Supporting information

Cross-species molecular imaging of bile salts and lipids in liver: identification of molecular structural markers in health and disease.

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Special staining of frozen tissue sections

HE

- Fixation: 10 minutes in formalin 10% at 4°C
- Airdry, staining on room temperature
- Dip in demin water
- Haematoxyline Gill III (Prosan)(or Harris): 2 minutes
- Tap water: 8 dips
- Demin water: 8 dips
- Phosphotungstic acid 2 %: 4 dips
- Demin water: 8 dips
- Sodiumacetate 2%: 4 dips
- Demin water: 8 dips
- Eosin 1% (Prosan): 1 min
- Ethanol 96 %: 2 x 8 dips
- Ethanol absolute: 2 x 8 dips
- Xylene: 2 x 1 minute
- Mount with synthetic mounting medium

Masson's Trichrome

Preparation of reagents

- Ponceau de xylidine 1 %: solve 3 g of Ponceau de Xylidine in 300 ml cooking demin water and let it cook for 1 or 2 minutes, then add 3 ml acetic acid and filter the solution.
- Acid anilin blue 0.5 %: solve 1 g of anilin blue in 200 ml demin water. Add 4 ml acetic acid.
- Phosphotungstic acid 3 %: solve 6 g of phosphotungstic acid in 200 ml demin water.
- Acetic acid 1 %: dilute 2 ml acetic acid in 200 ml demin water
- Mayer's haematoxylin: bought solution (Sigma Aldrich)

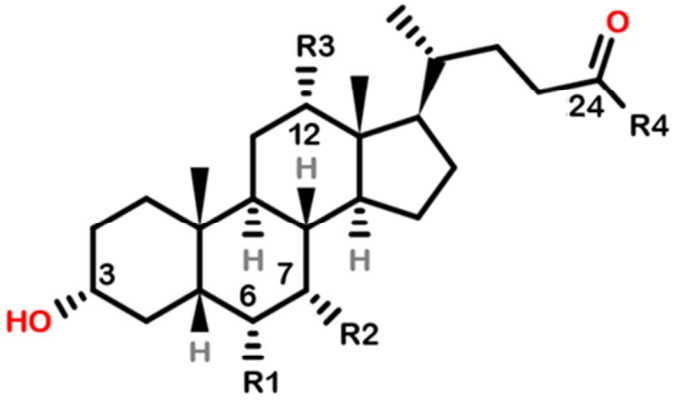
Staining:

- Fixation: 10 minutes in formalin 10 % at 4°C
- Airdry, staining on room temperature
- Mayer's haematoxylin: 10 minutes
- Running tap water: 10 minutes
- Rinse in demin water
- Phosphotungstic acid 3 %: 7 minutes
- Rinse in demin water
- Acid anilin blue 0.5 %: 4 minutes
- Acetic acid solution 1 %: dip a few times
- Ethanol 96 %: 2 x 2 minutes
- Ethanol absolute: 2 x 2 minutes
- Xylene: 2 x 2 minutes
- Mount with synthetic mounting medium

Immunohistochemistry

- Dry slides: 15-30 minutes
- Fix section: 10 minutes in acetone -20^{0}C
- Rinse with PBS
- Encircle tissue section with DAKO-Cytomation pen
- Rinse with PBS
- Block endogenous peroxidases with 0.3% H_2O_2 in methanol: 30 minutes
  1 ml 30% H_2O_2 + 100 ml methanol
- Rinse with PBS: 3 x 3 minutes
- Block with 5% BSA-PBS: 30 minutes
  2 ml PBS + 2 ml 10% BSA
- Incubate with 1:2000 α –Cytokeratin (DAKO Z0622) in DB: 1 hour
- Rinse with PBS: 3 x 3 minutes
- Incubate with 1:500 Goat- α –Rabbit-HRP in DB: 1 hour
- Rinse with PBS: 3 x 3 minutes
- Incubate with AEC
  19 ml 0.5M NaAc
  171 ml with MQ
  9 ml DMSO
  Before use; add 69 µl H_2O_2 + 1 ml 4% DMSO-AEC
- Rinse with demi water: ± 5 minutes
- Stain with Haematoxylin GIII staining: 1-2 min (liver)
- Rinse with tap water: ± 5 minutes
- Dry slides
- Mount with DAKO Faramount aqueous mounting medium ready-to-use S3025

A



B

Name	R1	R2	R3	R4
CA	H	α-OH	OH	OH
CDCA	H	α-OH	H	OH
LCA	H	H	H	OH
DCA	H	H	OH	OH
TCA	H	α-OH	OH	$NH(CH_2)_2SO_3H$
TCDCA	H	α-OH	H	$NH(CH_2)_2SO_3H$
TDCA	H	H	OH	$NH(CH_2)_2SO_3H$
GCA	H	α-OH	OH	$NHCH_2CO_2H$
GCDCA	H	α-OH	H	$NHCH_2CO_2H$
GDCA	H	H	OH	$NHCH_2CO_2H$

C

Name	Type	Formula	Mass ($[M-H]^-$)
CA	Primary	$C_{24}H_{39}O_5$	407.26
CDCA	Primary	$C_{24}H_{39}O_4$	391.26
LCA	Secondary	$C_{24}H_{39}O_3$	375.27
DCA	Secondary	$C_{24}H_{39}O_4$	391.26
TCA	Primary	$C_{26}H_{44}NO_7S$	514.28
TCDCA	Primary	$C_{26}H_{44}NO_6S$	498.28
TDCA	Secondary	$C_{26}H_{44}NO_6S$	498.28
GCA	Primary	$C_{26}H_{42}NO_6$	464.30
GCDCA	Primary	$C_{26}H_{42}NO_5$	448.30
GDCA	Secondary	$C_{26}H_{42}NO_5$	448.30

Figure S1. Information on bile acids. (A) Base structure of bile acids and (B) positions of the hydroxyl groups and site of conjugation. (C) Observed masses of bile acid standards in negative ion mode.

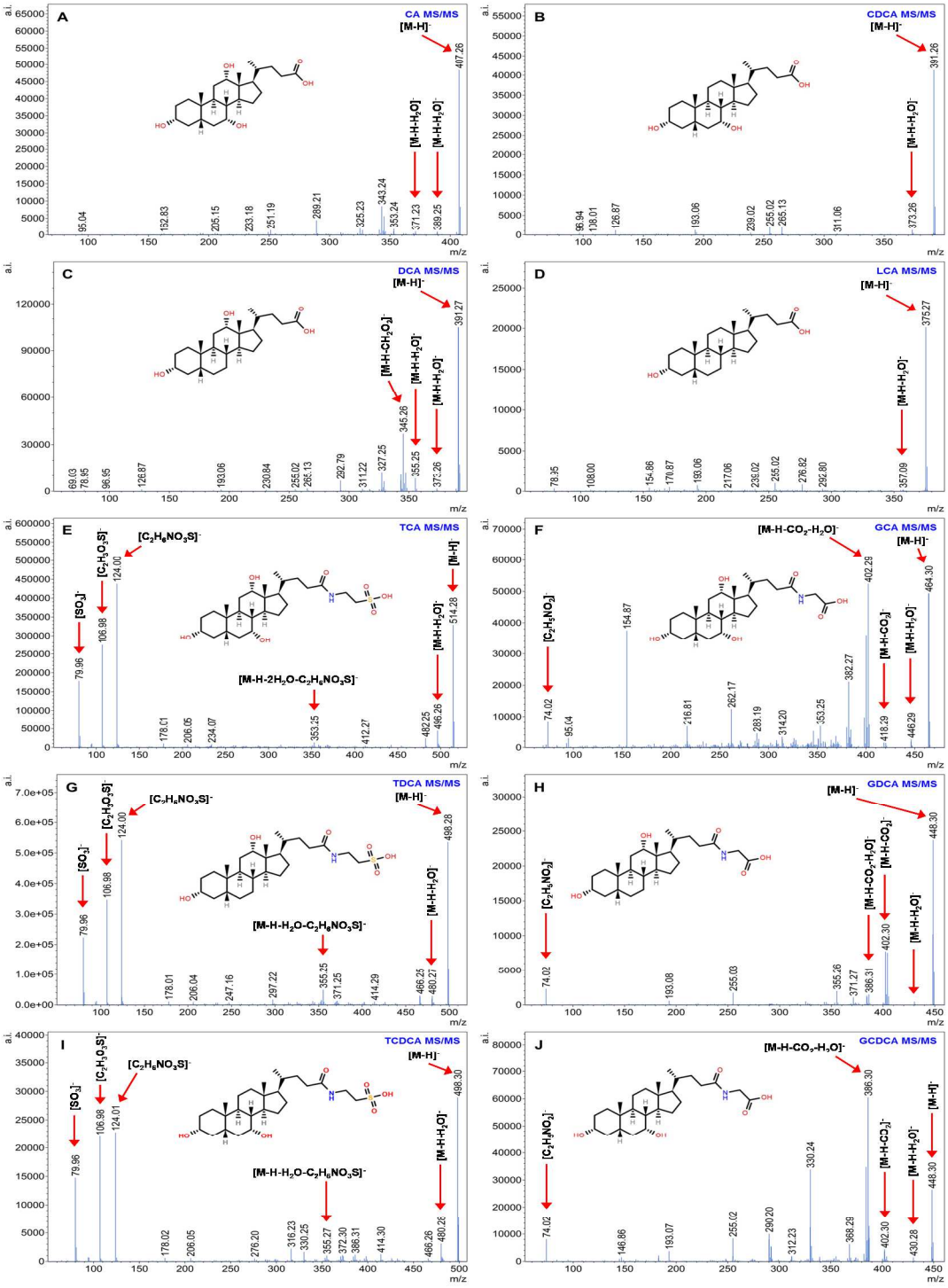


Figure S2. MALDI-MS/MS spectra of deprotonated ([M-H]$^-$) bile acid standards in negative ion mode. (A) cholic acid (*m/z* 407.26), (B) chenodeoxycholic acid (*m/z* 391.27), (C) deoxycholic acid (*m/z* 391.27), (D) lithocholic acid (*m/z* 375.27), (E) taurocholic acid (*m/z* 514.28), (F) glycocholic acid (*m/z* 464.30), (G) taurodeoxycholic acid (*m/z* 498.30), (H) glycodeoxycholic acid (*m/z* 448.30), (I) taurochenodeoxycholic acid (*m/z* 498.30), (J) glycochenodeoxycholic acid (*m/z* 448.30).

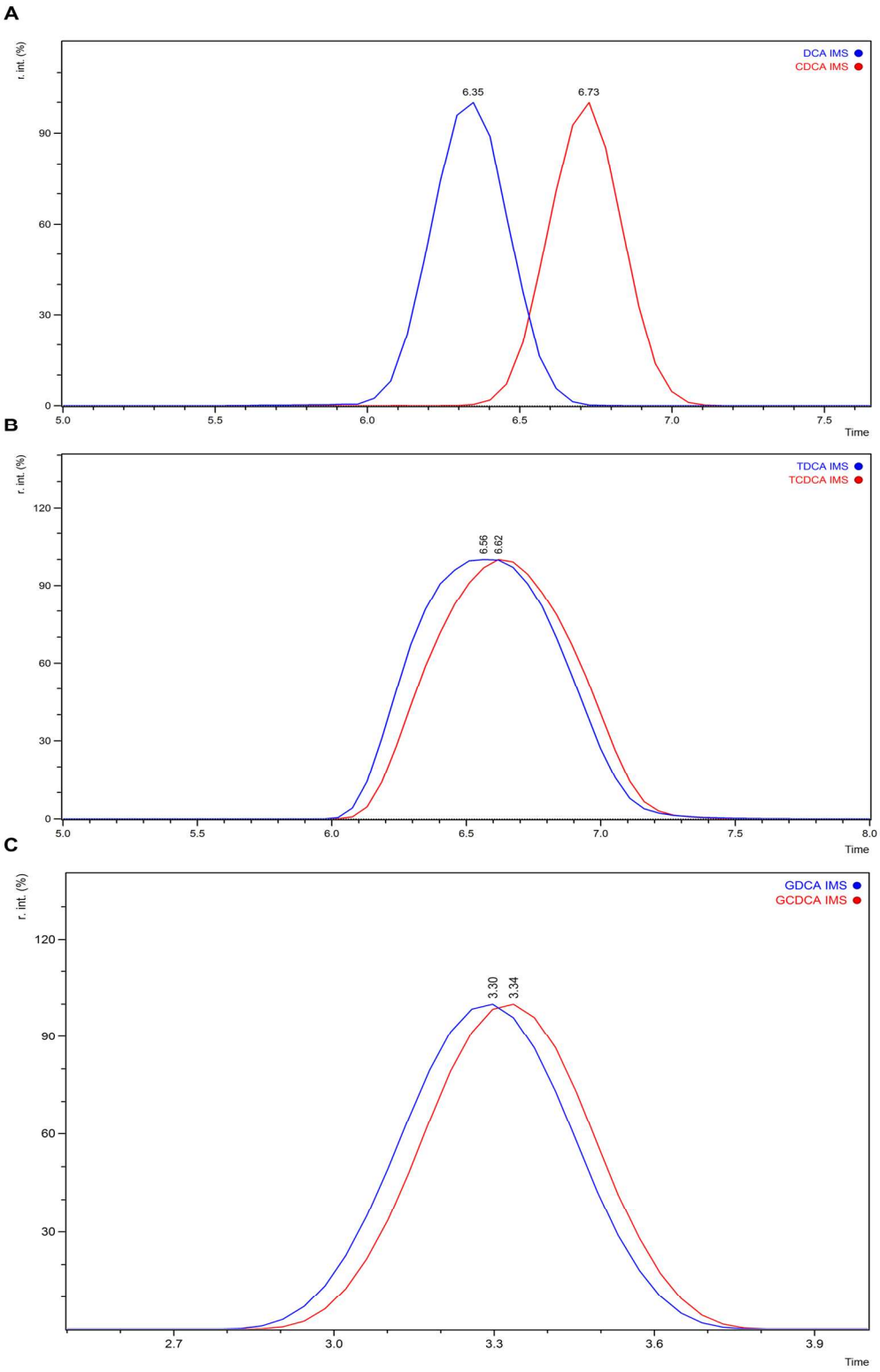


Figure S3. MALDI-IMS-MS of isomeric bile acids in negative ion mode. Mobilograms of (A) DCA/CDCA (*m/z* 391.27), (B) TDCA/TCDCA (*m/z* 498.28) and (C) GDCA/GCDCA (*m/z* 448.30).

