A toolbox of human iPSC-derived microglia in different genetic backgrounds and disease models for neurodegeneration drug discovery

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Abstract

Microglia are the tissue-resident macrophages of the brain. They survey neuronal function, play roles in neurogenesis and synaptic remodelling, and are the first responders to infection. Microglia have been implicated in a number of neurodegenerative diseases including Alzheimer's disease (AD). Due to the importance of microglia during development and disease, new models that can replicate the human phenotype are important to advance drug discovery.

Using opti-ox*, we have generated human induced pluripotent stem cell (iPSC)-derived microglia from both male and female backgrounds in a consistent manner, with the derived microglia expressing CD45, P2RY12, CD11b, CD14, IBA1, and TREM2.

Transcriptomically, male and female-derived ioMicroglia* show high similarity, and with a signature similar to primary microglia. Functionally, whilst both male and female-derived microglia demonstrate capacity to phagocytose various particles and exhibit a cytokine response to LPS and INFγ stimulation, background specific responses can be observed.

We have also demonstrated that ioMicroglia from both male and female-backgrounds can be co-cultured with excitatory neurons (ioGlutamatergic Neurons), highlighting their ability to be used to form more complex cellular model systems.

With an aim to provide a platform for investigation into mechanisms involved in neurodegeneration, we employed CRISPR/Cas9 gene editing to introduce specific point mutations in the TREM2 (R47H) and APOE (C112R, converting the wild-type APOE3 allele to APOE4) genes into the io Microglia male background; both genetic risk factors associated with late onset AD. The derived ioMicroglia maintain a microglia phenotype and remain functionally active, providing a platform for further phenotypic characterisation.

In addition, we provide multiple clones (up to three) per disease model to allow researchers to investigate phenotypes of interest across multiple clones of a specific disease model, originating from the same parental wild-type iPSC line. Studying multiple clones of a specific disease model can lead to a more comprehensive understanding of the impact of the disease-relevant mutation and cellular heterogeneity on associated disease phenotypes.

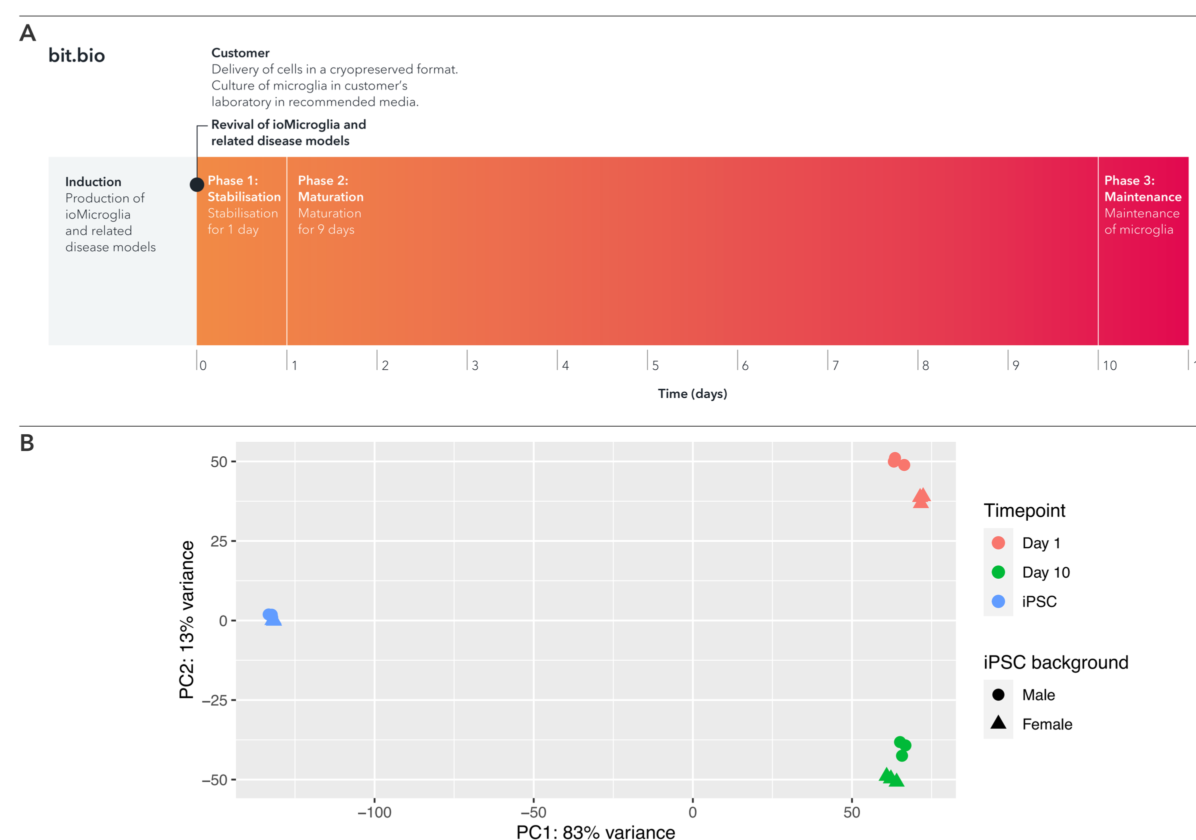
In conclusion, we have demonstrated that opti-ox mediated deterministic programming can generate microglia from iPSCs of different genetic backgrounds and can be used as a platform to create physiologically relevant disease model systems.

