## INVENTORY OF SUPPLEMENTAL INFORMATION

### SUPPLEMENTAL FIGURES AND LEGENDS

**Supplemental Figure 1.** Contains results of genomic DNA sequencing for IDH1 status in the tumor samples used in Fig.1.

**Supplemental Figure 2.** Contains images of the colony formation assay showing the density of the colonies in each sample, and a graph showing the quantification of the number of colonies per sample.

**Supplemental Figure 3.** Shows that Dvl2 does not induce apoptosis or senescence in U87 cells.

**Supplemental Figure 4.** Contains the quantitation of the change in Nestin, Tuj1 and GFAP upon Dvl2 depletion.

**Supplemental Figure 5.** Illustrates the ACGH analysis of two of the GBM cells used in this study.

**Supplemental Figure 6.** Contains additional data showing the effect of Dvl2 overexpression on proliferation and neurosphere formation in U87 glioma cell line.

**Supplemental Figure 7.** Contains additional data showing the effect of Dvl2 depletion on proliferation and colony formation in 4 more glioma cell lines.

**Supplemental Figure 8.** Illustrates the loss of tumorigenicity of U87 cells when injected in the brain of NOD/SCID mice.

**Supplemental Figure 9.** Contains additional results related to the block of canonical Wnt/β-catenin signaling in U87 cells. The images show a lack of morphological change or differentiation upon block of β-catenin signaling.

**Supplemental Figure 10.** Shows that active β-catenin (β-catS4A) is not able to rescue the differentiation event induced by Dvl2 depletion in U87 cells.

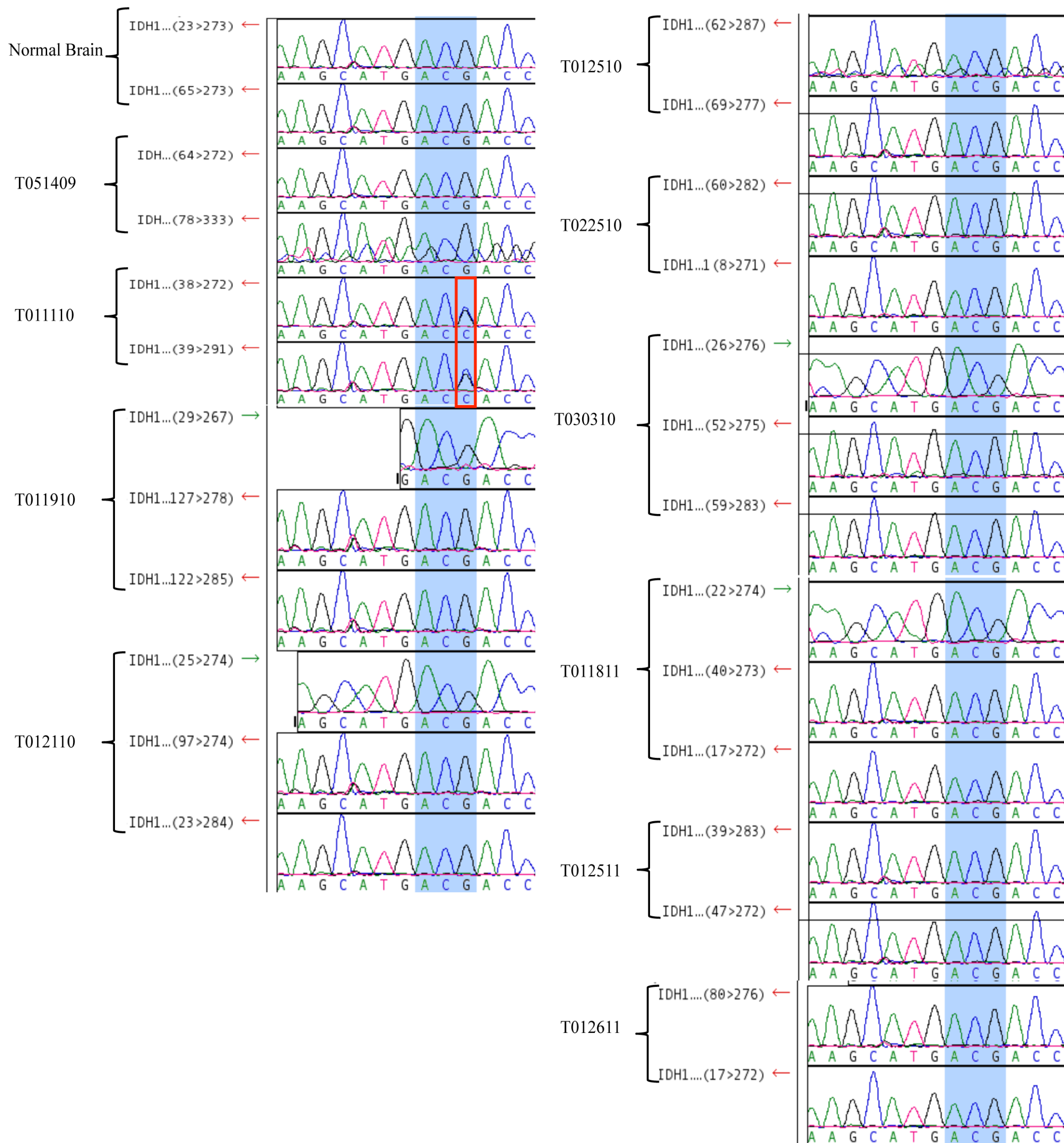
### SUPPLEMENTAL TABLES AND LEGENDS

Supplemental Table 1. Contains information about the patients and the tumors from which the GBM cells were derived.

