Figure S1

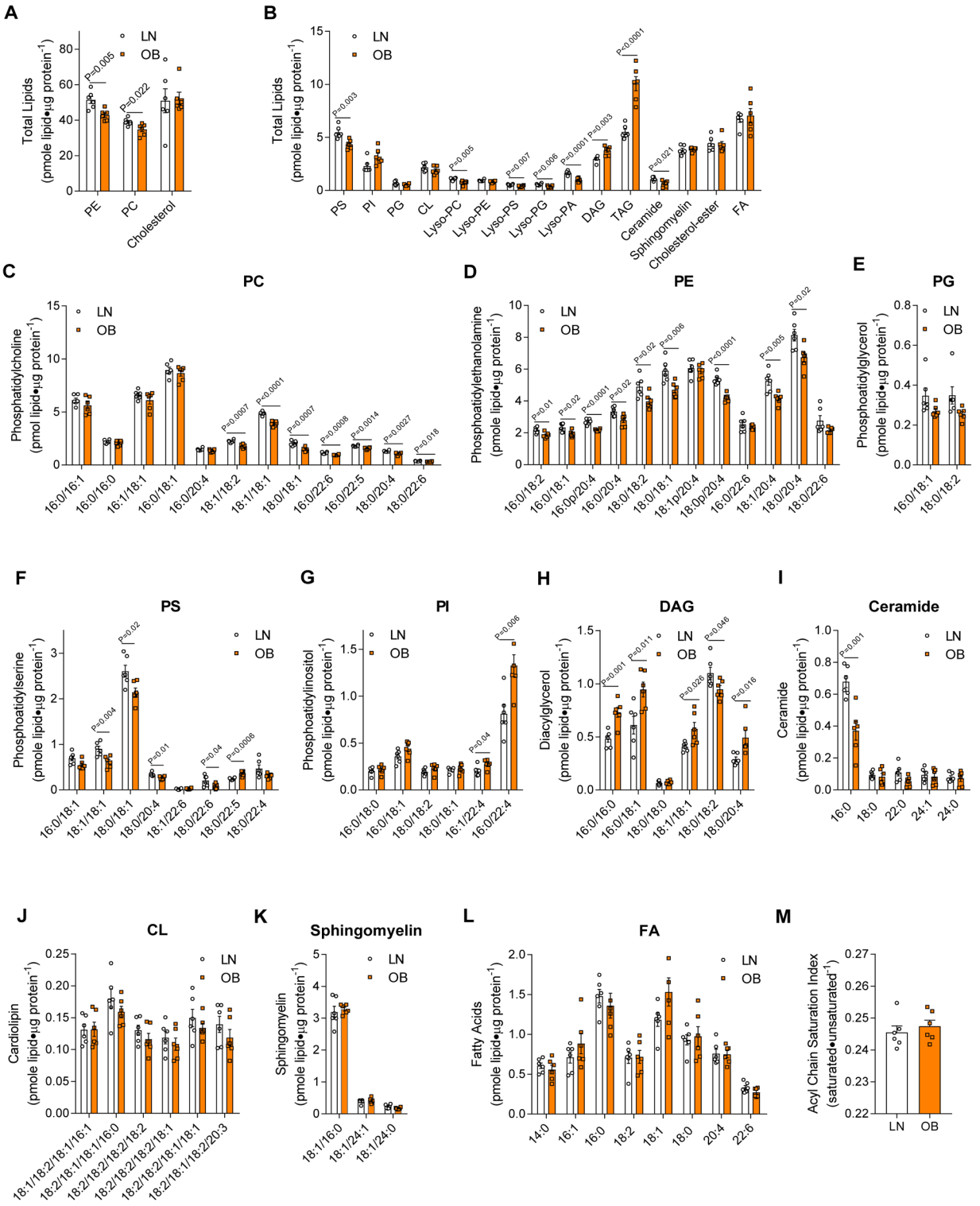

Figure S1: Lipid quantification in LN and OB HSkMC. (A-M) Muscle biopsies were taken from LN or OB human subjects and primary skeletal muscle cells were isolated and differentiated. Quantification of (A&B) total lipids by class, and species of (C) phosphatidylcholine (PC), (D) phosphatidylethanolamine (PE), (E) phosphatidylglycerol (PG), (F) phosphatidylserine (PS), (G) phosphatidylinositol (PI), (H) diacylglycerol (DAG), (I) ceramide, (J) cardiolipin (CL), (K) sphingomyelin, and (L) fatty acid (FA). (M) Quantification of the acyl chain saturation index of all detectable phospholipids. (n=6). Two-tailed t-tests were performed for all analyses. All data are represented as mean ± SEM.

