Supplementary Information

Methods

1. Materials and Reagents

Deionized water was produced by Barnstead™ MicroPure™ Water Purification system (ThermoFisher Scientific, serial No. 42034239) with the resistance value as 18.2 MΩ·cm at 23.5 °C and particles less than 0.2 μm. All of buffers in the experiments were prepared with deionized water. SYLGARD® 184 silicone elastomer kits (Dow Corning, Midland, MD) were used to make polydimethylsiloxane (PDMS) devices, with the kit containing elastomer base and curing agent. Silicon wafers were purchased from the Polishing Corporation of America (Santa Clara, CA). Negative photoresists, SU-8 2015 and SU-8 2050, were from Microchem (Westborough, MA); and positive photoresist, AZ 40XT-11D, was obtained from AZ Electronic Materials USA (Somerville, NJ). Heat inactivated HyClone™ Fetal Bovine Serum (FBS) was from Gel Healthcare Life Sciences, and Hanks' Balanced Salt Solution (HBSS) was purchased from Lonza. D-glucose, HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonicacid), penicillin-streptomycin, NaCl, $CaCl_2{\cdot}2H_2O$, KCl, $MgCl_2{\cdot}6H_2O$, resorufin sodium salt, cholortrimethylsilane, agarose (ultra-low gelling temperature, molecular biology grade), human insulin solution, 3-Isobutyl-1-methylxanthine, dexamethasone, and Glycerol Assay Kit (catalog#: MAK117-1KT) were purchased from Sigma-Aldrich (St. Louis, MO). Human insulin concentration was confirmed by protein absorbance at 280 nm (A_{280}) using a NanoDrop 1000 spectrophotometer (ThermoFisher). Isoproterenol hydrochloride (>98%, purity), Bovine serum albumin (BSA), and Accutase cell detachment solution were from VWR (West Chester, PA). Dulbecco's Modified Eagle Medium (DMEM; low glucose, L-glutamine, sodium pyruvate, and phenol red; catalog # 11885084), DMEM (no glucose, no glutamine, no phenol red; catalog # A11430-01), MEM Non-Essential Amino Acids Solution (100X; catalog # 111400502), Sodium Pyruvate (100 mM; catalog # 11360070), Fetal Bovine Serum (qualified, USDA-approved regions; catalog # 10437010), Penicillin-Streptomycin (10,000 U/mL; catalog # 15140122), and Dulbecco's Phosphate Buffered Saline (DPBS) were all purchased from ThermoFisher Scientific (Grand Island, NY). Pico-Surf surfactant (2% in Novec 7500) was purchased from Dolomite Microfluidics (Norwell, MA). Novec™ 7500 Engineered Fluid (HFE 7500) was obtained from 3M. Connexin 43 (gap junction) monoclonal antibody (CX-1B1) labeled by Alexa Fluor 488 (cat. # 138388), Image-IT Fixative solution (4% formaldehyde in PBS, methanol-free), proteinase K solution (20 mg/mL), HCS LipidTox Green Neutral Lipid Stain reagent (1000X) for cellular imaging, HCS LipidTox Red phospholipidosis detection reagent (10000X), 5-carboxyfluorescein diacetate acetoxymethyl ester (5-CFDA AM) were also purchased from ThermoFisher. TH Antibody

(F-11) Alexa Fluor™ 594 (catalog # sc-25269), 6-Hydroxydopamine hydrobromide (CAS 636-00-0), and Nile Red (CAS: 7385-67-3) were obtained from Santa Cruz Biotechnology (Dallas, TX).

2. Fabrication of master wafers

Microdevice photomasks were designed in Adobe Illustrator and printed at Fineline Imaging (Colorado Spring, CO) at 50,800 DPI resolution. The microfluidic device design includes two layers: the pneumatic valve control channels are on the bottom layer, and the fluidic flow channels are in the upper layer. Three masks were designed to allow two depths of fluidic channels (black and orange channels in **Fig. 1A**) and one pneumatic control layer (red channels in **Fig. 1A**). Master wafers for molding these devices were fabricated by commonly used photolithography. Silicon wafers were ready to use after shaking in diluted sulfuric acid (1 M) at 220 rpm for 30 min, washing and shaking in D.I. water for 30 min, then drying by an air stream.

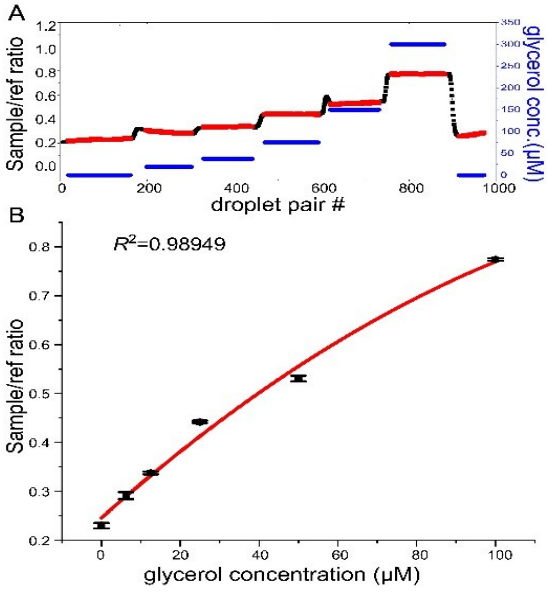

Figure S-1. Example calibration of the fluorescent enzyme assay specific for glycerol in droplets. **A)** The sample-to-reference ratio (from raw data in **Fig. S-4**) for each droplet pair responded quantitatively to the different concentrations of glycerol loaded into the cell culture inlet. Red labeled data were used in the standard curve calculation. **B)** Corresponding calibration curve, which was fitted to a quadratic polynomial model, where R^2 = 0.98949. A similar calibration was carried out at least once per day during the cell or tissue sampling expeirments.

For the pneumatic control layer, negative photoresist (SU-8 2015) was spin-coated onto the wafer at 2000 rpm for 45 seconds (~20 μm thickness), followed by soft baking on a hotplate for 5 min at 95 °C, then passive slow cooling to room temperature. UV exposure through the photomask was carried out for 2 min (~ 200 mJ/cm²) by an in-house built ultraviolet lithography light source[58], and the wafer was then hard baked for 5 min at 95 °C then developed in SU-8 developer solution for 7 min, making it ready for molding PDMS devices.

