ADDITIONAL FILES TO:

The expression of pro-prion, a transmembrane isoform of the prion protein, leads to the constitutive activation of the canonical Wnt/β-catenin pathway to sustain the stem-like phenotype of human glioblastoma cells

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Content:

Table S1

Clinical and pathological features of patients, GBMs, and *in vivo* tumorigenicity of GBM-derived stem cells

CODE	SEX	AGE (years)	Ki-67 (%)	WHO grade	Status/ Molecular subtype	LOCALIZATION	*IN VIVO* TUMORIGENESIS (NOD-SCID mice survival)
GBM 1	F	40	40	IV	Primary Neural	Multicentric	Yes (100 days)
GBM 2	M	48	60%	IV	Primary Neural	Left hemisphere and sub-cortical development	Yes (120 days)
GBM 3	M	67	35	IV	Primary Neural	Right hemisphere and cortical development	Yes (55 days)
GBM 4	M	73	40	IV	Primary Neural	Right hemisphere and sub-cortical development	Yes (150 days)
GBM 5	F	41	30	IV	Secondary Mesenchymal	Evolution from oligodendroglioma, right fronto-temporal, cortical diffusion	Yes (100 days)

Table S2

mRNA levels of stem and differentiation markers in GSC 1-5 cultures expressing different levels of PrP^C.

		SOX 2	NANOG	OCT 4	NESTIN	GFAP
GBM 1	SCR	100	100	100	100	100
	KO	41.3**	23**	39.6**	33.1**	305**
	OV	115°°	174°°	106	87.3°°	127°°
	REV	102	223**	217*	90.7**	41.3**
GBM 2	SCR	100	100	100	100	100
	KO	51.1**	48.0**	72.2**	80.1**	159**
	OV	102°°	109°°	124°°	61.2°	42.7°°
	REV	107	130**	159,4**	193**	64.4**
GBM 3	SCR	100	100	100	100	100
	KO	39.5**	10.1**	20.2**	20.0**	179**
	OV	100.5	300.9°	322°	46.7°	47°°
GBM 4	SCR	100	100	100	100	100
	KO	8.03**	28.1**	13.2**	13.2**	189**
	OV	106.9	258.1°°	126.4°°	70.2%°°	34.8°°
GBM 5	SCR	100	100	100	100	100
	KO	11.2**	30.7**	23.5**	36.0**	194**
	OV	66.1°	89°	135°°	51°°	89.0°°

Results are expressed as percentage of SCR cells, set as 100%; data represent the means ± SEM (n=4).

*** p < 0.05 and **p < 0.01 vs. respective GBM SCR cells**

° p < 0.05 and °°p< 0.01 vs. respective GBM KO cells

Table S3

Primary and secondary antibodies used in the study

PRIMARY ANTIBODIES
• anti-PrP: clones 3F4 (mouse) (Signet Lab) and Saf 32 (mouse) (SPIbio), dil. 1:5000 (Wb ,IF)
• anti- β-actin (mouse) (Sigma-Aldrich), dil. 1:1000 (Wb)
• anti-α-tubulin (rabbit) (Cell Signaling Technology), dil. 1:1000 (Wb)
• anti-GFAP (rabbit) (Cell Signaling Technology), dil. 1:1000 (Wb)
• anti-NANOG (rabbit) (Abcam), dil. 1:1000 (Wb)
• anti-Oct4 (rabbit) (Cell Signaling Technology), dil. 1:1000 (Wb)
• anti-Sox2 (rabbit) (Cell Signaling Technology), dil. 1:1000 (Wb)
• anti-CD44 (rabbit) (Abcam), dil. 1:1000 (Wb)
• anti-β-catenin (rabbit) (Cell Signaling Technology), dil. 1:1000 (Wb)
• anti-Dvl2 (rabbit) (Cell Signaling Technology), dil. 1:1000 (Wb)
• anti-Axin2 (rabbit) (Cell Signaling Technology), dil. 1:1000 (Wb)…….
• anti-Wnt5a/b (rabbit) (Cell Signaling Technology), dil. 1:1000 (Wb)
SECONDARY ANTIBODIES:
• Horseradish peroxidase-linked anti-mouse and anti-rabbit IgG antiserum (GE Healthcare), dil. 1:5000, used in Western blots.
• AlexaFluor 488-conjugated anti-rabbit secondary Ab (Molecular Probes), dil. 1:5000, used in immunofluorescence.

