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Supplemental Information

LINC00680 and TTN-AS1 Stabilized by EIF4A3 Promoted Malignant Biological Behaviors of Glioblastoma Cells

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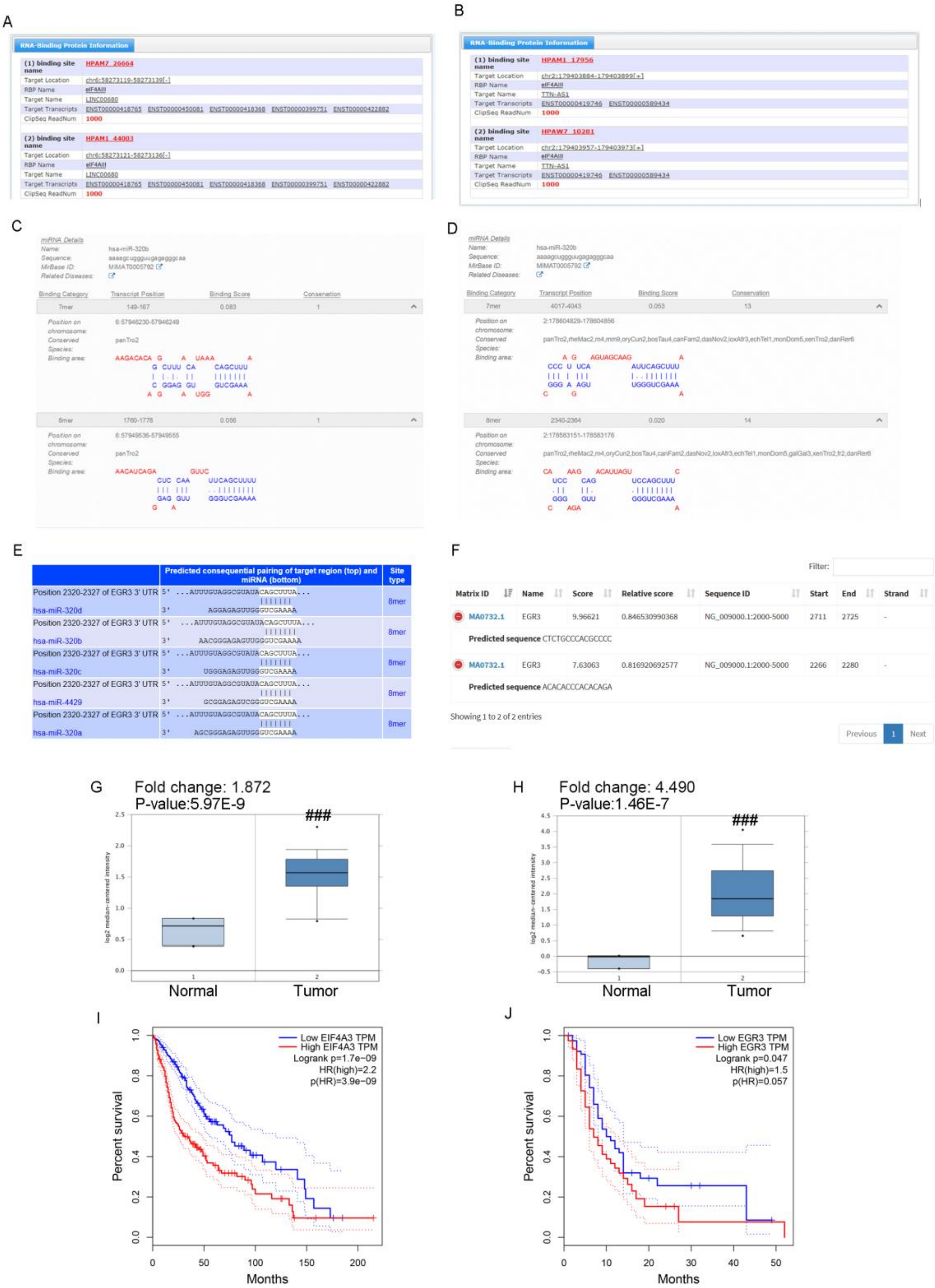


