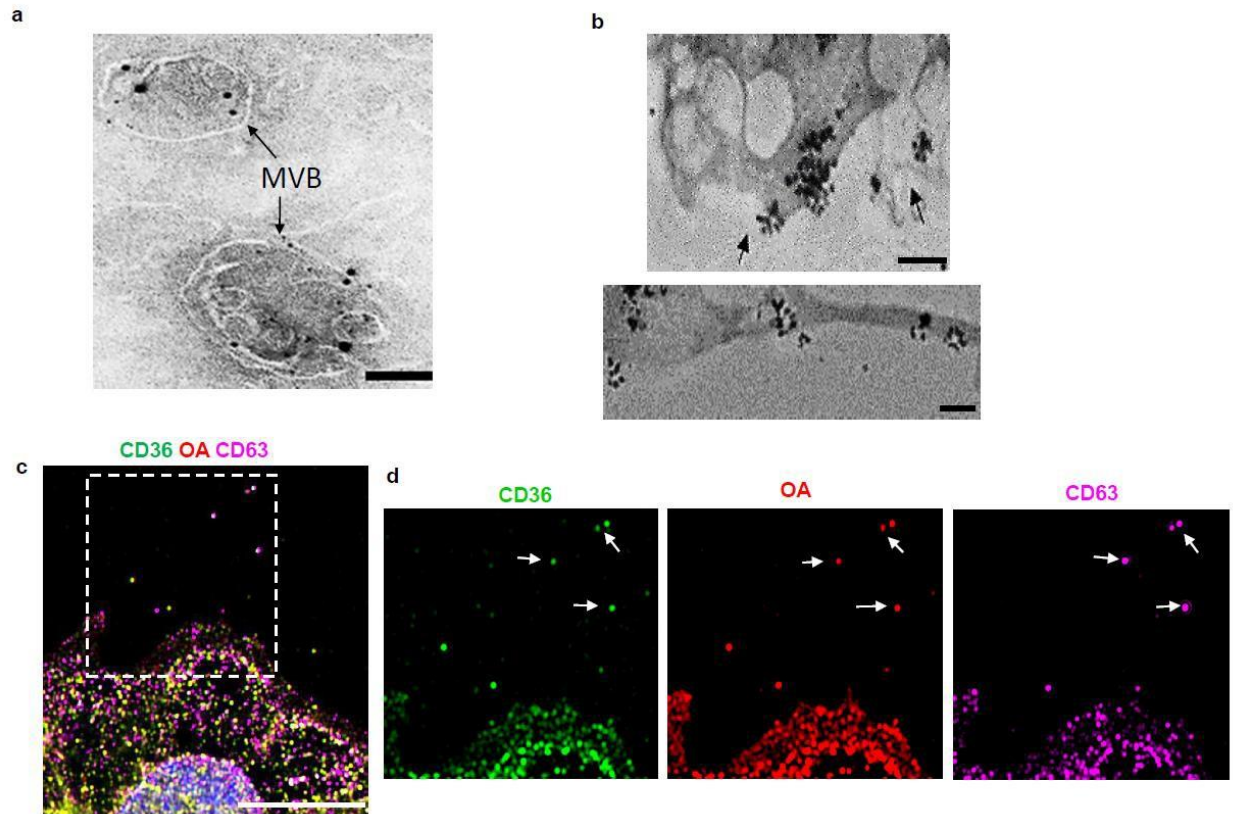
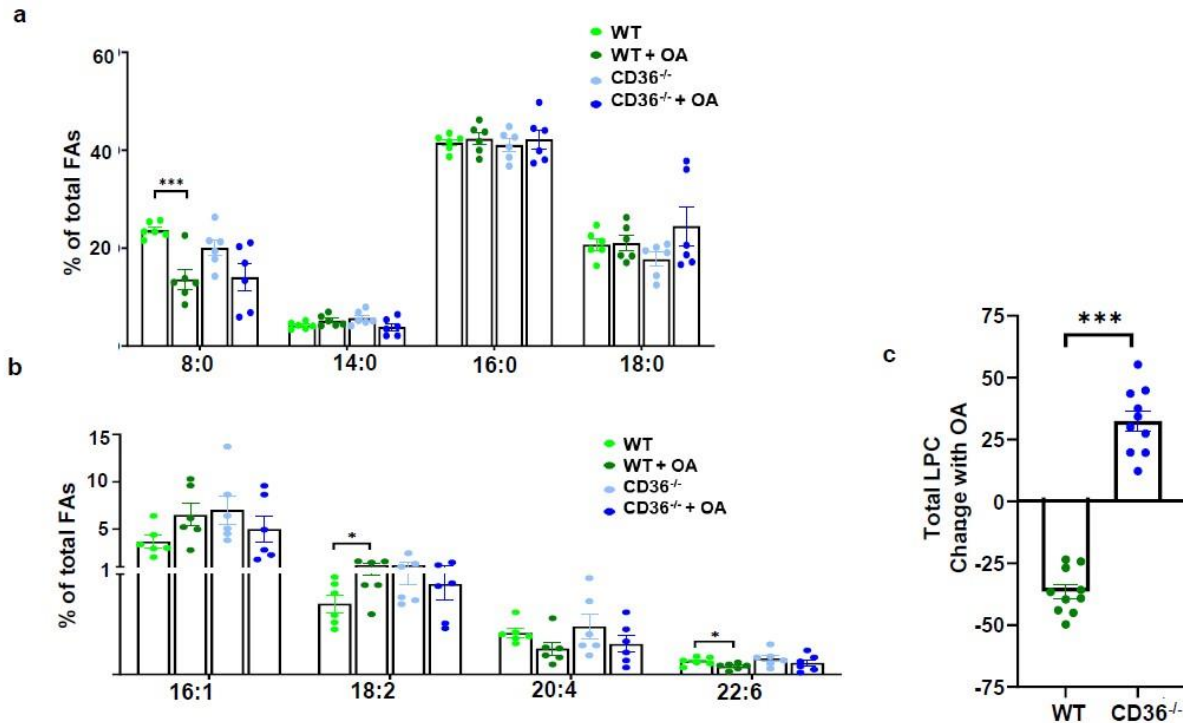


