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Supplementary Materials for

**Microglial PCGF1 alleviates neuroinflammation associated depressive behavior
in adolescent mice**

- The PDF file includes:**
- Supplementary Materials and Methods
 - Figs. S1 to S7
 - Table S1, S2

19 **Supplementary Materials and Methods**

20 **Behavioral Testing**

21 All behavioral tests were conducted in an isolated behavioral testing room with test
22 personnel keeping the identity of the test group confidential. All behavioral experiments
23 were recorded, measured, and analyzed using a tracking system (TopScan 3.0). Mice
24 from the same cohort were tested in the following behavioral order.

25 **Forced swimming experiment**

26 The experiment was conducted in an open glass cylinder (25 cm high, 18 cm diameter)
27 filled with 18 cm of water at a temperature of 22°C-25°C. Each mouse was forced to
28 swim for 6 min. The water in the glass cylinder was replaced after each mouse
29 swimming to prevent behavioral changes between animals. Mice were considered
30 immobile when they stopped swimming and remained motionless, making only the
31 necessary movements to keep their heads above water. The immobility time was
32 recorded for the last 5 minutes of the 6-minute procedure.

33 **Open field test**

34 Mice were acclimated to the experimental room for 1 h and then placed in the center
35 (20 × 20) of a 35 cm × 35 cm × 25 cm chamber. The mice were allowed to freely explore
36 the field for 10 min to observe their locomotor activity. The total travel distance, time
37 spent and number of entries in the central areas were recorded and analyzed as measures
38 of locomotor activity and anxiety-like behavior. The floor of the open field was cleaned
39 with a 75% ethanol solution between tests to prevent the transmission of olfactory cues.

40 **Tail suspension test**

41 Mice were suspended individually with tape. The tape was placed 1 cm from the tail
42 tip. The test procedure was videotaped for 6 min and analyzed by a blind observer. Mice
43 that climbed the tail were excluded from the experimental analysis. Mobility was
44 defined as movement of the hind legs.

45 **Elevated positive maze test**

46 The EPM apparatus consisted of two open and two closed arms and a central platform.
47 EPM was elevated 50 cm above the floor and the mice were placed in the central area
48 and allowed to explore freely in the EPM for 6 min. A tracking system was used to
49 record and analyze the mice staying time and number of entries in the open arms as a
50 measure of anxiety-like behavior. Between tests, the floor of both arms was cleaned
51 with a 75% ethanol solution to prevent the spread of olfactory cues.

52 **Sucrose preference test**

53 Mice were placed individually in cages with two bottles of sucrose solution (1%) for
54 the first 24 h, and then one bottle of sucrose solution was replaced with tap water for
55 the second 24 h. After this stage, mice were deprived of food and water in small
56 amounts for 24 h and then placed for 3 h in two bottles, one containing 100 ml of 1%
57 sucrose solution and the other containing 100 ml of tap water. Sucrose preference was
58 defined as sucrose consumption / [water consumption + sucrose consumption] × 100%.

59 **Western blotting**

60 Mice were anesthetized with an intraperitoneal injection of sodium phenobarbital
61 (28 mg/kg) as based on their body weight. Bilateral CA1 tissue samples were isolated
62 and lysed in RIPA buffer containing a cocktail of protease/phosphatase inhibitors.
63 Microglia rinsed with PBS and lysed directly using RIPA. After centrifugation (20 min,
64 12,000 rpm, 4 °C), cleared lysates containing the isolated proteins were harvested.
65 Protein concentrations were determined using the Pierce BCA protein kit. Proteins
66 (30 µg) from each tissue sample were loaded in each lane, electrophoretically separated
67 on SDS-PAGE gels and then transferred onto PVDF membranes for primary antibody
68 incubation at 4 °C for overnight. Membranes were incubated with the following
69 antibodies: mouse anti-β-actin (HC201, Transs), mouse anti-PCGF1 (sc-515371, Santa
70 Cruz Biotechnology), rabbit anti-MMP10 (ab261733, abcam), rabbit anti-H3K4me3
71 (#9751, CELL SIGNALING TECHNOLOGY), rabbit anti-H2AK119ub (#8240,
72 CELL SIGNALING TECHNOLOGY), rabbit anti-H3K27me3 (#9733, CELL
73 SIGNALING TECHNOLOGY), rabbit anti-H3K9me3 (#13969, CELL SIGNALING
74 TECHNOLOGY), rabbit anti-H3K9/K14ac (#9677, CELL SIGNALING
75 TECHNOLOGY), rabbit anti-H3K18ac (#9675P, CELL SIGNALING
76 TECHNOLOGY), rabbit anti-H3 (#4499, CELL SIGNALING TECHNOLOGY),
77 rabbit anti-p65 (#8242S, CELL SIGNALING TECHNOLOGY), rabbit anti-p-p65
78 (#3033T, CELL SIGNALING TECHNOLOGY), rabbit anti-p44/42 MAPK(Erk1/2)
79 (#4695, CELL SIGNALING TECHNOLOGY), rabbit anti-p-p44/4IN2 MAPK(Erk1/2)
80 (#4370, CELL SIGNALING TECHNOLOGY), rabbit anti-JNK (#9252, CELL
81 SIGNALING TECHNOLOGY), rabbit anti-p-JNK (#4668, CELL SIGNALING

TECHNOLOGY), rabbit anti-p38 (#8690, CELL SIGNALING TECHNOLOGY), rabbit anti-p-p38 (#4511, CELL SIGNALING TECHNOLOGY), rabbit anti-RING1B (16031-1-AP, proteintech), rabbit anti-EZH2 (21800-1-AP, proteintech). Image-J software was used to perform pixel quantification of the images. Intra-run normalization against the internal actin control was performed for each sample.