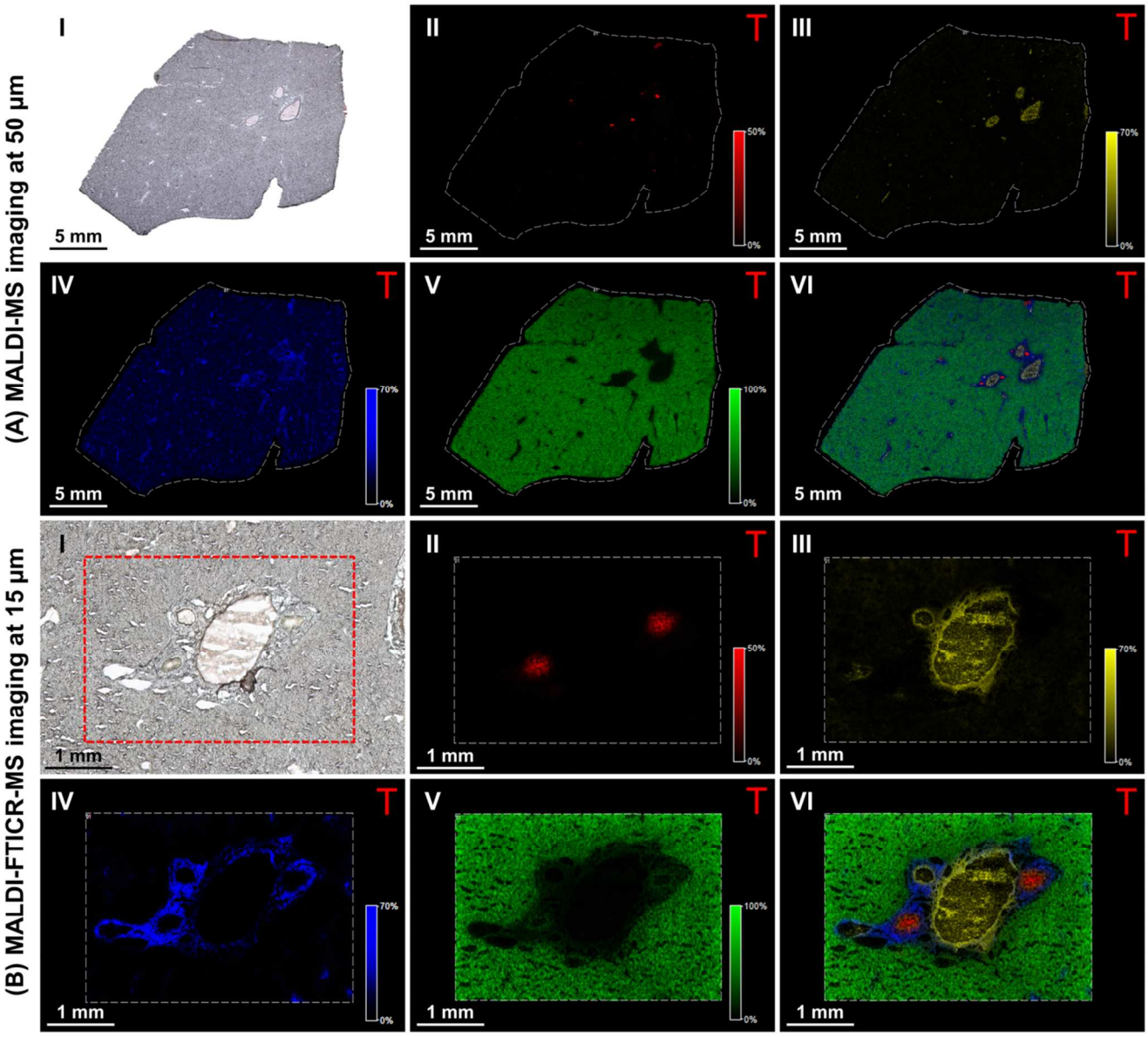
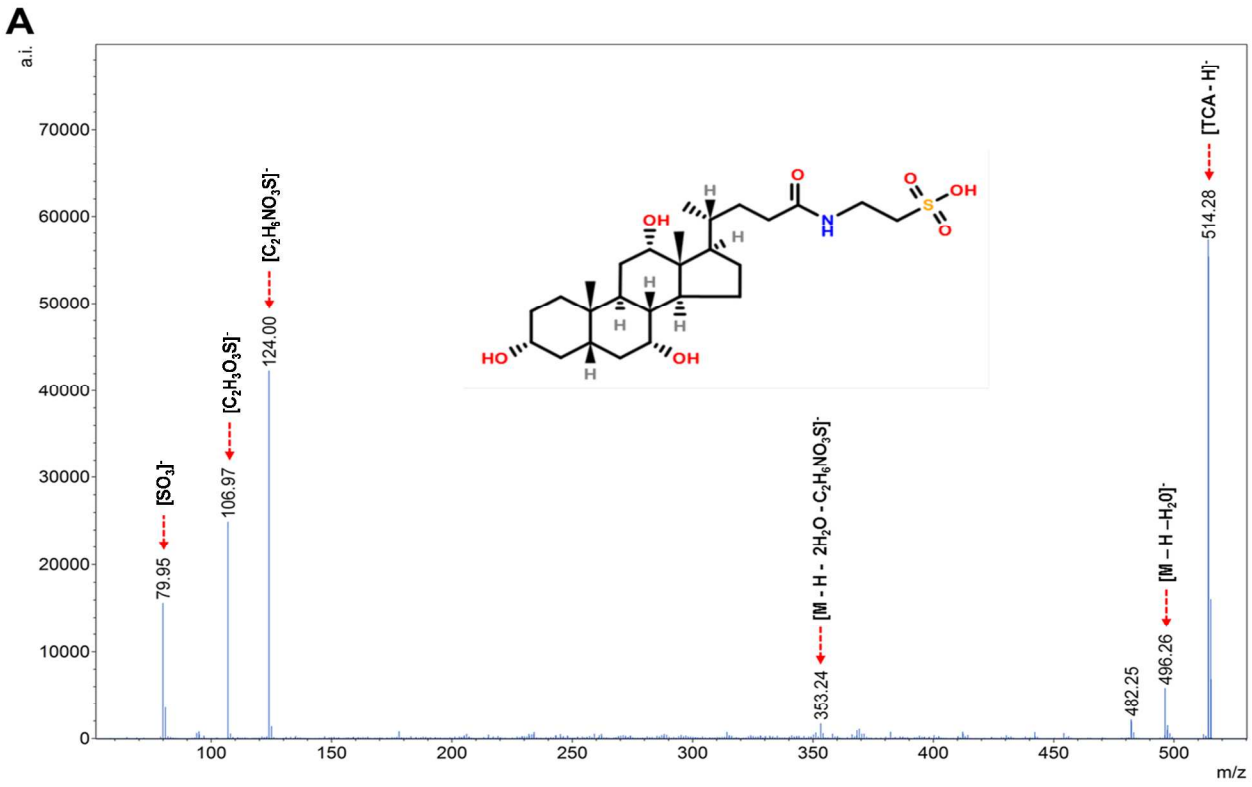