Supplemental Information.



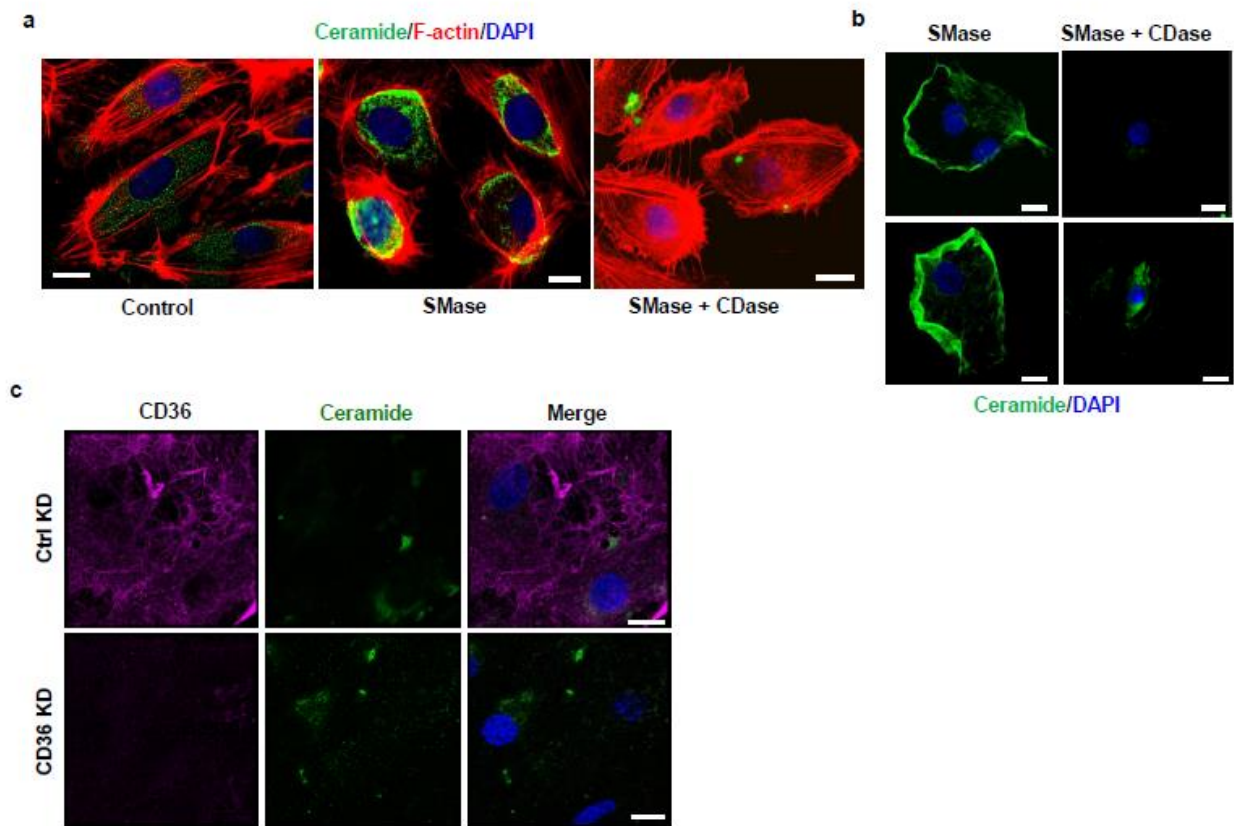
Supplement Figure 1. Electron microscopy of nanogold palmitic acid in hMECs

