SUPPLEMENTARY MATERIAL

Metabolic landscape of the mouse liver by ^{31}P-NMR quantitative analysis of the phosphorome

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PROTOCOL FOR METABOLITE EXTRACTION AND NMR PEAK DECONVOLUTION, ASSIGNMENT AND QUANTIFICATION

1. Extraction of metabolites from liver tissue samples

For each set of experiments 3 different mice were used to ensure robustness and statistical significance. We used the following weighted livers.

Target	Porphyria			NASH			
Mice models	**Control CEP** (6 mice)	**CEP** (7 mice)	**CEP+CPX** (7 mice)	**Control MAT1A-KO** (6 mice)	**MAT1A-KO** (9 mice)	**Control CDHF** (6 mice)	**CDHF** (9 mice)
Weighted liver (mg)	**302**	**308**	**301**	**303**	**362**	**309**	**107**
	329	**312**	**279**	**340**	**319**	**304**	**103**
	308	**283**	**294**	**322**	**319**	**317**	**111**
	315.5	**196**	**299**	**309.5**	**311**	**307**	**129**
	318	**292**	**313**	**302**	**331.6**	**318**	**99**
	317	**251**	**298**	**317**	**313**	**301**	**104**
		301	**321**		**306**		**98**
					314.4		**100**
					303		**101**

Table S1. Liver weights used for the extraction for the different animals employed.

The metabolic extraction procedure was done according to the protocol described in Figure S1:

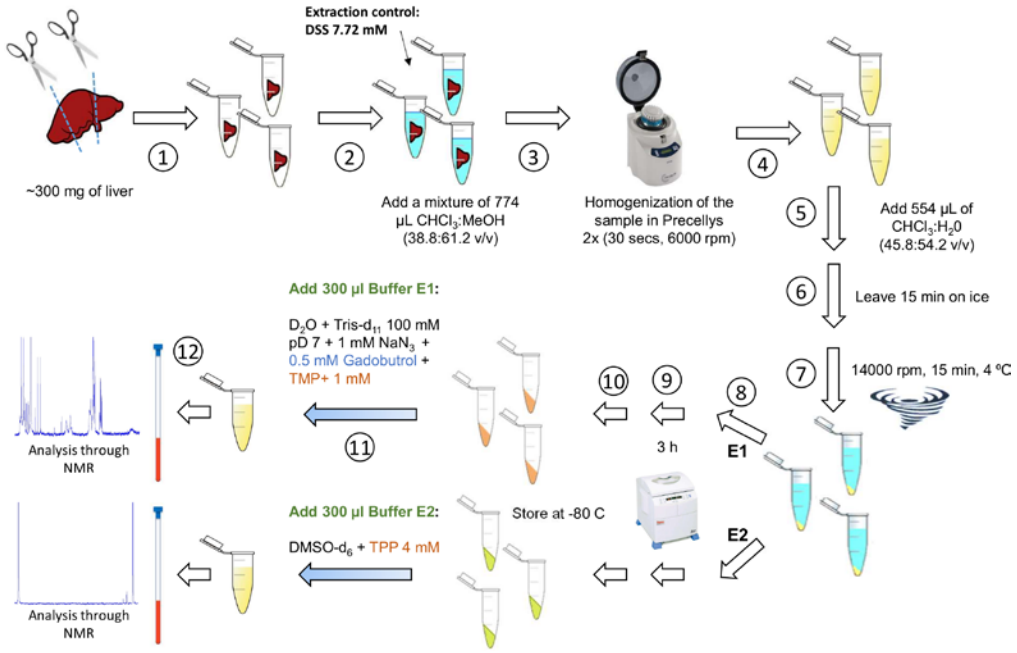

Figure S1. Extraction procedure. The protocol details how the metabolic extraction was performed for each liver.

1) An aliquot of about ~300 mg of mouse liver was weighted.

2) This amount of liver tissue was divided in 100 mg pieces and placed into 3 different Eppendorfs.
3) The extraction was carried out considering previously reported ternary mixtures of $CHCl_3$:MeOH:H_2O (Treatment 1[1] and Treatment 2[2], Figure S2). During liver extraction it is assumed that 90% of liver's weight will contribute to the water phase (indicated as a shift to darker spheres). Thus, 55:25:20 w/w/w $CHCl_3$:MeOH:H_2O mixture ensures a equilibrated extraction with a marked biphasic extraction of $CHCl_3$:MeOH:H_2O (indicated as a solid red line). Dashed green line indicates the maximum estimated chloroform "tie-line". Thus, for every 100 mgs of liver 774 µL of $CHCl_3$:MeOH (38.8:61.2 v/v**) was added. During this step 7.72 mM of DSS was added for the determination of yield extraction.
** For the sake of clarity we transform weights to volumes.

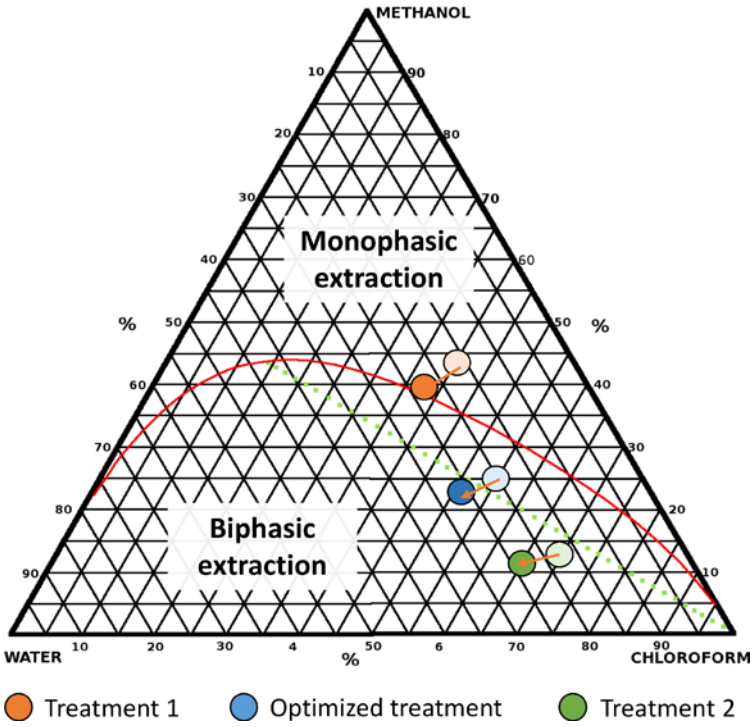

Figure S2. Ternary phase diagram of $CHCl_3$:MeOH:H_2O. *Treatment 1* (orange sphere) corresponds to an extraction at 40:44:16[1], *Treatment 2* (green sphere) corresponds to mixture of 70:13:17[2], and *Optimzed treatment* corresponds to a proportion of 55:25:20 w/w/w of $CHCl_3$:MeOH:H_2O. Dashed green line indicates the maximum estimated chloroform "tie-line". Red line indicated the biphasic separation region. During liver extraction it is considered that a 90% of liver's weight will contribute to the water phase (indicated as a shift to darker spheres).

