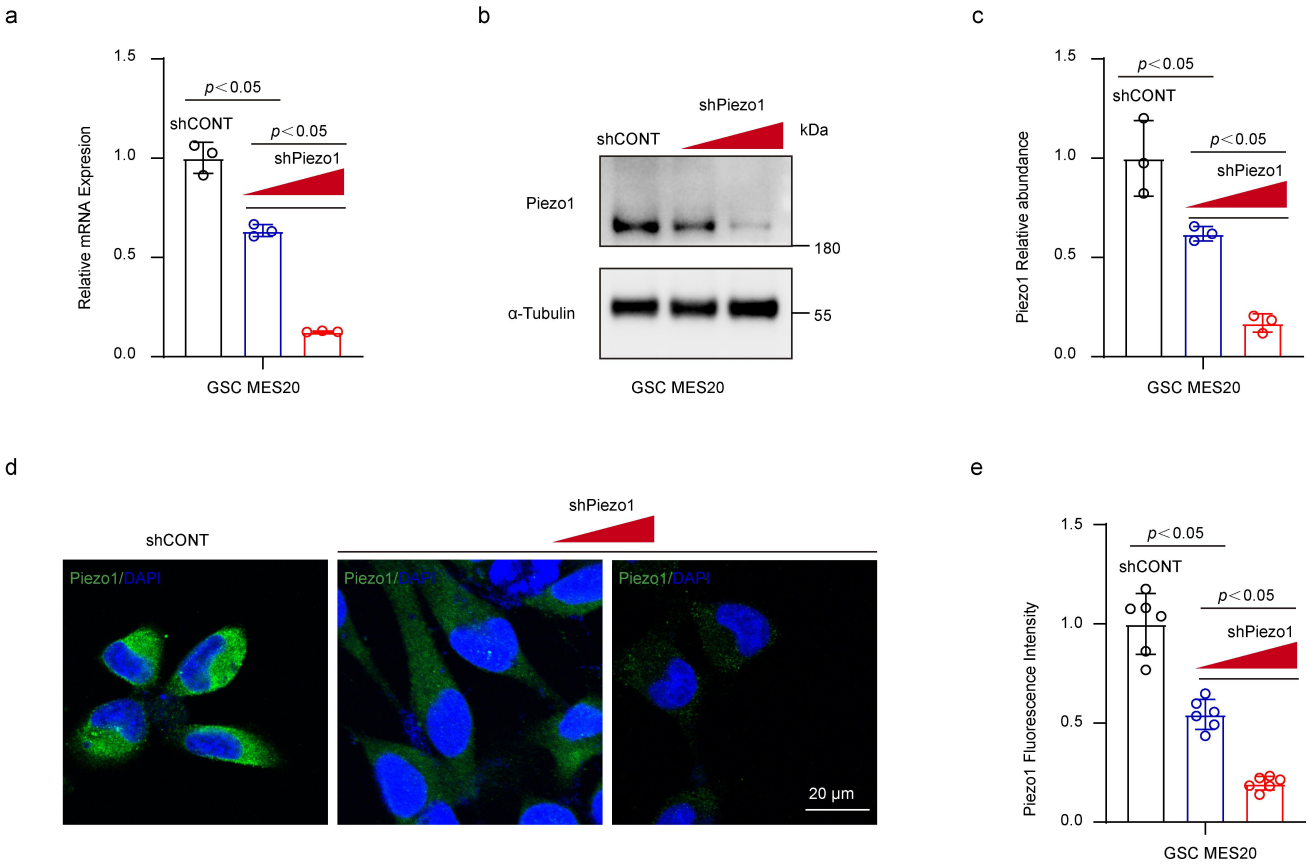


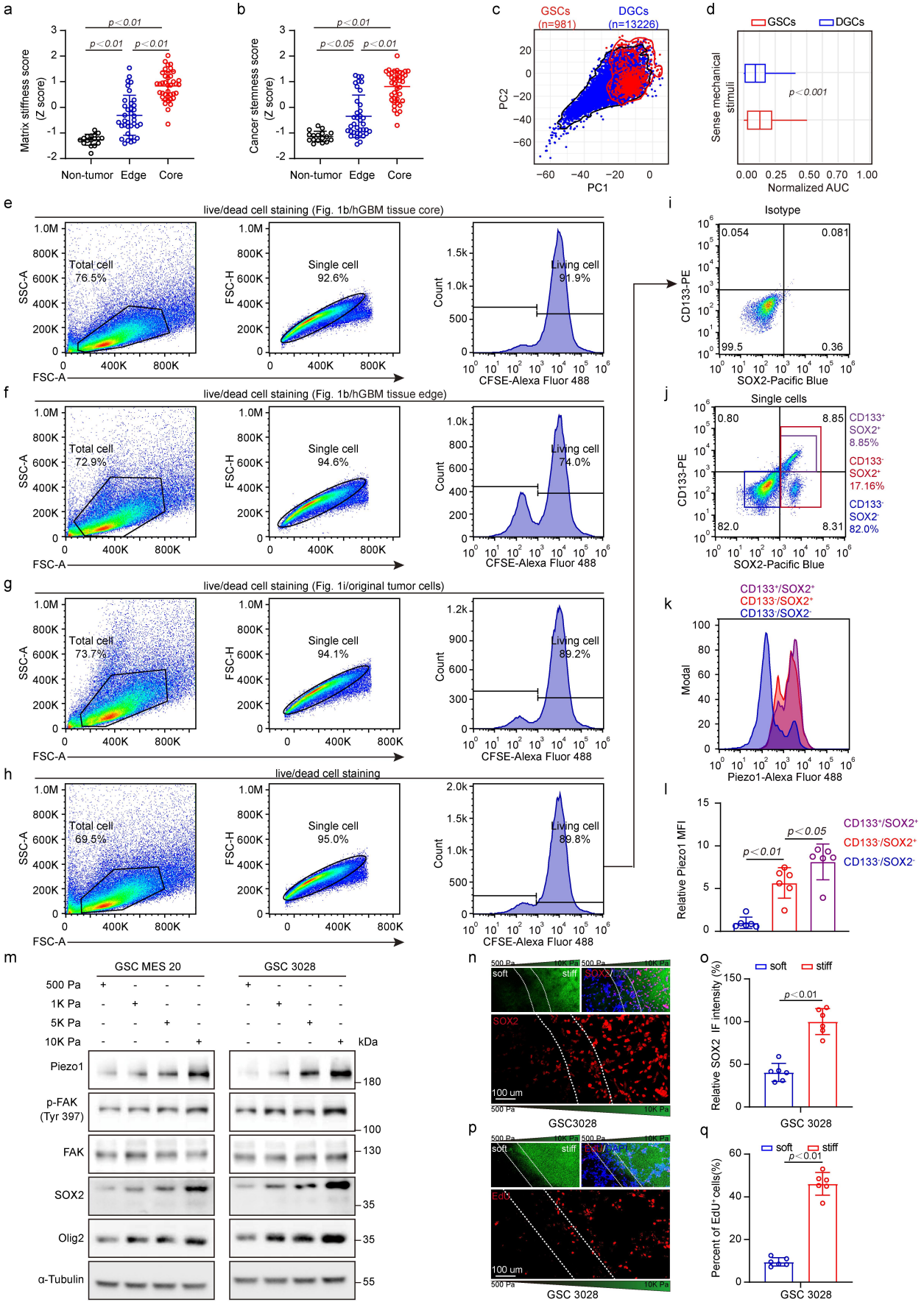
Supplementary Fig. 1

a, MES20 glioblastoma stem cells (GSCs) were transduced with either a non-targeting control shRNA (shCONT) or increasing concentrations of an shRNA targeting Piezo1 (shPiezo1). mRNA was collected, and Piezo1 gene expression relative to GAPDH levels was measured by qPCR.

b, c, Knockdown efficiencies of PIEZO1 in GSC MES20 are displayed, Cellular lysates for cells from (a) were collected and resolved by SDS-PAGE. Western blot analysis (**b**) and quantitative analysis (**c**) of GSCs (MES20) after PIEZO1 knockdown.

d, e, Immunofluorescence staining (**d**) and quantitative analysis (**e**, n = 6 randomly selected fields per group) of GSCs (MES20) following knockdown of PIEZO1 at different levels.

In **a**, **c** and **e**, data are presented as mean ± s.d. In **b** immunoblots are representative of three independent experiments with similar results. In **a**, **c** and **e** data are presented from three independent experiments. One-way ANOVA followed by multiple comparisons for **a**, **c** and **e**.


a
Matrix stiffness score (Z score)
p<0.01
p<0.01
p<0.01
Non-tumor
Edge
Core
b
Cancer stemness score (Z score)
p<0.01
p<0.05
p<0.01
Non-tumor
Edge
Core
c
GSCs (n=981)
DGCs (n=13226)
PC2
PC1
d
GSCs
DGCs
Sense mechanical stimuli
p<0.001
Normalized AUC
e
live/dead cell staining (Fig. 1b/hGBM tissue core)
Total cell 76.5%
Single cell 92.6%
Living cell 91.9%
SSC-A
FSC-A
FSC-H
Count
CFSE-Alexa Fluor 488
f
live/dead cell staining (Fig. 1b/hGBM tissue edge)
Total cell 72.9%
Single cell 94.6%
Living cell 74.0%
g
live/dead cell staining (Fig. 1i/original tumor cells)
Total cell 73.7%
Single cell 94.1%
Living cell 89.2%
h
live/dead cell staining
Total cell 69.5%
Single cell 95.0%
Living cell 89.8%
i
Isotype
0.054
0.081
99.5
0.36
CD133-PE
SOX2-Pacific Blue
j
Single cells
0.80
8.85
82.0
8.31
CD133+ SOX2+ 8.85%
CD133- SOX2+ 17.16%
CD133- SOX2- 82.0%
k
CD133+/SOX2+
CD133-/SOX2+
CD133-/SOX2-
Modal
Piezo1-Alexa Fluor 488
l
Relative Piezo1 MFI
p<0.01
p<0.05
m
GSC MES 20
GSC 3028
500 Pa
1K Pa
5K Pa
10K Pa
kDa
Piezo1
p-FAK (Tyr 397)
FAK
SOX2
Olig2
α-Tubulin
180
100
130
35
35
55
n
soft
stiff
SOX2
100 um
GSC3028
o
soft
stiff
p<0.01
Relative SOX2 IF intensity (%)
GSC 3028
p
Edu
100 um
GSC 3028
q
soft
stiff
p<0.01
Percent of EdU+ cells(%)
GSC 3028

Supplementary Fig. 2

a, b, Sequencing data from different regions of GBM samples were analyzed (GSE59612), and the matrix stiffness score (**a**) and stemness score (**b**) were quantified for the non-tumor (n = 17), tumor edge (n = 36), and tumor core (n = 39) regions.

c, d, Quantification of the ability to sense mechanical stimulation in scRNA-seq data from 14,207 malignant cells of seven GBM patients.

