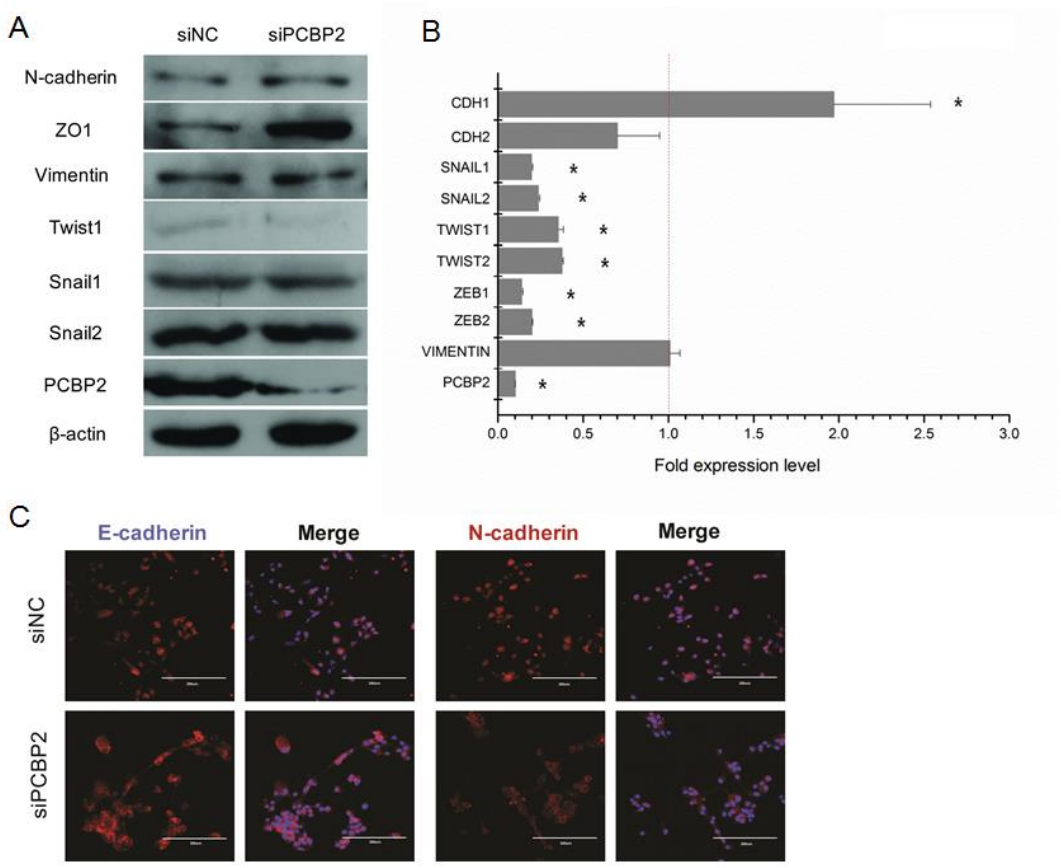
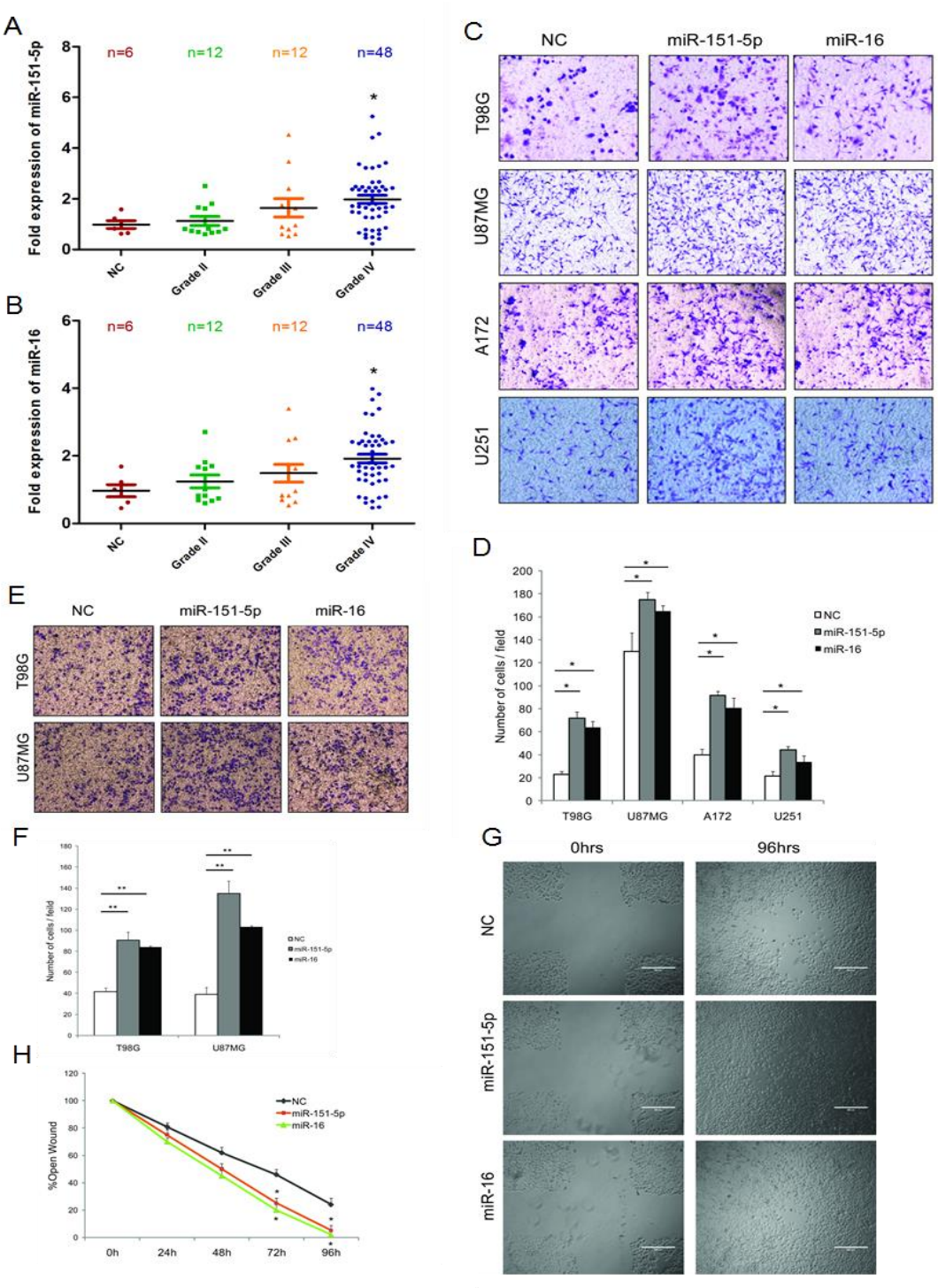
Interplay between PCBP2 and miRNA modulates *ARHGDIA* expression and function in glioma migration and invasion

