

Table S1. ABPP-MudPIT analysis of serine hydrolase activities in brains from ABHD4^{+/+} and ABHD4^{-/-} mice. Average spectral count values of serine hydrolases in brains from ABHD4^{+/+} and ABHD4^{-/-} mice were shown. Data are means $\pm$ SEM ($n = 4$ for ABHD4^{+/+}, $n = 5$ for ABHD4^{-/-}).

		ABHD4 ^{+/+}		ABHD4 ^{-/-}	
Protein	Abbreviation	Spectral count	SEM	Spectral count	SEM
Prolyl endopeptidase	PREP	2888	345	3193	489
Esterase D	ESD	56	4	278	78
Monoglyceride lipase (MAGL)	MGLL	866	133	692	159
Prolyl endopeptidase-like isoform C	PREPL	964	167	1175	66
Retinoblastoma-binding protein 9	RBBP9	160	23	174	54
Abhydrolase domain-containing protein FAM108B1	FAM108B1	85	8	70	7
FASN fatty acid synthase	FASN	2099	415	1999	510
Acylamino acid-releasing enzyme	APEH	939	157	1128	225
Acyl-CoA thioesterase 2	ACOT2	1022	146	1239	180
Acyl-protein thioesterase 1	LYPLA1	319	84	390	119
Protein phosphatase methylesterase 1	PPME1	133	46	182	64
Abhydrolase domain-containing protein FAM108C1	FAM108C1	48	6	43	7
Abhydrolase domain-containing protein 6	ABHD6	515	56	434	84
Abhydrolase domain-containing protein 12	ABHD12	280	59	275	49
Acyl-CoA thioesterase 1	ACOT1	1042	163	1153	174
Dipeptidyl peptidase 9	DPP9	431	90	407	102
Abhydrolase domain-containing protein 3	ABHD3	113	39	147	59
Platelet-activating factor acetylhydrolase 1B subunit gamma	PAFAH1B3	244	62	247	66
Fatty acid amide hydrolase 1	FAAH	474	102	498	139
Neutral cholesterol ester hydrolase 1	NCEH1	853	182	721	138
Dipeptidyl peptidase 8	DPP8	698	74	562	42
Acyl-protein thioesterase 2	LYPLA2	787	197	986	283
Abhydrolase domain-containing protein FAM108A	FAM108A	61	12	86	27
Carboxylesterase 1c	CES1C	470	88	605	133
Platelet-activating factor acetylhydrolase 2	PAFAH2	55	10	59	9
Platelet-activating factor acetylhydrolase, isoform 1b, subunit 2	PAFAH1B2	490	85	504	114
Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	PLA2G7	131	26	184	42
Isoamyl acetate-hydrolyzing esterase 1	IAH1	17	7	24	3

Neuropathy target esterase	PNPLA6	187	18	150	17
Abhydrolase domain containing 16A	ABHD16A	59	11	57	10
Lipase, hormone sensitive	LIPE	106	16	79	5
Abhydrolase domain-containing protein 10	ABHD10	358	108	368	125
Acetylcholinesterase	ACHE	137	18	184	38
Abhydrolase domain-containing protein 11	ABHD11	111	20	96	25
Patatin-like phospholipase domain containing 7	PNPLA7	61	8	47	6
Patatin-like phospholipase domain containing 8	PNPLA8	47	12	50	16
Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 3	SLC25A3	19	4	21	6
Dipeptidyl peptidase 7	DPP7	36	3	31	4
Phospholipase A2, group XV	PLA2G15	136	25	121	31
Butyrylcholinesterase	BCHE	34	7	30	3
Prolylcarboxypeptidase	PRCP	128	36	73	16
Cathepsin A	CTSA	69	21	91	36
Palmitoyl-protein thioesterase 2	PPT2	11	3	7	2
Retinoid-inducible serine carboxypeptidase	SCPEP1	29	7	40	13
Acyl-CoA thioesterase 6	ACOT6	61	58	94	42
Dipeptidyl peptidase 4	DPP4	26	6	25	9
Phospholipase A1 member A	PLA1A	5	2	5	2
Post-GPI attachment to proteins 1	PGAP1	15	2	12	3
Sn1-specific diacylglycerol lipase beta	DAGLB	13	3	9	2
Lysophospholipase-like protein 1	LYPLAL1	55	33	32	13
Serine hydrolase-like	SERHL	1	1	1	1
DDHD domain containing 2	DDHD2	5	3	7	4
Abhydrolase domain-containing protein 13	ABHD13	2	1	2	1
Abhydrolase domain-containing protein 4	ABHD4	14	2	0	0

