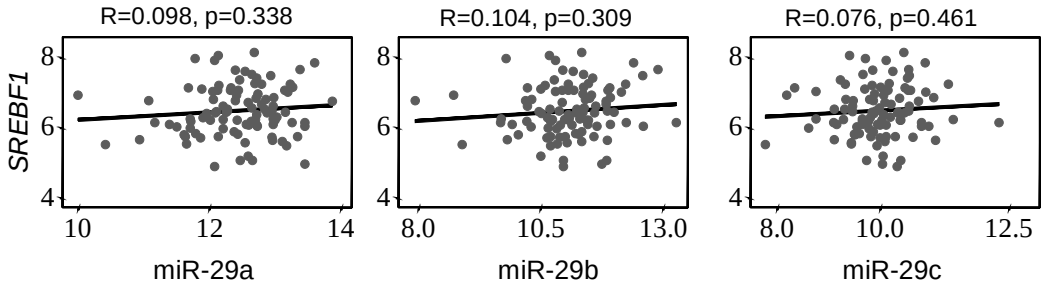
A

GBM patients with altered EGFR n=132

Genotype of EGFR	pair with SREBF1	Correlation coefficient (R)	p-value
Mut only (n=14)	miR-29a	0.184	0.528
	miR-29b	0.135	0.646
	miR-29c	0.362	0.204
Mut/AMP (n=52)	miR-29a	0.312	0.024
	miR-29b	0.319	0.021
	miR-29c	0.271	0.052
AMP only (n=66)	miR-29a	0.149	0.234
	miR-29b	0.206	0.098
	miR-29c	0.37	0.002
AMP with/without mut (n=118)	miR-29a	0.227	0.013
	miR-29b	0.261	0.004
	miR-29c	0.307	0.001

B

GBM patients with normal EGFR n=97



C

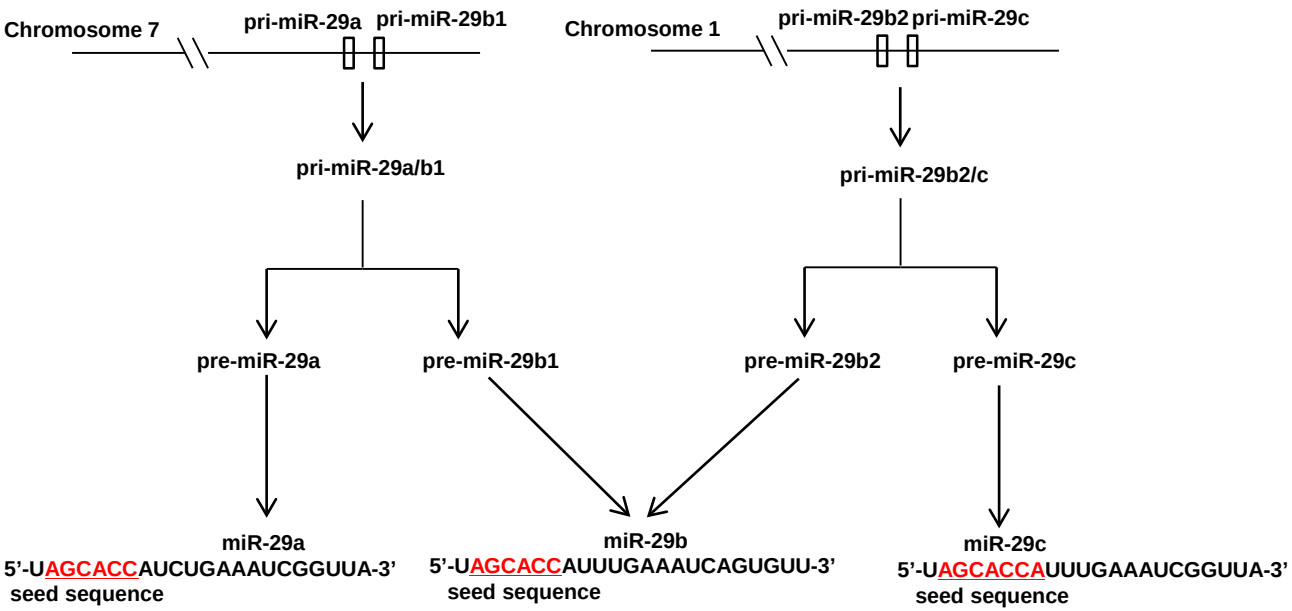


Figure S1 (related to Figure 1). The expression of *SREBF1* is correlated with miR-29 expression in GBM patients with EGFR alteration, but no significant correlation was observed in patients with normal EGFR.

(A) The table shows the results of the Pearson correlation analysis between *SREBF1 and* miR-29 expression (-29a, -29b and 29c) in GBM patients with different EGFR alteration status (amplification and/or mutation). The data were obtained from TCGA database. Correlation significance was indicated by the p-value shown in the last column.

(B) Pearson correlation analysis of GBM patients with normal genomic EGFR (no amplification and no mutation, n=97) from TCGA database.

(C) Schematic model illustrates the expression of miR-29 family. Pri-miR-29a and pri-miR-29b1 localize on chromosome 7 and are transcribed together by the same promoter to generate pri-miR-29a/b1, which then converts to pre-miR-29a and pre-miR-29b1 that further produce mature miR-29a and miR-29b. Similarly, pri-miR-29b2 and pri-miR-29c localize on chromosome 1 and share same promoter, which produces mature miR-29b and miR-29c. miR-29a, -29b and -29c share the same seed sequence that interacts with target genes.