4) Tissue was homogenized using two cycles of Precellys for 30 minutes at 6000 rpms.
5) Afterwards 554 µL of $CHCl_3$:H_2O (45.8:54.2 v/v) were added to each Eppendorf.
6) Samples were vortexed and leaved for 15 minutes on ice.
7) Tubes were centrifuged at 14000 rpms for 15 minutes at 4 ºC.
8) Phases were carefully divided into different Eppendorfs as hydrophilic (E1) and hydrophobic (E2) material.

9) Samples were dried using Speedvac for at least 3 h.
10) The remaining powder was stored at -80 ºC until further use.
11) For NMR measurements of the **E1** extraction of the liver (hydrophilic phase) the extracted material from the three dried Eppendorfs was combined and resuspended using 300 uL of E1 buffer (100 mM Tris-d11 at pD 7 + 1 mM NaN3 + 0.5 mM Gadobutrol + 1 mM TMP (internal reference) in D_2O). **E2** extraction is resuspended in 300 µL of buffer E2 (DMSO-d6 + TPP 4 mM) following the same procedure.
12) The resuspended extracts (300 µL) were filled into 5 mm Shigemi NMR tubes and NMR experiments were recorded.

2. NMR data acquisition and spectra processing

All NMR experiments were recorded at 298 K on a Bruker 600 MHz (12 T) Avance III spectrometer equipped with a QXI (^{1}H,^{13}C,^{15}N,^{31}P) probehead. For each sample three different experiments were collected (Figure S3 and S4):

(i) 1D ^{31}P-NMR *zgig* spectrum with inverse gated ^{1}H decoupling (11.5 h).

(ii) 1D ^{1}H-NMR *p3919gp* with water signals suppression using a binomial 3-9-19 pulse with echo gradient pair (7 min).

(iii) 2D ^{1}H-^{31}P-COLOC spectrum with coherence selection by gradients (13.5 h).

The 1D ^{31}P experiment was recorded with long interscan delays d1 > $3 \cdot T_{1,max}(^{31}P)$ for quantification, where the paramagnetic relaxation enhancer (0.5 mM of Gadobutrol) added only to the hydrophilic E1 fraction allowed to use a short d1 = 1 s while the hydrophobic E2 fraction required d1 = 5 s.

Altogether, considering all the experimental set-up we obtained a detection limit of ~200 pmol of ^{31}P-compound per mg of liver with a signal-to-noise ratio of 4. At this configuration we concluded that a minimum of 100 mg and an optimal value of 300 mg of liver tissue are required for a reliable metabolite quantification by this method. Yet, we reckon that an optimized NMR hardware set (i. e. direct observation for heteronuclei) would result in a 5-fold increase in sensitivity, equivalent to a demand of only 20 mg of liver tissue.

3. NMR Peak assignment

The same set of spectra were recorded for some 100 pure phosphorylated standard compounds (listed in Table S1) to facilitate the identification of phosphorylated liver metabolites. Each of ^{31}P standards was prepared at increased 5 mM concentration, in the appropriate solution corresponding to either E1 or E2 extract (see above), to allow faster NMR data acquisition.

The phosphorylated liver metabolites were identified by comparison with the ^{31}P chemical shifts of the pure standard compounds compiled in supplementary Table S1. In some cases, spiking experiments with selected pure standard compounds were performed to confirm the metabolite assignment. In figure S3 we show the use of the ^{1}H-^{31}P-COLOC experiment in the assignment of phosphorylated metabolites. In a complex mixture of phosphorylated compounds from a CEP

UROIIIS$^{P248Q/P248Q}$ mouse liver extraction we overlay 3 different ^{1}H-^{31}P-COLOCs from S7P, PEA and UDPG standards. The peaks overlap and automatically identify the compounds in the hydrophilic extract.

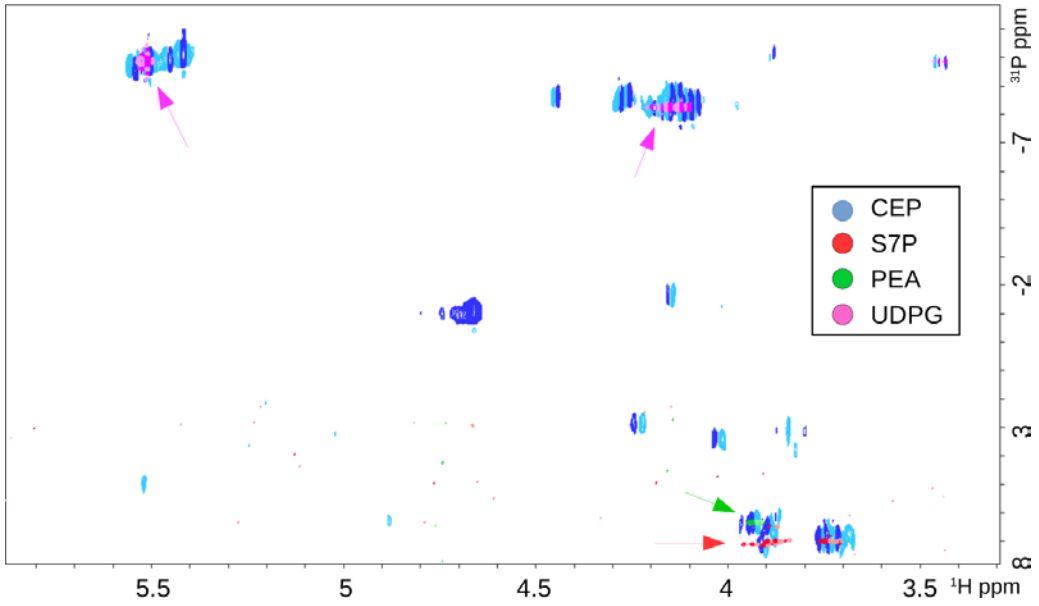

Figure S3. ^{1}H-^{31}P-COLOC spectrum CEP UROIIIS$^{P248Q/P248Q}$ hydrophilic extraction. Correlation spectroscopy via Long-range Coupling (^{1}H-^{31}P-COLOC) is a 2D heteronuclear experiment that correlates ^{31}P and ^{1}H nuclei. The addition of a second dimension facilitates the assignment of overlapping signals. In blue ^{1}H-^{31}P-COLOC spectrum CEP mouse liver E1 extraction. S7P (red), PEA (green) and UDPG (pink) ^{1}H-^{31}P-COLOC spectra are overlaid as an example of chemical shift assignment.