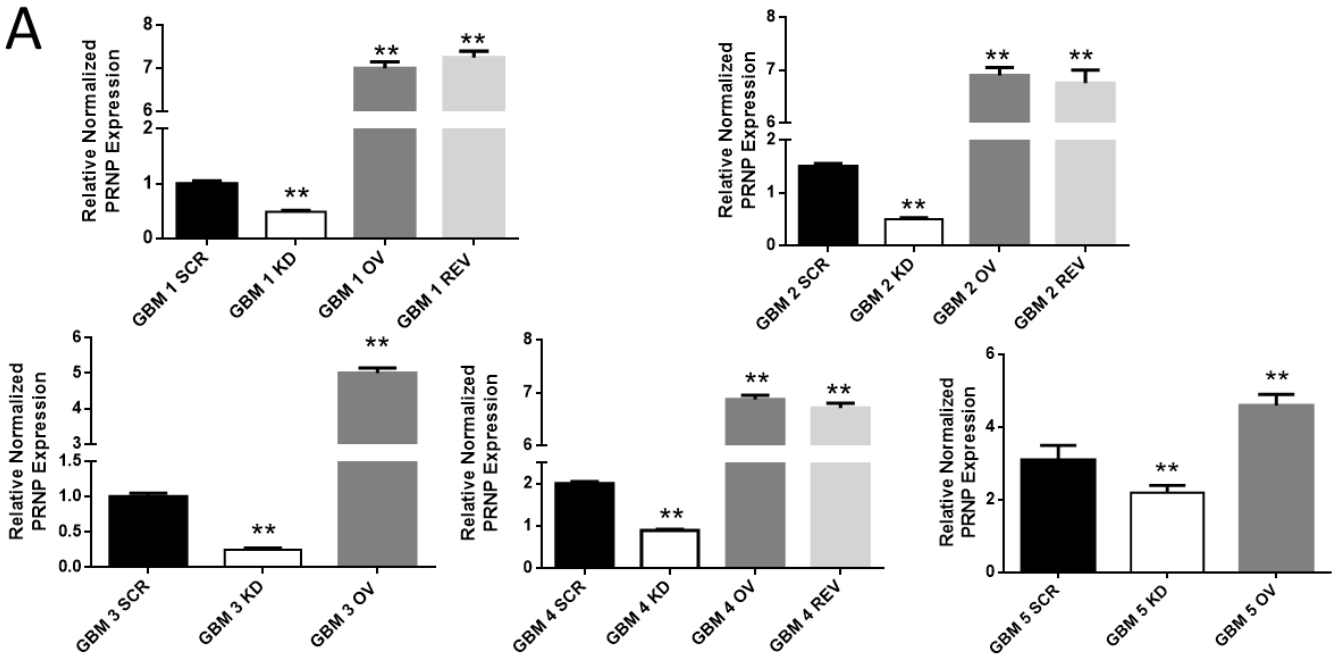
Table S4

Sequences of the primers used in qRT-PCR amplifications

	FORWARD	REVERSE
RPLP0	5’-TGTGGGCTCCAAGCAGATGCA-3’	5’-GCAGCAGTTTCTCCAGAGCTGGG-3’
28S	5'-CCCAGTGCTCTGAATGTCAA-3'	5'-AGTGGGAATCTCGTTCATCC-3'
PRNP	5'-AGTGGAACAAGCCGAGTAAGC-3'	5'-GTCACTGCCGAAATGTATGATG-3'
SOX-2	5'-CAGGAGTTGTCAAGGCAGAGA-3'	5'-TCCTAGTCTTAAAGAGGCAGCA-3’
NANOG	5'-GTCCCAAAGGCAAACAACCC-3'	5'-TGACCGGGACCTTGTCTTC-3'
NESTIN	5’-TGGCTCAGAGGAAGAGTCTGA-3’	5’-TCCCCCATTCACATGCTGTGA-3’
OCT-4	5’-CTTCGCAAGCCCTCATTTCAC-3’	5’-GAAGGCGAAATCCGAAGCCA-3’
GFAP	5’-TCCTGGAACAGCAAAACAAG-3’	5’-CAGCCTCAGGTTGGTTTCAT-3’
CD44	5’-TGAATATAACCTGCCGCTTTG-3’	5’-GCTTTCTCCATCTGGGCCAT-3’
WNT 3A	5’-CATGAACCGCCACAACAAC-3’	5’-TGGCACTTGCACTTGAGGT-3’
WNT 5A	5’-ATTGTACTGCAGGTGTACCTTAAAAC-3’	5’-CCCCCTTAGGCAGGTTGGC-3’
WNT 7A	5’-CTTCGGGAAGGAGCTCAAA-3’	5’-GCAATGATGGCGTAGGTGA-3’
WNT 7B	5’-CGCCTCATGAACCTGCATA-3’	5’-GCTGCATCCGGTCCTCTA-3’
FZD 1	5’-CGGCAAGACCCTCAACTC-3’	5’-CCTTGTTTGCTGTTGGTGAG-3’
FZD 3	5’-ACAGCAAAGTGAGCAGCTACC-3’	5’-CTGTAACTGCAGGGCGTGTA-3’
FZD 4	5’-TTCACACCGCTCATCCAGTA-3’	5’-TGCACATTGGCACATAAACA-3'

