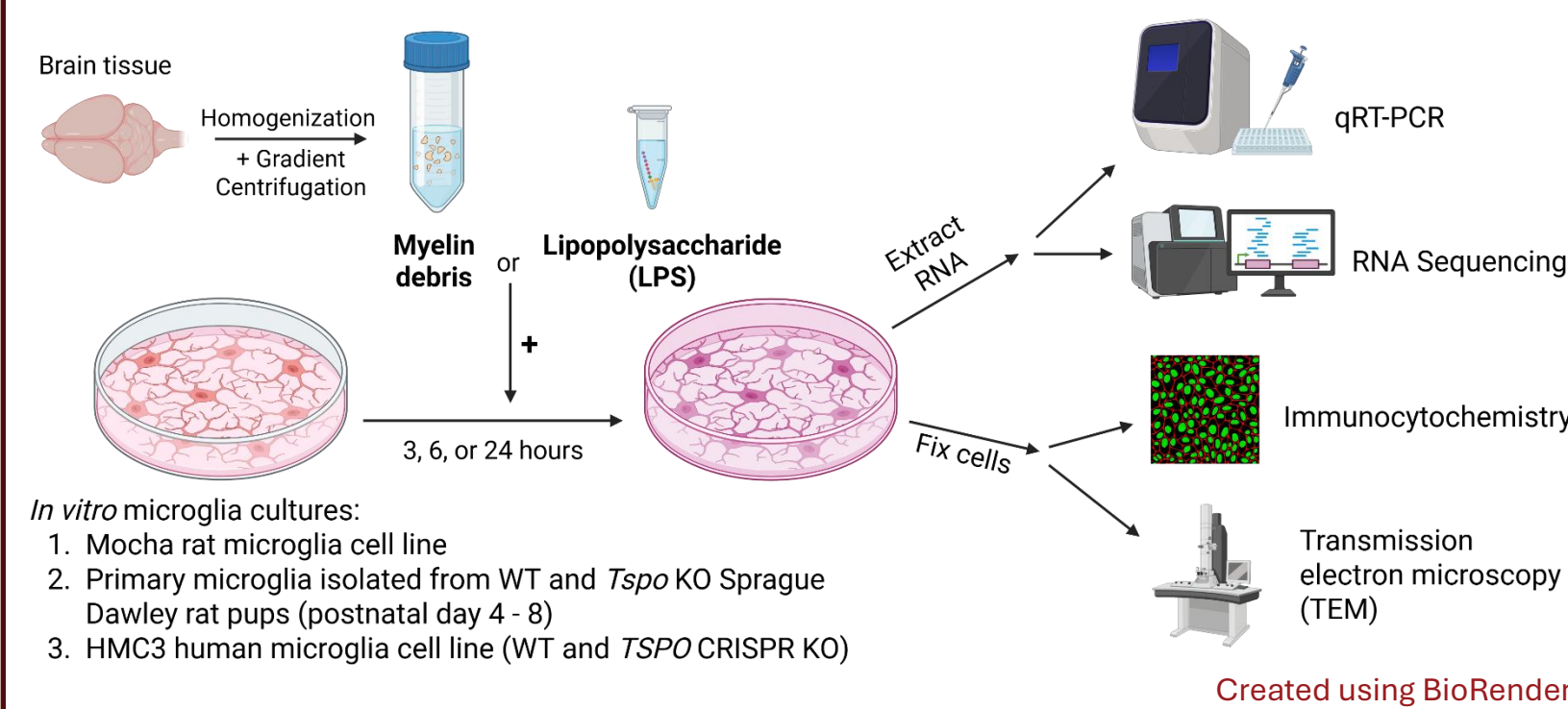


INTRODUCTION

Neurodegenerative diseases such as Alzheimer's Disease (AD) are commonly accompanied by neuroinflammation. Markers of neuroinflammation, such as translocator protein (TSPO), have been targeted in positron emission tomography to assist with diagnoses of AD; however, recent studies have challenged the relationship between TSPO and neuroinflammation. While TSPO levels were increased by pro-inflammatory stimulus lipopolysaccharide (LPS) in murine microglia and in activated microglia in murine neurodegenerative disease models, the same was not observed in human microglia nor in primate disease models. Therefore, we sought to investigate the reason for TSPO upregulation in AD brains beyond neuroinflammation. Given that degenerating brains give rise to excess lipid-rich myelin debris and TSPO is known to be involved in cholesterol homeostasis and phagocytosis, we hypothesize that TSPO is upregulated in microglia to meet cellular demands to phagocytose and recycle myelin debris.

