Science Advances
AAAS

Supplementary Materials for

Exercise mitigates flow recirculation and activates metabolic transducer SCD1 to catalyze vascular protective metabolites

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Sci. Adv. **10**, eadj7481 (2024)
DOI: 10.1126/sciadv.adj7481

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

SUPPLEMENTARY MATERIALS

SUPPLEMENTARY METHODS

Plasma and Tissue Collection

At the end of the experimental period, animals were fully anesthetized with isoflurane (VetFlo Vaporizer, Kent Scientific, Torrington, CT) before performing bilateral thoracotomy. Briefly, blood was collected by cardiac puncture with a 25G needle and heparinized syringe, followed by immediate opening of the right atria and transcardiac perfusion of ice-cold phosphate buffered saline solution (PBS) at a rate of 1 mL/min to perfuse the vasculature. The heart and entire thoracic aorta were dissected and washed in ice-cold PBS. Aortas were fixed for 1 hour in 4% paraformaldehyde (PFA) and transferred to PBS for further dissection. The blood samples were maintained in ice until centrifugation was performed for 5 min at 1,000 r.p.m. Plasma was separated and stored at -80°C for metabolomic analysis.

Endothelial Cell Culture

Human aortic endothelial cells (HAECs, S305, Cell Applications Inc.) were grown on bovine gelatin (Sigma Aldrich, MO)-coated-plates in humidified incubator at 37 °C and 5% CO_2. Endothelial cell growth media (Cell Applications Inc.) was supplemented with 5% fetal bovine serum and penicillin-streptomycin (Life Technologies, NY). HAECs were propagated and used for experiments between passages 3 and 8.

Transfection Assays

At ~50% confluency, FlexiTube^{TM} specific siRNAs targeting scrambled negative control (Scr), Scd1 (si-Scd1) or PPARγ (si-PPARγ) were transfected following the manufacturer's instructions. Lipofectamine^{TM} RNAiMAX (Thermofisher, MA) diluted with Dulbecco's Modified Eagle Medium (DMEM)/10% FBS and Opti-MEM media with reduced serum (Thermofisher, MA) was used for siRNA transfection. In a subset of experiments, HAECs were transfected with scrambled siRNA (Scr) or PPAR-γ (siPPAR-γ) and then were exposed to PSS for 4 hours.

PSS-Exposed HAECs for Western Blots

Confluent monolayers of HAECs cultured on 6-well plates were exposed to unidirectional PSS (6, 12 and 24 hours) using a modified flow device. Neutralized MCDB-131 medium (Sigma Aldrich, MO) containing 7.5% sodium bicarbonate solution, 10% FBS and 4% dextran from *Leuconostoc spp* (Sigma Aldrich, MO) was used for PSS exposure. Following exposure to PSS, the center of each monolayer was removed by using a cell scraper to collect only flow-aligned cells from the periphery of the well for RNA or protein analysis.

Western Blot

After exposure to PSS, HAECs were rinsed with PBS and lysed using RIPA buffer supplemented with protease and phosphatase inhibitors. Protein concentration was measured using the Bio-Rad DC assay and 50 μg of protein was loaded for Western blot with anti-SCD1 antibody (Cell Signaling) and anti-β-tubulin (Millipore, Inc.) for loading normalization. Densitometry was performed to quantify the intensity of the bands.

Fatty Acid Experiments

HAECs monolayers were treated with oleic acid (Sigma, #O3008) at 0.2 mM. HAECs monolayers were treated with or without 10 μM of the PPARγ agonist Rosiglitazone (Sigma, #L2654) for 4 hours. At the end of the experimental period, cells were lysed and processed for RNA extraction.

RNA extraction and Real-Time PCR

Cell cultures were lysed and processed for RNA extraction using the RNA Miniprep kit (Zymo Research) according to the manufacturer's instructions and the concentration and purity were assessed with BioDrop spectrophotometer. Total RNA (1-2 μg) was reverse-transcribed into cDNA using 5x All-In-One Reverse Transcription mix (Applied Biological Materials). Then, 2-5 ng of cDNA were amplified by quantitative PCR with iQ SYBR Green Supermix (BioRad) and specific primers designed using NCBI software. 18s rRNA or GAPDH were used as internal housekeeping control. Amplification reactions were performed, and the fluorescence was monitored in a Real Time PCR System, CFX Connect (Bio-Rad Laboratories). PCR thermal cycle parameters were: 5 minutes 95°C, 30 seconds at 60°C, and 30 seconds at 72°C. Melting curves were used to corroborate primer specificity for the intended templates. Fold expression changes were calculated using the delta-delta Ct method, and results from triplicates were averaged. The primer sequences used in this study are included in Table S3.