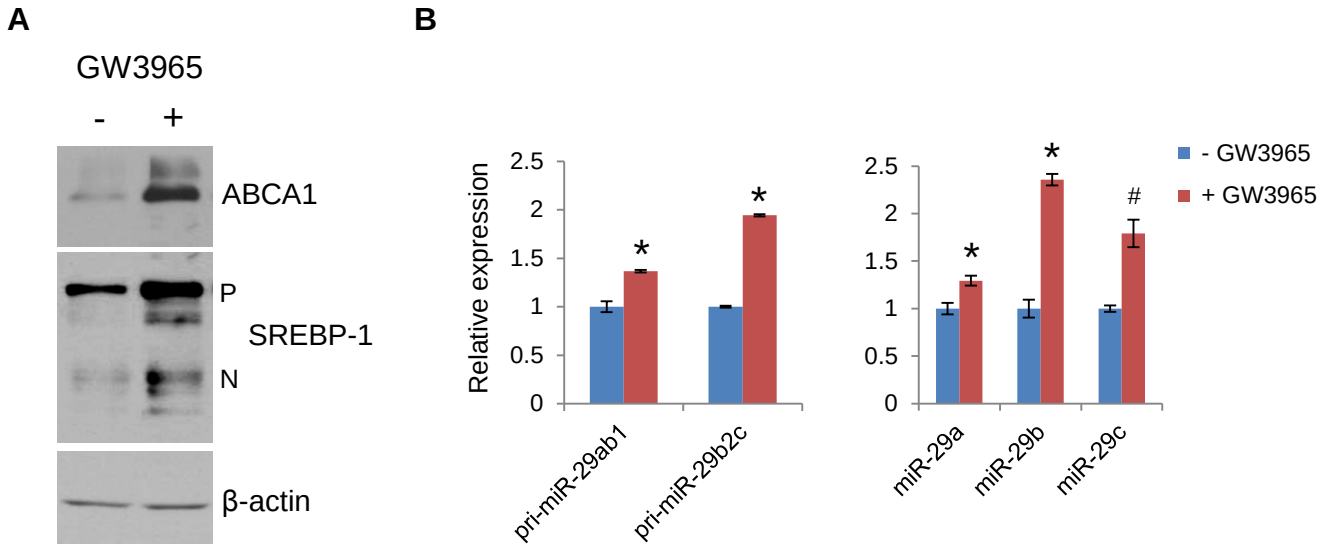


Figure S2 (related to Figure 2). Upregulation of SREBP-1 by activation of LXR using GW3965 enhances miR-29 expression.

(A) Western blot analysis of lysates from U87 cells after treatment with GW3965 (2 μM) for 24 hr.

(B) Real-time PCR analysis of pri-miR-29 and mature miR-29 expression in U87 cells after treatment with GW3965 (2 μM) for 24 hr.

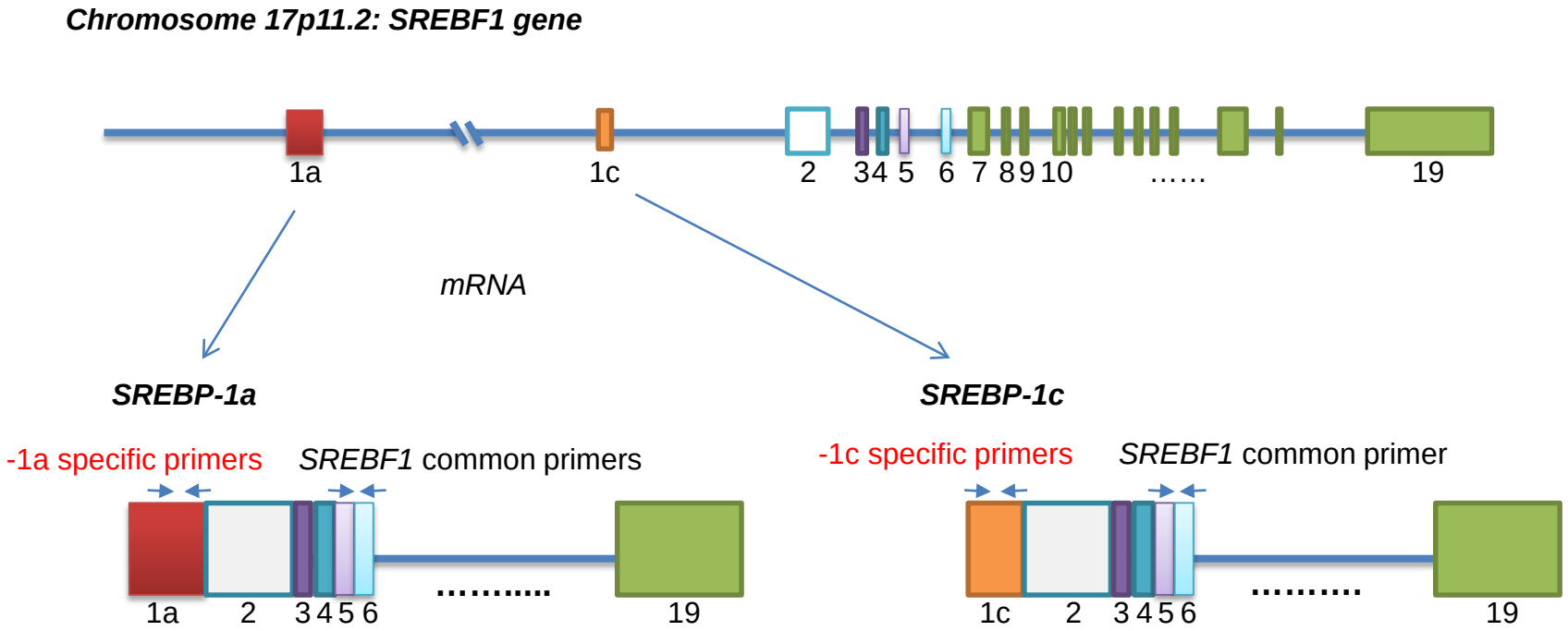
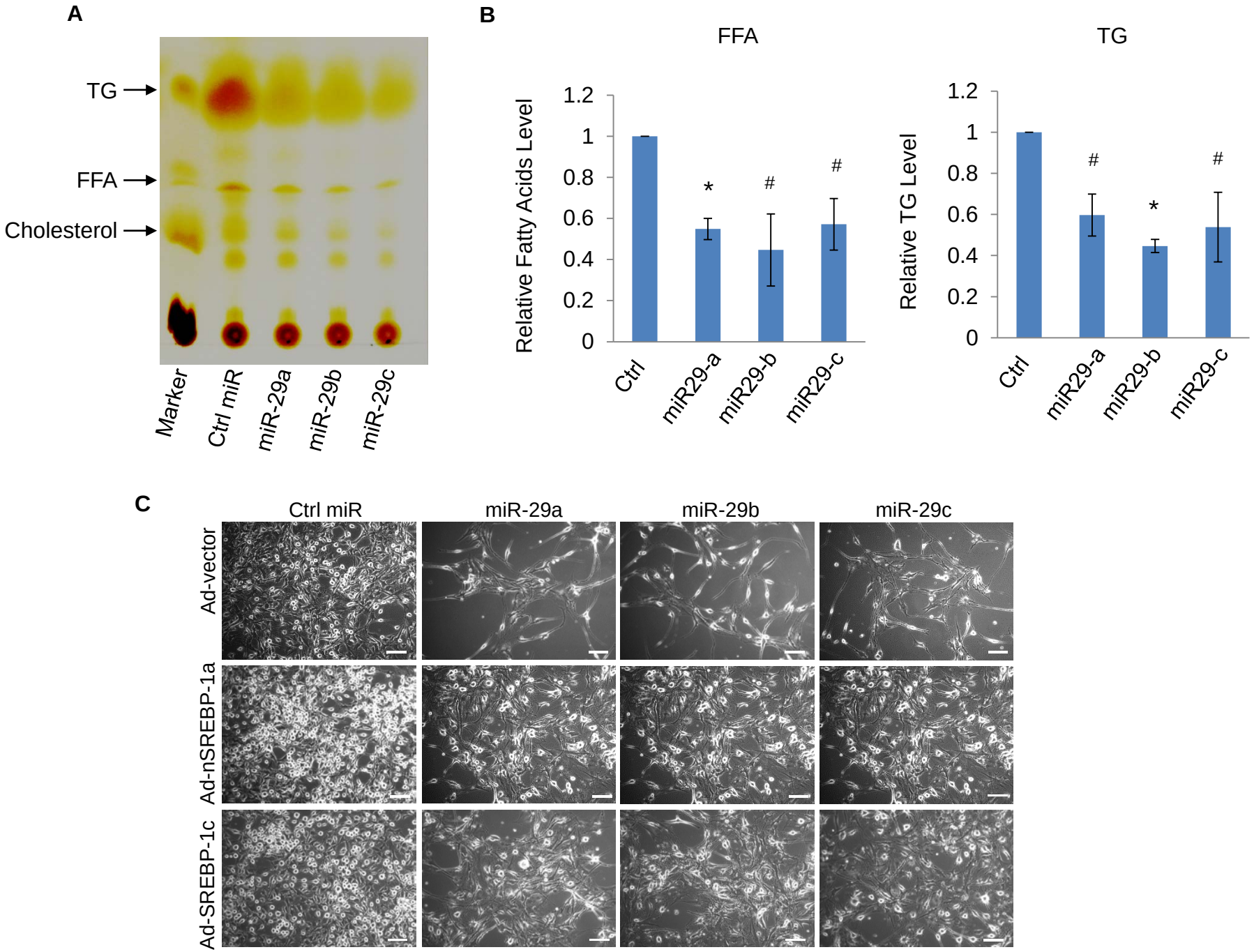


Figure S3 (related to Figure 3). Diagram of PCR primer design for SREBP-1a and -1c isoforms.
SREBF1 gene encodes two isoforms, SREBP-1a and SREBP-1c that differ in their first exon, by utilizing different transcriptional start sties. The primers were designed to specifically recognize SREBP-1a or SREBP-1c mRNA based on their distinct exon 1. *SREBF1* primers were designed based on the identical sequence between SREBP-1a and -1c that locates in their exons 5-6, which were able to amplify both isoforms.


A
TG
FFA
Cholesterol
Marker
Ctrl miR
miR-29a
miR-29b
miR-29c
B
FFA
Relative Fatty Acids Level
1.2
1
0.8
0.6
0.4
0.2
0
*
#
#
Ctrl
miR29-a
miR29-b
miR29-c
TG
Relative TG Level
1.2
1
0.8
0.6
0.4
0.2
0
#
*
#
Ctrl
miR29-a
miR29-b
miR29-c
C
Ctrl miR
miR-29a
miR-29b
miR-29c
Ad-vector
Ad-nSREBP-1a
Ad-SREBP-1c

