**B**) Assessment of the permeability of the Brain-Chip on culture day 7, in the absence or presence of microglia, astrocytes or neurons; n=4-8 independent chips; data are represented as mean ± S.E.M., ****P<0.0001, NS: Not Significant compared to full model (Brain-Chip), statistical analysis by one-way ANOVA with Sidak's multiple comparisons test.

(**C**) Comparison of the permeability of two different iPSC-derived microvascular endothelial-like cells (iBMECs) culture models, monoculture versus culture in the Brain-Chip (n=4-6 independent chips, ****P<0.0001). Data are represented as mean ± S.E.M, statistical analysis with Student's t-test.

(**D**) Representative merged confocal image of the vascular channel on culture day 7, stained for the tight junction protein marker (ZO-1, green) and Glucose transporter (GLUT-1, red) (bar, 100 µm).

(**E**) Representative merged confocal image of immunofluorescent staining for transferrin (transferrin conjugate, pHrodo, (red) in the cytoplasm of iBMECs, together with F-actin (green) and DAPI (blue) (bar, 100 µm).

(**F)** Flow cytometry analysis of the human brain endothelial-like cells labeled with antibodies for receptors and transporters, following culture on-chip for 7 days.

A

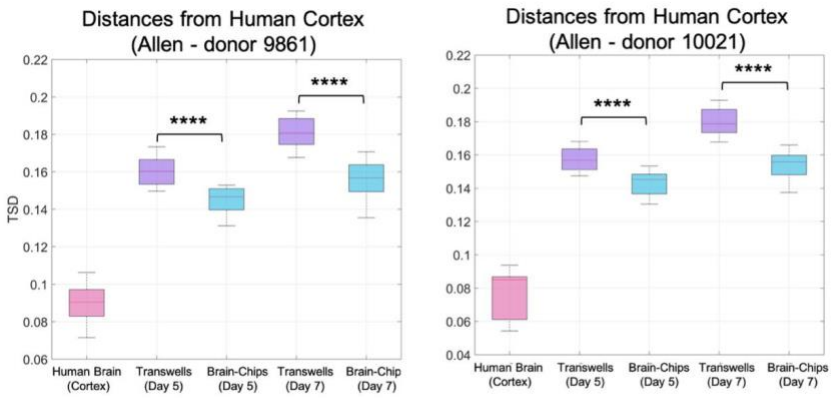

Figure S2, related to Figure 3. Comparative analyses of the Transcriptomic profiles of the Brain-Chip, adult cortex tissue and transwell culture.

(**A**) Boxplots summarizing the distributions of the corresponding pairwise TSD distances. In each pair, one sample belongs to the reference tissue (Human Brain-Cortex; Allen Brain Atlas) and the other either to the reference tissue or to one of our culture models, i.e., Brain-Chip or transwell, from culture days 5 and 7. The Brain-Chip and transwell cultures were run in parallel ****P<0.0001. Data are represented as mean ± S.E.M. Statistical analysis with two-sample t-test using a null hypothesis that the data from human tissue and the data from chips or transwells comes from independent random samples from normal distributions with equal means and equal but unknown variances.

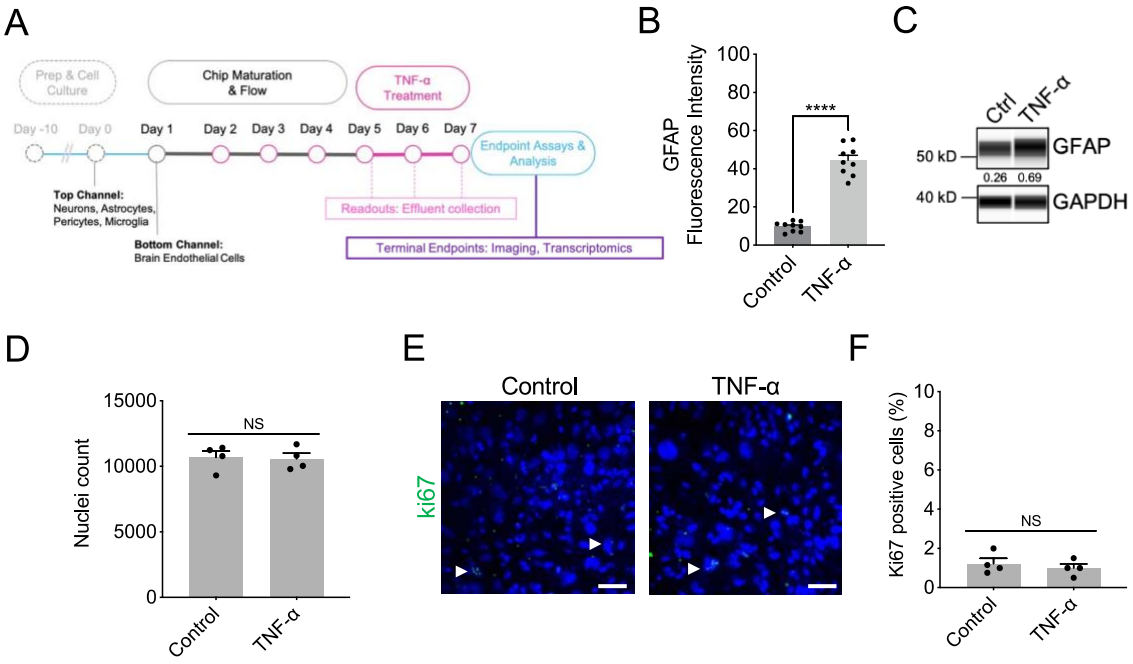

Figure S3, related to Figure 4. Brain-Chip response to TNF-α perfused through the brain channel

(**A**) Schematic of the timeline of a typical inflammation experiment.

