Supporting Material

Materials and Methods

Northern blot analysis. Northern blot analysis was performed according to standard methods as previously described (1). In brief, total RNA (20μg), isolated from human glioma cell lines and GFAP$^+$ cells micro-dissected from glioma tissues, was dissolved in 2×RNA Loading Buffer (95% formamide, 0.025% SDS, 0.025% bromophenol blue, 0.025% xylene cyanol, 0.025% ethidium bromide, 0.5mM EDTA), heated at 95°C for 3 min, loaded onto denaturing 15% TBE-Urea gels and transferred onto positively charged nylon membranes (Roche, Penzberg, Germany). Northern blots were prehybridized at 65 ºC for 1 h using Hybridization Buffer (Roche) and subjected to hybridization with 3'-DIG-labeled RNA probe (100 ng/ml) for *miR-30e** and DIG labeled U6 probe (Takara, Dalian, China) overnight at room temperature. Probe detection was performed using the DIG Luminescent Detection Kit (Roche, Penzberg, Germany) according to manufacturer's protocol. After equilibration in detection buffer, blots were incubated with chemiluminescent substrate CDP-Star and exposed to Kodak Biomax MR film.

Primers and Oligonucleotides

Primers	
① used for subcloning and plasmid construction	
pri-*miR-30e**-up	GCCAGATCTGCTGAATCAGAATCTCATG
pri-*miR-30e**-dn	GGCGAATTCGCTATCTTAGAATTTCAGGC
Anti-*miR-30e**-1-primer-up	GATCCCCCTTTATTTCGGATGTTTACAGCTTCAAGAGA GCTGTAAACATCCGACTGAAAGTTTTTA
Anti-*miR-30e**-1-primer-dn	AGCTTAAAAACTTTCAGTCGGATGTTTACAGCTCTCTT GAAGCTGTAAACATCCGAAATAAAGGGG

Anti-*miR-30e**-2-primer-up	GATCCCCCAAACAGTCGGATGTTTACAGCTTCAAGAG AGCTGTAAACATCCGACTGAAAGTTTTTA
Anti-*miR-30e**-2-primer-dn	AGCTTAAAAACTTTCAGTCGGATGTTTACAGCTCTC TTGAAGCTGTAAACATCCGACTGTTTGGGG
IκBα-3'UTR-GFP-up	TAGCTGCAGTGACACAGAGTCAGAGTTCACGGAGTTC
IκBα -3'UTR-GFP-down	TATCCGCGGGCAGTGTGGATATAAGTACACCC
IκBα -3'UTR-luc-up	ATACCGCGGTGACACAGAGTCAGAGTTCACGGAGTTC
IκBα -3'UTR-luc-down	GGCCTGCAGGCAGTGTGGATATAAGTACACCC
IκBα-3'UTR-mut-luc-up	CGTTATGAGTGCAAGGGGCTGGGAGAACATGGACTTG
IκBα-3'UTR-mut-luc-dn	CAAGTCCATGTTCTCCCAGCCCCTTTGCACTCATAACG
TNF promoter primer up	GCCGGTACCTAGCGGCTCTGAGGAATG
TNF promoter primer	GCCAAGCTTTGCCAACAACTGCCTTTAT
MYC promoter primer up	GCCCTCGAGTTTGCGGGTTACATACAGT
MYC promoter primer dn	GCCAGATCTAGTTCCCAATTTCTCAGCC
② used to amplify genes	
MYC-up	TCAAGAGGCGAACACACAAC
MYC-dn	GGCCTTTTCATTGTTTTCCA
*TNF*α-up	CCAGGCAGTCAGATCATCTTCTC
*TNF*α-dn	AGCTGGTTATCTCTCAGCTCCAC
IL-6- up	TCTCCACAAGCGCCTTCG
IL-6- dn	CTCAGGGCTGAGATGCCG
IL-8- up	TGCCAAGGAGTGCTAAAG
IL-8- dn	CTCCACAACCCTCTGCAC
Bcl-xL-up	TCCTTGTCTACGCTTTCCACG
Bcl-xL-dn	GGTCGCATTGTGGCCTTT

VEGFC-up	GTGTCCAGTGTAGATGAACTC
VEGFC-dn	ATCTGTAGACGGACACACATG
MMP1-up	TTCGGGGAGAAGTGATGTTC
MMP1-dn	TTGTGGCCAGAAAACAGAAA
MMP3- up	AGGGATTAATGGAGATGCCC
MMP3-dn	CAATTTCATGAGCAGCAACG
MMP9-up	ACGACGTCTTCCAGTACCGA
MMP9-dn	TTGGTCCACCTGGTTCAACT
MMP10-up	ATTTTGGCCCTCTCTTCCAT
MMP10-dn	CTGATGGCCCAGAACTCATT
MMP12-up	GAACAGCTCTACAAGCCTGGAA
MMP12-dn	TCTCCAGGGTAGATGTGTCCAGT
MMP13-up	TCAGGAAACCAGGTCTGGAG
MMP13-dn	TCACCAATTCCTGGGAAGTCT
IκBα-up	GTCAAGGAGCTGCAGGAGAT
IκBα-dn	CCATGGTCAGTGCCTTTTCT
GAPDH-up	ATTCCACCCATGGCAAATTC
GAPDH-dn	TGGGATTTCCATTGATGACAAG
Oligonucleotides	
IκBα siRNA#1	GCCAGAAAUUGCUGAGGCA
IκBα siRNA#1-mismatch	GUCAGAAAUUGCUGAGGUA
IκBα siRNA#2	GAGUCAGAGUUCACGGAGU
IκBα siRNA#2-mismatch	GGGUCAGAGUUCACGGAUU

Reference

1. Ramkissoon S H, Mainwaring L A, Sloand E M, Young N S, Kajigaya S. Nonisotopic detection of microRNA using digoxigenin labeled RNA probes. *Mol Cell Probes*.2006; 20(1):1-4.

Supplemental Figure Legends

Supplemental Figure 1. *miR-30e is upreregulated in the gliomas.** (A-C) Real-time PCR analysis of *miR-30e** expression in cells from eight pairs of glioma and adjacent tissues (A), or in micro-dissected samples containing only astrocytes from glioma and paired adjacent brain tissues (B), or in glioma cell lines and NHA (C). (D-E) The expressions of *miR-30e** in NHA cells, negative control (NC) transfected NHA cells and *miR-30e** mimic transfected cells were analyzed by ISH (D) and real-time RT-PCR (E). (F) Sister sections of a glioma (WHO grade III) were hybridized with the *miR-30e** probe or a control probe. (G) Representative images of *miR-30e** expression in normal brain tissues and WHO graded gliomas by ISH. (H) Real-time PCR analysis of *miR-30e** expression in indicated cells and WHO grading of glioma compared with that in normal brain tissues. Transcription levels were normalized by *U6* expression. The bounds of boxes represent the lower and upper quartile; lines within boxes and whiskers denote median and extremum, respectively. The experiments (A to H) were repeated at least three times with similar results. ** $P < 0.01$. Original magnification, ×1000 (D); ×200 (F and G).

Supplemental Figure 2. Ectopic expression of *miR-30e enhances the migratory speed of glioma cells in vitro.** (A-B) Biofunctions of *miR-30e**-regulated genes identified by microarray profiling in U87MG (A) and SNB19 (B) glioma cells. (C) Migration of indicated cells analyzed by the wound healing assay. (D) Representative images (left panel) and quantification (right panel) of indicated migrated cells were analyzed using a transwell assay (without Matrigel). The experiments (C-D) were repeated at least three times with similar results. ** $P < 0.01$. Original magnification, ×100 (C); ×200 (D).

Supplemental Figure 3. Ectopically expressing *miR-30e induces MMPs expression.** (A) Real-time PCR quantification of mRNA expression of *MMPs* in indicated cells. (B)

Gelatinase activity of MMP9 in indicated cells was accessed using gelatin zymography assays. (C) Representative micrographs of indicated cells cultured in a 3-D spheroid invasion assay. Original magnification, ×400. The experiments (A to C) were repeated at least three times with similar results. ** $P < 0.01$.

Supplemental Figure 4. Ectopically expressed *miR-30e activates NF↗B.** (A) The luciferase activities driven by *MYC*- (left), *TNF⇨*- (middle) or *MMP9*- (right) promoter were increased in *miR-30e**-transfected cells and were suppressed in *miR-30e** inhibitor-transfected cells. (B) The endogenous NF↗B activity in *miR-30e**-overexpressing glioma cell lines was dramatically increased as compared with that in control cells determined by EMSA. Oct-1 DNA-binding complexes served as a control. (C) The NF↗B reporter activities in the *miR-30e** cells with or without treatment by NF↗B inhibitor (JSH-23 or SN-50). (D) AP-1 reporter activities in the indicated cells. The experiments (A to C) were repeated at least three times with similar results. ** $P < 0.01$.