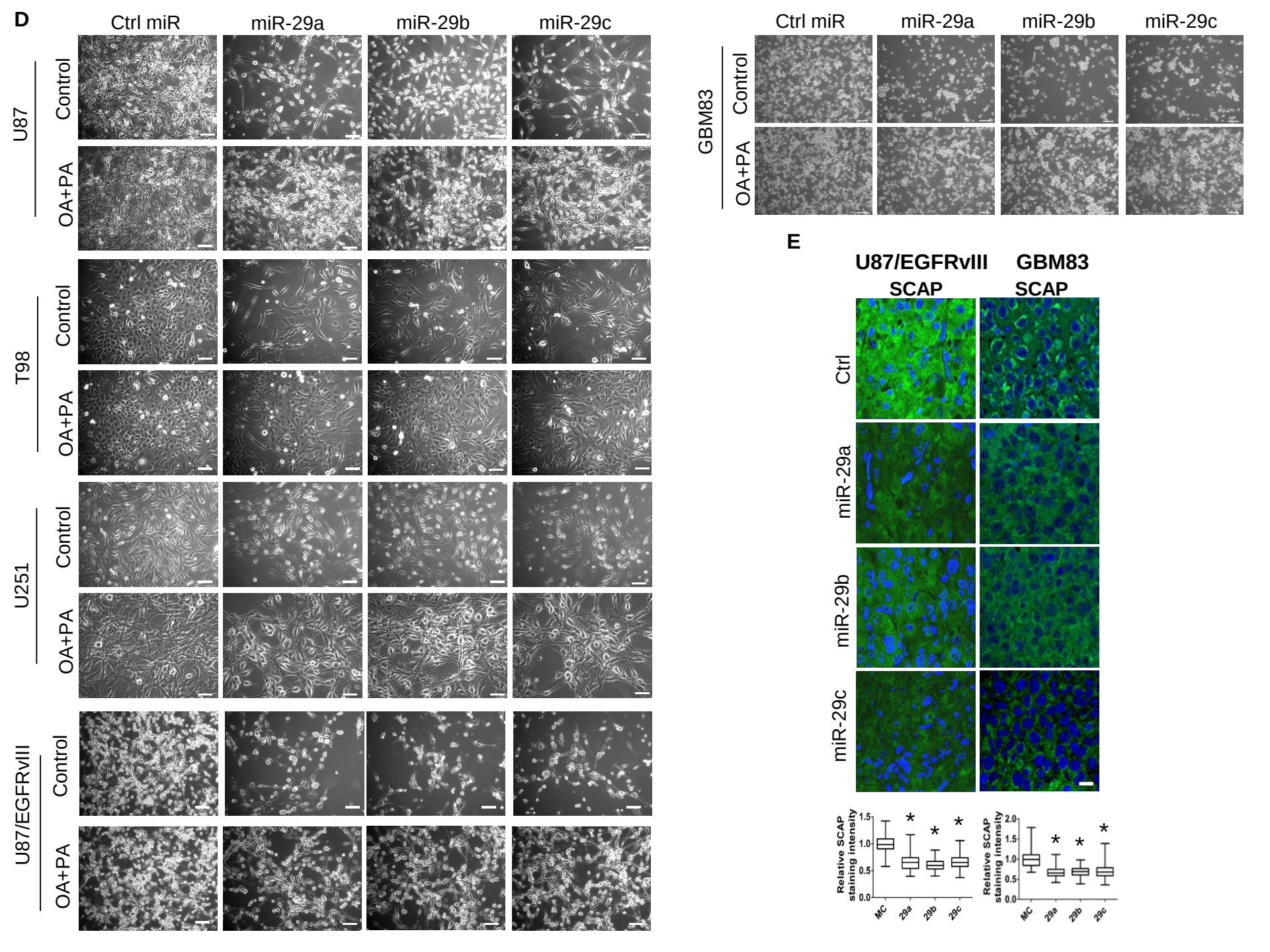

D
Ctrl miR
miR-29a
miR-29b
miR-29c
U87
Control
OA+PA
T98
U251
U87/EGFRvIII
GBM83
E
U87/EGFRvIII
GBM83
SCAP
Ctrl
miR-29a
miR-29b
miR-29c
Relative SCAP staining intensity
MC
29a
29b
29c

Figure S4 (related to Figure 4). miR-29 mimics transfection significantly reduces the levels of fatty acids and triglyceride and inhibits GBM cell growth.

(A, B) GBM U87 cells were transfected with control miRNA or miR-29a, -29b, or -29c mimics overnight, and then cells were replaced in fresh DMEM medium containing 1% LPDS for 72 hr. These cells were then collected and lipid were extracted from the same amount number of cells by using chloroform/methanol (2:1). Lipids were analyzed by thin layer chromatography (TLC) and results were photographed after iodine staining (left panel). Relative amount of fatty acids (FFA) and triglycerides (TG) were quantified by imageJ and normalized to the intensity of lipids in the control miRNA transfection group (right panel). Statistical significance was determined by an unpaired Student's t test; * $p<0.01$ and #$p<0.01$ in comparison with control cells.

(C, D) Micrographs show the growth of GBM cells transfected with miR-29 or scramble control miRNA (Ctrl miR) together with overexpression of adenovirus-mediated N-terminal active form of nSREBP-1a or -1c (A, U87 cells) or addition of PA (palmitate, 10 μM) and OA (oleic acid, 10 μM) for 3 days in 1% LPDS media (B, U87, T98, U251 cells) or 2 days (B, GBM83 cells). Scale bar, 100 μm.

(E) Imunofluorescence analysis of SCAP from U87/EGFRvIII-luc (left panels) or GBM83 (right panels) intracranial xenograft mouse model transfected with miR-29 or miRNA scramble control. Lower panels show the relative protein levels in tumor tissues quantified by the ImageJ software and averaged from 5 separate areas in each tumor. The results were normalized with control miRNA tumors (mean ± SEM). Statistical significance was determined by Student's t-test. *$p<0.001$ for comparison with scramble control miRNA transfection. Scale bar, 20 μm.