Figure S4. Multimodal imaging of healthy dog liver tissue. (I) Optical image of the tissue section prior to matrix application. Positive-ion mode (A) MALDI-MS and (B) MALDI-FTICR-MS images showing the distribution of selected molecular species in the (II) bile duct lumen (unknown at *m/z* 576.2418), (III) blood vessels (heme $[M]^+$ at *m/z* 616.1804), (IV) connective tissue ($[PC\ (32:0)+K]^+$ at *m/z* 772.5254), (V) parenchyma ($[PC\ (38:4)+K]^+$ at *m/z* 848.5564) and (VI) overlay of the selected species. (Designated masses are from MALDI-FTICR-MS imaging measurements, MALDI-MS image field of view 141828 pixels, area 124 mm^2 and spatial resolution 50 µm, MALDI-FTICR-MS image field of view 41700 pixels, 9.3 mm^2 area and spatial resolution 15 µm. Both images normalized with TIC).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[C2H3O3S]-
106.97
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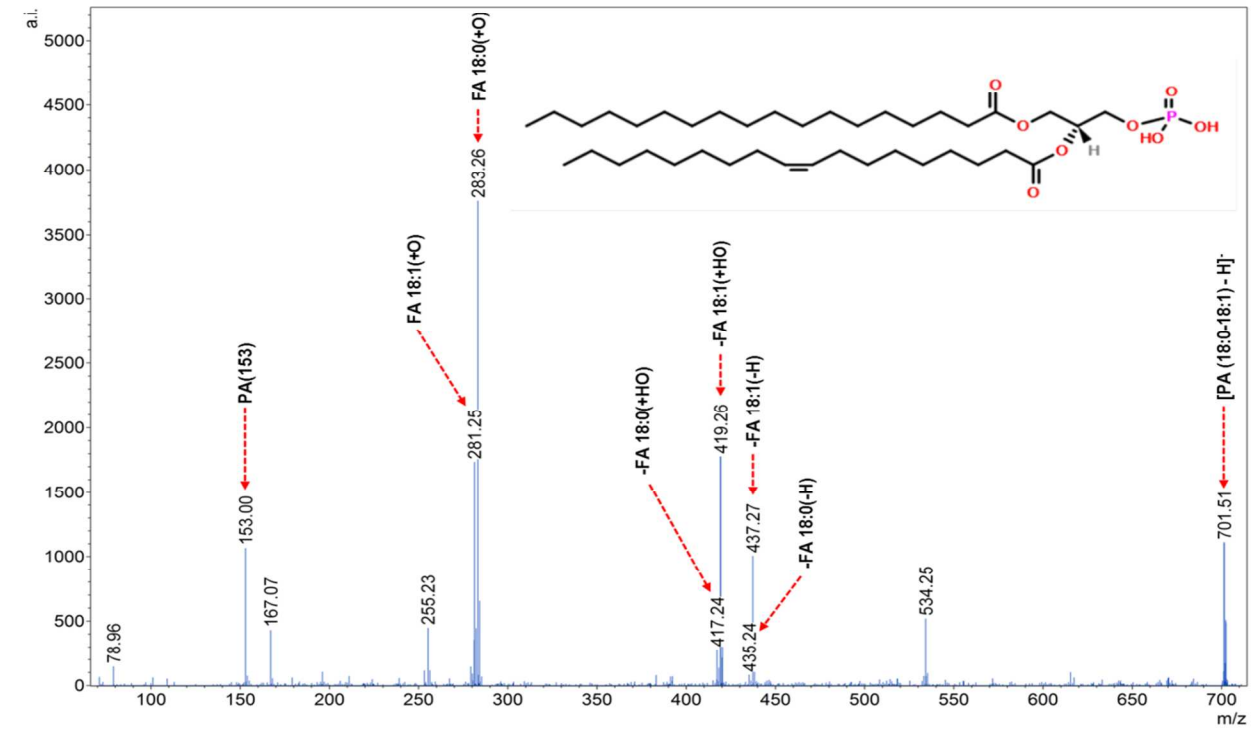
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m/z
FA 18:0(+O)
283.26
FA 18:1(+O)
281.25
PA(153)
153.00
167.07
78.96
255.23
-FA 18:0(+HO)
-FA 18:1(+HO)
419.26
417.24
-FA 18:1(-H)
437.27
435.24
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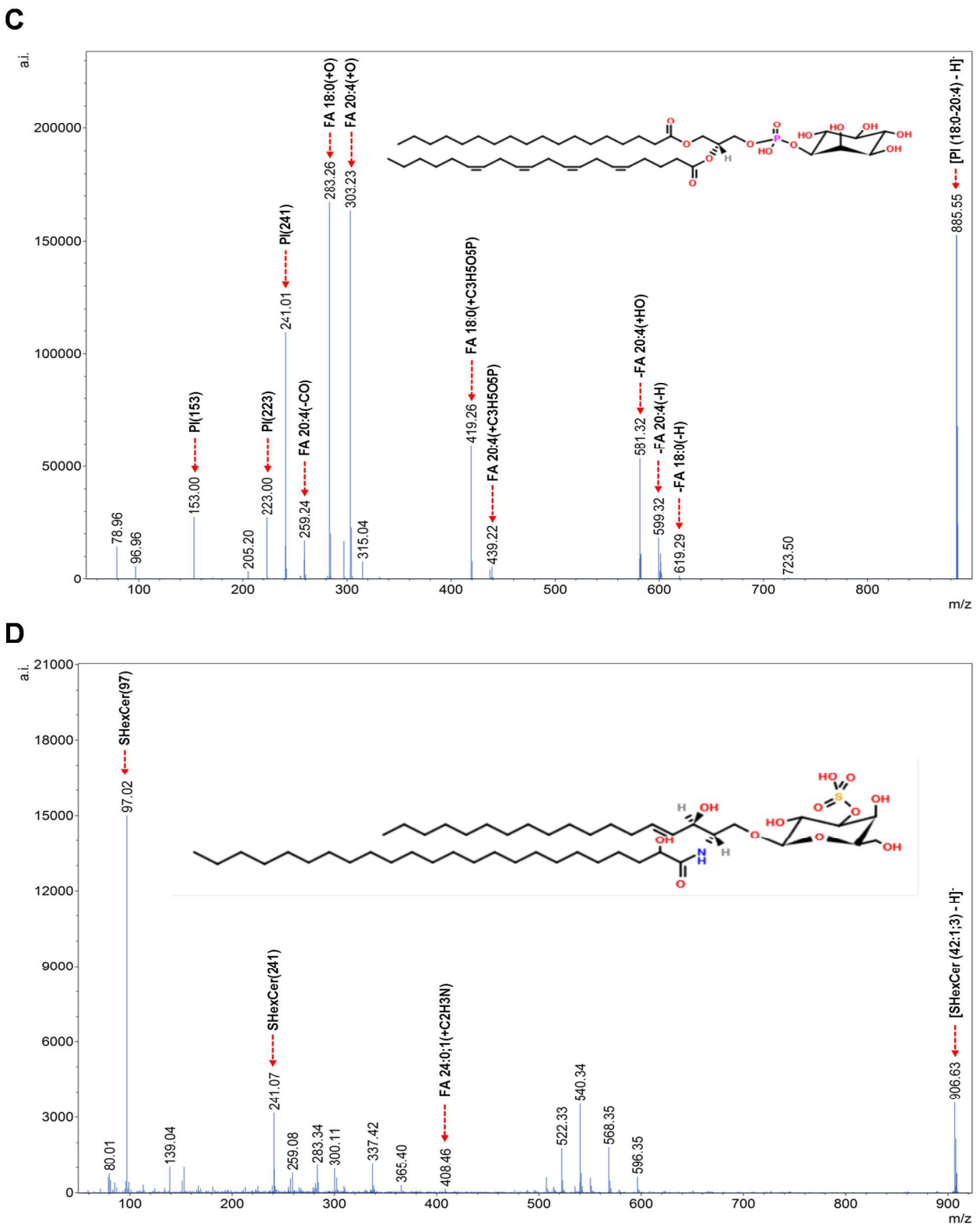


Figure S5. MALDI-MS/MS spectra of structural molecular markers for (A) bile duct lumen, (B) connective tissue, (C) parenchyma and (D) bile duct. Spectra obtained from healthy dog liver tissue.

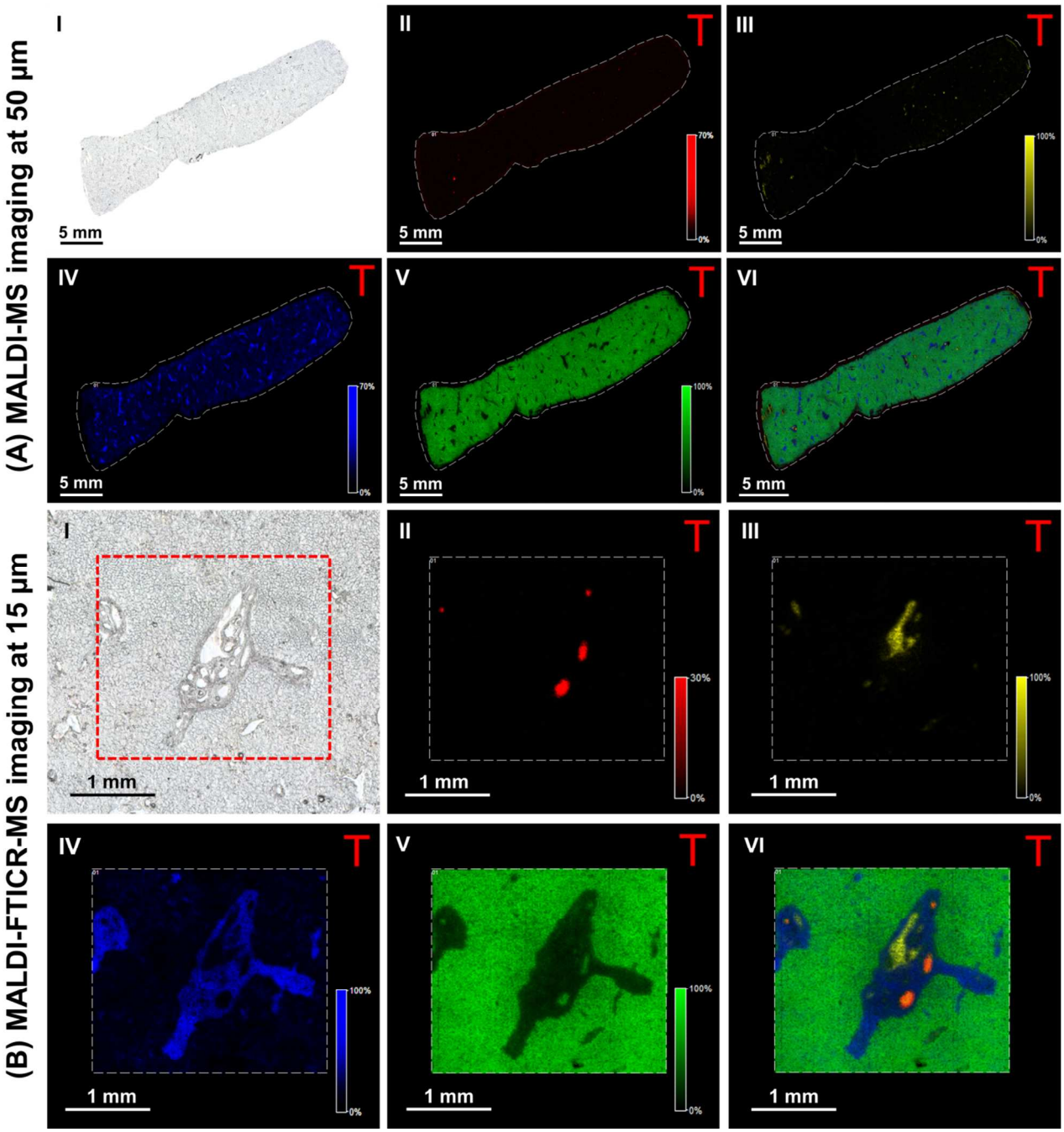


Figure S6. Multimodal imaging of healthy human liver tissue. (I) Optical image of tissue section prior to matrix application. Positive-ion mode (A) MALDI-MS and (B) MALDI-FTICR-MS images showing the distribution of selected molecular species in the (II) bile duct lumen (unknown at *m/z* 560.2427), (III) blood vessels (heme $[M]^+$ at *m/z* 616.1782), (IV) connective tissue ($[PC\ (32:0)+K]^+$ at *m/z* 772.5254), (V) parenchyma ($[PC\ (34:2)+K]^+$ at *m/z* 796.5252) and (VI) overlay of the selected species. (Designated masses are from MALDI-FTICR-MS imaging measurements, MALDI-MS image field of view 72058 pixels, area 110 mm^2 and spatial resolution 50 µm, MALDI-FTICR-MS image field of view 22338 pixels, 5 mm^2 area and spatial resolution 15 µm. Images normalized with TIC).

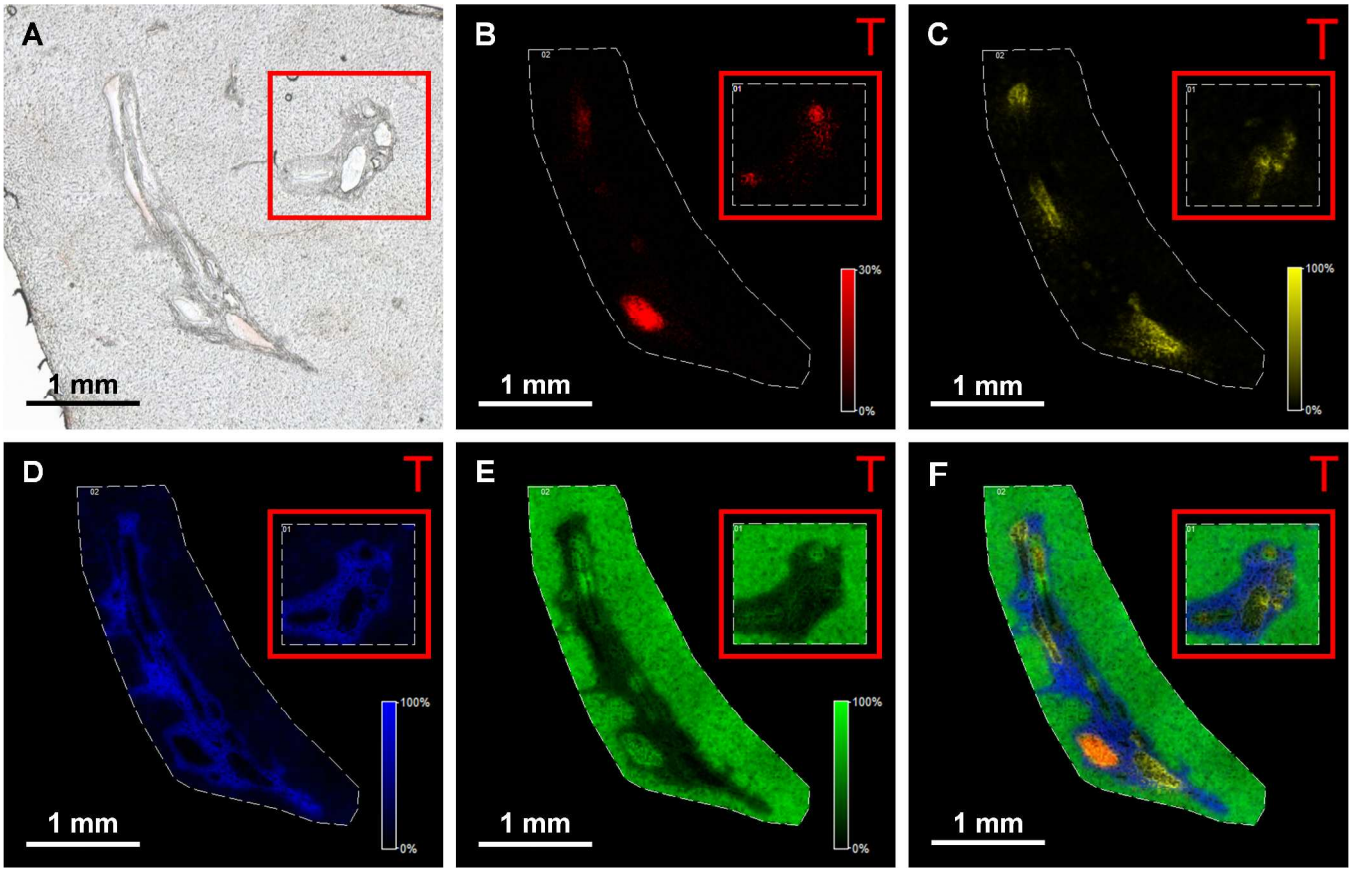


Figure S7. Multimodal imaging of liver from a patient with mild PSC (case 1). (A) Optical image of tissue section prior to matrix application. Positive-ion mode MALDI-FTICR-MS images showing the distribution of selected molecular species in the (B) bile duct lumen (unknown at *m/z* 560.2434), (C) heme ($[M]^+$) at *m/z* 616.1804, (D) connective tissue ($[PC\ (32:0)+K]^+$ at *m/z* 772.5251), (E) parenchyma ($[PC\ (34:2)+K]^+$ at *m/z* 796.5254) and (F) overlay of the selected species. (Designated masses are from MALDI-FTICR-MS imaging measurements, field of view 20238 pixels, area 10 mm^2 and spatial resolution 15 µm. Images normalized with TIC).

