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# Inflammation-dependent ISG15 upregulation mediates MIA-induced dendrite damages and depression by disrupting NEDD4/Rap2A signaling

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ABSTRACT

*Background:* Maternal immune activation (MIA) is an independent risk factor for psychiatric disorders including depression spectrum in the offsprings, but the molecular mechanism is unclear. Recent studies show that interferon-stimulated gene-15 (ISG15) is involved in inflammation and neuronal dendrite development; here we studied the role of ISG15 in MIA-induced depression and the underlying mechanisms.

*Methods:* By vena caudalis injecting polyinosinic: polycytidylic acid (poly I:C) into the pregnant rats to mimic MIA, we used AAV or lentivirus to introduce or silence ISG15 expression. Synaptic plasticity was detected by confocal microscope and Golgi staining. Cognitive performances of the offspring were measured by Open field, Forced swimming and Sucrose preference test.

*Results:* We found that MIA induced depression-like behaviors with dendrite impairments in the offspring with ISG15 level increased in the offsprings' brain. Overexpressing ISG15 in the prefrontal cortex of neonatal cubs (P0) could mimic dendritic pathology and depressive like behaviors, while downregulating ISG15 rescued these abnormalities in the offsprings. Further studies demonstrated that MIA-induced upregulation of inflammatory cytokines promoted ISG15 expression in the offspring' brain which suppressed Rap2A ubiquitination *via* NEDD4 and thus induced Rap2A accumulation, while upregulating NEDD4 abolished ISG15-induced dendrite impairments.

*Conclusions:* These data reveal that MIA impedes offsprings' dendrite development and causes depressive like behaviors by upregulating ISG15 and suppressing NEDD4/Rap2A signaling. The current findings suggest that inhibiting ISG15 may be a promising intervention of MIA-induced psychiatric disorders in the offsprings.

## 1. Introduction

Epidemiological studies indicate that maternal bacterial or viral infection during pregnancy increases the risk of developing several psychiatric disorders in the offsprings, such as depression, schizophrenia and autism spectrum disorder (ASD) [1–4]. Therefore, maternal immune activation (MIA) has been considered as a critical link between prenatal maternal infection and postnatal behavioral deficits. Neurochemical and behavioral abnormalities in the offspring caused by MIA have also been proved by rodents (mice and rats) or nonhuman primates (rhesus monkeys) exposed to lipopolysaccharide (LPS) or a viral mimic synthetic double-stranded RNA (polyinosinic:polycytidylic acid, poly I:C) during pregnancy [5–8]. However, the molecular mechanisms underlying MIA-induced neurodevelopmental disorders in the offspring are not fully understood. Current studies have mainly focused on investigating the effects of certain individual cytokine in MIA-induced abnormalities. For instance, it has been shown that pregnant dams injected with recombinant interleukin-6 (IL-6) alter fetal brain

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development with behavioral abnormalities [9]. Similarly, treatment with interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α) or IL-6 inhibits dendrite development in primary cultured neurons [10,11]. However, numerous pro-inflammatory and anti-inflammatory cytokines are increased during MIA including IL-1β, IL-6, CXCL8, TNF and IL-10, which may contribute to MIA-induced abnormalities in the offspring. It remained to be elucidated where these cytokines share a common signaling pathway?

Interferon-stimulated gene-15 (ISG15) is one of the first recognized ISG proteins upregulated in the brain after systemic interferon (IFN) exposure, viral infection, LPS, or retinoic acid administration [12–14]. As an antiviral protein, ISG15 is against influenza, sindbis, herpes and human immunodeficiency virus type 1 [15–17]. It can covalently conjugate to proteins (termed ISGylation) by similar processes to that of ubiquitin but exerts its biological effect by antagonizing ubiquitin-dependent target proteins [18]. Expression of free ISG15 or the ISGylation system (UbE1L and UbcH8) inhibits budding of Ebola virus VP40 VLPs by downregulating NEDD4 activity and VP40 ubiquitination [19]. As an E3 ubiquitin ligase, NEDD4 is abundantly expressed in the mammalian neurons and a crucial modulator for axon and dendrite branching [20,21]. Disrupting NEDD4 function severely inhibits axon branching in Xenopus retinal ganglion cells, and knockout NEDD4 impairs dendrite development in neurons [20,21].

Based on the above observations, we speculated that ISG15 might play a role in MIA-induced impairment in neurodevelopment and psychiatric disorders in the offsprings. To test this hypothesis, rats at gestation day 17 (GD17) received poly(I:C) injection through vena caudalis to mimic the acute phase response to viral infection, a well-established model of MIA [22–24]. Dendritic development and behavioral changes were observed in the offsprings, and the potential roles of ISG15 and NEDD4/Rap2A signaling were examined.

## 2. Methods and materials

### 2.1. Plasmids and virus

pCI-HA-NEDD4 plasmid was got from addgene (a gift donated by Dr. Joan Massague, Sloan Kettering Cancer Center, the Howard Hughes Institute for medicine), and then we constructed plasmid pCI-HA-NEDD4-EGFP (EGFP sequence is not fused with NEDD4 sequence). Adeno-associated virus AAV-EGFP-ISG15 and AAV-mCherry-ISG15 (EGFP or mCherry sequence is not fused with ISG15 sequence) were purchased from OBio Biologic Technology Co., Ltd. (Shanghai, China), and lentivirus lenti-shISG15-EGFP was purchased from Heyuan Biologic Technology Co., Ltd. (Shanghai, China).

### 2.2. Antibodies and reagents

Mouse monoclonal antibody (mAb) anti-Rap2A, rabbit polyclonal antibody(pAb)anti-GFP and anti-ISG15 were from Santa Cruz; mAb anti-GFAP and anti-STAT1 (phospho Y701), pAb anti-Ubiquitin, anti-NEDD4 and anti-PSD95 were from Abcam; mAb anti-α-tubulin (DM1A) was from Sigma; mAb anti-NeuN was from Millipore, mAb SMI-312R was from Convance; mAb anti-MAP2 and Synaphin I were from Millipore, mAb anti-STAT1 was from Cell Signaling. The dilution of the used antibodies is 1:1000 for Western Blotting or 1:300 for immunofluorescent staining. Poly(I:C) (Polyinosinic-polycytidylic acid sodium salt) and IFN-α Recombinant Rat Protein were purchased from Sigma. Recombinant inflammatory cytokines (IL-1, IL-2, IL-6, IL-10, IL-14, IL-17, TNF-α, INF-α, and IFN-β) were the gifts from Prof. Xiu-Fang Weng and Prof. Xiang-Ping Yang (Department of Immunology, Tongji Medical College, Huazhong University of Science and Technology). Human IFN-α (Interferon Alpha), human IFN-β (Interferon Beta), human IL-1α (Interleukin 1 Alpha) ELISA Kit, TNF-α (Tumor Necrosis Factor Alpha) ELISA Kit, ISG15 (Interferon Stimulated Gene 15) ELISA Kit, and Rat IFN-α (Interferon α), IL-1, IL-2, IL-6, IFN-β, TNF-α ELISA Kit were from Elabscience Biotechnology Co., Ltd.

### 2.3. Animal model

Sprague-Dawley rats were bred in the Experimental Animal Central of Tongji Medical College, Huazhong University of Science and Technology. Rats were mated at the age of 3 months and the first day after copulation was defined as day 1 of pregnancy, at gestational day (GD) 17, pregnant rats received a single injection of poly(I:C) or normal saline *via* caudal vein under the mild physical constraint with no apparent ill effects and body weight loss. Poly(I:C) was dissolved in saline to obtain a desired dosage (10 mg/kg, calculated based on the pure form poly(I:C), the volume of injection was 5 ml/kg). For IFN-α treatment, the recombinant protein (Sigma-Aldrich) in saline or same volume of saline was injected into dams *via* caudal vein. The animals were returned to the home cage after injection procedure. When experimental animals were used, adequate measures were taken to minimize pain or discomfort. All experiments were performed according to the 'Policies on the Use of Animals and Humans in Neuroscience Research' revised and approved by the Society for Neuroscience in 1995, and the animal study was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology.

### 2.4. Sample collection and cytokine measurements

At 12 h after poly(I:C) injection or P0d, 1 ml peripheral blood was collected from each pregnant dam, and then the blood was centrifuged to obtain serum at 4 °C for 20 min (12,000 g). The serums were stored at 80 °C until detection.

All samples (in duplicate) were assayed with ELISA kit (Elabscience). Assays were performed according to the manufacturer's instructions. Lyophilized cytokine standards were reconstituted first to a master standard stock using 500 μl of diluents, nine concentrations of the standards were made by eight, threefold serial dilutions of the master standard stock in serum diluent. Serum samples were diluted 1:3 prior to assay and 100 μl dilutions were assayed for each well. The plates were then read in the Bio-Rad 200 System and the data was analyzed using Curve Exert1.3 software with 5-parameter logistic regression (5PL) curve fitting to determine the standard curve and extrapolate the sample concentrations from the standard curve.

### 2.5. Western blotting and co-immunoprecipitation

Western blotting was performed as the method established in our lab [25]. Briefly, proteins extracted from cell lysis or brain tissues were separated and transferred to nitrocellulose membranes. Then the membranes were blocked and incubated with primary antibody at 4 °C overnight. Finally, the membranes were incubated with anti-rabbit or anti-mouse IgG conjugated to IRDye™ (800 CW) for 1 h and visualized using the Odyssey Infrared Imaging System (Licor biosciences, Lincoln, NE, USA). Gel-Pro Analyzer 4 (Toyobo, Osaka, Japan) software was used to measure the optical densities of targeted protein bands.

For co-immunoprecipitation (co-IP), cells were harvested in IP buffer, and the supernatants were obtained by centrifugation (17,500*g*, 15 min, 4 °C). Soluble fractions were combined with 50 μl Protein G-Dynabeads (100.04D, Invitrogen), pre-incubated with antibodies followed by overnight rotation at 4 °C. Dynabeads complexes were washed 3 times with IP buffer, which supplemented with 500 mM NaCl. Immunoprecipitates were eluted by heating (95 °C, 5 min) in sample buffer containing 5% 2-mercaptoethanol before Western blotting.

### 2.6. Cortical neurons culture

Primary cultured cortical neurons from 17 days embryonic (E17) rat cortex were isolated in HBSS (Invitrogen) containing 0.025% trypsin at 37 °C. Neurons were plated on poly-D-lysine and laminin (Bioscience)

pre-coated glass coverslips in Neurobasal medium (Invitrogen), supplemented with 2% B27/0.5 mM glutamine/25 mM glutamate, the cell density was controlled at 1–2 × $10^5$ cell/well in 12-well plate and 1–2 × $10^6$ cell/well in 6-well plate. Half the culture medium was changed every 3 days, and plates were maintained in a humid 5% $CO_2$ incubator at 37 °C. After transfection with virus or treatment with drugs, cells were either fixed for immunofluorescence studies or lysed for western blot analysis.