SUPPLEMENTARY FIGURES

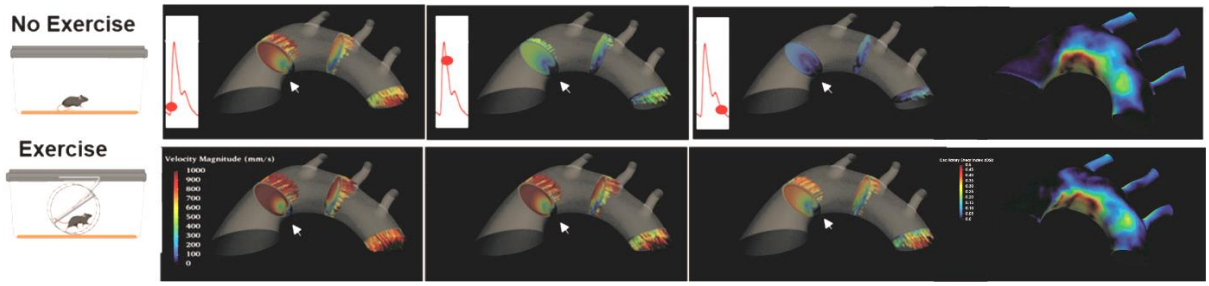

Figure S1. CFD analysis of blood flow in the mouse aortic arch. CFD analysis under no exercise (top) and exercise (bottom) conditions. The vectors and the flow contours in the cross sections are color-coded based on the velocity magnitude. OSI contour (right) is compared between the sedentary and exercise groups.

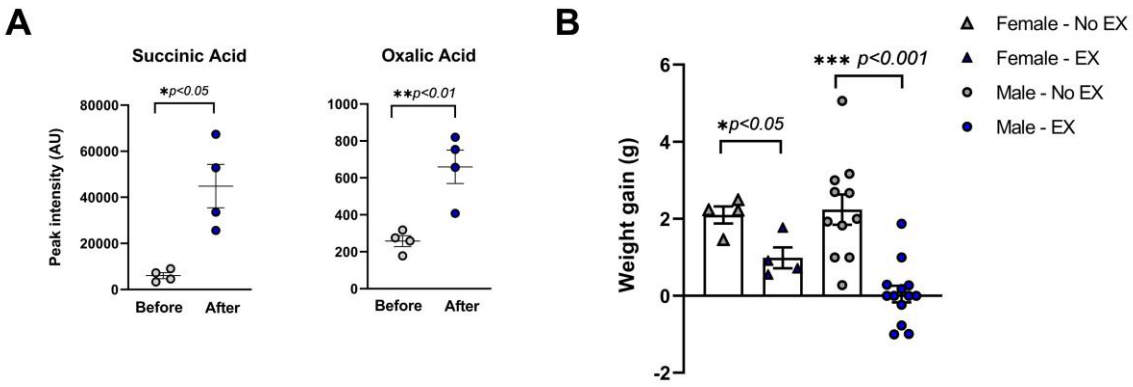

Figure S2. Exercise alters catalysis of lipid metabolites. (A) Elevated levels of metabolites succinic acid and oxalic acid, which have previously been shown to be regulated by physical activity. Data are shown as mean ± SEM and analyzed with paired two-tailed *t*-test. **(B).** Changes in body weight in female and male C57BL/6J mice after 14 days of voluntary wheel exercise (calculated as body weight at Day 14 – initial body weight at Day 0). Each point represents an individual animal. Results are expressed as mean ± SEM and compared using unpaired two-tailed *t*-test for each gender (females: n=4 No Exercise (Ex), N=4 EX; males: n=11 No EX, n=13 EX).