For the fluidic flow layer, both negative and positive photoresists were patterned on the same silicon wafer to give a two-depth master. As shown in **Fig. 1A**, the black channels represent the channels that cross over the valves, which are semi-circular in cross-section to allow valve closing; the mold for these channels was fabricated by AZ40XT-11D. Downstream incubation channels (orange) were made by molding from SU-8 2050 of rectangular cross-section, which is more appropriate for imaging droplets. The negative photoresist layer was fabricated first. SU-8 2050 was spin-coated on the pretreated wafer at 3000 rpm for 45 s (~50 μm thickness), followed by a soft bake on a hotplate for 5 min at 95 °C, then a slow cooling step. UV exposure through the photomask was carried out for 2 min (~ 200 mJ/cm²), a hard bake was done for 5 min at 95 °C, and the wafer was then treated by SU-8 developer for 7 min. After this pattern was developed, the wafer was put in an oven for 30 min at 60 °C. The second layer was then fabricated using positive photoresist (AZ40XT-11D). This photoresist was passively warmed to room temperature, spin-coated onto the wafer at 2000 rpm for 45 s, then soft baked on a hotplate (5 min at 65 °C, 5 min at 95 °C, and 5 min at 115 °C). The wafer was then allowed to cool down to room temperature very slowly. The fluidic photomask was aligned with the SU-8 patterns on the AZ photoresist-coated wafer, and this was exposed to UV for 90 s (~ 153 mJ/cm²). A post-exposure bake was done on the hotplate for 5 min at 65 °C, 5 min at 95 °C, and 90 s at 105 °C. This wafer was also cooled passively to room temperature then developed in AZ developer solution for 6 min. The last step was the reflow of AZ photoresist to create a semi-circular cross-section, which was done by heating the wafer on a hotplate for 10 min at 120 °C under the partial cover of petri dish. Ultimately, the negative photoresist layer was ~50 μm in thickness (orange channels in **Fig. 1A**), and the positive photoresist layer was ~40 μm thick (black channels in **Fig. 1A**). After the photolithography was done, the two wafer masters were ready to use for device molding.

3. Microchip fabrication

The photoresist-patterned silicon molds were exposed to chlorotrimethylsilane (TMCS) vapor for 30 min before the soft lithography of PDMS. One batch of PDMS precursor and curing agent was mixed at the ratio of 20:1, 10.5 g in total, and degassed and then spin coating on the valve channel master in the speed of 2400 rpm for 45 s. At the same time, another batch of PDMS prepolymers were mixed at the ratio of 5:1, 36 g in total, and degassed and poured on the flowing channel

master. Both were baked at 60 °C for 30 min in the oven, and the flow channel bulk chip were cut and aligned to the valve channel under observation by microscopy at 3X magnification. The PDMS was baked for another 4 hours in the oven. After PDMS stamps were peeled off the wafers, inlets and outlets punched with Miltex disposable biopsy punches, then the chips were washed by methanol, air dried, and irreversibly bound to the a cover slide (24 mm x 40 mm with 0.13-0.16 mm thickness) by plasma oxidization. The two-layer microchips were ready to use after thermally aging at 60 °C overnight to minimize uncured PDMS monomer leakage.

4. 3D-printed templates for the fabrication of cell culture reservoirs

3D-printed templates and inserts used in device fabrication and experiments were designed in the 3D modelling computer program, SketchUp 2017, and printed by a MakerBot Replicator 2 with PLA (polylactic acid) filament (HatchBox PLA, 1.75 mm diameter). The CAD design of 3D molds for the fabrication of cell culture reservoirs is shown in **Fig. 1B** (gray). During microchip fabrication (as described above), a 3D template was aligned with the wafer mold for the flow channel after pouring PDMS over the wafer, and the entire assembly was cured in the oven. After the bulk PDMS was peeled off from the 3D template and wafer, the cell culture reservoir was formed with 1.5-mm thick PDMS below the reservoir. A 0.75-mm ID punch (69039-07, Electron Microscopy Sciences, Hatfield, PA) was used to create a via between the tissue trapping/culturing reservoir and the inlet microfluidic channel for droplet sampling.

5. Automated flow control system with pneumatic valving

The 18 pneumatic control valves in the microfluidic chip were driven by solenoid switches (LHDA0533115H, the Lee Company, Westbrook, CT) assembled on a metal manifold and under the control of a data acquisition device (PCI-6259, National Instrument, Austin, TX) driven by LabVIEW programming software. A valve driver circuit was used as the buffer and current amplifier between the PCI and solenoid switches. A house nitrogen gas source was used to actuate these pneumatic solenoid valves by 26-psi pressure adjusted by a pressure regulator. The control valves on the microchip were connected to the corresponding solenoid valve with 90-degree angled 22-gauge blunt syringe needles (Jensen Global JG22-0.5HPX-90, Santa Barbara, CA) through Tygon microbore tubing (0.02″ I.D. X 0.06″ O.D., Cole-Parmer, Vernon Hills, IL). The valve inlets interfaced with the control system were punched by 0.75-mm ID punch (69039-07, Electron Microscopy Sciences, Hatfield, PA). An in-house written LabVIEW application was used to control the actuation of the valves. When a valve was set in closed status, the solenoid switch was activated to pressurize the nitrogen gas into the dead-end channel of the control layer to deflect the PDMS membrane up and close the fluidic

channel in the upper layer (i.e. push-up style valves). Control layer channels were dead-end-filled with deionized water to avoid air permeation through the PDMS membranes and into fluidic channels.

Microvalve pumping was controlled through LabVIEW using a 5-step pumping cycle, shown in **Fig. S-2** and supplementary video S-1. A pair of sample and reference droplets were formed by cycling through sample pumping, oil pumping, reference pumping, and oil pumping sequentially. The droplet size was controlled precisely by on-chip valves [25, 31], using the number of pumping cycles, and was chosen with consideration for the width of the incubation channel, since a widened "pancake" shape of droplets in the incubation channel facilitates microscopic imaging through a constant optical path length. Droplets with smaller size than the channel width flowed through the channel at different rates, and droplet order could be undesirably altered with small droplets. Since the volume for each pumping cycle is highly consistent, droplets could be generated at precise volumes. As shown in **Fig. S-5**, droplet sizes during a 50-minute test experiment were highly stable, with a deviation less than 1.5%. For all experiments reported in the paper, sample and reference solutions were each pumped through LabVIEW control to give 0.287 droplets s^{-1} (one droplet every 3.48 s), and oil segments were pumped at 0.575 segments s^{-1} (two segments every 3.48 s). This programming produced droplets with average volumes of 2.58 ± 0.04 nL (%CV = 1.5%) for the sample droplets and 2.18 ± 0.04 nL for the reference droplets (different volumes were intentional).

