

Targeting the Stress-Driven Loop in Glioma with Exosomes Co- loaded with BDNF Antibody and Propranolol

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Abstract

Chronic stress is a known risk factor for glioma progression. However, the specific mechanisms involved remain unclear, posing a major obstacle to the development of targeted therapies. Our study reveals that chronic stress drives glioma progression by activating a self-reinforcing feedback loop between intratumoral neurons and astrocytes. Under stress, neuron-derived norepinephrine (NE) upregulates (brain-derived neurotrophic factor) BDNF expression in astrocytes. BDNF then promotes tumor growth and invasion, which further elevates microenvironmental NE levels, creating a vicious cycle. To disrupt this loop, we developed iRGD/A2-co-modified exosomes (iRA2-MBP) to co-deliver a BDNF antibody (aBDNF) and propranolol for enhanced brain tumor targeting. The BDNF antibody neutralizes target and reprograms macrophages, while propranolol induces immunogenic cell death and alleviates T cell exhaustion. In stress-bearing glioma mice, iRA2-MBP achieved a 27.85-fold higher intratumoral antibody accumulation than free antibody, reversed T cell exhaustion, and promoted immune activation, leading to 40% tumor regression, prolonged survival, and long-term immunological memory. In conclusion, this work identifies a stress-associated NE–BDNF signaling mechanism in glioma progression and presents an exosome-based immunomodulatory therapeutic strategy for stress-associated glioma.

1. Introduction

Glioblastoma multiforme (GBM) remains one of the most lethal primary brain tumors, with a dire prognosis and an urgent unmet clinical need. Confronted with this survival threat, patients almost universally experience severe chronic psychological stress due to the diagnosis, aggressive treatment, and persistent risk of recurrence ^[1, 2]. Chronic stress has been increasingly recognized as an important systemic factor associated with accelerated disease progression and poorer clinical outcomes. However, its underlying mechanisms remain poorly defined ^[3]. To date, the specific molecular pathways through which this prevalent stress is translated into pro-tumorigenic signals within the tumor microenvironment remain unclear ^[4]. This knowledge gap significantly hinders the development of targeted therapeutic strategies.

Chronic stress activates the sympathetic nervous system, leading to the release of catecholamines from the adrenal glands and sympathetic nerve endings. In various peripheral solid tumors, NE has been shown to act on tumor cells and induce the expression of nerve growth factor (NGF) within the microenvironment, thereby promoting nerve infiltration and tumor progression ^[5, 6]. However, the glioma microenvironment in the central nervous system is highly innervated and features a neurotrophic signaling network orchestrated predominantly by BDNF, diverging from the NGF-dominant paradigm in peripheral cancers ^[7]. Within this niche, BDNF not only physiologically regulates neuronal survival and synaptic plasticity but has also been preliminarily implicated in the malignant behaviors of glioma ^[8]. Nevertheless, whether and how NE regulates BDNF expression in the glioma microenvironment—and

whether this signaling axis directly drives tumor proliferation, reshapes the neural niche, and establishes a self-sustaining malignant feedforward loop—remains entirely unexplored ^[9].

Glioma is characterized by an immunosuppressive "cold" tumor microenvironment, marked by significantly reduced infiltration and functional exhaustion of CD8⁺ T cells, along with unresponsiveness of residual CD4⁺ T cells to antigenic stimulation ^[10, 11]. The feedforward loop driven by the NE-BDNF signaling axis may further exacerbate this immunosuppressive state—under chronic stress, persistently elevated NE not only induces BDNF expression but also potentially accelerates T cell exhaustion through direct or indirect mechanisms, leading to abnormally low CD8⁺ T cell counts and impaired CD4⁺ T cell antigen responsiveness. Although various delivery systems—including small molecules, ultrasound-mediated approaches, and nanoparticles—have been extensively explored for blood–brain barrier traversal in recent years, the actual drug doses reaching the tumor core remain severely limited ^[10, 12, 13]. More critically, most existing studies have evaluated these delivery strategies under conventional conditions, lacking systematic investigation of their modulatory effects on the "cold" tumor microenvironment and T cell exhaustion under conditions that recapitulate the chronic stress state of clinical patients ^[14, 15]. Therefore, there is an urgent need to develop targeted intervention strategies that can both efficiently cross the blood–brain barrier and deeply penetrate tumors, while effectively reversing immunosuppression in the context of chronic stress ^[16].

Through single-cell transcriptomic profiling of human glioma, we uncovered a stress-driven feedforward loop in which intratumoral neurons release NE to activate astrocytic BDNF secretion, which in turn fuels tumor proliferation and neural infiltration—forming a self-perpetuating feedforward loop. Guided by this mechanistic discovery, we engineered a dual-targeting iRA2-MBP(iRGD/A2-co-modified MBP) co-modified with A2 and iRGD peptides for blood–brain barrier traversal and deep tumor penetration, and co-loaded with a BDNF-neutralizing antibody and propranolol to simultaneously block the circuit's core signal, antagonize adrenergic receptors, and induce immunogenic cell death. This platform disrupted the malignant loop, reactivated antitumor immunity, and achieved potent efficacy in stress-bearing glioma models, enabling a multi-level synergistic intervention spanning from systemic stress to local feedforward loop and ultimately to the immunosuppressive tumor microenvironment ^[17].

2. Results

2.1. Chronic Stress Accelerates Glioma via the NE-BDNF Signaling Axis

To investigate the impact of chronic stress on glioma progression, we subjected GL261 and U87 glioma-bearing mice to chronic restraint stress (CRS). CRS significantly accelerated tumor progression and shortened survival compared to non-stressed controls (Fig. 1a-c and Supplementary Fig. 1a-c). Transcriptomic analysis revealed that CRS markedly promoted neural axon and dendrite proliferation within tumor tissues and enhanced neurotransmitter transport-related pathway activity, while immune-

related pathways—including adaptive immune response and inflammatory response—were significantly suppressed (Fig. 1d and Supplementary Fig. 1d-e). A total of 1,020 upregulated and 847 downregulated genes were identified in response to chronic stress (Fig. 1e). Notably, genes associated with the β -adrenergic signaling pathway and *Bdnf* were significantly upregulated, whereas immune effector function-related genes were broadly downregulated, including cytotoxic effector molecules (*Prf1*, *Nkg7*), pro-inflammatory cytokines (*Il1b*, *Cxcl5*), and death receptor pathway genes (*Tnfrsf10b*), suggesting impaired T/NK cell function, suppressed inflammatory responses, and reduced apoptotic sensitivity. These results indicate that chronic stress exerts a dual regulatory effect by concurrently activating neuro-signaling pathways and suppressing antitumor immunity. At the protein level, DBH (Dopamine beta-hydroxylase) and CGA (Chromogranin A) expression increased in a time-dependent manner in tumor tissues under prolonged stress. Notably, BDNF expression remained unchanged in normal brain tissue with extended stress exposure, but exhibited significant time-dependent upregulation in U87, GL261, and U251MG glioma tissues (Fig. 1f and Supplementary Fig. 1f).

Analysis of single-cell RNA sequencing data from 11 glioma patients (Fig. 1g) identified a neuronal cluster co-expressing *TUBB3*⁺ and *DCC*⁺. These tumor-associated neurons primarily participate in axon guidance within tumor tissues and secrete NE. The released NE acts on adjacent astrocytes expressing β -adrenergic receptors, which concurrently highly express BDNF. BDNF subsequently exerts paracrine or autocrine effects on multiple cell types, including tumor stem cells, malignant glioma cells, neurons, and astrocytes themselves. Notably, a subset of this neuronal cluster expressed the immature neuron marker *DCX*⁺, and these neurons also highly expressed the BDNF receptor *TrkB*, suggesting that BDNF may participate in regulating neurogenesis within the tumor microenvironment through autocrine or paracrine mechanisms (Fig. 1h). Consistent with these observations, analysis of glioma patient data from the TCGA database revealed strong positive correlations between TH⁺ nerve fiber density and BDNF expression, as well as between DBH expression and BDNF expression (Fig. 1i), further supporting the relevance of the NE-BDNF axis in human gliomas.

We further conducted a prospective cohort study involving 13 patients with GBM. Patients were assessed for stress levels using the Hamilton Depression Rating Scale (HAMD-17) at the time of clinical treatment and subsequently stratified into stressed and non-stressed groups. MRI follow-up (Fig. 1j) demonstrated that, under the same chemotherapy regimen, tumor volume regression at approximately two months was significantly lower in the stressed group compared to the non-stressed group. Concurrently, immunofluorescence staining (Fig. 1k) revealed significantly elevated expression of BDNF and DBH in tumor tissues from stressed patients. Together, these results demonstrate that chronic stress drives glioma progression by elevating NE levels and subsequently promoting BDNF secretion.

2.2. The NE-BDNF Feedforward Axis in Neuron–Astrocyte Crosstalk Drives Glioma Progression and Immunosuppression

Given the activation of the stress-driven NE-BDNF axis in glioma, we sought to investigate the interaction between tumor-associated neurons and astrocytes. We established an in vitro coculture system using electrically stimulated, highly differentiated PC12 cells to mimic stress-activated neurons (Fig. 2a). Results showed that electrical stimulation significantly upregulated DBH expression in PC12 cells and increased NE levels in the supernatant (Fig. 2b). When GL261 cells were incubated with the supernatant collected before and after electrical stimulation, GL261 cell viability was markedly enhanced following stimulation (Supplementary Fig. 2a).

We next investigated whether NE modulates BDNF secretion from astrocytes. Stimulation of C8-D1A astrocytes with increasing concentrations of NE induced a dose-dependent upregulation of BDNF expression (Fig. 2c). ELISA results (Fig. 2d) confirmed that 10 μ M NE significantly promoted BDNF secretion, an effect that was abolished by propranolol. Concurrently, phosphorylation levels of PKA and ERK signaling pathways were markedly elevated (Fig. 2e and Supplementary Fig. 2b), indicating that adrenergic signaling mediates BDNF secretion via the PKA/ERK pathway.

To further assess the functional significance of NE-induced BDNF secretion, we employed a DRG and C8-D1A astrocyte coculture model and evaluated neurite outgrowth (Fig. 2f). Treatment with NE significantly enhanced DRG neurite outgrowth on days 3 and 5 in the coculture system (Fig. 2g-2i). Mechanistically, NE treatment markedly elevated ERK and MEK phosphorylation in the underlying DRG cells, an effect attributable to increased BDNF secretion from astrocytes. However, propranolol, by inhibiting BDNF secretion, almost completely abolished NE-induced neurite outgrowth and ERK/MEK phosphorylation (Fig. 2j and Supplementary Fig. 2c). Furthermore, in a coculture system of C8-D1A astrocytes and GL261 glioma cells, NE-pretreated C8-D1A cells significantly promoted GL261 cell viability through BDNF secretion (Fig. 2k). Notably, this effect was completely abrogated when the BDNF receptor TrkB was knocked out in GL261 cells using CRISPR-Cas9 technology (Supplementary Fig. 2d). ChIP-qPCR further confirmed that NE promotes p-CREB enrichment at the *Bdnf* promoter (Supplementary Fig. 2e).

To investigate the regulatory effects of neuron-derived NE on tumor cells and immune cells, we induced PC12 cells into low, medium, and high differentiation states (Supplementary Fig. 2f). As differentiation increased, the expression level of DBH, a key enzyme in NE synthesis, gradually rose (Fig. 2l). When PC12 cells at different differentiation states were co-cultured with GL261 glioma cells, GL261 cell viability was significantly enhanced in the high-differentiation PC12 group (Fig. 2m). In contrast, co-culture with primary T cells led to a marked upregulation of PD1 expression on T cells, as detected by flow cytometry, indicating T cell exhaustion (Fig. 2n). In BMDMs, BDNF treatment significantly induced polarization toward the M2 phenotype (CD206+), consistent with the suppression of inflammatory responses observed in the earlier GSEA analysis (Fig. 2o). Collectively, these results demonstrate that β 2-adrenergic receptor-mediated BDNF upregulation drives glioma malignancy by synergistically promoting axonogenesis and immunosuppression, enhancing neuro-tumor crosstalk while remodeling an immunosuppressive microenvironment.

2.3. Targeting the NE-BDNF Axis Suppresses Glioma Growth and Restores Antitumor Immunity

We next validated the function of the NE-BDNF axis *in vivo*. DBH-targeting siRNA (siDBH) encapsulated in lipid nanoparticles (LNP) was administered via intracranial injection to glioma-bearing mice (Fig. 3a), achieving silencing of DBH in the tumor microenvironment and subsequently blocking local NE synthesis and secretion. Serial MRI monitoring (Fig. 3b-3c and Supplementary Fig. 3a) revealed that tumor growth was significantly attenuated and survival was markedly prolonged in the siDBH+Stress group compared to the siNC+Stress group. At the molecular level, DBH silencing significantly suppressed downstream BDNF expression and reduced intratumoral infiltration of TH+ nerve fibers (Fig. 3d and Supplementary Fig. 3b).

Immunologically, siDBH administration markedly decreased the expression of immune exhaustion markers PD-1 and LAG-3 induced by chronic stress-driven NE overproduction, while ELISA demonstrated elevated levels of IFN- γ and TNF- α (Supplementary Fig. 3c). Notably, the reduction in BDNF expression was accompanied by a decreased proportion of M2 macrophages (Fig. 3e-3f), an increased proportion of M1 macrophages, and a consequent significant rise in the M1/M2 ratio (Supplementary Fig. 3d), consistent with our earlier findings (Fig. 2o). These results indicate that targeting DBH to inhibit NE synthesis relieves stress-induced immunosuppression and remodels the tumor immune microenvironment.

To verify the core pro-tumorigenic role of BDNF within this stress-driven neuronal–astrocytic feedforward loop, we further silenced BDNF expression (Fig. 3g). BDNF silencing significantly suppressed stress-induced tumor growth and prolonged mouse survival (Fig. 3h-3i and Supplementary Fig. 3e). Western blot analysis (Fig. 3j and Supplementary Fig. 3f) revealed that BDNF knockdown substantially reduced intratumoral TH+ nerve fiber infiltration and concurrently downregulated DBH expression, indicating that BDNF serves as a central hub in this loop, not only mediating downstream pro-tumor effects but also exerting feedback regulation on upstream neuronal signals. Immunologically, BDNF silencing similarly alleviated immune cell exhaustion (PD-1, LAG-3) induced by excessive NE, and immunofluorescence staining demonstrated an increased proportion of M1 macrophages alongside a decreased M2 population, indicating a pro-inflammatory remodeling of the tumor immune microenvironment (Fig. 3k-3l). Collectively, through dual targeting of the NE synthesis enzyme DBH and its downstream core effector BDNF, we demonstrate the central role of the NE-BDNF axis in driving glioma malignancy and immunosuppression, providing dual-target validation for intervention in this pathway.