Figure S2

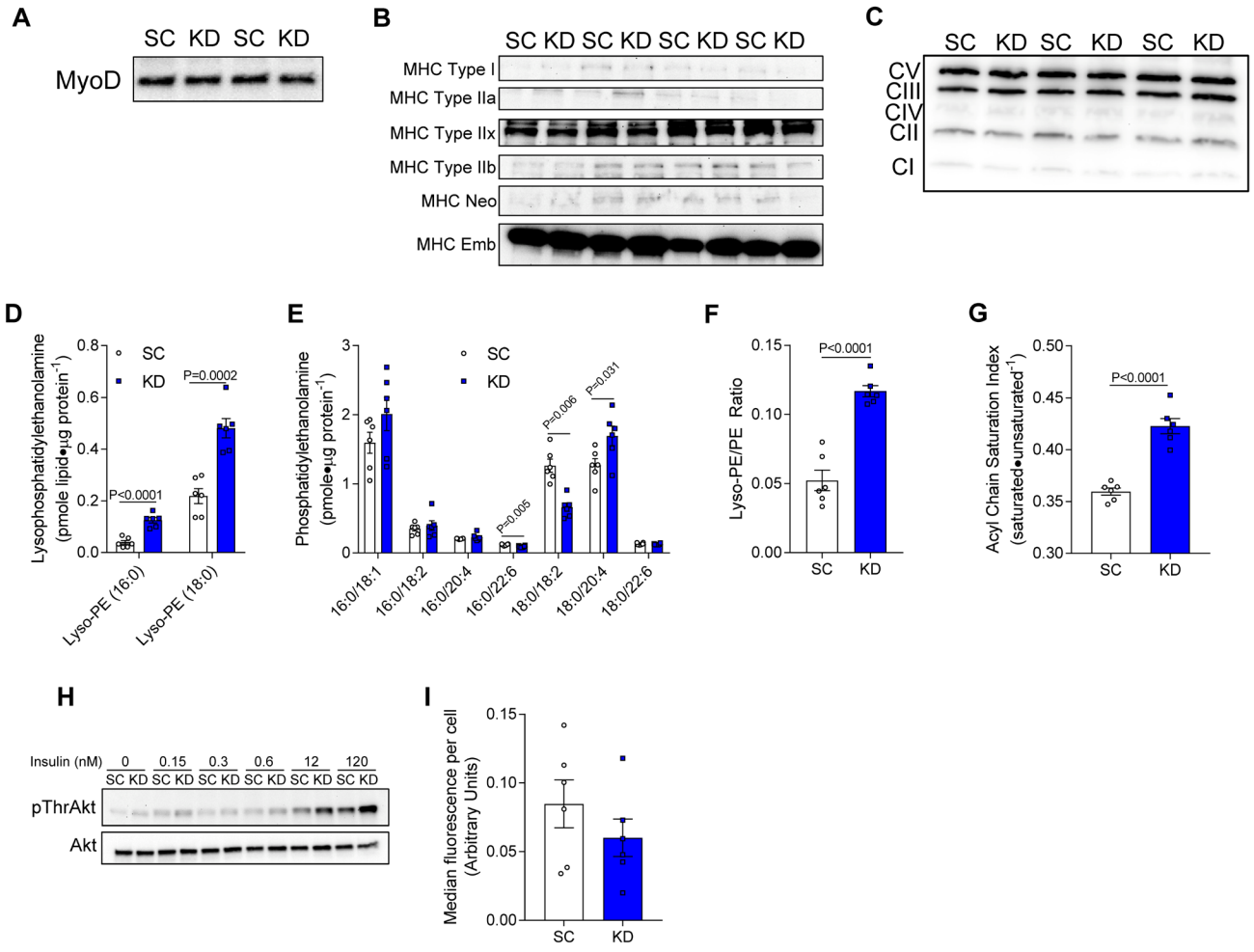

Figure S2: Knockdown of LPCAT3 in C2C12 myotubes. C2C12 myoblasts were infected with shRNA generating lentiviruses targeting scrambled (shScrambled; SC) or LPCAT3 (shLPCAT3; KD) to decrease LPCAT3 expression and cells were differentiated into myotubes. (A-C) Western blot in SC and KD cells probing for (A) MyoD, (B) myosin heavy chain isoforms, and (C) complexes I-V of the electron transport chain. (D-G) Lipids were extracted in SC and KD myotubes for quantification of (D) lyso-PE, (E) PE species, (F) total lyso-PE/PE, and (G) acyl chain saturation index of phospholipids (n=6). (H) Thr308 phosphorylation and total Akt from cells incubated (10 min) with various concentrations of insulin. (I) GM-1 microdomains were labeled with GFP and cross-linked to induce patching in SC and KD C2C12 myotubes. Total fluorescence in each cell was measured across 6 separate experiments (n=35-50/experiment) and the median of each experiment was used as a representative. Two-tailed t-tests were performed. All data are represented as mean ± SEM.

