Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information for:

3D-templated, fully automated microfluidic input/output multiplexer for endocrine tissue culture and secretion sampling

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Supporting Information (SI) Contents:

Supporting Videos:

Supplementary experimental materials and methods

General materials

All buffers were prepared with deionized, ultrafiltered water (BDH1168-5G, VWR, Radnor, PA). The following reagents were used as received: Polydimethylsiloxane (PDMS) precursors (Sylgard 184, Dow Corning, Midland, MD); SU-8 2015 photoresist (Microchem, Newton, MA); AZ-40-XT photoresist (MicroChem, Westborough, MA); insulin, D-glucose, 4−2−hydroxyethyl−1−piperazineethanesulfonicacid (HEPES), (3-Aminopropyl)trimethoxysilane, trimethylsilyl chloride (Me_3SiCl), sodium dodecanoate, fluorescein isothiocyanate (FITC), KH_2PO_4, NaH_2PO_4, and NaOH were all obtained from Sigma-Aldrich (St. Louis, Missouri); Bovine serum albumin (BSA), fetal bovine serum (FBS), NaCl, $CaCl_2 \bullet 2H_2O$, EtOH, MeOH, and DMF were purchased from VWR (West Chester, PA). 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid (BODIPY® FL C12, FFA*; "bodipy-laurate"), Minimal Essential Media (MEM) non-essential amino acids solution 100x, collagenase P, collagenase type I, Dulbecco's Modified Eagle Medium (DMEM), and $MgSO_4 \bullet 7H_2O$ were purchased from ThermoFisher Scientific (Grand Island, New York).

Microfluidic device master wafer fabrication

Two layer microfluidic devices were fabricated using standard multilayer soft lithography methods[1, 2] with 3D-printed templating[3, 4] of the tissue culture interfaces. Two different master wafers for fluidic channel and pneumatic/control channels were first fabricated by photolithography. The channel layout was designed in Adobe Illustrator and printed at 50800 dpi resolution by Fineline Imaging (Colorado Spring, CO) to serve as the photolithographic mask. For the control channel (thin lower layer), 30-µm thick negative photoresist (SU-8 2015) was spin coated onto a silicon wafer (Silicon Inc., Boise, ID, USA). The wafers were then baked at 105 °C for 5 min on a hotplate. UV exposure through the mask was done at ~330 mJ/cm^2 on an in-house built UV LED exposure unit[5]. The wafer was hard baked for 5 min at 105 °C then developed for 5 min in the SU-8 developer solution. For the fluidic channel layer, 50-µm thick positive photoresist (AZ 40 XT) was spun onto the silicon wafer. The wafers were then baked at 105 °C for 5 min followed by UV exposure at ~330 mJ/cm^2. After hard baking at 105 °C for 5 min and wafer development in AZ developer for about 5 min, the master wafer was baked at 115 °C for 6 min to anneal the AZ photoresist and round out the cross-section of the fluidic channel template (see Figure S-2). The silicon wafers were exposed to trimethylsilyl chloride vaper for 30 min before use to enhance PDMS removal. Channels were later characterized by slicing an assembled PDMS device and imaging the channel cross section (Figure S-2).

3D-printed interface templates for tissue culture regions

All 3D-printed templates and devices were designed in SketchUp 3D modeling software, error checked in NetFab, and printed on a MakerBot Replicator 2 (100 µm layer resolution in the z-direction) with polylactic acid filament (PLAF, 1.75 mm diameter). Six 3D-printed templates with varying tissue culture region heights (0.47, 0.48, 0.57, 0.72, 0.82, 1.24 mm) were design and printed for PDMS templating. In order to measure the height of the cell culturing regions, PDMS was cured with the 3D-template and a cross-section of the PDMS reservoir region was sliced with a razor blade and mounted onto a glass microscope slide. Images were captured on a Nikon Ti-E inverted fluorescence microscope at 2x magnification, operating in wide-field transmittance mode. The heights were measured with ImageJ software.

