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Supplementary Materials for

Activation of mTORC1 in skeletal muscle regulates whole-body metabolism through FGF21

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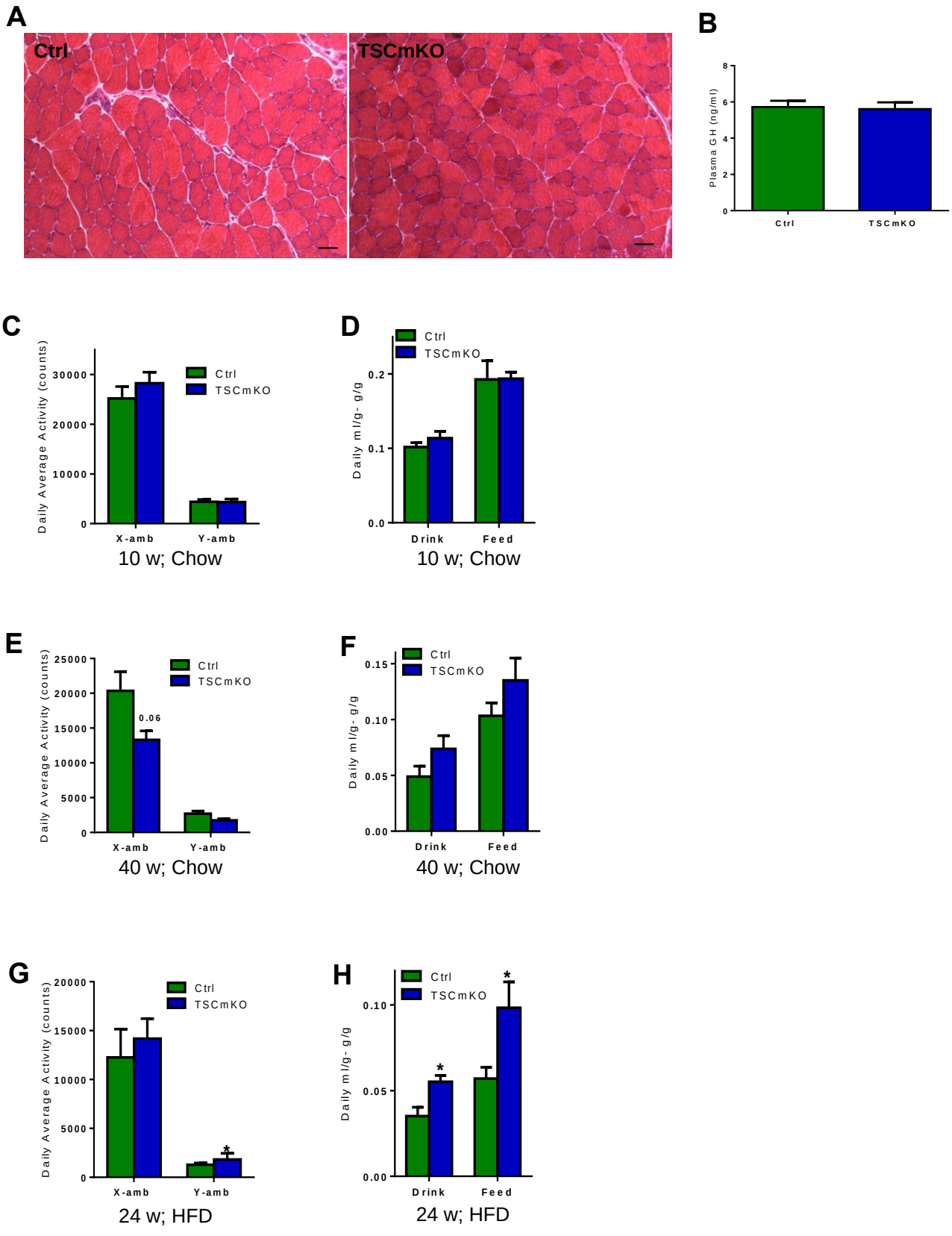
The PDF file includes:

Figure S1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X-amb
Y-amb
10 w; Chow
D
Daily ml/g- g/g
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0.1
0.2
Ctrl
TSCmKO
Drink
Feed
10 w; Chow
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40 w; Chow
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20000
Ctrl
TSCmKO
*
X-amb
Y-amb
24 w; HFD
H
Daily ml/g- g/g
0.00
0.05
0.10
Ctrl
TSCmKO
*
*
Drink
Feed
24 w; HFD