Figure S1

A



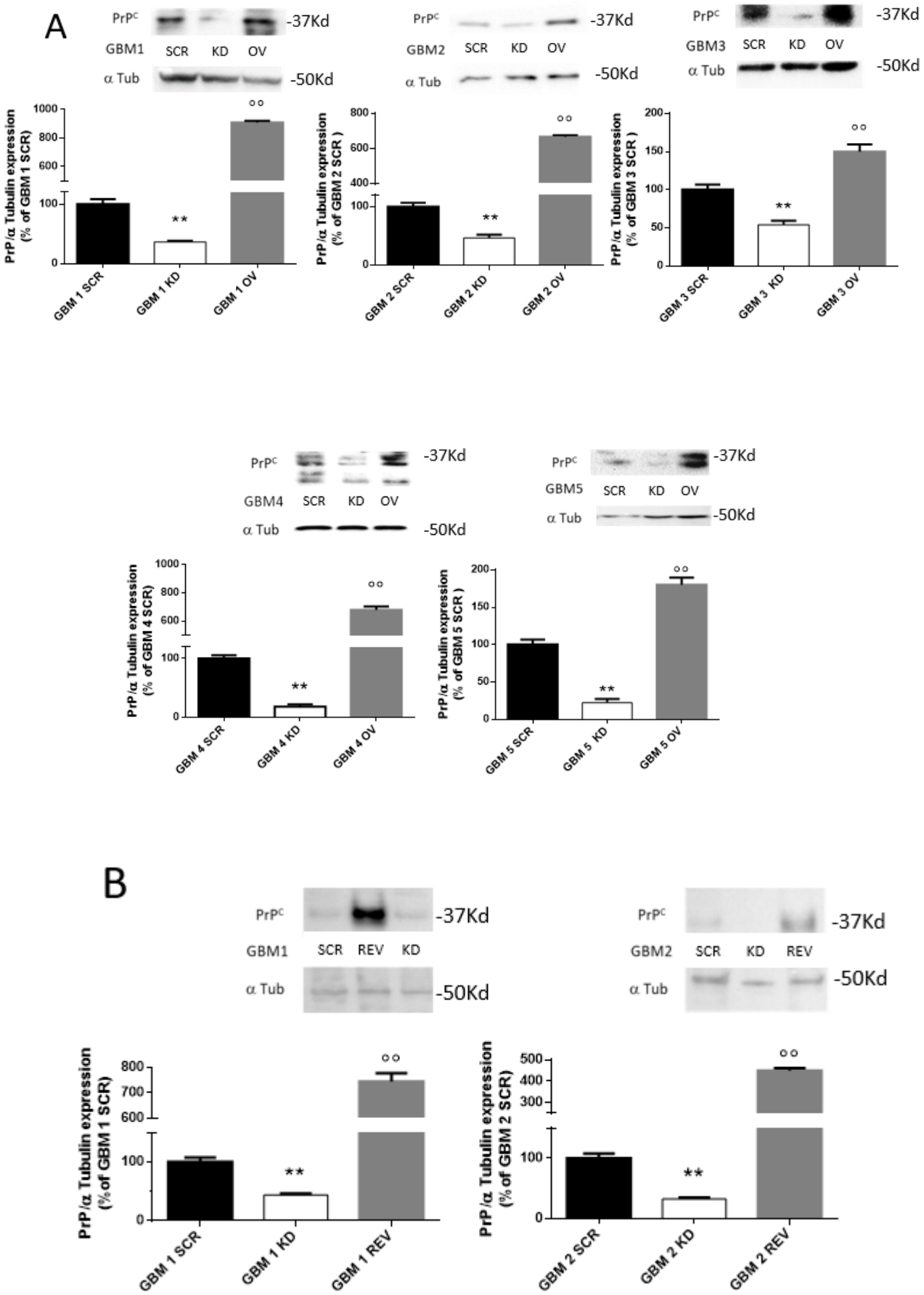
B

	SCR (%)	KD (%)	OV (%)	REV (%)
GBM 1	100	36.36**	909**	1080**
GBM 2	100	46.6**	666.2**	450**
GBM 3	100	62.3**	763.9**	
GBM 4	100	18.1**	679**	634**
GBM 5	100	64**	123**	

PrP^C mRNA expression in transfected GSCs

A) Normalized PrP^C mRNA levels in GBM 1-5 SCR, KO, OV, and REV evaluated by RT-qPCR. Results are expressed as means ± SEM (n=3). **p < 0.01 *vs*. respective GBM SCR cells (t-test).
B) Summary of PrP^C mRNA levels, represented as percentage versus respective GBM SCR cells. Results are as the mean ± SEM (n=3). **p <0.01 *vs*. respective GBM SCR (t-test).

