Supplementary Information for

Endothelial cell-derived SDF-1α elicits stemness traits of glioblastoma *via* dual-regulation of GLI1

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Running title: Stemness traits regulation by endothelial cells

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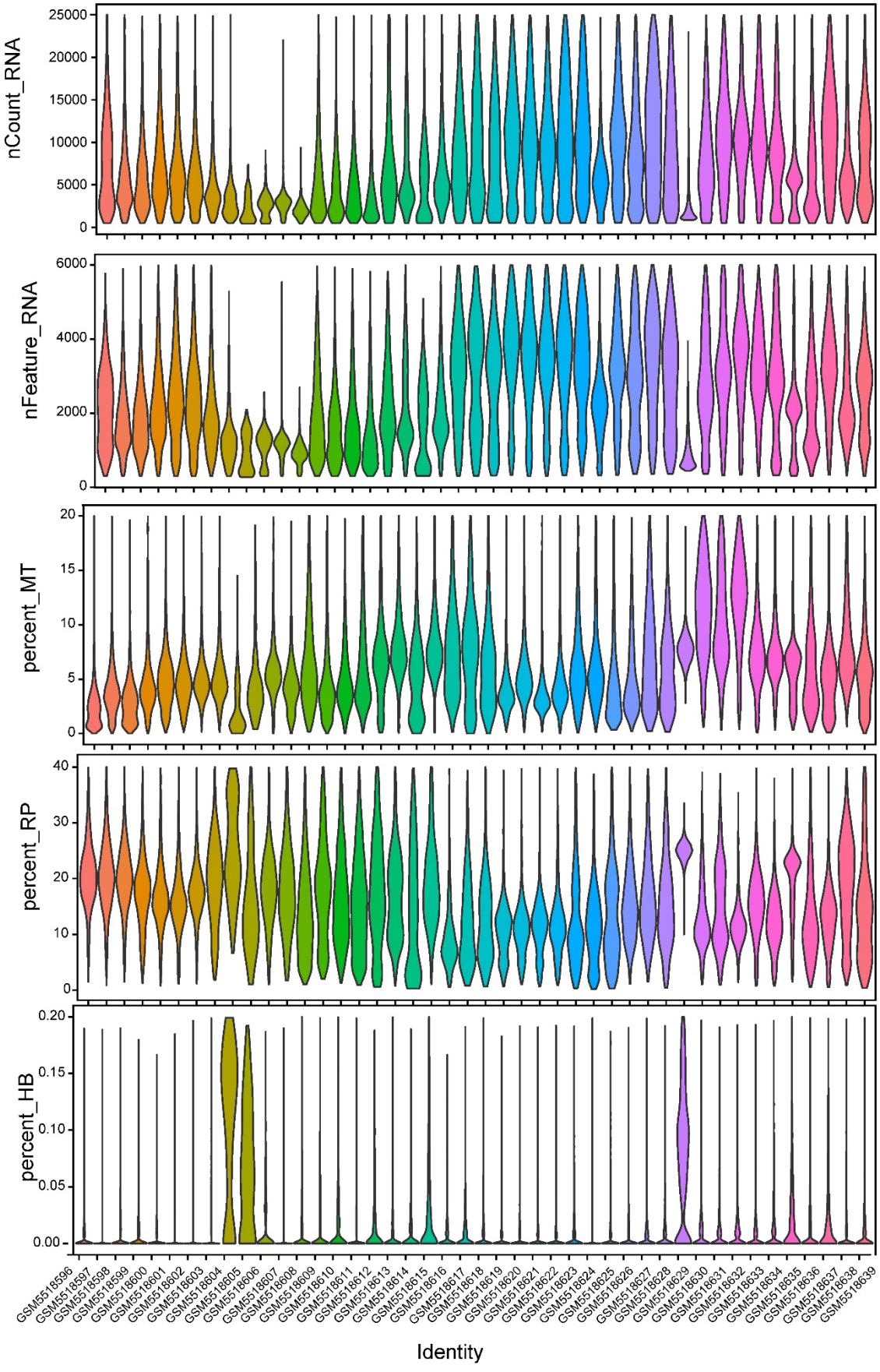

