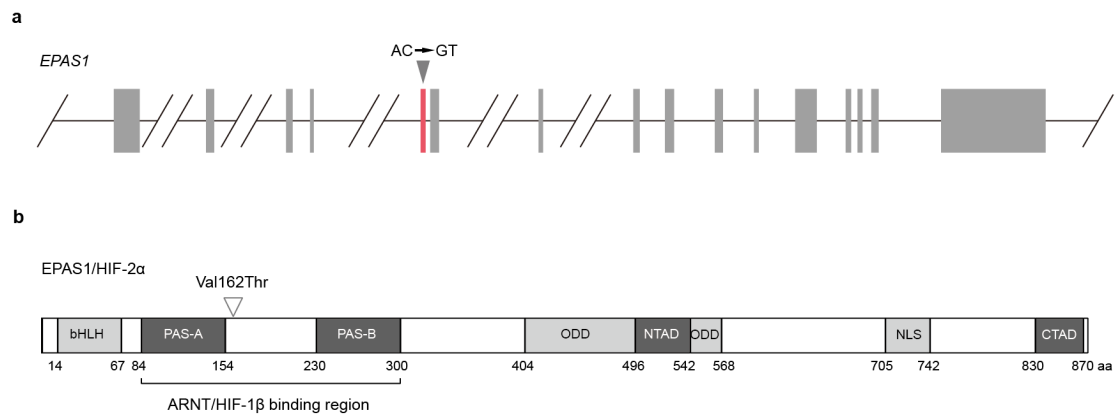
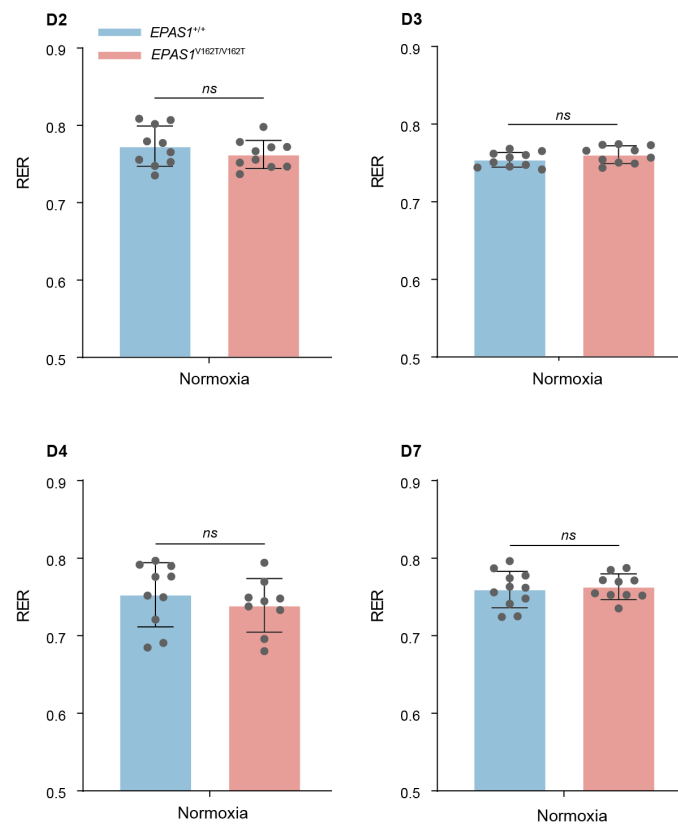


1 **Supplementary information for:**
2 **Homeostasis of glucose and lipid metabolism during physiological responses to a**
3 **simulated hypoxic high altitude environment**

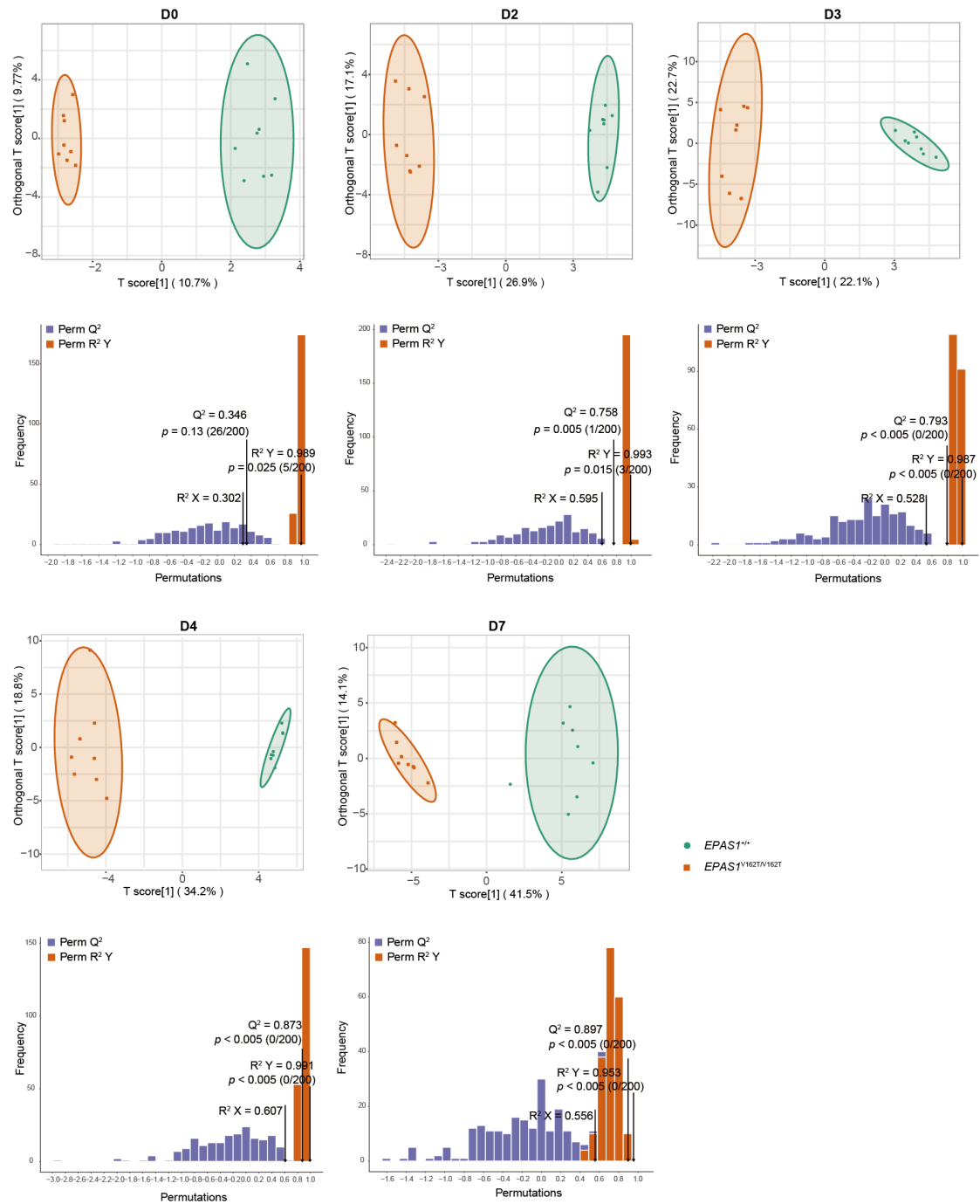
4
5 **Supplementary Figures**



6
7 **Supplementary Fig. 1** Generation of falconized mice by editing AC into GT in Exon
8 5 of the *EPAS1* gene on Chromosome 17. **a**, The gene structure: exon (grey boxes),
9 introns (horizontal lines), the mutation site (red). **b**, The resultant amino acid
10 substitution from Valine (Val) to Threonine (Thr) at the 162th residual (white triangle)
11 of EPAS1 protein, bHLH: basic helix-loop-helix, PAS-A/B: per-ARNT-Sim-A/B,
12 ODD: oxygen-dependent degradation domain, N/CTAD: N/C-terminal transactivation
13 domain, NLS: nuclear localization signal.



Supplementary Fig. 2 Comparison of respiratory exchange ratio (RER) values between *EPAS1*^{V162T/V162T} and *EPAS1*^{+/+} mouse individuals ($n = 10$ vs 10 on D2 and D3; 9 vs 10 on D4; 10 vs 11 on D7) under normoxia. Two-sided t tests were applied for all the comparisons. The bars display mean $\pm$ SD. *ns*: no significant difference. P values for each group were 0.3035, 0.1854, 0.4500 and 0.6967. Source data are provided as a **Source Data** file.



21

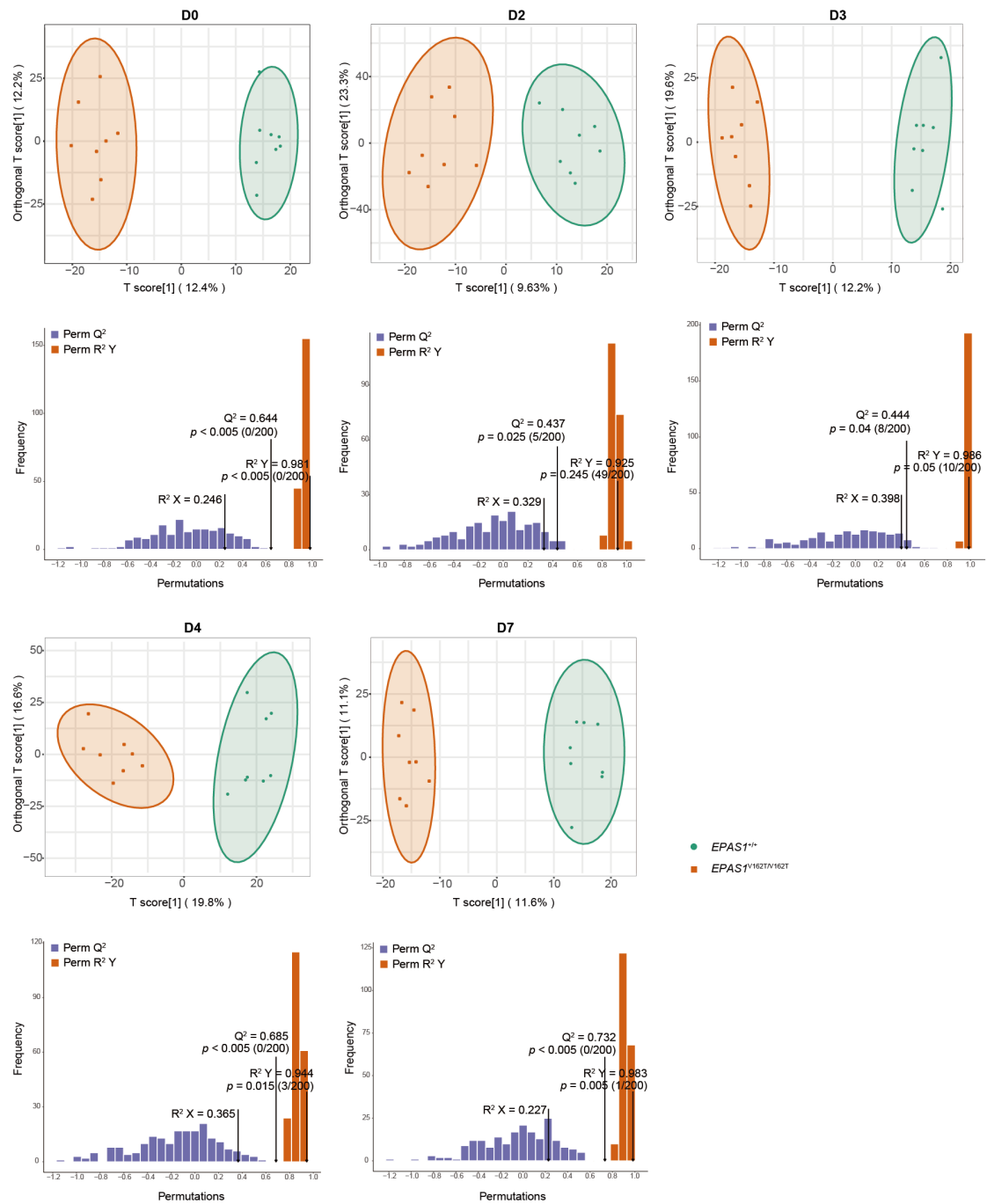
22 **Supplementary Fig. 3** Orthogonal partial least squares-discriminant analysis results

23 of carbohydrate metabolites in hepatic samples collected at each time point between

24 *EPAS1*^{V162T/V162T} and *EPAS1*^{+/+} mouse individuals ($n = 8$ for each group on D0-D7).

25 The x-axis represents the model's accuracy and the y-axis indicates the frequency of

26 classification effects within the model.



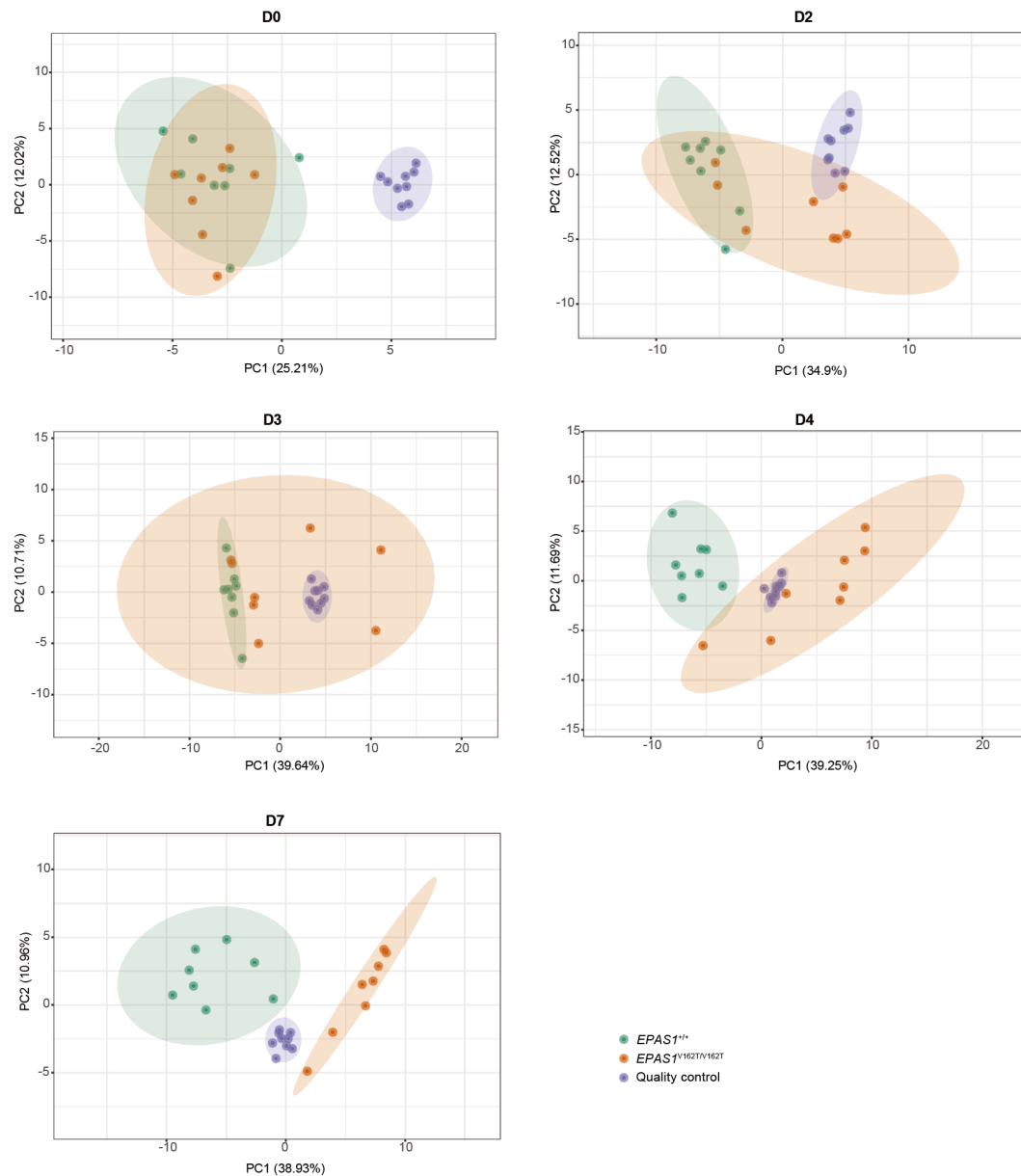
Supplementary Fig. 4 Orthogonal partial least squares-discriminant analysis results

of lipid metabolites in hepatic samples collected at each time point between

EPAS1^{V162T/V162T} and *EPAS1*^{+/+} mouse individuals ($n = 8$ for each group on D0-D7).