Figure S1: TSCmKO mice do not show overt behavioral changes

A. H&E staining of TA muscle from 12-week-old TSCmKO and control mice (n=3 mice per genotype). Scale bar, 100 µm.

B. Plasma growth hormone (GH) concentration is similar in 10-week-old TSCmKO (n= 7) and control mice (n= 9).

C-H. CLAMS (Comprehensive Lab Animal Monitoring System) analysis of 10- (C-D, n= 6 mice per genotype) and 40- (E-F, n=6 mice per genotype) week-old TSCmKO and control mice on a chow diet, and of 16-week-old mutant and control mice during HFD (G-H, n= 6 mice per genotype). Activity, drinking and feeding were measured and normalized to body weight and daily average.

Data represent mean ± SEM, *p<0.05, **p<0.01, ***p<0.001

Figure S2

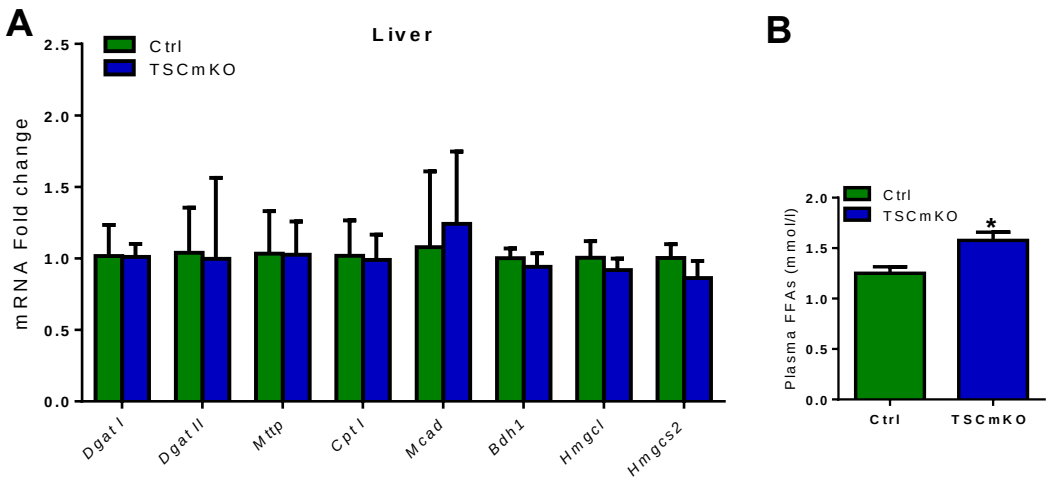


Figure S2: Higher plasma concentrations of free fatty acids in TSCmKO mice

A. Relative expression of genes involved in fatty acid metabolism in liver. mRNA was extracted from liver of 10-week-old TSCmKO and control mice (n= 4 mice per genotype).

B. Plasma free fatty acid concentrations after 24h starvation in 10-week-old TSCmKO and control mice (n= 4 mice per genotype).

Data represent mean ± SEM, *p<0.05.

