Supplementary Information

Lipid Droplets Sequester Palmitic Acid to Disrupt Endothelial Ciliation and Exacerbate Atherosclerosis in Male Mice

Yanjie Tan[1,8], Zhenzhou Huang[1,8], Yi Jin[2], Jiaying Wang[1], Hongjun Fan[1], Yangyang Liu[1], Liang Zhang[2], Yue Wu[1], Peiwei Liu[1], Tianliang Li[1], Jie Ran[1], He Tian[3], Sin Man Lam[3,4], Min Liu[5], Jun Zhou[1,6]*, Yunfan Yang[7]*

[1]Center for Cell Structure and Function, Haihe Laboratory of Cell Ecosystem, Collaborative Innovation Center of Cell Biology in Universities of Shandong, Shandong Provincial Key Laboratory of Animal Resistance Biology, College of Life Sciences, Shandong Normal University, Jinan 250014, China.

[2]Metabolism and Disease Research Centre, Central Hospital Affiliated to Shandong First Medical University, Jinan 250013, China.

[3]State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.

[4]LipidALL Technologies Company Limited, Changzhou 213022, China.

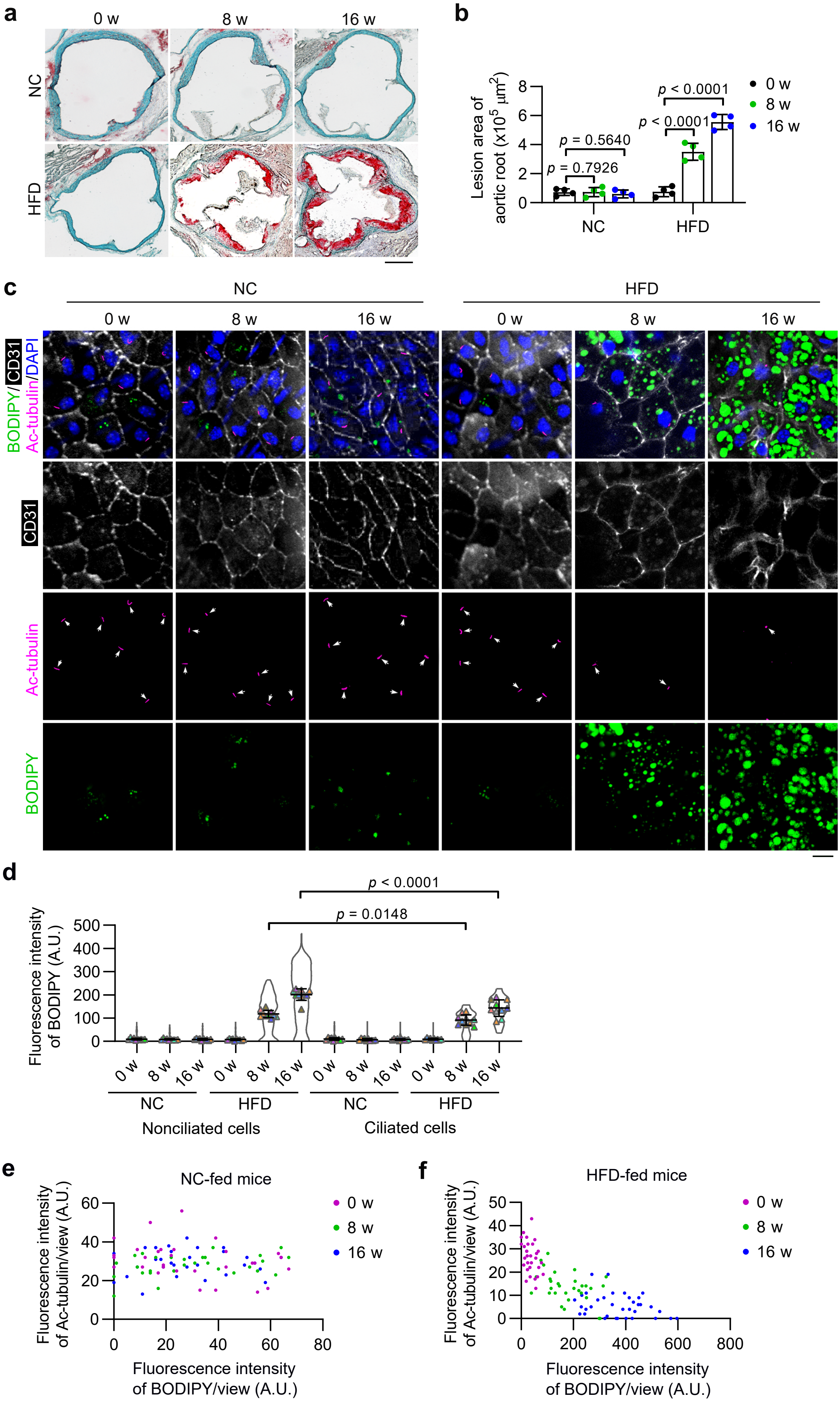
[5]Laboratory of Tissue Homeostasis, Haihe Laboratory of Cell Ecosystem, Tianjin 300462, China.

[6]State Key Laboratory of Medicinal Chemical Biology, College of Life Sciences, Nankai University, Tianjin 300071, China.

[7]Department of Cell Biology, School of Basic Medical Sciences, Cheeloo College of Medicine, Shandong University, Jinan 250012, China.

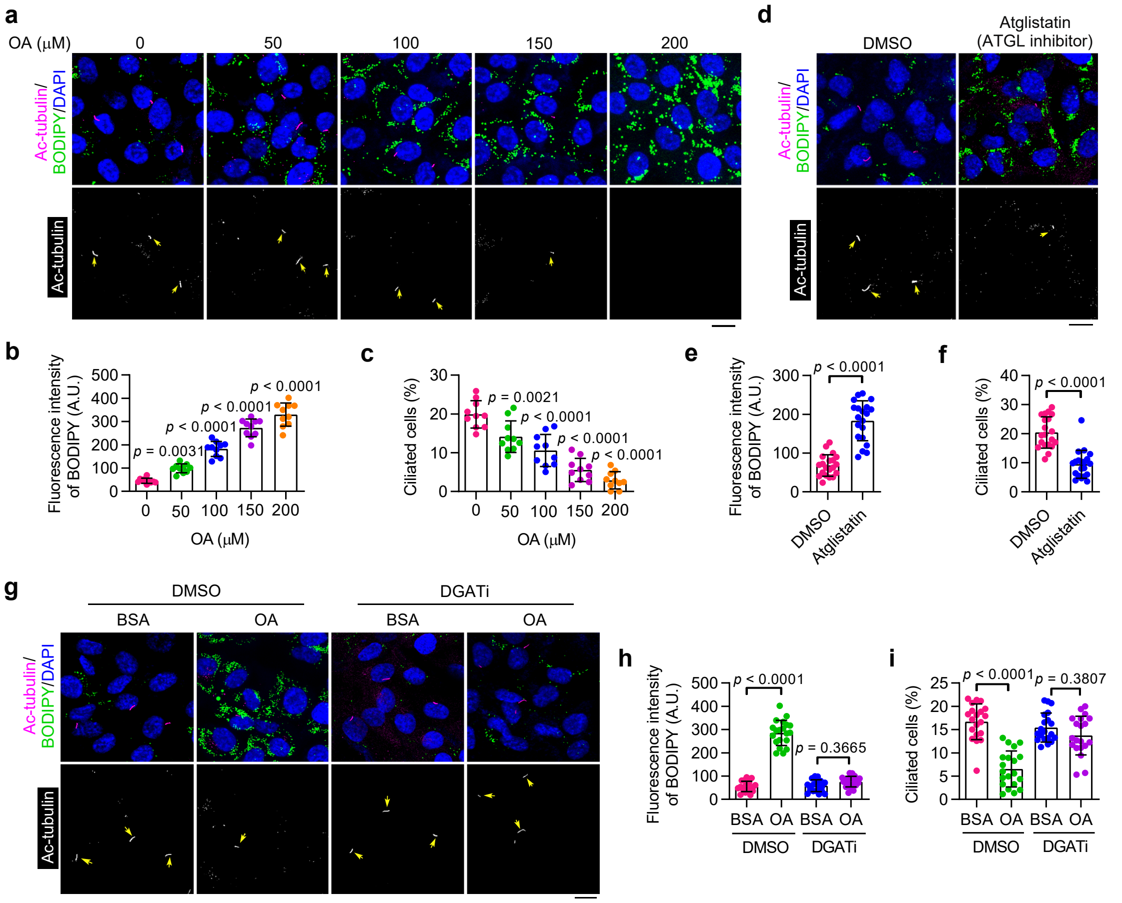
[8]These authors contributed equally to this work.

