Dissecting NLPR3 and DAP12/SYK Signaling Pathways Involved in Neuroinflammation using Human iPSC-derived Microglia

Alfonso Tedeschi, Michelle Curtis, Sarah Burton, Christie Savic, Madelyn Donegan, Rebecca Fiene, Jeanie Liu, Beatriz Freitas, Coby Carlson, and Scott Schachtele

FUJIFILM Cellular Dynamics, Inc., Madison, WI USA

FUJIFILM Value from Innovation

AD/PD™ 2026 ADVANCES IN SCIENCE & THERAPY

Poster #1900

Abstract

BACKGROUND: Microglia are the primary CNS immune cells and regulators of neuroinflammation.
- Dysregulated activation can lead to neurodegenerative diseases; e.g Alzheimer's disease (AD).
- Inflammatory pathways activate the NLRP3 inflammasome, IL-1β & IL-18 release, and caspase-1.
- AD risk genes (TREM2, APOE) influence microglial activation and inflammatory signaling.

OBJECTIVE: Develop a human iPSC-derived microglia model to:
- Interrogate NLRP3 inflammasome activation.
- Characterize TREM2–DAP12/SYK signaling.
- Illustrate the link between AD-associated genetic variants and microglial inflammatory responses.

METHODS: Cells, Stimuli, and Assays
- iCell® Microglia; Fujifilm Cellular Dynamics); Apparently healthy control (AHN); TREM2 variants R47H (patient-derived), HZ and HO Knock-outs (engineered); and APOE E4/4 (patient-derived)
- LPS, IFN-γ, Nigericin, ATP
- HTRF cytokine assays, Lumit immunoassays, AlphaLISA

CONCLUSIONS
- Human iPSC-derived microglia recapitulate key AD-linked neuroinflammatory pathways.
- Genetic risk variants (TREM2, APOE) significantly alter microglial inflammatory signaling.
- Human iPSC-derived microglia can drive therapeutic discovery by linking AD genetics and neuroinflammation.

