Response to Reviewer 2 Comments

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Point 1: The main concern about this work by Son et al. is that numerous sections (especially in the methods and results) are extremely confused, and it's hard for the reader to follow how the experiments have been performed and the significance of the results. Due to the relevance of the topic, i.e. the identification of functional food supplements useful for the reduction of cardiovascular risk, I suggest the authors to completely reconsider the paper and organize it better.

Response 1: We deeply appreciate the reviewer's point of view. As mentioned, all figures have been changed and restructured to help our readers understand it easily**.** The final manuscript contains 7 main figures, 3 supplementary figures and 3 tables. Figure legends and results have also been changed appropriately in the manuscript.

Figure and figure legends

Figure 1 (Original version)

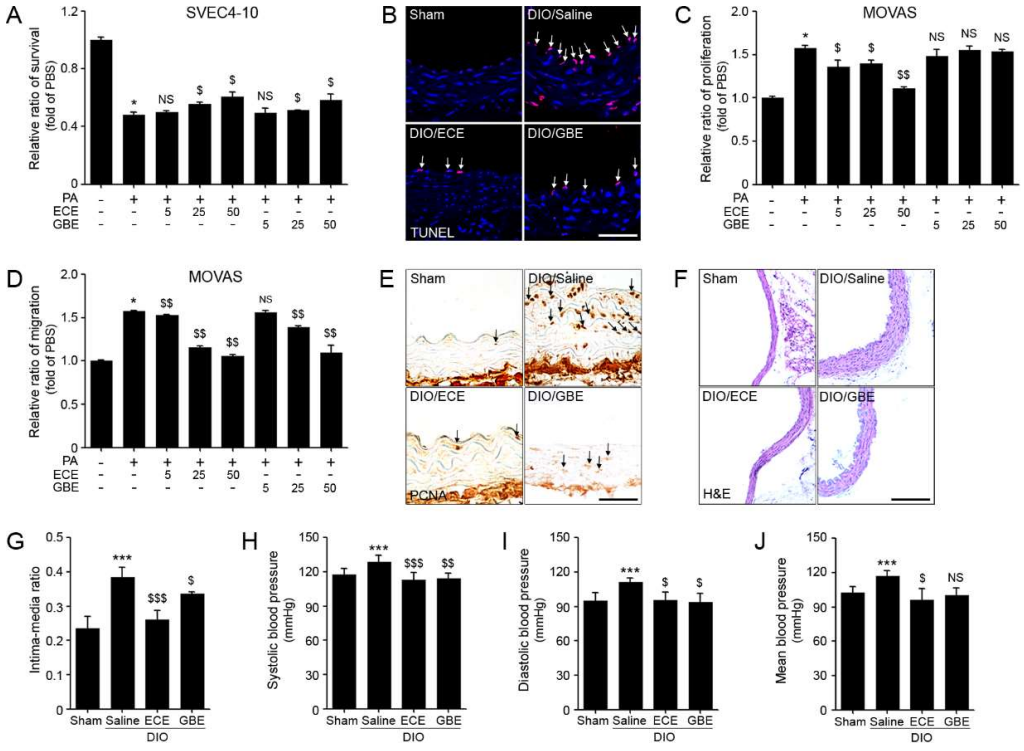

Figure 1. Comparative analysis of GBE and ECE on the improvement of vascular dysfunction in vitro and in vivo (A) PA/PBS, PA/ECE or PA/GBE to SVEC4-10 (mouse endothelial cells) and survival ratio was measured by cell survival assay. (B) Confocal microscopic images showing TUNEL positive cells (red; arrows) and DAPI stained nuclei (blue). Scale bar = 50 μm (c, d) PA/PBS, PA/ECE or PA/GBE to MOVAS and proliferating and migrating VSMCs ratio were measured by each assay. (E) Light microscopic images showing PCNA positive cells (brown, arrow) in obesity mice. Scale bar = 10 μm (f, g) H & E staining images indicate blood vessel thickness and intima-media ratio graph quantified from representative H & E images Scale bar = 200 μm (H) Blood pressure plots of systolic, diastolic, and mean artery blood pressure in obesity mice were shown. BSA plus PBS (PBS), PA plus PBS (PA/PBS), or PA plus ECE or GBE (PA/ECE or PA/GBE), *, P<0.05, **, P < 0.01 and ***, P < 0.001 vs. the PBS (Sham) group. $, P < 0.05; $$, P < 0.01 and $$$, P < 0.001 vs. PA (or obesity/Saline) group, NS, not significant. Data show mean ± SD. PA; palmitate conjugation to BSA, ECE; E. cava extract, GBE; GB extract ; PCNA; Proliferating cell nuclear antigen, H&E; Hematoxylin and eosin

Figure 1 (Modified version)

