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Supplementary Information for

Modulation of HIF-2α PAS-B domain contributes to physiological responses

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Supplementary Information Text

Materials and Methods

Reagents

PT2399 (1, 2) was provided by Peloton Therapeutics. The following antibodies were used: HIF-1α (Novus, NB100-123), HIF-2α (Novus, NB100-122), Goat Anti-Rabbit IgG (Jackson ImmunoResearch, 111-005-003), Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-005-003).

Animals

Mice expressing the *Epas1*^{S305M} Knock-In (KI) allele were generated by the UT Southwestern Transgenic Core Facility using CompoZr Custom Zinc Finger Nucleases (ZFN) designed by Sigma-Aldrich. The pronuclei of one-cell fertilized murine C57BL/6 eggs were injected with a cocktail containing mRNAs encoding two ZFNs and a DNA repair template oligonucleotide (5′-TGTGTGCTGCACACCACTCCTGCACCTCTCTTACCTCTGGCTCCTTTCTTCACTAGTGTGTACAAAGGGGCAGGTGGTAATGGGCCAGTACCGGATGCTAGCCAAACACGGAGGATATGTGTGGCTGGAGACCCAGGGGACG-3′) and the surviving eggs were transferred to the oviduct of pseudopregnant recipient females. DNA sequencing identified three founder mice, each containing one copy of the correctly repaired KI allele, used to establish independent lines. Mouse genomic DNA was isolated from tail biopsies following 20 min incubation at 100°C in buffer containing 25 mM NaOH and 0.2 mM EDTA followed by neutralization with an equal volume of 40 mM Tris-HCl. PCR (30 cycles at 98°C for 30 s, 62°C for 45 s, and 72°C for 90) was performed using the primer pairs to distinguish the *Epas1*^{S3045M} (oligo F; 5′- TACAAAGGGGCAGGTGGTAATG -3′ and oligo R; 5′-GCCGCTCATAGTCTTTCCAG -3′) and *Epas1* wild type (oligo F; 5′-AGGAGGTGAAGTCAGTAGGGTG-3′ and oligo R; 5′-TAGCATCCGGTACTGGCCAGA-3′) alleles.

Mice were housed at room temperature with a 12 h light-dark cycle and free access to food and water. Mice were fed either standard chow (10% fat; Research Diets, Inc; USA Cat # D12492) or a high fat diet (60% fat; Research Diets, Inc; USA Cat #

D12450J) beginning at 8 weeks of age. For the db/db obese mice model, 6-week-old male db/db mice with the C57BL/6J genetic background were purchased from the SLAC Laboratory Animal Co. Ltd (Shanghai, China) and housed under standard chow. After 2 weeks of acclimatization. Mice were administrated PT2399 for 4 weeks for measuring of body weight. PT2399 was suspended in 0.3% methylcellulose, 0.3% Tween 80, 30% PEG400, 10% ethanol by oral gavage as indicated. Measurements of mouse total body fat and lean mass were performed with the Bruker Minispec mq10 system (Bruker Corporation, Bilerica, MA). One week prior to initiation of PT2399 treatment, all mice were administered vehicle b.i.d. to acclimate them to the gavage procedure. To induce hypoxia, mice were housed with wet food in a dedicated chamber (25°C) in which O_2 was reduced in 3% decrements every 10 min to 9% O_2 for 6 h. Serum EPO levels were measured using the Mouse Erythropoietin Quantikine ELISA Kit (R&D Systems). All animal experiments were performed with the approval of the UTSW Institutional Animal Care and Use Committee.

Isolation of Adipose SVF and In Vitro Differentiation

Cells from the stromal vascular fraction (SVF) were isolated by collagenase digestion of minced inguinal WAT following published protocol (3). Cells were plated on collagen-coated dishes and cultured in growth medium (DMEM/F12 supplemented with 10% fetal bovine serum). After reaching 100% confluence for 48 h, cells were stimulated with an adipogenic cocktail (growth medium containing 5 μg/ml insulin, 1 μM dexamethasone, 0.5 mM isobutylmethyxanthine) for 48 h. Cells were maintained in growth medium containing 5 μg/ml insulin that was replaced every 48 h.

Immunoblotting

Protein extracts from cells or tissues were prepared with RIPA lysis buffer (Sigma, Cat # R0278). The lysates were centrifuged at 13,000 g for 6 min at 4°C. The supernatants were collected and protein concentrations were determined with a BCA Protein Assay kit (Pierce, Cat # 23225). Samples were resolved by SDS-PAGE. Immune complexes were detected by enhanced chemiluminescence followed by autoradiography and quantification by scanning densitometry.

HE staining

Tissues were dissected and fixed in 4% paraformaldehyde overnight. Paraffin processing, embedding, sectioning, and standard HE (Haematoxylin and Eosin) staining were performed by the UTSW Molecular Pathology Core Facility.

Systemic metabolic tests

Systemic metabolic tests in this study included OGTT, ITT, and lipid clearance study. Oral Glucose Tolerance Tests (OGGT) were performed by administration of 2 g/kg glucose by oral gavage following an overnight fast while Insulin Tolerance Tests (ITT) were performed by *i.p.* injection of 0.75 U/kg insulin (Sigma, Cat # I9278) following a 5 h of fast. Blood was collected from tail veins and glucose levels measured with test strips (Contour, Bayer Cat # 7099C). Lipid clearance studies were performed by administration 15 μl/g of a 20% Intra-lipid solution (Sigma, Cat # I141) by oral gavage following an overnight fast. Serum was separated from blood collected from tail veins following centrifugation at 6,000 rpm for 10 min. Triglyceride (TG) measurements were performed with a colorimetric kit (BioVision, Cat # K622) according to the manufacturer's protocol.

