Supporting Information

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SI Materials and Methods

Samples and Cell Lines. All human normal brain tissue and glioma samples were obtained from the Department of Neurosurgery, Beijing Tiantan Hospital. All human materials were used in accordance with the policies of the institutional review board at Beijing Tiantan Hospital. The four human glioma cell lines used here (U87MG, T98G, A172, and U251) were purchased from American Type Culture Collection. The human cervical carcinoma cell line HeLa was obtained from the China Center for Type Culture Collection (Wuhan, China). The human normal glial cell line (HEB) was kindly provided by Guangmei Yan (Department of Pharmacology, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, China).

HeLa, A172 and U251 cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% (vol/vol) fetal bovine serum (FBS), 5 mM L-glutamine, and 100 U/mL penicillin and 100 mg/mL streptomycin. T98G and U87MG were maintained in Modified Eagle Medium supplemented with 1 mM sodium pyruvate and 1% (vol/vol) NEAA, besides the normal FBS, L-glutamine and antibiotics. HEB was maintained in Dulbecco's Modified Eagle Medium with 1% (vol/vol) NEAA and the normal FBS, L-glutamine and antibiotics.

DNA Constructs and siRNA. To construct the human CREB expression vector, the ORF of *CREB* (CREB1, NM_004379) from T98G cells was amplified. For the luciferase reporter assay, the promoter of the *mir-23a-27a-24-2* gene cluster and the 3′ UTRs of FOXO3a (2 kb), FOXO4 (1.4 kb), PTEN-half1 (1.5 kb), and PTEN-half2 (1.5 kb) were amplified from the genomic DNA of T98G cells. The PCR products were then cloned into the firefly luciferase reporter vector (PGL3-basic or pcDNA3.1-Luc) as described (1). The double-stranded siRNA targeting human *CREB* (target sequence: 5′-CAA TAC AGC TGG CTA ACA AT-3′) were purchased from GenePharma. The shCREB vectors and adenoviral vectors were purchased from Genechem. The target sequence of shRNA for CREB was 5′-GAG AGA GGT CCG TCT AAT G-3′. The target sequences of the adenoviral-delivered shcreb were as follows: AD-shcreb (5′-GAG AGA GGT CCG TCT AAT G-3′); AD-shcreb#2 (5′-CAA TAC AGC TGG CTA ACA AT-3′).

Luciferase Reporter Assay. To study whether CREB can affect the transcriptional activity of the upstream regulatory sequences, T98G cells were transfected with 0.65 μg total amount of DNA containing 100 ng of the reporter vector (pGL3), 50 ng of phRL (Renilla Luciferase, for normalization) and 500 ng of the CREB expression construct, or 500 ng of the GFP control. To test the interactions between the 3′ UTR of TSGs and mir-23a in T98G cells, 100 ng of each of the TSG 3′ UTR-LUC was cotransfected with 50 ng of phRL and 50 nM of synthetic mir-23a mimics (mir-23a)/mir-23a antagomirs (anti-23a) or control miRNA mimics (mir-NC)/control antagomirs (anti-NC). Forty-eight hours later, lysates of HeLa or T98G cells from all treatment groups were collected by using Passive Lysis Buffer (Promega). Firefly luciferase activity was analyzed relative to Renilla luciferase activity in the same sample by using Dual-Luciferase Reporter Assay System (Promega). Luminescence was measured by using the GloMax-Multi Detection System (Promega). Three independent experiments were performed and assayed in quadruplicate per group.

Growth, Survival, Colony Formation, FACS and TUNEL assay. Cell growth was assayed by using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) standard method. Twenty-four hours after transfection, the cells were maintained in serum-free medium, and the surviving cell number was determined by using the MTT method. For colony formation assays, transfected or infected cells were plated in 12-well plates at 200 cells per well. Ten to 14 days later, the cell colonies were stained and counted. For cell cycle analysis, the transfected cells were harvested and assessed for cell cycle by flow cytometry as described (2). The TUNEL assay was performed by using the In Situ Cell Death Detection Kit, POD (Roche) according to the manufacturer's protocol.

Anchorage-independent Transformation Assay. Anchorage-independent transformation assays were performed by using the CytoSelect 96-Well Cell Transformation Assay Kit (Cell Biolabs). Briefly, the cells were plated in soft agar in a 96-well plate at 2,000 cells per well. The culture medium was changed every 3 d, and the cells were in culture for 7 d. To measure anchorage-independent growth, the agar layers were dissolved and lysed. Ten microliters of the lysed solutions of each well was mixed with CyQuant, and the fluorescence was read.

Quantitative PCR and Western Blotting. Quantitative real-time PCR was used to confirm the miRNA microarray data and to measure the CREB mRNA level in cell lines, as well as to determine the expression level and gene copy number of *CREB*. Stem-loop RT-PCR for mature miRNAs was performed as described (3).

