Supplementary Data

Lentiviral construction and Lentiviral-mediated overexpression of miR-129-1

A genomic fragment spanning the miR-129-1 coding region flanked by 190 and 140 base pairs from 5′ and 3′ end respectively was cloned into the *Xho* I/ *Mlu* I restriction site of a mammalian expression vector pLEX.jred downstream of the cytomegalovirus (CMV) promoter. Following PCR primers were used: miR-129-1 FW 5'-CCGCTCGAGACTCTCAGCTTTCTCTTCTC-3' and miR-129-1 RV 5'- CGACGCGTGTCACTCAGCAACTCTAAAG -3'. For control, empty vector without any cloned sequence (pLEX-Ctrl) and three different vectors with scramble sequences (pLEX-Scr) were used. Different scrambled sequences of miR-129-1 were designed using “InvivoGene”, “GenScript” and “Scramble sequence” websites and cloned in pLEX vector in shRNA formats. Scramble sequences were as follows: I) 5'-GGTCCGTGTTCGTTGTTCGCT-3', II) 5'-GGGTCGGTTTCCGCTTCTGTT-3', III) 5'-TCGTGTGTTGCCTTTCGGTCG-3'

Lentiviral particles were generated by transient calcium phosphate cotransfection of 293T cells with lentiviral vector DNA (or pLEX backbone), pPAX2 plasmid (packaging plasmid), and pMDG plasmid (containing vsv-G). Lentiviral supernatants were harvested two or three times, every 12 h and concentrated by ultracentrifuge at 47,000×g for 2 h at 4 °C. Lentivirus titer was determined by flow cytometry analysis of j.RED positive 293T cells (ABI).

To abrogated miR-129-1 overexpression, pLenti-III-miR-Off-129-1 construct containing GFP marker purchased from ABM Company and packaged into lentiviruses particles (pLenti-miR-Off-129-1 viruses) as mentioned for pLEX-jRED.

RNA extraction and RT-PCR

Total RNA was purified from cell lines or tissue specimens using Trizol (Invitrogen) according to the manufacturer's protocol and reverse transcribed to cDNA using random hexamers (for mRNA genes), stem-loop RT specific primers (for miR-129-1 and SNORD47) and M-MuLV Reverse Transcriptase (Promega).

Real-time PCR

Real-time PCR for target mRNAs was performed on ABI 157 PRISM 7500 real-time PCR System (Applied Biosystems) with 1 µl of cDNA product, 1×Quantitect SYBR Green PCR Master Mix (Takara) and 0.5 µl of each forward and reverse primer. Then, the reactions were incubated at 95° C for 3 min, followed by 40 cycles of 95° C for 30 sec and 60° C for 30 sec. QRT-PCR reactions were run in triplicate. The threshold cycle (Ct) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. The expression of mRNA targets relative to β-actin was determined using the $2^{-\Delta\Delta CT}$ method. QRT-PCR primer sequences are listed in supplemental Table S1.

Expression level of miR-129-1 was measured by universal reverse primer and specific miR-129-1 forward primer using SYBR Green PCR Master Mix (Takara). The relative expression level of miRNA was analyzed and normalized to endogenous expression of SNORD47 RNA as an internal control using the $2^{-\Delta\Delta CT}$ method. The quality of the quantification was calculated for miR-129-1 and SNORD47 RNA by analyzing different values of total RNA (1, 5, 10 and 50 ng).

The correlation coefficient R2 (0.978 and 0.983 respectively) and the efficiency of the reactions (98 and 96% respectively) were also determined.

In clinical samples, the mean of miR-129 and SNORD47 Cts was determined in normal samples and compared with obtained Cts of each patient. Based on that, patients were divided into two groups: A group that miR-129-1 gene expression level was lower than normal samples and the other that miR-129-1 gene expression level was higher than normal samples. Subsequently, relative gene expression of IGF2BP3, IGF1, and MAPK1 was determined using comparison of obtained Cts in patients with mean Cts in normal samples.

Cell proliferation curve of infected cells

A total of 5.0×10^5 cells were seeded into 10-ml plates and infected with control Lentiviruses (pLEX-Ctrl), and virus particles encoding for scramble (pLEX-Scr), miR-129-1 (pLEX-miR-129-1) and miR-Off -129-1 (pLenti-III-miR-Off-129-1).

Viable cells were collected and counted 2 - 4 days after infection by trypan blue exclusion using a ViCell counter (Beckman Coulter).