Figure S3

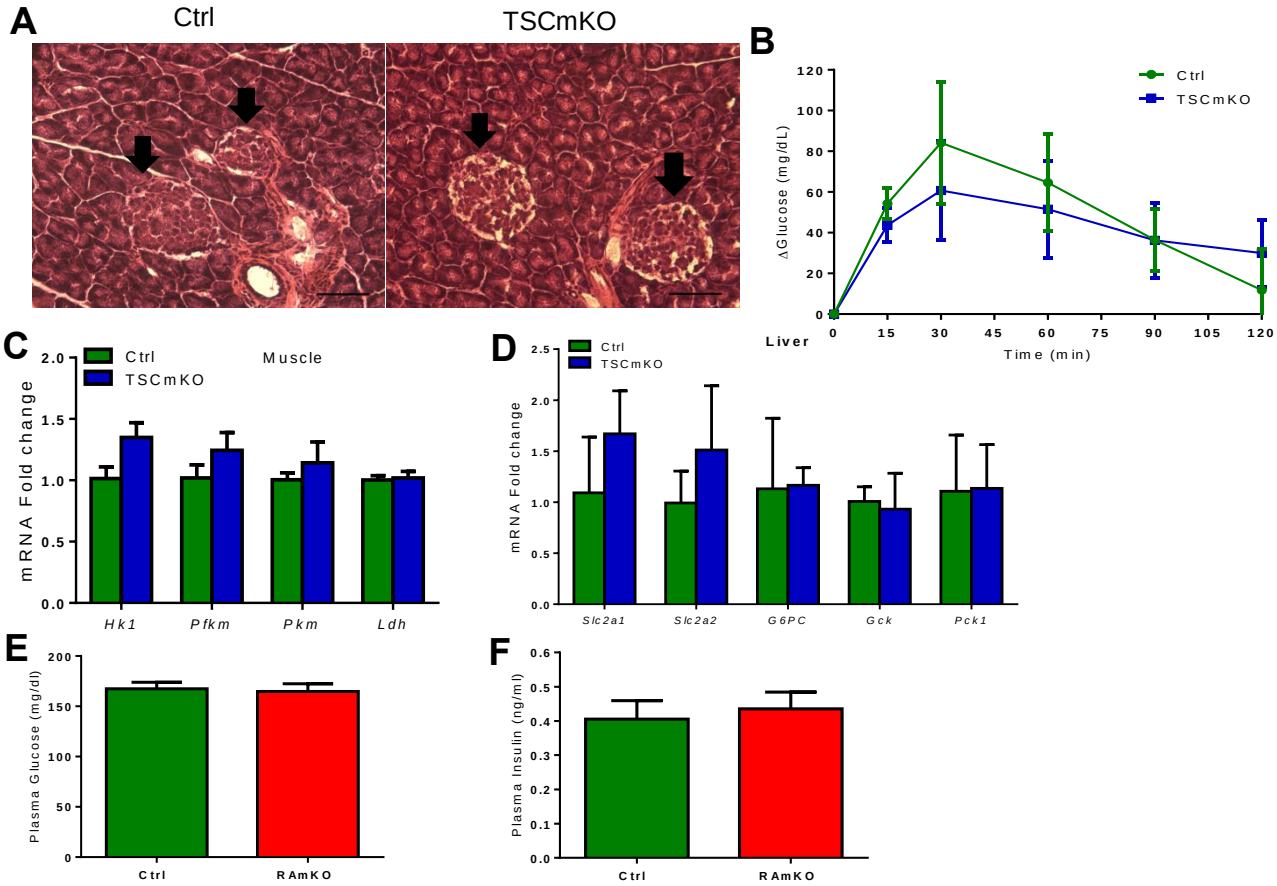


Figure S3: TSCmKO mice do not show changes in the expression of genes encoding enzymes involved in glycolysis

A. H&E staining of pancreas from 10-week-old TSCmKO and control mice (n=3 mice per genotype). Scale bar, 100 µm. Arrows point at Langerhans islets.

B. Pyruvate tolerance test in 10-week-old TSCmKO and control mice (n= 6 mice per genotype). Linear regression analysis shows that the differences between the groups are not significant ($p>0.05$).

C, D. Relative expression of genes involved in glucose metabolism in TA muscle (C) and in the liver (D) of 10-week-old TSCmKO and control mice (n=4 mice per genotype).

E, F. Plasma glucose (E) and insulin (F) concentrations in 10-week-old RAmKO and control mice (n= 6 mice per genotype). Data represent mean ± SEM, *$p<0.05$, **$p<0.01$.