MiRNA Microarray and ChIP & ChIP-chip. T98G cells were transected with siRNA for CREB or control siRNA and cultured for 48 h. Then, the cells were harvested for RNA extraction. The quality-controlled RNA samples were used for the TaqMan MicroRNA Assay (ABI 7900HT+TLDA low density chip). For the ChIP-chip assay, approximately 1×10^7 cells harvested in medium were fixed with 1% formaldehyde. Next, a glycine solution was added to the tube to quench the unreacted formaldehyde at a final concentration of 0.125 M. The fixed cells were then collected by spinning at 700 g for 5 min. Cells for chIP-chip assay were pelleted and were sent to the KangCheng Bio-tech lnc (Shanghai, China). We used the EZ-ChIPTM Chromatin Immunoprecipitation Kit (Millipore, catalog #17-371) according to the manufacturer's protocol. The ChIP products were used for quantitative real-time PCR.

Murine Tumor Model. Each one of the four-week-old BALB/C-nu mice was injected in the right flank with AD-shcreb-infected and in the left flank with AD-shNC-infected U251 or U87MG cells (2×10^6 cells in 100 μl physiological saline). The tumors were measured weekly or every 5 days thereafter. Tumor volume (V) was estimated using a caliper by measuring the length (L) and width (W), where $V = (L \times W^2)/2$.

Antibodies and Primers. The antibodies we used were anti-CREB (CST, #9197), anti-pCREB (CST, #9198), anti-PTEN (CST, #9552), anti-FOXO3a (Millipore, #07-702) and FOXO4 (CST, #9472). The primers used for cloning the 3′UTRs of TSGs, for detecting the miRNAs expression and for ChIP-QPCR are listed in Table S3.

Statistical Analysis. Data are expressed as mean ± SD. Student's t test was used for analysis, and P values <0.05 were considered significant.

Computational Prediction. Three target prediction databases (TargetScan, PicTar, and MiRDB) were used to analyze the

interaction between the oncogenic miRNAs and the TSGs. The transcription factor binding sites in the 2-kb upstream regulatory sequence of *mir-23a* were predicted with TFSEARCH (www.cbrc.jp/research/db/TFSEARCH.html) and the conservation track of the promoter region was obtained from the UCSC Genome browser (http://genome.ucsc.edu/).

1. Zhang Y, et al. (2009) MicroRNA-128 inhibits glioma cells proliferation by targeting transcription factor E2F3a. *J Mol Med (Berl)* 87:43–51.
2. Chao TF, et al. (2008) [MiR-9 regulates the expression of CBX7 in human glioma]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 30:268–274.
3. Chen C, et al. (2005) Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res* 33:e179.

