

SUPPLEMENTAL MATERIAL

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Isolation of human pre-adipocytes. Human pre-adipocytes were isolated from subcutaneous white adipose tissue (WAT) of healthy women undergoing elective fat removal at Sahlgrenska University Hospital in Gothenburg, Sweden and using an established protocol (1). All study subjects received written and oral information before giving written informed consent for the use of the tissue. The studies were approved by The Regional Ethical Review Board in Gothenburg, Sweden. Briefly, biopsies were thoroughly dissected, before digestion in Hank's Balanced Salt Solution with Mg^{2+}/Ca^{2+} containing collagenase II (300 U/ml) (4177, Worthington, Lakewood, NJ, USA) and fatty acid-free BSA (20 mg/ml) (A6003, Sigma-Aldrich, Darmstadt, Germany) for 45-60 min at 37°C with gentle shaking. Undigested tissue was removed by filtration through a mesh covered cell strainer (250 µm in size, (03-250/50, Sefar Nitex, Heiden, Switzerland). Wash buffer (KRHG/BSA) with 2 mmol/l glucose was added before adipocytes and buffer was poured into a separation funnel, allowing phase separation between mature adipocytes and the infranatant to be established, before emptying the infranatant into a sterile 1000 ml bottle. The resulting cell suspension was centrifuged 10 min at 200×g, and cells were re-suspended in adipocyte basal medium (BM-1, ZenBio, Durham, NC, USA) containing 10 % FBS (ThermoFisher), 1 % penicillin/streptomycin (ThermoFisher) and 17 ng/ml basic fibroblast growth factor (F0291, Sigma-Aldrich) before seeding at 19 000 cells/cm² in tissue culture-treated cell culture flasks. Cells were cultured at 37°C, 5 % CO₂ in air with 80 % humidity. Medium was changed the day after isolation, and on day 5, cells were detached using TrypLE Express (passage 1, ThermoFisher), and kept in nitrogen tanks until further usage.

Generation of immortalized human adipose derived stem cells (hASCs). Primary SVF cells were immortalized with hTERT as previously described (2). Primary SVF isolated from subject that had undergone population doublings were infected with a retrovirus containing the plasmid pBABEHygro-hTERT Retroviral Vector (Cell Biolabs; RTV-007), which expresses hTERT driven by a long-terminal-repeat promoter. The GP2-293 Packaging Cell Line which contains only the MoMuLV gag and pol genes, were transfected with pBABEpuro-hTERT Retroviral Vector using PolyJet DNA *in vitro* transfection reagent (SignaGen Laboratories, Rockville, MD). The viral envelope portion of the packaging function (env gene) was supplied by transiently co-transfecting pAmpho packaging vector (Clontech, 631530). Culture supernatants containing virus were collected at 24 h after transfection and filtered through a 0.45 µm filter. Primary SVF cells from human white fat at 80% confluence were infected with supernatants in the presence of 4 µg/ml Polybrene every day until cells reached 90% confluence. Cells were then treated with hygromycin (concentrations ranging from 400 µg/ml) in

Endothelial Cell Growth Medium MV2 (Promocell; containing. Once drug selection was finished, the cells were maintained in culture medium with 50 µg/ml hygromycin for 2 weeks.

AdipoR2 gene editing. Human immortalized pre-adipocyte ADIPOR2 KO line was generated by electroporating RNPs containing recombinant spCas9 (TrueCut™ Cas9 Protein v2, A36498, ThermoFisher) and sgRNA pair (CGAGCCAACAGAAAACCGAT and CAACTGGATGGTACACGAAG, TrueGuide Synthetic gRNA, ThermoFisher) targeting exon 1 of human ADIPOR2 gene, following a recently described protocol (3). Non-targeting sgRNA was used as a control (A35526, ThermoFisher). Briefly, 60 000 cells were transfected in a single electroporation reaction with 4.6 pmol of each sgRNA and 6 pmol of Cas9 v2 protein per reaction. Electroporation conditions were as follows: 1750V, 20 width, 1 pulse (Neon Transfection System, ThermoFisher). In total, 8 reactions were performed for both control and ADIPOR2-targeting conditions and pooled in a T75 flask. Cells were then expanded and frozen at 1 million cells/ml in 80:20 FBS:DMSO for future experiments. Before freezing, *ADIPOR2* genomic locus was analysed by PCR using the following primers: FW: TCTCCCAAGAAGAGGGGACA and RE: GGGCTCCAAATCTCCTTGGT; and the deletion was assessed at the *ADIPOR2* mRNA level using the following primers: FW: CTGGATGGTACACGAAGAGGT and RE: TGGGCTTGTAAGAGAGGGGAC.

Mice. All in vivo studies were permitted by the AstraZeneca internal committee for animal studies and the Gothenburg Ethics Committee for Experimental Animals in compliance with the EU directive 2010/63/EU on the protection of animals used for scientific purposes. All animal studies were designed in accordance with the principles of Good Statistical Practice, reported as per ARRIVE principles and be compliant with the Animal Care and Welfare Standard. The housing facility have full certification from the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Adult male mice were housed individually to prevent fighting while adult littermate females were housed in groups of two to five per cage (mixed genotypes). Unless otherwise indicated, mice were kept in temperature-regulated rooms (20-22 °C) with 50 % relative humidity and a 12:12 h light:dark cycle with ad libitum access to tap water and standard laboratory chow with an energy content of 12.6 kJ/g distributed as: 26 % protein, 12 % fat, and 66 % carbohydrates (R3, Lactamin, Kimstad, Sweden). Cages were environmentally enriched with straw, nesting cover and material, and chewing toys. Mice were weighed weekly from 4 weeks of age. Cohort litters with at least 4 WT and ADIPOR2 KO were deemed sufficient for studies. For exact numbers, see each respective study. The Adiponectin receptor 2 (ADIPOR2) knockout mice have been described previously (4, 5). All mice were bred on a C57BL/6N background (Charles River).

Isolation of mouse pre-adipocytes. Subcutaneous adipose tissue was removed post sacrifice, meticulously chopped and suspended in 10 ml digestion buffer containing 7 ml Hanks' Balanced Salt Solution (HBSS, H9269, Sigma), 0.23 g bovine serum albumin (BSA, A8806, Sigma), and 20 mg collagenase type II (C6885, Sigma), filtered through a 0.22 μ m membrane. Digestion was carried out at 37°C for 20 min, with horizontal shaking at 100 rpm. Afterwards, the digestion mixture was passed through a 100 μ m cell strainer (352360, Falcon) into a new tube, incubated at room temperature for 10 min, letting the adipocyte fraction to rise to the surface. The adipocyte fraction was removed and discarded by pipetting. The remaining suspension was centrifuged at 400 g, 4°C for 5 min and the pellet was re-suspended in 1 ml of pre-cooled (4°C) counting buffer (PBS, 1 mM EDTA, 3% heat-inactivated FBS). Cells were seeded into 75 cm² culture flasks (Falcon) containing growth medium (see cell culture below). When at 90% confluency, cells were detached using TrypLE express (passage 1, ThermoFisher), washed and re-suspended in cryopreservation medium (80% FBS, 20% DMSO) at 1 million cells/ml and cryopreserved, kept in nitrogen tanks until further usage.

Differentiation of mouse pre-adipocytes. Mouse pre-adipocytes were seeded to culture plates and kept in a growth medium which consisted of DMEM-high glucose (31966, ThermoFisher) supplemented with 10% FBS, 20 mM HEPES (ThermoFisher), and 1% penicillin/streptomycin. When cells reached ~90% confluency, differentiation was induced for 48 h using an induction medium: growth medium supplemented with 20 nM insulin, 1 nM T₃ (Sigma), 125 μ M indomethacin (Sigma), 500 nM dexamethasone, and 0.5 mM IBMX. After 48 h, cells were switched to a maintenance medium; growth medium supplemented with 20 nM insulin and 1 nM T₃ for 9 days with medium change every second day. Cells were differentiated for a total of 11 days followed by experiments.

Total lipid extraction and separation of phospholipids from DAG and TAG . Total lipid extraction and separation of polar (e.g. phospholipids) from lipophilic neutral lipids (e.g. diacylglycerols and triacylglycerols) was performed using the BUME method (6, 7). Briefly, cells or adipose tissues were homogenized and extracted in 0.5 ml of butanol:methanol (75:25) including either equiSPLASH (3310731, Avanti, AL, USA) or SPLASH (330707, Avanti) lipid internal standards. Separation of total lipids from non-lipids was achieved by liquid-liquid extraction following the addition of 0.5 mL aqueous buffer (1 % acetic acid) and 0.5 mL heptane:ethyl acetate (75:25). The obtained total lipid extract was subjected to evaporation of solvents under a gentle stream of nitrogen gas and the dried lipid extracts stored at -80 °C until direct lipidomics analysis or separation into polar and lipophilic neutral lipids prior to lipidomics analysis were performed. Briefly, separation of polar from lipophilic neutral lipids was achieved by first dissolving the dried total lipids in heptane:methanol (98:2). Separation of polar lipids from lipophilic neutral lipids was achieved by liquid-liquid extraction

following the addition of methanol:buffer (1 % NH₄OH in water) (95:5). The obtained upper lipophilic neutral lipid extract as well as the lower polar lipid extract were subjected to evaporation of solvents under a gentle stream of nitrogen gas and the dried lipid extracts were stored at -80 °C until lipidomics analysis.

HILIC-UPLC-MS/MS method for the determination of phospholipids molecular species composition in human cells and mouse tissues. Liquid chromatography and mass spectrometry.