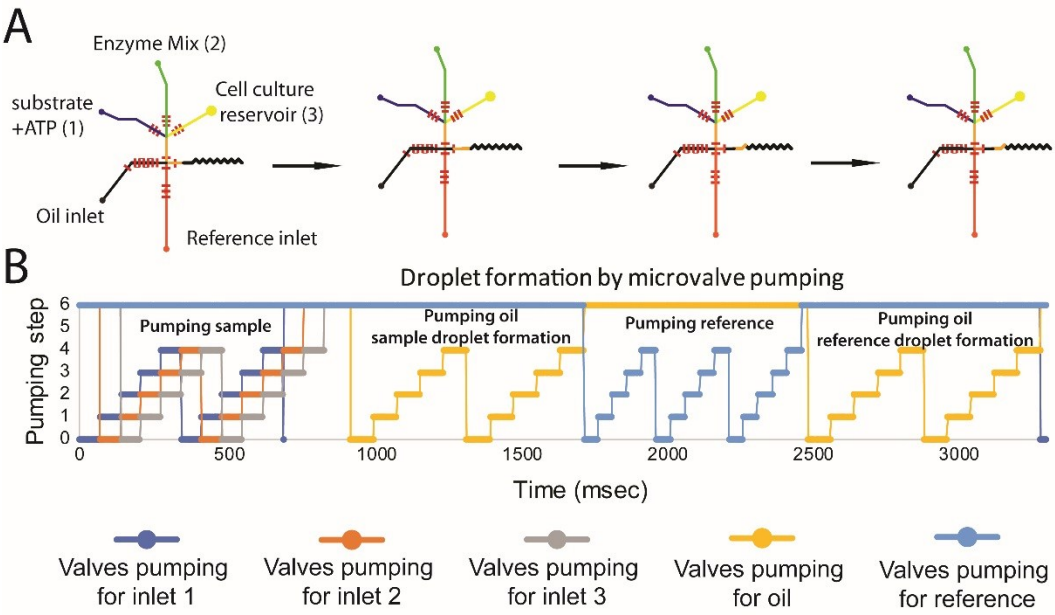

Figure S-2. In-house written LabVIEW application was run to control the formation of continuous pairs of sample and reference droplets. **A)** Schematic figure shows the open/closed states of microvalves in control layer for droplet formation. **B)** 5-step pumping was used to control the solution flowing into the channel sequentially to generate a pair

of droplets. Each micropump included three valves, and the pumping steps for each group of microvalves are labelled from 0 to 4, representing the 001,101, 100,110,111, (1 = closed and 0 = open; 5 is 000 and 6 means 111).

6. Extraction of murine epididymal adipose tissue (eWAT)

C57BL/6J male mice of different types from Jackson Laboratories were used in this project. All animal experiments were done in compliance with relevant laws and institutional guidelines, and protocols (2017-3101) were approved by the institutional animal care and use committee (IACUC) of Auburn University. 9-, 12-, and 17-week old male mice; 20-week old wild-type mice; and 20-week old diet-induced obese (DIO) mice were used in the experiments. Epididymal white adipose tissue (eWAT) pads were extracted from mice as described in previous papers [19, 24]. After extraction, they were transferred into 10 mL of phosphate-HEPES buffer (10 mM HEPES, 135.3 mM NaCl, 2.2 mM $CaCl_2 \cdot 2H_2O$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 0.4 mM KH_2PO_4, 2.2 mM Na_2HPO_4, 0.4 mM D-glucose, 2% BSA, pH 7.4). Fat pads were placed in a 60 mm Petri dish with phosphate-HEPES buffer, and extra vasculature was removed by Iris micro dissecting scissors. 2 mm explants were punched from fat pads by a 2 mm sterile disposable biopsy punch and collected into a glass tube with 4 mL phosphate-HEPES buffer. The explants were washed by the buffer 3 times by centrifuging at 1000 rpm for 3 min. 3 mL buffer was removed by the syringe after centrifuging, and 3 mL fresh buffer was added for washing. Then the phosphate-HEPES buffer was changed to fat serum media (DMEM + low glucose and phenol red with 12% fetal bovine serum, 120 units/mL Nystatin, 120 units/mL Penicillin-Streptomycin and 1.2X MEM NEAA), and the explants were washed in the media another 2 times. After washing, the explants were placed in the sterile 96-well plate with 200 µL fat serum media and 3D-printed anchor to counteract the buoyancy of the fat tissue, with each piece of explant in one well. The explants were cultured in the 37 ^{o}C incubator with 5% CO_2, which can be maintained up to 7-10 days if the serum media is refreshed twice per day. The explant for each experiment was pretreated in high glucose (25 mM) and high insulin (2 nM) DMEM media without serum for 30 min before loading onto the microchip.

7. 3T3-L1 cell culture, differentiation, and encapsulation

3T3-L1 fibroblasts (CL-173) were obtained from American Type Culture Collection (ATCC, Manassas, VA) and cultured in DMEM media with 10% FBS to 100% confluency in 25 cm^2 cell culture flask. Following this, an adipogenic cocktail (DMEM +10% FBS with 0.5 mM 3-isobutyl-1-methylxanthine, 250 nM dexamethasone, and 8.9 µg/mL insulin) was used to culture the cells for 4 days, and then the differentiation media was removed and changed to insulin media (DMEM +10% FBS with 8.9 µg/mL insulin) to culture the cells for another 3 days. The 3T3-L1 adipocytes were used in the sixth

day after differentiation. Adipogenesis of cells was validated by visualization of lipid droplet accumulation and staining of lipid droplets (**Fig. S-7**). Applying our device that is geared toward sampling tissue explants instead for glycerol secretion sampling from a cell line required that the adipocyte cells be interfaced to the microfluidic reservoir. However, it is difficult to manipulate single cells or small numbers of cells in this way. For this reason, we developed a method—using the same microfluidic device—to encapsulate 3T3-L1 adipocyte cells into agarose droplets to form 3D spheroids of cells with around 200 μm diameter (see **Fig. S-8** and supplementary video 2). Once gelled, these spheroids could then be easily loaded into and removed from the cell culture reservoir by pipetting—akin to manipulating adipose explants[59] or pancreatic islets[25].

