SUPPLEMENTARY INDEX

MATERIALS AND METHODS

Animals

As described previously (1), global HDAC11 KO mice on a C57BL/6J background were provided by Merck Research Laboratories. These mice were generated by a targeted deletion of floxed exon 3 of the HDAC11 gene utilizing Rosa26 promoter–driven Cre recombinase expression. Mice were maintained in a temperature- (22°C) and light-controlled facility with free access to water and standard rodent chow. All studies with HDAC11-KO or littermate WT mice were performed using 10-12 week-old male mice. Mice were sacrificed by isoflurane inhalation followed by cervical dislocation, and adipose tissues were harvested and subjected to pre-adipocyte isolation. For experiments with HDAC11-selective inhibitor, FT895, 8-week old C57BL/6J male mice (JAX stock #000664) were utilized. All animal studies were conducted using a protocol approved by the Institutional Animal Care and Use Committee of the University of Colorado Anschutz Medical Campus, following appropriate guidelines.

Human visceral adipose tissue acquisition and treatment

Human visceral adipose tissues (VAT) were acquired from laparoscopic surgeries under protocols approved by Colorado Multiple Institutional Review Board. All tissue used were de-identified prior to use in experiments. Patient characteristics are described in Table S3. Briefly, VAT explants were rinsed in pre-warmed DMEM and cut into fine pieces for culture on ThinCert™ Tissue Culture Inserts (Greiner Bio-One) submerged in DMEM. Explants were treated within 4h of harvest with FT895 (100 μM) for 12h. Vehicle (DMSO) treated explants were used as controls. Alkyne-tagged myristic acid (50μM, Cayman Chemical) was added to the explant culture 4h prior harvest. Tissues were flash frozen in liquid nitrogen and stored at -80^{o}C till further processing was performed for click assay and pulldown.

Cell culture

3T3-L1 preadipocytes purchased from ATCC (ATCC® CL-173™) were maintained and differentiated into mature adipocytes as per conventional protocol. Briefly, cells were seeded in 0.2% gelatin-coated tissue culture dishes and grown to confluency in DMEM supplemented with 10% newborn calf serum (NBCS; Gibco). Cells were then growth arrested in DMEM supplemented with 10% fetal bovine serum (FBS; GeminiBio) and 1% Penicillin-Streptomycin-L-Glutamine (PSG; Corning) for 48h prior to induction of differentiation. Differentiation was induced using a cocktail of 1µM dexamethasone (Sigma-Aldrich), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich), 1µg/mL insulin (Sigma-Aldrich) in 10% FBS-supplemented DMEM for 48h. Following this incubation, media was changed to adipocyte maintenance media (1µg/mL insulin, 10% FBS, 90% DMEM) and replenished every 48h for 8 days. At this time, 3T3-L1 adipocytes were treated as per experimental requirements. As described previously, HIB1B brown preadipocytes were maintained in DMEM with 10% FBS and differentiated into adipocytes by the addition of rosiglitazone (1 µM) for 4 days prior to experimental treatments (1). HEK293 cells were maintained in 10% FBS-supplemented DMEM and used at 60% confluency for all experiments. All cell culture was performed in a humidified cell culture incubator maintained at 37°C with 5% CO_2.

Mouse primary preadipocytes were isolated from stromal vascular fraction (SVF) of murine inguinal, epididymal and brown adipose depots (iWAT, eWAT and BAT) of 10 – 12 week old male C57BL/6J mice, based on an existing protocol (2). Briefly, adipose depots were dissected and washed in pre-warmed DMEM. Each adipose depot was transferred to separate petri dishes containing 10 mL of 0.2 % (w/v) collagenase II (Worthington), cut into approx. 1mm pieces with then further minced to allow opening up of the tissue using spring scissors (Fine Science Tools). This was then transferred 15 mL Falcon tubes with the lid loosely fastened and incubated in a shaker at 37 °C at 400rpm for 20 mins (iWAT and eWAT) or 30 mins (BAT), with vortexing every 5 min to prevent settling of the contents at the bottom of the tube. At the end of digestion, the tissue slurry was passed through a 70µm cell strainer into a 50 mL Falcon tube, followed by 30 mL preadipocyte media (5% NBCS, 5% FBS,1% PSG) to stop the enzymatic activity. After a brief (2-3 min) centrifugation at 200g at room temperature, the upper floating adipocyte-containing layer was discarded. The cell suspension was then centrifuged at 2000g for 5 min

at room temperature to pellet the SVF-derived preadipocytes. The supernatant was removed and the remaining cell pellet resuspended in pre-warmed preadipocyte media. Cells were plated directly onto 6-well cell culture plates (VWR) or onto 25 mm glass coverslips pre-coated with 2% gelatin (Sigma) in molecular biology grade water. Once 90 - 100 % preadipocyte confluency was reached, differentiation was induced by incubation with differentiation induction medium (DMEM, 10% FBS, 0.5% PSG, 1 µg/mL insulin, 0.5 µM dexamethasone, 0.25 mM 3-isobutyl-1- IBMX, 1 um rosiglitazone and 2 nM T3 for iBAT) for 72 hr. On day 3, induction medium was replaced with maintenance media (DMEM, 10 % FBS, 0.5% PSG, 1 ug/mL insulin, and 2 nM T3 for iBAT). All reagents other than DMEM were purchased from Sigma Aldrich. Maintenance media was replenished every 48hr and maturation followed by observing lipid droplet coverage by brightfield microscopy (EVOS FL, Life technologies), with 80 -90 % lipid droplet coverage considered fully differentiated.

Human subcutaneous adipose tissue samples were acquired from healthy volunteers as described previously (3) under protocols approved by Colorado Multiple Institutional Review Board (NCT02654925). Isolation of primary human preadipocytes from the stromal vascular fraction (SVF) has been described previously (4). Patient characteristics are described in Table S3. SVF-derived pre-adipocytes were isolated and plated onto 6-well tissue culture dishes in growth media (MEM α, 10% FBS, 0.1X Penicillin-Streptomycin). Once SVF-derived preadipocytes reached 90% confluency, media was changed to complete differentiation induction media for up to seven days, with media refreshed every 48 hr (DMEM, PSG, 33µM d-Biotin, 17µM pantothenate, 100nM dexamethasone, 1µM rosiglitasone, 0.5mM IBMX, 2nM T3, 10 µg/mL transferrin, 100nM insulin). All reagents other than DMEM were purchased from Sigma Aldrich. Following seven days, complete differentiation medium was switched to maintenance media (DMEM, PSG, 33µM d-Biotin, 17µM pantothenate, 10nM dexamethasone, 100 nM insulin). Differentiation was assessed by lipid droplet coverage by brightfield microscopy (EVOS FL, Life Technologies), with 80-90% lipid droplet coverage considered fully differentiated.

Stable isotope labeling using amino acids in cell culture (SILAC)

Lentivirus was generated by co-transfection of Hdac11 shRNA lentivirus plasmids, pCMV-dR8.2 and pMD2.G into HEK293T cells. The medium was collected 48 h after transfection and used to infect MEFs at MOI 10. After 72h of infection, cells were further treated with 1.5 μg/mL puromycin for an additional 72h to select for stable knockdown cells. shRNA targeting luciferase (Sigma, SHC007) was used as negative control. HDAC11 knockdown or control MEFs were then cultured in DMEM with [$^{13}C_6$, $^{15}N_2$]-L-lysine and [$^{13}C_6$, $^{15}N^4$]-L-arginine for six generations. Wild type cells were cultured in DMEM containing L-lysine and L-arginine for six generations. Then cells were treated with 50 μM Alk14 for 6 h. The cells were collected by centrifugation at 1000 × g for 5 min and then lysed in 4% SDS lysis buffer (50 mM triethanolamine at pH 7.4, 150 mM NaCl, 4% (w/v) SDS) with protease inhibitor cocktail (1:100 dilution) and nuclease (1:1000 dilution) at room temperature for 15 min. The protein concentrations were determined using the Pierce BCA Protein Assay Kit. Then 5 mg of the heavy lysate was mixed with 5 mg of the corresponding light lysate in a 50-ml tube and the volume was brought to 4.45 ml with 4% SDS lysis buffer. Biotin-N3 (100 μL 5 mM solution in DMF), Tris [(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (100 μL of 2 mM solution in DMF), $CuSO_4$ (100 μL of 50 mM solution in H_2O) and Tris(2-carboxyethyl)phosphine (50 μL of 50 mM solution in H_2O) were added into the reaction mixture. The click chemistry reaction was allowed to proceed at room temperature for 90 min. After incubation, 10 mL of methanol, 3.75 mL of chloroform, and 7.5 mL of water were added to each sample. After vortexing, the samples were centrifuged at 15,000 g for 20 min at 4 °C. The supernatant was gently removed by pipetting, and to each pellet was added 20 mL of methanol. The samples were again vortexed and spun down at 15,000 g for 10 min at 4 °C. The methanol was removed, and the protein pellets were washed again with 1 mL of methanol. After the second methanol wash, the protein pellets were air-dried for 15 min and then resolubilized in 1 mL of 4% SDS buffer. 2 mL of 1% Brij97 buffer (50 mM triethanolamine at pH 7.4, 150 mM NaCl, 1% (w/v) Brij97) was added to dilute the SDS concentration and then mixed with 100 μL streptavidin agarose beads (ThermoFisher). The mixture was rocked for 1 h at room temperature. After washing the beads three times with washing buffer (PBS with 0.2% SDS), the beads were further treated with 0.4 M hydroxylamine for 1 h at room temperature to remove any cysteine

modification. After washing beads three times with PBS, 100 µL of elution buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl) was added and the samples were boiled at 95 °C for 5 min. The supernatant was obtained and the reduction and alkylation of cysteines were then carried out. Disulfide reduction and protein denaturation were performed in 6 M urea, 10 mM DTT, 50 mM Tris-HCl, pH 8.0, at room temperature for 0.5 h. Then iodoacetamide was added (final concentration, 40 mM) and incubated at room temperature for 0.5 h. After that, 0.5 M of DTT was added to a final concentration of 15 mM and incubated at room temperature for 0.5 h to stop alkylation. The sample was diluted seven times with 50 mM TrisHCl, pH 8.0, and 1 mM CaCl2, before 2 µg trypsin was added and incubated at 37 °C for 18 h. The digestion was quenched with 0.1% trifluoroacetic acid and the mixture was desalted using Sep-Pak C18 cartridge. The lyophilized peptides were used for LC-MS/MS analysis.

