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Exosomes from high-altitude cerebral edema patients induce cognitive dysfunction by altering oxidative stress responses in mice

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The impact of exosomes derived from patients with High Altitude Cerebral Edema (HACE) on cognitive function in mice was investigated, along with the underlying mechanisms. Exosomes were extracted from HACE patients and injected into the dentate gyrus (DG) of mice. A series of behavioral tests assessed cognitive abilities. Results indicated that mice injected with HACE patient exosomes exhibited significant declines in exploratory behavior and object recognition, suggesting notable cognitive impairments. Additionally, these exosomes induced oxidative stress responses and abnormal activation of microglia, closely associated with neuronal death. Proteomic analysis revealed that the differentially expressed protein STAMBP, which is closely linked to neurodevelopment, may play a key role. In conclusion, our findings highlight the potential impact of exosomes from HACE patients on cognitive dysfunction in mice, providing new insights into the pathophysiological mechanisms of HACE.

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INTRODUCTION

HACE is a brain condition that occurs when individuals rapidly ascend from low to high altitudes without the ability to adapt to the hypoxic environment. Its primary symptoms include headache, vomiting [1, 2], and gait abnormalities, In severe cases, HACE can also lead to cognitive impairments, such as difficulty concentrating and memory loss [3]. Given the rapid onset of HACE and its potential for serious long-term effects, understanding its mechanisms is crucial for early intervention and improving patient outcomes.

Research indicates that cognitive dysfunction in HACE patients primarily results from pathological changes in brain tissue and functional impairments caused by hypoxia [4]. These changes include cerebral edema, increased blood-brain barrier permeability, and neuronal damage. The pathogenesis of HACE is complex, with vascular edema being one of the most widely accepted mechanisms [5]. In high-altitude hypoxic conditions, damage to endothelial cells disrupts the blood-brain barrier, which in turn leads to vascular edema. Moreover, the cytotoxic edema theory suggests that HACE may result from cell damage caused by cytotoxic factors [6]. This involves direct harm to neurons due to hypoxia and subsequent inflammatory responses. Furthermore, the severity and incidence of HACE may be influenced by physiological processes related to ubiquitination and polymorphisms of key enzymes [7].

Exosomes are extracellular vesicles with diameters ranging from 30–150 nanometers, containing various bioactive components that play a crucial role in intercellular communication. They have been extensively studied for applications in early diagnosis, disease monitoring, and drug delivery [8–11]. Recently, significant attention has been given to the positive effects of exosomes in treating conditions such as schizophrenia and depression [12, 13].

However, the potential negative impacts of the contents of patient-derived exosomes are often overlooked. Furthermore, research indicates that exosomes can cross the blood-brain barrier, raising the possibility that exosomes from HACE patients may play a role in the pathogenesis of HACE by disrupting oxidative stress responses and activating neuroinflammatory pathways [14, 15].

Based on these findings, we hypothesize that exosomes derived from HACE patients contribute to cognitive dysfunction in mice by altering oxidative stress responses and neuroinflammatory pathways. To test this hypothesis, we aim to: investigate the impact of HACE patient-derived exosomes on cognitive function in mice; and explore the molecular mechanisms underlying exosome-induced cognitive dysfunction, focusing on oxidative stress and neuroinflammation. Understanding the role of exosomes in the occurrence and progression of diseases is essential for developing effective therapeutic approaches.

MATERIALS AND METHODS

Animal preparation

Male C57BL/6 mice, aged 11 weeks and weighing 22 ± 1.5 g, were obtained from the Fang Yuanyuan Breeding Farm in Beijing, China. The mice were

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kept under controlled environmental conditions, with a temperature of 23 ± 1 °C and relative humidity of 50 ± 1%. They were housed on a 12-h light/dark cycle (lights on from 8 a.m.–8 p.m.) and given free access to a standard diet and water.

Ethics approval and consent to participate. All animal experiments within this investigation adhered strongly to the guidelines outlined by the National Institutes of Health for the Care and Use of Laboratory Animals (NIH Publication No. 80-23), receiving full endorsement from the Animal Care and Use Committee at Minzu University of China.

Participants

Eleven patients diagnosed with HACE by physicians at the General Hospital of Tibet Military Region were selected. An additional eleven healthy individuals, matched for age and gender with the HACE patients, were recruited as healthy controls (Ctr). The main characteristics of all participants are presented in Supplementary Table S1.

Ethics approval and consent to participate. Written informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of Minzu University of China and followed the guidelines of the Declaration of Helsinki. The samples had been previously used and published by Quan Tang et al. in another article [16].

Blood sample and exosome collection

In our experiment, blood samples were collected using BD Vacutainer™ Serum Tubes (Thermo Fisher Scientific, BD 367895), which do not contain any anticoagulant, allowing the blood to clot naturally. After collection, the samples were left at room temperature for 60 min to complete the coagulation process, followed by centrifugation at 1300 × g for 10 min to separate the serum. The separated serum was transferred to a new EP tube and immediately stored at −80 °C. Exosomes were extracted from the serum according to the manufacturer's instructions, using the qEVoriginal 70 nm Gen 2 system (IZON, ICO70-13030), and concentrated with the Vivaspin® 20 Ultrafiltration Unit (Sartorius, 30,000 MWCO PES) [13]. The size distribution of the exosomes was measured through nanoparticle tracking analysis using the NanoSight system (NanoSight, London, UK).

Exosome injection and animal grouping

To evaluate whether exosomes from patients with HACE impair learning, memory, and cognitive function, we administered continuous exosome injections to the bilateral dentate DG of mice. To reduce discomfort during the experiment, a cranial implant device was developed, enabling multiple exosome injections following a single surgical procedure [12]. The injection coordinates for the bilateral DG were: AP ± 2.1, ML + 1.7, DV −2.1. After a five-day recovery period, 1 μL of exosomes or saline was injected into each bilateral brain region every four days until euthanasia [17]. Specifically, for the HACE group, mice received 2.8×10^8 particles of exosomes per injection. In our study, human samples within the same group (HACE or healthy control) were pooled in sets of 2–4 samples to reduce individual variability and ensure group representativeness. In particular, we combined the serum from 2–4 patients and then extracted exosomes. The pooled exosomes were then evenly distributed among the corresponding group of mice, ensuring that the observed effects more accurately reflect the overall group characteristics rather than variability from individual human samples. The mice were randomly assigned to one of three groups, with eleven mice in each group:

1. 0.9% NaCl: Control group;
2. HACE: Exosomes from HACE patients (1.4E + 11 particles/mL);
3. Ctr: Exosomes from healthy individuals (1.7E + 11 particles/mL).