Supplemental Experiment Procedures

Antibodies and Reagents

Antibodies for p-Akt Ser473, p-Akt Thr308, Akt were purchased from Cell Signaling (#4051s, #9275, #9272); β-actin from Sigma (#A5316); SREBP-1 (IgG-2A4) and SREBP-2 from BD Pharmingen (#B557036, #B557037); p-EGFR Tyr1086 from Life technologies (#36-9700) and EGFR from Millipore (#04-338); SCAP (a.a 303-554) from Bethyl Laboratories, Inc. (#A303-554A); PDI from Santa cruz (#SC 30932); LDLR from Abcam (#ab30532). Reagents for human EGF (epidermal growth factor) were purchased from Sigma (#E9644); Erlotinib from LC lab (#E-4007); BKM120 and MK2206 from Selleckchem (#S2247, #S1078). Dulbecco's modified Eagle's medium (DMEM) was purchased from Cellgro. Neurobasal medium (Life Technologies, Cat# 21103-049). Recombinant Human FGF basic 145 aa (R&D, Cat# 4114-TC-01M). Human EGF (Sigma, Cat# E9644). Heparin (Sigma, Cat# H3393). B-27 Supplement (50X), minus vitamin A (Life Technologies, Cat# 12587-010). Glutamine (Life Technologies, Cat# 25030-081). TrypLE Express Enzyme (1X), no phenol red (Life Technologies, Cat# 12604-021). Poly-L-lysine hydrobromide (Sigma, Cat# P5899). Laminin (Sigma, Cat# L2020).

Transfection

U87/EGFR cells were transfected with control siRNA (10 nM) (Santa Cruz, #SC-37007), siSCAP (10 nM) (Santa Cruz, #SC-36462) or siSREBP-1 (10 nM) ((Santa Cruz, #SC-36557), respectively, using RNAiMAX transfection reagent (Life technologies, #13778) overnight, then changed to 5% FBS media for 12 hr. Cells were washed once with PBS and serum starved for 16-24 hr, then treated with EGF (50 ng/ml) for another 12 hr. Cell were then collected for western blot or real-time PCR analysis. For microRNA mimics transfection, GBM cells were transfected by mirVana miRNA mimic scramble control (Cat# 4464059), mirVana miRNA mimic hsa-mi-29a (Cat# 4464066, MC12499), mirVana miRNA mimic hsa-mi-29b (Cat#4464066, MC10103) or mirVana miRNA mimic hsa-mi-29c (Cat#4464066, MC10518) from Ambion with 50 nM (final concentration) for overnight. Cells were washed once with PBS and then changed to 1% lipoprotein deficient serum (Intracel, #RP-056) medium for 2-3 days. Cells were then collected for western blot or real-time PCR analysis, or cell number was counted.

To transfect GBM patient derived cells (GBM83), cells were seeded in 60 mm dish after coated with 2 ml of poly-lysine at 100ug/ml for 30 min or more at room temperature, then washed with sterile water and allowed to dry, then coated with 500 µl of laminin (50 µg/ml) for 30min at 37ºC. The laminin was removed and washed with PBS twice, air dry at least 45min before seeding cells. 8 x 10^5 GBM83 were plated into the coated dish culturing with fresh Neurobasal medium (Life technologies, cata#21103-49) including EGF, FGF, B27 and Glutamine (2mM) for 24 hr, then transfected with 50 nM miR-29 mimics or scramble miRNA by RNAiMAX transfection reagent for 12 hr and replaced with fresh medium for 36 hr (totally 48h for transfection). GBM38 cells were collected and analyzed by western blot, real-time PCR and cell proliferation. For fatty acid rescue assay, GBM83 cells were pre-treated with OA 10 µM/PA 10 µM for 4 hr before transfection.

Real-time RT-PCR

Total RNA was extracted from GBM cells using TRIzol reagent according to its protocol (Life Technologies, #15596). Total RNA (800 ng) was subjected to reverse transcription to cDNA using iScript cDNA Synthesis Kit (Bio-Rad, #170-8891) and amplified through a subsequent real-time PCR using IQ SYBR Green Supermix (Bio-Rad, #170-8884), and its values were normalized against the internal control gene *36B4* (*RPLP0*) for each replicate. The primers used were as follows:

