

Supplementary Information

The tumor suppressor TMEM127 regulates insulin sensitivity in a tissue-specific manner

Srikantan et al.

Supplementary Fig 1. **Global deletion of Tmem127 impairs growth and results in low adiposity** A) TMEM127 expression in different human tissues by RNA seq from the GTEX project (ENSG00000135956.4, data represent Log10 transformed TPM(transcript per million)+1 values; plots show median and 25th and 75th percentiles, points are outliers (above 1.5x the interquartile range), obtained from the GTEX portal, V7 release (including 11,688 samples from 714 donors) on 06/12/2019); B) Relative Tmem127 expression in tissues from WT and Tmem127 KO mice (n=7 and 12 per genotype, respectively), measured by RT-PCR and normalized to the Tfiib gene expression; C) Body weight of female wild-type (WT) and Tmem127 knockout (KO) mice (n=8 and 10 per genotype, respectively) from 6-22 months of age; D) fat and lean mass of male WT and KO mice at the indicated ages (n=5-14 per genotype, respectively); E) fat mass/lean mass ratio of female WT and KO mice across ages (n=8 or 10 per genotype, respectively); F) Plasma levels of norepinephrine and epinephrine of adult male WT (n=6) and KO (n=7) mice measured by gas chromatography/mass spectrometry (GC/MS); G) Western blot of adrenal lysates of WT and KO adult male mice (n=4 per genotype), probed for mTORC1 targets S6K (phosphorylated S6K, pS6K and total S6K) and p-4EBP1; TMEM127 and loading control antibodies; H) Plasma levels of Fgf21 of adult male WT and KO mice under regular fed conditions, after 24 h fasting and after 4h refeeding (n=6 per genotype); I) Relative inguinal (iWAT), epididymal (eWAT) and brown adipose tissue (BAT) mRNA expression of the Ucp1 gene expression in adult WT and KO mice (n=10 or 12 per genotype, respectively) measured by real-time PCR (RT-PCR) and normalized to Tfiib gene; J) Representative HE-stained sections of inguinal WAT of adult male WT and KO mice (scale bar is 100 μ M); K) Diameter of the designated adipocytes from adult male WT and KO mice (n=6 per genotype). Data were analyzed by Student's t-test. Values are expressed as mean +/- s.e.m. *P<0.05; **P<0.01; ***P<0.001. Adult mice were 9-12months of age. Source data are provided as a Source Data file.

Supplementary Table 1. Pre-birth and neonatal *Tmem127* knockout mouse fitness based on genotype distribution

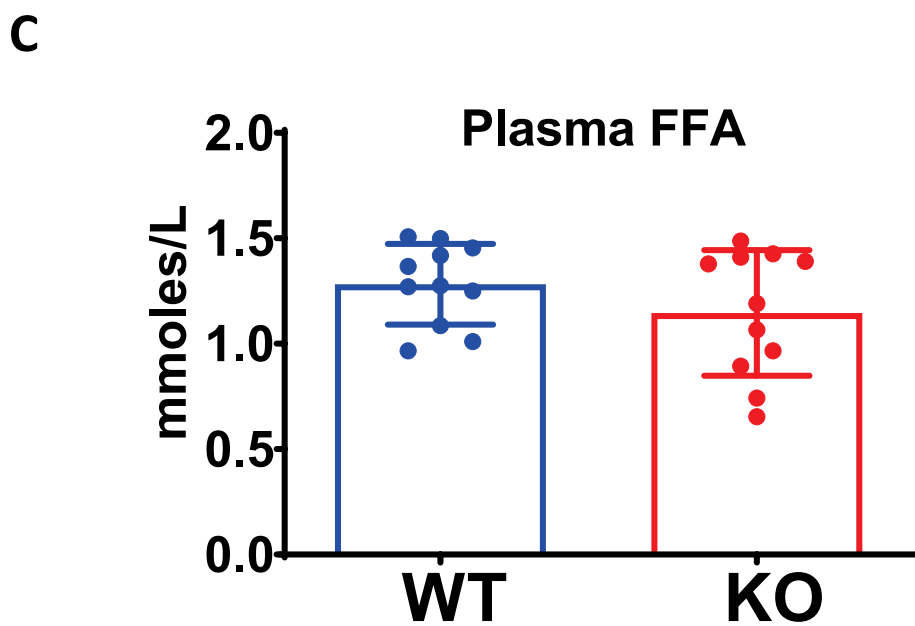
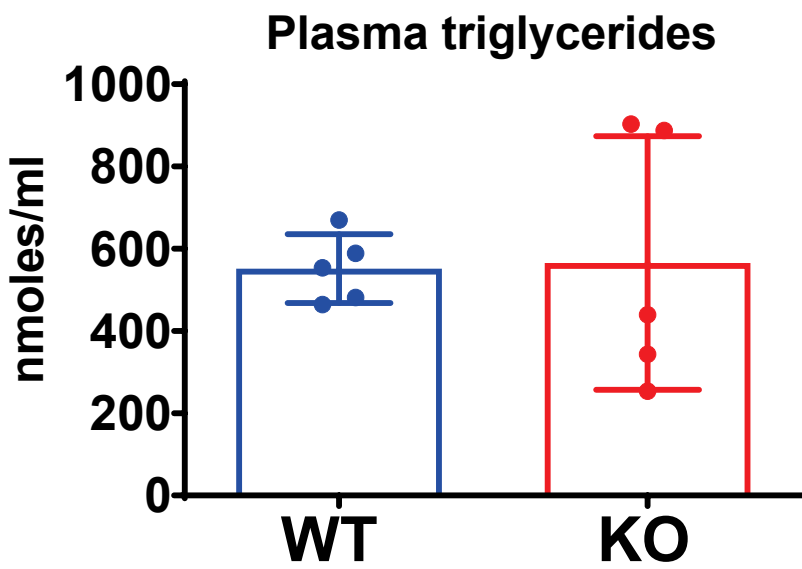
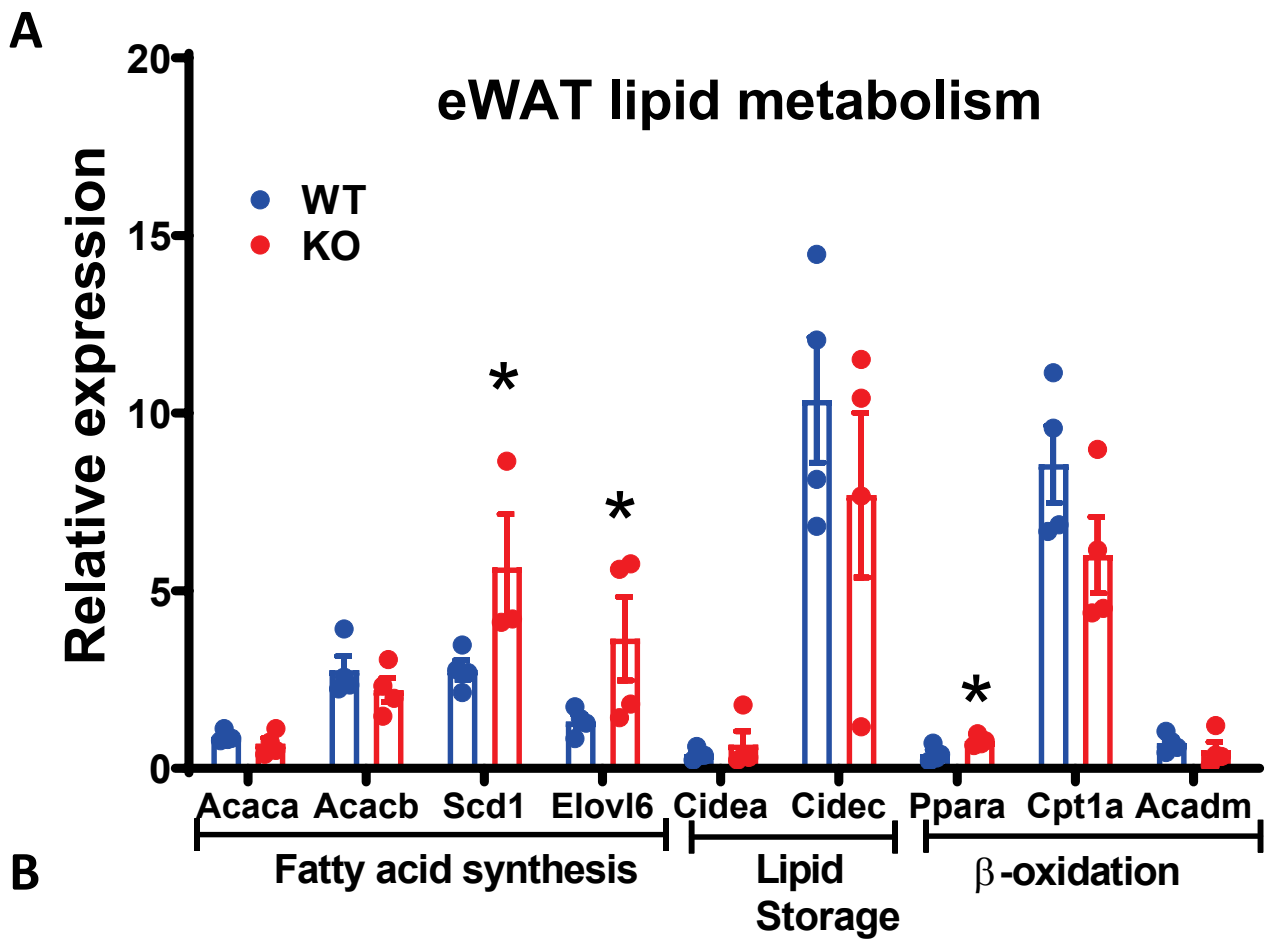
Age	Total number of litters	Total number of embryos	KOs (n)	Hets (n)	WT (n)	P value
E13.5	44	299	78	137	84	0.66
E18/E19	9	70	21	30	19	0.63
P0	23	154	27	95	32	0.03