Figure S3

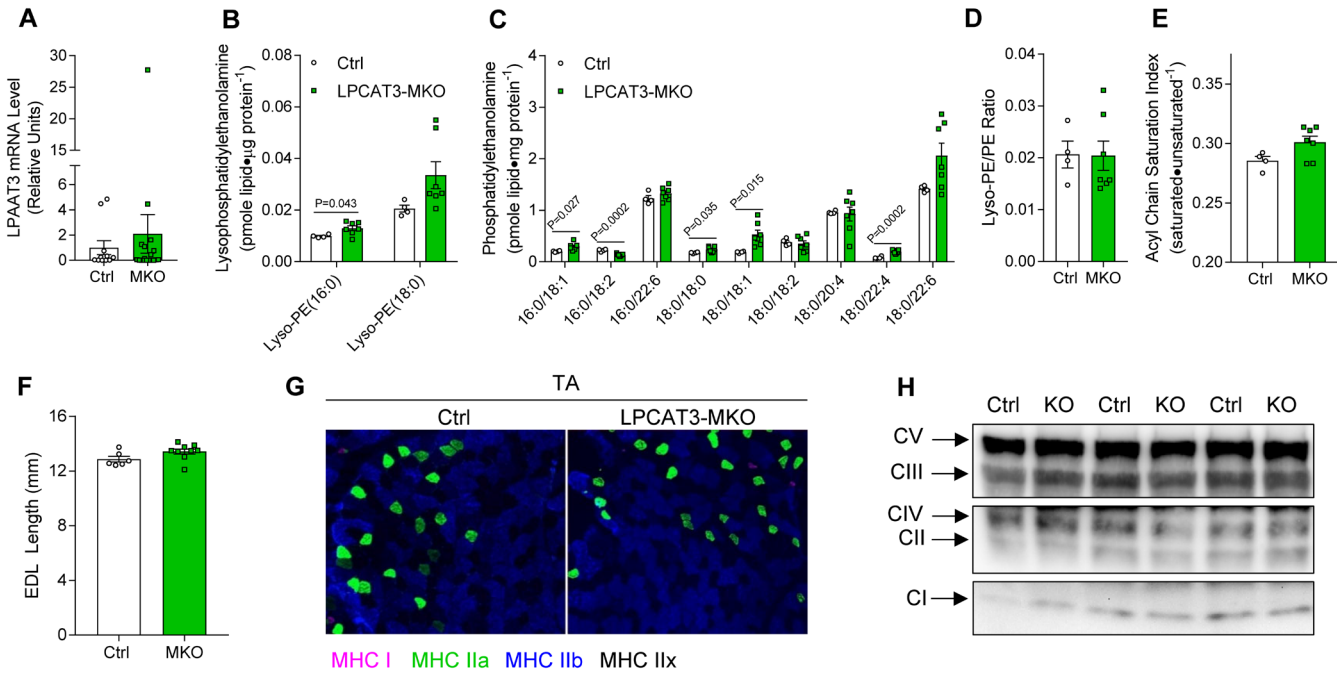

Figure S3: Additional data on muscles from HFD-fed Ctrl and LPCAT3-MKO mice. (A) Expression of LPAAT3 in TA muscle of control and LPCAT3-MKO mice (Ctrl n=11, MKO n=18). (B-E) Lipids were extracted from gastrocnemius muscles of Ctrl and LPCAT3-MKO mice for analysis. Quantification of (B) lyso-PE species, (C) PE species, (D) total lyso-PE/PE, and (E) phospholipid acyl chain saturation index (Ctrl n=4, MKO n=7). (F) Muscle lengths of extensor digitorum longus (EDL) muscles (Ctrl n=6, MKO n=9). (G) Skeletal muscle fiber-type (MHC I: pink, MHC IIa: green, MHC IIb:blue, and MHC IIx: negative) of tibialis anterior (TA) muscles. (H) Measurement of complexes I-V of the electron transport chain in TA muscles from Ctrl and LPCAT3-MKO mice. (A-F) Two-tailed t-tests. All data are represented as mean ± SEM.