SREBF1 forward: 5′-CGCTCCTCCATCAATGACA-3′

reverse: 5′-TGCGCAAGACAGCAGATTTA-3′

SREBP-1a forward: 5′-TCAGCGAGGCGGCTTTGGAGCAG-3′

reverse: 5′-CATGTCTTCGATGTCGGTCAG-3′

SREBP-1c forward: 5′-GGAGGGGTAGGGCCAACGGCCT-3′

reverse: 5′-CATGTCTTCGAAAGTGCAATCC-3′

SREBF2	forward: 5′-ATCTGGATCTCGCCAGAGG-3′
	reverse: 5′CCAGGCAGGTTTGTAGGTTG-3′
ACLY	forward: 5′-GAAGGGAGTGACCATCATCG-3′
	reverse: 5′-TTAAAGCACCCAGGCTTGAT-3′
ACACA	forward: 5′- GATGTGGATGATGGGCTACA-3′
	reverse: 5′-TGAGGCCTTGATCATTACTGG-3′
FASN	forward: 5′-GTTCACGGACATGGAGCAC-3′
	reverse: 5′-GTGGCTCTTGATGATCAGGTC-3′
HMGCR	forward: 5′-ATAATCCTGGGGAAAATGCC-3′
	reverse: 5′-TCTTCTTGGTGCAAGCTCCT-3′
HMGCS1	forward: 5′-AAAAAGATCCATGCCCAGTG-3′
	reverse: 5′-TCAGCAACATCCGAGCTAGA-3′
LDLR	forward: 5′-TCTTTACGTGTTCCAAGGGG-3′
	reverse: 5′-TGCAGTTTCCATCAGAGCAC-3′
SCD1	forward: 5′-TGCGATATGCTGTGGTGCT-3′
	reverse: 5′-GATGTGCCAGCGGTACTCA-3′
SCAP	forward: 5′-CAAGAGGCTGCGTGTTGTC-3′
	reverse: 5′-CCAGGATGCCAATCCAGA-3′
36B4	forward: 5′-AATGGCAGCATCTACAACCC-3′
	reverse: 5′-TCGTTTGTACCCGTTGATGA-3′
pri-miR-29a/b1	forward: 5′-TGCCAGGAGCTGGTGATTTCCT-3′
	reverse: 5′-ACGGGCGTACAGAGGATCCCC-3′
pri-miR-29b2/c	forward: 5′-GGCTGGGTCTTCCGATTGAATCTCC-3′
	reverse: 5′-TCCACCCTTGGCTGTGCTGCA -3′

Primers for pri-miR-29 were described before (Zhang et al., 2012). The amplification conditions were as follows: 95°C for 10 min, then denatured at 95°C for 30 s, annealed at 60°C for 30 s, and extended at 72°C for 30 s with 45 cycles.

Real-time RT-PCR Analysis for Mature miRNA:
Mature miRNA expression was determined by isolating total RNA using TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA (10 ng) was reversely transcribed using the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems) with miRNA-specific primers. To quantify the miRNA levels, the CFX96 (Bio-Rad) was used in conjunction with gene-specific TaqMan assays. Primers of Taqman MicroRNA Assays for hsa-miR-29a (#002112), hsa-miR-29b (#000413), hsa-miR-29c (#000587), U6 small nuclear RNA (#001973) were purchased from Applied Biosytems. Relative expression level of miR-29a, -29b, or -29c was calculated by normalization with U6 expression level in GBM cells.

Western Blot
Cells were washed with ice-cold PBS twice and scraped into a lysis buffer containing 10 mmol/L Tris, pH 7.4, 100 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L NaF, 20 mmol/L $Na_4P_2O_7$, 2 mmol/L Na_3VO_4, 0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate, 1% Triton X-100, 10% glycerol, 10 μg/mL leupeptin, 60 μg/mL aprotinin, and 1 mmol/L phenylmethanesulfonyl fluoride. Equal amounts of protein extracts were resolved by using 12% SDS-PAGE, and then transferred to a polyvinylidene difluoride membrane (BIO-RAD). After blocking for 1 hr in a Tris-buffered saline containing 0.1% Tween 20 and 5% nonfat milk, the membrane was probed with indicated primary antibodies overnight, followed by secondary

antibodies conjugated to horseradish peroxidase for 1~3 hr. The immunoreactivity was detected by an ECL kit (GE Healthcare Life Sciences).

ChIP Assay

ChIP assay was performed by using U87/EGFR cells with/without EGF stimulation (50 ng/ml) for 12 hr after serum starvation for 24 hr. Briefly, cells cultured in 15 cm plates were washed once using PBS, and scraped and pooled into 50 ml tube after crosslink for 5 minutes in 1% formaldehyde in PBS. After extensive sonication (20 minutes total sonication time in 30 second pulses), pre-clearing with protein-G sepharose, and removal of a 50 µl (1/20 input) fraction for normalization, soluble chromatin was split equally into two tubes for overnight immunoprecipitation (IP) with 2 µg of the following antibodies: mouse IgG (Millipore cat #12-371), or anti-SREBP-1 antibody (BD Pharmingen). DNA-Protein complexes were pulled down by incubation for 2 hr with protein-G sepharose, washed, and processed as previously described (Guo et al., 2009). 2 µl solution was used as template for PCR analysis.

PCR primers used for analysis of SREBP-1 binding motifs in miR-29 promoters:

miR-29a/b1	SRE-3/4	forward: 5′-GGGCTCTGTCTTAAATGACCA-3′
		reverse: 5′-TGGCTATAGGAGTCACTGGAA-3′
miR-29a/b1	SRE-2	forward: 5′-TGTAACCCTGGGCATGTCCTT-3′
		reverse: 5′-TCCGTCCATTCGTTTAATCTTCA-3′
miR-29a/b1	SRE-1	forward: 5′-TTCTTTTGCTGAGCTCGGTAT-3′
		reverse: 5′-TGTGATAGGATCAAAGGAACA-3′
miR-29b2/c	SRE	forward: 5′-CCAGCTCTGTACCAAGCCTAC-3′
		reverse: 5′-GGACATTCCTGATTTATCCAAG-3′

Promoter Luciferase Assay

After PCR, the fragment of promoter for miR-29ab1-4k, miR-29ab1-2k, miR-29b2c-4k or miR-29b2c-1.5k was cloned into pGL3-basic vector according to the instruction of In-Fusion® HD Cloning Plus Kit (Clontech, #638909). The fragment of miR-29ab1-3k or miR-29ab1-1k was cloned into pGL3-basic vector after digestion by KpnI (NEB, #R0142L) and NheI (NEB, #R0131L) or BgIII (NEB, #R0144L) endonuclease, and then inserted into pGL3-basic vector digested by the same endonucleases via ligation using T4 DNA ligase (NEB, #M0202) based on the construct of the miR-29ab1-4k. Promoter construct DNA (1 µg) was transfected into HEK293T cells together with active N-terminal SREBP-1a, -1c, or pC3.1 control vector (1 µg) and renilla (200 ng) in a 6-well plate by using X-tremeGENE™ HP DNA Transfection Reagent (Roche, #06366236001). Luciferase activity was measured 48 hr post-transfection by using firefly luciferase assay reagent (Promega, PRE2610) and renilla luciferase assay reagent (Promega, PRE2710) according to the kit instruction.