2.4. Construction and Characterization of iRA2-MBP: A Dual-Targeting Engineered Exosome Platform for Multimodal Anti-Glioma Therapy

First, monocytes isolated from bone marrow were differentiated into mature M1 macrophages using M-CSF and IFN- γ (Supplementary Fig. 4a) to maximize the yield of macrophage-derived exosomes. Pure exosomes (EM) were isolated from BMDMs via ultrahigh-speed differential centrifugation. Subsequently, we employed an integrated synthesis method-SFTE (Sonication, Freeze-Thaw cycling, and Extrusion)-to co-load the BDNF antibody (aBDNF) and propranolol into EM, significantly enhancing protein loading efficiency^[10] Compared to single-method preparations, SFTE increased the encapsulation efficiency of aBDNF by 1.65-fold (29.58% vs. 18.35%) (Supplementary Fig. 4b). Next, iRGD and A2 peptides were conjugated to the EM surface via click chemistry using the crosslinker N-Hydroxysuccinimide-Polyethylene Glycol4-Maleimide (NHS-PEG4-MAL), generating the dual-targeting engineered exosome iRA2-MBP with acid-responsive release properties (Fig. 4a). Within the acidic tumor microenvironment, iRA2-MBP efficiently releases the loaded aBDNF and propranolol: released aBDNF neutralizes BDNF, blocking the core signaling of the malignant loop, while propranolol antagonizes β -adrenergic receptors, inhibiting NE signaling and inducing immunogenic cell death. The surface-modified A2 peptide facilitates blood-brain barrier penetration by binding to LRP-1 (LDL receptor-related protein-1) on brain endothelial cells, triggering vesicle-mediated transcytosis. Meanwhile, iRGD targets $\alpha v \beta 3 / \alpha v \beta 5$ integrins on tumor cells, upon proteolytic cleavage, it exposes its CendR motif, which interacts with neuropilin-1 (NRP-1) to confer deep tumor penetration capability, thereby enhancing drug accumulation in the tumor core [18–20]. This design achieves a multi-level delivery strategy encompassing blood-brain barrier crossing, deep penetration, and synergistic therapy.

Subsequently, we systematically characterized iRA2-MBP. Transmission electron microscopy (TEM) revealed uniformly dispersed vesicular structures with a diameter of approximately 30 nm (Fig. 4b). Dynamic light scattering (DLS) measured the hydrodynamic diameter at approximately 107 nm. Zeta potential analysis showed that MBP exhibited a similar surface charge to native EM, whereas modification with A2 and iRGD peptides significantly increased the membrane potential (Fig. 4c). Stability assessment demonstrated that iRA2-MBP incubated in DMEM, DMEM containing 10% fetal bovine serum (FBS), and PBS (pH 7.4) for 7 days showed no significant changes in particle size, indicating excellent stability (Supplementary Fig. 4c). Quantitative analysis using a multifunctional microplate reader revealed that when the feed ratios of aBDNF and propranolol were 25% and 30%, respectively, their loading efficiencies were 30.59% and 10.53% (Supplementary Fig. 4d).

Furthermore, we monitored the in vitro release profile of aBDNF from iRA2-MBP under different pH conditions (6.0, 6.5, and 7.4) using dialysis to verify its acid-responsive dissociation properties. The results (Fig. 4d) showed that after 24 hours of incubation under physiological conditions (pH 7.4), the cumulative release of aBDNF was less than 30%, whereas under acidic conditions at pH 6.5 and pH 6.0, the release significantly increased to 58.3% and 62.4%, respectively, confirming the excellent acid-responsive release capability of iRA2-MBP. Concurrently, we analyzed key proteins associated with macrophage membrane (MM) and EM by Western Blot. The results (Fig. 4e) demonstrated that, compared to the MM group, EM(exosomes from M1 macrophages), MBP(aBDNF/propranolol-loaded-exosomes), and iRA2-MBP all highly expressed the exosomal marker TSG101, while showing no

expression of the endoplasmic reticulum marker Calnexin or the nuclear protein Histone H3, confirming the high purity of exosomes obtained by ultrahigh-speed differential centrifugation ^[21].

Further evaluation of the anti-tumor effects of MBP (aBDNF/propranolol-loaded-exosomes) revealed that it both directly inhibits tumor cell migration and induces immunogenic cell death. Flow cytometry further confirmed that MBP enhanced GL261 cell apoptosis after treatment (Fig. 4f). Wound healing assays (Supplementary Fig. 4e) showed that, compared to the control group, MBP treatment reduced the migration rate of GL261 cells by 8.25-fold within 24 hours. In a GL261 and BMDM coculture model (Fig. 4g), both the free propranolol group and the MBP group significantly upregulated HMGB1 expression, confirming that propranolol successfully induces tumor cell ICD and releases damage-associated molecular patterns (DAMPs). Concurrently, CD86 and CD80 expression were elevated, indicating effective BMDM activation and acquisition of antigen-presenting capacity, laying the foundation for initiating adaptive anti-tumor immunity. In a DRG and C8-D1A coculture model (Fig. 4h and Supplementary Fig. 4f), NE-pretreated C8-D1A promoted DRG neurite outgrowth, whereas MB and MP attenuated this effect, with MBP showing the strongest inhibitory activity. In conclusion, through the co-delivery of propranolol and BDNF antibody, synergistic effects were achieved in inhibiting tumor cell migration, inducing immunogenic cell death, activating antigen-presenting cells, and blocking neurite outgrowth, demonstrating a multi-layered anti-tumor effect.

2.5. In Vitro and In Vivo Targeting of Glioma by the Dual-Targeting Exosome iRA2-MBP

We next further evaluated the penetration capability of iRA2-MBP through the dense tumor layer and its ability to cross the blood–brain barrier. First, we established an in vitro tumor stroma model by seeding C8-D1A astrocytes in the upper Transwell chamber and GL261 glioma cells in the lower chamber (Fig. 5a) ^[11, 22]. aBDNF was labeled with FITC fluorescent dye and formulated into MBP, iR-MBP, and dual-targeting iRA2-MBP, which were added to the upper chamber. The results (Fig. 5b and Supplementary Fig. 5a) demonstrated that iRGD induces phosphorylation modification of tight junction proteins between C8-D1A cells via the NRP-1/PI3K γ δ pathway, thereby opening the intercellular barrier and allowing iRA2-MBP to successfully traverse the upper chamber and reach GL261 cells in the lower chamber [19, 23]. Compared to the FreeBP and MBP groups, the iR-MBP and iRA2-MBP treatment groups exhibited significantly enhanced fluorescence signals in the lower chamber, with iRA2-MBP achieving a penetration rate of up to 63.2% within 4 hours (Fig. 5c-5d and Supplementary Fig. 5b). At the molecular level, expression of the tight junction-associated proteins ZO-1 and Occludin in C8-D1A cells was significantly downregulated in the iRA2-MBP group compared to the FreeBP and MBP groups, confirming effective disruption of astrocytic tight junction structures (Fig. 5e and Supplementary Fig. 5c).

Next, we established an in vitro blood–brain barrier model by seeding bEnd.3 endothelial cells in the upper Transwell chamber and GL261 glioma cells in the lower chamber. aBDNF was labeled with FITC and formulated into MBP, iR-MBP, and iRA2-MBP, which were then added to the upper chamber. The results showed that iRA2-MBP, via its surface A2 peptide binding to LRP-1, triggered a transcytosis

mechanism, achieving a transmembrane delivery efficiency of 68.4% within 4 hours (Fig. 5f). At the molecular level, expression of Rab5 and Rab7 was significantly elevated in the A2-MBP and iRA2-MBP treatment groups (Fig. 5g and Supplementary Fig. 5d). Rab5 and Rab7 regulate early endosome formation and maturation, as well as the transport of endosomes toward lysosomes or the plasma membrane, respectively. Their upregulation indicates effective activation of the A2 peptide-mediated transcytosis pathway. To further mimic the complex physiological barrier environment *in vivo*, we co-seeded C8-D1A astrocytes with bEnd.3 endothelial cells in the upper chamber to construct a bilayer barrier model, with aBDNF labeled with CY5. Confocal laser scanning microscopy (CLSM) revealed that the fluorescence intensity in GL261 cells of the lower chamber was significantly higher in the iRA2-MBP group than in all other groups (Fig. 5h and Supplementary Fig. 5e), confirming its superior capability for traversing complex biological barriers.

We next evaluated the tumor accumulation and retention of iRA2-MBP in a GBM mouse model using an *in vivo* imaging system (IVIS). aBDNF labeled with CY5 fluorescent dye was encapsulated into exosomes to prepare Free BP, MBP, iR-MBP, A2-MBP, and iRA2-MBP, which were intravenously injected into tumor-bearing mice via the tail vein. Dynamic monitoring of fluorescence signals in the brain revealed that iRA2-MBP-treated mice exhibited significantly stronger brain fluorescence signals than the Free BP, iR-MBP, and A2-MBP groups, with signal intensity increasing in a time-dependent manner and peaking at 8–12 h post-injection (Fig. 5i–j). Importantly, strong fluorescence signals were still detectable in the tumor region of the iRA2-MBP group at 48 h post-injection, whereas signals in the other groups had markedly attenuated. *Ex vivo* brain imaging at 48 h post-injection further confirmed the superior blood–brain barrier crossing and deep tumor penetration capabilities of iRA2-MBP (Fig. 5k–l). Quantitative analysis showed that the CY5 fluorescence intensity of iRA2-MBP in the tumor site was 11.12-fold higher than that of Free BP and 2.31-fold higher than that of A2-MBP. Cryosection imaging further confirmed that iRA2-MBP distributed throughout the tumor core, whereas A2-MBP showed only weak perivascular signal and free aBDNF was undetectable (Supplementary Fig. 5f). This enhanced tumor accumulation was supported by pharmacokinetic analysis, which revealed a 3-fold longer circulation half-life of iRA2-MBP (7.9 h vs. 2.6 h for free aBDNF) and a shift from renal to hepatobiliary clearance (Supplementary Fig. 5g–i). Furthermore, biodistribution analysis in major organs (heart, liver, spleen, lungs, and kidneys) demonstrated that the uptake of iRA2-MBP in the liver and kidneys was significantly lower than that of Free BP (Fig. 5m–n), confirming the favorable *in vivo* targeting and biocompatibility of this nanoplatform. Furthermore, repeated iRA2-MBP administration under the therapeutic dosing regimen induced no significant alterations in serum biochemistry (ALT, AST, BUN, CRE) over 30 days, and H&E staining of major organs revealed no pathological lesions (Supplementary Fig. 5j–k). These data confirm the long-term biosafety of iRA2-MBP under repeated dosing.

2.6. iRA2-MBP Suppresses Glioma Growth and Prolongs Survival in Stressed Mice via Dual-Targeting Delivery

Under chronic restraint stress, GL261 tumor-bearing mice received iRA2-MBP or control formulations on days 5, 8, 12, and 16 post-implantation (Fig. 6a). The *in vitro* dosing concentrations of iRA2-MBP and its

components are summarized in Supplementary Table S1, and the in vivo administration doses are summarized in Supplementary Table S2. iRA2-MBP treatment produced the most pronounced tumor regression among all groups (Fig. 6b). Although the A2-MBP group showed a trend of tumor growth inhibition, its therapeutic efficacy remained limited, suggesting that A2-mediated BBB crossing alone is insufficient for deep tumor penetration without iRGD. Survival analysis revealed that 40% of iRA2-MBP-treated mice achieved complete tumor regression and remained tumor-free throughout the study period (Fig. 6c-d), highlighting the synergistic necessity of co-modification with iRGD and A2 peptides for effective dual-targeting delivery.

Furthermore, we evaluated the in vivo biosafety of iRA2-MBP. During the entire treatment period, the body weight of mice in the iRA2-MBP group gradually recovered as tumors regressed (Supplementary Fig. 6a). After treatment, H&E staining of major organs (Brain, liver, spleen, lungs, and kidneys) revealed no obvious histopathological abnormalities, and serum biochemical analysis showed no significant differences in liver function (ALT, AST) and renal function (BUN, CRE) parameters among all groups (Supplementary Fig. 6b), confirming the favorable biosafety of iRA2-MBP treatment.

At the molecular level, immunohistochemistry revealed that iRA2-MBP significantly suppressed tumor cell proliferation (Ki67) and induced pronounced apoptosis (TUNEL) compared to all other treatment groups (Fig. 6e). Immunofluorescence staining further demonstrated that iRA2-MBP markedly inhibited intratumoral NE production (DBH) and downregulated downstream BDNF expression (Fig. 6f). Meanwhile, the reduction in TH⁺ nerve fiber infiltration further confirmed that iRA2-MBP successfully disrupted this vicious cycle (Supplementary Fig. 6c).

2.7. iRA2-MBP Reverses Immunosuppression and Remodels the Immune Microenvironment in Stressed GBM

To investigate the immunomodulatory effects of iRA2-MBP on the tumor microenvironment, we further evaluated its impact on the infiltration of adaptive and innate immune cells in the GBM cold tumor microenvironment under chronic stress conditions. Under chronic stress, iRA2-MBP treatment significantly remodeled the tumor immune microenvironment. Specifically, iRA2-MBP markedly increased the proportion of CD4⁺ T cells and, more importantly, CD8⁺ T cells exhibited robust activation, comprising 33% of CD45⁺ cells—a 3.41-fold increase compared to the control group (Fig. 7a–b). Concurrently, the proportion of regulatory T cells (Tregs, Foxp3⁺ CD25⁺) significantly decreased from 13% to 2.46%, representing a 5.28-fold reduction (Fig. 7c). The significantly elevated CD8⁺ T cell/Treg ratio further confirmed (Supplementary Fig. 7a) that iRA2-MBP attenuated stress-associated immunosuppression and promoted a shift toward a more immune-active tumor microenvironment.

