Figure S1

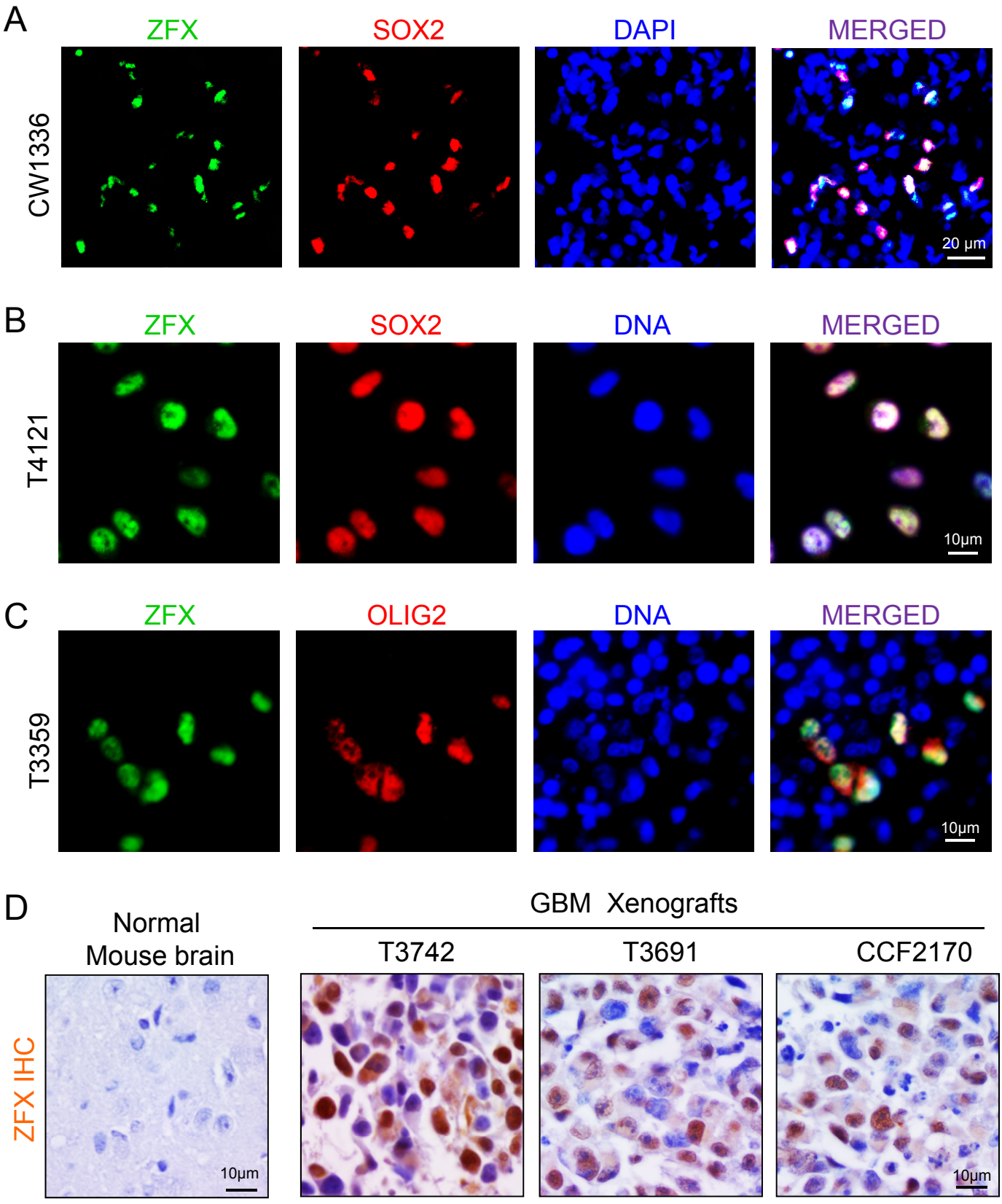

A
ZFX
SOX2
DAPI
MERGED
CW1336
20 μm
B
ZFX
SOX2
DNA
MERGED
T4121
10μm
C
ZFX
OLIG2
DNA
MERGED
T3359
10μm
D
Normal
Mouse brain
GBM Xenografts
T3742
T3691
CCF2170
ZFX IHC
10μm
10μm