Nano-LC-MS/MS analysis on Orbitrap fusion and data analysis

The SILAC tryptic digests were reconstituted in 50 µL of 0.5% Formic acid (FA) estimated at 0.1 µg/µL for nano-LC-ESI-MS/MS analysis, which was carried out using an Orbitrap Fusion™ Tribrid™ (ThermoFisher Scientific, San Jose, CA) mass spectrometer equipped with a nanospray Flex Ion Source, and coupled with a Dionex UltiMate 3000 RSL Cnano system (Thermo, Sunnyvale, CA). The tryptic peptide samples (5 µL) were injected onto a PepMap C-18 RP nano trap column (5 µm, 100 µm i.d x 20 mm, Dionex) with nanoViper™ Fittings at 20 µL/min flow rate for on-line desalting. The peptides were separated on a PepMap C-18 RP nano column (2 µm, 75 µm x 25 cm) at 35 °C, in a 120 min gradient of 5% to 38% acetonitrile (ACN) in 0.1% formic acid at 300 nL/min and followed by a 8 min ramping to 90% ACN-0.1% FA and a 9 min hold at 90% ACN-0.1% FA. The column was re-equilibrated with 0.1% FA for 25 min prior to the next run. The Orbitrap Fusion is operated in positive ion mode with spray voltage set at 1.6 kV and source temperature at 275°C. External calibration for FT, IT and quadrupole mass analyzers was performed. In data-dependent acquisition (DDA) analysis, the instrument was operated using FT mass analyzer in MS scan to select precursor ions followed by 3 second “Top Speed” data-dependent CID ion trap MS/MS scans at 1.6 m/z quadrupole isolation for precursor peptides with multiple charged

ions above a threshold ion count of 10,000 and normalized collision energy of 30%. MS survey scans at a resolving power of 120,000 (fwhm at m/z 200), for the mass range of m/z 375-1575. Dynamic exclusion parameters were set at 50 s of exclusion duration with ±10 ppm exclusion mass width. All data were acquired under Xcalibur 3.0 operation software (Thermo-Fisher Scientific). All MS and MS/MS raw spectra were processed and searched using Sequest HT software within the Proteome Discoverer 1.4.1.14 (PD 1.4, Thermo Scientific). The Homo sapiens NCBI UniprotKB.fasta database containing 20,153 entries downloaded on October 17, 2016 was used for database searches. The database search was performed under a search workflow with the "Precursor Ions Quantifier" node for SILAC 2plex (Arg10, Lys8) quantitation. The default setting for protein identification in Sequest node were: two mis-cleavages for full trypsin with fixed carbamidomethyl modification of cysteine, variable modifications of 10.008 Da on Arginine and 8.014 Da on lysine, N-terminal acetylation, methionine oxidation and deamidation of asparagine and glutamine residues. The peptide mass tolerance and fragment mass tolerance values were 10 ppm and 0.6 Da, respectively. Only high confidence peptides defined by Sequest HT with a 1% FDR by Percolator were considered for the peptide identification. The mass precision for expected standard deviation of the detected mass used to create extracted ion chromatograms was set to 3 ppm. The SILAC 2-plex quantification method within PD 1.4 was used with unique + razor peptides only to calculate the heavy/light ratios of all identified proteins without normalization. The final protein group list was further filtered with two peptides per protein in which only #1- ranked peptides within top scored proteins were used.

***In vitro* demyristoylation of gravin-α peptides**

The sequences for the gravin-α-Myr-K1502 and Myr-K1505 peptides were lLELETK(Myr)SSKLVQNIIQTAV and lLELETKSSK(Myr)LVQNIIQTAV, respectively, with N-terminal biotin groups. Both peptides were custom synthesized by Biomatik with 95% final purity. Myristoylated peptides (250 μM) were added to reaction buffer consisting of 20 mM ammonium bicarbonate and 50

mM NaCl, which was subsequently supplemented with 34 nM of purified HDAC11, prepared as previously reported paper, was added to the mixture to initiate the reaction (5). The mixture was then incubated at 37 °C for 1.5 hours. As a negative control, a reaction mixture without the enzyme was prepared. After quenching with acidic buffer (200 mM HCl, 330 mM acetic acid), the mixture was centrifuged at 17,000xg at room temperature for 10 minutes. The supernatant was injected into a Shimadzu HPLC LC20-AD and a Thermo Scientific LCQ Fleet Mass spectrometer, connected by a Kinetex 5u EVO C18 100 Å column (30 x 2.1 mm, 5 µm). Using HPLC grade water with 0.1% acetic acid as buffer A and HPLC-grade acetonitrile with 0.1% acetic acid, the mass traces were detected under positive ion mode.

***In vivo* fatty acid analog labeling**

FT895 (6) (5mpk, b.i.d.) or vehicle (5% DMA/1% Tween 80/94% sterile water) was administered via i.p. injections for five days. 200 µl of a 10x stock solution containing 1 mM alkynyl-myristic acid (in pre-warmed PBS containing 2% fatty acid-free BSA) was injected via the mouse tail vein as described (7). Adipose tissue depots were excised 4h post-injection, homogenized in 1% SDS buffer supplemented with protease and phosphatase inhibitors, and processed for click chemistry and pulldown assay.

Detection of lysine fatty acylation on Gravin-α using click chemistry

Cells or tissue explants were treated with 50 µM Alk12 (Cayman Chemical) for 4 h prior to harvest, then lysed in 1% SDS lysis buffer (50 mM Tris-HCl pH 8.0, 1% (w/v) SDS) with protease-phosphatase inhibitor cocktail (Thermo Scientific). Lysates were clarified via centrifugation at 13,000g at 4°C for 5 minutes. Supernatants were transferred to clean tubes, and protein concentrations was determined using the BCA assay (Thermo Scientific). Click chemistry reactions were performed using commercially available reagents as per manufacturer instructions (Thermo Scientific). Briefly, a total of upto 200µg of protein for each alkyne-labeled sample was used and the volume was brought to 50µl with 1% SDS lysis buffer. Biotin-azide (40 µM final concentration in Click-iT® reaction buffer), $CuSO_4$ (10 µL of 40 mM stock

solution) and other kit components were added into the reaction mixture. The click chemistry reactions were allowed to proceed at room temperature for 20-30 min with end-over-end rotation. After incubation, 600 µL of methanol, 150 µL of chloroform and 400 µL of 18 MΩ water were added to each sample. Samples were vortexed and centrifuged at 13,000 g for 5 min. The upper aqueous supernatant was gently removed and the interface layer containing the protein precipitate was left intact. 450 µL of methanol was added to the tubes, vortexed, and the samples were spun down at 13,000 g for 5 min to pellet the protein. The methanol was removed, and the protein pellets were washed again with 450 µL of methanol. The protein pellets were then air-dried for 15-30 min, resolubilized in 1% SDS buffer followed by BCA assay for determination of protein concentrations. 100ug labeled protein was incubated with 50 µL of streptavidin magnetic beads (ThermoFisher) overnight at 4°C with end-over-end rotation. Following incubation, the beads were collected and rinsed three times with washing buffer (Tris-buffered saline containing 0.1% Tween™-20). After the final wash, the supernatant was discarded, 50uL sample buffer was added to the beads and boiled at 95 °C for 5 min. The samples were further analyzed via western blotting using antibody specific for detection of endogenous gravin-α. In experiments where epitope (FLAG) tagged Gravin-α was used, western blotting was performed using an anti-FLAG (M2) antibody. Total Gravin-α amount was used for reference input.

Immunoblotting

Total protein was isolated from differentiated adipocytes using RIPA lysis buffer comprising 50mM Tris.HCl pH 8.0, 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS supplemented with protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Protein concentration was determined using BCA assay (Thermo Scientific), and proteins were resolved using SDS-PAGE and transferred onto nitrocellulose membranes (0.45 µm; Life Science Products). Membranes were probed with specific primary antibodies (in 2.5% BSA in TBS-T) overnight at 4°C. Following incubation with primary antibodies, blots were then probed with appropriate HRP-conjugated mouse or rabbit secondary

antibodies (Southern Biotech). Protein bands were visualized using enhanced chemiluminescent HRP substrate (Thermo Scientific) on a FluorChem HD2 Imager (Alpha Innotech) or ChemiDoc Imager (Biorad). A complete list of antibodies is available in Table S2.