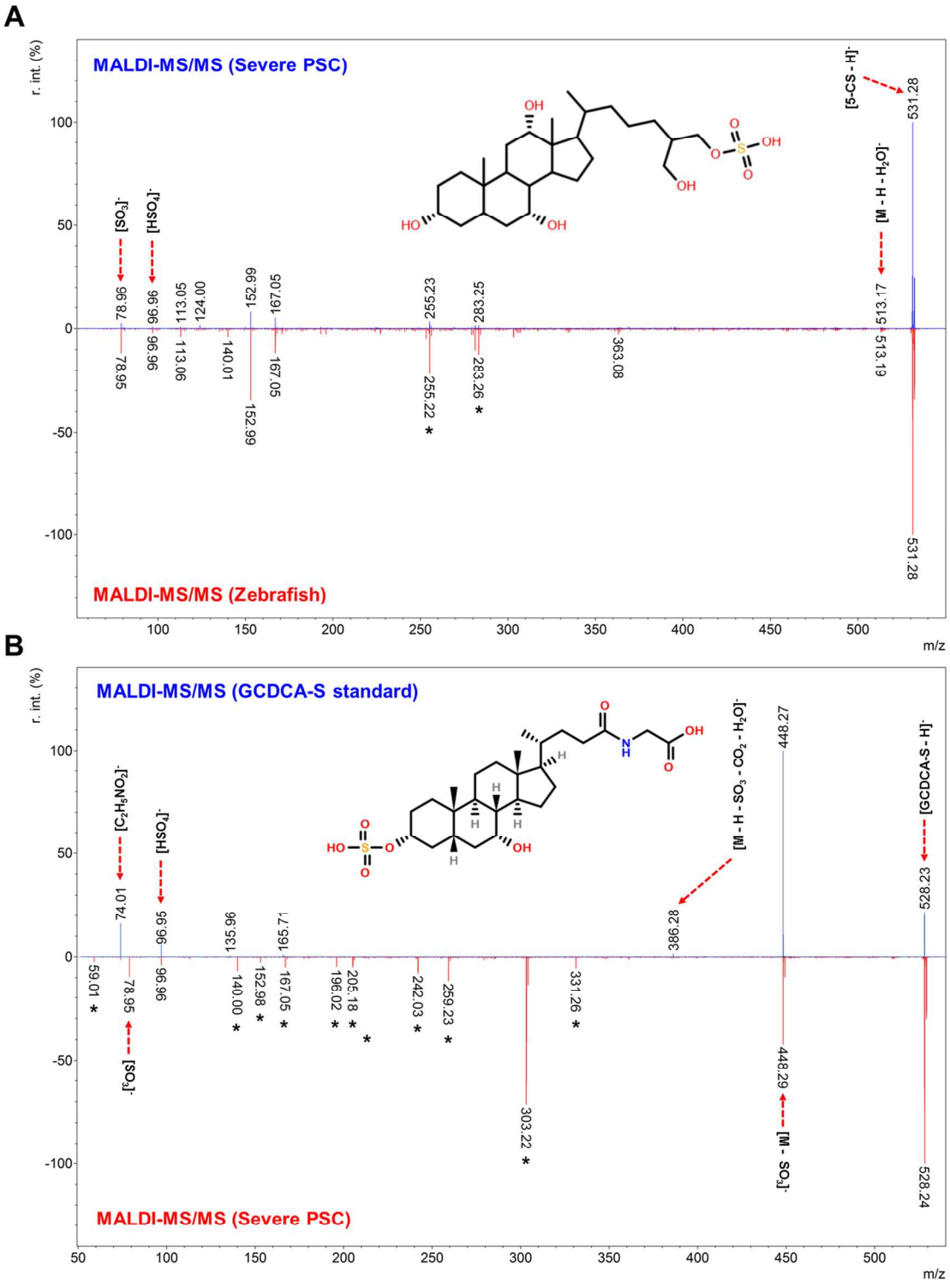


Figure S8. MALDI-MS/MS spectra of 5-CS and GCDCA-S in negative ion mode. A) MALDI-MS/MS spectra of 5-CS at *m/z* 531.3 obtained from severe PSC liver (blue) and whole-body zebrafish (red) tissue sections. B) MALDI-MS/MS spectra of G(C)DCA-S at *m/z* 528.2 obtained from pure standard (blue) and severe PSC liver tissue section (red). * indicated peaks come from unidentified endogenous species tentatively identified as fatty acids.

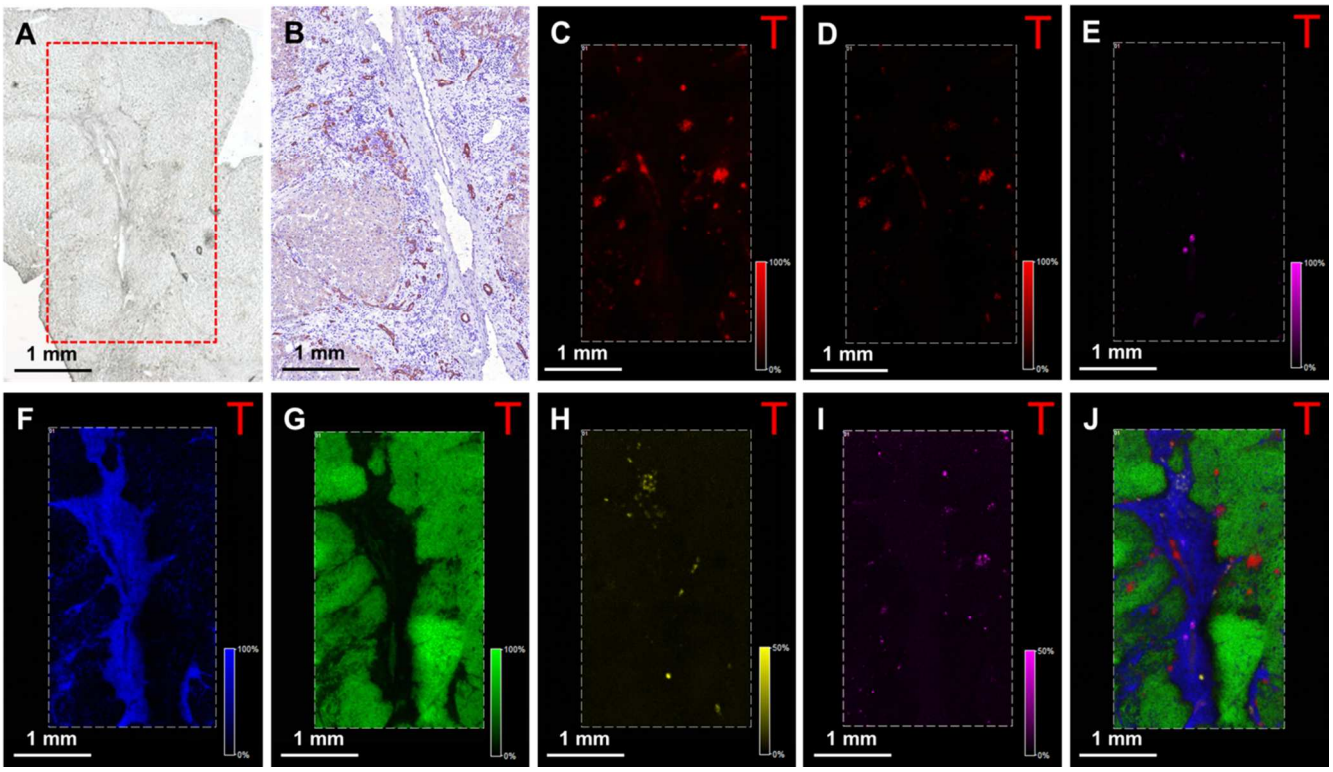


Figure S9. Comparison of immunohistochemical stain and MALDI-MS images from a patient with severe PSC (case 2). (A) Optical image of tissue section prior to matrix application and (B) cytokeratin staining of a consecutive tissue section. Negative-ion mode MALDI-MS images showing the distribution of (C) $[TCA\text{-}H]^-$ at *m/z* 514.28, (D) 5-cyprinolsulfate ($[M\text{-}H]^-$) at *m/z* 531.30, (E) heme ($[M\text{-}H]^-$) at *m/z* 615.17, (F) [PA (18:0_18:1)-H]$^-$ at *m/z* 701.51, (G) [PI (18:0_20:4)-H]$^-$ at *m/z* 885.55, (H) [ST-OH (18:1_24:0)-H]$^-$ at *m/z* 906.63 and (I) bilirubin diglucuronide ($[M\text{-}H]^-$) at *m/z* 935.32. (J) Overlay of the selected species. (Field of view 81186 pixels, area 8 mm^2 and spatial resolution 10 µm. Images normalized with TIC).

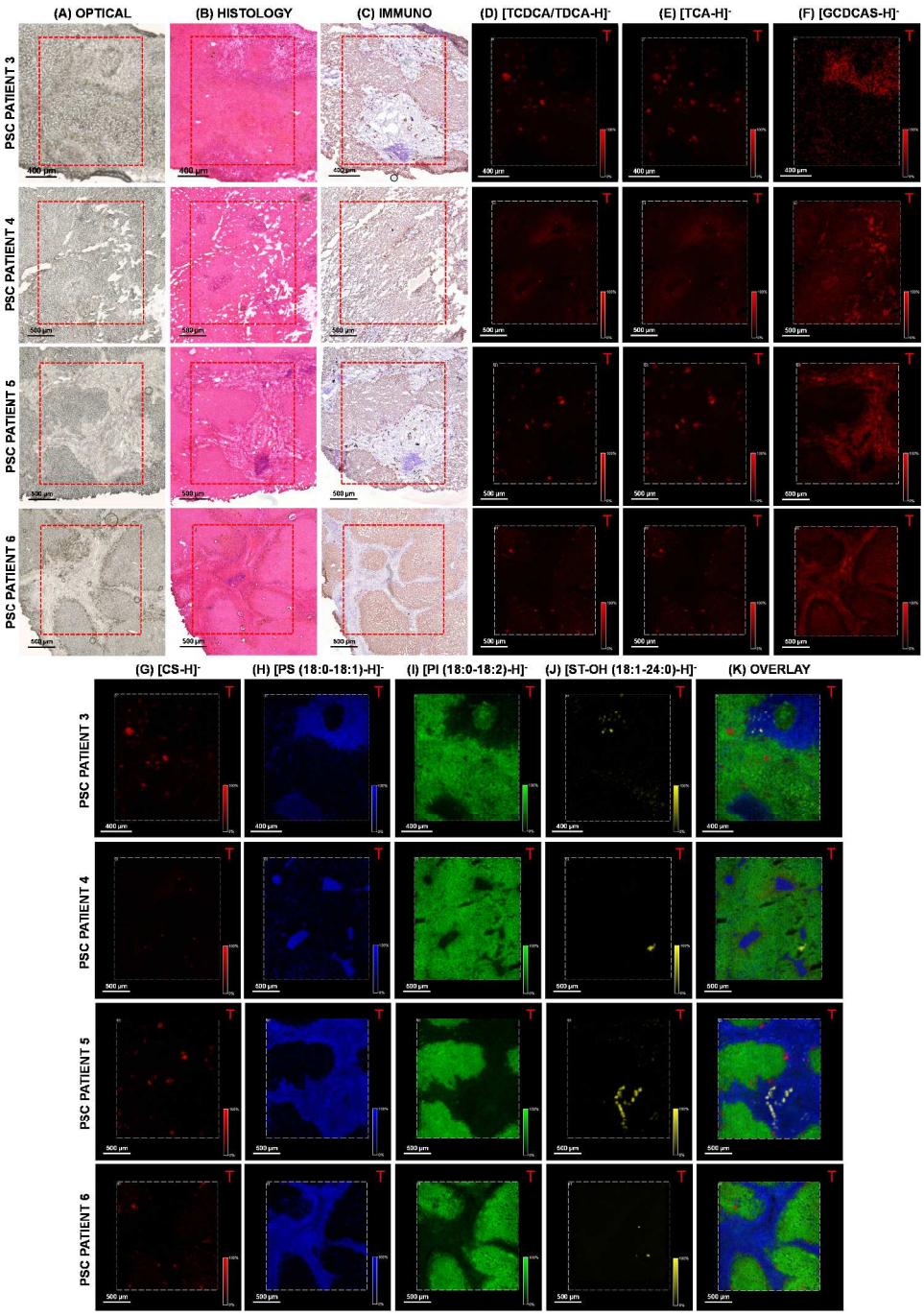


Figure S10. Multimodal imaging of liver tissue from patients with severe PSC (cases 3-6). (A) Optical image of tissue section prior to matrix application, (B) H&E staining of tissue sections post imaging and (C) cytokeratin staining of a consecutive tissue section. Negative-ion mode MALDI-FTICR-MS images showing the distribution of (D) [TCDCATDCA-H]$^-$ at *m/z* 498.2896, (E) [TCA-H]$^-$ at *m/z* 514.2844, (F) [G(C)DCAS-H]$^-$ at *m/z* 528.2639, (G) [5-CS-H]$^-$ at *m/z* 531.2998, (H) [PS (18:0_18:1)-H]$^-$ at *m/z* 788.5449, (I) [PI (18:0_18:2)-H]$^-$ at *m/z* 861.5502, (J) [ST-OH (18:1_24:0)-H]$^-$ at *m/z* 906.6352 and (K) overlay of the selected species. (PSC 1-4 fields of view 10356, 25482, 18987 and 21872 pixels and areas 2.3, 5.7, 4.2 and 4.8 mm^2 respectively. Spatial resolution of all images 15 µm, normalized with TIC).

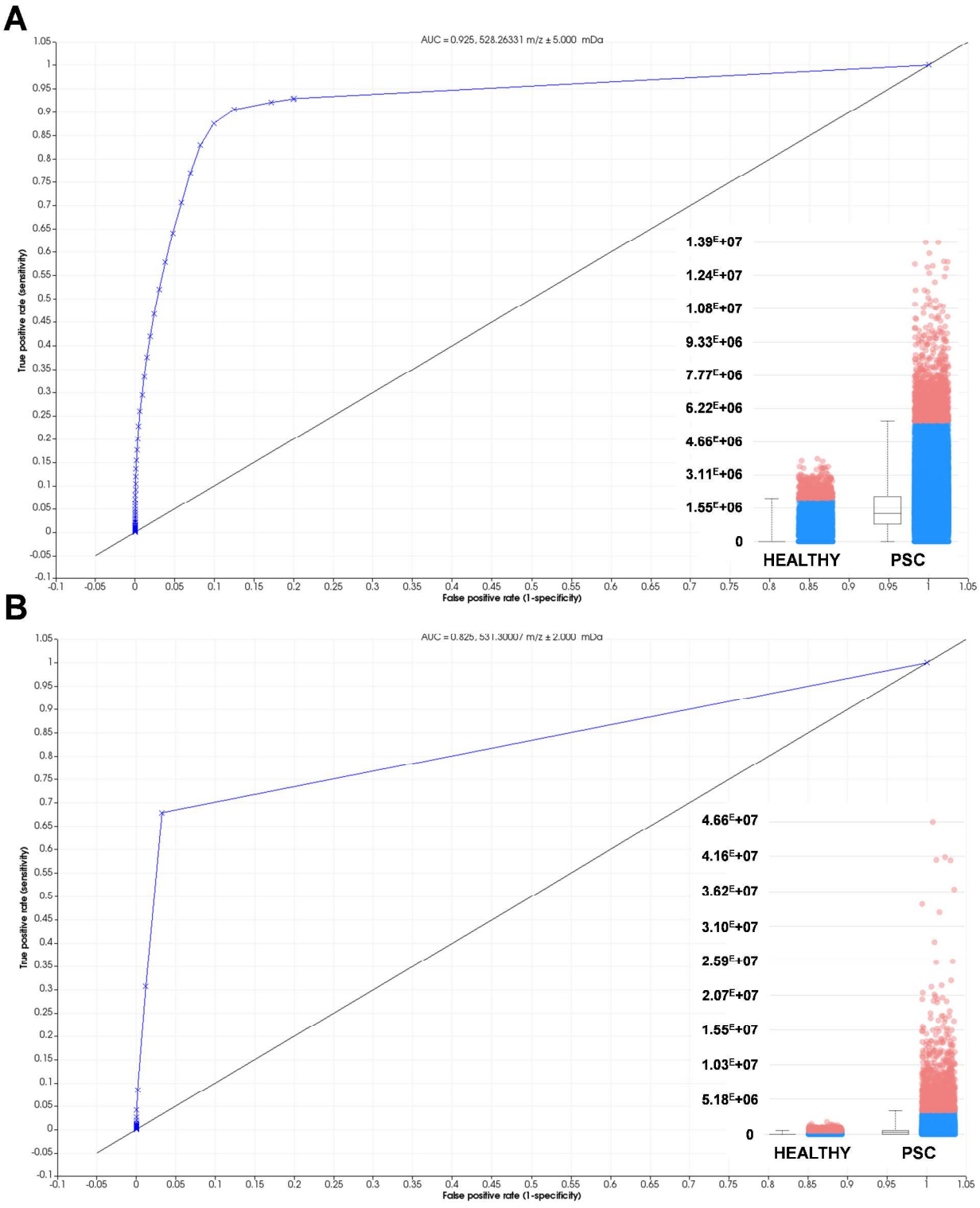


Figure S11. Receiver operating characteristic (ROC) analysis of A) G(C)DCA-S at *m/z* 528.2642 and B) 5-CS at *m/z* 531.2999 from healthy liver tissue and tissue from severe PSC cases. Inserts show the relative ion intensity of the displayed ions.

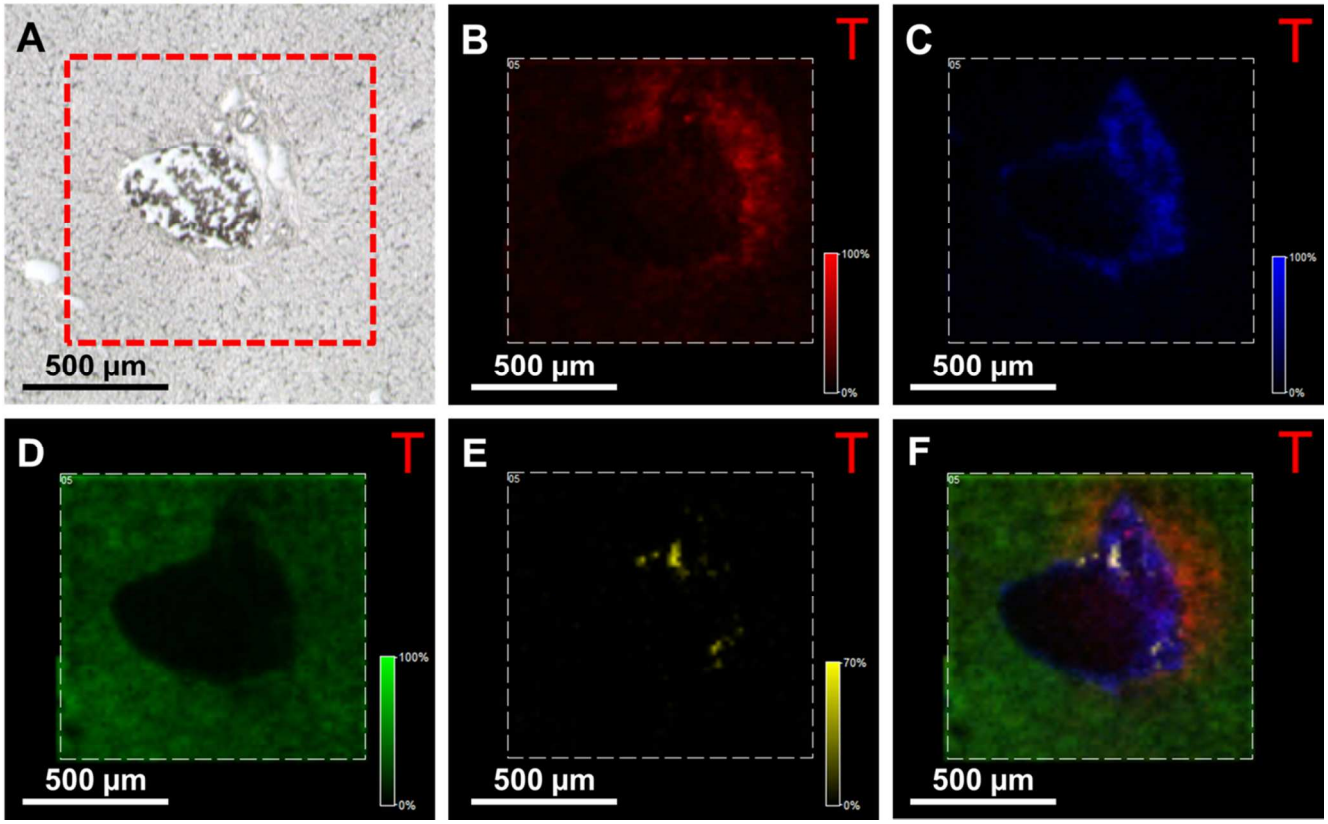


Figure S12. MALDI-FTICR-MS imaging of healthy rat liver tissue. (A) Optical image of tissue section prior to matrix application. Negative-ion mode MALDI-FTICR-MS images showing the distribution of selected molecular species in the (B) bile duct lumen ([TCA-H]$^-$ at *m/z* 514.2845), (C) connective tissue ([PA (18:0_18:1)-H]$^-$ at *m/z* 701.5129), (D) parenchyma ([PI (18:0_20:4)-H]$^-$ at *m/z* 885.5499), (E) bile duct ([ST-OH (18:1_24:0)-H]$^-$ at *m/z* 906.6348) and (F) overlay of the selected species. (Designated masses are from MALDI-FTICR-MS imaging measurements, field of view 4352 pixels, area 1 mm^2 and spatial resolution 15 µm. Images normalized with TIC).

