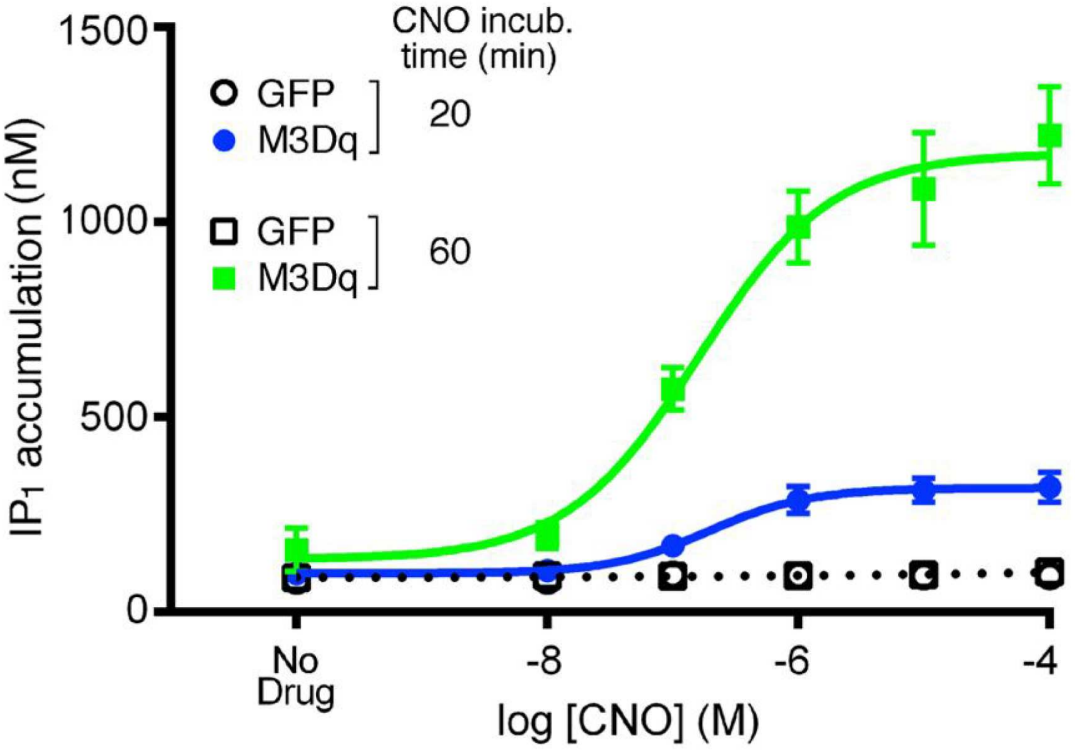
SUPPLEMENTARY DATA

Skeletal Muscle-Specific Activation of G_q Signaling Maintains Glucose Homeostasis

Derek B. J. Bone, Jaroslawna Meister, Jonas R. Knudsen, Diptadip Dattaroy, Amanda Cohen, Regina Lee, Huiyan Lu, Daniel Metzger, Thomas E. Jensen, and Jürgen Wess

SUPPLEMENTARY DATA

Supplementary Figure 1. CNO treatment of M3Dq-L6-GLUT4myc myoblasts leads to pronounced increases in inositol monophosphate (IP_1) production. L6-GLUT4myc myoblasts expressing either M3Dq or GFP (control) were incubated with increasing concentrations of CNO for 20 or 60 min in the presence of 50 mM LiCl. After CNO treatment, cells were lysed, and IP_1 content was determined using the IP-One Gq assay kit (Cisbio). Data are given as means ± SEM of three independent experiments.



