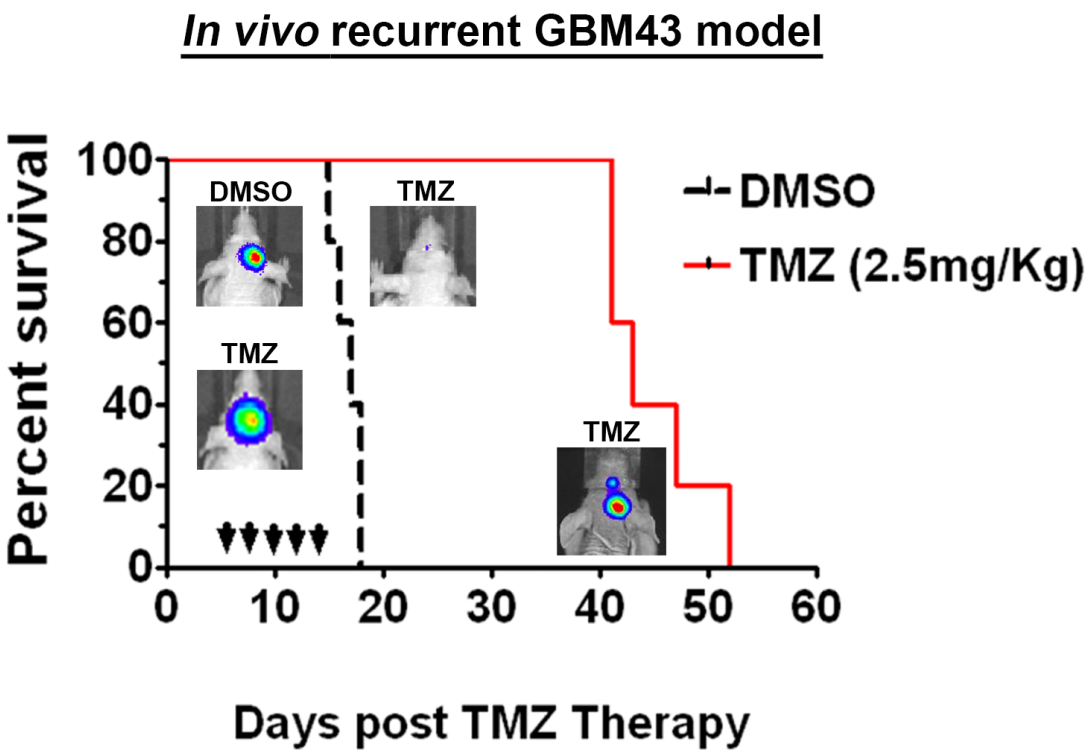
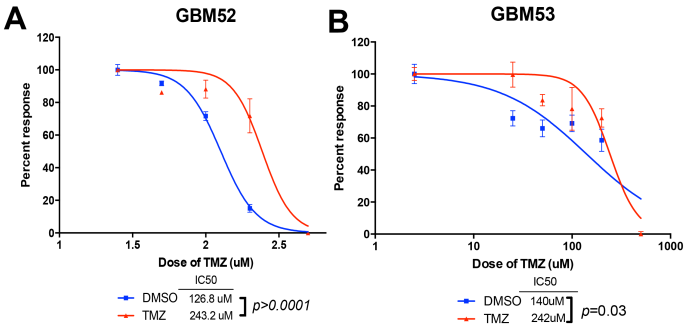
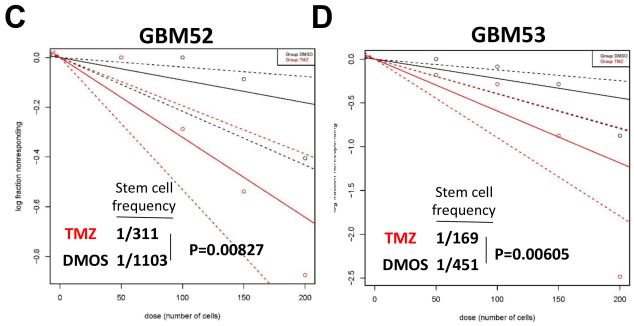
Supplementary Figure 1



Supplementary Figure 1. Expression of GSC markers increases with therapy *in vitro* and *in vivo*. Survival curve. GBM43 blue-tagged cells implanted intracranial and treated with or without TMZ (2.5 mg/kg/day for five consecutive days; indicated by arrows). One group of treated mice was sacrificed upon completion of primary chemotherapy and the other upon signs of disease relapse. Tumor burden was measured by bioluminescence imaging, which shows that tumor size decreases after chemotherapy however, 43 days later, it is much larger than the primary tumor.