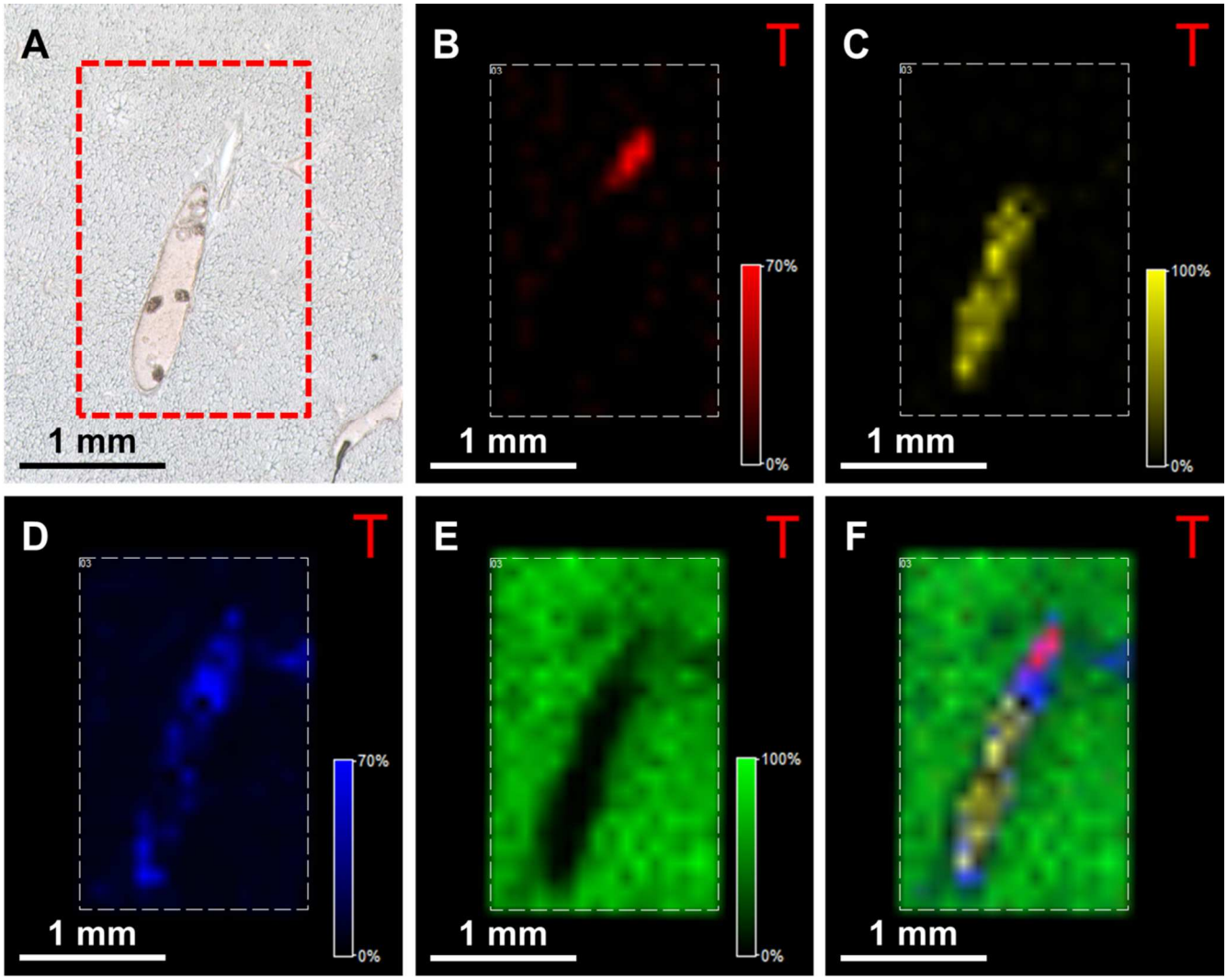


Figure S13. MALDI-FTICR-MS imaging of healthy rat liver tissue. (A) Optical image of tissue section prior to matrix application. Positive-ion mode MALDI-FTICR-MS images showing the distribution of selected molecular species in the (B) bile duct lumen (unknown at *m/z* 560.2414), (C) heme ($[M]^+$) at *m/z* 616.1775, (D) connective tissue ($[PC\ (32:0)+K]^+$ at *m/z* 772.5254), (E) parenchyma ($[PC\ (38:4)+K]^+$ at *m/z* 848.5566) and (F) overlay of the selected species. (Designated masses are from MALDI-FTICR-MS imaging measurements, field of view 400 pixels, area 3.8 mm^2 and spatial resolution 100 µm. Images normalized with TIC).

Sample	*Bilirubin (umol/L)*	*ALT (U/L)*	*AST (U/L)*	*ALP (U/L)*	*GGT (U/L)*	*INR*
1 (mild)	15	15	19	N/A	21	1.05
2 (severe)	48*	76*	79*	365*	229*	1.1
3 (mild)	7	48*	56*	52	61*	N/A
4 (mild/severe)	56*	39	46*	110	21	1.6*
5 (severe)	80*	56*	83*	373*	146*	N/A
6 (severe)	147*	157*	258*	240*	104*	1.4*

Key: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), international normalized ratio (INR). * Represents values above the reference range.

Table S1. Serum biochemistry of PSC patients.

RAT

PARENCHYMA

Mass	Intensity	Identification	Formula	Error (ppm)
848.5566	6.36E+09	[PC (38:4)+K]$^+$	C46H84NO8PK	0.015
820.5252	4.68E+09	[PC (36:4)+K]$^+$	C44H80NO8PK	-0.184
810.6008	2.93E+09	[PC (18:0_20:4)+H]$^+$	C46H85NO8P	0.113
782.5690	2.23E+09	[PC (16:0_20:4)+H]$^+$	C44H81NO8P	-0.578
796.5253	1.98E+09	[PC (34:2)+K]$^+$	C42H80NO8PK	-0.002
846.5413	1.55E+09	[PC (38:5)+K]$^+$	C46H82NO8PK	0.340
824.5565	1.54E+09	[PC (36:2)+K]$^+$	C44H84NO8PK	-0.129
844.5253	1.51E+09	[PC (38:6)+K]$^+$	C46H80NO8PK	-0.036
832.5627	1.45E+09	[PC (38:4)+Na]$^+$	C46H84NO8PNa	-0.019
798.5409	1.27E+09	[PC (34:1)+K]$^+$	C42H82NO8PK	-0.057

DOG

Mass	Intensity	Identification	Formula	Error (ppm)
832.5825	1.27E+11	[PC (38:4)+Na]$^+$	C46H84NO8PNa	-0.223
848.5564	9.51E+10	[PC (38:4)+K]$^+$	C46H84NO8PK	-0.304
808.5851	7.51E+10	[PC (18:0_20:5)+H]$^+$	C46H83NO8P	-0.012
810.6007	7.13E+10	[PC (18:0_20:4)+H]$^+$	C46H85NO8P	-0.048
780.5517	5.55E+10	[PC (34:2)+Na]$^+$	C42H80NO8PNa	0.385
824.5568	4.57E+10	[PC (36:2)+K]$^+$	C44H84NO8PK	0.244
796.5256	4.23E+10	[PC (34:2)+K]$^+$	C42H80NO8PK	0.343
782.5695	4.21E+10	[PC (16:0_20:4)+H]$^+$	C44H81NO8P	0.029
804.5517	4.05E+10	[PC (36:4)+Na]$^+$	C44H80NO8PNa	0.432
820.5255	2.81E+10	[PC (36:4)+K]$^+$	C44H80NO8PK	0.243

PARENCHYMA

Mass	Intensity	Identification	Formula	Error (ppm)
772.5254	5.37E+08	[PC (32:0)+K]$^+$	C40H80NO8PK	0.122
741.5310	4.66E+08	[SM (34:1)+K]$^+$	C39H79N2O6PK	0.396
851.6403	3.17E+08	[SM (42:2)+K]$^+$	C47H93N2O6PK	0.017
734.5694	2.85E+08	[PC (16:0_16:0)+H]$^+$	C40H81NO8P	-0.034
825.6249	2.24E+08	[SM (40:1)+K]$^+$	C45H91N2O6PK	0.288
756.5515	1.74E+08	[PC (32:0)+Na]$^+$	C40H80NO8PNa	0.174
725.5568	1.36E+08	[SM (34:1)+Na]$^+$	C39H79N2O6PNa	0.008
800.5569	1.13E+08	[PC (34:0)+K]$^+$	C42H84NO8PK	0.326
769.5623	8.69E+07	[SM (36:1)+K]$^+$	C41H83N2O6PK	0.314
762.6008	4.30E+07	[PC (16:0_18:0)+H]+	C42H85NO8P	0.131

CONNECTIVE TISSUE

Mass	Intensity	Identification	Formula	Error (ppm)
725.5570	1.61E+10	[SM (34:1)+Na]$^+$	C39H79N2O6PNa	0.289
756.5518	7.65E+09	[PC (32:0)+Na]$^+$	C40H80NO8PNa	0.556
835.6663	7.47E+09	[SM (42:2)+Na]$^+$	C47H93N2O6PNa	0.339
741.5311	6.84E+09	[SM (34:1)+K]$^+$	C39H79N2O6PK	0.445
753.5886	5.34E+09	[SM (36:1)+Na]$^+$	C41H83N2O6PNa	0.675
809.6510	4.44E+09	[SM (40:1)+Na]$^+$	C45H91N2O6PNa	0.327
851.6405	3.64E+09	[SM (42:2)+K]$^+$	C47H93N2O6PK	0.305
772.5254	3.34E+09	[PC (32:0)+K]$^+$	C40H80NO8PK	0.129
703.5750	3.02E+09	[SM (18:1_16:0)+H]$^+$	C39H80N2O6P	0.284
734.5695	2.67E+09	[PC (16:0_16:0)+H]$^+$	C40H81NO8P	0.136

HUMAN

PARENCHYMA

Mass	Intensity	Identification	Formula	Error (ppm)
796.5252	2.54E+11	[PC (34:2)+K]$^+$	C42H80NO8PK	-0.080
798.5410	1.44E+11	[PC (34:1)+K]$^+$	C42H82NO8PK	0.036
780.5513	1.42E+11	[PC (34:2)+Na]$^+$	C42H80NO8PNa	-0.094
824.5561	1.11E+11	[PC (36:2)+K]$^+$	C44H84NO8PK	-0.568
820.5248	8.76E+10	[PC (36:4)+K]$^+$	C44H80NO8PK	-0.581
808.5847	7.15E+10	[PC (18:0_20:5)+H]$^+$	C46H83NO8P	-0.471
758.5695	7.15E+10	[PC (16:0_18:2)+H]$^+$	C42H81NO8P	0.110
822.5406	6.76E+10	[PC (36:3)+K]$^+$	C44H82NO8PK	-0.487
806.5672	4.83E+10	[PC (36:3)+Na]$^+$	C44H82NO8PNa	0.199
848.5562	4.53E+10	[PC (38:4)+K]$^+$	C46H84NO8PK	-0.544

CONNECTIVE TISSUE

Mass	Intensity	Identification	Formula	Error (ppm)
741.5309	2.36E+10	[SM (34:1)+K]$^+$	C39H79N2O6PK	0.287
851.6399	2.34E+10	[SM (42:2)+K]$^+$	C47H93N2O6PK	-0.429
725.5568	2.24E+10	[SM (34:1)+Na]$^+$	C39H79N2O6PNa	0.045
835.6658	2.03E+10	[SM (42:2)+Na]$^+$	C47H93N2O6PNa	-0.285
772.5254	1.96E+10	[PC (32:0)+K]$^+$	C40H80NO8PK	0.078
756.5508	1.59E+10	[PC (32:0)+Na]$^+$	C40H80NO8PNa	-0.778
734.5691	7.59E+09	[PC (16:0_16:0)+H]$^+$	C40H81NO8P	-0.460
703.5749	6.53E+09	[SM (18:1_16:0)+H]$^+$	C39H80N2O6P	0.122
769.5626	6.18E+09	[SM (36:1)+K]$^+$	C41H83N2O6PK	0.632
762.6005	8.07E+08	[PC (16:0_18:0)+H]+	C42H85NO8P	-0.262

HUMAN (Mild PSC)

PARENCHYMA

Mass	Intensity	Identification	Formula	Error (ppm)
796.5254	8.38E+10	[PC (34:2)+K]$^+$	C42H80NO8PK	0.122
824.5567	4.01E+10	[PC (36:2)+K]$^+$	C44H84NO8PK	0.396
758.5697	3.33E+10	[PC (16:0_18:2)+H]$^+$	C42H81NO8P	0.017
820.5252	3.13E+10	[PC (36:4)+K]$^+$	C44H80NO8PK	-0.034
822.5410	2.31E+10	[PC (36:3)+K]$^+$	C44H82NO8PK	0.288
786.6008	1.58E+10	[PC (18:0_18:2)+H]$^+$	C44H85NO8P	0.174
806.5665	1.27E+10	[PC (36:3)+Na]$^+$	C44H82NO8PNa	0.008
784.5852	9.19E+09	[PC (18:1_18:2)+H]$^+$	C44H83NO8P	0.326
846.5414	7.99E+09	[PC (38:5)+K]$^+$	C46H82NO8PK	0.314
794.5099	6.93E+09	[PC (34:3)+K]$^+$	C42H78NO8PK	0.131

CONNECTIVE TISSUE

Mass	Intensity	Identification	Formula	Error (ppm)
741.5308	1.34E+10	[SM (34:1)+K]$^+$	C39H79N2O6PK	0.140
772.5251	1.05E+10	[PC (32:0)+K]$^+$	C40H80NO8PK	-0.322
851.6407	8.95E+09	[SM (42:2)+K]$^+$	C47H93N2O6PK	0.447
725.5569	7.83E+09	[SM (34:1)+Na]$^+$	C39H79N2O6PNa	0.183
756.5518	6.50E+09	[PC (32:0)+Na]$^+$	C40H80NO8PNa	0.559
825.6243	5.95E+09	[SM (40:1)+K]$^+$	C45H91N2O6PK	-0.356
835.6659	4.80E+09	[SM (42:2)+Na]$^+$	C47H93N2O6PNa	-0.213
734.5694	4.71E+09	[PC (16:0_16:0)+H]$^+$	C40H81NO8P	0.006
703.5744	3.78E+09	[SM (18:1_16:0)+H]$^+$	C39H80N2O6P	-0.575
809.6505	3.21E+09	[SM (40:1)+Na]$^+$	C45H91N2O6PNa	-0.253

HUMAN (Advanced PSC)

