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Activation of Sirt6 by icariside Ⅱ alleviates depressive behaviors in mice with poststroke depression by modulating microbiota-gut-brain axis

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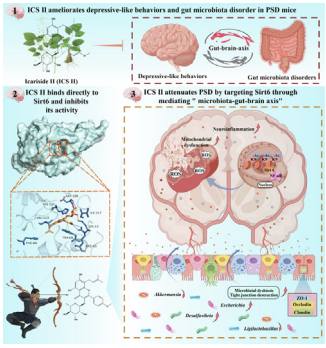
HIGHLIGHTS

- ICS Ⅱ protects against depressive-like behaviors induced by poststroke depression.
- ICS Ⅱ directly binds to Sirt6, suppressing oxidative stress and inflammation poststroke depression.
- ICS Ⅱ alleviates gut microbiota dysbiosis and enhances the production of gut microbial metabolites.
- ICS Ⅱ as a novel Sirt6 activator to overcome poststroke depression through modulation of the microbiota-gut-brain axis.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Sirt6-mediated gut microbiota plays a vital role in poststroke depression (PSD). Icariside Ⅱ (ICS Ⅱ) is a naturally-occurring neuroprotectant with Sirt6 induction potency. However, it is unknown whether ICS Ⅱ protects against PSD through modulation of gut microbiota.
Objective: This study aimed to reveal the effect and potential mechanisms of ICS Ⅱ on PSD, and the role of the microbiota-gut-brain axis was investigated.
Methods: Using middle cerebral artery occlusion (MCAO) and chronic unpredictable mild stress (CUMS) to establish post-stroke depression (PSD) mice, we assessed anti-depressant effects of ICS Ⅱ *via* behavioral tests, immunohistochemistry, and western blot. Transcriptome profiling, molecular docking, and surface plasmon resonance were used to identify key targets. 16S rDNA genomic-derived taxonomic profiling and fecal microbiota transplantation (FMT) were conducted to figure out the mechanistic role of the gut microbiota and short-chain fatty acids (SCFAs).
Results: ICS Ⅱ ameliorated depressive-like behaviors in PSD mice as evidenced by sucrose preference test, forced swimming test and tail suspension test. ICS Ⅱ restored mitochondrial function, reduced oxidative damage and pro-inflammatory cytokines both in brain and intestine through regulation of Sirt6/NF-κB pathway. ICS Ⅱ significantly increased the abundance of gut microbiota (such as Akkermansia and Ligilactobacillus), enhanced SCFAs concentrations, repaired intestinal barrier integrity and upregulated the tight junction protein expression. FMT from ICS II-treated mice replicated these benefits, confirming gut microbiota's role. Mechanistically, ICS Ⅱ directly bound to Sirt6 and enhanced its activity. However, ICS Ⅱ-mediated neuroprotection was neutralized in PSD mice or hydrogen peroxide-induced enteric glial cells when Sirt6 was absent.

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Conclusion: Our findings expand the pharmacological properties of ICS II by demonstrating its ability to ameliorate PSD through modulation of the microbiota-gut-brain axis. ICS Ⅱ, as a novel Sirt6 activator, could be translated into an alternative microbiota-targeted avenue for coping with PSD.

Introduction

Stroke is one of the foremost causes of grievous long-term physical disability and mortality worldwide [1]. Poststroke depression (PSD) is a prevalent and detrimental complication of stroke, which develops in almost a third of stroke survivors [2]. PSD is characterized by continuous anhedonia and despair, and contributes to pernicious consequences, including functional outcome, quality of life, recurrence rate and mortality [3]. Although antidepressants have demonstrated efficacy in relieving depressive symptoms in PSD patients, a battery of inevitable drawbacks are involved in delayed onset, severe adverse effects, drug-resistance and noncompliance in patients with PSD applying currently available drugs, which are a leading predicament in this psychiatric disorder [4]. There is a critical need for the development of more effective therapeutic interventions targeting PSD, particularly those capable of precisely addressing depressive-like syndromes associated with this neurological condition.

One significant gap in PSD treatment lies in the lack of targeted modulation of underlying biological pathways that contribute to disease pathogenesis, particularly those involving gut microbiota dysbiosis and oxidative stress. Accumulating evidence has identified that microbiota-gut-brain axis, a bidirectional coordinated interaction between the intestinal microbiota and brain, plays the crucial role in the PSD [5,6]. Preclinical trial findings have also revealed that the aberrant gut microbiome composition contributes to depression-like symptoms in rodents after PSD insult. Previous studies also demonstrate that PSD patients have abnormal digestive tract function and suffer disrupted gut microbial community and metabolites [7]. In addition, oxidative stress is considered as one of the crucial causes of cell damage in PSD, and probably associated with therapeutic response to pleiotropic antidepressants with anti-oxidation effect [8]. Besides the brain, intestine is also a tissue site of dysfunction affected by oxidative stress, and the intestinal barrier, gut microbiota composition and gut-brain axis communication were altered by oxidative stress in neurological disorders including PSD [9]. Thus, it is promising to develop new redox-based treatment that could defeat PSD by remodeling gut microbiota. Recent evidence support that sirtuin 6 (Sirt6), one of the mammalian homologues of Sir2 histone nicotinamide adenine dinucleotide $(NAD)^+$-dependent deacylase family, protects against oxidative stress-induced DNA damage and is involved in regulation of redox equilibrium [10]. Sirt6 is abundantly expressed in the cortex, and considered as a profitable mediator of stroke outcome [11,12]. Earlier studies found that Sirt6 knockout mice showed aggravated neurological dysfunction and increased cerebral infarct volume after middle cerebral artery occlusion (MCAO)-induced ischemic stroke mouse model. To the contrary, Sirt6 overexpression reduced infarct volume and ameliorated neurological impairment [13]. Intriguingly, a recent study reveals that the gut microbiota and Sirt6 interacts with each other to orchestrate the alteration of neurological disorders [14]. Under this scenario, we hypothesize that targeting Sirt6-mediated gut microbiome has vigoroso potency for the treatment of PSD by hindering the gut microbiota dysbiosis and oxidative stress.

Icariside Ⅱ (ICS Ⅱ) is a naturally-occurring bioactive flavonoids derived from traditional Chinese medicine Herba *Epimedii* [15]. Compared with another major active ingredient icariin of Herba *Epimedii*, ICS Ⅱ is not only absorbed more quickly, but also is the intestinal metabolite of icariin when oral administration. Emerging evidence confirms that ICS Ⅱ exerts pleiotropic neuroprotective effects with antioxidant and anti-inflammatory activities and a favorable safety profile [16]. Our previous studies reports that ICS II could reduce oxidative damage by increasing Sirt6 protein expression in acute liver injury mice [17], and it effectively protects against MCAO-induced ischemic stroke [18,19]. However, the therapeutic potential of ICS Ⅱ for alleviating depressive-like behaviors in PSD requires further validation. Concurrently, the mechanistic contribution of Sirt6-dependent gut microbiota modulation to ICS Ⅱ's antidepressant activity remains to be systematically investigated. In addition, chronic unpredictable mild stress (CUMS) combined with isolation after MCAO model is a typical animal model to mimic PSD, due to it induces a series of pathological and behavioral changes such as neuronal damage, anhedonia and psychosomatic symptoms [20,21]. Thus, we investigated the antidepressant effect of ICS Ⅱ in a PSD mouse model induced by MCAO combined with CUMS, and explored the intricate underlying mechanisms through which ICS Ⅱ exerts its neuroprotective effects on PSD. We found that ICS Ⅱ alleviated depressive-like behaviors and mitochondrial dysfunction and oxidative stress by targeting Sirt6 in PSD mouse model. Moreover, we used 16S rDNA gene sequencing and Ultra-high performance liquid chromatography-quadrupole-orbitrap high resolution mass spectrometry (UPLC-Q/Orbitrap/MS) to analyze alterations of gut microbiota and metabolites after the ICS Ⅱ treatment. Finally, fecal microbiota transplant (FMT) was administered with feces from the ICS Ⅱ-treated mice in a cocktail of antibiotics (ABX)-pretreated mice of PSD to validate the neuroprotective effect of ICS Ⅱ-remodeled gut microbiota. Collectively, our findings revealed that ICS Ⅱ evoked robust anti-PSD effect through a microbiota-gut-brain axis.

Materials and methods

Animal model with PSD

The PSD animal model was was established by superimposing chronic unpredictable mild stress (CUMS) on cerebral ischemia, a validated paradigm for assessing both established and novel therapeutic agents targeting neurological and behavioral deficits. The technology roadmap of the present study is shown in Fig. 1A. First, MCAO surgery was carried out to construct focal cerebral ischemia mouse model according to our previous study [18]. In brief, specific pathogen-free (SPF) male C57BL/6J mice were anesthetized *via* inhalation of 3.5 % isoflurane in a N_2O:O_2 (2:1) mixture and maintained in a supine position under sterile condition. Core body temperature was regulated at 37.0 ± 0.5 ℃ using a feedback-controlled heating pad throughout the procedure. MCAO was constructed using inserting a silicone-coated monofilament nylon (spherical diameter 0.20 ± 0.01 mm) into the internal carotid artery to intercept cerebral blood flow to the middle cerebral artery. Subsequently, the filament was withdrawn after 2 h to allow cerebral blood flow reperfusion. Then laser speckle contrast imaging was used to monitor the cerebral blood flow during the surgery. The mice in the sham group underwent the same procedure as mentioned above, including anesthesia and the same surgical procedure, except for inserting the monofilament (MCAO). A successful

