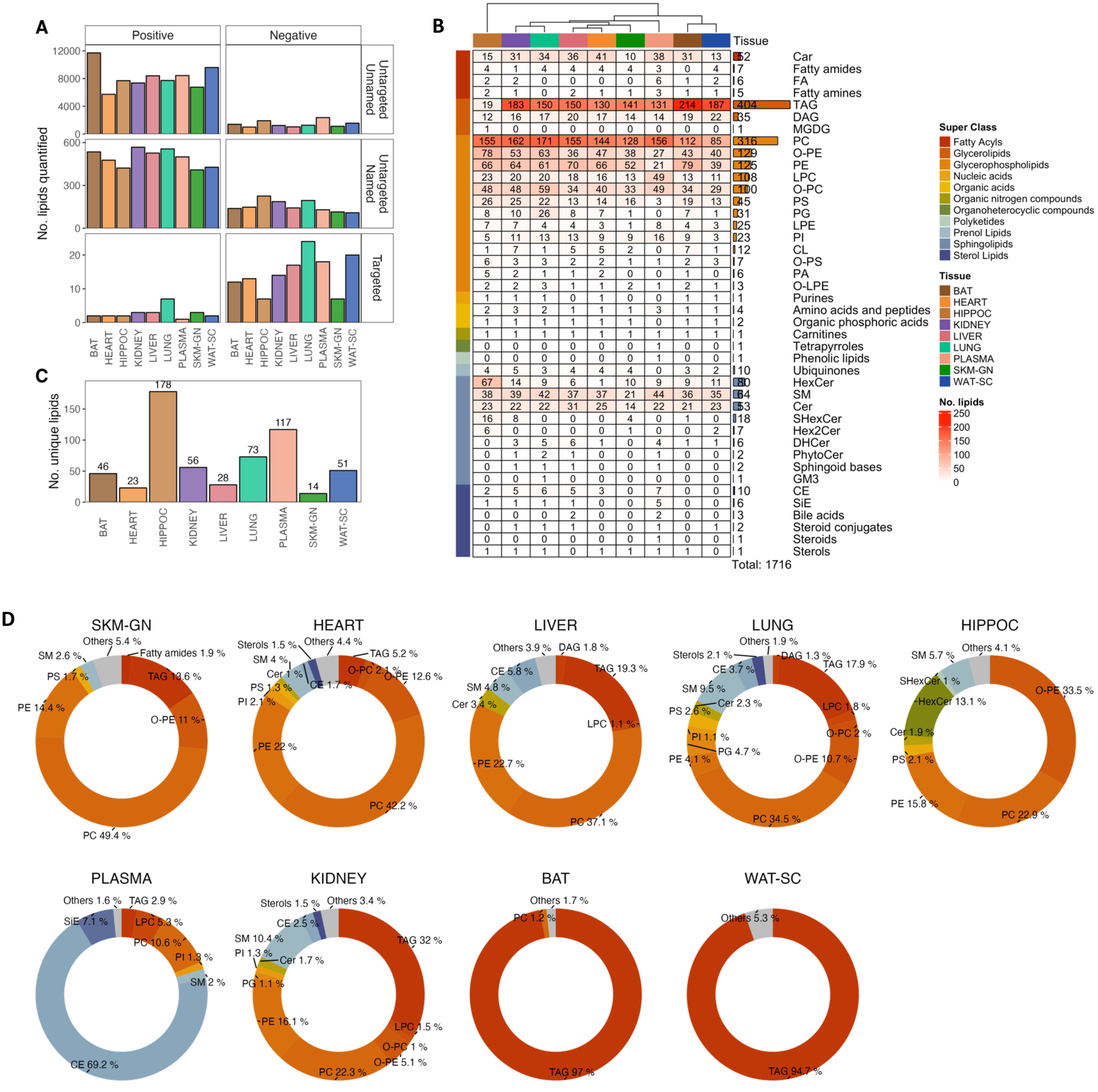


| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 15 | 31 | 34 | 36 | 41 | 10 | 38 | 31 | 13 | 52 | Car |
| 4 | 1 | 4 | 4 | 4 | 4 | 3 | 0 | 4 | 7 | Fatty amides |
| 2 | 2 | 0 | 0 | 0 | 0 | 6 | 1 | 2 | 6 | FA |
| 2 | 1 | 0 | 2 | 1 | 1 | 3 | 1 | 1 | 5 | Fatty amines |
| 19 | 183 | 150 | 150 | 130 | 141 | 131 | 214 | 187 | 404 | TAG |
| 12 | 16 | 17 | 20 | 17 | 14 | 14 | 19 | 22 | 35 | DAG |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | MGDG |
| 155 | 162 | 171 | 155 | 144 | 128 | 156 | 112 | 85 | 316 | PC |
| 78 | 53 | 63 | 36 | 47 | 38 | 27 | 43 | 40 | 129 | O-PE |
| 66 | 64 | 61 | 70 | 66 | 52 | 21 | 79 | 39 | 125 | PE |
| 23 | 20 | 20 | 18 | 16 | 13 | 49 | 13 | 11 | 108 | LPC |
| 48 | 48 | 59 | 34 | 40 | 33 | 49 | 34 | 29 | 100 | O-PC |
| 26 | 25 | 22 | 13 | 14 | 16 | 3 | 19 | 13 | 45 | PS |
| 8 | 10 | 26 | 8 | 7 | 1 | 0 | 7 | 1 | 31 | PG |
| 7 | 7 | 4 | 4 | 3 | 1 | 8 | 4 | 3 | 25 | LPE |
| 5 | 11 | 13 | 13 | 9 | 9 | 16 | 9 | 3 | 23 | PI |
| 1 | 7 | 1 | 5 | 5 | 2 | 0 | 7 | 1 | 12 | CL |
| 6 | 3 | 3 | 2 | 2 | 1 | 1 | 2 | 3 | 7 | O-PS |
| 5 | 2 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 6 | PA |
| 2 | 2 | 3 | 1 | 1 | 2 | 1 | 2 | 1 | 3 | O-LPE |
| 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | Purines |
| 2 | 3 | 2 | 1 | 1 | 1 | 3 | 1 | 1 | 4 | Amino acids and peptides |
| 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 2 | Organic phosphoric acids |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | Carnitines |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | Tetrapyrroles |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | Phenolic lipids |
