Drug Discovery Tools to Examine Neuroinflammation Signalling in Human iPSC-Derived Microglia

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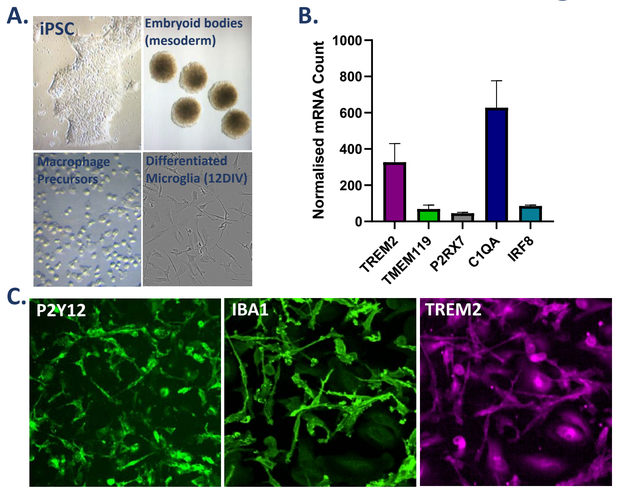
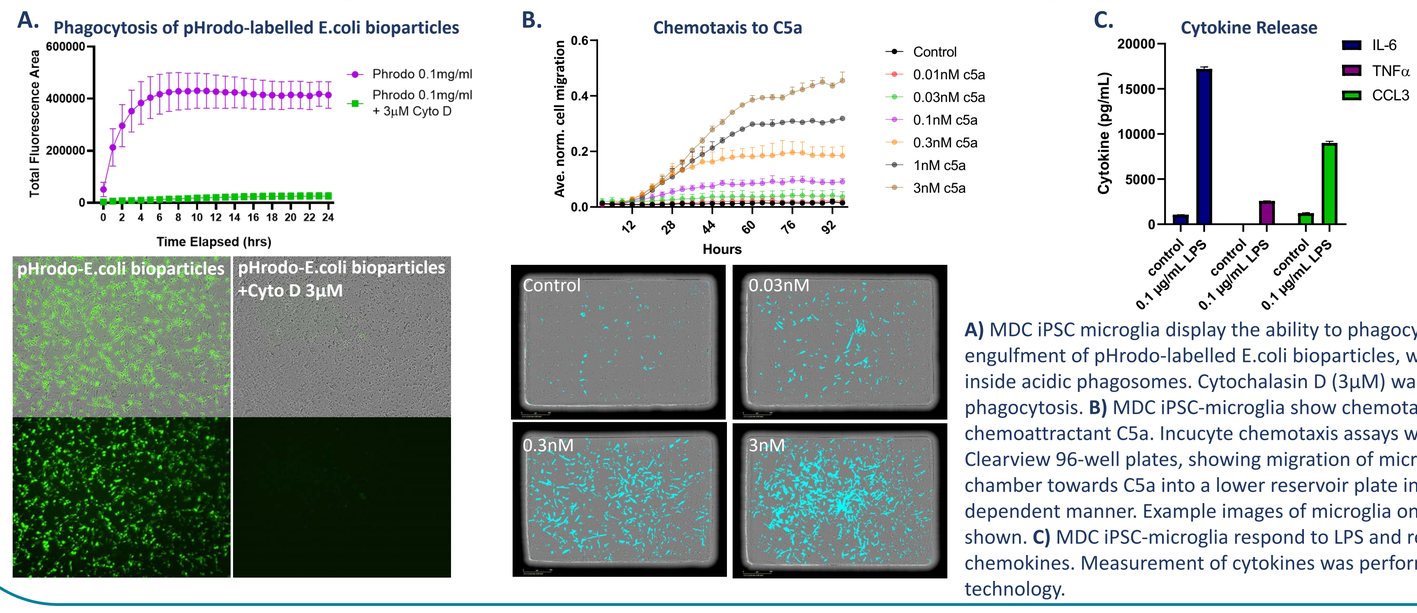
- **Huge challenges exist in central nervous system (CNS) drug discovery**: There is significant unmet need across the spectrum of CNS disorders.
- There has been renewed interest in developing novel CNS therapeutics and innovation with advances in human iPSC cell models, biomarker research and understanding immune system contribution.
- Neuroinflammation is proposed to play a major role in across the spectrum of CNS disorders, including neurodegenerative diseases such as Alzheimer's and Parkinson's.
- Microglia, the resident immune cells of the CNS, are key mediators of neuroinflammation in the CNS. Recently, recently there has been a significant effort directed towards developing human *in vitro* iPSC-microglia cell models, with the aim to improve the understanding of disease mechanisms and to increase clinical translation.
- At MDC, we have generated functional iPSC-microglia that respond to inflammatory stimuli.
- We have established lentiviral based reporters to interrogate inflammation signalling and inflammasome activation for drug discovery projects.
- Together these represent **invaluable drug discovery tools for investigating CNS and inflammatory diseases.**