Microdevice fabrication

The µMUX devices were fabricated as described in Figure S-1. 36 g of PDMS polymer mixture (5:1 ratio, monomer:curing agent) were mixed and degassed under vacuum and then poured onto the fluidic master wafer (AZ) wrapped within aluminum foil. The 3D-printed template was carefully aligned around the channel and set directly onto the wafer and into the layer of uncured PDMS. The entire assembly was baked in the oven at 50 °C undisturbed for 4 h. Following curing, the template was removed carefully, and the PDMS was peeled from the master, after which it was diced, access holes and vias were punched (1.5 mm inner diameter for interfacing with tubing, 1.0 mm for the via connection between the reservoir and channels), and each device was cleaned with methanol and dried with N_2 gas. Next, 5 g of degassed PDMS polymer mixture (20:1 ratio, monomer:curing agent) was spin coated onto the pneumatic control channel master wafer (SU-8) at 2300 rpm for 60 s and baked at 65 °C for 40 min to facilitate partial curing of the polymer. The freshly made fluidic layer PDMS, with access holes punched, was carefully aligned onto the partially cured control channel PDMS layer, and the two layers were permanently bonded by placing in the oven at 65 °C for at least 4 hours. The PDMS devices were then peeled from the wafer and diced, then access holes were punched where necessary. Finally, the assembled PDMS devices were plasma oxidized and bonded to a glass substrate. The µMUX devices were stored at room temperature. Immediately before use with islets or adipose explants, the devices were cleaned with an air plasma for 45 s then treated with PBS buffer with 1% BSA to generate a hydrophilic, biocompatible surface.

Pancreatic islet and adipose tissue explant isolation

Pancreatic islets[6, 7] and epididymal fat pads[3, 4, 8, 9] were isolated from C57BL/6 male mice as described previously. Following isolation, islets were placed in RPMI media (10% FBS, 11 mM glucose) at 37 °C and 5% CO_2 to incubate overnight. Fat pads were transferred to a 60 mm petri dish containing a few mL of fresh phosphate-HEPES buffer. Excess vasculature and other non-adipose tissue was excised using micro surgical scissors. 2- and 3-mm sterile biopsy punches were used to form aliquots of the fat tissue. As explants were punched, they were transferred with surgical tweezers into a glass tube with 3-4 mL of phosphate-HEPES buffer. Explants were centrifuged at 1000 rpm for 3 min. Infranatant was removed with an 18-gauge, 1.5-inch needle. 3-4 mL of phosphate-HEPES buffer was added back to the tube. Cells were centrifuged and washed in this fashion one additional time with phosphate-HEPES buffer and 2 additional times with fat serum media. After the final rinse, explants were transferred to individual wells on a sterile 96-well plate containing 200 µL of serum media in each well. The 96-well plate was incubated for 30 minutes at 5.0% CO_2 and 37 °C. 3D-printed explant traps were placed into each well and the plate was returned to the incubator. Prior to use in microfluidic stimulation experiments, explants were maintained for up to 7 days in the incubator with serum media replacement twice a day.