RNA isolation and real-time quantitative PCR

Total RNA was extracted using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) from CA1 and microglia. Total RNA of human plasma samples was extracted using MolPure Blood RNA Kit (Yeasen Biotechnology, Shanghai, China). The concentration of total RNA was then measured using a NanoDrop™ OneC spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Then cDNA was synthesized with a RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Quantitative Real-time PCR was performed with SYBR Green Realtime PCR Master Mix (TOYOBO CO, Ltd, Japan). Primer sequences used were listed in Table 1. Each sample was analyzed in duplicate. The expression of β -actin was regarded as a normalization control, and the $2^{-\Delta\Delta C_t}$ method was used to calculate changes of the gene expression levels. (Primer sequence reference Supplementary Table 1.)

Immunofluorescent staining and confocal microscopy

Frozen slices (15 μ m) were incubated overnight at 4°C with the following antibodies: Iba1 (Ab178680, Abcam), TNF- α (#11948, CELL SIGNALING TECHNOLOGY), IL-1 β (#12703, CELL SIGNALING TECHNOLOGY), p65 (#8242S, CELL SIGNALING

TECHNOLOGY), PCGF1 (sc-515371, Santa Cruz Biotechnology), MMP10 (ab261733, abcam), GFAP (#3670, CELL SIGNALING TECHNOLOGY), NeuN (#24307, CELL SIGNALING TECHNOLOGY). Sections were then incubated with Alexa Fluor 488 or 546 conjugated (Abcam), for 1 h at 37°C in a thermostatic oscillator. DAPI (Beyotime) was used for nuclear staining. Before each step, slices were washed three times in PBS. Images were captured using a confocal microscope (LSM880, Carl Zeiss, Germany) or fluorescence microscope (Olympus BX51; Olympus, Shinjuku, Japan) and processed using ZEN software. The fluorescent intensity analysis was performed using ImageJ (NIH, Bethesda, MD, USA).

Adeno-associated virus injection

The adeno-associated virus: AAV9-F40/80-PCGF1-P2A-GFP [7.45×10^{13} vg/ml] and control PCGF1 virus [6.47×10^{13} vg/ml], AAV9-F40/80-MMP10-P2A-mcherry [5.81×10^{13} vg/ml] and control MMP10 virus [3.40×10^{13} vg/ml] were packed by WZ biosciences Inc (Jinan, China). CA1 region (AP -1.68 mm, ML $\pm$ 1.33 mm, and DV -1.38 mm) was the local virus injection site. Mice were anesthetized by isoflurane, and AAV9 was administered by Hamilton micro- syringe coupled with a microinjection pump (KDS 200, KD Scientific, MA, USA) (Speed: 0.2 ul/min and injection volume: 0.4 ul/lateral, mixed virus injection volume 0.7ul/lateral). Infection efficiency assays were performed a minimum of 21 days after virus injection. Injection sites were verified and only data from mice with correct injection sites were included in the analyses. Panoramic scanning image were captured using an Olympus microscope (VS120,

Japan). The proportion of Iba1 and GFP double-labelled were counted in three consecutive brain slices per mouse. The value of each mouse was taken as the average of three brain slices. Animals that were incorrectly injected with virus were excluded from the analysis.

Transmission electron microscopy (TEM)

The ultrastructure of CA1 neurons was observed using transmission electron microscopy (Philips Tecnai 20 U-Twin, Holland). Bilateral CA1 tissues were carefully dissected ($1 \times 1 \times 1$ mm), fixed with 1% osmium tetroxide for 2 h, and dehydrated with graded ethanol. The tissue was infiltrated overnight with a semi-epoxy-propane mixture and then embedded in resin. Tissues were cut into ultrathin sections (70 nm), stained with 4% uranyl acetate for 20 min and then stained with 0.5% lead citrate on the copper grid. At least 30 micrographs were randomly selected from each mouse and analyzed using Image J analysis software (NIH, Scion Corporation, Frederick, MD).

Brain anatomy and tissue preparation

Twenty-four hours after behavioral tests, mice from each group were anesthetized with sodium phenobarbital (30 mg/kg) and transcardially perfused with 300 ml 0.9% NaCl containing heparin sodium salt followed by fixation with 4% paraformaldehyde (PFA). Brains were removed and post-fixed in 4% PFA overnight at 4°C followed by a graded dehydration. Brain tissue was encased in optimum cutting temperature compound and frozen serial coronal sections were cut. Sections, consisting of hippocampus were selected for immunofluorescent staining.

Plasmid transfection and lentivirus infection

Lentivirus-shPcgfl (hU6-MCS-CBh-gcGFP-IRES-puromycin, shPcgfl sequence: 5'-GCAGTTTTGACCACTCTAA-3') was designed by JiKai Gene (Shanghai, China). Pcgfl cDNA was PCR-amplified and cloned into GV493-gcGFP-Puro vector. For lentiviral infection, microglia were inoculated in 6-well plates (1×10^5 cells/well). After 12 h, the cells were incubated with 2 ml of virus-containing medium to which 10 μ l of polybrene (10 mg/ml, H8761, Solarbio) had been added. The effect of the virus on the expression was assessed at the mRNA and protein levels after 48 h. Plasmid-PCGF1 (pCMV-EGFP-Pcgfl(mouse)-HA-SV40-Neo) was obtained from MiaoLingBio, China. For transient transfection of plasmids into microglia, Lipofectamine 2000 reagent (Invitrogen) was used.

HE staining

Brains were fixed in 4% paraformaldehyde, subsequently embedded in paraffin and cut into 5- μ m-thick sections. Sections were stained with hematoxylin and eosin (H&E) to observe neuronal morphology in the hippocampus of the brain. The number of damaged neurons was counted in three non-overlapping regions per mouse and displayed as a percentage. The value for each mouse were averaged over the three regions.

