A versatile toolbox of human iPSC-derived microglia for disease modelling, CRISPR screens and multicellular in vitro models for neurodegeneration drug discovery

Authors

A. Byrne*, C. Fairbairn, P. Barton, E. Yates, M. Raman, A. Abram, R. Hickman, H. Garnett, L. Kowalski, S. Milde, M. Gamperl, B. Könye, S. Barbaria, S. Salic-Hainzl, T. Bürckstümmer, R. Northeast, F. Patell-Socha, K. Firth, W. Bernard, M. Metzakopian, M. Kotter

*Poster presenter (ann.byrne@bit.bio)

bit.bio

The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
info@bit.bio | www.bit.bio

Abstract

Microglia, the resident macrophages of the brain, play critical roles in neural function by regulating neurogenesis, synaptic remodelling, and serving as first responders to infection. They are also highly implicated in the pathology of neurodegenerative diseases, including Alzheimer's disease (AD). To advance drug discovery efforts in complex diseases such as AD, scientists need a diverse toolkit for advanced research applications to model disease, generate gene knockouts and track cells in co-culture.

Using opti-ox™, a deterministic cell programming technology, we have successfully generated human-induced pluripotent stem cell (iPSC)-derived microglia from both male and female genetic backgrounds in a consistent and scalable manner. These derived microglia express key markers, including CD45, P2RY12, CD11b, CD14, IBA1, and TREM2. Functionally, both male- and female-derived microglia exhibit robust phagocytic activity and secrete pro-inflammatory cytokines, however, background-specific responses are observed.

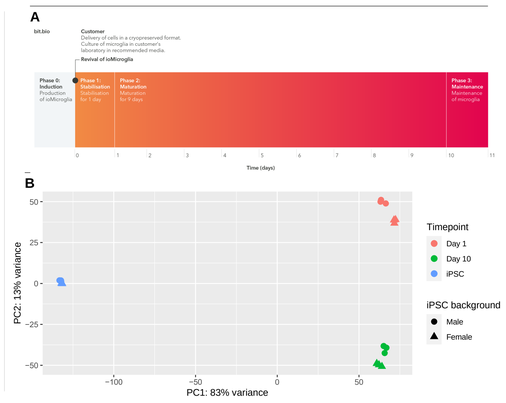
To provide new models for investigating mechanisms involved in neurodegeneration, we engineered ioMicroglia in the male genetic background with specific AD-relevant mutations. These include point mutations in TREM2 (R47H) and APOE (C112R), the latter of which exhibits a phagocytic phenotype associated with AD. Developing CRISPRko-compatible iPSC-derived cells has traditionally been a lengthy and complex process. To address this, we developed CRISPRko-Ready ioMicroglia, which constitutively express Cas9, enabling high-throughput CRISPR knockout screening and significantly reducing workflow duration from months to days. Proof-of-concept experiments validate the functionality of this system, demonstrating efficient single-gene knockouts and arrayed CRISPR screens.

To support the development of complex multicellular neurobiology models, we created GFP ioMicroglia, which constitutively express GFP throughout the cytosol for live-cell imaging, antibody-free cell sorting, and cell tracking in co-cultures. These microglia were successfully co-cultured with ioGlutamatergic Neurons and evaluated using live-cell imaging assays.

1. Human ioMicroglia are ready to use in 10 days and show high lot-to-lot consistency

A. Cells are shipped cryopreserved and are programmed to mature into microglia upon revival. The protocol is in 3 phases. Phase 0: an induction phase carried out at bit.bio. Phase 1: stabilisation for 24 hours with doxycycline. Phase 2: maturation for 9 days. Phase 3: the maintenance phase. Cells are ready to use from day 10 post-thaw.