1. Wildtype (WT) ioMicroglia from male and female backgrounds are ready to use within 10 days and show high lot-to-lot consistency

Generation of human microglia using a simplified 3-step protocol

A. Cells are shipped in a cryopreserved format and are programmed to mature into microglia upon revival and culture in the recommended media. The protocol for generation is in 3 phases. An induction phase that is carried out at bit.bio. Phase 1: Stabilisation for 24 hours with doxycycline. Phase 2: Maturation for a further 9 days. Phase 3: the Maintenance phase. Cells are ready to use within 10 days post-thaw.

B. Bulk RNA sequencing analysis was performed on three independent lots of male and female ioMicroglia at three different time points throughout the programming protocol. Principal component analysis represents the variance in gene expression between the lots of ioMicroglia and shows high consistency across each lot at each given timepoint.



2. ioMicroglia from male, female and associated AD disease model related backgrounds display typical morphology and express key markers

A. AD-associated mutations engineered into the WT ioMicroglia male background.

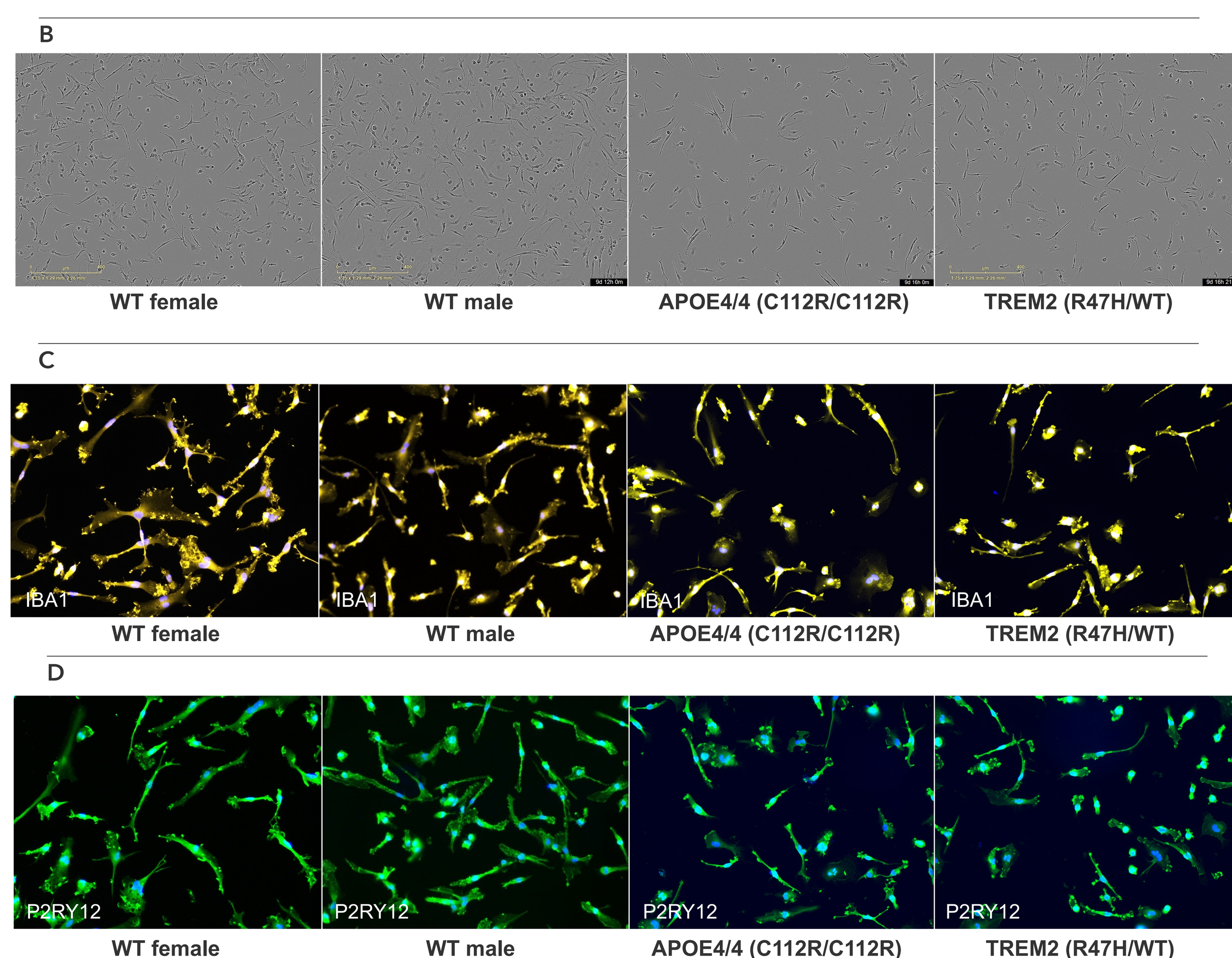
Disease models in WT ioMicroglia male background

Gene	Zygosity	Mutation
APOE	Het	C112R/WT (4/3)
	Hom	C112R/C112R (4/4)
TREM2	Het	R47H/WT
	Hom	R47H/R47H

*Note that ioMicroglia male (io1021S) is the genetically matched WT control for all the above AD disease models.

B. Brightfield images showing that ioMicroglia from WT male, female, and APOE4/4 Hom and TREM2 R47H Het disease model backgrounds display a key ramified morphology at day 10 post-thaw. 100x magnification.

