Supplementary information

Stem-cell-derived human microglia transplanted into mouse brain to study human disease

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Supplementary Information

Supplementary methods

Histological analysis

Perfusion of the mice, sectioning and histological analysis were performed as described in Mancuso et al., 2019^4 . In brief, mice were terminally anesthetized with an overdose of sodium pentobarbital and transcardially perfused with heparinized PBS. Brains were harvested, postfixed in 4% PFA overnight, embedded in agarose and cut in coronal or sagittal serial sections (40 µm thick) with a vibrating microtome (Leica). Sections were blocked with 5% normal donkey serum in PBS–0.2% Tween 20 for nonspecific binding. After rinses with PBS–0.1% Tween 20, sections were incubated overnight at 4 °C with primary antibodies (see table below). After washes with PBS–0.1% Tween 20, sections were incubated with the appropriated biotinylated (Vector Labs) or Alexa 488- and 594-conjugated secondary antibodies (Invitrogen) for 1 h at room temperature. Finally, sections were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted with Mowiol–DABCO (Sigma-Aldrich) mixture. Sections were visualized on a Nikon A1R Eclipse confocal system and analyzed on Fiji (ImageJ).

! CAUTION Pentobarbital sodium is harmful if swallowed. If administered, pentobarbital sodium may induce dizziness, sleepiness and confusion. Manipulate wearing proper personal protection equipment. Seek medical advice in case of intoxication.

! CAUTION Formaldehyde is a toxic and carcinogenic reagent. Upon contact and inhalation, formaldehyde might sensitize the skin, cause eye damage and induce respiratory issues. Wear protective gloves/protective clothing/eye protection/face protection and manipulate inside a fume hood.

Suggested antibodies for labeling microglial cells and working dilutions:

Primary Antibodies	Catalog number	Host	Final dilution
Anti-Iba1	130-109-285	Rabbit	1:500
Anti-hP2RY12	HPA014518	Rabbit	1:1000
Anti-hTMEM119	ab185333	Rabbit	1:500
Anti-GFP	ab13970	Chicken	1:500
STEM121 (hCyto)	Y40410	Mouse	1:500
Anti-Nuclei (HuNu)	MAB1281	Mouse	1:200

Supplementary Table 1

Supplementary Table 2

Secondary Antibodies	Catalog number	Host	Final dilution
Alexa 488 anti-rabbit	A-21206	Donkey	1:500
Alexa 488 anti-chicken	703-545-155	Goat	1:500
Alexa 488 anti-mouse	A-21202	Donkey	1:500
Alexa 594 anti-rabbit	A-21207	Donkey	1:500
Alexa 594 anti-mouse	A-21203	Donkey	1:500
Alexa 647 anti-rabbit	A-31573	Donkey	1:500

Supplementary Figures





Gating strategy used for FACS-based engraftment efficiency analysis reported in the manuscript (**Fig. 4**). Data were acquired with a MACSQuant® Tyto® Cell Sorter (Miltenyi) with thresholding on back-scatter blue (BSB) at 10¹ (upper left panel). CD11b positive viable cells were gated from the whole population (upper right panel) and microglia cells were further gated from these based either on hCD45 (human microglia) or mCD45 (mouse microglia) expression, excluding CD45 negative cells and mouse monocytes (mCD45^{high}, lower left panel). Human and mouse microglia populations were compared to determine engraftment efficiency (lower right panel).