Using Human iPSC-derived Microglia to Study Neuroinflammatory Biology

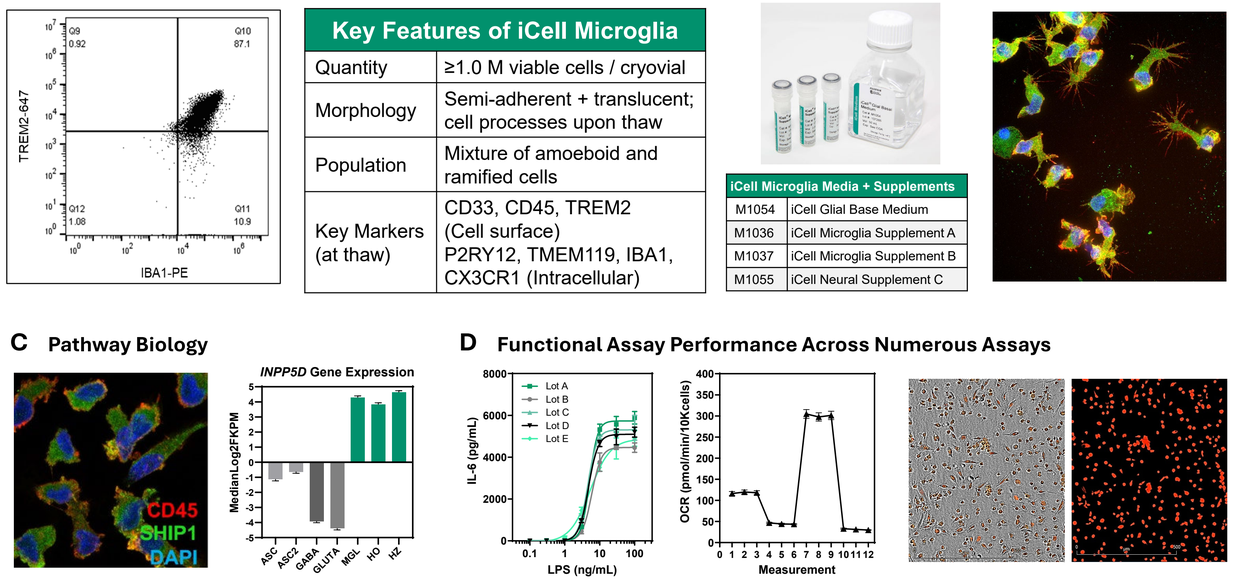


Figure 1. iPSC Microglia Characterization

(A) iCell Microglia[1] are highly pure human microglia (flow cytometry; TREM2 and IBA1) provided as a cryopreserved kit with optimized media for viability and function.
(B) Microglial morphology evidenced by typical ameboid and ramified structures.
(C) Microglia pathway biology evidenced by ICC, Western blot, and RNA-seq.
(D) Microglial functionality across cytokine release, neuroinflammation, metabolism, and phagocytosis.

