Supplementary material

Supplementary Table 1

Real-time PCR primer sequences and gene accession identities.

Supplementary Figure 1

Western showing Sox2 protein expression in glioma cell lines U-Cl2.6 MG, U-2987 MG, U-1242 MG, U-343 MG and U-87 MG. Sox2 expression is unchanged after stimulation with 250 μM of cGMP analog in *Prkg2*-expressing U-2987-P6 glioma cells at indicated time points.

Supplementary Figure 2

a) Western showing VASP (both phosphorylated and unphosphorylated bands), pAkt (Ser473) and pERK after 250 μM cGMP analog treatment for 4 hours of U-2987 MG, U-2987-P3 and U-2987-P6 glioma cells (*Prkg2*-transfected). Antibodies for Akt and ERK phosphorylation were incubated together. b) Filter stripped from Figure 4 showing phosphorylated PDK1 protein levels in U-2987 MG and U-2987-P6 cells as well as total VASP protein expression and phosphorylation. The phosphorylated form of VASP protein could be detected as a slowly migrating band. c) Western showing pAkt and total Akt protein levels for a U-87 MG *Prkg2* clone (U-87-P1) treated with 250 μM of cGMP analog at indicated time points.

Supplementary Figure 3

Phenotypes of U-2987 MG, U-2987-P6, U-87 MG and U-87-P1 (*Prkg2*-transfected) cells treated 4h with 10μM LY294002 or a combination of 250 μM cGMP analog and 0.5mM

IBMX. Photographed at 20x magnification (or 60x in small picture) when grown in cell culture media.

Supplementary Figure 4

a) Relative expression of *PDGFRα* of untreated glioma cell lines transfected with empty pcDNA vector as compared to corresponding *Prkg2* clones. *PDGFRα* expression in glioma cell clones transfected with empty pcDNA vector (denoted U-2987 MG, U-87 MG, U-343 MG and U-1242 MG) was set to 1 for each individual cell line (not to mistake with the absolute *PDGFRα* expression in the cell lines (not shown)). Expression was normalized to *GAPDH*. b) Chart of *SOX9* mRNA expression in U-2987 MG and U-2987-P6 cells after 4h of 250 µM cGMP analog treatment. c) Immunostaining of GFAP, TuJ1 and Sox9 in U2987 MG and U2987-P6 cells grown in serum with or without 72 h of cGMP analog treatment. Sox9 levels were apparently lower in cGMP-treated U-2987-P6 cells but any significantly different distribution patterns were not found after counting positively stained cells.

Supplementary Figure 5

a) Representative pictures of neurospheres of U-2987 MG and U-2987-P6 cells after 72 hours, 1 week and 3 weeks of culturing in Neurobasal media supplemented with EGF and FGF. b) Real-time PCR of *SOX9* expression levels in glioma cell lines cultured in 10% serum or 1 week in Neurobasal media (supplemented with EGF and FGF) as compared to total adult brain RNA. d) Distribution of cells counted positive for GFAP/Sox9 (co-stained), TuJ1 (only), TuJ1/Sox9 (co-stained), GFAP/TuJ1 (co-stained) and GFAP (only). Very few Sox9-only positive cells were found even when increasing the confocal Zeiss LSM 510 laser to maximum powers.

