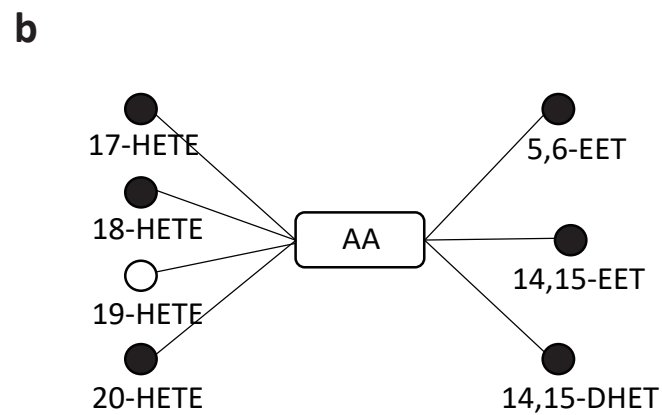
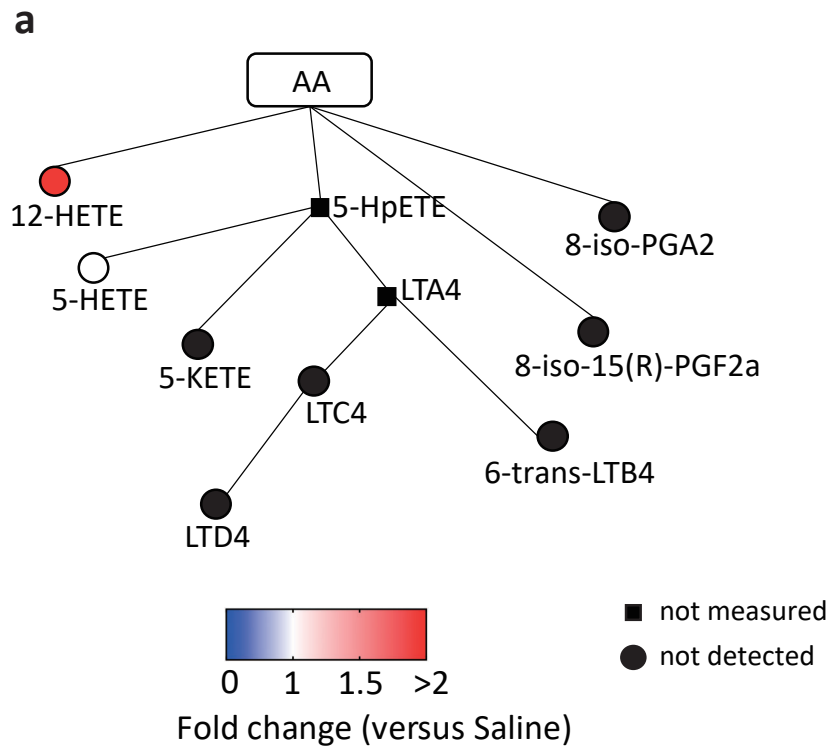


Supplementary Figure 1

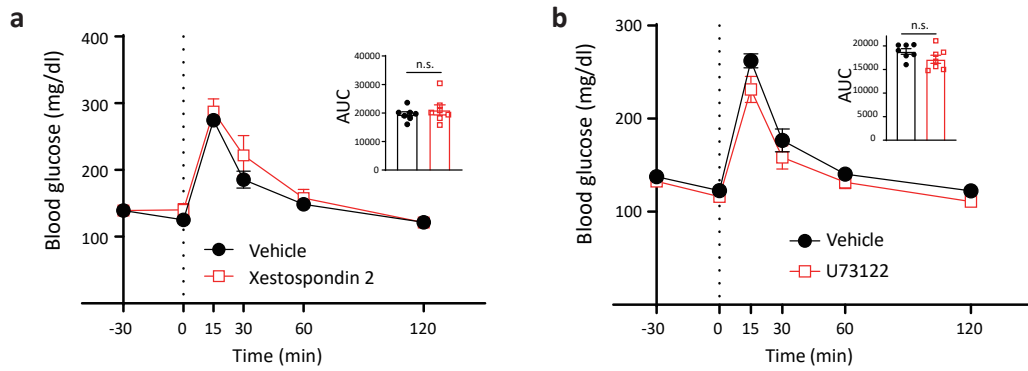
Relative intensities of lysospecies in the VMH (a) and ARC (b) by IMS after injection with saline (n=4) or glucose (n=4).

All data represent the mean  $\pm$  SEM. LPI: lysophosphatidylinositol, LPS: lysophosphatidylserine, LPE: lysophosphatidylethanolamine.