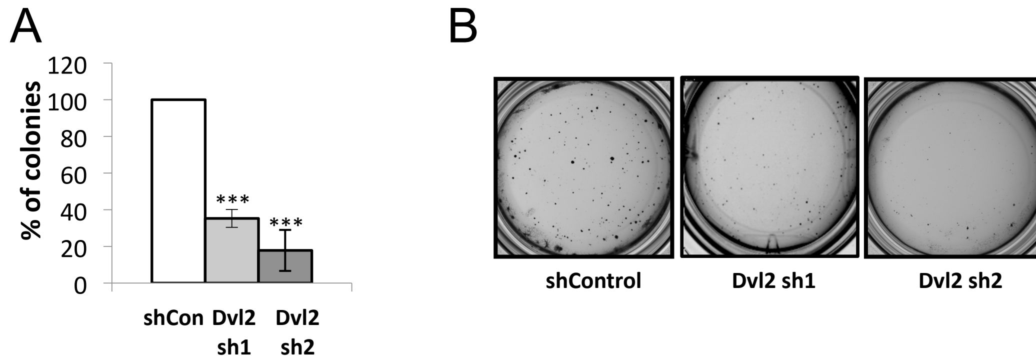
### SUPPLEMENTAL MOVIES AND LEGENDS

Movies S1 and S2 are related to Figure 1. Movie S1 shows control U87 cells; Movie S2 shows the morphological changes upon Dvl2 depletion in U87 cells.