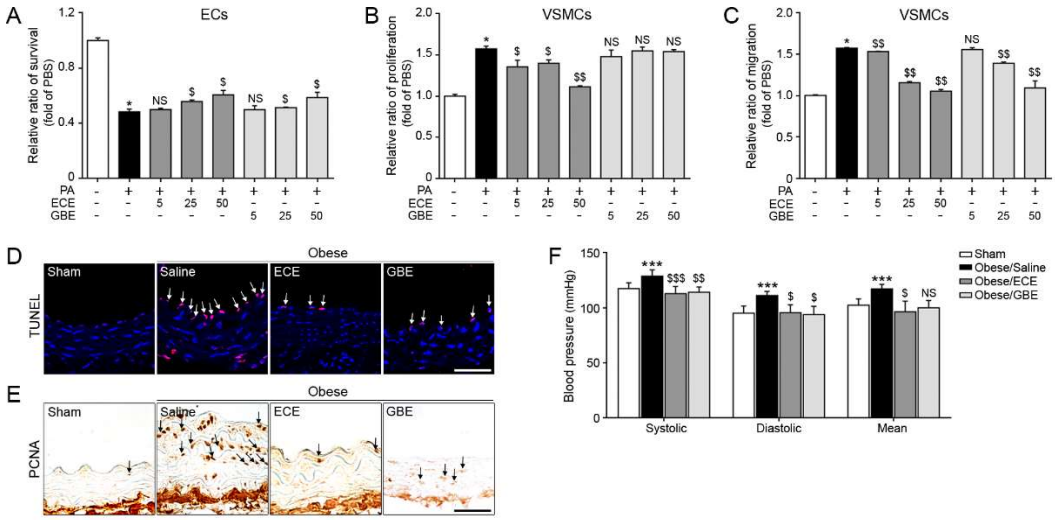

Figure 1. Comparative analysis of GBE and ECE on the improvement of vascular dysfunction *in vitro* and *in vivo*. (A) ECs (SVEC4-10; mouse endothelial cells) were exposed to Palmitate/PBS (PA/PBS), Palmitate/ECE (PA/ECE) or Palmitate/GBE (PA/GBE). The survival ratio of ECs was measured by the cell survival assay. (B, C) PA/PBS, PA/ECE or PA/GBE were administered to VSMCs (MOVAS; mouse vascular smooth muscle cells), and proliferating and migrating VSMC ratio were measured by the respective assays. (D) Confocal microscopic images showing TUNEL positive cells (red; arrows) and DAPI stained nuclei (blue). Scale bar = 50 μm. (E) Light microscopic images showing PCNA positive cells (brown, arrow) in obese mice. Scale bar = 10 μm. (F) Blood pressure plots of systolic, diastolic, and mean artery blood pressure in obese mice were shown. *, $P < 0.05$ and ***, $P < 0.001$ vs. the PBS (Sham) group; $, $P < 0.05$; $$, $P < 0.01$ and $$$, $P < 0.001$ vs. PA (or Obese/Saline) group; NS, not significant. Data show mean ± SD. PA, palmitate conjugation to BSA; ECE, *E. cava* extract; GBE, GB extract; PCNA, Proliferating cell nuclear antigen.

Figure 2 (Original version)

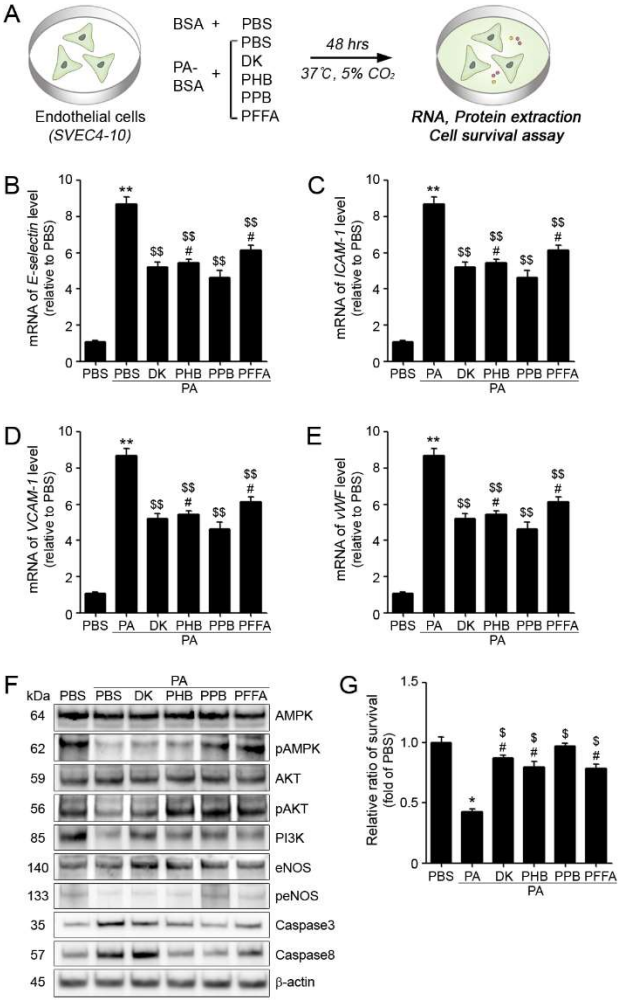

Figure 2. Comparison of the four phlorotannins isolated from ECE on EC adhesion molecule expression and cell survival in vitro (A) Illustration explains palmitate conjugation to BSA (PA) induced EC dysfunction model in vitro. (B-E) mRNA expression levels of the adhesion molecules E-selsectin, ICAM-1, VCAM-1 and VWF in ECs as determined by qRT-PCR. (F) Protein levels of the EC death-related molecules AMPK, pAMPK, AKT, pAKT, PI3K eNOS, peNOS, caspase 3, and caspase 8 were determined by western blotting. (G) Survival ratio of EC treated with PBS, PA/PBS, or PA/phlorotannins. BSA plus PBS (PBS), PA plus PBS (PA/PBS), or PA plus phlorotannins (PA/DK, PA/PHB, PA/PPB and PA/PFFA) **, $P < 0.01$ and ***, $P < 0.001$ vs. the PBS group, $, $P < 0.05$; $$, $P < 0.01$ and $$$, $P < 0.001$ vs. PA/PBS group, #, $P < 0.05$, ##, $P < 0.01$ and ###, $p < 0.001$ vs. PA/PPB. PA; palmitate conjugation to BSA, DK; dieckol, PHB; 2,7-phloroglucinol-6,6-bieckol, PPB; pyrogallol-phloroglucinol-6,6-bieckol, PFFA; phlorofucofuroeckol A, α-SMA; α-smooth muscle actin

Figure 2 (Modified version)

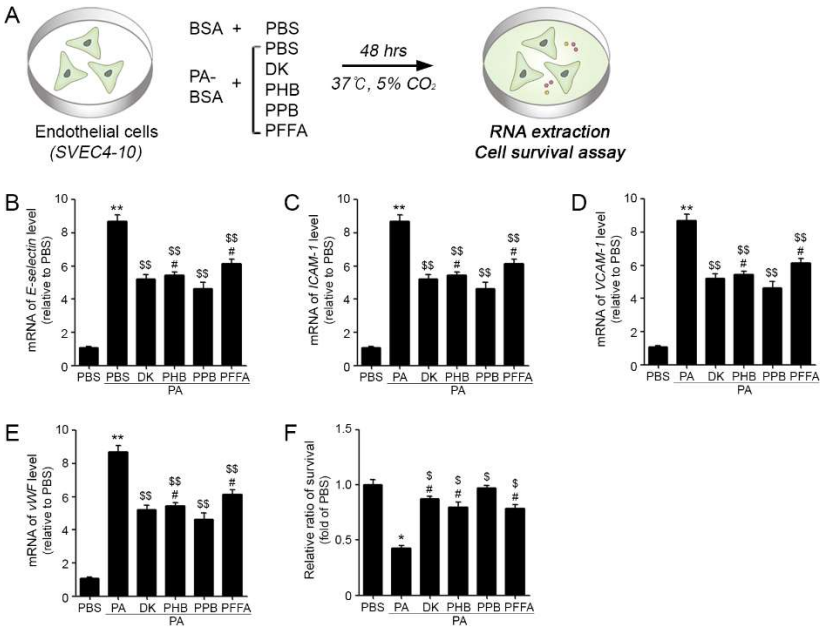

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Figure 3 (Original version)

