



Roles of miR-432 and circ_0000418 in mediating the anti-depressant action of ADAR1

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ABSTRACT

Adenosine deaminase acting on RNA1 (ADAR1) is a newly discovered epigenetic molecule marker that is sensitive to environmental stressors. A recent study has demonstrated that ADAR1 affects BDNF expression via miR-432 and is involved in antidepressant action. However, the detailed molecular mechanism is still unclear. We have uncovered a new molecular mechanism showing the involvement of miR-432 and circ_0000418 in mediating the antidepressant action of ADAR1. We demonstrate that the ADAR1 inducer (IFN- γ) alleviates the depressive-like behaviors of BALB/c mice treated with chronic unpredictable stress (CUS) exposure. Moreover, both in vivo and in vitro studies show that ADAR1 differently impacts miR-432 and circ_0000418 expressions. Furthermore, the in vitro results demonstrate that circ_0000418 oppositely affects BDNF expression. Together, our results indicate that ADAR1 affects CUS-induced depressive-like behavior and BDNF expression by acting on miR-432 and circ_0000418. Elucidation of this new molecular mechanism will not only provide insights into further understanding the important role of ADAR1 in stress-induced depressive-like behavior but also suggest a potential therapeutic strategy for developing novel anti-depressive drugs.

1. Introduction

At present, more than 300 million people worldwide suffer from depression according to the World Health Organization (de Aguiar Neto and Rosa, 2019). Depression patients have social dysfunction, cognitive impairment, and even suicide. Environmental stressors cause depressive-like behavior in both humans and animals (Barbon and Magri, 2020; Menard et al., 2016; Park et al., 2019; Zhang et al., 2021), but the detailed molecular mechanisms have not been fully elucidated. Recent studies suggest that adenosine deaminase acting on RNA1 (ADAR1) is a newly discovered epigenetic molecule marker that is sensitive to environmental stressors (Chen et al., 2016; Wang et al., 2004; Yu et al., 2018; Zhang et al., 2021). ADAR1 working as an RNA editing enzyme is involved in the pathogenesis of depression, which provides a new idea to elucidate the molecular mechanisms on

stress-induced depression. In vertebrates, ADARs are composed of ADAR1, ADAR2, and ADAR3 (Song et al., 2016). Alternative splicing of ADAR1 occurs during RNA maturation, which produces two isoforms of ADAR1p110 and ADAR1p150. It has been reported that ADAR1 is expressed in neurons and glial cells in the brain (Chen et al., 2016; Jacobs et al., 2009; Veno et al., 2012). ADARs play an important role in the development of the nervous system and the maintenance of normal immune function (Jacobs et al., 2009; Nakahama and Kawahara, 2020; Stellos et al., 2016; Veno et al., 2012). ADAR1 catalyzes the chemical transformation from adenosine (A) to inosine (I) editing of double-stranded RNAs in the nucleus (Song et al., 2016). ADAR1 prevents stress-induced apoptosis by editing one or more double-stranded RNAs. Owing to the essential role of ADAR1 on maintaining normal embryonic development (Jacobs et al., 2009; Veno et al., 2012), it is difficult for ADAR1 knockout mice to survive (Higuchi et al., 2000;

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Wang et al., 2000, 2004). Abnormal ADAR1 expression is closely related to human diseases (Gallo et al., 2017; Song et al., 2016), including cancer (Anadon et al., 2016; Chan et al., 2016; Han et al., 2015; Nemlich et al., 2013; Paz-Yaacov et al., 2015), autoimmune diseases (Nakahama and Kawahara, 2020), and cardiovascular diseases (Stellos et al., 2016). Recent studies have shown that ADAR1 is also involved in the pathogenesis of depression. The link between ADAR1 and depression is supported by the following evidence. Cerebral ADAR1 expression is increased in the patients with depression and suicide (Karanovic et al., 2015; Simmons et al., 2010). Moreover, cerebral ADAR1 expression is decreased in the BALB/c mice and is increased in Kunming mice enduring social isolation stress (Chen et al., 2016; Yu et al., 2018). Furthermore, cerebral RNA editing of the 5-HT_{2C} receptor is abnormal in depression patients (Dracheva et al., 2008; Iwamoto and Kato, 2003). 5-HT_{2C} receptor is the only serotonin receptor subtype to undergo A-to-I RNA editing, catalyzed by ADAR1, forming 24-amino acid isoforms of the 5-HT_{2C} receptor (Werry et al., 2008). The above evidence indirectly suggests that ADAR1, as an RNA editing enzyme, participates in the pathogenesis of depression via RNA editing of the 5-HT_{2C} receptor. One of the possible links between ADAR1 and depression is explained by that ADAR1 inducer alleviates the depressive-like behavior of the stressed mice by increasing cerebral brain-derived neurotrophic factor (BDNF) expression via miR-432 (Zhang et al., 2021). Interestingly, hsa-miR-432 is found to be related to hsa_circ_0000418 by circbase analysis (<http://circrna.org>). It is known that specific circular RNAs are involved in the occurrence, development, and prognosis of depression. Mmu_circ_0001223 expression (Zhang et al., 2018) and circDYM (Zhang et al., 2020) are involved in the antidepressant action in depressive mice. Mmu_circ_0001223 expression is decreased in the ventral medial prefrontal cortex and hippocampus of chronic unpredictable mild stressed mouse. Mmu_circ_0001223 over-expression enhances cAMP responsive element binding protein 1 (CREB1) and BDNF protein levels in PC12 cells (Zhang et al., 2018). CircDYM is significantly decreased both in the peripheral blood of patients with major depressive disorder (MDD) and in the two depressive-like mouse models: the chronic unpredictable stress and lipopolysaccharide (LPS) models. Restoration of circDYM expression significantly attenuates depressive-like behavior and inhibits microglial activation induced by CUS or LPS treatment (Zhang et al., 2020). Hsa_circRNA_103636 (Cui et al., 2016) and hsa_circ_0126218 (Bu et al., 2021) in peripheral blood mononuclear cells are significantly down-regulated in MDD patients, suggesting that these two circRNA are potential new biomarkers for the diagnosis and treatment of MDD. Nevertheless, the mechanism underlying the involvement of circular RNA in depression remains unclear. ADAR1 effectively binds and edits the reverse complementary Alu sequence of circRNA flanking sequence through double-stranded RNA binding sites and inhibits the formation and cyclization of the complementary structure of exon flanking sequence (Ivanov et al., 2015; Levanon et al., 2004; Osenberg et al., 2010; Ramaswami et al., 2012). However, the detailed molecular mechanisms underlying the involvement of ADAR1 in antidepressant action are not fully understood.

In this study, the CUS-elicited depressive mice were treated with ADAR1 inducer/inhibitor to elucidate the mechanism underlying the antidepressant action of ADAR1. Moreover, both *in vivo* and *in vitro* experiments were performed to investigate roles of miR-432 and circ_0000418 in mediating the link between ADAR1 and BDNF expression. Bio-informatics was also applied to analyze how the target molecules among miR-432, circ-0000418, and BDNF work in mediating the antidepressant action of ADAR1. This study provides the first evidence uncovering a new molecular mechanism mediating the anti-depressant function of ADAR1 in the stress-induced depressive-like behavior.

2. Methods

2.1. Experimental animals

The experimental animals used in this study were male BALB/c mice (provided by Dalian Medical University Experimental Animal Center, Dalian, n = 60), weighing 15–20 g. Room temperature was kept at 22 ± 1 °C, the humidity was 60 ± 10%. The regular light switch ensured the alternation of light and dark. All mice were treated with the food and water freely. All experimental protocols were approved by Animal Experiment Ethics Committee of Dalian Medical University (No: 12014021).

2.2. Chronic unpredictable stress mice (CUS) model preparation

Previously published procedures were used for preparing CUS-induced depressive mice (Zhang et al., 2021). Before the stress exposure, the BALB/c mice were adapted to the rearing environment for 1 week. Then they were treated with the CUS exposure, including overnight lighting for 10 h, fasting for 24 h, water deprivation for 24 h, single cage rearing for 24 h, tail clamping for 30 min (1 cm from the root of tail), forced swimming for 10 min, tilting cushion for 5 h, and restraint for 1 h. The mice were given two different stress exposures daily. The mice was not exposed with the same stress exposure in 2 d. The CUS-exposed mice were treated with the stress exposure for 4 weeks. The control mice lived in the normal feeding environment and were handled every day. After each stress exposure, the cage was cleaned to remove the stress odor.