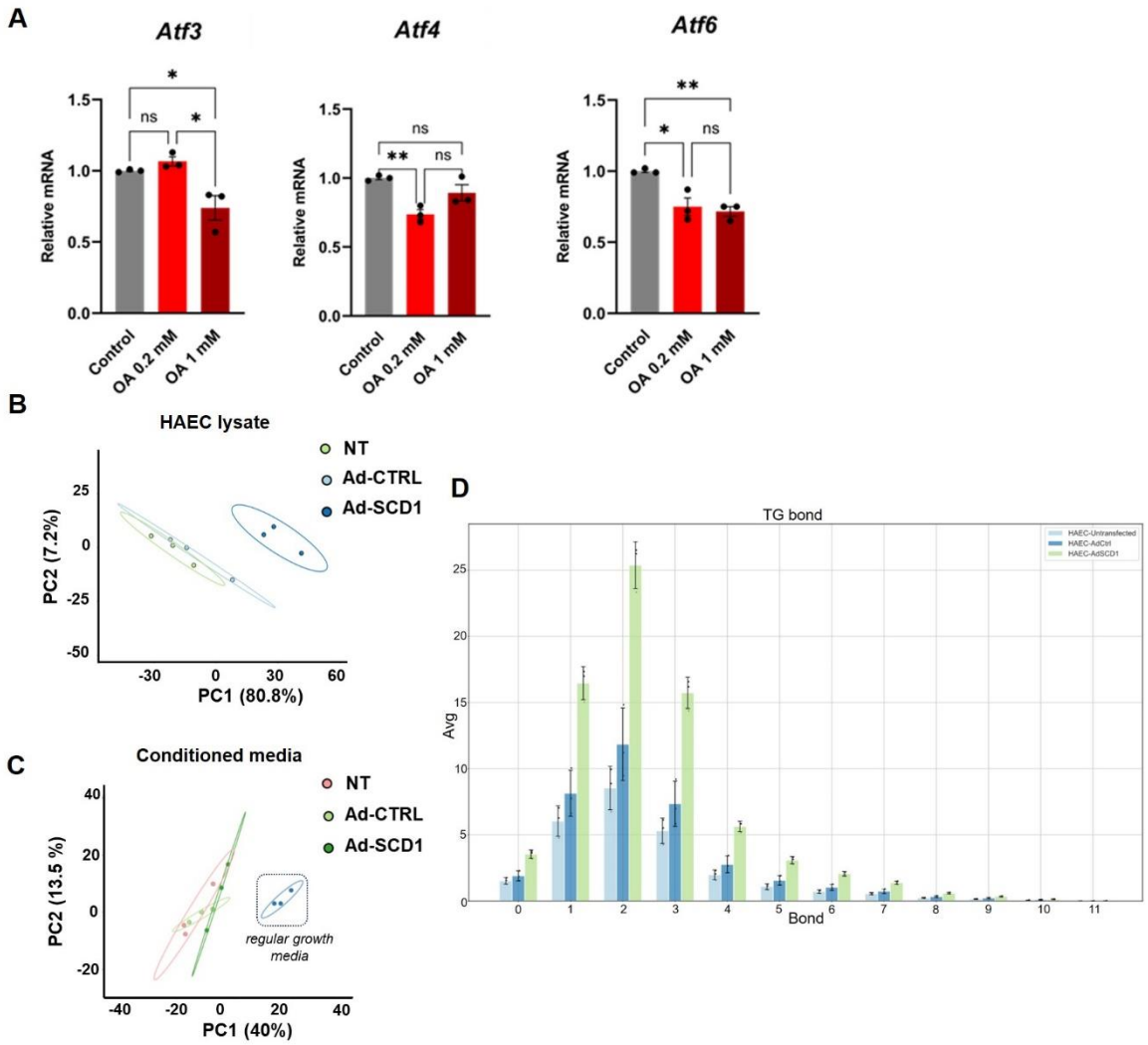

Figure S3. Oleic acid treatment or SCD1 overexpression mitigates pro-inflammatory mediators in HAEC. **(A)** OA treatment attenuated the ER stress-related genes *Atf3*, *Atf4* and *Atf6* expression (ns: non-significant; ** $p < 0.05$; ** $p < 0.01$* between the indicated groups, n=3). **(B, C)** PCA of individual lipids quantified in the cellular lysate **(B)** or conditioned media **(C)** from HAEC non-transfected (NT) or transfected with adenoviruses (Ad-CTRL or Ad-SCD1). PCA indicates the percentage of the total variance explained by individual principal components. Prediction ellipses are set at 95% probability. **(D)** Triglyceride (TG) bond analysis indicates that SCD1 overexpression most profoundly increased cellular TGs containing one, two or three double bonds total, corresponding with MUFA structure. TGs containing more than four double bonds correspond to polyunsaturated fatty acids.

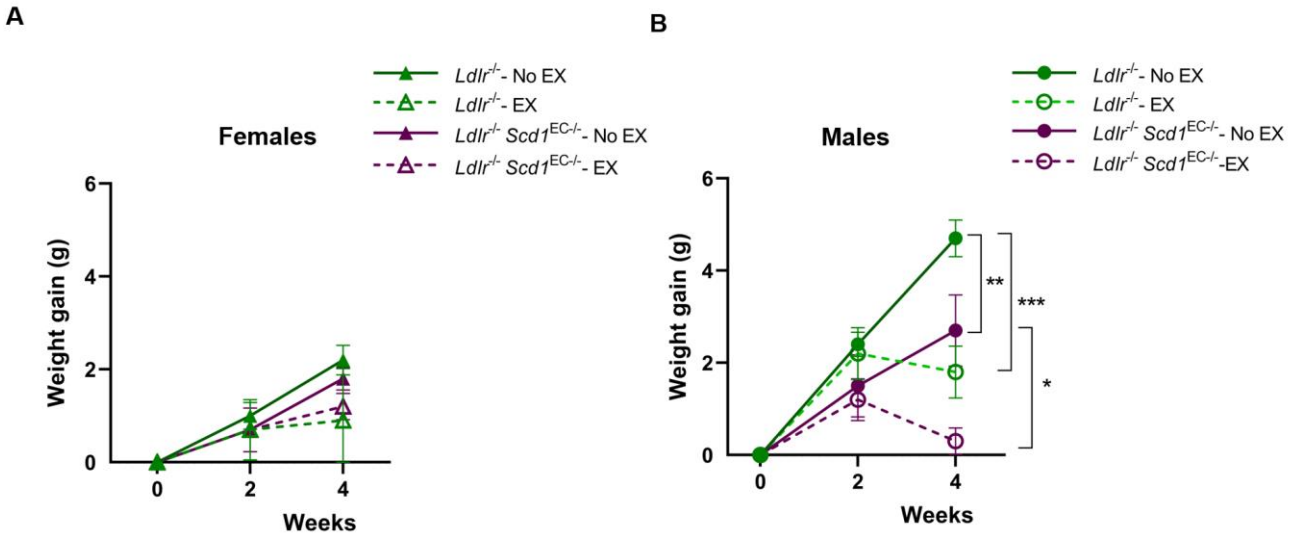

Figure S4. Body weight. Changes in body weight in female **(A)** and male **(B)** mice over the 28 days of HFD treatment with or without exercise. Results were analyzed by two-way ANOVA followed by multiple comparisons across time points and experimental groups using Tukey post-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n=5-12).