E=embryonic day; P=postnatal day; KO=knockout; WT=wild-type; Hets= heterozygotes; p-value: Z-statistic, significance level

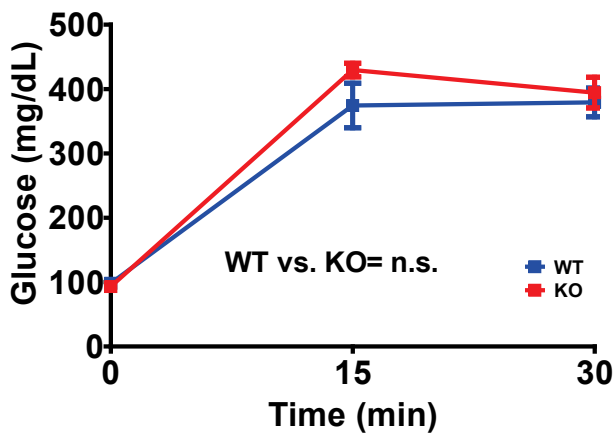
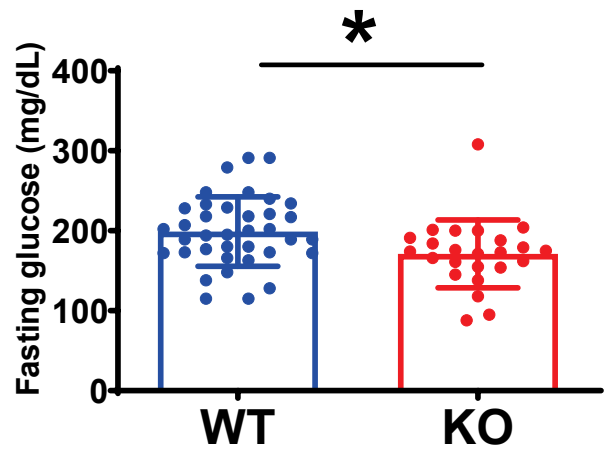
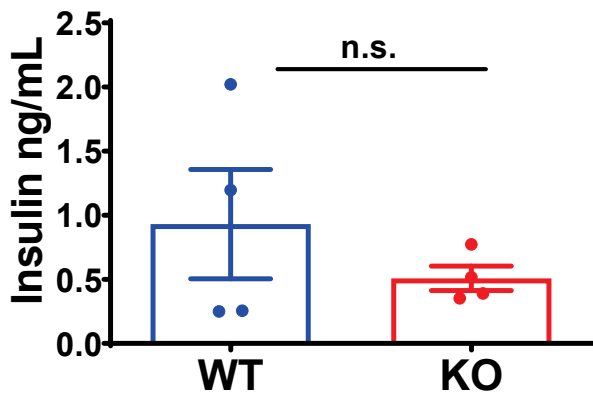
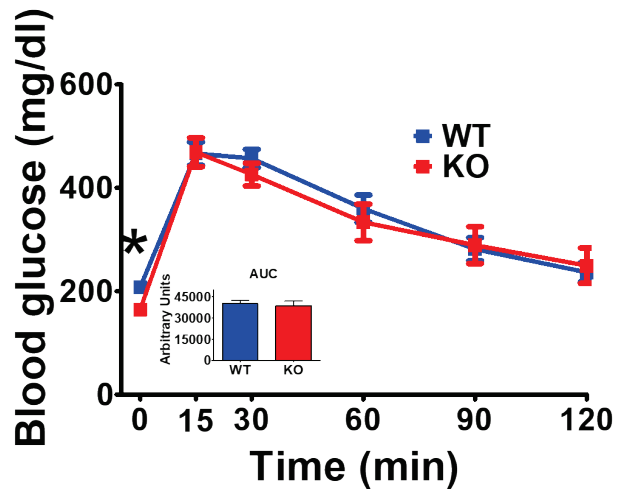
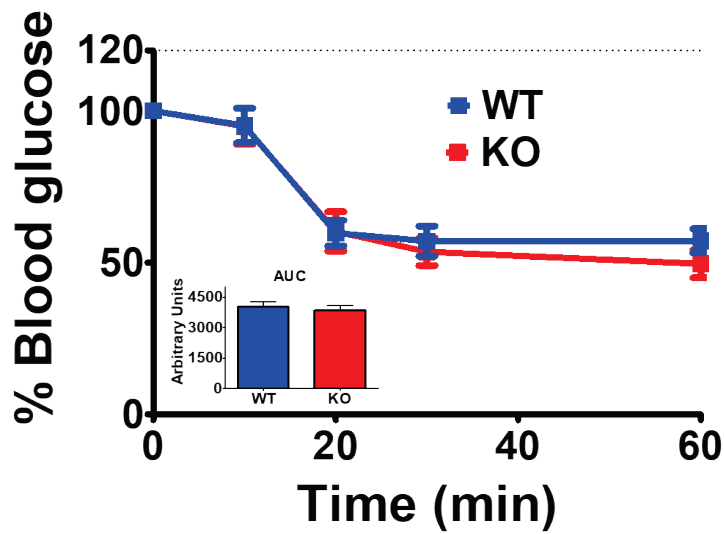
Supplementary Table 2. Perinatal (pre- and post-birth) body weight of *Tmem127* knockout mice

