Supplementary Materials

High-throughput screening of mouse gene knockouts identifies established and novel high body fat phenotypes

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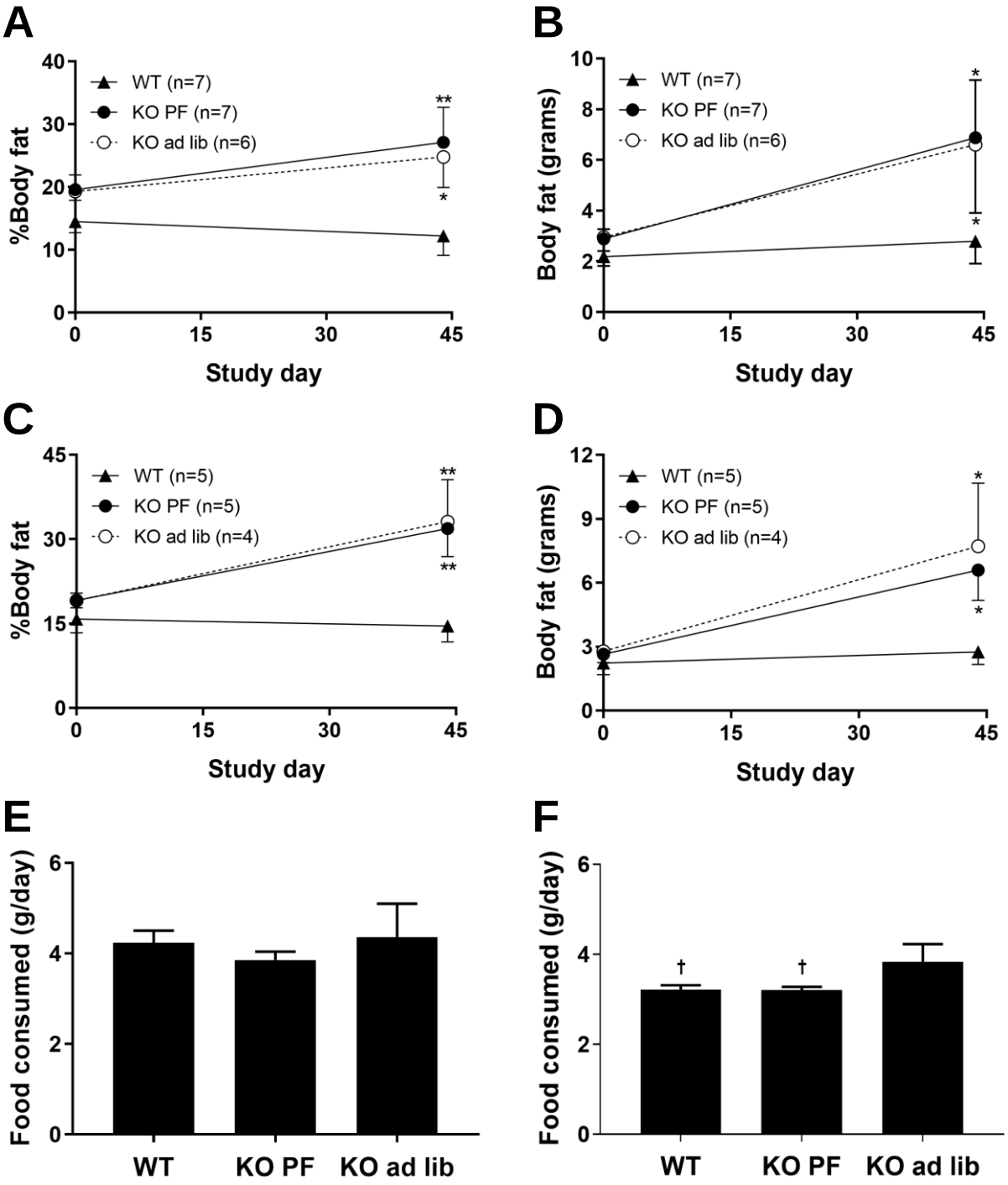
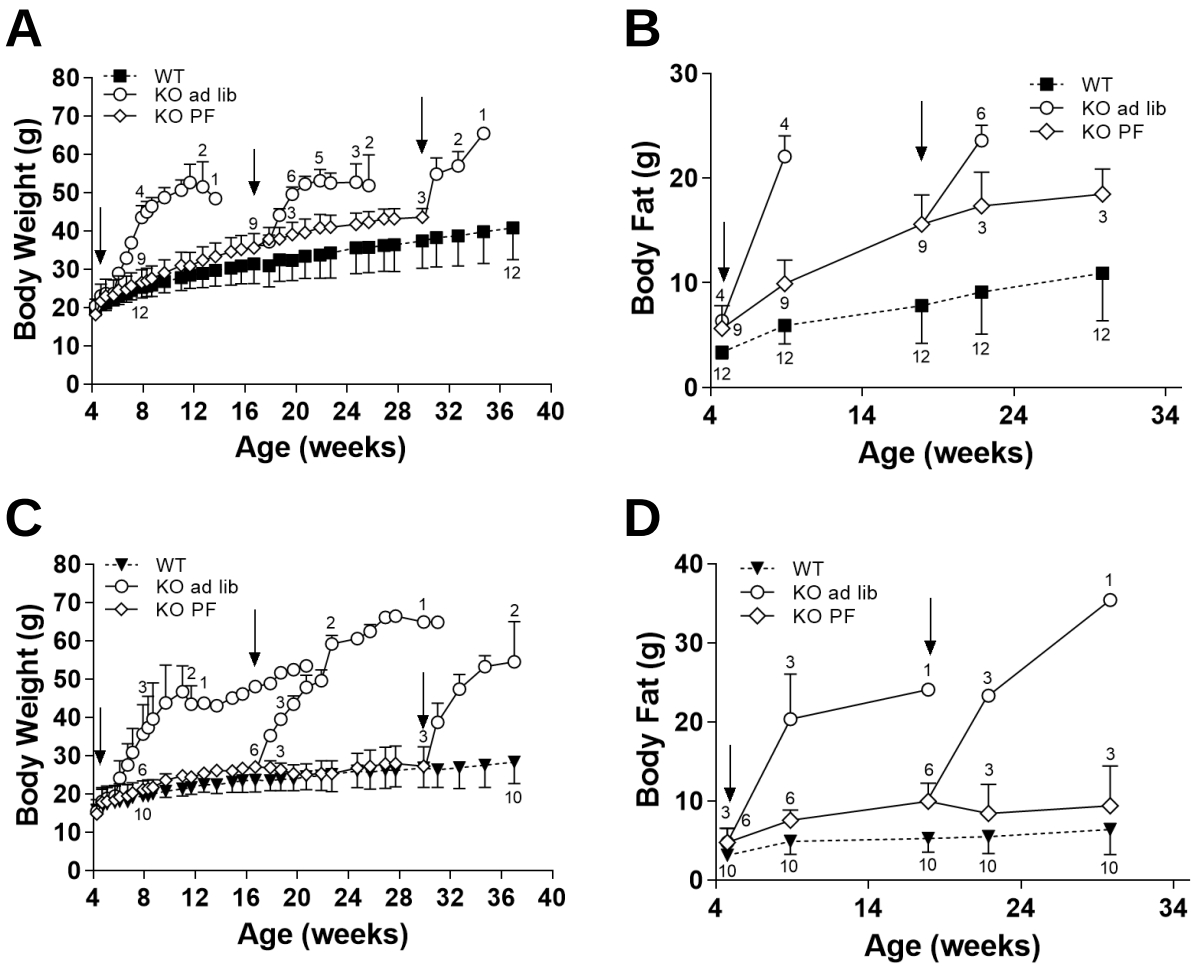
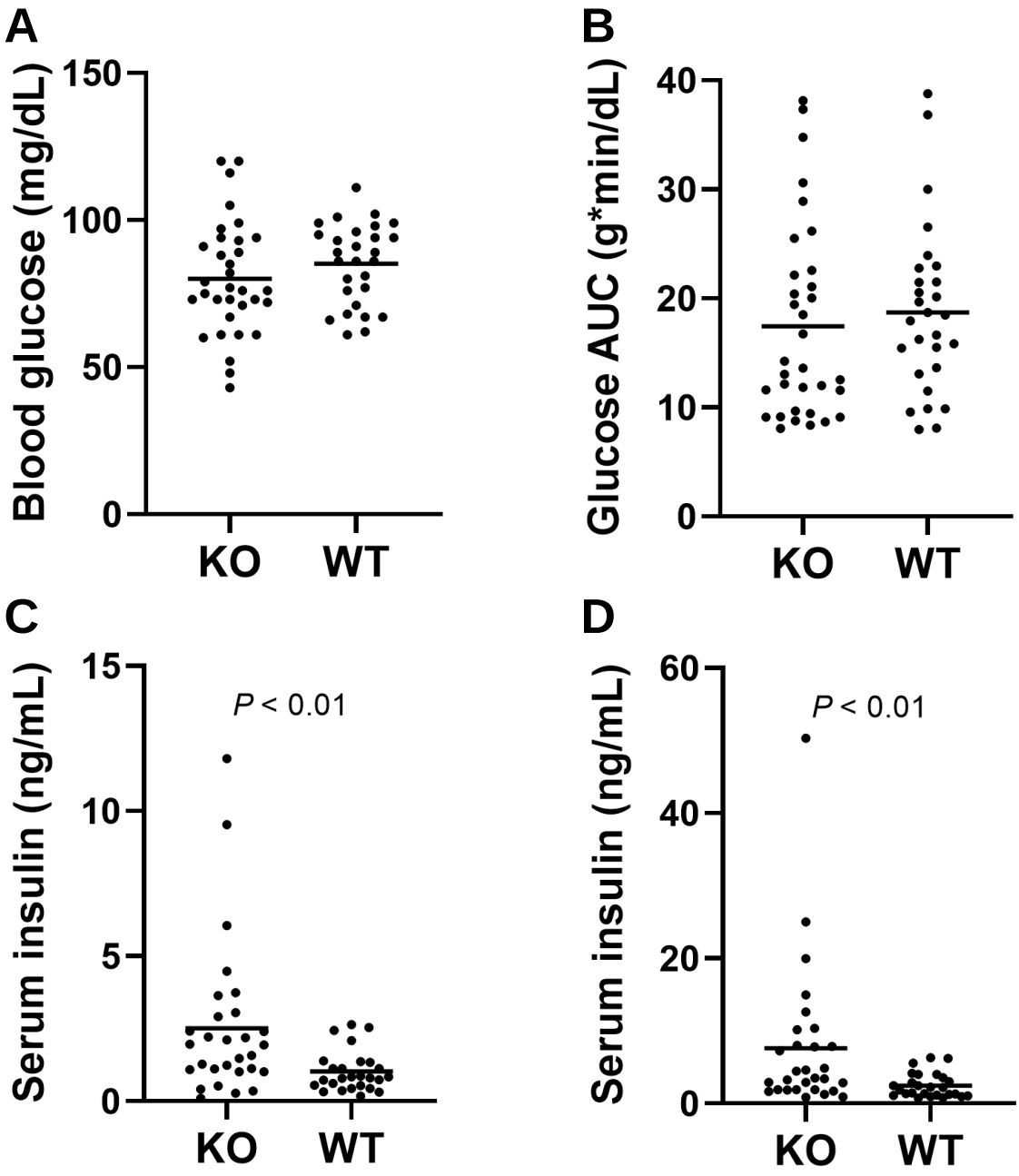