Supplementary Material

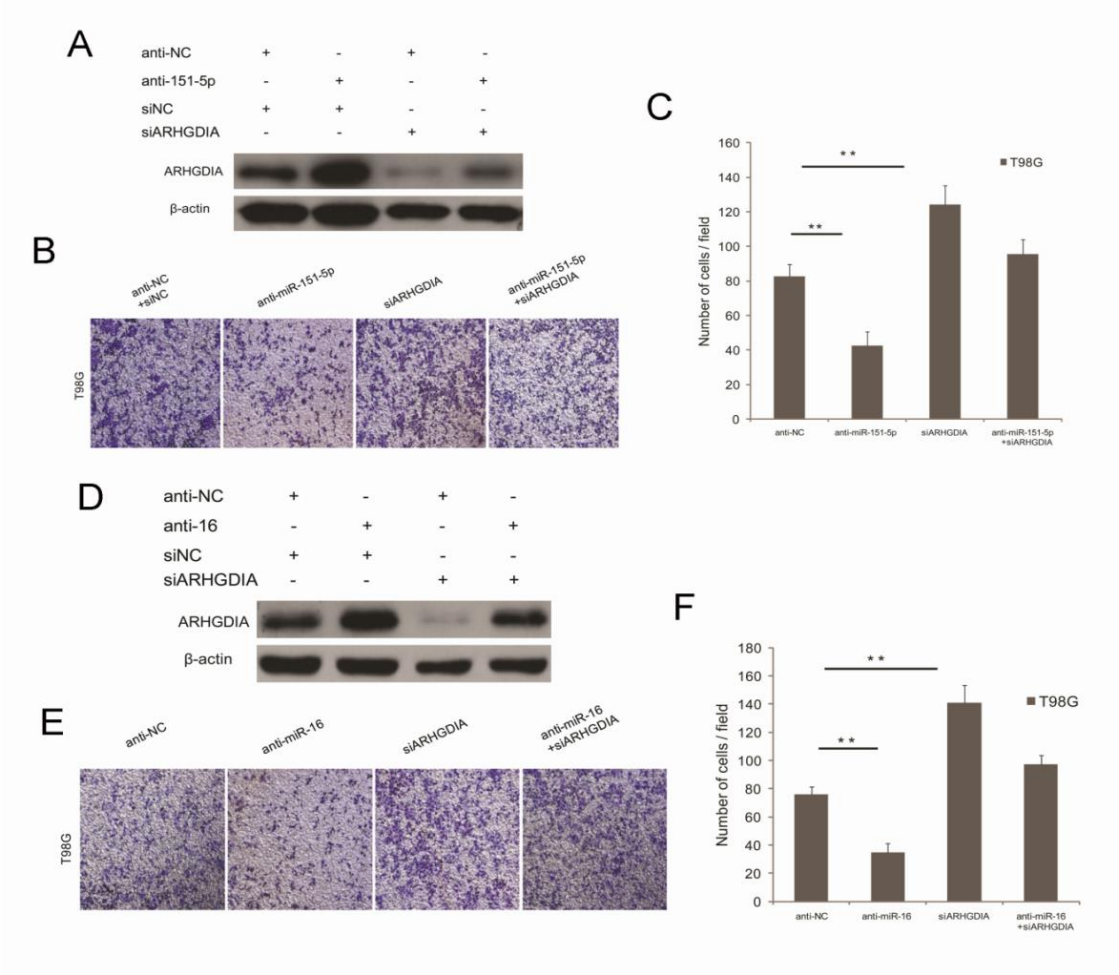


Supplementary Fig.1 Knockdown of PCBP2 reverses mesenchymal-like characteristics of glioma cells. **(A)** Immunoblot showing the down-regulation of mesenchymal markers and meanwhile up-regulation of epithelial markers after transfection with NC siRNA and PCBP2 siRNA for 48h into T98G cells. **(B)** Quantitative real-time PCR analysis of PCBP2 and EMT-related genes in T98G after transfection with NC siRNA and PCBP2 siRNA.Real-time PCR analysis values were normalized to GAPDH. data show the mean±S.D. *$P<0.05$,**$P<0.01$ by Student's *t*-test. **(C)** Immunofluorescence microscopy analysis of E-cadherin(red) and N-cadherin (red) , merge with nuclear DAPI staining (blue) in NC siRNA and PCBP2 siRNA transfection T98G cells. The scale bars represent 200 μm.