METHODS



RESULTS

Myelin debris upregulates TSPO in rat microglia

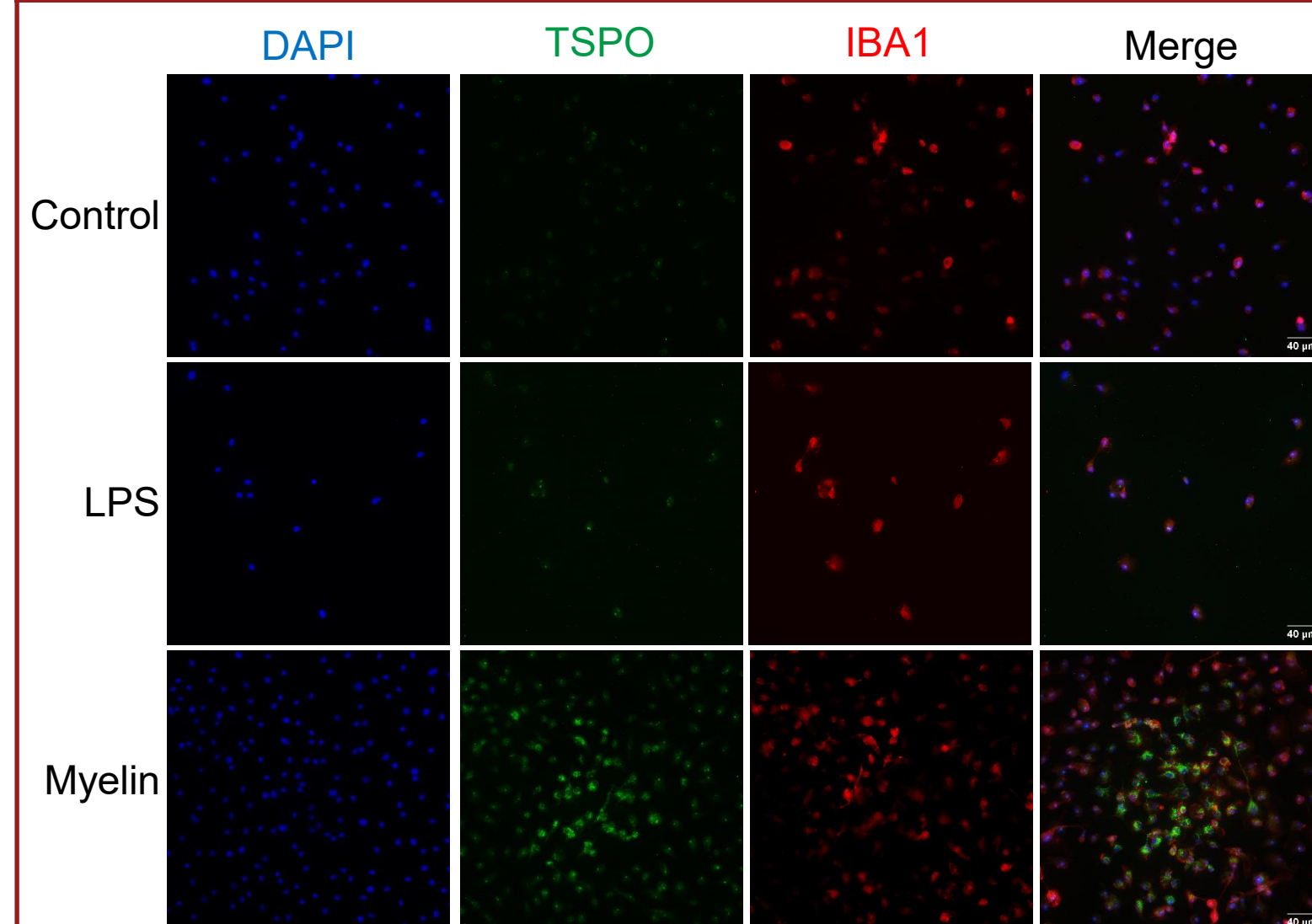


Figure 1: TSPO in LPS- or myelin-treated primary rat microglia. Representative immunofluorescence images of microglia treated with 10 ng/ml LPS or 1000 µg/ml myelin debris for 24 h. Cells were stained with TSPO (green) and IBA1 (red) antibodies, nuclei stained with DAPI (blue).