Regarding innate immune cells, immunofluorescence staining results (Fig. 7d) demonstrated that iRA2-MBP treatment was associated with reduced NE and BDNF expression, increased M1 macrophage infiltration, and decreased M2 macrophage abundance. The elevated M1/M2 ratio indicated successful activation of the innate immune system (Supplementary Fig. 7b). Subsequently, we analyzed mature

dendritic cells (mDCs) by flow cytometry (Supplementary Fig. 7c–e), and the results showed that the expression of MHC II⁺, CD86⁺ and CD80⁺ in the iRA2-MBP group was significantly higher than in the other groups. To explore the potential mechanism by which iRA2-MBP promotes mDC accumulation, we further measured HMGB1 levels in tumor tissues by ELISA (Supplementary Fig. 7f) and found that iRA2-MBP treatment significantly induced HMGB1 expression, suggesting that this strategy accelerates dendritic cell maturation by enhancing the ICD effect.

Regarding the induction of durable immune memory, the proportion of effector memory T cells (TEM, CD44⁺) was significantly increased in the iRA2-MBP group (Fig. 7e), representing a 1.96-fold increase compared to the Free BP group. Furthermore, ELISA analysis of tumor tissues revealed that iRA2-MBP treatment significantly upregulated the expression of pro-inflammatory cytokines TNF- α and IFN- γ , while downregulating the anti-inflammatory cytokine IL-10 (Supplementary Fig. 7g), further confirming its capacity to reverse immunosuppression and remodel the anti-tumor immune microenvironment.

Finally, to assess the contribution of iRA2-MBP-elicited immune responses to its antitumor efficacy, we depleted CD8⁺ T cells using anti-CD8 antibodies (aCD8) or macrophages using clodronate liposomes (Clo lip) to determine the functional necessity of distinct immune cell populations in this therapeutic effect. After aCD8 or Clo lip administration, we first validated the depletion efficiency by flow cytometry. The results (Fig. 7f and Supplementary Fig. 7h) showed that the proportions of CD8⁺ T cells and macrophages (F4/80⁺CD11b⁺) in the spleen were significantly reduced, confirming successful depletion of the respective cell populations. Subsequently, all groups of mice were subjected to 6 hours of daily chronic stress and received intravenous injections of iRA2-MBP on days 4, 8, 12, and 16 post-tumor inoculation. Tumor growth was dynamically monitored using the IVIS in vivo imaging system starting from day 5. The results (Fig. 7g–i) demonstrated that the iRA2-MBP monotherapy group exhibited marked tumor signal regression and significantly prolonged mouse survival. However, when iRA2-MBP was combined with aCD8 to deplete CD8⁺ T cells, the antitumor effect was markedly diminished, with only transient minor suppression observed on day 10, followed by rapid tumor progression. Similarly, iRA2-MBP combined with Clo lip to deplete macrophages showed only a modest tumor suppression trend, with limited therapeutic efficacy. These results indicate that the antitumor effect of iRA2-MBP is highly dependent on the synergistic involvement of both CD8⁺ T cells and macrophages. In conclusion, iRA2-MBP coordinately achieves tumor elimination and immune remodeling under chronic stress conditions, not only eradicating established tumors but also establishing durable immune memory to prevent GBM recurrence, highlighting its potential as a transformative immunotherapeutic strategy.

3. Conclusion

We identified a chronic stress-driven NE-BDNF feedforward loop between intratumoral neurons and astrocytes that promotes glioma progression. Our findings further support an important role for this signaling axis in tumor growth, neural remodeling, and immunosuppression [3, 24]. We further developed the iRGD/A2-engineered exosome platform iRA2-MBP to co-deliver BDNF antibody and propranolol,

which enables efficient brain tumor targeting, malignant loop blockade and robust anti-tumor and immunomodulatory efficacy in preclinical models. These findings advance the mechanistic understanding of stress-associated glioma and offer a promising translational immunotherapeutic strategy.

The clinical link between chronic stress and poor prognosis in glioblastoma has long been recognized, but the causal relationship remains unclear^[25–27]. Due to potential reverse causality and confounding factors, stress has been viewed more as a biomarker than a direct driver of tumor progression^[6, 28–30]. While mechanisms of stress-driven malignancy are well-established in peripheral cancers—largely through the sympathetic nervous system-catecholamine- β receptor axis—research in glioma has lagged^[5, 6, 31, 32]. Existing studies have only shown that NE promotes glioma cell proliferation in vitro or that chronic stress accelerates intracranial tumor growth in animal models^[4, 33, 34]. The multicellular interactions within the tumor microenvironment and the cascade relaying systemic stress signals to the intracranial niche remain unexplored^[35–37]. This mechanistic gap has confined chronic stress to the status of a prognostic marker, leaving targeted therapies for stress-associated glioma largely undeveloped^[7, 38, 39].

We found that under chronic stress, the β -adrenergic signaling pathway and BDNF expression were significantly upregulated in glioma tissues, whereas anti-tumor immune pathways were broadly suppressed. Integrating single-cell transcriptomic data from 11 glioma patients, we identified the core cellular subsets driving this malignant loop: intratumoral TUBB3⁺ DCC⁺ neurons serve as the primary source of NE under stress, while astrocytes expressing β -adrenergic receptors are the main source of BDNF. Elevated BDNF not only directly promotes glioma progression but also upregulates DBH, a key enzyme in NE synthesis, thereby exacerbating intratumoral NE accumulation and forming a self-reinforcing vicious cycle.

The NE-BDNF axis exerts dual functions: it directly promotes axonal growth and malignant tumor proliferation, while also orchestrating an immunosuppressive microenvironment by inducing T cell exhaustion and macrophage M2 polarization. In vivo gene silencing confirmed that blocking either DBH or BDNF significantly inhibits tumor growth and reverses immunosuppression. Notably, BDNF silencing feedback inhibits upstream NE synthesis, establishing its dual role as the core hub of this positive feedback loop. These findings directly link chronic stress, neural remodeling, and immunosuppression, providing a rationale for a dual-targeting strategy combining upstream NE blockade with downstream BDNF neutralization.

We addressed three key challenges in glioblastoma treatment: the blood-brain barrier restricting drug entry, dense tumor stroma limiting deep penetration, and stress-induced immunosuppression. Based on these clinical obstacles and our mechanistic insights, we developed iRA2-MBP, an engineered exosome co-modified with iRGD and A2 peptides and loaded with a BDNF antibody and propranolol, for synergistic therapy encompassing BBB crossing, deep penetration, loop blockade, and immune remodeling. The A2 peptide enables BBB traversal via LRP-1-mediated transcytosis, while iRGD drives deep tumor

penetration by targeting integrins and subsequently binding NRP-1 to open tight junctions [9, 40]. This dual-targeting mechanism significantly enhanced intratumoral drug accumulation and retention, establishing a foundation for effective treatment.

In vivo studies showed that iRA2-MBP crosses the blood–brain barrier and accumulates within GBM tissues. Through acid-responsive drug release, this platform enables dual blockade of the NE-BDNF loop at both upstream and downstream levels, along with coordinated regulation of the immune microenvironment. Specifically, propranolol antagonizes NE-mediated adrenergic signaling, thereby inhibiting BDNF secretion from astrocytes at the upstream source, while also inducing immunogenic cell death in glioma cells, leading to the release of damage-associated molecular patterns such as HMGB1, promoting dendritic cell maturation (CD80⁺CD86⁺), enhancing antigen presentation, and initiating adaptive antitumor immunity. Concurrently, aBDNF directly neutralizes BDNF in the microenvironment, blocking the core pro-tumor signal of the loop and reversing macrophage M2 polarization to reshape innate immune responses. Through this synergistic action, iRA2-MBP significantly enhances intratumoral CD8⁺ T cell infiltration and activation, reduces the proportion of regulatory T cells, and elevates the CD8⁺/Treg ratio, collectively confirming effective remodeling of the tumor immune microenvironment.

Several limitations of this study should be noted. Although our findings support a functional NE–CREB–BDNF signaling axis, additional assays including reporter assays, EMSA, and co-factor interaction studies will be valuable for further delineating the precise transcriptional regulatory mechanism. First, while we confirmed the core role of the NE-BDNF loop in stress-driven glioma progression, potential compensatory regulatory mechanisms within the glioma microenvironment cellular network remain to be clarified via spatial transcriptomics and lineage tracing. Second, our chronic restraint stress model cannot fully recapitulate the heterogeneous stress profiles and disease courses in clinical patients, requiring validation in additional preclinical models^[41]. Third, the large-scale production, storage stability, and drug-loading efficiency of the iRA2-MBP exosome platform—particularly for small hydrophilic molecules such as propranolol—need further optimization to facilitate clinical translation. Fourth, the contributions of non-CD8⁺ T cell/macrophage immune subsets to our therapeutic strategy have not been fully evaluated. Fifth, beyond this neuron–astrocyte–immune crosstalk, whether stress-driven BDNF directly impacts GSC self-renewal and immune evasion through MAPK-dependent mechanisms remains an open question for future investigation. Finally, our preclinical findings require validation in large animal models and clinical samples to support clinical trial development.

4. Experimental Section/Methods

4.1. Ethics Statement

This study strictly adhered to all applicable ethical regulations and requirements. All animal experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the Ethics Committee of Zhengzhou University (NO. ZZUIRB-2025-

206). For studies involving human samples, all experimental protocols were also reviewed and approved by the Ethics Committee of Zhengzhou University, following relevant ethical guidelines (NO. ZZUIRB-2024-191). A total of 13 tumor tissue samples were included in this study. Based on the presence or absence of depressive symptoms, participants were divided into two groups: a non-stress group (4 males, 2 females) and a stress group (5 males, 2 females). Depressive symptoms in all patients were assessed using the 17-item Hamilton Depression Rating Scale. All participants were informed and provided written informed consent prior to inclusion in the study.

4.2. Mice

Male C57BL/6 (RRID: IMSR_JAX:000664) and Balb/c (RRID: IMSR_JAX:000651) mice, aged six weeks, were obtained from Jiangsu Huachuang Xinnuo Pharmaceutical Technology Co., Ltd. (Nanjing, China). The mice were housed in a specific pathogen-free environment with controlled temperature (22–26°C), humidity (45% ± 5%), and a 12-hour light/dark cycle, with 4–5 mice per cage. Mice aged 7 to 8 weeks were used in the experiments. All mice were randomly allocated to experimental groups using a random number table prior to the start of experiments. For tumor implantation, the order of cell inoculation was also randomized. After tumor establishment, mice were further randomized into treatment groups to ensure comparable baseline body weight and tumor burden across groups.

4.3. Chronic Restraint Stress (CRS)

Mice were randomly assigned to either a home cage control group or a CRS group. Restraint was performed using transparent 50 mL centrifuge tubes modified with ventilation holes. Mice were placed into the tubes and restrained for 6 hours daily, during which food and water were withheld. After each session, mice were returned to their home cages. The model was validated by the tail suspension test after the final restraint. For the tail suspension test, video recordings were scored independently by two investigators who were blinded to the treatment conditions. MRI image acquisition and tumor volume quantification were performed by an operator aware of the animal identification numbers but blinded to the group allocation. Histological and immunofluorescence analyses were also conducted in a blinded manner.

4.4. Cytokine Analysis

Tumor tissues were weighed, homogenized in ice-cold PBS, and centrifuged at $12,000 \times g$ for 15 min at 4°C. Supernatants were collected for ELISA. Concentrations of TNF- γ (MM-0182M2), TNF- α (MM-47831M2), IL-10 (MM-0176M1), HMGB1 (E-EL-M0676), BDNF (MM-0176M1), and NE (MM-0876M2) were measured using ELISA kits (Jiangsu Meimian) following the manufacturer's protocols. Briefly, samples and standards were added to pre-coated plates, incubated, washed, and incubated with enzyme-linked detection antibodies. After washing, substrate was added for color development, and the reaction was stopped. Optical density was read at 450 nm, and concentrations were calculated from standard curves.

4.5. Flow Cytometry

To analyze tumor-infiltrating immune cells, tumor tissues were minced and digested in RPMI 1640 medium (Thermo Fisher, 11875-093) containing 3 mg/mL collagenase II/IV (Thermo Fisher, 17101-015) and 10 µg/µL DNase I (Thermo Fisher, EN0521) at 37°C for 60 min. Digestion was terminated by adding RPMI 1640 supplemented with 1% penicillin-streptomycin (Thermo Fisher, 15140-122), 0.5 mM EDTA (Thermo Fisher, R1021), and 10% FBS (Thermo Fisher, 10082-147, SCR_008988). The digested tissues were then passed through a 70 µm cell strainer (Corning Biosciences 352350) to obtain single-cell suspensions. Spleen tissues were mechanically disrupted using the plunger of a 2.5 mL syringe and filtered through a 70 µm cell strainer. To block non-specific binding, cells were incubated with mouse CD16/CD32 monoclonal antibody (eBioscience, 14-0161-82, AB_467133) (0.25 µg/100 µL) for 15 min at room temperature. Cells were then stained with appropriate antibodies against specific antigens in blocking buffer on ice, following the manufacturer's instructions. Viable cells were identified using a live/dead dye. Flow cytometry was performed on an LSR-Fortessa instrument (BD Biosciences). Cell gating was based on viability, and distinct immune cell populations were analyzed, including CD8⁺ T cells (CD45⁺CD3⁺CD8⁺), T cell subsets (CD45⁺CD3⁺Foxp3⁺CD25⁺CD44⁺), macrophages, and dendritic cells (CD45⁺F4/80⁺CD11c⁺CD86⁺CD206⁺CD80⁺), using FlowJo software (Tree Star). The gating strategy was performed according to the manufacturer's instructions and is presented in Supplementary Fig. 8–9. Fluorescence minus one controls for multicolour flow cytometry are shown in Supplementary Fig. 10.

4.6. RNA Sequencing Analysis

RNA sequencing analysis was performed on tumor tissues from stressed and non-stressed mice. Tumor samples were immediately frozen in liquid nitrogen, and RNA was isolated from purified tumor tissues according to the manufacturer's instructions. RNA concentration was quantified using a Qubit 2.0 fluorometer (Thermo Fisher) following the manufacturer's protocol. Libraries were prepared using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) and subsequently sequenced on a NovaSeq 6000 platform (Illumina) by the deep sequencing facility at Novogene. For data analysis, mapped reads for each sample were assembled using StringTie (v1.3.3b) with a reference-based approach, and mapped reads were quantified using featureCounts. Differential expression analysis between the two groups was performed using the DESeq2 R package (1.20.0).