The quantitative analysis was performed by HILIC-UPLC-ESI-MS/MS. The UPLC-MS/MS system was an Acquity I class, (Waters, Milford, MA, USA) coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters, Wilmslow, UK) with Waters Masslynx 4.1 Software. The analytical column was a BEH amide, 100 x 2.1 mm, 1.7 µm particle size (186004801, Waters). The mobile phases consisted of water (in house Milli-Q), Acetonitrile Optima (A955-212, LC-MS, Fisher Scientific, Gothenburg, Sweden), ammonium formate (70221-25G-F, Supelco, Bellefonte, PA, USA), and formic acid (00940, Supelco). Mobile phase A was acetonitrile:water 95:5, with 5 mM ammonium formate and mobile phase B was 10 mM ammonium formate (aq). The gradient was from 1% B in A, held for 1 min, and then to 30% B in A in 6 min, back to 1% B in 0.1 min and allowed to re-equilibrate for 3 min. Flow was 0.4 ml/min and injection volume was 5 µl. **MS parameters.** The mass spectrometer was operated in negative ESI mode with a source temp of 150 °C, a desolvation temp of 600 °C, a cone gas flow of 150 L/h, a desolvation gas flow of 1200L/h, and a collision gas flow of 0.15 mL/min. 20 transitions were monitored for PC, 21 for PE, 11 for PG, 16 for PI, 16 for LPC, and 16 for LPE with a total of 99 transitions, all transitions having a neutral loss corresponding to the R-COO fragment. The parent ion was M-1 except for PC which was measured as the formate adduct (M+HCOO)⁻. The cone voltage was kept at 30 V for all transitions and the collision energy was kept at 25 eV for all transitions. Phospholipids were quantified against their internal standard, one I S per lipid class.

UPLC-MS/MS method for the determination of diacylglycerol molecular species composition in human cells and mouse tissues. Liquid chromatography and mass spectrometry.

The quantitative analysis was performed by UPLC-ESI-MS/MS. The UPLC-MS/MS system was an Acquity I class, (Waters, Milford, MA, USA) coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters, Wilmslow UK) with Waters Masslynx 4.1 Software. The analytical column was a HSS T3, 50 x 2.1 mm, 1.8 µm particle size (186003538, Waters). The mobile phase consisted of methanol (A456-212, LC-MS, Fisher Scientific, Gothenburg, Sweden), Acetonitrile Optima (ACN) (A955-212, LC-MS, Fisher Scientific), ammonium formate (70221-25G-F, Supelco, Bellefonte, PA, USA), isopropanol (AC184130025, Fisher Scientific), and heptane (RH1004, Rathburn Chemicals, Walkerburn, Scotland). Mobile phase A was methanol with 5 mM ammonium formate and mobile phase B was a mixture of

40:50:10 of acetonitrile:isopropanol:heptane v/v/v. The gradient was from 5% B in A, held for 1 min, then to 30% B in A in 3 min, then held for 1 min and then back to 1% B in 0.1 min and allowed to re-equilibrate for 1 min. Flow was 0.3 ml/min and injection volume was 5 μ l. **MS parameters.** The mass spectrometer was operated in negative ESI mode with a source temp of 150 °C, a desolvation temp of 200 °C, a cone gas flow of 150 L/h, a desolvation gas flow of 1000L/h and a collision gas flow of 0.15 mL/min. 91 transitions were monitored for DAG, all transitions having a neutral loss corresponding to the R-COO fragment. The parent ion as the ammonium adduct ($M+NH_4$)⁺. The cone voltage was kept at 10 V for all transitions and the collision energy was kept at 13 eV for all transitions. Diacylglycerols were quantified against a deuterated internal standard, 15:0-18:1-d7 DG.

LC-MS/MS method for the determination of phospholipid, DAG and TAG molecular species composition in mouse adipocytes. Lipid extracts were reconstituted in 90:10 methanol:toluene, thoroughly mixed, and transferred to glass HPLC vials. Lipidomic characterization was performed on a Vanquish UHPLC – Orbitrap ID-X mass spectrometer (ThermoFisher). Chromatographic separation was performed on an Acquity CSH C18 column (1.7 μ m, 2.1 mm X 100 mm) (Waters) maintained at 65°C. Mobile phases A and B were 60:40 acetonitrile:water with 0.1% formic acid and 10 mM ammonium formate and 90:10 isopropanol:acetonitrile with 0.1% formic acid and 10 mM ammonium formate, respectively. Flow rate was maintained at 0.6 mL/min while gradient increased from 15% B to 30% B over 2 min, 30% B to 48% B over 0.5 min, 48% B to 82% B over 8.5 min, from 82% B to 99% B over 0.5 min, held at 99% B for 0.5 min, and equilibrated at 15% B for 3 min. LC-MS analysis was performed with an Orbitrap resolution of 60,000 and scan range of 120-1200 m/z on each individual sample. Lipid identification was performed by LC-MS/MS using HCD fragmentation with stepped collision energies of 25, 30, and 35% and quadrupole isolation window of 1 m/z . MS/MS spectra were acquired at Orbitrap resolution of 15,000 and auto scan range mode. AcquireX DeepScan data-dependent acquisition workflow (ThermoFisher) was performed on three iterative injections of a representative pooled sample from this study. LC-MS and LC-MS/MS data was analyzed using MS-DIAL v4.24 (8). Feature detection and alignment, adduct assignment, and lipid annotation were performed in MS-DIAL. Lipid annotations were assigned based on *in silico* spectral library provided in MS-DIAL with an identification score cutoff of 75%. Lipid quantitation was performed by normalizing each lipid species to the respective SPLASH internal standard amount and expressed as μ g lipid per million cells at extraction.

Gene expression analysis. Total RNA from cells and tissues were extracted using a RNeasy 96 kit following manufacturer's protocol (Qiagen). Total RNA was quantified using a Nanodrop (ThermoFisher) and cDNA synthesis was performed with 600 ng RNA input using a High-Capacity

cDNA Reverse Transcription Kit following manufacturer's protocol (ThermoFisher). Total cDNA was diluted to 0.533 ng/μl in RNase-free water and stored at –20°C until analysis. qRT-PCR was performed in 10 μL reactions with 3 μl of diluted cDNA, 5 μl of 2x TaqMan Fast Advanced Master Mix/Power SYBR Green Master Mix (ThermoFisher), 0.5 μl of 20x TaqMan assays/0.5 μM forward+reverse primer mix (ThermoFisher) and 1.5 μl of RNase-free water according to the default fast manufacturer's protocol (ThermoFisher). Reactions were run in technical duplicates for each sample and quantified using an ABI Prism 7900 sequence detection system (ThermoFisher). Duplicates were checked for reproducibility, and then averaged. A standard curve generated from a pool of all cDNA samples was used for quantification. The expression of genes of interest were normalized using the BestKeeper (BK) method to the geometric average of 3 housekeeping genes (human: *TBP*, *36B4* and *PPIA*; mouse: *Hprt*, *Tbp*, *Ppia*). Data was expressed as arbitrary units or normalized to the average of control group.

TaqMan assay probes used in this manuscript were:

TBP (Hs00427620_m1), *RPLP0* (Hs00420895_gH), *PPIA* (Hs04194521_s1), *HSPA5* (Hs00607129_gH), *XBPI* (Hs00231936_m1), *ATF4* (Hs00909569_g1), *ATF6* (Hs00232586_m1), *DDIT3* (Hs00358796_g1), *EIF2AK3* (Hs00984003_m1), *PPARG* (Hs01115513_m1), *SCD* (Hs01682761_m1), *FABP4* (Hs01086177_m1), *ADIPOQ* (00977214_m1), *SLC2A4* (Hs00168966_m1), *FASN* (Hs01005622_m1), *ACACA* (Hs01046047_m1), *ELOVL6* (Hs00907564_m1), *Hprt* (Mm03024075_m1), *Tbp* (Mm01277042_m1), *Ppia* (Mm02342430_g1), *Pparg* (Mm00440940_m1), *Scd1* (Mm00772290_m1), *Scd2* (Mm01208542_m1), *Scd4* (Mm01208549_m1), *Fabp4* (Mm00445878_m1), *Adipoq* (Mm04933656_m1), *Slc2a4* (Mm00436615_m1), *Lep* (Mm00434759_m1), *Srebf1* (Mm00550338_m1), *Fads1* (Mm00507605_m1), *Fads2* (Mm00517221_m1), *Acox1* (Mm01246834_m1), *Atf4* (Mm00515325_g1), *Atf6* (Mm01295319_m1), *Xbp1* (Mm00457357_m1), *Eif2ak3* (Mm00438700_m1), *Hspa5* (Mm00517691_m1), *Ddit3* (Mm01135937_g1), *Adipor2* (Mm01184032_m1), *Adipor1* (Mm01291337_mH), *Fasn* (Mm00662319_m1), *Cd36* (Mm00432403_m1), *Dgat1* (Mm00515643_m1), *Dgat2* (Mm00499536_m1), *Slc27a1* (Mm00449511_m1), *Acs11* (Mm00484217_m1), *Acs14* (Mm00490331_m1), *Cpt1a* (Mm01231183_m1), *Elovl6* (Mm00851223_s1), *Tnf* (Mm00443258_m1), *Adgre1* (Mm00802529_m1), *Mrc1* (Mm01329359_m1), *Ccl2* (Mm00441242_m1), *Ccl5* (Mm01302427_m1), *Il6* (Mm00446190_m1).

Immunofluorescence. For fluorescent analysis of lipid accumulation in differentiated adipocytes, live adipocytes were washed twice in PBS followed by fixation in 4% buffered formaldehyde containing 1 μg/ml of Hoechst 33342 (ThermoFisher) for 20 min at room temperature. Fixed cells were washed twice in PBS before adding PBS containing 1 μg/ml of BODIPY 493/503 and left to stain for 20 min. Cells were washed twice in PBS before proceeding with image acquisition using a robotic Yokogawa

CV8000 spinning disc confocal microscope (Wako Automation) at 60× (NA.95, 2 × 2 binning) using ZYLA 5.5 sCMOS cameras (Andor Technology). Images were acquired with 16 bits image depth and 1024 × 1024 resolution, using a pixel dwell of ~1.02 μs. Lipid morphology images were analyzed using Columbus 2.9.1.532 software (PerkinElmer).

