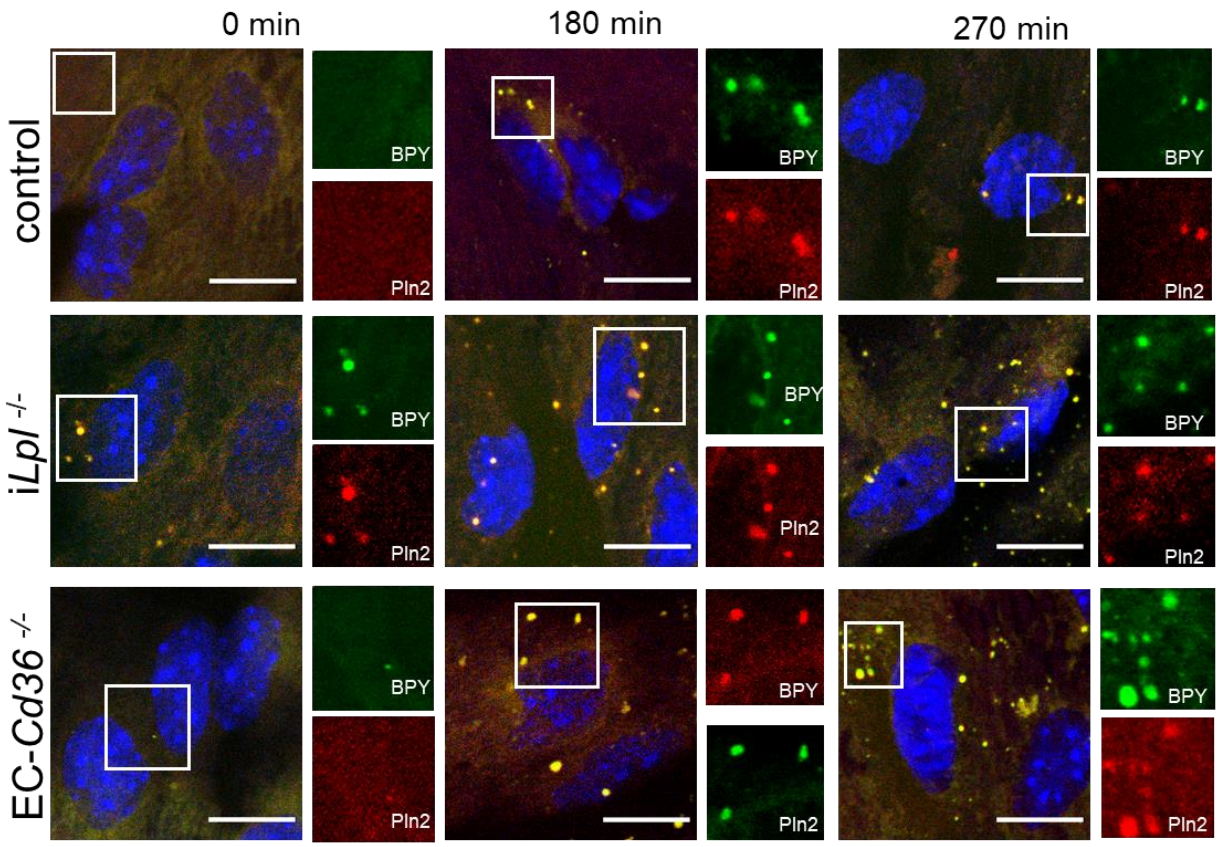
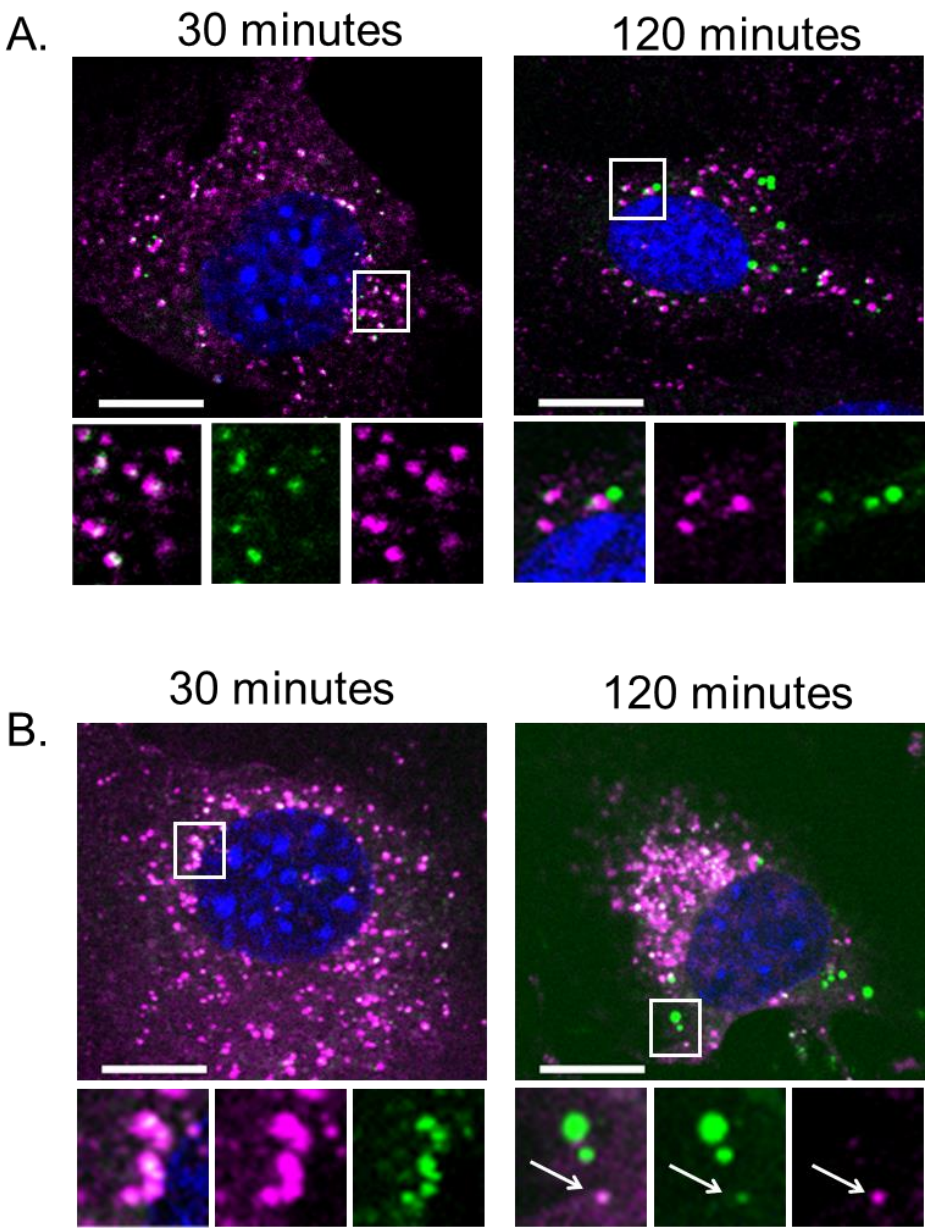
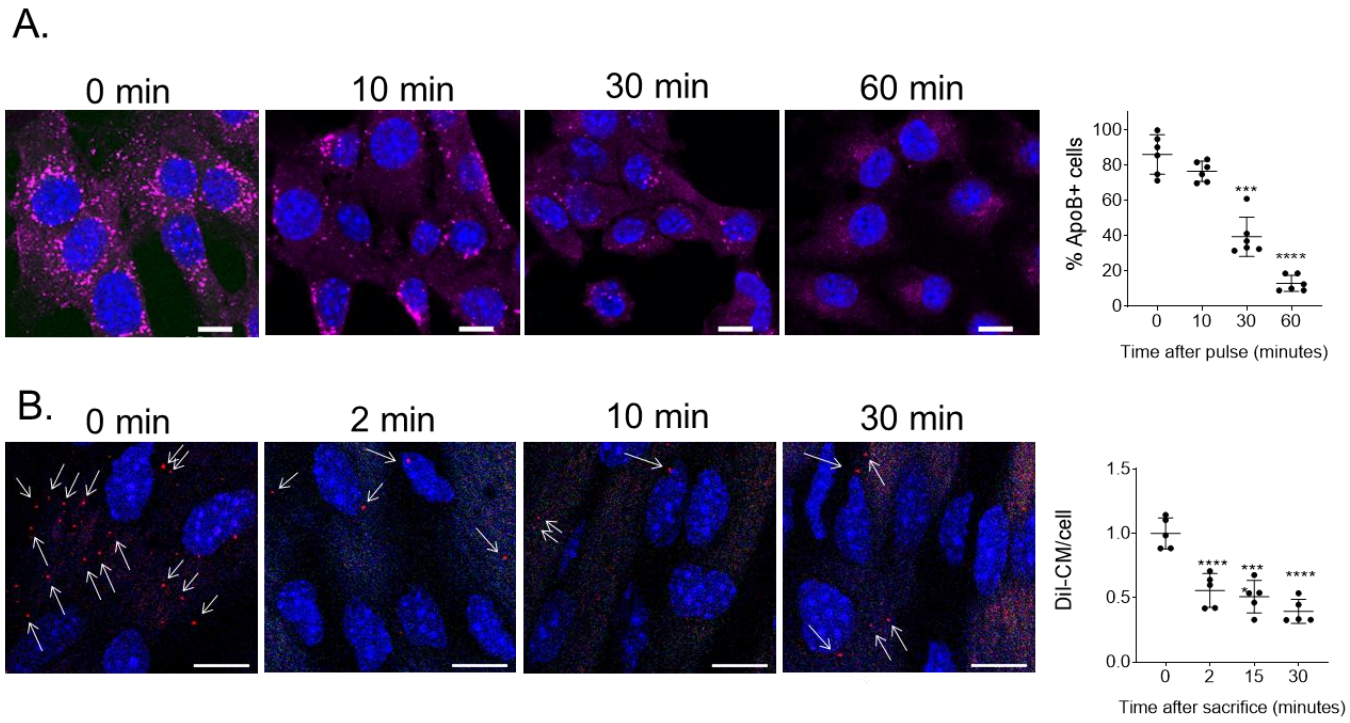