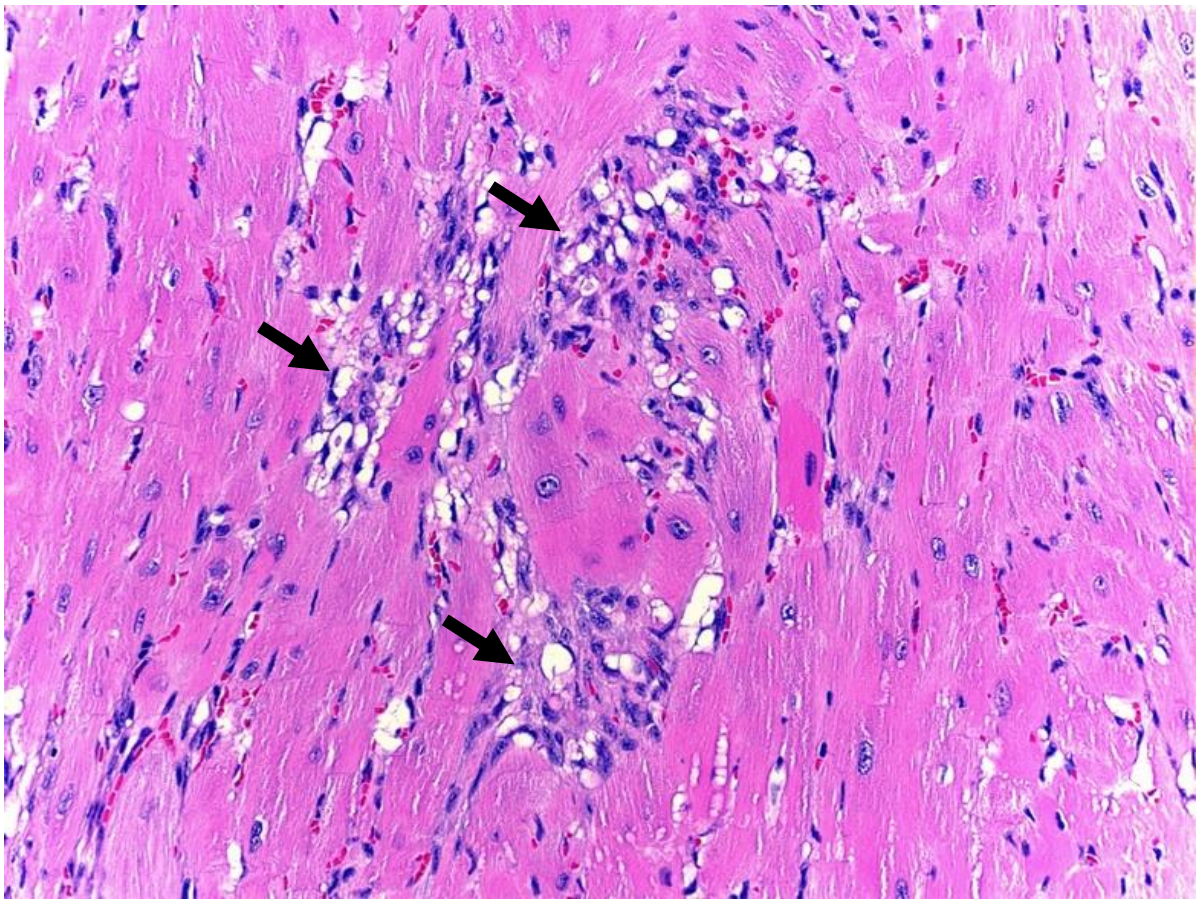
Supplementary Figure 1. *Gpr45* KO mice are obese due to decreased energy expenditure. Starting at weaning, 3-week old *Gpr45* KO and WT mice were individually housed for 44 days on chow diet. WT mice were fed ad libitum (ad lib) while KO mice were either fed ad lib (KO ad lib) or pair-fed to the WT mice (KO PF). Body composition was measured by QMR on the first and last study days, with changes in body fat analyzed by one-way ANOVA. Food consumption was measured daily. QMR data are shown for male **A)** %body fat and **B)** body fat (g), and for female **C)** %body fat and **D)** body fat (g). Also shown are mean daily food consumption of **E)** male and **F)** female mice. KO mice different from WT mice, $^{*}P < 0.05$, $^{**}P < 0.01$; WT and KO PF mice different from KO ad lib mice, $^{\dagger}P < 0.01$.