(**B**) Quantification of fluorescent intensity of GFAP in n=3 randomly selected different areas/chip, n=3 Brain-Chips; data are expressed as mean ± S.E.M., ****P<0.0001 compared to the untreated control group; statistical analysis with Student's t-test.

(**C**) Western blotting analysis of cell lysates from the brain channel shows increased expression of GFAP levels (MW: 50kDa) following exposure to TNF-α. For loading control, equal amounts of protein were immunoblotted with GAPDH antibody (MW: 37kDa). The numbers 0.26 and 0.69 indicate quantification for relative levels of signal intensity.

(**D**) Nuclei counts as per DAPI staining, are similar in control and TNF-α treated groups (n=4 Brain-Chips; data are expressed as mean ± S.E.M., NS: Not Significant to the untreated control group. Statistical analysis with Student's t-test.

(**E**) Immunocytochemical staining of Ki67-positive cells in control and TNF-α challenged groups (bar, 100 µm). White arrows show Ki67-positive cells.

(**F**) Ki67-positive cells in control and TNF-α challenged Brain-Chips, as percentage of the total number of cells (n=4 Brain-Chips; data are expressed as mean ± S.E.M., NS: Not Significant compared to the control group; statistical analysis with Student's t-test.

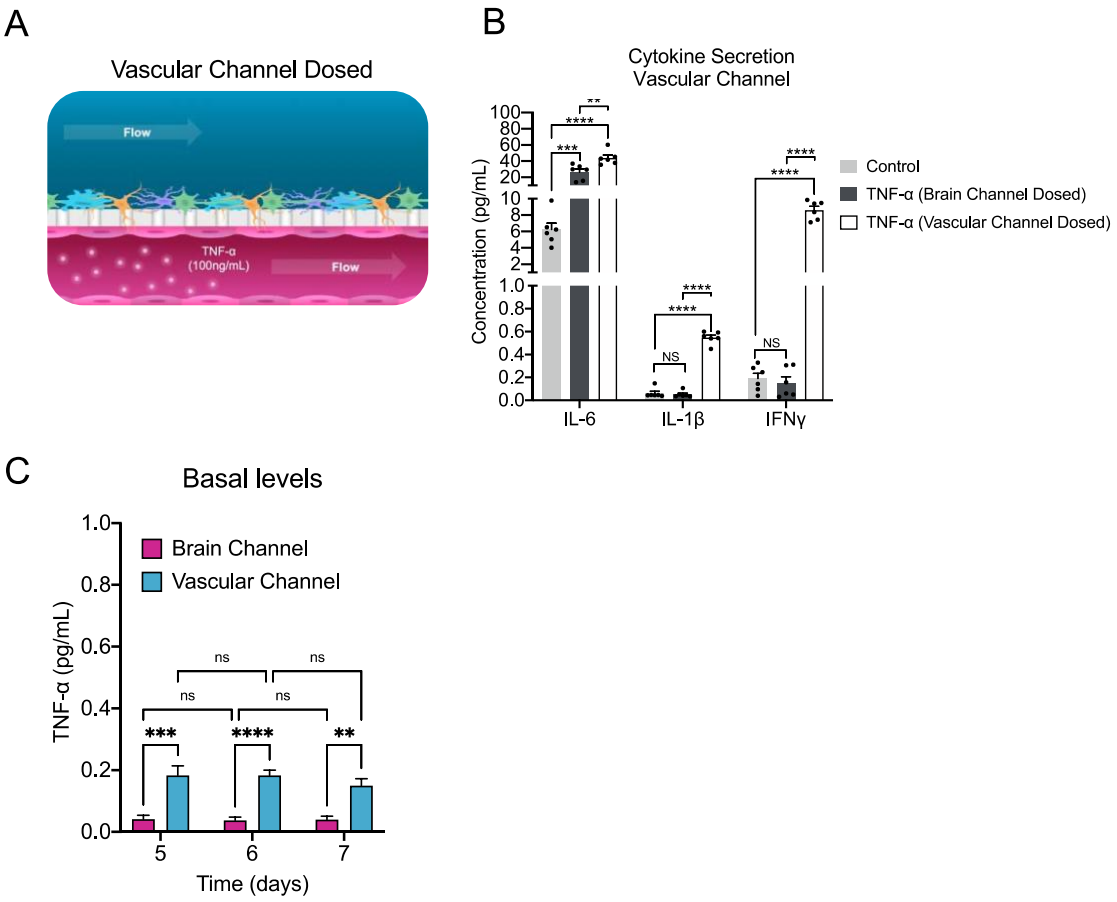

Figure S4, related to Figure 6. Brain-Chip response to TNF-α perfused through the vascular channel

(**A**) Schematic illustration of perfusion of TNF-α through the vascular channel (BBB) of the Brain-Chip.

(**B**) Secreted levels of proinflammatory cytokines (IL-6, IL-1β, and IFNγ) in the vascular channel of control or TNF-α treated Brain-Chips (either through the brain or vascular channel). n=6 independent chips, data are expressed as mean ± S.E.M., NS: Not Significant, *P***<0.01, *P****<0.001, *****P*<0.0001, statistical analysis with Student's t-test.

(**C**) Quantification of basal TNF-α levels present in the effluent of the Brain or Vascular Channel, on day 5, 6 and 7 of culture. n=3-5 independent chips; data are expressed as mean ± S.E.M. NS: Not Significant, **P<0.01, ***P<0.001, ****P<0.0001; statistical analysis with Student's t-test.