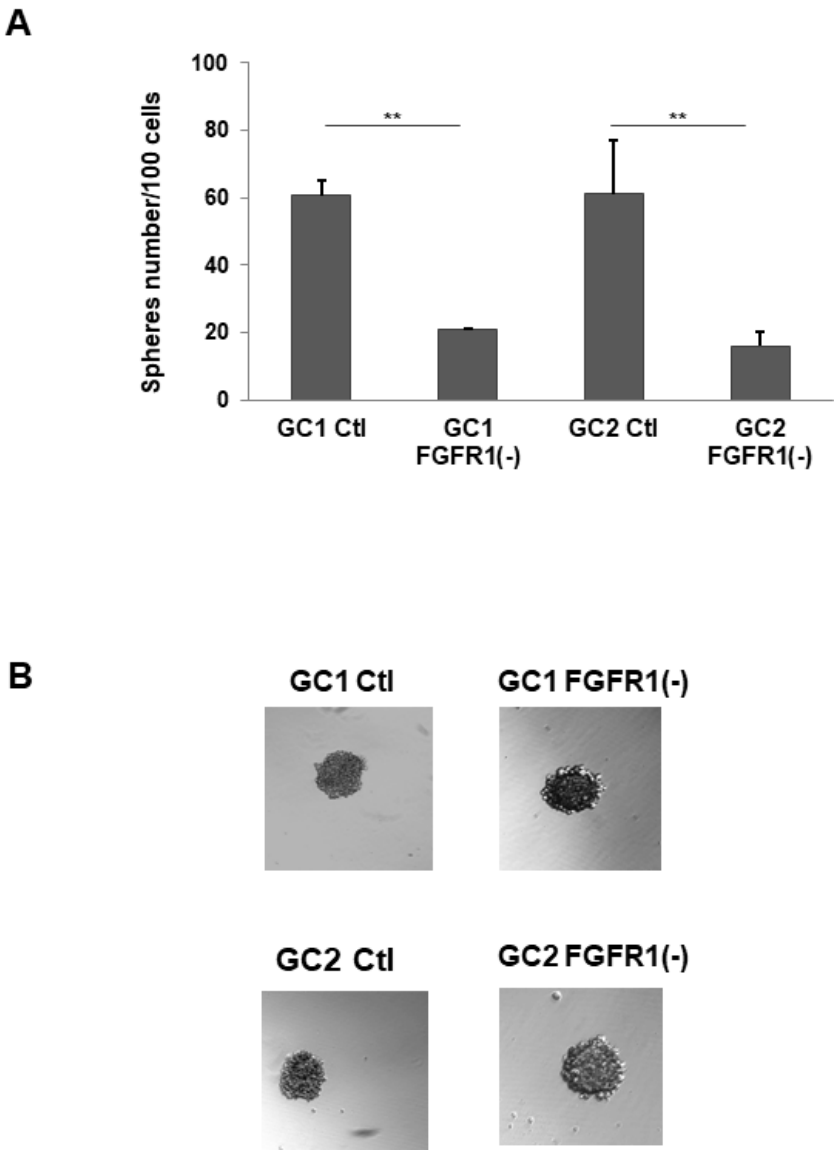
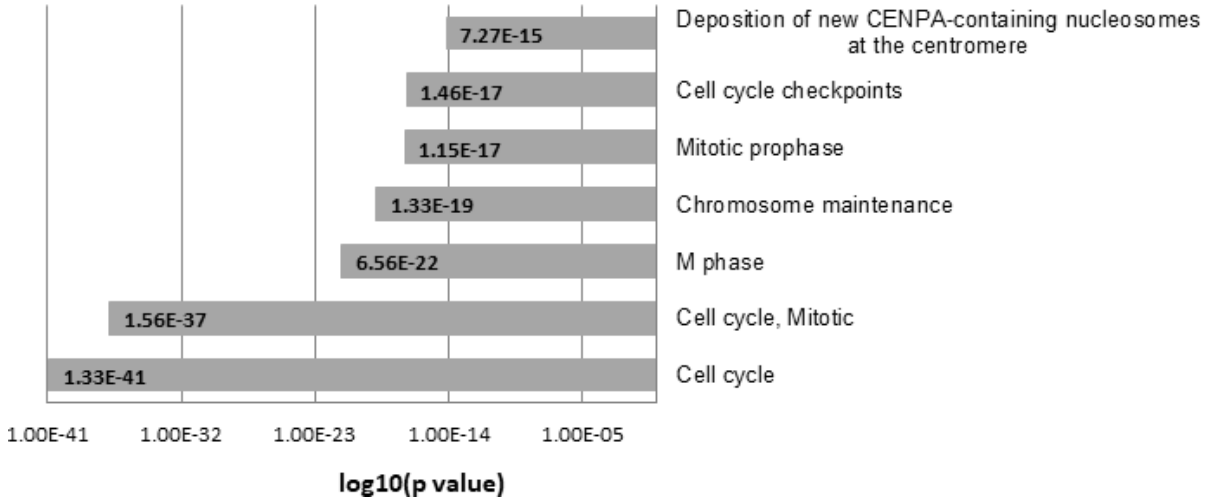
FGFR1/FOXM1 pathway: a key regulator of glioblastoma stem cells radioresistance and a prognosis biomarker

