Supplemental Data

Experimental Procedures

Brain tumors, primary cultures and gliomaspheres

All primary brain tumors were obtained fresh from the operating room following approved protocols, chopped manually, dissociated with Papain and plated in DMEM-F12 with FBS for adherent culture or seeded at a concentration of 50,000 cell/ml in 1/2-1/3 conditioned medium and DMEM-F12 serum-free medium with 20% BIT 9500 (Stem cell technologies Inc), 10 ng/ml of EGF and 10 ng/ml of FGF-2 for gliomasphere culture. GBM U87 cells (ATCC) were grown as suggested by the supplier. All experiments were done in triplicate. Error bars denoting s.e.m. are shown in all cases except when histograms show ratios. In this case, asterisks denote significative ($p<0.05$) changes.

Clonal assays and treatments

Gliomaspheres were dissociated and plated at 1 cell/well in 96-well plates containing DMEM-F12, 20% BIT 9500 media with EGF and FGF at a concentration of 10 ng/ml each, with 1/2-1/3 sphere-conditioned media for spheres. Clonal growth was assessed visually with an inverted microscope at 2 weeks (tumor adherent cells) or 10 days (gliomaspheres). ±400-600 cloning events in 4-6 96-well plates were performed per experiment and this was repeated in triplicate. N-SHH (100nM; R&D), Cyclopamine (TRC) or Temozolomide (Schering-Plough; kindly provided by P.-Y. Dietrich) were added at the beginning of the culture period for 7 days in media containing 1ng/ml EGF and FGF each. Control cells were treated in the same manner with PBS, tomatidine (Sigma) or DMSO (Fluka). For other uses, cells were treated with SHH, cyclopamine or tomatidine cells in low (2.5%) serum.

siRNAs, transfections and PCR

21nt-long double stranded siRNAs were purified and desalted (Dharmacon Inc). The sequences for the siRNAs were 5'-3': GLI1: AACUCCACAGGCAUACAGGAU; GLI2: AAGAUCUGGACAGGGAUGACU; GLI3: AAUGAGGAUGAAAGUCCUGGA. The second set (Ambion) was: GLI1: GCCCAGAUGAAUCACCAAA; GLI2: CCCUGUCGCCAUUCACAAG; GLI3: GGGCCGUUACCAUUACGAU. Control siRNA with or without an FITC tag: AACGUACGCGGAAUACAACGA. siRNA transfections (0.2µM) were performed with Oligofectamine (Invitrogen, Inc). Genes chosen for analyses and quantitative RT-PCR: The genes used to detect the stemness signature in human gliomas were chosen for their association with self-renewal and/or tumorigenicity. These were the following with indicative references: *CD133* (refs. 1-3); *OLIG2* (refs. 4,5); *BMI1* (refs. 6-10); *BREVICAN* (11,12; *BCAN*); *NANOG* and *OCT4* (refs. 13-17); *SOX2* (refs. 17-20); *PTEN* (21-26); *ABCG2* (refs. 27-29); *PDGFRA* (30-33); *NEUROD1* (*NRD1*;ref. 34); *NESTIN* (35-37); *NOTCH1* (refs. 38-41); *MELK* (42). qPCR primers were 5'->3': GLI2-F: CACCGCTGCTCAAAGAGAA, GLI2-R: TCTCCACGCCACTGTCATT; GLI3-F: CGAACAGATGTGAGCGAGAA, GLI3-R: TTGATCAATGAGGCCCTCTC; PTCH1-F: CCACAGAAGCGCTCCTACA, PTCH1-R: CTGTAATTTCGCCCCTTCC; SHH-2F: TCCAAGGCACATATCCACTG, SHH-2R: CCAGGAAAGTGAGGAAGTCG; HIP-F: CCCACACTTCAACAGCACCA, HIP-R: GCTTTGTCACAGGACTTTGC; CD133-F: GCCACCGCTCTAGATACTGC, CD133-R: TGTTGTGATGGGCTTGTCAT; NOTCH1-F: GGCCACCTGGGCCGGAGCTTC, NOTCH1-R: GCGATCTGGGACTGCATGCTG. SOX-2-F: ACACCAATCCCATCCACACT, SOX-2-R: GCAAACTTCCTGCAAAGCTC; MELK-F: CAGGCAAACAATGGAGGATT, MELK-R: TGTCTGTGAATGGGGTAGCA; CD44-F: AAGGTGGAGCAAACACAACC, CD44-R: AGCTTTTTCTTCTGCCCACA; BMI1SQ-F: GCAGCAATGACTGTGATG, BMI1SQ-R: AGTCCATCTCTCTGGTGAC; ABCG2-F: CACCTTATTGGCCTCAGGAA, ABCG2-R:

CCTGCTTGGAAGGCTCTATG; NANOG-F: GTCCCGGTCAAGAAACAGAA, NANOG-R: TGCGTCACACCATTGCTATT; OCT4-F: ATTCAGCCAAACGACCATCT, OCT4-R: TTGCCTCTCCACTCGGTTCTC; EGFR-F: CAGCGCTACCTTGTCATTCA, EGFR-R: AGCTTTGCAGCCCATTTCTA; PTEN-F: ACCAGGACCAGAGGAAACCT, PTEN-R: GCTAGCCTCTGGATTTGACG; PCNA-F: GGCTCTAGCCTGACAAATGC, PCNA-R: GCCTCCAACACCTTCTTGAG; YKL40-F: TCAAGAACAGGAACCCCAAC, YKL40-R: AAATTCGGCCTTCATTTCCT; BCAN-F: GGACTCAACGACAGGACCAT, BCAN-R: GCAGGTGTAGGACAGGTGGT; NCAM-F: GTGGACTCGACCAGAGAAGC, NCAM-R: CTTTGGGGCATATTGCACTT; GFAP-F: ACATCGAGATCGCCACCTAC, GFAP-R: ATCTCCACGGTCTTCACCAC; DCX-F: GACAGCCCACTCTTTTGAGC, DCX-R: TGGGTTTCCCTTCATGACTC; NEUD1-F: GCCCCAGGGTTATGAGACTA, NEUD1-R: GCTCCTCGTCCTGAGAACTG; OLIG2-F: CAGAAGCGCTGATGGTCATA, OLIG2-R: TCGGCAGTTTTGGGTTATTC; VIM-F: CCCTCACCTGTGAAGTGGAT, VIM-R: TCCAGCAGCTTCCTGTAGGT; at 60^{o}C and NESTIN-s: GGCAGCGTTGGAACAGAGGTTGGA, NESTIN-a: CTCTAAACTGGAGTGGTCAGGGCT; NUCLEOSTEMIN-s : CAAAGCCAAGTCGGGCAAAC, NUCLEOSTEMIN-a: CCTGAGGACATCTGCAACCAA at 56^{o}C, Primers for *GAPDH* and *GLI1* were as described respectively (30,43,44). Quantitative RT-PCR was performed using the iQ^{Tm} SYBR Green supermix (Biorad) according to manufacturers' instructions at 60°C.