PCR primers for promoter fragments:

miR-29ab1-4k

forward: 5'-ATTTCTCTATCGATAGGTACCGTCCCAACTACTCAAGAAACTG-3′

reverse: 5'-CACGCGTAAGAGCTCGGTACCGACTCAAGACGACCAACACT-3′

miR-29ab1-3k

forward: 5'-ATTTGGTACCTTCTCGCTGATGATGAAACTG-3′

reverse: 5'-TCTTGCTAGCGACTCAAGACGACCAACACT-3′

miR-29ab1-2k

forward: 5'-ATTTCTCTATCGATAGGTACCAACATCCAGCCTTAGAAATAGC-3′ reverse: 5'-CACGCGTAAGAGCTCGGTACCGACTCAAGACGACCAACACT-3′

miR-29ab1-1k

forward: 5'-CATAAGATCTCGTGGGCAAGGGCTGAGGAGA-3′

reverse: 5'-TCATAGATCTGACTCAAGACGACCAACACT-3′

miR-29b2c-4k

forward: 5'-ATTTCTCTATCGATAGGTACCGAAACAAAGCCTTGCTGAACG-3′

reverse: 5'-CACGCGTAAGAGCTCGGTACCCCCTCTGCATACCCATCTCCC-3′

miR-29b2c-1.5k

forward: 5'-CGAGCTCTTACGCGTGCTAGCAACAGCCGTGCTGCAGCAGAG-3’

reverse: 5'-AGATCTCGAGCCCGGGCTAGCCCCTCTGCATACCCATCTCCC-3′

3’-UTR Luciferase Assay

Wild type or mutant 3’-UTR was amplified by PCR, then cloned into pmiReport vector after digestion by SpeI (NEB, R0133s) and HindIII (NEB, R0104s) and ligation using T4 DNA ligase (NEB, M0202). pmiReport Plasmid DNA (200 ng) was transfected into HEK293T cells together with Renilla (20 ng) and miRNA mimics (50 nM) in a 12-well plate by using Lipofectamine 2000 (Life Technologies, 11668). Luciferase activity was measured 48 hr post-transfection by using firefly luciferase assay reagent (Promega, PRE2610) and renilla luciferase assay reagent (Promega, PRE2710) according to the kit instruction.

Primers used for:

SREBF1-3’-UTR-wild type

forward: 5′-GCACTAGTAGACCGGCGGCCATGATGGTGCTGACCTCT-3′ reverse: 5′-ACCAAGCTTTCTCTCCCACGACGGAGAGAGAGGCCTC-3′

SREBF1-3’-UTR-mutant

forward: 5′-TTAACTAGTACCTCTGGTGGCCGAT-3′

reverse: 5′-ACCAAGCTTTCTCTCCCACGACGGAGAGAGAGGCCTC-3′

SCAP-3’-UTR-wild type

forward: 5′-ACACTAGTCCCAGGCAGGAGGCTGGGGTGCTGTGTGGG -3′

reverse: 5′-GGGATATCGCCTGACAGATGATGATATGGTTTT -3′

SCAP-3’-UTR-mutant

forward: 5-TAACACTAGTCCCAGGCAGGAGGCTGGAACATCGTGTGGG -3′

reverse: 5′-GGGATATCGCCTGACAGATGATGATATGGTTTT -3′

Preparation of Cell Membrane Fractions

Isolation of cell membrane fractions to detect SCAP protein was performed as previously described (Cheng et al., 2015; Nohturfft et al., 1998). Cells were washed once with PBS, scraped into 1 ml PBS, and centrifuged at 1000x *g* for 5 min at 4°C. Cells were resuspended in an ice-cold buffer containing 10 mM HEPES-KOH (pH 7.6), 10 mM KCl, 1.5 mM $MgCl_2$, and 1 mM sodium EDTA, 1 mM sodium EGTA, 250 mM sucrose and a mixture of protease inhibitors (5 µg/ml pepstatin A, 10 µg/ml leupeptin, 0.5 mM PMSF, 1 mM DTT, and 25 µg/ml ALLN) for 30 min on ice. Extracts were then passed through a 22G x 1 1/2 needle 30 times and centrifuged at 890x g at 4°C for 5 min to isolate nuclei. Supernatant was used for the separation of membrane fractions. The supernatant from the original 890x g spin was centrifuged at 20,000x g for 20 min at 4°C. For subsequent western blot analysis, the pellet was dissolved in 0.1 ml of SDS lysis buffer (10 mM Tris-HCl pH 6.8, 100 mM NaCl, 1% (v/v) SDS, 1 mM sodium EDTA, and 1 mM sodium EGTA) and designated “membrane fraction”. The membrane fraction was incubated at 37°C for 30 min, and protein

concentration was determined. 1 µl 100x bromophenol blue solution was added before the samples were subjected to SDS-PAGE. SCAP protein was analyzed by western blot using membrane fractions.

