Advancing drug discovery: leveraging CRISPR-Ready Microglia* for functional genomics studies

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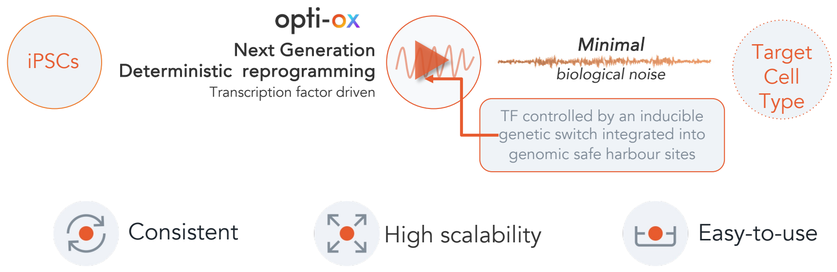
About bit.bio's opti-ox* technology

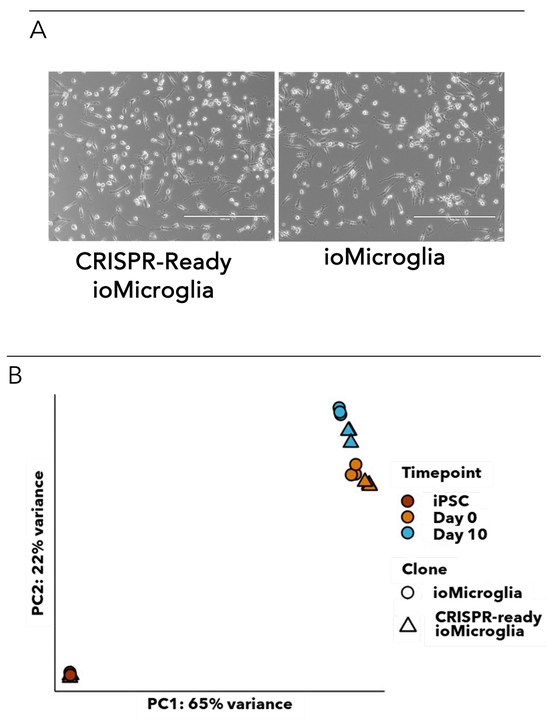


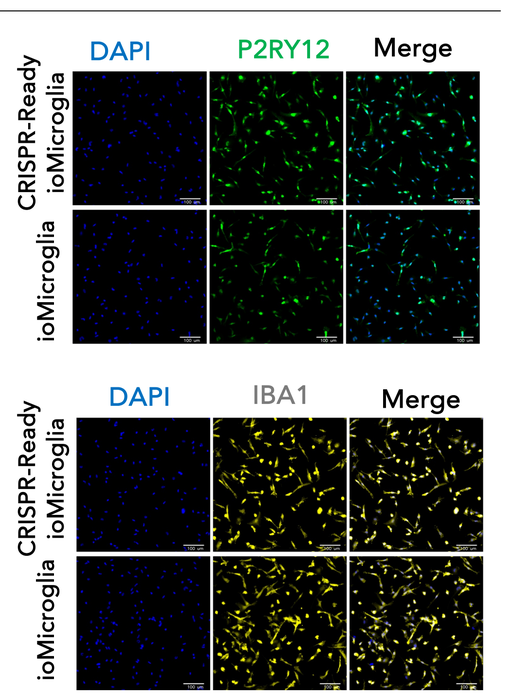
CRISPR-Ready ioMicroglia*

Microglia play a pivotal role in maintaining neural health, defending against pathogens, and maintaining the brain's functional equilibrium. Leveraging bit.bio's precision reprogramming technology, we introduce CRISPR-Ready ioMicroglia* for CRISPR/Cas9-based knockout screening. CRISPR-Ready ioMicroglia have been engineered to constitutively express Cas9, which is functional from day 1 post thaw. Using a guide RNA (sgRNA) targeting beta-2 microglobulin (B2M), we demonstrate >84% knockout efficiency at protein level as confirmed by flow cytometry.