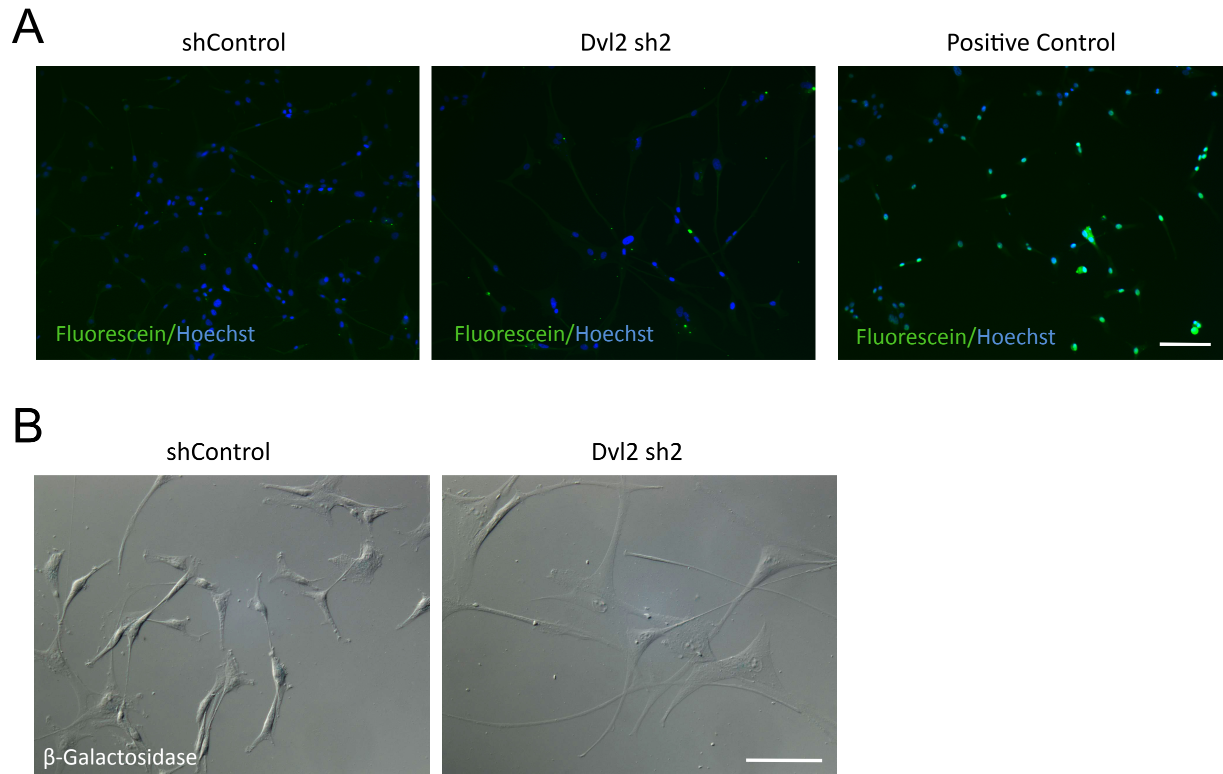
### SUPPLEMENTAL EXPERIMENTAL PROCEDURE



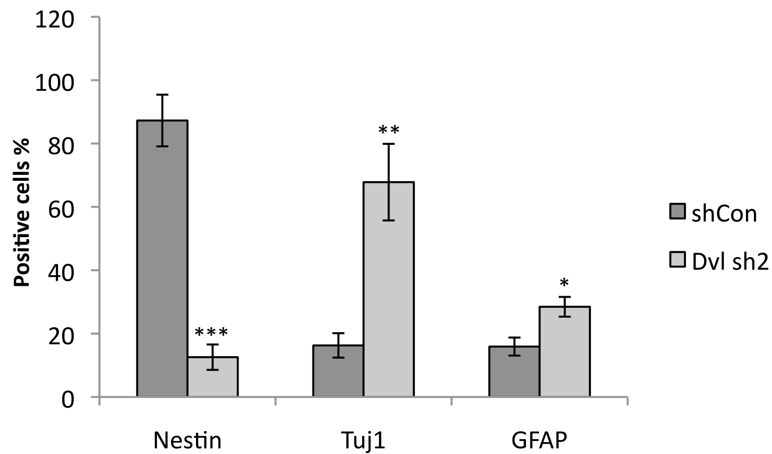

**Supplemental Figure 1. Isocitrate deihydrogenase 1 (IDH1) status in GBM samples.** Genomic DNA was extracted from the patient's derived samples used in Figure 1B for western blot analysis. Samples were sequenced to detect the status of the IDH1 gene. The most common IDH1 mutation in gliomas is R132H, specific for a single codon in the Arg132 residue. As shown in this figure, IDH1 sequence is wild type in 9 out of 10 samples. Sample T011110 carries a less common mutation, R132G, indicated here with a red box.




**Supplemental Figure 2. Dvl2 depletion inhibits U87 glioma cell proliferation and anchorage independent growth. (A,B)** U87 cells were infected with a lentivirus containing non-targeting (shCon) or two different Dvl2 targeting shRNAs (sh1 and sh2). Cells were selected in puromycin for 5d and grown for 14d in 1.2% methylcellulose in DMEM GlutaMax containing 10% FBS. The number of colonies was counted using an Optronix GelCount system. Data represent means ± SEM. ***P<0.001 relative to control cells (Student's t test).



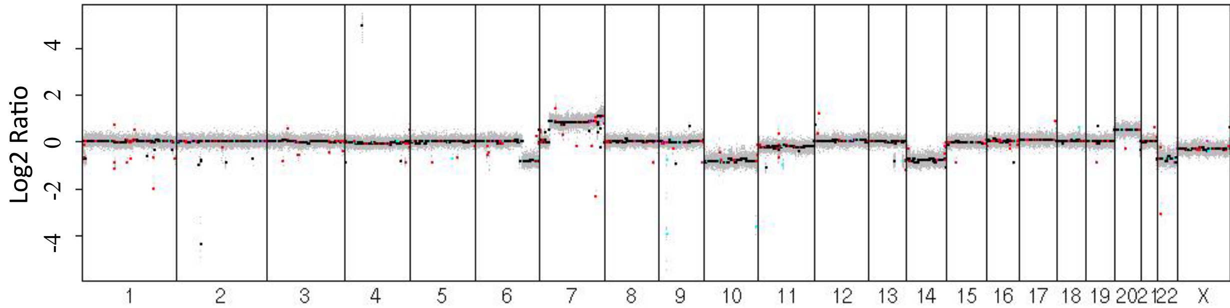

**Supplemental Figure 3. Dvl2 depletion does not induce apoptosis or senescence in U87 cells. (A)** U87 cells were infected with a lentivirus containing non-targeting (shControl) or Dvl2 targeting shRNAs (sh2). Cells were selected in puromycin for 5d and then fixed. Since DNA fragmentation is a hallmark of apoptosis, cells were processed for Tunel assay to check for any apoptotic event (green fluorescein). The positive control was obtained by treating fixed cells with recombinant DNAse I to induce DNA fragmentation. The results show that Dvl2 depletion does not induce apoptosis. (B) Cells were processed for a senescence assay, checking the activity of β-Galactosidase. The knockdown of Dvl2 does not induce senescence. Scale bar is 100µm.




**Supplemental Figure 4. Dvl2 depletion induces differentiation of U87 cells.**
U87 cells were infected with a lentivirus containing non-targeting (shCon) or Dvl2 targeting shRNAs (sh2). Cells were selected in puromycin for 5d and then fixed. Cells were stained for Nestin, Tuj1 and GFAP. The graph indicates the number of positive cells, as a percent of the total, before and after Dvl2 depletion. Data represent mean ± SEM. ***$P<0.001$,**$P<0.01$,*$P<0.05$, relative to control cells (Student's t test).

