FUJIFILM
Value from Innovation

Cellular Dynamics

Analysis of TREM2/DAP12/SYK signaling in iCell® Microglia

iCell Lab Note

Introduction.

Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) has been shown to play an important role in the pathophysiology of Alzheimer's Disease and recent data suggests that increasing TREM2 activation could have strong therapeutic effects. TREM2 associates with another transmembrane adapter protein called DAP12 and together they recruit spleen tyrosine kinase (SYK) to signal the microglial activation response. The goal of this study was to demonstrate that phosphorylation of SYK could be detected in human iPSC-derived microglia, and that biologically-relevant methods of stimulating TREM2, such as treatment with an anti-TREM2 antibody, could effectively activate the pathway. This Lab Note provides example data and guidance on how to measure p-SYK in iCell Microglia and the related TREM2 disease models.

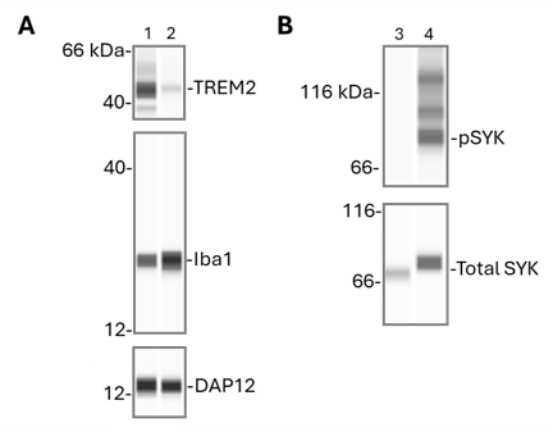

Figure 1. Verification of marker expression in iCell Microglia. Simple Western™ technology was used to analyze the expression of (A) TREM2, Iba1, and DAP12 in cells that were (1) untreated or (2) stimulated with 100 ng/ml LPS for 24 h. Complete loss of TREM2 was observed under these conditions, with minor changes to the other markers. (B) Data from (3) untreated cells or (4) microglia dose with 0.1 mM Na_3VO_4 for 30 min showed a robust increase in pSYK levels and a slight shift in gel migration of total SYK. *Data were kindly provided by ProteinSimple, a Bio-Techne brand.*

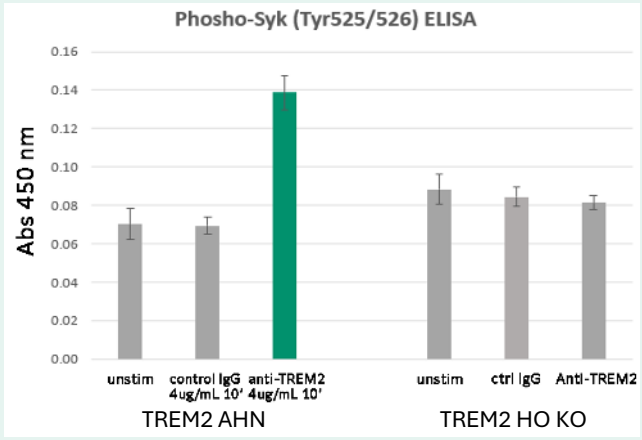

Figure 2. TREM2-dependent stimulation of iCell Microglia. Pathway activation was performed with an anti-TREM2 (EJ47A) rabbit mAb and detected with the Phospho-Syk (Tyr525/526) ELISA Kit. iCell Microglia treated with E4J7A for 10 min yielded a robust increase in ELISA signal (**green bar**), while none of the controls (**gray bars**) resulted in SYK phosphorylation, including unstimulated cells, treatment with an isotype control rabbit IgG, or dosing of an isogenic iCell Microglia line with a homozygous knockout (HO KO) of TREM2.

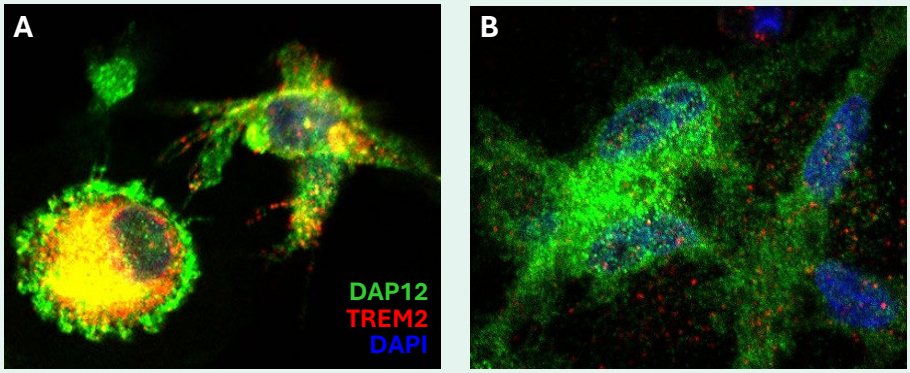

Figure 3. High Content Imaging to visualize changes in iCell Microglia. (A) Unstimulated iCell Microglia show a mixed population of amoeboid and ramified cells. DAP12 is localized at the membrane and there are high intracellular pools of TREM2. (B) Activation with IFNγ (20 ng/ml) and TNFα (50 ng/ml) for 24 h results in more rod-shaped morphology, movement of DAP12 into the cytoplasm, and full depletion of TREM2. *Images generated by Cell Signaling Technology (CST).*