CRISPR-Ready ioMicroglia retain all defining features of ioMicroglia, such as the expression of key markers (CD45, CD14, P2RY12, CX3CR1, CD11b and IBA1), and cytokine secretion profiles. Constitutive Cas9 expression does not adversely affect the reprogramming potential, transcriptional profile, or functionality of CRISPR-Ready ioMicroglia.

1. CRISPR-Ready ioMicroglia exhibit similar morphology and gene expression profiles as ioMicroglia





A. Brightfield images of CRISPR-Ready ioMicroglia and ioMicroglia 7 days post thawing show similar cell morphologies.

B. Principal component analysis (PCA) of bulk RNA sequencing data comparing CRISPR-Ready ioMicroglia and ioMicroglia at different time points during forward programming. Analysis shows comparable gene expression profiles.

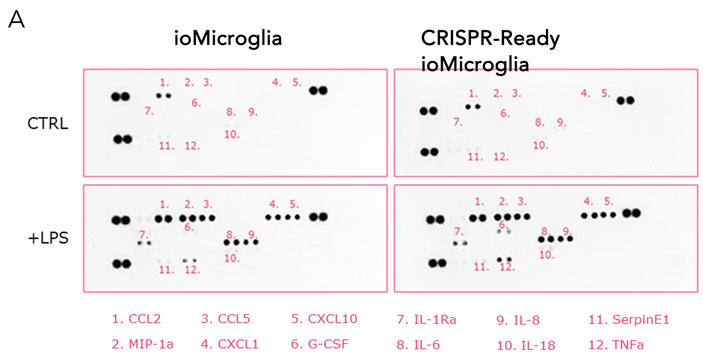
C. Immunofluorescent staining on day 10 post-revival demonstrates similar homogenous expression of the key microglia markers P2RY12 (upper panel) and IBA1 (lower panel) in CRISPR-Ready ioMicroglia compared to the genetically matched ioMicroglia. 100X magnification.

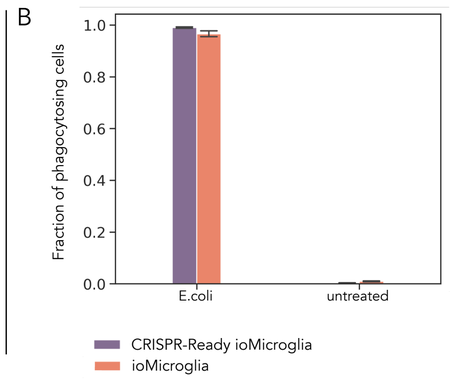
2. Functional Characterisation of CRISPR-Ready ioMicroglia

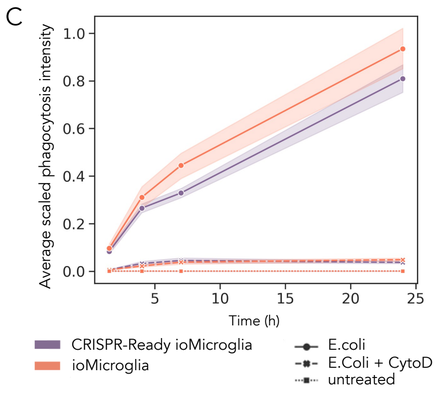
A. ioMicroglia and CRISPR-Ready ioMicroglia show comparable cytokine secretion profiles in resting and LPS (Lipopolysaccharide) - stimulated states at day 10 post-revival.

B. Phagocytosis assay using pHrodo™ E. coli BioParticles™ demonstrates efficient uptake of bacteria particles by CRISPR-Ready ioMicroglia and ioMicroglia after 4 hours of treatment.

C. An increase of fluorescence intensity of E.coli particles upon pH change in the phagosome, can be readily detected by fluorescence microscopy. A steep increase of fluorescence signal intensity was measured in the presence of E.coli particles alone, but not in combination with Cytochalasin D (CytoD), an inhibitor of actin polymerization.