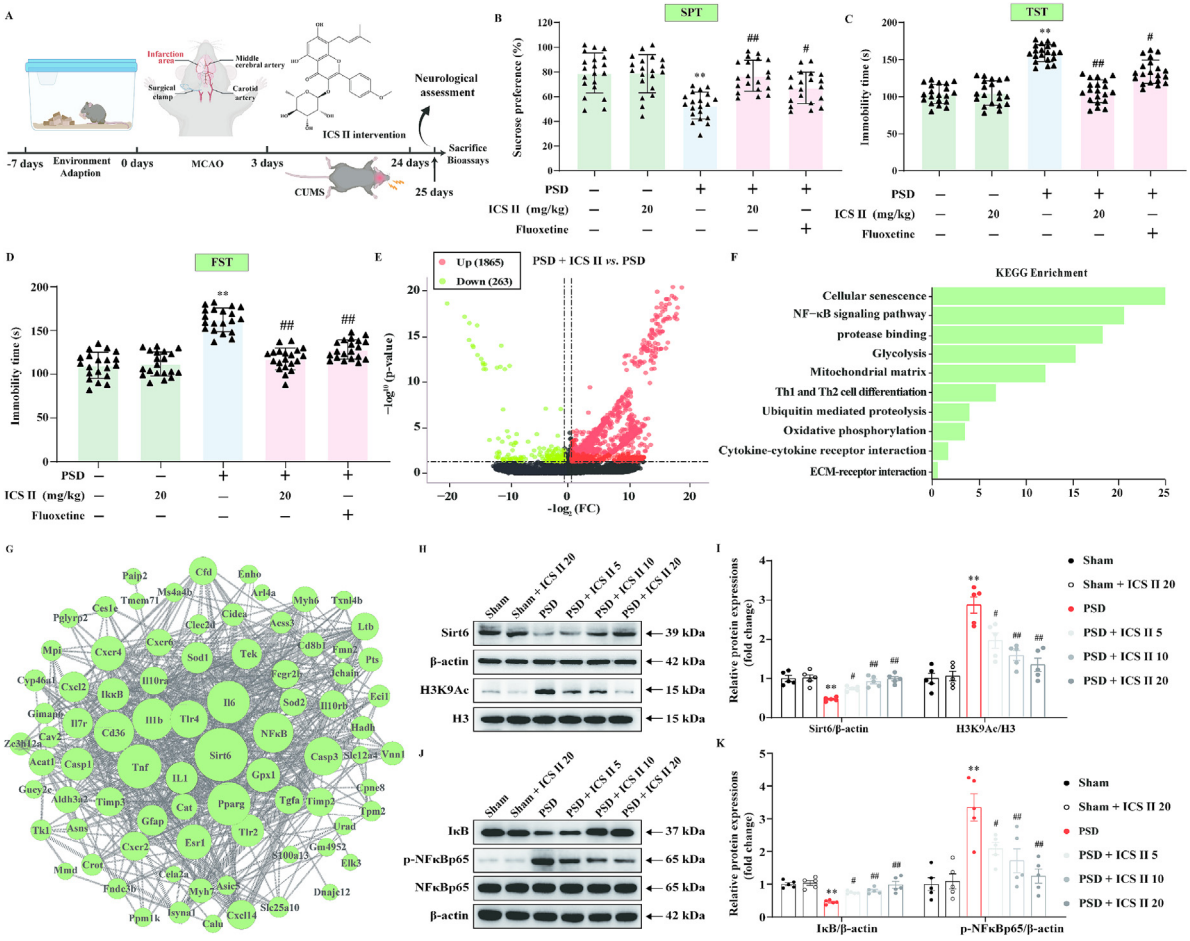

Fig. 1. ICS Ⅱ rescues depressive-like behaviors from PSD *via* Sirt6/NF-κB signaling pathway. Mice were pre-treated with ICS Ⅱ (2.5, 5, 10 and 20 mg/kg) for 7 days before MCAO superimposed with CUMS insult. (A) Schematic illustration on the PSD mouse model establishment and drug treatment. (B) Sucrose preference in SPT (n = 20/group). (C) Immobility time in TST (n = 20/group). (D) Immobility time in FST (n = 20/group). Transcriptome analysis of mPFC tissue by RNA-seq from PSD-treated mice and PSD plus ICS Ⅱ at the dose of 20 mg/kg. (E) The DEGs were identified by volcano plot. (F) Top 10 KEGG pathways were identified using Ingenuity Pathway Analysis. The results of transcriptome analysis were validated using Western blot. (G) PPI network was generated by GeneMANIA analysis. The respective node size presented the degree of linking in PPI network. Larger node Sirt6 shares more connection to others. (H) Representative images of Sirt6 and H3K9Ac. (I) Quantification of Sirt6 and H3K9Ac (n = 5/group). (J) Representative images of IκB and NF-κBp65. (K) Quantification of IκB and NF-κBp65 (n = 5/group). Data are shown as mean ± SD, $^{**}P < 0.01$ *vs.* Sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* PSD group.

MCAO mouse model was established when relative cerebral blood flow (rCBF) decreased less than or equal to 20 % and recovered greater than to 80 % of rCBF baseline. A 5-point scale was used to evaluate neurological function as previously described [19]. Thereafter, neurological function of the mice in MCAO groups were scored and the mice without neurological impairment were excluded (Fig.S1). After two days, the MCAO mice were underwent CUMS to construct the PSD animal model according to the previous study [22]. The procedure of CUMS with a random stressor including food/water deprivation (24 h), cage tilt (45°, 24 h), tail clamping (5 min), damp bedding (4 h), inverted light/dark cycle (24 h), forced suspension (5 min), and cold water swimming (4 ℃, 5 min). The modeling process was continued for 21 days. The Sham group were kept under normal conditions.

Experimental designs

Experiment 1: To evaluate the protective effect of ICS Ⅱ on PSD mouse model, the C57BL/6J mice were randomly allocated into eight experimental cohorts (n = 10/group): Sham group, Sham + ICS Ⅱ (20 mg/kg) group, PSD group, PSD + ICS Ⅱ (2.5 mg/kg), PSD + ICS Ⅱ (5 mg/kg), PSD + ICS Ⅱ (10 mg/kg), PSD + ICS Ⅱ (20 mg/kg), PSD + fluoxetine group. Adult male C57BL/6J mice (6–8 weeks old, weighing 20–25 g) were provided by Cyagen Biosciences Animal Co., Ltd (Suzhou, China; Certificate No. SCXK2021-003). All mice, except those in the Sham group, were subjected to MCAO combined with CUMS. The mice were received daily intragastrically gavage of ICS Ⅱ at doses of 2.5, 5, 10, or 20 mg/kg or fluoxetine (10 mg/kg) for seven days before PSD insult, and the mice of Sham and PSD groups received volume-matched vehicle.

Experiment 2: To verify whether Sirt6 is the potential target of ICS Ⅱ on PSD, the mice were randomly divided into eight groups (n = 10/group): Wild type (WT)-Sham group, WT-PSD group, WT-PSD + ICS Ⅱ group, WT-PSD + MDL800 (allosteric activator of Sirt6) group, Sirt6-deficient ($Sirt6^{-/-}$)-Sham group, $Sirt6^{-/-}$-PSD group, $Sirt6^{-/-}$-PSD + ICS Ⅱ group, $Sirt6^{-/-}$-PSD + MDL800 group. The $Sirt6^{-/-}$ male mice and littermate WT mice (6–8 weeks old, weighing 20–25 g) were provided by Cyagen Biology Co., Ltd. (Suzhou, China, Certificate No. SCXK2021-003). The verification results of $Sirt6^{-/-}$ mice is shown in Fig.S2. All mice, except for the Shams, were subjected to MCAO superimposed with CUMS. The mice were administered intragastrically with ICS Ⅱ at the dose of 20 mg/kg or MDL800 (80 mg/kg) once daily for seven days before PSD insult, and the mice of Sham and PSD groups received volume-matched vehicle.

Experiment 3: To elucidate whether gut microbiota is involved in the antidepressant effect of ICS Ⅱ on PSD, FMT was performed as described previously [23]. The donor mice were achieved with PSD induced by MCAO combined with CUMS. Simultaneously, the donor mice were administrated daily with ICS Ⅱ (20 mg/kg) for a duration of 21 days. At the end of ICS Ⅱ treatment after PSD insult, fresh feces (500 mg) were collected from ICS Ⅱ-treated donor mice, and then homogenized in 2 mL of sterile saline. Thereafter, the mixture was centrifuged at 3000 × g for 5 min under 4 °C to obtain the supernatant, which was used as a transplant material. The mice were randomly divided into four groups (n = 10/group): Sham group, Sham + FMT group, PSD group, PSD + FMT group. The indigenous gut microbiota of the mice was removed by intragastric administration of an ABX for 5 days as described in previous study [24]. The mice of FMT-treated groups were administered with fecal microbiota solution (0.2 mL) from WT donor mice once daily for seven days, then subjected to PSD. While the mice of Sham and PSD groups received volume-matched vehicle. pseudo-germ-free mice model treated with antibiotics were also applied, together with 16S ribosomal DNA (rDNA) genomic-derived taxonomic profiling.

Following MCAO combined with CUMS challenge, the mice were undergoing a series behavioral tests including sucrose preference test (SPT), forced swimming test (FST), tail suspension test (TST). Subsequently, mouse feces were collected under sterile conditions, and then mice were anaesthetized 3.5 % isoflurane in N_2O: O_2 (2:1) for blood collection, and immediately killed by cervical dislocation followed by removal of ileum tissue and the brain, and medial prefrontal cortex (mPFC) were isolated and quickly stored at −80 ℃ for the following experiments.

Statistical analysis

All data were presented as mean ± standard deviation (SD; n ≥ 5 biological replicates). Statistical analyses were performed using SPSS 29.0. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test with where appropriate. Correlation analysis between gut microbiota and short-chain fatty acids (SCFAs) was performed using Pearson correlation analysis. All experiments were performed randomly and blindly, and $P < 0.05$ was considered statistically significant.

Comprehensive methodological details for auxiliary assays, including behavioral tests, RNA-sequencing (RNA-seq), Molecular docking and molecular dynamic (MD) simulation, surface plasmon resonance (SPR) binding assay, microscale thermophoresis (MST) assay, transmission electron microscopy (TEM), analysis of NADPH/$NADP^+$ ratio and adenosine triphosphate (ATP), redox status, pro-inflammatory cytokines levels, lipopolysaccharide (LPS) contents, western blot analysis, 16S rDNA gene sequencing of fecal microflora analysis, haematoxylin and eosin (H&E) staining, immunohistochemistry (IHC), SCFAs concentration analysis were available in the Supplementary Materials.

Results

ICS Ⅱ ameliorates depressive-like behaviors in PSD mice

The antidepressant effect of ICS Ⅱ on PSD was examined using an MCAO model superimposed with CUMS-induced depression in mice. Fluoxetine, a classic antidepressant used clinically for PSD treatment, served as the positive control drug[25]. The results showed that the depressive-like behaviors occurred in PSD mice as evidenced by decreased sucrose preference, increased immobility time in FST and TST compared to the Sham group (Fig. 1B-D), indicating successful PSD mouse model establishment. Administration of ICS Ⅱ (5, 10, 20 mg/kg) or fluoxetine (10 mg/kg) significantly reversed these depressive-like behaviors (Fig. 1B-D, Fig. S3). Notably, ICS Ⅱ at the dose of 2.5 mg/kg showed no therapeutic effect (Fig. S3). while, ICS Ⅱ (20 mg/kg) alone did not alter baseline mouse behaviors (Fig. L). These findings suggest ICS Ⅱ rescues depressive-like behaviors from PSD in a dose-dependent manner, with 20 mg/kg demonstrating optimal efficacy. To investigate the underlying mechanisms, RNA-seq analysis of mPFC tissue revealed 2,128 differentially expressed genes (DEGs) between PSD + ICS Ⅱ (20 mg/kg) and PSD groups, comprising up-regulated 1,865 DEGs, and 263 down-regulated DEGs (Fig. 1E). Gene ontology analysis identified significant enrichment in cellular senescence, oxidative phosphorylation (OXPHOS) and nuclear factor kappa B (NF-κB) signaling pathway (Fig. 1F). Sirt6 emerged as the predominant regulatory gene, accompanied by NF-κB pathway components (Fig. 1G). Western blot validation confirmed,ICS Ⅱ-mediated upregulation of Sirt6 and inhibitor of NF-κB (IκB) protein expression, concurrent with decreased H3K9 acetylation and NF-κBp65 phosphorylation levels (Fig. 1 H-K). These findings indicate that ICS Ⅱ alleviates depressive-like behaviors in PSD through modulation of the Sirt6/NF-κB signaling pathway.