To accomplish encapsulation, culture media was first removed, and the cells were washed by 4 mL DPBS three times. 2 mL 1X LipidTox Green in DPBS was added into the flask and incubated at 37 °C for 30 min, and cellular imaging was collected through the FITC channel of a Nikon Ti-E fluorescence microscope. Staining solution was removed, and cells were washed by 4 mL DPBS three times. Cell detachment from the cell culture flask was induced by incubating cells with 2 mL Accutase cell detachment solution at 37 °C for 10 min. The cell suspension was transferred from the flask to 15 mL sterile conical tube and centrifuged at 1000 rpm for 3 min, then the upper layer of solution was aspirated, and the cell pellet was resuspended in 200 μL cell culture media. Around 10 μL of cell suspension was loaded in the second inlet on the microfluidic device. 0.125g agarose (ultra-low gelling temperature) was dissolved in 10 mL DPBS by heating in a microwave for 15 s to make 1.25% agarose solution. The agarose gel solution was loaded in the first and third inlets on the droplet-generating device (inlet 1 and inlet 3 in **Fig. 1**). HFE7500 with 0.5% Pico-Surf was utilized as the oil phase and loaded in the oil inlet. The in-house written LabVIEW program was set to run with 350 ms of sample pumping time for 20 sample pump cycles, and 450 ms of oil pump time for 2 oil pump cycles. The cells, now encapsulated in agarose droplets, were collected in the reference inlet which was set as the outlet in this specific application (long incubation channel not used here). The agarose droplet suspension was chilled on ice for 3 min to induce gel formation, then the droplet layer (upper layer) was transferred to adipocyte insulin media in a 3 mL Petri dish and conserved in the 37 °C incubator with 5% CO_2 until use. We found that adipocytes could be cultured within the gel for up to one week. Cell viability was usually reduced by about 15% after passing through the microvalves, which was estimated through cell viability test with a hemocytometer and 0.2% trypan blue for cell staining.

8. Glycerol release measurement, image acquisition, and analysis

The microfluidic chip was set up on the stage of a fluorescence microscope mounted with a Tokai Hit stage top incubator, with D.I. water back-filled into the microchip valve inlets. All experiments were operated at 37 °C in the

incubator. For the standard curve of glycerol assay on the microfluidic device, 1 µL of dye reagent and 1 µL of ATP from the glycerol assay kit were mixed with 135 µL glycerol assay buffer from the glycerol assay kit with 0.25% BSA, and 20 µL of the solution was added into the substrates inlet (**Fig. 1A**). 1 µL of enzyme mix reagent from the glycerol assay kit was mixed with 99 µL glycerol assay buffer with 0.25% BSA and 20 µL of the solution was loaded into the enzyme inlet (**Fig. 1A**). 85 µM resorufin was prepared in DMEM (no glucose, no glutamine, no phenol red) with 1% BSA and added into the reference inlet. HFE-7500 with 0.5% Pico-Surf was used as oil phase and loaded in the oil inlet (**Fig. 1A**, inlet 1). The glycerol standards (0 µM, 18.75 µM, 37.5 µM, 75 µM, 150 µM, and 300 µM) were diluted from 0.1 M glycerol stock by DMEM (no glucose, no glutamine, no phenol red) with 0.25% BSA, and 30 µL of the solution was added into the cell culture inlet (**Fig. 1A**, inlet 3) sequentially. An in-house written LabVIEW program was run to form droplets, generating 1 sample droplet and 1 reference droplet (of smaller volume) every 3.48 seconds. The following timing was used throughout this work: 350 ms sample pumping time for 2 pump cycles, 350 ms oil pumping time for 3 cycles, 250 ms of reference pumping time for 2 cycles, and another 350 ms oil pumping time for 3 cycles, with several delays of 10 to 70 ms built into the programming [total cycle time = (70 + 350)*2 + 10 + (350)*3 + 10 + (250)*2 + 10 + (350)*3 + 10 = 3480 ms = 3.48 s].

For every experiment with cells or tissues, the microdevice was calibrated with glycerol standards before cell sampling. Substrate and ATP were mixed and loaded in the left reservoir (see **Fig. 1A**), the enzyme mixture was added into the middle reservoir, and different concentrations of glycerol were added into the right reservoir in sequence. Each concentration of glycerol was loaded to form droplets for ~8 min, then the input (right reservoir) was changed to another concentration of glycerol by pipette. 85 µM of resorufin was utilized as the reference to use our phase-locked detection concept (µChopper) for correcting fluctuations of fluorescence measurements by imaging [30, 31, 34]. Other than the cell/tissue culture reservoir, the reagents in the other three reservoirs were sealed by mineral oil to avoid evaporation during hours-long experiments (see **Fig. 1D**). Generated droplets travelled in the long incubation channel for ~8 min to reach the plateau of enzyme reaction before the fluorescence images were taken. The BSA in the assay buffers was shown to obviate the cross-talk of resorufin [60] between droplets compared to the test without BSA (data not shown). Besides, BSA is also required for cell secretion experiments, since these proteins help carry lipolysis products such as fatty acids through solution and thereby reduce auto-inhibition effects, and they also reduce non-specific adsorption of biomolecules to the device surfaces [61]. Data indicate that enzyme reactions in droplets can reach equilibrium within about 1 min in static droplets (**Fig. S-6**), and the reaction rate is faster in moving droplets due to the mixing by forming counter rotating vortices [62].