Figure S2

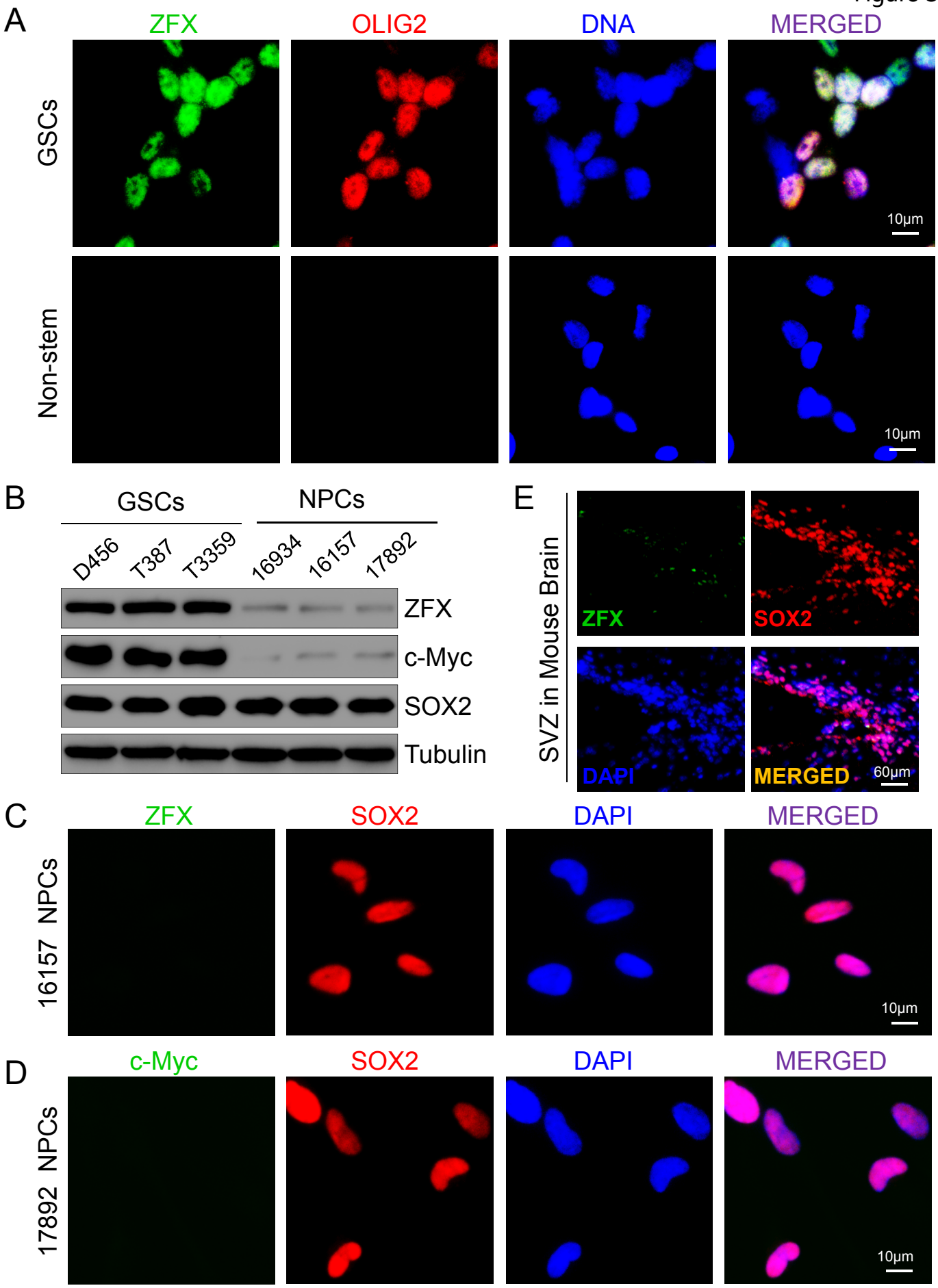
