

Supplemental Material

Supplemental Tables

Supplemental Table 1

Parameter	Concentration (%)	
	Normal Diet	High-cholesterol, High-fat Diet
Moisture	7.6	7.8
Crude ash	3.4	3.7
Crude protein	17.1	17.9
Total fat	4.5	18.7
Crude fibre	2.8	3.1
N-free substances	64.6	48.9
	Percentage of Total Energy (kcal%)	
Fat	11.1%	38.7%
Carbohydrates	70.4%	44.9%
Protein	18.5%	16.4%

Supplemental Table 1. Composition of experimental animal diets.

Nutritional composition of the two diet types used in this study: standard chow (normal diet) and high-cholesterol, high-fat diet. The table includes percentages of moisture, crude ash, crude protein, total fat, crude fibre, and nitrogen-free substances (% of total diet by weight). The lower section of the table details the percentage of total energy (kcal%), illustrating the caloric contribution of fat, carbohydrates, and protein across the two diet groups.

Supplemental Table 2

Fatty Acid	Concentration (% of total fatty acid)	
	Normal Diet	Milk Fat Based Diet
Caprylic Acid C8	<0.1	0.9
Capric Acid C10	0.1	2.4
Lauric Acid C12	0.1	3.7
Myristic Acid C14	0.4	11.6
Tetradecaenoic Acid C14:1	<0.1	1.1
Pentadecanoic Acid C15	<0.1	1.1
Palmitic Acid C16	11.4	31.7
Palmitoleic Acid C16:1	0.2	1.9
Heptadecanoic Acid C17	0.1	0.7
Stearic Acid C18	4.9	12.1
Oleic Acid C18:1	25.4	27
Linoleic Acid C18:2	49.7	2.3
Linolenic Acid C18:3	6.1	0.7
Octadecatetraenoic Acid C18:4	<0.1	0.8
Arachidic Acid C20	0.4	0.2
Eicosaenoic Acid C20:1	0.4	0.3
Eicosadienoic Acid C20:2	<0.1	<0.1
Eicosatrienoic Acid C20:3	<0.1	0.1
Arachidonic Acid C20:4	<0.1	0.1
Eicosapentaenoic Acid C20:5	<0.1	0.1
Behenic Acid C22	0.5	0.1
Docosaenoic Acid C22:1	<0.1	<0.1
Docosatrienoic Acid C22:3	<0.1	<0.1
Docosapentaenoic Acid C22:5	<0.1	0.1
Docosahexaenoic Acid C22:6	<0.1	<0.1
Tricosane Acid C23	0.1	<0.1

Tetracosanoic Acid C24	0.2	0.1
Nervonic Acid C24:1	<0.1	0.1

Supplemental Table 2. Fatty acid composition of animal diets.

Quantitative comparison of individual fatty acid species (% of total fatty acids) in the normal diet and the milk fat-based diet. Notable differences include a higher abundance of saturated fatty acids (e.g., C14:0, C16:0, C18:0) and lower linoleic acid (C18:2) in the milk fat-based diet. Fatty acids below 0.1% are denoted as <0.1%.

Supplemental Table 3

Group	Body weight(g)		Food consumption (g/day/mouse)	Energy intake (kcal/day/mouse)
	mean	95% CI		
Chow	35.82	(33.93, 37.71)	3.43	12.60
Chow_DR	32.82	(30.60, 35.04)	3.35	12.29
HCHF	39.75	(36.28, 43.22)	2.65	11.52
HCHF_DR	37.69	(34.14, 41.25)	2.50	10.88

Supplemental Table 3. Final body weight, average daily food consumption, and energy intake of mice in each experimental group.

Body weight is presented as mean with 95% confidence interval (95% CI). Food consumption and energy intake represent the average daily intake per mouse throughout the intervention. Energy intake was calculated from the measured food consumption and the metabolizable energy density of each diet.

Supplemental Table 4

Gene	Primer Sequence, 5' → 3'
<i>Gapdh</i> -F	AGGTGGTGTGAACGGATTG
<i>Gapdh</i> -R	TGTAGACCATGTAGTTGAGTCA
<i>Acat1</i> -F	ATGCCAGCACACTGAACGAT
<i>Acat1</i> -R	TGCTGCAATTGGTGCCAATG
<i>Acat2</i> -F	ACAAGTGCTGCCTGGATTGT
<i>Acat2</i> -R	AGGCAGCTAACATCCATCCC
<i>Ces1g</i> -F	GTGGCTCTGTGCTTTGAGTC
<i>Ces1g</i> -R	GGACTTTGCCATGAACCGTG
<i>CYP7A1</i> -F	ATGGAGAAGGCTAAGACGCAC
<i>CYP7A1</i> -R	GCTGCTTTCATTGCTTCAGG
<i>CYP8B1</i> -F	GGGCAGAAGATGATCACCAC
<i>CYP8B1</i> -R	TTCATCTCGCTGAGGGCAA
<i>Scd1</i> -F	GGATTTTCTACTATGACCAGCG
<i>Scd1</i> -R	ATTCGTACACGTCATTCTGGAAC

Supplemental Table 4. Primer sequences used for quantitative real-time PCR analysis.

Primer sequences (5'→3') used for quantitative real-time PCR (qPCR) analysis of genes involved in cholesterol metabolism and lipid homeostasis are listed. *Gapdh* served as the internal reference gene for normalization of gene expression.