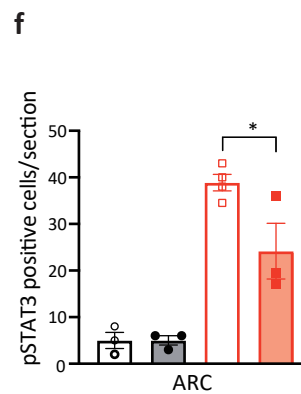
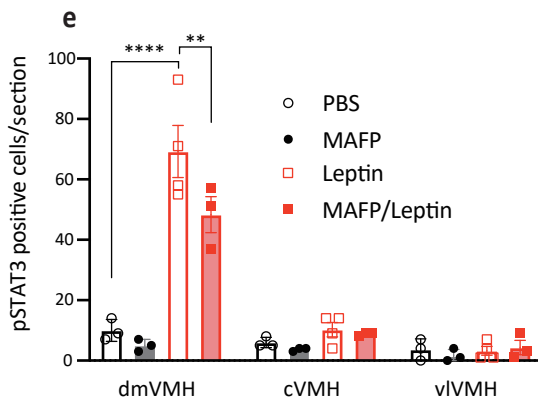
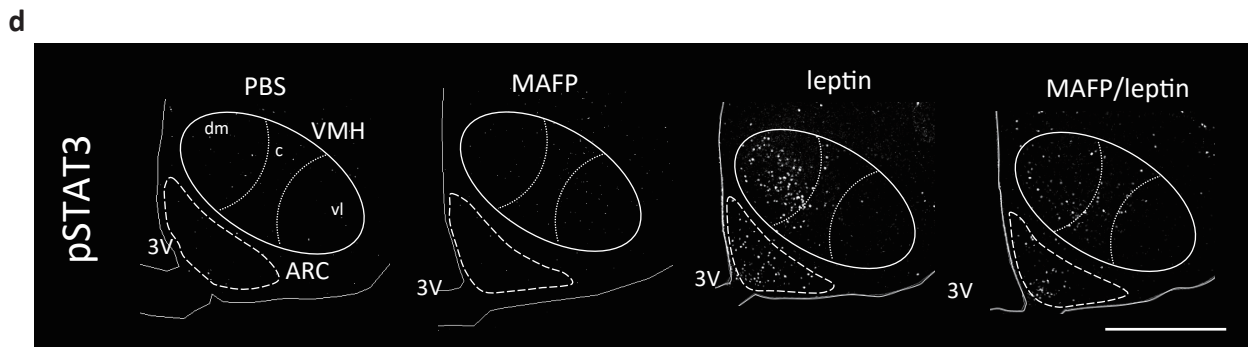
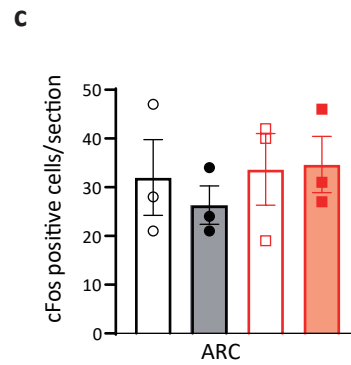
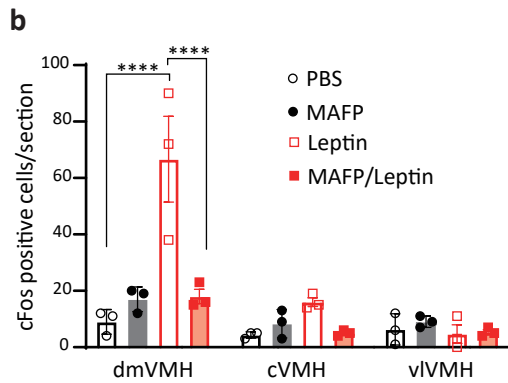
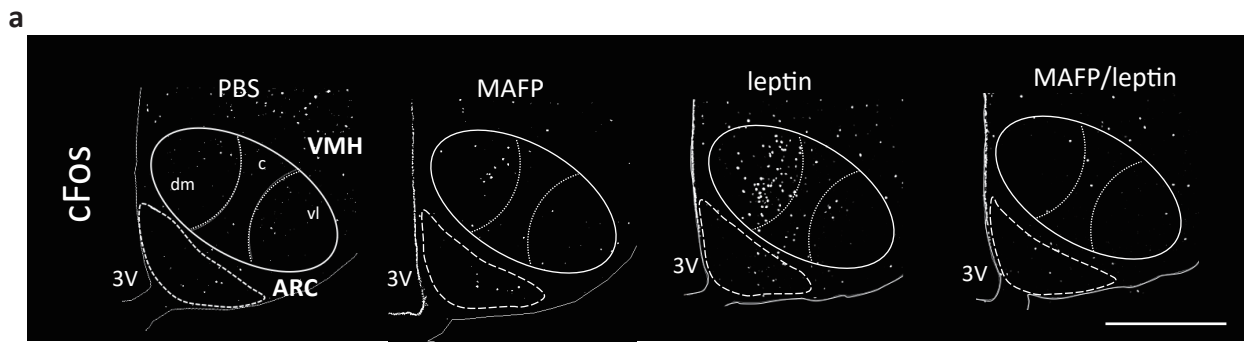
Supplementary Figure 2

Relative amounts of hypothalamic eicosanoids mediated by lipoxygenase (a) or cytochrome P450 (b) after the injection of glucose compared with saline injected mice. n=3 in each experimental group. Data represent the mean fold change in color.



Supplementary Figure 3

Hypothalamic PLC-mediated-pathway does not affect systemic glucose metabolism. (a) Glucose tolerance test (GTT) (0–120 min) after intra-hypothalamic injection (-30 min) of Xestospondin, an IP3 receptor antagonist, (n=7) or vehicle (n=7). (b) GTT (0–120 min) after intra-hypothalamic injection (-30 min) of U73122, a phospholipase C (PLC) inhibitor, (n=7) or vehicle (n=7). All data represent the mean  $\pm$  SEM

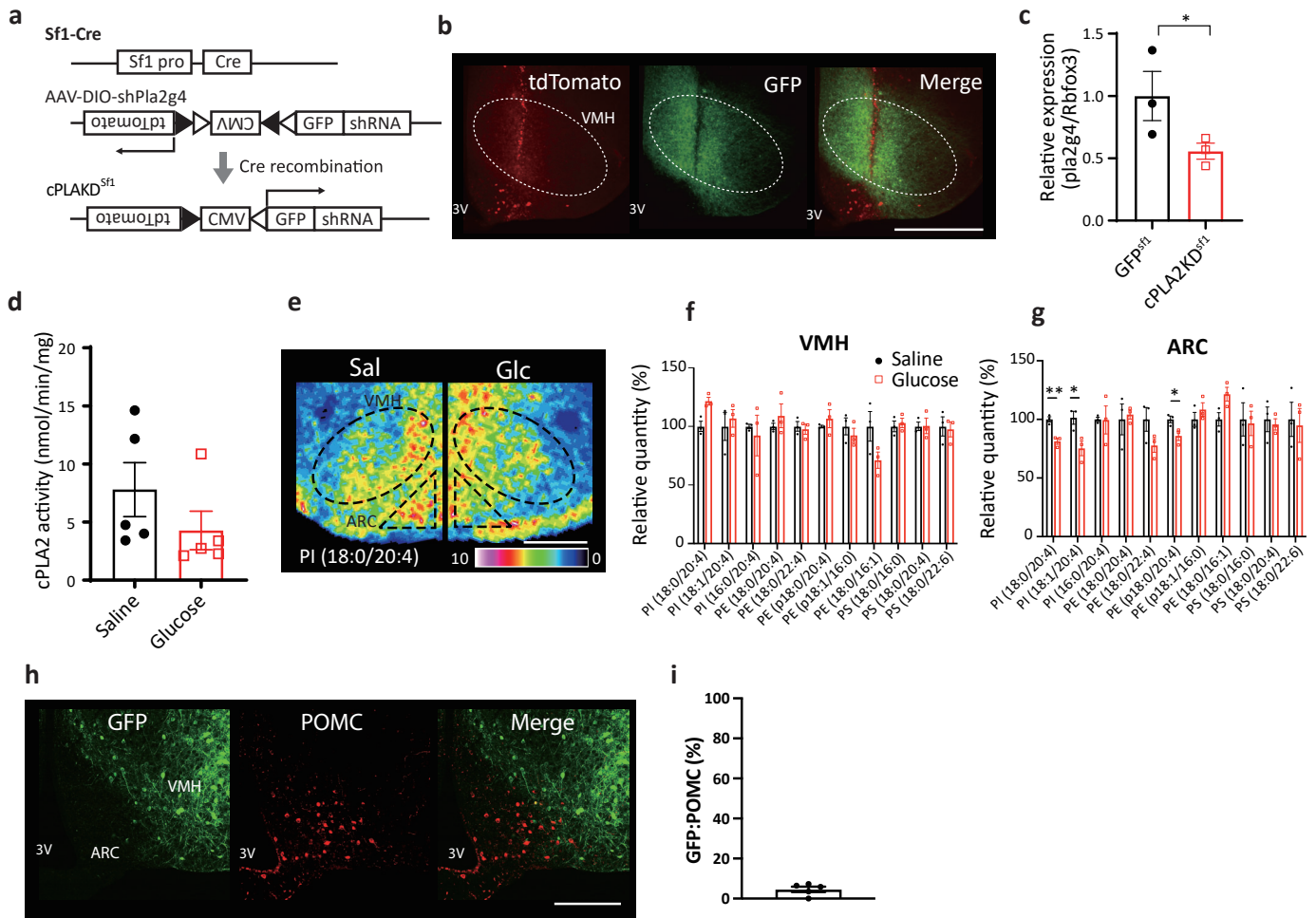


Supplementary Figure 4

Hypothalamic leptin responsiveness is regulated by cPLA2.