a. Nanogold PA, 10 μ M, 15min, added to hMECs is identified in multi-vesicular bodies (MVBs) inside the cells. Scale bar: 100nm. **b.** EM image showing nanogold PA vesicles being released at the basolateral membrane. Scale bar: 100nm. **c-d.** hMECs treated with alkyne OA, clicked with azide 555, stained for CD36 and CD63, and imaged by confocal microscopy. White arrows: sEVs positive for CD36, OA and CD63. Scale Bar: 10 μ m.



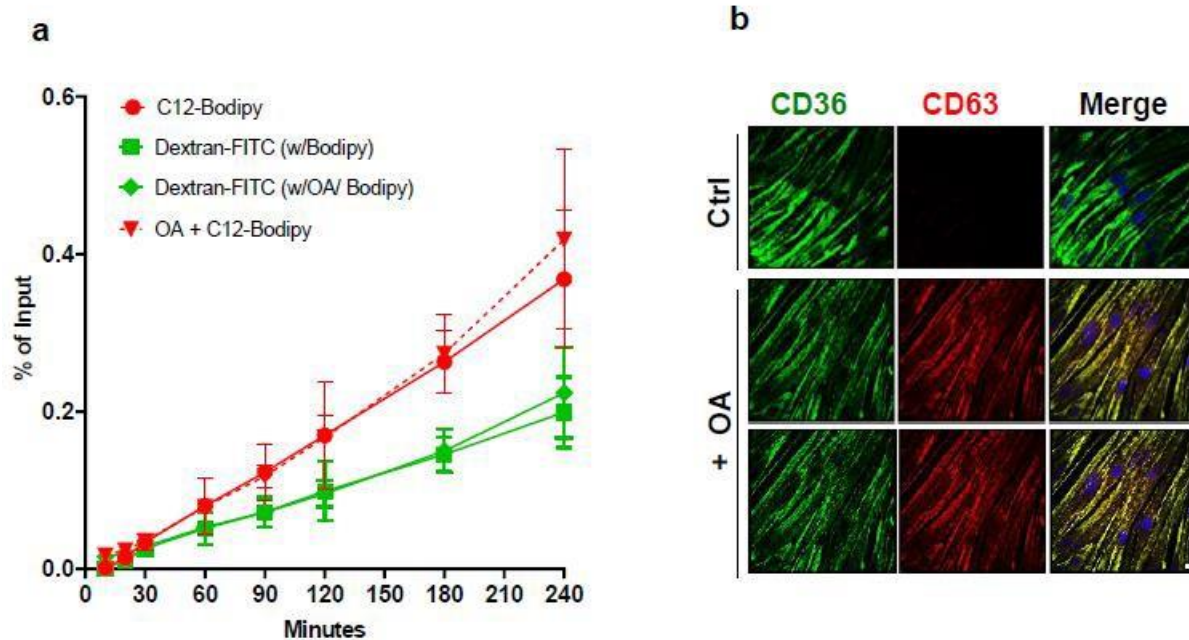
Supplement Fig. 2. Lipidomic analyses of sEV secreted by mMEC isolated from wildtype and *Cd36*^{-/-} mice with and without OA treatment.