MTT, Chemosensitivity assays and Apoptosis assay

MTT assay was performed using Vybrant® MTT Cell Proliferation Assay Kit according to manufacturer's protocol (Life Technologies). Absorbance was measured at 550nm using a multi-well spectrophotometer (Bio-Tek).

For chemosensitivity assay, U251 cells were seeded in 24 well plate and transduced by miR-Off-129-1. 48 hours after transduction, normal and transduced U251 cells were treated with the LY294002 (20 μM), or PD98059 (20 μM) and incubated at 37 ℃, 5% CO2 for 48 hours. At this point, MTT assay was performed as above.

Cell apoptosis was also detected using Caspase 3 colorimetric activity assay kit (Millipore) according to manufacturer's instruction. Luminescence of each sample was measured by a micro titer plate reader device (Eppendorf,). All reactions were performed in triplicate.

Western blot

Cells were lysed using cell lysis buffer (50Mm Tris, pH= 8.0, 150mM NaCl,1% NP-40, 0.5% sodium deoxycholat, 1mM sodium fluoride, 1mM sodium orthovanadate, 1mM EDTA) then 70 μg of protein was separated electrophoretically in 12% SDS polyacrylamide gel, transferred into poly vinyli den fluoride membrane and immersed in 5% non-fat milk powder over 1h at room temperature. Upon completion of the transfer, the membranes were incubated with primary antibodies for CDK6, MAPK1, IGF2 and β-actin (Abcam). After washing, the membranes were further probed with horseradish peroxidase-conjucated secondary antibodies (1:1,000, Abcam) and developed for detection by chemiluminescence on Kodak X-film.

Construction of the 3'-UTR-luciferase plasmid and luciferase assay

The partial lengths of IGF1, IGF2BP3 and MAPK1 3′-UTR, harboring potential miR-129-1 target sites, were amplified using U87-MG genomic DNA as the template and inserted at the

XhoI and NotI sites, downstream of the luciferase gene in the pSICHECK2 vector (Promega). The sequences of the primer are given in supplemental Table S2. For luciferase analysis, HEK293 cells were seeded in 96 well plates and transiently cotransfected with each of the IGF1-3′UTR, IGF2BP3-3′UTR or MAPK1-3′UTR luciferase vectors in combination with pLEX-miR-129-1 plasmid using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Empty vector of pSICHEK2 and expression plasmid of mutated miR-129-1 (Mut-miR-129-1) containing disrupted seed region were used in each test as blank and negative controls, respectively. For construction of Mut-miR-129-1 two mutations were created in the seed region of the pre-miR-129-1 and mutated stem-loop structure was cloned in pGFP-V-RS vector. Luciferase activity was measured 48 hr after transfection using the Dual Luciferase Reporter Assay System (Promega) and the Renilla luciferase signal normalized to the Firefly luciferase signal activity for control of transfection efficiency. All experiments were performed in triplicate.

RNA interference

To knockdown endogenous IGF2BP3 and MAPK1 the following target sequences were constructed in the small hairpin RNA (shRNA) vector pGFP-V-RS: shIGF2BP3-1: 5′-GCCTCATTCTTATTTCAAGAT-3′, shIGF2BP3-2: CGGTGAATGAACTTCAGAATT; shMAPK1-1: 5′-GGACCTCATGGAAACAGATCT-3′ and shMAPK1-2: GCTGCATTCTGGCAGAAATGC. An empty shRNA vector (shCtrl) was used as a negative control. U251 cells were transfected with shIGF2BP3, shMAPK1 and shCtrl using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. The transfection

efficiency was assessed by calculating the percentage of fluorescein-labeled cells using fluorescence microscopy. The transfection efficiency was above 80%. For construction of Mut-miR-129-1 as negative control in luciferase assays, two mutations were created in the seed region of the pre-miR-129-1 and mutated stem-loop structure was cloned in pGFP-V-RS vector.