The x-axis represents the model's accuracy and the y-axis indicates the frequency of

classification effects within the model.



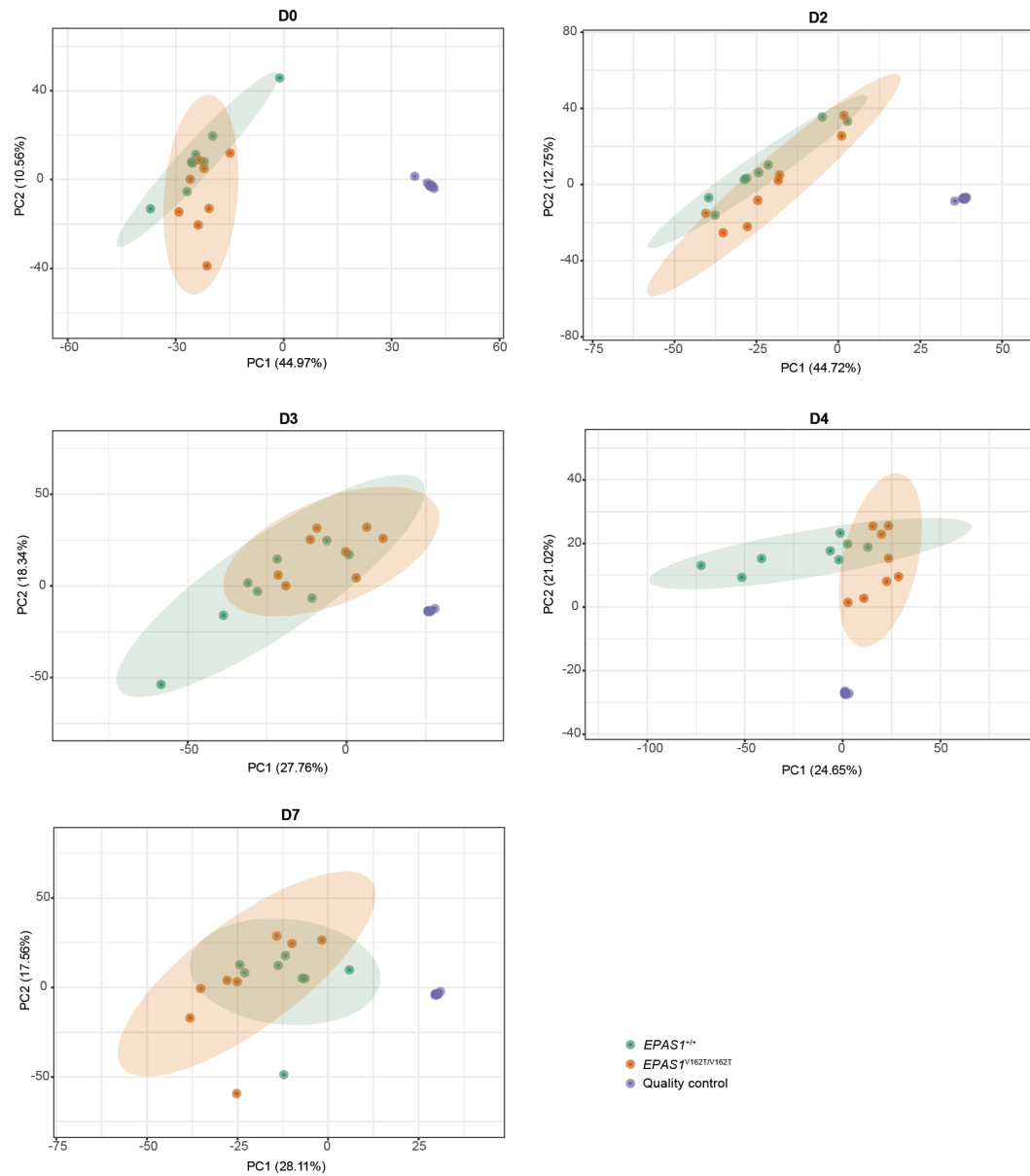
33

34 **Supplementary Fig. 5** Principal component analysis results of hepatic carbohydrate

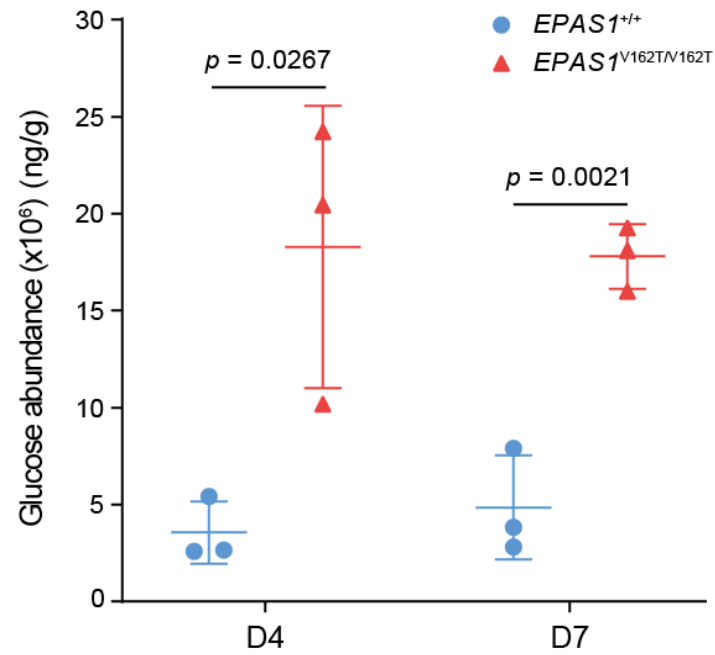
35 metabolites collected at each time point in *EPAS1*^{V162T/V162T}, *EPAS1*^{+/+} mouse

36 individuals ($n = 8$ for each group on D0-D7) and quality control samples ($n = 9$ on

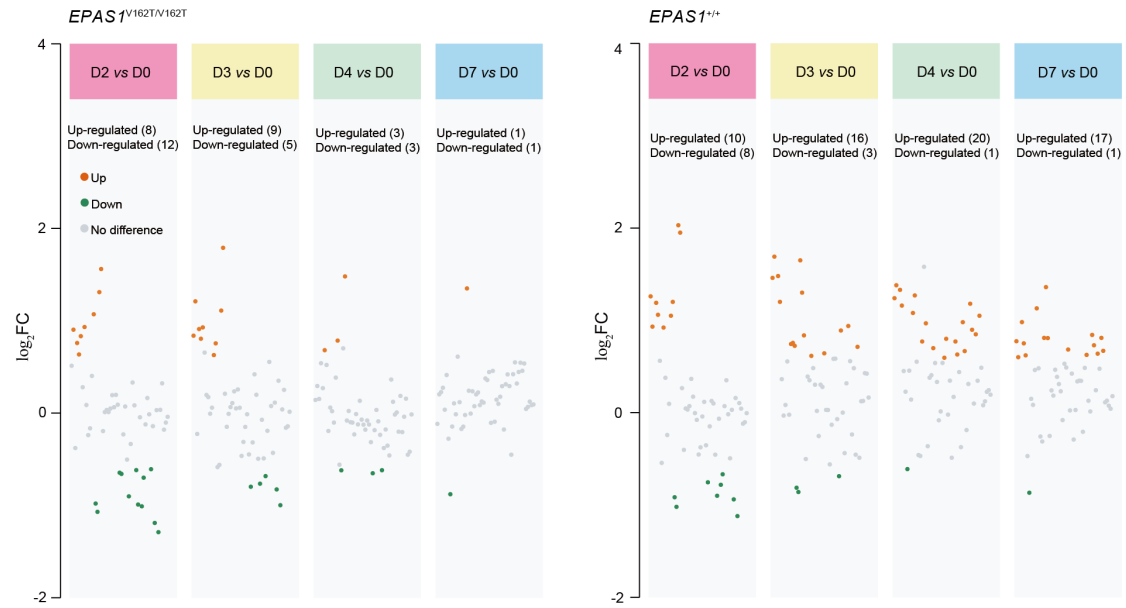
37 D0-D7).



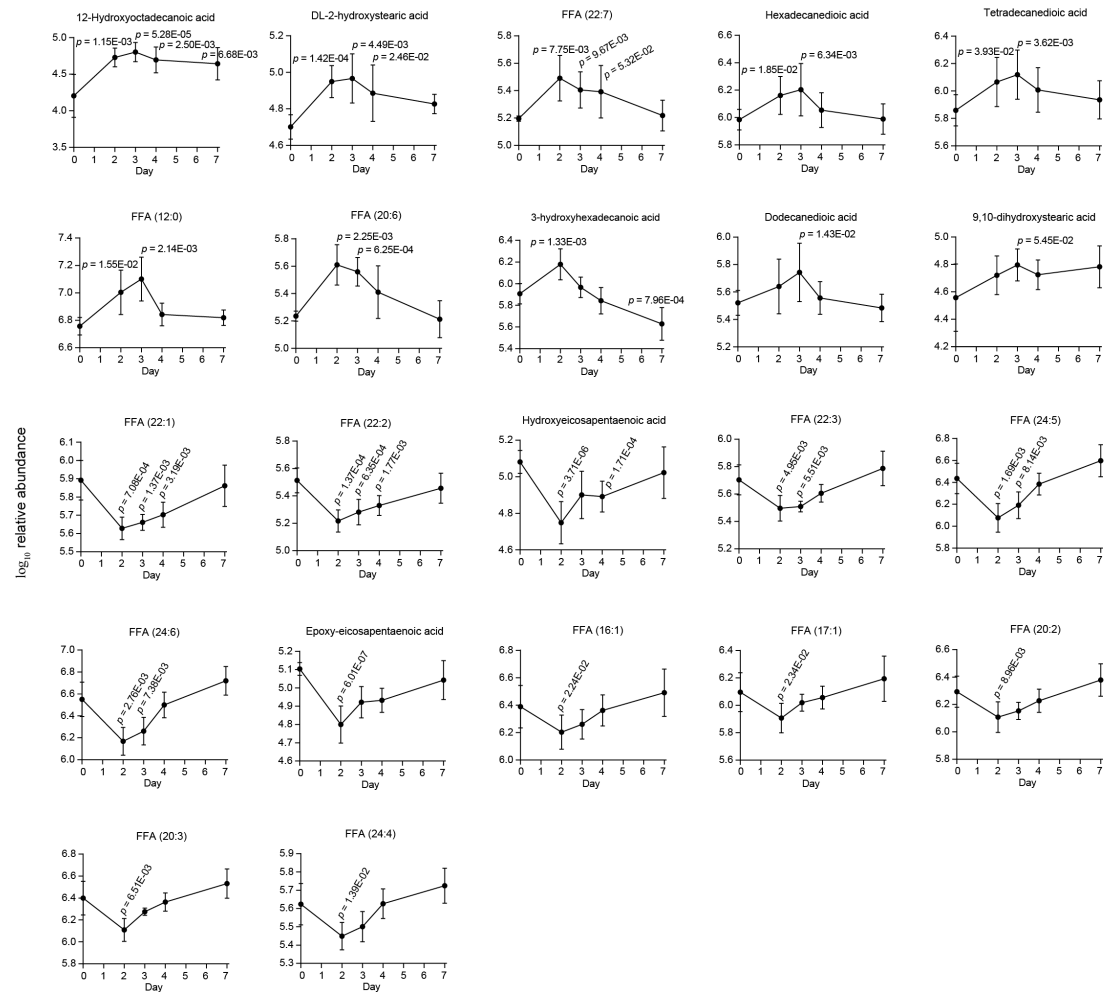
Supplementary Fig. 6 Principal component analysis results of hepatic lipid metabolites collected at each time point in *EPAS1*^{V162T/V162T}, *EPAS1*^{+/+} mouse individuals ($n = 8$ for each group on D0-D7) and quality control samples ($n = 9$ on D0-D7).



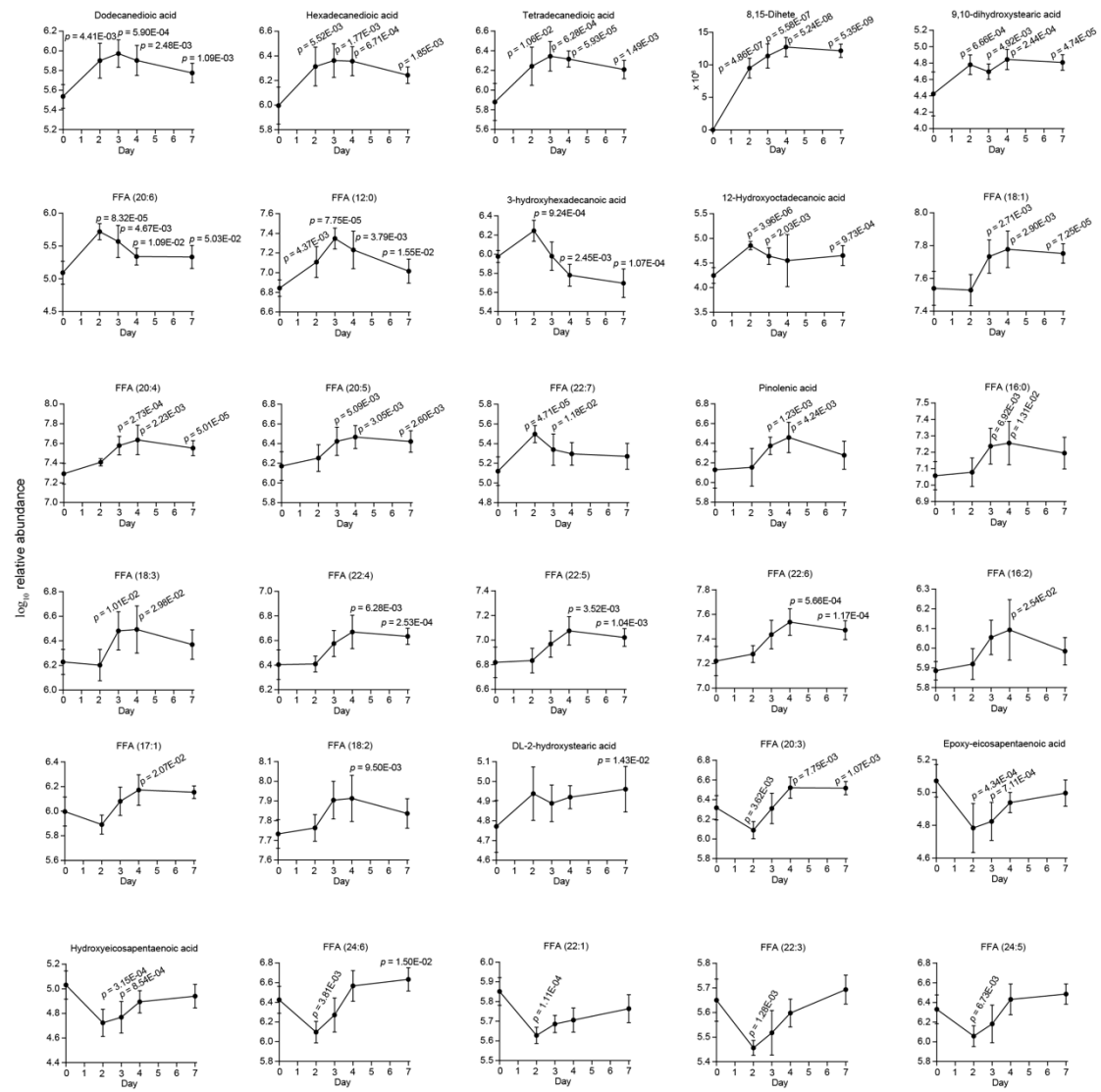
Supplementary Fig. 7 Comparisons of hepatic glucose content between *EPAS1*^{V162T/V162T} and *EPAS1*^{+/+} mouse individuals on D4 and D7 ($n = 3$ for each group) detected using targeted metabolomics in hepatic samples. The bars show mean $\pm$ SD. Two-sided t tests were applied. Source data are provided as a **Source Data** file.