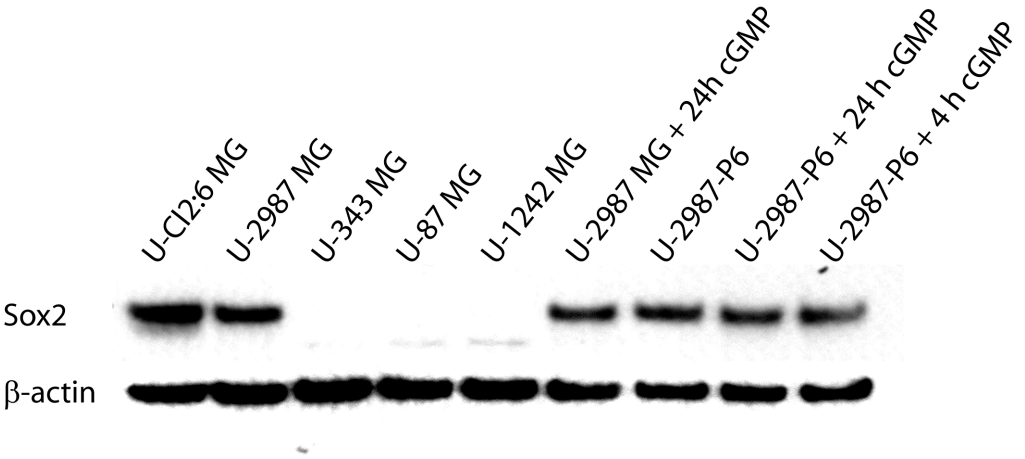
Supplementary Table 1

Table 1. Real-time PCR primers

Primer (gene)	Accession ID	Primer sequences (forward / reverse)
CD133	NM_006017	TACCAAGGACAAGGCGTTCACAGA
		GTGCAAGCTCTTCAAGGTGCTGTT
GAPDH	NM_002046	ACATCAAGAAGGTGGTGAAGCAGG
		TGTCGCTGTTGAAGTCAGAGGAGA
GFAP	NM_002055	TCTGGAGAGGAAGATTGAGTCGCT
		CATACTGCGTGCGGATCTCTTTCA
MAP2	NM_002374	TAACCAACCACTGCCAGACCTGAA
		AGCCACATTTGGATGTCACATGGC
NES	NM_006617	ACTGGAGTCTGTGGAAGTGAACCA
		TTGGTACTCTCCCTTTCCCAGGTT
PDGFRA	NM_006206	TGGTTGAAGGAACAGCCTATGG
		TGGCCGTGGGTTTTAGCAT
PLP	NM_000533	CTTCCCCAGCAAGACCTCTG
		AAAGCATTCCATGGGAGAACA
PRKG2	NM_006259	AGTAACACAGAGCACAGAAGGCCA
		CATCACGGTTCAGGTTTGCCACAT
SOX9	NM_000346	TCAACGGCTCCAGCAAGAACAAG
		ACTTGTAATCCGGGTGGTCCTTCT
TuJ1	NM_006086	ACAACGAGGCCTCTTCTCACAAGT
		TAGTGACCCTTGGCCCAGTTGTTG

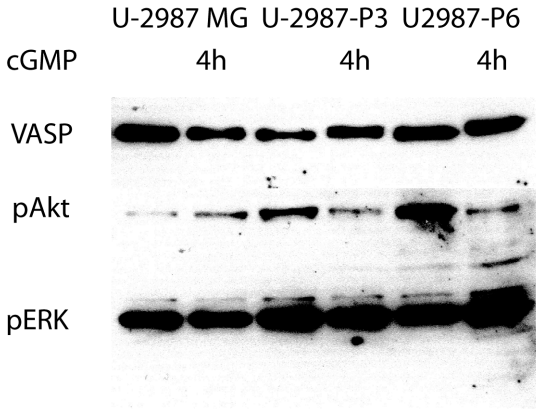
*Sequences were designed with Primer Express 1.5a software (Applied Biosystems) or by using the Integrated DNA Technology PrimerQuest (http://eu.idtdna.com/Scitools/Applications/Primerquest/). If possible, forward and reverse primers were designed aligning different exons. Absolute specificity was confirmed with National Center for Biotechnology Information (NCBI), BLAST and ENSEMBL genome browser (http://www.ensembl.org) searches.

