# Supplementary material

**Table S1.** List of primer for real-time quantitative real time polymerase chain reaction (qRT-PCR).

| Gene name (Gene ID) | Primer sequence | |
|---|---|---|
| ***Actb*** **(11461)** | Forward | 5' - ACA AAG CTG TTC AGT GTC TCC A - 3′ |
| | Reverse | 5' - CTC CGT TTC CAG AAT ACA CAC A- 3′ |
| *PKC* (18752) | Forward | 5′ - GAT CTC CAT CGG ACT GTT CTT C - 3' |
| | Reverse | 5′ - ATT TTG ATG TGC CCT TCT GAG T- 3' |
| *TGFβ* (21808) | Forward | 5' - CTG GCA GTA GCT CCC CTA TTT A-3′ |
| | Reverse | 5' - ACC AGG GTA AAA ATC GAG ATG A-3′ |
| *BMP2* (12156) | Forward | 5′ - ACA GTA GTT TCC AGC ACC GAA T- 3' |
| | Reverse | 5′ - TGT CCA ATA GTC TGG TCA CAG G- 3' |
| *NF-κB* (18033) | Forward | 5' - AGA AAT CCT ACC CAC AGG TCA A-3′ |
| | Reverse | 5' - CAT TTG TGA CCA ACT GAA CGA T-3′ |
| *CBFα1* (12393) | Forward | 5′ - CGG GAA TGA TGA GAA CTA CTC C- 3' |
| | Reverse | 5′ - TTG AAC CTG GCT ACT TGG TTT T- 3' |
| *osteocalcin* (12097) | Forward | 5′ - GTG TGA GCT TAA CCC TGC TTG T- 3' |
| | Reverse | 5′ - AGT GAT ACC GTA GAT GCG TTT G- 3' |

**Supplementary methods**

**Alkaline phosphatase (ALP) activity assay**

The Alkaline phosphatase (ALP) activity was determined in mouse serum using ALP activity assay kit (Abcam, UK). A quantity of 80 µl of serum was reacted with 50 µl of the 5 mM pnitrophenyl phosphate as the substrate for 60-minute before ending the reaction with the stop solution. After that the absorbance of sample was measured at 405 nm on a microplate reader.

**Calcium deposition assay**

Calcium quantification was measured by using a commercial kit (Abcam, UK). A quantity of 50 µl of serum sample was transferred into new wells. Chromogenic buffer was added and incubated for 5-minute. After that the absorbance of sample was detected at 575 nm on a microplate reader.

**Alizarin red S staining**

To validate calcium in aorta tissue, 0.2% alizarine red S staining conducted. The depariffinized slides washed with distilled water twice and then the slide incubated with 0.2% alizarine red S incubated for 5-minute and dehydrated in acetone. The stained slides imgersed with hematoxylin for nuclei counting and cleared with xylene and mounted with mounting medium (Sigma-Aldrich).

