Quantitative Analysis of TREM2 Signaling in iPSC-Derived Microglia

Using Simple Western Technology

Introduction

Alzheimer's disease (AD) remains a major public health challenge, affecting over 50 million individuals globally. While traditionally characterized by amyloid-beta plaques and neurofibrillary tangles, increasing evidence highlights the critical role of neuroinflammation and microglial dysfunction in AD pathogenesis. Microglia—the resident immune cells of the central nervous system—are highly dynamic responders to injury, infection, and degeneration. Among key molecular regulators, the triggering receptor expressed on myeloid cells 2 (TREM2) has emerged as a significant genetic risk factor for AD. TREM2 mutations can increase AD susceptibility by impairing ligand binding or downstream signaling, thus diminishing microglial responses to pathological stimuli.[1–3]

Studying TREM2 signaling in primary microglial cells is limited by scarce availability and phenotypic instability in vitro. The development of human induced pluripotent stem cell (iPSC)-derived microglia overcomes these limitations by providing renewable, disease-relevant models that recapitulate primary microglial features.

In this study, we utilized iCell® Microglia from FUJIFILM Cellular Dynamics to investigate TREM2-dependent signaling. The **Leo™ and Jess™ Systems**, powered by capillary-based immunoassay **Simple Western™ Technology** were employed to enable highly sensitive, multiplexed, and quantitative analysis of post-translational modifications and total protein levels. Highly-validated rabbit monoclonal antibodies from Cell Signaling Technology® (CST) were used to assess microglial responses, focusing on Syk and PLCγ2 phosphorylation as downstream readouts of TREM2 activation.

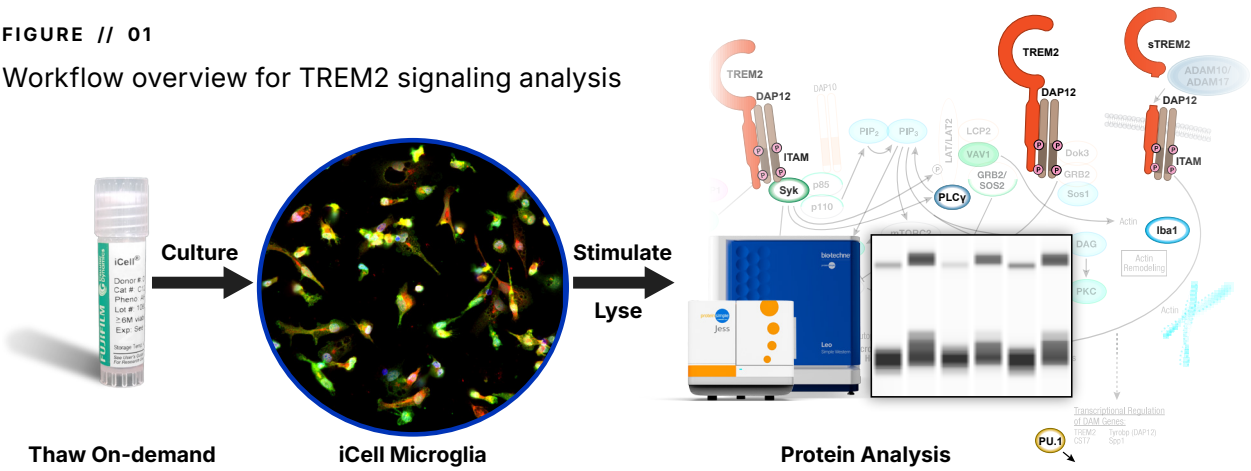
Materials and Methods

Cell Culture

Human iPSC-derived cell types (**Table 1**) were thawed and cultured according to the manufacturer's user guide. For iCell Microglia specifically, including the Apparently Healthy Normal (AHN) control (*Trem2*$^{+/+}$) and disease-relevant TREM2 variants: heterozygous (HZ) knockout (*Trem2*$^{+/-}$) and homozygous (HZ)

FIGURE // 01

Workflow overview for TREM2 signaling analysis



Name	Cat. #
iCell Microglia	C1110
iCell Microglia HZ TREM2 KO	C1134
iCell Microglia HO TREM2 KO	C1136
iCell Astrocytes 2.0	C1249
iCell Induced Excitatory Neurons	C1251
iCell GABANeurons	C1012
iCell GlutaNeurons	C1033