4.7. Western Blotting

Protein samples were extracted from cells or tumor tissues using RIPA lysis buffer (Beyotime, China) supplemented with protease and phosphatase inhibitor cocktails (Roche, Switzerland). Protein concentrations were determined using a BCA protein assay kit (Thermo Fisher Scientific, USA). Equal amounts of protein (20–40 µg per lane) were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, USA). Membranes were blocked with 5% skim milk in TBST (Tris-buffered saline containing 0.1% Tween-20) for 1 h at room temperature, followed by overnight incubation at 4°C with primary antibodies against the following targets: BDNF (Proteintech, 28205-1-AP, RRID: AB_2818960), DBH (Proteintech, 10777-1-AP, RRID: AB_2092084), TH (Proteintech, 66334-1-Ig, RRID: AB_2881623), CGA (Proteintech, 23342-1-AP, RRID: AB_2879213), TrkB (Proteintech, 13129-1-AP, RRID: AB_2877871), PKA C-α (Cell Signaling Technology, 4782, RRID: AB_2170170), phospho-PKA (HuaBio, HA721577, RRID:

AB_3072974), ERK (Proteintech, 16443-1-AP, RRID: AB_2878077), phospho-ERK (Cell Signaling Technology, 9101, RRID: AB_331772), MEK (Proteintech, 11049-1-AP, RRID: AB_2877635), phospho-MEK (Cell Signaling Technology, 9154, RRID: AB_2138017), CREB (Proteintech, 12208-1-AP, RRID: AB_2877803), phospho-CREB (Ser133) (Cell Signaling Technology, 9198, RRID: AB_2561044), HMGB1 (Proteintech, 10829-1-AP, RRID: AB_2877527), CD86 (Proteintech, 26903-1-AP, RRID: AB_2880721), CD80 (Proteintech, 66411-1-Ig, RRID: AB_3746566), PD-1 (Proteintech, 66220-1-Ig, RRID: AB_2881611), LAG-3 (Proteintech, 66667-1-Ig, RRID: AB_2881958), TSG101 (Proteintech, 28283-1-AP, RRID: AB_2881055), Calnexin (Proteintech, 66903-1-Ig, RRID: AB_2882231), Histone H3 (Proteintech, 17168-1-AP, RRID: AB_2878411), Rab5 (Proteintech, 11947-1-AP, RRID: AB_2269388), Rab7 (Proteintech, 55412-1-AP, RRID: AB_2881337), ZO-1 (Proteintech, 66452-1-Ig, RRID: AB_2881626), Occludin (Proteintech, 27260-1-AP, RRID: AB_2880820), VE-cadherin (Proteintech, 66834-1-Ig, RRID: AB_2881630), phospho-VE-cadherin (Cell Signaling Technology, 2500, RRID: AB_10839118), and β -actin (Proteintech, 66009-1-Ig, RRID: AB_2687938) or GAPDH (Cell Signaling Technology, 2118, RRID: AB_561053) as loading controls. After washing with TBST, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Proteintech) for 1 h at room temperature. Protein bands were visualized using enhanced chemiluminescence (ECL) substrate (Millipore, USA) and detected with a chemiluminescence imaging system. Densitometric analysis was performed using ImageJ software (NIH, USA, RRID: SCR_003070). All primary antibody dilutions followed the manufacturers' recommendations.

4.8. Immunofluorescence Staining

For immunofluorescence analysis, cells cultured on coverslips or frozen tumor tissue sections (8 μ m thick) were fixed with 4% paraformaldehyde for 15 min at room temperature, permeabilized with 0.3% Triton X-100 in PBS for 10 min, and blocked with 5% bovine serum albumin (BSA) in PBS for 1 h at room temperature. Samples were then incubated overnight at 4°C with primary antibodies against the following targets: CD86 (Proteintech, 26903-1-AP), CD206 (HuaBio, HA721192), TH (Proteintech, 66334-1-Ig), DBH (Proteintech, 15362-1-AP), BDNF (Proteintech, 28205-1-AP). After washing with PBS, samples were incubated with appropriate Alexa Fluor-conjugated secondary antibodies (Invitrogen, USA) for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI (Beyotime, China). Fluorescence images were captured using a confocal laser scanning microscope (Zeiss LSM 900, Germany) or a fluorescence microscope (Olympus BX53, Japan). Quantitative analysis of fluorescence intensity or positive cell counts was performed using ImageJ software (NIH, USA) from at least three random fields per sample.

4.9. Immunohistochemistry (IHC)

Tumor tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 4 μ m thickness. Sections were deparaffinized in xylene, rehydrated through graded ethanol, and subjected to antigen retrieval by heating in citrate buffer (pH 6.0) for 15 min. Endogenous peroxidase activity was blocked with 3% H₂O₂ for 10 min, followed by blocking with 5% BSA for 1 h at room temperature. Sections were then incubated overnight at 4°C with primary antibodies against Ki67 (Cell Signaling Technology, 12202, RRID: AB_2756525) or TUNEL (Roche, 11684795910) according to the

manufacturer's instructions. After washing, sections were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. Signal was developed using a DAB substrate kit (Vector Laboratories, USA), and sections were counterstained with hematoxylin. Images were captured using a light microscope (Olympus CKX53 or CX23, Japan). Quantification of Ki67⁺ or TUNEL⁺ cells was performed using ImageJ software (NIH, USA, RRID: SCR_003070) from at least five random fields per sample.

4.10. siRNA preparation

Small interfering RNAs (siRNAs) targeting mouse *Bdnf* and *Dbh* were chemically synthesized by Jin Ka Rui Biological Technology Co., Ltd. (Wuhan, China). The siRNA sequences were as follows:

Bdnf siRNA: F, 5'-GAGACCAAGUGUAAUCCCA-3'; R, 5'-UGGGAUUACACUUGGUCUC-3'.

Dbh siRNA: F, 5'-GGACAAGAUGGAGAACUUC-3'; R, 5'-GAAGUUCUCCAUCUUGUCC-3'.

Nc siRNA: F, 5'-UUCUCCGAACGUGUCACGUTT-3'; R, 5'-ACGUGACACGUUCGGAGAATT-3'.

4.11. Chromatin Immunoprecipitation qPCR (ChIP-qPCR)

ChIP assays were performed in C8-D1A astrocytes using antibodies against p-CREB (Ser133) and normal rabbit IgG as a negative control. Immunoprecipitated DNA was quantified by real-time qPCR with SYBR Green Master Mix using primers targeting the *Bdnf* promoter region containing the CRE element: forward, 5'-TCCACTGAGCAAAGCCGAAC-3'; reverse, 5'-GTCTCCCCAACAGATGCAGG-3' (product length, 77 bp). Enrichment was calculated by the percent input method (% Input = $100 \times 2^{-(Ct_{\text{adjusted input}} - Ct_{\text{ChIP}})}$).

4.12. Cell Culture

Mouse GL261 glioma cells (RRID: CVCL_Y003), mouse bEnd.3 endothelial cells (RRID: CVCL_0170), mouse C8-D1A astrocytes (RRID: CVCL_6379), rat PC12 adrenal pheochromocytoma cells (RRID: CVCL_0481), and human U87 glioma cells (RRID: CVCL_0022) were obtained from Procell Life Science & Technology Co., Ltd. (Wuhan, China). U87, bEnd.3, GL261, and C8-D1A cells were cultured in DMEM medium (HyClone, Cat# SH30243.01) supplemented with 10% fetal bovine serum (Gibco, Cat# 10082-147, RRID: SCR_008988) and 100 U/mL penicillin/streptomycin (Thermo Fisher Scientific, Cat# 15140-122). All cells were maintained at 37°C in a 5% CO₂ water-jacketed incubator (Thermo Fisher Scientific, MA, USA). A complete list of cell lines and their RRIDs is provided in Supplementary Table S3.

4.13. Neuron-astrocyte coculture model

For neurite outgrowth assessment, C8-D1A astrocytes were seeded in the lower chamber of 24-well Transwell plates (0.4 µm pore size, Corning, USA) at a density of 2×10^4 cells per well. After 24 h, DRG cells (5×10^3 cells per well) differentiated with 50 ng/mL nerve growth factor (BDNF, MCE) for 7 days were seeded onto coverslips placed in the lower chamber. Alternatively, for direct coculture, DRG cells were seeded directly onto the astrocyte monolayer. Cells were treated with 10 µM NE (NE, Sigma-Aldrich,

USA) $\pm$ 10 μ M propranolol (Sigma-Aldrich) for 48 h. At least 50 neurons per condition were analyzed from three independent experiments.

4.14. Astrocyte-glioma coculture model

C8-D1A astrocytes were seeded in 6-well plates (2×10^5 cells/well) and cultured for 24 h. After washing with PBS, GL261 glioma cells (1×10^5 cells/well) labeled with GFP were added directly onto the astrocyte monolayer. Cocultures were treated with 10 μ M NE $\pm$ 10 μ M propranolol for 48 h. GL261 cell viability was assessed by CCK-8 assay (Dojindo, Japan).

4.15. Macrophage-glioma coculture model

Bone marrow-derived macrophages (BMDMs) were differentiated from C57BL/6 mouse bone marrow cells using 20 ng/mL M-CSF (PeproTech, USA) for 7 days. BMDMs (2×10^5 cells/well) were seeded in 6-well plates. GL261 glioma cells (1×10^5 cells/well) labeled with GFP were added directly onto the BMDM monolayer. Cocultures were treated with free propranolol (25 μ M) or MBP formulations for 24 h. Cells were harvested for flow cytometry analysis of CD86 (Proteintech, 26903-1-AP) and CD80 (HuaBio, HA500344) expression on BMDMs (gated as F4/80⁺). Supernatants were collected for HMGB1 ELISA (IBL International, Germany) according to the manufacturer's instructions.

4.16. Primary DRG Neuron Culture

DRG neurons were isolated and cultured from embryonic mice according to established protocols. Pregnant C57BL/6 mice at embryonic day 13–15 were euthanized, and embryos were collected under sterile conditions and placed in ice-cold D-PBS. Under a dissecting microscope, the skin was incised along the midline of the embryonic back to expose the spinal column. A microdissecting scissors was inserted into the cervical vertebral canal, and the vertebral canal was cut bilaterally along the dorsal midline. After removal of the exposed spinal cord, the bead-like DRG were identified alongside the spinal column adjacent to the intervertebral foramina. DRG were carefully harvested using fine microdissecting forceps, with attached nerve fibers and capsules trimmed away as much as possible, and collected in ice-cold DMEM medium. Collected DRG were transferred to a centrifuge tube containing 0.25% trypsin-EDTA (Gibco) or an enzyme mixture (0.1% collagenase + 0.1% papain) and incubated at 37°C for 15–25 min with gentle agitation to facilitate digestion. Digestion was terminated by adding DMEM containing 10% FBS, followed by centrifugation at 1000 rpm for 5 min. The supernatant was discarded, and the pellet was resuspended in neuronal medium and gently triturated 10–15 times to obtain a single-cell suspension. The cell suspension was filtered through a 40 μ m cell strainer, counted, and adjusted to the desired density. Cells were seeded onto culture plates or coverslips pre-coated with poly-D-lysine (100 μ g/mL) and laminin (10 μ g/mL). After 4–6 h, once cells had adhered, the medium was replaced with neuronal maintenance medium: Neurobasal medium (Gibco) supplemented with 2% B27 (Gibco), 2 mM L-glutamine, and 1% penicillin/streptomycin. On days 2–3 post-seeding, mitotic inhibitors (5-fluorouracil and uridine, each at a final concentration of 5 μ M) were added to suppress non-neuronal cell

proliferation. Thereafter, half of the medium was replaced every 2–3 days. Neurons cultured for 7 days were used for subsequent experiments. All cultures were maintained at 37°C in a 5% CO₂ incubator.

4.17. Serum biochemical analysis

Blood was collected via cardiac puncture and centrifuged at 3,000 × g for 15 min at 4°C to obtain serum. Liver function markers (ALT, AST) and kidney function markers (BUN, CRE) were measured using an automated biochemical analyzer (Hitachi 7100, Japan) according to the manufacturer's protocols. All samples were analyzed in duplicate.

4.18. Establishment of TrkB-knockout GL261 cells

For stable knockout of TrkB in GL261 cells, short hairpin RNA (shRNA) targeting mouse TrkB (Ntrk2) was designed. The shRNA sequence was: 5'-

GAACATCAAGAGCATCCACTTCAAGAGAGTGGATGCTCTTGATGTTCTTTTTT-3'. This oligonucleotide was annealed and cloned into the pLKO.1-puro vector (Addgene, #8453) according to the standard protocol. The construct was verified by Sanger sequencing.

Lentivirus was produced by co-transfecting HEK293T cells with the pLKO.1-shTrkB plasmid, psPAX2 packaging plasmid, and pMD2.G envelope plasmid using Lipofectamine 3000. Viral supernatants were collected 48 and 72 h post-transfection, filtered through 0.45 µm filters, and concentrated by ultracentrifugation. GL261 cells were infected with lentivirus in the presence of 8 µg/mL polybrene. After 24 h, the medium was replaced with fresh DMEM containing 2 µg/mL puromycin for selection. Puromycin-resistant pools were expanded, and TrkB knockout efficiency was confirmed by Western blot analysis using an anti-TrkB antibody (Proteintech, RRID:AB_2155156).

4.19. Preparation of iRA2-MBP

M1 macrophage-derived exosomes (EM) were isolated from culture supernatants of BMDMs differentiated with M-CSF (PeproTech, 315-02) and IFN-γ (PeproTech, 315-05). Supernatants were collected and subjected to sequential centrifugation at 300 × g for 10 min, 2,000 × g for 20 min, and 10,000 × g for 30 min at 4°C to remove cells and debris. Exosomes were pelleted by ultracentrifugation at 100,000 × g for 70 min at 4°C (Beckman Coulter, USA, Optima XE-90, RRID: SCR_025331), washed with PBS, and re-pelleted under the same conditions. The resulting exosome pellets were resuspended in PBS and stored at – 80°C until use. Protein concentration of exosomes was determined using a BCA protein assay kit (Thermo Fisher Scientific, USA, Cat# 23225).