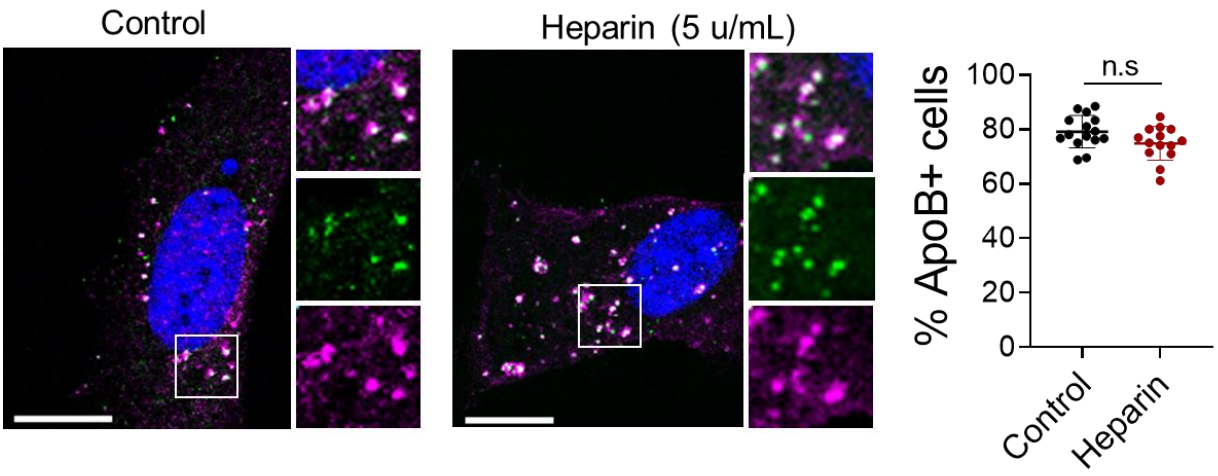
Supplementary Figure 1. 3-4 month old male control, iLpl-/- and EC-Cd36-/- mice were fasted for 16 hours and given olive oil by oral gavage (10ml/kg). At the indicated times, mice were sacrificed and their thoracic aortae were stained with BODIPY 493/503 (green) to label neutral lipids and immunostained for lipid droplet coat protein Perilipin 2 (Pln2, red). Co-localization of BODIPY 493/503 and Perilipin 2 in lipid droplets is shown in yellow. Scale bars = 10 µm. Additional inset magnification: x1.5 (top panels), x2 (middle and bottom panels).



