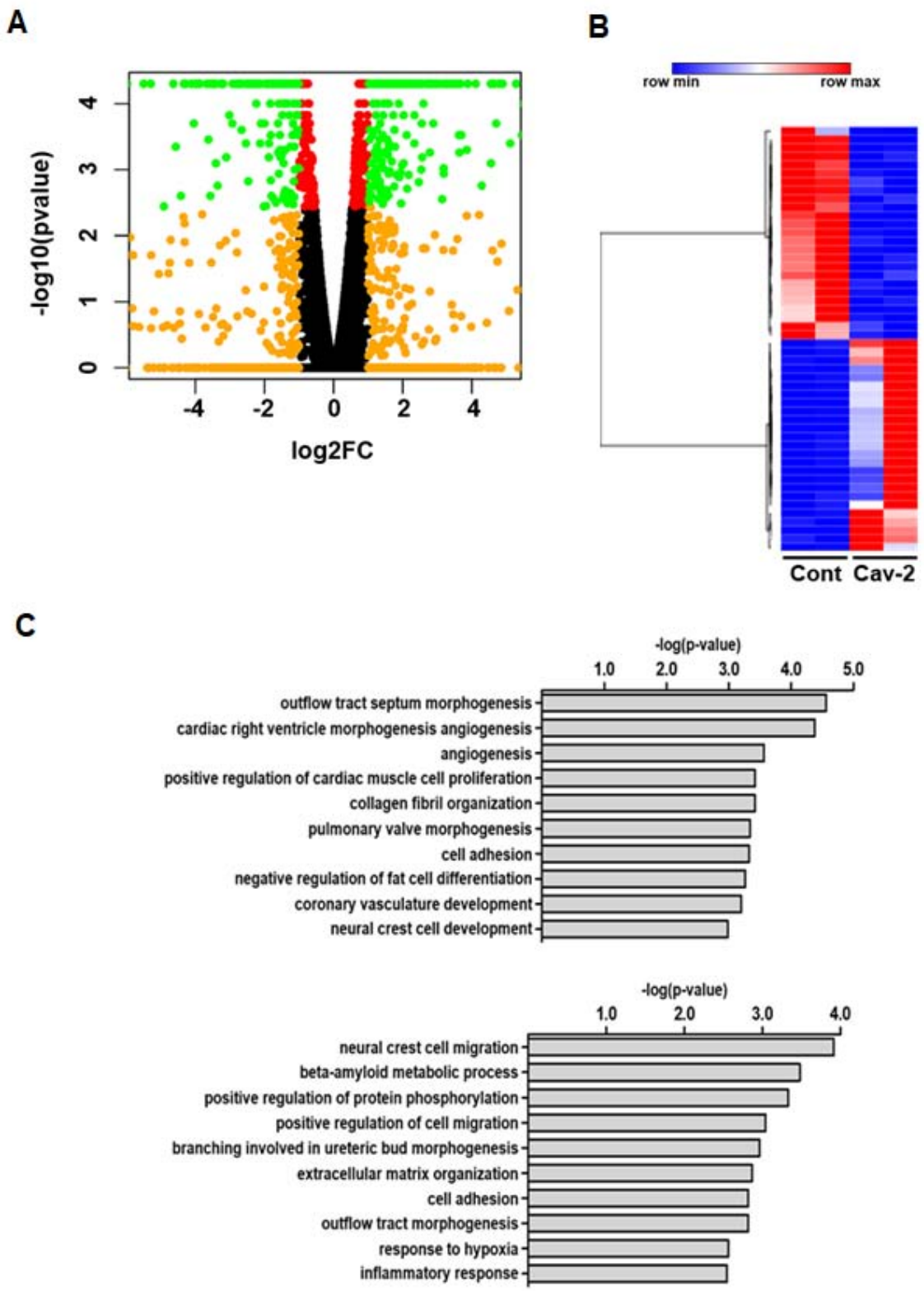
SUPPLEMENTARY MATERIALS AND METHODS

RNA extraction and RNA sequencing

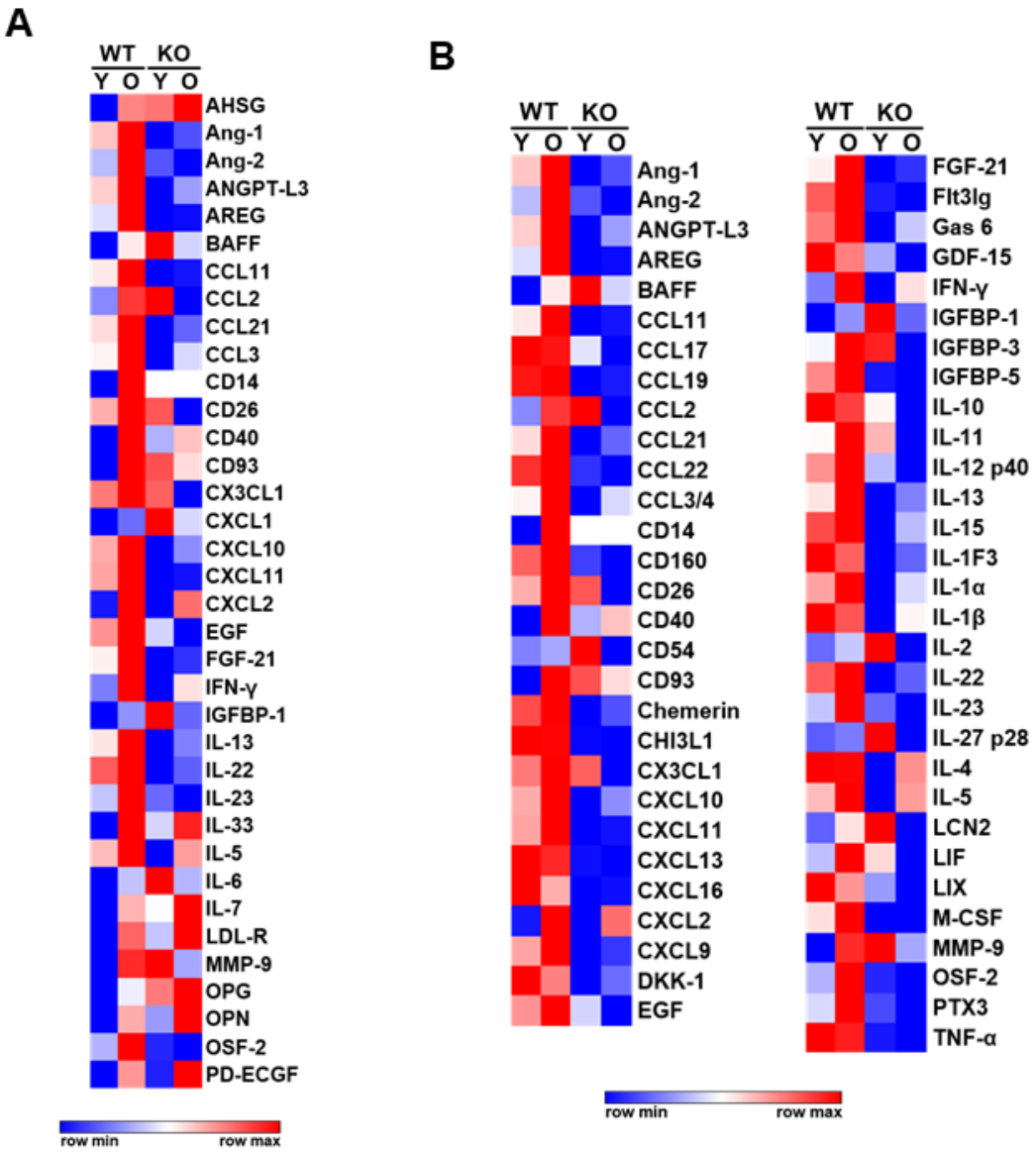
Total RNA from cells was extracted using the mirVana™ miRNA Isolation Kit (Ambion, USA) according to the manufacturer's instructions. mRNA sequencing libraries were prepared using the Illumina Truseq stranded mRNA library prep kit according to the manufacturer's instructions (Illumina, USA). mRNA was purified and fragmented from total RNA (1 μg) using poly-T oligo-attached magnetic beads. Cleaved RNA fragments were reverse transcribed into first-strand cDNA. A single 'A' base was added to these cDNA fragments and the adapter was ligated. The products were purified and enriched by PCR to create the final strand-specific cDNA library. After qPCR using SYBR Green