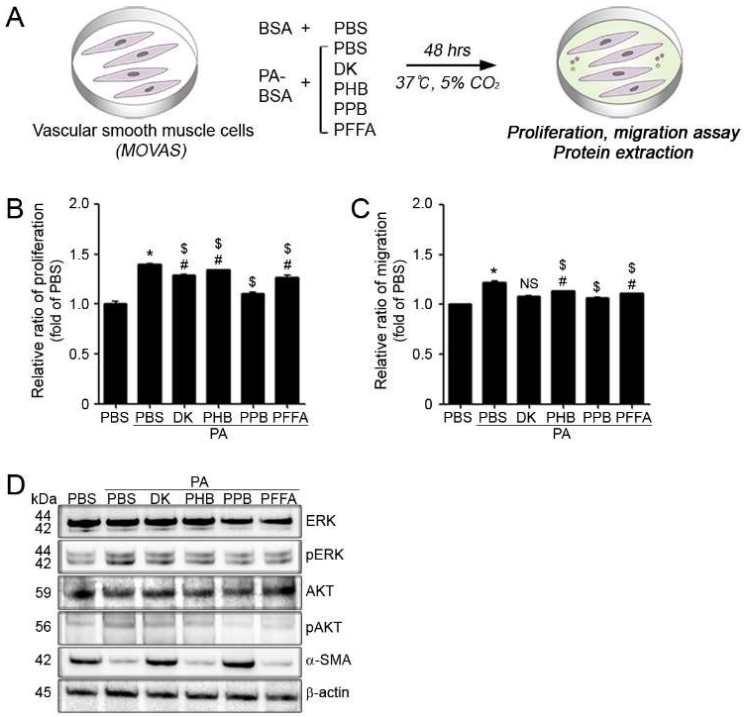


Figure 3. Comparison of the four phlorotannins isolated from ECE on excessive proliferation and migration of VSMCs in vitro (A) Illustration explains palmitate conjugation to BSA (PA) induced VSMC dysfunction model in vitro (B) Proliferations of VSMCs treated with PBS, PA or with PA plus each of the four phlorotannins were measured using a proliferation assay, and (C) migrations were measured using a transwell migration assay. (D) Protein levels of the proliferation and migration related molecules ERK1/2, pERK1/2, AKT, pAKT, and α-SMA were determined by western blotting. BSA plus PBS (PBS), PA plus PBS (PA/PBS), or PA plus phlorotannins (PA/DK, PA/PHB, PA/PPB and PA/PFFA). *, $P < 0.05$ vs. the PBS group, \$, $P < 0.05$ vs. PA group, and #, $P < 0.05$ vs. the PA/PPB group, NS, not significant. PA; palmitate conjugation to BSA, DK; dieckol, PHB; 2,7-phloroglucinol-6,6-bieckol, PPB; pyrogallol-phloroglucinol-6,6-bieckol, PFFA; phlorofucofuroeckol A, α-SMA; α-smooth muscle actin

Figure 3 (modified version)

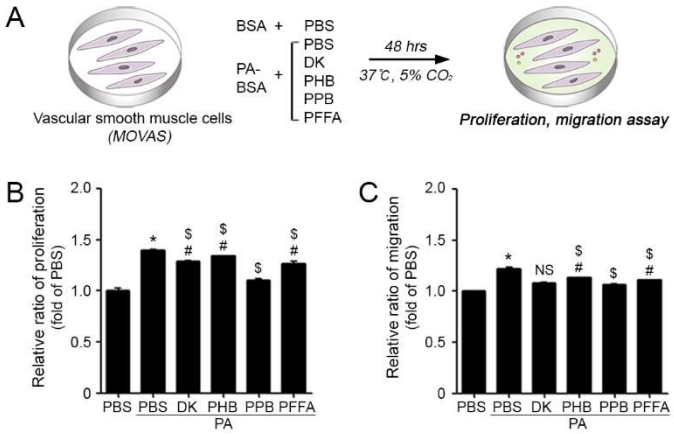


Figure 3. Comparison of the four phlorotannins isolated from ECE on excessive proliferation and migration of VSMCs *in vitro*. (A) Illustration explains palmitate conjugation to BSA (PA) induced VSMC dysfunction model *in vitro*. (B) PA with or without phlorotannin treated VSMCs were measured using a proliferation assay, and (C) trans-well migration assay. *, $P < 0.05$ vs. the PBS group; $, $P < 0.05$ vs. PA group; and #, $P < 0.05$ vs. the PA/PPB group. NS, not significant. PA, palmitate conjugation to BSA; DK, dieckol; PHB, 2,7-phloroglucinol-6,6-bieckol; PPB, pyrogallol-phloroglucinol-6,6-bieckol; PFFA, phlorofucofuroeckol A.

