Arrb2 promotes endothelial progenitor cell-mediated postischemic neovascularization

Supplemental Figures and Figure Legends:

**Figure S1:** Identification of EPCs from human umbilical cord blood.

**Figure S1:** A) After 15-day culture of mononuclear cells, spindle-shaped or cobblestone-like adherent cells were observed in bright field. Scale bar=400 µm. B) Representative images of Dil-ac-LDL and FITC-UEA-1 staining showing these cells were able to take up Dil-ac-LDL and bind FITC-UEA-1. Scale bar=100 µm. C) Representative images of flow cytometry showing these cells expressing progenitor cell–specific surface antigens CD34 and endothelial cell–specific surface antigen VEGFR2 and CD31.
Figure S2: The expression of Arrb1 and Arrb2 in EPCs after hind-limb ischemia induction or hypoxia treatment.

Figure S2: A) The protein expression of Arrb2 was profoundly reduced compared with non-ischemia group. However, Arrb1 expression was not altered. B) Arrb2 expression was overtly decreased in EPCs after hypoxia treatment, but hypoxia injury didn’t change the protein level of Arrb1 in EPCs. All data are expressed as mean±SD (n=3), **p<0.01, ***p<0.001 compared with control.
Figure S3: The effectiveness of Lv-Arrb2 and sh-Arrb2 in EPCs was confirmed.

A) Effect of Lv-Arrb2 on Arrb2 expression was determined by Western blot analysis and then quantitated by densitometric analysis.

B) Effect of sh-Arrb2 on Arrb2 expression was determined by Western blot analysis and then quantitated by densitometric analysis. Values are means±SD (n=3), **p<0.01 compared with control.
Figure S4: Arrb2 improves endothelial cells migration

A) Endothelial cells were infected with Lv-Arrb2 or Lv-GFP for 48 h. Representative images of the in vitro scratch-wound assay and quantification of the migration area are presented to show the effects of Lv-Arrb2 on cell migration. Scale bar=400 μm. 

B) Endothelial cells were infected with sh-Arrb2 or sh-Con for 48 h. Representative images of the in vitro scratch-wound assay and quantification of the migration area are presented to show the effects of sh-Arrb2 on cell migration. Scale bar=400 μm. All data are expressed as mean±SD (n=5), *p<0.05, **p<0.01, ***p<0.001 compared with control.
Figure S5: The effect of sh-Arrb2 and ERK1/2 inhibitor on the proliferation, migration and angiogenic function of EPCs.

**Figure S5:**

**A)** Representative images and quantification of Ki67 staining showing the decrease of proliferation in Arrb2-knockdown EPCs was aggravated by ERK1/2 inhibitor (PD98059). Scale bar=100 µm.

**B)** Representative images of Transwell migration assay and quantification of the migrated cells showing the decrease of migration in Arrb2-knockdown EPCs was aggravated by ERK1/2 inhibitor (PD98059). Scale bar=200 µm.

**C)** Representative images and quantification of tube formation showing the decrease of tube formation in Arrb2-knockdown EPCs was aggravated by ERK1/2 inhibitor (PD98059). Scale bar=400 µm. All data are expressed as mean±SD (n=5). *p<0.05, **p<0.01 compared with sh-Arrb2.
Figure S6: The effect of sh-Arrb2 and Akt inhibitor on the proliferation, migration and angiogenic function of EPCs.

**Figure S6:** A) Representative images and quantification of Ki67 staining showing the decrease of proliferation in Arrb2-knockdown EPCs was aggravated by Akt inhibitor (MK-2206 2HCL). Scale bar=100 µm. B) Representative images of Transwell migration assay and quantification of the migrated cells showing the decrease of migration in Arrb2-knockdown EPCs was aggravated by Akt inhibitor (MK-2206 2HCL). Scale bar=200 µm. C) Representative images and quantification of tube formation showing the decrease of tube formation in Arrb2-knockdown EPCs was aggravated by Akt inhibitor (MK-2206 2HCL). Scale bar=400 µm. All data are expressed as mean±SD (n=5). **p<0.01, ***p<0.001 compared with sh-Arrb2.
Figure S7: The expression of Arrb2 in EPCs from Arrb2\(^{-/-}\) mice and wild type mice.

**Figure S7:** The expression of Arrb2 in EPCs isolated from Arrb2\(^{-/-}\) mice and wild type (WT) mice was determined by Western blot analysis and then quantitated by densitometric analysis. Data are expressed as mean±SD (n=3). ***p<0.001 compared with WT.