

Membrane editing with proximity labeling reveals regulators of lipid homeostasis

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Supplementary Information

Table of Contents:

Supplementary Information	1
Supplementary Table 1. Summary of Feeding–Fishing hits related to lipid biology.	2
Supplementary Table 2. List of plasmids used in this study.....	9
Supplementary Table 3. List of oligonucleotides used in this study.	11
Supplementary Table 4. List of phospholipid mass and retention time.....	13
Supplementary Methods for Proteomics Workflow	14
Sample collection and Protein quantitation	14
Protein digestion and TMT16-plex labeling	14
High pH reverse phase (hpRP) fractionation of each set.....	15
Nano-scale reverse phase chromatography and tandem MS (nanoLC-MS/MS).....	16
Data processing and protein identification	17
Data analysis	17
Acknowledgements.....	18
Supplementary References.....	18

Supplementary Table 1. Summary of Feeding–Fishing hits related to lipid biology.

Gene	Description	Fold Change	P-value
Proteins enriched or depleted on the PA-fed plasma membrane (PM)			
Lipid localization			
ABCD3	ATP-binding cassette sub-family D member 3 isoform a [Homo sapiens]	0.801	0.012805847
ATP8A1	phospholipid-transporting ATPase 1A isoform X1 [Homo sapiens]	0.77	0.007368127
ATP8B2	phospholipid-transporting ATPase 1D isoform c [Homo sapiens]	0.81	0.014526777
CPT2	carnitine O-palmitoyltransferase 2, mitochondrial isoform 1 precursor [Homo sapiens]	1.883	1.67E-14
GRAMD1A	protein Aster-A isoform X4 [Homo sapiens]	1.177	0.046327294
NPC1	NPC intracellular cholesterol transporter 1 isoform X1 [Homo sapiens]	0.823	0.031899203
PDZD8	PDZ domain-containing protein 8 [Homo sapiens]	1.375	9.51E-05
PITPNM1	membrane-associated phosphatidylinositol transfer protein 1 isoform X1 [Homo sapiens]	1.386	0.001091586
PLIN2	perilipin-2 isoform X1 [Homo sapiens]	0.575	5.33E-09
SCP2	sterol carrier protein 2 isoform 1 precursor [Homo sapiens]	1.589	2.55E-06
SOAT1	sterol O-acyltransferase 1 isoform X1 [Homo sapiens]	1.496	2.86E-07
SPG11	spatacsin isoform 1 [Homo sapiens]	0.753	0.000914334
STARD3	stAR-related lipid transfer protein 3 isoform X1 [Homo sapiens]	0.734	0.000658561
STOML2	stomatin-like protein 2, mitochondrial isoform a [Homo sapiens]	1.199	0.027112535
TEX2	testis-expressed protein 2 isoform X1 [Homo sapiens]	1.252	0.004169375
VAPA	vesicle-associated membrane protein-associated protein A isoform 1 [Homo sapiens]	1.179	0.036579349
VPS13B	vacuolar protein sorting-associated protein 13B isoform 5 [Homo sapiens]	1.289	0.009721483
Lipid metabolism			
ABCD3	ATP-binding cassette sub-family D member 3 isoform a [Homo sapiens]	0.801	0.012805847
ACBD5	acyl-CoA-binding domain-containing protein 5 isoform X1 [Homo sapiens]	1.207	0.024593678
ACOT7	cytosolic acyl coenzyme A thioester hydrolase isoform hBACHb [Homo sapiens]	1.296	0.001737959
ADIPOR1	adiponectin receptor protein 1 [Homo sapiens]	0.561	4.27E-12
APP	amyloid-beta precursor protein isoform a precursor [Homo sapiens]	0.837	0.049915393
CAT	catalase [Homo sapiens]	1.206	0.022574891

CPT2	carnitine O-palmitoyltransferase 2, mitochondrial isoform 1 precursor [Homo sapiens]	1.883	1.67E-14
CYP51A1	lanosterol 14-alpha demethylase isoform 1 precursor [Homo sapiens]	1.203	0.023351774
DGKD	diacylglycerol kinase delta isoform X1 [Homo sapiens]	0.696	5.47E-05
DGKH	diacylglycerol kinase eta isoform 2 [Homo sapiens]	1.372	0.001857363
DHCR24	delta(24)-sterol reductase precursor [Homo sapiens]	1.325	0.015612696
EFR3A	protein EFR3 homolog A isoform 3 [Homo sapiens]	0.794	0.012160279
FAM135A	protein FAM135A isoform e [Homo sapiens]	1.161	0.044854878
GPAT3	glycerol-3-phosphate acyltransferase 3 isoform X1 [Homo sapiens]	1.224	0.034094178
INPP5B	type II inositol 1,4,5-trisphosphate 5-phosphatase isoform 1 precursor [Homo sapiens]	1.269	0.012882272
INPP5F	phosphatidylinositide phosphatase SAC2 isoform 1 [Homo sapiens]	1.237	0.007775227
LIPA	lysosomal acid lipase/cholesteryl ester hydrolase isoform 1 precursor [Homo sapiens]	1.357	0.000259402
LPIN1	phosphatidate phosphatase LPIN1 isoform 3 [Homo sapiens]	1.765	1.12E-10
LPIN2	phosphatidate phosphatase LPIN2 isoform X2 [Homo sapiens]	1.997	8.19E-06
MTM1	myotubularin isoform X3 [Homo sapiens]	1.179	0.047218447
MTMR9	myotubularin-related protein 9 [Homo sapiens]	1.238	0.042681907
MTMR9	myotubularin-related protein 9 [Homo sapiens]	1.238	0.042681907
NEU3	sialidase-3 isoform a [Homo sapiens]	1.74	1.22E-11
NPC1	NPC intracellular cholesterol transporter 1 isoform X1 [Homo sapiens]	0.823	0.031899203
PIK3CB	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform isoform X1 [Homo sapiens]	1.228	0.011427941
PITPNM1	membrane-associated phosphatidylinositol transfer protein 1 isoform X1 [Homo sapiens]	1.386	0.001091586
PRKAA2	5'-AMP-activated protein kinase catalytic subunit alpha-2 [Homo sapiens]	1.759	1.18E-10
PRKAB1	5'-AMP-activated protein kinase subunit beta-1 isoform X1 [Homo sapiens]	1.567	8.53E-09
PRKAB2	5'-AMP-activated protein kinase subunit beta-2 [Homo sapiens]	1.68	4.32E-10
PRKAG1	5'-AMP-activated protein kinase subunit gamma-1 isoform 1 [Homo sapiens]	1.858	5.95E-14
PRKD1	serine/threonine-protein kinase D1 isoform 1 [Homo sapiens]	0.681	0.000653551
PRKD3	serine/threonine-protein kinase D3 [Homo sapiens]	0.666	5.95E-06
PTPMT1	phosphatidylglycerophosphatase and protein-tyrosine phosphatase 1 isoform 1 [Homo sapiens]	1.78	1.36E-08
SCP2	sterol carrier protein 2 isoform 1 precursor [Homo sapiens]	1.589	2.55E-06