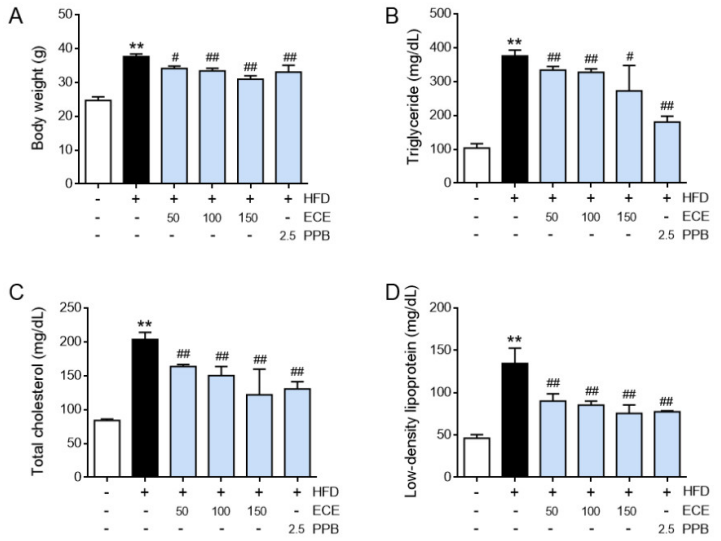


**Figure S1.** Changes of body weight, triglyceride, total cholesterol and LDL level of HFD-fed mice.C57BL/6N mice (male, 8 weeks of age) were bought and NFD or HFD-fed for 4 weeks and then 0.9% normal saline orally administered with NFD (NFD group) or HFD (HFD group) for the last 4 weeks. ECE (ECE group) of PPB (PPB group) orally administered with HFD for the last 4 weeks. At the end of the 8-week study period, (**A**) body weight measured and then all mice were sacrificed. **(B**) triglyceride, (**C**) total cholesterol and (**D**) LDL level measured in blood serum of mice. **, $p < 0.01$ vs. NFD/Saline group; #, $p < 0.05$, ##, $p < 0.01$ vs. HFD/Saline group. ECE, *Ecklonia cava* extract; HFD, high-fat diet; LDL, low-density lipoproteins; NFD, normal fat diet; PPB, pyrogallol-phloroglucinol-6,6-bieckol.

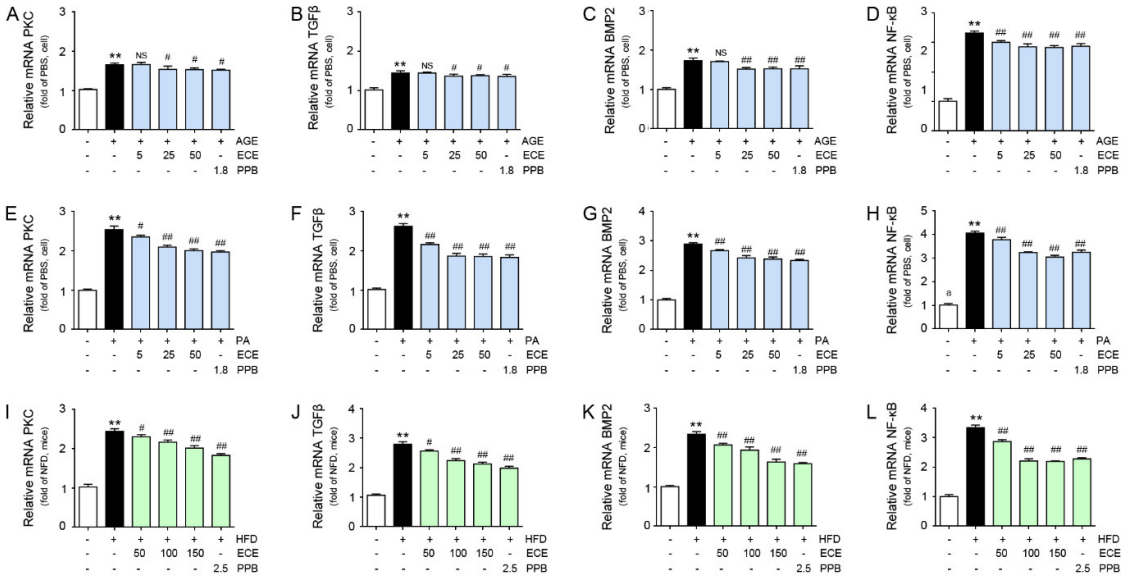


**Figure S2.** Inhibitory effects of ECE or PPB administration on vascular smooth muscle cell phenotypic switching to osteoblast-like cells mRNA levels of the phenotypic switching to osteoblast-like cell-related molecules PKC (**A, E, I**), TGFβ (**B,F,J**), BMP2 (**C,G,K**), NF-κB (**D,H,L**) were determined using qRT-PCR. 0, 25,50 ug/ml ECE or 1.8 ug/ml PPB were incubated with AGE or PA-treated VSMC (cell) and 50, 100, 250 mg/kg ECE or 2.5 mg/kg PPB administered with HFD-fed mice. **, $p < 0.01$ vs. PBS or NFD/Saline group; #, $p < 0.05$, ##, $p < 0.01$ vs. AGE, PA or HFD/Saline group. AGE, advanced glycation end products; BMP2, bone morphogenetic protein 2; ECE, *Ecklonia cava* extract; HFD, high-fat diet; NFD, normal fat diet; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NS, non-