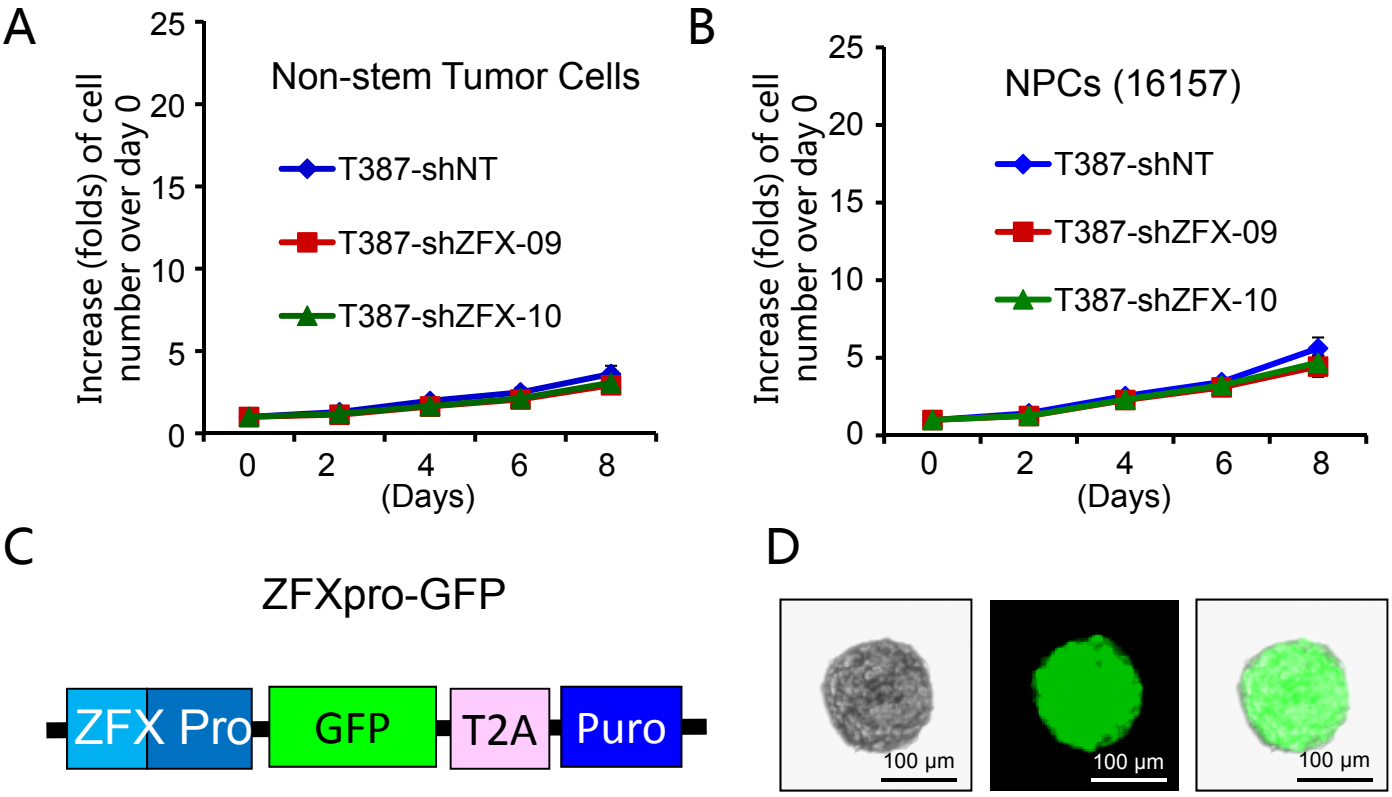

