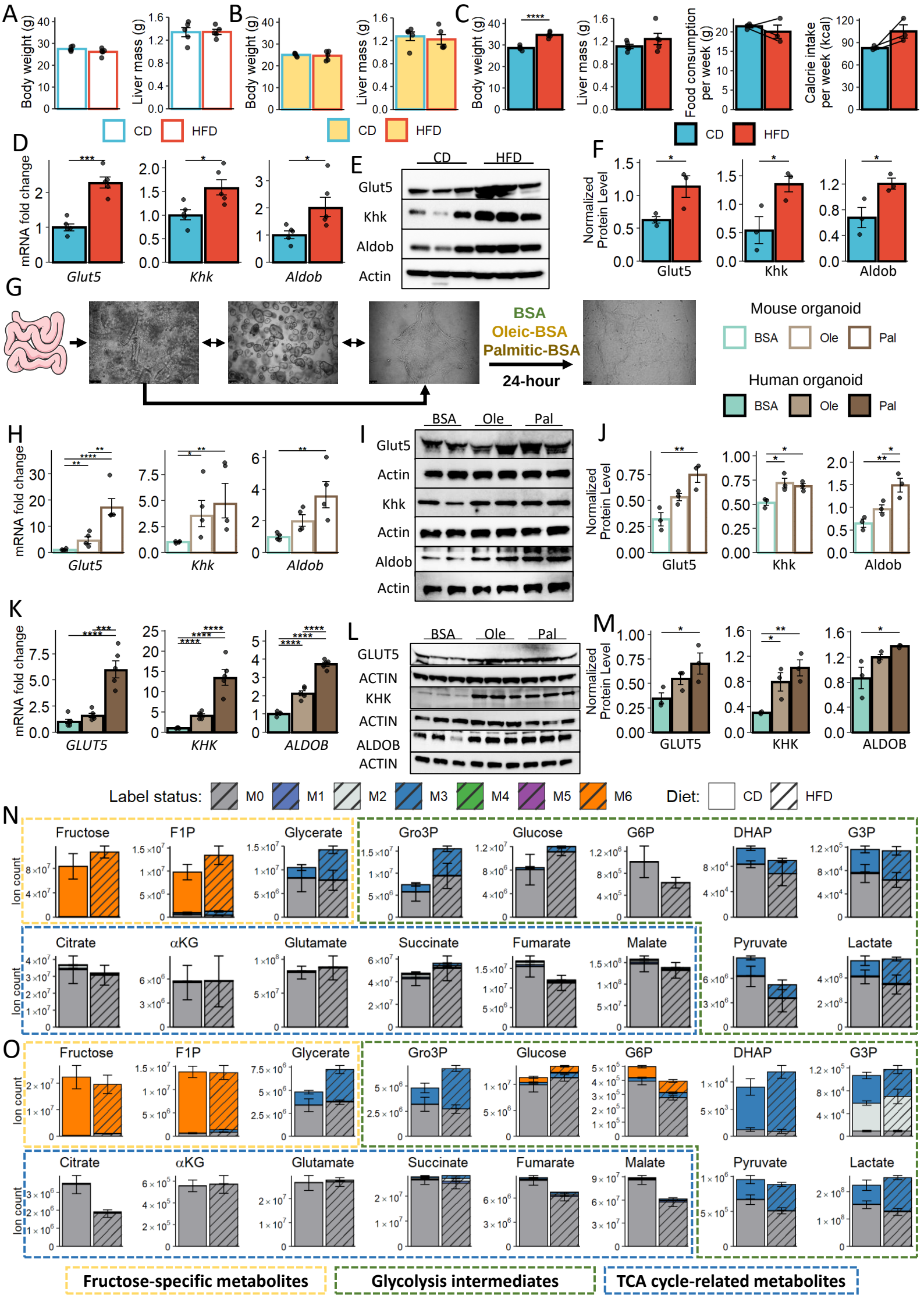
A
Body weight (g)
Liver mass (g)
CD
HFD
B
Body weight (g)
Liver mass (g)
CD
HFD
C
Body weight (g)
****
Liver mass (g)
Food consumption per week (g)
Calorie intake per week (kcal)
CD
HFD
D
mRNA fold change
***
*
*
Glut5
Khk
Aldob
E
CD
HFD
Glut5
Khk
Aldob
Actin
F
Normalized Protein Level
Glut5
Khk
Aldob
G
BSA
Oleic-BSA
Palmitic-BSA
24-hour
Mouse organoid
BSA
Ole
Pal
Human organoid
BSA
Ole
Pal
H
mRNA fold change
Glut5
Khk
Aldob
I
BSA
Ole
Pal
Glut5
Actin
Khk
Actin
Aldob
Actin
J
Normalized Protein Level
Glut5
Khk
Aldob
K
mRNA fold change
GLUT5
KHK
ALDOB
L
BSA
Ole
Pal
GLUT5
ACTIN
KHK
ACTIN
ALDOB
ACTIN
M
Normalized Protein Level
GLUT5
KHK
ALDOB
Label status:
M0
M1
M2
M3
M4
M5
M6
Diet:
CD
HFD
N
Ion count
Fructose
F1P
Glycerate
Gro3P
Glucose
G6P
DHAP
G3P
Citrate
αKG
Glutamate
Succinate
Fumarate
Malate
Pyruvate
Lactate
O
Ion count
Fructose
F1P
Glycerate
Gro3P
Glucose
G6P
DHAP
G3P
Citrate
αKG
Glutamate
Succinate
Fumarate
Malate
Pyruvate
Lactate
Fructose-specific metabolites
Glycolysis intermediates
TCA cycle-related metabolites

