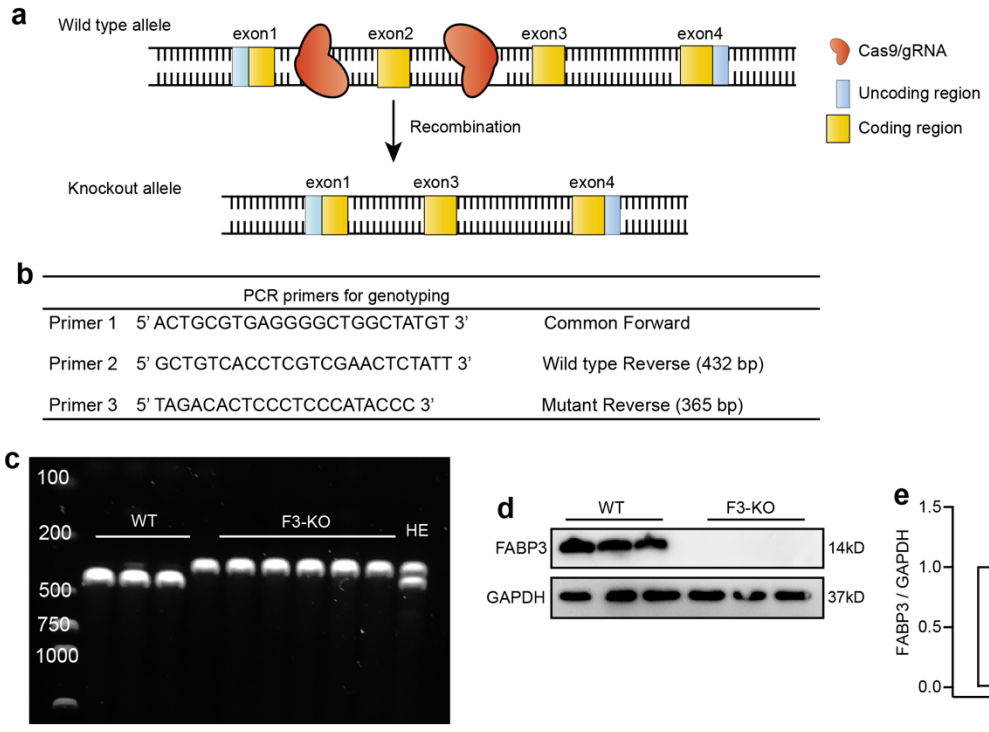
SUPPLEMENTAL MATERIALS

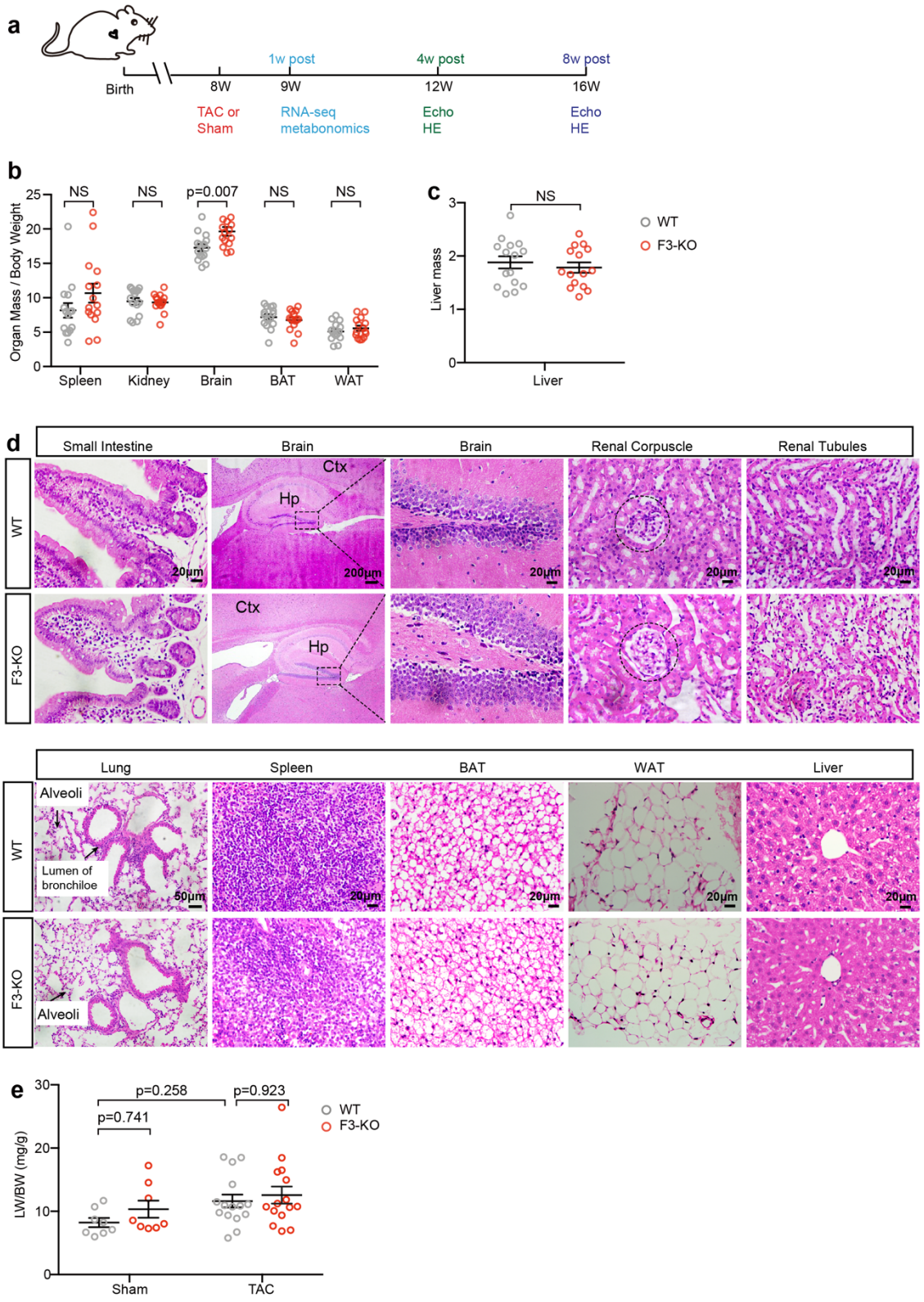
Supplementary Fig. 1-8

Supplementary Table. 1, 2

Supplementary Figures

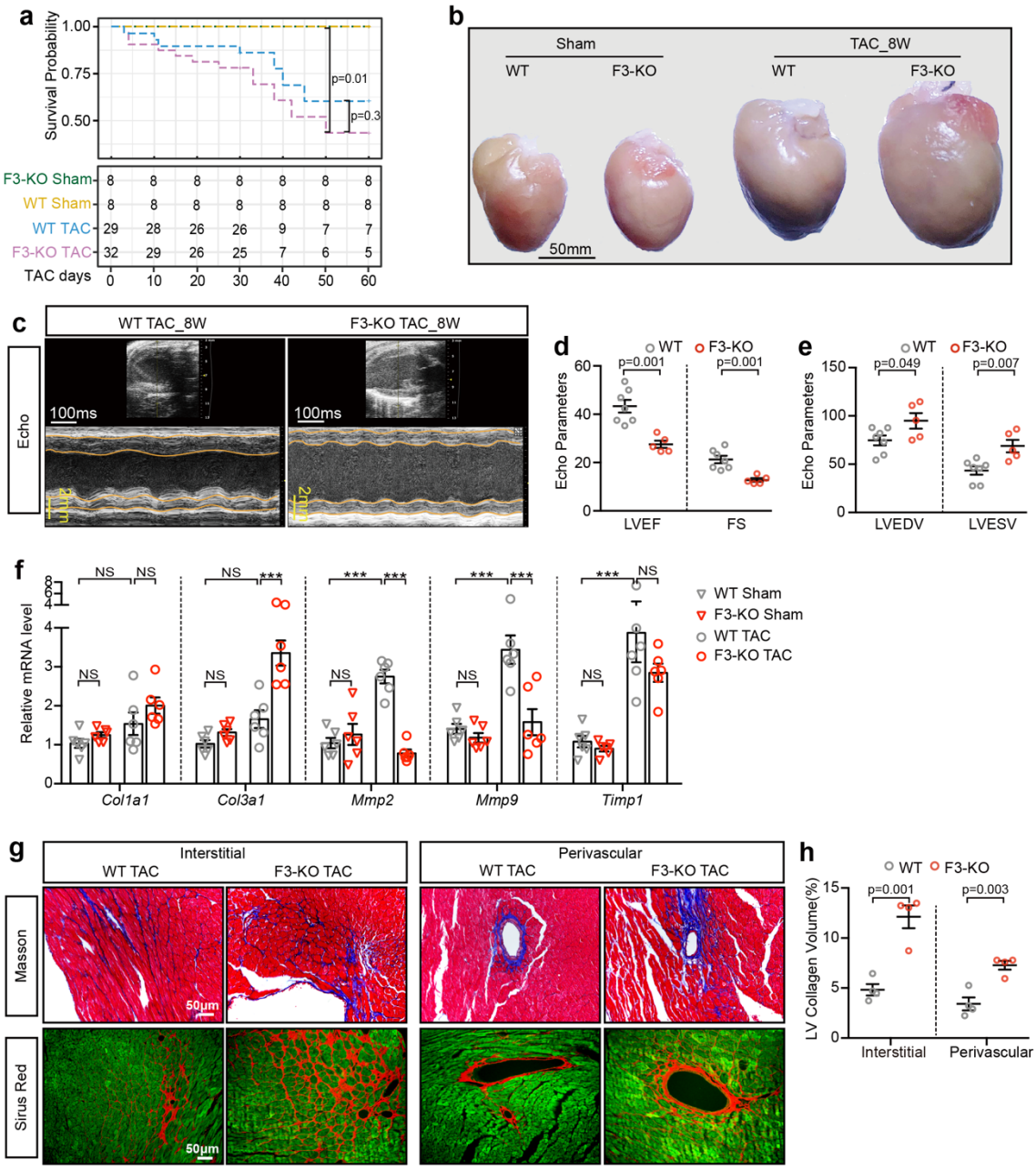


Supplementary Fig. 1 Construction of *Fabp3*-knock-out mice with the CRISPR/Cas9 method. **a** Schematic diagram of *Fabp3*-KO strategy. **b** Sequences of primers used for genotyping. Primers 1 and 2 amplify a 432-bp fragment in the wild-type (WT) allele, while primers 1 and 3 amplify a 365-bp fragment in the homozygous mutant allele. **c** Representative PCR genotyping images including WT, homozygous (F3-KO), and heterozygous mice (HE) mice. **d** Representative western blot images showing FABP3 expression in *Fabp3* deletion hearts (F3-KO) and its WT alleles. **e** Quantification of (**d**). [**e**, n = 3, Student's *t*-test.]