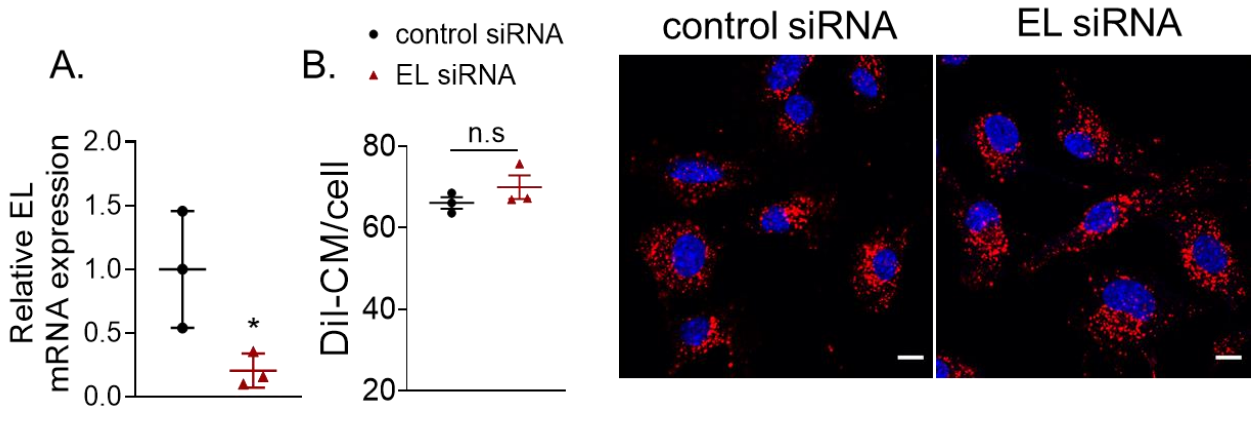
Supplementary Figure 2. ECs internalize chylomicrons from iLpl-/- mice and LpL-deficient humans: MECs were deprived of serum overnight and exposed to chylomicrons (4mg/dL TG) obtained from olive oil-gavaged iLpl-/- mice (A) or the plasma of an LpL-deficient patient (B) for 30 or 120 minutes. At 30 minute incubations, intracellular BODIPY493/503 puncta (green) fully co-localized with ApoB (magenta) (A and B, left panels). Co-localization at 120 minutes was partial, with only small BODIPY493/503 puncta co-localizing with ApoB (A and B, right panels). Scale bars = 10 µm. Additional inset magnification: x2.



