Cell viability assay

HAECs (8 *10^3/mL) were seeded into each of the 96-well culture plates overnight and kept in a humidified atmosphere of 5% CO2 and 95% air at 37°C. After 24h of incubation, the medium was exchanged for DMEM and added to catalpol at different concentrations (0, 5, 10, 20, 40, 80, 160, 320 μM) with or without TNF-α（50ng/mL）for 24h. Meanwhile, cells without any treatment were used as a control. Following this, the culture medium was removed, and 5 mg/mL methyl thiazolyl tetrazolium (MTT) was added to each well. The plates were then incubated for 4 h at 37°C. The supernatant were carefully removed, the formazan crystals in each well were dissolved in 200μL of DMSO for 30 min at 37℃, and optical density at 570 nm was read on a Microplate Reader (Thermo Fisher Scientific, MA, USA).

Fig. S1 chemical structure of catalpol.

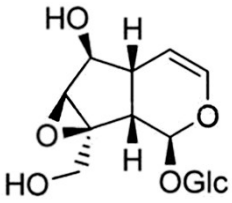

Fig.S2 Catalpol up-regulated SIRT1 expression and enhanced p65 deacetylation through activating AMPK

(A) Effect of catalpol on AMPK phosphorylation after treated with compound C.

(B) Effect of catalpol on SIRT1 protein expression after treating with compound C.

(C) Effect of catalpol on p65 acetylation after treated with compound C.

Data illustrated on the graph bar represent the mean ± SD from three independent experiments. **p < 0.05 vs. control group, ##p < 0.05 vs. TNF-α group. && p < 0.05 vs. compound C treatment, $$p < 0.05 vs. compound C with TNF-α treatment.

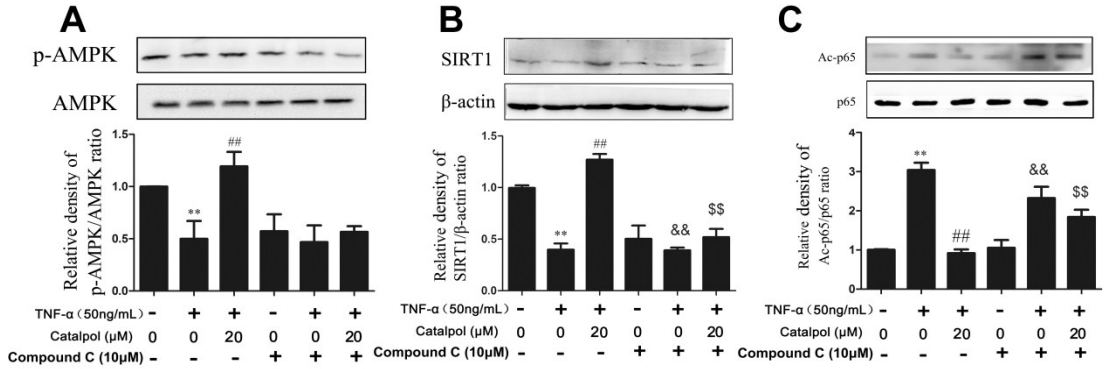

Fig.S3 Catalpol inhibited TNF-α-induced oxidative stress, cell apoptosis and autophagy deficiencythrough activating AMPK

Effect of catalpol on ROS production after treated with compound C. (B) Effect of catalpol on NOX4 expression after treated with compound C. (C) Effect of catalpol on LC3-Ⅱ/Ⅰ and p62/SQSTM expression after treated with compound C. (D) Effect of catalpol on ICAM-1 protein expression after treated with compound C. (E) Effect of catalpol on VCAM-1 protein expression after treated with compound C. (F) Effect of catalpol on Bcl-2 protein expression after treated with compound C. (G) Effect of catalpol on caspase-3 protein expression after treated with compound C. Data illustrated on the graph bar represent the mean ± SD from three independent experiments. **p < 0.05 vs. control group, ##p < 0.05 vs. TNF-α group. && p < 0.05 vs. compound C treatment, $$p < 0.05 vs. compound C with TNF-α treatment.