Immunohistochemistry in situ hybridization

Immunocytochemistry used mouse anti-BrdU (R&D Systems), rabbit anti-Capase-3 (Cell signaling), rabbit anti-GFAP (Sigma), mouse anti-Nestin (R&D Systems) and rabbit anti-GFP primary antibodies (Molecular Probes) with FITC- or rhodamine-conjugated secondary antibodies (Molecular Probes). Stained cells were observed by Zeiss Axiocam optical and LSM MEta 510 confocal microscopy. In situ hybridizations with digoxygenin-labeled antisense RNA probes for *GLI1*, *PTCH1* and *SHH* were as described (30).

Proliferation assays

U87 cells were given a 2 h pulse of BrdU (Sigma) at 4µg/ml, while primary tumor and gliomaspheres were given 16 h pulse. The length of the gliomasphere cell cycle length was estimated to be ±36h. Visualization of new DNA synthesis was revealed by anti-BrdU indirect immunofluorescence on adherent cultures directly with an Axiophot and in whole gliomaspheres with Confocal Z-stacks of an LSM510 Meta after placing them for 30-60' before fixation on adherent coated dishes. Cell viability was tested by the colorimetric MTT assay (Promega) in 96-well plates or by trypan blue exclusion after dissociation.

CD133 FACS staining sorting

Gliomasphere cultures were dissociated and labeled with CD133 antibody as previously described (1,3). Briefly, spheres were dissociated and labeled with CD133/2 (293C3)-PE antibody (Miltenyi Biotech) and the expression level was analysed on a Beckton Dickinson FACSCalibur (BD Bioscience) for measuring abundance. For sorting, dissociated sphere were labeled with CD133/1-microbeads (MiltenyiBiotech) at a concentration of 1ul/10^6 cells according to manufacturers'instructions. Magnetic bead-antibody-antigen separation was performed using the possel-s program on an autoMACS machine.

Mouse xenografts of human gliomas

Cells infected with appropriate lentiviral vectors (GFP- or beta gal-expressing) to allow tracing in vivo. ±72 after infection (to allow for expression of the integrated lentiviral vectors) cells were dissociated and adjusted at the desired concentration in 2µl in cold HBSS. For subcutaneous xenografts, U87 cells were injected (2x10^6 cells) on each back side of each mouse. As soon as the tumor was palpable, cyclodextrin-conjugated cyclopamine or cyclodextrin carrier alone (Sigma) at 10mg/kg were injected in the immediate vicinity or intratumorally when possible twice daily. For intracraneal grafts, gliomaspheres (10^4 or 10^5 cells) were implanted at coordinates X= -2, Y=0, Z=-2 relative to the bregma point with a sterotaxic apparatus. 2 weeks after injection, systemic intraperitoneal injection of cyclodextrin-conjugated cyclopamine or cyclodextrin carrier alone at 10mg/kg were done twice daily.

Lentiviral transduction and construction

pLVCTH lentiviral vectors containing an H1 promoter to drive shRNA expression were grown as described (45). The shRNA sequence targeting SMOH was: AGTGTTGACTGTGTCATTA. The VSV-G pseudotyped lentiviral vectors LV-control (pLV-CTH parental vector) and the derived LV-shSMOH were produced by transient cotransfection of three plasmids into 293T cells using approved protocols. Conditional expression of the shRNA from LV-shSMOH was accomplished after coinfection with LV-rtTA-KRAB (45). This lentiviral vector expresses a recombinant tet-repressor-KRAB protein that silences the promoters in LV-shSMOH. Only upon doxycyclin (DOX) addition (5µg/ml in the culture media renewed daily for in vitro use, or 2g/l in the drinking water with 5% sucrose plus 20µg/ml IP daily for in vivo use) does the trTR-KRAB protein is rendered nonfunctional and transcription of the shRNA begins.

Supplementary References

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Supplemental Figures

Supplemental Figure 1. Summary of brain tumors used in this study and their expression of SHH-GLI pathway components. A) Grade, age, gender and location of primary brain tumors, normal epileptic samples and the U87 cell line used.

B-D) Summary of the expression levels of *GLI1* and *PTCH1* (B), *GLI2* and *GLI3* (C) and *SHH* and *HIP1* (D) in different brain tumors as determined by qPCR. Note the ubiquitous activity of the HH-GLI pathway as denoted by the increased levels of GLI1 in all tumor types and two HH-GLI regulated genes: *PTCH1* and *HIP1*. The expression levels are the relative ratio of gene expression over that of *GAPDH*.

Supplemental Figure 2. Quantitative changes in gene expression in fresh tumors determined by qPCR. This data was used to generate Fig. 1A. All numbers denote relative fold expression over *GAPDH*, used as ubiquitous control. The bottom box includes the values of three normal cortical samples from a hemispherectomy of an epileptic patient used to determine the up and down thresholds for normal expression. The thresholds themselves were then arbitrarily set close to the up and down limit values observed for the control brain samples but always slightly expanding the normal range. This then allowed us to consider as within the normal range any value in tumor samples that was very close to the three normal values in order to avoid over-representing in the overexpressed set any value that was very close to the normal range.

Supplemental Figure 3. Poor correlation of a set of recently defined glioma markers with glioma grade or type. The three glioma classes reported (12) are mesenchymal, characterized by high *YKL40* and *CD44* expression, Proneural, characterized by high *BCAN* and low *MELK* expression and Proliferative, characterized by high *CD133*, *PCNA* and low *GFAP* expression (12). Thresholds were obtained as in Supplementary Fig. 2 from normal cortical samples. All numbers denote relative fold expression over *GAPDH*, used as ubiquitous control. Only 6/27 tumors matched: Mesenchymal: GBM5,6,7; Proneural: no match; Proliferative: GBM9.

