Supplemental tables and figures

Compound	Company	Cat # (Lot #)	Other
Cholic acid (CA; 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid)	Sigma-Aldrich	C-1129 (50K0240)	
Taurocholic acid (TCA; N-(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)-taurine)	Sigma-Aldrich	T4009-50 (S43S3752)	Sodium salt
Glycocholic acid (GCA; N-(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)-glycine)	Sigma-Aldrich	G-7132 (29H5236)	Sodium salt
Chenodeoxycholic acid (CDCA; 3α,7α-dihydroxy-5β-cholan-24-oic acid)	Sigma-Aldrich	C-9377 (124H0148)	
Taurochenodeoxycholic acid (TCDCA; N-(3α,7α-dihydroxy-5β-cholan-24-oyl)-taurine)	Toronto Research Chemicals	T008130	
Glycochenodeoxycholic acid (GCDCA; N-(3α,7α-dihydroxy-5β-cholan-24-oyl)-glycine)	Biosynth	G-5220 (0000011611)	
Lithocholic acid (LCA; 3α-hydroxy-5β-cholan-24-oic acid)	Sigma-Aldrich	L-6250 (100K2501)	
Taurolithocholic acid (TLCA; N-(3α-hydroxy-5β-cholan-24-oyl)-taurine)	Sigma-Aldrich	T-7515 (096H5019)	Sodium salt
Glycolithocholic acid (GLCA; N-[(3α,5β)-3-hydroxy-24-oxocholan-24-yl]-glycine)	Isosciences	13231UNL (SJ4-2014-276A1)	
Deoxycholic acid (DCA; 3α,12α-dihydroxy-5β-cholan-24-oic acid)	Sigma-Aldrich	D-6750 (30K0197)	
Taurodeoxycholic acid (TDCA; N-(3α,12α-dihydroxy-5β-cholan-24-oyl)-taurine)	Sigma-Aldrich	T-0875 (126H5022)	
Glycodeoxycholic acid (GDCA; N-(3α,12α-dihydroxy-5β-cholan-24-oyl)glycine)	Sigma-Aldrich	G-9910-1G (10K1024)	
Ursodeoxycholic acid (UDCA; 3α,7β-dihydroxy-5β-cholan-24-oic acid)	Sigma-Aldrich	U-5127 (129H1583)	
Glycoursodeoxycholic acid (GUDCA; N-(3α,7β-dihydroxy-5β-cholan-24-oyl)-glycine)	Calbiochem	362549 (D00032972)	Sodium salt
Allocholic acid (ACA; 3α,7α,12α-trihydroxy-5α-cholan-24-oic acid)	Toronto Research Chemicals	A545000	
Isoallolithocholic acid (IALCA; 3β-hydroxy-5α-cholan-24-oic acid)	Steraloids	C0700-000 (B1465)	
3-oxo-chol-4-enic acid	Steraloids	C2270-000 (B1338)	
3-oxo-cholic acid (3-oxo-CA; 3-oxo-7α,12α-dihydroxy-5α-cholan-24-oic acid)	Dr. James E. Polli's lab (UMB)	N/A	
7α -hydroxy-3-oxo-chol-4-en-24-oic acid	Toronto Research Chemicals	H951180 (1014-JQW-137)	
Cholic acid-d_4 (CA-d_4; 5β-cholanic acid-3α,7α,12α-triol-2,2,4,4-d_4)	Cambridge Isotope Laboratories	DLM-2611-0	
Glycochenodeoxycholic acid-d_4	Cambridge Isotope Laboroatories	DLM-7804-0.01	

(GCDCA-d_4; 5β-cholanic acid-3α,7α-diol N-[carboxymethyl]-amide-2,2,4,4-d_4)

Table S1. Sources of authentic standards for analytes and stable isotope-labeled internal standards.

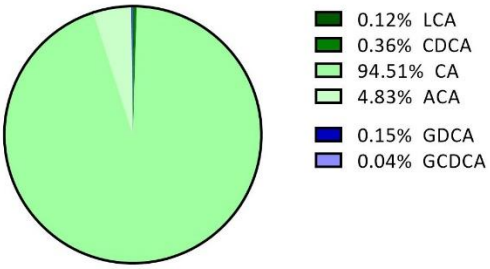
Internal Standard	Recovery	Intra-day precision (4 hr)	Intra-day accuracy (4 hr)	Inter-day precision (24 hr)	Inter-day accuracy (24 hr)	Benchtop stability (4 hr)
Plasma						
CA-d_4	45.5 (±13.2)%	8.2 (±10.8)%	95.6 (±11.9)%	8.1 (±10.8)%	88.2 (±19.7)%	11.2 (±9.0)%
GCDCA-d_4	114.9 (±28.6)%	14.6 (±4.7)%	117.7 (±5.6)%	14.5 (±4.2)%	108.4 (±20.9)%	3.9 (±5.4)%
Bile						
CA-d_4	100.0 (±28.9)%	12.7 (±5.0)%	119.5 (±26.8)%	12.4 (±1.7)%	113.3 (±2.0)%	10.5 (±8.4)%
GCDCA-d_4	100.0 (±25.7)%	7.3 (±4.1)%	98.9 (±8.8)%	5.7 (±8.1)%	95.4 (±9.7)%	11.3 (±8.9)%
Liver						
CA-d_4	73.1 (±4.2)%	8.0 (±5.6)%	80.4 (±25.8)%	9.8 (±5.8)%	108.4 (±9.9)%	13.2 (±0.2)%
GCDCA-d_4	73.3 (±4.5)%	2.4 (±2.6)%	91.3 (±26.9)%	2.6 (±1.1)%	100.2 (±3.6)%	2.8 (±1.1)%
Cell lysate						
CA-d_4	74.1 (±2.2)%	13.6 (±3.3)%	109.1 (±17.7)%	13.3 (±10.6)%	89.7 (±17.0)%	13.6 (±4.1)%
GCDCA-d_4	66.0 (±8.4)%	7.8 (±7.3)%	97.1 (±10.3)%	12.6 (±13.5)%	90.0 (±13.3)%	8.0 (±7.4)%
Cell medium						
CA-d_4	98.8 (±8.4)%	14.1 (±6.7)%	86.0 (±5.8)%	7.0 (±8.6)%	93.4 (±8.0)%	8.5 (±11.8)%
GCDCA-d_4	85.5 (±4.7)%	8.0 (±8.7)%	120.0 (±24.5)%	13.6 (±11.8)%	109.8 (±21.0)%	8.5 (±9.3)%

Table S2. Method validation – Apparent recovery, stability, precision, and accuracy. Values are presented as mean ± standard deviation; $n \geq 5$ for all validation experiments. Apparent recovery was obtained from the ratio of response from samples spiked with IS pre-extraction to responses from samples spiked with IS post-extraction for each matrix. Accuracy was determined by comparing calculated value with spike value of IS using calibrants run that day or 24 hours before. Intra-day studies were performed using 4 hour intervals for each matrix. Inter-day studies were performed using 24 hour intervals for each matrix. Benchtop stability was evaluated by leaving specified matrix with spiked IS at ambient room temperature (23±2°C) for 4 hours before performing extraction. COS-1 cells grown with DMEM were used for cell lysate and cell medium method validation.

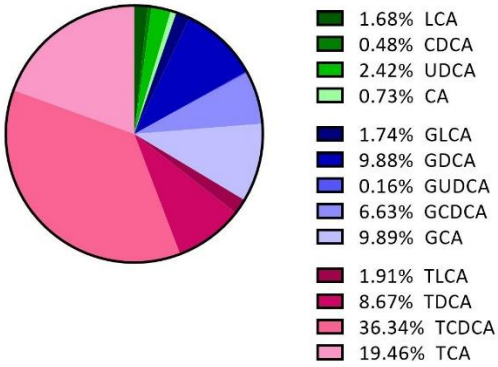
Analyte	*m/z* transition	t_R (min)	CE (eV)
Lithocholic acid (LCA)	375.3 > 375.3	21.99	18
Chenodeoxycholic acid (CDCA)	391.3 > 391.3	17.22	18
Deoxycholic acid (DCA)	391.3 > 391.3	17.76	18
Ursodeoxycholic acid (UDCA)	391.3 > 391.3	13.06	18
Cholic acid (CA)	407.3 > 407.3	12.72	18
Allocholic acid (ACA)	407.3 > 407.3	12.42	18
Glycolithodeoxycholic acid (GLCA)	432.3 > 74.1	18.35	35
Glycodeoxycholic acid (GDCA)	448.3 > 74.1	14.29	35
Glycoursodeoxycholic acid (GUDCA)	448.3 > 73.9	8.69	35
Glycochenodeoxycholic acid (GCDCA)	448.3 > 73.8	13.48	35
Glycocholic acid (GCA)	464.3 > 73.9	9.00	35
Taurolithocholic acid (TLCA)	482.3 > 80.0	17.55	50
Taurodeoxycholic acid (TDCA)	498.3 > 123.8	13.05	40
Taurochenodeoxycholic acid (TCDCA)	498.3 > 80.0	11.91	50
Taurocholic acid (TCA)	514.3 > 123.9	7.89	40
Isoallolithocholic acid (IALCA)	375.3 > 375.3	19.82	10
3-oxo-chol-4-enic acid	371.0 > 123.0	19.82	30
3-oxo-CA	405.0 > 289.0	11.88	30
7α-hydroxy-3-oxo-chol-4-en-24-oic acid	387.0 > 263.0	13.79	28
Internal standard			
Cholic acid-d_4 (CA-d_4)	411.3 > 347.3	12.72	35
Glycochenodeoxycholic acid-d_4 (GCDCA-d_4)	452.3 > 73.8	13.48	35

Table S3. Summary of *m/z* transitions, retention times, and collision energies used for analyte detection.

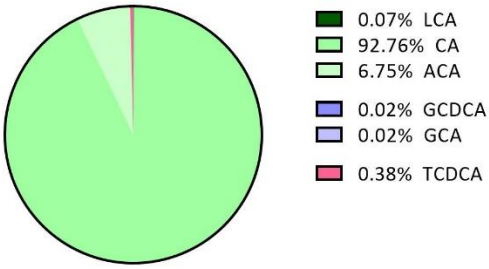
HepG2 Cells in HCM



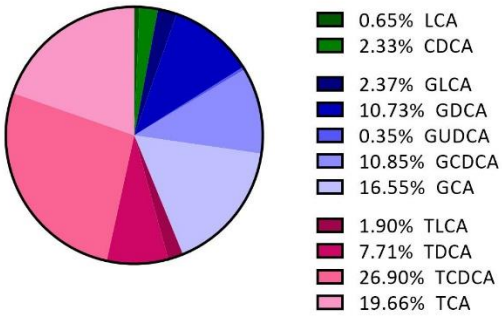
HepG2 Media (HCM)



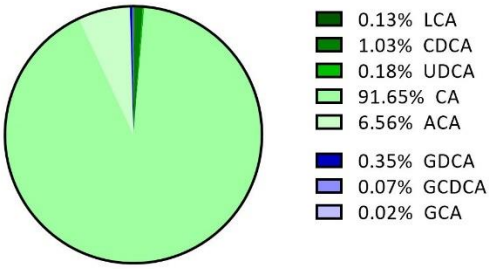
HepG2 Cells Grown in DMEM



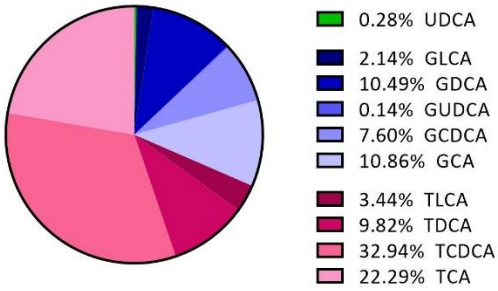
HepG2 Media (DMEM)



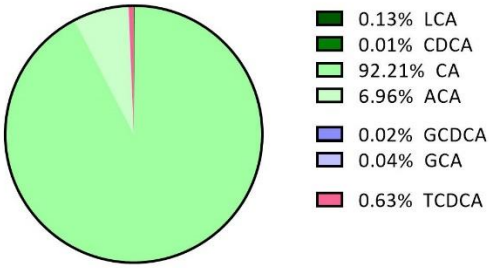
HuH-7 Cells Grown in HCM



HuH-7 Media (HCM)



HuH-7 Cells Grown in DMEM



HuH-7 Media (DMEM)

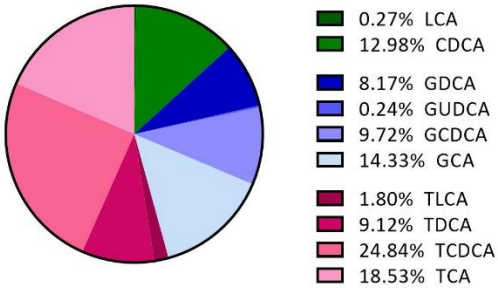


Figure S1. BA pool in immortalized cells and media, presented as average (n=3) percent of TBA. Unconjugated BAs are represented in shades of green, G-amidated BAs in shades of blue, and T-amidated BAs in shades of red. Constructed from data in Tables 2 and 3.

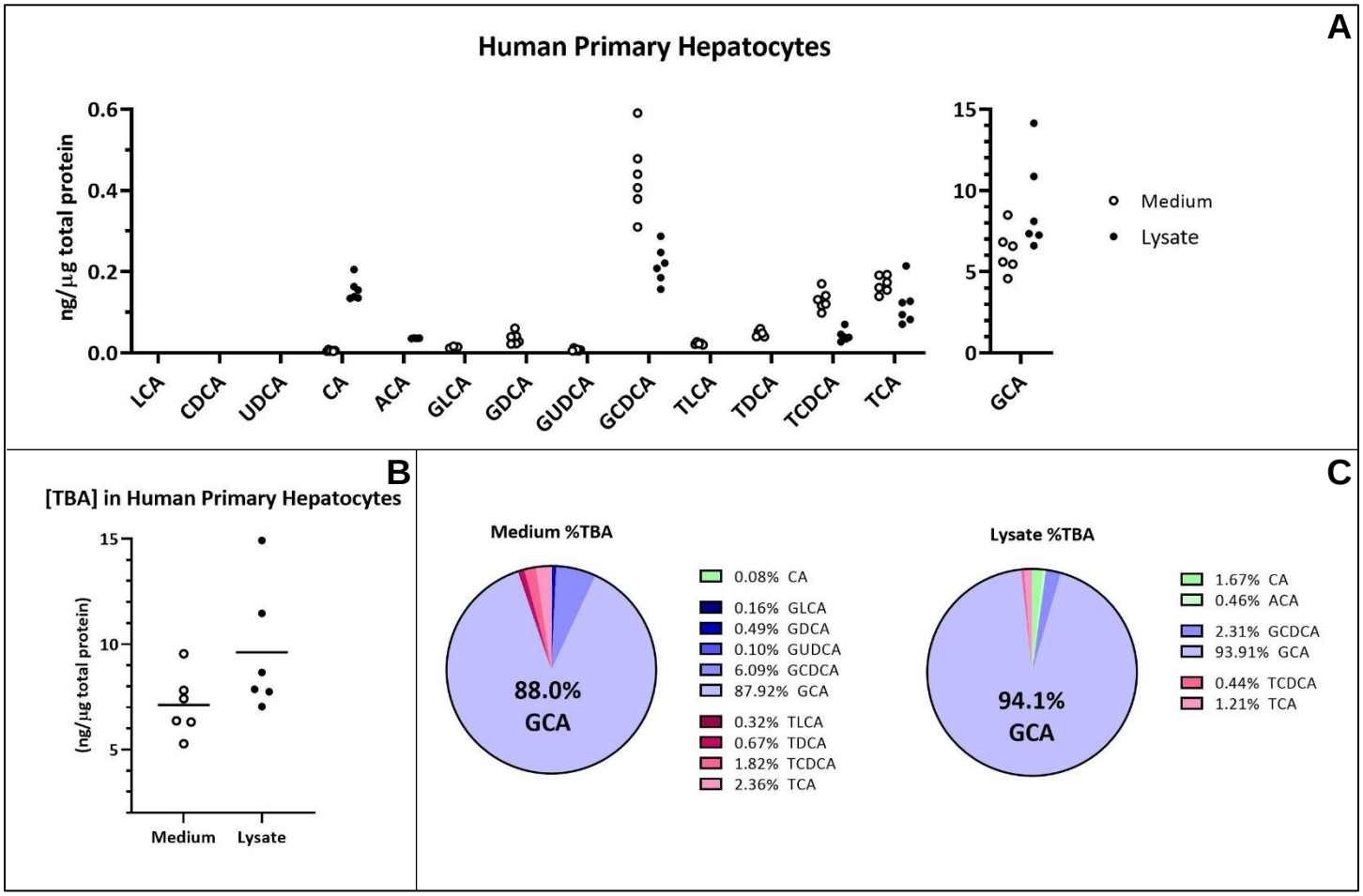


Figure S2. Concentrations of BAs determined in human primary hepatocytes. A: BAs measured in HPH cell media and lysates, presented as ng/µg total protein. B: Concentrations of TBA measured in HPH cell media and lysates, presented as ng/µg total protein. C: Composition of BA pool in HPHs, presented as average (n=6) percent of TBA. Unconjugated BAs are represented in shades of green, G-amidated BAs in shades of blue, and T-amidated BAs in shades of red. Constructed from data in Tables 2 and 3.