Figure S1. Effects of HFD on mouse and human intestinal fructose metabolism. Related to Figure 1.

A - C. Mice body weight and wet liver mass of individual mice during tissue harvest (N = 5) in the special diet feeding experiment for 3 days and 4 weeks.

D. mRNA expression of fructose metabolism genes in the jejunum as quantified by RT-PCR.

E - F. Protein expression of fructose metabolism genes in the jejunum by western blot and the associated quantification (N = 3).

G. Schematic diagram depicting the organoid culture and experimental designs.

H. mRNA expression of fructose metabolism genes in the mouse jejunal organoid as quantified by RT-PCR (N = 4 mice).

I - J. Protein expression of fructose metabolism genes in the mouse jejunal organoid by western blot and the associated quantification (N = 3 mice).

K. mRNA expression of fructose metabolism genes in the human jejunal organoid as quantified by RT-PCR (N = 5 from 2 human donors).

L - M. Protein expression of fructose metabolism genes in the human jejunal organoid by western blot and the associated quantification (N = 3 from 2 human donors).

N. Tracing data (3 days) of fructose metabolism in the jejunum; data are represented in the total ion count.

O. Tracing data (4 weeks) of fructose metabolism in the jejunum; data are represented in the total ion count.

BSA: Bovine serum albumin; Ole: oleic acid; Pal: Palmitic acid; TCA: tricarboxylic acid; F1P: fructose-1-phosphate; Gro3P: Glycerol-3-phosphate; G6P: Glucose-6-phosphate; F6P: fructose-6-phosphate; F1,6P: Fructose-1,6-biphosphate; DHAP: Dihydroxyacetone phosphate; G3P: D-Glyceraldehyde-3-phosphate; Glycerol-3-P: Glycerol-3-phosphate. Data are represented as mean ± SEM. T-test was used for comparison between 2 groups and the post hoc Tukey correction was performed after ANOVA was used for comparison more than 2 groups.*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