Morphologic analysis of microglia

The sholl analysis of the Fiji (Fiji is just ImageJ) was used to count the branches number of microglia.

Inhibitor treatment

Primary microglia were pretreated with or without SB203580 (20 μ M, HY-10256, MCE) and PDTC (50 μ M, HY-18738, MCE) for 1 h, (the supernatant was not discarded), then co-treated for 8 h with LPS (500 ng/ml).

Golgi staining

Mice brains were collected and processed for Golgi-Cox staining according to the protocol provided in the FD Rapid Golgi Staining Kit (PK401, FD NeuroTechnologies). Tissues were cut into 150- μ m coronal sections using a vibrating microtome (Leica VT1200S, Germany) and collected on gelatin-coated microscope slides, dehydrated with a series of graded alcohols, washed with xylene, and coverslipped. The slides were stored at room temperature protected from light. Stained pyramidal neurons in the hippocampal region were examined and images were captured using an Olympus microscope (VS120, Japan).

Immunoprecipitation and ELISA

Whole cells are lysed using pre-cooled IP lysis buffer and stirred for 5 minutes. For immunoprecipitation, 1 to 2 μ g of specific antibody was added to 1 mg of protein at 4°C to form immune complexes, which were then added to Protein A/G PLUS-Agarose (26146, Thermo Fisher Scientific). The complexes were washed to remove nonspecific binding, and 1 $\times$ conditioning buffer was used to separate the bound immune complexes from Protein A/G. Immunoprecipitates were separated by SDS-polyacrylamide gel

electrophoresis (SDS-PAGE) and detected using Western blot. ELISA kits for IL-1 β (MULTI SCIENCES) and Tnf- α (MULTI SCIENCES) were used to quantify the respective cytokines or compounds.

Behavior Measurements

A comprehensive clinical assessment of neurologic and psychiatric status was performed on all subjects. Depression was assessed using the children's depression inventory (CDI). The post-traumatic stress disorder (PTSD) scale were also used. (Participants' demographics and clinical characteristics reference Supplementary Table 2.)

Clinical sample collection and processing

The healthy group and MDD group completed the CDI and PTSD questionnaires. Then, at 8 am, 5 ml blood samples were taken from each fasted participant by a specialized physician and were maintained in anticoagulation tubes. Then, the samples were centrifuged at 4 °C and 2500 rpm for 10 min, and the upper plasma samples were collected. Total RNA from human plasma samples was extracted using a MolPure Blood RNA Kit (Yeasen Biotechnology, Shanghai, China). Total RNA was used for reverse transcription and quantitative real-time PCR assays.

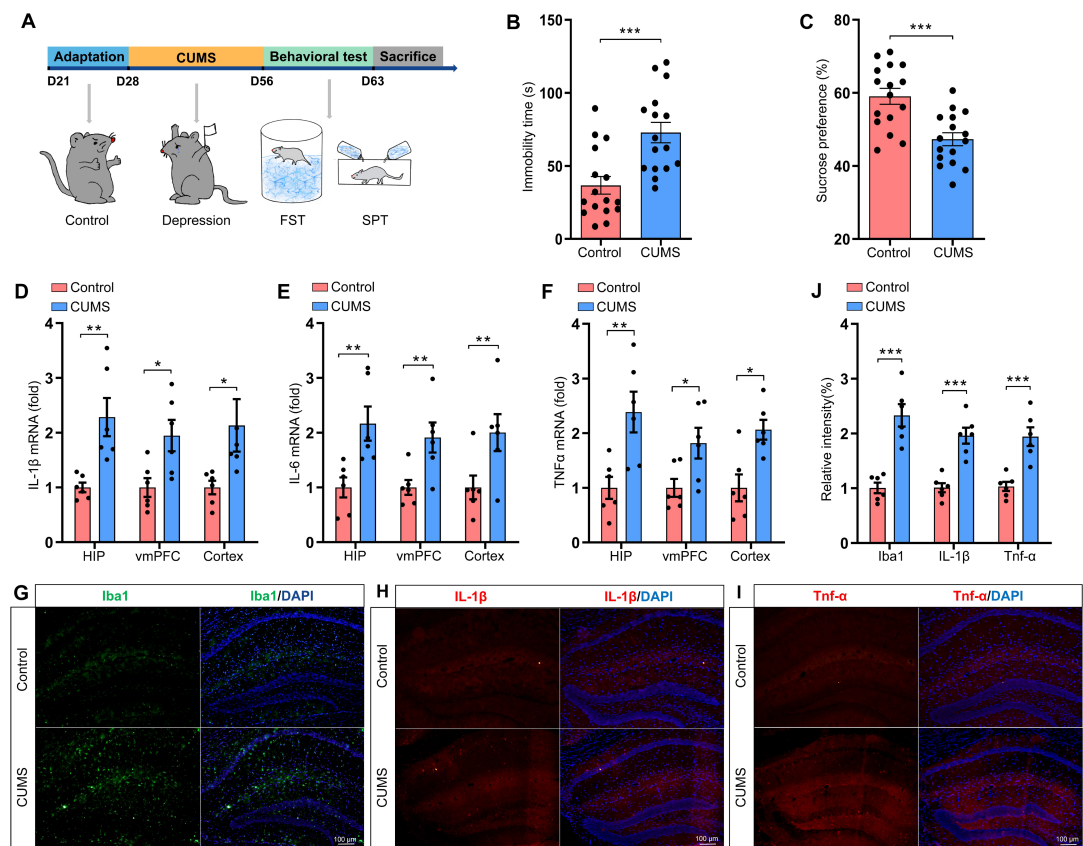
The Children's Depression Inventory (CDI)

CDI was used to assess the severity of the subject's depressive symptoms over the past two weeks. The scale consists of 27 items and includes five subscales: negative affect, relationship problems, anhedonia, negative self-esteem, and ineffectiveness. Each item

consists of three statements ranging in severity from 0 to 2. Negative statements are rated on a reverse scale. The total score indicates the severity of the depressive symptoms (0-54) with a cut-off point of 19, with higher scores indicating more severe depressive symptoms. The Chinese version of the CDI we used has good reliability and validity in measuring depressive symptoms.