Fluorescence imaging in the detection window (**Fig. 1A**) was operated continuously, and images were collected at 150 ms intervals using the TRITC filter cube (λ_{ex}=540 / 25 nm, λ_{em}=605 / 55 nm) by a CCD camera (CoolSnap HQ2; Photometrics Scientific) interfaced with Nikon inverted Ti-E fluorescence microscope (40X objective, 0.75 NA). After images were collected, the fluorescence intensity data of sample and reference droplets were obtained by image analysis in ImageJ (**Fig. S-4**), and the fluorescence intensity ratio between the sample droplet and reference droplet was determined continuously in response to glycerol concentration changes, shown in **Fig. S-1A**. In the calibration curve of glycerol (**Fig. S-1B**), the limit of detection was determined to be 0.74 μM in the droplets, which in one droplet (2.58 nL) corresponds to merely 1.92 fmol of glycerol. The average percent coefficient of variance (%CV) of the glycerol assay in droplets was about 1.7%. It can be observed that the fluctuations shown in raw data from reference droplets, due to optical perturbations during changing of glycerol solutions, could be corrected through our lock-in droplet analysis methodology[30, 31, 34]; **Fig. S-1** shows the corrected data.

For the glycerol secretion sampling from eWAT and encapsulated 3T3-L1 adipocytes, the experimental settings and droplet timing were equivalent to those during calibration, except that the cell culture inlet was loaded with tissue explants or cell encapsulations. The pretreated tissue explant or agarose gel spheroids with encapsulated cells were added into the 0.75-mm reservoir in the cell culture inlet (**Fig. 1C**, inlet 3). For the eWAT explant, a 3D-printed insert was used to help trap the tissue[19, 59] (red 3D CAD design shown in **Fig. 1B**). The treatments of HGHI [25 mM glucose and 2 nM insulin in DMEM (no glucose, no glutamine, no phenol red), with 0.25% BSA] and LGLIS [3 mM glucose, 50 pM insulin with 20 μM isoproterenol in DMEM (no glucose, no glutamine, no phenol red), with 0.25% BSA] were administrated to the cell culture inlet alternatively to test the glycerol secretion response from the samples. Fluorescent images were captured similarly as above. The fluorescence intensity data from the images were analyzed by ImageJ and data was further processed and analyzed in Microsoft Excel.

9. mRNA analysis

The RNeasy Mini Kit from Qiagen was used to extract total RNA from adipose tissue. The One-Step RT-PCR Kit from Qiagen was also purchased, and SYBR Green was added into the kit for quantitative PCR (qPCR) measurements. Beta-actin mRNA was quantified for the normalization of gene expression. The sequences of primers were from a previous publication [44] (listed below) and were obtained from IDT (Coralville, IA):

Beta-actin_Forward: GACGGCCAGGTCATCACTAT

Beta-actin_Reverse: CTTCTGCATCCTGTCAGCAA

AQ7_Forward: ATGGCCCCCAGGTCTGTGCTG

AQ7_Reverse: TTAGAAGTGCTCTAGAGGCACAGAGCC .

10. Photobleaching for gap junction studies in mouse eWAT

5-CFDA-AM (5-Carboxyfluorescein diacetate, acetoxymethyl ester) was dissolved in dry DMSO to obtain 5mg/mL stock solution (9.4mM). Three different treatment solutions were prepared: Control: 10 μM CFDA-AM in DPBS buffer; 2-APB treatment: 10 μM CFDA-AM with 100 μM 2-APB (2-aminoethoxydiphenyl borate) in DPBS buffer; 2-APB plus 6-OHDA treatment: 10 μM CFDA-AM with 100 μM 2-APB and 1.82 μg/μL 6-OHDA in DPBS buffer. The fat explants were washed in DPBS buffer, then set in the treatment solution for 1 h. After treating, the tissues were rinsed by DPBS 3 times and set in the DPBS buffer for imaging. In total, 6 explants from each mouse were used, with two for each treatment, and two mice were used for the test. Images were acquired by a Nikon A1R MP Confocal Microscope using a 10X objective, with a 488.0 nm laser line used for photobleaching and for excitation of fluorescence in the permeabilized 5-CFDA dye. This method allowed quantification of small molecule transfer rates between cells in the eWAT.

11. Immunostaining of mouse eWAT

Every piece of 3-mm WAT was washed by DPBS buffer and fixed in 200 μL Image-IT Fixative solution (4% formaldehyde in PBS, methanol-free) at 4 °C overnight. The tissues were washed by incubating in 200 μL DPBS at 4 °C for 2 h, then treated by 20 μg/mL proteinase K in 10 mM Tris-HCl for 5 min at room temperature. This was followed by incubating in 100% methanol at -20 °C for 5 min and PBS buffer with 1% BSA and 0.3% Triton X-100 at 37 °C overnight to permeabilize the tissues and block the non-specific binding. The tissues were incubated with antibody solutions at 37 °C for 5 h and 4 °C overnight. Either 1:250 diluted Connexin 43 monoclonal antibody (CX-1B1) labeled by Alexa Fluor 488 (0.5 mg/mL in stock solution), or 1:100 diluted TH antibody (F-11) Alexa Fluor™ 594 (0.2 mg/mL in stock solution) were used. The tissues were then washed by DPBS and incubated in 3 μg/mL Nile red (1 mg/mL in stock solution) or 1:1000 diluted LipidTox Green at 37 °C for 5 min to stain the lipid droplets in the adipocytes. The tissues were finally washed by DPBS, then set in DPBS and anchored at the bottom of culture plate using 3D-printed insert for imaging. Immunostained cell images were captured by the Nikon A1R MP Confocal Microscope using a 10X objective.

12. Spectrogram generation

Time traces of glycerol secretion, e.g. from **Fig. 2**, were first processed with a 57-point (198.36-second or 3.306-minute) moving average filter in Excel, which is essentially a high-pass filter with a cutoff frequency of 0.151 min^{-1} that removed the slow changes (baseline) and left the higher frequency bursts. Next, an in-house written LabVIEW application applied a 128-point (7.42-min), sliding window FFT analysis with a uniform windowing method, creating an image file

with time on the x-axis and frequency on the y-axis. These images were further processed in ImageJ to give a logarithmic intensity scale using a 16-color lookup table, as shown in **Fig. 3** and **Fig. 6**.

