Supplementary Material

Title: HMGB1-induced p62 overexpression promotes Snail-mediated epithelial–mesenchymal transition in glioblastoma cells via the degradation of GSK-3β

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Supplementary Methods

Reagents

Calcium phosphate coprecipitation method was used to transfect HEK 293T cells by using Mammalian Cell Transfection Kit (Chemicon International, Temecula, CA), while GBM cells were transfected with Lipofectamine (cat. no. 11668-019; Invitrogen, Carlsbad, CA, USA). Meanwhile, a stock solution of 100 mM rhHMGB1 (cat. no. T2577; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethylsulfoxide (DMSO; cat. no. D2650; Sigma-Aldrich) and stored at -20 °C. CQ (Sigma, C6628) was dissolved in sterile phosphate-buffered saline (PBS; Sigma, 08057) at a final concentration of 50 μM. All kinase inhibitors such as p38 MAPK inhibitor SB203580, JNK inhibitor SP600125, MEK inhibitor PD98059 and PI3K inhibitor LY294002 were obtained from Sigma-Aldrich and were added to the medium 24 h prior to rhHMGB1 treatment. Neutralizing anti-HMGB1 mAb (Novus Biologicals).

Immunohistochemical staining

The stained tissue sections were examined and scored independently by two pathologists blinded to clinical parameters. The immunostaining levels were scored as 0 (negative), 1+ (weakly positive, light yellow), 2+ (moderately positive, yellowish brown), and 3+ (strongly positive, brown). 0 and 1+ indicated low expression, whereas 2+ and 3+ indicated high expression in tumor cells.

Colony assay

Colony forming cell assay was performed as described previously[1].

Coimmunoprecipitation

For immunoprecipitation of GSK-3β and Rpt1 proteins, T98G and HEK 293T cells were lysed by IP lysis buffer (Thermo Scientific). The cell lysate was then incubated with 1 μg antibody or the corresponding normal IgG (as negative control) overnight at 4 °C with gentle rotation. Subsequently, the mixture was incubated with 50 μL of pre-washed protein A/G plus agarose (ThermoScientific) at 4 °C for 2 h. Immunoprecipitated complexes were

collected by centrifugation at 7,000 ×g for 1 min, and washed five times with 1 mL washing buffer. The immunoprecipitate was then eluted by incubating with 100 μL of elution buffer for 10 min at room temperature. Finally, the eluted proteins were subjected to immunoblot analysis.

ELISA

HMGB1 expression was dectected in supernatant by a specific anti-HMGB1 ELISA (Shino Test Corporation) according to the manufacturers instruction. The protocol was performed as described previously[2].

Wound healing and tanswell assays

Cell migration was assessed by wound healing assay. Briefly, cells were grown to 60% confluence in six-well plates coated with poly-L-Lysine (Sigma-Aldrich, St. Louis, MO, USA), and the culture medium was replaced with serum-free DMEM. After serum starvation for 12 h, cell monolayers were scratched with a 20-μL pipette tip to create a wound. The scratched monolayers were treated with rhHMGB1, and monitored under a microscope for 18h. Wound closure (%) was evaluated using ImageJ software (NIH, Bethesda, MD, USA).

Cell invasion was evaluated using a Transwell chamber (Costar, Corning, NY, USA) with a polycarbonic membrane (6.5 mm in diameter and 8-μm pore size). The cells were first treated with 1 μg/mL of rhHMGB1 for 24h, and then seeded onto the upper chamber of Matrigel-coated filters. Meanwhile, 600 μL of high-glucose DMEM medium supplemented with 5% FBS was added into the lower chamber as chemoattractant. After 24h of incubation at 37 °C, the invasive cells located on the lower surface of the membrane were visualized with 0.1% crystal violet (Sigma-Aldrich) staining and counted.

Western blotting

Western blotting was performed as described previously[3]. Commercially available primary antibodies were directed against snail (1:1,000 for IB, Cell Signaling 3896S), p62 (Cell Signaling 88588), Slug (1:1000, Cell Signaling 9585S), Zeb1 (1:1000, Cell Signaling

3396S), p-IKKα (1:500, Cell Signaling 2697), IκBα (1:1000, Cell Signaling 9242), p-IκBα (Santa Cruz sc-8404), IKKα (1:1000, Cell Signaling 2682), Axin2 (1:1000, Cell Signaling 2151), LEF-1 (1:1000, Cell Signaling 2230), CyclinD1 (1:1000, Cell Signaling 2098), Rabbit anti-Twist1 (Abcam ab175430), Mouse monoclonal anti-GSK-3β (Abcam ab93926), Rabbit anti-p-GSK-3β (Y216) (Abcam ab68476), Rabbit anti-Rpt1 (Santa Cruz sc-166972), Rabbit anti-Nrf2 (Santa Cruz sc-81342), T-cadherin (Millipore, ABT121), phospho-Akt (Ser473) (1:1,000, Cell Signaling 4060), phospho-p38 MAPK (Thr180/182) (1:1,000, Cell Signaling 4511), phospho-ERK1/2 (Thr202/204), phospho-JNK1/2 (Thr183/185), and Rabbit monoclonal anti-p-GSK-3β (S9) (Abcam ab75814), mouse anti-HA (1:1,000, Roche 11583816001),Rabbit anti-HA(1:500,Clontech 631207), HRP-conjugated anti-rabbit and anti-mouse IgG antibodies were obtained from Cell Signaling Technology (7074 and 7076, respectively), β-catenin(Cell Signaling Technology, 9587) , Occludin (Abcam, ab216327, 1:1000 dilution), Goat anti-Mouse IgG (H+L) Secondary Antibody, DyLight 488(Thermo Fisher Scientific 35502), Goat anti-Mouse IgG (H+L) Secondary Antibody, DyLight 594(Thermo Fisher Scientific 35510) , Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647(Thermo Fisher Scientific 35552) , Goat anti-Rabbit IgG (H+L) Secondary Antibody, DyLight 488(Thermo Fisher Scientific 35560), Goat anti-Rabbit IgG (H+L) Secondary Antibody, DyLight 594(Thermo Fisher Scientific A21235), Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647(Thermo Fisher Scientific A32733).