Post-Traumatic Stress Disorder (PTSD)

PTSD was measured by the Posttraumatic Stress Scale - Civilian Version (PCL-C), which is based on the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) (DSM-IV). The scale consists of 17 questions and includes three subscales: re-experiencing (5 items), avoidance and numbing (7 items), and hyperanxiety (5 items). The scale is rated on a 5-point scale, with 1 representing "not at all" and 5 representing "very much". The higher the score, the more severe the degree of PTSD. In the current study, the PCL-C had excellent internal reliability.



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228 **Supplementary Fig. 1 Neuroinflammation was observed in the HIP, vmPFC and**
229 **cortex of CUMS mice.**

230 **A** Schematic diagram of the experiment design. **B, C** Behavioral responses of FST and
231 SPT in control mice and CUMS mice (N = 16 per group). **D-F** QPCR analysis of IL-1 β ,
232 IL-6 and Tnf- α mRNA levels of HIP, vmPFC and cortex region (N = 6 per group). **G**
233 Representative images of Iba1 staining (green) within the HIP region from each group
234 of mice. Nuclei (blue) are stained with DAPI. Scale bar is 100 μ m (N = 6 per group).
235 **H-I** Representative images of IL-1 β and Tnf- α staining (red). Nuclei (blue) are stained
236 with DAPI. Scale bar is 100 μ m (N = 6 per group). **J** Fluorescence intensity analysis of

237 G-I. Statistical significance was determined by multiple unpaired two-sided Student's
238 t-tests or one-way ANOVA followed by Tukey post hoc test. Data are shown as the
239 mean±s.d. or as a representative result from three independent experiments. *P < 0.05,
240 **P < 0.01 and ***P < 0.001.

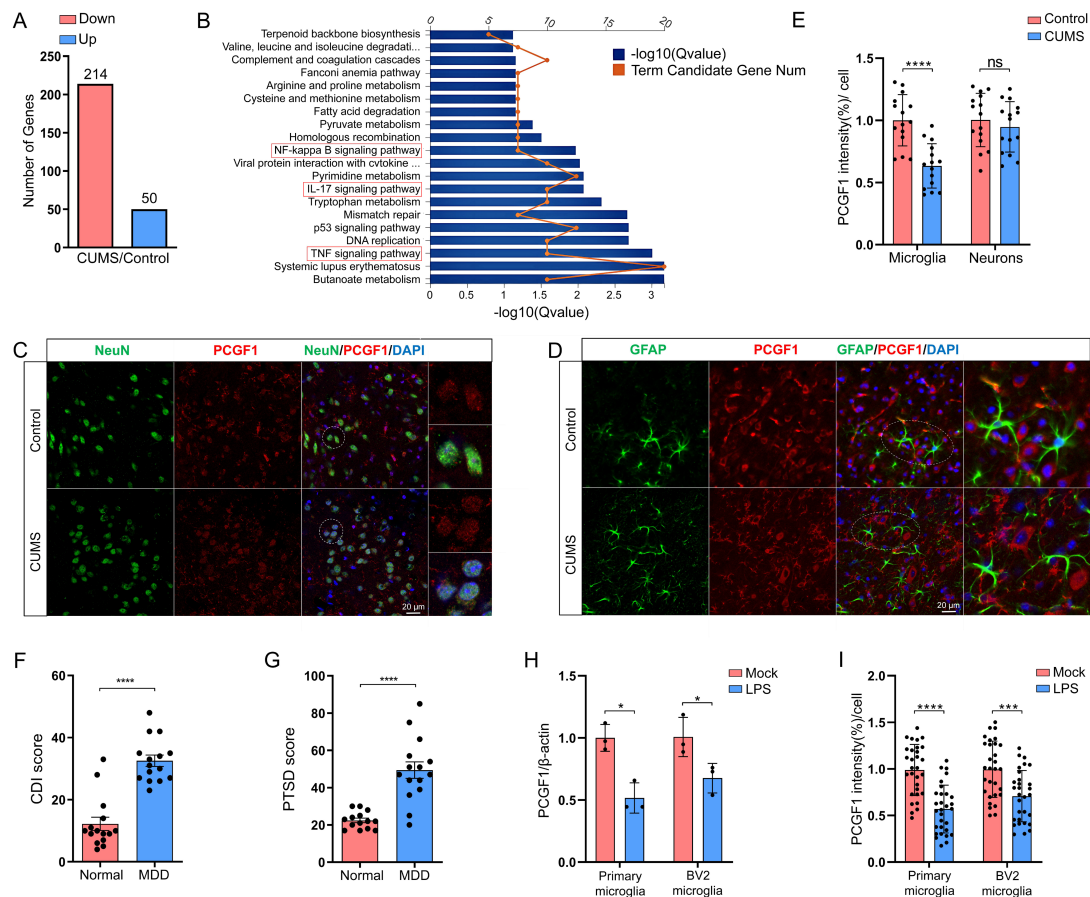
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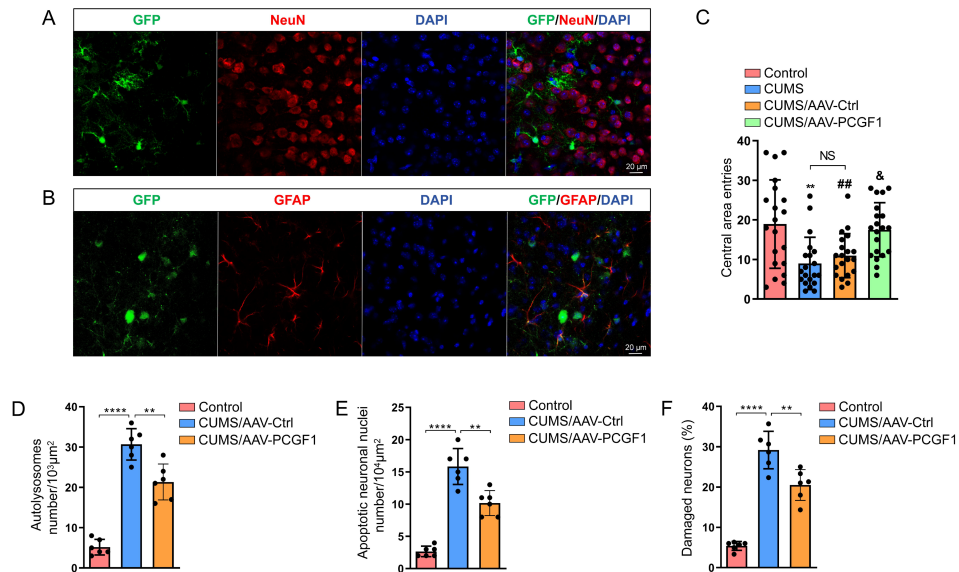


