# Ibrutinib alleviated LPS-induced neuroinflammation and synaptic defects in a mouse model of depression

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**Research article**

**Keywords:** Ibrutinib, Depression, Neuroinflammation, Inflammasome, synaptic defects

**Posted Date:** June 2nd, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-31118/v1

## Abstract

# Background

Previous studies indicate a close association between the altered immune system and major depressive disorders. and inhibition of neuroinflammation may represent an alternative mechanism to treat depression. Recently, the anti-inflammatory activity of ibrutinib has been reported, however, the effect of ibrutinib on neuroinflammation allied depression and its underlying mechanism has not been comprehensively studied. Therefore, we aimed to elucidate the potential anti-depressive role and mechanism of ibrutinib against neuroinflammation induced depression as well as synaptic defects.

# Methods

Adult C57BL/6J male mice weighing 25–30 g (age 7–8 weeks) were treated with LPS (2 mg/kg BW i.p) and 50 mg/kg BW ibrutinib, orally. Depressive-like behaviors were assessed by FST and SPT, cytokine levels were determined by ELISA, ROS, TBARs, and Nitric oxides were measured via biochemical assays, Iba-1 and GFAP expression were determined by immunofluorescence. Further, spine density was measured by Golgi staining, while NF-kB, Nrf2, SOD2, HO-1, NLRP3, P38, Caspase-1, BDNF, PSD95, and synaptophysin were measured by immunoblotting.

# Results

Our results showed that ibrutinib treatment significantly reduced LPS induced depressive-like behaviors and neuroinflammation via inhibiting NF-kB activation, decreasing pro-inflammatory cytokines level, normalizing redox signaling and it's a downstream component including Nrf2, HO-1, and SOD2 as well as glial cells activation markers such as Iba-1 and GFAP expression. Further, ibrutinib treatment inhibited LPS activated inflammasome activation by targeting NLRP3/P38/Caspase-1 signaling. Interestingly, LPS reduced dendritic spines numbers, expression of BDNF and synaptic related markers including PSD95, snap25, and synaptophysin were improved by ibrutinib treatment in the hippocampal area of the mice brain.

# Conclusion

In conclusion, our finding suggested that ibrutinib could alleviate neuroinflammation and synaptic defects, rendering its antidepressant potential against LPS induced neuroinflammation and depression.

## Background

Major depressive disorder (MDD) is a multifactorial severe psychiatric illness characterized by the lake of



ied by a disturbance in sleeping, eating, and

anhedonia [1–5]. It is estimated that MDD will be the second leading illness affecting 10% of the population world widely up to 2020 [6]. Although a variety of antidepressants are available to treat MDD and the pathogenesis of depression is illuminated widely, further investigation is still required to integrate the relevant molecular mechanisms properly. Numerous documented reports suggest a significant association between neuroinflammation and depression [7–9]. Pre-clinical and clinical researches postulate a key role of pro-inflammatory cytokines in the onset of depressive-like behaviors, as enhanced levels of cytokines including TNF-α, IL-1β, and IL-6 has been reported in the patients with MDD. Moreover, increased cytokine levels have also been detected in the brain sample of MDD patients committing suicide. Additionally, psychoneuroimmunologic perspectives also suggest that neuroinflammation can lead to depression [7, 10–13].

It is well known that microglia and astrocytes play a key role in neuroprotection [14, 15]. Under uncontrolled or chronic neuroinflammation induced by various sources including LPS, these cells are activated and subsequently accelerate neuro-inflammatory response by secreting pro-inflammatory cytokines [16]. Altered cytokines could lead to synaptic degeneration as well as neuronal cell death, providing a strong association of cytokine dysregulation and neurological disorders including neuro-inflammation allied depression [17]. LPS is one of the strong sources of microglia activation via its receptors (Toll-like receptor) present on the surface. Upon TLR activate, downstream signaling cascades including various transcription factors such as NF-kB can trigger and further facilitate pro-inflammatory cytokines production [16, 18–20]. Thus, dysregulated neuroinflammatory responses are potential therapeutic strategies against neuro-inflammation linked to depression. Accumulating studies support the notion that stress during depression causes a reduction in the length and branching of dendrites, followed by decreases in the number and function of spines synapsis which eventually results in decreased neuronal connection and leads to depressive-like behaviors [21, 22]. Furthermore, a decline in neurogenesis and synaptic dysfunction has been reported during chronic inflammation conditions after LPS exposure [23, 24]. Previous reports indicated that chronic LPS administration suppressed the expression of synaptic proteins, including PSD-95, synaptophysin (SYP), synaptosomal associated protein (SNAP-25) in the hippocampal area of mice via TLR4/NF-κB signaling [22, 25, 26].

Ibrutinib is an irreversible selective inhibitor of Bruton's tyrosine kinase (BTK) that selectively targets its kinase domain and modulates BTK downstream signaling by reducing its phosphorylating capacity [27]. Moreover, it can cross the blood-brain barrier as shown in several preclinical studies [28, 29]. As an immunomodulator, ibrutinib affects the function of the peripheral innate and acquired immune cells including macrophages, B cells, T cells, and natural killer cells [30, 31]. However, its role against center neuroinflammation and depression has not to be investigated comprehensively so far. In the current study, we have examined the role of ibrutinib on neuroinflammation allied depression and its underlying mechanisms. Our results showed that ibrutinib significantly reduced the LPS induced neuroinflammatory response as well as LPS induced depressive-like behavior by improving BDNF and synaptic related molecular expression.

## Material And Methods

# Experimental design drug treatment

Adult C57BL/6J male mice weighing 25–30 g (age 7–8 weeks) were purchased from Guangdong medical laboratory animal center, China. The experimental animals were housed at Laboratory Animal Research Center, Peking University Shenzhen Graduate School, under 12 h light /12 h dark cycle at 18–22 C, and had free access to diet and tap water throughout the study. The experimental procedures were set in such a way to minimize mice suffering. All experimental procedures were carried out according to the protocols approved by the Institutional Animal Care and Use Committee of Peking University Shenzhen Graduate School.

The experimental animals were divided into five groups (each group = 6): normal saline-treated (NC), LPS (2 mg/kg/day) treated (LPS), LPS + Ibrutinib (50 mg/kg/day) treated (LPS + IBR), and Ibrutinib (50 mg/kg/day) treated (IBR). The experimental design and drug treatment schedule were shown in Fig. 1A. LPS were administrated intraperitoneally while Ibrutinib (orally) was administrated 1hr before LPS administration. After 24 h of the final LPS administration, behavioral tests were performed. Finally, the mice were sacrificed and serum and tissues were collected and stored at freezing temperature (-80 °C ) until further use.

# Forced Swimming Test (FST)

The forced swimming test (FST) was performed according to previously developed protocols [32]. The experimental animals were trained for swimming and pre-experiment FST was performed to select healthy and normal mice. To perform the FST, the animals were placed in a Plexiglas cylinder (height: 70 cm, diameter: 30 cm) filled with water over the 30 cm level at a temperature of 23 ± 1°C. The video was taped for 6 minutes and the last 5 minutes were blindly analyzed. Mice were considered immobile when they remained floating motionless in the water or to make movements to keep their nose above the water surface. The horizontal movement of the animals throughout the cylinder was defined as swimming while vertical movement against the wall of the cylinder was defined as climbing.

# Sucrose Preference Test (SPT)

A sucrose preference test was performed while using a two-bottle free-choice paradigm. Mice were habituated with a 1% sucrose solution for 3 days and finally grouped randomly. To assess the individual sucrose intake, mice were deprived of water and food for 24 hours on the 3 days of drugs (LPS, Ibrutinib) administration. On the next day, each mouse had free access to two bottles containing sucrose and water, respectively. The position of water and sucrose-containing bottles were changed after 12 hours. Finally, the volume of consumed water and sucrose solution were recorded and calculated by the following

$$SPT = \frac{Sucroseconsumption}{waterandSucroseconsumtion} \times 100\%$$

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# ROS Measurement

Reactive oxygen species (ROS) were analyzed by a previously developed method [33, 34]. Briefly, hydrogen peroxide/serum/homogenates (5 µL/well) was added to 140 µL of 0.1M sodium acetate buffer (pH 4.8) in a 96-well microtiter plate. A mixture (100 µL) which was prepared from reagent R1 (100 µg/ml DEPPD in 0.1M Sodium acetate buffer, pH 4.8) and R2 (4.37 µM ferrous sulfate in 0.1M sodium acetate buffer) at a ratio of 1:25 was added in each well. Then, after free incubation of one minute, absorbance at 505 nm was measured using a plate reader (Envision 2104, PerkinElmer).

# Nitric Oxides And $H_2O_2$ Measurement

The level of NO and $H_2O_2$ was analyzed by a commercially available kit (Beyotime Institute of Biotechnology, China) [35, 36] and measured the absorbance at 540 nm using a microplate reader (Biorad-Benchmark, USA).