Table S1. Primers for qPCR of genes and miRNAs

Genes	Primer sequences
β-actin	FW, 5′-CTTCCTTCCTGGGCATG -3′; RW, 5′-GTCTTTGCGGATGTCCAC-3′
IGF1	FW, 5′-TCCTCCTCGCATCTCTTCTAC -3′; RW, 5′-CAATACATCTCCAGCCTCCTTAG-3′
IGF2BP3	FW, 5′-CTT CCT GGT GAA GAC TGG C -3′; RW, 5′-CCG AGT GCT CAA CTT CTA TG-3′
MAPK1	FW, 5′-CATGGTGTGCTCTGCTTATG -3′; RW, 5′-CTAGGTCTGGTGCTCAAAGG-3′
CDK6	FW, 5′-GCGACTTGAAGAACGGAG-3′; RW, 5′-ATCAAACAACCTGACCACG-3′

HDAC2	FW, 5′-AACCGACAACAGACTGATATGG-3′; RW, 5′-TTAGTAATTCAAGGATGGCAAGC-3′
MiR-129-1	FW, 5′-ATCTTTTTGCGGTCTGG -3′; RW, 5′-GAGCAGGGTCCGAGGT-3′
SNORD47	FW, 5′-ATCACTGTAAAACCGTTCCA-3′; RW, 5′-GAGCAGGGTCCGAGGT -3′

Table S2. Primers for 3′UTR cloning

Genes	**Primer sequences**
IGF1	FW, 5′-GCGGCTCGAGTTGCTCTGCACGAGTTACCTG-3′; RW, 5′-AAGCGGCCGCGATCCTTGAGGTGACCCAGTG-3
IGF2BP3	FW, 5′-GCGGCTCGAGACCACAGAGGCAGATGC -3′; RW, 5′-AAGCGGCCGCCCAGAAAGCCAGTCCATGA-3
MAPK1	FW, 5′-GCGGCTCGAGGTGGAAATACCTTGCTGATGT-3′; RW, 5′-TTGCGGCCGCCTATACATGCACGGTGCTGTGT-3

Table S3. Cell Cycle associated genes predicted by PANTHER. MiR-129-1 predicted target genes that further analyzed in current study are highlighted with yellow color.
ETV1, CELF2, KIF26A, MAST4, PABPC1, SMC1A, MAP3K13, RBMS2, GSK3B, NSFL1C, MAPK1, TTC28, SUN2, MAPRE1, CELF4, UBE2R2, RPS6KB1, MAK, PTP4A1, PAPD5, PDS5A, FOXP2, CDKN1C, CDK6, ETS1, HNRNPA1, CKAP4, FOXP4, DNAJC13, ETV6, JDP2, RBMS3, LPP, SCML4, A1CF, CELF1, RBMS1, KAT6B, IGF2BP3, ELK4, ELAVL4, NFASC, E2F7, YWHAB, HNRNPA3, PDIK1L, UBE2O, CDK5R1, NR2C2, CALN1, ZFP36L1, TSPYL4, ELAVL3, HDAC2, PCBP2, CALM1, L1CAM, C9orf69, ETV5, RBM14, IGF1, KRAS, E2F3, PIK3R1, TGFB2, PRKCB, FZD6, TCF7, PTEN, DAPK2, SHC4, CALML6.