*Correspondence: junzhou@sdnu.edu.cn (J.Z.); yunfanyang@sdu.edu.cn (Y.Y.)

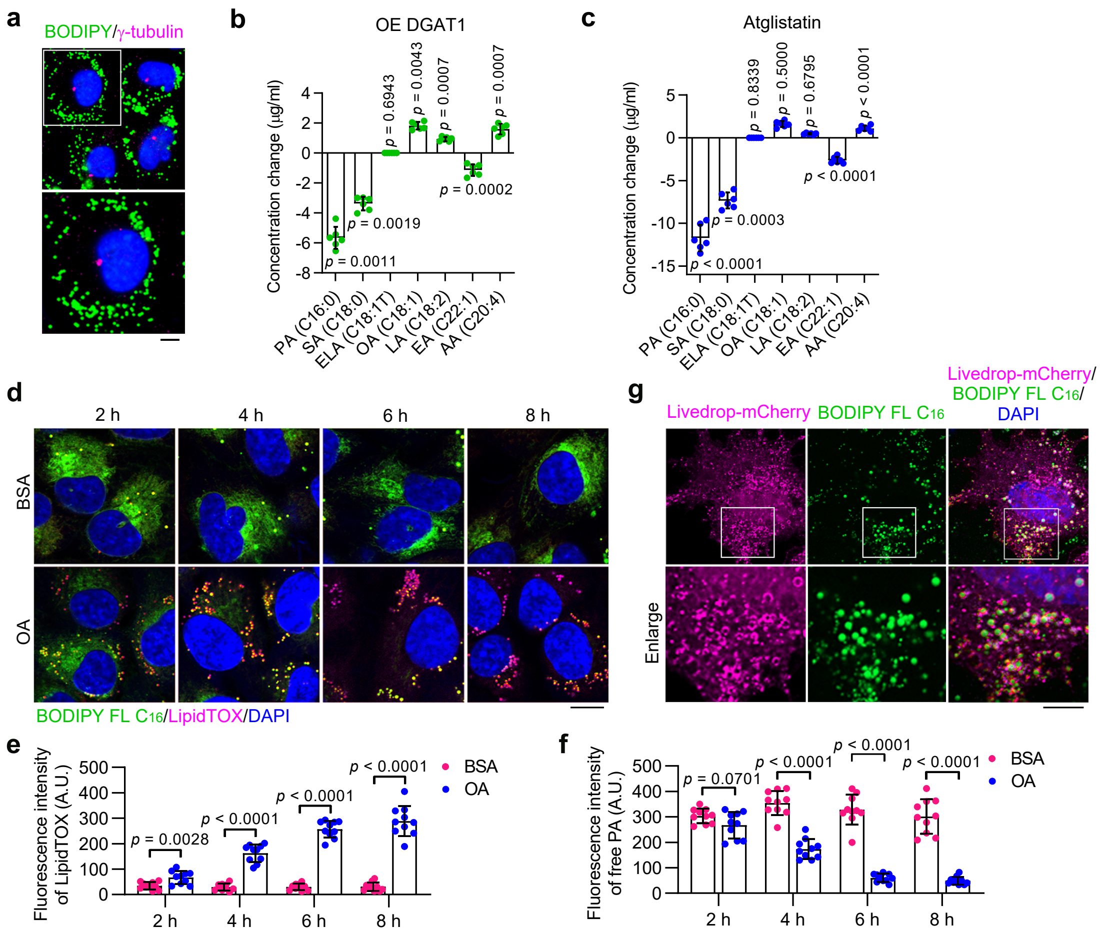


a
0 w
8 w
16 w
NC
HFD
b
Lesion area of aortic root (x10^5 μm^2)
p = 0.7926
p = 0.5640
p < 0.0001
p < 0.0001
0 w
8 w
16 w
NC
HFD
c
NC
HFD
0 w
8 w
16 w
BODIPY/CD31/Ac-tubulin/DAPI
CD31
Ac-tubulin
BODIPY
d
Fluorescence intensity of BODIPY (A.U.)
p < 0.0001
p = 0.0148
0 w
8 w
16 w
NC
HFD
Nonciliated cells
Ciliated cells
e
NC-fed mice
Fluorescence intensity of Ac-tubulin/view (A.U.)
Fluorescence intensity of BODIPY/view (A.U.)
0 w
8 w
16 w
f
HFD-fed mice
Fluorescence intensity of Ac-tubulin/view (A.U.)
Fluorescence intensity of BODIPY/view (A.U.)
0 w
8 w
16 w

Supplementary Figure 1. HFD feeding induces atherosclerosis in mice. **a** Representative Oil Red O (ORO) staining of aortic root sections from ApoE^{KO} mice fed normal chow (NC) or high fat diet (HFD) for indicated periods. Scale bar, 200 μm. **b** Quantitative analysis of panel **a** (n = 4 mice). **c** *En face* immunofluorescence images of aortic arch VECs stained with antibodies against cluster of differentiation 31 (CD31, white) and ac-tubulin (magenta). LDs were stained with BODIPY (green), and nuclei were stained with DAPI (blue). Scale bar, 10 μm. **d** Quantification of the fluorescence intensity of BODIPY in non-ciliated and ciliated VECs of the aortic arch shown in Fig. 1c (n = 10 mice). **e**, **f** Scatter plots showing the relationship between ciliary signal and LD signal of randomly chosen locations in the inner curvature of the aortic arch (n = 10 mice). Data are presented as mean ± SEM. Statistical significance was determined by unpaired two-tailed Student's t-test (**d**) or one-way ANOVA with post hoc analysis (**b**). Source data are provided as a Source Data file.