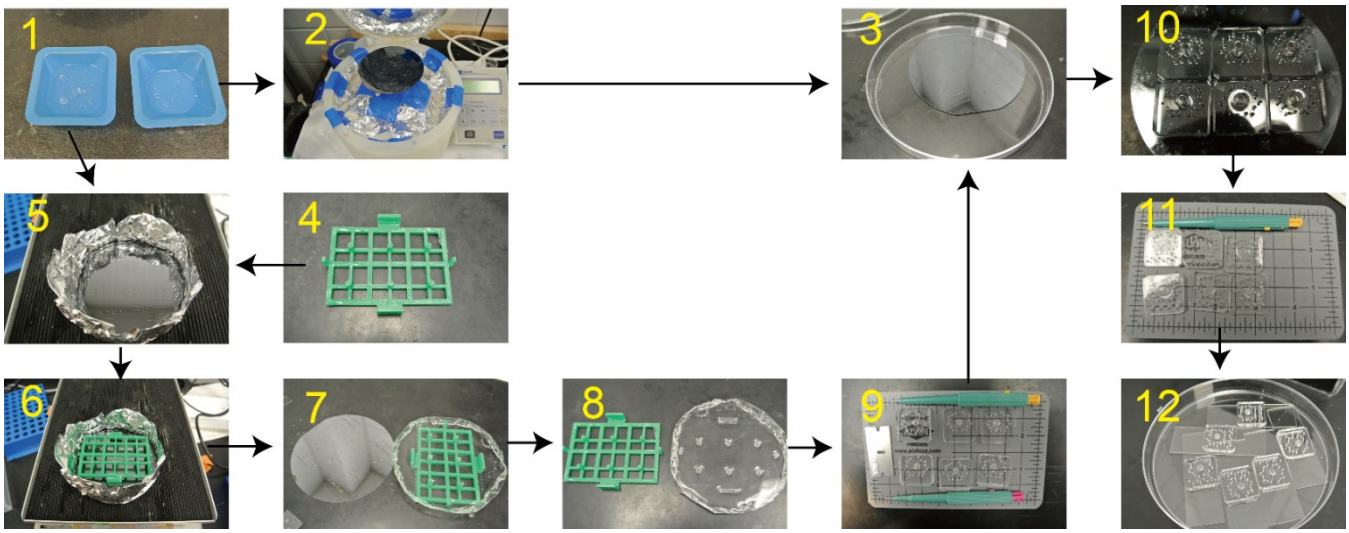

Figure S-1. Fabrication procedure of µMUX devices including 3D-templated reservoir molding. (1) 36 g of PDMS in 5:1 ratio (left) and 10.5 g of PDMS in a 20:1 ratio (right) were mixed and degassed under vacuum. (2) PDMS (20:1) was spin-coated over the control wafer. (3) The thin layer of PDMS on the control wafer was partially cured at 65 °C for 40 min, and fluidic channel layers (from step 9) were aligned for bonding. (4) 3D-printed template for sculpting tissue culture reservoirs. (5) PDMS (5:1) was poured onto the fluidic channel master in an aluminum foil boat. (6) 3D-printed insert was carefully aligned around the channel and set directly onto the wafer into the layer of uncured PDMS. The entire assembly was baked in the oven at 50 °C undisturbed for 4 h. (7) The cured PDMS with template was peeled from the wafer. (8) The 3D-printed template was removed. (9) Devices were diced, and holes were punched to prepare for bonding with the control channel PDMS layer. (10) After careful alignment (in step 3), the fluidic layer PDMS and partially cured control layer were permanently bonded together by placing in the oven at 65 °C for at least 4 hours. (11) Completed PDMS devices were diced, peeled from the wafer, and holes were punched for control channel pressure lines. (12) PDMS devices were finally plasma oxidized and bonded onto glass substratea, and these µMUX chips were ready to use.

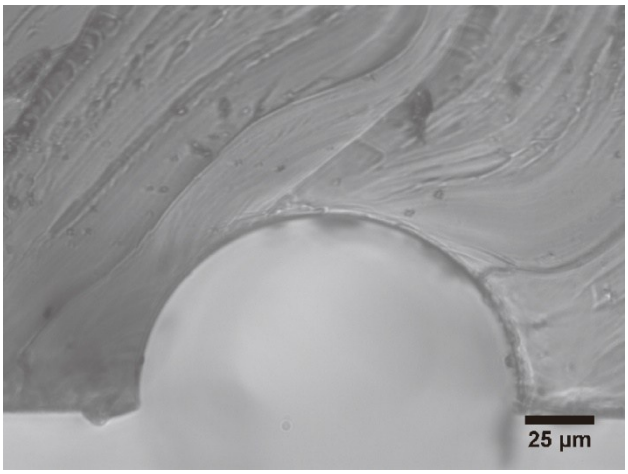

Figure S-2: Image of a representative cross-section of a µMUX fluidic channel. Using ImageJ software, the cross-sectional area of the channel was measured as 7580 ± 150 µm^2.