# TBARs Assay

TBARS level was estimated [37] to determine the damage to lipids caused by reactive oxygen species in various experimental groups. Briefly, 0.1 ml of sample, 0.1 ml FeSO4, 0.1 ml Tris-HCl, 0.6 ml distilled water, and 0.1 ml Ascorbic Acid were incubated at 37 °C in a test tube for 15 minutes and then 1 ml TCA and 2 ml TBA were added. These plugged test-tubes were incubated for 15 minutes at 100 °C followed by centrifugation at 3000 rpm for 10 minutes. The supernatant O.D. was determined at 532 nm and the following formula was applied to estimate TBARS as nM/mg protein: TBARS (nM/mg protein) = O.D × Total volume × Sample volume × 1.56 × 105 × mg protein/ml (1.56 × 105 = Molar Extinction Coefficient)

# ELISA

The frozen hippocampal and cortical tissue was lysed with RIPA buffer and homogenized on ice. Supernatants were collected after centrifugation and stored at freezing temperature for further analysis. The expression of cytokines was quantified using ELISA kits (ABclonal) according to the manufacturer's protocols. Briefly, after washing the wells of 96-well plate, 100 µL standard/sample was added and

incubated for 2 hours at 37° °C. The plate was then washed and a biotin-conjugated antibody (1:30) was added to each well. The plate was incubated for 1 hour at 37° °C. streptavidin-HRP was added for 30 minutes at 37° °C. Finally, the reaction was stopped, and the optical density was measured accordingly.

# Immunofluorescence

Immunofluorescence staining was performed according to previously reported protocols [38]. Briefly, brain tissue sections (20 μm thick) were washed with PBS for 15 minutes (5 min × 3). After washing, the sections were treated with blocking buffer (10% Goat serum in 0.3% Triton X-100 in PBS) for 1 hour at room temperature. After blocking the tissue was treated with primary antibodies (Iba1, GFAP) overnight at 4 °C. Next day secondary antibodies (Alexa Flour secondary antibodies, ThermoFisher) were applied at room temperature for 1 hour. The sections were washed with PBS for 5 minutes three times. After washing, sections were transferred to slides, and glass coverslips were mounted using the mounting medium. The images were taken under inverted fluorescence microscope IX73 Olympus.

# Golgi Staining

The FD Rapid GolgiStain Kit (FD NeuroTechnologies, Ellicott City, MD) was used to perform Golgi staining. Briefly, after removing the animal brain were rinse quickly in double distilled water, immersed impregnation solutions (A/B) (5 ml solution for each tissue), and store at room temperature for 2 weeks. The brain tissues were transferred to solution C and store for 72 hrs (the solution was replaced after 24 hrs), followed by freezing. After that, 100- to 200-μm sections were prepared using a sliding microtome, and mount to gelatin-coated microscope slides. Then the brain tissue was placed in staining solution for 10 min, rinse with double distilled water, followed by dehydration (sequential rinse 50%, 75%, and 95% ethanol) and xylene treatment. Finally, examined under inverted fluorescence microscope IX73 Olympus.

# Western Blotting

Western blotting was performed as previously reported [39]. Briefly, Denatured samples (boiled at 100° C for 10 minutes) were separated on SDS-PAGE and then transferred to the nitrocellulose membrane. The membrane was blocked in with non-fat milk in TBST (Tris-buffered saline, 0.1% Tween 20), then incubated in primary antibody (1: 500; 1:1,000) overnight at 4 °C. The next day membrane was treated with a secondary antibody (1:1,000) for 1 hr at 4 °C. For detection, the ECL Super signal chemiluminescence kit was used according to the manufacturer's protocol. Blots were developed using Chemidoc mp Bio-red. The densitometry analysis of the bands was performed using image lab software.

# Statistical analysis

Western blot bands and morphological data were analyzed using ImageJ and image lab software (Image J 1.30) and analyzed by SPSS Statistics 21 (IBM, US) and GraphPad Prism 8 software. Data were presented as mean ± SEM. One way ANOVA followed by posthoc Tukey Multiple Comparison tests were performed to compare different groups. $P < 0.05$ was regarded as significant. *: $p < 0.05$, **: $p < 0.001$ and ***: $p < 0.0001$.

## Results

# Ibrutinib attenuated LPS induced depressive-like behaviors

LPS was administered to induced neuroinflammation and depressive-like behaviors [16, 39]. The sickness and depressive-like behaviors were determined by FST, SPT, and relative body weight were measured. Our results showed LPS treatment significantly reduced animal body weight (Fig. 1B) and the sucrose preference of 65% for 1% (Fig. 1C), as compared to the control group. Besides, the immobility time during FST was longer for the LPS-treated mice than the vehicle (Fig. 1D). However, ibrutinib treatment (both pre **Fig. S1, and post** Fig. 1B, C, **and D)** significantly improved immobility time, body weight, and sucrose preference reduction by LPS, suggesting the antidepressant potential of ibrutinib.

# Ibrutinib Regulated Redox Signaling

Altered redox signaling participates in the progression of all major diseases including depressive disorders. Imbalance of oxidants and antioxidants occurs during inflammation, followed by redox signaling-dependent gene expression of proinflammatory mediators [40–45]. It has been shown that LPS administration accelerates ROS/RNS production in the brain tissues [32, 46, 47]. Moreover, antioxidants modulate the pathophysiology of chronic inflammation, indicating a beneficial role in neuroinflammation [48–51]. Herein, the ROS (Fig. 2A), NO (Fig. 2B), and TBARs (Fig. 2C) level were measured and analyzed. LPS treatment continuously accelerated ROS ($H_2O_2$), and NO production, while ibrutinib significantly reduced the effects of LPS on ROS and NO production, strongly suggesting its antioxidant potential. To further validate the anti-oxidative potential of ibrutinib, redox signaling and its associated molecules including Nrf2, HO-1, and SOD2 expression were measured (Fig. 2E, **and F)**. Interestingly, Ibrutinib administration significantly normalized LPS-mediated expression of these molecules. Aberrant PI3K/Akt signaling contributes to ROS production. Activated Akt may phosphorylate and inhibit Gsk3β activities, thus supporting ROS production [40, 52]. We then extend our line of investigation by measuring redox associated and affected signaling molecules including p-Akt, p-Gsk3β, and PI3k. As shown in Fig. 3, altered PI3K/p-Akt expression was observed upon LPS treatment, which was normalized by ibrutinib treatment (Fig. 3A, 3B, 3D). Similarly, enhanced p-GSK3β expression was detected in LPS treated mice hippocampus, and was reversed by ibrutinib treatment (Fig. 3A, **and C)**. These finding strongly suggests redox signaling regulatory potential of ibrutinib.

# Ibrutinib Alleviated LPS Induced Neuroinflammation

LPS is a widely used pro-inflammatory agent [32, 47, 53]. Herein, the protective role of ibrutinib against LPS-induced inflammation was examined both in the peripheral and central systems. Enhanced pro-inflammatory cytokines including TNF-α (serum), IL-1β (Hippocampus), and IL-6 (both serum and hippocampus) levels were detected in LPS treated animals compared to control group, however, ibrutinib treatment significantly attenuated LPS-induced cytokine productions (Fig. 4A, **and B, Fig. S2E)**. Moreover, NF-κB is a central mediator of pro-inflammatory gene regulation which plays a key role in both innate and adaptive immunity. Our results showed LPS treatment significantly enhanced NF-κB phosphorylation, an effect reduced by ibrutinib administration (Fig. 4C). Furthermore, activated glial cells including microglia and astrocytes are the key sources of neuroinflammation and central cytokines [9, 10, 54]. To examine the involvement of microglia and astrocytes in LPS induced neuroinflammation, glial cell activation markers of glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule (Iba-1) expression was determined by immunofluorescence. Interestingly, ibrutinib treatment significantly abolished the stimulating effect of LPS on microglia (Fig. 5, **Fig. S2A, and B)** and astrocytes (Fig. 6, **Fig. S2A, and B)**. Overall these finding supports the hypothesis that ibrutinib could inhibit neuroinflammatory response and reduced gliosis by regulating inflammatory mediators.

# Ibrutinib Alleviated LPS Effect On Inflammasome Activation

LPS may activate inflammasome via NRLP3 and its linked signaling molecules including p38 and caspase-1 regulation [55–60], which subsequently play a significant role in neuroinflammation and neurotoxicity. In our results, enhanced p-p38, caspase-1, and NRLP3 expression were detected in LPS treated mice hippocampus (Fig. 7A, B, C, **and D)**. Interestingly, ibrutinib treatment significantly reduced inflammasome activation as demonstrated by decreased expressions of NLRP3, p38, and caspase-1, suggesting that ibrutinib may exert its anti-inflammatory effects via the inflammasomesignaling pathways.