RNA isolation and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cultured cells using TRIzol reagent (cat. no. 15596-026; Invitrogen) according to the recommended protocol. cDNA was reverse transcribed from 2 μg of total RNA by using PrimeScript RT reagent kit (cat. no. RR047A; Takara, Shiga, Japan). qRT–PCR was carried out using Maxima SYBR Green/ROX qPCR Master Mix (cat. no. K0222; Thermo Scientific). The primers used were listed in **Table S1**. All samples were run in triplicate.

Tumor orthotopic assays in nude mice

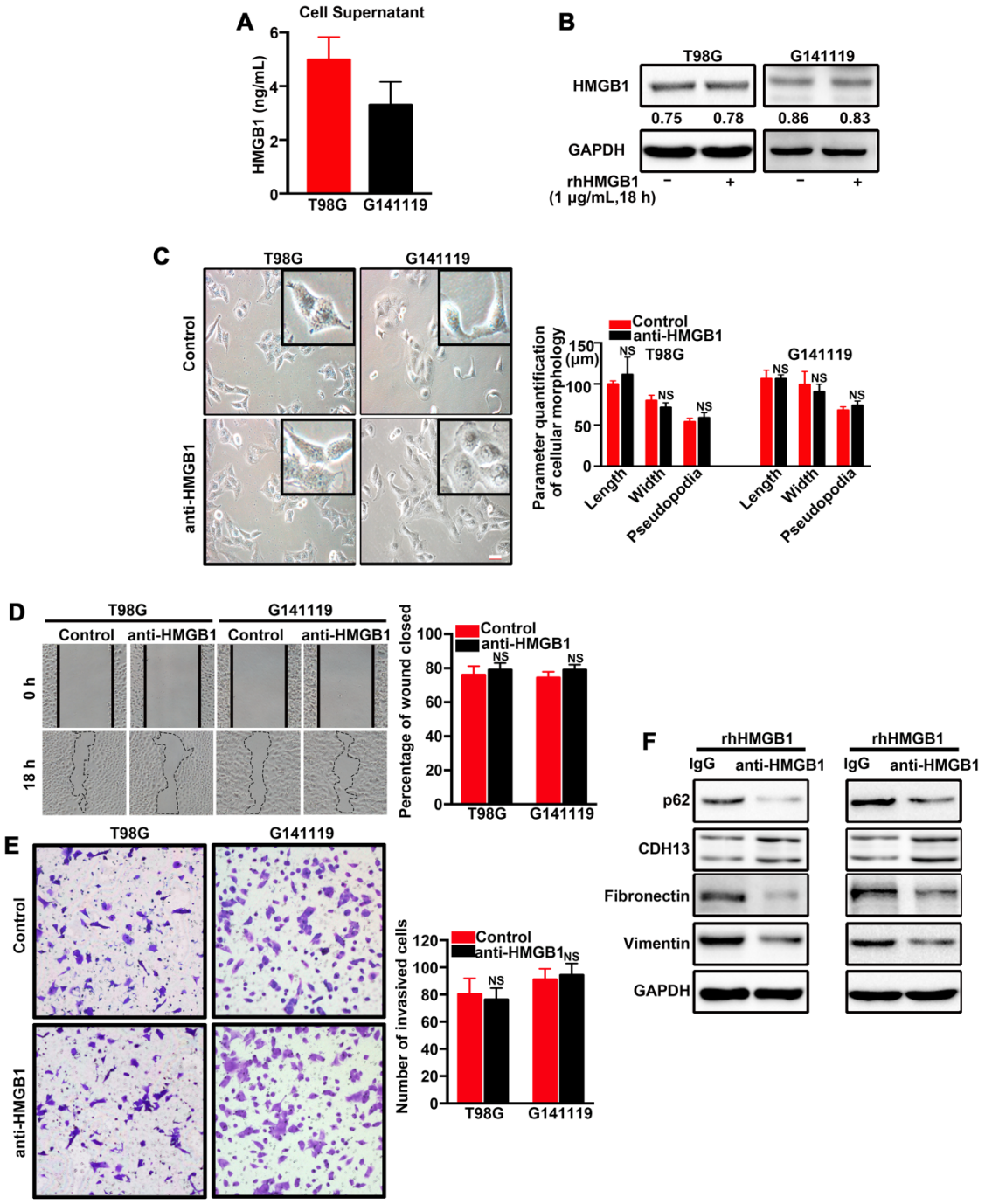
All experimental protocols were approved by the Animal Research Committee of Southern Medical University and were conducted in accordance with the guidelines of Southern Medical University. The mice were divided into four groups: G141119/Control/Control shRNA, G141119/rhHMGB1/Control shRNA, G141119/Control/p62-#2 shRNA and G141119/rhHMGB1/p62-#2 shRNA. Both G141119/p62-#2 shRNA and G141119/ Control shRNA-transfected cells were either untreated or pretreated with rhHMGB1 for 48 h. After incubation, G141119 cells (1×10^6 in 5 μL PBS) were stereotactically implanted into the brain of mice in order to determine tumorigenicity and invasiveness. For survival analysis, mice were monitored regularly and euthanized when they showed neuropathological symptoms. To observe the invasiveness of tumors, mice were regularly euthanized after 4 weeks of G141119 cell implantation. Mouse brains were harvested, fixed in 4% paraformaldehyde for 48 h, embedded into paraffin blocks and prepared for immunohistochemical analysis.

To evaluate the implication of rhHMGB1 in invasion and growth in vivo, we applied the neutralize antibody of HMGB1 to pretreat the cells with rhHMGB1 or Control. Nude mice were randomly divided into four groups: Control + Ig G group, rhHMGB1 + Ig G group, Control + anti-HMGB1 group, and rhHMGB1 + anti-HMGB1 group. In this model,6×10^5 cells were stereotactically implanted into the right striatum of the mice. Four weeks after injection, tumor burden of mice was assessed using magnetic resonance imaging (MRI) scanner (Bruker Medical Inc., Billerica, MA, USA). The tumor volume was performed as described previously [4].