Activation of Sirt6 contributes to the antidepressant effect of ICS Ⅱ on PSD

Based on the above-mentioned results, we hypothesized that Sirt6 was underlying therapeutic target of ICS Ⅱ against PSD. Molecular docking analysis revealed that ICS Ⅱ binds to Sirt6 with a binding energy of −9.008 kcal/mol. The binding mode showed that ICS Ⅱ enters the Sirt6 pocket via three hydrogen bonds with PHE-84 (3.58 Å), VAL-113 (3.76 Å) and TRP-69 (2.88 Å) (Fig. 2A). Free energy landscape (FEL) analysis confirmed the stability of the ICS Ⅱ-Sirt6 complex, as indicated by the compact minimal energy area (blue region) in Gibbs FEL plots (Fig. 2B, C). Furthermore, RMSD and RMSF were conducted by MD trajectories to ascertain the ICS Ⅱ-Sirt6 complex structural stability. The steady RMSD of the protein backbone indicated that ICS Ⅱ-Sirt6 complex displayed a favorable profile of equilibrium (Fig. 2D). The flexibility of different Sirt6 binding sites were assessed by RMSF analysis, and the results showed that most Sirt6-binding residues presented less flexibility with RMSF less than 0.5 Å, validating the stability of the ICS Ⅱ-Sirt6 complex (Fig. 2E). The binding affinity of ICS Ⅱ to Sirt6 was validated by SPR and MST. SPR analysis yielded a dissociation constant (K_D) of 7.568×10^{-8} M (Fig. 2F), while MST shouwed a K_D of 6.06 ± 2.26 μM (Fig. 2G, H). These findings suggested that ICS Ⅱ directly bound to Sirt6. Subsequently, behavioral assays in $Sirt6^{-/-}$ mice revealed exacerbated depressive-like behaviors compared to WT mice. However, the antidepressant effect of ICS Ⅱ and MDL800 were largely counteracted in $Sirt6^{-/-}$ mice (Fig. 2I-K). Mitochondrial ultrastructure analysis in mPFC showed that ICS Ⅱ rescued PSD-induced mitochondrial swelling and cristae disorganization in WT mice but had no effect in $Sirt6^{-/-}$ mice (Fig. S4A-C). ICS Ⅱ increased mitochondrial NADPH/$NADP^+$ ratio and ATP levels in WT mice post-PSD. but these effects were absent in $Sirt6^{-/-}$ mice (Fig. S4D, E). Additionally, ICS Ⅱ reduced mitochondrial reactive oxygen species (ROS) and enhanced antioxidant capacity in WT mice by elevating superoxide dismutase (SOD) 2 and glutathione peroxidase (GPx) 1 activities and glutathione (GSH)/oxidized glutathione (GSSG) ratio, whereas no changes were observed in $Sirt6^{-/-}$ mice (Fig. S4F-I). Pro-inflammatory cytokines including interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α and IL-18 were suppressed by ICS Ⅱ in WT mice but not in $Sirt6^{-/-}$ mice (Fig. S4J-M). These results reveal that Sirt6 activation is essential for the antidepressant effects of ICS Ⅱ in PSD, positioning ICS Ⅱ as a natural Sirt6 activator with therapeutic potential.

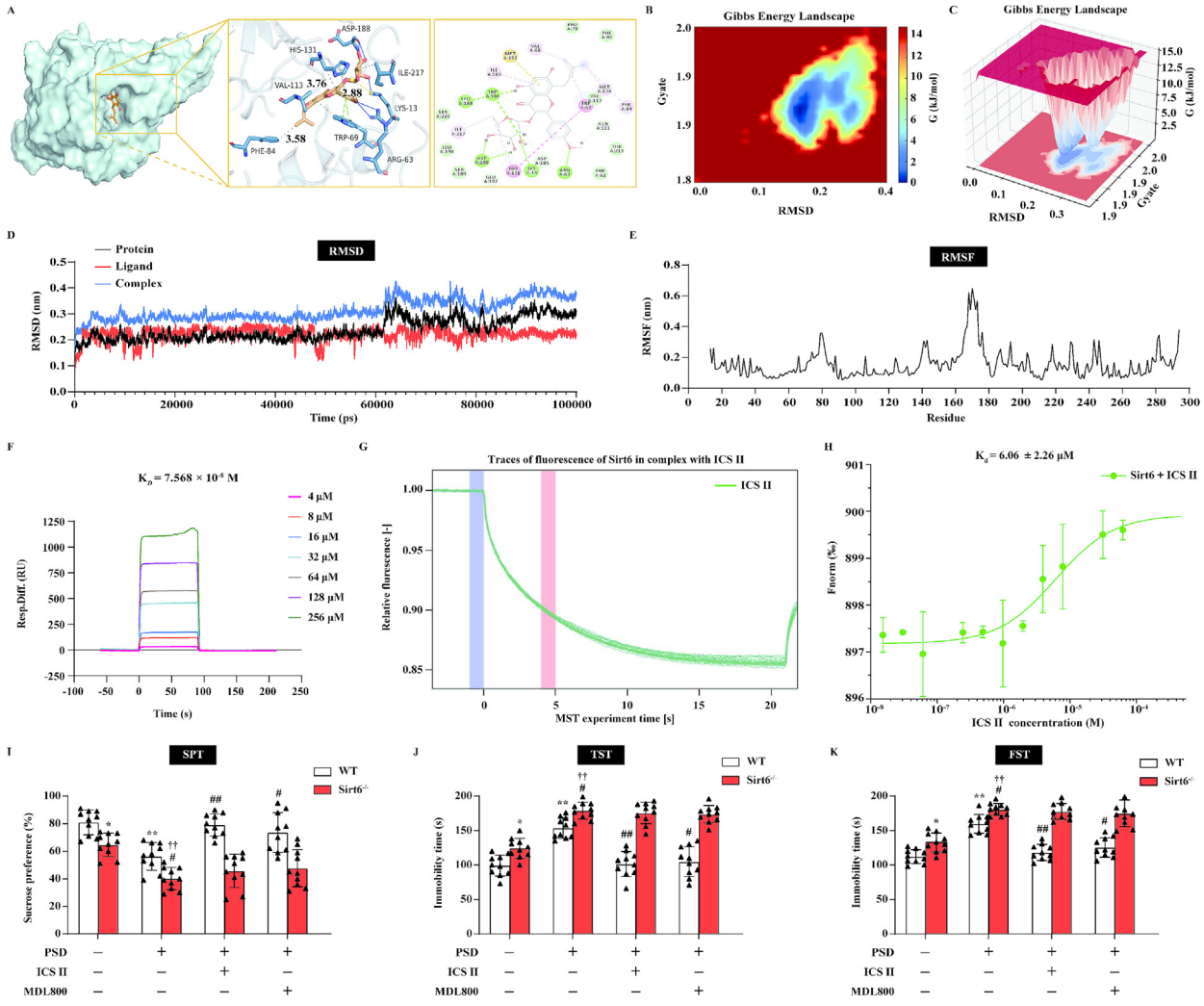

Fig. 2. Activation of Sirt6 contributes to the antidepressant effect of ICS Ⅱ on PSD. (A) ICS Ⅱ bound with Sirt6. Crystal structure of ICS Ⅱ (yellow) displaying Sirt6 (green) bound to the docking pocket. H-bond (grey): PHE-84 (3.58 Å), VAL-113 (3.76 Å) and TRP-69 (2.88 Å). (B) 2D Gibbs energy landscape profile of ICS Ⅱ-Sirt6 complex. (C) 3D Gibbs energy landscape profile of ICS Ⅱ-Sirt6 complex. (D) The RMSD values of the ICS Ⅱ-Sirt6 complex. (E) RMSF values of residues of the whole protein in the ICS Ⅱ-Sirt6 complex. (F) The binding affinity between ICS Ⅱ and Sirt6 was confirmed by SPR. (G) The procession of MST with relative fluorescence time curve. (H) Dissociation curve of ICS Ⅱ and Sirt6 protein. WT or Sirt6$^{-/-}$ mice were pre-treated with ICS Ⅱ (20 mg/kg) for 7 days before MCAO superimposed with CUMS insult. (I) Sucrose preference in SPT (n = 10). (J) Immobility time in TST (n = 10). (K) Immobility time in FST (n = 10). Data are shown as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* Sham group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* PSD group. $^{\dagger\dagger}P < 0.01$ *vs.* Sirt6$^{-/-}$-Sham group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ICS Ⅱ exerts antidepressant effect against PSD through remodeling gut microbiota composition

To explore the effect of ICS Ⅱ, which can ameliorate depressive-like behaviors, on gut microbiota, we performed 16S rDNA sequencing to analyze microbial composition in fecal samples from mice. α-diversity analysis showed no significant differences among Sham, PSD and ICS Ⅱ-treated groups, as indicated by Chao1, Simpson and Shannon indices (Fig. S5). However, the beta diversity analysis revealed significant separation between groups in principal coordinate analysis (PCoA), suggesting distinct gut microbiota profiles accross the treatment groups (Fig. 3A). At the phylum level, the Firmicutes/Bacteroidetes ratio was elevated in PSD mice compared to Sham controls, whereas ICS Ⅱ treatment significantly reversed this increase (Fig. 3B-D). At genus level, PSD mice exhibited increased abundance of Firmicutes-related genera (e.g. *Escherichia* and *Desulfovibrio*) and decreased Bacteroidetes-associated taxa (e.g. *Ligilactobacillus* and *Akkermansia*), which were normalized by ICS Ⅱ intervention (Fig. 3E-H). Histopathological analysis demonstrated ileal tissue damage in PSD mice compared to the Sham group, whereas ICS Ⅱ mitigated this damage (Fig. 3I). Ultrastructural evaluation revealed disrupted tight junction (TJ) architecture, abnormal microvilli morphology, and mitochondrial injury in PSD mice, all of which were ameliorated by ICS Ⅱ treatment (Fig. 3J). Consistently, Western blot analysis showed that ICS Ⅱ upregulated TJ protein expression (claudin, occludin, and ZO-1) in the ileum of PSD mice (Fig. 3K). Notably, Sirt6 protein expression was reduced and H3K9Ac levels were elevated in the ileum of PSD mice, mirroring changes observed in the mPFC. ICS Ⅱ restored Sirt6 expression and suppressed H3K9Ac hyperacetylation (Fig. 3L). Furthermore, ICS Ⅱ increased the intestinal NADPH/NADP ratio (Fig. 3M) and ATP levels (Fig. 3N), while reducing mitochondrial ROS (Fig. 3O), enhancing SOD2 (Fig. 3P) and GPx1 (Fig. 3Q) activities, and elevating the GSH/GSSG ratio (Fig. 3R).

