1 **Human iPSC-derived astrocytes transplanted into the mouse brain display three**

2 **morphological responses to amyloid-β plaques**

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52 **ABSTRACT**

53 **Background:** Increasing evidence for a direct contribution of astrocytes to neuroinflammatory
54 and neurodegenerative processes causing Alzheimer's disease comes from molecular studies
55 in rodent models. However, these models may not fully recapitulate human disease as human
56 and rodent astrocytes differ considerably in morphology, functionality, and gene expression.
57 **Methods:** To address these challenges, we established an approach to study human astroglia
58 within the context of the mouse brain by transplanting human induced pluripotent stem cell
59 (hiPSC)-derived glia progenitors into neonatal brains of immunodeficient mice.
60 **Results:** Xenografted (hiPSC)-derived glia progenitors differentiate into astrocytes that
61 integrate functionally within the mouse host brain and mature in a cell-autonomous way
62 retaining human-specific morphologies, unique features and physiological properties. In
63 Alzheimer´s chimeric brains, transplanted hiPSC-derived astrocytes respond to the presence
64 of amyloid plaques with various morphological changes that seem independent of the *APOE*
65 allelic background.
66 **Conclusion:** In sum, this chimeric model has great potential to analyze the role of patient-
67 derived and genetically modified astroglia in Alzheimer's disease.
68 **Keywords:** human induced pluripotent stem cells (hiPSCs), astrocytes, chimeric mouse
69 models, Alzheimer's disease, amyloid plaques, *APOE*
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77 **BACKGROUND**

78 Astrocytes are essential to maintain the homeostasis of the brain, provide trophic support,
79 stimulate synaptogenesis and neurotransmission, and regulate blood-brain-barrier
80 permeability (1,2). Impaired astroglial function contributes to neurological and
81 neurodegenerative disorders including Alzheimer's disease (AD) (3–8). Genome-wide
82 association studies (9,10) show that genetic risk of AD is also associated with genes mainly
83 expressed in astroglia such as Clusterin (*CLU*), Fermitin family member 2 (*FERMT2*) and
84 Apolipoprotein E (*APOE*) (11), highlighting the potential importance of these cells in the
85 disease. Different types of astroglial pathology have been described in the AD brain (12–14).
86 Among those, hypertrophic (15), quiescent and degenerating morphologies (16,17) were
87 found.

88

89 Transgenic models have provided invaluable tools to study the role of astroglia in AD (18–21).
90 However, these models of AD might insufficiently mimic the human disease, as there are major
91 differences between rodent and human astrocytes. Morphologically, human astrocytes are
92 larger and more complex, having around 10 times more processes than their rodent
93 counterparts (22). Molecularly, human astrocytes and mouse astrocytes display different,
94 although overlapping, gene expression profiles (11). Functionally, human astrocytes propagate
95 calcium waves four-fold faster than rodent ones (11,22,23), and human and mouse astrocytes
96 show very different responses when exposed to inflammatory stimuli (24,25).

97

98 The ability to generate induced pluripotent stem cells (iPSCs) from patients and differentiate
99 them into astrocytes and other CNS cell types has generated exciting opportunities to examine
100 AD associated phenotypes *in vitro* (39) and unravel the contribution of astroglial risk genes to
101 AD (26–29). Yet, human iPSC (hiPSC)-derived astrocytes grown in culture lack essential
102 components present in the brain which can induce altered phenotypes and gene expression
103 signatures significantly different from that of primary resting astroglia in the brain (11,30).

104 Therefore, it has proved challenging to advance understanding of human astroglial function in
105 AD.
106
107 To address these challenges, we aimed at developing a chimeric model that allows studying
108 hiPSC-derived astrocytes in an *in vivo* AD context. We and others have generated chimeric
109 models to study AD by transplanting human PSC-derived neurons or microglia into the brains
110 of immunodeficient AD mice and wild-type littermates (31–33). These models revealed that
111 human neurons and microglia transplanted into the mouse brain respond to pathology
112 differently than their murine counterparts, showing specific vulnerability and transcriptional
113 signatures when exposed to amyloid-β (Aβ) (31,32). Moreover, human glia chimeric mice have
114 been generated by Goldman and collaborators to investigate the function of engrafted human
115 glia, mainly NG2 cells and lower proportions of oligodendrocytes and astrocytes, in disease
116 relevant conditions such as Huntington disease, Schizophrenia or hypomyelination (34–36).
117 Yet, to date no studies have analyzed the phenotype and functional responses of xenografted
118 human astrocytes exposed to Aβ and AD-associated pathology *in vivo.*
119
120 We established here a chimeric model to investigate survival, integration, properties and
121 responses to Aβ species of human astrocytes expressing *APOE* ε3 (E3) vs *APOE* ε4 (E4)
122 variants. We document here engraftment of astrocytes that integrate in a functional way in the
123 mouse host brain and display human-specific morphologies and properties. When transplanted
124 human astrocytes are exposed to Aβ plaques, they display hypertrophic and atrophic
125 responses similar to the ones seen in AD patients' brains (12,16,17). Our results validate the
126 use of chimeric mice as a potential powerful tool for studying astrocyte contribution to AD. We
127 also discuss one of the major hurdles to fully capture the strength of this approach, which is,
128 in our hands, the variable and often low degree of chimerism obtained with human astrocytes
129 from different hiPSC lines after several months of transplantation.
130

131 **METHODS**

132 **Generation of isogenic CRISPR/Cas9 gene-edited hiPSCs**

133 Eight hiPSC lines were generated from three *APOE* ε4 carriers diagnosed with AD (Table 1)
134 as described previously by the 'CORRECT' scarless gene-editing method (37). The correct
135 *APOE* sgRNA sequence orientation was confirmed by Sanger sequencing and CRISPR/Cas9-
136 *APOE* sgRNA plasmid cleavage efficiency was determined using the Surveyor mutation
137 detection kit in 293T cells. The single-strand oligo-deoxynucleotide (ssODN) was designed to
138 convert *APOE* ε4 to *APOE* ε3 with a protospacer adjacent motif (PAM) silent mutation to
139 prevent recurrent Cas9 editing. hiPSCs (70-80% confluent) dissociated by Accutase
140 supplemented with 10 µM Thiazovivin (Tzv) (Millipore), were harvested (200 x g, 3 min), and
141 electroporated (Neon®, ThermoFisher) according to the manufacturer's instructions. In brief,
142 cells resuspended in 10µl Neon Resuspension Buffer R, 1µg CRISPR/Cas9-*APOE* sgRNA
143 plasmid and 1µl of 10µM of ssODN were electroporated plated on Matrigel-coated plates in
144 mTeSR media with 10 µM Tzv for 72h. GFP-expressing hiPSC were isolated by FACS (BD
145 FACSAria). Sorted single cells were suspended in mTeSR with Tzv and plated into 96 well
146 plates containing MEFs (4,000 cells/well). Clones were expanded and transferred to a replicate
147 plate for gDNA isolation and Sanger sequencing to identify genome edited clones.

148

149 **Table 1. Information on the hiPSC lines.**

hiPSC line	hiPSC name	Ethnicity	Gender	Age of onset	Age at skin biopsy	Disease status (CDR at biopsy)	APOE genotype	Genetic modification
1	TCW1E33-1F1	Caucasian	F	64	72	AD (2)	E4/E4	E3/E3
2	TCW1E44-2C2	Caucasian	F	64	72	AD (2)	E4/E4	E4/E4
3	TCW2E33-3D11	Caucasian	M	77	80	AD (0.5)	E4/E4	E3/E3
4	TCW2E44-4B12	Caucasian	M	77	80	AD (0.5)	E4/E4	E4/E4
5	TCW2E33-2E3	Caucasian	M	77	80	AD (0.5)	E4/E4	E3/E3

6	TCW2E44-4B1	Caucasian	M	77	80	AD (0.5)	E4/E4	E4/E4
7	TCW3E33-H-2	Caucasian	M	80	83	AD (0.5)	E4/E4	E3/E3
8	TCW3E44-F-2	Caucasian	M	80	83	AD (0.5)	E4/E4	E4/E4

150

151 The table shows hiPSC name, patient ethnicity, gender, age of onset, age at skin biopsy,
152 disease status (CDR at biopsy), original *APOE* genotype and genetic modification. F female,
153 M male, AD Alzheimer's disease, APOE apolipoprotein, CDR clinical dementia rating, hiPSC
154 human induced pluripotent stem cells. These cells were previously generated and
155 characterized by (29).