# Ibrutinib Reduced LPS Induced Neurotoxicity And Synaptogenesis Defects

LPS induces neurotoxicity via neuroinflammation, resulting in cell damage and neuronal loss which subsequently, contribute to dysregulated synaptogenesis [21, 61]. Further, both basic and clinically studies demonstrated that cortical and hippocampal synaptic loss is strongly associated with depression [21, 22]. In our study, a significant decline in spine density was detected in the LPS treated animal hippocampus compared to that in the control group (Fig. 8A **and B)** without any significant changes in NeuN expression (Fig. 9). Furthermore, synaptic defects were explored, and the results showed LPS treatment significantly suppressed PSD95, snap25, and synaptophysin expression in the hippocampus (Fig. 8C-D, **and E)**. However, it is interesting to report that ibrutinib significantly attenuated LPS-induced

changes, indicating that ibrutinib treatment could reduce synaptic defects generated during LPS-induced stress conditions. Additionally, LPS treatment significantly reduced NMDA receptors (NR2A **(**Fig. 10A **and B)**, NR2B **(**Fig. 10A **and C)**, and BDNF **(**Fig. 10A **and D)** expression, while increase p-EEF2 **(**Fig. 10A **and E)** expression, which could reverse by ibrutinib treatment. Overall, the current data suggest that ibrutinib could reduce LPS induced synaptogenesis defects.

# Discussion

In the current study, we have investigated the effect of ibrutinib on central inflammation-induced depression and its underlying mechanisms **(**Fig. 11**).** Several key observations were made. First, ibrutinib significantly suppressed LPS induced neuroinflammation by reducing pro-inflammatory cytokines production, NF-κB phosphorylation, GFAP, and Iba-1 expression as well as inflammasome activation through NLPR3 regulation. Second, ibrutinib treatment significantly alleviated redox signaling changes including Nrf2, HO-1, and SOD2 altered by LPS. Additionally, ibrutinib reversed LPS-induced synaptic defects of reduced PSD95, snap25, and synaptophysin expression, improved spine density loss, and BDNF decrease altered upon LPS administration. Together, these data suggested that ibrutinib could significantly prevent neuroinflammation and relieve depression by attenuating synaptic defects.

LPS is widely used to induce neuroinflammation [2, 3, 49, 54, 62]. Numerous studies showed that LPS treatment could induce central inflammatory response associated with proinflammatory cytokines production, glial cell activation, as well as ROS and RNS generation [2, 5, 7, 40, 44, 47, 54, 63–69]. Furthermore, activated NF-κB also promotes neurotoxic cytokines and ROS production of glial cells [2, 3, 5]. Enhanced NF-B activation, pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6β level, GFAP/Iba-1 expression along with altered ROS and NO concentration upon LPS treatment confirmed the brain neuroinflammation. Interestingly, both pre and post LPS of ibrutinib treatment significantly restrained LPS induced neuroinflammatory changes, suggesting the anti-inflammatory potential of ibrutinib.

The association of neuroinflammation with depression has been widely acknowledged as increased circulatory cytokines level has been determined in patients with mood disorders [70–73]. Peripheral immune activation leads to the transportation of cytokines to the central nervous system (CNS), which stimulates glial cell activation and subsequently accelerates central cytokine production via a positive feedback mechanism [13, 72]. Interestingly, highly activated microglia have also been reported in the brain of individuals who committed suicide [72, 74], indicating a crucial role of neuroinflammation in brain disorders including depression. Thus it is postulated that increased cytokine levels might evoke depressive-status through inflammation elicited synaptic changes [2, 46, 53, 75, 76]. Consistent with previous reports, our results showed that LPS induced neuroinflammation could lead to depressive-like status, as demonstrated by increased immobility and decreased sucrose preference after LPS administration. However, it was interesting to reveal that ibrutinib treatment significantly decreased LPS induced depressive behaviors and attenuated LPS induced neuroinflammation.

ROS is an essential secondary messenger in innate as well as in adaptive immunity, and impaired redox signaling components such as enhanced ROS level can result in hyperactivation of the inflammatory response, leading to tissue damage and pathology [65, 77–79]. Cells exposed to $H_2O_2$ induced intracellular ROS production, and showed potent activation of NF-κB as an oxidative stress sensor [80, 81]. In addition, oxidative stress-dependent PI3K/Akt signaling plays a critical role in the regulation of pro-inflammatory protein expression [82, 83]. However, these redox signaling alterations are counterbalanced by the body defense system. Nfr2 is one of the transcription factors playing a key role in maintaining redox homeostasis of the cell by regulating the expression of key components of antioxidants [84, 85]. Additionally, the Nrf2/p38/MAPK pathway has also been considered a target of HO-1 induction [86–88]. In our study, ibrutinib treatment significantly recovered the LPS induced decline of Nrf2, ultimately increased its target protein expression including HO-1 and SOD2. Finally, the ibrutinib treatment also normalized altered Pi3k, Akt, and its downstream target GSK3β expression in LPS treated groups, showing that ibrutinib might play a significant role in redox homeostasis under stress conditions.

The inflammasome is a multi-protein platform that contributes to cytokines production after activation [89]. An inflammatory agent such LPS can activate inflammasome by inducing assemble of NRLP3 inflammasome complex, which then recruits and processed Caspase-1, subsequently leading to the release of mature cytokines [87, 89–92]. LPS induced inflammasome activation is accompanied by an alteration in synaptic related protein expression and spine morphology. Moreover, cytokine overproduction leads to neuronal damage and synaptic dysfunction via activation of microglia, which then contributes to depressive behaviors [26]. In the present investigation, increased p-p38, NLRP3, and caspase-1 expression, as well as synaptic defects, were detected in the hippocampus of LPS treated mice, which could tremendously reverse by ibrutinib treatment. Selective inhibition of NLRP3 has been demonstrated as an important target to improve depressive-like behaviors. Moreover, fast-acting antidepressant drugs like ketamine could reverse depression by inhibiting the NMDA receptor and rapidly increasing synaptogenesis and spine formation [21]. In our results, ibrutinib treatment reversed LPS induced inflammasome activation and increased spine numbers and synapse related proteins like snap25, PSD95, and synaptophysin, suggesting a strong antidepressant potential of ibrutinib.

# Conclusion

In conclusion, the present study elucidated that LPS activated inflammasome could accelerate neuroinflammation, which subsequently contributed to depression by inducing synaptic defects. However, ibrutinib could reverse LPS induced pathological changes by decreasing neuroinflammation, normalizing redox signaling components expression as well as reducing inflammasome activation. Ibrutinib also increased dendritic spines and reversed synaptic defects induced by LPS. Taken together, our data suggest that neuroinflammation and inflammation-induced synaptic defects could be a significant therapeutic target for depression and ibrutinib might serve as a promising therapeutic candidate for depression by targeting neuroinflammation.

## List Of Abbreviations

LPS: Lipopolysaccharides; CNS: Central nervous system; MDD: Major depressive disorder; ROS: Reactive oxygen species; AKT: Serine-threonine protein kinase; GSK3: Glycogen synthase kinase 3; PIK3: phosphatidylinositol 3-kinase; NF-κB: Nuclear Factor Kappa B Subunit; Nrf2: Nuclear Factor, Erythroid 2 Like 2; HO-1: Heme Oxygenase 1; BDNF: Brain-derived neurotrophic factors; MAPK: Mitogen-Activated Protein Kinase; TLR4: Toll-like receptor 4; IL-1: Interleukin 1 beta; IL-6: Interleukin 6; TNF-α: Tumor Necrosis Factor-alpha; FST: Force swimming test; TBARs: Thiobarbituric Acid Reactive Substance; ELISA: enzyme-linked immunosorbent assay; GFAP: Glial Fibrillary Acidic Protein; IBA1: Allograft inflammatory factor 1; MT1/2: Melatonin receptor 1/2; ANOVA: Analysis of variance.

## Declarations

# Acknowledgments

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# Availability of data and materials.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

# Ethical Approval and Consent to participate

All experimental procedures were carried out according to the protocols approved by the Institutional Animal Care and Use Committee of Peking University Shenzhen Graduate School.

**Consent for publication.**

Not applicable

**Competing interests:**

The authors declare no competing financial interests.

# Funding

This work was supported by Grants Science and Technology Innovation Committee of Shenzhen No: JCYJ20170810163329510; Shenzhen-Hong Kong Institute of Brain Science No: 2019SHIBS0004.

# Author Contributions:

WL designed and, performed the experiments, TA data analysis, and wrote the manuscript. TA, KH, and ZL helped with experimental work. FA, QR, YL, and AJ help with the manuscript. SL supported the study, corresponding authors, reviewed and approved the manuscript, and held all the responsibilities related to this manuscript. All authors reviewed and approved the manuscript.