1. Production of MDC iPSC-Microglia



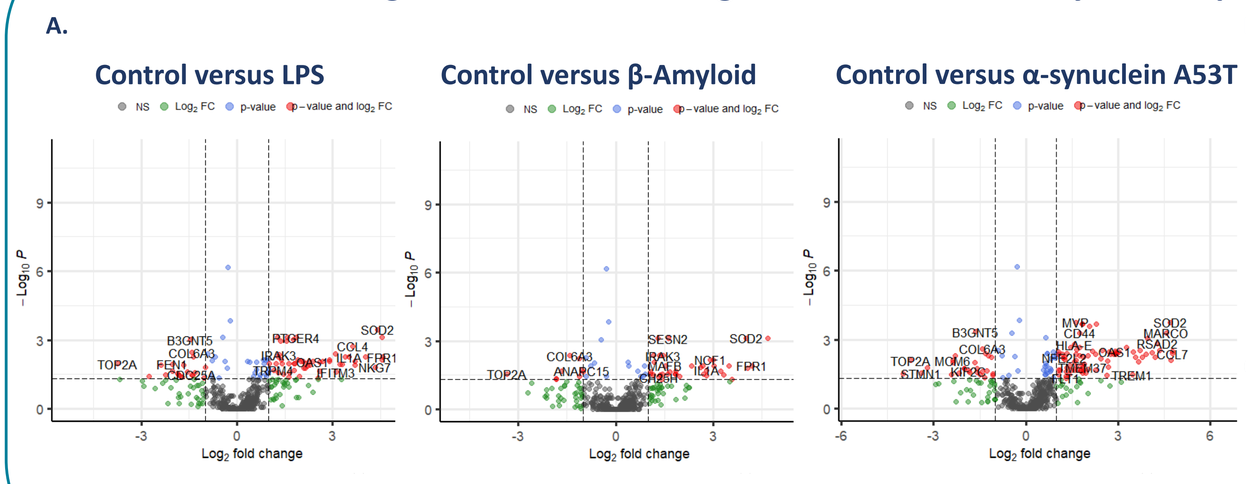
A) Brightfield images showing stages of differentiation of MDC iPSC-microglia (Haenseler et al 2017).**B)** mRNA analysis demonstrates expression of common microglia genes. **C)** Immunocytochemistry analysis of MDC iPSC-microglia showing expression of P2Y12, IBA1 and TREM2.

2. MDC iPSC-Microglia are Functional and Release Inflammatory Cytokines



A) MDC iPSC microglia display the ability to phagocytose. Here shown is engulfment of pHrodo-labelled E.coli bioparticles, which fluoresce once inside acidic phagosomes. Cytochalasin D (3µM) was added to inhibit phagocytosis. **B)** MDC iPSC-microglia show chemotaxis towards the chemoattractant C5a. Incucyte chemotaxis assays were performed in Clearview 96-well plates, showing migration of microglia from an upper chamber towards C5a into a lower reservoir plate in a concentration-dependent manner. Example images of microglia on the lower reservoir are shown. **C)** MDC iPSC-microglia respond to LPS and release cytokines and chemokines. Measurement of cytokines was performed using Luminex technology.

