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Supplementary Tables

Table S1. Proteins defined as hits in at least 8 out of 12 affinity enrichments and on-bead tryptic digestion (overlapped proteins are highlighted in grey).

Gene Name	Protein Name
Abhd12	Monoacylglycerol lipase ABHD12
Acads	Short-chain specific acyl-CoA dehydrogenase, mitochondrial
Acbd5	Acyl-CoA-binding domain-containing protein 5
Acin1	Apoptotic chromatin condensation inducer in the nucleus
Acp1	Low molecular weight phosphotyrosine protein phosphatase
Acsl6	Long-chain-fatty-acid--CoA ligase 6
Agk	Acylglycerol kinase, mitochondrial
Agpat5	1-acyl-sn-glycerol-3-phosphate acyltransferase epsilon
Aifm1	Apoptosis-inducing factor 1, mitochondrial
Aifm2	Apoptosis-inducing factor 2
Aldh3a2	Aldehyde dehydrogenase;Fatty aldehyde dehydrogenase
Aldh3b1	Aldehyde dehydrogenase family 3 member B1
Aldh7a1	Alpha-aminoadipic semialdehyde dehydrogenase
Alg2	Alpha-1,3/1,6-mannosyltransferase ALG2
Anapc7	Anaphase-promoting complex subunit 7
Api5	Apoptosis inhibitor 5
Apob	Apolipoprotein B-100;Apolipoprotein B-48
APP	Amyloid beta A4 protein;N-APP
Arl1	ADP-ribosylation factor-like protein 1
Arl8a	ADP-ribosylation factor-like protein 8A
Arl8b	ADP-ribosylation factor-like protein 8B
Asah1	Acid ceramidase;Acid ceramidase subunit alpha; beta
Atad2	ATPase family AAA domain-containing protein 2
Atad2b	MKIAA1240 protein
Atp13a1	Manganese-transporting ATPase 13A1
Atp5e	ATP synthase subunit epsilon, mitochondrial
Atp5l	ATP synthase subunit g, mitochondrial
Bax	Apoptosis regulator BAX
Bcas2	Pre-mRNA-splicing factor SPF27
BcL-xL	Bcl-2-like protein 1
Bcl2l13	Bcl-2-like protein 13
Bclaf1	Bcl-2-associated transcription factor 1
Bik	BCL2-interacting killer (apoptosis-inducing)
Birc5	Baculoviral IAP repeat-containing protein 5
Bud31	Protein Bud31 homolog
Bysl	Bystin
Cand2	Cullin-associated NEDD8-dissociated protein 2
Cbr1	Carbonyl reductase [NADPH] 1
Cdk5	Cyclin-dependent-like kinase 5
Cdk5rap2	CDK5 regulatory subunit-associated protein 2
Celf1	CUGBP Elav-like family member 1

Celf4	CUGBP Elav-like family member 4
Cers2	Ceramide synthase 2
Cers4	Ceramide synthase 4
Chd7; Chd6; Chd9	Chromodomain-helicase-DNA-binding protein 7; 6; 9
Cog7	Conserved oligomeric Golgi complex subunit 7
Coq9	Ubiquinone biosynthesis protein COQ9, mitochondrial
Cpsf3	Cleavage and polyadenylation specificity factor subunit 3
Cpt2	Carnitine O-palmitoyltransferase 2, mitochondrial
Csnk1e; Csnk1d	Casein kinase I isoform epsilon;Casein kinase I isoform delta
Ctps1	CTP synthase 1
Ctsb	Cathepsin B;Cathepsin B light chain;Cathepsin B heavy chain
Cyc1	Cytochrome c1, heme protein, mitochondrial
Dazap1	DAZ-associated protein 1
Dcakd	Dephospho-CoA kinase domain-containing protein
Ddx23	Protein Ddx23
Ddx24	ATP-dependent RNA helicase DDX24
Ddx54	ATP-dependent RNA helicase DDX54
Degs1	Sphingolipid delta(4)-desaturase DES1
Desi1	Desumoylating isopeptidase 1
Dhx36	ATP-dependent RNA helicase DHX36
Ecm29; AI314180	Proteasome-associated protein ECM29 homolog
Eif1; Eif1b	Eukaryotic translation initiation factor 1b
Emc8	ER membrane protein complex subunit 8
Erap1	Endoplasmic reticulum aminopeptidase 1
Ergic1	Endoplasmic reticulum-Golgi intermediate compartment protein 1
Erh	Enhancer of rudimentary homolog
Exoc5	Exocyst complex component 5
Fads1	Fatty acid desaturase 1
Fads2	Fatty acid desaturase 2
Fam162a	Protein FAM162A
Fam208b	Protein FAM208B
Fam210a	Protein FAM210A
Gbf1	Prostaglandin E synthase 2
Gemin5	Gem-associated protein 5
Gfpt1	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] 1
Gga2	ADP-ribosylation factor-binding protein GGA2
Ghdc	GH3 domain-containing protein
Glipr2	Golgi-associated plant pathogenesis-related protein 1
Glmn	Glomulin
Glyr1	Putative oxidoreductase GLYR1
Gnai1;Gnai3Gnai2	Guanine nucleotide-binding protein G(i/k) subunit alpha-1

Gnpat	Dihydroxyacetone phosphate acyltransferase
Golga5	Golgin subfamily A member 5
Got2	Aspartate aminotransferase, mitochondrial
Gpx4	Glutathione peroxidase;Phospholipid hydroperoxide glutathione peroxidase
Hccs	Cytochrome c-type heme lyase
Htra2	Serine protease HTRA2, mitochondrial
Idh2	Isocitrate dehydrogenase [NADP], mitochondrial
Ilvbl	Acetolactate synthase-like protein
Imp4	U3 small nucleolar ribonucleoprotein protein IMP4
Impa1	Inositol monophosphatase 1
Incenp	Inner centromere protein
Ing4	Inhibitor of growth protein 4
Ing5	Inhibitor of growth protein 5
Ints4	Integrator complex subunit 4
Itga1	Integrin alpha-1
Kpna1; Kpna6	Importin subunit alpha-5, N-terminally processed
Kras	GTPase KRas;GTPase KRas, N-terminally processed
Lars2	Probable leucine--tRNA ligase, mitochondrial
LASS2	LAG1 homolog, Ceramide synthase 2
LASS4	LAG1 homolog, Ceramide synthase 4
LASS6	LAG1 homolog, Ceramide synthase 6
Lgals1	Galectin-1
Lgals3	Galectin-3
Lrif1	Ligand-dependent nuclear receptor-interacting factor 1
Ltn1	E3 ubiquitin-protein ligase listerin
Lypla1	Acyl-protein thioesterase 1
Lyplal1	Lysophospholipase-like protein 1
Maged1	Melanoma-associated antigen D1
Mccc1	Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial
Mcu	Calcium uniporter protein, mitochondrial
Meaf6	Chromatin modification-related protein MEAF6
Mif	Macrophage migration inhibitory factor
Mlec	Malectin
Mon2	Protein MON2 homolog
Mospd2	Motile sperm domain-containing protein 2
Mpc2	Mitochondrial pyruvate carrier 2
Mphosph8	M-phase phosphoprotein 8
Mrpl47	39S ribosomal protein L47, mitochondrial
Mtch2	Mitochondrial carrier homolog 2
Mtor	Serine/threonine-protein kinase mTOR
Mtx1	Metaxin-1
Mtx2	Metaxin-2
Mxra7	Matrix-remodeling-associated protein 7
Mycbp	C-Myc-binding protein
Myg1	UPF0160 protein MYG1, mitochondrial