A

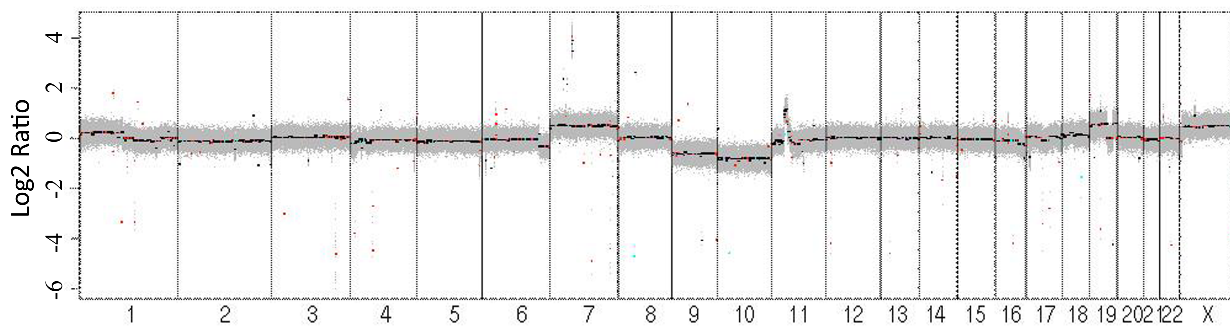
GBM1




CGH Summary: ++PDGFRA, +[EGFR,MET,CDK6], -PTEN, --CDKN2A

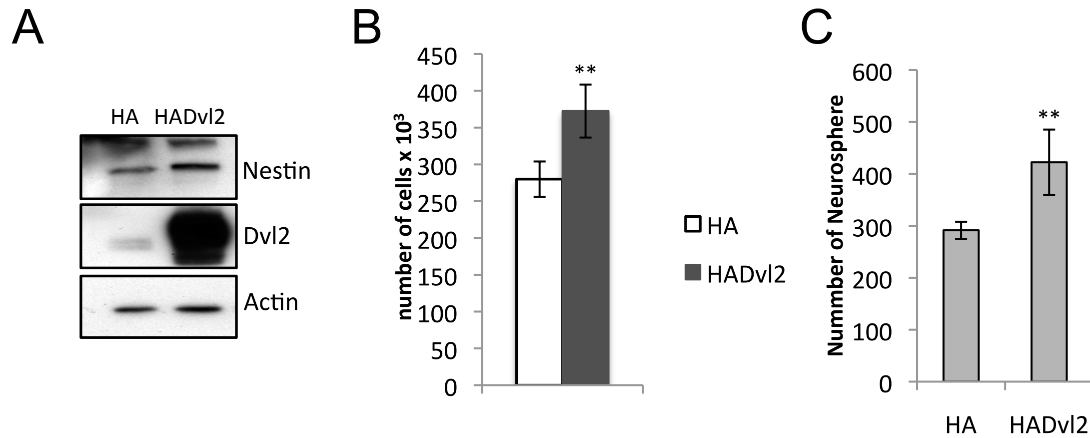
B

GBM3

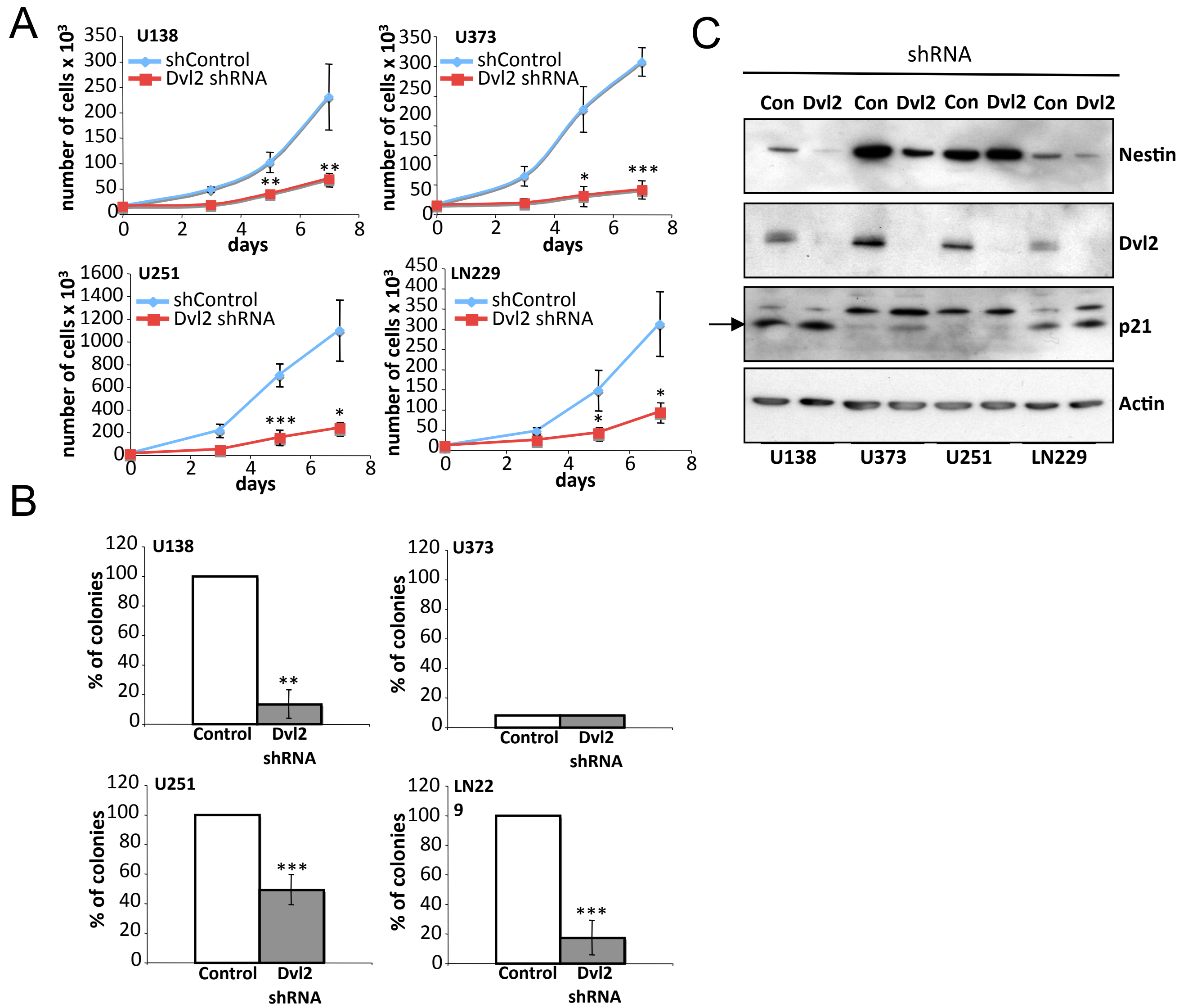



CGH Summary: ++EGFR, +[MET,CDK6], -[CDKN2A,PTEN]

