Characterization of human iPSC-derived microglial activation using high-content immunofluorescence

V. E. Bain[1], K. LeFrancois[1], R. Gray[1], S.Coveney[1], S.Favazza[1], A.Copelas[1], S. Singh[1], M. Curtis[2], C. Carlson[2], S.Schachtele[2], R. W. Cho[1]

1 Cell Signaling Technology, Inc., Danvers, MA 01923
2 FUJIFILM Cell. Dynamics, Madison, WI 53711

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INTRODUCTION

Microglia, the native immune cells of the brain, are keen responders and critical players in numerous neurological conditions including an increasingly recognized role in Alzheimer's disease (AD) pathology and neurodegeneration (1). In particular, triggering receptor expressed on myeloid cells 2 (TREM2) is a receptor protein localized at the membrane of microglia that has been genetically linked to late-onset AD and has become an interest as a therapeutic target (2-5). Loss of TREM2 through frameshift and nonsense mutations has also been associated with frontotemporal dementia (FTD) and Nasu-Hakola disease (NHD) (6). Investigating the role of microglial TREM2 and downstream signaling cascades within human-relevant *in vitro* models has been historically challenging but is now accessible using human induced pluripotent stem cell (iPSC) technology and protocols for differentiating these into microglia.

In this study we used commercially available iPSC-derived microglia (iCell® Microglia) to investigate mechanisms of TREM2 signaling using high-content immunofluorescence analysis (HCA) and specific monoclonal antibodies. We first validated iPSC-microglia by staining for established microglial markers (Iba1, SHIP1, CD45, PU.1), showing that the cells were highly pure, correctly differentiated, and absent of markers for neurons (NeuN) and astrocytes (GFAP). TREM2, upon ligand binding and activation, interacts with the tyrosine kinase-binding protein DNAX-activating protein 12 (DAP12, TYROBP) to form a receptor-signaling complex (7-8). DAP12 then recruits protein tyrosine kinase Syk and phosphorylation within the activation loop (Tyr 525 and 526) can be used as a reliable readout of this signaling (9). To investigate TREM2 signaling in microglia in more detail, we compared TREM2 and DAP12 localization in apparently healthy normal (AHN) iPSC- derived microglia with and without stimulation with lipopolysaccharide (LPS) or pro-inflammatory cytokines (IFNγ / TNFα) and looked at readouts of TREM2 activation. We then compared the effects of TREM2 on microglial activation using AD-relevant iPSC-microglia, engineered with a homozygous frameshift deletion of TREM2 (AD TREM2 HO KO).

The identification and implementation of antibodies for neuroscience research is not a trivial task. The antibodies used and validated in this study can be leveraged to further characterize iPSC-derived human cultures, especially within the context of inflammation. Together, these data demonstrate the utility of HCA and iPSC-derived microglia for investigating the TREM2-signaling cascade and can be applied to AD therapeutic research, targeting the benefits of upregulating or downregulating TREM2-dependent microglial activation to attenuate AD pathology.

REFERENCES

[1] Paolicelli, R.C. et al (2022). Neuron, 110, 3458–3483
[2] Panicker, N. et al. (2021). *J Cell Biol*, 220(4): e202012095
[3] Nguyen, A.T. et al. (2020). Acta Neuropathol, 140, 477-493
[4] Gratuze, M. et al. (2018). Mol Neurodegener, 13, 66
[5] Jonsson, T. et al. (2013). N Engl J Med, 368, 107-16
[6] Guerreiro, R. et al. (2013). JAMA Neurol, 70, 78-84
[7] Jay, T.R. et al. (2017). Mol Neurodegener, 12, 56
[8] McQuade, A. et al. (2020). Nat Commun, 11, 5370
[9] Lengerich, B. et al. (2023). Nat Neurosci, 26, 416-429
[10] Lively, S. et al. (2018). Front Cell Neurosci, 12, 215