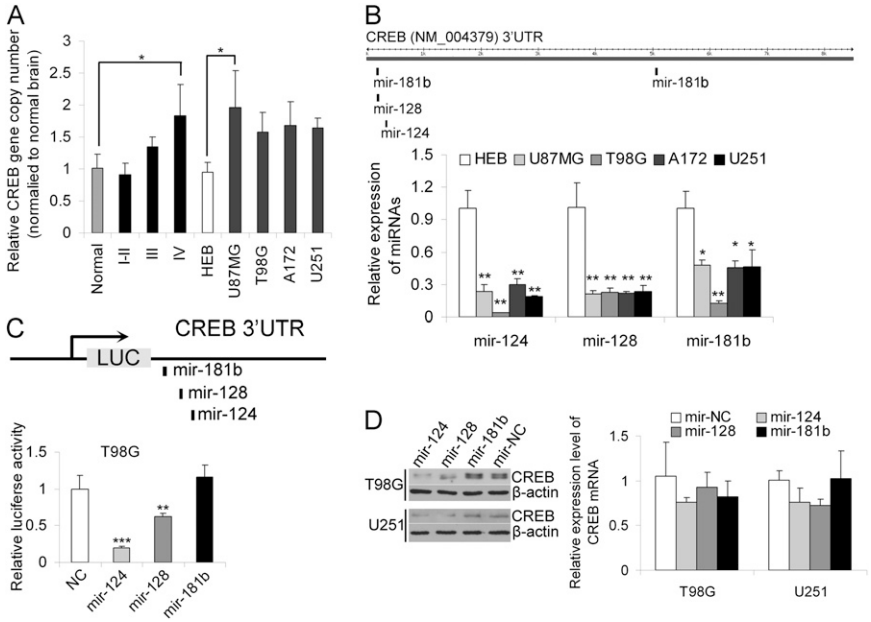

Fig. S1. The down-regulations of mir-124 and mir-128 contribute to the high CREB protein level in glioma cells. (*A*) The whole genome DNA was extracted from a normal brain tissue and three glioma tissues; HEB cell line and the four glioma cell lines; and the gene copy number of *CREB* was analyzed by quantitative real-time PCR. The data are expressed as the mean ± SD, $*P < 0.05$, two-tailed unpaired Student *t* test, relative to normal brain tissue or HEB. (*B*) The frequently down-regulated miRNAs (mir-124, mir-128, and mir-181b) in gliomas are predicted to target the 3′ UTR of *CREB* by using the Targetscan and PicTar algorithms. The short black bars indicate the miRNA binding sites in the *CREB* 3′ UTR. (*C*) The 1-kb *CREB* 3′ UTR containing the binding sites of mir-124, mir-128, and mir-181b was inserted downstream of the luciferase cassette. The luciferase reporter constructs were cotransfected with synthetic miRNA mimics (mir-124, mir-128, and mir-181b) or control mimics (mir-NC) in T98G cells, and the normalized luciferase activity was determined (mean ± SD, $n = 4$). $**P < 0.01$, $***P < 0.001$, two-tailed unpaired Student's *t* test, relative to control. (*D*) The T98G and U251 cells were transfected with synthetic miRNA mimics or control, and the *CREB* protein and mRNA were detected by Western blotting (*Left*) and quantitative RT-PCR (*Right*; mean ± SD, $n = 3$), respectively.

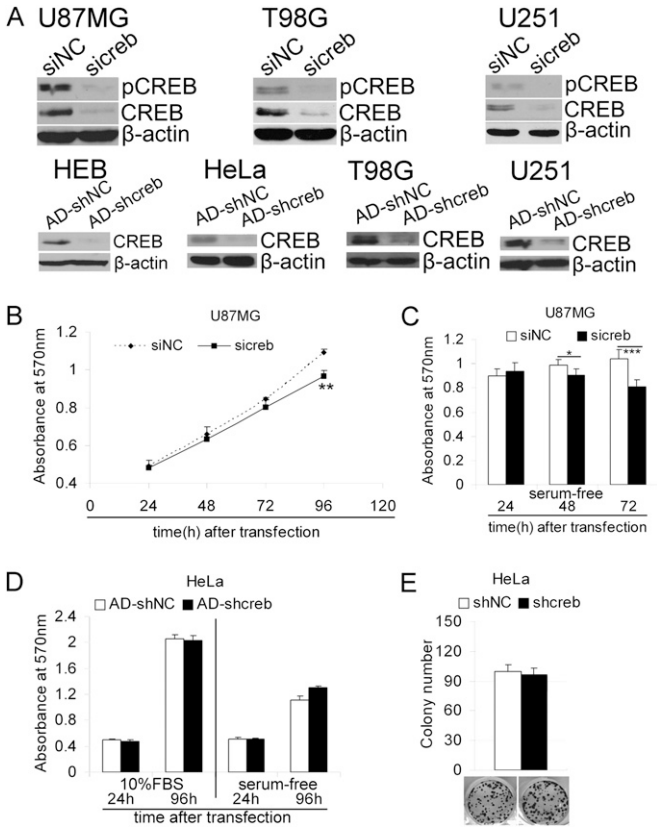

Fig. S2. (*A*) Western blotting was used to validate the CREB knockdown effect by using specific siRNA or adenovirus-delivered shRNA in glioma cell lines (U87MG, T98G, and U251), HEB, and HeLa cells. (*B* and *C*) Knocking down CREB with sicreb inhibits the growth (*B*) and survival (*C*) of U87MG cells. The data are expressed as the mean ± SD, $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, two-tailed unpaired Student's *t* test, relative to control. (*D* and *E*) CREB knockdown with AD-shcreb has no effect on the growth, survival (mean ± SD, $n = 5$) (*D*) and colony formation (mean ± SD, $n = 4$) (*E*) of HeLa cells.