Supplemental Figure 4. Inhibitory effects of cyclopamine treatment on glioma stem cell cultures and U87 glioma cells. A) Growth inhibition of three glioma stem cell cultures treated over 9 days and measured by the viability MTT assay. Histograms show cyc/tom ratios each at 10µM and asterisks denote significative ($p<0.05$) changes. B) Inhibition of *GLI1* transcription in U87 cells 4h after treatment with 10µM cyclopamine. C) Death of U87 cells induced by treatment with cyclopamine (10µM) and recovery of the culture following removal of the drug after 4 days for its ethanol solvent.

Supplemental Figure 5. Effects of an independent set of siRNAs targeting different regions in *GLI1*, *GLI2* and *GLI3* mRNAs and effects on U87 survival. A) Inhibition of cell proliferation shown as a percentage ratio of reduction of BrdU+ cells in each individual GLI siRNA over a control, unrelated siRNA (siC). Asterisks denote significative ($p<0.05$) changes. B) Reduction of *GLI1*, *GLI2* or *GLI3* mRNA levels in U87 cells and GBM-8 adherent cells after lipofection with the appropriate siRNAs and measured by qPCR, over *GAPDH* values, shown as ratios over a control siRNA. Gliomaspheres cannot be lipofected to usable levels. C) Long-term treatment of U87 glioma cells with siRNA GLI1 (siGLI1), but not siRNA GLI2 (siGLI2) or sRNA control (siC), leads to cell death as measured by retention of trypan blue. Cells were lipofected anew every other day to maintain high levels of siRNAs in them. Asterisks denote significative ($p<0.05$) changes.

Supplemental Figure 6. Effects of temozolomide on glioma stem cell cultures.
A-D) Concentration-dependent inhibition of cell proliferation (A,C) and increase in apoptotic death (B,D), measured by BrdU incorporation (A,C) and activated Caspase-3 immunostaining (B,D), by temozolomide (TMZ) treatment in U87 cells (A,B) and three glioma stem cell cultures (C,D). E) Additive and synergistic effects on the induction of apoptotic cell death after treatment with intermediate doses of TMZ and cyclopamine on three glioma stem cell cultures and U87. Histograms show ratios of TMZ versus DMSO treated samples (A-D) or over tomatidine treated samples for cycloapmine (E). Asterisks denote significative ($p<0.05$) changes as compared to cyclopamine alone. F,G) Long-term treatment and recovery of gliomasphere cultures after treatment with cyclopamine (F) or TMZ (G) for different number of days (d) as measured by trypan blue exclusion in live cells. Note that only after cyclopamine treatment there is significant ($p<0.05$) non-recovery of the cultures (asterisks).

Supplemental Figure 7. In situ hybridization of intracerebral human gliomas in nude mice.
Dissociated GBM-8 gliomaspheres were injected into the brains of nude mice. The brains harboring the infiltrative gliomas were collected after ±2-months, sectioned with a cryostat and frozen sections hybridized with human antisense RNA probes to localize the expression of *GLI1*, *SHH* and *PTCH1*. The human probes do not pick up the mouse mRNAs. Specific hybridization was revealed with NBT/BCIP yielding a blue precipitate (top row). As control, sense probes gave no signal (not shown) and the control, uninjected side of the mouse brain implanted with gliomaspheres contralaterally also did not show expression (bottom row). H&E staining was used (left column) to show cellularity, which increased in the tumor (top).

Scale bar = 70µm for all panels.

Supplemental Figure 8. Gene expression in glioma stem cell cultures determined by qPCR. The data shown here were used to generate Fig. 3A. Calculation of the mean included all samples except outliers when marked by an asterisk. Gene expression values are shown relative to that of *GAPDH*.

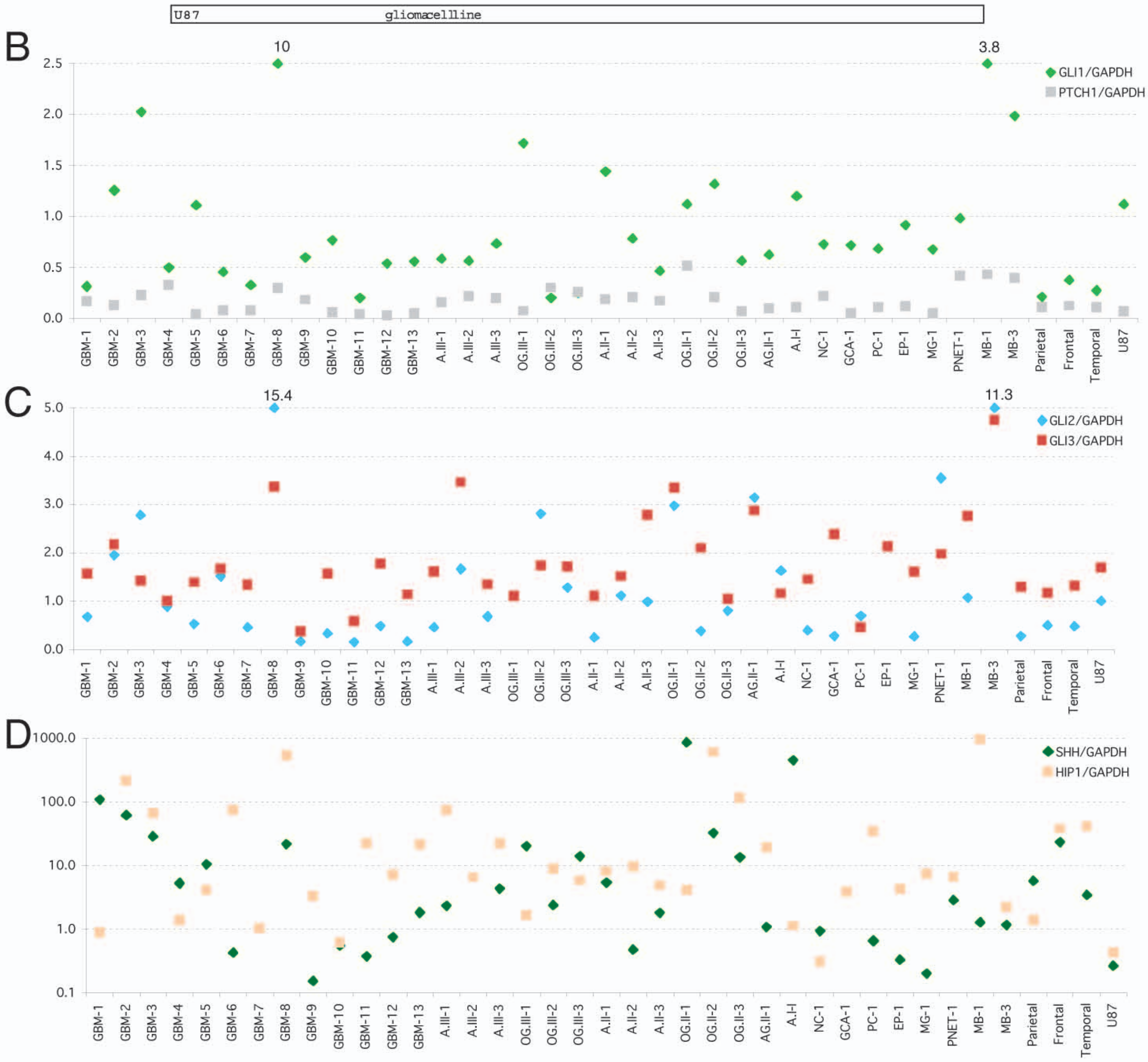
Supplemental Figure 9. Effects of SHH on glioma stem cell self-renewal. N-SHH (at 100nM; R&D Systems) or PBS used as control were used to treat whole glioma stem cell cultures (A) or CD133+ cells glioma stem cells (B) for 7 days. The gliomaspheres were then washed and tested for the formation of secondary spheres without SHH after dissociation. Histograms show ratios of SHH- versus PBS-treated samples and asterisks denote significant ($p<0.05$) changes. CD133+ cells were FACS-sorted from existing stem cell cultures (cultured) or from a fresh GBM from the operating room (fresh).