Supplementary Fig. 8 Volcano plots of differentially expressed free fatty acid (FFA) in the hepatic samples of mutant and wild type mice at each treatment interval in comparison with D0, respectively. The x-axis is the fold change ($\log_2FC$), and y-axis is the variable important in projection (VIP). A significant difference was determined based on $VIP \geq 1$, and $FC \geq 1.5$ or $FC \leq 0.67$.



Supplementary Fig. 9 Changes of FFA contents in *EPASI*^{V162T/V162T} mice at different time points in comparison with D0. A significant difference was determined based on VIP ≥ 1 , and FC ≥ 1.5 or FC ≤ 0.67 . The p values were for reference. The bars show mean $\pm$ SD. A two-sided t test was used.



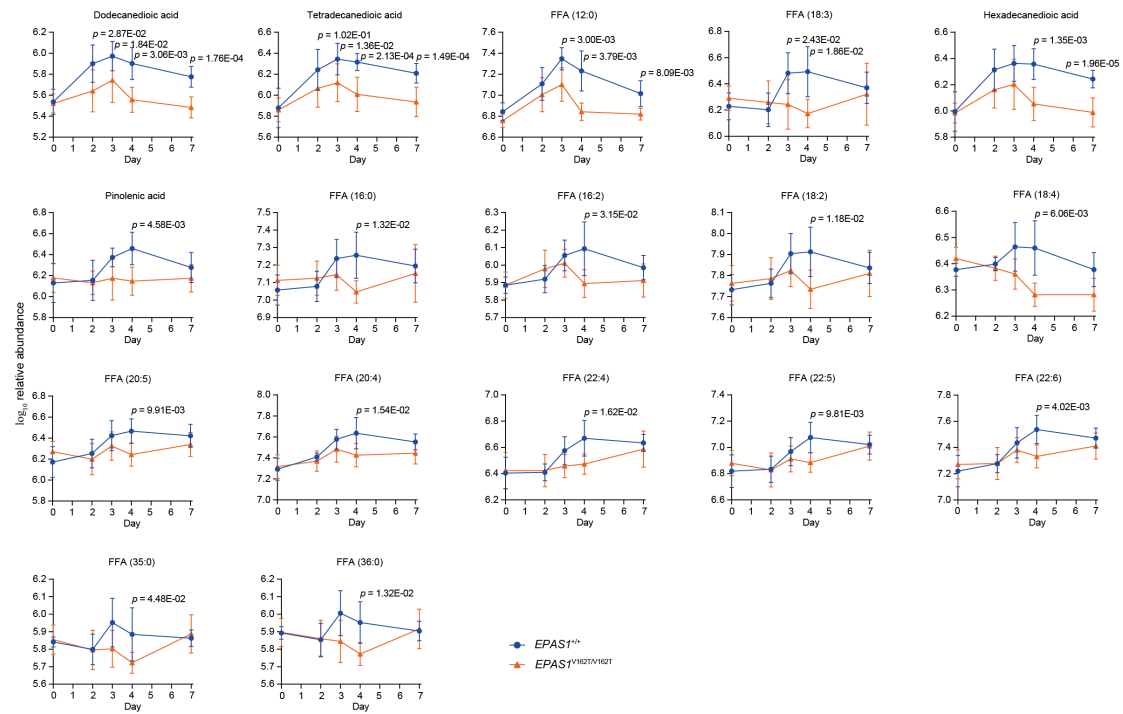
59

60 **Supplementary Fig. 10** Changes of FFA contents in *EPAS1*^{+/+} mice at different time

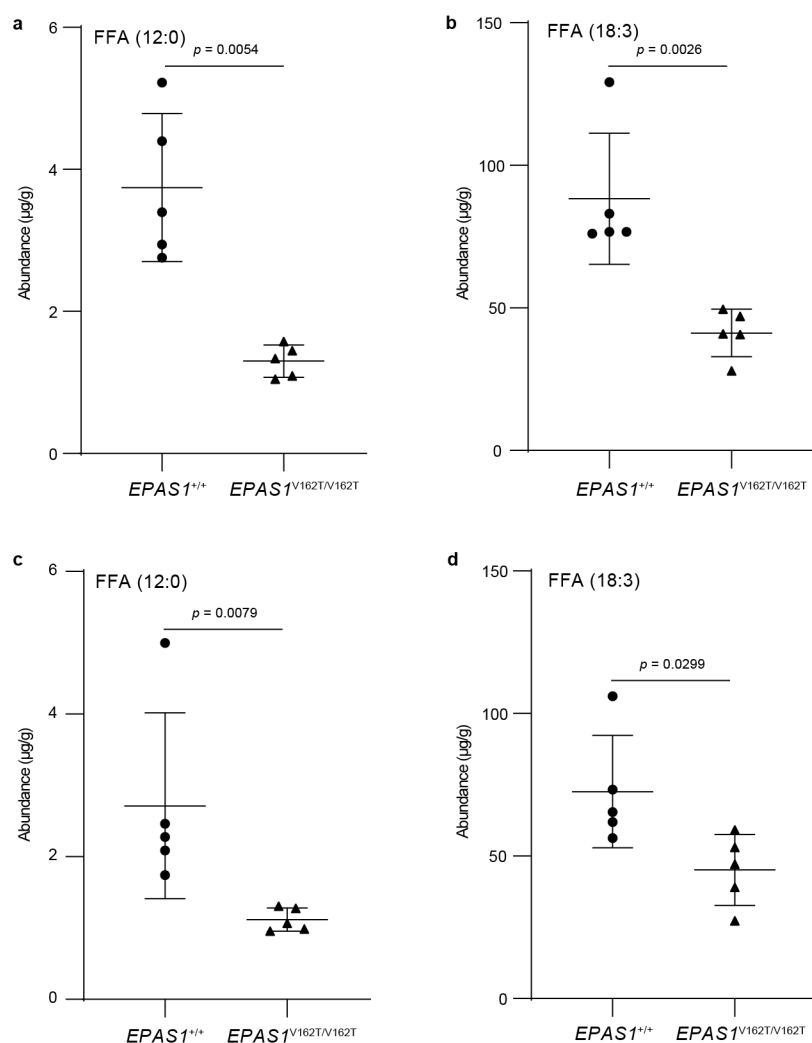
61 points in comparison with D0. A significant difference was determined based on VIP

62 ≥ 1 , and $FC \geq 1.5$ or $FC \leq 0.67$. The p values were for reference. The bars show mean

63 $\pm$ SD. A two-sided t test was used.



Supplementary Fig. 11 Content changes of FFA expressed both in mutant and wild type mice at different time points. A significant difference was determined based on $VIP \geq 1$, and $FC \geq 1.5$ or $FC \leq 0.67$. The p values were for reference. The bars show mean $\pm$ SD. A two-sided t test was used.



69

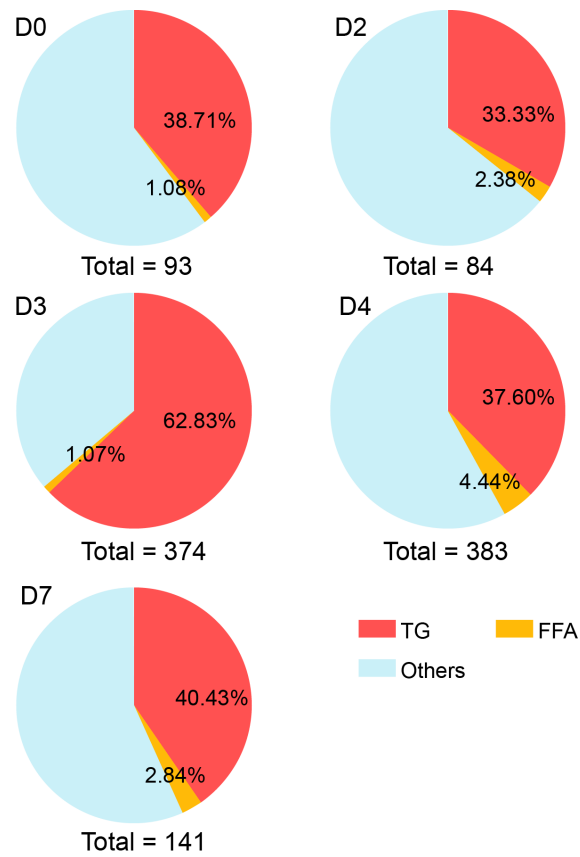
70 **Supplementary Fig. 12** Changes of FFA contents in mutant and wild type mice on

71 D3 (**a** and **b**) and D4 (**c** and **d**) detected using targeted lipidomics ($n = 5$ for each

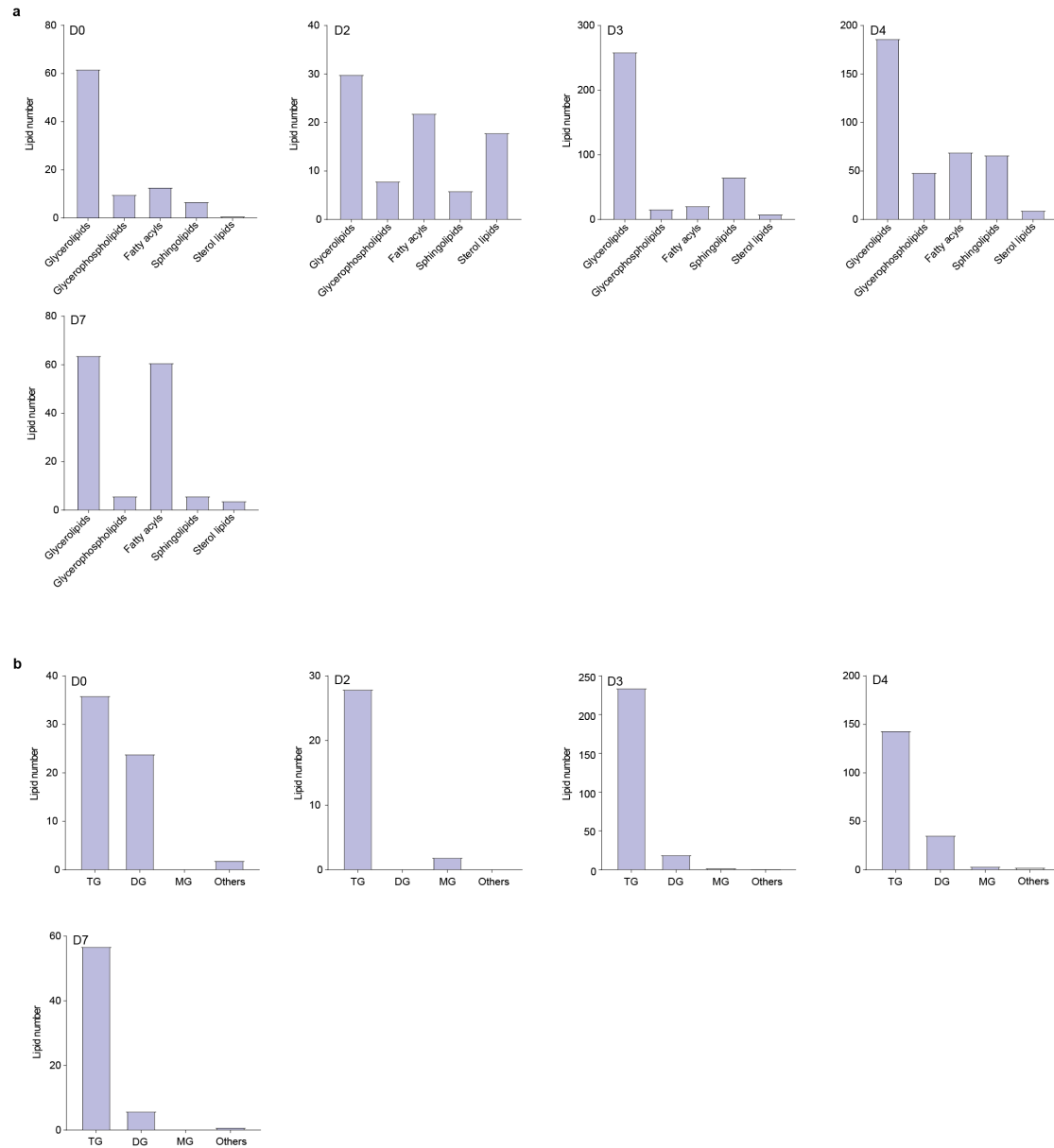
72 group). The bars show mean $\pm$ SD. Two-sided Welch's t test was applied for **a**, two-

73 sided t test for **b**, and two-sided Mann-Whitney U tests for **c** and **d**. Source data are

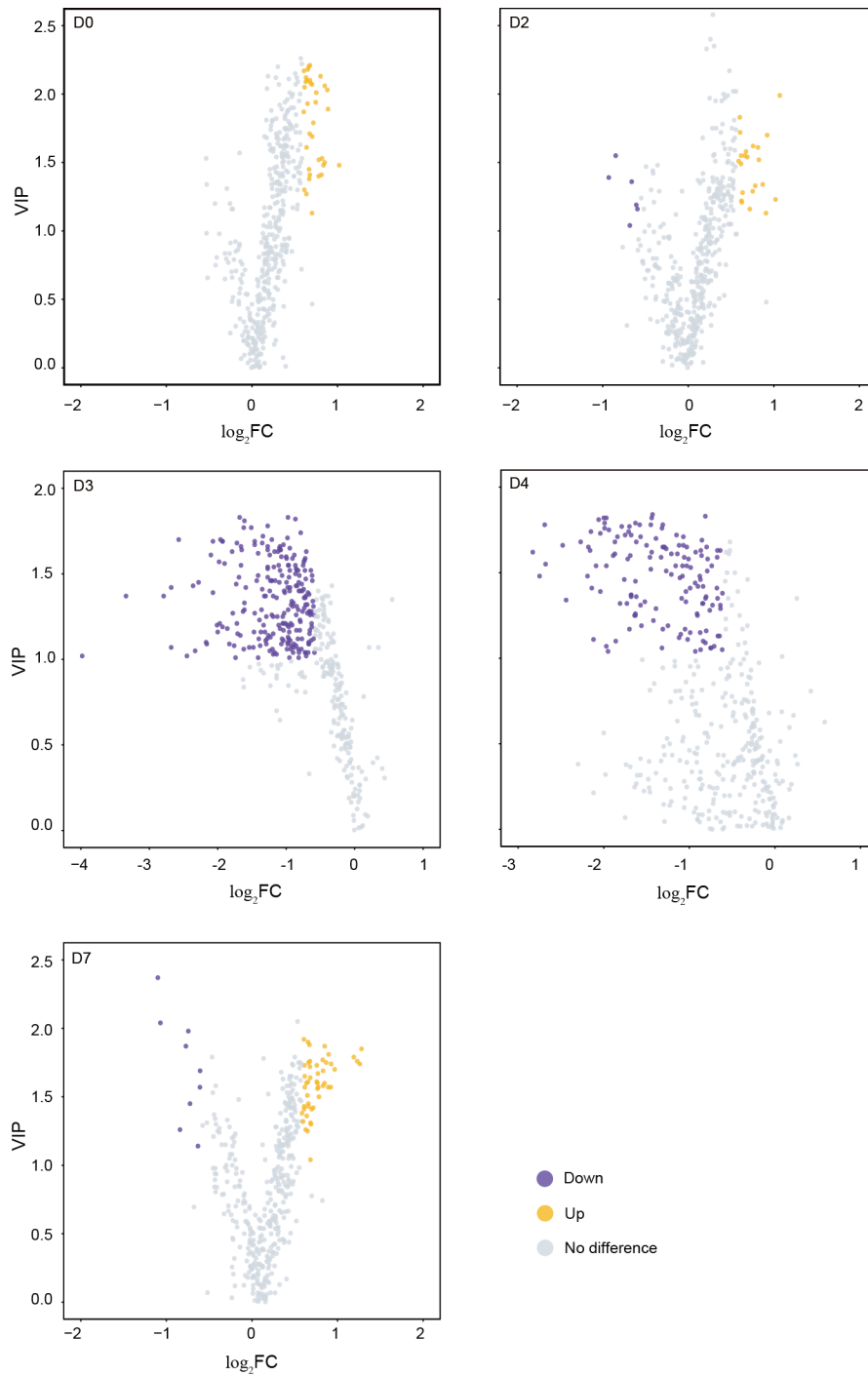
74 provided as a **Source Data** file.