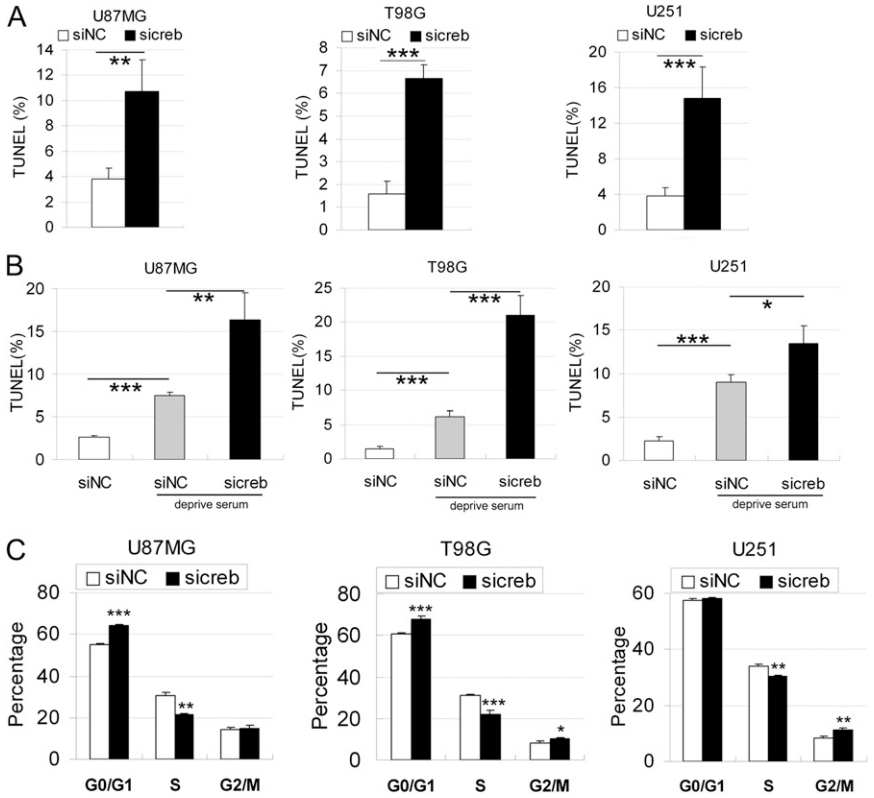

Fig. S3. Knocking down CREB induces apoptosis and cell cycle retardance of glioma cells. (*A*) Apoptosis cells were detected by TUNEL assays after transfected with sicreb or control siRNA for 72 h. The data are expressed as the mean ± SD, $n = 4$. (*B*) Forty-eight hours after transfected with sicreb or control siRNA, cells were serum-starved for another 24 h and then the TUNEL assay was performed to detect the apoptosis cells. The data are expressed as the mean ± SD, $n = 4$. (*C*) Glioma cells were transfected with sicreb or control siRNA, 48 h later, the cell-cycle profile was determined by FACS assays. The data are expressed as the mean ± SD, $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, two-tailed unpaired Student's *t* test, relative to control.

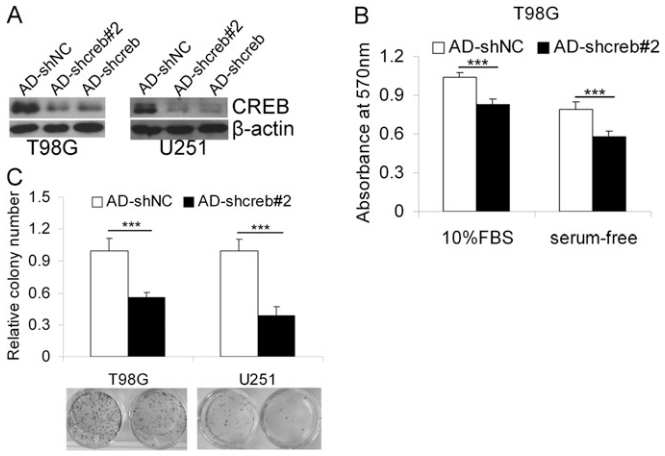

Fig. S4. Knocking down CREB with AD-shcreb#2 inhibits the growth, survival, and colony formation of T98G and U251 cells. (*A*) T98G and U251 cells were infected with AD-shcreb and AD-shcreb#2, and then the *CREB* protein levels were detected by Western blotting. (*B*) AD-shcreb#2 infected T98G cells were subjected to MTT and survival assays. Ninety-six hours after infection, the T98G cells' growth and survival were determined by absorbance measurement (mean ± SD, $n = 5$). $^{***}P < 0.001$, two-tailed unpaired Student *t* test. (*C*) AD-shcreb#2-infected T98G and U251 cells were subjected to colony formation assays. The cell colonies were counted and plotted (mean ± SD, $n = 4$). $^{***}P < 0.001$, two-tailed unpaired Student's *t* test.