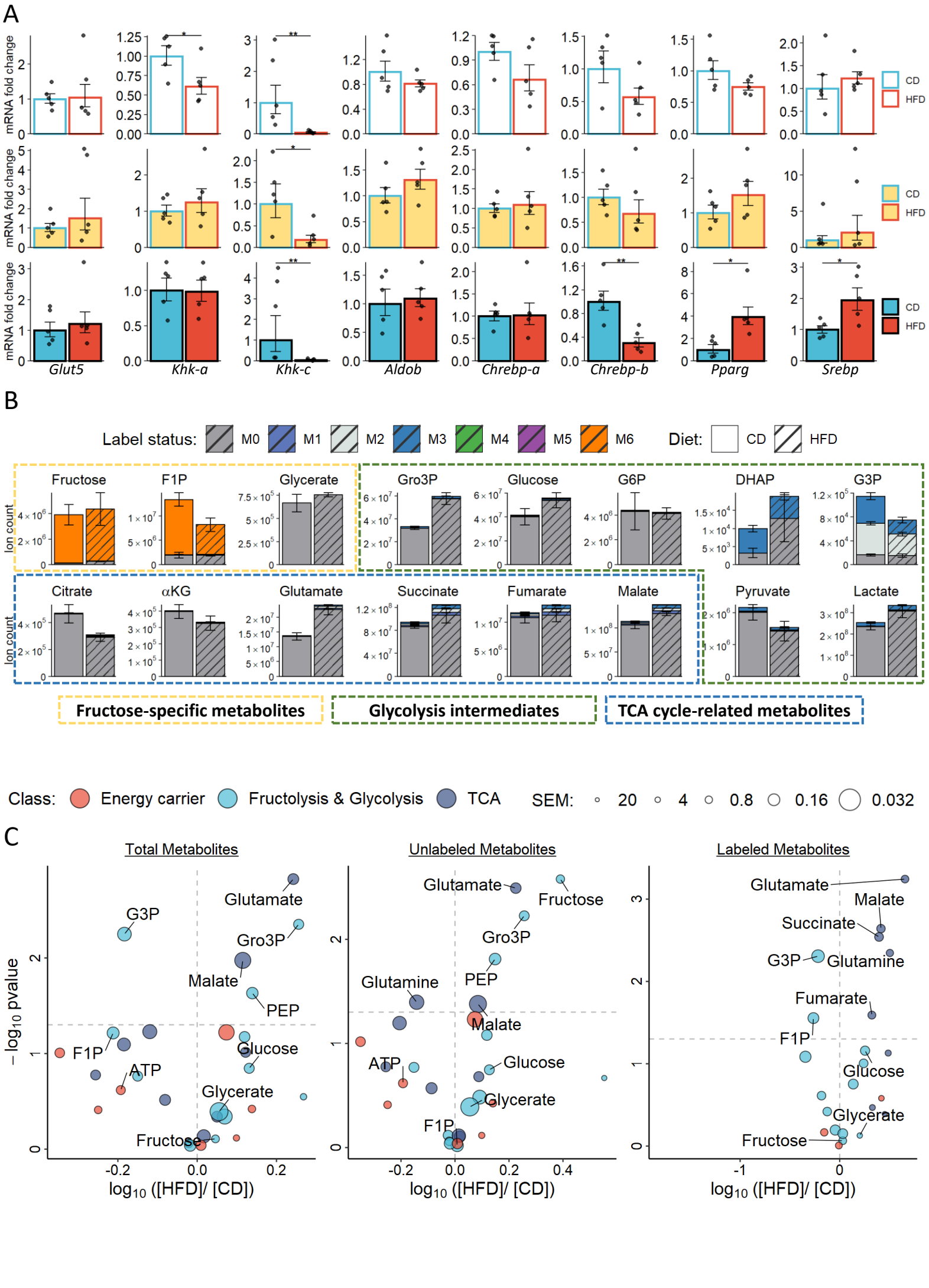
A
mRNA fold change
CD
HFD
Glut5
Khk-a
Khk-c
Aldob
Chrebp-a
Chrebp-b
Pparg
Srebp
B
Label status:
M0
M1
M2
M3
M4
M5
M6
Diet:
CD
HFD
Ion count
Fructose
F1P
Glycerate
Gro3P
Glucose
G6P
DHAP
G3P
Citrate
αKG
Glutamate
Succinate
Fumarate
Malate
Pyruvate
Lactate
Fructose-specific metabolites
Glycolysis intermediates
TCA cycle-related metabolites
Class:
Energy carrier
Fructolysis & Glycolysis
TCA
SEM:
20
4
0.8
0.16
0.032
C
Total Metabolites
Unlabeled Metabolites
Labeled Metabolites
− log10 pvalue
log10 ([HFD]/ [CD])
G3P
Glutamate
Gro3P
Malate
PEP
F1P
Glucose
ATP
Glycerate
Fructose
Glutamine
Succinate
Fumarate