Figure S4

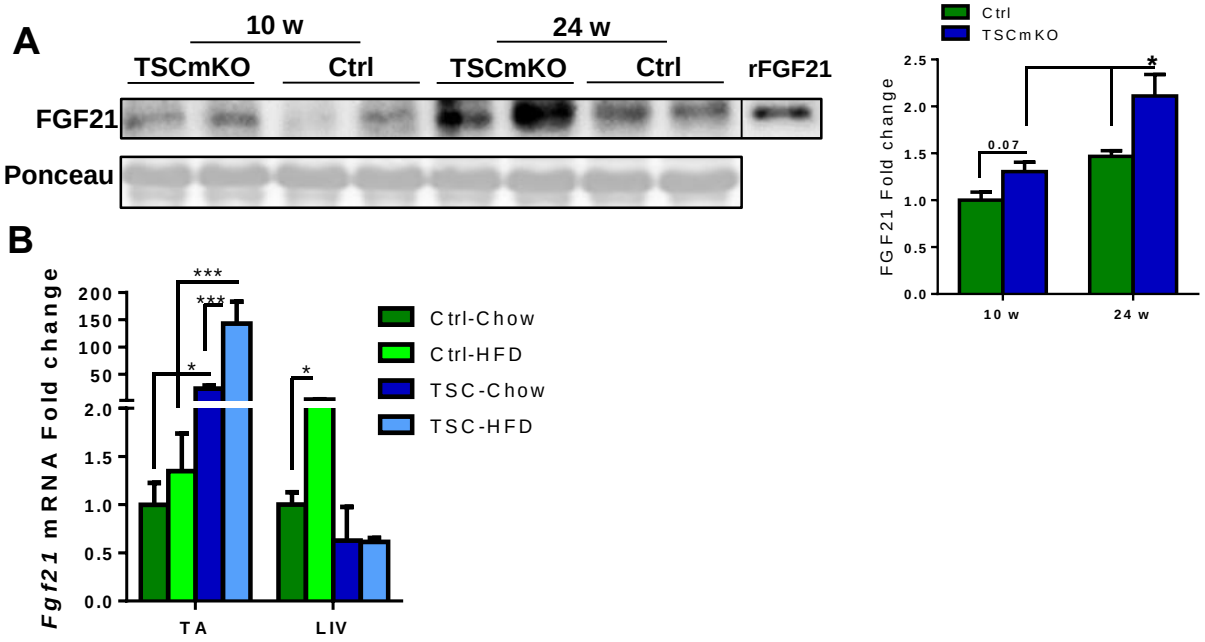


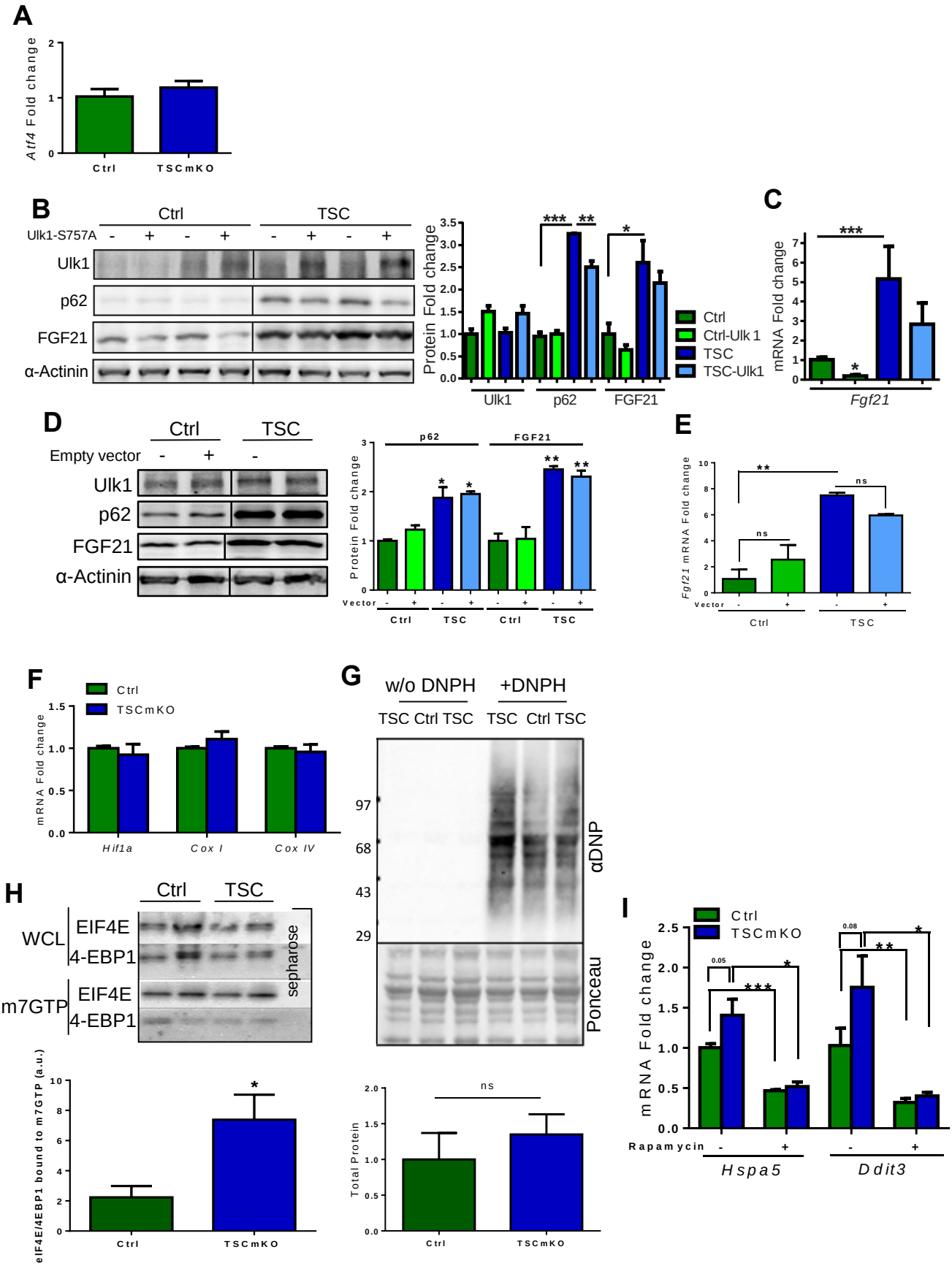
Figure S4: FGF21 abundance in muscle is increased in TSCmKO mice