Serum and liver chemistry

Serum was prepared from whole blood samples of sacrificed mice by centrifugation at 6,000 rpm for 10 min. Samples were then analyzed for TG, free fatty acid (BioVision, Cat # K612), and alanine aminotransferase (ALT) activity (Sigma, Cat # MAK052) with colorimetric kits according to the manufacturer's protocols. Frozen liver tissues (~100 mg) were homogenized in 1 ml of 5% NP-40 then slowly heated to 100℃ for 5 min. Insoluble material was separated by centrifugation and the remaining supernatants were diluted 10 fold with water and assayed for total TG analysis (BioVision, Cat # K622).

Lipidomics analysis

Frozen pieces of fresh liver were isolated from WT and KI mice fed a HFD for 12 weeks. Mice were administered vehicle or PT2399 treatment for the final 8 weeks. Frozen samples (50-100 mg) were placed in 2 mL tubes containing 2.4 mm ceramic beads (Omni) and maintained in liquid N_2. Samples were homogenized in 1 mL of 2:1 CH_2Cl_2/MeOH using an Omni Bead-Ruptor 24 (Kennesaw, GA) and were transferred

into 16 × 100 mm glass tubes containing 2:1 DCM/MeOH to yield a final lipid concentration of 10 mg/mL. Lipids were isolated by Bligh-Dyer based liquid-liquid extraction using an automatic Hamilton STARlet Robot (Reno, NV). Lipidomic infusion-based MS/MS^{ALL} analysis was performed according to (4). The final values of identified individual lipids were reported as relative peak intensity among the total lipid intensity, allowing for a representation of the relative lipid composition within the total lipid pool that can be used to compare different samples with similar characteristics.

RNA extraction and real-time quantitative PCR (qPCR)

Total RNA was extracted from cells and homogenized tissues using Trizol (Invitrogen) and RNA Extraction Mini Kit (Bio-Rad, Hercules, CA) according to manufacturer's protocols and quantitated using a Nanodrop Spectrophotometer (Thermo Scientific). Reverse transcription was performed using the iScript cDNA Synthesis Kit (Bio-Rad). Synthesized cDNAs were amplified using the iTaq Universal SYBR Green Supermix and CFX384^{TM} Real-Time System (Bio-Rad). Relative expression levels were normalized to ribosomal protein S18 and were analyzed by the $2^{-\Delta\Delta CT}$ method using Bio-Rad CFX Manager 3.1. All primer sets were purchased from the Sigma validated primer database and are listed in *SI Appendix*, Table S1.

Statistical analysis

GraphPad Prism 5.0 (GraphPad, Inc., La Jolla, CA) was used to perform the statistical analysis. Student's *t*-test was performed for comparison of two independent groups. Two-way analysis of variance (ANOVA) was performed for comparison of two groups over time followed by Sidak's multiple test. Data are presented as Mean ± S.E.M. For all analyses, values of $p<0.05$ were considered significant.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary Figures

Fig. S1. Introduction of a HIF-2α PAS-B cavity-filling mutation (S305M) into the mouse *Epas1* locus. (**a**) Sequence of the *Epas1*^{S305M} (KI) allele. The WT murine *Epas1* exon 8 (capital letters) and flanking intronic sequences (lowercase letters) are shown. ZFN binding sites are underlined. The S305M mutation introduced in the KI allele is highlighted in green. Silent mutations to prevent cutting of the repaired KI allele by a ZFN are highlighted in red. (**b**) An *Epas1* sequence fragment from a mouse homozygous for the KI allele confirms the presence of these mutations.

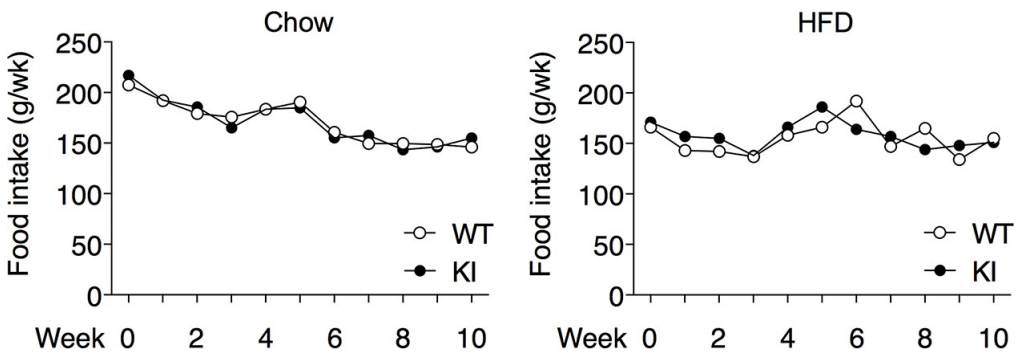

Fig. S2. WT and KI consume the same amount of food on when fed either standard chow or HFD. The total food intake was measured for mice in Fig 2a.

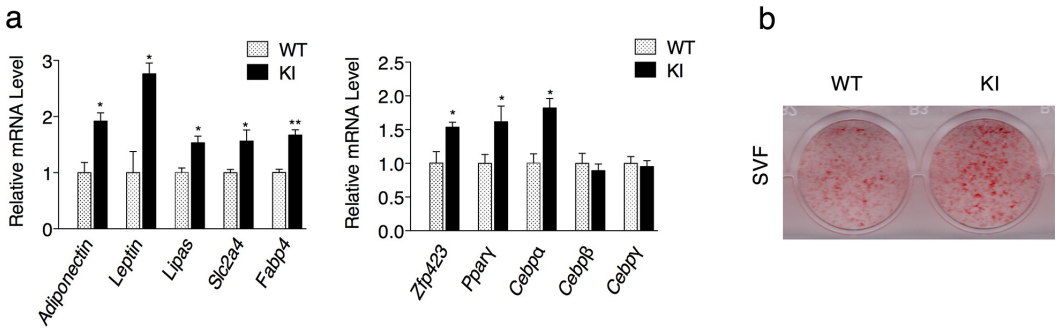

Fig. S3. SVF from KI WAT presents higher adipogenesis. (**a**) qPCR analysis and (**b**) Oil red staining following 10 days of differentiation of WT or KI SVF from iWAT into adipocytes. *p< 0.05