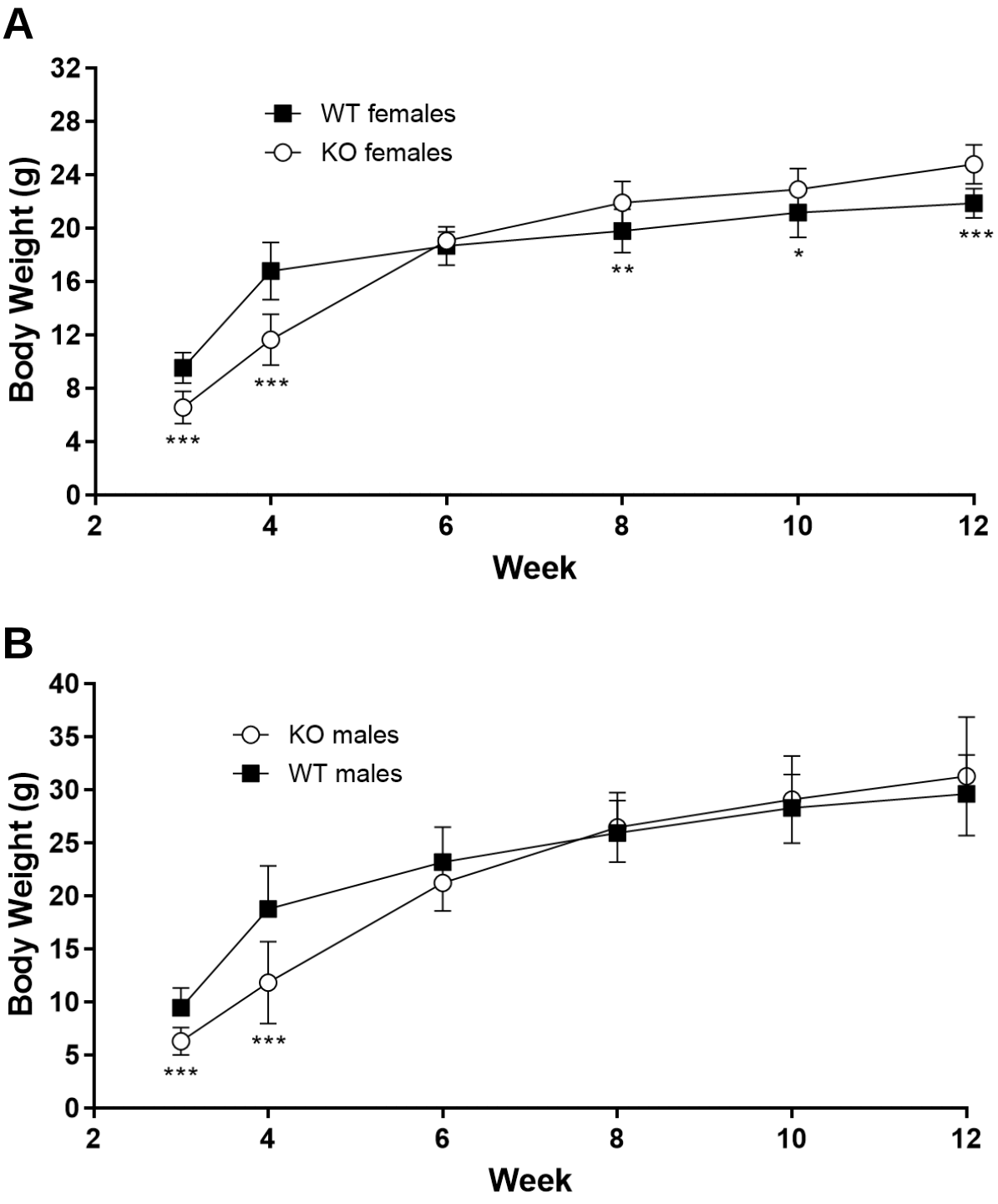
Supplementary Figure 2. The obesity of *Ksr2* KO mice results from increased energy intake and decreased energy expenditure. Male mice: 13 KO mice and 12 WT littermates were weaned onto chow diet. WT mice were fed ad lib throughout the study. At weaning, 4 KO mice were fed ad lib and 9 KO mice were pair-fed (PF) to the WT mice. At 17 weeks, 6 PF KO mice were switched to ad lib feeding, and at 30 weeks all remaining KO mice were switched to ad lib feeding. **A) Body weights**, and **B) Body fat in grams (g) by QMR**, were measured frequently during the study. Female mice: 9 KO mice and 10 WT littermates were weaned onto chow diet. WT mice were fed ad libitum (ad lib) throughout the study. At weaning, 3 KO mice were fed ad lib and 6 KO mice were pair-fed (PF) to the WT mice. At 17 weeks, 3 PF KO mice were switched to ad lib feeding, and at 30 weeks all remaining KO mice were switched to ad lib feeding. **C) Body weights**, and **D) Body fat in grams (g) by QMR**, were measured frequently during the study. For all panels, arrows indicate when feeding schedules were changed, and the numbers above the KO mouse groups and below the WT mouse group indicate the number of surviving mice in that group.