156

157 **Karyotyping**

158 Karyotyping was performed by Wicell Cytogenetics (Madison, WI). Karyotypes are shown in
159 Additional file 2, Figure S1.

160

161 **Generation of reporter hiPSC-astrocytes**

162 The consent for reprogramming human somatic cells to hiPSC was carried out on ESCRO
163 protocol 19-04 at Mount Sinai (J.TCW.). hiPSCs maintained on Matrigel (Corning) in mTeSR1
164 (StemCell Technologies) supplemented with 10 ng/ml FGF2 StemBeads (StemCultures) were
165 differentiated to neural progenitor cells (NPCs) by dual SMAD inhibition (0.1µM LDN193189
166 and 10µM SB431542) in embryoid bodies (EB) media (DMEM/F12 (Invitrogen, 10565), 1x N2
167 (Invitrogen, 17502-048), and 1x B27-RA (Invitrogen, 12587-010)). Rosettes were selected at
168 14 DIV by Rosette Selection Reagent (StemCell Technologies) and patterned to forebrain
169 NPCs with EB media containing 20ng/ml FGF2 (Invitrogen). NPCs ($CD271^-$/$CD133^+$) were
170 enriched by magnetic activated cell sorting (Miltenyi Biotec) (38) and validated
171 immunocytochemically using SOX2, PAX6, FoxP2 and Nestin (Additional file 1, Table S1).
172 Dissociated single cell forebrain NPCs were plated 1,000,000 cells/well on 12 well plates and
173 transfected with lentiGuide-tdTomato (Addgene #99376) plasmid and selected by

174 hygromycine. Pure fluorescent expressing NPCs were plated at low density (15,000 cells/cm^2)
175 on matrigel coated plates and differentiated to astrocytes in astrocyte medium (ScienCell,
176 1801) as described (39). Cells were cultured and harvested as astroglia progenitors at DIV 40-
177 44, validated immunocytochemically and/or by FACS for the astrocyte-specific markers and
178 used for subsequent experiments.
179

180 **AD and WT Immunodeficient Mice**

181 Mice were generated as described previously (31). Briefly, APP PS1 tg/wt mice (expressing
182 KM670/671NL mutated APP and L166P mutated PS1 under the control of the Thy1.2
183 promoter1.1) (40) were crossed with the immunodeficient NOD-SCID mice (NOD.CB17-
184 Prkdc^{scid}) that carry a single point mutation in the Prkdc gene (41). APP PS1 tg/wt Prkdc$^{scid/+}$
185 mice from the F1 generation were crossed with NOD-SCID mice to generate APP PS1 tg/wt
186 Prkdc$^{scid/scid}$ immunodeficient mice. APP PS1 tg/wt Prkdc$^{scid/scid}$ mice were subsequently
187 crossed with NOD-SCID mice to generate either APP PS1 tg/wt Prkdc$^{scid/scid}$ (AD mice) or APP
188 PS1 wt/wt Prkdc$^{scid/scid}$ (WT mice) used for transplantations. Mice were housed in IVC cages in
189 a SPF facility; light/dark cycle and temperature were always monitored. After weaning, no more
190 than five animals of the same gender were kept per cage. Genotyping was done as previously
191 described (31). Transplantation experiments were performed in both male and female
192 littermates at P0-P4. Mouse work was performed in accordance with institutional and national
193 guidelines and regulations, and following approval of the Ethical Committee of the KUL. All
194 experiments conform to the relevant regulatory standards.
195

196 **Intracerebral Grafting**

197 Grafting experiments of hiPSC-derived glial progenitors using neonatal APP PS1 tg/wt NOD-
198 SCID (AD mice) and APP PS1 wt/wt NOD-SCID (WT mice) at postnatal days P0-P4 were
199 performed as described previously (31) with some modifications. Briefly, hiPSC-derived glia
200 progenitor cells at DIV 44 were enzymatically dissociated, supplemented with HB-EGF (100-

47, Peprotech) and RevitaCell (A2644501, ThermoFisher) and injected into the frontal cortex of AD or WT mice. The pups were anesthetized by hypothermia and about 200,000 cells were injected with Hamilton syringes into the forebrain at two locations: 1 mm posterior Bregma, 1.5 mm bilaterally from the midline and 1.2 mm from the pial surface. Transplanted pups were returned to their home cages until weaning age.

Electrophysiological Characterization of Human Glia in Chimeric Mice

Four to five month-old WT mice were anesthetized with isoflurane and decapitated. Acute 300 μm-thick coronal slices were cut on a Leica VT1200 vibratome in a sucrose-based cutting solution consisting of (mM): 87 NaCl, 2.5 KCl, 1.25 NaH2PO4, 10 glucose, 25 NaHCO3, 0.5 CaCl2, 7 MgCl2, 75 sucrose, 1 kynurenic acid, 5 ascorbic acid, 3 pyruvic acid (pH 7.4 with 5% CO2/ 95% O2). Slices were allowed to recover at 34ºC for 45 minutes and maintained at room temperature (RT) in the same solution for at least 30 minutes before using. During recordings, slices were submerged in a chamber (Warner Instruments) perfused with 3-4mL/min artificial cerebrospinal fluid (ACSF) consisting of (mM): 119 NaCl, 2.5 KCl, 1 NaH2PO4, 26 NaHCO3, 4 MgCl2, 4 M CaCl2, 11 glucose at pH 7.4 with 5% CO2/ 95% O2. Recordings were done at 34ºC. hiPSC-astrocytes were identified based on the td-Tomato fluorescence with a 40x objective in an epifluorescent microscope (Zeiss Axio Examiner.A1). Whole-cell current clamp recordings were made from 17 hiPSC-astrocytes (hiPSC lines #1 to #4, n=6 mice) with borosilicate glass recording pipettes (resistance 3-6MΩ). Pipettes were pulled on a horizontal micropipette puller (Sutter P-1000) and filled with a K-gluconate based internal medium consisting of (mM): 135 K-Gluconate, 4 KCl, 2 NaCl, 10 HEPES, 4 EGTA, 4 MgATP, 0.3 NaATP (pH 7.25). To post-hoc identify the patched astrocyte and analyze its potential to form gap-junctions, 40 μM Alexa Fluor hydrazide dye 488 (Invitrogen) was included in the internal medium. Current steps of incrementing 20 pA were injected starting from 50 pA up to 150 pA. Resting membrane potential was calculated using Clampfit 10.7 (Axon Instruments). Currents were sampled at 20 kHz and stored after 3 kHz low-pass Bessel filtering. The data was low-pass filtered at 1 kHz (Molecular devices DigiData 1440A and Multiclamp 700B). Pipette series

229 resistance and membrane holding current were monitored throughout all recordings to ensure
230 stability of the recording.
231
232 **Immunofluorescence (IF) in Chimeric Mice**

233 For IF analysis, mice were anesthetized with CO2 and perfused with phosphate-buffered saline
234 followed by 4% paraformaldehyde solution. The brain was then removed, post-fixed in the
235 same fixative overnight to 48 hr and cut into 40 μm slices on a Leica VT1000S vibratome. IF
236 on grafted brains was performed as described previously (31) using primary and secondary
237 antibodies (Additional file 1, Table S1). Antigen retrieval was performed by microwave boiling
238 the slides in 10mM tri-Sodium Citrate buffer pH 6.0 (VWR). Aβ plaques were detected by
239 staining with Thioflavin (SIGMA). Briefly, for Thioflavin staining brain sections were incubated
240 with a filtered 0.05% aqueous Thioflavin-S (SIGMA) solution in 50% ethanol for 5 min at RT
241 and rinsed gradually with 70%, 95% ethanol and water. Nuclei staining was performed using
242 a specific anti-human Nuclear Antigen antibody (hNuclei) (Additional file1, Table S1), the pan-
243 nuclear staining TOPRO3 (Invitrogen), or DAPI (SIGMA). The sections were mounted with
244 Glycergel (DAKO). Confocal images were obtained using a Nikon Ti-E inverted microscope
245 equipped with an A1R confocal unit driven by NIS (4.30) software. The confocal was outfitted
246 with 20x (0.75 NA), 40x oil (1.4 NA) and 60x oil (1.4 NA) objectives lenses. For excitation 405
247 nm, 488 nm, 561 nm, 638 nm laser lines were used.
248
249 **Quantification and Statistical Analysis**

