Materials and methods

Experimental animals

All animal procedures were performed following protocols that the local Institutional Animal Care and Use Committee approved. C57/BL6J mice aged 6-8 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Mice were housed at a 12 h/12 h light-dark cycle, 20–22 °C, and 50%–60% humidity, with adequate water and food.

Establishment of mouse demyelination model

A mouse model of central nervous system demyelination was established by feeding rodent chow mixed with 0.2% (w/w) CPZ (Sigma-Aldrich, USA) for 6 weeks. Food and water were available ad libitum during the experiment, and litter was changed twice weekly.

In vitro culture of cBMMSC and establishment of sc-cBMMSC

cBMMSC and sc-cBMMSC were obtained from cranial bones. Briefly, the cranial bones from the clinic were excised into chips of approximately 4-8 mm^3, from which mononuclear cell content was flushed out using αMEM (Cellgro, USA). Collected mononuclear cells (~1×10^6) were seeded in a 10 cm tissue culture-treated petri dish (Sigma-Aldrich) and grown at 37°C, 5% CO2 and 95% humidity in αMEM supplemented with 10% FBS (Gibco, USA), 2mM L-glutamine (Sigma-Aldrich), 100 U/mL penicillin (Sigma-Aldrich) and 100 μg/mL streptomycin (Sigma-Aldrich). After 48 hours incubation, nonadherent cells were completely removed by thoroughly changing the medium and remaining adherent cells were continuously cultured.

To establish sc-cBMMSC, mononuclear cell content from cranial bones was seeded in first 10cm dish for 6h. The supernatant was transferred to a new dish and incubated for another 6h. nonadherent cells were then transferred to a new dish and incubated for 36h. Finally, adherent cells in the three dishes were cultured in αMEM supplemented with 10% FBS for 14d, and well-separated cell colonies appeared. Individual colonies were identified under phase-contrast microscope and enclosed using sterile cloning cylinders (10 mm in diameter; Sigma-Aldrich). Of note, selected colonies were well apart from each other to avoid multiple colonies within single cloning cylinder. After removing culturing medium within cloning cylinders, 50 μL of 0.25% trypsin (Gibco) was added to harvest and transferred sc-cBMMSC (~100 cells) into a 48-well tissue culture plate (Sigma-Aldrich) for further culturing and expansion under the same condition detailed above.

MSCs transplantation

The CPZ-induced mice were randomly divided into different groups: model group, vehicle group, cBMMSC group, and Olig2+ sc-cBMMSC group. A typical control group of age-matched health mice was also included. 1×10^6 cells were suspended in 100 μL PBS and administrated via tail vein injection once a week for two consecutive weeks. The mice of the vehicle group received two doses of 100 μL PBS.

Animal behavior tests

The rotarod test was conducted to assess the coordination and balance ability of the mice of various groups. The mice were trained to maintain balance and exercise on the rotary rod instrument (ZH-ZQ, Zhenghua, Huaibei), and the rotation speed slowly increased from 0 r/min to

45 r/min. The dwell time of individual mice on the rotarod was recorded. For the suspension experiment, fasten both ends of a piece of cotton string to the rack and straighten it. Next, hang the mouse's front paws horizontally on the line. If the mouse can catch itself with both paws, it scores 3 points; if the mouse can catch itself with only one paw, it scores 2 points; and if the mouse cannot catch itself with either paw, it scores 0 points.

For the Morris water maze experiments, the animals were trained to find a platform in a pool of water. The escape latency and the path length required to locate the platform were measured (ZH0065, Zhenghua, Huaibei). On day 1, the mice were manually guided to the visible platform if they failed to locate the platform within 90 seconds and remained on the platform for 20 seconds. Beginning on day 2, the submerged platform was hidden and the trial was repeated. Each animal performed 4 trials per day, and there was a 20-second interval between two trials. Each trial started from a different quadrant. On day 6, the number of passages within 90 seconds was measured after the platform was removed.

Detection of immune cells in peripheral blood

Venous blood was collected from various groups of mice, and lymphocytes were then separated by density gradient centrifugation and incubated with flow antibodies (Biolegend, USA). The ratio of CD3 and CD4 cells in peripheral blood-derived lymphocytes was determined by flow cytometry (BD FACSVerse).

Detection of inflammatory factors

Mouse serum was separated from the venous blood using standard protocol. Meanwhile, these mice were sacrificed, and brain tissue samples were collected and lysed using tissue lysis solution. ELISA kits were used to measure inflammation-related factors, TNFα (Boster, China), IL-1β (mibio, China), and IL-6 (mibio, China).