Supplemental Figure 5. *miR-30e is involved in NF↗B activation and induction of aggressiveness of primary glioma cells (PGC).** (A) Western blotting analysis of I↗B⇨ expression in indicated cells transfected with *miR-30e** or *miR-30e** inhibitor. ⇨-Tubulin was detected as a loading control. (B and C) Overexpression of *miR-30e** increased and inhibition of *miR-30e** decreased the NF↗B -driven luciferase activity (B) and expression of nine classical NF↗B target genes (C). (D) Representative images (left) and quantification (right) of indicated invaded cells analyzed in a TMPA with Matrigel. (E) Representative images and quantification of HUVEC cultured on Matrigel-coated plates with conditioned medium from indicated cells. The experiments (A to E) were repeated at least three times with similar results. ** $P < 0.01$. Original magnification, ×200 (D); ×100 (E).

Supplemental Figure 6. Silencing I↗B⇨ activates NF↗B. (A) Ectopically expressing _miR-30e*_ upregulated _I↗B⇨_ mRNA. The _I↗B⇨_ mRNA was increased in _miR-30e*_-transfected cells and suppressed in _miR-30e*_ inhibitor-transfected cells. (B) Western blotting analysis of I↗B⇨ protein in glioma cells U87MG transfected with scrambled siRNA, two different _I↗B⇨_ siRNAs and two mismatched _I↗B⇨_ siRNAs. ⇨-Tubulin was detected as a loading control. (C) NF↗B reporter activity in the indicated cells. (D) Real-time PCR analysis of mRNA expression levels of _MMP1_, _MMP3_, _MMP9_ and _MMP13_ in the indicated cells. (E and F) Real-time PCR analysis of mRNA expression levels of _IL-8_ and _VEGF-C_ in the indicated cells. Expression levels were normalized by _GAPDH_. The experiments (A to F) were repeated at least three times with similar results. ** $P < 0.01$.

Supplemental Figure 7. _miR-30e*_ enhances invasiveness of gliomas cells through downregulation of I↗B⇨. (A) Concomitant overexpression of _I↗B⇨_ ORF (without 3'UTR) and _miR-30e*_ could abolish the stimulatory effect of _miR-30e*_ on the invasive ability of the indicated glioma cells, while transfection of _I↗B⇨_ cDNA-3'UTR only partially inhibited the _miR-30e*_-mediated invasiveness. (B) Invasion abilities of _miR-30e*_ inhibitor-transfected glioma cells, as shown in a 3-D spheroid invasion assay, were rescued by downregulation of I↗B⇨. The experiments (A and B) were repeated at least three times with similar results. Original magification, ×200 (A and B).

Supplemental Figure 8. Stably expressing _miR-30e_ has no effect on the invasiveness of glioma cells. (A and B) Real-time PCR analysis of _miR-30e_ (A) or _miR-30e*_ (B) in glioma cells transfected with negative control (NC) or with _has-miR-30e_ mimic oligonucleotides. (C) Upregulation of miR-30e did not enhance the invasion ability of glioma cells. Representative images (left panel) and quantification (right panel) of indicated invaded cells were analyzed using TMPA. (D) Representative micrographs of indicated cells cultured in the 3-D spheroid invasion assay. (E) Representative images (left panel) and quantification (right panel) of indicated invaded cells in a TMPA. (F) Representative micrographs of indicated cells cultured in a 3-D spheroid invasion assay. Results from E and F indicated that inhibition of *miR-30e* did not change the invasion ability of *miR-30e/30e**-transduced glioma cells. (G) Relative expression of *miR-30e* in indicated cells. The experiments (A to G) were repeated at least three times with similar results. ** $P < 0.01$. Original magnification, ×200 (C, D, E and F) .

Supplemental Figure 9. Inhibition of *miR-30e in *miR-30e** stably expressing glioma cells reduces NF↗B transactivation and invasion of glioma cells.** (A) Real-time PCR analysis of *miR-30e** expression in indicated cells transduced with *miR-30e** inhibitors. (anti-*miR-30e**#1 and anti-*miR-30e**#2). Expression levels were normalized to *U6* transcripts. (B) Western blotting analysis of I↗B⇨ expression in indicated cells. (C) Real-time PCR quantification of changes in *MMP9* mRNA levels in indicated cells. (D) NF↗B reporter activity was reduced in anti-*miR-30e**-transduced cells. (E and F) Downregulation of *miR-30e** in the *miR-30e**-expressing glioma cells resulted in the reduction of invasion analyzed by TMPA (with Matrigel) (E) and the 3-D spheroid invasion assay (F). Original magnification, ×200 (F). The experiments (A to F) were repeated at least three times with similar results. ** $P < 0.01$.

Supplemental Figure 10. Microvascular densities (MVD) correlate with glioma progression and poor prognosis of glioma patients. (A) Representative MVD IHC staining was performed on each tumor specimen at least twice and generated similar staining patterns. Original magnification, ×200 (left panel); ×400 (right panel). (B) MVD increased with increasing grade of the gliomas. Quantification and statistical analysis of vessel numbers in 10 randomized fields in WHO graded glioma tissues. WHO grade I, 12 cases; WHO grade II, 33 cases; WHO grade III, 59 cases; WHO grade IV, 23 cases. ** $P < 0.01$. (C)

Kaplan-Meier analysis of MVD levels in WHO grade I to IV and survival of patients with malignant gliomas ($P < 0.001$, log-rank test). (D) Significant correlation was observed between *miR-30e** expression and MVD indicated by CD31 staining in 127 glioma specimens ($P < 0.01$). Experiment was repeated at least three times with similar results.

Supplemental Figure 11. ***miR-30e**** **promotes angiogenesis through the I↗B⇨/NF↗B pathway.** (A) The HUVEC cell migration assay was performed by culturing HUVEC with the conditioned media derived from indicated glioma cells treated with negative control (NC), *miR-30e** mimic, *miR-30e** inhibitor, or *miR-30e** mimic plus NF↗B inhibitor. (B and C) Expression of VEGF-C in indicated cells quantified by Real-time PCR analysis (B) and ELISA assay (C). The experiments were repeated at least three times with similar results. ** $P < 0.01$.

Supplemental Figure 12. Expression of *I↗B⇨* mRNA is upregulated in gliomas. Real-time RT-PCR analysis of *I↗B⇨* expression in 2 normal brain tissues and 10 glioma tissue samples. *GAPDH* was used as a loading control. The experiment was repeated at least three times with similar results. ** $P < 0.01$.

Supplemental Tables

Supplemental Table 1. Clinicopathological characteristics of studied patients and expression of *miR-30e** in gliomas

Factor	No.	(%)
Gender		
Male	92	72.4
Female	35	27.6
Age (years)		
≤ 45	85	66.9
> 45	42	33.1
Glioma histopathology (WHO grading)		
Grade I	12	9.4
Grade II	33	26.0
Grade III	59	46.5
Grade IV	23	18.1
Patient survival (n=127)		
Alive	41	32.3
Deceased	86	67.7
Expression of *miR-30e and survival**		
Low expression	48	37.8
Median survival time = 52 months		
High expression	79	62.2
Median survival time = 23 months		