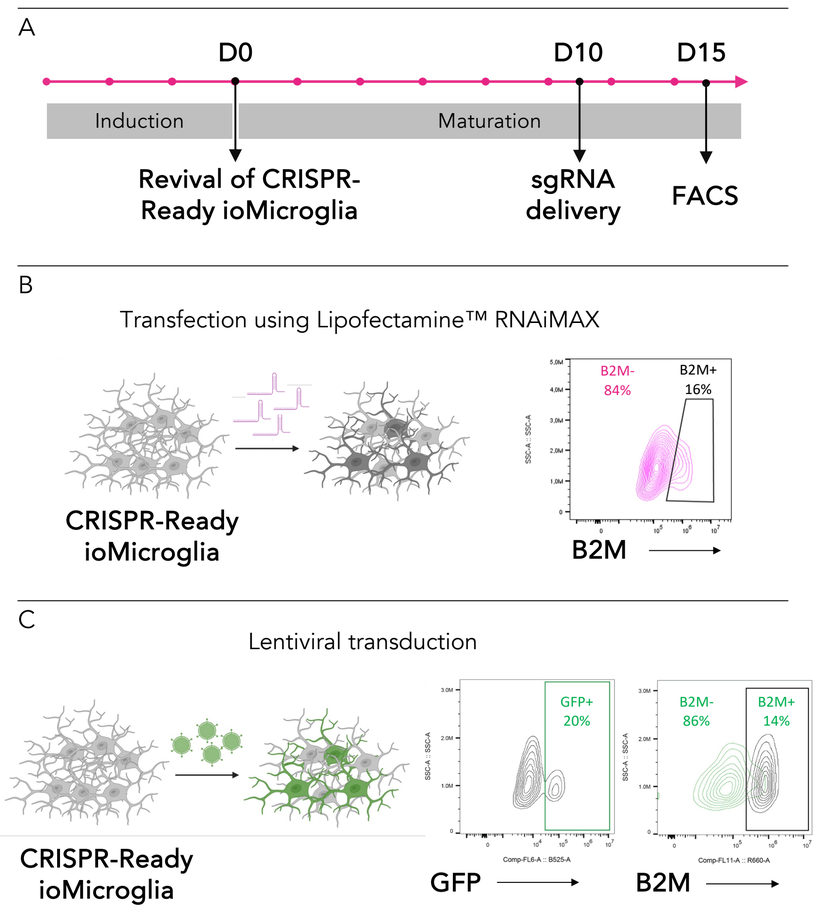


3. Lentiviral-based and Lipid-based sgRNA delivery result in high gene knockout efficiency in CRISPR-Ready ioMicroglia

A. CRISPR-Ready ioMicroglia are delivered as a cryopreserved product. The protocol for culturing these cells requires a 10-day maturation phase. sgRNA targeting B2M was delivered either by lipid-based transfection or by lentiviral transduction on day 10 post thawing, followed by FACS analysis on day 15.

B. Flow cytometry analysis of CRISPR-Ready ioMicroglia at day 15 following lipid-based transfection with sgRNA targeting B2M. High knockout efficiency of 84% was observed.

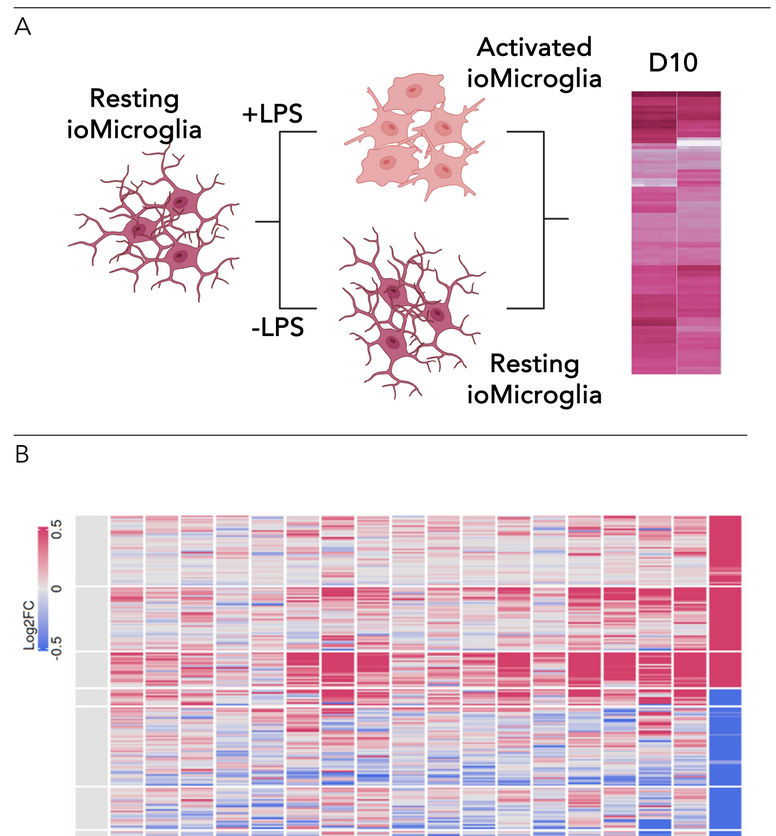
C. Flow cytometry analysis of CRISPR-Ready ioMicroglia on day 15 following lentiviral transduction. 20% of cells received the B2M sgRNA, as measured by GFP expression. High knockout efficiency of 86% was observed in these cells.