Table S2. Untargeted lipidomic profiling of ABHD4^{+/+} and ABHD4^{-/-} brain tissue.

Representative group of phospholipids, lysophospholipids, free fatty acids and MAGs from ABHD4^{+/+} and ABHD4^{-/-} mice were quantified by manual integration. MAG, PC, and LPC species were measured in positive ionization mode, and *m/z* values represent [M+H]⁺ ions. All other lipids were measured in negative ionization mode, and *m/z* values represent [M-H]⁻ or [M-2H]²⁻ ions. Relative lipid abundance in ABHD4^{-/-} vs. ABHD4^{+/+} brain lipids (KO/WT) is presented as a ratio of the average integrated peak area (*n* = 4 per genotype). Statistical analysis was performed by the unpaired, two-tailed Welch's *t* test. MAG; monoacylglycerol, PC; phosphatidylcholine, LPC; lysoPC, PE; phosphatidylethanolamine, LPE; lysoPE, PS; phosphatidylserine, LPS; lysoPS, PI; phosphatidylinositol, LPI; lysoPI, PA; phosphatidic acid, LPA; lysosPA, FFA; free fatty acid.

Lipid	<i>m/z</i>	Ion	Retention time (min)	Fold Change (KO/WT)	<i>P</i> value
MAG					
16:0	331.3	[M+H] ⁺	39.6	1.0	0.9578
18:1	357.3	[M+H] ⁺	40.1	0.8	0.2802
18:0	359.3	[M+H] ⁺	42.1	0.9	0.6320
20:4	379.3	[M+H] ⁺	38.9	1.0	0.6541
22:6	403.3	[M+H] ⁺	39.0	1.0	0.9624
PC					
32:1	732.4	[M+H] ⁺	46.7	1.0	0.7988
32:0	734.4	[M+H] ⁺	47.1	0.9	0.3152
34:1	760.4	[M+H] ⁺	47.6	0.8	0.0866
34:0	762.4	[M+H] ⁺	48.4	1.0	0.8233
36:4	782.4	[M+H] ⁺	47.0	1.0	0.7305
36:2	786.4	[M+H] ⁺	47.8	0.8	0.1566
36:1	788.4	[M+H] ⁺	48.8	1.1	0.4810
38:6	806.3	[M+H] ⁺	46.8	1.0	0.6351
38:4	810.4	[M+H] ⁺	48.2	0.8	0.2388
40:6	834.4	[M+H] ⁺	48.0	0.9	0.3005
LPC					
16:0	496.3	[M+H] ⁺	38.6	1.1	0.1547

18:1	522.4	[M+H] ⁺	39.2	1.0	0.7577
18:0	524.4	[M+H] ⁺	41.3	1.1	0.3615
20:4	544.3	[M+H] ⁺	37.1	1.1	0.4146
22:6	568.3	[M+H] ⁺	37.1	1.3	0.0154
PE					
34:1	716.5	[M-H] ⁻	41.7	0.9	0.1289
38:5	764.5	[M-H] ⁻	40.7	0.9	0.0261

Table S2. Cont.

38:4	766.5	[M-H] ⁻	42.8	0.9	0.0962
LPE					
16:0	452.3	[M-H] ⁻	30.0	0.9	0.3186
18:1	478.3	[M-H] ⁻	30.5	0.9	0.4558
18:0	480.3	[M-H] ⁻	32.1	1.0	0.8361
20:4	500.3	[M-H] ⁻	29.4	0.9	0.3901
22:6	524.3	[M-H] ⁻	29.4	1.0	0.9917
PS					
34:1	760.5	[M-H] ⁻	33.4	0.9	0.1213
36:2	786.5	[M-H] ⁻	33.9	1.0	0.8118
36:1	788.5	[M-H] ⁻	36.3	1.0	0.6112
38:5	808.5	[M-H] ⁻	33.0	0.8	0.0098
38:4	810.5	[M-H] ⁻	34.9	0.8	0.0016
40:6	834.5	[M-H] ⁻	35.8	1.0	0.8236
LPS					
16:0	496.3	[M-H] ⁻	24.5	1.1	0.5422
18:1	522.3	[M-H] ⁻	25.0	1.1	0.7584
18:0	524.3	[M-H] ⁻	26.1	0.9	0.4912
20:4	544.3	[M-H] ⁻	24.0	0.9	0.4566
22:6	568.3	[M-H] ⁻	24.0	1.1	0.5790
Lyso-NAPS					
34:0	380.8	[M-2H] ²⁻	29.9	0.04	0.0001
34:0	762.5	[M-H] ⁻	29.9	0.07	0.0002
PI					
38:4	885.5	[M-H] ⁻	37.1	1.0	0.2827
LPI					
18:0	599.3	[M-H] ⁻	28.9	1.1	0.5322
20:4	619.3	[M-H] ⁻	26.4	1.0	0.8941

Table S2. Cont.

PA					
32:0	647.5	[M-H] ⁻	32.3	1.8	0.3790
34:1	673.5	[M-H] ⁻	32.3	1.1	0.9093
36:4	695.5	[M-H] ⁻	31.7	1.1	0.8900
LPA					
16:0	409.2	[M-H] ⁻	23.3	1.0	0.7699
18:1	435.3	[M-H] ⁻	23.6	1.1	0.7955
18:0	437.3	[M-H] ⁻	24.9	1.1	0.3680
20:4	457.2	[M-H] ⁻	22.7	1.1	0.7650
FFA					
16:0	255.2	[M-H] ⁻	27.2	1.1	0.3846
18:1	281.2	[M-H] ⁻	27.6	1.0	0.9500
18:0	283.3	[M-H] ⁻	28.9	1.0	0.6083
20:4	303.2	[M-H] ⁻	26.9	1.0	0.7364
22:6	327.2	[M-H] ⁻	27.0	1.1	0.2803

Supporting Figure Legends

Figure S1. NAE, lyso-NAPE, GP-NAE, NAPE, and pNAPE content of brain tissue from ABHD4^{+/+} and ABHD4^{-/-} mice

(A) Targeted mass spectrometry (MS)-based measurements of NAE species of brain tissue from ABHD4^{+/+} and ABHD4^{-/-} mice. No significant differences were observed. Data represent mean values $\pm$ SEM ($n = 5$). (B) Targeted MS-based estimates of GP-NAE levels from lipids extracted under acidic conditions from brains from ABHD4^{+/+} and ABHD4^{-/-} mice. Lipids were extracted with 8 mL of 2:1:1 (vol/vol/vol) CHCl₃: MeOH:1% formic acid and GP-NAE levels were estimated by targeted MRM and comparison to internal standard. (C) Targeted MS-based estimates of lyso-NAPE abundance in brain tissue from ABHD4^{+/+} and ABHD4^{-/-} mice. Data represent mean values $\pm$ SEM ($n = 5$). **, $P < 0.01$. Unpaired, two-tailed t test was used. Data represent mean values $\pm$ SEM ($n = 5$). *, $P < 0.05$; ***, $P < 0.001$. Unpaired, two-tailed t test was used. (D, E) Targeted MS-based estimates of NAPE (D) and pNAPE (E) species from brain tissue from ABHD4^{+/+} and ABHD4^{-/-} mice. Data represent mean values $\pm$ SEM ($n = 5$). ***, $P < 0.001$. Unpaired, two-tailed t test was used.

Figure S2. NAS content of brain tissue from ABHD4^{+/+} and ABHD4^{-/-} mice.

(A, B, C) MS/MS fragmentation pattern of endogenous *N*-16:0 serine ([M - H]⁻ at m/z 342.3) (A), *N*-20:4 serine ([M - H]⁻ at m/z 390.3) (B), and synthetic *N*-17:1 serine ([M - H]⁻ at m/z 354.3) (C). Key daughter ions in the MS/MS analysis include 74.0 ([M - H - RCO - CH₂O]⁻), 104.0 ([M - H - RCO]⁻) and [M - H - CH₂O]⁻ as described previously.⁴² (D) Targeted MS-based estimates of the abundance of NAS species of brain tissue from ABHD4^{+/+} and ABHD4^{-/-} mice. No significant differences were observed. *N*-18:1

and N-20:4 serine levels were under detection. Data represent mean values $\pm$ SEM ($n = 4$).

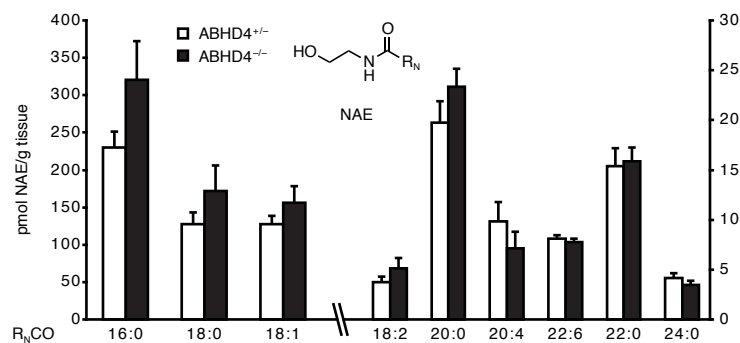
Figure S3. (Lyso) *N*-acyl phospholipid lipase activities of ABHD4-transfected cells and brain tissue from ABHD4^{+/+} and ABHD4^{-/-} mice.

(A, B) Soluble fractions of mock- and ABHD4-transfected COS-7 cell extracts (A) or brain tissue from ABHD4^{+/+} and ABHD4^{-/-} mice (B) were incubated with NAPS (1-stearoyl-2-oleoyl-*sn*-glycero-3-phospho (*N*-palmitoyl) serine) and hydrolytic activity was quantified by measuring lyso-NAPS (1-stearoyl-2-oleoyl-*sn*-glycero-3-phospho (*N*-palmitoyl) serine) release in the absence (A) or presence (A, B) of 0.1% of Triton X-100 expressed in arbitrary units. Data represent mean values $\pm$ SEM ($n = 4$ for A, $n = 5$ for B). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Unpaired, two-tailed t test was used.

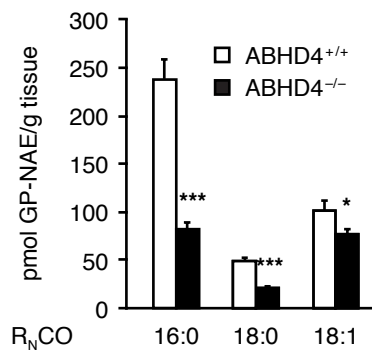
(C) Soluble fractions of brain tissue from ABHD4^{+/+} and ABHD4^{-/-} mice were incubated with NAPE (1,2-dioleoyl-*sn*-glycero-3-phospho (*N*-arachidonoyl) ethanolamine) or lyso-NAPE (1-oleoyl-2-hydroxy-*sn*-glycero-3-phospho (*N*-palmitoyl) ethanolamine) in the presence of 0.1% of Triton X-100 and hydrolytic activity was quantified by measuring lyso-NAPE or oleic acid release, respectively. Data represent mean values $\pm$ SEM ($n = 5$). **, $P < 0.01$; ***, $P < 0.001$. Unpaired, two-tailed t test was used.

Figure S1

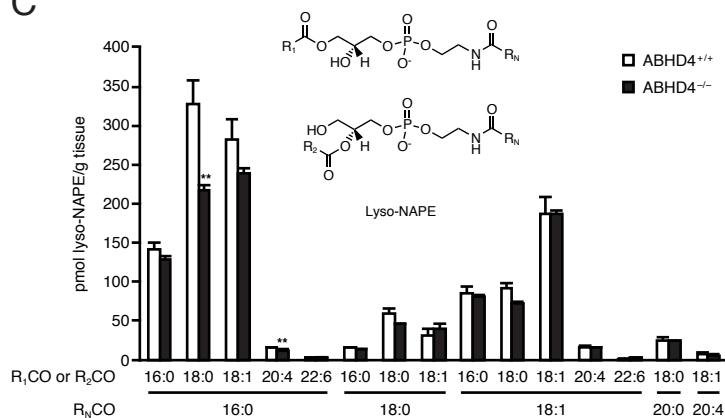
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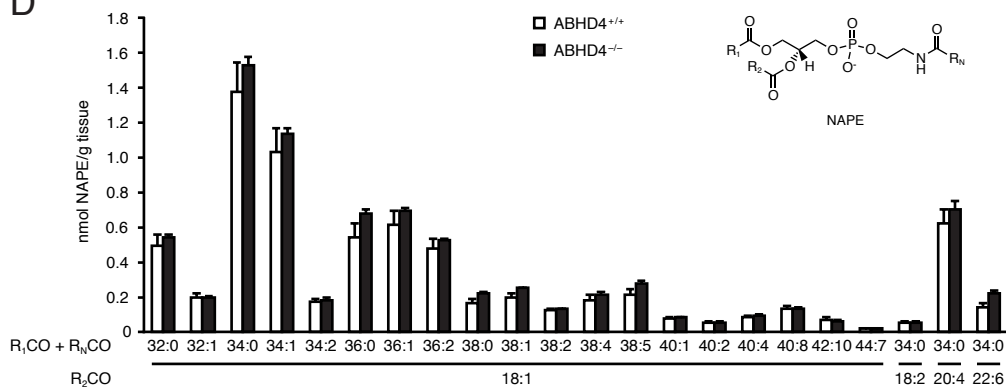
B