2.3. ADAR1 target intervention and grouping

Sixty mice were divided into control group (C1) and chronic unpredictable stress model group (CUS1) randomly (n = 30/group). The CUS1 group were treated with CUS exposure, while the C1 group mice were handled only. After 4 weeks of CUS exposure, open field test (OFT), tail suspension test (TST), and forced swimming test (FST) were performed to confirm whether the CUS-induced mice showed the depressive-like behavior. After CUS procedure, mice were randomly divided into 6 groups (n = 10/group), including control group (C), CUS group (CUS), ADAR1 inducer treatment only group (C + ADAR1 inducer), ADAR1 inhibitor treatment only group (C + ADAR1 inhibitor), CUS-exposed mice treated with ADAR1 inducer group (CUS + ADAR1 inducer), CUS-exposed mice treated with ADAR1 inhibitor group (CUS + ADAR1 inhibitor). Based on the pilot study and our previous report (Zhang et al., 2021), the mice were treated with ADAR1 inducer (IFN- γ , PeproTech, USA, 2.0 × 10⁵ U/kg/d, ip) or ADAR1 inhibitor (Pentostatin, MCE-USA, 1 mg/kg/d, ip.) continuously for 7 d. The C group and CUS group mice were treated with vehicle (normal saline, 20ml/kg/d, ip.) for 7 d. OFT, TST, and FST were performed after the drug intervention. The detail schedule for the behavior tests and ADAR1 target intervention is shown in the Fig. 1.

2.4. Behavior tests

2.4.1. Open field test

The open field test (Zhang et al., 2021) was performed as follows: Mice were put into the open field test box (50 cm × 50 cm × 40 cm), the spontaneous movement track (5 min), the time and distance for staying in the specific area of the open field were recorded. The open field test box was a black opaque box with 25 small squares (10 cm × 10 cm) on the bottom and 9 small squares in the middle as the central area. The open field lighting was artificial lighting (40 LX). At the end of each test, the floor of the box was thoroughly cleaned with 75% alcohol solution to avoid the interference of odor and excreta.

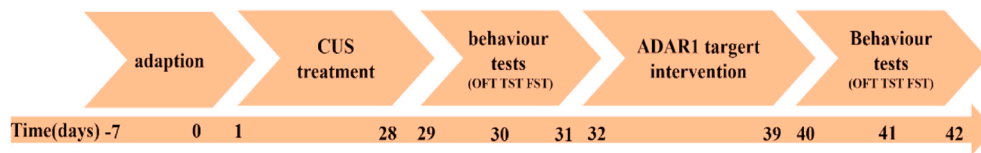


Fig. 1. The time schedule for stress-induced depressive mice model preparation, behavior tests, and ADAR1 target intervention.

2.4.2. Tail suspension test

Tail suspension test protocol (Porsolt et al., 1977; Yamawaki et al., 2012) was shown as follows: the tail of the experimental mouse was lifted, and the tail was fixed and suspended, so that the mouse was kept upside down, and the head was about 5 cm away from the ground. The immobility time of the experimental mouse was recorded. The total time length of the experiment was 6 min, the data collected in the last 4 min were analyzed in a double-blind manner.

2.4.3. Forced swimming test

Forced swimming test was performed following the previous reported methods (Porsolt et al., 1977; Yamawaki et al., 2012). The experimental mouse was forced to swim in a transparent glass container. The water temperature was 22 ± 1 °C. Immobility is defined when the mouse floated passively in the water with its nose above the water. Each test lasted for 6 min. The results were measured during the period of the last 4 min of the test in a double-blind manner.

2.5. Cell culture and RNA oligo transfection

The hsa-miR-432 high/low-expression cells, hsa-circ_0000418 high/low-expression cells, and hsa-adar1 low-expression cells were constructed by transfecting specific RNA oligonucleotides in SH-SY5Y cells. The culture was carried out in MEM/F12 medium with 10% fetal bovine serum at 37 °C and 5% CO₂. SH-SY5Y cells were put on 6-well plates and transfected when the cell density reached 60–80%. Transfection siRNA alone dose was 100 pmol and plasmid DNA dose was 4.0 μg, the co-transfection siRNA dose was 50 pmol and plasmid DNA dose was 2.0 μg. The transfection was performed according to the instructions of the supplier (Geneparma, China).

2.6. Immunofluorescence staining

Immunofluorescence staining was performed following the previously reported procedures (Zhang et al., 2021). SH-SY5Y cells were fixed with 4% paraformaldehyde, then were incubated with 0.2% Triton X-100/PBS for 5 min and were blocked with 3% bovine serum albumin for 1 h. The samples were incubated with the polyclonal primary antibody of ADAR1 (1:70, proteotech, USA) and BDNF (1:70, BIOSS, China) at 4 °C overnight. The samples were washed three times with $1 \times$ PBS and incubated with the fluorescent Alexa fluor 594 labeled secondary antibody (1:100, proteotech, China) for 2 h at room temperature. The nuclei were stained with 4', 6-diamino-2-phenylindole (DAPI, 1 μg/ml, Beyotime, China). Fluorescence microscope (Leica, Germany) was used to observe the expression of immune reactive-positive signals, and image analysis software (ImageJ, v1.4, USA) was used to analyze the optical density of the target molecule immune reactive-positive signals.

2.7. Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed as follows. Total RNA was extracted from frontal cortex of mouse and SH-SY5Y cells by TransZol reagent. The purity of RNA (Transgen biotech, China) was determined by spectrophotometry, and the total RNA was reversely transcribed into cDNA by using the TransScript Top Green qPCR kit (Transgen biotech, China). qRT-PCR system was used for PCR quantitative detection (ThermoFisher, China).

Synthetic cDNA was used for PCR quantity evaluation (Bori, China). GAPDH and U6 gene expressions were used as endogenous controls, and the target RNA expression difference was calculated by $2^{-\Delta\Delta CT}$ method. The primer sequences are shown in Table 1.

2.8. Western blot

The protein samples of SH-SY5Y cells were extracted with protein extraction kit (Keygen Biotech, China). BCA protein detection kit (Keygen Biotech, China) was used to detect the protein concentration. The protein of each sample was 30 μg. After protein denaturation, the sample was added into 10% SDS polyacrylamide gels. The protein was transferred to polyvinylidene difluoride membranes and blocked with 5% bovine serum albumin for 1 h (Beyotime, China). The membranes with the target protein were incubated with ADAR1 polyclonal antibody (1:1000, Proteintech, USA), BDNF polyclonal antibody (1:1,000, Bioss, China) and GAPDH monoclonal antibody (1:5000, Immunoway, USA) at 4 °C overnight. After that, the samples were washed with Tris-buffered saline containing Tween-20 (TBST), goat anti rabbit or goat anti mouse IgG (1:10000, Bioss, China) labeled with horseradish peroxidase was incubated with the membranes for 1 h at room temperature. Then the samples were washed with TBST, the target protein was detected by enhanced chemiluminescence Kit (Solar bio, China), and the optical density of each band was analyzed by image analysis system (Bio-Rad, USA), which was normalized to GAPDH protein level for data analysis.

2.9. RNA binding protein immunoprecipitation

RNA binding protein immunoprecipitation (RIP) was performed according to the instruction (Magna, Germany) and the modifications as reported by Keene et al. (2006). In brief, 2×10^7 SH-SY5Y cells were lysed in complete lysis buffer containing RNase inhibitor. The sample was centrifuged at 14000 rpm for 10 min at 4 °C. The magnetic beads for immunoprecipitation were prepared. 50 μl of magnetic beads suspension to each sample were transferred, then were added 0.5 ml of RIP wash buffer and were under vortex movement. The tubes were placed on the magnetic separator. The supernatant of each sample was discarded. The magnet was removed, and the beads in 100 μl of the RIP wash buffer were re-suspended. 5 μg of the antibody was added, including IgG and AGO2 (1:1000, Magna, Germany) respectively, then the sample was incubated with the target antibody under rotation for 30 min at room temperature. 100 μl of the RIP lysate supernatants were removed, and beads-antibody complex was added into the RIP immunoprecipitation buffer. All the tubes were incubated under rotation overnight at 4 °C. The tubes containing the samples were centrifuged and were placed on the magnetic separator. After that, the supernatant was discarded. The purification sample of target RNA was isolated with TransZol reagent for qRT-PCR analysis.

2.10. Statistical analysis

All the data were analyzed by using GraphPad Prism 7.0 (San Diego, USA) and IBM SPSS Statistics 23.0 (Aramonk, USA). Two-way ANOVA was used to determine whether there is an interaction between chronic unpredictable stress and ADAR1 target intervention (two independent variables) on ADAR1 mRNA expression, BDNF mRNA expression, circ_0000418 expression, and miR-432 expression (dependent variable).

Table 1
The primer sequence of qRT-PCR.