Myl12b	Myosin regulatory light chain 12B
Myo1c	Unconventional myosin-Ic
Napa	Alpha-soluble NSF attachment protein
Nceh1	Neutral cholesterol ester hydrolase 1
Ncoa5	Nuclear receptor coactivator 5
Ndc1	Nucleoporin NDC1
Ndufa4	Cytochrome c oxidase subunit NDUFA4
Ndufa7	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 7
Ndufa8	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8
Ndufc2	NADH dehydrogenase [ubiquinone] 1 subunit C2
Ndufs5	NADH dehydrogenase [ubiquinone] iron-sulfur protein 5
Nfxl1	Nfxl1 protein
Nol8	Nucleolar protein 8
Nop9	Nucleolar protein 9
Nras;Hras	GTPase NRas;GTPase HRas;GTPase HRas, N-terminally processed
Nsun5	Probable 28S rRNA (cytosine-C(5))-methyltransferase
Nubpl	Iron-sulfur protein NUBPL
Nup155	Nuclear pore complex protein Nup155
Nup85	Nuclear pore complex protein Nup85
Nusap1	Nucleolar and spindle-associated protein 1
Oxa1l	Mitochondrial inner membrane protein OXA1L
Pa2g4	Proliferation-associated protein 2G4
Pam16	Mitochondrial import inner membrane translocase subunit TIM16
Pbrm1	Protein polybromo-1
Pcnt	Pericentrin
Pdxk	Pyridoxal kinase
Pebp1	Phosphatidylethanolamine-binding protein 1
Pfn2	Profilin-2;Profilin
Phf14	PHD finger protein 14
Phip	PH-interacting protein
Plrg1	Pleiotropic regulator 1
Pmpca	Mitochondrial-processing peptidase subunit alpha
Ppan	Suppressor of SWI4 1 homolog
Pphln1	Periphilin-1
Ppig	Peptidyl-prolyl cis-trans isomerase G;Peptidyl-prolyl cis-trans isomerase
Ppox	Protoporphyrinogen oxidase
Ppp2r1b	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta
Ppp2r5a	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit alpha
Ppt1	Palmitoyl-protein thioesterase 1
Prdx5	Peroxiredoxin-5, mitochondrial
Prkar2b	cAMP-dependent protein kinase type II-beta regulatory subunit
Prpf38a	Pre-mRNA-splicing factor 38A
Psmg2	Proteasome assembly chaperone 2
Psph	Phosphoserine phosphatase
Ptar1	Protein Ptar1

Ptges2	Prostaglandin E synthase 2;Prostaglandin E synthase 2 truncated form
Ptpn1	Tyrosine-protein phosphatase non-receptor type 1
Rae1	mRNA export factor
Rala	Ras-related protein Ral-A
Rars2	Probable arginine--tRNA ligase, mitochondrial
Rfc2	Replication factor C subunit 2
Rngtt	mRNA-capping enzyme;Polynucleotide 5-triphosphatase
Rpl18a	60S ribosomal protein L18a
Rpl37a	60S ribosomal protein L37a
Rps29; Gm10126	40S ribosomal protein S29
Rtca;RtcA	RNA 3-terminal phosphate cyclase
Sar1b	GTP-binding protein SAR1b
Sdcbp	Syntenin-1
Sec14l1	SEC14-like protein 1
Sec24a	Protein transport protein Sec24A
Sec24d	Protein Sec24d
Sec62	Translocation protein SEC62
Sgpl1	Sphingosine-1-phosphate lyase 1
Slc1a4	Neutral amino acid transporter A
Slc1a5	Neutral amino acid transporter B(0)
Smpd3	Sphingomyelin phosphodiesterase 3
Smpd4	Sphingomyelin phosphodiesterase 4
Snap25	Synaptosomal-associated protein 25
Snrpd1	Small nuclear ribonucleoprotein Sm D1
Snx8	Sorting nexin-8
Spcs3	Signal peptidase complex subunit 3
Spg7	Paraplegin
Spryd7	SPRY domain-containing protein 7
Srrm1	Serine/arginine repetitive matrix protein 1
Sun1	SUN domain-containing protein 1
Tamm41	Phosphatidate cytidylyltransferase, mitochondrial
Tbc1d15	TBC1 domain family member 15
Tbl2	Transducin beta-like protein 2
Tcf20	Transcription factor 20
Tfip11	Tuftelin-interacting protein 11
Tmed4	Transmembrane emp24 domain-containing protein 4
Tmem263	Transmembrane protein 263
Tmpo	Lamina-associated polypeptide 2, isoforms alpha/zeta
Tmx4	Thioredoxin-related transmembrane protein 4
Tnpo3	Transportin-3
Top2b	DNA topoisomerase 2-beta
Tor1aip1	Torsin-1A-interacting protein 1
Tor1b	Torsin-1B
Tor3a	Torsin-3A

Trim32	E3 ubiquitin-protein ligase TRIM32
Trip12	E3 ubiquitin-protein ligase TRIP12
Trmt1l	TRMT1-like protein
Trmt2a	tRNA (uracil-5-)-methyltransferase homolog A
Ttc39b	Tetratricopeptide repeat protein 39B
Tuba1b; Tuba4a	Tubulin alpha-1B chain;Tubulin alpha-4A chain
Tubb6	Tubulin beta-6 chain
Tubgcp3	Gamma-tubulin complex component 3
Ube3c	Ubiquitin-protein ligase E3C
Ubl5; Gm16381	Ubiquitin-like protein 5
Ubxn4	UBX domain-containing protein 4
Uchl5	Ubiquitin carboxyl-terminal hydrolase isozyme L5
Uqcc2	Ubiquinol-cytochrome-c reductase complex assembly factor 2
Utp20	Small subunit processome component 20 homolog
Utp6	U3 small nucleolar RNA-associated protein 6 homolog
Xrcc1	DNA repair protein XRCC1

Table S2. Proteins defined as hits in the Protein microarray analysis (overlapped proteins are highlighted in grey).

Gene Name	**Protein Name**
PLA2G15	1-O-acylceramide synthase
ABHD10	abhydrolase domain containing 10 (ABHD10)
ABHD1	Abhydrolase domain-containing protein 1
ACAT1	acetyl-Coenzyme A acetyltransferase 1 (acetoacetyl Coenzyme A thiolase) (ACAT1)
ACP1	acid phosphatase 1, soluble (ACP1), transcript variant 1
ACOT11	Acyl-CoA thioesterase 11 (ACOT11), transcript variant 1, mRNA
ACBD7	acyl-Coenzyme A binding domain containing 7 (ACBD7)
ACOT4	Acyl-coenzyme A thioesterase 4
ARL4C	ADP-ribosylation factor-like 4C (ARL4C)
ALDH3B1	Aldehyde dehydrogenase 3 family, member B1 (ALDH3B1), transcript variant 2, mRNA
ORM1	Alpha-1-acid glycoprotein 1
APP	amyloid beta (A4) precursor protein (peptidase nexin-II, Alzheimer disease) (APP)
APOA1BP	apolipoprotein A-I binding protein (APOA1BP)
APOA2	apolipoprotein A-II (APOA2)
APOA4	Apolipoprotein A-IV
APOBEC3C	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C (APOBEC3C)
APOBEC3H	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3H (APOBEC3H)
APOC4	apolipoprotein C-IV (APOC4)
APOF	Apolipoprotein F
APOL3	Apolipoprotein L, 3 (APOL3), transcript variant alpha/a, mRNA
APOL6	apolipoprotein L, 6 (APOL6)
APOL2	Apolipoprotein L2
APOM	apolipoprotein M (APOM)