A
ZFX
OLIG2
DNA
MERGED
GSCs
Non-stem
10μm
10μm
B
GSCs
NPCs
D456
T387
T3359
16934
16157
17892
ZFX
c-Myc
SOX2
Tubulin
E
SVZ in Mouse Brain
ZFX
SOX2
DAPI
MERGED
60μm
C
ZFX
SOX2
DAPI
MERGED
16157 NPCs
10μm
D
c-Myc
SOX2
DAPI
MERGED
17892 NPCs
10μm

Figure S3



Differentiated Cells Derived from ZFXPro-GFP-transduced GSCs
(Day 6 after Differentiation Induction)

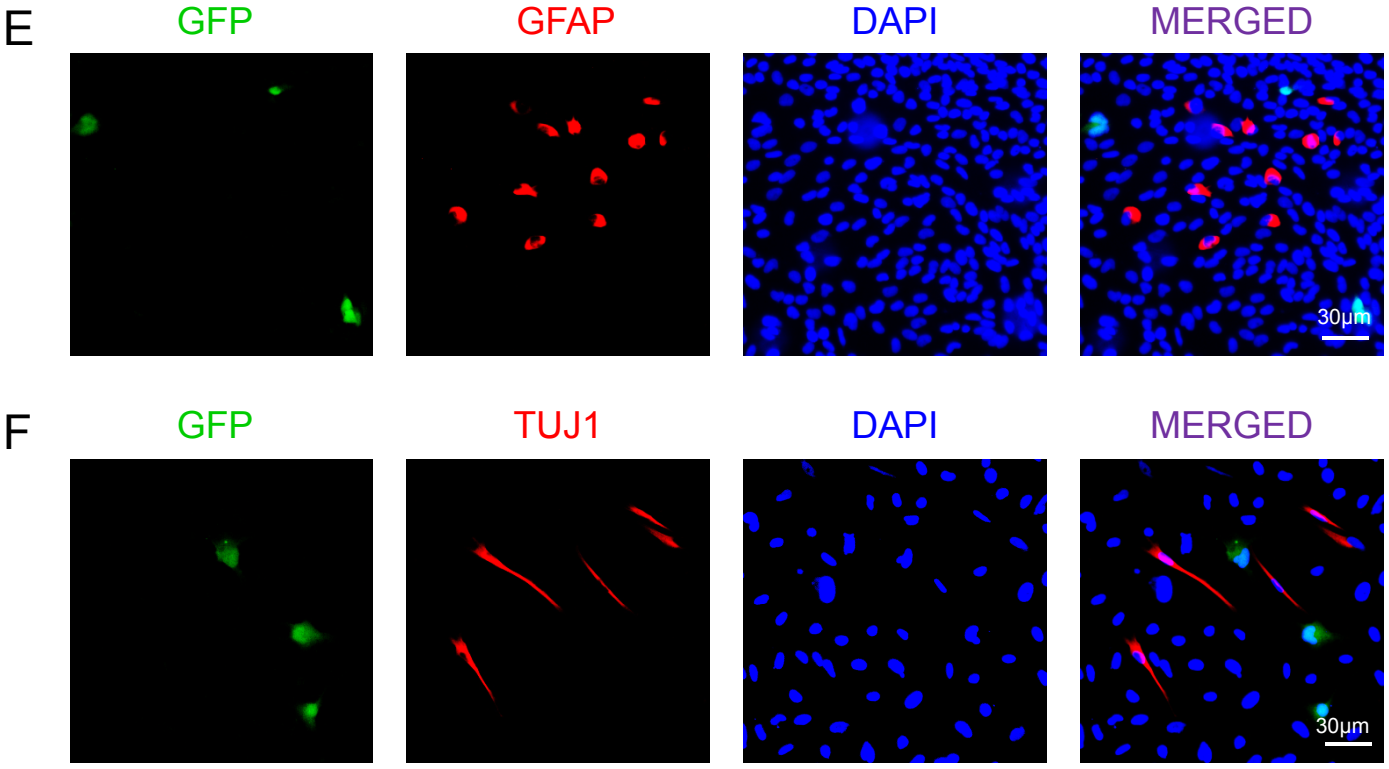
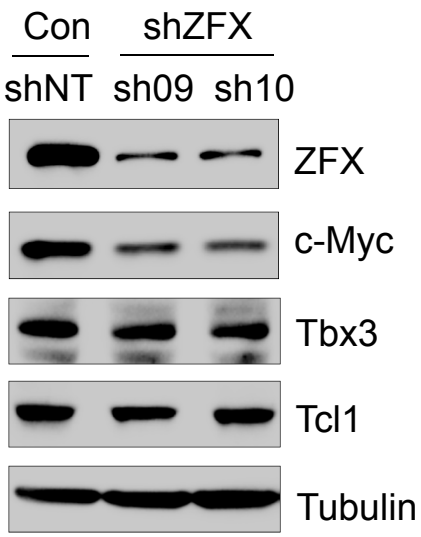