Target molecules	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
hsa-adar1	CTGAGACCAAAAAGAAACGCAGA	GCCATTGTAATGAACAGGTGGTT
hsa-bdnf	TAACGGCGGCAGACAAAAAGA	TGCACTTGGTCTCGTAGAAGTAT
hsa_circ_0000418	AGCTAGGAGGAAGTTTGCCA	GGATAATGTTGTGCCCGCAA
hsa-GAPDH	CATGTTTCGTCATGGGTGTGAA	GGCATGGACTGTGGTCATGAG
hsa-miR-432	CTGCCGTTCTTGAGTAGGTCA	CAGAGCAGGGTCCGAGGT
hsa-U6	CGCTTCGGCAGCACATATAC	TTCACGAATTTGCGTGTCAATC
mmu-adar1	AGCCACAGGTGCTCAATGC	GTCCCTTTTACACAGCGATT
mmu-bdnf	CACACACAGCGTCCCTTA	AGTGGTGGTCTGAGGTTGG
mmu_circ_0000418	TGACTGGATCTTGTAGTACCCTTA	ACCATGCCTGCTTCTAGAGGTT
mmu-GAPDH	GCCACCCAGAAGACTGTGGAT	GGAAGCCATGCCAGTGA
mmu-miR-432	GGGGTCTTGGAGTAGATCAGTGG	CCAGTGCAGGGTCCGAGGT
mmu-U6	CGCTTCGGCAGCACATACTA	GGAACGCTTACGAATTTGC

mmu: *Mus musculus*, hsa: *Homo sapiens*

respectively among the mice. $p < 0.05$ was considered to be statistically significant. All the data were expressed as the means \pm SD. *U* test was used to analyze the behavior data before and after ADAR1 inducer/inhibitor administration. *T* test was used to analyze the variance for the groups with and without CUS exposure, the groups with and without ADAR1 target intervention in the mice, as well as the groups with and without transfection in SH-SY5Y cells.

3. Results

3.1. ADAR1 inducer alleviates depressive-like behavior in CUS mice

The BALB/c mice of the CUS1 group were treated with CUS exposure for 4 weeks, and then were evaluated by behavior tests. The results showed that in the open field test (OFT), the time the mice of CUS1 group spent in central area (Fig. 2a, $p < 0.01$) and the total distance walked in the central area (Fig. 2b, $p < 0.01$) were significantly reduced, as compared with those of C1 control group mice. The immobility time

values of the CUS1 group mice in tail suspension test (TST) (Fig. 2c, $p < 0.01$) and forced swimming test (FST) (Fig. 2d, $p < 0.01$) were significantly increased, as compared with those of C1 group mice. These results demonstrate that the CUS1 group mice show the depressive-like behaviors and are in anxiety and despair state.

To determine whether ADAR1 was involved in the occurrence of depressive-like behavior, the CUS1 group mice were administered with an ADAR1 inducer (IFN- γ) or an ADAR1 inhibitor (Pentostatin) respectively, then the behavior tests were performed again. In the open field test (Fig. 2e and f), CUS-exposed mice spent less time in the central area ($p < 0.01$) and walked shorter distance ($p < 0.01$) than those in the control mice. CUS-exposed mice treated with IFN- γ spent more time in central area ($p < 0.01$) and walked longer distance ($p < 0.05$) than those in the CUS-exposed mice. In tail suspension test (Fig. 2g) and forced swimming test (Fig. 2h), CUS-exposed mice showed much more immobility time (both $p < 0.01$) than those of control mice. CUS-exposed mice treated with ADAR1 inducer showed less immobility time than those of CUS-exposed mice (both $p < 0.01$). Additionally, no difference between

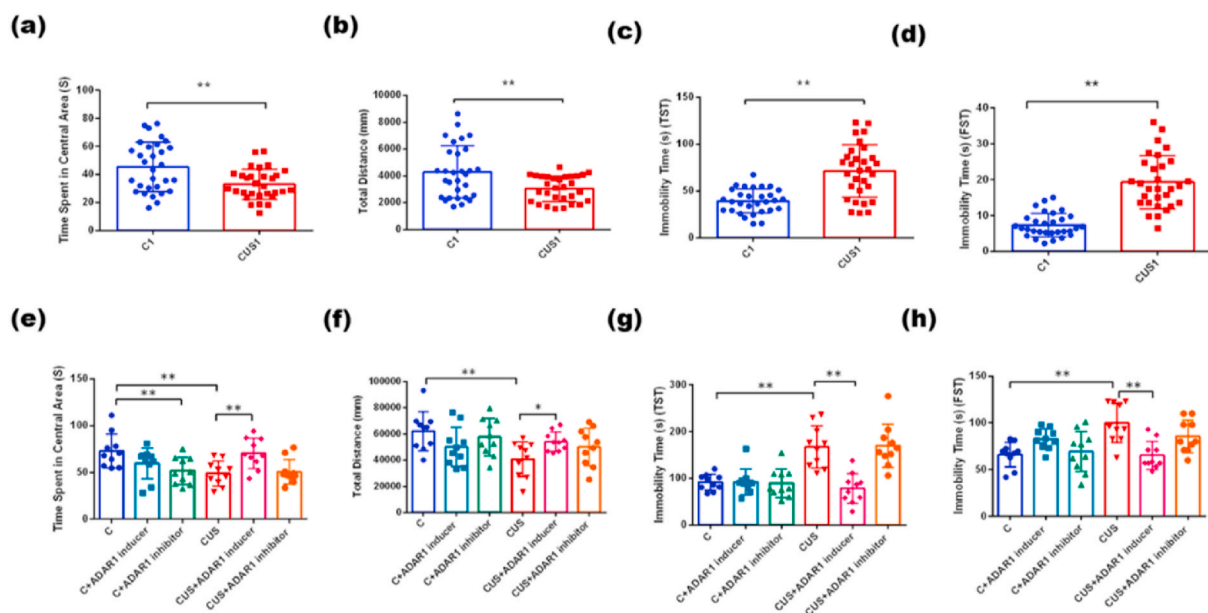


Fig. 2. ADAR1 inducer alleviates the depressive-like behavior of CUS-elicited depressive mice.

(a-d) CUS1 group mice showed the depressive-like behavior ($n = 30$ /group). (a) The time the mice spent in central area in the open field. (b) The total distance the mice walked in the open field. (c) The immobility time values of the mice in the TST. (d) The immobility time values of the mice in the FST. (e-h) ADAR1 inducer alleviates the depressive-like behavior of CUS-elicited depressive mice ($n = 10$ /group). (e) The time the mice spent in central area in the open field. (f) The total distance the mice walked in the open field. (g) The immobility time values of the mice in the TST. (h) The immobility time values of the mice in the FST. C1 and CUS1 represent the mice treated with CUS exposure and the relative control mice before the drug administration. C: control group, CUS: chronic unpredictable stress group, C + ADAR1 inducer: The mice treated with ADAR1 inducer, C + ADAR1 inhibitor: The mice treated with ADAR1 inhibitor, CUS + ADAR1 inducer: The depressive mice treated with ADAR1 inducer, CUS + ADAR1 inhibitor: The depressive mice treated with ADAR1 inhibitor. The data were expressed as mean \pm standard deviation. * $p < 0.05$, ** $p < 0.01$.

CUS-exposed mice and CUS mice treated with ADAR inhibitor was observed in all behavior results. These results demonstrate that ADAR1 inducer alleviates the depressive-like behavior of CUS mice.

3.2. ADAR1 inducer restores BDNF, circ_0000418, and miR-432 expressions in the frontal cortex of CUS mice

To investigate the detailed molecular mechanisms underlying the ADAR1 involvement in the depressive-like behavior, the effects of ADAR1 inducer on the expression of ADAR1, BDNF, mmu-miR-432, and mmu_circ_0000418 in the frontal cortex of mice were evaluated by qRT-PCR. The results showed that mmu-adar1 mRNA (Fig. 3a, $p < 0.01$), mmu-bdnf (Fig. 3b, $p < 0.01$) mRNA, and mmu-miR-432 (Fig. 3d, $p < 0.01$) expressions were significantly decreased, and mmu_circ_0000418 expression (Fig. 3c, $p < 0.05$) was significantly increased in CUS mice as compared with those in the control mice. Following the IFN- γ treatment, the reduction of mmu-adar1 (Fig. 3a, $p < 0.05$ vs CUS group data) and mmu-bdnf (Fig. 3b, $p < 0.05$ vs CUS group data) mRNA expressions and increase in mmu_circ_0000418 expression (Fig. 3c, $p < 0.05$ vs CUS group data) were fully returned to the control levels. CUS-elicited decrease in mmu-miR-432 expression was partially recovered (Fig. 3d, $p < 0.05$ vs CUS group data). By contrast, mmu-adar1 mRNA expression was further decreased in CUS mice treated with ADAR1 inhibitor mice (Fig. 3a, $p < 0.05$) as compared with that in CUS mice. Additionally, no difference between CUS-exposed mice and CUS mice treated with ADAR inhibitor was found in miR-432, mmu-bdnf, and mmu_circ_0000418 expression results. These results demonstrate that ADAR1 inducer rescues the abnormal levels of mmu-bdnf mRNA, mmu_circ_0000418, and mmu-miR-432 expression in the frontal cortex of CUS-elicited depressive BALB/c mice.