signal; PA, palmitate; PKC, protein kinase C; PPB, pyrogallol-phloroglucinol-6,6-bieckol; qRT-PCR, real-time quantitative real time polymerase chain reaction; TGFβ, transforming growth factor beta; VSMC, vascular smooth muscle cell.

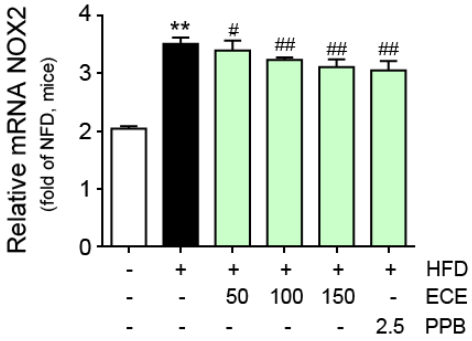


**Figure S3.** Inhibitory effects of ECE or PPB administration on NOX2 expression**.** mRNA levels of NOX2 was determined using qRT-PCR. 50, 100, 150 mg/kg ECE or 2.5 mg/kg PPB were administrated with HFD-fed mice. The asterisk (*) indicates difference between some groups vs. NFD group and the sharp (#) indicates difference between some groups vs. HFD group. **, $p < 0.01$ vs. NFD/Saline group; #, $p < 0.05$, ##, $p < 0.01$ vs. HFD/Saline group. ECE, *Ecklonia cava* extract; HFD, high-fat diet; NFD, normal fat diet; NOX2, nicotinamide adenine dinucleotide phosphate oxidase 2; PPB, pyrogallol-phloroglucinol-6,6-bieckol; qRT-PCR, real-time quantitative real time polymerase chain reaction.

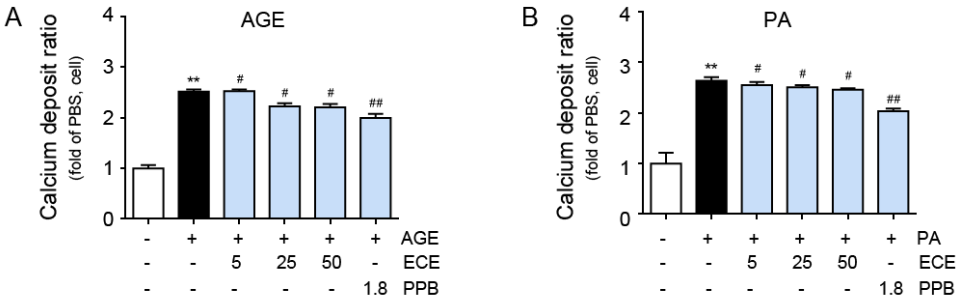


**Figure S4.** Inhibitory effects of ECE or PPB administration on calcium deposition of vascular smooth muscle cells. 0, 25,50 ug/ml ECE or 1.8 ug/ml PPB were incubated with AGE (A) or PA-treated VSMC (B) and Calcium deposition was calculated using the calcium deposition assay. **, $p < 0.01$ vs. PBS group; #, $p < 0.05$, ##, $p < 0.01$ vs. AGE or PA or group. AGE, advanced glycation end products; ECE, *Ecklonia cava* extract; PA, palmitate; PPB, pyrogallol-phloroglucinol-6,6-bieckol; VSMC, vascular smooth muscle cell.