METHODS

- Human iPSC-derived neural cells: iCell® Microglia (Catalog #C1110, #C1136), iCell® Astrocytes 2.0 (Catalog #C1247), and iCell® GlutaNeurons (Catalog #C1033) were acquired from FUJIFILM Cellular Dynamics. Cells were grown according to manufacturer's protocol and fixed in formaldehyde at 3DIV or 7DIV.
- Recombinant monoclonal antibodies against various cellular markers and signaling pathways were analyzed in iPSC-derived microglia by immunofluorescence utilizing the CST Immunofluorescence Protocol.
- Images were captured at 630X and 200X using the Operetta CLS HCA system in confocal and widefield mode. Additional images were captured at 630X using the Leica SP8 confocal.
- Image quantification was performed using Harmony high-content analysis software as well as Cell Profiler [3].

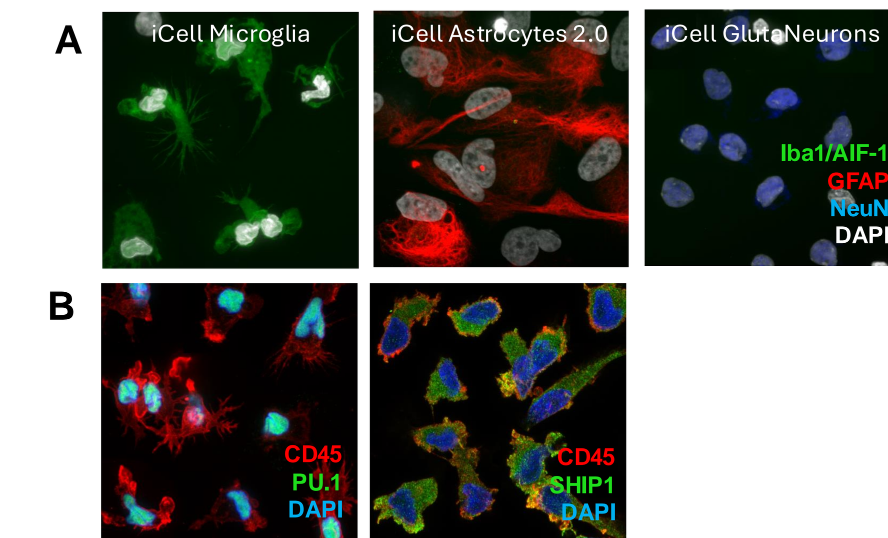

Figure 1: Validation of iPSC-derived Neural Cells using Cell-specific Antibodies.
(A) Validation that only iCell Microglia and not iCell Astrocytes or iCell GlutaNeurons express the microglia-specific marker, Iba1. Similarly, iCell Astrocytes and iCell GlutaNeurons are solely positive for the astrocyte marker, GFAP, and the neuron marker, NeuN, respectively. Cell nuclei are labeled with DAPI. **(B)** iCell Microglia also express microglia markers PU.1 and SHIP1. CD45 and DAPI are provided for additional cellular context.

Table 1:

Key Products Used	Catalog #	Lot #
PU.1 (9G7) Rabbit mAb	2258	4
CD45 (Intracellular Domain) (D9M8I) XP® Rabbit mAb (Alexa Fluor® 555 Conjugate)	62267	5
Iba1/AIF-1 (E4O4W) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate)	78060	3
NF-κB p65 (D14E12) XP® Rabbit mAb	8242	16
Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb	2710	25
TREM2 (E4F5G) Mouse mAb	29715	1
PathScan® RP TREM2 (Extracellular Amino-terminal Antigen) Sandwich ELISA Kit	90595	1
DAP12 (E7U7T) Rabbit mAb	97415	1
SHIP1 (C40G9) Rabbit mAb	2727	3
Additional antibody markers can be found at cellsignal.com		

We continue to develop a comprehensive portfolio of monoclonal antibodies to further characterize disease-associated cellular processes to understand the cellular changes in microglial activation in neurodegenerative diseases, such as AD and PD.