Figure S2. Effects of HFD on mouse hepatic fructose metabolism. Related to Figure 2.
A. mRNA expression of sugar and lipid metabolism genes in the liver as quantified by RT-PCR in mice fed on high fat diet (HFD) for 3 days with no fructose gavage (top panel), mice fed on HFD for 3 days, and tissue harvesting 10 minutes after fructose gavage (middle panel), and mice fed on HFD for 4 weeks and tissue harvesting 10 minutes after fructose gavage (bottom panel).
B. Tracing data (4 weeks) of fructose metabolism in the liver; data are represented in the total ion count.
C. Metabolomic data of fructose metabolism in the liver; data are represented in the log-ratio between the HFD and CD groups after mice were fed for 4 weeks.
TCA: tricarboxylic acid; F1P: fructose-1-phosphate; Gro3P: Glycerol-3-phosphate; G6P: Glucose-6-phosphate; F6P: fructose-6-phosphate; F1,6P: Fructose-1,6-biphosphate; DHAP: Dihydroxyacetone phosphate; G3P: D-Glyceraldehyde-3-phosphate; Glycerol-3-P: Glycerol-3-phosphate., Fructose-Bisphosphate B. NAD+: nicotinamide adenine dinucleotide; NADP+: nicotinamide adenine dinucleotide