Additionally, ICS Ⅱ lowered lipopolysaccharide (LPS) levels in both ileum (Fig. 3S) and serum (Fig. 3T), and decreased pro-inflammatory cytokines including IL-1β (Fig. 3U), IL-6 (Fig. 3V), TNF-α (Fig. 3W), and IL-18 (Fig. 3X). These findings suggest that ICS Ⅱ alleviates PSD-induced depressive-like behaviors, at least partially, by restoring gut microbiota homeostasis and attenuating oxidative stress and inflammation. These findings suggest that ICS Ⅱ alleviates PSD-induced depressive-like behaviors, at least

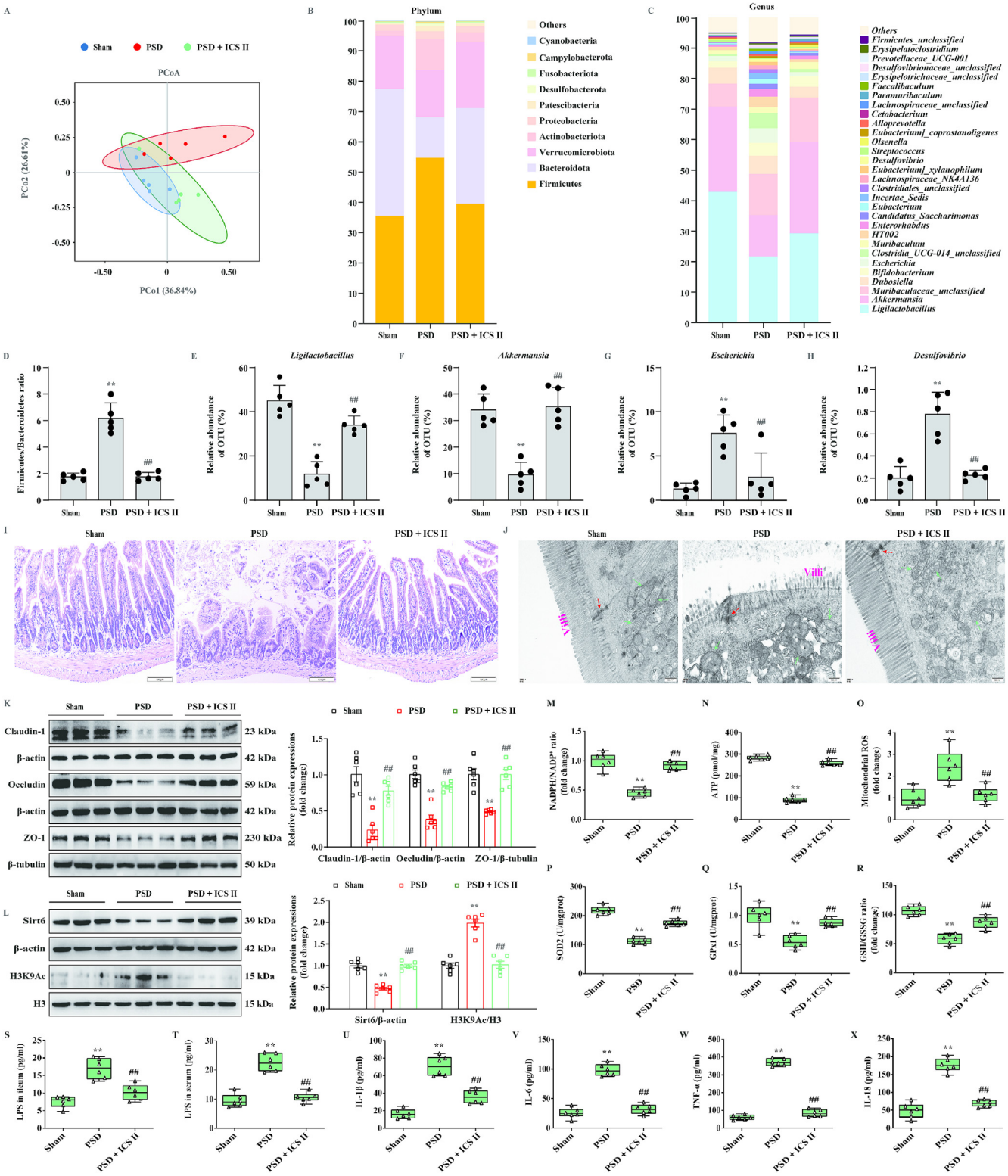

Fig. 3. ICS Ⅱ exerts antidepressant effect from PSD through modulating gut microbiota. (A) PCoA plot of beta diversity analysis. Percentages of variance elucidated using first two axes explain were provided, accounting for 36.84 % and 26.61 %, respectively. (B) Relative enrichment of gut microbiota at the phylum level. (C) Relative enrichment of gut microbiota at the genus level. (D) The ratio of *Firmicutes*/*Bacteroidetes* (n = 5/group). (E) *Ligilactobacillus* level (n = 5/group). (F) *Akkermansia* level. (G) *Escherichia* level (n = 5/group). (H) *Desulfovibrio* level. (I) Representative images of ileum in mice observed using H&E staining. Magnification 100 ×; bar scale = 100 μm. (J) TJ proteins injury (red arrow), villi loss (pink marker) and mitochondria (green marker) were detected using TEM. Bar scale = 500 nm. (K) Representative western blot images and quantification of claudin-1, occludin and ZO-1 proteins expression (n = 6/group). (L) Representative western blot images and quantification of Sirt6 protein expression and H3K9Ac protein level. (M) NADPH/NADP$^+$ ratio (n = 6/group). (N) ATP (n = 6/group). (O) Mitochondrial ROS production (n = 6/group). (P) SOD2 activity (n = 6/group). (Q) GPx1 activity (n = 6/group). (R) GSH/GSSG ratio (n = 6/group). (S) LPS in ileum (n = 6/group). (T) LPS in serum (n = 6/group). (U) IL-1β (n = 6/group). (V) IL-6 (n = 6/group). (W) TNF-α (n = 6/group). (X) IL-18 (n = 6/group). Data are shown as mean ± SD. $^{**}P < 0.01$ *vs*. Sham group, $^{\#\#}P < 0.01$ *vs*. PSD group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

partially, by restoring gut microbiota homeostasis and attenuating oxidative stress and inflammation.

The protective effect of ICS Ⅱ on hydrogen peroxide (H_2O_2)-induced injury in enteric glial cells (EGCs) is dependent on Sirt6

To investigate the role of Sirt6-mediated mitochondrial oxidative stress in the antidepressant effect of ICS Ⅱ on PSD, an *in vitro* model of H_2O_2-induced EGCs injury was used to mimic intestinal oxidative stress. We found that ICS Ⅱ (2, 4, 8 μM), NAC or MDL800 significantly attenuated H_2O_2-induced EGCs injury, as evidenced by MTT assay and LDH release (Fig. 4A, B). Of note, the protective effect of ICS Ⅱ (8 μM) against H_2O_2-induced EGCs injury was more pronounced than those of NAC and MDL800 (Fig. 4A, B). Sirt6 deficiency exacerbated EGCs injury compared to WT cells upon H_2O_2 exposure. However, the protective effect of ICS Ⅱ on EGCs injury was largely offset in the absence of Sirt6 (Fig. 4C, D). Furthermore, mitochondrial parameters including Mito-tracker intensity, NADPH/NADP ratio, mitochondrial DNA (mtDNA) copy number and ATP level were significantly reduced in Sirt6-deficient EGCs after H_2O_2 challenge, whereas mitochondrial ROS production and 8-hydroxy-2′-deoxyguanosine (8-OHdG) content were elevated compared to WT cells (Fig. 4E-K). H_2O_2 treatment increased in pro-inflammatory cytokine levels (IL-1β, IL-6 and TNF-α), indicating oxidative stress-induced intestinal cell inflammation. Sirt6 deficiency further aggravated this H_2O_2-triggered inflammatory on oxidative stress-related intestinal inflammation were nearly abolished in Sirt6-deficient EGCs. Importantly, the anti-inflammatory effects of ICS Ⅱ on oxidative stress-related intestinal cell inflammation were nearly abolished in Sirt6-deficient EGCs (Fig. 4L-N). Additionally, Sirt6 deficiency exacerbated mitochondrial bioenergetics dysfunction, as reflected by reduced oxygen consumption rate (OCR). The salutary effect of ICS Ⅱ on mitochondrial oxidative stress and bioenergetic impairement were also abrogated in Sirt6-deficient cells (Fig. 4O, P). Consistent with the *in vivo* findings, these results validate that Sirt6-mediated mitigation of intestinal mitochondrial oxidative stress and inflammation underlies the antidepressant of ICS Ⅱ on PSD.

ICS Ⅱ increases production of SCFAs in PSD mice

Since SCFAs act as potential regulators of the effects of gut microbiota on intestinal antioxidant ability, we subsequently investigated the effect of ICS Ⅱ-mediated gut microbiota on SCFAs production through targeted metabolomics analysis. PCA results showed distinct separation of SCFAs profiles among Sham, PSD and PSD + ICS Ⅱ groups, suggesting that ICS Ⅱ significantly influenced SCFA levels in PSD mice (Fig. 5A). UPLC-Q/Orbitrap/MS analysis identified reduced valeric acid, propionic acid, isovaleric acid, isobutyric acid, hexanoic acid, butyric acid, acetic acid and 2-Methylbutyric acid in PSD mice (Fig. 5B, C; Fig. S6; Fig. S7). However, ICS Ⅱ treatment increased the concentrations of valeric acid, propionic acid, isovaleric acid, isobutyric acid, hexanoic acid, butyric acid, acetic acid and 2-Methylbutyric acid (Fig. 5D-K). However, ICS Ⅱ increased the concentrations of valeric acid, propionic acid, isobutyric acid, butyric acid, acetic acid and 2-Methylbutyric acid and acetic acid (Fig. 5D-I), but had no effect on isovaleric acid and hexanoic acid levels (Fig. 5J, K). These results suggest that ICS Ⅱ effectively alleviates intestinal oxidative stress and inflammation in PSD mice. To elucidate the correlation between SCFAs levels, gut microbiota, oxidative stress and inflammatory response, we performed Pearson correlation analysis (Fig. 5L). These results demonstrated that ICS Ⅱ effectively enhanced SCFAs production, particularly of acetic acid, propionic acid, and butyric acids. These three SCFAs showed significant positive correlation with the NADPH/NADP ratio (r = 0.74. 0.72 or 0.87), ATP (r = 0.71, 0.80 or 0.87), SOD2 (r = 0.77, 0.88 or 0.88), GSH/GSSG ratio (r = 0.71, 0.83 or 0.77), and GP_X1 (r = 0.70, 0.85 or 0.86), while exhibiting negative correlations with LPS (r = –0,67, –0.70 or –0.80), ROS (r = -0.62, –0.72 or –0.77), and proinflammatory cytokines (TNF-α, IL-6, IL-1β and IL-18) in the ileum. Notably, analogous correlation patterns were observed between these SCFAs and biochemical indicators in the brain. Specifically, *Ligilactobacillus* and *Akkermansia* showed positive correlations with with acetic acid (r = 0.66 or 0.52), propionic acid (r = 0.80 or 0.69), and butyric acid (r = 0.80 or 0.70). Conversely, *Desulfovibrio* was negatively associated with propionic acid (r = –0.52) and butyric acid (r = –0.53), while *Escherichia* displayed negative correlations with both propionic acid (r = –0.76) and butyric acid (r = -0.67). Intriguingly, *Ligilactobacillus* and *Akkermansia* were positively linked to antioxidant indicators, but inversely associated with LPS, ROS, and pro-inflammatory cytokines in both ileal and brain tissues. In contrast, *Desulfovibrio* and *Escherichia* exhibited opposing correlation profiles. These findings suggest that ICS Ⅱ-mediated reshaping of gut microbiota and subsequent enhancement of acetic acid, propionic acid, and butyric acid production are strongly associated with improved oxidative status and attenuated inflammation in both intestinal and cerebral tissues. The evidence indicates that the intestinal antioxidant and anti-inflammatory effects of ICS Ⅱ primarily result from its ability to modulate gut microbiota and thereby promote the biosynthesis of these key SCFAs.

ICS Ⅱ-FMT rescues depressive-like behaviors in PSD mice

To validate the involvement of gut microbiota in ICS Ⅱ-mediated antidepressant effects on PSD, we performed FMT experiments (Fig. 6A). Results demonstrated that ICS Ⅱ-FMT administration significantly alleviated depression-like behaviors induced by PSD, as evidenced by decreased sucrose preference in the SPT, prolonged immobility time in both the FST and TST (Fig. 6B-D). Furthermore, histological analysis revealed that ICS Ⅱ-FMT treatment effectively mitigated PSD-induced intestinal barrier damage, showing preserved TJ architecture and reduced microvilli abnormalities (Fig. 6E, F). At the molecular level, western blot analysis confirmed that ICS Ⅱ-FMT upregulated ileal expression of key TJ proteins including claudin, occludin, and ZO-1 in PSD-challenged mice (Fig. 6G, H). These findings collectively suggest that the antidepressant effects of ICS Ⅱ in PSD may be mediated through gut microbiota modulation, with mechanistic pathways involving both restoration of microbial homeostasis and protection against intestinal barrier dysfunction.