Western blotting

Brain tissues were harvested and homogenized with cold tissue lysis solution(150 mM NaCl, 1% NP-40, 50 mM Tris–HCL, pH 8.0, supplemented with a complete set of protease inhibitors) (Roche, Basel, Switzerland). The concentration of extracted brain tissue proteins was measured with the BCA method (Beyotime Institute of Biotechnology, China). Whole protein extracts were added to Laemmli loading buffer and incubated at 99°C for 10 min. Samples (20 μg) were separated by 12% SDS-PAGE under reducing conditions. The gel was then transferred to a nitrocellulose membrane and immunoblotted with a primary antibody (anti-MBP antibody, Abcam, UK), a secondary antibody labeled with horseradish peroxidase (HRP, Beyotime Institute of Biotechnology), and an electrochemiluminescence system to display protein signals. ImageJ software was adopted to analyze the grayscale values obtained by western blotting.

Immunofluorescence staining

Mice were sacrificed, and the brain tissues were fixed with 4% paraformaldehyde and embedded in paraffin. Paraffin-embedded sections were stained with hematoxylin and eosin (H&E) and analyzed histologically. The remaining sections were blocked with 5% goat serum at 37°C for 1 h, and then stained with primary Abs (anti-Olig2， anti-Syn, Abcam, UK). After washing, the intestinal paraffin slices were incubated with an HRP-conjugated anti-rabbit/mouse IgG Ab

(Biolegend, USA) at 37°C for 1 h. Finally, the sections were incubated with DAB peroxidase substrate (Sigma, St. Louis, MO, USA). The staining results were observed under a light microscope (Olympus BX51, Tokyo, Japan).

Transmission electron microscope

The experimental animals were sacrificed, and the brain tissues were harvested and fixed in 2.5% glutaraldehyde at 4°C for 4 hours, then washed three times with PBS, dehydrated with ethanol gradient (40%–100%), and embedded in resin. The samples were sectioned at 90 nm thickness and stained with 1% uranyl acetate for 2 minutes and lead citrate for 5 minutes. The samples were finally observed and imaged using a transmission electron microscope (Leo Libra 120; Carl Zeiss SMT AG).

Statistical analysis

Graphpad 7.0 was used for statistical analyses. All data are presented as mean±standard deviation (SD). Student's t-tests evaluated statistical differences between two groups. Statistical significance was defined as $P<0.05$.

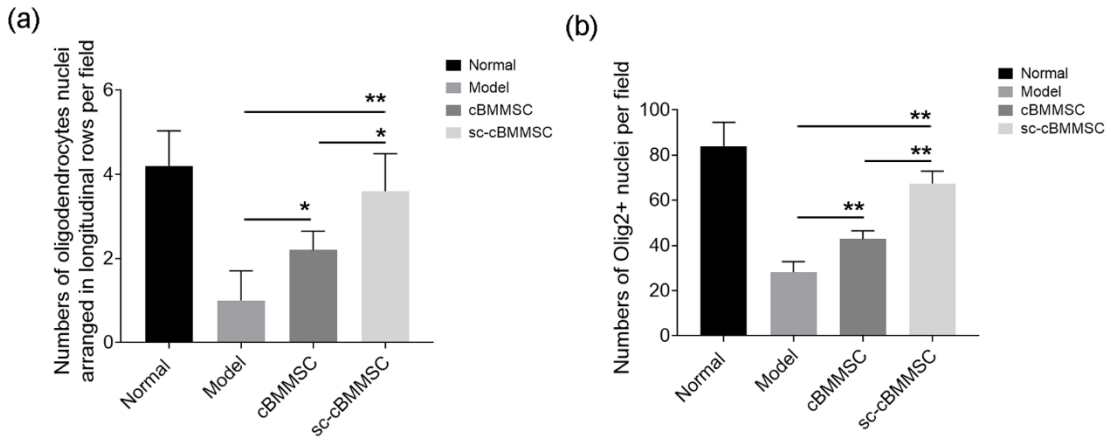

Fig. S1 Statistical results of the quantifition in arranged oligodendrocytes nuclei and Olig2$^+$ nuclei. (a) Numbers of oligodendrocytes nuclei arranged in longitudinal rows per field in brain tissue of HE staining were quantified; (b) Numbers of Olig2$^+$ nuclei per field in brain tissue of immunohistochemical staining were quantified. * $P<0.05$; ** $P<0.01$.