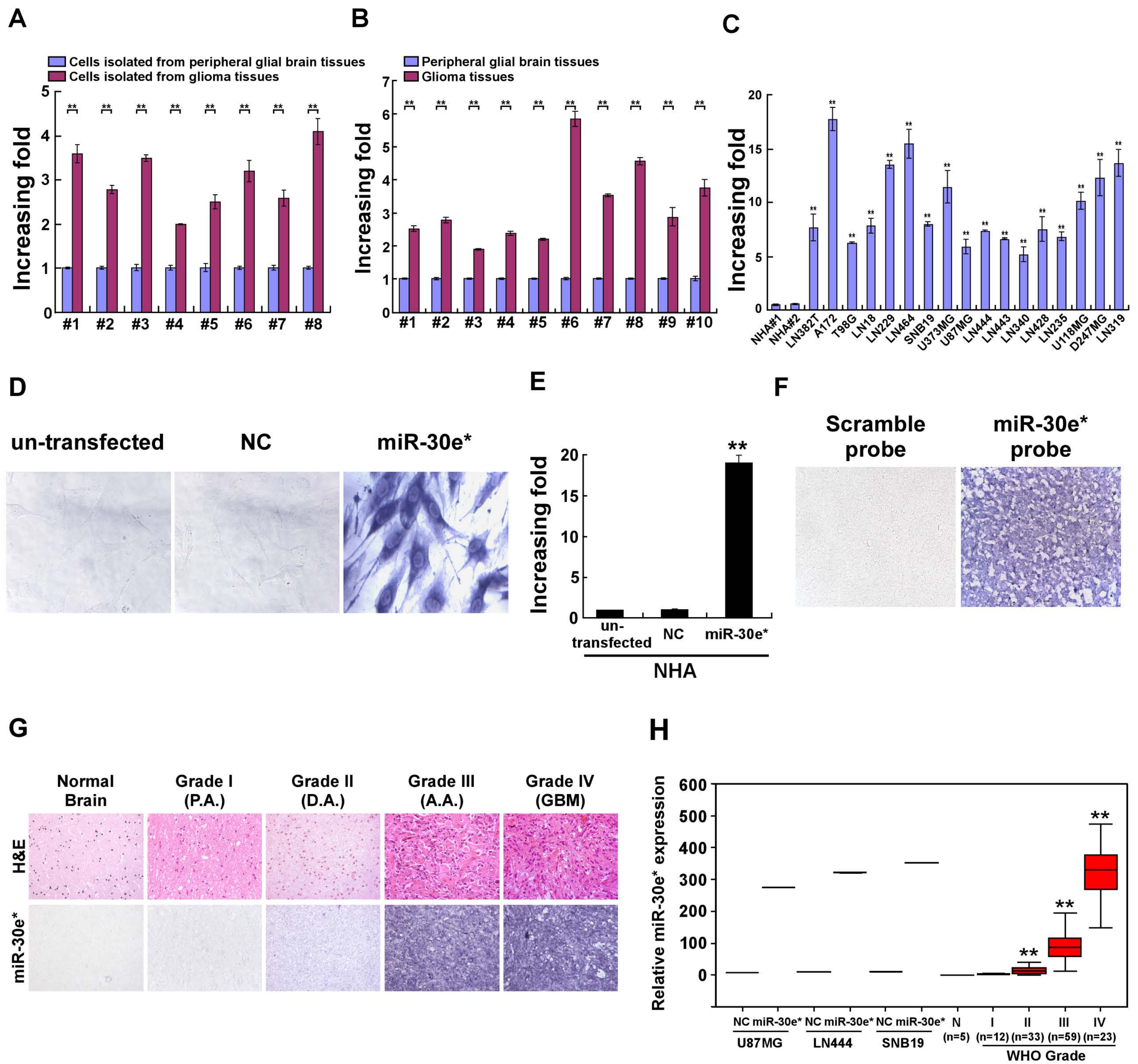
Supplemental Table 2. Correlation between the clinicopathological features and expression of *miR-30e**

Patient characteristics		*miR-30e** expression		*P*-value
		Low or none	High	
Sex	Male	41	51	0.011
	Female	7	28	
Age (years)	≤45	38	47	0.022
	>45	10	32	
Glioma histology (WHO grading)	I	7	5	<0.001
	II	27	6	
	III	14	45	
	IV	0	23	
Survival (n=127)	Alive	29	12	<0.001
	Deceased	19	67	

Supplemental Table 3. Univariate and multivariate analysis of different prognostic parameters in patients with glioma by Cox-regression analysis

	Univariate analysis		Multivariate analysis			
	No. patients	*P*	Regression coefficient (SE)	*P*	Relative risk	95% confidence interval
Age						
≤45	85	0.006	0.221	0.475	1.179	0.751-1.852
＞45	42					
Glioma histology (WHO grade)						
I	12	<0.001	0.281	0.001	2.863	1.543-5.312
II	33					
III	59					
IV	23					
*miR-30e** expression						
Low	48	<0.001	0.265	0.002	2.427	1.371-4.295
High	79					

Supplemental Figure 1



Supplemental Figure 1. ***miR-30e**** **is upregulated in the gliomas.**

(A-C) Real-time PCR analysis of *miR-30e expression in cells from eight pairs of glioma and adjacent tissues (A), or in micro-dissected samples containing only astrocytes from glioma and paired adjacent brain tissues (B), or in glioma cell lines and NHA (C).**

(D-E) The expressions of *miR-30e in NHA cells, negative control (NC) transfected NHA cells and *miR-30e** mimic transfected cells were analyzed by ISH (D) and real-time RT-PCR (E).**

(F) Sister sections of a glioma (WHO grade III) were hybridized with the *miR-30e probe or a control probe.**

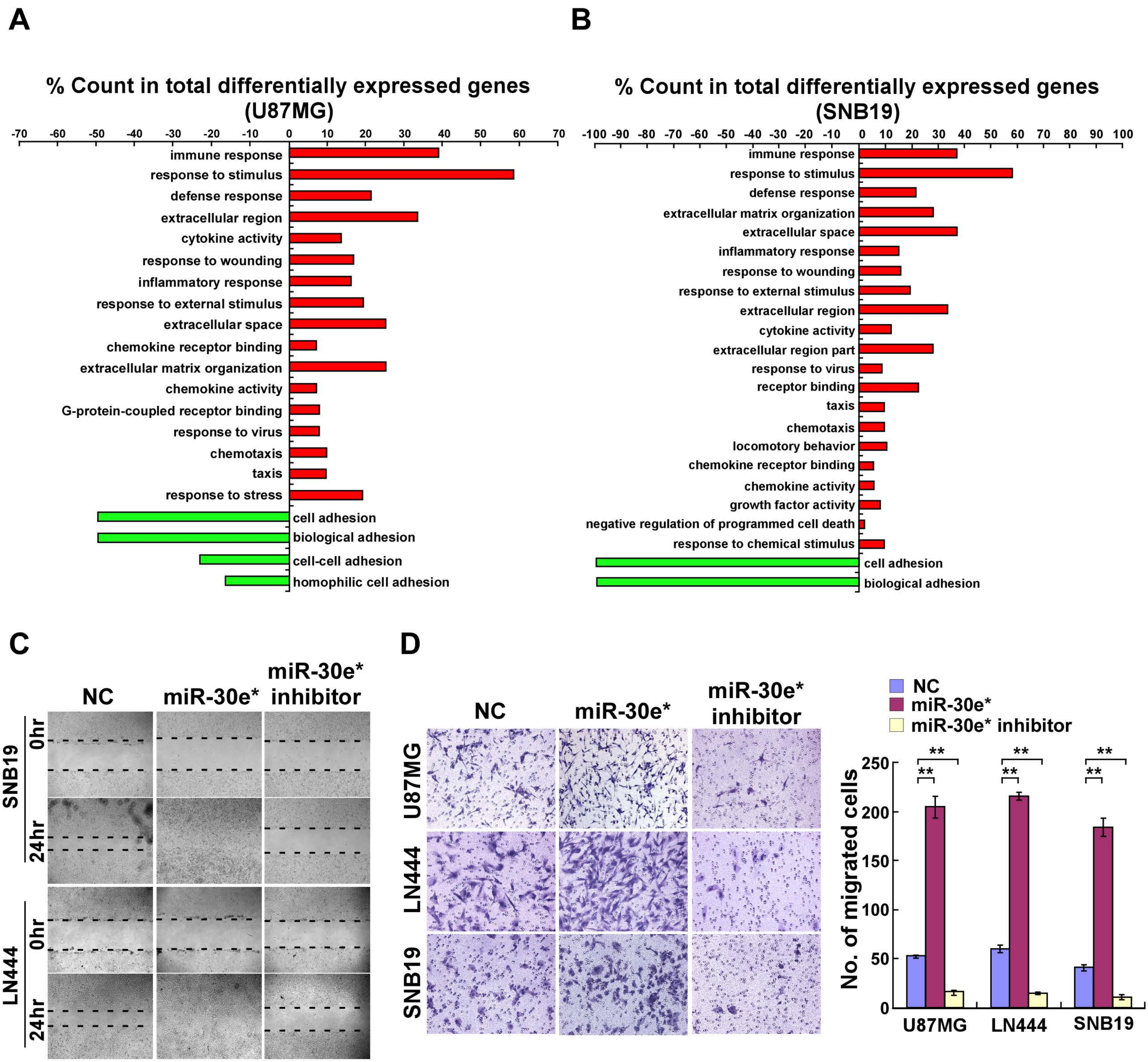
(G) Representative images of *miR-30e expression in normal brain tissues and WHO graded gliomas by ISH.**

(H) Real-time PCR analysis of *miR-30e expression in indicated cells and WHO grading of glioma compared with that in normal brain tissues. Transcription levels were normalized by *U6* expression. The bounds of boxes represent the lower and upper quartile ; lines within boxes and whiskers denote median and extremum, respectively.**

The experiments (A to H) were repeated at least three times with similar results. ** $P < 0.01$.