Figure S4



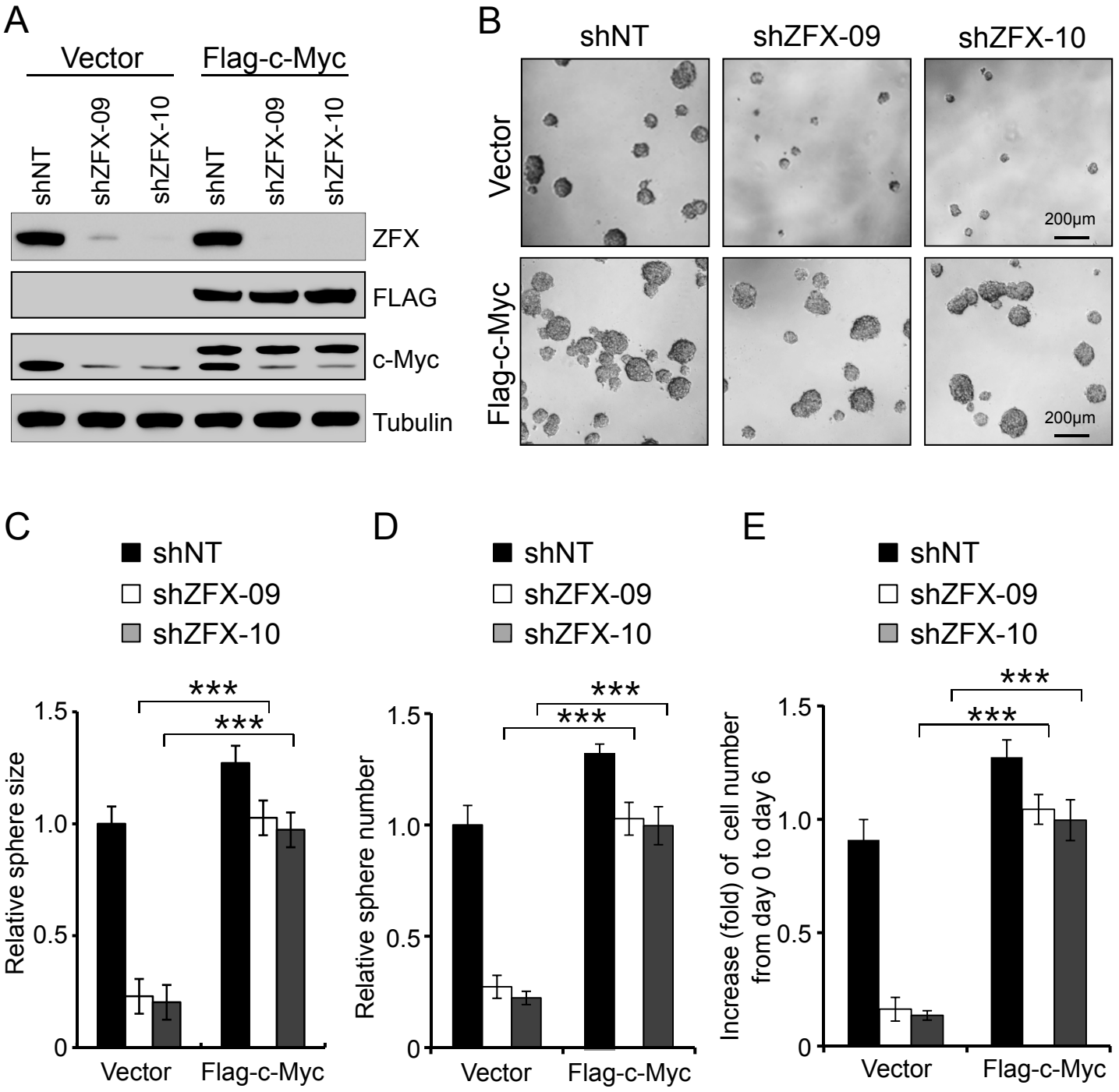

A
Vector
Flag-c-Myc
shNT
shZFX-09
shZFX-10
shNT
shZFX-09
shZFX-10
ZFX
FLAG
c-Myc
Tubulin
B
shNT
shZFX-09
shZFX-10
Vector
Flag-c-Myc
200μm
200μm
C
shNT
shZFX-09
shZFX-10
Relative sphere size
1.5
1.0
0.5
0
***
***
Vector
Flag-c-Myc
D
shNT
shZFX-09
shZFX-10
Relative sphere number
1.5
1.0
0.5
0
***
***
Vector
Flag-c-Myc
E
shNT
shZFX-09
shZFX-10
Increase (fold) of cell number from day 0 to day 6
1.5
1.0
0.5
0
***
***
Vector
Flag-c-Myc

Table S1

A summary of ZFX expression in 74 human primary GBMs in tissue microarrays by IHC Staining

ZFX+ cells / total cells (%)	**ZFX(+) Cells: none**	**ZFX(+) Cells: (≤1%)**	**ZFX(+) Cells: (1%-5%)**	**ZFX(+) Cells: (>5%)**
Positive case/ total cases	5/74	15/74	23/74	31/74
Positive cases (%)	6.75%	20.27%	31.08%	41.89%

Supplemental figure legends

Figure S1. ZFX is preferentially expressed in a fraction of cancer cells expressing GSC markers in GBM tumors.

(A-C): Immunofluorescence (IF) staining of ZFX and the GSC markers in human GBM tumors or xenografts (CW1336, T4121 and T3359). Frozen sections of GBM tumors were co-immunostained with specific antibodies against ZFX (in green) and a GSC marker (SOX2 or OLIG2, in red) and then counterstained with DAPI to show nuclei (in blue). ZFX is expressed in the cancer cells expressing the GSC markers.

(D): Immunohistochemical (IHC) staining of ZFX in mouse normal brain and GBM xenografts derived from human GSCs (CCF2170, T3742 and T3691). Tissue sections were counterstained with hematoxylin to mark nuclei. ZFX-positive cells are shown in brown color.

Scale bars represent 10 μm (**A**, **C** and **D**) and 20 μm (**B**).

Figure S2. ZFX and c-Myc are differentially expressed in GSCs relative to non-stem tumor cells and neural progenitor cells.

(A): IF staining of ZFX (in green) and the GSC marker OLIG2 (in red) in matched GSCs and non-stem tumor cells derived from GBM tumor (CCF2467). Nuclei were counterstained with DAPI. ZFX and OLIG2 are preferentially expressed in the GSC population.

(B): Immunoblot analysis of ZFX, c-Myc and SOX2 protein levels in GSC populations and human neural progenitor cells (NPCs). GSCs expressed much more ZFX and c-Myc than normal NPCs, while GSCs and NPCs expressed similar levels of SOX2.