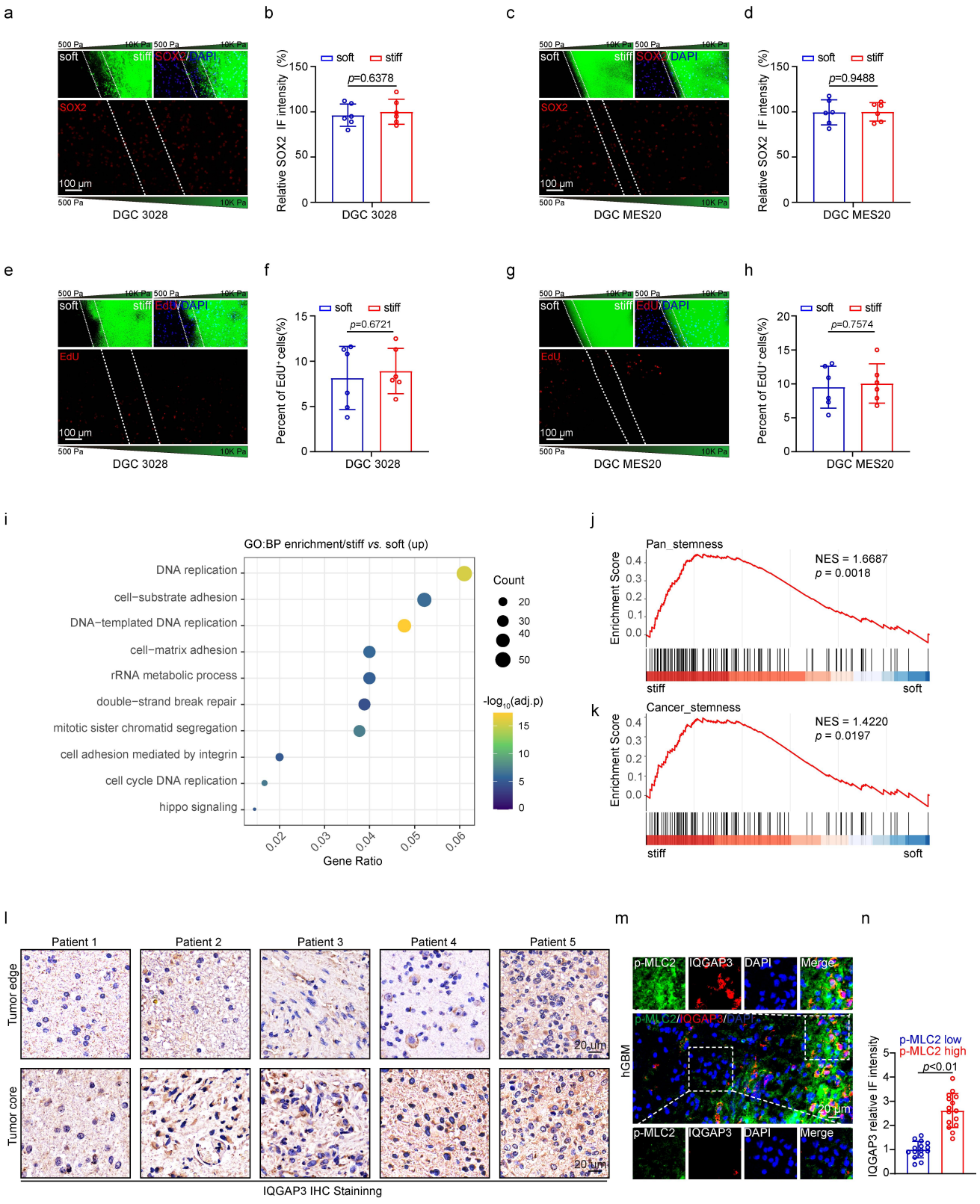
e - h, Live/dead cell staining analysis by flow cytometry.

i - l, Gating strategy (**i** and **j**), representative histogram plots (**k**), and statistical quantification **l**, n = 6 GBM samples per group) of Piezo1 flow cytometric analysis for the indicated cell populations in GBM samples. MFI, median fluorescence intensity.

m, Immunoblot of GSCs cultured on matrices with different stiffness levels.

n - q, GSC 3028 cultured on diffusion-based polyacrylamide stiffness gradient gels were subjected to immunofluorescence staining (**n**) and statistical quantification (**o**, n = 6 randomly selected fields per group) as well as quantification of EdU incorporation (**p** and **q**, n = 6 randomly selected fields per group). Stronger green fluorescence indicates higher matrix stiffness. IF: Immunofluorescence.

In **a, b, l, o** and **q**, data are presented as mean ± s.d. In **m** immunoblots are representative of three independent experiments with similar results. In **o** and **q**, data are presented from three independent experiments. Two-tailed paired t-test for **o** and **q**. One-way ANOVA followed by multiple comparisons for **a**, **b** and **l**.


a
b
c
d
e
f
g
h
i
j
k
l
m
n
soft
stiff
SOX2/DAPI
SOX2
EdU/DAPI
EdU
100 μm
500 Pa
10K Pa
DGC 3028
DGC MES20
Relative SOX2 IF intensity (%)
Percent of EdU+ cells(%)
p=0.6378
p=0.9488
p=0.6721
p=0.7574
GO:BP enrichment/stiff vs. soft (up)
DNA replication
cell−substrate adhesion
DNA−templated DNA replication
cell−matrix adhesion
rRNA metabolic process
double−strand break repair
mitotic sister chromatid segregation
cell adhesion mediated by integrin
cell cycle DNA replication
hippo signaling
Count
-log10(adj.p)
Gene Ratio
Pan_stemness
NES = 1.6687
p = 0.0018
Cancer_stemness
NES = 1.4220
p = 0.0197
Enrichment Score
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Tumor edge
Tumor core
20 μm
IQGAP3 IHC Staininng
hGBM
p-MLC2
IQGAP3
DAPI
Merge
p-MLC2/IQGAP3/DAPI
p-MLC2 low
p-MLC2 high
p<0.01
IQGAP3 relative IF intensity

Supplementary Fig. 3

a - h, DGCs (3028 and MES20) cultured on diffusion-based polyacrylamide stiffness gradient gels were subjected to immunofluorescence staining (**a** and **c**) and statistical quantification (**b** and **d**, n = 6 randomly selected fields per group) as well as quantification of EdU incorporation (**e** - **h**, n = 6 randomly selected fields per group). Stronger green fluorescence indicates higher matrix stiffness.

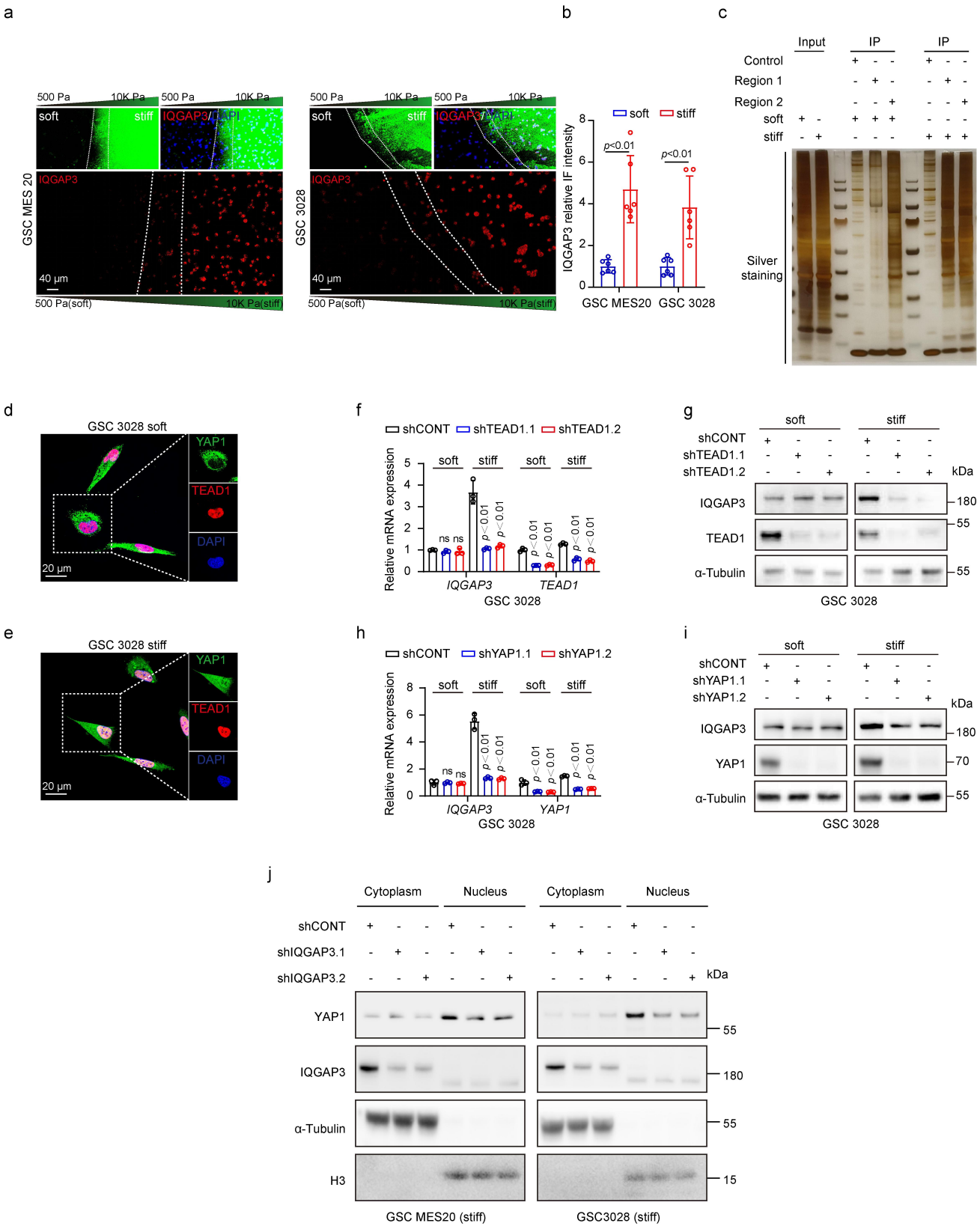
i, GO enrichment analysis of biological processes (GOBP) for genes upregulated in GSCs (stiff) compared to GSCs (soft).

