Supplemental Information

Loss of ABHD15 Impairs the Anti-lipolytic Action of Insulin by Altering PDE3B Stability and Contributes to Insulin Resistance

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Figure S1 related to Figure 1. ABHD15 expression profile and Abhd-15 ko mouse characterization
(A) ABHD15 expression in cardiac muscle (CM), skeletal muscle (SM), liver (Liv), pancreas (Pan), brown AT (BAT), eWAT, inguinal WAT (iWAT), dorsal WAT (dWAT), and mesenteric WAT (mWAT) of chow fed C57BL6/J mice and in 3T3-L1 adipocytes. One representative replicate is shown. (B) ABHD15 expression in murine 3T3-L1 cells during...
differentiation from day 0 to 7 (D0-D7). One representative replicate is shown. (C) ABHD15 expression in mature adipocytes and SVCs isolated from sWAT of 10 weeks old female C57BL6/J mice. SVCs were differentiated into adipocytes and harvested on day 7 (n = 3). (D-I) Body weight and body mass composition of mice at the age of 20 weeks on chow, HGD and HFD when fed ab libitum (n = 6-13). (J-R) Food intake was measured over 3 days, energy expenditure (EE), respiratory exchange ratio (RER) were measured over 3 days including overnight fasting and 6 hours refeeding period (except for HFD) (n = 6). D-I,J,M,P: Data are shown as mean ± SD. Unpaired two-tailed Student’s t test was performed.
Figure S2 related to Figure 2&3
Figure S2, related to Figure 2 and 3. ABHD15 associates with PDE3B and regulates its stability and expression

(A) Electroporation of 3T3-L1 cells on day 5 of differentiation with siRNA of non-targeting control or mix of siAbhd15-1 and siAbhd15-2; cells were harvested 72 hours after electroporation. (B-C) Protein quantification of ABHD15 and PDE3B depicted in (A) and normalized to BACTIN (n = 4). (D) Pull-down of ABHD15 and PDE3B from co-transfected BnlCl.2 hepatocytes. (E) Western blots of membrane fractions from 3T3-L1 adipocytes and eWAT. (F-G) Electroporation of 3T3-L1 cells on day 5 of differentiation with siRNA of non-targeting control or siPde3b; cells were harvested for mRNA expression 48 hours after electroporation and normalized to ß-actin (n = 3). (H) Electroporation of 3T3-L1 cells on day 5 of differentiation with siRNA of non-targeting control or siPde3b; cells were harvested for protein analysis 72 hours after electroporation. (I-J) Protein quantification of ABHD15 and PDE3B depicted in (H) and normalized to BACTIN (n = 8). (K) 72 hours after electroporation cells were serum starved for 6 hours, treated with or without 100nM insulin for 15 min and then harvested for western blotting. Arrow indicate the specific band of HSL. (L-K) Free fatty acids and glycerol release from cells 72 hours after electroporation; cells were incubated with 2% BSA medium for 3 hours (basal) or pre-treated with 100nM insulin in 2% BSA medium for 1 hour and incubated with insulin for 2 hours (n = 3).

B-C, F-G, I-J, L-K: Data are shown as mean ± SD. Unpaired two-tailed Student’s t test was performed, statistical significance is shown as *p < 0.05.
Figure S3 related to Figure 3&4
Figure S3, related to Figure 3 and 4. Abhd15-ko mice show disruption of insulin-mediated suppression of lipolysis

(A-B) Blood from overnight fasted and then saline or insulin injected mice (0.3 U/kg insulin) was used for lipid extraction and classes of FA determined using UPLC-MS (n = 4-5). (C) 44-week-old chow diet-fed mice were fasted overnight, then 0.3 U/kg insulin was injected, and plasma FA determined at indicated time points (n = 5). (D) cAMP levels measured from eWAT and sWAT of overnight fasted 20-week-old mice on chow diet (n = 4). (E-F) Overnight fasted mice (20-week-old on chow diet) were injected with saline or 0.6 U/kg insulin and tissues harvested 20 min later. (E) PKA substrate phosphorylation, (F) phospho-HSL and HSL expression in sWAT. (G) pHSL quantification of (F) normalized to total HSL (n = 6). (H) Free glycerol release from eWAT explants of saline or insulin injected mice. Explants were treated with 25 μM HSL inhibitor (iHSL) or 40 μM ATGL inhibitor (iATGL) for 1 hour (n = 4-7). (I) mRNA expression of genes involved in glucose and lipid metabolism in eWAT (n = 4-5). (J) Incorporation of 14C-oleic acid into TG of differentiated Abhd15-ko SVCs upon isoproterenol stimulation (n = 3).

