Supplementary information

Supplemental Table 1. Mutations of the ATRX, *TERT*, *TP53*, IDH1, IDH2 and *EGFR* in the four glioma stem cell lines.

Cell lines	Mutation of *TERT*		Loss of ATRX	Mutation of *TP53*				*EGFR vIII* mRNA	Mutation of IDH1		Mutation of IDH2 R172
	C228T	C250T		Exon 5	Exon 6	Exon 7	Exon 8		R100	R132	
GSC2	No	No	No	No	No	No	No	Yes	No	No	No
GSC5	Yes	No	No	No	No	No	No	Yes	No	No	No
U251 SLC	Yes	No	No	No	No	No	No	Yes	No	No	No
U87MG SLC	Yes	No	No	No	No	No	No	Yes	No	No	No

Supplemental Table 2. **IC_{50} values of all 13 compounds in four GSCs, and human normal cells, H1-NSC and 293ET.** "——" stands for that IC_{50} value> 10^6 μM.

Comp.	Names	Status	Biological activity	IC_{50} in different cell lines (μM)					
				U251SLC	GSC5	U87MG SLC	GSC2	H1-NSC	293ET
1#	PIMOZIDE	clinical use	antipsychotic	38.09	3.75	2.57	12.56	239.34	66.52
2#	2,5-DI-t-BUTYL-4-HYDROXYANISOLE	experimental	antioxidant	7.62	18.9	7.93	19.25	1123.31	52.5
3#	SULCONAZOLE NITRATE	clinical use	antifungal	2.83	1.24	0.19	5.24	59.32	31.03
4#	TOTAROL	undetermined activity		5.54	3.61	3.88	16.15	1023.27	16.77
5#	SULOCTIDIL	clinical use	peripheral vasodilator	18.68	3.99	23.74	11.73	544.83	32.86
6#	CHOLESTAN-3beta, 5alpha,6beta-TRIOL	Undetermined Activity		0.19	0.68	1.5	6.88	47.02	8.09
7#	IVERMECTIN	clinical use	antiparasitic	3.21	6.64	1.54	8.38	60.02	21.54
8#	PHENYLMERCURIC ACETATE	clinical use	antifungal	0.29	0.02	0.1	0.03	——	0.02
9#	CETRIMONIUM BROMIDE	clinical use	antiinfectant	3.52	2.02	2.29	4.19	378.54	6.2
10#	GAMBOGIC AMIDE	experimental	caspase inhibitor	0.64	0.57	0.28	0.3	——	0.17
11#	TETRACHLOROISO-PHTHALONITRILE	experimental	antifungal	0.25	0.25	1.79	0.02	91.32	3.15
12#	CLOFOCTOL	clinical use	antibacterial	6.95	7.67	12.17	4.98	1084.6	18.93
13#	TOMATINE	experimental	antifungal, antibacterial, antiinflammatory agent	2.06	0.79	0.86	1.84	462.23	76.95

Supplemental Table 3. **In silico predicted ADMET properties (BBB) of all 13 compounds.** "0" indicates compounds with very high BBB penetration; "1" indicates compounds with high BBB penetration; "2" indicates medium, "3" indicates low, and "4" indicates undefined BBB penetration.