Supplementary Fig.2 . High expression of miR-151-5p/miR-16 promotes glioma cell migration and invasion (A,B) Relative miR-151-5p and miR-16 expression were determined by TaqMan real-time PCR in in 6 control brain tissues, 12×II grade, 12×III grade, and 48×IV glioma tissues, U6 snRNA was used as a control. (C-F) Transwell migration in 4 glioma cells (T98G, U87 MG, A172, U251) and invasion assays in 2 glioma cells (T98G, U87 MG) were performed after transfection with control NC, miR-151-5p or

miR-16 mimics in 48-72h.The results are representatives of at least three independent experiments. Graphs indicate the average number of cells per field at the indicated cell lines in migration assays. Data show the mean±S.D. *$P<0.05$,**$P<0.01$. (G, H) The wound-healing assays in T98G cells transfection with control NC, miR-151-5p and miR-16 mimics for 0-96h. Pictures were taken every 24h. A representative picture was shown on time 0h and 96h.Graph represents the width of the remaining open wound calculated in relation to time 0 separation, *$P<0.05$, **$P<0.01$.



Supplementary Fig.3 The rescue assays of miR-151-5p and miR-16.**(A-C)** The co-knockdown of ARHGDIA and miR-151-5p for a rescue study. Western blotting and transwell assays after transfection with ARHGDIA siRNA and miR-151-5p inhibitor (anti-miR-151-5p) individually or cotransfected into T98G cells. Graphs indicate the average number of cells per field at the indicated cell lines in migration assays. Data show the mean±S.D. *$P<0.05$,**$P<0.01$. **(D-F)** The co-knockdown of ARHGDIA and miR-16 for a rescue study. Western blotting and transwell assays after transfection with ARHGDIA siRNA and miR-16 inhibitor (anti-miR-16) individually or cotransfected into T98G cells. Graphs indicate the average number of cells per field at the indicated cell lines in migration assays. Data show the mean±S.D. *$P<0.05$,**$P<0.01$.

Supplementary Table 1

Glioma clinical data

No.	Age	Gender	Grade	Recurrence	Hystotype
1	22	M	III	YES	oligo-astrocytoma
2	68	M	IV	NO	glioblastoma multiforme
3	49	M	II	NO	oligodendroglioma
4	50	F	III	YES	glioblastoma multiforme
5	48	M	IV	YES	glioblastoma multiforme
6	37	F	III	NO	oligodendroglioma
7	46	M	IV	NO	glioblastoma multiforme
8	57	F	IV	NO	oligo-astrocytoma
9	44	F	IV	YES	glioblastoma multiforme
10	22	M	IV	YES	glioblastoma multiforme
11	44	M	IV	YES	glioblastoma multiforme
12	34	F	II	NO	diffuse astrocytoma
13	36	F	IV	NO	glioblastoma multiforme
14	58	M	IV	NO	glioblastoma multiforme
15	46	M	IV	NO	glioblastoma multiforme
16	47	F	III	NO	astrocytoma

17	39	F	IV	NO	anaplastic astrocytoma
18	34	M	III	NO	diffuse oligodendroglioma
20	37	M	III	YES	astrocytoma
21	62	M	IV	NO	glioblastoma multiforme
22	28	F	IV	NO	anaplastic astrocytoma
23	43	M	III	YES	oligo-astrocytoma
24	45	M	IV	YES	glioblastoma multiforme
25	35	M	III	NO	oligodendroglioma
27	62	M	IV	NO	glioblastoma multiforme
28	34	M	IV	YES	glioblastoma multiforme
29	39	M	III	NO	astrocytoma
30	42	M	IV	YES	glioblastoma multiforme
31	38	M	IV	YES	glioblastoma multiforme
32	39	M	IV	YES	glioblastoma multiforme
36	43	F	IV	NO	glioblastoma multiforme
37	56	M	III	NO	oligo-astrocytoma
39	30	F	IV	YES	anaplastic oligo-astrocytoma
41	48	F	IV	YES	glioblastoma multiforme
42	34	M	II	NO	astrocytoma
44	60	M	IV	NO	glioblastoma multiforme

