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Supplemental information

Silencing alanine transaminase 2 in diabetic liver attenuates hyperglycemia by reducing gluconeogenesis from amino acids

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Supplemental Table 1. Subject characteristics *Related to Figure 1.*

	Pre-surgery	Post-surgery
BMI (kg/m^2)	57 ± 10	36 ± 7*
Change in BMI (%)	-	-36 ± 4
Basal glucose (mg/dL)	121 ± 55	87 ± 8*
Basal insulin (mU/L)	17 ± 10	4 ± 1*
HOMA-IR	4.2 ± 2.2	0.8 ± 0.3*
HISI (1000/(μmol/min x mU/L)	0.08 ± 0.04	0.28 ± 0.11*
Change in HISI (%)	-	500 ± 295

*p<0.05 versus Pre-surgery.

Supplemental Table 2. Plasma amino acid concentrations (μM) *Related to Figure 6.*

	db/+ shLacZ	db/+ shGpt2	db/db shLacZ	db/db shGpt2
threonine	130.6 ± 17.3	149.4 ± 16.5	122.8 ± 8.7	137.2 ± 19.1
methionine	62.6 ± 6.2	68.2 ± 4.7	55.9 ± 3.2	51.6 ± 3.6[b]
lysine	241.9 ± 17.0	249.4 ± 16.2	245.1 ± 18.0	248.9 ± 36.4
histidine	55.5 ± 3.4	58.5 ± 2.9	67.9 ± 2.9[a]	84.0 ± 12.2[a]
phenylalanine	78.3 ± 4.3	86.6 ± 2.7	111.2 ± 2.7[a]	118.8 ± 9.0[a]
tyrosine	90.6 ± 8.4	96.5 ± 7.0	87.8 ± 2.4	92.3 ± 14.7
serine	116.8 ± 7.0	133.8 ± 7.4	108.7 ± 5.8	118.6 ± 15.9
glutamine	435.9 ± 24.6	467.4 ± 9.1	411.5 ± 45.9	404.1 ± 20.9[b]
proline	79.3 ± 7.2	93.6 ± 6.5	79.8 ± 6.9	88.3 ± 11.5
ornithine	51.6 ± 5.9	77.0 ± 9.1[c]	70.1 ± 2.6[a]	85.5 ± 3.5[ac]
aspartate	25.0 ± 1.4	28.4 ± 1.0	23.3 ± 0.9	26.3 ± 3.6
asparagine	45.3 ± 5.2	61.8 ± 6.5	41.5 ± 4.1	50.2 ± 7.2
citrulline	50.6 ± 4.0	53.5 ± 3.5	57.2 ± 5.4	63.0 ± 6.1

[a]p<0.05 versus db/+ shLacZ mice.
[b]p<0.05 versus db/+ shGpt2.
[c]p<0.05 versus shLacZ mice of the same genotype.

Supplemental Table 3. Human Primer Sequences for Real-Time Quantitative PCR *Related to STAR Methods.*

Gene	Forward	Reverse
RPLP0	GTGATGTGCAGCTGATCAAGACT	GATGACCAGCCCAAAGGAGA
GPT2	GTGATGGCACTATGCACCTAC	TTCACGGATGCAGTTGACACC
GPT	CAAGAAGGTGCTCATGGAGATGG	TGTTCACCACCTCCACATAGC
SFXN	TTAACATCAAGGAACCTCGATGG	TGGGGTCAGTTACAGTGAAGA
SLC25A2	GGGACAGCGTGTGTACTGAC	ACTTGGGCGTATGTCTTCAGG
SLC25A39	TCGTGAAGATCGTGAGGCAC	GGCTCGACCACACAGGAAG
SLC25A18	CTCATCAATGGAGGTGTAGCAG	GAGCCGTCTTCATCAGGCA
SLC25A38	ATGATTCAGAACTCACGTCCGT	CAGGCGTGTTTTAAGGAGATCC
SLC25A40	ATTAGACTCCAAGCCCAAAACAA	GGAAGGCCACTCCATAGAGATT
ASNS	CAGCGGGGACCCAATAGTAG	GTGTAGGACGTGAGCAGAAAA
FGF21	ACCAGAGCCCCGAAAGTCT	CTTGACTCCCAAGATTTGAATAAC
TRIB3	AAGCGGTTGGAGTTGGATGAC	CACGATCTGGAGCAGTAGGTG
DDIT4	TGAGGATGAACACTTGTGTGC	CCAACTGGCTAGGCATCAGC
PSAT1	TGCCGCACTCAGTGTTGTTAG	GCAATTCCCGCACAAGATTCT