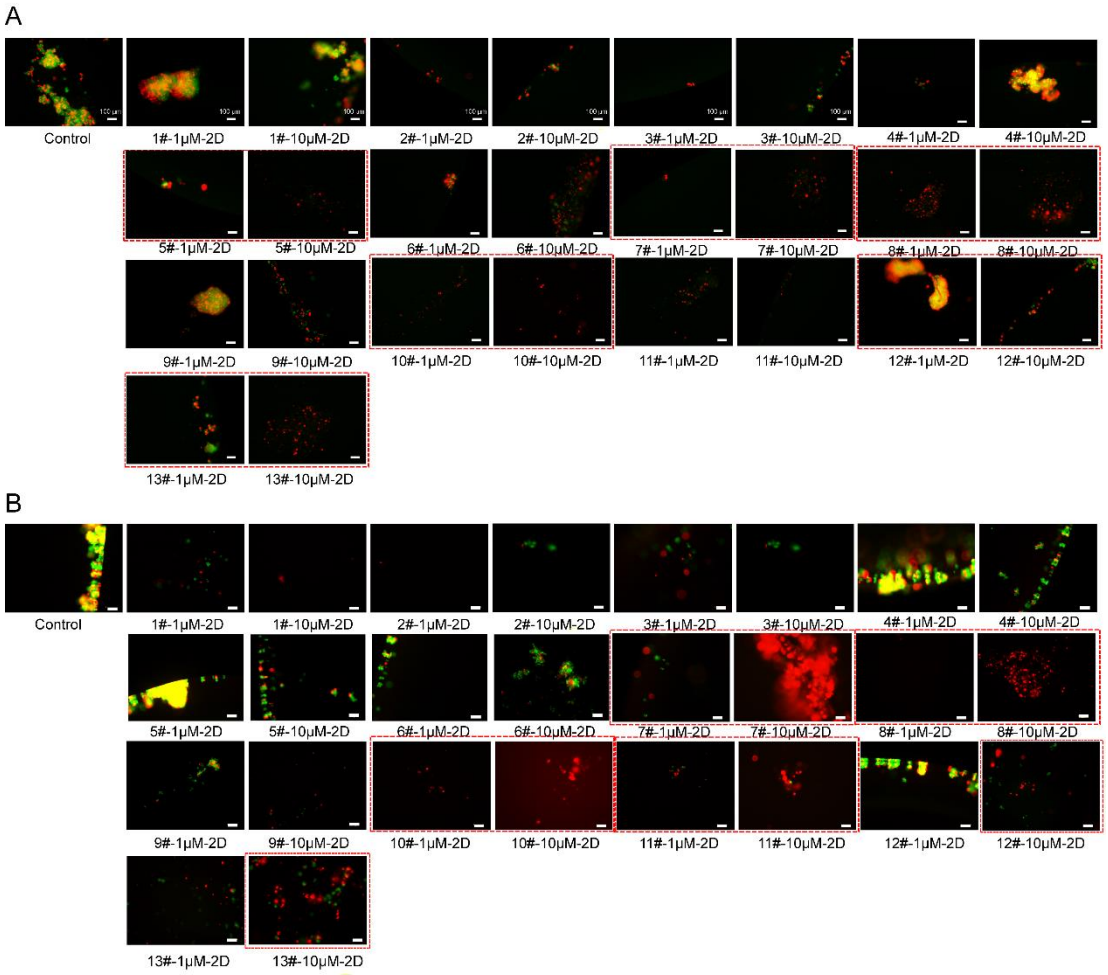
Comp.	Names	BBB
1#	PIMOZIDE	0
2#	2,5-DI-t-BUTYL-4-HYDROXYANISOLE	0
3#	SULCONAZOLE NITRATE	0
4#	TOTAROL	0
5#	SULOCTIDIL	0
6#	CHOLESTAN-3beta, 5alpha,6beta-TRIOL	1
7#	IVERMECTIN	3
8#	PHENYLMERCURIC ACETATE	1
9#	CETRIMONIUM BROMIDE	0
10#	GAMBOGIC AMIDE	4
11#	TETRACHLOROISO- PHTHALONITRILE	1
12#	CLOFOCTOL	4
13#	TOMATINE	4

Supplemental Table 4. Information of tumor specimens for PDXs.

PDX No.	Histology	Grage	Gender	Age
PDX1	Glioblastoma, primary	WHO IV	Male	66
PDX2	Oligodendrocytoma, relapsed	WHO III	Female	15
PDX3	Glioblastoma, relapsed	WHO IV	Male	62

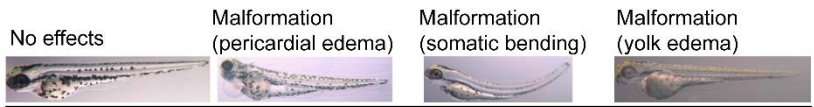
Supplemental Table 5. Segment of enriched proteins in the clofoctol-treated DARTS sample.