3. Molecular Profiling of MDC iPSC-Microglia Demonstrates they are Responsive to Inflammatory and Disease-causing Stimuli

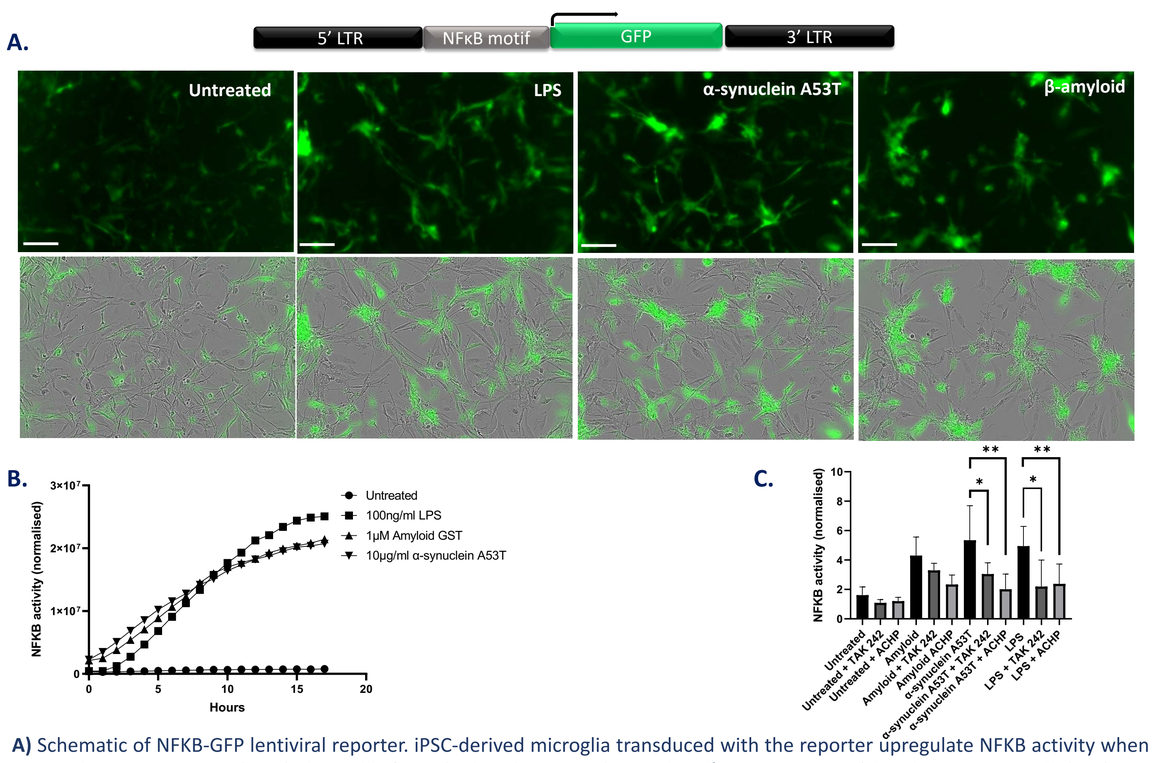


C.

	Number of Signif DEGs
Control vs LPS	73
Control vs β-Amyloid	46
Control vs α-Synuclein A53T	106