Figure S1 **Filtering of low-quality cells.**

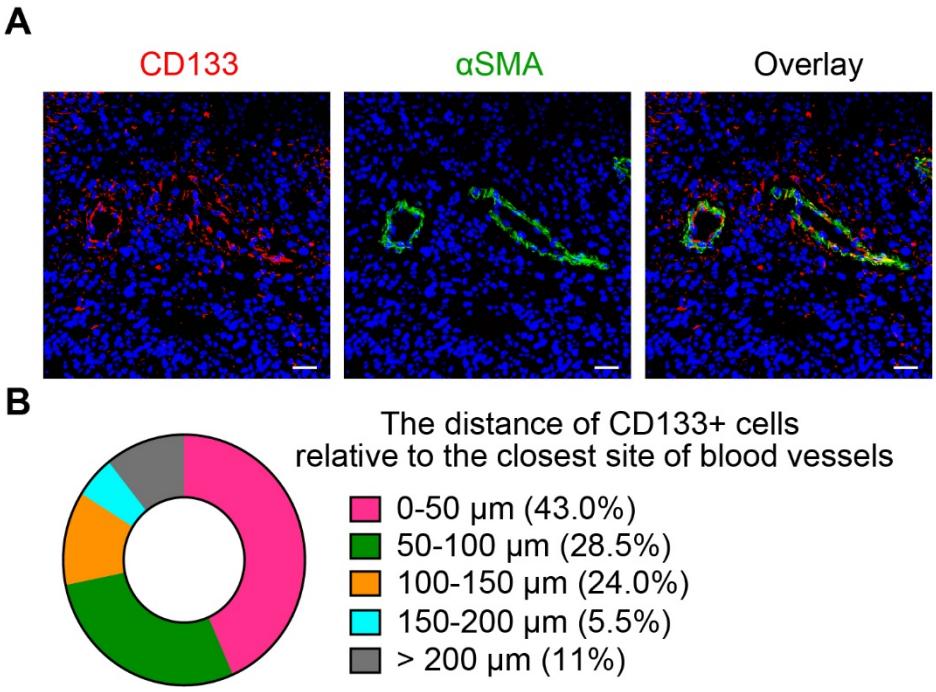

Figure S2 Spatial tissue localization of GSCs relative to microvessels.

(A) Immunofluorescent staining image illustrating co-localization of GSC marker CD133 (red) and vascular pericyte marker αSMA (green) in human GBM tissue. Scale bar = 50 μm. **(B)** The overall perivascular distribution of GSCs was defined by calculating the distances between $CD133^{+}$ cells and the closest sites in the blood vessel ring, data were collected from analyzing 200 GSCs.

