

PCBP2 siRNA* (with a different target sequence from PCBP2 siRNA) also inhibits PCBP2 expression. Western blot of PCBP2 expression in 3 glioma cell lines (T98G, U87MG, and U251) transiently transfected with control siRNA* or PCBP2 siRNA* for 48-72 h using Lipofectamine 2000. β -Actin was used as a loading control.



zVAD-FMK (pancaspase inhibitor) inhibits glioma cell apoptosis induced by knockdown of PCBP2 or overexpression of FHL3. (A) Representative western blot showing (cleaved) Caspase-3 and substrate its (cleaved) poly-(ADP-ribose) polymerase (PARP) in 3 glioma cell lines (T98G, U87MG, and U251) transiently transfected with control siRNA or PCBP2 siRNA for 48 h and supplemented with or without 10 µM zVAD-FMK. (B) Representative Western blot showing (cleaved) Caspase-3 and its substrate (cleaved) PARP in 3 glioma cell lines (T98G, U87MG, and U251) transiently transfected with control siRNA* or PCBP2 siRNA* for 48 h and supplemented with or without 10µM zVAD-FMK for 24 h. (C) Representative western blot showing (cleaved) Caspase-3 and its substrate (cleaved) PARP in T98G cells transfected with Flag-FHL3 for 48 h and supplemented with or without 10 µM zVAD-FMK for 24 h.



Efficiency of BAP-PCBP2 and BAP-GFP recovery from T98G cell lysates. (A) T98G cells were transfected transiently with BAP-GFP or BAP-PCBP2 supplemented with 100 μ M or 1 mM biotin. BAP-tagged proteins were biotinylated *in vivo* by the co-transfected *h*BirA enzyme. Lysates from cells were probed with streptavidin-HRP by western blotting. (B) Lysates from cells were incubated with streptavidin-sepharose beads. Samples from the input, supernatant (sup), and IP (beads) were loaded on SDS-PAGE gel. Western blots were probed with streptavidin-HRP. The absence of signal in the supernatant lanes and the strong signal visualized in the IP lanes together indicate excellent protein recovery from the precipitations.



Classification of PCBP2-associated mRNAs based on biological processes. PCBP2-associated mRNAs were annotated to the gene ontology term "biological processes" using the web-based tool SBC analysis system. We found that approximately 54% of our mRNAs (19 genes) were related to the 15 biological processes shown; the numbers in the pie chart represent the percentages of mRNAs identified in the groups. Note that the same gene may be assigned to more than one process, and thus, the sum of the annotations will be above 100%.

ARHGDIA-3'A sequence: 5'gacugacggacggacggacggacggacggacggauguguc<u>ccccccagccccuccccca</u>uaccaaagug cugacaggccouccgug<u>cccuccca</u>cccuggucogccucccuggccugguucaaccgagugccuccga<u>cc</u> <u>cccuccucagcccucccca</u>cccacaggcccagccuccucggucuccuguugcugcuucugccu gugcugggggggagaggggcgccagccaggccucugcugcccuuucugug<u>cccccca</u>gguucuaucuccc cgucaca-3'

CSF1-3'B sequence:

5'uaauucugucaucuccguageccucccaguugugccuccugcacauugaugagugccugcuguugucu uugccauguuguugauguagcugugacccuauuguuccucaccccugecceccaccaacccageuggce ca<u>cucuucceceucca</u>acccaageccacageccauccaggaagecuuccuggeuucuccacaaccu ucugacugcucuuucagucauge<u>uccuccu</u>gcucuu uugu-3'

FHL3-3'A sequence: 5'accacgggcccaggacuguggcuccuuuucuaaaccaccucugggacucagcuccccgccaaaaaaa augggucuccuucugggcuccaggauugucuccccacuccagcauccccaaacugguacuccugaccc ggcccccaaaccugggcuccuggguucccagacugagucaagcccacaacugguacuccagaau ucacccucuccccugcagucuggguucccagacugagucacuccccaaaucagggcucuagacccgagc ccuccaaaccugggcucugggacuuaggccccccuaaaucuagacuucucuuuauagguuuccaggucucc uaacacucucucucuuuaagguucccagacugagucaaaucuagacuucucuuuaagguuuccaggac uaugggugc-3