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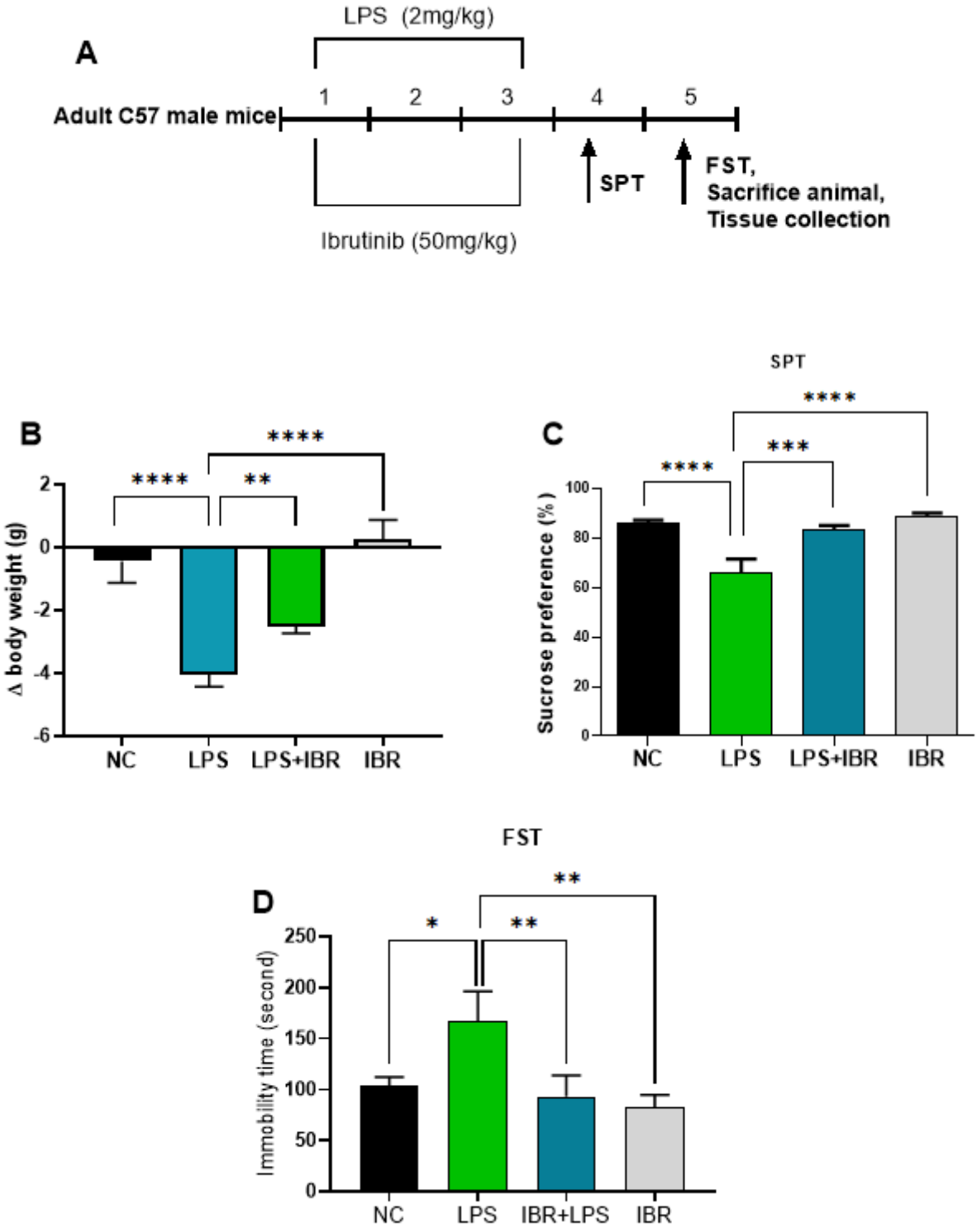
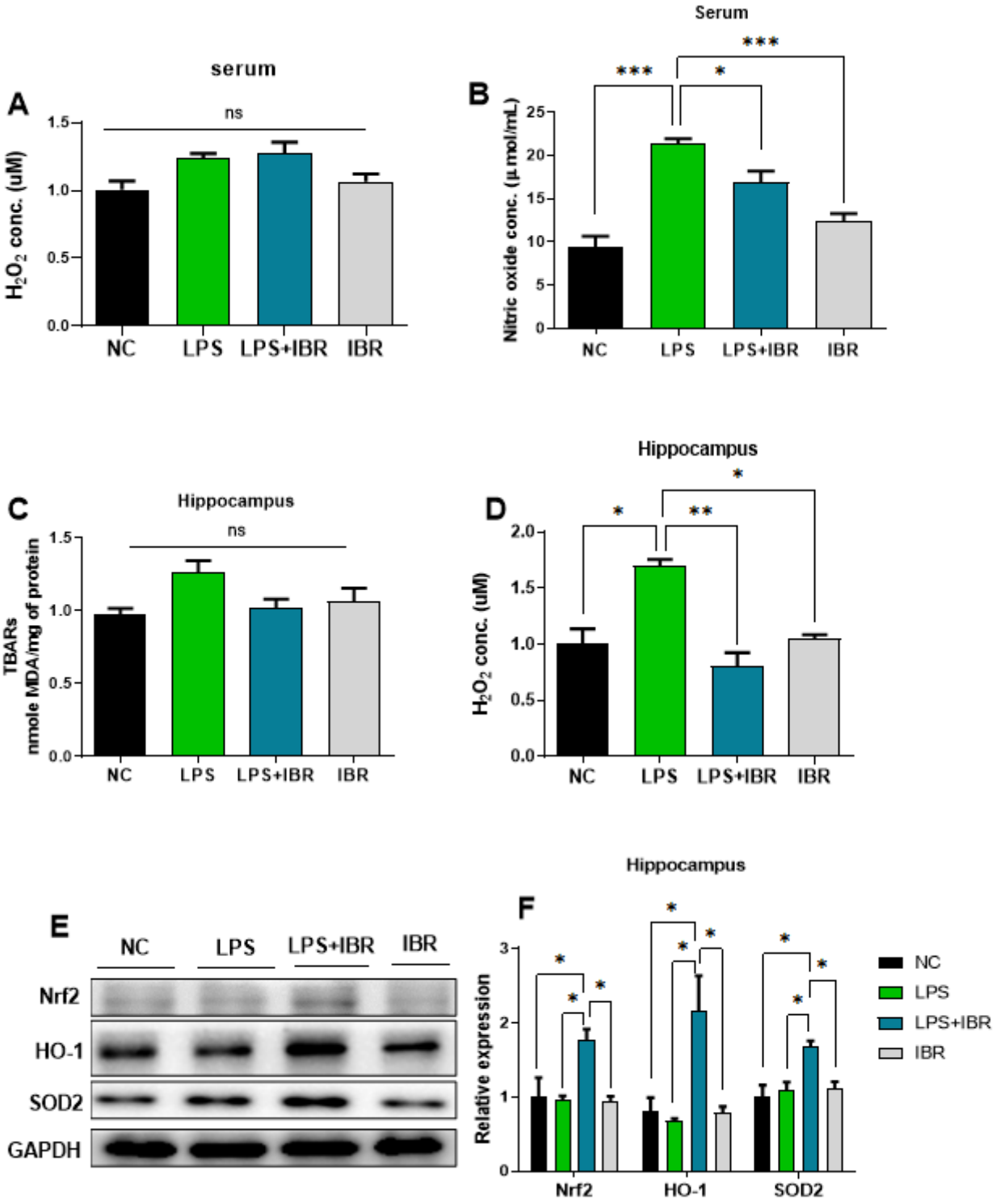
# Figures




## Figure 1

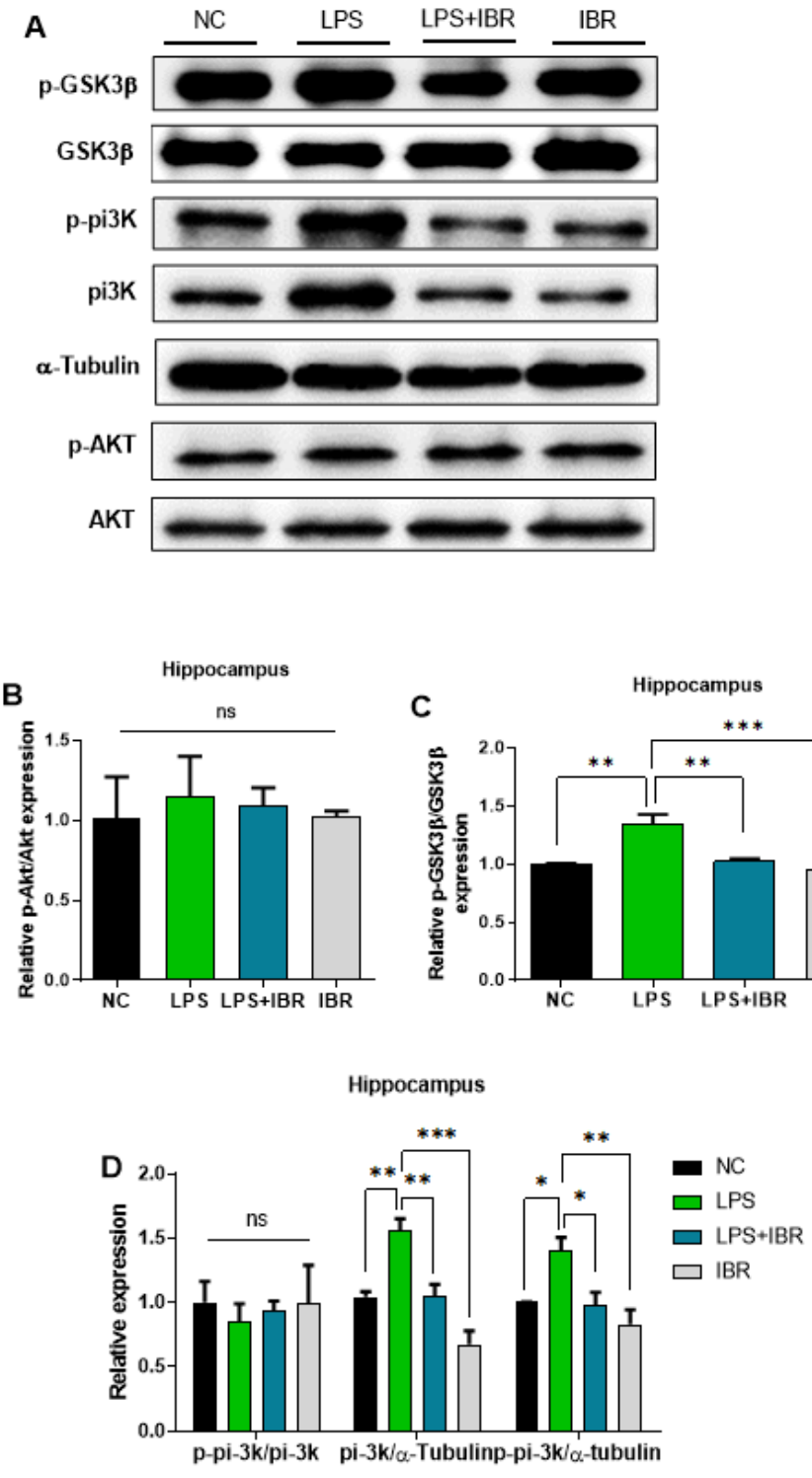
Drug treatment schedule and behavior analysis. A: Drugs treatment schedule, B: Body weights, C: sucrose preference test, D: Forced swimming test. All the values are expressed as mean ±SEM: ANOVA followed by post-hoc analysis. (*): p <0.05, (**): p <0.01), (***): p <0.001