a-b. Oleic acid treatment increases levels of free linoleic acid (FA, 18:2, n=6 independent preparations, *p<0.05 by one way ANOVA adjusted for multiple comparison, Data are are means +/- SEM) and decreases levels of free docosahexanoic acid (FA, 22:6, n=6 independent preparations, *p<0.05, one way ANOVA adjusted for multiple comparison, Data are presented as mean values +/- SEM) and caprylic acid (FA, 8:0, n=6 independent preparations, ***p<0.001, one way ANOVA adjusted for multiple comparison, Data are presented as mean values +/- SEM) in sEVs from WT mMECs. n=6 preparations. **c.** Total lysophosphatidylcholine levels (LPC) increased in mMEC sEVs from *Cd36*^{-/-} mMECs compared to wildtype mMECs. n= 10 independent preparations. *p <0.05, ***p<0.001 ny unpaired t test. Data are presented as mean values +/- SEM.



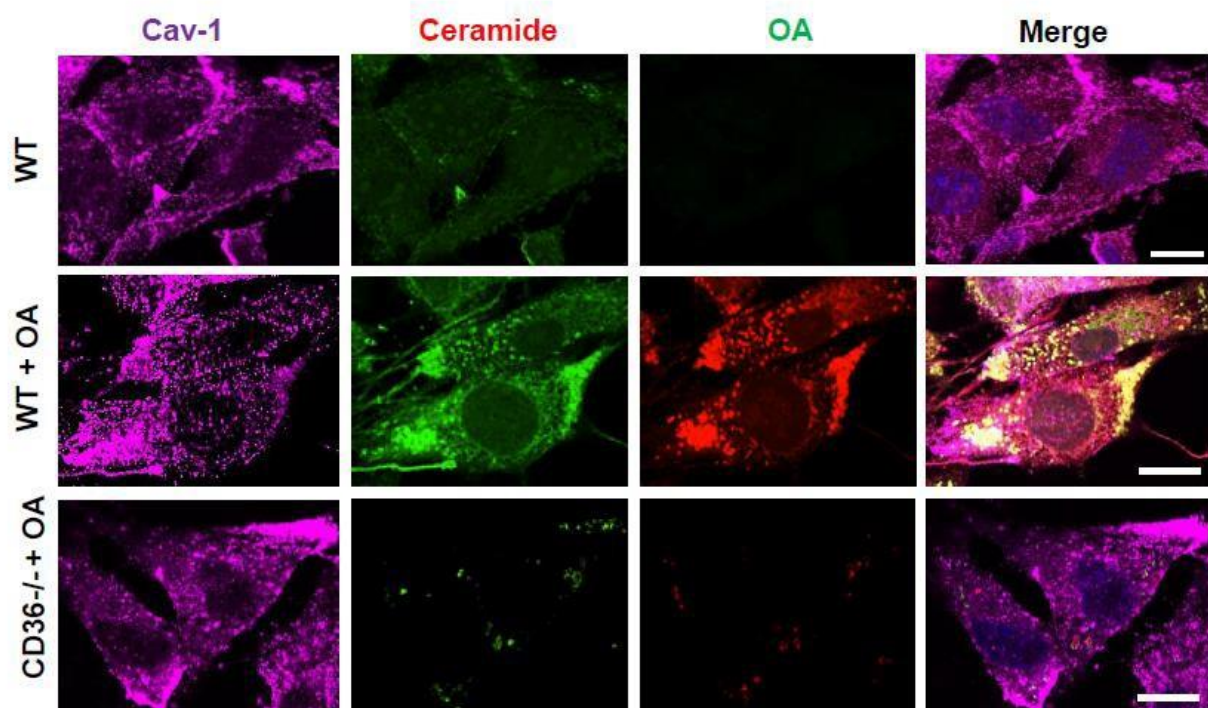
Supplement Fig. 3. Specificity of ceramide immunostaining.

a-b. hMEC were treated with bacterial Smase (25milliunits,10min) without or with subsequent treatment by ceramidase (CDase, 20milliunits,10min). Cells were fixed, permeabilized and processed for immunofluorescence. Mouse monoclonal ceramide antibody was used followed by goat anti-mouse antibody conjugated to Alexa Fluor 488 (green). F-actin and nuclei visualized with TRITC-Phalloidin and DAPI, respectively. Scale bar: 10 μ m. **c.** Low basal levels of ceramide in unstimulated hMECs (-OA alkyne). Nuclei were visualized with DAPI. Scale bar: 10 μ m. Representative of more than 3 preparations.