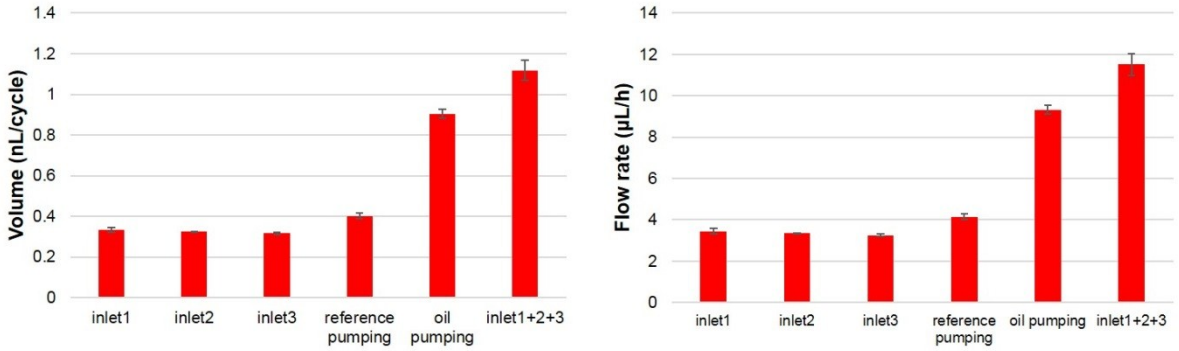

Figure S-3. Measurement of the valve pumping volumes. **A)** Volume for each pumping cycle from the various inlets; 3 tests were done for each group of valves, with timing as described in SI, section 8. **B)** Flow rate measurement by pumping each group of valves.

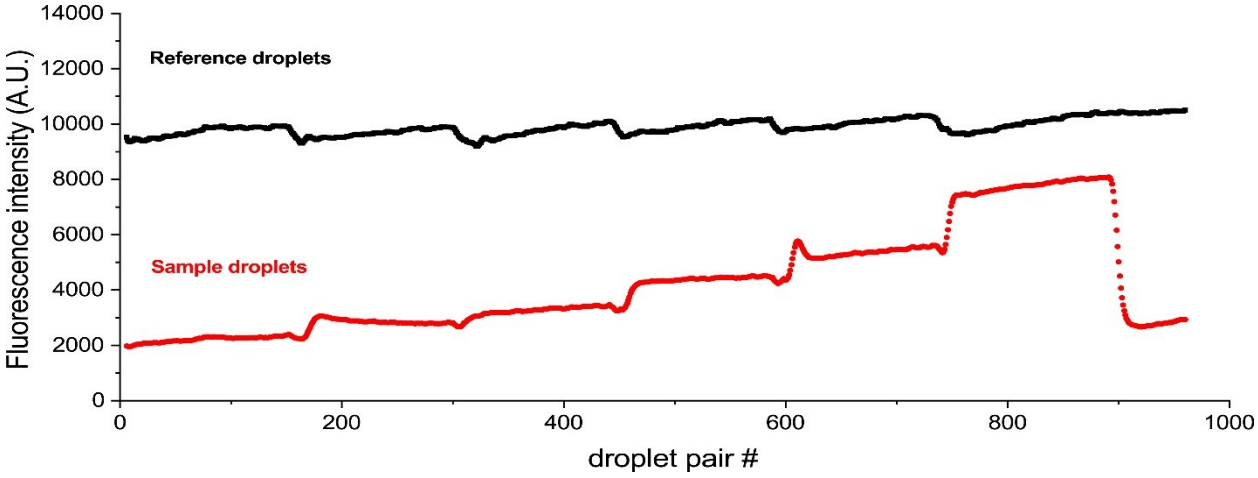

Figure S-4. Raw data of fluorescence intensity for each droplet during continuous, on-chip calibration. The fluorescence images were analyzed by ImageJ. Based on the droplet size and sequence, reference droplets could be separated from the sample droplets. The number of each droplet pair is shown on the x-axis, corresponding to about 55 minutes total for ~1000 droplets.

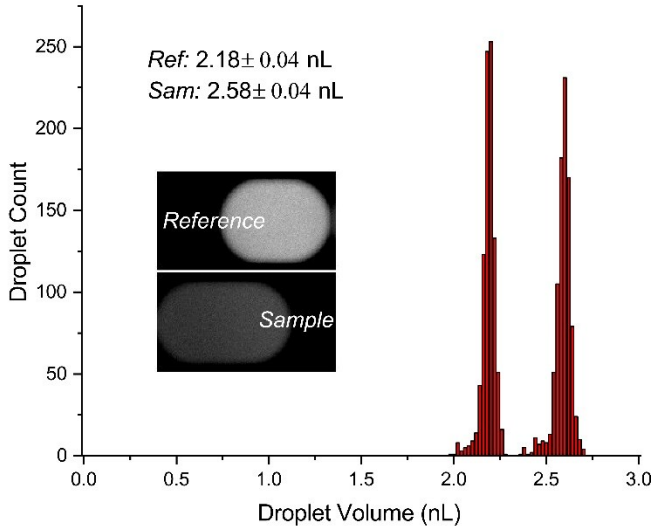

Figure S-5. Droplet volume measurement shows high precision control with our device, using 914 droplets of each type.

The volumes of droplets were calculated by $= A \cdot h + \frac{\pi \cdot h^2 \cdot P}{8}$, where h is the channel depth, A is the droplet area from top view images, and P is the perimeter of the droplet.

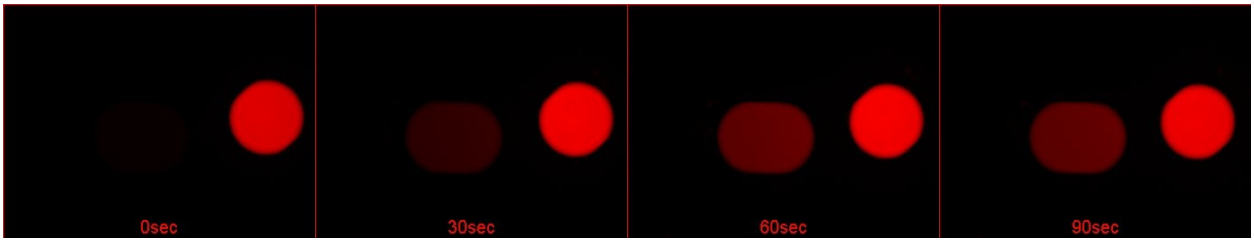

Figure S-6. To confirm efficient droplet-based enzyme reaction efficiency in early tests, the progress of the detection kit's enzyme reaction was monitored in static droplets. The smaller droplet was the reference droplet with 85 μM resorufin, and the larger droplet was the sample droplet containing 100 μM glycerol, enzymes, substrate, and ATP. Imaging started as soon as the droplets were formed. The produced fluorogenic molecule, resorufin, in the static sample droplet reached its highest concentration within about one minute.