Table 1. FUJIFILM Cellular Dynamics materials used in the study. KO: knockout.

knockout (*Trem2*$^{-/-}$). Cells were cultured for 3 days post-thaw prior to treatment with pervanadate for 30 minutes, lipopolysaccharide (LPS) (100 ng/mL) for 24 hours, and interferon-gamma (IFN-γ) plus tumor necrosis factor-alpha (TNF-α) (20 ng/mL and 50 ng/mL, respectively) for 24 hours. Post-stimulation, cells were washed with PBS and lysed on ice using RIPA buffer supplemented with 1X DNase I buffer (EDTA-free), Halt™ Protease and Phosphatase Inhibitor Cocktail, and 20 µg/mL Nuclease A. Lysates were clarified by centrifugation and stored at -80 °C until analysis.

Simple Western Protein Analysis

Leo and Jess Systems powered by Simple Western Technology were used to analyze samples listed in **Table 2**. Samples were prepared under reducing conditions using 1X Master Mix at 95 °C for 5 minutes. Multiplex analysis was performed using the RePlex™ Module. Detection was performed using CST antibodies diluted in Antibody Diluent 2 included in the Anti-Rabbit Detection Module (**Table 3**).

Name	Cat. #
12-230 kDa Separation Module (Jess)	SM-W004
12-230 kDa Separation Module (Leo)	SWSM-W015
Anti-Rabbit Detection Module	DM-001
Total Protein Detection Module	DM-TP01
RePlex Module	RP-001

Table 2. Simple Western materials used in the study, available from Bio-Techne.

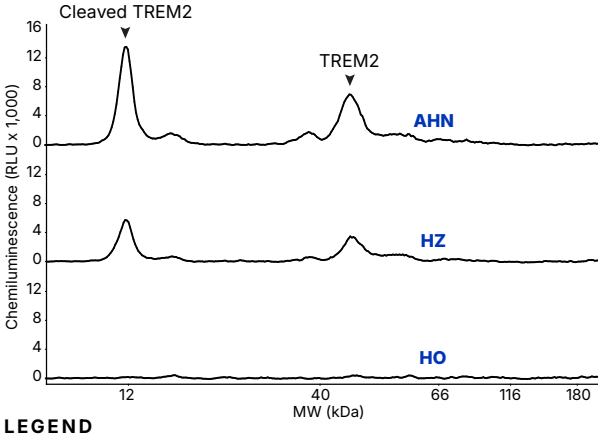
Name	Cat. #	Dil.
TREM2 (D8I4C) Rabbit mAb	91068	1:25
DAP12 (D7G1X) Rabbit mAb	12492	1:10
Iba1/AIF-1 (E4O4W) XP® Rabbit mAb	17198	1:10
PU.1 (9G7) Rabbit mAb	2258	1:10
Tyrosine Hydroxylase (A8Y7R) Rabbit mAb	13106	1:50
Doublecortin (F6K9E) Rabbit mAb	56130	1:50
PSD95 (D27E11) XP® Rabbit mAb	3450	1:25
β3-Tubulin (D65A4) XP® Rabbit mAb	5666	1:50
PLCγ2 (E5U4T) Rabbit mAb	55512	1:10
Phospho-PLCγ2 (Tyr759) (E9E9Y) Rabbit mAb	50535	1:50
Syk (D3Z1E) XP® Rabbit mAb	13198	1:10
Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb	2710	1:10

Table 3. Cell Signaling Technology antibodies and dilutions used in the study.

Results

Validation of Microglial Identity

Simple Western analysis confirmed genotype-dependent TREM2 expression. Unmodified cells exhibited robust expression of both full-length TREM2 (47 kDa) and cleaved TREM2 (12 kDa). HZ cells displayed reduced levels consistent with gene dosage



LEGEND
AHN Normal
HZ Heterozygous TREM2 KO
HO Homozygous TREM2 KO

Figure 2. Validation of TREM2 expression levels in heterozygous and homozygous TREM2 knockouts by Simple Western analysis.