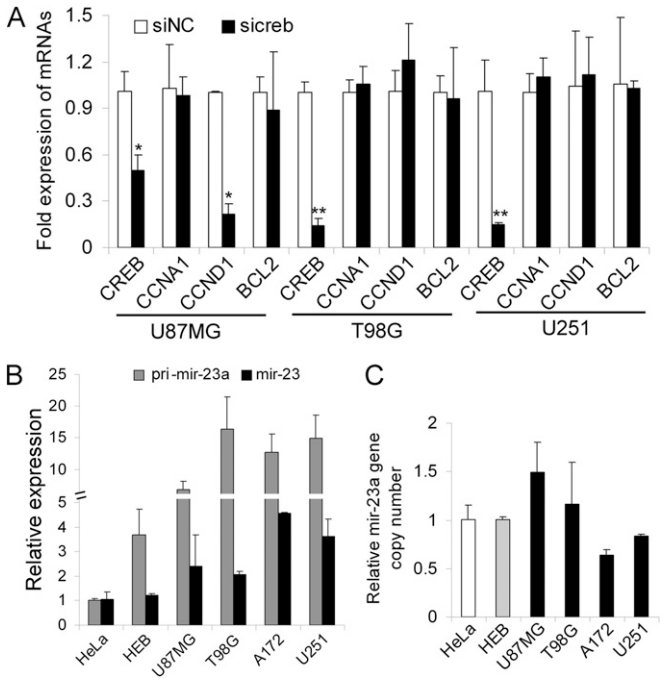

Fig. S5. (*A*) CCNA1, CCND1, and BCL2 are not actual targets of CREB in glioma cells. The glioma cells (U87MG, T98G, and U251) were transfected with sicreb or siNC. Forty-eight hours later, cells were harvested for total RNA extraction, and the mRNAs of these acknowledged CREB target genes as well as CREB were determined by quantitative RT-PCR. The data are expressed as the mean ± SD, $n = 3$. *$P < 0.05$, **$P < 0.01$, two-tailed unpaired Student's t test, relative to control. (*B* and *C*) The pri-mir-23a and mir-23a levels as well as the gene copy number of mir-23a were detected by quantitative real-time PCR in HeLa cells, HEB cells, and the four glioma cell lines (U87MG, T98G, A172, and U251).

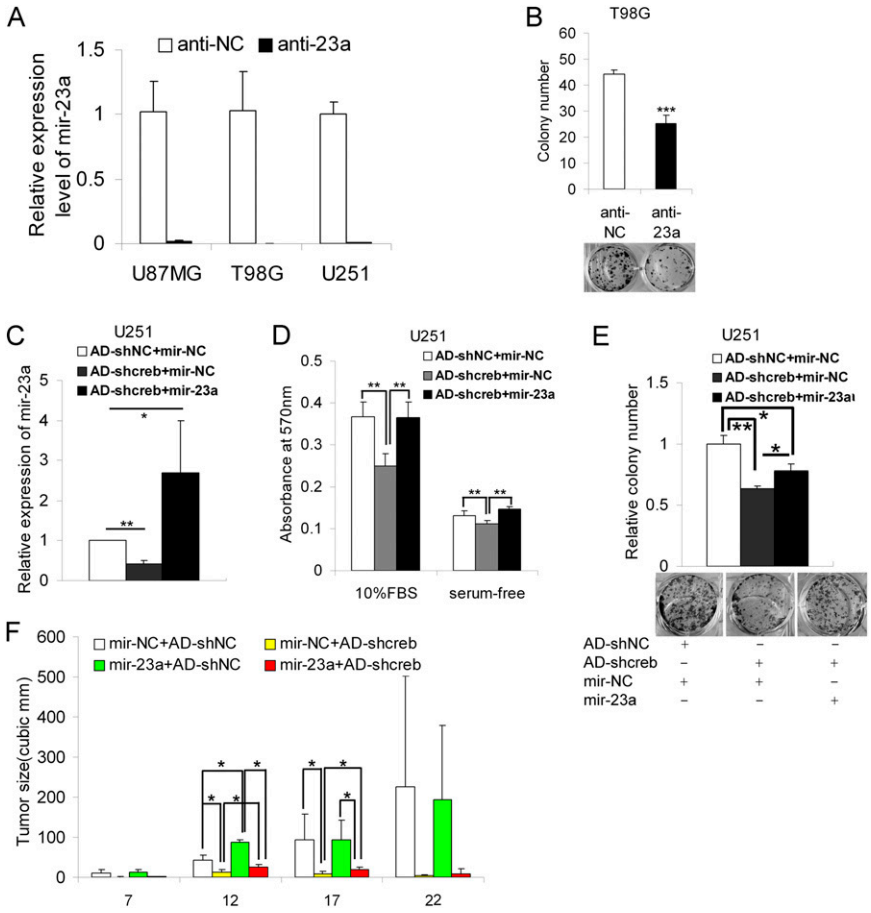

Fig. S6. mir-23a is a functional downstream target of CREB. (*A*) Knocking down mir-23a with specific miRNA antagomirs in U87MG, T98G, and U251 cells were verified by quantitative RT-PCR (mean ± SD, $n = 3$). (*B*) mir-23a knockdown significantly inhibited the colony formation ability of T98G cells (mean ± SD, $n = 4$). $^{***}P < 0.001$, two-tailed unpaired Student's *t* test, relative to control. (*C–E*) mir-23a partly rescued the effect of CREB knockdown in U251 cells. U251 cells were transfected with mir-23a mimics or control miRNA mimics after infection with AD-shcreb or AD-shNC. The expression of mir-23a was measured by quantitative RT-PCR (*C*). The transfected/infected cells were subjected to MTT assays (*D*) and colony formation assays (*E*). Colony numbers were normalized to control (mir-NC+AD-shNC). The data are expressed as the mean ± SD, $n = 4$. $^{**}P < 0.01$, $^{***}P < 0.01$, two-tailed unpaired Student's *t* test. A representative photograph of cell colonies was shown on the bottom. (*F*) Nude mice were injected s.c. with U251 cells that were transfected with synthetic mir-23a mimics or control miRNA mimics following infection with AD-shcreb or AD-shNC. The tumor volume was measured 1 wk later and was then measured every 5 d. The data are expressed as the mean ± SD $n = 5$. $^{*}P < 0.05$, two-tailed unpaired Student's *t* test.