Supplementary Figure 1



Supplementary Figure 2

a)



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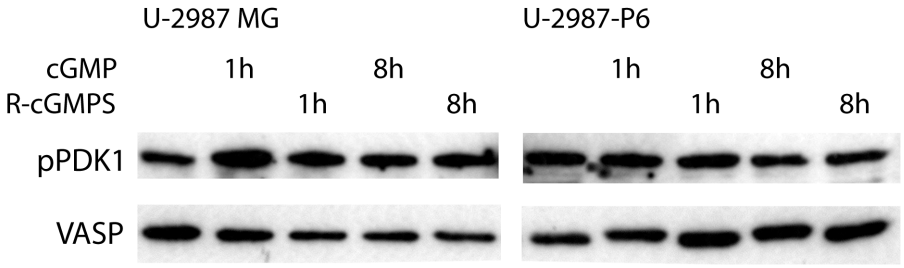

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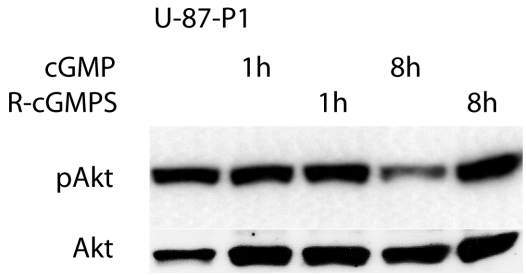
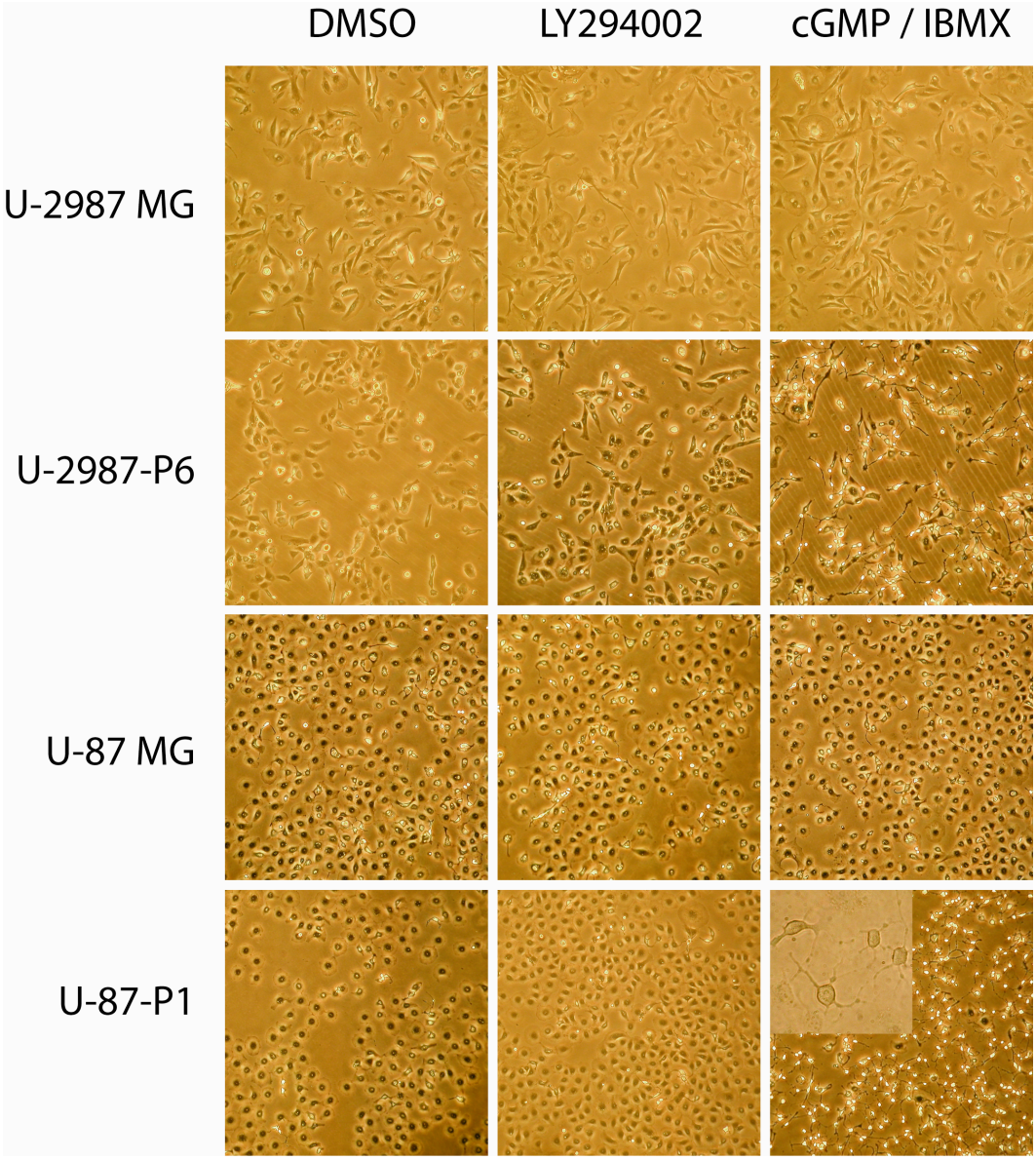
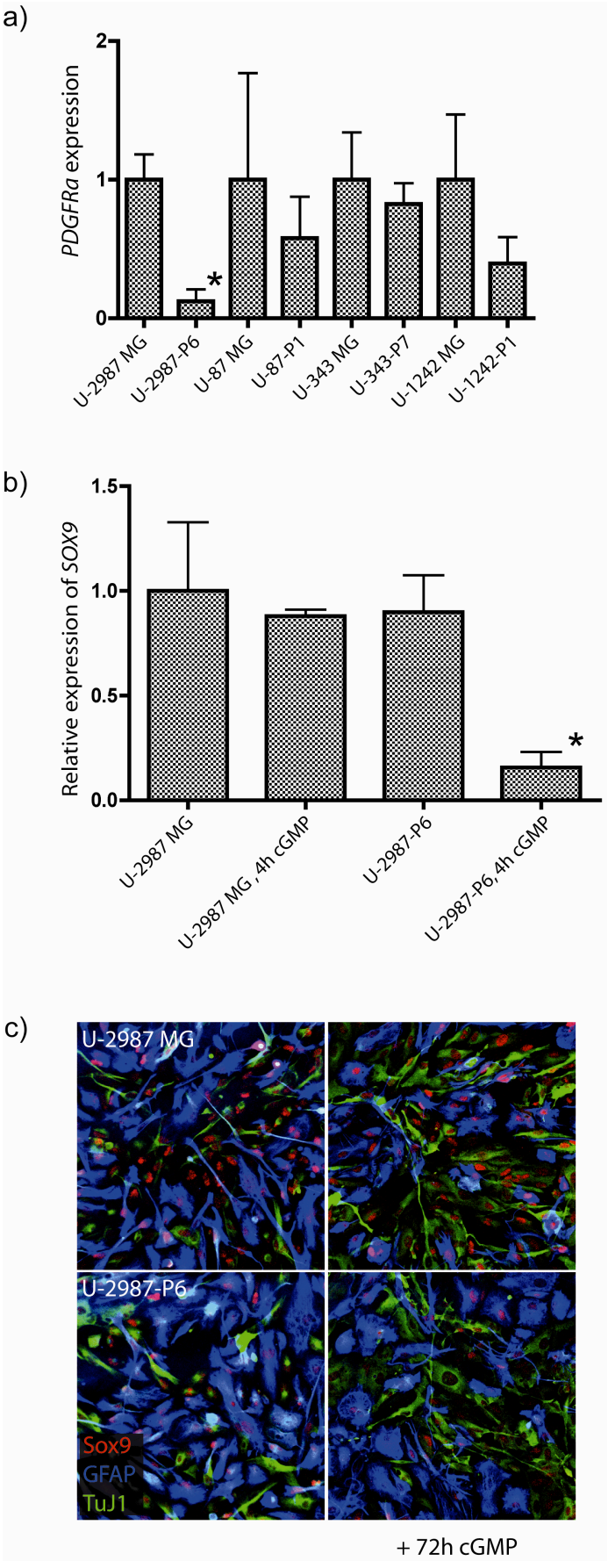


Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5