Figure S4

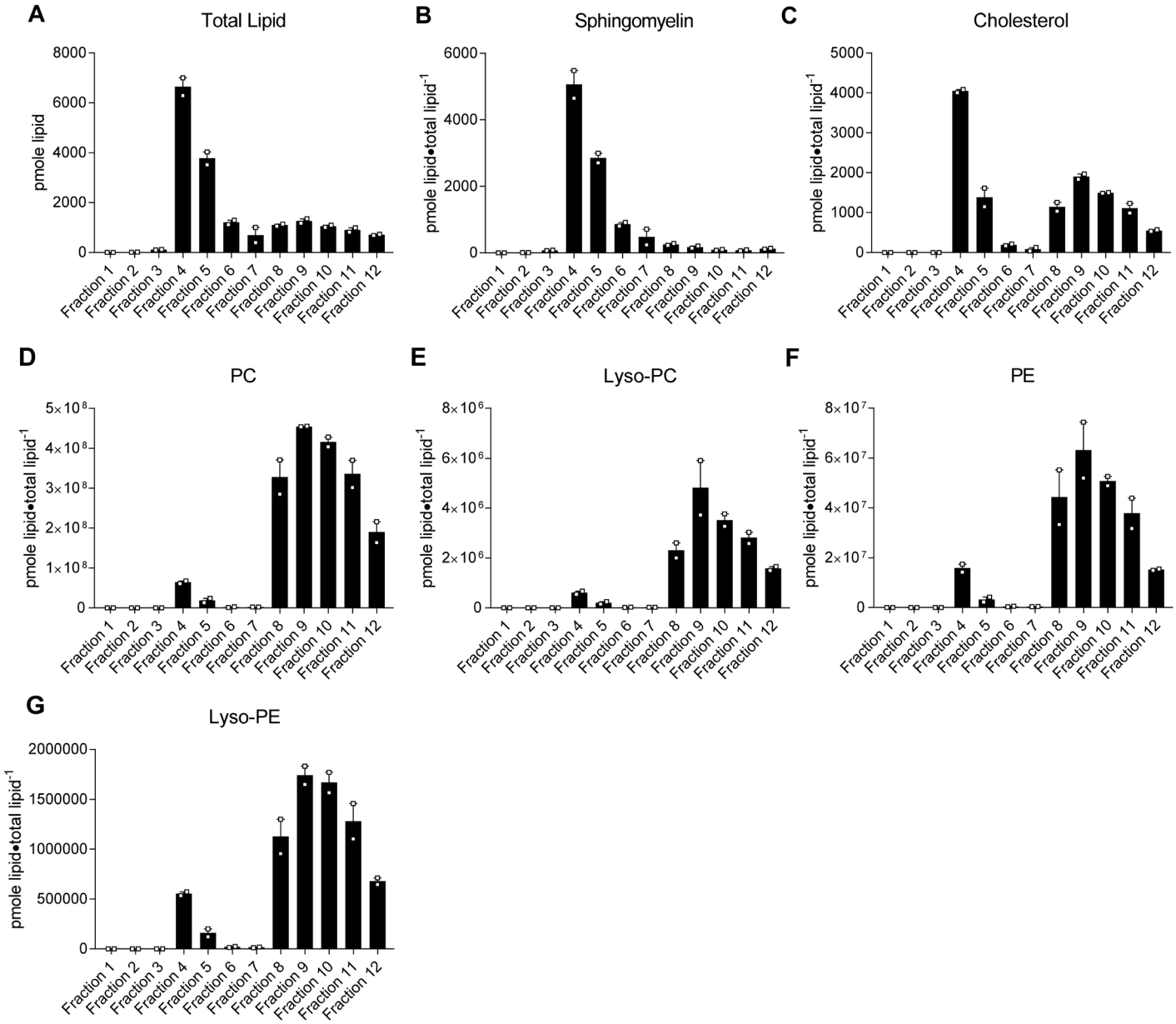

Figure S4: Lipid contents of detergent-resistant and detergent-soluble membrane fractions. (A-G) Wildtype C2C12 myotubes were suspended in a sucrose gradient and purified via ultracentrifugation to separate detergent-resistant membrane (DRM) fractions from detergent soluble membrane (DSM) fractions. After ultracentrifugation fractions were analyzed for (A) total lipid content, (B) sphingomyelin, (C) cholesterol, (D) PC, (E) lyso-PC, (F) PE, and (G) lyso-PE (n=2). All data are represented as mean ± SEM.

