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Supplemental Information

circ-SHKBP1 Regulates the Angiogenesis of U87

Glioma-Exposed Endothelial Cells through

miR-544a/FOXP1 and miR-379/FOXP2 Pathways

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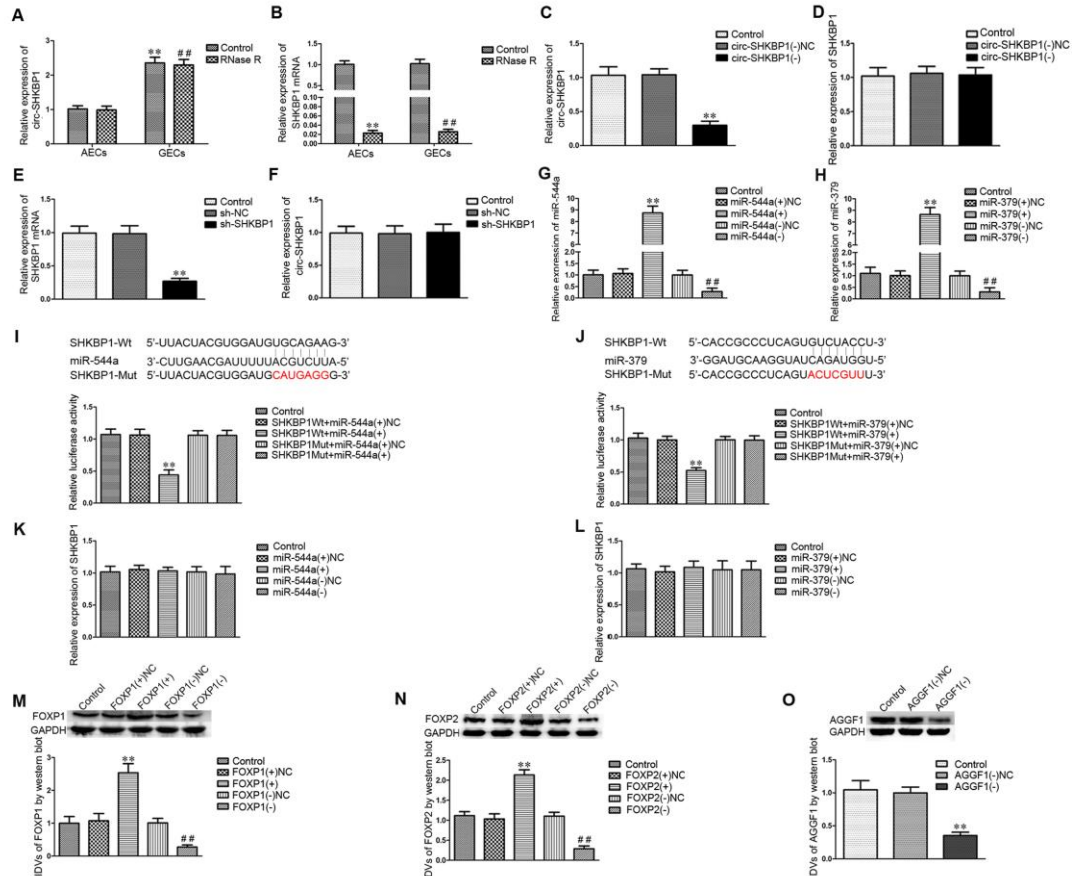


Figure S1 Correlation between SHKBP1 and miR-544a/miR-379 as well as the transfection efficiency of circ-SHKBP1, SHKBP1, miR-544a/miR-379, FOXP1/FOXP2 and AGGF1 were evaluated. (A) The expression of circ-SHKBP1 in GECs with RNase R treatment. Data represent means \pm SD ($n = 5$, each group). ***P* < 0.01 versus control group in AECs; ##*P* < 0.01 versus RNase R group in AECs. (B) The mRNA expression of SHKBP1 in GECs with RNase R treatment. Data represent means \pm SD ($n = 5$, each group). ***P* < 0.01 versus control group in AECs; ##*P* < 0.01 versus control group in GECs. (C) The transfection efficiency of circ-SHKBP1 knockdown was detected by qRT-PCR. Data represent means \pm SD ($n = 5$, each group). ***P* < 0.01 versus circ-SHKBP1 (-) NC group. (D) The expression of SHKBP1 was measured after knockdown of circ-SHKBP1. Data represent means \pm SD ($n = 5$, each group). (E) The mRNA expression of SHKBP1 was detected by qRT-PCR after SHKBP1 knockdown. Data represent means \pm SD ($n = 5$, each group). ***P* < 0.01 versus sh-NC group. (F) The expression of circ-SHKBP1 was measured after knockdown of SHKBP1. Data represent means \pm SD ($n = 5$, each group). (G and H) The transfection efficiency of miR-544a (G) and miR-379 (H) agomir or antagomir were evaluated by qRT-PCR. Data represent means \pm SD ($n = 5$, each group). ***P* < 0.01 versus miR-544a/miR-379 (+) NC group, ##*P* < 0.01 versus miR-544a/miR-379 (-) NC group. (I and J) The binding sites between SHKBP1 and miR-544a (I) or miR-379 (J) were predicted, and the relative luciferase activity was evaluated in HEK293T cells. Data represent means \pm SD ($n = 5$, each group). ***P* < 0.01 versus SHKBP1 Wt + miR-544a/miR-379 (+) NC group. (K and L) The relative SHKBP1 expression was detected by qRT-PCR after miR-544a (K) and miR-379 (L) overexpression or silencing. Data represent means \pm SD ($n = 5$, each group). (M and N) The transfection efficiencies of FOXP1 (M)