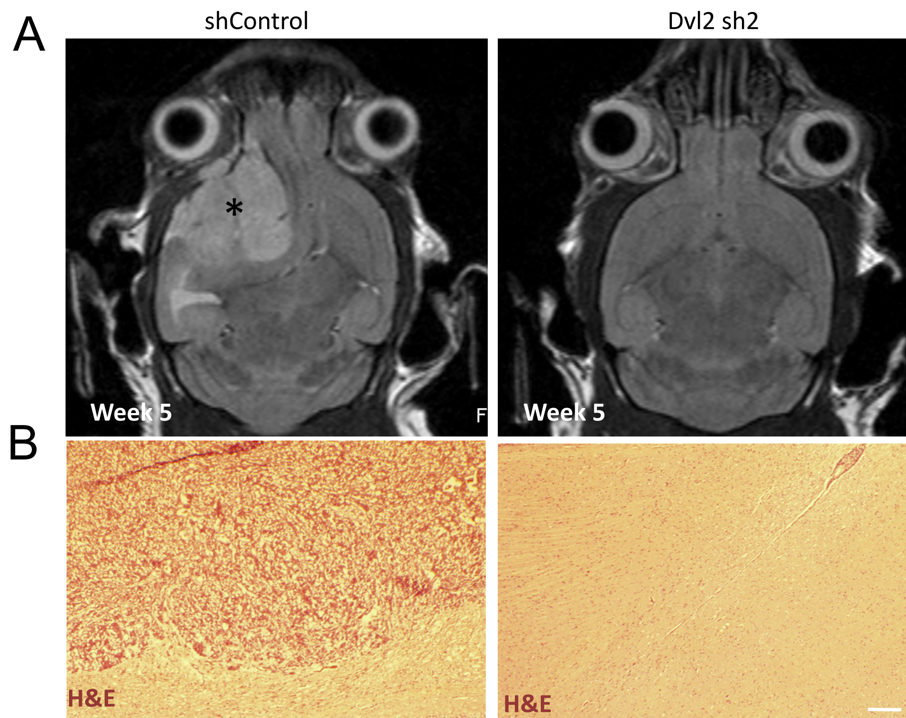
**Supplemental Figure 5. Comparative genomic hybridization (CGH) profiles.** Whole genome profiles of two GBM specimens used in this study (GBM1 **(A)** and 3 **(B)**; no remaining specimen was available for GBM2). The tumors shown include a variety of GBM-typical copy number aberrations including PDGFRA, EGFR, MET, CDK6 amplification and PTEN and CDKN2A deletion.




**Supplemental Figure 6. Ectopic expression of Dvl2 increases proliferation and self-renewal of U87 cells.** U87 cells were infected with a retrovirus containing an empty vector (HA) or a vector expressing mouse Dvl2 (HADvl2). **(A)** 10d after retroviral infection, the levels of mDvl2 and Nestin where analyzed by western blot. Cells expressing mDvl2 showed an increase in Nestin levels. **(B)** The cells were plated and grown in medium containing 10% FCS and then counted after 7d, or **(C)** plated and grown for 10d in serum-free neural stem cell medium containing 0.7% methylcellulose to asses their ability to form neurospheres. mDvl2 expression increased the proliferation of U87 cells and their ability to from neurospheres. Data represent mean ± SEM. **$P<0.01$, relative to control cells (Student's t test).




**Supplemental Figure 7. Dvl2 depletion inhibits proliferation and induces differentiation in four additional glioma cell lines.** U138, U373, U251 and LN229 glioma cell lines were infected with a lentivirus containing either a non-targeting (Con), or a Dvl2 shRNA (sh2). Cells were selected in puromycin for 5d. **(A)** Cells were plated in medium containing 10% FCS and cell numbers determined at 3d, 5d and 7d. **(B)** Cells were grown for 14d in 1.2% methylcellulose in DMEM GlutaMax containing 10% FBS. The number of colonies was counted using an Optronix GelCount system. **(C)** Western blot analysis of Dvl2 depleted cells. A decrease in the stem/progenitor marker Nestin is found in U138, U373 and LN229, while an increase in $p21^{WAF}$ is seen in U138, U373 and LN229. Data represent means ± SEM. ***P<0.001,**P<0.01,*P<0.05, relative to control cells (Student's t test).