Supplemental Figure 10. Gene expression in CD133+ FACS-sorted glioma stem cells. Expression profile of selected genes in CD133+ gliomasphere cells as compared with the $CD133^-$ population. The qPCR data shown in (A) were used to generate (B). The numbers in (A) represent the ratio of gene expression values in CD133+ over CD133- cells. In each case, gene expression was expressed as the relative value over that of *GAPDH*. B) White denotes values within 30% of equal expression in CD133+ vs CD133- cells, red for 40% enrichment or more and light blue for 40% impoverishment or more in CD133+ cells. *SHH* and *NOTCH1* as well as stemness genes are highlighted and values boxed. Values for clonogenicity and number of CD133+ cells is also given for the different stem cell cultures (B, bottom).

A

Table of brain tumors and normal tissues used in this study

GBM-1	Giloblastomamulitforme	52	M	righttemporal
GBM-2	"	n/a	n/a	n/a
GBM-3	"	50	M	frontal
GBM-4	"	51	F	parieto-occipital(ventriculartrigone)
GBM-5	"	56	M	occipitaleft
GBM-6	"	64	F	frontaleft
GBM-7	"	51	M	occipitalright
GBM-8	"	51	F	parietalright
GBM-9	"	70	M	temporalright
GBM-10	"	66	F	n/a
GBM-11	"	74	M	temporalleft
GBM-12	"	50	M	temporalleft
GBM-13	"	46	M	n/a
A.III-1	AstrocytomagradeIII	33	M	leftfrontal
A.III-2	"	30	M	n/a
A.III-3	"	34	F	temporalleft
OG.III-1	OligodendrogliomagradeIII	54	M	frontalleft(recurrence)
OG.III-2	"	68	M	temporalright(recurrence)
OG.III-3	"	83	M	n/a
A.II-1	AstrocytomagradeII	24	M	temporalrightlobe
A.II-2	"	31	F	n/a
A.II-3	"	47	M	n/a
OG.II-1	OlogodendrogliomagradeII	41	F	frontalleft
OG.II-2	"	n/a	n/a	n/a
OG.II-3	"	55	M	frontalright
AG.II-1	GenistocyticastrocytomagradeII	56	M	fronto-tempo-insular(peri-sylivan)
A.I-I	Juvenilepylocyticastrocytoma(gradeI)	n/a	n/a	n/a
NC-1	neurocytoma	2	M	3rdventricle
GCA-1	giantcellastrocytoma	12	M	sub-ependymallateralventricule
PC-1	pineacytoma	n/a	n/a	n/a
EP-1	ependymoma	6	M	cerebellum
EP-2	"	6	M	cerebellum
MG-1	meningioma	70	M	n/a
PNET-1	PNET	10	n/a	frontallobe
MB-1	medulloblastoma	17	M	cerebellum
MB-2	"	3	F	cerebellum
MB-3	"	n/a	n/a	cerebellum
Parietalcortex	epilepticnormalbrain	5	M	parietal
Frontal "	"	5	M	frontal
Temporal "	"	5	M	temporal
U87	gliomacellline			