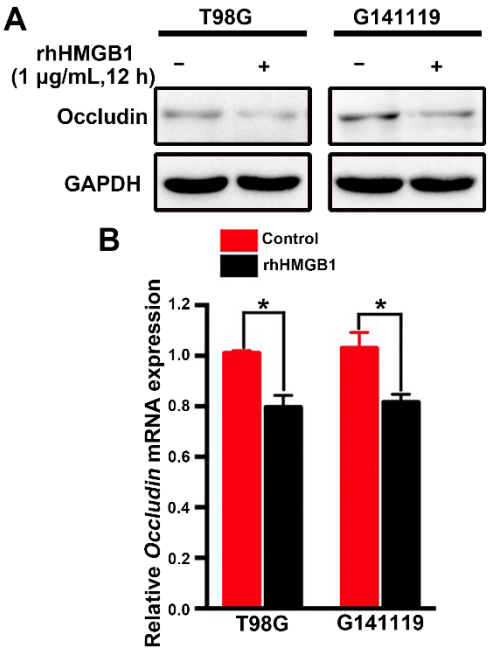
Patient samples

In this study, a total of 110 human GBM specimens were obtained from Department of Neurosurgery at Nanfang Hospital of Southern Medical University, Guangzhou, China (**Table S2**). The study is approval by the Ethics Committees of Nanfang Hospital. All patients were written informed consent in our study. The diagnoses of all specimens were based on pathological evidence. Subsequently, tissue sections were fixed and immunohistochemically stained with anti-p62 and anti-GSK-3β antibodies (cat. no. 4445 and 12649, respectively; Cell Signaling Technology, Danvers, MA, USA).