## Figure 2

Ibrutinib attenuated LPS effects on ROS, NO, Nrf2, SOD2, and HO-1. A: Serum H2O2 bar graph, B: NO, concentration in the hippocampus of the brain, C: Representative graph for TBARs, D: Bar graph showing H2O2 concentration in the hippocampus, E: Representative blots show Nrf2, SOD2, and HO-1 expression, F: Representative bar graph for Nr2, SOD2, HO-1 expression analysis. Quantified results were normalized

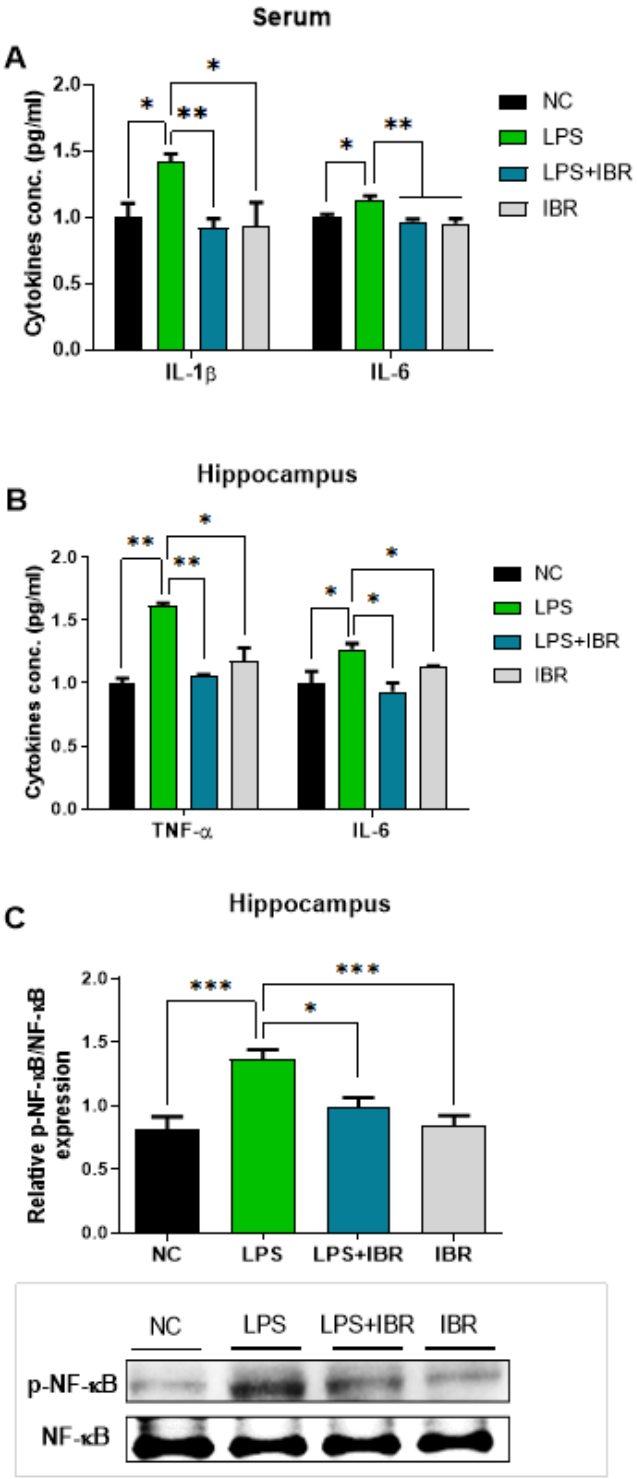
to GAPDH. All the values are expressed as mean ±SEM: ANOVA followed by post-hoc analysis. (*): p <0.05, (**): p <0.01), (***): p <0.001




## Figure 3

Ibrutinib reduced LPS effects on Akt, GSK3β, and PI3k expression. A: Representative blots show Akt, GSK3β, and PI3K expression, B: representing p-Akt relative expression, C: shows p-GSK3β expression in p-PI3K. Quantified results were normalized to

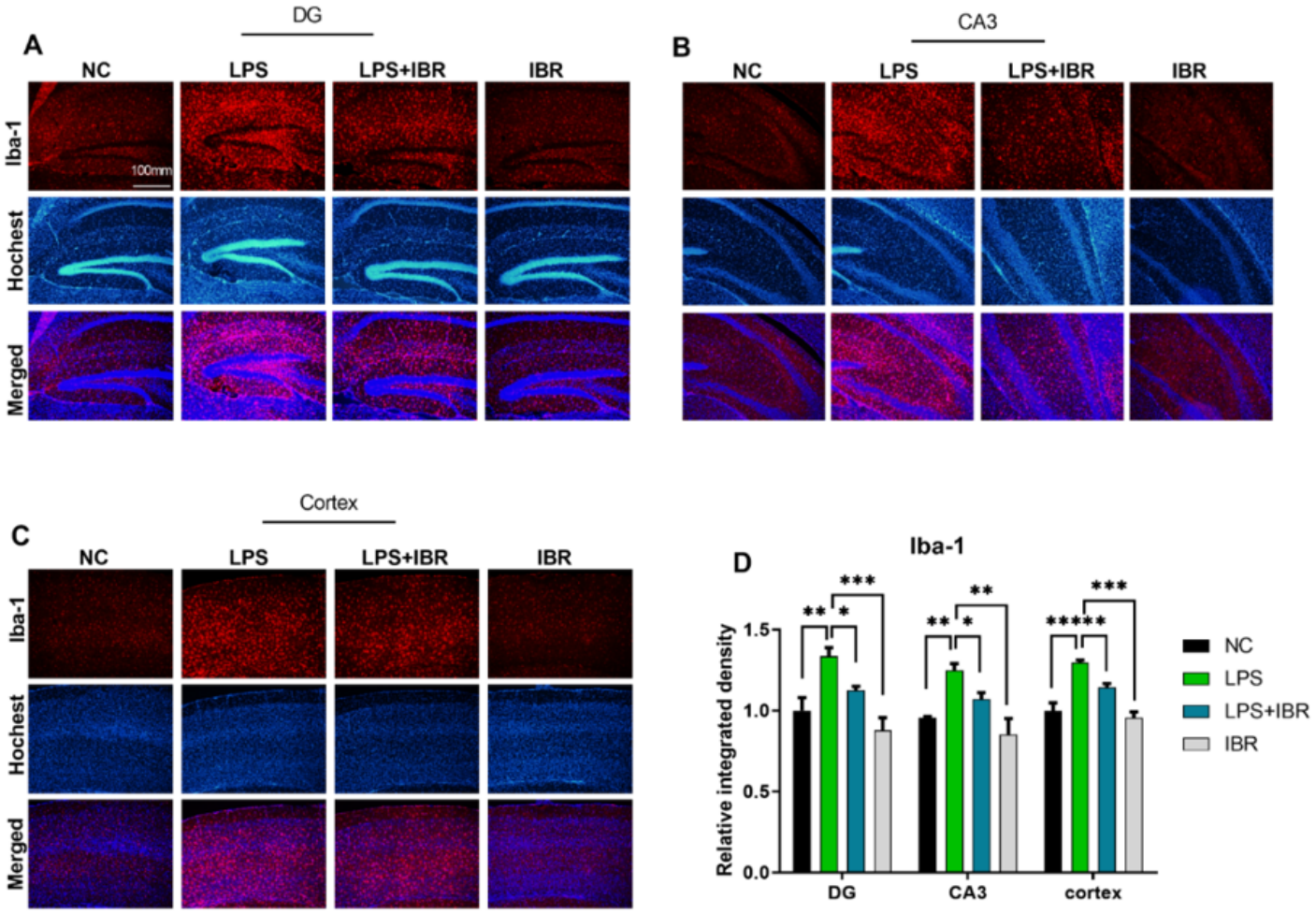
Tubulin. All the values are expressed as mean ±SEM: ANOVA followed by post-hoc analysis. (*): p <0.05, (**): p <0.01), (***): p <0.001