Supplemental Table 4. Mouse Primer Sequences for Real-Time Quantitative PCR *Related to STAR Methods.*

Gene	Forward	Reverse
Rplp0	GCAGACAACGTGGGCTCCAAGCAGAT	GGTCCTCCTTGGTGAACACGAAGCCC
Gpt	AGCCTTTTACTGAGGTTATCCGT	TCAGAAGATTGGGGTAGACACA
Gpt2	GAGGTAATCCGAGCCAACATTG	GGTTTGGGTAGGTGCAGAGTG
Atf4	CGAGTTAAGCACATTCCTGGAATC	TTCGCTGTTCAGGAAGCTCAT
Sfxn1	GTGCCACCCAACATTAACATCA	ACCACTTTCCTCGCATTCTCTA
Slc25a2	GTGGCCTTTACAGGGGAACC	TTCCTGACAAACTGTTGGCAA
Slc25a39	CTCGGCAACCAGCGAATTG	CCATTGCAGTATAGGAGGCACT
Slc25a18	ATCTCCGCAAAACTCATCAATGG	GGTTCTGTAGTCGGGTCTTGG
Slc25a38	TCCCCAGTGATCGAGAAGG	CTGGAAGAGGAGCGTGGAAC
Slca2540	TGGTGACTCCCCTGGATGTT	ATGTCCCACGGAAGTTTCCTG
Asns1	GTTTGGGAAAAGGTAGACCTCAC	TTGTATGGCTCGTTTTGGCAG
Fgf21	GTGTCAAAGCCTCTAGGTTTCTT	GGTACACATTGTAACCGTCCTC
Trib3	TGCAGGAAGAAACCGTTGGAG	CTCGTTTTAGGACTGGACACTTG
Ddit4	CAAGGCAAGAGCTGCCATAG	CCGGTACTTAGCGTCAGGG
Psat1	CAGTGGAGCGCCAGAATAGAA	CCTGTGCCCCTTCAAGGAG
Pck1	GGGTGCAGAATCTCGAGTTG	CACCATCACCTCCTGGAAGA
Mpc1	GACTTTCGCCCTCTGTTGCTA	GAGGTTGTACCTTGTAGGCAAAT
Mpc2	ACCTACCACCGACTCATGGAT	TGTAAAGCGGCCTCAATTTCT
Gdh1	TAAGATCAACCCCAAGAACTATACA	ATAGTGCCCTATGGTGCTGGCATAG
Got1	ACCAATCTACGTATCATCACCAA	TGGAGGACAAAGATGGAGAACTC

Supplemental Figure Legends

Supplementary Figure 1. *Gpt2* expression is regulated by obesity and ATF4. *Related to Figure 3.* **(A)** mRNA expression of *Gpt* and other genes encoding amino acid transporters in diet-induced obese mice. **(B)** mRNA expression of *Gpt* and ATF4-target genes following ATF4 overexpression or knockdown in *db/db* mice. **(C)** Gene expression in hepatocytes isolated from lean C57BL/6J mice treated with 1 μl (low; n=3) or 5 μl (high; n=3) of an adenovirus overexpressing ATF4 or 5 μl of an adenovirus overexpressing β-gal (n=3). Data are presented as mean ± SEM. * indicates $p<0.05$.

Supplemental Figure 2. Loss of ALT2 does not affect glutamine metabolism in isolated hepatocytes. *Related to Figure 4.* **(A)** Intracellular citrate and α-ketoglutarate (aKG) enrichment from ^{13}C-glutamine is shown. A representative experiment performed in triplicate is shown. **(B)** Media glucose enrichment from ^{13}C-glutamine is shown. **(C)** Blood glucose area under the curve during ATT, QTT, and PTT in lean WT or LS-*Gpt2*-/- mice. Data are presented as mean ± SEM.

Supplementary Figure 3. Loss of ALT2 in the livers of lean mice does not affect plasma organic and amino acids levels. *Related to Figure 5.* Plasma **(A)** organic acids and **(B)** amino acids from fed and fasted LS-*Gpt2*-/- and WT mice measured by mass spectrometry. Data analysis and quantification involved comparisons of individual ion peaks to that of the internal standard for each amino or organic acid. Data is presented as box and whisker plots with the whiskers indicating minimum and maximum values, the bottom of the box as the lower quartile, the line bisecting the box as the median, and the top of the box as the upper quartile.