Supplementary Fig. 13 Percent of differentially expressed metabolites numbers of lipid between *EPASI*^{V162T/V162T} and *EPASI*^{+/+} mice at checked time points. “Total” refers to the total number of differentially expressed lipid metabolite subclasses detected between mutant and wild type mice.

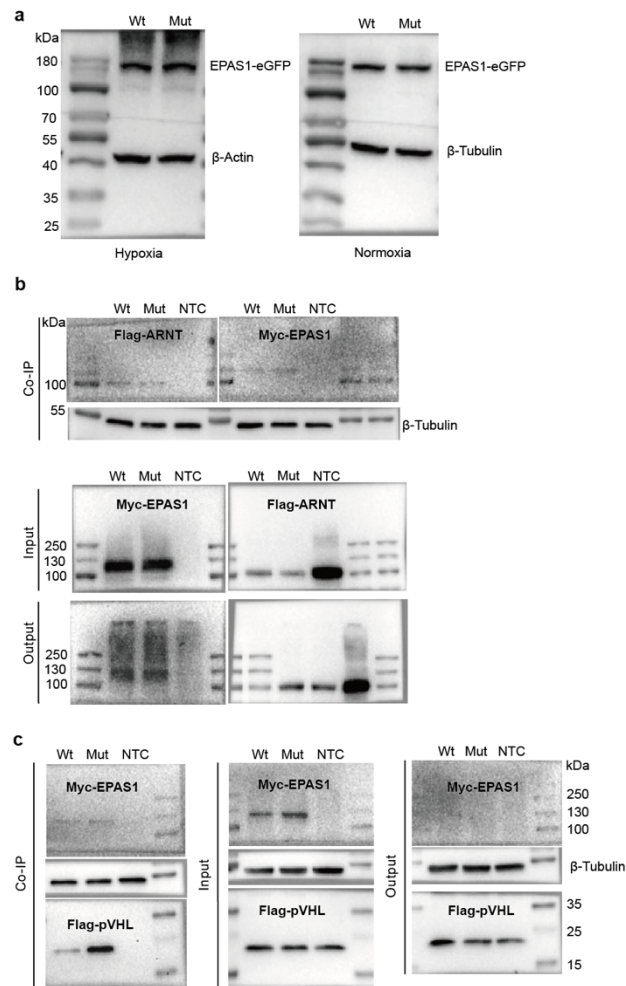


Supplementary Fig. 14 The lipid number of differentially expressed lipid metabolites (a) and glycerolipids (b) between *EPASI*^{V162T/V162T} and *EPASI*^{+/+} mice at each time point. TG: Triglyceride, DG: Diacylglycerol, MG: Monoacylglycerol.

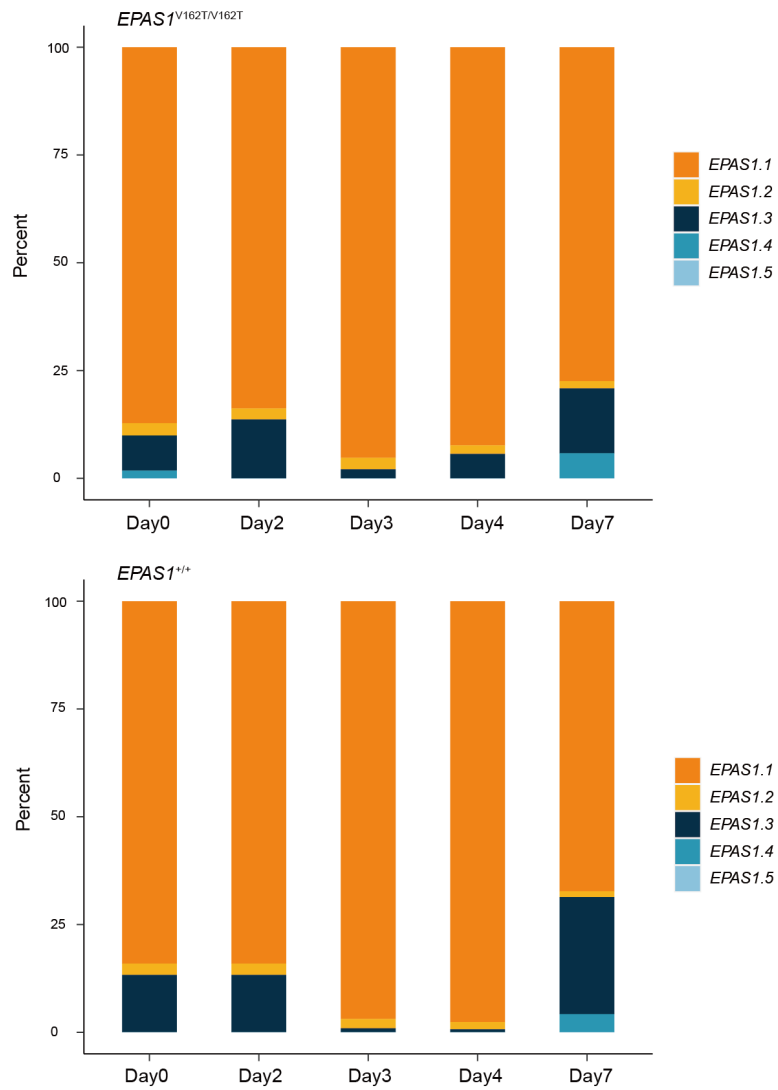


84

85 **Supplementary Fig. 15** Volcano plots of differentially expressed triglycerides in
 86 liver samples between mutant and wild mice at each time point. The x-axis is the fold
 87 change (FC), and y-axis is the variable important in projection (VIP). A significant
 88 difference was determined based on $VIP \geq 1$, and $FC \geq 1.5$ or $FC \leq 0.67$.



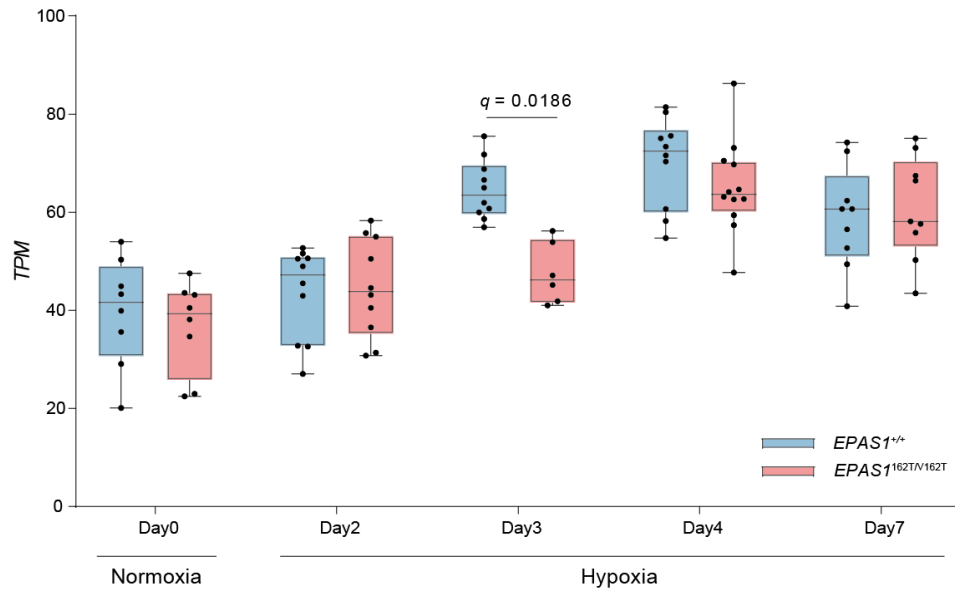
Supplementary Fig. 16 Expression of mutant and wild type EPAS1 proteins in HeLa cells under normoxia and hypoxia conditions using GFP antibody, respectively (**a**). The EPAS1-eGFP fusion proteins internal control β-Actin and β-Tubulin protein are about 124 kDa, 43 kDa and 55 kDa, respectively. Co-immunoprecipitation (Co-IP) assay between EPAS1 and ARNT proteins (**b**). Co-IP assay between EPAS1 and VHL proteins (**c**). Myc-EPAS1 protein, Flag-ARNT protein and Flag-VHL protein are about 100 kDa, 88 kDa and 22 kDa, respectively. Wt: EPAS1^{+/+}, Mut: EPAS1^{V162T/V162T}, NTC: negative control.



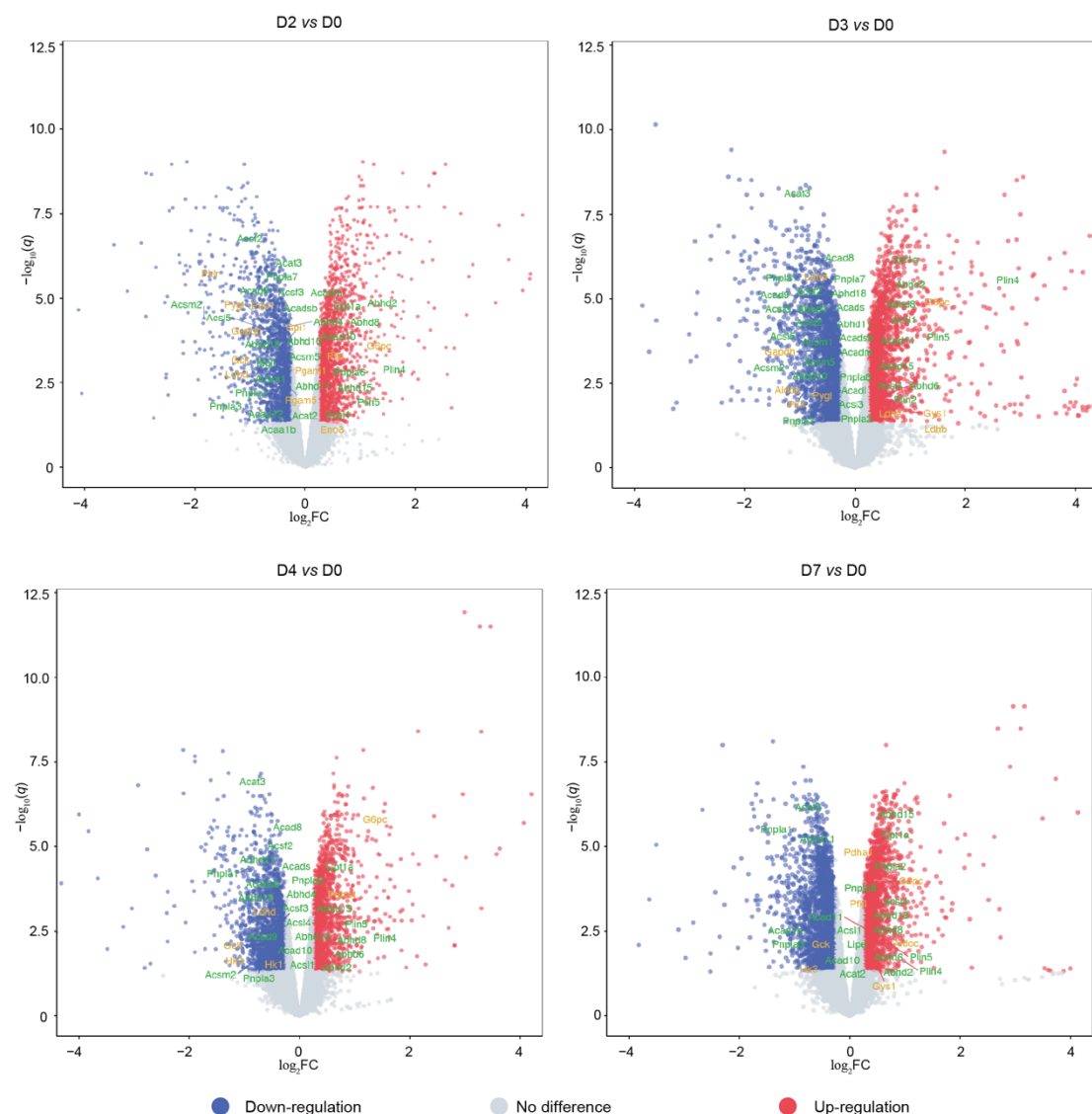
98

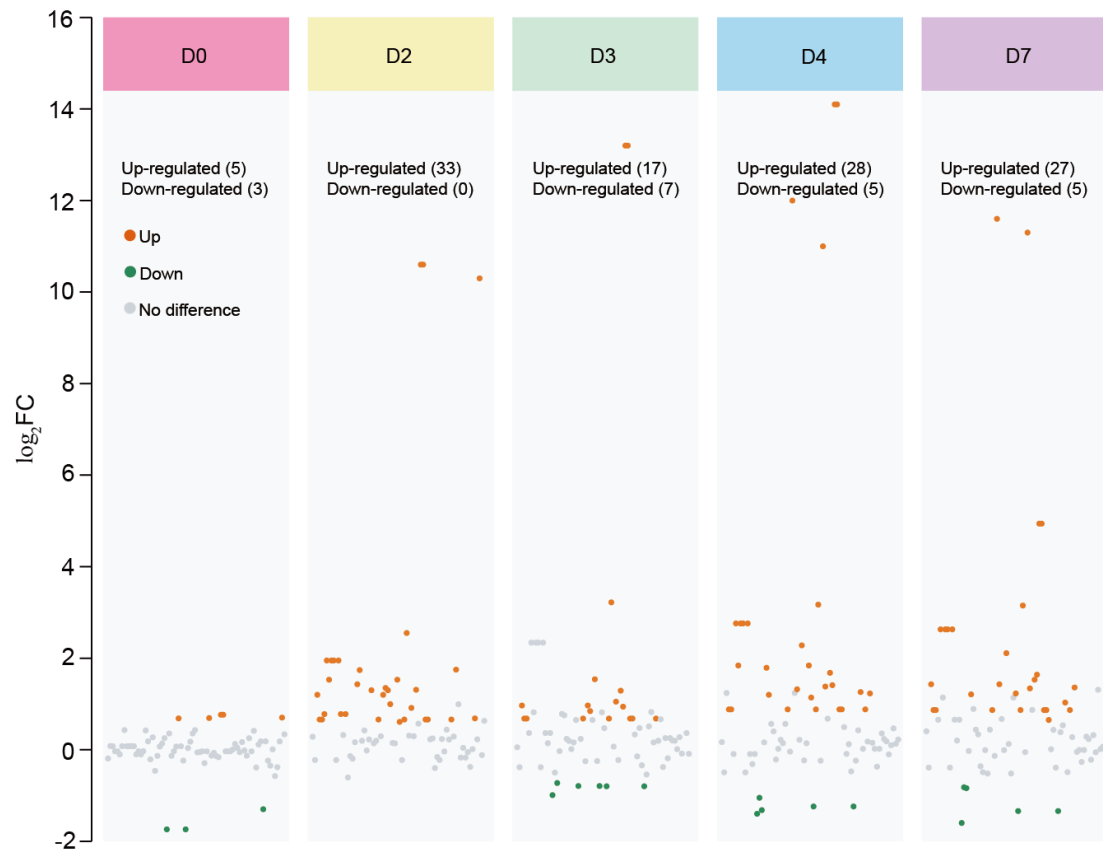
99 **Supplementary Fig. 17** Percentages of each *EPAS1* transcript expression in mutant

100 and wild type mice at each time point. Source data are provided as a **Source Data** file.