| 4 | 5 | 3 | 4 | 4 | 4 | 0 | 3 | 2 | 10 | Ubiquinones |
| 67 | 14 | 9 | 6 | 1 | 10 | 9 | 9 | 11 | 80 | HexCer |
| 38 | 39 | 42 | 37 | 37 | 21 | 44 | 36 | 35 | 64 | SM |
| 23 | 22 | 22 | 31 | 25 | 14 | 22 | 21 | 23 | 53 | Cer |
| 16 | 8 | 0 | 0 | 0 | 4 | 0 | 1 | 0 | 18 | SHexCer |
| 6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 7 | Hex2Cer |
| 0 | 3 | 5 | 6 | 1 | 0 | 4 | 1 | 1 | 6 | DHCer |
| 0 | 1 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | PhytoCer |
| 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | Sphingoid bases |
| 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | GM3 |
| 2 | 5 | 6 | 5 | 3 | 0 | 7 | 0 | 0 | 10 | CE |
| 1 | 1 | 1 | 1 | 0 | 0 | 5 | 0 | 0 | 6 | SiE |
| 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 3 | Bile acids |
| 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 2 | Steroid conjugates |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | Steroids |
| 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | Sterols |

Total: 1716

**Fig S1. Lipidomics profiling of eight tissues and plasma**

(A) Number of detected lipid features, including named and unnamed features.
(B) Number of annotated lipid features in each tissue, separated by lipid classes.
(C) Number of lipids that were unique (i.e., detected only once) within each tissue.
(D) Tissue lipid class abundance. Mean lipid class concentrations were calculated based on mean of male/female group medians and were reported as percentages of total lipids (sum of all lipids). Lipid classes <1% were summed and shown as ‘Others’.