45	76	M	IV	NO	glioblastoma multiforme
47	73	F	IV	NO	glioblastoma multiforme
49	24	F	IV	NO	glioblastoma multiforme
50	29	M	IV	NO	anaplastic oligo-astrocytoma
51	29	F	II	NO	oligodendroglioma
53	22	F	IV	NO	glioblastoma multiforme
54	40	F	IV	NO	glioblastoma multiforme
55	48	M	III	NO	glioneuronal tumor
56	41	F	II	NO	oligo-astrocytoma
57	72	F	IV	NO	glioblastoma multiforme
59	60	M	IV	NO	anaplastic astrocytoma
60	28	F	IV	YES	glioblastoma multiforme
61	50	F	IV	NO	glioblastoma multiforme
62	30	F	II	NO	astrocytoma
63	46	F	III	NO	anaplastic oligo-astrocytoma
65	24	M	IV	YES	glioblastoma multiforme
66	46	F	II	NO	oligodendroglioma
67	42	F	II	NO	oligo-astrocytoma
69	49	M	IV	NO	glioblastoma multiforme
71	45	F	IV	NO	glioblastoma multiforme

72	53	M	IV	NO	glioblastoma multiforme
73	49	M	IV	NO	glioblastoma multiforme
74	48	M	III	YES	anaplastic oligodendroglioma
75	26	F	III	NO	oligo-astrocytoma
76	70	M	IV	NO	glioblastoma multiforme
77	68	F	IV	NO	glioblastoma multiforme
78	37	M	III	YES	anaplastic astrocytoma
79	40	M	II	NO	oligodendroglioma
82	54	F	III	NO	anaplastic oligodendroglioma
84	55	M	III	YES	anaplastic oligo-astrocytoma
87	28	F	III	NO	anaplastic astrocytoma
88	29	F	III	NO	oligodendroglioma
90	40	M	II	YES	oligodendroglioma
93	69	F	II	NO	oligodendroglioma
98	32	M	II	NO	oligo-astrocytoma

Supplementary Table 2

miRNAs primers	
miR-151-5p RT primer	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTAGA-3'
miR-16 RT primer	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCGGTT-3'
u6 RT primer	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAGGCG-3'
miR-16 rltm primer forward	5'-GCCCCTTCGTCGTTAGA-3'
miR-151-5p rltm primer forward	5'-CGCGTCGAGGAGCTCACA-3'
U6 rltm primer forward	5'-CTGGTTAGTACTTGGACGGGAGAC-3'
Universal primer reverse	5'-GTGCAGGGTCCGAGGT-3'
ARHGDIA PCR primers	
ARHGDIA 3'UTR forward primer	5'-CCCTCGAGAGGAGGCACCCAAGGGTAT-3'
ARHGDIA 3'UTR reverse primer	5'-GCTCTAGAAAAGGCAGAGGCAGGACAA-3'
ARHGDIA CDS forward primer	5'-GCGGGGATCCTTGAGCATGGCTGAGCAG-3'
ARHGDIA CDS reverse primer	5'-GCCGCTCGAGGTCCTTCCAGTCCTTCTTGAT-3'
ARHGDIA rltm forward primer	5'-TTTCCGCAGACCCCAACG-3'
ARHGDIA rltm reverse primer	5'-ATCTCTCGGTTAACCCGGAAAG-3'
Biotin pull-down probe primers	
ARHGDIA-3' UTR-A forward	5'--GGAATTCGACTGACGGACGGACGAC-3'