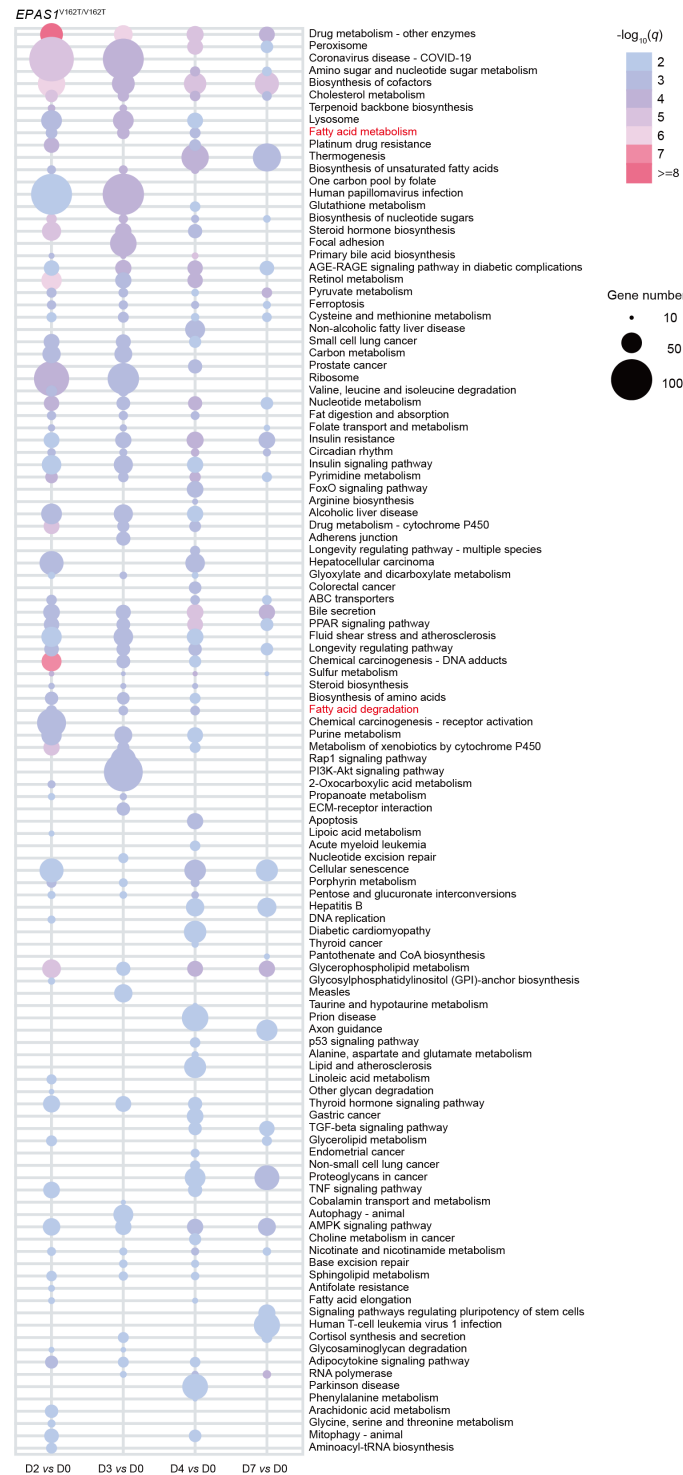


Supplementary Fig. 18 Expression differences of *EPAS1.1* transcript between mutant and wild type mice at each time point. *TPM*: the transcripts per million. A significant difference was determined by fold change ≥ 1.2 , and $q < 0.05$. Source data are provided as a **Source Data** file.





Supplementary Fig. 23 Volcano plots of differentially expressed carbohydrate metabolites in mutant and wild type mice under normoxia (D2-D7) and hypoxia (D0). The y-axis is fold change (log₂FC). A significant difference in expression level between mouse types was determined by variable important in projection value ≥ 1 , and FC being ≥ 1.5 or FC ≤ 0.67 .

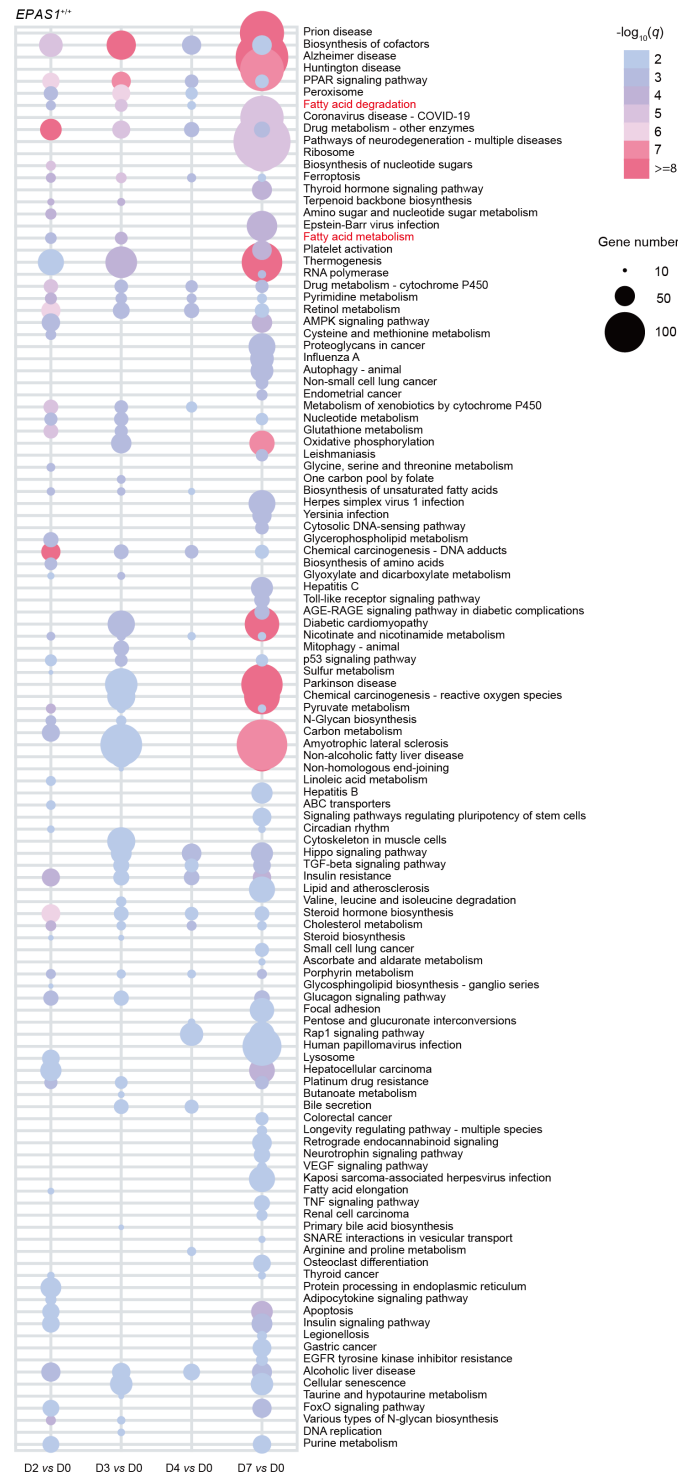


Supplementary Fig. 24 KEGG pathway enrichment on all DEGs in mutant mice

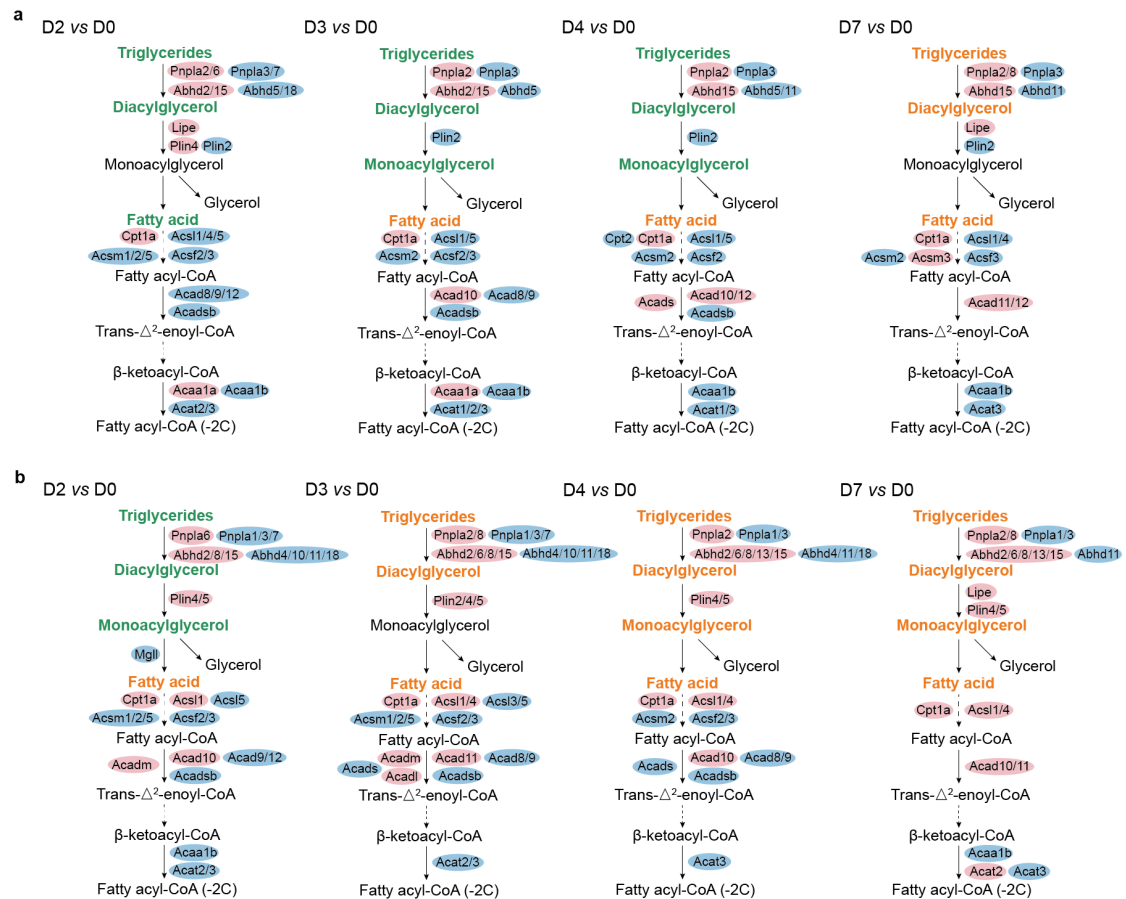
liver in comparison with D0 at each time point. Circle size represents the gene

number enriched in each pathway. A hypergeometric test and FDR adjustment were

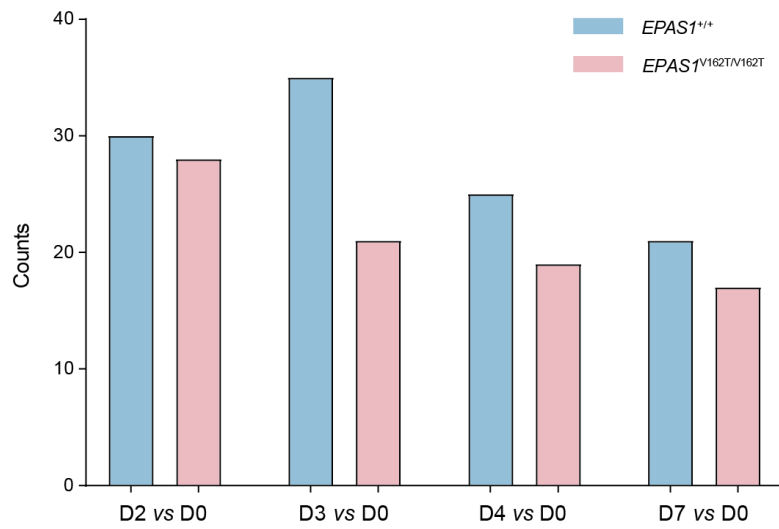
applied. Source data are provided as a **Source Data** file.



Supplementary Fig. 25 KEGG pathway enrichment on all DEGs in wild type mice liver in comparison with D0 at each time point. Circle size represents the gene number enriched in each pathway. A hypergeometric test and FDR adjustment were applied. Source data are provided as a **Source Data** file.



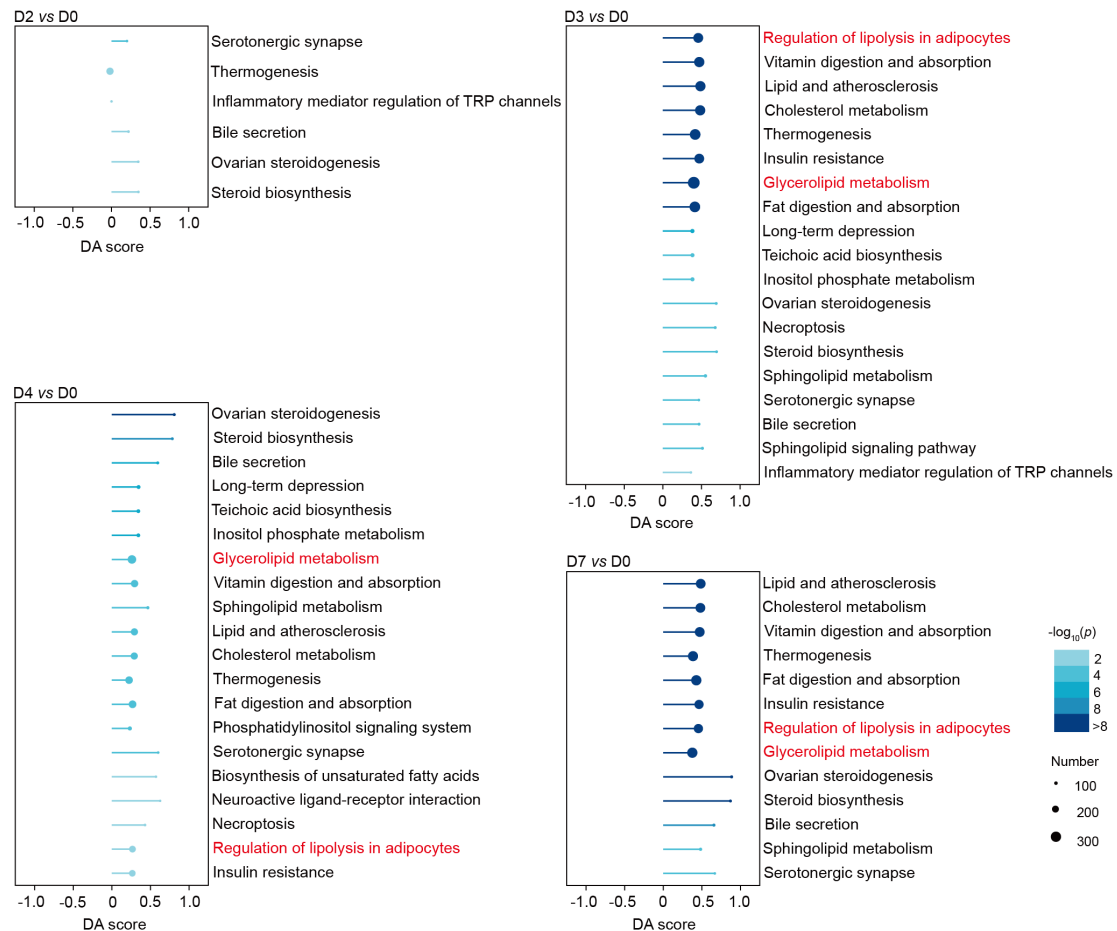
Supplementary Fig. 26 Significantly up-/down-regulated genes or metabolites in the FFA metabolic pathways in mutant and (a) wild type mice livers (b) from D2 to D7 in comparison with D0, respectively. Up- and down-regulated genes in both types of mice are in pink and blue, respectively, and up- and down-regulated lipid metabolites are in orange and green, respectively.