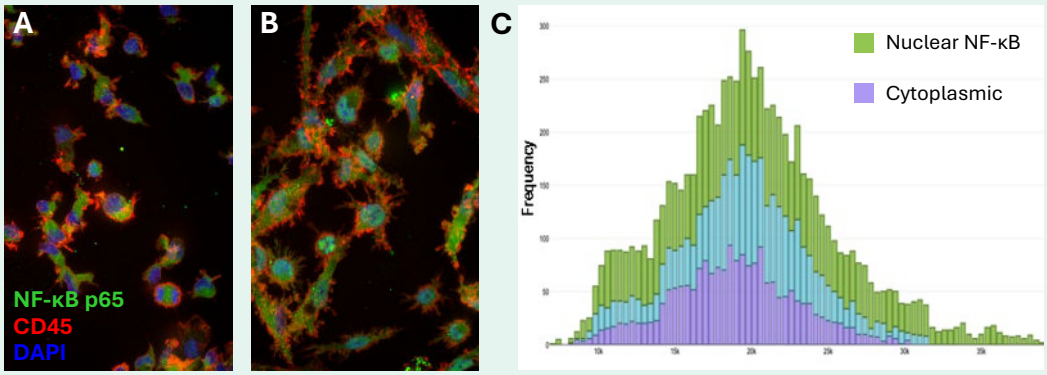

Figure 4. Activation of NF-κB pathway. When comparing (A) unstimulated microglia to (B) cells dosed with IFNγ (20 ng/ml) and TNFα (50 ng/ml) for 24 h, there is (C) a dramatic increase in nuclear signal for NF-κB p65. Stimulation of iCell Microglia can also modulate the NF--κB pathway. *Images and analysis provided by Cell Signaling Technology (CST).*

Methods.

Prepare iCell Microglia Complete Maintenance Medium and thaw cells according to the Quick Guide.

- On Day 0, thaw iCell Microglia and add cells into a PDL-coated 96-well plate at a density of 15-20K cells per well.
- Incubate iCell Microglia at 37ºC / 5% CO_2 for 2-3 days after plating to recover from thaw.
- On the day of assay, stimulate cells with desired agonist(s) for recommended amounts of time.
  - Activation with TREM2 mAb for p-SYK ELISA = 5-10 min
  - Treatment with LPS or IFNγ/TNFα for WB/IF = 18-24 h
- Collect cell culture supernatants for ELISA and either test immediately or store at -20ºC for later analysis.
- Lyse cells with RIPA buffer for Simple Western/WB analysis or fix cells and perm/stain for fluorescent imaging (IF).

Please visit these links for more detailed protocol information:

- www.cst-science.com/protocol
- www.bio-techne.com/simplewestern

Table 1. Materials Needed

Product	Vendor	Cat. #
iCell Microglia Kit, 01279	FCDI	R1131
LPS from *E. coli* O127:B8	Sigma	L4516
Activated Sodium Orthovanadate	Abcam	ab301822
Human IFN-gamma (PeproTech®)	Thermo	300-03
Human TNF-alpha (PeproTech®)	Thermo	300-01A
FastScan™ Phospho-Syk (Tyr525/526) ELISA Kit	CST	51426
TREM2 (E4J7A) Rabbit mAb *	CST	55739
TREM2 (D8I4C) Rabbit mAb †	CST	91068
TREM2 (E4F5G) Mouse mAb ‡	CST	29715
Iba1/AIF-1 (E4O4W) XP® Rabbit mAb †	CST	17198
DAP12 (D7G1X) Rabbit mAb †	CST	12492
DAP12 (E7U7T) Rabbit mAb ‡	CST	97415
NF-kB p65 (D14E12) XP® Rabbit mAb ‡	CST	8242
CD45 (D9M8I) XP® Rabbit mAb ‡	CST	62267

* This TREM2 mAb was used for pathway activation in the Phospho-Syk ELISA.
† These antibodies were used for Simple Western experiments.
‡ These antibodies were used for immunofluorescence (IF) staining and the catalog number may refer to the directly conjugated version.
Please inquire about additional materials not listed here, including Simple Western reagents, secondary antibodies, etc.

Summary.

iCell Microglia express markers relevant to the TREM2/DAP12/SYK signaling pathway and the expression levels can be modulated with different stimulation protocols. Various detection methods, including Simple Western, phospho-ELISA, and High Content Imaging, were utilized to dissect the cascade of events that occur upon activation of iCell Microglia. This iCell Lab Note provides guidance for handling cells from FUJIFILM CDI, features technologies from Bio-Techne for protein multiplexing of phospho/total isoforms and validates TREM2 pathway-focused antibody toolkit from Cell Signaling Technology.

Highlights.

iCell Microglia express expected markers, incl. TREM2, Iba1, DAP12, and SYK, as shown by Simple Western analysis.

Imaging of iCell Microglia not only shows various cell morphologies but also robust staining of key proteins in TREM2/SYK/DAP12 pathway.

Anti-TREM2 Ab engagement and detection of p-SYK is a biologically-relevant means to measure activation of iCell Microglia.

Scan here to download the iCell Microglia Quick Guide.

Contact **Technical Support** for more protocol details and supportive data.
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