PARENCHYMA

Mass	Intensity	Identification	Formula	Error (ppm)
796.5254	1.40E+10	[PC (34:2)+K]$^+$	C42H80NO8PK	0.125
782.5692	1.39E+10	[PC (16:0_20:4)+H]$^+$	C44H81NO8P	-0.277
798.5410	1.33E+10	[PC (34:1)+K]$^+$	C42H82NO8PK	0.125
780.5514	1.24E+10	[PC (34:2)+Na]$^+$	C42H80NO8PNa	0.128
808.5851	9.35E+09	[PC (18:0_20:5)+H]$^+$	C46H83NO8P	0.055
824.5568	8.08E+09	[PC (36:2)+K]$^+$	C44H84NO8PK	0.242
804.5512	5.72E+09	[PC (36:4)+Na]$^+$	C44H80NO8PNa	-0.248
820.5252	5.66E+09	[PC (36:4)+K]$^+$	C44H80NO8PK	-0.121
822.5411	4.79E+09	[PC (36:3)+K]$^+$	C44H82NO8PK	0.122
760.5851	4.49E+09	[PC (16:0_18:1)+H]$^+$	C42H83NO8P	0.054

CONNECTIVE TISSUE

Mass	Intensity	Identification	Formula	Error (ppm)
756.5512	3.05E+09	[PC (32:0)+Na]$^+$	C40H80NO8PNa	-0.264
835.6666	2.98E+09	[SM (42:2)+Na]$^+$	C47H93N2O6PNa	0.359
772.5253	2.74E+09	[PC (32:0)+K]$^+$	C40H80NO8PK	-0.078
725.5569	2.50E+09	[SM (34:1)+Na]$^+$	C39H79N2O6PNa	0.138
851.6406	2.14E+09	[SM (42:2)+K]$^+$	C47H93N2O6PK	0.352
741.5308	1.89E+09	[SM (34:1)+K]$^+$	C39H79N2O6PK	0.135
734.5695	9.94E+08	[PC (16:0_16:0)+H]$^+$	C40H81NO8P	0.136
703.5750	5.28E+08	[SM (18:1_16:0)+H]$^+$	C39H80N2O6P	0.284
800.5570	3.89E+08	[PC (34:0)+K]$^+$	C42H84NO8PK	0.499
769.5623	3.51E+08	[SM (36:1)+K]$^+$	C41H83N2O6PK	0.389

NOTES

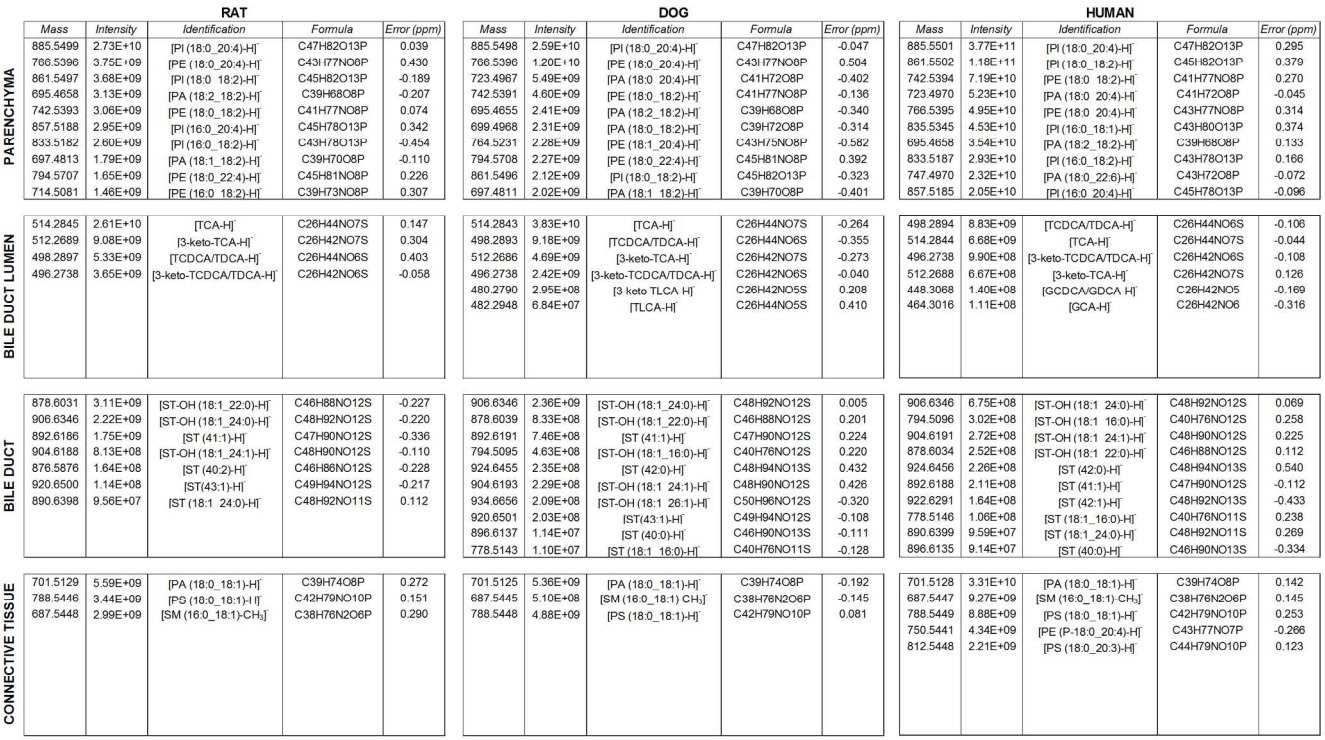
All peaks identified by MALDI-FTICR-MS, MALDI-MS/MS and databases/literature.

Species in positive ion mode were observed as protonated ([M+H]$^+$), sodium ([M+Na]$^+$) and potassium ([M+K]$^+$) adducts.

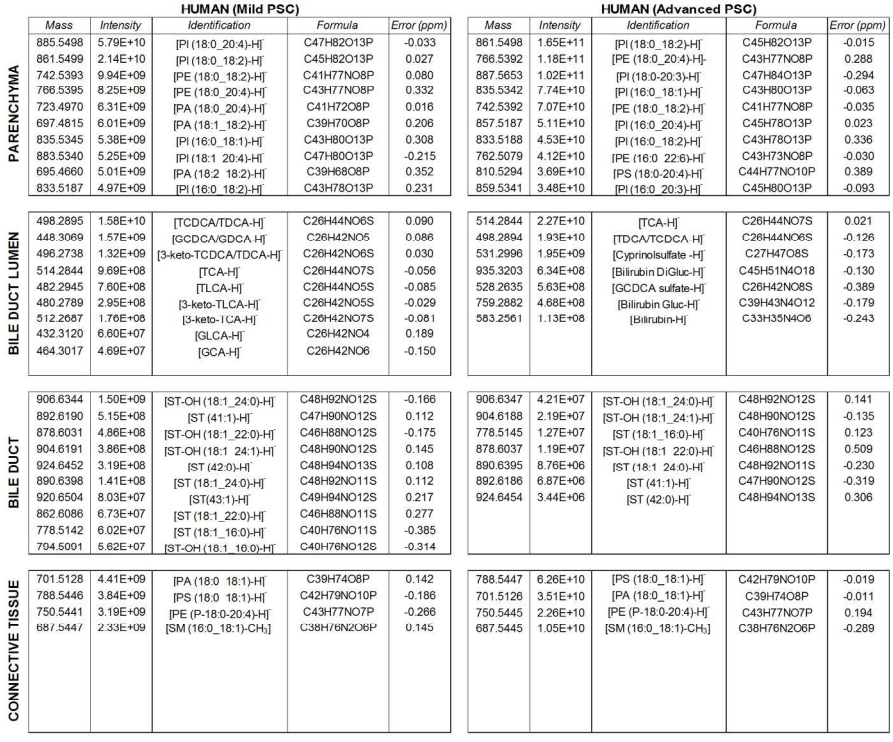
Fatty acid composition of positive ion could only be obtained from MS/MS of protonated species.

Key: Phosphatidylcholine (PC), Sphingomyelin (SM).

Table S2. Comparison of lipids present in the different areas of the liver across different species in positive ion mode.



	RAT				DOG				HUMAN			
	Mass	Intensity	Identification	Formula	Error (ppm)	Mass	Intensity	Identification	Formula	Error (ppm)	Mass	Intensity	Identification	Formula	Error (ppm)
PARENCHYMA	885.5499	2.73E+10	[PI (18:0_20:4)-H]⁻	C47H82O13P	0.039	885.5498	2.59E+10	[PI (18:0_20:4)-H]⁻	C47H82O13P	-0.047	885.5501	3.77E+11	[PI (18:0_20:4)-H]⁻	C47H82O13P	0.295
	766.5396	3.75E+09	[PE (18:0_20:4)-H]⁻	C43H77NO8P	0.430	766.5396	1.20E+10	[PE (18:0_20:4)-H]⁻	C43H77NO8P	0.504	861.5502	1.18E+11	[PI (18:0_18:2)-H]⁻	C45H82O13P	0.379
	861.5497	3.68E+09	[PI (18:0_18:2)-H]⁻	C45H82O13P	-0.189	723.4967	5.49E+09	[PA (18:0_20:4)-H]⁻	C41H72O8P	-0.402	742.5394	7.19E+10	[PE (18:0_18:2)-H]⁻	C41H77NO8P	0.270
	695.4658	3.13E+09	[PA (18:2_18:2)-H]⁻	C39H68O8P	-0.207	742.5391	4.60E+09	[PE (18:0_18:2)-H]⁻	C41H77NO8P	-0.136	723.4970	5.23E+10	[PA (18:0_20:4)-H]⁻	C41H72O8P	-0.045
	742.5393	3.06E+09	[PE (18:0_18:2)-H]⁻	C41H77NO8P	0.074	695.4655	2.41E+09	[PA (18:2_18:2)-H]⁻	C39H68O8P	-0.340	766.5395	4.95E+10	[PE (18:0_20:4)-H]⁻	C43H77NO8P	0.314
	857.5188	2.95E+09	[PI (16:0_20:4)-H]⁻	C45H78O13P	0.342	699.4968	2.31E+09	[PA (18:0_18:2)-H]⁻	C39H72O8P	-0.314	835.5345	4.53E+10	[PI (16:0_18:1)-H]⁻	C43H80O13P	0.374
	833.5182	2.60E+09	[PI (16:0_18:2)-H]⁻	C43H78O13P	-0.454	764.5231	2.28E+09	[PE (18:1_20:4)-H]⁻	C43H75NO8P	-0.582	695.4658	3.54E+10	[PA (18:2_18:2)-H]⁻	C39H68O8P	0.133
	697.4813	1.79E+09	[PA (18:1_18:2)-H]⁻	C39H70O8P	-0.110	794.5708	2.27E+09	[PE (18:0_22:4)-H]⁻	C45H81NO8P	0.392	833.5187	2.93E+10	[PI (16:0_18:2)-H]⁻	C43H78O13P	0.166
	794.5707	1.65E+09	[PE (18:0_22:4)-H]⁻	C45H81NO8P	0.226	861.5496	2.12E+09	[PI (18:0_18:2)-H]⁻	C45H82O13P	-0.323	747.4970	2.32E+10	[PA (18:0_22:6)-H]⁻	C43H72O8P	-0.072
	714.5081	1.46E+09	[PE (16:0_18:2)-H]⁻	C39H73NO8P	0.307	697.4811	2.02E+09	[PA (18:1_18:2)-H]⁻	C39H70O8P	-0.401	857.5185	2.05E+10	[PI (18:0_20:4)-H]⁻	C45H78O13P	-0.096
BILE DUCT LUMEN	514.2845	2.61E+10	[TCA-H]⁻	C26H44NO7S	0.147	514.2843	3.83E+10	[TCA-H]⁻	C26H44NO7S	-0.264	498.2894	8.83E+09	[TCDCA/TDCA-H]⁻	C26H44NO6S	-0.106
	512.2689	9.08E+09	[3-keto-TCA-H]⁻	C26H42NO7S	0.304	498.2893	9.18E+09	[TCDCA/TDCA-H]⁻	C26H44NO6S	-0.355	514.2844	6.68E+09	[TCA-H]⁻	C26H44NO7S	-0.044
	498.2897	5.33E+09	[TCDCA/TDCA-H]⁻	C26H44NO6S	0.403	512.2686	4.69E+09	[3-keto-TCA-H]⁻	C26H42NO7S	-0.273	496.2738	9.90E+08	[3-keto-TCDCA/TDCA-H]⁻	C26H42NO6S	-0.108
	496.2738	3.65E+09	[3-keto-TCDCA/TDCA-H]⁻	C26H42NO6S	-0.058	496.2738	2.42E+09	[3-keto-TCDCA/TDCA-H]⁻	C26H42NO6S	-0.040	512.2688	6.67E+08	[3-keto-TCA-H]⁻	C26H42NO7S	0.126
						480.2790	2.95E+08	[3 keto TLCA H]⁻	C26H42NO5S	0.208	448.3068	1.40E+08	[GCDCA/GDCA H]⁻	C26H42NO5	-0.169
						482.2948	6.84E+07	[TLCA-H]⁻	C26H44NO5S	0.410	464.3016	1.11E+08	[GCA-H]⁻	C26H42NO6	-0.316
BILE DUCT	878.6031	3.11E+09	[ST-OH (18:1_22:0)-H]⁻	C46H88NO12S	-0.227	906.6346	2.36E+09	[ST-OH (18:1_24:0)-H]⁻	C48H92NO12S	0.005	906.6346	6.75E+08	[ST-OH (18:1 24:0)-H]⁻	C48H92NO12S	0.069
	906.6346	2.22E+09	[ST-OH (18:1_24:0)-H]⁻	C48H92NO12S	-0.220	878.6039	8.33E+08	[ST-OH (18:1_22:0)-H]⁻	C46H88NO12S	0.201	794.5096	3.02E+08	[ST-OH (18:1 16:0)-H]⁻	C40H76NO12S	0.258
	892.6186	1.75E+09	[ST (41:1)-H]⁻	C47H90NO12S	-0.336	892.6191	7.46E+08	[ST (41:1)-H]⁻	C47H90NO12S	0.224	904.6191	2.72E+08	[ST-OH (18:1 24:1)-H]⁻	C48H90NO12S	0.225
	904.6188	8.13E+08	[ST-OH (18:1_24:1)-H]⁻	C48H90NO12S	-0.110	794.5095	4.63E+08	[ST-OH (18:1_16:0)-H]⁻	C40H76NO12S	0.220	878.6034	2.52E+08	[ST-OH (18:1 22:0)-H]⁻	C46H88NO12S	0.112
	876.5876	1.64E+08	[ST (40:2)-H]⁻	C46H86NO12S	-0.228	924.6455	2.35E+08	[ST (42:0)-H]⁻	C48H94NO13S	0.432	924.6456	2.26E+08	[ST (42:0)-H]⁻	C48H94NO13S	0.540
	920.6500	1.14E+08	[ST(43:1)-H]⁻	C49H94NO12S	-0.217	904.6193	2.29E+08	[ST-OH (18:1 24:1)-H]⁻	C48H90NO12S	0.426	892.6188	2.11E+08	[ST (41:1)-H]⁻	C47H90NO12S	-0.112
	890.6398	9.56E+07	[ST (18:1 24:0)-H]⁻	C48H92NO11S	0.112	934.6656	2.09E+08	[ST-OH (18:1 26:1)-H]⁻	C50H96NO12S	-0.320	922.6291	1.64E+08	[ST (42:1)-H]⁻	C48H92NO13S	-0.433
						920.6501	2.03E+08	[ST(43:1)-H]⁻	C49H94NO12S	-0.108	778.5146	1.06E+08	[ST (18:1_16:0)-H]⁻	C40H76NO11S	0.238
						896.6137	1.14E+07	[ST (40:0)-H]⁻	C46H90NO13S	-0.111	890.6399	9.59E+07	[ST (18:1_24:0)-H]⁻	C48H92NO11S	0.269
						778.5143	1.10E+07	[ST (18:1 16:0)-H]⁻	C40H76NO11S	-0.128	896.6135	9.14E+07	[ST (40:0)-H]⁻	C46H90NO13S	-0.334
CONNECTIVE TISSUE	701.5129	5.59E+09	[PA (18:0_18:1)-H]⁻	C39H74O8P	0.272	701.5125	5.36E+09	[PA (18:0_18:1)-H]⁻	C39H74O8P	-0.192	701.5128	3.31E+10	[PA (18:0_18:1)-H]⁻	C39H74O8P	0.142
	788.5446	3.44E+09	[PS (18:0_18:1)-H]⁻	C42H79NO10P	0.151	687.5445	5.10E+08	[SM (16:0_18:1)-CH3]⁻	C38H76N2O6P	-0.145	687.5447	9.27E+09	[SM (16:0_18:1)-CH3]⁻	C38H76N2O6P	0.145
	687.5448	2.99E+09	[SM (16:0_18:1)-CH3]⁻	C38H76N2O6P	0.290	788.5448	4.88E+09	[PS (18:0_18:1)-H]⁻	C42H79NO10P	0.081	788.5449	8.88E+09	[PS (18:0_18:1)-H]⁻	C42H79NO10P	0.253
											750.5441	4.34E+09	[PE (P-18:0_20:4)-H]⁻	C43H77NO7P	-0.266
											812.5448	2.21E+09	[PS (18:0_20:3)-H]⁻	C44H79NO10P	0.123