j, k, GSEA analysis of GOBP. NES, normalized enrichment score.

l, Immunohistochemical staining shows the expression of IQGAP3 protein in different regions of GBM.

m, n, Immunofluorescence staining (**m**) and quantitative analysis (**n**, n = 15 randomly selected fields examined over 5 GBM sample per group) of GBM tissue sections. IF: Immunofluorescence

In **b, d, f, h** and **n**, data are presented as mean ± s.d. In **b, d, f** and **h**, data are presented from three independent experiments. Two-tailed unpaired t-test for **b, d, f, h** and **n**. Weighted Kolmogorov–Smirnov statistic test for **j** and **k**. In **i - k**, transcriptomic data are from three biologically independent samples per group. IF: Immunofluorescence.


a
500 Pa
10K Pa
soft
stiff
IQGAP3/F-Actin/DAPI
IQGAP3
GSC MES 20
GSC 3028
40 μm
500 Pa(soft)
10K Pa(stiff)
b
soft
stiff
IQGAP3 relative IF intensity
p<0.01
GSC MES20
GSC 3028
c
Input
IP
Control
Region 1
Region 2
soft
stiff
Silver staining
d
GSC 3028 soft
YAP1
TEAD1
DAPI
20 μm
e
GSC 3028 stiff
f
shCONT
shTEAD1.1
shTEAD1.2
Relative mRNA expression
ns
p<0.01
IQGAP3
TEAD1
GSC 3028
g
shTEAD1.1
shTEAD1.2
kDa
IQGAP3
TEAD1
α-Tubulin
180
55
h
shYAP1.1
shYAP1.2
YAP1
i
shYAP1.1
shYAP1.2
70
j
Cytoplasm
Nucleus
shIQGAP3.1
shIQGAP3.2
H3
15
GSC MES20 (stiff)
GSC3028 (stiff)

Supplementary Fig. 4

a, b, GSCs (MES20 and 3028) cultured on diffusion-based polyacrylamide stiffness gradient gels were subjected to immunofluorescence staining (a) and statistical quantification (b, n = 6 randomly selected fields per group). Stronger green fluorescence indicates higher matrix stiffness.

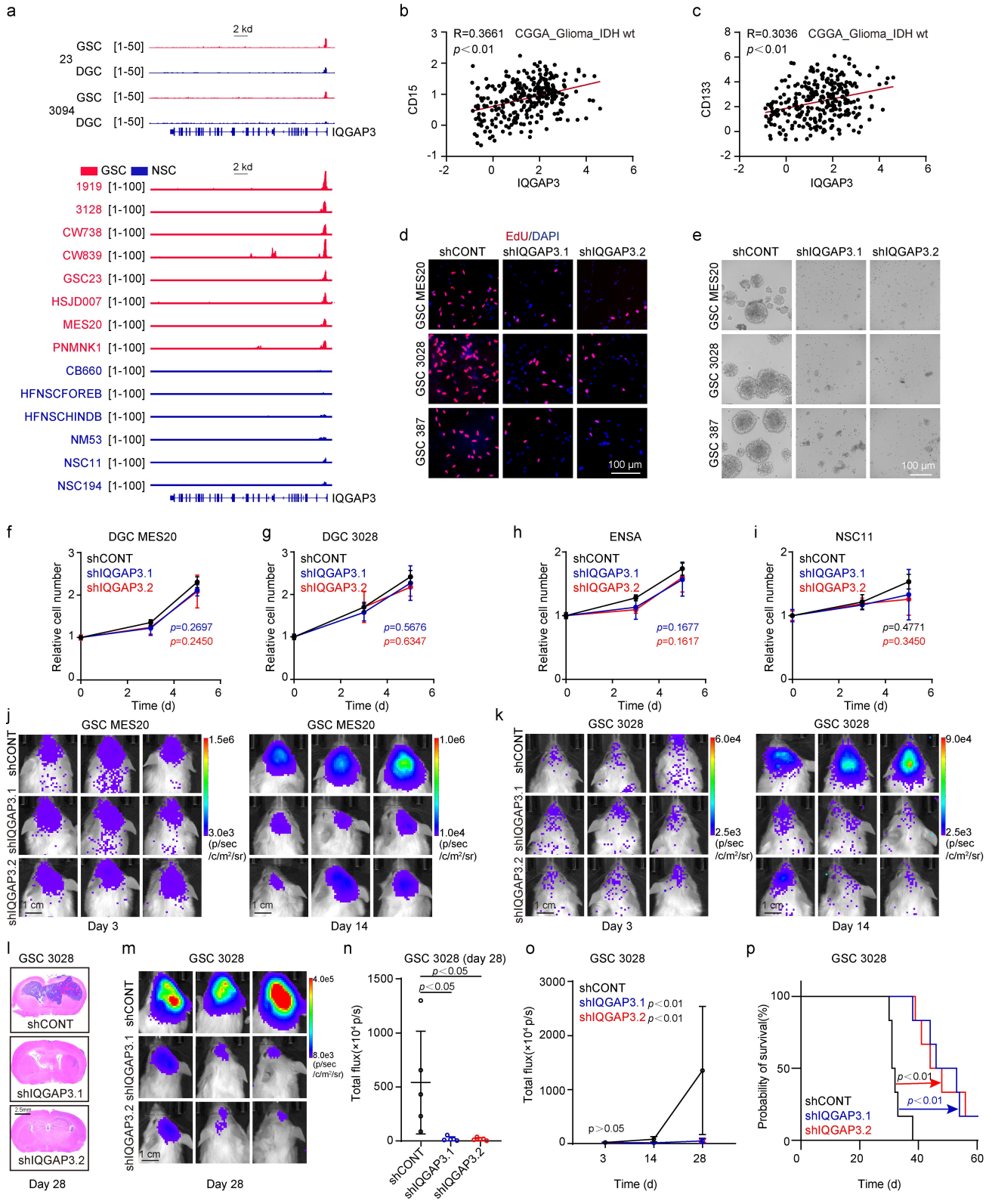
c, Silver staining shows protein binding to the IQGAP3 promoter region in GSCs cultured on substrates with different stiffness.

d, e, Immunofluorescence shows the nuclear localization of YAP1 protein and the expression of IQGAP3 protein in GSCs (3028) cultured on soft substrate (**d**) and stiff substrate (**e**).

f - i, RT-qPCR (**f** and **h**) and immunoblots (**g** and **i**) show that YAP1 and TEAD1 regulate the expression of IQGAP3 protein only in GSC (3028) cultured on stiff substrates.

j, GSCs (MES20 and 3028) were transduced with either a non-targeting control shRNA sequence (shCONT) or one of two shRNAs targeting IQGAP3 (designated shIQGAP3.1 or shIQGAP3.2), then cultured the cells of stiff matrices. Cytoplasmic and nuclear lysates were collected and resolved by SDS-PAGE. Western blot analysis showed the changes in YAP1 following IQGAP3 knockdown in GSCs cultured on stiff matrix gels.

In **b**, **f** and **h**, data are presented as mean ± s.d. In **g**, **i** and **j**, immunoblots are representative of three independent experiments with similar results. In **b**, **f** and **h**, data are presented from three independent experiments. Two-way ANOVA followed by multiple comparisons for **b**, **f** and **h**.


a
2 kd
GSC [1-50]
23
DGC [1-50]
GSC [1-50]
3094
DGC [1-50]
IQGAP3
GSC NSC
2 kd
1919 [1-100]
3128 [1-100]
CW738 [1-100]
CW839 [1-100]
GSC23 [1-100]
HSJD007 [1-100]
MES20 [1-100]
PNMNK1 [1-100]
CB660 [1-100]
HFNSCFOREB [1-100]
HFNSCHINDB [1-100]
NM53 [1-100]
NSC11 [1-100]
NSC194 [1-100]
IQGAP3
b
R=0.3661 CGGA_Glioma_IDH wt
p<0.01
CD15
IQGAP3
c
R=0.3036 CGGA_Glioma_IDH wt
p<0.01
CD133
IQGAP3
d
EdU/DAPI
shCONT
shIQGAP3.1
shIQGAP3.2
GSC MES20
GSC 3028
GSC 387
100 μm
e
shCONT
shIQGAP3.1
shIQGAP3.2
GSC MES20
GSC 3028
GSC 387
100 μm
f
DGC MES20
shCONT
shIQGAP3.1
shIQGAP3.2
p=0.2697
p=0.2450
Relative cell number
Time (d)
g
DGC 3028
shCONT
shIQGAP3.1
shIQGAP3.2
p=0.5676
p=0.6347
Relative cell number
Time (d)
h
ENSA
shCONT
shIQGAP3.1
shIQGAP3.2
p=0.1677
p=0.1617
Relative cell number
Time (d)
i
NSC11
shCONT
shIQGAP3.1
shIQGAP3.2
p=0.4771
p=0.3450
Relative cell number
Time (d)
j
GSC MES20
shCONT
shIQGAP3.1
shIQGAP3.2
1.5e6
3.0e3
(p/sec /c/m²/sr)
1 cm
Day 3
GSC MES20
1.0e6
1.0e4
(p/sec /c/m²/sr)
1 cm
Day 14
k
GSC 3028
shCONT
shIQGAP3.1
shIQGAP3.2
6.0e4
2.5e3
(p/sec /c/m²/sr)
1 cm
Day 3
GSC 3028
9.0e4
2.5e3
(p/sec /c/m²/sr)
1 cm
Day 14
l
GSC 3028
shCONT
shIQGAP3.1
shIQGAP3.2
Day 28
m
GSC 3028
shCONT
shIQGAP3.1
shIQGAP3.2
4.0e5
8.0e3
(p/sec /c/m²/sr)
1 cm
Day 28
n
GSC 3028 (day 28)
p<0.05
p<0.05
Total flux(×10⁴ p/s)
shCONT
shIQGAP3.1
shIQGAP3.2
o
GSC 3028
shCONT
shIQGAP3.1 p<0.01
shIQGAP3.2 p<0.01
p>0.05
Total flux(×10⁴ p/s)
Time (d)
p
GSC 3028
p<0.01
p<0.01
shCONT
shIQGAP3.1
shIQGAP3.2
Probability of survival(%)
Time (d)
q
GSC MES20
shCONT
shIQGAP3.1
shIQGAP3.2
IQGAP3/Ki67/DAPI
Xenograft tumor
40 μm
r
GSC MES20
shCONT
shIQGAP3.1
shIQGAP3.2
p<0.01
p<0.01
Ki67 positive cell (%)
s
GSC 3028
shCONT
shIQGAP3.1
shIQGAP3.2
p<0.01
p<0.01
Ki67 positive cell (%)

Supplementary Fig. 5

a, ChIP-seq analysis of H3K27ac signal on IQGAP3 promoter in indicated cells.

b, c, Pearson correlations between IQGAP3 and CD15 (**b**), CD133 (**c**), were analyzed using RNA-seq data from the CGGA dataset (primary, glioma_IDH_wt, n = 282 patients). Red lines indicate linear regression. IDH_wt: IDH wild type.

d, e, Representative images of EdU incorporation assay (**d**) and sphere formation assay (**e**) in GSCs (MES20, 3028 and 387) with or without IQGAP3 knockdown.