Protein Symbol	Protein Name	Enrichment
IKKB	inhibitor of nuclear factor kappa B kinase subunit beta	Clo-sample specifically
UNR	Upstream of N-ras	Clo-sample specifically
CIP2A	cell proliferation regulating inhibitor of protein phosphatase 2A	Clo-sample specifically
RPS6KA3	ribosomal protein S6 kinase A3	11.5
PPP4R3A	protein phosphatase 4 regulatory subunit 3A	9.0
CUL1	cullin1	6.6
DHX15	DEAH-box helicase 15	6.5
APLP2	amyloid beta precursor like protein 2	6.4



Supplemental Figure 1. **Coculture assay revealed that compound 7#, 8#, 10#, 12#, and 13# could selectively inhibit GSCs. (A and B)** GSC2 **(A)** and U87MG SLC **(B)** cells were labeled with GFP, and HA cells were labeled with RFP by lentivirus infection as indicated and sorted by flow cytometry. Cells were individually dissociated, mixed 1:1 and suspended as hanging drops in culture media with or without compounds at 1 μM or 10 μM concentrations for 48 h, and then imaged. Scale bars: 100 μm.

A



Zebrafish（1~2 cell stage） toxicity analysis

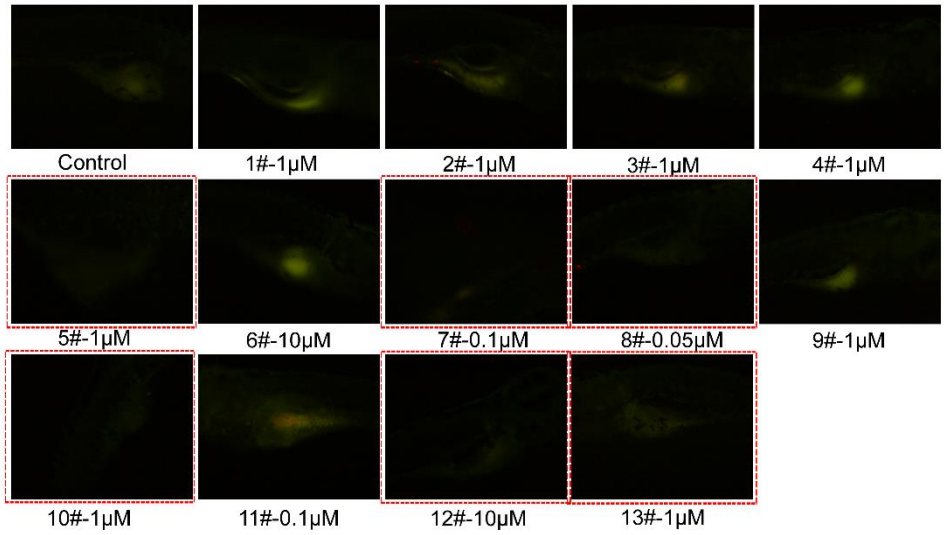
Comp.	1#	2#	3#	4#	5#	6#	7#	8#	9#	10#	11#	12#	13#
1μM	MF	NF	NF	NF	MF	NF	D	D	NF	NF	D	NF	D
5μM	MF	NF	DC	NF	MF	NF	D	D	D	D	D	NF	D
10μM	MF	NF	DC	NF	D	NF	D	D	D	D	D	NF	D
50μM	MF	D	D	D	D	NF	D	D	D	D	D	NF	D