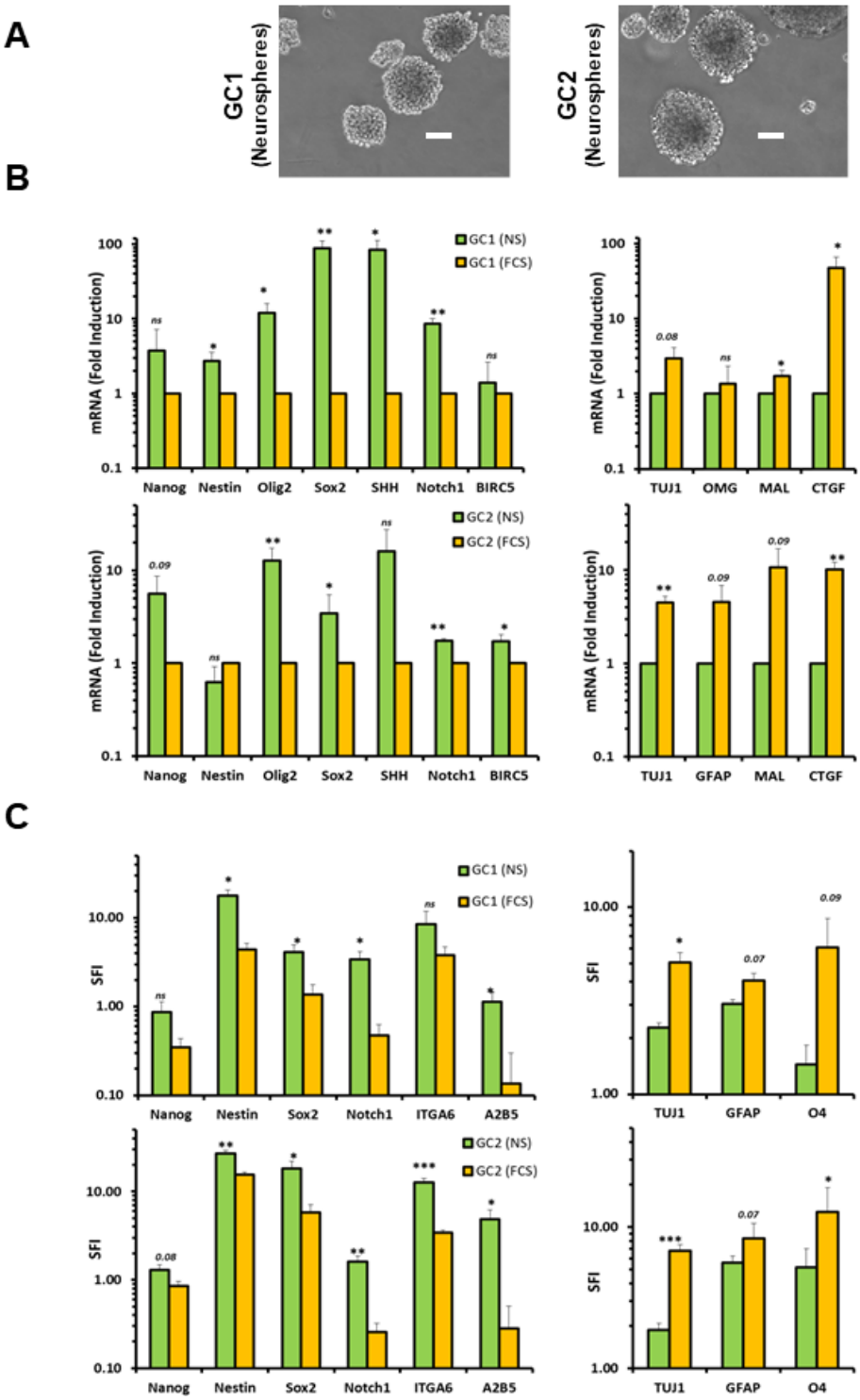
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Cells derived from human GBM biopsy specimens inhibited for FGFR1 form neurospheres. Cells derived from 2 GBM biopsy specimen (GC1 and GC2) were transfected with siFGFR1(11) (GC1FGFR1(-) or GC2FGFR1(-)) or a scramble control (Ctl). (**A**) Cells were analyzed for their capacity to form neurospheres as described in "Materials and Methods". Quantifications of 3 experiments are presented as means ± SD. $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}0.01 < p < 0.05$. (**B**) Phase contrast photomicrographs of GSLC-enriched neurosphere cell lines, magnification: ×10, scale bar: 6 µm.