Age	KO			WT			p value
	mean (g)	SEM	n	mean (g)	SEM	n	
E18.5	1.00	0.06	8	1.02	0.07	8	0.55
P7	3.28	0.21	10	3.79	0.12	18	0.03
P14	5.22	0.26	10	6.24	0.17	18	0.00
P21	6.60	0.47	10	8.85	0.20	18	< 0.0001
P30	11.48	1.00	9	15.77	0.34	16	< 0.0001
P45	17.00	0.75	7	21.48	0.35	15	< 0.0001
P60	20.19	0.99	5	24.57	0.37	14	< 0.0001

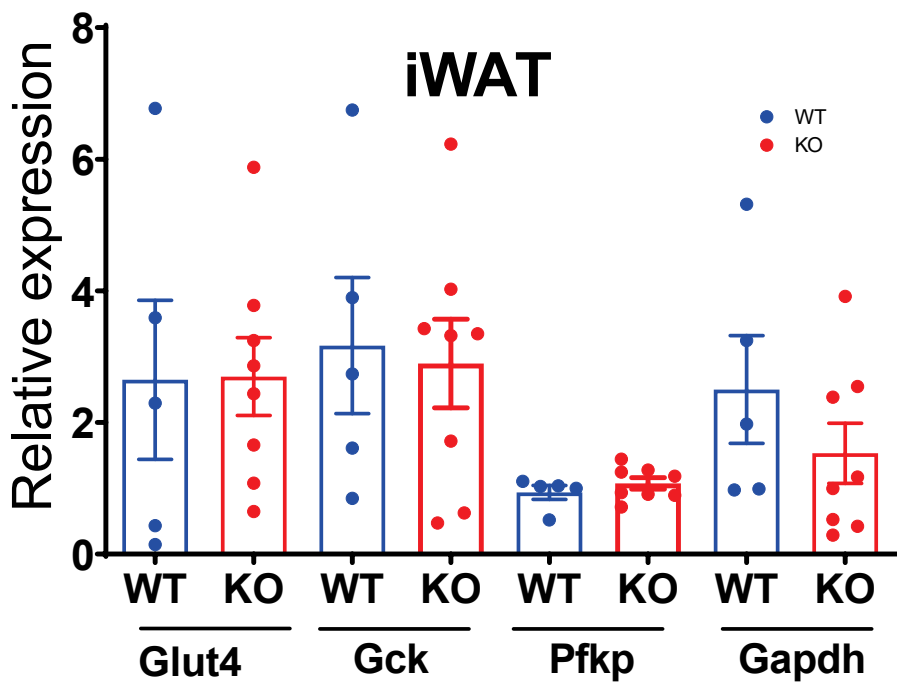
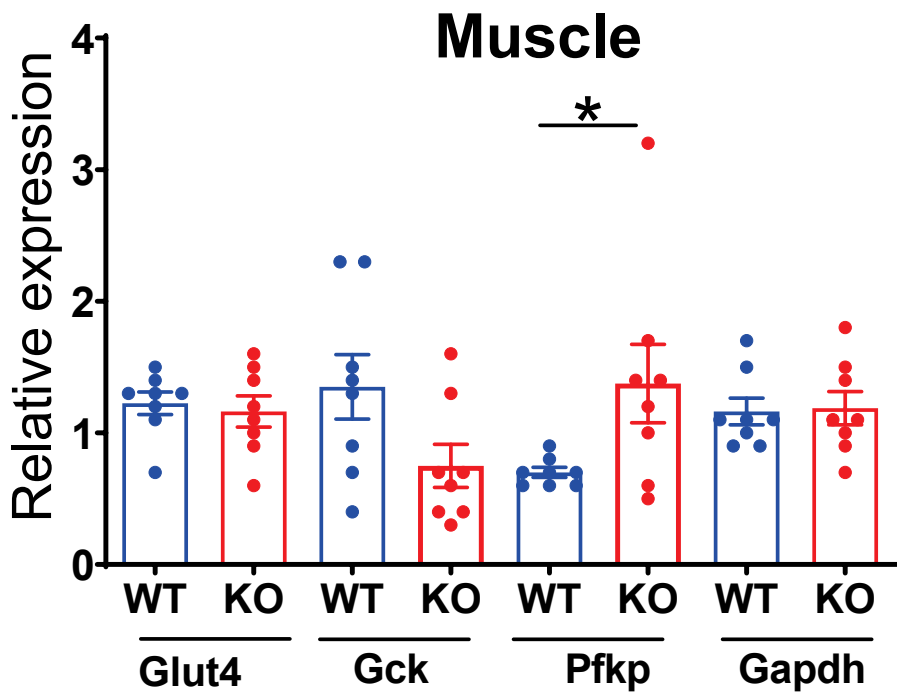
E=embryonic day; P=postnatal day; E=embryonic day; P=postnatal day; KO=knockout; WT=wild-type; Hets=heterozygotes; SEM=standard error of the means; p-value: Student's t-test