5. Targeted single-cell RNA sequencing uncovers genes involved in microglia activation following a pooled CRISPR/Cas9 based knockout screen

A. 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.

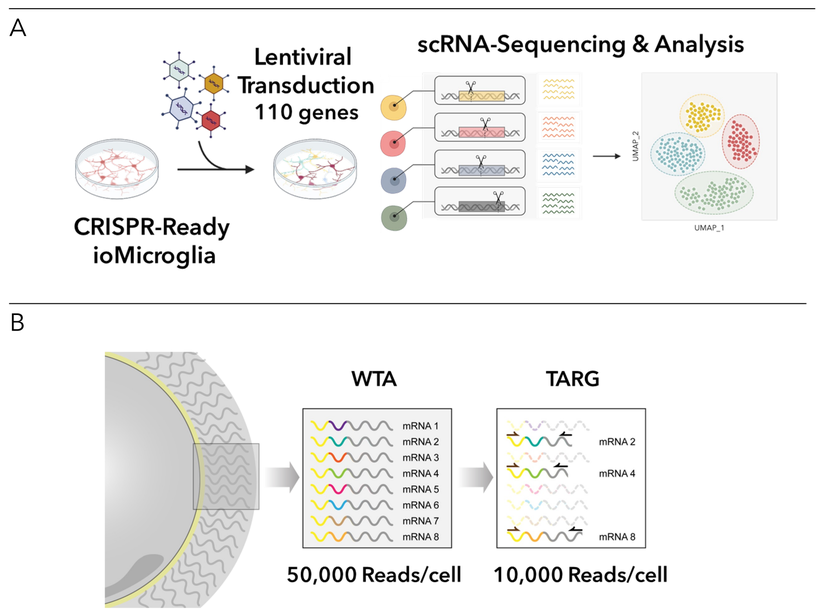
B. 110 candidate genes were selected for the pooled scCRISPR screen based on their known roles in neurodegeneration and neuroinflammation. Guide RNAs were delivered via lentiviral transduction on day 10, aiming for a single integration per cell. The cells were treated with +/- LPS for 24 hours before 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.



4. CRISPR-Ready ioMicroglia can be used in pooled CRISPR/Cas9 based knockout screens

A. CRISPR-Ready ioMicroglia are used in CRISPR/Cas9-based knockout screening workflows. A screen was performed targeting 110 genes involved in LPS-induced activation of microglia, with scRNA sequencing as a readout.

B. scRNA-seq can be conducted as Whole Transcriptome Analysis (WTA) or Targeted (TARG) scRNA sequencing. The latter offers a lower-cost alternative by focusing only on genes of interest.



Summary & conclusions

Functional CRISPR-Ready ioMicroglia

Well characterised and functional human microglia with constitutive Cas9 expression.

High knockout efficiency

Deliver sgRNA by lipid-based transfection or lentiviral-based transduction.

Compatible with knockout screening workflows

Perform large scale experiments for target identification and validation