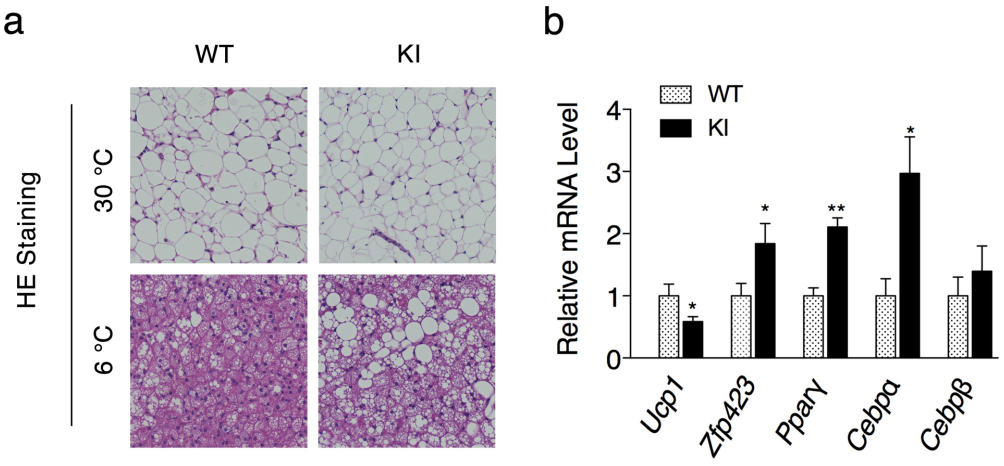

Fig. S4. KI mice retain a higher potential for adipogenesis than WT mice even when fed standard chow. Six weeks old WT or KI mice fed a standard chow diet were maintained for 1 week at 30 ℃ or 6 ℃ to induce the browning of fat. Following browning, iWAT from KI mice retains increased adipogenic potential as indicated by HE staining (**a**) and qPCR (**b**). *p< 0.05, **p< 0.01; n = 6/group

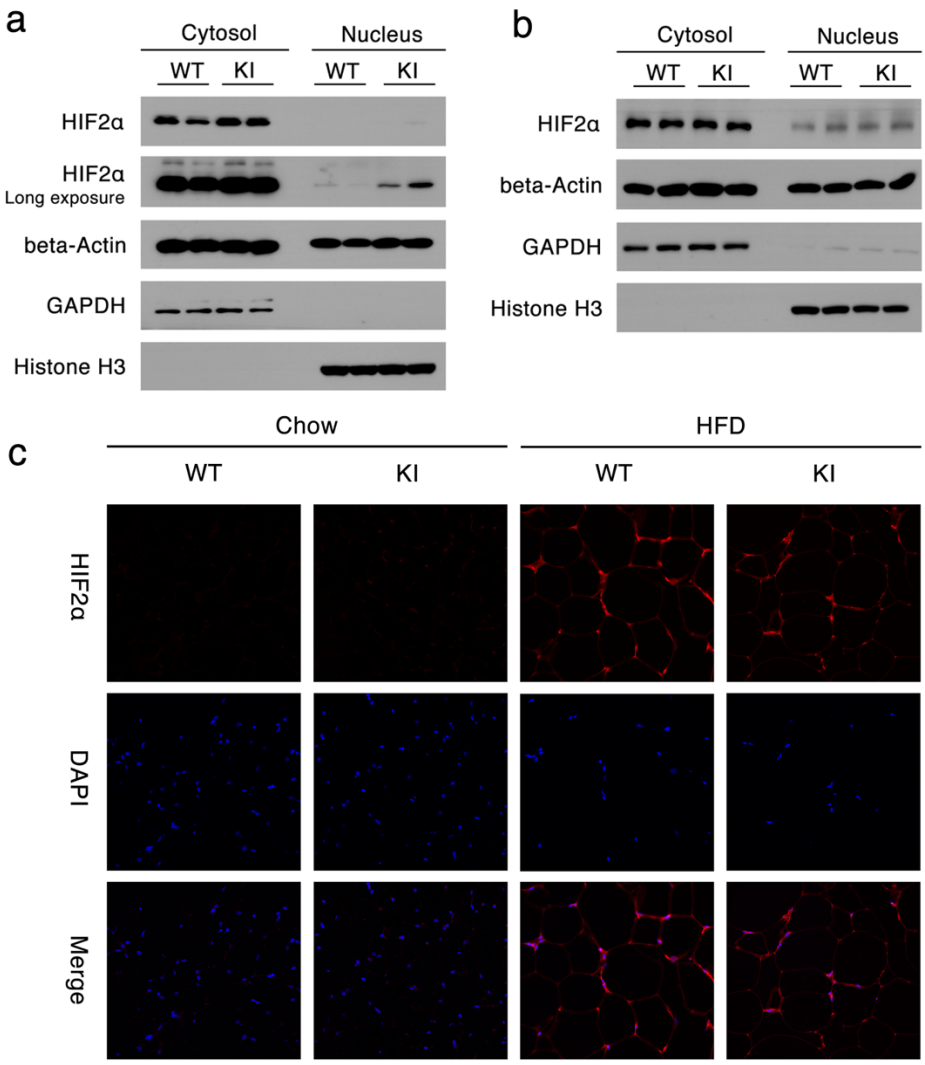

Fig. S5. KI mice present higher HIF-2 nuclear localization in adipose tissue. (**a**) Nucleus HIF-2α level in the adipose tissues of WT and KI mice under normal chow. (b) Nucleus HIF-2α level in the kidney tissues of WT and KI mice under normal chow. (**c**) Immunostaining of adipose HIF-2α from WT and KI mice under 8 weeks feeding of both normal chow and HFD.