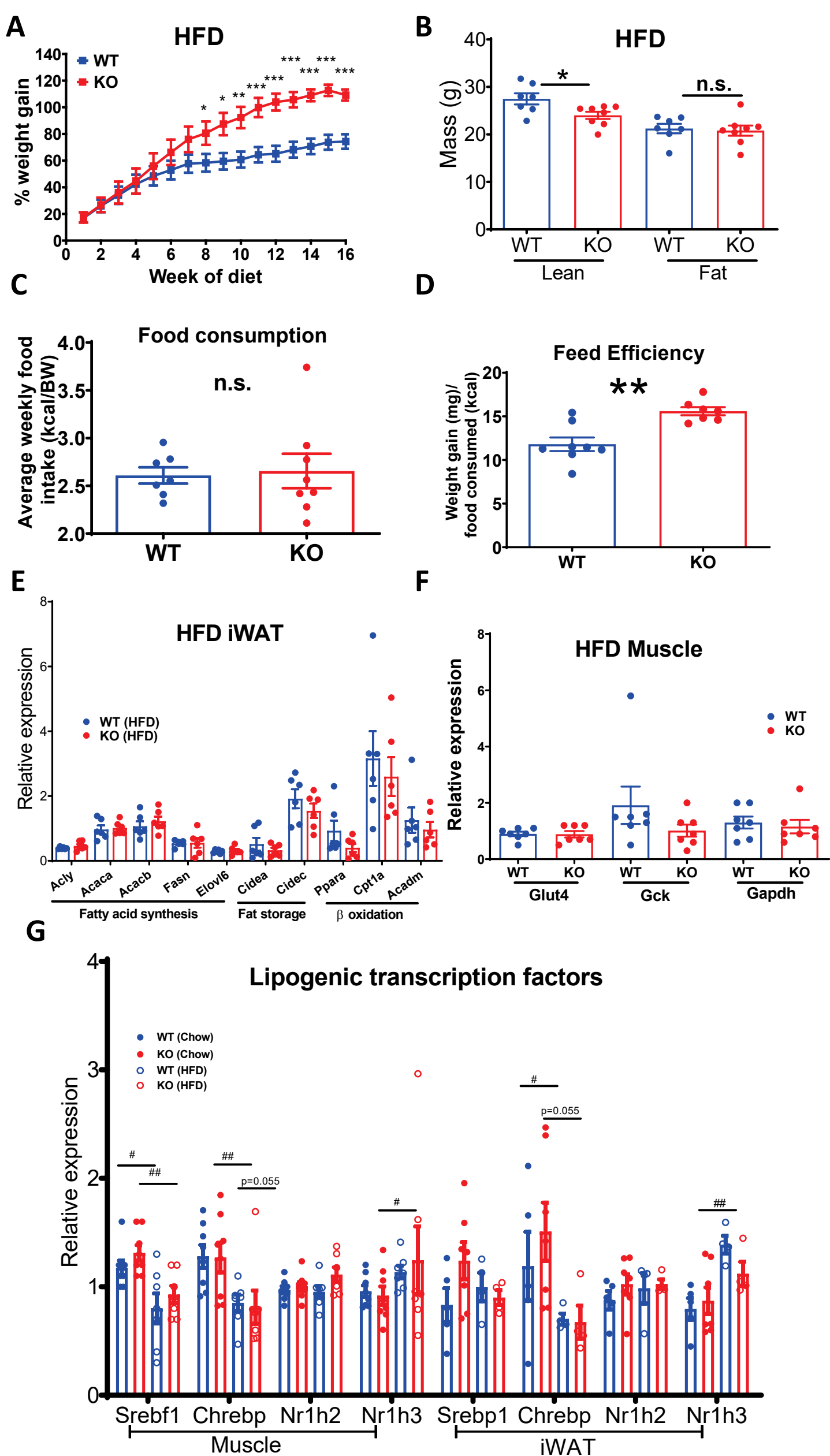
Supplementary Fig 2. **Global Tmem127 deletion impairs hepatic lipogenesis.** A) Relative eWAT mRNA expression of the indicated fatty acid synthesis, storage, oxidation and transport gene expression in adult WT and KO mice (n=10 or 12 per genotype, respectively) measured by real-time PCR (RT-PCR) and normalized to Tff1b gene; B) Plasma triglyceride content in fed adult WT and KO mice (n=5 per genotype); C) Plasma free fatty acids in fed adult WT and KO mice (n=13 per genotype). Data were analyzed by Student's t-test. Values are expressed as mean +/- s.e.m. *P<0.05; **P<0.01; ***P<0.001. Adult mice were 9-12months of age. Source data are provided as a Source Data file.

A**B****C****D****E**

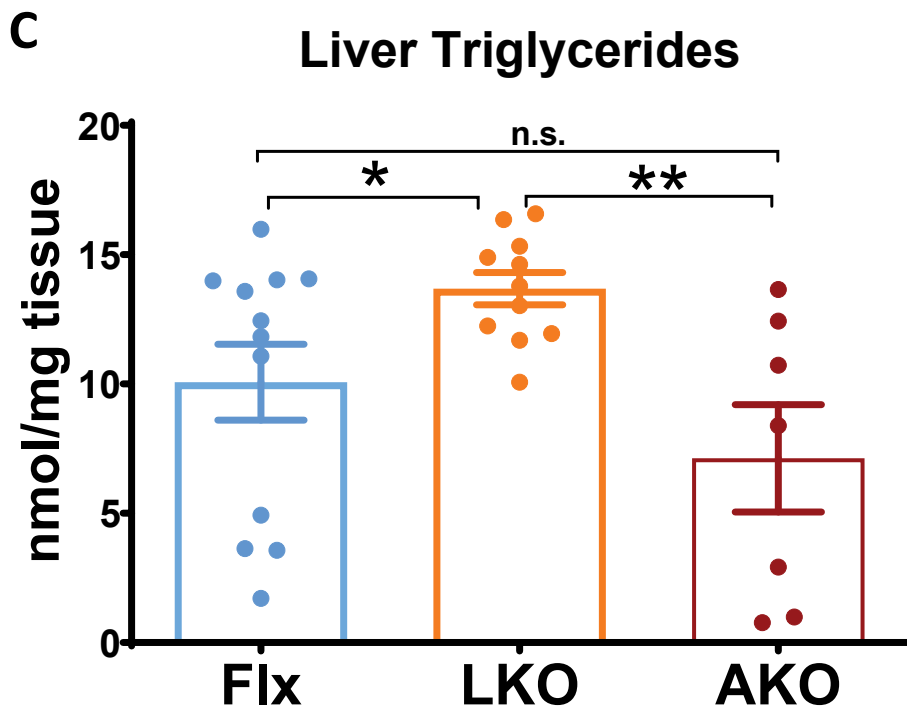
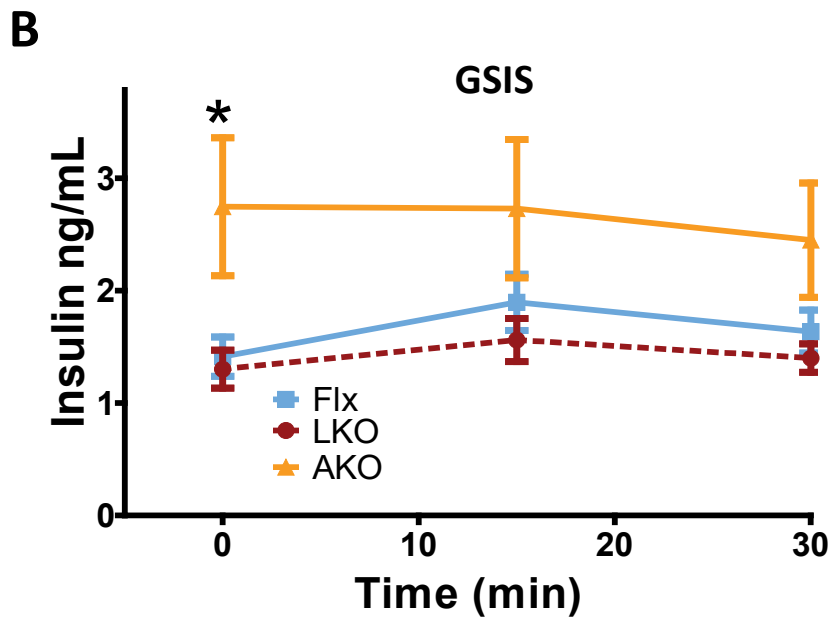
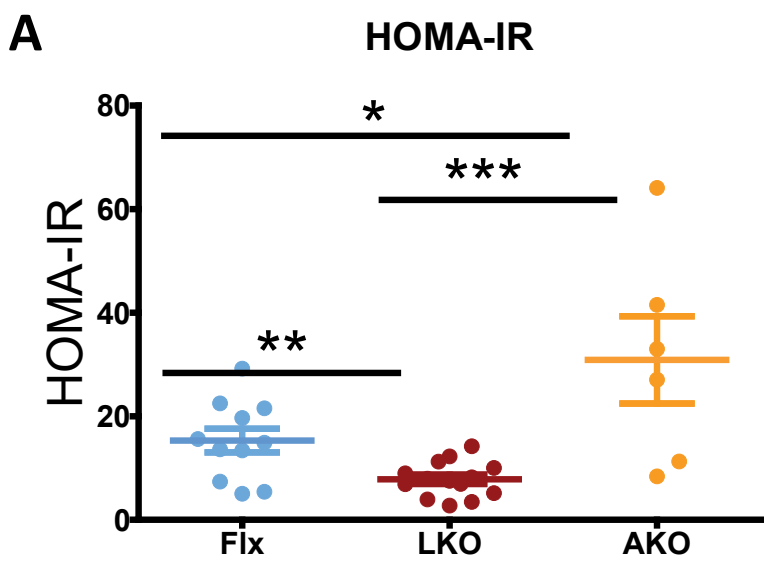
Supplementary Fig 3. **Tmem127 deletion leads to hypoinsulinemia and improved insulin tolerance.** A) Blood glucose levels from the GSIS test from Fig 2D-performed in adult male WT and KO mice (n=4 per genotype; B)Fasting (6 hours) blood glucose of 3-6 month-old (young) WT and Tmem127 KO male mice (n=37 and 24 per genotype, respectively); C) Fasting (6 hours) serum insulin of 3-6 month-old (young) WT and Tmem127 KO male mice (n=4 per genotype); D) Glucose tolerance test (GTT) and area under the curve (AUC, inset graph) of 3-6 month-old (young) WT and Tmem127 KO male mice (n=7 and 8 per genotype, respectively); E) Insulin tolerance test (ITT) and AUC (inset) of 3-6 month-old (young) WT and Tmem127 KO male mice (n=7 and 8 per genotype, respectively). Data were analyzed by Student's t-test or two-way ANOVA (in the case of GSIS). Values are expressed as mean +/- s.e.m. *P<0.05; **P<0.01; ***P<0.001. Adult mice were 9-12months of age. Source data are provided as a Source Data file.