4. NMR Peak integration, deconvolution and quantification

All NMR data processing and analysis was done using TopSpin 4.0.7 (Bruker Biospin GmbH) and in-house MatLab scripts. Assigned ^{31}P-metabolites were then quantified by referencing their ^{31}P peak integral against the added internal reference compound as shown in figure S4. In case of signal overlap, we applied peak deconvolution (command LDCON) to assign corresponding peak areas and thus determine the final concentration. For example, in the lipophilic phase of a Chow mouse liver PE and CL are overlapping. After peak deconvolution we can determine the area for each compound within the overlapping signal, finally determining the concentration of each. In the phosphorous spectra we can easily find our internal reference on the left which will be integrated and calibrated to a determined concentration value (1 mM for TMP^{+} and 4 mM for TPP). Considering this, metabolic quantification is done by peak integration and comparison with the reference taking into the account the spin system of the peak.

For the ^{1}H-spectra we identify, integrate and calibrate the reference's peak and we perform the same operation as for phosphorous metabolites but in this case for the lactate and choline quantification in the hydrophilic phase and for triglycerides and total cholesterol determination in the lipophilic phase.

The extraction yield was calculated through the comparison of the remaining peak observed at 0 ppms that corresponds to the extraction reference (DSS) that integrates 9 H and the absolute integral of the peak that integrates 12 H of the reference compound of the E1 (TMP^+).

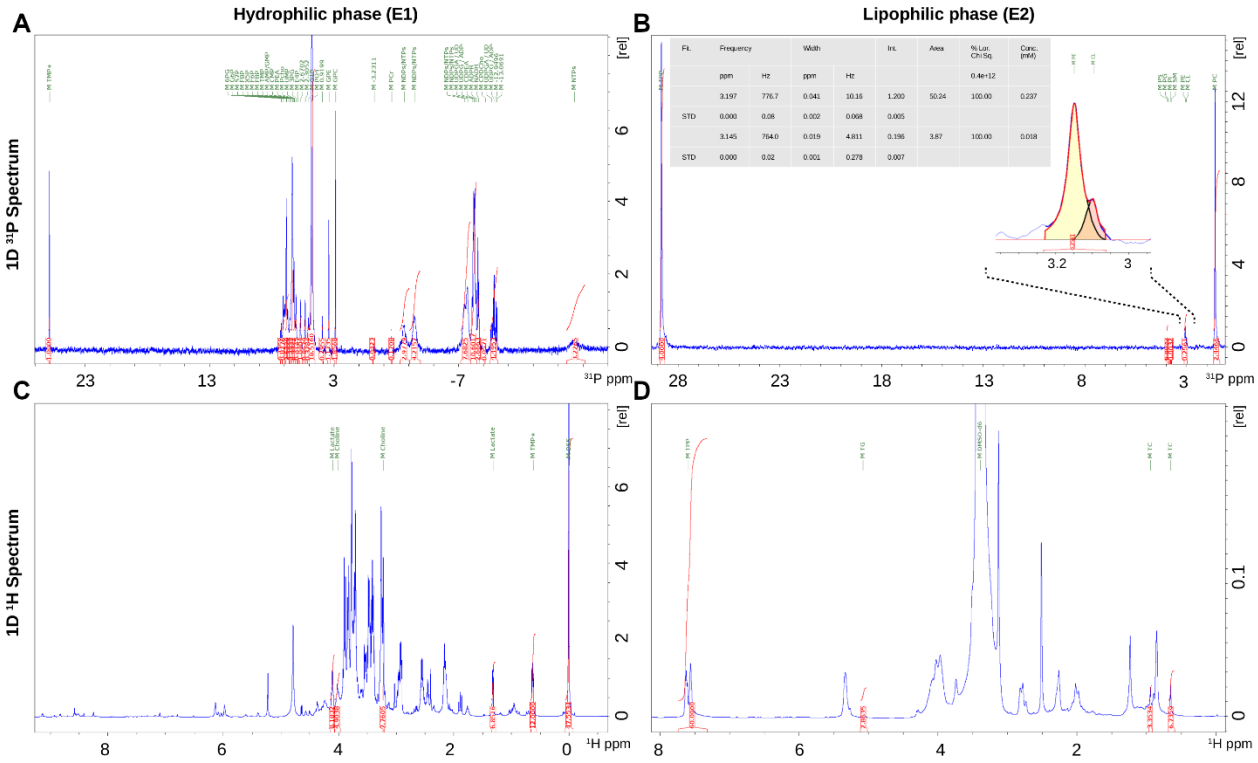

Figure S4. Peak quantification. ^{31}P-NMR *zgig* spectra (first row) and ^{1}H-NMR *p3919gp* spectra (second row) of the hydrophilic (first column) and the lipophilic (second column) of a WT mouse liver extraction. Peak assignment, deconvolution and quantification is performed as mentioned above.

References

1. Bligh, E.G. and Dyer, W. J. Canadian Journal of Biochemistry and Physiology. *Can. J. Biochem. Physiol.* **37**, (1959).
2. Nagana Gowda, G. A. & Raftery, D. Analysis of Coenzymes and Antioxidants in Tissue and Blood Using 1D 1H NMR Spectroscopy. in *Methods in Molecular Biology* vol. 2037 97–110 (Humana Press Inc., 2019).

