Supplementary Information

Bifidobacterium adolescentis-fermented supernatant preparation attenuates vascular senescence and age-related phenotypes by suppressing NF-κB-related signaling

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Author contributions

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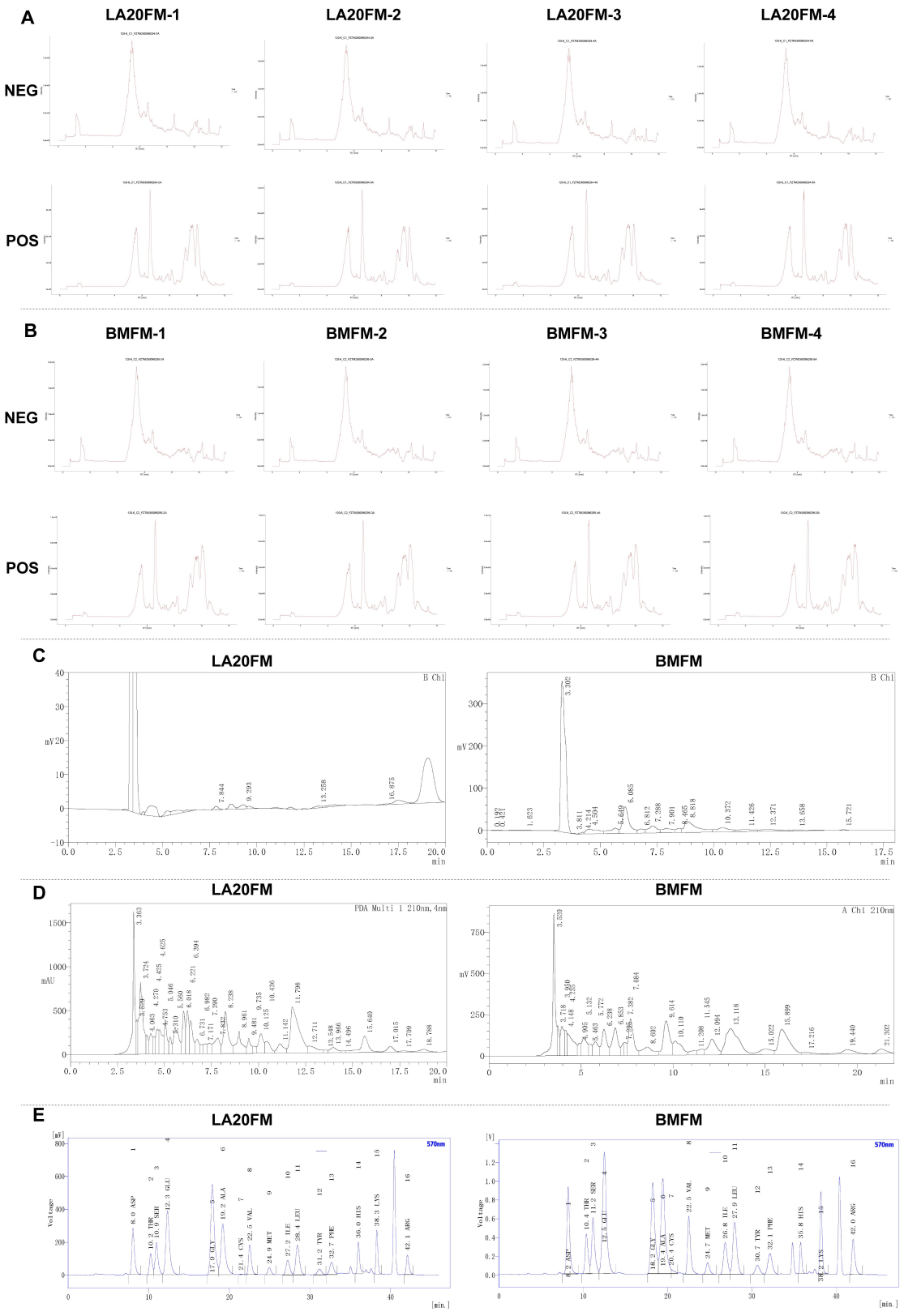
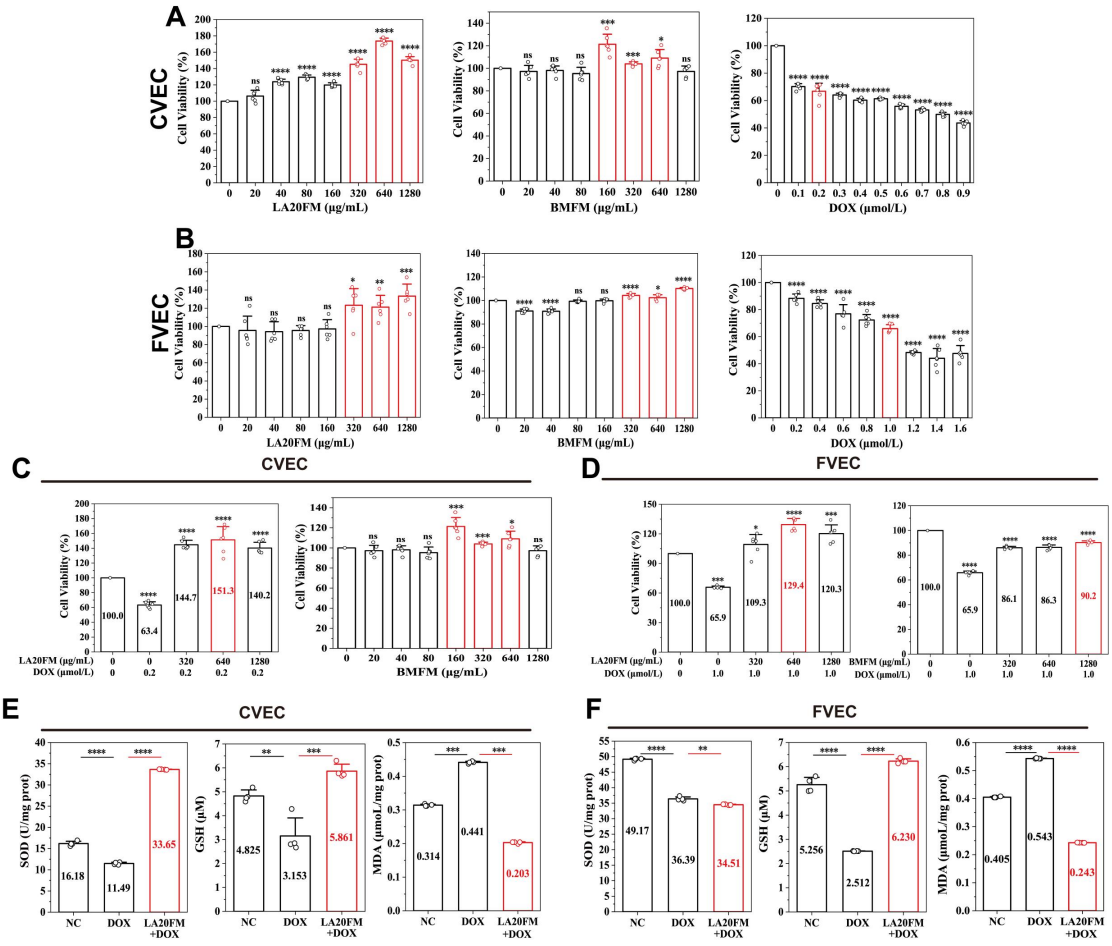