Supplementary Figure 2





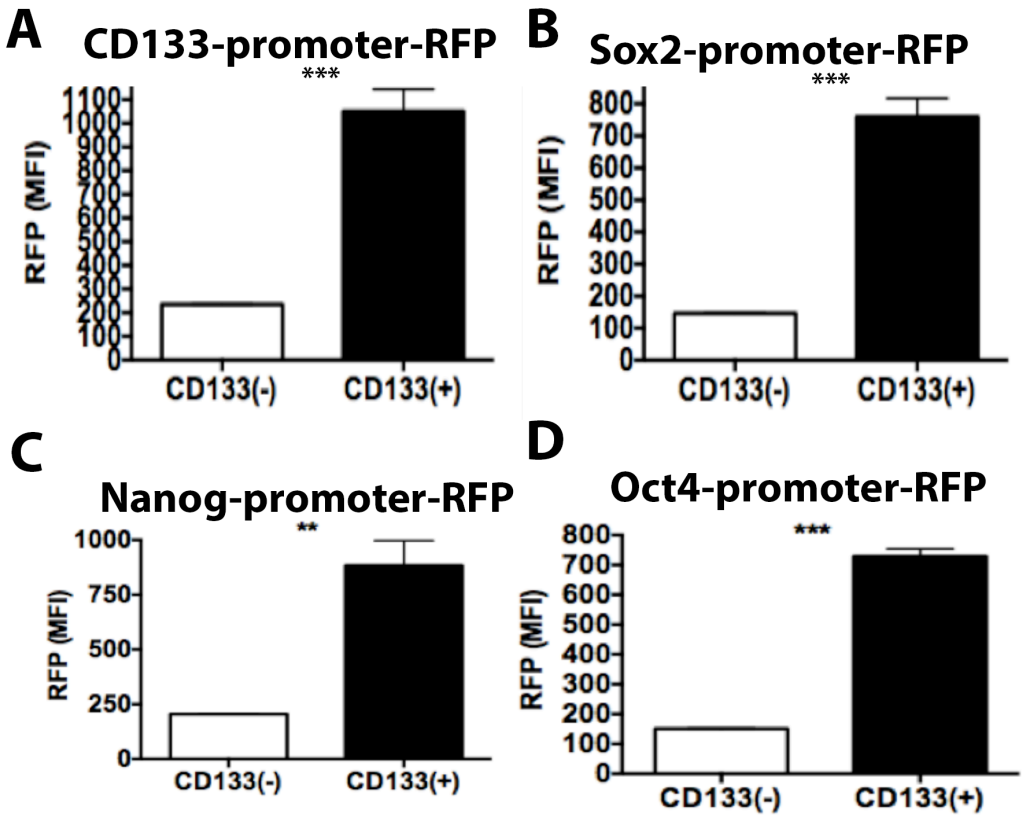
E

Sorted population	100 cells		1000 cells	
	DMSO	TMZ	DMSO	TMZ
CD133⁺ GSCs	1/3	1/3	5/5	5/5
Non-GSCs	0/3	1/3	3/5	5/5

Supplementary Figure 2. ***In vitro*** **evaluation of stemness and TMZ sensitivity in the TMZ rechallenged model.** Freshly isolated patient GBM samples were used to create a single cells suspension (GBM52 and 53). These lines were expose to 50µM TMZ for 8 days. After 8 days cells were re-plated in the presence of escalating dose of TMZ (5 to 750 µM of TMZ) for 7 days. Cell viability was measured by MTT assay, and the IC50 was measured by a dose response curve filtered by the sigmoidal model for GBM52 (A) and GBM53 (B) lines. The TMZ treated GBM cells were also evaluated for their self-renewing capacity by using tumorsphere assay. Both GBM52 (C) and GBM53 (D) demonstrated enhance self-renewing capacity post TMZ therapy as compared to DMSO-treated control. Next, to investigate the tumor engraftment efficiency *in*