A. Western blot analysis of plasma FGF21 after immunodepletion in 12- and 24-week-old fed TSCmKO and control mice (n= 4 mice per genotype and age). Ponceau staining was used as a loading control. 100 pg of recombinant FGF21 (rFGF21) were loaded as a control.

B. Relative expression of *Fgf21* in TA muscle and liver of Chow or HFD-fed 24-week-old control (Ctrl) and TSCmKO mice (n=3 mice per genotype and condition).

Data represent mean ± SEM, *p<0.05.

Figure S5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Cox I
Cox IV
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w/o DNPH
+DNPH
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TSC Ctrl TSC
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68
43
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αDNP
Ponceau
Total Protein
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Ctrl
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H
Ctrl
TSC
WCL
EIF4E
4-EBP1
m7GTP
EIF4E
4-EBP1
sepharose
eIF4E/4EBP1 bound to m7GTP (a.u.)
Ctrl
TSCmKO
I
Ctrl
TSCmKO
mRNA Fold change
Rapamycin
Hspa5
Ddit3

Figure S5: FGF21 in TSCmKO mice is not induced upon autophagy impairment or mitochondrial dysfunction.

A. Relative *Atf4* mRNA expression in TA muscle of 10-week-old TSCmKO and control mice (n= 4 mice per genotype).

B. Immunoblots of TA muscle electroporated with Ulk1-S757A (+) and non-electroporated contralateral muscle (-) from 20-week-old TSCmKO (TSC) and control (Ctrl) mice are shown for the indicated proteins (n= 3 mice per genotype and condition). Protein abundance is normalized to α-actinin.

C. *Fgf21* expression is significantly decreased in TA electroporated with Ulk1-S757A in control mice, but not in the electroporated TSCmKO muscle when compared to respective contralateral legs (n= 3 mice per genotype and condition).

D. Immunoblots of TA muscle electroporated with an empty vector (+) and of the non-electroporated contralateral muscle (-) from 20-week-old TSCmKO (TSC) and control (Ctrl) mice are shown for the indicated proteins (n= 3 mice per genotype and condition). Protein abundance is normalized to α-actinin.

E. *Fgf21* mRNA expression was quantified in TA muscle electroporated with an empty vector (+) and in the non-electroporated contralateral muscle (-) from 20-week-old TSCmKO (TSC) and control (Ctrl) mice (n=3 mice per genotype and condition).

F. Relative expression of mitochondrial genes in TA muscle from 10-week-old TSCmKO and control mice (n= 4 mice per genotype).

G. Protein oxidation status was quantified by Oxiblot assay in 10-week-old TSCmKO and control mice (n= 4 mice per genotype).

H. m7GTP binding assay reveals an increase in translation initiation in 10-week-old TSCmKO muscle, compared to control (n= 6 mice per genotype).

I. Relative expression of *Hspa5* and *Ddit3* in gastrocnemius muscle of 12-week-old TSCmKO and control mice with (+) and without (-) 3-day rapamycin treatment (n= 4 mice per genotype and treatment).

