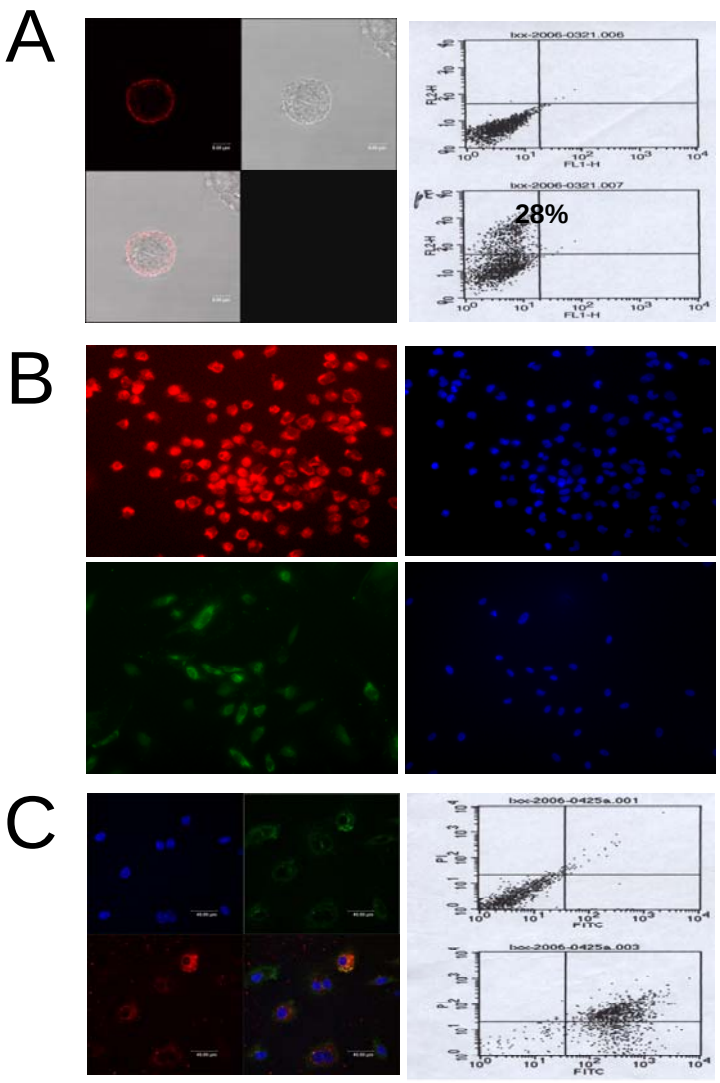
Suppl Fig. 1

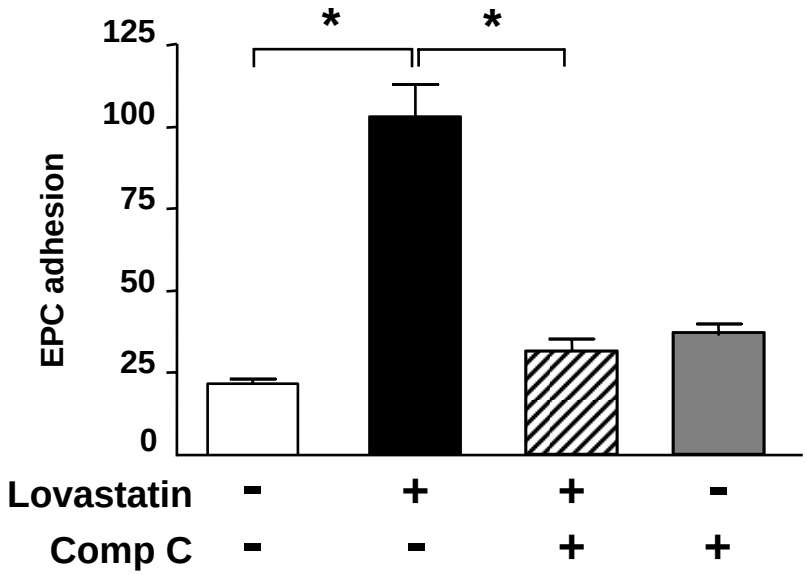


Suppl Fig 1. Identification of human cord blood-derived EPCs. After 2 passages, the EPCs were stained with antibodies against CD34-FITC and analyzed by confocal fluorescent microscopy (A, left) and fluorescent activating cell sorter (FACS) (A, right) analysis, the upper figure is isotype control. Scale bar=8 μ m (B) EPCs were stained with Hochest and antibodies against VEGFR-2 (KDR) (red) and vWF (green) and analyzed by confocal fluorescent microscopy. (C)Uptake of Dil-acLDL (red) for 4 hr at 37℃ before binding of UEA (green) for 1hr, and stained with Hochest (blue), the figures shown are confocal fluorescent microscopy analysis(left) and FACS analysis (right). Scale bar=40 μ m. The presented data were representative from 3 separate experiments.

Methods for EPC Identifications. Cells were incubated with 1,1-dioctadecyl-3,3,3,3-tetramethylindo-carbocyanine perchlorate-labeled acetylated LDL (DiI-acLDL; 10μg/ml; Sigma) at 37 ℃ for 4 hr. After fixed with 2% paraformaldehyde, the cells were incubated with FITC-labeled Ulex europeus agglutinin (UEA, 10μg/ml; Sigma) for 1hr. The cells were also incubated with FITC conjugated primary antibodies against CD34 (BD Pharmingen, San Diego, CA), and VEGF receptor 2 (KDR, Sigma), vWF (Santa Cruz Biotech) were also detected. The results were obtained by Leica confocal fluorescence microscope.

Fluorescence-Activated Cell Sorting (FACS) Analysis. The percentage of cells expressing EPC markers was quantified by FACS assay. Briefly, the cells were trypsinizied and re-suspended in PBS staining buffer to obtain a concentration of $1x10^5$ cells/ml, and then fixed with 2% paraformaldehyde. The markers of EPC detected were phycoerythrin (PE)-conjugated mouse anti-human CD34 (BD Pharmingen), and Dil-acLDL, FITC-conjugated UEA. Isotype-identical antibodies served as negative controls. 10,000 events per sample were analyzed with a Beckman Coulter EPICS XL flow cytometer with EXPO32 ADC software. The data was reported as the percentage of EPCs that expressed these markers.

Suppl Fig.2



Suppl Figure 2. AMPK is involved in the lovastatin-induced EPC adhesion.

EPCs were plated onto fibronectin-coated 24-well plates and cultured until confluent, and then were pretreated with or without Compound C (10 μM) followed by the addition of lovastatin (10 μM) for 24 hr. The cells were trypsinizated and reseeded in fibronectin-coated wells at $5x10^5$ cells/well. After incubation for 1 hr at 37° C, the non-adherent cells were removed by washing three times with PBS. Then the rest of cells were labeled with Hoechst 33342. To quantify the number of adhesion cells, 5 random fields of each group were captured under a fluorescence microscope and the numbers of stained cells were counted respectively. Results are mean ± S.D. from 3 independent experiments, each performed in triplicate (* indicates $p<0.05$).