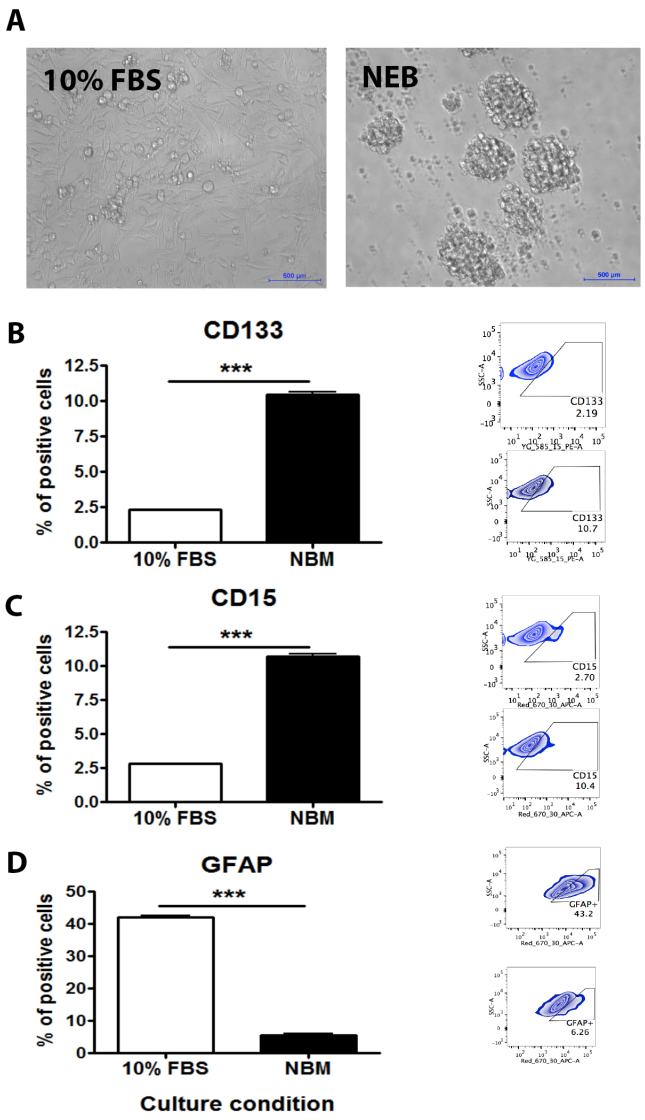
vivo, we have treated the U251 cells with 50μM TMZ for 8 days. Next, the CD133+ GSCs and CD133CD15 negative non-GSC glioma cells were sorted out by FACS, intracranially implanted (100, n=3 and 1000, n=5 cells) in the nude mice and animals were monitored for the end point survival. Table showing the number animals in each group developed tumor.

Supplementary Figure 3



Supplementary Figure 3. ***In vitro*** **Evaluation of GSC-specific reporter system.** FACS analysis of U251 cells expressing the GSC-specific promoter RFP reporter was performed to compare RFP expression with CD133 surface protein expression. The Analysis was performed on **A)** the CD133-promoter-RFP reporter, **B)** the Sox2-promoter-RFP reporter, **C)** the Nanog-promoter-RFP reporter, **D)** the Oct4-promoter-RFP reporter. Data represent results performed in triplicate. Where applicable, unpaired t-tests and survival analysis were applied, and statistical significance was defined as **P<0.01, and ***P<0.001.

