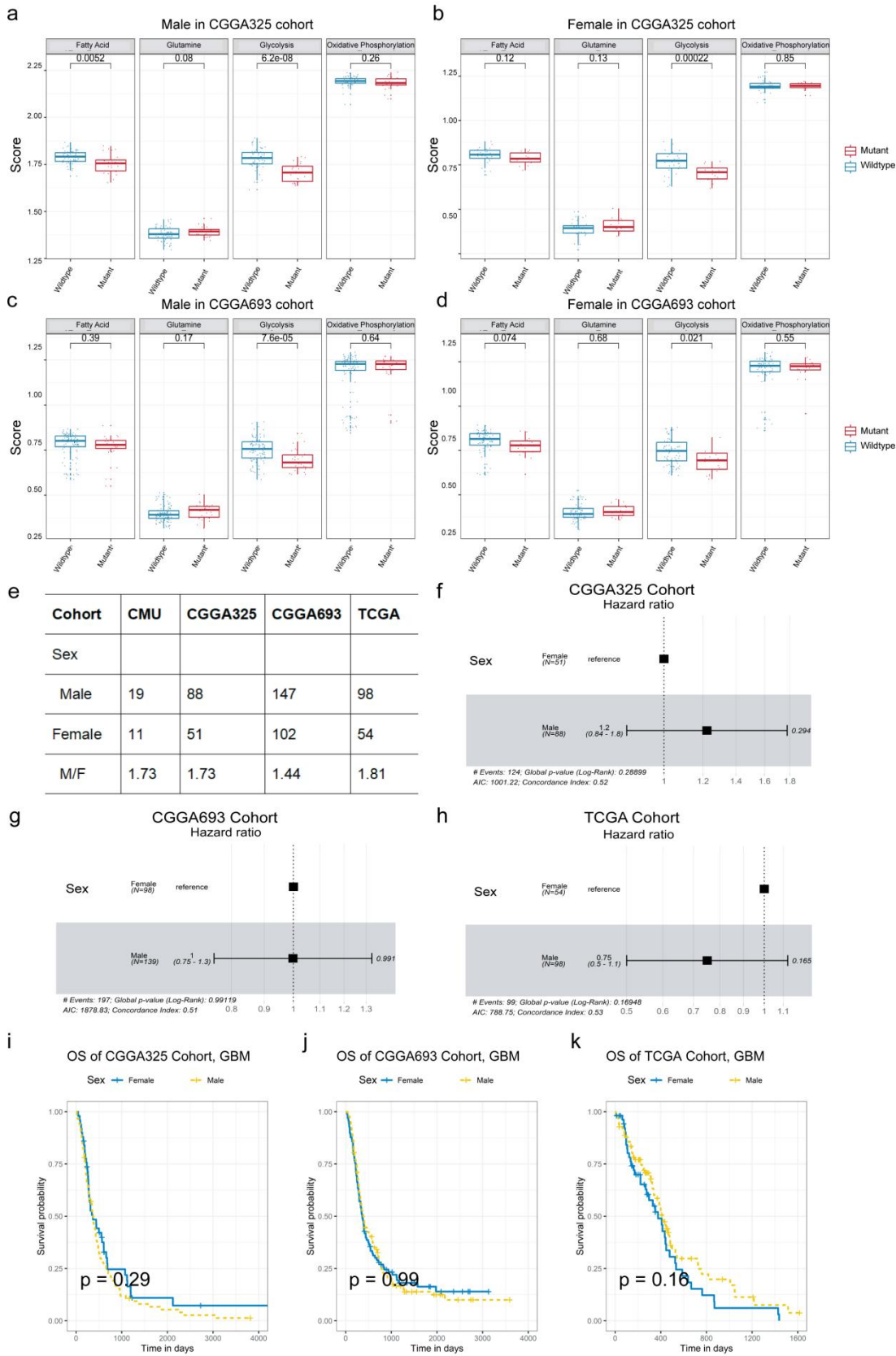


Supplementary Figure and Figure legends



Supplementary Fig. 1. The analysis of indicated metabolic modes as well as the association between sexes and patient survival in grade IV glioma.

a-d The differential analysis of indicated metabolic modes between IDH1 wild type and IDH1 mutant grade IV glioma based on sex in indicated glioma cohorts (a: male in CGGA325 cohort n = 88 (IDH1 wild type, n = 64; IDH1 mutant, n = 24), b: female in CGGA325 n = 51 (IDH1 wild type, n = 34; IDH1 mutant, n = 17), c: male in CGGA693 n = 132 (IDH1 wild type, n = 106; IDH1 mutant, n = 26), d: female in CGGA693 cohort n = 95 (IDH1 wild type, n = 76; IDH1 mutant, n = 19)).

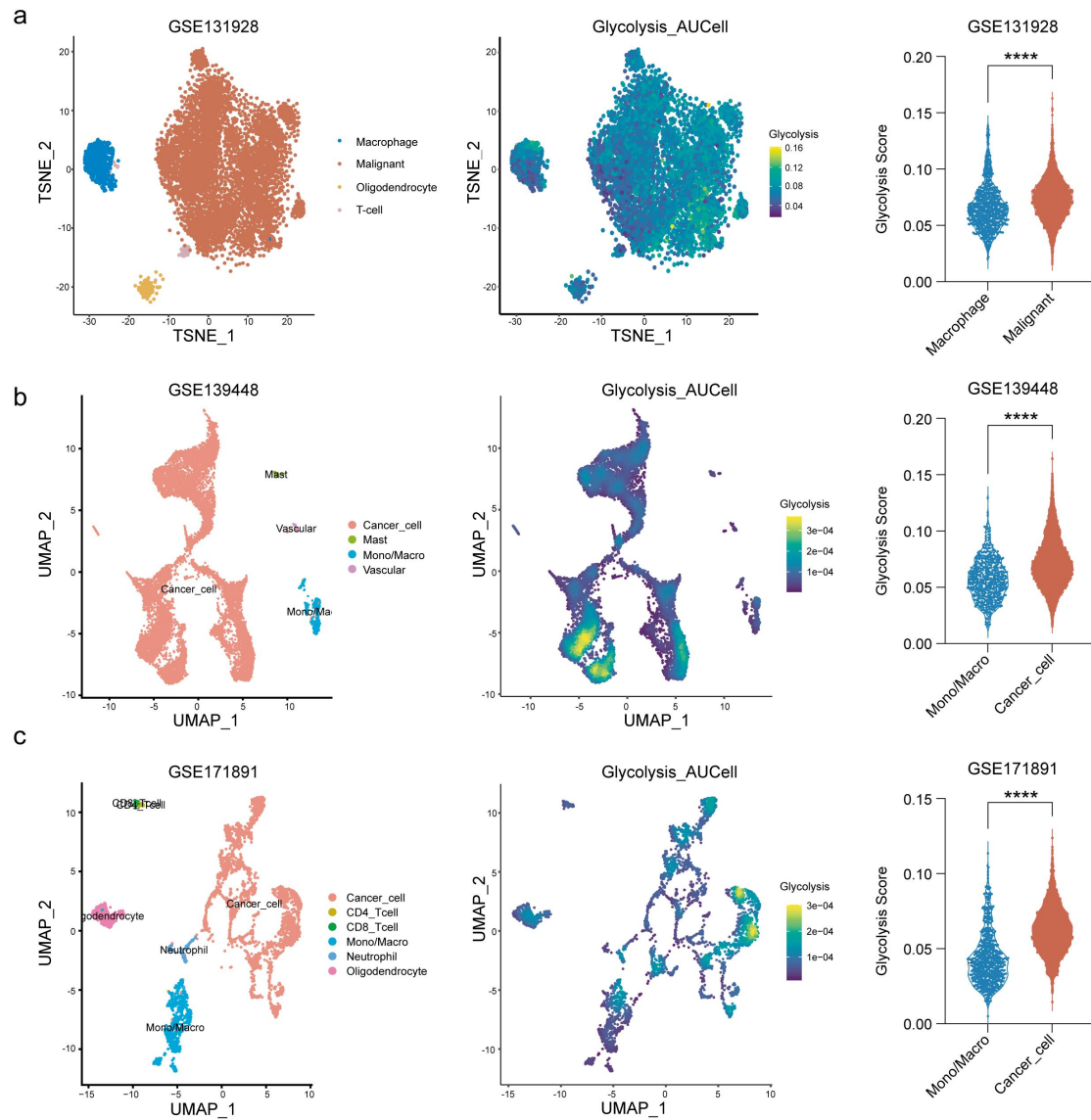
e Sex distribution analysis of grade IV glioma in indicated four glioma datasets.

f-h Univariate Cox regression analysis of the association between sex and survival of grade IV glioma in indicated three cohorts (f: CGGA325 n = 139, g: CGGA693 n = 237, h: TCGA n = 152).

i-k Overall survival curves of grade IV glioma stratified by sex in indicated three glioma cohorts (Log-rank; CGGA 325 cohort n = 139 (male, n = 88; female, n = 51), CGGA 693 cohort n = 237 (male, n = 139; female, n = 98), TCGA cohort n = 152 (male, n = 98; female, n = 54), including 4 cases with unknown IDH1 status).

(ns $P \geq 0.05$, * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$).

Data were presented as mean $\pm$ SEM. Statistical analyses were determined by two-tailed Student's t-test (a-d), and log-rank test (f-k). Source data are provided as a Source Data file.