Supplementary Figure 3. Impaired glucose homeostasis in adult male *Dpp8* Knockout mice. OGTT levels of **A) fasting blood glucose at baseline** and **B) OGTT AUC** in 33 knockout (KO) and 28 wild-type (WT) mice. In these same OGTTs, **insulin levels at C) baseline** (predose; T = 0 minutes) and **D) 30 minutes** in 29 KO and 26 WT mice.



Supplementary Figure 4. Myocardial lesions in *Aqp7* Knockout mice. Interstitial infiltrates of vacuolated macrophages are associated with scattered small foci of myocardial degeneration (arrows).



Supplementary Figure 5. Body weights of young *Tle4* knockout (KO) and wild-type (WT) littermate mice. Body weights were measured at multiple time points between weaning and 12 weeks of age for **A) female mice (**8 KO, 16 WT) and **B) male mice** (6 KO, 9 WT). KO mice different from WT mice, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Supplementary Table 1. List of 75 mouse genes knocked out

Gene symbol	Official gene name	Taconic catalog[a]
Adamts4	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 4	TF0170
Adamts18	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 18	TF3156
Adcy3	Adenylate cyclase 3	TF0019
Adm2	Adrenomedullin 2	TF4022
Ak5	Adenylate kinase 5	TF2909
Ankk1	Ankyrin repeat and kinase domain containing 1	TF2000
Aoc3	Amine oxidase, copper containing 3	TF0957
Apln	Apelin	TF3533
Aqp7	Aquaporin 7	TF0023
AU040320	Expressed sequence AUO40320	NL
Asnsd1	Asparagine synthetase domain containing 1	TF1796
Brs3	Bombesin-like receptor 3	TF1854
Ccn5	Cellular communication network factor 5	TF3923
Cxxc4	CXXC finger 4	TF0303
Ddah1	Dimethylarginine dimethylaminohydrolase 1	TF1669
Dgkg	Diacylglycerol kinase, 11	TF1946
Dpp8	Dipeptidylpeptidase 8	TF1778
Enox1	Ecto-NOX disulfide-thiol exchanger 1	TF2961
Ffar4 (Gpr120)	Free fatty acid receptor 4	TF0224
G2e3	G2/M-phase specific E3 ubiquitin ligase	NL
Glrx2	Glutaredoxin 2 (thioltransferase)	NL
Gpr45	G protein-coupled receptor 45	NL
Gpr61	G protein-coupled receptor 61	TF2683
Gpx7	Glutathione peroxidase 7	TF3532
Hdac5	Histone deacetylase 5	TF2997

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Hdac6	Histone deacetylase 6	TF2765
Herc1	HECT and RLD domain containing E3 ubiquitin ligase protein ligase family member 1	TF1657
Htr2c	5-hydroxytryptamine (serotonin) receptor 2C	TF1240
Igdcc4	Immunoglobulin superfamily, DCC subclass, member 4	TF3828
Igfbp2	Insulin-like growth factor binding protein 2	TF0321
Itih1	Inter-alpha trypsin inhibitor, heavy chain 1	TF1059
Kdm3a	Lysine demethylase 3A	NL
Kiss1	KiSS-1 metastasis-suppressor	TF3885
Kiss1r	KiSS-1 receptor	TF1853
Ksr2	Kinase suppressor of ras 2	TF3052
Lrrn2	Leucine rich repeat protein 2, neuronal	TF3414
Lrrtm1	Leucine rich repeat transmembrane neuronal 1	TF3653
Mc3r	Melanocortin 3 receptor	TF0402
Mc4r	Melanocortin 4 receptor	TF2556
Mfap3l	Microfibrillar-associated protein 3-like	TF3906
Ncoa1	Nuclear receptor coactivator 1	TF1666
Ncs1	Neuronal calcium sensor 1	TF0217
Npvf	Neuropeptide VF precursor	TF2353
Nr4a1	Nuclear receptor subfamily 4, group A, member 1	TF1559
Ntm	Neurotrimin	TF3441
Oprm1	Opioid receptor, mu 1	NL
P2rx6	Purinergic receptor P2X, ligand-gated ion channel, 6	TF0515
Pecr	Peroxisomal trans-2-enoyl-CoA reductase	TF0959
Pnpla2	Patatin-like phospholipase domain containing 2	NL
Prdx6	Peroxiredoxin 6	TF1709
Prlhr	Prolactin releasing hormone receptor	TF1878
Prmt7	Protein arginine N-methyltransferase 7	TF2986
Prok2	Prokineticin 2	TF3969