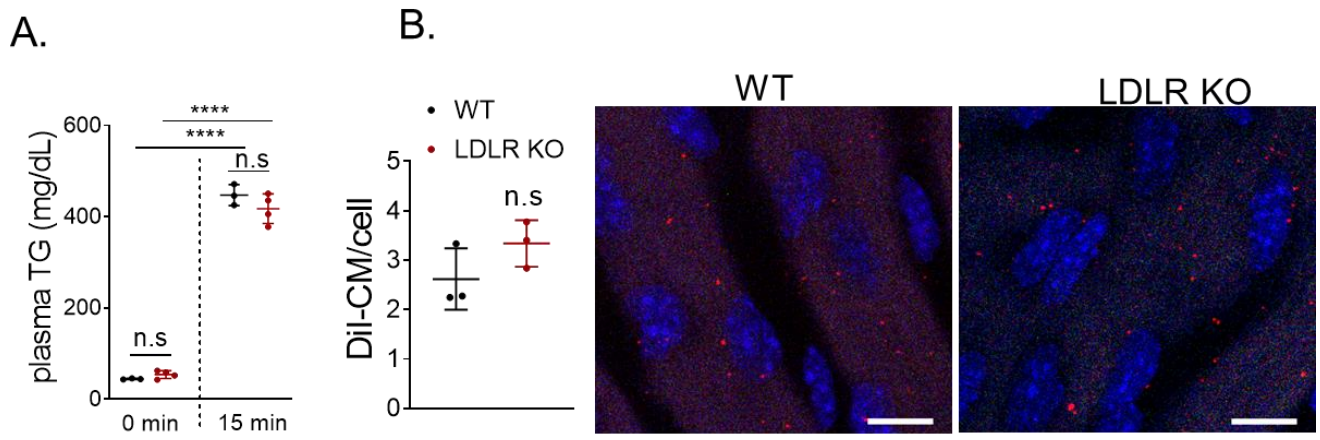
Supplementary Figure 3. **Intracellular chylomicron degradation in cultured MECs and aortic ECS.** (A) Cultured mouse ECs were deprived of serum overnight and exposed to human chylomicrons (4mg/dl in FBS-free medium) After a 30 minute pulse, cells were either fixed (0 min) or maintained in FBS-free medium for 10-60 minutes. The percentage of ApoB-positive cells at each time point is quantified in the graph (right panel). After a 30-minute pulse with chylomicrons, ≈ 80% cells exhibited intracellular ApoB puncta. When samples were switched to FBS-free medium, intracellular ApoB signal was gradually lost over time. Data represent the mean ± SD of 6 independent experiments. All comparisons are to 0 min after pulse. ***p<0.001, ****p<0.0001 (one-way ANOVA). (B) DiI-labeled chylomicrons (DiI-CM, red) were administered retro-orbitally to wild type mice (5 per group). Aortas were harvested 15 minutes post injection and either fixed (0 min) or maintained in FBS-free medium for 2-30 minutes. The number of DiI-labeled chylomicrons within aortic ECs rapidly declined over time, with a 50% reduction happening within 2 minutes (B, quantified in right panel). All comparisons are to 0 minutes. ****p<0.0001 (one-way ANOVA). White arrows indicate DiI-chylomicrons, Scale bars = 10 µm.