Figure S2


A
PrPC
-37Kd
GBM1 SCR KD OV
α Tub
-50Kd
GBM2 SCR KD OV
GBM3 SCR KD OV
PrP/α Tubulin expression (% of GBM 1 SCR)
PrP/α Tubulin expression (% of GBM 2 SCR)
PrP/α Tubulin expression (% of GBM 3 SCR)
GBM 1 SCR
GBM 1 KD
GBM 1 OV
GBM 2 SCR
GBM 2 KD
GBM 2 OV
GBM 3 SCR
GBM 3 KD
GBM 3 OV
GBM4 SCR KD OV
GBM5 SCR KD OV
PrP/α Tubulin expression (% of GBM 4 SCR)
PrP/α Tubulin expression (% of GBM 5 SCR)
GBM 4 SCR
GBM 4 KD
GBM 4 OV
GBM 5 SCR
GBM 5 KD
GBM 5 OV
B
GBM1 SCR REV KD
GBM2 SCR KD REV
PrP/α Tubulin expression (% of GBM 1 SCR)
PrP/α Tubulin expression (% of GBM 2 SCR)
GBM 1 SCR
GBM 1 KD
GBM 1 REV
GBM 2 SCR
GBM 2 KD
GBM 2 REV

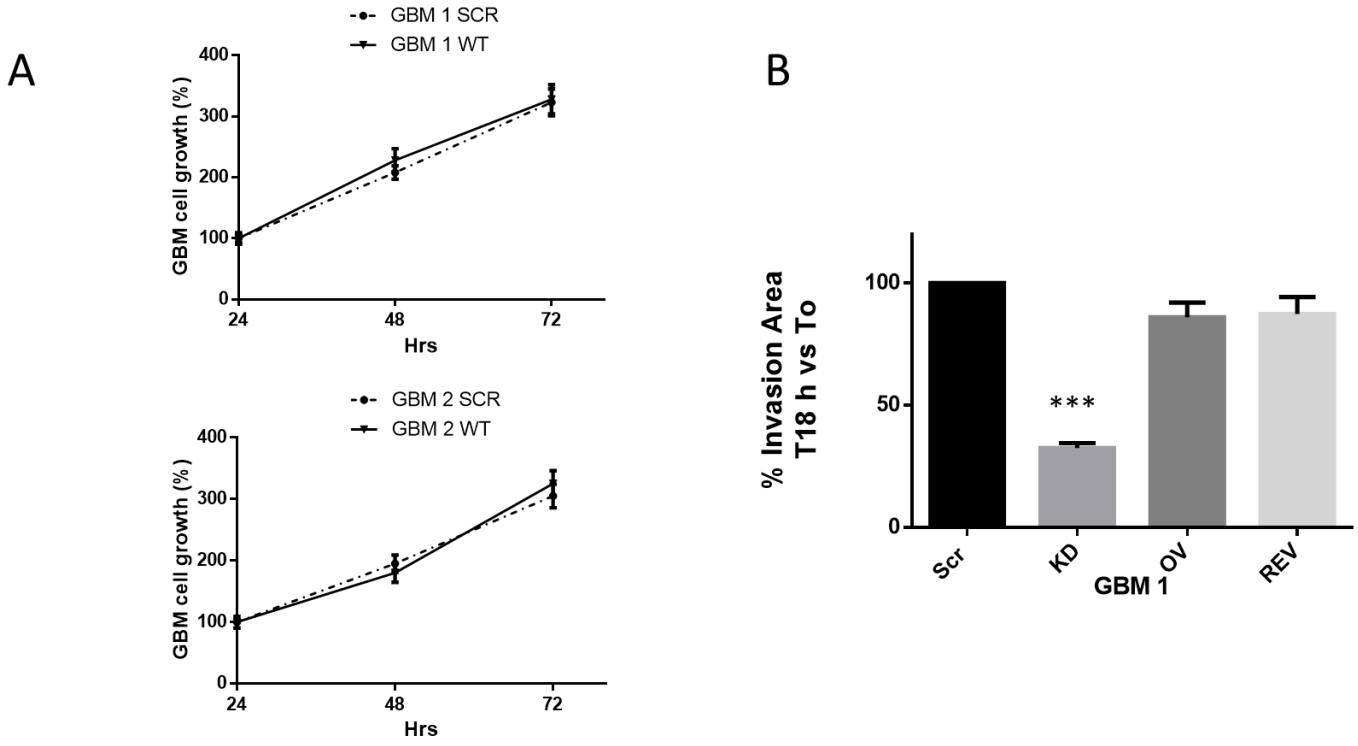
C

	SCR (%)	KD (%)	OV (%)	REV (%)
GBM 1	100	45**	950**	1191**
GBM 2	100	32**	543**	433**
GBM 3	100	42**	580**	
GBM 4	100	16**	230**	
GBM 5	100	35**	170**	

PrP^C protein expression in transfected GSCs

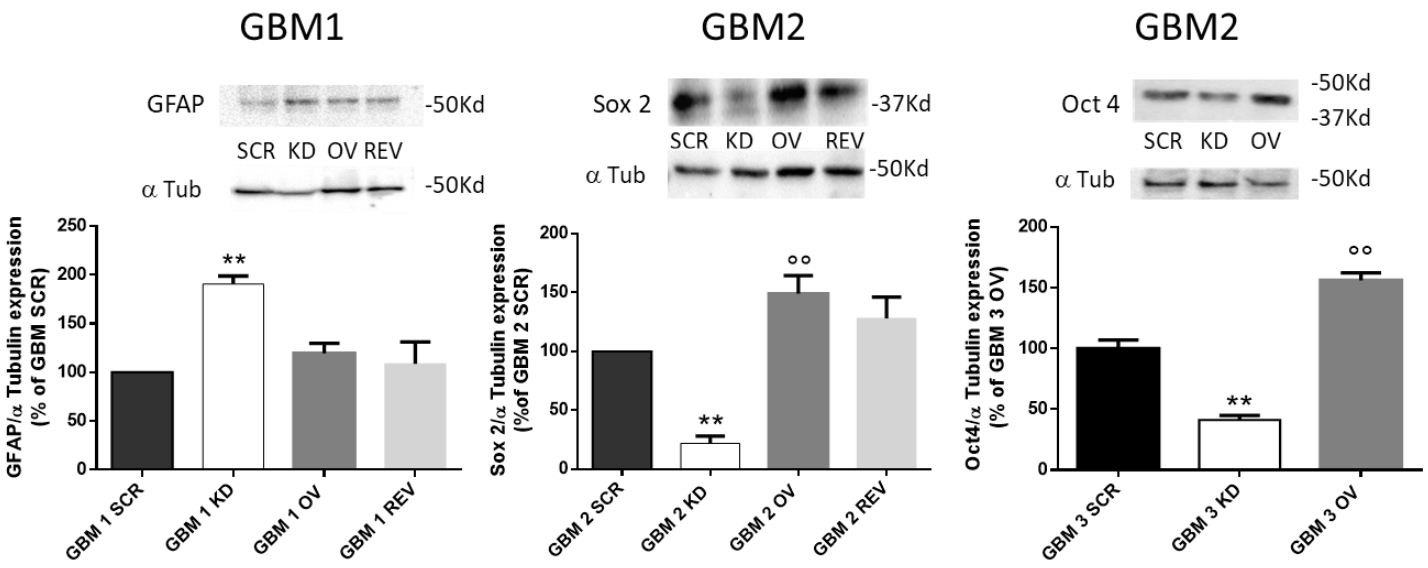
A) Immunoblots of PrP^C protein in GBM 1-5 SCR, KD, and OV.
B) Immunoblots of PrP^C protein in GBM1-2 SCR, KD, and REV.
PrP^C content was determined by 3F4 immunoreactivity by densitometric analysis, normalized to α-tubulin, and reported percentage of GBM SCR cells. Results are expressed as means ± SEM (n=3) **$p < 0.01$ vs respective GBM SCR, °° $p < 0.01$ vs respective GBM KO. (ANOVA, Tukey's post-test)
C) Summary of PrP^C levels, represented as percentage of respective GBM SCR cells. Each value is the mean ± SEM (n=3) and expressed as percentage of GBM SCR. **$p<0.01$ *vs*. respective GBM SCR cells (t-test).

Figure S3



A) Comparison of cell proliferation rate between GSC wild-type (WT) and SCR
Growth curves of GBM 1 and GBM 2 WT and respective SCR cells were obtained by MTT assay after 24, 48, and 72 hrs in culture. Each point reports the mean ± SD (n=8).
B) Quantification of Matrigel invasion by GBM 1 SCR, KD, OV, and REV cultures.
***p<0.001, *vs*. GBM SCR, OV AND REV (ANOVA, Tukey's post-test).