Original magnification, x1000 (D); x200 (F and G).

Supplemental Figure 2



Supplemental Figure 2. Ectopic expression of *miR-30e** enhances the migratory speed of glioma cells in vitro.

(A-B) Biofunctions of *miR-30e**-regulated genes identified by microarray profiling in U87MG (A) and SNB19 (B) glioma cells.

(C) Migration of indicated cells analyzed by the wound healing assay.

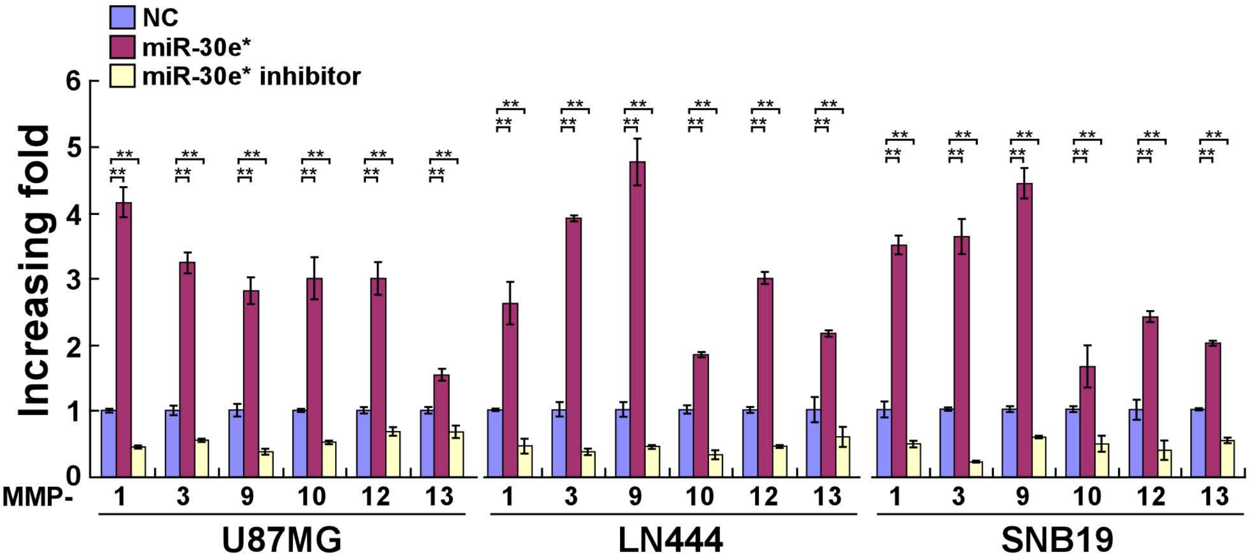
(D) Representative images (left panel) and quantification (right panel) of indicated migrated cells were analyzed using a Transwell assay (without Matrigel).

The experiments (C-D) were repeated at least three times with similar results. ** *P* < 0.01.

Original magnification, x100 (C); x200 (D).

Supplemental Figure 3

A



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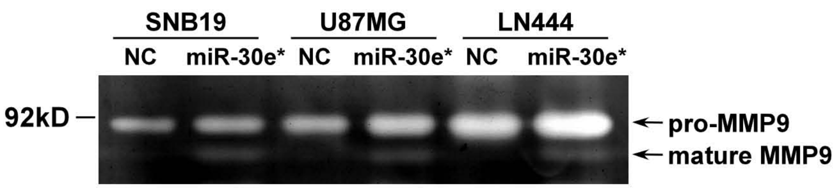

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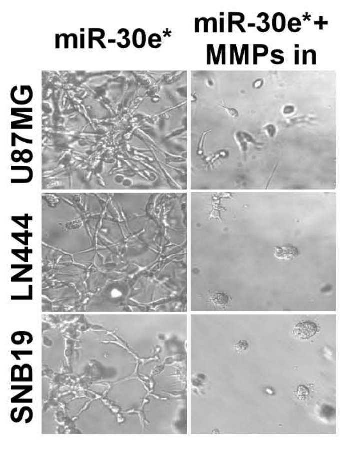


Supplemental Figure 3. Ectopically expressing *miR-30e** induces MMPs expression.

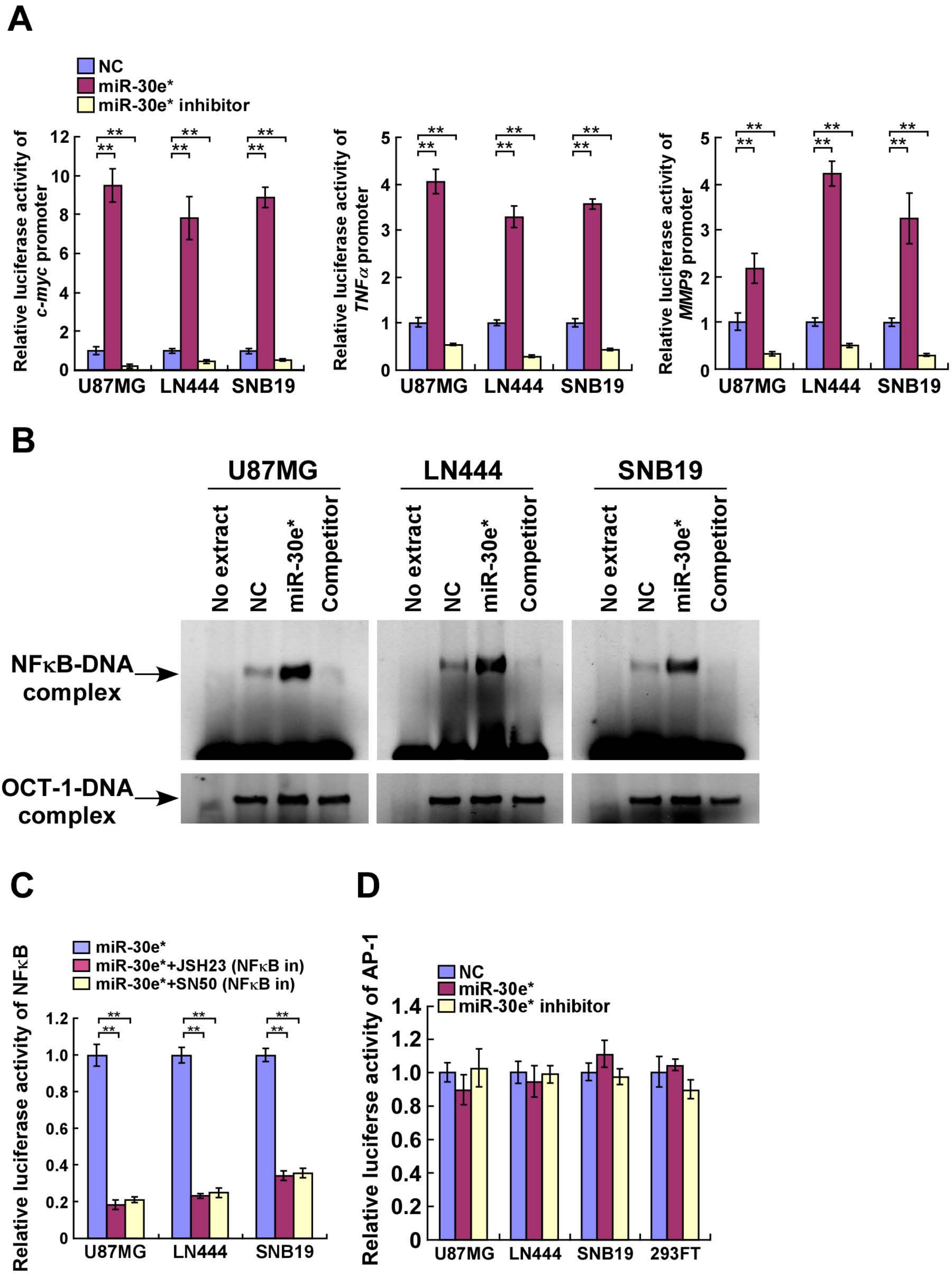
(A) Real-time PCR quantification of mRNA expression of *MMPs* in indicated cells.

(B) Gelatinase activity of MMP9 in indicated cells was accessed using gelatin zymography assays.

(C) Representative micrographs of indicated cells cultured in a 3-D spheroid invasion assay. Original magnification, x400.

The experiments (A to C) were repeated at least three times with similar results. ** $P < 0.01$.

Supplemental Figure 4



Supplemental Figure 4. Ectopically expressed *miR-30e** activates NFκB.