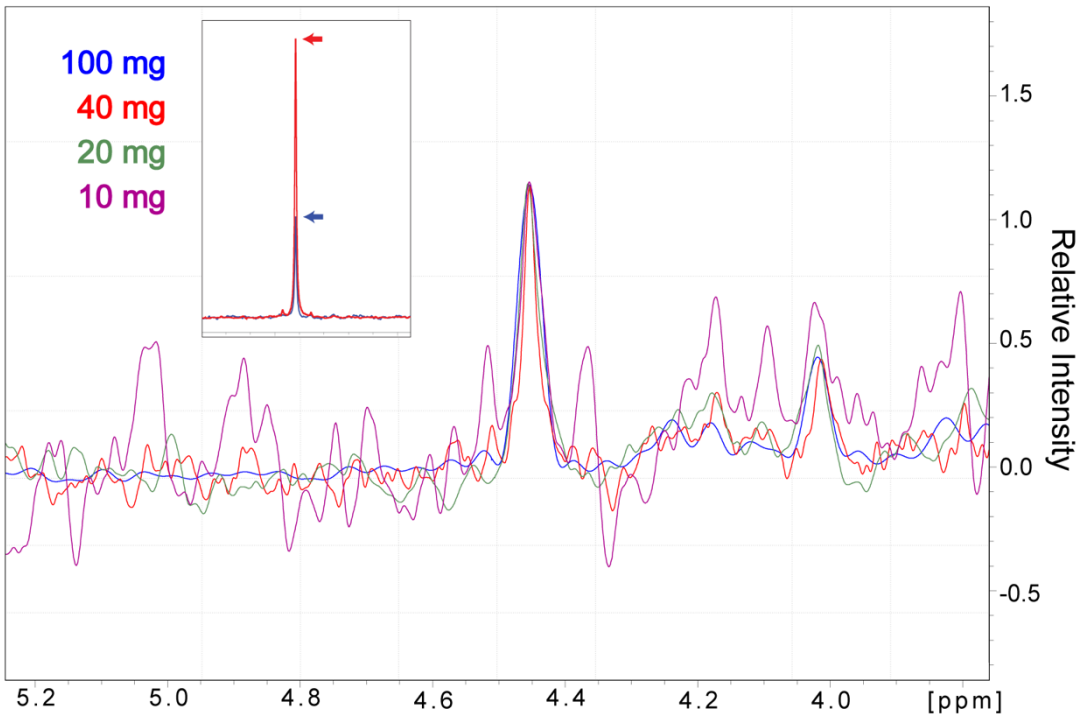

Figure S5. Signal-to-noise ratio (S/N) as a function of the amount of tissue initially used. The spectrum can be quantified using 20 mg of tissue or more. Inset: comparison of the same signal with two different NMR probes.

Compound	Company	Reference
1-(9Z-octadecenoyl)-sn-glycero-3-phospho-L-serine	Avanti	858143P
1,2-diheptadecanoyl-sn-glycero-3-phosphate (sodium salt)	Avanti	830856P
1,2-dimirystoyl-sn-glycero-3-phospho-1´-rac-glycerol	Avanti	840445
1,2-dimirystoyl-sn-glycero-3-phosphocholine	Avanti	850345
1-hexanoyl-2-hydroxy-sn-glycero-3-phosphate (ammonium salt)	Avanti	857119C
1-oleyl-2-hydroxy-sn-glycero-3-phosphocholine	Avanti	845875
1-oleyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	Avanti	846725
1-palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	Avanti	856705P
1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine	Avanti	850457
1-palmitoyl-2-oleyl-sn-glycero-3-phosphoethanolamine	Avanti	850757
2,3-Diphospho-D-glyceric acid pentasodium salt	Sigma	D5764
3-Deoxy-2-keto-6-phosphogluconic acid lithium salt	Sigma	79156
6-Phosphogluconic acid trisodium salt	Sigma	P7877
Adenosine 3´,5´-cyclic monophosphate sodium salt	Sigma	A6885
Adenosine 5´diphosphate sodium salt	Sigma	A2754
Adenosine 5´-diphosphoglucose disodium salt	Sigma	A0627
Adenosine 5´-diphosphoribose sodium salt	Sigma	A0752
Adenosine 5´-monophosphate sodium salt	Sigma	A1752
Adenosine 5´-triphosphate disodium salt	Sigma	A2383
Cardiolipine (Heart,Bovine) (sodium salt)	Avanti	840012
CDP-ethanolamine sodium salt hydrate	Sigma	90756
Cytidine 5´-diphosphate sodium salt	Sigma	C9755
Cytidine 5´-diphosphocholine sodium salt	Sigma	C0256
Cytidine 5´-monophosphate disodium salt	Sigma	C1006
Cytidine 5´-triphosphate disodium salt	Sigma	C1506
D-(-)-3-phosphoglyceric acid disodium salt	Sigma	79473
D-(+)-2-phosphoglyceric acid disodium salt	Sigma	79470
D-Erythrose 4-phosphate sodium salt	Sigma	E0377
D-Fructose 1,6-bisphosphate trisodium salt	Sigma	F6803
D-Fructose 6-phosphate disodium salt	Sigma	F3627
D-Glucose 6-phosphate disodium salt	Sigma	G7879
Dihydroxiacetone phosphate lithium salt / Glyceraldehyde 3-phosphate	Sigma	37442
D-Ribose 5-phosphate disodium salt	Sigma	R7750
D-Ribulose 5´-phosphate disodium salt	Sigma	83899
D-Sedoheptulose 7-phosphate lithium salt	Sigma	78832
D-Xylulose 5´-phosphate lithium salt	Sigma	15732
Farnesyl pyrophosphate	Sigma	F6892
Flavin adenine dinucleotide disodium salt	Sigma	F6625
Geranyl pyrophosphate	Sigma	G6772
Guanosine 5´-diphosphate sodium salt	Sigma	G7127
Guanosine 5´-diphosphoglucose sodium salt	Sigma	G7502
Guanosine 5´-monophosphate disodium salt	Sigma	G8377
Guanosine 5´-triphosphate sodium salt	Sigma	G8877
Lithium 6-phospho-D-galactonate	Sigma	55962
Lithium carbamoylphosphate dibasic hydrate	Sigma	C5625
Lithium potassium acetyl phosphate	Sigma	A0262
L-α-Glycerophosphocholine	Sigma	G5291
L-α-Phosphatidylcholine	Avanti	840054
L-α-Phosphatidylglycerol (Egg,Chicken) (sodium salt)	Avanti	841138

L-α-Phosphatidylinositol(Liver,Bovine) (sodium salt)	Avanti	840042
L-α-Phosphatidylserine(Brain,Porcine) (sodium salt)	Avanti	840032
O-Phosphorylethanolamine	Sigma	P0503
Phospho(enol)pyruvic acid monopotassium salt	Sigma	860077
Phosphocholine chloride calcium salt tetrahydrate	Sigma	P0378
Phosphocreatine disodium salt hydrate	Sigma	P6502
Riboflavin 5´-monophosphate sodium salt	Sigma	F2253
sn-Glycerol 1-phosphate lithium salt	Sigma	92034
sn-Glycerol 3-phosphate bis(cyclohexylammonium) salt	Sigma	G4631
Sodium phosphate	Sigma	342483
Sodium pyrophosphate tetrabasic	Sigma	322466
Spingomyelin (Egg,Chicken)	Avanti	860061
Tetramethylphosphonium chloride	Sigma	288276
Thymidine 5´-diphosphate disodium salt	Sigma	T7004
Thymidine 5´-monophosphate disodium salt	Sigma	T7004
Thymidine 5´-triphosphate sodium salt	Sigma	T0251
Triphenylphosphine oxide	Sigma	T84603
Uridine 5´-diphosphate disodium salt	Sigma	94330
Uridine 5´-diphosphogalactose disodium salt	Sigma	U4500
Uridine 5´-diphosphoglucose disodium salt	Sigma	94335
Uridine 5´-diphosphoglucuronic acid trisodium salt	Sigma	U6751
Uridine 5´-monophosphate disodium salt	Sigma	U6375
Uridine 5´-triphosphate trisodium salt hydrate	Sigma	94370
α-D-Glucose 1-phosphate disodium salt	Sigma	G7000
β- Nicotinamide adenine dinucleotide	Sigma	N0632
β-Glycerophosphate disodium salt	Sigma	G9422
β-Nicotinamide adenine dinucleotide 2´-phosphate reduced tetrasodium salt	Sigma	N7505

Table S2. Phosphorilated metabolites tested.