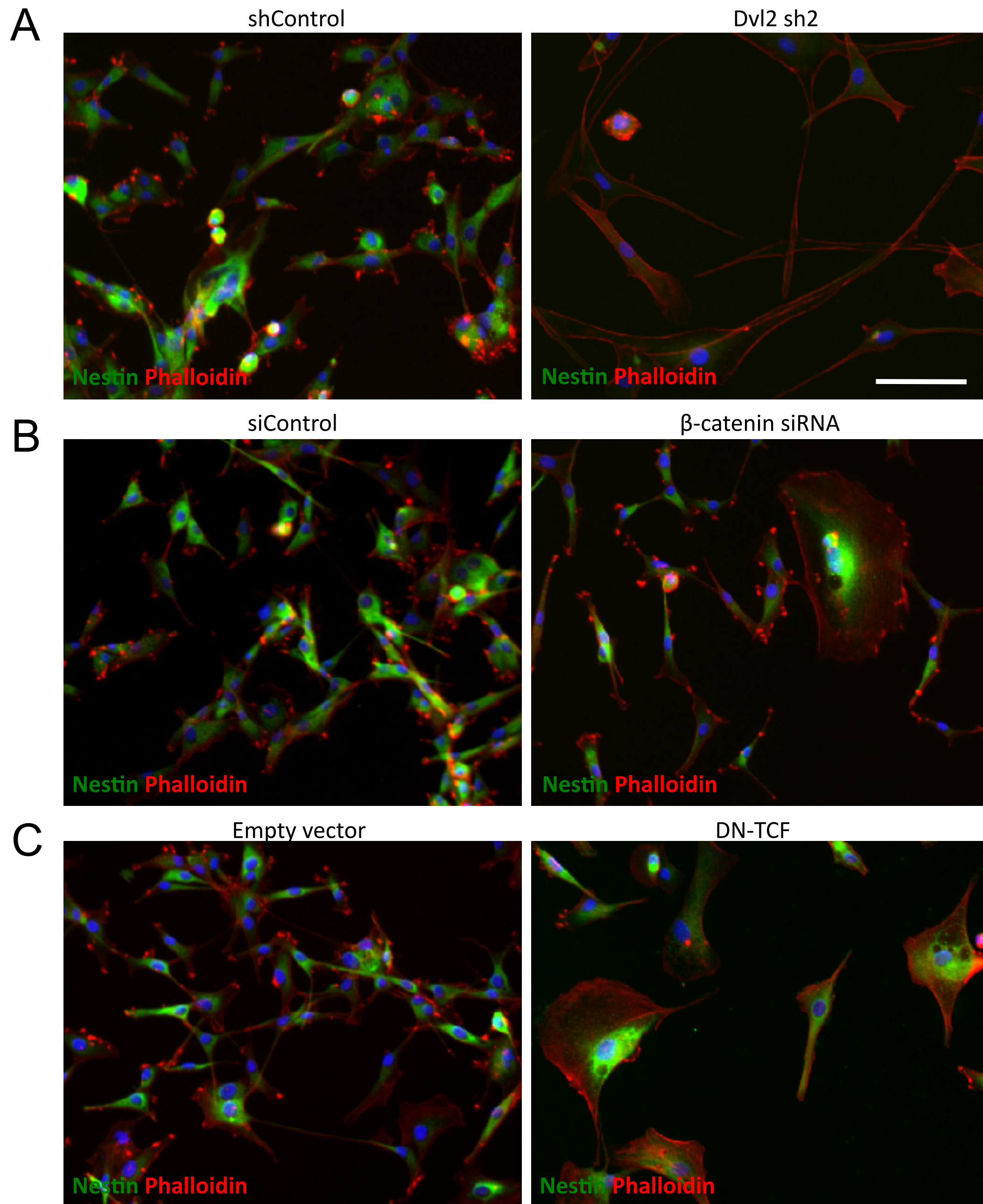



**Supplemental Figure 8. Dvl2 depletion blocks tumorigenicity of U87 in NOD/SCID mice and improves survival.**

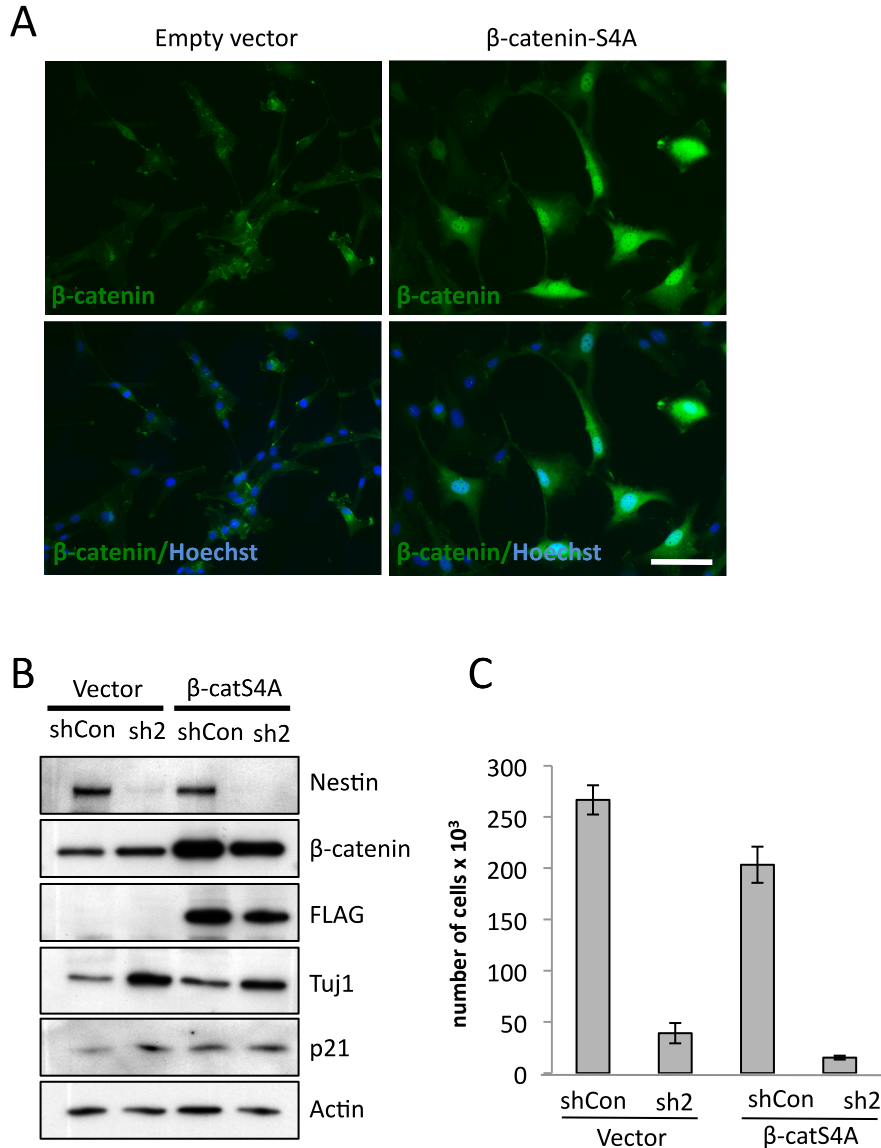
U87 cells were infected with non-targeting (shControl) or Dvl2 targeting shRNA (sh2) lentivirus and selected in purmoycin for 5d. Two hundred thousand cells were stereotactically injected into the striatum of NOD/SCID mice (5 mice for control and 9 mice for Dvl2 depletion).