SOAT1	sterol O-acyltransferase 1 isoform X1 [Homo sapiens]	1.496	2.86E-07
STARD3	stAR-related lipid transfer protein 3 isoform X1 [Homo sapiens]	0.734	0.000658561
TEX2	testis-expressed protein 2 isoform X1 [Homo sapiens]	1.252	0.004169375
VAPA	vesicle-associated membrane protein-associated protein A isoform 1 [Homo sapiens]	1.179	0.036579349
Regulation of lipid localization			
ATP8A1	phospholipid-transporting ATPase 1A isoform X1 [Homo sapiens]	0.77	0.007368127
PRKCD	protein kinase C delta type isoform a [Homo sapiens]	0.815	0.0277653
SCP2	sterol carrier protein 2 isoform 1 precursor [Homo sapiens]	1.589	2.55E-06
Regulation of lipid metabolism			
ADIPOR1	adiponectin receptor protein 1 [Homo sapiens]	0.561	4.27E-12
IDH1	isocitrate dehydrogenase [NADP] cytoplasmic [Homo sapiens]	1.247	0.041033899
LPIN1	phosphatidate phosphatase LPIN1 isoform 3 [Homo sapiens]	1.765	1.12E-10
LSR	lipolysis-stimulated lipoprotein receptor isoform 2 precursor [Homo sapiens]	0.788	0.011971172
MTMR9	myotubularin-related protein 9 [Homo sapiens]	1.238	0.042681907
NCOR1	nuclear receptor corepressor 1 isoform X1 [Homo sapiens]	0.764	0.037450675
PHB2	prohibitin-2 isoform 1 [Homo sapiens]	1.387	5.38E-05
PRKCD	protein kinase C delta type isoform a [Homo sapiens]	0.815	0.0277653
PRKD1	serine/threonine-protein kinase D1 isoform 1 [Homo sapiens]	0.681	0.000653551
SCP2	sterol carrier protein 2 isoform 1 precursor [Homo sapiens]	1.589	2.55E-06
STOML2	stomatin-like protein 2, mitochondrial isoform a [Homo sapiens]	1.199	0.027112535
Response to lipids			
ABL2	tyrosine-protein kinase ABL2 isoform b [Homo sapiens]	1.225	0.00841391
AKAP12	A-kinase anchor protein 12 isoform 1 [Homo sapiens]	1.936	1.33E-15
ARG1	arginase-1 isoform 1 [Homo sapiens]	1.435	0.00023827
CAT	catalase [Homo sapiens]	1.206	0.022574891
FKBP4	peptidyl-prolyl cis-trans isomerase FKBP4 [Homo sapiens]	1.263	0.005298457
GRAMD1A	protein Aster-A isoform X4 [Homo sapiens]	1.177	0.046327294
IDH1	isocitrate dehydrogenase [NADP] cytoplasmic [Homo sapiens]	1.247	0.041033899
NCOR1	nuclear receptor coactivator 4 isoform 1 [Homo sapiens]	0.657	1.30E-05
NPC1	NPC intracellular cholesterol transporter 1 isoform X1 [Homo sapiens]	0.823	0.031899203
PHB2	prohibitin-2 isoform 1 [Homo sapiens]	1.387	5.38E-05

PRKAA2	5'-AMP-activated protein kinase catalytic subunit alpha-2 [Homo sapiens]	1.759	1.18E-10
S100A7	protein S100-A7 [Homo sapiens]	1.808	3.45E-12
S100A9	protein S100-A9 [Homo sapiens]	1.358	0.002259506
SNW1	SNW domain-containing protein 1 isoform 2 [Homo sapiens]	0.825	0.036528865
TXNIP	thioredoxin-interacting protein isoform X1 [Homo sapiens]	0.68	1.69E-05
UBE2L3	ubiquitin-conjugating enzyme E2 L3 isoform 4 [Homo sapiens]	1.227	0.035283369
YAP1	transcriptional coactivator YAP1 isoform X1 [Homo sapiens]	1.305	0.00260548
Proteins enriched or depleted on the PA-fed lysosomes (Lyso)			
Lipid localization			
ABCD3	ATP-binding cassette sub-family D member 3 isoform a [Homo sapiens]	0.773	0.002840834
ATP8B2	phospholipid-transporting ATPase ID isoform c [Homo sapiens]	0.798	0.010892761
AUP1	lipid droplet-regulating VLDL assembly factor AUP1 [Homo sapiens]	0.811	0.015426767
LDAH	lipid droplet-associated hydrolase isoform X1 [Homo sapiens]	1.209	0.031554662
LIMA1	LIM domain and actin-binding protein 1 isoform 1 [Homo sapiens]	1.144	0.040899045
OSBPL1A	oxysterol-binding protein-related protein 1 isoform X1 [Homo sapiens]	1.774	5.77E-15
OSBPL3	oxysterol-binding protein-related protein 3 isoform X1 [Homo sapiens]	0.766	0.000674283
OSBPL6	oxysterol-binding protein-related protein 6 isoform X1 [Homo sapiens]	0.76	0.001384209
PDZD8	PDZ domain-containing protein 8 [Homo sapiens]	2.492	1.00E-17
PLIN2	perilipin-2 isoform X1 [Homo sapiens]	0.693	2.37E-05
PNPLA8	calcium-independent phospholipase A2-gamma isoform X1 [Homo sapiens]	1.347	0.010309324
SCARB1	scavenger receptor class B member 1 isoform 3 [Homo sapiens]	0.822	0.031950259
STX12	syntaxin-12 [Homo sapiens]	1.213	0.0120282
TEX2	testis-expressed protein 2 isoform X1 [Homo sapiens]	1.545	1.84E-08
TEX264	testis-expressed protein 264 isoform 1 [Homo sapiens]	1.344	0.014436466
VAPA	vesicle-associated membrane protein-associated protein A isoform 1 [Homo sapiens]	1.157	0.030518225
VPS13C	vacuolar protein sorting-associated protein 13C isoform 2A [Homo sapiens]	1.235	0.003168102
Lipid metabolism			
ABCD3	ATP-binding cassette sub-family D member 3 isoform a [Homo sapiens]	0.773	0.002840834