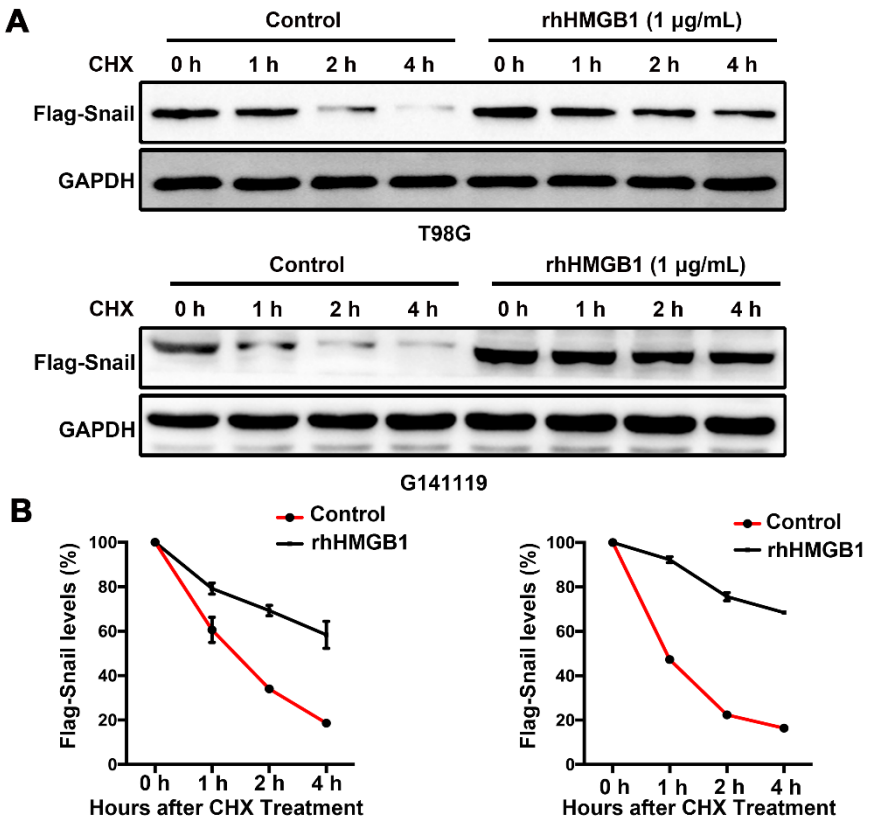
Supplementary Figures



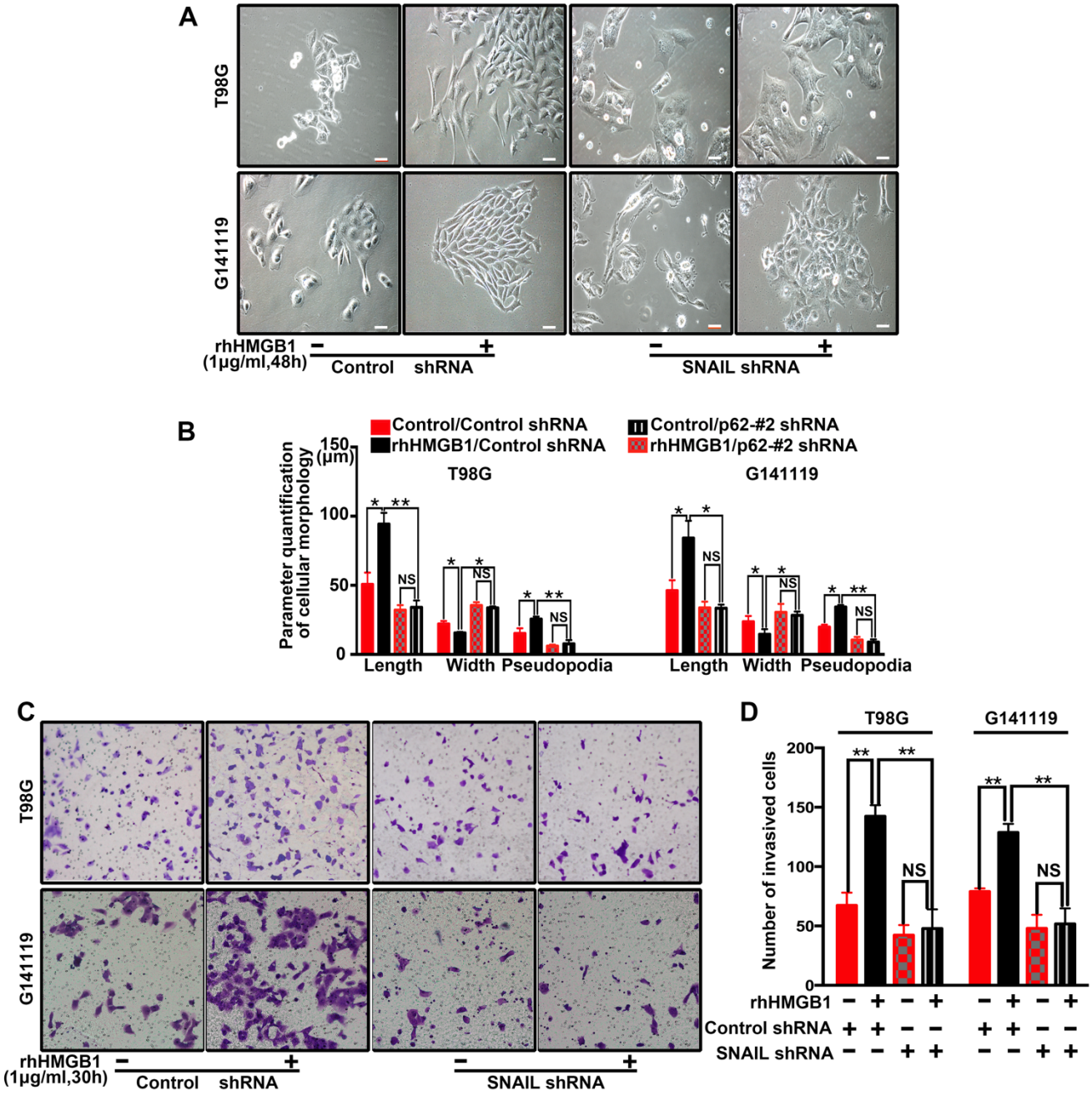
Supplementary Figure S1. The endogenous HMGB1 has little effect in exogenous HMGB1-induced EMT in GBM cells. (A) HMGB1 released into the supernatant was assessed by ELISA. **(B)** Representative blots of HMGB1 was shown in T98G and G141119 cells treated with rhHMGB1 for 18 h. The effect of anti-HMGB1 neutralizing Ab on morphology **(C)**, migration **(D)**, invasion **(E)** of GBM cells (Scale bar: 40 μm). **(F)** Anti-HMGB1 neutralizing Ab blocked the EMT induced-by exogenous rhHMGB1.NS, no statistical significance by unpaired Student's t test.



Supplementary Figure S2. HMGB1 affects the expression of Occludin in GBM cells. qRT-PCR **(A)** and Western blot **(B)** analysis in T98G and G141119 cells treated with rhHMGB1. **P*<0.05 by unpaired Student's t test.

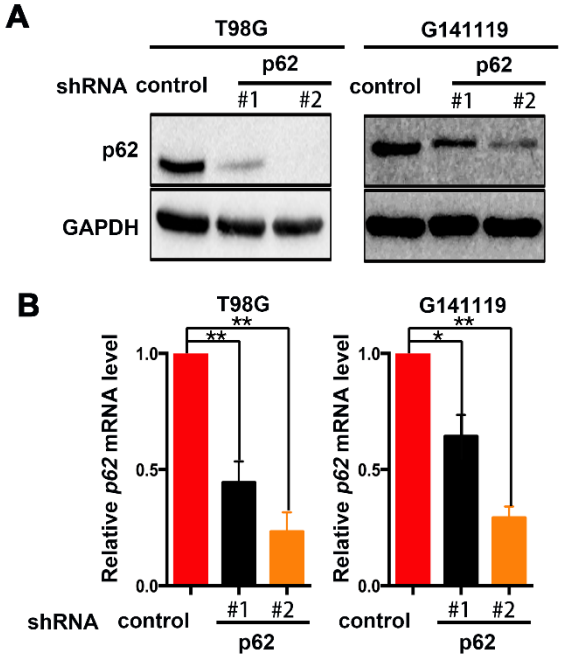


Supplementary Figure S3. HMGB1 stabilizes the snail protein. (A) Following the control or rhHMGB1 treatment, Snail protein half-life was determined in GBM cells after treating with CHX for 0, 1, 2 or 4 h. **(B)** The half-life of Snail was measured from the slope of densitometric protein abundance.

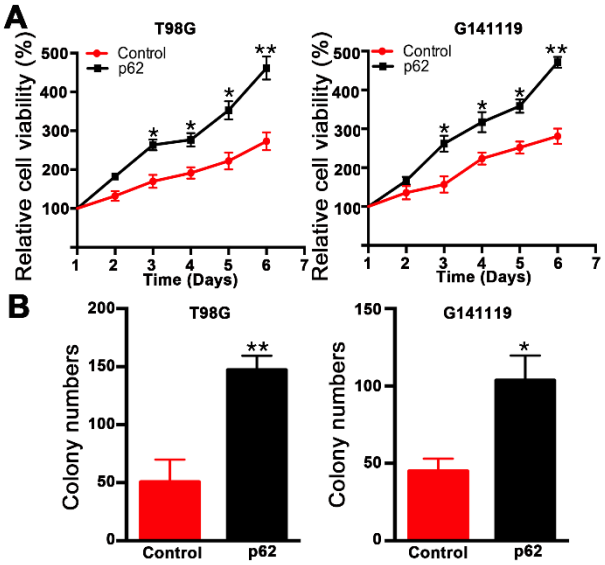


Supplementary Figure S4. Depletion of snail influences HMGB1-induced EMT of GBM cells. GBM cells were transduced with shRNA to knocked down *SNAIL*, and then the cells were treated with rhHMGB1. The morphology of pretreated GBM cells was determined by the inverted microscope (Scale bar: 40 μm) **(A-B)** and the invasiveness of pretreated GBM cells was determined by the Transwell assays (magnification, × 40) **(C-D)**. Statistical analysis used one-way ANOVA. ***P*<0.01,

*P<0.05, NS, no statistical significance. The results are shown as mean ± SEM.