250 Morphometry and measurements were performed with Fiji/ImageJ software on animals at five
251 months after transplantation. At least 4-5 different coronal brain sections comprising the
252 transplanted astrocytes and the mouse host tissue were included per animal.
253 Immunofluorescence (IF) sections were imaged by confocal microscopy (Nikon Ti-E inverted
254 microscope) using a 20x (0.75 NA) objective lens to image Z-stacks (8-10 optical sections with
255 a spacing of 1 μm). All images were acquired using identical acquisition parameters as 16-bit,

256 1024x1024 arrays. Maximum intensity projections and threshold were applied using
257 Fiji/ImageJ to isolate specific fluorescence signals.
258 For analyses of **cell integration**, brains were sectioned and stained with the antibodies against
259 RFP and hNuclei (human Nuclear antigen). The number of hNuclei+ and RFP+ cells was
260 counted manually on IF images of astrocytes derived from the eight hiPSC lines used on the
261 study (#1 to #8, Table 1). Final counts were corrected for series number (1:6) to get an estimate
262 of the total number of hNuclei+ and RFP+ cells per animal (Additional file 2, Figure S1d).
263 For analyses of **cell identity**, brains were sectioned and stained with the following antibodies:
264 RFP and hNuclei (human Nuclear antigen), GFAP (astroglia marker), NeuN (neuronal marker)
265 or APC (marker of oligodendrocytes). Results are shown for four hiPSC lines (#1, #2, #7 and
266 #8, Table 1). Total percentages of RFP+ cells co-localizing with GFAP (n=14 mice), hNuclei
267 (n=15 mice), NeuN or APC (n=9 mice each) were manually determined on IF images using
268 Fiji/ImageJ. Data are represented as mean ± SEM. Statistical analyses were done with
269 Student's t test (Fig. 1 and Additional file 3, Figure S2).
270 To analyze the **morphological subtypes of hiPSC-astrocytes**, brains were sectioned and
271 stained with antibodies against RFP and hNuclei (human Nuclear antigen) and morphometry
272 analyses were manually performed on IF images using Fiji/ImageJ. Results are shown for two
273 hiPSC lines (#1 and #2, Table1) in WT mice (n=9). Data are represented as mean ± SEM (Fig.
274 3).
275 For quantification of the average **cell area**, brains were stained with RFP and GFAP, and the
276 NIS-elements software was used (version 5.21.01 build 1483, Nikon Instruments). All the z-
277 stacks were first denoised (denoise.ai tool) and then projected on a 2D image using an
278 extended focus operation (EDF, zero-based, balanced). The resulting 2D image was used for
279 further quantification with a General Analysis (GA3) protocol. In short, to count the number of
280 cells, a spot detection approach was used (average size 11 µm). For detection of the cell area,
281 we first applied a rolling ball filter (6 µm) and, consequently, a thresholding step. Both the
282 settings for the threshold and the spot detection were adjusted per image to compensate for
283 differences in intensity due to a change of acquisition parameters. Results are shown for four

284 hiPSC lines (#1, #2, #3 and #4, Table1) in WT mice (n=12). Data are represented as mean ±
285 SEM. Statistical analysis was done with Student's t test (Fig. 3).
286 To analyze the **morphological responses to Aβ plaques**, brains were sectioned and stained
287 with RFP and Thioflavin and morphometry analyses were manually performed on IF images
288 using Fiji/ImageJ. Results are shown for two hiPSC lines (#1 and #2, Table1) in AD mice (n=7).
289 Data are represented as mean ± SEM. Statistical analysis was performed with Chi-square t
290 test (Fig. 5).
291

292 **Neuropathology on Human Brain Samples**

293 Brain tissue samples from 4 AD, 5 pre-AD and 3 non-demented control patients were included
294 in this study (Table 2). The autopsies were performed with informed consent in accordance
295 with the applicable laws in Belgium (UZ Leuven) and Germany (Ulm, Bonn and Offenbach).
296 The use of human tissue samples for this study was approved by the UZ Leuven ethical
297 committee (Leuven, Belgium). Brain tissues were collected as described in previous studies
298 (42) with an average post-mortem interval (PMI) of 48 h. Briefly, after autopsy, the brains were
299 fixed in 4% aqueous solution of formaldehyde for 2–4 weeks. Samples of the anterior
300 entorhinal cortex and hippocampus were dissected coronally, dehydrated and embedded in
301 paraffin. The paraffin blocks were microtomed at 10 μm, mounted on Flex IHC adhesive
302 microscope slides (Dako), and dried at 55 °C before storing. For neuropathological analysis,
303 sections from all blocks were stained with anti-pTau (AT8), anti-Aβ (4G8) (Additional file 1,
304 Table S1), and with the Gallyas and the Campbell-Switzer silver techniques for detection of
305 neurofibrillary changes and amyloid deposits (43).

306 The post-mortem diagnosis of AD pathology was based upon the standardized clinico-
307 pathological criteria, including the topographical distribution of Aβ plaques in the medial
308 temporal lobe (AβMTL phase) based on Aβ immunohistochemistry (43), and the Braak
309 neurofibrillary tangle (NFT) stage based on pTau immunohistochemistry (44). The study
310 comprised 12 cases with an average age of 77 years and a female to male ratio of 4:8. The

311 cases were divided in three groups based on the clinical and neuropathological diagnosis: (1)
312 AD = high-intermediate degree of AD pathology and signs of cognitive decline during life (CDR
313 ≥ 0.5); (2) p-preAD = cases with intermediate-low degrees of AD pathology lacking clinical
314 signs of cognitive decline (CDR = 0); (3) non-AD = low-no pathological signs of AD pathology
315 (CDR = 0).

316

317 **Table 2. Details of Human Cases.**

Case number	Age	Gender	Aβ phase	Braak stage	PMI	Neuropathological Diagnosis	Type of dementia
1	82	M	5	3	72	AD	AD
2	81	F	5	5	48	AD, CAA, I	AD
3	85	M	5	3	48	AD, CAA, MI	AD-VaD
4	83	M	5	5	24	AD, CAA, I, B	AD-VaD
5	83	F	4	4	24	p-preAD, AGD, CM	0
6	85	F	4	3	24	p-preAD	0
7	87	M	4	3	96	p-preAD, CAA	0
8	72	M	2	3	72	p-preAD, I	0
9	66	F	0	0	48	non-AD control, AGD	0
10	62	M	0	0	48	non-AD control	0
11	75	M	1	2	48	non-AD control, AGD	0
12	64	M	0	0	24	non-AD control	0

318

319 The table shows the human subjects studied for histology of astrocytes. Indicated are: the age
320 in years, the gender, the Aβ-MTL phase representing the distribution of Aβ deposits in the
321 subfields of the MTL (43), the stage of neurofibrillary tangle pathology according to Braak and
322 Braak (44) (NFT stage), PMI, neuropathological diagnosis and type of dementia. F female, M
323 male, AD Alzheimer's disease, AD-VaD Alzheimer's disease plus signs of vascular dementia,
324 p-preAD preclinical AD, non-AD non-demented control, AGD argyrophilic grain disease, B

325 bleeding, CAA cerebrovascular angiopathy, CM carcinoma metastasis, I infarction, MI
326 microinfraction, MTL medial temporal lobe, NFT neurofibrillary tangle, PMI post-mortem
327 interval.
328

329 **Immunohistochemistry and Immunofluorescence on Human Samples**

330 The distribution of astrocytes and Aβ deposits was examined in human samples of the
331 entorhinal cortex and hippocampus using immunohistochemical and immunofluorescence
332 techniques. Immunohistochemical detection of Aβ deposits and astrocytes was performed
333 after formic acid pretreatment. For double-labeling, a monoclonal anti-$A\beta_{17-24}$ antibody (4G8,
334 Additional file 1, Table S1) was subsequently combined with a polyclonal anti-GFAP (DAKO,
335 Additional file 1, Table S1) as described previously (43). The anti-$A\beta_{17-24}$ antibody was
336 detected with biotinylated secondary antibodies and ABC, and visualized with
337 3,3´diaminobenzidine-HCl. After peroxidase blocking, the anti-GFAP was applied, detected
338 with biotinylated secondary antibodies, and ABC, and visualized with the Vector peroxidase kit
339 SG (blue staining). Microscopy analysis was performed using a light Leica DM2000 LED
340 microscope (Leica Microsystems) and images were captured with a Leica DFC7000 T camera
341 (Leica Microsystems).