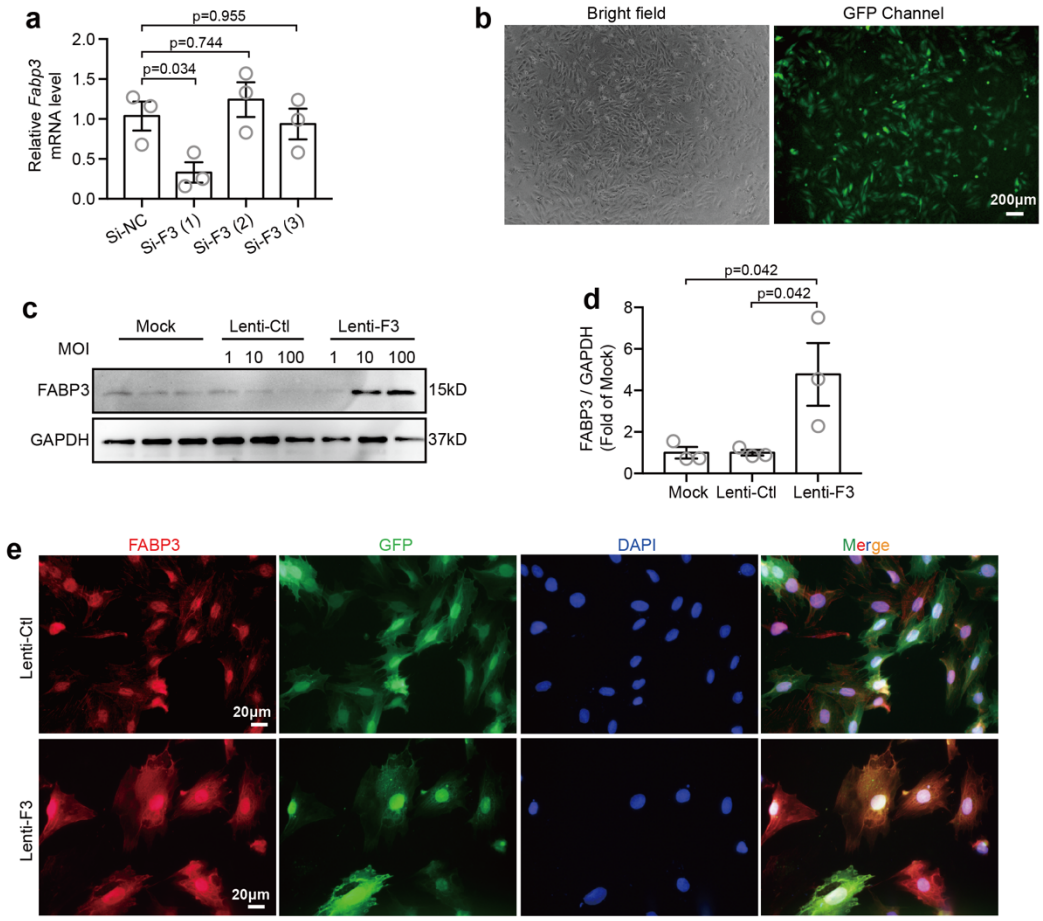
Supplementary Fig. 2 FABP3 defect does not affect tissue mass or their morphology except for hearts. a Experimental schematic diagram illustrating the strategy for multi-omics analysis,

echocardiography, and morphology analysis of WT and F3-KO mice after TAC or sham surgery. **b** The ratio of organ mass to body weight in WT and F3-KO mice at 4 weeks after TAC operations. **c** Liver mass in F3-KO mice and its WT littermates. **d** Representative H&E images of tissue organs, including the small intestine, brain, kidney, lung, brown adipose tissue (BAT), and white adipose tissue (WAT), from WT and F3-KO mice at 4 weeks after TAC operations. **e** The ratio of lung weight to body weight from sham- or TAC-operated WT and F3-KO mice. [**b**, **c**, n = 15, Student's *t*-test; **e**, n = 8, 8, 15, 15, respectively, Tukey post-hoc test.]

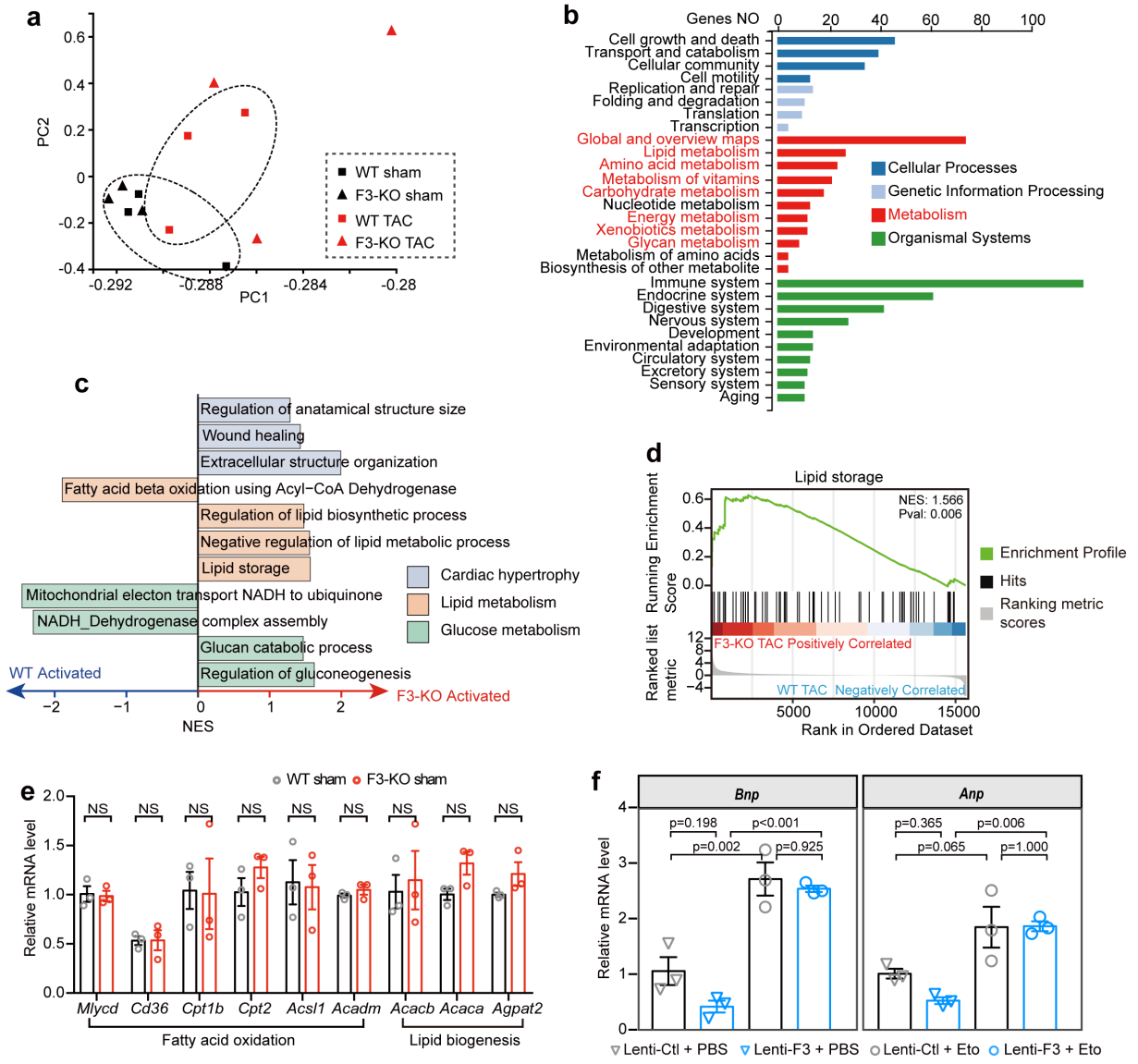


Supplementary Fig. 3 FABP3-null exacerbates TAC-induced cardiac dysfunction and fibrosis. a Survival probability of WT and F3-KO mice up to 8 weeks after TAC or sham surgery. **b** Gross heart morphology images in WT and F3-KO mice at 8 weeks after surgery. **c** Representative echo images of WT and F3-KO mice at 8 weeks after TAC operation. **d** Quantification of left ventricular ejection fraction (LVEF) and fractional shortening (FS) in panel