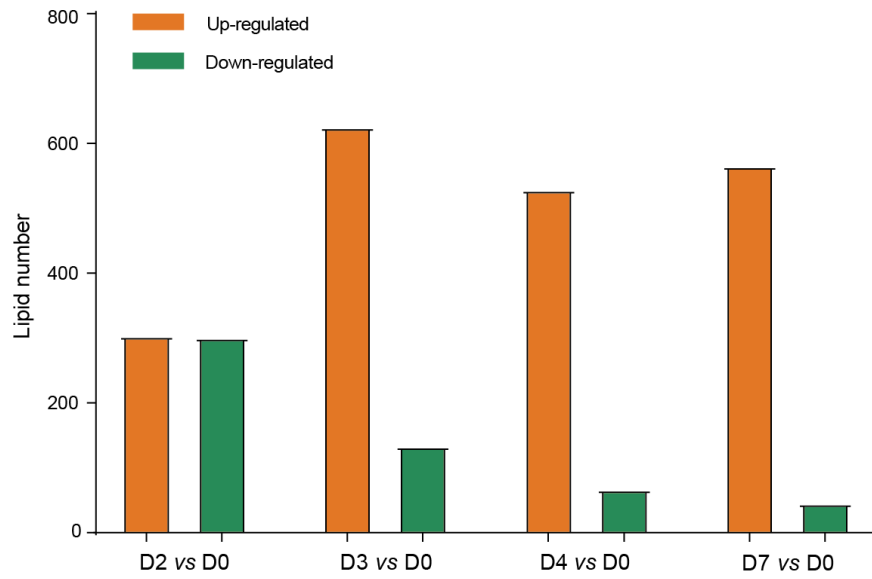
150

151 **Supplementary Fig. 27** Count comparison of DEGs that were related to FFA

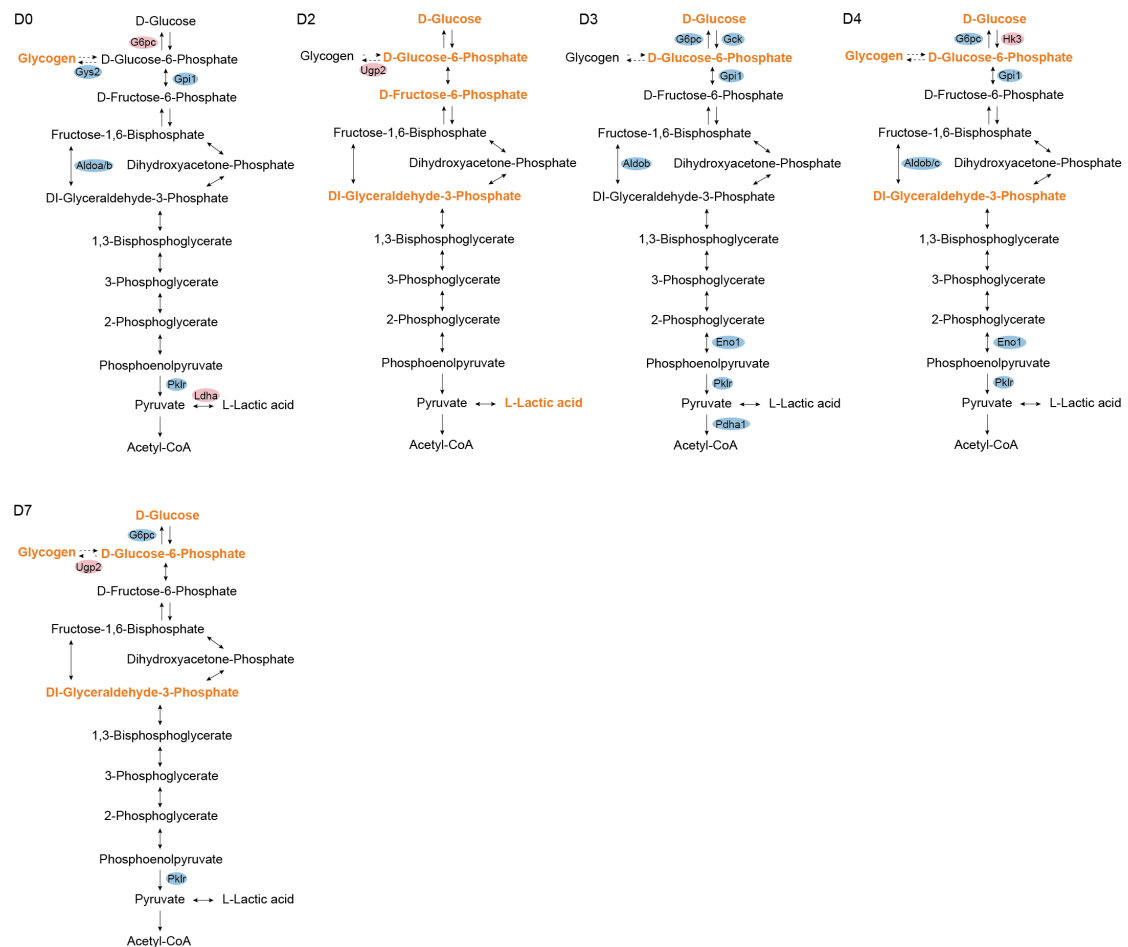
152 metabolic pathways in comparison with D0 between mutant and wild type mice.



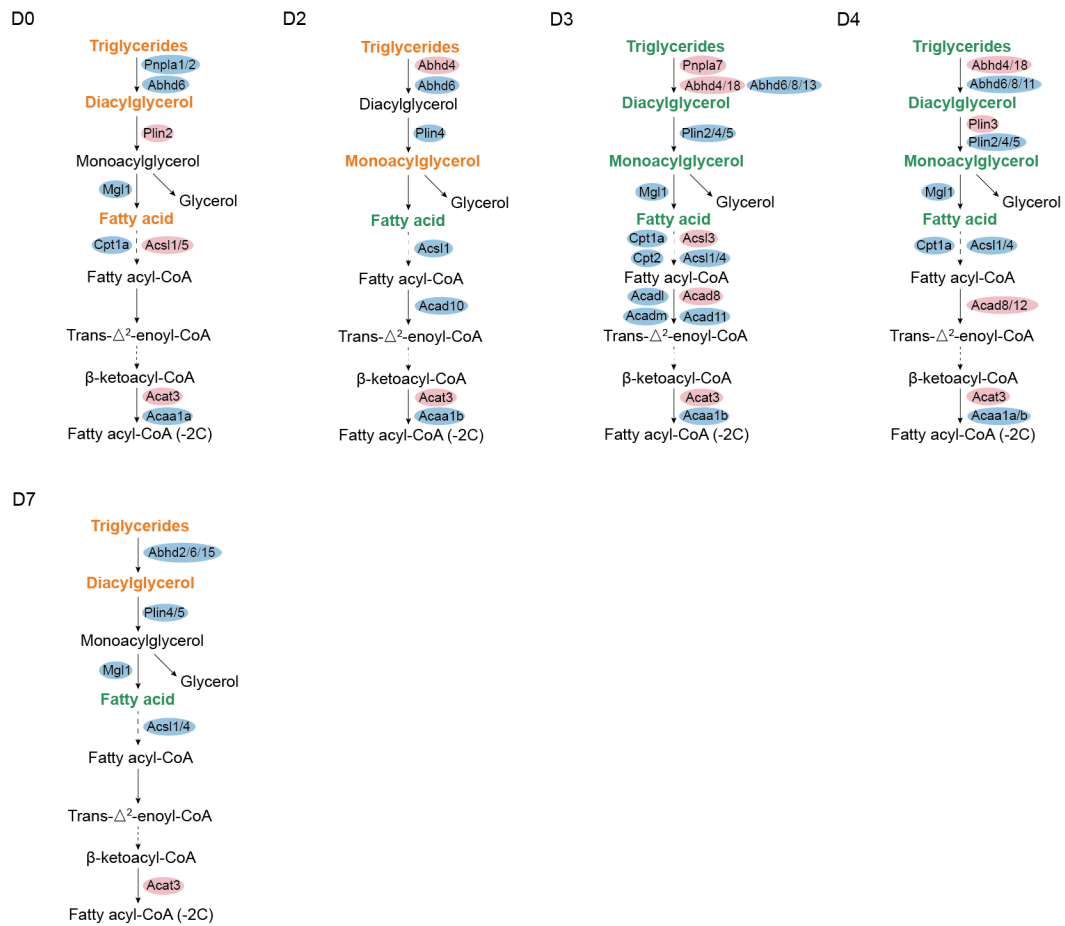
Supplementary Fig. 28 KEGG enrichment analysis on all differentially expressed lipid metabolites in wild type mice at each time point in comparison with D0. X-axis shows the enrichment score. Circles represent the number of metabolites enriched. Source data are provided as a **Source Data** file.



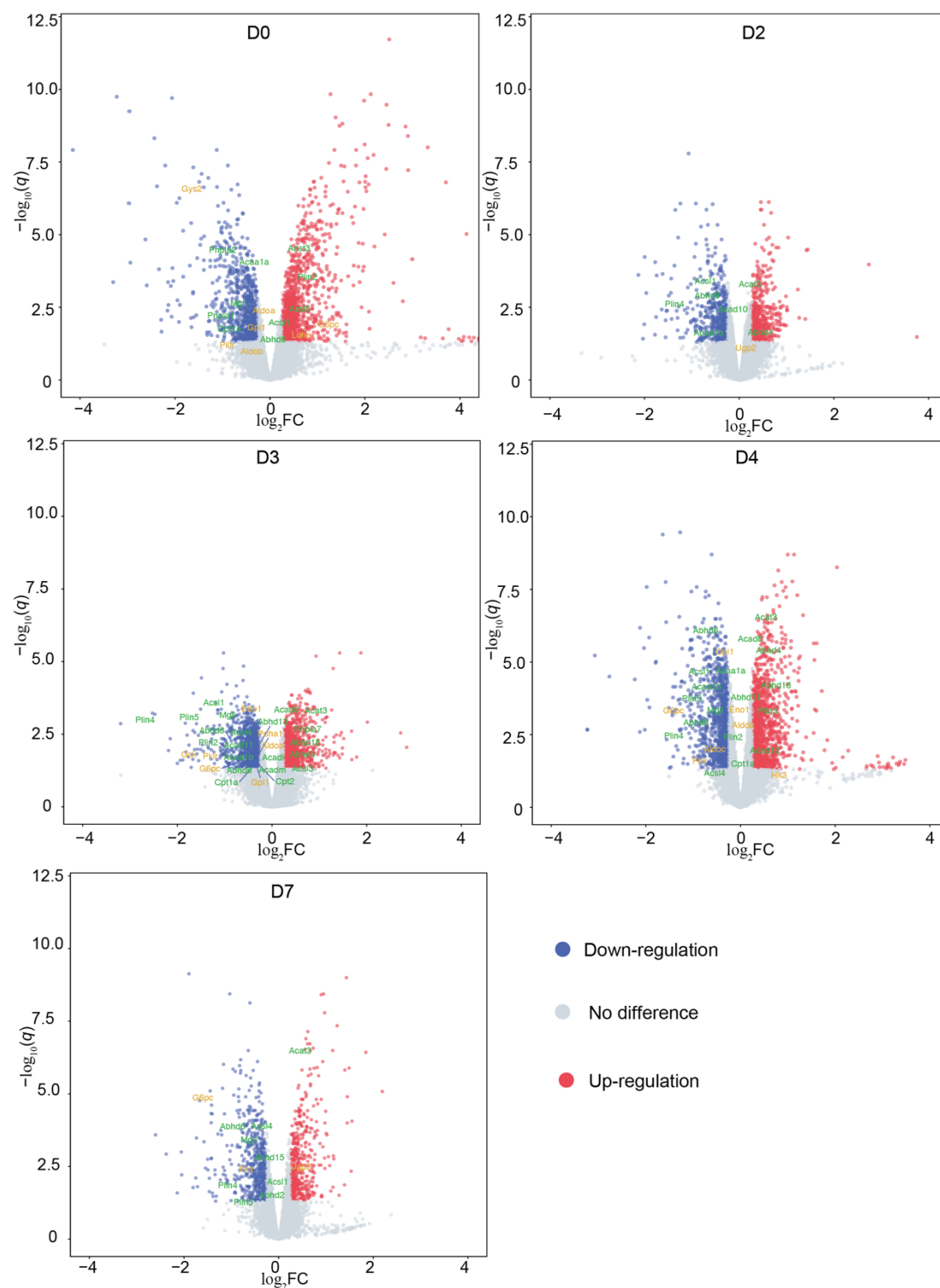
Supplementary Fig. 29 The number of differentially expressed lipid metabolites in wild type mice at each time point in comparison with D0. The total number means the number of differentially expressed lipid metabolites detected between hypoxic treatments and normoxic controls.



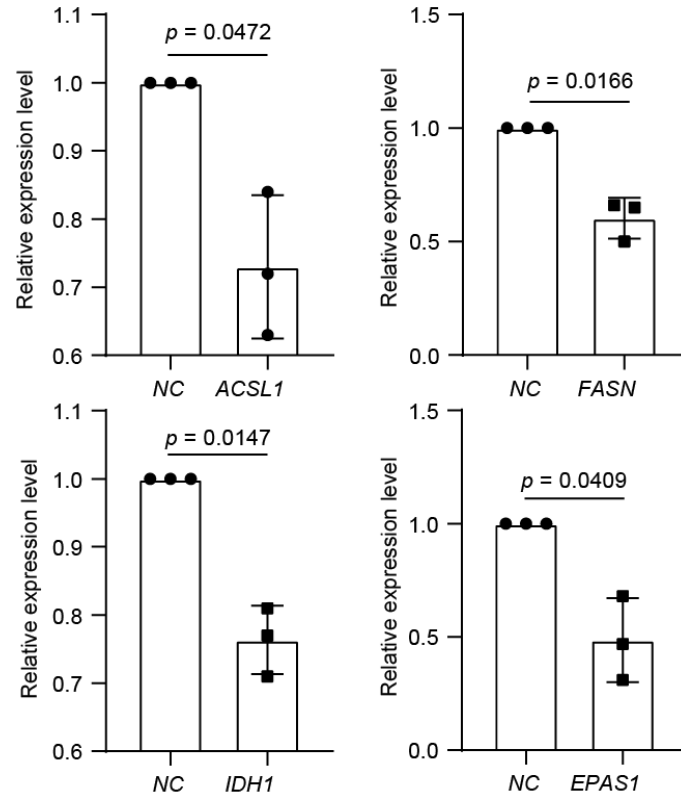
Supplementary Fig. 30 Significantly up-/down-regulated genes (pink and blue) or metabolites (orange and green) in the glucose metabolic pathways between mutant and wild type mice livers from D0 to D7.



Supplementary Fig. 31 Significantly up-/down-regulated genes (pink and blue) or metabolites (orange and green) in the lipid metabolic pathways between mutant and wild type mice livers from D0 to D7.



Supplementary Fig. 32 Volcano plots for differential expression level of each gene in hepatic samples between mutant ($EPAS1^{V162T/V162T}$) and wild type ($EPAS1^{+/+}$) mice from D0 to D7. The x-axis is the fold change ($\log_2FC$), $|\log_2FC| \geq 0.26$ and y-axis is the q value, the probability that a gene has a statistical significance.



181

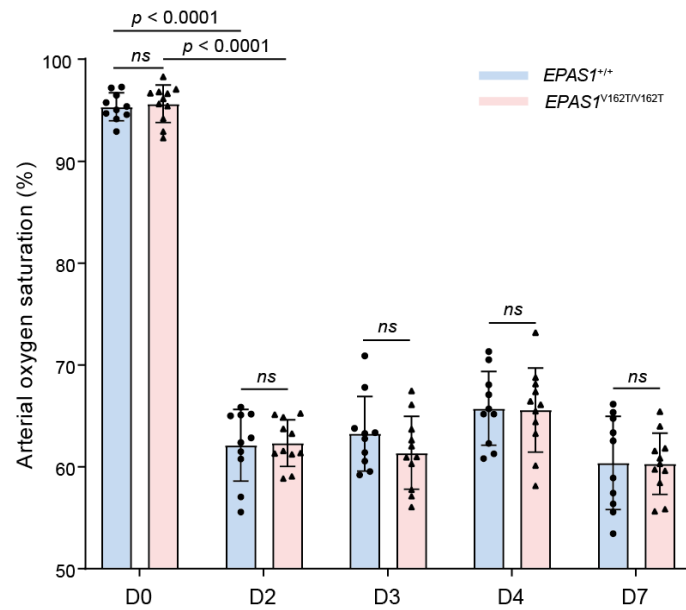
182 **Supplementary Fig. 34** qPCR results of relative expression level of genes involved

183 in lipid metabolism. *ACSL1*: acyl-CoA synthetase long chain family member 1,

184 *FASN*: fatty acid synthase, *IDH1*: isocitrate dehydrogenase (NADP (+)) 1. The bars

185 show mean $\pm$ SD and two-sided *t* tests were applied. Source data are provided as a

186 **Source Data** file.