342 For double-labeling immunofluorescence, sections were pre-treated as mentioned above and
343 incubated with formic acid for 3 min, when required. Immunostainings were performed with an
344 antibody cocktail and primary antibodies were detected with species-specific fluorescent-
345 conjugated secondary antibodies (Additional file 1, Table S1). Images were captured via Nikon
346 NIS-Elements software using a Nikon A1R laser scanning confocal system coupled to a Nikon
347 Eclipse Ti inverted microscope (Nikon Instruments, Inc.). Acquired data were further processed
348 using ImageJ software (National Institutes of Health).
349

350 **RESULTS**

351 **Human iPSC-Derived Glial Progenitors Engraft the Mouse Brain and Differentiate into**
352 **Astrocytes**

353 To generate human-mouse astroglia chimeras, we differentiated human iPSCs (hiPSCs) into
354 glial progenitor cells (hGPCs) *in vitro* (39) (Fig. 1a). After 44 days in culture, td-Tomato
355 expressing hGPCs, which expressed several astroglia markers (Additional file 2, Figure S1b),
356 were xenografted into the brains of newborn mice (Fig. 1a). We used transgenic Tg (Thy1-
357 APPSw,Thy1-PSEN1*L166P) 21Jckr, also called APP/PS1-21 mice (40) crossed with
358 immunodeficient NOD.CB17-Prkdc^{scid}/J, further called NOD-SCID mice (41), to generate AD
359 mice or wild-type (WT) littermates suitable for grafting experiments (31). We transplanted
360 hiPSC lines from AD patients carrying the *APOE E4/E4* alleles and the corresponding
361 corrected *APOE E3/E3* isogenic lines (Table 1).

362 Five months after transplantation, immunofluorescence (IF) analysis revealed engraftment of
363 human cells throughout the forebrain (Fig. 1b, Additional file 2, Figure S1c). Human cells were
364 identified based on the expression of the td-Tomato marker RFP and of the human nuclear
365 antigen hNuclei. RFP+ cells infiltrate the cortex, corpus callosum and subcortical areas such
366 as the hippocampus, striatum, thalamus or hypothalamus (Fig. 1c-e). Assessment of the
367 engraftment capacity revealed considerable variation across cell lines (Additional file 2, Figure
368 S1d): we show here examples of robust engraftment, with RFP+ cells both in clusters as well
369 as integrated individually within the mouse brain (Fig. 1b, c), but these results were variable
370 with often lower engraftment capacity at 5 months after transplantation (Additional file 2, Figure
371 S1c, d). Variation was independent of the *APOE* genetic background or the patient (overview
372 in Additional file 2, Figure S1d).

373 Further analyses revealed that at this stage, human RFP+ cells strongly express the astroglia
374 markers GFAP, S100b, Vimentin and Aquaporin-4 (Fig. 1f-i), the latter largely concentrated at
375 the astrocytic end-feet along the blood vessels (Figure 1i). Staining with human specific GFAP
376 antibody (hGFAP), confirms the human origin of the cells (Additional file 3, Figure S2a).
377 Quantification showed that 93% of the RFP+ hiPSC-cells express the astroglia marker GFAP

378 (Fig. 1j) and 95% of the hNuclei+ hiPSC-cells co-express RFP (Fig. 1k). Thus, the RFP marker
379 is not downregulated, and most of the transplanted cells indeed differentiated into human
380 astroglia. This was further confirmed as no or only minimal expression (less than 3%) of
381 neuronal or oligodendroglial markers was observed in RFP+ cells (Fig. 1l, Additional file 3,
382 Figure S2b, c). No differences were observed between *APOE E4/E4* and *APOE E3/E3* lines
383 (Additional file 3, Figure S2d-f). A subset of RFP+ cells identified by their distinct radial glia-
384 like morphology and not expressing GFAP (Additional file 3, Figure S2g-i) often coexisted with
385 RFP+ cells with more complex structures and expressing main astroglia markers. These cells
386 are likely in a progenitor state which was also described previously (23,45).

387 **Transplanted iPSC-Derived Astrocytes Integrate Functionally Within the Mouse Brain**

388 We assessed morphological and electrophysiological features of individual hiPSC-derived
389 astrocytes in the chimeric brain. We observed hiPSC-astrocytes extending processes that
390 terminated in end-feet contacting mouse host vasculature in the chimeric brains (Fig. 2a)
391 similar to human astrocytes in the human brain (Fig. 2b). Moreover, hiPSC-astrocytes strongly
392 expressed the gap-junction marker Connexin-43 in their processes (Fig. 2c). The gap junctions
393 were functioning, as the Alexa488 dye loaded through the patch clamp pipette on RFP+
394 astrocytes diffused into neighboring mouse host cells (Fig. 2d-h). Electrophysiological
395 analyses on acute brain slices of chimeric mice at 4-5 months showed that transplanted RFP+
396 astrocytes displayed properties resembling human astrocytes (46). Specifically, their non-
397 excitable responses to stimulations with current injection in current clamp mode (Fig. 2i),
398 resting membrane potentials (Fig. 2j), and linear current to voltage (I/V) curves (Fig. 2k).
399 Human iPSC-astrocytes do not replace the endogenous murine astrocytes and both cell types
400 are found in the chimeric mouse brains (Additional file 3, Figure S2j). These data reveal that
401 the transplanted hiPSC-astrocytes are able to integrate functionally within the mouse host
402 brain, show human-like physiological features and co-exist with endogenous mouse
403 counterparts.

404 **Human iPSC-Derived Astrocytes Acquire Human-Specific Morphologies and Features**
405 **In Vivo**

406 An advantage of low engraftment capacity is that it favors the assessment of morphological
407 details of the transplanted astrocytes. Five months after transplantation, four main
408 morphological subtypes of hiPSC-derived astrocytes were identified in the chimeric brains of
409 the control animals. RFP+ interlaminar astrocytes were frequently observed in superficial
410 layers of the cortex and close to the ventricles, with their small and round cell bodies near the
411 pial surface and their long, unbranched and sometimes tortuous processes descending into
412 deeper layers (Fig. 3a-c). Varicose-projection astrocytes were relatively sparse but easily
413 identified by their bushy appearance and the presence of long processes with regularly spaced
414 beads or varicosities (Fig. 3d, e). Protoplasmic astrocytes were found in deeper layers of the
415 brain and showed the characteristic star-shaped morphology and shorter processes extending
416 in all directions and often contacting the vasculature (Fig. 3f, g). Fibrous astrocytes were found
417 in white matter tracts and presented the typical morphology with small soma and fine, straight
418 and radially oriented processes (Fig. 3h-j). Interlaminar astrocytes were the most abundant
419 subtype of hiPSC-astrocytes in the mouse brain, summing up to 62% of the RFP+ cells, and
420 similar proportions of fibrous and protoplasmic astrocytes were found (16% and 13% of the
421 RFP+ cells respectively). The varicose-projection astrocytes are the less frequent subtype,
422 constituting 9% of RFP+ cells found in the host brain (Fig. 3k). Interestingly, we found the same
423 astroglia subtypes in the human entorhinal cortex and white matter tracts of various control
424 individuals (Table 2, subjects 10-12), when staining with the astrocyte marker GFAP: subpial
425 interlaminar astrocytes with their soma in superficial layers of the cortex (molecular layer to
426 pre-α) and long processes extending into deeper layers (Fig. 4a-c), protoplasmic (Fig. 4a, d)
427 and varicose-projection astrocytes (Fig. 4e-f) in deeper layers of the cortex (pri-α to pri-γ), and
428 fibrous astrocytes in white matter tracts (Fig. 4g-i). Of note, hiPSC-astrocytes covered about
429 15-fold larger areas than mouse astrocytes and displayed more complex structures (Fig. 3l-m,
430 Additional file 3, Figure S2j). Thus, transplanted hiPSC-astrocytes were able to keep their

431 intrinsic properties and develop in a cell-autonomous way adopting human-specific features
432 and morphologies within the mouse host brain.