(**c**). **e** Quantification of left ventricular volume at diastole and systole (LVEDV, LVESV) in panel (**c**). **f** mRNA expression of fibrosis-related genes (*Col1a1*, *Col3a1*, *Mmp2*, *Mmp9*, and *Timp1*) from WT and F3-KO hearts at sham or 8 weeks post-surgery. NS, not significant, *** $p < 0.001$. **g** Representative images of Masson (top) and Sirus Red staining (bottom) in interstitial and perivascular area of WT and F3-KO hearts. **h** Quantification results of collagen volume in panel (**g**). [**d**, **e**, n = 7, 5, respectively; **f**, n = 6, Tukey's post-hoc test; **h**, n = 4; (**d**, **e**, and **h**): Student's *t*-test.]

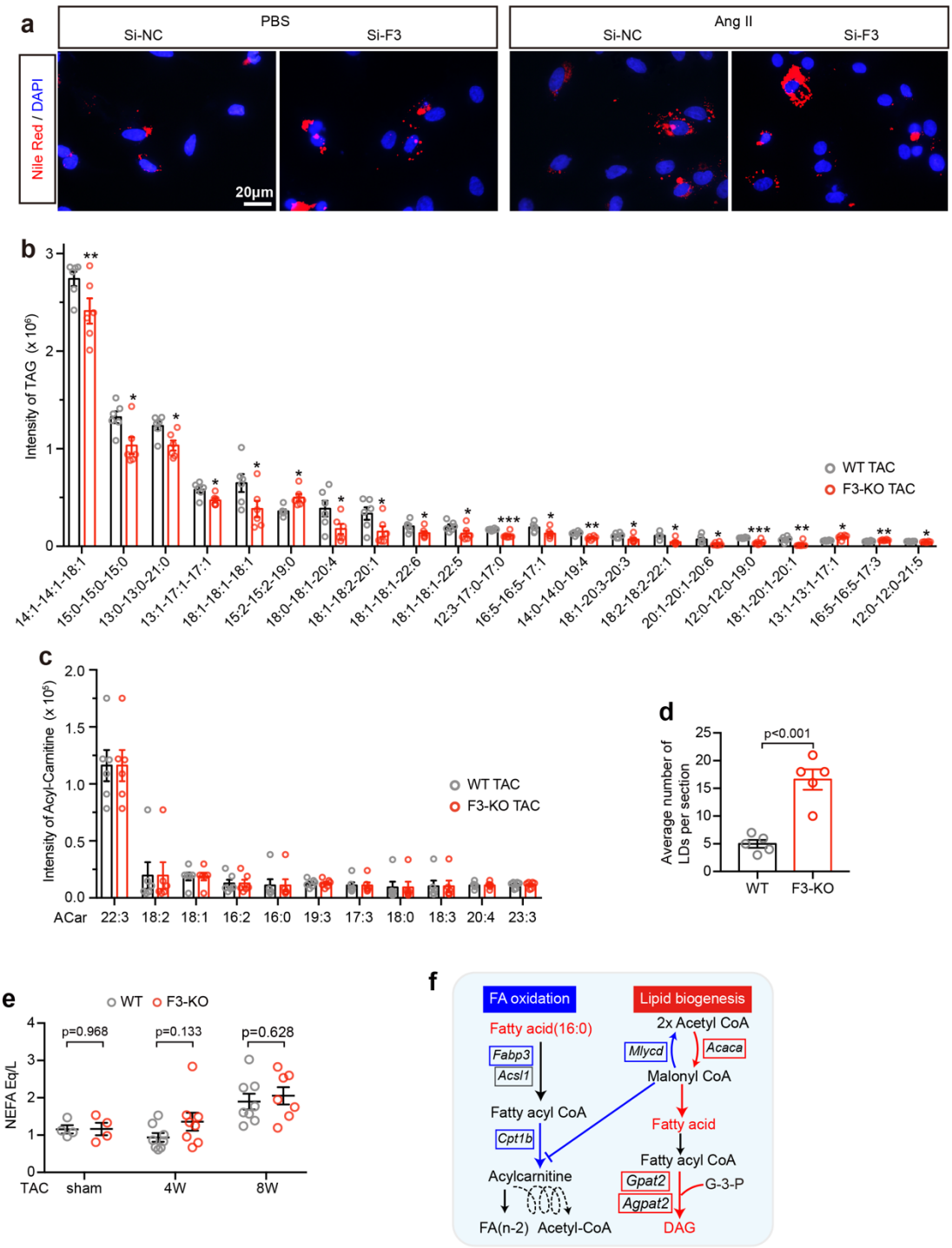


Supplementary Fig. 4 Manipulating *Fabp3* expression with siRNA or lentivirus. a H9C2 cells were transfected with si-RNA targeting *Fabp3* (Si-F3(1), Si-F3(2), Si-F3(3)) or its negative control (Si-NC), then total RNA was extracted for qPCR analysis to determine *Fabp3* mRNA expression. **b** H9C2 cells were transfected with lentivirus and visualized using a microscope to determine the transfection efficiency; (Left) bright field of cultured cells, (Right) GFP channel of the same field corresponding to the left. **c** H9C2 cells were transfected with *Fabp3*-lentivirus or its control virus (Lenti-Ctl) at different MOIs (1, 10, 100). The whole cell lysates were then analyzed with western blotting. **d** Quantification results of (**c**). **e** Immunofluorescence co-staining of FABP3 (red), GFP (green), and DAPI (blue) after Lenti-Ctl or Lenti-F3 transfection. [**a**, **d**, n = 3; (**a**, **d**): Dunnett's post-hoc test.]