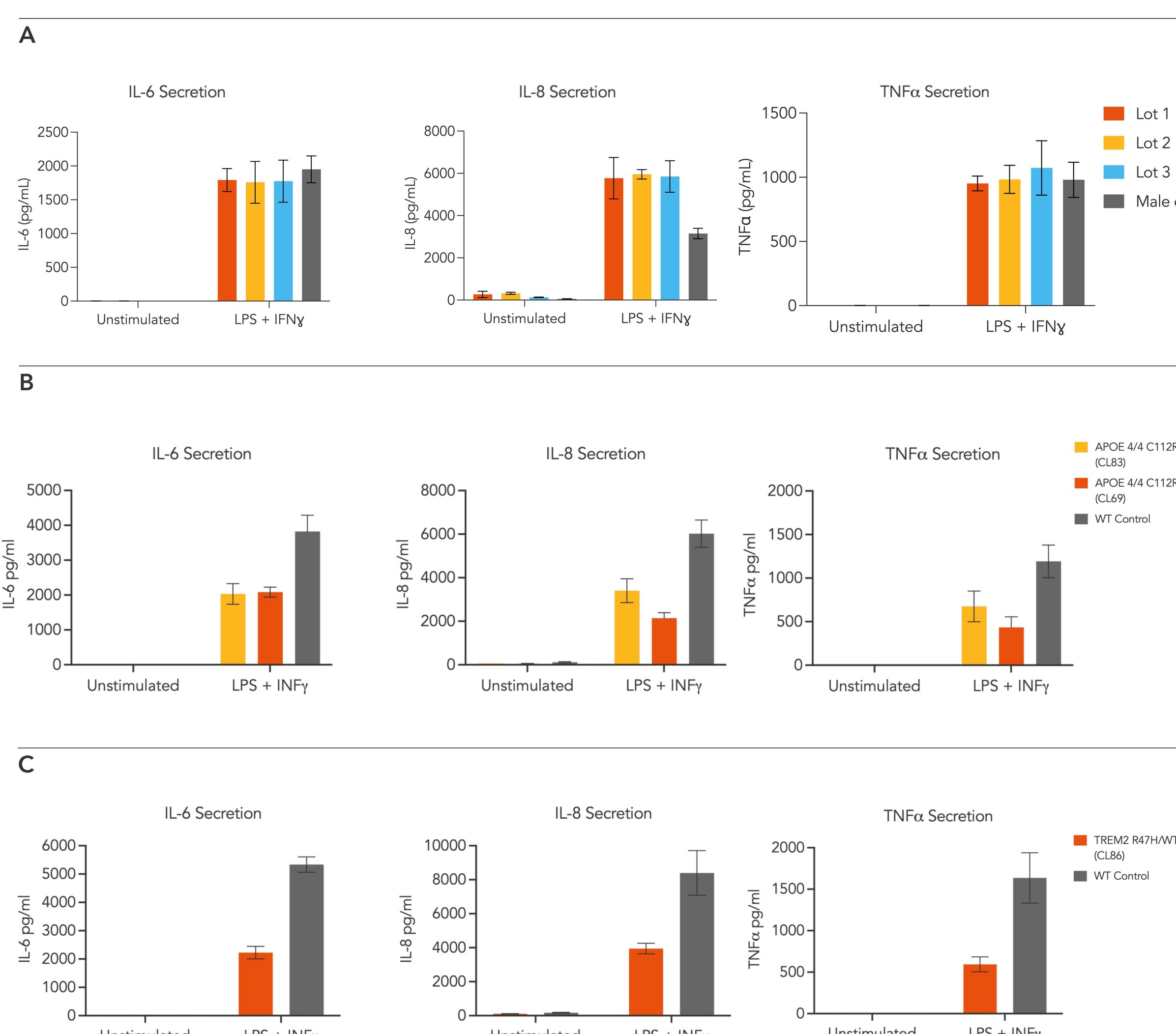
C and D. Immunofluorescent staining on day 10 post-revival demonstrates similar homogenous expression of microglia markers IBA1 (panel C) and P2RY12 (panel D) and ramified morphology in ioMicroglia from WT male, female, and APOE4/4 Hom and TREM2 R47H Het disease model backgrounds. 100X magnification.



3. ioMicroglia from male, female and associated AD disease model related backgrounds show key cytokine responses

A. Female donor-derived ioMicroglia display a different pro-inflammatory cytokine response to male donor-derived cells. Day 10 female donor-derived ioMicroglia from three independent lots were stimulated with LPS (100 ng/ml) and IFNγ (20 ng/ml) for 24 hours alongside male donor-derived ioMicroglia. Female donor-derived ioMicroglia secrete mainly pro-inflammatory cytokines, TNFα, IL-6, IL-8 (and IL-1b, IL-12p70 and IL-10 - data not shown) in response to stimuli, with lot-to-lot consistency. Female donor-derived ioMicroglia show a higher level of secretion of IL-8, IL-1β (data not shown), and a lower level of IL-12p70 cytokine (data not shown) than male donor-derived ioMicroglia.

B and C. Disease model cells display reduced secretion of pro-inflammatory cytokines upon activation compared to the genetically matched wild-type control. Cytokine response after stimulation with LPS and IFNγ was measured in the APOE4/4 Hom and TREM2 R47H Het disease model alongside the WT control, at day 10 post-revival. Both APOE4/4 disease model clones (panel B) secrete the IL-6, IL-8 and TNFα cytokines (also IL-10, IL-12p70, IL-1β – data not shown) at a lower-level compared to the WT control. The TREM2 R47H Het disease model clone (panel C) secretes the predominantly pro-inflammatory cytokines, IL-6, IL-8 and TNFα cytokines (also IL-10, IL-12p70, IL-1β – data not shown) at a lower level than the WT control.

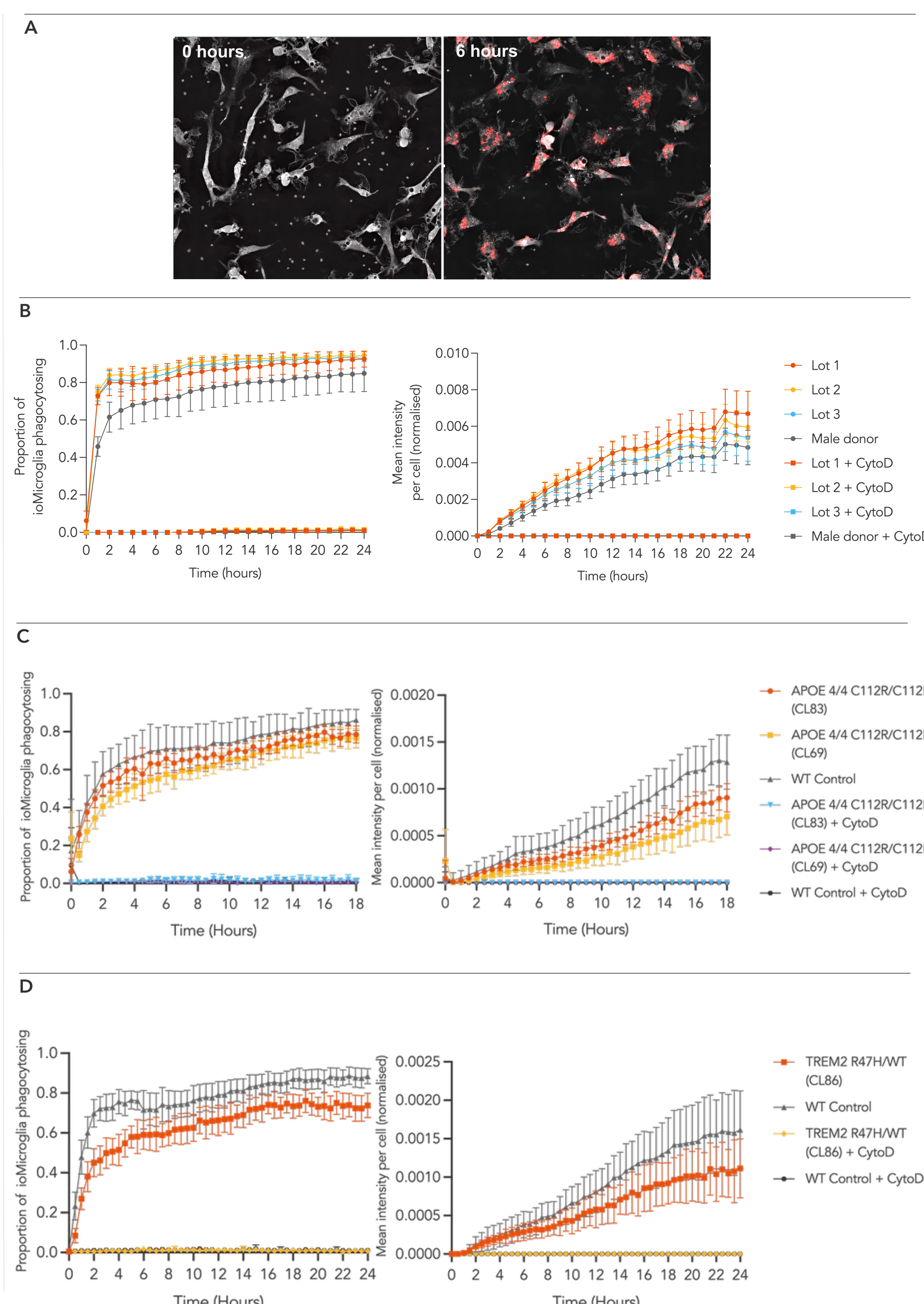


4. ioMicroglia from male, female and associated AD disease model related backgrounds show key phagocytic function

A. Representative image stills from video showing phagocytosis of pHrodo™ RED labelled Zymosan particles by female donor-derived ioMicroglia. When female donor-derived ioMicroglia engulf these particles, this causes the particles to fluoresce red, within the cells, due to the drop in pH in the phagolysosome. Live imaging was performed in 2-minute intervals over a time period of 2 hours using the 3D Cell Explorer 96focus Nanolive Imaging system.

B. Female donor-derived ioMicroglia display a consistent degree of phagocytosis across lots and display a different level of phagocytosis than male donor-derived cells. Day 10 female donor-derived ioMicroglia (io1029) from three independent lots and male donor-derived ioMicroglia (io1021) from one lot were incubated with pHrodo™ RED labelled Zymosan particles for 24 hours +/- cytochalasin D (CytoD) control. The graph on the left displays that the proportion of cells phagocytosing Zymosan particles over 24 hours is consistent across three independent lots and that female donor-derived ioMicroglia cells display a higher proportion of phagocytosis than male donor-derived cells. The graph on the right displays that the degree of cells phagocytosing Zymosan particles over 24 hours is consistent across three independent lots. Images were acquired every 30 mins on the Incucyte® looking at red fluorescence and phase contrast. Three technical replicates were performed per lot.

C and D. Disease model cells show a reduced proportion and degree of phagocytosis of E. coli particles compared to the genetically matched wild-type (WT) control. Phagocytosis of APOE4/4 Hom and TREM2 R47H Het mutant ioMicroglia was analysed at day 10 post-revival after incubation with 1 μg/0.33 cm2 pHrodo™ RED labelled E. coli particles for 24 hours +/- cytochalasin D control. The graphs on the left displays the proportion of cells phagocytosing E. coli particles over 24 hours and the graphs on the right displays the fluorescence intensity per cell displaying degree of phagocytosis per cell. These graphs show that shows that all the APOE 4/4 Hom (panel C) and TREM2 R47H Het disease clones (panel D) show a reduced proportion and degree of phagocytosis compared to the WT control. Images were acquired every 30 mins on the Incucyte® looking at red fluorescence and phase contrast. Three technical replicates were performed per experiment.