Interrogating Mechanisms of Microglia NLRP3 Inflammasome Activation

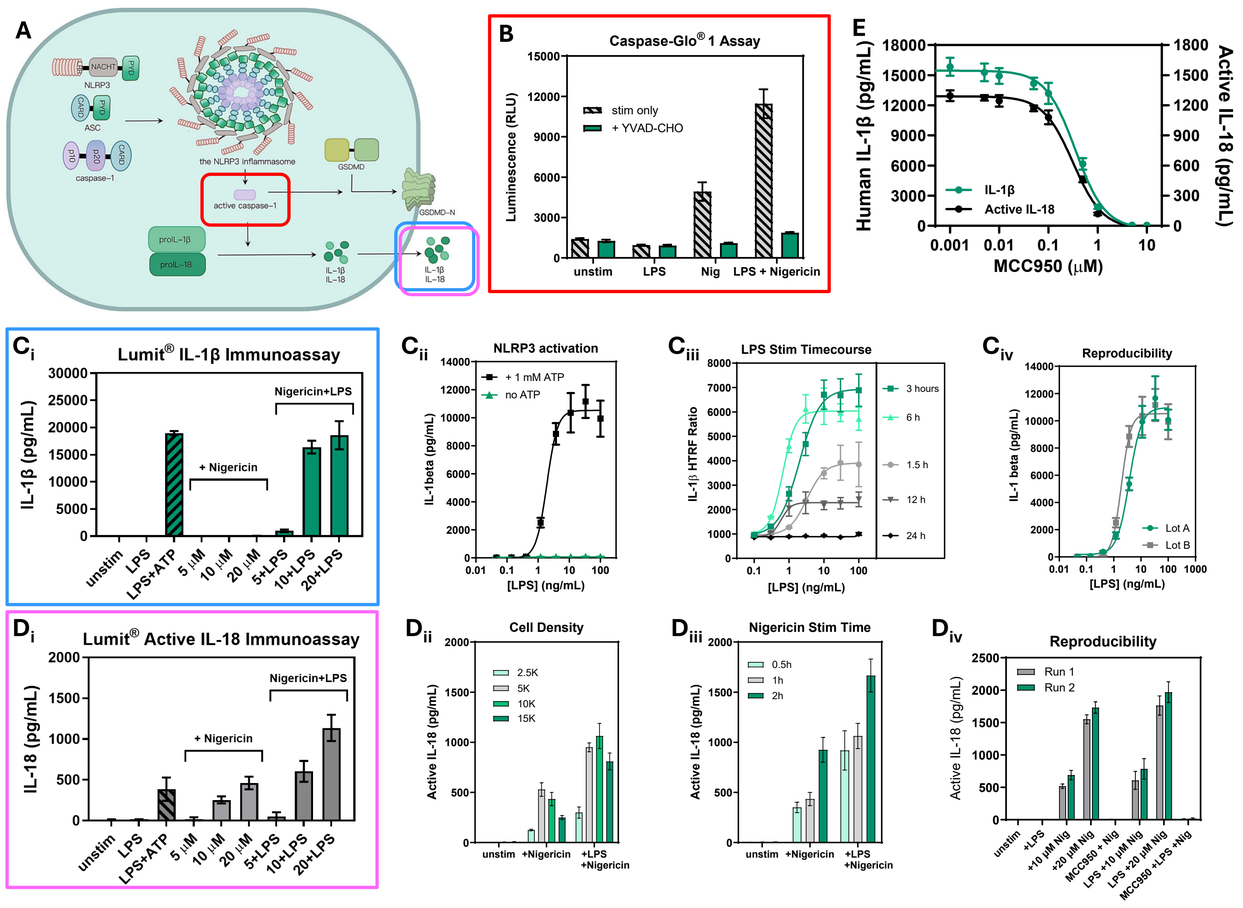


Figure 2. Microglial Inflammasome Activation and Modulation

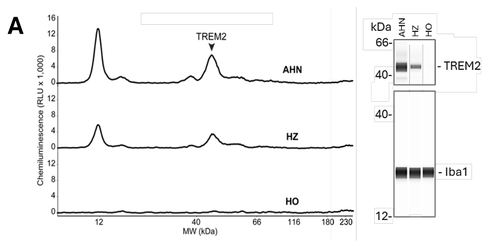
(A) Inflammasome activation causes caspase-1 activity (red) and release of IL-1β (blue) and IL-18 (pink).
(B) Inflammasome activation requires both LPS priming and Nigericin (Caspase-1 activity. Pathway specificity demonstrated by YVAD-linked inhibition
(C) IL-1β secretion requires LPS (100ng/mL) with ATP (1mM) or Nigericin (10–20μM). C_{ii-iv} illustrate the LPS-dose and time dependency of Il1β secretion and reproducibility across cell lots.
(D) IL-18 secretion requires LPS (100ng/mL) with ATP (1mM). Nigericin alone causes IL-18 secretion and is augmented by LPS priming. D_{ii-iv} illustrate the impact of cell density and time dependency of Nigericin-based IL-18 secretion and reproducibility across runs.
(E) Inflammasome inhibition is demonstrated by pre-treatment with MCC950 (1hr) before Nigericin treatment (20μM, 2hrs) after LPS priming; IC50 values of 0.35μM and 0.32μM, respectively.

[1] iCell Microglia are derived from an apparently healthy iPSC donor, using licensed protocols from the Blurton-Jones lab (Abud et al. Neuron 2017).

Connecting AD Risk Factors to Microglial Neuroinflammation Cascades

AD Panel of iCell Microglia		
Description	**Donor**	**Cat. #**
AHN (control)	01279	C1110
TREM2 HZ KO	01279	C1134
TREM2 HO KO	01279	C1136
TREM2 R47H	11969	C1231
APOE 4/4	11995	C1227

- The R47H variant is the most studied TREM2 risk variant and is strongly linked to increased Alzheimer's risk with an impaired ability to clear toxic protein aggregates.
- The APOE-ε4 allele is the most significant known genetic risk factor for late-onset AD.