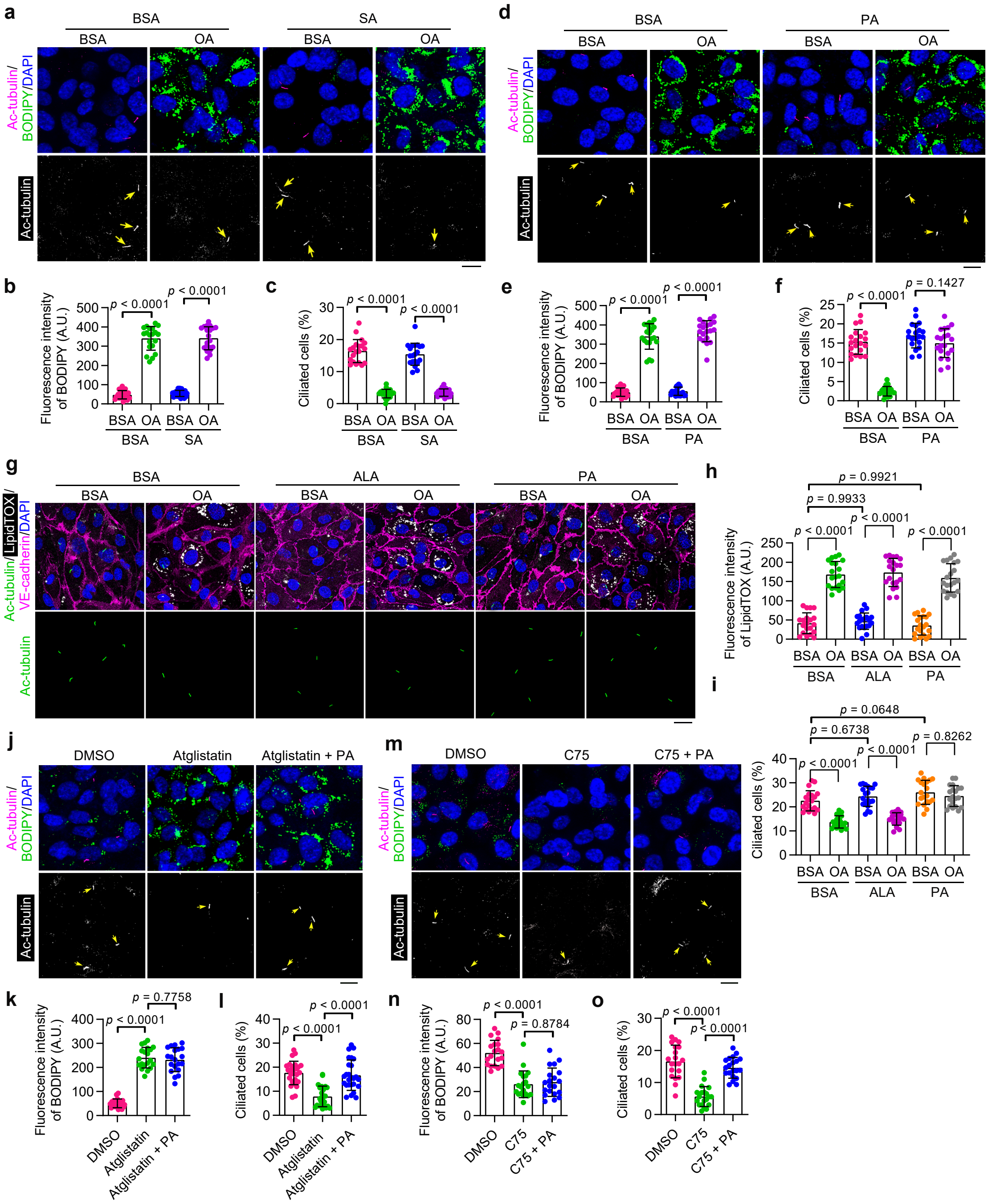


Supplementary Figure 2. Accumulation of LDs triggers ciliary loss in cultured VECs. **a-c** Immunofluorescence images (**a**) and quantifications of BODIPY staining (**b**) and ciliation (**c**) of cultured HUVECs treated with oleic acid (OA) at the indicated concentration for 12 h, followed by serum starvation for 48 h (n = 10 fields from 3 independent experiments). Scale bar, 10 μm. **d-f** Immunofluorescence images (**d**) and quantifications of BODIPY staining (**e**) and ciliation (**f**) of HUVECs treated with Atglistatin (10 μM) for 12 h, followed by serum starvation for 48 h (n = 20 fields from 3 independent experiments). Scale bar, 10 μm. **g-i** Immunofluorescence images (**g**) and quantifications of BODIPY staining (**h**) and ciliation (**i**) of HUVECs treated with DMSO or DGAT inhibitors (A922500, 10 μM and PF-06424439, 5 μM) for 24 h, exposed to BSA or OA (200 μM) for 12 h, and then serum-starved for 48 h (n = 20 fields from 3 independent experiments). BSA was used as a control treatment. Scale bar, 10 μm. Data are presented as mean ± SEM. Statistical significance was determined by unpaired two-tailed Student's t-test (**e**, **f**), one-way (**b**, **c**), or two-way (**h**, **i**) ANOVA with post hoc analysis. Source data are provided as a Source Data file.



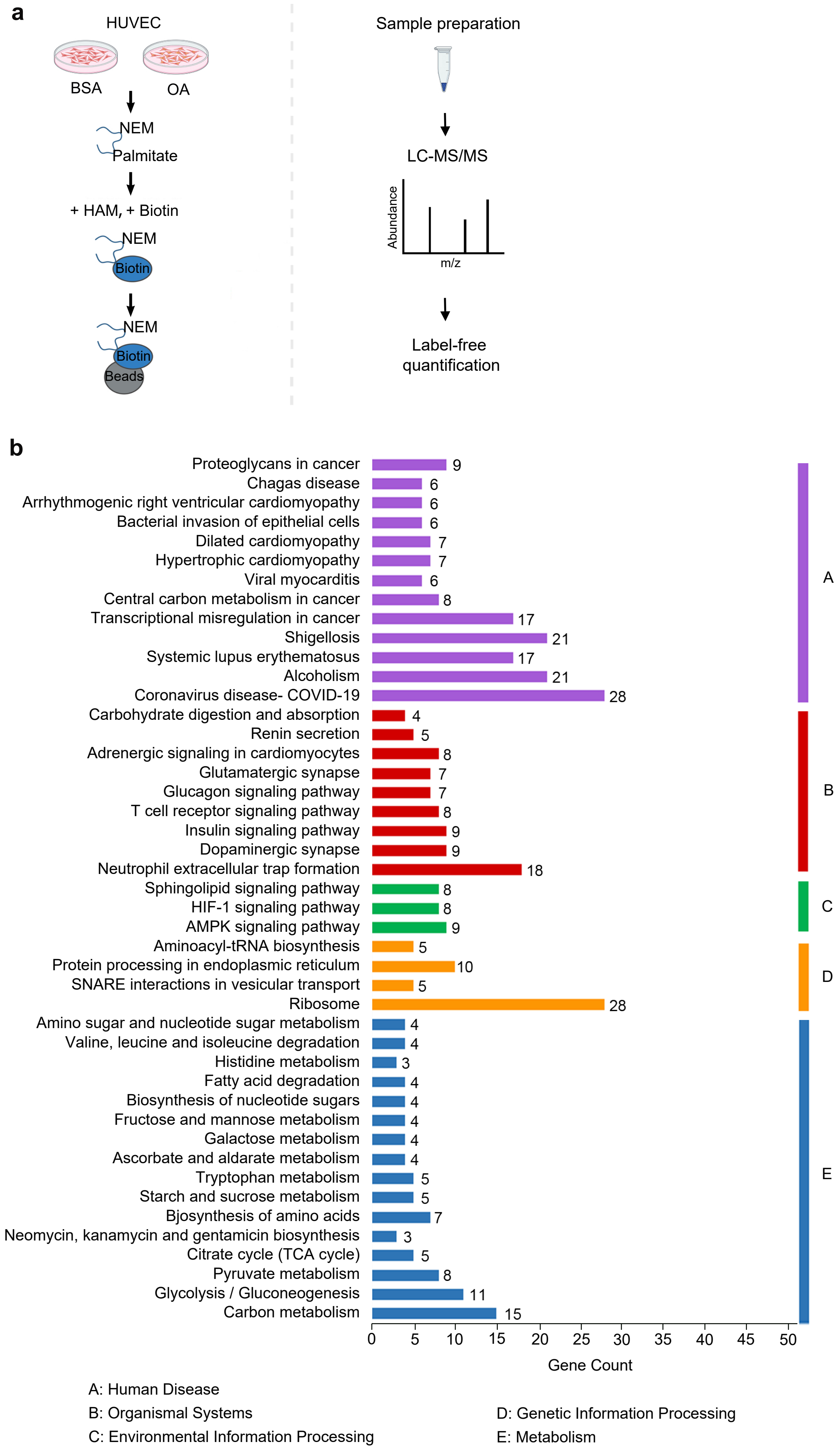
Supplementary Figure 3. LD accumulation in endothelial cells preferentially stimulates the sequestration of cytosolic PA. **a** Immunofluorescence images of HUVECs labeled with γ-tubulin antibody (magenta) and stained with BODIPY (green) and DAPI (blue). Boxed area is enlarged in the bottom panel. Scale bar (for enlarged image), 5 μm. **b**, **c** GC-MS analysis showing changes in levels of indicated free fatty acids in HUVECs exposed to DGAT1 overexpression for 48 h (**b**) or Atglistatin (10 μM) treatment for 24 h (**c**). PA: palmitic acid, SA: stearic acid, ELA: elaidic acid, OA: oleic acid, LA: linoleic acid, EA: erucic acid, AA: arachidonic acid. **d-f** HUVECs were pre-incubated with BODIPY FL C16 (0.5 μM) for 30 min and then treated with oleic acid (OA, 200 μM) or BSA for indicate periods (**d**). The fluorescence intensity of LipidTOX (**e**) and cytosolic PA (**f**) were quantified (n = 10 fields from 3 independent experiments). Scale bar, 10 μm. **g** HUVECs expressing livedrop-mCherry were pre-incubated with BODIPY FL C16 (0.5 μM) for 30 min and then treated with OA (200 μM) for 30 min. Boxed areas are enlarged in the bottom panel. Scale bar (for enlarged images), 5 μm.

Data are presented as mean ± SEM. Statistical significance was determined by unpaired two-tailed Student's t-test. Source data are provided as a Source Data file.

