The spastic paraplegia-associated phospholipase DDHD1 is a primary brain phosphatidylinositol lipase

Jordon M. Inloes, Hui Jing, Benjamin F. Cravatt†

Department of Chemistry, The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California 92037, United States

SUPPORTING INFORMATION

Methods

Western blotting

Rabbit polyclonal DDHD1 antibody (Thermo Fisher Scientific, Cat. No. PA5-62070) was used for Western Blotting at a concentration of 1:1000. Mouse monoclonal GAPDH antibody (Santa Cruz Biotechnology, Cat. No. sc-47724) was used for Western Blotting at a concentration of 1:200. Secondary antibodies IRDye® 800CW Goat anti-Mouse IgG (Cat. No. 926-32210) and IRDye® 680LT Donkey anti-Rabbit IgG (Cat. No. 926-68023) were used for Western Blotting at concentrations of 1:10,000.

Behavioral characterization of DDHD1$^{-/-}$ mice

All the behavioral tests were performed in The Scripps Research Institute mouse behavior assessment core facility. Mice were housed in groups in a temperature-controlled room with lights regulated on a 12-hr light/dark cycle (lights off at 0600 hours) and food and water were available ad libitum. Behavior was monitored during the dark phase when mice are most active. For our studies, a cohort of age- and sex-matched DDHD2$^{-/-}$, DDHD2$^{+/-}$, and DDHD2$^{+/+}$ mice from DDHD2$^{+/-}$ parents (n = 13 mice per genotype, mixed sex) were tested for locomotor activity and cognition at 6 months of age. Animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Footprint pattern test. Basic gait measures can be assessed using simple footprint pattern analysis [1,2.] For example, a mouse model of Ataxia telangiectasia had shorter stride lengths than WT mice [3]. Non-toxic paint was applied to each mouse's paws (a different color was used for front and back paws). The mouse was then placed at one end of a runway covered in paper and allowed to ambulate until their paws no longer left marks. Measurements were forelimb and hindlimb stride lengths (left and right). Three full strides were averaged for each mouse's values. Data were excluded from mice that did not make 3 measurable strides (i.e. they circled or stopped).

Hanging wire test. The hanging wire test allows for the assessment of grip strength and motor coordination[4,5]. Mice were held so that only their forelimbs contact an elevated metal bar (2 mm diameter, 45 cm long, 37 cm above the floor) held parallel to the table by a large ring stand and let go to hang. Each mouse was given three trials separated by 30 seconds. Each trial was scored as follows and the average for each mouse was calculated: 0 — fell off, 1 — hung onto the wire by two forepaws, 2 — hung onto the wire by two forepaws, but also attempted to climb onto the wire, 3 — hung onto the wire by two forepaws plus one or both hindpaws around the wire, 4 — hung onto the wire by all four paws plus tail wrapped, 5 — escaped (crawled to the ring stand and righted itself or climbed down the stand to the table). Latency to falling off was also measured up to a maximum of 30 s.

Locomotor activity. Locomotor activity was measured in polycarbonate cages (42 x 22 x 20 cm) placed into frames (25.5 x 47 cm) mounted with two levels of photocell beams at 2 and 7 cm above the bottom of the cage (San Diego Instruments, San Diego, CA). These two sets of beams allowed for the recording of both horizontal (locomotion) and vertical (rearing) behavior. A thin layer of bedding material was applied to the bottom of the cage. Mice were tested for 120 min and data were collected at 5 min intervals.

Rotarod test. Rotarod balancing requires a variety of proprioceptive, vestibular, and fine-tuned motor abilities as well as motor learning capabilities[1]. A Roto-rod Series 8 apparatus (IITC Life Sciences, Woodland Hills, CA) was used which records test results when the animal drops

onto the individual sensing platforms below the rotating rod. An accelerating test strategy was used whereby the rod started at 0 rpm and then accelerated at 10 rpm. The mice were tested in two sets of 3 trials per day for two days, for a total of 12 trials.

Y-maze test for spontaneous alternations. To determine spontaneous alternation behavior, a measure of spatial working memory, exploratory behavior and responsiveness to novelty[6,7] , we tested mice in a Y-maze with 34 x 8 x 14 cm arms. Each mouse received one 5 min trial during which arm choices and total numbers of arm entries were recorded. Spontaneous alternation, expressed as a percentage, refers to the ratio of arm choices differing from the previous two choices, to the total number of arm entries. Mice have the opportunity to do repeated entries into a single arm, resulting in a chance performance level of 22% (2/9) for spontaneous alternations[8,9]. Healthy young mice make 60-70% spontaneous alternations.

von Frey test. Tactile allodynia was tested on the plantar region of the hind paw and the lumbar spine using von Frey filaments with forces of 0.04, 0.16, 0.4, 1.0 and 4.0 g. Filaments were tested by applying them to the paw until they bent slightly in ascending order and repeated ten times. A positive response to the filament was defined as a hind paw flexion reflex. The number of positive responses to each filament was used to create a sensitivity curve. For this test, mice were placed in an open cylinder on top of an elevated wire platform, allowing access from below and acclimated for 5 min prior to testing.

Acetone test. Cold sensitivity was assessed by measuring the time spent in acetone-evoked behaviors for 1 minute after a drop (25 µl) of acetone was applied gently to the plantar surface of the left hind paw (behaviors = paw elevation, flinching, biting, licking, and scratching time).

Hot plate test. To examine thermal nociception (which requires circuitry in the brain as well as spinal cord), mice were placed in a glass cylinder on a 52-52.5 °C or 49.5-50°C hot plate[10]. Latency to a nociceptive response (hind paw lick, flick, or jump) was measured, and mice were immediately removed from the apparatus. If a nociceptive response was not seen within 30 s, the test was stopped.

Structural characterization of lipids altered in DDHD1$^{-/-}$ brain tissue

Fragmentation pattern analysis was performed with the Agilent 6520 Q-TOF. The same column chromatography conditions used for discovery metabolite profiling were used to compare retention times significantly altered DMP features and commercially available lipid standards. MS1 and MS2 spectra were collected with the following MS2 fragmentation parameters: (proposed metabolite, precursor ion, collision energy, fragmentor voltage) C38:4 PI, 883.5, 40 V, 100 V; C38:4 PI, 885.5, 40 V, 100 V; C20:4 LPI, 619.3, 40 V, 100 V.

Targeted lipidomic analysis

Phospholipids were quantified using multiple reaction monitoring (MRM) on an Agilent 6410 QQQ LC/MS instrument. The source temperature was 350 °C, capillary voltage was 3500 V and -3500V for positive and negative mode, respectively, gas flow was 9 L/min, nebulizer pressure was 35 psi. Chromatographic separations were performed using the same mobile phases and column as for discovery metabolite profiling. The following gradient was used: 0% B for 5 min, increase to 100% B over 19 min, hold 100% B for 4 min, switch to 0% B, hold 0%B for 3 min. The following table contains MRM acquisition parameters that were applied to generate lists of lipids based on defined fragmentation patterns. Data were analyzed with MassHunter Quantitative Analysis software (Agilent) and signals for endogenous lipids were normalized to the concentration of appropriate internal standards (C17:1 LPG, C17:1 LPS, C17:1 LPI, C17:0 LPA, C17:0 LPC, C17: 1 LPE, C17:0/C17:0 PS, C16:0 D31 /C18:1 PI, C17:0/C20:4 PI(3)P, C17:0/C20:4 PI(4,5)P_2, C17:0/C20:4 PI(3,4,5)P_3, C16:0 D31/C18:1 PG, C14:0/C14:0 BMP,

C12:0/C12:0 PC, C12:0/C12:0 PE, C16:0 D31/C18:1 PA, C17:1/C17:1/C17:1 TAG, C18:0/C20:4 D8 DAG),and wet weight of tissues or number of cells.

MRM parameters of various phospholipid classes.