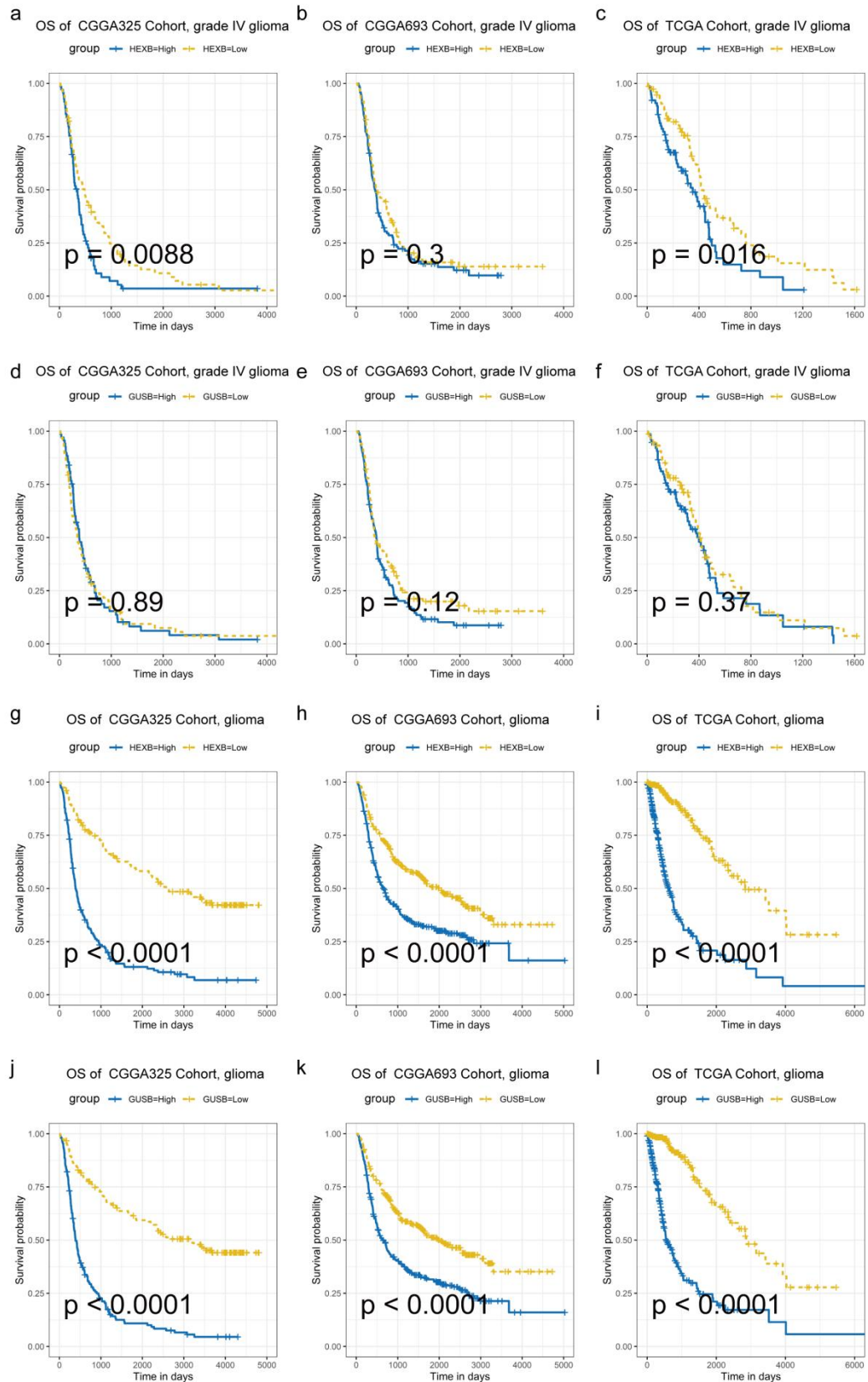


Supplementary Fig. 2. The comparative analysis based on glycolytic scores of macrophages and malignant cells in single-cell GBM RNA-sequencing datasets.

a-c Different cell annotations (Left), glycolysis score calculation and distribution in indicated cell components based on AUCell method (Middle), and the comparative analysis based on glycolysis scores of GBM cells and mono/macrophages (Right, t-test) in GSE131928 (a), GSE139448 (b) and GSE171891 (c) scRNA-seq GBM cohorts (GSE131928 $n = 7930$: Malignant (GBM cells) $n = 6863$; Macrophage $n = 754$, SMART-seq2; GSE139448 $n = 12444$: Cancer cell (GBM cells) $n = 11714$, Monocyte/Macrophage $n = 535$, 10x Genomics; GSE171891 $n = 4193$, 8 GBM sample data were included for the analysis: Cancer cell (GBM cells) $n = 2611$, Monocyte/Macrophage $n = 742$, 10x Genomics).

(**** $P < 0.0001$).

Data were presented as mean $\pm$ SEM. Statistical analyses were determined by two-tailed Student's t-test (a-c). Source data are provided as a Source Data file.

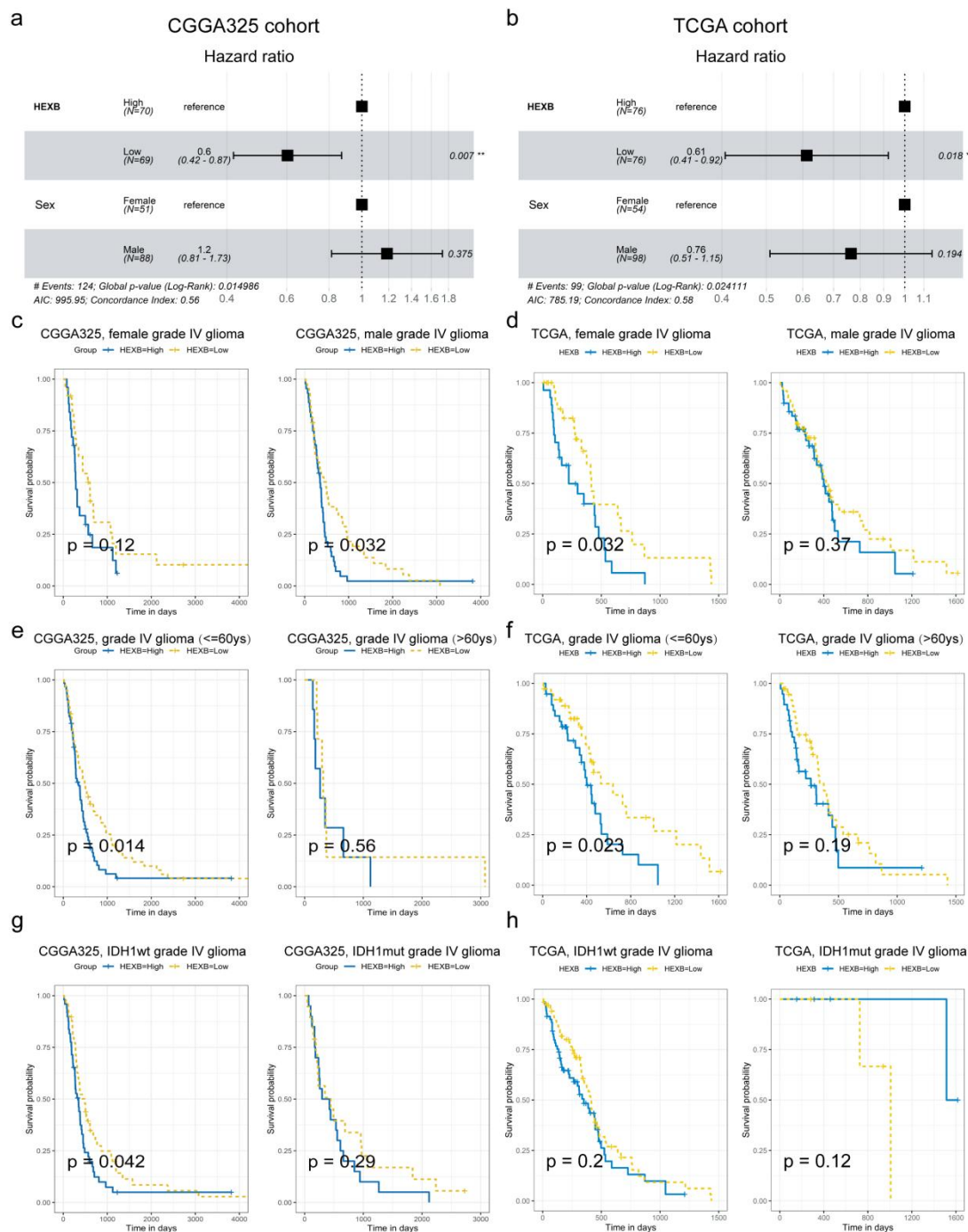


Supplementary Fig. 3. The survival analysis in indicated glioma and grade IV glioma according to HEXB and GUSB expression in indicated glioma datasets.

a-f Overall survival curves stratified by HEXB (a-c) and GUSB (d-f) expression in indicated three grade IV glioma cohorts, respectively (Log-rank analysis; a, d: CGGA 325 n = 137, including low group n = 68 and high group n = 69; b, e: CGGA 693 n = 237, including low group n = 118 and high group n = 119; c, f: TCGA n = 152, including low group n = 76 and high group n = 76).

g-l Overall survival curves stratified by HEXB (g-i) and GUSB (j-l) expression in indicated three glioma cohorts (Log-rank; g, j: CGGA 325 n = 313, including low group n = 156 and high group n = 157; h, k: CGGA 693 n = 657, including low group n = 328 and high group n = 329; i, l: TCGA cohort n = 603, including low group n = 301 and high group n = 302).

Statistical analyses were determined by log-rank test (a-l). Source data are provided as a Source Data file.



Supplementary Fig. 4. The survival analysis of HEXB considering sex in indicated grade IV glioma cohorts.

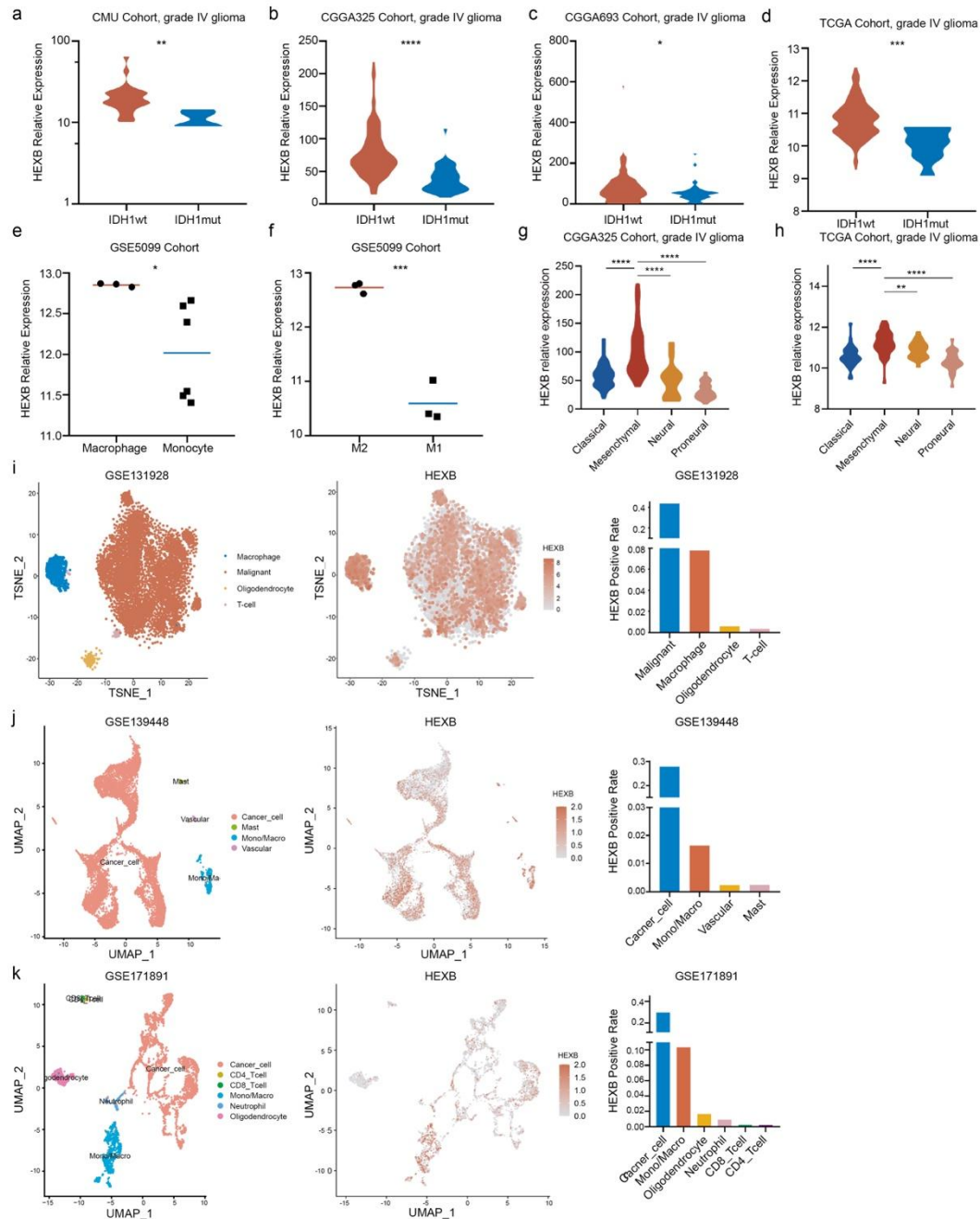
a, b Multivariate Cox analysis of HEXB expression according to the data of grade IV glioma obtained from indicated glioma datasets disclosing that HEXB as a prognostic factor independent of sex (CGGA325 cohort $n = 139$, TCGA cohort $n = 152$).

c-h The survival analysis of HEXB with high and low groups according to sex (c, d), ages (e, f) and IDH1 status (g, h) (CGGA325 cohort: male $n = 87$, low group $n = 43$, high $n = 44$; female $n = 50$, low group $n = 25$, high group $n = 25$; age ≤ 60 years $n =$

123, low group n = 61, high group n = 62; age > 60 n = 14, low group n = 7, high group n = 7; IDH1 wild type n = 98, low group n = 49, high group n = 49; IDH1 mutant n = 39, low group n = 19, high group n = 20; TCGA cohort: male n = 98, low group n = 49, high n = 49; female n = 54, low group n = 27, high group n = 27; age ≤ 60 years n = 76, low group n = 38, high group n = 38; age > 60 n = 76, low group n = 38, high group n = 38; IDH1 wild type n = 139, low group n = 69, high group n = 70; IDH1 mutant n = 9, low group n = 4, high group n = 5).