Supplemental Table 5

Lipid	PMID	Chow	Chow_DR	HCHF	HCHF_DR
SM(16:0)	PMID: 38734675	40.13 ± 3.12	34.36 ± 2.81	46.66 ± 5.23	86.33 ± 8.99
SM(24:1)	PMID: 38734675	63.76 ± 4.25	60.79 ± 5.17	46.92 ± 3.24	85.77 ± 9.17
PC(30:1)	PMID: 38734675	1.10 ± 0.65	2.67 ± 0.83	2.58 ± 0.87	3.40 ± 1.16
PC(30:0)	PMID: 37237999	4.47 ± 0.69	2.99 ± 0.38	7.73 ± 0.65	8.55 ± 1.65
PC(O 32:1)	PMID: 38734675	1.07 ± 0.38	0.87 ± 0.23	2.15 ± 0.45	5.34 ± 1.47
PC(O 32:0)	PMID: 38734675	5.02 ± 0.80	4.99 ± 0.64	6.32 ± 0.98	11.09 ± 1.36
PC(32:1)	PMID: 40176064	71.65 ± 5.86	69.54 ± 3.55	133.54 ± 6.71	201.03 ± 10.99
PC(32:0)	PMID: 38734675; PMID: 40176064	94.81 ± 7.68	80.79 ± 4.36	49.75 ± 3.31	72.31 ± 8.09
PC(O 34:1)	PMID: 38734675	11.64 ± 1.25	11.25 ± 0.56	35.77 ± 1.32	54.24 ± 2.89
PC(O 34:0)	PMID: 38734675	2.04 ± 0.58	1.63 ± 0.27	3.90 ± 0.27	4.75 ± 0.68
PC(34:2)	PMID: 40176064	1061.05 ± 119.51	1026.30 ± 52.74	387.46 ± 24.91	537.41 ± 35.22
PC(34:1)	PMID: 38734675; PMID: 40176064	585.10 ± 35.86	684.20 ± 30.53	1103.28 ± 56.80	1478.34 ± 78.46
PC(36:1)	PMID: 38734675	90.55 ± 10.50	92.63 ± 5.86	191.90 ± 13.81	276.46 ± 16.49
PC(O 38:5)	PMID: 38734675	12.28 ± 1.02	9.47 ± 1.17	10.61 ± 0.58	10.30 ± 1.61
PC(38:0)	PMID: 38734675	13.12 ± 1.26	14.25 ± 0.94	19.00 ± 1.54	28.98 ± 2.56
PE(32:1)	PMID: 38734675	4.75 ± 1.51	5.70 ± 1.06	16.60 ± 1.92	23.74 ± 1.60
PE(34:3)	PMID: 38734675	17.74 ± 2.87	15.99 ± 1.29	9.59 ± 1.50	13.09 ± 1.47
PE(34:2)	PMID: 38734675	158.12 ± 22.57	168.50 ± 20.86	95.91 ± 13.66	119.55 ± 10.19
PE(34:1)	PMID: 38734675	40.20 ± 5.82	43.16 ± 3.27	93.68 ± 9.95	117.76 ± 9.32
PE(O 36:2)	PMID: 38734675	4.17 ± 0.54	4.21 ± 0.46	4.95 ± 0.54	7.03 ± 1.03
PE(36:5)	PMID: 38734675; PMID: 24622385	23.91 ± 1.54	24.69 ± 1.59	27.65 ± 2.40	35.86 ± 2.96

		283.44 ±			
PE(36:4)	PMID: 38734675	16.26	291.39 ± 11.65	204.52 ± 14.68	249.21 ± 19.29
PE(36:3)	PMID: 38734675	87.71 ± 10.71	97.16 ± 6.08	87.75 ± 8.43	109.80 ± 10.70
		136.64 ±			
PE(36:2)	PMID: 38734675	16.84	135.56 ± 6.50	102.33 ± 8.40	123.05 ± 11.15
PE(36:1)	PMID: 38734675	17.05 ± 3.67	17.32 ± 0.92	31.73 ± 1.74	37.59 ± 3.27
PE(38:2)	PMID: 38734675	2.77 ± 0.58	3.24 ± 0.64	3.78 ± 0.33	4.49 ± 0.37
PE(40:1)	PMID: 38734675	0.32 ± 0.13	0.36 ± 0.15	0.38 ± 0.16	0.22 ± 0.11
LPC(16:1)	PMID: 40176064	4.79 ± 0.54	5.97 ± 0.41	5.54 ± 0.40	7.92 ± 0.56
LPC(22:5)	PMID: 40176064	3.29 ± 0.56	3.98 ± 0.23	2.01 ± 0.42	1.68 ± 0.13
LPC(22:4)	PMID: 40176064	0.59 ± 0.13	0.62 ± 0.05	0.32 ± 0.06	0.24 ± 0.06
Cer(16:0)	PMID: 38734675	9.34 ± 0.80	9.35 ± 0.63	17.21 ± 1.75	16.69 ± 1.24
Cer(18:0)	PMID: 38734675	2.08 ± 0.33	2.35 ± 0.35	3.51 ± 0.34	3.43 ± 0.23
Cer(24:1)	PMID: 38734675	22.70 ± 2.78	23.81 ± 1.70	36.83 ± 3.57	35.59 ± 2.64
CE(14:0)	PMID:38992128	25.09 ± 3.98	18.70 ± 1.49	60.03 ± 2.58	65.62 ± 1.79
CE(15:0)	PMID:38992128	6.55 ± 0.69	4.18 ± 1.03	32.00 ± 1.57	37.89 ± 1.33
		2227.51 ±	1713.64 ±	2224.21 ±	2607.70 ±
CE(16:1)	PMID: 40176064	290.40	193.35	143.15	84.40
CE(20:3)	PMID:35422138	15.29 ± 1.75	12.00 ± 1.87	19.41 ± 2.34	17.68 ± 0.92
CE(20:2)	PMID:35422138	4.53 ± 0.67	3.94 ± 0.56	6.10 ± 0.50	6.25 ± 0.22
CE(20:1)	PMID:35422138	13.56 ± 2.08	11.10 ± 1.39	18.48 ± 0.92	19.70 ± 0.63
CE(20:0)	PMID:35422138	14.22 ± 1.53	12.68 ± 1.05	14.32 ± 1.32	26.48 ± 4.95
CE(22:1)	PMID:35422138	4.97 ± 0.74	4.56 ± 0.79	5.66 ± 0.59	6.33 ± 0.42

Supplemental Table 5. Pro-atherogenic lipids in the liver (Mean ± SEM, pmol/mg tissue)

This table presents the mean ± SEM concentrations (pmol/mg tissue) of lipid species previously reported to promote atherosclerosis. Lipid classes include sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC), and ceramide (Cer). Lipid species were selected based on their pro-atherogenic potential in published studies (PMID: 38734675, 40176064, 37237999, 24622385, 38992128, 35422138). Lipid quantification was performed by targeted lipidomics, and values were compared across four dietary groups: Chow, Chow_DR, HCHF, and HCHF_DR.

