doi: 10.4149/gpb_2024037

Transcription factor Yy1 modulates Trem1 to control LPS-triggered neuroinflammation and oxidative stress in mouse astrocytes *via* the NF-κB pathway

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Abstract. Dysfunction of astrocytes has a crucial role in the pathology of depression. Here, we aimed to define the exact action of the ubiquitous transcription factor (TF) Yin Yang-1 (Yy1) in depression pathogenesis and astrocytic dysfunction. A chronic unpredictable mild stress (CUMS) mouse model was generated. Primary mouse astrocytes were exposed to lipopolysaccharide (LPS). Cell growth was determined by CCK-8 and EdU assays. The direct interaction of Yy1 and the Trem1 promoter was validated by chromatin immunoprecipitation (ChIP) and luciferase assays. In CUMS mice, the levels of Yy1 and inflammatory cytokines were augmented and oxidative stress was enhanced. Functionally, disruption of Yy1 or triggering receptor expressed on myeloid cell 1 (Trem1) relieved LPS-triggered pro-growth, pro-inflammation, and pro-oxidative stress effects in mouse astrocytes. Mechanistically, Yy1 directly promoted the transcription and expression of Trem1 by binding to the Trem1 promoter. Yy1 disruption exerted regulatory impacts in LPS-induced mouse astrocytes *via* down-regulation of Trem1. Additionally, the Yy1/Trem1 cascade could modulate the activation of the NF-κB signaling in mouse astrocytes. Our study defines that Yy1 disruption relieves LPS-triggered neuroinflammation and oxidative stress in mouse astrocytes *via* the NF-κB pathway by down-regulating Trem1, providing possible strategies for depression treatment.

Key words: Astrocytes — Depression — Transcription factor Yy1 — Trem1 — Neuroinflammation — Oxidative stress

Introduction

Depression is a widespread chronic blight in humans that leads to suffering, disability, and death, and causes diminished quality of life, family disruption, and medical comorbidities (Monroe and Harkness 2022). Nevertheless, so far clinical reports have unveiled that depression is frequently undiagnosed and the confirmed patients do not have a good therapeutic outcome due to the stigma and the lack of effective treatment (Monroe and Harkness 2022). Therefore, a large number of specialists focus on the neuropathological mechanisms underlying depression and seek to define depressive behaviors on cellular and molecular levels (Yang et al. 2022; Marwaha et al. 2023). Dysregulated neuroinflammatory responses and overactivated oxidative stress have established critical roles in the pathophysiology of major depression disorders (Bhatt et al. 2020; Troubat et al. 2021). Furthermore, recent evidence uncovers a crucial

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Electronic supplementary material. The online version of this article (doi: 10.4149/gpb_2024037) contains Supplementary material.
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role of astrocytes in depression where astrocytic dysfunction has been proved (Liao et al. 2021; Dolotov et al. 2022; Zhao et al. 2022). Exploring the mechanisms driving astrocytic dysfunction is indispensable to offer novel insight into treatment of clinical depression.

The Yin Yang-1 (YY1) is a ubiquitous transcription factor (TF) that possesses a fundamental function in normal development (especially central nervous system) by controlling gene expression (Verheul et al. 2020), and it has attracted wide attention in the research due to its implication in brain pathology (Pabian-Jewuła et al. 2022). Astrocytic Yy1 operates as a pivotal player for murine brain development and is essential in protecting against pathological neuroinflammation, apoptosis, and oxidative stress (Pajarillo et al. 2023). Importantly, YY1 has been confirmed to affect cognitive impairment in major depression diseases (Pérez-Granado et al. 2022). During stress-induced behavioral maladaptation, alteration of Yy1 expression is found, and its selective silencing in cortical excitatory neurons leads to increased sensitivity to stress in experimental mice by affecting related gene expression (Kwon et al. 2022). In patients with major depressive disorders, YY1 is enhanced in their plasma samples, and its increased expression predicts worse cognitive dysfunction, which may be related to the YY1–NF-κB–IL-1β inflammatory cascade (Lu et al. 2023). It is still undefined how YY1 modulates the pathogenesis of depression and astrocytic dysfunction.

To resolve this question, we first generated a chronic unpredictable mild stress (CUMS) mouse model to confirm Yy1's involvement during depression development. We then stimulated primary mouse astrocytes with lipopolysaccharide (LPS) to focus on Yy1's modulation in cell growth, inflammation and oxidative stress. Further, we identified the transcriptional targets of Yy1 and uncovered a novel Yy1/target gene cascade. Our study suggests a crucial role of Yy1 in LPS-triggered astrocytic neuroinflammation and oxidative stress.

Materials and Methods

Mice and CUMS induction

For induction of a CUMS mouse model, we obtained male C57BL/6 mice (7–8 weeks old, $n = 12$) from Cyagen (Suzhou, China) and maintained them under non-specific pathogen conditions of 20–24°C at 60% humidity with 12-h light/12-h dark periods. The use and treatment of mice were conducted following a protocol approved by Hubei Province Huangshi City Mental Hospital Institutional Animal Committee. We generated the CUMS mouse model ($n = 6$) as described elsewhere with minor modifications (Fu Q et al. 2023). In brief, mice in the CUMS group were stimulated with three randomly different stressors once daily for continuous 28 days, and these stressors included light inversion (24 h), food and water deprivation (24 h), cold water swimming (4°C for 5 min), warm swimming (4°C for 5 min), wet bedding (24 h), sleep deprivation (24 h), cage tilt (45° for 24 h), restraint (3 h). Mice in the control group did not subject to any stress.

Behavioral tests and cortical tissue collection

At the endpoint, we analyzed depressive-like behaviors in these mice by performing sucrose preference test, tail suspension, forced swimming test, and social interaction test under standard protocols described by previous work (Song et al. 2020; Fu Q et al. 2023). After that, we collected cerebral cortical tissue samples from the sacrificial mice and quick-froze them in liquid nitrogen for the subsequent assays.

Culture and LPS exposure of mouse astrocytes

For *in vitro* research, we purchased primary mouse astrocytes (CP-M157) from Procell (Wuhan, China) and propagated them at 37°C and 5% CO_2 using standard culture media made by Procell. LPS (Selleck, Shanghai, China) diluted in phosphate buffered saline (PBS) or vehicle solution alone was added into the cell culture media (the final concentration of LPS was 100 ng/ml) and applied for 24 h.

Constructs and transfection

We used these constructs in this study: pcDNA3.1-Yy1 (mouse)-3×HA (Miaoling Biology, Wuhan, China), pcDNA3.1 control (Miaoling Biology), triggering receptor expressed on myeloid cell 1 (Trem1) expression construct, mouse Yy1-siRNA (si-Yy1), mouse Trem1-siRNA (si-Trem1), and si-NC oligonucleotide mock. We generated Trem1 expression construct by inserting mouse Trem1 coding sequence into the pcDNA3.1 vector. Mouse siRNAs were generated by Tsingke (Xian, China). We introduced siRNA or/and plasmid construct into mouse astrocytes by utilizing Lipofectamine 3000 and the accompanying protocols (Life Technologies, Tokyo, Japan). We harvested transfected astrocytes for expression analysis after 48 h and performed LPS exposure after 24 h.