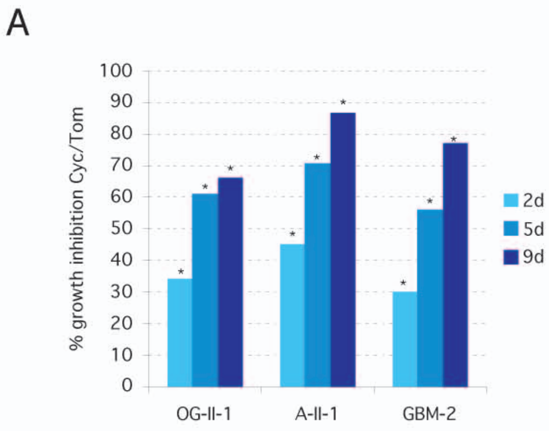
Suppl. Figure 1 Clement et al

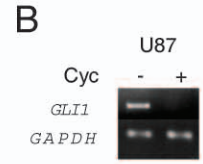
	GLI1	GLI2	GLI3	PTCH1	SHH	NESTIN	NOTCH1	CD133	OLIG2	BMI1	BCAN	OCT.4	NANOG	PTEN	ABCG2	PDGFRA	SOX2	NRD1	MELK	EGFR	GFAP	PCNA
GBM-12	0.54	0.49	1.77	0.03	0.75	1.17	0.96	ND	0.20	ND	ND	0.04	0.15	0.005	ND	ND	0.23	0.007	0.01	ND	ND	0.03
GBM-1	0.32	0.68	1.58	0.17	109.3	3.58	0.36	0.87	0.32	0.40	0.10	0.22	0.50	1.00	0.24	0.01	3.01	0.05	0.02	0.15	8.86	0.23
GBM-11	0.20	0.16	0.60	0.05	0.38	3.07	0.11	5.02	0.62	0.25	0.04	0.13	0.21	0.25	0.05	0.10	5.22	0.14	0.02	0.08	39.35	0.10
GBM-10	0.77	0.34	1.58	0.06	0.56	1.71	0.20	8.11	0.37	0.18	0.06	0.15	0.44	0.32	0.07	0.12	5.64	0.02	0.02	0.21	117.2	0.11
GBM-9	0.60	0.17	0.38	0.19	0.16	2.38	0.31	4.37	0.32	0.99	0.03	0.33	0.76	1.20	0.20	0.07	2.55	4.76	0.01	0.02	17.95	0.40
GBM-2	1.26	1.95	2.17	0.13	61.9	3.13	0.36	5.98	1.13	0.49	0.10	0.17	0.52	0.60	0.17	0.01	1.74	0.29	0.02	0.27	235.0	0.20
GBM-8	9.97	15.42	3.36	0.30	21.71	1.82	0.70	0.16	0.13	0.11	0.13	0.20	0.25	0.47	0.02	1.74	138.7	0.06	0.04	0.14	54.0	0.22
GBM-13	0.56	0.17	1.14	0.06	1.84	27.06	0.15	8.83	0.70	0.26	0.05	0.33	1.45	0.47	0.14	0.04	1.40	0.10	0.02	0.07	58.4	0.12
GBM-7	0.33	0.47	1.34	0.08	ND	19.60	1.06	43.55	0.88	0.61	0.20	0.25	0.49	0.87	0.19	0.15	4.15	0.09	0.08	0.28	254.8	0.35
GBM-3	2.02	2.79	1.42	0.23	28.3	11.10	0.10	3.67	7.38	0.60	0.15	0.23	0.47	0.78	0.28	0.13	5.16	0.96	0.05	0.34	206.1	0.32
OG.III-1	1.72	ND	1.11	0.08	20.1	7.84	7.23	ND	1.42	0.63	0.10	1.04	3.13	0.63	ND	0.27	2.79	0.83	0.10	0.02	74.3	0.51
GBM-5	1.11	0.53	1.39	0.04	10.56	2.41	0.62	4.70	1.26	0.41	0.18	0.36	0.65	1.12	0.41	0.45	5.33	0.25	0.03	0.12	220.0	0.53
GBM-4	0.50	0.89	1.02	0.33	5.30	2.43	1.21	1.47	1.17	0.42	0.35	0.44	1.02	1.25	0.53	0.46	6.72	0.22	0.09	0.19	179.0	0.38
GBM-6	0.46	1.52	1.67	0.08	0.43	13.93	0.51	11.42	1.74	1.02	0.25	0.73	1.44	1.61	0.83	0.86	50.72	1.12	0.07	0.28	553.5	0.74
A.III-3	0.73	0.68	1.35	0.20	4.35	6.62	0.88	2.66	2.69	0.69	0.29	0.48	1.37	1.75	0.53	1.05	4.85	0.74	0.03	0.24	134.5	0.41
A.III-1	0.59	0.46	1.62	0.16	2.34	8.44	2.00	9.50	10.38	2.09	0.63	0.96	2.27	2.19	0.70	1.64	8.06	1.74	0.35	0.32	651.6	0.79
A.III-2	0.56	1.67	3.46	0.22	ND	37.13	0.41	15.68	11.23	2.03	0.42	1.17	2.56	2.80	0.92	1.70	115.5	1.19	0.12	0.47	482.9	1.13
OG.III-2	0.20	2.81	1.74	0.30	2.42	4.23	1.00	20.22	18.02	2.04	0.50	0.74	1.52	3.47	1.53	1.75	13.40	1.62	0.07	0.30	444.4	0.74
OG.III-3	0.25	1.28	1.72	0.26	14.1	43.96	0.26	9.97	13.06	1.59	0.44	0.56	1.82	2.45	0.96	1.82	132.6	1.36	0.05	0.30	88.7	0.70
OG.II-3	0.57	0.81	1.05	0.07	13.6	6.78	1.84	12.45	10.80	1.73	0.86	1.52	2.64	3.37	1.20	6.31	20.04	2.58	0.04	0.66	561.6	1.00
AG.II-1	0.63	3.14	2.87	0.10	1.10	21.27	0.78	108.3	1.85	1.97	0.16	0.63	0.27	2.29	0.75	0.28	13.02	0.21	0.03	0.63	549.5	1.04
A.II-1	1.44	0.26	1.11	0.19	5.48	8.57	0.66	10.61	4.82	1.00	0.31	0.42	1.09	1.67	0.62	0.30	2.92	0.14	0.04	0.03	74.6	0.26
A.II-3	0.47	1.00	2.78	0.17	1.82	11.53	0.84	0.46	2.11	0.42	0.26	0.49	1.37	0.54	0.23	0.14	5.43	0.35	0.02	0.09	78.4	0.09
OG.II-1	1.12	2.98	3.35	0.52	858	15.05	0.54	3.42	8.73	0.81	0.31	0.38	0.77	1.18	0.44	0.07	7.71	0.39	0.03	0.39	273.5	0.25
A.II-2	0.79	1.12	1.52	0.21	0.48	5.99	1.47	ND	7.51	1.39	0.04	0.05	0.13	0.04	ND	0.01	0.31	2.50	0.02	ND	8.29	ND
OG.II-2	1.32	0.38	2.10	0.21	32.7	0.89	0.14	17.70	0.21	0.23	0.09	0.12	0.47	0.29	0.09	0.02	2.27	0.20	0.02	0.03	74.3	0.14
A.I-I	1.20	1.63	1.16	0.11	452	1.30	0.23	0.16	1.99	0.11	0.09	0.17	0.26	0.18	0.15	0.01	0.50	ND	0.004	0.06	283.6	0.20
MB-1	3.77	1.07	2.77	0.43	1.29	5.54	1.02	33.94	0.51	0.63	0.02	0.29	0.74	0.63	0.13	0.05	4.85	1.74	0.06	0.07	13.89	0.23
MB-3	1.99	11.27	4.76	0.39	1.17	0.21	0.89	ND	0.04	0.04	0.005	0.003	0.03	0.13	0.02	0.006	0.095	ND	0.05	0.001	0.79	ND
threshold up	*0.4*	*0.7*	*1.50*	*0.15*	*24*	*10*	*1.2*	*4.30*	*1.1*	*0.50*	*0.15*	*0.35*	*1.00*	*1.2*	*0.4*	*0.3*	*13*	*0.5*	*0.15*	*0.07*	*50*	*0.2*
threshold down	*0.15*	*0.20*	*1.15*	*0.06*	*3*	*3.5*	*0.5*	*0.8*	*0.2*	*0.20*	*0.03*	*0.1*	*0.3*	*0.5*	*0.18*	*0.15*	*6*	*0.2*	*0.005*	*0.01*	*20*	*0.05*
parietal ctx	0.21	0.28	1.31	0.11	5.77	4.99	0.93	1.28	0.46	0.38	0.08	0.28	0.77	0.88	0.34	0.20	10.03	0.37	0.02	0.04	25.77	0.14
frontal ctx	0.38	0.50	1.18	0.13	23.50	4.24	0.67	1.15	0.96	0.44	0.06	0.16	0.64	0.80	0.24	0.23	12.74	0.37	0.01	0.04	23.31	0.11
temporal ctx	0.28	0.48	1.33	0.11	3.44	8.52	1.04	4.02	0.58	0.31	0.10	0.28	0.86	0.66	0.33	0.24	12.11	0.32	0.10	0.03	38.52	0.08