APOOL	apolipoprotein O-like (APOOL)
AIFM2	Apoptosis-inducing factor 2
AIFM3	apoptosis-inducing factor, mitochondrion-associated, 3 (AIFM3)
C2orf28	Apoptosis-related protein 3
BIRC5	baculoviral IAP repeat-containing 5 (survivin) (BIRC5), transcript variant 1
BCL2L12	Bcl-2-related proline-rich protein
BCL2L2	BCL-W / BCL2L2 Protein (His Tag)
BIK	BCL2-interacting killer (apoptosis-inducing) (BIK)
BCL2L11	BCL2-like 11 (apoptosis facilitator) (BCL2L11), transcript variant 3
BCL2L14	BCL2-like 14 (apoptosis facilitator) (BCL2L14), transcript variant 1
BUD31	BUD31 homolog (S. cerevisiae) (BUD31)
MYCBP	c-myc binding protein (MYCBP)
CDK6	Cell division protein kinase 6
COQ6	coenzyme Q6 homolog, monooxygenase (S. cerevisiae) (COQ6), transcript variant 2
CDK5	cyclin-dependent kinase 5 (CDK5) and p25: CDK5 and p25 sequences are seperated by -- (in protein
CDK7	cyclin-dependent kinase 7 (MO15 homolog, Xenopus laevis, cdk-activating kinase) (CDK7)
CDKL1	Cyclin-dependent kinase-like 1
PLA2G4C	Cytosolic phospholipase A2 gamma
ERGIC1	endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1 (ERGIC1)
FAAH2	fatty acid amide hydrolase 2 (FAAH2)
FABP3	fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor) (FABP3)
FADS2	fatty acid desaturase 2 (FADS2)
FRAP1(mTOR)	Serine/threonine kinase
GABRA3	gamma-aminobutyric acid (GABA) A receptor, alpha 3 (GABRA3)
GABRA6	Gamma-aminobutyric acid (GABA) A receptor, alpha 6 (GABRA6), mRNA
GPX4	glutathione peroxidase 4 (phospholipid hydroperoxidase) (GPX4)
GDPD5	glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5)
HEATR2	HEAT repeat containing 2 (HEATR2)
IPO11	importin 11 (IPO11)
IPO4	Importin-4
IPO9	Importin-9
ICMT	Isoprenylcysteine carboxyl methyltransferase (ICMT), transcript variant 2, mRNA
CERS1	LAG1 homolog, ceramide synthase 1 (S. cerevisiae), mRNA (
LASS4	LAG1 homolog, ceramide synthase 4 (LASS4)
LASS6	LAG1 homolog, ceramide synthase 6, mRNA (cDNA clone IMAGE:4396549), complete cds.
LAS1L	LAS1-like (S. cerevisiae) (LAS1L)
LGALS1	lectin, galactoside-binding, soluble, 1 (galectin 1) (LGALS1)
LGALS14	lectin, galactoside-binding, soluble, 14 (LGALS14)
LGALS2	lectin, galactoside-binding, soluble, 2 (LGALS2)
LGALS3	lectin, galactoside-binding, soluble, 3 (LGALS3)
LGALS3	lectin, galactoside-binding, soluble, 3 (LGALS3)
LGALS3	LGALS3 / Galectin-3 Protein (Native)
ACSBG1	Long-chain-fatty-acid--CoA ligase ACSBG1
LYPLA2	lysophospholipase II (LYPLA2)
LYPLAL1	lysophospholipase-like 1 (LYPLAL1)

LAPTM5	Lysosomal-associated multispanning membrane protein-5 (LAPTM5), mRNA
LAPTM4A	lysosomal-associated protein transmembrane 4 alpha (LAPTM4A)
MARK2	MAP/microtubule affinity-regulating kinase 2 (MARK2), transcript variant 3
MAT1A	methionine adenosyltransferase I, alpha (MAT1A)
MAP1LC3A	microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A), transcript variant 1
MAP1LC3A	microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A), transcript variant 2
OXA1L	Mitochondrial inner membrane protein OXA1L
MAP2K1	mitogen-activated protein kinase kinase 1 (MAP2K1)
MYLC2PL	myosin light chain 2, precursor lymphocyte-specific (MYLC2PL)
MYLK	myosin light chain kinase
MYLK2	myosin light chain kinase 2, skeletal muscle (MYLK2)
MYL1	myosin, light chain 1, alkali; skeletal, fast (MYL1), transcript variant 3f
MYLK	myosin, light polypeptide kinase (MYLK), transcript variant 5
ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1 (ASAH1), transcript variant 1
ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1, mRNA (cDNA clone MGC:24572
OGT	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-
PPT1	palmitoyl-protein thioesterase 1 (ceroid-lipofuscinosis, neuronal 1, infantile) (PPT1)
PPT2	palmitoyl-protein thioesterase 2 (PPT2), transcript variant 2
PDE4DIP	phosphodiesterase 4D interacting protein (myomegalin) (PDE4DIP)
GALNT10	Polypeptide N-acetylgalactosaminyltransferase 10
GALNT13	Polypeptide N-acetylgalactosaminyltransferase 13
PQLC3	PQ loop repeat containing 3 (PQLC3)
PFN4	Profilin-4
PTGER3	Prostaglandin E receptor 3 (subtype EP3) (PTGER3), transcript variant 9, mRNA
PTGES2	prostaglandin E synthase 2 (PTGES2), transcript variant 2
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) (PTGS2)
PDXK	pyridoxal (pyridoxine, vitamin B6) kinase (PDXK)
RICTOR	rapamycin-insensitive companion of mTOR (RICTOR)
SAR1B	SAR1 gene homolog B (S. cerevisiae) (SAR1B), transcript variant 2
SEPT4	septin 4 (SEPT4), transcript variant 1
SEPT7	septin 7 (SEPT7), transcript variant 1
SUMO4	Small ubiquitin-related modifier 4
SPHK1	sphingosine kinase 1 (SPHK1), transcript variant 1
SPHK1	Sphingosine kinase 1, mRNA (cDNA clone MGC:15041 IMAGE:3831657), complete cds
SPHK2	sphingosine kinase 2 (SPHK2)
SGPL1	sphingosine-1-phosphate lyase 1 (SGPL1)
TBCEL	Tubulin-specific chaperone cofactor E-like protein
UBIAD1	UbiA prenyltransferase domain containing 1 (UBIAD1)
COQ9	Ubiquinone biosynthesis protein COQ9, mitochondrial
UBE3A	ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome)
UBE3C	ubiquitin protein ligase E3C (UBE3C)
UBE1	ubiquitin-activating enzyme E1 (UBE1), transcript variant 1
UBE1L	ubiquitin-activating enzyme E1-like (UBE1L)
UBE1L2	ubiquitin-activating enzyme E1-like 2 (UBE1L2)
UBE1C	ubiquitin-activating enzyme E1C (UBA3 homolog, yeast) (UBE1C), transcript variant 3

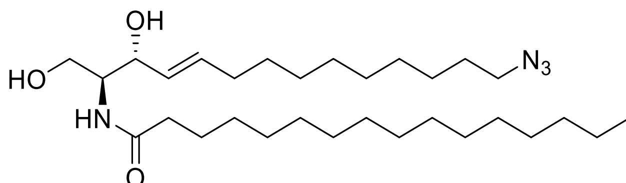
UBE2A	ubiquitin-conjugating enzyme E2A (RAD6 homolog) (UBE2A)
UBE2B	ubiquitin-conjugating enzyme E2B (RAD6 homolog) (UBE2B)
UBE2C	ubiquitin-conjugating enzyme E2C (UBE2C), transcript variant 1
UBE2C	ubiquitin-conjugating enzyme E2C (UBE2C), transcript variant 4
UBE2CBP	ubiquitin-conjugating enzyme E2C binding protein (UBE2CBP), mRNA.
UBL3	ubiquitin-like 3 (UBL3)
UBL4A	ubiquitin-like 4A (UBL4A)
UBL5	ubiquitin-like 5 (UBL5), transcript variant 1
UBL3	Ubiquitin-like protein 3
UBL7	Ubiquitin-like protein 7
YIPF1	Yip1 domain family, member 1 (YIPF1)

Chemistry.

Materials.