ICS Ⅱ-FMT reduces mitochondrial oxidative stress and inflammatory responses of brain and gut in PSD mice

Electron microscopy analysis revealed pronounced mitochondrial ultrastructural abnormalities in the mPFC of PSD mice, characterized by mitochondrial swelling and cristae disorganization. Notably, ICS Ⅱ-FMT administration effectively ameliorated these pathological alterations (Fig. 7A-C). Biochemical analysis demonstrated that PSD induction significantly reduced both NADPH/$NADP^+$ ratio and ATP levels in both cerebral and intestinal tissues, which were substantially normalized by ICS Ⅱ-FMT treatment (Fig. 7D-G). Furthermore, ICS Ⅱ-FMT intervention significantly attenuated mitochondrial ROS accumulation (Fig. 7H, I) and increased SOD2 activity (Fig. 7J, K), GPx1 activity (Fig. 7L, M) and GSH/GSSG ratio (Fig. 7N, O) in both mPFC and intestinal tissues. Moreover, the LPS elevation was observed across mPFC, serum, ileum and feces of PSD mice, which was significantly attenuated by ICS Ⅱ-FMT (Fig. 7P-S). Immunohistochemical analysis further revealed increased macrophage infiltration (F4/80 positive cells) in the ileal mucosa of PSD mice, a pathological feature that was

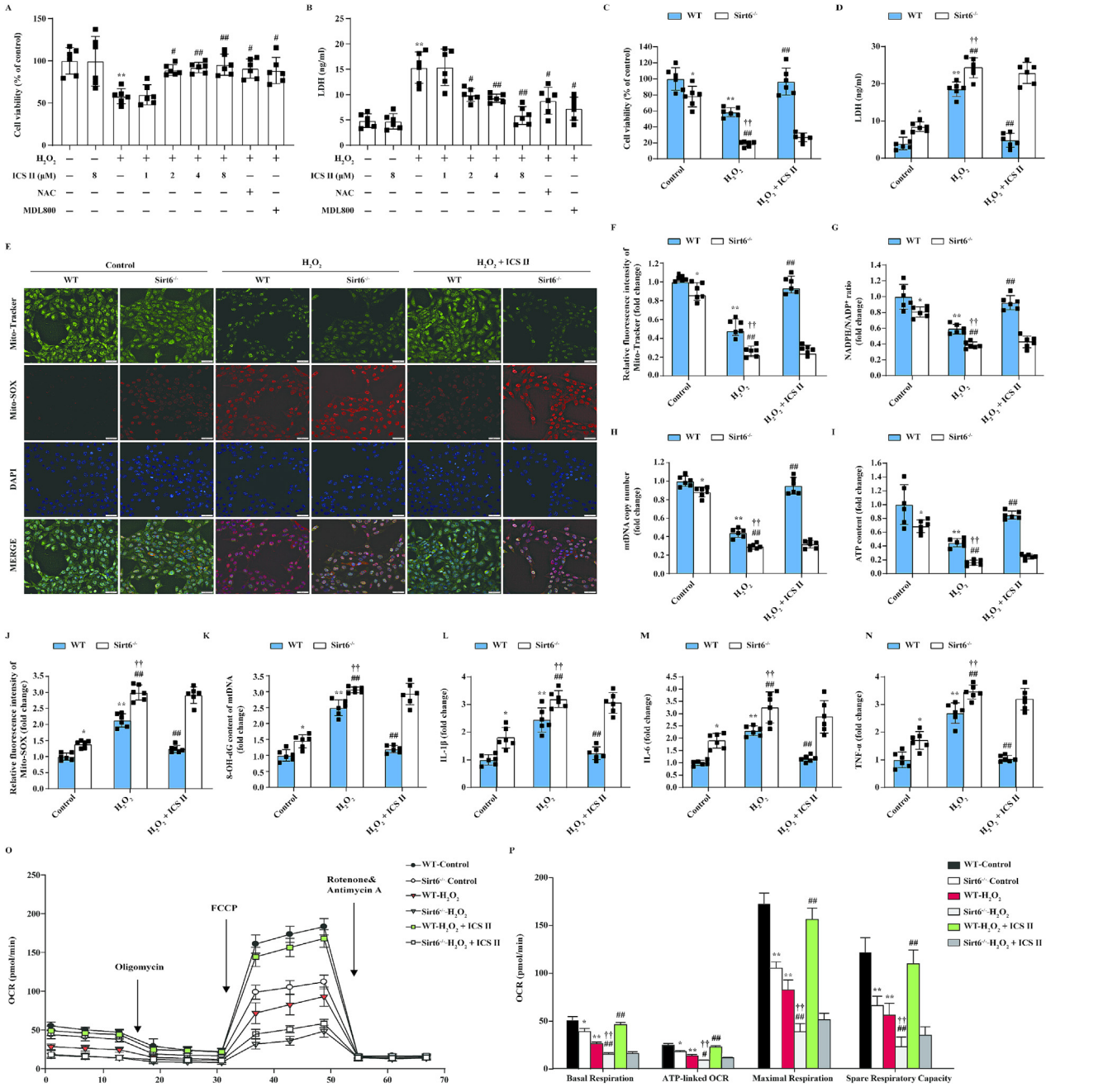

Fig. 4. Role of Sirt6-mediated mitochondrial oxidative stress and inflammation in the beneficial effect of ICS Ⅱ on H_2O_2-induced EGCs injury. The EGCs were pre-treated for 1 h with ICS Ⅱ (1, 2, 4, 8 μM) or NAC (6 mM) or MDL800 (10 μM), and then challenged with H_2O_2 (200 μM) for another 12 h. (A) cell viability. (B) LDH release. Sirt6 knockout EGCs were generated using CRISPR-Cas9 system. The WT or Sirt6 deficiency EGCs were pre-treated for 1 h with ICS Ⅱ (8 μM), and challenged with H_2O_2 (200 μM) for another 12 h. (C) Cell viability. (D) LDH release. (E) Representative images of Mito-tracker and Mito-SOX staining. Magnification 200 ×; bar scale = 20 μm. (F) Quantification of Mito-tracker intensity. (G) NADPH/NADP ratio. (H) mtDNA. (I) ATP content. (J) Quantification of Mito-SOX intensity. (K) 8-OHdG content. (L) IL-1β. (M) IL-6. (N) TNF-α. (O) Representative OCR profile. (P) Quantification of OCR. Data are shown as mean ± SD. n = 6/group. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* Control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* WT-H_2O_2 group. $^{\dagger\dagger}P < 0.01$ *vs.* Sirt6$^{-/-}$-Control group.

effectively reversed following ICS Ⅱ-FMT treatment (Fig. 7T, U). These collective findings indicate that ICS Ⅱ-modulated gut microbiota exerts protective effects against mitochondrial oxidative stress and associated inflammatory responses in both cerebral and intestinal tissues of PSD mice.

Discussion

The principal findings of the present study are: (1) ICS Ⅱ rescues depressive-like behaviors from PSD *via* Sirt6/NF-κB signaling pathway. (2) Structurally, ICS Ⅱ directly binds to Sirt6 to suppress oxidative stress and inflammation both in brain and intestine. (3) ICS Ⅱ restores gut microbiota dysbiosis and promotes the formation of gut microbial metabolites SCFAs. (4) Importantly, ICS Ⅱ-treated gut microbiota also remodels the gut microbiota and effectively equilibrates redox status and inhibits oxidation-induced intestinal inflammation. To our knowledge, this is the first report that Sirt6-mediated microbiota-gut-brain axis contributes to the oxidative stress and inflammation in PSD, and ICS Ⅱ may serve as a novel and naturally-occurring Sirt6 activator to rescue depressive-like behaviors from PSD through modulating microbiota-gut-brain axis.