Table S1. The up-regulated miRNAs in glioma in microarray assays

miRNAs up-regulated in glioma	Ciafre et al. (1)	Godlewski et al. (2)	Silber et al. (3)	Gal et al. (4)	Huse et al. (5)	Sasayama et al. (6)	Malzkorn et al. (7)	Jiang et al. (8)	Rao et al. (9)	Up-regulated in glioma cell lines	
										Zhou et al. (10)	Ciafre et al. (1)
mir-21	+	+	+	—	+	+	+	—	+	+	—
mir-10b	+	+	+	—	+	+	—	+	—	—	—
mir-25	+	—	—	—	+	—	+	+	+	—	—
mir-106b	—	—	—	—	+	+	—	—	+	+	—
mir-23a	—	—	—	—	+	—	—	—	+	+	+
mir-486	—	+	—	+	+	—	—	+	—	—	—
mir-16	—	—	—	+	—	—	+	—	+	—	—
mir-9/9*	+	—	—	—	+	—	+	—	—	—	—
mir-221/222	+	—	—	—	—	—	—	—	—	+	+
mir-451	—	—	—	+	+	—	—	+	—	—	—
mir-15b	—	—	—	—	+	—	—	—	—	+	—
mir-26a	—	+	—	—	+	—	—	—	—	—	—
mir-92b	—	—	—	—	+	+	—	—	—	—	—
mir-93	—	—	—	—	+	—	—	—	+	—	—
mir-182	—	—	—	—	+	—	—	+	—	—	—
mir-183	—	—	—	—	+	+	—	—	—	—	—

The microarray results from 10 references were summarized, and the miRNAs which were reported to be highly expressed in glioma tissues or glioma cell lines by at least two studies were listed in the table.

1. Ciafrè SA, et al. (2005) Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem Biophys Res Commun* 334:1351–1358.
2. Godlewski J, et al. (2008) Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. *Cancer Res* 68:9125–9130.
3. Silber J, James CD, Hodgson JG (2009) microRNAs in gliomas: Small regulators of a big problem. *Neuromolecular Med* 11:208–222.
4. Gal H, et al. (2008) MIR-451 and Imatinib mesylate inhibit tumor growth of Glioblastoma stem cells. *Biochem Biophys Res Commun* 376:86–90.
5. Huse JT, et al. (2009) The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. *Genes Dev* 23:1327–1337.
6. Sasayama T, Nishihara M, Kondoh T, Hosoda K, Kohmura E (2009) MicroRNA-10b is overexpressed in malignant glioma and associated with tumor invasive factors, uPAR and RhoC. *Int J Cancer* 125:1407–1413.
7. Malzkorn B, et al. (2010) Identification and functional characterization of microRNAs involved in the malignant progression of gliomas. *Brain Pathol* 20:539–550.
8. Jiang L, et al. (2010) miR-182 as a prognostic marker for glioma progression and patient survival. *Am J Pathol* 177:29–38.
9. Rao SA, Santosh V, Somasundaram K (2010) Genome-wide expression profiling identifies deregulated miRNAs in malignant astrocytoma. *Mod Pathol* 23:1404–1417.
10. Zhou X, et al. (2010) Downregulation of miR-21 inhibits EGFR pathway and suppresses the growth of human glioblastoma cells independent of PTEN status. *Lab Invest* 90:144–155.