Supplementary Figure 4. Characterization of blood and plasma parameters in fed and fasted LS-*Gpt2*-/- mice. *Related to Figure 5.* LS-*Gpt2*-/- and fl/fl mice were either given ad libitum access to food or fasted for 18h. Graphs depict plasma concentrations of **(A)** insulin, **(B-C)** lipids, **(D)** total ketone bodies, **(E)** glycerol, and **(F)** amino acids. Data are presented as mean ± SEM. *indicates $p<0.05$.

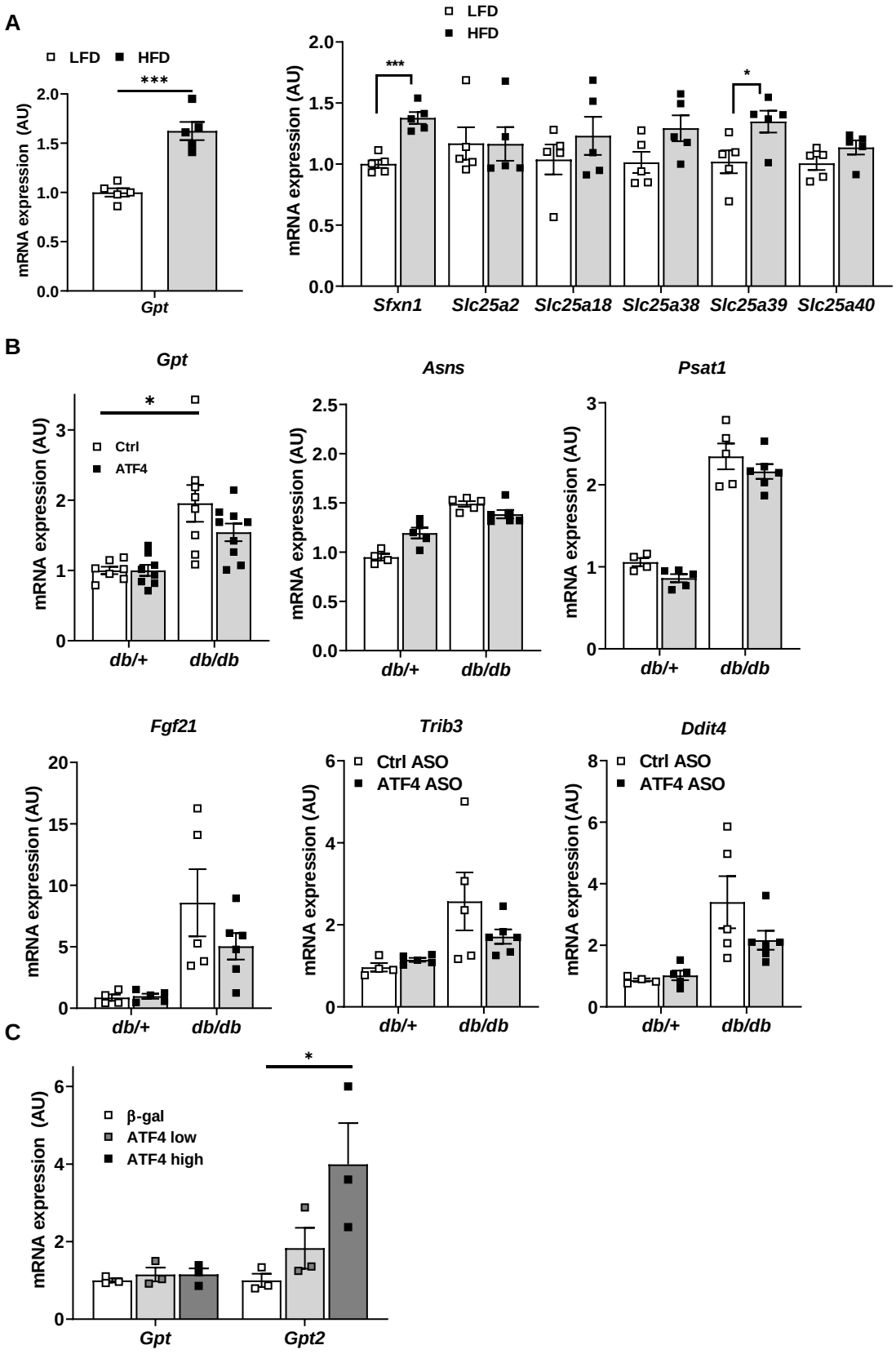
Supplementary Figure 5. Effect of hepatic loss of ALT2 on gene expression of potential compensatory pathways in fed and fasted conditions. *Related to Figure 5.* mRNA expression of liver **(A)** *Gpt*, **(B)** mitochondrial pyruvate carriers (*Mpc1* and *Mpc2*), **(C)** *Pck1* (gluconeogenic), **(D)** glutamate/glutamine metabolism-related genes, **(E)** mitochondrial amino acid transporters. Data generated by qPCR are presented as arbitrary units (AU) while data generated by RNA-seq is expressed as counts per million (cpm). Data is presented as mean ± SEM. *indicates $p<0.05$.