RESULTS

LPS and myelin debris lead to drastically different cellular responses in rat microglia

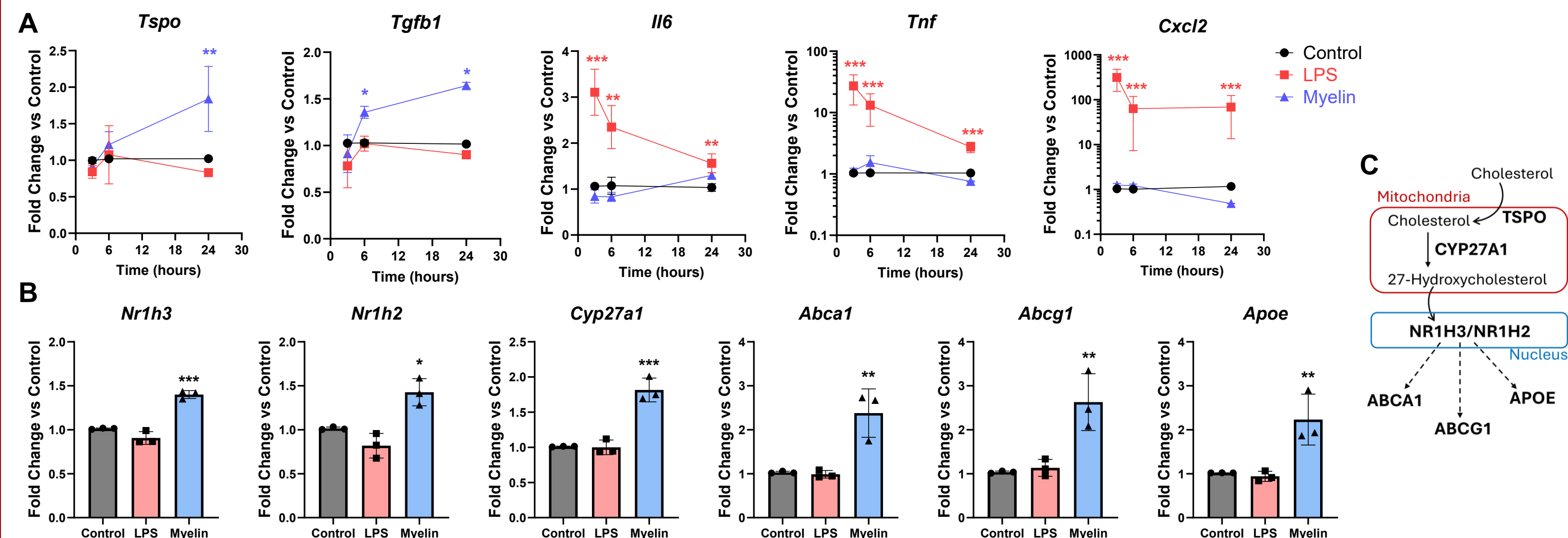


Figure 2: qRT-PCR analysis of inflammatory and cholesterol efflux gene expression in LPS- or myelin-treated Mocha rat microglia cells. Mocha cells were treated with 100 ng/ml LPS or 1000 µg/ml myelin debris for (A) 3, 6, and 24 h or (B) 24 h. Relative expression was normalized to α -tubulin and shown as fold change compared to the control condition of the same timepoint. Data are presented as mean $\pm$ SD, N = 3. Statistics performed using one-way ANOVA followed by Dunnett's multiple comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (C) Schematic of cholesterol efflux pathway.

TSPO deletion impairs myelin processing and dysregulates inflammatory states in rat microglia