(* $P < 0.05$, ** $P < 0.01$).

Data were presented as mean ± SEM. Statistical analyses were determined by log-rank test (a-h). Source data are provided as a Source Data file.



Supplementary Fig. 5. The analysis of HEXB expression in indicated bulk and scRNA-seq glioma and GBM datasets.

a-d The expression analysis of HEXB expression in grade IV IDH1 wild type and IDH1 mutant glioma samples. (a: CMU cohorts n = 30 (IDH1 wild type, n = 26; IDH1 mutant, n = 4), b: CGGA325 n = 139 (IDH1 wild type, n = 98; IDH1 mutant, n = 41), c: CGGA693 n = 239 (IDH1 wild type, n = 190; IDH1 mutant, n = 49), d: TCGA n = 148 (IDH1 wild type, n = 139; IDH1 mutant, n = 9)).

e, f The expression analysis of HEXB in Macrophages and Monocytes (e: Monocyte n = 6, Macrophage n = 3, t-test), as well as M2 and M1 macrophages (f: M2 n = 3, M1 n = 3) according to GSE5099 dataset.

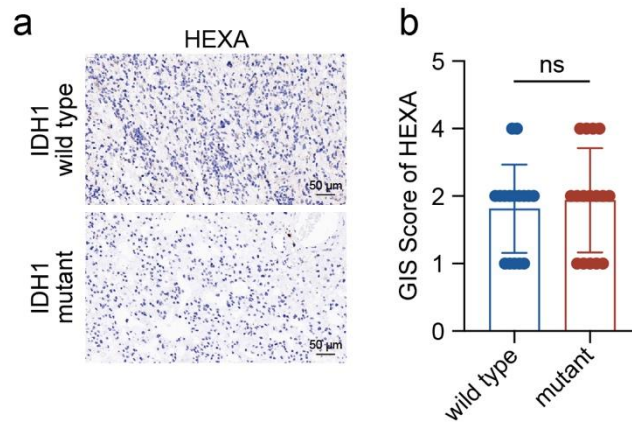
g, h The analysis of HEXB expression in indicated subgroups of grade IV glioma patients in CGGA325 and TCGA cohorts (CGGA325 cohort, Classical n = 46,

Mesenchymal n = 50, Neural n = 14, Proneural n = 29; TCGA cohort, Classical n = 39, Mesenchymal n = 48, Neural n = 26, Proneural n = 36).

i-k Different cell annotations (Left), and the proportion of HEXB positive cells (Count number > 0) (Middle) and the comparative analysis based on HEXB expression in indicated cell populations (Right, t-test) of GBM (i: GSE131928 scRNA-seq GBM cohort (n = 7930): Malignant (GBM cells) n = 6863, Macrophage n = 754, Oligodendrocyte n = 219, T cell n = 94, SMART-seq2; j: GSE139448 scRNA-seq GBM cohort (n = 12444): Cancer cell (GBM cells) n = 11714, Monocyte/Macrophage n = 535, Vascular n = 100, Mast n = 95, 10x Genomics; k: GSE171891 scRNA-seq glioma cohort (n = 4193, 8 GBM sample data were included for the analysis): Cancer cell (GBM cells) n = 2611, Monocyte/Macrophage n = 742, Oligodendrocyte n = 342, Neutrophil n = 134, CD8 T cell n = 45, CD4 T cell n = 41, unknown n = 278, 10x Genomics).

(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Data were presented as mean $\pm$ SEM. Statistical analyses were determined by two-tailed Student's t-test (a-f), and one-way ANOVA test (g-h). Source data are provided as a Source Data file.



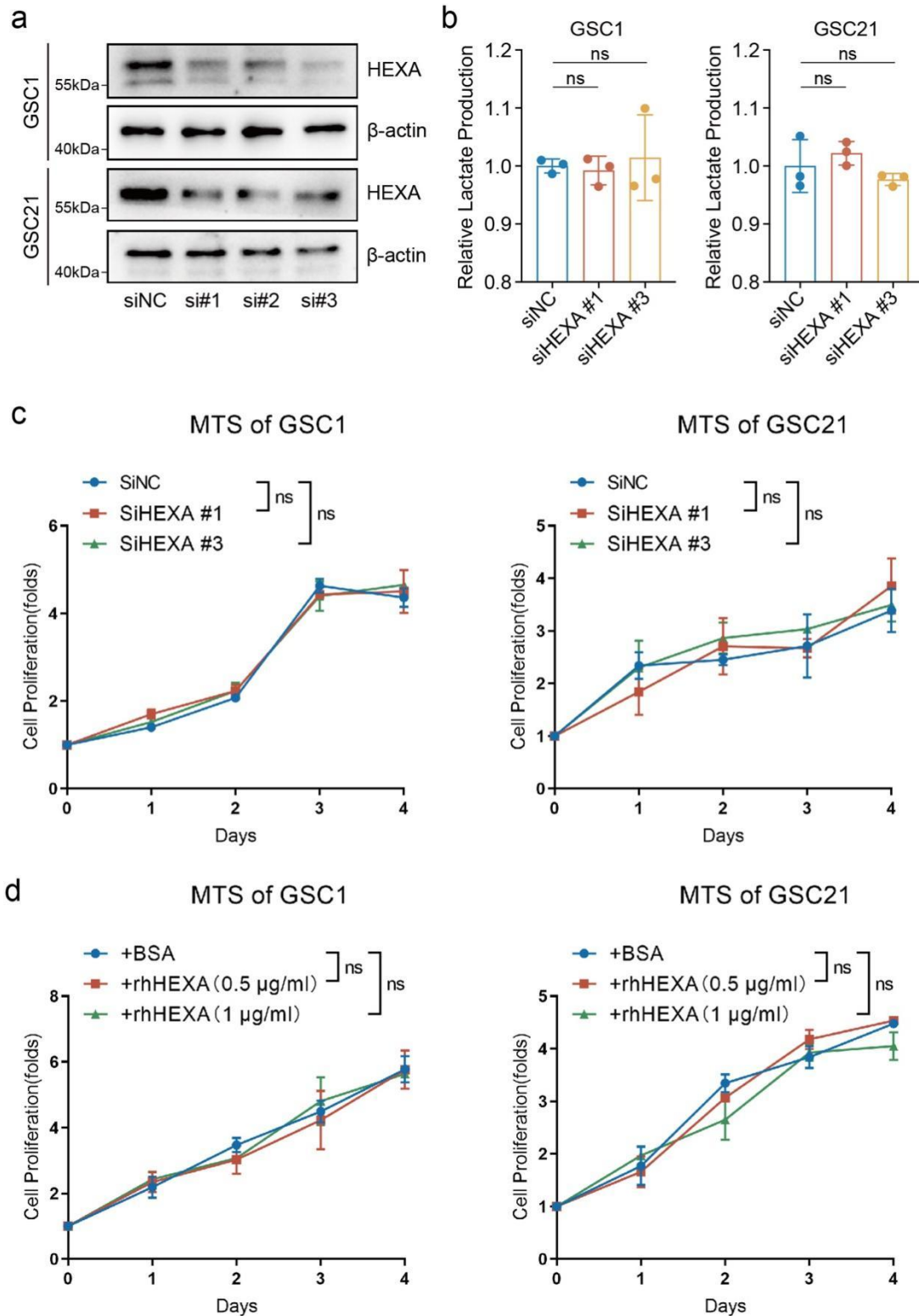
Supplementary Fig. 6. The IHC staining analysis of HEXA in clinical IDH1 wild type (GBM) and grade IV IDH1 mutant glioma tissue samples.

a Representative IHC images of HEXA staining in clinical grade IV IDH1 wild type (GBM) and IDH1 mutant glioma tissue samples.

b The analysis of HEXA in grade IV IDH1 wild type (GBM) and IDH1 mutant glioma tissue samples (n = 32, including IDH wild type n = 16 and IDH mutant n = 16).

(ns $P \geq 0.05$).

Data are representative of 3 independent experiments with similar results (a-b). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by two-tailed Student's t-test (b). Source data are provided as a Source Data file.



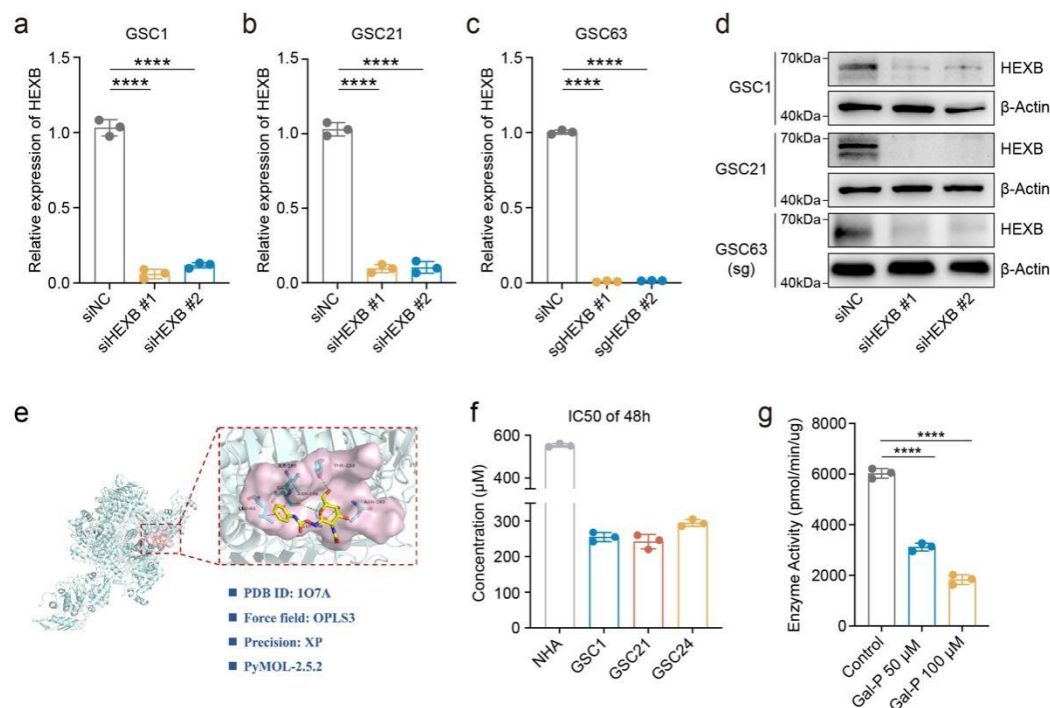
Supplementary Fig. 7. HEXA is not essential for the lactate and proliferation of GBM cells.

a Western blotting analysis of indicated GBM cell samples transduced with siHEXA or control vector, respectively (The samples derive from the same experiment but different gels for HEXA and β -actin were processed in parallel).

b, c The lactate production analysis (b) and MTS assays (c) of GSC1 and GSC21 samples after transduction of siHEXA or control vector, respectively (n = 3).

d MTS assays of GSC1 and GSC21 samples with indicated BSA or recombinant human HEXA protein (rhHEXA) treatment (24 h, 0.5 and 1 µg/ml; n = 3). (ns $P \geq 0.05$).