433 **Human Astroglia Display Differential Morphological Responses to Amyloid-β Plaques**

434 Interestingly, transplanted hiPSC-astrocytes adopt three clearly distinct morphologies in the
435 brains of chimeric AD mice five months after transplantation, when the Aβ load is high.
436 Immunofluorescence analyses with RFP revealed that about 25% of the astrocytes became
437 hypertrophic and showed thicker processes that surround Aβ deposits (Fig. 5a-c and 5a'-c',
438 Fig. 5g). 62% of the astrocytes seemed not to be morphologically affected at all, even when in
439 close contact with the Aβ plaques (Fig. 5d, 5d', 5g). Finally, about 13% of astrocytes showed
440 atrophic features, displaying thinner processes that sometimes even looked degenerating (Fig.
441 5e-f and 5e'-f', Fig. 5g). *APOE* E3/E3 or *APOE* E4/E4 genotype did not affect these proportions
442 (Fig. 5h). Hypertrophic, atrophic and quiescent phenotypes were also found in human
443 astrocytes in close proximity to Aβ deposits in the entorhinal cortex and hippocampus of
444 patients with AD (Table 2, subjects 1-4), both by immunohistochemistry (Fig. 6) and
445 immunofluorescence (Fig. 7).

446 In conclusion: engrafted hiPSC-astrocytes show differential morphological responses to Aβ
447 plaques that resemble that of human astrocytes in AD patients' brains. The potential of
448 astrocytes to become hyper- or a-trophic, or remain in a quiescent state, does not seem to be
449 influenced by the *APOE* genetic background.

450

451 **DISCUSSION**

452 A major challenge to model astroglia function in AD is the difference between mouse and
453 human astrocytes. A powerful approach to overcome this challenge is the use of hiPSC-
454 derived astrocytes to generate chimeric mice.
455 We investigate in the current study the potential of such experiments using patient derived
456 iPSC lines and isogenic counterparts (Table1). We include AD mice and control littermates.

457 We demonstrate integration of human glia into the mouse brain and differentiation of the
458 majority of cells into four main subtypes of astrocytes expressing main astroglial markers and
459 showing human-specific large, complex morphologies and electrophysiological properties.
460 Additionally, hiPSC-astrocytes contact blood vessels and couple via gap-junctions with mouse
461 cells, demonstrating functional integration in the host brain. In contrast to other glia chimeric
462 models (35), we do not see replacement of the endogenous murine counterparts.
463 hiPSC-astrocytes respond robustly to Aβ pathology showing hypertrophic, atrophic or
464 unaffected morphologies that are very similar to the morphological changes observed in
465 astrocytes in AD patients' brains (15–17). Such responses are not dependent on the *APOE*
466 genetic background. Further work is however needed to understand whether the different
467 *APOE* variants influence the molecular and functional states of human astrocytes surrounding
468 Aβ plaques.
469 While human astrocytes were consistently detected in every injected brain, the number of
470 engrafted cells varied largely from a few hundreds or thousands to >50,000 cells (Additional
471 file 2, Figure S1d). This, combined with the difficulty of recovering the engrafted cells from the
472 mouse brain for single-cell analysis, made further molecular analyses of the cellular
473 phenotypes, unfortunately, not possible at this moment.
474 Others have also observed variations in transplantation efficiencies of hiPSC-derived microglia
475 and neurons (47,48). While many successful reports on "glia" chimeric mice have been
476 reported (23,34–36,49), these glia chimeras develop, in addition to human astrocytes, a large
477 number of human NG2 cells and oligodendrocytes, whose relative ratios varied considerably
478 across different brain regions and animals (23,34,49). This suggests that in these other
479 experiments a different glia precursor state has been transplanted which maintains more 'stem
480 cell like' properties allowing these cells to spread over the brain and to compete with mouse
481 glia as shown before (49). We speculate that in our experimental conditions we have
482 transplanted more differentiated cells which are closer to a final astrocyte phenotype and
483 therefore not able to proliferate once they were injected in the brain of the host mice. It will now
484 be critical to define the optimal window for transplantation of differentiating hiPSCs in order to

485 maximize astrocyte colonization of the mouse brain. In other experiments we succeeded
486 already to determine this for microglia using the Migrate protocol (Fattorelli et al, 2020). In the
487 Migrate protocol there is a very critical window during the cell differentiation *in vitro* that results
488 in 60-80% chimerism. One week longer in culture results in < 5% chimerism although the cells
489 before transplantation look morphologically identical to the more efficiently transplanted ones.
490 Other possible improvements would be the use of RAG2-/- mice which can be maintained for
491 a much longer time period than the NOD-SCID mice we use here.
492

493 **CONCLUSIONS**

494 In conclusion, despite some intrinsic limitations, the approach to transplant human astroglia
495 into mouse brain to study astrocyte pathophysiology in AD is promising. We recapitulated here
496 typical morphological responses of human astrocytes to amyloid plaques *in vivo*. Moreover,
497 the combination of the model with isogenic *APOE* lines points out the potential use of this
498 approach to analyze the impact of patient-derived and genetically modified astroglia on human
499 CNS disease.
500

501 **LIST OF ABBREVIATIONS**

502 5 M: 5 months of age; AD: Alzheimer's disease; Aβ: amyloid-β; APOE: Apolipoprotein E; CDR:
503 clinical dementia rating, DIV: days in vitro; EB: embryoid bodies; GPCs: glia progenitor cells;
504 hiPSCs: human induced pluripotent stem cells; IF: immunofluorescence; IVC: individually
505 ventilated cages; NFT: neurofibrillary tangles; NPCs: neural progenitor cells; PAM: protospacer
506 adjacent motif; PMI: post-mortem interval; RFP: red fluorescent protein; RT: room temperature;
507 SPF: specific pathogen free; ssODN: single-strand oligo-deoxynucleotide; Tzv: Thiazovivin;
508 WT: Wild-type.
509

510 **DECLARATIONS**

Ethics approval and consent to participate

All animal experiments were conducted according to protocols approved by the local Ethical Committee of Laboratory Animals of the KU Leuven (governmental licence LA1210591) following governmental and EU guidelines. All experiments conform to the relevant regulatory standards. The consent for reprogramming human somatic cells to hiPSCs was carried out on ESCRO protocol 19-04 at Mount Sinai (J.TCW.). The autopsies were performed with informed consent in accordance with the applicable laws in Belgium (UZ Leuven) and Germany (Ulm, Bonn and Offenbach). The use of human brain tissue samples for this study was approved by the ethical committees of Leuven University and UZ Leuven.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Competing interests

BDS is a consultant for Eisai. PP, JTCW, AS, SC, MAT, NC, SM, DRT, AMG and AMA declare that they have no competing interests.

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543

544 **Authors' contributions**

545 AMA and BDS conceived the study and planned experiments. AMA, PP, JTCW, AS, SC, MAT,
546 NC, and SM performed the experiments. All authors interpreted data. AMA and BDS wrote the
547 first version of the manuscript. All authors contributed to and approved the final version.
548

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554

555 **REFERENCES**

556 1. Ferrer I (2018) Astrogliopathy in Tauopathies. Neuroglia 1:126–150. doi:
557 10.3390/neuroglia1010010

558 2. Verkhratsky A, Nedergaard M (2018) Physiology of Astroglia. Physiol Rev 98:239–389.
559 doi: 10.1152/physrev.00042.2016

560 3. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett
561 ML, Münch AE, Chung W-S, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar
562 M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA (2017)
563 Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541:481-487. doi:
564 10.1038/nature21029

565 4. Ouali Alami N, Schurr C, Olde Heuvel F, Tang L, Li Q, Tasdogan A, Kimbara A,
566 Nettekoven M, Ottaviani G, Raposo C, Röver S, Rogers-Evans M, Rothenhäusler B, Ullmer C,
567 Fingerle J, Grether U, Knuesel I, Boeckers TM, Ludolph A, Wirth T, Roselli F, Baumann B
568 (2018) NF-κB activation in astrocytes drives a stage-specific beneficial neuroimmunological
569 response in ALS. EMBO J. 37:e98697. doi: 10.15252/embj.201798697

570 5. Rothhammer V, Borucki DM, Tjon EC, Takenaka MC, Chao C, Ardura-fabregat A, Lima
571 KA De, Gutiérrez-vázquez C, Hewson P, Staszewski O, Blain M, Healy L, Neziraj T, Borio M,
572 Wheeler M, Dragin LL, Laplaud DA, Antel J, Alvarez JI, Prinz M, Quintana FJ (2018) Microglial
573 control of astrocytes in response to microbial metabolites. Nature 557:724-728. doi:
574 10.1038/s41586-018-0119-x