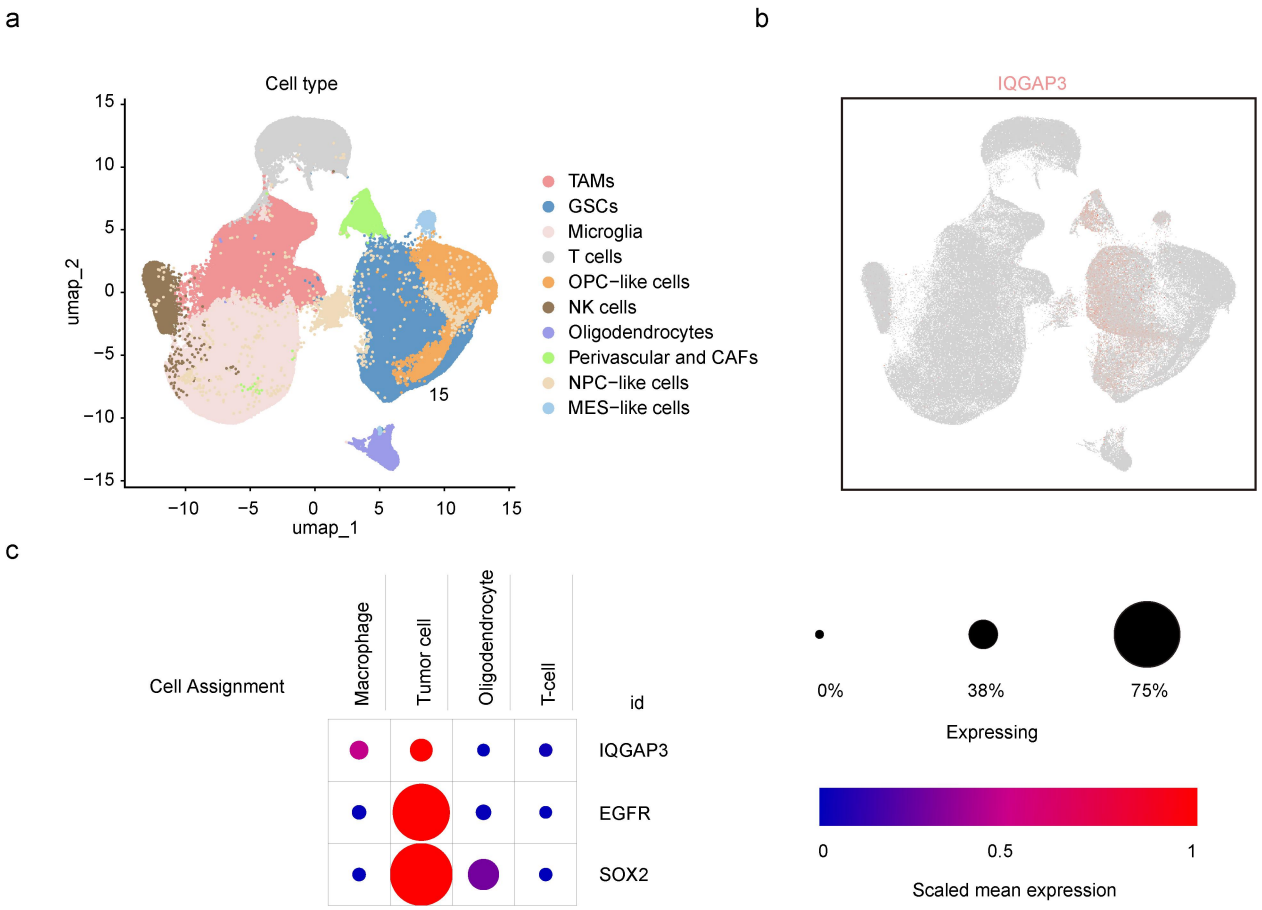
f - i, Cell viability in DGCs (**f** and **g**) and NSCs (**h** and **i**) with or without IQGAP3 knockdown. (n = 4)

j, k, Representative in vivo bioluminescence imaging at the indicated timepoints of mice bearing the indicated xenografts.

l - p, Representative images of hematoxylin and eosin (H&E)-stained brain sections at the indicated timepoint (**l**). Representative in vivo bioluminescence imaging (**m**) and quantification (**n**) at the indicated timepoint of mice bearing the specified xenografts (n = 5 mice per group). Tumor growth curve from in vivo bioluminescence analysis of mice bearing the indicated xenografts (**o**, n = 5 mice per group). Kaplan - Meier survival curves of mice bearing the indicated xenografts (**p**, n = 6 mice per group).

q - s, Representative images (**q**) and quantifications (**r** and **s**, n = 9 randomly selected fields examined across 3 mice per group) of Ki67 (green) and IQGAP3 (red) in brain sections derived from the indicated xenografts. DAPI (blue) was used to stain the nuclei.

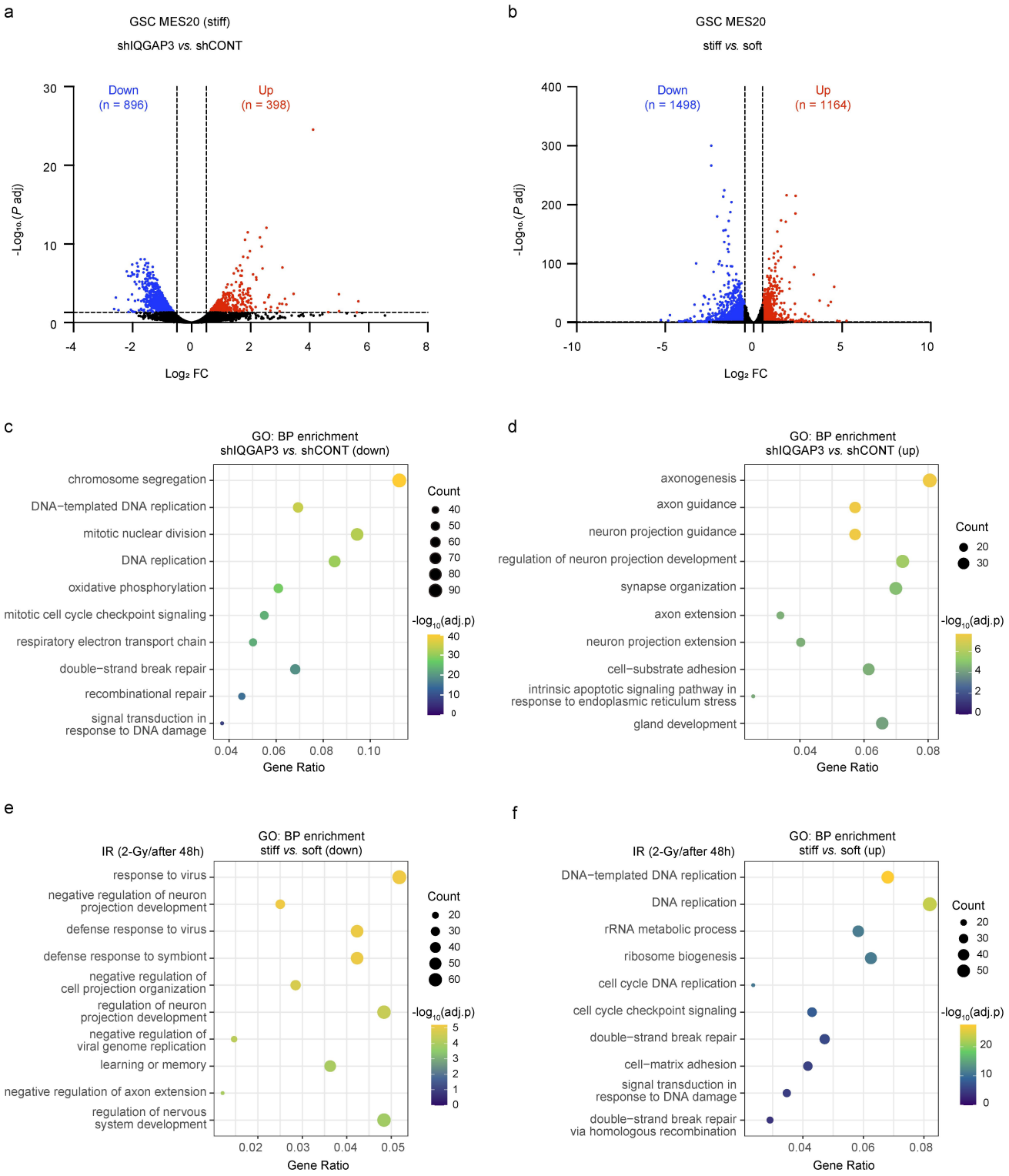
In **f - i**, **n**, **o**, **r** and **s**, data are presented as mean ± s.d. One-way ANOVA followed by multiple comparisons for **n**, **r and s**. Two-way ANOVA followed by multiple comparisons for **f - i** and **o.** Two-tailed Pearson correlation for **b** and **c.** Log-rank test for **p.**