			Congenital Erythropoietic Porphyria [nmol/mg]						NASH [nmol/mg]							
Peak	**Compound**	**Extract**	**Control CEP**	STDV	**UROS**	STDV	**UROS + CPX**	STDV	**Control MAT1A-KO**	STDV	**MAT1A-KO**	STDV	**Control CDHF**	STDV	**CDHF**	STDV
16	2PG	E1	0.44	0.06	0.38	0.06	0.43	0.08	0.28	0.00	0.24	0.02	0.17	0.01	0.54	0.03
14	3PG	E1	0.56	0.10	1.37	0.04	0.39	0.08	0.62	0.01	0.78	0.04	0.51	0.03	0.96	0.06
2	6PG	E1	0.13	0.02	0.03	0.01	0.06	0.01	0.40	0.02	0.17	0.02	0.26	0.02	0.23	0.01
30	ADPR	E1	0.54	0.07	0.97	0.11	0.46	0.05	1.12	0.04	1.55	0.13	0.39	0.05	1.81	0.19
9	AMP/GMP	E1	1.44	0.18	1.21	0.14	1.84	0.10	3.59	0.27	6.97	0.59	3.80	0.04	9.08	0.65
32	CDPCH	E1	0.15	0.02	0.31	0.04	0.14	0.01	0.39	0.02	0.17	0.02	0.27	0.00	0.04	0.01
29	CDPE	E1	1.14	0.12	0.93	0.18	0.86	0.08	3.53	0.08	1.57	0.35	1.48	0.07	1.36	0.12
	Cho	E1 (1H)	4.38	0.15	2.45	0.24	3.52	0.15	0.07	0.01	0.09	0.01	0.47	0.02	1.08	0.06
47	CL	E2	0.40	0.07	0.62	0.10	0.41	0.07	0.05	0.01	0.06	0.01	0.18	0.01	0.13	0.01
10	CMP	E1	1.06	0.14	0.80	0.10	1.33	0.08	1.26	0.10	1.58	0.16	1.46	0.07	1.17	0.07
15	E4P	E1	0.03	0.01	0.07	0.02	0.22	0.04	0.04	0.00	0.03	0.01	0.03	0.01	0.06	0.01
7	F6P	E1	0.02	0.01	0.10	0.04	0.32	0.05	0.04	0.01	0.11	0.02	0.05	0.00	0.16	0.02
28	FADH2	E1	0.78	0.13	0.37	0.03	1.17	0.24	2.74	0.39	0.60	0.21	1.76	0.36	0.80	0.16
5	FBP	E1	0.02	0.01	0.06	0.01	0.32	0.05	0.04	0.01	0.08	0.01	0.17	0.02	0.13	0.01
18	G1P	E1	0.94	0.09	0.49	0.04	0.88	0.07	0.46	0.02	0.66	0.03	0.71	0.06	0.83	0.07
3	G6P	E1	0.12	0.03	0.06	0.01	0.82	0.15	0.56	0.02	0.85	0.06	0.55	0.01	1.52	0.23
22	GPC	E1	5.58	1.03	3.22	0.49	2.29	0.76	2.74	0.12	0.79	0.09	1.53	0.15	2.16	0.18
21	GPE	E1	3.23	0.38	1.65	0.41	1.40	0.47	1.57	0.13	0.39	0.06	0.65	0.07	0.68	0.04
17	Impurity	E1	1.27	0.06	1.38	0.11	1.38	0.13	1.21	0.05	1.01	0.10	1.27	0.03	3.16	0.20
	LAC	E1 (1H)	3.30	0.15	4.45	0.16	2.36	0.16	2.73	0.22	2.41	0.43	4.57	0.08	8.53	0.63
40	LPA/LPE	E2	0.02	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.01	1.00
44	LPC	E2	0.07	0.01	0.12	0.01	0.06	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00
31	NADH	E1	0.56	0.07	0.21	0.03	0.30	0.02	4.21	0.09	2.10	0.23	0.43	0.04	1.96	0.24
20	NADPH	E1	0.81	0.09	0.20	0.04	0.52	0.08	0.39	0.03	0.16	0.02	0.19	0.00	1.09	0.12
25	NDPs	E1	1.81	0.08	1.31	0.05	2.07	0.15	3.08	0.14	2.89	0.25	1.27	0.06	3.39	0.24
35	NTPs	E1	0.23	0.05	0.13	0.03	0.07	0.02	5.86	0.17	1.69	0.23	0.42	0.02	1.43	0.19
50	PC	E2	2.78	0.18	1.53	0.26	3.00	0.28	2.25	0.17	2.58	0.26	2.64	0.11	5.61	0.68
12	PCH	E1	0.15	0.01	0.11	0.01	0.67	0.10	0.53	0.11	0.43	0.06	0.95	0.07	0.17	0.01
24	PCr	E1	0.25	0.03	0.19	0.02	0.16	0.02	0.16	0.01	0.19	0.03	0.12	0.01	0.16	0.01
46	PE	E2	0.62	0.08	0.84	0.10	0.64	0.10	0.25	0.01	0.40	0.03	0.31	0.01	0.97	0.12
11	PEA	E1	0.13	0.02	0.11	0.01	0.54	0.09	0.67	0.11	0.27	0.05	0.89	0.04	0.37	0.03
23	PEP	E1	0.04	0.01	0.04	0.01	0.04	0.01	0.11	0.01	0.03	0.00	0.02	0.00	0.05	0.02
41	PG	E2	0.10	0.01	0.18	0.07	0.11	0.05	0.01	0.00	0.01	0.00	0.02	0.01	0.06	0.01
42	PI	E2	0.07	0.02	0.05	0.01	0.06	0.02	0.00	0.00	0.01	0.00	0.02	0.00	0.04	0.00
19	PO_4	E1	63.00	1.74	52.82	4.45	84.80	6.20	21.54	2.07	29.49	2.28	47.68	0.16	23.62	1.26
39	PS	E2	0.36	0.06	0.25	0.06	0.27	0.07	0.13	0.01	0.13	0.02	0.03	0.01	0.18	0.04
4	S7P	E1	0.35	0.04	0.28	0.09	1.44	0.20	3.71	0.14	3.28	0.24	4.38	0.06	0.69	0.04
43	SM	E2	0.07	0.01	0.05	0.01	0.10	0.04	0.00	0.00	0.01	0.00	0.05	0.01	0.02	0.00
	TC	E2 (1H)	3.23	0.03	4.25	0.05	2.85	0.11	3.28	0.08	3.91	0.47	3.17	0.22	8.23	0.98
	TG	E2 (1H)	23.14	1.31	29.88	1.89	18.98	1.96	9.10	0.38	12.02	0.40	12.62	0.18	38.89	4.31
8	TMP	E1	0.18	0.03	0.28	0.05	0.52	0.06	0.51	0.07	0.64	0.07	0.53	0.00	0.43	0.10
1	TMP+	E1	0.99	0.01	1.00	0.01	1.01	0.02	0.93	0.05	1.06	0.06	1.00	0.00	1.00	0.00
36	TPP	E2	3.79	0.06	4.46	0.32	4.00	0.06	3.54	0.15	3.84	0.04	3.87	0.02	4.00	0.00
27	NDPG	E1	0.32	0.05	0.87	0.10	0.33	0.07	1.73	0.08	2.09	0.24	0.36	0.06	1.87	0.10
26	UDPGA / UDPgal	E1	0.14	0.01	0.57	0.03	0.17	0.05	0.95	0.07	0.47	0.06	0.28	0.01	0.72	0.03
13	UMP	E1	0.07	0.01	0.06	0.01	0.42	0.09	0.59	0.08	0.39	0.05	0.87	0.05	0.14	0.01
6	X5P	E1	0.24	0.03	0.15	0.05	1.05	0.14	0.77	0.03	0.48	0.04	0.75	0.02	0.17	0.01
33	Unknown	E1	0.09	0.01	0.13	0.03	0.07	0.01	0.27	0.01	0.07	0.01	0.07	0.00	0.16	0.02
34	Unknown	E1	0.44	0.07	0.67	0.10	0.36	0.07	1.69	0.05	0.65	0.08	0.54	0.01	0.95	0.03
37	Unknown	E2	0.06	0.01	0.17	0.08	0.07	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00
38	Unknown	E2	0.12	0.03	0.31	0.08	0.19	0.05	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00
45	Unknown	E2	0.16	0.05	0.21	0.07	0.21	0.08	0.00	0.00	0.01	0.00	0.03	0.01	0.12	0.01
48	Unknown	E2	0.12	0.03	0.54	0.16	0.25	0.09	0.00	0.00	0.02	0.00	0.00	0.00	0.06	0.01
49	Unknown	E2	0.15	0.04	0.65	0.13	0.30	0.09	0.00	0.00	0.01	0.00	0.00	0.00	0.05	0.01

Table S3. Quantification of the metabolites and standard deviations (STDV) for the different compounds and mouse models under consideration. The following number of mice were used in each experiment: Control CEP, n=6; CEP, n=7; CEP+CPX, n=7; Control MAT1A-KO, n=6; MAT1A-KO, n=9; Control MAT1A-KO, n=6; CDHF, n=9.

E1 (Hydrophilic)	^{31}P (ppm)	STDV
TMP+ (REF)	25.83	0.01
6PG	6.86	0.36
G6P	6.79	0.28
S7P	6.66	0.25
FBP	6.65 6.39	0.25 0.21
X5P	6.51	0.23
F6P	6.39	0.22
TMP	6.22	0.28
AMP/GMP	6.14	0.26
CMP	6.13	0.22
PEA	5.97	0.24
PCH	5.96	0.23
UMP	5.95	0.22
3PG	5.77	0.24
E4P	5.60	0.24
2PG	5.40	0.25
G1P	4.71	0.37
PO4	4.45	0.41
NADPH	3.90	0.05
GPE	3.41	0.01
GPC	2.87	0.01
PEP	-0.23	0.06
PCr	-1.62	0.00
NDPs/NTPs	-2.86 -3.94 -7.17 -7.68	0.29 0.49 0.27 0.04
UDPGA / UDPgal	-8.20 -9.65	0.05 0.02
UDPG / ADPG / GDPG / FADH2	-8.24 -9.83	0.01 0.01
CDPE	-8.26	0.02
ADPR	-8.34	0.11
NADH	-8.61	0.05
CDPCH	-9.07	0.09
NTPs	-16.72	0.37

E2 (Hydrophobic)	^{31}P (ppm)	STDV
TPP (REF)	28.60	0.09
PS	4.05	0.08
LPA/LP	4.02	0.06
PG	3.99	0.07
PI	3.84	0.10
SM	3.80	0.10
LPC	3.46	0.04
PE	3.15	0.07
CL	3.19	0.10
PC	1.67	0.09

Table S4. ^{31}P Chemical shifts of assigned metabolites in the hydrophilic (E1) and hydrophobic phase (E2).

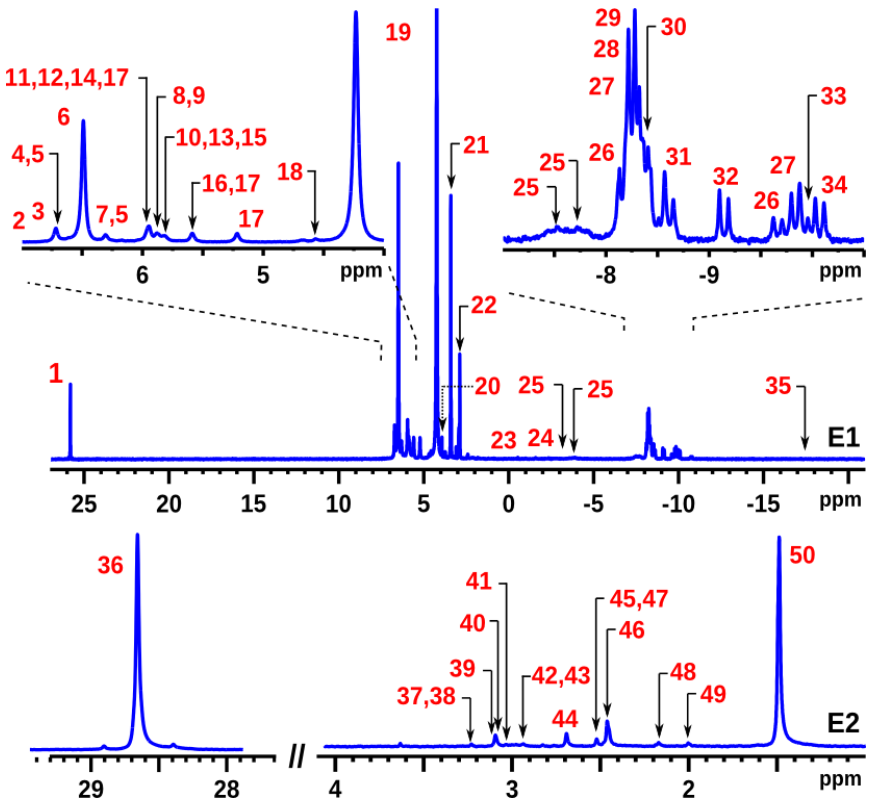

Figure S6. ^{31}P-NMR spectrum of a sample of human liver tissue (hepatocellular carcinoma) obtained from biopsy. E1 (E2) stands for the hydrophilic (lipophilic) extract. A total of 50 different peaks are identified within the two extracts. For the abbreviation meaning, see Table 1.