Data are representative of 3 independent experiments with similar results (a-d). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by one-way ANOVA test (b-d). Source data are provided as a Source Data file.



Supplementary Fig. 8, related to Figure 2. HEXB is a critical modulator for GBM cell proliferation.

a-c qPCR analysis of HEXB knockdown (a: GSC1 and b: GSC21) and knockout (c: GSC63) efficiency in indicated GBM cell samples (n = 3).

d Western blotting analysis of HEXB protein expression in indicated GBM cell samples.

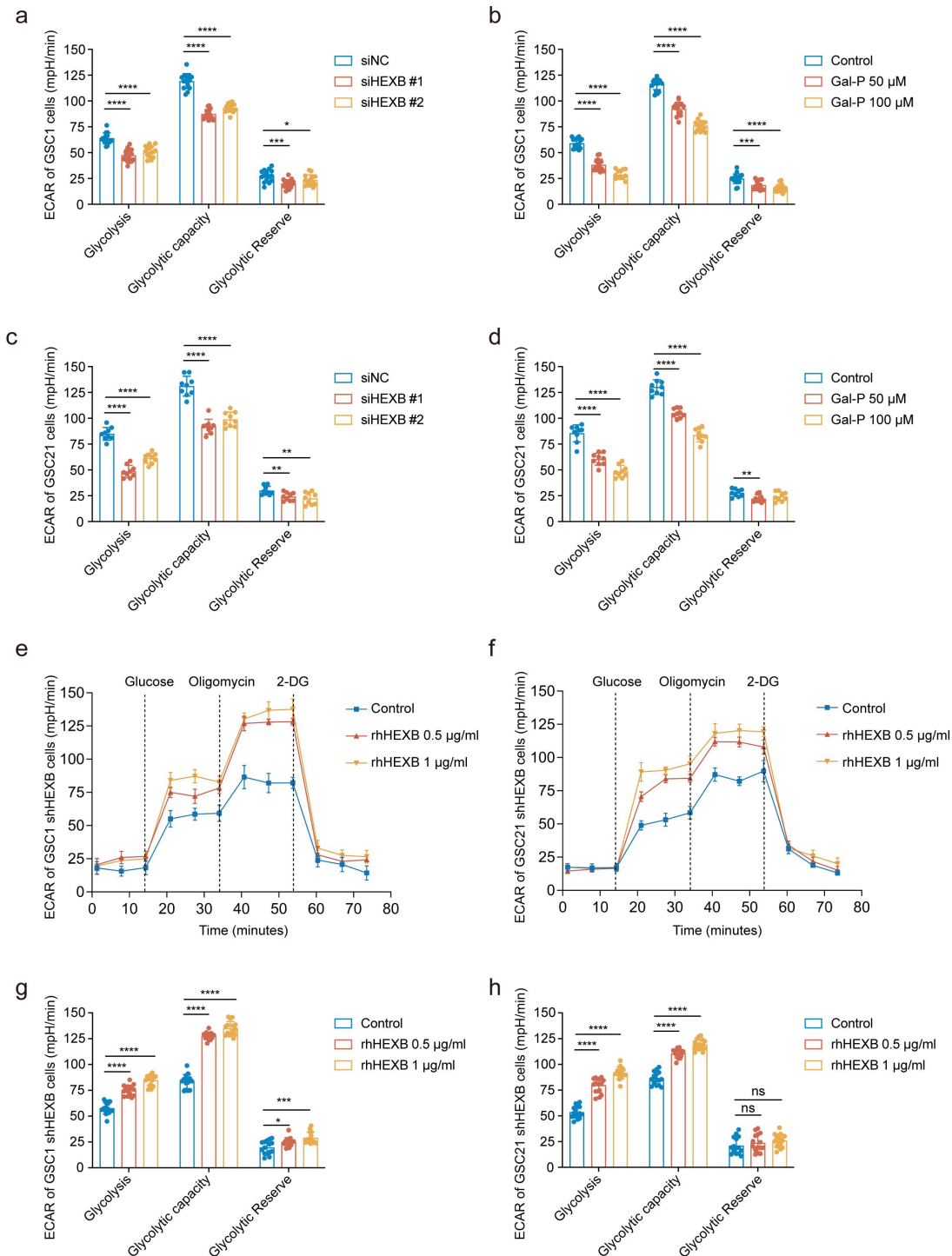
e 3D molecular simulation docking between HEXB and Gal-P based on Swiss-model and HDock software.

f The IC₅₀ analysis of indicated NHA and GSC samples treated with Gal-P (n = 3).

g The analysis of HEXB enzyme activity of recombinant human HEXB protein *in vitro* treated with indicated dose of Gal-P (n = 3).

(**** $P < 0.0001$).

Data are representative of 3 independent experiments with similar results (a-d, f-g). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by one-way ANOVA test (a-c, f-g). Source data are provided as a Source Data file.



Supplementary Fig. 9, related to Figure 2. HEXB serves as a key regulator enhancing glycolytic activity in GBM cells.

a-d The glycolytic rate analysis (ECAR) of indicated GSC samples (a, b: GSC1 n = 5 independent samples and 3 biological replicates for each sample; c, d: GSC21 n = 3 independent samples and 3 biological replicates for each sample) transfected with HEXB silencing or control vector (a, c), or with indicated Gal-P treatment (b, d).

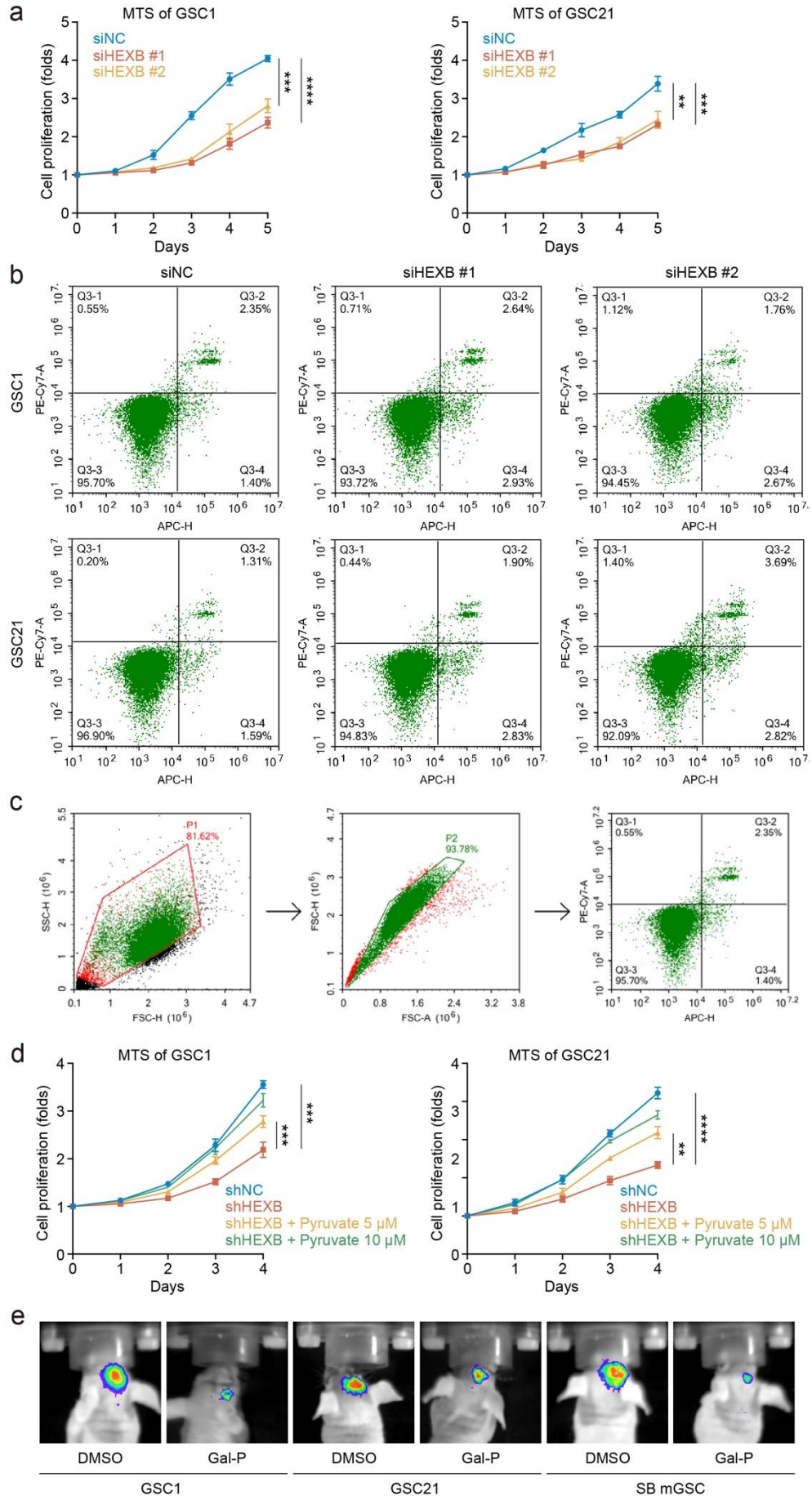
e, f The glycolytic rate (ECAR) analysis of GSC1 (e) and GSC21 (f) transduced with shHEXB vector, and then treated with indicated concentration of recombinant human

HEXB protein or BSA (n = 5 independent samples).

g, h The glycolytic rate (ECAR) analysis of GSC1 (g) and GSC21 (h) transduced with shHEXB vector, and then treated with recombinant human HEXB protein (n = 5 independent samples and 3 biological replicates for each sample).

(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

Data are representative of 3 independent experiments with similar results (a-h). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by one-way ANOVA test (a-d, g-h). Source data are provided as a Source Data file.



Supplementary Fig. 10. HEXB functions as a critical modulator for the proliferation of GBM cells.

a The cell proliferation assays of GSC1 (Left) and GSC21 (Right) transduced with shHEXB or control vector (n = 3).

b Detection of apoptosis level in indicated HEXB knockdown and negative control GBM cell samples.