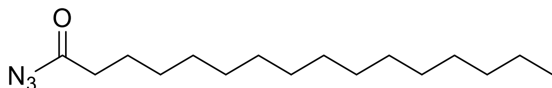
All commercially available compounds were used as provided without further purifications. Chemicals and solvents were purchased from the companies Sigma Aldrich, Alfa Aesar, Acros Organics. Merck Chemicals, Life Technologies and Fisher Scientific. Dry solvents for reactions and solvents for column chromatography (laboratory reagent grade or HPLC grade) were used as commercially available. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel aluminium plates with F-254 indicator. Compounds were visualized by irradiation with UV light or potassium permanganate staining or cerium ammonium molybdate (CAM). Column chromatography was performed using silica gel Acros 60 A (particle size 0.035-0.070 nm). NMR were recorded on a Bruker Avance DRX-400 or 500 (400 or 500 MHz) or on a Agilent Technologies Mercury Inova-600 (600MHz). Deuterated solvents were used as internal standards ($CDCl_3$: δ = 7.26 ppm for ^{1}H, δ = 77.16 ppm for ^{13}C; acetone-D_6: δ = 2.05 ppm for ^{1}H, δ = 29.84 ppm for ^{13}C). Chemical shift (δ) values are reported in ppm. Signal characterization is described using the next abbreviations: s (singlet), d (doublet), t (triplet), q and m (multiplet). Coupling constant values are presented in Hertz (Hz). High resolution mass spectra were recorded on a LTQ Orbitrap mass spectrometer coupled to an Accela HPLC-System (flow injection: 50% (containing 0.1% formic acid) water and 50% (containing 0.1% formic acid) acetonitrile, flow rate: 250 µL/ min). Preparative HPLC-MS was performed on a Agilent Series 1100/LC/MSD VL system, using a Macherey & Nagel Nucleodur VP C4 or C18 column (Solution A: 0.1% TFA in water and Solution B containing 0.1% TFA in acetonitrile. Analytical HPLC-MS was recorded on a Ultimate 3000 (Thermo Fisher Scientific) using a Nucleodur C4 (EC 125/3) or C18 (EC 50/3) column. Mass detection was performed through a LCQ Fleet (Thermo Fisher Scientific) using HESI as ionization technique.

Synthesis of ωN_3C16Cer (1)



ωN_3Sphingosine was prepared following a previously reported procedure. To prepare ωN_3C16Cer (**1**) 20 mg of ωN_3Sphingosine were dissolved in 1 mL of dry THF and added to a 0°C cold solution of 50% sodium acetate in water. The mixture was stirred for 10 min and 0.023 mL palmitoyl chloride (0.08 mmol, 1.1 eq) were added dropwise under vigorous stirring. The reaction mixture was stirred for 6 hours at room temperature. The solvent was removed under reduced pressure and the residue was solved in 3 mL diethyl ether. The organic phase was washed with 2 mL of a 2 N ammonium hydroxide solution, water, brine and dried over $MgSO_4$ yielding 20 mg (0.04 mmol, 62 %) of ωN_3C16Cer (**1**). HPLC-MS: m/z calcd. for $C_{30}H_{58}N_4O_3$: 523,45 $[M+H]^+$, found 523,24 $[M+H]^+$; HR-MS $[M+H]^+$: m/z calcd. for $C_{30}H_{58}N_4O_3$: 523.4581 $[M+H]^+$, found 523.4577 $[M+H]^+$; ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.04 (s, 1H), 5.57 (m, 2H), 4.53 (d, *J* = 8.3 Hz, 1H), 3.62 – 3.42 (m, 3H), 2.23 (s, 2H), 1.63 – 1.46 (m, 4H), 1.41 – 1.09 (m, 42H), 0.87 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.4, 133.8, 129.6, 69.5, 62.4, 53.9, 51.0, 36.6, 34.4, 28.4, 26.6, 25.7, 22.4, 14.0.

Palmitoyl azide (2)



Palmitoyl chloride (5.1 mmol, 0.6 eq) was added dropwise to a pre-chilled solution of 500 mg sodium azide (7.7 mmol, 1 eq) in acetone at 0°C. The reaction was stirred for 30 min, filtered and the resulting solid was dried under vacuum and used without further purification.

HPLC-MS: m/z calcd. for $C_{16}H_{31}N_3O$: 282.43 $[M+H]^+$, found 282.25 $[M+H]^+$; HR-MS $[M+H]^+$: m/z calcd. for $C_{16}H_{31}N_3O$: 282.2539 $[M+H]^+$, found 282.2538 $[M+H]^+$; ^{1}H-NMR (400 MHz Acetone-d_6): δ 2.41 (t, 2H), 1.62 (m, 2H), 1.35 – 1.24 (m, 24H), 0.88 (s, 3H); ^{13}C-NMR (101 MHz, Acetone-d_6): δ 180.2, 38.7, 33.9, 32.2, 31.9, 31.6, 31.5, 31.4, 31.3, 31.1, 30.9, 30.7, 30.3, 26.7, 24.7, 15.8.

Biology

Materials

C16-ceramide was acquired from Santa Cruz Biotechnologies and 1,2-dipalmitoyl-*sn*-glycerol was purchased from Sigma Aldrich. C16-*threo*-Ceramide was prepared following reported procedures.[44] DBCO-PEG4-TAMRA was acquired from Sigma Aldrich. Dibenzocyclooctyne (DBCO) Agarose was purchased from Jena Bioscience, Germany. Complete protease Inhibitor (EDTA free) and PhosSTOP (Phosphatase inhibitor) were purchased from Roche Eagle's Minimum Essential Medium (MEM), Pyruvate, *L*-Glutamine and PenStrep-mix were purchased from PAN BioTech. Foetal calf serum (FCS) was purchased from GIBCO-Invitrogen. LysC and Trypsin (proteomic grade) were acquired from Wako and Roche respectively. Cell culture flasks were purchased from Sarstedt. The ProtoArray® Human protein microarray was purchased from Invitrogen, Thermo Fisher Scientific. SDS-PAGE was performed for standard protein separation using the MiniProtean 3 Gel electrophoresis system (Bio-Rad, Germany); SDS Separating Gel (12%, 20 mL) contains 6.60 mL H_2O, 8.00 mL Acrylamide mix (30%), 5.00 mL Tris (1.5 M, pH 8.8), 0.20 mL SDS (10%), 0.20 mL Ammonium persulfate (10%) and 0.008 mL TEMED (N, N, N', N'-Tetramethylethylendiamine). SDS Stacking Gel (5%, 5 mL) contains 3.40 mL H_2O, 0.83 mL acrylamide mix (30%), 0.63 mL Tris (1.0 M, pH 6.8),0.05 mL SDS (10% (w/v), 0.05 mL Ammonium persulfate (10% (w/v) and 0.005 mL TEMED.

Cell culture. Neuro-2a cells (ATCC®CCL-131™, mus musculus, mouse) were cultured in Eagle's Minimum Essential Medium (MEM, PAN BioTech, Aidenbach, Germany), supplemented with 10% fetal calf serum (FCS, GIBCO-Invitrogen, Darmstadt, Germany), sodium pyruvate (PAN BioTech, Aidenbach, Germany), *L*-Glutamine and PenStrep-mix (100 U/mL penicillin/100 µg/mL streptomycin (PAN Biotech, Aidenbach Germany) and grown at 37°C in a 5% CO_2 incubator.

Preparation of cell lysates

The Neuro-2a cells were cultured and harvested after they reached confluence. After washing steps with PBS, the cells were incubated with trypsin/EDTA solution for 3-5 min at 37°C in a 5% CO_2 incubator, washed with PBS and lysed in lysis buffer (50 mM NaCl, 5 mM $MgCl_2$, 5 mM EGTA, 0.1 % NP-40, 0.1 % Tween-20, 0.1 % TritonX-100, Protease-/phosphatase-inhibitors (Complete protease Inhibitor (EDTA free) and PhosSTOP (Phosphataseinhibitor) Roche), pH 7.4) (protein concentration >2 mg/mL). The cells were unlocked by repeating freeze-thaw cycles and a final ultra-sonication step. The protein concentration of the supernatant was determined by the Bradford method

Probe Immobilization on DBCO Agarose

Homogenised DBCO Agarose (500 µL, *9.38* µmol DBCO-groups/mL resin*, Jena BioScience GmbH, Germany*) were washed two times with 1 mL PBS *(*140 mM NaCl, 10 mM Phosphate buffer, 3 mM KCl, pH 7.4*)* and 500 µL of a 2 mM solution of either ωN_3C16Cer ***(1)*** or palmitoyl azide (**2**) in PBS (stock solution 10 mM in DMSO) were added to the beads and incubated for 60 min at 4 °C with rotary shaking. The resulting modified beads were then collected and washed twice with PBS.