Data represent mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

Figure S6

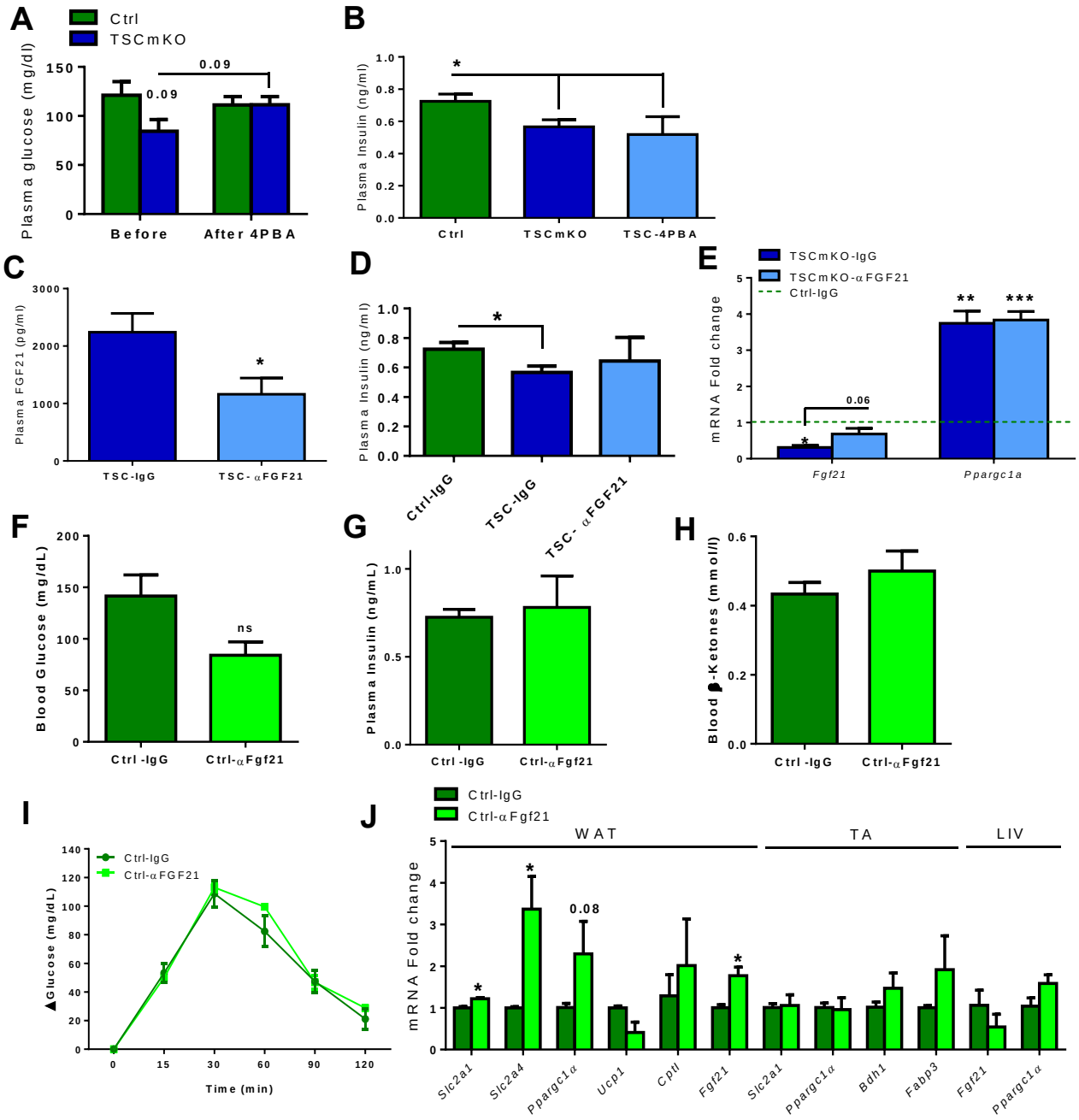

Figure S6: FGF21 blockade does not alter plasma insulin concentration in TSCmKO mice or metabolism in control mice

A. Plasma glucose concentrations from 10-week-old TSCmKO mice before and after 4-PBA treatment (n= 5 mice per genotype and treatment).

B. Plasma insulin concentrations in 14-week-old TSCmKO mice after 4-PBA treatment (n= 5) compared to untreated TSCmKO (n= 6) and control mice (n= 8).

C. Plasma FGF21 concentrations in 24-week-old TSCmKO mice treated with FGF21-neutralizing antibody and TSCmKO mice treated with non-immune IgG (n= 5 mice per genotype and treatment).