3.3. ADAR1 impacts circ_0000418 and BDNF expressions in SH-SY5Y cells

To further investigate how ADAR1 affects the expression of circ_0000418 and BDNF, hsa-adar1 si-RNA transfected SH-SY5Y cells, which showed 70% reduction in mRNA expression (Fig. 4a, $p < 0.01$) was used. qRT-PCR analysis showed that hsa-bdnf (Fig. 4b, $p < 0.01$) mRNA expression was significantly decreased and hsa_circ_0000418 expression (Fig. 4c, $p < 0.01$) was significantly increased in the ADAR1-si group as compared with those in the C group respectively. The immunofluorescence staining results showed that the optical density values of ADAR1 and BDNF immunoreactive-positive signals were significantly decreased in the ADAR1-si group (Fig. 4d-g, both $p < 0.01$) as compared with those in the C group respectively. There is no difference between C group and empty plasmid NC group in the above results.

These in vitro results showing that hsa-adar1 low-expression

increases hsa_circ_0000418 expression and decreases hsa-bdnf mRNA expression and immunoreactivity of BDNF immunoreactive positive signals in the SH-SY5Y cells, are consistent with in vivo data and further confirm a critical role of ADAR1 in impacting circ_0000418 and BDNF expressions.

3.4. Circ_0000418 impacts BDNF expression in SH-SY5Y cells

To determine the effects of circ_0000418 on BDNF expression, hsa_circ_0000418 high/low-expression SH-SY5Y cells were used to measure ADAR1 and BDNF expressions by qRT-PCR, western blot, and immunofluorescence staining. The results showed that hsa_circ_0000418 expression was significantly increased ($p < 0.01$) in the circ_0000418 pcDNA group and was significantly decreased ($p < 0.01$) in the hsa_circ_0000418 inhibitor group (Fig. 5a) as compared with those in the C group. Hsa-adar1 (Fig. 5b, $p < 0.01$) and hsa-bdnf (Fig. 5c, $p < 0.01$) mRNA expressions were significantly decreased in the circ_0000418 pcDNA group as compared with those in the C group. Furthermore, hsa-adar1 (Fig. 5b, $p < 0.01$) and hsa-bdnf mRNA expressions (Fig. 5c, $p < 0.01$) were significantly increased in the circ_0000418 inhibitor group as compared with those in the C group. ADAR1 and BDNF protein expressions in SH-SY5Y cells by western blot were shown in Fig. 5d. The gray values of ADAR1 (Fig. 5e, $p < 0.05$) and BDNF protein expressions (Fig. 5f, $p < 0.01$) were also significantly decreased in the circ_0000418 pcDNA group. In addition, the gray values of ADAR1 (Fig. 5e, $p < 0.05$) and BDNF (Fig. 5f, $p < 0.05$) protein expressions were significantly increased in the circ_0000418 inhibitor as compared with those in the C group. Moreover, the optical density of ADAR1 and BDNF immunoreactive positive signals were analyzed in hsa_circ_0000418 high/low-expression SH-SY5Y cells (Fig. 5g, i) as compared with those in the C group. The optical density values of ADAR1 (Fig. 5h, $p < 0.01$) and BDNF (Fig. 5j, $p < 0.01$) immunoreactive positive signals were significantly decreased in the hsa_circ_0000418 pcDNA group, and the optical density values of ADAR1 (Fig. 5h, $p < 0.01$) and BDNF (Fig. 5j, $p < 0.01$) immunoreactive positive signals were increased in the hsa_circ_0000418 inhibitor group as compared with those in the C group. There is no difference between C group and empty plasmid NC group in the above related data. Together, these results show that hsa_circ_0000418 impacts both the expression of BDNF and ADAR1.

3.5. miR-432 and circ_0000418 affect BDNF expression in an interactive manner in SH-SY5Y cells

In vitro study showed a negative feedback regulatory relationship between circ_0000418 and miR-432. Hsa-miR-432 expression was significantly decreased/increased (Fig. 6a, both $p < 0.01$) in the

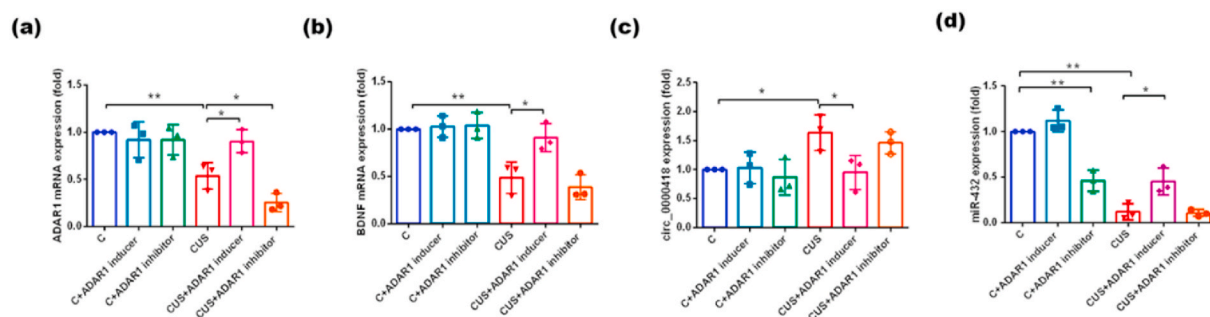


Fig. 3. ADAR1 inducer recovers the abnormal mmu-bdnf, mmu_circ_0000418, and mmu-miR-432 expressions in the frontal cortex of CUS-elicited depressive BALB/c mice.

(a) mmu-adar1 mRNA expression. (b) mmu-bdnf mRNA expression. (c) mmu_circ_0000418 expression. (d) mmu-miR-432 expression. C: control group, CUS: chronic unpredictable stress group, C + ADAR1 inducer: The mice treated with ADAR1 inducer, C + ADAR1 inhibitor: The mice treated with ADAR1 inhibitor, CUS + ADAR1 inducer: The depressive mice treated with ADAR1 inducer, CUS + ADAR1 inhibitor: The depressive mice treated with ADAR1 inhibitor. The data were expressed as mean \pm standard deviation. * $p < 0.05$, ** $p < 0.01$ ($n = 3$ /group).

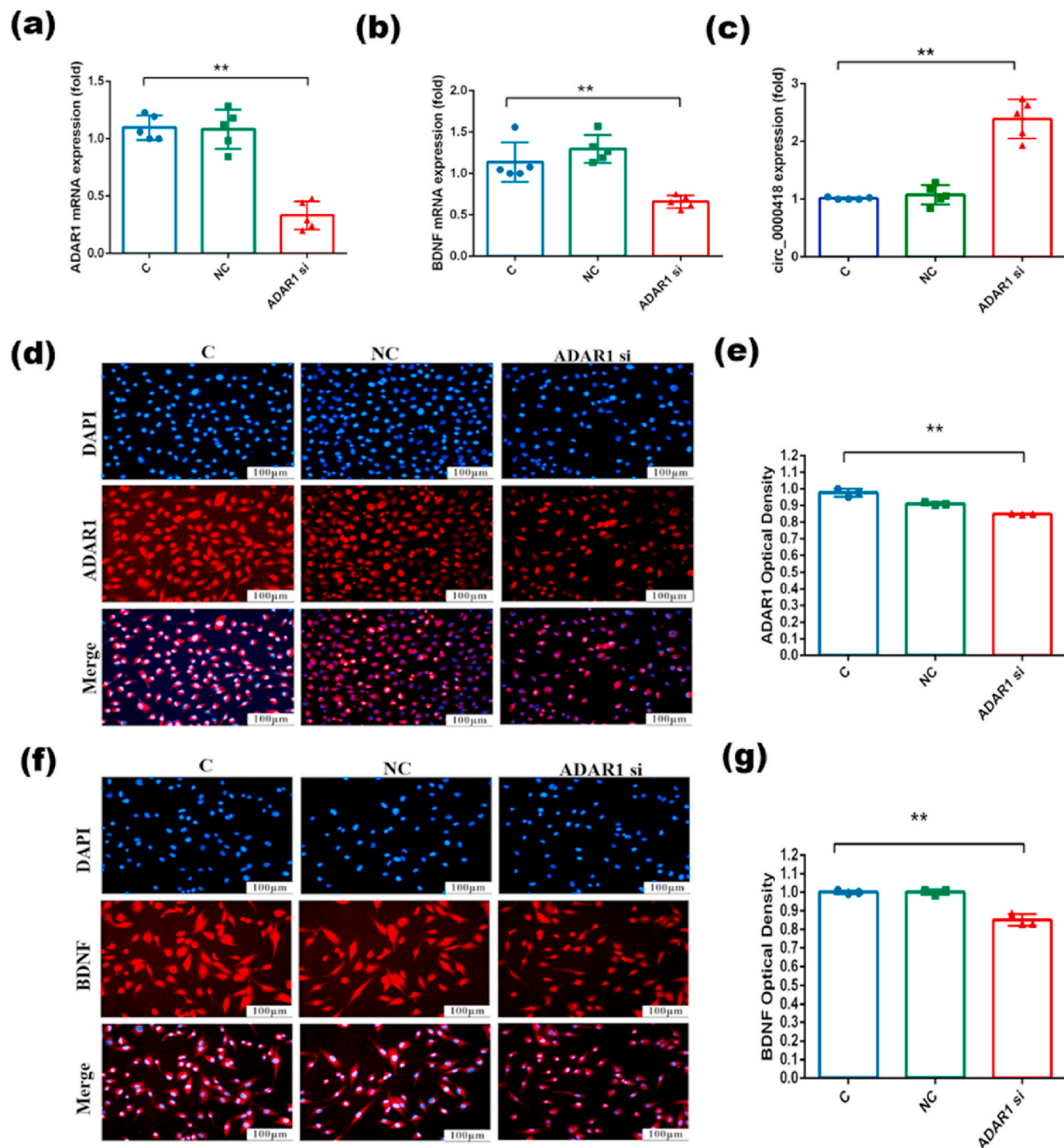


Fig. 4. ADAR1 impacts circ_0000418 and BDNF expressions in SH-SY5Y cells.