A-D,G-J: Data are shown as mean ± SD. Unpaired two-tailed Student’s t test was performed; statistical significance is shown as *p < 0.05, **p < 0.01, ***p < 0.001.
Figure S4, related to Figure 4 and 5. Abhd15-ko mice show impaired insulin-dependent glucose metabolism and develop insulin resistance

(A) C/EBPa and ABHD15 expression in SVCs during differentiation. One representative replicate is shown. (B) Representative oil-red-O staining of fully differentiated SVCs (day 7), scale bar: 100 μm. (C) 14C-glucose incorporation into TAG and CE of fully differentiated SVCs treated with or without 100 nM insulin (n = 4). (D) Overnight fasted mice were injected with saline or 0.6 U/kg insulin and tissues were harvested 20 min later. sWAT was used for phospho-AKT (pAKT) and panAKT expression. (E) pAKT (S473) quantification of (D) normalized to panAKT (n = 6). (F) ITT of 13-week-old mice on chow diet with 0.3 U/kg insulin (n = 6-8). (G) GTT of 14-week-old mice on chow diet with 1.5 g/kg...
glucose (n = 6-7). (H) ITT of 20-week-old mice on HGD with 0.3 U/kg insulin (n = 6). (I) GTT of 21-week-old mice on HGD with 1.5 g/kg glucose (n = 6). (J) ITT of 20-week-old mice on HFD with 0.35 U/kg insulin (n = 4-6). (K) GTT of 21-week-old mice on HFD with 1.5 g/kg glucose (n = 7-8).

C, E-K: Data are shown as mean ± SD. Unpaired two-tailed Student’s t test or two-way ANOVA (multiple datasets) was performed; statistical significance is shown as **p < 0.01, ***p < 0.001.
Figure S5, related to Figure 6. AT-specific ablation of ABHD15 is responsible for the phenotype observed in global Abhd15-ko mice

(A-F) Abhd15 flox+/− and Abhd15 flox+/AdipoQ-cre mice on chow diet were used at the age of 20-22 weeks. (A) 20-week-old Abhd15 flox+/− and Abhd15 flox+/AdipoQ-cre were fasted overnight and injected with 0.3 U/kg insulin. FA levels were measured at indicated time points (n = 6-8). (B) Body mass composition of mice at indicated states (n = 5-7). (C-D) Food intake and energy expenditure (EE) were measured over 3 days, EE includes overnight fasting and 6 hours refeeding (n = 5-7). (E-F) Plasma insulin (E) and adiponectin (F) levels were measured after overnight fasting and then 1 hour refeeding.
(n = 5-7). (G-L) Abhd15 flox+/+ and Abhd15 flox+/+AdipoQ-cre mice on HGD were used at the age of 20-22 weeks (n = 3-5).

(G) Body weight. (H) Body mass composition. (I) Plasma insulin levels were measured after overnight fasting and then 1 hour of refeeding. (J) Energy expenditure was measured over 3 days including overnight fasting and 6 hours refeeding.

(K) Lipids metabolism genes and PDE3 expressions in eWAT of Abhd15 flox+/+ and Abhd15 flox+/+AdipoQ-cre mice. (L) PDE3B expression in eWAT of Abhd15 flox+/+ and Abhd15 flox+/+AdipoQ-cre mice. (M) Protein quantification of PDE3B depicted in (L) and normalized to βACTIN (n = 3).

A-C, E-I, L: Data are shown as mean ± SD. Unpaired two-tailed Student’s t test was performed; statistical significance is shown as *p < 0.05, **p < 0.01, ***p < 0.001.
| Measurement                              | fasted                         | refed                          |
|-----------------------------------------|--------------------------------|--------------------------------|
| Blood glucose (mg/dL)                   | WT 128 ± 10.3 Abhd15-k0 120.6 ± 17.3 p value 0.41 | WT 198.2 ± 18.9 Abhd15-k0 183.5 ± 35.4 p value 0.41 |
|                                        | High glucose diet 90.9 ± 11.5 90.4 ± 8.9 p value 0.95 | High glucose diet 194.3 ± 20.2 265.1 ± 32.7 p value 0.0004 |
|                                        | High fat diet 172.7 ± 14.8 171.4 ± 26.6 p value 0.93 | High fat diet 163.7 ± 24.5 171.2 ± 36.2 p value 0.72 |
| Plasma free fatty acids (mM)            | Chow diet 1.34 ± 0.18 1.24 ± 0.14 p value 0.33 | Chow diet 0.62 ± 0.15 0.79 ± 0.15 p value 0.08 |
|                                        | High glucose diet 1.00 ± 0.07 0.95 ± 0.09 p value 0.3 | High glucose diet 0.73 ± 0.16 0.68 ± 0.10 p value 0.58 |
|                                        | High fat diet 1.16 ± 0.14 1.04 ± 0.12 p value 0.21 | High fat diet 0.60 ± 0.12 0.67 ± 0.17 p value 0.5 |
| Plasma triacylglycerol (mM)             | Chow diet 2.01 ± 0.24 2.22 ± 0.36 p value 0.28 | Chow diet 1.8 ± 0.24 2.82 ± 0.58 p value 0.003 |
|                                        | High glucose diet 1.18 ± 0.42 0.80 ± 0.09 p value 0.07 | High glucose diet 0.79 ± 0.35 1.08 ± 0.44 p value 0.23 |
|                                        | High fat diet 1.55 ± 0.70 1.84 ± 0.30 p value 0.42 | High fat diet 0.90 ± 0.20 1.32 ± 0.26 p value 0.02 |