Behavioral assessments

Sixteen days after the initial exosome injection, the mice underwent a series of behavioral tests. Exosome injections continued every four days until euthanasia. All assessments were carried out in a quiet environment to minimize external stressors. To ensure accurate behavior readings, the mice were relocated to the testing area at least three hours prior to the assessments, allowing for sufficient acclimatization to the new environment.

Open field test. The open field test (OFT) is a commonly used method for evaluating the locomotor activity and exploratory behavior of mice [18, 19]. The apparatus is divided into 16 equal squares, and mice are initially placed in the center to acclimate for 2 min. This period allows the mice to adjust to their new environment, ensuring natural responses. After acclimation, the following metrics are recorded for 3 min: total distance traveled, time spent in the central zone, and the number of center entries. To maintain the objectivity of the analysis, the test is conducted by an investigator blinded to the treatment groups.

Novel object recognition test. The novel object recognition (NOR) test assesses memory based on the rodents' natural tendency to explore unfamiliar objects [20, 21]. During the experiment, mice are allowed to explore two identical objects for 10 min. Following a one-hour rest period, one of the objects is replaced with a novel object, and the mice are reintroduced to the arena for an additional 10 min. The exploration times and interaction frequencies with both the new and familiar objects are recorded to evaluate the mice's recognition memory.

Y-maze test. The Y-maze test measures spatial learning and memory by tracking the exploratory behavior of animals within the maze [22, 23]. Mice are placed in the starting arm of the maze and allowed to explore freely. Metrics such as time spent in each arm and the sequence of arm entries are recorded. The spontaneous alternation percentage, an indicator of working memory, is calculated using the following formula: [(number of alternations)/(total arm entries - 2)] × 100.

Novelty-suppressed feeding. The novelty-suppressed feeding (NSF) test evaluates the motivation and anxiety levels of mice when exposed to a novel environment following a 24-h fasting period [24]. n this test, food is placed on a piece of white filter paper in the center of a 50 × 50 × 45 cm apparatus. Each mouse is individually placed in a corner of the arena and allowed to explore. The latency to begin feeding is recorded, providing insights into the animal's anxiety and motivation in response to the novel surroundings.

Fear conditioning. The fear conditioning reflex involves two chambers: a freeze monitor box (23 × 23 × 30 cm) within a larger soundproof chamber (30 × 30 × 37 cm). The freeze monitor box is equipped with a metal grid for foot shocks and records the animals' vertical and horizontal movements. The first day focuses on conditioning training, following a specific protocol: the animals remain still for 60 s, after which they receive 12 stimulations. Each stimulation consists of a 30-s conditional stimulus at 75 dB, followed by a 30-s inter-stimulation interval, and concludes with a 2-s foot shock (30 mA) and 15 s of inactivity. Six hours later, a short-term memory test is conducted, reducing the number of stimulations to six. Each trial during this test includes the conditional stimulus and a 30-s tracking interval, concluding without foot shocks. The long-term memory test occurs on the second day, one day after the short-term test, using the same methodology to evaluate long-term memory quality [25].

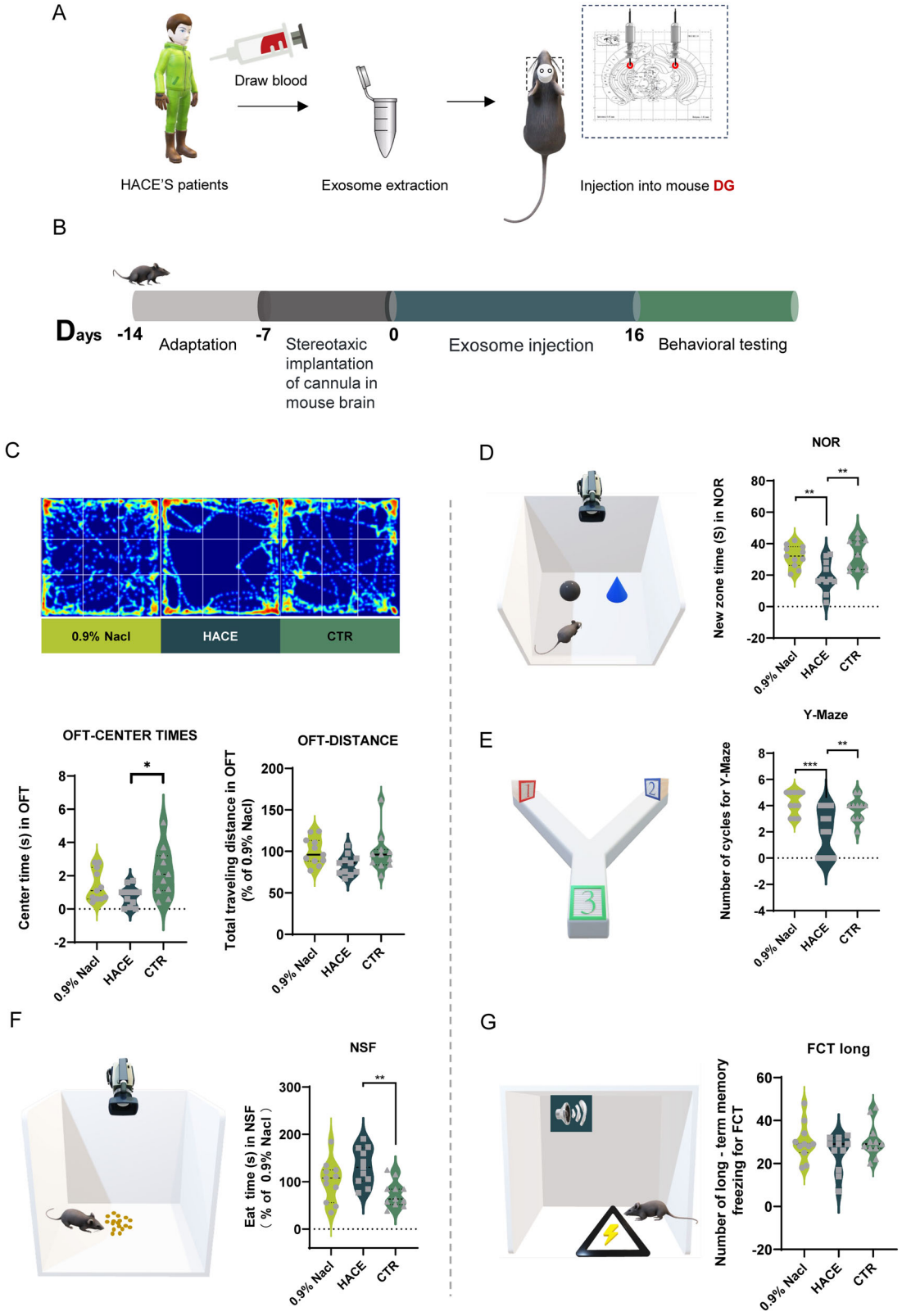
Detection of oxidative markers

The animals were first anesthetized with intraperitoneal pentobarbital (100 mg/kg), which was administered using a solution prepared with a 10% pentobarbital sodium (Sigma-Aldrich) solution dissolved in sterile saline. Once the animals were fully anesthetized and unresponsive to external stimuli, we proceeded with the procedure of eyeball blood collection. After blood collection, the intracardiac perfusion was performed with saline to ensure the removal of blood from the circulatory system. No heparin was used in this procedure. Once perfusion was completed, the animals were euthanized, and tissues were collected for further analysis.