Supplementary Fig. 6

a, b, Single-cell analysis of glioblastoma (GSE182109) revealed that IQGAP3 was predominantly expressed in tumor cells.

c, Single-cell analysis (GSE131928) revealed that IQGAP3, EGFR, and SOX2 were all highly expressed in tumor cells.


a
GSC MES20 (stiff)
shIQGAP3 vs. shCONT
Down
(n = 896)
Up
(n = 398)
-Log10(P adj)
Log2 FC
b
GSC MES20
stiff vs. soft
Down
(n = 1498)
Up
(n = 1164)
-Log10(P adj)
Log2 FC
c
GO: BP enrichment
shIQGAP3 vs. shCONT (down)
chromosome segregation
DNA−templated DNA replication
mitotic nuclear division
DNA replication
oxidative phosphorylation
mitotic cell cycle checkpoint signaling
respiratory electron transport chain
double−strand break repair
recombinational repair
signal transduction in response to DNA damage
Count
-log10(adj.p)
Gene Ratio
d
GO: BP enrichment
shIQGAP3 vs. shCONT (up)
axonogenesis
axon guidance
neuron projection guidance
regulation of neuron projection development
synapse organization
axon extension
neuron projection extension
cell−substrate adhesion
intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress
gland development
Count
-log10(adj.p)
Gene Ratio
e
IR (2-Gy/after 48h)
GO: BP enrichment
stiff vs. soft (down)
response to virus
negative regulation of neuron projection development
defense response to virus
defense response to symbiont
negative regulation of cell projection organization
regulation of neuron projection development
negative regulation of viral genome replication
learning or memory
negative regulation of axon extension
regulation of nervous system development
Count
-log10(adj.p)
Gene Ratio
f
IR (2-Gy/after 48h)
GO: BP enrichment
stiff vs. soft (up)
DNA−templated DNA replication
DNA replication
rRNA metabolic process
ribosome biogenesis
cell cycle DNA replication
cell cycle checkpoint signaling
double−strand break repair
cell−matrix adhesion
signal transduction in response to DNA damage
double−strand break repair via homologous recombination
Count
-log10(adj.p)
Gene Ratio

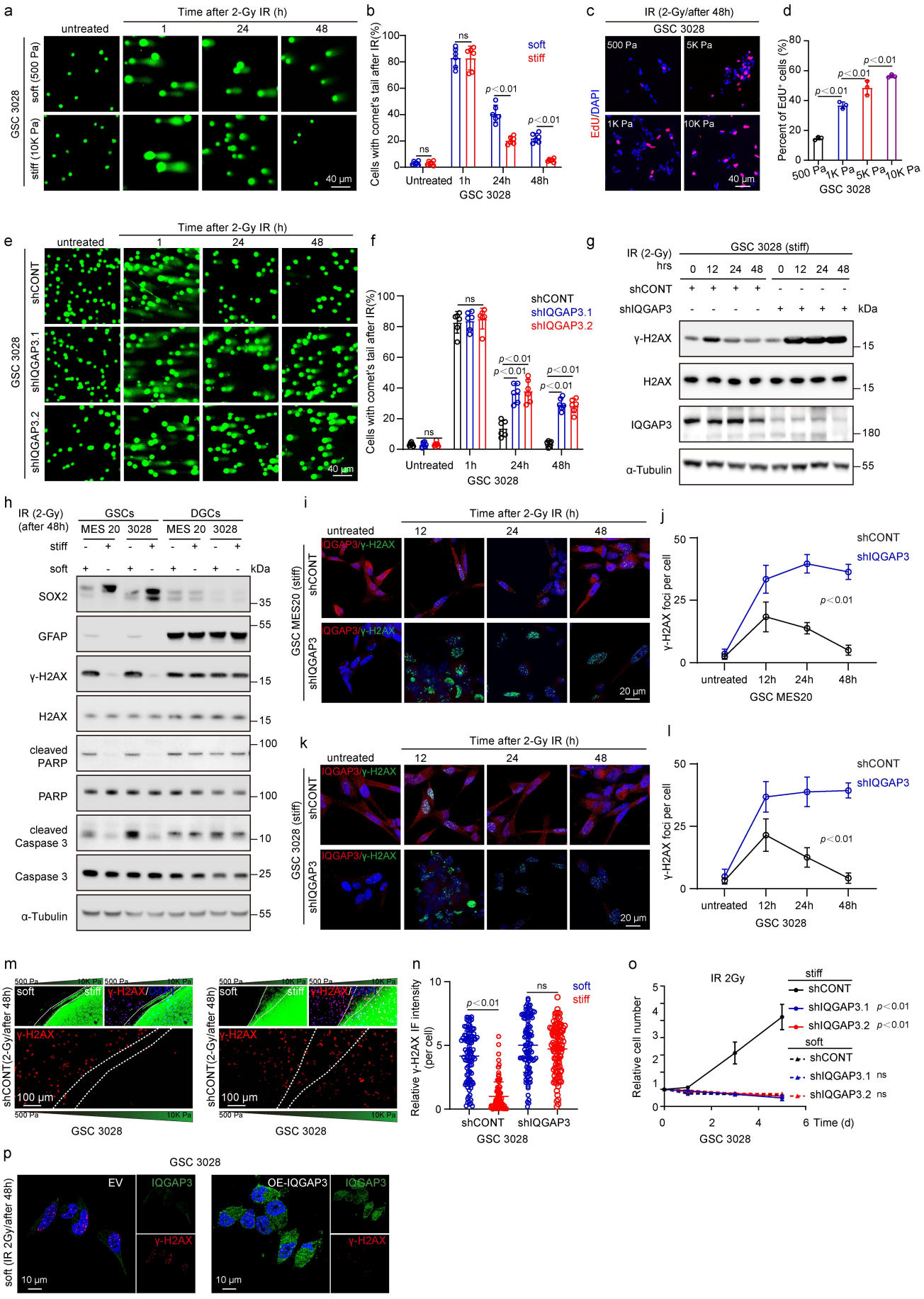
Supplementary Fig. 7

a, Volcano plot showing differentially expressed genes (cutoff, |log2 (fold change (FC)) > 0.585 false discovery rate (FDR) < 0.05) in RNA-seq data of GSCs (shIQGAP3) *vs.* GSCs (shCONT). Red dots indicate upregulation, and blue dots indicate downregulation in GSCs (shIQGAP3).

b, Volcano plot showing differentially expressed genes (cutoff, |log2 (fold change (FC)) > 0.585 false discovery rate (FDR) < 0.05) in RNA-seq data of GSCs (stiff) *vs.* GSCs (soft) after irradiation. Red dots indicate upregulation, and blue dots indicate downregulation in GSCs (stiff).

c, d, GO enrichment analysis of biological processes (GOBP) for genes that are downregulated (c) and upregulated (d) in shIQGAP3 compared to shCONT in GSCs (MES20). shCONT: short hairpin (sh) Control.

e, f, GO enrichment analysis of biological processes (GOBP) for genes that are downregulated (c) and upregulated (d) in GSCs (stiff) compared to GSCs (soft) after irradiation. IR: Ionizing Radiation (X-ray).


a
Time after 2-Gy IR (h)
untreated
1
24
48
GSC 3028
soft (500 Pa)
stiff (10K Pa)
40 μm
b
Cells with comet's tail after IR(%)
soft
stiff
ns
p<0.01
Untreated
1h
24h
48h
GSC 3028
c
IR (2-Gy/after 48h)
GSC 3028
500 Pa
5K Pa
1K Pa
10K Pa
EdU/DAPI
40 μm
d
Percent of EdU+ cells (%)
p<0.01
500 Pa
1K Pa
5K Pa
10K Pa
GSC 3028
e
Time after 2-Gy IR (h)
untreated
1
24
48
GSC 3028
shCONT
shIQGAP3.1
shIQGAP3.2
40 μm
f
Cells with comet's tail after IR(%)
shCONT
shIQGAP3.1
shIQGAP3.2
ns
p<0.01
Untreated
1h
24h
48h
GSC 3028
g
IR (2-Gy)
hrs
GSC 3028 (stiff)
0 12 24 48 0 12 24 48
shCONT
shIQGAP3
kDa
γ-H2AX
H2AX
IQGAP3
α-Tubulin
15
15
180
55
h
IR (2-Gy)
(after 48h)
GSCs
DGCs
MES 20
3028
MES 20
3028
stiff
soft
kDa
SOX2
GFAP
γ-H2AX
H2AX
cleaved PARP
PARP
cleaved Caspase 3
Caspase 3
α-Tubulin
35
55
15
15
100
100
10
25
55
i
Time after 2-Gy IR (h)
untreated
12
24
48
GSC MES20 (stiff)
shCONT
shIQGAP3
IQGAP3/γ-H2AX
20 μm
j
γ-H2AX foci per cell
shCONT
shIQGAP3
p<0.01
untreated
12h
24h
48h
GSC MES20
k
Time after 2-Gy IR (h)
untreated
12
24
48
GSC 3028 (stiff)
shCONT
shIQGAP3
IQGAP3/γ-H2AX
20 μm
l
γ-H2AX foci per cell
shCONT
shIQGAP3
p<0.01
untreated
12h
24h
48h
GSC 3028
m
500 Pa
10K Pa
soft
stiff
γ-H2AX
shCONT(2-Gy/after 48h)
100 μm
GSC 3028
n
Relative γ-H2AX IF intensity (per cell)
soft
stiff
p<0.01
ns
shCONT
shIQGAP3
GSC 3028
o
IR 2Gy
Relative cell number
stiff
shCONT
shIQGAP3.1 p<0.01
shIQGAP3.2 p<0.01
soft
shCONT
shIQGAP3.1 ns
shIQGAP3.2 ns
Time (d)
GSC 3028
p
GSC 3028
soft (IR 2Gy/after 48h)
EV
IQGAP3
γ-H2AX
OE-IQGAP3
IQGAP3
γ-H2AX
10 μm

Supplementary Fig. 8

a, GSC 3028 (soft) and GSC 3028 (stiff) cells were irradiated with 2 Gy of IR. DNA damage at sequential time points post-irradiation was assessed using a single-cell gel electrophoresis assay under alkaline conditions (alkaline comet assay).

b, Quantification of the percentages of cells with comet tails at different time points after IR in GSC 3028 (soft) and GSC 3028 (stiff) populations. (n = 6 randomly selected fields per group)

c, d, GSC 3028 cells were irradiated with 2 Gy of IR. Representative images of the EdU incorporation assay (**c**) and statistical quantification (**d**) under different matrix stiffness conditions. (n = 3 randomly selected fields per group)

e, GSC 3028 (stiff) cells, with or without IQGAP3 knockdown, were irradiated with 2 Gy of IR. DNA damage at sequential time points post-irradiation was assessed using a single-cell gel electrophoresis assay under alkaline conditions (alkaline comet assay).

f, Quantification of the percentages of cells with comet tails at different time points after IR in GSC 3028 (stiff) populations, with or without IQGAP3 knockdown. (n = 6 randomly selected fields per group).

g, Immunoblotting shows changes in γ-H2AX after radiotherapy in GSC 3028 (stiff) cells, with or without IQGAP3.

h, Immunoblotting showed protein changes in GSCs (MES20 and 3028) and their corresponding DGCs (MES20 and 3028) cultured on soft and stiff matrix gels after IR treatment.

i - l, GSCs (MES20 and 3028) cultured on stiff matrix gel, with or without IQGAP3 knockdown, were irradiated with 2 Gy of IR. γ-H2AX foci at sequential time points post-irradiation were assessed using immunofluorescence (**i** and **k**) and statistically quantified (**j** and **l**, n = 100 cells per group).

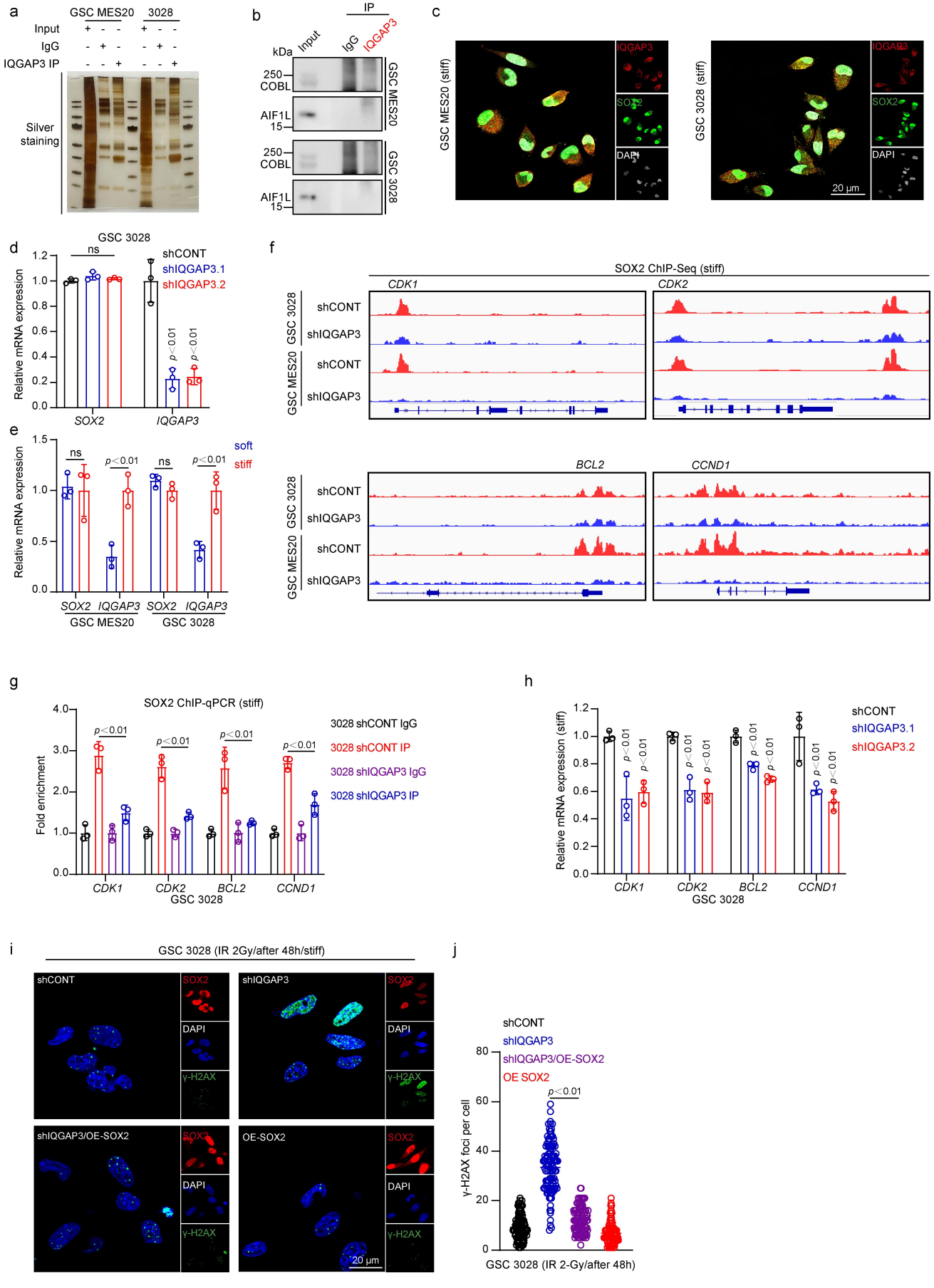
m, n, GSCs (3028), with or without IQGAP3 knockdown, were cultured on diffusion-based polyacrylamide stiffness gradient gels and subjected to radiotherapy. Immunofluorescence experiments (**m**) and statistical quantification (**n**) were performed 48 hours post-treatment to assess γ-H2AX fluorescence intensity. (n = 100 cells per group).

o, GSCs (3028), with or without IQGAP3 knockdown, were cultured on soft or stiff matrix gels and subjected to radiotherapy, followed by quantification of cell proliferation. (n = 4)

p, Immunofluorescence staining shows that GSCS (3028), with or without IQGAP3 overexpression (OE), were cultured on soft matrix gel and treated with IR.

In **b, d, f, j, l, n** and **o**, data are presented as mean ± s.d. In **g** and **h** immunoblots are representative of three

independent experiments with similar results. In **b, d, f, j, l** and **n** data are presented from three independent experiments. One-way ANOVA followed by multiple comparisons for **d**. Two-way ANOVA followed by multiple comparisons for **b, f, j, l, n** and **o**. shIQGAP3: shIQGAP3.1 and shIQGAP3.2. IR: Ionizing Radiation (X-ray). ns, not significant.


a
GSC MES20
3028
Input
IgG
IQGAP3 IP
Silver staining
b
IP
kDa
Input
IgG
IQGAP3
250
COBL
AIF1L
15
GSC MES20
GSC 3028
c
GSC MES20 (stiff)
GSC 3028 (stiff)
IQGAP3
SOX2
DAPI
20 μm
d
GSC 3028
ns
shCONT
shIQGAP3.1
shIQGAP3.2
Relative mRNA expression
p<0.01
SOX2
IQGAP3
e
soft
stiff
ns
p<0.01
SOX2
IQGAP3
GSC MES20
GSC 3028
f
SOX2 ChIP-Seq (stiff)
CDK1
CDK2
BCL2
CCND1
GSC 3028
GSC MES20
shCONT
shIQGAP3
g
SOX2 ChIP-qPCR (stiff)
Fold enrichment
3028 shCONT IgG
3028 shCONT IP
3028 shIQGAP3 IgG
3028 shIQGAP3 IP
CDK1
CDK2
BCL2
CCND1
GSC 3028
h
Relative mRNA expression (stiff)
shCONT
shIQGAP3.1
shIQGAP3.2
i
GSC 3028 (IR 2Gy/after 48h/stiff)
shCONT
shIQGAP3
shIQGAP3/OE-SOX2
OE-SOX2
SOX2
DAPI
γ-H2AX
20 μm
j
shCONT
shIQGAP3
shIQGAP3/OE-SOX2
OE SOX2
γ-H2AX foci per cell
GSC 3028 (IR 2-Gy/after 48h)

Supplementary Fig. 9

a, IP was performed for IQGAP3 protein in GSCs (MES20 and 3028) cultured on stiff matrix gel, followed by analysis using immunoblotting and silver staining.

b, Immunoblot of IQGAP3 protein immunoprecipitates in GSCs (MES20 and 3028) cultured on stiff matrix gel.

c, GSCs (MES20 and 3028) were cultured on stiff matrix gel and subjected to immunofluorescence staining.

d, RT-qPCR analysis of GSCs (3028) cultured on stiff matrix gel with or without IQGAP3 knockdown.

e, RT-qPCR analysis was performed on GSCs (MES20 and 3028) cultured on soft and stiff matrix gels.