Table S1 (related to Figure 1). Blood parameters of global Abhd15-k0 compared to WT mice on chow, HGD and HFD. Blood was taken from 20 weeks old either overnight fasted mice (fasted) or 1 hour refeed mice after overnight fasting (refed) (n = 6-8). Data are shown as mean ± SD. Unpaired two-tailed Student’s t test was performed. P values are mentioned.
### Table S2 (related to Figure 4). mRNA expression of ABHD and PDE family members in diabetic versus glucose-tolerant obese women.

mRNA expression as fold-change (FC) and p values (p) in visceral AT from microarray data (GSE16415) comparing diabetic with glucose-tolerant obese women (n = 5).

|        | Member   | 1FC | 2p   | Member   | FC  | p   |
|--------|----------|-----|------|----------|-----|-----|
| **ABHD Family** |          |     |      |          |     |     |
|        | **down-regulated** |     |      | **up-regulated** |     |     |
|        | **Member** | 1FC | 2p   | **Member** | FC  | p   |
|        | ABHD1    | -2.02 | 0.067 | ABHD3    | 1.28 | 0.54 |
|        | ABHD5    | -3.03 | **0.0012** | ABHD4    | 1.14 | 0.61 |
|        | ABHD6    | -1.17 | 0.54  | ABHD8    | 1.09 | 0.71 |
|        | ABHD10   | -1.26 | 0.27  | ABHD11   | 1.01 | 0.94 |
|        | ABHD12B  | -2.14 | 0.081 | ABHD12   | 1.23 | 0.57 |
|        | ABHD13   | -1.27 | 0.42  | ABHD16A  | 1.46 | 0.084|
|        | ABHD14A  | -1.1  | 0.75  | ABHD17A  | 1.53 | 0.16 |
|        | ABHD14B  | -1.69 | 0.11  |          |      |     |
|        | **ABHD15** | -1.98 | **0.0376** |          |      |     |
|        | ABHD16B  | -1.08 | 0.74  |          |      |     |
|        | ABHD17B  | -1.09 | 0.78  |          |      |     |
| **PDE Family** |          |     |      |          |     |     |
|        | **Member** | FC  | p   | **Member** | FC  | p   |
|        | PDE2A    | -1.48 | 0.38  | PDE1A    | 1.72 | 0.21 |
|        | PDE3B    | **-4.19** | **0.001** | PDE3A    | 1.62 | 0.16 |
|        | PDE4C    | -1.54 | 0.08  | PDE4A    | 1.41 | 0.18 |
|        | PDE4D    | -1.82 | 0.98  | PDE4B    | 1.54 | 0.25 |
|        | PDE6A    | -3.03 | 0.67  | PDE5A    | 1.16 | 0.73 |
|        | PDE8A    | -1.38 | 0.28  | PDE7     | 1.63 | 0.23 |
|        | PDE8B    | **-3.55** | **0.013** | PDE10    | 1.12 | 0.78 |