Plasmids and lentivirus generation

p3XFLAG-CMV-14 expression vector (Sigma-Aldrich) encoding rat gravin-α was a gift from Dr. Joseph Miano, Augusta University, U.S.A. (8). This base vector was used to generate truncated constructs of gravin-α via PCR using specific primers. pcDNA3.1(+)-based plasmids encoding human HDAC11 WT and H143A (catalytically inactive) fused in-frame to an N-terminal Myc epitope tag were previously described (1). For HDAC11 NES, oligonucleotides encoding the nuclear export sequence from HIV-REV (LQLPPLERLTLD) were annealed and fused in-frame 3' of the Myc tag. PCR with Pfu Turbo polymerase (Agilent Technologies) was employed to create the final construct encoding the Myc-NES fused in-frame to codon 2 of HDAC11. A similar approach was employed to fuse HDAC11 to a nuclear localization signal (NLS) peptide from SV40 large T antigen (TPPKKKRKVEDP) using the oligonucleotides shown in Table S1. Site-directed mutagenesis reactions were performed to create p3XFLAG-CMV-14 encoding rat gravin-α (G2A, G2A K1502R, G2A K1505R, G2A KK/RR, KK/RR) and pcDNA3.1 encoding human HDAC11 (H143A) using the QuickChange method (Agilent Technologies). Lentiviral constructs were generated by cloning complementary DNAs encoding rat Gravin-α, KK/RR mutant, human HDAC11 and H143A mutant (NES and NLS) into pLenti CMV Hygro DEST. pLKO.1 plasmids (Millipore Sigma) encoding shRNA for murine gravin-α and a negative control (SHC002) were obtained through the Functional Genomics Facility at the University of Colorado Cancer Center. Lentivirus was generated by co-transfection of shRNA or pLenti lentivirus plasmid, psPAX2 and pMD2.G into L293 cells. The viral supernatant was collected 48 h after transfection, filtered and used to infect adipocytes. After 24h or 48h post-infection, cells were used for experiments as indicated. pLenti CMV Hygro DEST (w117-1) was a gift from Eric Campeau & Paul Kaufman (Addgene plasmid # 17454) (9). psPAX2 and pMD2.G were gifts

from Didier Trono (Addgene plasmids # 12260, #12259). A complete list of primers is available in Table S2.

Co-immunoprecipitation assay (Co-IP)

3T3-L1 white adipocytes were infected with lentiviruses encoding FLAG-tagged WT rat Gravin-α or lysine-myristoylation inactive mutant form (KK/RR) for 24h. In an alternate experiment, 3T3-L1 white adipocytes were infected similarly with lentiviruses encoding Myc-tagged human HDAC11 (WT), HDAC11 with nuclear export signal (NES) or with nuclear localization signal (NLS). pLenti (empty vector; Mock) infected cells were used as controls. HEK293 cells were transfected with epitope-tagged cDNA expression vectors encoding rat Gravin-α (WT; 3xFLAG-tagged), deletion constructs of Gravin-α (3xFLAG-tagged), human β_3-AR (HA-tagged), human HDAC11 (MYC-tagged) or deletion constructs of HDAC11 (MYC-tagged), using polyethylenimine (PEI) for 24 hours. Uninfected 3T3-L1 white adipocytes were used for pulldown of endogenous Gravin-α. Cells were treated with 10μM FT895 (or vehicle) as indicated. For all experiments, cells were lysed in IP lysis buffer (Thermo Scientific) containing 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol, supplemented with 2x protease-phosphatase inhibitor cocktail, using a syringe with a 25-gauge needle. Total protein was collected after centrifugation of lysates at 12000g at 4°C for 10 minutes, and protein concentrations were determined using BCA assay (Thermo Scientific). Protein homogenates (0.5 mg) were diluted in lysis buffer and were immunoprecipitated using precipitating antibody (gravin-α or IgG; Abcam) coupled to magnetic beads or epitope-tagged magnetic beads (FLAG or Myc; Thermo Scientific) overnight at 4°C with end-over-end rotation. Following incubation, beads were collected and washed three times, denatured in sample loading buffer, and resolved through SDS polyacrylamide gels. Whole-cell lysates were used as input controls. Proteins were transferred to nitrocellulose membranes (0.45 μm; Life Science Products) and immunoblotted. The membranes were blocked with 5% non-fat milk (in TBS-T) and incubated with primary antibodies (untagged or HRP-tagged) overnight at 4°C. Membranes were incubated with

secondary antibodies (where required) and protein bands were visualized using enhanced chemiluminiscence reagents (Thermo Scientific) on a FluorChem HD2 Imager (Alpha Innotech) or ChemiDoc Imager (Biorad). The antibodies used in this assay are listed in Table S2.

cAMP pulldown assay

3T3-L1 adipocytes were infected with lentiviruses encoding FLAG-tagged WT rat Gravin-α or lysine-myristoylation inactive mutant form (KK/RR) for 24h followed by treatment with Vehicle (DMSO) or FT895 (10μM; 12h). Cells were then trypsinized and lysed using IP lysis buffer (Thermo Scientific) supplemented with protease and phosphatase inhibitor cocktail (Thermo Scientific) and 3-Isobutyl-1-Methylxanthine (IBMX; Sigma Aldrich). Lysates were passed through 263/8 gauge needle ten times followed by gentle agitation on a rocking platform at 4°C for 10 minutes. The lysates were clarified via centrifugation at 10000g for 10minutes and supernatants were transferred to 1.5mL Eppendorf tubes. Following protein estimation using BCA assay (Thermo Scientific), 400ug protein lysate was used for all pulldown reactions. 50μL packed Adenosine 3′,5′-cyclic monophosphate (cAMP)–Agarose (Sigma Aldrich) beads was used for each reaction. Briefly, beads were resuspended in 1x TBS-T buffer, then washed with 1mL IP lysis buffer three times. All residual buffer was removed after the washes, and the beads were incubated with protein lysate overnight at 4°C with end-over-end rotation. For control reactions, parallel incubations were performed with Protein A/G PLUS-Agarose (Santa Cruz Biotechnology). Following incubations, the beads were pelleted by centrifugation at 5000g for 30s at 4°C. The beads were rinsed twice with 1x PBS and collected by centrifugation as above. All residual PBS was removed and the beads were incubated in 50uL sample buffer at 90°C for 10 minutes. The beads were then spun down at 5000g for 30s and the supernatant was transferred to a new tube. The proteins were resolved via SDS-PAGE, transferred onto nitrocellulose membrane followed by blocking (5% non-fat milk) and incubation with anti-FLAG-HRP antibody for detection of PKA-bound Gravin-α. Total protein (15μg) was used as input control and subjected to SDS-PAGE and immunoblotting as above.

Cellular fractionation

3T3-L1 adipocytes were infected with lentiviruses encoding Myc-tagged WT human HDAC11 for 24h. Cells were then harvested using trypsin-EDTA (Corning) and centrifuged at 500g for 5min. Cell pellets were rinsed once with 500µL PBS, split into two parts (1:4 by volume) and pelleted by centrifugation as above. The supernatants were carefully removed and discarded. Total protein was harvested using RIPA buffer supplemented with protease and phosphatase inhibitor cocktail (Thermo Scientific). The remaining cell pellets were subjected to cytoplasmic and nuclear protein extraction using commercially available reagents as per manufacturer's protocol (Thermo Scientific). All extraction buffers were supplemented with protease and phosphatase inhibitor cocktail (Thermo Scientific). Briefly, cytoplasmic extraction reagents were added to the pellets, vortexed, incubated on ice, vortexed again and then centrifuged for 5 min at 16000g at 4^{o}C. Cytosolic fraction supernatant was transferred to pre-chilled tubes. The pellets were again rinsed once with ice-cold PBS and centrifuged at 1000g for 5 min, supernatant was then collected and discarded. Nuclear extraction reagent was added to these pellets, vortexed and incubated on ice. The vortexing was repeated every 10 min for a total of 40 min, followed by centrifugation at 16000g at 4^{o}C for 15min. The supernatants were then immediately transferred to pre-chilled tubes. BCA assay was performed to estimate protein concentrations. Whole cell, cytoplasmic and nuclear protein extracts were resolved using SDS-PAGE and transferred onto nitrocellulose membranes. Following blocking (5% non-fat milk), membranes were incubated with anti-Myc antibody for detection of HDAC11. Purity of compartment fractions were confirmed by probing blots with antibodies specific for α-tubulin (cytosolic) or Lamin A/C (nuclear).