Oxidative markers were detected using enzymatic colorimetric tests according to the manufacturer's instructions. Peripheral serum samples (0.8 mL) were collected from the retroorbital vessels of the mice and centrifuged for 20 min at 4 °C and 4000 rpm to obtain 300 μL of serum [26]. To assess oxidative stress levels, hippocampal tissue samples were also collected. The levels of malondialdehyde (MDA) (A003-1-2), glutathione peroxidase (GSH-Px) (A005-1-2), catalase (CAT) (A007-1-1), nitric oxide (NO) (A012-1-2), superoxide dismutase (SOD) (A001-3-2), corticosterone, and total antioxidant capacity (T-AOC) (A015-2-1) were measured using a kit from the Nanjing Jiancheng Bioengineering Institute.

Immunofluorescence analysis

Mice were first anesthetized with intraperitoneal pentobarbital (100 mg/kg). Once the animals were deeply anesthetized and unresponsive to



stimuli, intracardiac perfusion with saline was performed to euthanize the animals and fix tissues. The fixed specimens were dehydrated in sucrose solutions (20% followed by 30%) prepared in phosphate-buffered saline (PBS). Coronal brain sections, 35 μm thick, were then prepared and washed with PBS. To reduce nonspecific binding, the sections were blocked for one hour at room temperature using a solution of 1% bovine serum albumin, 0.3% Triton X-100, and 10% goat serum in PBS, followed by three PBS washes. The sections were incubated overnight at 4 °C with the following primary antibodies: mouse anti-ionized calcium-binding adapter molecule 1 (IBA-1, Cell Signaling Technology, #14082, 1:400) and rabbit anti-microtubule-associated protein 2 (MAP2, Cell Signaling Technology, #14082, 1:400). Afterward, the sections were incubated for an additional

Fig. 1 Cognitive dysfunction induced in mice by exosomes from HACE patients. A Experimental Design: Schematic Representation of Exosome Injection. **B** Flowchart of Animal Experiments. **C** In the open field test, exosomes from HACE patients reduced the time mice spent exploring the central area. **D** In the novel object recognition test, HACE group mice spent less time exploring the new object. **E** In the Y-maze experiment, HACE group mice showed a decrease in the number of entries into different arms. **F** In the novel environment feeding suppression test, HACE group mice took longer to eat food. **G** Performance of HACE in Contextual Fear Conditioning Experiments. All values are presented as the means ± SEMs. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. The normality of the data was assessed using the Shapiro-Wilk test, and F test was used to compare variances between groups. n = 11 per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (OFT open field test, NOR novel object recognition, NSF novelty-suppressed feeding test, NFT fear conditioning).

two hours at room temperature with fluorophore-conjugated secondary antibodies: goat anti-rabbit IgG (Alexa Fluor® 594 conjugate, Invitrogen, #A11008, 1:1000) and goat anti-mouse IgG (Alexa Fluor® 594 conjugate, Invitrogen, #A21422, 1:1000). Following three final washes, the sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) to visualize the nuclei. Imaging was conducted using a Leica TCS SP8 confocal microscope (Leica Microsystems, Germany), facilitating detailed observation and documentation of labeled cellular and subcellular structures [27].

Golgi-cox impregnation

Golgi-Cox impregnation, a classic histological technique, was employed to investigate neuronal structures and dendritic trees in the brain [28, 29]. Following euthanasia, brains were submerged in Golgi-Cox solution for a 14-day impregnation phase in darkness. Specimens were then dehydrated in 30% sucrose, and 100 µm coronal sections were prepared using a vibratome. After mounting on gelatin-coated slides and rinsing to remove excess reagents, the sections were dehydrated, cleared in xylene, and sealed with a coverslip. Bright-field microscopy visualized the Golgi-stained sections, revealing detailed images of neuronal and synaptic formations, which enhanced our understanding of neural functionality and pathology.

Olink

Protein concentrations were quantified using a multiplex immunoassay developed by Olink Proteomics (Uppsala, Sweden), based on PEA technology. This assay converts protein concentration measurements into Ct values via qPCR, subsequently reporting concentrations as normalized protein expression (NPX) on a log2 scale through a normalization procedure [30]. OLINK technology was utilized to assess proteomic indicators in hippocampal tissue, with detailed information available in Supplementary Table S2.

Functional enrichment analysis

The Database for Annotation, Visualization, and Integrated Discovery function annotation tool (https://david.ncifcrf.gov) was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis on differentially expressed proteins (DEPs) [31]. GO terms and KEGG pathways with Benjamini-corrected p-values < 0.05 and false discovery rates <0.01 were considered significant.

Data processing and analysis

Statistical analysis was conducted using GraphPad Prism (version 5.0). One-way ANOVA was employed to detect group differences, and Tukey's multiple comparisons test was performed for post hoc analysis ($P < 0.05$). Results are presented as mean ± SEM to convey central tendency and variability. The quantification of dendritic spines in the article was performed by counting the number of dendritic spines within the same visual field using ImageJ software.

RESULTS

Cognitive dysfunction induced in mice by exosomes from HACE patients

The potential negative impact of exosomes secreted by patients with HACE on cognitive function needs to be evaluated through behavioral testing. To investigate this, we extracted exosomes from these patients and injected them into the DG of mice. Using a specialized device, we administered continuous injections after an initial injury. Following 16 days of exosome infusion, we conducted a series of behavioral tests.

Figure 1A shows the schematic of exosome extraction and injection, while Fig. 1B outlines the timeline for model establishment. Mice injected with exosomes from HACE patients exhibited reduced exploration time in the center area of the OFT (Fig. 1C), indicating impaired exploratory behavior. In contrast, mice injected with exosomes from healthy individuals or with saline demonstrated higher activity levels. Among them, the mice receiving healthy exosomes performed better in exploratory behavior than those injected with saline. In the NOR test, the HACE mice took significantly longer to recognize a new object, clearly demonstrating that exosomes from HACE patients negatively affected their learning, memory, and cognitive functions (Fig. 1D). The Y-maze test, used to assess learning and cognitive abilities, revealed that HACE mice completed fewer successful alternations, further reflecting deficits in cognition and memory (Fig. 1E). In the NSF test, HACE mice took longer to approach food in the center area, further indicating a notable reduction in exploratory ability (Fig. 1F). During the fear conditioning test, HACE mice showed fewer freezing responses to auditory stimuli, while no abnormalities were observed in the other groups (Fig. 1G).