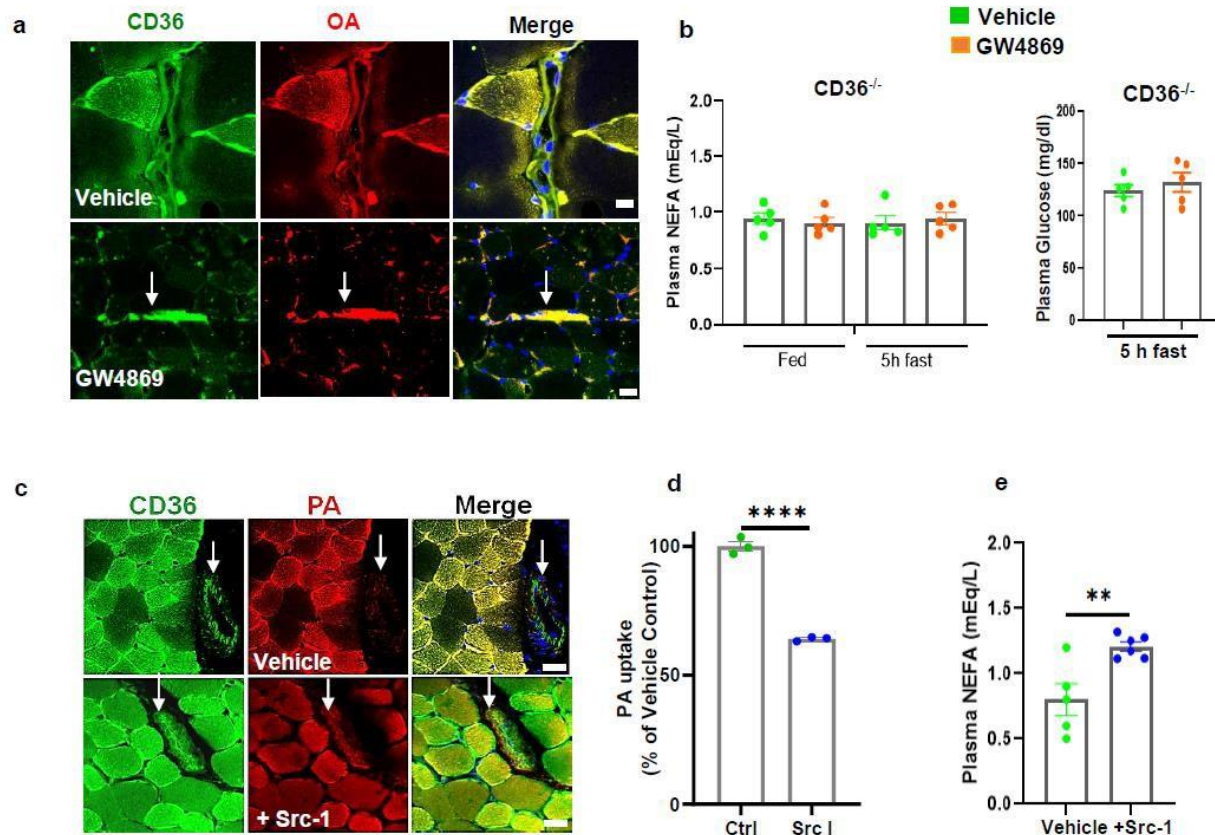
Supplement Fig. 4. Barrier integrity of hMECs grown on transwells.

a. FITC dextran (1mg/ml) or C12 Bodipy (20uM) were added to the upper chamber and fluorescence for both monitored in the lower chamber. Transfer of dextran or C12 Bodipy was calculated as percent of input to the upper chamber. Dextran transfer was low ~0.02% of input at 30 min and remained <0.2% at 240min. The % dextran transferred was unaffected by addition of oleic acid, OA: albumin, 200:40μM. n=3 independent experiments. Data are means +/- SEM. **b.** OA induces transfer of the exosome marker RFP-CD63 from hMEC to myotubes. The hMECs transduced with RFP-CD63 lentivirus were grown on transwell filters until 3 days post confluence. Then human myoblasts grown and differentiated into myotubes on coverslips were placed in the bottom wells. OA was added, OA-BSA:100μM:50μM (30min) then myotubes processed for CD36 immunostaining and confocal microscopy. Scale bar: 10μm.