To load BDNF antibody (aBDNF) and propranolol into exosomes, an integrated synthesis method termed SFTE was employed. Briefly, purified EM (100 µg protein) were mixed with aBDNF (Millipore Sigma, AB1779SP, RRID: AB_2106783) and propranolol (Sigma-Aldrich, P0884) at feed ratios of 25% and 30% (w/w), respectively. The mixture was subjected to sonication (20 kHz, 20% amplitude, 6 cycles of 30 s on/30 s off) on ice, followed by three freeze-thaw cycles (– 80°C to room temperature). The suspension was then extruded through 200 nm polycarbonate membranes (Avanti Polar Lipids, USA) using a mini-

extruder. Unloaded drugs were removed by ultracentrifugation at $100,000 \times g$ for 70 min at 4°C. The resulting MBP (aBDNF/propranolol-loaded exosomes) were resuspended in PBS.

For dual peptide modification, iRGD peptide (Cys-iRGD, GL Biochem, China) and A2 peptide (Cys-A2, GL Biochem) were conjugated to the surface of MBP via click chemistry using the crosslinker NHS-PEG4-MAL (Thermo Fisher Scientific, Cat# 22102). Briefly, MBP (100 µg) were incubated with 10 µM NHS-PEG4-MAL for 30 min at room temperature. Excess crosslinker was removed by ultrafiltration (Amicon Ultra, 100 kDa MWCO, Millipore). Activated MBP were then incubated with 50 µM iRGD and 50 µM A2 peptides overnight at 4°C with gentle shaking. Unconjugated peptides were removed by ultracentrifugation at $100,000 \times g$ for 70 min at 4°C. The resulting dual-targeting exosomes (iRA2-MBP) were resuspended in PBS and stored at 4°C for short-term use or at – 80°C for long-term storage.

Encapsulation efficiency (EE) of aBDNF and propranolol was determined by measuring unloaded drugs in the supernatant after ultracentrifugation. aBDNF concentration was quantified by ELISA (R&D Systems, USA) according to the manufacturer's instructions, and propranolol concentration was measured by high-performance liquid chromatography (HPLC, Agilent, USA). EE (%) was calculated as $[(\text{total drug} - \text{free drug}) / \text{total drug}] \times 100\%$. Drug loading content (LC) was calculated as $(\text{loaded drug weight} / \text{exosome protein weight}) \times 100\%$.

The acid-responsive release profile of aBDNF from iRA2-MBP was evaluated using dialysis. iRA2-MBP suspensions (containing 10 µg aBDNF) were placed into dialysis bags (MWCO 100 kDa, Spectra/Por, USA) and immersed in 20 mL of PBS at pH 7.4, 6.5, or 6.0 at 37°C with gentle shaking. At predetermined time points (0, 1, 2, 4, 6, 8, 12, 24 h), 1 mL of release medium was collected and replaced with fresh pre-warmed buffer. aBDNF concentration in collected samples was measured by ELISA. Cumulative release percentage was calculated and plotted against time.

4.20. Primary BMDM culture

BMDMs were isolated from 6–8 week old C57BL/6 mice (RRID: IMSR_JAX:000664). Mice were euthanized and immersed in 70% ethanol for 5 min. Femurs and tibias were aseptically removed and dissected free of surrounding muscle tissue. Both ends of the bones were cut, and the bone marrow cavity was flushed with ice-cold PBS containing 2% FBS (Gibco, Cat# 10082-147, RRID: SCR_008988) using a 23-gauge needle. The collected bone marrow cells were centrifuged at $300 \times g$ for 5 min at 4°C, and red blood cells were lysed with ACK lysis buffer (Beyotime, Cat# C3702) for 3 min on ice. After washing with PBS, cells were resuspended in DMEM medium (HyClone, Cat# SH30243.01) supplemented with 10% fetal bovine serum (Gibco, Cat# 10082-147, RRID: SCR_008988), 100 U/mL penicillin/streptomycin (Thermo Fisher Scientific, Cat# 15140-122), and 20 ng/mL recombinant murine M-CSF (PeproTech, Cat# 315-02). Cells were seeded in 6-well plates at a density of 1×10^6 cells per well and cultured at 37°C in a 5% CO₂ incubator. On day 3, half of the medium was replaced with fresh M-CSF containing medium. After 7 days of differentiation, adherent cells were confirmed as BMDMs and used for subsequent experiments. For M1 polarization, BMDMs were stimulated with 100 ng/mL LPS (Sigma-Aldrich, Cat# L4391) and 20 ng/mL IFN-γ (PeproTech, Cat# 315-05) for 24 h.

4.21. Single-cell RNA sequencing analysis

Public scRNA-seq data of 11 glioma patients were obtained from the GEO database under accession GSE189357. Raw data were processed with Cell Ranger (10x Genomics) and analyzed using the Seurat package (v4.1.0) in R. Cells with low quality (< 200 genes, > 4000 genes, or > 20% mitochondrial UMIs) were excluded. After normalization and scaling, PCA was performed and the first 20 principal components were used for graph-based clustering. UMAP was applied for dimensional reduction. Cell types were annotated based on canonical markers. A neuronal cluster co-expressing TUBB3 and DCC was identified. This cluster was further analyzed for expression of DBH, TH, DCX (immature neuron marker), and the BDNF receptor NTRK2. Differential expression between clusters was determined using the Wilcoxon rank-sum test with Bonferroni correction (adjusted $P < 0.05$).

4.22. MRI and IVIS imaging

Tumor progression was monitored by magnetic resonance imaging (MRI) using a 7.0 T small animal MRI scanner (Bruker, Germany) on days 7, 14, and 21 post-implantation. Mice were anesthetized with 1.5% isoflurane during imaging. Tumor volumes were calculated from T2-weighted images using ImageJ software.

For in vivo fluorescence imaging, aBDNF was labeled with CY5 dye and formulated into Free BP, MBP, iR-MBP, A2-MBP, and iRA2-MBP as described. Glioma-bearing mice were injected intravenously via the tail vein with equivalent doses of each formulation. At predetermined time points (1, 2, 4, 6, 8, 12, 24, and 48 h post-injection), mice were anesthetized with isoflurane and imaged using an IVIS Spectrum imaging system (PerkinElmer, USA). Fluorescence signals were acquired with appropriate excitation/emission filters for CY5. At 48 h post-injection, mice were euthanized, and brains as well as major organs (heart, liver, spleen, lungs, kidneys) were harvested for ex vivo imaging. Total radiant efficiency was quantified using Living Image software (PerkinElmer). For immune cell depletion studies, tumor progression in mice treated with iRA2-MBP alone or in combination with aCD8 or Clo lip was monitored by IVIS imaging at indicated time points.

4.23. In vivo immune cell depletion

For CD8⁺ T cell depletion, glioma-bearing mice received intraperitoneal injections of 200 µg anti-CD8α antibody (clone 2.43, BioXCell) on day – 1 and every 5 days thereafter. For macrophage depletion, mice received intravenous injections of 200 µL clodronate liposomes (Clo lip, Liposoma) on the same schedule. Depletion efficiency was confirmed by flow cytometry analysis of splenocytes using anti-CD8-FITC for CD8⁺ T cells and anti-F4/80-APC/anti-CD11b-PE for macrophages, with > 95% reduction considered successful. Following depletion, mice were treated with iRA2-MBP under chronic stress conditions, and tumor growth and survival were monitored as described.

4.24. Bioinformatics analysis

TCGA data were accessed and processed following established bioinformatics workflows for cancer genomics data. Gene expression data and clinical information for glioma patients (GBM) were obtained from The Cancer Genome Atlas (TCGA) through the Genomic Data Commons (GDC) Data Portal (<https://portal.gdc.cancer.gov>). Level 3 RNA-seq data (gene expression quantification) were downloaded using the TCGAbiolinks R package (version 2.25.0) following standard query and download procedures.

For correlation analysis between TH/DBH expression and BDNF expression, gene expression values ($\log_2$ -transformed) were extracted for TH, DBH, and BDNF. The TH-positive nerve fiber density data were obtained from previously published datasets or quantified from histology images as described in the corresponding figure legends. Pearson correlation coefficients were calculated to assess the relationship between TH nerve fiber density and BDNF expression, as well as between DBH expression and BDNF expression. Correlation plots were generated using the ggplot2 package in R (version 4.1.2).

4.25. RNA sequencing analysis

Total RNA was isolated from glioma tissues using TRIzol reagent (Invitrogen, USA) following the manufacturer's instructions. RNA purity and integrity were verified prior to library construction. Sequencing libraries were generated using a standard mRNA library preparation kit and sequenced on an Illumina sequencing platform to generate paired-end reads.

Raw sequencing data were processed to remove adapter sequences and low-quality reads. Clean reads were aligned to the *Mus musculus* reference genome (mm10/GRCm38) using HISAT2. Gene expression levels were quantified as read counts and normalized to FPKM for subsequent analysis.

Differentially expressed genes (DEGs) between groups were identified using the edgeR package with thresholds of adjusted P-value < 0.05 and $|\log_2(\text{fold change})| > 1$. Volcano plots, PCA plots, and sample correlation heatmaps were generated to visualize expression patterns and sample distribution. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to explore the biological functions and pathways of DEGs.

4.26. Statistical analysis

Data were expressed as mean $\pm$ SEM from at least three independent experiments. Statistical analyses were performed using GraphPad Prism 9.0. Comparisons between two groups were analyzed by unpaired two-tailed Student's t-test. Multiple group comparisons were performed by one-way ANOVA with Tukey's post hoc test, and two-way ANOVA with Bonferroni's post hoc test for time-course experiments. Survival curves were analyzed by log-rank test. Correlation was assessed by Pearson correlation coefficient. For scRNA-seq data, differential expression was determined by Wilcoxon rank-sum test with Bonferroni correction. $P < 0.05$ was considered statistically significant. Exact P values are indicated in the figures.

Declarations

Author Contributions

M.L. and J.W. conducted the synthesis, characterization, cell studies, animal experiments, and data analysis. M.L., J.W., B.Z. and J.C.. performed the construction of an orthotopic brain tumor model. M.L., J.W., B.Z., H.J., H.L., B.J. and J.C. wrote the manuscript. Z.W., Q.L. Z.Y. J.W. and Y.Z. contributed to the data analysis and discussed the data. M.L., H.J., H.L. and J.C. conceived and designed the experiments. J.C., and H.L. supervised the entire project. M.L., J.W., B.Z., B.J. and Y.Z. revised the manuscript. All authors reviewed the manuscript. Meng Li[#] and Junfan Wei[#] contributed equally to this work.

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Competing interests

The authors declare that they have no competing interests.

Data Availability Statement

The data that support the findings of this study are openly available. The RNA sequencing data have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE325672. All other remaining data are available within the article, Supplementary Information, or from the corresponding author upon reasonable request.

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References

1. Kuchen R, Singer S, Schranz M, et al. Distress Screening in Patients with High-Grade Glioma: Diagnostic Accuracy in Relation to a Structured Clinical Interview in a Multicenter Cluster-Randomized Controlled Trial [J]. Support Care Cancer. 2025;33(8):737.
2. Wang Y, Wang X, Wang K, et al. Chronic Stress Accelerates Glioblastoma Progression Via Drd2/Erk/B-Catenin Axis and Dopamine/Erk/Th Positive Feedback Loop [J]. J Exp Clin Cancer Res. 2023;42(1):161.
3. Wang X, Zhang L, Zhou Y, et al. Chronic Stress Exacerbates the Immunosuppressive Microenvironment and Progression of Gliomas by Reducing Secretion of Ccl3 [J]. Cancer Immunol Res. 2024;12(5):516–29.
4. Ge M, Zhang W, Zhang W et al. Depression Remodels Tumor Microenvironment to Drive Tumor Progression: Bio-Behavioural Signalling Pathways and Clinical Interventions [J]. J Adv Res, 2025.
5. Renz BW, Takahashi R, Tanaka T, et al. B2 Adrenergic-Neurotrophin Feedforward Loop Promotes Pancreatic Cancer [J]. Cancer Cell. 2018;33(1):75–e907.

6. Kobayashi H, Iida T, Ochiai Y, et al. Neuro-Mesenchymal Interaction Mediated by a B2-Adrenergic Nerve Growth Factor Feedforward Loop Promotes Colorectal Cancer Progression [J]. *Cancer Discov.* 2025;15(1):202–26.
7. Taylor KR, Barron T, Hui A, et al. Glioma Synapses Recruit Mech Adapt Plast [J] *Nat.* 2023;623(7986):366–74.
8. Xu L, Chen S, Fu Y, et al. Neuro-Immune-Tumor Axis in Gliomas: A Review of Mechanisms, Models, and Translational Opportunities [J]. *Front Immunol.* 2025;16:1682322.
9. Xu N, Bian S, Lyu P, et al. Dynamic Interplay of Neuroendocrine Signaling and Immuno-Surveillance in Tumor Niche Remodeling [J]. *Crit Rev Oncol Hematol.* 2025;216:104958.
10. Xu X, Zhang Z, Du J, et al. Recruiting T-Cells toward the Brain for Enhanced Glioblastoma Immunotherapeutic Efficacy by Co-Delivery of Cytokines and Immune Checkpoint Antibodies with Macrophage-Membrane-Camouflaged Nanovesicles [J]. *Adv Mater.* 2023;35(25):e2209785.
11. Mansurov A, Ishihara J, Hosseinchi P, et al. Collagen-Binding IL-12 Enhances Tumour Inflammation and Drives the Complete Remission of Established Immunologically Cold Mouse Tumours [J]. *Nat Biomed Eng.* 2020;4(5):531–43.
12. Zhang Z, Xu X, Du J, et al. Redox-Responsive Polymer Micelles Co-Encapsulating Immune Checkpoint Inhibitors and Chemotherapeutic Agents for Glioblastoma Therapy [J]. *Nat Commun.* 2024;15(1):1118.
13. Zhu D, Zhu Y, Yang X, et al. Enhancing Tim-3 Immunotherapy with Epirubicin-Loaded Ph Sensitive Fusion Membrane Nanoparticles for Effective Glioblastoma Treatment [J]. *J Control Release.* 2025;388(Pt 2):114367.
14. Globig AM, Zhao S, Roginsky J, et al. The B(1)-Adrenergic Receptor Links Sympathetic Nerves to T Cell Exhaustion [J]. *Nature.* 2023;622(7982):383–92.
15. Zheng C, Liu S, Fazel Modares N, et al. Cholinergic T Cells Revitalize the Tumor Immune Microenvironment: Time to Chat [J]. *Nat Immunol.* 2025;26(5):665–77.
16. De Hurtado T, Mose ES, Botta GP, et al. Tumor-Penetrating Therapy for B5 Integrin-Rich Pancreas Cancer [J]. *Nat Commun.* 2021;12(1):1541.
17. Ye L, Hou Y, Hu W, et al. Repressed Blautia-Acetate Immunological Axis Underlies Breast Cancer Progression Promoted by Chronic Stress [J]. *Nat Commun.* 2023;14(1):6160.
18. Teesalu T, Sugahara KN, Kotamraju VR, et al. C-End Rule Peptides Mediate Neuropilin-1-Dependent Cell, Vascular, and Tissue Penetration [J]. *Proc Natl Acad Sci U S A.* 2009;106(38):16157–62.
19. Pan Y, Xue Q, Yang Y, et al. Glycoengineering-Based Anti-Pd-1-Irgd Peptide Conjugate Boosts Antitumor Efficacy through T cell Engagement [J]. *Cell Rep Med.* 2024;5(6):101590.
20. Zou Y, Sun X, Yang Q, et al. Blood-Brain Barrier-Penetrating Single Crispr-Cas9 Nanocapsules for Effective and Safe Glioblastoma Gene Therapy [J]. *Sci Adv.* 2022;8(16):eabm8011.
21. Vestad B, Llorente A, Neurauder A, et al. Size and Concentration Analyses of Extracellular Vesicles by Nanoparticle Tracking Analysis: A Variation Study [J]. *J Extracell Vesicles.* 2017;6(1):1344087.