For fluorescent measurement of membrane order using Laurdan, PA treated pre-adipocytes were washed twice in PBS before staining with 200 μM laurdan dye (6- dodecanoyl-2-dimethylaminonaphthalene) (ThermoFisher) at 10 μM (HUVECs) or 15 μM (HEK293) for 60 min. Image acquisition was done using a robotic Yokogawa CV8000 spinning disc confocal microscope (Wako Automation) with temperature (37 °C) and carbon dioxide levels (5%) controlled during live cell imaging. Images were acquired at 20× (NA.95, 2 × 2 binning) using a 405 nm laser excitation and 445/45 (ordered phase) plus 525/50 (disordered phase) bandpass filters using ZYLA 5.5 sCMOS cameras (Andor Technology). Images were acquired with 16 bits image depth and 1024 × 1024 resolution, using a pixel dwell of ~1.02 μs. Images were analyzed using ImageJ version 1.47 software, following published guidelines (9).

Bioenergetics. Oxygen consumption rate (OCR) was measured using a Seahorse XF96 instrument (Agilent). Briefly, cell medium composition during the assay was: Seahorse XF base medium (Agilent) supplemented with 5 mM glucose, 1 mM pyruvate and 1 mM L-glutamine. A Seahorse XF Cell Mito Stress Test was run using 1 μM oligomycin (Sigma-Aldrich), 200 μM DNP (as uncoupler; Sigma-Aldrich), 1 μM rotenone (Sigma-Aldrich) and 1 μM antimycin A (Sigma-Aldrich) as per the manufacturer's instructions. Afterwards, cells were stained with both Hoechst 33342 (ThermoFisher) and propidium iodide (ThermoFisher) to assess nuclear viability as measured by fluorescence microscopy. OCR values were normalized against viable cells.

PA treatments. All PA treatments on cells were prepared in each respective maintenance medium by diluting a 1.5 mM palmitate-BSA conjugate solution (6:1 molar ratio) to the desired treatment concentration. Control treatment was BSA alone at equivalent concentration. Cells were washed twice in PBS before adding PA or control treatment to cells. Cells were left to incubate for 24 h before assessing endpoint analysis. PA treatments for NanoSIMS imaging was done for 4 h before removing treatments and adding the same concentration of U-13C-labeled PA (Cambridge Isotope Laboratories) for 2 h prior to fixation.

Cell Viability Assay. Cell viability in response to palmitate treatment was assessed by measuring cellular ATP levels using CellTiter-Glo® 2.0 Cell Viability Assay according to the manufacturer's protocol (G9241, Promega). Briefly, cells were lysed in the presence of luciferin and Ultra- Glo®

rLuciferase, which oxidises luciferin to oxyluciferin in the presence of ATP, producing light. Luminescence was measured using a SpectraMax 190 (Molecular Devices).

Adiponectin treatments. All adiponectin treatments on cells were prepared in human specific maintenance medium by diluting a 0.18 mg/ml human recombinant adiponectin stock (1065-AP-050, R&D Systems) to 0.6 µg/ml. Control treatment was BSA alone at equivalent concentration. Cells were washed twice in PBS before adding adiponectin or control treatment to cells. Cells were left to incubate for 24 h before assessing endpoint analyses.

SCD inhibition treatments. All SCD inhibition treatments on cells were prepared in human specific maintenance medium by diluting a 10 mM small molecule SCD inhibitor (10012562, Cayman Chemical Company) to 1 µM in either BSA or 600 µM PA aliquots as described in PA treatments. Control treatment was DMSO at equivalent concentration. Cells were washed twice in PBS before adding treatments to cells. Cells were left to incubate for 24 h before assessing endpoint analyses.

Sample preparation for NanoSIMS imaging. For NanoSIMS imaging, WT and cells were seeded on 35mm glass-bottom dishes (MatTek Life Science, USA) at a density of 56 250 cells/cm² per dish and differentiated for 30 days prior to PA treatment. Following compound incubation, cells were washed twice with PBS and then fixed with a modified Karnovsky's fixative (2.5% glutaraldehyde (Agar Scientific, UK), 2% formaldehyde (Sigma Aldrich), 0.02% sodium azide (BDH, UK) in 0.05M Na-cacodylate buffer) for 30 min at room temperature. Cells were then incubated in fixative at 1:10 in 0.1M Na-cacodylate buffer overnight at 4°C. The dishes were then washed twice with 0.1M Na-cacodylate buffer, once with 50mM glycine (Sigma Aldrich) in 0.1M Na-cacodylate buffer, and then twice with 0.1M Na-cacodylate buffer. The dishes were then incubated with 1% osmium tetroxide and 1% potassium ferricyanide (Sigma Aldrich) in water for 45 min in the dark and then washed three times with H₂O. The dishes were then incubated for 30 min with 1% tannic acid and washed three times with H₂O. The dishes were then incubated for 30 min with 1% uranyl acetate (Merck, Sigma-Aldrich, Sweden) in the dark and washed three times with H₂O. The cells were then sequentially dehydrated with 30%, 50%, 70%, 85%, 95% and absolute ethanol. Embedding was performed in Agar 100 premix kit hard (Agar Scientific Ltd, UK). Agar 100 epoxy resin was mixed with pre-mixed hardeners (DDSA and MNA), but not with accelerator (BDMA), for 30 min to produce mixture A. For BSEM sample preparation, samples were exposed at room temperature to 25% mixture A and 75% absolute ethanol for 10 min, 50% mixture A and 50% absolute ethanol for 10 min, 75% mixture A and 25% absolute ethanol for 10 min and 100% mixture A for 10 min, 3 times. Mixture A was mixed with accelerator

(BDMA) for 30 min to produce mixture B. BEEM capsules were filled with 100 % mixture B. Resin was decanted from MatTek dishes and the BEEM capsules were inverted onto dishes. The tube and dish were polymerised at 60°C for 16h. After polymerization, resin blocks were dislodged from MatTek dishes by using liquid nitrogen and sectioned with an ultramicrotome (Leica EM UC6). 300 nm thick sections of resin embedded cells were mounted onto silicon wafers (Ted Pella). Backscattered electron (BSE) images were obtained with a Gemini 300 (Zeiss) scanning electron microscope to assess the quality of the sample preparation and select cells of interest (Figure S4A).

NanoSIMS imaging and data analysis. Samples were gold coated and measurements were performed on a NanoSIMS 50L (CAMECA, France) at the Chemical Imaging Infrastructure at Chalmers University of Technology and University of Gothenburg (Sweden) on the same sections from which SEM images were acquired. Samples were scanned with a focused 16 keV Cs⁺ beam, and secondary ions (¹²C₂⁻, ¹³C¹²C⁻, ¹²C¹⁴N⁻, ³¹P⁻ and ³²S⁻) were collected. Prior to each image acquisition, a saturation fluence of $\approx 1 \times 10^{17}$ Cs⁺.cm⁻² (D1.1) was implanted at area of interest, ensuring a secondary ion sputtering steady state. 18 μm^2 or 16 μm^2 regions of interest (ROIs) were imaged with a 2 pA beam (D1.3, FCp \approx 15 nA, L1 (10) \approx 18500, ES4, AS3) with a dwell time of 500 $\mu\text{s} \cdot \text{pixel}^{-1}$. 512 x 512 pixels images containing 16 cycles were obtained. NanoSIMS images were processed using WinImage (CAMECA). Sequential image cycles were drift corrected and accumulated. For data analysis, ROIs were manually defined based on the ¹²C¹⁴N⁻ images that allow to visualize the cell morphology. ¹³C¹²C⁻/¹²C₂⁻ and ³¹P/¹²C₂⁻ ratios were extracted for each ROIs. For evaluation of the carbon isotopic enrichment ($\delta^{13}\text{C}$) in the sample, ¹³C¹²C⁻/¹²C₂⁻ ratio images were expressed as deviations relative to the isotopic ratio of the Vienna Pee Dee Belemite reference (VPDB=0,0112372) in per mille (‰) using the following equation:

$$\delta^{13}\text{C}(\text{‰}) = \frac{{}^{13}\text{C}^{12}\text{C}^{-}/{}^{12}\text{C}_2^{-}}{2 \times 0,0112372} \times 1000 - 1000$$

Then, $\delta^{13}\text{C}_{\text{ROIs}}$ enrichments were corrected by the enrichment in the resin ($\delta^{13}\text{C}_{\text{resin}}=20,1 \text{ ‰} \pm 4,4 \text{ ‰}$).

Glucose and palmitate uptake. Differentiated human and mouse adipocytes on 96w CytoStar plates (PerkinElmer) were washed twice in PBS before adding insulin starvation medium (human; BM-1 (ZenBio), 3 % TET-free FBS (ThermoFisher), 1 % penicillin/streptomycin (ThermoFisher), mouse; DMEM (31966, ThermoFisher), 3 % FBS, 1 % penicillin/streptomycin) with or without PA overnight (16 h). Adipocytes were washed twice in PBS before adding a low glucose medium (DMEM-no glucose (11966, ThermoFisher), 25 mM HEPES, 0.1% FA-free BSA, (Sigma), and 0.1 mM glucose (D-glucose, Agilent)) followed by incubation at 37 °C for 3 h. Cells were then stimulated with varying concentrations of insulin for 30 min before addition of either 2-Deoxy-[U-¹⁴C]-glucose (PerkinElmer)

or 50 μ M cold PA spiked with [$1\text{-}^{14}\text{C}$]-palmitic acid (PerkinElmer) at 0.5 μ Ci/ml final concentration followed by incubation, reading the plate after 15 and 90 min on a MicroBeta2 reader (PerkinElmer).