(a) *hsa-adar1* mRNA expression in SH-SY5Y cells. (b) *hsa-bdnf* mRNA expression in SH-SY5Y cells. (c) *hsa_circ_0000418* expression in SH-SY5Y cells. (d) The immunofluorescence staining results of ADAR1 immunoreactive positive signals in SH-SY5Y cells (bar = 100 μ m). (e) The optical density analysis of ADAR1 immunoreactive positive signals in SH-SY5Y cells. (f) The immunofluorescence staining results of BDNF immunoreactive positive signals in SH-SY5Y cells. (bar = 100 μ m). (g) The optical density analysis of BDNF immunoreactive positive signals in SH-SY5Y cells. C: control group. NC: negative control empty plasmid group. ADAR1-si group: *hsa-adar1* low-expression group. The data were expressed as mean \pm standard deviation. ** $p < 0.01$, ($n = 5$ /group in a,b,c; $n = 3$ /group in e,g).

hsa_circ_0000418 high/low-expression cells respectively. Conversely, the *hsa_circ_0000418* expression was significantly decreased/increased (Fig. 6b, both $p < 0.01$) in the *hsa-miR-432* high/low-expression cells, respectively. In addition, in *hsa-miR-432* and *hsa_circ_0000418* co-high/low cells, as compared with that *hsa-miR-432* high/low-expression alone cells respectively, *hsa-miR-432* expression was significantly decreased/increased (Fig. 6a, both $p < 0.01$). Similarly, in *hsa-miR-432* and *hsa_circ_0000418* co-high/low cells, *hsa_circ_0000418* expression was decreased/increased significantly (Fig. 6b, both $p < 0.01$), as compared to that in *hsa_circ_0000418* high/low-expression alone cells respectively (Fig. 6b, $p < 0.01$). These results further confirm that there

is a negative regulatory effect between *circ_0000418* and *miR-432*.

When *hsa_circ_0000418* and *hsa-miR-432* were co-high/low expressed, they showed an interactive and synergistic effect on *hsa-bdnf* mRNA expression. In *hsa_circ_0000418* and *hsa-miR-432* were co-high/low cells, *hsa-bdnf* mRNA decreased/increased significantly, as compared with that in *hsa_circ_0000418* high/low-expression alone cells (Fig. 6c, both $p < 0.05$), in the meanwhile, *hsa-bdnf* mRNA increased as compared to that in *hsa-miR-432* low-expression alone cells (Fig. 6c, $p < 0.05$). When *hsa_circ_0000418* and *hsa-miR-432* were co-high expressed, they showed an interactive and synergistic effect on *hsa-adar1* mRNA expression. In *hsa_circ_0000418* and *hsa-miR-432* co-

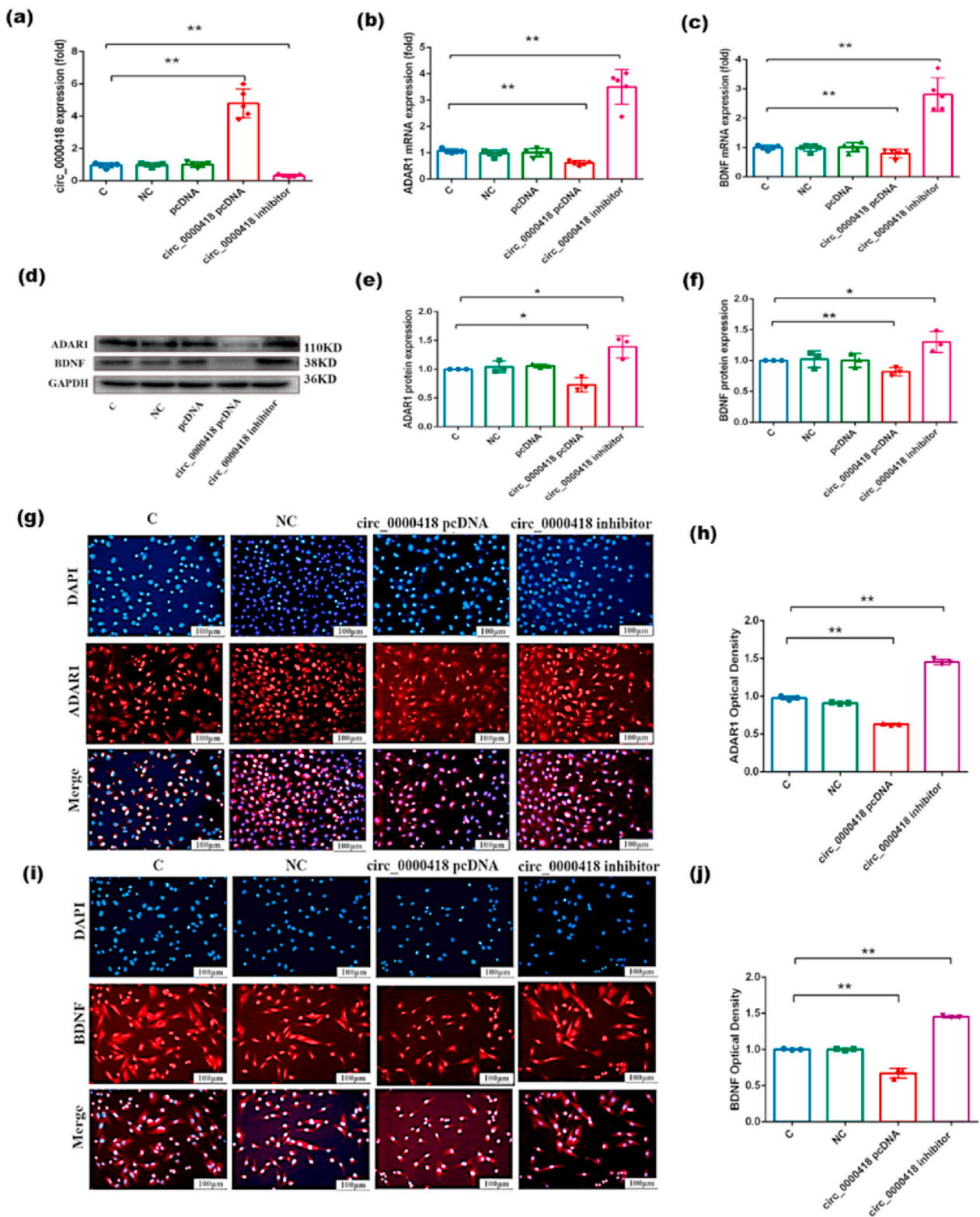


Fig. 5. High/low hsa_circ_0000418 expressions impact ADAR1 and BDNF expressions in SH-SY5Y cells. (a) hsa_circ_0000418 expression. (b) hsa-adar1 mRNA expression. (c) hsa-bdnf mRNA expression. (d) ADAR1 and BDNF protein expressions. (e) The gray values of ADAR1 protein expression were analyzed by using image analysis system. (f) The gray values of BDNF protein expression were analyzed by using image analysis system. (g) The immunofluorescence staining results of ADAR1 immunoreactive-positive signals (bar = 100µm). (h) The optical density analysis of ADAR1 immunoreactive-positive signals. (i) The immunofluorescence staining results of BDNF immunoreactive-positive signals (bar = 100µm). (j) The optical density analysis of BDNF immunoreactive positive signals. C: control group, NC: negative control empty plasmid group, circ_0000418 pcDNA group: hsa_circ_0000418 high-expression group, circ_0000418 inhibitor group: hsa_circ_0000418 low-expression group. The data were expressed as mean ± standard deviation. **p* < 0.05, ***p* < 0.01, (n = 5/group in a,b,c; n = 3/group in e,f,h,j).