Supplement Fig. 5. Oleic acid addition induces ceramide formation in primary mouse lung microvascular cells.

mMECs isolated from lungs of WT and *Cd36*^{-/-} mice were treated 10min with 15μM alkyne OA, fixed, clicked for visualizing OA then immunostained for Cav-1 and ceramide. Upper: mMEC controls, middle: OA-treated mMECs. lower: OA-treated *Cd36*^{-/-} mMECs. n= 3 preparations per genotype. Scale bar: 10μm



Supplement Fig. 6. Endothelial cells transfer FA to muscle via sEVs.

a. Blocking sEV secretion by GW4899 reduces muscle FA uptake in WT mice. The mice (n=3/group) were given intraperitoneally vehicle or GW4869 (2.5µg/g) prior to retro-orbital injection of alkyne OA, and euthanized 30min later. Skeletal muscles were fixed, clicked for OA and stained for CD36. Scale bar 20µm. **b.** GW4869 does not affect blood nonesterified FAs (NEFAs) or glucose in Cd36^{-/-} mice. Mice were tested, either fed (end of dark period) or after a 5h fast. NEFAs or glucose were measured in tail vein blood. NEFA and plasma glucose: n=5/group, representative of 2 cohorts. p=ns by two-way Anova adjusted for multiple comparison for NEFA, and unpaired t test for glucose. Data are means +/- SEM. **c.** Src inhibitor 1 reduces PA uptake by muscle fibers and associates

with more OA remaining in blood vessels. Wildtype mice were given vehicle (DMSO) or Src inhibitor-1 (2mg/kg intraperitoneally for 4 days) then were retro-orbitally injected with alkyne PA and euthanized 30min later. Skeletal muscle sections were processed for click reaction and immunostaining. Upper: Skeletal muscle from a vehicle treated mouse shows strong alkyne-PA uptake into fibers with no detectable PA in a nearby CD36-expressing vessel (arrows). Lower: Skeletal muscle from a Src-1 treated mouse showing reduced PA uptake into fibers and PA retention within a nearby CD36-expressing vessel (arrows). Scale bar 20µm. **d.** PA uptake quantified for data in c. n= 3 mice per condition, $p<0.0001$ by unpaired t test. **e.** *Src inhibition increases blood nonesterified FA (NEFA).* Plasma NEFA were measured in tail blood. n=5-6 mice/group, $**p<0.01$ by unpaired t test. Data are means +/- SEM.

Supplementary Table 1: Changes in expression of genes related to endocytosis, vesicular traffic, and cell structure remodeling in human microvascular endothelial cells with CD36 knockdown.

Gene Name	Gene details	KD/Ctrl fold change	p value
FSCN3	Fascin, actin-bundling protein 3	17.105	0.0000903
ITGAD	Integrin subunit alpha D	7.115	0.034874
ADCY8	Adenylate cyclase 8	5.447	0.042693
ITGA4	Integrin subunit alpha 4	3.411	0.006861
ADCY7	Adenylate cyclase 7	3.092	0.015564
CLDN14	Claudin 14, cell migration	2.253	0.021665
RAB36	RAB36, vesicular traffic	2.145	0.0000402
ADCY1	Adenylate cyclase 1	1.946	0.0263
PI3KCD	PI-4,5-bisphosphate 3-kinase catalytic delta	1.715	0.00072
PTP4A3	Protein tyrosine phosphatase type IVA, member 3	1.691	0.007661
ITGAV	Integrin subunit alpha V	1.668	0.000216
ITGB5	Integrin subunit beta 5	1.654	0.000818
EHD3	EH domain containing 3, endocytosis	1.564	0.004321
MLCK	Myosin light chain kinase, actin fibers organization	1.535	0.003951
VPS37B	VPS37B, ESCRT-I subunit, cargo sorting into MVBs	1.48	0.01177
PFN2	Profilin 2, actin polymerization	1.446	0.000669
DNM3	Dynamin 3, endocytosis, vesicular traffic	1.415	0.037675
CLDN12	Claudin 12, cell migration	1.395	0.00106
CORO7	Coronin 7, Actin binding, Golgi to endosome transport	1.368	0.014585
TFAM	Transcription factor A, mitochondrial, late endosomes/lysosomes	0.433	0.0000408
RAB3C	RAB3C, vesicle exocytosis	0.397	0.021952
FBN2	Fibrillin 2, elastic fiber assembly	0.362	0.018666