Immunoblotting

Through the modified RIPA buffer reported by Tsoi et al. (2018), we obtained total protein from homogenizing cortical tissues and treated mouse astrocytes. After being resolved by SDS-PAGE and blotted to nitrocellulose, membranes were subjected to immunoblot analysis with mouse anti-Yy1 mAb (RRID:AB_2881664, #66281-1-Ig, 1:25000, Proteintech,

Wuhan, China), rabbit anti-GFAP pAb (RRID:AB_305808, #ab7260, 1:10000, Abcam, Cambridge, UK), rabbit anti-Trem1 pAb (RRID:AB_2208556, #11791-1-AP, 1:500, Proteintech), rabbit anti-p-IκBα mAb (RRID:AB_2801653, #ab133462, 1:1000, Abcam), mouse anti-IκBα mAb (RRID:AB_2881790, #66418-1-Ig, 1:30000, Proteintech), rabbit anti-p-p65 mAb (RRID:AB_1524028, #ab76302, 1:1000, Abcam), rabbit anti-p65 pAb (RRID:AB_2178878, #10745-1-AP, 1:3000, Proteintech), and mouse anti-β-actin mAb (RRID:AB_2687938, #66009-1-Ig, 1:60000, Proteintech), diluted in TBST. Hyper-sensitive ECL detection reagent (Servicebio, Wuhan, China) was applied for HRP detection on immunoblots after incubation by goat anti-rabbit (RRID:AB_955447, #ab6721, 1:15000) or anti-mouse (RRID:AB_955439, #ab6789, 1:5000) secondary antibody (Abcam) linked with HRP. Densitometric analyses were performed using the Image Gauge program (Fujifilm, Tokyo, Japan). All original images for immunoblotting were provided in Supplementary materials.

ELISA for IL-1β, TNF-α and IL-6

For analysis of production levels of IL-1β, TNF-α and IL-6 in homogenizing cortical tissues and the medium supernatant of treated mouse astrocytes, we applied the colorimetric Mouse IL-1β ELISA Kit, Mouse TNF-α ELISA Kit, and Mouse IL-6 ELISA Kit, as described by the vendor (Multi Sciences, Hangzhou, China).

Reactive oxygen species (ROS) detection

For evaluation of the amount of ROS, we employed the ROS Fluorometric Assay Kit (Elabscience, Wuhan, China) and conducted this detection referring to the recommendations. After being subjected to the relevant transfection or/and LPS stimulation, mouse astrocytes were incubated with 10 μM DCFH-DA probe diluted in non-serum media for 50 min at an incubator of 37°C. Following non-serum media washing, single-cell suspensions were loaded into a flow cytometer (FACSCanto II). For ROS detection in cells of homogenizing cortical tissues, single-cell suspensions were first prepared, and the kit reagents was used, which was followed by absorbance measurement at wavelengths of 500 nm excitation and 525 nm emission.

Detection of cell viability and proliferation

We determined the effect of LPS stimulation and the combination of the relevant transfection and LPS stimulation on growth of mouse astrocytes by CCK-8 and EdU assays. In brief, mouse astrocytes grown in 96-well culture plates were introduced with or without the relevant construct for 24 h prior to LPS exposure for additional 24 h. In CCK-8 experiment, CCK-8 reagent (Solarbio, Beijing, China) at a 10 μl volume was added to the medium and applied for 3 h at 37°C, followed by absorbance measurement at 450 nm. In EdU assay using the EdU Assay kit (Servicebio), we replaced half of the medium with 2×EdU working reagent (the final concentration of EdU was 10 μM) and subsequently incubated for 2 h. After fixation, the prepared iF488 working solution was applied for additional 30 min at room temperature free of the light. Using fluorescence microscopy, we scored the ratio of the EDU-positive cells after nucleus staining by DAPI reagent (Beyotime).

Determination of malondialdehyde (MDA) content

We determined the content of MDA in treated mouse astrocytes using the MDA Assay Kit and its suggestions (Beyotime, Shanghai, China). The supernatant of mouse astrocytes subjected to the relevant transfection or/and LPS stimulation was collected and processed by MDA content detection. Results were presented relative to the control group.

mRNA expression analysis by qPCR

We isolated total RNA from mouse astrocytes subjected to the relevant transfection by the use of TRIzol (Life Technologies). cDNA generated by reverse transcription (RT) using isolated RNA and QuantiTect RT Kit (Qiagen, Frankfurt, Germany) was used as the template for qPCR. The qPCR mixture for a reaction consisted of 10 μl SYBR Green Mix (Servicebio), 1 μl template, 0.4 μl of each primer (Trem1-sense: 5'-GCGTGTTCTTTGTCTCAGGT-3' and Trem1-antisense: 5'-GGGTAGGGATCGGGTTGTAG-3'), and 8.2 μl nuclease-free water. We analyzed the data using the $2^{-\Delta\Delta Ct}$ method with normalization to β-actin (sense: 5'-CGATATCGCTGCGCTGGTC-3' and antisense: 5'-AGGTGTGGTGCCAGATCTTC-3').

Chromatin immunoprecipitation (ChIP)

We evaluated the direct interaction of Yy1 and the Trem1 promoter by ChIP experiment using ChIP^{TM} Enzymatic Assay Kit as recommended by the vender (Beyotime). Briefly, after formaldehyde (1%) fixation and glycine incubation, the nucleus of mouse astrocytes was prepared, followed by DNA fragmentation using MNase. After that, lysates of the nucleus were incubated with mouse anti-Yy1 mAb (RRID:AB_2881664, #66281-1-Ig, 1:3000, Proteintech) or rabbit anti-IgG mAb (RRID:AB_2687931, #ab172730, 1:1000, Abcam), which was followed by incubation of Protein A/G Magnetic Beads. The antibody precipitates were harvested, and the co-precipitated DNA was subjected to PCR analysis for the enrichment of the Trem1 promoter fragment harboring the predicted binding sequence using specific

primer sets (sense: 5'-GATCCAACAGGCCTCTGTCTC-3' and antisense: 5'-GGGTGCGGATTCTACCCATC-3').

Luciferase assay

For assessment of the direct interaction of TF Yy1 and the Trem1 promoter by the predicted binding sequence (CTAAATGG), we cloned the Trem1 promoter fragment (73 bp) harboring the predicted binding sequence or mutated seed region (AGCCCGTT), synthesized by Tsingke, into the pGL3-basic vector (HonorGene, Changsha, China). Mouse astrocytes were co-transfected with pRL-TK *Renilla* control plasmid (Promega, Mannheim, Germany), each reporter construct, and si-Yy1 or si-NC by utilizing Lipofectamine 3000. We measured luciferase activity of Firefly&*Renilla* after 48 h by the use of Dual-Lumi Assay Kit (Servicebio).