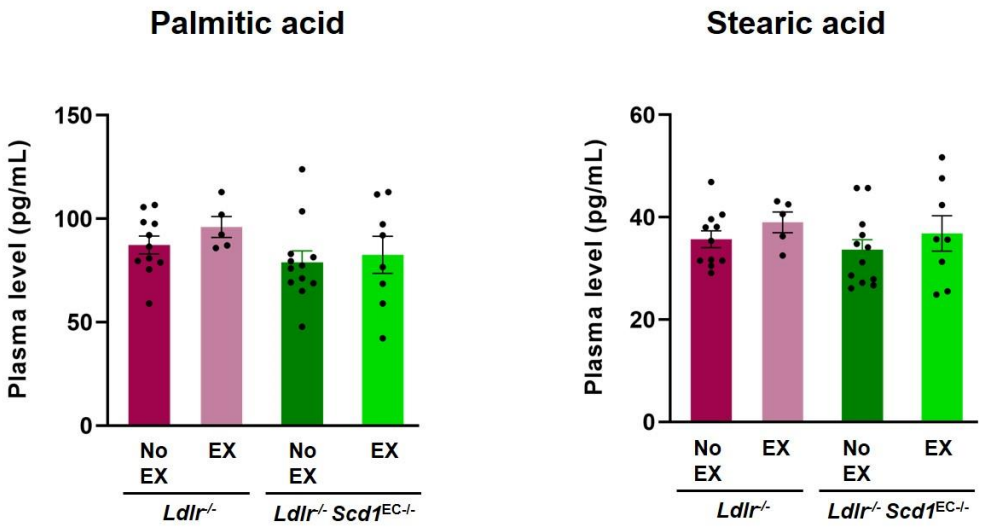

Figure S5. Circulating levels of saturated fatty acids. The absolute plasma concentration of saturated FA palmitic and stearic in the *Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ *Scd1*$^{EC-/-}$ mice. (ns; non-significant; n= 5-12; each dot represents an individual animal).

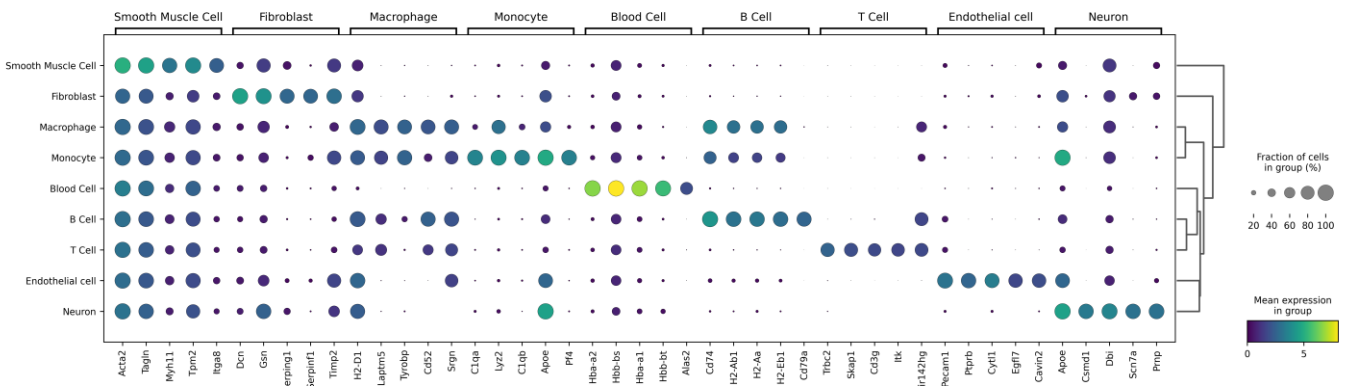

Figure S6. Lineage markers used for cell clustering. Dot plot of the major conserved marker genes per cluster.