Supplemental Figure 6. Liver amino acid transporter expression and plasma organic acids in *db/db* mice after shRNA treatment. *Related to Figure 6.* **(A)** mRNA expression of mitochondrial amino acid transporters in livers collected from *db/db* mice after a 5h fast. Data are presented as mean ± SEM. *$p<0.05$. **(B)** Plasma organic acid concentrations in *db/+* or *db/db* mice 7 days after administration of adenovirus expressing shRNA against *LacZ* or *Gpt2*. Plasma was collected at sacrifice after a 4 h fast (n=4-5 per group). *$p<0.05$ versus *shLacZ* mice of the same genotype.

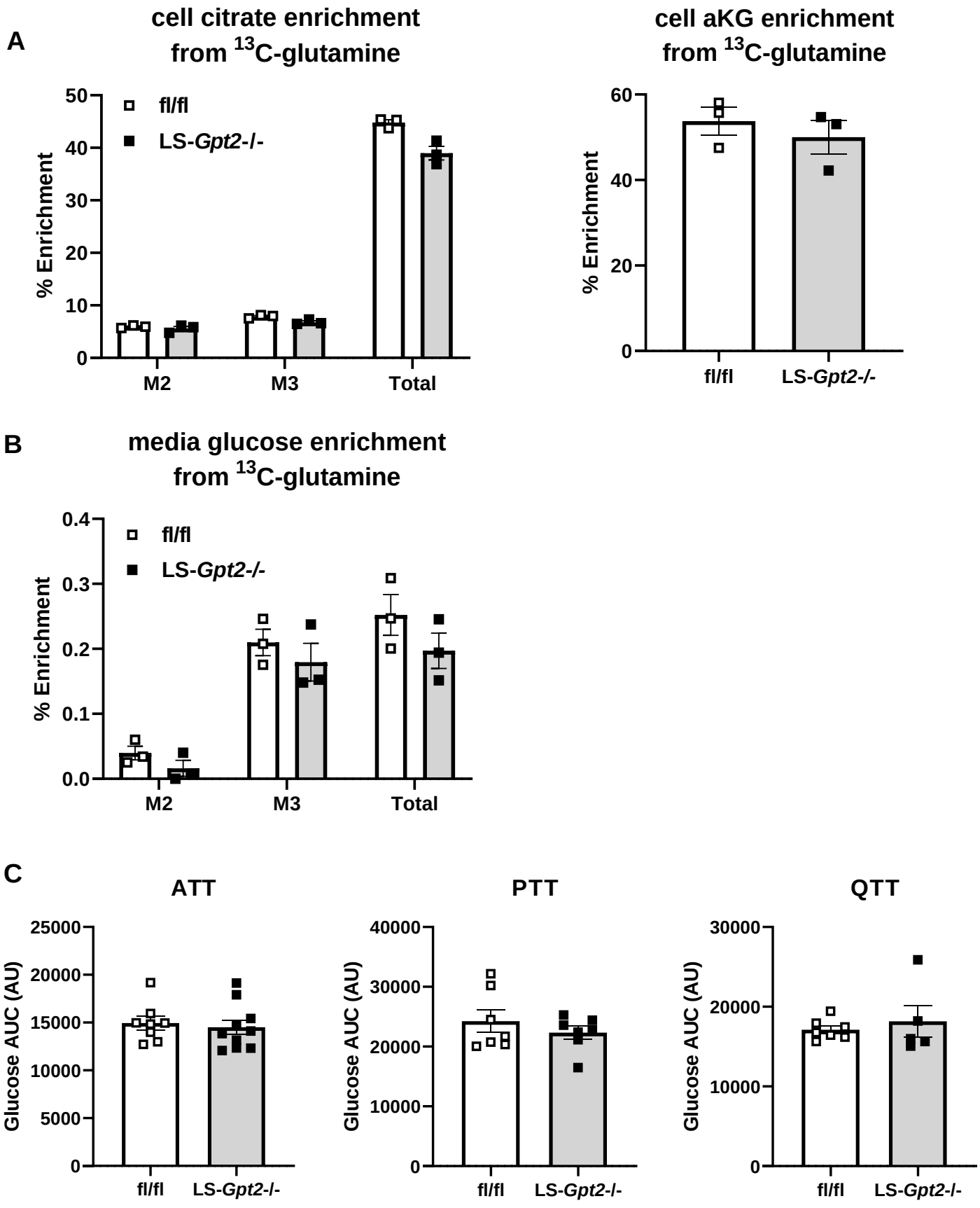
Supplemental Figure 7. Plasma lipids, insulin-stimulated AKT phosphorylation, and liver mitochondrial amino acid transporters are not affected by *Gpt2* knockdown in *db/db* mice. *Related to Figure 7.* **(A)** Plasma triglyceride and cholesterol concentrations 7 days after