Indirect immunofluorescence

3T3-L1 adipocytes were infected with lentiviruses encoding Myc-tagged human HDAC11 (WT), HDAC11 with nuclear export signal (NES) or with nuclear localization signal (NLS) for 24h. Mouse primary

preadipocytes were isolated as described and differentiated on glass coverslips. Cells were fixed with 4% paraformaldehyde (methanol-free, Polysciences) at room temperature for 20 minutes, permeabilized using PBS-T (0.2% Triton X-100) for 15 minutes and blocked with PBS containing 5% BSA for 1 hr at room temperature. These were then incubated with primary antibodies (HDAC11, 1:500, or c-Myc, SantaCruz Biotechnology, 1:250) overnight in 2.5% BSA. Secondary antibodies (anti-rabbit Alexa Fluor 594, 1:500, or anti-mouse Alexa Fluor 594, 1:500; Invitrogen) were applied for 45 minutes at room temperature. Cells were counterstained with DAPI and mounted on glass slides using anti-fade mounting reagent (Vector Laboratories, H-1400-10). Images were acquired on a Zeiss LSM780 confocal microscope (Advanced Light Microscopy Core, University of Colorado Anschutz Medical Campus; Fig 1A) and processed through ZEN Black software (Zeiss) or on a Keyence BZ-X710 All-in-One Fluorescence Microscope (Fig 1C). For human VAT whole mount tissue immunofluorescence, explants were fixed in 4% paraformaldehyde (methanol-free, Polysciences) in phosphate buffered saline (PBS) for one hour whilst rotating at room temperature, followed by permeabilization in PBS-T (0.5% Triton X100) for 30 minutes with rotation at room temperature. Tissues were rinsed once in PBS and blocked in 5% fatty acid-free bovine serum albumin (BSA, Sigma Aldrich) in PBS for one hour at room temperature. Lipid droplet staining was performed using BODIPY (493/503 neutral lipid stain, Thermo Fisher Scientific, Molecular Probes™) at a final concentration of 5μM in PBS, rotating overnight at 4°C. Tissues were rinsed twice in PBS then mounted in Lab-Tek II Chamber slides (Nunc) in anti-fade mounting reagent (Vector Laboratories, H-1400-10). Whole mount imaging was perfomed on a Zeiss LSM780 confocal microscope (Advanced Light Microscopy Core, University of Colorado Anschutz Medical Campus) and processed through ZEN Black software (Zeiss). Antibodies used are listed in Table S3.

Super-resolution stimulated emission depletion (STED) nanoscopy

3T3-L1 adipocytes plated on #1.5 glass coverslips and infected with lentiviruses encoding FLAG-tagged WT rat Gravin-α or lysine-myristoylation inactive mutant form (KK/RR) for 24h, followed by treatment with HDAC11 inhibitor (FT895, 10μM, 1hr). Cells were then washed twice with PBS and fixed with 4% paraformaldehyde for 10 min. Coverslips were rinsed thrice with PBS on an orbital shaker platform. Cells were permeabilized with PBS-T (0.2% Triton X-100), rinsed with PBS (thrice for 5min each) and then blocked overnight with filter-sterilized 5% BSA/PBS. Adipocytes were incubated with primary antibody in 5% BSA/PBS at room temperature (mouse anti-FLAG M2, 1:500; rabbit anti-Caveolin 1, 1:400), then washed thrice PBS and incubated at room temperature for 1 h with secondary antibodies (goat anti-rabbit-Atto 647N 1:500 and goat anti-mouse-Atto 594 1:500; Rockland). Coverslips were rinsed again with PBS, mounted on glass slides with ProLong Glass Antifade Mountant (Invitrogen) and allowed to cure for 24h at room temperature in the dark. Images were acquired using a 100x objective lens on a custom built two-color STED microscope (10). The point spread functions for each STED channel were determined from images of 40nm beads (custom) that contained fluorophores in the red and far red channel. Deconvolution was performed using the Richardson-Lucy algorithm (five iterations) on Deconvolutionlab2 (11). Colocalization analysis was performed using the Coloc 2 plugin in FIJI (12), and values from pixel intensity correlation over space method of Pearson was plotted. A complete list of antibodies is available in Table S2.

Detergent resistant lipid raft isolation

Detergent resistant lipid raft isolation was based on an existing protocol (13). Differentiated 3T3-L1 adipocytes were trypsinized and pelleted by gentle centrifugation at 1000g for 5 mins. The cell pellets were resuspended in 10 mL ice-cold sterile PBS and pelleted at 1000 g for 5 mins. This was repeated once. The resulting cell pellet was gently resuspended in 1.4 mL TNEV buffer with 1%Triton X-100 and 1 mM PMSF (TNEV buffer: 10 mM Tris-HCL pH 7.5, 150 mM NaCl, 5 mM EDTA and 1 mM Na_3VO_4, autoclaved and ice cold) and incubated for 1 h at 4 °C. Suspended cells were then transferred to a pre-

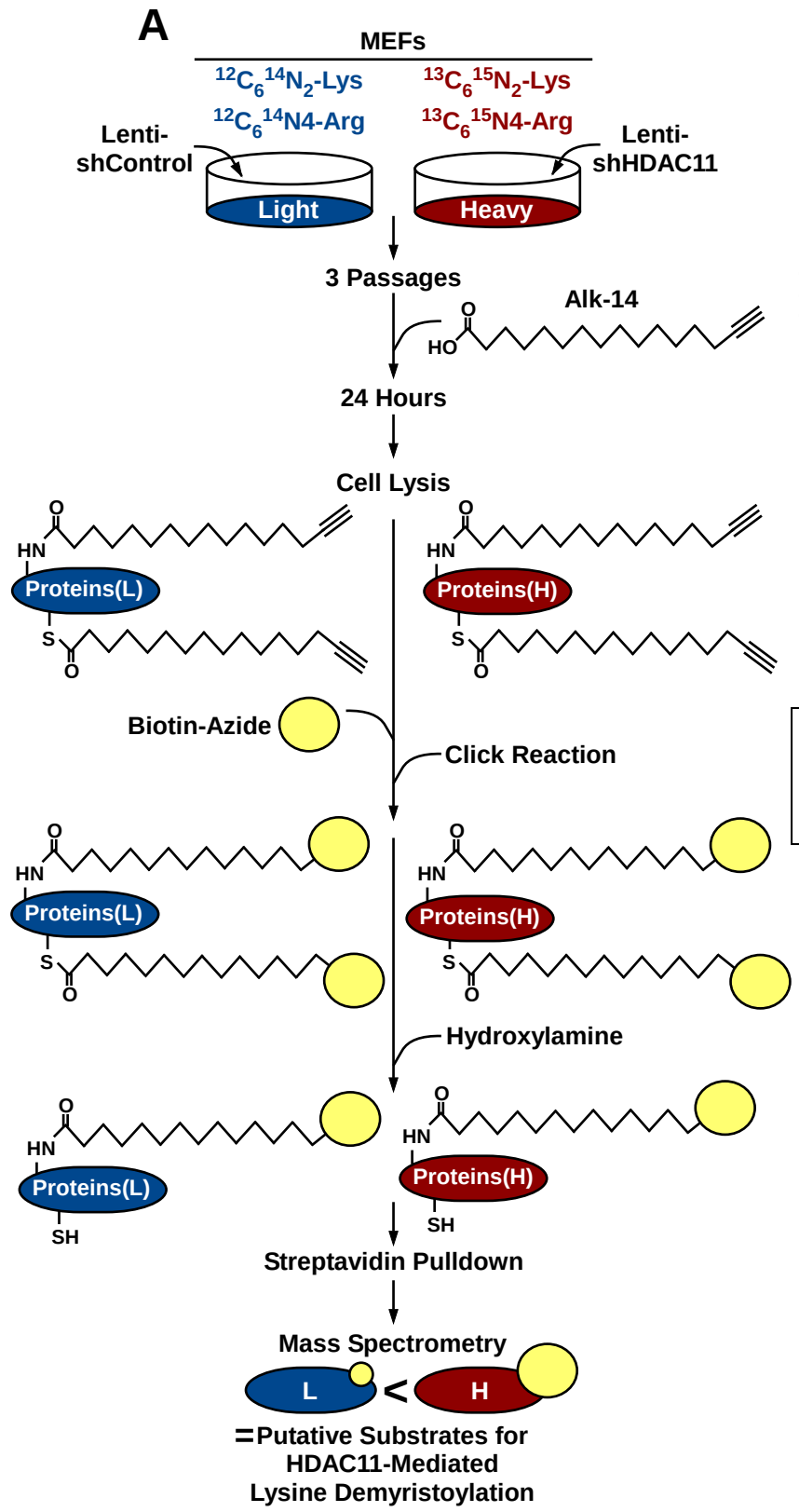
cooled 5 mL glass homogenizer (Cole-Parmer #EW-44468-14). Cells were homogenized with 10–15 strokes of the glass homogenizer. Homogenized cells were transferred into a 1.5 mL Eppendorf tube and centrifuged at 1000g for 5 min at 4 °C in a microfuge (Eppendorf). The supernatant, comprising the membrane fraction, was removed while the residual pellet, representing unbroken cells, nuclei, and cellular debris, was discarded. 1 mL of the membrane-containing supernatant was mixed 1ml of 85 % sucrose (w/v; Sigma Aldrich) in TNEV buffer and transferred to the bottom of a 14 mL 95-mm thin-wall ultraclear round-bottom centrifuge tube (Beckman Coulter Life Sciences) without touching the sides of the ultracentrifuge tube. 6ml 35% (w/v) sucrose in TNEV buffer was placed on top, using a 25 g needle and syringe, bent to allow carefully layering of the 35 % sucrose buffer onto the lower 85 % sucrose buffer containing the cell fraction. A final 3.5 ml 5% (w/v) sucrose in TNEV buffer layer was poured on top to form a discontinuous sucrose gradient. The cell suspension underwent ultracentrifugation at 38000g for 18h at 4°C in a Sw40 Ti ultracentrifuge rotor. Following centrifugation, the ultracentrifuge tubes were carefully removed and 1mL fractions taken from the top (fraction 1 being the top 1mL post-centrifugation), down to fraction 12 at the very bottom of the tube. Cell fractions in suspension were prepared for immunoblotting by directly adding 4x Laemmeli buffer, to a final concentration of 1x and boiled at 95 °C for 5 min. Proteins were resolved by SDS-PAGE and immunoblotting was performed as described.