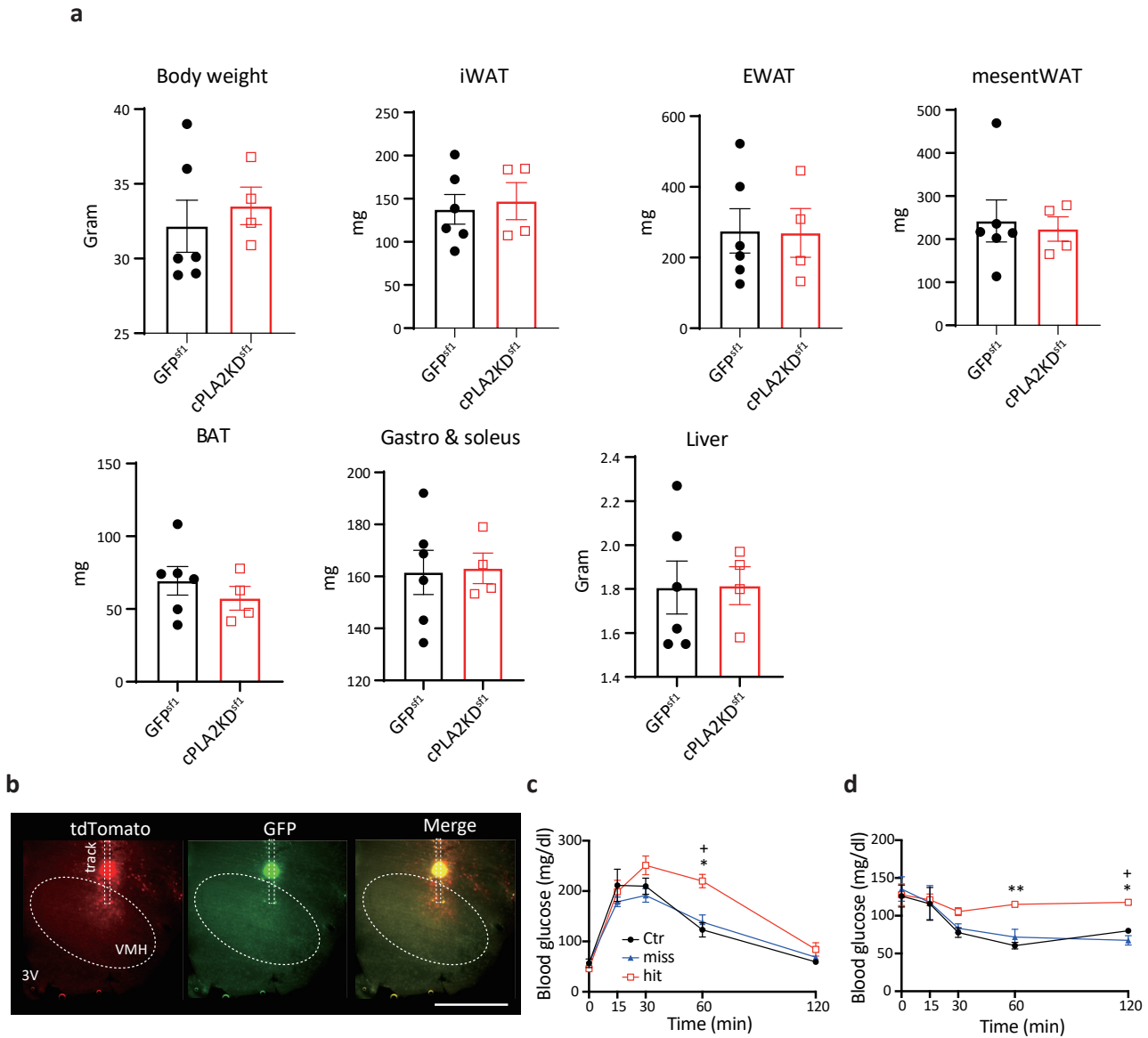
a, Representative micrographs showing immunofluorescent cFos staining in the hypothalamus of mice i.c.v. injected with PBS, MAFP, leptin or both MAFP and leptin. Scale bar: 500  $\mu$ m. b, c, Quantification of cFos expression in the dmVMH, cVMH, vVMH (b), and ARC (c) after i.c.v. injection of PBS, MAFP, leptin or both MAFP and leptin (n=3 in each experimental group) (VMH: two-way ANOVA followed by Sidak multiple comparison test, in b,  $p < 0.0001$  PBS vs Leptin,  $p < 0.0001$  Leptin vs MAFP/Leptin.). d, Representative micrographs showing immunofluorescent pSTAT (Tyr 705) staining in the hypothalamus of mice i.c.v. injected with PBS, MAFP, leptin or both MAFP and leptin. Scale bar: 500  $\mu$ m. e, f, Quantification of pSTAT3 (Tyr 705)-positive cells in the dmVMH, cVMH, vVMH (e), and ARC (f) after i.c.v. injection of PBS, MAFP, leptin or both MAFP and leptin (n=4 in each experimental group) (VMH: two-way ANOVA followed by Sidak multiple comparison test, in e,  $p < 0.0001$  Leptin vs PBS,  $p = 0.0095$  Leptin vs MAFP/Leptin. In f, one-way ANOVA followed by Sidak multiple comparison test,  $p = 0.0408$  Leptin vs MAFP/Leptin.).

All data represent the mean  $\pm$  SEM; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ ; \*\*\*\* =  $p < 0.0001$ .