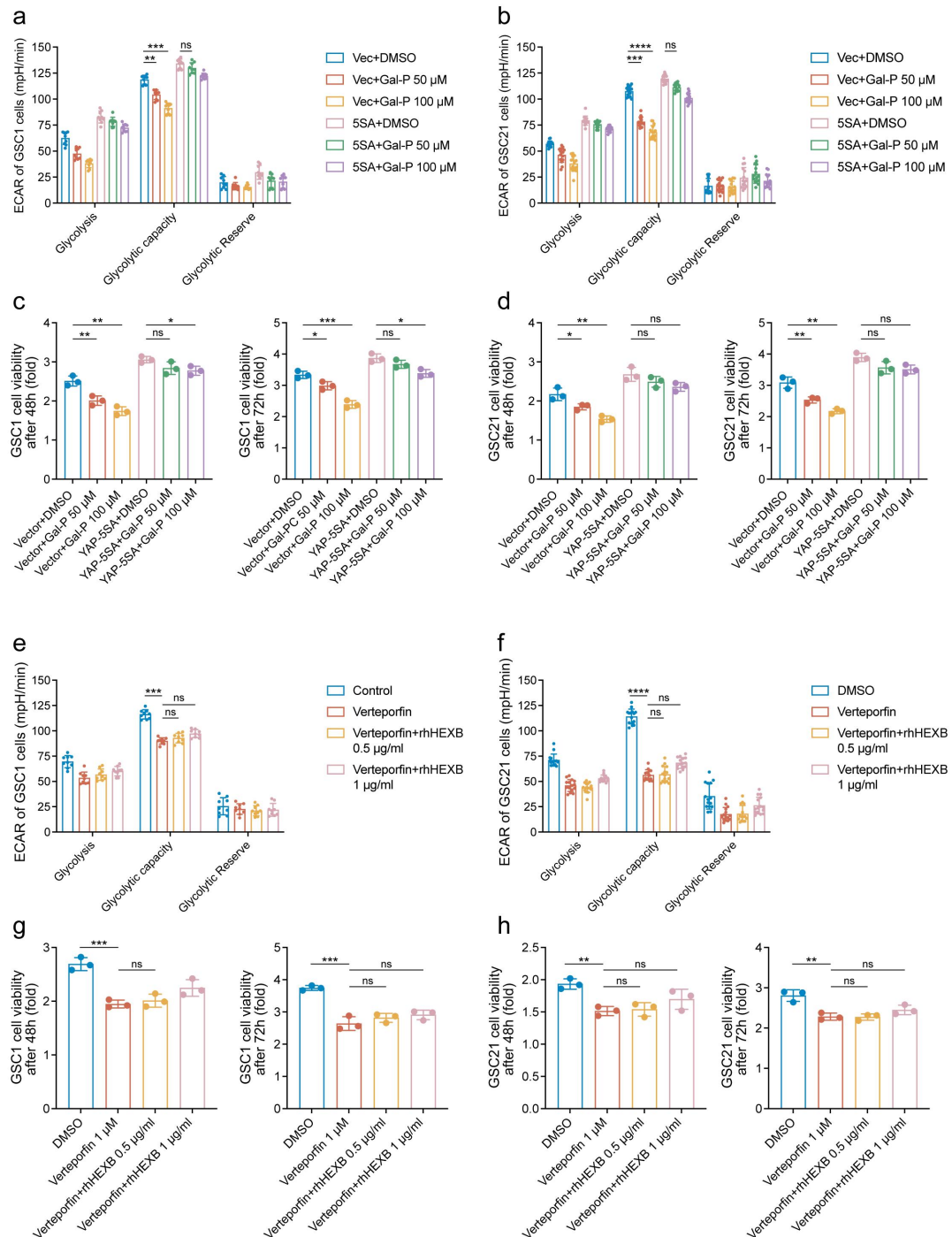
c FACS sorting strategies of data in Supplementary Fig. 10b.

d The cell proliferation assays of GSC1 and GSC21 transduced with shHEXB or control vector, and then treated with pyruvate at indicated concentrations (n = 3).

e Representative *in vivo* bioluminescence images (21 days) of indicated Gal-P treated (100 μ mol/kg) and control orthotopic xenograft models (Left: GSC1; middle: GSC21; right: SB mGSC).

(ns $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

Data are representative of 3 independent experiments with similar results (a-e). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by one-way ANOVA test (a, d). Source data are provided as a Source Data file.



Supplementary Fig. 11, related to Fig. 2. HEXB facilitates the glycolysis of GBM cells by activating YAP1.

a, b The glycolytic activity analysis (ECAR) of indicated YAP-5SA or control vector transfected GBM cells with Gal-P or DMSO administration (a: GSC1 n = 3 independent samples and 3 biological replicates for each sample, b: GSC21 n = 5 independent samples and 3 biological replicates for each sample).

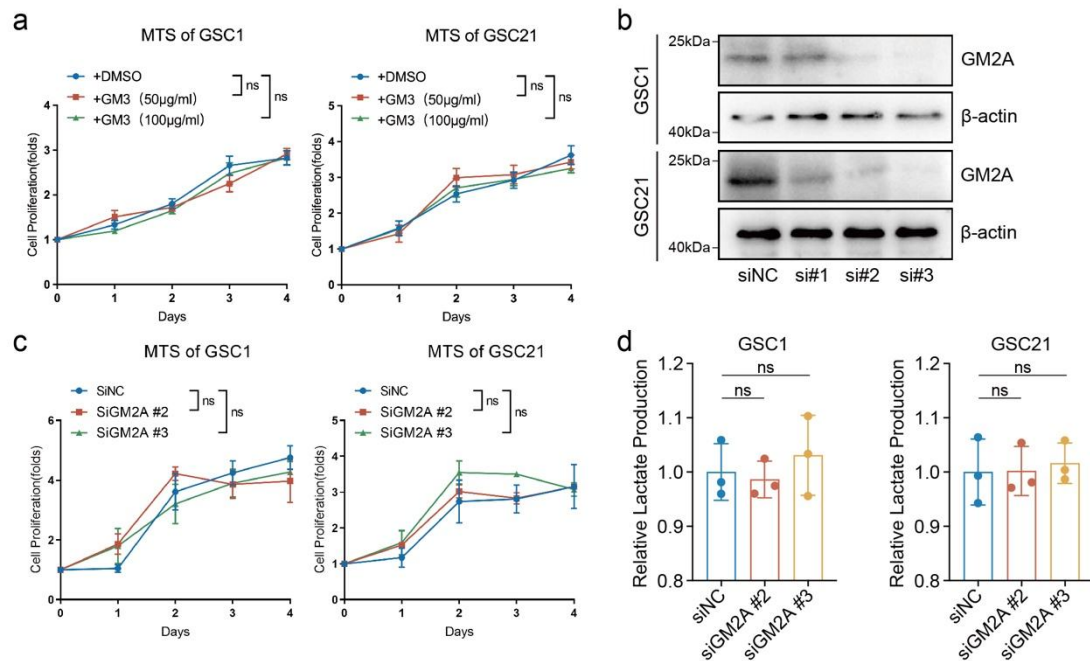
c, d The MTS assays of YAP-5SA or control vector transfected GBM cells with indicated Gal-P treatment (c: GSC1, d: GSC21; Left: 48h, right: 72h; n = 3 independent samples).

e, f The glycolytic activity analysis (ECAR) of YAP1 inhibitor verteporfin pretreated and corresponding control GBM cells with indicated recombinant human HEXB protein treatment (e: GSC1 n = 3 independent samples and 3 biological replicates for each sample, f: GSC21 n = 5 independent samples and 3 biological replicates for each sample).

g, h The MTS assays of YAP1 inhibitor verteporfin pretreated and corresponding control GBM cells with indicated recombinant human HEXB protein treatment (g: GSC1, h: GSC21; Left: 48h, right: 72h; n = 3 independent samples).

(ns $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

Data are representative of 3 independent experiments with similar results (a-h). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by one-way ANOVA test (a-h). Source data are provided as a Source Data file.



Supplementary Fig. 12. GM3 and GM2A are not essential for promoting the proliferation and lactate production of GBM cells.

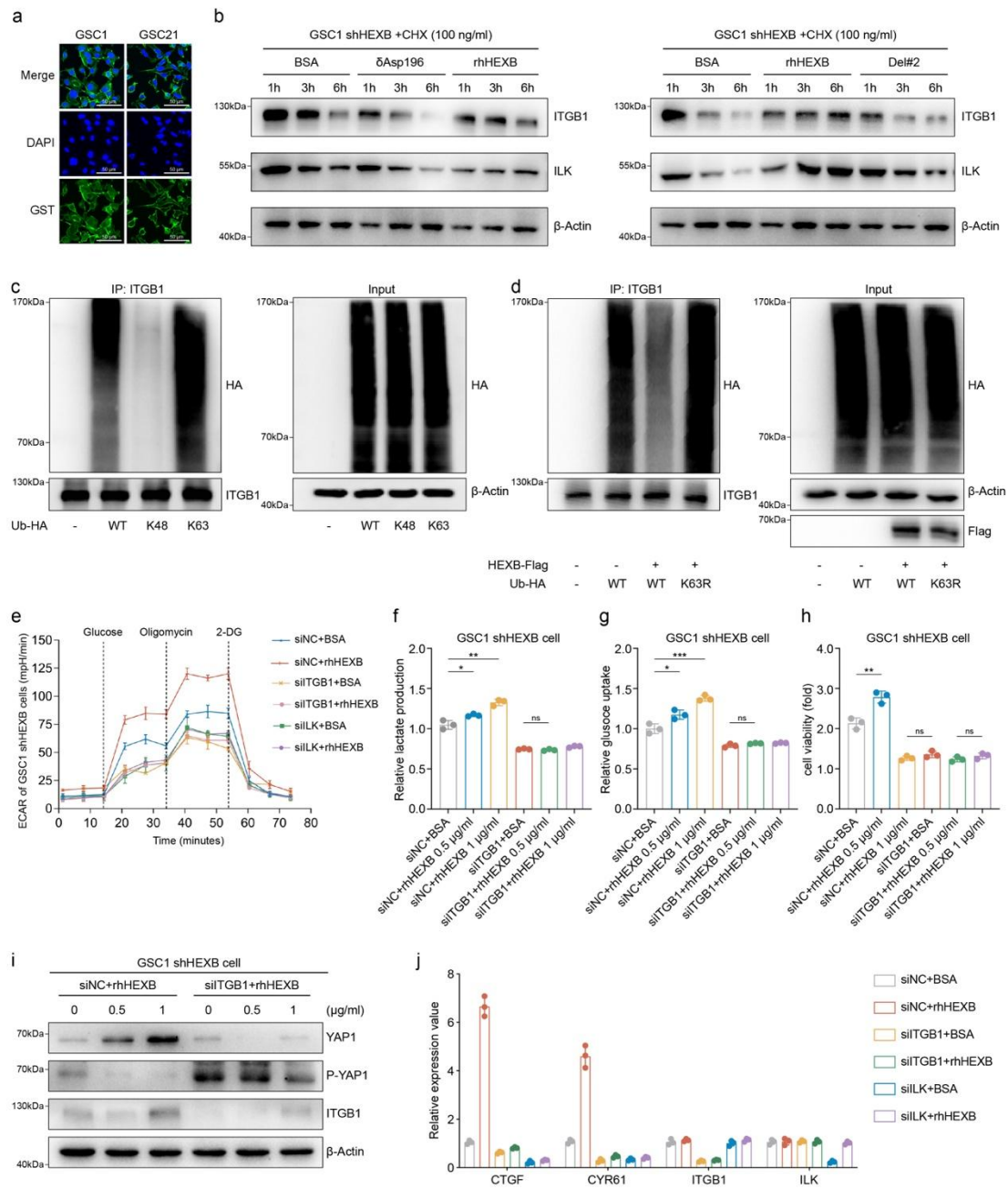
a MTS assays indicating that the proliferation ability of indicated GBM cells (Left: GSC1, and right: GSC21) was not significantly restrained by indicated concentrations of GM3 treatment (50 ng/ml and 100 ng/ml; $n = 3$ independent samples).

b Western blotting analysis of indicated GBM cells (GSC1 and GSC21) transduced with indicated siGM2A or non-targeted vectors (The samples derive from the same experiment but different gels for GM2A and β-actin were processed in parallel).

c, d MTS (c) and lactate production analysis (d) of indicated GBM cells (Left: GSC1, and right: GSC21) transduced with indicated siGM2A or non-targeted vectors ($n = 3$ independent samples).

(ns $P \geq 0.05$).