Bioinformatics and statistics

Through the Jaspar online web, the motif of mouse TF Yy1 was predicted at https://jaspar.elixir.no/. For all analyses of two groups, we applied two-tailed unpaired *t*-test. For significance calculation of multiple groups, we utilized Tukey's *post hoc* test following one-way ANOVA. Mean ± SD was used to present the data from at least three replicates, and significance was defined as $p < 0.05$. We provided a figure describing the experimental design and workflow in the Supplementary material (Fig. S1).

Results

Levels of mouse Yy1 and inflammatory cytokines are upregulated and oxidative stress is enhanced in CUMS mice

C57BL/6 mice were exposed to several stressors to induce a CUMS mouse model. The sucrose preference test revealed that relative to control mice, CUMS mice had less sucrose solution to drink (Fig. 1A). In the tail suspension and forced swimming experiments, CUMS mice had a longer time of tail suspension and forced swimming (Fig. 1B,C). Conversely, CUMS mice had more difficult social interaction than control mice, revealed by the reduced social interaction time in CUMS mice (Fig. 1D). These behavioral observations indicated that CUMS mice were intermittently motionless and

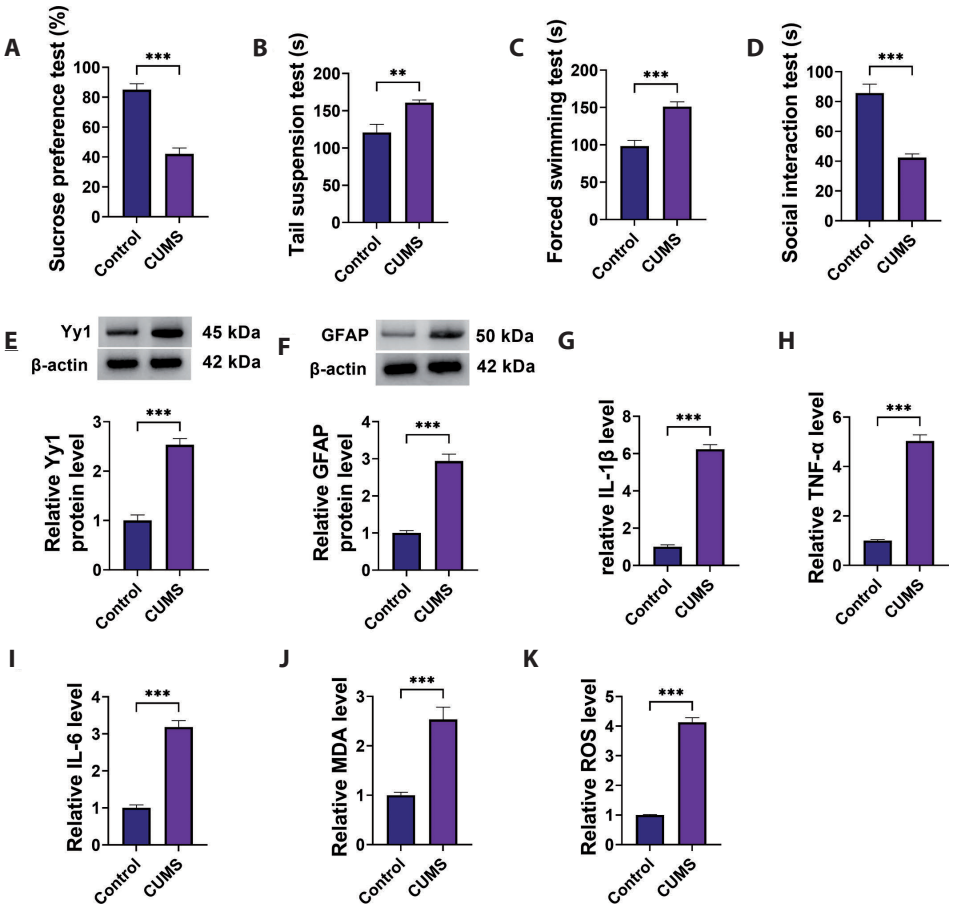

Figure 1. Involvement of TF Yy1, neuroinflammation, and oxidative stress in depression in CUMS mice. C57BL/6 mice were exposed to several stressors to induce the CUMS mouse model. Behavioral tests, including sucrose preference (**A**), tail suspension (**B**), forced swimming (**C**) and social interaction (**D**) tests, were performed. Immunoblotting for the TF Yy1 (**E**) and the specific astrocyte marker GFAP (**F**) in mouse cortical tissues. ELISA for IL-1β (**G**), TNF-α (**H**), and IL-6 (**I**) production levels in mouse cortical tissues. **J.** MDA content in mouse cortical tissues using corresponding assay kit with ELISA instrument. **K.** ROS production in mouse cortical tissues using corresponding assay kit. ** $p < 0.01$, *** $p < 0.001$, Student's *t*-test.

could not get out of the situation, validating the successful establishment of the CUMS mouse model.

We then examined the expression pattern of the TF Yy1 in cerebral cortical tissues of CUMS mice. As expected, CUMS mice showed high levels of mouse Yy1 protein in cortical tissues than control mice (Fig. 1E), suggesting the implication of Yy1 in depression pathogenesis. A specific astrocyte marker GFAP was elevated in cortical tissues of CUMS mice (Fig. 1F), suggesting the key role of astrocytes in depression development.

Examination of three pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) revealed overactivated inflammatory response, evidenced by a striking augmentation of their production levels in cortical tissues of CUMS mice (Fig. 1G–I). We next evaluated oxidative stress in cortical tissues of CUMS mice by examining the contents of MDA and ROS. CUMS mice showed higher levels of MDA and ROS in their cortical tissues than control mice (Fig. 1J,K), confirming the involvement of enhanced oxidative stress in depression.

Yy1 disruption relieves LPS-triggered pro-growth, pro-inflammation, and pro-oxidative stress effects in mouse astrocytes

Next, we generated LPS-stimulated astrocytes to examine the biological function of TF Yy1 in depression development. Based on the up-regulation of Yy1 in CUMS mice, we wanted to elucidate the consequences following Yy1 disruption. In primary mouse astrocytes, Yy1 protein was repressed by Yy1-siRNA (si-Yy1) introduction (Fig. 2A). Elevation of Yy1 protein was confirmed by immunoblotting in LPS-stimulated astrocytes (Fig. 2B). Stimulation of LPS led to a significant enhancement in astrocyte growth, evidenced by elevated GFAP expression (Fig. 2C), enhanced cell viability (Fig. 2D), and increased ability to proliferate (Fig. 2E). Importantly, pro-inflammatory cytokines IL-1β (Fig. 2F), TNF-α (Fig. 2G) and IL-6 (Fig. 2H) secretion levels as well as oxidative stress markers MDA (Fig. 2I) and ROS (Fig. 2J) contents were upregulated in mouse astrocytes after LPS stimulation. Therefore, LPS stimulation induces growth, neuroinflammation, and oxidative stress in astrocytes.