C



D



E

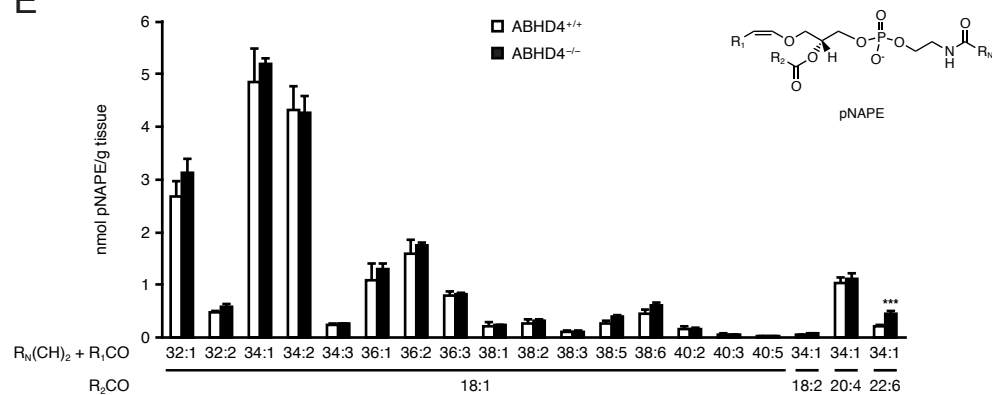


Figure S2

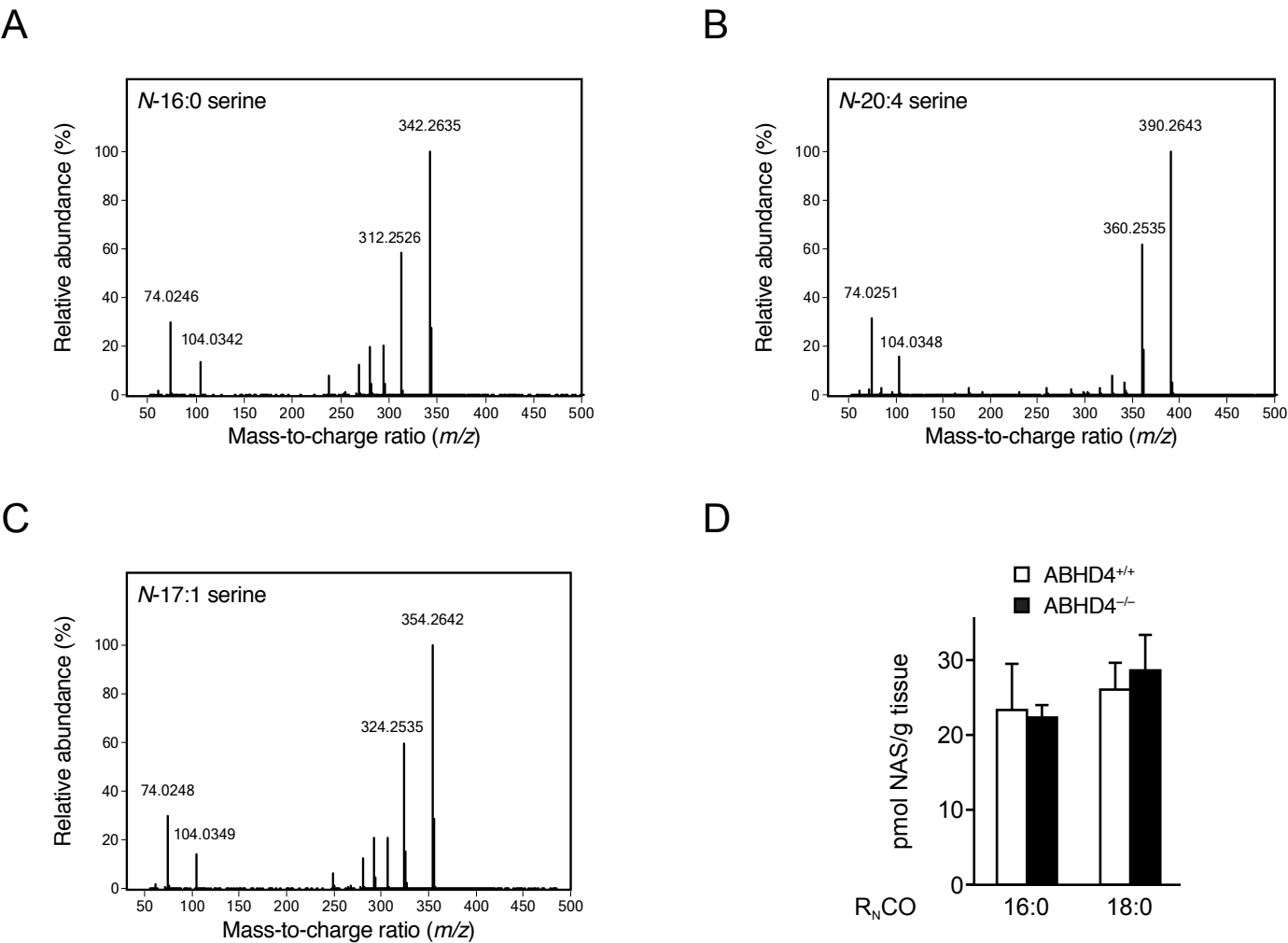


Figure S3