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Prokr2	Prokineticin receptor 2	TF3011
Ptp4a1	Protein tyrosine phosphatase 4a1	NL
Ptprn	Protein tyrosine phosphatase, receptor type, N	TF0601
Pyy	Peptide YY	TF3812
Resp18	Regulated endocrine-specific protein 18	TF4045
Retn	Resistin	TF3517
Retsat	Retinol saturase (all trans retinol 13,14 reductase)	TF3526
Rgs10	Regulator of G-protein signalling 10	TF0059
Scg3	Secretogranin III	TF3191
Sik2	Salt inducible kinase 2	TF2778
Slc6a4 (Sert)	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	TF2861
Sost	Sclerostin	TF3444
Srpk2	Serine/arginine rich protein specific kinase 2	TF1921
St3gal2	ST3 beta-galactoside alpha-2,3-sialyltransferase 2	TF2992
Tenm3	Teneurin transmembrane protein 3	TF3824
Tle4	Transducin-like enhancer of split 4	TF2902
Tnfsf13b	TNF superfamily member 13b	TF0903
Tsn	Translin	TF0827
Tusc3	Tumor suppressor candidate 3	TF2125
Usp13	Ubiquitin specific peptidase 13 (isopeptidase T-3)	TF2533
Usp38	Ubiquitin specific peptidase 38	TF2838
Wnt8b	Wingless-type MMTV integration site family, member 8B	TF3868

Abbreviations: NL, not listed

[a]More information available at the Taconic Biosciences webpage: https://www.taconic.com/find-your-model/

Supplementary Table 2. Mouse gene KO lines generated by homologous recombination (N=38)

Mouse Gene	Protein class	Mouse chromosome	Site of deletion	NCBI Reference Sequence
Adamts4	Enzyme	1	Coding exon 4	NM_172845
Adamts18	Enzyme	8	Coding exons 1-3	NM_172466
Adm2	Secreted	15	Both coding exons	NM_182928
Ak5	Enzyme	3	Coding exons 3-5	AK053807
Ankk1	Enzyme	9	Coding exons 1-5	NM_172922
Apln	Secreted	X	Both coding exons	NM_013912
Aqp7	Transporter	4	Coding exon 1	NM_007473
AU040320	Secreted	4	Coding exon 2	NM_001035525
Ccn5	Secreted	2	Coding exons 2-3	NM_016873
Cxxc4	Intracellular zinc finger	3	Coding exon 1	NM_001004367
Dgkg	Enzyme	16	Coding exons 3-4	NM_138650
Dpp8	Enzyme	9	Last 6 coding exons	NM_028906
Gpr45	G protein-coupled receptor	1	Single coding exon	AF139642
Gpr61	G protein-coupled receptor	3	Single coding exon	NM_175470
Gpx7	Enzyme	4	Coding exons 2-3	NM_024198
Itih1	Secreted	14	Coding exons 1-6	NM_008406
Lrrn2	Membrane protein	1	Single coding exon	NM_010732
Lrrtm1	Membrane protein	6	Single coding exon	NM_028880
Mfap3l	Enzyme	8	Last coding exon	NM_027756
Ncs1	Calcium binding protein	2	Coding exon 1	NM_019681
Npvf	Secreted	6	Coding exons 1-2	NM_021892
Ntm	Cell adhesion	9	Coding exon 1	BC023307
Oprm1	G protein-coupled receptor	10	Coding exon 2	NM_001039652
P2rx6	Channel	16	Coding exons 1-2	NM_011028
Pnpla2	Enzyme	7	Coding exons 2-4	NM_025802
Prok2	Secreted	6	Coding exons 1-2	NM_015768

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Prokr2	G-protein coupled receptor	2	Coding exon 1	NM_144944
Ptprn	Membrane protein	1	Last 8 coding exons	NM_008985
Pyy	Secreted	11	All 3 coding exons	NM_145435
Resp18	Secreted	1	Coding exons 2-5	NM_009049
Retn	Secreted	8	Coding exons 1-2	NM_022984
Retsat	Enzyme	6	Coding exons 1-4	AF466400
Scg3	Secreted	9	Coding exons 1-4	NM_009130
Sik2	Enzyme	9	Coding exons 2-3	NM_178710
Tnfsf13b	Secreted	8	Coding exon 1	NM_033622
Usp13	Enzyme	3	Coding exon 8	NM_001013024
Usp38	Enzyme	8	Second to last coding exon	NM_027554
Wnt8b	Secreted	19	Coding exons 3-6	NM_011720

Abbreviations: KO, knockout; N, number of KO lines; NCBI, National Center for Biotechnology Information

Supplementary Table 3. Mouse gene KO lines generated by gene trapping (N=24)