Mice	Control CEP (n = 6)	CEP (n = 6)	CEP+CPX (n = 6)
NAS score	**0** (100%)	**2** (66%); **3** (33%)	**0** (100%)
Fibrosis stage	**0** (100%)	**1** (66%); **0** (33%)	**0** (66%); **1** (33%)

Table S5. NAS score for CEP. Reference values for NAS scores: 0-2 considered as not diagnostic of NASH, 3-4 borderline dignostic of NASH, 5-8 positive diagnostic of NASH.

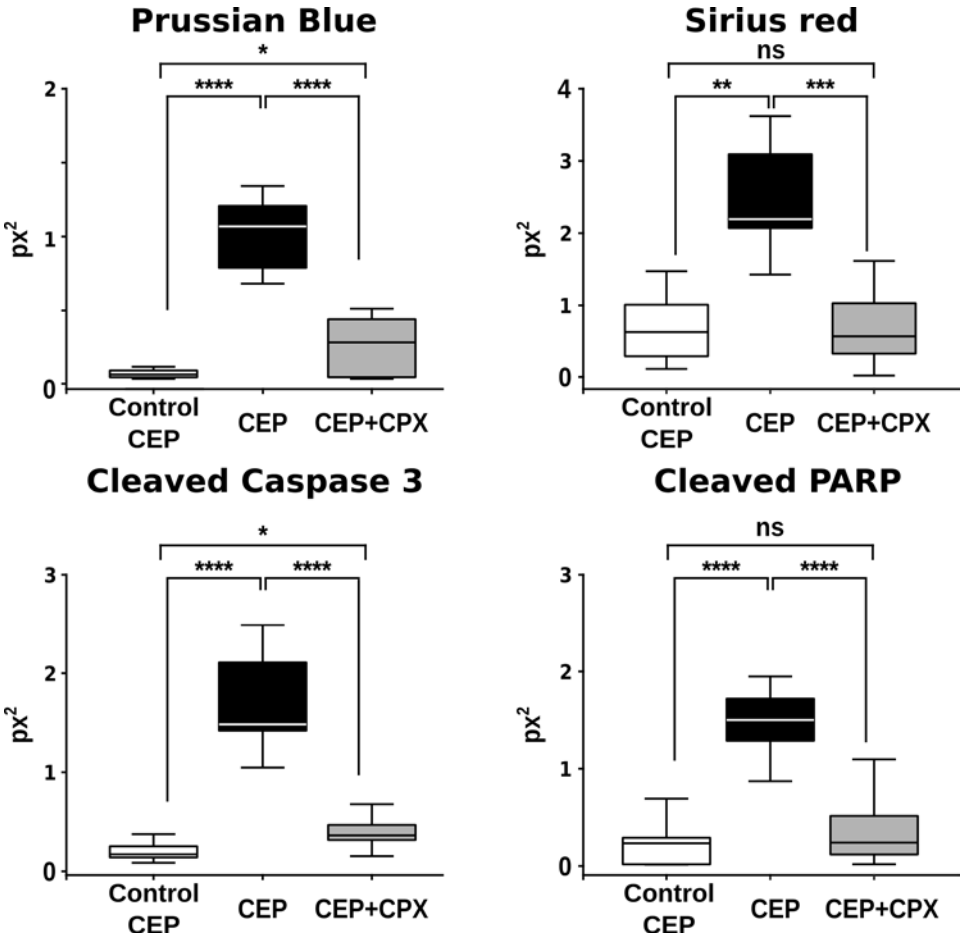

Figure S7. Quantification of the different histological stainings. P-values of < 0.1, 0.05, 0.01, 0.001 and 0.0001 are represented by ·, *, **, *** and **** respectively while ns accounts for not significant.