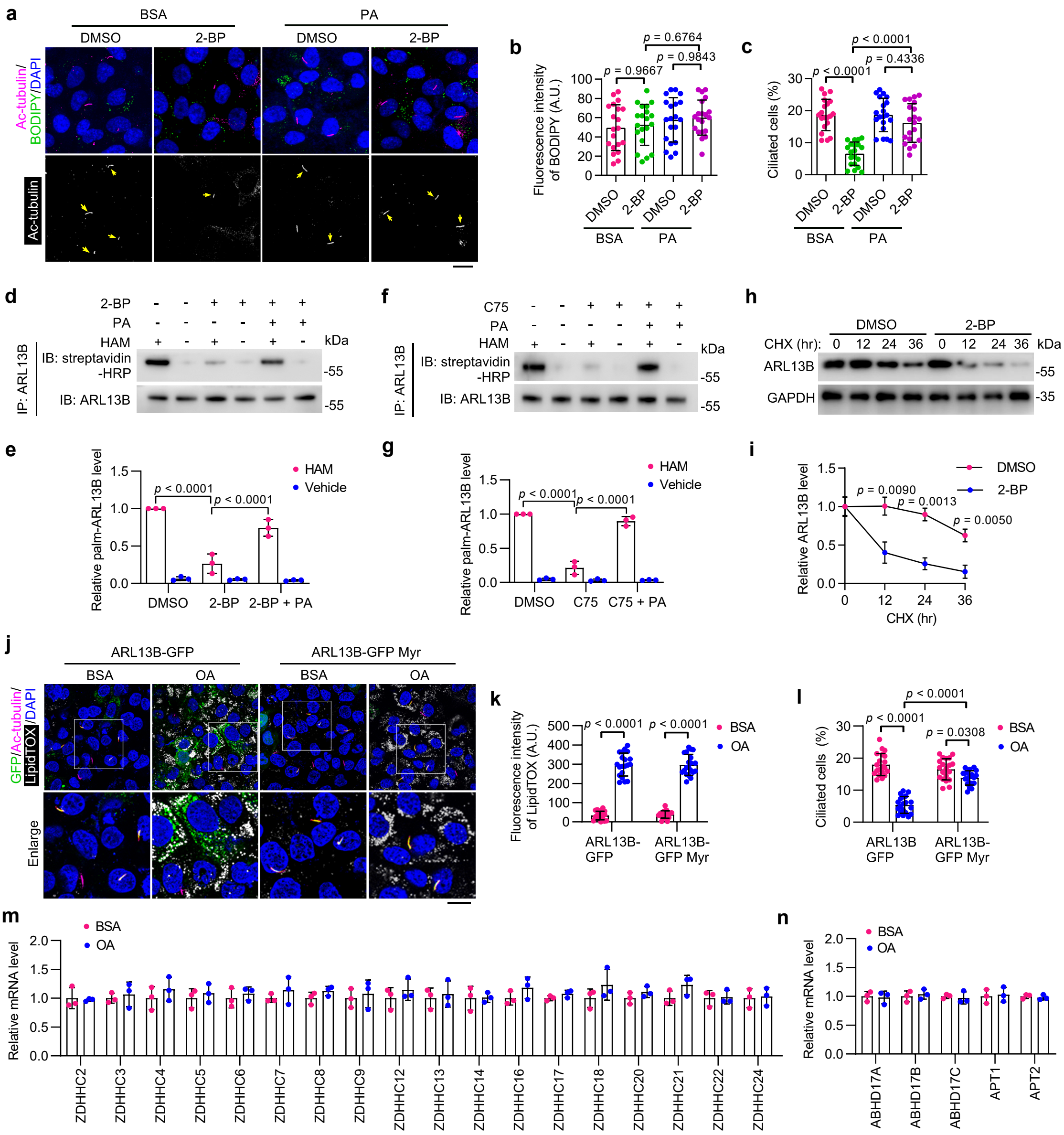


Supplementary Figure 4. LD accumulation disrupts ciliary homeostasis by reducing cytosolic PA. **a**-**f** Immunofluorescence images (**a**, **d**) and quantifications of BODIPY staining (**b**, **e**) and ciliation (**c**, **f**) of HUVECs first treated with BSA or oleic acid (OA, 200 μM) for 12 h, then supplemented with stearic acid (SA, 200 μM) or palmitic acid (PA, 200 μM) for 12 h, and finally serum-starved for 48 h (n = 20 fields from 3 independent experiments). Scale bars, 10 μm. **g**-**i** Immunofluorescence images (**g**) and quantifications of LipidTOX staining (**h**) and ciliation (**i**) of HAECs serum-starved for

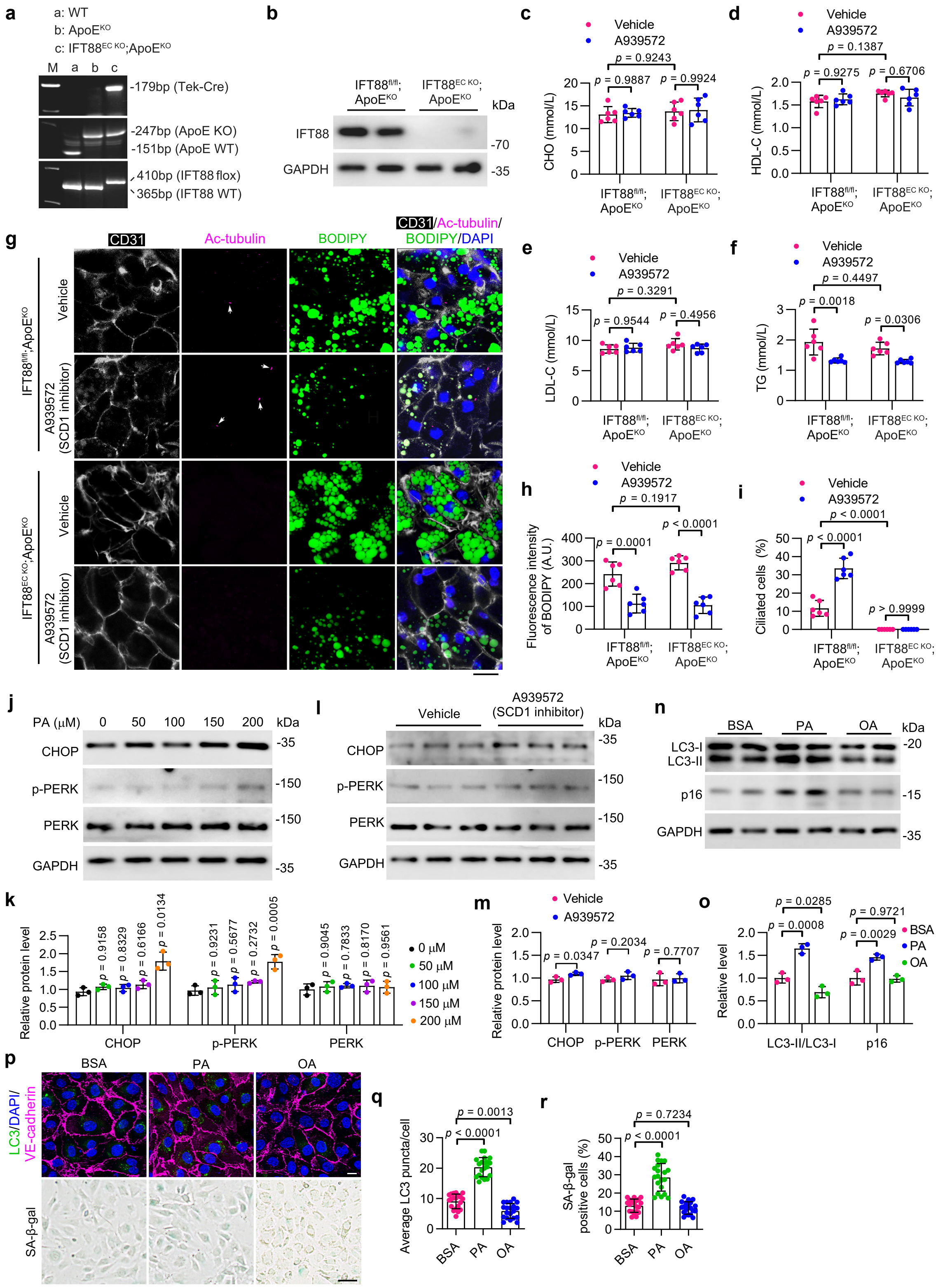
48 h, then exposed to OA (100 μM) and/or α-linolenic acid (ALA) or PA (100 μM) for 16 h (n = 20 fields from 3 independent experiments). Scale bar, 20 μm. **j**-**l** Immunofluorescence images (**j**) and quantifications of BODIPY staining (**k**) and ciliation (**l**) of HUVECs treated with Atglistatin (10 μM) for 12 h, exposed to PA (200 μM) for 12 h, and then serum-starved for 48 h (n = 20 fields from 3 independent experiments). Scale bar, 10 μm. **m**-**o** Immunofluorescence images (**m**) and quantifications of BODIPY staining (**n**) and ciliation (**o**) of HUVECs treated with an FASN inhibitor (C75, 10 μM) for 24 h, exposed to PA (200 μM) for 12 h, and then serum-starved for 48 h (n = 20 fields from 3 independent experiments). Scale bar, 10 μm. Data are presented as mean ± SEM. Statistical significance was determined by one-way (**k**, **l, n, o**), or two-way (**b**, **c**, **e**, **f, h, i**) ANOVA with post hoc analysis. Source data are provided as a Source Data file.


