SUPPORTING INFORMATION

Lipidomic characterization of exosomes isolated from human plasma using various mass spectrometry techniques

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Fig. S-1 Quality control for plasma, using absolute intensities for **(A)** UHPSFC/MS, **(B)** UHPLC/MS, and **(C)** MALDI-MS, visualize by Levey-Jennings graphs.

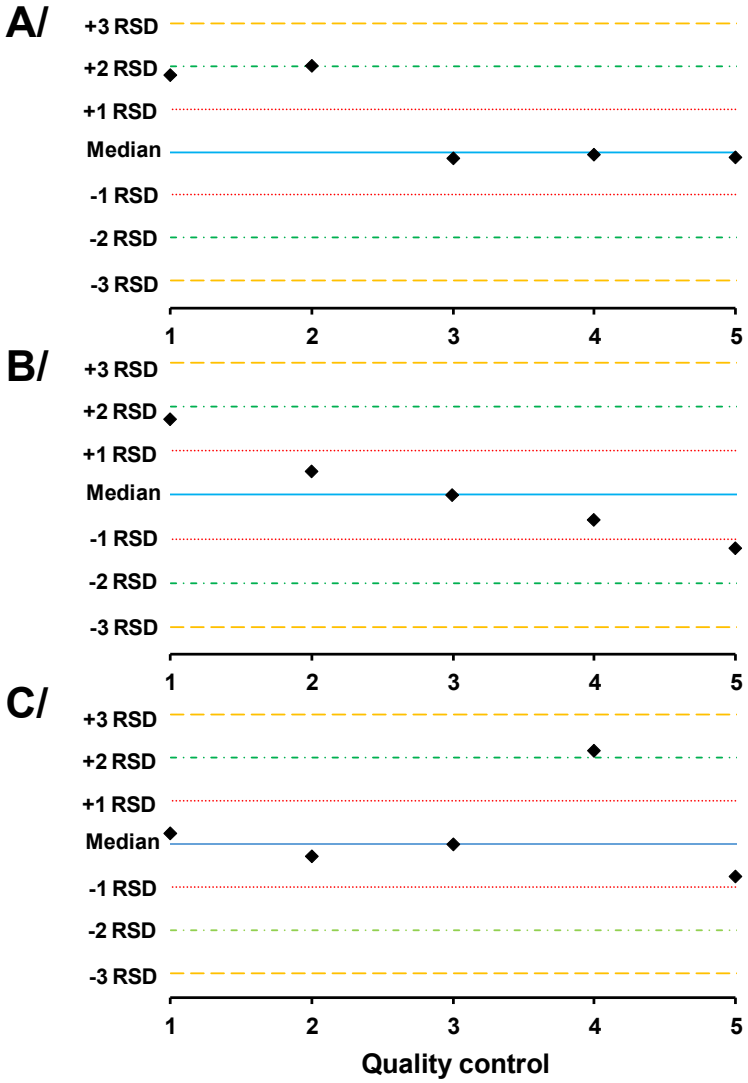
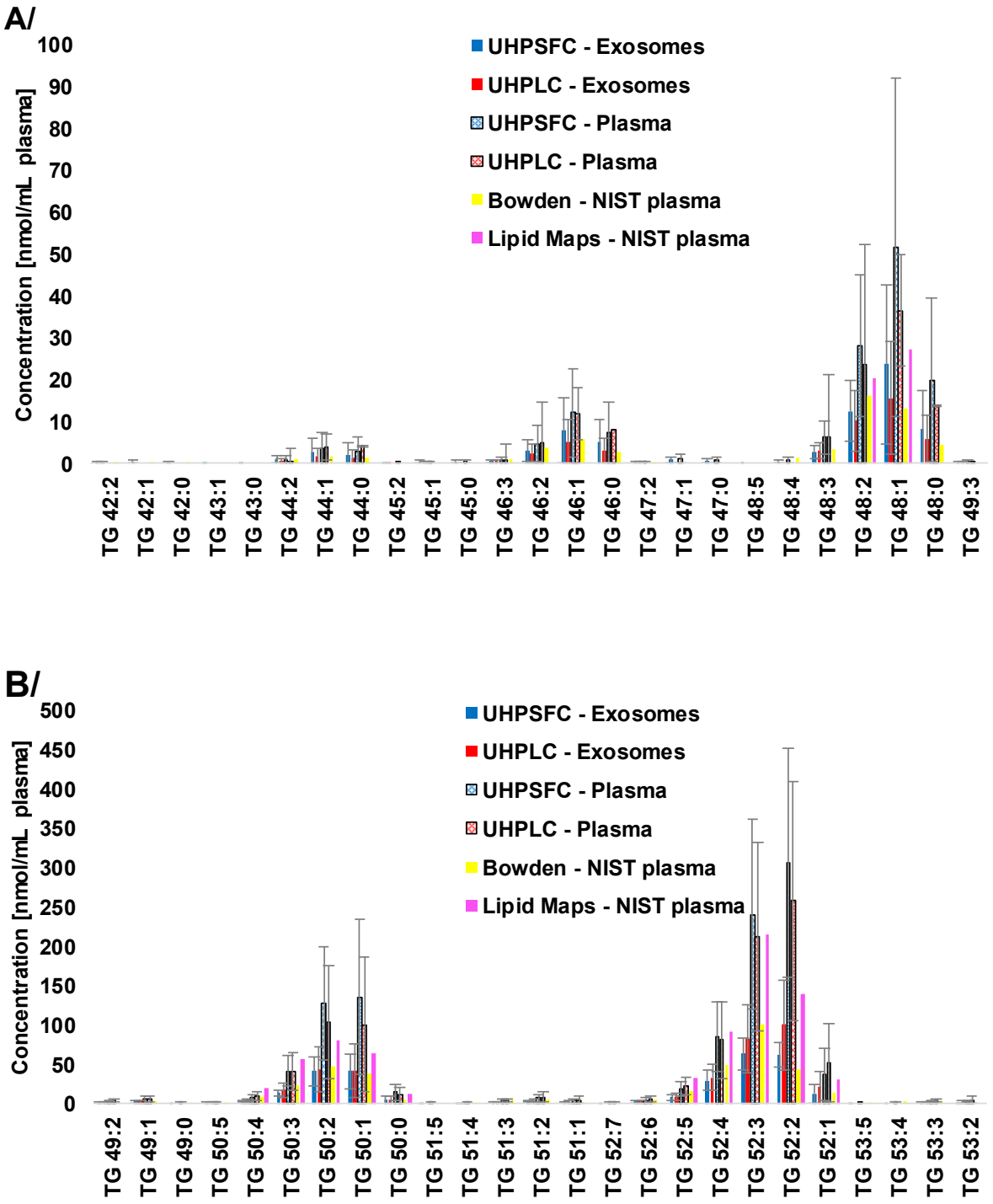
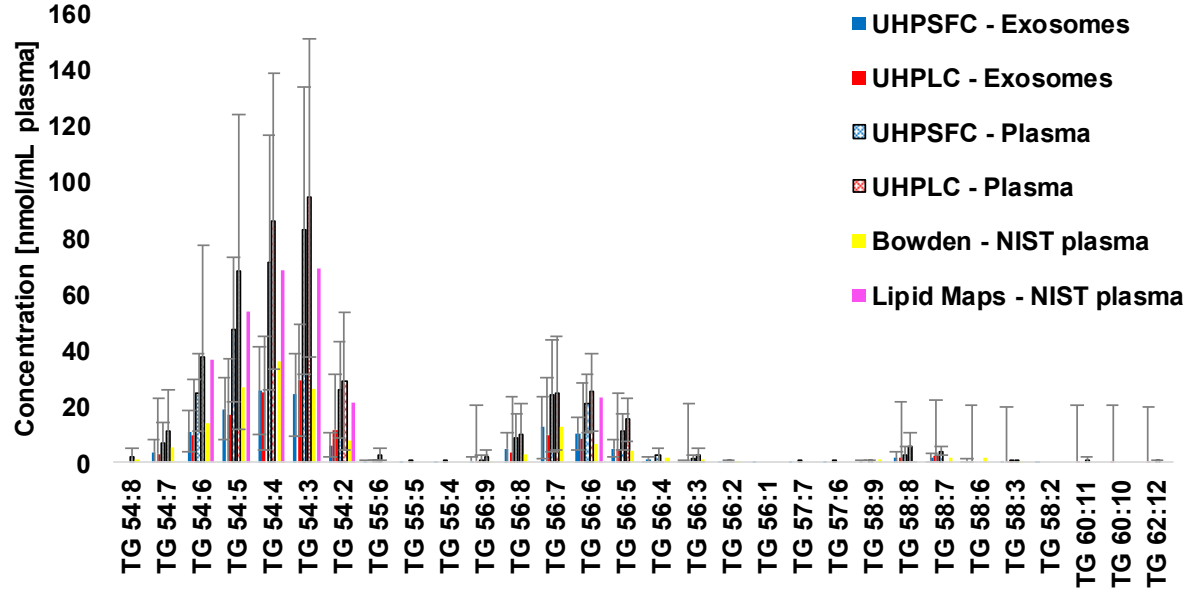


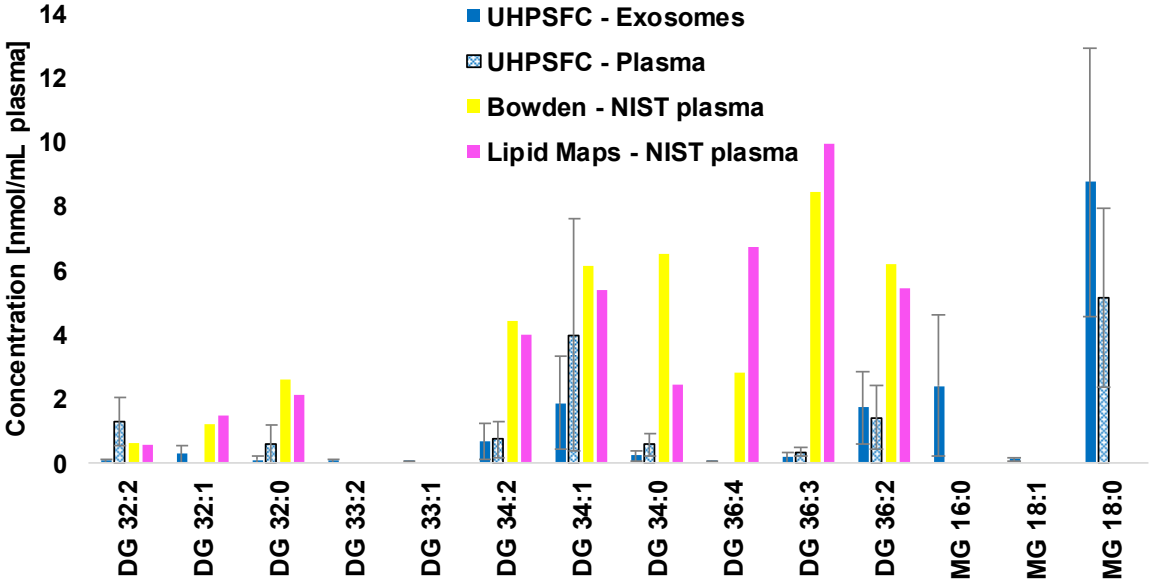
Fig. S-2 Absolute molar concentrations of lipids in exosomes and plasma measured by UHPSFC/MS, UHPLC/MS, MALDI-MS, and references data. **(A-C)** triacylglycerols, **(D)** diacylglycerols and monoacylglycerols, **(E)** ceramides, **(F)** phosphatidylethanolamines, **(G-H)** phosphatidylcholines, **(I)** sphingomyelins, **(J)** lysophosphatidylcholines, **(K)** phosphatidylinositols, and **(L)** sulfatides.



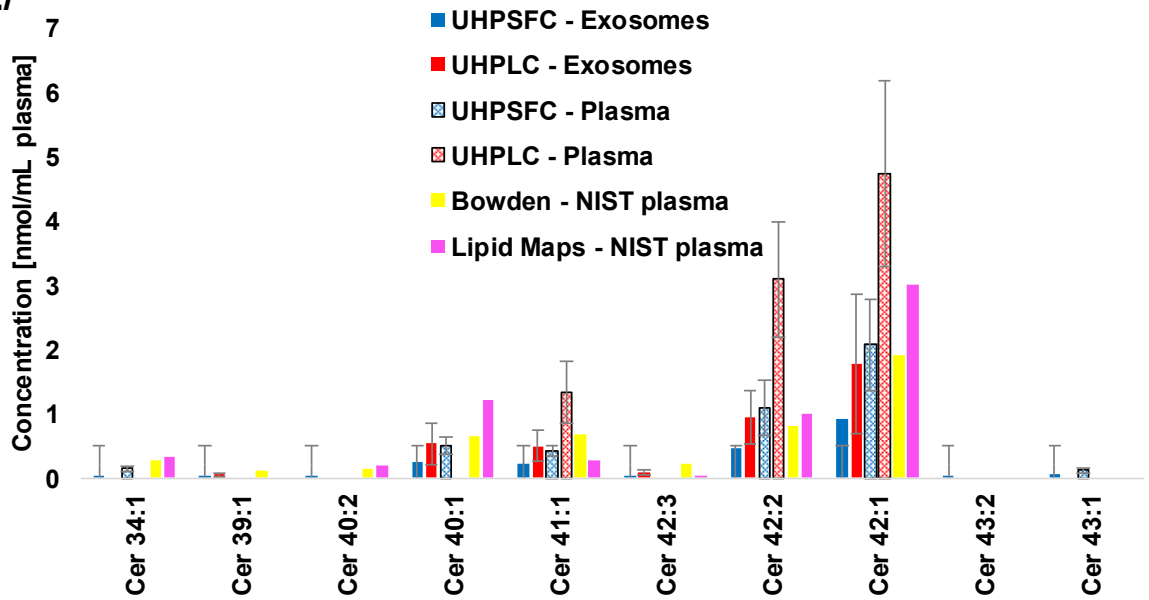
C/


Concentration [nmol/mL plasma]
160
140
120
100
80
60
40
20
0
UHPSFC - Exosomes
UHPLC - Exosomes
UHPSFC - Plasma
UHPLC - Plasma
Bowden - NIST plasma
Lipid Maps - NIST plasma
TG 54:8
TG 54:7
TG 54:6
TG 54:5
TG 54:4
TG 54:3
TG 54:2
TG 55:6
TG 55:5
TG 55:4
TG 56:9
TG 56:8
TG 56:7
TG 56:6
TG 56:5
TG 56:4
TG 56:3
TG 56:2
TG 56:1
TG 57:7
TG 57:6
TG 58:9
TG 58:8
TG 58:7
TG 58:6
TG 58:3
TG 58:2
TG 60:11
TG 60:10
TG 62:12

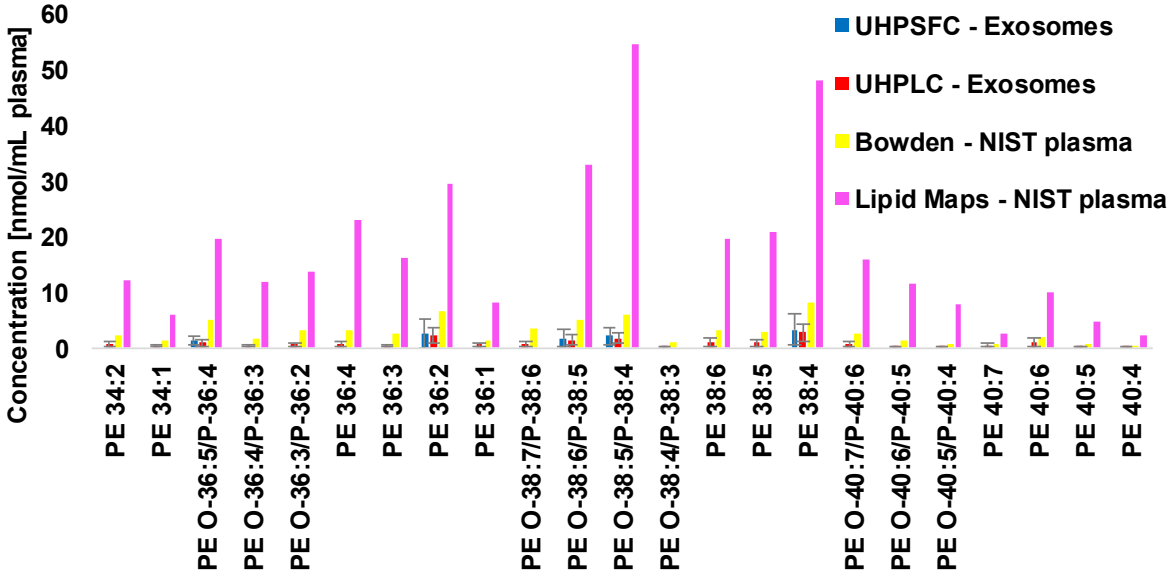
D/


Concentration [nmol/mL plasma]
14
12
10
8
6
4
2
0
UHPSFC - Exosomes
UHPSFC - Plasma
Bowden - NIST plasma
Lipid Maps - NIST plasma
DG 32:2
DG 32:1
DG 32:0
DG 33:2
DG 33:1
DG 34:2
DG 34:1
DG 34:0
DG 36:4
DG 36:3
DG 36:2
MG 16:0
MG 18:1
MG 18:0

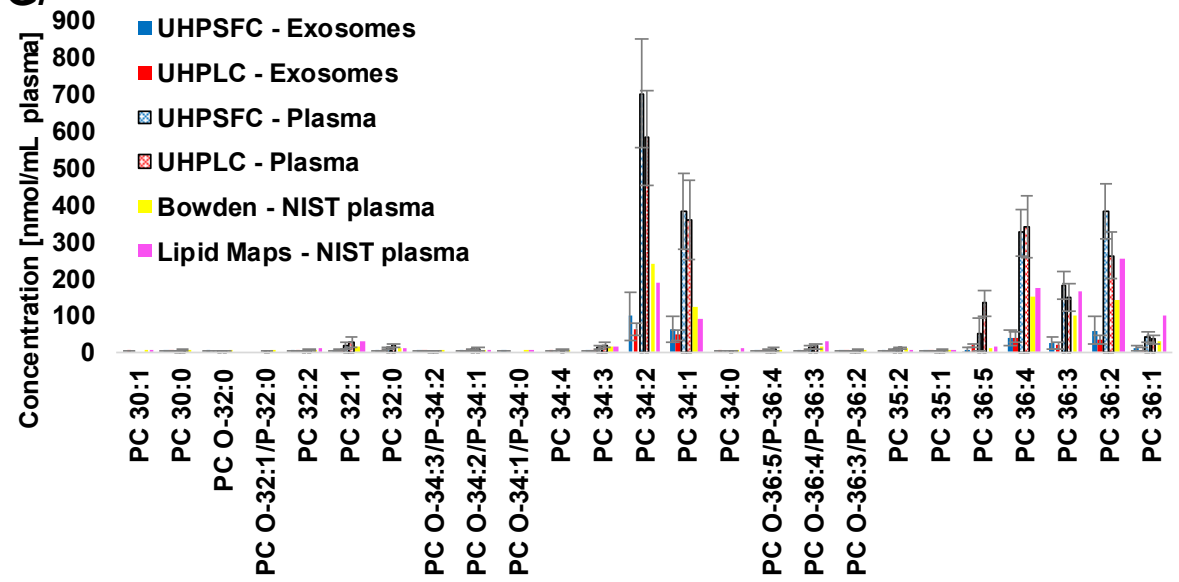
E/


UHPSFC - Exosomes
UHPLC - Exosomes
UHPSFC - Plasma
UHPLC - Plasma
Bowden - NIST plasma
Lipid Maps - NIST plasma
Concentration [nmol/mL plasma]
0
1
2
3
4
5
6
7
Cer 34:1
Cer 39:1
Cer 40:2
Cer 40:1
Cer 41:1
Cer 42:3
Cer 42:2
Cer 42:1
Cer 43:2
Cer 43:1

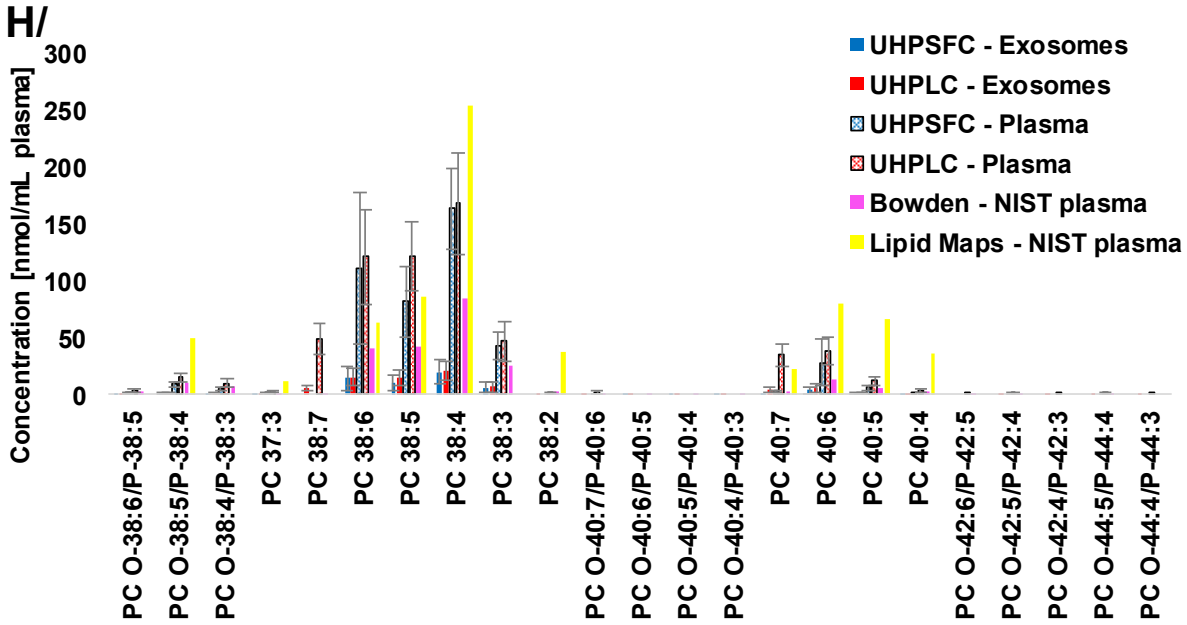
F/

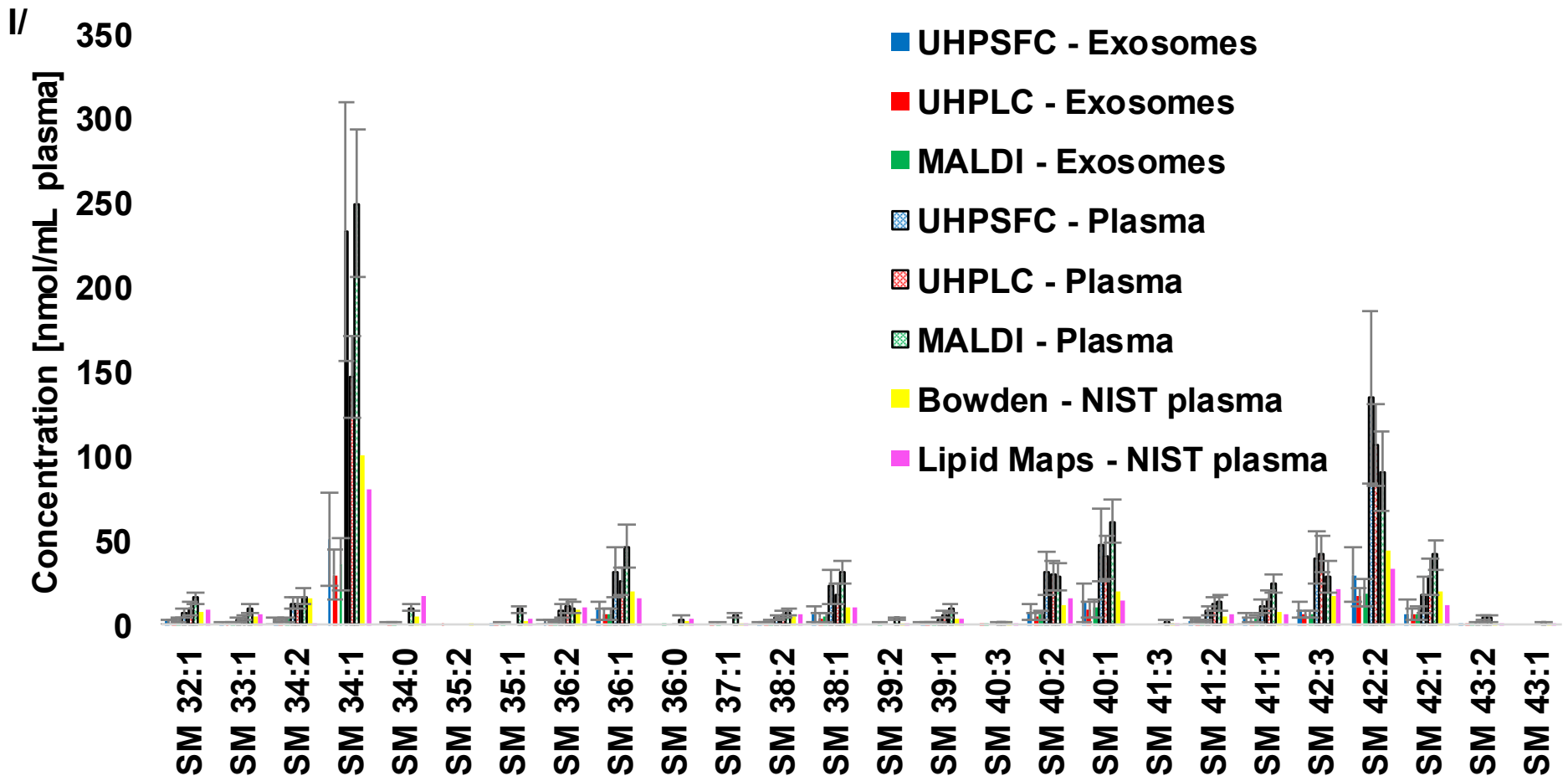

UHPSFC - Exosomes
UHPLC - Exosomes
Bowden - NIST plasma
Lipid Maps - NIST plasma
Concentration [nmol/mL plasma]
0
10
20
30
40
50
60
PE 34:2
PE 34:1
PE O-36:5/P-36:4
PE O-36:4/P-36:3
PE O-36:3/P-36:2
PE 36:4
PE 36:3
PE 36:2
PE 36:1
PE O-38:7/P-38:6
PE O-38:6/P-38:5
PE O-38:5/P-38:4
PE O-38:4/P-38:3
PE 38:6
PE 38:5
PE 38:4
PE O-40:7/P-40:6
PE O-40:6/P-40:5
PE O-40:5/P-40:4
PE 40:7
PE 40:6
PE 40:5
PE 40:4

G/

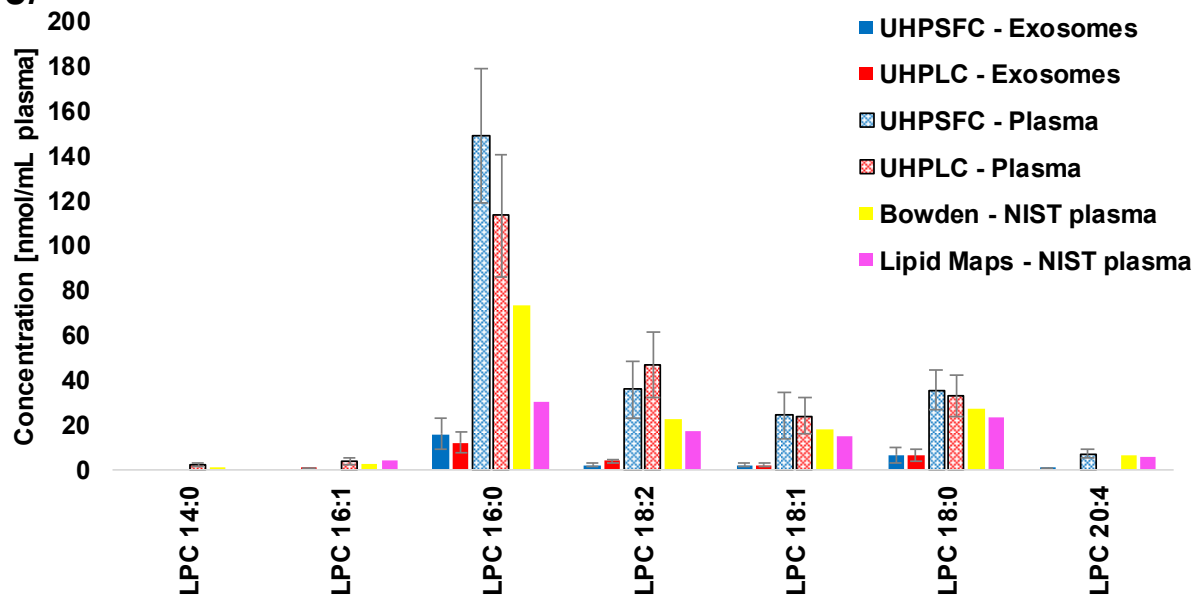

Concentration [nmol/mL plasma]
900
800
700
600
500
400
300
200
100
0
UHPSFC - Exosomes
UHPLC - Exosomes
UHPSFC - Plasma
UHPLC - Plasma
Bowden - NIST plasma
Lipid Maps - NIST plasma
PC 30:1
PC 30:0
PC O-32:0
PC O-32:1/P-32:0
PC 32:2
PC 32:1
PC 32:0
PC O-34:3/P-34:2
PC O-34:2/P-34:1
PC O-34:1/P-34:0
PC 34:4
PC 34:3
PC 34:2
PC 34:1
PC 34:0
PC O-36:5/P-36:4
PC O-36:4/P-36:3
PC O-36:3/P-36:2
PC 35:2
PC 35:1
PC 36:5
PC 36:4
PC 36:3
PC 36:2
PC 36:1

H/

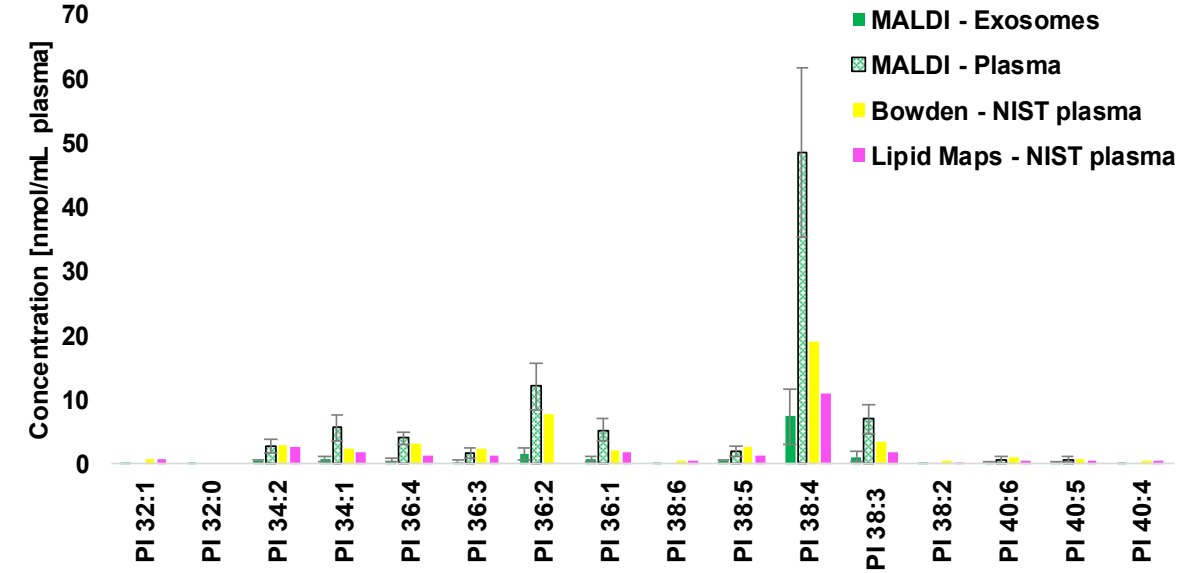

Concentration [nmol/mL plasma]
300
250
200
150
100
50
0
UHPSFC - Exosomes
UHPLC - Exosomes
UHPSFC - Plasma
UHPLC - Plasma
Bowden - NIST plasma
Lipid Maps - NIST plasma
PC O-38:6/P-38:5
PC O-38:5/P-38:4
PC O-38:4/P-38:3
PC 37:3
PC 38:7
PC 38:6
PC 38:5
PC 38:4
PC 38:3
PC 38:2
PC O-40:7/P-40:6
PC O-40:6/P-40:5
PC O-40:5/P-40:4
PC O-40:4/P-40:3
PC 40:7
PC 40:6
PC 40:5
PC 40:4
PC O-42:6/P-42:5
PC O-42:5/P-42:4
PC O-42:4/P-42:3
PC O-44:5/P-44:4
PC O-44:4/P-44:3


I/
Concentration [nmol/mL plasma]
350
300
250
200
150
100
50
0
UHPSFC - Exosomes
UHPLC - Exosomes
MALDI - Exosomes
UHPSFC - Plasma
UHPLC - Plasma
MALDI - Plasma
Bowden - NIST plasma
Lipid Maps - NIST plasma
SM 32:1
SM 33:1
SM 34:2
SM 34:1
SM 34:0
SM 35:2
SM 35:1
SM 36:2
SM 36:1
SM 36:0
SM 37:1
SM 38:2
SM 38:1
SM 39:2
SM 39:1
SM 40:3
SM 40:2
SM 40:1
SM 41:3
SM 41:2
SM 41:1
SM 42:3
SM 42:2
SM 42:1
SM 43:2
SM 43:1

J/


Concentration [nmol/mL plasma]
200
180
160
140
120
100
80
60
40
20
0
UHPSFC - Exosomes
UHPLC - Exosomes
UHPSFC - Plasma
UHPLC - Plasma
Bowden - NIST plasma
Lipid Maps - NIST plasma
LPC 14:0
LPC 16:1
LPC 16:0
LPC 18:2
LPC 18:1
LPC 18:0
LPC 20:4

K/


Concentration [nmol/mL plasma]
70
60
50
40
30
20
10
0
MALDI - Exosomes
MALDI - Plasma
Bowden - NIST plasma
Lipid Maps - NIST plasma
PI 32:1
PI 32:0
PI 34:2
PI 34:1
PI 36:4
PI 36:3
PI 36:2
PI 36:1
PI 38:6
PI 38:5
PI 38:4
PI 38:3
PI 38:2
PI 40:6
PI 40:5
PI 40:4

L/

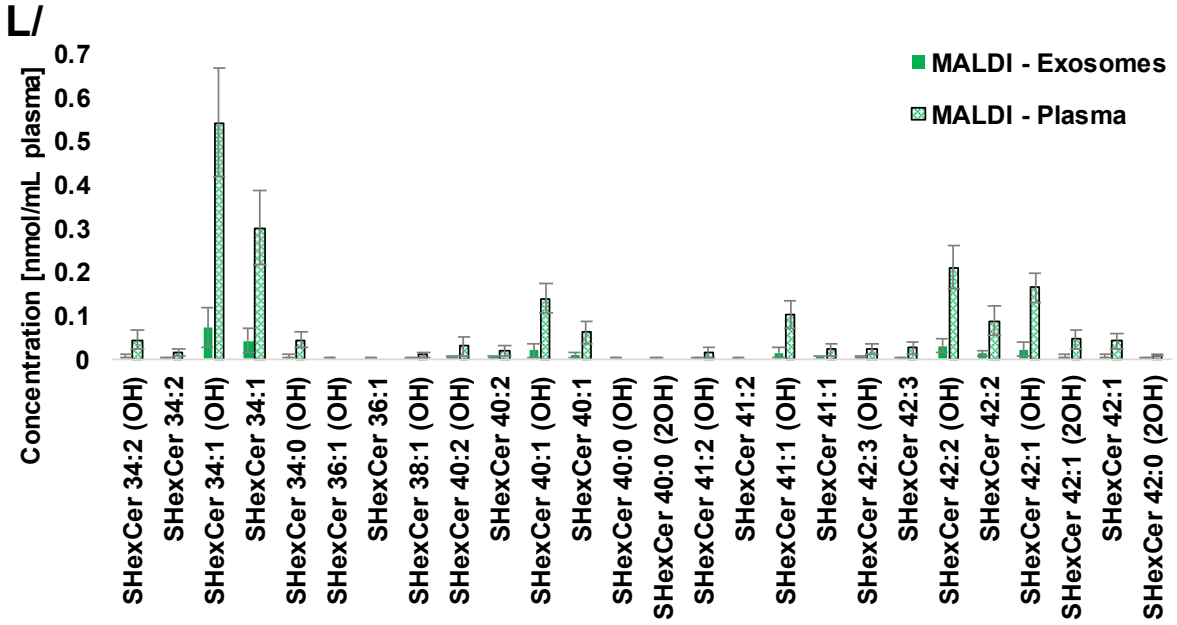

Concentration [nmol/mL plasma]
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
MALDI - Exosomes
MALDI - Plasma
SHexCer 34:2 (OH)
SHexCer 34:2
SHexCer 34:1 (OH)
SHexCer 34:1
SHexCer 34:0 (OH)
SHexCer 36:1 (OH)
SHexCer 36:1
SHexCer 38:1 (OH)
SHexCer 40:2 (OH)
SHexCer 40:2
SHexCer 40:1 (OH)
SHexCer 40:1
SHexCer 40:0 (OH)
SHexCer 40:0 (2OH)
SHexCer 41:2 (OH)
SHexCer 41:2
SHexCer 41:1 (OH)
SHexCer 41:1
SHexCer 42:3 (OH)
SHexCer 42:3
SHexCer 42:2 (OH)
SHexCer 42:2
SHexCer 42:1 (OH)
SHexCer 42:1 (2OH)
SHexCer 42:1
SHexCer 42:0 (2OH)

Fig. S-3 Correlation of sphingomyelins between methods for exosomes (**A – C**) and plasma (**D – F**). Graphs **G and H** show correlation between UHPSFC/MS and UHPLC/MS for all lipid classes. This comparison shows only lipids detected by both methods.

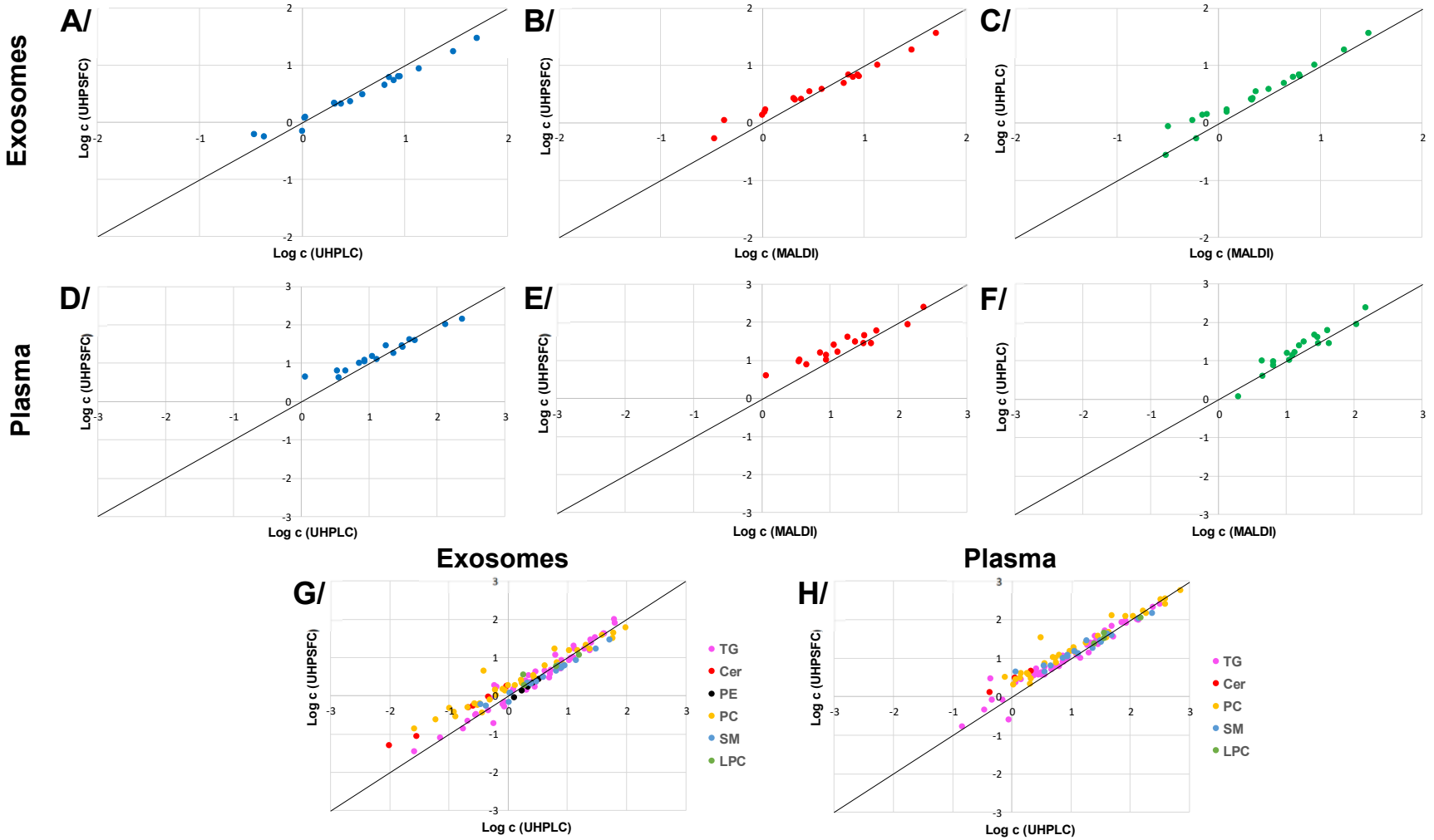


Fig. S-4 Multivariate data analysis of normalized data (pareto scaling and logarithmic transformation) for exosomes (blue), plasma (red), quality control for exosomes (yellow), and quality control for plasma (green) measured by UHPSFC/MS. Unsupervised PCA score plot of **(A)** absolute molar concentrations (nmol/mL plasma) and **(B)** relative concentrations (% lipid abundances within the class).

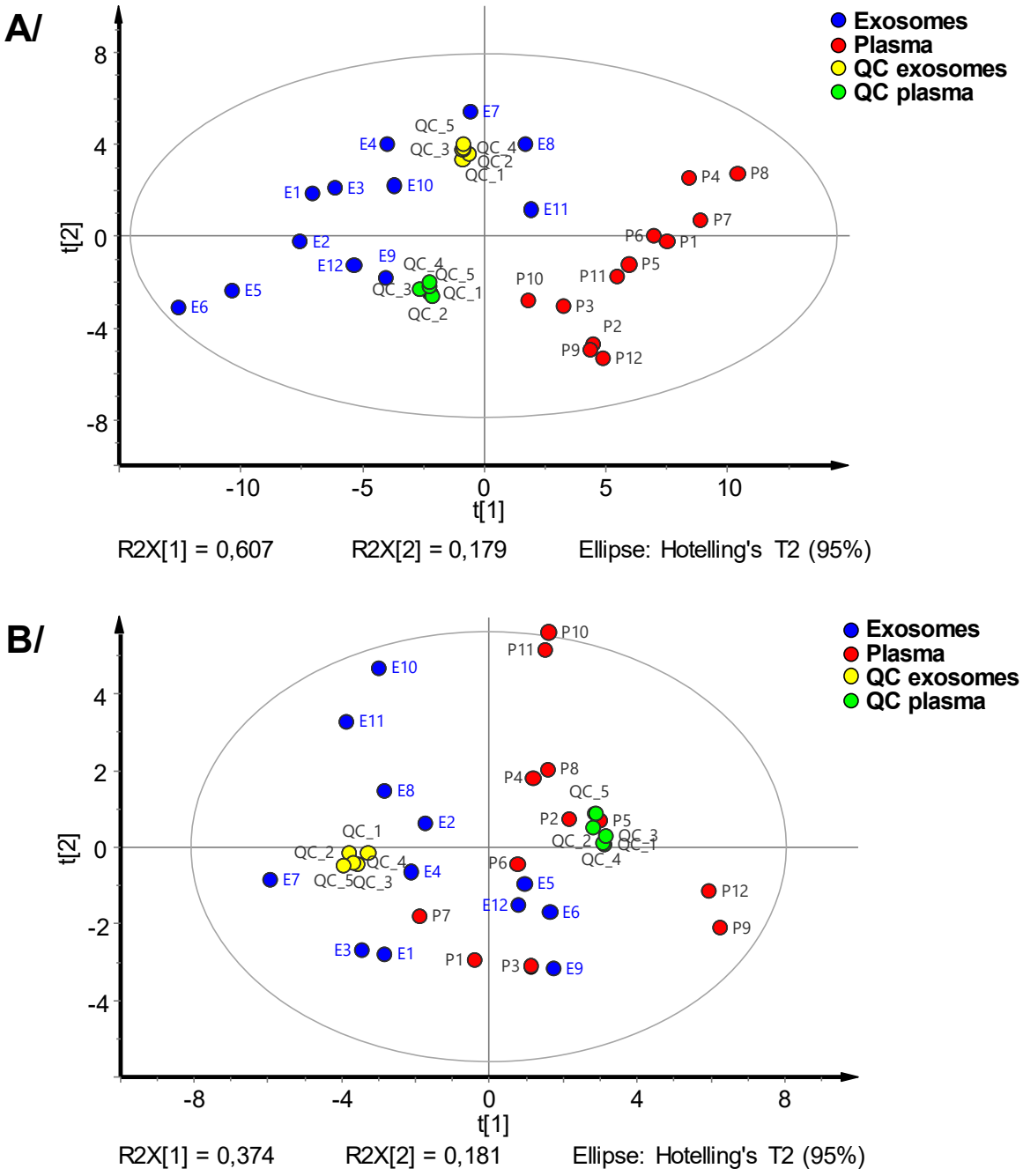


Fig. S-5 S-plot of relative molar abundances with the annotation of most up- and down-regulated lipids generated from supervised OPLS-DA model. **(A)** UHPSFC/MS, **(B)** UHPLC/MS, and **(C)** MALDI-MS.

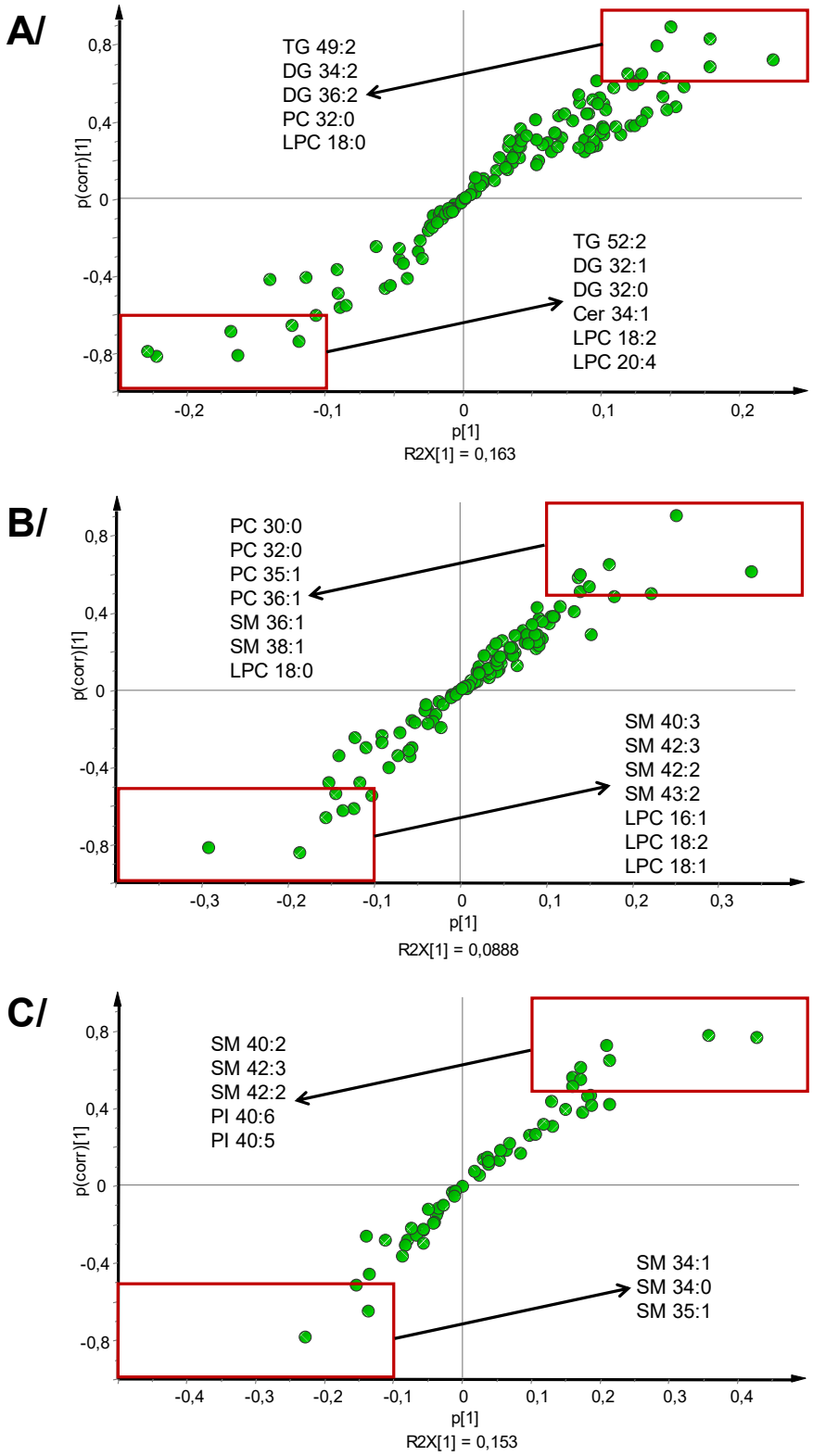


Fig. S-6 Comparison of particular changes of acylglycerols and membrane lipids in **(A - C)** exosomes and **(D - F)** human plasma by UHPSFC/MS and UHPLC/MS techniques. Relative concentration of **(A, D)** TG, DG, and MG related to the total concentration of acylglycerols, **(B, C, E, and F)** Cer, PC, SM, and LPC related to the total concentration of sphingomyelins and phospholipids. This comparison shows only lipids detected in both sample types.

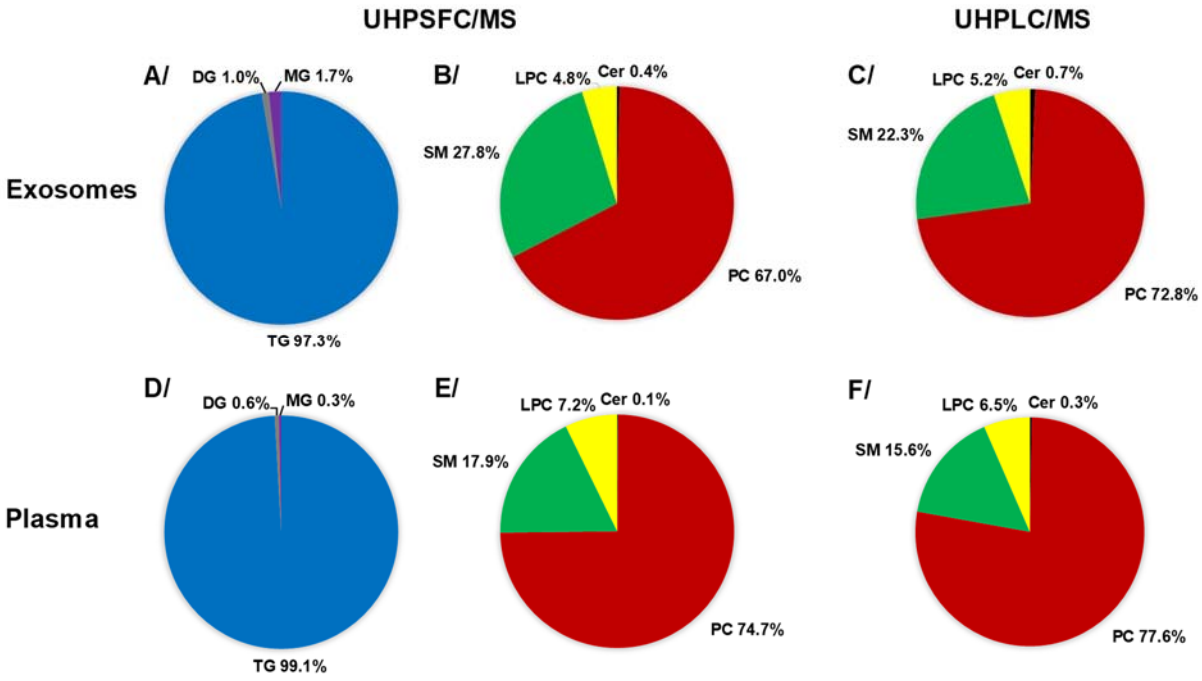


Table S- 1 Concentration of internal standards for exosomes and plasma in IS Mix.

Internal standard	Stock solution [µg/µL]	Concentration for exosomes [nmol/mL plasma]	Concentration for plasma [nmol/mL plasma]
TG 19:1/19:1/19:1	2	5.52	113.29
DG 12:1/12:1	2	2.26	46.42
MG 19:1/0:0/0:0	2	5.53	113.42
D7-CE 16:0	2	10.80	443.30
D7-Chol	2	52.00	1066.81
D7-PI 18:1/15:0	1	1.32	13.23
LPC 17:0/0:0	2.1	2.81	57.72
LPE 14:0/0:0	2	0.64	13.17
PC 14:0/14:0	2	12.09	123.99
PE 14:0/14:0	2	1.07	8.81
PG 14:0/14:0	2	0.10	4.07
PS 14:0/14:0	2	0.80	7.98
Cer 18:1/12:0	2	1.70	11.63
SM 18:1/12:0	2	4.22	43.31
SHexCer 18:1/12:0	0.25	0.01	0.07

Table S-2 Molar concentrations of individual lipids in exosomes and plasma measured by individual methods (table is uploaded separately as Excel sheet).

Table S-3 Basic information on individual subjects of healthy volunteers.

Sample	Gender	Age	BMI[1]
1	Male	45	28.81
2	Male	62	22.75
3	Male	44	26.47
4	Male	46	24.96
5	Male	50	21.30
6	Male	44	23.77
7	Male	51	29.91
8	Male	52	28.31
9	Male	53	29.40
10	Male	57	28.39
11	Male	51	25.34
12	Male	45	26.30
Median[2]		50.5 ± 5.3	26.4 ± 2.6

[1]Body max index

[2]Median ± standard deviation

Table S-4 Number of quantified lipids and agreement between exosomes and plasma by UHPSFC/MS, UHPLC/MS, and MALDI-MS.

Lipid class	Exosomes			Plasma			Present in both materials		
	UHPSFC	UHPLC	MALDI	UHPSFC	UHPLC	MALDI	UHPSFC	UHPLC	MALDI
TG	76	55	-	66	50	-	66	47	-
DG	11	-	-	7	-	-	7	-	-
MG	3	-	-	1	-	-	1	-	-
Cer	9	6	-	6	3	-	6	3	-
PE	5	23	-	0	0	-	0	0	-
PC	36	43	-	32	40	-	32	40	-
SM	18	22	23	17	18	25	17	18	23
LPC	5	5	-	5	6	-	5	5	-
PI	-	-	16	-	-	11	-	-	11
SHexCer	-	-	26	-	-	21	-	-	21
Sum	163	154	65	134	117	57	134	113	55

Table S-5 Number of lipids quantified by two or three methods.

Lipid class	UHPSFC and UHPLC		UHPSFC, UHPLC, and MALDI	
	Exosomes	Plasma	Exosomes	Plasma
TG	52	45	-	-
DG	-	-	-	-
MG	-	-	-	-
Cer	6	3	-	-
PE	5	0	-	-
PC	34	32	-	-
SM	18	17	18	17
LPC	4	4	-	-
PI	-	-	-	-
SHexCer	-	-	-	-
Sum	119	101	18	17