## Figure 4

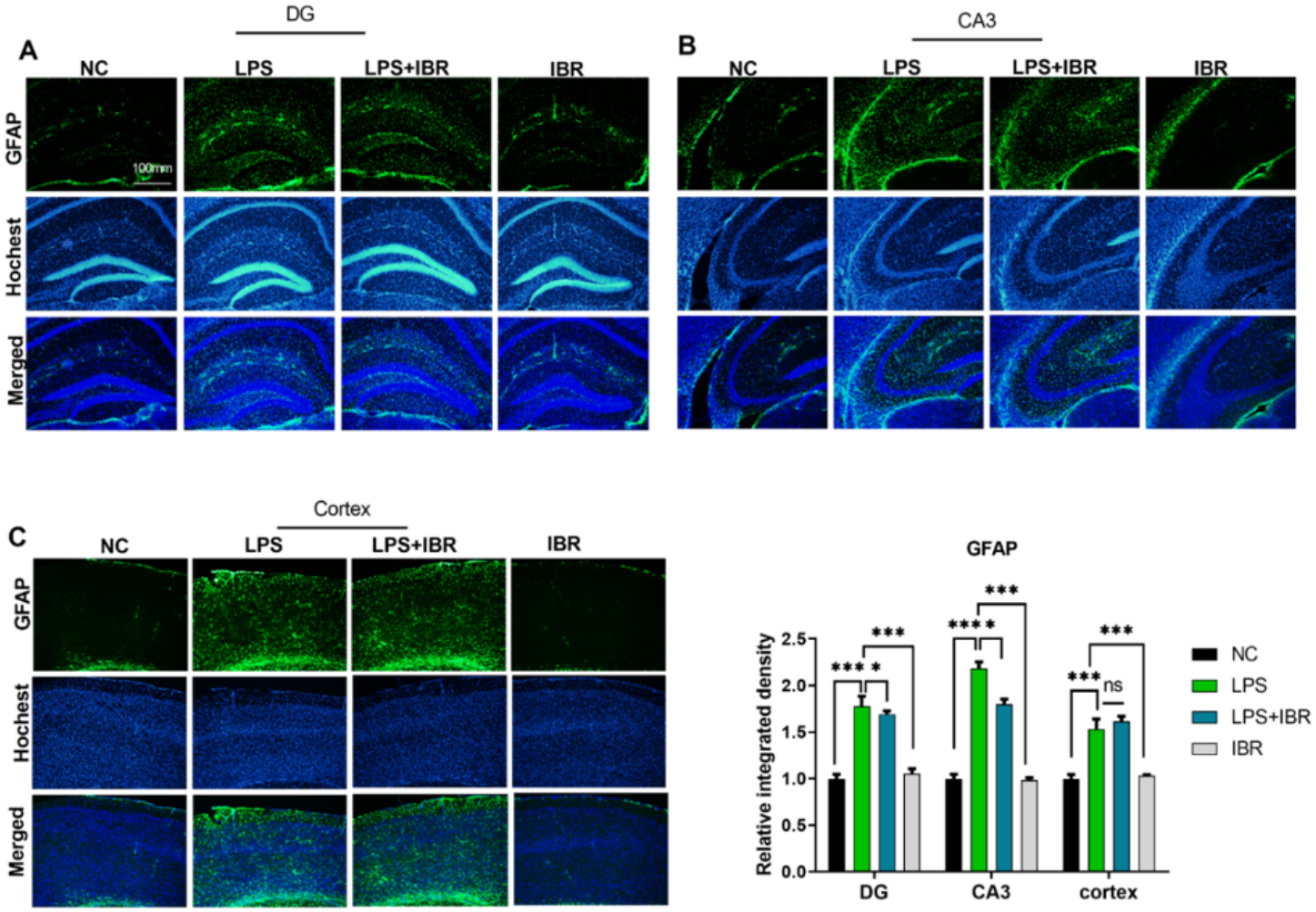
Ibrutinib reduced LPS-induced cytokines level. A: Serum IL-1β and IL-6 expression, B: TNFa and IL-6 level in the hippocampus of the mice, C: Represents immunoblots indicating the expression of p-NF-κB /NF-κB

in the hippocampus of experimental mice. All the values are expressed as mean ±SEM: ANOVA followed by post-hoc analysis. (*): p <0.05, (**): p <0.01), (***): p <0.001



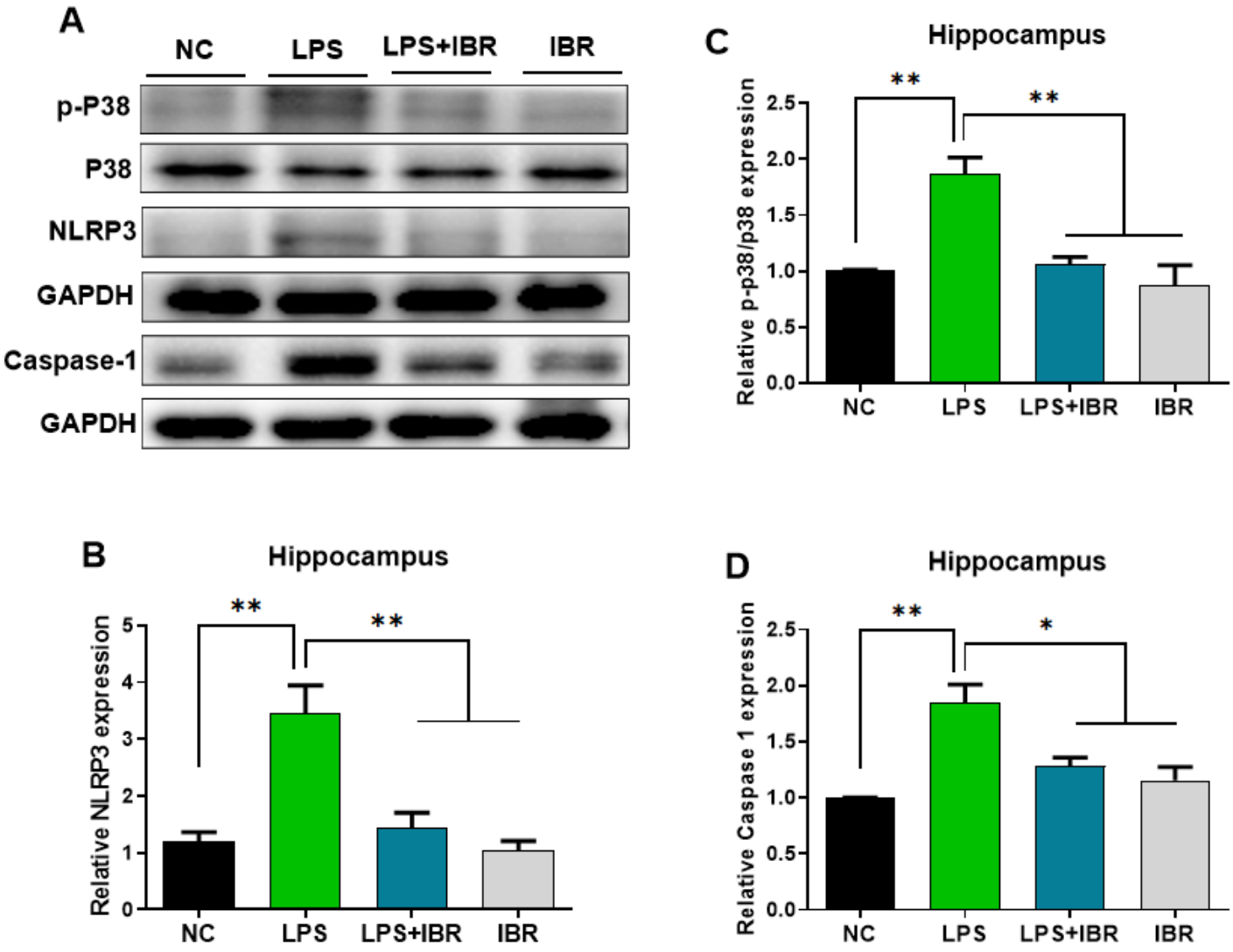

## Figure 5

Ibrutinib alleviated LPS effects on Iba-1 expression. A, B, and C: Representative immunofluorescence of Iba-1 in the hippocampus, D: Column graphs representing the Iba-1 expression. (*): p <0.05, (**): p <0.01), (***): p <0.001