In summary, we identified significant cognitive impairments in HACE mice through various behavioral tests, while no such deficits were observed in mice receiving healthy exosomes. In fact, some cognitive indicators were even better in the healthy exosome group compared to the saline group. This suggests that exosomes from HACE patients may contain specific substances that contribute to cognitive dysfunction. We further conducted physiological assessments on the mice's brain tissue and blood.

Exosomes from high-altitude cerebral edema patients induce oxidative stress responses in mice

Research has shown that oxidative stress homeostasis imbalance is involved in the pathogenesis of HACE [32]. This imbalance primarily arises from excessive production of reactive oxygen species (ROS) or insufficient antioxidant defenses. These factors contribute to abnormal microglial activation and the disruption of endothelial tight junctions, ultimately compromising the integrity of the neurovascular unit [33]. Such disruption leads to irreversible neuronal death and subsequent damage to the blood-brain barrier, resulting in cerebral edema [34]. To assess whether HACE exosomes induce abnormalities in oxidative capacity and ROS levels, we measured the expression levels of NO, SOD, GSH-Px, MDA, CAT, and T-AOC in the serum and two brain regions—the hippocampus (Hip) and medial prefrontal cortex (mPFC)—of mice [35].

NO is a redox-active molecule that plays a key role in regulating vascular endothelial tone, improving blood flow and oxygen supply, and influencing oxidative stress and inflammatory responses. These functions significantly impact the occurrence and progression of HACE [36]. We measured NO levels in the serum, hippocampus, and prefrontal cortex of the mice. The results showed a marked increase in NO levels in both the serum and hippocampus of mice injected with HACE exosomes (Fig. 2A, G).

SOD is an antioxidant enzyme that catalyzes the conversion of superoxide into hydrogen peroxide, thereby affecting oxidative stress levels. Research has demonstrated significantly elevated SOD expression in the brain tissues of rats exposed to high-altitude cerebral edema [37]. We conducted a detailed examination of SOD levels in mice injected with HACE exosomes, finding abnormal elevations in the hippocampus, prefrontal cortex, and serum of the HACE group (Fig. 2B, H). GSH-Px, an antioxidant that neutralizes excessive ROS and prevents oxidative damage [38, 39], was significantly reduced in the hippocampus of the HACE group mice (Fig. 2C, I).

MDA, a byproduct of lipid peroxidation, is an indirect marker of oxidative stress. Ruzanna A. Shushanyan et al. observed a significant increase in MDA levels in the hippocampus and cortex of rats with HACE [40]. Similarly, we noted a significant rise in MDA levels in the blood, prefrontal cortex, and hippocampal tissues of the HACE group mice (Fig. 2D, J).

CAT primarily decomposes hydrogen peroxide into water and oxygen, protecting cells from oxidative stress. A decrease in CAT levels leads to heightened oxidative stress [41, 42]. In the HACE group, we observed a slight increase in CAT expression (Fig. 2E, K).

T-AOC reflects the overall antioxidant status of various substances and enzymes, serving as a critical indicator of oxidative stress [43]. The T-AOC levels in the hippocampus of the HACE group showed significant changes. Overall, the HACE group displayed active lipid peroxidation and redox imbalance in both serum and brain tissues (Fig. 2F, L).

Exosomes from HACE patients cause abnormal death of mouse hippocampal neurons

Neuroinflammatory responses are closely linked to oxidative stress, and excessive oxidative stress can inhibit neurotransmission and synaptic plasticity, ultimately leading to neuronal damage [44, 45]. To assess whether exosomes from HACE patients cause such damage, we performed Golgi staining on the DG region of the mouse hippocampus. The results showed a significant reduction in dendritic spine density in the neurons of the HACE group (Fig. 3A, B). Furthermore, MAP2 immunofluorescence staining, which labels neurons in the hippocampal DG region, indicated a substantial decrease in the number of neurons in the HACE group (Fig. 3C, D). These findings suggest that exosomes from HACE patients severely impair hippocampal neurons in mice.

Exosomes from HACE induce proliferation and activation of microglia in mouse hip and mPFC

Zhao et al. found that the activation of microglia increases inflammation levels and exacerbates brain edema in rats [46]. This abnormal activation releases inflammatory factors that impact surrounding neurons, resulting in inflammatory damage to them [47]. To further investigate the quantity and activation status of microglia in the hippocampus, we labeled them using IBA1 and CD68. The results indicated an increase in the number of microglia in the DG (Fig. 4A), CA1 (Fig. 4C), CA2 (Fig. 4D), CA3 (Fig. 4E), and mPFC (Fig. 4F) regions of the HACE group mice, with a significant rise in the number of activated microglia (Fig. 4B). Additionally, these microglia displayed abnormal characteristics, such as enlarged cell bodies and shortened processes.

Exosomes from HACE patients affect mouse cognitive function by depleting neuronal development proteins

To investigate the mechanisms underlying cognitive dysfunction in HACE, we examined the impact of exosomes from HACE patients on protein expression in the hippocampus of mice. We performed Olink proteomic sequencing on the hippocampal tissues from both HACE and control groups, each consisting of six independent samples, totaling twelve mice. We utilized Uniform Manifold Approximation and Projection (UMAP) for dimensionality reduction, which presented the overall protein group characteristics (Fig. 5A). Next, we quantified the NPX alues of the differentially expressed proteins (Fig. 5B). The volcano plot revealed a total of 25 genes with significant differences in expression (Fig. 5C). Heatmap analysis further demonstrated substantial differences between the HACE and control groups (Fig. 5D). Specifically, 15 proteins were upregulated in the HACE group, including Ribokinase (RBKS), Protein Transport Factor (PRTFDC1), Fc Receptor-Like Protein 6 (FCRL6), C-C Motif Chemokine Ligand 27 (CCL27), Cathepsin S (CTSS), Defensin Beta 4 A (DEFB4A), Interleukin-1 Receptor-Associated Kinase 4 (IRAK4), Tenascin-R (TN-R), Corneodesmosin (CDSN), SPARC-related Modular Calcium-Binding Protein 2 (SMOC2), Ezrin (EZR), Galectin-8 (gal-8), A Disintegrin and Metalloproteinase 15 (ADAM15), and FK506 Binding Protein 7 (FKBP7). Conversely, 10 proteins were downregulated, including STAM Binding Protein (STAMBP), Neurotrophic Receptor Tyrosine Kinase 2 (NTRK2), Axin Protein 1 (AXIN1), Delta/Notch EGF Repeat-Containing (DNER), Ephrin Type-B Receptor 6 (EPHB6), Neutral Endopeptidase (NEP), Fibroblast Growth Factor 5 (FGF-5), Neuronal Cell Adhesion Molecule (Nr-CAM), Spondin 1 (SPOCK1) and S SH2 Domain Containing 1 A (SH2D1A).