a
HUVEC
BSA
OA
NEM
Palmitate
+ HAM, + Biotin
NEM
Biotin
NEM
Biotin
Beads
Sample preparation
LC-MS/MS
Abundance
m/z
Label-free
quantification
b
Proteoglycans in cancer 9
Chagas disease 6
Arrhythmogenic right ventricular cardiomyopathy 6
Bacterial invasion of epithelial cells 6
Dilated cardiomyopathy 7
Hypertrophic cardiomyopathy 7
Viral myocarditis 6
Central carbon metabolism in cancer 8
Transcriptional misregulation in cancer 17
Shigellosis 21
Systemic lupus erythematosus 17
Alcoholism 21
Coronavirus disease- COVID-19 28
Carbohydrate digestion and absorption 4
Renin secretion 5
Adrenergic signaling in cardiomyocytes 8
Glutamatergic synapse 7
Glucagon signaling pathway 7
T cell receptor signaling pathway 8
Insulin signaling pathway 9
Dopaminergic synapse 9
Neutrophil extracellular trap formation 18
Sphingolipid signaling pathway 8
HIF-1 signaling pathway 8
AMPK signaling pathway 9
Aminoacyl-tRNA biosynthesis 5
Protein processing in endoplasmic reticulum 10
SNARE interactions in vesicular transport 5
Ribosome 28
Amino sugar and nucleotide sugar metabolism 4
Valine, leucine and isoleucine degradation 4
Histidine metabolism 3
Fatty acid degradation 4
Biosynthesis of nucleotide sugars 4
Fructose and mannose metabolism 4
Galactose metabolism 4
Ascorbate and aldarate metabolism 4
Tryptophan metabolism 5
Starch and sucrose metabolism 5
Bjosynthesis of amino acids 7
Neomycin, kanamycin and gentamicin biosynthesis 3
Citrate cycle (TCA cycle) 5
Pyruvate metabolism 8
Glycolysis / Gluconeogenesis 11
Carbon metabolism 15
A
B
C
D
E
0 5 10 15 20 25 30 35 40 45 50
Gene Count
A: Human Disease
B: Organismal Systems
C: Environmental Information Processing
D: Genetic Information Processing
E: Metabolism

Supplementary Figure 5. ABE-MS detection of S-palmitoylated proteins. **a** Schematic describing the enrichment strategy and mass spectrometry-based workflow used for the identification of S-acylated proteins in HUVECs treated with BSA or oleic acid (OA, 200 μM) for 12 h. NEM, N-Ethylmaleimide; HAM, hydroxylamine. **b** KEGG analysis of differentially S-palmitoylated proteins between BSA- and OA-treated HUVECs. Panel **a** was created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license.

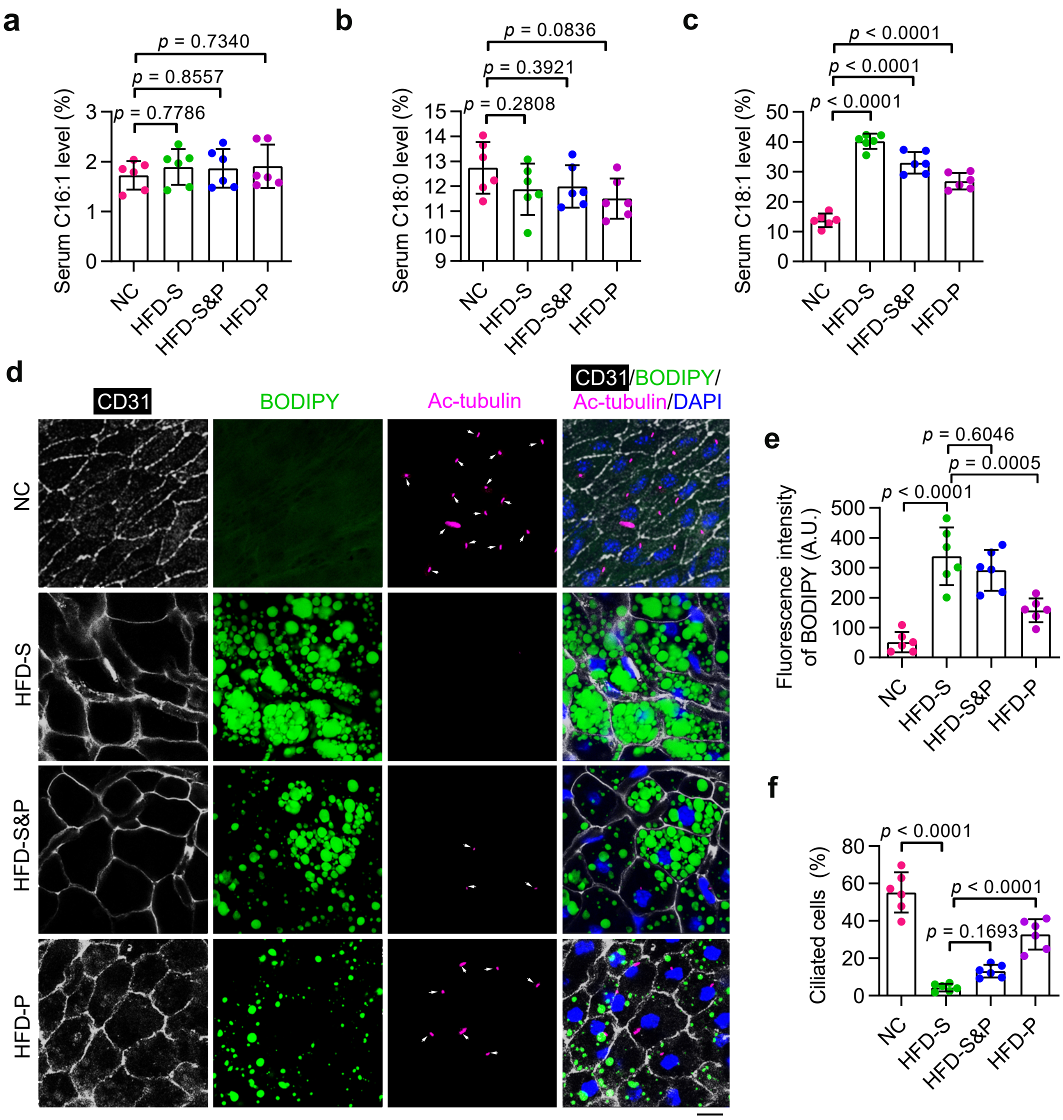


Supplementary Figure 6. PA is essential for ARL13B S-palmitoylation. **a-c** Immunofluorescence images (**a**) and quantifications of BODIPY staining (**b**) and ciliation (**c**, n = 20 fields from 3 independent experiments) of HUVECs first treated with DMSO or 2-Bromopalmitate (2-BP, 50 μM) for 24 h, exposed to BSA or palmitic acid (PA, 200 μM) for 12 h, and then serum-starved for 48 h. Scale bar, 10 μm. **d**, **e** MAECs were first treated with DMSO or 2-BP (50 μM) for 24 h and then supplemented with either BSA or PA (200 μM) for another 12 h. The level of ARL13B S-palmitoylation was examined by IP-ABE and immunoblotting (**d**) and quantified by densitometry (**e**) (n = 3 samples). HAM, hydroxylamine. **f**, **g** MAECs were pre-treated with DMSO or the FASN inhibitor C75 (10 μM) for 24 h and then supplemented with either BSA or PA (200 μM) for another 12 h. The level of ARL13B S-palmitoylation was then examined by IP-ABE and immunoblotting (**f**) and quantified by densitometry (**g**) (n = 3 samples). **h**, **i** HUVECs were treated with DMSO or 2-BP (50 μM) for 24 h and then treated with CHX (20 mg/mL) for indicated periods. The level of ARL13B was examined by immunoblotting (**h**) and quantified by densitometry (**i**) (n = 3 samples). **j**-**l** Immunofluorescence images (**j**) and quantifications of LipidTOX staining (**k**) and ciliation (**l**) of HAECs overexpressed with ARL13B-GFP or ARL13B-GFP Myr and treated with BSA or oleic acid (OA, 200 μM) (n = 20 fields from 3 independent experiments). Boxed areas are enlarged in the bottom panel. Scale bar (for enlarged images), 10 μm. **m**, **n** Transcriptional levels of palmitoyl acyltransferases (ZDHHCs) (**m**) and depalmitoylases, including APT1, APT2, ABHD17A, ABHD17B, and ABHD17C (**n**) in MAECs treated with BSA or OA (200 μM) for 12 h (n = 3 samples). Data are presented as mean ± SEM. Statistical significance was determined by unpaired two-tailed Student's t-test (**i**, **m**, **n**) or two-way ANOVA with post hoc analysis (**b**, **c**, **e**, **g**, **k**, **l**). Source data are provided as a Source Data file.