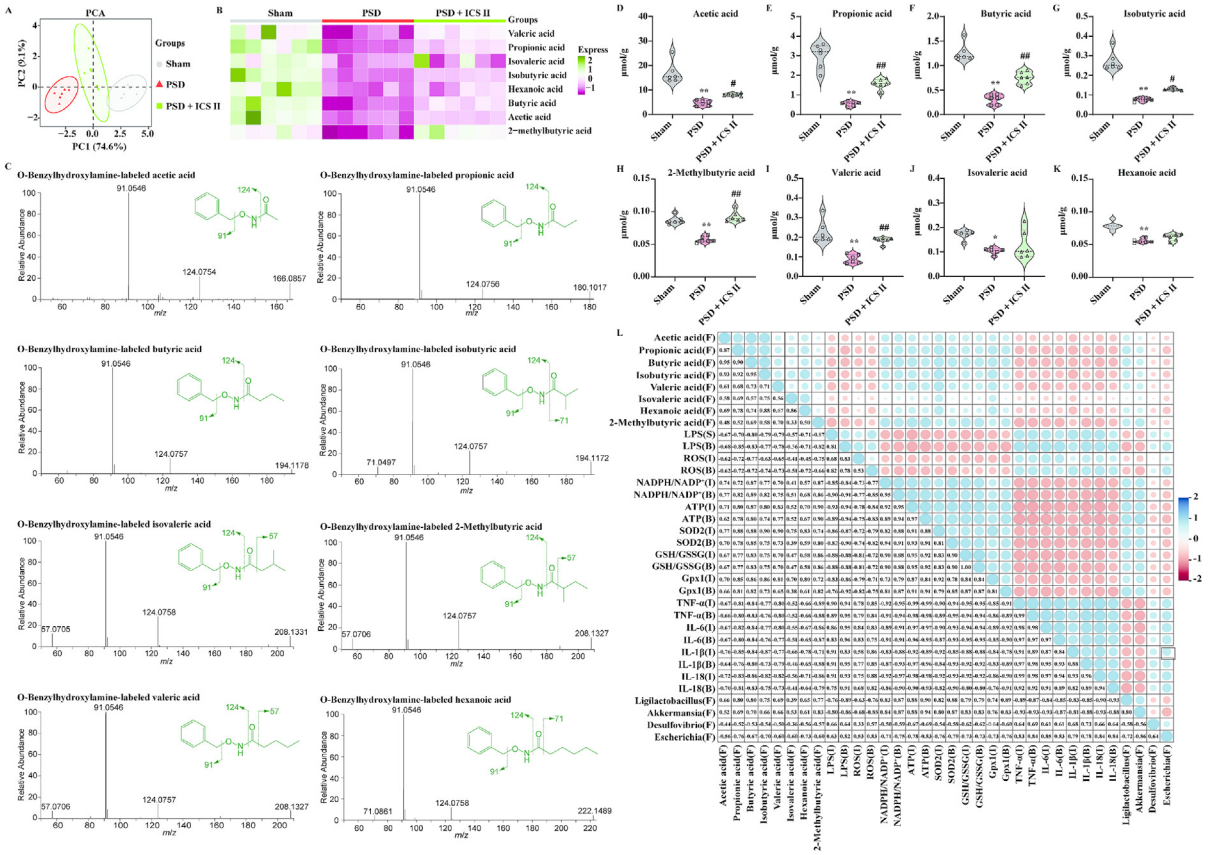

Fig. 5. Effect of ICS Ⅱ on production of SCFAs in PSD mice. (A) PCA plot of SCFAs analysis. (B) The productions of SCFAs were assayed using hierarchical clustering analysis. (C) Product ion mass spectra of SCFAs. Quantification of (D) acetic acid (E) propionic acid (F) butyric acid (G) isobutyric acid (H) 2-Methylbutyric acid (I) valeric acid (J) isovaleric acid and (K) hexanoic acid. (L) Correlation analysis of SCFAs levels in feces, abundance of gut microbiota in feces, and other biochemical index in the ileum, serum and mPCF. In the right of the matrix, the size and color of the circles indicate the degree of correlation. Blue represents a positive correlation, and red represents a negative correlation, and the corresponding value of the correlation index can be found on the right. The parameters for mPCF of brain, serum, ileum and feces were labeled as B, S, I and F, respectively. Data are shown as mean ± SD. n = 6/group. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* Sham group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* PSD group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Oxidative stress is defined as a disequilibrium between the generation of ROS and antioxidants, which results in an interruption of redox status and/or cellular damage [9]. Emerging evidence supports that oxidative stress is considered as a culprit of depressive-like behaviors during PSD [26]. Thus, inhibiting oxidative stress to manage psychiatric disorders is a prospective approach to overcome PSD. Encouragingly, ICS Ⅱ, a botanical substance, has been proved to have robust neuroprotection due to its excellent anti-oxidant effect [18,19,27]. In this study, we clearly demonstrated for the first time that ICS Ⅱ effectively ameliorated depressive-like behaviors with PSD animal model, which discovers a new property of ICS II that might have potential as a clinical agent to treat PSD. Subsequently, data of transcriptomics pinpointed that the DEGs were mainly enriched in cellular response to oxidative stress, OXPHOS, and NF-κB signaling pathways. Of note, there is a potential interaction between the Sirt6 signaling molecules and the DEGs induced by ICS Ⅱ. Earlier studies demonstrate that Sirt6 hinders NF-κB-targeted gene expression by deacetylation of histone H3K9, and expedites negative feedback loop that decreases NF-κB activity by up-regulating the protein expression of IκBα (a vital inhibitor of NF-κB), thereby reducing ROS production and pro-inflammatory cytokines release including TNF-α, IL-1β, and IL-6 [13]. In agreement with transcriptomics results, ICS Ⅱ changed the protein expressions of Sirt6/NF-κB signaling pathway, which suggested that the antidepressant effect of ICS Ⅱ on PSD, at least partly, through mediating Sirt6/NF-κB signaling pathway. Based on aforementioned results, we then explored whether ICS Ⅱ can interact with Sirt6. We found that ICS Ⅱ directly bound to Sirt6, which suggested Sirt6 might be a potential pharmacological target of ICS Ⅱ on PSD. To validate these results, Sirt6 knockout mice and classic Sirt6 activator were used. Consistent with our hypothesis, ICS Ⅱ demonstrated superior efficacy to MDL800 in alleviating PSD symptoms, with its enhanced therapeutic performance being mechanistically linked to concurrent mitigation of mitochondrial oxidative stress and coordinated suppression of pro-inflammatory cytokines. Most importantly, Sirt6 deficiency counteracted the neuroprotective effect of ICS Ⅱ on PSD in mice. These findings validate that ICS Ⅱ rescues depressive-like behaviors from PSD by targeting Sirt6.

Previous studies have paid more attention to the direct protective effect of ICS Ⅱ on neurological disorders, neglecting that the human body is a coordinated and unified whole. Recently, the gut microbiota status has increasingly been focused and is deemed as reasonable outcome of neurological diseases[28]. Concurrently, in view of the low bioavailability of ICS Ⅱ and its capacity to exist and reach the intestine [29], we suppose that ICS Ⅱ protects against depressive-like behaviors from PSD through remodeling the disrupted gut microbiota. As we expected, ICS Ⅱ reshaped gut microbiota composition in mice with PSD, as evidenced by the increase in abundance of Bacteroidetes including *Ligilactobacillus* and *Akkermansia*, and the decrease in abundance of Firmicutes, *Escherichia* and *Desulfovibrio*. Of note, the gut microbiota dysbiosis elicits oxidative stress and damage the intestinal barrier integrity, facilitating the pernicious bacteria or substances translocate into the

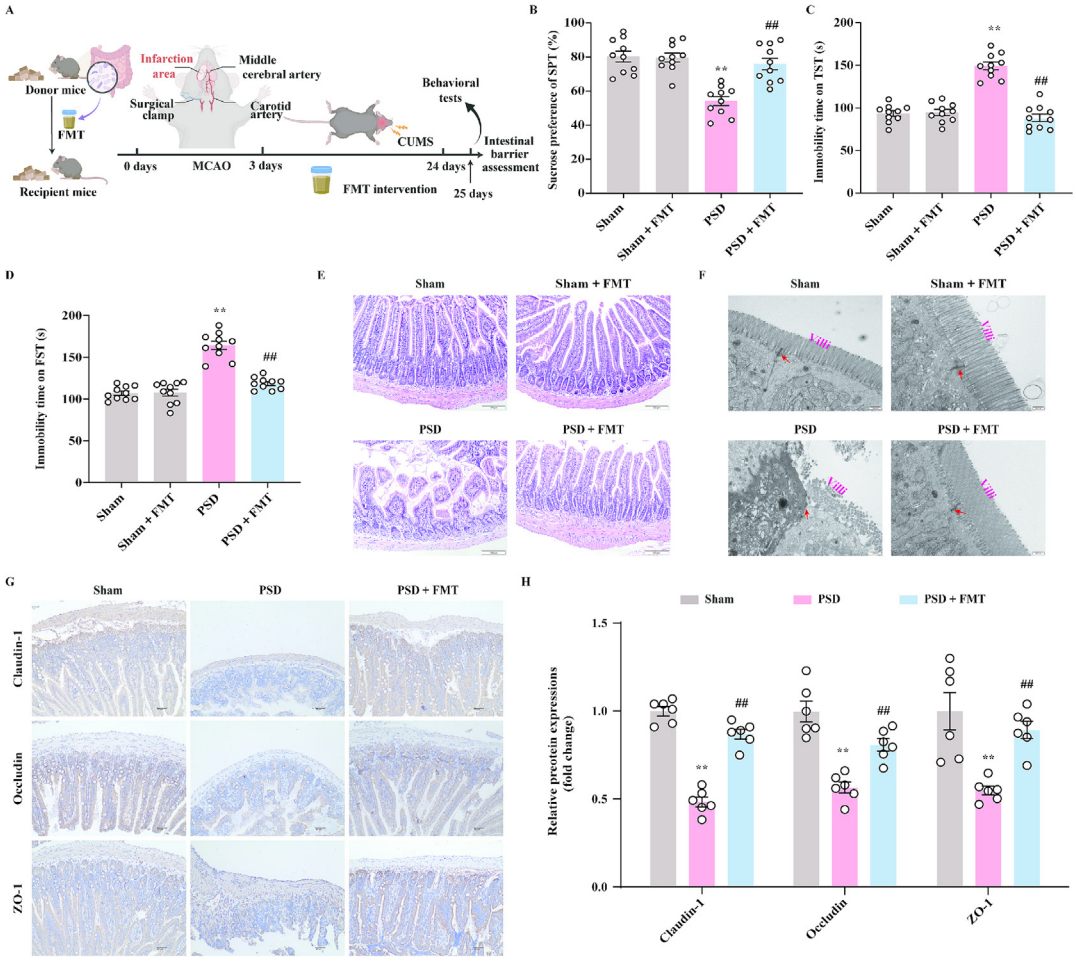

Fig. 6. Effect of ICS Ⅱ-FMT on depressive-like behaviors in PSD mice. Mice were pre-treated with FMT for 7 days before MCAO superimposed with CUMS insult. (A) Schematic illustration on the PSD mouse model treated with or without FMT. (B) Sucrose preference in SPT (n = 5/group). (C) Immobility time in TST (n = 5/group). (D) Immobility time in FST (n = 5/group). (E) Representative images of ileum in mice observed using H&E staining. Magnification 100 ×; bar scale = 100 μm. (F) TJ proteins injury (red arrow) and villi loss (pink marker) were detected using TEM. Bar scale = 500 nm. (G) Representative images of claudin-1, occludin and ZO-1 protein expressions in ileum. (H) Quantification of claudin, occludin and ZO-1 (n = 5/group). Data are shown as mean ± SD, $^{**}P < 0.01$ *vs.* Sham group; $^{\#\#}P < 0.01$ *vs.* PSD group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

blood, and promoting the "waterfall effect" release of pro-inflammatory cytokines [30]. Our results showed that in parallel with gut microbiota dysbiosis, intestinal barrier damage and oxidative stress were observed after challenged by PSD, in keeping with the theory of previous studies [6]. However, ICS Ⅱ significantly attenuated intestinal barrier damage through increasing the TJ protein expressions, which are mainly comprised of claudin, occludin and ZO-1 and maintain the intestinal barrier integrity. In addition, ICS Ⅱ effectively repaired the mitochondrial damage, restored the redox status and reduced oxidation-induced inflammation. To validate the causative role of gut microbiota in the anti-depressive properties of ICS Ⅱ against PSD, the mouse model with experimental PSD with gut microbiota deletion, and FMT from untreated or ICS Ⅱ-treated mice were conducted. We found that ICS Ⅱ-FMT effectively ameliorated the depressive-like behavior and repaired intestinal barrier integrity through increasing TJs protein expressions. ICS Ⅱ-FMT effectively reversed mitochondrial dysfunction, reduced oxidative stress and oxidation-induced inflammation both in brain and intestine. SCFAs are metabolites generated by gut microbiota, which have been considered modulators of gut microbiota influencing intestinal antioxidant ability and act as a bridge of microbiota-gut-brain axis [7,31]. SCFAs also have salutary effects on retaining gut barrier integrity through increasing the TJ proteins expression [32]. As a consequence, we assume that ICS Ⅱ remodels gut microbiota through its metabolic SCFAs to counteract mitochondrial dysfunction and ROS production from mitochondria to safeguard the psychiatric disorders in PSD. As predicted, the level of SCFAs were significantly decreased after PSD insult, in keeping with previous reports [33]. However, ICS Ⅱ markedly increased the SCFAs concentration, particularly acetic, propionic, and butyric acids, which are the major types of SCFAs [34]. The correlation analysis further validated that ICS Ⅱ increased the levels of SCFAs production-related microbiota, such as *Akkermansia*. Importantly, SCFAs were positively correlated with antioxidant indicators and negatively associated with LPS, ROS, and proinflammatory cytokines in the serum or ileum and brain. These findings confirm that SCFAs-producing microbiota, such as *Akkermansia*, and subsequent SCFAs production conduces to the anti-oxidative state of ICS Ⅱ-mediated neuroprotection in the PSD mice. Our results confirm that ICS Ⅱ rescues depressive-like behaviors from PSD *via* modulating microbiota-gut-brain axis due to its anti-oxidative capacity.