Lipid class	Precursor ion	Fragment ion	MS1 resolution	Collision energy (V)	MS2 resolution	Fragmentor (V)	Dwell (ms)	Polarity
PE	$[M+H]^+$	Neutral loss of 141	Unit	20	Unit	100	50	Positive
LPE	$[M+H]^+$	Neutral loss of 141	Unit	15	Unit	100	50	Positive
PC	$[M+H]^+$	184.1	Unit	25	Unit	100	50	Positive
LPC	$[M+H]^+$	184.1	Unit	20	Unit	100	50	Positive
PIP	$[M-H]^-$	240	Unit	28	Unit	172	50	Negative
PIP_2	$[M-H]^-$	Neutral loss of 98	Unit	24	Unit	218	50	Negative
PI	$[M-H]^-$	[fatty acid–H]$^-$	Unit	50	Unit	260	50	Negative
LPI	$[M-H]^-$	[fatty acid–H]$^-$	Unit	36	Unit	260	50	Negative
PS	$[M-H]^-$	[fatty acid–H]$^-$	Wide	35	Unit	100	50	Negative
LPS	$[M-H]^-$	Neutral loss of 87	Wide	15	Unit	100	50	Negative
PG	$[M-H]^-$	[fatty acid–H]$^-$	Unit	40	Unit	150	50	Negative
BMP	$[M+NH4]^+$	[MAG–H2O +H]$^+$	Wide	20	Unit	100	50	Positive
LPG	$[M-H]^-$	[fatty acid–H]$^-$	Unit	40	Unit	100	50	Negative
PA	$[M-H]^-$	[fatty acid–H]$^-$	Unit	40	Unit	150	50	Negative
LPA	$[M-H]^-$	79	Unit	40	Unit	100	50	Negative
PG	$[M+NH4]^+$	neutral loss of 189	Wide	15	Unit	100	50	Positive

Diagnostic fragments that provide structural information about lipid classes are were targeted in MRM mode on an Agilent 6410B QQQ. MS1 resolutions were chosen to avoid contamination of signals with lipids related by unsaturation (± 2 amu). Fragmentor energy and collision energy were optimized for each lipid class using representative internal standards.

Supplementary Figures

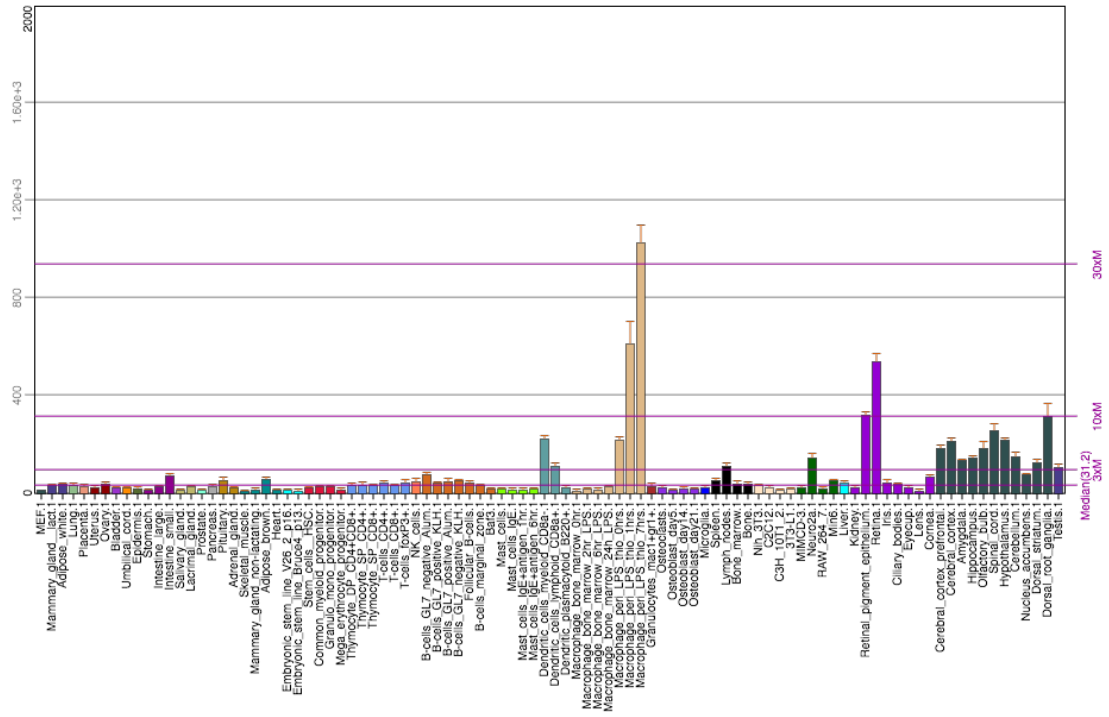

Figure S1. DDHD1 expression in mouse tissues from BioGPS database. Shown are transcriptional profiles of DDHD1 across the indicated mouse tissue and cell types as measured by microarray analysis using Affymetrix GeneChip Mouse Genome U74A array. Note that DDHD1 is highly expressed in brain regions and peritoneal macrophages. Data courtesy of BioGPS.org.

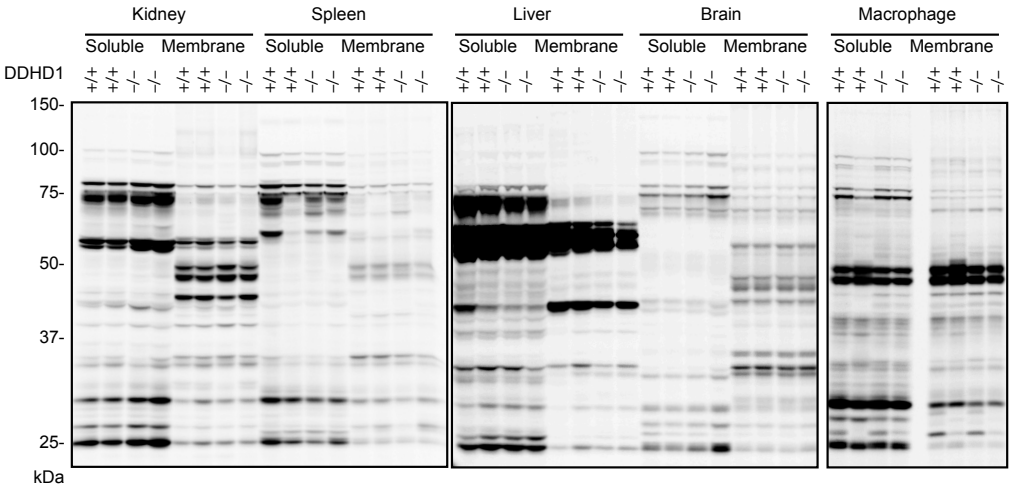

Figure S2. Gel-based ABPP with the general serine hydrolase probe FP-Rh does not detect DDHD1 signals in mouse tissue lysates. Kidney, spleen, liver, brain, and macrophage proteomes were separated into soluble and membrane fractions, labeled with the general serine hydrolase activity-based probe, fluorophosphate-rhodamine (FP-Rh), and separated by SDS-PAGE. DDHD1 has a predicted molecular weight of ~100 kDa, but an FP-Rh-labeled protein of this size was not observed to be selectively expressed in DDHD1$^{+/+}$, but not DDHD1$^{-/-}$ tissues. Instead, loss of DDHD1 activity in DDHD1$^{-/-}$ tissues was confirmed by MS-based ABPP (see **Figure 1E**).