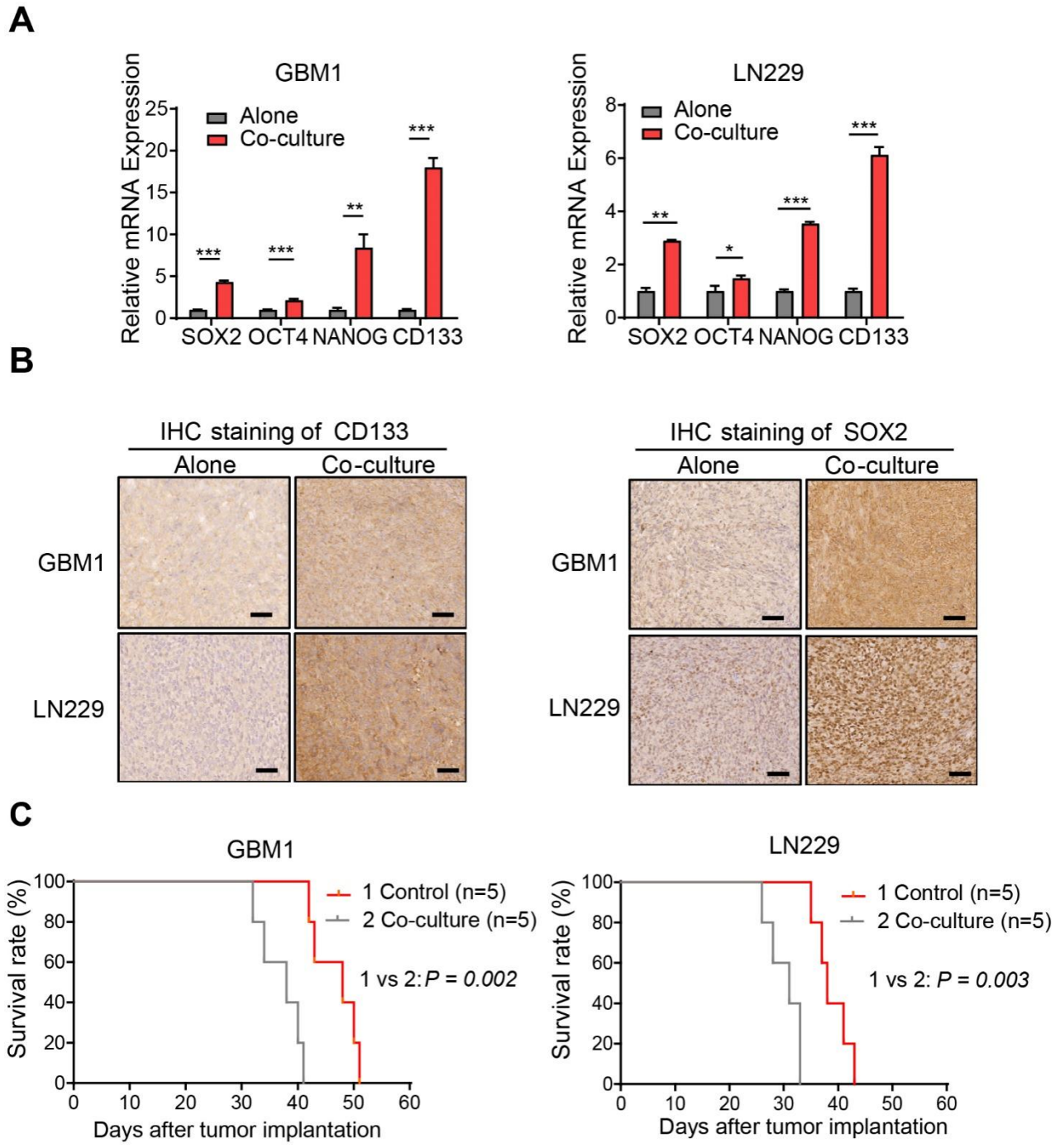

Figure S3 ECs induce the expression of stemness markers and traits in co-cultured non-GSC populations.

(A) qRT-PCR assay tested the expression of SOX2, OCT4, NANOG, and CD133 in ECs co-cultured and control GBM cells. **(B)** Representative images depicting IHC staining of CD133 and SOX2 in xenografts from mice bearing GBM cells, both co-cultured with ECs and in control conditions. Scale

bar = 40 um. **(C)** Kaplan-Meier survival analysis of the mice bearing xenograft tumors of GBM cells, comparing those with prior co-culture with ECs to those with GBM cells alone. Data are means ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