1FC: fold change; 2p: p value
| Gene   | Forward                | Reverse                       |
|--------|------------------------|-------------------------------|
| Dgat1  | GACGGCTACTGGGATCTGA    | TCACCACACACCAATTCAGG          |
| Acsf1  | TCCTACAAAGAGGTGGCAGAACT | GGCTTGAACCCCCCTTCTGGAT        |
| Acot3  | GCTCAGTCACCCCTACGTTA   | AAGTTTCCGGCGATTTGGA           |
| Acot4  | ACATCCAAAGGTTAAAAGGCACA | TCCACTGAATGCAGAGCCATT         |
| Ldlr   | GCTTCATGTACTGGACAG     | CTGAAAGATCTAGTGGTA            |
| Abcg1  | CTCTATATCAGATACAGG     | CTCTGACTTCTGGGAAGTG           |
| Hmgcs1 | GTTCTATCCCCCTTGGTG     | GGTAAAGAGCTGTGGTA             |
| Gck    | CCGTGATCCGGAGAGAGA      | GGGAAACCTGACACGGGTAGAG        |
| Pck    | GGCCACAGCTGCTGCAG       | GGTCGCATGCGAAAGG             |
| Fas    | GCTGTAGCAGCTACCGTACG    | TCGTGCTCTCGTTCCAGGATC         |
| AceCS  | GCTGCCGACGGGATCGA       | TCCAGACACATTGAGCATGTCTAT      |
| AceCC  | TGACAGACTGCTGACAGAAGA   | TGGAGAGCCACACACACA            |
| Gpat   | GCGGAAAAACTACGTTACG     | TCTGACTCTGGCTTCTAAATATTCCT    |
| Srebp-1c | GGAGCCATGGATTGCACATT  | GCTTCCAGAGAGGAGCCAG           |
| Scd1   | ATCGCCTCTGGAGCCACAC     | ACACGTCATTCTGGAAACGC          |
| Acsf1  | TCCTACAAAGGTTGGCAGAACT | GGCTTGAACCCCCCTTCTGGAT        |
| Pde3b  | ATTCAATGGCAAAGCCAATG    | AATTGATGCACACCTGGCAG          |
| Pde3a  | GTCTGTGTCATCTAGTACGA   | GTGTCATCTGCTTCTGTTTG          |
| Abhd15 | TATGAACGTGGTGTTCTTGCT   | TTTGTGTGCACAGAAGGG            |
| ABHD15 | CCGTGTCGTGGCCTGGAGAGGTTG | GGCTGTGGCATACCTGTCAGGGCG     |
| TFIIβ  | TCAATAACTCGTCCCCTAACA  | GTCACATGTCGGAATCATCCA         |

Table S3 (related to all qRT-PCR data). Murine and human primer sequences used for qRT-PCR.
Supplemental Experimental Procedures

Reagents. All chemicals were obtained from Sigma-Aldrich unless otherwise stated. All cell culture reagents were purchased from Invitrogen. The following primary antibodies were used in this study: Anti-Flag and anti-βACTIN (Sigma), anti-HIS (GE Healthcare), anti-ABHD15 (ProteinTech). Anti-GAPDH, Anti-phospho-PKA substrate, anti-AKT, anti-phospho-AKT (Ser473), anti-HSL, anti-phospho-HSL (Ser660) antibodies were from Cell Signaling Technology. Anti-PDE3B antibody and pAcSG2-mPde3b plasmid were generous gifts from Dr. Vincent Manganiello (NHLBI, NIH) and Dr. Eva Degerman (Lund University). Abhd15-his and LacZ-his plasmids were constructed in our lab. Enhanced chemiluminescence (ECL) reagents were purchased from GE Healthcare. EDTA-free protease inhibitor cocktail (PIC) tablets, phosphatase inhibitor cocktail (PhosSTOP) tablets, Collagenase D and Dispase II were purchased from Roche Diagnostics.

Western blot analysis. Cell were lysed by scraping with RIPA buffer supplemented with PIC. Frozen tissues were minced and homogenized with an electrical homogenizer in RIPA buffer supplemented with PIC and PhosSTOP. Cell or tissue lysates were incubated on ice for 30 min, then centrifuged at 16,000 g, 4 °C for 30 min and the clean middle layer were collected and frozen at -20 °C until usage. Protein concentrations were determined with the BCA protein assay kit ( Pierce). Protein samples were diluted in sodium dodecyl sulfate (SDS) loading buffer and boiled for 10 min at 80 °C. Proteins were resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Individual proteins were detected with the specific antibodies and visualized on film using horseradish peroxidase-conjugated secondary antibodies (DAKO) and ECL reagents with the G:Box detection system (Syngene).