and FOXP2 (N) were evaluated with western blot. Data represent means \pm SD ($n = 5$, each group). $**P < 0.01$ versus. FOXP1/FOXP2 (+) NC group, $##P < 0.01$ versus. FOXP1/FOXP2 (-) NC group. (O) The transfection efficiency of AGGF1 was investigated with western blot. Data represent means \pm SD ($n = 5$, each group). $**P < 0.01$ versus. AGGF1 (-) NC group.

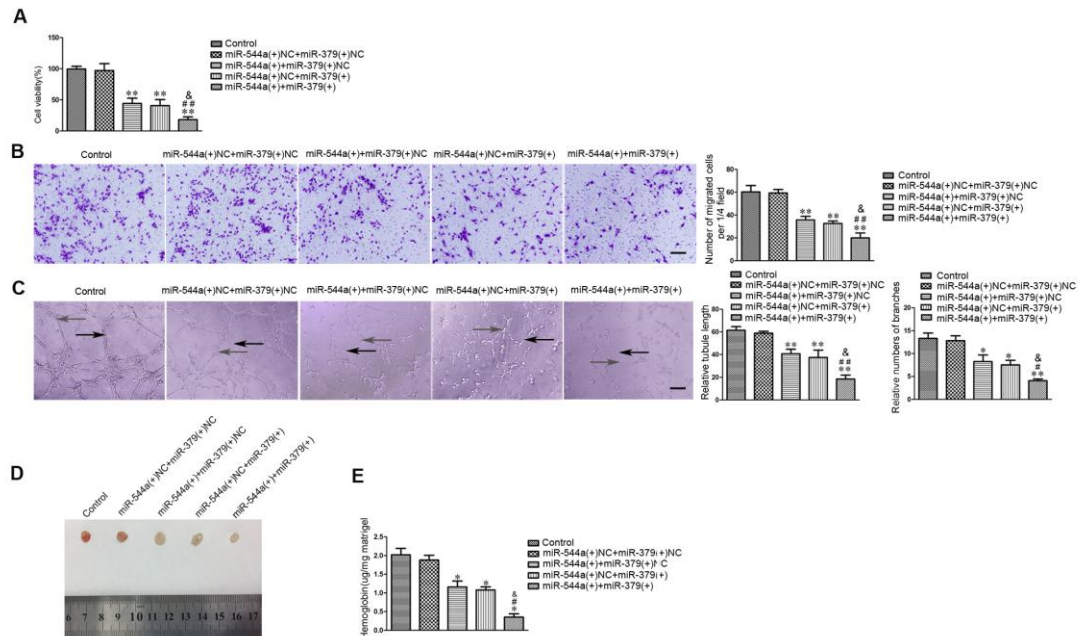


Figure S2 The co-effects of miR-544a/miR-379 on the angiogenesis of U87 glioma *in vivo*. (A) The co-effects of miR-544a/miR-379 on the viability of GECs were evaluated by CCK8 assay. Values are means \pm SD ($n = 3$, each group). $**P < 0.01$ versus. miR-544a (+) NC + miR-379 (+) NC group; $##P < 0.01$ versus. miR-544a (+) + miR-379 (+) NC group. $&P < 0.05$ versus. miR-544a (+) NC + miR-379 (+) group. (B) The co-effects of miR-544a/miR-379 on the migration of GECs was evaluated were evaluated by Transwell assay. Values are means \pm SD ($n = 3$, each group). $**P < 0.01$ versus. miR-544a (+) NC + miR-379 (+) NC group; $##P < 0.01$ versus. miR-544a (+) + miR-379 (+) NC group. $&P < 0.05$ versus. miR-544a (+) NC + miR-379 (+) group. Scale bar represents 30 μ m. (C) The co-effects of miR-544a/miR-379 on the tube formation of GECs were evaluated by Matrigel tube formation assay (black arrow, tube structures; grey arrow, tube branches). Values are means \pm SD ($n = 3$, each group). $*P < 0.05$, $**P < 0.01$ versus. miR-544a (+) NC + miR-379 (+) NC group; $#P < 0.05$, $##P < 0.01$ versus. miR-544a (+) + miR-379 (+) NC group. $&P < 0.05$ versus. miR-544a (+) NC + miR-379 (+) group. Scale bar represents 30 μ m. (D) The co-effects of miR-544a and miR-379 on the angiogenesis *in vivo* was evaluated by Matrigel plug assay. (E) The amount of hemoglobin was measured after combination of miR-544a and miR-379. Values are means \pm SD ($n = 3$, each group). $*P < 0.05$ versus. miR-544a (+) NC + miR-379 (+) NC group; $#P < 0.05$ versus. miR-544a (+) + miR-379 (+) NC group. $&P < 0.05$ versus. miR-544a (+) NC + miR-379 (+) group.