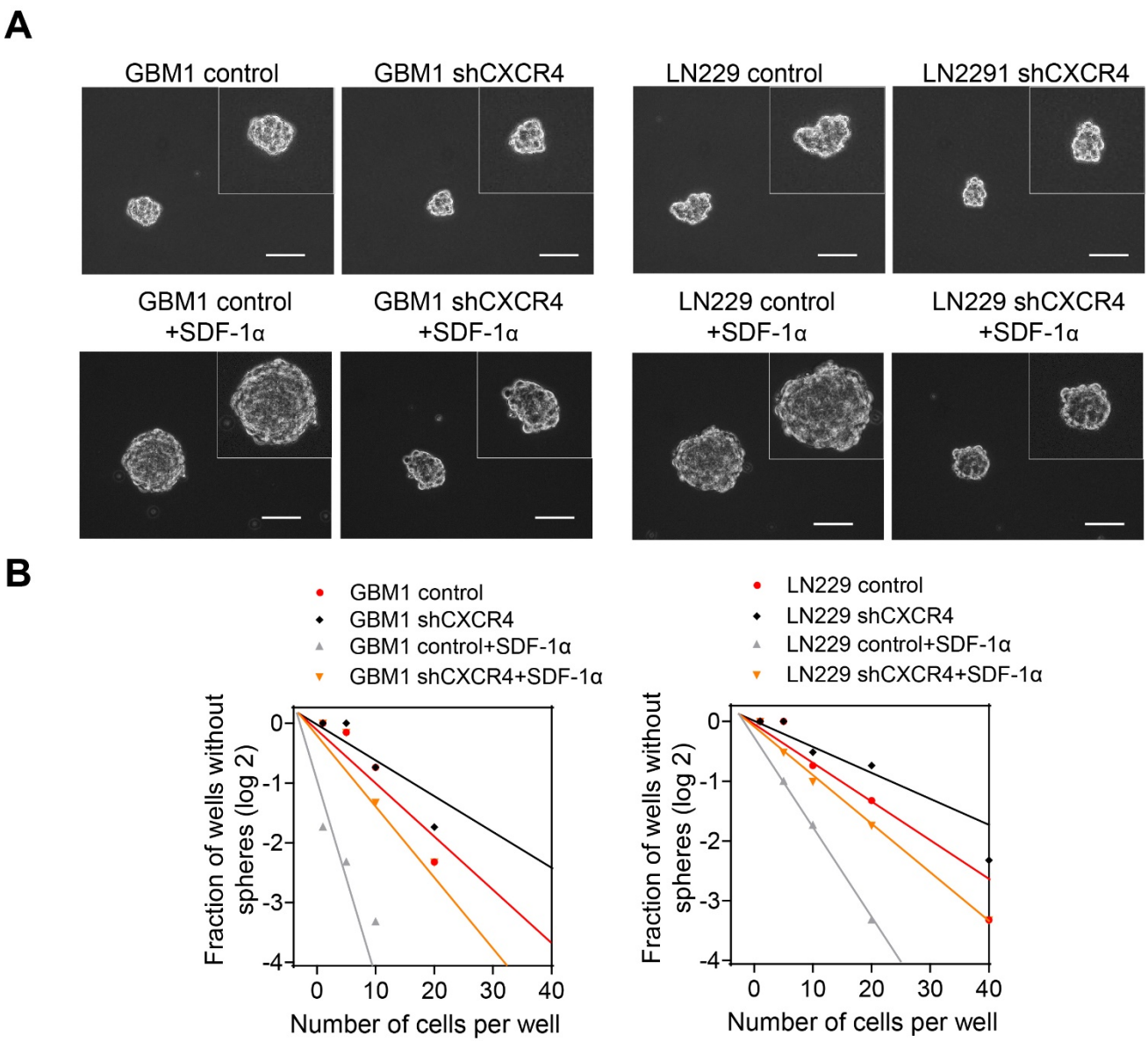

Figure S4 Disrupting CXCR4 attenuated the SDF-1α dependent stemness in GBM cells.

(A-B) Limiting dilution sphere-forming assays conducted on GBM1 **(A)** and LN229 **(B)** cells treated with either SDF-1α, shCXCR4, or a combination of both, along with representative images of the resulting tumor spheres.