### 2.7. Virus injection in neonatal pups

The male neonatal pups (on the day of birth, designated as P0) were individually cryoanesthetized by placed on ice (with a thin cushion) for 5 min. After sterilized with 75% alcohol, 2 μl of virus were slowly infused into each lateral ventricle with a specially designed 10-μl Hamilton syringe with a 30-gauge/0.5-inch/hypodermic cemented needle (Halmiton Syringe Co., Reno, NV, USA) [26]. The pups were placed back to mother rats after warmed up at 37 °C.

### 2.8. Immunofluorescent staining

For brain sections, animals were sacrificed and perfused, brains were removed and cryoprotected by overnight immersion in 30% sucrose, then they were embedded in OCT compound. The frozen brain was coronally sectioned at 25 μm with a cryostat (HM505E, Microm, Germany). For primary neuron cultures, cells were fixed in 4% paraformaldehyde. Brain sections and cells were permeabilized in 1% Triton X-100-PBS for 20 min, then they were incubated with 5% BSA for 1 h to block nonspecific sites and probed with primary antibodies at 4 °C overnight. The immunoreactivity was detected by incubated with Rhodamine Red-X- (Molecular Probes, R6393, Eugene) or Oregon Green 488-conjugated secondary antibodies (Molecular Probes, O6383, Eugene) at 37 °C for 1 h. All fluorescence images were captured with a Zeiss LSM 710 laser-scanning confocal fluorescence microscope (Zeiss, Jena, Germany) equipped with Zen software.

### 2.9. Neuronal morphometry

Neuronal morphometry was carried out as below. Briefly, dendritic marker MAP2 and axonal marker SIM-312R were used to identify dendrites and axons of neurons. For dendrites, three parameters were measured: (i) the number of primary dendritic, where the primary dendrites were defined as those MAP2-positive processes directly emerging from the soma. (ii) the total length of dendrite, including the length of all primary dendrites and dendritic branches, and (iii) the dendritic branch tips number, which represents all dendritic branch ends number. For axons, the length of the longest processes emerging from soma with a SMI-312R-positive signal was measured. All images were analyzed using Image-Pro Plus 6.0 software. Repeated experiments were implemented for statistical analysis.

Animals were anaesthetized and transcardially perfused. Brain tissue was dissected out and fixed in 4% PFA at 4 °C overnight, washed three times, and sunk in 30% sucrose-PBS. 30% Agar-embedded 30 μm coronal sections were cut on LEICACM1900 and coverslipped with Immuno mount (Shandon). Confocal fluorescence images were obtained using the ZEISS LSM confocal microscope.

### 2.10. Relative quantitative RT-PCR

Trizol reagent was used to extract RNA from primary cultured neurons and prefrontal cortex of rat brain, and reverse transcription was carried out according to manufacturer's instruction (TaKaRa, Dalian, China). The PCR system contains $MgCl_2$ (3 mM), forward and reverse primers (0.5 μM), SYBR Green PCR master mixes (2 μl) and cDNA (2 μl). The mix was assayed on a Rotor Gene 300 Real-time Cycler (Corbett Research, Sydney, Australia). The interest gene expression level was normalized by the housekeeping gene β-actin (PCR primers were listed in Table S1).

### 2.11. Behavioral tests

Behavioral testing was commenced when male offsprings were weaned at postnatal day 30 (P30). Each prenatal treatment group consisted of subjects derived from multiple independent litters as previously reported.

#### 2.11.1. Open field test

The open field was made of black acrylic (120 × 120 cm) with 22-cm-high walls. The floor was divided into 16 squares by computer, and 4 squares in the middle of the arena called the center zone. Rats were placed individually in the center of open field and total distance (total moving distance in the field), center duration (time stay in the center zone) and center distance (moving distance in the center zone) were recorded for 5 min. The apparatus was thoroughly cleaned using 70% ethanol after each animal finished the test.

#### 2.11.2. Forced swimming test

Animals were individually placed in a Plexiglas cylinder (46 cm tall × 20 cm in diameter) containing water (23–25 °C) with 30 cm deep, and were videotaped for 6 min. Active (swimming, climbing, struggling) or passive (immobility) behaviors were scored using a time sampling technique to rate the predominant behavior in each 5-s interval. In contrast to protocols designed to detect reductions in immobility (for example, scoring minutes 2–6 of testing, when immobility is very high in controls, to detect antidepressant effects), the first 2 min of the test was scored separately to better detect potential increases in immobility. The latency to become immobile for the first time was also measured. After the swim session, animals were dried and placed in a cage surrounded by a heating pad. The water was changed between each animal.

#### 2.11.3. Sucrose preference test

For the sucrose preference test, animals were placed in separate cages, and water was removed from the cages for 24 h, then two bottles were given to rats for 2 h; one containing tap water and the other containing a 2% sucrose solution. The bottles were counterbalanced to avoid side preferences for 1 h. Percent sucrose preference was calculated as sucrose consumption (g) × 100%/sucrose consumption (g) + water consumption (g).

### 2.12. Golgi staining

Golgi staining was performed by using a GD Rapid Golgi Stain Kit (FD Neurotechnologies) [27]. Mice were deeply anaesthetized and brains were dissected out and immersed in impregnation solution (Golgi-Cox solution, including mercuric chloride, potassium dichromate and potassium chromate). The brain samples were soaked in dark for 21 days at room temperature and replaced to a fresh impregnation solution every 2 days. After 3 weeks, brain tissues were transferred to a solution of 3% sucrose in PBS for 5 days. The brain was sectioned sagittally (100 μm thick) using a Leica VT1000S vibratome and sections were mounted on gelatin-coated slides. After drying, slides were rinsed and then placed in ammonia hydroxide for 30 min (in dark) and Kodak black-and-white fixing solution for another 30 min. The sections were rinsed and dehydrated, placed into CXA solution for 15 min, and cleared in xylene two times. When sections were semidry, one drop of mounting medium was added on the slide and slides were covered with cover glass and sealed with Neutral gum.

### 2.13. Statistical analysis

All data were collected and analyzed in a blinded manner. Data

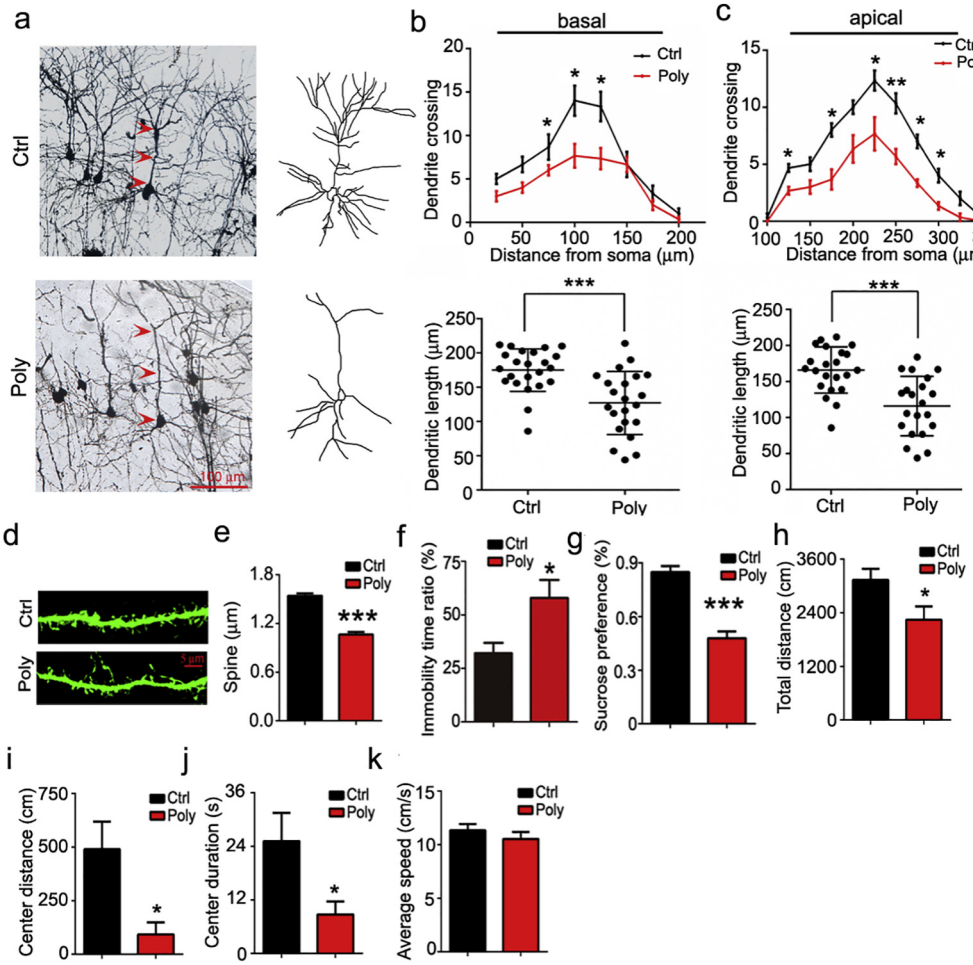



**Fig. 1.** MIA induces offsprings' dendrite damage and depression behaviors.
The pregnant rats were injected through vena caudalis with poly(I:C) (10 mg/kg) to mimic MIA or normal saline as control (Ctrl) at gestational day 17 (GD17), the offsprings were infused into the each lateral ventricle with AAV-EGFP ($9.77 \times 10^{12}$ V.G./ml, 2 μl) at P0 by cryoanesthetized on ice for 5 min, and the depression-like behaviors were detected in 1 m-old offsprings.
(a–c) The representative morphology of dendritic arbors in prefrontal cortex neurons imaged by Golgi staining (a), and the decreased basal and apical dendrite numbers and length in poly(I:C) group were analyzed by Sholl analyses (b, c). At least 20 neurons from 4 rats per group were analyzed.
(d, e) The representative images of dendritic spines in the prefrontal cortex neurons and the quantitative analysis ($n$ = 40 from 4 rats).
(f) The increased mean immobility time in the poly(I:C) offsprings measured by forced swimming test ($n$ = 9 rats).
(g) The decreased sucrose consumption in the poly(I:C) offsprings measured by sucrose preference test (n = 9 rats).
(h–k) The MIA offsprings show decreased total distances (h), center distance (i) and the center duration (j) with unchanged average speed (k) in open field test ($n$ = 12 rats).
The Data were expressed as mean ± SD (b, c, e) or mean ± SEM (f-k). *, $p < .05$, **, $p < .01$, ***, $p < .001$ *vs* Ctrl or Healthy; unpaired two-tailed student's *t*-test.

were expressed as mean ± SD or mean ± SEM and statistical analysis was performed using unpaired two-tailed student's *t*-test (two-group comparison), or two-way ANOVA with Bonferroni tests. All analyses were performed using SPSS 19 software, and significance was set at $p < .05$.