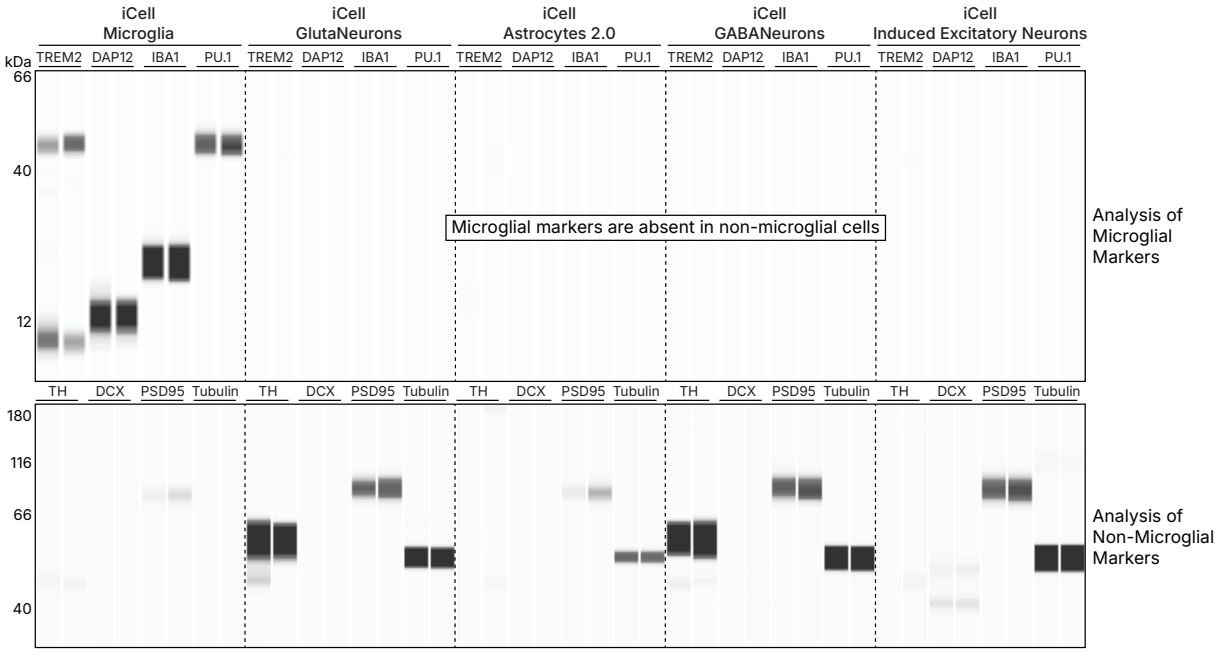
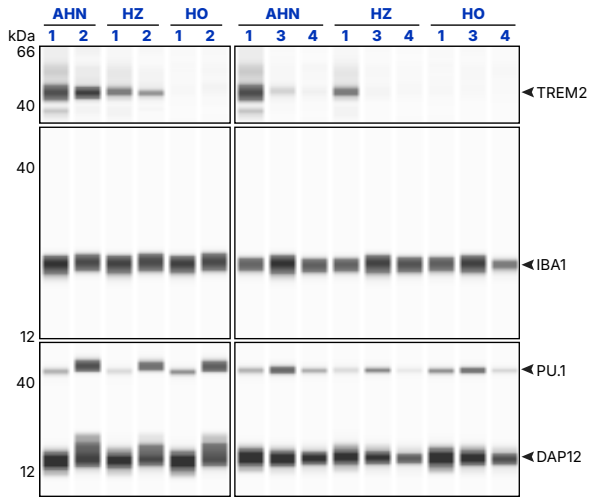


Figure 3. Validation of microglial identity on Leo. The top panel shows microglial markers (TREM2, DAP12, IBA1, PU.1) in iPSC-derived microglia versus non-microglial iPSC-derived neuronal cell types. The bottom panel shows non-microglial markers (TH, DCX, PSD95, β3-Tubulin). Each sample was analyzed in duplicate. TH: tyrosine hydroxylase; DCX: doublecortin; Tubulin: β3-tubulin.

effects, while HO cells lacked detectable expression of either form, validating complete knockout of TREM2 (Fig. 2).

Experiments with the Leo System demonstrated clear expression of microglial markers (TREM2, DAP12, IBA1, PU.1) exclusively in iCell Microglia, while neuronal markers (tyrosine hydroxylase, doublecortin, PSD95, β3-tubulin) were absent from microglia but generally present in other neural cell types (Fig. 3). This confirms lineage specificity and validates microglial identity. Importantly, the 96-sample capacity of Leo allowed detection of all microglial and non-microgial targets with duplicate replicates in a single run.