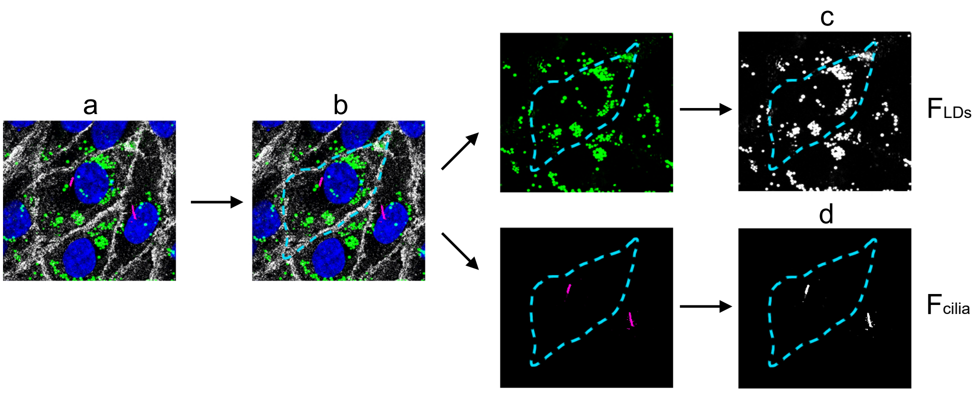


Supplementary Figure 7. Effects of SCD1 inhibition on circulating lipid levels. **a** Genotyping of indicated mouse lines. M: marker, a: WT, b: ApoE^{KO}, c: IFT88$^{EC\ KO}$;ApoE^{KO}. **b** Immunoblotting of IFT88 protein level in MAECs isolated from IFT88$^{EC\ KO}$;ApoE^{KO} and littermate ApoE^{KO} mice. **c-f** Levels of total cholesterol (CHO, **c**), high-density lipoprotein cholesterol (HDL-C, **d**), low-density lipoprotein cholesterol (LDL-

C, **e**), and triglycerides (TG, **f**) in the serum of IFT88$^{EC\ KO}$;ApoE^{KO} and littermate ApoE^{KO} mice intravenously injected with A939572 (5 mg/kg body weight/2 days) or vehicle for 4 weeks (n = 6 mice). **g-i**, *En face* immunofluorescence images (**g**) and quantifications of BODIPY staining (**h**) and ciliation (**i**) of aortic arch VECs of IFT88$^{EC\ KO}$;ApoE^{KO} and littermate ApoE^{KO} mice intravenously injected with A939572 (5 mg/kg body weight/2 days) or vehicle for 4 weeks after an 8-week HFD feeding (n = 6 mice). Scale bar, 20 μm. **j**, **k** Immunoblotting (**j**) and quantification (**k**) of the protein levels of CHOP in MAECs treated with PA at the indicated concentration for 12 h (n = 3 samples). **l**, **m** Immunoblotting (**l**) and quantification (**m**) of the protein levels of CHOP, p-PERK, and PERK in the aortic arch of ApoE^{KO} mice intravenously injected with A939572 (5 mg/kg body weight/2 days) or vehicle for 4 weeks after an 8-week HFD feeding (n = 3 mice). **n**, **o** Immunoblotting (**n**) and quantification (**o**) of LC3 lipidation and p16 protein level in HAECs (n = 3 samples). **p-r** Images (**p**) and quantifications of LC3 puncta (**q**) and SA-β-gal-positive cells (**r**) in HAECs treated with OA or PA (200 μM) for 12 h (n = 20 fields from 3 independent experiments). Scale bar for immunofluorescence images, 20 μm. Scale bar for SA-β-gal staining, 50 μm. Data are presented as mean ± SEM. Statistical significance was determined by unpaired two-tailed Student's t-test (**m**), one-way (**k, o, q, r**), or two-way (**c**, **d-f**, **h**, **i**) ANOVA with post hoc analysis. Source data are provided as a Source Data file.



Supplementary Figure 8. Examination of ApoE^{KO} mice challenged with the indicated diets. **a-c** Levels of palmitoleic acid (POA, C16:1) (**a**), stearic acid (SA, C18:0) (**b**), and oleic acid (OA, C18:1) (**c**) in the serum of ApoE^{KO} mice fed with the indicated diets (n = 6 mice). **d-f** *En face* immunofluorescence images (**d**) and quantifications of BODIPY staining (**e**) and ciliation (**f**) of aortic arch VECs of ApoE^{KO} mice treated as described in Fig. 7a (n = 6 mice). NC, normal chow; HFD, high fat diet; S, soybean oil; P, palm oil. Scale bar, 10 μm. Data are presented as mean ± SEM. Statistical significance was determined by one-way ANOVA with post hoc analysis. Source Data are provided as a Source Data file.



Supplementary Figure 9. Scheme depicting the method for measuring the fluorescence intensity of LDs and cilia in individual endothelial cells. **a** VE-cadherin immunofluorescence staining was performed to label the cell membrane of endothelial cells for delineating each cell's region. **b** Individual cell regions were outlined with cyan dashed lines based on the VE-cadherin fluorescence signal using ImageJ software. **c**, **d** The fluorescence intensities in the indicated channels within the selected regions were measured as F_{LDs} (**c**) and F_{cilia} (**d**) using the "measurement" tool by ImageJ software.

Supplementary Table 1. The fractional abundance of $^{13}C_{16}$-labeled triglycerides in different samples.

M+16	BSA-whole cells			OA-whole cells			OA-LDs		
TAG48:2	0.163405	0.140884	0.185191	0.236564	0.24418	0.24481	0.179437	0.175597	0.251498
TAG48:1	0.133255	0.108331	0.148846	0.162038	0.16666	0.181186	0.223402	0.219806	0.236626
TAG50:4	0.270872	0.272719	0.295094	0.159974	0.174798	0.165927	0.087491	0.091527	0.172058
TAG50:3	0.217719	0.20615	0.231091	0.254389	0.25386	0.251559	0.14854	0.146998	0.245513
TAG50:2	0.213248	0.196108	0.213866	0.275503	0.278028	0.281241	0.207748	0.207184	0.278488
TAG50:1	0.173794	0.152298	0.177299	0.210769	0.21647	0.219362	0.251337	0.241416	0.251099
TAG52:5	0.304058	0.273027	0.319822	0.200709	0.193125	0.187368	0.080159	0.082367	0.169051
TAG52:4	0.196854	0.194903	0.213205	0.265444	0.271142	0.255509	0.112302	0.109937	0.214767
TAG52:3	0.260636	0.257817	0.268264	0.333745	0.333669	0.318588	0.142007	0.142768	0.260656
TAG52:2	0.276268	0.265487	0.260854	0.348132	0.362746	0.338519	0.189363	0.187206	0.289902
TAG52:1	0.229631	0.209673	0.221455	0.272057	0.271254	0.265788	0.248434	0.247549	0.282431
TAG54:5	0.177626	0.151246	0.184732	0.069116	0.069776	0.070739	0.035273	0.033478	0.066342
TAG54:4	0.356134	0.356609	0.345143	0.100932	0.10288	0.101387	0.045831	0.04631	0.100994
TAG54:3	0.230521	0.226928	0.258715	0.175291	0.174768	0.174862	0.083825	0.080754	0.168302
TAG54:2	0.253488	0.251025	0.236269	0.275982	0.279534	0.276803	0.158218	0.164177	0.258844
TAG54:1	0.214008	0.203386	0.206958	0.261238	0.26528	0.263942	0.229373	0.227609	0.280147
TAG56:3	0.211615	0.21252	0.241579	0.180619	0.176942	0.177904	0.100975	0.099145	0.185721
TAG56:2	0.227159	0.223742	0.234967	0.262233	0.264163	0.258539	0.17035	0.162028	0.243013
TAG58:3	0.212519	0.215991	0.241857	0.194496	0.199587	0.207026	0.118243	0.116621	0.206348