Data are representative of 3 independent experiments with similar results (a-d). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by one-way ANOVA test (a, c-d). Source data are provided as a Source Data file.



Supplementary Fig. 13. HEXB promotes YAP1 activation by stabilizing the ITGB1/ILK complex.

a The immunofluorescence staining of indicated GBM cells treated with GST-recombinant human HEXB (Green: GST; Blue: DAPI).

b Western blotting analysis of ITGB1 and ILK protein degradation rates in HEXB silenced GSC1 cells treated with BSA, HEXB exogenous protein, Asp196 mutant protein (Left), or fragment deletion HEXB protein (Right). (The samples derive from the same experiment but different gels for ITGB1, ILK, and β-actin were processed in parallel).

c IP analysis of ITGB1 ubiquitination in HEK293T cells transduced with indicated ubiquitin or control vectors (The samples derive from the same experiment but

different gels for ITGB1, HA, Flag, and β -actin were processed in parallel).

d IP analysis of ITGB1 ubiquitination in HEK293T cells transduced with indicated HEXB overexpression or control vector, and followed by the transfection of ubiquitin K63 or K63R mutant vector, respectively.

e, f The glycolytic activity (ECAR, **e**), and lactate production analysis (**f**) of indicated GSC1 cells treated with recombinant human HEXB protein or BSA, respectively ($n = 3$ independent samples).

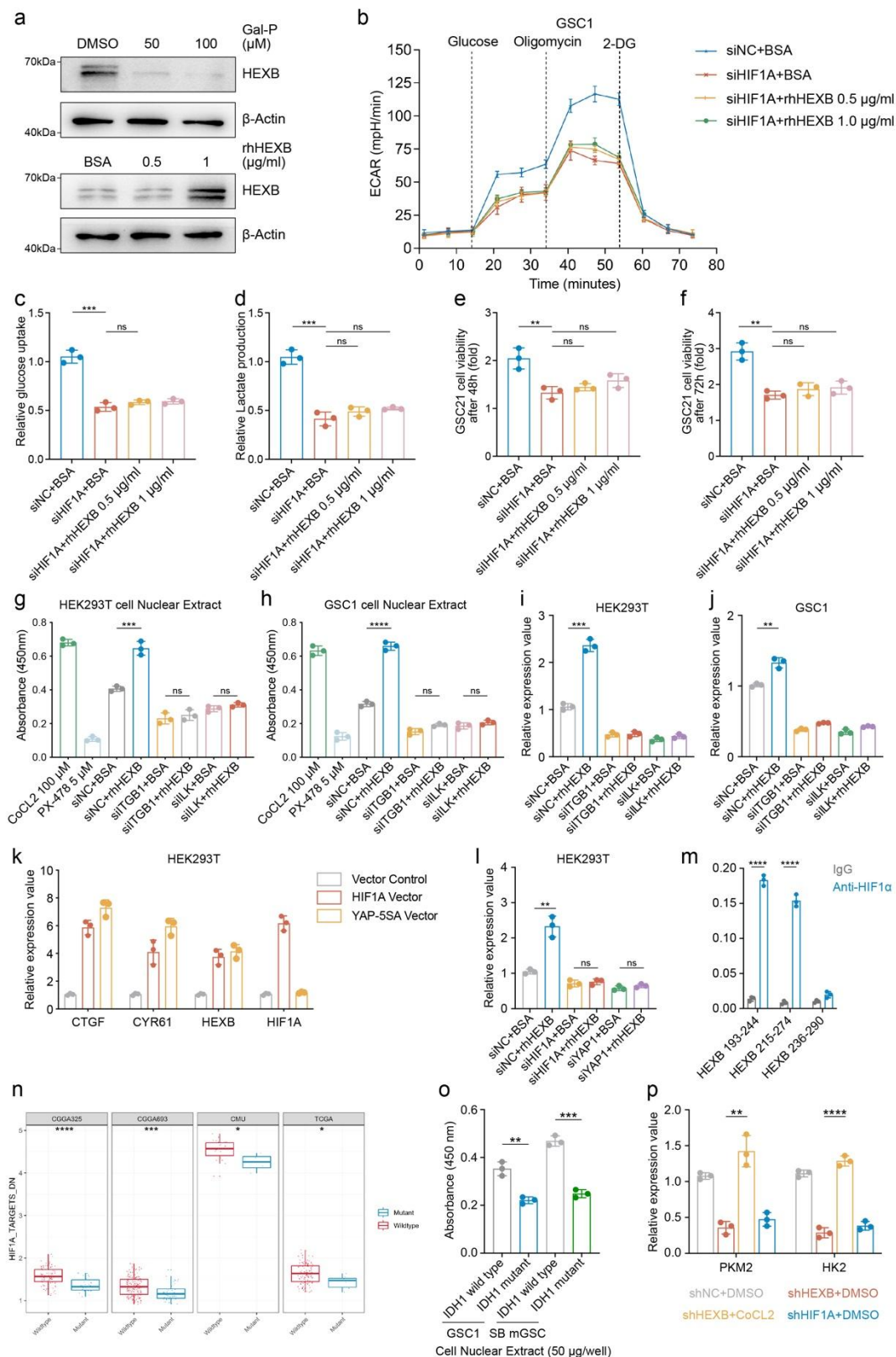
g, h The glucose uptake (**g**) and proliferation rate analysis (**h**) of indicated GSC1 shHEXB cells treated with recombinant human HEXB protein or BSA ($n = 3$ independent samples).

i Western blotting analysis of YAP1 and p-YAP1 in HEXB-knockdown GSC1 transduced with control or siITGB1 vector, respectively, and then treated with indicated concentration of recombinant human HEXB protein (The samples derive from the same experiment but different gels for ITGB1, YAP1, P-YAP1, and β -actin were processed in parallel).

j qPCR analysis of CTGF, CYRB1, ITGB1 and ILK mRNA in HEXB-knockdown GSC1 transduced with control, siITGB1 or siILK vector, respectively, and then treated with recombinant human HEXB protein (1 μ g/ml) or BSA ($n = 3$ independent samples).

(ns $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Data are representative of 3 independent experiments with similar results (a-j). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by one-way ANOVA test (f-h, j). Source data are provided as a Source Data file.



Supplementary Fig. 14. YAP1/HIF1 α regulates HEXB transcription in GBM cells.

a Western blotting analysis of HEXB in GSC1 treated with indicated concentration of Gal-P (Upper panel, 24 h) or HEXB silencing GSC1 treated with indicated

recombinant human HEXB protein (Lower panel, 24 h).

b The glycolytic analysis (ECAR) in HIF1 α knockdown GSC1 with indicated recombinant human HEXB protein administration (n = 5 independent samples).

c-f The analysis of glucose uptake (c), lactate production (d), and proliferation rate (e: 48 h, f: 72 h) in HIF1 α knockdown GSC1 with indicated recombinant human HEXB protein or bovine serum albumin (BSA) administration (n = 3 independent samples).

g, h The analysis of HIF1 α transcriptional activity in indicated HEK293T (g) or GSC1 samples (h) with indicated recombinant human HEXB protein or bovine serum albumin (BSA) administration (n = 3 independent samples).

i, j The qPCR analysis of HEXB mRNA in HEK293T (i) or GSC1 samples (j) with indicated recombinant human HEXB protein or bovine serum albumin (BSA) administration (n = 3 independent samples).

k The analysis of transfection efficiency in indicated HEK293T cells with HIF1 α or YAP-5SA vector (n = 3 independent samples).

l The analysis of HEXB in HEK293T cell with indicated recombinant human HEXB protein or bovine serum albumin (BSA) administration (n = 3 independent samples).

m ChIP analysis of indicated HEXB promoter fragments in HEK293T cells precipitate pull-downed by HIF1 α antibody (n = 3 independent samples).

n The analysis of HIF1 α downstream signaling pathway activation in IDH1 wild type (GBM) and IDH1 mutant grade IV glioma based on a HIF1 α score established by ssGSEA method according to a HIF1 α downstream target gene set (ELVIDGE_HIF1A_TARGETS_DN,

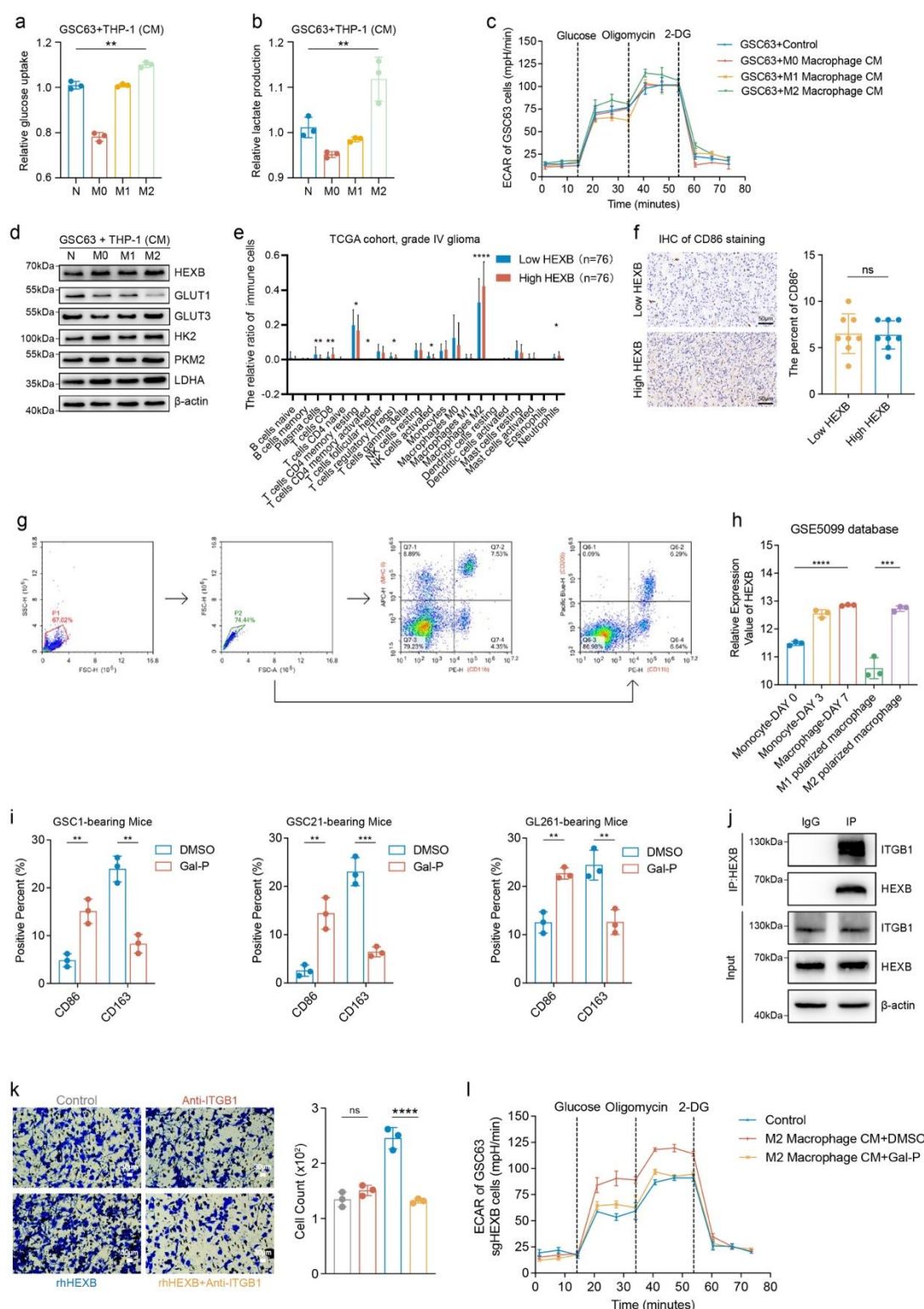
https://www.gsea-msigdb.org/gsea/msigdb/cards/ELVIDGE_HIF1A_TARGETS_DN.html) in indicated glioma cohorts (CMU cohorts n = 30 (IDH1 wild type, n = 26; IDH1 mutant, n = 4), CGGA325 n = 139 (IDH1 wild type, n = 98; IDH1 mutant, n = 41), CGGA693 n = 239 (IDH1 wild type, n = 190; IDH1 mutant, n = 49), TCGA n = 148 (IDH1 wild type, n = 139; IDH1 mutant, n = 9)).

o The analysis of HIF1 α transcriptional activities in indicated IDH1 wild type and mutant glioma cell samples (n = 3 independent samples).

p The qPCR analysis of PKM2 and HK2 mRNA in GSC1 pretreated with DMSO or CoCL₂ (100 μ M) and then separately transduced with non-target, shHEXB or shHIF1 α vector (n = 3 independent samples).