ACSL4	long-chain-fatty-acid--CoA ligase 4 isoform X2 [Homo sapiens]	1.159	0.032262249
ADIPOR1	adiponectin receptor protein 1 [Homo sapiens]	0.656	1.14E-07
BBS1	Bardet-Biedl syndrome 1 protein [Homo sapiens]	0.702	0.0025055
CAT	catalase [Homo sapiens]	1.244	0.022222953
DGKD	diacylglycerol kinase delta isoform X1 [Homo sapiens]	0.7	1.63E-05
EFR3A	protein EFR3 homolog A isoform 1 [Homo sapiens]	0.762	0.001566603
ERLIN2	erlin-2 isoform 1 [Homo sapiens]	0.82	0.026732363
FAM126B	protein FAM126B isoform 2 [Homo sapiens]	1.2	0.020748292
FDFT1	squalene synthase isoform 3 [Homo sapiens]	0.762	0.00453892
FDPS	farnesyl pyrophosphate synthase isoform X1 [Homo sapiens]	1.345	0.009377695
IDI1	isopentenyl-diphosphate Delta-isomerase 1 isoform a [Homo sapiens]	0.831	0.040652326
LDAH	lipid droplet-associated hydrolase isoform X1 [Homo sapiens]	1.209	0.031554662
LIMA1	LIM domain and actin-binding protein 1 isoform 1 [Homo sapiens]	1.144	0.040899045
LIPA	lysosomal acid lipase/cholesteryl ester hydrolase isoform 1 precursor [Homo sapiens]	1.301	0.002048992
LPIN1	phosphatidate phosphatase LPIN1 isoform 3 [Homo sapiens]	1.99	9.00E-09
LRP2	low-density lipoprotein receptor-related protein 2 precursor [Homo sapiens]	1.225	0.004045353
MTMR12	myotubularin-related protein 12 isoform 1 [Homo sapiens]	1.28	0.017342437
MTMR12	myotubularin-related protein 12 isoform 1 [Homo sapiens]	1.28	0.017342437
OSBPL1A	oxysterol-binding protein-related protein 1 isoform X1 [Homo sapiens]	1.774	5.77E-15
OSBPL3	oxysterol-binding protein-related protein 3 isoform X1 [Homo sapiens]	0.766	0.000674283
OSBPL6	oxysterol-binding protein-related protein 6 isoform X1 [Homo sapiens]	0.76	0.001384209
PI4K2A	phosphatidylinositol 4-kinase type 2-alpha [Homo sapiens]	1.209	0.038523203
PI4K2B	phosphatidylinositol 4-kinase type 2-beta [Homo sapiens]	1.213	0.008535005
PIKFYVE	1-phosphatidylinositol 3-phosphate 5-kinase isoform X1 [Homo sapiens]	1.262	0.001262648
PNPLA8	calcium-independent phospholipase A2-gamma isoform X1 [Homo sapiens]	1.347	0.010309324
PRKAA2	5'-AMP-activated protein kinase catalytic subunit alpha-2 [Homo sapiens]	1.447	1.95E-07
PRKAB1	5'-AMP-activated protein kinase subunit beta-1 isoform X1 [Homo sapiens]	1.319	0.000155093
PRKAB2	5'-AMP-activated protein kinase subunit beta-2 [Homo sapiens]	1.307	0.000593424

PRKAG1	5'-AMP-activated protein kinase subunit gamma-1 isoform 3 [Homo sapiens]	1.564	2.91E-09
PRKD1	serine/threonine-protein kinase D1 isoform 2 [Homo sapiens]	0.531	8.66E-15
PRKD2	serine/threonine-protein kinase D2 isoform A [Homo sapiens]	0.816	0.023160328
PRKD3	serine/threonine-protein kinase D3 [Homo sapiens]	0.633	3.54E-08
SC5D	lathosterol oxidase [Homo sapiens]	0.789	0.04447631
SCARB1	scavenger receptor class B member 1 isoform 3 [Homo sapiens]	0.822	0.031950259
SCPEP1	retinoid-inducible serine carboxypeptidase precursor [Homo sapiens]	1.304	0.024532044
SLC25A1	tricarboxylate transport protein, mitochondrial isoform b [Homo sapiens]	0.809	0.021552203
SNX17	sorting nexin-17 isoform 1 [Homo sapiens]	1.287	0.000513549
SREBF2	sterol regulatory element-binding protein 2 [Homo sapiens]	0.78	0.038221953
TEX2	testis-expressed protein 2 isoform X1 [Homo sapiens]	1.545	1.84E-08
TEX264	testis-expressed protein 264 isoform 1 [Homo sapiens]	1.344	0.014436466
VAPA	vesicle-associated membrane protein-associated protein A isoform 1 [Homo sapiens]	1.157	0.030518225
Regulation of lipid localization			
ACSL4	long-chain-fatty-acid--CoA ligase 4 isoform X2 [Homo sapiens]	1.159	0.032262249
AKT1	RAC-alpha serine/threonine-protein kinase [Homo sapiens]	0.694	0.001340709
ANXA2	annexin A2 isoform 1 [Homo sapiens]	1.181	0.01783528
IRS2	insulin receptor substrate 2 [Homo sapiens]	0.776	0.003955893
OSBPL6	oxysterol-binding protein-related protein 6 isoform X1 [Homo sapiens]	0.76	0.001384209
SREBF2	sterol regulatory element-binding protein 2 [Homo sapiens]	0.78	0.038221953
Regulation of lipid metabolism			
ADIPOR1	adiponectin receptor protein 1 [Homo sapiens]	0.656	1.14E-07
AKT1	RAC-alpha serine/threonine-protein kinase [Homo sapiens]	0.694	0.001340709
APPL2	DCC-interacting protein 13-beta isoform 2 [Homo sapiens]	1.324	0.000226293
ERLIN2	erlin-2 isoform 1 [Homo sapiens]	0.82	0.026732363
IRS2	insulin receptor substrate 2 [Homo sapiens]	0.776	0.003955893
LPIN1	phosphatidate phosphatase LPIN1 isoform 3 [Homo sapiens]	1.99	9.00E-09
LSR	lipolysis-stimulated lipoprotein receptor isoform X1 [Homo sapiens]	0.768	0.008023682
PRKCE	protein kinase C epsilon type isoform X1 [Homo sapiens]	0.515	2.22E-16