Supplementary Fig. 35 Comparisons of arterial oxygen saturation between

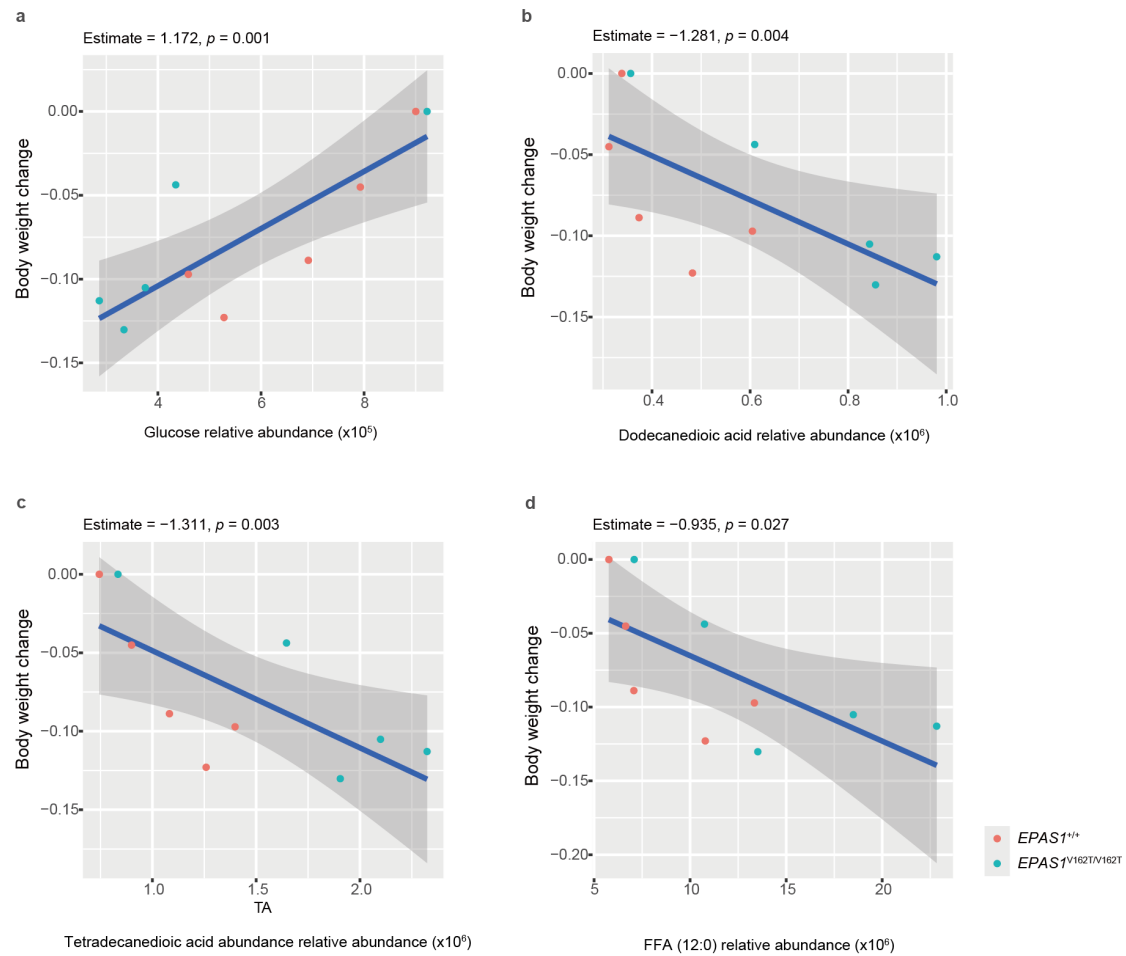
EPAS1^{V162T/V162T} ($n = 11$) and *EPAS1*^{+/+} ($n = 10$) mouse individuals under normoxia

and hypoxia. The bars display mean $\pm$ SD. Two-sided Welch's t test was applied for

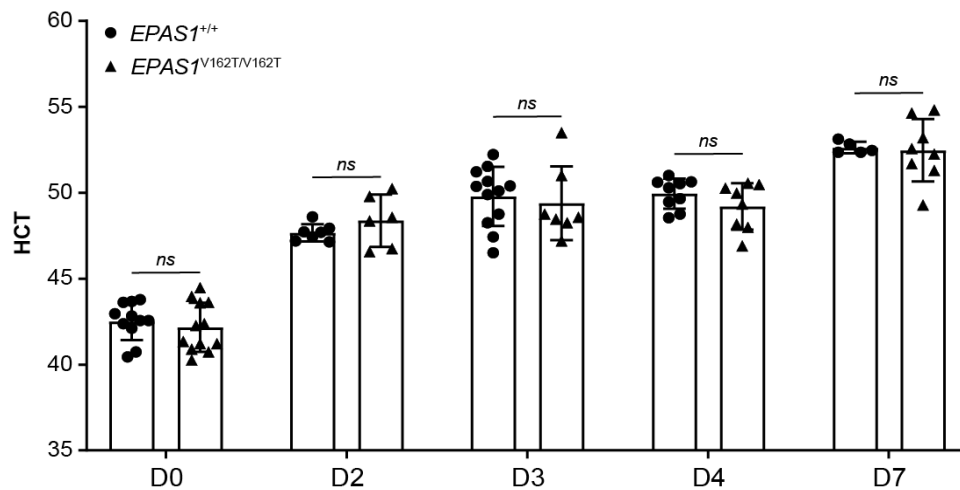
the testing D2 vs D0 in wild type mice and two-sided t tests for the remaining. *ns*: no

significant difference. P values for each group were 0.6723, 0.8691, 0.2483, 0.9249

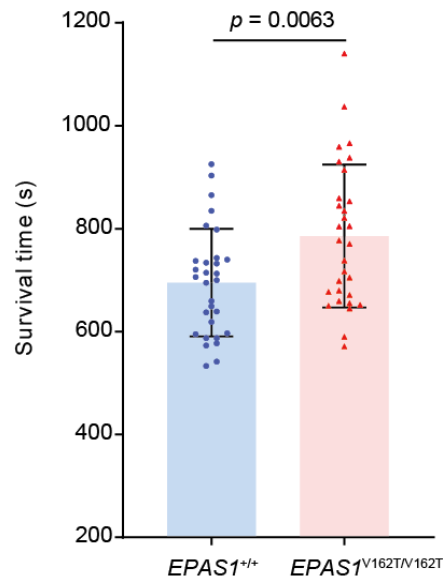
and 0.9606. Source data are provided as a **Source Data** file.



Supplementary Fig. 36 The correlation analysis between mouse body weight changes (a comparison of body weight between D0 and Dx, $x = 2, 3, 4, 7$) and metabolite levels (**a**: hepatic glucose; **b-c**: FFA). The mean body weight change was set as the dependent variable, the mean metabolite abundance as the fixed effect, and the genotype as a random effect.



Supplementary Fig. 37 Comparisons of haematocrit (HCT) measurements between *EPAS1^{V162T/V162T}* and *EPAS1^{+/+}* mouse individuals ($n = 12$ vs 11 on D0, 6 vs 7 on D2, 7 vs 12 on D3, 8 vs 9 on D4 and 8 vs 5 on D7) under different treatments. The bars display mean $\pm$ SD and two-sided t tests were applied. *ns*: no significant difference. P values for each group were 0.5205, 0.2704, 0.6692, 0.2004, and 0.8522. Source data are provided as a **Source Data** file.



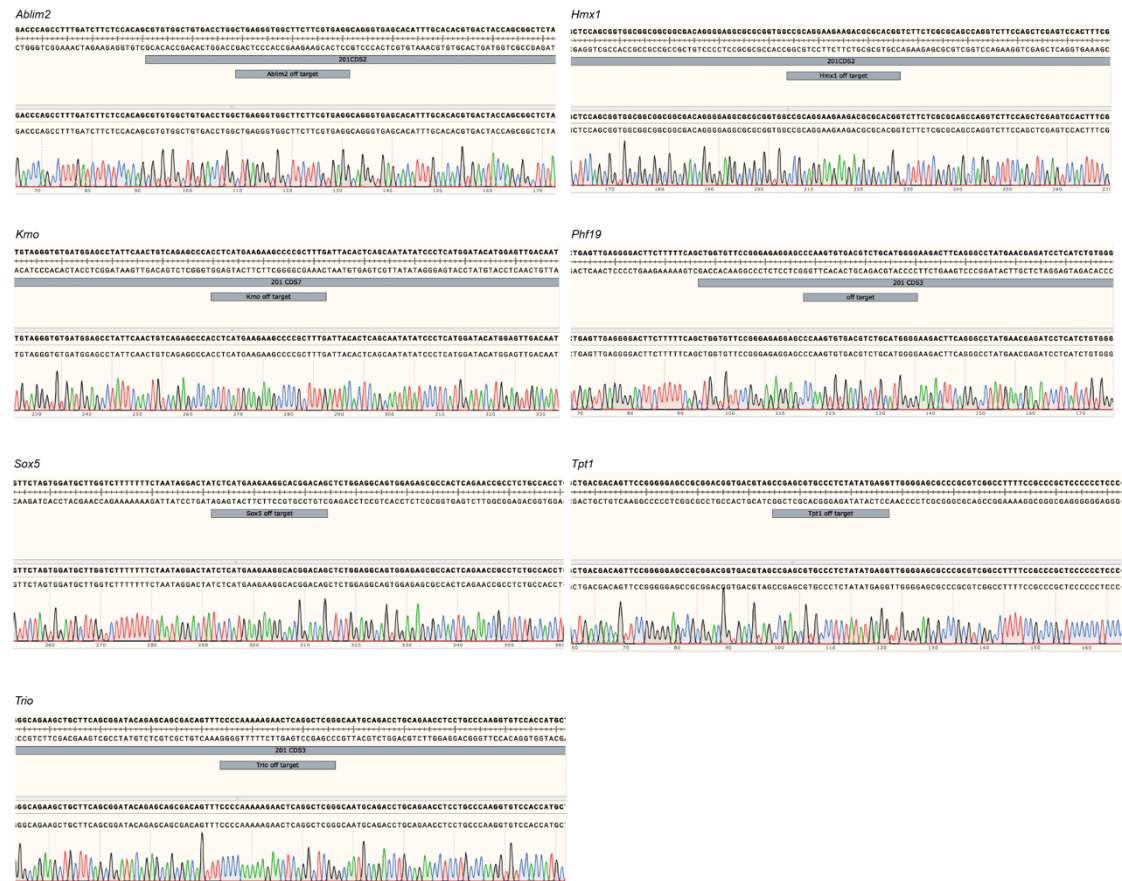
207

208 **Supplementary Fig. 38** Comparison of survival time between $EPAS1^{+/+}$ and

209 $EPAS1^{V162T/V162T}$ mouse individuals ($n = 30$ for each group) under acute hypoxia (4%

210 O_2). The bars display mean $\pm$ SD and a two-sided t test was applied. Source data are

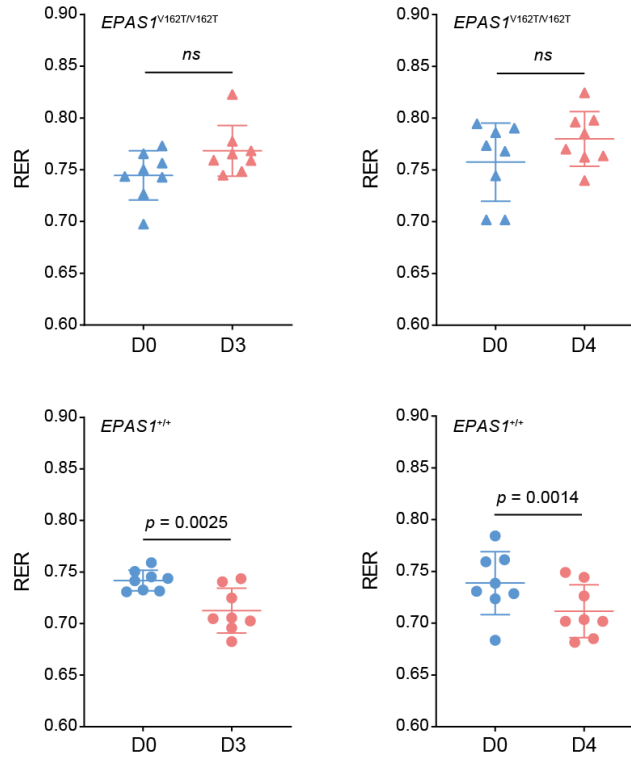
211 provided as a **Source Data** file.



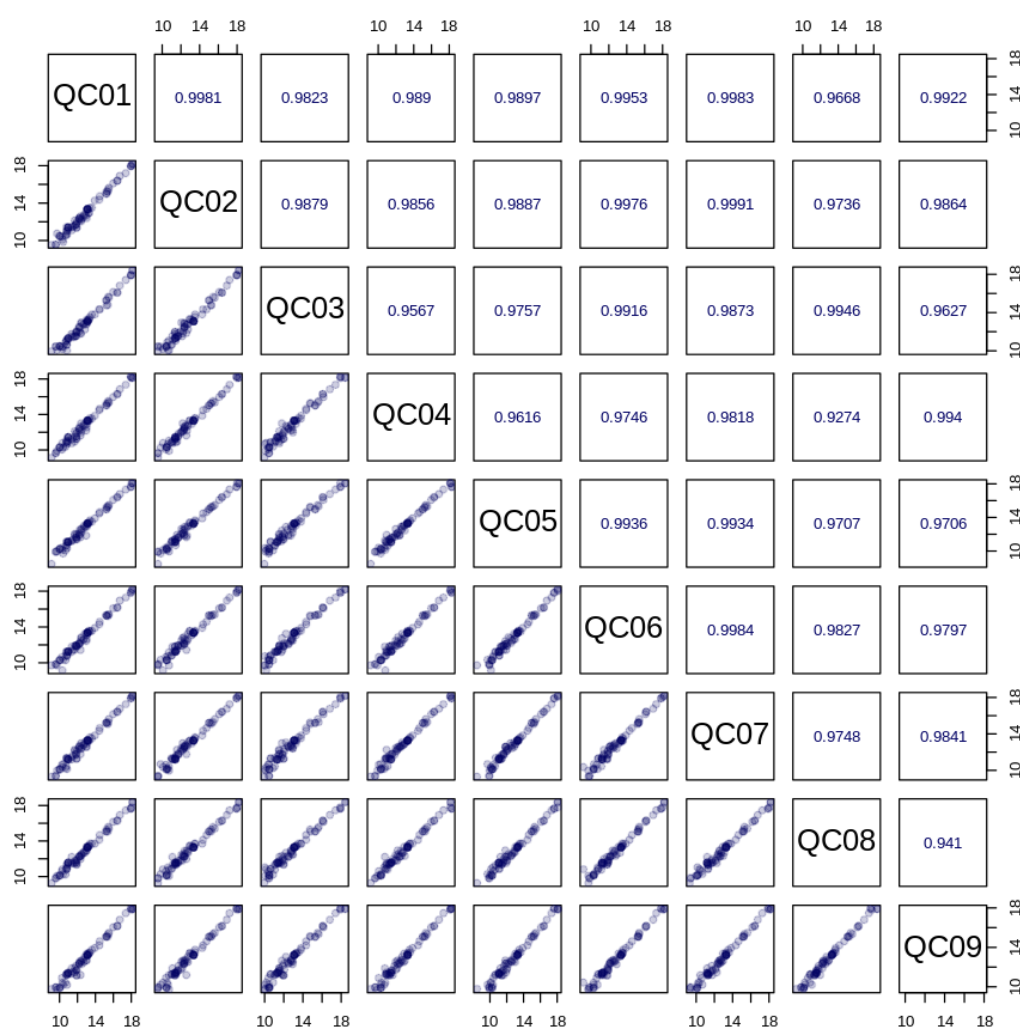
Supplementary Fig. 39 Sanger sequencing results of potential off-target genetic loci (exons).