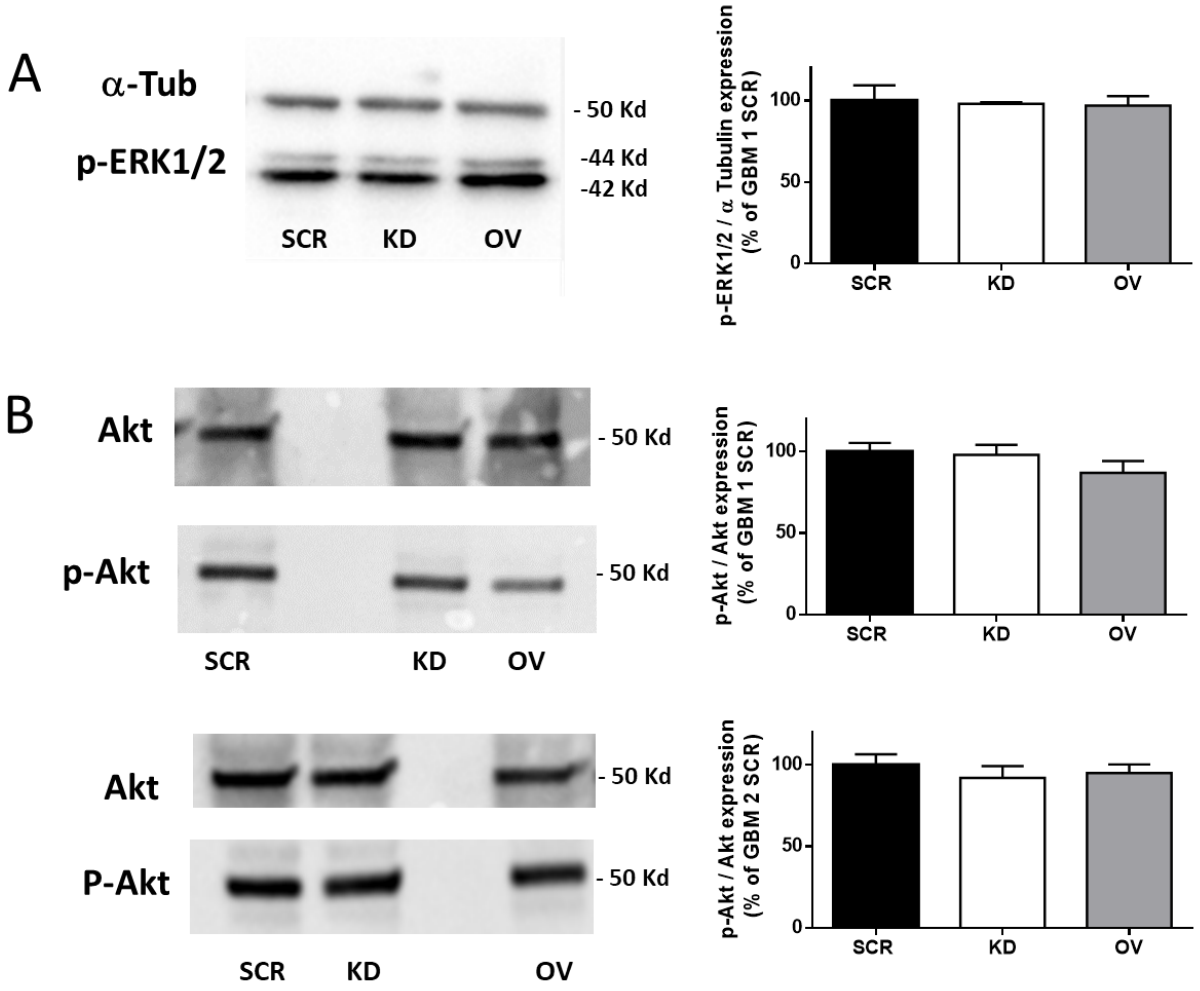
Figure S4



Expression of differentiation and stemness marker in GSCs upon modulation of PrP^C expression

Representative immunoblots of proteins GFAP, Sox 2, and Oct-4 proteins in GSCs. Quantification of protein levels by densitometric analysis, were normalized to α-tubulin, is reported as percentage of GBM SCR. Results are expressed as mean ± SEM (n=3) **p<0.01, °°p<0.01 *vs*. respective GBM SCR (ANOVA, Tukey's post-test).