PRKD1	serine/threonine-protein kinase D1 isoform 2 [Homo sapiens]	0.531	8.66E-15
SCARB1	scavenger receptor class B member 1 isoform 3 [Homo sapiens]	0.822	0.031950259
SREBF2	sterol regulatory element-binding protein 2 [Homo sapiens]	0.78	0.038221953
Response to lipids			
AKAP12	A-kinase anchor protein 12 isoform 1 [Homo sapiens]	1.462	3.74E-07
PRKCE	protein kinase C epsilon type isoform X1 [Homo sapiens]	0.515	2.22E-16
ADAM9	disintegrin and metalloproteinase domain-containing protein 9 isoform X1 [Homo sapiens]	1.227	0.005335348
CCNA2	cyclin-A2 [Homo sapiens]	0.741	0.025855108
RHOA	transforming protein RhoA isoform 1 precursor [Homo sapiens]	1.161	0.046853363
PPP5C	serine/threonine-protein phosphatase 5 isoform X1 [Homo sapiens]	1.158	0.040302963
SCARB1	scavenger receptor class B member 1 isoform 3 [Homo sapiens]	0.822	0.031950259
S100A8	protein S100-A8 isoform a [Homo sapiens]	1.319	0.001062323
YAP1	transcriptional coactivator YAP1 isoform X1 [Homo sapiens]	1.214	0.011647086
AKT1	RAC-alpha serine/threonine-protein kinase [Homo sapiens]	0.694	0.001340709
PRKAA2	5'-AMP-activated protein kinase catalytic subunit alpha-2 [Homo sapiens]	1.447	1.95E-07
UBE2L3	ubiquitin-conjugating enzyme E2 L3 isoform 4 [Homo sapiens]	1.244	0.009388023
FOXO1	forkhead box protein O1 isoform X2 [Homo sapiens]	1.353	0.009323432
CAT	catalase [Homo sapiens]	1.244	0.022222953
ARG1	arginase-1 isoform 1 [Homo sapiens]	1.295	0.000455554
NEDD4L	E3 ubiquitin-protein ligase NEDD4-like isoform X11 [Homo sapiens]	1.237	0.006149986

Supplementary Table 2. List of plasmids used in this study.

	Name	Source	Notes
1	PITPNM1	DNASU: HsCD00870184	Nir2-encoding gene used as a template for cloning
2	SCP2	DNASU: HsCD00871173	SCP2-encoding gene used as a template for cloning
3	PDZD8	DNASU: HsCD00438812	PDZD8-encoding gene used as a template for cloning
4	TEX2	DNASU: HsCD00351688	TEX2-encoding gene used as a template for cloning
5	OSBPL1A	DNASU: HsCD00877998	ORP1L-encoding gene used as a template for cloning
6	pEGFP-Spo20	Vitale Lab (U Penn)	PA-binding probe used as a template for cloning
7	pEGFP-PASS	Addgene: 193970	PA-binding probe used as a template for cloning
8	pCDNA-CRY2-mCherry-PLD ^{dead} -P2A-CIBN-Sac1	Tei and Baskin. 2020	For transient expression of ER-targeted deadPLD
9	pCDNA-CRY2-mCherry-PLD ^{dead} -P2A-CIBN-CAAX	Tei and Baskin. 2020	For transient expression of plasma membrane-targeted deadPLD
10	pCDNA-CRY2-mCherry-PLD ^{x30} -P2A-CIBN-CAAX	Tei et al. 2023	For transient expression of plasma membrane-targeted superPLD
11	pCDNA-p18-CIBN-P2A-CRY2-mCherry-PLD ^{dead}	Tei et al. 2023	For transient expression of lysosome-targeted deadPLD
12	pCDNA-p18-CIBN-P2A-CRY2-mCherry-PLD ^{x30}	Tei et al. 2023	For transient expression of lysosome-targeted superPLD
13	pCDNA-C1(1-29)-TurboID-V5	Addgene: 107173	For transient expression of ER-targeted TurboID
14	pCDNA-TurboID-V5-CAAX	This study	For transient expression of plasma membrane-targeted TurboID
15	pCDNA-p18-TurboID-V5	This study	For transient expression of lysosome-targeted TurboID
16	pCDNA-TMEM192-TurboID-V5	This study	For transient expression of lysosome-targeted TurboID candidate
17	pCDNA-miRFP-Nir2	This study	For transient expression of miRFP-Nir2
18	pCDNA-miRFP-Nir2(T59A)	This study	For transient expression of miRFP-Nir2 with T59A mutation
19	pEGFP-ORP1L	This study	For transient expression of EGFP-ORP1L
20	pCDNA-PDZD8-EGFP	This study	For transient expression of PDZD8-EGFP
21	pCDNA-TEX2-EGFP	This study	For transient expression of TEX2-EGFP
22	pCDH-CRY2-mCherry-PLD	Tei et al. 2023	For lentiviral expression of optoPLD