Inducing inflammation in iPSC-derived microglia

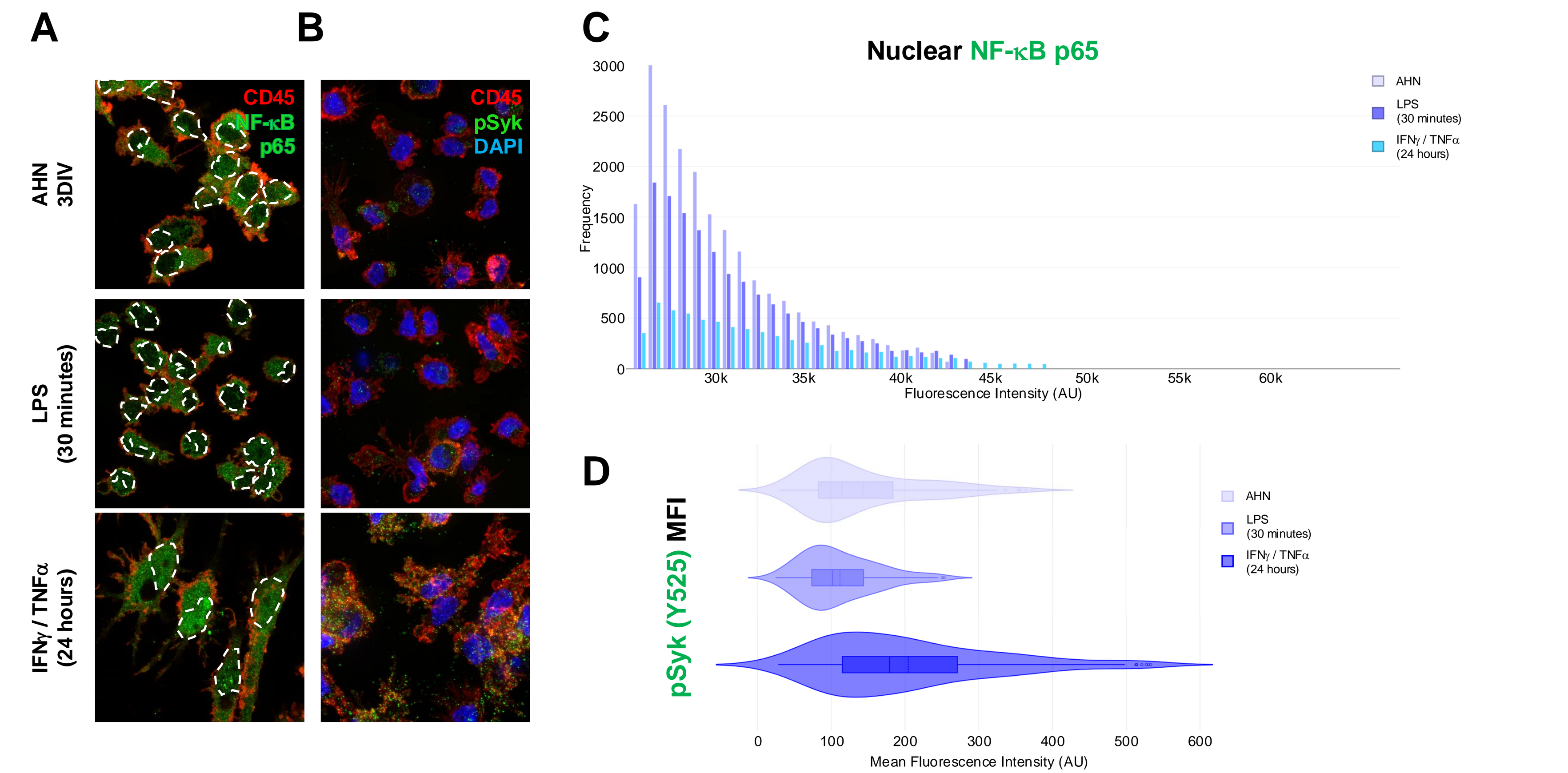

Figure 2: Differential Activation of iCell Microglia from LPS or IFNγ / TNFα stimulation.
Transcription factor NF-κB p65 (**A**) and activating kinase phospho-Syk (Y525/Y526) (**B**, pSyk) are imaged in AHN iCell Microglia, cells treated with 50 ng/ml of Lipopolysaccharides (LPS) for 30 minutes, or cells treated with cytokines (20 ng/ml IFNγ + 50 ng/ml TNFα) for 24 hours. The images are gated on positive nuclear signal (**C**) or quantified for mean fluorescent intensity (**D**).