Zebrafish（96-hour-old） toxicity analysis

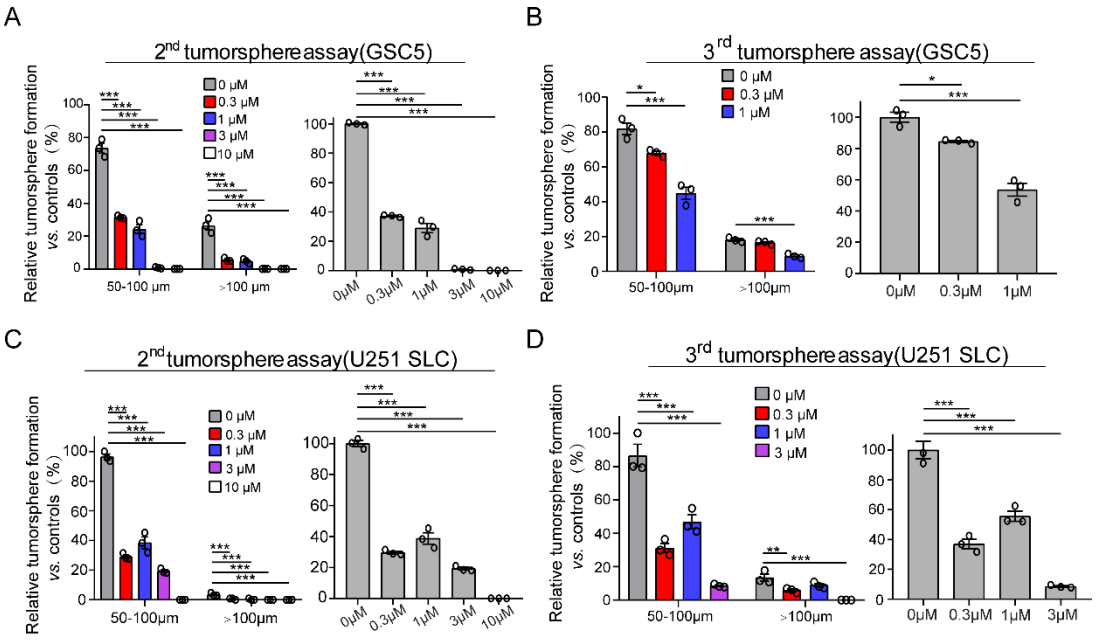
Comp.	1#	2#	3#	4#	5#	6#	7#	8#	9#	10#	11#	12#	13#
0.05μM	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
0.1μM	NF	NF	NF	NF	NF	NF	NF	D	NF	NF	NF	NF	NF
1μM	D	NF	NF	NF	NF	NF	MF	D	D	NF	NF	NF	NF
5μM	D	NF	D	NF	D	NF	D	D	D	D	D	NF	D
10μM	D	NF	D	D	D	NF	D	D	D	D	D	NF	D

NF No effects
DC Decayed
MF Malformation
D Dead

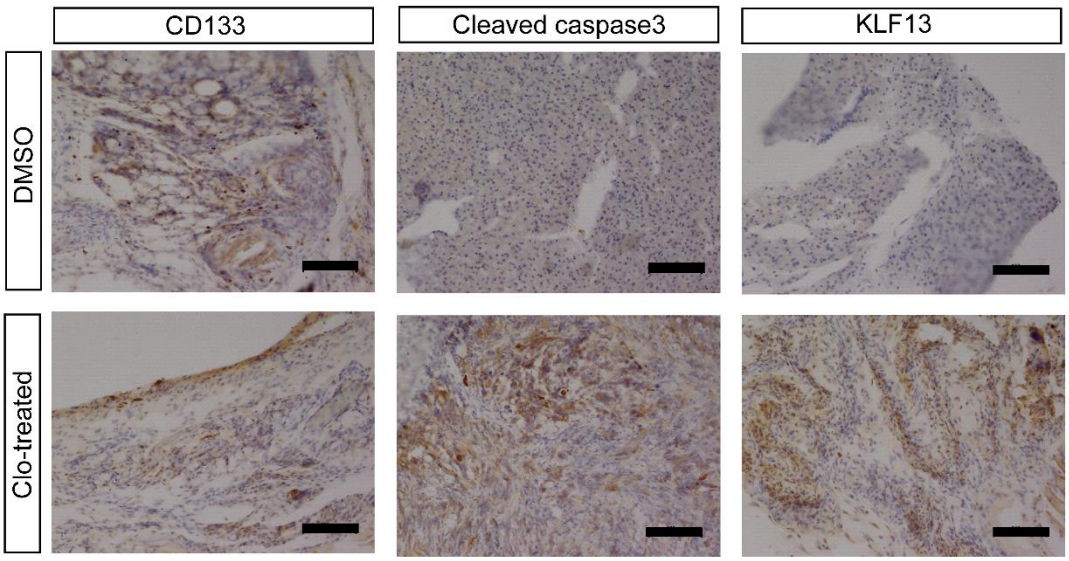
B



Supplemental Figure 2. Toxicity and in vivo efficacy in zebrafish xenograft models. (A) Phenotypes displayed by zebrafish larvae (top) or adult zebrafish (bottom) after treatment with compounds at indicated concentrations. Images of larvae exposed to different compounds. **(B)** Forty-eight-hour zebrafish were injected with 3000 U87MG SLC-GFP cells into the yolks; 48 h later, they were treated with different compounds applied to the egg water at maximum safe concentrations. Imaging revealed that compounds 5#, 7#, 8#, 10#, 12#, and 13# could markedly inhibit tumor growth.