	HUMAN (Mild PSC)					HUMAN (Advanced PSC)				
	Mass	Intensity	Identification	Formula	Error (ppm)	Mass	Intensity	Identification	Formula	Error (ppm)
PARENCHYMA	885.5498	5.79E+10	[PI (18:0_20:4)-H]⁻	C47H82O13P	-0.033	861.5498	1.65E+11	[PI (18:0_18:2)-H]⁻	C45H82O13P	-0.015
	861.5499	2.14E+10	[PI (18:0_18:2)-H]⁻	C45H82O13P	0.027	766.5392	1.18E+11	[PE (18:0_20:4)-H]-	C43H77NO8P	0.288
	742.5393	9.94E+09	[PE (18:0_18:2)-H]⁻	C41H77NO8P	0.080	887.5653	1.02E+11	[PI (18:0-20:3)-H]⁻	C47H84O13P	-0.294
	766.5395	8.25E+09	[PE (18:0_20:4)-H]⁻	C43H77NO8P	0.332	835.5342	7.74E+10	[PI (16:0_18:1)-H]⁻	C43H80O13P	-0.063
	723.4970	6.31E+09	[PA (18:0_20:4)-H]⁻	C41H72O8P	0.016	742.5392	7.07E+10	[PE (18:0_18:2)-H]⁻	C41H77NO8P	-0.035
	697.4815	6.01E+09	[PA (18:1_18:2)-H]⁻	C39H70O8P	0.206	857.5187	5.11E+10	[PI (16:0_20:4)-H]⁻	C45H78O13P	0.023
	835.5345	5.38E+09	[PI (16:0_18:1)-H]⁻	C43H80O13P	0.308	833.5188	4.53E+10	[PI (16:0_18:2)-H]⁻	C43H78O13P	0.336
	883.5340	5.25E+09	[PI (18:1 20:4)-H]⁻	C47H80O13P	-0.215	762.5079	4.12E+10	[PE (16:0 22:6)-H]⁻	C43H73NO8P	-0.030
	695.4660	5.01E+09	[PA (18:2 18:2)-H]⁻	C39H68O8P	0.352	810.5294	3.69E+10	[PS (18:0-20:4)-H]⁻	C44H77NO10P	0.389
	833.5187	4.97E+09	[PI (16:0_18:2)-H]⁻	C43H78O13P	0.231	859.5341	3.48E+10	[PI (16:0_20:3)-H]⁻	C45H80O13P	-0.093
BILE DUCT LUMEN	498.2895	1.58E+10	[TCDCA/TDCA-H]⁻	C26H44NO6S	0.090	514.2844	2.27E+10	[TCA-H]⁻	C26H44NO7S	0.021
	448.3069	1.57E+09	[GCDCA/GDCA H]⁻	C26H42NO5	0.086	498.2894	1.93E+10	[TDCA/TCDCA H]⁻	C26H44NO6S	-0.126
	496.2738	1.32E+09	[3-keto-TCDCA/TDCA-H]⁻	C26H42NO6S	0.030	531.2996	1.95E+09	[Cyprinolsulfate -H]⁻	C27H47O8S	-0.173
	514.2844	9.69E+08	[TCA-H]⁻	C26H44NO7S	-0.056	935.3203	6.34E+08	[Bilirubin DiGluc-H]⁻	C45H51N4O18	-0.130
	482.2945	7.60E+08	[TLCA-H]⁻	C26H44NO5S	-0.085	528.2635	5.63E+08	[GCDCA sulfate-H]⁻	C26H42NO8S	-0.389
	480.2789	2.95E+08	[3-keto-TLCA-H]⁻	C26H42NO5S	-0.029	759.2882	4.68E+08	[Bilirubin Gluc-H]⁻	C39H43N4O12	-0.179
	512.2687	1.76E+08	[3-keto-TCA-H]⁻	C26H42NO7S	-0.081	583.2561	1.13E+08	[Bilirubin-H]⁻	C33H35N4O6	-0.243
	432.3120	6.60E+07	[GLCA-H]⁻	C26H42NO4	0.189					
	464.3017	4.69E+07	[GCA-H]⁻	C26H42NO6	-0.150					
BILE DUCT	906.6344	1.50E+09	[ST-OH (18:1_24:0)-H]⁻	C48H92NO12S	-0.166	906.6347	4.21E+07	[ST-OH (18:1_24:0)-H]⁻	C48H92NO12S	0.141
	892.6190	5.15E+08	[ST (41:1)-H]⁻	C47H90NO12S	0.112	904.6188	2.19E+07	[ST-OH (18:1_24:1)-H]⁻	C48H90NO12S	-0.135
	878.6031	4.86E+08	[ST-OH (18:1_22:0)-H]⁻	C46H88NO12S	-0.175	778.5145	1.27E+07	[ST (18:1_16:0)-H]⁻	C40H76NO11S	0.123
	904.6191	3.86E+08	[ST-OH (18:1 24:1)-H]⁻	C48H90NO12S	0.145	878.6037	1.19E+07	[ST-OH (18:1 22:0)-H]⁻	C46H88NO12S	0.509
	924.6452	3.19E+08	[ST (42:0)-H]⁻	C48H94NO13S	0.108	890.6395	8.76E+06	[ST (18:1 24:0)-H]⁻	C48H92NO11S	-0.230
	890.6398	1.41E+08	[ST (18:1_24:0)-H]⁻	C48H92NO11S	0.112	892.6186	6.87E+06	[ST (41:1)-H]⁻	C47H90NO12S	-0.319
	920.6504	8.03E+07	[ST(43:1)-H]⁻	C49H94NO12S	0.217	924.6454	3.44E+06	[ST (42:0)-H]⁻	C48H94NO13S	0.306
	862.6086	6.73E+07	[ST (18:1_22:0)-H]⁻	C46H88NO11S	0.277					
	778.5142	6.02E+07	[ST (18:1_16:0)-H]⁻	C40H76NO11S	-0.385					
	704.5001	5.62E+07	[ST-OH (18:1_16:0)-H]⁻	C40H76NO12S	-0.314					
CONNECTIVE TISSUE	701.5128	4.41E+09	[PA (18:0_18:1)-H]⁻	C39H74O8P	0.142	788.5447	6.26E+10	[PS (18:0_18:1)-H]⁻	C42H79NO10P	-0.019
	788.5446	3.84E+09	[PS (18:0_18:1)-H]⁻	C42H79NO10P	-0.186	701.5126	3.51E+10	[PA (18:0_18:1)-H]⁻	C39H74O8P	-0.011
	750.5441	3.19E+09	[PE (P-18:0-20:4)-H]⁻	C43H77NO7P	-0.266	750.5445	2.26E+10	[PE (P-18:0-20:4)-H]⁻	C43H77NO7P	0.194
	687.5447	2.33E+09	[SM (16:0_18:1)-CH3]⁻	C38H76N2O6P	0.145	687.5445	1.05E+10	[SM (16:0_18:1)-CH3]⁻	C38H76N2O6P	-0.289

NOTES
All peaks identified by MALDI-FTICR-MS, MALDI-MS/MS and databases/literature.
Species in negative ion species were observed exclusively as deprotonated ([M-H]-) species.
Key: Bile acids (BA), Phosphatidic acid (PA), Phosphatidylethanolamine (PE), Phosphatidylserine (PS), Phosphatidylinositol (PI), Phosphatidylcholine (PC), Sphingomyelin (SM), Sulfatide (SM) and Hydroxyl-sulfatide (ST-OH).

Table S3. Comparison of lipids present in the different areas of the liver across different species in negative ion mode.

Identity	*Mass*	*ROC value*
$[TCDCA/TDCA-H]^-$	498,2896	0.626
$[TCA-H]^-$	514,2844	0.721
$[Bilirubin-H]^-$	583,2561	0.568
$[PS\ (18:0-18:1)-H]^-$	788,5449	0.684
$[PI\ (18:0-18:2)-H]^-$	861,5502	0.551
$[ST\text{-}OH\ (18:1-24:0)-H]^-$	906,6352	0.439

Table S4. ROC values for other molecular markers.