B. Bulk RNA sequencing analysis on three independent lots of male and female ioMicroglia at three different time points of the reprogramming 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 and female backgrounds display typical morphology and showcase key functionalities

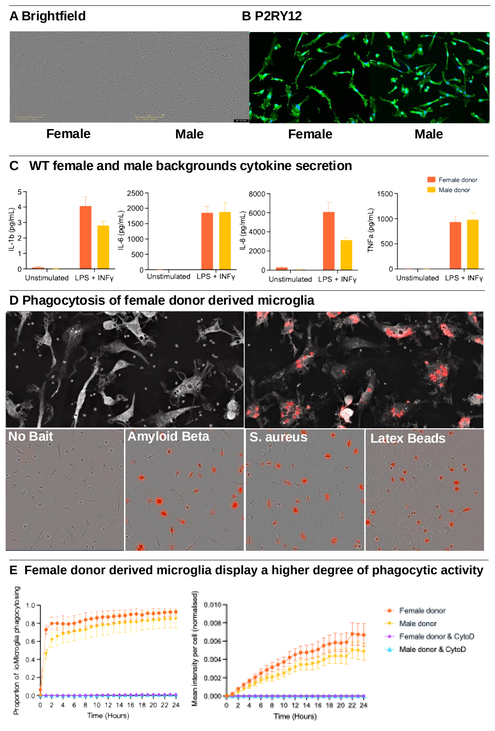
A. Brightfield images of ioMicroglia from WT female and male backgrounds displaying ramified morphology at day 10 post-thaw. 100x magnification.

B. Immunocytochemistry (ICC) on day 10 post-thaw with homogenous expression of microglia marker P2RY12 (green), with Hoechst counterstain (blue). 10x magnification.

C. Day 10 female and male donor-derived ioMicroglia were stimulated with LPS (100 ng/ml) and IFNɣ (20 ng/ml) for 24 hours. ioMicroglia secrete IL-1b, IL-6, IL-8 and TNFα (as well as IL-12p70 and IL-10 - data not shown) in response to stimuli, predominantly producing a pro-inflammatory response. Female donor-derived ioMicroglia show a higher level of secretion of IL-8 and IL-1β cytokines than male donor-derived ioMicroglia.

D. Left: NanoLive Imaging video showing phagocytosis of pHrodo™ RED labelled Zymosan particles by female donor-derived ioMicroglia. Right: Phagocytosis assay performed on day 10 male donor-derived ioMicroglia using pHrodo RED Amyloid-beta, pHrodo™ RED S.aureus and pHrodo RED latex beads. Representative images of phagocytosis post-incubation.

E. Day 10 female and male donor-derived ioMicroglia were incubated with pHrodo™ RED labelled Zymosan particles for 24 hours +/- cytochalasin D (CytoD) cotrol. ioMicroglia phagocytose pHrodo™ RED labelled Zymosan particles. Images were acquired every 30 mins on the Incucyte® looking at red fluorescence and phase contrast. Three technical replicates were performed per lot.



3. ioMicroglia Alzheimer's disease models demonstrate relevant disease-related phenotypes

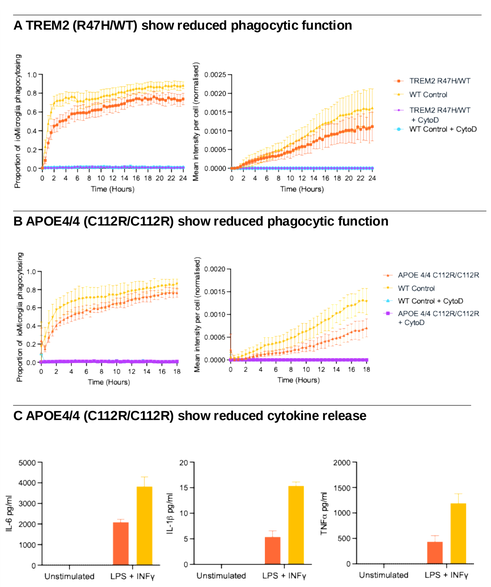
Disease models		
Gene	Zygosity	Mutation
APOE	Het	C112R/WT (4/3)
	Hom	C112R/C112R (4/4)
TREM2	Het	R47H/WT
	Hom	R47H/R47H

ioMicroglia Alzheimer's disease models with early onset disease mutations in APOE and TREM2.