Figure S5

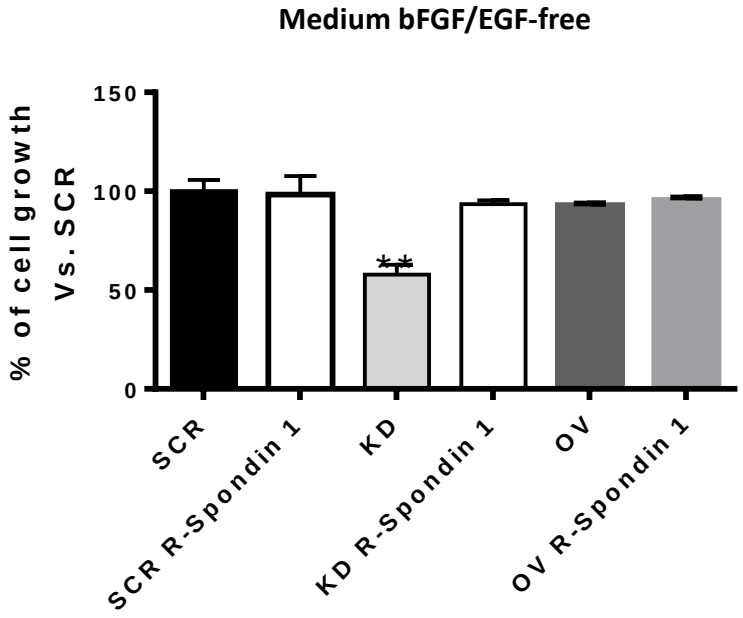


Downregulation of PrP^C does not influence amount of p-ERK1/2 or p-AKT

A) Representative immunoblot showing the amount of p-ERK1/2 protein in in GBM1 SCR, KD, and OV (left). Quantification of p-ERK1/2 amount normalized for α-Tubulin content (right). Bars represent the mean ± SD (n=3).

B) Representative immunoblot showing the content of p-AKT protein in GBM1, 2 SCR, KD, and OV (left). Quantification of p-AKT amount normalized for AKT content (right). Bars represent the mean ± SD (n=3).

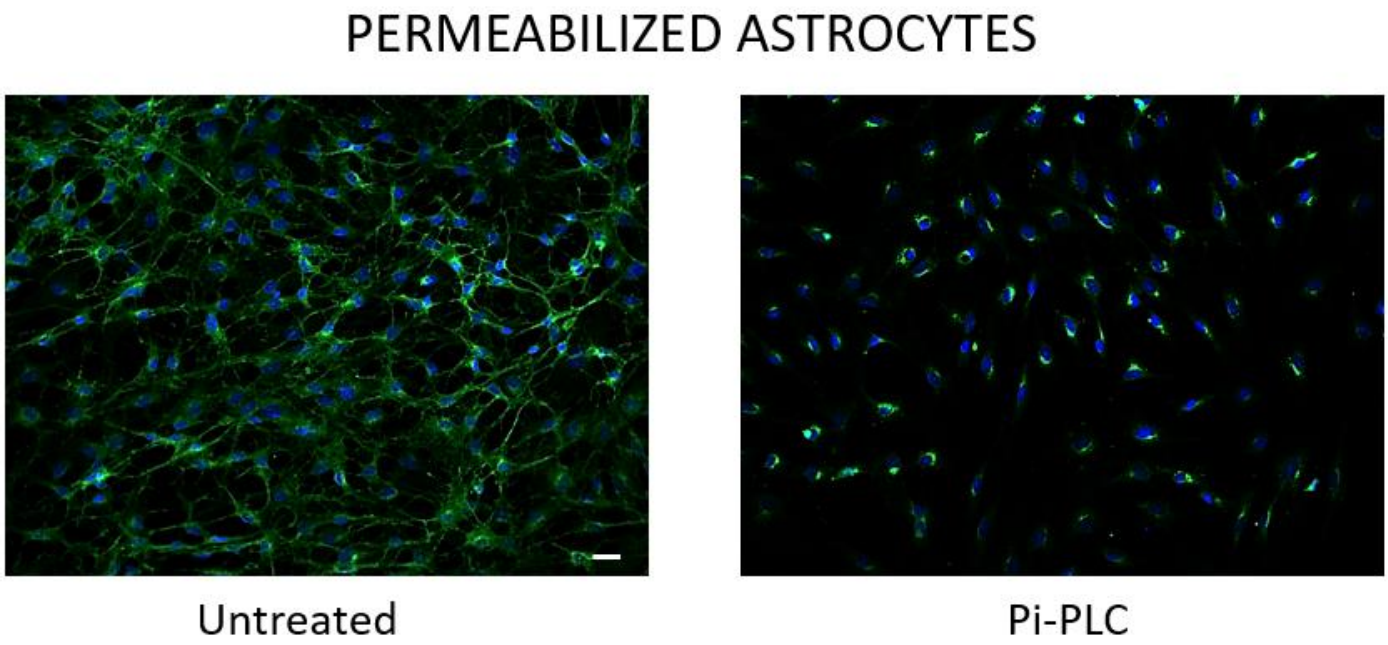
Figure S6



The absence of bFGF/EGF does not influence R-Spondin1-dependent recover of proliferation rate in GBM KD cells.