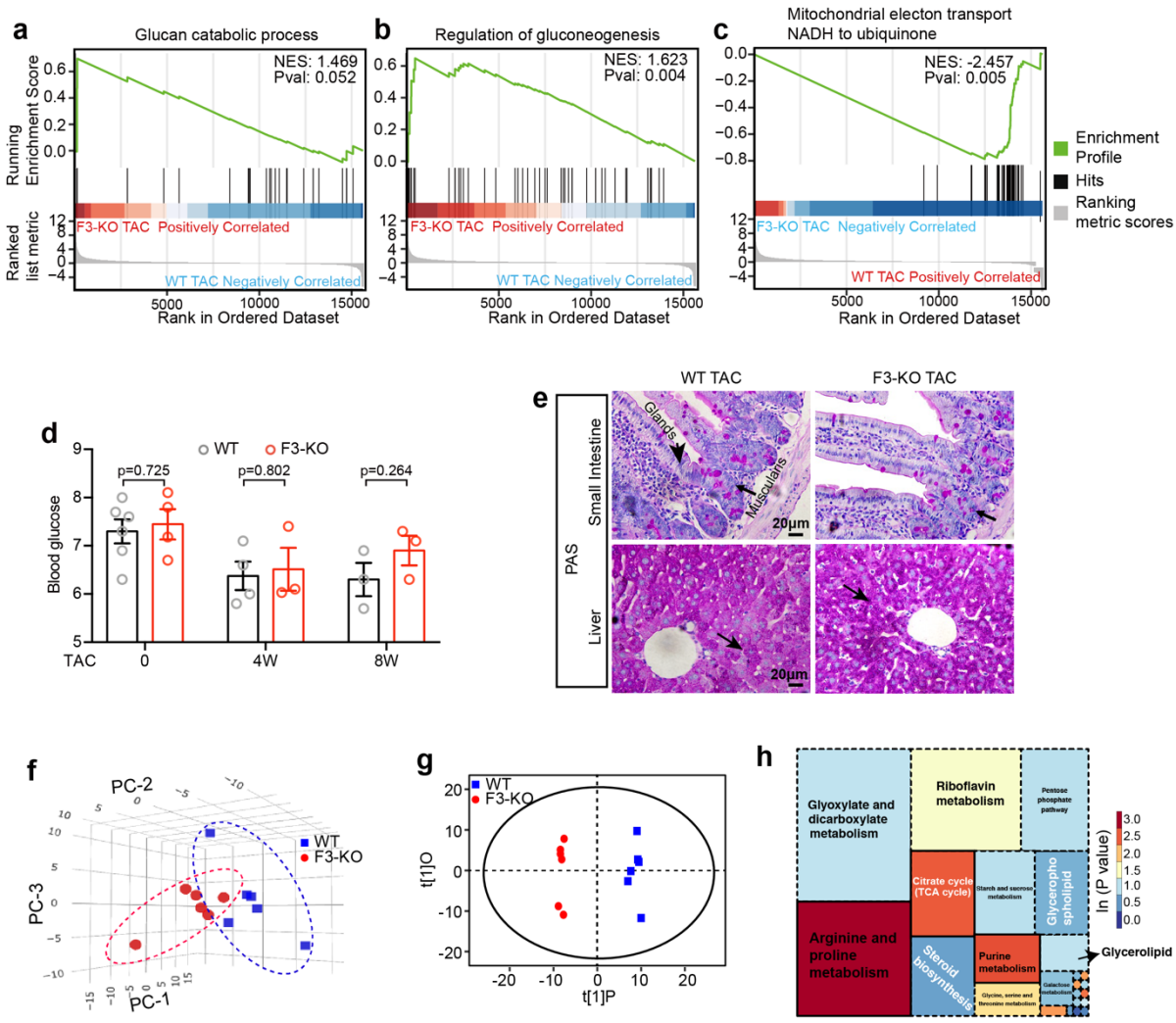
Supplementary Fig. 5 FABP3-defect hearts exhibit deranged metabolic pathways. a PCA plot comprising all samples in RNA-seq analysis (n = 3 biological replicates / per group). **b** KEGG functional enrichment results including four biological terms (cellular process, genetic information processing, metabolism, and organismal systems) between TAC-operated F3-KO and WT hearts. **c** GSEA analysis based on GO terms comparing differentially activated pathways between TAC-operated F3-KO and WT hearts. Results are displayed according to the normalized enrichment score (NES). Pathways with positive NES indicated more upregulated genes in F3-KO hearts. **d** GSEA plot showed that *Fabp3*-null hearts are positively correlated with lipid storage. **e** The mRNA expression of FAO and lipid biogenesis genes in WT and F3-KO hearts after sham operations was

determined by qPCR assay. NS, not significant. **f** NRVMs with knocking-in expression of *Fabp3* were treated with or without etomoxir (Eto), then the mRNA expression of *Bnp* and *Anp* was determine by qPCR assay. [**e**, n = 3, Student's *t*-test; **f**, n = 3, Tukey's post-hoc test.]