Supplementary Figure 2: FGFR1 inhibition induces a downregulation of cell cycle pathways. The genes list regulated in GCS2FGFR1(-) cells was compared to Reactome database according to "Materials and Methods".



Supplementary Figure 3: Characterization of the stem and differentiated phenotypes in GSLC-enriched neurospheres and in differentiated GBM cultures (FCS). (**A**) Phase contrast photomicrographs of GSLC-enriched neurosphere cell lines isolated from 2 patient tumors (GC1, GC2). Magnification: ×10, scale bar: 6 µm. (**B**–**C**) GSLC-enriched neurosphere cell lines (GC1, GC2) were kept in stem cell medium or allowed to differentiate as adherent GBM cells for at least 15 days in FCS medium. (B) Real-time Quantitative PCR analysis of the stem (left panel) and differentiation (right panel) markers in neurospheres or GBM differentiated cells for both GC1 and GC2 cell lines. Shown are the fold inductions expressed as means ± SEM of at least 3 independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the related control. ns: not significant. (C) Immunofluorescence FACS analysis of the stem (left panel) and differentiation (right panel) markers in neurospheres or GBM differentiated cells. The results, expressed as SFI (Specific Fluorescence Index), were representative of at least 3 independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the related control.

Supplementary Table 1: Forward and reverse primer sequences used for quantitative PCR detection