phosphate; ATP: adenosine triphosphate; GTP: guanosine triphosphate; UTP: uridine triphosphate; SEM: standard error mean. Data are represented as mean ± SEM. T-test was used.*: $p < 0.05$, **: $p < 0.01$.

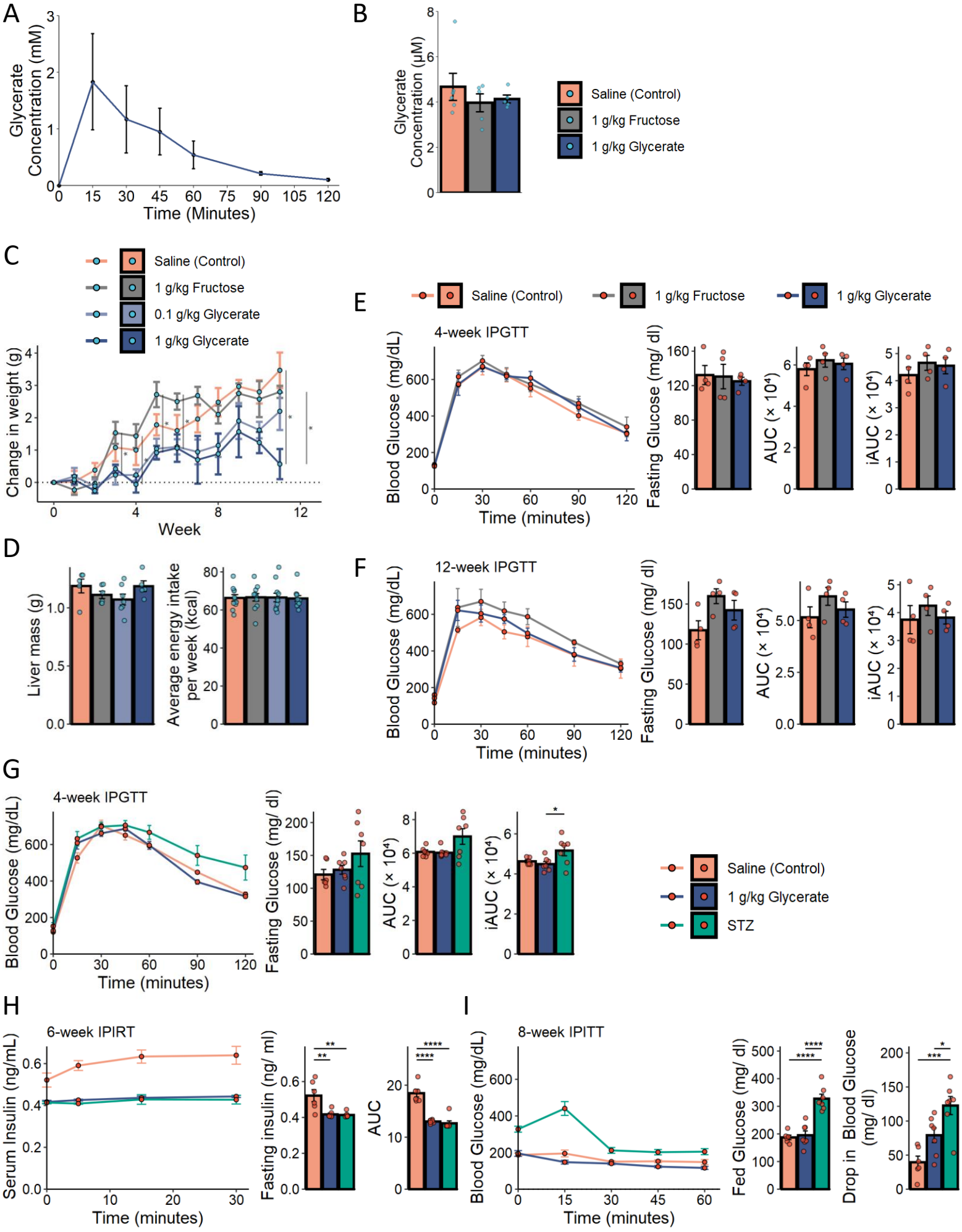
A
Glycerate Concentration (mM)
Time (Minutes)
B
Glycerate Concentration (μM)
Saline (Control)
1 g/kg Fructose
1 g/kg Glycerate
C
Saline (Control)
1 g/kg Fructose
0.1 g/kg Glycerate
1 g/kg Glycerate
Change in weight (g)
Week
D
Liver mass (g)
Average energy intake per week (kcal)
E
Saline (Control)
1 g/kg Fructose
1 g/kg Glycerate
4-week IPGTT
Blood Glucose (mg/dL)
Time (minutes)
Fasting Glucose (mg/ dl)
AUC (× 10⁴)
iAUC (× 10⁴)
F
12-week IPGTT
Blood Glucose (mg/dL)
Time (minutes)
Fasting Glucose (mg/ dl)
AUC (× 10⁴)
iAUC (× 10⁴)
G
4-week IPGTT
Blood Glucose (mg/dL)
Time (minutes)
Fasting Glucose (mg/ dl)
AUC (× 10⁴)
iAUC (× 10⁴)
Saline (Control)
1 g/kg Glycerate
STZ
H
6-week IPIRT
Serum Insulin (ng/mL)
Time (minutes)
Fasting insulin (ng/ ml)
AUC
I
8-week IPITT
Blood Glucose (mg/dL)
Time (minutes)
Fed Glucose (mg/ dl)
Drop in Blood Glucose (mg/ dl)