Lipid Synthesis Assay

The cells were grown in serum-free media (containing 5 mM glucose and 2 mM glutamine) for 24 hr, which was then replaced with fresh serum-free media (containing 5 mM glucose and 2 mM glutamine) and stimulated by EGF (50 ng/ml) for 10 hr. After switching to new serum-free media (containing 2 mM glucose and 2 mM glutamine plus 50 ng/ml EGF), 0.5 µCi ^{14}C- glucose was added to media and incubated for 2 hr. The cells were washed twice with PBS and lipids were extracted with 500 µl hexane:isopropanol (3:1) for 1 hr. The liquid phase was collected in 1.5 ml tube and left overnight to air-dry and 200 µl chloroform was used to dissolve the lipids for 0.5 -1 hr, then analyzed with a scintillation counter (Beckman coulter).

Thin Layer Chromatography for Lipids

Cellular total lipid extract was obtained by scraping cells from the 10 cm culture dish into 2 ml PBS containing protease inhibitor and 1 mM phenylmethylsulphonyl fluoride (PMSF) and adding 4 ml of chloroform/methanol (2:1, v/v) with 0.01% butylated hydroxytoluene (Sigma). The solution was vortexed and centrifuged at 1500 g for 5 min. The organic phase was collected and 2.5 ml of chloroform was added to the residual aqueous phases which was vortexed and centrifuged at 1500 g for 5 min. The organic phase was pooled with the previous extraction. Thin layer chromatography (TLC) was performed by spotting the cellular total lipid extract on a 5 × 10 cm silica gel aluminum sheet (EMD Chemicals) and developed with hexane/diethyl ether/acetic acid (80:20:2, v/v/v). Lipids were visualized with iodine vapor and imaged using a desktop scanner (Guo et al., 2009; Watson, 2006).\

Immunohistochemistry (IHC)

Tissue sections were cut from paraffin blocks of mouse brain growing orthotropic xenograft GBM tumor at 5 µm. The tissue slides were melted in oven at 60^{o}C for 30 min, and then deparaffinized by xylenes 3 times for 5 min each followed by dipping in graded alcohols (100%, 95%, 80% and 70%) 3 times for 2 min each. Slides were washed with distilled water (dH_2O) 3 x 5 min, and then immersed in 3% Hydrogen Peroxide for 10 min followed by being washed thoroughly with dH_2O. Slides were transferred into pre-heated 0.01M Citrate buffer (pH 6.0) in a steamer for 30 min, and then washed with dH_2O and PBS after cooling. Slides were blocked with 3% BSA/PBS for 1 hr at room temperature, and then incubated with primary antibody overnight at 4°C, followed by incubation with secondary antibody for 30 min at room temperature. After incubation with avidin-biotin ABC complex (#PK-4000, Vector labs, Burlingame, CA) followed by PBS wash 3 x 5 min and staining with DAB solution (#SK-4105, Vector labs, Burlingame, CA), slides were washed thoroughly with tap water, counterstained with hematoxylin (#H-3401, Vector labs, Burlingame, CA) and dipped briefly in graded alcohols (70%, 80%, 95% and 100%), in xylenes 2 x 5 min. Finally slides were mounted and imaged.

Mice luminescent Imaging

Mice implanted with cells expressing luciferase were injected Luciferin (Perkin Elmer) solution (15 mg/ml in PBS, dose of 150 mg/kg) by an intraperitoneal route that is allowed to distribute in awake animals for about 5-15 min. The mice are placed into a clear Plexiglas anesthesia box (2.0-3.0% isoflurane) that allows unimpeded visual monitoring of the animals; animals are then placed on non-fluorescent black paper on the imaging platform of an IVIS Lumina II to reduce background noise. The imaging chamber is continuously infused with 1-1.5% of isoflurane, and imaging platform is heated at 37°C to keep the mice warm. Animals are imaged 10 min after Luciferin injection to ensure consistent photon flux. Image was analyzed using Living Image software and quantified by measuring the luminescence signal intensity on the regions of interest (ROIs). The total numbers of photons within each ROI were calculated. ROI size was helping constant across all images. This imaging experiment was conducted at OSU Small Animal Imaging Core.

Supplemental References

Cheng, C., Ru, P., Geng, F., Liu, J., Yoo, J.Y., Wu, X., Cheng, X., Euthine, V., Hu, P., Guo, J.Y., *et al.* (2015). Glucose-Mediated N-glycosylation of SCAP Is Essential for SREBP-1 Activation and Tumor Growth. Cancer Cell *28*, 569-581.

Guo, D., Prins, R.M., Dang, J., Kuga, D., Iwanami, A., Soto, H., Lin, K.Y., Huang, T.T., Akhavan, D., Hock, M.B., *et al.* (2009). EGFR signaling through an Akt-SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to antilipogenic therapy. Sci Signal *2*, ra82.

Nohturfft, A., Brown, M.S., and Goldstein, J.L. (1998). Topology of SREBP cleavage-activating protein, a polytopic membrane protein with a sterol-sensing domain. The Journal of biological chemistry *273*, 17243-17250.

Watson, A.D. (2006). Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Lipidomics: a global approach to lipid analysis in biological systems. J Lipid Res *47*, 2101-2111.

Zhang, X., Zhao, X., Fiskus, W., Lin, J., Lwin, T., Rao, R., Zhang, Y., Chan, J.C., Fu, K., Marquez, V.E., *et al.* (2012). Coordinated silencing of MYC-mediated miR-29 by HDAC3 and EZH2 as a therapeutic target of histone modification in aggressive B-Cell lymphomas. Cancer Cell *22*, 506-523.