(A) The luciferase activities driven by *MYC*- (left), *TNFα*- (middle) or *MMP9*- (right) promoter were increased in *miR-30e**-transfected cells and were suppressed in *miR-30e** inhibitor-transfected cells.

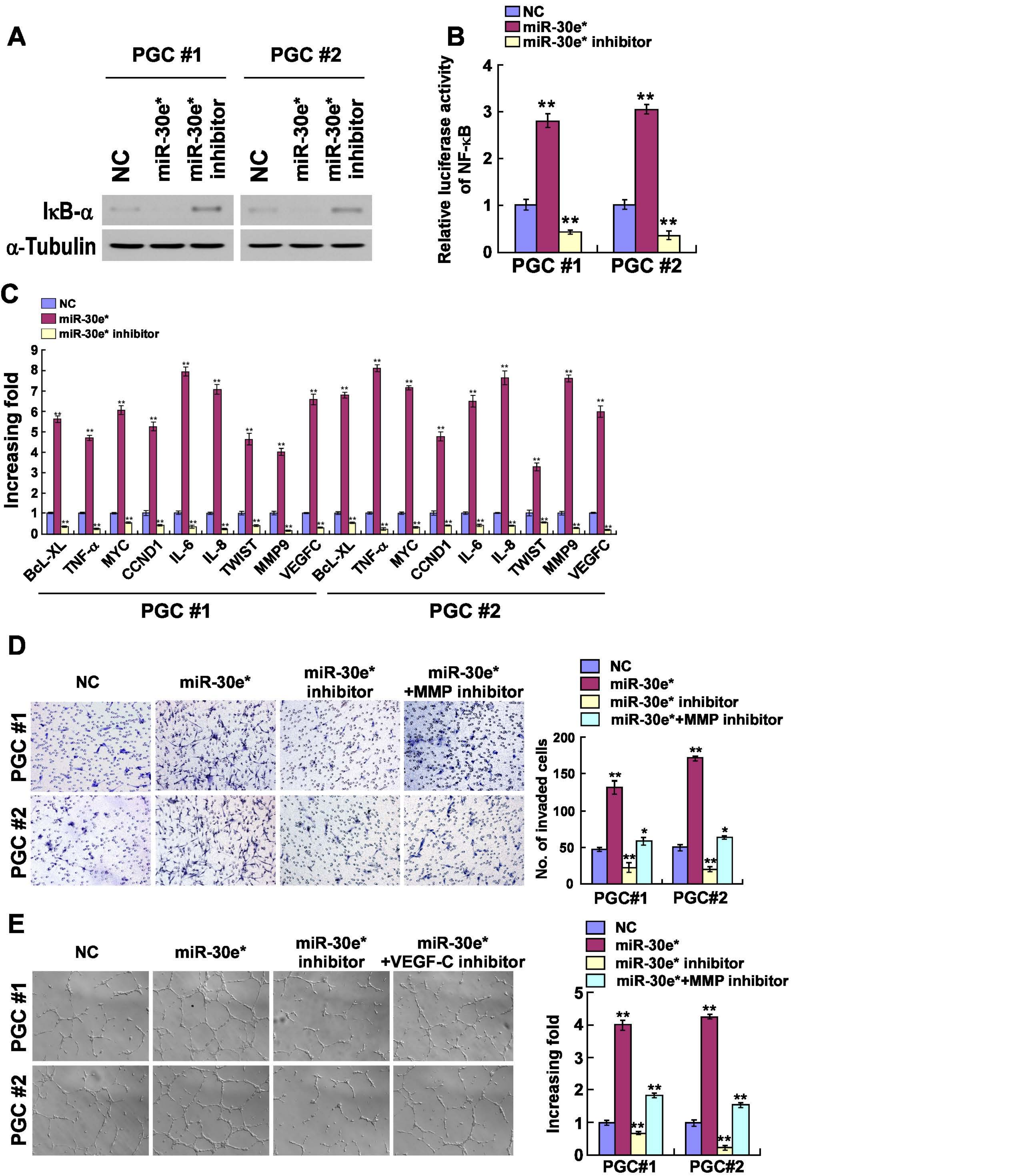
(B) The endogenous NFκB activity in *miR-30e**-overexpressing glioma cell lines was dramatically increased as compared with that in control cells determined by EMSA. Oct-1 DNA-binding complexes served as a control.

(C) The NFκB reporter activities in the *miR-30e** cells with or without treatment by NFκB inhibitor (JSH-23 or SN-50).

(D) AP-1 reporter activities in the indicated cells.

The experiments (A to C) were repeated at least three times with similar results. ** $P < 0.01$.

Supplementary Figure S5



Supplemental Figure 5. *miR-30e is involved in NFκB activation and induction of aggressiveness of primary glioma cells (PGC).**

(A) Western blotting analysis of IκBα expression in indicated cells transfected with *miR-30e or *miR-30e** inhibitor. α-Tubulin was detected as a loading control.**

(B and C) Overexpression of *miR-30e increased and inhibition of *miR-30e** decreased the NFκB-driven luciferase activity (B) and expression of nine classical NFκB target genes (C).**

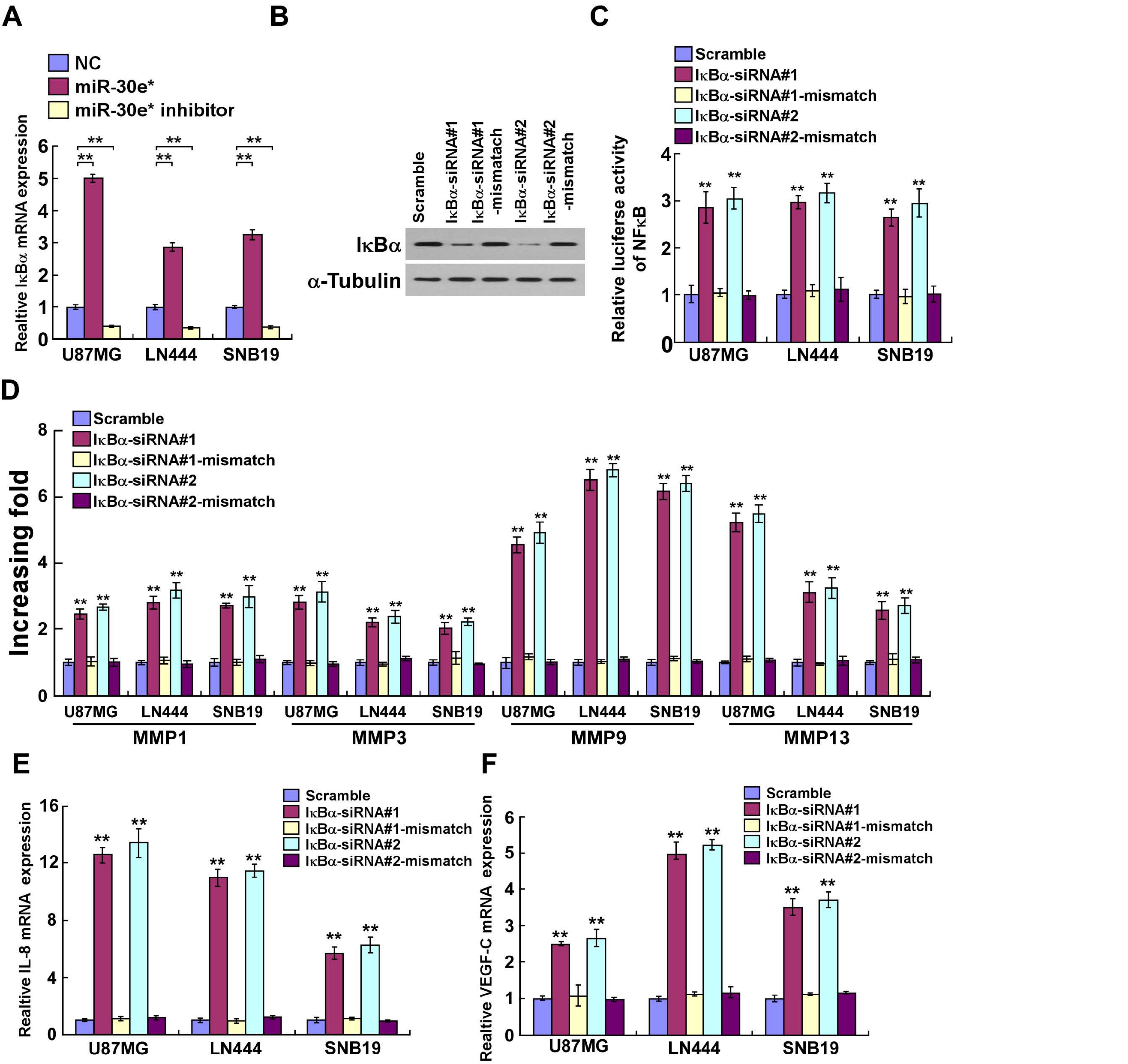
(D) Representative images (left) and quantification (right) of indicated invaded cells analyzed in a TMPA with Matrigel.