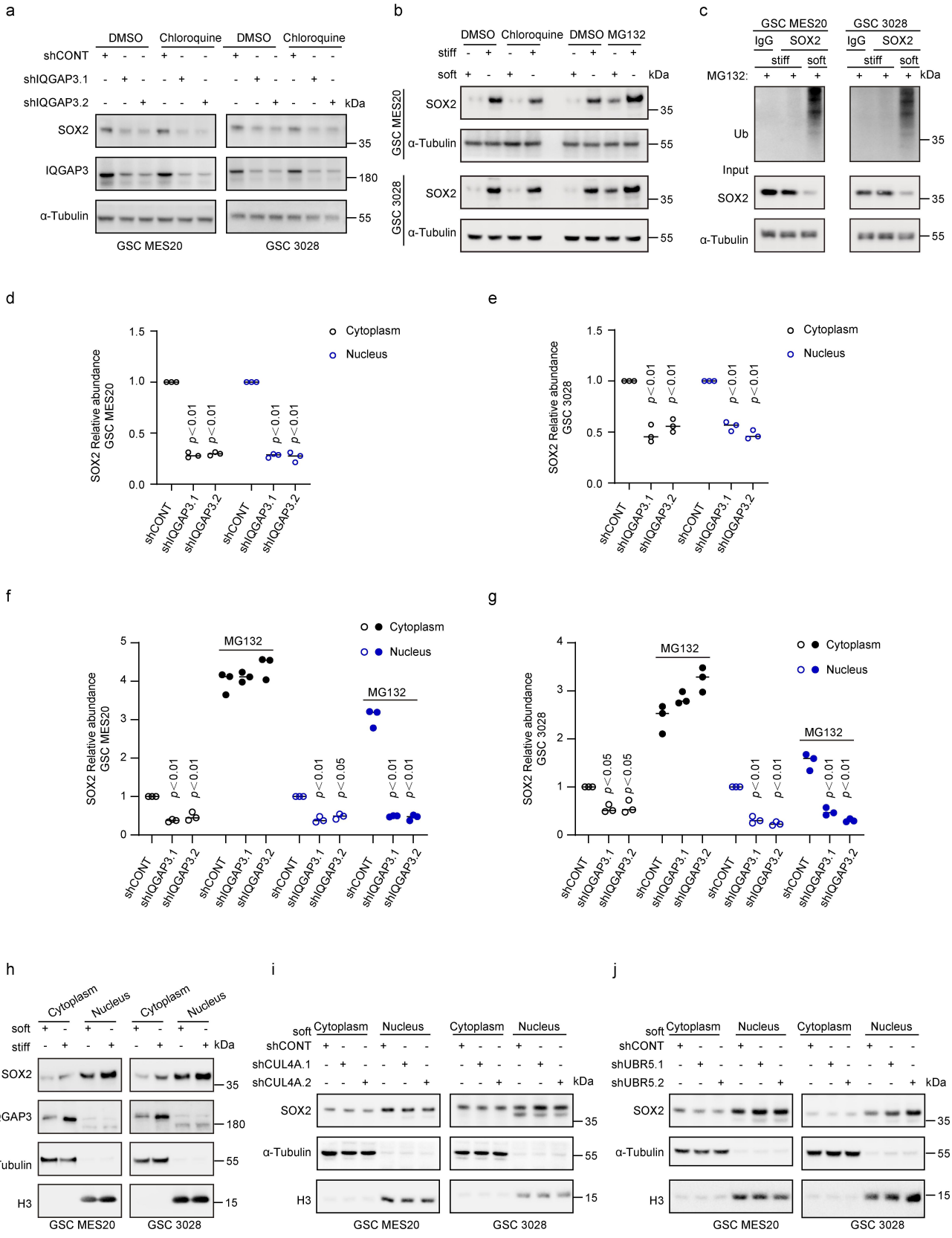
f, SOX2 ChIP-seq tracks at CDK1, CDK2, BCL2 and CCND1 gene loci.

g, SOX2 ChIP-qPCR analysis in GSC 3028 cultured on stiff matrix gel, with or without IQGAP3 knockdown.

h, RT-qPCR analysis of GSC 3028 cultured on stiff matrix gel with or without IQGAP3 knockdown.

i, j, GSCs (3028) were cultured on a stiff matrix gel, with or without IQGAP3 knockdown, followed by SOX2 overexpression after IQGAP3 knockdown. Following IR treatment, immunofluorescence (**i**) and quantification (**j**) were performed 48 hours later (n = 100 cells per group).

In **d, e, g, h** and **j,** data are presented as mean ± s.d. In **b**, immunoblots are representative of three independent experiments with similar results. In **d, e, g, h** and **j,** data are presented from three independent experiments. One-way ANOVA followed by multiple comparisons for **j**. Two-way ANOVA followed by multiple comparisons for **d, e, g** and **h**. IP: immunoprecipitation. shIQGAP3: shIQGAP3.1 and shIQGAP3.2. IR: Ionizing Radiation (X-ray). ns: not significant.


a
DMSO
Chloroquine
DMSO
Chloroquine
shCONT
shIQGAP3.1
shIQGAP3.2
kDa
SOX2
IQGAP3
α-Tubulin
35
180
55
GSC MES20
GSC 3028
b
DMSO
Chloroquine
DMSO
MG132
stiff
soft
kDa
GSC MES20
GSC 3028
SOX2
α-Tubulin
35
55
c
GSC MES20
GSC 3028
IgG
SOX2
stiff
soft
MG132:
kDa
Ub
Input
SOX2
α-Tubulin
35
55
d
SOX2 Relative abundance
GSC MES20
Cytoplasm
Nucleus
p < 0.01
shCONT
shIQGAP3.1
shIQGAP3.2
e
SOX2 Relative abundance
GSC 3028
Cytoplasm
Nucleus
p < 0.01
f
SOX2 Relative abundance
GSC MES20
Cytoplasm
Nucleus
MG132
p < 0.01
p < 0.05
g
SOX2 Relative abundance
GSC 3028
Cytoplasm
Nucleus
MG132
p < 0.05
p < 0.01
h
Cytoplasm
Nucleus
soft
stiff
kDa
SOX2
IQGAP3
α-Tubulin
H3
35
180
55
15
GSC MES20
GSC 3028
i
soft
Cytoplasm
Nucleus
shCONT
shCUL4A.1
shCUL4A.2
kDa
SOX2
α-Tubulin
H3
35
55
15
GSC MES20
GSC 3028
j
soft
Cytoplasm
Nucleus
shCONT
shUBR5.1
shUBR5.2
kDa
SOX2
α-Tubulin
H3
35
55
15
GSC MES20
GSC 3028

Supplementary Fig. 10

a, Immunoblot of GSCs (MES20 and 3028) cultured on a stiff matrix gel, with or without IQGAP3 knockdown, after treatment with chloroquine to inhibit lysosomal degradation.

b, Immunoblot of GSCs (MES20 and 3028) cultured on matrigel with different stiffness, treated with chloroquine and MG132 to inhibit lysosomal and proteasomal degradation, respectively.

c, Immunoblot of SOX2 ubiquitination levels in GSCs (MES20 and 3028) cultured on Matrigel with different stiffness.

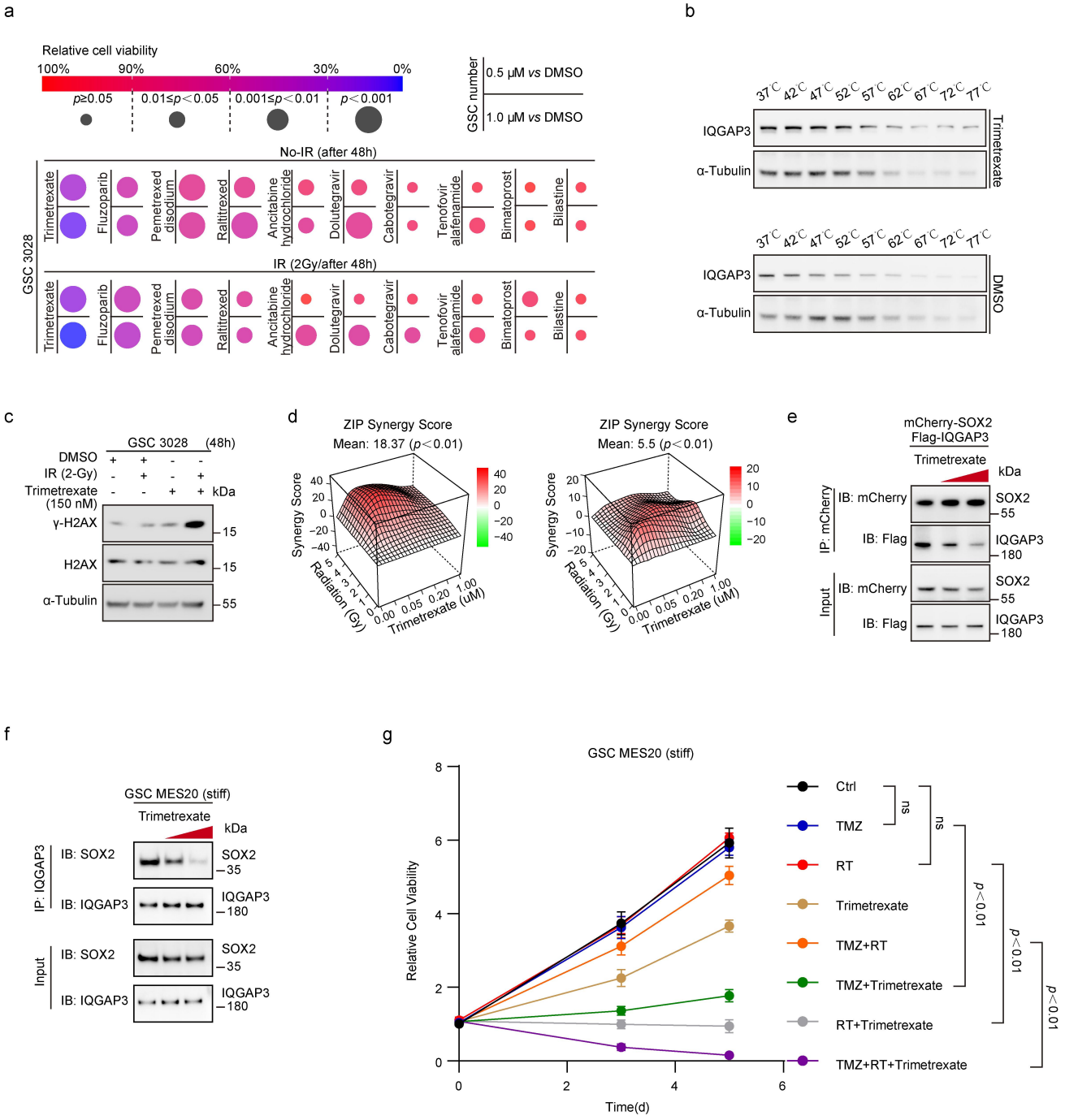
d, e, Immunoblot quantification of nuclear and cytoplasmic SOX2 fractions in stiff matrix gel-cultured MES20 and 3028 GSCs with/without IQGAP3 knockdow.

f, g, Immunoblot quantification of nuclear and cytoplasmic SOX2 fractions in stiff matrix gel-cultured MES20 and 3028 GSCs with/without IQGAP3 knockdown following proteasomal inhibition by MG132.

h, Immunoblot of nuclear and cytoplasmic fractions of GSCs cultured on matrigel with different stiffness.

i, j, Immunoblot of nuclear and cytoplasmic fractions of GSCs (MES20 and 3028) cultured on a soft matrix gel after knockdown of UBR5 (**e**) and CUL4A (**f**).