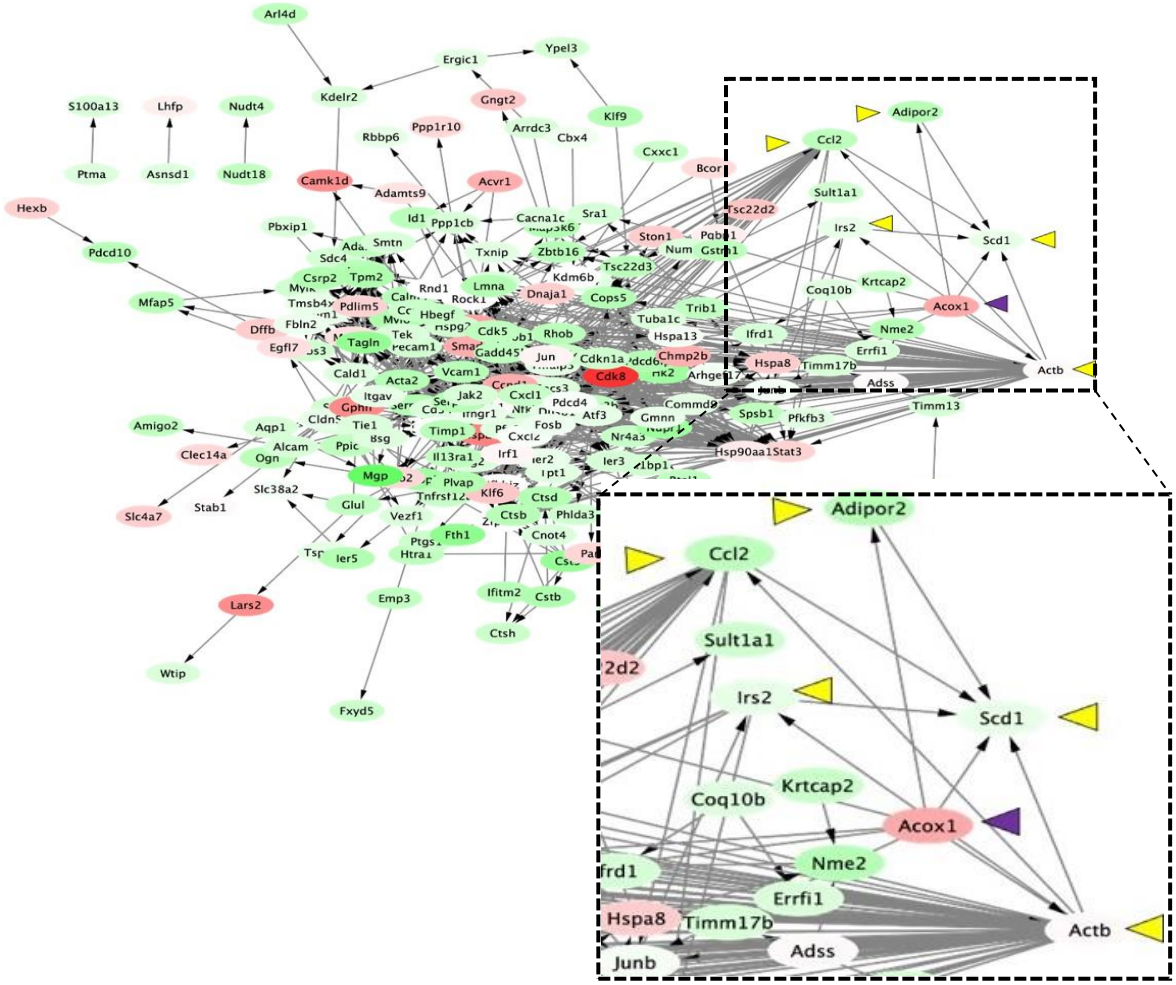

Figure S7. Gene network interactions. Gene network generated with Cytoscape software depicting interconnections of EC transcriptomics and mechanosensitive genes involved in lipid metabolism pathways (yellow arrows).