Role of TREM2 in microglial activation

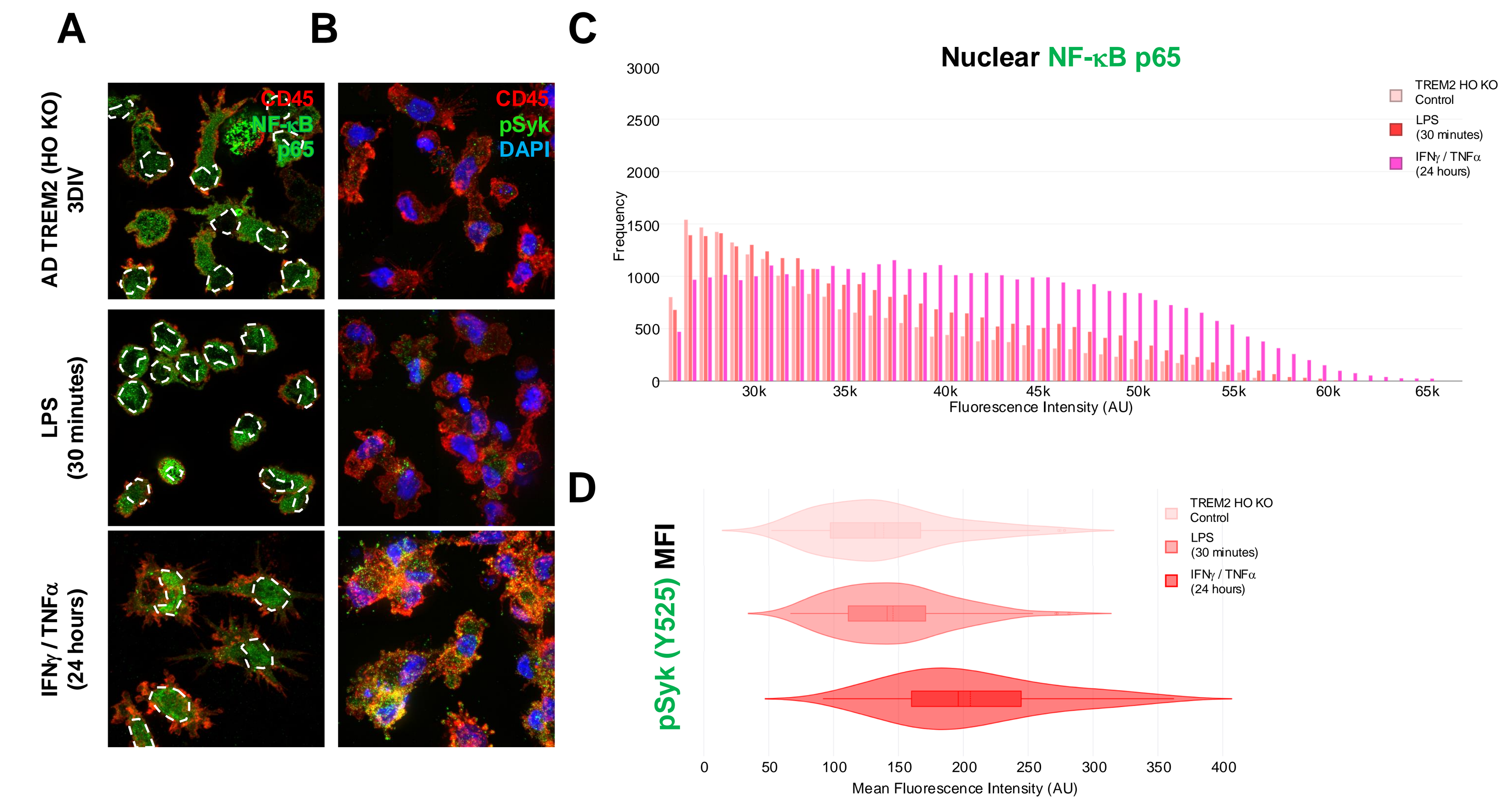

Figure 6: Loss of TREM2 does not prevent activation of microglia by LPS and IFNγ / TNFα treatments.
iCell microglia and engineered TREM2 Homozygous Knockout (HO KO) microglia were treated with LPS or cytokines, to assess how lack of TREM2 impacts **(A)** NF-κB and (**B**) pSyk. The