Figure 4 (Original version)

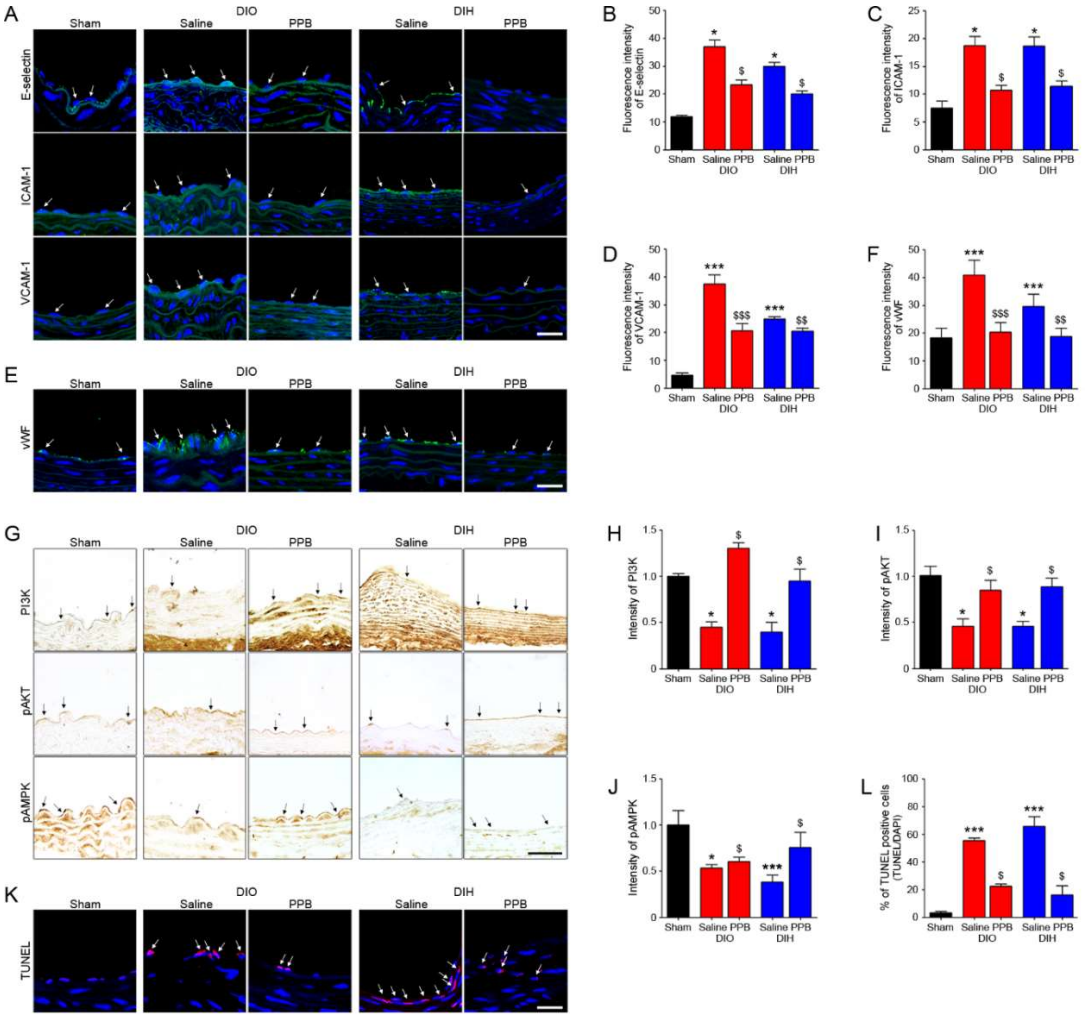


Figure 4. Inhibitory effects of the PPB isolated from ECE on EC dysfunction in diet-induced mouse models of obesity and hypertension (A-E) Arrows in confocal microscopic images show adhesion molecule expressions (green, arrow; E-selectin, ICAM-1, VCAM-1, VWF) or nuclei (DAPI, blue) in obesity and hypertension mouse models. Quantitative graphs show fluorescence intensity from representative images (red bar; obesity mouse model, blue bar; obesity mouse model). Scale bar = 50 μm (G-J) Light microscopic images showing the EC survival related molecules such as PI3K, pAKT and pAMPK (brown, arrow). Quantitative graphs show intensity from representative images (red bar; obesity mouse model, blue bar; obesity mouse model). Scale bar = 10 μm (K,L) TUNEL positive cells (red, arrow) indicate apoptotic cells and nuclei stained DAPI (blue). Quantitative graphs show percentage of TUNEL positive cell from representative images. Scale bar = 50 μm *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$ vs. the Sham group. $, $P < 0.05$, $$,$P < 0.01$ and $$$, $P < 0.001$ vs. obesity/Saline or hypertension/Saline group. PPB; pyrogallol-phloroglucinol-6,6-bieckol obesity; diet-induced obesity, hypertension; diet-induced hypertension, ICAM-1; Intercellular Adhesion Molecule 1, VCAM-1; Vascular cell adhesion protein 1, VWF; von Willebrand factor, TUNEL; Terminal deoxynucleotidyl transferase dUTP nick end labeling

Figure 4 (modified version)

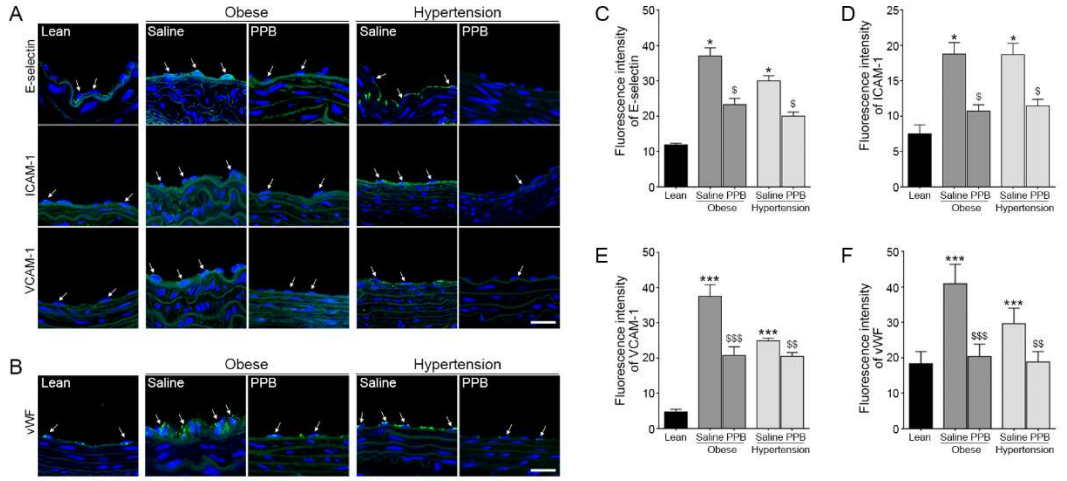


Figure 4. Inhibitory effects of the PPB isolated from ECE on EC dysfunction in diet-induced mouse models of obese and hypertension. (A, B) Arrows in confocal microscopic images show adhesion molecules including E-selectin, ICAM-1, VCAM-1 and vWF expressions (arrow, green) or nuclei (DAPI, blue), in the Obese and Hypertension mouse models. (C-F) Quantitative graphs show fluorescence intensity from representative images (dark gray bar, obese mouse model; gray bar, hypertension mouse model). Scale bar = 50 μm. *, $P < 0.05$ and ***, $P < 0.001$ vs. the lean group; $, $P < 0.05$, $$, $P < 0.01$ and $$$, $P < 0.001$ vs. obese/Saline or hypertension/Saline group.