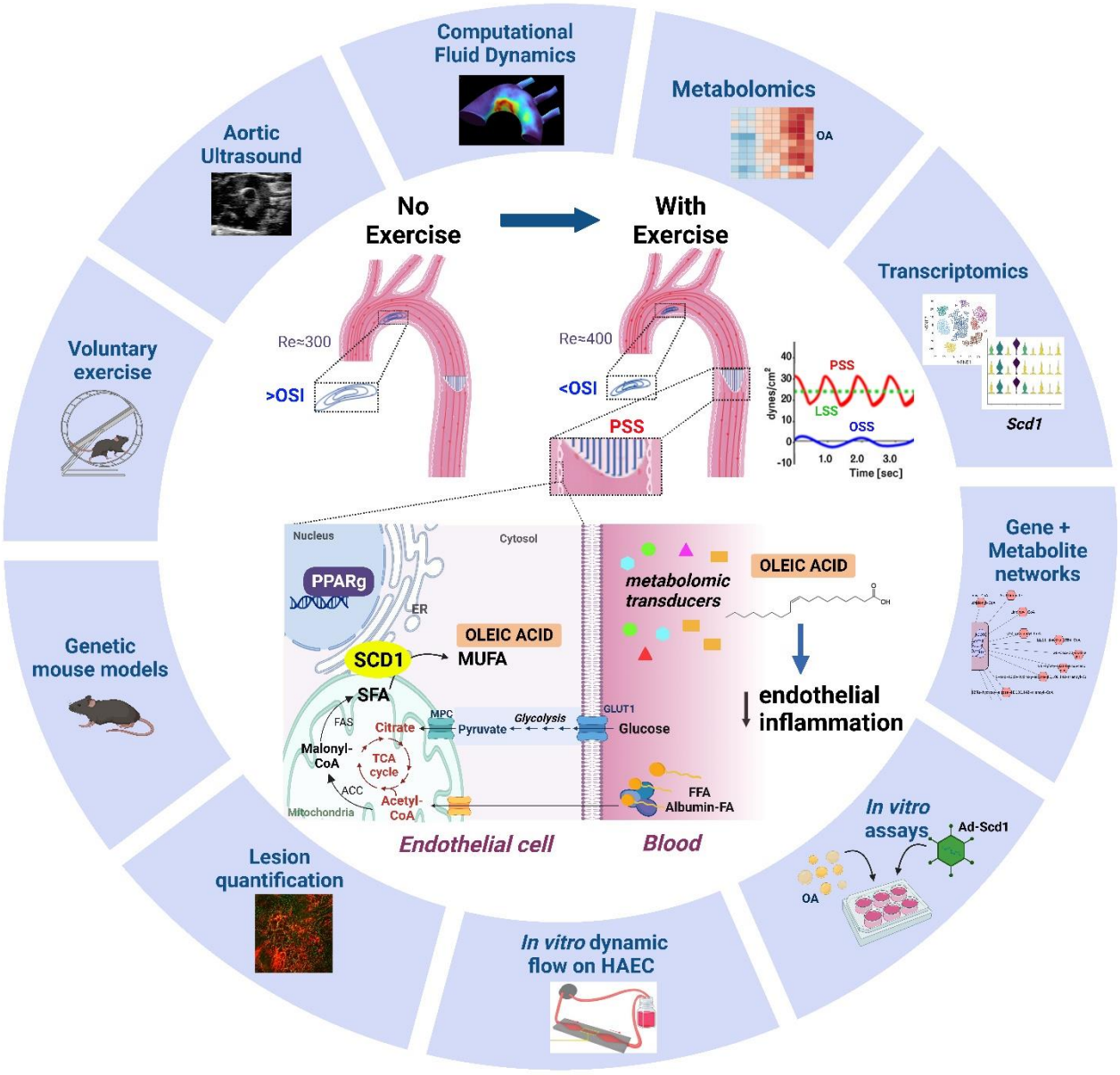

Figure S8. Schematic model for exercise-augmented PSS in the aorta and activation of the flow-responsive PPARγ-SCD1 pathway for atheroprotective metabolites. Integration of *in vivo* and *in vitro* biological data with *in-silico* computational modeling to support our model for exercise-induced PSS in the aorta and activation of the flow-responsive PPARγ-SCD1 pathway for vascular atheroprotection. Exercise-induced PSS reduces flow recirculation and OSI in the aortic arch. EC can uptake and metabolize either free fatty acids (FFA) or albumin-bound FAs. SCD1 located in the ER membrane adjacent to the mitochondrial membrane is the limiting step in the conversion of SFA into MUFA. SCD1 product OA reduces NF-κB-mediated inflammatory markers. MPC: mitochondrial pyruvate carrier; TCA: tricarboxylic acid. ACC: acetyl-CoA carboxylase; FAS: fatty acid synthase.

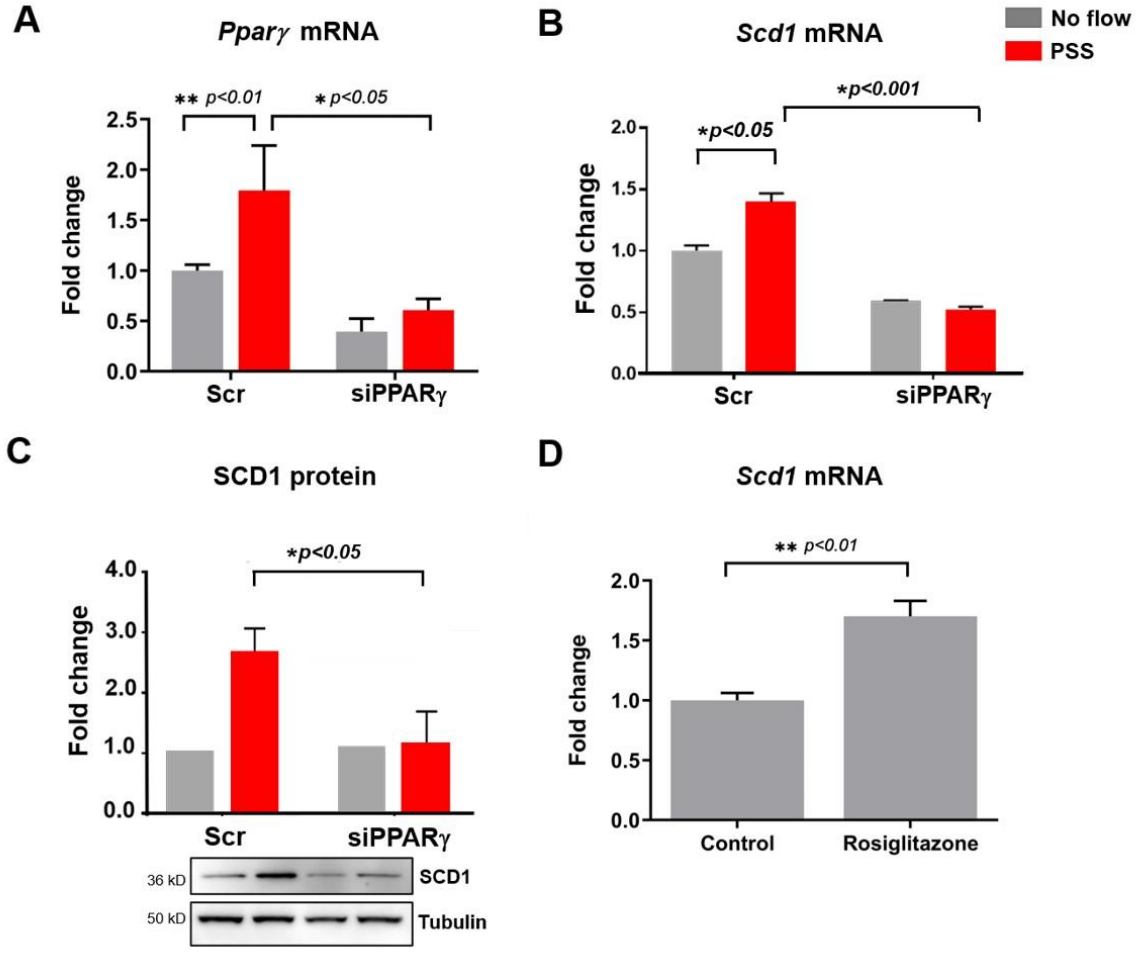

Figure S9. PSS-activated SCD1 expression is PPARγ-dependent. (A, B) HAECs were transfected with scrambled (Scr) or PPARγ (siPPARγ) siRNA and then subjected to PSS for 4 hours. PSS increased PPARγ and *Scd1* mRNA. Silencing PPARγ with siRNA completely blocked PSS-induced *Scd1* expression. **(C)** Western Blot with anti-SCD1 antibody demonstrating that PSS-induced SCD1 protein expression was blocked after siPPARγ treatment. **(D)** HAECs were treated with the PPARγ agonist, Rosiglitazone (10μM), for 4 hours. Rosiglitazone significantly increased *Scd1* mRNA expression as compared to the untreated control ($P < 0.01$, n=3), supporting the interpretation that *Scd1* is flow-responsive and PPARγ-dependent.

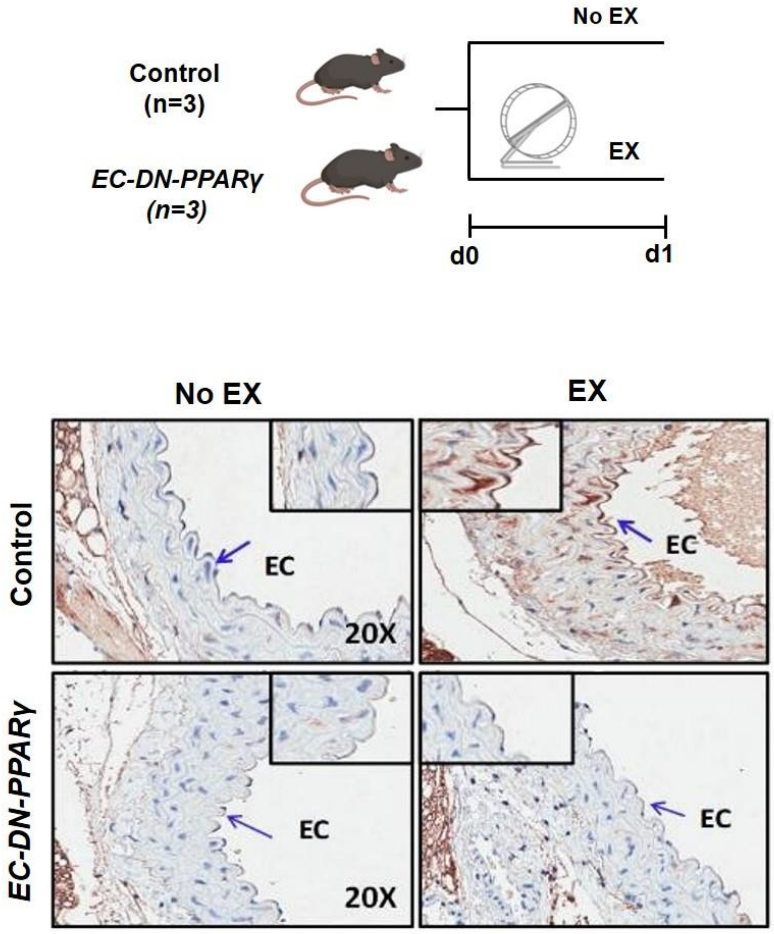

Figure S10. Exercise-activated endothelial SCD1 expression was absent in mice with endothelial dominant negative PPARγ. Wild type C57BL/6J (control) or EC-dominant negative (EC-DN)-PPARγ mice underwent 24-hr voluntary exercise. Representative images of transversal sections of the descending aorta stained with anti-SCD1 antibody. SCD1 staining was prominent the endothelial cells in wild type mice after exercise but was nearly absent in the EC-DN-PPARγ mice (n=3 males). Note that SCD1 is also present in the periaortic adipocytes

SUPPLEMENTAL TABLES

	C57BL/6J female	C57BL/6J male
Distance (km)	144 ± 22	170 ± 11
Active Time (hr)	85 ± 9	87 ± 5
Average Speed when running (m/min)	27 ± 2	32 ± 1
Maximum Speed (m/min)	101 ± 6	110 ± 3
Maximum Acceleration (m/(min.s))	38 ± 4	42 ± 1

Table S1. Exercise parameters in wild type C57BL/6J mice. Exercise parameters collected over a continuous 2-week recording period using the BIO-ACTIVWHEEL SOFT software (Bioseb) for wild type C57BL/6J mice. Results are expressed as mean ± SEM and compared using unpaired two-tailed *t*-test (ns, females n=5, males n=8).