images are gated on positive nuclear signal (**C**) or quantified for mean fluorescent intensity(**D**).

Confirming microglia morphology in inflammation models

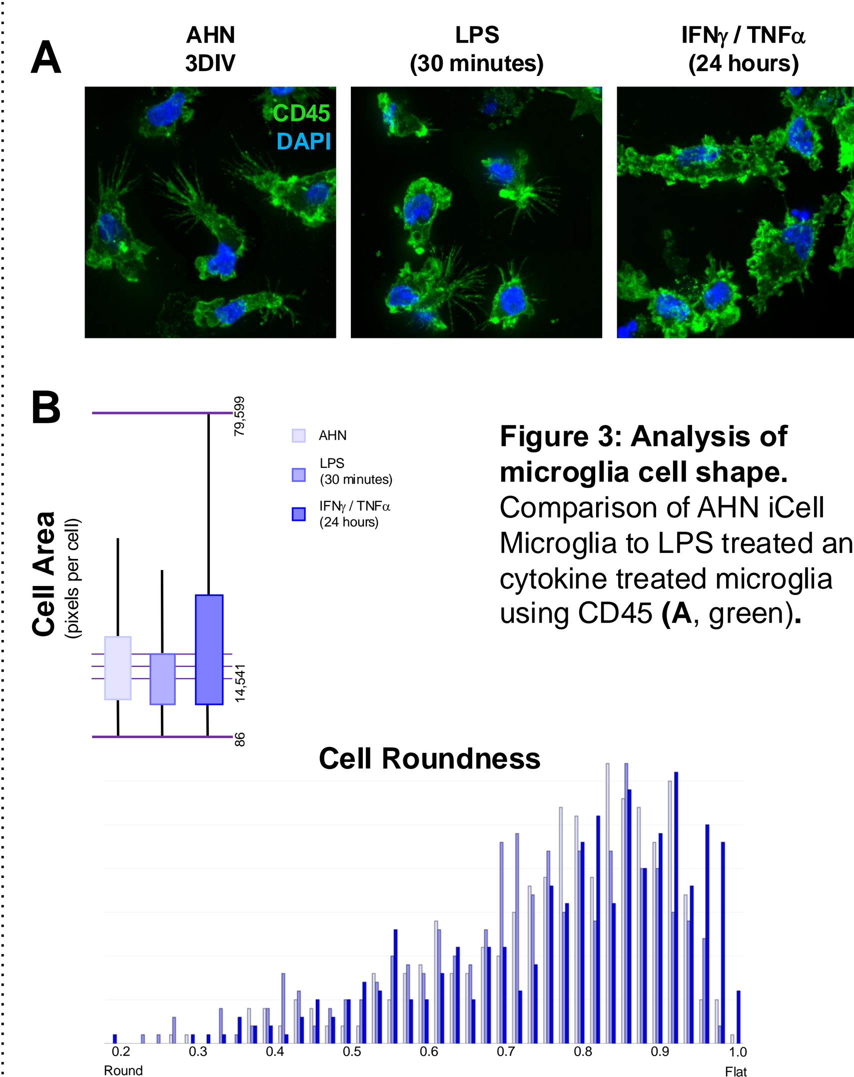

Figure 3: Analysis of microglia cell shape. Comparison of AHN iCell Microglia to LPS treated and cytokine treated microglia using CD45 (**A**, green).