Figure 5 (Original version)

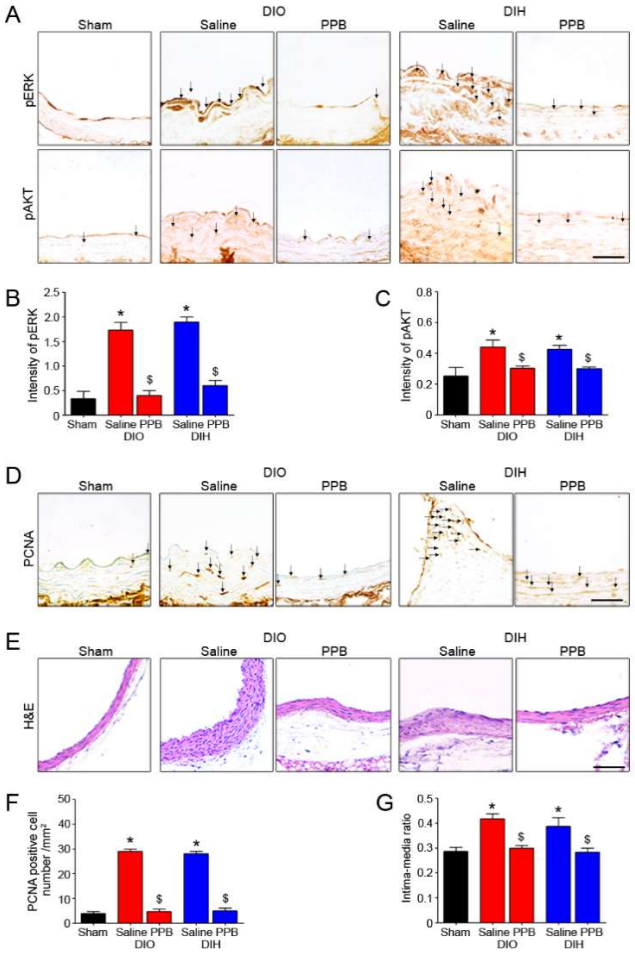


Figure 5. Inhibitory effects of the PPB isolated from ECE on VSMC dysfunction in diet-induced mouse models of obesity and hypertension (A-C) Light microscopic images showing abnormally high expressions of proliferation and migration related molecules (pERK1/2 and pAKT) in VSMCs in obesity and in hypertension. Quantitative graphs show intensity from representative images (red bar; obesity mouse model, blue bar; obesity mouse model). Scale bar = 10 μm (D-G) Light microscopic images showing proliferation marker (PCNA) and H&E stained blood vessels. Plots showing numbers of PCNA positive cells and intima-media ratios obtained using representative H&E images (red bar; obesity mouse model, blue bar; obesity mouse model). Scale bar = 10 μm (PCNA) and 200 μm (H&E) *, $P < 0.05$ vs. Sham group. $, $P < 0.05$ vs. obesity/Saline or hypertension/Saline mice group. PPB; pyrogallol-phloroglucinol-6,6-bieckol obesity; diet-induced obesity, hypertension; diet-induced hypertension, VSMCs; vascular smooth muscle cells, PCNA; Proliferating cell nuclear antigen, H&E; Hematoxylin and eosin

Figure 5 (modified version)

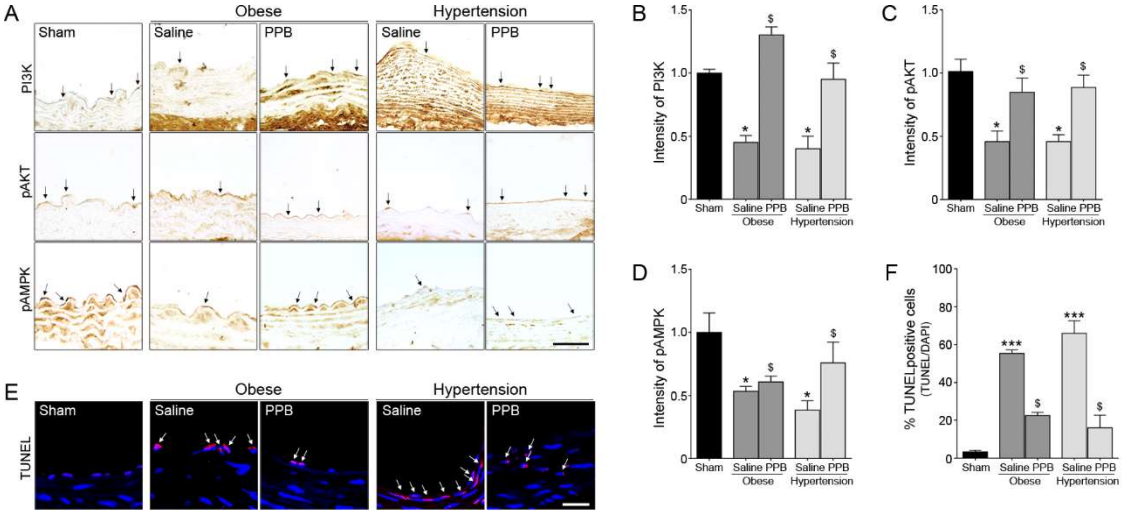


Figure 5. Inhibitory effects of the PPB isolated from ECE on EC dysfunction in diet-induced mouse models of obese and hypertension. (A) Light microscopic images showing the EC survival related molecules such as PI3K, pAKT and pAMPK (brown, arrow). (B-D) Quantitative graphs show intensity from representative images (dark gray bar, obese mouse model; gray bar, hypertension mouse model). Scale bar = 10 μm. (E, F) TUNEL positive cells (red, arrow) indicate apoptotic cells, and nuclei stained DAPI (blue). Quantitative graphs show percentage of TUNEL positive cells from representative images. Scale bar = 50 μm. *, $P < 0.05$ and ***, $P < 0.001$ vs. the lean group; $, $P < 0.05$ vs. Obese/Saline or Hyertension/Saline group. PPB, pyrogallol-phloroglucinol-6,6-bieckol.