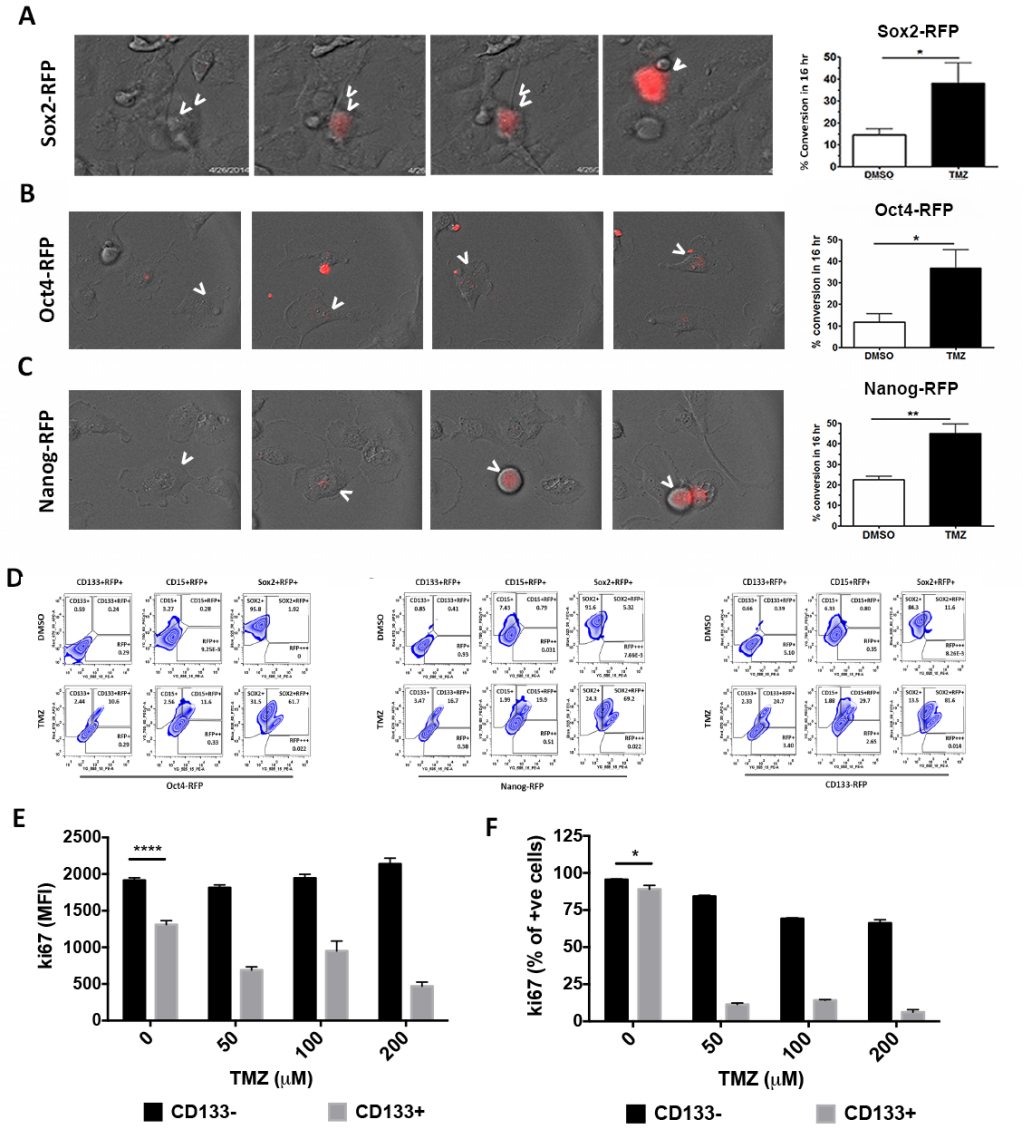
Supplementary Figure 4



Supplementary Figure 4. Differentiation of PDX GBM line after culturing in the media with 10% FBS. A) Representative morphology of GBM43 PDX line under light a microscope. The cells cultured in media with 10% FBS grew as adhere monolayer as compared to cells form neurosphere like structure when grown in the neurobasal media containing EGF and FGF growth factors. **B-D)**

FACS analysis for the GSC specific markers CD133 (B) and CD15 (C) as well as differentiation markers GFAP (D) demonstrating that 10% FBS significantly decreased CD133 and CD15 expression and increased differentiation markers GFAP expression within 72h post culture. Data represent results performed in triplicate. Where applicable, unpaired t-tests and survival analysis were applied, and statistical significance was defined as ***$P<0.001$.