Figure S5

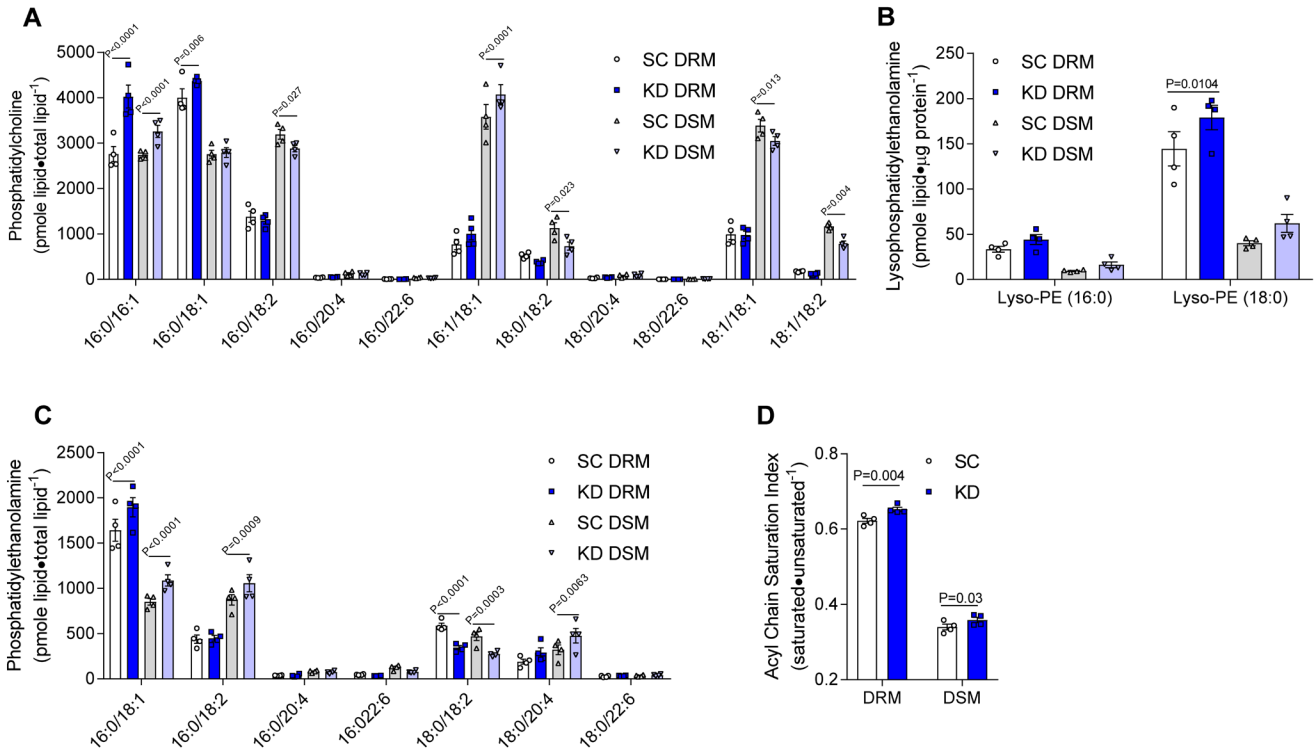

Figure S5: Plasma membrane microdomains and phospholipid composition in membrane fractions with LPCAT3 inhibition. (A-D) SC and KD C2C12 myotubes were suspended in a sucrose density gradient and purified with ultracentrifugation. (A) PC, (B) lyso-PE, (C) PE species, and (D) acyl chain saturation index of all phospholipids were quantified in DRM and DSM fractions (n=4). (D) Two-tailed t-tests or (A-C) 2-way ANOVA with Sidak's multiple comparisons test were performed. All data are represented as mean ± SEM.

Table S1: Characteristics of subjects that are insulin-sensitive and lean (LN) or insulin-resistant with obesity (OB) (*n*=6/group).

	LN	OB	p
Age (year)	30.2±3.5	35.8±3.1	0.25
Height (cm)	162.4±2.8	166.9±2.6	0.26
Weight (kg)	62.9±2.4	126.1±8.0	<0.0001
BMI ($kg \cdot m^{-2}$)	23.85±0.67	45.0±1.85	<0.0001
Glucose ($mg \cdot dL^{-1}$)	81.3±1.0	92.3±4.3	0.031
Insulin ($\mu U \cdot mL^{-1}$)	6.4±1.3	15.9±1.3	0.0004
HOMA-IR	1.29±0.27	3.6±0.44	0.001