(E) Representative images and quantification of HUVEC cultured on Matrigel-coated plates with condi tioned medium from indicated cells.

The experiments (A to E) were repeated at least three times with similar results. ** $P < 0.01$.

Original magnification, x200 (D); x100 (E).

Supplemental Figure 6



Supplemental Figure 6. Silencing IκBα activates NFκB.

(A) Ectopically expressing *miR-30e upregulated *IκBα* mRNA. The *IκBα* mRNA was increased in *miR-30e**-transfected cells and suppressed in miR-30e* inhibitor-transfected cells.**

(B) Western blotting analysis of IκBα protein in glioma cells U87MG transfected with scrambled siRNA, two different *IκBα* siRNAs and two mismatched *IκBα* siRNAs. α-Tubulin was detected as a loading control.

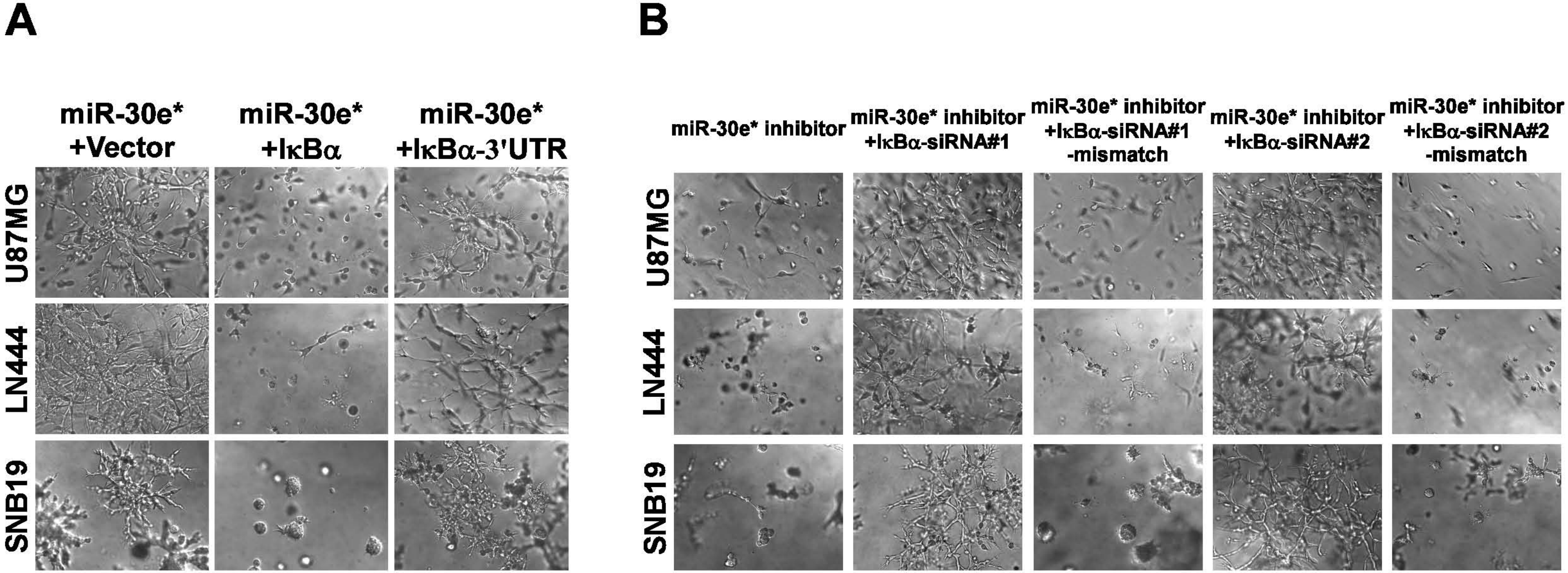
(C) NFκB reporter activity in the indicated cells.

(D) Real-time PCR analysis of mRNA expression levels of *MMP1*, *MMP3*, *MMP9* and *MMP13* in the indicated cells.

(E and F) Real-time PCR analysis of mRNA expression levels of *IL-8* and *VEGF-C* in the indicated cells. Expression levels were normalized by *GAPDH*.

The experiments (A to F) were repeated at least three times with similar results. ** $P < 0.01$.

Supplemental Figure 7



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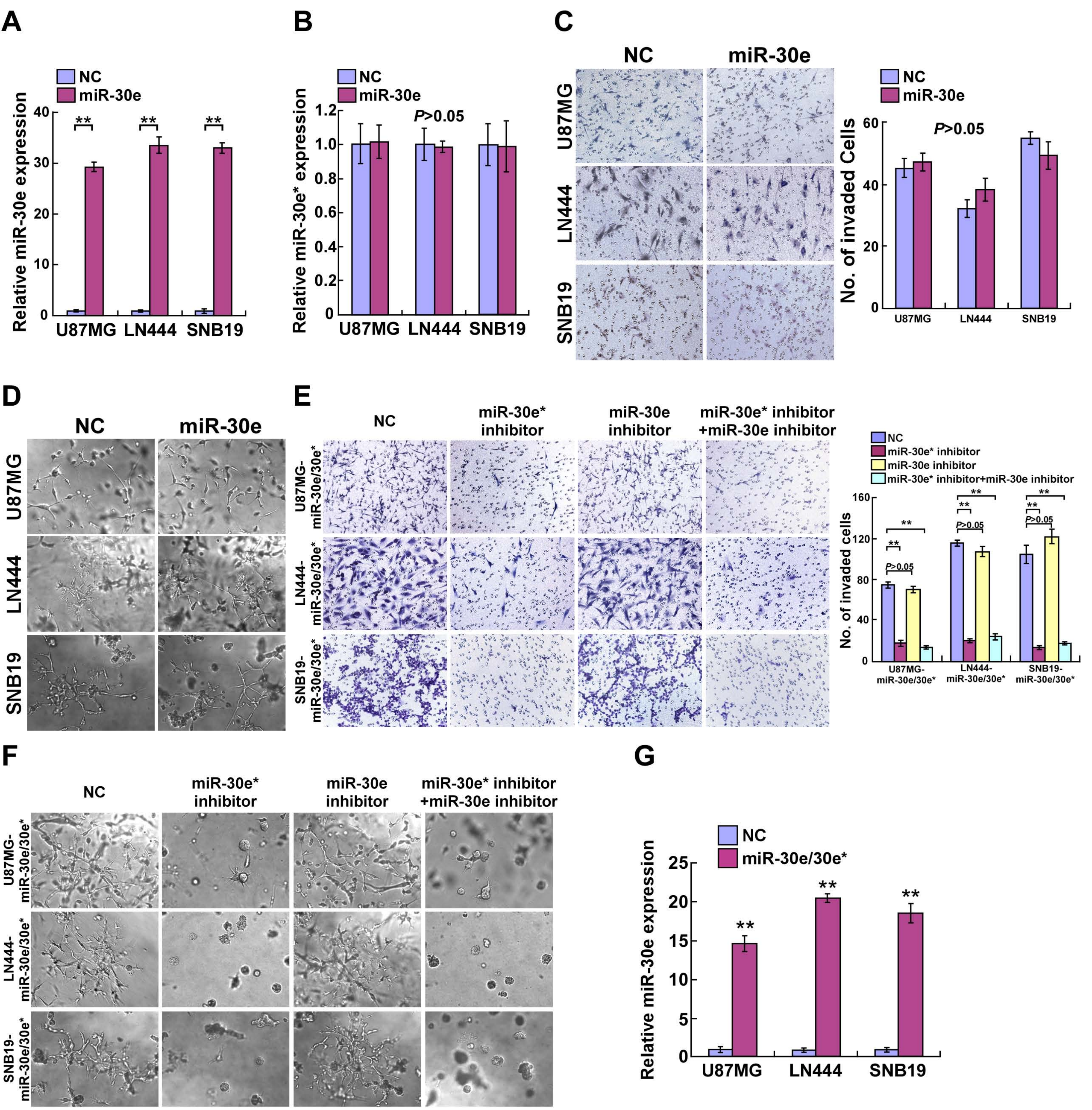
(A) Concomitant overexpression of *IκBα* ORF (without 3'UTR) and *miR-30e** could abolish the stimulatory effect of *miR-30e** on the invasive ability of the indicated glioma cells, while transfection of *IκBα* cDNA-3'UTR only partially inhibited the *miR-30e**-mediated invasiveness.