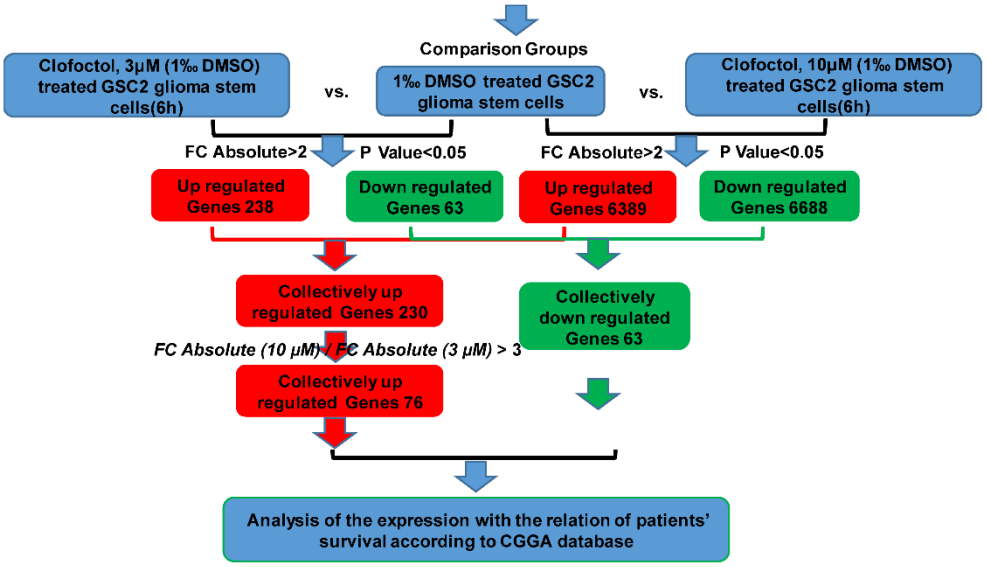


Supplemental Figure 3. Clofoctol could inhibit the colony-forming ability of glioma stem cells. (A and C) Secondary tumorsphere assay showing that treatment with clofoctol for 6 h could significantly suppress the colony-forming capability of GSC5 cells **(A)** and U251 SLC cells **(C)**. **(B and D)** Third tumorsphere assay of GSC5 **(B)** and U251 SLC **(D)** cells from second tumorspheres after treatment with clofoctol for 6 h. Data are presented as the mean ± SEM and are analyzed by ANOVA. Samples were assayed in triplicate.



Supplemental Figure 4. Expression of CD133, cleaved caspase3 and KLF13 in PDXs after clofoctol treated. Immunohistochemical analysis of sections from PDXs stained with antibody to CD133 (left), cleaved caspase3 (middle) and KLF13 (right). Three individual samples were analyzed. Scale bars: 100 μm.