Cholesterol ($mg \cdot dL^{-1}$)	177.7±13.0	177±12.9	0.97
HDL ($mg \cdot dL^{-1}$)	55.2±2.3	47.0±2.9	0.050
LDL ($mg \cdot dL^{-1}$)	105.2±11.9	109.5±8.6	0.78
Triglycerides ($mg \cdot dL^{-1}$)	86.8±19.8	102.7±23.7	0.62

Table S2: Primers used for quantitative-RT-PCR.

Gene	Species	F/R	Sequence (5'→3')
L32	Mouse	F	TTCCTGGTCCACAATGTCAA
		R	GGCTTTTCGGTTCTTAGAGGA
LPCAT1	Mouse	F	CACGAGCTGCGACTGAGC
		R	ATGAAAGCAGCGAACAGGAG
LPCAT2	Mouse	F	ACCTGTTTCCGATGTCCTGA
		R	CCAGGCCGATCACATACTCT
LPCAT3	Mouse	F	GGCCTCTCAATTGCTTATTTCA
		R	AGCACGACACATAGCAAGGA
LPCAT4	Mouse	F	GAGTTACACCTCTCCGGCCT
		R	GGCCAGAGGAGAAAGAGGAC
LPAAT3	Mouse	F	ACCTATACCGCCGTATCAACTGC
		R	AGTCGATCTCGAAGTTGTGGTTG
Caveolin3	Mouse	F	GGATCTGGAAGCTCGGATCAT
		R	TCCGCAATCACGTCTTCAAAAT
L32	Human	F	GTCAAGGAGCTGGAAGTGCT
		R	CTCTTTCCACGATGGCTTTG
LPCAT1	Human	F	CAGGCCAGCAGCATCAT
		R	TCAGCGCCCTGCAGAAG
LPCAT2	Human	F	TTGCCTGTTTCAGATGTCTTG
		R	GCCAGGCCAATCACATACTC
LPCAT3	Human	F	AGCCTTAACAAGTTGGCGAC
		R	TGCCGATAAAACAAAGCAAA
LPCAT4	Human	F	CCCTTCGTGCATGAGTTACA
		R	ATAAAGGCCAGAAGCACTCG