Cell culture. 3T3-L1 fibroblasts (American Type Culture Collection) were cultured and differentiated as described previously (Bogner-Strauss et al., 2010). Control non-targeting siRNA and siRNA directed against Abhd15 were purchased from Sigma (MISSION siRNA NM_026185). Control non-targeting siRNA (cat no. D-001810-10) and siRNA corresponding to murine Pde3b mRNA (cat no. L-043781-00) were purchased from (Dharmacon). 450,000 differentiated 3T3-L1 cells (5 days after differentiation start) were electroporated per 100 μL reaction with control siRNA or a mixture of siAbhd15 #1 and #2 (400 nM) using the Neon Transfection System (Invitrogen), at 1400 V, 20 ms, 2 pulse. Cells were harvested for lipolysis and western blotting 72 hours after electroporation. The cDNA of mouse Pde3b-flag was cut from pAcSG2-mPde3b plasmid with Xho1 and inserted into a murine stem cell virus vector (pMSCVpuro; BD Biosciences Clontech). The generation of Pde3b-flag recombinant retrovirus was described before (Walenta et al., 2013). Viral supernatants were supplemented with 8 μg/ml polybrene and added to 3T3-L1 cells (30 % confluence) for infection for 18-24 hours. Cells were selected with 3 μg/mL puromycin, expanded, and seeded for further experiments. If not otherwise stated, cells were used 7 days after induction of differentiation. Successful overexpression of PDE3B protein was confirmed by Western blot analysis. COS7 and Bnl Cl.2 cells (American Type Culture Collection) were maintained in DMEM (4.5 g/L glucose, glutamine) supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin at 37 °C, 5% CO2. For transfection, 200,000 cells per well were seeded into 12-well plates, the cells were transfected with 1 μg DNA together with 2 μL metafectene overnight in complete DMEM medium. 48 hours after transfection, cells were harvested for co-immunoprecipitation or treated with 5 μg/mL cycloheximide (CHX) for indicated times.

Primary cell culture. The isolation of adipocytes and stromal vascular cells (SVCs) was described previously (Aune et al., 2013) with the following modifications. 1g subcutaneous WAT from 8-10 weeks old, female mice was dissected, washed, minced, and digested in 1 mL PBS containing 0.125 U/mL Collagenase D, 2.4 U/mL Dispase II, 10 mM CaCl2 (added just prior to digestion of the tissue) at 37 °C with constant agitation at 180 rpm for 25-30 min. To stop digestion, complete DMEM/F12 media containing Glutamax (LifeTechnology), 10% FBS, P/S was added to the digestion mixture then filtered through a 100-μm cell strainer to remove undigested tissue. The flow-through was centrifuged for 10min at 200 g, the floating adipocyte layer was collected for protein isolation, while the left medium and cells was re-suspended and filtered through a 70-μm cell strainer. After centrifugation for 10 min at 700 g, the cell pellet containing the SVCs was resuspended in complete DMEM/F12 and seeded on a 10-cm cell culture dish. At a confluency of ~80%, cells were propagated and seeded for further experiment. To re-express Abhd15 in KO-SVCs, 50,000 cells in 12-well plates were subjected to either pMSCV-puro or pMSCV-Abhd15 retrovirus medium (prepared as described above). 48 hours after reaching confluency, adipocyte differentiation was induced by using complete DMEM/F12 media supplemented with 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine (IBMX), 5 μg/mL insulin, and 1 μM rosiglitazone. Three days after induction, medium was changed to complete DMEM/F12 supplemented with 5 μg/mL insulin for two days, afterwards cells were maintained in complete DMEM/F12 medium. On day 7, fully differentiated cells were harvested for Western Blotting.

Membrane isolation. Cells from 10-cm dishes were washed with ice-cold PBS, then scraped in 1 mL hypotonic lysis medium (HLM) containing 50 mM HEPES, 50 mM sucrose, 1 mM EDTA, 100 mM NaCl and 1 x PIC and were lysed using a Dounce homogenizer (~50 strokes). Around 50 mg frozen tissue were minced in 1 mL HLM, and thoroughly dounced. Lysates were centrifuged at 5000 g, 4 °C for 10 min. The supernatant was centrifuged at 100,000 g, 4 °C for 30 min. The supernatant was collected and frozen at -20 °C until usage. Protein concentrations were determined with the BCA protein assay kit (Pierce). Protein samples were diluted in sodium dodecyl sulfate (SDS) loading buffer and boiled for 10 min at 80 °C. Proteins were resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Individual proteins were detected with the specific antibodies and visualized on film using horseradish peroxidase-conjugated secondary antibodies (DAKO) and ECL reagents with the G:Box detection system (Syngene).
min. The resulting supernatant represented the cytosolic fraction; membrane pellets were resuspended in RIPA buffer with 1 x PIC for Western blotting.

Co-immunoprecipitation. Pde3b-Flag overexpressing cells in 35-mm dishes were washed twice with ice-cold PBS and then lysed in 1mL pulldown buffer (50 mM Tris-HCl pH 7.4-7.5; 300 mM NaCl; 1% Triton X-100; 1x PIC and PhosSTOP). Lysates were cleared from cell debris via centrifugation and protein content was measured by BCA as described above. 1 mg protein lysate was used for pulldown with Anti-FLAG M2 affinity gel (Sigma Aldrich) according to manufacturer’s guidelines. After overnight incubation, beads were washed thoroughly and affinity-bound proteins were eluted by boiling the samples with 2x SDS lysis buffer (100 mM Tris/HCl pH 6.8, 10% glycerol, 2.5% SDS, 1 x PIC). Pulldown-products were directly subjected to Western blot analysis.