However, introduction of si-Yy1, but not si-NC control, exerted a counteracting impact on LPS-induced Yy1 augmentation in mouse astrocytes (Fig. 2B). The increase in GFAP expression (Fig. 2C), cell viability (Fig. 2D) and proliferation ability (Fig. 2E) following LPS stimulation was repressed by Yy1 disruption. Furthermore, the augmentation in IL-1β (Fig. 2F), TNF-α (Fig. 2G) and IL-6 (Fig. 2H) secretion levels as well as MDA (Fig. 2I) and ROS (Fig. 2J) contents following LPS stimulation was decreased by Yy1 disruption. These data suggest the promoting impact of LPS on astrocytic neuroinflammation and oxidative stress, in part, *via* Yy1 up-regulation.

Trem1 disruption alleviates LPS-triggered pro-growth, pro-inflammation, and pro-oxidative stress effects in mouse astrocytes

Consistently, stimulation of LPS resulted in a strong elevation in Trem1 protein expression in mouse astrocytes (Fig. 3A), and this effect was diminished by Trem1-siRNA (si-Trem1) transfection (Fig. 3B). More importantly, reduction of Trem1 markedly counteracted LPS-triggered promotion in GFAP expression (Fig. 3C), viability (Fig. 3D), and proliferation (Fig. 3E) in mouse astrocytes. Disruption of Trem1 also counteracted LPS-triggered pro-inflammation and pro-oxidative stress effects in mouse astrocytes, evidenced by down-regulation of IL-1β (Fig. 3F), TNF-α (Fig. 3G), IL-6 (Fig. 3H), MDA (Fig. 3I), and ROS (Fig. 3J) levels following Trem1 disruption. Thus, Trem1 is a crucial player of LPS-triggered neuroinflammation and oxidative stress in astrocytes.

Yy1 directly promotes the transcription and expression of Trem1

We further assessed the transcriptional targets of Yy1 in affecting astrocytic inflammation and oxidative stress under LPS stimulation. Through the Jaspar online web, the motif of mouse Yy1 was predicted and shown in Figure 4A. Our above *in vitro* findings demonstrate that Yy1 and Trem1 have same functions in LPS-triggered mouse astrocytes. We therefore attempted to predict the direct binding of Yy1 and Trem1 and examined whether Yy1 could control Trem1 in mouse astrocytes. As expected, when we set the relative profile score threshold to 85%, we found that Yy1 harbored a potential binding sequence (CTAAATGG) for the Trem1 promoter (Fig. 4A). The ChIP-qPCR experiments revealed the enrichment of the Trem1 promoter in the Yy1-specific antibody precipitate (Fig. 4B), demonstrating the direct relationship of Yy1 and the Trem1 promoter. We subsequently assessed whether Yy1 could bind to the Trem1 promoter by cloning the sequence into the pGL3 vector ($Trem1^{wt}$) and mutating it ($Trem1^{mut}$). Yy1 disruption diminished the luciferase activity of astrocytes introduced with $Trem1^{wt}$, while no significant effect on the luciferase of $Trem1^{mut}$-transfected astrocytes was observed following Yy1 disruption (Fig. 4C), indicating the repressive effect of Yy1 disruption on Trem1 transcriptional activity. Regulation of Yy1 in Trem1 expression was also examined in mouse astrocytes. Increased Yy1 expression by the Yy1 cDNA construct, evidenced by immunoblotting (Fig. 4D), enhanced Trem1 expression at both mRNA and protein (Fig. 4E,F). On the other hand, both Trem1 mRNA level and its protein expression were diminished by Yy1 disruption (Fig. 4E,F). Therefore, in mouse astrocytes, Yy1 can enhance the transcription and expression of Trem1 by binding to the Trem1 promoter.