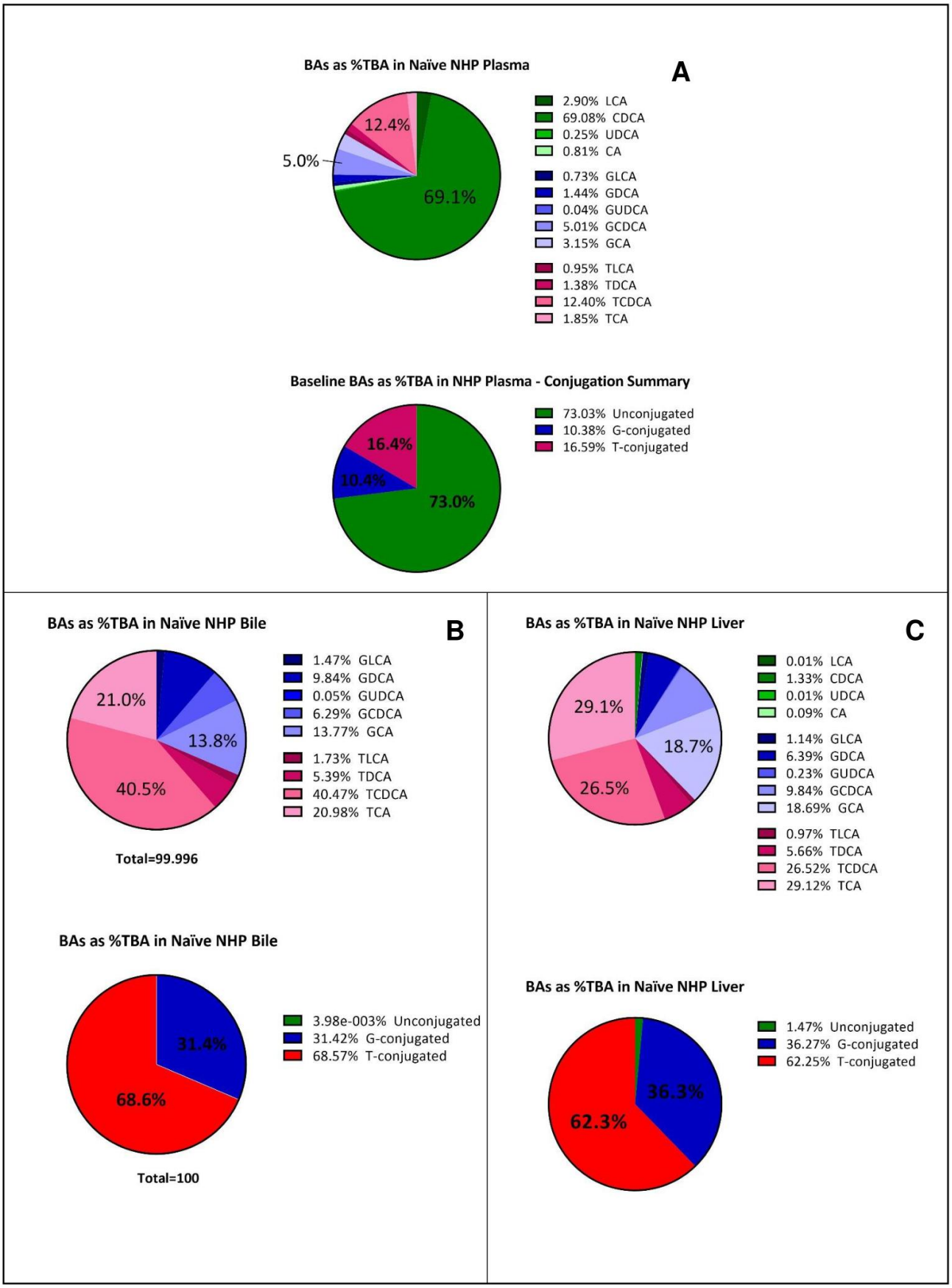


Figure S3. BA pools and amidation summaries in *M. mulatta* tissue and biofluids, presented as average percent TBA. A:

BA pool in plasma. B: BA pool in bile. C: BA pool in liver. The amounts of three largest portions of the BA pool are explicitly stated within the pie charts. Unconjugated BAs are represented in shades of green, G-amidated in shades of blue, and T-amidated in shades of red.