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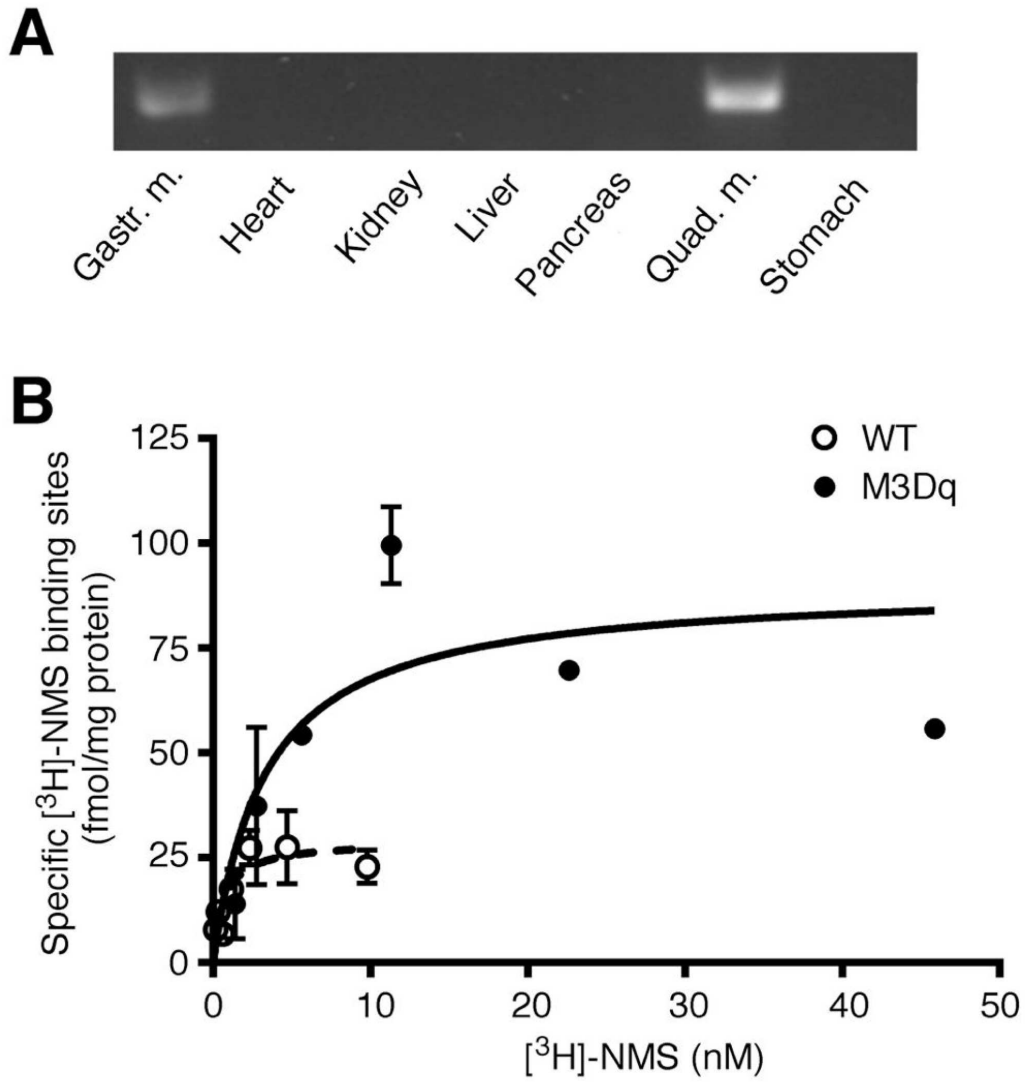
Supplementary Figure 2. Selective expression of M3Dq in SKM of transgenic mice. *A:* RT-PCR studies. Tissues were harvested from SKM-M3Dq transgenic mice, and total RNA was isolated as described under Methods. RT-PCR was performed, and PCR products were resolved by agarose gel electrophoresis. Only RNA prepared from SKM tissues (gastrocnemius and quadriceps muscles) gave an M3Dq-specific band. Gastr. m., gastrocnemius muscle; Quad. m., quadriceps muscle. *B:* Expression of the M3Dq designer receptor in SKM of SKM-M3Dq transgenic mice. [^{3}H]-NMS saturation binding studies were carried out using membranes prepared from quadriceps muscle of SKM-M3Dq transgenic mice and WT littermates (for details, see Methods). Data are means ± SEM of 2-4 independent experiments, each performed in duplicate. For all experiments, adult male mice were used.



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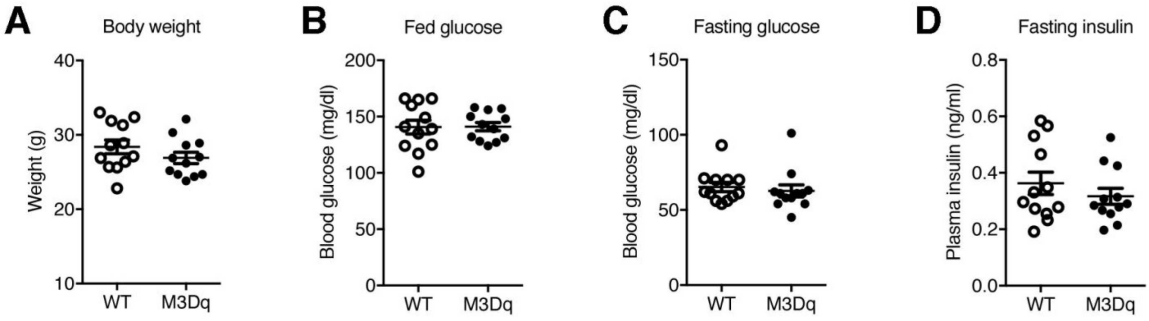
Supplementary Figure 3. SKM-M3Dq transgenic mice show normal body weight, blood glucose, and plasma insulin levels. SKM-M3Dq transgenic mice and their WT littermates were maintained on regular chow. *A:* Body weight. *B:* Blood glucose levels in freely fed mice. *C:* Blood glucose levels in mice that had been fasted overnight for 14-16 hr. *D:* Plasma insulin levels in mice that had been fasted overnight for 14-16 hr. All measurements were carried out with male littermates that were at least 8-12 weeks old. Data are means ± SEM.



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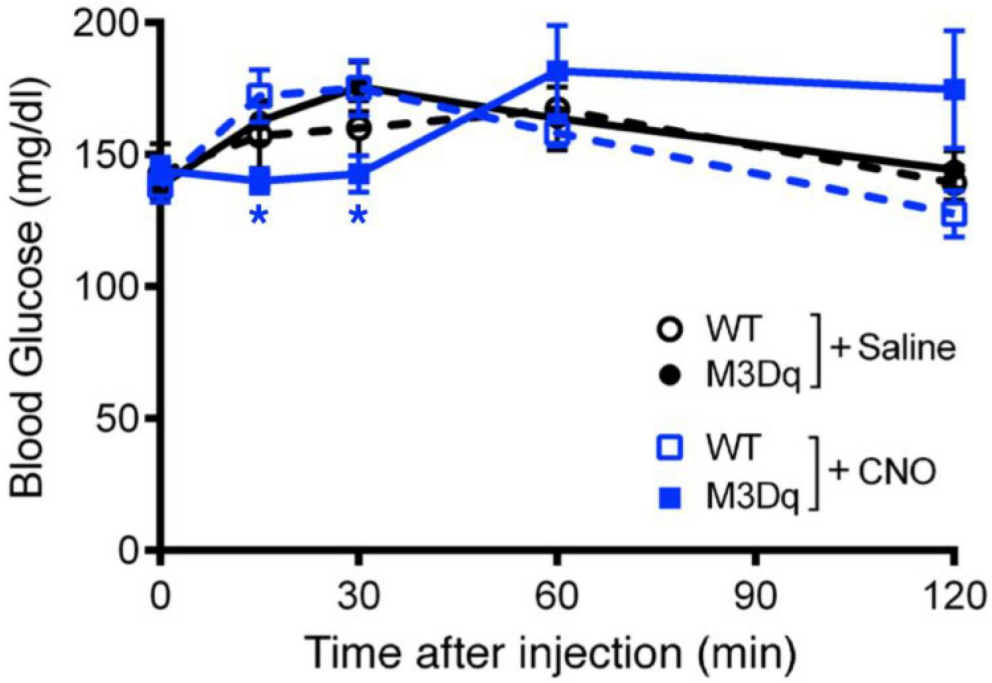
Supplementary Figure 4. Effect of acute CNO treatment of SKM-M3Dq transgenic mice on blood glucose levels. SKM-M3Dq transgenic mice and their WT littermates were injected i.p. with either saline or CNO (1 mg/kg), and blood glucose levels were determined at the indicated time points. Experiments were carried out with male littermates that were at least 8 weeks old. Data are means ± SEM (6 mice per group). **P*<0.05 (M3Dq/CNO vs. M3Dq/saline).



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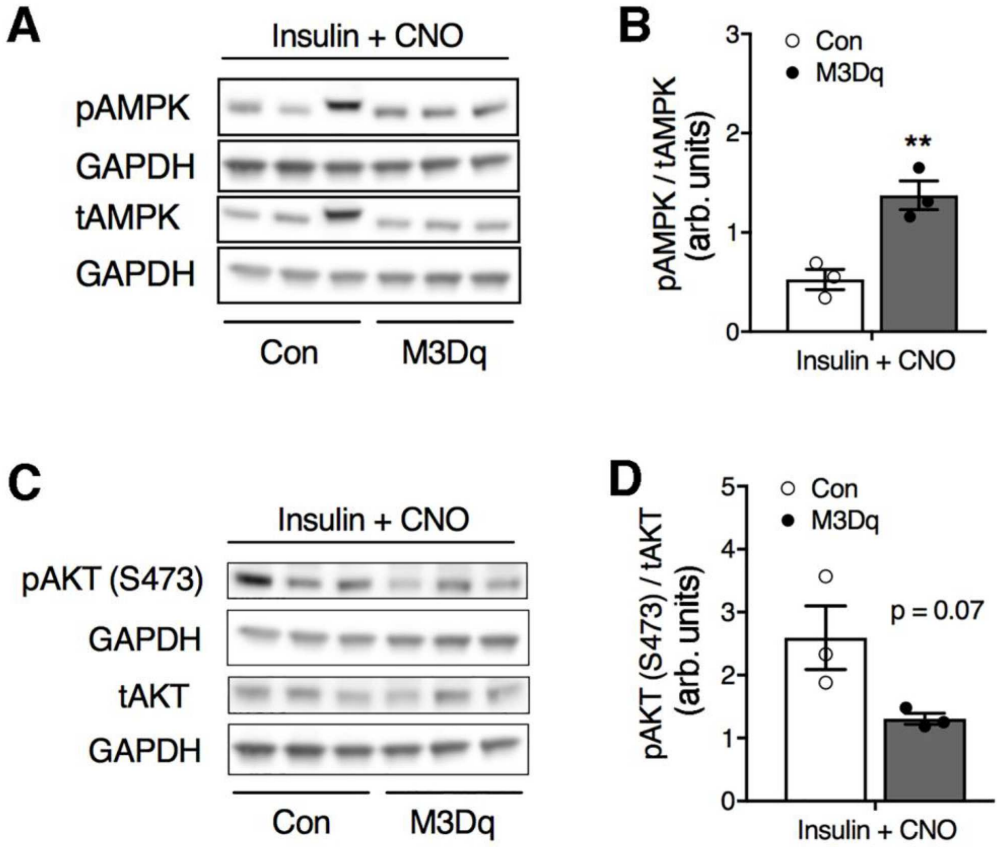
Supplementary Figure 5. Western blotting studies carried out with skeletal muscle from control and HSA-M3Dq mutant mice. HSA-M3Dq transgenic mice and their control littermates (males) that had been fasted for 5 hr were co-injected i.p. with insulin (0.75 IU/kg) and CNO (1 mg/kg). Gastrocnemius muscles were harvested 20 min post-injection. Tissue lysates were subjected to Western blotting analysis (see Methods for details). *A:* Western blots showing the expression of pAMPK and total AMPK (tAMPK). *B:* Quantification of the data shown in (*A*). *C:* Western blots showing the expression of pAKT (S473) and total AKT (tAKT). *D:* Quantification of the data shown in (*C*). Protein expression levels were normalized to the amount of loaded GAPDH (control). Blots were probed with the indicated antibodies. Data are presented as means ± SEM. ***P* <0.01, versus control (Student's t-test).



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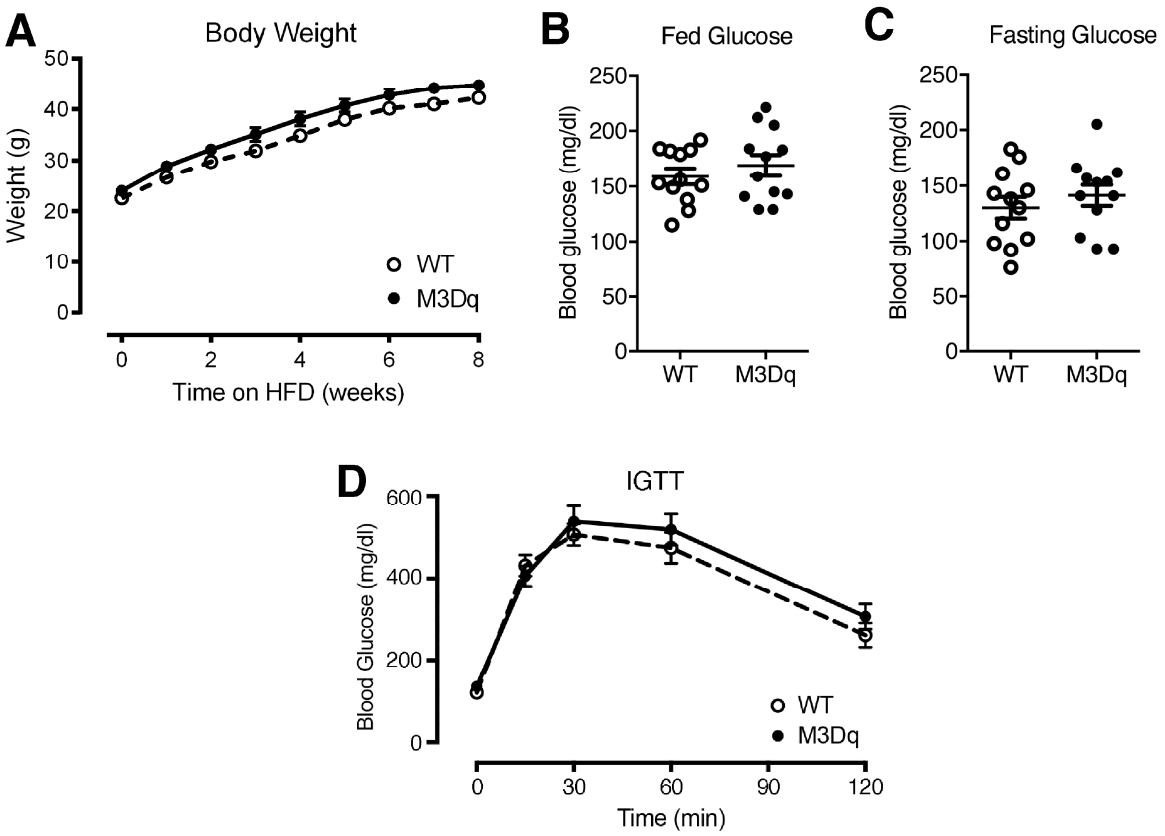
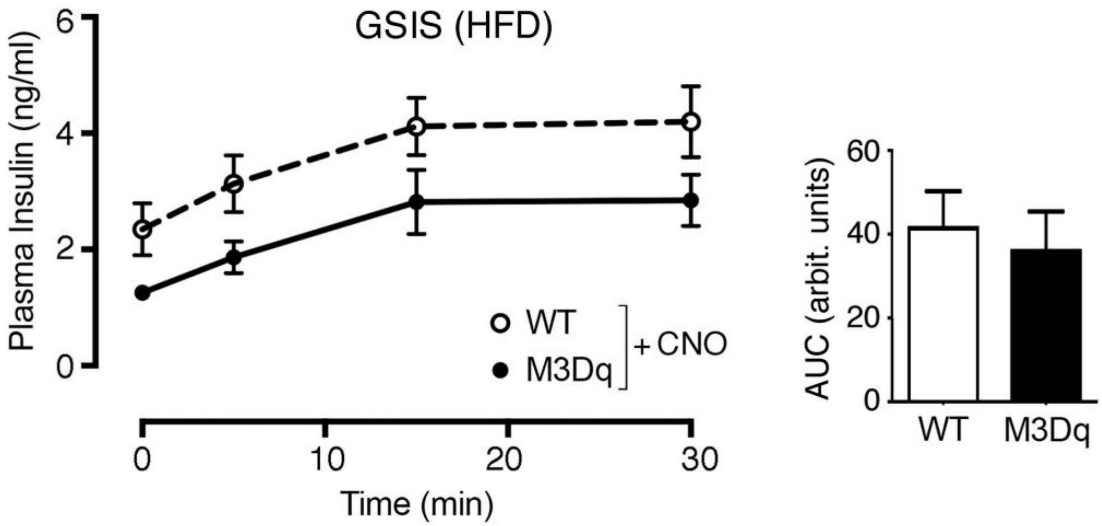
Supplementary Figure 6. SKM-M3Dq mice and WT littermates consuming a high-fat diet (HFD) show similar body weights, glycemia, and glucose tolerance. SKM-M3Dq transgenic mice and their WT littermates were maintained on a HFD for 8 weeks. *A:* Body weight measurements. *B:* Blood glucose levels in freely fed mice after 8 weeks of HFD feeding. *C:* Blood glucose levels in mice maintained for 8 weeks on a HFD after an overnight fast. *D:* IGTT. HFD mice were fasted overnight and then injected with glucose (2 g/kg i.p.). All measurements were carried out with male littermates. Data are given as means ± SEM (6 mice per group).



SUPPLEMENTARY DATA

Supplementary Figure 7. Glucose-stimulated insulin secretion (GSIS) in CNO-treated HFD mice. SKM-M3Dq transgenic mice and their WT littermates were maintained on a HFD for 8 weeks. Mice were fasted overnight and then injected i.p. with glucose (2 g/kg) plus CNO (1 mg/kg), followed by the measurement of plasma insulin levels at the indicated time points. Experiments were carried out with male littermates that were at least 12 weeks old. Data are presented as means ± SEM (6 mice per group). AUC, area under the curve.



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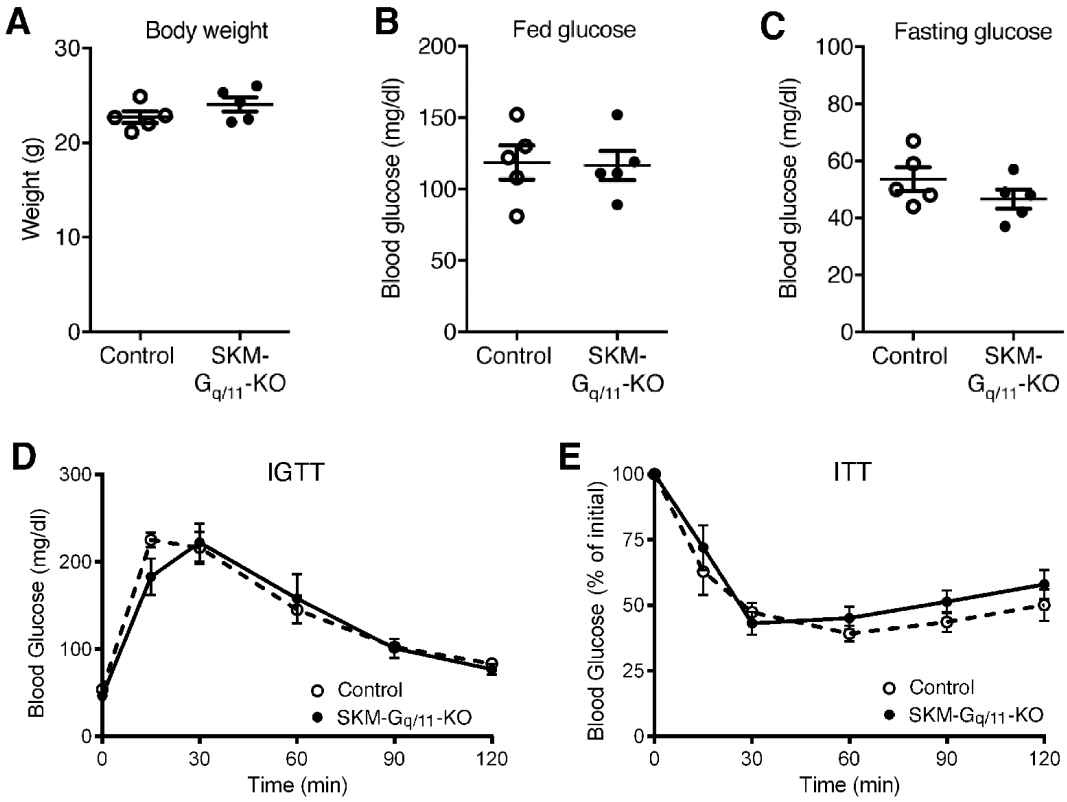
Supplementary Figure 8. Metabolic studies with SKM-$G_{q//11}$ KO mice maintained on regular chow. SKM-$G_{q//11}$ KO mice were obtained after tamoxifen treatment of mice of the following genotype: HSA-Cre(ER^{T2}) $Ga_q^{flox/flox}$ $Ga_{11}^{-/-}$ (for details, see Methods). TMX-treated $Ga_q^{flox/flox}$ $Ga_{11}^{-/-}$ mice littermates that lacked the Cre(ER^{T2}) transgene served as control animals. All mice consumed regular chow. *A:* Body weight at 8 weeks. *B:* Blood glucose levels in freely fed mice. *C:* Blood glucose levels in mice after an overnight fast. *D:* IGTT. Mice were fasted overnight and then injected with glucose (2 g/kg i.p.). *E:* ITT. Mice were fasted for 5 hr and then injected with insulin (0.75 U/kg i.p.). All experiments were carried out with male littermates that were at least 8 weeks old. Data are presented as means ± SEM of (5 mice per group).



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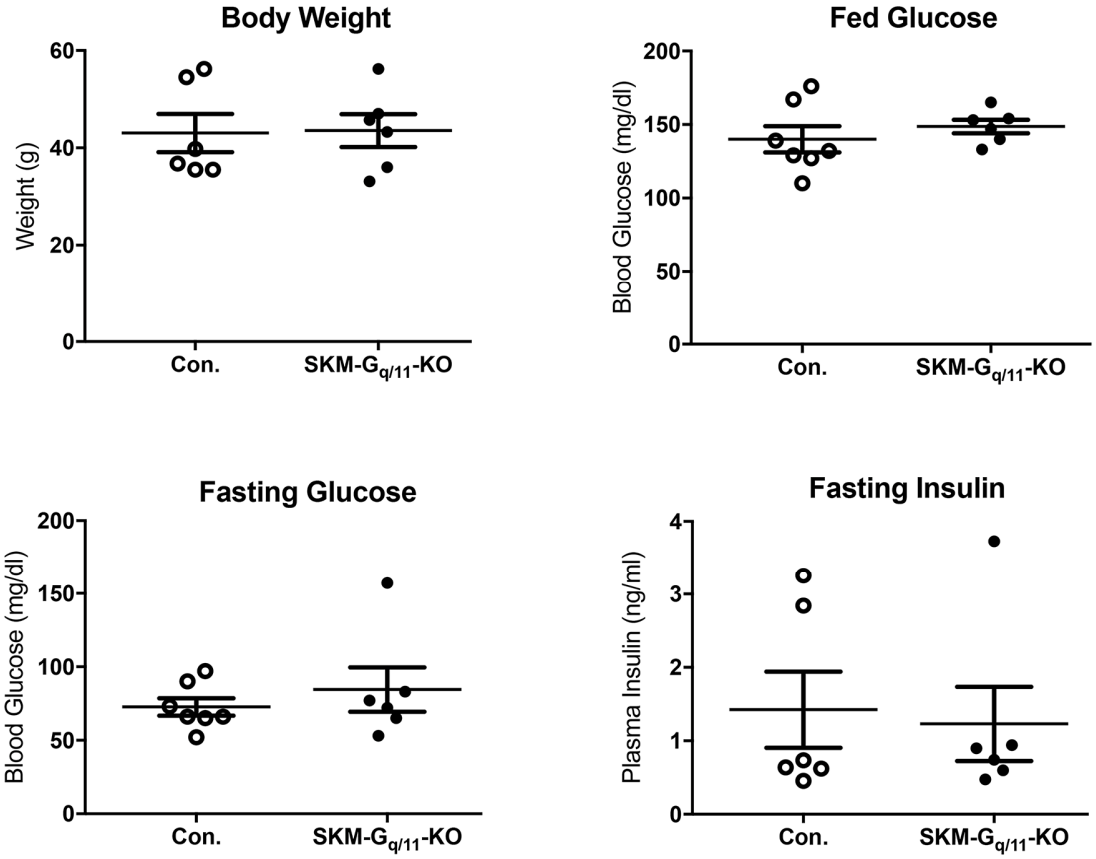
Supplementary Figure 9. Body weight, blood glucose, and plasma insulin levels of SKM-$G_{q//11}$ KO and control mice maintained on HFD. SKM-$G_{q//11}$ KO and control mice (see legend to Supplementary Fig. 7) were maintained on a HFD for at least 8 weeks. All experiments were carried out with male littermates that were at least 12 weeks old. Data are presented as means ± SEM of (6 or 7 mice per group).



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Supplementary Table 1. List of human SKM GPCRs that are either up- or down-regulated in type 2 diabetes (T2D)

Name	Description	Gene Name	base Mean	log2 Fold Change	p value	padj
ENSG00000148680.11	HTR7	5-HT receptor 7	68.5473	0.675419	2.61E-10	2.98E-07
ENSG00000115353.6	TACR1	tachykinin receptor 1	35.871	0.516471	1.56E-07	4.29E-05
ENSG00000122420.5	PTGFR	prostaglandin F receptor	233.554	0.444000	2.10E-07	5.19E-05
ENSG00000150594.5	ADRA2A	adrenoceptor alpha 2A	95.745	0.396211	1.89E-06	0.000255
ENSG00000160013.4	PTGIR	prostaglandin I2 (prostacyclin) receptor (IP)	125.179	0.339715	9.40E-05	0.003597
ENSG00000174944.4	P2RY14	purinergic receptor P2Y14	121.692	-0.27023	0.000113	0.004052
ENSG00000163485.11	ADORA1	adenosine A1 receptor	99.5072	0.336926	0.000235	0.006606
ENSG00000180758.10	GPR157	G protein-coupled receptor 157	2073.03	-0.236199	0.000256	0.006935
ENSG00000180914.6	OXTR	oxytocin receptor	24.704	0.313895	0.000325	0.008026
ENSG00000069696.6	DRD4	dopamine receptor D4	37.6932	0.281554	0.000803	0.014629
ENSG00000184451.5	CCR10	chemokine (C-C motif) receptor 10	125.862	0.205065	0.000875	0.015433
ENSG00000149295.9	DRD2	dopamine receptor D2	37.423	0.296943	0.00133	0.020155
ENSG00000181408.2	UTS2R	urotensin 2 receptor	12.392	0.32372	0.00247	0.029881
ENSG00000178623.7	GPR35	G protein-coupled receptor 35	65.7146	0.197650	0.00286	0.032612
ENSG00000121764.7	HCRTR1	hypocretin receptor 1	13.093	-0.282355	0.00324	0.035264

Using a data set of all differentially expressed genes in human SKM from the GTEx database (7), we identified all GPCRs that are either up- or down-regulated in T2D SKM ($p<0.05$). GPCRs represented 15 of the >2,400 differentially expressed genes in T2D SKM. Of these 15 GPCRs, only three are primarily coupled to G_q-type G proteins (prostaglandin F receptor, oxytocin receptor, and urotensin 2 receptor).