Supplemental Methods

Clinical specimens

A total of 8 human normal brain tissues (NBTs) and 56 glioma tissue specimens were used in this study. All specimens were collected at the Department of Neurosurgery, Shengjing Hospital of China Medical University. Specimens were placed in liquid nitrogen for cryopreservation immediately after surgical resection. NBTs were collected from patients with severe brain trauma, cerebral hemorrhage, or epilepsy and used as negative controls. All glioma specimens were histologically graded by two experienced clinical pathologists according to the World Health Organization guidelines for tumors of the CNS (2021) and divided into four groups according to the grade: 1. Grade I (WHO I, n=6); 2. Grade II (WHO II, n=10); 3. Grade III (WHO III, n=14); 4. Grade IV (WHO IV, n=26). All specimens used in this study were approved by the Ethics Committee of the Shengjing Hospital of China Medical University.

Cell culture

Normal human astrocytes (NHA) were purchased from Sciencell Research Laboratories (Carlsbad, CA, USA). Human bone marrow mesenchymal stem cells (hBMSCs) were purchased from Shenyang Cell Center. Human glioma cell lines U251 and U373 and human embryonic kidney cell line HEK-293T were purchased from the Cell Resource Center of Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences. All cell lines were authenticated by short tandem repeat (STR) profiling and confirmed to be mycoplasma negative before the beginning of the study. NHA cells and hBMSCs were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and Dulbecco's modified eagle medium/nutrient mixture F-12 (DMEM/F12) medium supplemented with 10% FBS (Thermo Fisher Scientific, USA), respectively; human glioma cell lines U251 and U373 and human embryonic kidney cell lines HEK-293T were cultured in DMEM high glucose medium supplemented with 10% FBS at 37 °C in an atmosphere containing 5%CO_2.

Cell transfection

The miR-1208 overexpression and its negative control lentivirus were purchased from GenePharma (Shanghai, China). The METTL3 overexpression and its negative control plasmids were constructed by GenScript (Piscataway, NJ, USA). The short-hairpin RNA (shRNA) against METTL3, NUP214 and IGF2BP2 (every gene designed three target sequences) and their negative controls were purchased from GenePharma. The shRNA template sequence was provided in Table.S1. In pre-experiments, sh-METTL3-1, sh-NUP214-2, and sh-IGF2BP2-1 were the target sequences with the highest knockdown efficiency screened by western blot after 48 h of transient transfection (results showed in Fig.S6), then the stable transfection was conducted with the above plasmids. Cells with good growth conditions were seeded into 24-well plates. When the cell confluency reached about 50-60%, the cells were transfected with corresponding lentiviruses or plasmids according to the optimal infection conditions determined in the pre-experiment. Media were replaced with fresh complete medium 12 h after transfection, and cells were cultured for 48 h. The fluorescence expression of the cells was observed under a fluorescence microscope. G418, puromycin, and hygromycin (Sigma-Aldrich, USA) were used to further screen the cells and establish stable transfection cell lines.

Separation, extraction, and identification of Exos

When cultured hBMSCs reached 60–70% confluence, the original culture medium was discarded; the cells were washed thrice with phosphate buffered saline (PBS) and cultured in Exos-free serum medium for 48 h, and the cell supernatant was collected. The cell supernatant was centrifuged at 300 × *g* for 10 min, 2000 × *g* for 20 min, and 10000 ×*g* for 30 min, and the supernatant was subsequently transferred into a new centrifuge tube. The precipitation was discarded, and the supernatant was centrifuged at 100000 × *g* for 70 min. Next, the supernatant was discarded, and 1 ml of PBS was added to resuspend the precipitation. The suspended precipitates in multiple tubes were transferred into new centrifugal tubes to form pairs of sample tubes which were

centrifuged at 100,000 *g* for 70 min. The supernatant was then discarded, and the pellet was resuspended in the desired amount of PBS and stored in aliquots at -80°C for later use. The properties of Exos were confirmed by performing transmission electron microscopy (TEM), NTA, and western blot analysis on the obtained Exos and detecting the expression of Exos-specific markers TSG101, CD81, CD9, and calnexin.

RNA extraction, reverse transcription, and qRT-PCR

Total RNA was extracted from clinically obtained tissue samples, NHA, U251 and U373 cell lines using the Trizol reagent, and RNA in Exos was extracted using the Trizol LS reagent (Thermo Fisher Scientific, USA). The obtained RNA samples were diluted to determine the RNA concentration and OD260/OD280 value. The RNA samples can be directly reverse transcribed or stored in a -80°C refrigerator for later use. The RNA was reverse transcribed into cDNA using the reverse transcription kit of TaKaRa company, and the reaction system and reaction conditions were configured according to the manufacturer's instructions. Real-time quantitative PCR analysis was performed using the TB Green® Fluorescence Quantitative PCR Kit of TaKaRa Company. The primers are exhibited in Table S2. U6 was used as the endogenous control, the average ΔCt of the control group was calculated, and the relative expression levels of each detection indicators were calculated by the $2^{-\Delta\Delta Ct}$ assay.

Western blot analysis

Each sample was lysed on ice using RIPA protein lysis buffer containing 1% PMSF. After the mixture was centrifuged at 14,000 × *g* for 50 min at 4 °C, the supernatant was aspirated to obtain total protein. The nuclear protein of the cell samples was extracted using the nuclear protein extraction kit of Thermo Company according to the manufacturer's instructions. The protein concentration was determined using the BCA method. The same amount of protein samples were loaded, separated on the SDS-PAGE gel via electrophoresis, and transferred to the poly vinylidene fluoride (PVDF) membrane (Millipore, Shanghai, China). After transfer, the membrane was

quickly washed and placed in tris buffered saline with tween (TBST) solution containing 5% nonfat milk for blocking at room temperature for 2 h. After blocking, the PVDF membrane was removed from the solution and immersed in the primary antibody (METTL3, 1:1000, abcam, ab195352, USA; NUP214, 1:1000, abcam, ab70497, USA; IGF2BP2, 1:1000, abcam, ab128175, USA; p-Smad2, 1:1000, abcam, ab280888, USA; p-Smad3, 1:1000, abcam, ab52903, USA; GAPDH, 1:5000, Proteintech, 10494–1-AP, USA; TBP, 1:500, Proteintech, 22006-1-AP, USA) working solution for overnight incubation at 4 °C on a shaker. After the primary antibody incubation, the membrane was washed thrice with TBST buffer for 10 min each and incubated with goat anti-rabbit secondary antibody for 2 h on a shaker at room temperature. After the secondary antibody incubation, the membrane was washed again with TBST buffer three times for 10 min each. The ECL luminescent solution (Beyotime, China) was dropped on the surface of the PVDF membrane, and the visualized protein bands were analyzed after the chemiluminescence imaging system was used to emit light. Finally, the relative expression levels of the corresponding detection indicators in each protein sample were calculated.

Cell counting kit-8 (CCK-8) assay

Cultured U251 and U373 cells were prepared into single cell suspensions and seeded in 96-well plates at a density of 100 μl per well (2000 cells). Cells were treated correspondingly according to different experimental conditions in each group. Five replicate wells were set up in each experimental group, and all experiments were repeated independently at least three times. The CCK-8 kit (Beyotime, China) was used to detect the cell viability after 48 h; CCK-8 solution (10 μl) was added to each well; the cells were then incubated at 37°C for 2 h; the optical density (OD) of each well was measured at 450nm,and cell viability was calculated.

Clone formation experiments

The cells of each experimental group in a good growth state were prepared into single cell suspension, seeded in a 60 mm petri dish at a density of 1000 cells per dish, and cultured in complete medium supplemented with 30% FBS. The cells were monitored,

and the medium was changed once every three days. The petri dishes were collected when the number of cells in most single clones was greater than 100. Cultured cells were fixed and stained with crystal violet, washed with ddH_2O for several times, dried, and photographed to count the clones. Three independent experiments were conducted for each experimental group.

Cell migration and invasion experiments

In vitro migration and invasion abilities of cells were detected using Transwell chambers (Costar, Corning, USA), with a diameter of 6.5 mm and a pore size of 8.0 μm. In the migration experiment, the cells of each experimental group in a good growth state were digested with trypsin, and the cell density was adjusted to 5×10^5 cells/mL after counting. Cells were subsequently pipetted into a single cell suspension with serum-free medium. Next, 100 μl of the suspension was added to the upper chamber and 600 μl of complete culture medium containing 10% FBS was added to the lower chamber, following which the cells were cultured in a constant temperature incubator at 37°C with 5% CO_2. After 48 h, the chamber was retrieved, the culture medium of the upper chamber was discarded, and the chamber was fixed after PBS washing. After fixation for 30 min, the non-migratory cells on the upper surface of the membrane were scraped off with cotton swabs. After being completely dried, the cells were dyed in 10% Giemsa stain solution for 60 min. After being washed with PBS, the chamber was placed under an inverted microscope to observe and count the number of transmembrane cells in 5 random high-power fields, and the average value was calculated. Three independent experiments were repeatedly performed for each experimental group. In the invasion assay, the steps were similar to the migration assay, except that the chambers need to be pretreated. The polycarbonate membrane on the upper surface of the chamber was covered with Matrigel solution (BD biosciences, USA) with a concentration of 500 ng/μl and incubated at 37 °C for 4 h. The subsequent steps were the same as in the migration experiment.

Apoptosis assay

Cells in each experimental group were collected and double-stained with Annexin

V-PE/7-AAD using the Annexin V-PE/7-AAD double staining apoptosis kit (BD biosciences, USA) according to the manufacturer's instructions. Sample detection were performed using a flow cytometer and experimental data was analyzed.

Immunohistochemical (IHC) experiments

Fresh tissue was fixed with 4% paraformaldehyde for 48 h and immunohistochemistry was performed using the Ki-67 antibody (1:10000, Proteintech, 27309-1-AP, USA) and IHC Kit (MXB, China) according to the manufacturer's instructions. Briefly, paraffin-embedded sections were deparaffinized in different concentrations of alcohol and xylene using an automatic dewaxing machine, and then heated in Tris-EDTA antigen retrieval solution for antigen retrieval. After cooling, operations were conducted according to the steps of IHC Kit. Finally, DAB was used for color development; slides were counterstained with hematoxylin, dehydrated, and mounted for imaging under a microscope.

Dual-luciferase reporter gene assay

TargetScan online bioinformatics software was used to predict the potential binding sites of miR-1208 and METTL3. The m^6A binding site of 3'UTR of NUP214 mRNA was predicted by m^6A bioinformatics analyzing software SRAMP and RMVar. METTL3 3'UTR wild-type and mutant plasmid were constructed using pmirGLO vector, and NUP214 3'UTR wild-type and mutant double luciferase plasmids were purchased from Genscript Company. The day before the experiment, HEK-293 or U251 cells were seeded in a 96-well plate. The cells were observed to grow well and achieve about 70-80% confluence on the day of transfection. The dual-luciferase empty plasmid and the corresponding wild-type or mutant dual-luciferase plasmid were co-transfected with miR-1208 mimics and their negative controls or sh-NC and sh-METTL3; after 48 h of culture, the luciferase activity was detected by a dual-luciferase reporter gene detection system (Promega, USA).

RNA-binding protein immunoprecipitation assay (RIP)

The RIP experiment was performed using the EZ-Magna RIP kit (Millipore, USA)

according to the manufacturer's instructions. After glioma cells were lysed with RNA lysis buffer, the whole cell lysate was collected and incubated with RIP buffer containing human anti-IGF2BP2 antibody and magnetic beads with gentle shaking overnight. The anti-IgG group was used as the control group. The samples were incubated and treated with protease K, followed by RNA concentration determination with a spectrophotometer. Finally, the resulting RNA was purified and tested for binding using qRT-PCR.

RNA methylation co-immunoprecipitation (MeRIP)

The MeRIP Kit (BersinBio, China) was used to carry out the experiment according to the manufacturer's instructions. First, 2×10^7 of glioma cells were collected and total RNA was extracted, then the RNA samples were fragmented into fragments of about 100 nt long, and the fragmented samples were divided into Input group, IP group and IgG group. A total of 5 μg of anti-m^6A antibody was added to the IP group, and 5 μg of anti-IgG antibody was added to the IgG group, and the mixtures were placed in a vertical mixer at 4 °C and incubated for 2-4 h. The prepared protein A/G magnetic beads were mixed and incubated with antibody hybrid solutions of IP or IgG group. After incubation, the sample was digested with proteinase K. Finally, the magnetic beads were collected and the supernatant was transferred to a new RNase-free tube. Finally, the resulting RNA was purified and tested for binding using qRT-PCR.

RNA stability detection

RNA stability assays were performed using actinomycin D according to the manufacturer's instructions. HEK293 cells were transfected with METTL3 silencing plasmid or its negative control plasmid, and treated with actinomycin D (final concentration: 5 mg/ml) or DMSO. The total RNA of cells was extracted and analyzed by qRT-PCR at 0, 2, 4, 6, 8, and 10 h after transfection.

