

Supplementary Materials

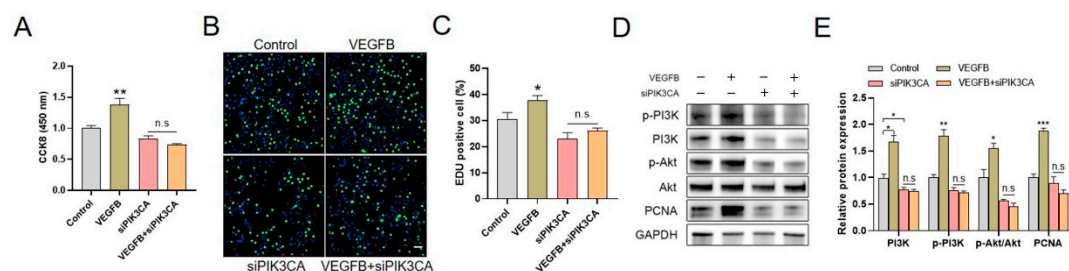


Figure S1. Inhibition of PI3K/Akt by knockdown of PIK3CA blocked the promotion of C2C12 proliferation induced by VEGFB. (A) Effect of 100 ng/mL VEGFB and/or siPIK3CA on the proliferation of C2C12 after 48 h culture was determined by CCK8 analysis ($n = 6$). (B) Effects of 100 ng/mL VEGFB and/or siPIK3CA on C2C12 proliferation were assessed by using EdU incorporation assay ($n = 3$). The nuclei were stained with Hoechst, and the scale bar = 200 μ m. (C) Percentage of EdU positive cells in panel B. (D) Western blot analysis of p-PI3K, PI3K, p-Akt, Akt, and PCNA in C2C12 after 48 h culture. GAPDH was used as loading control. (E) Mean \pm SEM of immunoblotting bands of p-PI3K, PI3K, p-Akt, Akt, and PCNA; the results are expressed as arbitrary units ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group. n.s = not significant. siPIK3CA, small interfering RNA for PIK3CA.

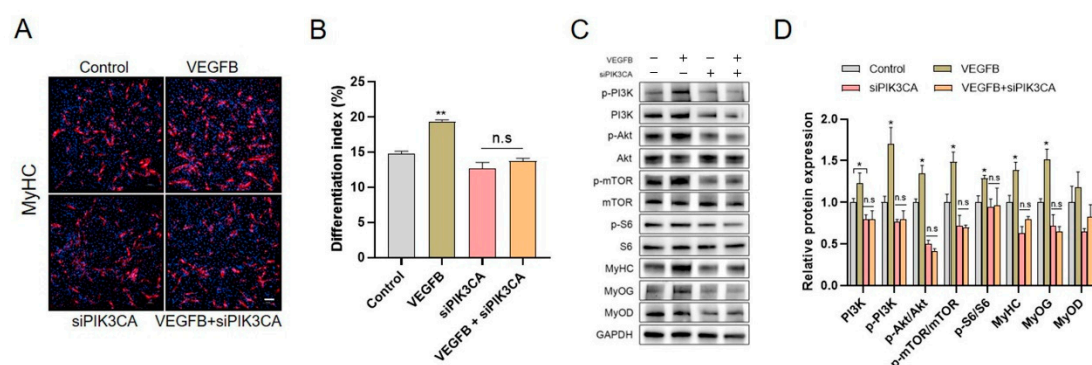


Figure S2. Inhibition of PI3K/Akt by knockdown of PIK3CA blocked the promotion of C2C12 differentiation induced by VEGFB. (A) Effect of 100 ng/mL VEGFB and/or siPIK3CA on the differentiation of C2C12 after 5 days differentiation was determined by immunofluorescence of MyHC ($n = 3$). The nuclei were stained with Hoechst and the scale bar = 200 μ m. (B) The differentiation index was counted by comparing the MyHC-positive cells to total nuclei in panel A. (C) Western blot analysis of p-PI3K, PI3K, p-Akt/Akt, p-mTOR/mTOR, p-S6/S6, MyHC, MyoG, and MyoD in C2C12 after 5 days differentiation. GAPDH was used as loading control. (D) Mean \pm SEM of immunoblotting bands of p-PI3K, PI3K, p-Akt/Akt, p-mTOR/mTOR, p-S6/S6, MyHC, MyoG, and MyoD; the results are expressed as arbitrary units ($n = 3$). * $P < 0.05$, ** $P < 0.01$ versus control group. n.s = not significant. siPIK3CA, small interfering RNA for PIK3CA.