23	pCDH-CRY2-mCherry-deadPLD	Tei et al. 2023	For lentiviral expression of catalytically-dead optoPLD
24	pCDH-CRY2-mCherry-superPLD ^{x30}	Tei et al. 2023	For lentiviral expression of a superPLD
25	pCDH-CIBN-CAAX	Tei et al. 2023	For plasma membrane-recruitment of optoPLD
26	pCDH-p18-CIBN	Tei et al. 2023	For lysosomal recruitment of optoPLD
27	pCDH-TurboID-V5-CAAX	This study	For lentiviral expression of PM-TurboID
28	pCDH-p18-TurboID-V5	This study	For lentiviral expression of Lyso-TurboID
29	pCDH-EGFP-Spo20	This study	For lentiviral expression of PA-binding probe
30	pCDH-V5-Nir2	This study	For lentiviral expression of V5-tagged PITPNM1
31	pCDH-V5-SCP2	This study	For lentiviral expression of V5-tagged SCP2 (SCP-x)
32	pCDH-V5-mSCP2	This study	For lentiviral expression of V5-tagged mSCP2
33	pCDH-SCP2	This study	For lentiviral expression of untagged SCP2 (SCP-x)
34	pCDH-proSCP2	This study	For lentiviral expression of untagged proSCP2 (405–547 aa)
35	pCDH-PDZD8-V5	This study	For lentiviral expression of V5-tagged PDZD8
36	pCDH-TEX2-V5	This study	For lentiviral expression of V5-tagged TEX2
37	pCDH-TEX2 Δ PH-V5	This study	For lentiviral expression of V5-tagged TEX2 lacking PH domain
38	pCDH-V5-ORP1L	This study	For lentiviral expression of V5-tagged ORP1L
39	pCDH-V5-ORP1L(H651A/H652A)	This study	For lentiviral expression of V5-tagged ORP1L with HH/AA mutations
40	pCDH-V5-ORP1L(Y583A)	This study	For lentiviral expression of V5-tagged ORP1L with Y583A mutation
41	pCDH-V5-ORP1L(F476A/Y477A)	This study	For lentiviral expression of V5-tagged ORP1L with FY/AA mutations
42	pCDNA3-Lyn10-mCherry-LOVPLD	Li et al. 2024	For transient expression of plasma membrane-targeted LOVPLD
43	pCDNA3-p18-mCherry-LOVPLD	Li et al. 2024	For transient expression of lysosome-targeted LOVPLD
44	pCDNA3-C1-mCherry-LOVPLD	Li et al. 2024	For transient expression of ER-targeted LOVPLD
45	pCDH-Lyn10-mCherry-LOVPLD	This study	For lentiviral expression of plasma membrane-targeted LOVPLD
46	pCDH-p18-mCherry-LOVPLD	This study	For lentiviral expression of lysosome-targeted LOVPLD
47	pCDH-C1-mCherry-LOVPLD	This study	For lentiviral expression of ER-targeted LOVPLD
48	pET-6xHis-mSCP2	This study	For purification of mSCP2

Supplementary Table 3. List of oligonucleotides used in this study.

	Name	Sequence	Notes
1	KpnI-Turbo-S	ctGGTACCATGAAAGACAATACTGTGCCTCTG	For cloning pCDNA-TurboID-V5-CAAX
2	AgeI-V5-AS	gtACCGGTGGTGTCTGTCCAGGCC	
3	HindIII-p18-S	atcAAGCTTGCCACCATGGGGTGCTGCTAC	For cloning pCDNA-p18-TurboID-V5
4	NotI-p18-AS	atcGCGGCCGCCGTAGTTGGGCTCGGCTCCATTG	
5	HindIII-TMEM192-S	gtcAAGCTTGCCACCATGGCGGCGGGGG	For cloning pCDNA-TMEM192-TurboID-V5
6	NotI-TMEM192-AS	atcGCGGCCGCCACTACCTCCACCTCCCGTTC	
7	KpnI-miRFP-S	gtGGTACCGCCACCATGGTAGCAG	For cloning pCDNA3-miRFP-C1
8	EcoRI-miRFP-AS	gtcaGAATTCCGCTCTCAAGCGCGGTG	
9	EcoRV-PITPNM1-S	atgcaGATATCCACCATGCTCATCAAGGAATACCAC	For cloning pCDNA-miRFP-Nir2
10	NotI-PITPNM1-AS	gtGCGGCCGCCTCCTCGCTGTCCAGC	
11	PITPNM1-T59A-S	GCAATACGCCCACAAGGTGTACC	For cloning pCDNA-miRFP-Nir2(T59A) by overlap PCR
12	PITPNM1-T59A-AS	CCTTGTGGGCGTATTGCCCCG	
13	BglII-OSBP-S	gtcAGATCTATGAACACAGAAGCGGAGC	For cloning pEGFP-ORP1L
14	KpnI-OSBP-AS	gtGGTACCTTAATAAAATGTCAGGCAAATTGAAGTAG	
15	XhoI-EGFP-S	gtcaTCGAGGTATGGTGAGCAAGGGCG	For cloning pCDNA3-EGFP-N1
16	ApaI-XbaI-EGFP-AS	tGGGCCCTCTAGATTACTTGTACAGCTCGTCCATG	
17	EcoRV-PDZD8-S	atgcaGATATCCACCATGGGGCTGCTGCTCATG	For cloning pCDNA-PDZD8-EGFP
18	NotI-PDZD8-AS	gtGCGGCCGCCACAGACTCGGATGGGC	
19	EcoRI-TEX2-S	gtcaGAATTCTGCCACCATGACAAGTCTGTATGGTCGCC	For cloning pCDNA-TEX2-EGFP
20	KpnI-TEX2-AS	gtGGTACCCCTGGCTGATCAGCAGCCTC	
21	EcoRI-TurboID-S	gtcaGAATTCGCCACCATGAAAGACAATACTGTG	For cloning pCDH-TurboID-V5-CAAX
22	BamHI-CAAX-AS	gtcaGGATCCCTACATAATTACACACTTTGTCTTTG	
23	EcoRI-p18-S	gtcaGAATTCGCCACCATGGGGTGCTGC	For cloning pCDH-p18-TurboID-V5
24	BamHI-Turbo-AS	gtcaGGATCCTTAGGTGCTGTCCAGGCC	
25	EcoRI-EGFP-S	gtcaGAATTCGCCACCATGGTGAGCAAG	For cloning pCDH-EGFP-Spo20 and pCDH-EGFP-PASS
26	BamHI-Spo20-AS	gtcaGGATCCTTAAGTCTTAGTGGCGTCATC	
27	AgeI-PITPNM1-S	gtACCGGTATGCTCATCAAGGAATACCAC	For cloning pCDH-V5-Nir2
28	NotI-PITPNM1-AS	gtcaGCGGCCGCTTACTCCTCGCTGTCCAGC	