A

RNA-Binding Protein Information

(1) binding site name	HPAM7_26664
Target Location	chr6:58273119-58273139[-]
RBP Name	eIF4AIII
Target Name	LINC00680
Target Transcripts	ENST00000418765 ENST00000450081 ENST00000418368 ENST00000399751 ENST00000422882
ClipSeq ReadNum	1000
(2) binding site name	HPAM1_44003
Target Location	chr6:58273121-58273136[-]
RBP Name	eIF4AIII
Target Name	LINC00680
Target Transcripts	ENST00000418765 ENST00000450081 ENST00000418368 ENST00000399751 ENST00000422882
ClipSeq ReadNum	1000

B

RNA-Binding Protein Information

(1) binding site name	HPAM1_17956
Target Location	chr2:179403884-179403899[+]
RBP Name	eIF4AIII
Target Name	TTN-AS1
Target Transcripts	ENST00000419746 ENST00000589434
ClipSeq ReadNum	1000
(2) binding site name	HPAW7_10281
Target Location	chr2:179403957-179403973[+]
RBP Name	eIF4AIII
Target Name	TTN-AS1
Target Transcripts	ENST00000419746 ENST00000589434
ClipSeq ReadNum	1000

C

miRNA Details
Name: hsa-miR-320b
Sequence: aaaagcugguugagagggcaa
MirBase ID: MIMAT0005792
Related Diseases:

Binding Category	Transcript Position	Binding Score	Conservation
7mer	149-167	0.083	1

Position on chromosome: 6:57946230-57946249
Conserved Species: panTro2
Binding area:

```
AAGACACA G    A  UAAA        A
         G CUUU CA    CAGCUUU
         | |.|. ||    |||||||
         C GGAG GU    GUCGAAA
        A G    A  UGG        A
```

Binding Category	Transcript Position	Binding Score	Conservation
8mer	1760-1778	0.056	1

Position on chromosome: 6:57949536-57949555
Conserved Species: panTro2
Binding area:

```
AACAUCAGA        GUUC
          CUC CAA    UUCAGCUUUU
          ||| |||    ..||||||||
          GAG GUU    GGGUCGAAAA
         G   A
```

D

miRNA Details
Name: hsa-miR-320b
Sequence: aaaagcugguugagagggcaa
MirBase ID: MIMAT0005792
Related Diseases:

Binding Category	Transcript Position	Binding Score	Conservation
7mer	4017-4043	0.053	13

Position on chromosome: 2:178604829-178604856
Conserved Species: panTro2,rheMac2,rn4,mm9,oryCun2,bosTau4,canFam2,dasNov2,loxAfr3,echTel1,monDom5,xenTro2,danRer6
Binding area:

```
    A G  AGUAGCAAG         A
 CCC U UCA         AUUCAGCUUU
 ||| | |||         |..|||||||
 GGG A AGU         UGGGUCGAAA
C     G                    A
```

Binding Category	Transcript Position	Binding Score	Conservation
8mer	2340-2364	0.020	14

Position on chromosome: 2:178583151-178583176
Conserved Species: panTro2,rheMac2,rn4,oryCun2,bosTau4,canFam2,dasNov2,loxAfr3,echTel1,monDom5,galGal3,xenTro2,fr2,danRer6
Binding area:

```
CA    AAG   ACAUUAGU        C
  UCC    CAG        UCCAGCUUU
  .||    ||.        .||||||||
  GGG    GUU        GGGUCGAAA
 C    AGA                   A
```