Table S2. ChIP-chip results

mi_name	miBLOCK	S1	S2	S3	Positive sample count	mi_chr/strand	mi_accession
hsa-mir-132	−2000, 0	1	1	—	2	chr17-	MI0000449
hsa-mir-130b	−10000, -8000	1	—	1	2	chr22+	MI0000748
hsa-mir-130b	−26000, -24000	—	1	1	2	chr22+	MI0000748
hsa-mir-148a	−2000, 0	1	1	1	3	chr7-	MI0000253
hsa-mir-188	−38000, -36000	1	—	1	2	ChrX+	MI0000484
hsa-mir-199b	−12000, -10000	1	—	1	2	chr9-	MI0000282
hsa-mir-199b	−10000, -8000	1	—	1	2	chr9-	MI0000282
hsa-mir-210	−48000, -46000	1	1	—	2	chr11-	MI0000286
hsa-mir-23a	0, 2000	1	1	2	3	chr19-	MI0000079
hsa-mir-23a	−42000, -40000	2	1	1	3	chr19-	MI0000079
hsa-mir-23a	−16000, -14000	1	1	1	3	chr19-	MI0000079
hsa-mir-23a	−14000, -12000	1	1	1	3	chr19-	MI0000079
hsa-mir-23a	−34000, -32000	2	—	2	2	chr19-	MI0000079
hsa-mir-23a	−48000, -46000	—	1	1	2	chr19-	MI0000079
hsa-mir-23a	−10000, -8000	2	2	—	2	chr19-	MI0000079
hsa-mir-23a	−4000, -2000	2	1	—	2	chr19-	MI0000079
hsa-mir-23a	−38000, -36000	1	1	—	2	chr19-	MI0000079
hsa-mir-23a	−30000, -28000	1	1	—	2	chr19-	MI0000079
hsa-mir-23a	−8000, -6000	1	1	—	2	chr19-	MI0000079
hsa-mir-23b	−2000, 0	1	—	1	2	chr9+	MI0000439
hsa-mir-27a	0, 2000	1	1	2	3	chr19-	MI0000085
hsa-mir-27a	−42000, -40000	3	1	1	3	chr19-	MI0000085
hsa-mir-27a	−16000, -14000	1	1	1	3	chr19-	MI0000085
hsa-mir-27a	−14000, -12000	1	1	1	3	chr19-	MI0000085
hsa-mir-27a	−34000, -32000	2	—	1	2	chr19-	MI0000085
hsa-mir-27a	−36000, -34000	1	—	1	2	chr19-	MI0000085
hsa-mir-27a	−48000, -46000	—	1	1	2	chr19-	MI0000085
hsa-mir-27a	−10000, -8000	2	1	—	2	chr19-	MI0000085
hsa-mir-27a	−38000, -36000	1	1	—	2	chr19-	MI0000085
hsa-mir-27a	−30000, -28000	1	1	—	2	chr19-	MI0000085
hsa-mir-27a	−8000, -6000	1	1	—	2	chr19-	MI0000085
hsa-mir-362	−42000, -40000	2	—	1	2	chrX+	MI0000762
hsa-mir-516a-1	−4000, -2000	1	—	1	2	chr19+	MI0003180
hsa-mir-516a-2	−10000, -8000	1	—	1	2	chr19+	MI0003181
hsa-mir-516a-2	−8000, -6000	1	—	1	2	chr19+	MI0003181
hsa-mir-671	−12000, -10000	1	1	—	2	chr7+	MI0003760
hsa-mir-9–1	−16000, -14000	2	1	—	2	chr1-	MI0000466
hsa-mir-9–2	6000, 8000	—	1	1	2	chr5-	MI0000467
hsa-mir-9–3	−36000, -34000	1	2	—	2	chr15+	MI0000468
hsa-mir-106b	4000, 6000	1	1	—	2	chr7-	MI0000734
hsa-mir-93	4000, 6000	1	1	—	2	chr7-	MI0000095
hsa-mir-25	4000, 6000	1	1	—	2	chr7-	MI0000082

The results of three ChIP-chip arrays were analyzed. The up-regulated miRNAs in glioma that contain CREB binding peaks in the upstream regulatory sequences were confirmed by at least two arrays were listed. mi_name, the name of the miRNA that the peak is overlapping; miBLOCK, a serial number for each peak searching regions that are 2 kb tiling from −50 kb to +10 kb of premiRNAs; S1/S2/S3, represents peak number overlapping the promoter region for the corresponding sample; positive sample count, the number of samples which contain at least a peak overlapping its promoter. mi_chr/strand, tells which chromosome the gene lies in and strand of the transcript; mi_accession, Ref_seq accession number of the miRNA transcript.

Table S3. The primers used for cloning the TSGs 3′ UTR and the promoter of *mir-23a*, and the real-time PCR primers are all listed in the table