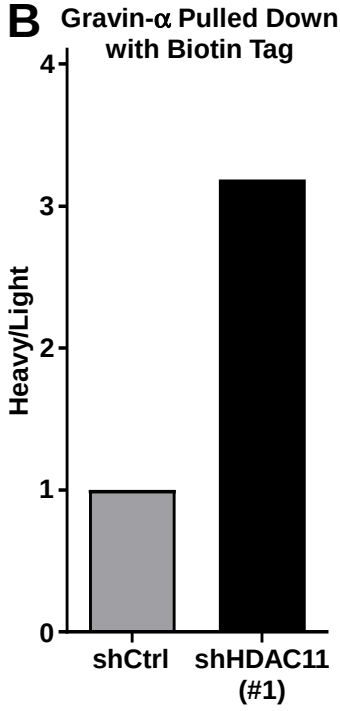
Statistical analysis

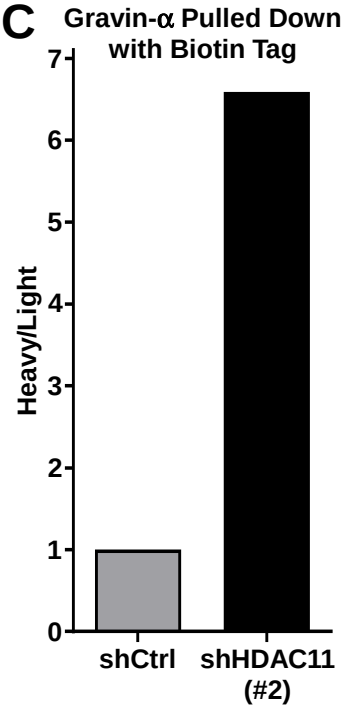
All data are presented as mean +SEM. Statistical significance ($P<0.05$) was determined using unpaired t-test (two groups) or one-way ANOVA with correction for multiple comparisons via a Tukey post-hoc test (GraphPad Prism 9).

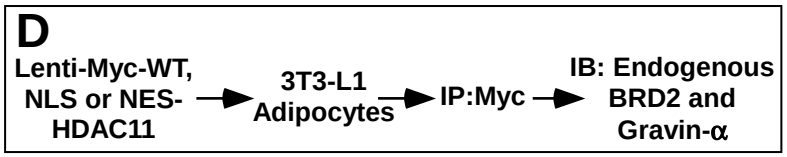
SUPPLEMENTARY FIGURES

Figure S1


A
MEFs
$^{12}C_6{}^{14}N_2$-Lys
$^{12}C_6{}^{14}$N4-Arg
$^{13}C_6{}^{15}N_2$-Lys
$^{13}C_6{}^{15}$N4-Arg
Lenti-shControl
Lenti-shHDAC11
Light
Heavy
3 Passages
Alk-14
24 Hours
Cell Lysis
Proteins(L)
Proteins(H)
Biotin-Azide
Click Reaction
Hydroxylamine
SH
Streptavidin Pulldown
Mass Spectrometry
L
H
=Putative Substrates for HDAC11-Mediated Lysine Demyristoylation


B
Gravin-α Pulled Down with Biotin Tag
Heavy/Light
shCtrl
shHDAC11 (#1)


C
Gravin-α Pulled Down with Biotin Tag
Heavy/Light
shCtrl
shHDAC11 (#2)


D
Lenti-Myc-WT, NLS or NES-HDAC11
3T3-L1 Adipocytes
IP:Myc
IB: Endogenous BRD2 and Gravin-α

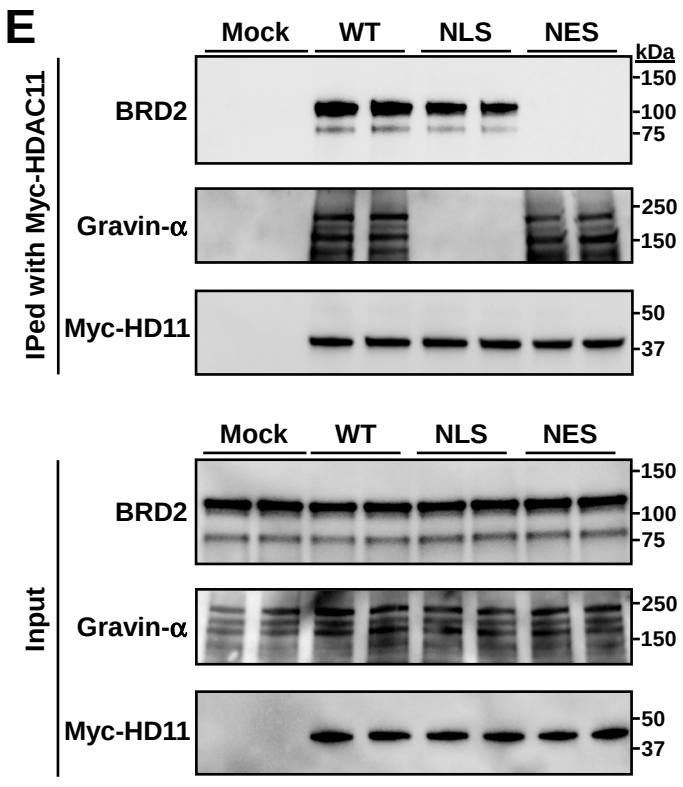

E
Mock
WT
NLS
NES
kDa
IPed with Myc-HDAC11
BRD2
Gravin-α
Myc-HD11
Input

Figure S1. Gravin-α is a putative substrate of HDAC11

(A) Schematic of the SILAC and Alk14 labeling experiment to identify proteins with increased acylation in mouse embryonic fibroblasts (MEFs) with HDAC11 knockdown (shHDAC11) compared with control (shControl) cells.

(B) Histogram representing heavy/light ratio obtained from mass spectrometry analysis of gravin-α in HDAC11 knockdown ((shHDAC11 (#1)) MEFs compared to control cells.

(C) Histogram representing heavy/light ratio obtained from mass spectrometry analysis of gravin-α in independent HDAC11 knockdown ((shHDAC11 (#2)) MEFs compared to control cells.

(D) Schematic depiction of the co-immunoprecipitation experiment.

(E) Immunoblot analysis showing co-immunoprecipitation, or lack of co-immunoprecipitation, of ectopically expressed Myc-epitope tagged HDAC11 constructs with endogenous BRD2 and gravin-α in 3T3-L1 adipocyte homogenates. Input levels of each protein, prior to immunoprecipitation, are shown in the lower panel. WT, wildtype; NLS, nuclear localization signal; NES, nuclear export signal; IPed, immunoprecipitated.