Differential Response to Stimulation

TREM2 signals via the adaptor protein DAP12, which recruits spleen tyrosine kinase (Syk) following ITAM phosphorylation. Activated Syk then propagates downstream signaling through phospholipase C gamma 2 (PLCγ2) (Fig. 1), influencing phagocytosis, cytokine secretion, and cell survival.[3–5]

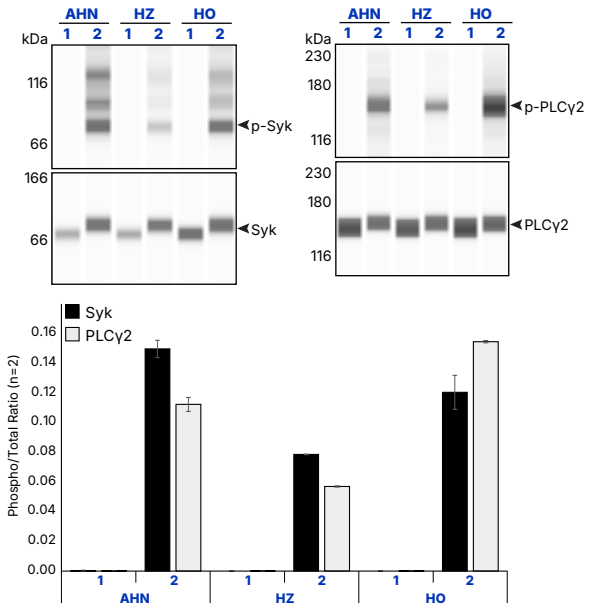
Stimulation of the various TREM2 variants revealed differences in TREM2 expression and downstream signaling. Overnight treatment with LPS and IFN-γ + TNF-α reduced TREM2 expression compared to untreated samples. Increases in the apparent molecular weights of DAP12 and PU.1 were observed in microglia treated with pervanadate, possibly as a result of phosphorylation of these proteins.

Figure 4. Analysis of TREM2 and TREM2-dependent signaling proteins following stimulation of iPSC-derived microglia.

LEGEND

1 Untreated
2 Pervanadate (30 mins)
3 LPS (24 hrs)
4 IFN-γ + TNF-α (24 hrs)

AHN Normal
HZ Heterozygous TREM2 KO
HO Homozygous TREM2 KO



LEGEND

1 Untreated
2 Pervanadate (30 mins)

AHN Normal
HZ Heterozygous TREM2 KO
HO Homozygous TREM2 KO

Figure 5. Quantification of phospho and total protein isoform ratios of Syk and PLCγ2 in untreated and treated iPSC-derived microglial. Error bars represent standard deviations from the means.

LPS and IFN-γ + TNF-α treatments demonstrated less pronounced effects on the TREM2 pathway, highlighting the specificity of receptor engagement for optimal pathway activation (Fig. 4).

Pervanadate broadly increased tyrosine phosphorylation, but only partially restored Syk phosphorylation in HO TREM2 knockouts, indicating that full activation requires TREM2. Interestingly, PLCγ2 phosphorylation remained elevated in HO TREM2 knockouts, suggesting alternative kinase compensation or Syk-independent pathways (Fig. 5).

Conclusion

iCell Microglia were characterized for unique marker expression and TREM2 levels were quantified across the different disease model lines. Cells responded to various stimuli and analysis of the TREM2/Syk signaling pathway was performed. Validation of phospho-specific antibodies like p-Syk and p-PLCγ2 will be useful for TREM2-dependent activation.

CST antibodies combined with Simple Western Technology provide an efficient, scalable platform for dissecting microglial signaling with assay capacity ranging from 60 to 720 runs per vial of iCell Microglia (Table 4). The inclusion of the Leo System further ensured validation of lineage specificity across iPSC-derived neural populations.

Parameter	Value
iCell Microglia/vial	1×10^6
Experimental conditions/vial	2 (0.5×10^6 cells each)
Cell lysate volume per condition	200 µL
Protein concentration	~2 mg/mL
# of assays/condition Simple Western (duplicate)	30–180
# of assays/condition RePlex/Multiplex (duplicate)	60–360
Total assays per vial	60–720

Table 4. Efficiency of Simple Western with iPSC-derived Samples.

References

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3. Gratuze, M. et al. (2018). Mol Neurodegener, 13, 66.
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