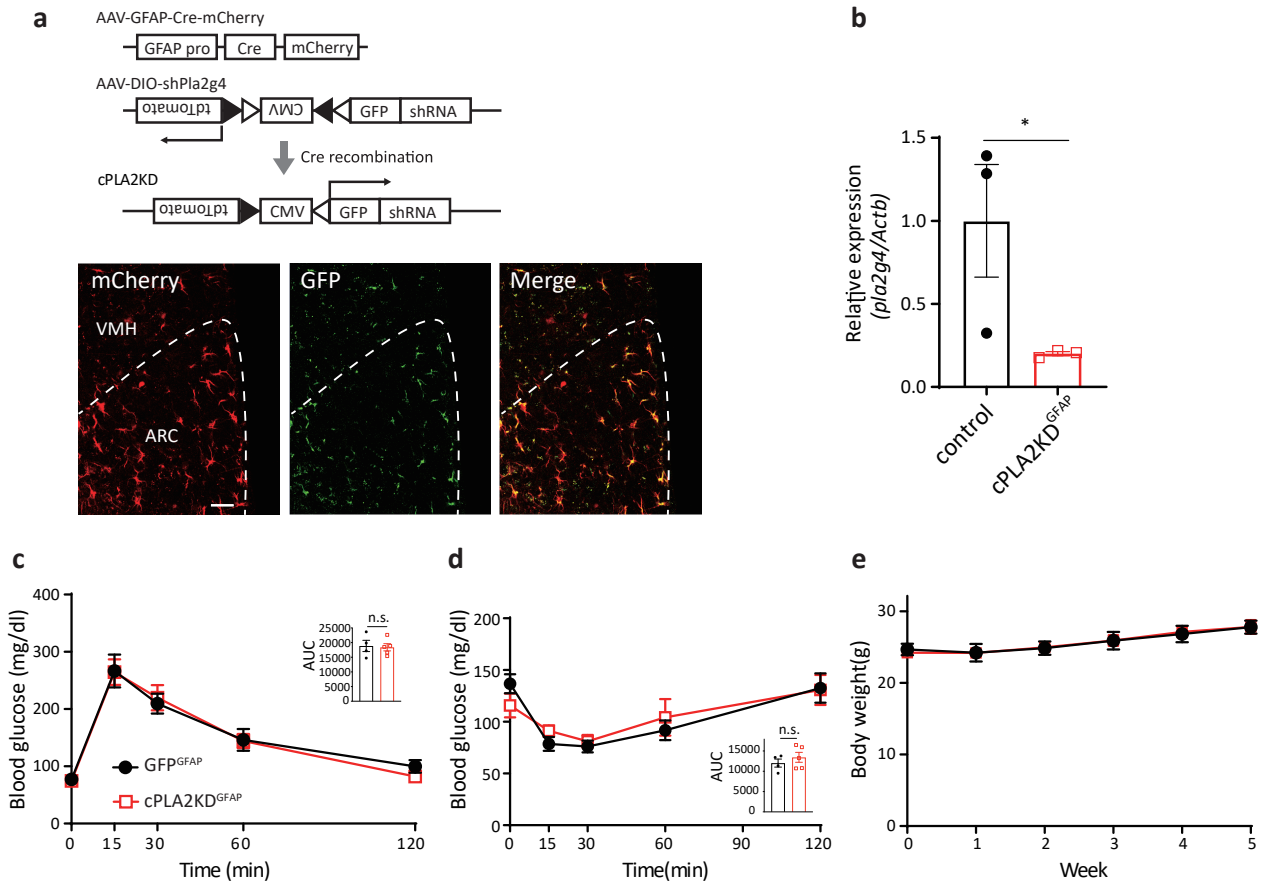
Supplementary Figure 5

a, Construct of AAV8-DIO (CreOn)-shRNA against *mpla2g4*, containing DIO (Double-floxed Inverted Open reading frame) to express shRNA Cre-dependently. b, Representative micrographs showing virus infected (tdTomato) and shRNA expressing (GFP) Sf1-neurons. Scale bar: 500  $\mu$ m. c, Expression of *pla2g4* mRNA in the whole hypothalamus injected with AAV8-DIO-shRNA against *mpla2g4* (cPLA2KD<sup>Sf1</sup>; n=3) compared with control mice (GFP<sup>Sf1</sup>; n=3) (two-tailed t test, p = 0.0497, cPLA2KD<sup>Sf1</sup> vs GFP<sup>Sf1</sup>). d, Enzymatic activity of cPLA2 after injection of saline (n=5) or glucose (2g/kg) (n=5) in C57BL/6J mice. e-g, Distributions of phospholipids in the hypothalamus of cPLA2KD<sup>Sf1</sup> mice after i.p. injection of saline or glucose. e, Representative results of IMS on hypothalamic phosphatidylinositol (PI) (18:0/20:4) of cPLA2KD<sup>Sf1</sup> mice after saline (left half) or glucose injection (right half). Scale bar: 500  $\mu$ m. f, g, Relative intensities of phospholipids in the VMH (f) and ARC (g) of cPLA2KD<sup>Sf1</sup> mice after injection of saline (n=3) or glucose (n=3). (two-tailed t test for each molecule, in g, p = 0.0056 for PI (18:0/20:4), p = 0.0343 for PI (18:1/20:4), p = 0.0453 for PE (p18:0/20:4)). h, Representative micrographs showing immunofluorescence of POMC ( $\alpha$ -MSH) and GFP in Sf1-cre mice injected with AAV-DIO-GFP. Scale bar: 200  $\mu$ m. i, Ratio of GFP expressed POMC neurons (n=5). All data represent the mean  $\pm$  SEM; \* = p<0.05; \*\* = p<0.01