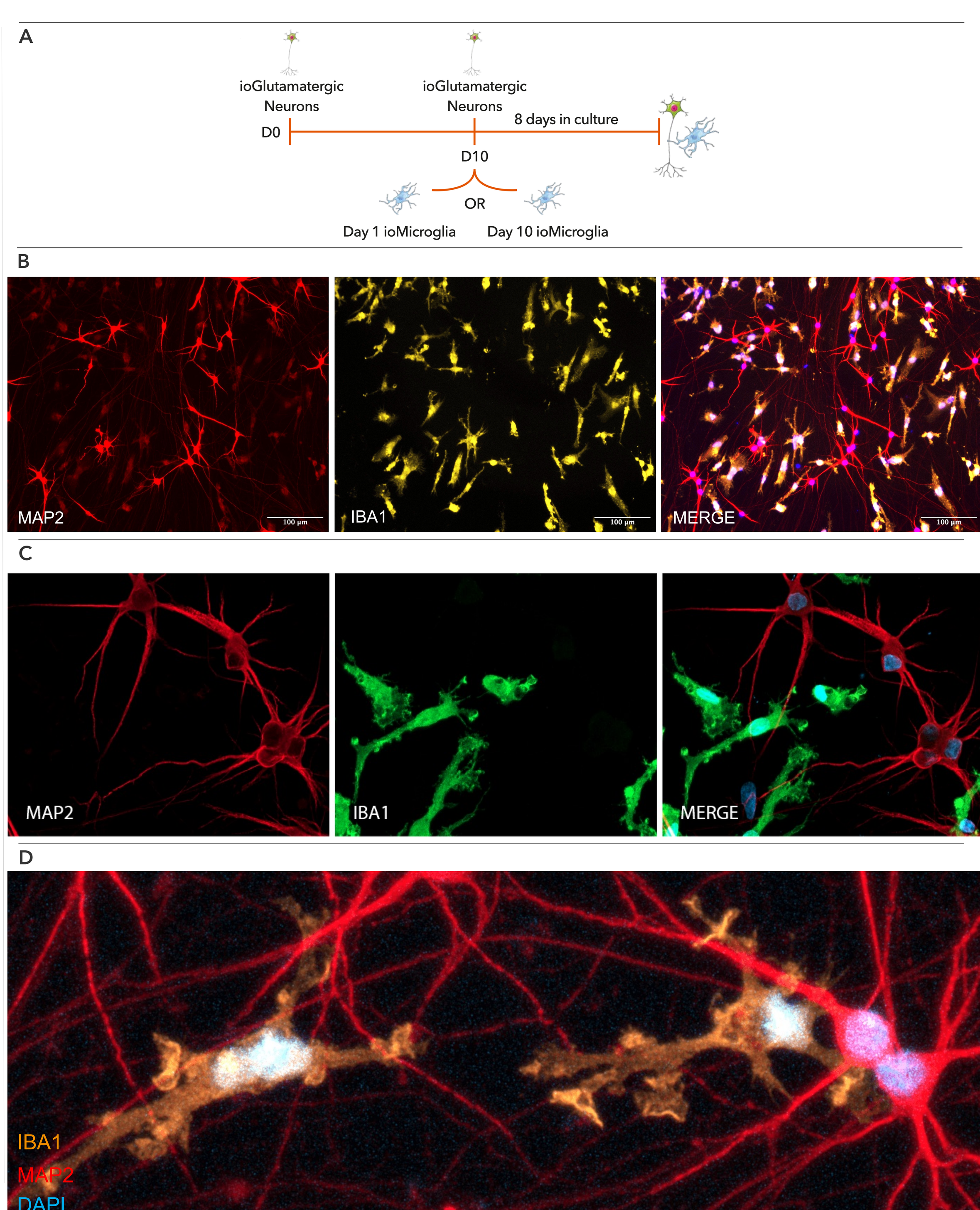


5. ioMicroglia from male and female backgrounds form stable co-cultures with ioGlutamatergic Neurons*

A. Schematic showing the co-culture protocol. ioGlutamatergic Neurons were cultured to day 10. ioMicroglia, cultured to either day 1 or day 10, were added directly to day 10 ioGlutamatergic Neurons. The co-cultures were maintained for a further 8 days.

B and C. ICC analysis at day 8 of the male donor-derived (panel B) ioMicroglia and female donor-derived (panel C) co-cultures shows homogeneous expression of the microglial marker, IBA1 (male; yellow, female; green) and the pan-neuronal marker, MAP2 (red). This enables the study of complex intercellular interactions. Representative images at 10x with 100μm scale bar.

D. Confocal image at 40x, 20μm scale bar. Male donor-derived ioMicroglia (left) display a ramified morphology (stained with IBA1, orange) and show indications of interactions with neurons, (stained with MAP2, red). Co-culture with ioGlutamatergic Neurons appears to enhance the maturation of ioMicroglia as indicated by the more pronounced ramified morphology.



Summary & conclusions

ioMicroglia have been deterministically programmed from human iPSCs from male and female donors into consistent, mature, functional microglia showing a high level of transcriptomic similarity between lots.

A panel of disease model cells carrying Alzheimer's disease-relevant mutations within the APOE (C112R, converting the wild-type APOE3 allele to APOE4) and TREM2 (R47H) genes were generated from the male donor using CRISPR/Cas9 gene engineering.

The disease model cells showed similar protein expression of key microglia markers to the genetically matched wild-type control, ensuring biological comparability of the disease models.

The ioMicroglia APOE4/4 (C112R/C112R) and TREM2 (R47H/WT) disease models also show reduced cytokine secretion levels and phagocytic activity compared to the genetically matched wild-type control.

Using opti-ox technology and CRISPR/Cas9 gene engineering, we have produced a panel of hiPSC-derived Alzheimer's disease model cells for research and drug discovery.

The panel offers an accessible, consistent, and functional system for investigating the impact of AD-relevant mutations in human microglial cells enabling research into molecular mechanisms and treatments for AD.

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