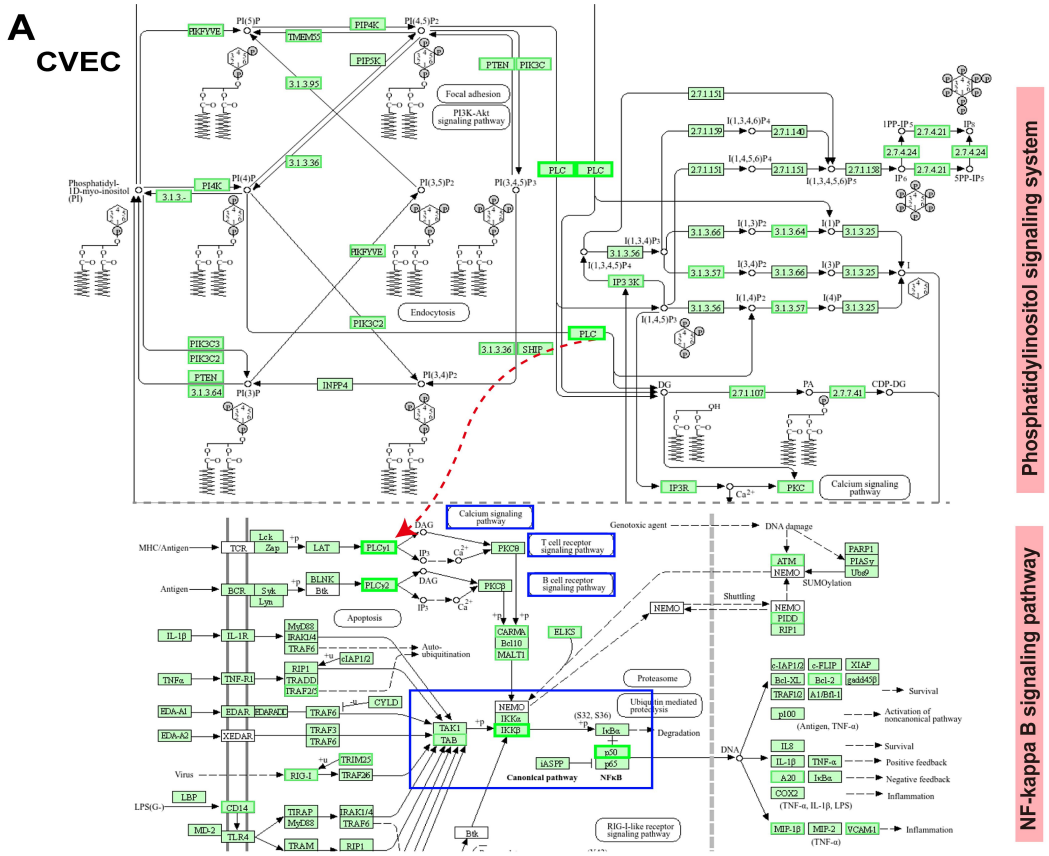
Supplementary Fig. 1. Chromatographic characterization of LA20FM and BMFM. (A) Representative LC–MS/MS total ion chromatograms (TICs) of LA20FM samples in negative ion mode (NEG) and positive ion mode (POS). (B) Representative LC–