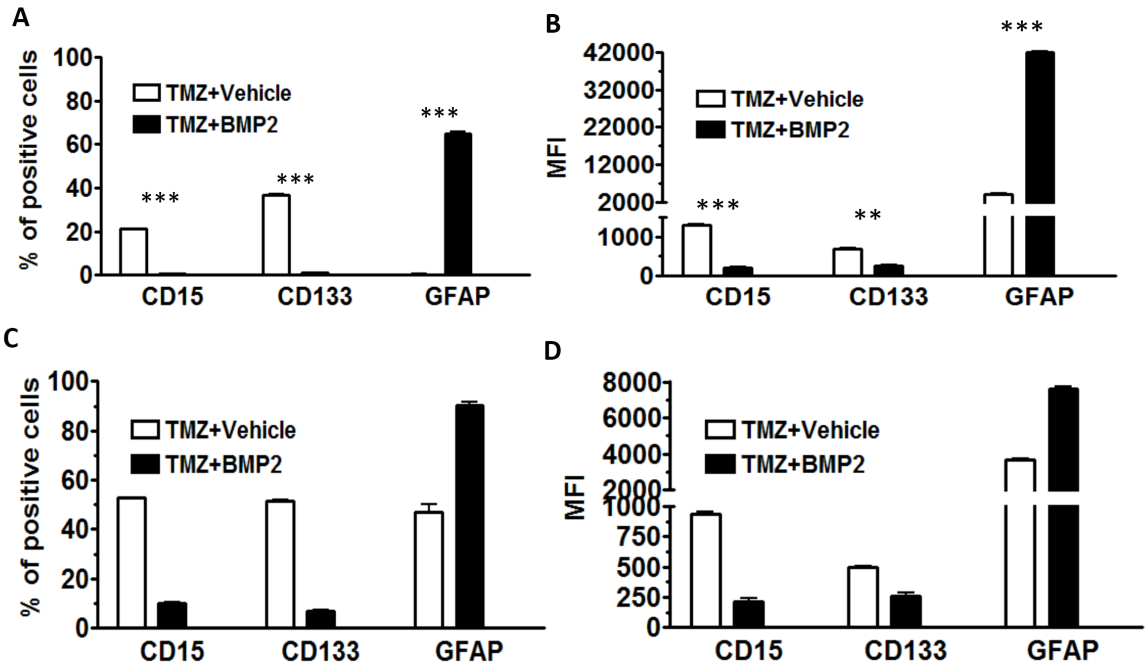
Supplementary Figure 5



Supplementary Figure 5. GSC-specific promoters functionally represent the GSC subpopulation. A, B and C) Real-time microscopy images of a single cell undergoing activation of CSC-specific promoters as a representation of conversion from non-GSC to a stem-like state after treatment with TMZ, visualized by the RFP and indicated by the arrows for Sox2-RFP (A), Oct4-RFP (B) and Nanog-RFP (C) reporter expressing U251 cells. **Right.** Graph depicting the increased frequency of conversion

events when treating with TMZ as compared to control. The control group (DMSO) received DMSO (n=250 RFP negative cells). **D) Left,** Flow cytometry analysis of Oct4 promoter driving RFP expression with or without 8 days TMZ (50μM). In the presence of chemotherapy, CD133+RFP+, CD15+RFP+, and Sox2+RFP+ expressions increase significantly compared to the control. **In the middle,** same flow cytometry analysis mentioned in **A**, but utilizing the Nanog-RFP reporter system. **Right panel,** same flow cytometry analysis mentioned in **A**, but utilizing the CD133-RFP reporter system. **E, F)** The Frequency of proliferating cells in the CD133+ GSC compartment and CD133- non-GSC compartment measured by Ki67 expression. GBM43 cells were treated with or without 8 days TMZ (0, 50, 100 and 200 μM) and subject to FACS analysis with co-staining with CD133 and Ki67. D) mean fluorescent intensity (MFI) and E) percent of Ki67 positive cells in the CD133+ and CD133- negative compartment. Data represent results performed in triplicate. Where applicable, unpaired t-tests and survival analysis were applied, and statistical significance was defined as ****$P<0.0001$.