PCR Master Mix (Applied Biosystems, USA), libraries that were index tagged in equimolar amounts in the pool were combined. Cluster generation occurred in the flow cell on the cBot automated cluster generation system (Illumina). The flow cell was loaded on the HISEQ 2500 sequencing system (Illumina), and sequencing was performed using a 2 × 100 bp read length.

Identification of DEGs and functional enrichment analysis

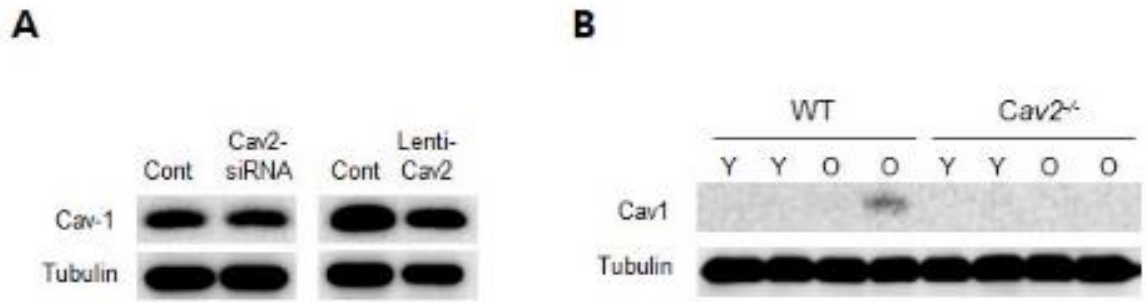
Reads for each sample were mapped to the reference genome (Human hg19) by TopHat (v2.0.13) [PMID: 19289445]. The aligned results were added to Cuffdiff (v2.2.0) to report differentially expressed genes (DEGs) [PMID: 23222703]. Functional analysis of genes was performed using the DAVID bioinformatics resources 6.8 (https://david.abcc.ncifcrf.gov/home.jsp).