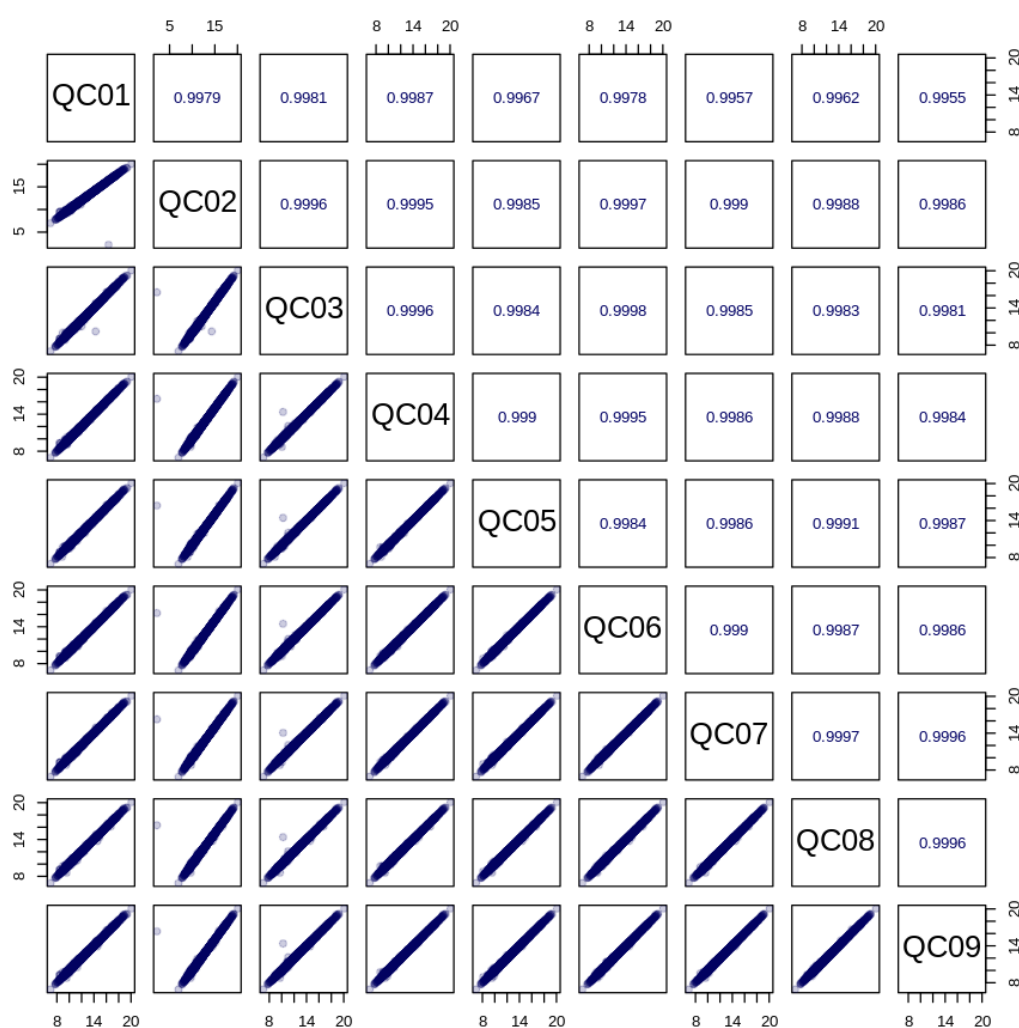
Supplementary Fig. 40 Sanger sequencing results of potential off-target genetic loci (introns).



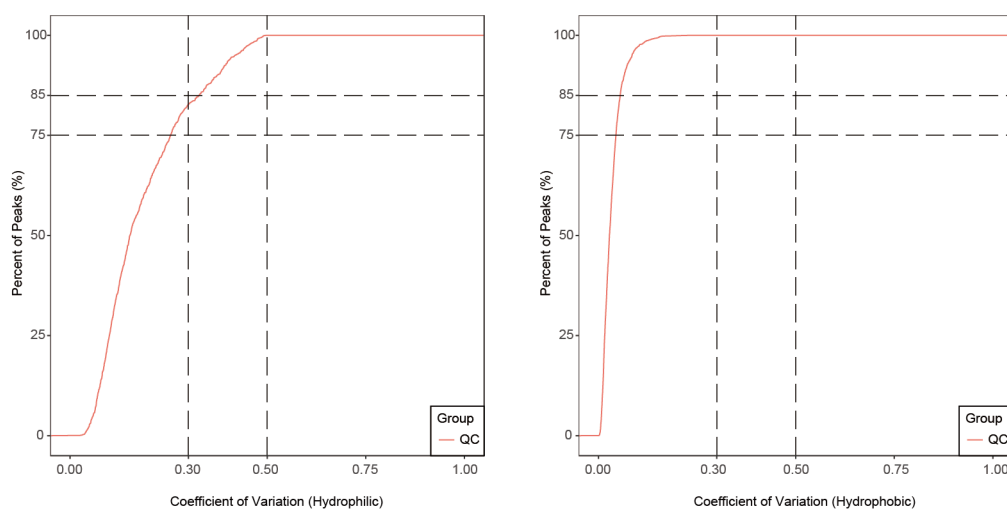
Supplementary Fig. 41 Measurements of respiratory exchange ratio (RER) in mutant (an additional independently derived knock-in mice) and wild type mice under hypoxia and normoxia ($n = 8$ for each group). The bars show mean $\pm$ SD and two-sided t tests were applied. *ns*: no significant difference. P values were 0.1198 (D3 vs D0) and 0.2037 (D4 vs D0) in mutant mice. Source data are provided as a **Source Data** file.



Supplementary Fig. 42 Pearson correlation plot of quality control (QC) samples used for hydrophilic metabolite detection in mouse livers. The diagonal squares represent the names of the QC samples for hydrophilic metabolite detection. The squares in the lower-left corner of the diagonal contain the corresponding scatter plots of the QC sample correlations, where the x and y axes represent the metabolite concentrations (Log-transformed). Each point in the plot represents a metabolite. The squares in the upper-right corner of the diagonal display the Pearson correlation coefficients for the corresponding QC samples.



Supplementary Fig. 43 Pearson correlation plot of QC samples used for hydrophobic metabolite detection in mouse livers. The diagonal squares represent the names of the QC samples for hydrophobic metabolite detection. The squares in the lower-left corner of the diagonal contain the corresponding scatter plots of the QC sample correlations, where the x and y axes represent the metabolite concentrations (Log-transformed). Each point in the plot represents a metabolite. The squares in the upper-right corner of the diagonal display the Pearson correlation coefficients for the corresponding QC samples.



243

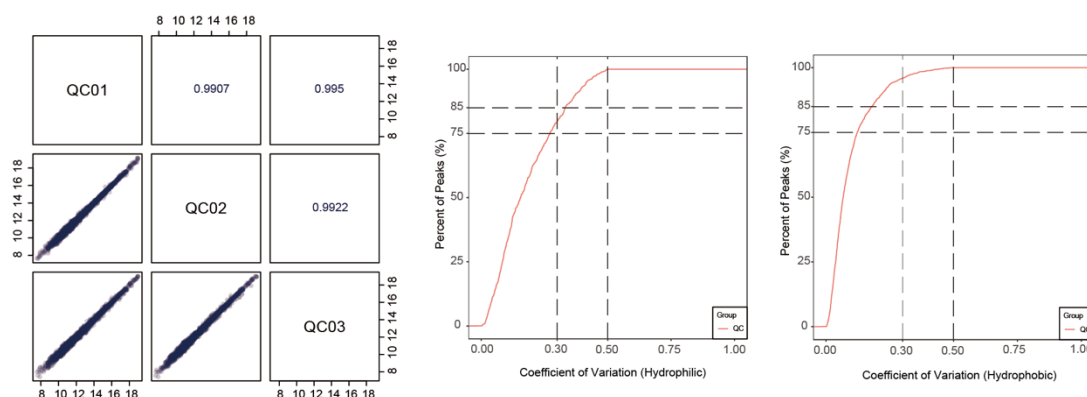
244 **Supplementary Fig. 44** Coefficient of variation (CV) distribution map of QC

245 samples used for hydrophilic and hydrophobic extract detection in mouse livers. The

246 x-axis represents the CV value, while the y-axis indicates the proportion of

247 metabolites with a CV value lower than the corresponding value, relative to the total

248 number of metabolites.



Supplementary Fig. 45 Pearson correlation plot (left) and CV distribution maps

(middle and right) of QC samples used for hydrophilic and hydrophobic extract detection in plasmas of saker falcon. The diagonal squares represent the names of the QC samples for the detection. The squares in the lower-left corner of the diagonal contain the corresponding scatter plots of the QC sample correlations, where the x and y axes represent the metabolite concentrations (Log-transformed). Each point in the plot represents a metabolite. The squares in the upper-right corner of the diagonal display the Pearson correlation coefficients for the corresponding QC samples. In the CV distribution maps, the x-axis represents the CV value and the y-axis indicates the proportion of metabolites with a CV value lower than the corresponding value, relative to the total number of metabolites.

Supplementary Tables

Supplementary Table 1. Summary of glucose and lipid metabolic DEGs within the same mouse type and DEGs between the two mouse types.

<i>EPAS1</i> ^{V162T/V162T}	<i>EPAS1</i> ^{+/+}	<i>EPAS1</i> ^{V162T/V162T} vs <i>EPAS1</i> ^{+/+}
<i>G6pc</i>	<i>G6pc</i>	<i>G6pc</i>
<i>Gck</i>	<i>Gck</i>	<i>Gck</i>
<i>Hk3</i>	<i>Hk1/3</i>	<i>Hk3</i>
<i>Pygl</i>	<i>Pygl</i>	
<i>Gys2</i>	<i>Gys1/2</i>	<i>Gys2</i>
<i>Upg2</i>		<i>Upg2</i>
		<i>Gpi1</i>
<i>Pfkl</i>	<i>Pfkl</i>	
<i>Aldoa/c</i>	<i>Aldoc</i>	<i>Aldoa/b/c</i>
<i>Gapdh</i>	<i>Gapdh</i>	
<i>Pgam1/5</i>	<i>Pgam1/5</i>	
<i>Eno1</i>	<i>Eno1/3</i>	<i>Eno1</i>
<i>Pklr</i>	<i>Pklr</i>	<i>Pklr</i>
<i>Ldha/d</i>	<i>Ldha/b/d</i>	<i>Ldha</i>
<i>Pdha1</i>	<i>Pdha1</i>	<i>Pdha1</i>
<i>Pnpla2/3/6/7/8</i>	<i>Pnpla1/2/3/6/7/8</i>	<i>Pnpla1/2/7</i>
<i>Abhd2/5/11/15/18</i>	<i>Abhd2/4/6/8/10/11/13/15/18</i>	<i>Abhd2/4/6/8/11/13/15/18</i>
<i>Lipe</i>	<i>Lipe</i>	
<i>Plin2/4</i>	<i>Plin2/4/5</i>	<i>Plin2/3/4/5</i>
	<i>Mgl1</i>	<i>Mgl1</i>
<i>Cpt1a, Cpt2</i>	<i>Cpt1a</i>	<i>Cpt1a, Cpt2</i>
<i>Acs11/4/5; Acs1/2/3/5; Acsf2/3</i>	<i>Acs11/3/4/5; Acs1/2/5; Acsf2/3</i>	<i>Acs11/3/4/5</i>
<i>Acad8/9/10/11/12; Acadsb</i>	<i>Acad8/9/10/11/12; Acadsb; Acads/m/l</i>	<i>Acad8/10/11/12; Acadm/l</i>
<i>Acaa1a/b; Acat1/2/3</i>	<i>Acaa1b; Acat2/3</i>	<i>Acaa1a/b; Acat3</i>

Supplementary Table 2. Summary of explanatory degrees of different principal component analysis (PCA) components on behavioral parameters (breathing rate, food intake, food absorption, locomotion).

	PC1	PC2	PC3	PC4
Standard deviation	1.5186	1.0011	0.7826	0.2816
Proportion of variance	0.5765	0.2505	0.1531	0.0198
Cumulative proportion	0.5765	0.8271	0.9802	1.0000

269 **Supplementary Table 3.** Summary of the *Vegf* gene expression in falconized mice under hypoxia.

Gene ID	Full name	Up- or down-regulation	<i>q</i> value	Day
<i>Vegfa</i>	Vascular endothelial growth factor A	Down	2.24E-04	4
<i>Vegfb</i>	Vascular endothelial growth factor B	Down	2.28E-03	4

270

Supplementary Table 4. The qPCR primers for investigated candidate genes involved in lipid metabolism.

Gene ID	Full name	Primer sequence (5' to 3')
<i>ACSL1</i>	<i>Acyl-CoA synthetase</i>	F: CTTTTCGAGCACTCACCACC
	<i>long chain family</i>	R: TCTTCGTGCACCACCACTAC
	<i>member 1</i>	
<i>FASN</i>	<i>Fatty acid synthase</i>	F: GTCTTGAACTCCTTGGCGGA
		R: AGGAAGATAGCCATGCCGAG
<i>IDH1</i>	<i>Isocitrate dehydrogenase</i>	F: AGGGTTGGCCTTTGTATCTG
	<i>(NADP (+)) 1</i>	R: CATGTCGTCGATGAGCCTATG
<i>EPAS1</i>	<i>Endothelial PAS domain</i>	F: CCTCCATCATGCGACTGGCAA
	<i>protein 1</i>	R: CACCACGGCAATGAAACCCTC
<i>ACTB</i>	<i>Actin beta</i>	F: GATGAGATTGGCATGGCTTT
		R: GTCACCTTCACCGTTCCAGT

274 **Supplementary Table 5.** The internal standards for hydrophilic compound detection
 275 in mouse liver tissues using UPLC-MS/MS.

Internal standard	Manufacture	Catalog No.	Concentration
Testosterone-[d3]	IsoReag	IR-15240S	1 µg/mL
Lidocaine	Dr. Ehrenstorfer	CDCT	1 µg/mL
L-2-chlorophenylalanine	J&K Scientific	106151-100mg	1 µg/mL
Sulfaquinoxaline-13C6	ANPEL	CDAA-540001-10mg	1 µg/mL
[2H5]-Phenoxy acetic Acid	IsoReag	IR-22227-250mg	1 µg/mL
L-Phenylalanine (2-13C, 99%)	TCI	ZTO-P0134-25g	1 µg/mL
succinic acid d4	Sigma	293075-5G	1 µg/mL

276

277 **Supplementary Table 6.** The internal standards for hydrophobic compound detection
 278 in mouse liver tissues using UPLC-MS/MS.

Internal standard	Manufacture	Catalog No.	Concentration
PC (13:0/13:0)	Avanti	850340P-25MG	0.5 μ M
Cer (d18:1/4:0)	TRC	ZTR-C262550-50mg	0.2 μ M
PE (12:0/12:0)	Cayman	15089	0.2 μ M
PE (16:0-d31/18:1)	Avanti	860374P-10mg	0.2 μ M
LPE (0:0/14:0)	Avanti	856735P-25mg	0.2 μ M
2-Chloro-L-phenylalanine-1	J&K Scientific	106151-100mg	0.5 μ M
PE (16:0_d31/18:1)	Avanti	860374P-10mg	0.2 μ M
PC (13:0/13:0)	Avanti	850340P-25MG	0.5 μ M

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Supplementary Table 7. The external standards used for targeted metabolomics in mouse liver tissues.

External standard	Manufacture	Catalog No.	Concentration
Glucose	Sigma	158968-25g	0.1 µg/ml~150000 µg/ml
FFA (12:0)	Zzstandard	ZC-59052-100mg	0.01 µg/ml~50 µg/ml
FFA (18:3)	Zzstandard	ZC-59052-100mg	0.01 µg/ml~50 µg/ml

283 **Supplementary Table 8.** The internal standards for hydrophilic compound detection
 284 using UPLC-MS/MS for saker falcon plasma samples.

Internal standard	Manufacture	Catalog No.	Concentration
Sulfaquinoxaline-13C6	ANPEL	CDAA-540001-10mg	1 µg/mL
L-2-chlorophenylalanine	J&K Scientific	106151-100mg	1 µg/mL
4-Fluoro-L-α-phenylglycine	TCI	ZTO-F0862-1g	1 µg/mL
L-tryptophan-d5	Sigma	615862-500MG	1 µg/mL
[2H5]-Phenoxy acetic Acid	IsoReag	IR-22227-250mg	1 µg/mL
L-Phenylalanine (2-13C, 99%)	TCI	ZTO-P0134-25g	1 µg/mL
[2H5]-Hippuric Acid	TRC	ZTR-H356702-10mg	1 µg/mL

285

286 **Supplementary Table 9.** The internal standards for hydrophobic compound detection

287 using UPLC-MS/MS for saker falcon plasma samples.

Internal standard	Manufacture	Catalog No.	Concentration
LPE (0:0/14:0)	Avanti	856735P-25mg	0.2 µM
CUDA	Zzstandard	ZC-23994-5mg	0.5 µM
PC (13:0/13:0)	Avanti	850340P-25MG	0.5 µM
PE (12:0/12:0)	Cayman	15089	0.2 µM
PE (16:0_d31/18:1)	Avanti	860374P-10mg	0.2 µM
PC (13:0/13:0)	Avanti	850340P-25MG	0.5 µM
PE (12:0/12:0)	Cayman	15089	0.2 µM
2-Chloro-L-phenylalanine-1	J&K Scientific	106151-100mg	0.5 µM

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