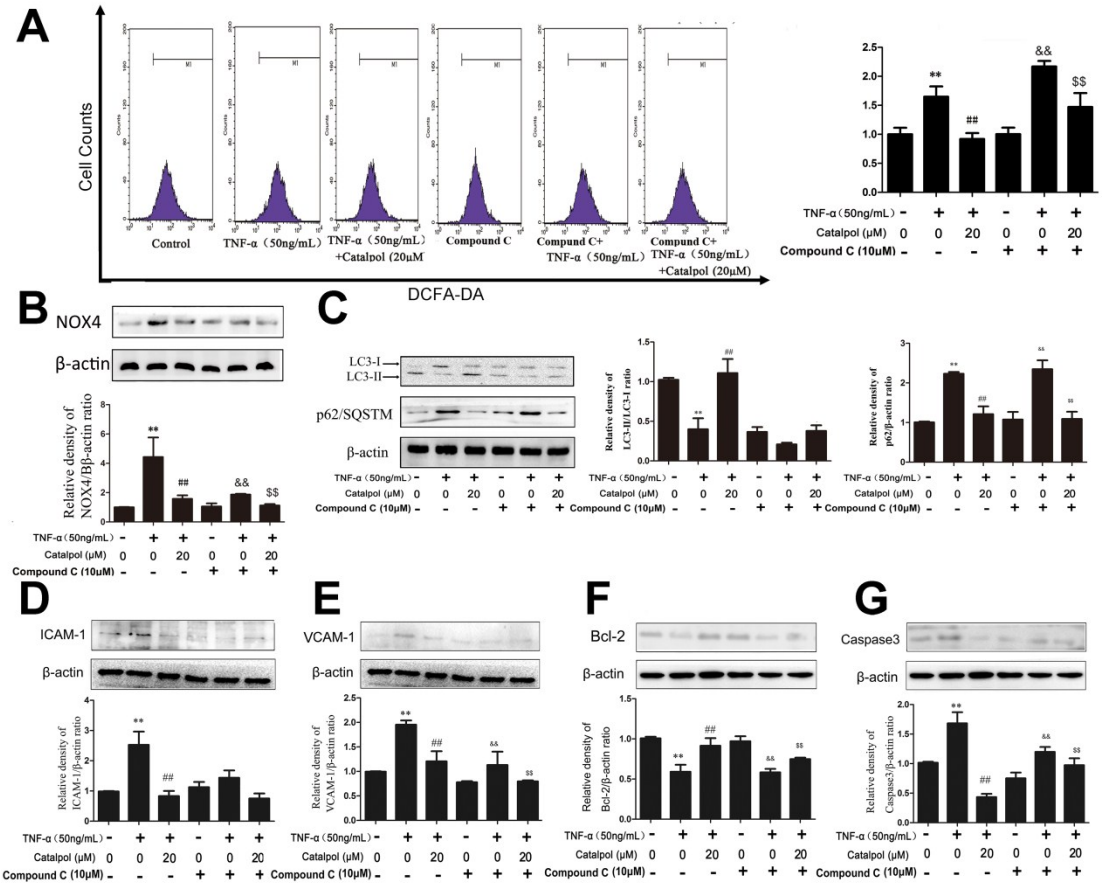

Fig.S4 Effect of catalpol on cell viability with or without TNF-α. (A) Effect of catalpol on cell viability in a concentration-dependent manner. (B) Effect of catalpol on cell viability on TNF-α-treated HAECs. Data illustrated on the graph bar represent the mean ± SD from three independent experiments. **p < 0.05 vs. control group, ##p < 0.05 vs. TNF-α group. && p < 0.05 vs. compound C treatment, $$p < 0.05 vs. compound C with TNF-α treatment.

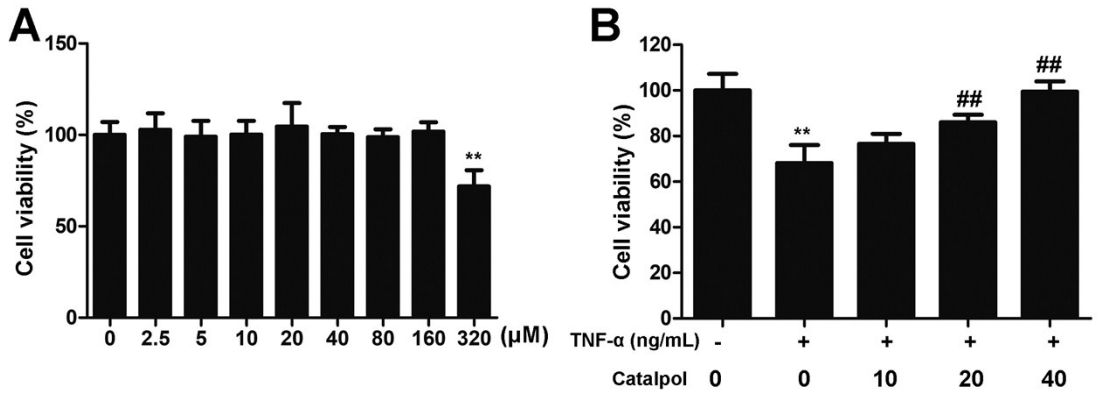

Fig.S5 Graphic summary for the mechanism that catalpol protect HAECs against TNF-α induced oxidative stress and decrease of autophagy through activating AMPK.