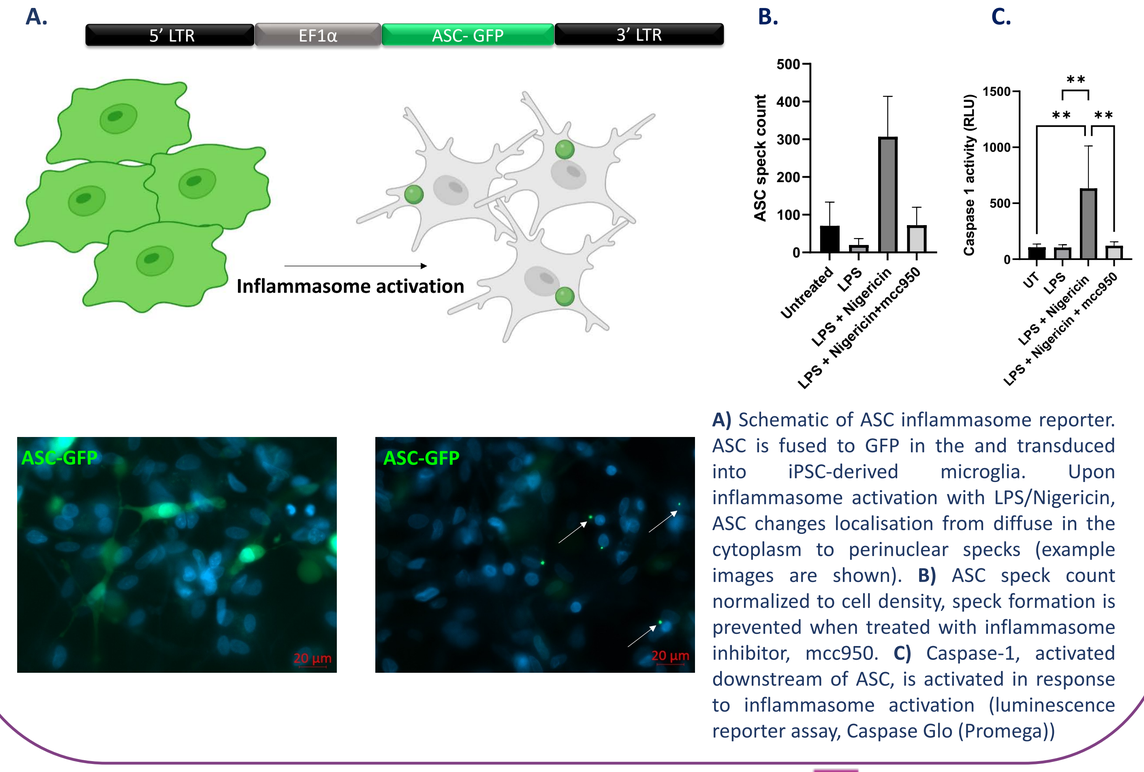
A) Volcano plots to display gene expression changes in MDC iPSC-microglia following treatment with LPS (100ng/ml), β-Amyloid (1µM) or α-Synuclein A53T (10µg/ml) (24 hours) using the Nanostring nCounter Neuroinflammation panel (770 genes) **B)** Graphs showing differences in mRNA levels in selected genes involved in cytokine/chemokine signalling and inflammation pathways upon treatment with stimuli. **C)** Table displaying the number of differentially expressed genes (DEGs) in MDC iPSC-microglia arising with the different treatments

4. Use of a NFκB-GFP lentiviral Reporter in MDC iPSC Microglia Demonstrates Response to Multiple Inflammatory Stimuli



A) Schematic of NFKB-GFP lentiviral reporter. iPSC-derived microglia transduced with the reporter upregulate NFKB activity when exposed to various stimuli including LPS, β-amyloid and α- synuclein A53T. **B)** Live imaging of NFKB activity over 24hr shows upregulation within hours of stimulation **C)** Attenuation of activation by NFKB inhibitors 12 hr post stimulation

5. Tools to Examine Inflammasome Activation



A) Schematic of ASC inflammasome reporter. ASC is fused to GFP in the and transduced into iPSC-derived microglia. Upon inflammasome activation with LPS/Nigericin, ASC changes localisation from diffuse in the cytoplasm to perinuclear specks (example images are shown). **B)** ASC speck count normalized to cell density, speck formation is prevented when treated with inflammasome inhibitor, mcc950. **C)** Caspase-1, activated downstream of ASC, is activated in response to inflammasome activation (luminescence reporter assay, Caspase Glo (Promega))

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