E

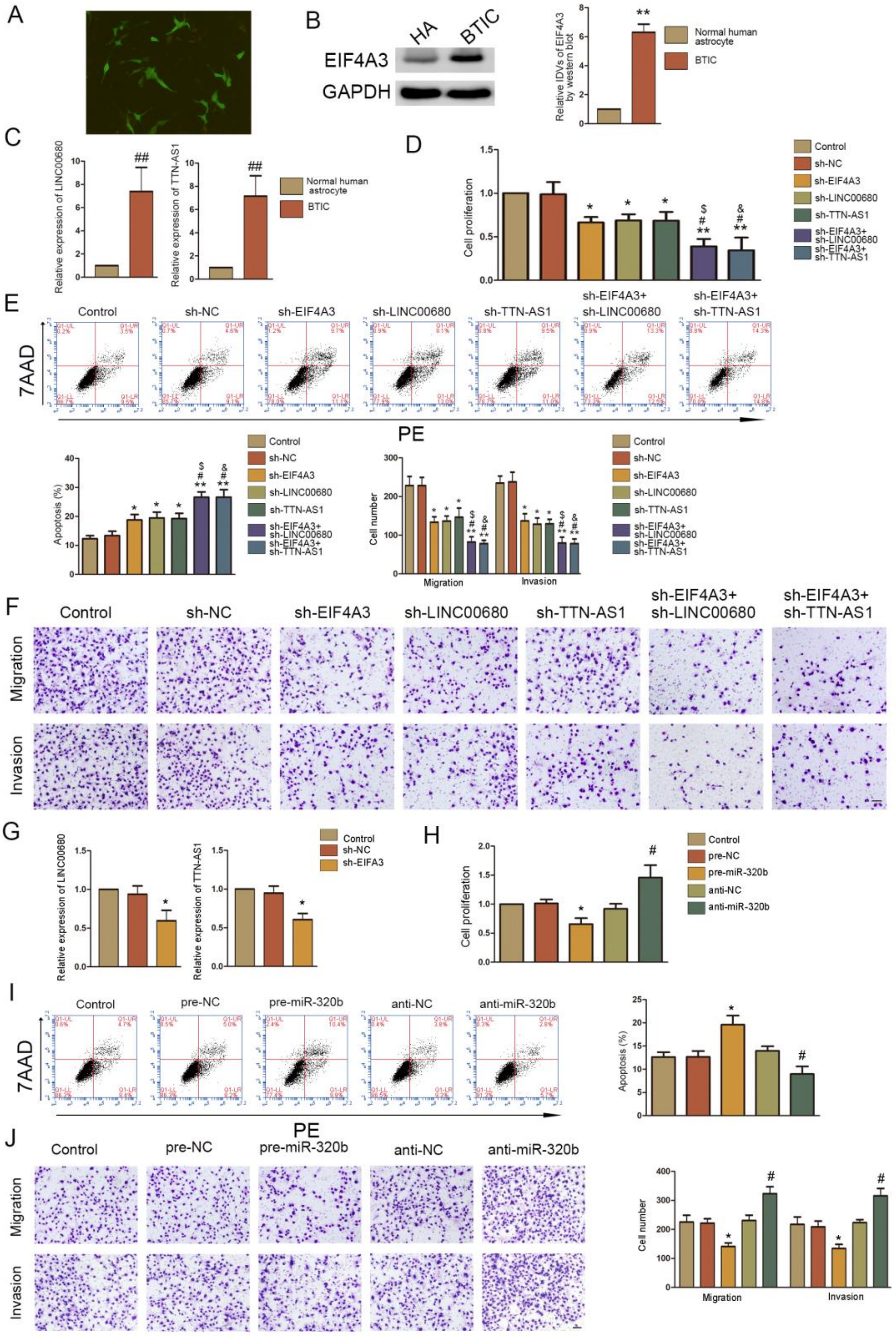
	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type
Position 2320-2327 of EGR3 3' UTR hsa-miR-320d	5' ...AUUUGUAGGCGUAUACAGCUUUA... ||||||| 3' AGGAGAGUUGGGUCGAAAA	8mer
Position 2320-2327 of EGR3 3' UTR hsa-miR-320b	5' ...AUUUGUAGGCGUAUACAGCUUUA... ||||||| 3' AACGGGAGAGUUGGGUCGAAAA	8mer
Position 2320-2327 of EGR3 3' UTR hsa-miR-320c	5' ...AUUUGUAGGCGUAUACAGCUUUA... ||||||| 3' UGGGAGAGUUGGGUCGAAAA	8mer
Position 2320-2327 of EGR3 3' UTR hsa-miR-4429	5' ...AUUUGUAGGCGUAUACAGCUUUA... ||||||| 3' GCGGAGAGUCGGGUCGAAAA	8mer
Position 2320-2327 of EGR3 3' UTR hsa-miR-320a	5' ...AUUUGUAGGCGUAUACAGCUUUA... ||||||| 3' AGCGGGAGAGUUGGGUCGAAAA	8mer