A**B**

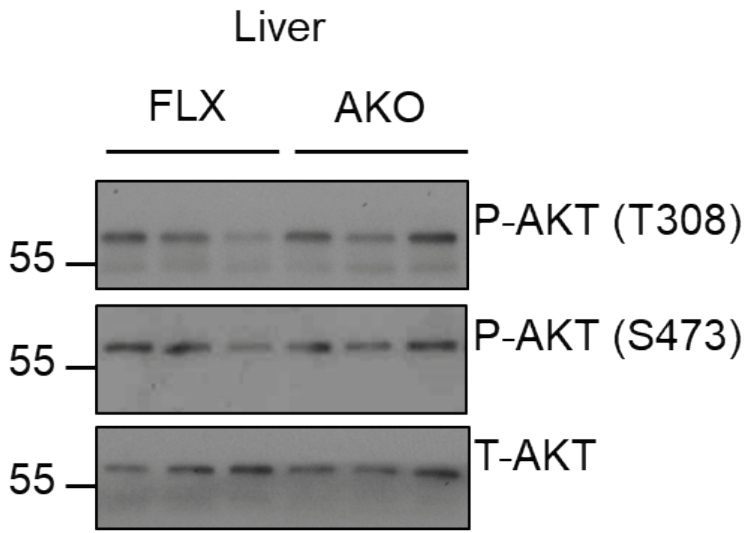
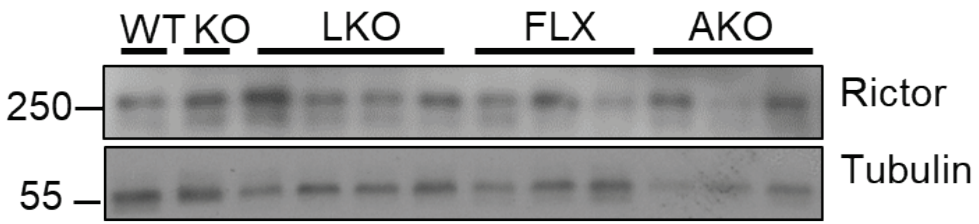
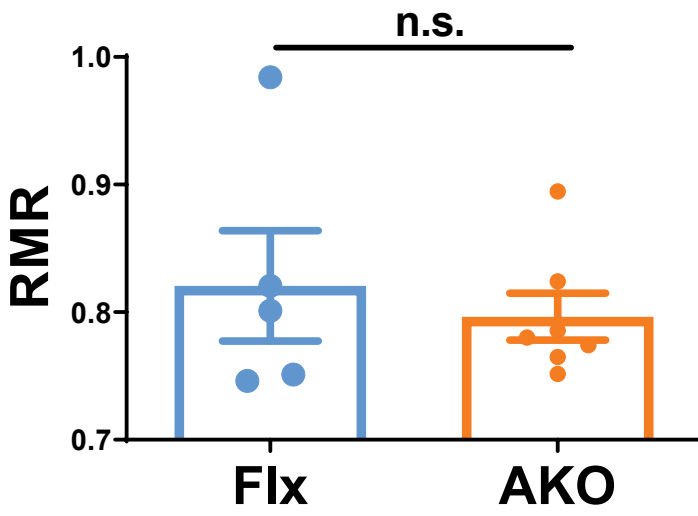
Supplementary Fig 4. **Tmem127 deletion decreases hepatic gluconeogenesis and increases insulin signaling.** A) Relative iWAT mRNA expression of the indicated glucose transporter, glycolysis and gluconeogenesis genes from adult WT and KO mice (n=5 or 8 per genotype, respectively); B) Relative muscle mRNA expression of the indicated glucose transporter, glycolysis and gluconeogenesis genes from adult WT and KO mice (n=7 or 8 per genotype, respectively). Data were analyzed by Student's t-test or two-way ANOVA (in the case of GSIS). Values are expressed as mean +/- s.e.m. *P<0.05; **P<0.01; ***P<0.001. Adult mice were 9-12months of age. Source data are provided as a Source Data file.