Interestingly, we also found that there was a significant increase of Sirt6 protein expression of intestine in mice with PSD, which drives us to explore the role of Sirt6-mediated oxidative stress in intestine. Since damage to the intestinal epithelium is often closely related to mitochondria, and H_2O_2 often leads to increased mitochondrial mtROS and dysfunction [35]. EGCs are one major cell

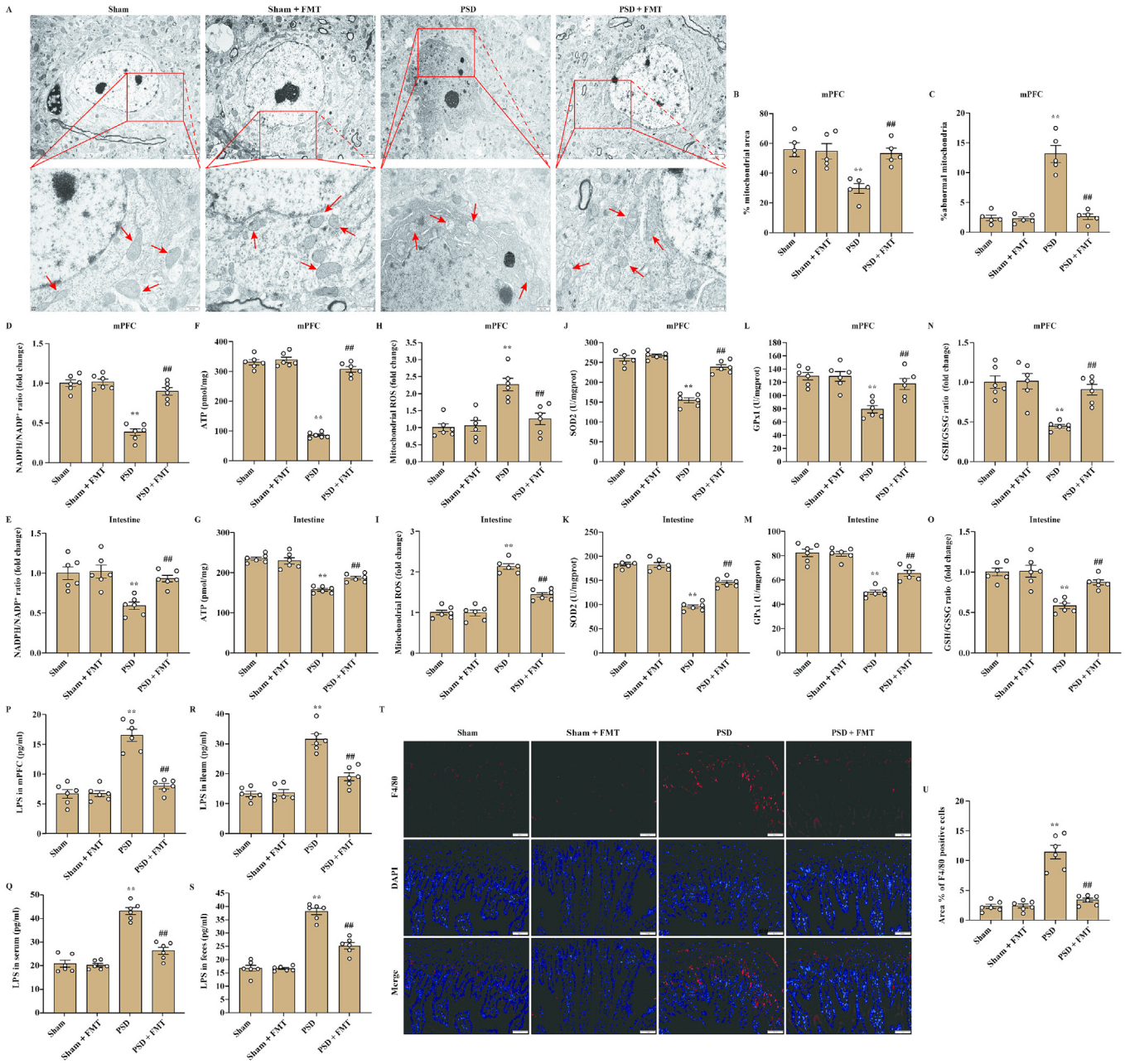

Fig. 7. Effect of ICS Ⅱ-FMT on mitochondrial oxidative stress associated with inflammation of brain and gut in PSD mice. (A) Ultrastructure of mitochondria shown by the red arrow. Bar scale = 500 nm. Quantification of mitochondria-related parameters including (B) % mitochondria area refers to the ratio of mitochondrial area to image area. (C) % abnormal mitochondria refer to the abnormal mitochondria ratio in mPFC (n = 5/group). (D) NADPH/$NADP^+$ ratio in mPFC (n = 6/group). (E) NADPH/$NADP^+$ ratio in gut (n = 6/group). (F) ATP in mPFC (n = 6/group). (G) ATP in gut (n = 6/group). (H) Mitochondrial ROS production in mPFC (n = 6/group). (I) Mitochondrial ROS production in gut (n = 6/group). (J) SOD2 activity in mPFC (n = 6/group). (K) SOD2 activity in gut (n = 6/group). (L) GPx1 activity in mPFC (n = 6/group). (M) GPx1 activity in gut (n = 6/group). (N) GSH/GSSG ratio in mPFC (n = 6/group). (N) GSH/GSSG ratio in gut (n = 6/group). (P) LPS in mPFC (n = 6/group). (Q) LPS in serum (n = 6/group). (R) LPS in ileum (n = 6/group). (S) LPS in feces (n = 6/group). (T) Representative images of F4/80 staining in ileum. Magnification 200 ×; bar scale = 50 μm. (U) Area % of F4/80 positive cells (n = 6/group). Data are shown as mean ± SD. $^{**}P < 0.01$ *vs*. Sham group, $^{\#\#}P < 0.01$ *vs*. PSD group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

type of the enteric nervous system, which plays a crucial role in maintaining intestinal barrier integrity [36]. Thus, we used H_2O_2-induced EGCs injury *in vitro* to mimic intestinal oxidative stress. A multitude of evidence supports that mitochondria are the center of ROS production, and generate ATP synthesis through OXPHOS. The disequilibrium redox status elicits oxidative stress, where excessive ROS damage mitochondrial function *via* compromising mitochondrial biogenesis, especially OXPHOS and mtDNA, and ATP synthesis[37,38]. Our results found that ICS Ⅱ potently suppressed H_2O_2-induced EGCs injury by reducing mitochondrial ROS production and pro-inflammatory cytokines, while concurrently enhancing the antioxidant enzyme activities, in keeping with our *in vivo* observations. Furthermore, ICS Ⅱ mitigated oxidative stress-induced mitochondrial dysfunction by augmenting OXPHOS and preventing mtDNA damage, ultimately facilitating ATP synthesis. However, Sirt6 deficiency aggravated oxidative stress-induced mitochondrial dysfunction and inflammation in EGCs, and ICS Ⅱ failed to improve the mitochondrial function, and oxidation-induced inflammation in EGCs in the absence of Sirt6. These findings provide validation that ICS Ⅱ effectively

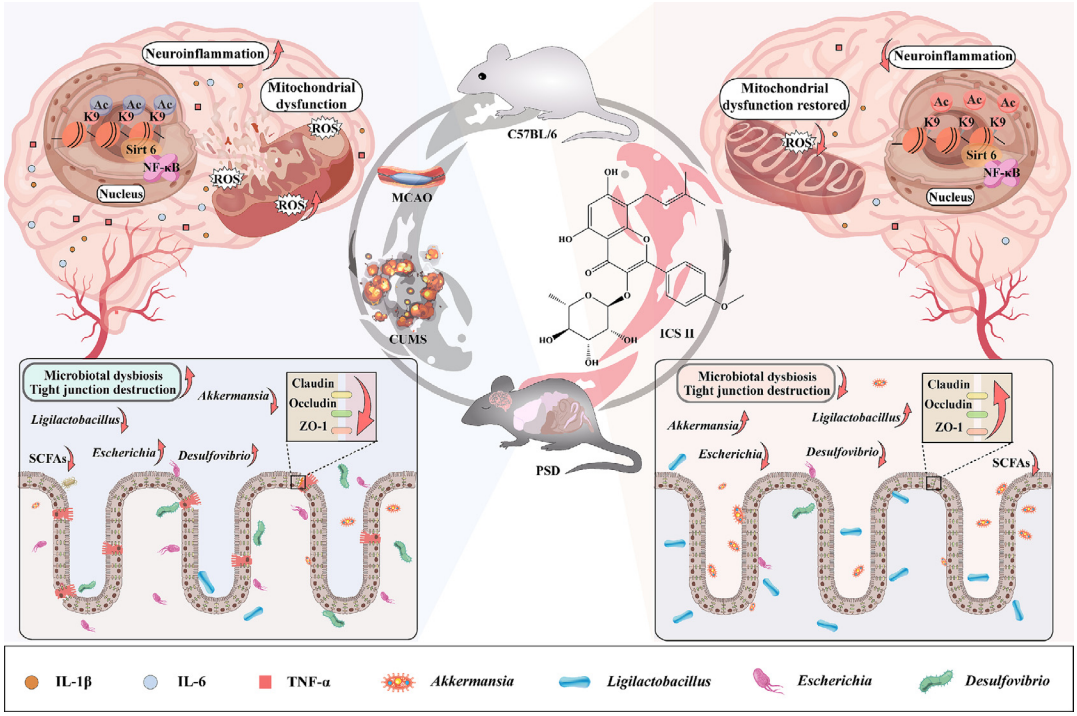

Fig. 8. Schematic diagram of a proposed mechanism for the antidepressant effect of ICS Ⅱ on poststroke depression (PSD) mice. PSD is a prevalent and detrimental complication of stroke. Sirt6-mediated gut microbiota plays a crucial role in the PSD. Icariside Ⅱ (ICS Ⅱ), a naturally-occurring bioactive flavonoids derived from traditional Chinese medicine Herba *Epimedii*, exerts powerful anti-PSD effect due to suppression of oxidative stress and oxidation-induced inflammation both in brain and intestine by targeting Sirt6. Concurrently, ICS Ⅱ significantly increased the abundance of gut microbiota (such as Akkermansia and Ligilactobacillus), enhanced SCFAs concentrations, repaired intestinal barrier integrity and upregulated the tight junction protein expression. Collectively, ICS Ⅱ may serves as a novel and naturally-occurring Sirt6 activator to rescue depressive-like behaviors from PSD through modulating microbiota-gut-brain axis.

mitigates intestinal oxidation-induced inflammation by targeting Sirt6. By integrating the *in vitro* and *in vivo* results mentioned above, it can be seen that Sirt6-mediated mitochondrial oxidative stress plays a vital role in gut-brain axis, and it might be the potential target of ICS Ⅱ to treat PSD through modulating gut microbiota. Considering that ICS II operates through a different mechanism than existing treatments, it could offer an alternative option for patients who do not adequately respond well to current therapies. Furthermore, given that numerous patients with PSD experience significant cognitive impairments, the potential of ICS II to improve cognitive function could exert a profound impact on patients' daily lives and overall functioning. Additionally, several patients also suffer intolerable adverse effects from existing treatments. Since ICS II boasts a more favorable safety profile, it could emerge as a preferred choice for these patients.

Dspite our experimental findings being encouraging, there are still pending issues to be addressed. Firstly, the objective and findings of the present study were just to investigate the neuroprotective effect of ICS Ⅱ on male PSD mice. In light of the significant gender difference in PSD, whether the antidepressant effects of ICS Ⅱ on Sirt6-mediated mitochondrial oxidative stress and its impact on the gut-brain axis differ between males and females will be investigated in-depth. We will perform sex-specific analyses using male and female PSD animal models to determine if there are any gender-related variations in efficacy or mechanisms of action. Secondly, despite the recognized safety profile of ICS Ⅱ, the long-term toxicity is required to be evaluated. We will implement long-term animal studies to evaluate the toxicity and safety of ICS Ⅱ over extended periods, including assessments of reproductive and developmental toxicity. Last, but not least, large clinical trials, and the pharmacokinetics, the distribution and metabolism of ICS Ⅱ in the brain also need to explore the potential druggability of ICS Ⅱ. To addressed this issue, Phase I trials will be initiated to establish the safety, tolerability, and pharmacokinetic profile of ICS Ⅱ in healthy volunteers. Subsequently, phase II and phase III clinical trials will be conducted to assess the efficacy and safety of ICS Ⅱ in reducing PSD symptoms and its impact on gut microbiota and gut-brain axis function in patients with PSD.