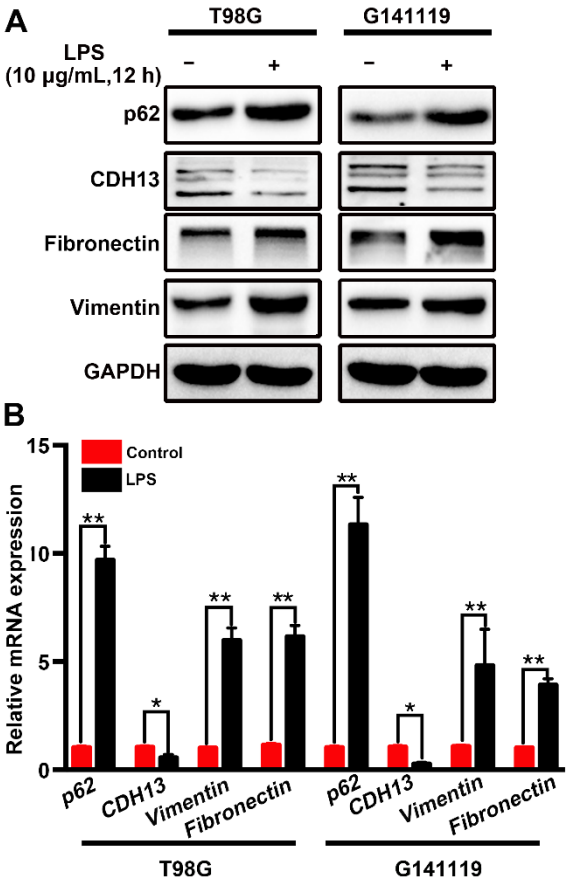


Supplementary Figure S5. Efficiencies for knockdown of p62 in GBM cells were tested by western blot assay**(A)** and qRT-PCR assay **(B)**. **P<0.01, *P<0.05 compared to Control. Error bars represent mean ± SEM.

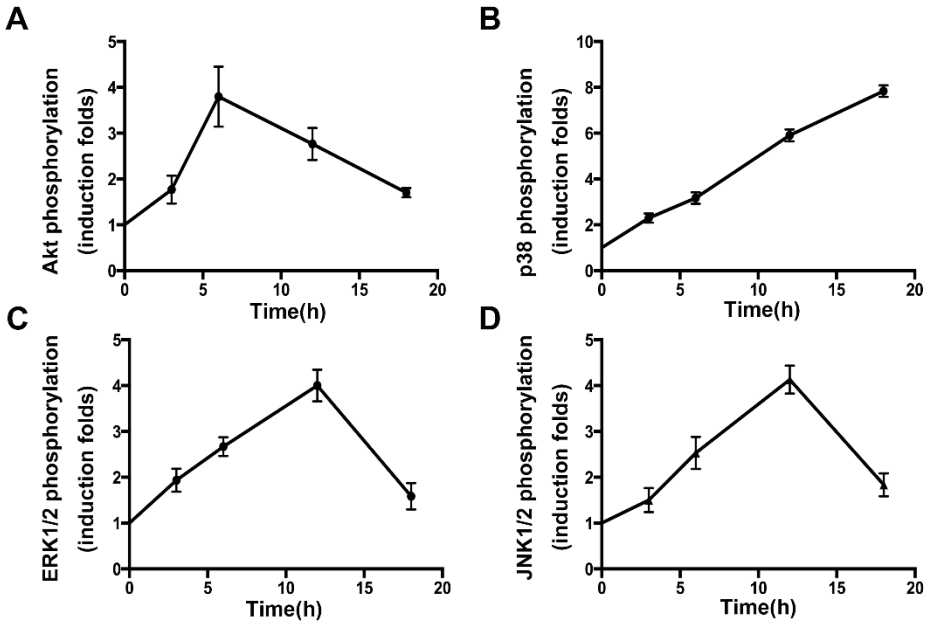


Supplementary Figure S6. p62 promotes GBM cell proliferation. Proliferating

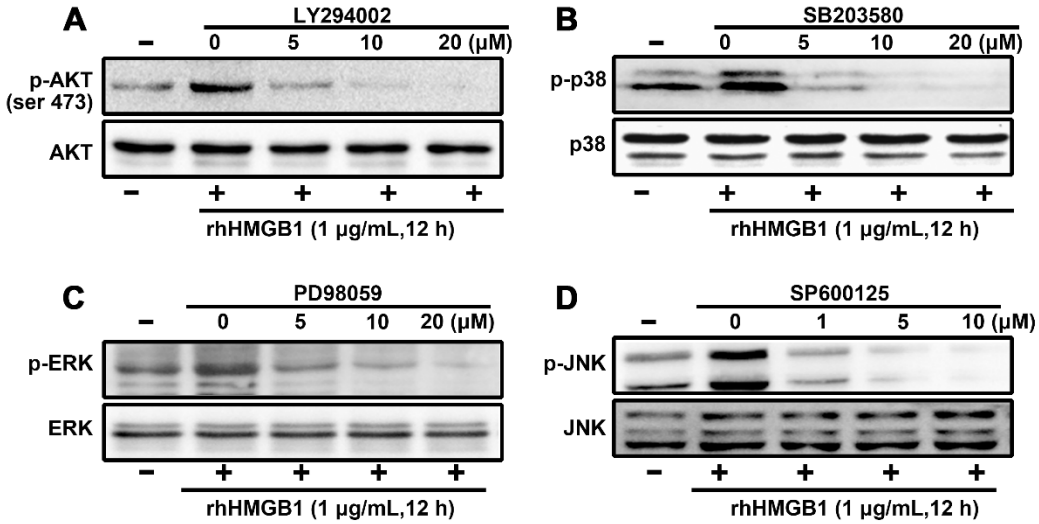
cells were measured by MTT assays **(A)** after T98G and G141119 cells expressing either control vector or Flag-tagged p62-expressing plasmid were cultured for 6 days. （**P<0.01, *P<0.05; two-way ANOVA post-hoc Sidak's test ）. **(B)** Quantification of T98G and G141119-control vector/p62-expressing plasmid cells colony formation at days 15 after plating. (*P< 0.05; **P < 0.01, two-sided unpaired t-test with Welch's correction being applied when homoscedasticity was not verified).