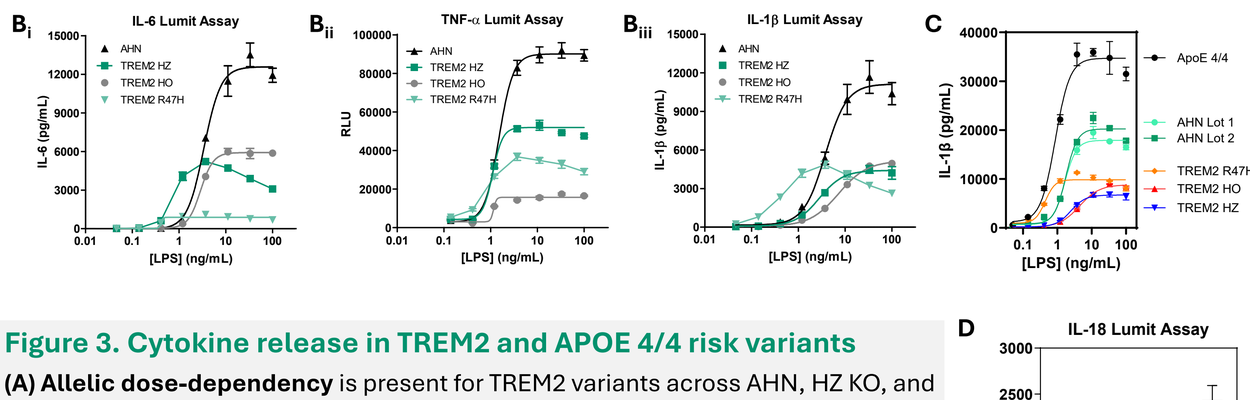
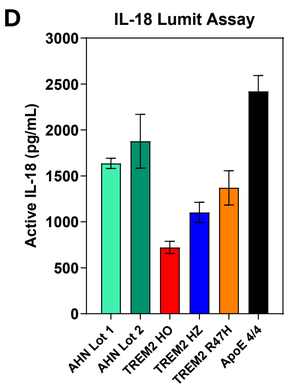


Figure 3. Cytokine release in TREM2 and APOE 4/4 risk variants

(A) Allelic dose-dependency is present for TREM2 variants across AHN, HZ KO, and HO KO microglia (Promega Lumit Immunoassays).
(B) Genotype-specific cytokine release (after stimulation). B_i IL-6 (24hr LPS), B_{ii} TNF-α (24hr LPS + IFNγ), B_{iii} IL-1β (3hr LPS + 30 min ATP).
TREM2 variants generally show reduced cytokine release vs. AHN, with variant-specific differences. For example, R47H produced less IL-6 but exhibited the highest IL-1β sensitivity (lowest EC50) and intermediate TNF-α levels.
(C,D) APOE 4/4 increases cytokine release versus AHN and TREM2 variants. patient-derived lines were more sensitive to LPS priming (data not shown).



Modulating TREM2-SYK Signaling for Drug Discovery

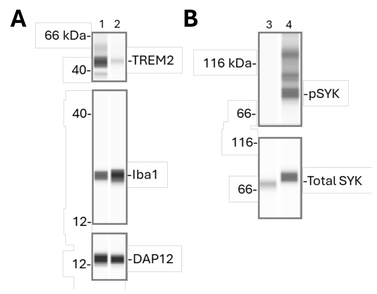


Figure 4. Marker Expression Verification

(A) LPS down-regulates TREM2 levels in iCell Microglia untreated (1) or after 24 h LPS stimulation (2) as shown by simple Western analysis of TREM2, Iba1, and DAP12.
(B) 0.1 mM Na_3VO_4 modulates pSYK levels in as shown by simple Western analysis in untreated (3) vs. 30 min. treatment (4). Data courtesy of Protein Simple, a Bio-Techne brand.