Supplementary Fig 5. **Tmem127 deletion protects against high-fat diet (HFD)-induced hepatic steatosis and insulin resistance.** A) Weight gain relative to start body weight of WT and Tmem127 KO male mice on a 60% kcal fat diet for 16 weeks (n=8 per genotype, starting age 3-5 months); B) Relative lean and fat mass of WT and KO mice after 16 weeks of HFD (n=7 or 8 per genotype, respectively); C) Average weekly calorie intake per body weight of singly housed WT and KO mice on a HFD (n=8 or 7 per genotype, respectively); D) Feed efficiency calculated as the weight gain per kcal food intake, averaged per week) of singly housed WT and KO mice on a HFD (n=8 per genotype); E) Relative iWAT mRNA expression of the indicated fatty acid synthesis, storage and oxidation gene expression from HFD-fed male WT and KO mice (n=8 per genotype, 9-11 mo-old) measured by RT-PCR; F) Relative muscle mRNA expression of the indicated glucose transport and metabolism gene expression from HFD-fed male WT and KO mice (n=7 per genotype, 9-11 mo-old) measured by RT-PCR; G) Relative muscle and iWAT mRNA expression of the indicated lipogenic transcription factor genes from chow or HFD-fed male WT and KO mice (n=8 per genotype, 9-11 mo-old); data were analyzed by Student's t-test. Srebf1, Chrebp values are significantly different per diet, but not per genotype in both tissues. Other values are non-significant. Data were analyzed by Student's t-test and/or ANOVA. Values are expressed as mean +/- s.e.m. *P<0.05; **P<0.01; ***P<0.001; # comparisons between diet; * comparisons between genotype # or *P<0.05; ## or **P<0.01; ### or ***P<0.001. Source data are provided as a Source Data file.

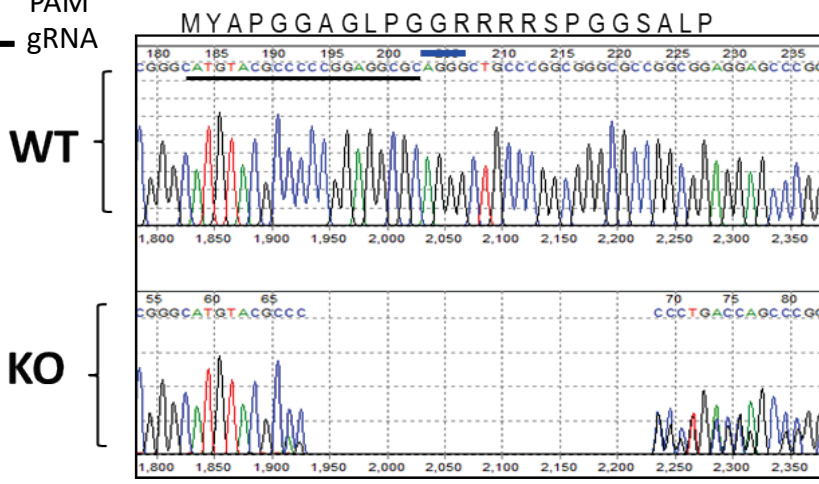


Supplementary Fig 6. **Liver-specific *Tmem127* deficient mice show improved glucose metabolism whereas adipose-specific *Tmem127* deficient mice are insulin resistant.** A) Homeostatic model assessment -insulin resistance index (HOMA-IR) of adult male Flx, LKO and AKO mice (n= 11 Flx, 14 LKO, 6 AKO), data were analyzed by Student's t-test. Values are expressed as mean +/- s.e.m. *P<0.05; **P<0.01; ***P<0.001. B) Glucose-stimulated insulin secretion (GSIS) of adult Flx, LKO and AKO mice (n=9 Flx, n=6 LKO, n=5 AKO); e); C) Liver triglyceride content of fed Flx, LKO and AKO (n=12, 11 and 7, respectively). Data were analyzed by ANOVA; *p<0.05. Source data are provided as a Source Data file.

A**B****C**

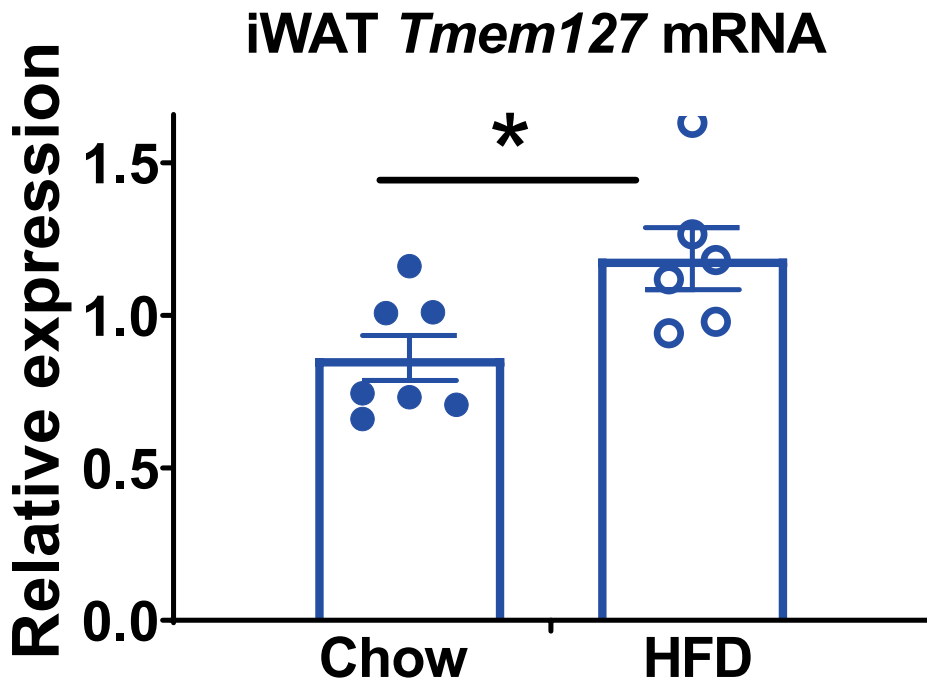
Supplementary Fig 7. **Adipose-specific *Tmem127* deletion and hepatic insulin signaling.** A) Western blot of liver lysates of Flx or AKO chow-fed, adult male (n=3 per genotype), probed with phosphorylated Akt at Ser473 or Thr308 or total Akt; B) Western blot of liver lysates of Flx (n=3); LKO (n=4); AKO (n=3), and one WT and one KO adult male, chow fed samples, probed with Rictor or Tubulin; C) Resting metabolic rate (RMR) calculated from the five lowest values of the adjusted oxygen consumption (VO₂) in mL/hr/g lean body mass in 24h of Flx (n=5) and AKO (n=7) chow-fed, adult male mice. Data were analyzed by two-way ANOVA (n.s., non-significant). Source data are provided as a Source Data file.