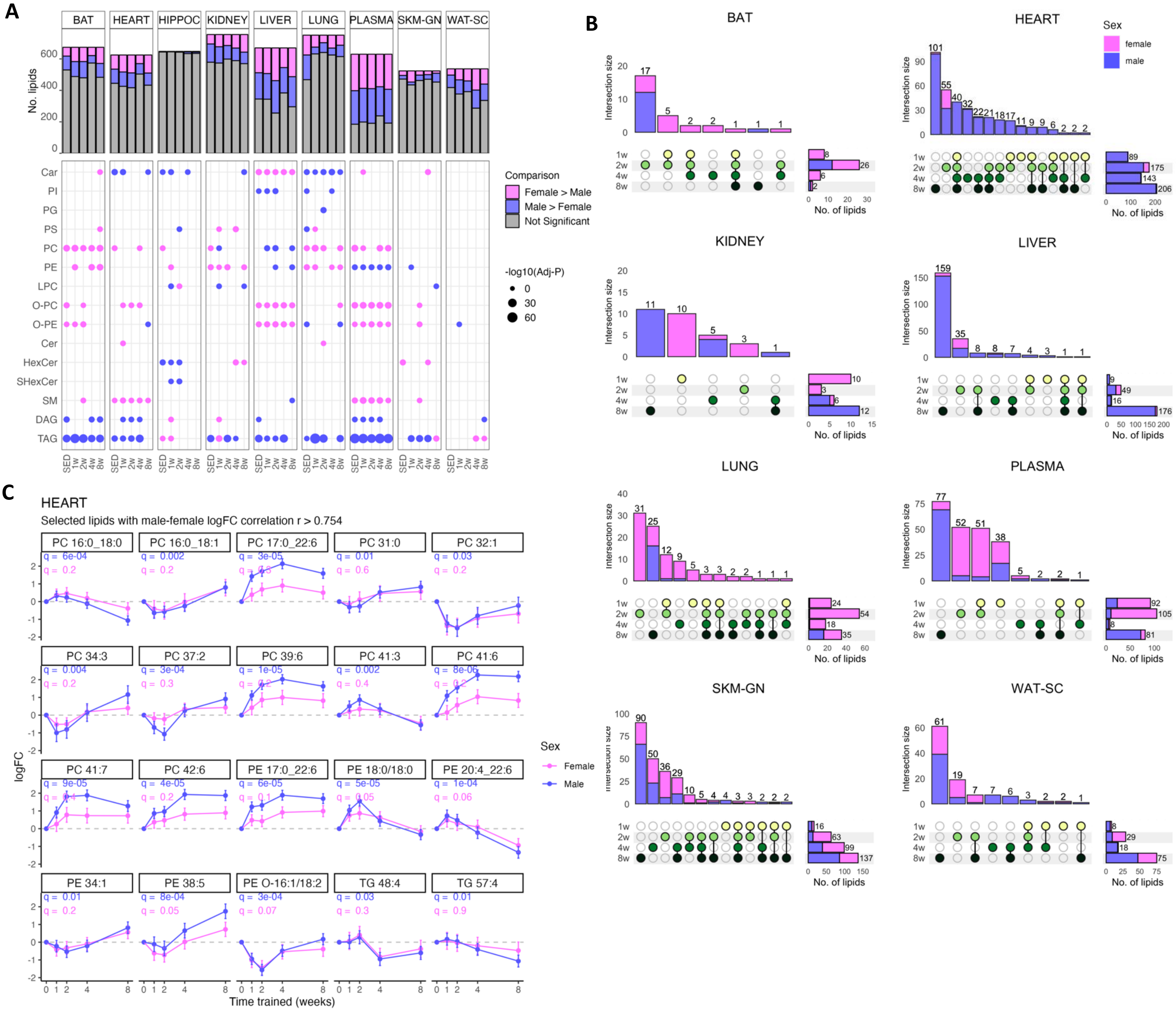

A
BAT
HEART
HIPPOC
KIDNEY
LIVER
LUNG
PLASMA
SKM-GN
WAT-SC
No. lipids
Car
PI
PG
PS
PC
PE
LPC
O-PC
O-PE
Cer
HexCer
SHexCer
SM
DAG
TAG
SED 1w 2w 4w 8w
Comparison
Female > Male
Male > Female
Not Significant
-log10(Adj-P)
0
30
60
B
BAT
HEART
KIDNEY
LIVER
LUNG
PLASMA
SKM-GN
WAT-SC
Sex
female
male
Intersection size
No. of lipids
1w
2w
4w
8w
C
HEART
Selected lipids with male-female logFC correlation r > 0.754
PC 16:0_18:0
PC 16:0_18:1
PC 17:0_22:6
PC 31:0
PC 32:1
PC 34:3
PC 37:2
PC 39:6
PC 41:3
PC 41:6
PC 41:7
PC 42:6
PE 17:0_22:6
PE 18:0/18:0
PE 20:4_22:6
PE 34:1
PE 38:5
PE O-16:1/18:2
TG 48:4
TG 57:4
logFC
Time trained (weeks)
Sex
Female
Male

**Fig S2. Sex-specific temporal *ExT responses* in lipidomes**

(A) Number of lipids and enrichment plot of lipid classes that were significantly different between sexes at each timepoint (FDR < 0.05). Color and dot size indicate sex comparison and enrichment significance, respectively.

(B) UpSet plots of the number of significant lipids from each training timepoint relative to sex-matched sedentary control (FDR < 0.05).

(C) Trajectory plots of logFC of top 20 lipids that were significantly correlated between male vs female logFC ($r > 0.754$) but showed significant training response only in male heart in Fig 1E. Color indicates sex. Number indicates training differential abundance FDR.