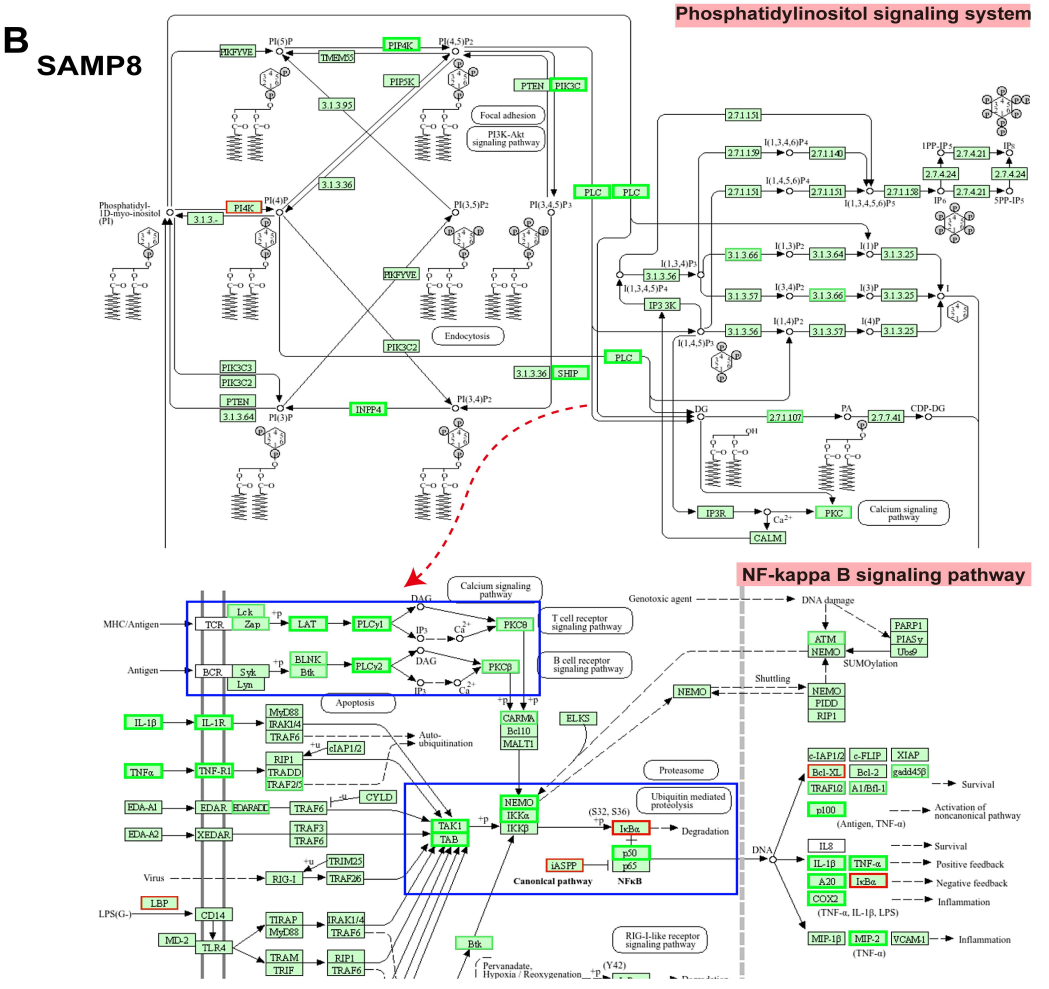
MS/MS TICs of BMFM samples in NEG and POS modes. (C) Representative chromatographic profiles of carbohydrates in LA20FM and BMFM. (D) Representative chromatographic profiles of organic acids in LA20FM and BMFM. (E) Representative chromatographic profiles of amino acids in LA20FM and BMFM. LA20FM, *Bifidobacterium adolescentis* Life Age 20 fermentation supernatant preparation; BMFM, blank matrix fermentation preparation prepared under the same matrix conditions without *Bifidobacterium adolescentis* LA20.