— PAM
— gRNA

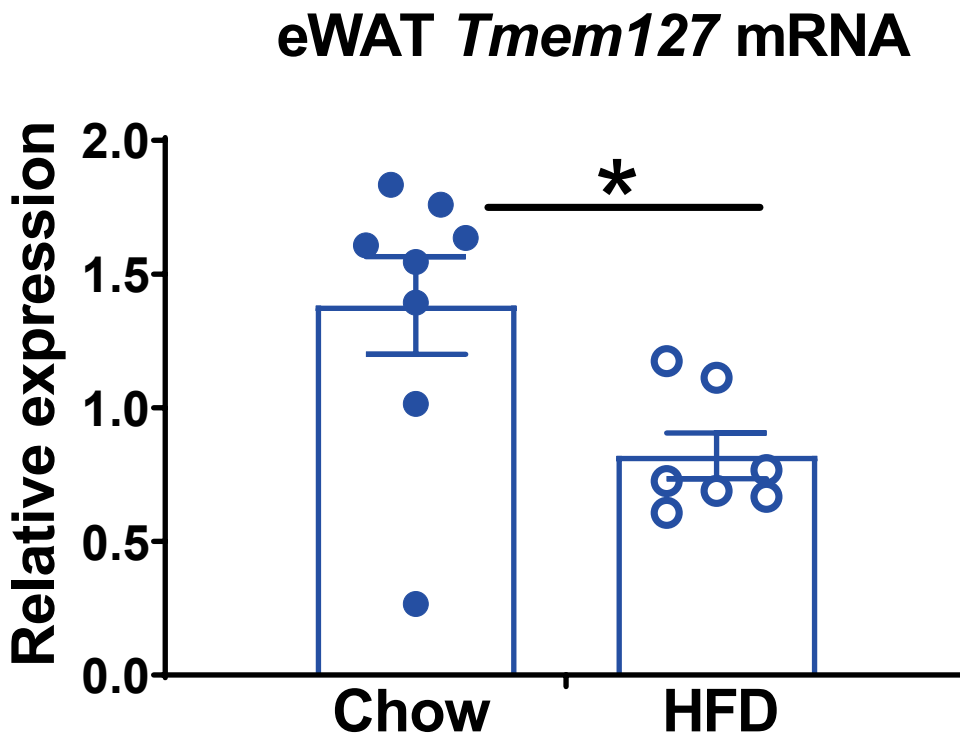


Supplementary Fig 8. **Characterization of CRISPR-Cas9 mediate-*TMEM127*KO HepG2 cells.** Sanger sequencing of a HepG2 clone carrying a heterozygous deletion of exon 2 of the *TMEM127* gene (KO). Guide RNA sequence is indicated with a black bar and PAM sequence is indicated with a blue bar.

A



B



Supplementary Fig 9. *Tmem127* mRNA expression of in fat tissues of wild-type adult male mice under chow or high-fat diet (HFD), quantified by real time PCR and displayed as relative expression normalized to the *Tfiib* gene: A) results from inguinal white adipose tissue (iWAT; n= chow n=7; HFD n=6; B) results from epididymal white adipose tissue (eWAT; chow n=8; HFD n=7). Data were analyzed by Student's t test; *p<0.05. Source data are provided as a Source Data file.

Supplementary Table 3- Liver TMEM127 mRNA levels and metabolic parameters in patients with fatty liver disease

Variable	Overall P	NAFLD+NASH P	NASH P
Age	0.052	0.058	0.201
BMI	0.019	0.038	0.041
AST	0.798	0.703	0.679
ALT	0.877	0.796	0.379
Insulin	0.005	0.018	0.036
Hba1C	0.019	0.004	0.544
Cholesterol	0.138	0.294	0.904
Triglyceride	0.015	0.075	0.307
INR	0.716	0.323	0.656
Glucose	0.096	0.277	0.934
Weight	0.838	0.693	0.894
HOMA-IR	0.011	0.050	0.203
NAFLD SCORE*	0.349	0.297	0.449

P values (Spearman's Correlation Coefficient) of TMEM127 expression: 'Overall P': all groups combined controls vs. nonalcoholic fatty liver disease (NAFLD) vs. nonalcoholic steatohepatitis (NASH); 'NAFLD + NASH P', combined NAFLD and NASH vs. control; 'NASH P', NASH only vs control; HOMA-IR= homeostatic model assessment of insulin resistance, BMI= body mass index; AST= aspartate amino transferase, ALT= alanine amino transferase; *As reported in Ref (29) Harrison et al. Gut 57, 1441-7 (2008)

Supplementary Table 4. Clinical, metabolic and molecular features of the cohort of patients with fatty acid liver disease

Disease Status	Fibrosis Stage	TMEM127 read counts*	T2DM	Insulin (μ U/L)	HOMA-IR	BMI
Control	0	496.23	Yes	7.90	2.03	36.27
Control	0	147.16	No	9.40	2.20	35.42
Control	0	416.00	Yes	10.80	0.00	31.00
Control	0	491.94	No	15.80	4.41	31.01
Control	0	283.59	No			28.23
Control	0	343.43	No	1.20	0.29	26.38
Control	0	385.07	No	5.40	0.00	
NAFLD	0	490.08	No	69.70	16.69	26.07
NAFLD	0	426.05	No	10.70	3.09	36.77
NAFLD	0	489.00	No	14.70	3.70	24.69
NAFLD	0	530.73	No	19.10	4.53	32.61
NAFLD	0	495.23	No	7.00	1.69	29.99
NAFLD	0	426.00	No	17.40	3.87	33.12
NAFLD	0	359.48	No	11.40	2.42	32.38
NAFLD	0	400.56	No	44.40	10.63	25.60
NAFLD	0	467.38	No	7.60	1.71	26.31
NAFLD	0	444.05	No	18.20	4.45	27.80
NAFLD	0	160.92	No	13.10	3.49	29.66
NAFLD	0	471.48	No	9.50	2.56	29.02
NAFLD	0	280.86	No	14.00	3.28	30.27
NAFLD	0	438.38	No	7.40	1.74	28.41
NAFLD	0	456.75	No	6.30	1.28	30.38
NAFLD	0	438.77	No	23.70	5.79	31.75
NAFLD	0	407.13	No	16.60	3.89	28.17
NAFLD	0	394.36	Yes	21.70	7.34	30.02
NAFLD	0	490.61	No	9.60	2.20	
NAFLD	0	503.62	No	16.40	4.01	
NAFLD	0	438.35	No	9.50	2.58	
NASH	1	495.40	No	15.00	3.93	27.61
NASH	1	451.00	No	26.70	6.13	32.93
NASH	1	524.18	No	116.40	38.23	37.49
NASH	1	556.00	No	52.60	12.08	36.71
NASH	1	469.22	No	27.30	6.40	31.16
NASH	1	550.35	No	41.30	11.63	36.67
NASH	1	500.57	Yes	35.20	13.99	37.27
NASH	1	349.03	No	32.50	9.15	35.44
NASH	1	475.02	Yes	235.20	66.79	36.65
NASH	1	412.65	No	25.70	6.79	27.55
NASH	1	456.46	No	8.70	2.08	34.49
NASH	1	435.18	No	65.70	14.60	32.61
NASH	1	471.56	Yes	4.60	0.99	26.88
NASH	1	410.39	No	8.40	5.08	
NASH	1	500.28	Yes	26.10	15.79	
NASH	1	484.91	No			
NASH	2	510.76	No	17.60	3.69	32.61
NASH	2	494.87	No	13.10	3.11	25.62
NASH	2	500.71	Yes	41.00	10.43	27.53
NASH	2	498.30	Yes	28.90	7.99	
NASH	2	488.55	Yes	24.70	9.51	
NASH	3	437.54	Yes	22.60	13.23	31.35
NASH	3	540.56	No	39.20	10.55	31.47
NASH	3	554.93	Yes	12.70	3.54	37.56