Figure S3. Effect of elevated glycerate on mouse physiology. Related to Figure 3.
A. Serum glycerate concentration quantification upon single IP injection of 1 g/kg glycerate (N = 5). Tail vein blood was collected at the specified time-point among treatment-naive mice.
B. Serum glycerate concentration upon nutrient injection at 24-hour (N = 5 – 6). Cardiac blood was collected after treatment for 12 weeks.
C - D. Anthropometric measurement of CD mice on different daily nutrient injection cohort (N = 5 – 6).
E - F. Blood glucose levels, AUC, and iAUC quantification in IPGTT experiments among mice fed on HFD (N = 4).
G. In a separate cohort of mice, blood glucose levels, AUC, and iAUC quantification in IPGTT experiment at 4th week (N = 6 – 7).
H. Serum insulin levels and associated AUC quantification in IPIRT experiment (N = 6 – 7).
I. Blood glucose levels and changes in blood glucose level in IPITT experiment (N = 6 – 7).
Data are represented as mean ± SEM. T-test was used for comparison between 2 groups and the post hoc Tukey correction was performed after ANOVA was used for comparison more than 2 groups.*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

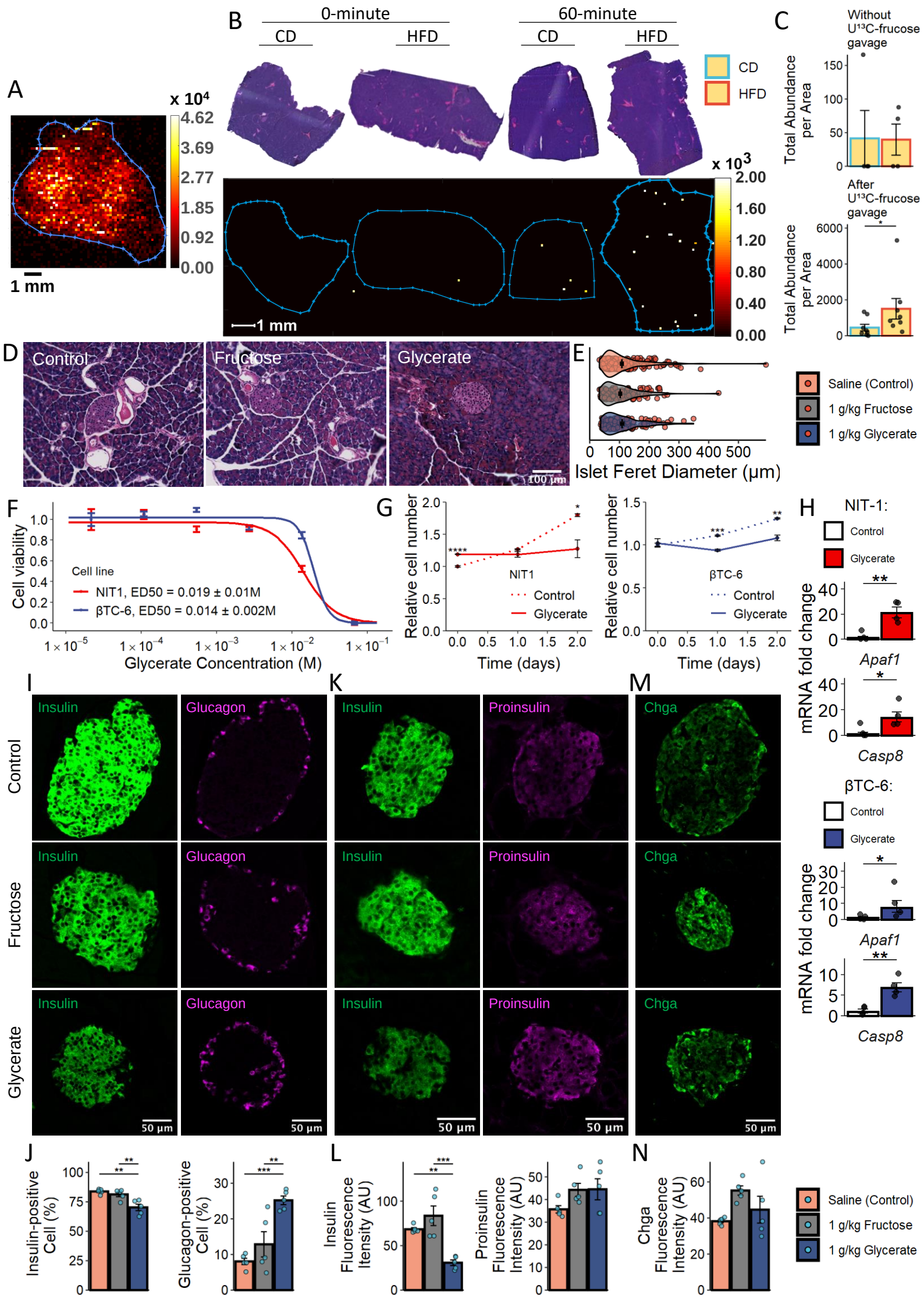
A
x 10^4
4.62
3.69
2.77
1.85
0.92
0.00
1 mm
B
0-minute
60-minute
CD
HFD
CD
HFD
CD
HFD
x 10^3
2.00
1.60
1.20
0.80
0.40
0.00
1 mm
C
Without U13C-frucose gavage
Total Abundance per Area
After U13C-frucose gavage
Total Abundance per Area
D
Control
Fructose
Glycerate
100 μm
E
0 100 200 300 400 500
Islet Feret Diameter (μm)
Saline (Control)
1 g/kg Fructose
1 g/kg Glycerate
F
Cell viability
Cell line
NIT1, ED50 = 0.019 ± 0.01M
βTC-6, ED50 = 0.014 ± 0.002M
Glycerate Concentration (M)
G
Relative cell number
NIT1
Control
Glycerate
Time (days)
Relative cell number
βTC-6
Control
Glycerate
Time (days)
H
NIT-1:
Control
Glycerate
mRNA fold change
Apaf1
Casp8
βTC-6:
Control
Glycerate
mRNA fold change
Apaf1
Casp8
I
K
M
Control
Fructose
Glycerate
Insulin
Glucagon
Insulin
Proinsulin
Chga
50 μm
J
Insulin-positive Cell (%)
Glucagon-positive Cell (%)
L
Insulin Fluorescence Itensity (AU)
Proinsulin Fluorescence Intensity (AU)
N
Chga Fluorescence Intensity (AU)
Saline (Control)
1 g/kg Fructose
1 g/kg Glycerate