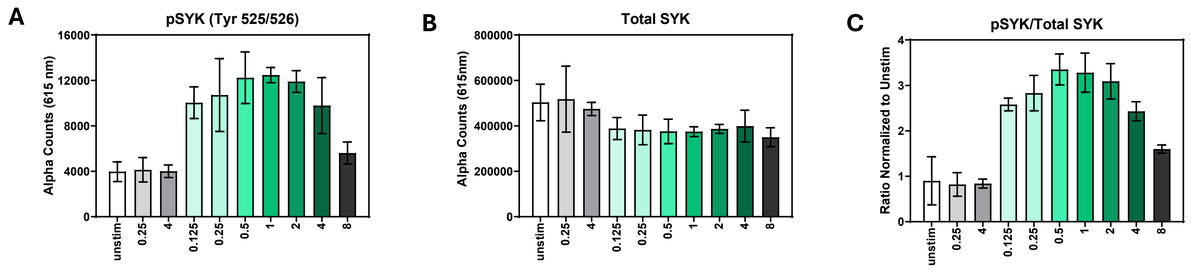


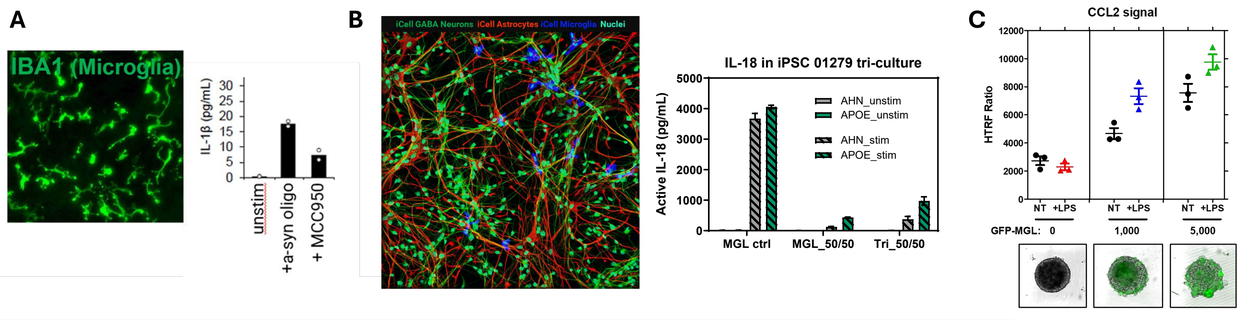
Figure 5. pSYK Levels in TREM2 Patient-derived Microglia

(A-C) pSYK Assay Development. Microglia seeded in 96-well plates were stimulated on day 2 with control IgG (gray) or anti-TREM2 antibody (green; clone E4J7A; CST #55739). Lysates were analyzed by AlphaLISA using a CLARIOstar Plus plate reader (BMG LABTECH). Maximal pSYK activation occurred at 0.5–1 μg/mL anti-TREM2. (A) pSYK (Tyr525/526), (B) total SYK, and (C) normalized phospho/total SYK ratio.
(D) TREM2 R47H microglia show reduced pSYK levels as demonstrated by the response of the patient-derived micrglia (gray) relative to AHN control microglia (green)
Revvity kits (Cat. # ASU-PSYK-A500, ALSU-TSYK-A500).

Summary

- Human iPSC-derived microglia model **neuroinflammation and Alzheimer's disease in vitro**
- **iCell Microglia** are well-characterized, functionally robust, and maintain key microglia activation pathways.
- **NLRP3 inflammasome activation** in these cells triggers **caspase-1 activity** and secretion of pro-inflammatory cytokines.
- FUJIFILM CDI provides engineered and patient-derived iPSC microglia models carrying AD-linked **TREM2** and **APOE variants**, with relevant cytokine release, phagocytosis, and metabolic profiles.
- These models enable exploration of **genetic risk factors and inflammatory signaling** in AD, aiding identification of novel therapeutic targets.

FUTURE DIRECTIONS: Alt. Agonists, Advanced Co-Culture, and 3D Systems



What's next?

(A) Explore alternate NLRP3 inflammasome activation by agonists such as alpha-synuclein forms (monomers, oligomers, and pre-formed fibrils).
(B) Develop advanced models through complex culture formats and media optimization.
(C) Implement markers, such as iCell GFP-Microglia and luciferase-based components, that can be integrated into 3D spheroids for cutting-edge interrogation.

Thanks to our collaborators on this Poster!

bio-techne Cell Signaling TECHNOLOGY® Promega revvity