Insulin signaling. Differentiated human and mouse adipocytes were washed twice in PBS before serum and insulin starving cells for 4 h in DMEM (31966, ThermoFisher) supplemented with 25 mM HEPES (ThermoFisher) and 0.1% FA-free BSA (Sigma). Cells were then stimulated with varying concentrations of insulin (Actrapid) for 10 min (human) or 50 min (mouse) followed by lysis. Cells were lysed in lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EGTA (Sigma), 1mM EDTA (Sigma), 1% Triton X-100 (Sigma), 0.1% SDS (Sigma), Complete Mini protease inhibitor (Roche), PhosSTOP (Roche)) supplemented with 4 % FA-free BSA (Sigma). pAKT/AKT, pERK/ERK, pIR/IR, pIRS1/IRS1, and pIGF-1/IGF-1 protein levels were quantified using MSD phospho(Ser473)/Total Akt Whole Cell Lysate Kit (K15100D-3, Meso Scale Diagnostics), Phospho/Total ERK1/2 Whole Cell Lysate Kit (K15107D, Meso Scale Diagnostics), Insulin Signaling Panel (Phospho Protein & Total Protein) Kits (K15151C-1 & K15152C-1, Meso Scale Diagnostics) respectively according to manufacturer's instructions.

To measure pAKT/AKT protein levels in mouse tissues, they were homogenized on ice in T-PER™ Tissue Protein Extraction Reagent (ThermoFisher) supplemented with protease and phosphatase inhibitors. Protein concentration in tissue lysates was determined using Pierce™ BCA Protein Assay Kit (ThermoFisher) according to manufacturer's protocol. Lysates were then diluted to 0.8 mg/ml, and pAKT/AKT protein levels were quantified using a MSD phospho(Ser473)/Total Akt Whole Cell Lysate Kit (K15100D-3, Meso Scale Diagnostics) according to manufacturer's instructions.

Histopathology and image analysis. Adipose tissue (eWAT) from WT and homozygous AdipoR2 KO 12-week old male mice were fixed in 4 % paraformaldehyde for 24 h. After formalin fixation, dehydration and paraffin embedding, 4 μ m sections were taken at three levels, 300 μ m apart. Slides were stained with Hematoxylin and Eosin (H&E), and scanned using a Panoramic scan II device (3DHistec Ltd., Hungary). Image analysis was performed on digital images using Visiopharm Integrator System software; version 2019.2 (Visiopharm, Hørsholm, Denmark).

In the H&E-stained slides, the adipose lipid droplets are unstained, while the cell membrane and other tissue is stained blue. A first software application setup, based on artificial intelligence, detected the adipose tissue, removing non-adipose tissue from the analysis. Minor manual correction was done to adjust the adipose tissue segmentation. Following segmentation, a threshold method was used to distinguish lipid droplets from each other, classifying them into droplet areas of 500 up to 4000 μm^2 in

intervals of 500 μm^2 . A counting frame was simultaneously incorporated to avoid duplicate classification of lipid droplets.

High fat diet studies. To provide a diet intervention rich in saturated fat with minimal lipid oxidation in brown adipose tissue (BAT), 7 WT and 7 ADIPOR2 KO male mice were transferred at four weeks of age to cages in a temperature- and humidity-controlled room set at 30 °C with 50 % relative humidity and a 12:12 h light:dark cycle. For the first four weeks, mice were fed chow (R3, Lactamin) followed by a switch to a high-saturated fat diet with an energy content of 5.56 kcal/g distributed as: 17 % protein, 25 % carbohydrates, and 58 % from fat (86 % being saturated) (D12331, Research Diets, New Brunswick, NJ, USA) starting at 8 weeks of age for 16 weeks. Oral glucose tolerance test (oGTT) was performed at 23 weeks of age.

oGTT was performed on fasted mice, during which they were acclimatized to study room (6 h) at 13.00 h by administering glucose at 2 g/kg by oral gavage. Duplicate blood samples (2 μl) were taken from the tail vein prior to gavage and 15, 30, 60, and 120 min after administration for the determination of blood glucose levels (2 μl , Accu-Chek, Roche Diagnostics, Mannheim, Germany) and insulin ELISA performed according to manufacturer's protocol (2 x 2 μl , 90060, Crystal Chem, Zaandam, Netherlands). The mice were left to recuperate for 1 week followed by termination.

Mouse identity and genotype was blinded for all participants in the in vivo experiments except for study leaders H.P & K.P. Genotypes were re-confirmed based on qPCR looking at *AdipoR2* expression.

Statistical analysis. Most data are presented as averages, with error bars showing the standard error of the mean. Replicate numbers for a given experiment are specified in each figure legend. Where replicate numbers are high, data is expressed as box and whisker plots. Some data is visualized as fold-change, where the underlying normalization is described in the figure legend. All statistical tests are described in each respective figure legend. Student's t-tests were used to compare two groups. Multiple t-tests (one per row) with correction for multiple comparisons were used to compare gene expression in mice tissues and lipogenic markers in human and mouse cells. In cases where more than one variable influenced the outcome, a 2-way ANOVA was used to test for the significance of each factor, and the interaction between factors with a Sidak's multiple comparisons post-hoc test to analyze differences between genotype in a group. No mice were excluded from a study nor analysis. Statistical tests and graphs were generated using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). CRISPR-targeting visualization were generated using Geneious Prime v2021.1.1 (Biomatters Ltd., Auckland, New Zealand). Figures and graphs were edited using Adobe Illustrator 2019 (Adobe, San Jose, CA, USA). Schematic illustrations and graphical abstract were created with BioRender.com.

SUPPLEMENTARY FIGURES

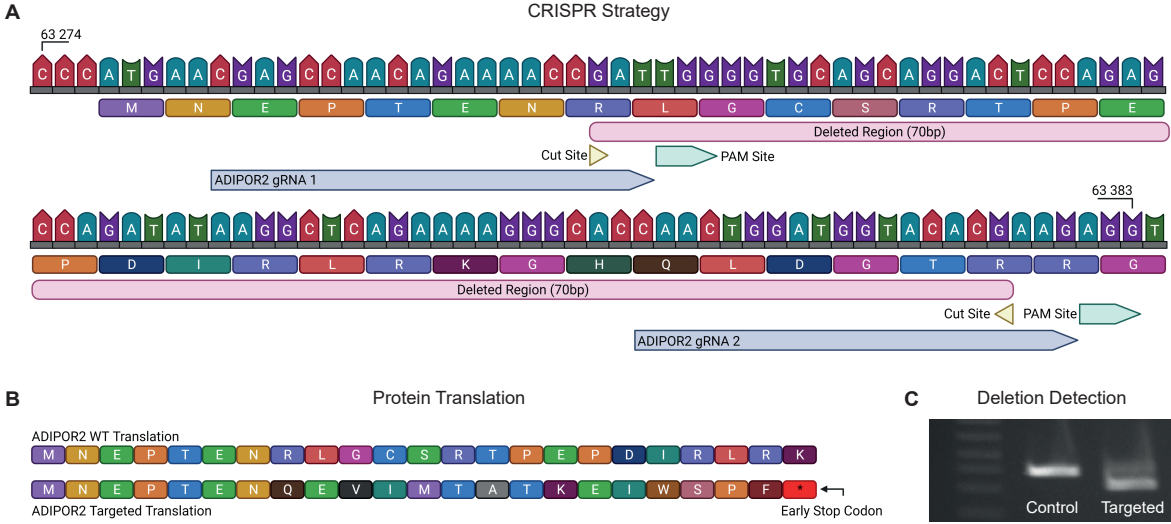


Fig. S1. Generation of human immortalized pre-adipocyte ADIPOR2 KO pool. A, CRISPR strategy highlighting the editing of exon 1 of *ADIPOR2* locus in human immortalized pre-adipocytes. B, predicted protein translation of the ADIPOR2 N terminus in WT (above) and edited (below) highlighting a premature stop codon in red. C, DNA validation of the edited ADIPOR2 KO pre-adipocyte pool showing the WT pool PCR band (left) and the ADIPOR2 KO PCR band (right).