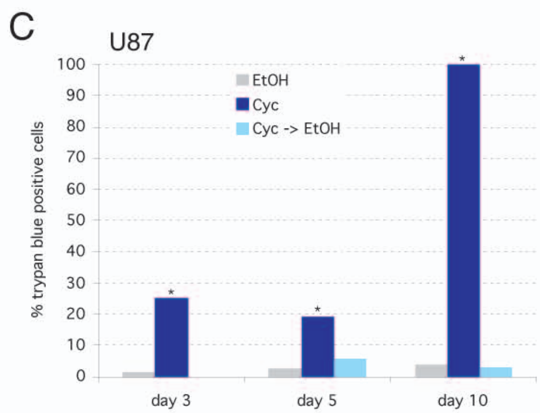
Suppl. Fig. 2 Clement et al.

	Mesenchymal		Proneural		Proliferative		
	high YKL40	high CD44	high BCAN	low MELK	high CD133	low GFAP	high PCNA
GBM-1	0.35	0.06	0.10	0.02	0.87	8.86	0.23
GBM-2	0.30	0.08	0.10	0.02	5.98	234.98	0.20
GBM-3	0.04	0.07	0.15	0.05	3.67	206.12	0.32
GBM-4	0.01	0.05	0.35	0.09	1.47	179.01	0.38
GBM-5	0.34	0.20	0.18	0.03	4.70	220.04	0.53
GBM-6	0.41	0.18	0.25	0.07	11.42	553.54	0.74
GBM-7	0.43	0.18	0.20	0.08	43.55	254.80	0.35
GBM-8	0.19	0.08	0.13	0.04	0.16	53.95	0.22
GBM-9	0.01	0.02	0.03	0.01	4.37	17.95	0.40
GBM-10	0.14	0.06	0.06	0.02	8.11	117.17	0.11
GBM-11	0.22	0.04	0.04	0.02	5.02	39.35	0.10
GBM-12	ND	ND	ND	0.01	ND	ND	0.03
GBM-13	0.38	0.04	0.05	0.02	8.83	58.43	0.12
A.III-1	0.02	0.17	0.63	0.35	9.50	651.55	0.79
A.III-2	0.07	0.14	0.42	0.12	15.68	482.94	1.13
A.III-3	0.02	0.06	0.29	0.03	2.66	134.46	0.41
OG.III-1	0.28	0.25	0.10	0.10	ND	74.34	0.51
OG.III-2	0.06	0.12	0.50	0.07	20.22	444.45	0.74
OG.III-3	0.02	0.06	0.44	0.05	9.97	88.69	0.70
A.II-1	0.02	0.03	0.31	0.04	10.61	74.56	0.26
A.II-2	ND	ND	0.04	0.02	ND	8.29	ND
A.II-3	0.01	0.04	0.26	0.02	0.46	78.37	0.09
OG.II-1	0.01	0.06	0.31	0.03	3.42	273.53	0.25
OG.II-2	0.02	0.02	0.09	0.02	17.70	74.25	0.14
OG.II-3	0.04	0.17	0.86	0.04	12.45	561.59	1.00
AG.II-1	0.05	0.13	0.16	0.03	108.31	549.51	1.04
A.I-I	0.03	0.04	0.09	0.004	0.16	283.56	0.20
threshold up	*0.03*	*0.17*	*0.15*	*0.15*	*4.30*	*50*	*0.2*
threshold down	*0.005*	*0.005*	*0.03*	*0.01*	*0.8*	*20*	*0.05*