D. Plasma insulin concentrations from 12-week-old TSCmKO mice treated with FGF21-neutralizing antibody (n= 5) compared to TSCmKO (n= 6) and control mice (n= 8) treated with non-immune IgG.

E. Expression of *Fgf21* and *Ppargc1a* in liver from 10-week-old TSCmKO mice treated with FGF21-neutralizing antibody and TSCmKO and control mice treated with non-immune IgG (n= 4 mice per genotype and treatment). Control line (Ctrl-IgG) represents normalized *Fgf21* and *Ppargc1a* expression of the IgG treated littermates.

F-H. Plasma glucose (F), insulin (G) and ketone body (H) concentrations in anti-FGF21 or IgG antibody treated 10-week-old control (Ctrl) mice (n= 3 mice per genotype and treatment).

I. Pyruvate tolerance test in anti-FGF21 or IgG antibody treated 24-week-old control (Ctrl) mice (n= 3 mice per genotype and treatment). Linear regression analysis shows that the differences between the groups are not significant ($p>0.05$).

J. Expression of genes involved in glucose, fatty acid or ketone metabolism in WAT, TA and Liver of anti-FGF21 or IgG antibody treated 10-week-old control (Ctrl) mice (n= 3 mice per genotype and treatment).

Data represent mean ± SEM, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table S1. Weight and size analysis in 10- and 40-week-old mice

	10 weeks			40 weeks		
	Ctrl	TSCmKO		Ctrl	TSCmKO	
Weight (g)	25.3 ± 0.4	21.5 ± 0.7	***	27.8 ± 1.1	19.2 ± 0.7	***
Tibia (mm)	17.8 ± 0.1	17 ± 0.1	***	18.1 ± 0.1	17.3± 0.2	***

p values determined by Student's test are indicated by asterisks (n= 6-11 mice per genotype).

Values represent mean ± SEM. ***p<0.001

Table S2. COBAS plasma analysis of 10-week-old mice

	Ctrl	TSCmKO
Triglycerides (mmol/l)	1.48 ± 0.14	1.84 ± 0.24
HDL-Cholesterol (mmol/l)	2.25 ± 0.09	2.08 ± 0.06
LDL-Cholesterol (mmol/l)	0.17 ± 0.01	0.22 ± 0.04
Cholesterol (mmol/l)	2.47 ± 0.08	2.28 ± 0.10

Values represent mean ± SEM (n= 5 mice per genotype)