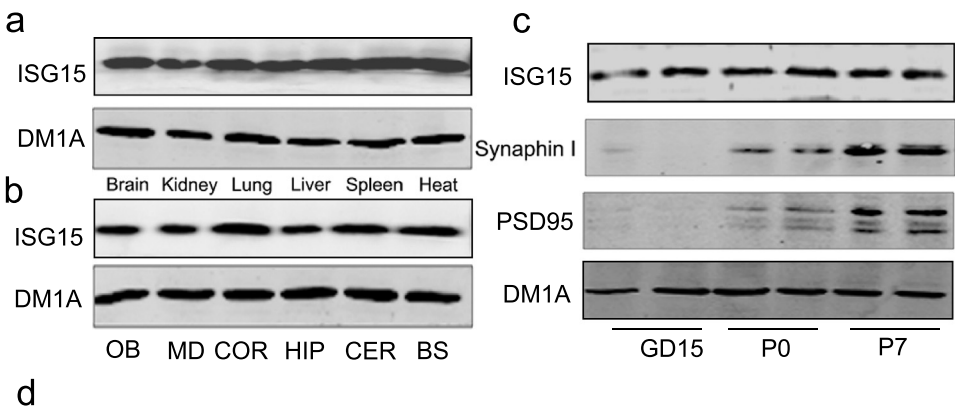
## 3. Results

### 3.1. MIA induces depression and dendrite impairment in the offspring

Rats at gestation day 17 (GD17) were injected with poly(I:C) (10 mg/kg) *via* vena caudalis to mimic MIA, AAV-EGFP virus was infused into the offsprings' lateral ventricle to label the neurons at postnatal 0 day (P0), and dendritic complex and spine density in the prefrontal cortex neurons were analyzed in 1 m-old offspring by Golgi staining and direct fluorescence imaging. The results showed that total basal dendrite length, basal or apical dendritic crossing, and spine density decreased in the prefrontal cortex neurons of the MIA-offspring as compared with the controls (Fig. 1a–e). Dendrite impairment has been considered as the structural bases of depression and most of depression patients show dendrite loss [28–30], therefore depression-like behaviors in the offsprings were examined by forced swimming test and sucrose preference test at P30. MIA offsprings showed an increased mean immobility time and decreased percentages of sucrose consumption compared to the controls (Fig. 1f, g). In open field test, the MIA offsprings showed shorter total exploring distance and distance in the center area, and stayed less time in the center area than the normal saline controls (Fig. 1h-j) with no motor dysfunction as measure by average speed (Fig. 1k). These data together demonstrated that MIA induced depression-like behaviors and impeded dendrite development in the offspring.

### 3.2. MIA augments ISG15 in maternal serum and the offsprings' brain

ISG15 is actively involved in inflammation and neural development [15–17,20,21]. To explore the involvement of ISG15 in MIA-induced



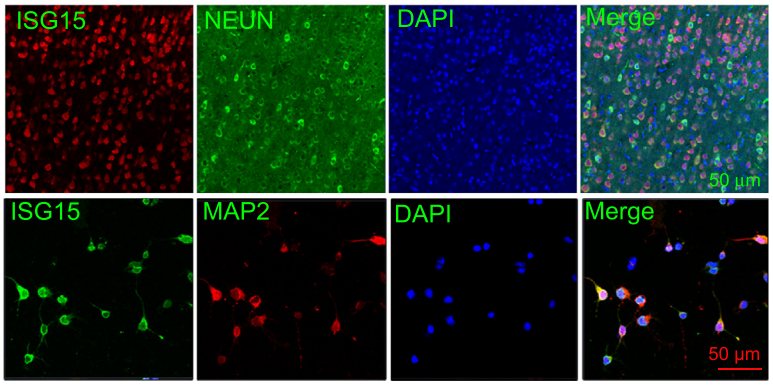




**Fig. 2.** Expression pattern of ISG15 in different periphery tissues and central nerves system.
(a, b) ISG15 was widely expressed in different organs and different brain regions of rats measured by Western blotting (OB, olfactory bulb; MD, midbrain; COR, cortex; HIP, hippocampus; CER, cerebellum; BS, brainstem).
(c) ISG15 was detected in embryo (GD15) and early postnatal days, while Synapsin 1 and PSD95 (postsynaptic density) were not detected at GD15. DM1A was used as loading control.
(d) Co-localization of ISG15 with NEUN and MAP2 (neuronal markers) in adult rat cortex slices and in 2 div primary cultured cortical neurons.

dendrite impairment, we first measured the expression pattern of ISG15. The results showed that ISG15 was widely expressed in the brain, kidney, lung, live, spleen and heart (Fig. 2a). In various brain subsets, ISG15 levels were abundantly expressed in the cortex and the brainstem with relatively low level in the hippocampus (Fig. 2b), and was also detected in the rat brains from gestation day 15 (GD15) to postnatal day 7 (P7) (Fig. 2c). At the cellular level, ISG15 was largely expressed in neuronal soma and dendrite (Fig. 2d). These data provided fundamental evidence for the possible involvement of ISG15 in neurodevelopment.

Then, we measured serum level of ISG15 in the pregnant rats with or without poly(I:C) exposure. The results showed that protein level of ISG15 in matrix serum was significantly increased at 12 h and P0 after poly(I:C) exposure (Fig. 3a). By collecting serum samples from healthy and infected pregnant women at gestation 28–32 weeks, we also detected significantly increased ISG15 in the infected group (Fig. 3b), in which white blood cell counts were generally higher than $20 \times 10^9$/L. We further studied whether the change of ISG15 was also occurred in the offsprings' brain cortex after poly(I:C) exposure. Remarkably increased ISG15 mRNA and protein were detected at 12 h and P0 in offsprings' prefrontal cortex after poly(I:C) injection of the dams (Fig. 3c, d). These human and rodents data demonstrated that MIA upregulated ISG15 expression in both dams' serum and the offsprings' brain.

### 3.3. ISG15 regulates dendrite development and overexpression of ISG15 induces dendrite impairments and behavior deficits

To explore the role of ISG15 in dendrite development, AAV-ISG15-EGFP or lenti-shISG15-EGFP or the empty vectors was transfected into the culture neurons at 1 *div* and dendrite developments were analyzed at various stages. The efficiency of ISG15 overexpression (5-fold of the normal level) or knockdown (0.11 of the control) was confirmed by Western blotting (Fig. 4a). By using anti-MAP2 to stain dendrites at 5 *div*, we found that ISG15 overexpression decreased total dendrite length and the number of branch tips without affecting primary branch number compared with the controls (Fig. S1a-c), while downregulating ISG15 increased total dendrite length with no significant effects on the number of branch tips and primary branches (Fig. S1a–c). The influence of ISG15 on dendrite development was more significant when measured by using anti-MAP-2 co-staining at 7 *div* (Fig. S1d-g), while no significant change on axon length was detected by using SMI-312R staining (Fig. S2). Remarkably changed dendrite branches and spine density were also shown by overexpressing or downregulating ISG15 at 17 *div* neurons (Fig. 4b–g). These data indicate that ISG15 negatively regulates dendrite development in physiological condition.

To verify the role of ISG15 in MIA-induced dendritic impairment and depression *in vivo*, AAV-ISG15-EGFP or empty vector were infused into the lateral ventricle of the neonatal rats at P0, the results showed that ISG15 protein level increased by 6.9-fold of the control level by expressing AAV-ISG15-EGFP (Fig. 5a). Simultaneously, the dendritic complexity and spine density in prefrontal cortex neurons were significantly decreased in ISG15-overexpressing rats by direct fluorescence imaging and Golgi staining (Fig. 5b–h). The rats manifested depression-like behaviors showing decreased total exploring distance, less time stayed in the center area and reduced distance in the center area, with no motor dysfunction in open field test (Fig. 5i–l). Overexpressing ISG15 also increased the mean immobility time in forced swimming test and decreased the sucrose preference in sucrose preference test (Fig. 5m, n). These data suggest that upregulation of ISG15 is sufficient to induce depression and dendrite impairment, which mimic the effects of MIA offspring.

### 3.4. Knockdown of ISG15 attenuates MIA-induced dendrite and behavioral damages in offsprings

To investigate whether ISG15 knockdown ameliorates the offspring's abnormal behaviors induced by MIA, rats were injected with

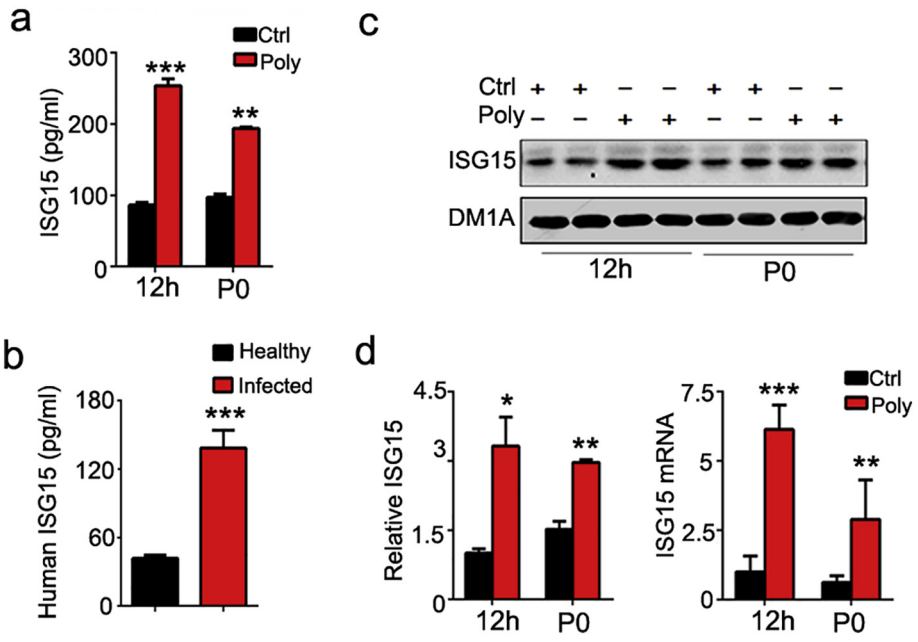



**Fig. 3.** A remarkable augmentation of ISG15 in matrix serum and the offsprings' brain induced by MIA. (a) MIA increases ISG15 protein level in maternal peripheral serum, measured respectively at 12 h and the delivery day (P0) after poly(I:C) injection by ELISA. At least 6 samples from 3 rats per group were measured.
(b) MIA increases serum protein level of ISG15 in the infected pregnant human measured at gestational 28–32 weeks by ELISA ($n$ = 12–14 cases per group).
(c, d) MIA increases mRNA and protein levels of ISG15 in the offsprings' prefrontal cortex measured at 12 h and P0 after poly(I:C) injection by qRT-PCR and Western blotting, respectively. At least 6 samples from 3 rats per group were measured.
The Data were expressed as mean ± SD. *, $p < .05$, **, $p < .01$, ***, $p < .001$ *vs* Ctrl or Healthy; unpaired two-tailed student's *t*-test.

poly(I:C) through vena caudalis at GD17 and infused with lenti-shISG15-EGFP into the offsprings' brain at P0. Dendritic plasticity and depression-like behaviors were measured at 1 m-old. Downregulation of ISG15 in the offspring by 0.25 of the control (Fig. 6a, Fig. S3) ameliorated decreased dendritic complexity and spine density (Fig. 6b–h). Meanwhile, MIA-induced depression was improved as shown by increased total exploring distance, staying time and distance in the center area (Fig. 6i–l), as well as decreased mean immobility time and increased sucrose preference (Fig. 6m, n). These data suggest that ISG15 knockdown ameliorates dendritic impairments and cognitive deficits in the offspring induced by MIA.