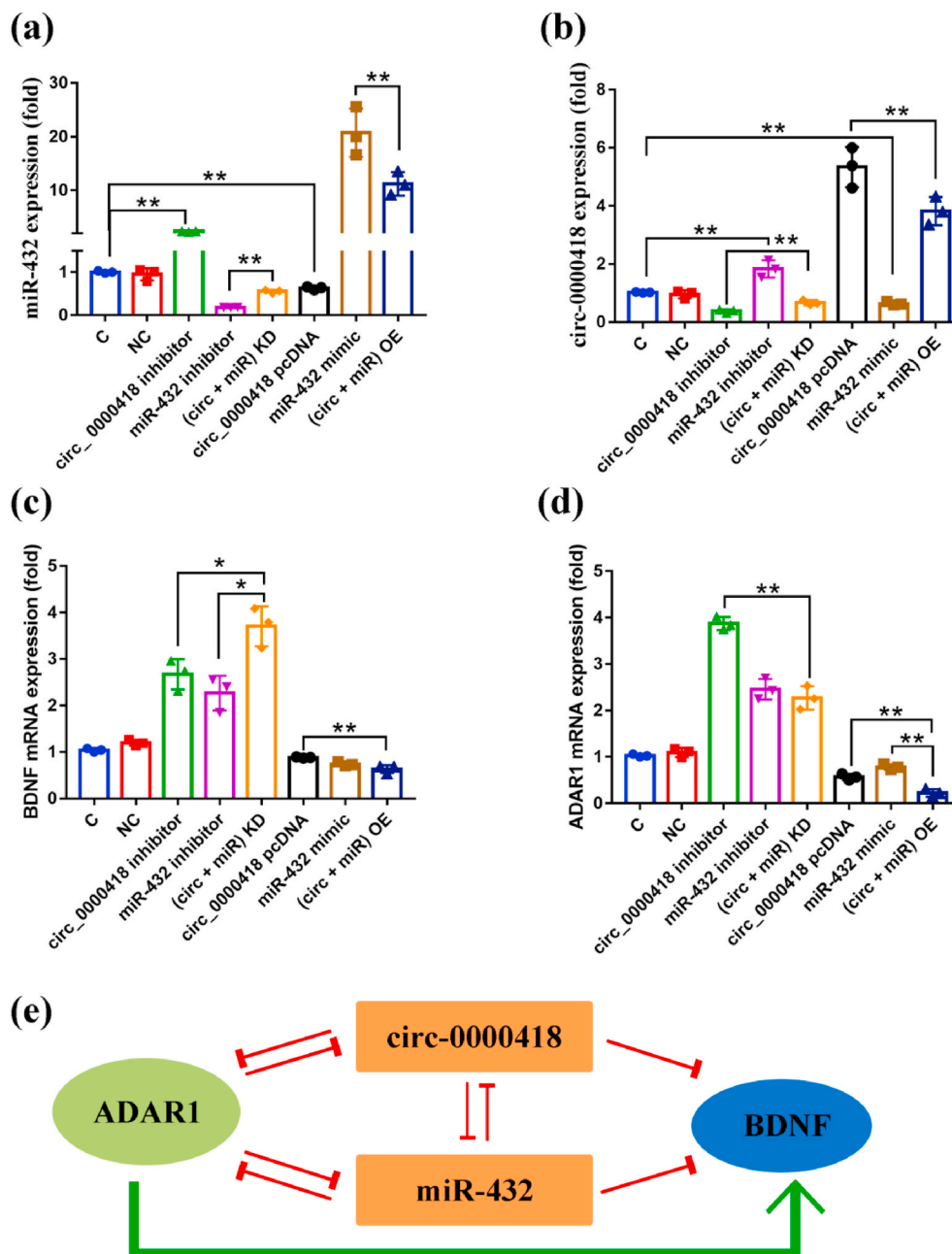


Fig. 6. Interactive effects of miR-432 and circ_0000418 co-expression on BDNF expression. (a) hsa-miR-432 expression (b) hsa_circ_0000418 expression (c) hsa-bdnf mRNA expression (d) hsa-adar1 mRNA expression. The above target RNA expressions were analyzed in hsa-miR-432 high/low-expression cells, hsa_circ_0000418 high/low-expression cells, hsa-miR-432 and hsa_circ_0000418 co-high/low expression cells. (n = 3/group). C: control group; NC: negative control group; circ_0000418 pcDNA: hsa_circ_0000418 high-expression group; circ_0000418 inhibitor: hsa_circ_0000418 low-expression group, (circ + miR) OE: hsa_circ_0000418 and hsa-miR-432 co-high expression group, (circ + miR) KD: hsa_circ_0000418 and hsa-miR-432 co-low expression group. The data were expressed as mean ± standard deviation. **p* < 0.05 and ***p* < 0.01. (e) The relationships among ADAR1, circ_0000418, miR-432, and BDNF. "T" means inhibition, arrow means promotion.

high cells, hsa-adar1 mRNA expression was significantly decreased (Fig. 6d, both *p* < 0.01), as compared to that in hsa_circ_0000418 high-expression alone cells and hsa-miR-432 high-expression alone cells respectively. Interestingly, in hsa_circ_0000418 and hsa-miR-432 co-low cells, hsa-adar1 mRNA expression was significantly decreased (Fig. 6d, *p* < 0.01), as compared to that in hsa_circ_0000418 low-expression alone cells. This may be that when hsa_circ_0000418 and hsa-miR-432 decrease together, it will cause other potential molecules to affect hsa-adar1 mRNA expression, which needs further research and confirmation.

Additionally, hsa-miR-432 binding ability to AGO2 was significantly increased (IgG: 1.00 ± 0.07, AGO2: 5.26 ± 0.91, *p* < 0.01) and hsa_circ_0000418 binding ability to AGO2 was significantly decreased (IgG: 1.00 ± 0.07, AGO2: 0.63 ± 0.04, *p* < 0.01) as compared with that in IgG control group. These results suggest that sponge mechanism is not involved in the interaction between miR-432 and circ_0000418.

3.6. Potential target molecules involved in mediating the regulatory function of ADAR1 on BDNF, miR-432 and circ_0000418: A bio-informatics analysis

Bio-informatics analyses showed that RNA binding proteins that bind to hsa_circ_0000418 include: AGO1, C22ORF28, DGCR8, EWSR1, FUS, FXR2, LIN28A, LIN28B, TNRC6, ZC3H7B, AUF1, EIF4A3, FMRP, HNRNPC, HuR, IGF2BP1, IGF2BP2, IGF2BP3, PTB, SFRS1, U2AF65, and TAF15. The proteins associating with hsa-miR-432 include: IGF2BP2, IGF2BP3, DGCR8, FUS, LIN28B, STAT2, and AGO1 by checking in "targetscan human". The proteins related to ADAR1 include: DROSHA, DICER1, LIN28B and STAT2 by checking in "<https://string-db.org>". Based on the above bio-informatics results, human recombinant protein (LIN28B) is the potential molecule being involved in the interaction among ADAR1, hsa-miR-432, and hsa_circ_0000418 (Fig. 7a).

Another bio-informatics analysis indicated that molecules closely related to BDNF are predicted to be: NTRK2, FMR1, HNRNPC, HNRNPK,

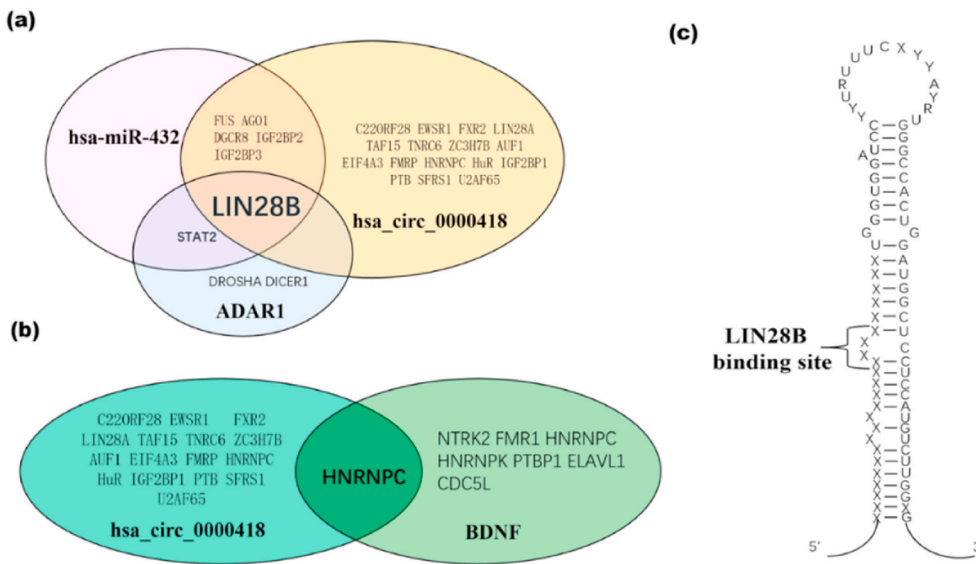


Fig. 7. Potential target molecules involved in mediating the regulatory function of ADAR1 on BDNF, miR-432 and circ_0000418.

Bio-informatics analysis was performed by the <https://circinteractome.nia.nih.gov/> link and <http://asia.ensembl.org/index.html> link. (a) LIN28B is the potential molecule involved in the interaction among ADAR1, miR-432, and circ_0000418. The link <https://string-db.org> was used. (b) HNRNPC is the potential target RNA binding protein closely related to both circ_0000418 and BDNF. “http://asia.ensembl.org/Gorilla_gorilla/Gene/SecondaryStructure?db=core;g=ENSGGOG000000030221;r=14:84901729-84901822;t=ENSGGOT00000036390” link was use. (c) Secondary structure of hsa-pri-miR-432, X represents the potential nucleotide base, Y represents C (Cytosine) or U (Uracil), R represents A (Adenine) or G (Guanine). There is the potential motif “GGAG” in the stem loop structure of hsa-pri-miR-432, which binds to LIN28B.