Figure 6 (Original version)

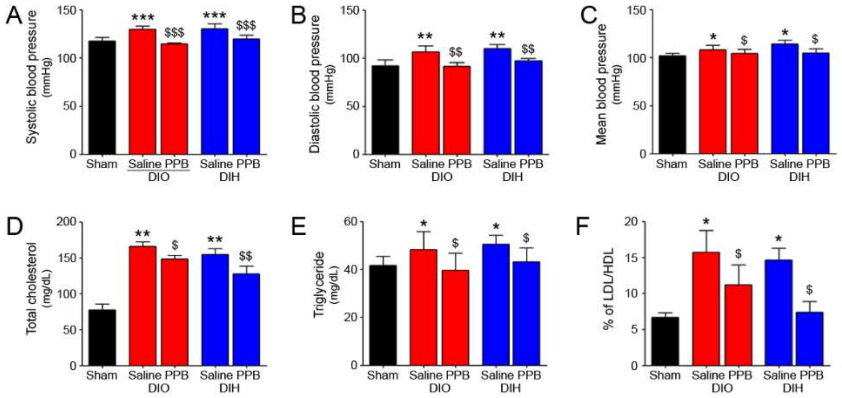


Figure 6. Effects of PPB isolated from ECE on blood pressure and serum lipoprotein, cholesterols in diet-induced mouse models of obesity and hypertension Blood pressures such as (A) systolic, (B) diastolic and (C) mean artery pressure in obesity and hypertension mouse models. Serum levels of (D) total cholesterol and (E) triglyceride and (F) LDL/HDL ratios (red bar; obesity mouse model, blue bar; obesity mouse model) *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$ vs. the Sham group. $, $P < 0.05$, $$, $P < 0.01$ and $$$, $P < 0.001$ vs. the obesity/Saline or hypertension/Saline group. PPB; pyrogallol-phloroglucinol-6,6-bieckol obesity; diet-induced obesity, hypertension; diet-induced hypertension

Figure 6 (modified version)

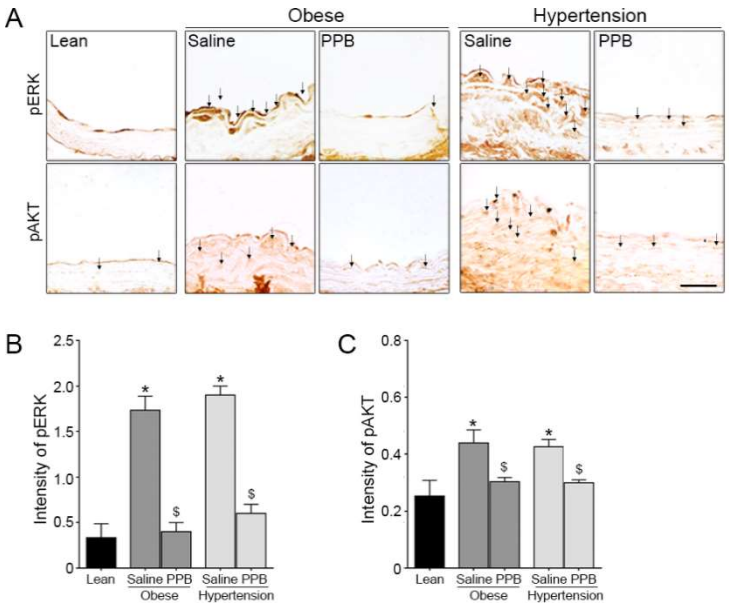


Figure 6. Inhibitory effects of the PPB isolated from ECE on VSMC dysfunction related molecules in diet-induced mouse models of obese and hypertension. (A) Light microscopic images showing abnormally high expressions of proliferation and migration related molecules (pERK1/2 and pAKT) in VSMCs in Obese and in Hypertension models. (B, C) Quantitative graphs show intensity from representative images (dark gray, Obese mouse model; gray bar, Hypertension mouse model). Scale bar = 10 μm.

Figure 7 (original version)

None

Figure 7 (modified version)

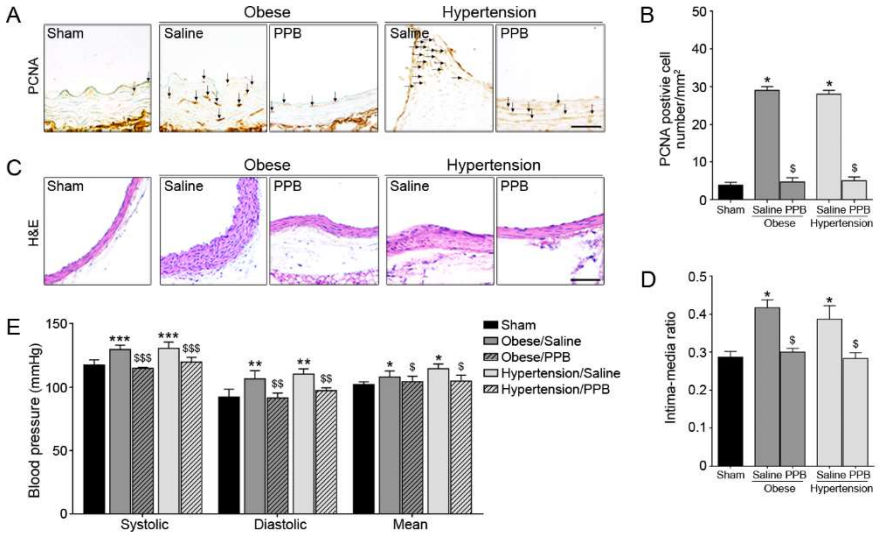


Figure 7. Effects of PPB isolated from ECE on vascular dysfunction and blood pressure in diet-induced mouse models of obese and hypertension (A,C) Light microscopic images showing proliferation marker (PCNA) and H&E stained blood vessels. (B,D) Plots showing numbers of PCNA positive cells and intima-media ratios obtained using representative H&E images (dark gray bar, Obese mouse model; gary bar, Hypertension mouse model). (E) Blood pressures such as systolic, diastolic and mean artery pressure in Obese and Hypertension mouse models. Scale bar = 10 μm (PCNA) and 200 μm (H&E). *, $P < 0.05$ vs. lean group; $, $P < 0.05$ vs. Obese/Saline or Hypertension/Saline mice group. PCNA, Proliferating cell nuclear antigen; H&E, Hematoxylin and eosin.