Human study. Omental white adipose tissue (OWAT) samples from severely obese human subjects (BMI > 40 kg/m²; n = 11) were collected as part of previous trial of a collaborator group (Itariu et al., 2012). As described, anthropometric parameters and blood sampling for laboratory analysis were determined at inclusion. Patients underwent a 75 g standardized 2 hours oral glucose tolerance test (OGTT). Estimators of systemic insulin sensitivity and insulin resistance were calculated such as the oral glucose insulin sensitivity (OGIS) (Mari et al., 2001), the composite insulin sensitivity index (ISI) (Matsuda and DeFronzo, 1999), the clamp-like index (CLIX) (Anderwald et al., 2007), first and second phase response to glucose challenge, area under the curve (AUC) during OGTT and HOMA-IR (Matthews et al., 1985). The study was performed in accordance with the Helsinki Declaration of 1975 as revised in 1983 and with Good Clinical Practice guidelines and was approved by the Ethics Committee of the Medical University of Vienna and Göttinger Heiland Hospital (EK Nr. 963/2009, EK Nr. 488/2006 and E10-N01-01). All subjects provided written informed consent.

Animal study. We flanked exon 2 of the Abhd15 gene with 2 loxP sites and cloned the homologous regions into a targeting vector that was electroporated into 129 HM-1 embryonic stem (ES) cells. Homologous and Cre recombinant ES cells harboring the floxed allele were injected into C57BL/6 blastocysts, and chimeric males were tested for germ-line transmission (Figure 1A upper panel). Heterozygous floxed mice were bred with CMV-Cre mice (Su et al., 2002) and Adiponectin-Cre (Eguchi et al., 2011) mice to gain heterozygous total knock-out and adipose tissue specific knock-out mice (Figure 1A lower panel). Mice were backcrossed to the C57BL/6J background for at least 10 generations. Homozygous Abhd15-ko and Abhd15-flox+/AdipoQ-cre mice were fertile and were used for breeding. Mice were housed in groups of 2–4 in filter-top cages in a pathogen-free barrier facility. The animals were maintained in a 14 hours light/ 10 hours dark cycle, light on at 7:00 a.m., and had ad libitum access to food and water, except when food was restricted during fasting. The overnight fasting is around 12 to 14 hours during the dark cycle. At the age of 8-10 weeks, they were either fed a chow diet (calories 11 kJ% from fat, 53kJ% from carbohydrates, and 36 kJ% from protein, #V1126, Ssniff Spezialdiäten, Germany) or put, at the age of 8-10 weeks on HGD (calories 7 kJ% from fat, 72 kJ% from carbohydrates, and 21 kJ% from protein, #E15629-34, Ssniff Spezialdiäten, Germany) or on HFD (Sniff, Germany, #E15744-34, 45 kJ% calories from fat, 35 kJ% from carbohydrates, and 20 kJ% from protein) until experiments were done. Experiments were performed after 12 weeks on the according diet, or mice were maintained on diets until experiments were finished. If not otherwise stated, age matched male Abhd15-ko and wild-type (WT) mice, Abhd15-flox and Abhd15-flox+/AdipoQ-cre mice (except for SVC isolation we used female, 10 weeks old mice) were used for each experiments in this study (age and number of mice used are noted in figure legends). The study was approved by the institutional ethics committee and experiments were performed according to the guidelines of the Austrian Federal Ministry of Science and Research. Experiment licenses were granted under BMWF-68.205/0258-II/3b/2011, BMWF-66.007/0026-WF/V/3b/2015 and BMWF-66.007/0008-WF/V/3b/2016.

Microarray experiments and functional annotation. Male mice at age of 14 weeks on chow diet after overnight fasting and one hour of refeeding were harvested for microarray analysis. Total RNA was isolated from eWAT as described above. Two-hundred ng of total RNA were prepared for Affymetrix hybridizations on Mouse Gene 2.1 ST arrays. Raw array data was analyzed using the R package oligo, normalized using the robust multi-array average (RMA) method, and log10-transformed. Data was deposited in NCBI gene expression omnibus (GEO) with the accession number GSE98321. Probe sets were filtered for inter-quartile range (IQR) > 0.5 and Refseq annotation. Significantly differentially expressed genes were identified using the R package limma and p-values were adjusted for multiple testing according to the Benjamini-Hochberg method and considered if p<0.01 (FDR<0.1) and fold-change >1.5. Functional annotation (gene ontology biological process and KEGG pathways) were performed using DAVID (Huang et al., 2009). Heatmap was generated using Genesis (Sturn et al., 2002) based on gene-wise z-score of expression levels.