Affinity enrichment/Pull-down (PD) experiments

100 µL of the corresponding modified DBCO Agarose were collected, washed twice with PBS and 100 µL of cell lysate (2 mg total protein) were added to the beads (ratio cell lysate: beads = 1:1 (v/v)). The mixture was incubated at 4°C overnight with rotary shaking. To remove unbound proteins, the beads were washed twice with lysis buffer (without protease and phosphatase inhibitors) and three more times using increasing concentrations of $MgCl_2$ (25-75 mM). Finally, the beads were washed two more times for 15 minutes with PBS to remove detergents to facilitate the subsequent mass analysis.

Sample Preparation for Mass Spectrometry

On-bead digestion. 50 µL of a 8 M denaturing/reducing buffer (8 M urea in 50 mM Tris pH 7.9; 1 mM DTT) were added to 25 µL of the beads and the suspension was incubated for 30 min on a shaker at 350 rpm (37°C). For alkylation, 5 µL of 50 mM chloracetamide in denaturing/reducing buffer were added to the beads and the mixture was incubated for 30 min on a shaker at 350 rpm at room temeprature. 5 pM BSA were used as a positive control of the tryptic digestion. 1 µg of LysC was added to each sample and incubated for 60 min at 37°C on a shaker at 350 rpm to pre-digest the samples. The supernatant was transferred into a new low-binding Eppendorf tube. 150 µL Tris-buffer (50 mM, pH 7.5) containing 1 µg Trypsin were added to the beads and incubated for 60 min on a shaker at 350 rpm (37°C). The supernatants were combined in a new low-binding Eppendorf tube and 2 µg Trypsin (1 mg/mL in 10 mM HCl) were then added. The final digestion was run overnight on a shaker at 350 rpm (37°C). The reaction was stopped by adding 2 µL of concentrated trifluoroacetic acid.

Stage tip purification of on-bead digested samples. Proteins were desalted in C18 stage tips. Briefly, the tips were prepared as follows. Two 3M high performance extraction disks (C18, 47 mm diameter, cat no: 22152) were placed in an ordinary pipette tip. The stage tips were activated by adding 100 µL methanol and centrifuged. They were then washed twice with 100 µL buffer B (20% water/ 80% acetonitrile with 0.1% formic acid), centrifuged, and finally washed with 100 µL buffer A (water with 0.1% formic acid) twice and centrifuged again. After that, the samples were loaded onto the tips, in multiple portions if necessary, incubated for 1 min and centrifuged. The tips were washed once with 100 µL buffer A and peptides were eluted by using 20 µl buffer B twice, centrifuged and collected into new low-binding Eppendorf tubes. The samples were dried in a SpeedVac concentrator and analysed

Mass spectrometry analysis

The tryptic peptides were separated and analysed using a nano-HPLC/MS/MS. The separations were carried out on an UltiMateTM 3000 RSLCnano system (Dionex, Germany). The MS and MS/MS experiments were carried out on a Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer equipped with a nano-spray source (Nanospray Flex Ion Source, Thermo Scientific). All solvents employed were LC-MS grade. The dried tryptic peptides were dissolved in 20 µL of a 0.1 % TFA solution in water. 3 µL of sample were injected onto a pre-column cartridge (5 µm, 100 Å, 300 µm ID * 5 mm, Dionex, Germany) using 0.1 % TFA in water as eluent with a flow rate of 30 µL/min. Desalting was performed for 5 min with eluent flow to waste followed by back-flushing of the sample during the whole analysis from the pre-column to the PepMap100 RSLC C18 nano-HPLC column (2 µm, 100 Å, 75 µm ID × 50 cm, nanoViper, Dionex, Germany) using a linear gradient starting with 95 % water containing 0.1 % formic acid / 5 % acetonitrile containing 0.1 % formic acid and increasing to 70 % water containing 0.1 % formic acid / 30 % acetonitrile containing 0.1 % formic acid after 95 min using a flow rate of 300 nL / min.

The nano-HPLC was online coupled to the Quadrupole-Orbitrap Mass Spectrometer using a standard coated SilicaTip (ID 20 µm, Tip-ID 10 µM, New Objective, Woburn, MA, USA). Mass range of m/z 300 to 1650 was acquired with a resolution of 70000 for full scan, followed by up to ten high energy collision dissociation (HCD) MS / MS scans of the most intense at least doubly charged ions with a resolution of 17500. Experiments were performed in 4 biological replicates with three technical replicates, each. Data evaluation was performed for each biological replicate separately using the MaxQuant software[56] (v.1.5.2.8) including the Andromeda search algorithm and searching the mouse reference proteome of the Uniprot database together with the contaminant databased included in MaxQuant. The search was performed for full enzymatic trypsin cleavages allowing two miscleavages. For protein modifications carbamidomethylation was chosen as fixed and oxidation of methionine and acetylation of the N-terminus as variable modifications. The mass accuracy for full mass spectra was set to 5 ppm and for MS/MS spectra 20 ppm. The false discovery rates for peptide and protein identification were set to 1 %. Only proteins for which at least two peptides were quantified were chosen for further validation. Proteins which were not identified with at least two razor and unique peptides in at least one technical replicate, those from the search of the reverse database used to determine the false discovery rate and known contaminants corresponding to the contaminant database included in MaxQuant were filtered off. Relative quantification of proteins was carried out using the label-free quantification algorithm implemented in MaxQuant. Label-free quantification (LFQ) intensities were logarithmized (log2), the active samples were grouped together and the negative controls as well. Proteins which were not quantified in at least two technical replicates in at least one group were deleted. After that the medians of the LFQ of each protein per group were calculated (if just one LFQ value exist, this was used as median). All proteins only quantified in the active group or with a difference of at least 2 (1 in the biological experiment using azide instead of palmitoyl azide as negative

control) in the median of the logrithmized LFQ intensities were considered as potential hit of a single biological replicate. In a final step the potential hits of all 12 replicates (4 biological replicates with 3 technical ones, each) were taken together and proteins which were potential hits in at least 8 out of the 12 experiments were listed in Table S1 and merged with the hits resulting from the microarray assay. All proteins quantified in the negative group in the median of the logrithmized LFQ intensities were considered as potential negative hits of a single biological replicate. In a final step the potential hits of all nine replicates (3 biological replicates with 3 technical ones, each) were taken together and proteins which were potential hits in at least 6 out of the 9 experiments were listed as potential hydrophobic binders and removed from the final hit list.

Protein Microarray

The identification of ceramide binding proteins was done by following the vendor's instructions. The microarray was pre-chilled from -20 °C to 4°C for around 20 min and washed with 5 mL SMI-buffer (50 mM Tris-HCl pH 7.5, 5 mM $MgSO_4$, 0.1% Tween 20, 1x Roti®Block (Carl Roth, Germany)) for 60 min at 4°C in a humidity chamber under mild orbital shaking (50 rpm). After removal of the buffer, 600 µL of the corresponding ceramide analogue (10 µM in SMI-buffer) were added dropwise to the microarray and the slide was incubated for 90 min at 4 °C in a humidity chamber under orbital shaking at 50 rpm. Afterwards, the slide was washed 3 times with 5 mL SMI-buffer for 5 min (50 rpm, 4°C). For the cupper-free click reaction 5 mL of a 100 µM DBCO-PEG4-TAMRA solution in SMI-buffer were added dropwise to the microarray. After incubation for 45 min at 4°C under orbital shaking (50 rpm) in a dark humidity chamber, all solutions were removed and the slide was washed 3 times with 5 mL SMI-buffer. Finally, the microarray was dipped in ddH_2O, to remove remaining salts, and completely dried under argon. To ensure the drying process, the slides were additionally centrifuged at 200 x g for 1 min at room temperature. The chip was protected from light until being analysed using a Typhoon®-Microarray Scanner system (Abs/Em = 545/567nm).