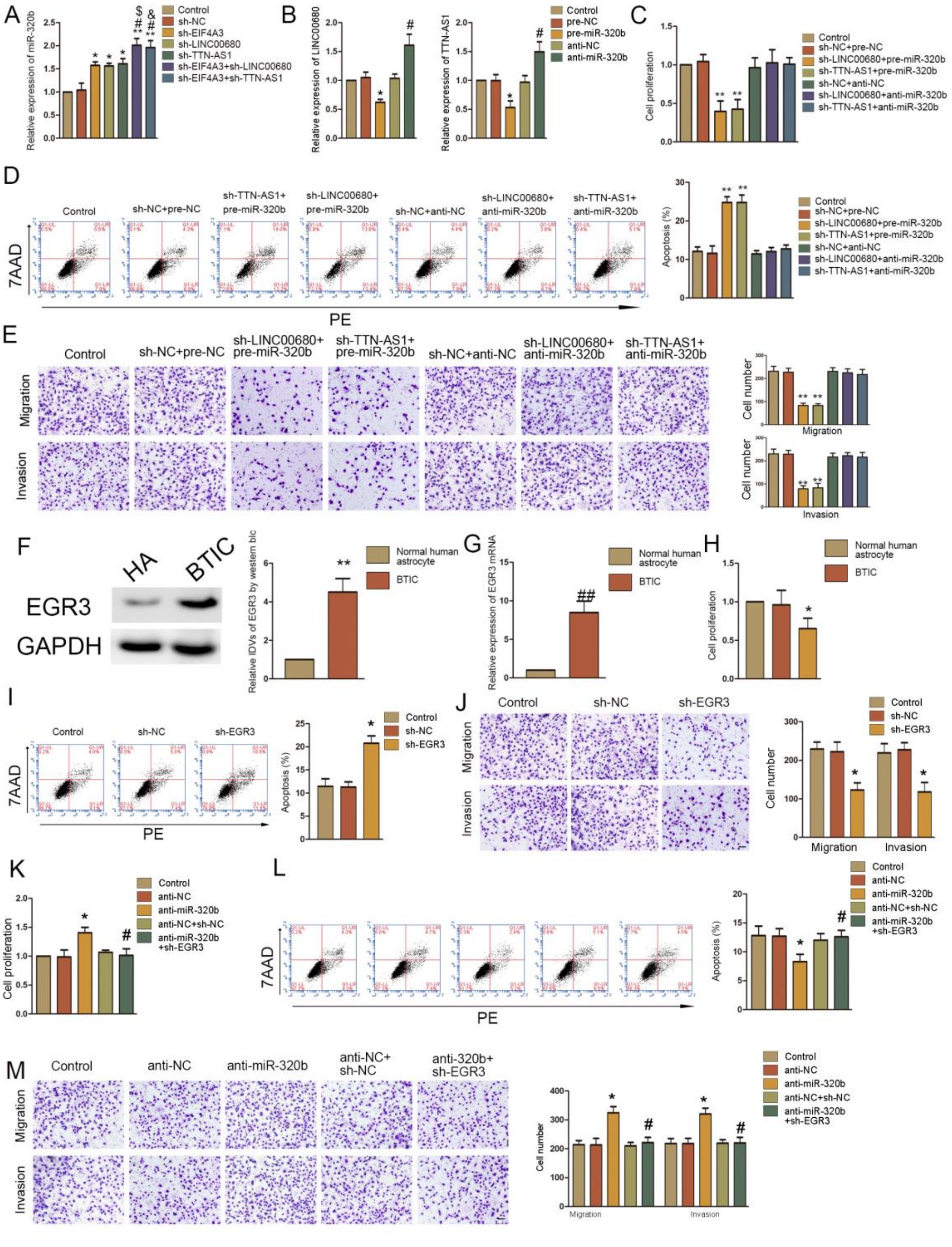
F

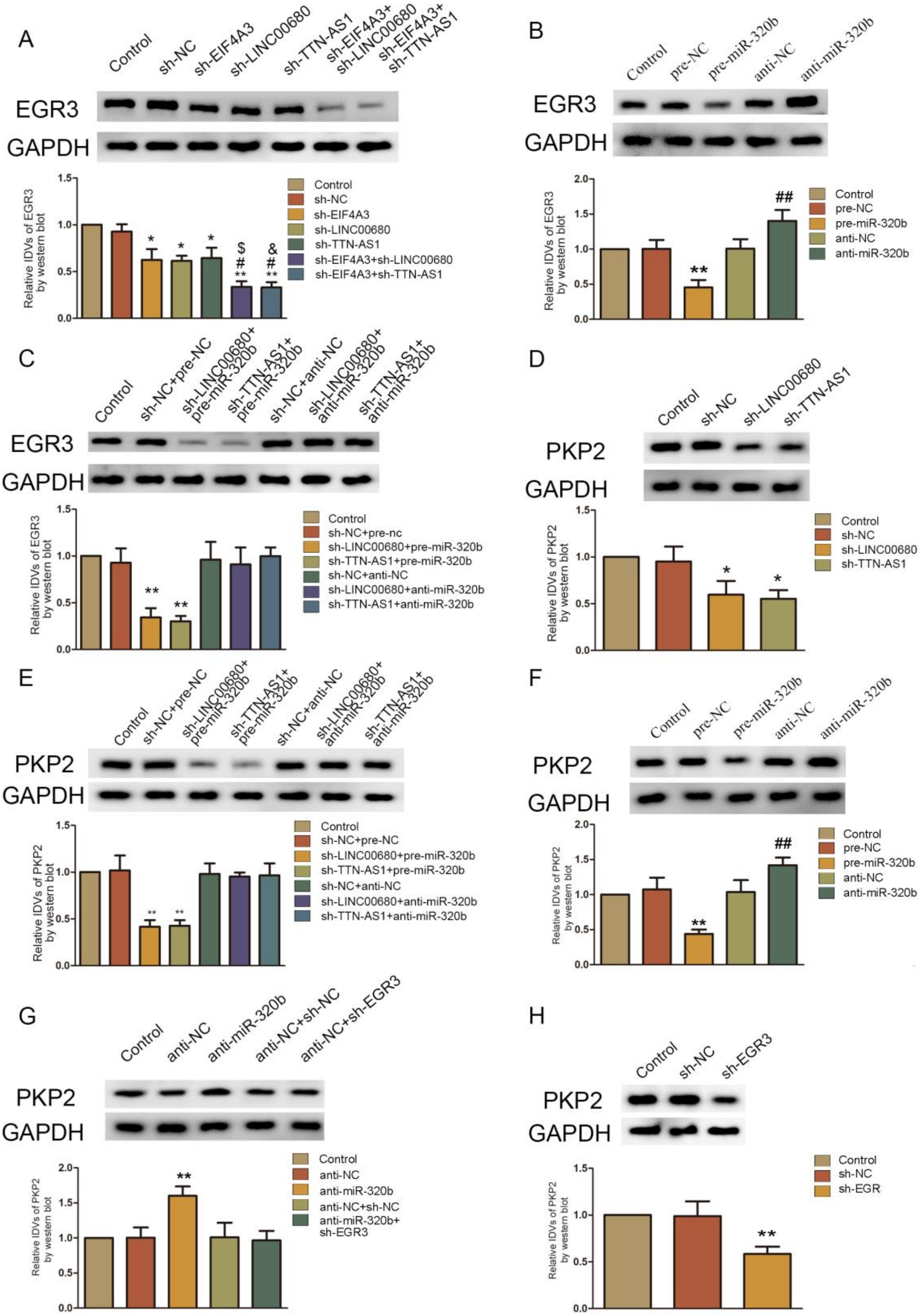
Filter:

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand
MA0732.1	EGR3	9.96621	0.846530990368	NG_009000.1:2000-5000	2711	2725	-
Predicted sequence CTCTGCCCACGCCCC							
MA0732.1	EGR3	7.63063	0.816920692577	NG_009000.1:2000-5000	2266	2280	-
Predicted sequence ACACACCCACACAGA							

Showing 1 to 2 of 2 entries


A
B
HA
BTIC
EIF4A3
GAPDH
Relative IDVs of EIF4A3 by western blot
**
Normal human astrocyte
BTIC
C
Relative expression of LINC00680
Relative expression of TTN-AS1
##
##
Normal human astrocyte
BTIC
D
Cell proliferation
Control
sh-NC
sh-EIF4A3
sh-LINC00680
sh-TTN-AS1
sh-EIF4A3+ sh-LINC00680
sh-EIF4A3+ sh-TTN-AS1
E
Control
sh-NC
sh-EIF4A3
sh-LINC00680
sh-TTN-AS1
sh-EIF4A3+ sh-LINC00680
sh-EIF4A3+ sh-TTN-AS1
7AAD
PE
Apoptosis (%)
Cell number
Migration
Invasion
F
Control
sh-NC
sh-EIF4A3
sh-LINC00680
sh-TTN-AS1
sh-EIF4A3+ sh-LINC00680
sh-EIF4A3+ sh-TTN-AS1
Migration
Invasion
G
Relative expression of LINC00680
Relative expression of TTN-AS1
Control
sh-NC
sh-EIFA3
H
Cell proliferation
Control
pre-NC
pre-miR-320b
anti-NC
anti-miR-320b
I
Control
pre-NC
pre-miR-320b
anti-NC
anti-miR-320b
7AAD
PE
Apoptosis (%)
J
Control
pre-NC
pre-miR-320b
anti-NC
anti-miR-320b
Migration
Invasion
Cell number
Migration
Invasion