Area and roundness were measured using CellProfiler (**B**). While LPS treated microglia are slightly smaller (P=2E-2) and rounder (P=2E-3) than AHN cells, microglia treated with cytokines are larger (P=3E-2) and flatter (P=3E-6) than AHN cells.

Analysis of TREM2 and DAP12 levels in healthy and activated microglia

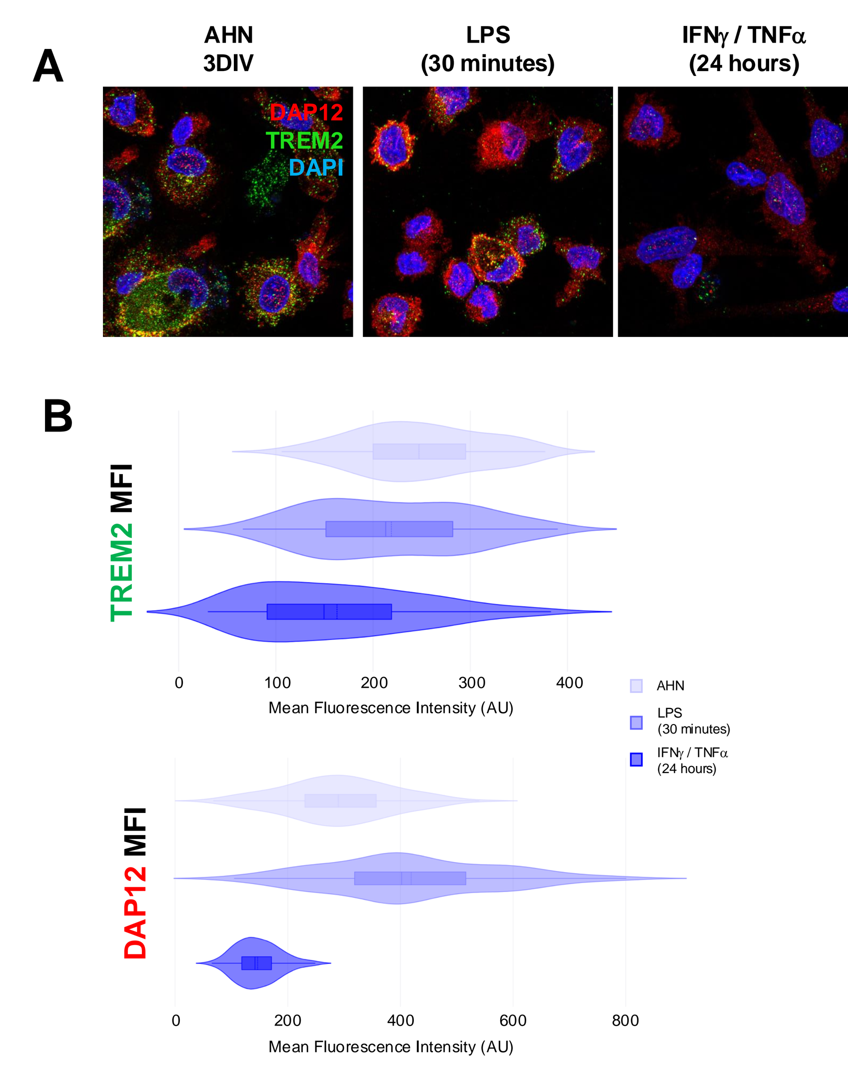

Figure 4: Analysis of TREM2 and DAP12.
Staining of iCell Microglia with TREM2 and DAP12 antibodies shows colocalization in AHN cells (**A**). LPS stimulation results in reduced MFI for TREM2 (P=2E-3) but not DAP12, while cytokine treatment results in decreased MFI for both (P=2E-8, P=1E-15) (**B**).