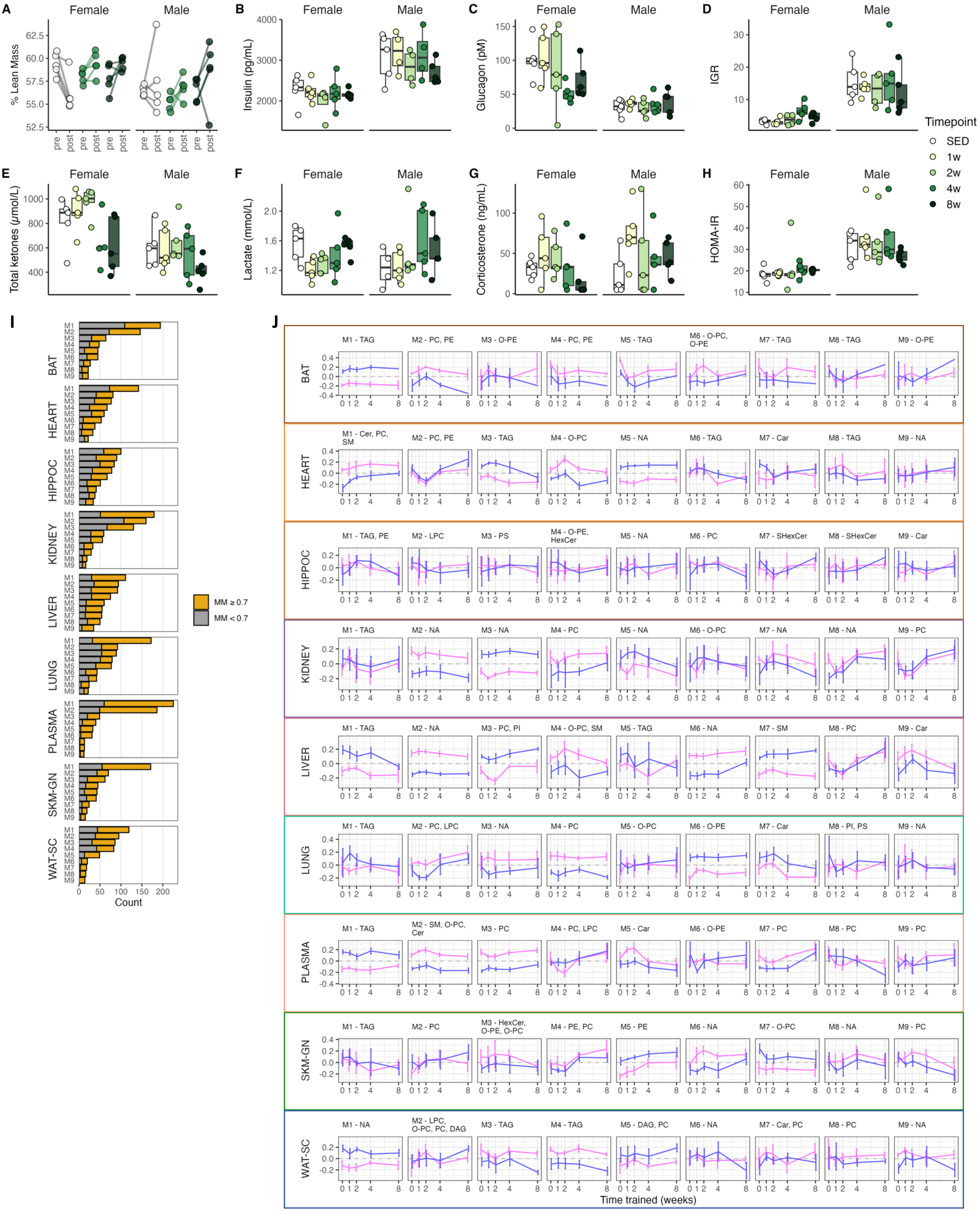

A
Female
Male
% Lean Mass
62.5
60.0
57.5
55.0
52.5
pre
post
B
Insulin (pg/mL)
4000
3000
2000
C
Glucagon (pM)
150
100
50
0
D
IGR
30
20
10
Timepoint
SED
1w
2w
4w
8w
E
Total ketones (μmol/L)
1000
800
600
400
F
Lactate (mmol/L)
2.0
1.6
1.2
G
Corticosterone (ng/mL)
100
50
0
H
HOMA-IR
60
50
40
30
20
10
I
BAT
HEART
HIPPOC
KIDNEY
LIVER
LUNG
PLASMA
SKM-GN
WAT-SC
M1
M2
M3
M4
M5
M6
M7
M8
M9
MM ≥ 0.7
MM < 0.7
0
50
100
150
200
Count
J
BAT: M1 - TAG; M2 - PC, PE; M3 - O-PE; M4 - PC, PE; M5 - TAG; M6 - O-PC, O-PE; M7 - TAG; M8 - TAG; M9 - O-PE
HEART: M1 - Cer, PC, SM; M2 - PC, PE; M3 - TAG; M4 - O-PC; M5 - NA; M6 - TAG; M7 - Car; M8 - TAG; M9 - NA
HIPPOC: M1 - TAG, PE; M2 - LPC; M3 - PS; M4 - O-PE, HexCer; M5 - NA; M6 - PC; M7 - SHexCer; M8 - SHexCer; M9 - Car
KIDNEY: M1 - TAG; M2 - NA; M3 - NA; M4 - PC; M5 - NA; M6 - O-PC; M7 - NA; M8 - NA; M9 - PC
LIVER: M1 - TAG; M2 - NA; M3 - PC, PI; M4 - O-PC, SM; M5 - TAG; M6 - NA; M7 - SM; M8 - PC; M9 - Car
LUNG: M1 - TAG; M2 - PC, LPC; M3 - NA; M4 - PC; M5 - O-PC; M6 - O-PE; M7 - Car; M8 - PI, PS; M9 - NA
PLASMA: M1 - TAG; M2 - SM, O-PC, Cer; M3 - PC; M4 - PC, LPC; M5 - Car; M6 - O-PE; M7 - PC; M8 - PC; M9 - PC
SKM-GN: M1 - TAG; M2 - PC; M3 - HexCer, O-PE, O-PC; M4 - PE, PC; M5 - PE; M6 - NA; M7 - O-PC; M8 - NA; M9 - PC
WAT-SC: M1 - NA; M2 - LPC, O-PC, PC, DAG; M3 - TAG; M4 - TAG; M5 - DAG, PC; M6 - NA; M7 - Car, PC; M8 - PC; M9 - NA
0.4
0.2
0.0
-0.2
-0.4
0 1 2 4 8
Time trained (weeks)

**Figure S3. Integrative phenotypic-lipidomic responses to ExT**

- (A-H) Measurements of selected phenotypic traits: (A) %lean mass, circulating plasma levels of (B) insulin, (C) glucagon, (D) insulin:glucagon ratio (IGR), (E) total ketones, (F) lactate, (G) corticosterone, (H) HOMA-IR.
- (I) Number of total and hub lipids (membership ≥ 0.7) in each tissue module.
- (J) Plots of the module eigenvalues (MEs) from each tissue module.