ARHGDIA-3' UTR-A reverse	5'--CGGGATCCTGTGACGGGGAGATAGAACCT-3'
ARHGDIA-3' UTR-A-① forward	5'-GGAATTCACGGACAGGCGGATGTGT-3'
ARHGDIA-3' UTR-A-① reverse	5'-CCCAAGCTTAGCACTTTGGTATGGGGAGG-3'
ARHGDIA-3' UTR-A-② forward	5'--GGAATTCCATACCAAAGTGCTGACAGGC-3'
ARHGDIA-3' UTR-A-② reverse	5'-CCCAAGCTTTCGGTTGAGCCAGGCCA-3'
ARHGDIA-3' UTR-A-③ forward	5'-GGAATTCTCAACCGAGTGCCTCCGA-3'
ARHGDIA-3' UTR-A-③ reverse	5'-CCCAAGCTTTGGGTGGGGGAGGGCT-3'
ARHGDIA-3' UTR-A-④ forward	5'-GGAATTCGCCTGGCTCAACCGAGTG-3'
ARHGDIA-3' UTR-A-④ reverse	5'-CCCAAGCTTACGAGACAGGAGACCGAGGA-3'
α-globin-3' UTR forward	5'-GGAATTCGCTGGAGCCTCGGTGG-3'
α-globin-3' UTR reverse	5'-CCCAAGCTTGCCGCCCACTCAGACTT-3'
mutation primers	
miR-151-5p mt forward	5'-ACCCACAGGCCCAGCCAATCCGGTCTCCT-3'
miR-151-5p mt reverse	5'-TTGGCTGGGCCTGTGGGTGGGGGAGGG-3'
miR-16 mt forward	5'-GTCTCCTGTCTCGTTGAAGCTTCTGCCTGTG-3'
miR-16 mt reverse	5'-TTCAACGAGACAGGAGACCGAGGAGGCTGGG-3'
ARHGDIA-3' UTR-A-①-mt forward	5'-TGTGTCCCCCCCAGCCGGAGGCCTCCCCATACCAAAGTGCT-3'
ARHGDIA-3' UTR-A-①-mt reverse	5'-AGCACTTTGGTATGGGGAGGCCTCCGGCTGGGGGGGACACA-3'
ARHGDIA-3' UTR-A-③-mt forward	5'-AACCGAGTGCCTCCGACCGGGGACCTCAGCCCTCCCCCAC-3'
ARHGDIA-3' UTR-A-③-mt reverse	5'-GTGGGGGAGGGCTGAGGTCCCCGGTCGGAGGCACTCGGTT-3'

EMT-related genes primers	
CDH1 forward	5'-GCCCCATCAGGCCTCCGTTT-3'
CDH1 reverse	5'-ACCTTGCCTTCTTTGTCTTTGTTGGA-3'
CDH2 forward	5'-ACAGTGGCCACCTACAAAGG-3'
CDH2 reverse	5'-CCGAGATGGGGTTGATAATG-3'
SNAI1 forward	5'-TGCGCTACTGCTCGGCGAAT-3'
SNAI1 reverse	5'-AGGGCTGCTGGAAGGTAAACTCTGG-3'
SNAI2 forward	5'-CTGGGCGCCCTGAACATGCAT-3'
SNAI2 reverse	5'-GGCTTCTCCCCCGTGTGAGTTCTA-3'
TWIST1 forward	5'-CACGAGCGGCTCAGCTACGC-3'
TWIST1 reverse	5'-ACAATGACATCTAGGTCTCCGGCCC-3'
TWIST2 forward	5'-GGCGCGCCAGGAGGAGATTCT-3'
TWIST2 reverse	5'-TGCTCACTCCCGCCAACGTT-3'
ZEB1 forward	5'-AGTGGTCATGATGAAAATGGAACACCA-3'
ZEB1 reverse	5'-AGGTGTAACTGCACAGGGAGCA-3'
ZEB2 forward	5'-GACAGATCAGCACCAAATGC-3'
ZEB2 reverse	5'-GCTGATGTGCGAACTGTAGG-3'
VIMENTIN forward	5'-GAGAACTTTGCCGTTGAAGC-3'
VIMENTIN reverse	5'-GCTTCCTGTAGGTGGCAATC-3'
GAPDH forward	5'-GGAGTCAACGGATTTGGTCGTA-3'

GAPDH reverse	5'-GGCAACAATATCCACTTTACCAGAGT-3'
PCBP2 siRNA	5'-GGCCTATACCATTCAAGGA-3'
ARHGDIA siRNA	5'-GGAAAGGCGUCAAGAUUGAdTdT-3'