TREM2 Knockout Microglia

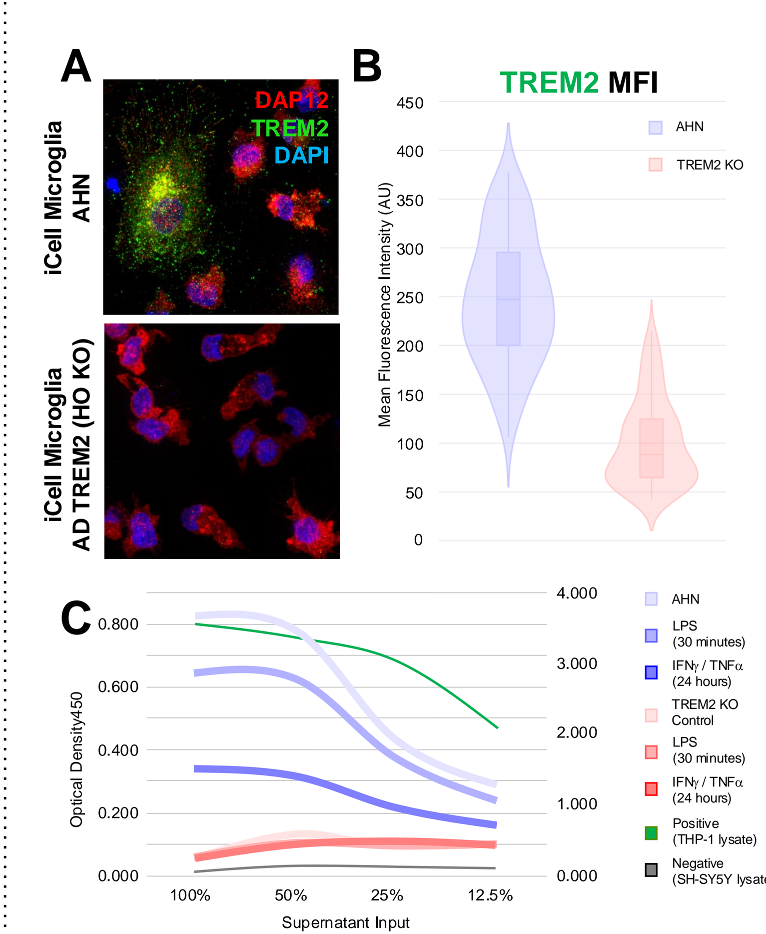

Figure 5: iCell Microglia AD TREM2 do not express TREM2.
Staining for TREM2 confirms that iCell Microglia TREM2 HO KO have little-to-no expression of TREM2 compared to AHN iCell Microglia (P=1E-4) (**A**,**B**).
Both healthy and disease microglia express DAP12 (**A**). TREM2 ELISA on cell supernatant confirms lack of TREM2 (**C**). Endogenous controls for comparison (right axis).

CONCLUSIONS

- A toolkit has been developed for characterizing inflammation in human iPSC-derived microglia (iCell Microglia) through nuclear translocation of NF-κB p65 (D14E12), activation of Phospho-Syk (Tyr525/526) (C87C1), and decreased TREM2 (E4F5G).
- Induction of neuroinflammation through LPS treatment results in subtle changes to microglia including decreased TREM2 and increased nuclear NF-κB.
- Morphological changes occur in microglia treated with cytokines IFNγ and TNFα for 24 hours. Both the morphological changes and the TREM2 reduction are congruent with the literature (10). Cytokine treated cells also exhibit more intense nuclear NF-κB staining, increased pSyk, and decreased DAP12 levels.
- In the absence of TREM2, both levels and frequency of nuclear NF-κB is higher suggesting that part of the response to these treatments are TREM2 independent.
- Complementary data interrogating iCell Microglia using CST antibodies in multiplex Western blot can be found in SFN Poster #8455.

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