	Ldlr$^{-/-}$	*Ldlr*$^{-/-}$*Scd1*$^{EC-/-}$
Distance (km)	108 ± 20	118 ± 7
Active Time (hr)	72 ± 12	81 ± 3
Average Speed when running (m/min)	24 ± 2	24 ± 1
Maximum Speed (m/min)	84 ± 2	86 ± 1
Maximum Acceleration (m/(min.s))	32 ± 2	33 ± 1

Table S2. Exercise parameters in *Ldlr*$^{-/-}$ mice. Exercise parameters collected over a continuous 2-week recording period using the BIO-ACTIVWHEEL SOFT software (Bioseb) for *Ldlr* $^{-/-}$and *Ldlr* $^{-/-}$*Scd1*$^{EC-/-}$ mice subjected to exercise concomitantly with HFD feeding (week 2 to week 4). The data for males and females were pooled together as there were no differences among genders. Results are expressed as mean ± SEM and compared using unpaired two-tailed *t*-test (ns, n=5 for *Ldlr* $^{-/-}$, n=8 for *Ldlr* $^{-/-}$*Scd1*$^{EC-/-}$).

Gene name	Species	NCBI Accession Number	Forward (5'-3')	Reverse (5'- 3')
Ccl2 (Mcp1)	human	NM_002982.4	AGAATCACCAGCAGCAAGTGTCC	TCCTGAACCCACTTCTGCTTGG
E-Sel	human	NM_000450.2	TCAAGGGCAGTGGACACAGCAA	GGAAACTGCCAGAAGCACTAGG
Icam1	human	NM_000201.3	AGCGGCTGACGTGTGCAGTAAT	TCTGAGACCTCTGGCTTCGTCA
IL-6	human	NM_000600.5	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
IL-8 (Cxcl8)	human	NM_000584.4	GAGAGTGATTGAGAGTGGACCAC	CACAACCCTCTGCACCCAGTTT
Pparγ	human	NM_001330615	AGCCTGCGAAAGCCTTTTGGTG	GGCTTCACATTCAGCAAACCTGG
Scd1	human	NM_005063.5	CCTGGTTTCACTTGGAGCTGTG	TGTGGTGAAGTTGATGTGCCAGC
Vcam1	human	NM_001078	GATTCTGTGCCCACAGTAAGGC	TGGTCACAGAGCCACCTTCTTG
Gapdh	mouse	NM_001289726.1	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG
Mcp1	mouse	NM_011333.3	GCTACAAGAGGATCACCAGCAG	GTCTGGACCCATTCCTTCTTGG
Pparg	mouse	NM_001127330	GTACTGTCGGTTTCAGAAGTGCC	ATCTCCGCCAACAGCTTCTCCT
Rn18s (18sRNA)	mouse	NR_003278.3	ACCCGTTGAACCCCATTCGTGA	GCCTCACTAAACCATCCAATCGG
Scd1	mouse	NM_009127	GCAAGCTCTACACCTGCCTCTT	CGTGCCTTGTAAGTTCTGTGGC
Vcam1	mouse	NM_011693.3	GCTATGAGGATGGAAGACTCTGG	ACTTGTGCAGCCACCTGAGATC

Table S3. Oligonucleotide sequences used for qPCR amplification. Sequences are listed by alphabetical order. Common synonyms for some genes are included between brackets.

Table S4. Metabolomic analysis of HAECs exposed to flow conditions. Primary data for untargeted metabolomic analysis of HAECs exposed to static condition, OSS or PSS for 4 h. Detectable peaks are identified by retention times and mass spectra from the Mass Bank of North America. Data are reported as relative peak intensities normalized to the peak intensity in quality control samples.

Table S5. Metabolomic analysis of mouse plasma before and after exercise. Plasma samples were collected from wild-type male C57BL/6J before and after 24 h of voluntary wheel running for untargeted metabolomic analysis. Detectable peaks are identified by retention times and mass spectra from the Mass Bank of North America. Data are reported as relative peak intensities normalized to the peak intensity in quality control samples.

Table S6. Metabolomic analysis of mouse plasma after HFD and EX treatments. Plasma samples were collected from male (M) and female (F) *Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$*Scd1*$^{EC-/-}$ after 4 weeks of HFD or HFD +EX for metabolomic analysis. Detectable peaks are identified by retention times and mass spectra from the Mass Bank of North America. Targeted analysis for selected metabolites provides the absolute quantification using stable isotope-labeled internal standards. For untargeted analysis, data are reported as relative peak intensities normalized to the peak intensity in quality control samples.