SP2-3'A sequence: 5'gaggccugaagaugcaguc<u>cccca</u>ccuguguccucccugggccccuguggaaaggagcccuguggcu gccuugggccugccucagccccacuccuguucugcacuguccccacaggaaggggcucuguucccugu uugu<u>ccuccuuc</u>ugaagccccuugggccuugggcccuagggaaggggcuccuguuccaaag gacuguggacacuggccgugcccaaugagacguucuaaaccaggacggugggaacccugaucccggcugccca gacuguggacacuggccguccucggaggagcaaagugaggccccua<u>cccca</u>ccccgauccccgauccccaa cacugccggagucgucaugccaug<u>ccccu</u>uccccuggcccugccgccugccgcucccaa cacugcggagucgucaugccaug<u>ccccu</u>uccccuggcccugccggccugcugccca ccuggggcuuggcacccaccucuggaggaaacucggg-3'

MAP2K5-5'B sequence: 5'ucgcucaacuccagaaccuuccgaccuccgcuaguuccugcgggccuuugccgcuucccggugca <u>ccccggggagacaccucagacccccggacagccugggcaggugcguuccuggugcguuccugaucaccc</u> <u>cuccccucuucccuccacucc</u>auccuccauucccuuguuuucacccucuguccucggugcguuccugaucac<u>ucc</u> uugucaccucuuggagccccuucuaaccagcggccaguggguuucccauaccccaggaugugagggugg

Figure S5

Nucleotide sequences of the mRNA segments identified. Nucleotide sequences of 8 mRNA segments identified above. CU-rich patches are underlined.



Co-knockdown of PCBP2 and FHL3 rescues the phenotype induced by knockdown of PCBP2 alone using PCBP2 siRNA* and FHL3 siRNA*. (A) Western blot of FHL3 expression in 3 glioma cell lines (T98G, U87MG, and U251) transiently transfected with control siRNA* or FHL3 siRNA* (different target sequence from FHL3 siRNA) for 48-72 h using Lipofectamine 2000. β -actin was used as a loading control. (**B**) Representative western blot showing PCBP2 and FHL3 protein levels in glioma cell lines transiently transfected with control siRNA*, PCBP2 siRNA*, or PCBP siRNA* plus FHL3 siRNA*. (C) MTT assay of the 3 glioma cell lines after transfection with control siRNA*, PCBP2 siRNA*, or PCBP2 siRNA* plus FHL3 siRNA* (*P<0.05 compared with control siRNA*-transfected cells and co-transfected cells by a t test). (D) Approximately 48 h after transfection, the above cells were analyzed by flow cytometry. The proportions of cells in the G1, G2, and S phages of the cell cycle are depicted in the histograms (*P<0.05 compared with control siRNA by a t test). (E) Nuclear TUNEL staining for apoptotic glioma cells approximately 72 h after transfection. The percentage of TUNEL positive cells was calculated (N=5) and plotted on the histogram (*P<0.05 compared with control siRNA*-transfected cells and co-transfected cells by a t test). (F) Representative western blot showing (cleaved) Caspase-3 and its substrate (cleaved) poly (ADP-ribose) polymerase (PARP) in the glioma cell lines approximately 48-72 h after transfection.



Transfection and infection efficiencies of the U87MG stable transfectants adenovirus-FHL3 and adenovirus-shFHL3. (**A**) Representative western blot showing PCBP2 protein levels in shNT-U87MG and shPCBP2-U87MG stable transfectants. β -actin was used as a loading control. (**B**) Representative western blot showing FHL3 protein levels in U87MG and U251 cells after infection with adenovirus-FHL3. β -actin was used as a loading control. (**C**) Representative western blot showing FHL3 protein levels in vector-U87MG and FHL3-U87MG stable transfectants. β -actin was used as a loading control. (**D**) Representative western blot showing FHL3 and PCBP2 protein levels in U87MG and U251 cells after infection with adenovirus-shFHL3 or co-infection with adenovirus-shPCBP2 and adenovirus-shFHL3. β -Actin was used as a loading control.