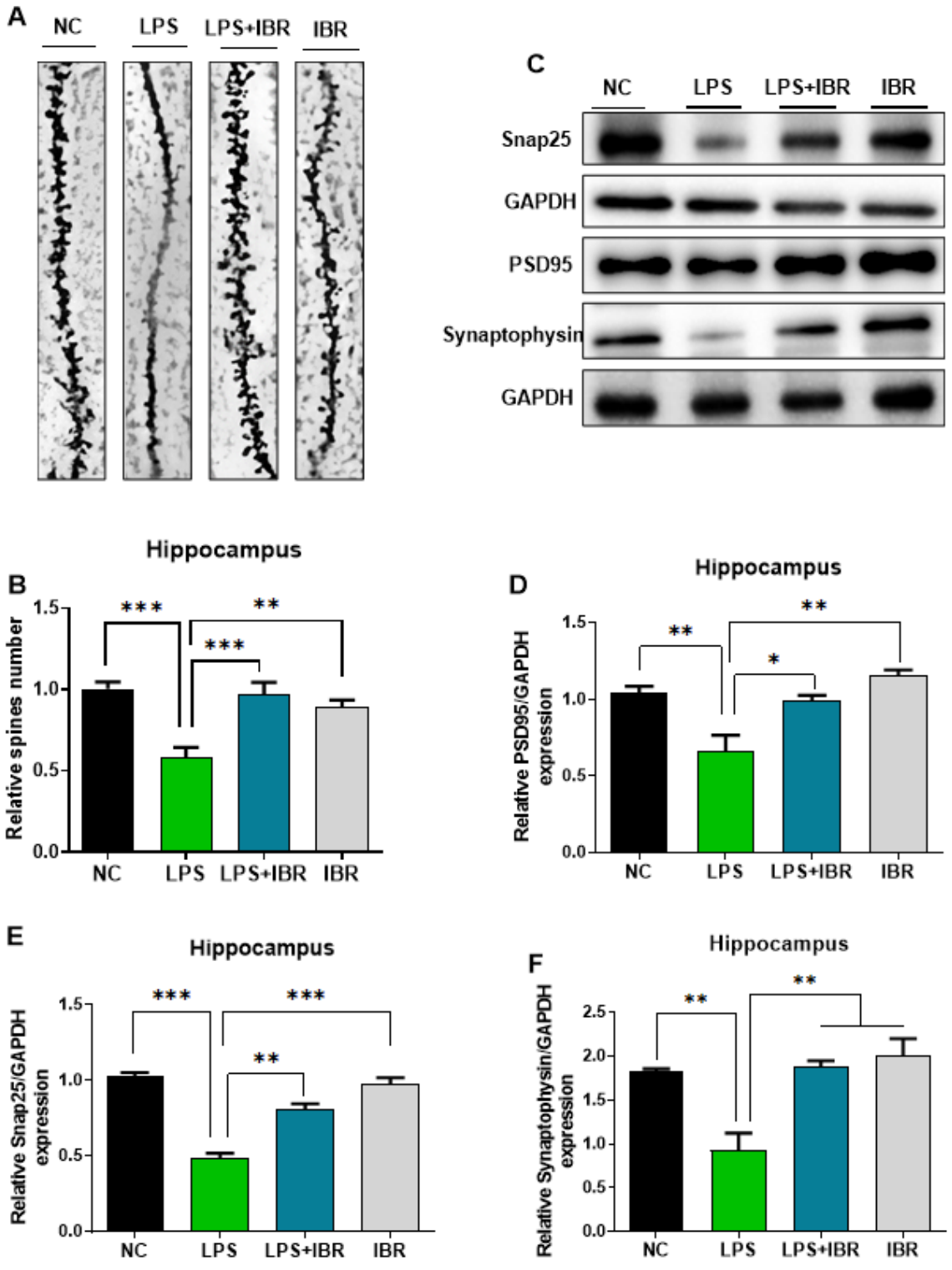

## Figure 6

Ibrutinib attenuated LPS effects on GFAP expression. A, B, and C: Representative immunofluorescence of GFAP in the hippocampus, D: Column graphs representing the GFAP expression. (*): p <0.05, (**): p <0.01), (***): p <0.001




## Figure 7

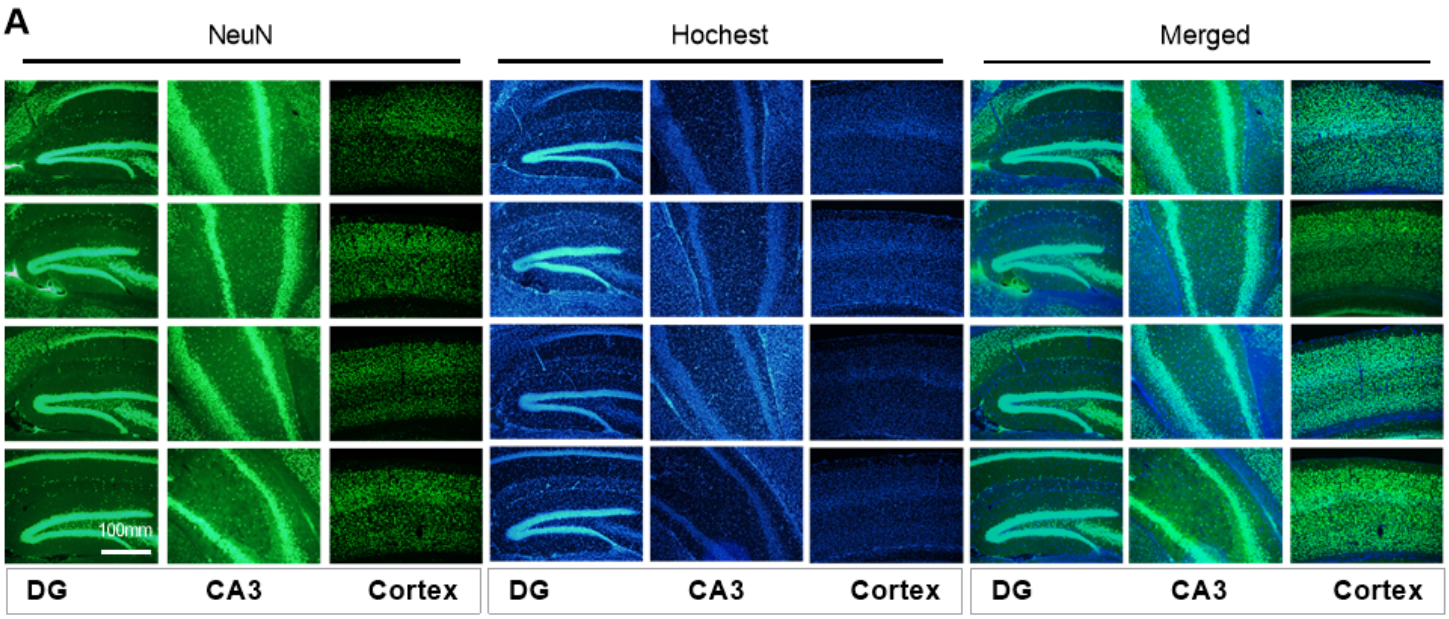
Ibrutinib reduced LPS effects on inflammasome activation. A: Representative blots show P38, NLRP3, Caspase-1 expression. C: representing p-p38 relative expression, D: shows NLRP3 expression in the hippocampus of the animal model. D: Quantitative analysis of Caspase-1. Quantified results were normalized to GAPDH. All the values are expressed as mean ±SEM: ANOVA followed by post-hoc analysis. (*): p <0.05, (**): p <0.01), (***): p <0.001

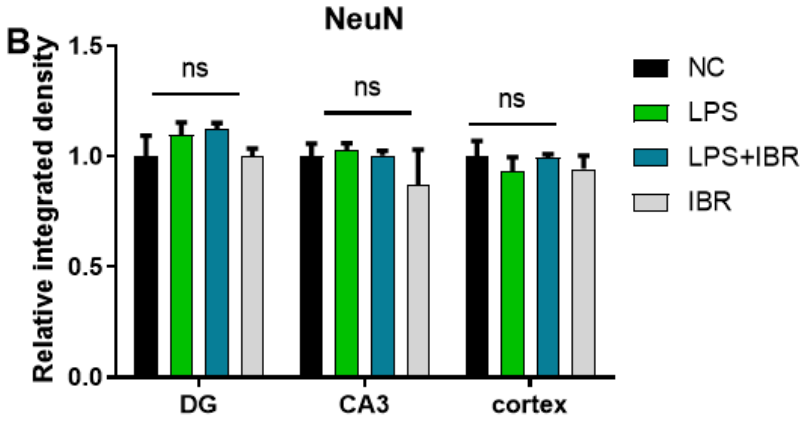



## Figure 8

Ibrutinib enhanced LPS-reduced spin density and synaptogenesis gene expression. A: Micrograph of Golgi staining shows apical dendrites from the hippocampus. B: Column graphs representing relative spin numbers, C: Representative blots show snap25, PSD95, and Synaptophysin expression. D, E, and F: Represents expressional analysis of PSD95, snap, and Synaptophysin in the hippocampus of the