Mouse gene	Protein class	Omnibank sequence tag	Mouse Chr	Insertion site	NCBI reference sequence
Adamts4	Enzyme	106997	1	Intron between coding exons 1 & 2	NM_172845
Adcy3	Enzyme	68174	12	Intron between coding exons 2 & 3	NM_138305
Aoc3	Enzyme	179698	11	Intron between coding exons 1 & 2	NM_009675.1
Ddah1	Enzyme	7734	3	Intron between coding exons 1 & 2	NM_026993.1
Enox1	Enzyme	GST_3658_B3	14	Intron between coding exons 1 & 2	NM_172813
Glrx2	Enzyme	108649	1	Intron between coding exons 1 & 2	NM_023505.1
Hdac6	Enzyme	244126	X	5' noncoding	BC041105.1
Herc1	Enzyme	443596	9	5' noncoding	AK083823
Igdcc4	Membrane protein	244907	9	Intron between coding exons 1 & 2	NM_020043
Igfbp2	Secreted	365171	1	Intron between coding exons 1 & 2	NM_008342
Kdm3a	Enzyme	227748	6	Intron between coding exons 8 & 9	AU035888
Ncoa1	Nuclear receptor coactivator	455417	12	5' noncoding	NM_010881.1
Nr4a1	Nuclear receptor	GST_5707_C8	15	5' noncoding	NM_010444
Pecr	Enzyme	63826	1	Intron between coding exons 1 & 2	NM_023523
Prdx6	Enzyme	519147	1	Intron between coding exons 2 & 3	NM_007453
Prmt7	Enzyme	415307	8	5' noncoding	NM_145404
Ptp4a1	Enzyme	165257	1	5' noncoding	BC031734.1
Rgs10	GTPase activator	24334	7	Intron between coding exons 3 & 4	NM_026418
Scg3	Secreted	17542	9	Intron between the last 2 coding exons	NM_009130
Srpk2	Enzyme	464130	5	Intron between coding exons 1 & 2	NM_009274
St3gal2	Enzyme	213584	8	5' noncoding	NM_178048

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Tenm3	Membrane protein	99683	8	Intron between coding exons 1 & 2	NM_011857
Tle4	Transcription corepressor	19477	19	Intron between coding exons 10 & 11 from 3' end	NM_011600.2
Tusc3	Enzyme subunit	24273	8	Intron between coding exons 3 & 4 from 3' end	NM_030254.2

Abbreviations: KO, knockout; N, number of KO lines; Chr, chromosome; NCBI, National Center for Biotechnology Information; GTPase, guanosine triphosphatase

Supplementary Table 4. Body composition of *Mc3r* KO, *Mc4r* KO and *Mc3r*/*Mc4r* DKO mice

Genotype (n)	Body fat (g)	Lean body mass (g)
WT (12)	8 ± 4	26 ± 2
Mc3r KO (14)	14 ± 3	25 ± 4
Mc4r KO (12)	23 ± 5	37 ± 3
Mc3r/*Mc4r* DKO (12)	30 ± 7	34 ± 4
Two-way ANOVA	***P* value**	***P* value**
Mc3r KO vs WT	<0.0001	0.074
Mc4r KO vs WT	<0.0001	<0.0001
Interaction	0.928	0.165

Notes: Data are from 20-week-old mice fed HFD from weaning
Abbreviations: n, number of mice; g, grams; WT, wild-type; KO, knockout; DKO, double knockout; ANOVA, analysis of variance

Supplementary Table 5. Body composition of *Gpr45* KO and WT mice

Diet	Age	Sex	Genotype (n)	Body fat (g)	% body fat	LBM (g)
Chow	3 weeks	Male	WT (7)	2.19 ± 0.37	14.5 ± 1.8	12.9 ± 1.6
			KO (13)	2.92 ± 0.45**	19.4 ± 1.9***	12.1 ± 1.2
		Female	WT (5)	2.23 ± 0.54	15.8 ± 2.5	11.7 ± 0.9
			KO (9)	2.71 ± 0.32	19.1 ± 1.3**	11.5 ± 1.2
	7-38 weeks	Male	WT (40)	5.5 ± 3.8	16.4 ± 7.5	25.9 ± 3.5
			KO (49)	12.4 ± 5.4*** †	32.2 ± 8.3***	24.4 ± 4.1
		Female	WT (36)	4.8 ± 2.8	19.4 ± 6.5	18.3 ± 2.7
			KO (51)	15.7 ± 6.4*** †	42.7 ± 6.8***	19.8 ± 3.9
	57 weeks	Male	WT (19)	9.2 ± 4.7	21.6 ± 7.5	31.4 ± 3.3
			KO (17)	25.9 ± 5.9***	42.1 ± 5.5***	35.1 ± 3.1**
		Female	WT (16)	9.9 ± 4.2	29.2 ± 8.1	22.8 ± 2.1
			KO (18)	28.4 ± 8.6*** †	51.2 ± 9.1***	25.6 ± 2.8**
	87 weeks	Female	WT (15)	8.1 ± 4.8	24.2 ± 9.8	23.3 ± 2.4
			KO (10)	29.8 ± 14*** †	51.4 ± 12.9***	24.8 ± 2.1
HFD	14 weeks	Male	WT (10)	12.2 ± 2.9	32.3 ± 6.1	25.2 ± 1.5
			KO (13)	18.9 ± 1.7***	40.2 ± 2.3***†	28.1 ± 2.2**
		Female	WT (7)	7.3 ± 3.6	26.6 ± 9.2	19.1 ± 1.5
			KO (5)	21.8 ± 3.2***	49 ± 1.6**†	22.7 ± 3**

Notes: KO mice different from WT mice, ***P* < 0.01; ****P* < 0.001; † Statistical analysis by Mann–Whitney test.