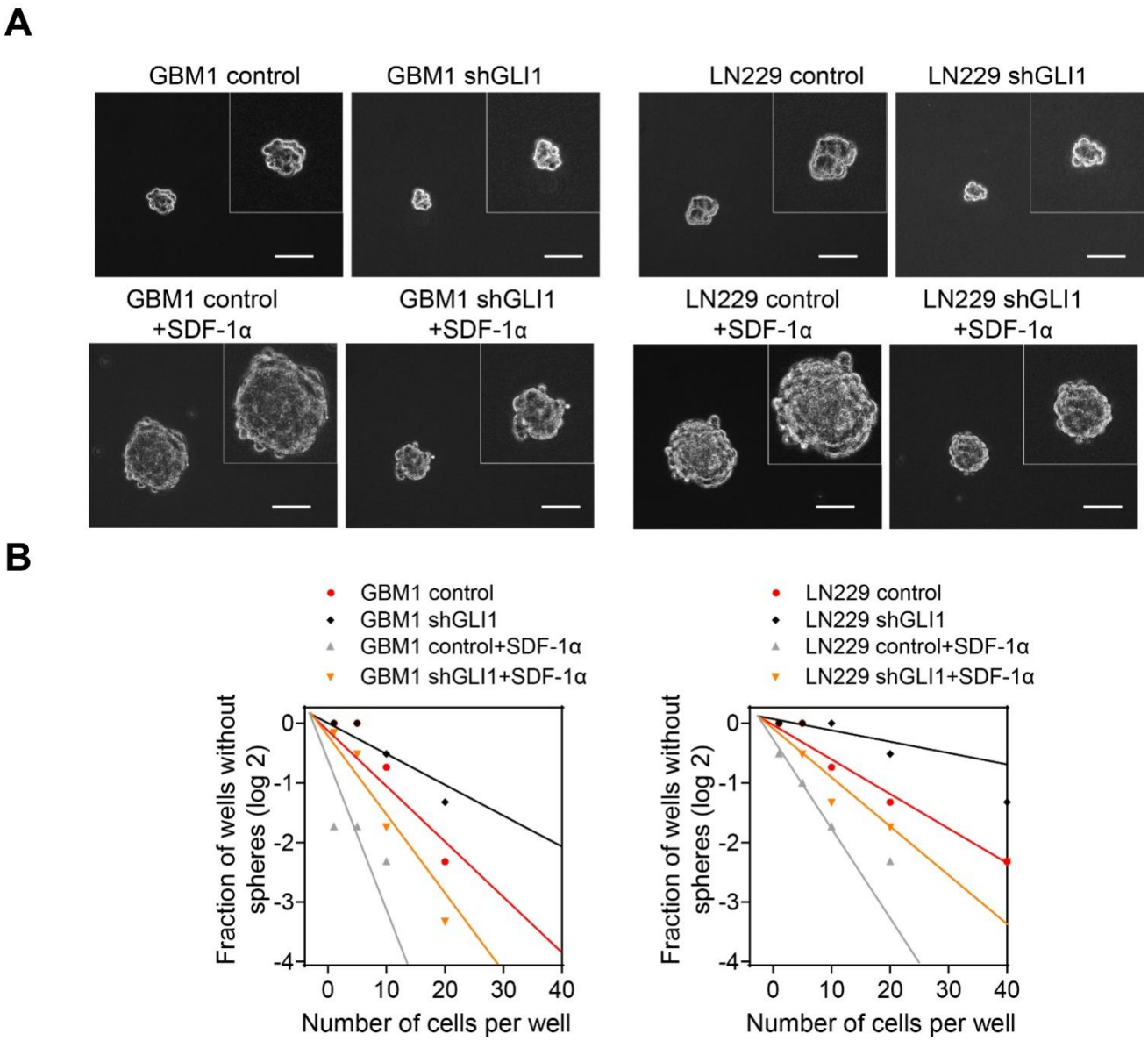

Figure S5 Disrupting GLI1 attenuates SDF-1α dependent stemness in GBM cells.

(A-B) Limiting dilution sphere-forming assays conducted on GBM1 **(A)** and LN229 **(B)** cells treated with either SDF-1α, shGLI1, or a combination of both, along with representative images of the resulting tumor spheres.