**(A)** Tumor growth as determined by Magnetic Resonance Imaging (MRI) five weeks after injection. The asterisk in the left panel indicates the tumor. Left panel - control cells; right panel, Dvl2-depleted cells.

**(B)** Intracranial tumor characterization by H&E staining of brain cryo-sections. Scale bar is 100µm.




**Supplemental Figure 9. Suppression of canonical Wnt/β-catenin signaling does not induce differentiation. (A)** U87 cells were infected with non-targeting (shControl), or Dvl2 targeting shRNA (Dvl2 sh2) lentivirus and selected in purmoycin for 5d. **(B)** U87 cells were transfected with a non-targeting siRNA oligonucleotide (siControl) or an siRNA oligonucleotide targeting β-catenin. **(C)** U87 cells were infected with a control retrovirus, or retrovirus expressing Delta-N TCF (DN-TCF). Cells were fixed and stained for Nestin (green) and phalloidin (red) to monitor differentiation (i.e. loss of Nestin) and morphological changes, respectively. Cells expressing DN-TCF appeared flatter and more spread compared to control cells. Scale bar is 50µm.




**Supplemental Figure 10. Expression of β-catenin-S4A in U87 cells.** U87 cells were infected with empty vector, or β-catenin-S4A expressing retrovirus and selected in hygromycin for 7d. **(A)** Cells were fixed and stained for β-catenin (green) and Hoechst (blue). Once β-catenin is stabilized (active), it should translocate to the nuclei and interact with TCF to activate transcription. As shown in this figure, non-phosphorylatable/stabilized β-catenin-S4A localizes in the nuclei of U87 cells, suggesting that in these cells the transcription pathway downstream to Wnt/β-catenin pathway is activated. **(B)** Cells were treated as in (A) and subsequently infected with a lentivirus containing a non-targeting shRNA (shCon), or a shRNA against Dvl2 (sh2) as previously described. Levels of Nestin, Tuj1 and p21 are visualized by western blot. No rescue was observed in cells expressing β-catS4A. **(C)** Cells were treated as in (B) and then plated and grown in medium containing 10%FCS and counted after 7d. Scale bar is 50μm.

| GBM | gender | grade | age | status | EGFR | Chromosome 7 |
|---|---|---|---|---|---|---|
| GBM1 | M | GBM | 69 | new | N/A | N/A |
| GBM2 | F | GBM | 47 | recurrent | >20 copies in 85% of cells | 2-4 copies in 92% of cells |
| GBM3 | F | GBM | 54 | new | >40 copies in 100% of cells Express EGFRvIII | 2-6 copies in 100% of cells |

**Supplemental Table 1.** Three GBM specimens used in this study. Patient data reflect status at the time of tissue acquisition. Data on EGFR and chromosome 7 amplification is obtained via FISH analysis. EGFRvIII is detected by RT-PCR. These analyses were performed by a certified clinical facility at Memorial Sloan-Kettering Cancer Center.

## SUPPLEMENTAL MOVIES AND LEGENDS

**MovieS1 related to Figure1. Time-lapse live video microscopy of U87 glioma cells infected with control shRNA.** U87 cells were infected with non-targeting (shControl) and imaged after 72h. Images were captured every 5 min for 24h using a Zeiss Axiovert 200M microscope.

**MovieS2 related to Figure1. Time-lapse live video microscopy showing morphological changes induced after Dvl2 depletion in U87 glioma cells.** U87 cells were infected with Dvl2 targeting shRNA (sh2) lentivirus and imaged after 72h. Images were captured every 5 min for 24h using a Zeiss Axiovert 200M microscope.