Gene expression analysis. Human ABHD15 mRNA expression raw data from the microarray with accession number GSE16415 published by Agarwal et al was analyzed with GEO2R online tool (Davis and Meltzer, 2007). As output, fold change and p-value were obtained. Tissue RNA was isolated with TRizol® reagent (Invitrogen) according to the manufacturer’s protocols. cDNA was generated using the cDNA Reverse Transcription Kit (Thermo Fisher Scientific). mRNA expression was assessed using real-time PCR using the StepOne Plus Detector system and SYBR Green PCR master mix (Invitrogen). Gene expression was normalized to TβIIIβ in murine tissues and βACTIN in human tissues. Relative
mRNA expression levels were calculated using averaged 2^−ΔΔCt values for each biological replicate (Livak and Schmittgen, 2001). Primers are listed in Table S3.

**Blood parameters.** Whole blood was taken from facial vein and blood glucose was measured with a glucose meter (Calla light, Wellion) from the tail vein. Plasma was collected after centrifugation at 1200 rpm, 4 °C for 10 min. Plasma triglycerides (TG) and fatty acids (FAs) levels were measured with Infinity™ Triglycerides kit (Thermo Fisher) and NEFA kit (WAKO). Plasma insulin levels were measured with the Mouse Ultrasensitive Insulin ELISA (Alpco Diagnostics, Salem, NH, USA) and the Adiponectin and Leptin with Mouse ELISA (Crystal Chem, Downers Grove, IL, USA) kits.

**In vivo and ex vivo lipolysis.** To determine in vivo lipolysis, circulating plasma FA levels were measured after overnight fasting (12-14 hours). Mice were then given an intraperitoneal (i.p.) injection of human insulin (Sigma) at a dose of 0.3 U/kg for HGD, 0.35 U/kg for HFD or an intragastric gavage of glucose at a dose of 2.5 g per kg body weight. During both procedures, mice were continuously fasted. Plasma FAs were measured after 15, 30, 60, 90 min post injection or gavage. To determine ex vivo lipolysis, the release of FA and free glycerol from AT explants was measured as previously described (Schweiger et al., 2014). For chow diet mice, overnight-fasted mice were injected intraperitoneally with 0.6 U/kg insulin or saline. Twenty minutes thereafter, mice were sacrificed and eWAT was excised. The fat pads were washed in prewarmed 2% BSA (FFA free)-DMEM medium (BSA medium). AT explants (20 mg) were incubated in 200 µL BSA medium in the presence or absence of 25 µM HSL inhibitor 76-0079 (NCN 0076-0000-0079, Novo Nordisk) or 40 µM Atglistatin (Schweiger et al., 2017) for 1 hour at 37 °C, 5% CO2 and 95% humidified atmosphere. After this pre-incubation, the AT explants were transferred into identical, fresh medium containing the appropriate lipolysis inhibitors and incubated for another hour at 37°C. For HFD mice, eWAT and sWAT were excised from overnight fasted mice. The fat pads were washed in prewarmed BSA medium. AT pieces (20 mg) were incubated in 200 µL BSA medium with 5 µM Triacsin C (Sigma) in the presence or absence of 100 nM insulin for 4 hours at 37 °C. Thereafter, the medium was removed and used to measure FA (NEFA Kit, WAKO) and glycerol (Free glycerol Kit, Sigma-Aldrich) release. For protein determination, AT explants were first incubated in 400 µL extraction solution (chloroform:methanol, 2:1) for 1 hour. Then the tissue pieces were transferred in 400 µL lysis solution (NaOH/SDS, 0.3N/0.1%) and incubated overnight at 56 °C under vigorous shaking. Protein content was determined using BCA reagent (Pierce) and BSA as standard.

**Histology and Oil red O staining.** H&E staining and Oil-Red-O staining was performed as previously described by us elsewhere (Pessentheiner et al., 2017).

**Glucose uptake and incorporation assay.** Mice were fasted overnight for 14-16 hours, glucose spiked with [3H]-deoxy-glucose was administrated by intragastric gavage (2.5 g/kg glucose, ~10 µCi per mouse). 20 min post-gavage, the mice were perfused with ice-cold PBS and tissue were collected and snap frozen until further processing. The accumulation of [3H]-deoxy-glucose-phosphate (deoxy-gluc-P) in different organs was determined as described (Schreiber et al., 2015). SVCs isolated from WT and Abhd15-ko mice were seeded in 12-well plates and used for glucose incorporation assay that was performed as described by us elsewhere (Pessentheiner et al., 2013).