Supplementary Table 2: Pathway / Gene Set	Adjusted P-value
Regulation of Cyclin Dependent Protein S/T Kinase Activity (GO)	0.01
Regulation of Cyclin Dependent Protein Kinase Activity (GO)	0.01
Vesicle Tethering Complex (GO)	0.01
Focal Adhesion (KEGG)	0.02
Cell Projection Membrane (GO)	0.02
ERK1 and ERK2 Cascade (GO)	0.02
Arachidonic Acid Metabolism (KEGG)	0.03
Secretory Granule Membrane (GO)	0.03
Actin Cytoskeleton (GO)	0.03
Cortical Actin Cytoskeleton (GO)	0.03
Regulation of Actin Cytoskeleton (KEGG)	0.04
PI3K-Akt Signaling Pathway (KEGG)	0.04
GABAergic Synapse (KEGG)	0.04
ECM-receptor Interaction (KEGG)	0.04
Glutamergic Synapse (KEGG)	0.05
Endocytosis (KEGG)	0.07

Supplement Table 3: Primary antibodies

Antibody	Species	Source, Clone and Cat No	WB	IF
Mouse CD36	Goat	R&D Systems (#AF2519)	1:100	1:50
Human CD36	Goat	R&D Systems (#AF1955)	1:100	1:50
Caveolin 1	Rabbit	CST (#3238)	1:1000	1:100
pCaveolin1 ^{Y14}	Mouse	BD Biosciences (#611338)	1:1000	-
AKT	Rabbit	CST (pan) (C67E7) (#4691)	1:1000	-
pAKT ^{S473}	Rabbit	CST (D9E) (#4060)	1:1000	-
Actin	Mouse	SCBT (C4) sc-47778	1:1000	-
pERM	Rabbit	CST (#3141)	1:1000	-
Ceramide	Mouse	Sigma (#C8104)	-	1:100
Src	Rabbit	CST (36D10) (#2109)	1:1000	-
Cofilin	Mouse	Sigma (#SAB2702206)	1:1000	-
pCofilin ^{S3}	Rabbit	CST (77G2) (#3313)	1:1000	-
Rab7	Rabbit	CST (D95F2) (#9367)	1:1000	-
CD9	Rabbit	CST (E8L5J) (#98327)	1:1000	-
CD81	Mouse	SCBT(B-11) (#sc-166029)	1:1000	-
Calnexin	Rabbit	Enzo Life Sciences (#ADI-SPA-860)	1:1000	-
CD63, hybridoma	Mouse	DSHB	-	1:2

Supplement Table 4. Primer sequences

Primer	Sense	Antisense
CPT1a	GCAAAAATCAATCGGACTCTGGA	TCAGGGAGTAGCGCATGGT
FABP3	GCGGGAGCTAATTGATGGAA	CTCATAAGTGCGAGTGCAAAGT
ACSL3	AAAAGTGGGAGGAAGGTGGATAC	CTTTGGCCCCCAATAAGAATT
Glut1	CAGCAGCCCTAAGGATCTCA	CTACAGTCGGCTCGGCC
HK2	GAGTTTGACCTGGATGTGGTTGC	CCTCCATGTAGCAGGCATTGCT
eNOS	ATCTTCAGCCCCAAACGGAG	CTGGAACATCTTCCGCCTGT
VEGFR2	TGGTTGTGTATGTCCCACCC	TACCAGTGGATGTGATGCGG
PPAR δ	AGAGGAGGAGAAAGAGGAAGTG	ATGAACACCGTAGTGGAAGC
IRS1	AGTCTGTCGTCCAGTAGCACCA	ACTGGAGCCATACTCATCCGAG
36B4	GTGATGTGCAGCTGATCAAGACT	GATGACCAGCCCCAAAGGAGA