A. Phagocytosis of TREM2 (R47H/WT) 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 graph on the left displays the proportion of cells phagocytosing E. coli particles over 24 hours. The graph on the right displays the fluorescence intensity per cell displaying degree of phagocytosis per cell. Images were acquired every 30 mins on the Incucyte® looking at red fluorescence and phase contrast. Three technical replicates were performed per experiment.

B. As above for the APOE4/4 (C112R/C112R) mutant ioMicroglia.

C. Cytokine response after stimulation with LPS (100 ng/ml) and IFNɣ (20 ng/ml) was measured in the APOE4/4 (C112R/C112R) disease model alongside the WT control, at day 10 post-revival. Secretion of IL-6, IL-8 and TNFα (also IL-10, IL-12p70, IL-1β – data not shown) in the disease model was at a lower-level compared to the WT control.



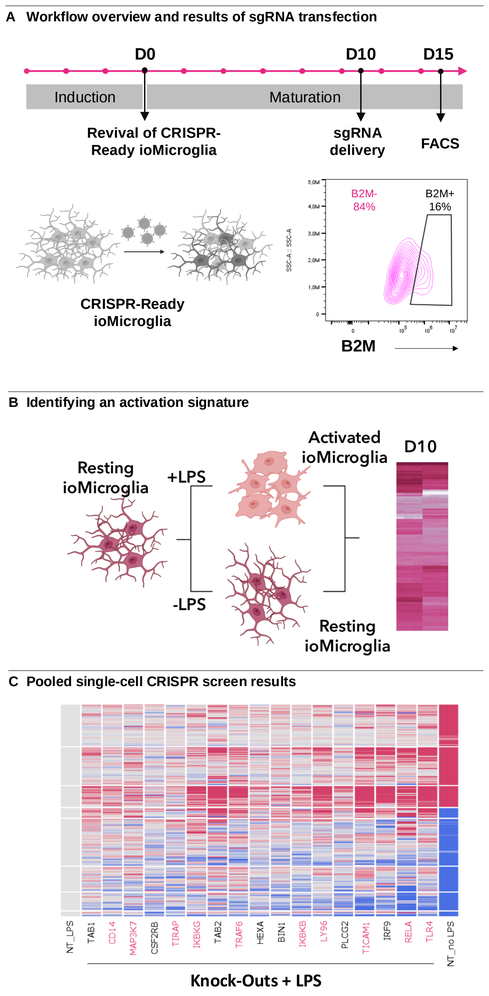
4. Leveraging CRISPR-Ready ioMicroglia for functional genomics studies

CRISPR-Ready ioMicroglia constitutively express Cas9 for routine gene knockouts and CRISPR screens.

A. These cells are delivered as a cryopreserved product. The protocol for culturing these cells requires a 10-day maturation phase. sgRNA targeting B2M was delivered by lipid-based transfection on day 10 post thawing, followed by FACS analysis on day 15. FACS analysis of CRISPR-Ready ioMicroglia following sgRNA transfection revealed 84% knockout efficiency of B2M.

B. An activation signature of LPS-treated CRISPR-Ready ioMicroglia was identified using bulk RNA sequencing. A total of 1,610 genes were differentially expressed between the LPS-treated and untreated conditions. Out of these, 258 genes were selected for the targeted sequencing readout. This signature served as a benchmark in the pooled scCRISPR knockout screen to identify modulators of LPS-induced activation.