## SUPPLEMENTAL EXPERIMENTAL PRODECURES

### Virus production

HEK 293 FT cells were plated at confluency ($3x10^6$ cells in 100mm plastic dishes) and 1d later transfected with 5μg of shRNA DNA construct, 5μg of VSV-G and 5μg of pDeltaR8.9 (packaging vectors) using lipofectamine (Invitrogen). cDNAs were mixed in 3ml of OptiMem (Gibco). After 3h, 3ml of complete medium were added to the cells. 1d later, the medium was replaced with fresh complete medium. The medium containing the virus was collected every day starting from the second day. At day 5, the medium was filtered through a 0.45μm filter and centrifuged at 19,000 rpm for 2h at 4$^o$C. The supernatant was discarded and the pellet resuspended in 1ml of PBS. The concentrated virus was used for the infection, or stored at -80$^o$C. A similar protocol was used for the retroviral production, namely transfecting HEK 293 FT cells with 5μg of the cDNA retroviral construct, 5μg of VSV-G and 5μg of Gag-Pol as retroviral packaging vectors. DN-TCF4 (deltaN31) in pPGS vector was purchased from Addgene (plasmid # 19284).

### Cell infection and transfection.

Lentivirus and retrovirus vectors were produced by transfecting HEK 293 FT cells, as described above. 24h after plating, the cells were rinsed three times in 1x PBS and the medium replaced with serum and antibiotic free medium. Cells were infected with 50μl of each virus directly diluted in serum free/antibiotic free medium containing polybrene (8μg /ml). The infection of primary GBM cells was carried out in the absence of polybrene. After 6h, the medium was replaced with complete culture medium. 2d after infection, cells were selected for 5d in 1μg/ml puromycin, 800μg/ml G418 or 100μg/ml

hygromycin depending on the selection marker carried by the expression vector. Alternatively, 24h after plating cells were transfected with 100nmole (in 5μl) of SMARTpool siRNA (Dharmacon) using Oligofectamine (Invitrogen). The serum-free medium was replaced with complete medium 6h after transfection.

Mouse Dvl2 was subcloned in pBABE HAII retroviral vector using BamH1 and EcoRI restriction enzymes. In order to make this construct resistant to Dvl2 shRNA2, three silent mutations were inserted in the sequence using a Stratagene site-directed mutagenesis kit (QuikChange II, 200523-5). The primers pair used for the site-directed mutagenesis was: GCC AAG TGT TGG GGC CCG TCT CCC; GTA GCC CTG GGG AGA CGG GCC CCA.

Unphosphorylatable/active Xenopus β-catenin (β-catenin-S35/37/41/45A or β-catenin-S4A) in pBabe vector was a gift of Giancotti F., Cell Bology Program, MSKCC (1).

**shRNA and siRNA sequences**

shRNA sequences from Sigma Mission were as follows:

Non targeting control vector (SHC002),

Dvl2 (sh1, GCACCATTACATCTGGATCGT, TRCN0000033343; sh2, GAGACAGAAACCGAGTCAGTA, TRCN0000033341).

siRNA duplexes, from Dharmacon, were as follows: Non targeting SMARTpool ( D-001206-14, Thermo Fisher Scientific ); β-catenin SMARTpool (D-003482, Thermo Fisher Scientific).

**BrdU incorporation**

U87 cells were infected with viral vectors and selected as described above. Cells were treated with BrdU (10μM) for 6h and then processed for BrdU incorporation analysis using the BrdU Flow Kit (BD Pharmingen, Cat. no. 559619). Samples were analyzed using a FACScalibur instrument and data analysis was performed using FlowJo software.

**Colony formation assay**

The glioma cell lines were infected and selected for 5d in puromycin. Cells were trypsinized and resuspended in MEM GlutaMax, containing 10% FCS and 1.2% methylcellulose. $5x10^3$ cells were plated in each well of an ultra-low attachment 24-well plate (Corning, 3473) in quadruplicate. After 15d, colonies were stained with 25μl MTT 0.5mg/ml in 1xPBS for 2h at 37$^o$C and counted using an Optronix GelCount system.

**Protein analysis**

Cells were plated 24h before use. Following infection and selection, cells were washed three times with ice-cold PBS and collected immediately at 4°C in lysis buffer (1% NP-40, 0.1% SDS, 50mM Tris-HCl, pH 7.4, 150mM NaCl, 2mM EDTA, 5mM $Na_3VO_4$, 10mM NaF) containing protease inhibitors (Roche, 11697498001). Cell lysates were centrifuged at 18,000*g* for 10min at 4°C and the supernatant was immediately processed for SDS–PAGE and western blotting. The following antibodies were used: Rabbit anti-Dvl2 (Cell Signaling 3224, 1:1000), rabbit anti-EGFR (Santa Cruz sc-03), rabbit anti-p53 (Cell Signaling 9282), mouse anti-p21 (Santa Cruz sc-6246, 1:250), mouse anti-Nestin

(Abcam ab22035, 1:1000), mouse anti Tuj1 (Millipore MAB1637, 1:1000), rabbit anti-GFAP (DAKO Z0334, 1:4000), rabbit anti-β-catenin (Zymed 71-2700, 1:2000).

**CGH studies**.

Comparative genomic hybridization (CGH) assay was performed by hybridizing genomic DNA from frozen banked whole tumor on 1M human genome CGH arrays (commercial arrays from Agilent). DNA extraction, purification, labelling and hybridization were performed at Sloan Kettering Cancer Center's Genomics Core Facility according to the manufacturer's instructions. Log2 ratios were normalized by Lowess against probe intensity and mean %GC of the genomic region mapped to by the probe. Segmentation of normalized log2 ratios was by Circular Binary Segmentation (CBS, R package DNAcopy).

SUPPLEMENTAL REFERENCE

1. Okada T, Lopez-Lago M, Giancotti FG. Merlin/NF-2 mediates contact inhibition of growth by suppressing recruitment of Rac to the plasma membrane. J Cell Biol. 2005;171:361-71.