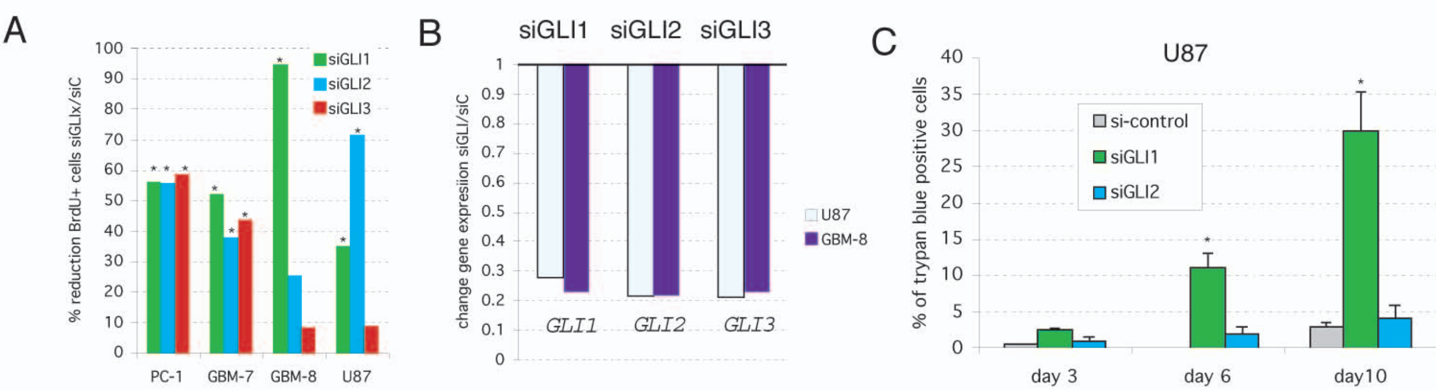
Supp Fig 3 Clement et al



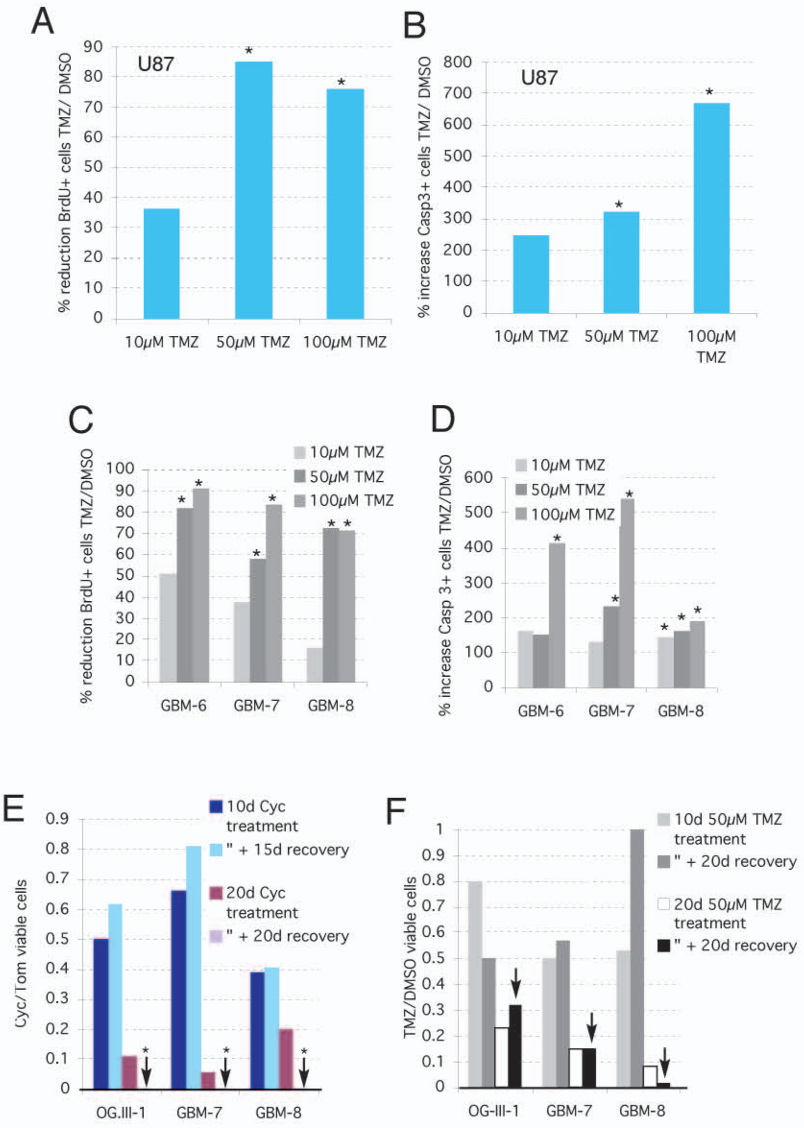




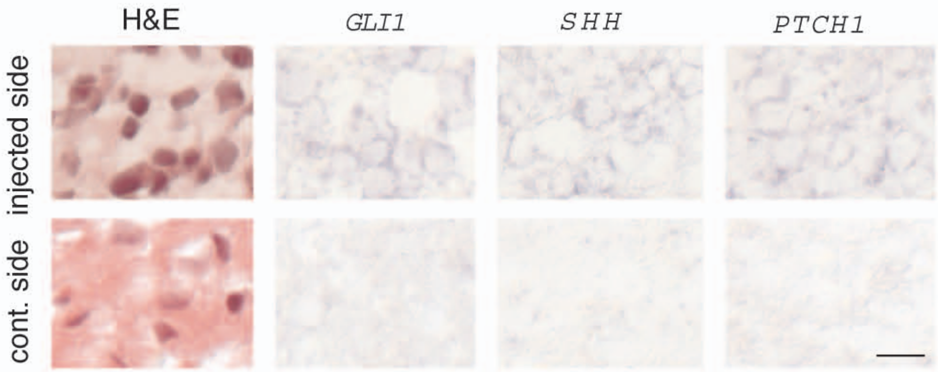
Suppl. Figure 4. Clement et al



Suppl. Figure 5. Clement et al



Suppl. Figure 6. Clement et al

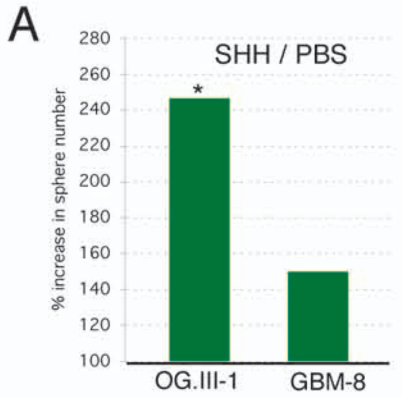


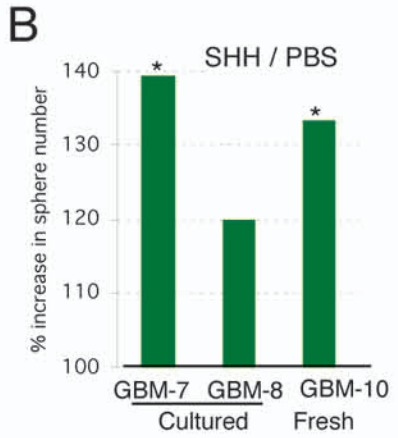
Suppl. Fig 7 Clement et al

	NANOG	*OCT.4*	*SOX2*	*BMI1*	*PCNA*	*Gli3*	*NCAM*	*GLI1*	*PTCH1*	*PDGFRA*	*NESTIN*	*GFAP*	*BCAN*	*PTEN*	*NOTCH1*	*OLIG2*
GBM-14	0.32	0.23	0.19	0.03	0.21	1.78	0.20	1.17	0.32	0.18	2.32	0.13	0.03	0.09	0.46	0.08
GBM-10	0.43	0.27	0.28	0.02	0.19	1.64	0.34	0.77	0.35	0.22	2.18	0.24	0.17	0.03	0.75	0.29
GBM-8	0.23	0.29	0.31	0.03	0.23	3.47	0.34	7.74	1.18	0.57	5.02	0.19	0.21	0.27	3.43	1.64
GBM-7	0.30	0.32	0.34	0.03	0.28	2.00	0.35	0.83	0.40	0.36	2.28	0.37	0.21	0.08	1.90	0.32
GBM-6	0.37	0.15	0.33	0.05	0.27	2.02	0.17	0.53	0.47	0.11	3.82	0.05	0.11	0.01	0.39	0.22
A.III-2	0.24	0.52	0.45	0.03	0.35	1.28	0.34	0.93	0.30	0.45	0.68	0.20	0.11	0.03	0.67	0.29
OG.III-1	0.41	0.27	0.32	0.02	0.27	1.30	0.38	1.09	0.57	0.34	1.36	0.27	0.25	0.10	2.58	2.67
mean	*0.32*	*0.29*	*0.32*	*0.03*	*0.25*	*1.93*	*0.3*	*0.8**	*0.50*	*0.32*	*2.52*	*0.20*	*0.17**	*0.05**	*1.12**	*0.28**