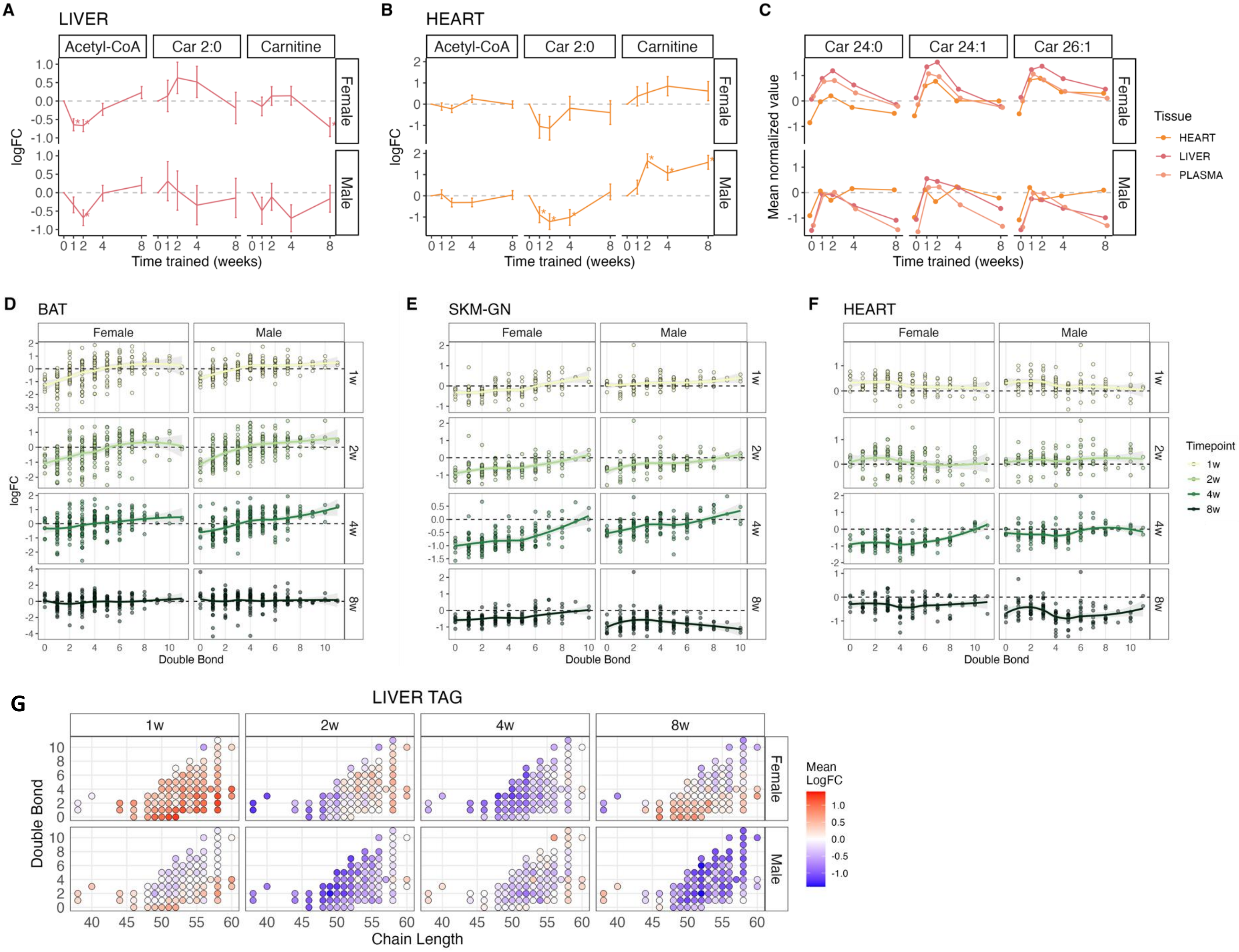

A
LIVER
Acetyl-CoA
Car 2:0
Carnitine
Female
Male
logFC
Time trained (weeks)
B
HEART
Acetyl-CoA
Car 2:0
Carnitine
Female
Male
logFC
Time trained (weeks)
C
Car 24:0
Car 24:1
Car 26:1
Female
Male
Mean normalized value
Time trained (weeks)
Tissue
HEART
LIVER
PLASMA
D
BAT
E
SKM-GN
F
HEART
Female
Male
1w
2w
4w
8w
logFC
Double Bond
Timepoint
1w
2w
4w
8w
G
LIVER TAG
1w
2w
4w
8w
Female
Male
Double Bond
Chain Length
Mean LogFC
1.0
0.5
0.0
-0.5
-1.0

**Figure S4. ExT effects on lipids in energy metabolism**

- (A-B) Trajectory plots of logFC of acetylcarnitine, free carnitine, and acetyl-CoA in (A) liver and (B) heart.
- (C) Trajectory plots of standardized abundances of very-long-chain acylcarnitines in heart, liver, and plasma.
- (D-F) Timewise logFC *versus* the number of double bonds of TAG in (D) BAT, (E) SKM-GN, (F) heart.
- (G) Liver TAG logFC. Row and column indicate numbers of total carbon and double bond respectively. Color indicates mean logFC (when there were multiple TAG species with the same acyl composition).