(C and D): IF staining of ZFX and SOX2 (**C**) or c-Myc and SOX2 (**D**) in human neural

progenitor cells (NPCs). ZFX and c-Myc were shown in green, and SOX2 was shown in red. Nuclei were counterstained with DAPI (in blue). NPCs express little ZFX and c-Myc.

(E): IF staining of ZFX (in green) and SOX2 (in red) in the subventricular zone (SVZ) of mouse brain from an adult mouse. Nuclei were counterstained with DAPI (in blue). The brain SVZ region enriched with neural stem/progenitor cells expressed abundant SOX2 but showed low expression of ZFX.

Scale bars represent 10 μm (**A**, **C** and **D**) and 60 μm (**E**).

Figure S3. ZFX is not required for the growth of non-stem tumor cells and NPCs and the ZFX promoter is inactivated in differentiated cells derived from GSCs.

(A and B): Growth curves of non-stem tumor cells (**A**) and NPCs (**B**) expressing shZFX (sh09 or sh10) or NT shRNA. Non-stem tumor cells (T387) and human NPCs (16157) transduced with shZFX or NT shRNA through lentiviral infection and then measured for cell viability over a time course.

(C): A schematic representation of a lentiviral construct for the ZFX promoter-driven expression of GFP (ZFXpro-GFP).

(D): Images of phase contrast and GFP fluorescence of tumorspheres derived from T387 GSCs transduced with the ZFX promote-driven GFP expression.

(E and **F):** Fluorescence analysis of GFP and IF staining of GFAP (an astrocyte marker) or TUJ1 (a marker for neuronal lineage) in differentiated cells derived from the T387 GSCs transduced with the ZFX promote-driven GFP (ZFXpro-GFP). Nuclei were counterstained with DAPI (in blue). GFAP and TUJ1-positive cells (in red) lost GFP expression.

Scale bars represent 100 μm (**D**) and 30 μm (**E** and **F**). Data are means ± SD.

Figure S4. ZFX disruption down-regulates expression of c-Myc but not Tbx3 and Tcl1 expression in GSCs**.** Immunoblot analysis of ZFX, c-Myc, Tbx3 and Tcl1 in T387 GSCs expressing shZFX (sh09 and sh10) or NT shRNA was shown. ZFX knockdown reduced c-Myc protein but showed no effect on Tbx3 and Tcl1 protein levels.

Figure S5. Ectopic expression of c-Myc to its endogenous level in GSCs rescued the phenotype caused by ZFX disruption in vitro.

(A): Immunoblot analysis of ZFX, Flag-c-Myc, and endogenous c-Myc in T387 GSCs transduced with Flag-c-Myc or vector control in combination with shZFX (sh09 or sh10) or NT shRNA. The ectopic expression of c-Myc (Flag-c-Myc) was similar to the endogenous level of c-Myc. ZFX knockdown reduced the endogenous c-Myc but not the ectopic Flag-c-Myc.

(B-D): Tumorsphere formation of GSCs transduced with Flag-c-Myc or vector control in combination with shZFX (sh09 or sh10) or NT shRNA. Representative images (phase contrast) of tumorspheres are shown (**B**). Ectopic expression of c-Myc in the GSCs rescued the impaired tumorsphere formation caused by ZFX disruption. Quantifications indicate that ectopic expression of c-Myc (Flag-c-Myc) in the GSCs rescued the reduction in tumorsphere number (**C**) and size (**D**) caused by ZFX down-regulation.

(E): Effects of c-Myc ectopic expression on cell growth of GSCs expressing shZFX or NT shRNA. GSCs were transduced with Flag-c-Myc or vector control in combination with shZFX (sh09 or sh10) or NT shRNA. The relative increase (folds) of cell number from day 0 to day 6 among the six groups (Flag-Myc/Vector and shZFX09/shZFX10/NT shRNA) was compared.

Scale bars represent 200 μm (**B**). Data are means ± SD.