Supplementary Figure S7. LPS induces EMT-associated markers and p62 expression in T98G cells. T98G cells were incubated with LPS (100 ng/mL) for 12 h. **(A)** Western blot analysis of CDH13, fibronectin, vimentin and p62. **(B)** CDH13, fibronectin, vimentin and p62 mRNA expression were examined by qPCR.

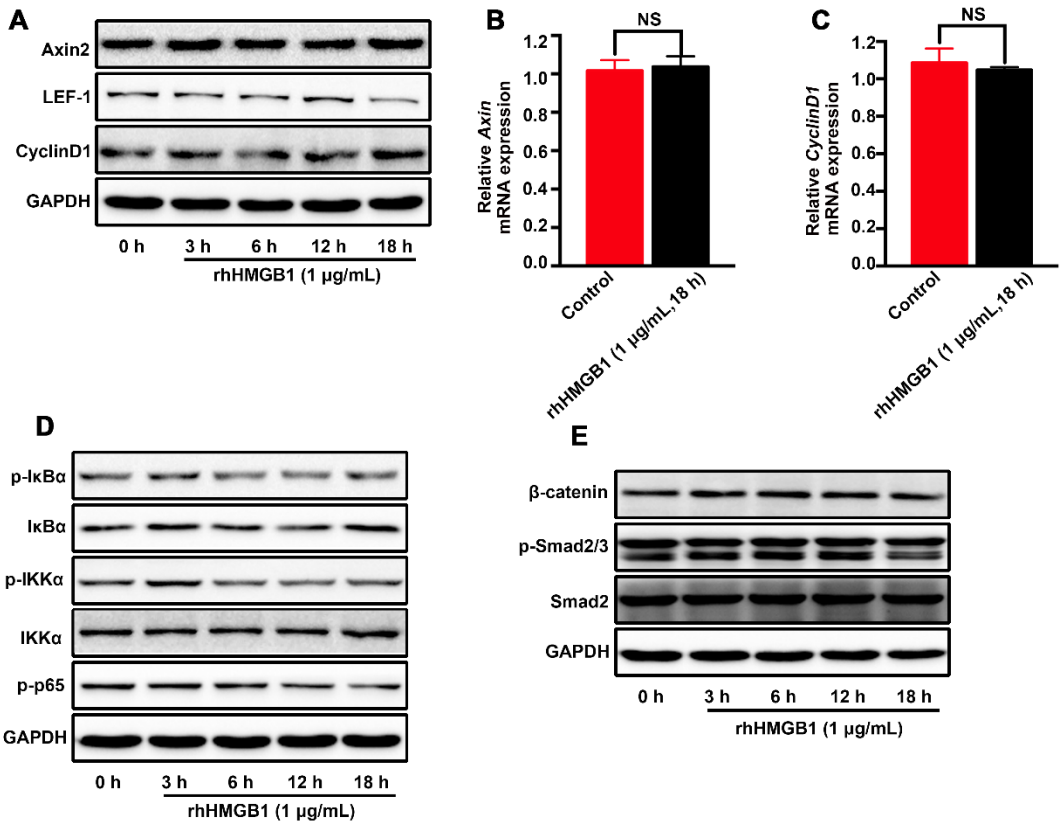


Supplementary Figure S8. Phosphorylation density of Akt **(A)**, p38 **(B)**, ERK1/2 **(C)** and JNK1/2 **(D)** protein were relatively quantified to corresponding total protein content. The ratios at indicated time points were normalized with that of control.



Supplementary Figure S9. The efficiency of SB203580, PD98059, SP60012 and LY294002. Cells were grown to 70% confluence and serum starved for 3 h. The cells were then incubated for 12 h with various concentrations of each inhibitor and

stimulated with 1 μg/mL rhHMGB1. Cell proteins were resolved by SDS-PAGE and probed with anti-phospho-AKT and anti-AKT **(A)**, anti-phospho-p38, anti-p38 **(B)**, anti-phospho-ERK1/2 (Thr202/Tyr204)(p-ERK1/2),anti-ERK1/2 **(C)**,anti-phospho-JNK and anti-JNK **(D)**.



Supplementary Figure S10. Effects of HMGB1 on the Wnt/β-catenin, TGF-β and NF-kB pathways in GBM cells. (A) T98G cells were treated with rhHMGB1 (1 μg/ml) for 3, 6, 12, and 18 h, and the Axin2, LEF-1, CyclinD1 were detected by western blot. The mRNA of *Axin2* **(B)** and **(C)** *Cyclin D1* after 18 h treated with rhHMGB1.NS, no statistical significance by unpaired Student's t test. The activation of phospho-NF-κB p65 **(D)** and phospho-Smad2/3 **(E)** in T98G cells were detected by western blot.

Supplementary Tables

Table S1: Sequences of the oligonucleotides for siRNA, qRT-PCR assays.

Assays	Sequences
siRNA	Targeting sequences
TLR4	5’-GGAACAACAUUAGAACAGC-3’
Nrf2	5’-GGAGAAUUCCUCCCAAU-3’
Negative Control	5’-GAAATGGCCACAACAAGTTGT-3’
qRT-PCR	Primer sequences
GSK-3β	Forward: 5’-TCGAGCCAAGCAGACACTCC-3’; Reverse: 5’-ACATTG GGCTCTCCTCGGAC-3’
p62	Forward: 5’-AGGCGCACTACCGCGAT-3’; Reverse: 5’-CGTCACTGGAAAAGGCAACC-3’
CDH13	Forward: 5’-TACAATGCCGCCATCGCTTAC-3’; Reverse: 5’-ATCAGCCGCTTTCAGATT-3’
Vimentin	Forward: 5’-AATGACCGCTTCGCCAACT-3’; Reverse: 5’-CGATGTAGTTGGCGAAGC-3’
Fibronectin	Forward: 5’-GTGCCTGGGCAACGGA-3’; Reverse: 5’-CCCGAC CCTGACCGAAG-3’
SNAIL	Forward: 5’-TTCTCTAGGCCCTGGCTGCTACAA-3’; Reverse:5’-TCTCTGACATCTGAGTGGGTCTGGA-3’
Nrf2	Forward: 5’-CCTCAACTATAGCGATGCTGAATCT-3’; Reverse: 5’-AGGAGTTGGGCATGAGTGAGTAG-3’
GAPDH	Forward: 5’-AATCCCATCACCATCTTCC-3’; Reverse: 5’-CTCGCTCCTGGAAGATGG-3’
Occludin	Forward: 5’-TGGCATACTCTTCCAATGGC-3’; Reverse: 5’-GTCATCCACAGGCGAAGTTA-3’