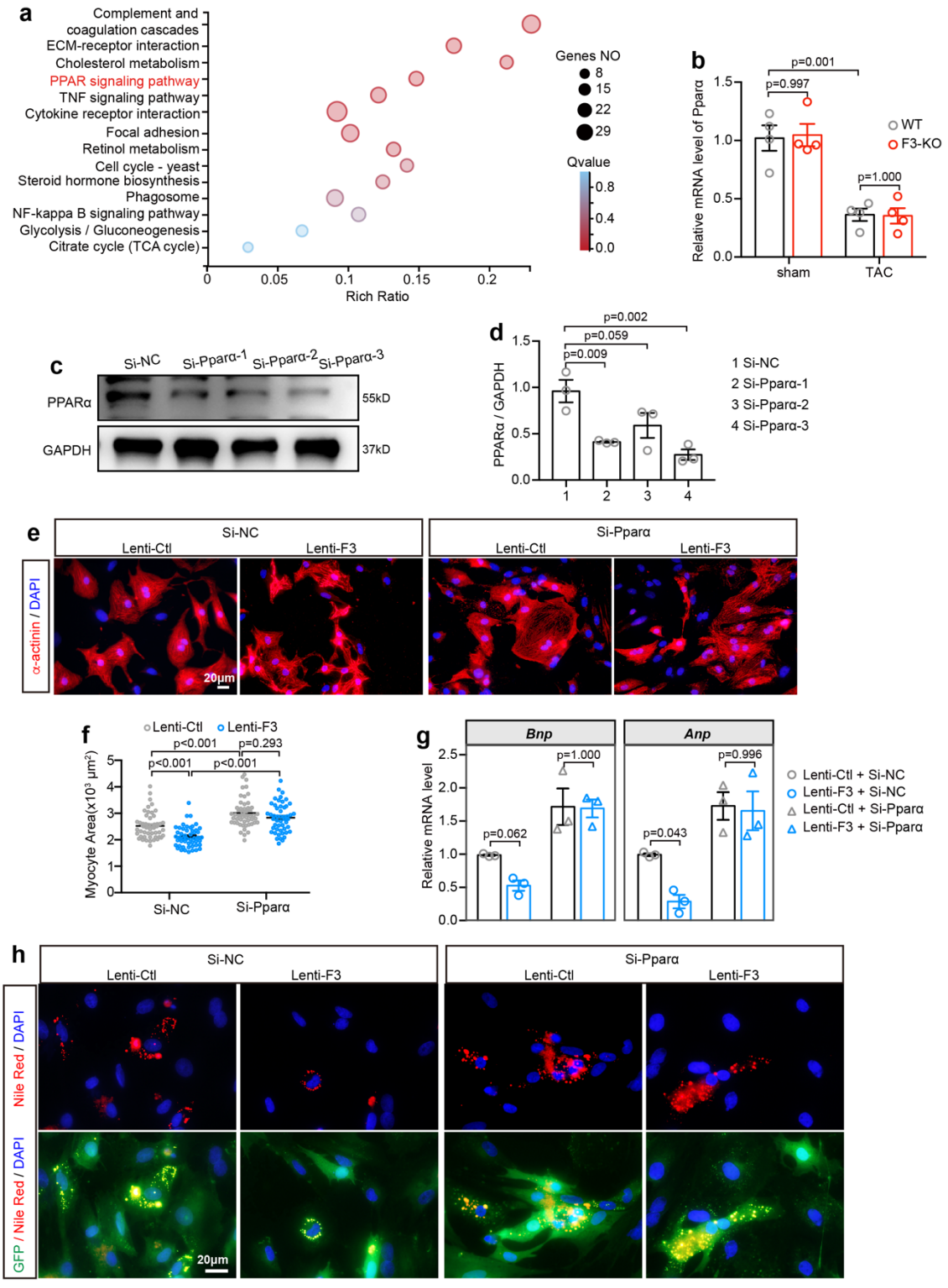
Supplementary Fig. 6 FABP3-depletion leads to excessive cardiac lipid accumulation after TAC operation. a NRVMs with knocking-down expression of *Fabp3* or its scrambled control were stained with nile red and DAPI (blue). **b** The level of triglyceride (TAG) in WT and F3-KO hearts was determined by LC-MS analysis, 6 biological replicates / per group. * $p < 0.05$, ** $p <$

0.01. **c** The level of Acyl-Carnitine (ACar) in WT and F3-KO hearts was determined by LC-MS analysis. **d** Quantification the number of lipid droplet in Fig. 4l. **e** Blood non-esterified fatty acid (NEFA) levels were measured in F3-KO and WT littermates at 0, 4, and 8 weeks after TAC operations. **f** Schematic diagram including FAO/lipid biogenesis genes and metabolites shows defective FAO and activated lipid biogenesis in F3-KO hearts. Red font and rectangles denote upregulated metabolites or genes, and blue represents those that are downregulated. [**d**, n = 5; **e**, n = 4, 4, 8, 8, 8, 7, respectively; (**b**, **d** and **e**): Student's *t*-test.]



Supplementary Fig. 7 FABP3-null hearts show increased glycolysis after TAC surgery. a-c GSEA plot showed *Fabp3*-defect heart was positively correlated with "glucan catabolic process" (**a**), "Regulation of gluconeogenesis" (**b**), while was negatively correlated with "Mitochondrial electon transport NADH to ubiquinone" (**c**). **d** Blood glucose level was measured in F3-KO mice and WT littermates at 0-week, 4-weeks, and 8-weeks after TAC operation. **e** Periodic acid Schiff glycogen staining (PAS) of the small intestine and liver sections was performed on TAC-operated WT and F3-KO mice at 4 weeks post-surgery. (**f-h**) Non-targeted metabolomics analysis was performed on TAC-operated WT and F3-KO hearts. **f** PCA plot showing metabolic differences in the above groups, n = 6 biological replicates /group. **g** Orthogonal partial least-squares

discrimination analysis (OPLS-DA) based on differential metabolomics. **h** KEGG pathway analysis of differential metabolites. [**d**, n = 6, 4, 4, 3, 3, 3, respectively, Student's *t*-test.]


a
Complement and coagulation cascades
ECM-receptor interaction
Cholesterol metabolism
PPAR signaling pathway
TNF signaling pathway
Cytokine receptor interaction
Focal adhesion
Retinol metabolism
Cell cycle - yeast
Steroid hormone biosynthesis
Phagosome
NF-kappa B signaling pathway
Glycolysis / Gluconeogenesis
Citrate cycle (TCA cycle)
Rich Ratio
Genes NO
Qvalue
b
Relative mRNA level of Pparα
p=0.001
p=0.997
p=1.000
WT
F3-KO
sham
TAC
c
Si-NC
Si-Pparα-1
Si-Pparα-2
Si-Pparα-3
PPARα
GAPDH
55kD
37kD
d
PPARα / GAPDH
p=0.002
p=0.059
p=0.009
1 Si-NC
2 Si-Pparα-1
3 Si-Pparα-2
4 Si-Pparα-3
e
Si-NC
Si-Pparα
Lenti-Ctl
Lenti-F3
α-actinin / DAPI
20μm
f
Lenti-Ctl
Lenti-F3
Myocyte Area(x10³ μm²)
p<0.001
p=0.293
p<0.001
p<0.001
Si-NC
Si-Pparα
g
Bnp
Anp
Relative mRNA level
p=0.062
p=1.000
p=0.043
p=0.996
Lenti-Ctl + Si-NC
Lenti-F3 + Si-NC
Lenti-Ctl + Si-Pparα
Lenti-F3 + Si-Pparα
h
Si-NC
Si-Pparα
Lenti-Ctl
Lenti-F3
Nile Red / DAPI
GFP / Nile Red / DAPI
20μm

