SUPPLEMENTAL INFORMATION

MiR-33a Promotes Glioma Initiating Cell Self-renewal via PKA/Notch Pathways

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Supplemental Figures

Supplemental Figure S1. Characterization of CD133-enriched GICs from xenografted glioma lines D456MG and 11-0040. (*A, B*) CD133 IF staining was performed on frozen tissue sections from xenografted tumors with different passages and percentage of CD133+ cells in the tumor sections was measured by ImageJ. For each frozen tissue, 8 images were viewed from one section. (**$p<0.01$) (*C, E*) Neurosphere formation assay was performed on CD133+ and CD133-negative cells isolated from D456MG and 11-0040 xenograft lines. 50 cells from either D456MG and 11-0040 were plated in each well of 24-well plates. (*D, F*) Limiting dilution assay with the measurement on formation of intracranial tumors was performed using varied number of CD133+ and CD133-negative cells as indicated. Each group contained 3 mice that were monitored for up to 160 days after implantation. Incidences of intracranial tumor formation and median survival days were shown. For CD133-negative cells derived from both gliloma lines, inoculation with 1,000 cells led to termination of one mouse in each group at 73 and 121 days due to tumor growth-caused neurological symptoms, respectively. The remaining two mice in both groups were healthy until the end of experiment at 160 days.

Supplemental Figure S2. Expression levels of stemness markers in GICs and non-GICs. qRT-PCR analysis was performed to determine relative mRNA levels of indicated stemness markers in GICs and non-GICs selected by defined media from 12 patient-derived samples.

Supplemental Figure S3. Impact of overexpressing four miRNAs on the formation of neurospheres in CD133+ cells. Neurosphere formation assays were performed using CD133+ D456MG GICs expressing vector control or indicated individual primary miRNAs. 50 cells were plated in each well of 24-well plates. (**p<0.01 compared to control group, NS: not significant.)

Supplemental Figure S4. Correlation between expression levels and methylation status of the promoter for SREBP2/miR-33a. (*A*) qRT-PCR was performed to measure the levels of SREBP2 and miR-33a in CD133+ and CD133- cells isolated from D456MG and 11-0040. (*B*) Bisulphite genomic sequencing analysis of CpG islands on the *SREBP2* promoter in CD133+ and CD133- cells isolated from D456MG and 11-0040. For each sample, eight colonies were picked for sequencing and the indicated methylation sites were labeled on the basis of the status of sequenced CpG islands. Black circles and open circles represent methylated and unmethylated CpG dinucleotides, respectively.

Supplemental Figure S5. Expression profiles of miR-33a in GICs with miR-33a-sponge or non-GICs ectopically expressing miR-33a. (*A, B*) qRT-PCR analysis was performed to determine the levels of miR-33a and miR-15a (used as a control) in CD133+ D456MG (*A*) or 11-0040 (*B*) cells with miR-33a knockdown by sponge or miR-Zip. (*C, D*) qRT-PCR analysis was performed to determine the miR-33a levels in CD133-negative D456MG (*C*) or 11-0040 (*D*) cells with miR-33a ectopic expression (CD133- 33a) compared to scramble control (CD133- scr control) or CD133+ cells isolated from the same tumor sample. (**p<0.01.)

Supplemental Figure S6. miR-33a promotes self-renewal and tumor progression of GICs. (*A, D, G*) Neurosphere formation assays were performed in CD133+ 11-0040 or D456MG cells expressing vector control or the miR-33a sponge, Zip-NT control or Zip-33a as indicated. For D456MG GICs, 20 cells were plated per well in 24-well plates; for 11-0040 GICs, 50 cells were plated per well in 24-well plates. (*B*) qRT-PCR analysis was performed to determine expression level of stemness-associated genes in CD133+ 11-0040 cells expressing scramble control or the miR-33a sponge. β-actin was used as internal control. (*C, E, H*) Kaplan-Meier curve were shown to measure progression of tumors initiated by 11-0040 or D456MG cells expressing vector control or the miR-33a sponge, Zip-NT control or Zip-33a. 5,000 CD133+ D456MG cells and 10,000 CD133+ 11-0040 cells were implanted respectively in each mouse. Each group contained 5 animals in C, H and 10 animals in E. (*F*) Neurosphere formation assays were performed in SSEA-1+ D456MG cells expressing scramble control or the miR-33a sponge as indicated. 50 cells were plated per well in 24-well plates. (*I*) Representative H&E staining of sections of intracranial tumors derived from CD133+ D456MG or 11-0040 cells expressing Zip-NT control and Zip-33a. There were three animals in each group. Pictures were taken at tumors' maximum cross sections from each brain. Scale bar: 5.0mm. (*J*) Neurosphere formation assay of CD133-negative 11-0040 cells with miR-33a overexpression compared to vector control. 100 cells were plated in each well of 24-well plates. (* $p<0.05$, ** $p<0.01$ compared to control group.)

Supplemental Figure S7. miR-33a exerts detectable positive effect on cell proliferation but little effect on apoptosis or multi-lineage differentiation. (*A*) Edu incorporation assay was performed on CD133+ D456MG cells with or without miR-33a knockdown. Cells were incubated with 10 μg/ml Edu for 2 hours, stained with FITC conjugated Edu antibody and analyzed by FACS. (*B, C*) Caspase 3/7 activities were measured in CD133+ D456MG/11-0040

cells with or without miR-33a knockdown using an apoptosis kit from Promega (G8090). (*D*) D456MG CD133+ cells expressing scramble control or the miR-33a sponge were induced to differentiation in 5% serum for 10 days. Then immunocytochemistry staining was performed to assess the expression pattern of differentiation markers as indicated. Scale bar: 50um. (*E*) Quantification of the cells expressing differentiation markers O4, GFAP, or TuJ-1 as indicated.

Supplemental Figure S8. Impact of changing miR-33a expression on H9 ES cell-derived neural stem cells and hTERT-E6/E7/Ras transformed human astrocytes. (*A*) qRT-PCR analysis was performed to determine the miR-33a levels in H9 ES cell-derived NSCs, D456MG CD133+ or CD133-negative cells. (*B*) qRT-PCR analysis was performed to determine the expression level of stemness-associated genes in H9 NSCs expressing scramble control or the miR-33a sponge. β-actin was used as internal control (*C*) qRT-PCR analysis was performed to determine the miR-33a levels in normal human astrocytes (NHA) and hTERT-E6/E7/Ras transformed astrocytes as indicated. (*D*) Neurosphere formation assays were performed in hTERT E6/E7/Ras transformed cells expressing scramble control or the miR-33a sponge as indicated. 100 cells were plated per well in 24-well plates. A representative sphere is shown in the window, scale bar: 50um. (**$p<0.01$ compared to either of the other two groups).

Supplemental Figure S9. MiR-33a over-expression leads to preferential down-regulation of predicted miR-33a targets. Microarray data of CD133-negative D456MG cells with miR-33a over-expression were analyzed to generate Cumulative Probability Density Function plots of LogFC for total microarray probes (11107) and the top 634 predicted miR-33a targets from TargetScan 6.0. ($p<0.0001$.)

Supplemental Figure S10. Predicted binding sites of miR-33a in the 3'UTR of PDE8A and UVRAG, respectively.

Supplemental Figure S11. Overexpression/knockdown of PDE8A and UVRAG compromised/enhanced self-renewal and tumor progression of CD133+ GICs. (*A, B*) Immunoblots were performed to verify overexpression of PDE8A and UVRAG in CD133+ D456MG cells (*A*) or their knockdown with two independent shRNAs in CD133-negative D456MG cells (*B*), respectively. γ-tubulin was used as internal control. (*C*) Neurosphere formation assays were performed in CD133+ D456MG cells expressing vector control, PDE8A or UVRAG. 50 cells were plated in each well of 24-well plate. (*D*) Growth rate of CD133+ D456MG cells expressing vector control, PDE8A and UVRAG were measured by cell titer assay. (*E*) Kaplan-Meier curve were shown to measure the tumor progression ability of CD133+ D456MG cells expressing vector control, PDE8A or UVRAG. 5,000 D456MG GICs were implanted in each mouse. Each group contained 5 animals. (*F*) Neurosphere formation assays were performed in CD133-negative D456MG cells expressing a specific shRNA for PDE8A or UVRAG as indicated. 100 cells were plated in each well of 24-well plate. (*G*) Neurosphere formation assays were performed in CD133+ 11-0040 cells expressing miR-33a sponge or scramble control with stable PDE8A and/or UVRAG knockdown or scramble control as indicated. 50 cells were plated in each well of 24-well plate. (*$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to control group.)

Supplemental Figure S12. cAMP levels in miR-33a overexpressing and PDE8A knockdown cells. cAMP levels were measured by cAMP-Glo™ Assay (Promega) in CD133-negative D456MG (*A*) and 11-0040 (*B*) cells after miR-33a overexpression or PDE8A knockdown. (*$p<0.05$ compared to control group).

Supplemental Figure S13. MiR-33a overexpression leads to preferential up-regulation of genes associated with PKA and Notch pathways. Microarray data of CD33-negative D456MG cells over-expressing miR-33a were analyzed to generate the Cumulative Probability Density Function plots of LogFC for total microarray probes (11107) and 80 cAMP/PKA pathway genes (*A*) or 68 Notch pathway target genes (*B*). $p<0.01$ in both sets.

Supplemental Figure S14. mRNA levels of Notch1 and subunits of γ-secretase. (*A, C, E*) qRT-PCR was performed to measure mRNA levels of Notch1 (*A*) and different subunits of γ-secretase from CD133-negative cells expressing vector control or miR-33a in D456MG (*C*) and 11-0040 (*E*). (*B, D, F*) qRT-PCR was performed to measure mRNA levels of Notch1 (*B*) and different subunits of γ-secretase from CD133+ cells expressing scramble control or miR-33a sponge in D456MG (*D*) and 11-0040 (*F*).