M+16	OE NC-whole cells			OE DGAT1-whole cells			OE DGAT1-LDs		
TAG48:2	0.138486	0.144759	0.184901	0.298536	0.291484	0.302097	0.222096	0.220194	0.213472
TAG48:1	0.123746	0.111851	0.112117	0.243293	0.245156	0.246452	0.209667	0.200324	0.210549
TAG50:4	0.134483	0	0	0.200369	0.198914	0.194727	0.107559	0.106975	0.114875
TAG50:3	0.153641	0.171981	0.195577	0.281578	0.285318	0.282087	0.203154	0.211518	0.198209
TAG50:2	0.199014	0.184033	0.206456	0.278445	0.290024	0.282052	0.249752	0.252344	0.248286
TAG50:1	0.134318	0.122303	0.133834	0.236725	0.255959	0.242436	0.237025	0.23584	0.236763
TAG52:5	0.189553	0.191486	0.220712	0.265073	0.276498	0.279581	0.120602	0.126175	0.114227
TAG52:4	0.380094	0.428654	0.465981	0.351299	0.34037	0.353721	0.19108	0.199025	0.179858
TAG52:3	0.213316	0.210598	0.224125	0.371344	0.367622	0.374638	0.229666	0.238397	0.220708
TAG52:2	0.259173	0.248051	0.2749	0.37532	0.367614	0.37349	0.266382	0.271643	0.255172
TAG52:1	0.184758	0.18057	0.193859	0.284097	0.300889	0.303599	0.252855	0.252812	0.247736
TAG54:5	0.120927	0.117768	0.119701	0.11774	0.112481	0.118788	0.041573	0.040936	0.04206
TAG54:4	0.321198	0.33856	0.403657	0.117128	0.120552	0.121685	0.045396	0.046482	0.046409
TAG54:3	0.225172	0.226271	0.249056	0.182219	0.18004	0.189655	0.091172	0.096595	0.095902
TAG54:2	0.198173	0.194974	0.205162	0.27487	0.280729	0.277564	0.195336	0.201938	0.19718
TAG54:1	0.194106	0.186483	0.188276	0.280938	0.292415	0.292787	0.260179	0.267272	0.259276
TAG56:3	0.167581	0.165164	0.182104	0.161829	0.171837	0.166324	0.107924	0.115875	0.111249
TAG56:2	0.176795	0.168922	0.180764	0.250141	0.271678	0.269895	0.195404	0.196843	0.19842
TAG58:3	0.185304	0.176038	0.195985	0.201705	0.20717	0.209003	0.132993	0.139649	0.13531

M+16	DMSO-whole cells			Atglistatin-whole cells			Atglistatin-LDs		
TAG48:2	0.197089	0.193064	0.160215	0.296072	0.292791	0.299318	0.236047	0.224751	0.200558
TAG48:1	0.154902	0.147066	0.134205	0.255376	0.251816	0.251585	0.216987	0.214282	0.202605
TAG50:4	0	0	0	0.192255	0.180752	0.171468	0.123915	0.10971	0.098899
TAG50:3	0.227229	0.222466	0.193693	0.282001	0.275743	0.284665	0.216281	0.218003	0.194154
TAG50:2	0.235501	0.230555	0.208364	0.301196	0.29491	0.279496	0.264472	0.261821	0.240169
TAG50:1	0.162371	0.160719	0.147772	0.262689	0.258462	0.242786	0.239448	0.237188	0.224183
TAG52:5	0.234946	0.229021	0.21989	0.283645	0.261169	0.272281	0.143126	0.144788	0.103563
TAG52:4	0.46233	0.472976	0.446192	0.34755	0.344522	0.352414	0.207763	0.203884	0.171035
TAG52:3	0.248689	0.239179	0.242079	0.369047	0.365399	0.376065	0.247882	0.240322	0.21029
TAG52:2	0.272732	0.268579	0.254489	0.364588	0.366028	0.373423	0.284912	0.27944	0.248394
TAG52:1	0.207343	0.22238	0.197393	0.305763	0.302722	0.2922	0.271473	0.247543	0.235008
TAG54:5	0.131931	0.125915	0.128887	0.124181	0.117741	0.117824	0.044915	0.050619	0.039263
TAG54:4	0.346216	0.352868	0.347214	0.132762	0.128967	0.117736	0.04798	0.053834	0.041929
TAG54:3	0.232809	0.250992	0.231703	0.201591	0.190322	0.178359	0.102208	0.10268	0.087108
TAG54:2	0.218337	0.211062	0.206882	0.286419	0.277941	0.280508	0.208202	0.202375	0.184275
TAG54:1	0.205171	0.215112	0.204543	0.301148	0.29399	0.291344	0.274463	0.26705	0.255507
TAG56:3	0.171666	0.173814	0.177557	0.177584	0.170012	0.165306	0.1123	0.11685	0.098649
TAG56:2	0.188946	0.18981	0.179838	0.268964	0.260068	0.253688	0.197951	0.209111	0.188429
TAG58:3	0.181075	0.17317	0.180067	0.212989	0.210598	0.20622	0.142611	0.153851	0.120386

Supplementary Table 2. Palmitoylated cilia-related proteins identified by IP-ABE in HUVECs.

Symbol	Entrez Gene Name
ARL13B	ADP ribosylation factor like GTPase 13B
EHD1	EH domain-containing protein 1
ARF4	ADP-ribosylation factor 4
SEPTIN2	Septin-2
EHD2	EH domain-containing protein 2
HDAC6	Histone deacetylase 6
SEPTIN7	Septin-7
DYNLL1	Dynein light chain 1
BBS7	Bardet-Biedl syndrome 7
NECTIN2	Nectin-2
FLNA	Filamin-A
AURKA	Aurora kinase A
MAP4	Microtubule-associated protein 4
RP2	Protein XRP2
ATXN10	Ataxin-10
RAB8A	Ras-related protein Rab-8A
SLC9A3R1	Na (+) / H (+) exchange regulatory cofactor NHE-RF; Na (+) / H (+) exchange regulatory cofactor NHE-RF1
GSN	Gelsolin
KIF3A	Kinesin-like protein KIF3A
WDR54	WD repeat-containing protein 54
ACTR3	Actin-related protein 3
PTPN23	Tyrosine-protein phosphatase non-receptor type 23
CEP131	Centrosomal protein of 131 kDa
DNMBP	Dynamin-binding protein
DCTN1	Dynactin subunit 1
RO60	60 kDa SS-A/Ro ribonucleoprotein
HSPB11	Intraflagellar transport protein 25 homolog
CFAP20	Cilia- and flagella-associated protein 20
MAPRE1	Microtubule-associated protein RP/EB family member 1
ACTR2	Actin-related protein 2
GBF1	Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1
PCM1	Pericentriolar material 1 protein
DNAAF5	Dynein assembly factor 5

Supplementary Table 3. Compositions of diets used in this study.

		CD		HFD-S		HFD-S & P		HFD-P	
		gm	kcal	gm	kcal	gm	kcal	gm	kcal
Protein	Casein	200	800	200	800	200	800	200	800
	L-Cystine	3	12	3	12	3	12	3	12
Carbohydrate	Corn Starch	375	1500	0	0	0	0	0	0
	Maltodextrin	125	500	125	500	125	500	125	500
	Sucrose	200	800	68.75	275	68.75	275	68.75	275
Fat	cocoa butter	20	180	20	180	20	180	20	180
	Soybean Oil	25	225	250	2250	115	1035	25	225
	Palm Oil	0	0	0	0	135	1215	225	2025
Fatty acid composition	C16:0	7.96	-	31.59	-	76.14	-	105.84	-
	C16:1	0.04	-	0.04	-	0.44	-	0.71	-
	C18:0	8.24	-	18.14	-	13.27	-	17.91	-
	C18:1	17.86	-	119.56	-	107.95	-	100.21	-
	SFA	16.64	-	51.74	-	97.23	-	127.56	-
	MUFA	12.3	-	63.6	-	82.77	-	95.55	-
	PUFA	14.98	-	144.81	-	78.91	-	35.91	-
Vitamins and Minerals	cellulose	50	0	50	0	50	0	50	0
	Mineral Mix, S10021	10	0	10	0	10	0	10	0
	Calcium Phosphate	13	0	13	0	13	0	13	0
	Calcium Carbonate	5.5	0	5.5	0	5.5	0	5.5	0
	Potassium Citrate	16.5	0	16.5	0	16.5	0	16.5	0
	Vitamin Mix, v10001	10	40	10	40	10	40	10	40
	Choline Bitartrate	2	0	2	0	2	0	2	0
	cholesterol	5.3	0	5.3	0	5.3	0	5.3	0
Summary		gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
	Protein	19	20	26	20	26	20	26	20
	Carbohydrate	67	70	26	20	26	20	26	20
	Fat	4	10	35	60	35	60	35	60

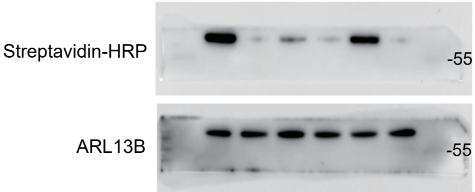
Supplementary Table 4. Primers used for qRT-PCR.