Supplementary Fig. 8 Requirement of PPARα in FABP3-driven metabolic regulation and cardiac hypertrophic response. **a** KEGG analyses of top enriched pathways based on differentially expressed genes between TAC-operated WT and F3-KO hearts in RNA-seq analysis. **b** The mRNA expression of *Pparα* in WT and F3-KO hearts after sham- or TAC-operation. **c** NRVMs were transfected with siRNA targeting *Pparα* or its negative control and its protein expression was determined by western blot assay. **d** The quantification results in panel (**c**). **e** NRVMs with knocking-in expression of FABP3 were transfected with Si-Pparα or its negative control. After Ang II treatment, cell area was determined by α-actinin staining. **f** The quantification results of cell area in (**e**), 50 cells /group were calculated. **g** The mRNA expression of *Bnp* and *Anp* was determined in the indicated groups by qPCR assay. **h** The level of neutral lipid was measured by nile red staining in the aforementioned groups. [**d**, n = 3, Dunnett's post-hoc test; **f**, Tukey's post-hoc test; **g**, n = 3, Games-Howell post-hoc test.]

Supplementary Tables

Supplementary Table. 1 Expression of PPARα target genes in RNA-seq

Supplementary Table. 2 Primer pairs used in the current study

Supplementary Table. 1 Expression of PPARα target genes in RNA-seq

MUS ID	Terms	log2(F3-KO TAC vs WT TAC)	Qvalue(F3-KO TAC vs WT TAC)	log2(F3-KO sham vs WT sham)	Qvalue(F3-KO sham vs WT sham)
Gck	glycolysis	1.694E+00	2.000E-181	1.201E+00	4.060E-54
Pklr	glycolysis	3.834E+00	1.774E-02	NA	NA
Pck1	glycolysis	3.696E+00	2.420E-08	-1.656E+00	4.542E-01
G6pc	glycolysis	3.419E+00	5.425E-02	NA	NA
Fbp1	glycolysis	2.319E+00	3.020E-04	-4.861E-01	7.884E-01
Pcx	glycolysis	-1.220E-01	6.733E-02	-1.657E-02	8.640E-01
G6pc3	glycolysis	-9.954E-02	6.387E-02	-9.750E-02	9.396E-02
Fbp2	glycolysis	-1.531E+00	1.800E-84	-6.448E-01	9.570E-20
Pdk4	glycolysis	-1.856E+00	0.000E+00	-4.201E-01	2.250E-52
Eno1	glycolysis	-1.871E-01	1.010E-14	1.613E-01	3.140E-09
Cpt1b	Mitochondrial β-Oxidation	-2.654E-01	6.160E-100	1.183E-01	9.380E-21
Mlycd	Mitochondrial β-Oxidation	-4.021E-01	1.030E-31	1.103E-02	8.401E-01
Acaa2	Mitochondrial β-Oxidation	-5.587E-01	0.000E+00	2.349E-01	7.740E-85
Acadl	Mitochondrial β-Oxidation	-2.831E-01	4.260E-165	-1.081E-01	4.980E-23
Acadm	Mitochondrial β-Oxidation	-2.022E-01	1.130E-82	6.708E-02	7.260E-11
Acads	Mitochondrial β-Oxidation	-1.543E-01	1.000E-07	9.716E-02	6.040E-04
Acadvl	Mitochondrial β-Oxidation	-3.518E-01	2.400E-211	2.211E-01	1.990E-98
Cpt2	Mitochondrial β-Oxidation	-2.880E-01	3.630E-33	5.199E-02	4.732E-02
Crat	Mitochondrial β-Oxidation	-2.193E-01	1.030E-53	5.248E-02	2.990E-04
Ehhadh	Mitochondrial β-Oxidation	-2.113E-01	1.672E-02	1.330E-01	2.390E-01
Hadha	Mitochondrial β-Oxidation	-3.991E-01	0.000E+00	1.681E-01	3.690E-102
Hadhb	Mitochondrial β-Oxidation	-1.304E-01	3.480E-54	2.792E-01	4.190E-295
Slc25a20	Mitochondrial β-Oxidation	-3.290E-01	7.040E-37	4.489E-01	2.170E-76
Aldh9a1	ω-Hydroxylation/Oxidation	-3.810E-01	6.690E-14	4.407E-02	5.159E-01
Cpt1a	Mitochondrial β-Oxidation	2.983E-01	2.310E-14	4.334E-01	2.800E-24
Acaca	Lipogenesis	3.354E-01	5.150E-05	-6.874E-02	5.780E-01
Agpat2	Lipogenesis	1.148E-01	2.359E-02	2.451E-01	1.540E-06
Elovl6	Lipogenesis	3.091E-01	3.578E-01	6.277E-01	1.130E-01
Fads1	Lipogenesis	2.607E-01	1.100E-05	-6.364E-02	5.038E-01
Fads2	Lipogenesis	5.966E-01	7.180E-08	8.289E-02	6.642E-01