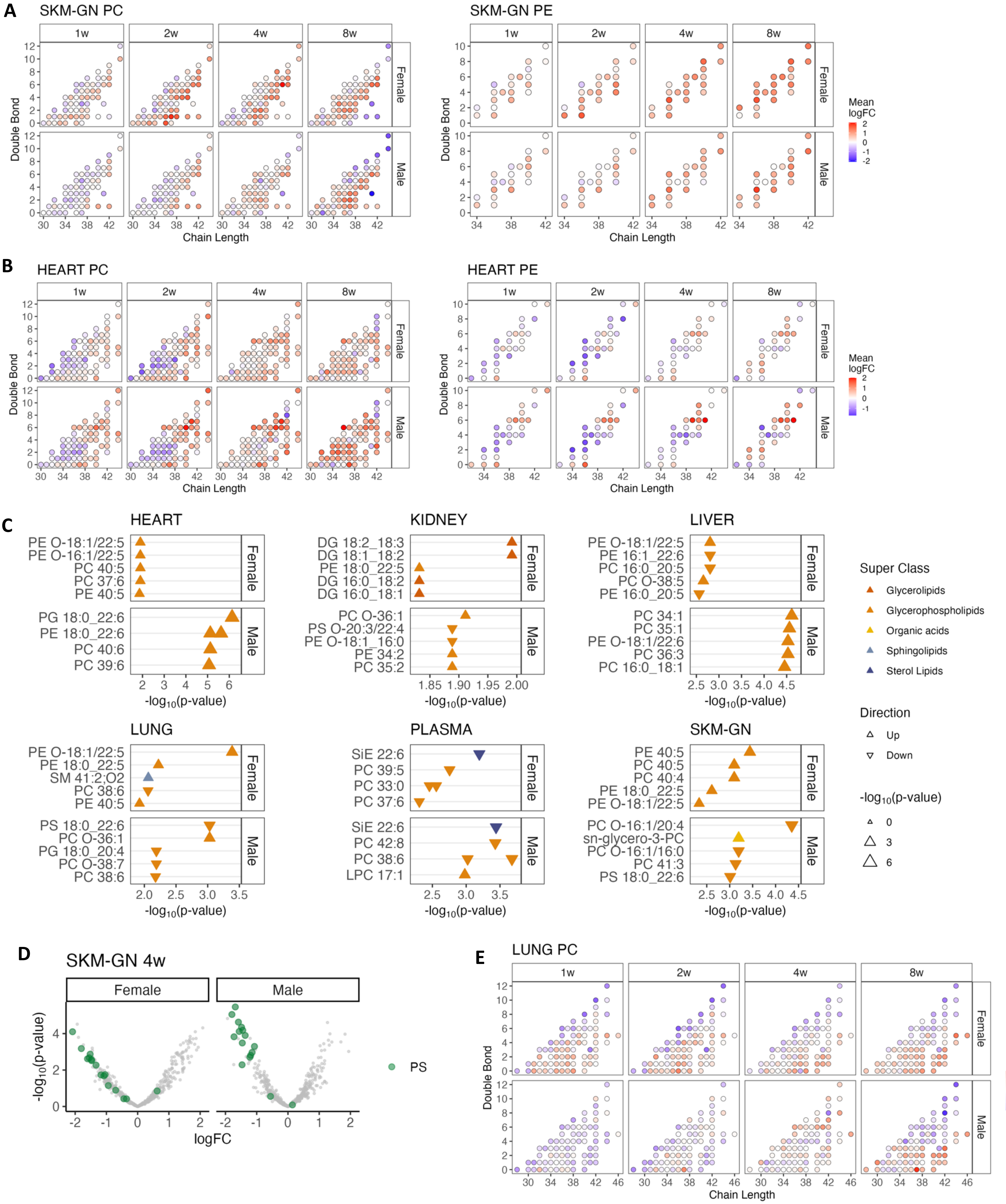

A
SKM-GN PC
SKM-GN PE
1w
2w
4w
8w
Female
Male
Double Bond
Chain Length
Mean logFC
B
HEART PC
HEART PE
C
HEART
PE O-18:1/22:5
PE O-16:1/22:5
PC 40:5
PC 37:6
PE 40:5
PG 18:0_22:6
PE 18:0_22:6
PC 40:6
PC 39:6
KIDNEY
DG 18:2_18:3
DG 18:1_18:2
PE 18:0_22:5
DG 16:0_18:2
DG 16:0_18:1
PC O-36:1
PS O-20:3/22:4
PE O-18:1_16:0
PE 34:2
PC 35:2
LIVER
PE O-18:1/22:5
PE 16:1_22:6
PC 16:0_20:5
PC O-38:5
PE 16:0_20:5
PC 34:1
PC 35:1
PE O-18:1/22:6
PC 36:3
PC 16:0_18:1
LUNG
PE O-18:1/22:5
PE 18:0_22:5
SM 41:2;O2
PC 38:6
PE 40:5
PS 18:0_22:6
PC O-36:1
PG 18:0_20:4
PC O-38:7
PC 38:6
PLASMA
SiE 22:6
PC 39:5
PC 33:0
PC 37:6
SiE 22:6
PC 42:8
PC 38:6
LPC 17:1
SKM-GN
PE 40:5
PC 40:5
PC 40:4
PE 18:0_22:5
PE O-18:1/22:5
PC O-16:1/20:4
sn-glycero-3-PC
PC O-16:1/16:0
PC 41:3
PS 18:0_22:6
-log10(p-value)
Super Class
Glycerolipids
Glycerophospholipids
Organic acids
Sphingolipids
Sterol Lipids
Direction
Up
Down
D
SKM-GN 4w
logFC
PS
E
LUNG PC

**Figure S5. ExT effects on phospholipids**

(A) SKM-GN PC and PE logFC. Row and column indicate numbers of total carbon and double bond respectively. Color indicates mean logFC (when there were multiple PC species with the same total acyl chain; red, positive; blue, negative).
(B) Heart PC and PE logFC.
(C) Top 5 lipids that responded to exercise ranked by p-values, with all timepoints combined. Direction of the triangle indicates the direction of change, and color indicates lipid super class.
(D) Volcano plots displaying comparisons of trained animals against sex-matched sedentary controls in SKM-GN at 4w. Colors highlight PS (grey: all other lipids).
(E) Lung PC logFC.