These differentially expressed proteins may collectively contribute to cognitive dysfunction through multi-pathway interactions. In the neuroinflammatory response induced by HACE exosomes, pro-inflammatory factors including CTSS, IRAK4, and CCL27 were significantly upregulated, potentially driving microglial hyperactivation via NF-κB signaling pathway [48–51]. Concurrently, downregulation of STAMBP and NEP may impair protein clearance mechanisms, exacerbating proteotoxic stress and β-amyloid (Aβ) deposition [52], ultimately triggering neuronal apoptosis, which aligns with our previous observation of reduced MAP2-positive neurons. Regarding neuroplasticity regulation, suppressed Notch/DNER signaling and dysregulated Wnt/AXIN1 pathway may compromise hippocampal neurogenesis [26, 53, 54], while disrupted NTRK2-BDNF signaling and impaired Nr-CAM-mediated cell adhesion could lead to decreased synaptophysin synthesis and dendritic complexity [55]. These alterations resulted in significant exploratory behavior deficits in novel object recognition and open field tests, corroborated by reduced synaptic density in Golgi-stained HACE model mice. Furthermore, aberrant expression of FCRL6 and DEFB4A may induce autoimmune-like damage [56, 57], whereas SMOC2 and EZR overexpression could disrupt blood-brain barrier integrity, promoting vascular leakage and cerebral edema [58, 59]. Metabolic disturbances involving RBKS and PRTFDC1 may further aggravate cerebral energy crisis [60, 61]. These multi-omics findings demonstrate that HACE-associated cognitive impairment essentially stems from synergistic effects of neuroinflammatory storms, synaptic remodeling dysfunction, and proteostasis imbalance.

GO enrichment analysis further elucidated functional pathway dysregulation in hippocampal tissues following HACE exosome intervention. Significant downregulation of interleukin-1 receptor binding activity correlated with low STAMBP expression (a deubiquitinase regulating IL-1R signaling), suggesting compromised anti-inflammatory feedback that exacerbates IL-1β-mediated neural damage [62]. Impaired magnesium ion binding and carbohydrate kinase activities corresponded with dysfunctional RBKS and PRTFDC1, indicating TCA cycle disruption and mitochondrial oxidative phosphorylation inefficiency [63, 64]. Notably, aberrant activation of transmembrane receptor protein kinase and protein tyrosine kinase activities reflected EGFR/VEGFR signaling dysregulation, forming a pro-inflammatory/vascular leakage loop with IRAK4 (IL-1R-associated kinase) and ADAM15 (extracellular matrix protease) [65, 66]. Additionally, upregulated Notch binding and metallopeptidase activities matched expression changes of TN-R (Notch-regulated ECM protein) and CTSS, potentially aggravating blood-brain barrier disruption via Notch-dependent microglial activation [67, 68] (Fig. 5E) .