Microarray analysis

Spot and background intensity were measured for each spot. Data were normalised against background and only signals that were at least 2-fold or more than the background were considered to be potential interacting candidates. Two additional criteria were used to define a significant interaction between a protein and ceramide: A *Z*-score greater than 3.0 (Z-score was calculated by subtracting the overall average intensity from the raw intensity data for each spot, and dividing that result by the SD of the measured intensities) and a replicate spot CV less than 50%, where CV is the coefficient of variation for the assay from duplicate spots, determined by dividing the SD of the spot signals by their mean intensity.

Immunoblotting

Cells were collected and lysed following the procedure described above. Protein concentration was determined using the Bradford method. DBCO-beads were modified and treated with cell lysates as described above. To release the proteins from the beads, samples were adjusted to the same protein concentration, mixed with SDS loading buffer (5x, 50% (v/v) glycerol, 250 mM Tris (pH 6.8), 350 mM SDS, 500 mM DTE, 360 µM bromophenol blue) and boiled for 5-10 min at 96°C prior to loading onto the gel. Protein samples were loaded onto a SDS gel. The separation of the proteins was conducted in SDS running buffer (2.5 M glycine, 250 mM Tris, 35 mM SDS) at 40 mA per gel for 1 h at room temperature. Estimation of the protein size was performed in reference to the PageRulerTM Plus Prestained Protein Ladder (Thermo Fisher Scientific). To stain the proteins, the SDS gel was incubated with a fixing Solution (10% (v/v) glacial acetic acid, 40% (v/v) ethanol, in water) for 15 min and immediately transferred into a Coomassie Staining Solution (0.25% (w/v) Coomassie brilliant blue G-250, 45% (v/v) methanol, 10% (v/v) acetic acid) and stained for 1 h. Afterwards the SDS gel was de-stained using a de-staining Solution (30% (v/v) methanol, 10% (v/v) acetic acid) and rinsed with ddH_2O. The gel was imaged using the Gellogic 200 Imaging System (KODAK) and KODAK-Mi software.
The proteins separated by SDS-PAGE were transferred by means of semi-dry electroblotting to a polyvinylidene difluoride (PVDF) membrane (Immobilon® FL- Transfer Membrane, Thermo Scientific, Schwerte, Germany) with a Bio-Rad Trans-Blot semi-dry blotter for 60 min at 25 V prior to the electrotransfer, all the layers were soaked in freshly prepared transfer buffer (14.41 g/L glycin, 3.03 g/L Tris, 20% (v/v) MeOH, in water). Membranes were blocked for 30 min using 2% (w/v) non-fat dry milk powder in PBS-buffer containing 0.05 % (v/v) Tween-20, pH 7.5 (PBS-T). The appropriate primary antibody was incubated overnight at 4°C in the buffer following vendor's instructions. Anti-α-tubulin was employed to detect α-tubulin as loading control. Unbound antibodies were removed by washing with PBS-T buffer for 10 min (3 times). The

corresponding secondary antibody was added according to vendor's instructions and incubated for 1 h at room temperature at constant agitation in the dark. The membrane was again washed twice with PBS-T buffer (10 min) and twice with PBS (10 min) to decrease the detergent concentration. Signals were detected using Super-Signal West Pico Chemiluminescent Substrate (Thermo Scientific) and scanned employing an Odyssey® Fc Imaging System (Li-COR® Bioscience, Bad Homburg, Germany).

Primary antibodies for immunoblotting

Antibody	Organism	Order No	Dilution	Supplier
Anti-Tubulin	mouse	# F2168	1:1000	Sigma Aldrich
Anti-Bcl-xL	rabbit	# 2764	1:1000	CellSignaling Technology®
Anti-mTOR	rabbit	# 2983	1:1000	CellSignaling Technology®
Anti-mTOR	mouse	# 4517	1:1000	CellSignaling Technology®
Anti-Aifm2	rabbit	# PA5-24562	1:1000	Thermo Scientific
Anti-PPT1	rabbit	# WA-AP2538b	1:1000	Biomol
Anti-Bax	rabbit	# sc-526	1:200	Santa Cruz Biotechnology
Anti-Ergic1	goat	# sc-107206	1:200	Santa Cruz Biotechnology
Anti-Oxa1L	mouse	# sc-365534	1:200	Santa Cruz Biotechnology
Anti-CERT	rabbit	# PA5-28788	1:500	InvitrogenTM
Anti-BUD31	mouse	# sc-374163	1:200	Santa Cruz Biotechnology
Anti-APP	rabbit	# A8717	1:200	Sigma Aldrich
Anti-Gal1	mouse	# 40103	1:1000	CellSignaling Technology®
Anti-Gal3	rabbit	# 87985	1:1000	CellSignaling Technology®

Secondary antibodies for immunoblotting

Secondary Antibody	Reactivity	Order No	Supplier
donkey-anti-goat HRP	Goat	# sc-2033	Santa Cruz Biotechnology
goat-anti-mouse HRP	Mouse	# 31430	Thermo Scientific
goat-anti-rabbit HRP	Rabbit	# 31460	Thermo Scientific
donkey-anti-goat IR680	Goat	# 926-68074	Li-Cor
donkey-anti-goat IR800	Goat	# 926-32214	Li-Cor
donkey-anti-mouse IR680	Mouse	# 926-68072	Li-Cor
goat-anti-mouse IR800	Mouse	# 926-32210	Li-Cor
donkey-anti-rabbit IR680	Rabbit	# 926-68073	Li-Cor
donkey-anti-rabbit IR800	Rabbit	# 926-32213	Li-Cor
goat-anti-rat IR800	Rat	# 926-32219	Li-Cor

Competition experiments

For the competitive experiments, increasing concentrations (50 –500 µM) of C16-Cer were added to the cell lysates (2 mg of total protein). The mixture was incubated at 4°C for 2 h with rotary shaking. The corresponding modified DBCO Agarose (ration cell lysates: beads= 1:1 (v/v)) was added to the preincubated mixture and incubated at 4°C overnight with rotary shaking. To remove unbound proteins, the beads were washed twice with PBS, boiled with 5xSDS-loading buffer and further processed by SDS-gel and immunoblot analysis as described above.

Cellular thermal shift assay

Cells were harvested, lysed and divided into two equal portions of 1300 µL. 13 µL of a 10 mM stock solution of C16-Cer in DMSO or a 10 mM solution of Palmitic acid in DMSO were added to 1300 µL of cell lysate (protein concentration 8 mg/mL), resulting in a final concentration of 100 µM of C16-Cer and 1% DMSO. Analogously, 13 µl of DMSO were added to 1300 µL of cell lysate. The samples were pre-incubated for 10 min at room temperature and divided into ten equal samples (130 µL). Samples were heated for 5 min to the target temperature (range: 42.0-63.40 °C) and then incubated for 5 min at room temperature. Samples were then centrifuged and the supernatant was transferred into a new eppendorf tube, boiled with 5xSDS-loading buffer and further processed by SDS-gel and immunoblot analysis. Data was normalized to protein content in the samples at 42.0 °C. Data represent mean values ± S.D. n=3 and was analysed by a Student's two-tailed t-test with GraphPad Prism® software version 5.0 (GraphPad, San Diego, CA, USA) and it is represented as mean ± SD.