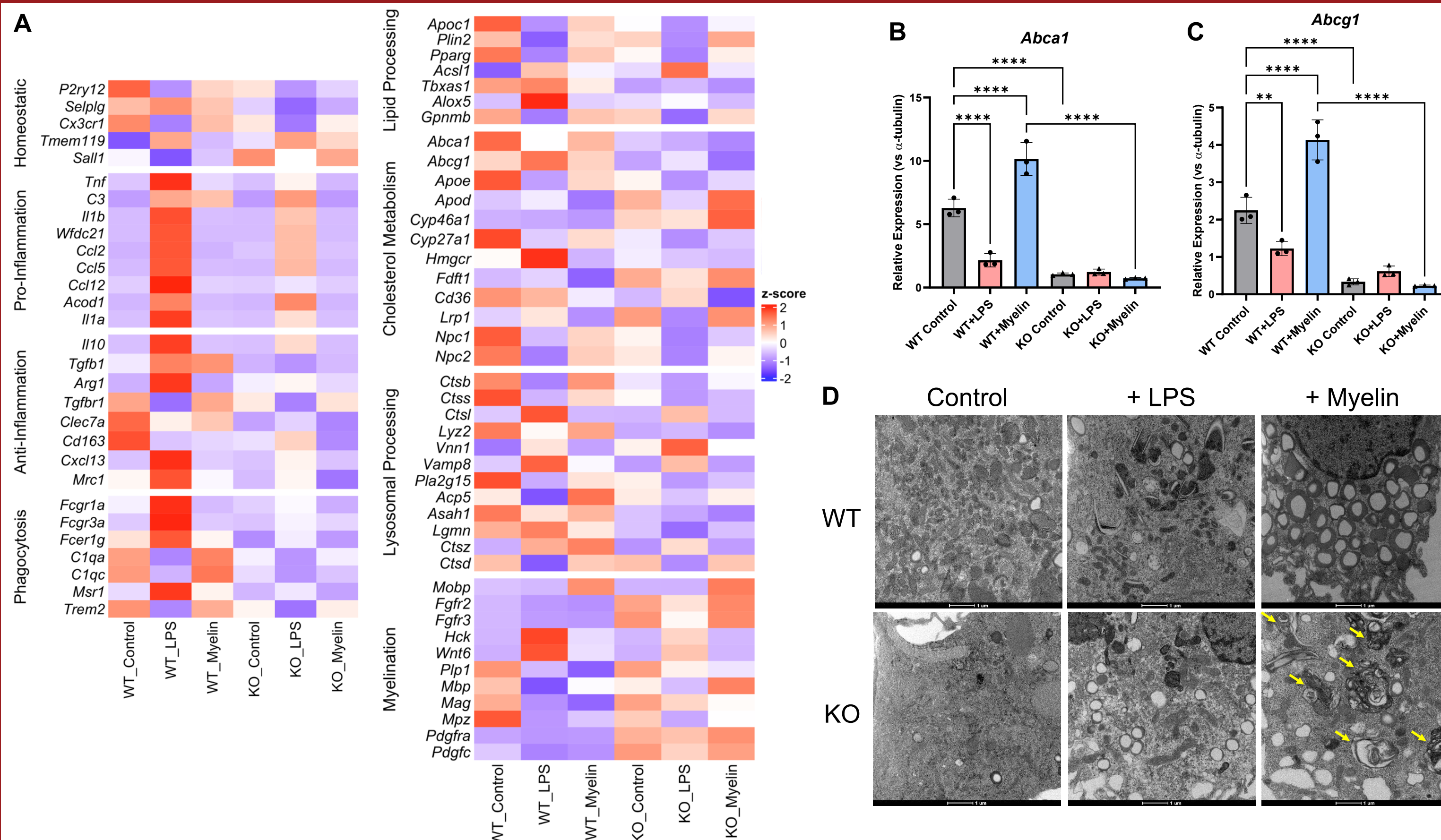


Figure 3: Transcriptomic and subcellular changes in WT and *Tspo*-KO primary rat microglia. Cells were treated with vehicle, 10 ng/ml LPS, or 1000 µg/ml myelin debris for 24h. (A) Heatmaps depicting changes in inflammation, phagocytosis, and lipid processing genes measured by RNA sequencing. (B, C) qRT-PCR analysis of cholesterol efflux genes. Relative expression was normalized to α -tubulin. Data are presented as mean $\pm$ SD, N = 3. Statistics performed using one-way ANOVA followed by Dunnett's multiple comparison test (** $p < 0.01$, **** $p < 0.0001$). (D) TEM images of treated microglia cells.