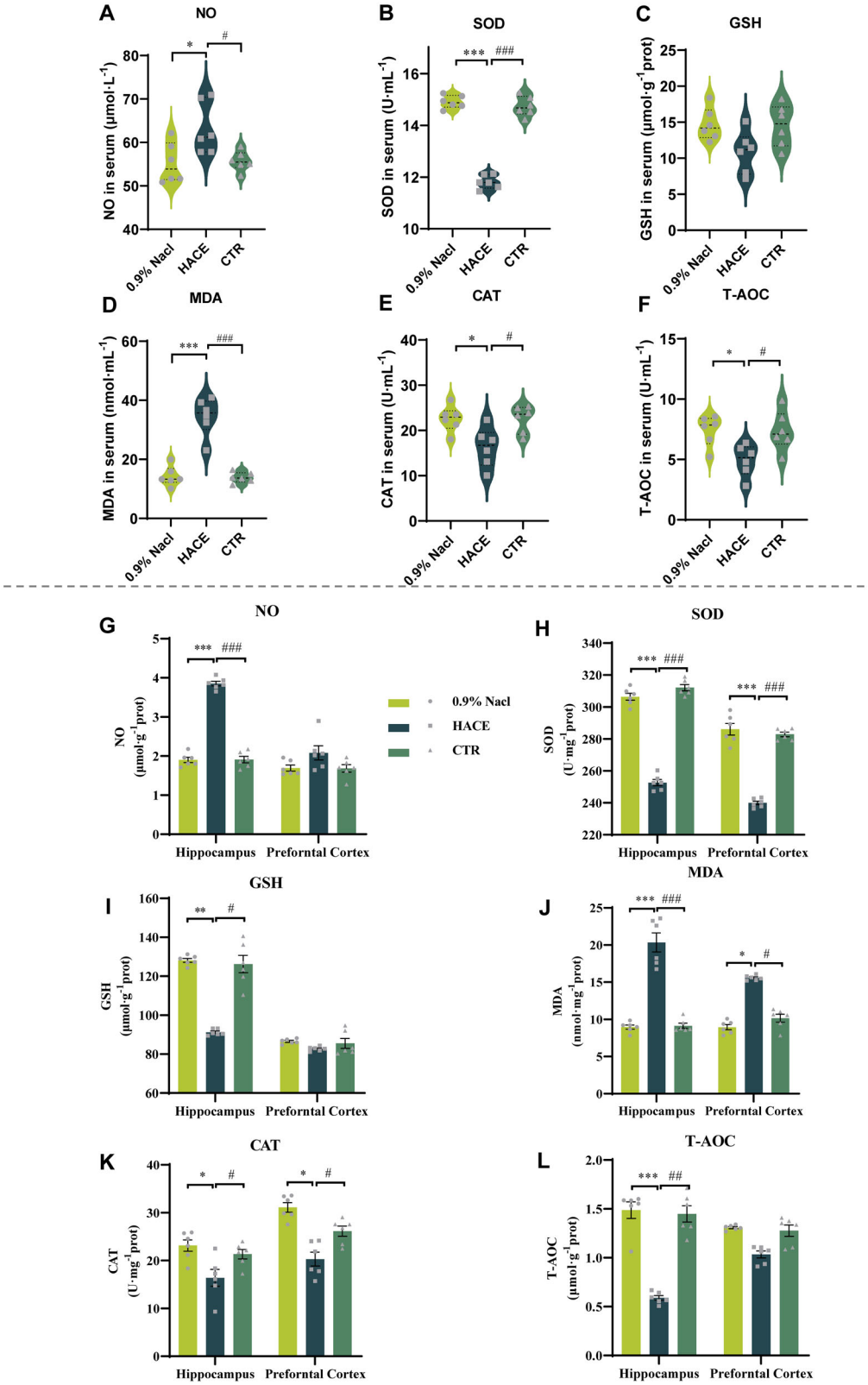


Fig. 2 Exosomes from high-altitude cerebral edema patients induce oxidative stress responses in mice. A–L Exosomes from HACE patients can induce abnormal expression of oxidative stress-related factors in the blood, hippocampus, and prefrontal cortex of mice. These factors include NO, MDA, GSH, SOD, CAT and T-AOC. All values are presented as the means ± SEMs. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. The normality of the data was assessed using the Shapiro-Wilk test, and F test was used to compare variances between groups. n = 6 per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (NO nitric oxide, MDA malondialdehyde, GSH glutathione peroxidase, SOD superoxide dismutase, CAT catalase, T-AOC total antioxidant capacity).

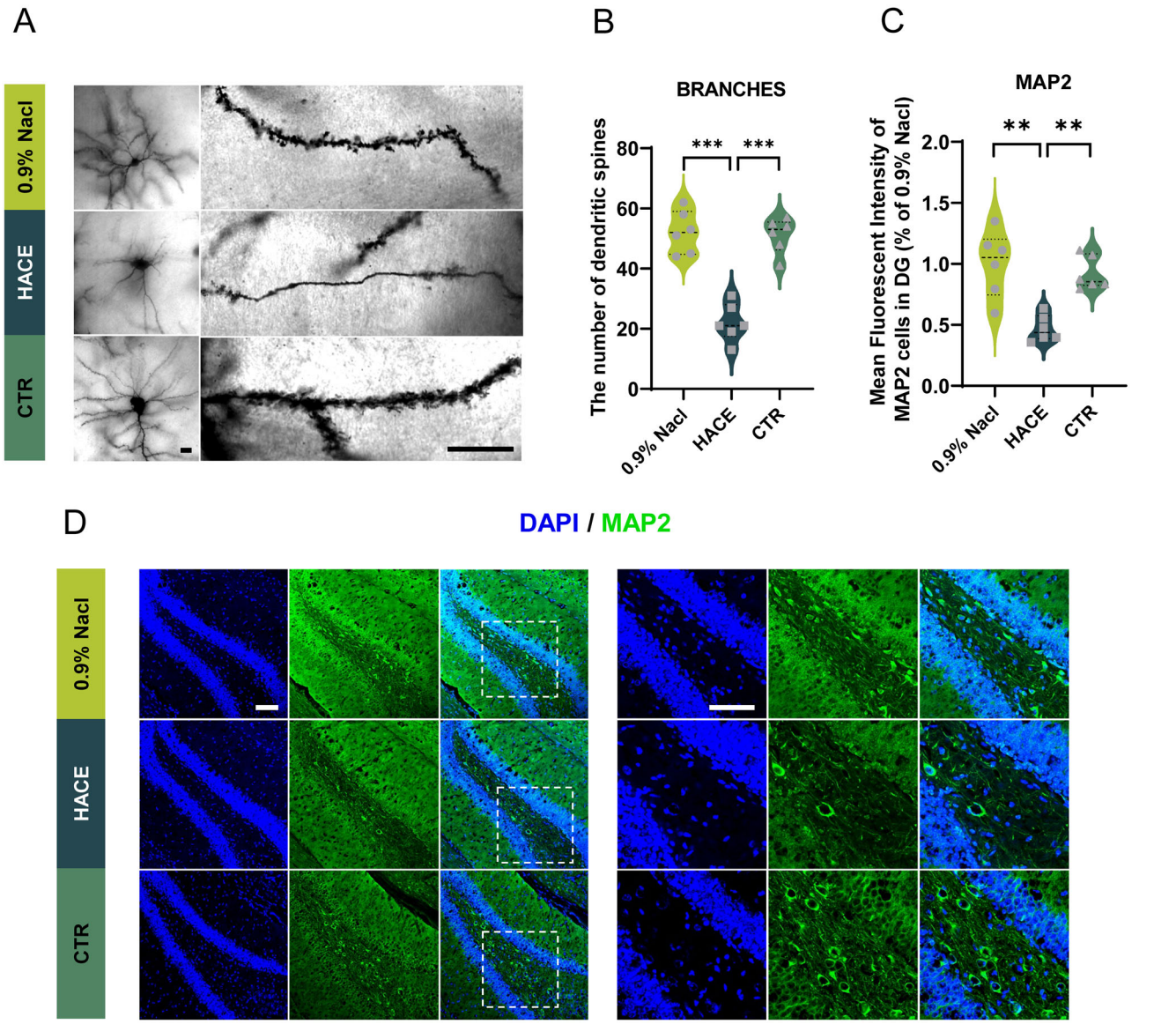


Fig. 3 Exosomes from HACE patients cause abnormal death of mouse hippocampal neurons. A Schematic representation of Golgi staining in the DG region. **B** Significant reduction in the number of dendritic spines in the DG region of HACE group mice. **C** Noticeable decrease in the number of MAP2-positive cells in the DG region of HACE group mice. **D** Schematic representation of MAP2 immunofluorescence in the DG region. All values are presented as the means ± SEMs. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. The normality of the data was assessed using the Shapiro-Wilk test, and F test was used to compare variances between groups. Immunofluorescence images were analyzed using ImageJ. n = 6 per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Scale bars in A and D = 50 μm. (DAPI 2-(4-amidinophenyl)-6-indoleca rbamidine dihydrochloride, DG dentate gyrus, MAP2 Microtubule-Associated Protein 2).

DISCUSSION

Exosomes derived from patients with HACE significantly impact cognitive function in mice. Experimental results showed that mice injected with exosomes from HACE patients exhibited notable cognitive impairments in various behavioral tests, particularly in the Y-maze, NOR and NSF tests. These findings align with recent research on the role of exosomes in neurodegenerative diseases, where patient-derived exosomes have been shown to contain pathological components affecting neuronal function, such as tau protein and α-synuclein [69].