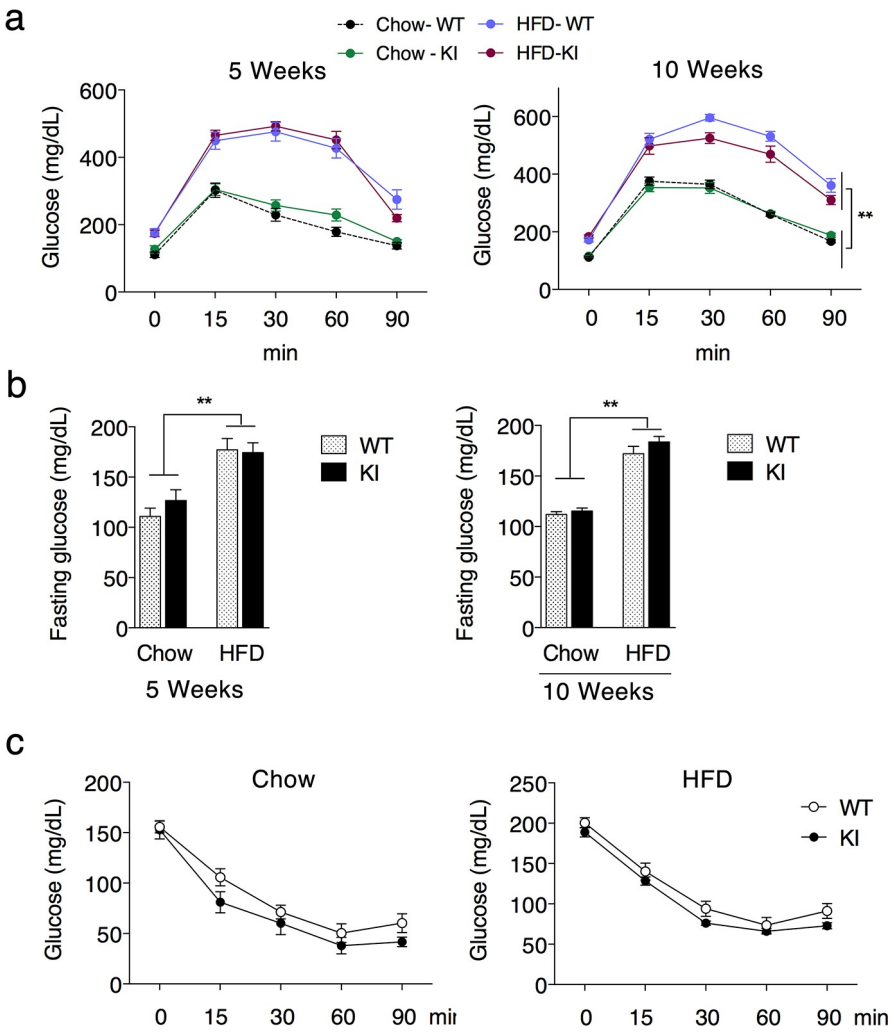

Fig. S6. There is no difference in glucose tolerance or insulin sensitivity in obese WT and KI mice. (**a**) Fasting glucose of WT and KI mice after 5 or 10 weeks of feeding on standard chow or HFD. (**b**) ITT of WT and KI mice after 9 weeks of feeding on standard chow or HFD. **p< 0.01; n = 10/group

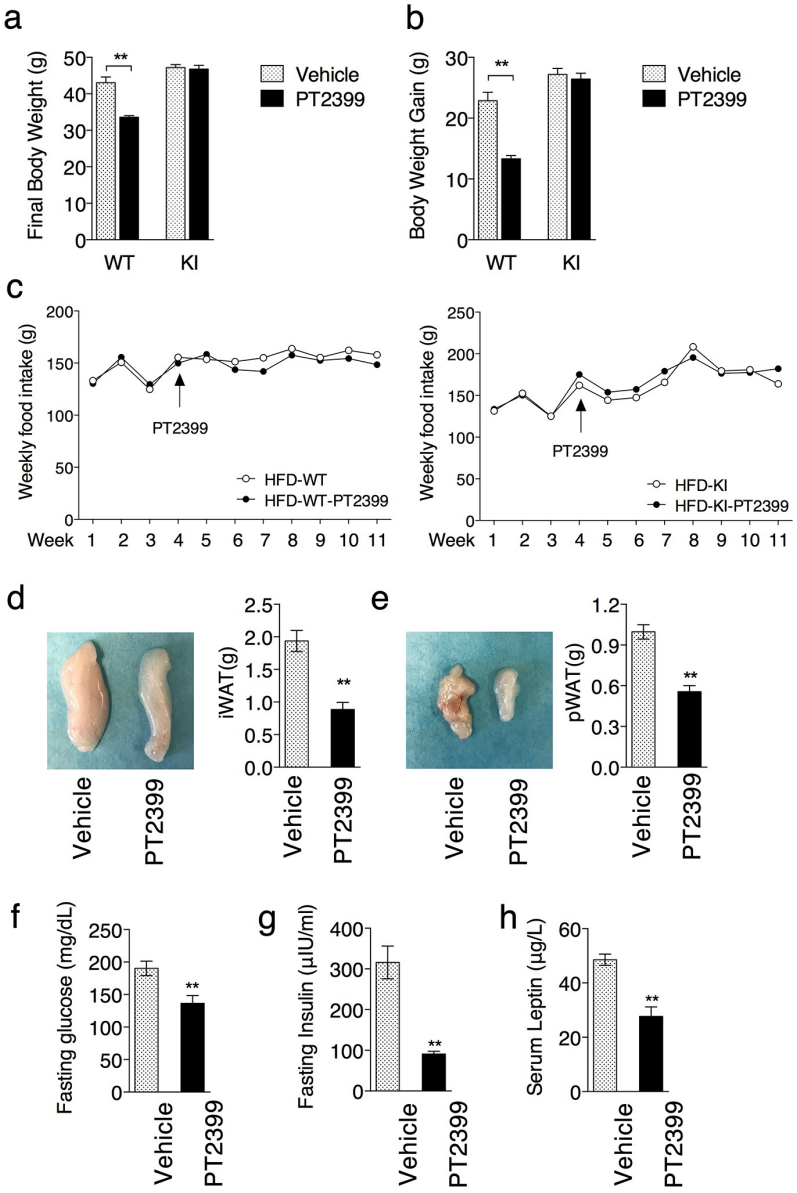

Fig. S7. PT2399 treatment ameliorates metabolic dysfunction in WT mice fed HFD. WT mice fed a HFD for 12 weeks beginning at 8 weeks of age were administered vehicle or 30 mg/kg PT2399 *b.i.d.* for the final 8 weeks. Measurements of (**a**) final body weight, (**b**) body weight gain, (**c**) total food intake (arrows indicate the initiation of PT2399 treatment), (**d**) iWAT mass, (**e**) pWAT mass, (**f**) fasting glucose, (**g**) fasting insulin, and (**h**) serum leptin were taken following treatment. **p< 0.01; n = 8/group

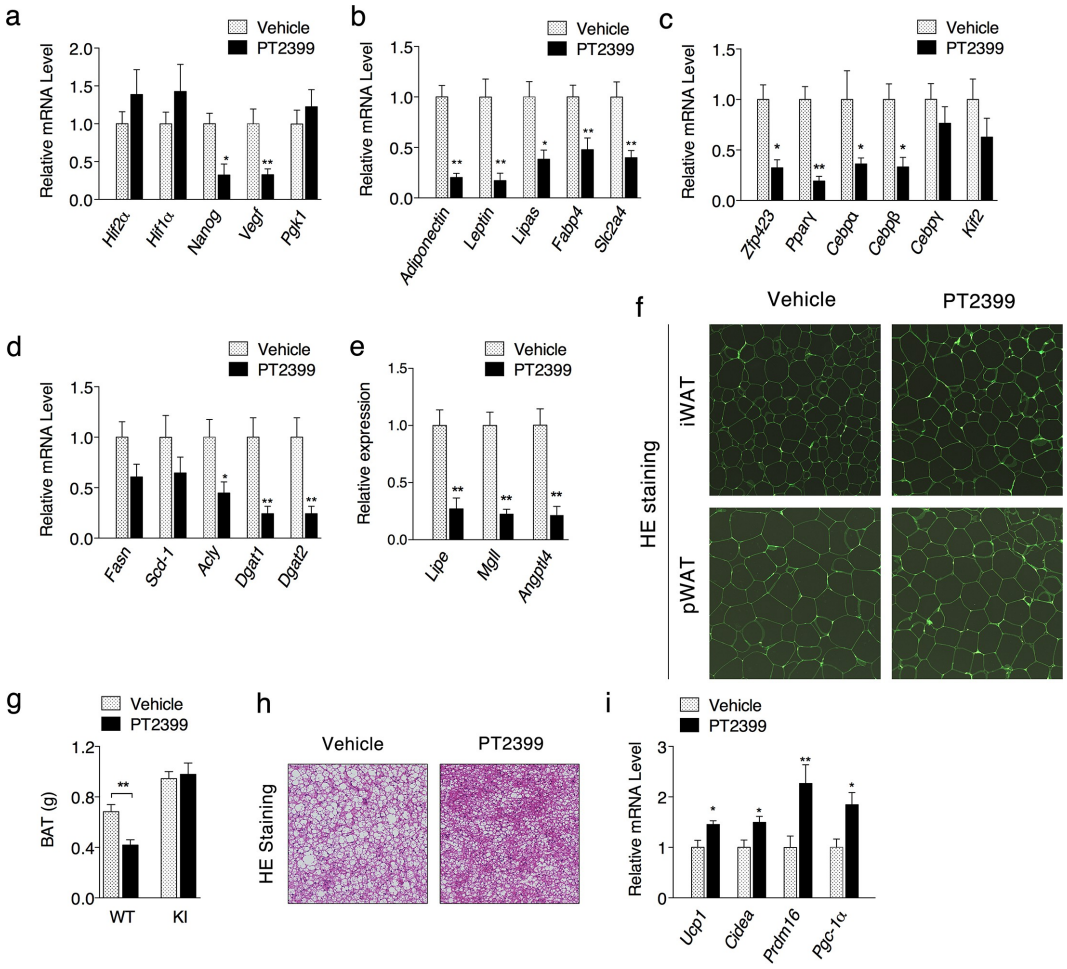

Fig. S8. HIF-2 inhibition suppresses adipogenesis *in vivo*. WT mice fed HFD for 12 weeks were administered vehicle or 30 mg/kg PT2399 *b.i.d.* for the final 8 weeks. mRNA expression levels of genes in iWAT encoding (**a**) hypoxia markers, (**b**) adipocyte markers, (**c**) adipogenesis promoting factors, (**d**) lipogenic enzymes, and (**e**) lipolysis enzymes were measured by qPCR. (**f**) HE staining of iWAT and pWAT. **(g)** Mass of BAT, (**b**) HE stains of BAT from WT mice, and (**c**) qPCR analysis of BAT specific genes from WT mice. *p< 0.05, **p< 0.01; n = 8/group.

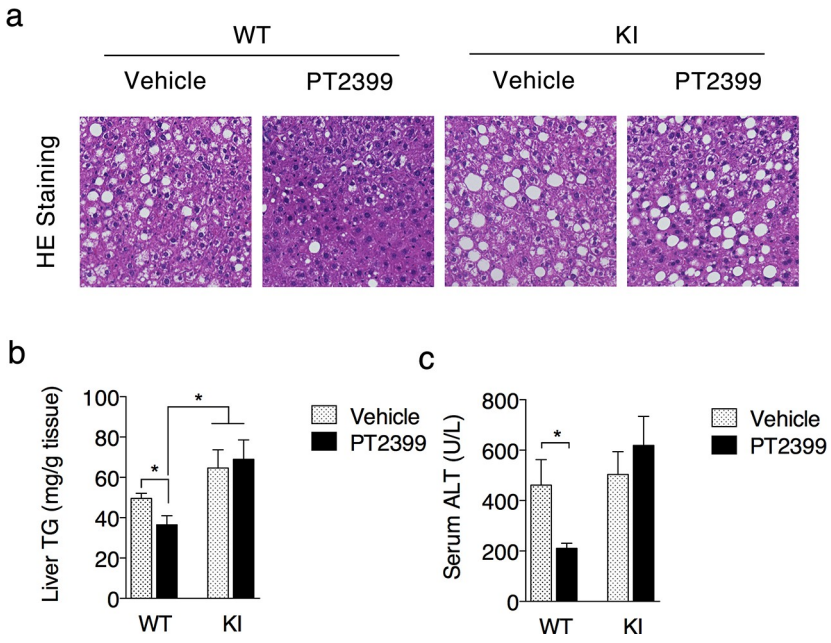

Fig. S9. PT2399 treatment reduces fatty liver only in obese WT mice. Obesity was induced in WT and KI mice fed HFD for 9 weeks. Mice were transitioned to standard chow diet for 1 week and then administered vehicle or 30 mg/kg PT2399 *b.i.d.* for 2 weeks. (**a**) HE staining of liver tissues from WT and KI mice. Liver TG levels (**b**) and serum ALT levels (**c**) from WT or KI mice. *p< 0.05, **p< 0.01; n = 7-8/group

Table S1. Lipidomics analysis of liver DAG and TAG levels in WT and KI mice fed on HFD for 12 weeks.

Name	Fold Change	*p* value	Mean WT	Mean KI
DAG|28:2|(NL14:1,14:1)[NH4]	0.646	0.1169	0.0057	0.0037
DAG|32:0|(NL-16:0,16:0)[NH4]	0.5375	0.0141	0.0053	0.0029
DAG|34:0|(NL-16:0,18:0)[NH4]	0.5993	0.0191	0.0043	0.0026
DAG|34:1|(NL-16:0,18:1)[NH4]	0.694	0.0781	0.0229	0.0159
DAG|34:2|(NL-18:2,16:0)[NH4]	0.5023	0.023	0.0286	0.0144
DAG|34:3|(NL-16:1,18:2)[NH4]	0.6157	0.0349	0.0053	0.0033
DAG|36:0|(NL-18:0,18:0)[NH4]	0.8717	0.5964	0.0042	0.0037
DAG|36:1|(NL-18:0,18:1)[NH4]	0.557	0.0866	0.0043	0.0024
DAG|36:2|(NL-18:1,18:1)[NH4]	0.3788	0.0199	0.0449	0.017
DAG|36:3|(NL-18:1,18:2)[NH4]	0.2785	0.0174	0.0295	0.0082
DAG|36:4|(NL-18:2,18:2)[NH4]	0.3035	0.0442	0.0163	0.0049
DAG|38:1|(NL-22:0,16:1)[NH4]	0.7551	0.295	0.0032	0.0024
DAG|38:5|(NL-20:4,18:1)[NH4]	0.5631	0.0356	0.0046	0.0026
DAG|38:6|(NL-20:4,18:2)[NH4]	0.6226	0.1274	0.0036	0.0023
DAG|40:1|(NL-24:0,16:1)[NH4]	0.7872	0.4926	0.0035	0.0028
DAG|40:7|(NL-22:6,18:1)[NH4]	0.5871	0.0088	0.0046	0.0027
TAG|48:0|(NL-16:0,,)[NH4]	1.5274	0.2076	0.0585	0.0894
TAG|48:1|(NL-16:0,,)[NH4]	1.5281	0.021	0.1252	0.1913
TAG|48:2|(NL-14:0,,)[NH4]	1.6	0.0236	0.0424	0.0679
TAG|48:3|(NL-18:2,,)[NH4]	1.6753	0.056	0.0102	0.0172
TAG|50:1|(NL-16:0,,)[NH4]	1.4195	0.0149	1.5831	2.2471
TAG|50:2|(NL-14:0,,)[NH4]	1.3908	0.0292	0.0851	0.1183
TAG|50:3|(NL-18:2,,)[NH4]	1.3127	0.0133	0.4457	0.585
TAG|50:4|(NL-18:3,,)[NH4]	1.2351	0.0113	0.0198	0.0245
TAG|51:1|(NL-17:0,,)[NH4]	1.2285	0.0851	0.0179	0.022
TAG|51:2|(NL-17:1,,)[NH4]	1.0516	0.544	0.055	0.0579
TAG|51:3|(NL-17:0,,)[NH4]	1.2452	0.0578	0.0044	0.0055
TAG|52:1|(NL-18:0,,)[NH4]	1.469	0.1534	0.1621	0.2382
TAG|52:2|(NL-18:0,,)[NH4]	1.3414	0.2978	0.1863	0.2499
TAG|52:3|(NL-20:1,,)[NH4]	1.2449	0.0817	0.0056	0.0069
TAG|52:4|(NL-14:0,,)[NH4]	1.2761	0.0664	0.0069	0.0088
TAG|52:5|(NL-14:0,,)[NH4]	1.3216	0.1196	0.0057	0.0076
TAG|53:2|(NL-19:0,,)[NH4]	1.0698	0.5268	0.0049	0.0053
TAG|53:3|(NL-17:1,,)[NH4]	1.1713	0.0573	0.0306	0.0358
TAG|54:0|(NL-16:0,,)[NH4]	0.549	0.0359	0.0072	0.0039
TAG|54:1|(NL-18:1,,)[NH4]	1.5956	0.2947	0.0162	0.0259
TAG|54:2|(NL-20:1,,)[NH4]	1.3052	0.0384	0.1731	0.2259
TAG|54:3|(NL-20:2,,)[NH4]	1.1914	0.0442	0.0991	0.1181
TAG|54:4|(NL-20:3,,)[NH4]	1.2364	0.0373	0.199	0.2461
TAG|54:5|(NL-20:2,,)[NH4]	1.3146	0.0495	0.0126	0.0166
TAG|54:6|(NL-14:0,,)[NH4]	1.028	0.8915	0.003	0.003
TAG|54:7|(NL-22:5,,)[NH4]	1.0487	0.789	0.0034	0.0036

TAG|56:2|(NL-18:0,,)[NH4]	1.0485	0.752	0.0122	0.0128
TAG|56:3|(NL-20:3,,)[NH4]	1.0338	0.8197	0.003	0.0031
TAG|56:4|(NL-20:2,,)[NH4]	1.3739	0.0433	0.034	0.0468
TAG|56:5|(NL-18:1,,)[NH4]	1.0404	0.7723	0.1625	0.1691
TAG|56:6|(NL-20:3,,)[NH4]	1.1309	0.1331	0.0605	0.0685
TAG|56:7|(NL-22:6,,)[NH4]	1.1391	0.2748	0.4166	0.4745
TAG|58:6|(NL-16:0,,)[NH4]	1.0306	0.86	0.0104	0.0107
TAG|58:7|(NL-16:0,,)[NH4]	1	0.9999	0.0138	0.0138
TAG|58:8|(NL-16:0,,)[NH4]	1.0513	0.6948	0.009	0.0095
TAG|58:9|(NL-18:2,,)[NH4]	0.7662	0.1421	0.0176	0.0135

Table S2. Lipidomics analysis of liver DAG and TAG levels in WT and KI mice fed on HFD for 12 weeks with vehicle or PT2399 treatment in the final 8 weeks.

WT vs. WT-PT

Name	Fold Change	*p* value	Mean WT	Mean WT-PT
DAG|28:2|(NL-14:1,14:1)[NH4]	1.709	0.0195	0.0057	0.0098
DAG|34:0|(NL-16:0,18:0)[NH4]	1.7366	0.0417	0.0043	0.0075
DAG|34:1|(NL-20:0,14:1)[NH4]	1.6203	0.0137	0.0033	0.0053
DAG|34:2|(NL-18:1,16:1)[NH4]	1.3806	0.0473	0.005	0.0069
DAG|36:0|(NL-18:0,18:0)[NH4]	2.35	0.0363	0.0042	0.0099
DAG|36:1|(NL-18:1,18:0)[NH4]	1.7489	0.0368	0.0036	0.0062
DAG|36:3|(NL-18:2,18:1)[NH4]	1.8628	0.0299	0.032	0.0597
DAG|36:4|(NL-18:2,18:2)[NH4]	2.3672	0.0472	0.0163	0.0385
DAG|38:1|(NL-22:0,16:1)[NH4]	5.1014	0.0461	0.0032	0.0162
DAG|38:6|(NL-20:4,18:2)[NH4]	2.661	0.0144	0.0036	0.0097
DAG|40:1|(NL-24:0,16:1)[NH4]	7.4227	0.067	0.0035	0.0261
DAG|40:7|(NL-22:6,18:1)[NH4]	1.9451	0.0282	0.0046	0.0089
TAG|48:0|(NL-16:0,,)[NH4]	0.846	0.0986	0.037	0.0313
TAG|48:1|(NL-16:1,,)[NH4]	0.5993	0.0711	0.0385	0.0231
TAG|48:2|(NL-16:1,,)[NH4]	0.5088	0.0789	0.0529	0.0269
TAG|48:3|(NL-16:1,,)[NH4]	0.7293	0.0473	0.0178	0.013
TAG|50:1|(NL-16:0,,)[NH4]	0.4928	0.014	1.5831	0.7801
TAG|50:2|(NL-18:1,,)[NH4]	0.4328	0.0097	0.7509	0.325
TAG|50:3|(NL-16:1,,)[NH4]	0.4175	0.0069	0.6241	0.2606
TAG|50:4|(NL-16:1,,)[NH4]	0.4862	0.053	0.1003	0.0488
TAG|51:1|(NL-17:0,,)[NH4]	0.7472	0.0302	0.0179	0.0134
TAG|51:2|(NL-17:1,,)[NH4]	0.4553	0.0034	0.055	0.025
TAG|51:3|(NL-17:1,,)[NH4]	0.5685	0.0069	0.0458	0.0261
TAG|52:1|(NL-20:1,,)[NH4]	0.6211	0.0372	0.0105	0.0065
TAG|52:2|(NL-16:0,,)[NH4]	0.4358	0.0027	6.2922	2.7423
TAG|52:3|(NL-16:1,,)[NH4]	0.4274	0.0361	0.753	0.3219
TAG|52:4|(NL-20:3,,)[NH4]	0.5283	0.0151	0.0163	0.0086
TAG|52:5|(NL-20:4,,)[NH4]	0.5798	0.0255	0.0264	0.0153
TAG|53:2|(NL-19:1,,)[NH4]	0.5947	0.0357	0.019	0.0113
TAG|53:3|(NL-17:1,,)[NH4]	0.7077	0.1976	0.0194	0.0137
TAG|54:0|(NL-16:0,,)[NH4]	0.6381	0.0903	0.0072	0.0046
TAG|54:1|(NL-20:0,,)[NH4]	0.5356	0.061	0.0446	0.0239
TAG|54:2|(NL-16:0,,)[NH4]	0.3958	0.0001	0.2053	0.0813
TAG|54:3|(NL-16:1,,)[NH4]	0.3949	0.0103	0.0372	0.0147
TAG|54:4|(NL-20:3,,)[NH4]	0.345	0.0005	0.199	0.0687
TAG|54:5|(NL-20:4,,)[NH4]	0.4704	0.0045	0.3661	0.1722
TAG|54:6|(NL-16:1,,)[NH4]	0.448	0.0012	0.0346	0.0155
TAG|54:7|(NL-16:1,,)[NH4]	0.5855	0.0289	0.0204	0.0119
TAG|56:2|(NL-22:1,,)[NH4]	0.3777	0.0276	0.016	0.0061
TAG|56:3|(NL-16:0,,)[NH4]	0.4562	0.0596	0.0202	0.0092
TAG|56:4|(NL-22:3,,)[NH4]	0.4557	0.0138	0.0134	0.0061