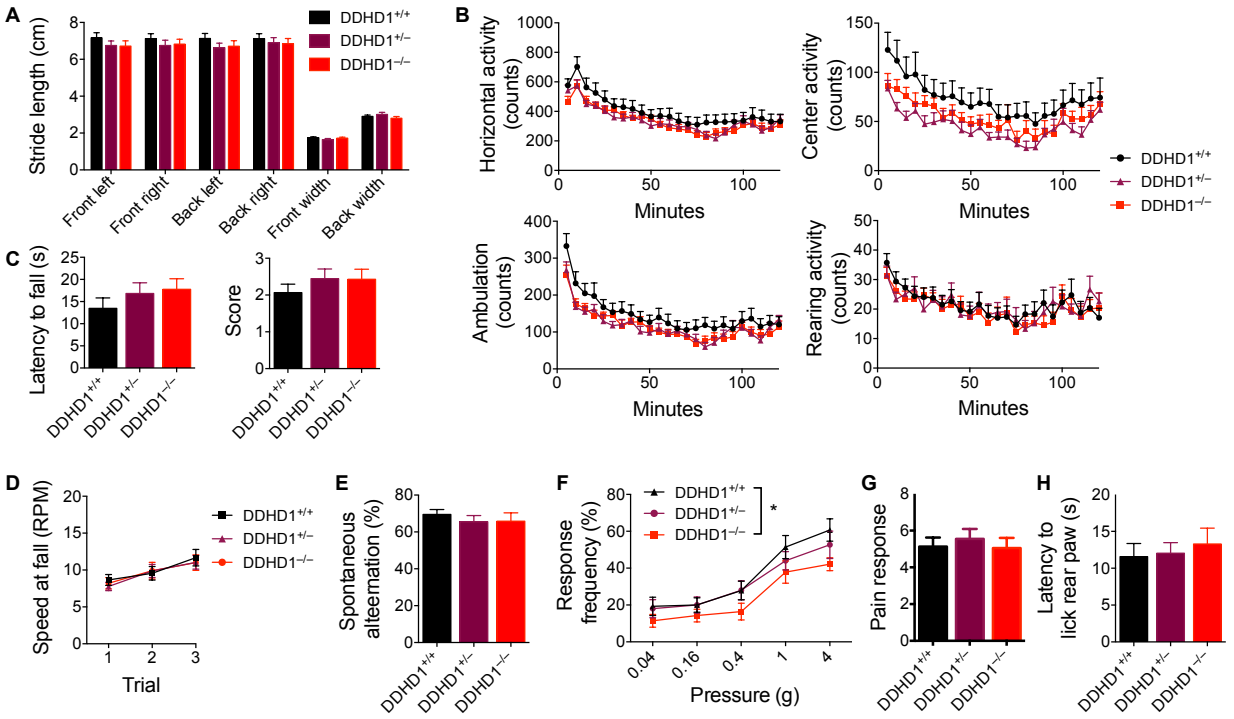

Figure S3. Behavioral characterization of DDHD1$^{-/-}$ mice. (**A**) Gait analysis measuring distance between subsequent foot placement of DDHD1$^{-/-}$, DDHD1$^{+/-}$, and DDHD1$^{+/+}$ mice. (**B**) Activity levels of mice, as measured by photobeam breaks. Horizontal activity denotes the sum of fine movements and ambulation. Ambulation refers to concerted forward motion. Center activity correlates with lower anxiety levels and rearing denotes vertical movements. (**C**) Motor function as assessed by the time before falling and the score assigned to mouse performance during the hanging wire test. (**D**) The rotarod test of coordinated movement. (**E**) The Y-Maze test of working memory measuring the propensity of mice to explore a new arm of the maze. (**F**) Paw withdrawal in response to forces applied underside of the paw by Von Frey filaments among DDHD1 genotypes. (**G and H**) Thermal pain sensitivity as measured by response to cold stimulus (**G**) in the acetone evaporation test, or response to heat (**H**) in the hotplate test. Data represents mean± S.E.M. for 14-16 mice per group. * $p<0.05$ for DDHD1$^{-/-}$ and DDHD1$^{+/+}$ mice.

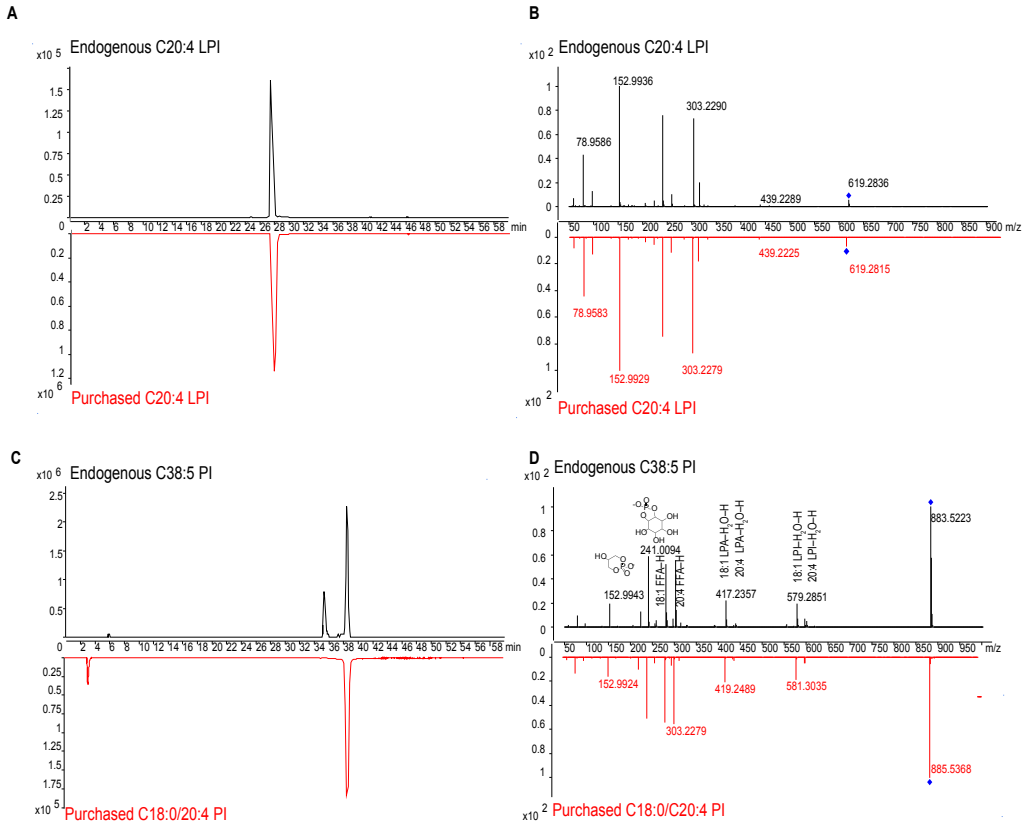

Figure S4. Structural assignment of DDHD1-regulated metabolites by comparison of retention time and fragmentation spectra to lipid standards. Metabolites altered in DDHD1$^{-/-}$ brain were eluted from a Gemini C18 50 cm x 4.6 cm, 5µm particle column using the gradient described in the Methods and isolated and fragmented with 40 V collision energy using an Agilent 6520 Q-TOF. (**A**) Extracted ion chromatogram of a feature corresponding to the [M-H]$^-$ ion of C20:4 LPI (*m/z* 619.29) lipid from mouse brain (black) is equivalent to that of a purchased C20:4 LPI standard (red). (**B**) Equivalent MS2 spectra were observed for the endogenous lipid from mouse brain (black) and a purchased standard (red). (**C**) Extracted ion chromatogram of a feature corresponding to the [M-H]$^-$ ion of C38:5 PI (*m/z* 883.53) from mouse brain (black) is similar to that of commercially available C18:0/C20:4 PI standard (red). (**D**) The MS2 spectrum of endogenous and commercially available PI lipids are similar, with a diagnostic PI peak of *m/z* 241. Endogenous C18:0/C20:4 PI was not significantly altered in DDHD1$^{-/-}$ brain, however it was used as a standard because C18:1/C20:4 PI is not commercially available and both PI lipids have a similar retention time and fragmentation pattern. The fragment ions we observed were consistent with published studies of collision-induced fragmentation of PI[11].

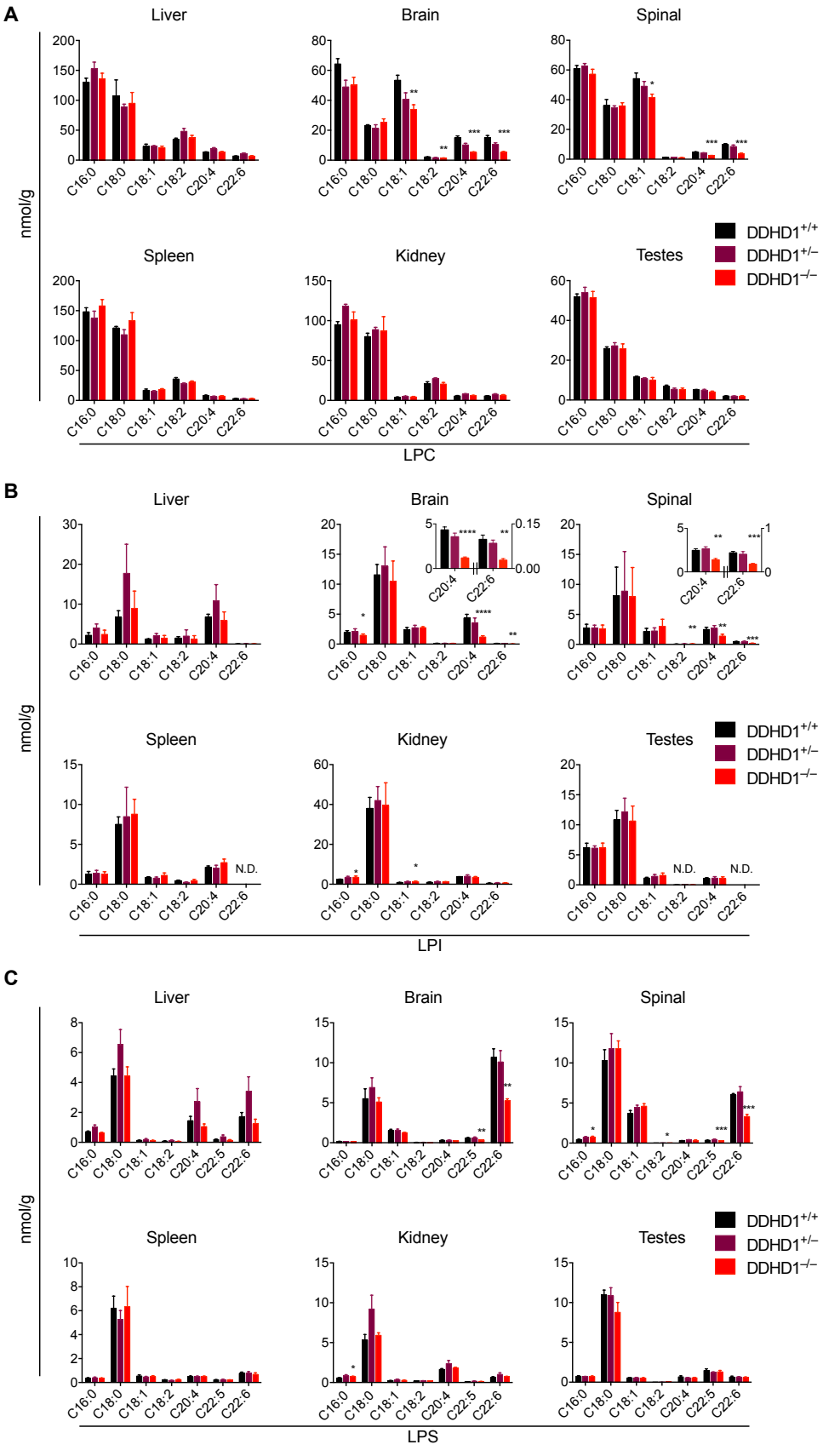
A
Liver
Brain
Spinal
Spleen
Kidney
Testes
nmol/g
C16:0
C18:0
C18:1
C18:2
C20:4
C22:6
DDHD1+/+
DDHD1+/–
DDHD1–/–
LPC
B
N.D.
LPI
C
C22:5
LPS

Figure S5. Targeted LC-M-based quantification of lysophospholipids in tissues from DDHD1$^{-/-}$ mice. Frozen tissues were extracted in organic solvents in the presence of lipid internal standards to quantify lysophospholipid levels. (**A-C**) Targeted LC-M-based quantification of the indicated lysophosphatidylcholine (LPC) (**A**), lysophosphatidylinositol (LPI) (**B)**, and lysophosphatidylserine (LPS) (**C**) lipids in DDHD1$^{-/-}$ brain and spinal cord tissues from DDHD1$^{+/+}$, DDHD1$^{+/-}$, and DDHD1$^{-/-}$ mice N = 4, error bars represent mean ± SEM. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001, DDHD1$^{+/+}$ vs. DDHD1$^{-/-}$.

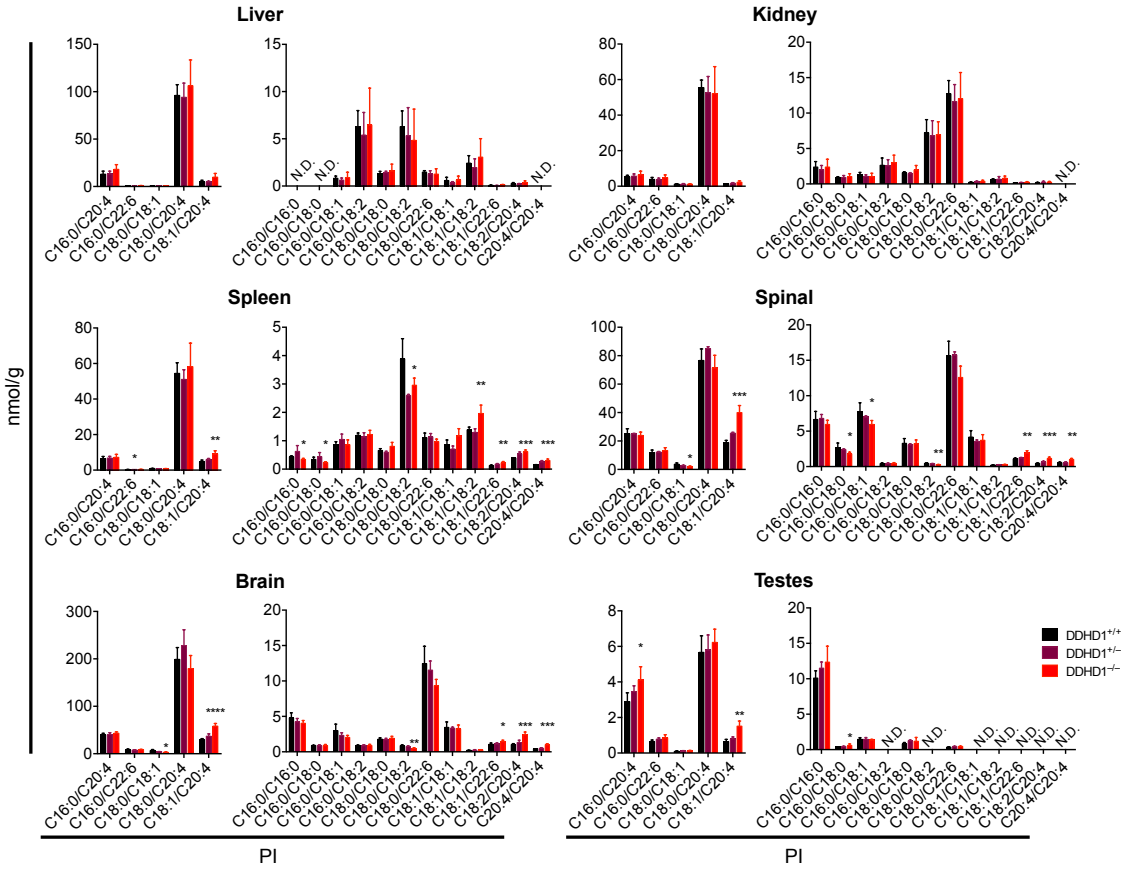

Figure S6. Targeted LC-M-based quantification of PI lipids in tissues from DDHD1$^{-/-}$ and DDHD1$^{+/+}$ mice. N.D. denotes lipids that could not be quantified in the indicated tissue. N = 4. Error bars represent averages ± SEM. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001, DDHD1$^{+/+}$ vs. DDHD1$^{-/-}$.

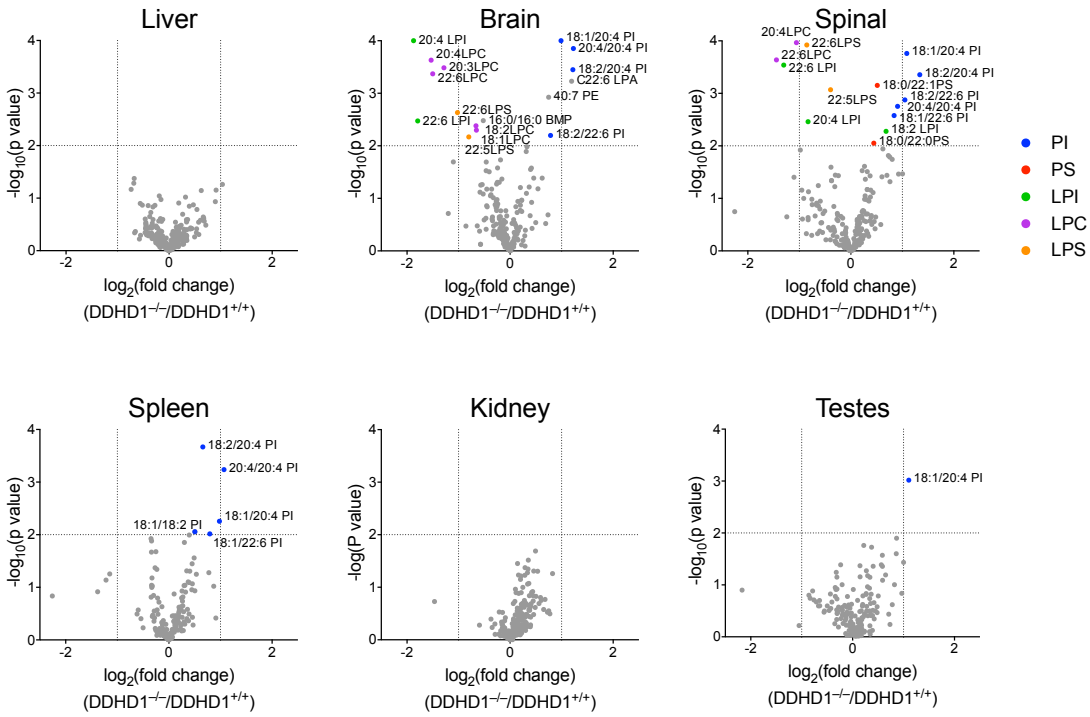

Figure S7. Metabolomic analysis identifies phospholipid changes in tissues from DDHD1$^{-/-}$ mice. Volcano plots showing relative abundance of metabolites (x-axis) versus significance of the observed changes (y-axis) in the indicated tissues from DDHD1$^{-/-}$ and DDHD1$^{+/+}$ mice. Structural assignments for significantly changing metabolites (*P*<0.001*)* with log2 transformed fold changes of <-1 or >1 are shown next to data points. For integrated peak areas of all extracted metabolites see (**Table S1**).

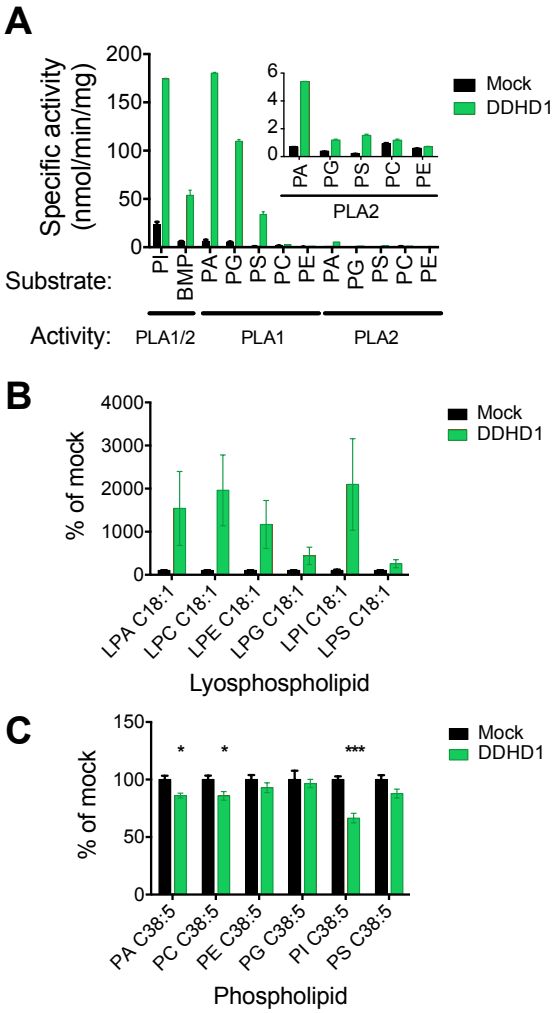

Figure S8. Measurement of phospholipase substrate selectivity of recombinantly expressed DDHD1. (**A**) Phospholipase activity of soluble lysates from mock or DDHD1-transfected HEK293T cells. Lysates were incubated with 100 μM of various phospholipid substrates and generation of lysophospholipids was measured by quantification to an appropriate internal standard. When lipids with two unique acyl chains were used, cleavage of the acyl chain at the SN-1 or SN-2 position are denoted as PLA1 and PLA2 activity, respectively. The inset depicts PLA2 activity for various phospholipid classes. Phospholipids used as substrates: for PA, PC, PE, PS, and PG, 16:0-D31/18:1; for PI, 16:0/16:0; for BMP, 14:0/14:0. (**B**) Mock- or DDHD1-transfected HEK293T cells were harvested 48 h after transfection and lipids extracted and analyzed by untargeted LC-MS analysis as described in the **Methods** section. For additional lipids see **Table S2**. (**C**) Phospholipids analyzed as in part **B**. N = 4, error bars represent mean ± SEM., * P<0.05, *** P<0.001, Mock vs. DDHD1.

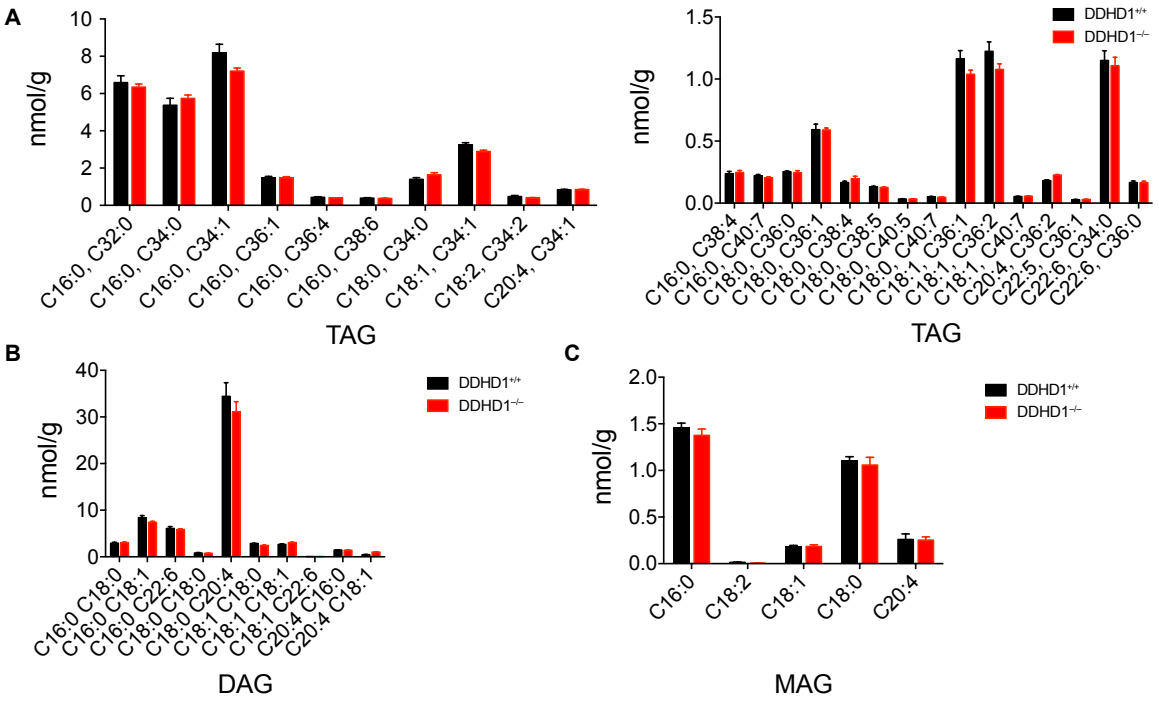

Figure S9. DDHD1 does not regulate neutral lipids in the brain. (**A-C**) Targeted LC-M-based quantification of triacylglycerol (TAG) (**A**), diacylglycerol (DAG) (**B**), and monoacylglycerol (MAG) (**C**) lipids in brain tissue from DDHD1$^{+/+}$ and DDHD1$^{-/-}$ mice. Data were normalized to an unnatural TAG, DAG or MAG internal standards. N=4, Error bars represent means ± SEM.

Lipid	*m/z*	Retention time (min)	DDHD1+/+ Mean area	DDHD1+/+ Standard deviation	DDHD1–/– Mean area	DDHD1–/– Standard deviation	Fold change (KO/WT)	P value
LPI								
18:0	595.3	28.9	1758900	443837	1393793	169020	0.79	0.21
18:1	597.3	28.6	329006	24041	407975	50509	1.24	0.02
20:4	619.3	23.9	2222019	260361	530594	854529	0.24	1.55E-05
22:6	643.3	24.4	210077	37230	42326	16178	0.2	1.70E-04
PI								
30:0	781.5	37.7	480844	90279	607474	281370	1.26	0.42
32:0	809.5	38.6	1677567	155219	1664508	424958	0.99	0.96
32:1	807.5	34.2	4620576	769133	4769340	1240167	1.03	0.85
32:2	805.5	37.6	439980	89604	569652	207015	1.29	0.29
34:0	837.5	39.5	1011548	101032	848154	123192	0.84	0.09
34:1	835.5	37.2	91592280	6725953	88279325	4141556	0.96	0.43
34:2	833.5	37.9	194752	37859	387261	208556	1.99	0.12
36:0	865.6	40.7	1151863	56928	1024111	407333	0.89	0.56
36:1	863.6	39.4	1572362	214807	1127877	161422	0.72	0.02
36:2	861.5	38.7	501590	121841	481462	166555	0.96	0.85
36:4	857.5	38.1	5529619	3418665	7692761	353156	1.39	0.25
36:5	855.5	37.5	113248	26352	206549	24783	1.82	2.10E-03
38:4	885.6	39	32688523	3284052	30262983	2694934	0.93	0.3
38:5	883.5	35.1	3157351	334626	5343551	679204	1.69	1.18E-03
38:6	881.5	38.2	252742	71598	388442	51024	1.54	0.02
40:5	911.6	37.9	61789	13700	113772	10090	1.84	8.76E-04
40:7	907.5	37	132176	13758	158044	21899	1.2	0.09
40:8	905.5	36.4	123473	11673	264885	22427	2.15	3.05E-05
42:10	929.5	37.7	52206	4951	59215	6722	1.13	0.14
42:8	933.6	38.7	5755	1339	15981	2383	2.78	2.94E-04
LPA								
16:0	409.2	23.7	166648	22113	136166	32533	0.82	0.19
18:0	437.3	25.4	383947	188769	413234	157957	1.08	0.83
18:1	435.3	24.1	193521	54536	180243	62189	0.93	0.77
20:4	458.2	23.9	330530	49068	202258	30597	0.61	0.07
22:6	482.2	24.6	355913	75885	760981	112872	2.14	0.02
PA								
32:0	647.5	30.2	788715	81686	804994	81838	1.02	0.88
34:1	673.5	30.5	16644352	1669284	13838513	1725163	0.83	0.13
36:2	699.5	30.8	6753078	536432	7001198	721776	1.04	0.76
36:4	695.5	32.0	2572640	425587	2021062	253697	0.79	0.14
38:4	723.5	31.4	5490703	574444	5118959	370356	0.93	0.44
38:5	721.5	30.5	1337435	151119	1534191	296923	1.15	0.31

			DDHD1 +/+		DDHD1 –/–			
Lipid	*m/z*	Retention time (min)	Mean area	Standard deviation	Mean area	Standard deviation	Fold change (KO/WT)	P value
LPS								
18:1	522.3	25.6	343250	64793	323915	72729	0.94	0.71
20:4	544.3	29.9	62852	13318	48683	17530	0.77	0.13
22:6	568.3	29.7	1504264	388482	695896	306398	0.46	0.01
PS								
34:1	760.5	35.2	9014291	526812	8988885	237590	1	0.97
36:1	788.5	33.6	30697627	4359626	21543115	1428819	0.7	0.17
36:2	786.5	32.6	29284282	1907439	31564988	3394892	1.08	0.39
38:4	810.5	33	12300099	640272	11453722	814592	0.93	0.28
LPE								
16:0	452.3	30.4	700371	33017	726423	109117	1.04	0.66
16:0-O	438.3	31.3	42105	6692	38994	7775	0.93	0.57
16:0-P	436.3	31.3	296642	34663	314429	53683	1.06	0.6
16:1	450.3	30.6	14492	4508	18802	8670	1.3	0.41
18:0	480.3	32.7	1026208	244034	1172925	469445	1.14	0.6
18:0-O	466.3	32.5	102090	10562	118270	23700	1.16	0.26
18:0-P	464.3	33.5	276401	25202	262445	75594	0.95	0.74
18:1	478.3	30.9	1448716	146816	1141351	246536	0.79	0.08
18:2	476.3	29.2	43542	11502	30374	3529	0.7	0.07
20:1	506.3	32.9	69878	22083	72124	29284	1.03	0.91
20:4	500.3	29.3	375532	64085	261336	63138	0.7	0.04
22:1	534.4	30.1	64997	27202	71468	34871	1.1	0.78
22:4	528.3	30.9	345528	86539	351525	109649	1.02	0.93
22:5	526.3	30.8	30491	7126	29894	9172	0.98	0.92
22:6	524.3	30.1	1178518	230432	800906	54228	0.68	0.02
24:1	562.4	31.2	49957	37027	65008	48401	1.3	0.64
PE								
32:0	690.5	42	46245	2216	60436	4888	1.31	1.85E-03
34:1	716.5	42.8	1806423	43354	1767054	126717	0.98	0.58
36:0	746.6	42.6	7179293	457877	8127542	775492	1.13	0.08
36:1	744.6	45.2	1127326	65758	1012111	31811	0.9	0.02
36:2	742.5	43.2	3100347	122632	3569201	299103	1.15	0.03
36:4	738.5	41.2	1540055	139595	1372891	187153	0.89	0.2
38:1	772.6	43	3537352	130419	3526938	149509	1	0.92
38:2	770.6	41.2	55444	10081	62514	15240	1.13	0.47
38:4	766.5	44	13852668	669151	13526577	765000	0.98	0.54
38:5	764.5	41.8	2441739	161301	2909175	328789	1.19	0.04
38:6	762.5	41.3	5270460	575255	5805312	920471	1.1	0.36
38:7	760.5	43.4	262293	10426	264595	17327	1.01	0.83
40:9	784.5	41.7	1951	1008	2047	549	1.05	0.87
44:10	838.5	42.5	251380	26761	418024	24321	1.66	9.21E-05

			DDHD1+/+		DDHD1–/–			
Lipid	*m/z*	Retention time (min)	Mean area	Standard deviation	Mean area	Standard deviation	Fold change (KO/WT)	P value
LPC								
16:0	496.3	37.74	245783592	2.4E+07	234445915	1.9E+07	0.95	0.48
18:1	522.4	38.37	87717158	9915646	82890663	5848960	0.94	0.43
18:0	524.4	40.25	149075713	3.5E+07	127837799	2.6E+07	0.86	0.37
20:4	544.3	36.44	78835397	8024187	29248696	4436710	0.37	3.70E-05
22:6	568.3	36.54	65223078	9825983	27242193	2011848	0.42	2.75E-04
PC								
30:0	706.5	46.35	65561552	5024104	71642795	4161032	1.09	0.11
32:2	730.6	46	24217588	5147551	23950791	4360615	0.99	0.94
32:1	732.6	46.58	105745527	1.1E+07	103669491	9443638	0.98	0.79
32:0	734.6	47.18	95713173	7852701	101804735	3623232	1.06	0.21
34:4	754.5	46.01	7285364	1869232	8350524	1460932	1.15	0.4
34:2	758.6	46.86	24925293	3042091	28812667	899316	1.16	0.05
34:1	760.6	47.46	142514220	5872401	140591720	8760210	0.99	0.73
34:0	762.6	48.03	65288484	2388355	71939312	3067575	1.1	0.01
36:5	780.6	46.16	19571447	3115133	21875204	1966216	1.12	0.26
36:4	782.6	46.88	71389428	8565519	72252424	2139975	1.01	0.85
36:2	786.6	47.6	27043319	1138140	31300377	2606126	1.16	0.02
36:1	788.6	48.31	164341608	5962806	149316623	1.3E+07	0.91	0.08
36:0	790.6	48.31	23387748	725432	21283780	1959482	0.91	0.09
38:6	806.6	46.7	81008350	9902512	82596127	4604431	1.02	0.78
38:5	808.6	46.98	31669459	5037768	39555106	1698688	1.25	0.03
38:4	810.6	47.78	53021904	4739104	52354176	3830040	0.99	0.83
40:9	828.6	46.03	1094742	239519	1300543	415933	1.19	0.42
40:8	830.6	46.38	7711966	677320	9701983	889809	1.26	0.01
40:7	832.6	46.96	13660468	2202452	16502744	851033	1.21	0.05
40:5	836.6	47.83	9401237	980921	10253389	396455	1.09	0.16
42:10	854.6	46.31	13282957	1403749	11928955	985643	0.9	0.17
42:9	856.6	46.3	2792281	193979	2534081	312402	0.91	0.21
42:8	858.6	47.03	289258	43374	462441	66885	1.6	4.85E-03
42:7	860.6	47.85	1111201	147931	1405596	121517	1.26	0.02

			DDHD1+/+		DDHD1–/–			
Lipid	*m/z*	Retention time (min)	Mean area	Standard deviation	Mean area	Standard deviation	Fold change (KO/WT)	P value
MAG								
16:0	331.3	39.96	8206386	2102937	6664246	1302537	0.81	0.26
18:0	359.3	42.23	19687447	3727909	17765358	4750939	0.9	0.55
22:6	403.3	38.96	911122	226214	758152	246547	0.83	0.4
DAG								
32:0	586.5	48.38	104891	10845	112316	18630	1.07	0.52
34:1	612.6	48.48	584710	51903	570747	99439	0.98	0.81
36:4	634.6	48	369709	37891	335626	34770	0.91	0.23
36:3	636.6	48	84571	10840	94905	5343	1.12	0.14
36:2	638.6	48.58	379437	26906	422308	48189	1.11	0.17
36:1	640.6	48.76	1134547	55043	1185680	94943	1.05	0.39
38:6	658.6	47.95	329084	45046	305911	17824	0.93	0.38
38:4	662.6	48.73	5115157	270615	4842337	1502030	0.95	0.73
40:8	682.6	46.19	2408831	557205	1714214	710004	0.71	0.17
40:7	684.6	48.03	36640	2315	36867	3830	1.01	0.92
40:6	686.6	48.68	291996	29120	252503	69215	0.86	0.33
42:6	706.6	46.39	65610402	5124767	71807834	4146256	1.09	0.11
44:12	730.5	46.08	13054367	3986165	16109875	2642913	1.23	0.25
44:11	732.6	46.61	105046959	1.1E+07	104297222	9274042	0.99	0.92
44:10	734.6	47.19	94785745	7894738	99805191	5434203	1.05	0.34
TAG								
48:0	824.8	50.76	9244383	1690217	12190604	3218739	1.32	0.21
51:0	860.8	58.38	3090615	842951	2435344	438583	0.79	0.22
52:2	876.8	51.67	21770565	1.5E+07	38036098	6280757	1.75	0.09
52:1	878.8	55.64	33803981	2.3E+07	53001701	7921520	1.57	0.16
54:5	898.7	51.32	15142545	9567122	15889656	5243154	1.05	0.9
54:3	902.8	52.2	10869020	7602945	21210369	2008499	1.95	0.04
54:0	908.9	57.99	1067136	1078642	2057441	342419	1.93	0.13
58:8	948.8	50.69	198670	46806	185997	47587	0.94	0.72
56:6	948.8	51.95	868891	420229	1123257	466915	1.29	0.45
58:7	950.8	51.85	3695984	2432715	6714258	1403798	1.82	0.08
60:12	968.8	56.71	22579323	3194489	20612573	3802660	0.91	0.46
66:15	1046.9	52.85	6277533	1567581	6400716	1708879	1.02	0.92
FFA								
16:0	255.3	28.47	33381115	2028324	32039390	1659230	0.96	0.25
18:0	283.3	30.20	53189734	4193931	50317370	5074643	0.95	0.39
18:1	281.3	28.84	32650530	3735455	29538169	3318839	0.90	0.17
20:4	303.2	27.85	63305484	4952993	63467577	3790926	1.00	0.96
22:0	339.3	33.82	106345	21628	143434	64885	1.35	0.30
24:0	367.4	35.64	64790	18563	70332	23157	1.09	0.66

			DDHD1+/+		DDHD1–/–			
Lipid	*m/z*	Retention time (min)	Mean area	Standard deviation	Mean area	Standard deviation	Fold change (KO/WT)	P value
LPG								
16:0	483.3	28.3	870284	212625	835604	202918	0.96	0.81
18:0	511.3	29.8	21980	20477	61338	30752	2.79	0.05
18:1	509.3	28.7	330258	47914	304196	38714	0.92	0.44
20:4	533.3	28.3	145147	11207	150527	9536	1.04	0.49
22:6	557.3	29.3	150003	36388	144619	21676	0.96	0.81
PG								
30:0	693.5	37.2	14876	624	15248	3436	1.02	0.84
32:0	721.5	36.6	591048	80310	516466	41927	0.87	0.23
32:0	721.5	36.6	617711	70926	538111	82240	0.87	0.19
32:1	719.5	37.8	49156	1612	50611	7480	1.03	0.72
32:2	717.5	32.9	70361	9468	59286	14464	0.84	0.25
34:0	749.5	39.3	213314	26073	256315	110870	1.20	0.48
34:1	747.5	38.7	3310455	336112	3147471	63487	0.95	0.48
34:1	747.5	38.7	3332667	261274	3135355	259917	0.94	0.33
36:0	777.6	39.5	80147	11013	101167	50190	1.26	0.44
36:1	775.6	39.5	413498	50489	492510	110451	1.19	0.24
36:4	769.5	36.8	562330	54265	569474	36043	1.01	0.82
36:4	769.5	36.8	606440	35100	606069	21899	1.00	0.99
38:4	797.5	39.3	1118627	105684	1135643	101990	1.02	0.82
38:5	795.5	39.2	1027196	88288	856860	193461	0.83	0.16

Table S1. Semiquantitative analysis of brain lipids identifies phospholipids affected by DDHD1 disruption. Untargeted lipidomics using the XCMS algorithm for data analysis was used to identify features with significantly altered levels in DDHD1$^{-/-}$ versus DDHD1$^{+/+}$ brain tissue. Lists of *m/z* values of [M+H]+ or [M-H]– ions corresponding to complex lipids were manually extracted and the area under the curve of each chromatogram was calculated. Means and standard deviations of peak areas are reported for each genotype. Significantly altered features (P-value <0.05) with fold change >1.33 or <0.75 are highlighted in red. PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidyl ethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol, PS, phosphatidylserine. The prefix “L” before the phospholipid class denotes lysophospholipids, which are monoacyl-glycerophospholipids. FFA, free fatty acids; MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol. [M-H]– ions of FFA, LPA, PA, LPG, PG, LPS, PS, LPI and PI were measured. [M+H]+ ions of LPE, PE, LPC PE and MAG and [M+NH4]+ ions of DAG and TAG were measured. N=4 biological replicates.

Table S2. Quantitative analysis of lipids in tissues from DDHD1$^{+/+}$ and DDHD1$^{-/-}$ mice.
Targeted LC-MS quantification of lipids from indicated tissues in DDHD1$^{+/+}$ and DDHD1$^{-/-}$ mice. N = 4, error bars represent mean ± SEM. In spreadsheet, asterisks for significance mark lipids that changed > 1.5 fold in DDHD1$^{-/-}$ tissues and displayed I values of < 0.05

See accompanying Excel sheet.

Lipid	*m/z*	Retention time (min)	Mock		DDHD1		Fold change (DDHD1/Mock)	P value
			Mean area	Standard deviation	Mean area	Standard deviation		
LPI								
16:0	571.3	24.5	21800	4079	75198	33477	3.45	0.02
18:0	595.3	28.9	582237	79486	550569	43045	0.95	0.51
18:1	597.3	28.6	38061	16653	798411	808573	20.98	0.11
20:4	619.3	23.9	61772	5314	1173921	1231420	19	0.12
22:6	643.3	24.4	4031	1540	25540	16603	6.34	0.04
PI								
32:0	809.5	38.6	340012	28323	452699	31222	1.33	1.75E-03
32:1	807.5	34.2	1634333	189215	2269272	337332	1.39	0.02
32:2	805.5	37.6	256964	29429	306576	34851	1.19	0.07
34:0	837.5	39.5	64069	6445	145660	5437	2.27	1.23E-06
34:1	835.5	37.2	3295414	247073	3677920	316656	1.12	0.11
34:2	833.5	37.9	2562391	277697	2124119	317734	0.83	0.08
34:4	829.5	38.8	52562	11790	93784	35127	1.78	0.07
36:0	865.6	40.7	54580	7785	57621	2818	1.06	0.49
36:1	863.6	39.4	4206386	335101	4735405	227784	1.13	0.04
36:2	861.5	38.7	3094829	182940	2574094	204314	0.83	0.01
36:4	857.5	38.1	1114944	136692	1200974	53915	1.08	0.29
36:5	855.5	37.5	189405	13292	235775	73852	1.24	0.26
38:4	885.6	39	8561602	827942	5754510	415795	0.67	9.16E-04
38:5	883.5	35.1	1614948	89634	1073511	136396	0.66	5.66E-04
38:6	881.5	38.2	245157	27027	194050	41112	0.79	0.08
40:5	911.6	37.9	301371	20275	255012	16311	0.85	0.01
40:7	907.5	35	71690	1615	54990	6320	0.77	2.18E-03
40:8	905.5	36.4	24378	7417	16230	5668	0.67	0.13
42:10	929.5	37.7	17871	7739	17397	1704	0.97	0.91
LPS								
16:0	496.3	24	23257	5256	23484	3317	1.01	0.94
18:0	524.3	25.7	54631	24884	640019	750037	11.72	0.17
18:1	522.3	25.6	51404	5389	132883	96294	2.59	0.14
20:4	544.3	24.9	73819	14936	88077	45268	1.19	0.57
22:6	568.3	24.7	5378	3854	44065	53424	8.19	0.2
PS								
34:1	760.5	35.2	4050801	112077	3582551	170515	0.88	3.73E-03
34:4	754.5	35.6	99055	16188	98558	3953	0.99	0.95
36:1	788.5	33.6	3208340	445264	2653311	285500	0.83	0.08
36:2	786.5	32.6	3526376	78736	2593825	114141	0.74	1.05E-05
36:4	782.5	34	300199	22076	243647	46401	0.81	0.07
36:5	780.5	36.7	70819	11414	124369	22174	1.76	0.01
38:4	810.5	33	363663	12333	393953	23561	1.08	0.06
38:5	808.5	34.4	477991	310080	262624	48329	0.55	0.22
38:6	806.5	34.1	259927	22354	246075	12230	0.95	0.32
40:6	834.5	34.6	598612	46507	444986	19243	0.74	8.81E-04
40:7	832.5	34.6	458699	31982	396434	24388	0.86	0.02