While previous studies have indicated the crucial role of exosomes in disease progression, the potential negative effects of exosomes from HACE patients are seldom reported. Results suggest that these exosomes may increase oxidative stress in neurons, lead to abnormal activation of microglia, and cause neuronal death by releasing specific bioactive molecules. This supports the view that oxidative stress plays a key role in HACE's pathological processes. Specifically, elevated levels of ROS were observed in mice, accompanied by decreased antioxidant enzyme activity, consistent with oxidative damage and neuronal dysfunction observed in high-altitude environments [70, 71]. Compared to literature on oxidative stress-induced microglial activation and neuroinflammatory responses, these findings emphasize the potential bridging role of exosomes in this process, deepening understanding of the mechanisms underlying HACE and its cognitive dysfunction [72]. Proteomic analysis results corroborate these findings. The differential protein CCL27, an important chemokine from the C-C motif family [73], promotes the migration of leukocytes, especially T cells, to sites of inflammation by binding to its receptors (e.g., CCR10). Its expression in the brain may be closely related to neuroinflammation and injury [74]. GO analysis revealed that proteins extracted from exosomes were

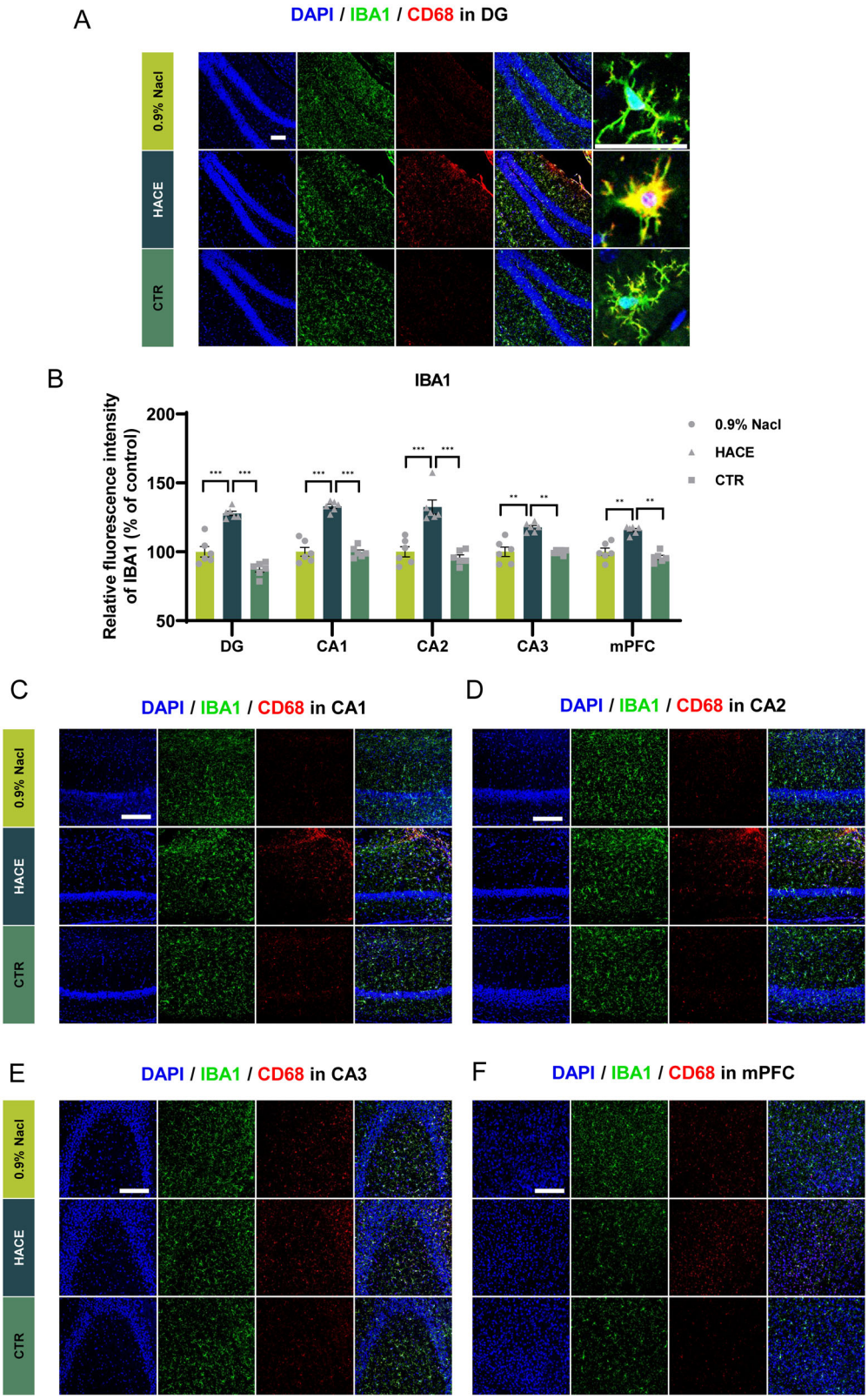


Fig. 4 Exosomes from HACE induce proliferation and activation of microglia in mouse hip and mPFC. A Schematic representation of IBA1 immunofluorescence in the DG region. **B** Increased number of microglial cells in the hippocampus and prefrontal cortex of HACE group mice. **C**–**F** Schematic representations of IBA1 immunofluorescence in the CA1, CA2, CA3, and mPFC regions. All values are presented as the means ± SEMs. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. The normality of the data was assessed using the Shapiro-Wilk test, and F test was used to compare variances between groups. Immunofluorescence images were analyzed using ImageJ. n = 6 per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Scale bars in A, C, D, E and F = 50 μm. (DAPI 2 - (4 - amidinophenyl) – 6 - indolecarbamidine dihydrochloride, IBA1 ionized calcium-binding adaptor molecule 1, DG dentate gyrus, CA1 cornu ammonis 1 of the hippocampus, CA2 cornu ammonis 2 of the hippocampus, CA3 cornu ammonis 3 of the hippocampus, mPFC medial prefrontal cortex).