Supplementary Figure 6

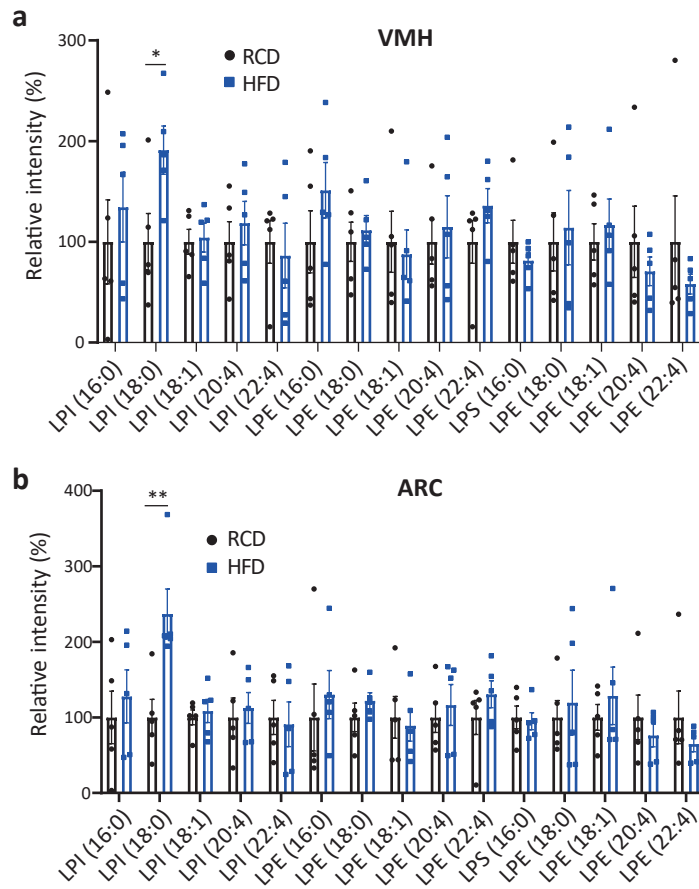
a, Body weight and tissue weight in cPLA2KD<sup>Sf1</sup> mice (n=4) and GFP<sup>Sf1</sup> mice (n=5) 8 weeks after the AAV injection. (iWAT: inguinal white adipose tissue. EWAT: epididymal white adipose tissue. mesentWAT: mesenteric white adipose tissue. BAT: brown adipose tissue). b-d, Phenotype of mice with “hit” and “miss” injection of AAV8-DIO-shRNA against *mpla2g4* into the VMH in Sf1-cre mice. b, Representative micrographs showing the “miss” injection of virus into the VMH. c, Glucose tolerance test on the Sf1-cre mice received “miss” (n=3) or “hit” (n=4) injection of AAV8-DIO-shRNA against *mpla2g4* or hit injection of AAV-DIO-GFP (Ctr) (n=3) (two-way ANOVA followed by Sidak multiple comparison test, p = 0.0156 at time = 60 Ctr vs hit, p = 0.0302 at time =60 miss vs hit). d, Insulin tolerance test on the mice receive “miss” (n=3) or “hit” (n=3) injection or hit injection of AAV-DIO-GFP (Ctr) (n=3) (two-way ANOVA followed by Sidak multiple comparison test, p = 0.0022 at time = 60 Ctr vs hit, p = 0.0117 at time = 120 Ctr vs hit, p = 0.0141 at time = 120, miss vs hit). Data represent the mean ± SEM. \* = p<0.05 (Ctr versus hit) ; \*\* = p<0.01 (Ctr versus hit) ; + = p<0.05 (hit versus miss).



Supplementary Figure 7

Knockdown of astrocytic cPLA2 in hypothalamus (cPLA2KD<sup>GFAP</sup>) did not change body weight and glucose metabolism.

a, Construct of AAV8-GFAP-Cre-mCherry and AAV8-DIO-shRNA against *mpla2g4* and representative micrographs showing virus infected (tdTomato) and shRNA expressing (GFP) astrocytes in the ARC. Scale bar: 25  $\mu$ m. b, Relative expression of cPLA2 in the hypothalamus of cPLA2KD<sup>GFAP</sup> and control mice. n=3 in each experimental group (two-tailed t test, p = 0.039, Ctr vs cPLA2KD<sup>GFAP</sup>). c, Glucose tolerance test in cPLA2KD<sup>GFAP</sup> mice (n=5) and GFP<sup>GFAP</sup> mice (n=4). d, Insulin tolerance test in cPLA2KD<sup>GFAP</sup> mice (n=5) and GFP<sup>GFAP</sup> mice (n=4). e, Body weight change in cPLA2KD<sup>GFAP</sup> mice (n=5) and GFP<sup>GFAP</sup> mice (n=4) after viral injection. All data represent the mean  $\pm$  SEM. \* = p<0.05



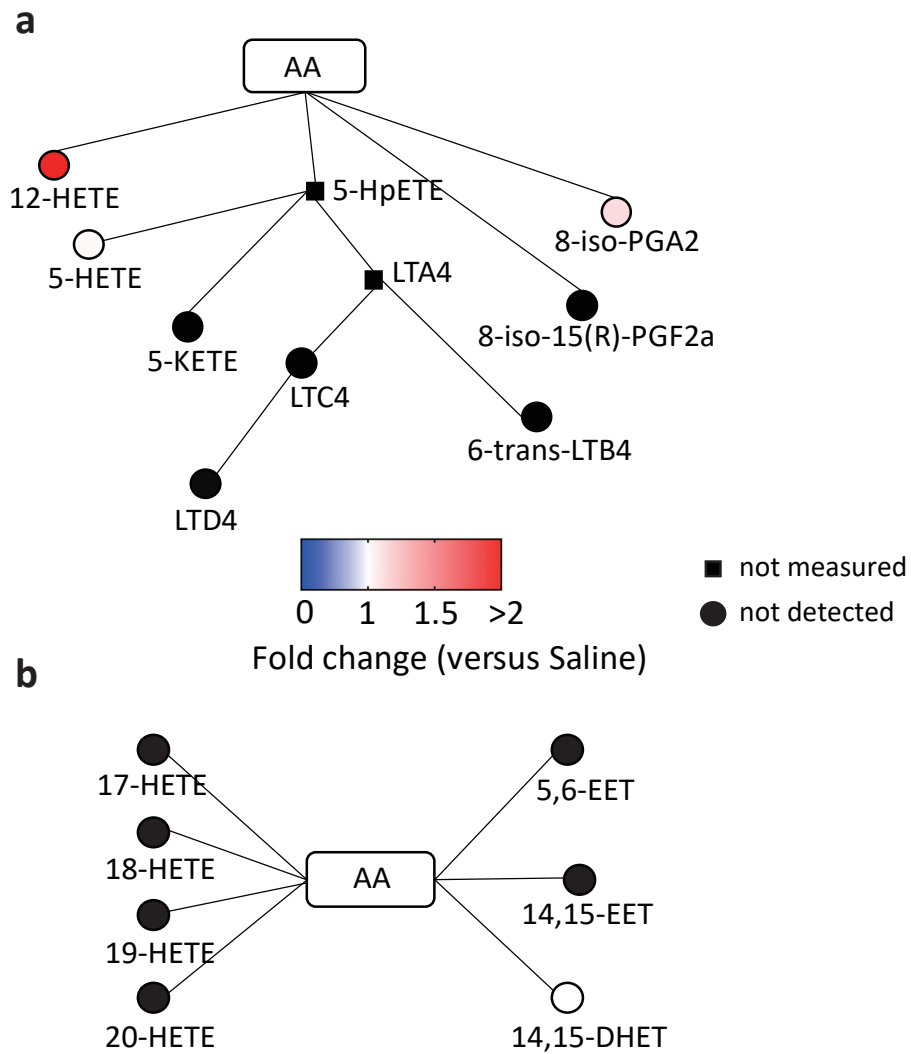
Supplementary Figure 8

Relative intensities of lipospecies in the VMH (a) and ARC (b) by IMS from RCD (n=5) or HFD(n=5) fed mice. (two-tailed t test for each molecule, LPI(18:0) in VMH,  $p = 0.0389$  RCD vs HFD, in ARC  $p=0.0099$  RCD vs HFD).

All data represent the mean  $\pm$  SEM; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ .

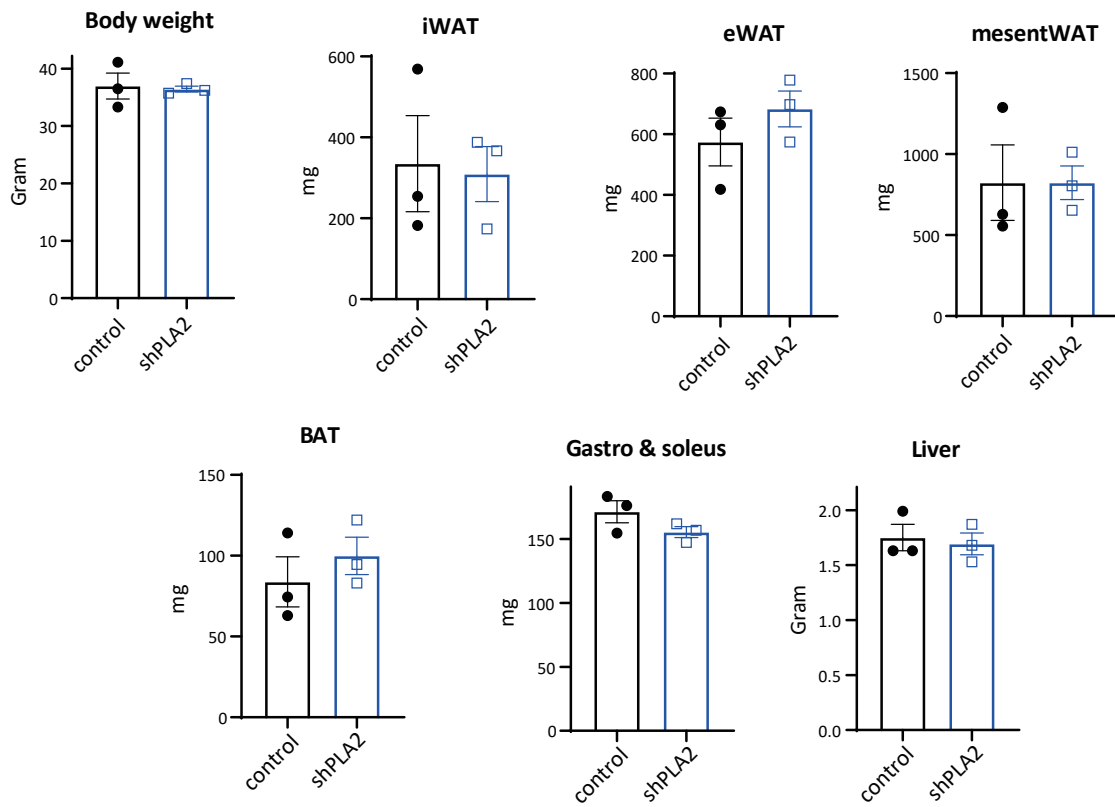
LPI: lysophosphatidylinositol, LPS: lysophosphatidylserine, LPE: lysophosphatidylethanolamine.





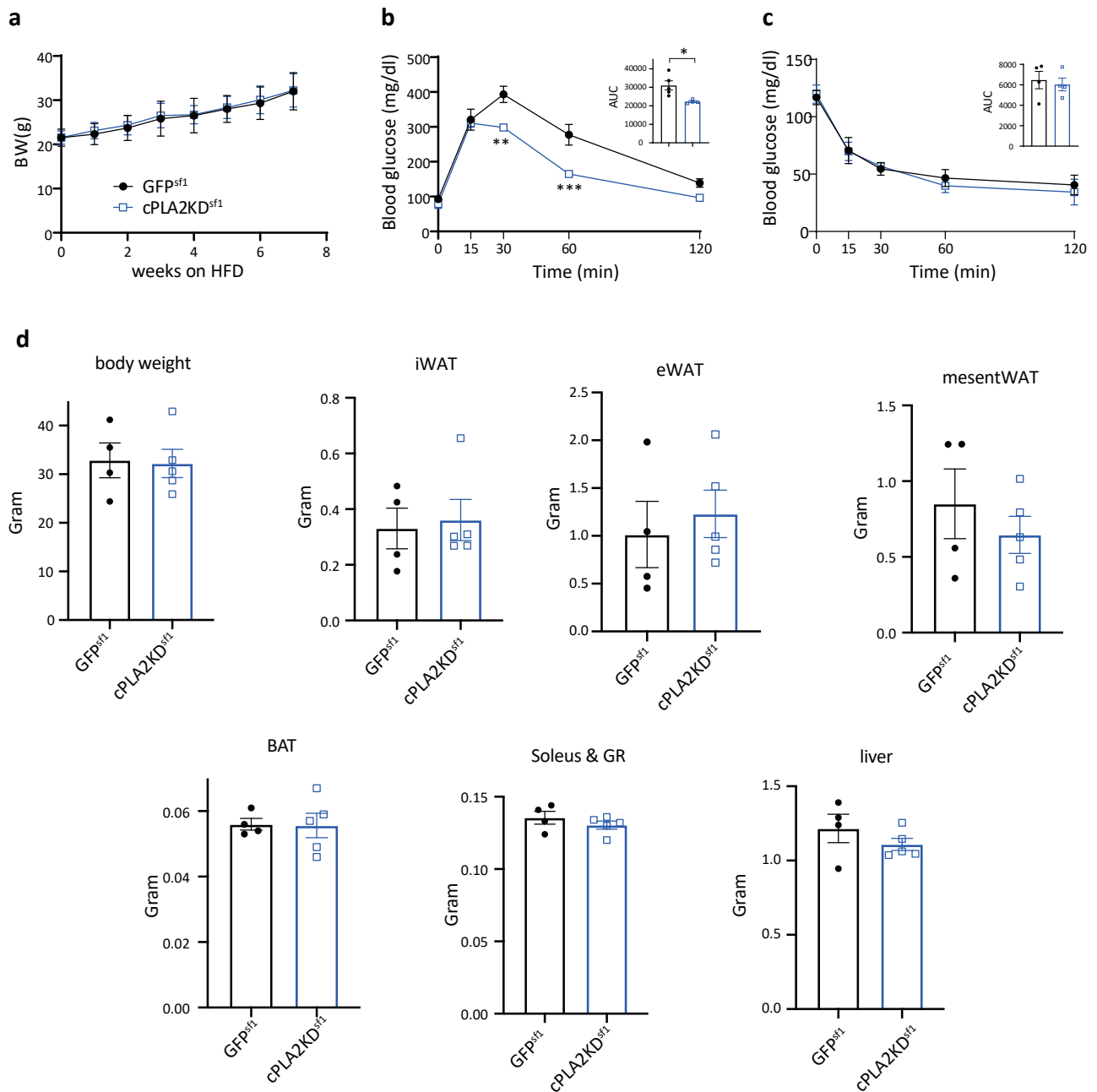
Supplementary Figure 9

Relative amounts of hypothalamic eicosanoids mediated by lipoxygenase (a) or cytochrome P450 (b) in RCD or HFD fed mice. n=3 each experimental group. Data represent the mean fold change in color.