Gene Name	Primer Sequence 5′-3′
ZDHHC2	Forward: TCCCGGTGGTGTTCATCAC Reverse: CAACTTGTTCGCCAGTGTTTTC
ZDHHC3	Forward: CCACTTCCGAAACATTGAGCG Reverse: CCACAGCCGTCACGGATAAA
ZDHHC4	Forward: CCTGACTTGTGGAACCAATCC Reverse: GCACCTCACGTTCTTTGGAAAC
ZDHHC5	Forward: GTTTGGCTTTGGCCTCCTTTA Reverse: ACACACATTACTGCCATTGTGAC
ZDHHC6	Forward: GTTGTGGTATTGGCCCTTACA Reverse: AAAGCCCGGACCGACAAAC
ZDHHC7	Forward: CTGACCGGGTCTGGTTCATC Reverse: CATGACGAAAGTCACCACGAA
ZDHHC8	Forward: CTCAAACCCGCCAAGTACATC Reverse: ACACAGCTCGTGTCAACCAC
ZDHHC9	Forward: CCTGGGTGGGGAATTGTGTT Reverse: ACGACGGACCAGAGTGTAAAG
ZDHHC12	Forward: GTGCTGACCTGGGGAATCAC Reverse: CTGCACATTCACGTAGCCA
ZDHHC13	Forward: AGGAAGCCATTAAGGTCACTCC Reverse: GCCAAAACCTATGCACCGTC
ZDHHC14	Forward: TGTGATAACTGCGTAGAACGGT Reverse: CGTGGGTGATAACGAATGCAA
ZDHHC16	Forward: CGAAAGGCACATCAACAAGAAG Reverse: AGTTGTCCAAGCAGCCGTAG
ZDHHC17	Forward: GGCCCGGATGAGTACGATAC Reverse: TCCAAGAGGTTCACCATATCCA
ZDHHC18	Forward: CACCCCGAACCTCACACTG Reverse: TGAAGGCCGTCAGGAATGAGA
ZDHHC20	Forward: TTCGTGGTCGTCTGGTCCTA Reverse: AGGTAAACAACGGTCTTTCCATT
ZDHHC21	Forward: TGTTGTTGACCCACATGGTTG Reverse: GAGGCCCTCACTAAGGCAA
ZDHHC22	Forward: GGGGCGCTCTTCCTATTCC Reverse: GCAGAAGTGGGTGCTAGGTG
ZDHHC24	Forward: CTGGCACAGTTTGCCTTGG Reverse: CAGGGACCCAGGTCATAGGAG

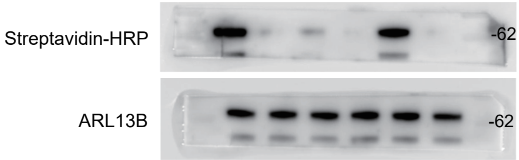
ABHD17A	Forward: GGTACACGGTCCTCTTCTCG Reverse: CGTAGCCGGAGTAGTCGTAG
ABHD17B	Forward: TCTTGGTCAAATGAGCAGCTTT Reverse: GCGAATGCCATATCTTGTCCT
ABHD17C	Forward: GGTTTGCGTGTGGCTTTTCC Reverse: CATGAATGACCAACACAGGAGA
APT1	Forward: CCTTTGCAGGTATCAGAAGTTCA Reverse: GCTGCCTGTTTAATCCCAGAT
APT2	Forward: AGGCAGCTAATGGCAGTGC Reverse: AGGTGTGACAACAGACCGGA
SCD1	Forward: TTCTTGCGATACACTCTGGTGC Reverse: CGGGATTGAATGTTCTTGTCGT
IL1B	Forward: GCAACTGTTCCTGAACTCAACT Reverse: ATCTTTTGGGGTCCGTCAACT
IL6	Forward: TAGTCCTTCCTACCCCAATTTCC Reverse: TTGGTCCTTAGCCACTCCTTC
Tgfb1	Forward: CTCCCGTGGCTTCTAGTGC Reverse: GCCTTAGTTTGGACAGGATCTG
Vcam1	Forward: AGTTGGGGATTCGGTTGTTCT Reverse: CCCCTCATTCCTTACCACCC
Sele	Forward: ATGCCTCGCGCTTTCTCTC Reverse: GTAGTCCCGCTGACAGTATGC
Tnfaip3	Forward: GAACAGCGATCAGGCCAGG Reverse: GGACAGTTGGGTGTCTCACATT
Hmox1	Forward: AAGCCGAGAATGCTGAGTTCA Reverse: GCCGTGTAGATATGGTACAAGGA
iNOS	Forward: AGGGACAAGCCTACCCCTC Reverse: CTCATCTCCCGTCAGTTGGT
GAPDH (human)	Forward: AGGTCGGTGTGAACGGATTTG Reverse: TGTAGACCATGTAGTTGAGGTCA
GAPDH (mouse)	Forward: AGGTCGGTGTGAACGGATTTG Reverse: TGTAGACCATGTAGTTGAGGTCA

Uncropped Immunoblots of Supplementary Figures

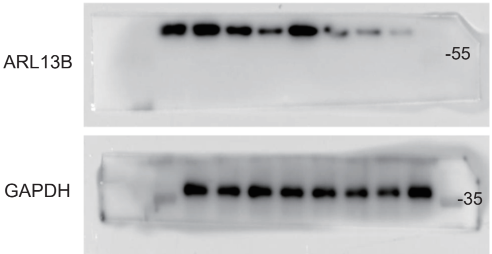
Supplementary Figure 6d:



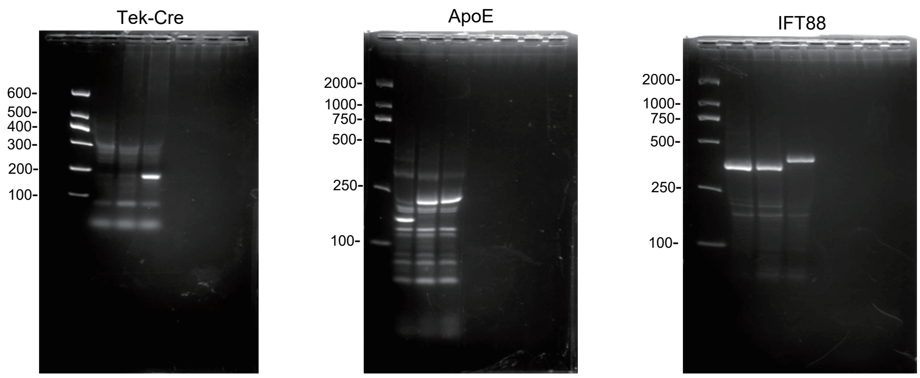
Supplementary Figure 6f:



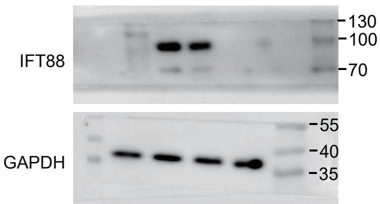
Supplementary Figure 6h:



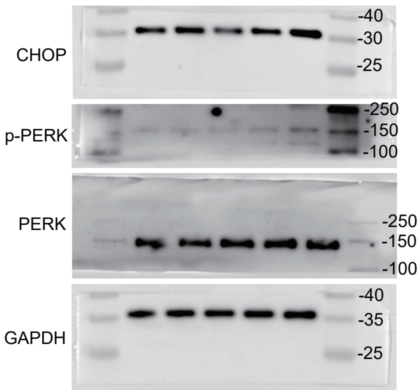
Supplementary Figure 7a:



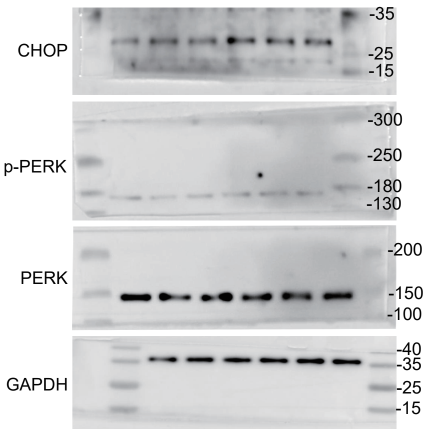
Supplementary Figure 7b:



Supplementary Figure 7j



Supplementary Figure 7i:



Supplementary Figure 7n: