Supplemental data

Inhibiting serotonin signaling through HTR2B in visceral adipose tissue improves obesity related insulin resistance

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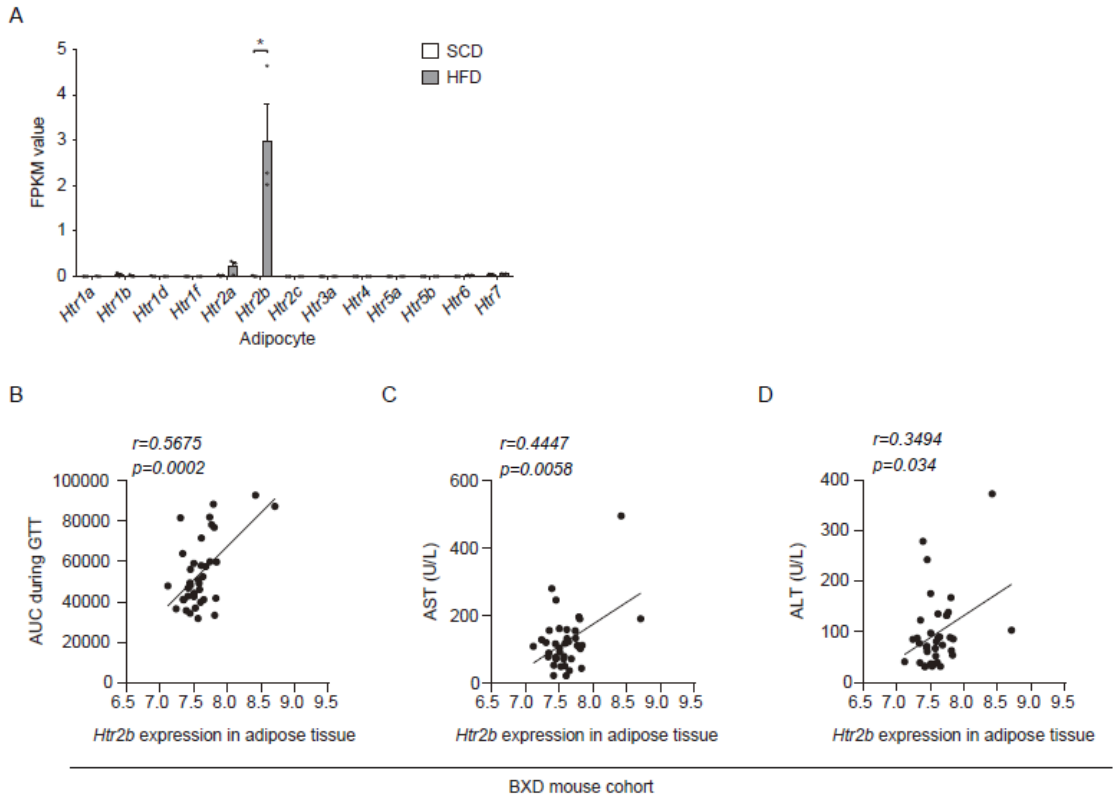
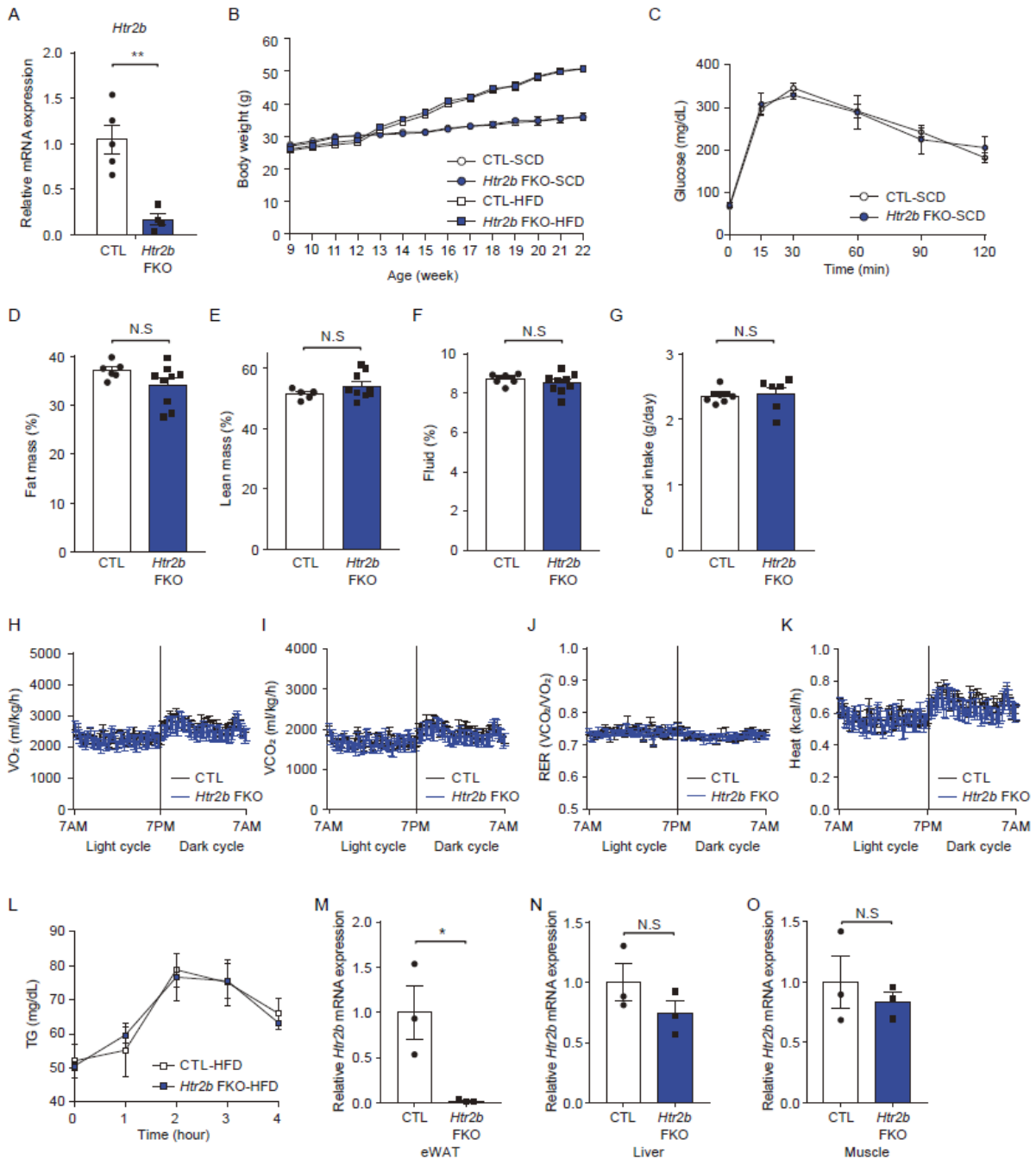
Supplemental Figure 1.



Supplemental Figure 1. *Htr2b* expression was increased in visceral adipose tissue upon HFD.

(A) FPKM values of *Htrs* in adipocytes isolated from eWAT of SCD- or HFD-fed 17-week-old C57BL/6J mice (n = 3/group), as assessed by RNA-seq (GEO accession number: GSE129665). (B–D) (Related to Figure 1G) Correlation of *Htr2b* expression in WAT with AUC during GTT in HFD (B), AST level in HFD (C), and ALT level in HFD (D). Pearson's r correlation coefficient with corresponding *p* values. Data were expressed as means ± SEM (*$P < 0.05$, Student's *t*-test [A]).

Supplemental Figure 2.

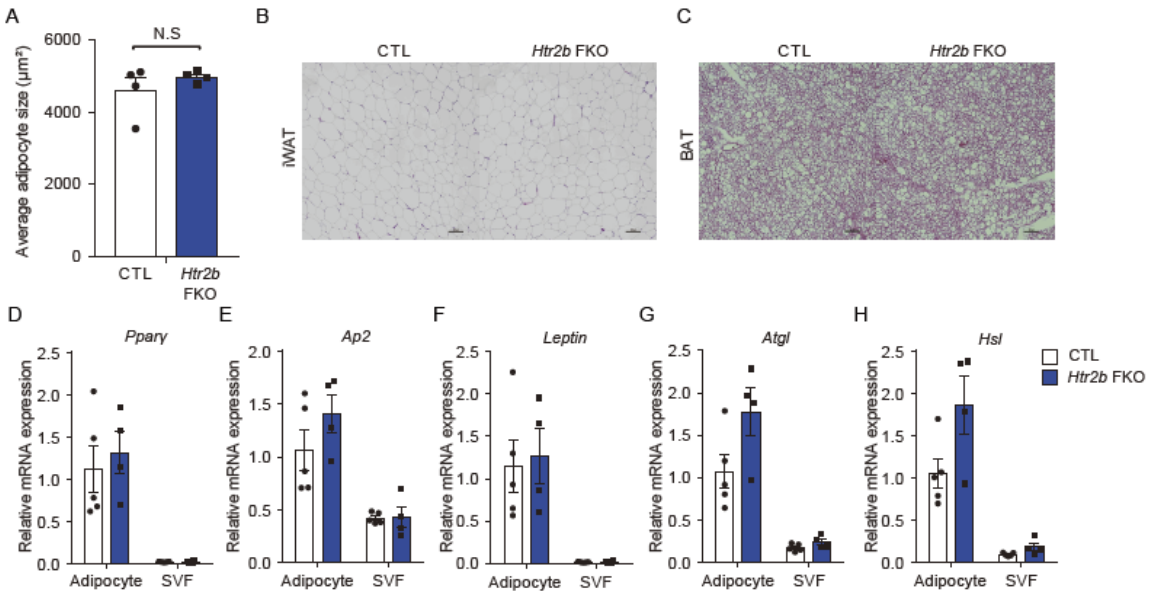


Supplemental Figure 2. Metabolic phenotypes of *Htr2b* FKO mice.

(A) *Htr2b* mRNA expression in adipocytes isolated from eWAT of HFD-fed control (n = 5) and *Htr2b* FKO (n = 4) mice, as assessed by qRT-PCR. (B and C) Twelve-week-old control and *Htr2b* FKO mice were fed a SCD or HFD for 10 weeks. (B) Body weight trends for control-SCD (n = 5), *Htr2b* FKO-SCD (n = 3), control-HFD (n = 11), and *Htr2b* FKO-HFD (n = 11)

mice. (C) Intraperitoneal glucose tolerance test (IPGTT) after a 16-hour fast in control-SCD (n = 9) and *Htr2b* FKO-SCD (n = 6) mice. (D–F) Percent fat body mass (D), lean body mass (E), and fluid mass (F) of HFD-fed control (n = 6) and *Htr2b* FKO (n = 9) mice. (G) Food intake of HFD-fed control and *Htr2b* FKO mice (n = 6/group for both). (H–K) Metabolic parameters of HFD-fed control and *Htr2b* FKO mice (n = 6/group for both). (L) Oral lipid tolerance test after 16-hour fast in HFD-fed control and *Htr2b* FKO mice (n = 7/group for both). (M–O) (Related to Figure 3, F–H) *Htr2b* mRNA expression in eWAT (M), liver (N), and skeletal muscle (O) of HFD-fed control and *Htr2b* FKO mice, as assessed by qRT-PCR (Control, n = 3; *Htr2b* FKO, n = 3). Data were expressed as means ± SEM ((*$P < 0.05$, **$P < 0.01$, Student's *t*-test [A–O]).

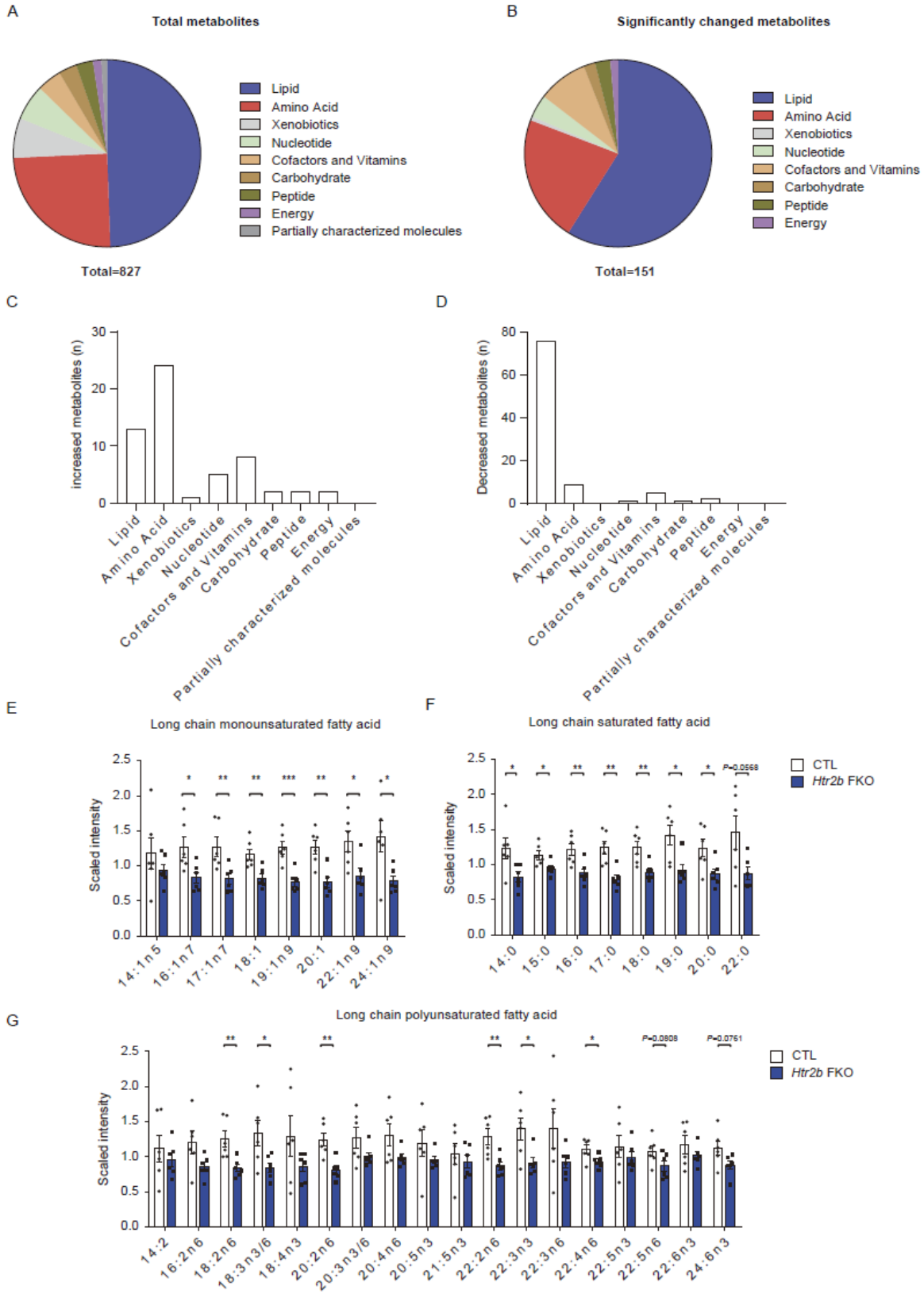
Supplemental Figure 3.

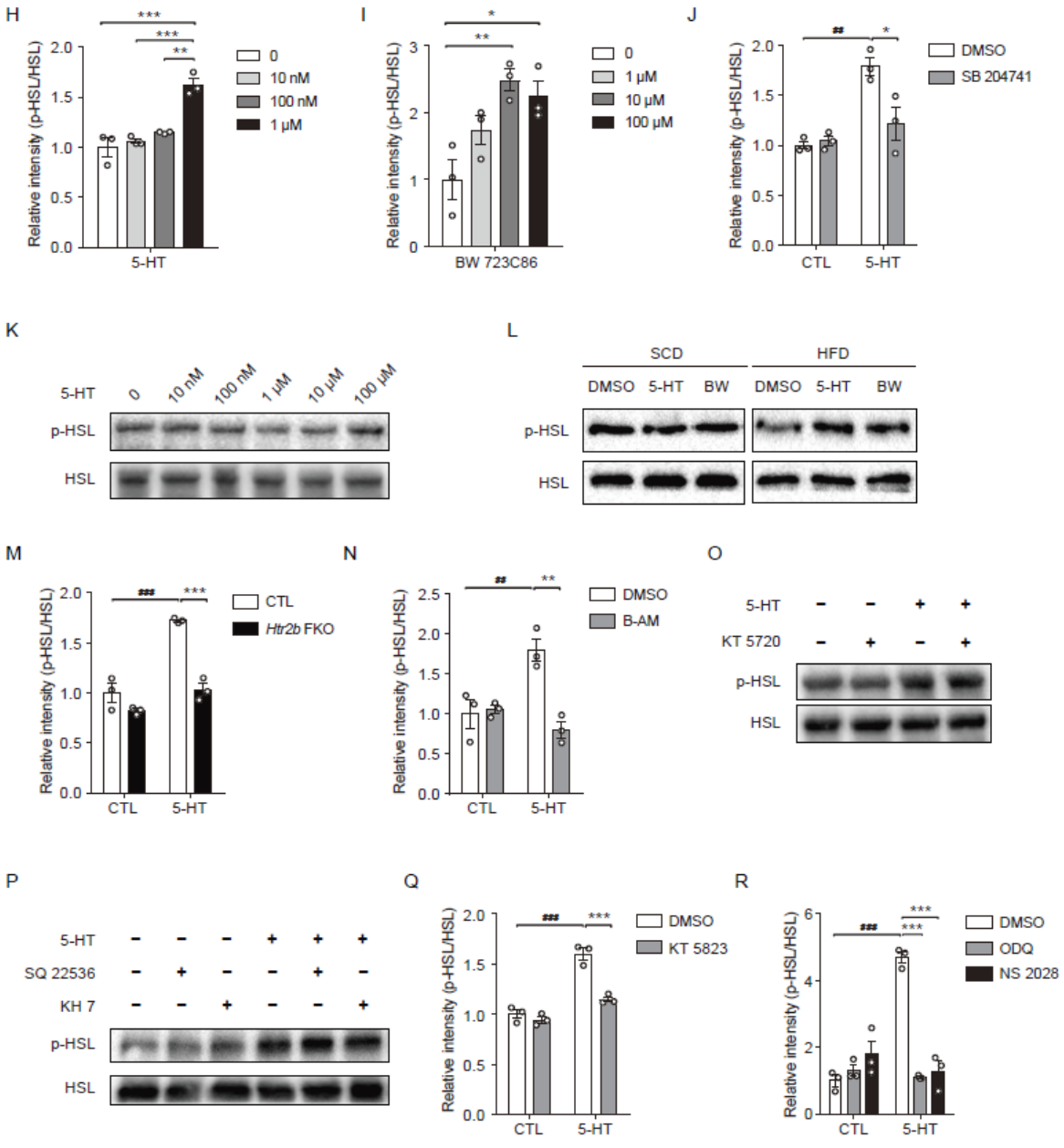


Supplemental Figure 3. Adipose tissue phenotypes of *Htr2b* FKO mice.

(A) Average adipocyte size in eWAT from HFD-fed control and *Htr2b* FKO mice (n = 4 mice/group for both, 5 images per mouse). (B and C) Representative histology of iWAT (B) and BAT (C) from HFD-fed control and *Htr2b* FKO mice, as assessed by H&E staining. Scale bars, 100 μm. (D–H) Relative mRNA expression of genes involved in adipogenesis (D–F) or lipolysis (G and H) in adipocytes and SVF isolated from eWAT, as assessed by qRT-PCR (Control, n = 5/group; *Htr2b* FKO, n = 4/group). Data were expressed as means ± SEM (Student's *t*-test [A, D–H]).

Supplemental Figure 4.



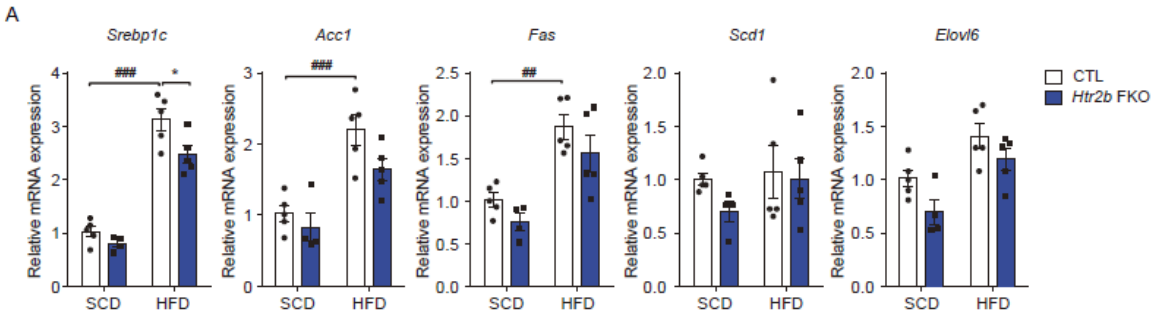


Supplemental Figure 4. Metabolomics analysis and underlying mechanism of adipose HTR2B.

(A–G) Twelve-week-old control and *Htr2b* FKO mice were fed a HFD for 10 weeks. Global metabolomics profiling was performed on plasma. (A and B) Pathway categories of the 827 detected metabolites (A) and 151 significantly changed metabolites (B). (C and D) Pathway categories of significantly increased (C) or decreased (D) metabolites. (E–G) Relative

abundance of long-chain monounsaturated FAs (E), long-chain saturated FAs (F), and long-chain polyunsaturated FAs (G) in plasma (n = 6/group for both). (H–J, M, N, Q, R) Quantification of relative pHSL/HSL ratio using adipocytes isolated from eWAT of HFD-fed mice. (H and I) (Related to Figure 5, C and D) Treatment with different concentrations of 5-HT (H) or BW 723C86 (I) for 15 minutes. (J) (Related to Figure 5E) Treatment with 1 μM 5-HT for 15 minutes, with or without pretreatment for 30 minutes with 10 μM SB 204741. (K) Western blot showing concentration-dependent 5-HT–induced changes in HSL phosphorylation after a 15-minute treatment of primary adipocytes isolated from iWAT of HFD-fed mice. (L) Western blot showing HSL phosphorylation in adipocytes isolated from eWAT of SCD- or HFD-fed mice after treatment with 1 μM 5-HT or 10 μM BW 723C6 for 15 minutes. (M) (Related to Figure 5G) Treatment with 4 μg/kg 5-HT (i.p.) for 15 minutes. (N) (Related to Figure 5H) Treatment with 1 μM 5-HT for 15 minutes, with pretreatment with 1 μM BAPTA-AM for 30 minutes. (O and P) Western blot showing HSL phosphorylation in primary adipocytes isolated from eWAT of HFD-fed mice after treatment with 1 μM 5-HT for 15 minutes, with 1 μM KT 5720 (PKA inhibitor) (O) or 10 μM SQ 22536 (AC inhibitor) and 10 μM KH 7 (AC inhibitor) (P) pretreatment for 30 minutes. (Q) (Related to Figure 5I) Treatment with 1 μM 5-HT for 15 minutes, with pretreatment with 1 μM KT 5823 for 30 minutes. (R) (Related to Figure 5J) Treatment with 1 μM 5-HT for 15 minutes, with pretreatment with 10 μM ODQ and 10 μM NS 2028 for 30 minutes. Data were expressed as means ± SEM (*$P < 0.05$, $^{\#\#}$, **$P < 0.01$, $^{\#\#\#}$, ***$P < 0.001$, Student's *t*-test [E–G], one-way ANOVA with *post-hoc* Tukey's test [H, I], or two-way ANOVA with *post-hoc* Tukey's test [J, M, N, Q, R]).

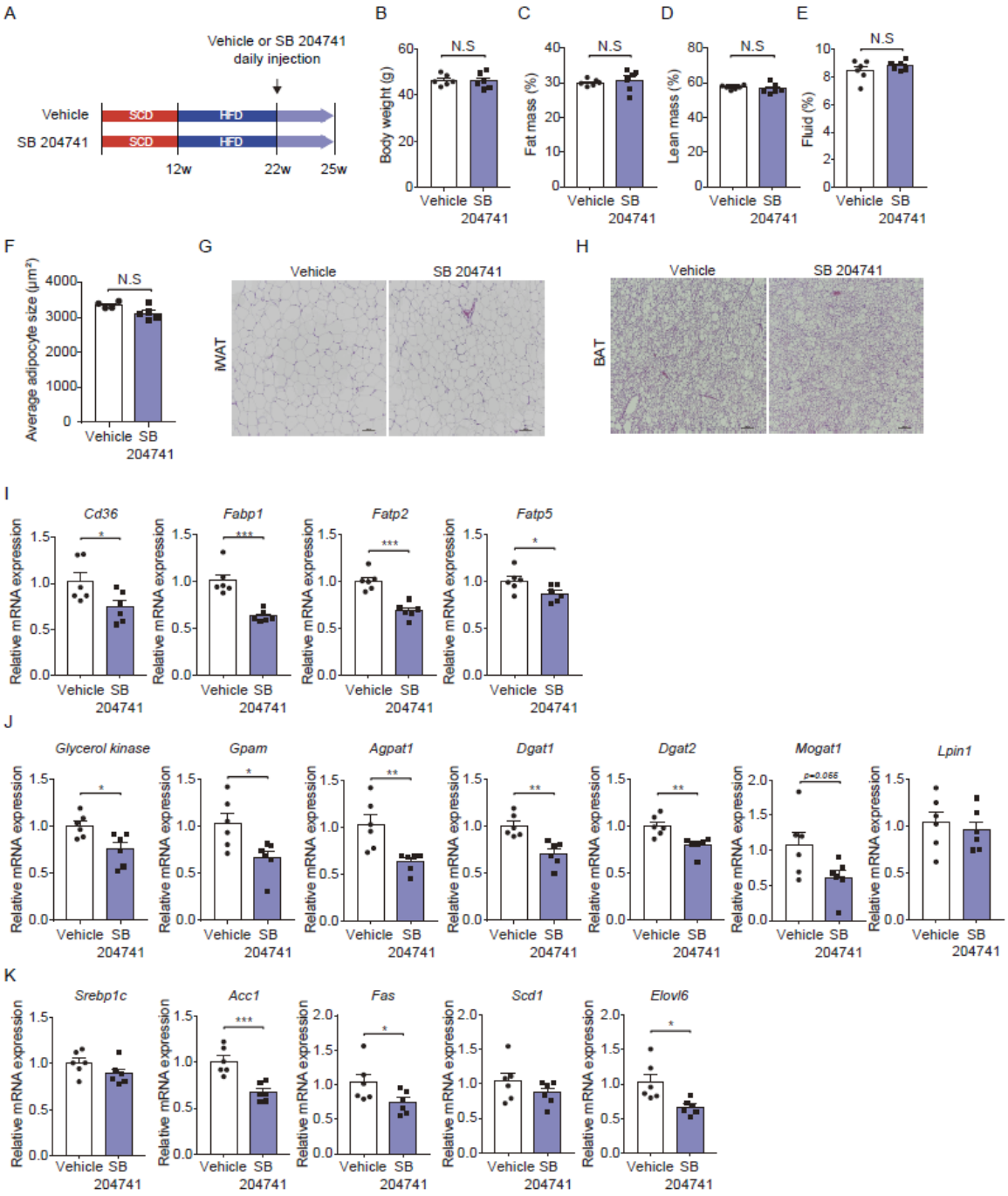
Supplemental Figure 5.



Supplemental Figure 5. Relative expression of mRNA for genes involved in *de novo* lipogenesis in the liver of *Htr2b* FKO mice.

Twelve-week-old control and *Htr2b* FKO mice were fed a SCD or HFD for 10 weeks. (A) Relative expression of mRNA for genes involved in *de novo* lipogenesis in liver. Data were expressed as means ± SEM ($^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, two-way ANOVA with *post-hoc* Tukey's test [A]).

Supplemental Figure 6.

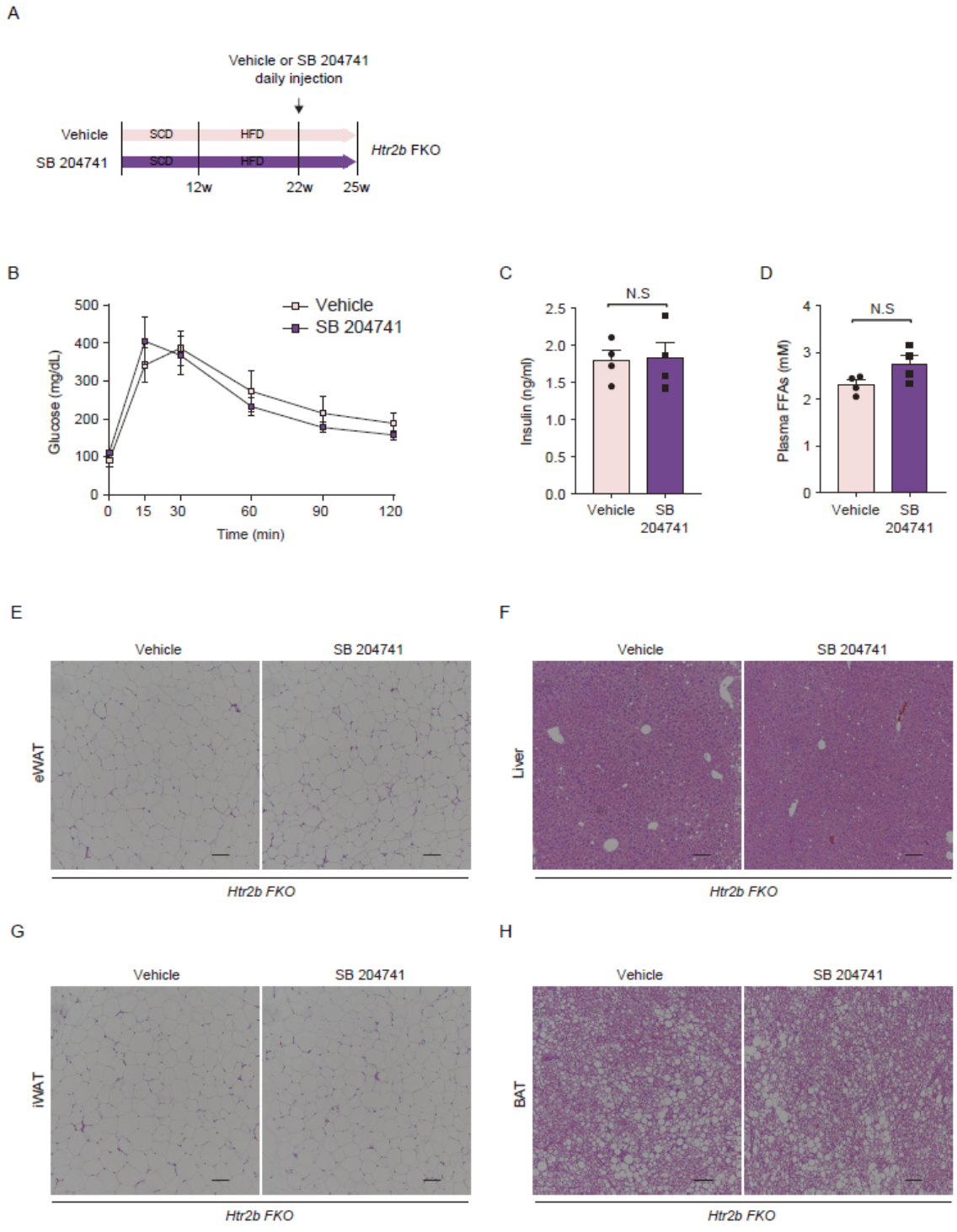


Supplemental Figure 6. Metabolic phenotypes of HTR2B antagonism on obese mice.

(A–K) Twelve-week-old mice were fed a HFD for 10 weeks and treated with vehicle or SB 204741 daily via intraperitoneal injection for 3 weeks while continuing HFD feeding. (A)

Experimental scheme. (B) Body weight (n = 6/group). (C–E) Percent fat body mass (C), lean body mass (D), and fluid mass (E) (n = 6/group). (F) Average adipocyte size in eWAT (Vehicle, n = 4; SB 204741, n = 5, 5 images per mouse). (G and H) Representative iWAT (G) and BAT (H) histology, as assessed by H&E staining. Scale bars, 100 μm. (I–K) Relative expression of mRNA for genes involved in FA uptake (I), TG synthesis (J) and *de novo* lipogenesis (K) in the liver, as assessed by qRT-PCR (n = 6/group). Data were expressed as means ± SEM (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, Student's *t*-test [B–F, I–K]).

Supplemental Figure 7.



Supplemental Figure 7. Protective effect of SB 204741 is adipocyte specific mechanism in metabolic dysfunction. (A) Experimental scheme. (B–H) Twelve-week-old *Htr2b* FKO mice

were fed a HFD for 10 weeks and treated with vehicle or SB 204741 daily via i.p. injection for 3 weeks while continuing HFD feeding. (B) IPGTTs of HFD-fed vehicle (n = 4) and SB 204741 (n = 5) treated *Htr2b* FKO mice. (C) Plasma insulin levels in HFD-fed *Htr2b* FKO mice treated with vehicle or SB 204741 (n = 4/group). (D) Plasma FFA levels (n = 4/group). (E–H) Representative histology of eWAT (E), liver (F), iWAT (G) and BAT (H) in HFD-fed *Htr2b* FKO mice treated with vehicle or SB 204741, as assessed by H&E staining. Scale bars, 100 μm. Data were expressed as means ± SEM (Student's *t*-test [B-D]).

Supplemental Table 1. Resource table.

Reagent or resource	Source	Identifier
Antibodies		
Anti-HSL	Cell Signaling	4107S
Anti-p-HSL (660)	Cell Signaling	4126S
Anti-AKT	Cell Signaling	9272S
Anti-AKT (473)	Cell Signaling	9271S
Anti-beta actin (HRP conjugation)	Cell Signaling	5125S
Anti-F4/80	Abcam	Ab16911
Anti-perilipin	Fitzgerald	20R-PP004
FITC-conjugated goat anti-rat antibody	Jackson ImmunoResearch	112-095-167
TRITC-conjugated goat anti-guinea pig antibody	Jackson ImmunoResearch	106-025-003
Anti-mouse IgG, HRP-linked antibody	Cell Signaling	7076P2
Anti-rabbit IgG, HRP-linked antibody	Cell Signaling	7074P2
Chemicals, Peptides and Recombinant proteins		
TRIzol reagent	Ambion	15596-018
Collagenase type II	Worthington	LS004176
BSA (western blot)	Sigma-Aldrich	1.08E+10
HBSS	Welgene	LB003-02
DMEM high glucose	Hyclone	SH30243.01
Penicillin/streptomycin	Hyclone	SV30010
Human insulin	Roche	11376497001
Isopropyl alcohol	Merck	1.09634.2511
Formalin	Sigma-Aldrich	HT501640
Calf serum	Gibco	16170-078
Fetal bovine serum	Gibco	16000-044
Reverse transcriptase	ABI	4368813
Triton X-100	Sigma-Aldrich	T8787
Paraformaldehyde	Sigma-Aldrich	158127
Goat or donkey serum	Jackson ImmunoResearch	005-000-121, 017-000-121
DAPI	Sigma-Aldrich	D9542
Glucose Injection 20% Daihan	Dai Han Pharm	645100954

NP-40	Sigma-Aldrich	74385
RIPA buffer	Pierce	89901
Protease and phosphatase inhibitors	Thermo	78440
BCA Protein Assay Kit	Pierce	23228
Polyvinylidene difluoride membrane	Millipore	IPVH00010
Glycerol reagent	Sigma-Aldrich	F6428
Fatty acid-free BSA	Roche	10775835001
Triglyceride Reagent	Sigma-Aldrich	T2449
HTR2B antagonist (SB 204741)	R&D Systems	1372
ODQ	Tocris	0880
NS 2028	Tocris	4517
Serotonin	Sigma-Aldrich	14927
KT 5823	Tocris	1289
BAPTA-AM	Sigma	A1076
SQ 22536	Tocris	1435
KH 7	Tocris	3834
KT 5720	Tocris	1288
HTR2B agonist (BW 723C86)	Tocris	1059
Teklad global 18% protein rodent diet (sterilizable)	ENVIGO	2018S
Rodent diet with 60 kcal% fat	Research Diets	D12492
Software		
Prism 7		
AdipoCount		
QuantStudio™ Software V1.2.4	Applied Biosystems	
Image Lab (Chemi-doc)	Bio-Rad	
Hyperinsulinemic-euglycemic Clamp		
Small animal one flow (O2) Anesthesia system	L.M.S. KOREA	L-PAS-01
Microdialysis Syringe Pump	Harvard Apparatus	CMA/102

Glucose analyzer	Analox Instruments	GM9
Liquid scintillation counter	Perkin Elmer	Tri-carb 4910
Vacuum oven	JEIO TECH	OV-11
Cold trap bath	JEIO TECH	CTB-10
Vacuum pump	WOOSUNG VACUUM	MVP6
Fiber optic illuminator	Olympus	LG-PS2
Heating pad	JEUNG DO BIO	JD-OT-06
2-Deoxy-D-[1-14C] glucose	Perkin Elmer	NEC-495-1
[3-3H] glucose	Perkin Elmer	NET-331C-3
Ultima Gold	Perkin Elmer	3013329
Humulin R	Lilly	HI-210
20% Dextrose	Daihan	
Isotonic sodium chloride	Daihan	
Poly-Prep prefilled chromatography columns	Bio-Rad	#7316211
Barium hydroxide solution	Sigma	B4059
Zinc sulfate solution	Sigma	Z2876
Ammonium acetate	Sigma	A1542
Formic acid	Sigma	695076
Heparin sodium	Hanlim Pharm	P31075-15
I-Fran liquid	Hana Pharm	200711400
Mouse Insulin ELISA	Merck Millipore	EZRMI-13K
1cc syringe	BD	REF301321
30G needle	Jungrim	JRN-30G
Silicone tubing	HelixMark	REF 60-011-01
Micro-Hematocrit Capillary Tubes Heparinized	Kimble	41B2501
Surgical sutures	B. Braun	C0762121
Gauze	Daehan Medical Systems	DHG-338B

Supplemental Table 2. qPCR primer sequence.

Mouse Gene	Forward	Reverse
Htr2a	CGTGTCCATGTTAACCATCCT	ACTGGGATTGGCATGGATATAC
Htr2b	ATTGCCCTCTTGACAATCATGT	GGAATAACCAGGCAGGACAC
36B4	GAGGAATCAGATGAGGATATGGGA	AAGCAGGCTGACTTGGTTGC
iNOS	CACCTTGGAGTTCACCCAGT	ACCACTCGTACTTGGGATGC
Lpl	GTGGCCGAGAGCGAGAAC	AAGAAGGAGTAGGTTTTATTTGTGGAA
Mogat1	TTGACCCATGGTGCCAGTTT	GTGGCAAGGCTACTCCCATT
Dgat1	GGATCTGAGGTGCCATCGTC	ATCAGCATCACCACACACCA
Dgat2	CATCATCGTGGTGGGAGGTG	TGGGAACCAGATCAGCTCCAT
Cd36	TGGCCAAGCTATTGCGACAT	ACACAGCGTAGATAGACCTGC
Glycerol kinase	TGAACCTGAGGATTTGTCAGC	CCATGTGGAGTAACGGATTTCG
TNFα	CTGAACTTCGGGGTGATCGG	GGCTTGTCACTCGAATTTTGAGA
IL-1β	TGCCACCTTTTGACAGTGAT	GATTTGAAGCTGGATGCTCT
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Adiponectin	TCCTGGAGAGAAGGGAGAGAAAG	CAGCTCCTGTCATTCCAACATC
Pparγ	TTTAAAAACAAGACTACCCTTTACTGAAATT	AGAGGTCCACAGAGCTGATTCC
Ap2	GCGTGGAATTCGATGAAATCA	CCCGCCATCTAGGGTTATGA
Leptin	ACACACGCAGTCGGTATCC	GCAGCACATTTTGGGAAGGC
Atgl	TAGGAGGAATGGCCTACTGAA	GGCTGCAATTGATCCTCCTCT
Hsl	GCAGTGGTGTGTAACTAGGAT	CGCTGAGGCTTTGATCTTGC
Srebp1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
Acc1	CAGTAACCTGGTGAAGCTGGA	GCCAGACATGCTGGATCTCAT
Fas	CCTGGACTCGCTCATGGGT	ATTTCCTGAAGTTTCCGCAGC
Scd1	CCTGCGGATCTTCCTTATCATT	GATCTCGGGCCCATTCG
Elovl6	CAGCAAAGCACCCGAACTA	AGGAGCACAGTGATGTGGTG
Fabp1	TGAAGGCAATAGGTCTGCCC	GTCATGGTCTCCAGTTCGCA
Fatp2	CTTCGGGAACCACAGGTCTTC	CATAGCAAGGCCTGTCCCATAC
Fatp5	TTCGAAAGAACCAACCCTTCCT	GCGTCGTACATTCGCAACAA

Gpam	CCACAGAGCTGGGAAAGGTT	GTGCCTTGTGTGCGTTTCAT
Agpat1	GCGCAATGTCGAGAACATGA	TCATTCCAAGCAGGTCGAGG
Lpin1	CATACAAAGGCAGCCACACG	CGGGGTTCAGTCCCTTGTAG
Human Gene	Forward	Reverse
HTR2A	AGCAAGCTTTGTGCAGTCTG	ACAAAGTTATCATCGGCGAGTAA
HTR2B	AACTCACGGGCTACAGCATT	GTGTGAAGAAGGCAGCCAGT
Cyclophilin	TCTGCACTGCCAAGACTGAG	TCGAGTTGTCCACAGTCAGC

Supplemental Table 3. Baseline characteristics of enrolled human subjects.

	Normal (N = 10)	Obese non-DM (N = 9)	Obese DM (N = 9)	P-value	
				Normal vs. non-DM	Normal vs. DM
Age	36.8 ± 13.02	33.22 ± 8.91	38.78 ± 12.27	0.6251	0.7382
BMI	21.91 ± 2.42	38.95 ± 4.46	41.97 ± 9.61	< 0.0001	< 0.0001
Glucose (mg/dL)	118.2 ± 19.98	95.89 ± 9.09	161.44 ± 59.98	0.0069	0.0456
HbA1c (%)		5.5 ± 0.35	8.41 ± 2.19	0.0012	
BUN	12.1 ± 3.21	13.89 ± 4.65	13.88 ± 2.42	0.3386	0.2140
Cr	1.12 ± 0.2	0.87 ± 0.31	0.83 ± 0.09	0.0468	0.0014
AST	25.4 ± 8.4	44.33 ± 20.76	42.89 ± 17.29	0.0165	0.0110
ALT	17.2 ± 7.84	95 ± 61.41	67.78 ± 22.29	0.0009	< 0.0001

DM, Diabetes mellitus; BMI, body mass index; HbA1c, glycated hemoglobin; BUN, blood urea nitrogen; Cr, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase.