Supplementary Materials

for the manuscript by S.Sales, J.Graessler, S.Ciucci, R.Al-Atrib, T.Vihervaara, K.Schuhmann, D.Kauhanen, M.Sysi-Aho, S.R.Bornstein, M.Bickle, C.V.Cannistraci, K.Ekroos and A.Shevchenko ”Gender, Contraceptives and Individual Metabolic Predisposition Shape a Healthy Plasma Lipidome”

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Supplementary Tables S2, S3 and **S4** are Excel spreadsheets and uploaded as separate files.

Supplementary Table 1. Basal characteristics of the study cohort (extended table).

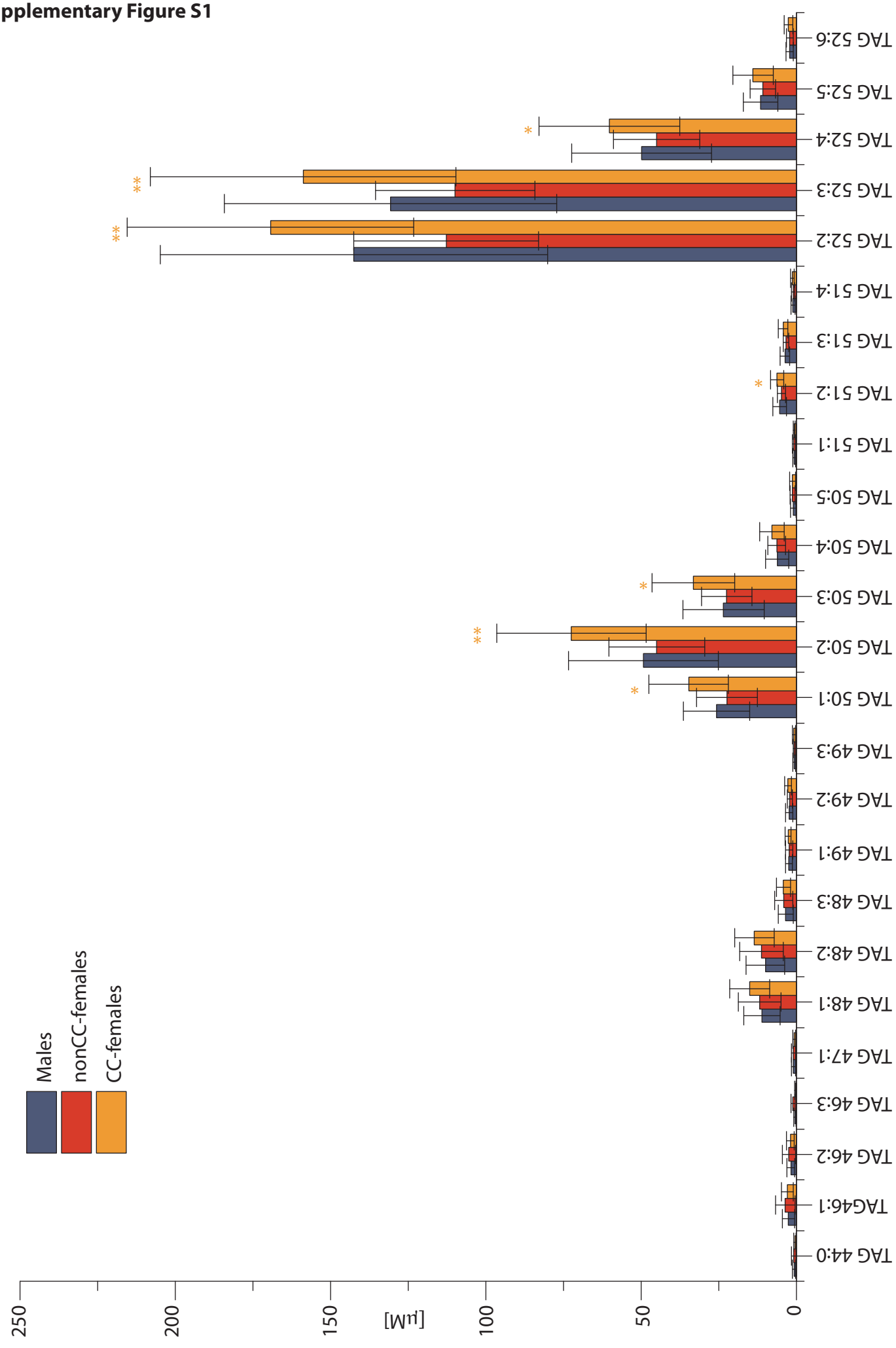
		Men (*n*=36)			Women (*n*=35)			
Parameter	**units**	**Mean**	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**Mann-WhitneyTest Benjamini corrected (*p*)**
Age	years	24.0	2.7	23.1-24.9	25.5	2.8	24.5-26.4	0.0244
Weight	kg	77.2	8.8	74.2-80.1	61.6	7.9	58.9-64.3	3.418E-08
Height	m	1.82	0.06	1.80-1.84	1.70	0.06	1.68-1.72	3.756E-08
BMI	Kg/m^2	23.4	2.4	22.6-24.2	21.3	2.2	20.6-22.0	0.001
Waist	cm	80.6	8.2	77.8-83.3	69.3	6.6	67.0-71.6	4.569E-08
Hip	cm	101.8	6.4	99.6-103.9	100.2	6.1	98.0-102.3	0.1374
Waist-to-hip ratio		0.79	0.05	0.77-0.81	0.69	0.06	0.67-0.71	5.021E-09
Systolic blood pressure	mmHg	122.5	9.4	119.3-125.7	113.4	8.7	110.4-116.4	0.0003
Diastolic blood pressure	mmHg	69.9	7.2	67.4-72.3	65.4	6.1	63.3-67.5	0.009
Triglycerides	mmol/L	0.78	0.27	0.69-0.87	0.83	0.26	0.74-0.92	0.205
Total cholesterol	mmol/L	4.23	0.55	4.04-4.41	4.57	0.47	4.41-4.73	0.205
LDL-cholesterol	mmol/L	2.44	0.54	2.25-2.62	2.40	0.49	2.23-2.57	0.377
HDL-cholesterol	mmol/L	1.55	0.31	1.45-1.66	1.93	0.31	1.83-2.04	2.262E-05
Hemoglobin	mmol/L	9.47	0.60	9.27-9.68	8.34	0.51	8.16-8.51	7.106E-09
Hematocrit		0.44	0.02	0.43-0.45	0.39	0.02	0.39-0.40	2.9E-09
Leukocytes	GPt/L	5.50	1.03	5.15-5.84	5.57	1.20	5.16-5.98	0.384
Platelets	GPt/L	216.4	45.1	201.2-231.7	245.8	49.5	228.8-262.8	0.009
Erythrocytes	TPt/L	5.05	0.25	4.97-5.14	4.49	0.26	4.40-4.58	7.859E-09
Creatinine	µmol/L	84.1	10.4	80.6-87.7	71.7	6.9	69.3-74.0	1.37E-06
Alanine aminotransaminase	µmol/L*s	0.37	0.14	0.32-0.41	0.25	0.10	0.22-0.29	0.0002
Gamma-glutamyltransferase	µmol/L*s	0.30	0.11	0.26-0.34	0.27	0.13	0.22-0.31	0.029
Uric acid	µmol/L	334.5	59.4	314.4-354.6	233.8	36.7	221.2-246.4	2.9E-09
C-reactive protein	mg/L	0.65	0.75	0.39-0.90	1.33	1.49	0.81-1.84	0.055
Hemoglobin A1c	%	5.17	0.31	5.07-5.28	5.21	0.21	5.14-5.28	0.328
Plasma glucose	mmol/L	4.54	0.36	4.42-4.66	4.49	0.30	4.39-4.60	0.282
Free fatty acids	mmol/L	0.31	0.16	0.26-0.37	0.37	0.19	0.30-0.43	0.137
Proinsulin	pmol/L	2.10	1.54	1.58-2.62	2.15	1.84	1.51-2.78	0.386
C-peptide	pmol/L	548.9	216.3	475.7-622.1	597.7	190.9	532.2-663.3	0.116
Insulin	pmol/L	48.3	26.6	39.3-57.3	58.3	40.8	44.3-72.3	0.115
Thyroid-stimulating-hormone	mU/L	2.84	1.81	2.22-3.45	2.60	1.15	2.21-2.99	0.382
Luteinizing hormone	U/L	3.37	1.12	2.99-3.75	3.16	2.77	2.21-4.11	0.054
Follicle-stimulating hormone	U/L	2.76	1.62	2.21-3.31	3.83	3.11	2.76-4.90	0.12
Cortisol	nmol/L	433.5	116.7	394.0-472.9	446.0	156.6	392.2-499.8	0.367
Dehydroepiandrosterone	nmol/L	26.5	10.2	23.0-29.9	24.5	12.3	20.3-28.8	0.176
Sex hormone-binding globulin	nmol/L	22.4	7.4	19.9-24.9	94.0	66.8	71.1-117.0	5.021E-09

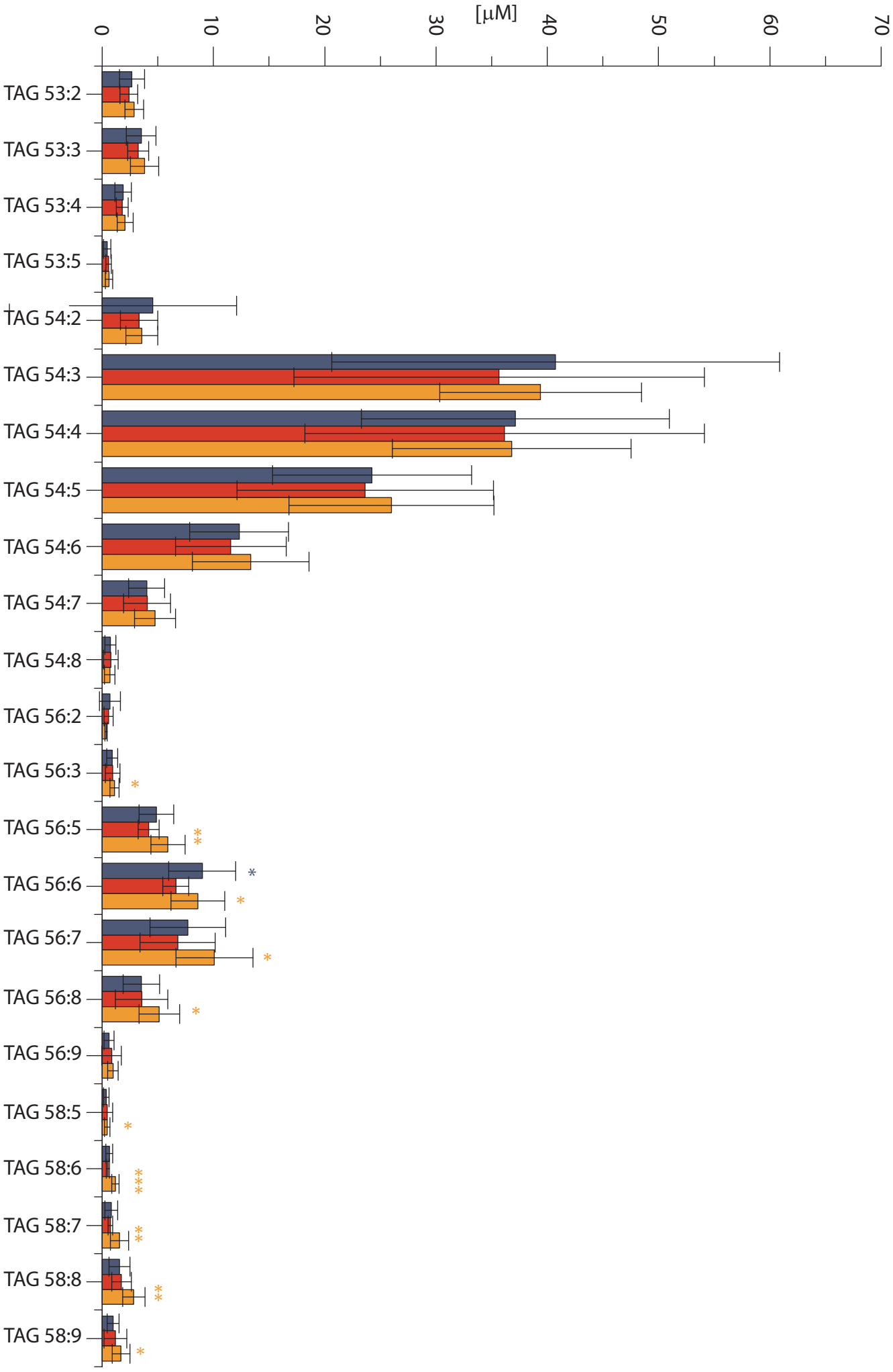
Supplementary Table S5.
Exclusion criteria and gender-specific causes for exclusion from the study

Parameter	**units**	**Cut-off values**	**Excluded Individuals (n)**	
			Males (12)	***Females (20)***
Blood pressure (systolic/diastolic)	mmHg	> 140 / 85		
ALAT	µmol/L*s	> 0.80		
γGT	µmol/L*s	> 0.80		
Serum creatinine	µmol/L	> 110 men / > 95 women		
C-reactive protein	mg/L	> 5	**3**	**2**
Hemoglobin	mmol/L	< 8.0 men / < 7.4 women		
HbA_{1C}	%	> 5.7		
Total cholesterol[1]	mmol/L	> 5.2	**5**	**14**
LDL-cholesterol[1]	mmol/L	> 4.0		
HDL-cholesterol[1]	mmol/L	< 0.9 men / < 1.1women		
Triglycerides[1]	mmol/L	> 1.7	**2**	
Plasma glucose[1]	mmol/L	> 5.5		
Plasma insulin[1]	pmol/L	> 250		**1**
Uric acid[1]	µmol/L*s	> 490	**1**	

[1] values after 12h overnight fasting; ALAT: alanine aminotransferase, γGT: gamma glutamyltransferase, HbA_{1C}: glycosylated hemoglobin
One male individual was excluded due to missing data; two females were excluded due to taking iron preparations; one due to lactation

Supplementary Figure S1

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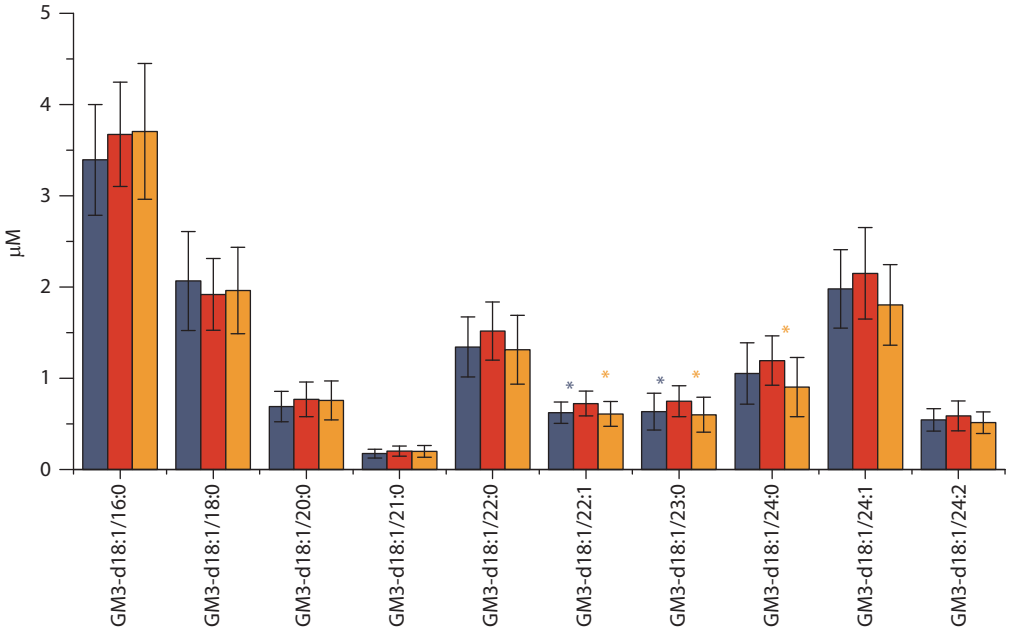
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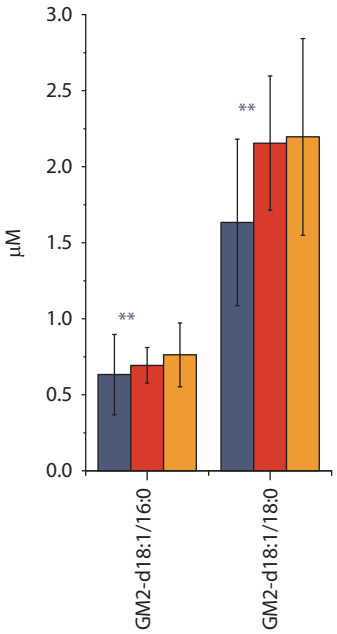
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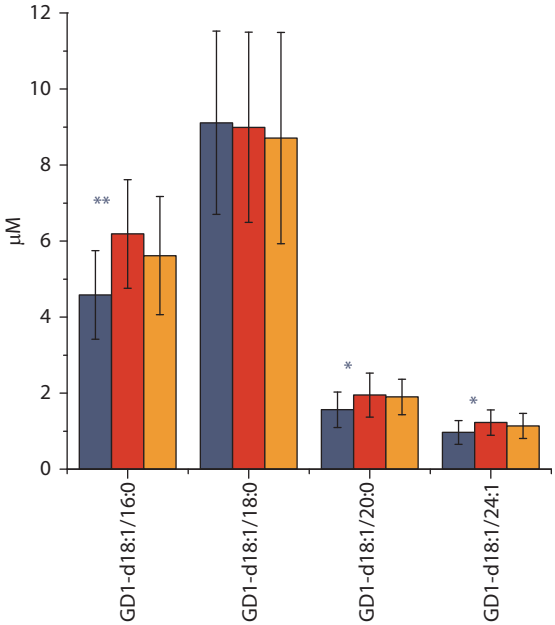
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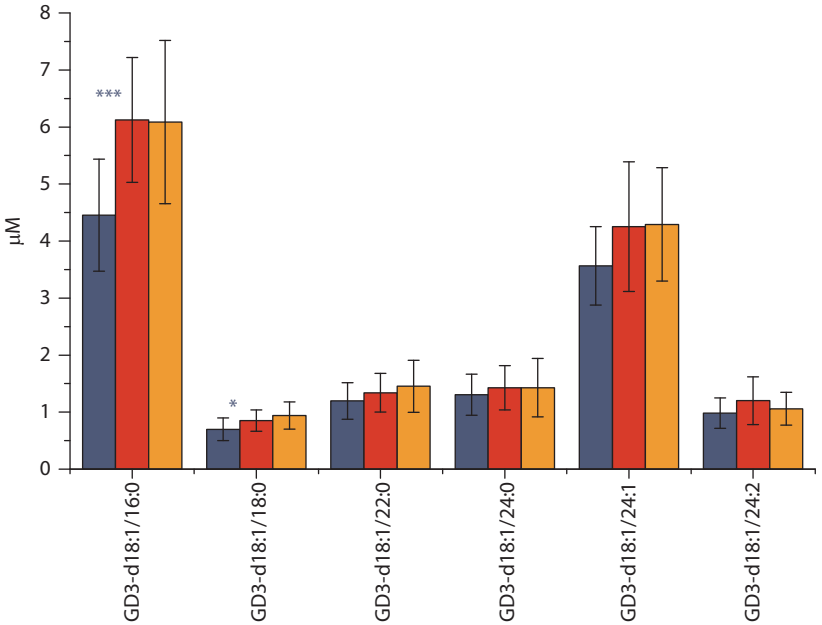
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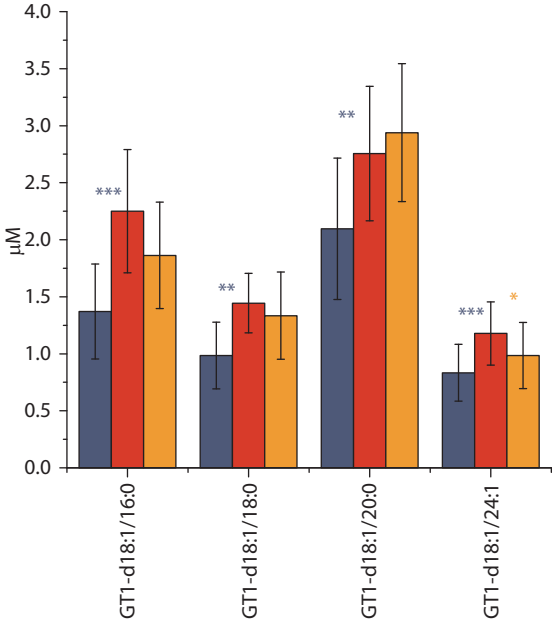
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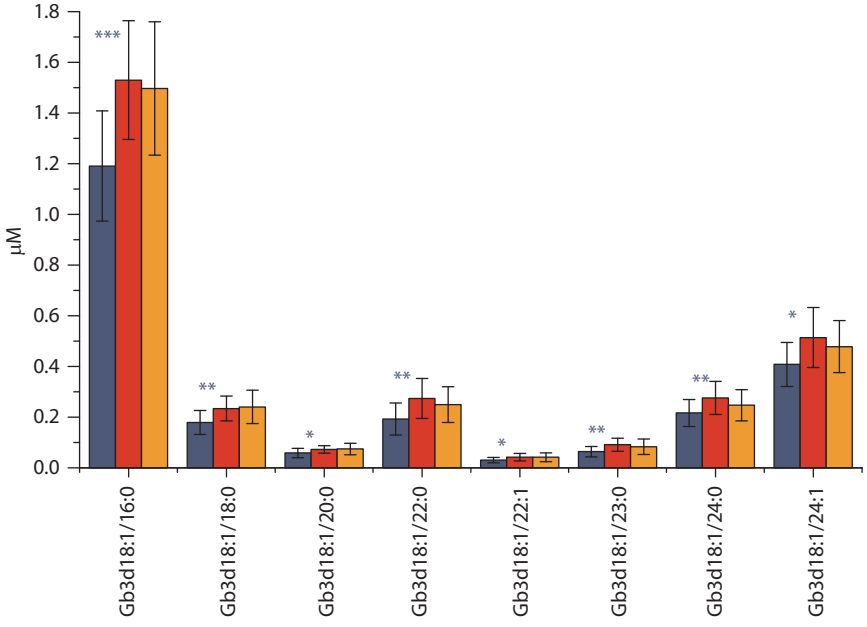
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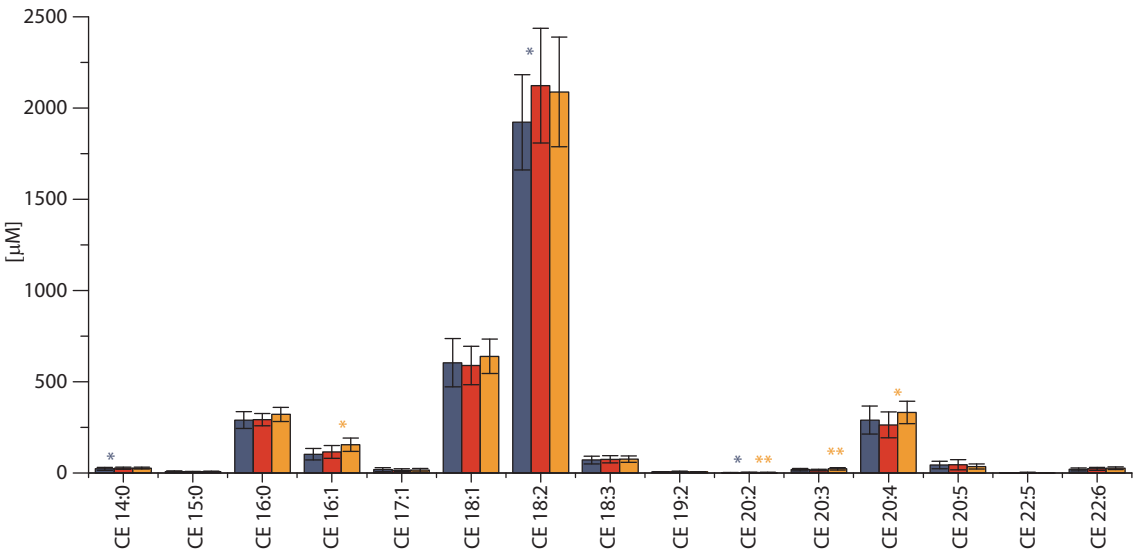
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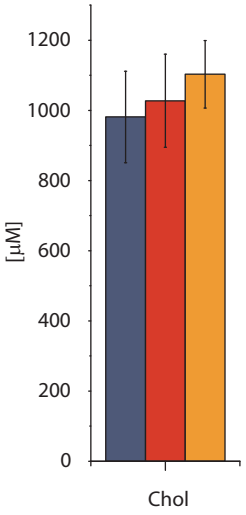
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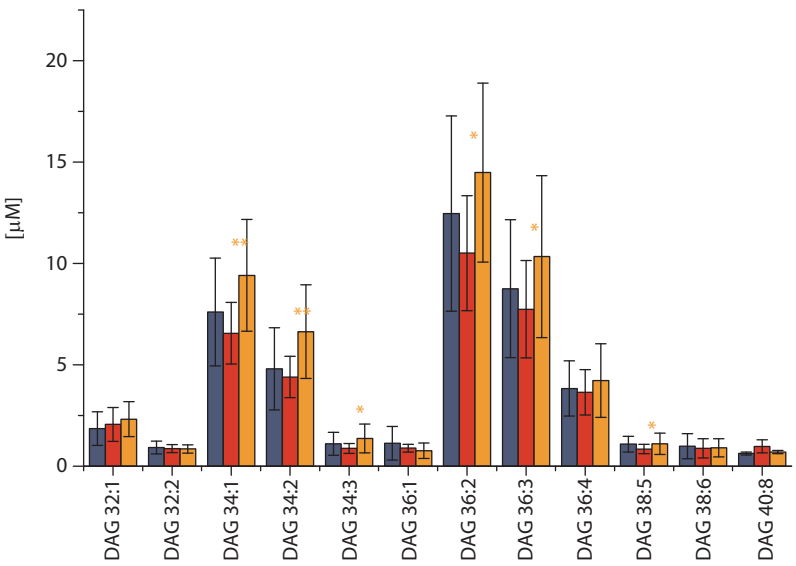
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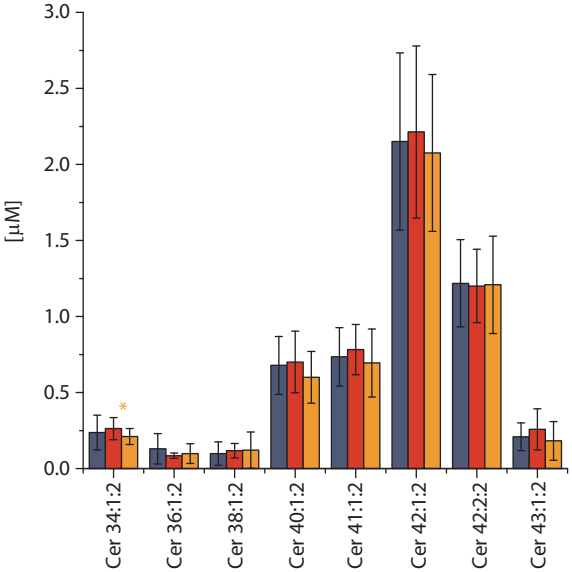
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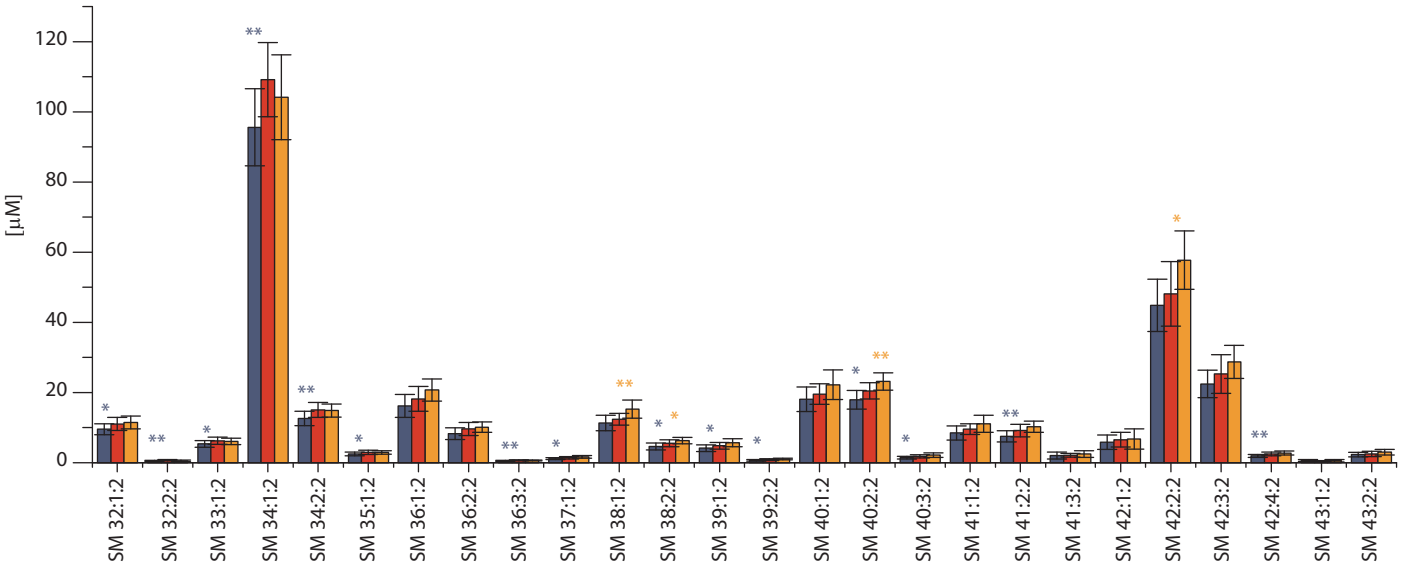
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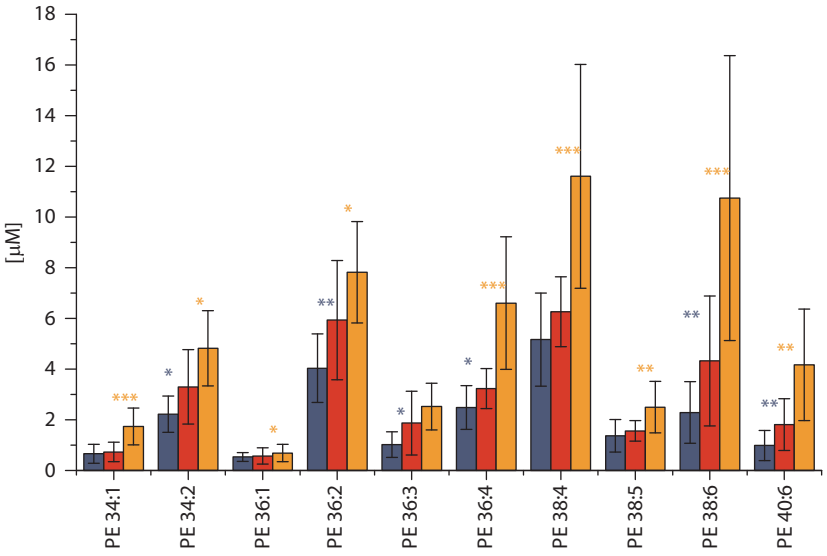
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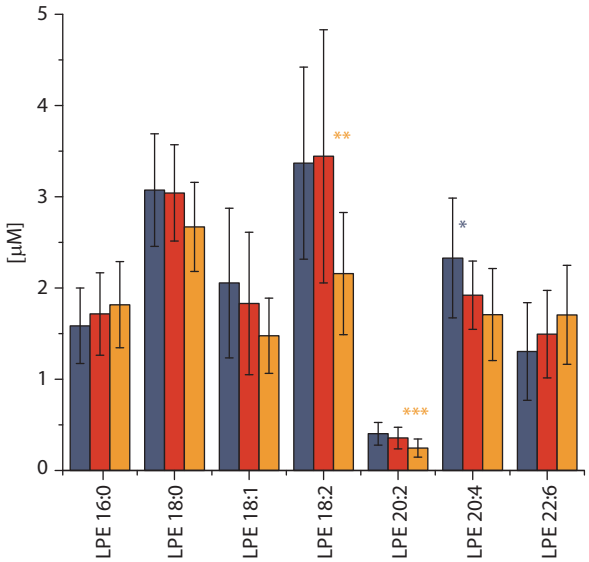
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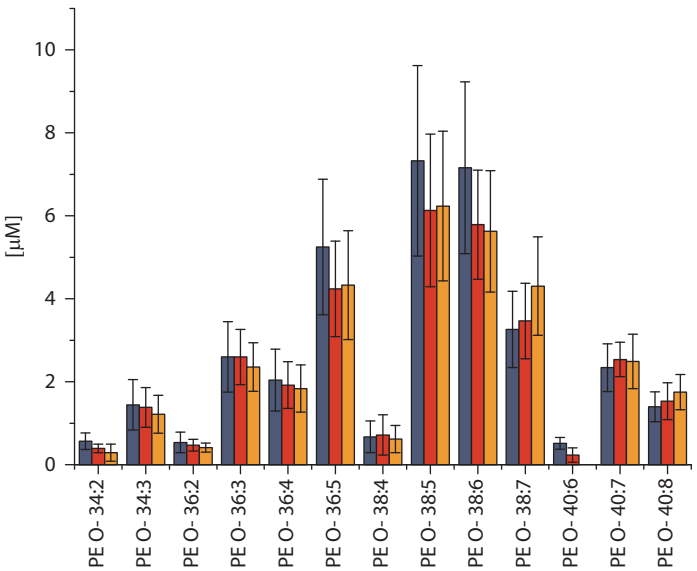
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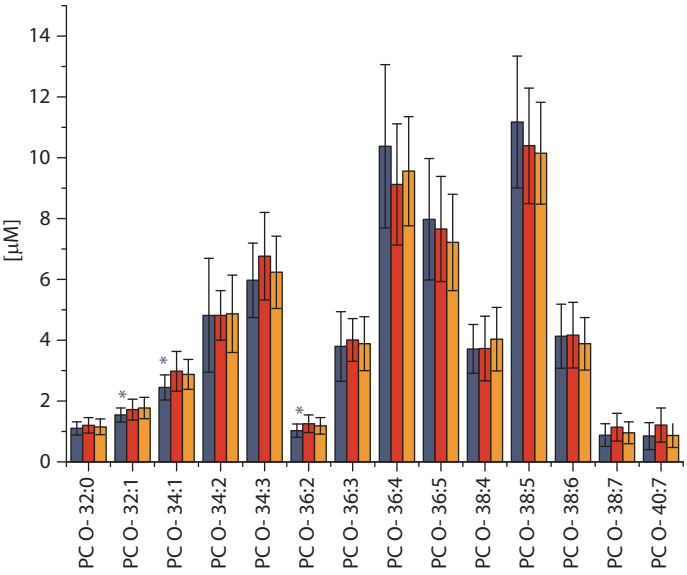
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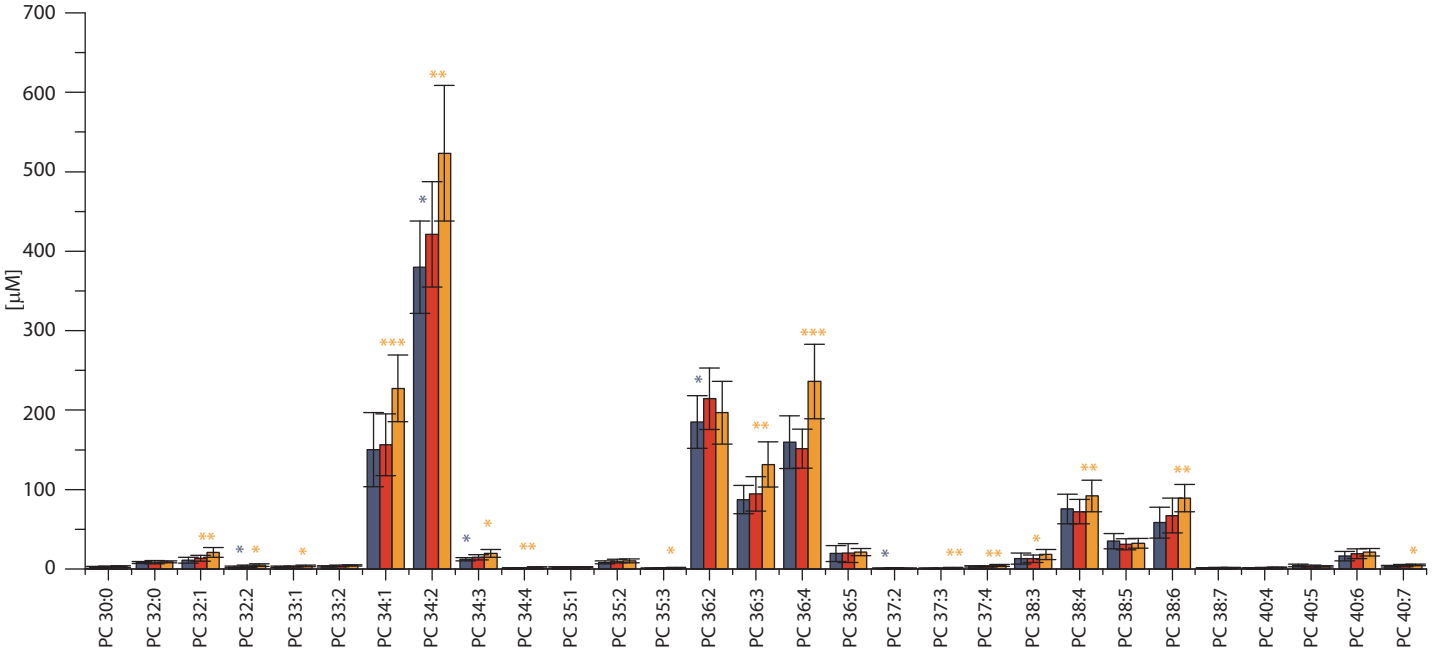
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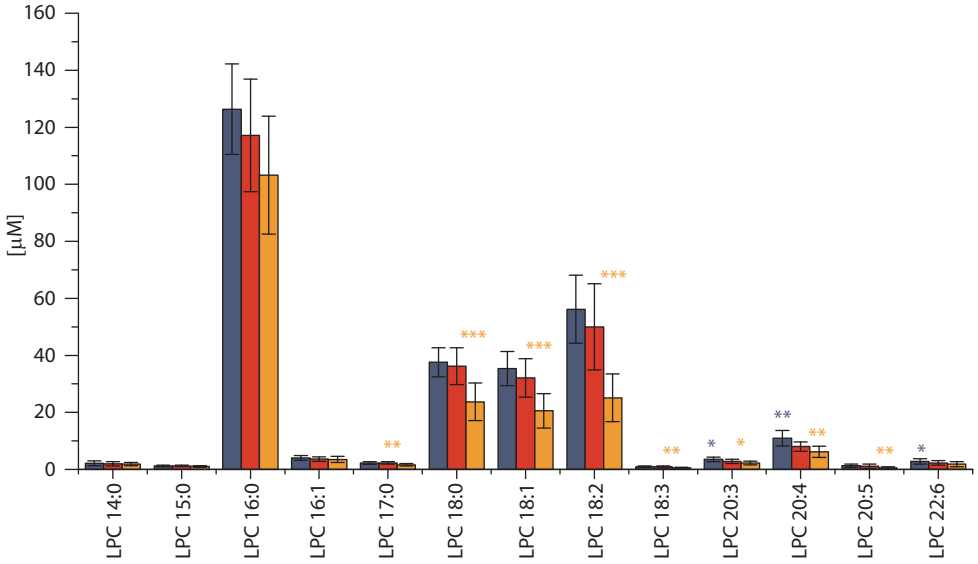
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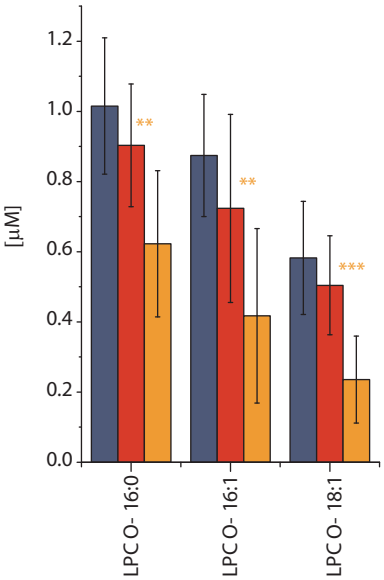
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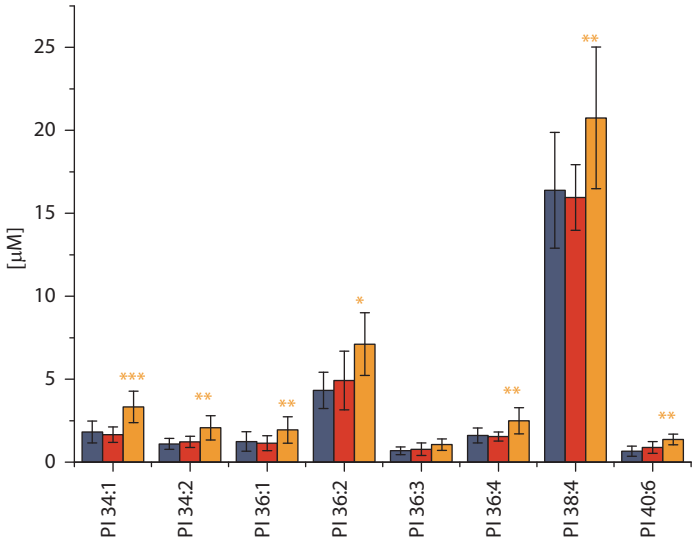
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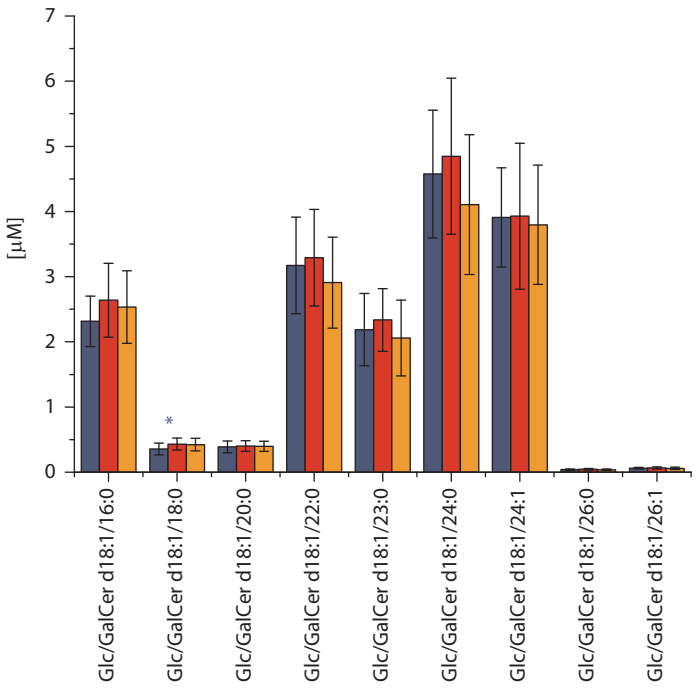
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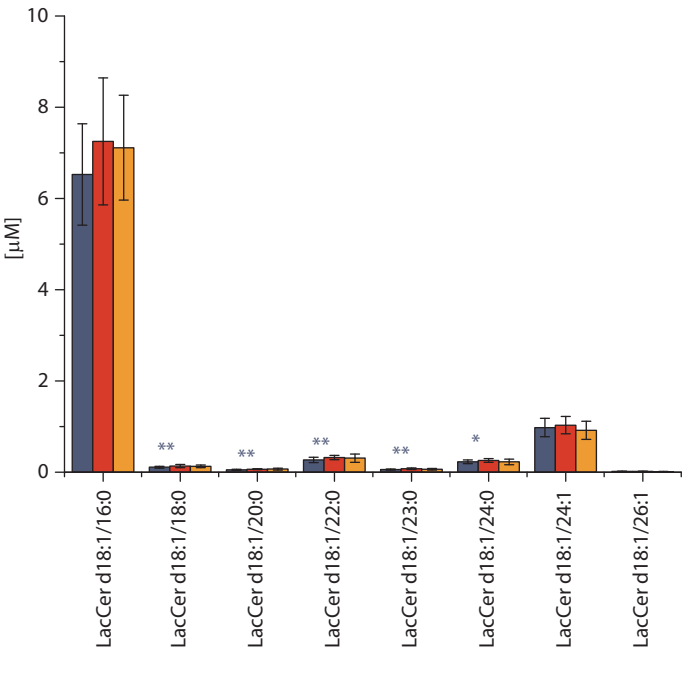
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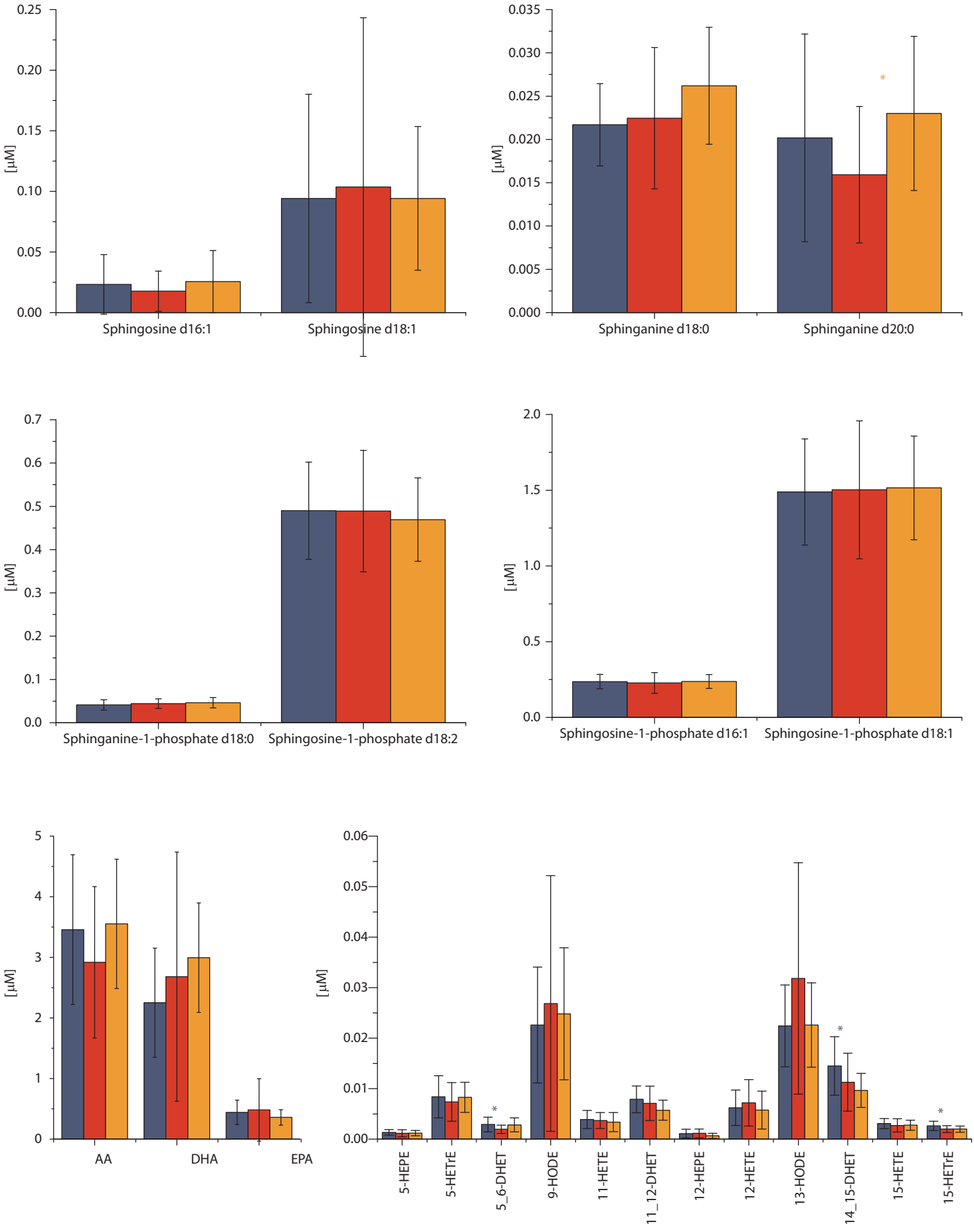
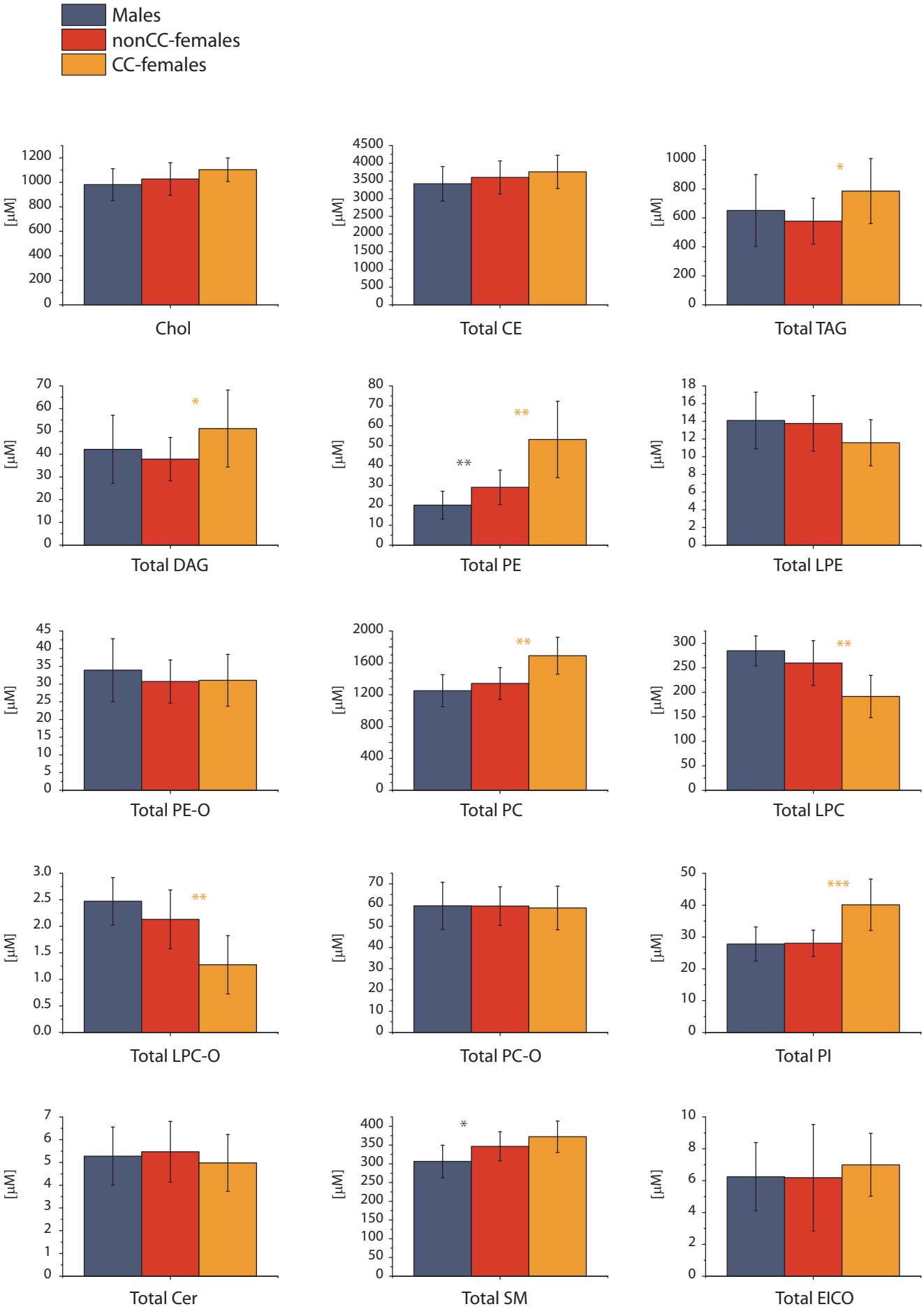
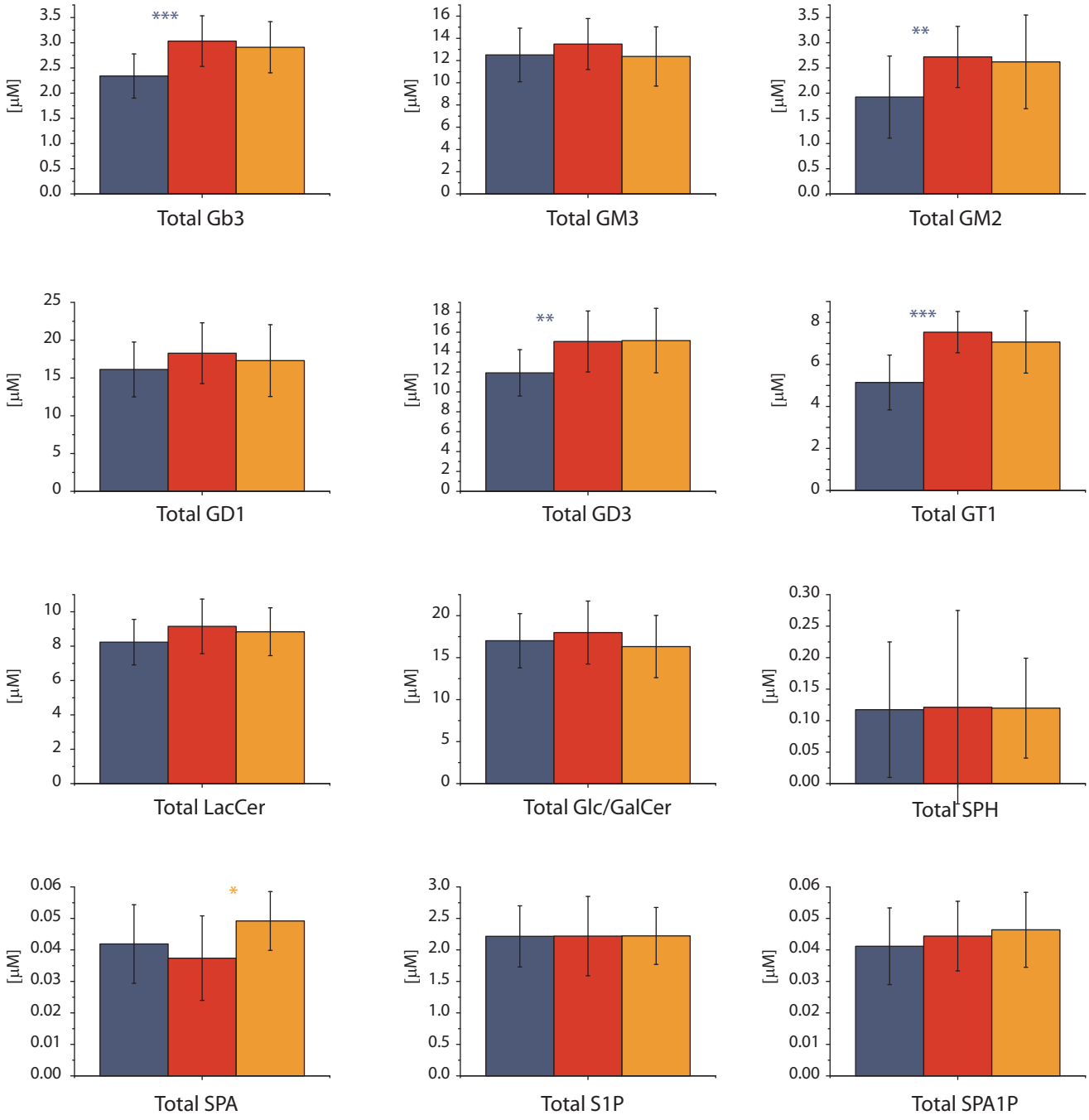