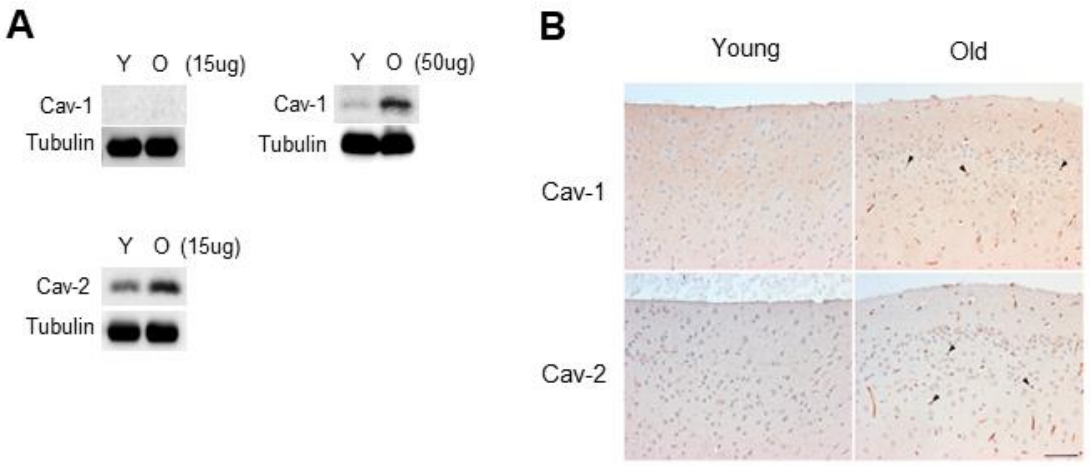
Supplementary Fig. S1. RNA-seq analysis of *Cav-2*-expressing human umbilical vein endothelial cells (HUVECs). (A) The volcano plot was constructed by plotting the log of the fold change between the two conditions on the x-axis and the negative log of the *P* value on the y-axis. Orange dots indicate the |log2FC| > 1; red dots indicate the FDR <0.05; green dots indicate both and represent DEGs. (B) Heatmap indicates the hierarchical clustering of the top 50 differentially expressed genes (DEGs) based on negative Pearson correlation. (C) DAVID Gene Ontology Biological Process analysis of up- (upper) and downregulated (lower) genes.



Supplementary Fig. S2. Aging and caveolin-2 (Cav-2) deficiency affect cytokine expression. (A) 36 chemokines were upregulated more than two-fold in aged wild-type mice than in young wild-type (WT) mice (out of 111 chemokines tested), whereas 59 chemokines showed over two-fold increase in aged wild-type mice compare with their expression in aged Cav-2 knockout (KO) mice (B).



Supplementary Fig. S3. The expression of Cav-1 was not affected altered Cav-2 expression in both HUVEC cell lines and mouse brain. (A) Fifteen micrograms of whole-cell lysates from the lenti-ctrl HUVECs, lenti-Cav-2 HUVECs, and (Cav-2 siRNA) HUVECs transfected with siRNA for Cav-2 were used for immunoblotting to determine Cav-1 expression. (B) Thirty-five micrograms of whole-cell lysates from the cerebral cortex of 3-month-old (young, Y) and 15-month-old (old, O) C57BL/6 mice were analyzed by immunoblotting against total Cav-1.



Supplementary Fig. S4. The expression of caveolin-1 (Cav-1) and caveolin-2 (Cav-2) is increased in the aged mice brain. (A) Fifteen and fifty micrograms of whole-cell lysates from the cerebral cortex of 3-month-old (young, Y) and 15-month-old (old, O) mice were used for western blotting to determine Cav-1 and Cav-2 expression. Cav-2 protein was well identified with 15 μg of brain lysates, but Cav-1 was not detectable even after long exposure. When 50 μg of brain lysates was used for immunoblotting, Cav-1 protein was successfully detected. (B) Immunohistochemical staining of Cav-1 and Cav-2 in the cerebral cortex of 3-month-old (young) and 15-month-old (old) C57BL/6 mice. Representative images show immunoreactivity of Cav-1 and Cav-2 (arrowheads).