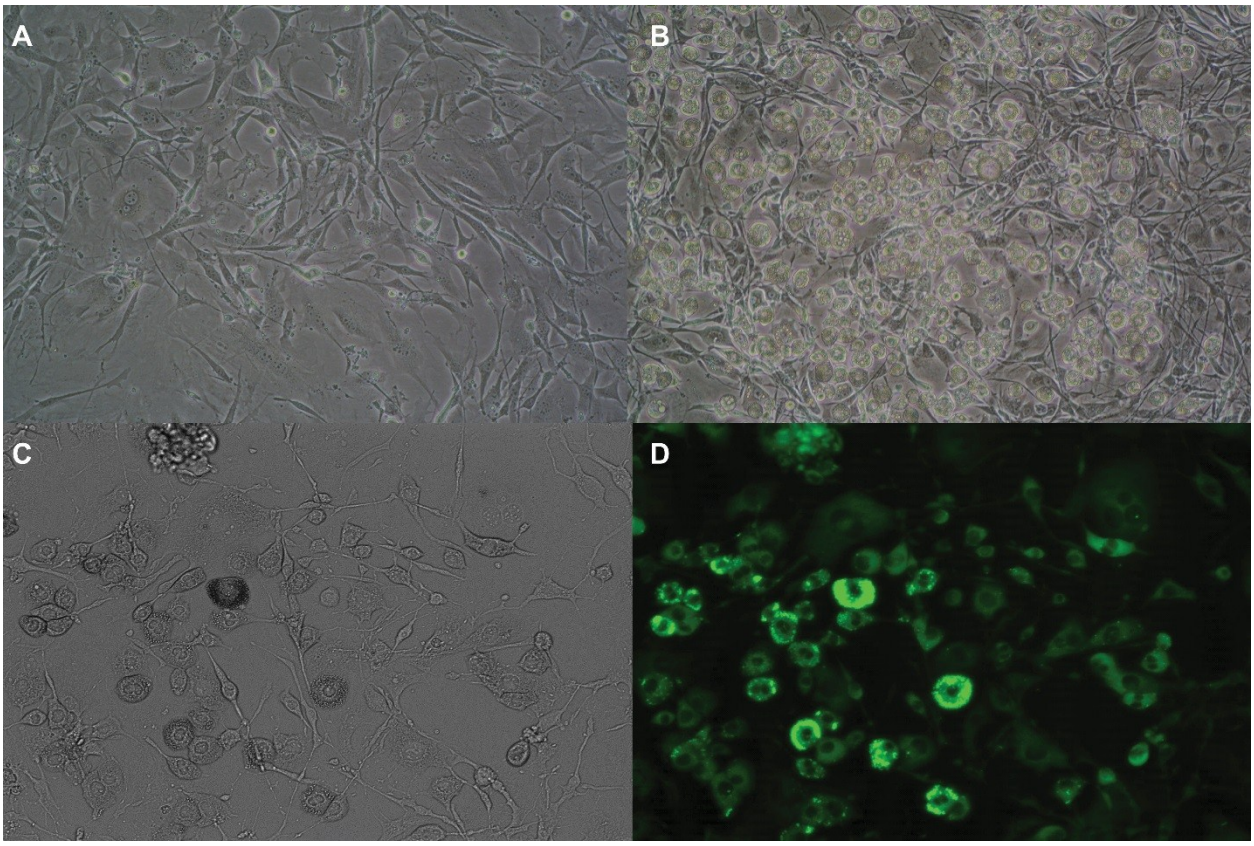

Figure S-7. Culture and differentiation of the 3T3-L1 cell line. **A)** Fibroblasts of 3T3-L1 at around 90% confluence. **B)** Differentiated 3T3-L1 adipocytes. The cell morphology was changed from fibroblast-like to circular shaped with visible lipid droplets formed in the cells. A and B were captured by a digital camera (Nikon J1) under 10X magnification. **C)** DIC image and **D)** corresponding fluorescence image of 3T3-L1 adipocytes by the objective with 10X magnification (Nikon Ti-E). The lipid droplets in the adipocytes were stained by LipidTox Green and detected in the FITC channel of the fluorescence microscope.

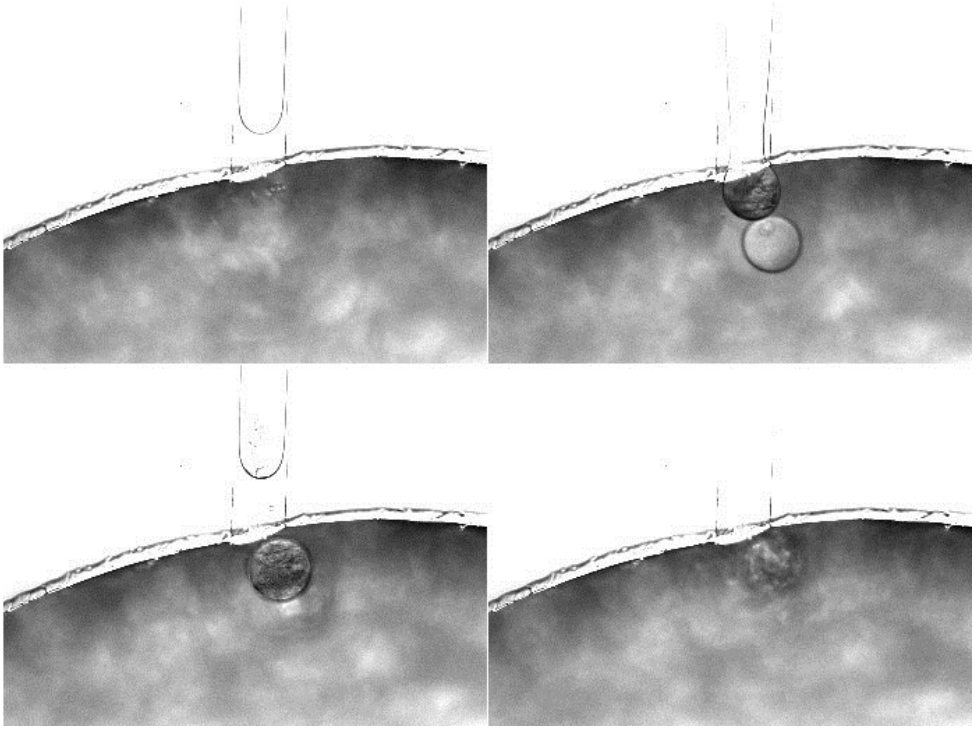

Figure S-8. Cell spheroid generation using 3T3-L1 adipocytes and agarose in the microfluidic droplet chip (see also supplementary video 2). Cells encapsulated in droplets of liquid agarose gel are shown as they exit the microfluidic channel (top) and enter the reservoir (bottom).