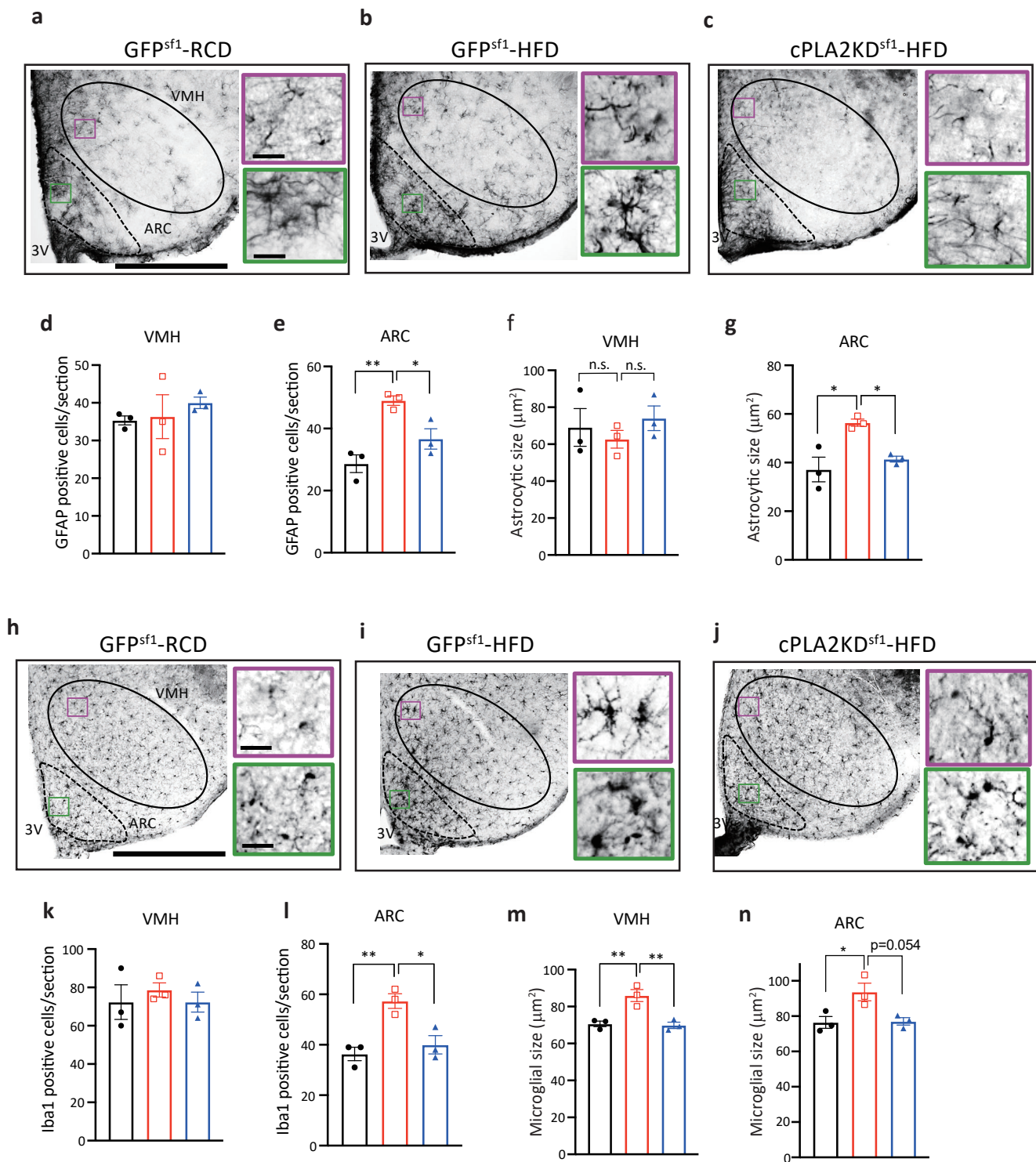
Supplementary Figure 10

Body weight and tissue weight in male cPLA2KD<sup>Sf1</sup> mice (n=3) and GFP<sup>Sf1</sup> mice (n=3) after 8 weeks of a HFD feeding. (iWAT: inguinal white adipose tissue. eWAT: epididymal white adipose tissue. mesentWAT: mesenteric white adipose tissue. BAT: brown adipose tissue). All data represent the mean  $\pm$  SEM.



Supplementary Figure 11

Knockdown of cPLA2 improves HFD-induced impairment of glucose metabolism in female mice. a, Body weight change in female cPLA2KD<sup>Sf1</sup> mice (n=4) and GFP<sup>Sf1</sup> mice (n=4) under HFD. b, Glucose tolerance test on female cPLA2KD<sup>Sf1</sup> mice (n=4) and GFP<sup>Sf1</sup> mice (n=5). (two-way ANOVA followed by Sidak multiple comparison test,  $p = 0.0059$  at time = 30,  $p = 0.0009$  at time = 60, GFP<sup>Sf1</sup> vs cPLA2KD<sup>Sf1</sup>. two-tailed t test in area under the curve (AUC) during GTT,  $p = 0.0177$ , GFP<sup>Sf1</sup> vs cPLA2KD<sup>Sf1</sup>). c, Insulin tolerance test on cPLA2KD<sup>Sf1</sup> (n=4) mice and GFP<sup>Sf1</sup> mice (n=4) after 8 weeks of HFD feeding. d, Body weight and tissue weight in cPLA2KD<sup>Sf1</sup> mice (n=5) and GFP<sup>Sf1</sup> mice (n=4) after 8 weeks of HFD feeding. (iWAT: inguinal white adipose tissue. eWAT: epididymal white adipose tissue. mesentWAT: mesenteric white adipose tissue. BAT: brown adipose tissue.) All data represent the mean  $\pm$  SEM; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ .



Supplementary Figure 12

Knockdown of cPLA2 prevents HFD-induced microgliosis and astrogliosis in female mice.