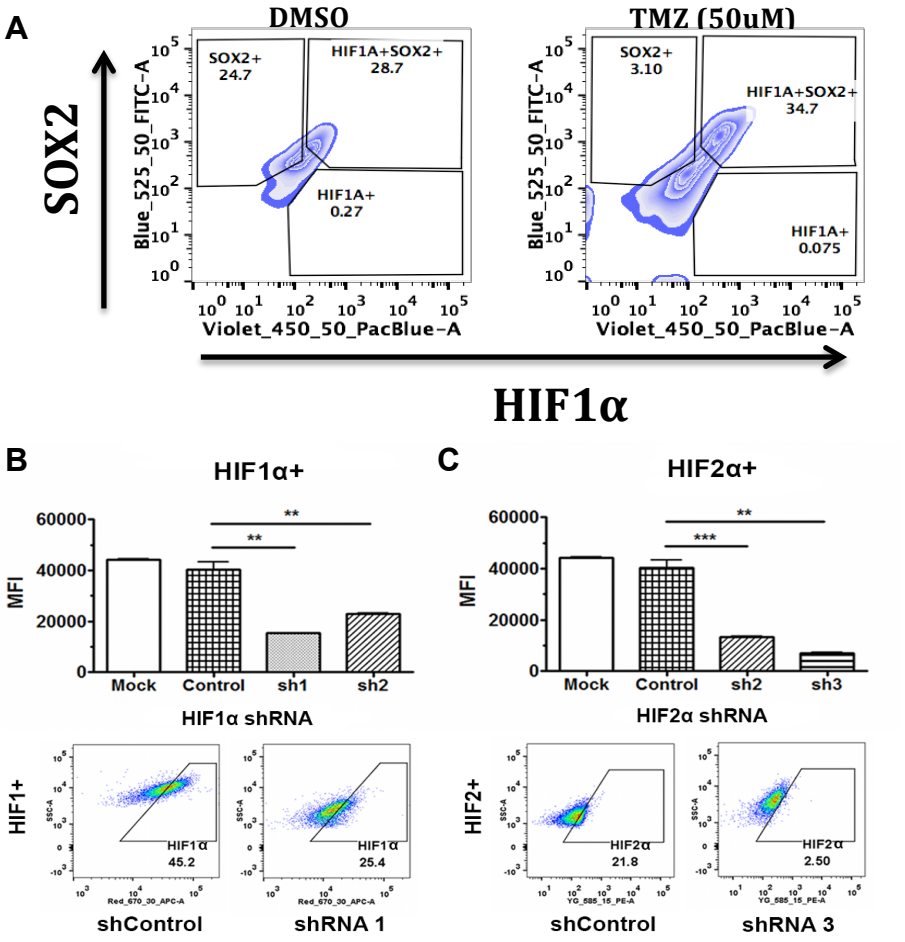
Supplementary Figure 6



Supplementary Figure 6. Differentiation of PDX GBM line after culturing in the media Bone Morphogenic Protein 2 (BMP2). A-B) GBM43 PDX line was cultured in serum free neurobasel media with 50μM TMZ for 8 days. At day 6, 50 ng/ml BMP2 was added in the culture and treated of 2 days. At 8 days post TMZ therapy (2 days post BMP2 therapy) cells were subject to FACS analysis for the GSC specific markers CD133 and CD15 as well as differentiation markers GFAP positive frequency (A) and the mean fluorescence intensity (MFI) (B). **C-D)** Same experiments described above was demonstrating that 10% FBS significantly decreased CD133 and CD15 expression and increased differentiation markers GFAP expression within 72h post culture. Data represent results performed in

triplicate. Where applicable, unpaired t-tests and survival analysis were applied, and statistical significance was defined as ***P<0.001.

Supplementary Figure 7



Supplementary Figure 7. HIFs can modulate the expression of GSC markers.

A) Co-staining of GBM43 cells treated with 50μM of TMZ for 8 days and analyzed of coexpression of SOX2 and HIF1α. **B, C**) Knockdown efficiencies of HIF1α (B) and HIF2α (C) shRNA validated by the respective protein expressions via flow cytometry analysis. Representative flow plots are shown at the bottom. Data represent results

performed in triplicate. Where applicable, unpaired t-tests and survival analysis were applied, and statistical significance was defined as **P<0.01, and ***P<0.001.