Abbreviations: qRT-PCR: quantitative reverse transcriptase-PCR;

1

2 **Table S2. Clinicopathological characteristics of tumor samples.**

Lab annotation	Age (Years)	Gender	Volume (cm^3)*	Side and Tumor location	Functional area	Tumor resection#	Survival (Days)	Survival status
GBM-2	68	Male	21.8	Right, Parietal lobe	Yes	PTR	145	Dead
GBM-4	53	Male	28.5	Left, Temporal lobe	Yes	GTR	460	Dead
GBM-6	46	Female	48.6	Right, Frontal lobe	No	GTR	512	Dead

GBM-7	62	Male	23.8	Left, Temporal lobe	Yes	GTR	398	Dead
GBM-8	45	Male	38.3	Right, Temporal lobe	Yes	STR	145	Dead
GBM-10	52	Female	22.6	Right, Frontal lobe	Yes	STR	156	Dead
GBM-11	36	Male	55.8	Right, Parietal lobe	Yes	GTR	269	Dead
GBM-12	38	Female	72.1	Right, Occipital lobe	Yes	GTR	196	Dead
GBM-14	46	Male	14.5	Right, Thalamus	Yes	PTR	337	Dead
GBM-15	58	Male	24.8	Left, Temporal lobe	No	GTR	880	Dead
GBM-17	46	Female	3.8	Left, Occipital lobe	No	GTR	277	Dead
GBM-20	36	Male	31.1	Right, Frontal lobe	No	GTR	692	Dead
GBM-22	52	Male	10.6	Left, Frontal lobe	Yes	GTR	330	Dead
GBM-23	55	Female	17.4	Right, Frontal lobe	Yes	STR	430	NA
GBM-24	47	Female	43.5	Right, Temporal lobe	No	GTR	1289	Dead
GBM-25	66	Female	35.2	Right, Temporal lobe	No	GTR	230	NA
GBM-27	42	Male	15.5	Right, Parietal lobe	Yes	STR	700	Dead
GBM-28	41	Male	94.4	Left, Frontal lobe	Yes	STR	576	Dead
GBM-31	48	Male	9	Right, Temporal lobe	No	GTR	598	Dead
GBM-32	48	Female	9.1	Right, Brain stem	Yes	PTR	573	Dead
GBM-33	68	Male	12.8	Left, Parietal lobe	Yes	GTR	558	Dead
GBM-35	56	Female	18.6	Left, Occipital lobe	No	GTR	356	Dead
GBM-36	56	Male	60.4	Left, Temporal lobe	Yes	STR	377	Dead
GBM-38	62	Female	24	Left, Occipital lobe	Yes	GTR	586	Dead
GBM-41	60	Female	25.5	Right, Temporal lobe	No	GTR	380	NA
GBM-42	57	Male	41.2	Left, Thalamus	Yes	GTR	825	NA
GBM-44	55	Male	37.8	Right, Temporal lobe	No	GTR	1267	Dead
GBM-49	57	Male	60.4	Left, Temporal lobe	Yes	STR	578	Dead
GBM-50	36	Female	19.2	Left, Temporal lobe	Yes	GTR	278	Dead
GBM-52	62	Female	20.1	Left, Thalamus	Yes	GTR	195	Dead
GBM-56	47	Male	56.7	Bilateral, Frontal lobe	Yes	STR	431	NA
GBM-57	42	Male	39.3	Left, Parietal lobe	Yes	GTR	750	Dead
GBM-59	38	Female	32.7	Right, Temporal lobe	No	GTR	678	NA
GBM-60	32	Male	56.1	Right, Thalamus	Yes	PTR	788	Dead
GBM-61	49	Male	3.5	Median, Brain stem	Yes	PTR	156	Dead
GBM-62	52	Female	11.2	Left, Thalamus	Yes	STR	567	Dead
GBM-64	50	Female	21.5	Left, Temporal lobe	Yes	STR	568	Dead
GBM-65	49	Female	28.3	Right, Parietal lobe	Yes	GTR	560	Dead
GBM-66	46	Male	18.25	Right, Frontal lobe	No	GTR	562	NA
GBM-69	52	Female	55.8	Right, Parietal lobe	Yes	GTR	444	Dead
GBM-70	58	Male	27.9	Left, Temporal lobe	Yes	STR	1578	Dead
GBM-72	56	Male	15.5	Right, Thalamus	Yes	PTR	606	Dead
GBM-74	36	Male	68.5	Left, Frontal lobe	Yes	GTR	294	Dead