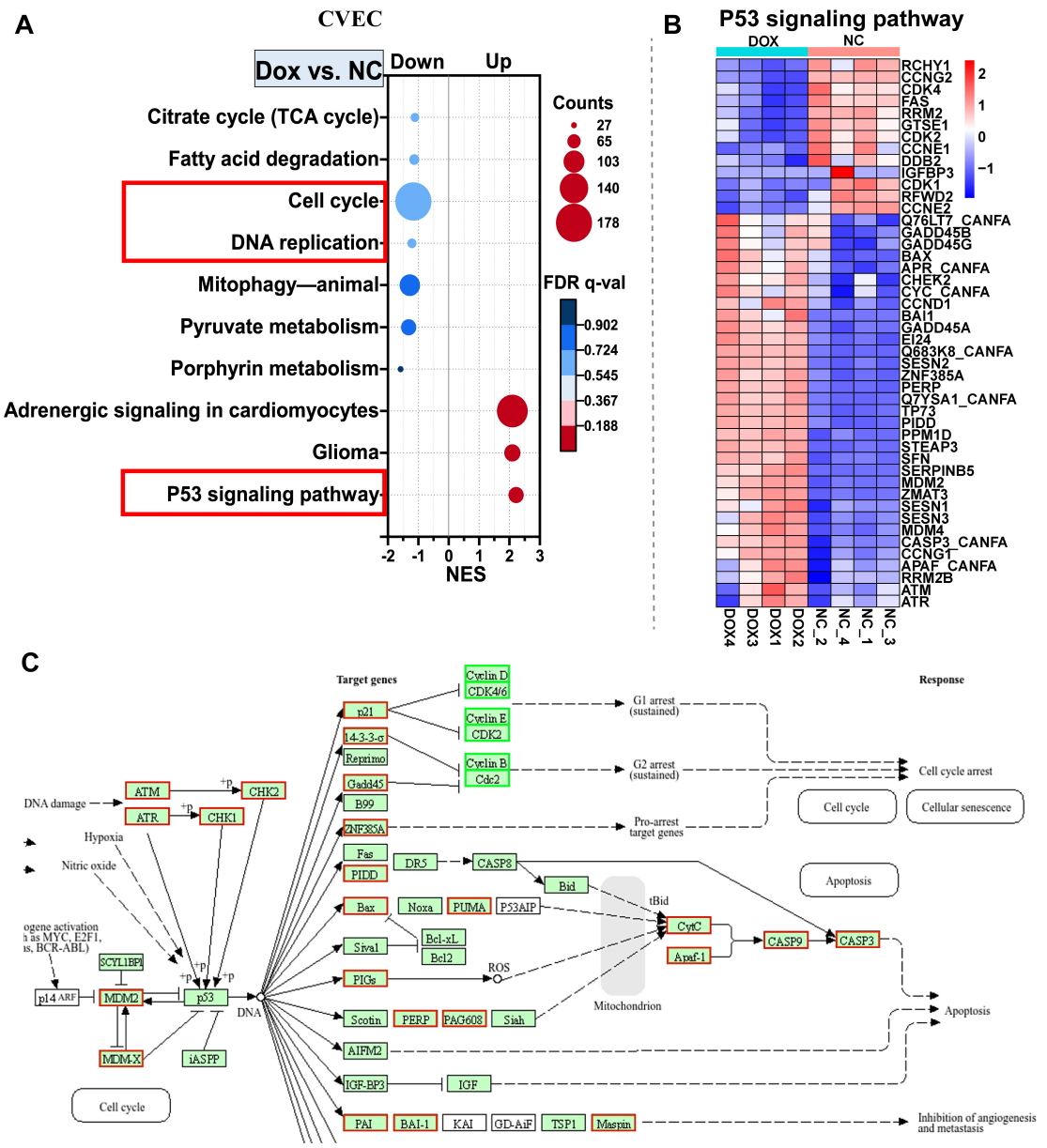
Supplementary Fig. 2. Evaluation of cell viability and oxidative stress markers in CVECs and FVECs under LA20FM treatment. (A, B) Cell viability of Canine (CVECs) (A) and Feline (FVECs) (B) vascular endothelial cells treated with varying concentrations of LA20FM (0–1280 μg/mL), BMFM (0–1280 μg/mL), or Doxorubicin (DOX). The red frames indicate the optimal DOX concentrations (0.2 μmol/L for CVECs and 1.0 μmol/L for FVECs) selected to establish the aging model (targeting 60–70% viability). (C, D) Protective effects of LA20FM and BMFM against DOX-induced cytotoxicity in CVECs (C) and FVECs (D). Cells were co-treated with the selected DOX concentration and indicated doses of LA20FM or BMFM. (E, F) Analysis of oxidative stress levels in CVECs (E) and FVECs (F). Intracellular superoxide dismutase (SOD) activity, glutathione (GSH) content, and malondialdehyde