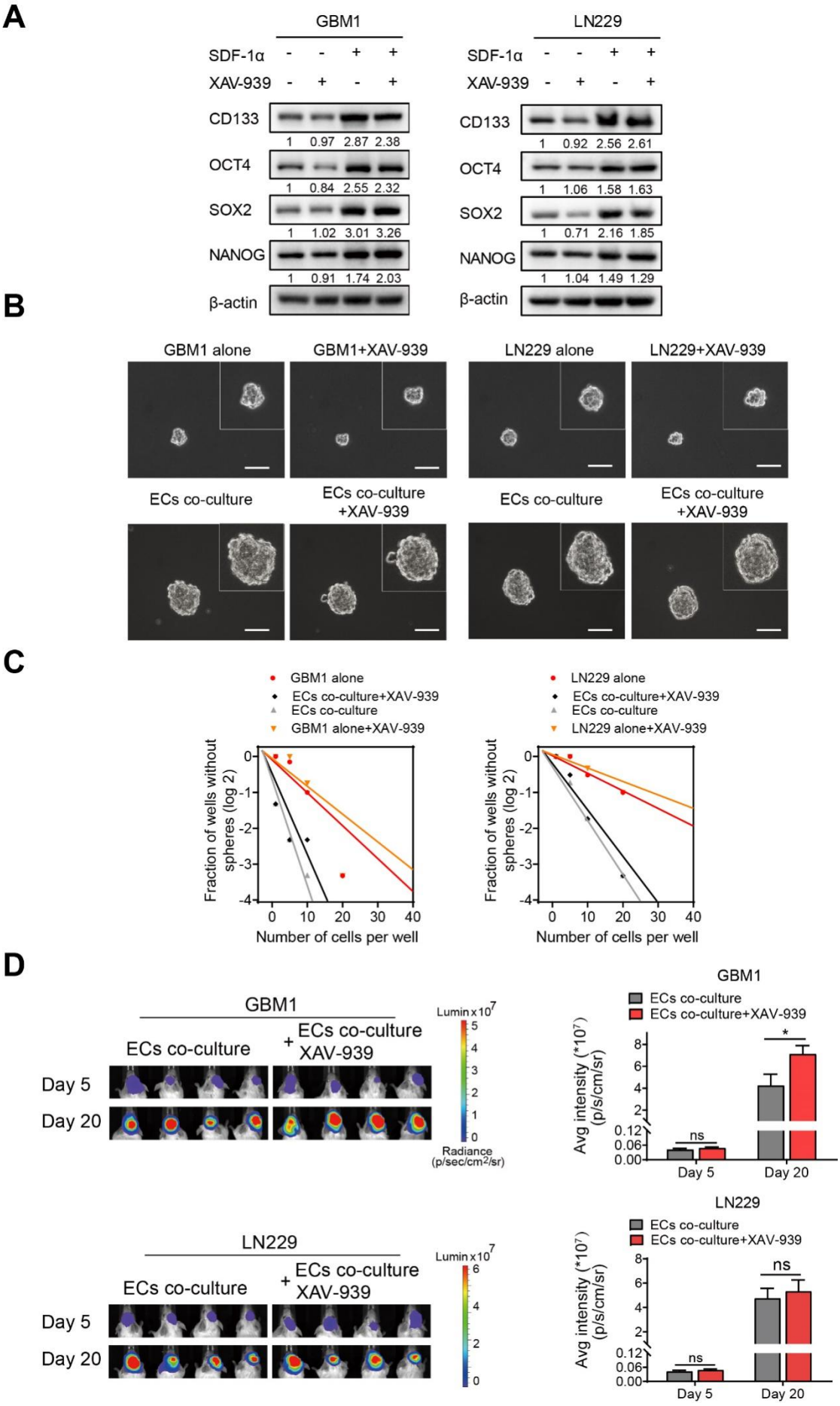
A
GBM1
LN229
SDF-1α - - + +
XAV-939 - + - +
CD133
1 0.97 2.87 2.38
1 0.92 2.56 2.61
OCT4
1 0.84 2.55 2.32
1 1.06 1.58 1.63
SOX2
1 1.02 3.01 3.26
1 0.71 2.16 1.85
NANOG
1 0.91 1.74 2.03
1 1.04 1.49 1.29
β-actin
B
GBM1 alone
GBM1+XAV-939
LN229 alone
LN229+XAV-939
ECs co-culture
ECs co-culture +XAV-939
C
GBM1 alone
ECs co-culture+XAV-939
ECs co-culture
GBM1 alone+XAV-939
LN229 alone
LN229 alone+XAV-939
Fraction of wells without spheres (log 2)
Number of cells per well
D
GBM1
ECs co-culture
+ ECs co-culture XAV-939
Day 5
Day 20
Lumin x10^7
Radiance (p/sec/cm²/sr)
Avg intensity (*10⁷) (p/s/cm/sr)
ECs co-culture
ECs co-culture+XAV-939
ns
*
LN229

Figure S6 XAV-939 demonstrated few or none inhibitory effect on SDF-1α dependent stemness maintaining in GBM cells.