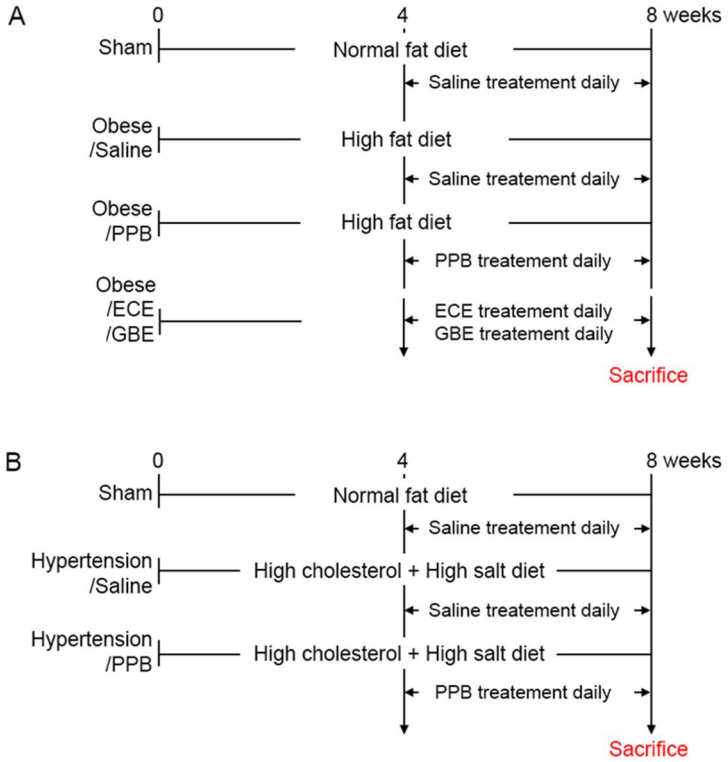
Point 2: Animal model: the mouse model should be better described and justified. Why using only 45% kcal from fat in the diet? Please explain the different models using a Table, not in the text.

Response 2: We appreciate and agree with comment. In order to describe and justify these results, we need to explain the different models using a table and figure, which have now been added in supplementary materials as Table S2 and Figure S3, and is accordingly mentioned in the main manuscript (page 9).

Table S2. Composition of experimental diets

Group / Conposition	Sham	Obese	Hypertension
Ingredient (% Kcal)			
Protein	24.7	20	15.5
Carbohydrate	13.2	35	41.9
Fat	62.1	45	42.6
Total	100	100	100
Kcal/gm	3.07	4.72	4.5
Total Cholesterol (% of diet)	0	0.12	1.25
Salt (mg/kg, daily)	0	0	0.03

Figure S3. Scheme of experimental animal models



(A) Scheme of this study. C57BL/6 mice fed high fat diet (HFD) for 8 weeks and some of the obese mice orally administrated 0.9% normal saline, PPB(2.5 mg/kg/day), ECE(70 mg/kg/day) and GBE (500mg/kg/day) after 4 weeks of HFD feeding. (B) C57BL/6 mice fed high cholesterol diet with salt for 8 weeks and some of the hypertension mice orally administrated 0.9% normal saline, PPB(2.5 mg/kg/day) after 4 weeks of high cholesterol diet with salt feeding.

Point 3: In vitro experiments: why not using primary cells obtained by mice to confirm the data?.

Response 3: We appreciate and agree with the comment. Experiments using primary cells are more relevant than cell lines. However, the purpose of cell experiments (*in vitro*) in this study was to facilitate the screening of the inhibitory efficacy for EC and VSMC dysfunction of the four compounds (DK, PHB, PPB and PFFA) isolated from *Ecklonia cava* extract, prior to animal experiments (*in vivo*). PPB was selected from among the four isolated compounds. The inhibitory efficacy of PPB was then confirmed in the obese- and hypertension- induced mouse models.

Point 4: The authors should first describe and discuss the in vitro experiments and then the in vivo findings.

Response 4: We understand and are in agreement with this comment. The order of *in vitro* and *in vivo* experiment findings has accordingly been changed. The *in vitro* findings are explained first and *in vivo* findings explained later to help readers understand. The revised section can be found through pages 3-7.

Point 5: Fig.2F: western blot. the last 3 proteins (caspase 3 and 8 and beta-actin) seem to be taken from a whole membrane, whereas the others seem to be obtained by a cleaved one. why?.

Response 5: We appreciate this comment. Our results are extracted from the following experimental results. The results seem to be truncated because the membrane background is darker than the other three proteins (caspase 3 and 8 and beta-actin). In this paper, it is thought that it is difficult for readers to understand because it includes many contents such as pathway analysis, including AMPK. Therefore, to facilitate the reader's understanding, we reorganized the main figure and results. Accordingly, Western blotting results have been removed in Figure 2 and Figure 3, and we have concentrated on EC death, VSMC proliferation and migration in obese- and hypertension-induced mouse models. The revised figures can be found through page 4.