Supplementary Figure S1. Concentrations of individual lipid species. Comparison between males (*n*=36), and nonCC-females (*n*=16) (blue stars), and between nonCC-females (*n*=16) and CC-females (*n*=19) (orange stars) . Data are the mean ± SD. Statistic was performed by nonparametric Mann-Whitney test; Benjamini corrected. **p*<0.05; ***p*<0.01; ****p*<0.001.

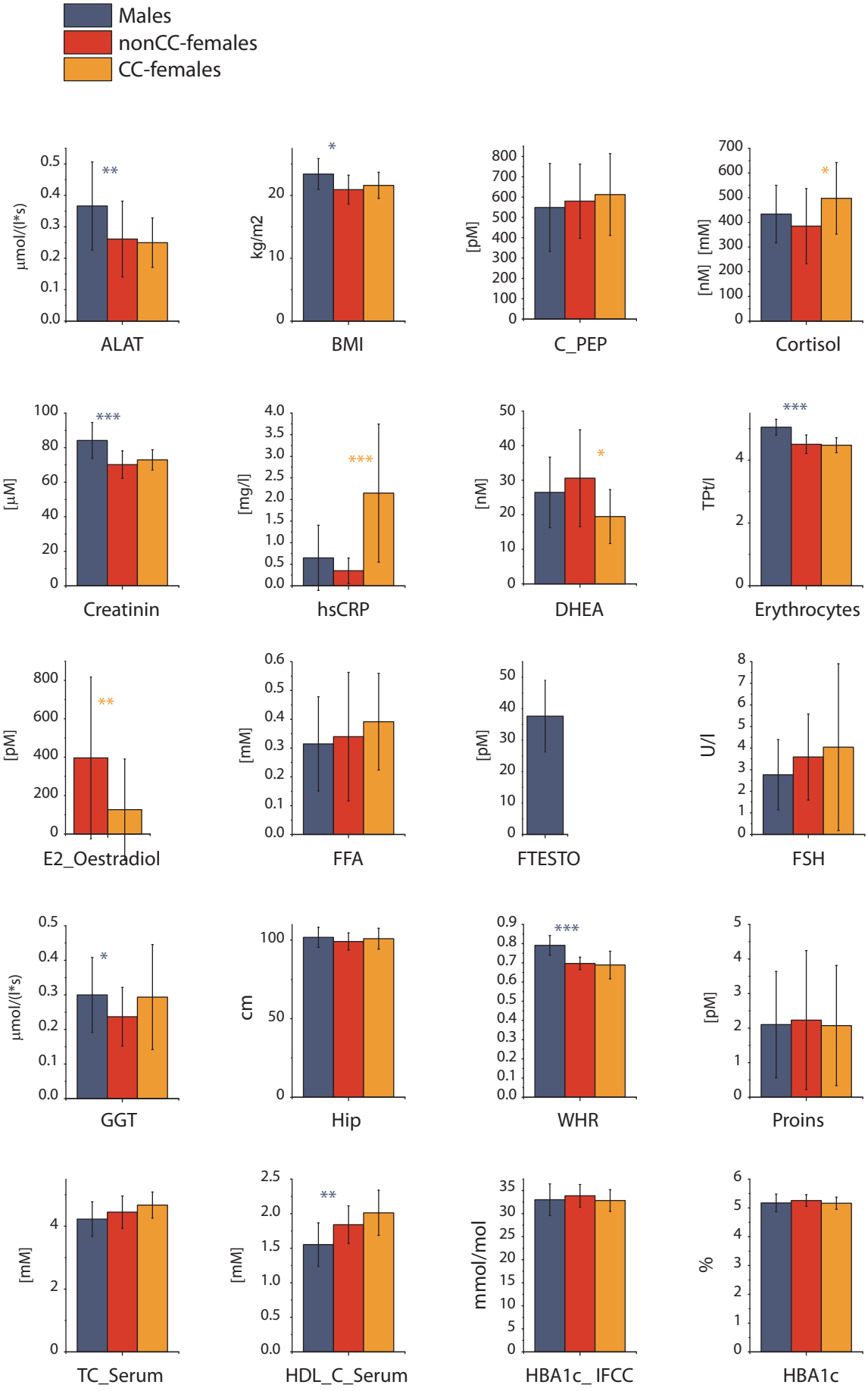
Supplementary Figure S2

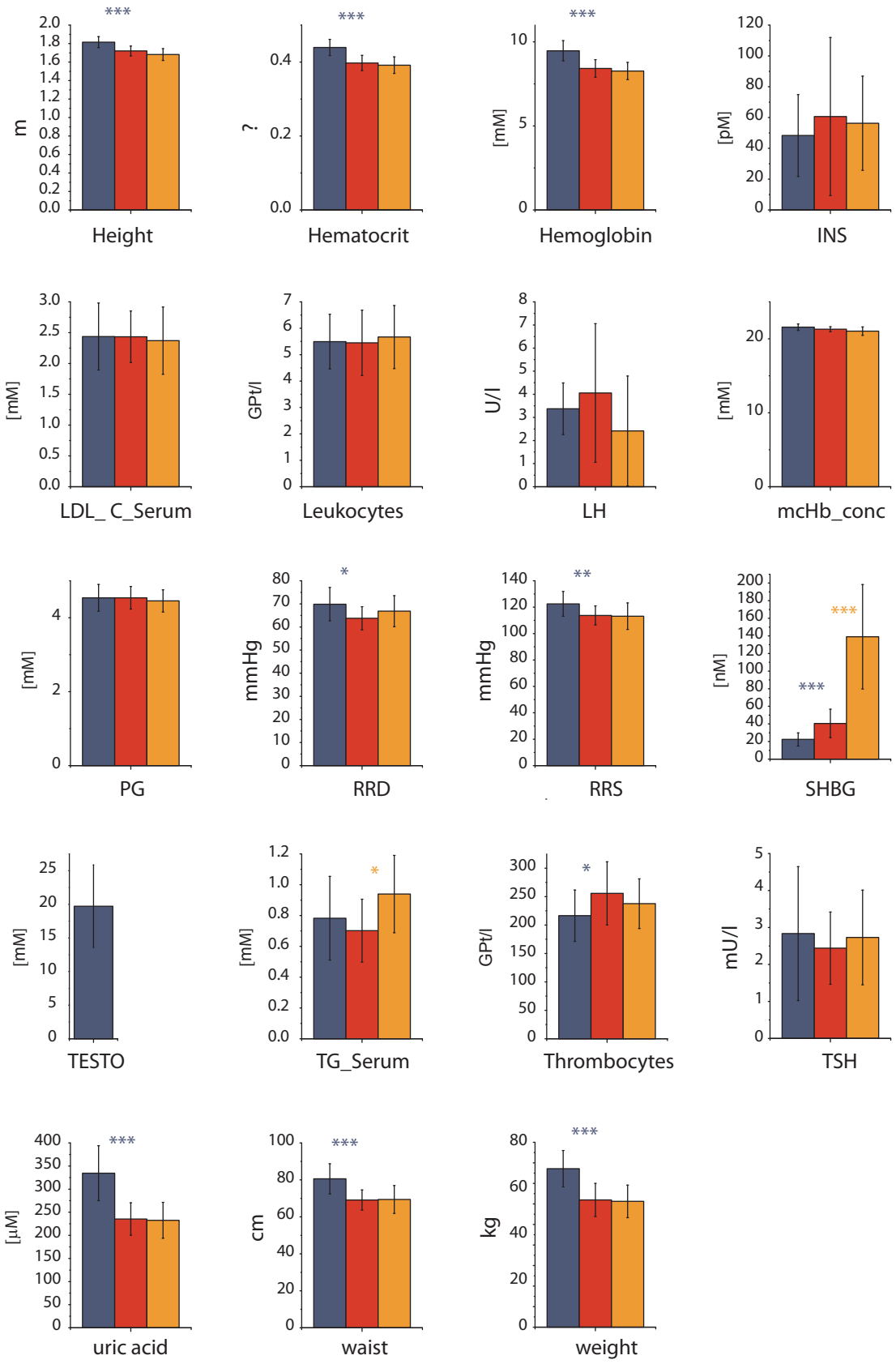




Supplementary Figure S2. Total concentrations of lipid classes. Comparison between males (*n*=36), and nonCC-females (*n*=16) (blue stars), and between nonCC-females (*n*=16) and CC-females (*n*=19) (orange stars) . Data are the mean ± SD. Statistics was performed by nonparametric Mann-Whitney test; Benjamini corrected. **p*<0.05; ***p*<0.01; ****p*<0.001.

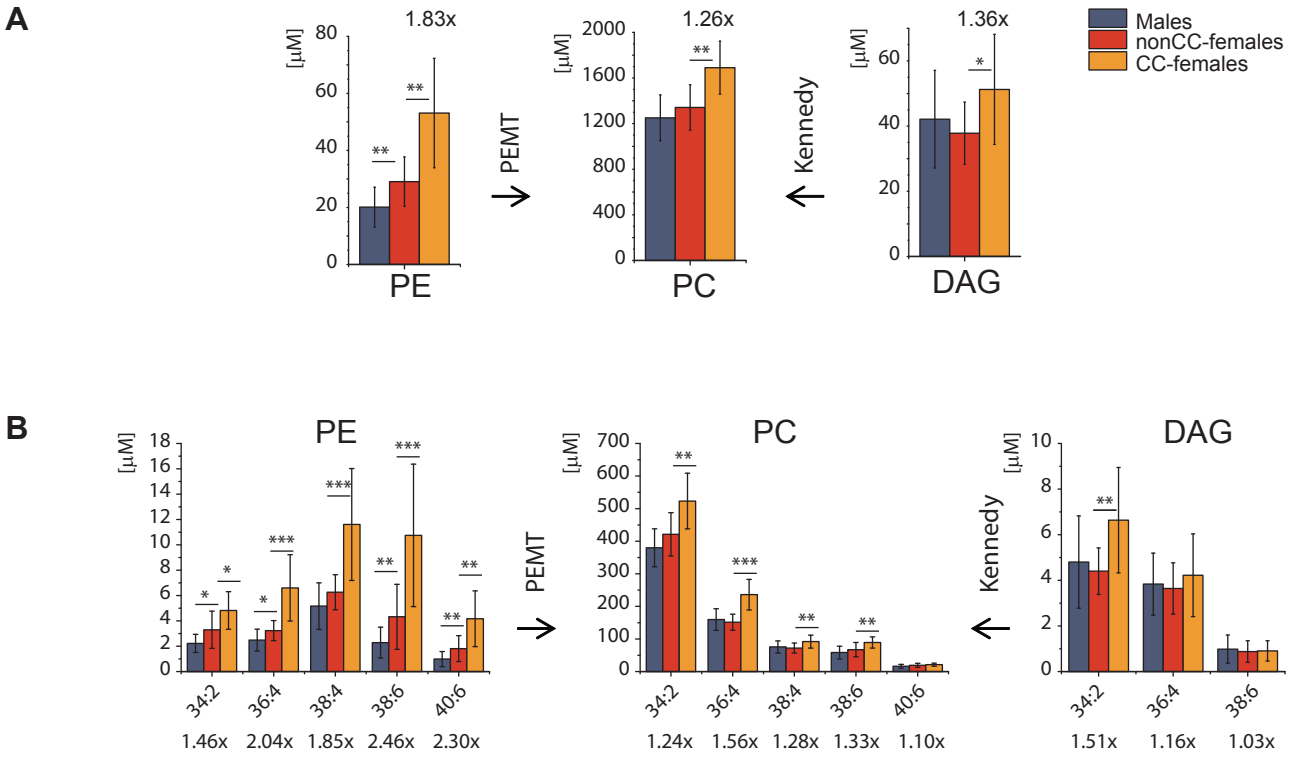
Supplementary Figure S3





Supplementary Figure S3. Values of clinical and anthropometric indices. Comparison between males (*n*=36), and nonCC-females (*n*=16) (blue stars), and between nonCC-females (*n*=16) and CC-females (*n*=19) (orange stars) . Data are the mean ± SD. Statistic was performed by nonparametric Mann-Whitney test; Benjamini corrected. **p*<0.05; ***p*<0.01; ****p*<0.001.

Supplementary Figure S4



Supplementary Figure S4. Estradiol-dependent and -independent regulation of lipid concentrations in plasma. (A) Total concentration of DAG, PE, and PC in males (*n*=36), nonCC-females (*n*=16), and CC-females (*n*=19). Fold differences between nonCC-females and CC-females are above the concentration bars. **(B)** Several abundat DAG, PE, and PC species and corresponding for differences (shown below concentration bars) between nonCC-females and CC-females. Conversion of polyunsaturated PE species into corresponding PC is faster in nonCC-females compared to CC-females. * $p<0.05$; ** $p<0.01$; $p<0.001$ (nonparametric Mann-Whitney test; Benjamini corrected). Data are the mean ± SD.