Supplementary Fig. 2 Microglial PCGF1 is downregulated in CUMS mice and *in vitro* LPS-induced experiments.

A Histogram plots represented differentially expressed genes in microglia of control mice and CUMS. The red color indicates downregulated genes, and the blue color indicates upregulated genes if they had a log2 fold change >1 or <-1 , Q-value < 0.05 .

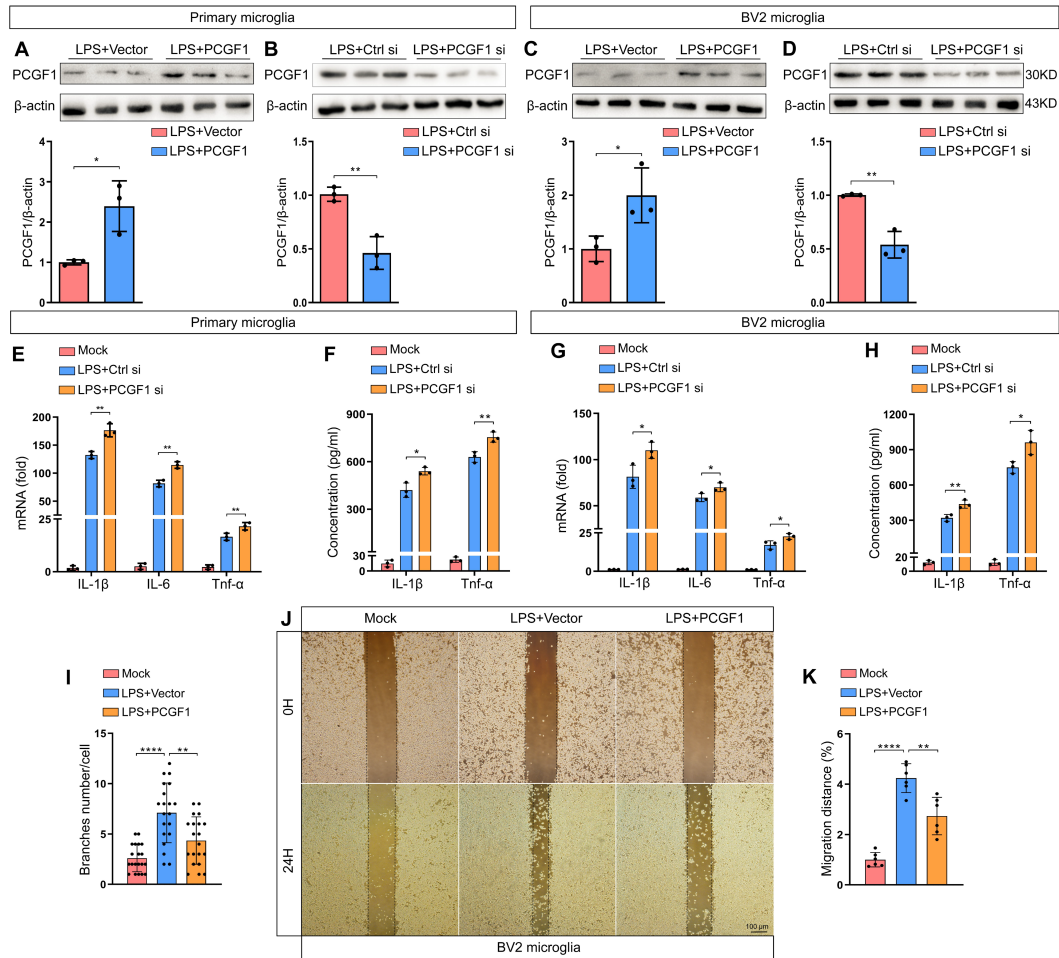
B KEGG analysis revealed that the differentially expressed genes were involved in inflammatory signaling pathways. **C** Representative images of PCGF1 staining (red) in the CUMS mice. Nuclei (blue) are stained with DAPI. Neurons (green) are stained with Anti-NeuN. Scale bar is 20 μm . **D** Representative images of PCGF1 staining (red) in the CUMS mice. Nuclei (blue) are stained with DAPI. Astrocytes (green) are stained

with Anti-GFAP. Scale bar is 20 μ m. **E** Fluorescence intensity analysis of 2E, S1C (N = 15 per group). **F** CDI scores of normal controls and MDD adolescent patients (N = 15 per group). **G** PTSD scores of normal controls and MDD adolescent patients (N = 14 or 15 per group). **H** Western bolt analysis of PCGF1 expression efficiency (N = 3 per group). **I** Fluorescence intensity analysis of 2K-L (N = 30 per group). Statistical significance was determined by multiple unpaired two-sided Student's t-tests or one-way ANOVA followed by Tukey post hoc test. Data are shown as the mean $\pm$ s.d. or as a representative result from three independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001.