A
Relative expression of miR-320b
Control
sh-NC
sh-EIF4A3
sh-LINC00680
sh-TTN-AS1
sh-EIF4A3+sh-LINC00680
sh-EIF4A3+sh-TTN-AS1
B
Relative expression of LINC00680
Relative expression of TTN-AS1
Control
pre-NC
pre-miR-320b
anti-NC
anti-miR-320b
C
Cell proliferation
Control
sh-NC+pre-NC
sh-LINC00680+pre-miR-320b
sh-TTN-AS1+pre-miR-320b
sh-NC+anti-NC
sh-LINC00680+anti-miR-320b
sh-TTN-AS1+anti-miR-320b
D
Control
sh-NC+pre-NC
sh-TTN-AS1+ pre-miR-320b
sh-LINC00680+ pre-miR-320b
sh-NC+anti-NC
sh-LINC00680+ anti-miR-320b
sh-TTN-AS1+ anti-miR-320b
7AAD
PE
Apoptosis (%)
E
Control
sh-NC+pre-NC
sh-LINC00680+ pre-miR-320b
sh-TTN-AS1+ pre-miR-320b
sh-NC+anti-NC
sh-LINC00680+ anti-miR-320b
sh-TTN-AS1+ anti-miR-320b
Migration
Invasion
Cell number
F
HA
BTIC
EGR3
GAPDH
Relative IDVs of EGR3 by western blot
Normal human astrocyte
BTIC
G
Relative expression of EGR3 mRNA
Normal human astrocyte
BTIC
H
Cell proliferation
Normal human astrocyte
BTIC
I
Control
sh-NC
sh-EGR3
7AAD
PE
Apoptosis (%)
J
Control
sh-NC
sh-EGR3
Migration
Invasion
Cell number
K
Cell proliferation
Control
anti-NC
anti-miR-320b
anti-NC+sh-NC
anti-miR-320b +sh-EGR3
L
7AAD
PE
Apoptosis (%)
Control
anti-NC
anti-miR-320b
anti-NC+sh-NC
anti-miR-320b +sh-EGR3
M
Control
anti-NC
anti-miR-320b
anti-NC+ sh-NC
anti-320b+ sh-EGR3
Migration
Invasion
Cell number
Control
anti-NC
anti-miR-320b
anti-NC+sh-NC
anti-miR-320b +sh-EGR3



Supplemental figure 1. Results of the in-silico experiments and expression and survival of EIF4A3 and EGR3 from databases

A. Putative binding sites of EIF4A3 and LINC00680 from Database: Starbase V2.0

(http://starbase.sysu.edu.cn/starbase2/). B. Putative binding sites of EIF4A3 and TTN-AS1 from Database: Starbase V2.0 (http://starbase.sysu.edu.cn/starbase2/).C. Putative binding sites of LINC00680 and miR-320b from Database: LncBase (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=lncBase/index). D. Putative binding sites of TTN-AS1 and miR-320b from Database: LncBase (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=lncBase/index). E. Putative binding sites of miR-320b and EGR3 mRNA from Database: TargetScan (http://www.targetscan.org/vert_72/). F. Putative binding sites of EGR3 and PKP2 promotor from Database: Jaspar (http://jaspar.genereg.net/). G. Expression of EIF4A3 from database Database: Oncomine (https://www.oncomine.org/resource/main.html), ###p<0.001 versus normal brain tissue. H. Expression of EGR3 from database Database: Oncomine, ###p<0.001 versus normal brain tissue. I. Survival data of EIF4A3 from Database: GEPIA (http://gepia.cancer-pku.cn/detail.php). J. Survival data of EGR3 from Database: GEPIA.