**Insulin tolerance test (ITT) and glucose tolerance test (GTT).** Mice were fasted prior to GTT and ITT for 6 or 4 hours, respectively. 1.0-2.5 g/kg glucose or 0.25-0.5 U/kg human insulin were i.p. injected and blood glucose levels from tail vein were monitored after 15, 30, 60, 90, and 120 min. The respective dose of glucose or insulin was indicated in the figure legends.

**Targeted Lipidomic analysis.** Total plasma lipids (70 µL) were extracted twice according to Folch et al. (Folch et al., 1957) using chloroform/methanol/water (2/1/0.6, v/v/v) containing 500 pmol butylated hydroxytoluene, 1% acetic acid, and 100 pmol of internal standards (ISTD, 17:0 FA, Avanti Polar Lipids) per sample. Extraction was performed under constant shaking for 60 min at room temperature (RT). After centrifugation at 1,000 x g for 15 min at RT the lower organic phase was collected. 2.5 mL chloroform were added to the remaining aqueous phase and the second extraction was performed as described above. Combined organic phases of the double-extraction were dried under a stream of nitrogen and resolved in 200 µL methanol/2-propanol/water (6/3/1, v/v/v) for UPLC-TQ analysis. Chromatographic separation was modified after (Knittelfelder et al., 2014) using an AQUITY-UPLC system (Waters Corporation), equipped with a Kinex C18 column (2.1 x 50 mm, 1.7 µm; Phenomenex) starting a 20 min gradient with 100% solvent A (MeOH/H2O, 1/1, v/v; 10 mM ammonium acetate, 0.1% formic acid). A EVOQ Elite™ triple quadrupole mass spectrometer (Bruker) equipped with an ESI source was used for detection. FA species were analyzed by selected reaction monitoring (FA: [M-H]- to [M-H]-, 0eV). Data acquisition was done by MS Workstation (Bruker). Data were normalized for recovery and extraction- and ionization efficacy by calculating analyte/ISTD ratios.

**Body composition and indirect calorimetric measurements.** Body mass composition was assessed in non-anesthetized mice by using the time-domain NMR minispec (LIVE Mice Analyzer system, Model LF90II, Bruker Optik). For indirect calorimetric measurements, mice were individually housed in metabolic cages for 3-4 days at 20 to 22 °C on a 14 hours light/10 hours dark cycle starting at 7:00 am. Prior to the experiment, mice were adapted to the metabolic cages for 2 days.
Food intake, locomotor activity, oxygen consumption, and carbon dioxide production of animals were monitored by using a laboratory animal monitoring system (PhenoMaster, TSE Systems). Respiratory exchange ratio (RER, VCO₂/VO₂) was measured by the system, and energy expenditure (EE) was calculated from indirect calorimetry data using the formula: EE (kcal per h) = (15.818*VO₂/5.176*VCO₂)/4.1868/1.000 (Elia and Livesey, 1992). Mice were provided with drinking water ad libitum during the whole measurement, and were either fed ad libitum or overnight fasted and then refed with the diet mentioned in the figures.

**Reestertification assay.** Primary stromal vascular cells (SCVs) were isolated, seeded and differentiated as described in the Main Methods. On day 7 of differentiation, cells were pre-incubated with 5 μM DGAT1 and DGAT2 inhibitors (iDGATs, Sigma) for 30 min in complete media. Thereafter, cells were washed twice with PBS, thereafter lipolysis was induced by 10μM isoproterenol in media without serum or BSA. Thirty minutes upon induction of lipolysis, 0.5 μCi 14C-labeled oleic acid (Cat#:MC406, American Radiochemicals) were added to each well. Two hours upon induction of lipolysis, cells were washed with PBS and lipids were extracted twice with hexane/isopropanol (3:2, v/v). Lipid extracts were dried using N₂, re-suspended in chloroform, and one half of the extracted lipids was separated by thin layer chromatography (TLC) using hexane/diethylether/acetic acid (80:20:1) as mobile phase. TLC spots corresponding to triacylglycerol (TG) were cut out and the co-migrating radioactivity was determined by scintillation counting. Cells were lysed in NaOH/SDS (0.3N/0.1%) for 3 hours and protein content was determined by BCA protein assay using BSA as standard. Reestertification was analyzed as the incorporation of 14C-labeled oleic acids into TG and is expressed as cpm/mg cellular protein. Analyses of wild-type (WT) and knockout cells (Abhd15-ko) were run in parallel.

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