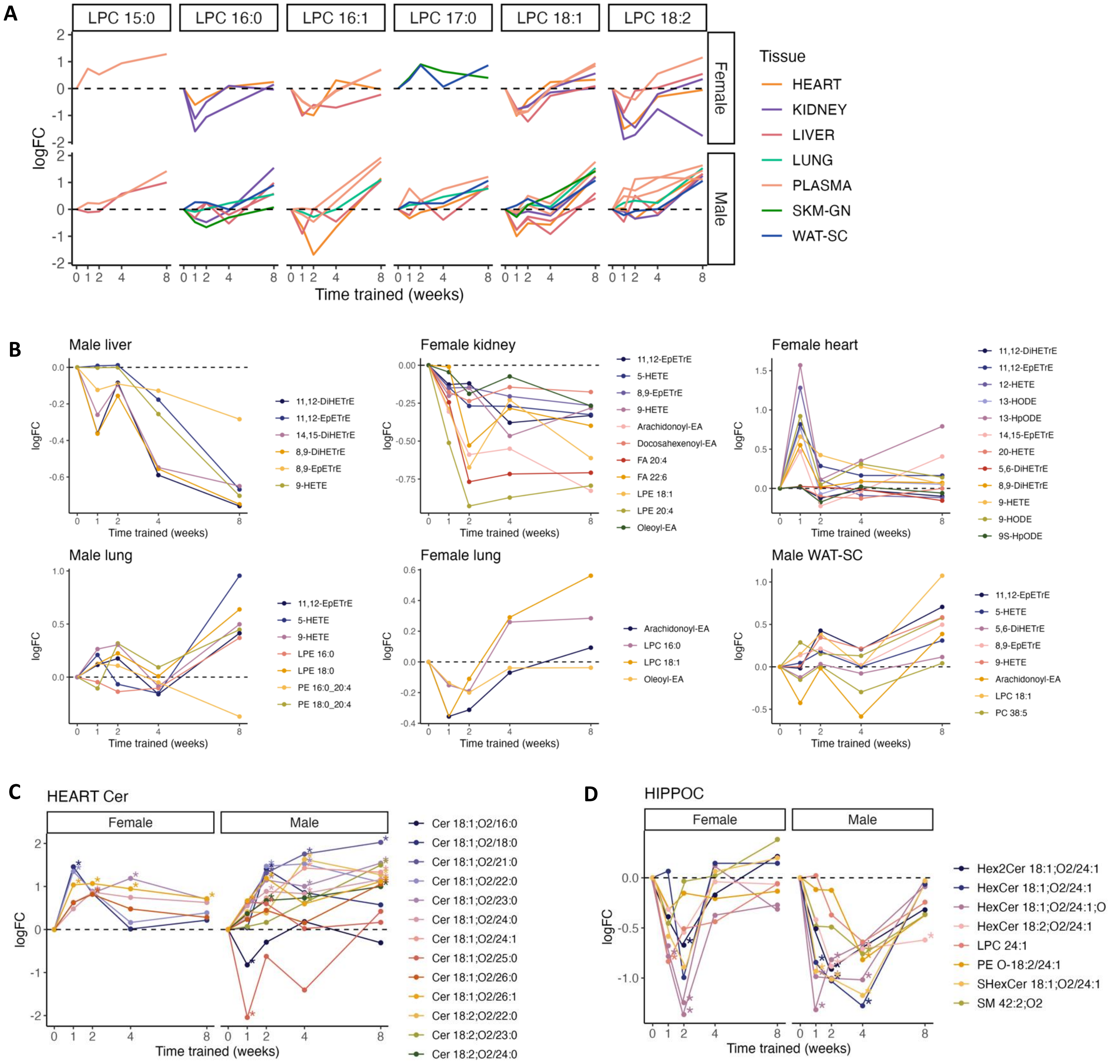
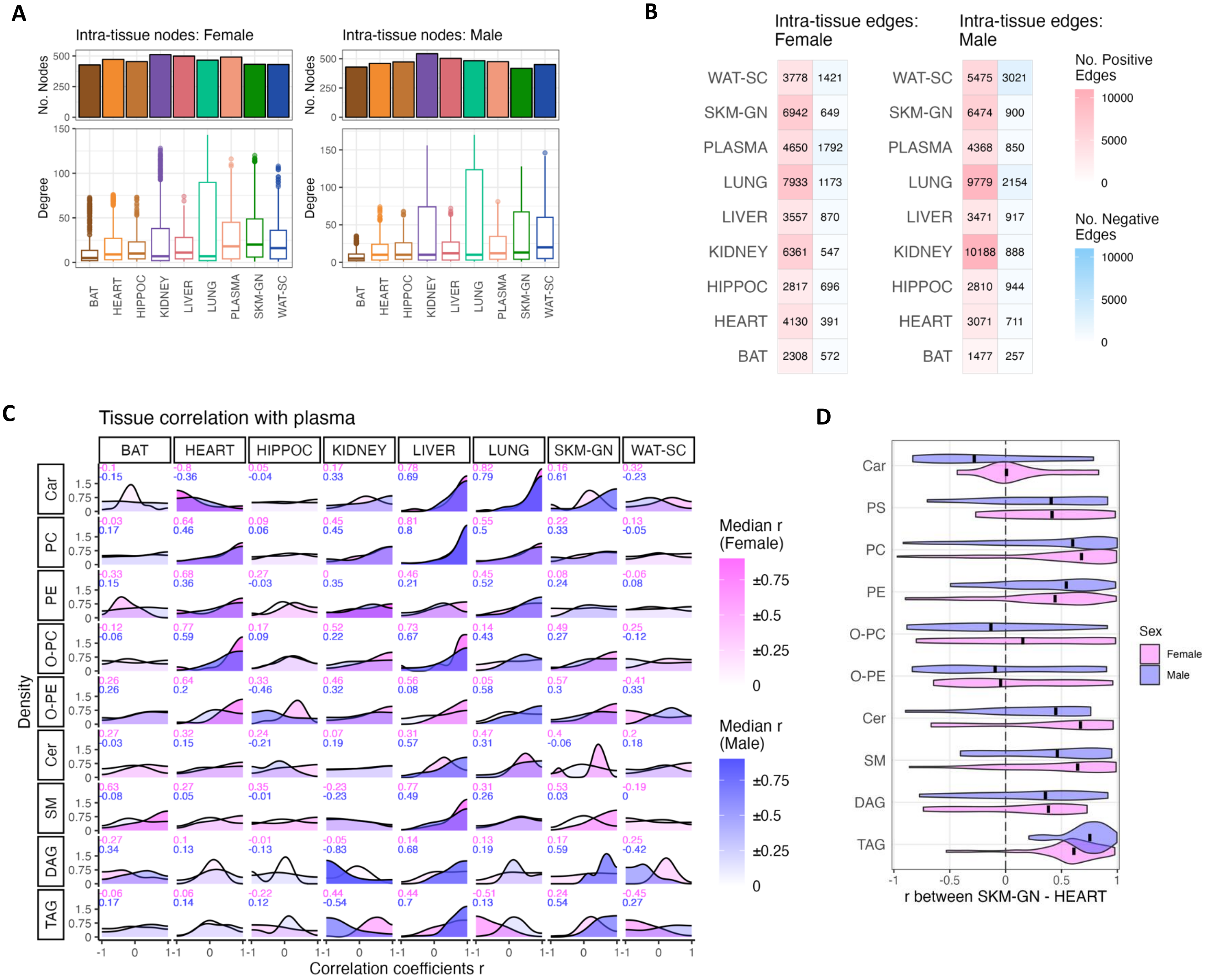