Protein expression and purification

Fully sequenced human cDNA constructs for BCL-XL was purchased from Addgene (#46972 pCDH-puro-BCL-XL, Addgene, USA). cDNA construct for AIFm2 was a kind gift of Dr S. Kapoor. The nucleotide sequence corresponding to the proteins was amplified and subcloned in a pOPIN-NHis-3C. vector. Proteins were expressed in *Escherichia coli* cells BL21 (DE3) after autoinduction at 25 °C in the presence of ampicillin and purified by means of Ni-sepharose affinity chromatography followed by size-exclusion chromatography. Gal-1 and iFABP were a kind gift of Prof. H. Waldmann and Dr. I. Vetter, respectively.

Differential scanning fluorimetry (DSF)

Differential scanning fluorimetry (DSF) assay was performed using a RT-PCR device (CFX96 Real-Time System; C1000 Touch Thermal Cycler (BIO-RAD)) as previously described. [40, 41]DSF was carried out by equilibrating the samples at 25 °C for 5 minutes and then increasing temperature to 90 °C at a rate of 2 °C/minute. Data points were collected at 0.5 °C intervals. Data evaluation for melting temperatures was performed using a pre-installed analysis software provided with the instrument (CFX Manager Version 3.1.1517.0823). Optimal concentrations of the protein and SYPRO orange dye were determined by performing DSF on a grid of varying concentrations of protein and SYPRO orange in PBS Buffer. The optimal conditions were ~0.25 mg/mL protein in PBS Buffer and 2X SYPRO orange (diluted from 5000X stock) within the reaction well, as determined by maximum range of relative Fluorescence Units observed in the melting curves. In a single well of a 96-well PCR plate, a 20 µL reaction was conducted by combining 10 µL of protein solution (SYPRO orange in PBS Buffer) and 10 µL of a solution containing lipids diluted from 10mM DMSO stock with SYPRO orange in PBS Buffer) or 2 % DMSO (v/v) in SYPRO orange in PBS Buffer (as vehicle control). All experiments were done in triplicates.

Supplementary References

Cox, J., and Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol *26*, 1367-1372.
Niesen, F.H., Berglund, H., and Vedadi, M. (2007). The use of differential scanning fluorimetry to detect ligand interactions that promote protein stability. Nat protoc *2*, 2212-2221.

Supplementary Figures

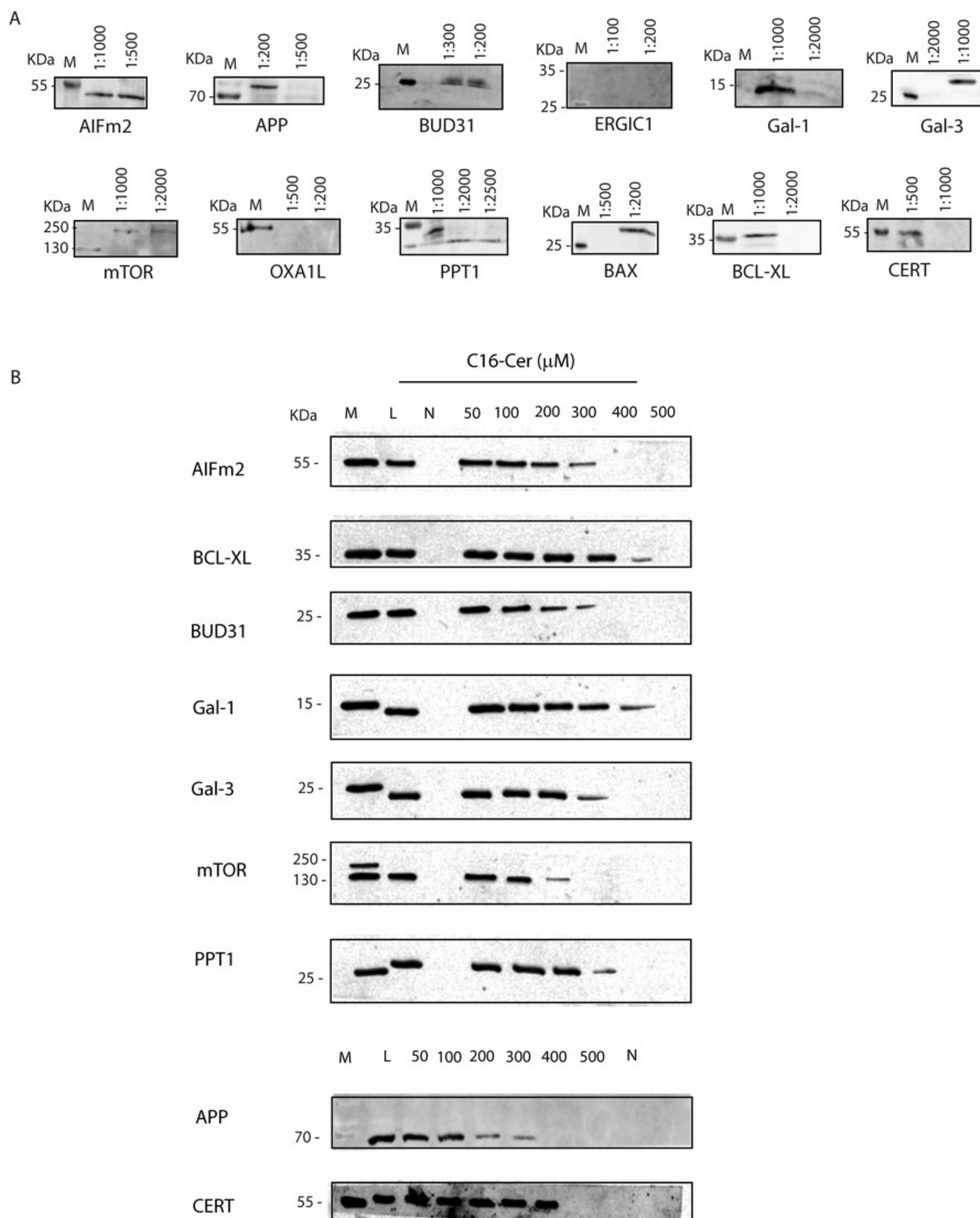


Figure S1. A) Experimental validation of representative ceramide targets. N2a cell lysates were treated with ceramide-modified DBCO agarose. Protein-lipid complexes presents were enriched and analysed by SDS-PAGE and Western blotting. Representative blots of 5

biological replicates. B) Representative blots of competition experiments with C16-ceramide. M; marker, L; lysate, N; negative control.