Figure S2

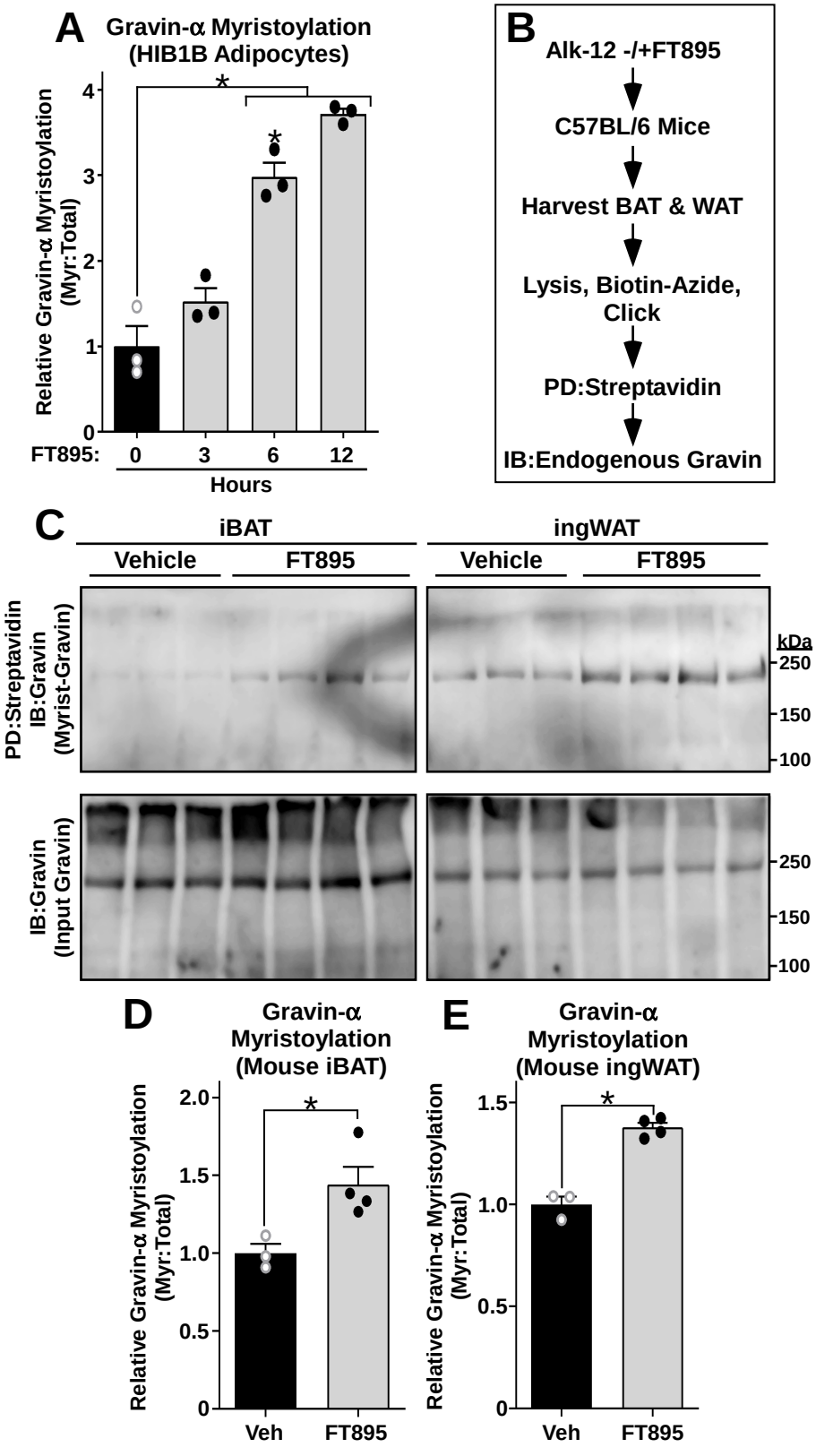

A
Gravin-α Myristoylation
(HIB1B Adipocytes)
Relative Gravin-α Myristoylation
(Myr:Total)
FT895:
0
3
6
12
Hours
B
Alk-12 -/+FT895
C57BL/6 Mice
Harvest BAT & WAT
Lysis, Biotin-Azide,
Click
PD:Streptavidin
IB:Endogenous Gravin
C
iBAT
ingWAT
Vehicle
FT895
Vehicle
FT895
PD:Streptavidin
IB:Gravin
(Myrist-Gravin)
IB:Gravin
(Input Gravin)
kDa
250
150
100
250
150
100
D
Gravin-α
Myristoylation
(Mouse iBAT)
Relative Gravin-α Myristoylation
(Myr:Total)
Veh
FT895
E
Gravin-α
Myristoylation
(Mouse ingWAT)
Relative Gravin-α Myristoylation
(Myr:Total)
Veh
FT895

Figure S2. Gravin-α/AKAP12 myristoylation is induced upon HDAC11 inhibition

(A) Quantification of gravin-α myristoylation in HIB1B brown adipocytes from Figure 2C. n=3 per condition; *p<0.05

(B) Schematic depiction of the experiment to determine if HDAC11 inhibition promotes gravin-α myristoylation in adipose tissue in vivo fatty. BAT, brown adipose tissue; WAT, white adipose tissue.

(C) Immunoblot analysis of adipose tissue homogenates showing myristoylated gravin-α in iBAT and ingWAT of vehicle or FT895-treated mice. iBAT, interscapular brown adipose tissue; ingWAT, inguinal white adipose tissue; PD; pulldown; IB, immunoblotting; Myrist, myristoylated.

(D) Densitometric quantification of myristoylated gravin-α in iBAT from panel C. n=3-4 per condition; *p<0.05

(E) Densitometric quantification of myristoylated gravin-α in ingWAT from panel C. n=3-4 per condition; *p<0.05

Figure S3

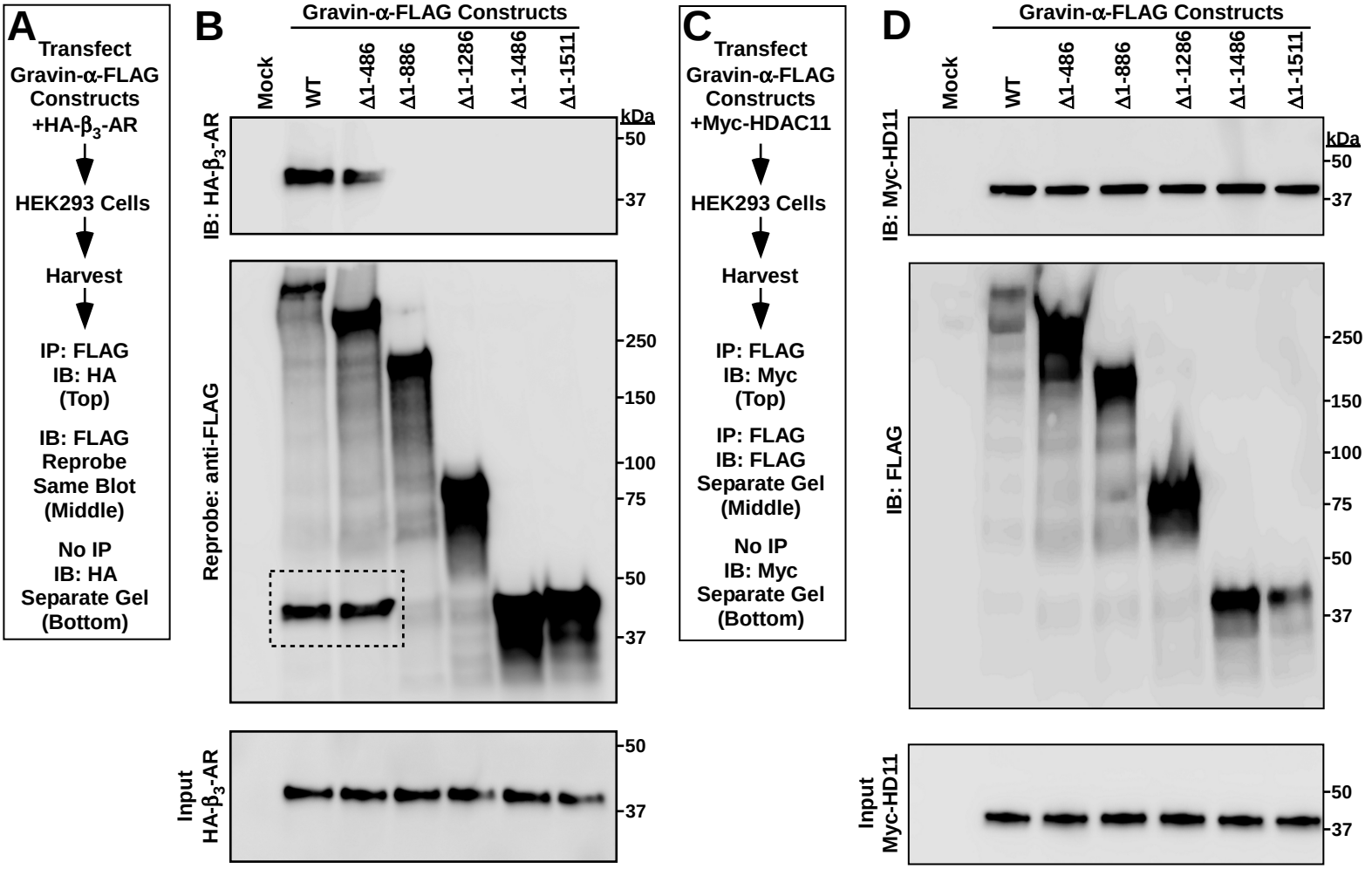

A
Transfect
Gravin-α-FLAG
Constructs
+HA-β3-AR
HEK293 Cells
Harvest
IP: FLAG
IB: HA
(Top)
IB: FLAG
Reprobe
Same Blot
(Middle)
No IP
IB: HA
Separate Gel
(Bottom)
B
Gravin-α-FLAG Constructs
Mock
WT
Δ1-486
Δ1-886
Δ1-1286
Δ1-1486
Δ1-1511
kDa
IB: HA-β3-AR
Reprobe: anti-FLAG
Input
HA-β3-AR
50
37
250
150
100
75
50
37
C
Transfect
Gravin-α-FLAG
Constructs
+Myc-HDAC11
HEK293 Cells
Harvest
IP: FLAG
IB: Myc
(Top)
IP: FLAG
IB: FLAG
Separate Gel
(Middle)
No IP
IB: Myc
Separate Gel
(Bottom)
D
Gravin-α-FLAG Constructs
Mock
WT
Δ1-486
Δ1-886
Δ1-1286
Δ1-1486
Δ1-1511
kDa
IB: Myc-HD11
IB: FLAG
Input
Myc-HD11
50
37
250
150
100
75
50
37

Figure S3. Discrete regions of gravin-α mediate associations with the β_3-AR and HDAC11

(A) Schematic depiction of the co-immunoprecipitation assay of ectopic gravin-α (WT or N-terminal truncation mutants) and ectopic β_3-AR in HEK293 cells.

(B) Immunoblot analysis reveals ectopic β_3-AR associates with a region of gravin-a between amino acids 487-886. For the gravin-α input blot, residual β_3-AR signal is indicated with a hatched box.

(C) Schematic depiction of the co-immunoprecipitation assay of ectopic gravin-α (WT or N-terminal truncation mutants) and ectopic HDAC11 in HEK293 cells.

(D) Immunoblot analysis reveals ectopic HDAC11 associates with a region of gravin-α between amino acids 1511-1687.

Figure S4

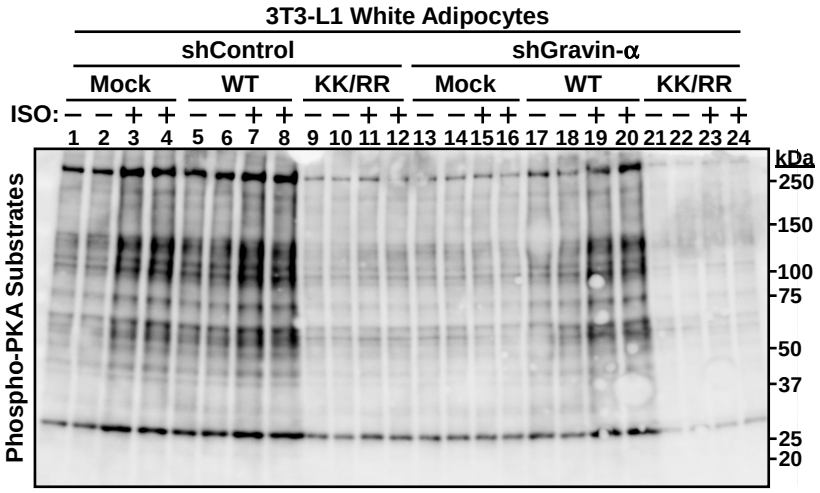

3T3-L1 White Adipocytes
shControl
shGravin-α
Mock
WT
KK/RR
Mock
WT
KK/RR
ISO: – – + + – – + + – – + + – – + + – – + + – – + +
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Phospho-PKA Substrates
kDa
250
150
100
75
50
37
25
20

Figure S4. Gravin-α lysine myristoylation promotes isoproterenol-induced PKA substrates phosphorylation

Immunoblot analysis of 3T3-L1 homogenates showing that β_2-AR-mediated PKA substrates phosphorylation is dependent on gravin-α and its myristoylation on lysine residues. ISO, Isoproterenol.

Figure S5

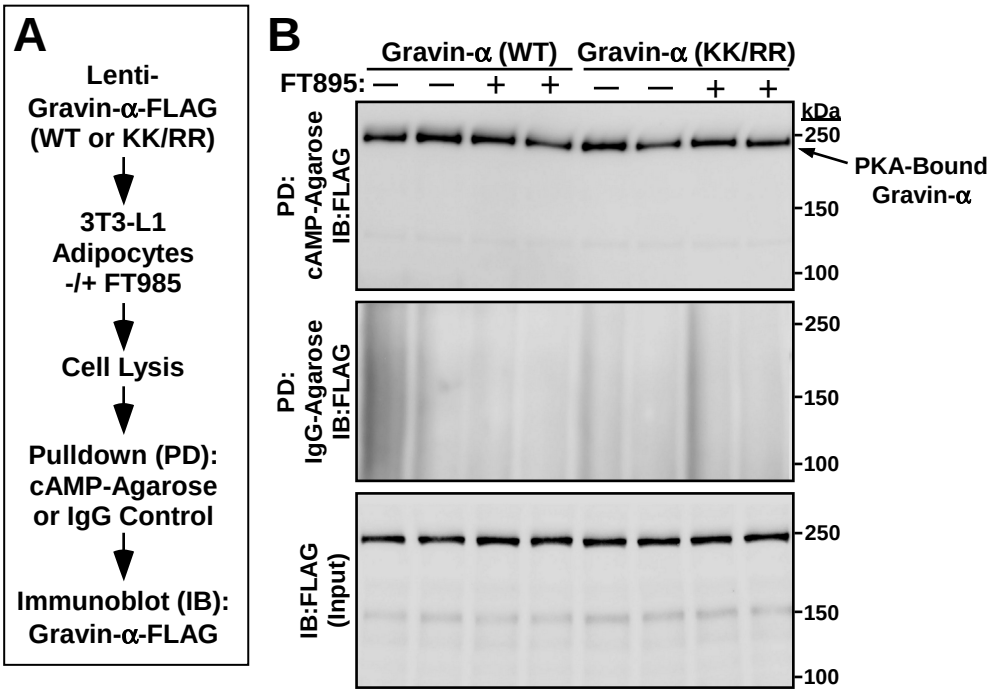

A
Lenti-
Gravin-α-FLAG
(WT or KK/RR)
3T3-L1
Adipocytes
-/+ FT985
Cell Lysis
Pulldown (PD):
cAMP-Agarose
or IgG Control
Immunoblot (IB):
Gravin-α-FLAG
B
Gravin-α (WT)
Gravin-α (KK/RR)
FT895: — — + + — — + +
kDa
250
150
100
PKA-Bound
Gravin-α
PD:
cAMP-Agarose
IB:FLAG
PD:
IgG-Agarose
IB:FLAG
IB:FLAG
(Input)

Figure S5. Gravin-α lysine myristoylation does not alter its binding to PKA

(A) Schematic depiction of the cAMP-agarose pulldown assay to assess binding of endogenous PKA complexes to ectopic gravin-α (WT or KK/RR) in 3T3-L1 adipocytes. cAMP, cyclic adenosine monophosphate

(B) Immunoblot blot analysis showing that PKA bound to gravin-a WT and KK/RR equivalently, and association of PKA with either form of gravin-α was unaffected by treatment of the cells with 10 μM FT895 for 12 hours.

Figure S6

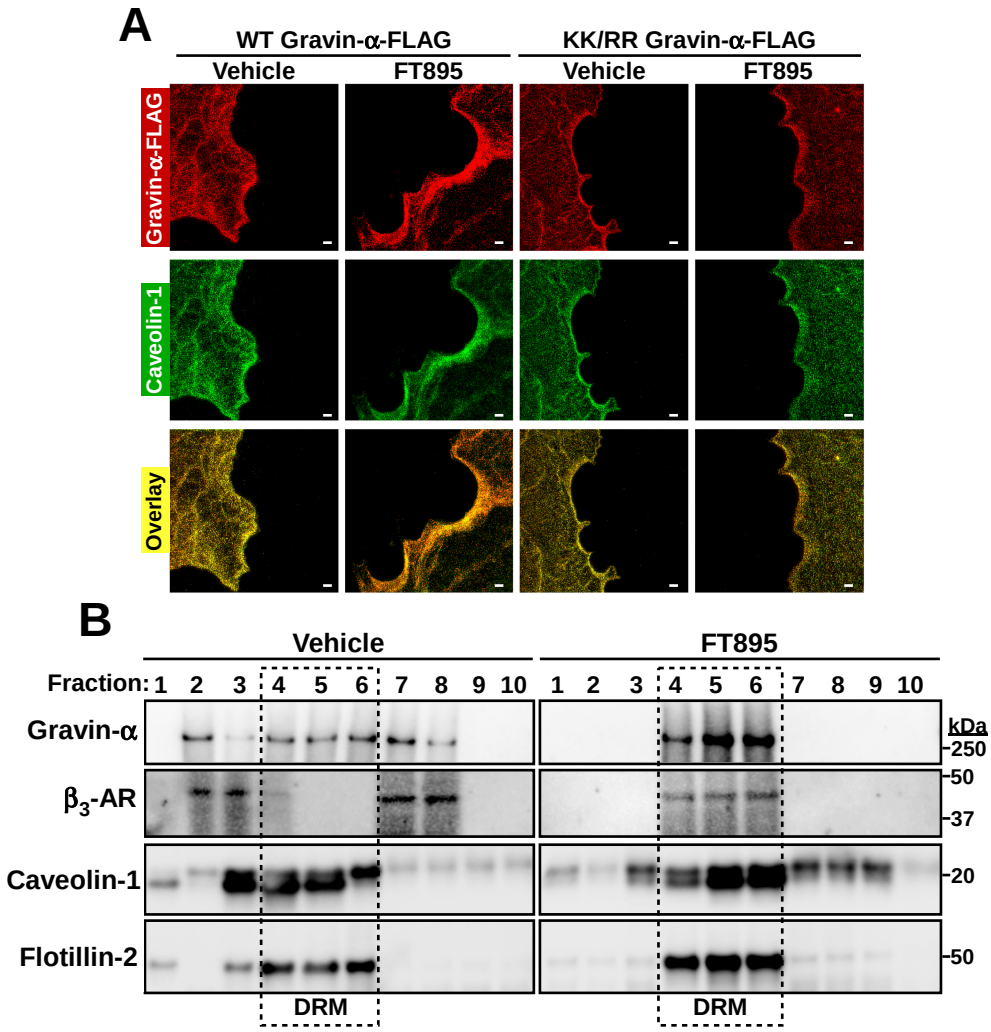

A
WT Gravin-α-FLAG
KK/RR Gravin-α-FLAG
Vehicle
FT895
Vehicle
FT895
Gravin-α-FLAG
Caveolin-1
Overlay
B
Vehicle
FT895
Fraction: 1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
Gravin-α
β3-AR
Caveolin-1
Flotillin-2
kDa
250
50
37
20
50
DRM
DRM

Figure S6. HDAC11 inhibition targets gravin-α to membrane domains indicative of lipid rafts

(A) Stimulated emission depletion (STED) microscopy of FLAG-tagged gravin-α (red) and endogenous caveolin-1 (green) in 3T3-L1 adipocytes treated with 10 μM FT895 or vehicle control for 1 hour. Scale bar = 1000 nm. WT, wildtype; KK/RR, myristoylation-deficient.

(B) Immunoblot analysis of endogenous gravin-α, β_3-AR, caveolin-1 and flotillin-2 in sucrose gradient fractions, with the fractions consisting of detergent resistant membranes indicated. Cells were treated with 10 μM FT895 or vehicle control of 12 hours. β_3-AR, β_3 adrenergic receptor; DRM, detergent-resistant membrane, FT, FT895.

Dataset 1. Identification of gravin-α by mass spectrometry.

All peptides and relevant quantification is provided.