### 3.5. MIA-induced cytokines increase ISG15 protein level

Serum levels of inflammatory cytokines in MIA rats and the infected pregnant women were also measured using ELISA. The results showed that levels of IL-1, IL-2, IL-6, TNF-α, IFN-β and IFN-α were remarkably increased in MIA rats and infected human (Fig. 7a–h, Fig. S4). To confirm the effect of the elevated cytokines on ISG15 expression, cultured neurons (1 *div*) were treated with various cytokines including IL-1, IL-6, IFN-β, TNF-α, IL-17, IL-2, IL-14 and IL-10 for 48 h. We found that all tested cytokines except IL-10, -14 and -17 could increase ISG15 protein level in the cultured neurons (Fig. 7i, j).

STAT1 is a recognized upstream regulatory gene of ISG15 [31], therefore we also examined the levels of total STAT1 and the phosphorylated STAT1 (p-STAT1, the active form). The ratio of p-STAT1/STAT1 in offsprings' brain cortex was significantly increased at 12 h and P0 after MIA (Fig. S5), suggesting that STAT1 activation may also be involved in MIA-induced ISG15 upregulation.

### 3.6. Downregulating ISG15 rescues inflammatory cytokine-induced dendrite impairments and depression

The data so far strongly suggest that the elevation of inflammatory cytokines during MIA induces ISG15 expression, and the latter in turn inhibits dendrite development and causes depression-like behaviors. To further verify the role of ISG15 in mediating cytokines' effects on dendrite outgrowth, we treated the primary neurons at 1 *div* with different cytokines for 48 h and transfected the neurons with lenti-shISG15-EGFP at 3 *div*, and then analyzed the dendritic complexity at 7 *div*. The results showed that ISG15 knockdown attenuated dendrite impairments induced by IL-2, IL-6, IFN-α and IFN-β (Fig. 7k–n).

To further explore whether knockdown ISG15 could rescue the cytokine-induced depression behaviors, we infused IFN-α into the pregnant rats at GD17 and infected the offsprings with lenti-shISG15-EGFP through ventricular infusion at P0. Behavioral changes of the offsprings were tested at 1 m-old. The results showed that matrix injection of IFN-α increased ISG15 level in offsprings' brain prefrontal cortex, while downregulating ISG15 (Fig. 7o, p) improved IFN-α-induced depression-like behaviors shown by the partially restored exploring behavior, mean immobility time and sucrose preference (Fig. 7q).

### 3.7. ISG15 impedes dendrite development by inhibiting NEDD4

Finally, we investigated the molecular mechanisms underlying the effects of ISG15 on dendrite development. ISG15 has domains similar to ubiquitin, and it can inhibit the activity of ubiquitin-protein ligase [17]. NEDD4 is an important E3 ubiquitin-protein ligase and ISG15 can interact with NEDD4 and thus inactivate the ligase [19,32]. Additionally, ubiquitination of Rap2A by NEDD4 can regulate neurite outgrowth and arborization in mammalian neurons [20,21]. Therefore, we studied whether NEDD4/Rap2A pathway is involved in ISG15-modulated dendrite development. We found that protein level of NEED4 rats was decreased in offsprings' prefrontal cortex with maternal poly(I:C) exposure (Fig. 8a, b). In primary cultured neurons, ISG15 overexpression decreased NEDD4 mRNA and protein levels (Fig. 8c–e), which confirms the inhibitory effect of ISG15 on NEDD4. By co-immunoprecipitation, we found that upregulating ISG15 increased association of ISG15 with NEDD4, which simultaneously decreased NEDD4 and Rap2A interaction and led to reduced Rap2A ubiquitination (Fig. 8f, g). These data suggest that MIA-induced ISG15 upregulation can suppress NEDD4 expression and thus decrease Rap2A ubiquitination, which may involve a competitive binding of ISG15 to NEDD4.

To further verify the role of NEDD4 suppression, we studied whether upregulating NEDD4 could rescue ISG15-induced dendritic impairment. By co-expressing AAV-ISG15-mCherry with pCI-HA-NEDD4-EGFP in primary cultured neurons, we observed that NEDD4 overexpression could rescue ISG15-induced dendrite impairments as shown by restored total dendrite length and branch numbers measured at 7 *div* (Fig. 8h–j). These data confirm the role of NEDD4 suppression in ISG15-induced dendrite impairments.

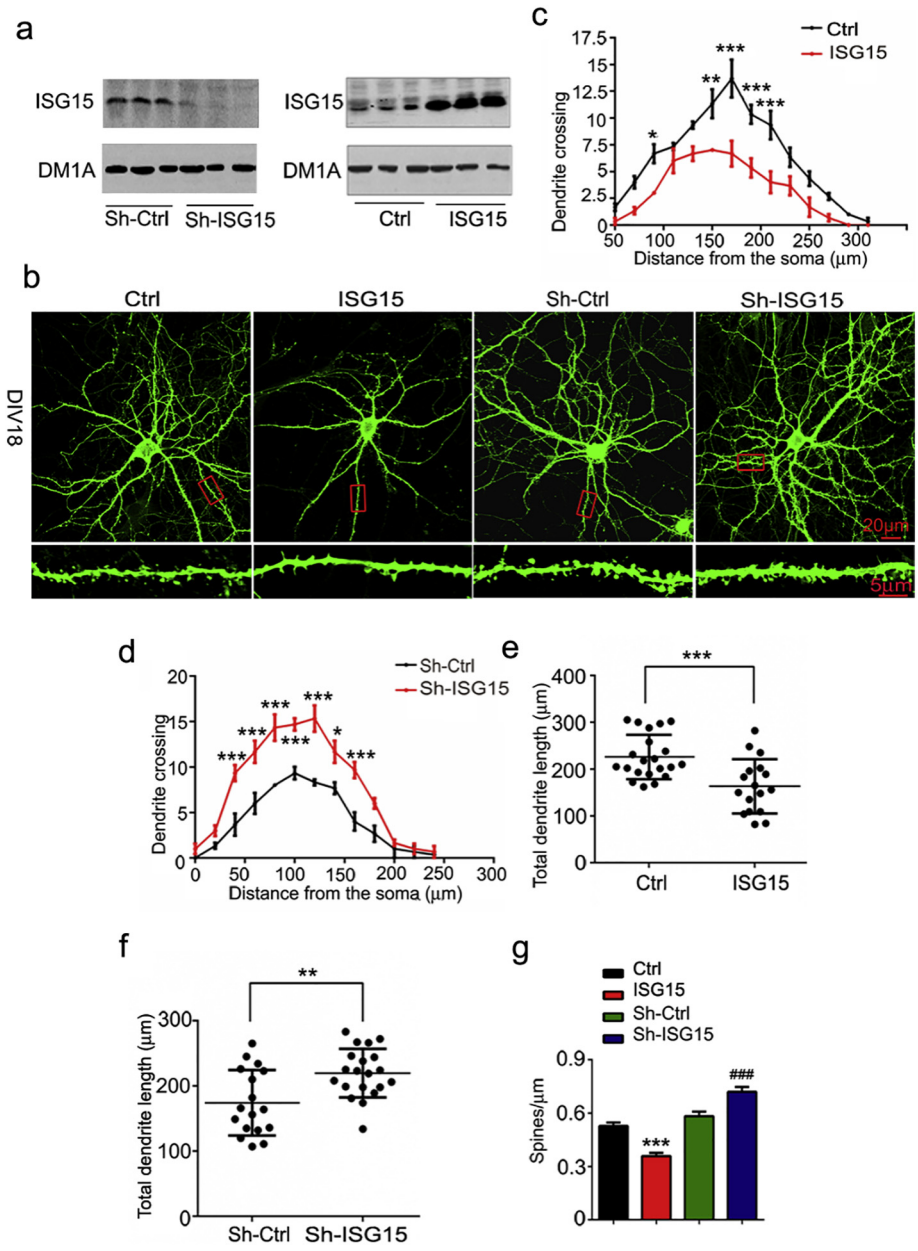



**Fig. 4.** ISG15 negatively regulates dendrite development in normal cultured neurons.

The primary cultured cortical neurons were infected with AAV-EGFP-ISG15 (ISG15, $6.69 \times 10^{12}$ V.G./ml), or lenti-shISG15-EGFP (Sh-ISG15, $7.45 \times 10^{8}$ TU/ml), or the empty AAV-vector (Ctrl, $9.77 \times 10^{12}$ V.G./ml), or the empty lenti-vector (Sh-Ctrl, $7.83 \times 10^{8}$ TU/ml) at 1 *div*, and the dendrite complexity was stained and analyzed using anti-MAP2 at 5 *div* and 7 *div* (see Fig. S1) and 18 *div*, respectively.

(a) Expressing AAV-ISG15 increases ISG15 protein level to 5-fold of the normal level, while expressing lenti-shIGF15 almost completely ablated ISG15 protein measured by Western blotting ($n = 3$).

(b) The representative images show reduced dendrite complexity and spine densities by overexpressing ISG15, and downregulating ISG15 enhances dendrite development and spine generation recorded in cultured cortex neurons at 18 *div*.

(c-f) Sholl analyses show the changed numbers of dendrite crossings and the length by overexpressing or knockdown of ISG15 in cultured cortex neurons at 18 *div*. At least 20 neurons were analyzed.

(g) Quantitative analyses show changed spine density at 18 *div*. At least 20 neurons per group were analyzed.

The Data were expressed as mean ± SD. *, $p < .05$, **, $p < .01$, ***, $p < .001$ *vs* Ctrl or Sh-Ctrl; unpaired two-tailed student's t-test.

## 4. Discussion

Epidemiological and animal studies indicate that MIA remarkably increases the risk for the offsprings to suffer from psychiatric disorders including depression spectrum, autism, schizophrenia and so on [33], but the molecular mechanisms are unclear. Based on the current knowledge, it is indicated that maternal inflammation and the impeded neuronal development play an important role in MIA-induced psychiatric disorders in the offsprings. As ISG15 is actively involved in inflammation and NEDD4 activity that plays a crucial role in neuronal development [15–19,34], we investigated the role of ISG15 in MIA-induced dendrite impairments and depression-like behaviors. We found that MIA significantly increased ISG15 level in matrix rats, human periphery blood, and the offsprings' brain. Furthermore, overexpression of ISG15 in neonatal brain induced an impeded dendrite development accompanied with depression-like behaviors, while ISG15 knockdown in the fetal brain attenuated the behavioral and dendritic deficits in MIA offspring rats. We also found that cytokines such as IL-1, IL-2, IL-6, TNFa, IFNβ and IFNα induced by MIA increased ISG15 level and inhibited dendrite development of the neurons. All these suggested that ISG15 plays a vital role in MIA-induced neurite growth deficits and abnormal behaviors in the offspring.

It is well known that dendrite branching and spine density are indispensable for neurons to carry out specialized cognitive functions [29,30,35,36], and loss of dendrite branching or spine density has been widely observed in neurodevelopmental disorders, including depression [29,35,37–39]. In agreement with our present finding that the offsprings of pregnant dams treatment with poly(I:C) induced depression behaviors and reduced dendrite branching and spine density, previous studies showed that MIA could cause depression in the offsprings [33,40], reduced dendrite branching and spine density in cortex and hippocampus [41,42]. In the present study, we also found that similar pathological and behavioral deficits of AAV-ISG15 virus injected offsprings. Furthermore, ISG15 knockdown in the prefrontal cortex reversed the morphology deficits and ameliorated depression-like behaviors, indicating the importance of ISG15 in the MIA-induced abnormal morphology of neurons and depressive-like behaviors. To our knowledge, this is the first evidence revealing that ISG15 plays a key role in

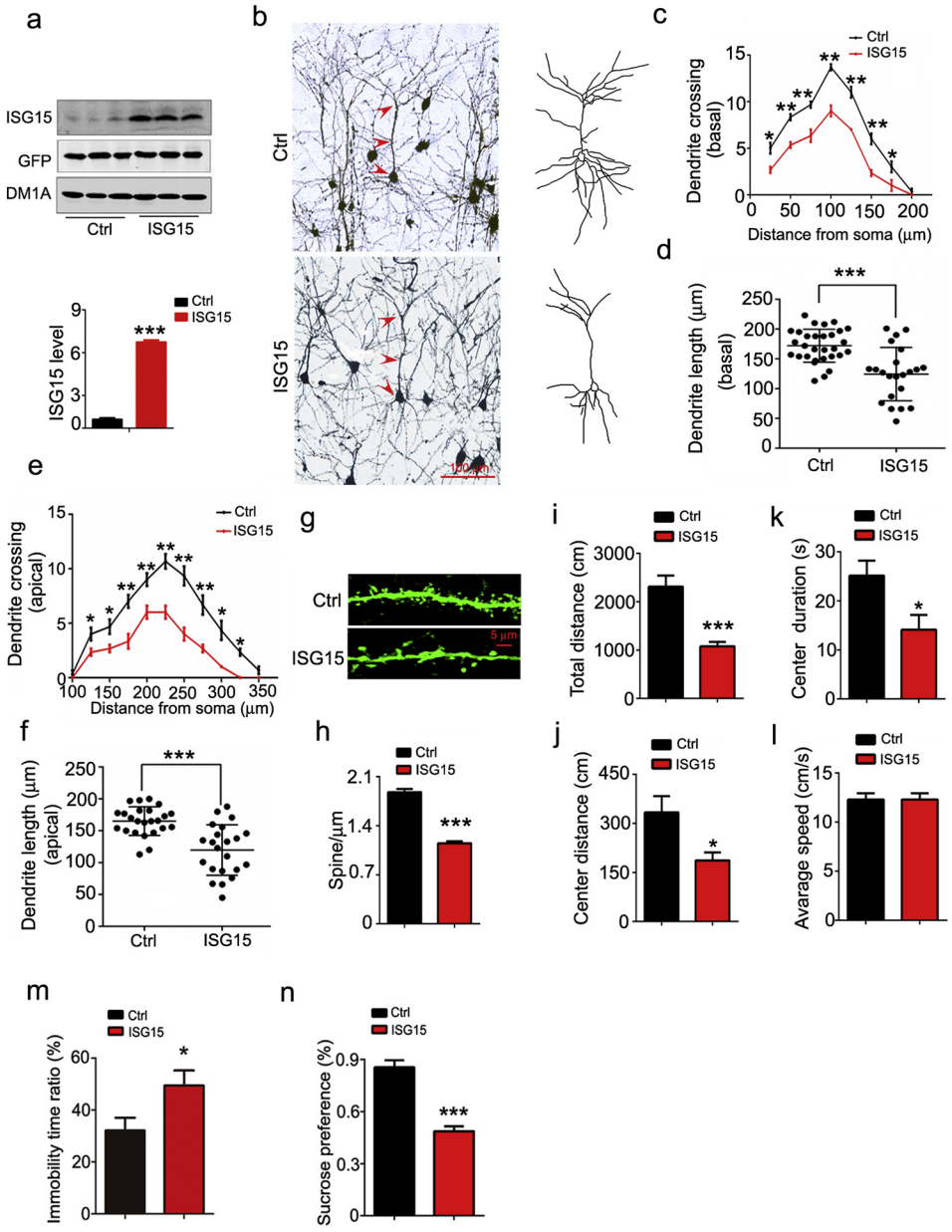



**Fig. 5.** Overexpressing ISG15 in newborn rats mimics MIA-effects on dendrite and behavioral deficits.

AAV-EGFP-ISG15 (2 μl, $6.69 \times 10^{12}$ V.G./ml) or the empty vector ($9.77 \times 10^{12}$ V.G./ml) was infused into the each lateral ventricle at P0 by cryoanesthetized on ice for 5 min, and the dendrite morphology and depression behaviors were detected at 1 m-old.

(a) Overexpression of ISG15 protein was confirmed by Western blotting (n = 3 rats).

(b–f) Overexpressing ISG15 in newborn rats impedes dendrite development in the prefrontal cortex neurons measured by Golgi staining (b) and the Sholl analyses (c-f) (At least 22 neurons from 4 rats per group were analyzed).

(g, h) Overexpressing ISG15 in newborn rats impedes spine generation in the prefrontal cortex neurons measured by direct fluorescence imaging (AAV-Ctrl, $n = 46$; AAV-ISG15, $n = 45$).

(i–l) Overexpressing ISG15 in newborn rats induces depression-like behaviors shown by the decreased total distance (i), the center distance (j) and center duration (k) with unchanged average speed (l) measured by open field test ($n = 10$–12 rats).

(m) In forced swimming test, the increased mean immobility time ratio was shown (n = 9 rats).

(n) In sucrose preference test, the decreased percentage of sucrose consumption was shown (n = 9 rats).

The data were expressed as mean ± SD (a, c–f, h) or mean ± SEM (i-n). *, $p < .05$, **, $p < .01$, ***, $p < .001$ *vs* AAV-Ctrl; unpaired two-tailed student's *t*-test.

MIA-induced dendrite damages and depression-like behavior in the offsprings.

Then, the mechanisms of the elevated ISG15 impeding dendrite development in MIA are investigated. We demonstrated that ISG15 elevation can suppress NEDD4 activity with a significantly reduced ubiquitination of Rap2A, and the mechanism involves an increased association of ISG15 with NEDD4, which can competitively decrease NEDD4-Rap2A interaction. It is well known that protein ubiquitination plays a critical role in establishing neural polarity, neurogenesis and synapse formation [43,44], and ubiquitination deficit can cause neurodevelopmental disorders [45,46]. NEDD4, an E3 ubiquitin ligase, can ubiquitinate and thus inhibit Rap2A activity, resulting in dendrite outgrowth and arborization [21], and ISG15 can inhibit NEDD4 [32]. This was further supported by our results that NEDD4 upregulation efficiently rescues ISG15-induced dendrite impairment. These findings reveal that ISG15 impedes dendrite development through inhibiting NEDD4 signaling. Interestingly, a previous study shows that NEDD4 can regulate axon outgrowth and arborization in Xenopuslaevis retinal ganglion cells by promoting phosphatidylinositol 3-kinase (PI3K)-induced cytoskeletal rearrangements [20], however, we have not detected significant axon change in the current study. This discrepancy may be partially explained by the different experimental objects used in the studies, *i.e.* Xenopuslaevis retinal ganglion cells *versus* mouse cortex neurons.

To investigate the underlying mechanisms *via* that maternal MIA augments the expression of ISG15 in the offsprings brain; we first measured the serum level of the inflammatory cytokines. Our results showed that the levels of IL-1, IL-2, IL-6, TNF-α, and IFN-α were remarkably increased in MIA-matrix rats and the infected pregnant human. Previous results also showed the elevation of the inflammatory cytokines in the maternal serum of MIA, and a single inflammatory cytokine injection, such as IL-6, IL-8 or IL-2, was sufficient to induce autism- or schizophrenia-like behaviors in the offsprings [9,11,47–51]. Therefore, we further investigated the role of inflammatory cytokines in ISG15 expression. Our results demonstrated that treating the primary neurons with inflammatory cytokines including IL-1, IL-2, IL-6, TNF-α, and IFN-β, but not IL-10, IL14, and IL-17, or injecting IFN-α into the pregnant dams remarkably stimulated ISG15 expression in cortex neurons of the fetal brain, whereas ISG15 knockdown attenuated cytokine-induced dendrite abnormality and behavior deficits. These data

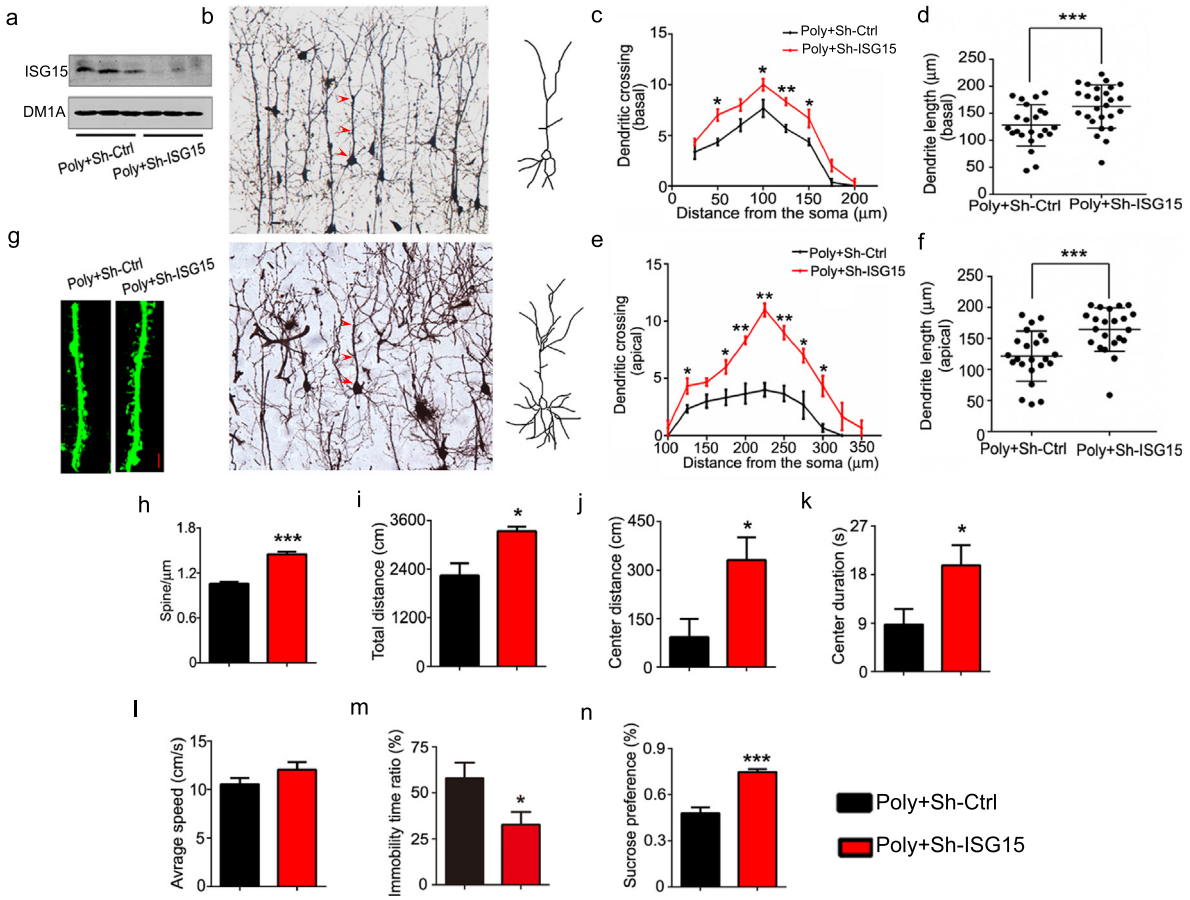



**Fig. 6.** Knockdown of ISG15 attenuates MIA-induced dendrite and behavioral damages in offsprings.
The pregnant rats were received poly(I:C) injection at GD17, and the offsprings were infused at P0 through bilateral ventricle with 2 μl lenti-shISG15-EGFP ($7.45 \times 10^8$ TU/ml) or its empty vector ($7.83 \times 10^8$ TU/ml), and the depression behaviors in the offspring were tested at 1 m-old.
(a) Brain infusion of lenti-shRNA in pups efficiently reduced ISG15 level in MIA-pup's prefrontal cortex measured at 1-m by Western blotting (n = 3 rats).
(b–f) Downregulating ISG15 attenuates MIA-induced dendrite impairment in prefrontal cortex neurons measured by Golgi staining (b) and the Sholl analyses (c–f). At least 22 neurons from 4 rats per group were analyzed.
(g, h) ISG15 knockdown restores MIA-induced reduction of spine density. At least 41 neurons from 4 rats per group were analyzed.
(i–l) Downregulating ISG15 attenuates MIA-induced depression-like behaviors shown by the increased total distances (i), the center distance (j) and center duration (k) with unchanged average speed (l) in open field test (n = 10–12 rats).
(m) In forced swimming test, the reduced mean immobility time ratio was shown (n = 9 rats).
(n) In sucrose preference test, the increased percentage of sucrose consumption was shown (n = 9 rats).
The data were expressed as mean ± SD (c-f, h) or mean ± SEM (i–n). *, $p < .05$, **, $p < .01$, ***, $p < .001$ *vs* Poly + Sh-Ctrl; unpaired two-tailed student's t-test.

reveal that MIA-augmented inflammatory cytokines are required for ISG15 elevation and its detrimental effects on neurodevelopment and behavioral function. Although overexpressing IL-10 in macrophages could partially prevent the detrimental effects of MIA in the offsprings [11,33], we did not see significant influence of IL-10 on ISG15 expression or dendrite development *in vitro*. These data suggest a complex role of the cytokines during MIA. STAT1 is a recognized upstream regulator of ISG15 [31]. Our data also show that STAT1 activation may also contribute to the MIA-induced ISG15 upregulation in the brain cortex of the offsprings.

During MIA, the stimulated inflammation or the increased cytokines play roles as a 'double-edged sword'. On one hand, increased inflammatory cytokines in the fetal brain can lead to abnormal brain development and long-term behavioral deficits. On the other hand, increased cytokines prevent further damages to the mothers and protect fetus from harms caused by viruses and bacteria. For instance, it is a good proof that no virus has been detected in fetal brain of the influenza model, and virus transmission does not occur in cases where IFN-α presents in the placenta and simultaneously in the maternal and fetal circulations [52]. These conflicts bring difficulties for the application of anti-inflammatory treatments to the matrix. As ISG15-deficient human individuals exhibit no overt susceptibility to viral diseases [53], downregulating ISG15 level may serve as a promising strategy for preventing or treating the MIA-induced psychiatric disorders in the offsprings. Additionally, previous studies show that MIA induced by poly(I:C), or LPS, or a single immune factor injection could result in adult (2–3 m-old) offsprings' depressive behaviors [9,33,40]. In the present study, we demonstrated that depression-like behaviors were already significant in the 1 m-old offsprings. These findings suggest that early intervention is important for preserving offsprings' neurodevelopment during or after MIA.

In summary, we found in the present study that MIA remarkably increased expression of ISG15 in the fetal brain, which impeded neurodevelopment and induced depression-like behaviors in 1 m-old offsprings with the molecular mechanism involving disruption of NEDD4/Rap2A pathway by the elevated ISG15 (Fig. 9). Knockdown of ISG15 ameliorated MIA-induced pathological and behavioral abnormalities in the pups, which suggests that inhibiting ISG15 may be a promising intervention of MIA-induced psychiatric disorders in the offsprings.

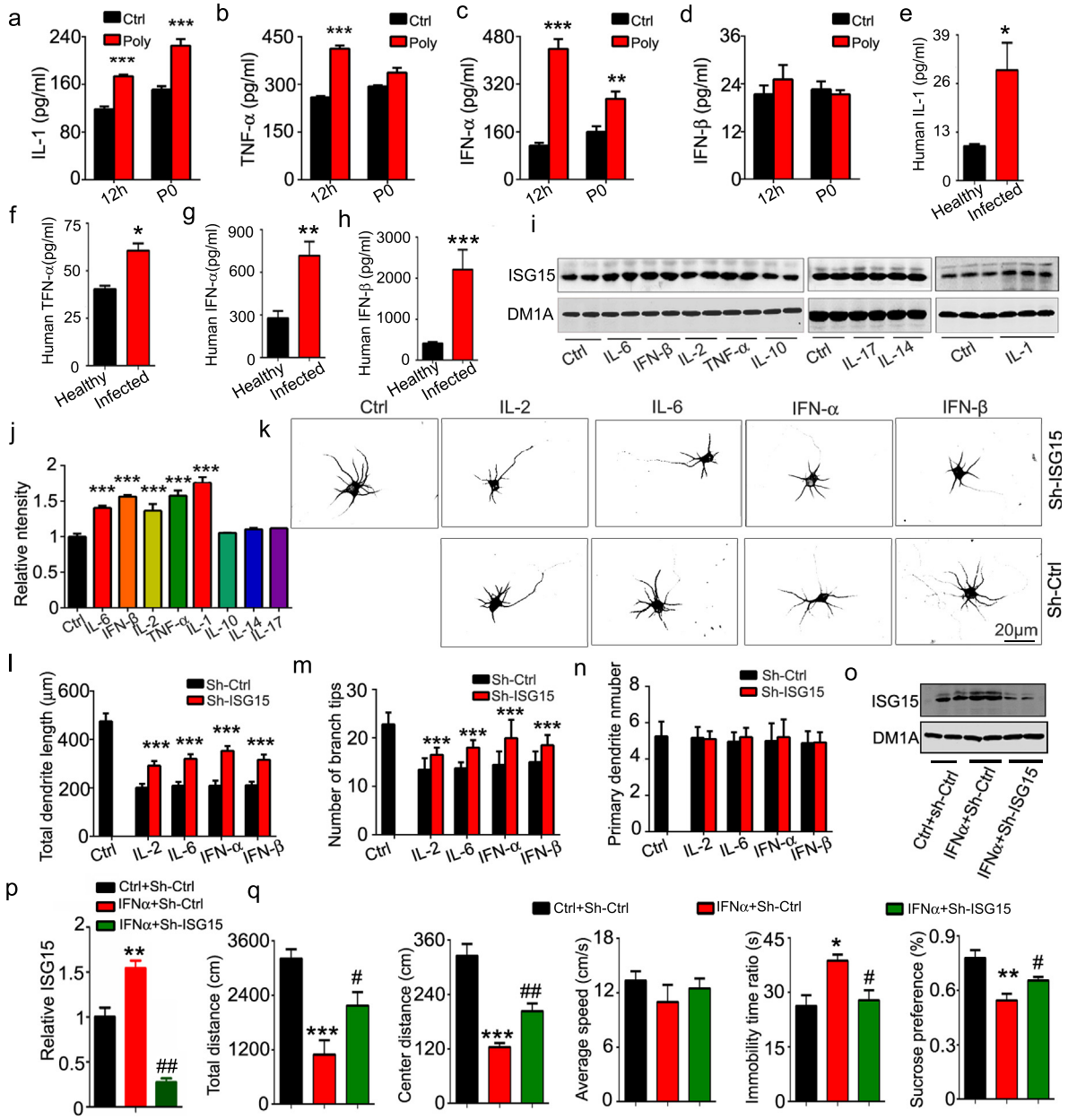



**Fig. 7.** MIA-augmented inflammation is required for offsprings' ISG15 upregulation and its toxic effects on dendrite development.

(a–d) MIA increases the inflammatory cytokines in maternal serum of rats. The pregnant rats were injected with poly(I:C) (10 mg/kg) or normal saline (Ctrl) at GD17, and the peripheral blood serum were collected respectively at 12 h and at the day of delivery (P0) for measurement of the inflammatory cytokines by ELISA ($n = 6$ rats).

(e–h) MIA increases the inflammatory cytokines in maternal human serum. Peripheral blood serum from healthy and infected pregnant mothers (gestation 28–32 weeks) were collected for measurement of the inflammatory cytokines by ELISA (n = 12–14 cases per group).

(i, j) The MIA-augmented inflammatory cytokines promote ISG15 expression. The primary cultured neurons in prefrontal cortex at 1 *div* were treated with the inflammatory cytokines as labeled (100 UI/ml) for 48 h, and the neurons were harvested at 7 *div* for measurement of ISG15 expression by Western blotting ($n = 5$).

(k–n) Representative images and the quantitative analyses show *in vitro* rescue of dendrite morphologies by ISG15 knockdown. Primary cortex neurons at 1 *div* were treated with 100 UI/ml cytokines for 48 h; then lenti-shISG15-EGFP ($7.45 \times 10^8$ TU/ml) or its empty vector ($7.83 \times 10^8$ TU/ml) was transfected and the neurons were fixed at 7 *div* for measurement of dendrite complexity (At least 25 neurons per group were analyzed).

(o, p) The pregnant rats were injected through vena caudalis with IFN-α (2000 UI/kg) or normal saline (Ctrl) at GD17, then lenti-shISG15-EGFP or its empty vector was injected into the offsprings brain *via* lateral ventricle at P0. Downregulation of ISG15 was confirmed by Western blotting in the brain of 1 m-old offsprings (n = 4 rats).

(q) Downregulation of ISG15 rescues IFN-α-induced depression-like behaviors shown by the increased total distance and center distance in open field test, the increased percentage of sucrose consumption in sucrose preference test, and decreased mean immobility time ratio in forced swimming test ($n = 15$ rats).

The data were expressed as mean ± SD (a–h, j, l–n, unpaired two-tailed student's t-test; p, two-way ANOVA with Bonferroni tests) or mean ± SEM (q, two-way ANOVA with Bonferroni tests). *, $p < .05$, **, $p < .01$, ***, $p < .001$ *vs* Ctrl or Healthy or or Sh-Ctrl or Ctrl + Sh-Ctrl; #, $p < .05$, ##, $p < .01$ *vs* IFN-α + Sh-Ctrl.

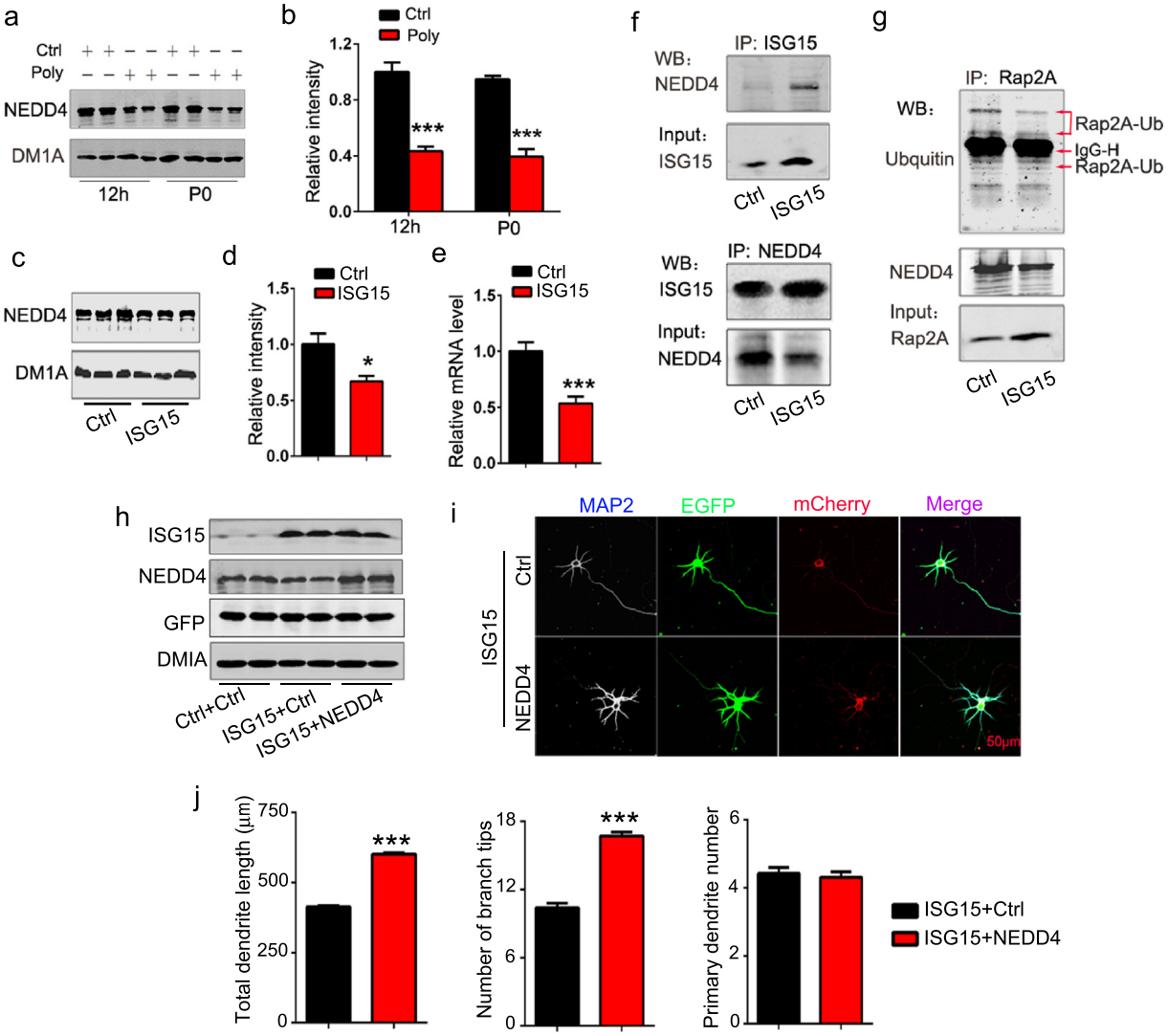



**Fig. 8.** ISG15 impedes dendrite development by inhibiting NEDD4-mediated ubiquitination of Rap2A.
(a, b) The pregnant dams were injected with poly(I:C) or normal saline at GD17, and the reduced protein level of NEDD4 in the offsprings cortex was detected by Western blotting at 12 h or P0 after poly(I:C) exposure (n = 4 rats).
(c–e) Primary cortical neurons were infected with AAV-EGFP-ISG15 ($6.69 \times 10^{12}$ V.G./ml) or its empty vector ($9.77 \times 10^{12}$ V.G./ml) at 1 *div*, and the reduced protein and mRNA levels of NEDD4 were detected respectively by Western blotting and qRT-PCR at 7 *div* (n = 4).
(f, g) The decreased interaction of ISG15 with NEDD4, or Rap2A with NEDD4, and the reduced ubiquitination of Rap2A were detected by co-immunoprecipitation and Western blotting by using the antibodies as labeled.
(h–j) AAV-mCherry-ISG15 ($6.54 \times 10^{12}$ V.G./ml) was co-transfected with pCI-HA-NEDD4-EGFP (NEDD4) or its empty vector (Ctrl) in primary neurons at 1 *div*, and the neurons were harvested at 7 *div*.
(h) The overexpression of ISG15 and NEDD4 proteins was confirmed by Western blotting (n = 4).
(i, j) Overexpression of NEDD4 rescued ISG15-induced dendrite impairments shown by the increased total dendrite length and number of branch tips (ISG15 + Ctrl, $n = 36$; ISG15 + NEDD4, $n = 32$).
The data was expressed as mean ± SD. *, $p < .05$, ***, $p < .001$ *vs* Ctrl or ISG15 + Ctrl, unpaired two-tailed student's t-test.

## Transparency document

The Transparency document associated with this article can be found, in online version.

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## Author contributions

J.Z.W. and G.P.L. conceived the project, designed the experiments, and wrote the manuscript. Y.H. and X.Y.H. designed and performed most of the experiments. X.G.L., D.S.S., and X.L. performed electrophysiological experiments. Q.F. and X.W. prepared primary neurons. H.L.W. and T.L. performed the immunohistochemical experiments. X.F·Y, J.F.Z., and R.H.M. assisted with *in vivo* and *in vitro* experiments. Q.W. and D.K assisted with data analysis.

## Conflicts of interest

All authors disclose: (a) no actual or potential conflicts of interest

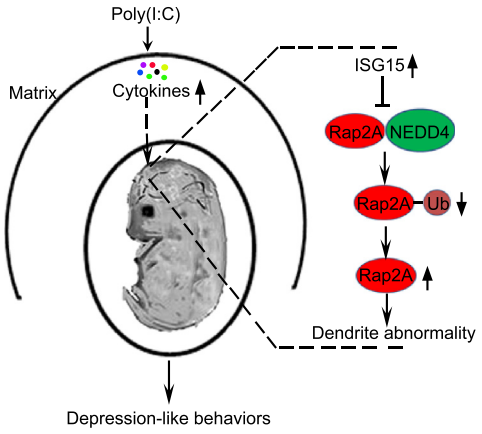


**Fig. 9.** The working model.
During MIA, the elevated cytokines in matrix circulating system stimulates ISG15 expression in the fetal brain, which in turn disrupts association of NEDD4 and Rap2A leading to an inhibited Rap2A ubiquitination and elevation of Rap2A. Elevation of Rap2A impedes dendrite development and induces depression behaviors in the offsprings.

including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work. (b) When applicable, provide statements verifying that appropriate approval and procedures were used concerning animals.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2019.02.020.

## References

[1] W. Adams, R.E. Kendell, E.H. Hare, P. Munk-Jørgensen, Epidemiological evidence that maternal influenza contributes to the aetiology of schizophrenia. An analysis of Scottish, English, and Danish data, Br. J. Psychiatry 163 (1993) 522–534.
[2] M. Cannon, D. Cotter, V.P. Coffey, P.C. Sham, N. Takei, C. Larkin, et al., Prenatal exposure to the 1957 influenza epidemic and adult schizophrenia: a follow-up study, Br. J. Psychiatry 168 (1996) 368–371.
[3] H.O. Atladóttir, P. Thorsen, L. Østergaard, D.E. Schendel, S. Lemcke, M. Abdallah, et al., Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders, J. Autism Dev. Disord. 40 (2010) 1423–1430, https://doi.org/10.1007/s10803-010-1006-y.
[4] A.S. Brown, Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism, Dev. Neurobiol. 72 (2012) 1272–1276, https://doi.org/10.1002/dneu.22024.
[5] C.J. Machado, A.M. Whitaker, S.E. Smith, P.H. Patterson, M.D. Bauman, Maternal immune activation in nonhuman primates alters social attention in juvenile offspring, Biol. Psychiatry 77 (2015) 823–832, https://doi.org/10.1016/j.biopsych.2014.07.035.
[6] U. Meyer, J. Feldon, To poly(I:C) or not to poly(I:C): advancing preclinical schizophrenia research through the use of prenatal immune activation models, Neuropharmacology 62 (2012) 1308–1321, https://doi.org/10.1016/j.neuropharm.2011.01.009.
[7] P. Coyle, N. Tran, J.N. Fung, B.L. Summers, A.M. Rofe, Maternal dietary zinc supplementation prevents aberrant behaviour in an object recognition task in mice offspring exposed to LPS in early pregnancy, Behav. Brain Res. 197 (2009) 210–218, https://doi.org/10.1016/j.bbr.2008.08.022.
[8] M.D. Bauman, A.M. Iosif, S.E. Smith, C. Bregere, D.G. Amaral, P.H. Patterson, Activation of the maternal immune system during pregnancy alters behavioral development of rhesus monkey offspring, Biol. Psychiatry 75 (2014) 332–341, https://doi.org/10.1016/j.biopsych.2013.06.025.
[9] S.E. Smith, J. Li, K. Garbett, K. Mirnics, P.H. Patterson, Maternal immune activation alters fetal brain development through interleukin-6, J. Neurosci. 27 (2007) 10695–10702.
[10] J.H. Gilmore, L.F. Jarskog, Exposure to infection and brain development: cytokines in the pathogenesis of schizophrenia, Schizophr. Res. 24 (1997) 365–367.
[11] J.H. Gilmore, L. Fredrik Jarskog, S. Vadlamudi, J.M. Lauder, Prenatal infection and risk for schizophrenia: IL-1beta, IL-6, and TNFalpha inhibit cortical neuron dendrite development, Neuropsychopharmacology 29 (2004) 1221–1229.
[12] S. Kitareewan, I. Pitha-Rowe, D. Sekula, C.H. Lowrey, M.J. Nemeth, T.R. Golub, et al., UBE1L is a retinoid target that triggers PML/RARalpha degradation and apoptosis in acute promyelocytic leukemia, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 3806–3811.
[13] L. Labrada, X.H. Liang, W. Zheng, C. Johnston, B. Levine, Age-dependent resistanceto lethal alphavirus encephalitis in mice: analysis of gene expression in the centralnervous system and identification of a novel interferon-inducible protective gene, mouse ISG12, J. Virol. 76 (2002) 11688–11703.
[14] K.J. Ritchie, D.E. Zhang, ISG15: the immunological kin of ubiquitin, Semin. Cell Dev. Biol. 15 (2004) 237–246.
[15] D.J. Lenschow, N.V. Giannakopoulos, L.J. Gunn, C. Johnston, A.K. O'Guin, R.E. Schmidt, et al., Identification of interferon-stimulated gene 15 as an antiviral molecule during Sindbis virus infection in vivo, J. Virol. 79 (2005) 13974–13983.
[16] W. Yuan, R.M. Krug, Influenza B virus NS1 protein inhibits conjugation of he interferon (IFN)-induced ubiquitin-like ISG15 protein, EMBO J. 20 (2001) 362–371.
[17] A. Okumura, G. Lu, I. Pitha-Rowe, P.M. Pitha, Innate antiviral response targets HIV-1 release by the induction of ubiquitin-like protein ISG15, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 1440–1445.
[18] J. Narasimhan, J.L. Potter, A.L. Haas, Conjugation of the 15-kDa interferon-induced ubiquitin homolog is distinct from that of ubiquitin, J. Biol. Chem. 271 (1996) 324–330.
[19] A. Okumura, P.M. Pitha, R.N. Harty, ISG15 inhibits Ebola VP40 VLP budding in an L-domain-dependent manner by blocking Nedd4 ligase activity, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 3974–3979, https://doi.org/10.1073/pnas.0710629105.
[20] J. Drinjakovic, H. Jung, D.S. Campbell, L. Strochlic, A. Dwivedy, C.E. Holt, E3 ligase Nedd4 promotes axon branching by downregulating PTEN, Neuron 65 (2010) 341–357, https://doi.org/10.1016/j.neuron.2010.01.017.
[21] H. Kawabe, A. Neeb, K. Dimova, S.M. Young Jr., M. Takeda, S. Katsurabayashi, et al., Regulation of Rap2A by the ubiquitin ligase Nedd4-1controls neurite development, Neuron 65 (2010) 358–372, https://doi.org/10.1016/j.neuron.2010.01.007.
[22] J.H. Gilmore, L.F. Jarskog, S. Vadlamudi, Maternal poly I:C exposure during pregnancy regulates TNF alpha, BDNF, and NGF expression in neonatal brain and the maternal-fetal unit of the rat, J. Neuroimmunol. 159 (1–2) (2005) 106–112.
[23] L. Zuckerman, I. Weiner, Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring, J. Psychiatr. Res. 39 (2005) 311–323.
[24] P. Boksa, Effects of prenatal infection on brain development and behavior: a review of findings from animal models, Brain Behav. Immun. 24 (6) (2010) 881–897, https://doi.org/10.1016/j.bbi.2010.03.005.
[25] G.P. Liu, W. Wei, X. Zhou, H.R. Shi, X.H. Liu, G.S. Chai, et al., Silencing PP2A inhibitor by lenti-shRNA interference ameliorates neuropathologies and memory deficits in tg2576 mice, Mol. Ther. 21 (2013) 2247–2257, https://doi.org/10.1038/mt.2013.189.
[26] X. Wang, J. Blanchard, I. Grundke-Iqbal, J. Wegiel, H.X. Deng, T. Siddique, et al., Alzheimer disease and amyotrophic lateral sclerosis: an etiopathogenic connection, Acta Neuropathol. 127 (2014) 243–256, https://doi.org/10.1007/s00401-013-1175-9.
[27] Y. Koyama, M. Tohyama, A modified and highly sensitive Golgi-Cox method to enable complete and stable impregnation of embryonic neurons, J. Neurosci. Methods 209 (2012) 58–61, https://doi.org/10.1016/j.jneumeth.2012.06.007.
[28] D. Ott, F. Wuchert, J. Murgott, C. Rummel, R. Gerstberger, J. Roth, The viral mimetic polyinosinic:polycytidylic acid (poly I:C) induces cellular responses in primary cultures from rat brain sites with an incomplete blood-brain barrier, Neurosci. Lett. 530 (2012) 64–68, https://doi.org/10.1016/j.neulet.2012.09.038.
[29] W.E. Kaufmann, H.W. Moser, Dendritic anomalies in disorders associated with mental retardation, Cereb. Cortex 10 (2000) 981–991.
[30] V.A. Kulkarni, B.L. Firestein, The dendritic tree and brain disorders, Mol. Cell. Neurosci. 50 (2012) 10–20, https://doi.org/10.1016/j.mcn.2012.03.005.
[31] N. Au-Yeung, R. Mandhana, C.M. Horvath, Transcriptional regulation by STAT1 and STAT2 in the interferon JAK-STAT pathway, JAKSTAT 22 (2013) e23931, , https://doi.org/10.4161/jkst.23931.
[32] O.A. Malakhova, D.E. Zhang, ISG15 inhibits Nedd4 ubiquitin E3 activity and enhances the innate antiviral response, J. Biol. Chem. 283 (2008) 8783–8787, https://doi.org/10.1074/jbc.C800030200.
[33] I. Knuesel, L. Chicha, M. Britschgi, S.A. Schobel, M. Bodmer, J.A. Hellings, et al., Maternal immune activation and abnormal brain development across CNS disorders, Nat. Rev. Neurol. 10 (2014) 643–660, https://doi.org/10.1038/nrneurol.2014.187.
[34] G. Lu, J.T. Reinert, I. Pitha-Rowe, A. Okumura, M. Kellum, K.P. Knobeloch, et al., ISG15 enhances the innate antiviral response by inhibition of IRF-3 degradation, Cell. Mol. Biol. (Noisy-le-Grand) 52 (2006) 29–41.
[35] A. Soetanto, R.S. Wilson, K. Talbot, A. Un, J.A. Schneider, M. Sobiesk, et al., Association of anxiety and depression with microtubule-associated protein 2- and synaptopodin-immunolabeled dendrite and spine densities in hippocampal CA3 of older humans, Arch. Gen. Psychiatry 67 (2010) 448–457, https://doi.org/10.1001/archgenpsychiatry.2010.48.
[36] F.C. de Anda, A.L. Rosario, O. Durak, T. Tran, J. Gräff, K. Meletis, et al., Autism spectrum disorder susceptibility gene TAOK2 affects basal dendrite formation in the neocortex, Nat. Neurosci. 15 (2012) 1022–1031, https://doi.org/10.1038/nn.3141.
[37] L. Eiland, B.S. an McEwen, Early life stress followed by subsequent adult chronicstress potentiates anxiety and blunts hippocampal structural remodeling, Hippocampus 22 (2012) 82–91, https://doi.org/10.1002/hipo.20862.
[38] R. Galvez, W.T. Greenough, Sequence of abnormal dendritic spine development inprimary somatosensory cortex of a mouse model of the fragile X mental retardationsyndrome, Am. J. Med. Genet. A 135 (2005) 155–160.
[39] M. Dierssen, G.J. Ramakers, Dendritic pathology in mental retardation: frommolecular genetics to neurobiology, Genes Brain Behav. 5 (Suppl. 2) (2006) 48–60.

[40] M.L. Estes, A.K. McAllister, Maternal immune activation: implications for neuropsychiatric disorders, Science 353 (2016) 772–777, https://doi.org/10.1126/science.aag3194.
[41] H. Soumiya, H. Fukumitsu, S. Furukawa, Prenatal immune challenge compromises development of upper-layer but not deeper-layer neurons of the mouse cerebral cortex, J. Neurosci. Res. 89 (2011) 1342–1350, https://doi.org/10.1002/jnr.22636.
[42] H.T. Ito, S.E. Smith, E. Hsiao, P.H. Patterson, Maternal immune activation alters nonspatial information processing in the hippocampus of the adult offspring, Brain Behav. Immun. 24 (2010) 930–941, https://doi.org/10.1016/j.bbi.2010.03.004.
[43] J.J. Yi, M.D. Ehlers, Emerging roles for ubiquitin and protein degradation in neuronal function, Pharmacol. Rev. 59 (2007) 14–39.
[44] H. Kawabe, N. Brose, The role of ubiquitylation in nerve cell development, Nat. Rev. Neurosci. 12 (2011) 251–268, https://doi.org/10.1038/nrn3009.
[45] T. Kishino, M. Lalande, J. Wagstaff, UBE3A/E6-AP mutations cause Angelman syndrome, Nat. Genet. 15 (1997) 70–73.
[46] T. Matsuura, J.S. Sutcliffe, P. Fang, R.J. Galjaard, Y.H. Jiang, C.S. Benton, et al., De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome, Nat. Genet. 15 (1997) 74–77.
[47] L.M. Ellman, R.F. Deicken, S. Vinogradov, W.S. Kremen, J.H. Poole, D.M. Kern, et al., Structural brain alterations in schizophrenia following fetal exposure to the inflammatory cytokine interleukin-8, Schizophr. Res. 121 (2010) 46–54, https://doi.org/10.1016/j.schres.2010.05.014.
[48] U. Meyer, Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems, Biol. Psychiatry 75 (2014) 307–315, https://doi.org/10.1016/j.biopsych.2013.07.011.
[49] G.B. Choi, Y.S. Yim, H. Wong, S. Kim, H. Kim, S.V. Kim, et al., The maternal interleukin-17a pathway in mice promotes autism-like phenotypes inoffspring, Science 351 (2016) 933–939, https://doi.org/10.1126/science.aad0314.
[50] N.M. Ponzio, R. Servatius, K. Beck, A. Marzouk, T. Kreider, Cytokine levels during pregnancy influence immunological profiles and neurobehavioral patterns of the offspring, Ann. N. Y. Acad. Sci. 1107 (2007) 118–128.
[51] B.E. Deverman, P.H. Patterson, Cytokines and CNS development, Neuron 64 (2009) 61–78, https://doi.org/10.1016/j.neuron.2009.09.002.
[52] L. Shi, S.H. Fatemi, R.W. Sidewell, P.H. Patterson, Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring, J. Neurosci. 23 (2003) 297–302.
[53] X. Zhang, D. Bogunovic, B. Payelle-Brogard, V. Francois-Newton, S.D. Speer, C. Yuan, et al., Human intracellular ISG15 prevents interferon-α/β over-amplification and auto-inflammation, Nature 517 (2015) 89–93, https://doi.org/10.1038/nature13801.