Gene (human)	Primer sequences	Type of marker
β2-microglobulin	forward 5′-ACCCCCACTGAAAAAGATGA-3′ reverse 5′-ATCTTCAAACCTCCATGATG-3′	endogenous control
OMG	forward 5′-TAGGGACTCCATGTTCTACCCA-3′ reverse 5′-TCTGCATCCCACTTACAGTGA-3′	Oligodendrocytic marker
MAL	forward 5′-CGCTGCCCTCTTTTACCTCAG-3′ reverse 5′-GAAGCCGTCTTGCATCGTGAT-3′	Oligodendrocytic marker
GFAP	forward 5′-GGCAAAAGCACCAAAGACGG-3′ reverse 5′-GGCGGCGTTCCATTTACAAT-3′	Astrocytic marker
TUJ1	forward 5′-GCTCAGGGGCCTTTGGACATCTCTT-3′ reverse 5′-TTTTCACACTCCTTCCGCACCACATC-3′	Neuronal marker
CTGF	forward 5′-CATCTCCACCCGGGTTACC-3′ reverse 5′-CAGGCGGCTCTGCTTCTCTA-3′	Differentiation marker
Nanog	forward 5′-GTCCCGGTCAAGAAACAGAA-3′ reverse 5′-TGCGTCACACCATTGCTATT-3′	Stemness-associated marker
Nestin	forward 5′-ATCGCTCAGGTCCTGGAAGG-3′ reverse 5′-AAGCTGAGGGAAGTCTTGGAG-3′	Stemness-associated marker
Sox2	forward 5′-GCACATGAACGGCTGGAGCAACG-3′ reverse 5′-TGCTGCGAGTAGGACATGCTGTAGG-3′	Stemness-associated marker
Olig2	forward 5′-CAGAAGCGCTGATGGTCATA-3′ reverse 5′-TCGGCAGTTTTGGGTTATTC-3′	Stemness-associated marker
SonicHH	forward 5′-GCGGAAGGTATGAAGGGAAG-3′ reverse 5′-GCCAAAGCGTTCAACTTGTC-3′	Stemness-associated marker
Notch1	forward 5′-TGGACCAGATTGGGGAGTTC-3′ reverse 5′-GCACACTCGTCTGTGTTGAC-3′	Stemness-associated marker
BIRC5/Survivin	forward 5′-CGAGGCTGGCTTCATCCA-3′ reverse 5′-AGAAGAAACACTGGGCCAAGTC-3′	Stemness-associated marker

Supplementary Table 2: Primary antibodies used for flow cytometry

Marker (human)	Antibodies and fluorochromes	Suppliers	FACS channel	Type of marker
A2B5	Mouse monoclonal antibody : A2B5 - APC Isotype control : IgM - APC	Miltenyi	FL4-H	Stemness-associated marker
ITGA6	Rat monoclonal antibody : CD49f - PE Isotype control : Igg2A -PE	eBioscience	FL2-H	Stemness-associated marker
GFAP	Mouse monoclonal antibody : GFAP - AF647 Isotype control : Igg2B - AF647	BD Biosciences	FL4-H	Astrocytic marker
Nanog	Goat polyclonal antibody : Nanog - PE Isotype control : IgG - PE	R&D Systems	FL2-H	Stemness-associated marker
Nestin	Mouse monoclonal antibody : Nestin -Fluorescein Isotype control : Igg1 - Fluorescein	R&D Systems	FL1-H	Stemness-associated marker
Notch1	Mouse monoclonal antibody : Notch1 - APC Isotype control : Igg1 - APC	R&D Systems	FL4-H	Stemness-associated marker
O4	Mouse monoclonal antibody : O4 - PE Isotype control : IgM - PE	Miltenyi	FL2-H	Oligodendrocytic marker
TUJ1	Mouse monoclonal antibody : Tuj1 - AF488 Isotype control : Igg2A - AF488	BD Biosciences	FL1-H	Neuronal marker
Sox2	Mouse monoclonal antibody : Sox2 - APC Isotype control : Igg2A - APC	R&D Systems	FL4-H	Stemness-associated marker

Supplementary Table 3: Six-genes set and their corresponding coefficients

	Coefficient
FGFR1	0.27084
FOXM1	0.63132
MELK	−0.45751
GLI2	−0.60469
TWIST1	−0.00581
ZEB1	−0.01317

Coefficients determined as described in “Materials and Methods”.