administration of adenovirus expressing shRNA against *LacZ* or *Gpt2*. Plasma was collected at sacrifice after a 4 h fast (n=7-8 per group). **(B)** Densitometric quantification of blots in Figure 7E from *db/db* mice 7 days after administration of adenovirus expressing shRNA against *LacZ* or *Gpt2*.

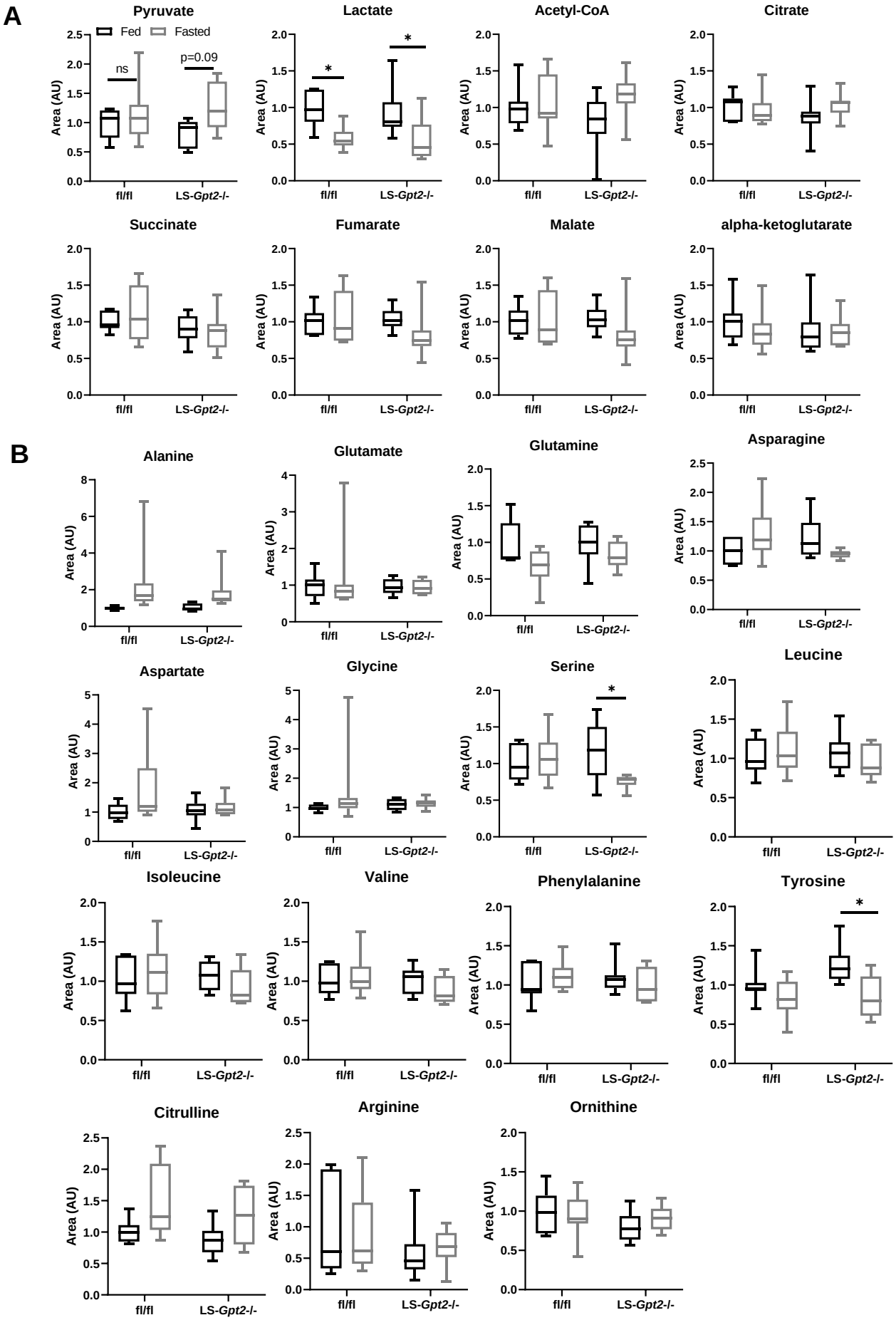
Supplemental Figure 1



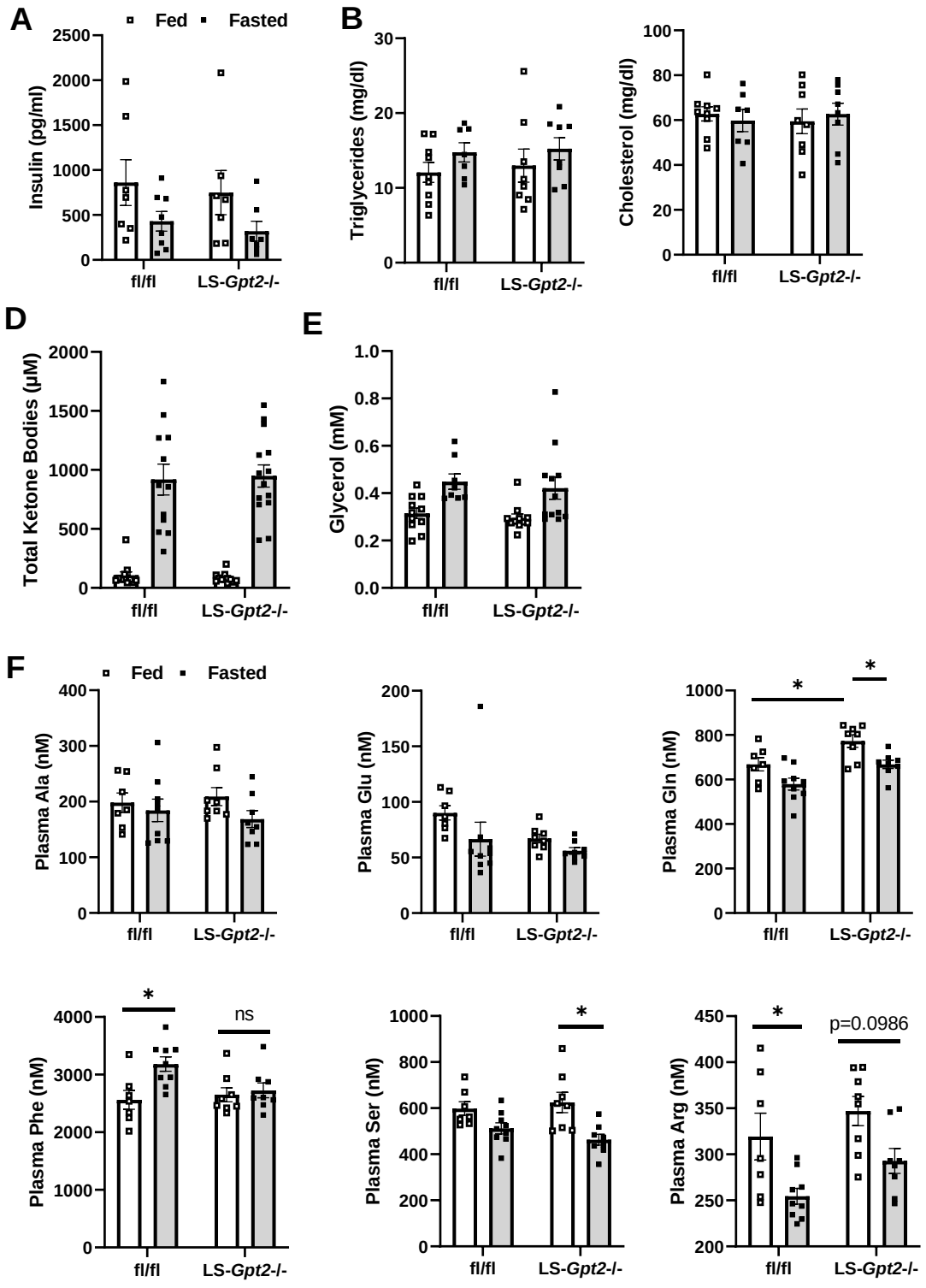
Supplemental Figure 2