TAG|56:5|(NL-16:0,,)[NH4]	0.5173	0.0075	0.0582	0.0301
TAG|56:6|(NL-16:0,,)[NH4]	0.565	0.0124	0.1071	0.0605
TAG|56:7|(NL-22:4,,)[NH4]	0.6639	0.009	0.0103	0.0068
TAG|58:6|(NL-24:5,,)[NH4]	0.6679	0.0045	0.0114	0.0076
TAG|58:7|(NL-24:6,,)[NH4]	0.5697	0.0732	0.0147	0.0084
TAG|58:8|(NL-24:6,,)[NH4]	0.6962	0.0715	0.0152	0.0106
TAG|58:9|(NL-20:3,,)[NH4]	0.7478	0.3092	0.0074	0.0055

KI vs. KI-PT

Name	Fold Change	*p* value	Mean KI	Mean KI-PT
DAG|28:2|(NL14:1,14:1)[NH4]	1.0776	0.8216	0.004	0.004
DAG|34:0|(NL-16:0,18:0)[NH4]	0.9643	0.8951	0.003	0.003
DAG|34:1|(NL-20:0,14:1)[NH4]	1.0419	0.9096	0.016	0.0165
DAG|34:2|(NL-18:1,16:1)[NH4]	0.9311	0.6593	0.013	0.0121
DAG|36:0|(NL-18:0,18:0)[NH4]	0.8098	0.3317	0.004	0.003
DAG|36:1|(NL-18:1,18:0)[NH4]	1.004	0.9799	0.002	0.0024
DAG|36:3|(NL-18:2,18:1)[NH4]	0.8605	0.5233	0.008	0.0071
DAG|36:4|(NL-18:2,18:2)[NH4]	1.0371	0.7996	0.003	0.003
DAG|38:1|(NL-22:0,16:1)[NH4]	0.9798	0.9127	0.002	0.0023
DAG|38:6|(NL-20:4,18:2)[NH4]	1.0082	0.9646	0.002	0.0023
DAG|40:1|(NL-24:0,16:1)[NH4]	1.0975	0.7707	0.003	0.003
DAG|40:7|(NL-22:6,18:1)[NH4]	0.9823	0.9388	0.003	0.0026
TAG|48:0|(NL-16:0,,)[NH4]	0.9286	0.7836	0.004	0.0038
TAG|48:1|(NL-16:1,,)[NH4]	0.9714	0.8769	0.061	0.0594
TAG|48:2|(NL-16:1,,)[NH4]	0.9351	0.747	0.085	0.079
TAG|48:3|(NL-16:1,,)[NH4]	1.0244	0.8949	0.014	0.014
TAG|50:1|(NL-16:0,,)[NH4]	1.0272	0.8735	0.82	0.8427
TAG|50:2|(NL-18:1,,)[NH4]	0.9684	0.8527	0.118	0.1146
TAG|50:3|(NL-16:1,,)[NH4]	0.9781	0.9093	0.005	0.005
TAG|50:4|(NL-16:1,,)[NH4]	1.0257	0.8439	0.03	0.0307
TAG|51:1|(NL-17:0,,)[NH4]	0.9422	0.6313	0.022	0.0207
TAG|51:2|(NL-17:1,,)[NH4]	1.0199	0.8825	0.019	0.0194
TAG|51:3|(NL-17:1,,)[NH4]	1.158	0.4032	0.004	0.0041
TAG|52:1|(NL-20:1,,)[NH4]	0.9835	0.8491	0.01	0.0093
TAG|52:2|(NL-16:0,,)[NH4]	1.0019	0.9901	0.006	0.006
TAG|52:3|(NL-16:1,,)[NH4]	1.0243	0.8422	0.007	0.0071
TAG|52:4|(NL-20:3,,)[NH4]	0.9782	0.7884	0.027	0.0263
TAG|52:5|(NL-20:4,,)[NH4]	0.9649	0.8458	0.008	0.0073
TAG|53:2|(NL-19:1,,)[NH4]	1.0045	0.9457	0.036	0.0364
TAG|53:3|(NL-17:1,,)[NH4]	0.991	0.9505	0.036	0.0354
TAG|54:0|(NL-16:0,,)[NH4]	1.0021	0.994	0.004	0.004
TAG|54:1|(NL-20:0,,)[NH4]	0.8786	0.7174	0.037	0.0327
TAG|54:2|(NL-16:0,,)[NH4]	0.9028	0.3321	0.244	0.2203
TAG|54:3|(NL-16:1,,)[NH4]	1.0533	0.7175	0.263	0.2772
TAG|54:4|(NL-20:3,,)[NH4]	1.1725	0.1723	0.27	0.3163
TAG|54:5|(NL-20:4,,)[NH4]	1.0393	0.8404	0.013	0.0136

TAG|54:6|(NL-16:1,,)[NH4]	0.9571	0.6896	0.107	0.1021
TAG|54:7|(NL-16:1,,)[NH4]	1.1987	0.2407	0.003	0.004
TAG|56:2|(NL-22:1,,)[NH4]	0.8683	0.5819	0.014	0.0123
TAG|56:3|(NL-16:0,,)[NH4]	1.048	0.8063	0.013	0.0132
TAG|56:4|(NL-22:3,,)[NH4]	1.0927	0.4828	0.013	0.0136
TAG|56:5|(NL-16:0,,)[NH4]	1.1336	0.3594	0.054	0.0617
TAG|56:6|(NL-16:0,,)[NH4]	0.915	0.5629	0.007	0.0064
TAG|56:7|(NL-22:4,,)[NH4]	1.0778	0.6864	0.003	0.0031
TAG|58:6|(NL-24:5,,)[NH4]	1.0265	0.8646	0.006	0.0061
TAG|58:7|(NL-24:6,,)[NH4]	1.1082	0.3923	0.006	0.0071
TAG|58:8|(NL-24:6,,)[NH4]	1.0612	0.847	0.032	0.0338
TAG|58:9|(NL-20:3,,)[NH4]	0.9358	0.8089	0.003	0.0028

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