Nascent RNA capture assay

The expression of NUP214 nascent RNA was detected using the Click-iT® Nascent RNA Capture Kit according to the manufacturer's instructions. First, nascent RNA in

cells was labeled with 0. 2 mM EU, and the cells were harvested after 4 h of incubation at room temperature, and total RNA was extracted. The Click-iT RNA reaction mixture was prepared and mixed with UltraPure™ glycogen, ammonium acetate and 75% ethanol, and the mixture was incubated overnight (-20°C, 12–16 h). After incubation, the supernatant was discarded after centrifugation, air-dried, and 30 μl of DEPC water was added and the RNA concentration was measured. The mixture was prepared by mixing 125 μl of Click-iT RNA reaction buffer, 2μl of RNAseOUT™ RNA recombinant ribonuclease inhibitor, 1μg of RNA and DEPC water, mixed well, and incubated at 70°C for 5 min. The magnetic beads were fixed using DynaMag™-2 magnets, and the SuperScript ® enzyme mixture was added and heated at 70°C; the cDNA was released from the magnetic beads by intermittent vortex oscillation. Finally, the supernatant was collected by centrifugation, and the expression of nascent RNA was detected using qRT-PCR.

EB extravasation assay and *In vivo* experiment on Exos combined with FUS on anti-tumor effect in nude mice

Female BALB/c nude mice (6 weeks old) were purchased from Vital River Laboratory Animal Technology (China) and housed in SPF-grade animal facility. All animal experiments were approved by the Laboratory Animal Management Committee of Shengjing Hospital of China Medical University. The experimental animals were randomly divided into two groups (control and FUS groups) with five animals in each group. Nude mice were subjected to abdominal anesthesia with 2% pentobarbital sodium and fixed to a stereotaxic brain locator. In the FUS group, FUS irradiation was performed after microbubbles (MBs, Bracco SonoVue®, Italy) were injected into the tail vein of nude mice. Ultrasonic parameters were set at a frequency of 650 KHz, an intensity of 1 W, and a duration time of 1 min. After irradiation, 2% Evans blue (EB) solution was injected, and only EB solution was injected in the control group. The nude mice were sacrificed 1 h later, and the brain was collected after perfusion. The successful opening of BBB was confirmed by observing EB exudation of brain tissue. The brain tissue was embedded and sectioned, and the safety was evaluated using hematoxylin and eosin (HE) staining.

Glioma U251 cells were transfected with Luciferase lentivirus to establish U251 stably transfected cell line with Luciferase tag. The orthotopic tumor model of nude mice was established by injecting 1×10^6 cells into the right striatum of nude mice. Seven days later, the xenograft tumor nude mice models were randomly divided into five groups, with five mice in each group. Each experimental group was treated accordingly every three days, and 200 μl of PBS or Exos (1500 μg/ml) was injected into the tail vein of nude mice until the nude mice died. The two groups combined with FUS were injected with Exos after BBB opening and irradiated with FUS again 12 h later. The tumor growth was dynamically monitored by the small animal *in vivo* imaging system every five days, and the survival of nude mice was recorded daily. Survival analysis was performed using Kaplan–Meier survival curve, and HE staining and Ki-67 immunohistochemical experiments were performed on tumor tissues of each experimental group.

Xenograft experiment in nude mouse

In the Xenograft subcutaneous tumor experiment in nude mouse, the experimental animals were randomly divided into five groups with five mice in each group. The corresponding U251 or U373 cell suspensions (200 μl of PBS containing 3×10^5 cells) were planted in the right armpit of nude mice. The growth of nude mice and changes of tumor sites were observed, and the tumor volumes were measured and recorded every four days. Tumor volume was calculated as follows: volume (mm^3) $= 0.5 \times$ (longest diameter) $\times$ (shortest diameter)^2, and the data were statistically analyzed after 44 days.

The grouping of orthotopic tumor transplantation experiment in nude mice is consistent with that of the subcutaneous experiment. Approximately 1×10^6 cells were injected into the right striatum of nude mice using a brain stereotaxic instrument (the death of nude mice within seven days of injection was considered not related to tumor formation, and new nude mice would be added). The survival of nude mice was recorded daily, and the mice were sacrificed on the 50^{th} day, and the Kaplan–Meier

278 survival curve was used for analysis.

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281 Committee of Shengjing Hospital of China Medical University.