Figure S4. Effects of in vitro and in vivo exposure to elevated glycerate. Related to Figure 4.
A. Representative mass spectrometry imaging of mouse jejunum with $U^{13}C$-fructose gavage. Glycerate were detected at m/z = 108.0293 ± 0.0003 Th (± 2.5 ppm).
B. Representative mass spectrometry imaging of mouse pancreas with $U^{13}C$-fructose gavage after 3 days of feeding with CD and HFD. Top row: H&E of the tissue section; Bottom row: matched mass spectrometry imaging heatmap. Glycerate were detected at m/z = 108.0293 ± 0.0003 Th (± 2.5 ppm), N = 4 – 8.
C. Quantification of M+3 glycerate abundance within the pancreatic tissue without $U^{13}C$-fructose gavage and 45 – 60 minutes after $U^{13}C$-fructose gavage. Wilcoxon rank sum test was used to compare the abundance quantification between CD and HFD groups.
D – E. H&E staining of pancreas and quantification after 12 weeks of control, fructose, or glycerate treatment of mice fed on HFD (Control: 222 islets; Fructose: 162 islets; Glycerate: 134 islets).
F. Dose-response curve of in vitro treatment of glycerate to β-cell lines, βTC-6 and NIT-1 (N = 4)
G. Proliferation assay of β-cell lines upon either PBS or 5 mM glycerate treatment. (N = 3 – 5)
H. mRNA expression of apoptosis-related genes in β-cell lines after either PBS or 5 mM glycerate treatment for 24 hours as quantified by RT-PCR (N = 4). Apaf1: apoptotic peptidase activating factor 1; Casp8: caspase 8.
I - J. Separate channel of Insulin-Glucagon staining and the quantification of the α-cell and β-cell.
K - L. Separate channel of Insulin-Proinsulin staining and the quantification of the insulin and proinsulin fluorescence intensity.
M - N. Chromogranin A (Chga) staining and the quantification fluorescence intensity.
N = 5 mice for all staining experiments. Data are represented as mean ± SEM. T-test was used for comparison between 2 groups and the post hoc Tukey correction was performed after ANOVA was used for comparison more than 2 groups.*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

Table S1. Association between the glycerate kinase mutation and diabetes. Related to Figure 3.

	Diabetes Mellites	
	Log Odds	*p-value*
(Intercept)	-3.68	$< 2.00 \times 10^{-16}$
Age	0.0427	$< 2.00 \times 10^{-16}$
Sex (Male)	0.484	$< 2.00 \times 10^{-16}$
Ethnicity (Caucasian)	-0.537	$< 2.00 \times 10^{-16}$
Overweight (BMI >25)	0.472	$< 2.00 \times 10^{-16}$
Trunk fat (%)	0.0693	1.27×10^{-10}
Total cholesterol	-0.925	$< 2.00 \times 10^{-16}$
High density cholesterol	-0.164	4.53×10^{-4}
Low density cholesterol	0.249	9.53×10^{-7}
Triglycerides	0.452	$< 2.00 \times 10^{-16}$
GLYCTK mutations	0.458	1.17×10^{-7}

Table S2. Primers used in the study. Related to STAR Methods.