Table S3: Primers used for quantitative polymerase chain reaction.			
Gene	**Forward primer**	**Reverse primer**	**Name of the gene**
Acac1	ACC TTA CTG CCA TCC CAT GTG	GTG CCT GAT GAT CGC ACG AAC AAA	*Acetyl-CoA carboxylase 1*
Acat1	GTGAAGGAAGTCTACATGGGC	TGTGGTGCATGGAGTGGAAAT	*Acetyl CoA acyltransferase 1*
Atf4	AGCAAAACAAGACAGCAGCC	ACTCTCTTCTTCCCCCTTGC	*Activating transcription factor 4*
Bdh1	TTGAGCTGGATGGTTCTCAGTC	TTTGCTGGCTGTTTGATGAAGG	*D-β-hydroxybutyrate dehydrogenase*
Cidea	TGGGATTGCAGACTAAGAAGGTC	CGGTCATGGTTTGAAACTCGAAA	*Cell death-inducing DFFA-like effector a*
Cox I	GGT CAA CCA GGT GCA CTT TT	TGG GGC TCC GAT TAT TAG TG	*Cytochrome C-oxidase 1*
Cox IV	TACTTCGGTGTGCCTTCGA	TGACATGGGCCACATCAG	*Cytochrome C-oxidase 4*
Cptlb	GGT CGA TTG CAT CCA GAG AT	GAC TCC GGT GGA GAA GAT GA	*Carnitine palmitoyltransferase 1*
Ddit3	CCACCACACCTGAAAGCAGAA	AGGTGAAAGGCAGGGACTCA	*DNA-Damage-Inducible Transcript 3-CHOP*
Dgat I	CATGCGTGATTATTGCATCC	ACAGGTTGACATCCCGGTAG	*Diglyceride acyltransferase I*
Dgat II	GCGCTACTTCCGAGACTACTT	GGGCCTTATGCCAGGAAACT	*Diglyceride acyltransferase II*
Fabp3	CCC CTCAGCTCAGCACCA	CAG AAA AAT CCC AAC CCA AGA AT	*Fatty acid binding protein 3*
Fasn	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT	*Fatty acid synthase*
Fgf21	TACACAGATGACGACCAAGA	GGCTTCAGACTGGTACACAT	*Fibroblast growth factor 21*
Gck	CCCTGAGTGGCTTACAGTTC	ACGGATGTGAGTGTTGAAGC	*Glucokinase*
G6pc	AGC GGA ATG GGA GCA ACT TG	CAG AAT GGG TCC ACC TTG ACA C	*Glucose-6-phosphatase*
Hif1α	CAGTACAGGATGCTTGCCAAAA	ATACCACTTACAACATAATTCACACACACA	*Hypoxia inducible factor 1α*
Hk1	CCCTGCCACCAGACGAAA	GACTTGAACCCCTTAGTCCATGA	*hexokinase*
Hmgcl	GATGCCGGGAAACTTCTGAATG	CCAGCTTTGTTTCTCCCAAGTG	*3-hydroxymethyl-3-methylglutaryl-CoA lyase*
Hmgcs2	CCACAAGGTGAACTTCTCTCCA	TGCATCTCATCCACTCGTTCA	*3-hydroxy 3-methylglutaryl CoA synthase 2*
Hspa5	TTCAGCCAATTATCAGCAAACTCT	TTTTCTGATGTATCCTCTTCACCAGT	*Heat shock 70kDa protein 5 -BiP*
β-Klotho	AGGGTCTCCGGGGAATGAAT	GATCTTTGCAGTGCCCTGTTG	
Ldh	TGTCTCCAGCAAAGACTACTGT	GACTGTACTTGACAATGTTGGGA	*Lactate dehydrogenase*
Mcad	TCTCGAAGACGTCAGAGTGC	TGCGACTGTAGGTCTGGTTC	*Medium-chain acyl-coenzyme A dehydrogenase*
Mttp	CGTCCACATACAGCCTTGAC	CCACCTGACTACCATGAAGC	*Microsomal triglyceride transport protein*
Oxct1	CCCATACCCACTGAAAGACGAA	CTGGAGAAGAAAGAGGCTCCTG	*3-oxoacid-CoA transferase 1*
Pck1	CAT CCA GGC AAT GTC ATC GC	GCA TAA CTA ACC CG AAG GCA AG	*Phosphoenolpyruvate carboxikinase*
Pdk4	AAA ATT TCC AGG CCA ACC AA	CGA AGA GCA TGT GGT GAA GGT	*Pyruvate dehydrogenase kinase, isozyme 4*
Pfkm	CAGATCAGTGCCAACATAACCAA	CGG GAT GCA GAG CTC ATC A	*Phosphofructokinase*
Pkm	CGATCTGTGGAGATGCTGAA	AATGGGATCAGATGCAAAGC	*Pyruvate kinase*
Ppargc1α	TGATGTGAATGACTTGGATACAGACA	GCTCATTGTTGTACTGGTTGGATATG	*Peroxisome proliferator activated receptor gamma coactivator 1-alpha*
Ppargc1β	CCATGCTGTTGATGTTCCAC	GACGACTGACAGCACTTGGA	*Peroxisome proliferator activated receptor gamma coactivator 1-beta*
Prdm16	AGCTGAGGAAGCATTTGAAGTTA	ATATGCCTGGTTCTTAGCCTGC	*PR domain containing 16*
Scd1	CAA GCTGGAGTACGTCTGGA	CAG AGC GCT GGT CAT GTA GT	*Stearoyl-CoA desaturase 1*
Slc2a1	CGAGGGACAGCCGATGTG	GCCGACCCTCTTCTTTCAT	*Glucose transporter1*
Slc2a4	GATGAGAAACGGAAGTTGGAGAGA	GCACCACTGCGATGATCAGA	*Glucose transporter4*
Trib3	GGACAAGATGCGAGCCACAT	CCACAGCAGGTGACAAGTCT	*Tribbles homolog 3*
Ucp1	GGCCTCTACGACTCAGTCCA	TAAGCCGGCTGAGATCTTGT	*Uncoupling protein 1*
Ucp2	TCCCCTGTTGATGTGGTCAA	CAGTGACCTGCGCTGTGGTA	*Uncoupling protein 2*
Xbp1	TGGCCGGGTCTGCTGAGTCCG	GTCCATGGGAAGATGTTCTGG	*X-box binding protein 1*