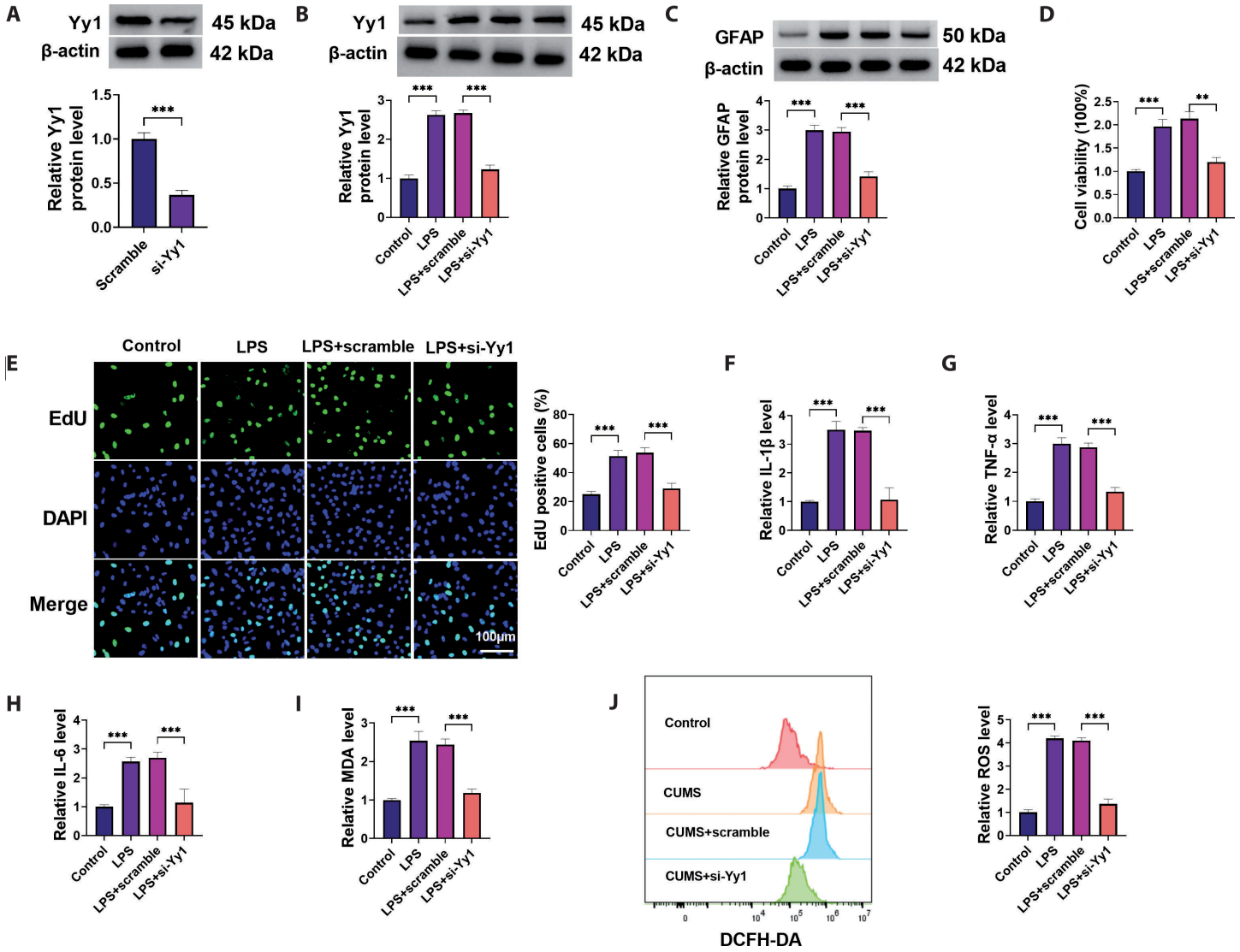

Figure 2. Yy1 disruption attenuates LPS-triggered enhancement in growth, inflammation and oxidative stress of mouse astrocytes. **A.** Immunoblotting for the TF Yy1 in si-Yy1-transfected or si-NC-introduced mouse astrocytes. **B**. Mouse astrocytes were introduced with si-Yy1 or si-NC for 24 h prior to stimulation with LPS (100 ng/ml; 24 h) or mock, with Yy1 protein detection by immunoblotting. **C.** Immunoblotting for GFAP in mouse astrocytes treated as B. **D**. Measurement of viability of mouse astrocytes treated as B by CCK-8. **E.** Evaluation of proliferation of mouse astrocytes treated as B by EdU assay. Measurement of TNF-α (**F**), IL-1β (**G**), and IL-6 (**H**) production levels in mouse astrocytes treated as B by ELISA. **I.** MDA content assessment in mouse astrocytes treated as B using orresponding assay kit with ELISA instrument. **J.** ROS production in mouse astrocytes treated as B using corresponding assay kit with flow cytometry. ** $p < 0.01$, *** $p < 0.001$, Student's *t*-test or one-way ANOVA.

Yy1 disruption relieves LPS-triggered effects in mouse astrocytes by Trem1 down-regulation

Next, we elucidated if involvement of Yy1 in LPS-triggered effects in mouse astrocytes is due to its regulation in Trem1. In mouse astrocytes, Trem1 expression was elevated by introduction of the Trem1 cDNA construct (Fig. 5A). In LPS-induced mouse astrocytes, disruption of Yy1 reduced Trem1 expression, and this effect was abolished by co-introduction of the Trem1 cDNA construct (Fig. 5B). The suppression of Yy1 disruption on GFAP expression (Fig. 5C), cell viability (Fig. 5D), cell proliferation (Fig. 5E), IL-1β production (Fig. 5F), TNF-α secretion (Fig. 5G), and IL-6 level (Fig. 5H), as well as MDA content (Fig. 5I) and ROS level (Fig. 5J) were reversed by restoration of Trem1. These observations suggest Yy1 disruption exerts its impacts in LPS-induced mouse astrocytes, in part, by down-regulation of Trem1.

The Yy1/Trem1 cascade affects the activation of the NF-κB signaling in mouse astrocytes

Finally, we set out to examine whether the Yy1/Trem1 cascade could affect this signaling pathway. In mouse astrocytes, LPS exposure led to a clear elevation in the ratio of p-IκBα/IκBα