Conclusion

In conclusion, the present study expands and discovers a novel pharmacological property of ICS Ⅱ with antidepressant effect on PSD. ICS Ⅱ suppresses oxidative stress and oxidation-induced inflammation both in brain and intestine by targeting Sirt6. Concurrently, ICS Ⅱ remodels gut microbiota and increases the production of its metabolites SCFAs due to its antioxidative capacity (Fig. 8). The modulation of gut microbiota and promotion of SCFAs production by ICS II represents a groundbreaking therapeutic approach in PSD treatment. This finding underscores the importance of the microbiota-gut-brain axis in neuropsychiatric disorders and paves the way for future research and development in this promising area.

Author contributions

Qihai Gong was responsible for the study concept, designed and revised the paper. Jianmei Gao, Fangqin Hou, Fuguo Shi, Xiaoyu Wu, Yang Yi, Yuandong Zhang were responsible for the experiments, acquisition of data and statistical analysis. Jianmei Gao was responsible for drafting of the manuscript. Approval of the final version of the manuscript on behalf of all authors was done by Qihai Gong.

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Compliance with ethics requirements

All animal experiments were approved by the Ethics Committee of Zunyi Medical University (approval number: ZMU[2020]2-134), and the experimental procedures were executed in compliance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2025.03.002.

References

[1] Mobley AR, Subramanian A, Champsi A, Wang X, Myles P, McGreavy P, et al. Thromboembolic events and vascular dementia in patients with atrial fibrillation and low apparent stroke risk. *Nat Med* 2024.
[2] Gomberg J, Stein LK, Dhamoon MS. Risk of recurrent stroke and mortality among black and white patients with poststroke depression. *Stroke* 2024;55 (5):1308–16.
[3] Diseases GBD, Injuries C. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet* 2024;403 (10440):2133–61.
[4] Richards LG, Cramer SC. Therapies targeting stroke recovery. *Stroke* 2023;54 (1):265–9.
[5] Cuartero MI, Garcia-Culebras A, Nieto-Vaquero C, Fraga E, Torres-Lopez C, Pradillo J, et al. The role of gut microbiota in cerebrovascular disease and related dementia. *Br J Pharmacol* 2024;181(6):816–39.
[6] Jeong S, Chokkalla AK, Davis CK, Vemuganti R. Post-stroke depression: epigenetic and epitranscriptomic modifications and their interplay with gut microbiota. *Mol Psychiatry* 2023;28(10):4044–55.
[7] Jiang W, Chen J, Gong L, Liu F, Zhao H, Yan Z, et al. Microbiota-derived short-chain fatty acids may participate in post-stroke depression by regulating host's lipid metabolism. *J Psychiatr Res* 2023;161:426–34.
[8] Yang Z, Zhao Y, Wang Y, Liu X, Jiang Y, Jiang Y, et al. Echinacoside ameliorates post-stroke depression by activating BDNF signaling through modulation of Nrf2 acetylation. *Phytomedicine* 2024;128:155433.
[9] Shandilya S, Kumar S, Kumar Jha N, Kumar Kesari K, Ruokolainen J. Interplay of gut microbiota and oxidative stress: perspective on neurodegeneration and neuroprotection. *J Adv Res* 2022;38:223–44.
[10] Casper E. The potential role of SIRT6 in regulating the crosstalk between Nrf2 and NF-kappaB pathways in cardiovascular diseases. *Pharmacol Res* 2022;182:106300.
[11] He T, Shang J, Gao C, Guan X, Chen Y, Zhu L, et al. A novel SIRT6 activator ameliorates neuroinflammation and ischemic brain injury via EZH2/FOXC1 axis. *Acta Pharm Sin B* 2021;11(3):708–26.
[12] Liberale L, Gaul DS, Akhmedov A, Bonetti NR, Nageswaran V, Costantino S, et al. Endothelial SIRT6 blunts stroke size and neurological deficit by preserving blood-brain barrier integrity: a translational study. *Eur Heart J* 2020;41 (16):1575–87.
[13] Ren SC, Chen X, Gong H, Wang H, Wu C, Li PH, et al. SIRT6 in vascular diseases, from bench to bedside. *Aging Dis* 2022;13(4):1015–29.
[14] Xu K, Guo Y, Wang Y, Ren Y, Low V, Cho S, et al. Decreased Enterobacteriaceae translocation due to gut microbiota remodeling mediates the alleviation of premature aging by a high-fat diet. *Aging Cell* 2023;22(2):e13760.
[15] Jiang W, Ding K, Yue R, Lei M. Therapeutic effects of icariin and icariside II on diabetes mellitus and its complications. *Crit Rev Food Sci Nutr* 2024;64 (17):5852–77.
[16] Liu MB, Wang W, Gao JM, Li F, Shi JS, Gong QH. Icariside II attenuates cerebral ischemia/reperfusion-induced blood-brain barrier dysfunction in rats via regulating the balance of MMP9/TIMP1. *Acta Pharmacol Sin* 2020;41 (12):1547–56.
[17] Gong MX, Wei JJ, Yi Y, Liu X, Hou FQ, Li YQ, et al. Targeting PPARα/γ by icariside II to rescue GalN/LPS-induced acute liver injury in mice: Involvement of SIRT6/NF-κB signaling pathway. *Phytomedicine* 2025;136:156250.
[18] Gao J, Long L, Xu F, Feng L, Liu Y, Shi J, et al. Icariside II, a phosphodiesterase 5 inhibitor, attenuates cerebral ischaemia/reperfusion injury by inhibiting glycogen synthase kinase-3beta-mediated activation of autophagy. *Br J Pharmacol* 2020;177(6):1434–52.
[19] Gao J, Ma C, Xia D, Chen N, Zhang J, Xu F, et al. Icariside II preconditioning evokes robust neuroprotection against ischaemic stroke, by targeting Nrf2 and the OXPHOS/NF-kappaB/ferroptosis pathway. *Br J Pharmacol* 2023;180 (3):308–29.
[20] Kronenberg G, Gertz K, Heinz A, Endres M. Of mice and men: modelling post-stroke depression experimentally. *Br J Pharmacol* 2014;171(20):4673–89.
[21] Qian L, Huang S, Liu X, Jiang Y, Jiang Y, Hu Y, et al. Morroniside improves the symptoms of post-stroke depression in mice through the BDNF signaling pathway mediated by MiR-409-3p. *Phytomedicine* 2024;123:155224.
[22] Wang AR, Mi LF, Zhang ZL, Hu MZ, Zhao ZY, Liu B, et al. Saikosaponin A improved depression-like behavior and inhibited hippocampal neuronal apoptosis after cerebral ischemia through p-CREB/BDNF pathway. *Behav Brain Res* 2021;403:113138.
[23] Chevalier G, Siopi E, Guenin-Macé L, Pascal M, Laval T, Rifflet A, et al. Effect of gut microbiota on depressive-like behaviors in mice is mediated by the endocannabinoid system. *Nat Commun* 2020;11(1):6363.
[24] Jiang Y, Wang X, Chen J, Zhang Y, Hashimoto K, Yang JJ, et al. Repeated (S)-ketamine administration ameliorates the spatial working memory impairment in mice with chronic pain: role of the gut microbiota-brain axis. *Gut Microbes* 2024;16(1):2310603.
[25] Almeida OP, Hankey GJ, Ford A, Etherton-Beer C, Flicker L, Hackett M. Assessment of fluoxetine in stroke recovery trial c: depression outcomes among patients treated with fluoxetine for stroke recovery: the AFFINITY randomized clinical trial. *JAMA Neurol* 2021;78(9):1072–9.
[26] Shao A, Lin D, Wang L, Tu S, Lenahan C, Zhang J. Oxidative stress at the crossroads of aging, stroke and depression. *Aging Dis* 2020;11(6):1537–66.
[27] Yan L, Deng Y, Gao J, Liu Y, Li F, Shi J, et al. Icariside II Effectively Reduces Spatial Learning and Memory Impairments in Alzheimer's Disease Model Mice Targeting Beta-Amyloid Production. *Front Pharmacol* 2017;8:106.
[28] Hong R, Luo L, Wang L, Hu ZL, Yin QR, Li M, et al. Lepidium meyenii Walp (Maca)-derived extracellular vesicles ameliorate depression by promoting 5-HT synthesis via the modulation of gut-brain axis. *iMeta* 2023;2(3):e116.
[29] Szabó R, Rácz CP, Dulf FV. Bioavailability improvement strategies for icariin and its derivates: a review. *Int J Mol Sci* 2022;23(14).
[30] Zhou J, Hao J, Zhong Z, Yang J, Lv T, Zhao B, et al. Fecal microbiota transplantation in mice exerts a protective effect against doxorubicin-induced cardiac toxicity by regulating Nrf2-mediated cardiac mitochondrial fission and fusion. *Antioxid Redox Signal* 2024;41(1–3):1–23.
[31] Fang Z, Chen M, Qian J, Wang C, Zhang J. The bridge between ischemic stroke and gut microbes: short-chain fatty acids. *Cell Mol Neurobiol* 2023;43 (2):543–59.
[32] Liu Q, Xi Y, Wang Q, Liu J, Li P, Meng X, et al. Mannan oligosaccharide attenuates cognitive and behavioral disorders in the 5xFAD Alzheimer's disease mouse model via regulating the gut microbiota-brain axis. *Brain Behav Immun* 2021;95:330–43.
[33] Sun M, Chen H, Dong S, Zhang G, Zhou X, Cheng H. Alteration of gut microbiota in post-stroke depression patients with Helicobacter pylori infection. *Neurobiol Dis* 2024;193:106458.
[34] Lei C, Liu C, Peng Y, Zhan Y, Zhang X, Liu T, et al. A high-salt diet induces synaptic loss and memory impairment via gut microbiota and butyrate in mice. *iMeta* 2023;2(2):e97.
[35] Song X, Huang Q, Yang Y, Ma L, Liu W, Ou C, et al. Efficient therapy of inflammatory bowel disease (IBD) with highly specific and durable targeted Ta (2) C modified with chondroitin sulfate (TACS). *Adv Mater* 2023;35(36): e2301585.
[36] Seguella L, Pesce M, Capuano R, Casano F, Pesce M, Corpetti C, et al. High-fat diet impairs duodenal barrier function and elicits glia-dependent changes along the gut-brain axis that are required for anxiogenic and depressive-like behaviors. *J Neuroinflammation* 2021;18(1):115.
[37] Long X, Liu M, Nan Y, Chen Q, Xiao Z, Xiang Y, et al. Revitalizing ancient mitochondria with nano-strategies: mitochondria-remedying nanodrugs concentrate on disease control. *Adv Mater* 2024;36(18):e2308239.
[38] Luo Y, Chatre L, Melhem S, Al-Dahmani ZM, Homer NZM, Miedema A, et al. Thiosulfate sulfurtransferase deficiency promotes oxidative distress and aberrant NRF2 function in the brain. *Redox Biol* 2023;68:102965.