PTBP1, ELAVL1, CDC5L. Based on the above bio-informatics results, heterogeneous nuclear ribonucleoprotein C (HNRNPC) (Fig. 7b) is the potential target nuclear protein closely related to both hsa_circ_0000418 and BDNF, and is used as the potential target protein involved in the interaction between BDNF and hsa_circ_0000418. Based on the published literature (Cho et al., 2012; Nowak et al., 2014; Oh et al., 2018), and the search from the gene bank, “GGAG” is the potential binding site for LIN28B. It is likely that hsa-pri-miR-432 may contain the “GGAG” motif in its pre-miRNA terminal loop.

4. Discussion

In this study, we uncover a new molecular mechanism underlying the

antidepressant action of ADAR1 in CUS-exposed mice. We demonstrate that an increase in the expression of ADAR1 after IFN- γ treatment alleviates depressive-like behavior. Further, in vivo studies reveal the involvement of miR-432 and circ_0000418 in mediating the action of ADAR1 in the expression of brain BDNF, which is a key factor participated in the pathogenesis of depression. A series of in vitro studies using SH-SY5Y cells with altered levels of expression of miR-432 and hsa_circ_0000418 further confirm critical roles of these two RNAs in bridging the interactions between ADAR1 and BDNF. Together, this study provides the first evidence indicating important roles of miR-432 and circ_0000418 in mediating the anti-depressant action of ADAR1. For further discussion, we construct a figure (Fig. 8) to illustrate possible molecular interactions mediating the antidepressant effect of ADAR1.

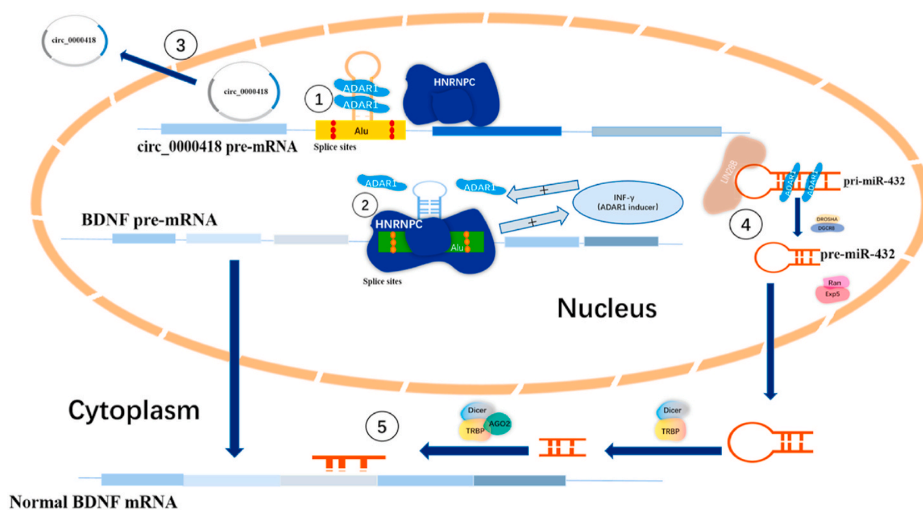


Fig. 8. The possible molecular mechanisms underlying the antidepressant action of ADAR1

① ADAR1 acts on the near Alu sequence of hairpin structure in circ_0000418 pre-mRNA through A-I editing and affects the bio-gensis of mature circ_0000418; ADAR1 is complementary with the flanking sequences of circ_0000418 exon, and inhibits the cyclization of circ_0000418, leading to the decreased circ_0000418 expression; HNRNPC acts on the hairpin structure of near Alu sequence in circ_0000418 pre-mRNA by affecting the exposure of splicing sites of circ_0000418 and affects the bio-gensis of mature circ_0000418. HNRNPC and ADAR1 are shown to be competitive inhibition with each other. ② ADAR1 acts on the hairpin structure near the Alu sequence of BDNF pre-mRNA and affects the maturation process of BDNF pre-mRNA through A-I editing, HNRNPC acts on the hairpin structure near the Alu sequence of BDNF pre-mRNA and affects the maturation process of BDNF pre-mRNA by affecting the exposure of splicing sites. Circ_0000418 consumes a large amount of HNRNPC during maturation, resulting in the decrease of interferon, and then leading to the decrease of

ADAR1 expression. ③ Circ_0000418 pre-mRNA is mainly located in the nucleus and exits to the cytosol via nuclear export to release mature circ_0000418, which contains both intron and exon. The effect of circ_0000418 on BDNF mRNA expression is due to that both circ_0000418 pre-mRNA and BDNF pre-mRNA bind with ADAR1 and HNRNPC in a competitive inhibition manner. ④ ADAR1 influences the formation of pri-miR-432 and pre-miR-432 through A-I editing, and affects miR-432 maturation process; LIN28B combines with pri-miR-432, and then degrades pri-miR-432, and affects the formation process of mature miR-432. Both LIN28B and ADAR1 affect the formation of mature miR-432. LIN28B promotes ADAR1 expression via reducing miR-432. ⑤ MiR-432 affects BDNF mRNA through RNA interference, and then reduces BDNF expression. MiR-432 and circ_0000418 affect BDNF expression in a synergistic and interactive manner with the regulatory role of ADAR1.

4.1. Roles of ADAR1 in CUS-induced depressive like behaviors

ADAR1, as an RNA editing enzyme, is involved in depressive behavior resulted from chronic exposure to a variety of environmental stressors through epigenetic regulation both in human and experimental animals. BALB/c mice show decreased cerebral ADAR1 when they are undergoing social isolation stress exposure (Chen et al., 2016; Yu et al., 2018; Zhang et al., 2021). In addition, ADARB1 variants are considered suicide attempt risk factors in psychiatric patients with exposures to a various childhood and recent stressful experiences (Karanovic et al., 2015). ADAR1 inducer exerts antidepressant effects by restoring the decrease of ADAR1 protein expression in the brain of chronic unpredictable stress exposed BALB/c mice (Zhang et al., 2021). These reports confirm that maintaining ADAR1 homeostasis is a core element in the treatment of stress-induced depressive-like behaviors. This study demonstrates that ADAR1 inducer alleviates depressive-like behavior and rescues the decreased frontal *adar1* mRNA expression in depressed mice, which are consistent with these published reports.

So far, the molecular mechanism as to how ADAR1 exerts antidepressant action remains unclear. A study found that cerebral 5-HT_{2C} receptor RNA editing at A and D sites is significantly enhanced in depression patients with suicide attempt (Iwamoto and Kato, 2003). Our results provides an alternative molecular pathway indicating a critical role of miR-432 and *circ_0000418* in linking the association between ADAR1 and BDNF which are involved the CUS-induced depressive behaviors. Together, results from this study provided additional information confirming the important role of ADAR1 in depressive behaviors.

4.2. Roles of miR-432 and *circ_0000418* in coupling ADAR1 and BDNF in exerting antidepressant effects

BDNF is known to be a biomarker of depression, which is closely related to the occurrence, development and prognosis of depression (Karpova, 2014; Peng et al., 2018; Rana et al., 2020; Tripp et al., 2012). Dwivedi Y et al. reported that BDNF expression in frontal cortex and hippocampus of suicide patients is significantly decreased (Dwivedi et al., 2003; Karege et al., 2005). Antidepressants improve stress-induced depressive-like behavior by promoting BDNF expression (Tian et al., 2019). miR-432 also decreases BDNF mRNA through RNAi, (Zhang et al., 2021). *Rno_circ_0001223* over-expression was reported to promote *rno-bdnf* mRNA and BDNF protein expressions in PC12 cells through cAMP response element binding protein (Zhang et al., 2018). Bu Q et al. reported that *mmu_circ_002381* acts on *mmu-miR-138-5p* and reduces BDNF expression in N2a cells via sponge mechanism (Bu et al., 2019). Our in vitro study confirms that *hsa_circ_0000418* high expression decreases the expression of mRNA and protein of *bdnf*. By contrast, *hsa_circ_0000418* low expression increases the expression of mRNA and protein of *bdnf*.