a-c, Left: Representative micrographs showing immunofluorescent GFAP staining in the hypothalamus of RCD-fed GFP<sup>Sf1</sup> mice (GFP<sup>Sf1</sup>-RCD) (a), HFD-fed GFP<sup>Sf1</sup> mice (GFP<sup>Sf1</sup>-HFD) (b) and HFD-fed cPLA2KD<sup>Sf1</sup> (cPLA2KD<sup>Sf1</sup>-HFD) mice (c). Scale bar: 500  $\mu$ m. Right: magnified rectangles showed in left. Scale bar: 30  $\mu$ m. d,e, Quantification of GFAP-positive cells in the VMH (d) or ARC (e) of GFP<sup>Sf1</sup>-RCD (n=3), GFP<sup>Sf1</sup>-HFD (n=3) and cPLA2KD<sup>Sf1</sup>-HFD (n=3) mice (one-way ANOVA followed by Sidak multiple comparison test, in e,  $p = 0.005$  GFP<sup>Sf1</sup> RCD vs GFP<sup>Sf1</sup> HFD,  $p = 0.0497$  GFP<sup>Sf1</sup> HFD vs cPLA2KD<sup>Sf1</sup> HFD). f,g, Size of GFAP positive cells in in the VMH (f) or ARC (g) of GFP<sup>Sf1</sup>-RCD (n=3), GFP<sup>Sf1</sup>-HFD (n=3) and cPLA2KD<sup>Sf1</sup>-HFD (n=3) mice (one-way ANOVA followed by Sidak multiple comparison test, in g,  $p = 0.0004$  GFP<sup>Sf1</sup> RCD vs GFP<sup>Sf1</sup> HFD,  $p = 0.0128$  GFP<sup>Sf1</sup> HFD vs cPLA2KD<sup>Sf1</sup> HFD). h-j, Left: Representative micrographs showing immunohistochemistry Iba1 staining in the hypothalamus of GFP<sup>Sf1</sup>-RCD (h), GFP<sup>Sf1</sup>-HFD (i) and cPLA2KD<sup>Sf1</sup>-HFD mice (j). Scale bar: 500  $\mu$ m. Right: magnified rectangles showed in left. Scale bar: 30  $\mu$ m. k,l, Quantification of Iba1-positive cells in the VMH (k) or ARC (l) of GFP<sup>Sf1</sup>-RCD (n=3), GFP<sup>Sf1</sup>-HFD (n=3) and cPLA2KD<sup>Sf1</sup>-HFD (n=3) mice (one-way ANOVA followed by Sidak multiple comparison test, in l,  $p = 0.0089$  GFP<sup>Sf1</sup> RCD vs GFP<sup>Sf1</sup> HFD,  $p = 0.0219$  GFP<sup>Sf1</sup> HFD vs cPLA2KD<sup>Sf1</sup> HFD). m,n, Size of Iba1 positive cells in in the VMH (m) or ARC (n) of GFP<sup>Sf1</sup>-RCD (n=3), GFP<sup>Sf1</sup>-HFD (n=3) and cPLA2KD<sup>Sf1</sup>-HFD (n=3) mice (one-way ANOVA followed by Sidak multiple comparison test, in m,  $p = 0.0162$  GFP<sup>Sf1</sup> RCD vs GFP<sup>Sf1</sup> HFD,  $p = 0.0077$  GFP<sup>Sf1</sup> HFD vs cPLA2KD<sup>Sf1</sup> HFD, in n,  $p = 0.0042$  GFP<sup>Sf1</sup> RCD vs GFP<sup>Sf1</sup> HFD,  $p = 0.0038$  GFP<sup>Sf1</sup> HFD vs cPLA2KD<sup>Sf1</sup> HFD). All data represent the mean  $\pm$  SEM; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ .

Supplementary table 1 List of reagents and resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Rabbit-anti-cFos	Santa Cruz	CAT#SC-52
Rabbit-anti-Iba1	FUJIFILM Wako	CAT#LKN5648
Rabbit-anti-GFAP	Sigma-Aldrich	CAT#HPA056030
Rabbit-anti-GFP	Frontier institute	AB_2571573
Rabbit-anti-pSTAT3 (Tyr 705) antibody	Cell Signaling Technologies	9145S
Phospho-cPLA2 (Ser505) Antibody	Cell Signaling Technologies	2831S
cPLA2 Antibody	Cell Signaling Technologies	2832S
Streptavidin, DyLight 488 Conjugated	Vector Biolabs	SA-5488
Biotinylated-Goat-Anti-Rabbit (IgG) secondary antibody	Thermo Fisher Scientific	A11034
Anti-rabbit IgG (H+L), F(ab') <sub>2</sub> Fragment (Alexa Fluor 647 Conjugate)	Cell Signaling Technologies	4414S
Anti-rabbit IgG (H+L), F(ab') <sub>2</sub> Fragment (Alexa Fluor® 488 Conjugate)	Cell Signaling Technologies	4412S
<b>Bacterial and Virus Strains</b>		
AAV8-DIO (Cre-On)-shRNA against <i>mpla2g4</i>	Vigene	Custom made
AAV8-GFAP-mcherry-Cre	UNC Vector Core	Lot# AV5056C
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
9-Aminoacridine hemihydrate	Thermo Fisher Scientific	134410010
Glucose	FUJIFILM Wako	049-31165
Novolin R 100 IU	Novo Nordisk	N/A
Glucose-D-[3- <sup>3</sup> H]	Muromachi Kikai	ART0124
2-Deoxy-D-glucose <sup>14</sup> C(U)	Muromachi Kikai	ARC0112A
Methyl arachidonyl fluorophosphonate (MAFP)	Sigma-Aldrich	M2939
Indomethacin (Indo)	Sigma-Aldrich	I7378
Ethanol	FUJIFILM Wako	056-03341
Dimethyl sulfoxide	Nacalai Tesque	13407
TRIzol™ reagent	Thermo Fisher Scientific	15596026
<b>Critical Commercial Assays</b>		
Mouse Insulin ELISA KIT	FUJIFILM Wako	633-23919
Cytosolic Phospholipase A2 Assay Kit	Abcam	Ab133090
Secretory-phospholipase-A2 Assay Kit	Abcam	Ab133089
TaqMan™ Gene Expression Master Mix	Thermo Fisher Scientific	4369016
M-MLV Reverse Transcriptase	Thermo Fisher Scientific	28025013
VECTASTAIN Elite ABC Kit	Vector Laboratories	PK-6100
DAB tablet	FUJIFILM Wako	045-22833

Supplementary table 2

Assignment of lipid molecular species by IMS negative ion mode

<u>Lipid assignment</u>	<u>[M-H]<sup>-</sup>(m/z)</u>
Palmitic acid	255.25
Oleic acid	281.3
Stearic acid	283.35
Arachidonic acid	303.3
DHA	327.33
PE (p18:1/16:0), plasmalogen	700.6
PE (18:0/16:1)	716.6
PE (p18:0/20:4), plasmalogen	750.5
PS (18:0/16:0)	762.6
PE (18:0/20:4)	766.5
PE (18:0/22:4)	794.5
PS (18:0/20:4)	810.6
PS (18:0/22:6)	834.6
PI (16:0/20:4)	857.6
PI (18:1/20:4)	883.55
PI (18:0/20:4)	885.77

LPE 16:0	452.3
LPE 18:0	480.3
LPE 18:1	478.3
LPE 20:4	500.3
LPE 22:4	528.3
LPI 16:0	571.3
LPI 18:0	599.3
LPI 18:1	597.3
LPI 20:4	619.3
LPI 22:4	647.3
LPS 16:0	496.3
LPS 18:0	524.3
LPS 18:1	522.3
LPS 20:4	544.3
LPS 22:4	572.3