SUPPLEMENTAL REFERENCES

1. Bagchi RA*, et al.* (2018) HDAC11 suppresses the thermogenic program of adipose tissue via BRD2. *JCI Insight* 3(15).
2. Cai J*, et al.* (2021) Manassantin B attenuates obesity by inhibiting adipogenesis and lipogenesis in an AMPK dependent manner. *FASEB J* 35(5):e21496.
3. Gavin KM*, et al.* (2016) De novo generation of adipocytes from circulating progenitor cells in mouse and human adipose tissue. *FASEB J* 30(3):1096-1108.
4. Lee MJ & Fried SK (2014) Optimal protocol for the differentiation and metabolic analysis of human adipose stromal cells. *Methods Enzymol* 538:49-65.
5. Son SI, Cao J, Zhu CL, Miller SP, & Lin H (2019) Activity-Guided Design of HDAC11-Specific Inhibitors. *ACS Chem Biol* 14(7):1393-1397.
6. Martin MW*, et al.* (2018) Discovery of novel N-hydroxy-2-arylisoindoline-4-carboxamides as potent and selective inhibitors of HDAC11. *Bioorg Med Chem Lett* 28(12):2143-2147.
7. Yap MC*, et al.* (2010) Rapid and selective detection of fatty acylated proteins using omega-alkynyl-fatty acids and click chemistry. *J Lipid Res* 51(6):1566-1580.
8. Streb JW, Kitchen CM, Gelman IH, & Miano JM (2004) Multiple promoters direct expression of three AKAP12 isoforms with distinct subcellular and tissue distribution profiles. *J Biol Chem* 279(53):56014-56023.
9. Campeau E*, et al.* (2009) A versatile viral system for expression and depletion of proteins in mammalian cells. *PLoS One* 4(8):e6529.
10. Meyer SA*, et al.* (2016) Super-resolution imaging of ciliary microdomains in isolated olfactory sensory neurons using a custom two-color stimulated emission depletion microscope. *J Biomed Opt* 21(6):66017.
11. Sage D*, et al.* (2017) DeconvolutionLab2: An open-source software for deconvolution microscopy. *Methods* 115:28-41.

12. Schindelin J*, et al.* (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9(7):676-682.
13. Gajate C & Mollinedo F (2021) Lipid Raft Isolation by Sucrose Gradient Centrifugation and Visualization of Raft-Located Proteins by Fluorescence Microscopy: The Use of Combined Techniques to Assess Fas/CD95 Location in Rafts During Apoptosis Triggering. *Methods Mol Biol* 2187:147-186.

Supplementary Table 1: Primers for cloning (lower case denotes mutated bases)

Target	Forward oligo (5'- 3')	Reverse oligo (5'- 3')
HIV-REV NES	CGATCTTCAGCTACCACCGCTTG AGAGACTTACTCTTGATAT	CGATATCAAGAGTAAGTCTCTCA AGCGGTGGTAGCTGAAGAT
SV40 NLS	CGATACTCCTCCAAAGAAGAAGA GAAAGGTAGAGGATCCTAT	CGATAGGATCCTCTACCTTTCTC TTCTTCTTTGGAGGAGTAT
pcDNA 3.1 HDAC11 NLS	CCATCGATCTACACACAACCCAG CTGTACCAGCAT	GCTCTAGATCAGGGCACTGCAG GGGGAAGCAGCGG
pcDNA 3.1 HDAC11 NES	CCATCGATCTACACACAACCCAG CTGTACCAGCAT	GCTCTAGATCAGGGCACTGCAG GGGGAAGCAGCGG
pCMV Gravin-α Δ1-486	GTCGAATTCCCACCATGGAGATG CTGTCCTCTCAGGAAAG	GTCGGATCCGGATTCTGCCAGG TCTCCCTTTG
pCMV Gravin-α Δ1-586	GTCGAATTCCCACCATGGGAGA GAAGAAGAGGGAAGGGATC	GTCGGATCCGGATTCTGCCAGG TCTCCCTTTG
pCMV Gravin-α Δ1-686	GTCGAATTCCCACCATGGATGAA GGAGGGCCAAGGACAC	GTCGGATCCGGATTCTGCCAGG TCTCCCTTTG
pCMV Gravin-α Δ1-786	GTCGAATTCCCACCATGTGGGTT TCCATTAAGAAATTCATCCCCG	GTCGGATCCGGATTCTGCCAGG TCTCCCTTTG
pCMV Gravin-α Δ1-886	GTCGAATTCCCACCATGGAAGAG CGGTCTCCTTCGTGG	GTCGGATCCGGATTCTGCCAGG TCTCCCTTTG
pCMV Gravin-α Δ1-1286	GTCGAATTCCCACCATGAGTCTT AGCCTGGAGGAGGGAG	GTCGGATCCGGATTCTGCCAGG TCTCCCTTTG
pCMV Gravin-α Δ1-1486	GTCGAATTCCCACCATGGAAAAA GTCCTCAAGGCTGAACC	GTCGGATCCGGATTCTGCCAGG TCTCCCTTTG
pCMV Gravin-α Δ1-1511	GTCGAATTCCCACCATGCAGACA GCCGTTGACCAGTTCG	GTCGGATCCGGATTCTGCCAGG TCTCCCTTTG
pCMV Gravin-α Δ1511- 1687 (1-1511)	GTCGAATTCCCACCATGATGGGC GCAGGCAGTTCCACCG	GTCGGATCCAATGACGTTCAGC ACAATCTTG
pCMV Gravin-α Δ1561- 1687 (1-1561)	GTCGAATTCCCACCATGATGGGC GCAGGCAGTTCCACCG	GTCGGATCCGGGTGTTTCATCT TGGCAACTTTCATTTTTGTCC
pCMV Gravin-α Δ1611- 1687 (1-1611)	GTCGAATTCCCACCATGATGGGC GCAGGCAGTTCCACCG	GTCGGATCCCTCCTTTTGATCTT TGGGTTGAGGAGGC
pCMV Gravin-α Δ1661- 1687 (1-1661)	GTCGAATTCCCACCATGATGGGC GCAGGCAGTTCCACCG	GTCGGATCCCATTTCTTCTTCCT GGACCTCAACTTTTGGG
Gravin-α G2A	CGAGGTCGACACATGGcCGCAG GCAGTTCCACCGAG	CTCGGTGGAACTGCCTGCGgCC ATGTGTCGACCTCG
Gravin-α K1502R	GATCCTGGAACTTGAGAGTAgGA GCAACAAGATTGTGCTGAAC	GTTCAGCACAATCTTGTTGCTCc TACTCTCAAGTTCCAGGATC
Gravin-α K1505R	GGAACTTGAGAGTAAGAGCAACA gGATTGTGCTGAACGTCATTCAG	CTGAATGACGTTCAGCACAATCc TGTTGCTCTTACTCTCAAGTTCC
Gravin-α KK/RR	GGAACTTGAGAGTAgGAGCAACA gGATTGTGCTGAACGTCATTCAG	CTGAATGACGTTCAGCACAATCc TGTTGCTCcTACTCTCAAGTTCC
HDAC11 H143A	GGGGGTGGCTTCCACgccTGCTC CAGCGACCGT	ACGGTCGCTGGAGCAggcGTGG AAGCCACCCCC

Supplementary Table 2: List of antibodies

Antibody/ Target	Manufacturer/ Supplier	Catalog No.
UCP-1	Abcam	ab10983 or ab209483
Lamin A/C	Proteintech	10298-1-AP
Gravin (AKAP12) for IB	Proteintech	25199-1-AP
Gravin (AKAP12) for IP	Abcam	ab49849
BRD2	Cell Signaling	5848
HDAC11	Edward Seto	N/A
FLAG® M2 HRP for IB	Sigma	A8592
FLAG® M2 for IF	Sigma	F1804
Myc (9E10) HRP for IB	Santa Cruz Biotechnology	sc-40 HRP
Myc tag (9E10) for IF	Santa Cruz Biotechnology	sc-40
Caveolin-1	Cell Signaling	3238
Flotillin-2	Cell Signaling	3436
β_3AR (ADRB3)	Abcam	ab94506
phospho- PKA substrates	Cell Signaling	9624
HA HRP	Cell Signaling	2999
FLAG (IF)	Sigma	F1804
α-tubulin HRP	Santa Cruz Biotechnology	sc-23948 HRP
Rabbit-HRP	Southern Biotech	4050-05
Mouse-HRP	Southern Biotech	1031-05
Mouse IgG (H&L) Antibody ATTO 594 Conjugated Pre-Adsorbed for STED	Rockland	610-155-121S
Rabbit IgG (H&L) Antibody ATTO 647N Conjugated Pre-Adsorbed for STED	Rockland	611-156-122S
Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 594	Invitrogen	A-11032
Goat anti-Rabbit IgG (H+L) Antibody, Alexa Fluor 594	Invitrogen	A-11012
Mouse IgG2a, kappa monoclonal [MG2a-53] - Isotype control for IP	Abcam	ab18415

Supplementary Table 3: Patient characteristics

Visceral White Adipose Tissue (Abdominal)

Patient ID	Age (years)	Gender	BMI (kg/m^2)
0582-05	30	Male	43.2
0582-07	38	Female	48.3
0582-08	40	Female	24.8
0582-19	62	Female	27.2
BSA-005	17	Female	32.4

Visceral White Adipose Tissue Pre-adipocytes

Patient ID	Age (years)	Gender	BMI (kg/m^2)
37526	32	Male	23.2