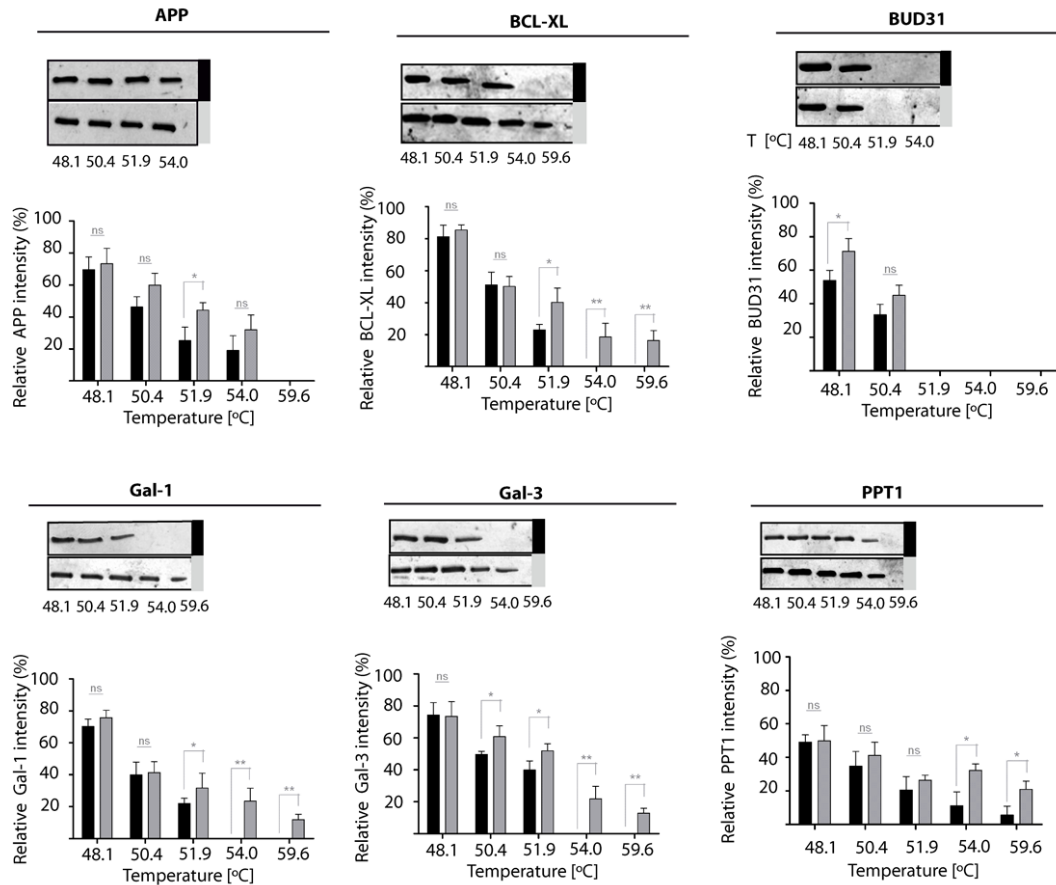


Figure S2. Cellular thermal shift assays were performed to measure the binding ability of C16-Cer to the tested proteins. Cell lysates were incubated with DMSO (1%), with C16-Cer (100 μM, 1% DMSO) or with palmitic acid (100 μM, 1% DMSO). Data was normalized to protein content (42 °C) and are presented as mean ± SD (n = 3). A student's two-tailed t test was used to determine P values *P < 0.05, **P < 0.01, NS: not significant.

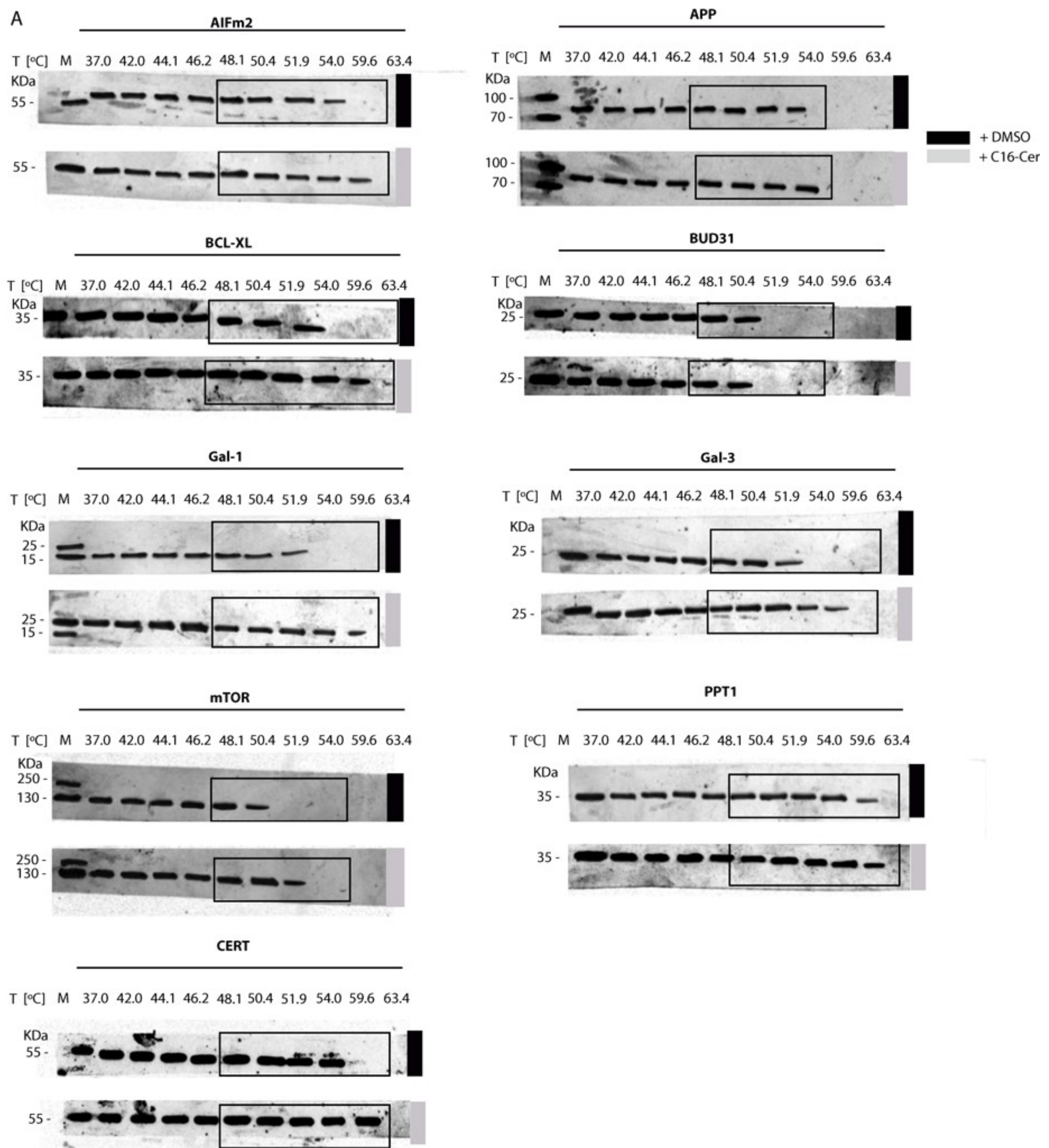


Figure S3. Cellular thermal shift assay was performed to measure the binding ability of C16-Ceramide (100 µM, 1% DMSO) to the tested proteins. Uncroppped images of the blots.

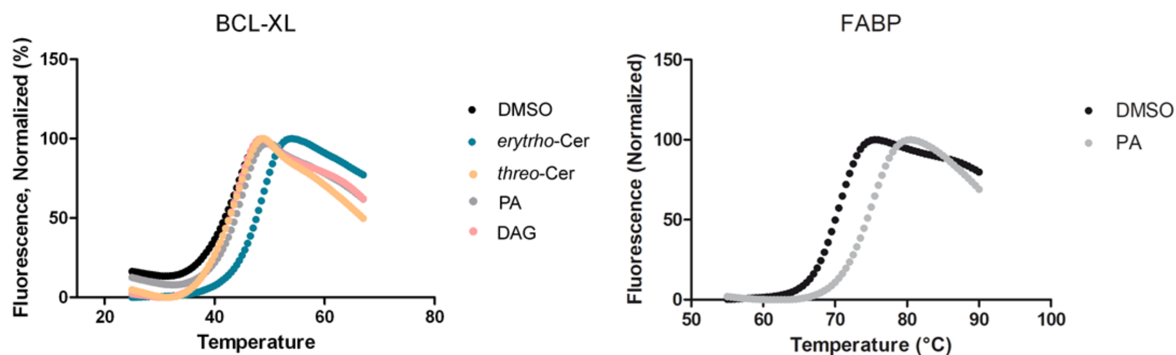


Figure S4. Differential scanning fluorimetry. Experiments were performed in the absence of any compounds as a negative control (DMSO) and in the presence (10 μM) of *erytrho*-Cer, *threo*-Cer, palmitic acid (PA) or diacylglycerol (DAG). Presence of *erytrho*-Cer and palmitic acid resulted in stabilization of BCL-XL (ΔT_m of 5.9 °C) and iFABP (ΔT_m of 4.7 °C) respectively. The inflection point of each transition curve is considered melting temperature T_m and the increase in ΔT_m is an indication of binding.