Primers	Primer sequences (5′-3′)
Real-time PCR primers (for detecting the mRNAs of CREB and the TSGs)	
CREB-rltm-F	GTCATGGCCTACGAGGAGAA
CREB-rltm-R	CACGTCAGGGAGAAGCAGAG
GAPDH-rltm-F	GGTCATCCATGACAACTTTGG
GAPDH-rltm-R	GGCCATCACGCCACAG
FoXO3a-rltm-F	TGCCAGGCTGAAGGATCACT
FoXO3a -rltm-R	GGGATTCACAAAGGTGTTAAGCTG
PTEN-rltm-F	GGGACGAACTGGTGTAATGA
PTEN -rltm-R	CGCCTCTGACTGGGAATAG
Real-time PCR primers (for detecting the gene copy numbers)	
gCREB-rltm-fwd	GTCATGGCCTACGAGGAGAA
gCREB-rltm-rev	CACGTCAGGGAGAAGCAGAG
gGAPDH-rltm-F	GGTCATCCATGACAACTTTGG
gGAPDH-rltm-R	GGCCATCACGCCACAG
gmir-23a-rltm-F	CCCTCACCCCTGTGCCA
gmir-23a-rltm-R	GGGTCGGTTGGAAATCCCTG
Real-time PCR primers (for detecting the miRNAs)	
Universal reverse	GTGCAGGGTCCGAGGT
U6-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAATATG
U6-R	GCGCGTCGTGAAGCGTTC
Hsa-mir-9-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCATAC
Hsa-mir-9-F	CGGCCGTCTTTGGTTATCTAGC
Hsa-mir-10b-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACAAA
Hsa-mir-10b-F	CTACCCTGTAGAACCGAATTTGTG
Hsa-mir-21-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAACA
Hsa-mir-21-F	GCGTAGCTTATCAGACTGATGTTG
Hsa-mir-23a-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGAAATC
Hsa-mir-23a-F	GCGATCACATTGCCAGGGA
Hsa-mir-25-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAGAC
Hsa-mir-25-F	CGCGCATTGCACTTGTCTC
Hsa-mir-26a-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCCTAT
Hsa-mir-26a-F	CGCCGCTTCAAGTAATCCAGG
Hsa-mir-106b-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATCTGC
Hsa-mir-106b-F	CGCGTAAAGTGCTGACAGTG
Hsa-mir-182-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGTGTG
Hsa-mir-182-F	CGCGGTTTGGCAATGGTAGA
Hsa-mir-221-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAAACC
Hsa-mir-221-F	GAGCTACATTGTCTGCTGGGT
Primers for cloning the TSGs 3′ UTRs	
FOXO3a 3′ UTR-F	CCGCTCGAGTGGAATGGAGTCCAGTTGGT
FOXO3a 3′ UTR-R	GCTCTAGACCCCAGAGCAACTCAGGAC
FOXO4 3′ UTR-F	CCGCTCGAGGTCGGGACATCATCTTCACC
FOXO4 3′ UTR-R	GCTCTAGAAGGCTTGGGCTAAGAGAAGG
PTEN 3′ UTR-half1-F	CCGCTCGAGGCCTTTCTCTTGCCTGCATA
PTEN 3′ UTR-half1-R	GCTCTAGAATAAGGGGCTGTGTTGCTTG
PTEN 3′ UTR-half2-F	CCGCTCGAGCACAAGCAGTTCCCTCCATT
PTEN 3′ UTR-half2-R	GCTCTAGAAAGCATACCGACTGGGATTG
PTEN 3′ UTR-half2-1-R	GCTCTAGACAACCACAGCCATCGTTATG
PTEN 3′ UTR-half2-2-F	GCTCTAGATTGAAATTTATATGCCACCTTGTCT
PTEN 3′ UTR-half2-1-MT-F	GGAGTACAACTACTATTGTAAAGCTTTCACATAGATATTATTAAAAAGGTTTTTTTTTCCAGA
PTEN 3′ UTR-half2-1-MT-R	TCTGGAAAAAAAAACCTTTTTAATAATATCTATGTGAAAGCTTTACAATAGTAGTTGTACTCC
PTEN 3′ UTR-half2-2-MT-F	TGCACTGTTATTATTTTTCCTTTGGTTCACATAGGTCTGAATGAGGGTTTTGAT
PTEN 3′ UTR-half2-2-MT-R	ATCAAAACCCTCATTCAGACCTATGTGAACCAAAGGAAAAATAATAACAGTGCA
Primers for ChIP-QPCR	
chIP-mir-23a-a-F	GCACTTTGGAAAGCCGAGGT
chIP-mir-23a-a-R	GCCACCACGCCCAGTTAAG
chIP-mir-23a-b-F	CTAAGCCCTGGCCACTGAG
chIP-mir-23a-b-R	CATGGGGAGAGGAAGCCAAG
chIP-mir-23a-1-F	CACACCACCGCACCTGG
chIP-mir-23a-1-R	AGTGGCTCATGCCTGTAATCC
chIP-mir-23a-2-F	TGTTTTGTAGAGACGGGGTTTCG
chIP-mir-23a-2-R	CAGGCACGGTGGCTCAC

Table S3. Cont.

Primers	Primer sequences (5′-3′)
chIP-mir-23a-3-F	ATAGGGCGCAGACACCAGT
chIP-mir-23a-3-R	GTACACCCCGTCATCAGGTC
chIP-mir-23a-4-F	CCCTTGGTATTTTCCGATCCCA
chIP-mir-23a-4-R	CCCATGCTCCAAGAATGAATCAG
chIP-mir-23a-5-F	TGATCCCCAACTCTGCAACTG
chIP-mir-23a-5-R	ATGGAAGGGCCACCCACTC
Primers for cloning the promoter of mir-23a gene	
P23a(-2000-0)-F	GAAGATCTCCACTCACTAAAGACTTAGGGCTG
P23a(-2000-0)-R	GAAGATCTGGGTCGGTTGGAAATCCCTG
P23a(-500-0)-F	GAAGATCTGGGTGCGTGTCTAGAAAGAGAG
P23a(-500-0)-R	CCCAAGCTTGGGTCGGTTGGAAATCCCTG
P23a(-500-0)-MT-R	GCAGGCGGGTGAGGAAGTCTTCAGACCCCAGCTGGCGCCA
P23a(-500-0)-MT-F	TGGCGCCAGCTGGGGTCTGAAGACTTCCTCACCCGCCTGC

F, forward primer; MT, mutation; R, reverse primer; RT, reverse transcription primers.