			Mock		DDHD1			
Lipid	*m/z*	Retention time (min)	Mean area	Standard deviation	Mean area	Standard deviation	Fold change (DDHD1/Mock)	P value
LPA								
16:0	409.2	23.7	61712	15285	85994	51830	1.39	0.4
18:0	437.3	25.4	122098	47347	82549	1630	0.68	0.15
18:1	435.3	24.1	449738	40904	6927828	7724562	15.4	0.14
20:4	458.2	23.9	135972	21712	170391	36432	1.25	0.16
22:6	482.2	24.6	1457745	151103	1260483	159085	0.86	0.12
PA								
34:1	673.5	37.5	1328459	117463	1483431	119008	1.12	0.11
34:4	668.4	36.9	51017	4455	46052	7086	0.9	0.28
34:6	664.4	37.1	74143	33108	38175	14774	0.51	0.09
36:2	699.5	39.8	2237396	54154	2241697	73503	1	0.93
36:4	695.5	38	69372	6305	58516	4193	0.84	0.03
36:5	694.5	39.4	505768	56727	373529	17357	0.74	4.29E-03
36:6	692.4	38.5	285076	59320	204540	36586	0.72	0.06
38:4	723.5	38.4	49534	2529	49786	8609	1.01	0.96
38:5	721.5	30.5	378354	25488	325465	16046	0.86	0.01
38:6	720.5	39.7	1562881	606968	1822022	30950	1.17	0.43
38:7	718.5	38.9	179941	17606	500162	46430	2.78	1.34E-05
40:6	748.5	40.9	10387424	626840	9794770	291946	0.94	0.14
40:7	746.5	39.3	4668921	800142	4802557	202878	1.03	0.76
40:8	744.5	39.1	652078	117529	624835	32407	0.96	0.67
42:10	768.5	38.6	107956	39845	68347	8769	0.63	0.1
42:9	742.5	38.8	56278	8621	65701	11875	1.17	0.25
44:12	792.5	38.8	167370	30187	192735	11115	1.15	0.17
LPG								
16:0	483.3	28.3	32891	1457	82252	48457	2.5	0.09
18:0	511.3	29.8	40107	4575	43730	7992	1.09	0.46
18:1	509.3	28.7	249345	12643	1097373	1005383	4.4	0.14
20:4	533.3	28.3	90770	29810	79956	23494	0.88	0.59
22:6	557.3	29.3	1106471	228095	1012716	195869	0.92	0.56
PG								
28:0	665.5	38	158912	45491	108188	30337	0.68	0.11
30:0	693.5	37.2	506970	57833	374117	16972	0.74	4.53E-03
32:0	721.5	36.6	95816	13897	89750	7742	0.94	0.47
32:0	721.5	36.6	350832	21529	328685	22014	0.94	0.2
32:1	719.5	37.8	2154405	144851	1826769	48323	0.85	0.01
32:2	717.5	32.9	185324	16479	516832	48466	2.79	1.30E-05
34:0	749.5	39.3	1205410	81780	1124995	37987	0.93	0.12
34:1	747.5	38.7	2429603	646268	2205849	207754	0.91	0.53
34:1	747.5	38.7	10382179	628154	9851439	330845	0.95	0.19
36:0	777.6	39.5	227433	12450	187520	7289	0.82	1.47E-03
36:1	775.6	39.5	1567400	158221	1417723	75252	0.9	0.14
36:4	769.5	36.8	472031	84988	469289	29560	0.99	0.95
38:4	797.5	39.3	948444	174926	945936	63852	1	0.98
38:5	795.5	39.2	281682	43004	271895	20448	0.97	0.7

			Mock		DDHD1			
Lipid	*m/z*	Retention time (min)	Mean area	Standard deviation	Mean area	Standard deviation	Fold Change (DDHD1/Mock)	P value
LPE								
16:0	454.3	37.5	259063	27339	441586	93939	1.7	0.01
18:1	480.3	37.5	376355	25903	4398356	4195680	11.69	0.1
18:0	482.3	39.7	410616	67522	738395	42199	1.8	1.73E-04
20:4	502.3	36.2	151439	35870	1046110	1587417	6.91	0.3
PE								
34:1	718.5	47	9675127	1699418	9110397	1296783	0.94	0.62
36:4	740.5	46.4	667944	67480	797300	74159	1.19	0.04
38:5	766.5	46.4	2469271	144047	2287479	213758	0.93	0.21
38:4	768.6	47.3	925184	53865	1166939	75984	1.26	4.57E-03
LPC								
16:0	496.3	37.8	1308863	69544	2512675	298141	1.92	2.24E-04
18:1	522.4	37.8	565532	42837	26293327	3.1E+07	46.49	0.15
18:0	524.4	39.7	426362	57825	903171	222271	2.12	0.01
20:4	544.3	36.1	246316	38035	1112201	184660	4.52	9.38E-05
PC								
34:0	762.6	47.5	5730886	354588	5942691	182936	1.04	0.33
36:1	789.6	48.6	12751865	1002567	14804117	556842	1.16	0.01
CER								
d18:1/16:0	538.5	48.5	7732191	327693	8192122	394345	1.06	0.12
d18:1/18:1	564.5	48.6	18850983	603083	18629539	1108122	0.99	0.74
SM								
d18:1/16:0	703.6	47.2	46767238	2529053	47841649	1531784	1.02	0.49
d18:1/18:1	729.6	47.5	1043957	44260	1290832	128546	1.24	0.01

			Mock		DDHD1			
Lipid	*m/z*	Retention time (min)	Mean area	Standard deviation	Mean area	Standard deviation	Fold Change (DDHD1/Mock)	P value
MAG								
16:0	331.3	39.4	1178598	319095	1453539	446051	1.23	0.35
18:0	359.3	41.5	2206507	324969	3464846	1523020	1.57	0.16
20:4	379.3	38.4	130622	12512	277982	152561	2.13	0.1
22:6	403.3	39.7	53326	11543	56019	8336	1.05	0.72
DAG								
32:0	586.5	47.8	202757	14126	253717	33876	1.25	0.03
34:1	612.6	48	972337	37328	1313923	198665	1.35	0.01
36:3	636.6	47.6	47503	5139	63311	12176	1.33	0.05
36:2	638.6	48.1	627086	44069	793328	143893	1.27	0.07
36:1	640.6	48.7	559517	51894	809958	170664	1.45	0.03
38:5	660.6	47.8	22443	6628	23795	5921	1.06	0.77
38:4	662.6	48	159008	55615	182823	112027	1.15	0.72
40:8	682.5	45.6	3915253	368758	3785313	153062	0.97	0.54
40:6	686.6	46	80749	1593	72929	11326	0.9	0.22
42:6	706.5	45.9	23046967	1440625	21328730	1076341	0.93	0.1
44:12	730.5	46	21684885	1282340	22847403	2847756	1.05	0.48
44:11	732.6	46.2	56433171	1944678	63829719	2674173	1.13	4.22E-03
44:10	734.6	46.8	6384190	374511	7314230	504241	1.15	0.03
TAG								
48:0	824.8	57.8	11971957	197720	10150418	683391	0.85	2.18E-03
50:1	850.8	58.3	32324356	7402668	33128954	4854971	1.02	0.86
52:0	880.8	58	4624233	205929	4961147	131616	1.07	0.03
52:2	876.8	58.5	18935819	6328844	22900847	4765489	1.21	0.36
52:1	878.8	58.3	3621900	1331250	4338805	1062840	1.2	0.43
54:5	898.8	57.7	2282397	145550	2066919	69758	0.91	0.04
54:3	902.8	58.1	1008810	81475	927183	212976	0.92	0.5
54:0	908.9	58.4	1477876	86693	1296933	286796	0.88	0.27

Table S3. Semiquantitative analysis of HEK293T lipids identifies phospholipids affected by DDHD1 disruption. Metabolic profiling was used to identify features with significantly altered levels in Mock transfected or DDHD1 transfected HEK293T cells. Lists of m/z values of $[M+H]^+$ or $[M-H]^-$ ions corresponding to complex lipids were manually extracted, and the area under the curve of each chromatogram was calculated. Means and standard deviations of peak areas are reported for each genotype. Significantly altered features (P-value <0.05) with fold change >1.33 or <0.75 are highlighted in red. $[M-H]^-$ ions of LPA, PA, LPG, PG, LPS, PS, LPI and PI were measured. $[M+H]^+$ ions of LPE, PE, LPC PE, and MAG and $[M+NH4]^+$ ions of DAG and TAG were measured. N=4 biological replicates.

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