RESULTS

Myelin debris upregulates TSPO, which is essential for lipid processing in human microglia

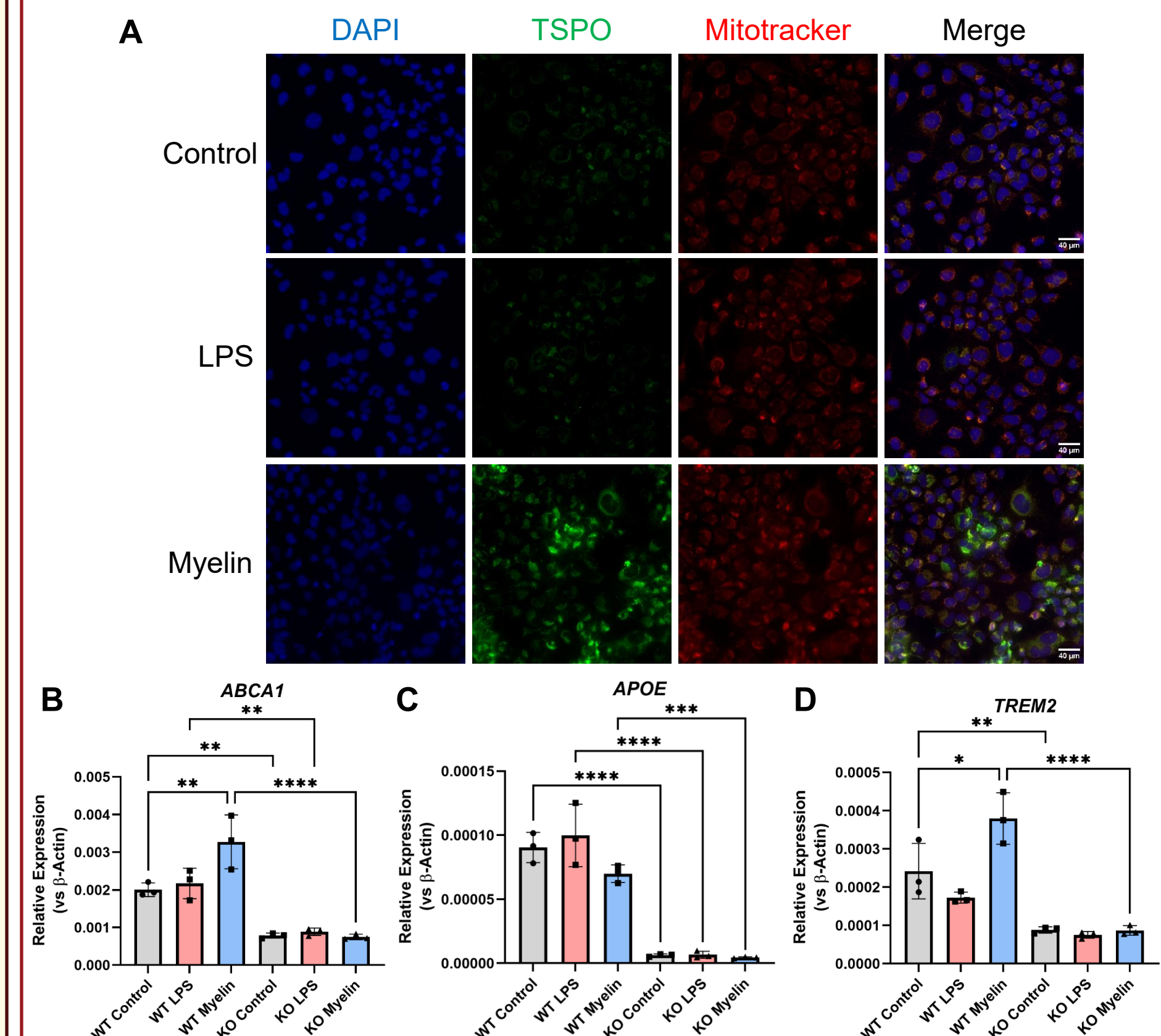


Figure 4: TSPO and lipid processing genes in WT and *Tspo*-KO HMC3 cells. Cells were treated with 100 ng/ml LPS or 1000 µg/ml myelin debris for 24 h. (A) Representative immunofluorescence images of WT HMC3 cells stained with TSPO (green), mitochondria stained with Mitotracker Red, and nuclei stained with DAPI (blue). (B – D) qRT-PCR analyses of lipid processing genes in WT and KO HMC3 cells. Relative expression was normalized to β -actin. Data are presented as mean $\pm$ SD, N = 3. Statistics performed using one-way ANOVA followed by Dunnett's multiple comparison test (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$).

CONCLUSIONS

- Myelin debris increases TSPO levels in rat and human microglia
- TSPO modulates the expression of multiple lipid processing genes and its deletion greatly impairs microglia's myelin processing capabilities
- TSPO is not required for microglia's inflammatory responses but its deletion causes dysregulation in basal and stimulated inflammatory states

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