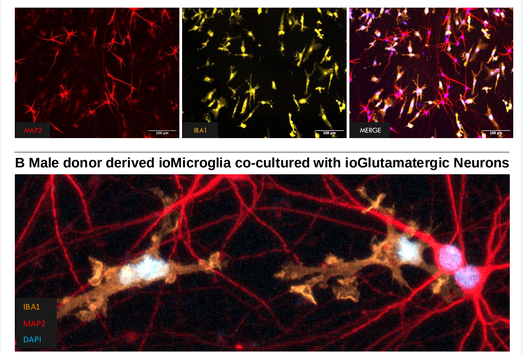
C. 110 candidate genes were selected for the pooled scCRISPR screen based on their known roles in neurodegeneration and neuroinflammation. Guide RNAs were delivered via lentiviral transduction on day 10, aiming for a single integration per cell. The cells were treated with +/- LPS for 24 hours before single cell processing on day 15. Cosine similarity analysis compared knockouts in LPS-treated CRISPR-Ready ioMicroglia to both resting and activated states. The analysis identified 17 gene knockouts that altered responses to LPS stimulation. The heatmap shows Log2FC profiles for gene knockouts that had a cosine similarity above 0.3 (arbitrarily chosen threshold) compared to cells with non-targeting guides in the unstimulated condition. Knockouts are sorted based on their cosine similarity to the non-LPS condition. CD14, MAP3K7, TIRAP, IKBKG, TRAF6, IKBKB, LY96, TICAM1, RELA, and TLR4 are genes known to be involved in LPS activation mediated via the TLR4 signalling pathway.



5. ioMicroglia form co-cultures with ioGlutamatergic Neurons

A. 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. Confocal images of ioGlutamatergic neurons (left) stained with MAP2 and male donor-derived ioMicroglia (middle) stained with IBA1. 100µm scale bars.

B. As above but at 40x, 20µm scale bar. Male donor-derived ioMicroglia display a ramified morphology and show indications of interactions with neurons, (stained with MAP2, red).



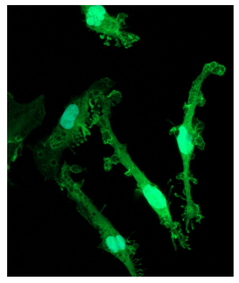
6. Utilising GFP ioMicroglia for live-cell imaging and easy co-culture tracking

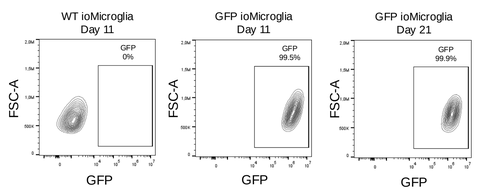
GFP ioMicroglia constitutively express GFP throughout the cytosol

A. Flow cytometry analysis demonstrating GFP expression in over 99.5% of cells for GFP ioMicroglia cultured until day 11, with no GFP expression seen in ioMicroglia Male (io1021). At day 21, the percentage of cells expressing GFP and the intensity does not decrease over time, indicating there is no silencing of the reporter gene.

B. GFP ioMicroglia were cultured to day 10 post-thaw and were added directly to day 11 ioGlutamatergic Neurons. The co-cultures were maintained for a further 3 days before live-cell imaging with Leica DMi8 (**C**).

C. Brightfield and fluorescence images were taken and merged, demonstrating distribution of GFP ioMicroglia within the co-culture.





Summary & conclusions

opti-ox-mediated deterministic programming of iPSC-derived microglia from diverse genetic backgrounds and serves as a versatile platform for disease modelling and the development of advanced co-culture systems.

Wild-type microglia from male and female backgrounds display key characteristics and functionalities as expected and provide a diverse panel to study complex neurogenerative diseases with a neuroinflammatory component.

A panel of disease model cells carrying Alzheimer's disease-relevant mutations within the APOE4/4 (C112R/C112R) & TREM2 (R47H/WT) display phenotypic differences in phagocytosis and cytokine secretion, indicating their utility in modelling AD for drug discovery research.

CRISPR-Ready ioMicroglia, constitutively express Cas9 nuclease for gene knockout and functional assays. In our proof-of-concept CRISPR screen, we identified modulators of microglial activation, illustrating the suitability of CRISPR-Ready ioMicroglia for advanced applications such as single-cell CRISPR screens.

ioMicroglia form functional co-cultures with ioGlutamatergic Neurons, supported by a fully optimised protocol.

GFP ioMicroglia enable easy tracking of cell motility and morphology, aid assessment of multi-cell culture integration via live-cell imaging and enable antibody-free cell sorting.

For information on bit.bio's trade marks, visit www.bit.bio/trademarks