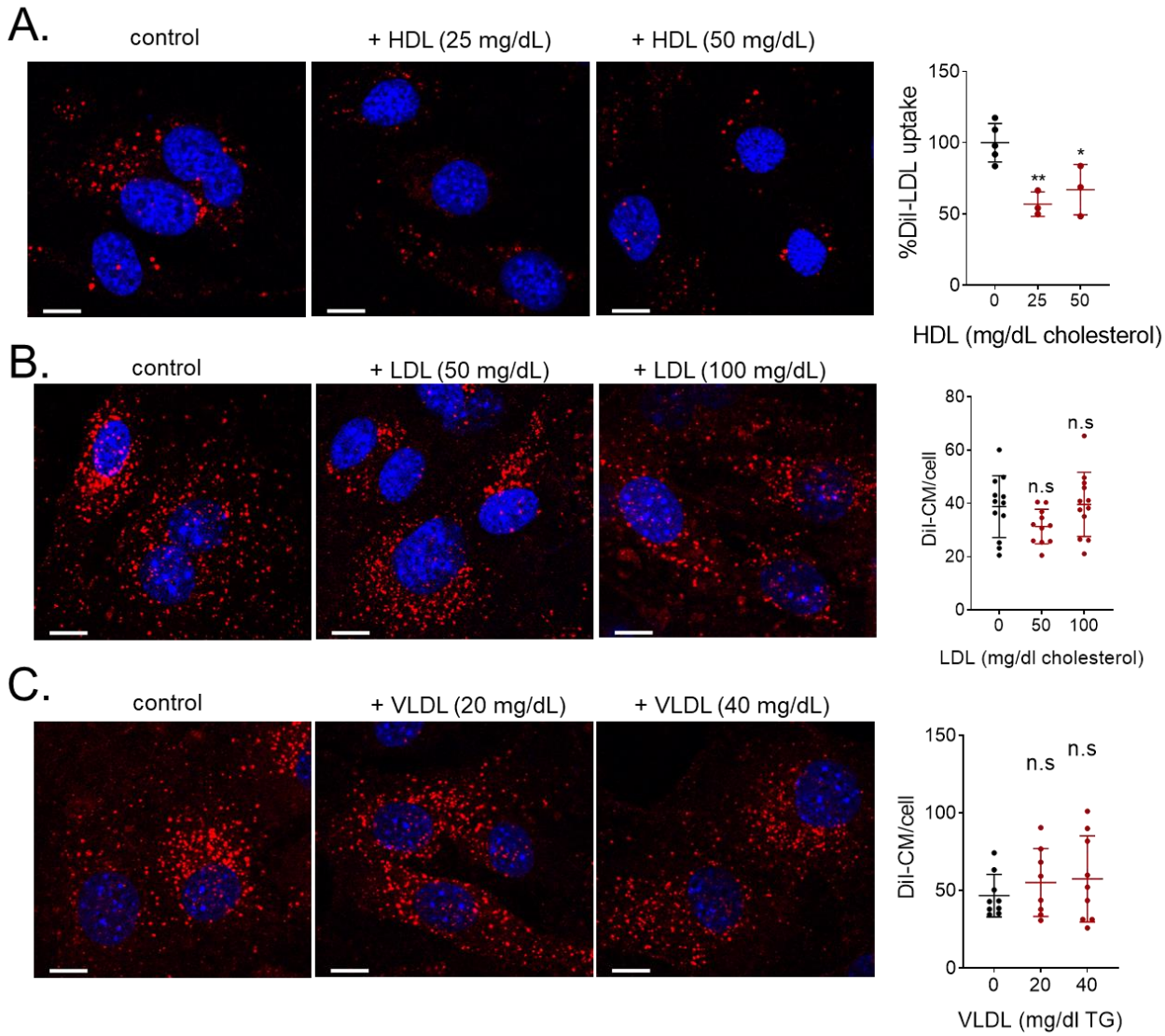
Supplementary Figure 4. Treatment with heparin to disrupt proteoglycan binding does not preclude EC chylomicron internalization. Cultured MECs were deprived of serum overnight and either left untreated (control) or pre-treated with 5 units/mL heparin for 1 hour. Cells were then exposed to a chylomicron pulse for 30 minutes (4 mg/dL TG), and internalized chylomicrons were assessed by the presence of ApoB /BODIPY 493/503-positive puncta. Treatment with heparin did not disrupt chylomicron internalization by MECs (quantification is shown in graph, right panel). Scale bars = 10 µm. Additional inset magnification: x2.