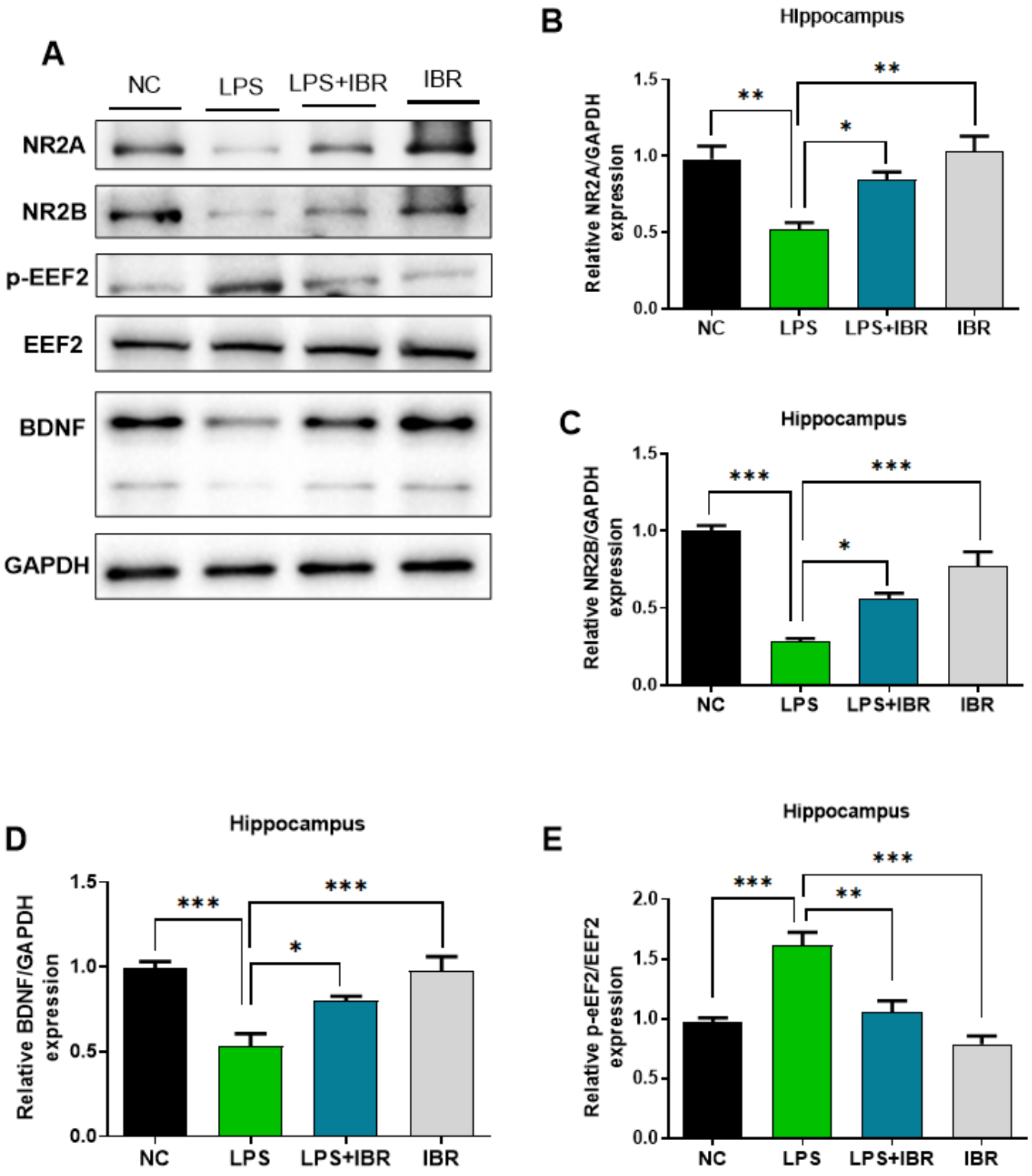
experimental mice. Quantified results were normalized to GAPDH. All the values are expressed as mean ±SEM: ANOVA followed by post-hoc analysis. (*): p <0.05, (**): p <0.01), (***): p <0.001




## Figure 9

LPS effects on NeuN expression. A: Representative immunofluorescence of NeuN in the hippocampus, D: Column graphs representing the NeuN expression. (*): p <0.05, (**): p <0.01), (***): p <0.001




## Figure 10

Ibrutinib abolished LPS effects on NR2A, NR2B, p-eEF2, and BDNF. A: Representative blots show NR2A, NR2B, and BDNF expression. B: representing NR2A relative expression, C: shows NR2B relative expression. D: Quantitative analysis of BDNF, E: Shows p-eEF2 relative expression. Quantified results were normalized to GAPDH. All the values are expressed as mean ±SEM: ANOVA followed by post-hoc

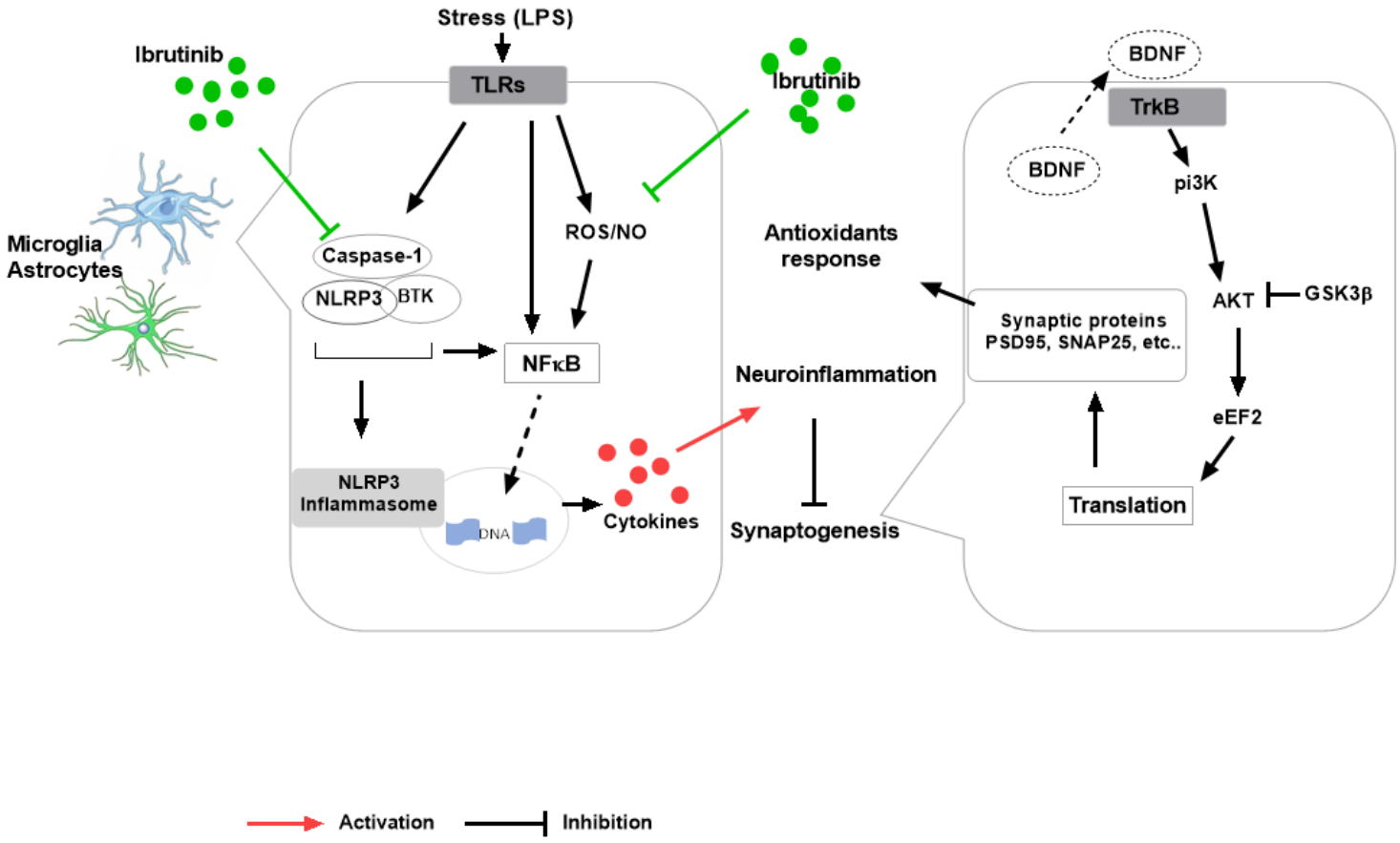



**Figure 11**

Diagram illustrating the action mechanism of ibrutinib by which it regulates neuroinflammatory and synaptogenesis molecules.

## Supplementary Files

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