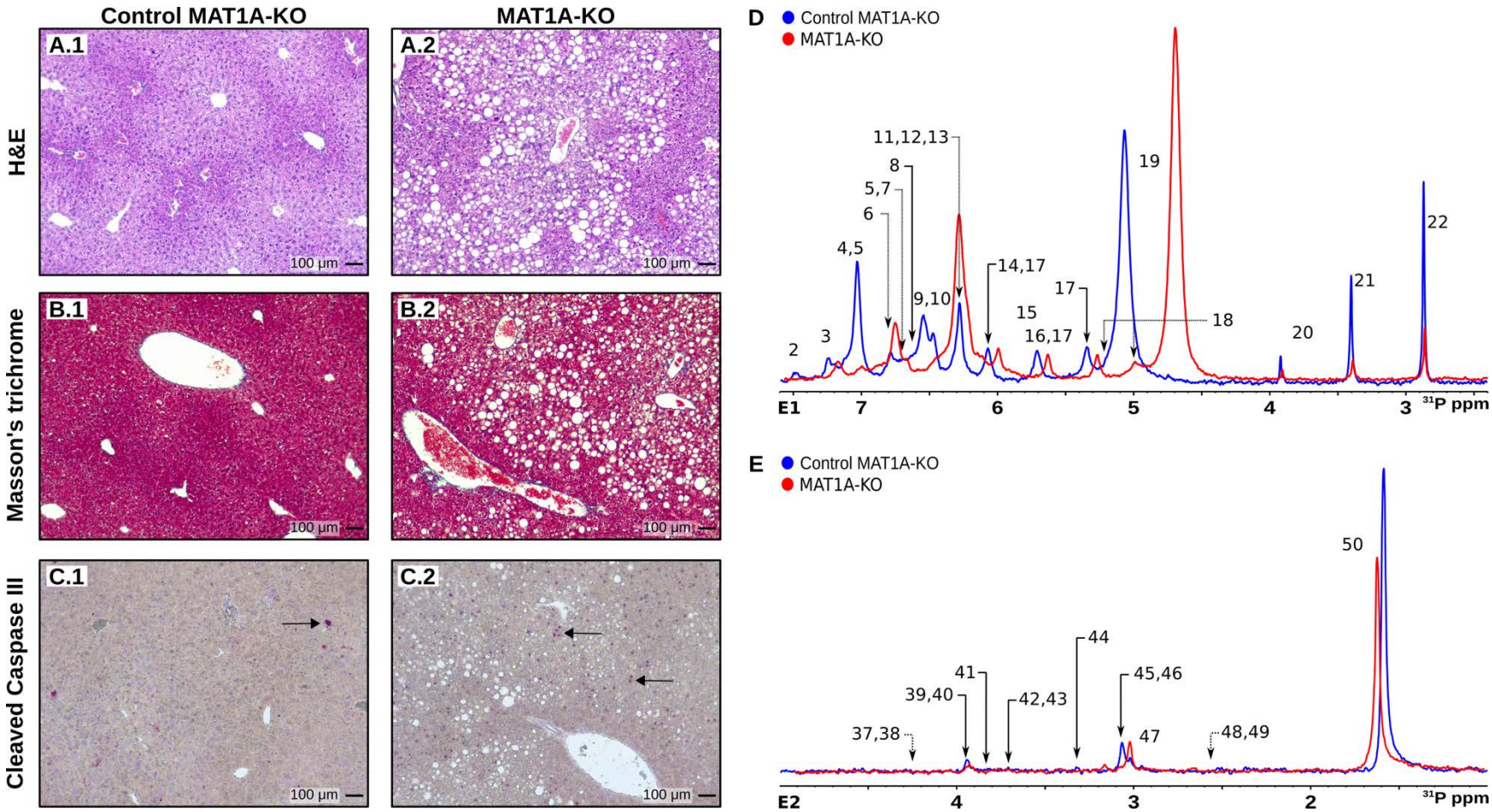

Figure S8. Characterization of the MAT1A-KO mice. A-C) Histology performed by Hematoxylin/Eosin (A.#) Masson's trichrome (B.#) and Cleaved Caspase III staining (C.#) of Control MAT1A-KO (#.1) and MAT1A-KO (#.2) mice models. MAT1A-KO mice is compatible with a steatotic liver with fibrosis. Arrows point to cells with Caspase III activity. D-E) ^{31}P-NMR spectra of hydrophilic (D) and lipophilic (E) phase for WT (blue) and MAT1A-KO (red) mice models. For the abbreviation meaning, see Table 1.

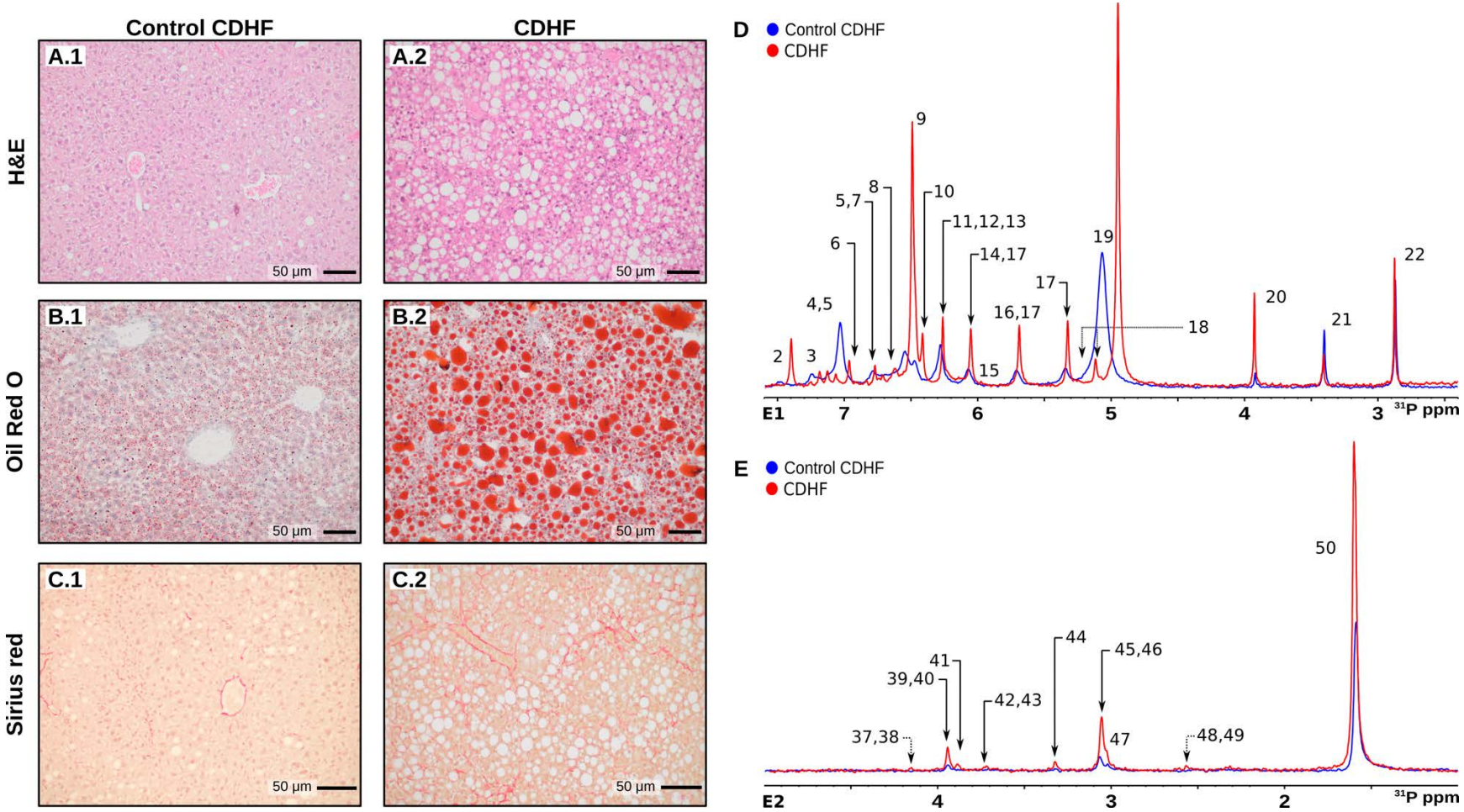

Figure S9. Characterization of the CDHF mice. A-C) Histology performed by Hematoxylin/Eosin (A.#), Oil Red-O (B.#) and Cleaved Caspase III staining (C.#) of Control CDHF (#.1) and CDHF (#.2) mice models. CDHF mice is compatible with a steatotic liver with fibrosis. Arrows point to cells with Caspase III activity. D-E) ^{31}P-NMR spectra of hydrophilic (D) and lipophilic (E) phase for WT (blue) and CDHF (red) mice models. For the abbreviation meaning, see Table 1.