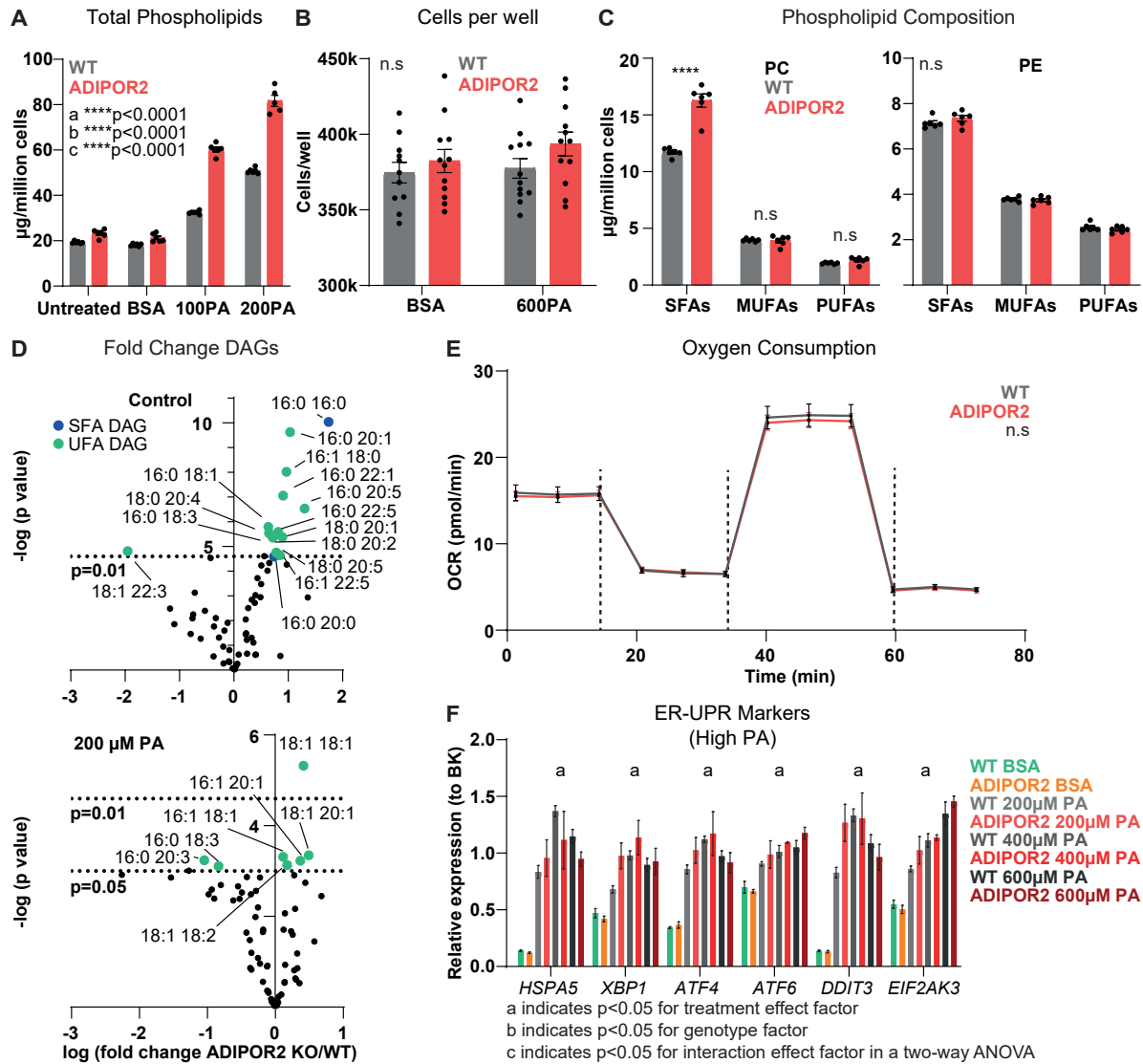


Fig. S2. Lipidomics and functional readouts from the human pre-adipocytes. A, Total phospholipid levels in human WT and ADIPOR2 KO pre-adipocyte pools in full medium or full medium with either BSA, 100 μM PA or 200 μM PA for 24 h. (n=5 for ADIPOR2 200 μM PA and n=6 for all other conditions). B, Representative amount of WT and ADIPOR2 KO pre-adipocytes per well in a confluent 6-well plate containing full medium treated with either BSA or 600 μM PA for 24 h. (n=12 for all conditions). C, Total phospholipid composition of PCs & PEs in WT and ADIPOR2 KO pre-adipocyte pools in full medium. (n=6 for all conditions). D, Volcano plots of DAG species of ADIPOR2 KO pre-adipocyte pools over WT in either full medium (control) or full medium with 200 μM PA for 24 h with color coding specifying saturation of lipid moieties. E, Seahorse based oxygen consumption rates (OCR) in human WT and ADIPOR2 KO pre-adipocyte pools in full medium. First dashed line indicates addition of oligomycin; second dashed line indicates addition of DNP, and third indicates addition of rotenone/antimycin A. (n>37 for all conditions). F, Relative expression of ER-UPR markers in human WT and ADIPOR2 KO pre-adipocyte pools in full medium with either BSA, 200 μM PA, 400 μM PA,

or 600 μ M PA for 24 h. (n=3 for all conditions). All graphs show means \pm SEM. In A & F, a indicates $p<0.05$ for treatment effect factor, b indicates $p<0.05$ for genotype factor and c indicates $p<0.05$ for interaction effect factor in a two-way ANOVA. In B & C, **** $p<0.0001$, and n.s indicates no significance from student's t-test comparisons. In E, n.s indicates no significance in a one-way ANOVA using repeated measures.

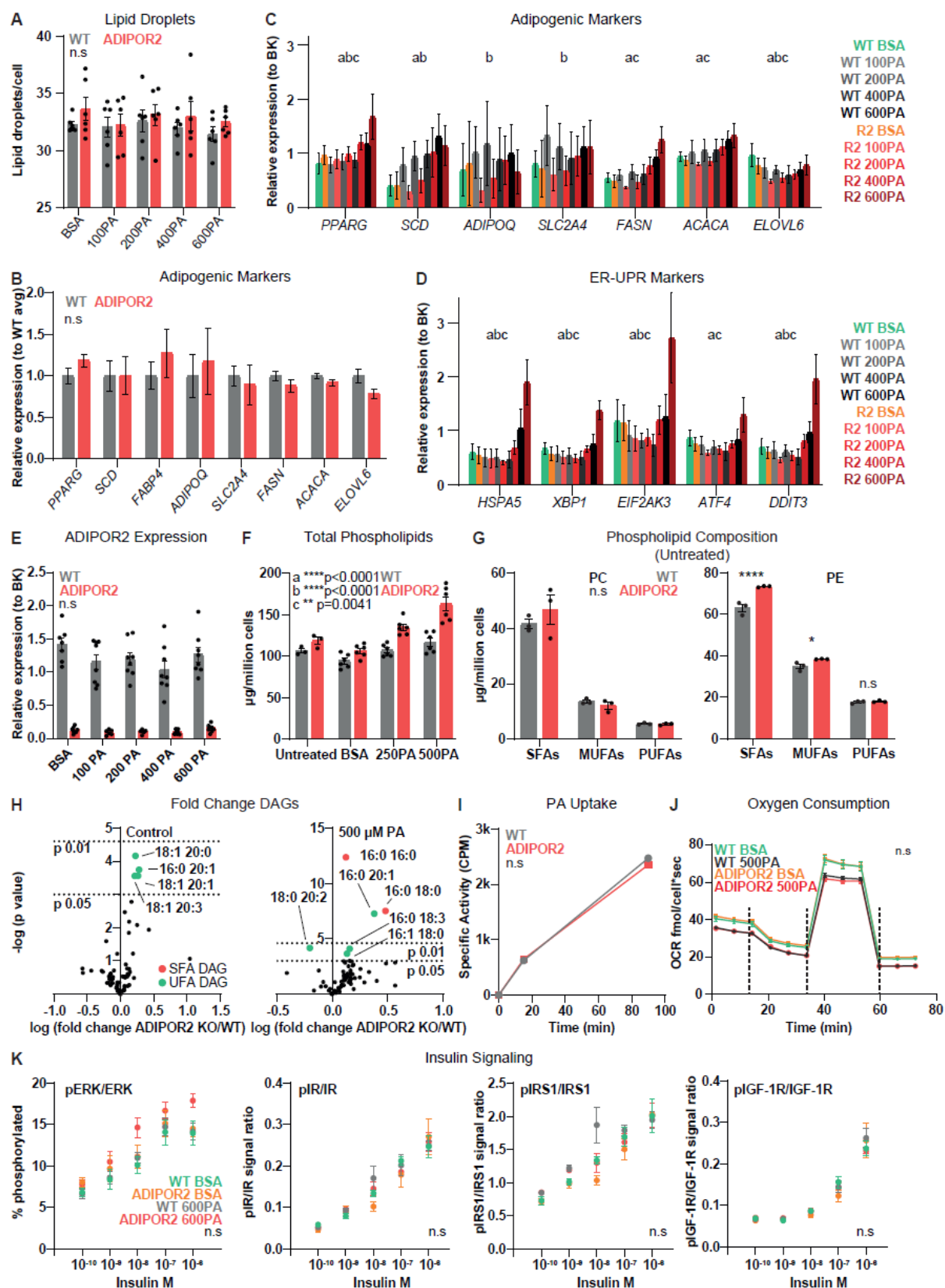


Fig. S3. Gene expression, lipidomics and functional readouts from the human adipocytes. A, Lipid droplets per cell analysis in human WT and ADIPOR2 KO adipocyte pools pre-treated in full medium with either: BSA, 100 µM, 200 µM, 400 µM or 600 µM PA for 24 h. (n=6 for all conditions). B, Relative expression of adipogenic markers in human WT and ADIPOR2 KO pool adipocytes. (n=6 for all

conditions). C, Relative expression of indicated adipogenic and lipogenic transcripts in human WT and ADIPOR2 KO adipocyte pools pre-treated in full medium with either: BSA, 100 μ M, 200 μ M, 400 μ M or 600 μ M PA for 24 h. (n=8 for all conditions). D, Relative expression of indicated ER-UPR transcripts in human WT and ADIPOR2 KO adipocyte pools pre-treated in full medium with either: BSA, 100 μ M, 200 μ M, 400 μ M or 600 μ M PA for 24 h. (n=8 for all conditions). E, Relative expression of *ADIPOR2* transcript in human WT and ADIPOR2 KO adipocyte pools pre-treated in full medium with either: BSA, 100 μ M, 200 μ M, 400 μ M or 600 μ M PA for 24 h. (n=8 for all conditions). n.s: no significant changes in WT with respect to treatment. F, Total phospholipid levels in human WT and ADIPOR2 KO adipocytes in full medium or full medium with either: BSA, 250 μ M or 500 μ M PA for 24 h. (n=6 for all other conditions). G, Total phospholipid composition of PCs and PEs in human WT and ADIPOR2 KO adipocytes in full medium. (n=6 for all conditions). H, Volcano plot of DAG species of ADIPOR2 KO adipocytes over WT in either full medium (control) or full medium containing 500 μ M PA for 24 h with color coding specifying saturation of lipid moieties. I, Cellular uptake of radiolabeled PA over time in human WT and ADIPOR2 KO adipocytes in full medium. (n=4 for all conditions). J, Seahorse based oxygen consumption rates (OCR) of human WT and ADIPOR2 KO adipocyte pools pre-treated in full medium with either BSA or 500 μ M PA. First dashed line indicates addition of oligomycin; second dashed line indicates addition of DNP, and third indicates addition of rotenone/antimycin A. (n=14 for all conditions). n.s.: no significant differences between BSA treatments nor PA treatments. K, Insulin-stimulated phospho-ERK/total ERK, phospho-IR/total IR, phospho-IRS1/total IRS1, and phospho-IGF-1/total IGF-1 ratio in human WT and ADIPOR2 KO adipocytes in either full medium or full medium containing 600 μ M PA for 24 h. Data expressed as % phosphorylation for pERK/ERK and as signal ratio for remaining graphs. (n=3-4 for all conditions). All graphs show means \pm SEM. In A, a indicates $p < 0.05$ for treatment effect factor, b indicates $p < 0.05$ for genotype factor, c indicates $p < 0.05$ for interaction effect factor, n.s indicates no significance in a two-way ANOVA. In B, n.s. indicates no significance from multiple t-test comparison (one per row). In C, D & F, a indicates $p < 0.05$ for treatment effect factor, b indicates $p < 0.05$ for genotype factor and c indicates $p < 0.05$ for interaction effect factor in a two-way ANOVA. In E, n.s indicates no significance in a one-way ANOVA comparing treatments for WT. In G, * $p < 0.05$, **** $p < 0.0001$, and n.s indicates no significance from student's t-test comparisons. In I & J, n.s indicates no significance from a one-way ANOVA with repeated measures with comparisons done for each treatment in J. In K, n.s. indicates no repeatable significance from a one-way ANOVA using repeated measures with comparisons done for each treatment.

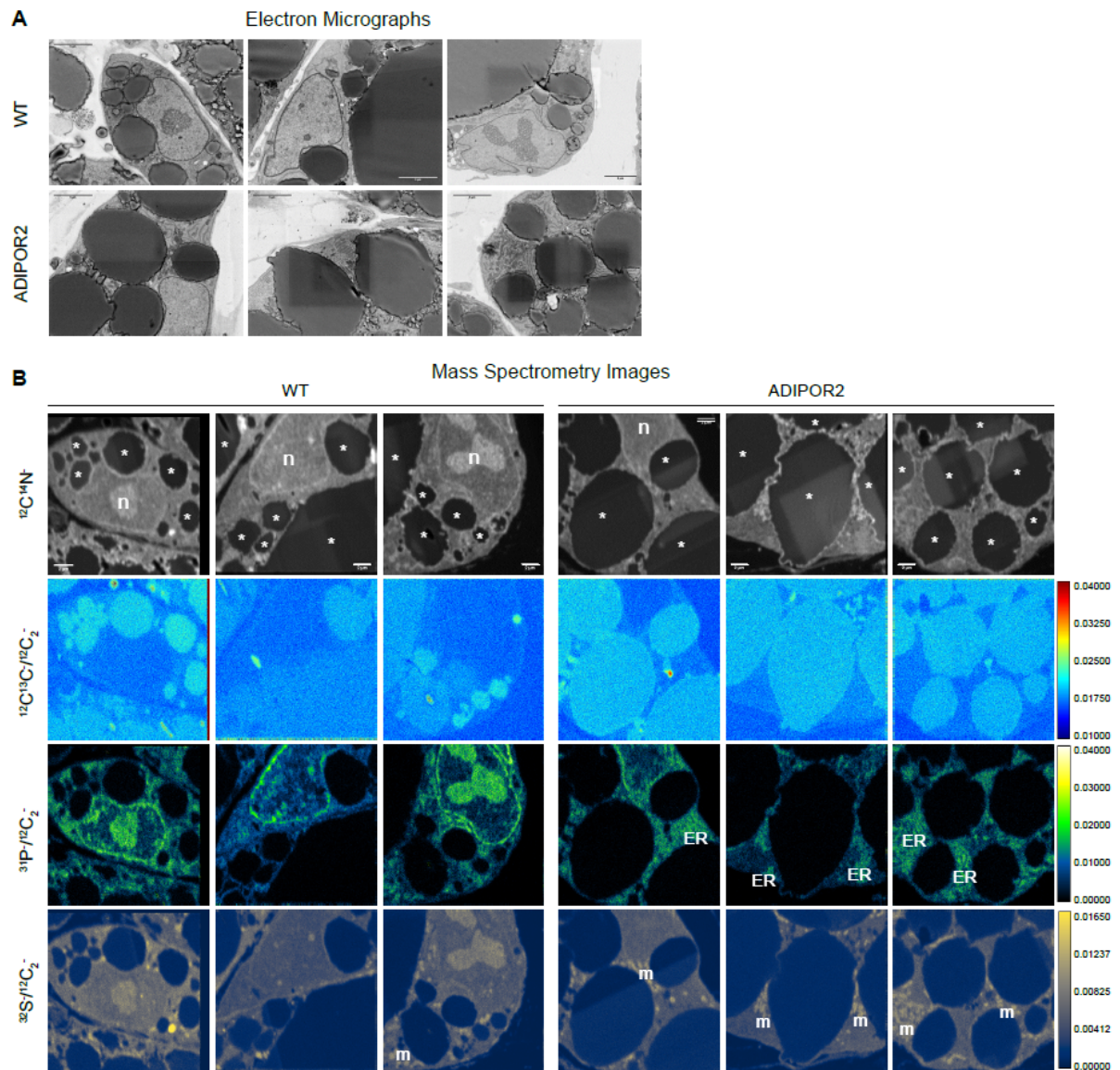


Fig. S4. Cellular distribution of ^{13}C -labeled palmitate in WT and ADIPOR2 KO adipocytes. A, Electron micrographs of the cells selected for NanoSIMS imaging. Scale bars: 5 μm. B, NanoSIMS imaging of adipocytes following treatment with $[\text{u}^{13}\text{C}]$ palmitate (n=3 cells per condition). $^{12}\text{C}^{14}\text{N}^-$ NanoSIMS images were used to visualize the cell morphology where lipid droplets (*) appear “black”. $^{12}\text{C}^{13}\text{C}^-/^{12}\text{C}_2^-$ ratio images revealed ^{13}C enrichments in lipid droplets and cytosol. $^{31}\text{P}^-/^{12}\text{C}_2^-$, $^{32}\text{S}^-/^{12}\text{C}_2^-$ ratio images combined with SEM micrographs (A) allow to identify specific organelles (mitochondria “m”, endoplasmic reticulum (ER)). Scale bars: 2 μm. C, Difference in $\delta^{13}\text{C}$ enrichments for different subcellular compartments for both WT and ADIPOR2 KO adipocytes. C shows means \pm SEM, each dot represents one region of interest (ROI), n=3 cells per genotype. In C, *p<0.05 from Welch’s t-test.

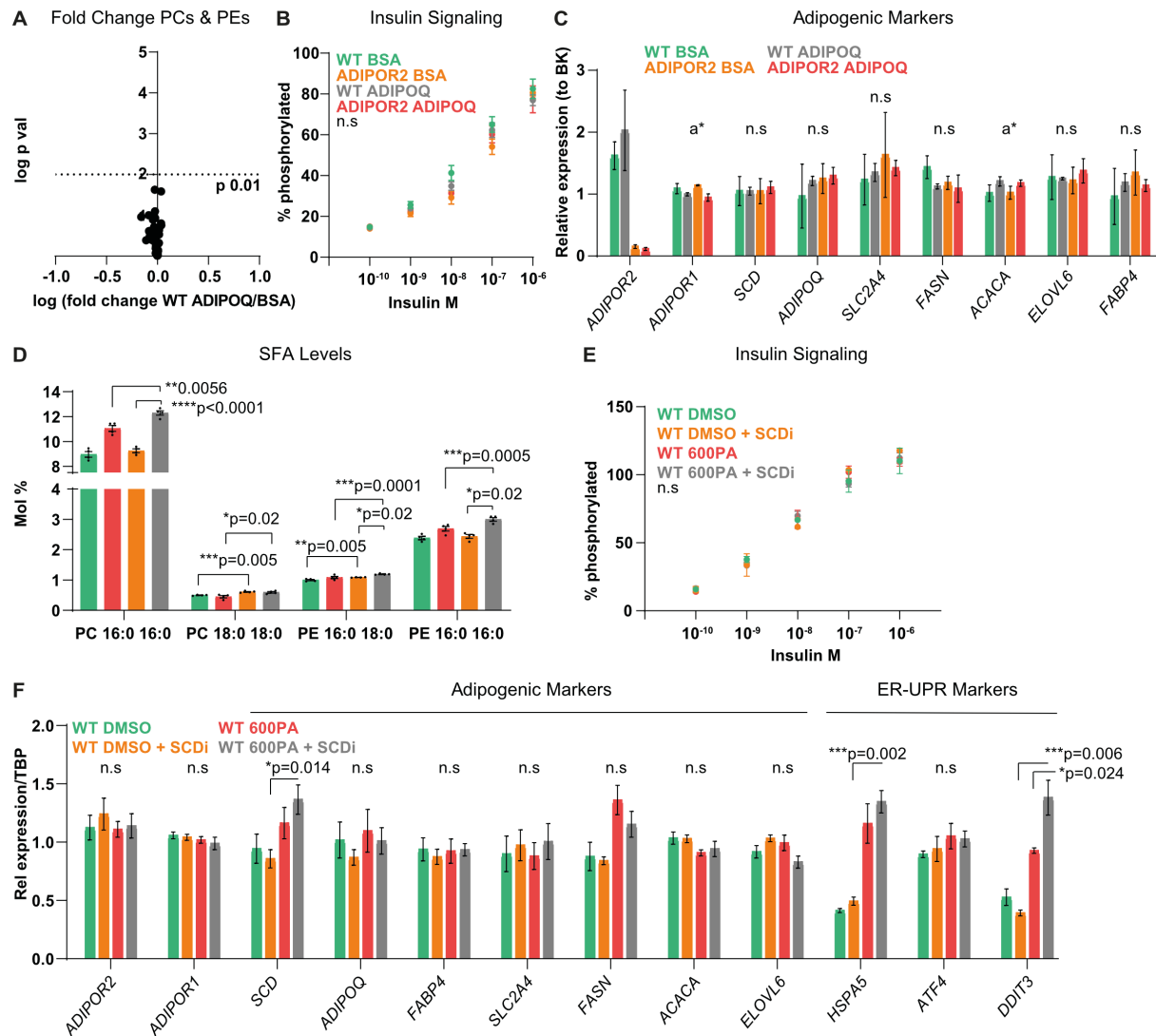


Fig. S5. The effects of exogenous adiponectin and SCD inhibition on adipocyte phospholipid composition and insulin signalling. A, Volcano plot of 55 PC and PE species species of human WT adipocytes in either full medium or full medium containing 6µg/ml recombinant adiponectin for 24 h. B, Insulin-stimulated phospho-ERK/total ERK ratio in human WT and ADIPOR2 KO adipocytes in either full medium or full medium containing 6µg/ml recombinant adiponectin for 24 h. Data expressed as % phosphorylation for pERK/ERK. (n=3-4 for all conditions). C, Relative expression of indicated adipogenic and lipogenic transcripts in human WT and ADIPOR2 KO adipocytes pre-treated in either full medium or full medium containing 6µg/ml recombinant adiponectin for 24 h. (n=4 for all conditions). D, Mol % of SFA-containing PCs and PEs in human WT pre-treated in full medium containing either: DMSO, 1 µM SCD inhibitor, 600 µM PA, or 600 µM PA plus 1 µM SCD inhibitor for 24 h. (n=4 for all conditions). E, Insulin-stimulated phospho-ERK/total ERK ratio in human WT adipocytes pre-treated in full medium containing either: DMSO, 1 µM SCD inhibitor, 600 µM PA, or 600 µM PA plus 1 µM SCD inhibitor for 24 h. (n=4 for all conditions). F, Relative expression of indicated adipogenic, lipogenic and ER-UPR transcripts in human WT adipocytes pre-treated in full

medium containing either: DMSO, 1 μ M SCD inhibitor, 600 μ M PA, or 600 μ M PA plus 1 μ M SCD inhibitor for 24 h. (n=4 for all conditions). All graphs show means \pm SEM. In B & E, n.s. indicates no repeatable significance from a one-way ANOVA using repeated measures with comparisons done for each treatment. In C & F, a indicates $p < 0.05$ for treatment effect factor, b indicates $p < 0.05$ for genotype factor and c indicates $p < 0.05$ for interaction effect factor in a two-way ANOVA. In D, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ from student's t-test comparisons.

ADIPOR2 KO adipocytes pre-treated in full medium with either: BSA, 250 μ M or 500 μ M PA for 24h. (n=5 for all conditions). F, Total phospholipid levels in mouse WT and ADIPOR2 KO adipocytes in full medium with either: BSA, 250 μ M or 500 μ M PA for 24h. (n=4 for WT BSA, WT 500 μ M PA and ADIPOR2 KO 500 μ M PA. n=5 for all other conditions). G, Total DAG levels in mouse WT and ADIPOR2 KO adipocytes in full medium with either: BSA, 250 μ M or 500 μ M PA for 24h. (n=4 for WT BSA and WT 500 μ M PA. n=5 for all other conditions). H, Volcano plots of DAG and TAG species of mouse ADIPOR2 KO adipocytes over WT pre-treated in full medium with either BSA or 500 μ M PA for 24h with color coding specifying neutral lipid classes. I, Relative expression of ER-UPR transcripts in mouse WT and ADIPOR2 KO adipocytes pre-treated in full medium with either: BSA, 250 μ M or 500 μ M PA for 24h. (n=5 for all conditions). All graphs show means \pm SEM. In B, C, D, E, F & H, n=different mice per genotype. In A, C, D, E, F & H, a indicates $p < 0.05$ for treatment effect factor, b indicates $p < 0.05$ for genotype factor, c indicates $p < 0.05$ for interaction effect factor, and n.s. indicates no significance in a two-way ANOVA. In B, n.s indicates no significance in a one-way ANOVA comparing treatments for WT.

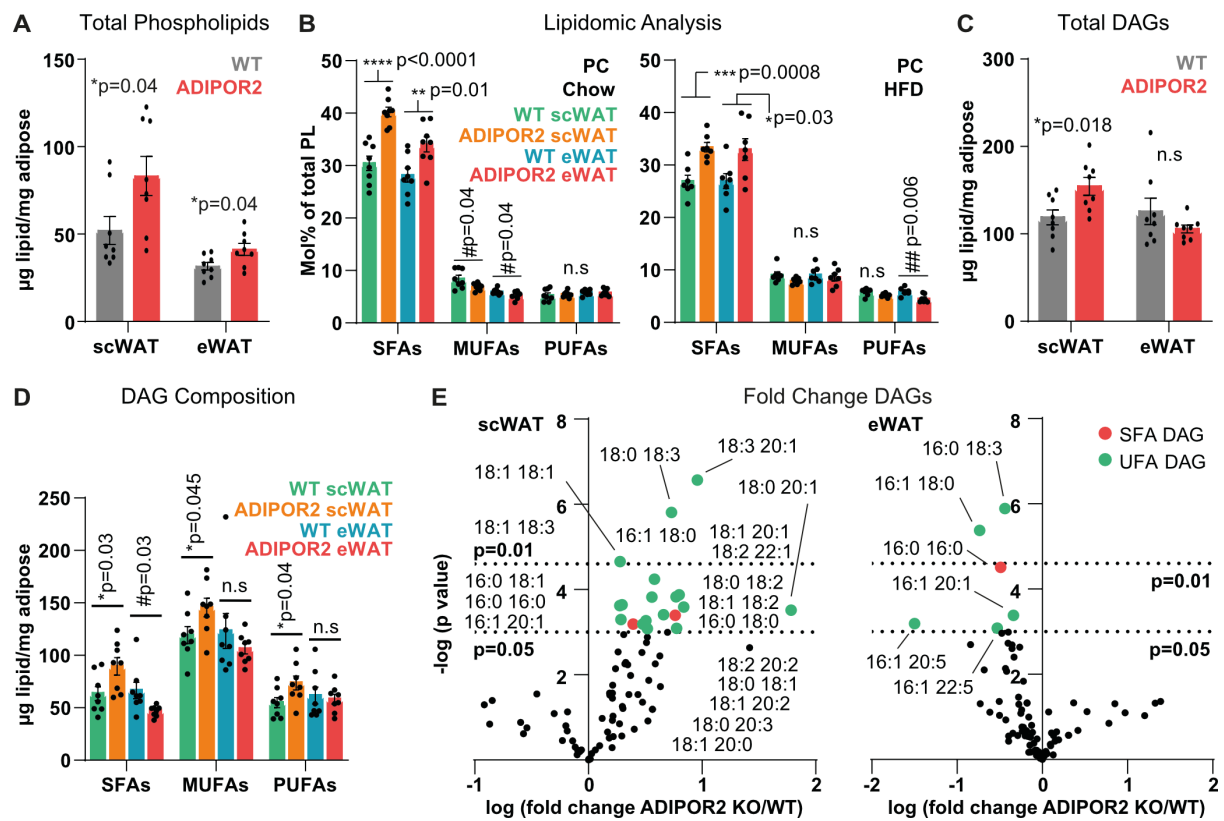


Fig. S7. Neutral lipid analysis of white adipose tissues isolated from chow-fed WT and ADIPOR2 KO mice. A, Total phospholipid levels in WT and ADIPOR2 KO mice adipose tissue at 6 months of age. (n=8 for all conditions). B, Total PC phospholipid composition normalized to total phospholipids of WT and ADIPOR2 KO mice adipose tissue at 6 months of age. (n=8 for all conditions). C, Total DAG levels in WT and ADIPOR2 KO mice adipose tissue after 16 weeks on chow. (n=8 for all conditions). D, Total DAG composition of WT and ADIPOR2 KO mice adipose tissue at 6 months of age. (n=8 for all conditions). E, Volcano plots of DAG species of WT and ADIPOR2 KO mice adipose tissue at 6 months of age. Fold change is shown as ADIPOR2 KO over WT with color coding specifying saturation of lipid moieties. (n=8 for all conditions). All graphs show means \pm SEM. Each n is one mouse. In A, B, C & D, */# $p < 0.05$ and n.s indicates no significance from student's t-test comparisons. * indicates an increase in ADIPOR2 KO compared to WT and # indicates a decrease in ADIPOR2 KO compared to WT.

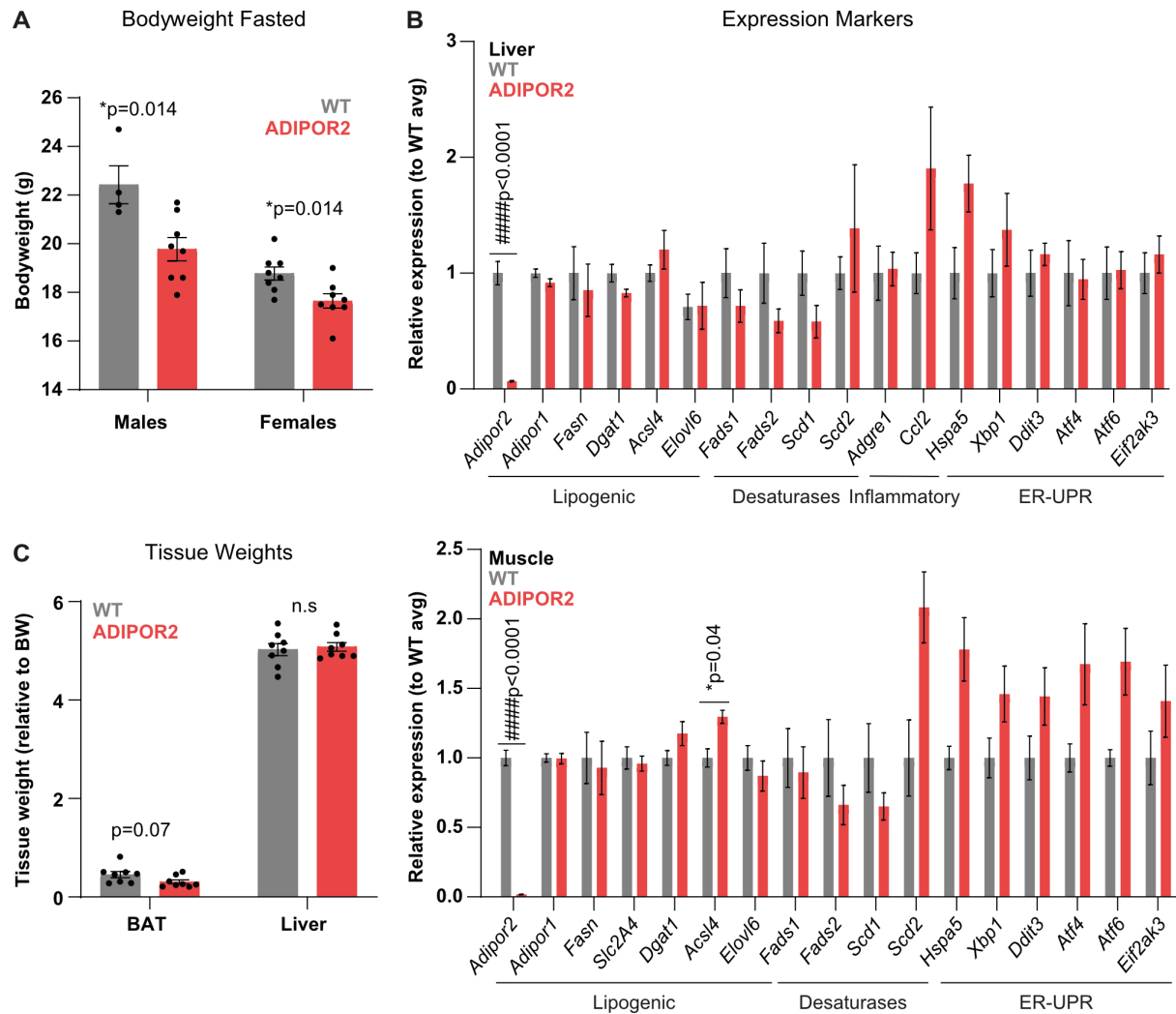


Fig. S8. Gene expression, body- and tissue weights of chow-fed WT and ADIPOR2 KO mice. A, Fasted bodyweight of WT and ADIPOR2 KO mice prior to acute insulin treatment. (n=5 male WT mice, n=8 male ADIPOR2 KO mice, n=6 for female mice, all mice were 12 weeks of age). B, Relative expression of indicated lipogenic, desaturase, inflammatory, and ER-UPR markers in liver and muscle of WT and ADIPOR2 KO mice at 6 months of age. Data is expressed as fold change of ADIPOR2 KO from WT averages. (n=8 for all conditions). C, BAT and liver tissue weight at termination of WT and ADIPOR2 KO mice adipose tissue at 6 months of age. (n=8 for all conditions). All graphs show means \pm SEM. Each n is one mouse. In A, *p<0.05, and n.s indicates no significance from student's t-test comparisons. In B, #####p<0.0001, *p<0.05, adjusted p-values from multiple t-test comparison (one per row) using the Holm-Sidak method. * indicates an increase in ADIPOR2 KO compared to WT and # indicates a decrease in ADIPOR2 KO compared to WT.

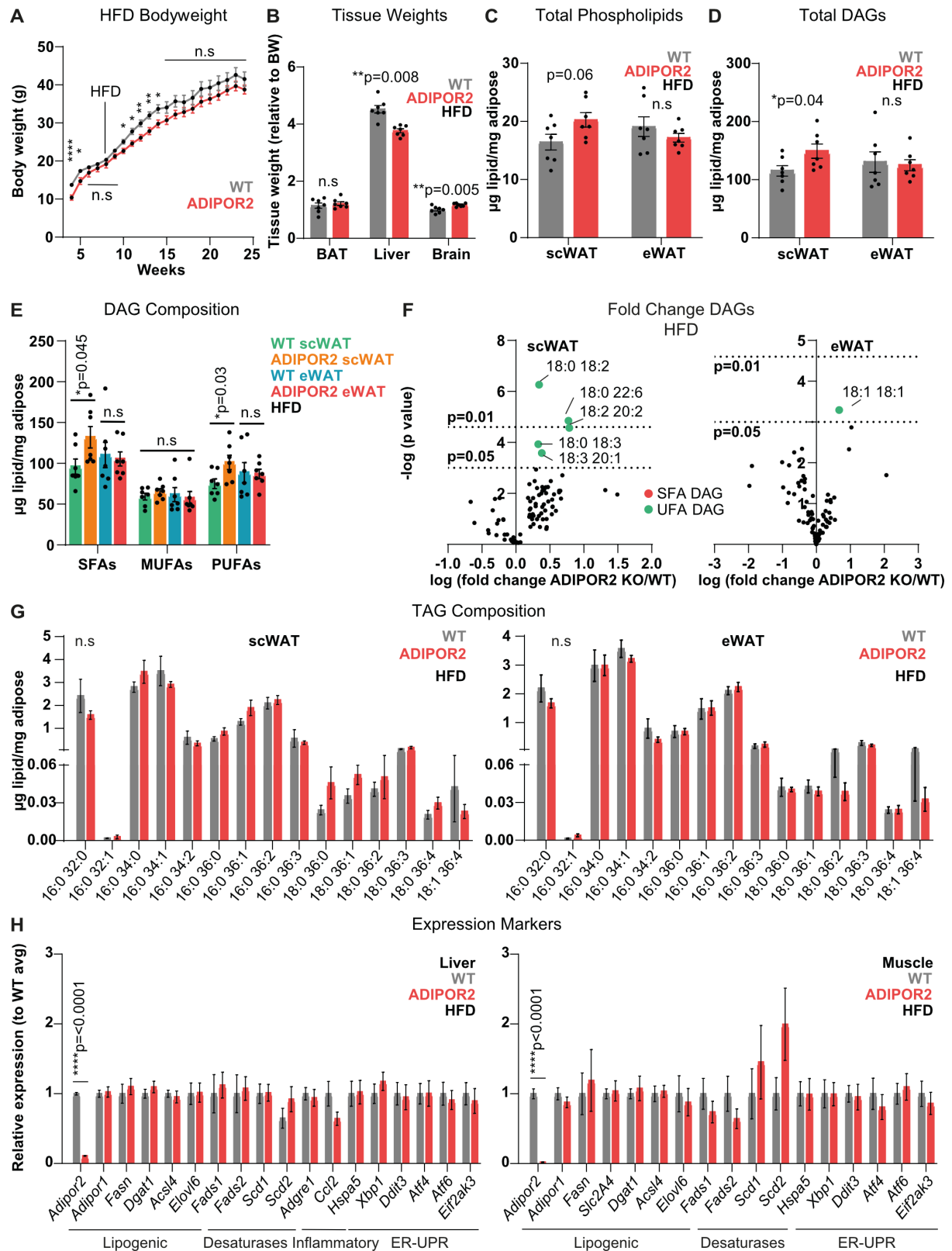


Fig. S9. Body- and tissue weights, neutral lipid analysis and gene expression for HFD- fed WT and ADIPOR2 KO mice housed in thermoneutrality. A, Body weight over time in WT and ADIPOR2 KO mice during HFD intervention which was introduced at age 8 weeks. Mice were transferred to thermoneutrality at 4 weeks of age. (n=7 for all conditions). B, BAT, liver and brain tissue

weight at termination of WT and ADIPOR2 KO mice adipose tissue after 16 weeks on a HFD. (n=7 for all conditions). C, Total phospholipid levels in WT and ADIPOR2 KO mice adipose tissue after 16 weeks on a HFD. (n=7 for all conditions). D, Total DAG levels in WT and ADIPOR2 KO mice adipose tissue after 16 weeks on a HFD. (n=7 for all conditions). E, Total DAG composition of WT and ADIPOR2 KO mice adipose tissue after 16 weeks on a HFD. (n=7 for all conditions). F, Volcano plot of DAG species of WT and ADIPOR2 KO mice adipose tissue after 16 weeks on a HFD. Fold change is shown as ADIPOR2 KO over WT with color coding specifying lipid saturation of lipid moieties. (n=7 for all conditions). G, Abundance of TAG species containing SFAs in WT and ADIPOR2 KO mice adipose tissue after 16 weeks on a HFD. (n=7 for all conditions). H, Relative expression of indicated lipogenic, desaturase, inflammatory, and ER-UPR markers in liver and muscle of WT and ADIPOR2 KO mice after 16 weeks on a HFD. Data is expressed as fold change of ADIPOR2 KO from WT averages. (n=7 for all conditions). All graphs show means \pm SEM. Each n is one mouse. In A, ****p<0.0001, **p<0.01 as adjusted p-values from a one-way ANOVA using repeated measures using the Holm-Sidak method. In B, C, D & E, **p<0.01, *p<0.05, and n.s indicates no significance from student's t-test comparisons. In G & H, ****p<0.0001 adjusted p-value and n.s. indicates no significance from multiple t-test comparison (one per row) using the Holm-Sidak method.

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