Fasn	Lipogenesis	3.305E-01	1.640E-06	-6.352E-02	5.088E-01
Gpam	Lipogenesis	3.065E-01	5.230E-39	1.468E-01	5.160E-09
Scd1	Lipogenesis	3.667E-01	1.920E-06	-3.301E-01	1.905E-03
Scd2	Lipogenesis	3.173E-01	7.250E-06	-8.918E-02	3.801E-01
Acacb	Lipogenesis	-1.605E-01	6.250E-19	8.186E-02	6.280E-06
Apoa1	Lipid Binding & Transport / Lipoproteins	5.076E+00	3.440E-50	NA	NA
Apoa2	Lipid Binding & Transport / Lipoproteins	3.238E+00	4.910E-39	7.292E-01	1.633E-01
Apoa5	Lipid Binding & Transport / Lipoproteins	5.589E+00	7.080E-07	NA	NA
Apoc3	Lipid Binding & Transport / Lipoproteins	5.921E+00	2.080E-08	NA	NA
Lipc	Lipid Binding & Transport / Lipoproteins	5.709E-01	5.528E-01	-7.110E-02	9.869E-01
Lpl	Lipid Binding & Transport / Lipoproteins	-9.695E-02	2.320E-132	-2.253E-01	0.000E+00
Pltp	Lipid Binding & Transport / Lipoproteins	3.134E-01	1.670E-11	-6.226E-02	3.442E-01
Slc27a1	Lipid Binding & Transport / Lipoproteins	-9.675E-01	4.640E-264	-4.333E-01	8.650E-72
Slc27a2	Lipid Binding & Transport / Lipoproteins	2.082E+00	1.330E-07	-6.878E-01	4.459E-01
Slc27a4	Lipid Binding & Transport / Lipoproteins	-1.165E-02	9.063E-01	-1.734E-01	2.560E-02
Vldlr	Lipid Binding & Transport / Lipoproteins	1.692E-01	1.920E-23	6.631E-02	4.440E-04
Hmgcs2	Ketogenesis	-6.357E-01	3.470E-24	-6.318E-01	3.350E-16
Abca1	Cholesterol Metabolism	6.041E-01	3.630E-40	-6.307E-02	3.825E-01
Abca4	Cholesterol Metabolism	-2.034E+00	3.870E-29	2.817E-01	9.389E-02
Cyp7a1	Cholesterol Metabolism	2.834E+00	3.998E-02	-2.067E+00	2.870E-01
Cyp8b1	Cholesterol Metabolism	1.156E+00	1.737E-02	-6.561E-01	5.556E-01
Nr1h2	Cholesterol Metabolism	-9.100E-02	6.145E-02	-8.460E-02	1.108E-01
Nr1h3	Cholesterol Metabolism	-8.302E-02	4.458E-01	-6.667E-01	1.460E-14
Fgf12	growth factor activity	1.303E+00	7.757E-02	3.888E-01	6.388E-01
Acaa1a	Peroxisomal β-Oxidation	-1.459E-01	4.556E-03	-9.915E-03	9.047E-01
Acaa1b	Peroxisomal β-Oxidation	4.564E+00	2.370E-11	NA	NA
Acox1	Peroxisomal β-Oxidation	-7.232E-02	3.100E-05	-8.613E-02	5.550E-07
Ech1	Peroxisomal β-Oxidation	-5.972E-01	0.000E+00	-7.171E-02	5.500E-14

Supplementary Table. 2 Primer pairs used in the current study

Primers for mouse qPCR		
Gene	Forward primers (5'- 3')	Reverse primers (5'- 3')
Fabp3	AGCCTGGACCCAGTTCCTAC	GGTGGCCTTGGTTCTGCTTTAT
Anp	GGGTAGGATTGACAGGATTGG	CTCCTTGGCTGTTATCTTCGG
Bnp	TGGGAGGTCACTCCTATCCT	GGCCATTTCCTCCGACTTT
Acta1	CCAAAGCTAACCGGGAGAAG	GACAGCACCGCCTGGATAG
Myh7	CGGACCTTGGAAGACCAGAT	GACAGCTCCCCATTCTCTGT
Col1a1	CATAAAGGGTCATCGTGGCT	TTGAGTCCGTCTTTGCCAG
Col3a1	ACGTAGATGAATTGGGATGCAG	GGGTTGGGGCAGTCTAGTG
Mmp2	ACCAAGAACTTCCGATTATCCC	CAGTACCAGTGTCAGTATCAGC
Mmp9	TCCCCAAAGACCTGAAAACC	CTGCTTCTCTCCCATCATCTG
Timp1	CTCAAAGACCTATAGTGCTGGC	CAAAGTGACGGCTCTGGTAG
Gapdh	GCCTTCCGTGTTCCTACC	CCTCAGTGTAGCCCAAGATG
Mlycd	CGCCTATCCCTGGATTCACC	ATCCCTGAGGTGCCAAACAC
Cd36	ATTCCCTTGGCAACCAACCA	TACGTGGCCCGGTTCTACTA
Cpt1b	GCACACCAGGCAGTAGCTTT	CAGGAGTTGATTCCAGACAGGTA
Cpt2	TGATGGCTGAGTGCTCCAAA	GAACACCAATGTTCATGAGGAAGAA
Acsl1	CGGCCGCGACTCCTTAAATA	ATAGGGCTGGTTTGGCTTCC
Acadm	GCGGCCATTAAGACCAAAGC	GAAGACAGGTTCCTCCGCCA
Acaca	ATGCACAGGACTGAGAAGGC	GTGATAAGGTGGTGGCAGGG
Acacb	TCCAAGTGGCCCTAGTGAGT	CGGATCCAGAGTGTTCGAGG

Agpat2	CAGCCAGGTTCTACGCCAAG	TGATGCTCATGTTATCCACGGT
Gck	TAGCGGGGGTCATAAATCGC	GCAGCCCTTACTCTTCTGGG
Pck1	TTGAACTGACAGACTCGCCC	GGCACTTGATGAACTCCCCA
Slc2a1	GCTTGTAGAGTGACGATCTGAGC	AAGCCAAACACCTGGGCAAT
Slc2a4	GCTCTGACGATGGGGAACC	CACCGAGACCAACGTGAAGA
Pparα	ACACGCGTGCGAGTTTTCA	TCGCCGAAAGAAGCCCTTAC
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

Primers for Rat qPCR

Gene	Forward primers (5'- 3')	Reverse primers (5'- 3')
Fabp3	ATGAAGTCACTCGGTGTGGG	TCCCACTTCTGCACATGGAC
Anp	GAAGATGCCGGTAGAAGATGAG	AGAGCCCTCAGTTTGCTTTTC
Bnp	GGTGCTGCCCCAGATGATT	CTGGAGACTGGCTAGGACTTC
Myh7	GCCCCAAATGCAGCCAT	CGCTCAGTCATGGCGGAT
Gapdh	TGACAACTCCCTCAAGATTGTCA	GGCATGGACTGTGGTCATGA