22. Xiang Q, Yang X, Zhang Z, et al. Fe/Mo-Based Lipid Peroxidation Nanoamplifier Combined with Adenosine Immunometabolism Regulation to Augment Anti-Breast Cancer Immunity [J]. *Adv Mater.* 2025;37(8):e2419120.
23. Ding N, Zou Z, Sha H, et al. Irgd Synergizes with Pd-1 Knockout Immunotherapy by Enhancing Lymphocyte Infiltration in Gastric Cancer [J]. *Nat Commun.* 2019;10(1):1336.
24. Lopes M, Vieira De Castro J, Pojo M, et al. Chronic Stress Does Not Influence the Survival of Mouse Models of Glioblastoma [J]. *Front Oncol.* 2022;12:856210.
25. Zhao K, Gu J, Zhao B. Impact of Gliomas in Different Brain Hemispheres on Anxiety and the Prognosis of Neurosurgical Patients [J]. *Sci Rep.* 2025;15(1):39280.
26. Kim-Fuchs C, Le CP, Pimentel MA, et al. Chronic Stress Accelerates Pancreatic Cancer Growth and Invasion: A Critical Role for Beta-Adrenergic Signaling in the Pancreatic Microenvironment [J]. *Brain Behav Immun.* 2014;40:40–7.
27. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: Globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries [J]. *CA Cancer J Clin.* 2021;71(3):209–49.
28. Faulkner S, Jobling P, March B, et al. Tumor Neurobiology and the War of Nerves in Cancer [J]. *Cancer Discov.* 2019;9(6):702–10.
29. Silverman DA, Martinez VK, Dougherty PM, et al. Cancer-Associated Neurogenesis and Nerve-Cancer Cross-Talk [J]. *Cancer Res.* 2021;81(6):1431–40.
30. Bapat AA, Hostetter G, Von Hoff DD, et al. Perineural Invasion and Associated Pain in Pancreatic Cancer [J]. *Nat Rev Cancer.* 2011;11(10):695–707.
31. Park H, Poo MM. Neurotrophin Regulation of Neural Circuit Development and Function [J]. *Nat Rev Neurosci.* 2013;14(1):7–23.
32. Boilly B, Faulkner S, Jobling P, et al. Nerve Dependence: From Regeneration to Cancer [J]. *Cancer Cell.* 2017;31(3):342–54.
33. Chen J, Zhang Y, Liu Y, Ma J, Zhao S. Norepinephrine Promotes Glioblastoma Cell Proliferation and Invasion via the β 2-adrenergic Receptor/ERK/MMP-9 Signaling Pathway [J]. *Oncol Rep.* 2019;41:1817–26.
34. Wang X, Wang Y, Xie F, et al. Norepinephrine Promotes Glioma Cell Migration through up-Regulating the Expression of Twist1 [J]. *BMC Cancer.* 2022;22(1):213.
35. Lei Y, Tang L, Xie Y, et al. Gold Nanoclusters-Assisted Delivery of Ngf Sirna for Effective Treatment of Pancreatic Cancer [J]. *Nat Commun.* 2017;8:15130.
36. Zhu Y, Zhang G, Yang Y, et al. Perineural Invasion in Early-Stage Cervical Cancer and Its Relevance Following Surgery [J]. *Oncol Lett.* 2018;15(5):6555–61.
37. Nejo T, Krishna S, Yamamichi A, et al. Glioma-Neuronal Circuit Remodeling Induces Regional Immunosuppression [J]. *Nat Commun.* 2025;16(1):4770.
38. Shi LL, Qiao X, Hu Y et al. Niban2/Flii/Rreb1 Axis Drives Glioma Stem Cell Malignancy Via Tlr3 Pathway Activation [J]. *Adv Sci (Weinh)*, 2026: e19382.

39. Gonzales CN, Negussie MB, Krishna S, et al. Malignant Glioma Remodeling of Neuronal Circuits: Therapeutic Opportunities and Repurposing of Antiepileptic Drugs [J]. Trends Cancer. 2024;10(12):1106–15.
40. Qian J, Zhou S, Lin P, et al. Recent Advances in the Tumor-Penetrating Peptide Internalizing Rgd for Cancer Treatment and Diagnosis [J]. Drug Dev Res. 2023;84(4):654–70.
41. He Z, Liu Z, Wang Q, et al. Single-Cell and Spatial Transcriptome Assays Reveal Heterogeneity in Gliomas through Stress Responses and Pathway Alterations [J]. Front Immunol. 2024;15:1452172.

Figures

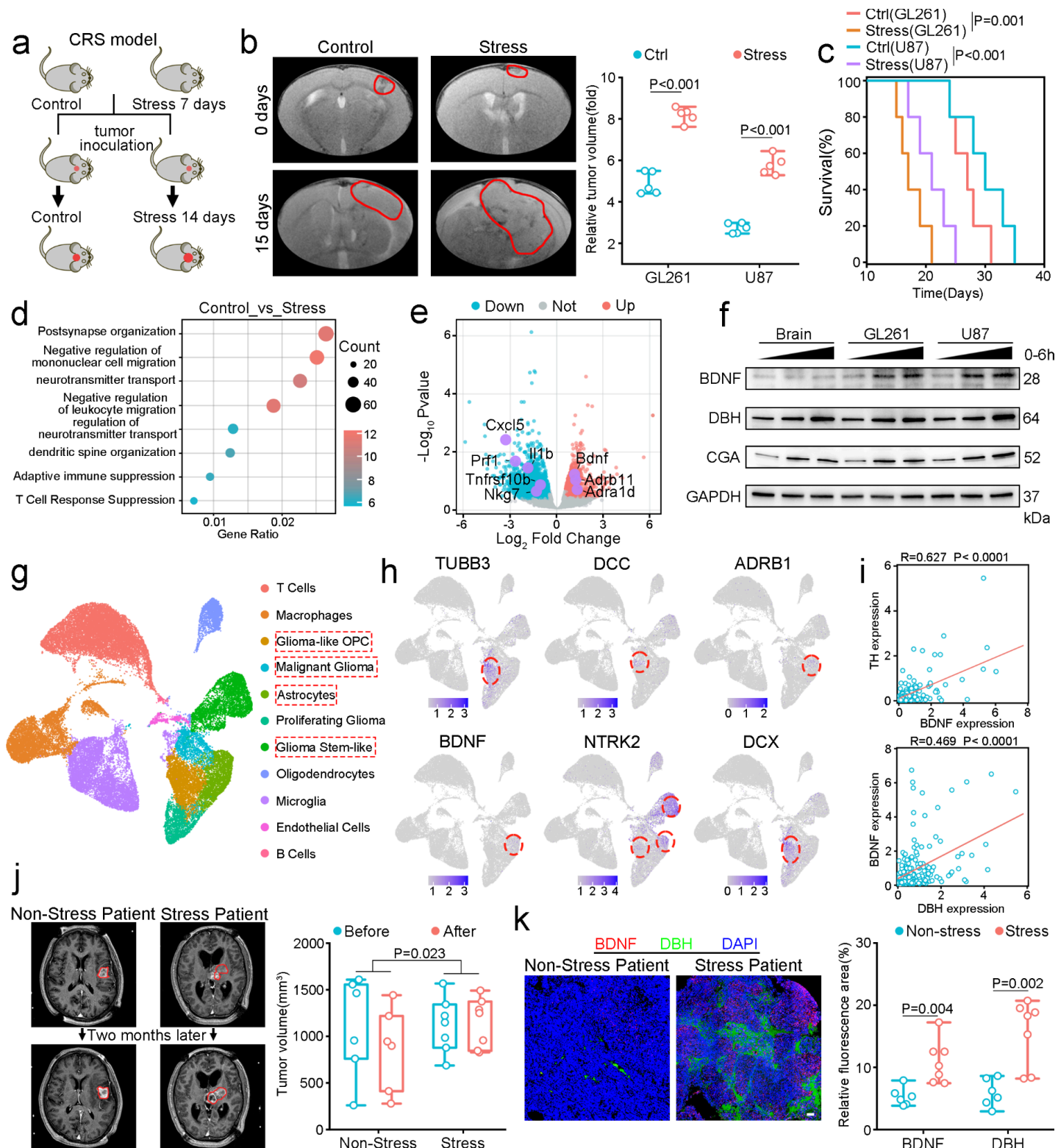


Figure 1

Chronic stress promotes glioma progression in mouse models. a-c, MRI monitoring and survival analysis of GL261 and U87 glioma-bearing mice subjected to chronic restraint stress (CRS) for 21 days, showing accelerated tumor growth and shortened survival compared to non-stressed controls (n=5). d-e, Transcriptomic analysis of GL261 tumors under chronic stress. GO show enrichment of neuro-related pathways and suppression of immune pathways. (d), GO and GSEA show enrichment of neuro-related

pathways and suppression of immune pathways. (e), DEG analysis reveals upregulation of β -adrenergic signaling genes and *Bdnf*, and downregulation of immune effector molecules (*Prf1*, *Nkg7*, *Il1b*). f, Western blot analysis of BDNF, DBH, and CGA expression in tumor tissues under chronic stress. BDNF showed time-dependent upregulation specifically in glioma tissues, while DBH and CGA levels progressively increased with stress duration. g, UMAP visualization of single-cell transcriptomes from 11 glioma samples, revealing a neuronal population co-expressing *TUBB3* and *DCC*. h, Expression profiles demonstrate that a subset of these neurons express *DCX* and the BDNF receptor *NTRK2*, implicating BDNF signaling in tumor-associated neurogenesis. i, TCGA database analysis reveals strong positive correlations of TH nerve fiber density and DBH expression with BDNF expression in glioma patients. j, MRI follow-up of GBM patients stratified by stress level (HAMD-17 score). Tumor volume regression at approximately two months after chemotherapy is significantly lower in the stressed group compared to the non-stressed group. k, Immunofluorescence staining of tumor tissues from stressed versus non-stressed patients showing significantly elevated expression of BDNF and DBH in the stressed group. Scale bar, 50 μ m. Data are representative of three independent experiments. Data are presented as mean $\pm$ SEM. Statistical significance was determined by unpaired two-tailed Student's t-test or log-rank test. Exact P values are indicated in the figures.

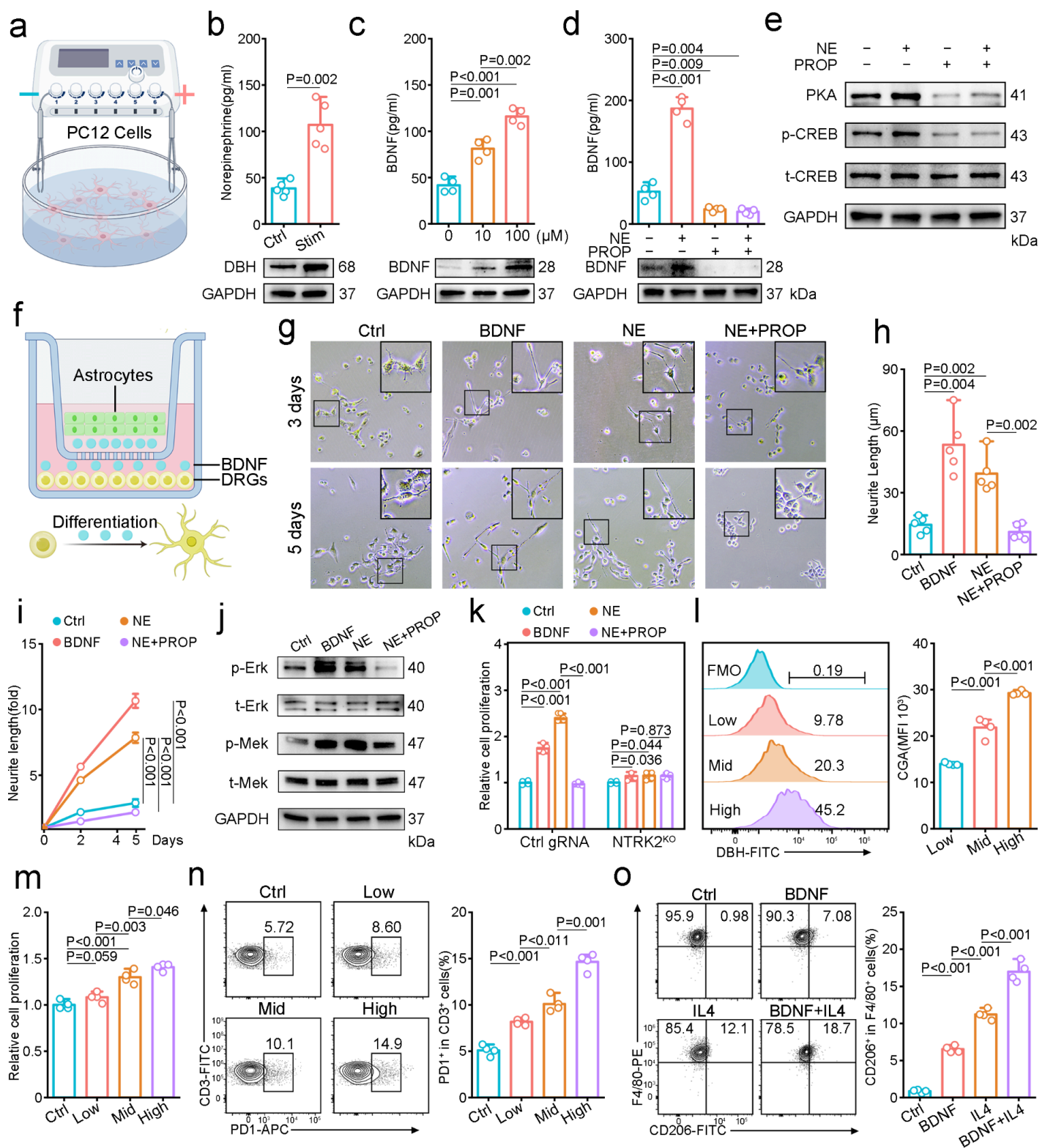


Figure 2

NE-BDNF axis drives neuron-astrocyte crosstalk, promoting glioma cell proliferation and immunosuppression.