Supplemental Figure S15. Protein levels of PDE8A and UVRAG in subcutaneously xenografted tumors treated with the miR-33a LNA inhibitor. (*A*) Protein levels of PDE8A and UVRAG were measured by immunoblots in CD133+ D456MG or 11-0040 cells after treatment of miR-33a inhibitor or scramble control. γ-tubulin was used as internal control. (*B, C*) Representative Images of IHC staining of PDE8A (*B*) and UVRAG (*C*) in harvested subcutaneous 11-0040 tumors. Scale bars: 150μm. Quantification of these results was measured from five random fields of the samples by ImageJ. (* $p<0.05$, ** $p<0.01$ compared to control group)

Supplemental Figure S16. Scramble control for miR-33a expression analysis used for in situ hybridization of GBM tissues. Two representative images are shown. Scale bars: 100 μm.

Supplemental Tables S1. Summary of the up- and down-regulated miRNAs in three GBM patient-derived sample sets, as well as in three xenografted tumor samples derived from patients but maintained in nude mice in serial passages. 16 miRNAs that were commonly deregulated significantly in both patient specimens and xenografts are shown with miR-17, miR-18a, miR-20a, miR-20b, miR-106b, and miR-93 belonging to the miR-17-92 cluster (please refer to Supplemental Table S2 for the whole dataset).

microRNA	Adjusted P value	Fold Change
hsa-miR-345	0.0038	6.39
hsa-miR-101	0.0090	66.41
hsa-miR-17	0.0090	24.22
hsa-miR-93	0.0090	19.86
hsa-miR-18a	0.0110	26.11
hsa-miR-20b	0.0110	21.56
hsa-miR-106b	0.0112	41.07
hsa-miR-130a	0.0124	13.24
hsa-miR-15a	0.0124	21.21
hsa-miR-33a	0.0127	15.94
hsa-miR-20a	0.0162	26.85
hsa-miR-301a	0.0240	45.10
hsa-miR-181a	0.0292	12.57
hsa-miR-103	0.0322	13.33
hsa-miR-150	0.0380	0.37
hsa-miR-208a	0.0380	0.46

Supplemental Tables S2. A separate excel file is attached. LogFC and p value of 80 miRNAs of GICs compared to non-GICs in three GBM patient specimens (11-0236, 11-0296, 12-0133) and three xenografted glioma lines (D456MG, 10-0228, 11-0040).

Supplemental Table S3. Fold change of commonly down-regulated genes in 11-0040 and D456MG CD133-negative cells with miR-33a overexpression compared to control.

Gene	11-0040 CD133-	D456MG CD133-
ABHD2	0.722	0.792
BACH1	0.547	0.390
CDR2	0.398	0.440
CPT1A	0.415	0.185
GALNT10	0.763	0.689

IRS1	0.953	0.556
KCNJ2	0.613	0.779
KCTD9	0.536	0.596
MAP4K5	0.566	0.436
NCAM1	0.616	0.160
PDE8A	0.785	0.501
PRDM10	0.664	0.537
SEMA6A	0.914	0.903
SERP-INF	0.904	0.841
SETD4	0.354	0.770
SNAI2	0.193	0.371
TMED5	0.707	0.975
UVRAG	0.688	0.487

Supplemental Table S4. Clinical and pathological features of GBM patients and relationship with expression profiles of miR-33a.

Variables	**All Cases (n=107)**	**Low expression (n=37)**	**High expression (n=70)**	***p* value***
Age (years)				
≥45	46	16	30	0.565
< 45	61	21	40	
Sex				
Female	50	18	32	0.465
Male	57	19	38	
Tumor location				
Frontal	49	14	35	0.159
Nonfrontal	58	23	35	
Extend of resection				
Total and subtotal	58	20	38	0.571
Partial	49	17	32	
Karnofsky score				
≥70	51	15	36	0.193
< 70	56	22	34	
Smoking status				
Ever or current	53	22	31	0.098
Never	54	15	39	
Alcohol consumption				
Ever or current	54	16	38	0.189
Never	53	21	32	
Hypertension				
Yes	47	19	28	0.179
No	60	18	42	

*$\chi 2$ Test.

Supplemental Materials and Methods

Plasmids. miR-33a sponge containing five repeats of anti-sense miR-33a (TGCAAT GCAACTACAATGCAC), or a scramble sequence (AAGUUUUCAGAAAGCUAACA, Ebert et al. 2007) was synthesized by GenScript and cloned into pWPXLD vector (Addgene). Zip-NT and Zip-33a plasmids were purchased from System Biosciences. Primary miR-33a was cloned into pWPXLD vector to overexpress miR-33a. A scramble sequence was cloned into the same vector as a control. PDE8A and UVRAG cDNA were cloned into pLenti vector (Addgene) to overexpress these two genes. All these vectors were co-transfected with the packaging vectors psPAX2 and pCI-VSVG (Addgene) into 293T cells by Lipofectamine 2000 (Invitrogen) to produce virus. Virus-infected GICs or differentiated glioma cells were selected by GFP marker or puromycin (1ug/ml) according to the plasmids used for infection.

qRT-PCR. Total RNA was prepared using the mirVana^{TM} miRNA siolation Kit (Ambion). To measure messenger RNA, total RNA was reverse transcribed into cDNA using iScript cDNA synthesis kit (BioRad) and mRNA levels were measured using specific primers (sequences were shown in following table) with SYBR Green (Qiagen) and a Mastercycler® Realplex system (Eppendorf). To measure miRNA, total RNA was reverse transcribed into cDNA by miScript RT Kit (Qiagen). Specific miRNA levels were measured by miScript primers (Qiagen) with the miScript SYBR Green PCR Kit (Qiagen).

Primer sequences for qRT-PCR analyses

Gene	Forward primer	Reverse primer
β-actin	CATCCACGAAACTACCTTCAACTCC	GAGCCGCCGATCCACACG
Lin28A	TGTAAGTGGTTCAACGTGCG	CCTCACCCTCCTTCAAGCTC
Oct-4	CATAGTCGCTGCTTGATCGCTTG	GAGAACCGAGTGAGAGGCAACC

Nanog	GATTTGTGGGCCTGAAGAAA	TTGGGACTGGTGGAAGAATC
UVRAG	TCCAAAAGGAGGGGAGAAGT	TGAAGTTTTTCAGGTTGGGAA
PDE8A	CCTCTTGGAGTCGGAGCTT	CCCTGCAGAATCCATTACATT
Pim1	CGGGAAGCTGGAGACAGAAG	CCCAGCAAATAGCAGCCTTT
ABCA1	GACGCAAACACAAAAGTGGA	AACAAGCCATGTTCCCTCAG
Cpt1a	TGCTTTACAGGCGCAAACTG	GCAGATGTGTCAGGACCGAGT
PRKAA1	TCCTACCACATCAAGGCTCC	TACATTCTGGGTGACACGCT
CROT	TGTTCACCACAGGGATACAAGCCT	TACCTTGGCCTCCCACCGTGCTAA
IRS1	GCTCAACTGGACATCACAGC	AATGGATGCATCGTACCATCT
CDK6	CTGAATGCTCTTGCTCCTTT	AAAGTTTTGGTGGTCCTTGA
IRS2	ACCTCAGTTCAAGGTAAAGCCGGA	AGGTACCTGCACTGGAATCCAACA
ABCG1	CAGGAAGATTAGACACTGTGG	GAAAGGGGAATGGAGAGAAGA
CCND1	ACGAAGGTCTGCGCGTGTT	CCGCTGGCCATGAACTACCT

Cell viability assay and caspase 3/7 activity assay. Cells with indicated modification or treatment were plated in white with clear bottom 96-well plates (Costar) at 2000 cells per well. For cell viability assay, plates were examined every 24 hours by Cell Titer-Glo® Luminescent Cell Viability Assay kit (Promega). For caspase 3/7 activity assay, plates were examined by the Caspase-Glo 3/7 kit from Promega (G8090). Results are reported from at least triplicate samples as the mean ± standard deviation.

Cell proliferation assay. Proliferation rates were determined using Click-iT® EdU Alexa Fluor® 647 Flow Cytometry Assay Kit (Invitrogen) according to the manufacturer's instructions. Briefly, cells were incubated with 10μM EdU for two hours, fixed and processed. Analyses were done using flow cytometry. Results are reported from at least triplicate samples as the mean ± standard deviation.

Multi-lineage differentiation assay. D456MG cells differentiation and immunocytochemistry were performed as described previously (Pollard *et al*. 2009). Briefly, six well plates with one 22 mm coverslip in each well were coated with Laminin (Sigma) for 3 hr at 10 ug/ml prior to use. 0.5x105 cells were seeded into a well in TNC culture media without EGF or FGF-2 and with 5% fetal calf serum for 10 days. Media was changed every 2-3 days. Cells was then fixed with 4% PFA for 15 min and permeabilized with 0.1% Triton X-100. For immunostaining, cells were incubated overnight with eFluor 660 conjugated Anti-GFAP (eBioscience, 50-9892), , eFluor 570 conjugated anti-Tuj1 (eBioscience, 41-4510) or mouse anti-O4 (R&D Systems, MAB1326) antibodies. Alexa fluor 594 goat anti-mouse IgM (Invitrogen, A21044) was used as secondary antibody for O4 antibody staining. Nuclei were counterstained with DAPI. Images were captured with Zeiss Axio Imager and analyzed using ImageJ software.

Bisulphite modification and genomic sequencing. The methylation status of the CpG dinucleotides close to the SERBF-2 promoter was analyzed. A bisulfite sequencing assay was performed on 1.0 mg of bisulfite-treated genomic DNA from cells. Bisulfite conversion was performed using the MethylDetector Bisulfite Modification Kit (Active Motif, 55001) according to the manufacturer's instructions. The fragments of interest were amplified using the following specific primer pairs designed with the MethPrimer software (Li *et al*. 2002): forward, 5′ - GGAGGGTGGTTTGTTAATTTTTTAT-3 ′ ; reverse, 5 ′ - TCAATAAAACCAAACTCATCTCAAC-3′. PCR products were cloned into the pCR2-TOPO TA vectors (Invitrogen). Individual bacterial colonies were picked and sequenced using the M13 Reverse primer (5′ - CAGGAAACAGCTATGACC-3′) to analyze DNA methylation.

Intracranial tumor assay. GICs with indicated cell number were implanted into the left or right frontal lobes of athymic BALB/c nu/nu mice under a Duke University Institutional Animal Care

and Use Committee approved protocol. Mice were maintained for 100 days or until the development of neurologic symptoms. Brains of euthanized mice were collected, fixed in formalin, paraffin embedded, and sectioned for H&E staining.

Microarray and analysis. Total RNA was prepared using the mirVanaTM miRNA siolation Kit (Ambion). After the RNA samples passed quality control, microarray was performed by Duke Microarray facility using GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix). Microarray data was analyzed by Duke Microarray facility using Partek Genomics Suite.

Immunoblot. Quantified protein lysates were resolved on Novex SDS-PAGE (Invitrogen), transferred onto PVDF membrane (Millipore, Billerica, MA) and probed with rabbit anti-human PDE8A (1:1000; Abcam), rabbit anti-human UVRAG (1:500; Cell Signaling),), rabbit anti-human p-CREB(1:1000; Cell Signaling),), rabbit anti-human CREB (1:1000; Cell Signaling),), rabbit anti-human NICD (1:1000; Cell Signaling) and mouse anti-human γ-tubulin (1:10000; Sigma) followed by incubation with secondary HRP-conjugated antibodies (1:5000; Invitrogen).

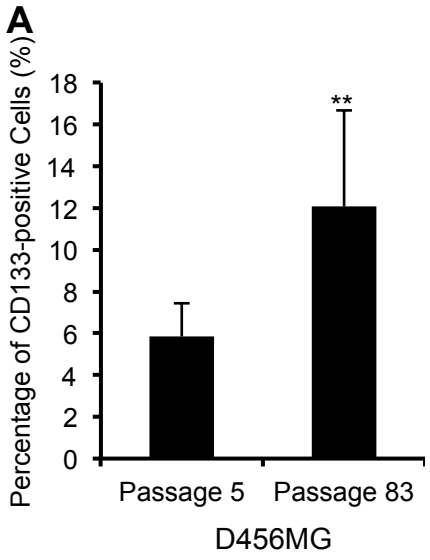
Luciferase reporter assays. 3'UTR of PDE8A (916bp, Forward primer: GACGCTAGCTGGG AGACACCACCCAGAGCCC; Reverse primer: GACGTCGACTGTTAAGTCCTAACTTTTCT TT) and UVRAG (1060bp, Forward primer: GACGCTAGCGTTCCATCTCTTCTAACCAGCC; Reverse primer: GACGTCGACCCACCCACACTGACTCCAAAAT) containing the predicted binding sites of miR-33a was cloned into NheI and SalI sites of a firefly luciferase reporter of pmirGLO (Promega). For the luciferase assay, 293T cells were co-transfected with 0.1 μg of firefly luciferase constructs and 0 μg, 0.5 μg or 1μg of miR-33a over-expressing plasmid respectively with 1 μg, 0.5 μg and 0 μg empty vector plasmid. And 50ng pRL-TK Renilla luciferase plasmid

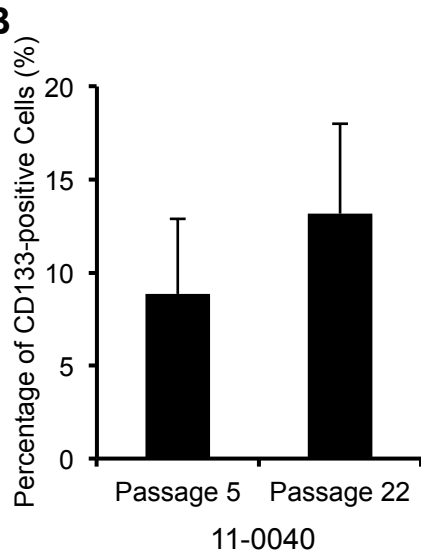
(Promega) was co-transfected as an internal control. Two days after transfection, firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay (Promega). The results were normalized as relative luciferase activity (firefly luciferase/Renilla luciferase) and reported from at least triplicate samples as the mean ± standard deviation.

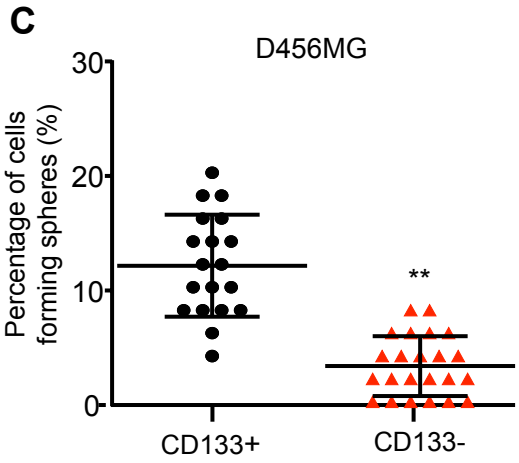
Lentiviral mediated shRNA targeting. Lentiviral shRNA clones (Sigma Mission RNAi) targeting PDE8A, UVRAG and scramble control (SHC002) were purchased from Sigma. Detailed information of shRNA constructs are provided in a supplemental table. These vectors were co-transfected with the packaging vectors psPAX2 and pCI-VSVG (Addgene) into 293T cells by Lipofectamine 2000 (Invitrogen) to produce virus.

Sequence of shRNA constructs

Name of constructs	TRC number	Clone ID	Sequence
sh-PDE8A	TRCN0000301061	NM_002605.2-1335s21c1	CCGGCCGGATACATTCCATGACAATCTCGAGATTGTCATGGAATGTATCCGGTTTTTG
sh-PDE8A	TRCN0000048874	NM_002605.1-1793s1c1	CCGGGCTAAGATCATGGTTACAAATCTCGAGATTTGTAACCATGATCTTAGCTTTTTG
sh-UVRAG	TRCN0000368916	NM_003369.3-2403s21c1	CCGGACTTAACCCTTTGTGATAATGCTCGAGCATTATCACAAAGGGTTAAGTTTTTTG
sh-UVRAG	TRCN0000005201	NM_003369.2-1265s1c1	CCGGGCCCTTGGTTATACTGCACATCTCGAGATGTGCAGTATAACCAAGGGCTTTTT

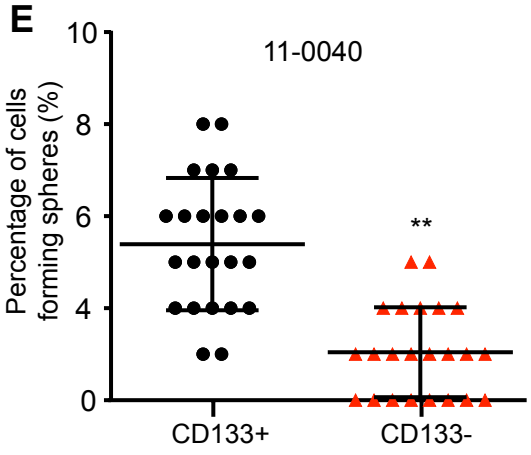






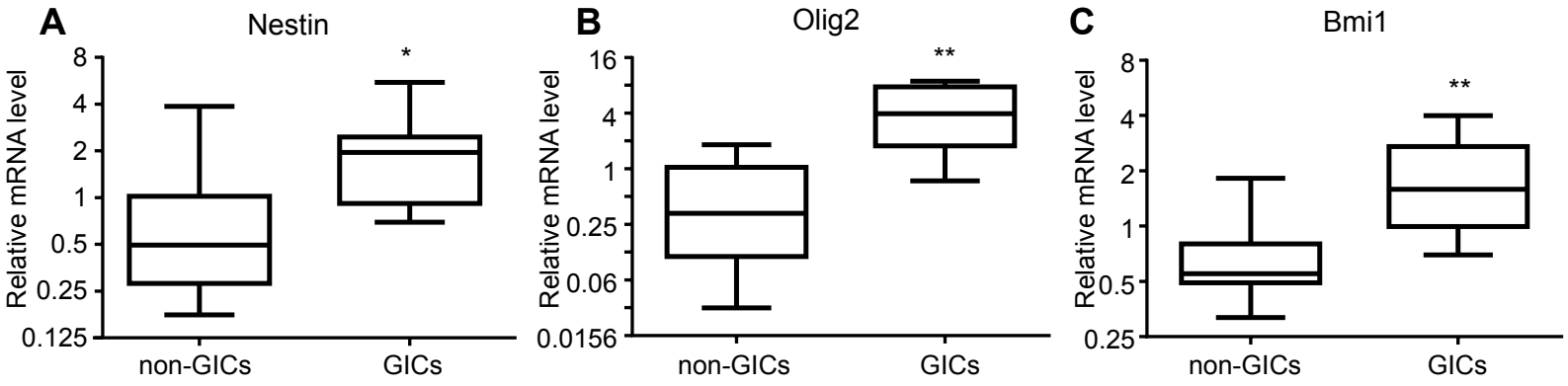
D

	D456MG CD133+		D456MG CD133-	
Cell number	10^4	10^3	10^4	10^3
Incidence	3/3	3/3	2/3	1/3
Median survival days	27	43	61	>160



F

	11-0040 CD133+		11-0040 CD133-	
Cell number	10^4	10^3	10^4	10^3
Incidence	3/3	3/3	2/3	1/3
Median survival days	74	78	80	>160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4
2
1
0.5
0.25
0.125
*
non-GICs
GICs
B
Olig2
Relative mRNA level
16
4
1
0.25
0.06
0.0156
**
non-GICs
GICs
C
Bmi1
Relative mRNA level
8
4
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1
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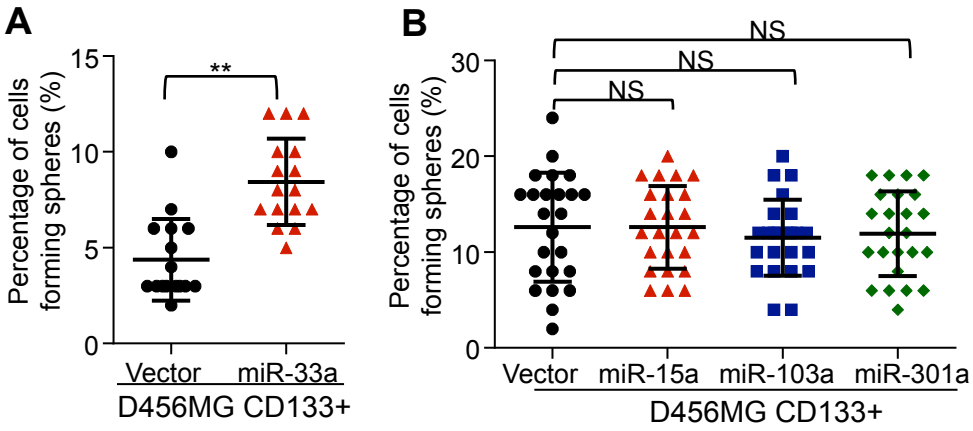
non-GICs
GICs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10
5
0
**
Vector
miR-33a
D456MG CD133+
B
Percentage of cells forming spheres (%)
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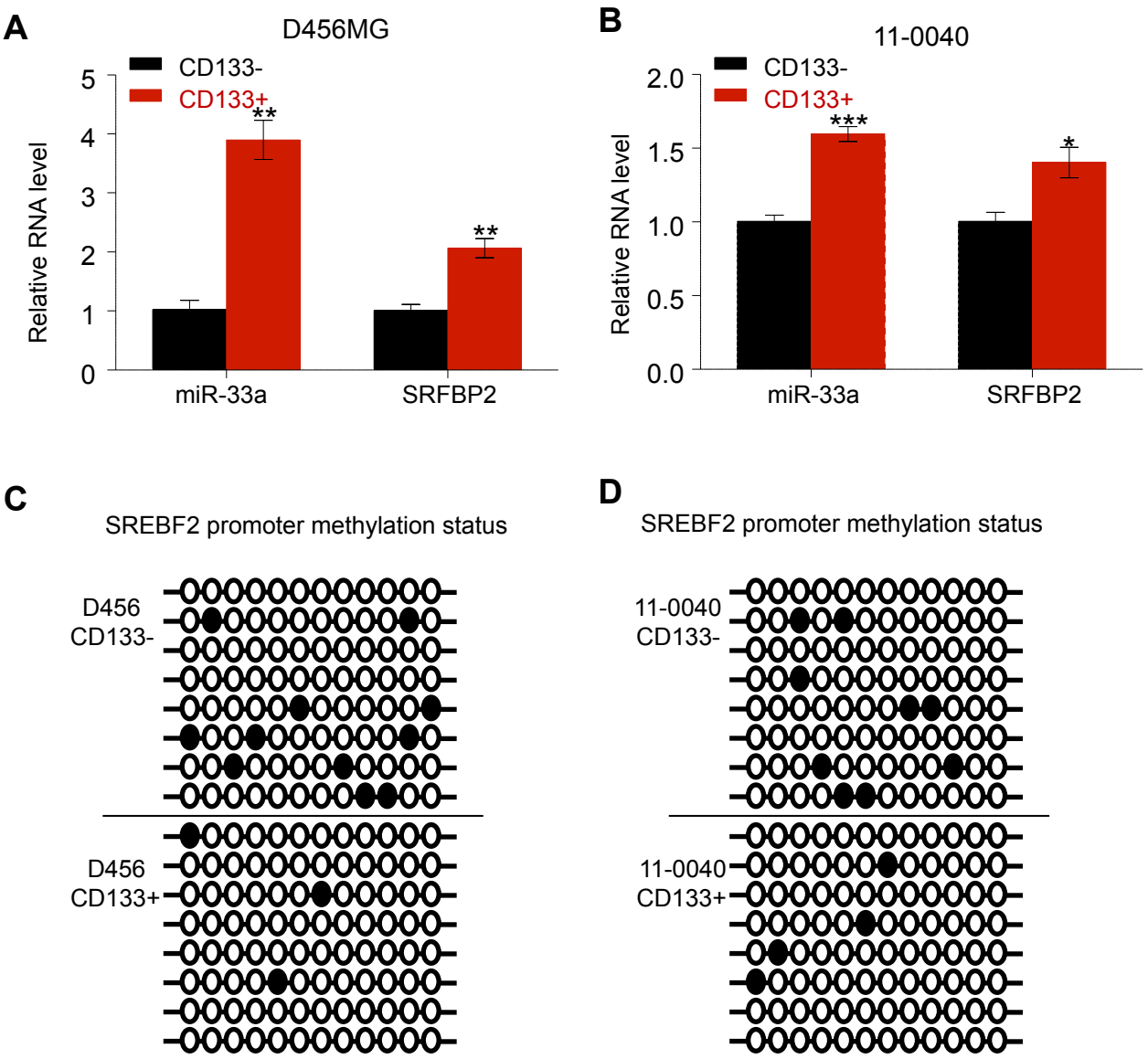
20
10
0
NS
NS
NS
Vector
miR-15a
miR-103a
miR-301a
D456MG CD133+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CD133-
CD133+
Relative RNA level
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2
3
4
5
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miR-33a
SRFBP2
B
11-0040
CD133-
CD133+
Relative RNA level
0.0
0.5
1.0
1.5
2.0
***
*
miR-33a
SRFBP2
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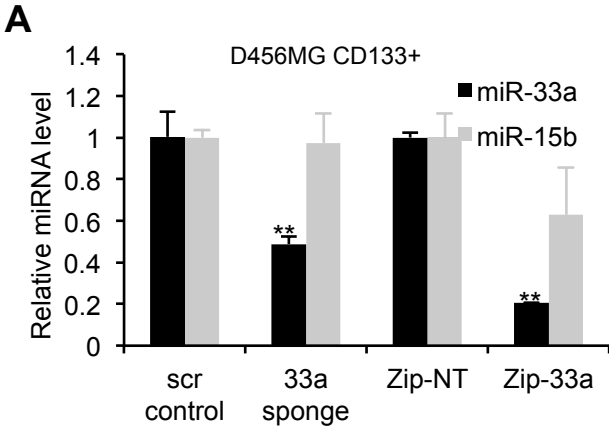
SREBF2 promoter methylation status
D456
CD133-
D456
CD133+
D
SREBF2 promoter methylation status
11-0040
CD133-
11-0040
CD133+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miR-33a
miR-15b
Relative miRNA level
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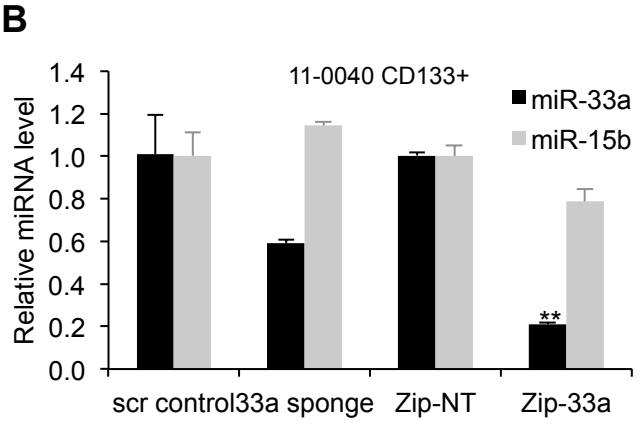
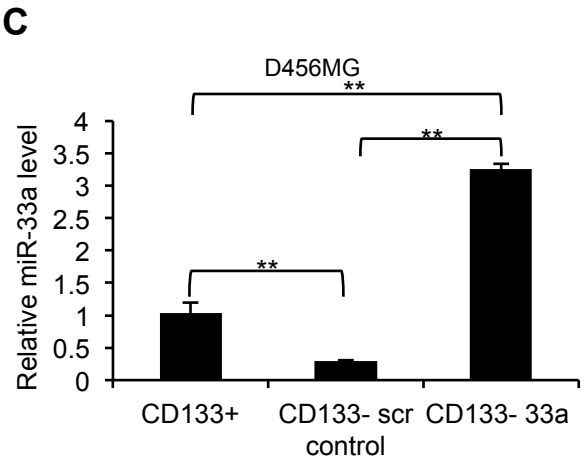
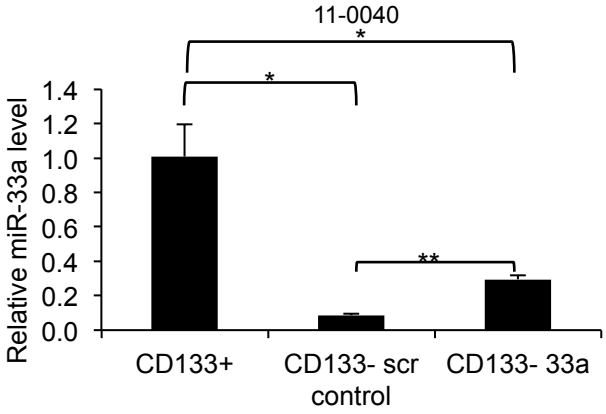
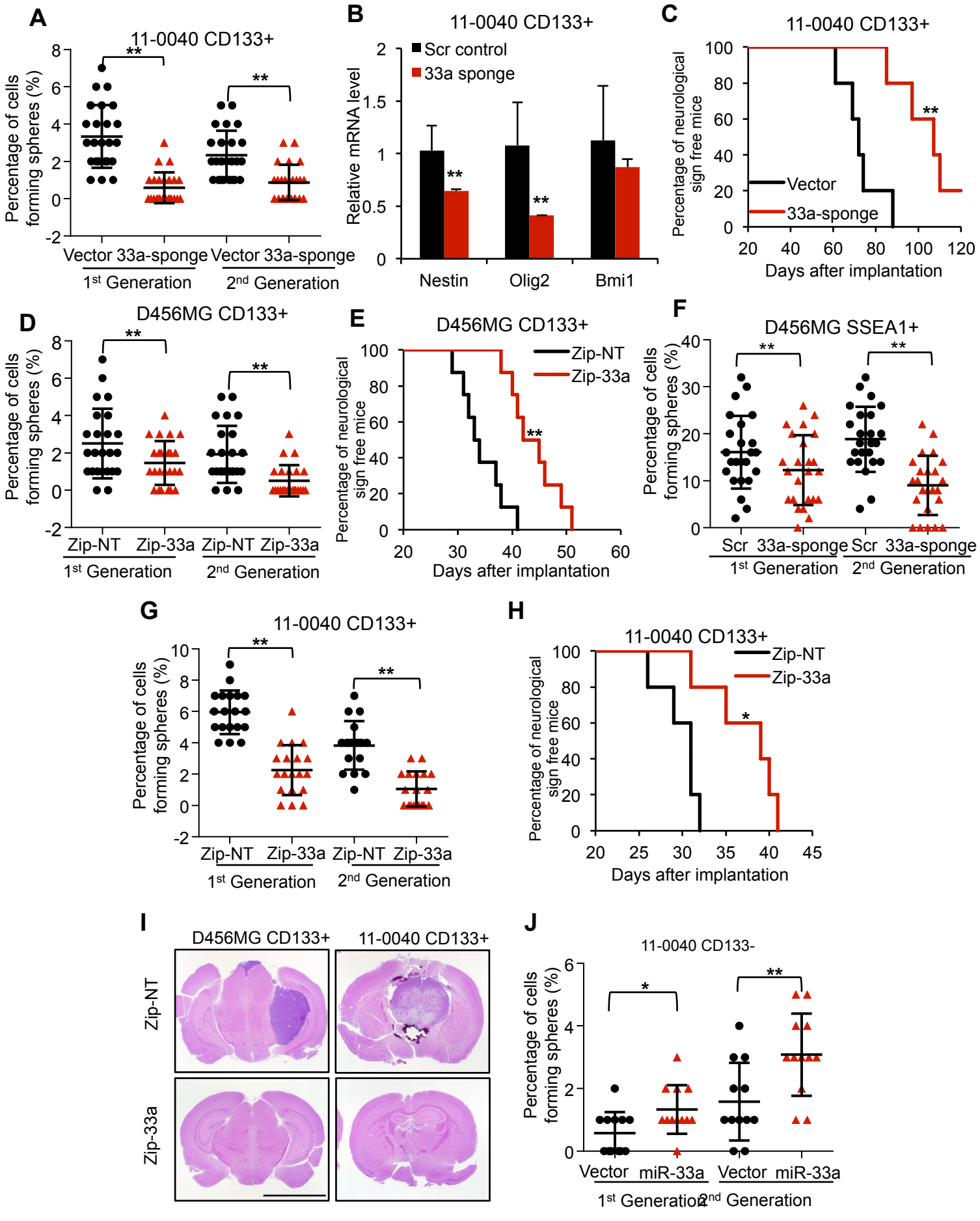
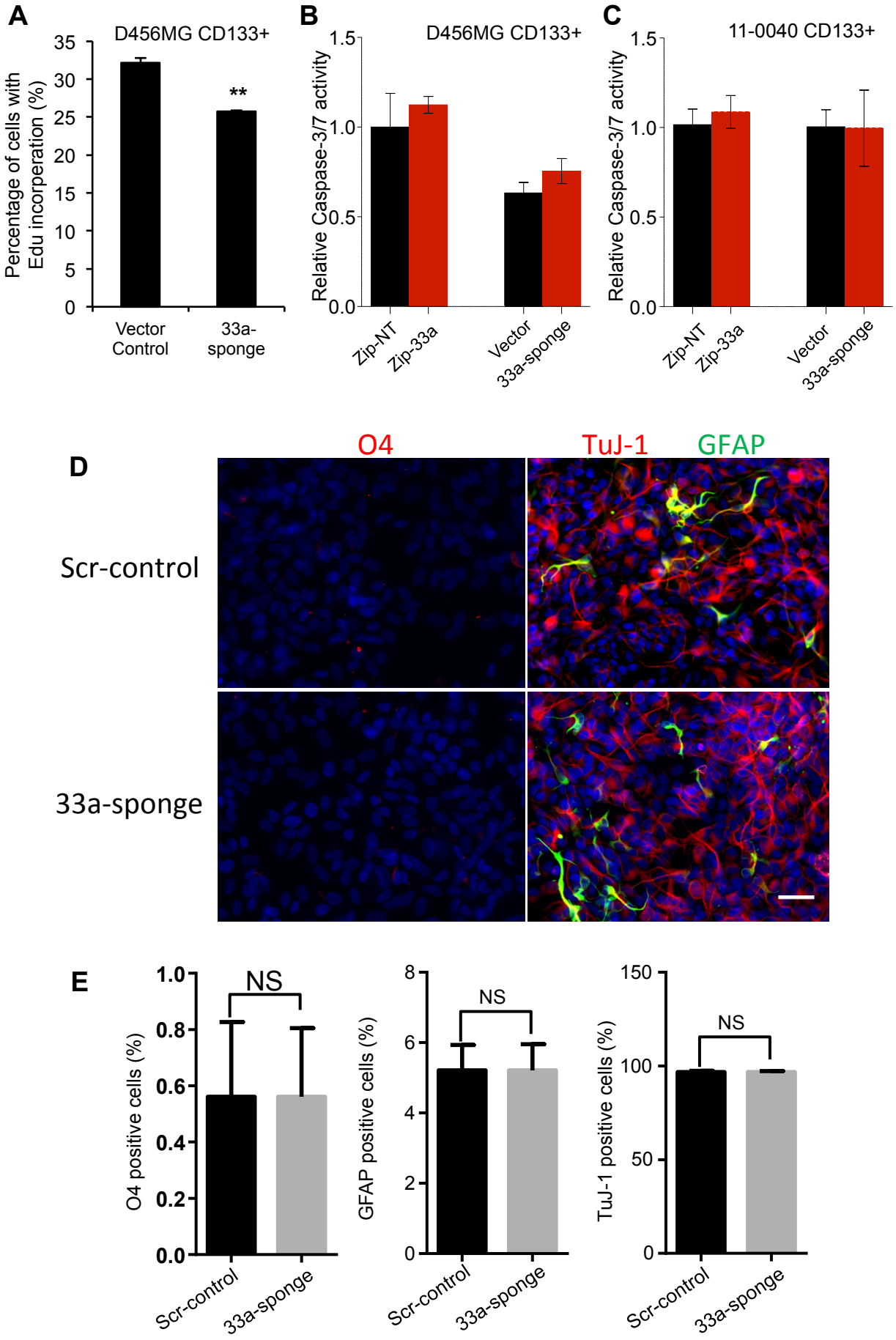
0
**
**
scr control
33a sponge
Zip-NT
Zip-33a

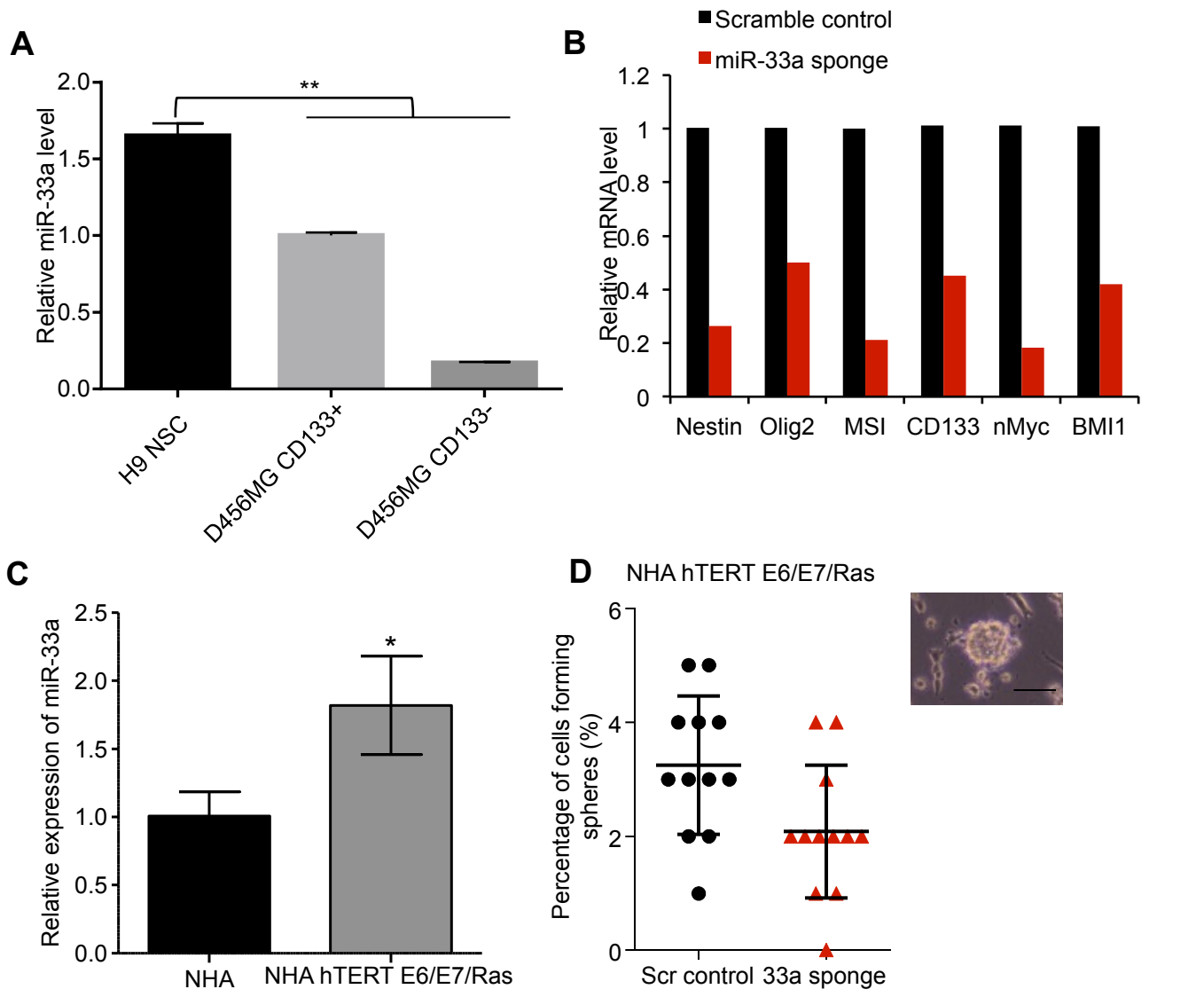

B
11-0040 CD133+
miR-33a
miR-15b
Relative miRNA level
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
scr control
33a sponge
Zip-NT
Zip-33a

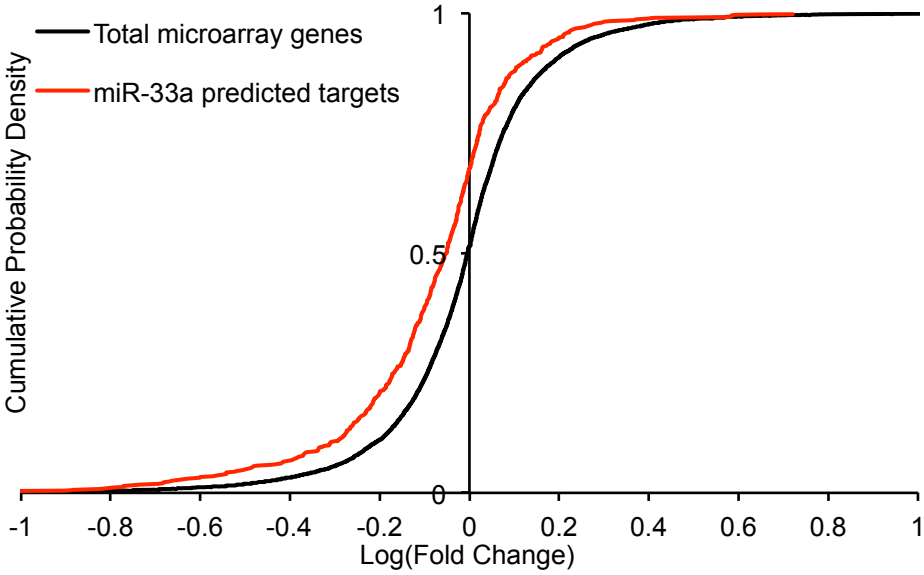

C
D456MG
**
**
**
Relative miR-33a level
4
3.5
3
2.5
2
1.5
1
0.5
0
CD133+
CD133- scr control
CD133- 33a


D
11-0040
*
*
**
Relative miR-33a level
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CD133+
CD133- scr control
CD133- 33a


A
11-0040 CD133+
Percentage of cells forming spheres (%)
Vector 33a-sponge Vector 33a-sponge
1st Generation
2nd Generation
B
11-0040 CD133+
Scr control
33a sponge
Relative mRNA level
Nestin
Olig2
Bmi1
C
11-0040 CD133+
Percentage of neurological sign free mice
Vector
33a-sponge
Days after implantation
D
D456MG CD133+
Percentage of cells forming spheres (%)
Zip-NT Zip-33a Zip-NT Zip-33a
1st Generation
2nd Generation
E
D456MG CD133+
Zip-NT
Zip-33a
Percentage of neurological sign free mice
Days after implantation
F
D456MG SSEA1+
Percentage of cells forming spheres (%)
Scr 33a-sponge Scr 33a-sponge
1st Generation
2nd Generation
G
11-0040 CD133+
Percentage of cells forming spheres (%)
Zip-NT Zip-33a Zip-NT Zip-33a
1st Generation
2nd Generation
H
11-0040 CD133+
Zip-NT
Zip-33a
Percentage of neurological sign free mice
Days after implantation
I
D456MG CD133+
11-0040 CD133+
Zip-NT
Zip-33a
J
11-0040 CD133-
Percentage of cells forming spheres (%)
Vector miR-33a Vector miR-33a
1st Generation
2nd Generation


A
D456MG CD133+
Percentage of cells with Edu incorporation (%)
35
30
25
20
15
10
5
0
**
Vector Control
33a-sponge
B
D456MG CD133+
Relative Caspase-3/7 activity
1.5
1.0
0.5
0.0
Zip-NT
Zip-33a
Vector
33a-sponge
C
11-0040 CD133+
Relative Caspase-3/7 activity
1.5
1.0
0.5
0.0
Zip-NT
Zip-33a
Vector
33a-sponge
D
O4
TuJ-1
GFAP
Scr-control
33a-sponge
E
O4 positive cells (%)
1.0
0.8
0.6
0.4
0.2
0.0
NS
Scr-control
33a-sponge
GFAP positive cells (%)
8
6
4
2
0
NS
Scr-control
33a-sponge
TuJ-1 positive cells (%)
150
100
50
0
NS
Scr-control
33a-sponge


A
Relative miR-33a level
2.0
1.5
1.0
0.5
0.0
**
H9 NSC
D456MG CD133+
D456MG CD133-
B
Scramble control
miR-33a sponge
Relative mRNA level
1.2
1
0.8
0.6
0.4
0.2
0
Nestin
Olig2
MSI
CD133
nMyc
BMI1
C
Relative expression of miR-33a
2.5
2.0
1.5
1.0
0.5
0.0
*
NHA
NHA hTERT E6/E7/Ras
D
NHA hTERT E6/E7/Ras
Percentage of cells forming spheres (%)
6
4
2
0
Scr control
33a sponge


Total microarray genes
miR-33a predicted targets
Cumulative Probability Density
1
0.5
0
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Log(Fold Change)

A

PDE8A (NM_002605)

3338bp-3358bp (21bp)

5'-AGACUUAUUUUCAUAAUGCAA-3'

3'-ACGUUACGUUGAUGUUACGUG-5'

B

UVRAG (NM_003369)

3190bp-3212bp (23bp)

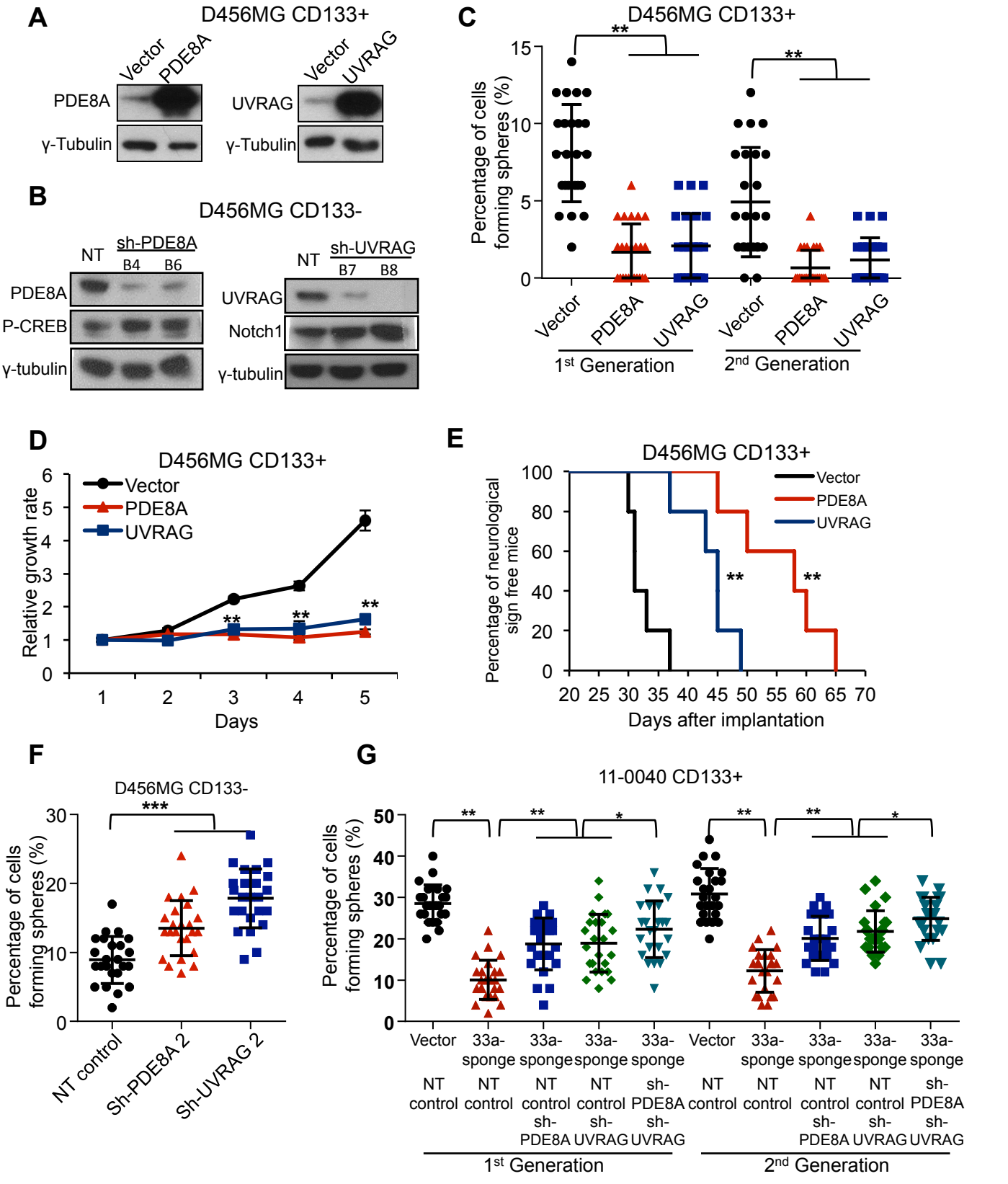
5'-AGGCAUGCUUUCUGGCAAUGCAC-3'

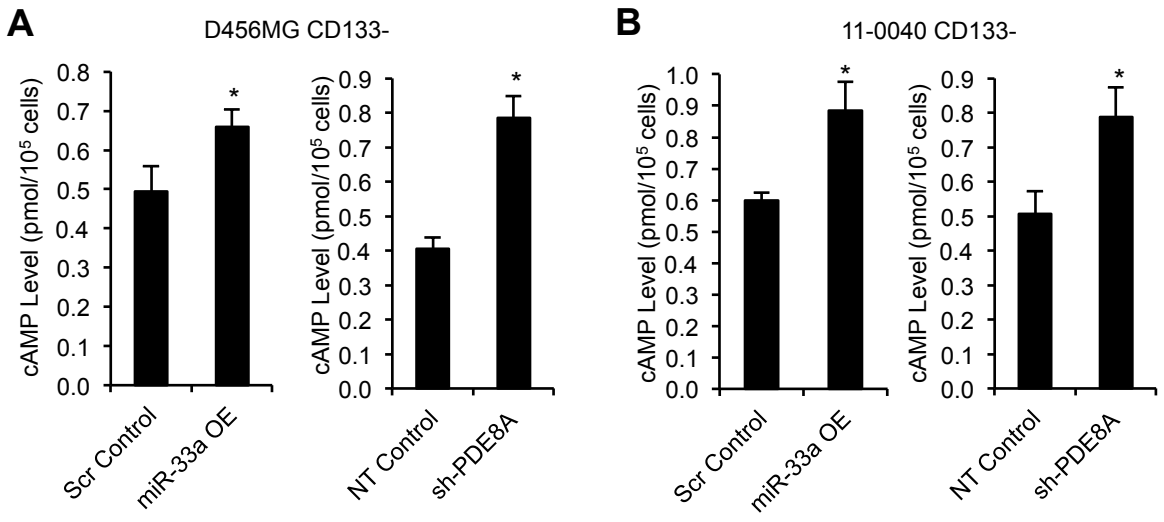
3‘-ACGUUACGUU_GAU_GUUACGUG-5‘

3831bp-3851bp (21bp)

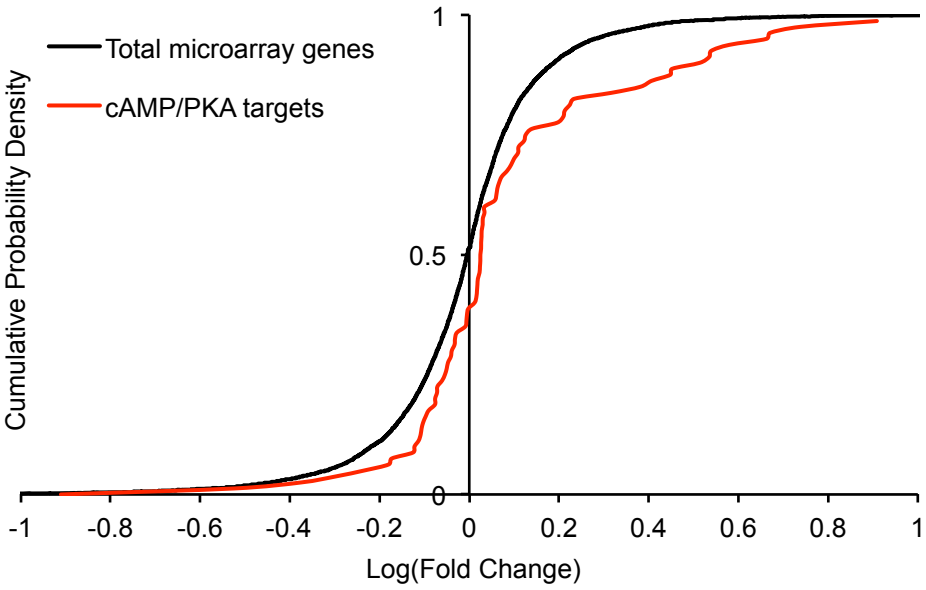
5‘-CUG_GGCUCAAGUU_UAAUGUAU-3'

3'- ACGUUACGUU_GAUGUUACGUG-5'

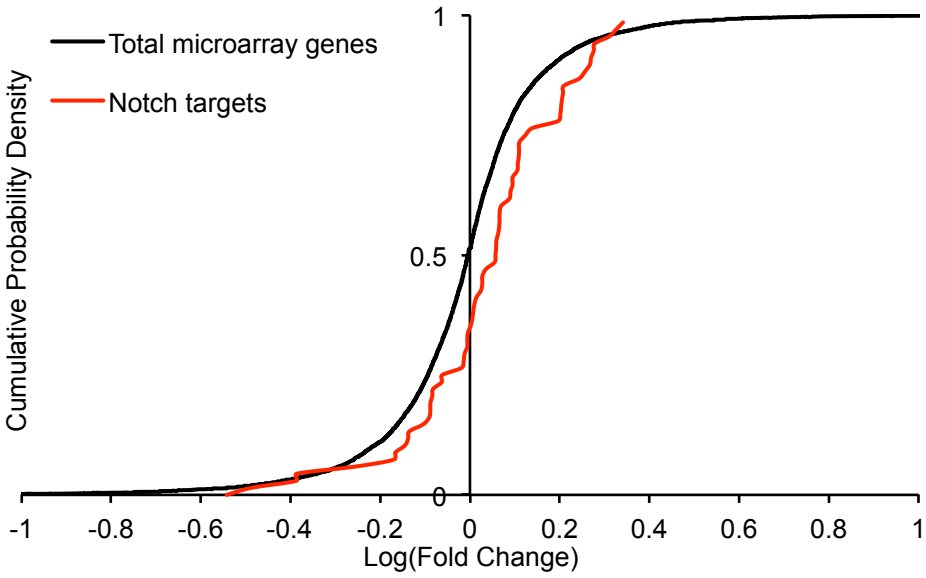

A
D456MG CD133+
Vector
PDE8A
PDE8A
γ-Tubulin
Vector
UVRAG
UVRAG
γ-Tubulin
B
D456MG CD133-
NT
sh-PDE8A
B4
B6
PDE8A
P-CREB
γ-tubulin
NT
sh-UVRAG
B7
B8
UVRAG
Notch1
γ-tubulin
C
D456MG CD133+
Percentage of cells forming spheres (%)
Vector
PDE8A
UVRAG
Vector
PDE8A
UVRAG
1st Generation
2nd Generation
D
D456MG CD133+
Relative growth rate
Vector
PDE8A
UVRAG
Days
E
D456MG CD133+
Percentage of neurological sign free mice
Vector
PDE8A
UVRAG
Days after implantation
F
D456MG CD133-
Percentage of cells forming spheres (%)
NT control
Sh-PDE8A 2
Sh-UVRAG 2
G
11-0040 CD133+
Percentage of cells forming spheres (%)
Vector
33a-sponge
NT control
sh-PDE8A
sh-UVRAG
1st Generation
2nd Generation

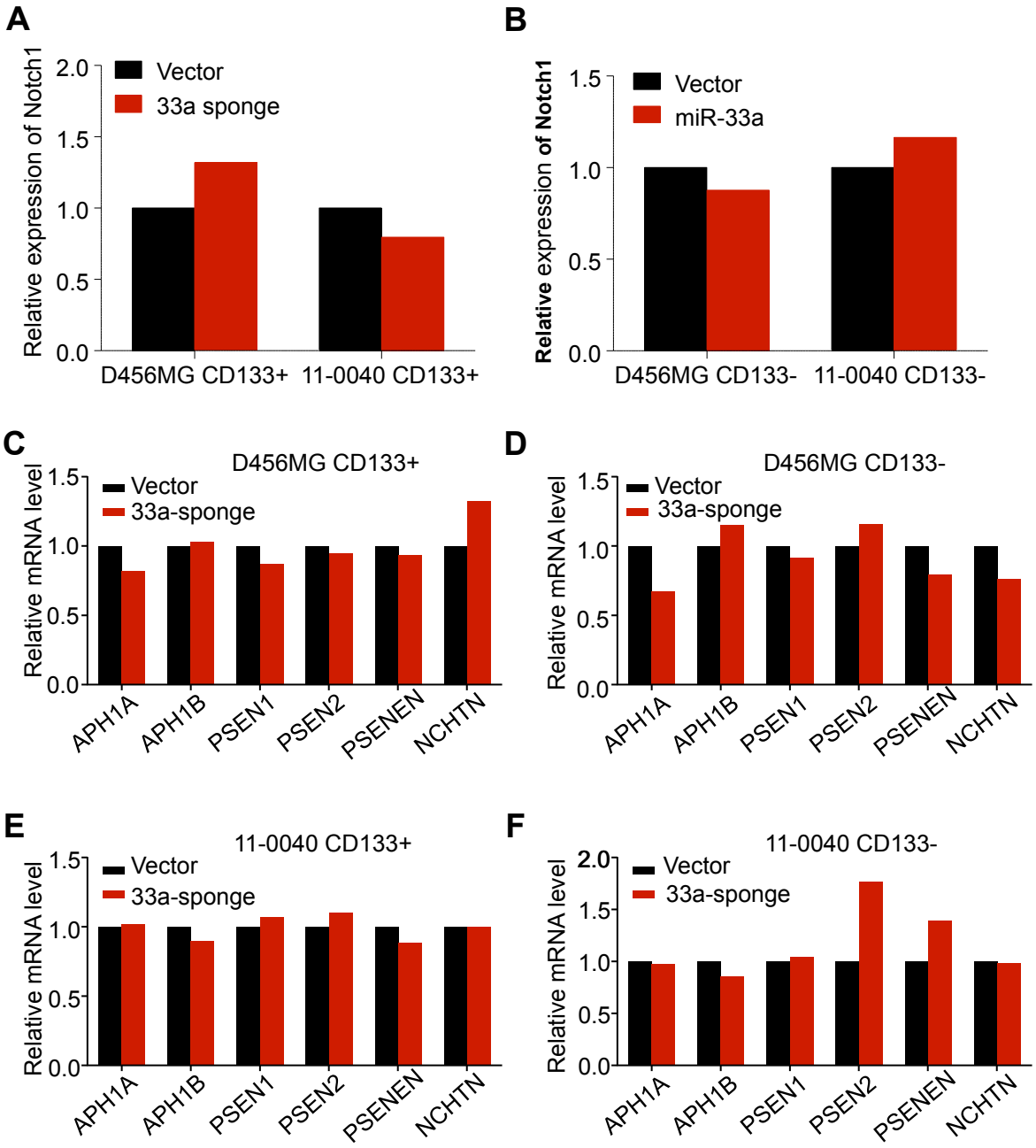

A
D456MG CD133-
cAMP Level (pmol/10^5 cells)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
*
Scr Control
miR-33a OE
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
*
NT Control
sh-PDE8A
B
11-0040 CD133-
cAMP Level (pmol/10^5 cells)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
*
Scr Control
miR-33a OE
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
*
NT Control
sh-PDE8A

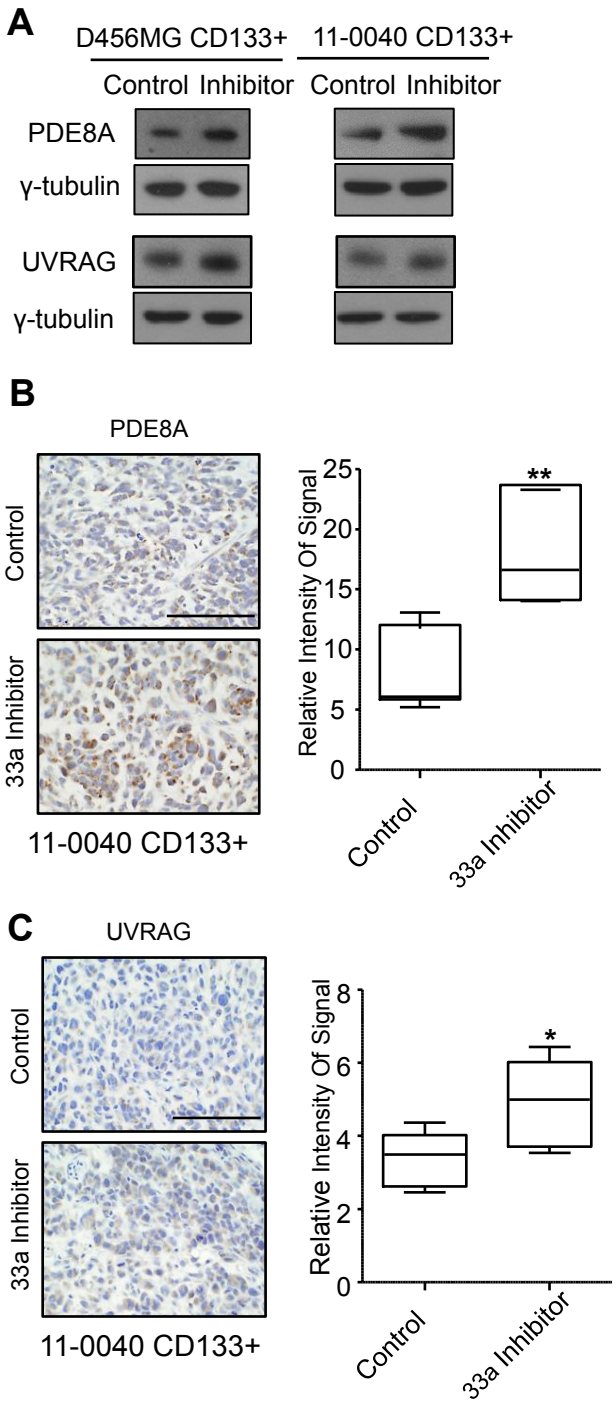
A

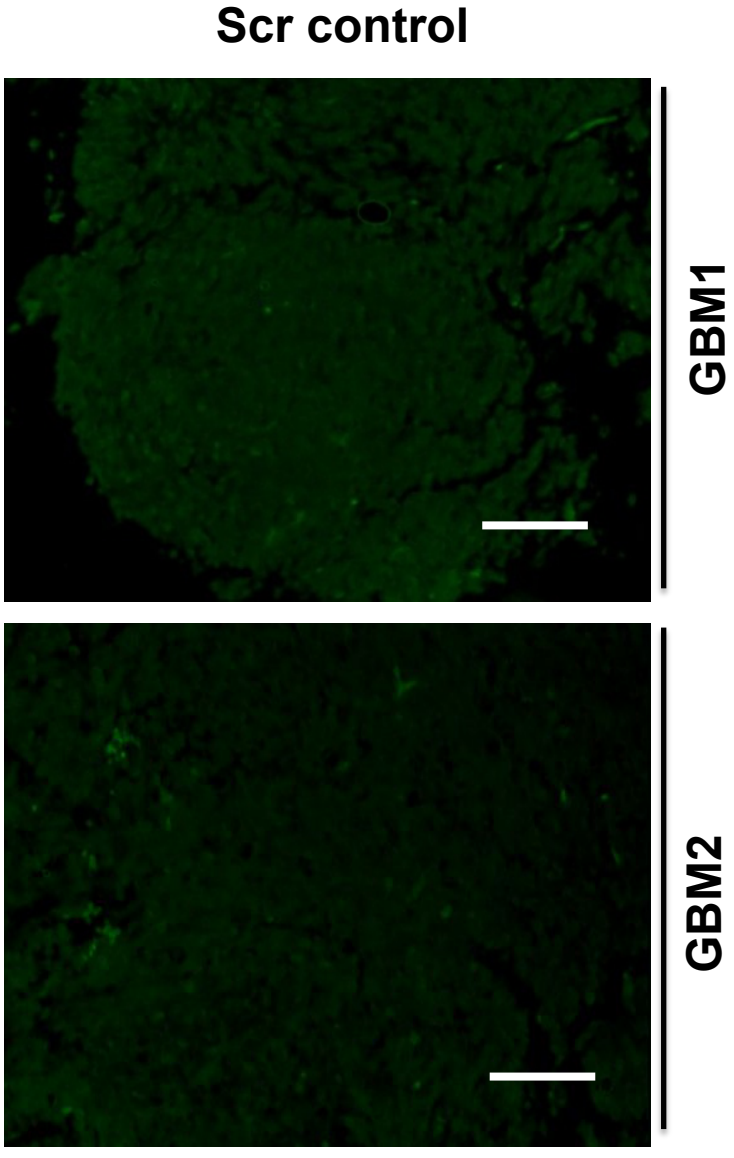

Total microarray genes
cAMP/PKA targets
Cumulative Probability Density
1
0.5
0
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Log(Fold Change)

B


Total microarray genes
Notch targets
Cumulative Probability Density
1
0.5
0
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Log(Fold Change)


A
Relative expression of Notch1
2.0
1.5
1.0
0.5
0.0
Vector
33a sponge
D456MG CD133+
11-0040 CD133+
B
Relative expression of Notch1
1.5
1.0
0.5
0.0
Vector
miR-33a
D456MG CD133-
11-0040 CD133-
C
D456MG CD133+
Relative mRNA level
Vector
33a-sponge
APH1A
APH1B
PSEN1
PSEN2
PSENEN
NCHTN
D
D456MG CD133-
Relative mRNA level
Vector
33a-sponge
APH1A
APH1B
PSEN1
PSEN2
PSENEN
NCHTN
E
11-0040 CD133+
Relative mRNA level
Vector
33a-sponge
APH1A
APH1B
PSEN1
PSEN2
PSENEN
NCHTN
F
11-0040 CD133-
Relative mRNA level
Vector
33a-sponge
APH1A
APH1B
PSEN1
PSEN2
PSENEN
NCHTN


A
D456MG CD133+
11-0040 CD133+
Control
Inhibitor
Control
Inhibitor
PDE8A
γ-tubulin
UVRAG
γ-tubulin
B
PDE8A
Control
33a Inhibitor
11-0040 CD133+
Relative Intensity Of Signal
25
20
15
10
5
0
**
Control
33a Inhibitor
C
UVRAG
Control
33a Inhibitor
11-0040 CD133+
Relative Intensity Of Signal
8
6
4
2
0
*
Control
33a Inhibitor


Scr control
GBM1
GBM2