In **d - g**, data are presented as mean ± s.d. In **a - c** and **h - j** immunoblots are representative of three independent experiments with similar results. One-way ANOVA followed by multiple comparisons for **d - g**.


a
Relative cell viability
100%
90%
60%
30%
0%
p≥0.05
0.01≤p<0.05
0.001≤p<0.01
p<0.001
GSC number
0.5 μM vs DMSO
1.0 μM vs DMSO
No-IR (after 48h)
IR (2Gy/after 48h)
GSC 3028
Trimetrexate
Fluzoparib
Pemetrexed disodium
Raltitrexed
Ancitabine hydrochloride
Dolutegravir
Cabotegravir
Tenofovir alafenamide
Bimatoprost
Bilastine
b
37°C
42°C
47°C
52°C
57°C
62°C
67°C
72°C
77°C
IQGAP3
α-Tubulin
Trimetrexate
DMSO
c
GSC 3028 (48h)
DMSO
IR (2-Gy)
Trimetrexate (150 nM)
kDa
γ-H2AX
H2AX
α-Tubulin
15
15
55
d
ZIP Synergy Score
Mean: 18.37 (p<0.01)
Mean: 5.5 (p<0.01)
Synergy Score
Radiation (Gy)
Trimetrexate (uM)
e
mCherry-SOX2
Flag-IQGAP3
Trimetrexate
kDa
IP: mCherry
IB: mCherry
IB: Flag
Input
SOX2
IQGAP3
55
180
f
GSC MES20 (stiff)
Trimetrexate
kDa
IP: IQGAP3
IB: SOX2
IB: IQGAP3
Input
SOX2
IQGAP3
35
180
g
GSC MES20 (stiff)
Relative Cell Viability
Time(d)
Ctrl
TMZ
RT
Trimetrexate
TMZ+RT
TMZ+Trimetrexate
RT+Trimetrexate
TMZ+RT+Trimetrexate
ns
ns
p<0.01
p<0.01
p<0.01

Supplementary Fig. 11

a, Effects of top 10 compounds on cell viability in GSC 3028 cells.

b, Immunoblot of IQGAP3 from CETSA in HEK293T cells overexpressing Flag-IQGAP3, pretreated with 200 nM Trimetrexate.

c, Immunoblot of GSCs (MES20) cultured on a stiff matrix gel after treatment with IR or Trimetrexate.

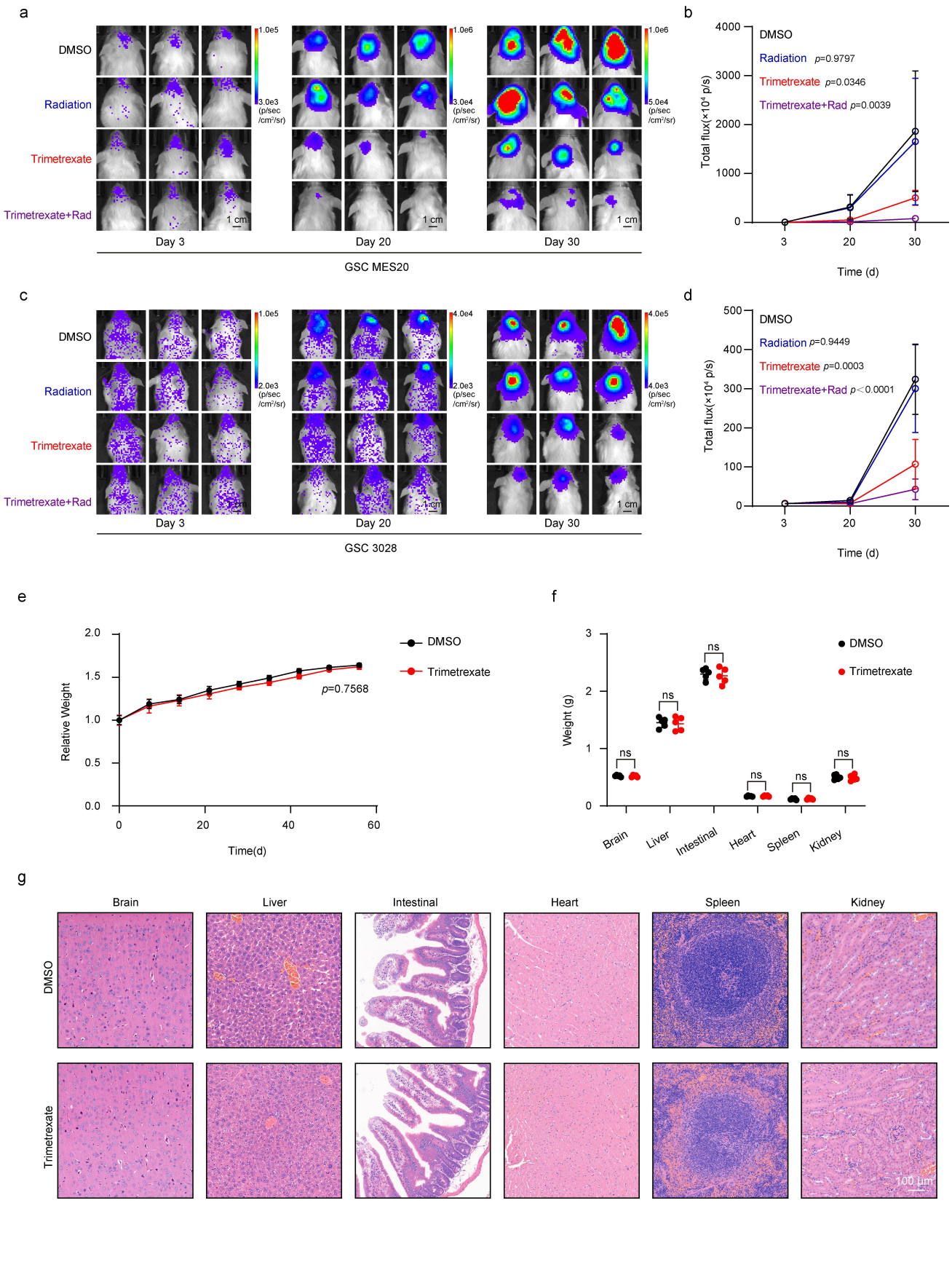
d, 3D surface plot showing the effects of combined Trimetrexate and IR treatment on GSCs (MES20) cultured on stiff (left) and soft (right) matrix gels after 48 hours.

e, Immunoblot of mCherry-IP was performed on HEK293T cells overexpressing mCherry-SOX2 and Flag-IQGAP3 after treatment with gradually increasing concentrations of Trimetrexate (0 nM, 100 nM, 200 nM) for 6 hours.

f, Immunoblot of IQGAP3-IP was performed on GSCs (MES20) after treatment with gradually increasing concentrations of Trimetrexate (0 nM, 100 nM, 200 nM) for 6 hours.

g, Cell viability assay of GSCs (MES20) under different treatment conditions (n = 4).

In **g**, data are presented as mean ± s.d. In **b, c, e** and **f**, immunoblots are representative of three independent experiments with similar results. In **a** and **d,** data are presented from three independent experiments. Two-tailed unpaired t-test for **a**. Two-way ANOVA followed by multiple comparisons for **g**. IP: immunoprecipitation. IR: Ionizing Radiation (X-ray).


a
DMSO
Radiation
Trimetrexate
Trimetrexate+Rad
Day 3
Day 20
Day 30
GSC MES20
b
DMSO
Radiation p=0.9797
Trimetrexate p=0.0346
Trimetrexate+Rad p=0.0039
Total flux(×10⁴ p/s)
Time (d)
c
GSC 3028
d
Radiation p=0.9449
Trimetrexate p=0.0003
Trimetrexate+Rad p<0.0001
e
Relative Weight
Time(d)
p=0.7568
f
Weight (g)
ns
Brain
Liver
Intestinal
Heart
Spleen
Kidney
g
100 μm

Supplementary Fig. 12

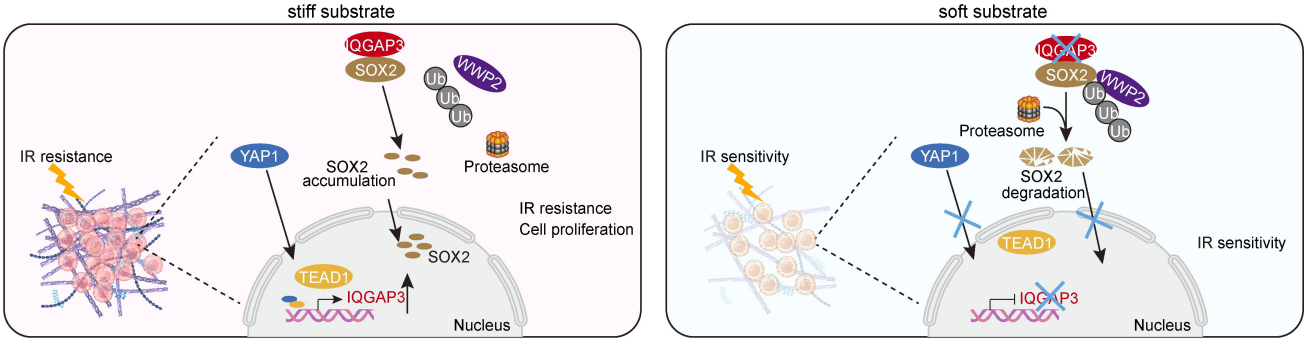
a - d, Representative in vivo bioluminescence imaging at the specified time point (**a** and **c**) and tumor growth curves from in vivo bioluminescence analysis of mice carrying the indicated xenografts (**b** and **d**, n = 5 mice per group).

e, Body weight change trend in mice receiving 5 consecutive days of intraperitoneal DMSO or trimetrexate (10 mg/kg) injection.

f, Organ weight in mice receiving 5 consecutive days of intraperitoneal DMSO or trimetrexate (10 mg/kg) injection.

g, Hematoxylin-eosin (HE) staining of mouse organs after 5 consecutive days of intraperitoneal DMSO or trimetrexate (10 mg/kg) injection.

In **b, d,e** and **f,** data are presented as mean ± s.d. Two-way ANOVA followed by multiple comparisons for **b, d** and **f**. Two-tailed unpaired t-test for e. ns, not significant.



Supplementary Fig. 13. Graphic abstract.

Mechanical stimulation in stiff matrix induced expression of IQGAP3 in GSCs, leading to stabilization of SOX2 in the cytoplasm, and increased SOX2-mediated transcription of stemness and treatment resistance-related pathways in the nucleus