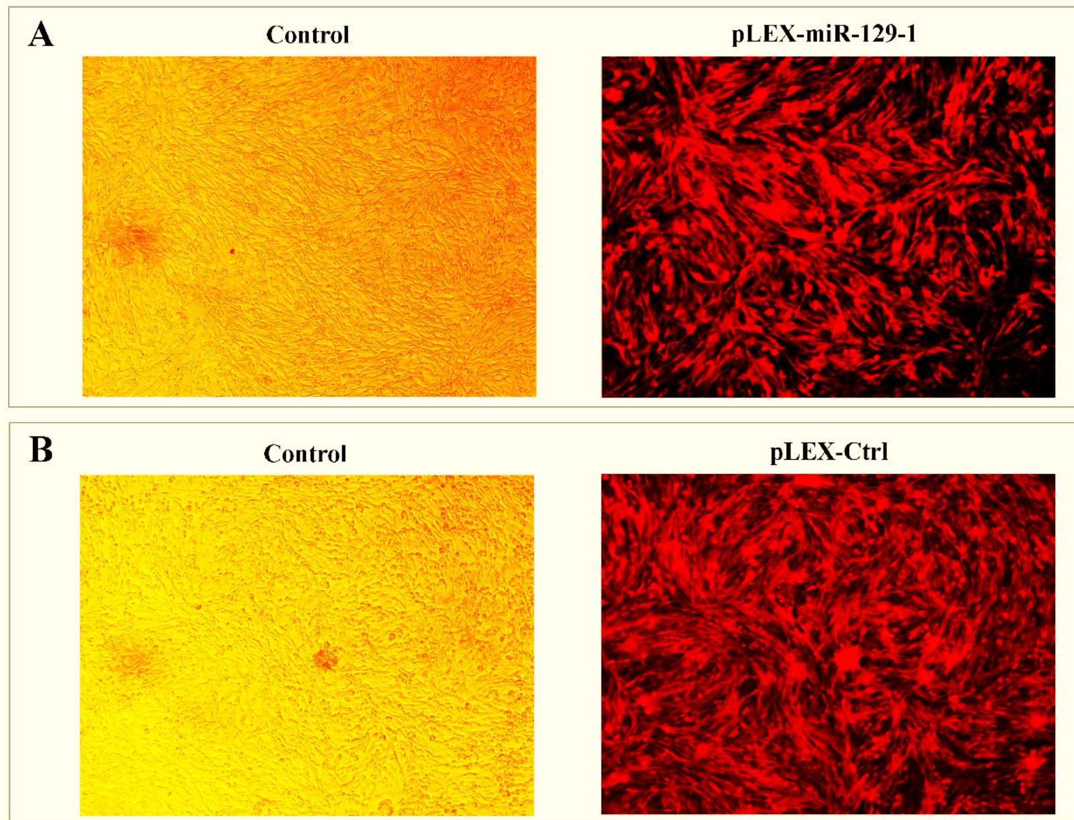


Figure S1. U87-MG cells were successfully transduced by pELX-miR-129 (upper row) or pLEX-Ctrl viruses (bottom row). Transduction efficiency was above 70% in each experiment.

Fig. S2

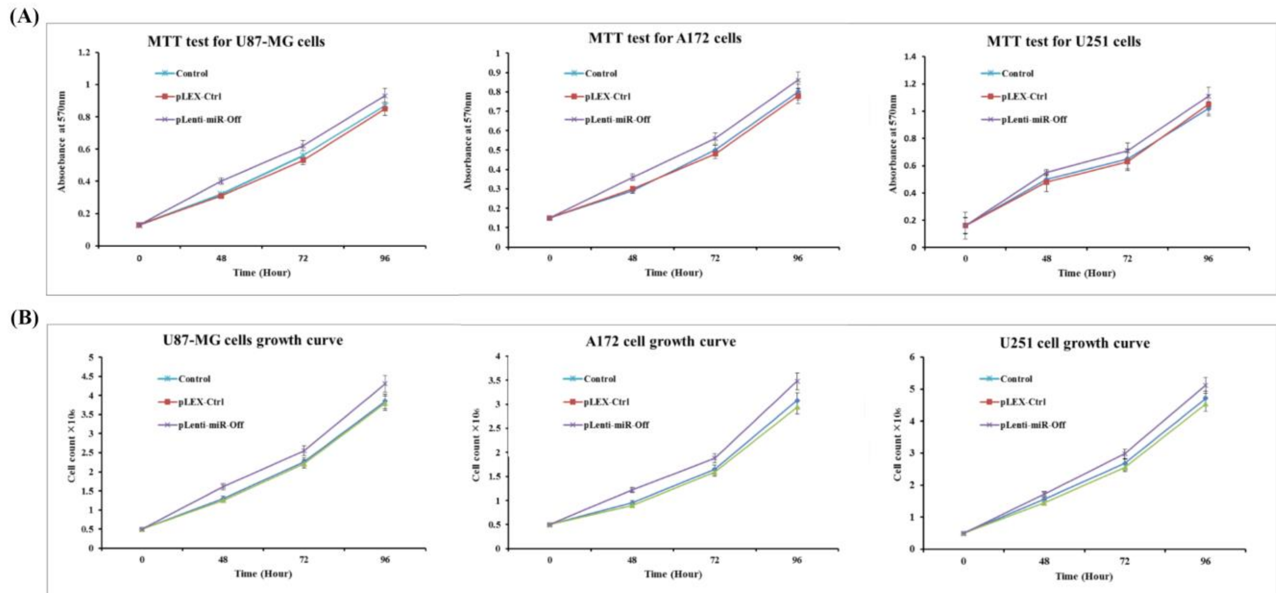
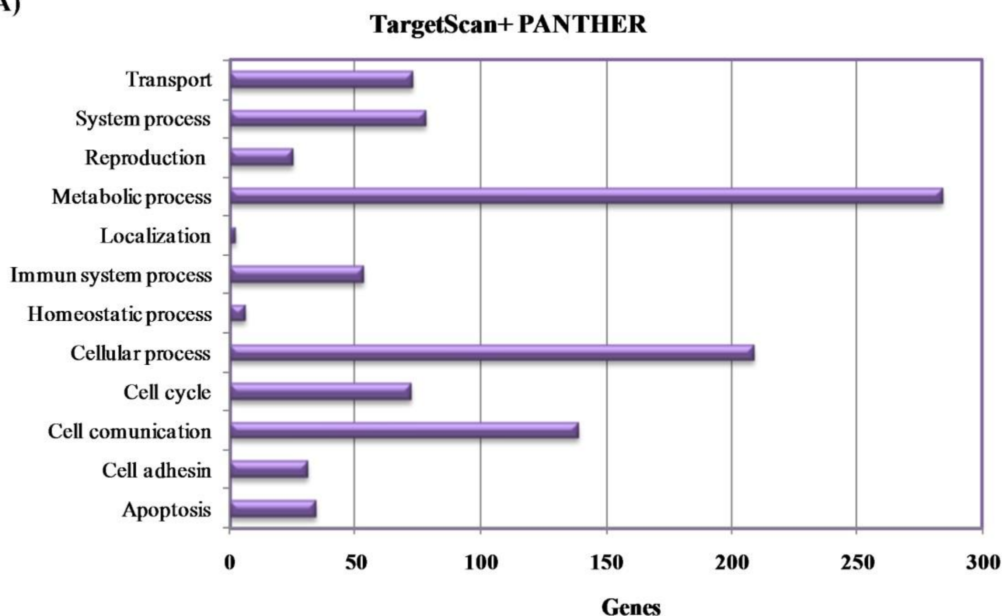