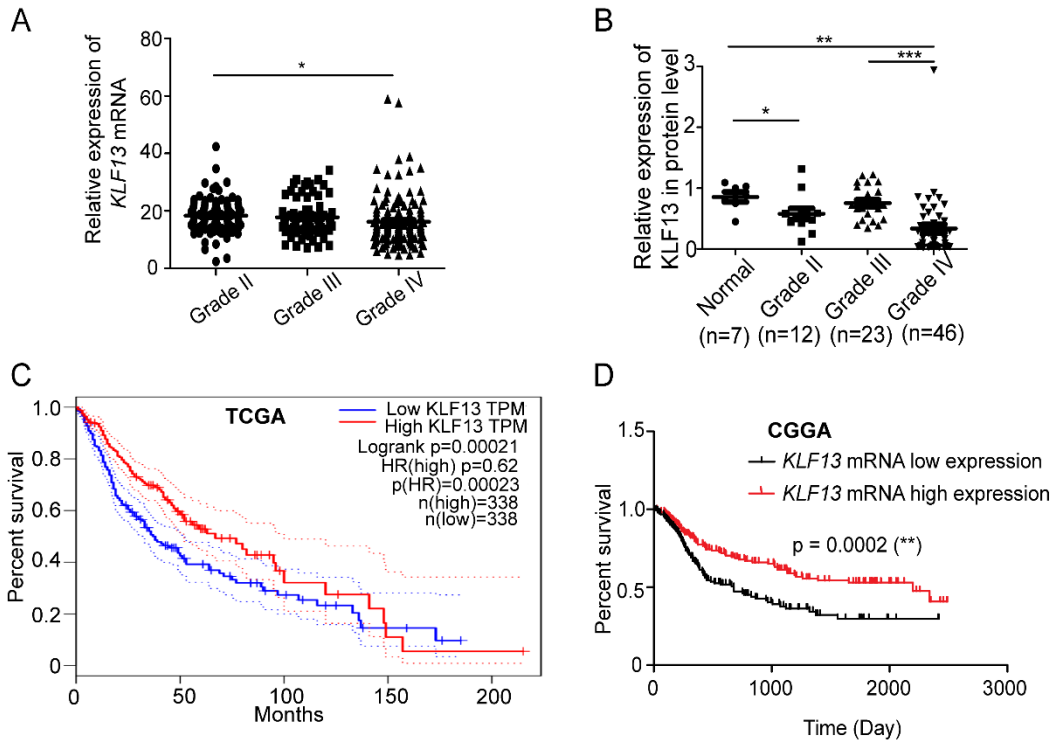
A



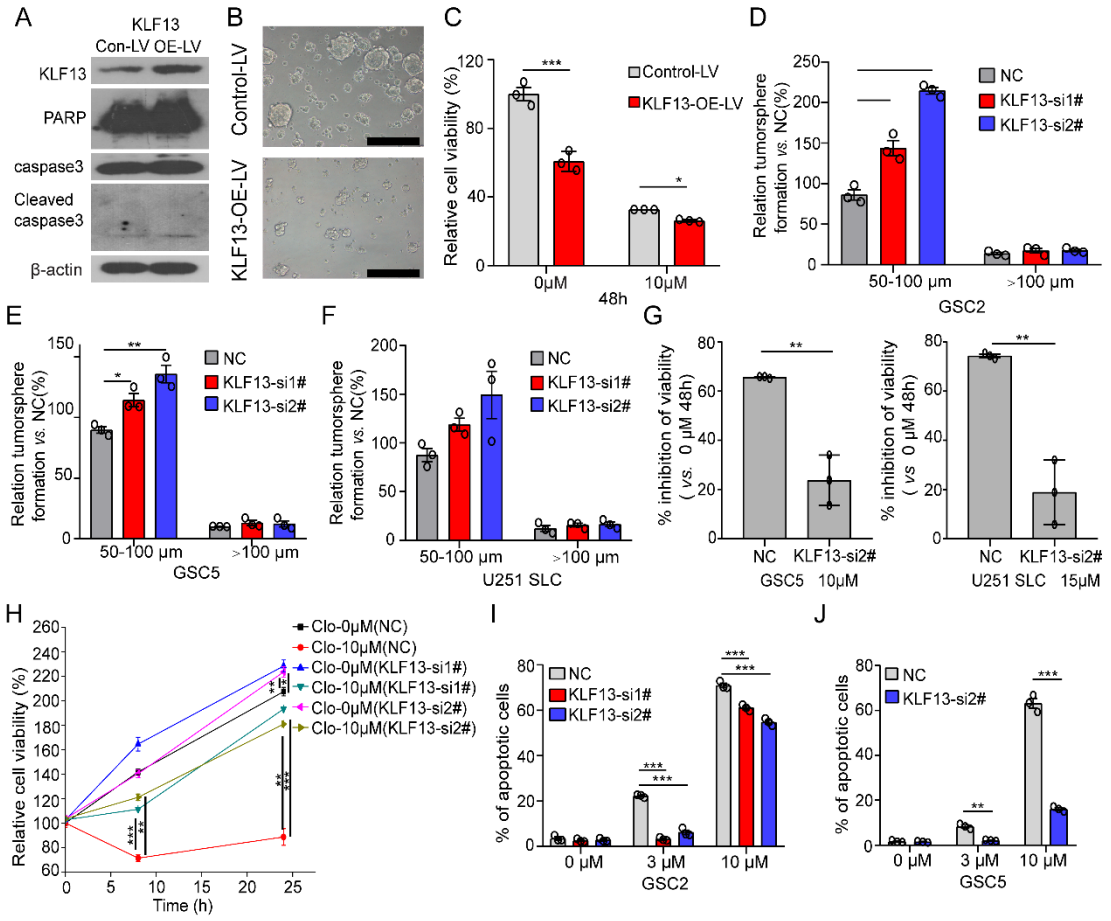
B

Selected assay	Tumorsphere assay(siRNA)			Clofoctol recovery assay(siRNA)			Limited dilution assay	
	GSC2	GSC5	U251 SLC	GSC2	GSC5	U251 SLC	GSC2	GSC5
LDHB	√	√	√	-	X	√	-	-
FGD5-AS1	X	√	√	-	√	X	-	-
PDZRN4	√	√	√	√	√	√	X	X
KLF13	√	√	√	√	√	√	√	√
NRSN1	X	X	X	-	X	√	-	-

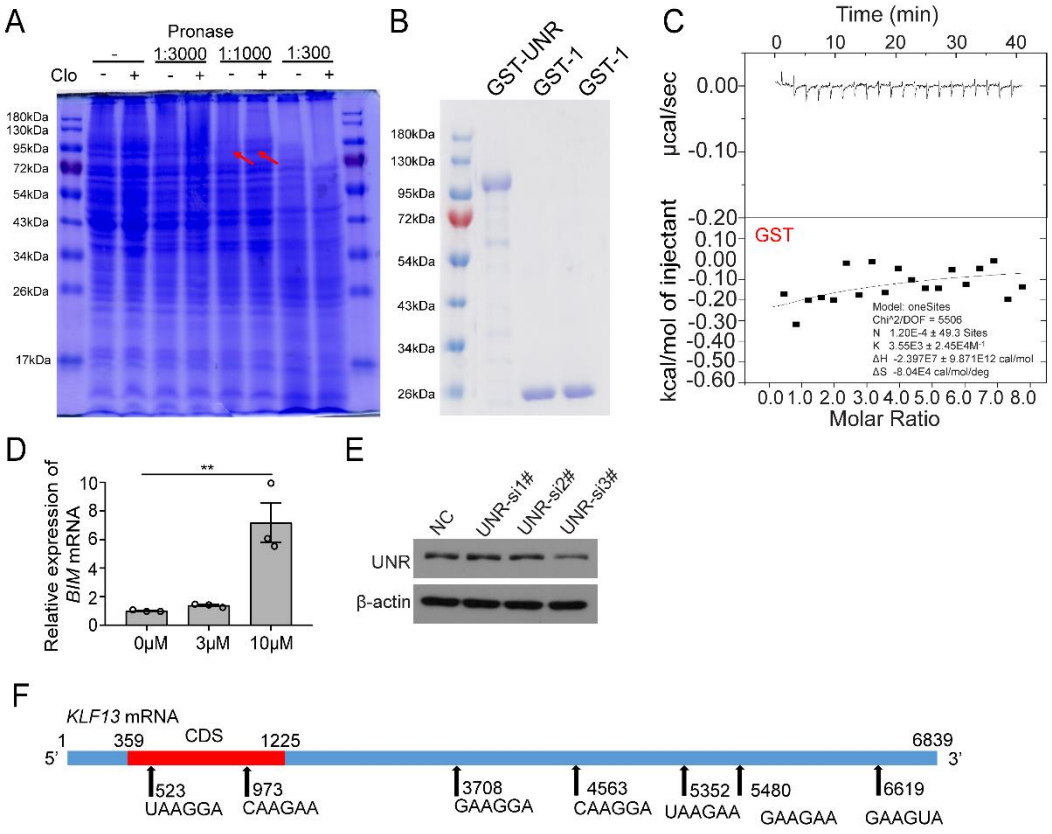
Supplemental Figure 5. Process of analysis expression profiling identified KLF13 as a target of clofoctol in GSCs. (A) Process of analysis of mRNA expression changes after GSC2 cells were treated with 1‰ DMSO or 3 μM or 10 μM clofoctol for 6 h. Analysis of differentially expressed genes related to patients survival according the CGGA database. Real-time PCR analysis of the expression change in GSC2, GSC5, and U251 SLC cells. See also Figure 4E. **(B)** Selection criteria for identifying KLF13 as the crucial gene underlying the effects of clofoctol in GSCs. For "tumorsphere assays", "√" indicates that after GSC transfection with relevant siRNA, the tumorsphere formation ability was increased; "X" indicates that it was not. For "clofoctol recovery assays", "√" indicates that after GSCs transfection with relevant siRNA, clofoctol effects were reversed; "X" indicates that they were not. For the "limited dilution assay", "√" indicates that after GSC transfection with relevant siRNA, limiting dilution tumor-sphere ability was increased; "X" indicates that it was not.