a, Schematic of the in vitro coculture system using electrically stimulated PC12 cells to model stress-activated neurons. **b**, NE secretion (ELISA, top) and DBH expression (Western blot, bottom) in PC12 cells with/without electrical stimulation. **c**, BDNF secretion (ELISA, top) and expression (Western blot, bottom) in C8-D1A astrocytes treated with increasing NE concentrations. **d**, BDNF

secretion/expression (top/bottom) in C8-D1A cells treated with 10 μ M NE $\pm$ propranolol (n=4). e, Western blot analysis of PKA and p-CREB/t-CREB phosphorylation in C8-D1A astrocytes treated with 10 μ M NE $\pm$ propranolol (corresponding to Fig. d). f-i, Assessment of neurite outgrowth in DRG and C8-D1A coculture model. f, Schematic illustration of the experimental setup. g, Representative immunofluorescence images of DRG neurite outgrowth under indicated treatments. Scale bar, 10 μ m. h, Quantification of neurite length under control, NE, and NE + propranolol conditions (n=5). i, Time course analysis of neurite outgrowth measured on days 0, 2, and 5 (n=5). j, Western blot analysis of ERK and MEK phosphorylation in the underlying DRG cells from the coculture system under indicated treatments. k, CCK-8 assay of GL261 cell viability in coculture with C8-D1A astrocytes. l, Flow cytometry analysis of DBH expression in PC12 cells at low, medium, and high differentiation states. DBH levels increased with differentiation (n=4). m, CCK-8 assay of GL261 cell viability after coculture with PC12 cells at indicated differentiation states (n=4). n-o, Flow cytometry analysis. n, PD1+ T cells after coculture with PC12 cells at indicated differentiation states (n=4). o, CD206+ M2 macrophages in BMDMs treated with BDNF. BDNF significantly induced M2 polarization (n=4). Data are mean $\pm$ SEM. Statistical significance was assessed by unpaired t-test or one-way ANOVA followed by post hoc test (for multiple groups). Exact P values are shown.

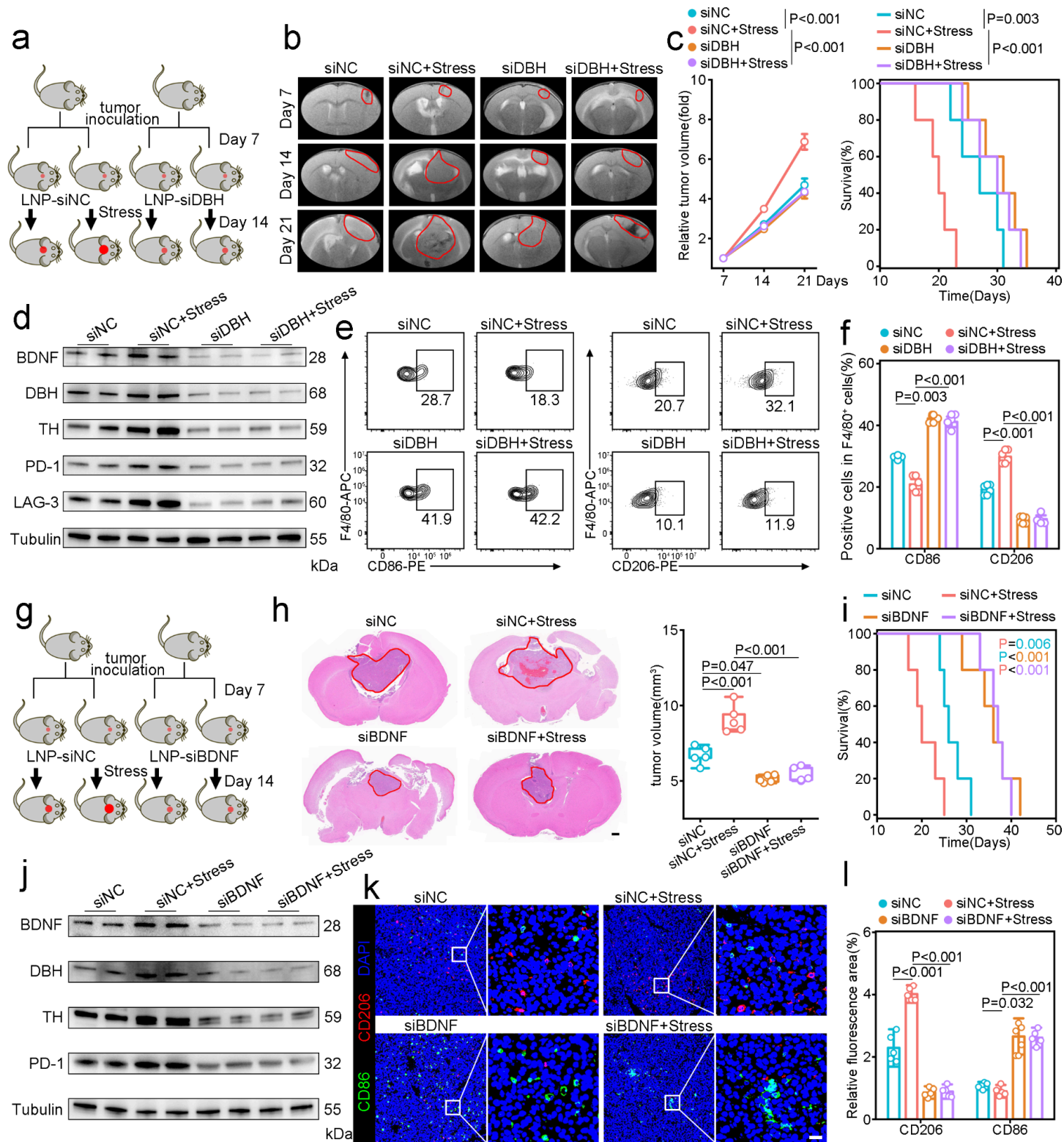


Figure 3

In vivo validation of targeting the NE-BDNF axis suppresses glioma growth and restores antitumor immunity. a, Schematic illustration of the experimental design. GL261 tumor-bearing mice were subjected to chronic restraint stress and received intravenous injections of LNP-siNC or LNP-siDBH on days 7, 12, 17 post-tumor implantation. b, MRI monitoring and survival analysis of tumors from each treatment group on days 7, 14, and 21 post-implantation. c, Left: Quantification of tumor growth kinetics

presented as fold change relative to day 7. Right: Kaplan–Meier survival curves of mice in each group (n=5). d, Western blot analysis of BDNF, DBH, TH, PD-1, and LAG-3 expression in tumor tissues. e-f, Flow cytometric analysis of tumor-associated macrophages. e, Representative CD86+ (M1) and CD206+ (M2) staining. f, Quantification of M1 and M2 ratio. g, Schematic illustration of BDNF silencing experiment. h, H&E staining (left) and tumor size quantification (right) on day 23. i, Kaplan–Meier survival curves of mice in each group (n=5). j, Western blot analysis of BDNF, DBH, TH, PD-1, and LAG-3 expression in tumor tissues. k-l, Immunofluorescence staining of macrophage markers. k, Representative images of CD86+ (M1) and CD206+ (M2) in tumor sections. Scale bar, 50 μ m. Quantification of fluorescence intensity in tumor tissues. Data are presented as mean $\pm$ SEM. Statistical significance was determined by unpaired two-tailed Student's t-test or log-rank test. Exact P values are indicated in the figures.

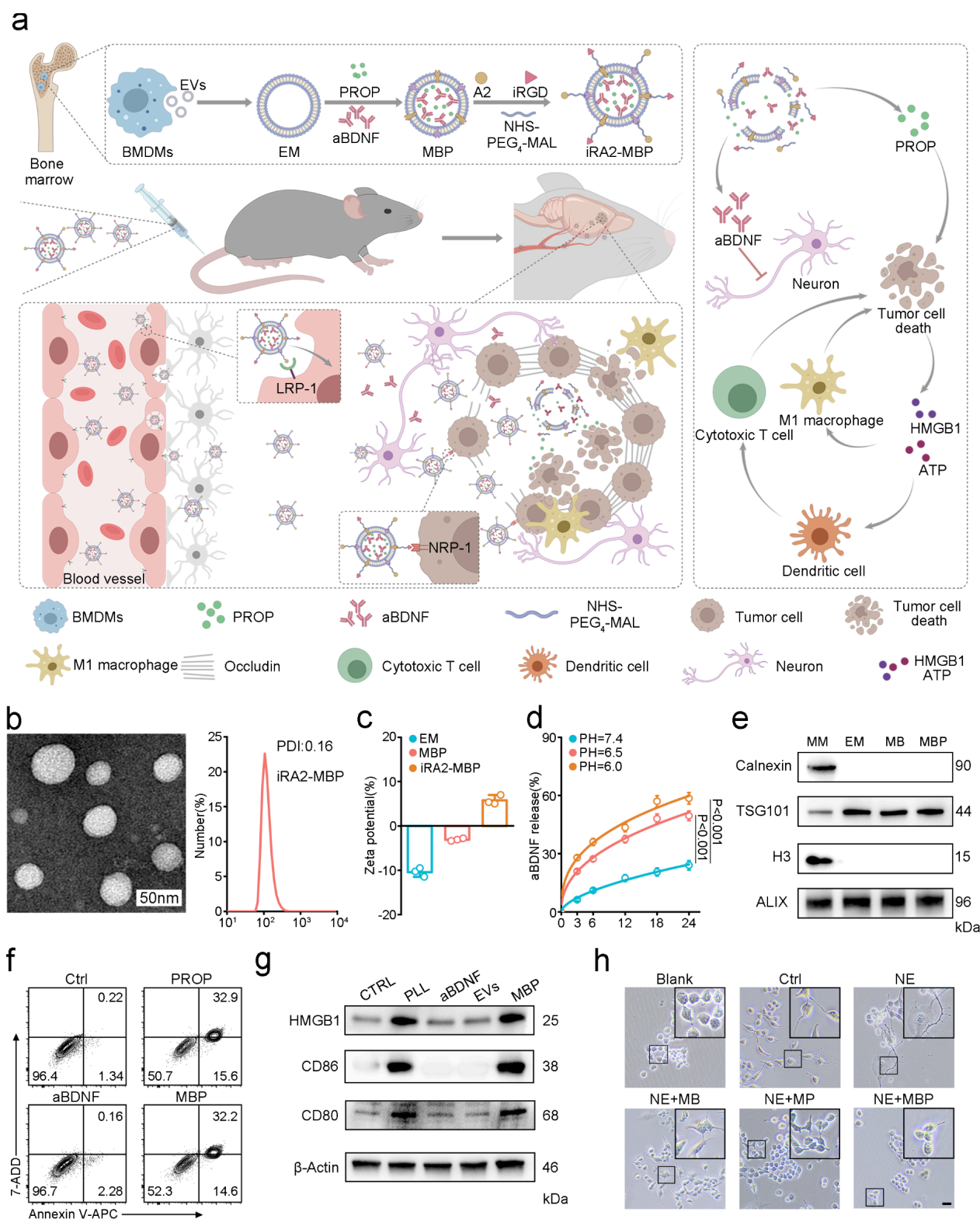


Figure 4

Preparation and characterization of iRA2-MBP. a, Schematic showing iRA2-MBP preparation and its dual-targeting mechanism for BBB penetration and deep tumor infiltration. b, Representative transmission electron microscopy (TEM) image (left) and dynamic light scattering (DLS) size distribution (right) of iRA2-MBP. c, Zeta potential analysis of EM, MBP, and iRA2-MBP. d, In vitro release profile of aBDNF from iRA2-MBP under different pH conditions (7.4, 6.5, and 6.0) over 24 hours. e, Western blot

analysis of calnexin (endoplasmic reticulum marker), TSG101 (exosomal marker), and histone H3 (nuclear protein) in macrophage membrane (MM), exosomes (EM), MBP, and iRA2-MBP. f, Flow cytometry analysis of GL261 cell apoptosis after treatments. g, Western blot analysis of HMGB1, CD86, and CD80 expression in the GL261 and BMDM coculture model under treatments. h, Representative microscopy images of DRG neurite outgrowth in coculture with C8-D1A astrocytes under treatments. Scale bar, 20 μm . Data are presented as mean $\pm$ SEM. Statistical significance was determined by one-way ANOVA. Exact P values are indicated.

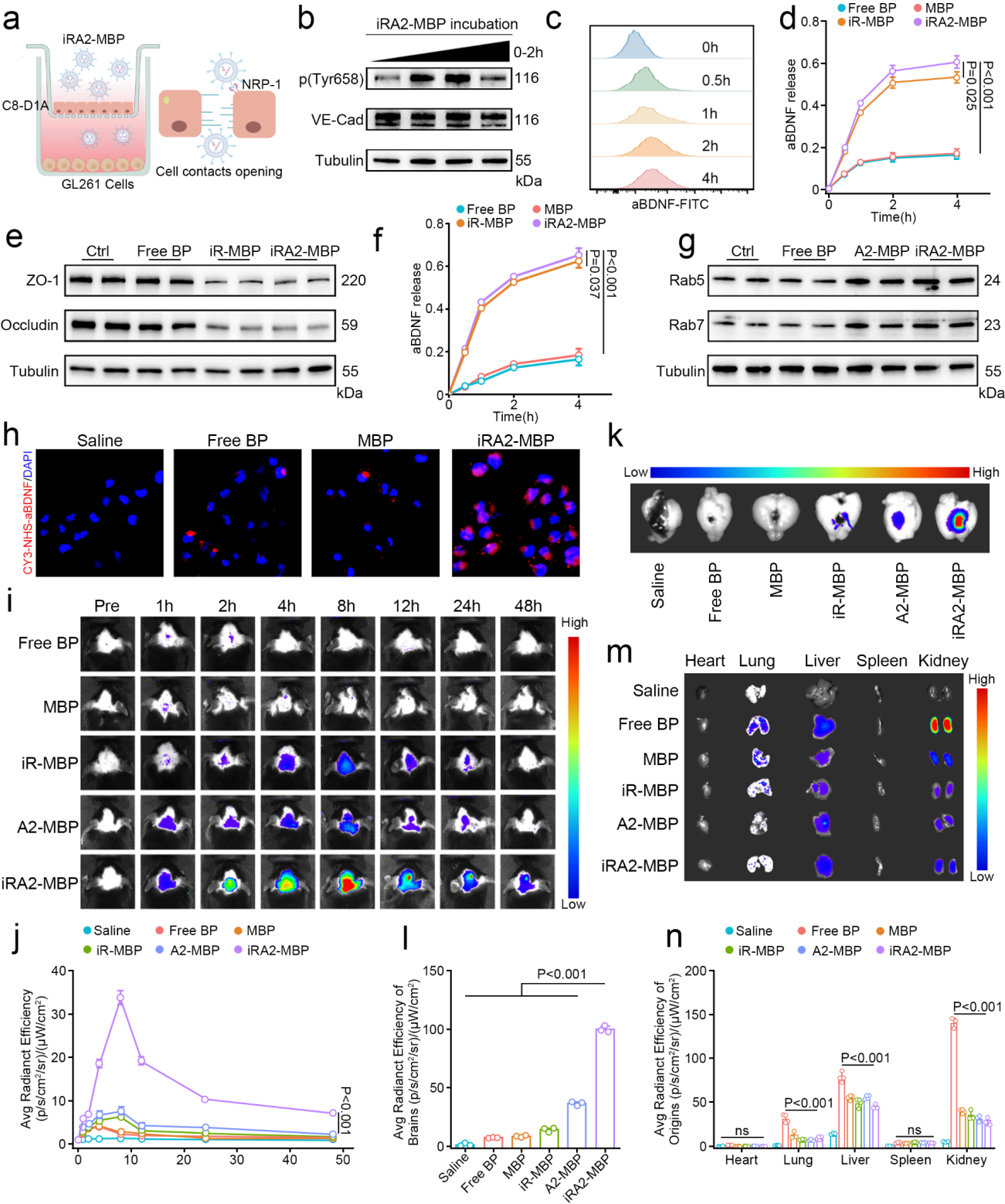


Figure 5

Glioma-targeting evaluation of iRA2-MBP in vitro and in vivo. a, Schematic illustration of the proposed mechanism by which iRGD facilitates barrier penetration. b, Western blot analysis of VE-cadherin phosphorylation in C8-D1A cells at indicated time points after treatment. c-d, Time-dependent increase and the accumulation of fluorescence intensity for 4 h in basolateral chamber cultured with aBDNF-encapsulated formulations (n = 3). e, Western blot of ZO-1 and Occludin in C8-D1A cells. iRGD downregulated their expression, confirming tight junction opening. f, Time-dependent fluorescence intensity and cumulative penetration of aBDNF-loaded formulations in the basal chamber over 4 hours. g, Western blot analysis of Rab5 and Rab7 expression in bEnd.3 cells after indicated treatments. h, CLSM images of GL261 cells in the lower chamber showing uptake of CY5-labeled aBDNF formulations (n = 3). i, Time course of in vivo fluorescence imaging signal intensity at the tumor site in GL261 glioma-bearing mice after intravenous injection of Free BP, MBP, or iRA2-MBP. Images were acquired at 1, 2, 4, 6, 8, 12, 24, and 48 hours post-injection (n = 4). j, Quantification of fluorescence signal intensity at the tumor site from the IVIS imaging in (i) at indicated time points (n = 3). k, Ex vivo fluorescence imaging of brains isolated from glioma-bearing mice 48 hours after intravenous injection of indicated formulations. l, Quantification of ex vivo fluorescence intensity in tumor-bearing brains from (k) (n = 3). m, Ex vivo fluorescence images of major organs (heart, liver, spleen, lungs, kidneys) harvested from glioma-bearing mice 48 hours after intravenous injection of indicated formulations. n, Quantification of fluorescence intensity in each organ from (m) (n = 3). Data are presented as mean $\pm$ SEM. Statistical significance was determined by one-way ANOVA. Exact P values are indicated.

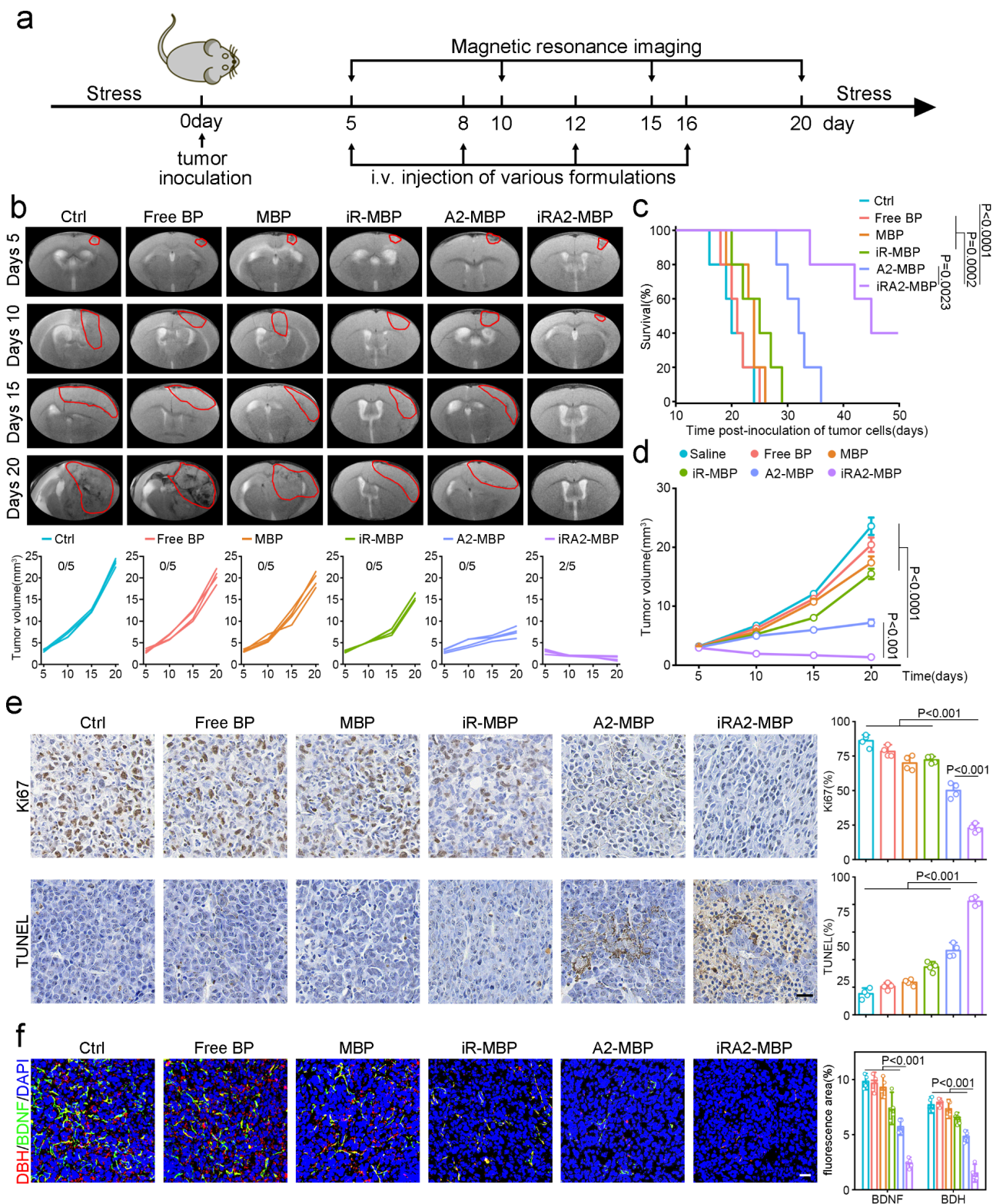


Figure 6

In vivo therapeutic effect of iRA2-MBP on GL261 tumor-bearing mice under chronic stress. a, Schematic diagram of the treatment regimen. b-c, MRI monitoring and survival analysis of tumors from indicated groups (PBS, Free BP, MBP, iR-MBP, A2-MBP, and iRA2-MBP) on day 21 post-implantation and quantification of tumor growth ($n = 5$). d, Kaplan–Meier survival curves of GL261 tumor-bearing mice in indicated groups under chronic stress ($n = 5$). e, IHC staining of Ki67 and TUNEL in tumor tissues and

quantification (n = 4). f, Immunofluorescence co-staining of DBH and BDNF in tumor tissues from indicated groups. Scale bar, 50 μ m (n = 4). Data are presented as mean $\pm$ SEM. For continuous variables, statistical significance was determined by one-way ANOVA, for survival data, the log-rank test was used. Exact P values are indicated.

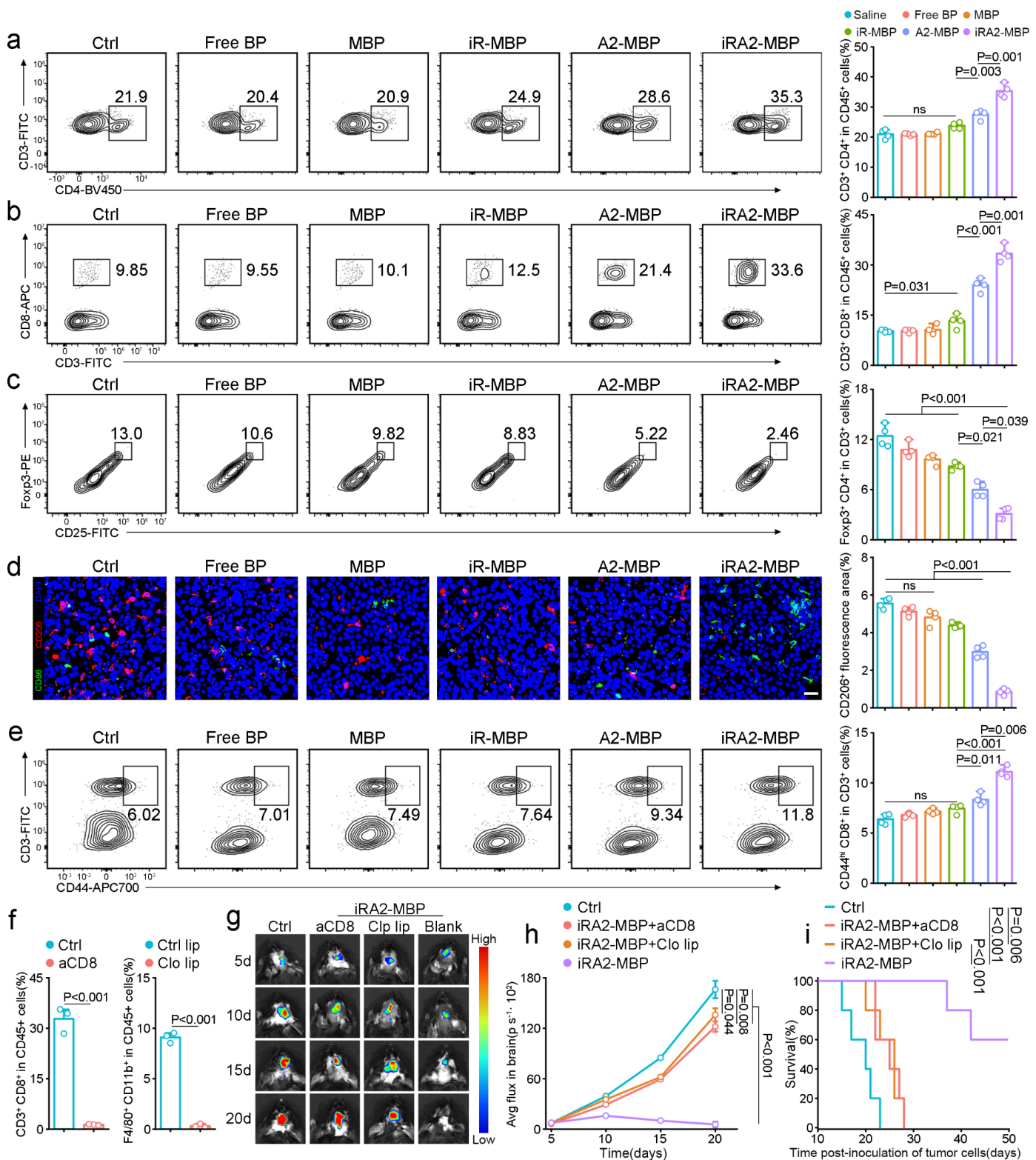


Figure 7

iRA2-MBP reverses immunosuppression and remodels the immune microenvironment in stressed GBM. a-b, Flow cytometry analysis of tumor-infiltrating T cells in indicated groups under chronic stress. a, Representative plots and quantification of CD4⁺ T cells (n=4). b, Representative plots and quantification of CD8⁺ T cells (n=4). c, Flow cytometry analysis of regulatory T cells (Tregs, Foxp3⁺ CD25⁺) in tumors from groups (n=4). d, Immunofluorescence co-staining of CD86⁺ and CD206⁺ macrophage in tumor tissues from indicated groups (n=4). Scale bar, 50 μ m. e, Flow cytometry analysis of effector memory T cells (TEM, CD44⁺) in tumors from indicated groups (left) and quantification (right) (n=4). f, Flow cytometry validation of immune cell depletion efficiency in spleens after anti-CD8 antibody (aCD8) or clodronate liposome (Clo lip) treatment (n=3). g, IVIS images of stressed glioma-bearing mice treated with iRA2-MBP $\pm$ aCD8 or Clo lip. h, Quantification of fluorescence signal intensity from IVIS imaging in (g) (n=5). i, Kaplan–Meier survival curves of stressed glioma-bearing mice treated with iRA2-MBP alone or in combination with aCD8 or Clo lip (n=5). Data are mean $\pm$ SEM. Statistical significance was assessed by unpaired t-test or one-way ANOVA followed by post hoc test. Exact P values are shown.

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