GBM-76	48	Female	19.6	Right, Temporal lobe	No	GTR	329	Dead
GBM-79	28	Female	51	Left, Occipital lobe	Yes	GTR	578	Dead
GBM-84	39	Male	16.7	Right, Thalamus	Yes	STR	478	NA
GBM-86	35	Female	14.9	Right, Temporal lobe	Yes	GTR	414	Dead
GBM-89	51	Male	68.3	Right, Frontal lobe	Yes	GTR	371	Dead
GBM-91	52	Male	30.8	Right, Frontal lobe	Yes	PTR	465	Dead
GBM-94	50	Male	34.2	Right, Parietal lobe	Yes	PTR	663	Dead
GBM-95	56	Female	56.3	Left, Parietal lobe	Yes	STR	197	Dead
GBM-96	63	Male	22.8	Left, Frontal lobe	Yes	STR	979	Dead
GBM-100	58	Female	34.7	Right, Frontal and Temporal lobe	Yes	GTR	1304	NA
GBM-102	34	Female	35.9	Right, Frontal lobe	Yes	GTR	441	Dead
GBM-103	47	Female	25.2	Right, Frontal lobe	Yes	GTR	348	Dead
GBM-107	60	Female	19.2	Left, Temporal lobe	Yes	STR	348	Dead
GBM-108	55	Male	5.36	Left, Parietal lobe	Yes	GTR	254	Dead
GBM-110	15	Male	75	Bilateral, Frontal lobe	Yes	PTR	182	Dead
GBM-112	68	Male	11.9	Left,Temporal lobe	Yes	GTR	196	Dead
GBM-113	51	Male	20.1	Right, Frontal lobe	Yes	STR	408	Dead
GBM-115	55	Male	23.9	Right,Temporal lobe	YES	GTR	287	Dead
GBM-118	51	Male	7.54	Right, Frontal lobe	Yes	GTR	243	Dead
GBM-119	49	Female	18.6	Left,Temporal lobe	Yes	GTR	409	NA
GBM-120	38	Male	17.9	Right, Frontal lobe	Yes	GTR	246	Dead
GBM-121	51	Female	10	Right,Temporal lobe	Yes	GTR	234	NA
GBM-123	31	Male	9	Left, Frontal lobe	Yes	GTR	221	Dead
GBM-125	46	Male	70.2	Right,Parietal lobe	Yes	GTR	292	Dead
GBM-126	25	Male	5	Right, Frontal lobe	Yes	GTR	389	Dead
GBM-128	80	Male	30.3	Left,Temporal lobe	Yes	GTR	368	Dead
GBM-129	64	Male	60	Right, Frontal lobe	Yes	GTR	238	Dead
GBM-130	49	Female	8.4	Right, Frontal lobe	Yes	GTR	111	NA
GBM-131	32	Male	36.8	Left,Temporal lobe	Yes	GTR	189	Dead
GBM-132	37	Male	9	Left,Temporal lobe	Yes	GTR	1443	NA
GBM-134	13	Male	69.2	Left, Frontal lobe	Yes	GTR	225	NA
GBM-146	53	Female	45.3	Right, Occipital lobe	Yes	GTR	99	Dead
GBM-147	56	Male	37.8	Right,Temporal lobe	Yes	GTR	298	Dead
GBM-150	66	Male	42.1	Left,Temporal lobe	Yes	GTR	223	Dead
GBM-152	41	Male	16.8	Left,Temporal lobe	Yes	GTR	168	Dead

GBM-153	42	Female	65.3	Right, Frontal lobe	Yes	GTR	368	Dead
GBM-157	59	Male	40	Left, insular lobe	Yes	GTR	377	Dead
GBM-159	63	Female	12	Left,Temporal lobe	Yes	GTR	458	Dead
GBM-160	15	Female	8.4	Left, Frontal lobe	Yes	GTR	236	Dead
GBM-161	65	Male	24.3	Left,Temporal lobe	Yes	GTR	268	Dead
GBM-163	63	Male	33	Right,Temporal lobe	Yes	GTR	220	Dead
GBM-164	41	Female	52.1	Left, Frontal lobe	Yes	GTR	68	Dead
GBM-165	65	Male	34.6	Left, Parietal lobe	Yes	GTR	334	Dead
GBM-166	66	Male	33.1	Left, Frontal lobe	Yes	GTR	1530	NA
GBM-167	48	Male	18.4	Right,Parietal lobe	Yes	GTR	456	Dead
GBM-168	56	Male	24.7	Left, Frontal lobe	Yes	GTR	268	Dead
GBM-169	37	Male	36.4	Right,Hippocampal area	No	STR	578	Dead
GBM-170	26	Male	53.8	Left,Frontal lobe	Yes	STR	198	Dead
GBM-171	67	Male	29.7	Left, Frontal lobe	Yes	GTR	187	Dead
GBM-172	43	Male	57.2	Left, Parietal lobe	Yes	GTR	2008	NA
GBM-173	54	Male	26.4	Left,Temporal lobe	Yes	PTR	342	Dead
GBM-174	62	Male	43.1	Right, Frontal lobe	Yes	GTR	297	Dead
GBM-176	66	Male	38.9	Right, Frontal lobe	No	GTR	343	Dead
GBM-177	53	Male	42.7	Right, Frontal lobe	Yes	GTR	467	Dead
GBM-178	55	Male	34.7	Left,Temporal lobe	Yes	GTR	128	Dead
GBM-179	51	Female	25.8	Right,Temporal lobe	Yes	STR	423	Dead
GBM-180	37	Female	34	Right, Frontal lobe	Yes	GTR	142	Dead
GBM-181	16	Male	3	Left,Parietal lobe	Yes	GTR	1107	Dead
GBM-182	51	Male	7.5	Right, Frontal lobe	Yes	STR	760	NA
GBM-183	48	Male	33.1	Left,Temporal lobe	Yes	STR	389	Dead
GBM-188	67	Female	26.3	Right, Frontal lobe	Yes	GTR	398	Dead
GBM-189	63	Male	10.1	Right,Parietal lobe	Yes	PTR	1208	Dead
GBM-191	62	Male	34	Right,Parietal lobe	Yes	GTR	456	Dead
GBM-192	35	Female	16.5	Right,Temporal lobe	Yes	GTR	38	NA
GBM-194	55	Female	12.4	Right,Temporal lobe	Yes	STR	298	Dead
GBM-195	51	Male	10.8	Right, Frontal lobe	Yes	GTR	298	Dead
GBM-198	62	Female	7.3	Right, Frontal lobe	Yes	GTR	214	Dead

*, Tumor volume was measured according to the T1WI scan before surgery using the Coniglobus formula as “a × b × c / 2”, which is widely used to evaluate the volume of intracrnial hemorrhage. “a” indicates the maximal diameter in the tumor cross section, “b” indicates the maximal diameter perpendicular to “a”, and “c” indicates the slice thickness of the tumor

#, The degree of tumor resection. GTR, gross total tumor removal, indicating no residual tumor in th postsurgical MRI; STR, subtotal tumor removal, indicating that more than 90% of tumor was removed; PTR, partial tumor removal, indicating that less than 90% of the tumor was removed

Supplementary references

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