(A) Western blot assay tested the expression of SOX2, OCT4, NANOG, and CD133 in GBM cells treated with SDF-1α/XAV-939 alone or together. **(B-C)** Limiting dilution sphere-forming assays were performed on ECs co-cultured with control GBM cells, with and without XAV-939 treatment, accompanied by representative images of tumor spheres. **(D)** *In vivo* bioluminescent imaging and quantification of xenograft tumor burdens were carried out in mice bearing ECs-GBM cells from the co-cultured group, with or without XAV-939 treatment. Data are means ± SD. ns: no significance; *, $P < 0.05$.

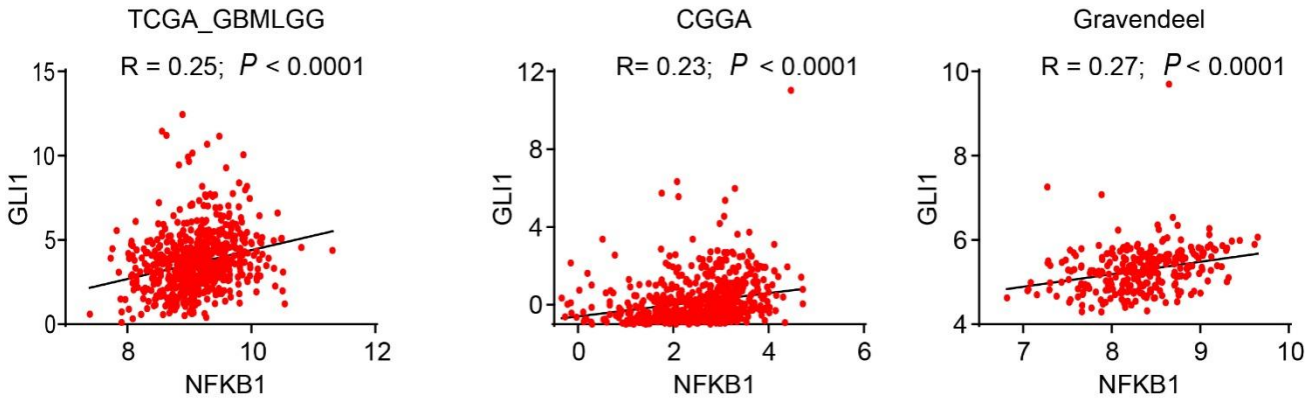

Figure S7 The correlation between expressions of NFKB1 and GLI1 in GBM databases.

The correlation plots showing relevance of NFKB1 to GLI1 from TCGA_GBMLGG, CGGA and Gravendeel datasets.