29	AgeI-SCP2-S	gtACCGGTATGTCTCTTCCCCGTG	For cloning pCDH-V5-SCP2
30	NotI-SCP2-AS	gtcaGCGGCCGCTTAGAGCTTAGCGTTGCCTG	
31	AgeI-mSCP2-S	gtACCGGTATGTCTGCCAGTGATGGATTTAAG	For cloning pCDH-V5-mSCP2
32	NheI-SCP2-S	gtcaGCTAGCGCCACCATGTCTCTTCCCCGTG	For cloning pCDH-SCP2
33	NheI-proSCP2-S	gtcaGCTAGCGCCACCATGGGTTTTCCGGAAGC	For cloning pCDH-proSCP2
34	pCDH-PDZD8-S	ttgacctccatagaagattgccaccATGGGGCTGCTGCTC	For cloning pCDH-PDZD8-V5 by Gibson cloning
35	PDZD8-V5-AS	gggttggggatgggcttgccCACAGACTCGGATGGGCC	
36	ORT666-pCDH-TEX2-S	tttgacctccatagaagattgccaccATGACAAGTCTGTATGGTCG C	For cloning pCDH-TEX2-V5 by Gibson cloning
37	ORT667-TEX2-V5-AS	ggttggggatgggcttgcc TGGCTGATCAGCAGCCTC	
38	ORT701-TEX2-PHdel-AS	CTTGATTTCCGA AGGTTCTTTGATATCCAGAGATC	For cloning pCDH-TEX2ΔPH-V5 by overlap PCR
39	ORT702-TEX2-PHdel-S	GATATCAAAGAACCT TCGGAATCAAGAAGTCATCG	
40	AgeI-OSBPL1A-S	gtACCGGTATGAACACAGAAGCGGAGC	For cloning pCDH-V5-ORP1L
41	NotI-OSBPL1A-AS	gtcaGCGGCCGCTTAATAAATGTCAGGCAAATTGAA GTAG	
42	OSBPL1A-FY/AA-AS	GCATC AGCGGC TTCGTCCTCGGACAG	For cloning pCDH-V5-ORP1L(H651A/H652A)
43	OSBPL1A-FY/AA-S	GACGAA GCCGCT GATGCACTGTCCGAC	
44	OSBPL1A-Y583A-AS	GTTCCAT GGC CTCGGTCAGCC	For cloning pCDH-V5-ORP1L(Y583A)
45	OSBPL1A-Y583A-S	GACCGAG GCC ATGGAACACACATAC	
46	OSBPL1A-HH/AA-AS	GGGG AGCTGC GGAGACCTGCTC	For cloning pCDH-V5-ORP1L(F476A/Y477A)
47	OSBPL1A-HH/AA-S	GTCTCC GCAGCT CCCCCTATTAGCG	
48	pCDH-LOVPLD-S	gaagattctagagctagcg CAAGCTTgccaccATG	For cloning pCDH-mCherry-xx-LOVPLD
49	pCDH-LOVPLD-AS	GATCCTTcGCGGCCGC CACTATAGAATAGGGCCC	
50	6xHis-mSCP-S	cgcGGATCCATGCATCATCATCATCACagcTCTGC CAGTGATGGATTTAAGGCC	For cloning pET-6xHis-mSCP2
51	6xHis-mSCP-AS	ccGCTCGAGTTAGAGCTTAGCGTTGCCTGGC	
52	Nir2 siRNA #1–3	hs.Ri.PITPNM1.13.1, hs.Ri.PITPNM1.13.2, hs.Ri.PITPNM1.13.3 (purchased from IDT)	
53	SCP2 siRNA #1–3	hs.Ri.SCP2.13.1, hs.Ri.SCP2.13.2, hs.Ri.SCP2.13.3 (purchased from IDT)	
54	PDZD8 siRNA #1–3	hs.Ri.PDZD8.13.1, hs.Ri.PDZD8.13.2, hs.Ri.PDZD8.13.3 (purchased from IDT)	
55	Negative control siRNA	Scramble DsiRNA #51-01-14-03 (purchased from IDT)	

Supplementary Table 4. List of phospholipid mass and retention time.