NAFLD=nonalcoholic fatty liver disease, NASH= nonalcoholic steatohepatitis, T2DM= type 2 diabetes, HOMA-IR= homeostatic model assessment of insulin resistance, BMI= body mass index, *normalized read counts

Species	Primer Name	Primer sequence
mouse	Acly_F	GCCAGCGGAGCACATC
mouse	Acly_R	CTTTGCAGGTGCCACTTCATC
mouse	Acaca_F	GCC TCT TCC TGA CAA ACG AG
mouse	Acaca_R	TGA CTG CCG AAA CAT CTC TG
mouse	Acacb_F	ACTCGCTTTGGAGGCAACAG
mouse	Acacb_R	CGCAGCGATGCCATTGTGTG
mouse	Fasn_F	CAG CAG AGT CTA CAG CTA CCT
mouse	Fasn_R	ACC ACC AGA GAC CGT TAT GC
mouse	Scd1_F	CTGACCTGAAAGCCGAGAAG
mouse	Scd1_R	GCGTTGAGCACAGAGTGTA
mouse	Elov16_F	TGC TGC ATC CAG TTG AAG AC
mouse	Elov16_R	TGC CAT GTT CAT CAC CTT GT
mouse	Cidea_F	GCCTGCAGGAATTATCAGC
mouse	Cidea_R	TGCTTGACACTGGGACATA
mouse	Cidec_F	ATGGACTACGCCATGAAGTCT
mouse	Cidec_R	CGGTGCTAACACGACAGGG
mouse	Ppara_F	AACATCGAGTGTGAATATGTGG
mouse	Ppara_R	CCGAATAGTTCCGCCGAAAGAA
mouse	Cpt1a_F	AGGGCCGATCATGGTTAACA
mouse	Cpt1a_R	CGTGTGGATGGTGTCTGTC
mouse	Acadm_F	AGGTTTCAAGATCGCAATGG
mouse	Acadm_R	ACCTTCATGCCATTCTGC
mouse	Cd36_F	ATTTCATGCCAGTCGGAGA
mouse	Cd36_R	TGGGTTTTGCACATCAAGA
mouse	Glut2_F	GGCTAATTTCAGACTGGTT
mouse	Glut2_R	TTTCTTTGCCCTGACTTCCT
mouse	Gck_F	ATGGCTGTGGATACTACAAGGA
mouse	Gck_R	TTCAGGCCACGGTCCATCT
mouse	G6Pase_F	TGCTGCTCACTTTCCACCAG
mouse	G6Pase_R	TCTCAAAGTCCACAGGAGGT
mouse	Pepck1_F (Pck1)	GACAGCCTGCCCCAGGCAGTGA
mouse	Pepck1_R (Pck1)	CTGGCCACATCTCGAGGGTCAG
mouse	Glut4_F	GTGACTGGAACTGGTCTTA
mouse	Glut4_R	CCAGCCACGTTTGATTGTAG
mouse	Srebf1_F	GATCAAAGGAGGACAGTG
mouse	Srebf1_R	TAGATGGTGGCTGTGAGTG
mouse	Chrebptotal_F	CACTCAGGGAATACACGCCTAC
mouse	Chrebptotal_R	ATCTTGCTTTAGGGTCTCAGG
mouse	Chrebpa_F	CGACACTCACCCACCTCTTC
mouse	Chrebpa_R	TTGTTACGCCGATCTTGTC
mouse	Chrebpb_F	TCTGCAGATCGCGTGGAG
mouse	Chrebpb_R	CTTGTCGCCGATAGCAAC
mouse	Nr1h2_F	ATAGTGGGTACGAAGCAGC
mouse	Nr1h2_R	AGGGCAACAGAGTCGGAGAC
mouse	Nr1h3_F	AGGAGTGTGACTTCGCAAA
mouse	Nr1h3_R	CTCTTCTGCCGCTTCAGTTT
mouse	Cebpa_F	CAAGAACAGCAACGAGTACCG
mouse	Cebpa_R	GTCACTGGTCACTCCAGCAC
mouse	Tfiib_F	TGGAGATTTGCCACCATGA
mouse	Tfiib_R	GAATTGCCAAACTCATCAAACT
mouse	Pdk1_F	GGCGGCTTTGTGATTGTAT
mouse	Pdk1_R	ACCTGAATCGGGGATAAAC
mouse	Pfkip-F	AGGAGGGCAAGGAGGTGTT
mouse	Pfkip-R	TTGGCAGAAATCTTGGTTCC
mouse	Pgd-F	AGACAGGCAGCCACTGAGTT
mouse	Pgd-R	AAGTTCTGGGTTTCGCTCAA
mouse	Gapdh_F	AGGTGCGGTGAAACGGAATTTG
mouse	Gapdh_R	TGTAGACCATGTAGTTGAGGTCA
mouse	Tmem127_3F	TCGGTATGCCTTTGCTCACATCCT
mouse	Tmem127_4R	ACTTCTATGCTGTGCTGCTGGG
human	ACC1_R	AGTGGGTCAACCCATTGTT
human	ACC1_F	TTCTAACAGGAGCTGGAGCC
human	FASN_R	TCTCCGACTCTGGCAGCTT
human	FASN_F	GCTCCAGCCTCGCTCTC
human	SCD1_R	GCAGCCGAGCTTTGTAAGAG
human	SCD1_F	GTCTACACCTGGCTTTGGG
human	ELOVL6_R	GTCTCTGACCCTTGCACTCTT
human	ELOVL6_F	CCTGGTCAAACTGACTGCT
human	SREBF1c_F	AAAAATCCGCCGCGCT
human	SREBF1c_R	GCATGTCTTCGAAAGTGCAA
human	CHREBPb_F	AGCCAGCCTCAAGGAGC
human	CHREBPb_R	CTGCTGGCAGGTTAATGG
human	LXRB_F	CGCTACAACCAGGACAGA
human	LXRB_R	GAACCTGAAGATGGGGTTGA
human	LXRA_F	GAAGAACTGAAGCGGCAAGA
human	LXRA_R	ACTCGAAGCGGTCAGAAAA
human	TBP_F	TATAATCCCAAGCGTTTGTGCG
human	TBP_R	AATTGTTGGTGGTGAGCACAAAGG
human	G6PASE_F (G6PC)	ACTGGCTCAACCTCGTCTTTA
human	G6PASE_R (G6PC)	CGGAAAGTGTGCTGTAGTAGTCA
human	TMEM127_F	TCGGTATGCCTTTGCTCACATCCT
human	TMEM127_R	ACTTCTATGCTGTGCTGCTGGG