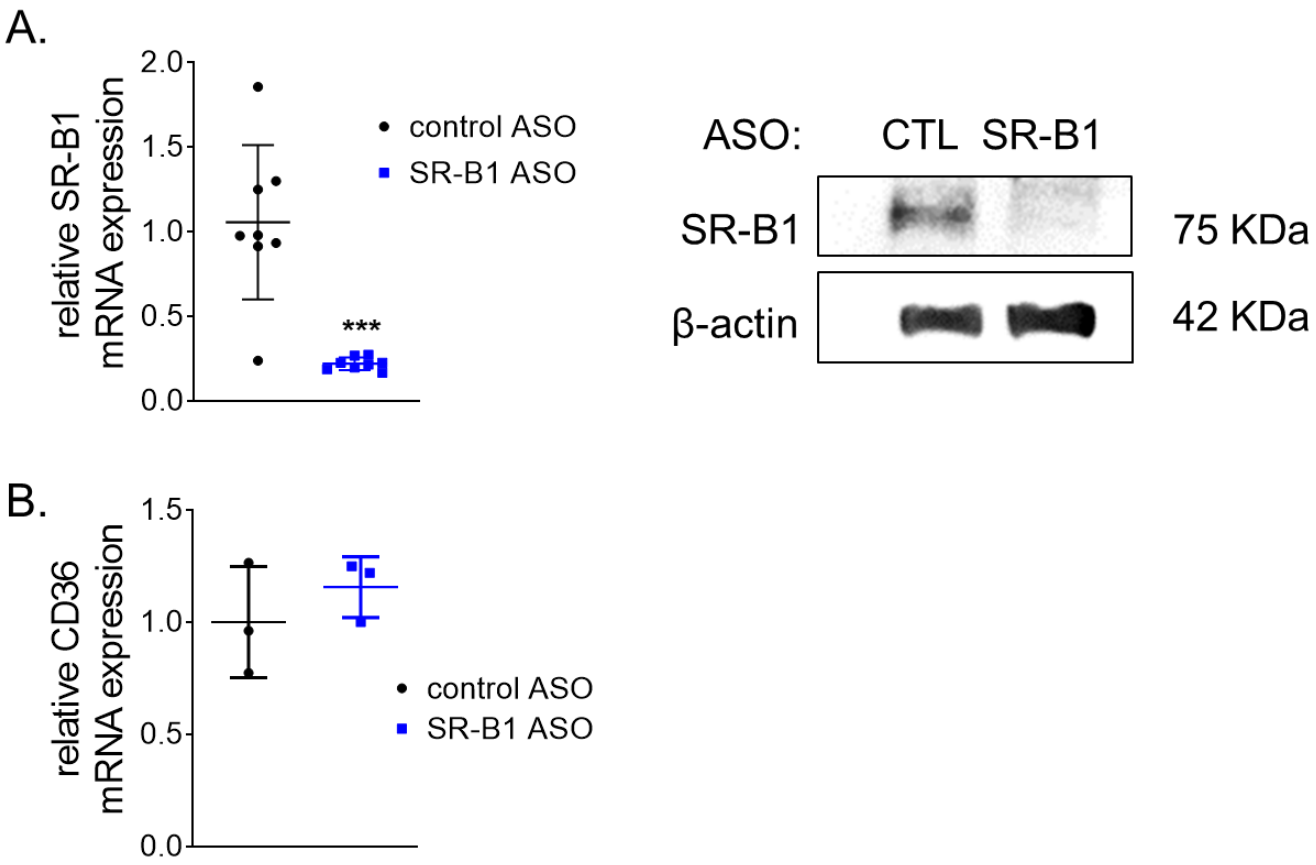
Supplementary Figure 5. Inhibition of endothelial lipase (EL) with siRNA does not preclude uptake of DiI-labeled chylomicrons by ECs. Cultured MECs were treated with control siRNA or siRNA against EL, deprived of serum overnight, and incubated with DiI-labeled chylomicrons (4mg/dL TG in FBS-free medium). EL siRNA significantly inhibited the expression of the lipase, as monitored by qRT-PCR (A), but had no significant defect on the uptake of DiI-labeled chylomicrons (B). Data represents the mean +/- SD of 3 independent experiments. *$p<0.05$ (Student's t test). Scale bars = 10 µm.