Figure and figure legends

Figure 2 (Original version)

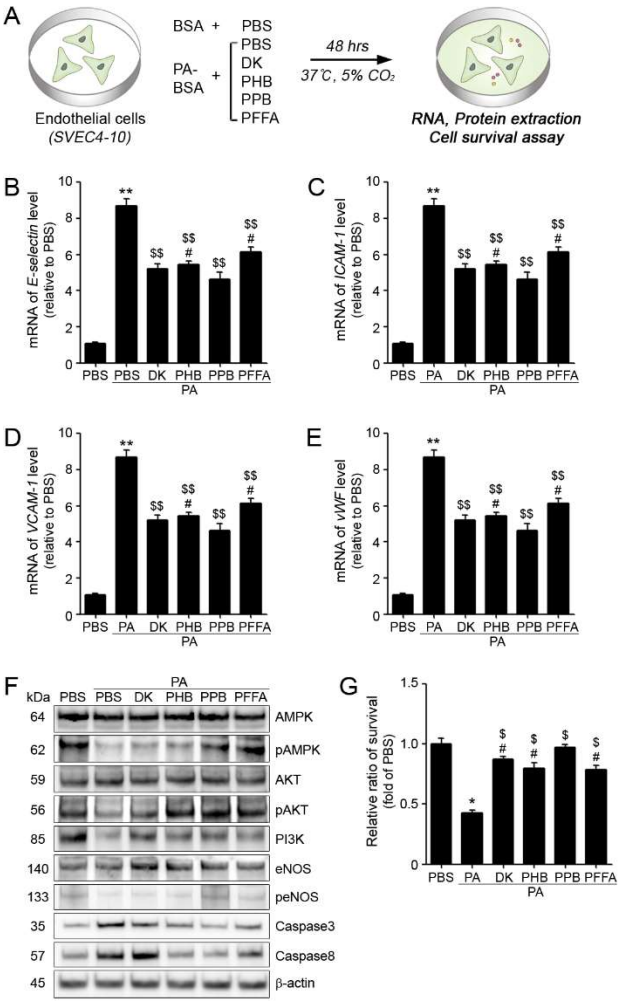


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Figure 2 (Modified version)

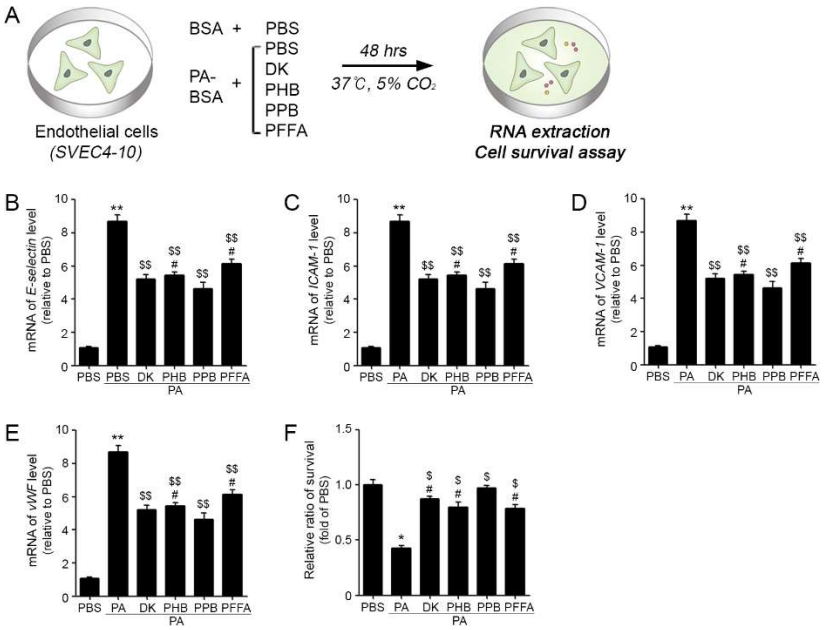

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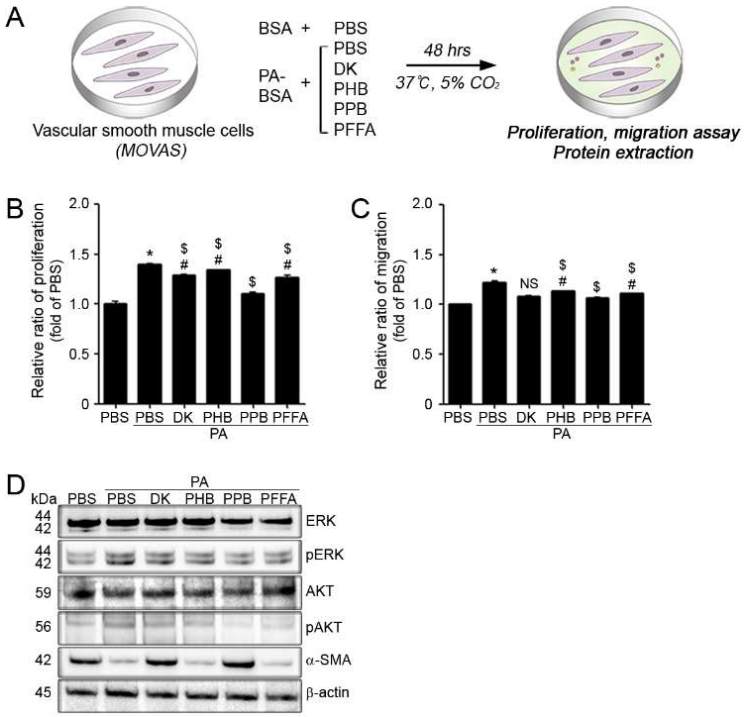
Figure 3 (Original version)



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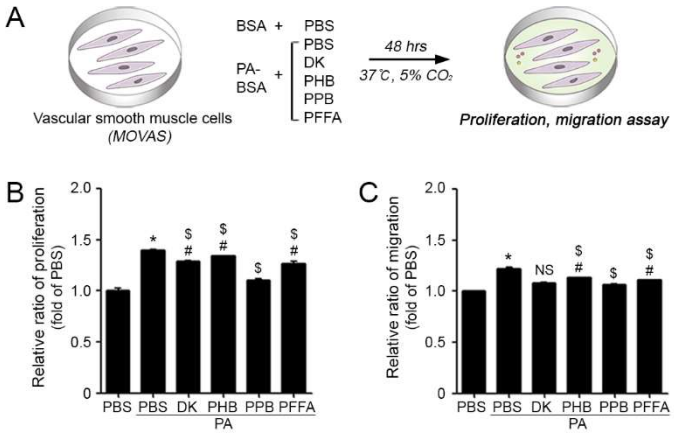

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