Supplemental figure 2. Results of experiments using BTICs

A. GFAP staining by Immunofluorescence. Secondary antibody was labeled with FITC. Green fluorescence demonstrated GFAP in BTICs. B. Expression of EIF4A3 determined by western blot. **p < 0.01 versus normal human astrocytes (HA). C. Expression of LINC00680 and TTN-AS1 determined by PCR (n=3). ##p < 0.01 versus HA. D. CCK-8 assay was conducted to investigate the effect of EIF4A3, LINC00680 and TTN-AS1 on proliferation in BTICs. *p < 0.05 versus sh-NC group (empty vector);

**p < 0.01 versus sh-NC group; #p < 0.05 versus sh-EIF4A3 group; $p < 0.05 versus sh-LINCC0680 group, &p < 0.05 versus sh-TTN-AS1 group. E. Flow cytometry analysis of EIF4A3, LINC00680 and TTN-AS1 knockdown in BTICs. *p < 0.05 versus sh-NC group (empty vector); **p < 0.01 versus sh-NC group; #p < 0.05 versus sh-EIF4A3 group; $p < 0.05 versus sh-LINCC0680 group, &p < 0.05 versus sh-TTN-AS1 group. F. Transwell assays were used to measure the effect of EIF4A3, LINC00680 and TTN-AS1 on cell migration and invasion in BTICs. Representative images and statistical plots were presented. *p < 0.05 versus sh-NC group (empty vector); **p < 0.01 versus sh-NC group; #p < 0.05 versus sh-EIF4A3 group; $p < 0.05 versus sh-LINCC0680 group, &p < 0.05 versus sh-TTN-AS1 group. Scale bars represent 80 μm. G. Expression of LINC00680(left) and TTN-AS1(right) were detected in BTICs with EIF4A3 knockdown by PCR. *p < 0.05 versus sh-NC group. H. CCK-8 assay was conducted to investigate the effect of miR-320b on proliferation in BTICs. *p < 0.05 versus pre-NC group, #p<0.05 versus anti-NC group. I. Flow cytometry analysis effect of miR-320b on apoptosis in BTICs. *p < 0.05 versus pre-NC group, #p<0.05 versus anti-NC group. J. Transwell assays were used to measure the effect of miR-320b on cell migration and invasion in BTICs. Representative images and statistical plots were presented. *p < 0.05 versus pre-NC group, #p<0.05 versus anti-NC group. Scale bars represent 80 μm. Data are presented as the mean ± SD (n = 3 in each group).

Supplemental figure 3. Results of experiments using BTICs

A. Real-time PCR analysis for EIF4A3, LINC00680 and TTN-AS1 regulating miR-320b expression in BTICs. *p < 0.05 versus sh-NC group; **p < 0.01 versus sh-NC group; #$p < 0.05$ versus sh-EIF4A3 group; \$$p < 0.05$ versus sh-LINCC0680 group, &$p < 0.05$ versus sh-TTN-AS1 group. B. Real-time PCR analysis for miR-320b modulating LINC00680 and TTN-AS1 expression in BTICs. *$p < 0.05$ versus pre-NC group, #$p<0.05$ versus anti-NC group. C. CCK-8 assay was conducted to investigate the effect of LINC00680/TTN-AS1 and miR-320b on proliferation in BTICs. **$p < 0.01$ versus sh-NC+ pre-NC group. D. Flow cytometry analysis of LINC00680/TTN-AS1 and miR-320b in BTICs. **$p < 0.01$ versus sh-NC+ pre-NC group. E. Transwell assays were used to measure the effect of LINC00680/TTN-AS1 and miR-320b on cell migration and invasion in BTICs. Representative images and statistical plots were presented. **$p < 0.01$ versus sh-NC+ pre-NC group. Scale bars represent 80 μm. Data are presented as the mean ± SD ($n = 3$). F. Western blot was used to detect expression of EGR3 in BTICs. G. Expression of EGR3 mRNA in BTICs was detected by Real-time PCR. H. CCK-8 assay was conducted to investigate the effect of EGR3 on proliferation in BTICs. *$p < 0.05$ versus sh-NC group. I. Flow cytometry analysis of EGR3 in BTICs. *$p < 0.05$ versus sh-NC group. J. Transwell assays were used to measure the effect of EGR3 on cell migration and invasion in BTICs. Representative images and statistical plots were presented. Data are presented as the mean ± SD ($n = 3$ in each group). *$p < 0.05$ versus sh-NC group. Scale bars represent 80 μm. Data are presented as the mean ± SD ($n = 3$). K. CCK-8 assays were performed in BTICs with the altered expression of miR-320b and EGR3. *$p < 0.01$ versus anti-NC group; #$p < 0.05$ versus anti-miR-320b group. L. Flow cytometry analysis of BTICs with the altered expression of miR-320b and EGR3. *$p < 0.05$ versus anti-NC group; #$p < 0.05$ versus anti-miR-320b group. M.