Abbreviations: HFD, high fat diet; (g), grams; KO, knockout; WT, wild-type; n, number of mice; LBM, lean body mass

Supplementary Table 6. Metabolic parameters measured in *Gpr45* KO and WT mice

Assay		Sex	Age	Genotype (n)	Mean ± SD
OGTT	Glucose area under the curve (mg*min/dL)	Male & female	37-39 weeks	WT (30)	13,596 ± 3,976
				KO (30)	11,591 ± 4,151
	Fasting blood glucose (mg/dL)	Male & female	37-39 weeks	WT (30)	86 ± 24
				KO (30)	93 ± 22
	Fasting serum insulin (ng/mL)	Male & female	37-39 weeks	WT (29)	0.87 ± 1.22
				KO (30)	2.56 ± 0.87***
	30' serum insulin (ng/mL)	Male & female	37-39 weeks	WT (30)	2.33 ± 3.33
				KO (30)	6.61 ± 4.17***
	Composite Insulin Sensitivity Index[a]	Male & female	37-39 weeks	WT (29)	11.9 ± 16.4
				KO (30)	1.2 ± 1.0***†
	HOMA Insulin Sensitivity Index[b]	Male & female	37-39 weeks	WT (29)	38.7 ± 61.7
				KO (30)	2.7 ± 2.6***†
Serum leptin (ng/mL)		Male & female	20 weeks	WT (6)	8.7 ± 4.5
				KO (12)	44.1 ± 16.7***†
Serum total cholesterol (mg/dL)		Male & female	14 weeks	WT (6)	122 ± 27
				KO (14)	159 ± 26*
Serum triglycerides (mg/dL)		Male & female	14 weeks	WT (6)	84 ± 27
				KO (14)	142 ± 51*

Notes: KO mice different from WT mice, *$P < 0.05$; ***$P < 0.001$; † Statistical analysis by Mann–Whitney test.

Abbreviations: n, number of mice; SD, standard deviation; KO, knockout; WT, wild-type

[a]Higher values indicate increased insulin sensitivity. From: Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462-70. doi: 10.2337/diacare.22.9.1462.

[b]Higher values indicate increased insulin sensitivity. From: Turner RC, Holman RR, Matthews D, Hockaday TD, Peto J. Insulin deficiency and insulin resistance interaction in diabetes: estimation of their relative contribution by feedback analysis from basal plasma insulin and glucose concentrations. *Metabolism* 1979;28:1086-96. doi: 10.1016/0026-0495(79)90146-x.

Supplementary Table 7. Physical activity and VO_2 measurements in *Gpr45* KO and WT mice

Assay		Sex	Age	Genotype (n)	Mean ± SD
Mini Mitter System	Normalized gross motor activity (%), light phase	Male	32-33 weeks	WT (6) KO (12)	100 ± 23 119 ± 25
	Normalized gross motor activity (%), dark phase	Male	32-33 weeks	WT (6) KO (12)	100 ± 51 83 ± 26
Oxymax	Ambulatory counts/hour	Male & female	14-16 weeks	WT (8) KO (16)	6.4 ± 6.7 8.6 ± 8.7
	VO_2, mL/hour	Male & female	14-16 weeks	WT (8) KO (16)	70.2 ± 10.8 78.4 ± 8.4

Abbreviations: n, number of mice; SD, standard deviation; KO, knockout; WT, wild-type; VO_2, oxygen consumption

Supplementary Table 8. Body composition of *Kiss1* and *Kiss1r* KO and WT mice

Gene	Sex	Genotype (n)	Normalized body weight	Normalized body fat (g)	Normalized % body fat	Normalized LBM (g)
Kiss1	Male	WT (12)	100 ± 13	100 ± 46	100 ± 34	100 ± 4
		KO (17)	94 ± 15	125 ± 41	134 ± 32*	83 ± 7***
	Female	WT (12)	100 ± 18	100 ± 41	100 ± 27	100 ± 9
		KO (12)	105 ± 21	123 ± 54	115 ± 28	100 ± 12
	Combined	WT (24)	100 ± 15	100 ± 43	100 ± 30	100 ± 7
		KO (27)	98 ± 18	124 ± 46*	127 ± 31**	90 ± 12***
Kiss1r	Male	WT (5)	100 ± 10	100 ± 13	100 ± 4	100 ± 10
		KO (7)	80 ± 11**	96 ± 25	124 ± 27	75 ± 11**
	Female	WT (8)	100 ± 6	100 ± 19	100 ± 17	100 ± 5
		KO (11)	112 ± 28	165 ± 72**	151 ± 32***	96 ± 17
	Combined	WT (13)	100 ± 8	100 ± 16	100 ± 13	100 ± 7
		KO (18)	99 ± 28	139 ± 67	140 ± 32***	88 ± 18*

Notes: KO mice different from WT mice, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$
Abbreviations: n, number of mice; g, grams; LBM, lean body mass; WT, wild-type; KO, knockout