(B) Invasion abilities of *miR-30e** inhibitor-transfected glioma cells, as shown in a 3-D spheroid invasion assay, were rescued by downregulation of *IκBα*.

The experiments (A and B) were repeated at least three times with similar results.

Original magnification, x200 (A and B).

Supplemental Figure 8



Supplemental Figure 8. Stably expressing *miR-30e* has no effect on the invasiveness of glioma cells.

(A and B) Real-time PCR analysis of *miR-30e* (A) or *miR-30e (B) in glioma cells transfected with negative control (NC) or with *hsa-miR-30e* mimic oligonucleotides.**

(C) Upregulation of *miR-30e* did not enhance the invasion ability of glioma cells.Representative images (left panel) and quantification (right panel) of indicated invaded cells were analyzed using TMPA.

(D) Representative micrographs of indicated cells cultured in the 3-D spheroid invasion assay.

(E) Representative images (left panel) and quantification (right panel) of indicated invaded cells in a TMPA.

(F) Representative micrographs of indicated cells cultured in a 3-D spheroid invasion assay.

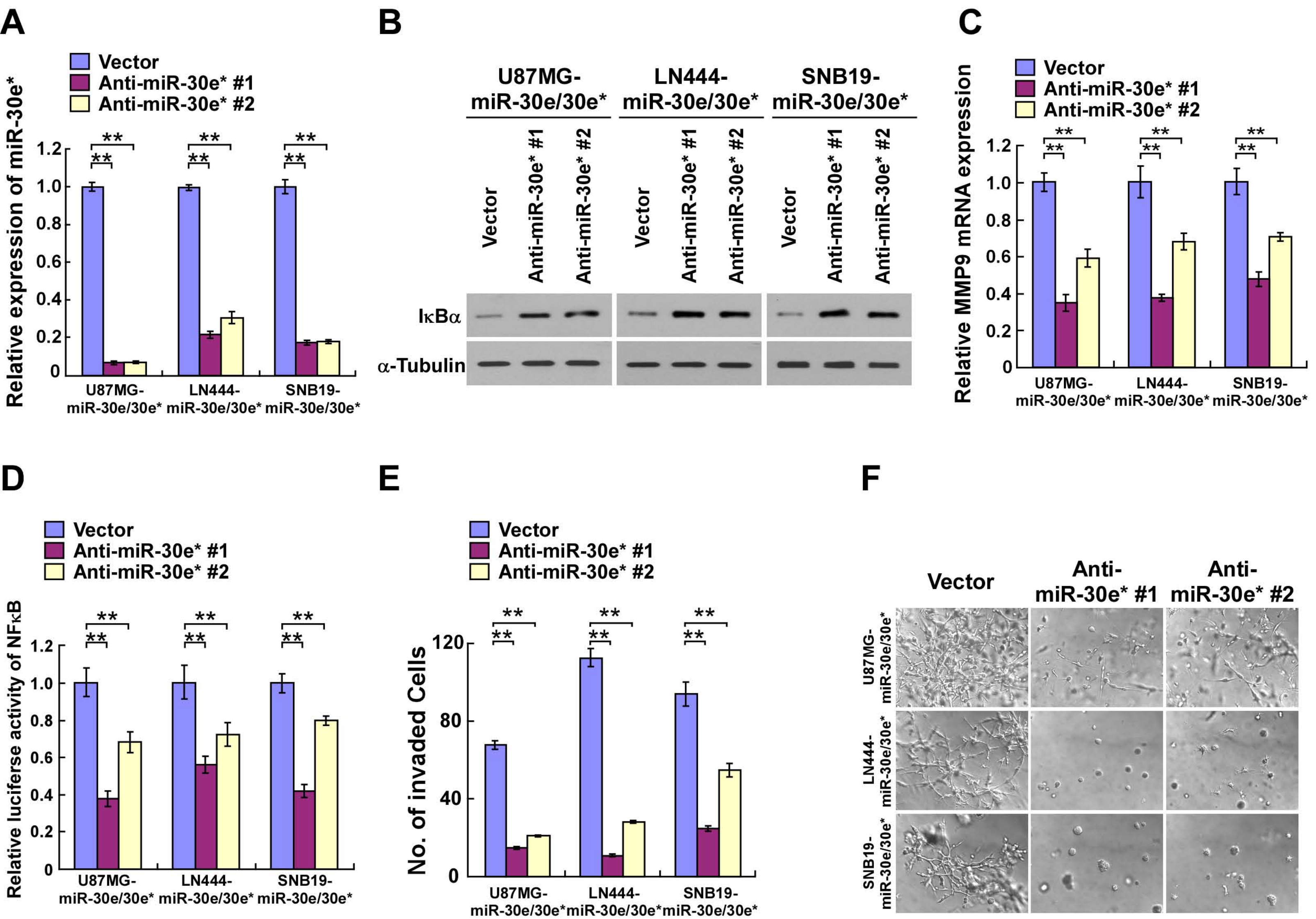
Results from E and F indicated that inhibition of *miR-30e* did not change the invasion ability of *miR-30e/30e-transduced glioma cells.**

(G) Relative expression of *miR-30e* in indicated cells.

The experiments (A to G) were repeated at least three times with similar results. ** *P* < 0.01.

Original magnification, x200 (C, D, E and F) .

Supplemental Figure 9



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(A) Real-time PCR analysis of *miR-30e expression in indicated cells transduced with *miR-30e** inhibitors . (anti-*miR-30e**#1 and anti-*miR-30e**#2). Expression levels were normalized to *U6* transcripts.**

(B) Western blotting analysis of IκBα expression in indicated cells.

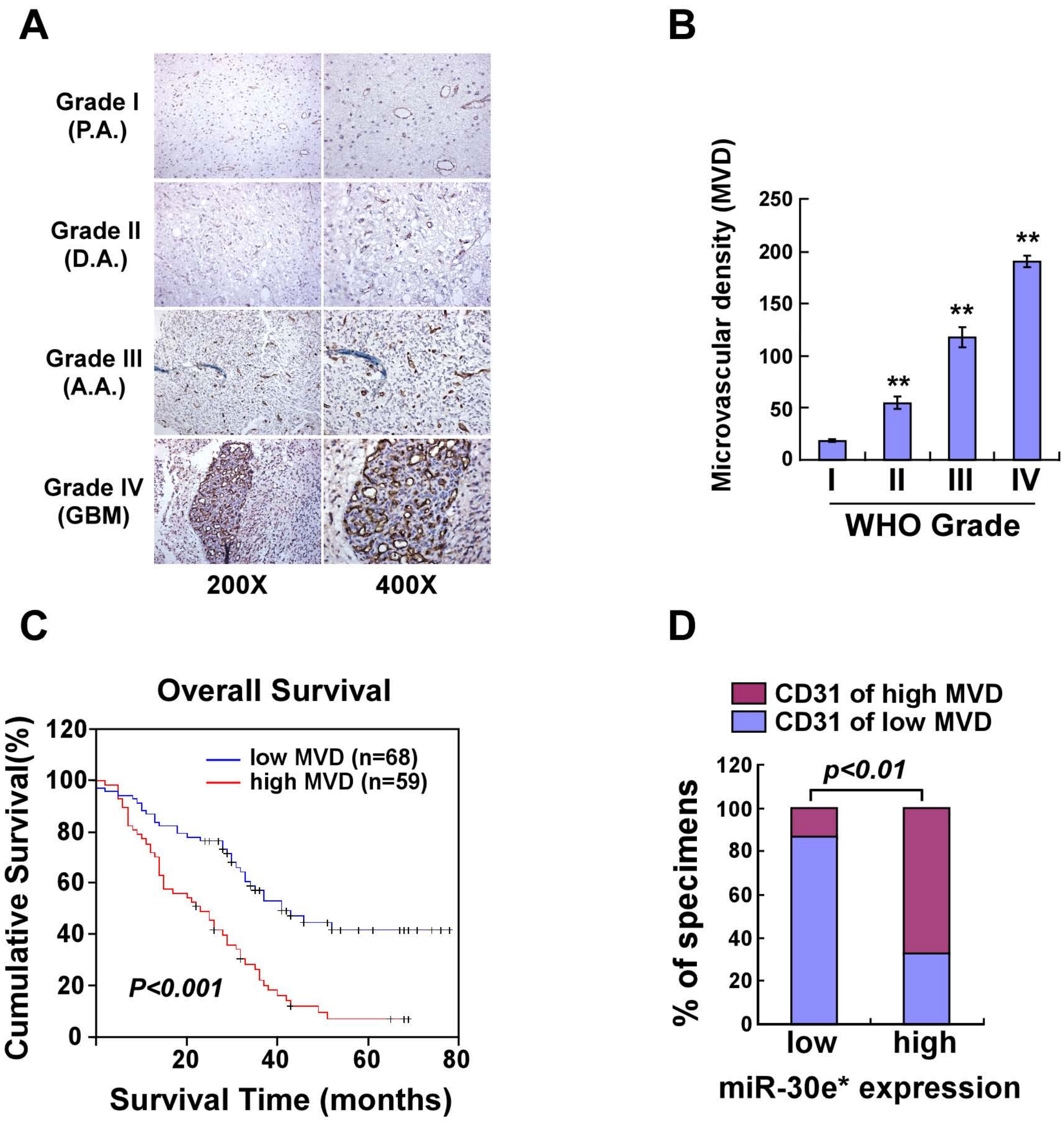
(C) Real-time PCR quantification of changes in *MMP9* mRNA levels in indicated cells.

(D) NFκB reporter activity was reduced in anti-*miR-30e-transduced cells.**

(E and F) Downregulation of *miR-30e in the *miR-30e**-expressing glioma cells resulted in the reduction of invasion analyzed by TMPA (with Matrigel) (E) and the 3-D spheroid invasion assay (F). Original magnification, x200 (F).**

The experiments (A to F) were repeated at least three times with similar results. ** *P* < 0.01.

Supplemental Figure 10



Supplemental Figure 10.

Microvascular densities (MVD) correlate with glioma progression and poor prognosis of glioma patients.

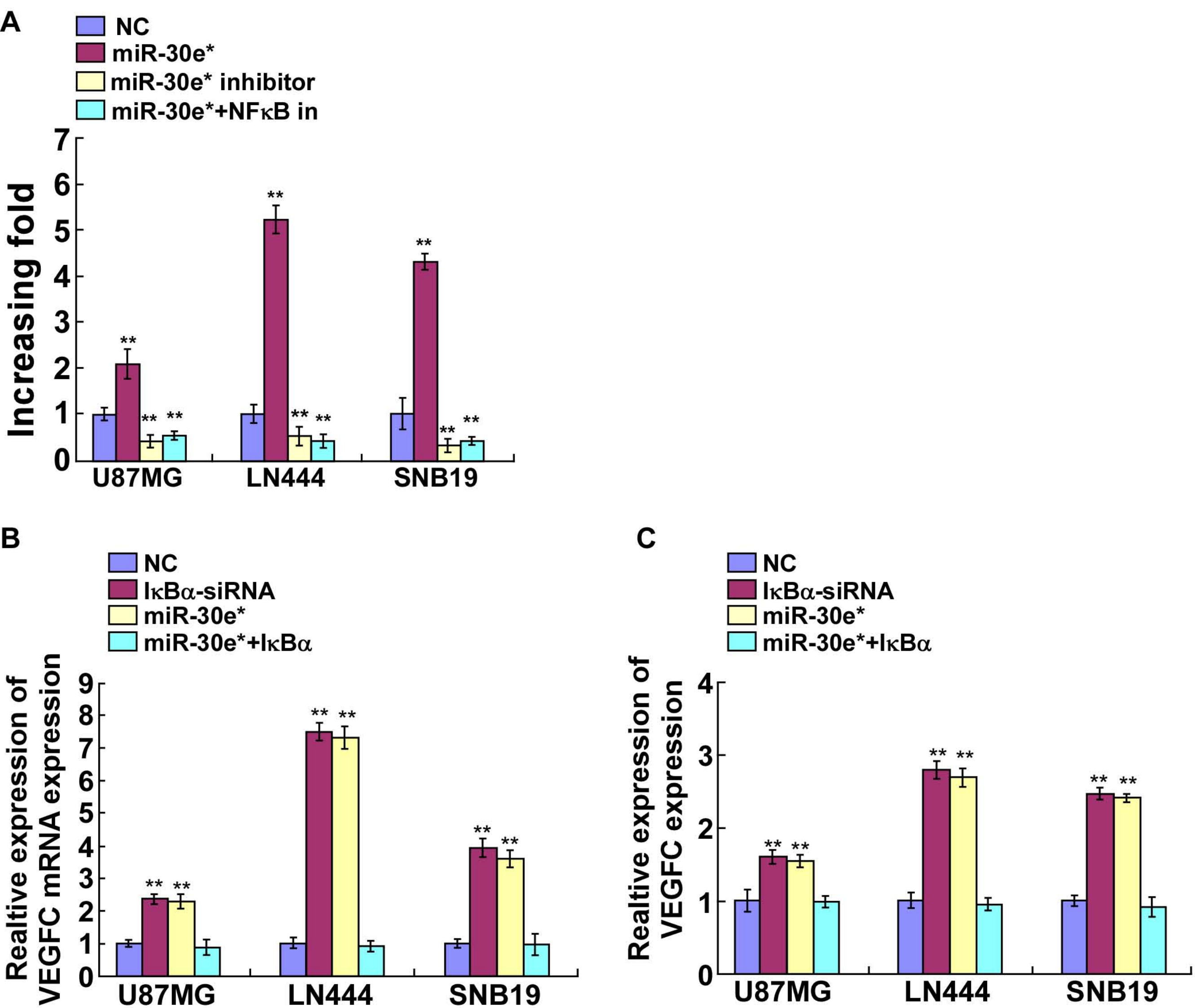
(A) Representative MVD images assessed with IHC for CD31 expression specimens of varying WHO grades. IHC staining was performed on each tumor specimen at least twice and generated similar staining patterns. Original magnification, x200 (left panel); x400 (right panel).

(B) MVD increased with increasing grade of the gliomas. Quantification and statistical analysis of vessel numbers in 10 randomized fields in WHO graded glioma tissues. WHO grade I, 12 cases; WHO grade II, 33 cases; WHO grade III, 59 cases; WHO grade IV, 23 cases. ** $P < 0.01$.

(C) Kaplan-Meier analysis of MVD levels in WHO grade I to IV and survival of patients with malignant gliomas ($P < 0.001$, log-rank test).

(D) Significant correlation was observed between *miR-30e** expression and MVD indicated by CD31 staining in 127 glioma specimens ($P < 0.01$). Experiment was repeated at least three times with similar results.

Supplemental Figure 11



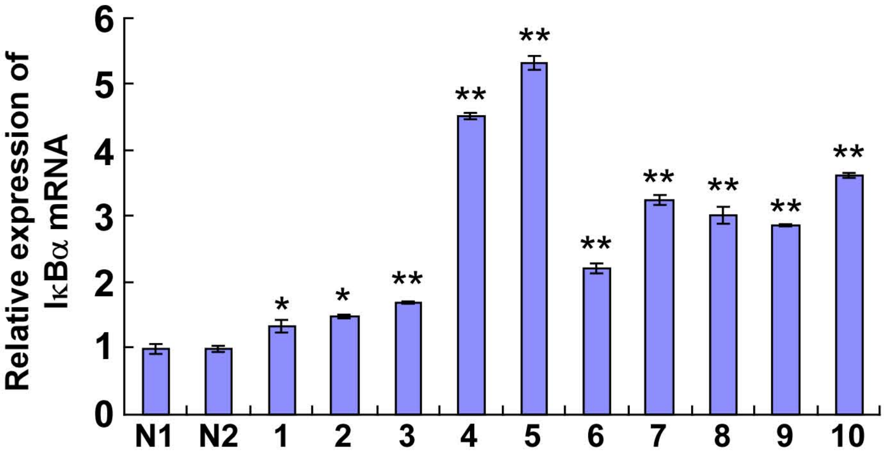
Supplemental Figure 11. ***miR-30e**** **promotes angiogenesis through the IκBα/NFκB pathway.**

(A) The HUVEC cell migration assay was performed by culturing HUVEC with the conditioned media derived from indicated glioma cells treated with negative control (NC), miR-30e* mimic, *miR-30e inhibitor, or *miR-30e** mimic plus NFκB inhibitor.**

(B and C) Expression of VEGF-C in indicated cells quantified by real-time PCR analysis (B) and ELISA assay (C).

The experiments were repeated at least three times with similar results. ** *P* < 0.01.

Supplemental Figure 12



Supplemental Figure 12. Expression of *IκBα* mRNA is upregulated in gliomas.

Real-time RT-PCR analysis of *IκBα* expression in 2 normal brain tissues and 10 glioma tissue samples.

***GAPDH* was used as a loading control.**

The experiment was repeated at least three times with similar results. ** *P* < 0.01.