Supplemental Table 6

Lipid	PMID	Chow	Chow_DR	HCHF	HCHF_DR
SM(23:1)	PMID: 38734675	4.89 ± 1.08	3.22 ± 0.86	2.91 ± 0.59	7.41 ± 1.77
SM(23:0)	PMID: 38734675	11.49 ± 0.81	11.75 ± 0.91	9.00 ± 0.92	18.19 ± 2.81
SM(24:0)	PMID: 38734675	33.75 ± 2.98	32.04 ± 2.84	16.83 ± 1.85	24.44 ± 2.79
PC(32:2)	PMID: 38734675	12.09 ± 1.99	9.95 ± 1.45	14.74 ± 1.55	21.22 ± 2.02
PC(36:5)	PMID: 38734675	58.32 ± 3.83	62.65 ± 2.69	46.21 ± 1.54	71.51 ± 5.91
PC(36:4)	PMID: 38734675	849.82 ± 37.40	814.51 ± 20.41	303.26 ± 18.46	356.39 ± 25.35
PC(38:7)	PMID: 38734675	30.53 ± 1.97	29.27 ± 1.81	18.17 ± 1.54	25.07 ± 2.04
PC(38:6)	PMID: 38734675	679.11 ± 47.66	690.49 ± 18.57	346.44 ± 24.58	398.46 ± 34.63
PC(38:5)	PMID: 38734675	197.68 ± 8.17	205.64 ± 11.28	126.76 ± 6.19	174.47 ± 12.61
PC(38:4)	PMID: 37237999; PMID: 38734675	487.25 ± 26.26	436.21 ± 20.52	165.91 ± 8.24	210.33 ± 10.77
PC(38:3)	PMID: 40176064; PMID: 38734675	79.55 ± 6.22	89.10 ± 6.62	76.69 ± 4.57	100.18 ± 6.68
PC(40:7)	PMID: 40176064; PMID: 38734675	101.27 ± 6.62	112.52 ± 7.44	89.31 ± 4.79	114.86 ± 10.43
PC(40:6)	PMID: 38734675	243.65 ± 18.57	232.86 ± 10.21	121.17 ± 9.06	130.34 ± 9.62
PE(36:6)	PMID: 38734675	0.96 ± 0.31	0.34 ± 0.15	1.23 ± 0.29	2.04 ± 0.63
PE(O 38:7)	PMID: 38734675	0.57 ± 0.21	0.40 ± 0.20	0.32 ± 0.14	0.49 ± 0.20
LPC(16:0)	PMID: 38734675	160.70 ± 13.73	210.36 ± 9.84	120.25 ± 7.12	144.33 ± 10.52
LPC(18:2)	PMID: 38734675	59.30 ± 6.68	66.51 ± 4.33	14.40 ± 0.91	17.27 ± 1.37
LPC(18:1)	PMID: 38734675	46.74 ± 4.19	60.67 ± 3.27	77.81 ± 4.80	96.23 ± 5.80

LPC(18:0)	PMID: 38734675	112.50 ± 11.17	134.35 ± 6.29	66.89 ± 3.96	83.82 ± 6.58
LPC(20:4)	PMID: 38734675	73.96 ± 7.36	75.32 ± 3.57	22.88 ± 1.52	24.81 ± 1.59
Cer(23:0)	PMID: 38734675	3.87 ± 0.48	4.12 ± 0.32	6.34 ± 0.51	6.70 ± 0.44
Cer(24:0)	PMID: 38734675	11.23 ± 1.41	12.34 ± 1.02	10.32 ± 0.69	9.73 ± 0.58
CE(18:2)	PMID: 40176064	408.68 ± 54.41	355.95 ± 28.58	96.04 ± 2.99	92.77 ± 2.63

Supplemental Table 6. Anti-atherogenic lipids in the liver (Mean ± SEM, pmol/mg tissue)

This table summarizes mean ± SEM concentrations (pmol/mg tissue) of hepatic lipid species associated with anti-atherogenic effects, based on literature-reported functions (PMID: 38734675, 40176064, 37237999, 24622385, 38992128, 35422138). Included classes are SM, PC, PE, LPC, ceramide (Cer), and cholesteryl ester (CE). Lipid concentrations were compared across the same four dietary groups.

Supplemental Table 7

Lipid	PMID	Chow	Chow_DR	HCHF	HCHF_DR
SM(16:0)	PMID: 38734675	2.00 ± 1.33	4.00 ± 1.63	3.00 ± 1.53	8.00 ± 1.33
SM(24:1)	PMID: 38734675	0.00 ± 0.00	1.00 ± 1.00	0.00 ± 0.00	4.00 ± 1.63
PC(30:1)	PMID: 38734675	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 1.33	3.00 ± 1.53
PC(30:0)	PMID: 37237999	1.00 ± 1.00	1.00 ± 1.00	6.00 ± 1.63	5.00 ± 1.67
PC(O 32:1)	PMID: 38734675	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 1.00	3.00 ± 1.53
PC(O 32:0)	PMID: 38734675	1.00 ± 1.00	1.00 ± 1.00	1.00 ± 1.00	2.00 ± 1.33
PC(32:1)	PMID: 40176064	68.00 ± 9.75	82.00 ± 13.06	256.00 ± 52.77	261.00 ± 49.20
PC(32:0)	PMID: 38734675; PMID: 40176064	24.00 ± 3.71	23.00 ± 3.67	19.00 ± 3.14	30.00 ± 6.32
PC(O 34:1)	PMID: 38734675	5.00 ± 1.67	7.00 ± 1.53	55.00 ± 11.95	57.00 ± 11.93
PC(O 34:0)	PMID: 38734675	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.00 ± 1.63
PC(34:2)	PMID: 40176064	1251.00 ± 181.05	1784.00 ± 223.91	817.00 ± 210.01	739.00 ± 133.07
PC(34:1)	PMID: 38734675; PMID: 40176064	500.00 ± 59.31	715.00 ± 92.79	1855.00 ± 450.99	1606.00 ± 264.60
PC(36:1)	PMID: 38734675	36.00 ± 4.27	36.00 ± 3.71	104.00 ± 20.67	103.00 ± 13.09
PC(O 38:5)	PMID: 38734675	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 1.00	5.00 ± 1.67
PC(38:0)	PMID: 38734675	7.00 ± 1.53	8.00 ± 2.49	18.00 ± 2.91	22.00 ± 4.42
PE(32:1)	PMID: 38734675	20.97 ± 10.27	6.73 ± 1.98	48.08 ± 10.26	44.32 ± 6.86
PE(34:3)	PMID: 38734675	13.09 ± 4.60	11.34 ± 2.98	16.45 ± 5.75	9.33 ± 2.60

PE(34:2)	PMID: 38734675	192.63 ± 46.94	184.35 ± 30.73	194.03 ± 68.52	144.22 ± 24.02
PE(34:1)	PMID: 38734675	101.00 ± 22.82	73.59 ± 10.68	254.34 ± 55.38	206.18 ± 34.12
PE(O 36:2)	PMID: 38734675	3.49 ± 1.14	1.62 ± 0.58	6.00 ± 2.01	6.96 ± 1.98
PE(36:5)	PMID: 38734675; PMID: 24622385	11.65 ± 2.93	6.13 ± 1.62	16.81 ± 4.21	15.65 ± 3.54
PE(36:4)	PMID: 38734675	93.60 ± 13.55	67.62 ± 12.34	95.48 ± 20.39	95.90 ± 18.71
PE(36:3)	PMID: 38734675	50.32 ± 10.06	42.44 ± 8.76	58.75 ± 15.11	51.15 ± 10.98
PE(36:2)	PMID: 38734675	99.50 ± 15.98	88.39 ± 11.93	112.70 ± 26.71	131.82 ± 20.30
PE(36:1)	PMID: 38734675	53.74 ± 22.04	30.09 ± 3.93	46.24 ± 10.82	61.57 ± 14.41
PE(38:2)	PMID: 38734675	0.61 ± 0.21	0.60 ± 0.23	4.09 ± 1.71	5.41 ± 1.72
PE(40:1)	PMID: 38734675	0.71 ± 0.33	2.02 ± 1.60	1.24 ± 0.57	1.09 ± 0.64
LPC(16:1)	PMID: 40176064	0.84 ± 0.17	1.80 ± 0.77	2.97 ± 0.74	5.38 ± 1.29
LPC(22:5)	PMID: 40176064	0.26 ± 0.11	0.33 ± 0.16	0.16 ± 0.06	0.28 ± 0.10
LPC(22:4)	PMID: 40176064	0.03 ± 0.03	0.06 ± 0.03	0.01 ± 0.01	0.02 ± 0.01
Cer(16:0)	PMID: 38734675	48.39 ± 10.54	28.91 ± 3.28	40.01 ± 12.92	58.68 ± 14.26
Cer(18:0)	PMID: 38734675	8.17 ± 1.41	5.32 ± 0.75	5.40 ± 0.76	10.19 ± 2.25
Cer(24:1)	PMID: 38734675	15.59 ± 2.47	9.37 ± 1.38	14.26 ± 2.04	25.48 ± 5.02
CE(14:0)	PMID:38992128	0.07 ± 0.07	0.15 ± 0.12	0.23 ± 0.06	0.46 ± 0.17
CE(15:0)	PMID:38992128	0.88 ± 0.48	1.41 ± 0.65	0.49 ± 0.06	1.07 ± 0.35
CE(16:1)	PMID: 40176064	0.70 ± 0.27	0.82 ± 0.27	3.79 ± 1.24	10.66 ± 5.38
CE(20:3)	PMID:35422138	0.07 ± 0.05	0.00 ± 0.00	0.06 ± 0.04	0.18 ± 0.08

CE(20:2)	PMID:35422138	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.07 ± 0.05
CE(20:1)	PMID:35422138	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.04	0.26 ± 0.14
CE(20:0)	PMID:35422138	17.35 ± 9.10	30.59 ± 14.60	10.12 ± 2.41	18.97 ± 8.53
CE(22:1)	PMID:35422138	0.12 ± 0.08	0.27 ± 0.17	0.12 ± 0.04	0.24 ± 0.11

Supplemental Table 7. Pro-atherogenic lipids in the bile (Mean ± SEM, nmol/ml bile)

This table provides mean ± SEM concentrations (nmol/ml bile) of bile lipid species linked to increased atherosclerotic risk. Quantified classes include PC, PE, SM, and Cer. Lipid identities were curated from prior publications (PMID: 38734675, 40176064, 37237999, 24622385, 38992128, 35422138). based on their atherogenicity. Comparisons were made among mice receiving different diets (Chow, Chow_DR, HCHF, HCHF_DR).

Supplemental Table 8

Lipid	PMID	Chow	Chow_DR	HCHF	HCHF_DR
PC(32:2)	PMID: 38734675	12.00 ± 2.91	15.00 ± 4.01	31.00 ± 6.90	32.00 ± 6.80
PC(36:5)	PMID: 38734675	37.00 ± 5.78	50.00 ± 6.67	49.00 ± 10.38	50.00 ± 10.33
PC(36:4)	PMID: 38734675	469.00 ± 60.23	558.00 ± 67.00	266.00 ± 58.56	227.00 ± 36.03
PC(38:7)	PMID: 38734675	7.00 ± 1.53	9.00 ± 2.33	13.00 ± 3.67	10.00 ± 2.11
PC(38:6)	PMID: 38734675	189.00 ± 24.79	240.00 ± 31.59	184.00 ± 41.05	153.00 ± 27.49
PC(38:5)	PMID: 38734675	60.00 ± 8.43	60.00 ± 9.43	60.00 ± 13.17	65.00 ± 10.35
PC(38:4)	PMID: 37237999; PMID: 38734675	88.00 ± 8.92	82.00 ± 9.04	55.00 ± 8.20	57.00 ± 10.33
PC(38:3)	PMID: 40176064; PMID: 38734675	9.00 ± 2.77	10.00 ± 2.58	19.00 ± 5.04	20.00 ± 3.94
PC(40:7)	PMID: 40176064; PMID: 38734675	11.00 ± 1.80	10.00 ± 2.58	20.00 ± 3.33	19.00 ± 4.82
PC(40:6)	PMID: 38734675	12.00 ± 2.49	10.00 ± 2.58	14.00 ± 2.67	13.00 ± 2.60
PE(36:6)	PMID: 38734675	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
PE(O 38:7)	PMID: 38734675	2.97 ± 1.70	0.00 ± 0.00	0.52 ± 0.37	0.64 ± 0.52
LPC(16:0)	PMID: 38734675	11.09 ± 1.24	17.57 ± 3.48	11.00 ± 1.95	15.05 ± 2.18
LPC(18:2)	PMID: 38734675	19.47 ± 3.90	41.48 ± 14.92	8.34 ± 1.84	14.37 ± 2.71
LPC(18:1)	PMID: 38734675	8.78 ± 1.38	18.73 ± 5.79	26.36 ± 5.41	41.86 ± 8.75
LPC(18:0)	PMID: 38734675	5.25 ± 0.61	6.11 ± 0.77	4.10 ± 0.68	5.12 ± 0.52
LPC(20:4)	PMID: 38734675	10.55 ± 2.86	16.56 ± 5.84	4.72 ± 0.96	7.81 ± 1.65
Cer(23:0)	PMID: 38734675	2.07 ± 0.56	0.87 ± 0.23	1.64 ± 0.33	2.93 ± 0.64
Cer(24:0)	PMID: 38734675	6.94 ± 1.52	3.44 ± 0.49	3.76 ± 0.90	6.49 ± 1.17

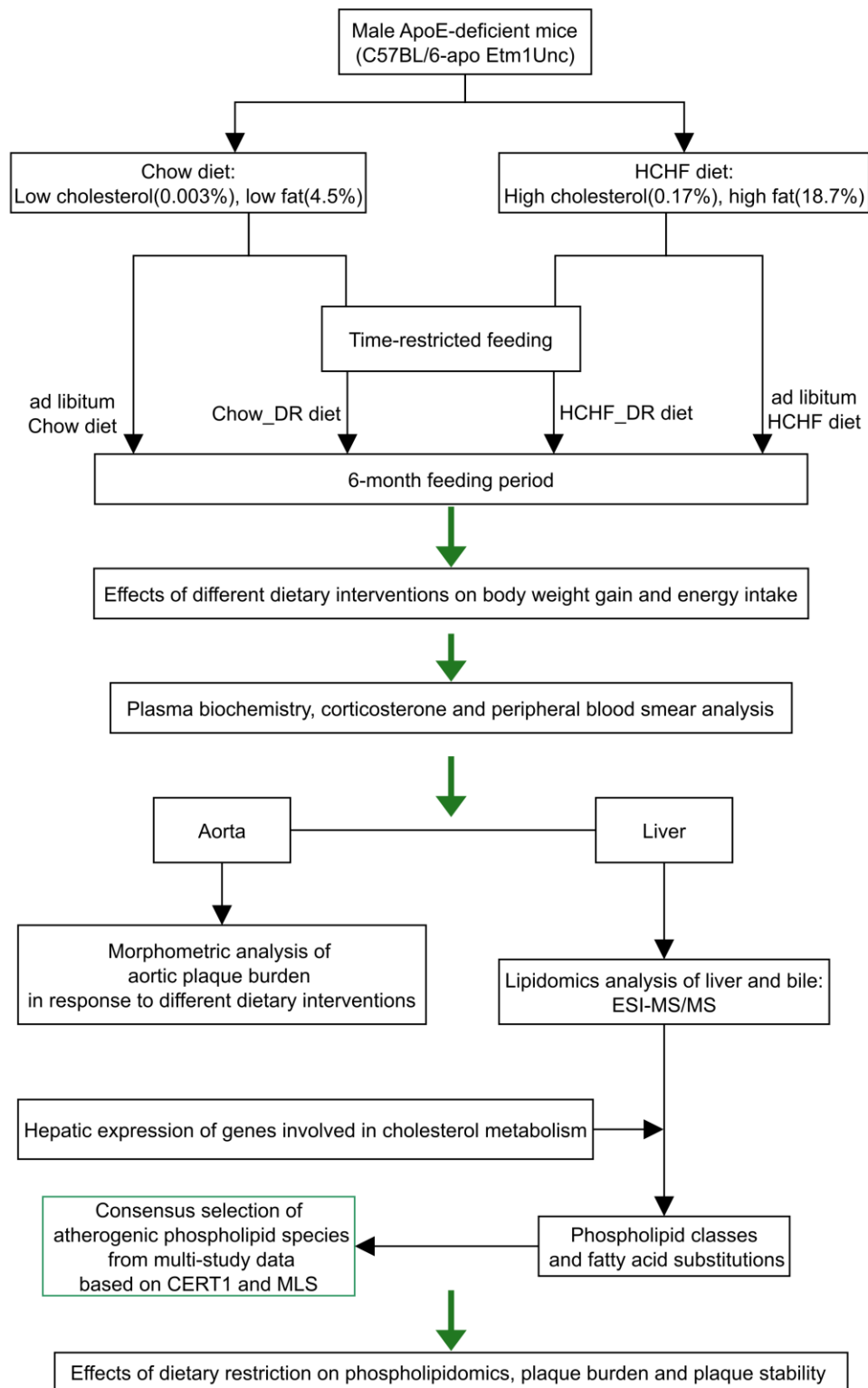
CE(18:2)	PMID: 40176064	0.56 ± 0.18	0.46 ± 0.09	0.99 ± 0.29	1.61 ± 0.71
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Supplemental Table 8. Anti-atherogenic lipids in the bile (Mean ± SEM, nmol/ml bile)

This table shows mean ± SEM concentrations (nmol/ml bile) of lipid species with potential atheroprotective roles, as reported in previous studies (PMID: 38734675, 40176064, 37237999, 24622385, 38992128, 35422138). Lipid classes include PC, LPC, PE, Cer, and CE. Group comparisons were made between the four dietary interventions.

Supplemental Figures

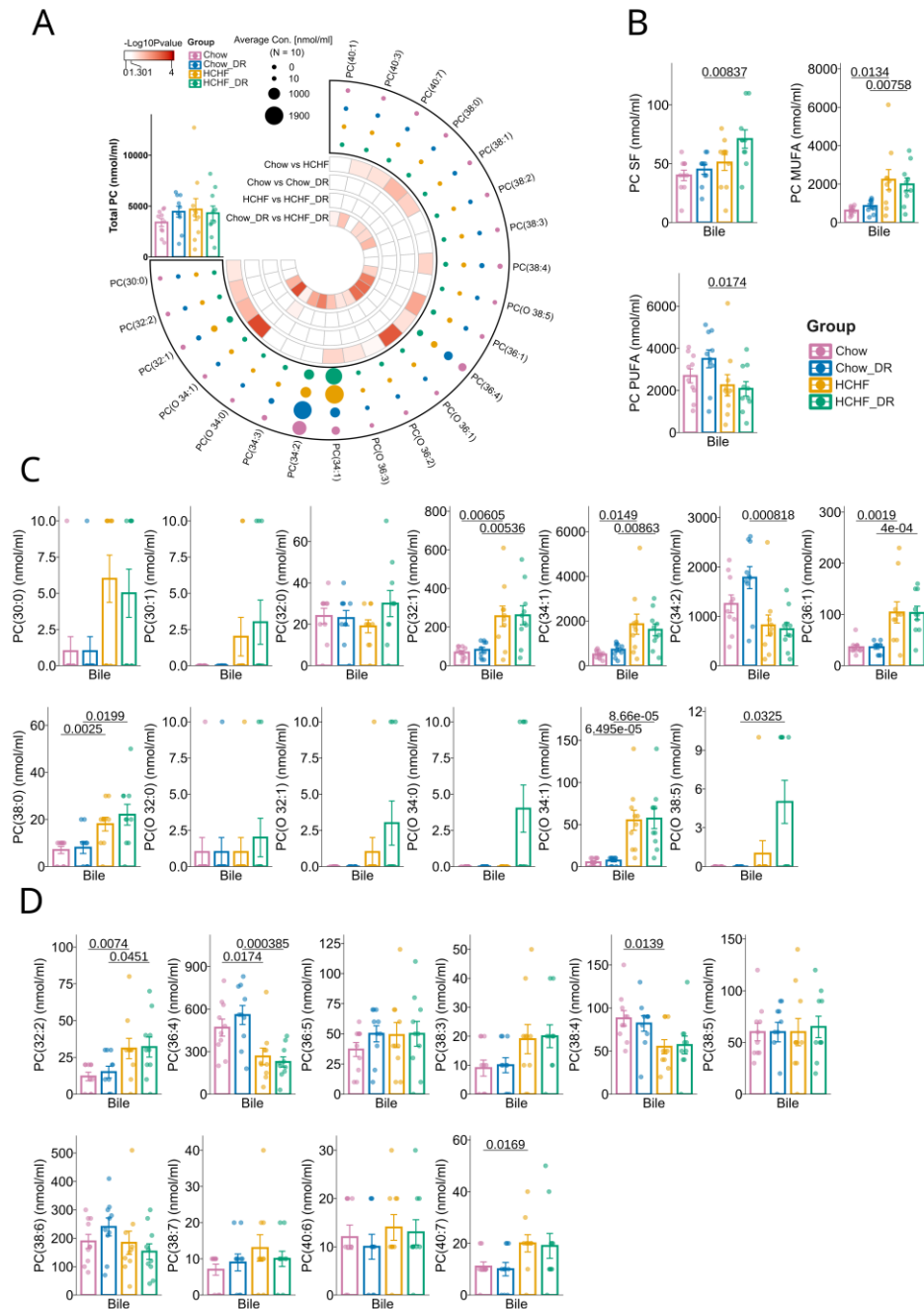
Supplemental Figure 1



Supplemental Figure 1. Experimental design and analytical workflow.

Male ApoE-deficient mice were fed either a chow diet or a high-cholesterol high-fat (HCHF) diet under ad libitum feeding or time-restricted feeding conditions for 6 months, generating four experimental groups (Chow, Chow_DR, HCHF, and HCHF_DR). Body weight, food intake, and energy intake were monitored during the intervention. At the end of the study, plasma biochemistry, corticosterone, and peripheral blood smears were analyzed. Aortic tissues were used for quantitative morphometric assessment of atherosclerotic lesions, whereas liver and bile samples were analyzed by ESI-MS/MS lipidomics together with hepatic gene expression analysis. Lipidomic profiling was integrated with CERT1- and machine learning-based lipid score (MLS) analyses to identify consensus atherogenic phospholipid species and determine the effects of dietary restriction on phospholipid composition, atherosclerotic plaque burden, and plaque stability.