# Formula	RT	Mass	Cpd
C33H65O8P	7.7	620.4417	PA 30:0
C33H63O8P	7.7	618.4261	PA 30:1
C35H69O8P	7.7	648.473	PA 32:0
C35H67O8P	7.7	646.4574	PA 32:1
C37H71O8P	7.6	674.4887	PA 34:1
C37H69O8P	7.6	672.473	PA 34:2
C39H75O8P	7.6	702.52	PA 36:1
C39H73O8P	7.6	700.5043	PA 36:2
C38H77CINO8P	6.5	741.5076	PC 30:0
C38H75CINO8P	6.5	739.4919	PC 30:1
C40H81CINO8P	6.5	769.5389	PC 32:0
C40H79CINO8P	6.5	767.5232	PC 32:1
C42H83CINO8P	6.4	795.5545	PC 34:1
C42H81CINO8P	6.4	793.5389	PC 34:2
C44H87CINO8P	6.3	823.5858	PC 36:1
C44H85CINO8P	6.3	821.5702	PC 36:2
C44H83CINO8P	6.3	819.5545	PC 36:3
C44H81CINO8P	6.3	817.5388	PC 36:4
C46H85CINO8P	6.2	845.5702	PC 38:4
C37H72NO8P	4.9	689.4996	PE 32:1
C39H76NO8P	4.9	717.5309	PE 34:1
C39H74NO8P	4.9	715.5152	PE 34:2
C41H80NO8P	4.8	745.5622	PE 36:1
C41H78NO8P	4.8	743.5465	PE 36:2
C41H74NO8P	4.8	739.5152	PE 36:4
C43H78NO8P	4.7	767.5465	PE 38:4
C43H76NO8P	4.7	765.5309	PE 38:5
C38H73O10P	3.6	720.4941	PG 32:1
C40H77O10P	3.5	748.5254	PG 34:1
C40H75O10P	3.5	746.5098	PG 34:2
C42H81O10P	3.4	776.5567	PG 36:1
C42H79O10P	3.4	774.5411	PG 36:2
C41H77O13P	6.6	808.5102	PI 32:1
C43H81O13P	6.5	836.5415	PI 34:1
C43H79O13P	6.5	834.5258	PI 34:2
C45H85O13P	6.4	864.5728	PI 36:1
C45H83O13P	6.4	862.5571	PI 36:2

C45H79O13P	6.4	858.5258	PI 36:4
C47H85O13P	6.3	888.5728	PI 38:3
C47H83O13P	6.3	886.5571	PI 38:4
C47H81O13P	6.3	884.5415	PI 38:5
C49H87O13P	6.2	914.5884	PI 40:4
C38H72NO10P	7.2	733.4894	PS 32:1
C40H76NO10P	7.1	761.5207	PS 34:1
C40H74NO10P	7.1	759.505	PS 34:2
C42H80NO10P	7	789.552	PS 36:1
C42H78NO10P	7	787.5363	PS 36:2
C44H80NO10P	6.9	813.552	PS 38:3
C46H82NO10P	6.9	839.5676	PS 40:4

Supplementary Methods for Proteomics Workflow

Sample collection and Protein quantitation

Protein concentration for each sample was determined by running on a precast 4-20% Criterion TGX (BioRad) gel along with *E. coli* lysates (0.5, 1, 2, 3 µg/lane) serving as a standard curve. The SDS gel was visualized with SyproRUBY protein gel stain (Invitrogen) and imaged on a ChemiDoc MP system (Bio-Rad), followed by quantification using Image Lab 6.1 (Bio-Rad).

Protein digestion and TMT16-plex labeling

Further processing of the proteins was then performed according to Thermo Scientific's TMTpro Mass Tagging Kits and Reagents protocol (Lot number: VL313890) with a slight modification¹⁻⁴. A total of 20 µg protein of each sample in the final buffer concentration: 50mM triethylammoniumbicarbonate (TEAB) pH 8.5, 7 M Urea, 2 M Thiourea, 1% SDS was reduced with 10 mM tris(2-carboxyethyl) phosphine (TCEP) for 1 h at 34 °C, alkylated with 20 mM iodoacetamide for 45 min in the dark, and then quenched with a final concentration of 27 mM dithiothreitol (DTT). Each sample was digested separately using the S-Trap Micro Spin column (Protifi, Huntington NY)⁵. After quenching, 12% phosphoric acid was added to a final concentration of 1%. Followed by 1:7 dilution (v/v) with 90% methanol, 0.1M TEAB pH 8.5. The samples were then placed into the S-Trap Micro spin column and centrifuged at 4000g for 30 sec. Then washed three times with 150 µL of 90% methanol, 0.1 M TEAB (pH 8.5). Digestion

was performed with 25 μ L trypsin at 100 ng/ μ L (1:10 w/w) in 50 mM TEAB (pH 8.5) added to the top of the spin column. The digest solution was absorbed into the highly hydrophilic matrix, and the spin columns were incubated overnight (16 h) at 37 °C. Following incubation, the digested peptides were eluted off the S-Trap column sequentially with 40 μ L each of 50 mM TEAB (pH 8.5), 0.2% formic acid, 50% acetonitrile (ACN)-0.2% formic acid. Three eluates were pooled together and dried by a Speedvac SC110 (Thermo Savant, Milford, MA). The dried peptides were reconstituted in 100 μ L water and re-dried in the speedvac to ensure complete removal of residual formic acid.

Immediately before labeling, each sample was reconstituted in 30 μ L of 0.1 M TEAB pH 8.5. The TMTpro16 plex labels (dried powder) were reconstituted with 15 μ L of anhydrous ACN prior to labeling and added to each of the 30 μ L tryptic digest samples (1:6.6 w/w ratio peptide to TMT label) and incubated for 1 hour at room temperature. The labeled peptides from each set were pooled together. The pooled peptides of each set were then evaporated to dryness and subjected to cleanup by solid phase extraction (SPE) on MCX Cartridges (Waters, Milford, MA). Labeling incorporation was then checked using Orbitrap Eclipse (Thermo Fisher Scientific, San Jose, CA). The eluted tryptic peptides were evaporated to dryness and then fractionated via a high pH reverse phase chromatography as described below.