Figure S2. MiR-129-1 down-regulation via utilizing antimiR led to an increase in proliferation of GBM cell lines. (A) Measurement of cell absorbance by MTT assay after transduction with pLenti-miR-Off-129-1. Impacts of miR-129-1 down-regulation on cell proliferation were measured up to 96 h after transduction. Each time point was expressed as total absorbance at 570 nm after background subtraction (Y axis). Points, mean of three experiments; bars, SD. (B) Cell growth curves for U87-MG, A172 and U251 cells transdused with pLenti-miR-Off-129-1 and their respective controls. Transduction of pLenti-miR-Off-129-1 slightly increased cell proliferation, as compared with the cells transduced with pLEX-Ctrl or pLEX-Scr. Points, mean of three experiments; bars, SD.

Fig S3

(A)



(B)

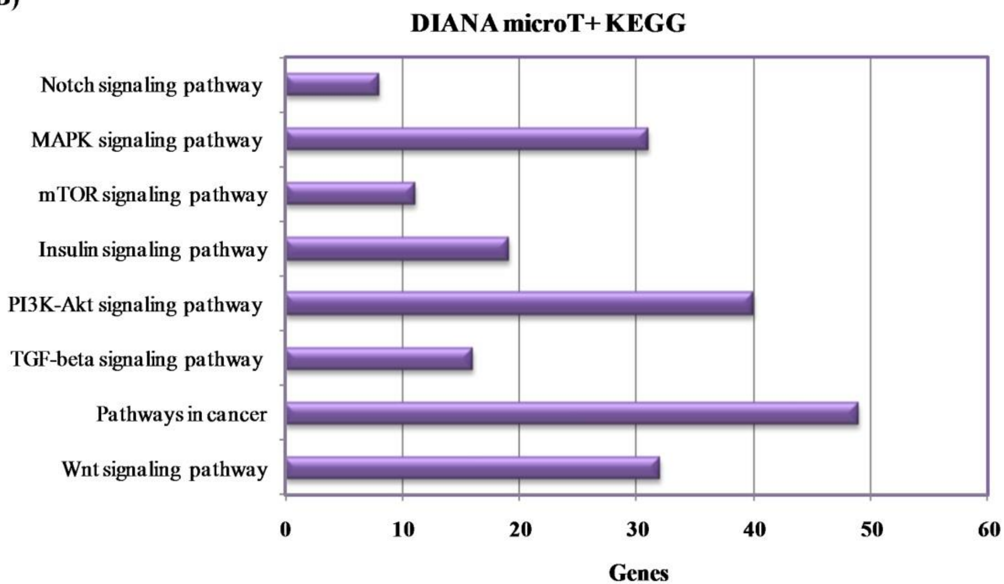


Figure S3. MiR-129-1 predicted target genes in biological processes. (A) PANTHER gene classification program was used to analyze biological process of TargetScan-predicted conserved targets for miR-129-

1. (B) DIANA-microT program was used to generate a stringent list of miR-129-1 targets. Items shown are KEGG categories associated with cancer.