So far, the contribution of circRNA in the occurrence and development of depression is still unclear. Only a few reports have been published. Zhang et al. reported that *mmu_circ_0001223* expression is significantly decreased in the ventral medial prefrontal cortex and hippocampus in chronic unpredictable mild stress-exposed depressive mice (Zhang et al., 2018). Panax notoginseng saponins, a herb medicine, alleviates the depressive like behavior of chronic unpredictable mild stress-exposed depressive mice by recovering decreased *mmu_circ_0001223* expression in the brain. The same paper also shows that over-expression of *mmu_circ_0001223* enhances CREB1 and BDNF protein expressions in cultured PC12 cells (Zhang et al., 2018). Another report shows that *circDYM* levels are significantly decreased both in the peripheral blood of patients with MDD and in the two depressive-like mouse models, including the chronic unpredictable stress and lipopolysaccharide models. Restoration of *circDYM* expression significantly attenuates depressive-like behavior and inhibits microglial activation in the above-mentioned two depressive mouse models (Zhang et al., 2020). Cui et al. found that *hsa_circRNA_103636* in peripheral blood

mononuclear cells is significantly decreased in MDD patients and is recovered 4-week and 8-week after treatment with antidepressant regimens (Cui et al., 2016). The expression level of *hsa_circ_0126218* displays up-regulation in peripheral blood mononuclear cells of female patients with MDD. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses state that some of the enriched pathways downstream of *hsa_circ_0126218* are closely related to MDD (Bu et al., 2021). In agreement with the above evidence, our study found that *mmu_circ_0000418* expression was significantly increased in CUS mice. IFN- γ treatment restored the increased levels of *mmu_circ_0000418* expression back to normal values in the frontal cortex of CUS depressive BALB/c mice. Moreover, our in vitro results confirmed that *hsa_circ_0000418* impacts both the expression of BDNF and ADAR1 in SH-SY5Y cells. We also demonstrated a negative feedback regulatory relationship between *hsa_circ_0000418* and *hsa_miR-432*. When *hsa_circ_0000418* and *hsa-miR-432* were co-high/low expressed, they showed an interactive effect on *hsa-bdnf* mRNA and *hsa-adar1* mRNA expressions respectively. Together, our results indicate that *circ_0000418* interacts with miR-432 and mediates the antidepressant effects between ADAR1 and BDNF.

In addition, our in vitro study shows a negative feedback regulation relationship between *hsa_circ_0000418* and *hsa_miR-432*. As for the detailed molecular mechanism of negative feedback regulation between *circ_0000418* and miR-432, we speculated that it was the sponge mechanism because of that the specific circRNAs can be used as competitive inhibitors to inhibit the ability of corresponding miRNAs to bind to their target mRNA through sponge mechanism, thereby affecting the corresponding target proteins expressions (Ebert and Sharp, 2010; Hansen et al., 2013). Unexpectedly, the interaction between miR-432 and *circ_0000418* is not via sponge mechanism, which is supported by the RIP results of this study.

4.3. LIN28B and HNRNPC are potential key molecules participated in the action of ADAR1

Based on our bio-informatics prediction results, LIN28B and HNRNPC are potentially involved in the action of ADAR1 on miR-432 and *circ_0000418* affecting BDNF expression. It has been known that ADAR1 up-regulates LIN28B expression (Zipeto et al., 2016). LIN28B binds to the "GGAG" motif of pre-miRNA, resulting in miRNAs degradation (Cho et al., 2012; Nowak et al., 2014; Oh et al., 2018). According to the information from gene bank, *hsa-pri-miR-432* contains the "GGAG" motif in its pre-miRNA terminal loop (Fig. 7c). Thus, it is highly likely that LIN28B will combine with pre-miR-432, and then process pre-miR-432 and release mature miR-432. HNRNPC has been shown to bind and obscure Alu sequences on pre-mRNAs and protect against Alu exonization (Zarnack et al., 2013). Alu sequences are known to generate long double-stranded RNAs edited by ADAR1 (Liddicoat et al., 2015). In addition, decreased HNRNPC levels cause that the splice sites in intronic Alu sequences are exposed, then result in the inappropriate exonization. The double-stranded RNA structures derived from Alu sequences trigger the induction of interferon. ADAR1 is an interferon-inducible double-stranded RNA-specific adenosine deaminase (Sarbanes et al., 2018).

Based on these reports, we propose a mechanism how HNRNPC interacts with *circ_0000418*. HNRNPC may act on the hairpin structure of near Alu sequence in *circ_0000418* pre-mRNA by affecting the exposure of splicing sites of *circ_0000418*, and then enhance the production of mature *circ_0000418*. The maturation of both *circ_0000418* and *bdnf* mRNA requires the presence of *adar1* and HNRNPC molecules in a competitive manner, thus the increase of *circ_0000418* would decrease the amount of *adar1* and HNRNPC. This will result in the decrease of the mature *bdnf* mRNA production. The detailed mechanism on the effect of *circ_0000418* on BDNF mRNA expression may be due to that both *circ_0000418* pre-mRNA and BDNF pre-mRNA have binding sites with ADAR1 and HNRNPC, so they play a competitive role in binding with ADAR1 and HNRNPC. We also found that *circ_0000418* negatively

affects ADAR1 expression. However, the possible mechanism underlying this negative regulation is not clear from our study. We postulate that circ_0000418 requires a large number of HNRNPC to participate in its maturation process. Increased HNRNPC is known to reduce endogenous interference production (Sarbanes et al., 2018), which may decrease ADAR1 expression. Circ_0000418 may also affect ADAR1 expression by acting on the transcription of ADAR1 gene and the translation of ADAR1 mRNA. This hypothesis remains to be further studied by more related molecular network prediction and analysis.

In summary, our results indicate that ADAR1 affects CUS-induced depressive-like behavior and BDNF expression mediated through miR-432 and circ_0000418. Elucidation of this new molecular mechanism will not only provide insights into further understanding the important role of ADAR1 in stress-induced depressive-like behavior, but also suggest a potential therapeutic strategy for developing novel anti-depressive drugs.

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Ethical statement

All experimental procedures are approved by the Tab of Animal Experimental Ethical Inspection, Dalian Medical University. The treatment of animals in all experiments conforms to the ethical standards of experimental animals.

CRediT authorship contribution statement

Xiaonan Zhang: contributed to the study design. **Wei Yan:** Writing – review & editing, Formal analysis. **Ying Xue:** Formal analysis. **Hong Xu:** conducted the behavior experiments and drug treatment. **Jinying Li:** performed western blot, and cell culture and RNA oligo transfection. **Ziwei Zhao:** Formal analysis. **Ye Sun:** contributed to the study design, Supervision. **Yanfeng Wang:** contributed to the data collection and analysis, performed the qRT-PCR. **Jiaqian He:** performed immunofluorescence staining. **Yuyue Huang:** performed immunofluorescence staining. **Deqin Yu:** conducted the behavior experiments and drug treatment. **Zhaoyang Xiao:** Formal analysis, contributed to the study design, performed western blot, and cell culture and RNA oligo transfection, contributed to the data collection and analysis, performed the qRT-PCR, contributed to the bioinformatic prediction, contributed to the writing—original draft, Writing – original draft. **Shengming Yin:** performed western blot, and cell culture and RNA oligo transfection.

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.

Abbreviations

A	Adenine
ADAR	Adenosine deaminase acting on RNA
AGO1	Argonaute 1
A-to-I RNA editing	Adenosine-to-inosine RNA editing
AUF1	AU-rich element RNA-binding factor 1
BDNF	Brain-derived neurotrophic factor
C	Cytosine
C22ORF28	Chromosome 22 open reading frame 28
CDC5L	Cell division cycle 5-like
Circ RNA	Circular RNA

CUS:	Chronic unpredictable stress
CREB1	cAMP responsive element binding protein 1
DGCR8	DiGeorge syndrome critical region 8
Drosha	Drosha ribonuclease III
EIF4A3	Eukaryotic translation initiation factor 4A3
ELAVL1	ELAV-like RNA-binding protein1
EWSR1	EWS RNA-binding protein1
FMR1	Fragile X mental retardation1
FMRP	Fragile X mental retardation protein
FST	Forced swimming test
FUS:	Fused in sarcoma
FXR2	Fragile X-related protein2
G	Guanine
HNRNPC	Heterogeneous nuclear ribonucleoprotein C
HNRNPK	Heterogeneous nuclear ribonucleoprotein K
Hsa	<i>Homo sapiens</i>
HuR	Human antigen R
5-HT2C	Serotonin2C
IGF2BP1	Insulin-like growth factor2 mRNA-binding protein1
IGF2BP2	Insulin-like growth factor2 mRNA-binding protein2
IGF2BP3	Insulin-like growth factor2 mRNA-binding protein3
IFN- γ :	Interferon-gamma
LIN28A	Lin-28 homolog A
LIN28B	Lin-28 homolog B
Mmu	Mus musculus
miR	microRNA
NTRK2	Neurotrophic receptor tyrosine kinase2
NC	Negative control
OFT	Open field test
PTB	Phosphotyrosine binding
PTBP1	Polypyrimidine tract-binding protein1
pri-miRNAs	primary microRNAs
RIP	RNA immunoprecipitation
SFRS1	Splicing factor Arg/Ser rich 1
STAT2	Signal transduction and activator of transcription 2
TNRC6	Trinucleotide repeat containing 6
TAF15	TATA-box binding protein associated factor 15
TST	Tail suspension test
U2AF65	U2 small nuclear ribonucleoprotein auxiliary factor 65
U	Uracil
ZC3H7B	Zinc finger CCCH-type containing 7B

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