Supplemental Figure 6. KLF13 is aberrantly downregulated in gliomas and its downregulation is connected with poor prognosis of patients. (A) The KLF13 mRNA expression levels in whole-genome profiling of 325 glioma samples from CGGA database. Data are presented as the mean ± SEM. *P <0.05, **P <0.01, ***P <0.001 by ANOVA. **(B)** Relative KLF13 protein levels in 7 control brain tissues and in 12 grade II, 23 grade III and 46 grade IV glioma tissues; β-actin was used as a loading control. Related to Figure 5E. Data are presented as the mean ± SEM. *P <0.05, **P <0.01, ***P <0.001 by ANOVA. **(C-D)** Low expression of KLF13 correlated with poor survival of patients by analyzing information from GEPIA website **(C)** and CGGA database **(D)**. Mantel-Cox test was used.



Supplemental Figure 7. Clofoctol Inhibits Growth of GSCs by upregulation of KLF13. (A) Western blotting analysis of KLF13 overexpression by related lentivirus and the cleavage of cell apoptosis proteins (PARP, caspase 3, cleaved caspase 3) after KLF13 overexpressed. **(B)** The relative graphs under microscope of Figure 6C. Scale bars: 100 μm. **(C)** Control-LV and KLF13-LV-overexpressing GSC2 cells were treated with 0 μM and 10 μM clofoctol for 48 h, and relative cell viability was measured by MTS. **(D-F)** Colony-formating ability was increased by KLF13 knockdown in GSC2 **(D)**, GSC5 **(E)** and U251 SLC **(F)** cells. **(G)** Percent cell viability inhibition of GSC5 and U251 SLC cells transiently transfected with KLF13-siRNA2# and control siRNA treated with clofoctol. **(H)** GSC2 cells transfected with KLF13-si1# and si2# were treated with 0 μM and 10 μM clofoctol, and viability was measured by MTS at 0 h, 8 h, and 24 h. **(I)** The quantitative analysis of Figure 6J. **(J)** The quantitative analysis of Figure 6K. Data are presented as the mean ± SEM. Data in Supplemental Figure 7C, G, J and H were analyzed by 2-tail Student's t test. Data in Supplemental Figure 7D, E, F and I were analyzed by ANOVA.



Supplemental Figure 8. UNR is a clofoctol binding protein, according to DARTS analysis, that regulates expression of KLF13. (A) Lysates from untreated human GSC2 cells were incubated with DMSO control or clofoctol (100 µM) for 1 h at room temperature. Each sample was then split into 4 aliquots that underwent digestion with various concentrations of pronase, relative to the total amount of protein per sample, for 30 mins at room temperature. Digestion was stopped by adding 6× loading buffer and boiling immediately. 8 µL of each sample was then loaded onto one of two 10% SDS/PAGE gels for Coomassie staining. **(B)** Purity analysis of purified fushion protein, GST-UNR and GST by Coomassie staining. **(C)** ITC binding curves for GST with clofoctol**. (D)** The expression of *BIM* mRNA after clofoctol treatment. Samples were assayed in triplicate and data were analyzed by ANOVA. **(E)** Analysis of the effects of UNR siRNA treatment by western blotting. **(F)** Possible binding sites of UNR in KLF13 mRNA.