A) Cell proliferation of GBM 2 SCR, KD, and OV, after 48 hrs of treatment with R-Spondin1 (500 ng/ml), measured by MTT assay. Bars represent the mean ± SD (n=3) **p<0.01 (t-test).

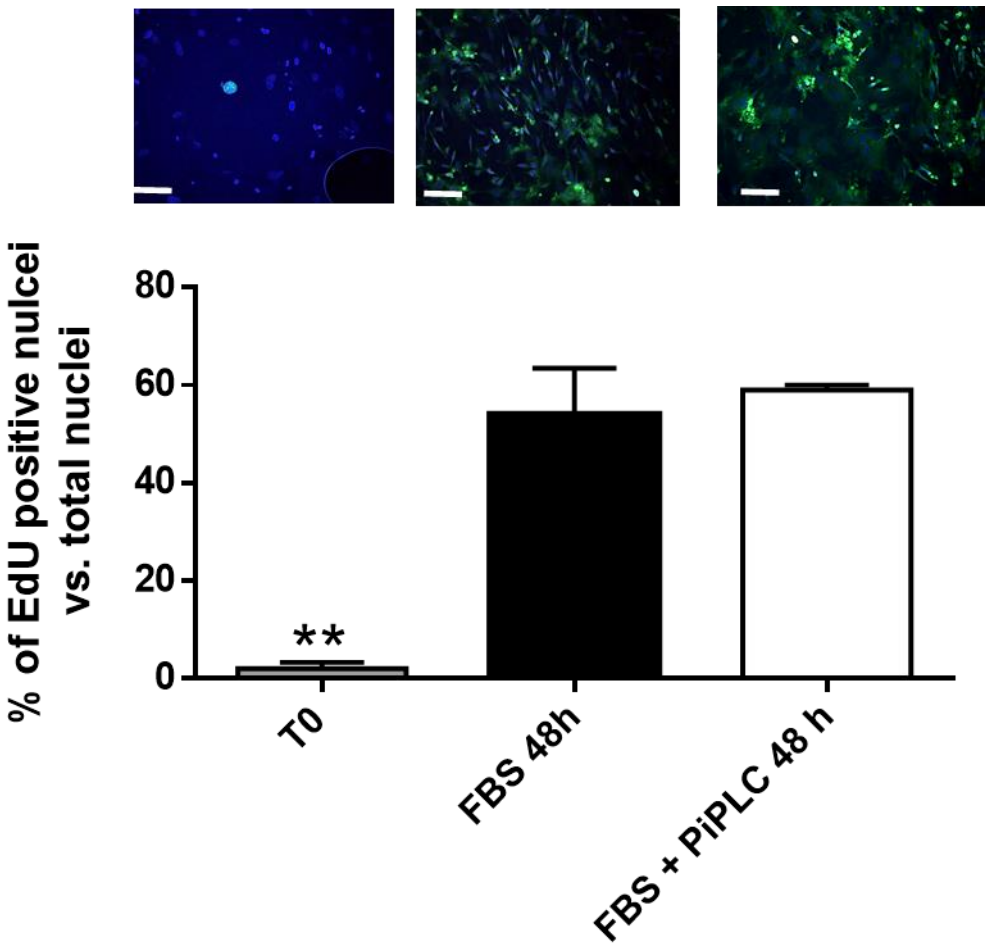
Figure S7



PrP^C expression in astrocytes

Representative images of PrP^C immunofluorescence (green) obtained with the anti-PrP^C antibody SAF 32 in astrocytes, in control conditions (untreated) and after treatment with 1U/ml Pi-PLC at 37 °C for 30 min. After fixation, astrocytes have been permeabilized to evidence intracellular PrP^C immunoreactivity. Nuclei were counterstained with DAPI (blue). Scale bar = 100µM

Figure S8



Pi-PLC treatment does not affect astrocytes proliferation

A) Representative images of proliferating astrocytes (green) in absence ore presence of Pi-PLC treatment, assessed by EdU–incorporation. Scale bar =200 μm.
B) Histograms show the percentage of labelled (proliferating) cells obtained by ImageJ analysis of 7 microscopy fields for each replica experiment (n=3). Bars represent the mean ± SD.