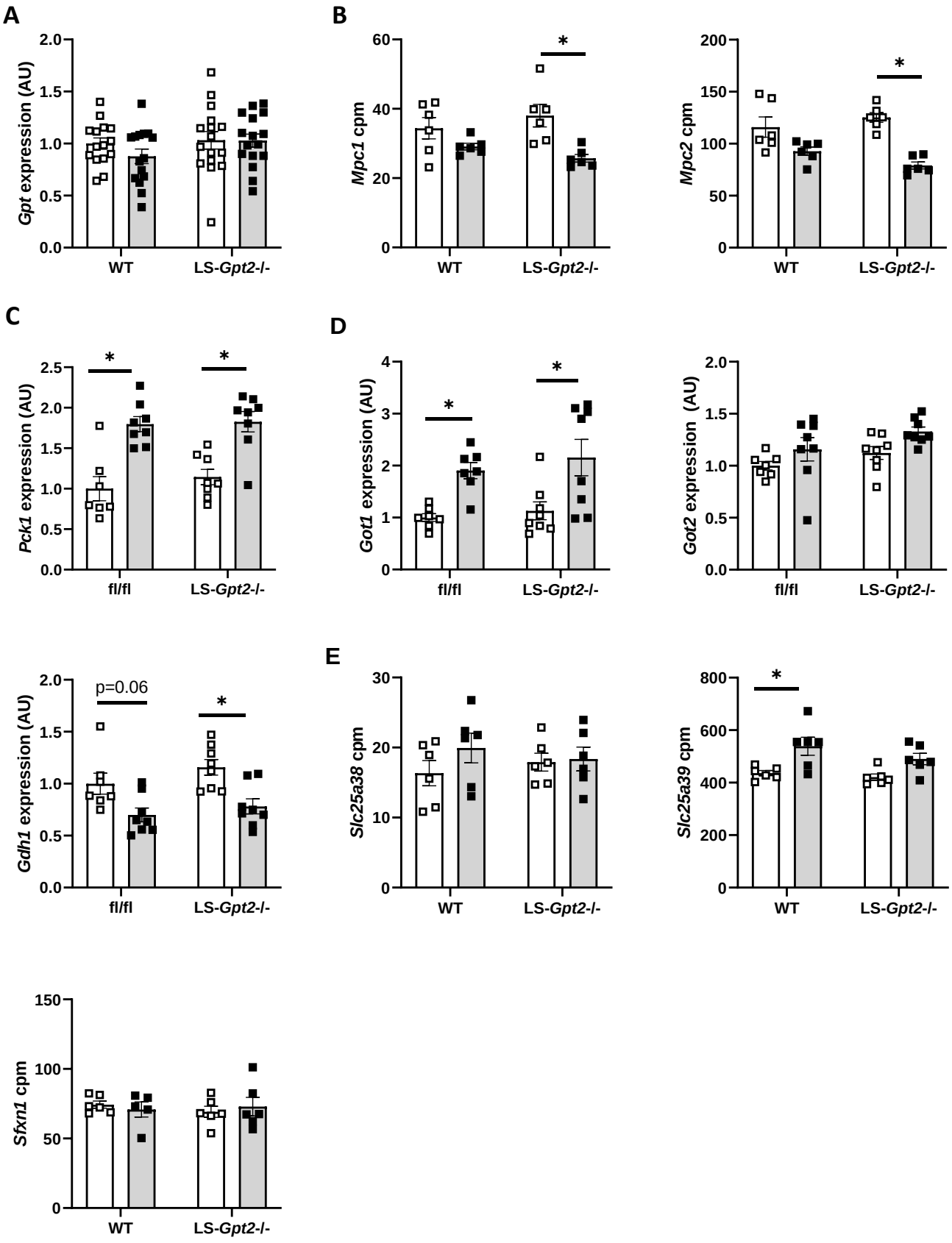
Supplemental Figure 3



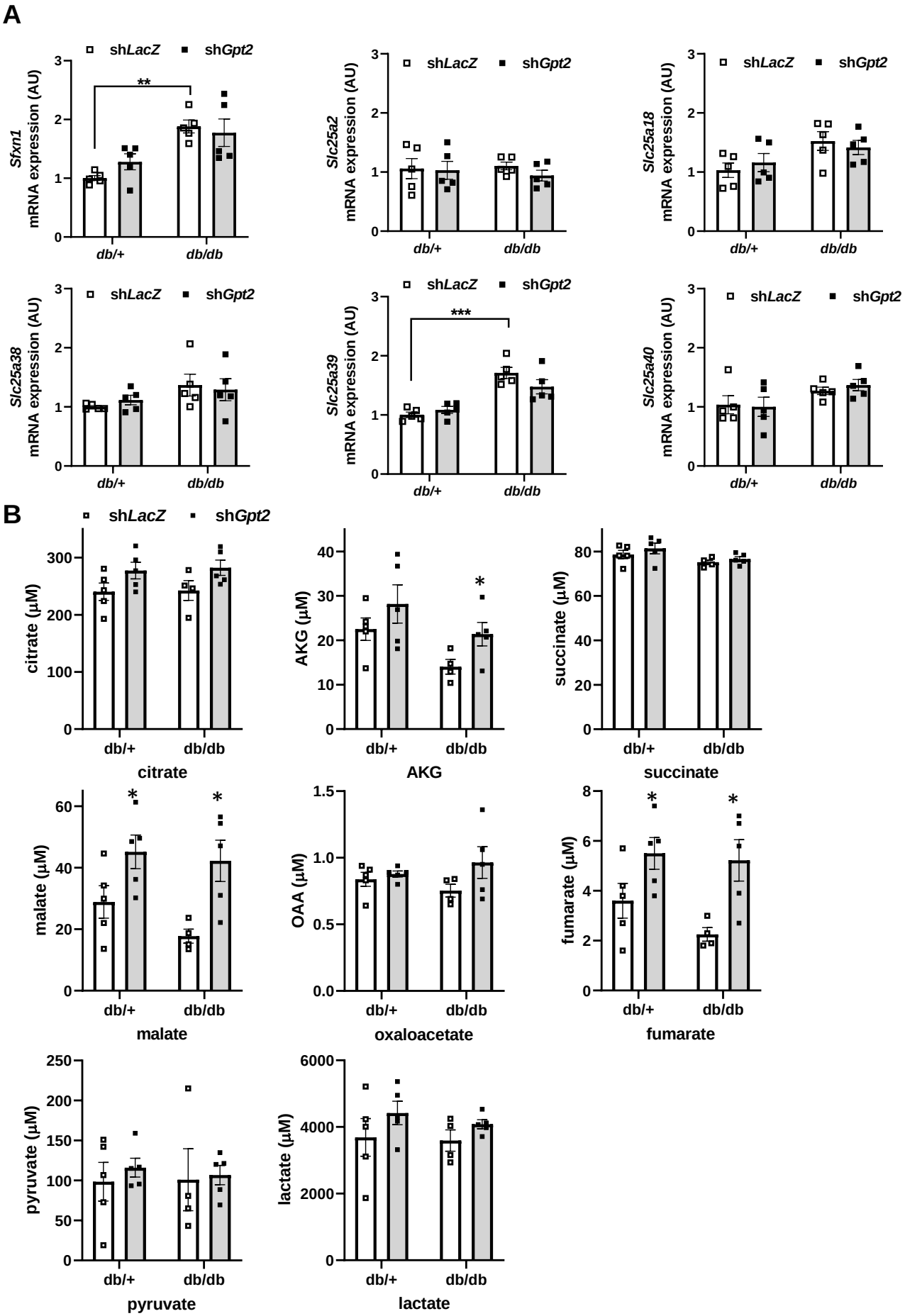
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7