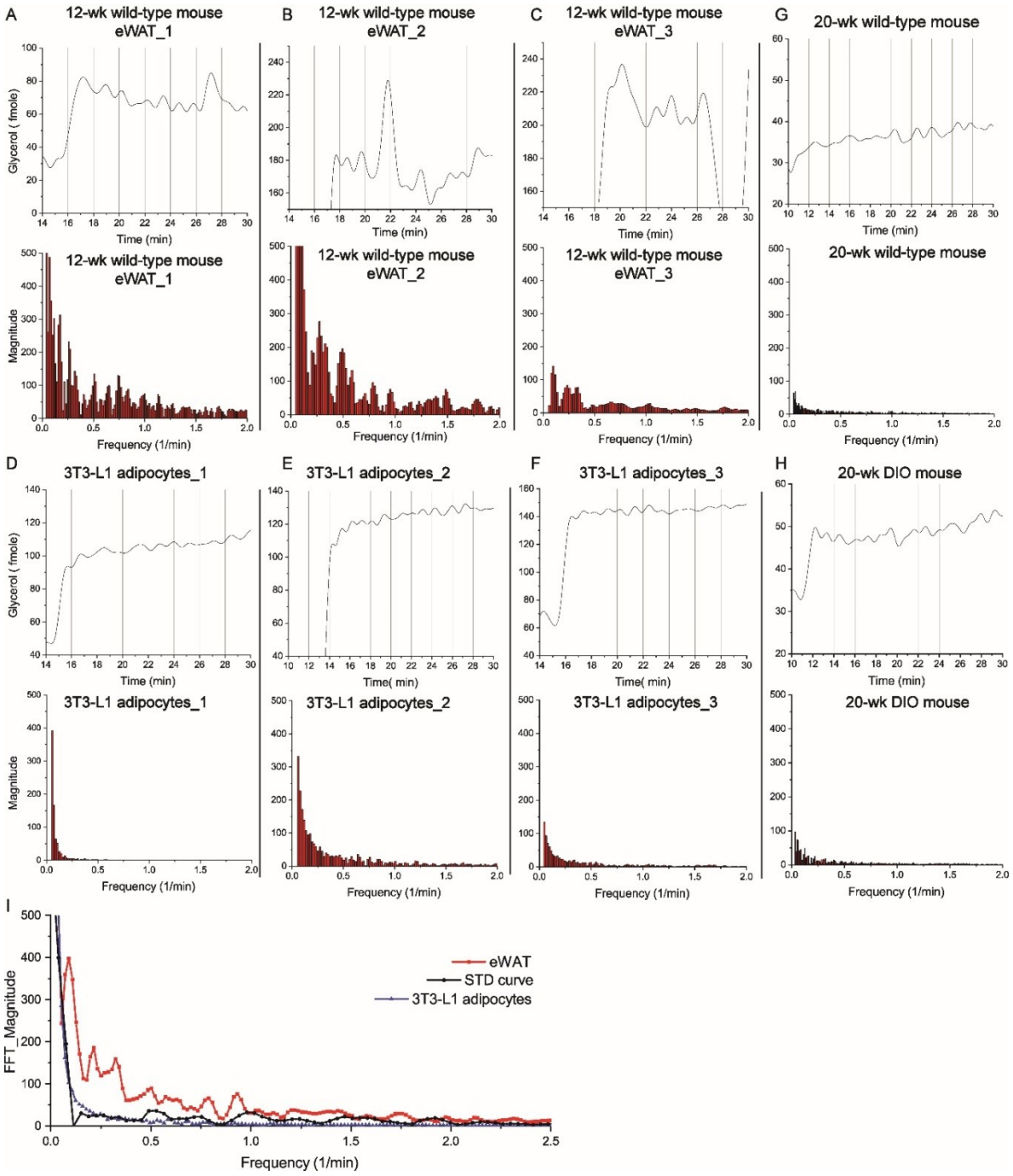

Figure S-9. A-H) Oscillation analyses of glycerol release from primary white adipose tissues and 3T3-L1 adipocytes. For each group of data from **Fig. 2**, the upper figure shows part of the plot after analyzed by low-pass filter with 1.0 min^{-1} frequency cutoff. The lower plot displays the FFT results. Overall, we observed fewer oscillations/bursts from 3T3-L1 adipocytes and eWAT of old mice than from eWAT of 12-week wild-type mice. **I)** Average magnitude in FFT analysis to show the difference between the tissues and cell clusters, proven by comparing with the FFT analysis of standard curve (n =3).

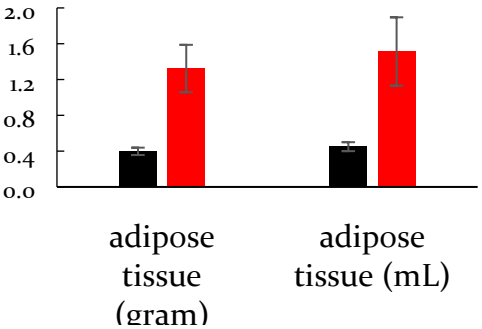

Figure S-10. Adipose tissue mass and volume for 9-12-week old mice and 20-week old mice. Data included three mice for each age group. Note that DIO mice eWAT weight was 2.30 grams.

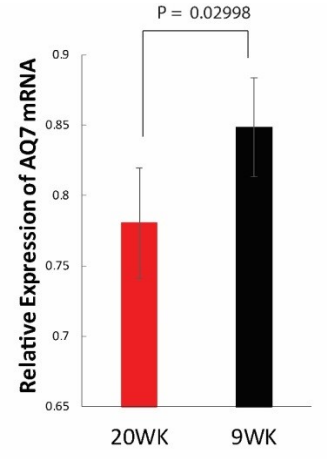

Figure S-11. Aquaporin 7 gene expression in mice of different ages, normalized to that of beta-actin expression. Data included three or more samples from each mouse.