575 6. Yun SP, Kam T, Panicker N, Kim S, Oh Y, Park J, Kwon S, Park YJ, Karuppagounder
576 SS, Park H, Kim S, Oh N, Kim NA, Lee S, Brahmachari S, Mao X, Lee JH, Kumar M, An D,
577 Kang S, Lee Y, Lee KC, Na DH, Kim D, Lee SH, Roschke V V, Liddelow SA, Mari Z, Barres
578 BA, Dawson VL, Lee S (2018) Block of A1 astrocyte conversion by microglia is neuroprotective
579 in models of Parkinson ' s disease. Nat Med. 24:931-938. doi: 10.1038/s41591-018-0051-5

580 7. Wheeler MA, Clark IC, Tjon EC, Li Z, Zandee SEJ, Couturier CP, Watson BR, Scalisi
581 G, Alkwai S, Rothhammer V, Rotem A, Heyman JA, Thaploo S, Sanmarco LM, Ragoussis J,
582 Weitz DA, Petrecca K, Moffitt JR, Becher B, Antel JP, Prat A, Quintana FJ (2020) MAFG-driven
583 astrocytes promote CNS inflammation. Nature 578:593–599. doi: 10.1038/s41586-020-1999-
584 0

585 8. Arranz AM, De Strooper B (2019) The role of astroglia in Alzheimer's disease:
586 pathophysiology and clinical implications. Lancet Neurol 18:406–414. doi: 10.1016/S1474-
587 4422(18)30490-3

588 9. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, et al.
589 (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's
590 disease. Nat Genet 45:1452–8. doi: 10.1038/ng.2802

591 10. Verheijen J, Sleegers K (2018) Understanding Alzheimer Disease at the Interface
592 between Genetics and Transcriptomics. Trends Genet 34:434–447. doi:
593 10.1016/j.tig.2018.02.007

594 11. Zhang Y, Sloan SA, Clarke LE, Caneda C, Plaza CA, Blumenthal PD, Vogel H,
595 Steinberg GK, Edwards MSB, Li G, Duncan JA, Cheshier SH, Shuer LM, Chang EF, Grant
596 GA, Gephart MGH, Barres BA (2016) Purification and Characterization of Progenitor and
597 Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse.
598 Neuron 89:37-53. doi: 10.1016/j.neuron.2015.11.013

599 12. Thal DR, Schultz C, Dehghani F, Yamaguchi H, Braak H, Braak E (2000) Amyloid β-
600 protein (Aβ)-containing astrocytes are located preferentially near N-terminal-truncated Aβ
601 deposits in the human entorhinal cortex. Acta Neuropathol 100:608–617. doi:
602 10.1007/s004010000242

603 13. Thal DR (2012) The role of astrocytes in amyloid β-protein toxicity and clearance. Exp
604 Neurol 236:1–5. doi: 10.1016/j.expneurol.2012.04.021

605 14. Mulder SD, Veerhuis R, Blankenstein MA, Nielsen HM (2012) The effect of amyloid
606 associated proteins on the expression of genes involved in amyloid-β clearance by adult
607 human astrocytes. Exp Neurol 233:373–379. doi: 10.1016/j.expneurol.2011.11.001

608 15. Pike CJ, Cummings BJ, Cotman CW (1995) Early association of reactive astrocytes
609 with senile plaques in Alzheimer's disease. Exp Neurol 132:172–179. doi: 10.1016/0014-
610 4886(95)90022-5

611 16. Colombo JA, Quinn B, Puissant V (2002) Disruption of astroglial interlaminar processes
612 in Alzheimer's disease. Brain Res Bull 58:235–242. doi: 10.1016/S0361-9230(02)00785-2

613 17. Hsu ET, Gangolli M, Su S, Holleran L, Stein TD, Alvarez VE (2018) Astrocytic
614 degeneration in chronic traumatic encephalopathy. Acta Neuropathol. 136:955-972. doi:
615 10.1007/s00401-018-1902-3

616 18. Orre M, Kamphuis W, Osborn LM, Jansen AHP, Kooijman L, Bossers K, Hol EM (2014)
617 Isolation of glia from Alzheimer's mice reveals inflammation and dysfunction. Neurobiol Aging.
618 35:2746-2760. doi: 10.1016/j.neurobiolaging.2014.06.004

619 19. Lian H, Yang L, Cole A, Sun L, Chiang ACA, Fowler SW, Shim DJ, Rodriguez-Rivera
620 J, Taglialatela G, Jankowsky JL, Lu HC, Zheng H (2015) NFκB-Activated Astroglial Release
621 of Complement C3 Compromises Neuronal Morphology and Function Associated with
622 Alzheimer's Disease. Neuron 85:101-115. doi: 10.1016/j.neuron.2014.11.018

623 20. Lian H, Litvinchuk A, Chiang AC-A, Aithmitti N, Jankowsky JL, Zheng H (2016)
624 Astrocyte-Microglia Cross Talk through Complement Activation Modulates Amyloid Pathology
625 in Mouse Models of Alzheimer's Disease. J Neurosci 36:577–589. doi:
626 10.1523/JNEUROSCI.2117-15.2016

627 21. Diniz LP, Tortelli V, Matias XI, Morgado J, Be AP, Melo XHM, Seixas XGS, Alves-leon
628 XS V, Souza XJM De, Ferreira XST, Felice XFG De, Gomes A (2017) Astrocyte Transforming
629 Growth Factor Beta 1 Protects Synapses against Aβ Oligomers in Alzheimer's Disease Model.
630 Journal of Neuroscience 37:6797–6809. doi: 10.1523/JNEUROSCI.3351-16.2017

631 22. Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W,
632 Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely Hominid Features of
633 Adult Human Astrocytes. J Neurosci. 29:3276-87. doi: 10.1523/JNEUROSCI.4707-08.2009

634 23. Han X, Chen M, Wang F, Windrem M, Wang S, Shanz S, Xu Q, Oberheim NA, Bekar
635 L, Betstadt S, Silva AJ, Takano T, Goldman SA, Nedergaard M (2013) Forebrain engraftment
636 by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. Cell
637 Stem Cell 12(3):342–53. doi: 10.1016/j.stem.2012.12.015

638 24. Tarassishin L, Suh HS, Lee SC (2014) LPS and IL-1 differentially activate mouse and
639 human astrocytes: Role of CD14. Glia 62:999–1013. doi: 10.1002/glia.22657

640 25. Lundin A, Delsing L, Clausen M, Ricchiuto P, Sanchez J, Sabirsh A, Ding M,
641 Synnergren J, Zetterberg H, Brolén G, Hicks R, Herland A, Falk A (2018) Human iPS-Derived
642 Astroglia from a Stable Neural Precursor State Show Improved Functionality Compared with
643 Conventional Astrocytic Models. Stem Cell Reports 10:1030–1045. doi:
644 10.1016/j.stemcr.2018.01.021

645 26. Zhao J, Davis MD, Martens YA, Shinohara M, Graff-radford NR, Younkin SG, Wszolek
646 ZK, Kanekiyo T, Bu G (2017) APOE e 4 / e 4 diminishes neurotrophic function of human iPSC-
647 derived astrocytes. Hum. Mol. Genetics. 26:2690–2700. doi: 10.1093/hmg/ddx155

648 27. Oksanen M, Petersen AJ, Naumenko N, Puttonen K, Lehtonen Š, Gubert Olivé M,
649 Shakirzyanova A, Leskelä S, Sarajärvi T, Viitanen M, Rinne JO, Hiltunen M, Haapasalo A,
650 Giniatullin R, Tavi P, Zhang SC, Kanninen KM, Hämäläinen RH, Koistinaho J (2017) PSEN1
651 Mutant iPSC-Derived Model Reveals Severe Astrocyte Pathology in Alzheimer's Disease.
652 Stem Cell Reports 9:1885–1897. doi: 10.1016/j.stemcr.2017.10.016

653 28. Lin YT, Seo J, Gao F, Feldman HM, Wen HL, Penney J, Cam HP, Gjoneska E, Raja
654 WK, Cheng J, Rueda R, Kritskiy O, Abdurrob F, Peng Z, Milo B, Yu CJ, Elmsaouri S, Dey D,
655 Ko T, Yankner BA, Tsai LH (2018) APOE4 Causes Widespread Molecular and Cellular
656 Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain
657 Cell Types. Neuron 98:1141-1154.e7. doi: 10.1016/j.neuron.2018.05.008

658 29. TCW J, Liang SA, Qian L, Pipalia NH, Chao MJ, Bertelsen SE, Kapoor M, Marcora E,
659 Sikora E, Holtzman D, Maxfield FR, Zhang B, Wang M, Poon WW, Goate AM (2019)
660 Cholesterol and Matrisome Pathways Dysregulated in Human APOE ϵ4 Glia. bioRxiv. 713362.
661 doi: 10.2139/ssrn.3435267

662 30. Perriot S, Mathias A, Perriard G, Canales M, Jonkmans N, Merienne N, Meunier C, El
663 Kassar L, Perrier AL, Laplaud DA, Schluep M, Déglon N, Du Pasquier R (2018) Human
664 Induced Pluripotent Stem Cell-Derived Astrocytes Are Differentially Activated by Multiple

665 Sclerosis-Associated Cytokines. Stem Cell Reports 11:1199–1210. doi:
666 10.1016/j.stemcr.2018.09.015

667 31. Espuny-Camacho I, Arranz AM, Fiers M, Snellinx A, Ando K, Munck S, Bonnefont J,
668 Lambot L, Corthout N, Omodho L, Vanden Eynden E, Radaelli E, Tesseur I, Wray S, Ebneth
669 A, Hardy J, Leroy K, Brion JP, Vanderhaeghen P, De Strooper B (2017) Hallmarks of
670 Alzheimer's Disease in Stem-Cell-Derived Human Neurons Transplanted into Mouse Brain.
671 Neuron 93:1066–1081.e8. doi: 10.1016/j.neuron.2017.02.001

672 32. Mancuso R, Van Den Daele J, Fattorelli N, Wolfs L, Balusu S, Burton O, Liston A,
673 Sierksma A, Fourne Y, Poovathingal S, Arranz-Mendiguren A, Sala Frigerio C, Claes C,
674 Serneels L, Theys T, Perry VH, Verfaillie C, Fiers M, De Strooper B (2019) Stem-cell-derived
675 human microglia transplanted in mouse brain to study human disease. Nat Neurosci. 22:2111-
676 2116. doi: 10.1038/s41593-019-0525-x

677 33. Hasselmann J, Coburn MA, England W, Figueroa Velez DX, Kiani Shabestari S, Tu
678 CH, McQuade A, Kolahdouzan M, Echeverria K, Claes C, Nakayama T, Azevedo R, Coufal
679 NG, Han CZ, Cummings BJ, Davtyan H, Glass CK, Healy LM, Gandhi SP, Spitale RC, Blurton-
680 Jones M (2019) Development of a Chimeric Model to Study and Manipulate Human Microglia
681 In Vivo. Neuron 103:1016–1033.e10. doi: 10.1016/j.neuron.2019.07.002

682 34. Benraiss A, Wang S, Herrlinger S, Li X, Chandler-Militello D, Mauceri J, Burm HB, Toner
683 M, Osipovitch M, Jim Xu Q, Ding F, Wang F, Kang N, Kang J, Curtin PC, Brunner D, Windrem
684 MS, Munoz-Sanjuan I, Nedergaard M, Goldman SA (2016) Human glia can both induce and
685 rescue aspects of disease phenotype in Huntington disease. Nat Commun. 7:11758. doi:
686 10.1038/ncomms11758

687 35. Windrem MS, Schanz SJ, Guo M, Tian GF, Washco V, Stanwood N, Rasband M, Roy
688 NS, Nedergaard M, Havton LA, Wang S, Goldman SA (2008) Neonatal Chimerization with
689 Human Glial Progenitor Cells Can Both Remyelinate and Rescue the Otherwise Lethally
690 Hypomyelinated Shiverer Mouse. Cell Stem Cell. 2:553-565. doi: 10.1016/j.stem.2008.03.020

691 36. Windrem MS, Osipovitch M, Liu Z, Bates J, Chandler-Militello D, Zou L, Munir J, Schanz
692 S, McCoy K, Miller RH, Wang S, Nedergaard M, Findling RL, Tesar PJ, Goldman SA (2017)
693 Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia. Cell Stem
694 Cell 21:195–208.e6. doi: 10.1016/j.stem.2017.06.012

695 37. Paquet D, Kwart D, Chen A, Sproul A, Jacob S, Teo S, Olsen KM, Gregg A, Noggle S,
696 Tessier-Lavigne M (2016) Efficient introduction of specific homozygous and heterozygous
697 mutations using CRISPR/Cas9. Nature 533:125–129. doi: 10.1038/nature17664

698 38. Bowles KR, Julia TCW, Qian L, Jadow BM, Goate AM (2019) Reduced variability of
699 neural progenitor cells and improved purity of neuronal cultures using magnetic activated cell
700 sorting. PLoS One 14:1–18. doi: 10.1371/journal.pone.0213374

701 39. TCW J, Wang M, Pimenova AA, Bowles KR, Hartley BJ, Lacin E, Machlovi SI, Abdelaal
702 R, Karch CM, Phatnani H, Slesinger PA, Zhang B, Goate AM, Brennand KJ (2017) An Efficient
703 Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells. Stem Cell
704 Reports 9:600–614. doi: 10.1016/j.stemcr.2017.06.018

705 40. Radde R, Bolmont T, Kaeser SA, Coomaraswamy J, Lindau D, Stoltze L, Calhoun ME,
706 Jäggi F, Wolburg H, Gengler S, Haass C, Ghetti B, Czech C, Hölscher C, Mathews PM, Jucker
707 M (2006) Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust
708 pathology. EMBO Rep 7:940–6. doi: 10.1038/sj.embor.7400784

709 41. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B,
710 McKenna S, Mobraaten L, Rajan T V, Greiner DL (1995) Multiple defects in innate and adaptive
711 immunologic function in NOD/LtSz-scid mice. J Immunol 154:180–91

712 42. Koper MJ, Van Schoor E, Ospitalieri S, Vandenberghe R, Vandenbulcke M, von Arnim
713 CAF, Tousseyn T, Balusu S, De Strooper B, Thal DR (2020) Necrosome complex detected in
714 granulovacuolar degeneration is associated with neuronal loss in Alzheimer’s disease. Acta
715 Neuropathol 139:463–484. doi: 10.1007/s00401-019-02103-y

716 43. Thal DR, Rüb U, Schultz C, Sassin I, Ghebremedhin E, Del Tredici K, Braak E, Braak
717 H (2000) Sequence of Aβ-protein deposition in the human medial temporal lobe. J Neuropathol
718 Exp Neurol 59:733–748. doi: 10.1093/jnen/59.8.733

719 44. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Tredici K (2006) Staging of Alzheimer
720 disease-associated neurofibrillary pathology using paraffin sections and
721 immunocytochemistry. Acta Neuropathol 112:389–404. doi: 10.1007/s00401-006-0127-z

722 45. Chen H, Qian K, Chen W, Hu B, Blackbourn LW, Du Z, Ma L, Liu H, Knobel KM, Ayala
723 M, Zhang SC (2015) Human-derived neural progenitors functionally replace astrocytes in adult
724 mice. J Clin Invest 125:1033–1042. doi: 10.1172/JCI69097

725 46. Sosunov AA, Wu X, Tsankova NM, Guilfoyle E, McKhann GM, Goldman JE (2014)
726 Phenotypic heterogeneity and plasticity of isocortical and hippocampal astrocytes in the human
727 brain. J Neurosci 34:2285–2298. doi: 10.1523/JNEUROSCI.4037-13.2014

728 47. Xu R, Li X, Boreland AJ, Posyton A, Kwan K, Hart RP, Jiang P (2020) Human iPSC-
729 derived mature microglia retain their identity and functionally integrate in the chimeric mouse
730 brain. Nat Commun 11: 1577. doi: 10.1038/s41467-020-15411-9

731 48. Kirkeby A, Nolbrant S, Tiklova K, Heuer A, Kee N, Cardoso T, Ottosson DR, Lelos MJ,
732 Rifes P, Dunnett SB, Grealish S, Perlmann T, Parmar M (2017) Predictive Markers Guide
733 Differentiation to Improve Graft Outcome in Clinical Translation of hESC-Based Therapy for
734 Parkinson's Disease. Cell Stem Cell 20:135–148. doi: 10.1016/j.stem.2016.09.004

735 49. Windrem MS, Schanz SJ, Morrow C, Munir J, Chandler-Militello D, Wang S, Goldman
736 SA (2014) A Competitive Advantage by Neonatally Engrafted Human Glial Progenitors Yields
737 Mice Whose Brains Are Chimeric for Human Glia. J Neurosci. 34:16153–16161. doi:
738 10.1523/JNEUROSCI.1510-14.2014

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741 **TABLES AND FIGURE LEGENDS**

742 **Table 1. Information on the hiPSC lines.** The table shows hiPSC name, patient ethnicity,
743 gender, age of onset, age at skin biopsy, disease status (CDR at biopsy), original *APOE*
744 genotype and genetic modification. F female, M male, AD Alzheimer's disease, APOE
745 apolipoprotein, CDR clinical dementia rating, hiPSC human induced pluripotent stem cells.
746 These cells were previously generated and characterized by [37].

747 **Table 2. Details of the Human Cohort.** The table shows the human subjects studied for
748 histology of astrocytes. Indicated are: the age in years, the gender, the Aβ-MTL phase
749 representing the distribution of Aβ deposits in the subfields of the MTL (43), the stage of
750 neurofibrillary tangle pathology according to Braak and Braak (44) (NFT stage), PMI,
751 neuropathological diagnosis and type of dementia. F female, M male, AD Alzheimer's disease,
752 AD-VaD Alzheimer's disease plus signs of vascular dementia, p-preAD preclinical AD, non-AD
753 non-demented control, AGD argyrophilic grain disease, B bleeding, CAA cerebrovascular
754 angiopathy, CM carcinoma metastasis, I infarction, MI microinfraction, MTL medial temporal
755 lobe, NFT neurofibrillary tangle, PMI post-mortem interval.

756 **Fig. 1 hiPSC-glia progenitors engraft the mouse brain and differentiate into astrocytes.**
757 **(a)** Schematics of the differentiation and transplantation procedures. hiPSCs: human induced
758 pluripotent stem cells, NPCs: neural progenitor cells, GPCs: glia progenitor cells, SB:
759 SB431542, LDN: LDN193189, FGF2: fibroblast growth factor 2, AGS: astrocyte growth
760 supplement. Scale bars: 100 µm. **(b)** RFP staining (red) shows the distribution of hiPSC-
761 derived astrocytes on a coronal brain section of a chimeric mouse at five months after
762 transplantation. Scale bar: 200 µm. **(c)** Dot map displaying the widespread distribution of the
763 hiPSC-derived astrocytes (RFP, red) in four coronal sections of this mouse brain. **(d-e)** RFP
764 (red) and hNuclei (green) expressing hiPSC-astrocytes depict a complex fine structure in the
765 cortex (CTX) and corpus callosum (CC) of chimeric mice. Scale bars: 50 µm (d), 25 µm (e).
766 **(d'-e')** Enlarged images of the inserts in d and e. **(f-i)** Engrafted hiPSC-astrocytes (RFP+, red)
767 express GFAP (f), S100b (g), Vimentin (h) and AQP4 (i) (green) five months after

transplantation. Scale bars: 25 µm. **(j)** Percentage of RFP+ cells expressing GFAP (n=14 mice). **(k)** Percentage of hNuclei+ cells expressing RFP (n=15 mice). **(l)** Percentage of RFP+ cells expressing NeuN and APC (n=9 mice). Data are represented as mean ± SEM

Fig. 2 hiPSC-astrocytes integrate functionally within the mouse brain. (a-b) A xenografted hiPSC-astrocyte in the chimeric mouse brain (a, red) and a GFAP+ cortical astrocyte in the human brain (b, brown) contacting blood vessels with their end-feet. Scale bars: 25 µm. **(c)** hiPSC-astrocyte processes (RFP, red) express the gap junction marker Cx43 (green, arrows). Scale bar: 2 µm. **(d)** The gap-junction dye Alexa488 loaded on a hiPSC-astrocyte (RFP+, red) diffuses into RFP- neighboring host cells. Scale bar: 25 µm. **(e-h)** Enlarged views of the area selected in d. (e) RFP+ hiPSC-astrocyte, (f) Alexa488 dye, (g) Nuclei stained with DAPI, (h) Overlay. Arrows point to Alexa488+ RFP- host cells. **(i-k)** Representative traces of current injection steps of 20mV (i), resting membrane potentials (j) and current-voltage (I/V) curves (k) of hiPSC-astrocytes in the host brain (n=17 cells from 6 mice). Data are represented as mean ± SEM

Fig. 3 hiPSC-astrocytes recapitulate human morphological subtypes and retain human specific features within the mouse brain. (a-j) Representative images of RFP+ (white) interlaminar (a-c), varicose-projection (d-e), protoplasmic (f-g) and fibrous astrocytes (h-j) in the brain of wild-type mice five months after transplantation. Scale bars: 25 µm. **(k)** Histogram showing the percentage of RFP+ cells of each astroglial subtype on the mouse brain (n=9 mice). Data are represented as mean ± SEM. **(l)** Representative image showing mouse (green, arrows) and hiPSC-astrocytes (red) on a chimeric mouse brain five months after transplantation. Scale bar: 25 µm. **(m)** Histogram plotting the size of hiPSC-derived astrocytes vs mouse astrocytes on the host brain (n=12 mice). Data are represented as mean ± SEM, Student's t test: ****p<0.0001

Fig. 4 Four subtypes of morphologically defined GFAP+ astrocytes in the human entorhinal cortex and white matter. (a) Overview of human entorhinal cortex layers stained with GFAP (brown) to detect astrocytes. Layers molecular to lamina dissecans are mainly

795 composed of subpial interlaminar astrocytes, while layers pri-α to pri-γ are rich in protoplasmic
796 astrocytes (arrows). **(b-f)** Representative images of subpial interlaminar astrocytes (b) and
797 their tortuous processes (c), protoplasmic astrocytes (d), varicose-projection astrocytes (e) and
798 their beaded processes (f). **(g-i)** Overview of human white matter (g) and GFAP+ fibrous
799 astrocytes (h-i). mol: molecular layer, diss: lamina dissecans. Scale bars: 50 μm in (a) and (g);
800 25 μm in (b) and (h); 10 μm in (c-f) and (i)

801 **Fig. 5 hiPSC-astrocytes show differential morphological responses to Aβ plaques within**
802 **the chimeric mouse brain. (a-f, a'-f')** hiPSC-astrocytes (RFP+, red) exposed to Aβ plaques
803 (Thioflavin, green) show hypertrophic (a-c, a'-c'), quiescent (d, d') and atrophic (e-f, e'-f')
804 morphologies in AD chimeric mice five months after transplantation. Scale bars: 25 μm. **(g-h)**
805 Percentage of hiPSC-astrocytes showing differential morphologies as a group (g, n=7 mice)
806 and per ApoE genotype (h, n=3 mice for APOE3/3; n=4 mice for APOE4/4) five months post-
807 transplantation. Data are represented as mean ± SEM, Chi-square test: n.s., non-significant

808 **Fig. 6 Astrocytes display differential responses to Aβ in the human AD-patient brain. (a-**
809 **f)** Representative immunohistochemistry images of GFAP+ astrocytes (brown) around
810 amyloid-deposits (blue, dashed lines) in the cortex and hippocampus of AD-patient brains. **(a-**
811 **d)** Overviews (a, b) and enlarged views (c, d) of the insets in a, b respectively. **(c-f)** GFAP+
812 hypertrophic (red arrows) and quiescent or atrophic (green arrows) astrocytes around amyloid-
813 deposits. Scale bars: 25 μm in (a, b); 10 μm in (c-f)

814 **Fig. 7 Hypertrophic, quiescent and atrophic astrocytes close to amyloid deposits in the**
815 **human AD-patient brain. (a-l)** Representative immunofluorescence images of GFAP+
816 astrocytes (red) around amyloid-deposits (4G8, green) in the cortex and hippocampus of AD
817 patient brains. **(c-l)** GFAP+ astrocytes (red) show hypertrophic (d-e, i-j), quiescent (f, k) and
818 atrophic (g, l) morphologies close to amyloid deposits. (d-g, i-l) Enlarged views of the insets in
819 c and h respectively. Scale bars: 50 μm in (a, b) and 25 μm in (c, h)

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