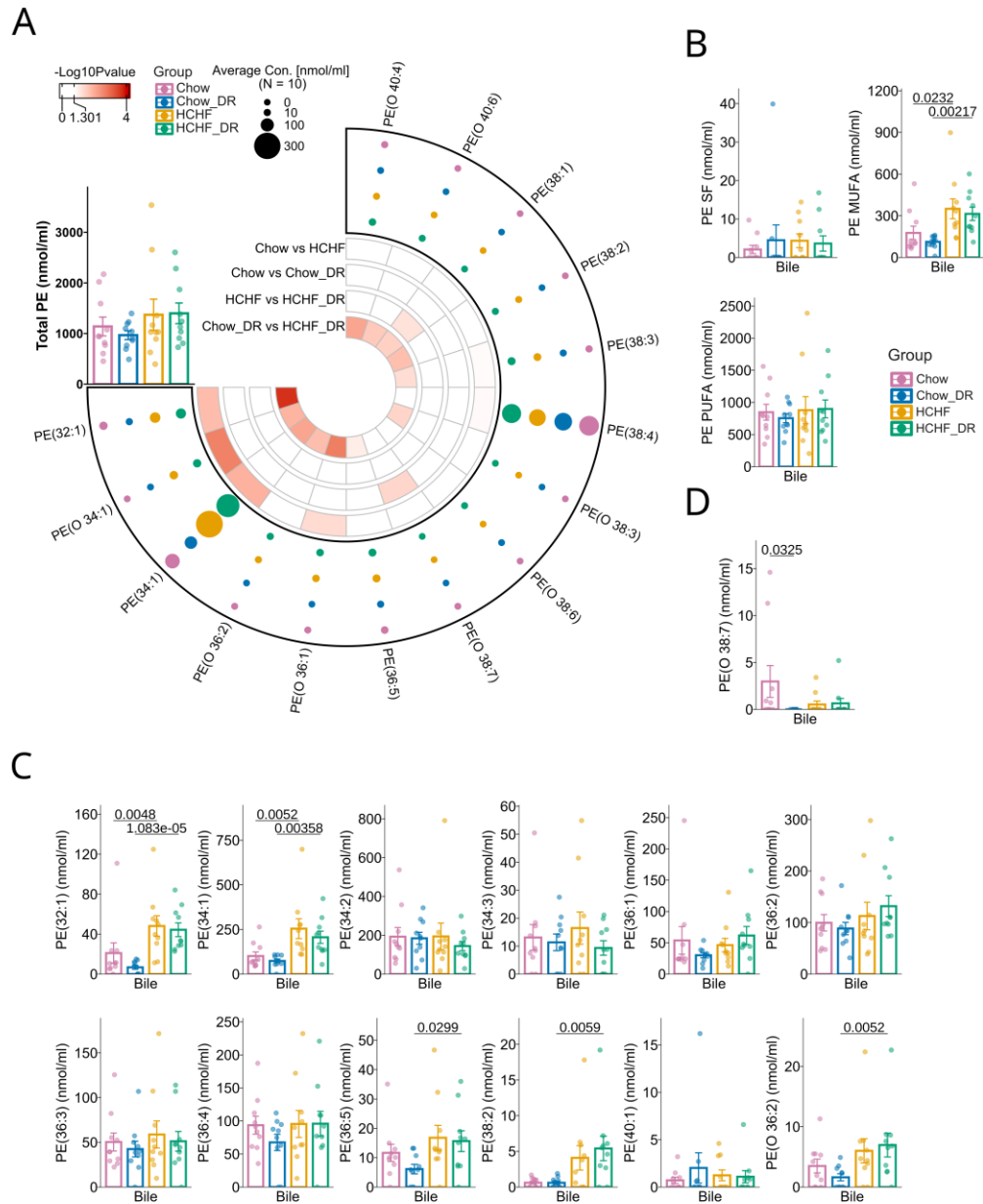
Supplemental Figure 2



Supplemental Figure 2. Absolute quantification of phosphatidylcholine (PC) species in bile.

(A) Circular bubble heatmap showing concentrations (nmol/ml) of individual PC species across dietary groups. (B) Total bile PC species containing saturated (SFA), monounsaturated (MUFA), or polyunsaturated (PUFA) fatty acids. (C) Quantification of pro-atherogenic PC species in bile. (D) Quantification of anti-atherogenic PC species in bile. Data are presented as mean $\pm$ SEM ($n = 10$). Statistical significance was determined using an unpaired two-tailed Student's *t* test or the two-sided Wilcoxon rank-sum (Mann–Whitney U) test, as appropriate. Exact *P* values are shown in the figure.

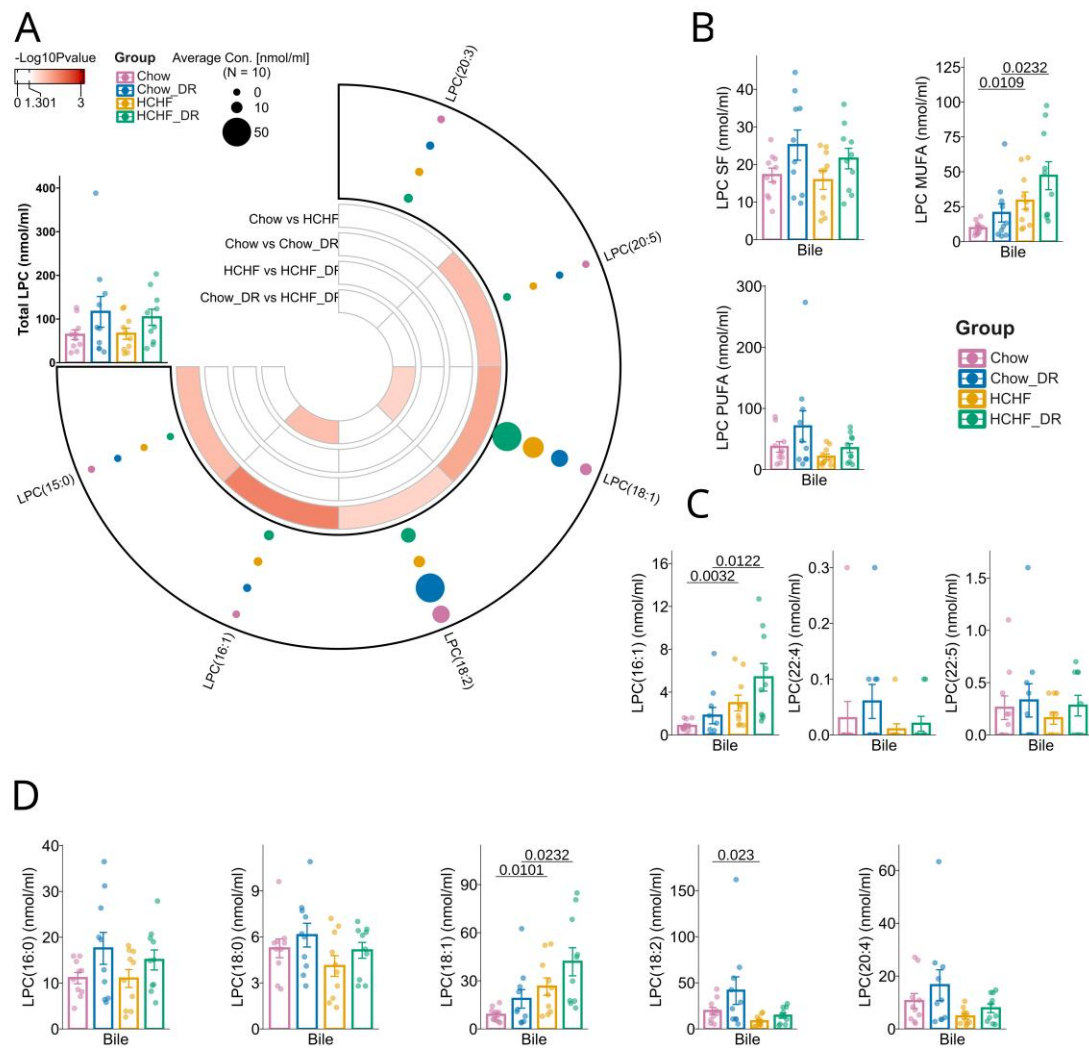
Supplemental Figure 3



Supplemental Figure 3. Quantification of phosphatidylethanolamine (PE) species in bile.