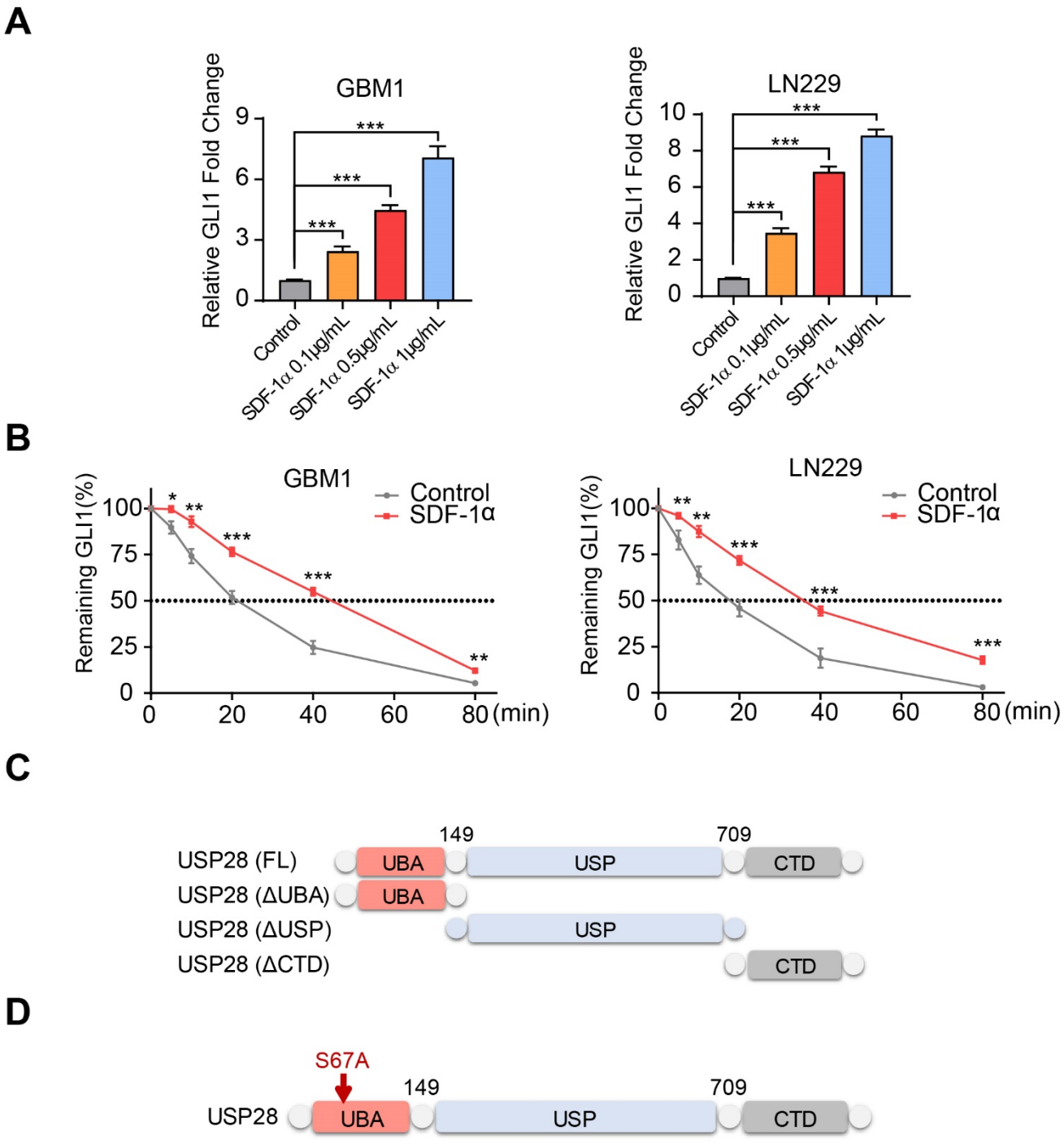

Figure S8 SDF-1α activates USP28 to regulate the turnover of GLI1 protein.

(A) qRT-PCR assay tested the expression of GLI1 in GBM cells treated with different concentrations of SDF-1α recombinant protein. **(B)** The halftime of GLI1 in GBM cells treated with SDF-1α recombinant protein or not at the indicated time. **(C)** Schematic representation of USP28 truncation mutants. **(D)** Schematic representation of USP28 point mutant

Table S1 The sequences of mRNA primers used in qPCR.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
β-actin	AGATGTGGATCAGCAAGCA	GCGCAAGTTAGGTTTTGTCA
SOX2	ACATGAACGGCTGGAGCAACG	CTGCGAGCTGGTCATGGAGTTG
CD133	TCGCCTCAAGCCAGCCTCAG	ACTTGGTGCCTCCTGCCTCAG
OCT4	CTGGGTTGATCCTCGGACCT	CCATCGGAGTTGCTCTCCA
NANOG	TTTGTGGGCCTGAAGAAAACT	AGGGCTGTCCTGAATAAGCAG
GLI1	AGCGTGAGCCTGAATCTGTG	CAGCATGTACTGGGCTTTGAA