	Table S1. Biotin pull-down Probe Primers	
RNA	Primer(Forward)	Primer(Reverse)
ARHGDIA-3'A	5'- GGAATTCGACTGACGGACGGACGAC- 3'	5'-CGGGATCCTGTGACGGGGAGATAGAACCT-3'
ARHGDIA-3'B	5'- GGAATTCTGTGTCTGCATGAGCATGTG-3'	5'- CGGGATCCGTGGGTGAGGCTGTCCAT-3'
ARHGDIA-3'C	5'-GGAATTCCGCCTGGCCATCAGTATTTATT-3'	5'-CGGGATCCGCATTTATTTCCCGGTCAGAAAAG-3'
C16orf57-3'A	5'- GGAATTCCGATGGAGATGCTTCTAGCCT-3'	5'- CGGGATCCAACAGCCGAGACAGGAGAG-3'
C16orf57-3'B	5'- GGAATTCGTTGTGGTCCTTCCCAGAAAC-3'	5'- CGGGATCCAGCAACATGCTTCAAGGAGAG-3'
C16orf57-3'C	5'- GGAATTCGCCACTTCAAAGCCAAGGTA-3'	5'- CGGGATCCTGTCCATCACCAGAAATCCAG-3'
CRTC2-3'UTR	5'- CGGGATCCCCTCATCACCATCCCTCTTC-3'	5'- CCCAAGCTTCTCTCTCCTCCACTGCAAG-3'
CSF1-5'UTR	5'- GGAATTCCGCAGAAGACAGAGGGTGA-3'	5'- CGGGATCCGTCCTGGGATGCGGAGAG-3'
CSF1-3'A	5'- CGGGATCCTAAGACCCCTCACCATCCTG-3'	5'- CCCAAGCTTCCAACAAGGTGGCTTTAACAAA-3'
CSF1-3'B	5'-CGGGATCCTAATTCTGTCATCTCCGTAGCCC-3'	5'- CCCAAGCTTACAAAAGAGCAGGAGGAGCA-3'
FCGR2A-3'A	5'- GGAATTCGCTTGCTGAGTGGATGACAA-3'	5'- CCCAAGCTTCAGTGGCAAAATGTCACAGG-3'
FCGR2A-3'B	5'- GGAATTCATCTTGGCTCACTGCAAACC-3'	5'- CCCAAGCTTGGAGCTTCCGACTGCATAAG-3'
FCGR2A-3'C	5'- GGAATTCGAGCCCAATTACCAGAACCA-3'	5'- CCCAAGCTTTGCATCACCTTGAAACAATCAC-3'
FHL3-3'A	5'- CGGGATCCCAGGACTGTGGCTCCTTTTC-3'	5'- CCCAAGCTTGCACCCATAGGAGACCTGAA-3'
FHL3-3'B	5'- CGGGATCCGGACTGTTCTCAGGCTTGAC-3'	5'- CCCAAGCTTCACACCGCTTTATTGCAGAATC-3'
MAP2K5-5'A	5'- GGAATTCAGTAACAGTGTCGCCGCC-3'	5'- CGGGATCCACGCTATGGTGAAGGTCTCTG-3'
MAP2K5-5'B	5'- GGAATTCTCGCTCAACTCCAGAACCTT-3'	5'- CGGGATCCAGGCTCACATCCTGGGGTA-3'
SP2-3'A	5'- GGAATTCGAGGCCCTGAAGATGCAGT-3'	5'- CGGGATCCCCCGAGTTTCTCCAGAGGTG-3'
SP2-3'B	5'- GGAATTCCAACCTCTCCTCCAGCAC-3'	5'- CGGGATCCGCCTCAAAAATAACAAGCAATGT-3'
α-globin-3'UTR	5'- GGAATTCGCTGGAGCCTCGGTGG-3'	5'- CCCAAGCTTGCCGCCCACTCAGACTT-3'
FHL3-3'A-1	5'- CGGGATCCCAGGACTGTGGCTCCTTTTC-3'	5'- CCCAAGCTTGCTGGAGTGGGGAGACAAT-3'
FHL3-3'A-2	5'- CGGGATCCCAGGACTGTGGCTCCTTTTC-3'	5'- CCCAAGCTTCTGTAAGAGCCCAGGATTGG-3'
FHL3-3'A-3	5'- CGGGATCCCAGGACTGTGGCTCCTTTTC-3'	5'- CCCAAGCTTAGGGGAGAGGGTGAATTCTG-3'
FHL3-3'A-4	5'- CGGGATCCCAGGACTGTGGCTCCTTTTC-3'	5'- CCCAAGCTTCCTGATTTGGGGAGAGGACT-3'