Primer	Source
Primer: *Glut5* Forward for total mouse cDNA: CCAATATGGGTACAACGTAGCTG	(Chen et al., 2018)
Primer: *Glut5* Reverse for total mouse cDNA: GCGTCAAGGTGAAGGACTCAATA	(Chen et al., 2018)
Primer: *Khk* Forward for total mouse cDNA: ATGTGGTGGACAAATACCCAGA	(Zhao et al., 2020)
Primer: *Khk* Reverse for total mouse cDNA: CAAGCAAGGAAAGGACAGTGC	(Zhao et al., 2020)
Primer: *Khk-a* Forward for total mouse cDNA: TTGCCGATTTTGTCCTGGAT	(Diggle et al., 2010)
Primer: *Khk-a* Reverse for total mouse cDNA: CCTCGGTCTGAAGGACCACAT	(Diggle et al., 2010)
Primer: *Khk-c* Forward for total mouse cDNA: TGGCAGAGCCAGGGAGAT	(Diggle et al., 2010)
Primer: *Khk-c* Reverse for total mouse cDNA: ATCTGGCAGGTTCGTGTCGTA	(Diggle et al., 2010)
Primer: *Aldob* Forward for total mouse cDNA: GAAACCGCCTGCAAAGGATAA	(Zhao et al., 2020)
Primer: *Aldob* Reverse for total mouse cDNA: GAGGGTCTCGTGGAAAAGGAT	(Zhao et al., 2020)
Primer: *Chrebp-a* Forward for total mouse cDNA: CGACACTCACCCACCTCTTC	(Zhao et al., 2020)
Primer: *Chrebp-a* Reverse for total mouse cDNA: TTGTTCAGCCGGATCTTGTC	(Zhao et al., 2020)
Primer: *Chrebp-b* Forward for total mouse cDNA: TCTGCAGATCGCGTGGAG	(Zhao et al., 2020)
Primer: *Chrebp-b* Reverse for total mouse cDNA: CTTGTCCCGGCATAGCAAC	(Zhao et al., 2020)
Primer: *Pparg* Forward for total mouse cDNA: CGGTTTCAGAAGTGCCTTG	(Fujiki et al., 2009)
Primer: *Pparg* Reverse for total mouse cDNA: GGTTCAGCTGGTCGATATCAC	(Fujiki et al., 2009)
Primer: *Srebp-1c* Forward for total mouse cDNA: GAGCCATGGATTGCACATTT	(Rahtu-Korpela et al., 2014)
Primer: *Srebp-1c* Reverse for total mouse cDNA: CTCAGGAGAGTTGGCACCTG	(Rahtu-Korpela et al., 2014)
Primer: *Apaf1* Forward for total mouse cDNA: AGTGGCAAGGACACAGATGG	(Hauck et al., 2017)
Primer: *Apaf1* Reverse for total mouse cDNA: GGCTTCCGCAGCTAACACA	(Hauck et al., 2017)
Primer: *Casp8* Forward for total mouse cDNA: TGCTTGGACTACATCCCACAC	(Hauck et al., 2017)
Primer: *Casp8* Reverse for total mouse cDNA: TGCAGTCTAGGAAGTTGACCA	(Hauck et al., 2017)
Primer: *Ins1+2* Forward for total mouse cDNA: AGCGTGGCTTCTTCTACACACC	(Pujadas et al., 2016)
Primer: *Ins1+2* Reverse for total mouse cDNA: CCAGCTCCAGTTGTGCCACT	(Pujadas et al., 2016)
Primer: *Gcg* Forward for total mouse cDNA: AGGAATTCATTGCGTGGCTG	(Pujadas et al., 2016)
Primer: *Gcg* Reverse for total mouse cDNA: CAATGGCGACTTCTTCTGGG	(Pujadas et al., 2016)
Primer: *β-Actin* Forward for total mouse cDNA: GGCTGTATTCCCCTCCATCG	(Chen et al., 2018)
Primer: *β-Actin* Reverse for total mouse cDNA: CCAGTTGGTAACAATGCCATGT	(Chen et al., 2018)
Primer: *GLUT5* Forward for total human cDNA: ACGTTGCTGTGGTCTGTAACC	(Weng et al., 2018)
Primer: *GLUT5* Reverse for total human cDNA: CATTAAGATCGCAGGCACGATA	(Weng et al., 2018)
Primer: *KHK* Forward for total human cDNA: CTAAGGAGGACTCGGAGATAAGG	(Wang et al., 2020)
Primer: *KHK* Reverse for total human cDNA: CATTGAGCCCATGAAGGCAC	(Wang et al., 2020)
Primer: *ALDOB* Forward for total human cDNA: GGCAGTTCCGAGAAATCCTCT	This paper
Primer: *ALDOB* Reverse for total human cDNA: CTCCTTGGTCTAACTTGATTCCC	This paper
Primer: *β-ACTIN* Forward for total human cDNA: AGAGCTACGAGCTGCCTGAC	(Weng et al., 2018)
Primer: *β-ACTIN* Reverse for total human cDNA: AGCACTGTGTTGGCGTACAG	(Weng et al., 2018)