High pH reverse phase (hpRP) fractionation of each set

The HpRP chromatography was carried out using a Dionex UltiMate 3000 HPLC system with a built-in micro fraction collection option in its autosampler and UV detection (Thermo Scientific, Sunnyvale, CA) as reported previously¹⁻⁴. Specifically, the TMTpro 16 plex tagged tryptic peptides were reconstituted in buffer A (20 mM ammonium formate, pH 10, in water), filtered using 0.22 mm cellulose acetate filters (Costar Spin-X, Corning Inc., Salt Lake City, UT), and injected onto a XBridge C18 column (3.5 μ m, 2.1 x 150 mm) from Waters, (Milford, MA) with 20 mM ammonium formate (NH₄FA), pH 10, as buffer A and 85% ACN/20% 20 mM NH₄FA as buffer B. The LC was performed using a linear gradient: 5-5-14-50-90-90-5-5 %B, 0-0.25-1-40-42-45-45.5-49 min; at a flow rate of 200 μ L/min. The column temperature was maintained at 30 °C and the collection plate at 5 °C. Forty-eight fractions were collected at 1-minute intervals beginning at 1-minute gradient runtime, ending at 49 minutes and pooled into 10 fractions based on the UV absorbance at 214 nm, using a multiple fraction concatenation strategy⁶. Each of the 10

pooled fractions was dried, re-dried from 200 mL water (Optima LC-MS, Fisher Scientific, Fair Lawn, NJ) and reconstituted in 100 μ L of 2% ACN/0.5% FA for nanoLC-MS/MS analysis.

Nano-scale reverse phase chromatography and tandem MS (nanoLC-MS/MS)

The nanoLC-MS/MS analysis was carried out using an Orbitrap Eclipse (Thermo Fisher Scientific, San Jose, CA) mass spectrometer equipped with a nanospray Flex Ion Source coupled with the UltiMate 3000 RSLCnano (Dionex, Sunnyvale, CA). Each reconstituted fraction (2 μ L for set 1 and set 2, 1.5 μ L for set 3) was injected onto a PepMap C-18 RP nano trap column (5 μ m, 100 μ m $\times$ 20 mm, Dionex) at 20 μ L/min flow rate for rapid sample loading, and separated on a PepMap C18 RP nano column (2 μ m, 75 μ m $\times$ 25 cm). The column was equilibrated with 2% ACN in 0.1% aqueous formic acid (eluant A) prior to each run. The labeled peptides were eluted in a 120 min gradient of 5% to 32% eluant B containing 95% ACN in 0.1% formic acid at 300 nL/min, followed by an 8-min ramp to 90% B, a 7-min hold, and a 21-min re-equilibration with 2% ACN-0.1% FA prior to the next run. The Orbitrap Eclipse was operated in positive ion mode with a nanospray voltage set of 1.7 kV and a source temperature of 300 $^{\circ}$ C. External calibration for FT, IT, and quadrupole mass analyzers was performed. Raw MS data files for all the fractions were acquired using a real-time search (RTS) synchronous precursor selection (SPS) MS³ workflow as reported previously⁴. Specifically, the RTS MS³ workflow consisted of 2.5 second “Top Speed” data-dependent CID-MS/MS scans (for peptide identifications by RTS) that enabled to trigger SPS of 10 MS² product ions for subsequent MS³ in FT. In RTS mode, the *Homo sapiens* NCBI database downloaded April 2021 which contains 81,785 entries were imported as the FASTA database with trypsin as the enzyme for RTS for the samples from corresponding species. The search parameters included: TMTpro modifications on lysine and N-terminal amines (Δ mass 304.2071), carbamidomethyl modification of cysteine (Δ mass 57.0215), and maximum 2 variable methionine oxidation per peptide, and 1 missed cleavage. A maximum search time for 35 ms allowed for the RTS MS³ searching. The MS³ scan was carried out using a mass range of 110-500 m/z, an MS isolation window of 1.1 m/z and MS² isolation window of 2.0 m/z were used. A resolving power of 50,000 at MS³ with a normalized collision energy of 55% was used for acquisition of reporter ions used for peptide quantitation. Other parameters included 200% normalized AGT target and 120 ms for maximum injection time. Dynamic exclusion parameters

were set at 1 within 50 s exclusion duration with ± 10 ppm exclusion mass window. All data were acquired under Xcalibur 4.3 operation software in Orbitrap Eclipse (Thermo Fisher Scientific).

Data processing and protein identification

All raw MS spectra were processed and searched using the Sequest HT search engine within the Proteome Discoverer 2.5 (PD 2.5, Thermo). The same NCBI databases used for RTS in data acquisition was used for post-MS database searches. The default search settings used for TMTpro 16 plex quantitative processing and protein identification in PD 2.5 searching software were: two mis-cleavage for full trypsin with fixed carbamidomethyl modification of cysteine, fixed 16-plex TMT modifications on lysine and N-terminal amines along with variable modifications of methionine oxidation, deamidation on asparagine/glutamine residues and protein N-terminal acetylation, M-loss and M-loss+acetylation. The peptide mass tolerance and fragment mass tolerance values were 10 ppm for MS survey scan, 0.6 Da for MS², and 20 ppm for MS³, respectively. Identified peptides were further filtered for maximum 1% FDR using the Percolator algorithm in PD 2.5 along with additional peptide confidence set to high and peptide mass accuracy ≤ 5 ppm. The TMTpro 16plex quantification method within the Proteome Discoverer 2.5 software was used to calculate the reporter ion abundances in MS³ spectra that were corrected for the isotopic impurities. Both unique and razor peptides were used for quantitation. Signal-to-noise (S/N) values were used to represent the reporter ion abundance with a co-isolation threshold of 50% and an average reporter S/N (intensity) threshold of ≥ 10 used for quantitation spectra. The intensities of peptides, which were summed from the intensities of the peptide-spectrum match (PSM), were summed to represent the abundance of the proteins. For relative ratio between the two groups, normalization on total peptide amount for each sample was applied. Student's t-test was used for p value calculation of the reported ratios. The search results including ratios, p values, peptide abundance, and other parameters for each sample were exported to a Microsoft Excel spreadsheet for further data analysis.

Data analysis

The proteomics data contained the abundance of different proteins detected in each sample. We screened for proteins that showed significant increase (fold change > 1 and fold change p -value < 0.05) or decrease (fold change < 1 and fold change p -value < 0.05) based on protein

abundance ratio. Gene ontology enrichment analysis was performed to identify proteins linked to biological process related to lipids. Corneocyte proteins, keratin and filaggrin, which were considered to be detected due to contamination, were removed from the search.

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Supplementary References

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