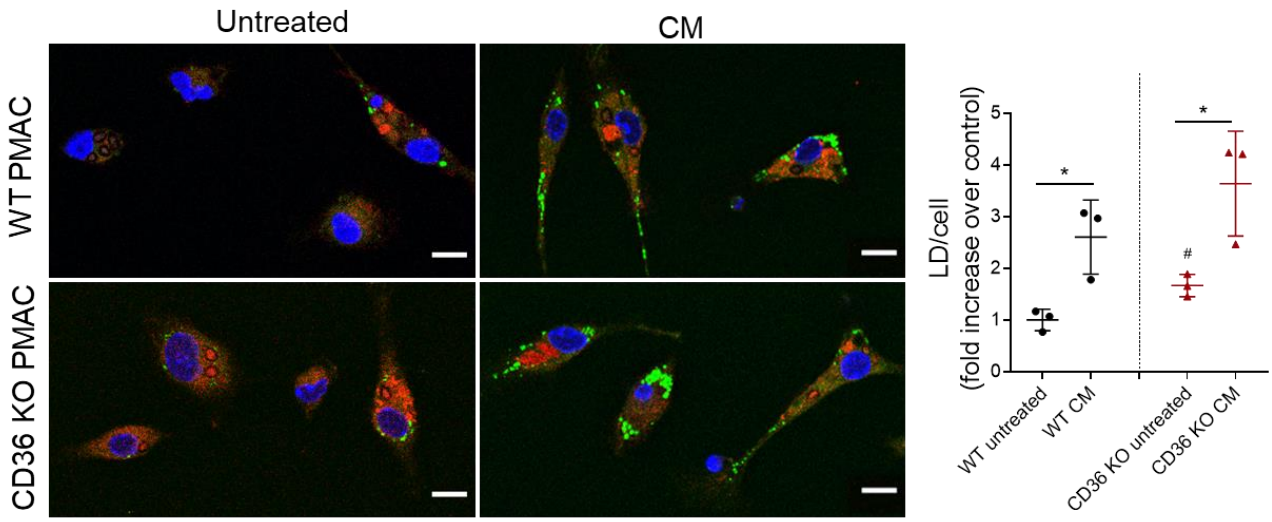
Supplementary Figure 6. Uptake of DiI-labeled chylomicrons by aortic ECs is not inhibited in LDLR-deficient mice. WT and LDLR KO mice (3-4 month old males) were fasted overnight, and DiI-chylomicrons (0.5 mg/g TG) were administered retro-orbitally. 15 minutes after DiI-chylomicron injection, mice were sacrificed and their thoracic aortas were harvested for confocal microscopy visualization of DiI-chylomicrons within aortic ECs. (A) Both WT and LDLR KO mice exhibited a significant increase in plasma TG levels 15 minutes after intravenous DiI-chylomicron administration. (B) LDLR deficiency did not decrease the number of DiI-chylomicrons (red) visible within aortic ECs. Data represent the mean ± SD of 3 independent experiments. ****p <0.00001 (one-way Anova, Tukey's multiple comparisons test). Scale bars = 10 µm.



Supplementary Figure 7. Competition studies. (A) DiI-labeled LDL uptake by cultured MECs is inhibited by co-incubation with unlabeled HDL. MECs were deprived of serum overnight, then pulsed with DiI-labeled LDL (DiI-LDL, red) in the absence (control) or presence of the indicated concentrations of unlabeled HDL. The number of DiI-LDL particles per cell is quantified in the graph (right panel). Data represent mean ± SD of 3 independent experiments. All comparisons are to control. *p<0.05, **p<0.01 (one-way Anova, Dunnet's multiple comparisons test). (B, C) MECs were deprived of serum overnight, then pulsed with DiI-labeled chylomicrons (DiI-CM, red) for 30 minutes and in the absence (control) or presence of the indicated concentrations of unlabeled LDL (B) or VLDL (C). The number of DiI-CM per cell is quantified in the graphs (right panels). Co-incubation with LDL or VLDL did not affect DiI-CM uptake in cultured MECs. Data represent mean ± SD of 6-8 independent experiments. All comparisons are to control (one-way Anova, Dunnet's multiple comparisons test). Scale bars = 10 μm.



Supplementary Figure 8. Knockdown of SR-B1 with antisense oligonucleotides (AS0). (A) Cultured MECs were treated with control ASO or ASO against SR-B1. SR-B1 ASO significantly reduced SR-B1 mRNA expression (left panel). SR-B1 knockdown was also apparent at the protein level, as assessed by immunoblotting (right panel, *unspecific band). Data represent mean ± SD of 4 independent experiments. ***p<0.001 (Student's t test). (B) Wild type mice (6 per group) were injected with either control or SR-B1 ASO (100 mg/kg) once a week for 3 weeks. 72 hours after the last injection, livers were harvested and CD36 expression was monitored by RT PCR. Despite their close molecular similarity, SR-B1 ASO did not reduce CD36 mRNA expression. Data represent mean ± SD (Student's t test).



Supplementary Figure 9. MECs were grown to confluency in transwell inserts and deprived of FBS for 24h. On the day of the experiment, MECs were either left untreated (control) or exposed to a 30-minute pulse with chylomicrons, thoroughly washed, and switched to fresh FBS-free medium. Inserts were then immediately placed into wells containing freshly harvested primary peritoneal macrophages (PMAC) obtained from wt or CD36 KO mice and co-cultured for 4h. PMACs were immunostained for macrophage marker CD68 (red), and LDs were labeled with BODIPY493/503 (green). PMAC from both wt and CD36 KO mice exhibited a significant increase in lipid droplet content when co-cultured with chylomicron-pulsed MECs as compared to control MECs maintained in FBS-free media. Data represent mean ± SD of 3 independent experiments.*$p<0.05$ (Student's t test). Scale bars = 10 µm.

Supplementary Table 1

MATERIALS

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Goat polyclonal to Apolipoprotein B	Abcam	ab7616
Rabbit Polyclonal to Perilipin-2/ADFP antibody	Novus Biologicals	NB110-40877
Rabbit Polyclonal to SR-B1 antibody	Novus Biologicals	NB400-104
Rabbit Polyclonal to VE Cadherin	Abcam	ab33168
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 568)	Abcam	ab175471
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)	Abcam	ab150077
Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 633	Invitrogen	A-21082
Peptides, siRNA and ASO		
SR-B1 ASO (GCTTCAGTCATGACTTCCTT)	Ionis Pharmaceuticals, Inc.	ISIS No: 353382
Human Recombinant ApoB18 peptide	Courtesy of Dr M. Mahmood Hussain (NYU Winthrop, Long Island), obtained as described (1).	N/A
ALK1 siRNA	Dharmacon	D-005302-06
Primary cells and cell lines		
Mouse cardiac microvascular endothelial cells	Angiocrine Bioscience	mVera-hrt-01
Human umbilical vein endothelial cells (HUVEC)	Yale University Vascular Biology and Therapeutics (VBT) Core facility	N/A
Mouse primary peritoneal macrophages (PMAC)	Obtained from Zymogen-treated WT C57BL/6 mice	N/A
Animal models		
Mouse: C57BL/6J	Jackson Laboratory	Stock No: 000664
Mouse: *Lpl*$^{fl/fl}$ and i*Lpl*$^{-/-}$	Generated in Ira Goldberg's lab as described (2).	N/A
Mouse: *Cd36*$^{fl/fl}$ and EC-*Cd36*$^{-/-}$	Generated in Ira Goldberg's lab as described (3).	N/A
Tissue culture media and supplements		
Advanced DMEM/F-12 (Dulbecco's Modified Eagle Medium/Ham's F-12)	ThermoFisher Scientific	12634028
Endothelial Cell Growth supplement, bovine hypothalamus, BT-203	ThermoFisher Scientific (Alfa Aesar)	J64516-MF
Recombinant Murine VEGF 165	ThermoFisher Scientific (Peprotech)	50-162-6093
Recombinant Human FGF-basic (154 a.a.)	ThermoFisher Scientific (Peprotech)	50-398-430
GlutaMAX™ Supplement	Gibco	35050061
SB 431542 (4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]benzamide)	Tocris	16-141-0
Gibco™ HEPES (1M)	Gibco	15-630-106
Heparin sodium salt from porcine intestinal mucosa	Sigma-Aldrich	H3149-50KU
Fetal Bovine Serum	Life Technologies	10437028
Antibiotic-Antimycotic (100X)	Life Technologies	15240-062

RPMI 1640, 1x with L glutamine	Corning	10-040-CV
Commercial lipoproteins, lipid dyes, and lipid quantification reagents		
Chylomicrons (ULDL) from Human Plasma	Lee Biosolutions	194-14-0.1
Low Density Lipoprotein from Human Plasma, DiI complex (DiI LDL)	Invitrogen	L3482
High Density Lipoprotein (HDL)	Lee Biosolutions	361-25-1
Very Low Density Lipoprotein (VLDL)	Lee Biosolutions	361-10-0.01
BODIPY™ 493/503	Invitrogen	D3922
DiI Stain (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate ('DiI'; DiIC18(3)))	Invitrogen	D282
Infinity Triglyceride Reagent	ThermoFisher Scientific	TR22421
NEFA HR (2) Color Reagent A	Wako Diagnostics	NC9517308
NEFA HR (2) Solvent A		NC9517309
NEFA HR (2) Color Reagent B		NC9517310
NEFA HR (2) Solvent B		NC9517311
Enzymes and pharmacological inhibitors		
Lipoprotein Lipase (LpL) from Bovine Milk	Sigma-Aldrich	L2254-1KU
Heparin sodium salt from porcine intestinal mucosa	Sigma-Aldrich	H3149-50KU
Atglistatin	Sigma-Aldrich	SML1075
Bafilomycin A1	Sigma-Aldrich	19-148
Dynasore hydrate	Sigma-Aldrich	D7693

Supplementary references

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2. Noh, H.-L., Okajima, K., Molkentin, J. D., Homma, S. & Goldberg, I. J. Acute lipoprotein lipase deletion in adult mice leads to dyslipidemia and cardiac dysfunction. Am. J. Physiol. Endocrinol. Metab. 291, E755-60 (2006).

3. Son, N. H. et al. Endothelial cell CD36 optimizes tissue fatty acid uptake. J. Clin. Invest. 128, 4329–4342 (2018).