Quantification number of migration and invasion cells treated with anti-miR-320b and sh-EGR3. Representative images and statistical plots were presented. Scale bars represent 80 μm. *p < 0.05 versus anti-NC group; #p < 0.05 versus anti-miR-320b group. Data are presented as the mean ± SD (n = 3).

Supplemental figure 4. Results of experiments using BTICs

A. Expression of EGR3 was regulated in BTICs lines with knockdown of EIF4A3 and/or LINC00680/TTN-AS1. *p < 0.05 versus sh-NC group; **p < 0.01 versus sh-NC group; #p < 0.05 versus sh-EIF4A3 group; $p < 0.05 versus sh-LINCC0680 group, &p< 0.05 versus sh-TTN-AS1 group. B. Expression of EGR3 was regulated in BTICs lines with knockdown or over-expression of miR-320b. **p < 0.01 versus pre-NC group, ##p<0.01 versus anti-NC group. C. Expression of EGR3 was regulated by LINC00680/TTN-AS1 and miR-320b. **p < 0.01 versus sh-NC+ pre-NC group. Data are presented as the mean ± SD (n = 3). D. Western blot analysis of PKP2 in BTICs knockdown of LINC00680 or TTN-AS1. Data are presented as the mean ± SD (n = 3 in each group). *p < 0.05 versus sh-NC group. E.Western blot analysis of PKP2 in BTICs regulated by LINC00680/TTN-AS1 and miR-320b. **p < 0.01 versus sh-NC+ pre-NC group. F. Western blot analysis of PKP2 in BTICs regulated by miR-320b. **p < 0.01 versus pre-NC group, ##p<0.01 versus anti-NC group. G.Western blot analysis of PKP2 in BTICs regulated by miR-320b and EGR3. **p < 0.01 versus anti-NC group; ##p < 0.05 versus anti-miR-320b group. H. Western blot analysis of PKP2 in BTICs regulated by EGR3. **p < 0.01 versus sh-NC group. Data are presented as the mean ± SD (n = 3).

Table S1. Primers used for RT-qPCR.

EIF4A3	Forward primer	5'-GATGCCGATGAACGTTGCTG-3'
	Reverse primer	5'-GGTGGTGGCACCTTAGAAGTAT-3'
LINC00680	Forward primer	5'-CCATCGACTGGCTCATCACAA-3'
	Reverse primer	5'-GGGGCAAGGCAAATCAATACC -3'
TTN-AS1	Forward primer	5'-ACACTCATCATCCAGCCTGC-3'
	Reverse primer	5'-TGGCCATCATTGCAAGCTAAC-3'
miR-320b	Forward primer	5'-AATTAATCCCTCTCTTTCTAGTTCT-3'
	Reverse primer	5'-AGTTAATTTGTTTGCCCTCTCAA-3'
EGR3	Forward primer	5'-GGTGACCATGAGCAGTTTGC-3'
	Reverse primer	5'-TAGGTCACGGTCTTGTTGCC-3'

Table S2. Sequences for sh-RNA, agomir and antagomir

sh-EIF4A3	5'-GGATATTCAGGTTCGTGAAAC-3'
sh-LINC00680	5'-GCACTTGAAGAGAGGTTTACC-3'
sh-TTN-AS1	5'-GCAATGATGGGCCACTAAATTG-3'
sh-EGR3	5'-CTAAACCAACTGCCTGACAAT-3'
miR-320b agomir	5’- AAAGCUGGGUUGAGAGGG CAA -3’
miR-320b antagomir	5’- UUGCCCUCUCAACCCAGCUUUU-3’

Table S3. Primers used for ChIP experiments

Binding site or Control		Sequence	Product size (bp)	Annealing temperature (°C)
PCR1	Forward primer	5'-GTGACTCTCGAGGGCTCACC-3'	192	60.8
	Reverse primer	5'-CGGCCTTCCCTGAGGAGG-3'		
PCR2	Forward primer	5'-GCAGTGGCTCACGCCTGTAATC-3'	261	61.2
	Reverse primer	5'-GAGTGCAGTGGTGTGATCTCAGC-3'		