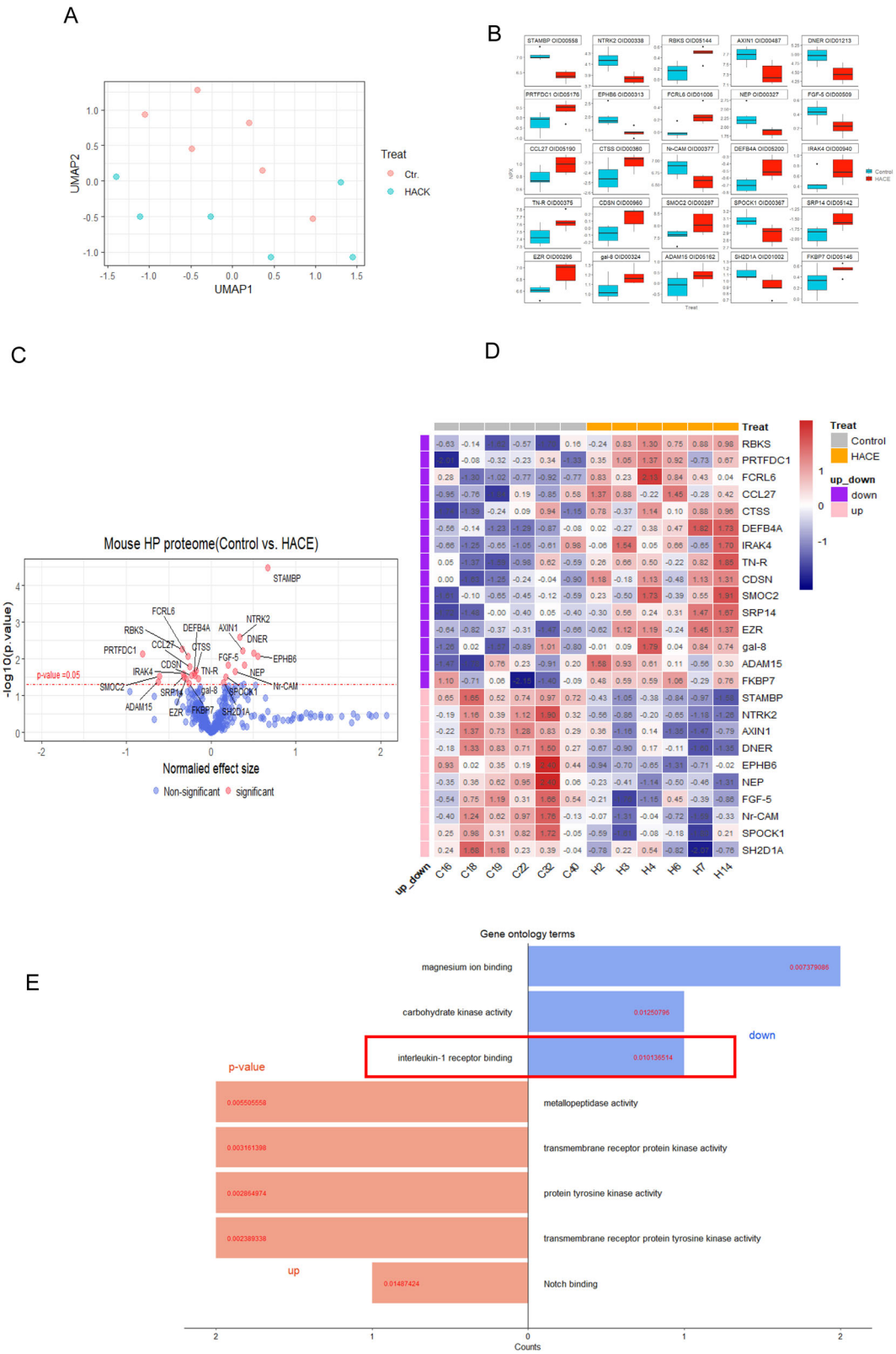


Fig. 5 Exosomes from HACE patients affect mouse cognitive function by depleting neuronal development proteins. A Dimensionality reduction and overall proteomic feature visualization using UMAP for 12 samples. **B** Quantification of NPX values for differential proteins. **C** Volcano plot of differentially expressed proteins. **D** Heatmap of significantly differentially expressed proteins between the HACE and CTR groups. **E** GO functional clustering analysis of differential proteins. n = 6 per group.

enriched in functions related to "interleukin-1 receptor binding," closely associated with the pathological mechanisms of HACE. Interleukin-1 (IL-1), a key pro-inflammatory cytokine, participates in neuroinflammation and oxidative stress processes [75]. Previous studies indicate that excessive expression of IL-1 can lead to neuronal damage and dysfunction, potentially exacerbating cerebral edema by promoting microglial activation and inflammatory responses [76, 77]. Therefore, exploring intervention strategies targeting exosomes in inflammation and oxidative stress may provide new insights for HACE treatment.

Olink proteomic detection revealed that exosomes from HACE patients may be associated with altered expression of the STAMBP protein, which is related to neurodevelopment, and this alteration may contribute to cognitive dysfunction in mice. STAMBP is involved in deubiquitination, and mutations in this gene have been reported to reduce the proliferation capacity of neural stem cells (NSCs) [78]. The role of STAMBP in maintaining normal NSC proliferation is crucial; its downregulation may potentially influence brain development and function, which could lead to learning and memory deficits. While these findings suggest that cognitive impairments in HACE could be linked to STAMBP downregulation, additional studies are needed to confirm this association [78].

In addition, proteins related to neurodevelopment, such as Nr-CAM, NTRK2, and DNER, also showed significant differences. Nr-CAM, a cell adhesion molecule, is involved in interactions between nerve cells, and its abnormal expression may lead to changes in neuronal adhesion and signaling, affecting cognitive function and learning abilities [79]. NTRK2, a neurotrophic factor receptor, participates in neuronal growth and synaptic plasticity [80]. DNER plays a crucial role in neuronal development; changes in its expression levels may relate to nerve injury and regeneration processes, thereby influencing cognitive function and the formation of neural networks [81]. The abnormal expression of these proteins provides evidence that exosomes from HACE patients may contribute to disruptions in neurodevelopment, thereby affecting cognitive function.

Despite significant findings, some limitations remain. The water maze is an excellent behavioral tool for assessing learning, memory, and cognitive dysfunction; however, relevant data could not be obtained due to the constraints of the head apparatus used on experimental animals. Additionally, establishing HACE mouse models (such as hypoxia-induced models or chronic high-altitude exposure models) would facilitate more robust comparisons with mice injected with patient exosomes.

In summary, exosomes from HACE patients potentially impact cognitive dysfunction by inducing oxidative stress, promoting neuroinflammation, and disrupting neurodevelopment. These findings provide new insights into the pathological mechanisms of HACE and offer novel strategies for future therapeutic approaches.

CONCLUSION

The findings of this study demonstrate that exosomes derived from patients with HACE significantly impair cognitive function in mice. The depletion of STAMBP protein was observed in association with disrupted oxidative stress responses, which may contribute to the cognitive dysfunction. Additionally, the activation of neuroinflammatory pathways was noted to further exacerbate neuronal damage and cognitive impairments. These insights provide valuable information for understanding the pathophysiological processes associated with HACE and suggest that targeting exosomal components may offer new therapeutic strategies for managing cognitive dysfunction in affected individuals.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article and its Supplementary Information files.

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AUTHOR CONTRIBUTIONS

Yong Cheng conceived the study; Zhongyong Jiang and Yong Cheng designed the study; Qiang Fu, Rui Qiu, Quan Tang, Xiaodong Li, Yaobo Li, Yuxiang Qin, Qiaosheng Li, Jia Yao and Huan Xu performed the experiments; All the authors analyzed and interpreted the data; Qiang Fu drafted the manuscript with critical revisions from Yong Cheng.

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COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ADDITIONAL INFORMATION

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