Supplementary Fig.3 Microglial PCGF1 in the CA1 region is associated with OFT test in CUMS mice.

A Colocalization of GFP (green) and NeuN (red), scale bar, 20 μm. **B** Colocalization of GFP (green) and GFAP (red), scale bar, 50 μm. **C** In CUMS mice, Microglial PCGF1 increase the number of entries in the central area during the OFT test (N = 20 per group). **D** Statistics on autolysosomes number (N = 6 per group). **E** Statistics on apoptotic neuronal nuclei number (N = 6 per group). **F** Statistics on the percentage of damaged neurons in HE staining (N = 6 per group). Statistical significance was determined by multiple unpaired two-sided Student's t-tests or one-way ANOVA followed by Tukey post hoc test. Data are shown as the mean±s.d. or as a representative result from three independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001.



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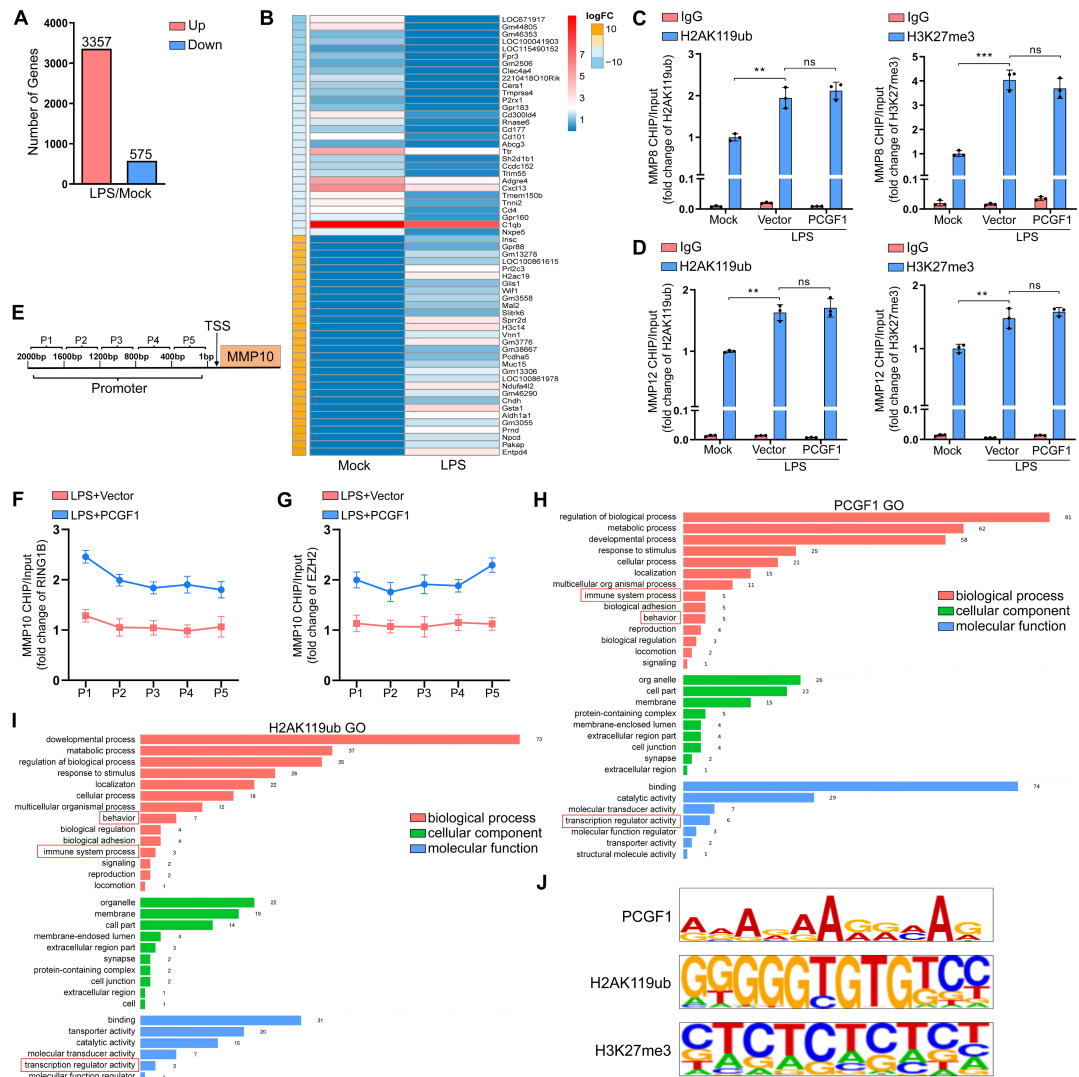
283 **Supplementary Fig. 4 PCGF1 regulates LPS-induced neuroinflammation in**
284 **microglia.**

285 **A-D** Western bolt analysis of PCGF1 overexpression or knock down efficiency in
286 primary and BV2 microglia (N = 3 per group). **E, F** QPCR analysis and ELISA analysis
287 of IL-1β, IL-6 and Tnf-α level in primary microglia (N = 3 per group). **G, H** QPCR
288 analysis and ELISA analysis of IL-1β, IL-6 and Tnf-α level in BV2 microglia (N = 3
289 per group). **I** Statistics on branches number of primary microglia (N = 20 per group). **J**
290 The migration ability of BV2 microglia was measured by scratch assay. Scale bar is
291 100 μm. **K** Statistics on migration distance of BV2 microglia (N = 6 per group).

292 Statistical significance was determined by multiple unpaired two-sided Student's t-tests
293 or one-way ANOVA followed by Tukey post hoc test. Data are shown as the mean $\pm$ s.d.
294 or as a representative result from three independent experiments. *P < 0.05, **P < 0.01
295 and ***P < 0.001.

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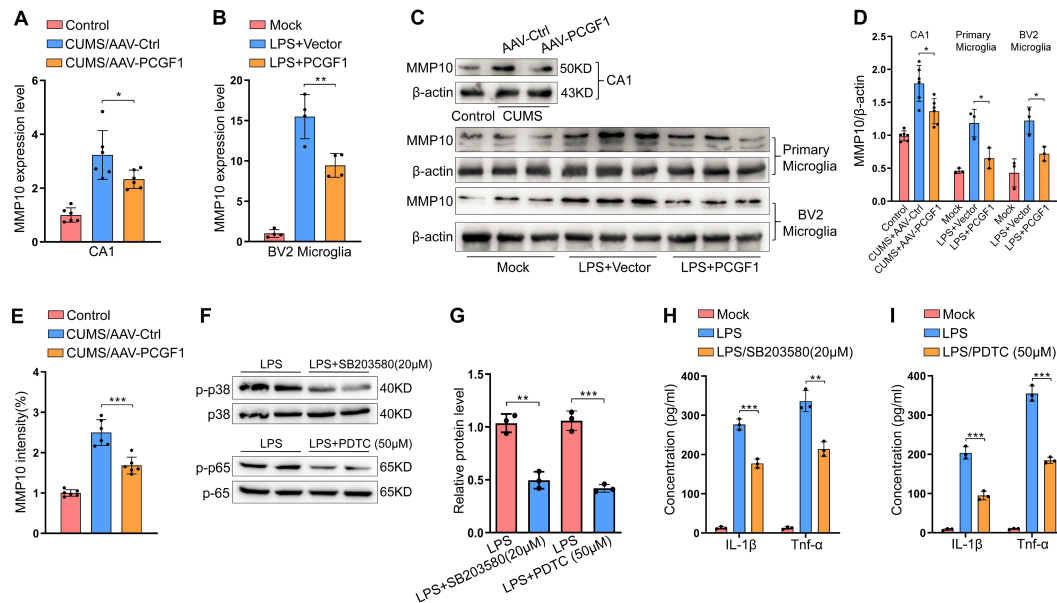
Supplementary Fig. 5 PCGF1 regulates histone modification in the MMP10 promoter region.

A Histogram plots represented differentially expressed genes in primary microglia of Mock and LPS. The red color indicates upregulated genes, and the blue color indicates downregulated genes if they had a log2 fold change >1 or <-1, Q-value < 0.05. B Heatmap of top 30 up- and down-regulated genes in primary microglia of Mock and LPS. C ChIP-QPCR analysis identified the expression of H2AK119ub and H3K27me3 at the promoters of MMP8 in BV2 microglia (N = 3 per group). D ChIP-QPCR analysis

identified the expression of H2AK119ub and H3K27me3 at the promoters of MMP12 in BV2 microglia (N = 3 per group). **E** The MMP10 promoter region is divided into five parts (P1-P5). **F, G** The binding tracks of RING1B and EZH2 in the MMP10 promoter region from ChIP-QPCR (N = 3 per group). **H, I** GO analysis showed that PCGF1 and H2AK119ub binding differential genes were enriched in the immune system, behavior, and transcriptional regulation. **J** Motif analysis showed base characteristics of PCGF1/H2AK119ub/H3K27me3 binding. Statistical significance was determined by multiple unpaired two-sided Student's t-tests or one-way ANOVA followed by Tukey post hoc test. Data are shown as the mean±s.d. or as a representative result from three independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001.

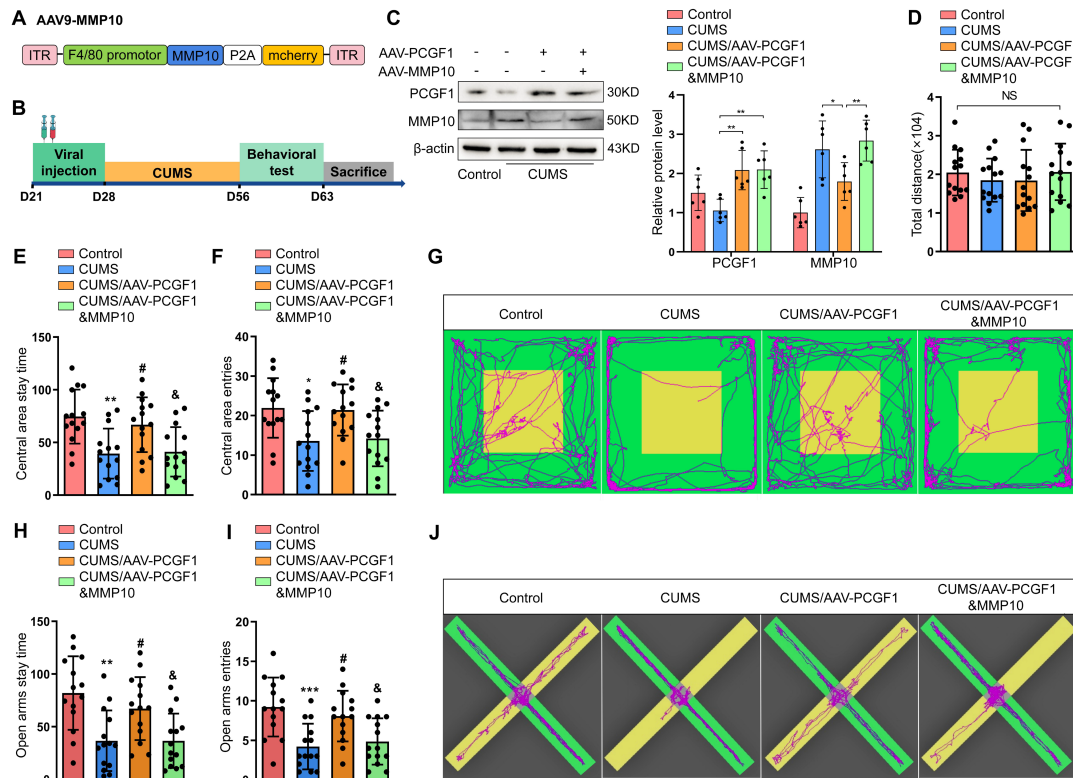
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Supplementary Fig. 6 The phosphorylation inhibitors of p38 and p65 inhibits inflammation in primary microglia.

A QPCR analysis of MMP10 mRNA levels in CA1 region (N = 6 per group). **B** QPCR analysis of MMP10 mRNA levels in BV2 microglia (N = 3 per group). **C, D** Representative western blot images showing the expression of MMP10 in CA1 region (N = 6 per group), primary microglia and BV2 microglia (N = 3 per group). **E** MMP10 fluorescence intensity statistics (N = 6 per group). **F, G** Representative western blot images of the phosphorylation of p38 and p65 in primary microglia after using SB203580 and PDTC (N = 3 per group). **H, I** ELISA analysis of IL-1β and Tnf-α secretion in primary microglia after using SB203580 and PDTC (N = 3 per group). Statistical significance was determined by multiple unpaired two-sided Student's t-tests or one-way ANOVA followed by Tukey post hoc test. Data are shown as the mean±s.d. or as a representative result from three independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001.



Supplementary Fig. 7 Microglial MMP10 is associated with depression-like behavior in CUMS mice.

A Schematics of AAV vectors engineered to overexpress MMP10. **B** Experimental paradigm for determining behavioral responses of mice infected with the virus. **C** The fold change of the protein level of PCGF1 and MMP10 in the CA1 region of mice (N = 6 per group). **D** Total distance traveled in the OFT was not significantly different (N = 14 per group, * CUMS vs. Control; # CUMS/AAV-PCGF1 vs. CUMS; & CUMS/AAV-PCGF1/MMP10 vs. CUMS/AAV-PCGF1; the same below). **E, F** MMP10 reduce the stay time and the number of entries in the central area during the OFT test (N = 14 per group). **G** Representative activity tracking in the OFT. **H, I** MMP10 reduced the stay time and the number of entries in the open arms during the EPM test (N = 14 per group). **J** Representative activity tracking in the EMP. Statistical

significance was determined by multiple unpaired two-sided Student's t-tests or one-way ANOVA followed by Tukey post hoc test. Data are shown as the mean±s.d. or as a representative result from three independent experiments. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. # and & follows the same rules.

374 **Supplementary Table 1: Primer sequence information.**

Gene	Forward primer	Reverse primer
IL-1 β	AGCATCCAGCTTCAAATC	CTTCTCCACAGCCACAAT
TNF- α	TCTCATTCCTGCTTGTGGC	CACTTGGTGGTTTGCTACG
IL-6	ACAACCACGGCCTTCCCTAC	CATTTCACGATTTCCCAGA
MMP8	AACGGTCTTCAGGCTGCTTA	ATCAATGGCTTGGACACTCC
MMP10	TGACCCCACTCACTTTCTCC	GGGTGCAAGTGTCCATTTCT
MMP12	TTTCTTCCATATGGCCAAGC	GGTCAAAGACAGCTGCATCA
MMP13	TTTATTGTTGCTGCCCATGA	TTTTGGGATGCTTAGGGTTG
MMP27	CAAATCCATCCACACACTCG	TCTGTGGGAATCCTCTGTCC
PCGF1	GCCGGCTACTTCGTGGAT	GGTCCAGTTTGAGGTTGAGC
PCGF1 (Human)	GGCTGAGTTCTGGCAAAGAC	TGTAAGCAAGGGGATGG
PCGF2	ACTCCTTTCTTACCGTAGGTTTCTC	AATCGTTAAGACGGGGGAGCC
PCGF3	AGGACAACGACTACCACCGCAG	TCTTCAGATGCAAGACGGTCGC
PCGF4	CGCCCGCTCAGATCGCTC	ACCCTCCACACAGGACACACATT
PCGF5	ATGGCTACCCAAAGGAAACA	AACTTGGTTGCCACACCTTG
PCGF6	GGAGAAGCAACTATCGGGCA	CCAGTAAGTGATCCCCACAGA
MMP10 promotor	ATCCTTCCTACCCCTCCTCA	GATCCAGAGCCTAGCACCAG
MMP8 promotor	GCCCCGATTAAGTAGCACAA	GAAGTGGGGGAGTCATTCAA
MMP12 promotor	CTTCAAGGACCCTCATTCCA	CCTTGAAGGCCTCAGAAATG

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380 **Supplementary Table 2: Participants' demographics and clinical characteristics**

	Normal (N=15)	Depression (N=15)	p
	Mean±SEM	Mean±SEM	
Age (years)	16.22±0.65	14.75±0.49	0.0851
Height (cm)	172.3±2.04	165.8±1.36	0.0126
Weight (kg)	63.33±2.70	61.07±4.57	0.6727
Gender (Male) (% , N)	53.33% (10)	13.33% (2)	
Family history	0/15	0/15	
Infected with COVID-19	100% (15)	100% (15)	
Divorced parents	6.67% (1)	26.67% (4)	
Eating disorder	0	40% (6)	

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