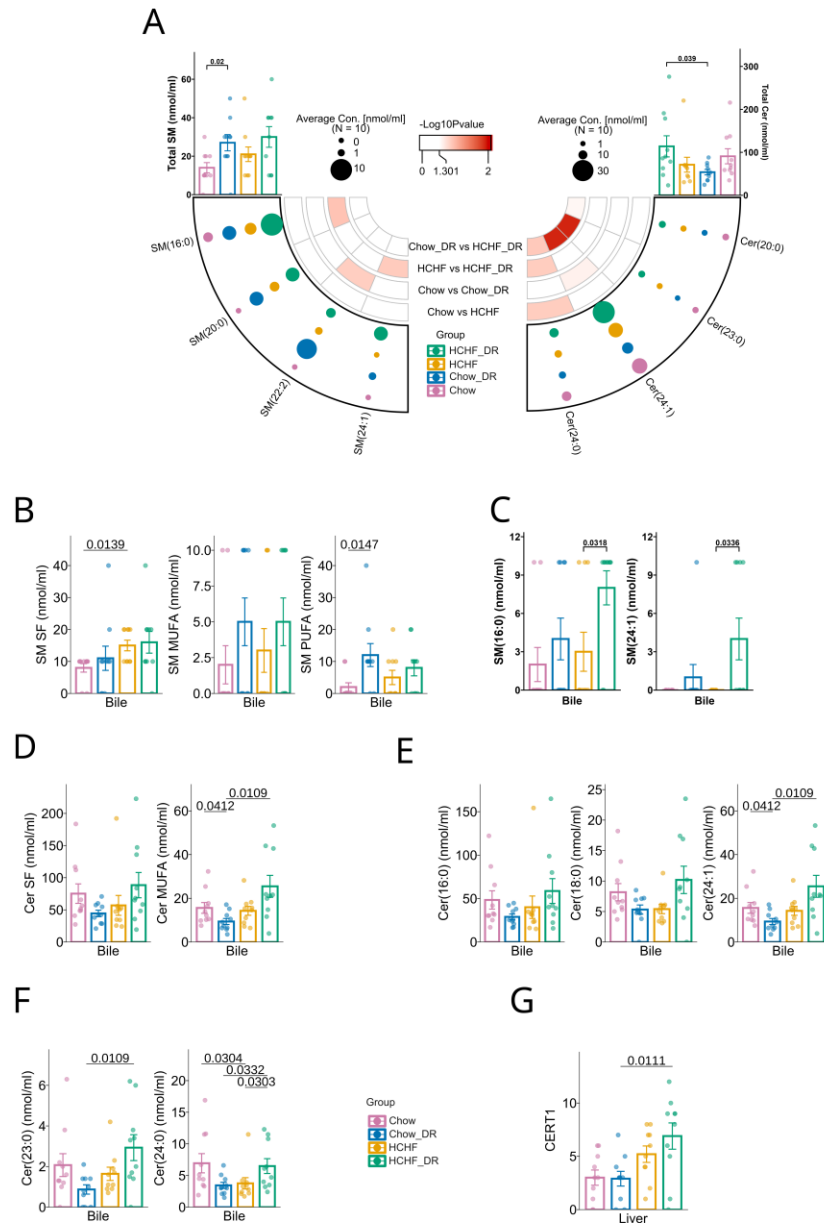
(A) Circular bubble heatmap showing concentrations (nmol/ml) of PE species in bile. (B) Total PE subclass concentrations (SFA, MUFA, PUFA). (C) Quantification of pro-atherogenic PE species in bile. (D) Quantification of anti-atherogenic PE species in bile. Data are presented as mean $\pm$ SEM ($n = 10$). Statistical significance was determined using an unpaired two-tailed Student's *t* test or the two-sided Wilcoxon rank-sum (Mann–Whitney U) test, as appropriate. Exact *P* values are shown in the figure.

Supplemental Figure 4



Supplemental Figure 4. Quantification of lysophosphatidylcholine (LPC) species in bile. (A) Circular bubble heatmap displaying concentrations (nmol/ml) of LPC species across diets. (B) Total LPC subclasses (SFA, MUFA, PUFA). (C) Quantification of pro-atherogenic LPC species in bile. (D) Quantification of anti-atherogenic LPC species in bile. Data are presented as mean $\pm$ SEM ($n = 10$). Statistical significance was determined using an unpaired two-tailed Student's *t* test or the two-sided Wilcoxon rank-sum (Mann–Whitney *U*) test, as appropriate. Exact *P* values are shown in the figure.

Supplemental Figure 5



Supplemental Figure 5. Quantification of sphingomyelin (SM), ceramide (Cer), and CERT1 in bile.

(A) Circular bubble heatmaps of SM (left panel) and Cer (right panel) species concentrations in bile; bubble size reflects average values, red arcs denote $-\log_{10}$ -transformed P values. (B) Quantification of SM subclasses and representative SM species. (C) Quantification of pro-atherogenic SM species in bile. (D) Total Cer subclasses (SFA, MUFA). (E) Quantification of pro-atherogenic Cer species in bile. (F) Quantification of anti-atherogenic Cer species in bile. (G) Ceramide risk score of CERT1 in bile. Data are presented as mean $\pm$ SEM ($n = 10$). Statistical significance was determined using an unpaired two-tailed Student's t test or the two-sided Wilcoxon rank-sum (Mann–Whitney U) test, as appropriate. Exact P values are shown in the figure.