	GLI2	*DCX*	*NCS*	*YKL40*	*ABCG2*	*EGFR*	*SHH*	*CD133*	*NEUROD1*
GBM-14	2.56	0.56	2.55	0.40	0.19	0.11	0.72	16.88	0.02
GBM-10	2.49	0.35	0.88	0.04	0.05	0.05	31.35	17.81	0.004
GBM-8	8.88	0.16	0.19	0.20	0.50	0.43	4.78	0.70	0.035
GBM-7	0.86	0.99	0.93	0.42	0.08	0.19	6.54	10.04	0.003
GBM-6	1.76	1.64	3.60	0.001	0.01	0.01	0.33	62.27	0.02
A.III-2	1.76	0.29	1.77	0.14	0.03	0.08	13.91	0.67	0.013
OG.III-1	ND	0.06	0.26	0.74	0.06	2.36	2.71	1.10	ND
mean	*1.88**	*0.58*	*1.45*	*0.32**	*0.7**	*0.14**	*4.7**	*14*	*0.025**

Suppl. Fig 8 Clement et al





Suppl. Figure 9. Clement et al

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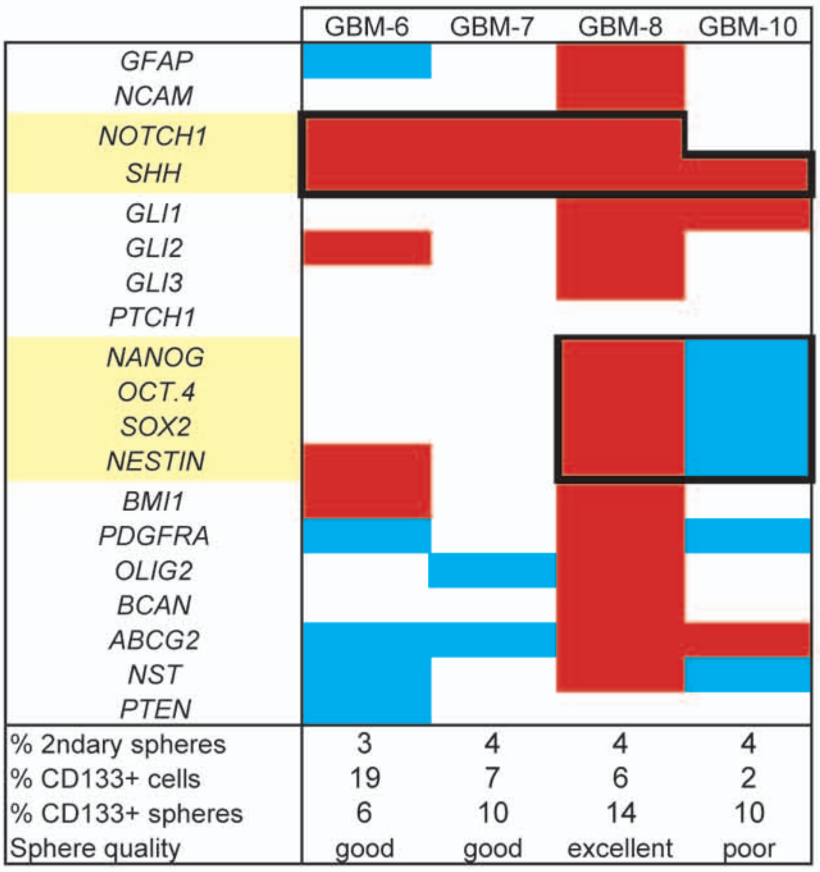
	GBM-6	GBM-7	GBM-8	GBM-10
GFAP	0.6	1.0	2.0	1.1
NCAM	1.1	0.9	2.0	0.8
NOTCH1	2.0	1.6	1.5	1.1
SHH	1.4	1.8	2.1	1.6
GLI1	0.7	1.1	1.4	1.4
GLI2	1.6	1.1	1.8	0.9
GLI3	0.8	1.2	1.4	1.0
PTCH1	0.9	0.8	1.1	0.7
NANOG	1.2	1.3	1.7	0.6
OCT.4	1.2	1.1	1.4	0.6
SOX2	0.7	0.8	1.9	0.6
NESTIN	1.8	1.1	3.5	0.4
BMI1	1.4	1.0	1.4	0.7
PDGFRA	0.3	1.0	1.6	0.02
OLIG2	0.8	0.6	2.9	0.7
BCAN	0.8	0.8	2.7	0.8
ABCG2	ND	0.7	2.4	1.8
NST	0.5	0.9	1.8	0.6
PTEN	0.5	1.1	1.2	0.7

B



Suppl. Fig. 10 Clement et al