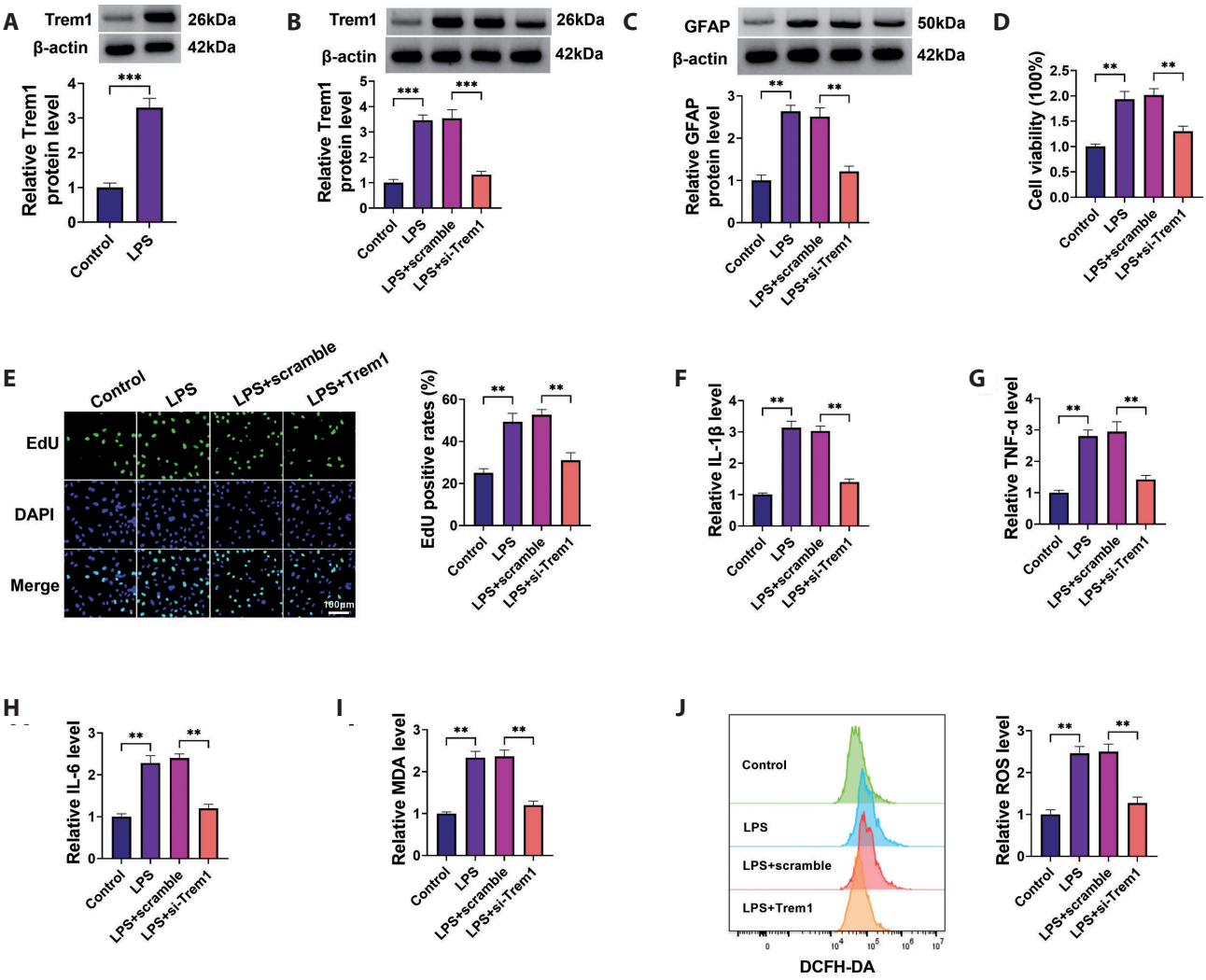

Figure 3. Trem1 disruption reverses LPS-triggered pro-growth, pro-inflammation, and pro-oxidative stress effects in mouse astrocytes. **A.** Immunoblotting for Trem1 in mouse astrocytes after introduction of si-Trem1 or si-NC. **B.** Immunoblotting for Trem1 in mouse astrocytes subjected to transfection of si-Trem1 or si-NC for 24 h before stimulation with LPS (100 ng/ml; 24 h) or mock. **C.** Immunoblotting for Trem1 in mouse astrocytes treated as B. **D.** Measurement of viability of mouse astrocytes treated as B by CCK-8. **E.** Evaluation of proliferation of mouse astrocytes treated as B by EdU assay. Measurement of TNF-α (**F**), IL-1β (**G**), and IL-6 (**H**) production levels in mouse astrocytes treated as B by ELISA. **I.** MDA content assessment in mouse astrocytes treated as B using orresponding assay kit with ELISA instrument. **J.** ROS production in mouse astrocytes treated as B using corresponding assay kit with flow cytometry. ** $p < 0.01$, *** $p < 0.001$, Student's *t*-test or one-way ANOVA.

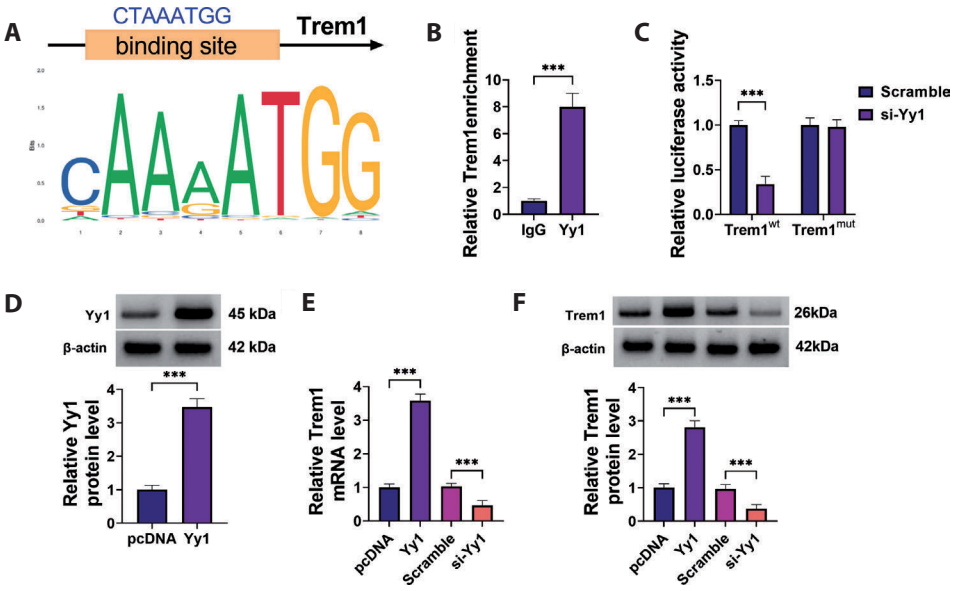

Figure 4. Positive regulation of Yy1 in the transcription and expression of Trem1. **A.** The motif of mouse Yy1 and the predicted binding sequence of Yy1 and the Trem1 promoter on the Jaspar online web. **B.** Chromatin of mouse astrocytes was obtained and incubated with the Yy1-specific antibody or IgG control (ChIP experiment). DNA in the precipitates was purified and assayed for the Trem1 promoter enrichment level by qPCR. **C.** The predicted binding sequence of Yy1 and the Trem1 promoter ($Trem1^{wt}$) or the mutation ($Trem1^{mut}$) was cloned into the pGL3 vector and transfected into mouse astrocytes along with si-Yy1 or si-NC, followed by evaluation of luciferase activity. **D.** Immunoblotting for Yy1 in pcDNA- or Yy1 cDNA construct-transfected mouse astrocytes. qPCR for Trem1 mRNA (**E**) and immunoblotting for Trem1 protein (**F**) in mouse astrocytes after introduction by Yy1 cDNA construct, si-Yy1, pcDNA, or si-NC. *** $p < 0.001$, Student's *t*-test or one-way ANOVA.

and p-p65/p65 (Fig. 6A), which indicated that LPS activates the NF-κB signaling, and this effect was diminished by Trem1 disruption (Fig. 6A), which suggested the blockade of Trem1 disruption on LPS-induced activation of this pathway. Consistent with the effect of Trem1 disruption, Yy1 depletion resulted in a marked inhibition in this signaling activation induced by LPS (Fig. 6B). More importantly, restoration of Trem1 counteracted Yy1 depletion-driven blockade of this signaling activation in LPS-stimulated mouse astrocytes (Fig. 6B). Thus, we conclude that the Yy1/Trem1 cascade affects the NF-κB signaling, a crucial pathway in depression pathogenesis.

Discussion

By functioning as essential transcriptional modulators of gene expression, some TFs closely associated with brain development and function have been shown to participate in major depressive disorder and depression progression (Wang and Mao 2019; He et al. 2021; Soga et al. 2021). As an example, the TF NF-κB is detected to be a crucial player in depression pathogenesis (Caviedes et al. 2017). The forkhead box O TFs have emerged as important participators in the etiology of depression (Wang et al. 2015). These findings suggest the potential of TFs as novel antidepressants. Interestingly, high TF YY1 expression is negatively linked to cognitive dysfunction in patients with major depressive disorder (Lu et al. 2023). Here, we decided to define the function and mechanism of Yy1 in LPS-triggered neuroinflammation and oxidative stress in mouse astrocytes. Using functional analysis, regulation analysis, and rescue experiments, we establish the crucial implication of Yy1 in mouse astrocytes under LPS exposure *via* the Trem-1/NF-κB pathway. Our findings suggest a novel Yy1/Trem-1/NF-κB mechanism that affects LPS-induced depression.

It has recently been described that astrocytic Yy1 can protect against pathological neuroinflammation, apoptosis, and oxidative stress and thus is crucial for murine brain development (Pajarillo et al. 2023). In the pathology of major depressive disorder, YY1 is closely associated with central inflammation and cognitive dysfunction (Lu et al. 2023). In accordance with its high expression in plasma of patients with major depressive disorder (Lu et al. 2023), our data uncovered the up-regulation of Yy1 in cerebral cortical tissues of the CUMS mouse model, suggesting the involvement of Yy1 in depression pathogenesis. Dysfunction of astrocytes contributes to depression development (Liao et al. 2021; Dolotov et al. 2022; Zhao et al. 2022). Suppression of activated astrocytes is able to mitigate depressive behaviors triggered by LPS by down-regulating iNOS, IL-1β, and TNF-α (Wang et al. 2019). Thus, suppressing activated astrocytes may be a promising strategy for depression prevention and treatment. By using LPS-challenged dysfunction of mouse astrocytes, we demonstrate that disruption of Yy1 relieves LPS-triggered pro-growth, pro-inflammation, and pro-oxidative stress effects in astrocytes.

As a proinflammatory receptor player, the TREM-1 is linked to overactivated inflammatory response, and its

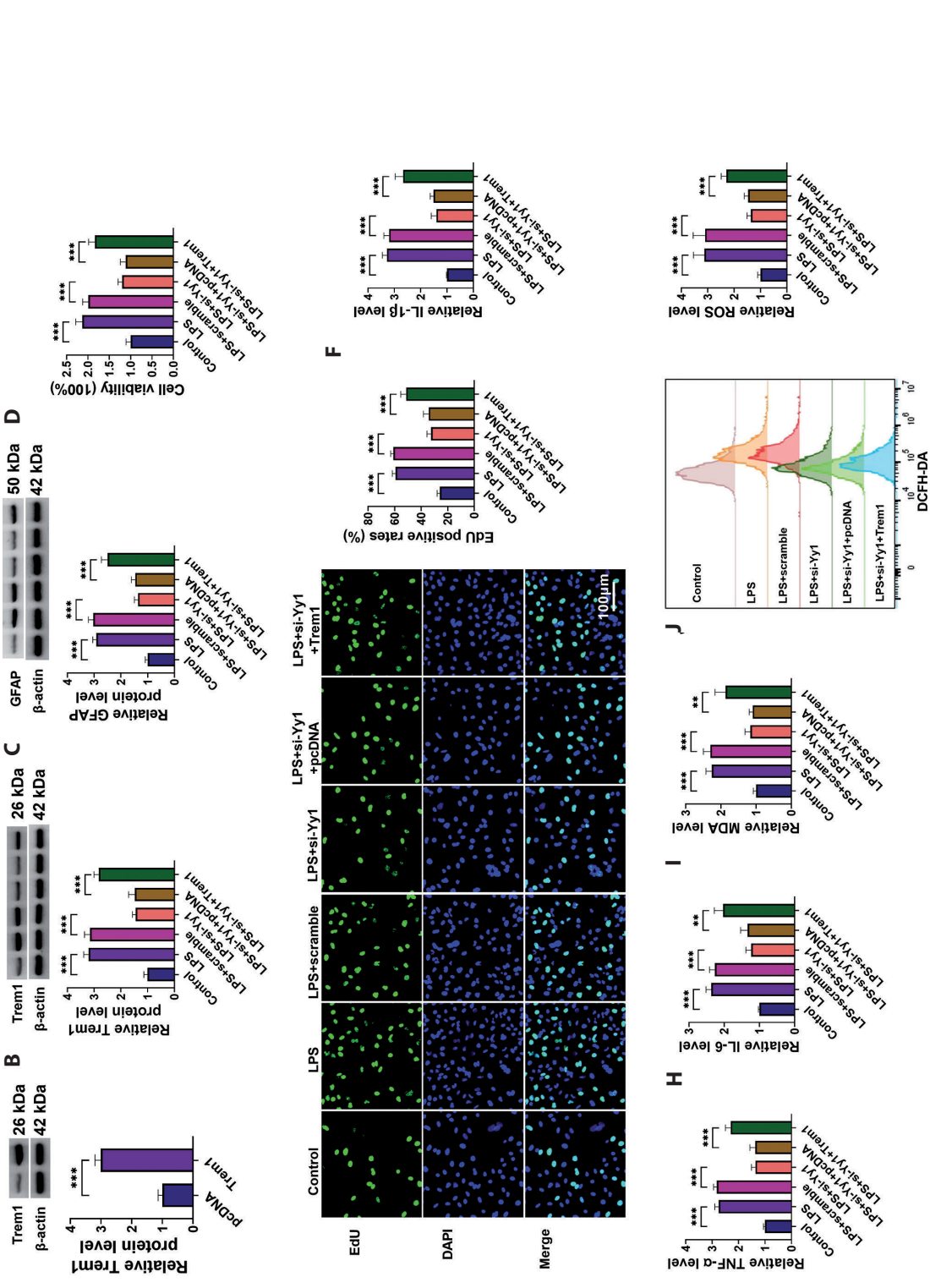

Figure 5. Yy1 disruption affects LPS-triggered neuroinflammation and oxidative stress in astrocytes *via* Trem1. **A.** Immunoblotting for Trem1 in pcDNA- or Trem1 cDNA construct-transfected mouse astrocytes. **B.** Immunoblotting for Trem1 in mouse astrocytes transfected with or without si-Yy1, si-NC, si-Yy1+pcDNA, or si-Yy1+Trem1 before LPS (100 ng/ml; 24 h) stimulation. **C.** Immunoblotting for GFAP in mouse astrocytes treated as B. **D.** Viability measurement of mouse astrocytes treated as B by CCK-8. **E.** Evaluation of proliferation of mouse astrocytes treated as B by EdU assay. Measurement of TNF-α production (**F**), IL-1β secretion (**G**), and IL-6 level (**H**) in mouse astrocytes treated as B by ELISA. **I.** MDA content assessment in mouse astrocytes treated as B using orresponding assay kit with ELISA instrument. **J.** ROS production in mouse astrocytes treated as B using corresponding assay kit with flow cytometry. $^{**}p < 0.01$, $^{***}p < 0.001$, Student's *t*-test or one-way ANOVA.

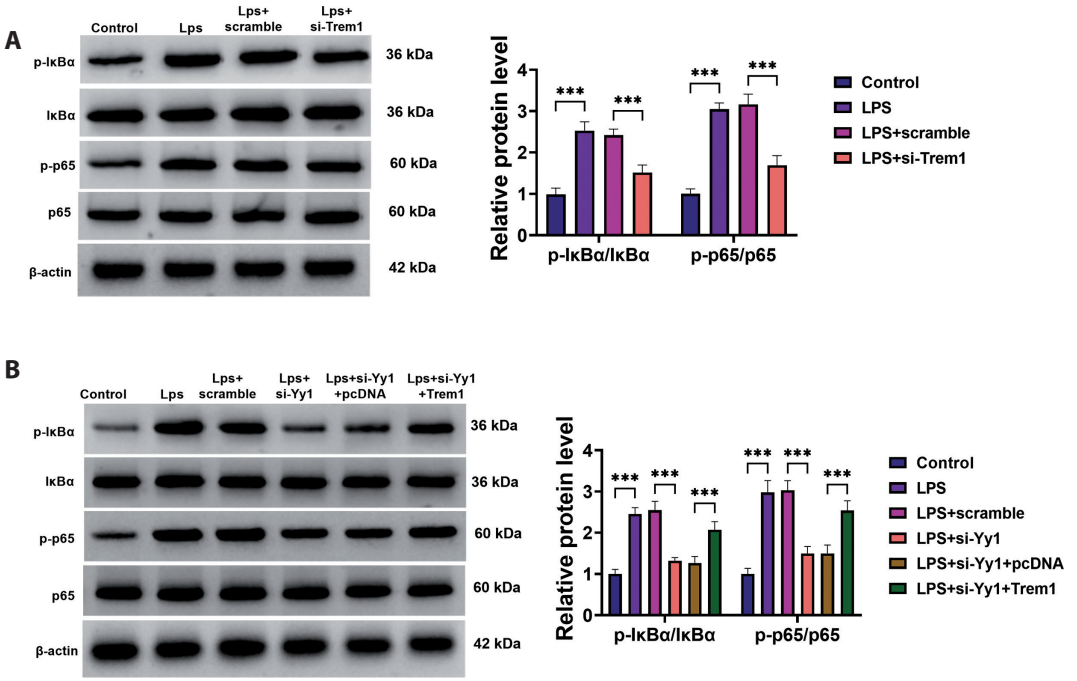

Figure 6. The Yy1/Trem1 cascade affects the NF-κB signaling in LPS-stimulated mouse astrocytes**. A.** Immunoblotting for p-IκBα, IκBα, p-p65 and p65 levels in mouse astrocytes subjected to transfection of si-Trem1 or si-NC for 24 h before stimulation with LPS (100 ng/ml; 24 h) or mock. **B.** Immunoblotting for p-IκBα, IκBα, p-p65 and p65 levels in mouse astrocytes transfected with or without si-Yy1, si-NC, si-Yy1+pcDNA, or si-Yy1+Trem1 before LPS (100 ng/ml; 24 h) stimulation. *** $p < 0.001$, one-way ANOVA.

alteration is bound to a variety of inflammatory diseases, including neurodegenerative disorders (Natale et al. 2019; Matos et al. 2021; Siskind et al. 2022). In a DSS-evoked colitis model, Trem-1, expressed in anterior cingulate cortex, is capable of driving depressive-like behaviors by affecting neuroinflammatory responses (Wu et al. 2023). Through the PI3K/Akt signaling, Trem-1 disruption is demonstrated to relieve depressive behaviors triggered by LPS in a mouse model (Fu A et al. 2023). Similarly, our siRNA knockdown experiments unveiled that Trem1 is a crucial player of neuroinflammation and oxidative stress triggered by LPS in astrocytes.

TFs are well-known to control gene expression and thus possess critical activities in cellular biology and depression development (Wang et al. 2015; Soga et al. 2021; Lu et al. 2023). Further investigations using the Jaspar database, ChIP-qPCR experiment, luciferase assay and expression regulation analysis confirm the direct regulation of Yy1 in the transcription and expression of Trem1. Furthermore, we provide, for the first time, the direct evidence that Yy1 disruption exerts regulatory effects in LPS-challenged mouse astrocytes in part by down-regulation of Trem1, suggesting the implication of the Yy1/Trem1 cascade in depression pathogenesis. Additionally, BACE1 plays a key role in various cognitive impairments, such as Alzheimer's disease and age-related comorbidities (Hampel et al. 2021; Bao and Shen 2023). YY1 has been identified as a strong activator of BACE1 (Nowak et al. 2006; Rossner et al. 2006). Our data showed that BACE1 is enhanced in LPS-stimulated mouse astrocytes (Fig. S2), suggesting the involvement of the Yy1/BACE1 axis in LPS-induction depression. We speculate that the Yy1/Trem1 and Yy1/BACE1 axes may form a complex network in controlling LPS-induction depression.

The NF-κB signaling, a pro-inflammatory pathway in neuroinflammation, actively participates in the etiology of depression (Jiang et al. 2021; Bian et al. 2022). Inhibition of this pathway has been validated to be fundamental mechanism for several potential anti-depressive agents, such as polydatin, arctigenin, and quercitrin (Xu et al. 2020; Sun et al. 2021; Bian et al. 2022). Interestingly, we point out the regulation of the Yy1/Trem1 cascade in this pathway and the suppression of Yy1 depletion in LPS-induced activation of this pathway. By combining these previous findings of this signaling function in depression, we conclude that the NF-κB pathway may be responsible for the regulation of the Yy1/Trem1 cascade in affecting neuroinflammation and oxidative stress in LPS-challenged astrocytes. However, the direct evidence is lacking in the current research.

In summary, our observations demonstrate that Yy1 disruption relieves LPS-triggered growth, neuroinflammation, and oxidative stress in astrocytes via the NF-κB pathway by down-regulating Trem1. This finding is a crucial complement

for the action of Yy1 in depression pathogenesis and suggests possible strategies for this disease treatment.

Conflict of interest. The authors declare that they have no conflicts of interest.

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Received: January 12, 2024
Final version accepted: August 7, 2024

doi: 10.4149/gpb_2024037

Supplementary Material

Transcription factor Yy1 modulates Trem1 to control LPS-triggered neuroinflammation and oxidative stress in mouse astrocytes *via* the NF-κB pathway

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Supplementary Figures

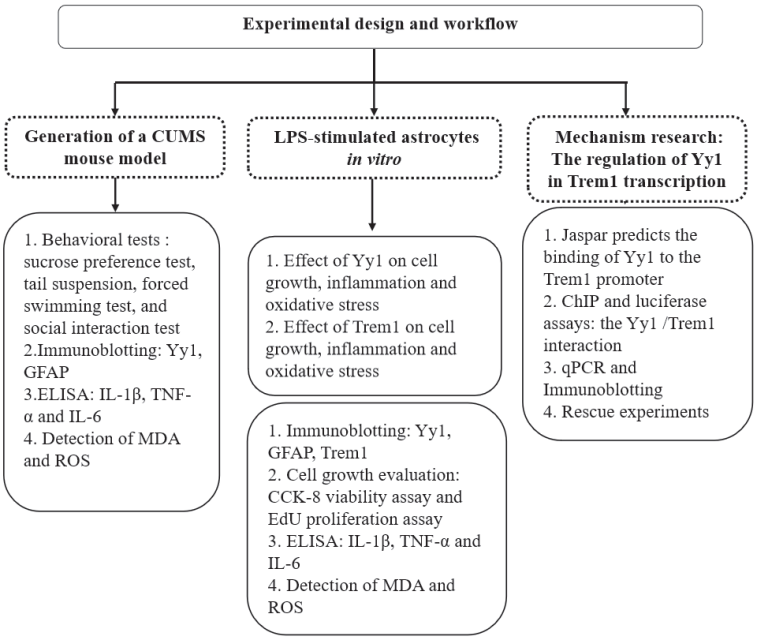

Figure S1. Schematic of the experimental design and workflow in this study.

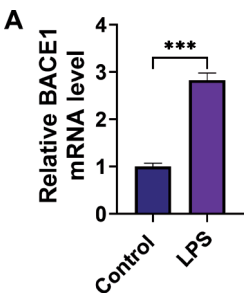

Figure S2. Expression of BACE1 mRNA in mouse astrocytes stimulated with or without LPS. *** $p < 0.001$.