(MDA) levels were measured in the Negative Control (NC), DOX-induced aging model (DOX), and DOX + LA20FM treatment groups. Data are expressed as mean ± SD from six independent biological replicates (n = 6) for cell viability assay and four independent biological replicates (n = 4) for oxidative stress markers assay. P-values indicate specific comparisons between the indicated groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.


A
CVEC
Phosphatidylinositol signaling system
NF-kappa B signaling pathway
Phosphatidyl-1D-myo-inositol (PI)
Focal adhesion
PI3K-Akt signaling pathway
Endocytosis
Calcium signaling pathway
T cell receptor signaling pathway
B cell receptor signaling pathway
Apoptosis
Auto-ubiquitination
Proteasome
Ubiquitin mediated proteolysis
Canonical pathway
NFκB
Shuttling
SUMOylation
Degradation
Survival
Activation of noncanonical pathway
(Antigen, TNF-α)
Positive feedback
Negative feedback
Inflammation
(TNF-α, IL-1β, LPS)
MHC/Antigen
Antigen
LPS(G-)
Virus
Genotoxic agent
DNA damage
RIG-I-like receptor signaling pathway


B
SAMP8
Phosphatidylinositol signaling system
NF-kappa B signaling pathway
Phosphatidyl-1D-myo-inositol (PI)
Focal adhesion
PI3K-Akt signaling pathway
Endocytosis
Calcium signaling pathway
T cell receptor signaling pathway
B cell receptor signaling pathway
Apoptosis
Auto-ubiquitination
Proteasome
Ubiquitin mediated proteolysis
Canonical pathway
NFκB
Shuttling
SUMOylation
Degradation
Survival
Activation of noncanonical pathway
(Antigen, TNF-α)
Positive feedback
Negative feedback
Inflammation
(TNF-α, IL-1β, LPS)
MHC/Antigen
Antigen
LPS(G-)
Virus
Genotoxic agent
DNA damage
RIG-I-like receptor signaling pathway
Pervanadate, Hypoxia / Reoxygenation

Supplementary Fig. 3. Cascade network of the phosphatidylinositol signaling system and NF-κB signaling pathway regulated by LA20FM in CVECs and SAMP8 vascular tissues. (A) KEGG pathway–based cascade network illustrating transcriptional changes in the phosphatidylinositol signaling system and the NF-κB signaling pathway in canine vascular endothelial cells (CVECs). Differentially expressed genes identified by RNA sequencing in the DOX + LA20FM group relative to the DOX group were mapped onto the corresponding KEGG pathways. (B) KEGG pathway–based cascade network illustrating transcriptional changes in the phosphatidylinositol signaling system and the NF-κB signaling pathway in abdominal aortic vascular tissues from senescence-accelerated mouse prone 8 (SAMP8) mice. Differentially expressed genes identified from the OC + LA20FM group relative to the OC group were mapped onto the same pathway framework. Red and green boxes denote significantly upregulated and downregulated genes, respectively, following LA20FM treatment. Solid arrows represent established signaling relationships defined by KEGG pathway annotations, and dashed arrows indicate the cascade linkage between phosphatidylinositol-related signaling and NF-κB pathway components.



Supplementary Fig. 4. DOX activates the p53 signaling pathway and suppresses cell cycle–related programs in CVECs. (A) Gene set enrichment analysis (GSEA) of KEGG pathways in canine vascular endothelial cells (CVECs) comparing DOX-treated cells with the negative control (NC). Bubble plots display representative enriched pathways, including the p53 signaling pathway, cell cycle, DNA replication, and metabolic pathways. The normalized enrichment score (NES) indicates the direction and magnitude of enrichment, bubble size represents the number of genes contributing to each pathway, and color denotes the false discovery rate (FDR) q-value. (B) Heatmap

showing the expression profiles of genes involved in the p53 signaling pathway in CVECs under DOX and NC conditions. Rows represent individual genes and columns represent biological replicates. Color intensity reflects relative gene expression levels after normalization, with red indicating higher expression and blue indicating lower expression. (C) Schematic diagram of the p53 signaling network illustrating key transcriptional targets and downstream biological outcomes. Genes highlighted in red represent p53-responsive genes that were upregulated following DOX treatment, whereas genes highlighted in green represent genes that were downregulated. The diagram summarizes the involvement of p53 signaling in DNA damage response, cell cycle arrest, apoptosis, and cellular senescence in CVECs.

Supplementary Data Table S1

Summary of annotated differential metabolites between LA20FM and BMFM groups (provided in the Excel file "Supplementary Data Table.xlsx").

Supplementary Data Table S2

Composition analysis of the extract of brown algae (provided in the Excel file "Supplementary Data Table.xlsx").

Supplementary Data Table S3

Metabolite identification and quantification of canine vascular endothelial cells under different treatments (also provided in the Excel file "Supplementary Data Table.xlsx").