A
LPC 15:0
LPC 16:0
LPC 16:1
LPC 17:0
LPC 18:1
LPC 18:2
Female
Male
logFC
Time trained (weeks)
Tissue
HEART
KIDNEY
LIVER
LUNG
PLASMA
SKM-GN
WAT-SC
B
Male liver
11,12-DiHETrE
11,12-EpETrE
14,15-DiHETrE
8,9-DiHETrE
8,9-EpETrE
9-HETE
Female kidney
11,12-EpETrE
5-HETE
8,9-EpETrE
9-HETE
Arachidonoyl-EA
Docosahexenoyl-EA
FA 20:4
FA 22:6
LPE 18:1
LPE 20:4
Oleoyl-EA
Female heart
11,12-DiHETrE
11,12-EpETrE
12-HETE
13-HODE
13-HpODE
14,15-EpETrE
20-HETE
5,6-DiHETrE
8,9-DiHETrE
9-HETE
9-HODE
9S-HpODE
Male lung
11,12-EpETrE
5-HETE
9-HETE
LPE 16:0
LPE 18:0
PE 16:0_20:4
PE 18:0_20:4
Female lung
Arachidonoyl-EA
LPC 16:0
LPC 18:1
Oleoyl-EA
Male WAT-SC
11,12-EpETrE
5-HETE
5,6-DiHETrE
8,9-EpETrE
9-HETE
Arachidonoyl-EA
LPC 18:1
PC 38:5
logFC
Time trained (weeks)
C
HEART Cer
Female
Male
Cer 18:1;O2/16:0
Cer 18:1;O2/18:0
Cer 18:1;O2/21:0
Cer 18:1;O2/22:0
Cer 18:1;O2/23:0
Cer 18:1;O2/24:0
Cer 18:1;O2/24:1
Cer 18:1;O2/25:0
Cer 18:1;O2/26:0
Cer 18:1;O2/26:1
Cer 18:2;O2/22:0
Cer 18:2;O2/23:0
Cer 18:2;O2/24:0
logFC
Time trained (weeks)
D
HIPPOC
Female
Male
Hex2Cer 18:1;O2/24:1
HexCer 18:1;O2/24:1
HexCer 18:1;O2/24:1;O
HexCer 18:2;O2/24:1
LPC 24:1
PE O-18:2/24:1
SHexCer 18:1;O2/24:1
SM 42:2;O2
logFC
Time trained (weeks)

**Figure S6. ExT effects on *lipid mediators and sphingolipids***

(A) Trajectory plots of logFC of selected LPC species in tissues. Colors indicate tissue.
(B) Trajectory plots of selected oxylipins, lysophospholipids, fatty acid ethanolamides, and PC, PE species.
(C) Trajectory plots of logFC of selected heart ceramides that were significantly different compared to sedentary control in each sex (* indicates $p<0.05$).
(D) Trajectory plots of logFC of 24:1-containing lipids in the HIPPOC.



Intra-tissue edges: Female

| | | |
|---|---|---|
| WAT-SC | 3778 | 1421 |
| SKM-GN | 6942 | 649 |
| PLASMA | 4650 | 1792 |
| LUNG | 7933 | 1173 |
| LIVER | 3557 | 870 |
| KIDNEY | 6361 | 547 |
| HIPPOC | 2817 | 696 |
| HEART | 4130 | 391 |
| BAT | 2308 | 572 |

Intra-tissue edges: Male

| | | |
|---|---|---|
| WAT-SC | 5475 | 3021 |
| SKM-GN | 6474 | 900 |
| PLASMA | 4368 | 850 |
| LUNG | 9779 | 2154 |
| LIVER | 3471 | 917 |
| KIDNEY | 10188 | 888 |
| HIPPOC | 2810 | 944 |
| HEART | 3071 | 711 |
| BAT | 1477 | 257 |

**Figure S7. Tissue connectivity and networking during ExT**

(A) Number of nodes associated with each tissue in intra-tissue correlations. Boxplot of node degree (number of edges per node) of each lipid within a tissue. Box width is proportional to the number of nodes within each tissue.

(B) Number of intra-tissue edges. Color indicates correlation coefficient sign (red, positive; blue, negative), and the number and transparency level indicate the number of edges.

(C) Density plot of correlation coefficients between lipid timewise logFC, shown by lipid class. Correlations were performed using logFC trajectory of the same lipid between plasma and each tissue for each sex. Color indicates sex, and the number and transparency level indicate the median correlation coefficients.

(D) Violin plot of correlation coefficients of lipid timewise logFC between SKM-GN and heart, shown by lipid class. Color indicates sex. Black vertical bars indicate median correlation coefficients.