(ns $P \geq 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

Data are representative of 3 independent experiments with similar results (a-m, o-p). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by one-way ANOVA test (c-l, p), and two-tailed Student's t-test (m-o). Source data are provided as a Source Data file.



Supplementary Fig. 15. HEXB and M2 tumor associated macrophages form an intercellular positive feedback regulation loop.

a, b The glucose consumption (a) and lactate production analysis (b) in GSC63 cells treated with conditional medium (CM) from indicated macrophages (n = 3 independent samples).

c The glycolytic analysis (ECAR) of GSC63 with indicated treatment (n = 3

independent samples).

d Western blotting analysis of HEXB and indicated glycolytic markers in GSC63 treated with CM from indicated polarized macrophages derived from THP-1 cells (The samples derive from the same experiment but different gels for HEXB, GLUT1, GLUT3, HK2, PKM2, LDHA, and β -actin were processed in parallel).

e The analysis of relative immune cell ratio in grade IV glioma samples, according to TCGA (High HEXB $n = 76$, low HEXB $n = 76$)

f Representative IHC images (left) and positive cell ratio analysis (right) of CD86⁺ staining in GBM samples with low and high HEXB expression ($n = 16$, including low HEXB $n = 8$, high HEXB $n = 8$).

e FACS sorting strategies of data in Figure 6 d and e.

h The expression analysis of HEXB in indicated polarized stages of macrophage (GSE5099 cohort, $n = 3$).

i IHC analysis of macrophages polarization markers (CD86 and CD163) in indicated *in vivo* orthotopic glioma model with Gal-P or DMSO treatment (($n = 3$ independent samples)).

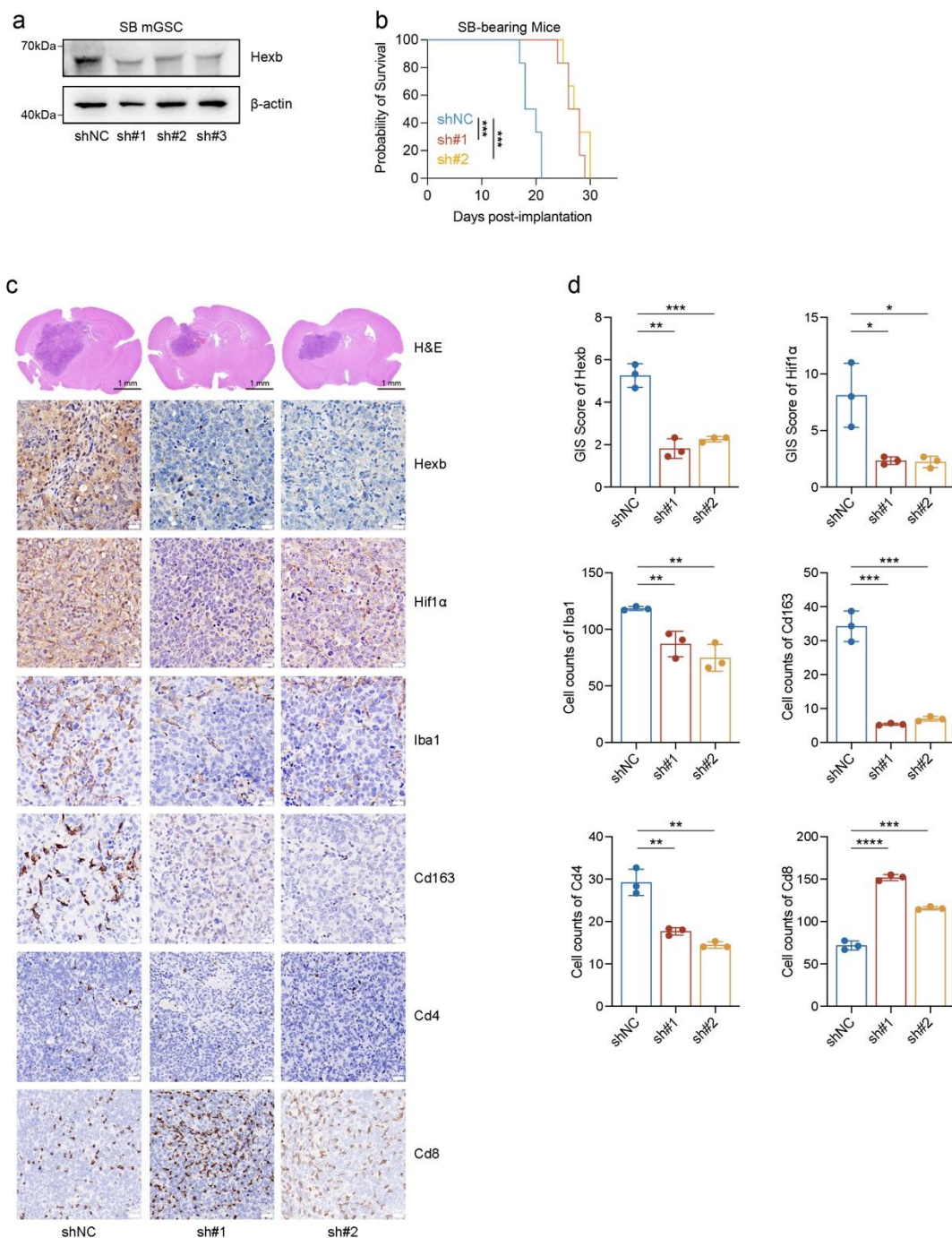
j Co-IP analysis of HEXB and ITGB1 interaction in THP1-derived macrophages revealed that HEXB could interact with ITGB1 (The samples derive from the same experiment but different gels for ITGB1, HEXB, and β -actin were processed in parallel).

k The migration ability analysis of THP-1 derived macrophages with indicated treatment (Transwell assays; ($n = 3$ independent samples)).

l The analysis of glycolytic rate (ECAR) of sgHEXB GBM cells (GSC63) with treatment of conditioned medium (CM) obtained from THP-1 derived M2 macrophages or HEXB inhibitor Gal-P ($n = 3$ independent samples).

(ns $P \geq 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

Data are representative of 3 independent experiments with similar results (a-d, f-l). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by one-way ANOVA test (a-b, h, k), and two-tailed Student's t-test (e-f, i). Source data are provided as a Source Data file.



Supplementary Fig. 16. Hexb knockdown in mGSCs reverses the immunosuppressive microenvironment in GBM.

a Western blotting analysis of Hexb expression in indicated SB mGSCs after Hexb-knockdown lentiviruses transfections (The samples derive from the same experiment but different gels for hexb and β -actin were processed in parallel).

b The survival analysis of tumor-bearing mice after intracranial implantation of SB mGSCs transduced with indicated Hexb knockdown or negative control vector (n = 6 mice).

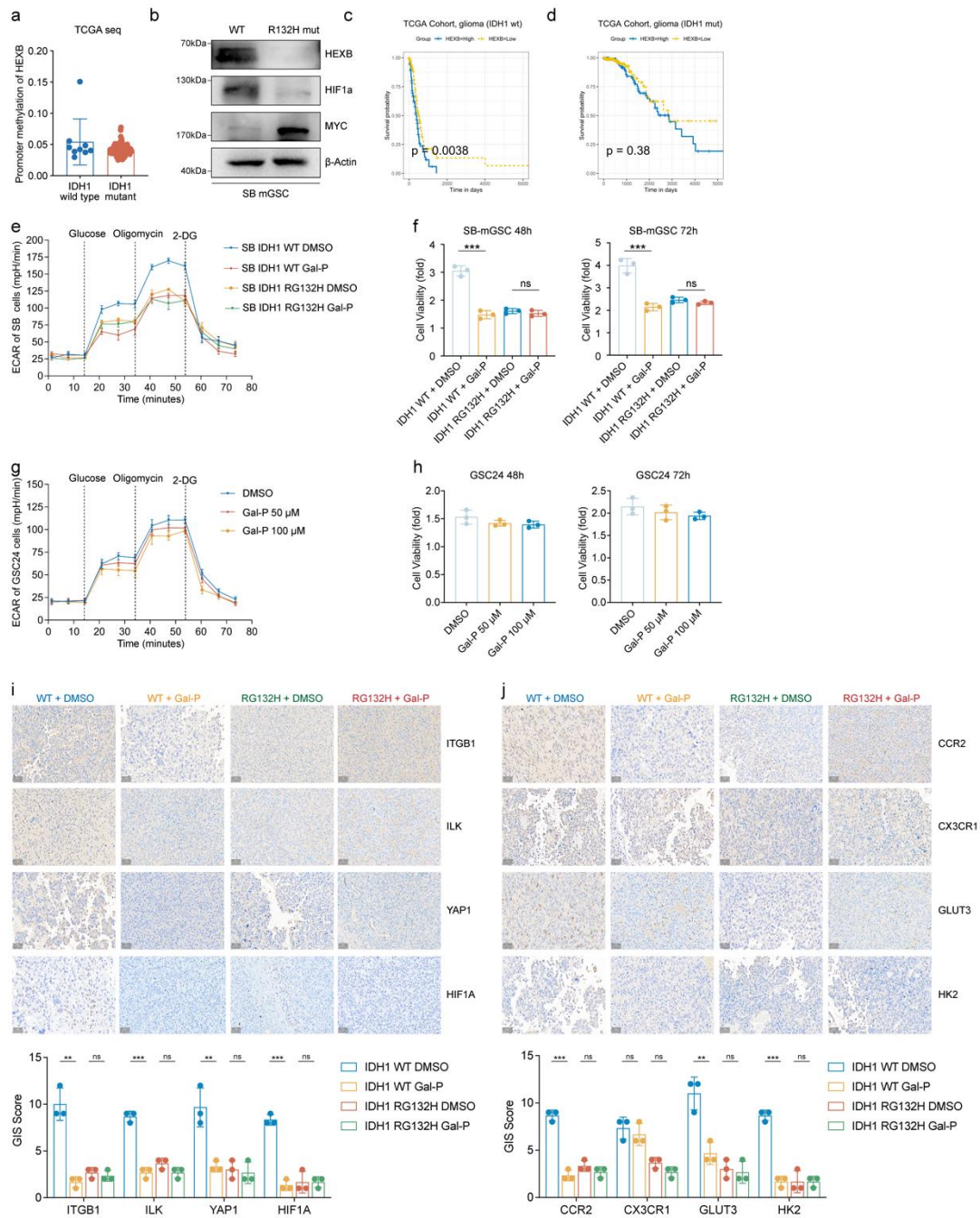
c Representative H&E (day 16) and IHC staining images (Cd4, Cd8, Hexb, Hif1 α ,

Iba1, and Cd163) of mice tumor section derived from intracranial implantation of SB mGSCs transduced with indicated Hexb knockdown or negative control vector.

d The IHC staining analysis of indicated markers (Hexb, Hif1 α , Cd4, Cd8, Iba1 and Cd163) in mice tumor section derived from intracranial implantation of SB mGSCs transduced with indicated Hexb knockdown or negative control vector (n = 3).

(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

Data are representative of 3 independent experiments with similar results (a-d). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by log-rank test (b) and one-way ANOVA test (d). Source data are provided as a Source Data file.



Supplementary Fig. 17. IDH1 mutation restrains the efficiency of HEXB-targeted intervention in glioma.

a The methylation analysis of HEXB promoter in grade IV IDH1 wild type (GBM) and mutant glioma (TCGA cohort, IDH1 wild type n = 135, mutant n = 8).

b Representative western blotting images of HEXB and HIF1α in IDH1 wild type and IDH1 mutant mGSCs derived from mouse GBM tumors established by IDH1 wild type and mutant Sleeping Beauty (SB) transposon vectors, respectively (The samples derive from the same experiment but different gels for HEXB, HIF1A, MYC, and β-actin were processed in parallel).

c, d Overall survival curves stratified by HEXB in IDH1 wild type and mutant glioma in TCGA cohort (IDH1 wild type, n = 224; IDH1 mutant, n = 373).

e ECAR assay showing the glycolytic rate in the IDH1 wild type and mutant SB mGSCs with indicated Gal-P treatment.

f MTS assay demonstrating the cell viability in the IDH1 wild type and mutant SB mGSCs with indicated Gal-P treatment (n = 3 independent samples).

g ECAR assay showed the glycolytic rate in GSC24 (IDH1 mutant) cell models with indicated Gal-P treatment (n = 5 independent samples).

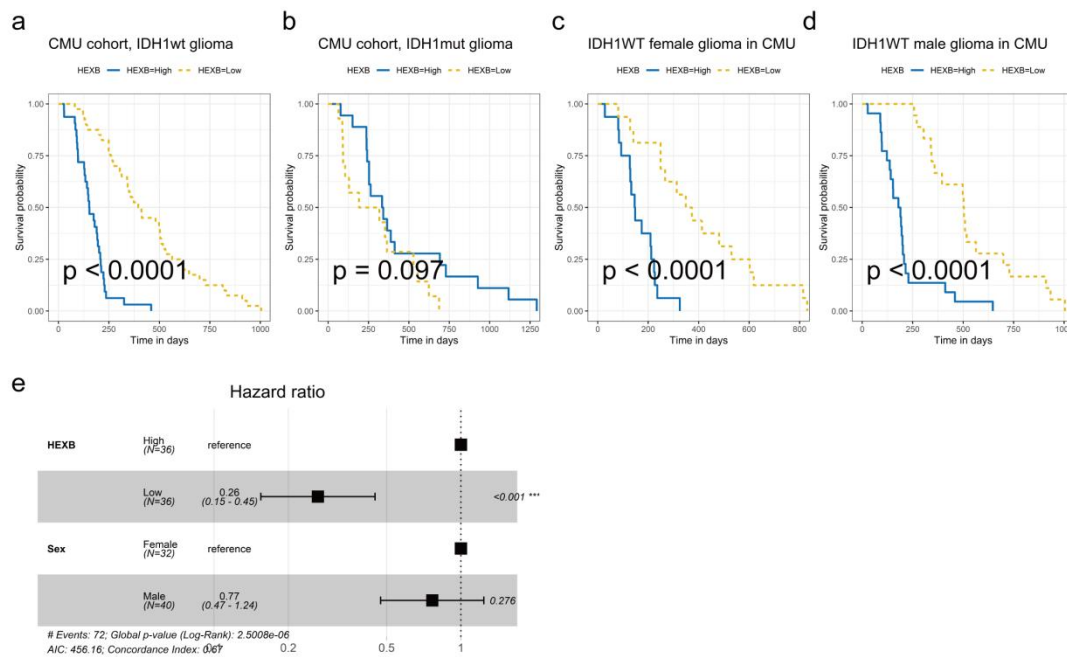
h MTS assays showed the cell viability in GSC24 (IDH1 mutant) cell models with indicated Gal-P treatment (n = 3 independent samples).

i Representative IHC images of ITGB1, ILK, YAP1 and HIF1 α staining in GBM tumor derived from GSC1 transduced with control or IDH1 RG132H vector, respectively, and then treated with Gal-P or DMSO (n = 3 independent samples).

j Representative IHC images of indicated macrophage and glycolytic markers staining (CCR2, CX3CR1, GLUT3 and HK2) in GBM tumor derived from GSC1 transduced with control or IDH1 RG132H vector, respectively, and then treated with Gal-P or DMSO (n = 3 independent samples).

(ns $P \geq 0.05$, *** $P < 0.001$, **** $P < 0.0001$).

Data are representative of 3 independent experiments with similar results (b, e-j). Data were presented as mean $\pm$ SEM. Statistical analyses were determined by two-tailed Student's t-test (a, f, i-j), log-rank test (c-d) and one-way ANOVA test (h). Source data are provided as a Source Data file.



Supplementary Fig. 18. HEXB as a risky prognostic factor in IDH1 wild type glioma independent of Sex.

a, b Overall survival curves stratified by HEXB expression in CMU IDH1 wild type and mutant glioma samples (IDH1 wild type, $n = 72$ (high group, $n = 32$; low group, $n = 40$); IDH1 mutant, $n = 32$ (high group, $n = 18$; low group, $n = 14$)).

c, d Overall survival curves of IDH wild type glioma patient stratified by HEXB in CMU glioma dataset according to sex (female, $n = 32$ (high group, $n = 16$; low group, $n = 16$); male, $n = 40$ (high group, $n = 22$; low group, $n = 18$)).

e Multivariate Cox analysis of HEXB expression and sex in CMU glioma dataset ($n = 72$) disclosing HEXB as a prognostic factor independent of sex in IDH1 wild type glioma.

(*** $P < 0.001$).

Data were presented as mean $\pm$ SEM. Statistical analyses were determined by log-rank test (a-e). Source data are provided as a Source Data file.

Supplementary Table 1. Clinical sample and related molecular information of CMU, CGGA325, CGGA693, and TCGA glioma datasets included in the study.

Cohort	CMU (n = 30)	CGGA325 (n = 139)	CGGA693 (n = 249)	TCGA (n = 152)
Age (years)	55.37 ± 16.25	46.55 ± 12.57	48.82 ± 13.86	59.86 ± 13.58
Sex				
Male	19	88	147	98
Female	11	51	102	54
Survival (days)	NA	570.9± 678.5	616.1± 634.5	351.4± 309.1
Available	0	137	237	152
IDH1 status				
Wild type	26	98	190	139
Mutant	4	41	49	9
Unavailable	0	0	10	4
MGMT Promoter Methylation Status				
Unmethylated	17	70	106	70
Methylated	11	66	93	52
Unavailable	2	3	50	30
1p19q Codeletion Status				
Non-codeletion	22	129	205	147
Codeletion	6	7	13	0
Unavailable	2	3	31	5

Supplementary Table 2. Survival analysis of HEXB and GUSB in glioma and grade IV glioma according to CGGA325, CGGA693 and TCGA datasets
(Statistical analyses were determined by log-rank test)

	CGGA325, Glioma	CGGA693, Glioma	TCGA, Glioma	CGGA325, GBM	CGGA693, GBM	TCGA , GBM
HEXB P value	< 0.0001 (Risk factor)	< 0.0001 (Risk factor)	< 0.0001 (Risk factor)	0.0088 (Risk factor)	0.3	0.016 (Risk factor)
GUSB P value	< 0.0001 (Risk factor)	< 0.0001 (Risk factor)	< 0.0001 (Risk factor)	0.89	0.12	0.37

Supplementary Table 3. The sequences information of shRNAs, siRNAs and primers for qPCR employed in present study.

shRNAs and siRNAs	Sense (5'-3')	Antisense (5'-3')
HEXB shRNA/(siRNA1)	CCAUCAUGAAUCCACCAUTT	AUGGUGGAUUCAUUGAUGGTT
HEXB siRNA2	CCGUAUGGUGAUUGAAUAUTT	AUAUCAAUCACCAUACGGTT
HEXB sgRNA1	CCACCATTATTGATTCTCCA	TGGAGAATCAATAATGGTGG
HEXB sgRNA	TCCTTAAGGCCAACAGAGTT	AACTCTGTTGGCCTTAAGGA
ITGB1 siRNA	GGCUCCAAAGAUUAAAAGATT	UCUUUAUAUCUUUGGAGCCTT
ILK siRNA	CAGCUUAACUUCUGACGATT	UCGUCAGGAAGUUAAGCUGTT
HIF1A siRNA	CUGAUGACCAGCAACUUGA	UCAAGUUGCUGGUCAUCAG
YAP1 siRNA	GACAUCUUCUGGUCAGAGATT	UCUCUGACCAGAAGAUGUCTT
HEXA siRNA1	GUUGGAUACAUCUCGCCAUUATT	UAAUGGCGAGAUGUAUCCAACTT
HEXA siRNA3	GCGAGAGGAUAUUCAGUGAATT	UUCACUGGAAUAUCCUCUCGCTT
GM2A siRNA2	GAUCCCAUGCACAGACUACAUTT	AUGUAGUCUGUGCAUGGGAUCTT
GM2A siRNA3	UCCUGGGAUAAACUGUGAUGAATT	UUCAUCACAGUUAUCCAGGATT
NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Primers for RT-qPCR	Forward (5'-3')	Reverse (5'-3')
HEXB	GTCAGAGTGTGATGCTTTCCC	TAAACCTCGTAATGCTCCCCA
18S	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
ITGB1	CCTACTTCTGCACGATGTGATG	CCTTTGCTACGGTTGGTTACATT
ILK	GACGACATTTTCACTCAGTGCC	ACGGTTCATTACATTGATCCGTG
CTGF	CCTGGTCCAGACCACAGAGT	ATGTCTTCATGCTGGTGCAG
CYR61	GGTCAAAGTTACCGGGCAGT	GGAGGCATCGAATCCCAGC
HIF1A	AGCACAGTTACAGTATTCCAGCAGAC	TCATCAGTGGTGGCAGTGGTAGT
YAP1	TAGCCCTGCGTAGCCAGTTA	TCATGCTTAGTCCACTGTCTGT
CD163	GCGGGAGAGTGGAAGTGAAAG	GTTACAAATCAGAGACCGCT
ARG1	CCCTGGGGAACACTACATTTTG	GCCAATTCCTAGTCTGTCCACTT
IL1B	TTCGACACATGGGATAACGAGG	TTTTTGCTGTGAGTCCCGGAG
MHCII	TGTCCACCAACCTGATCCGTA	CCACGGTGACAGGACTATCCA
PKM2	ATGTCGAAGCCCCATAGTGAA	TGGGTGGTGAATCAATGTCCA
HK2	GAGCCACCACTCACCTACT	CCAGGCATTCGGCAATGTG
HEXB 193-244	ATACCACCCAGATGGAAAAG	GTTTCTGCTGATATTTTGAGG
HEXB 215-274	TGTGAATCTGCCGCCTCT	ATGTGTTCTGGGCCTAGCA
HEXB 236-290	TGTTTATGCTCCTGTATGT	AGTATTTCTCTTGGGTT