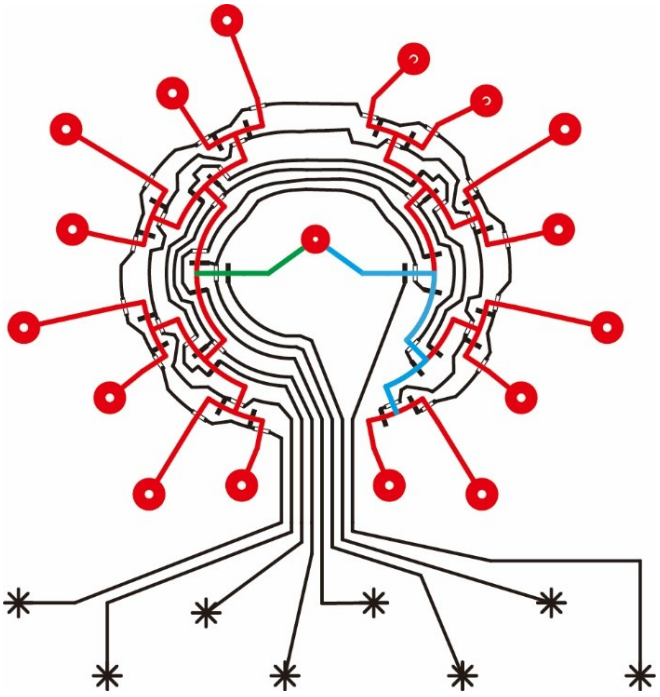

Figure S-3. Schematic of the µMUX channel design at higher resolution compared to that shown in **Figure 1A** in the manuscript. Control channels are shown in black and fluidic channels in red. Also highlighted here are the channel lengths representing the shortest (green, 4.08 mm) and longest (blue, 8.96 mm) possible plug lengths of dead volume when switching between input/output channels. As discussed in the text, these dead volumes represent a negligible amount (<0.4%) of solution compared to the tissue culture volume, even without washing steps included. Note that this dead volume analysis assumes that >9 channels are used.

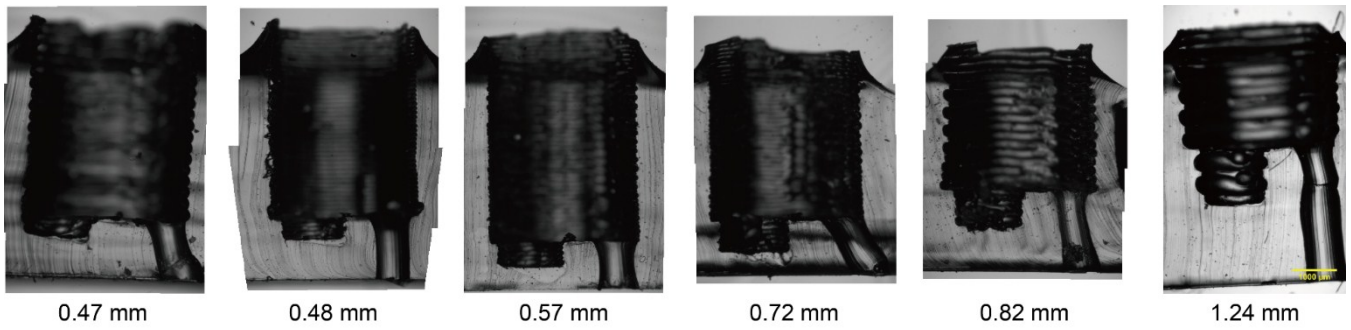

Figure S-4. Cross-sections of multiple devices with varying depths of tissue culture regions, facilitated by 3D-printed templating. Tissue culture region depths are included below each image. Scale bar = 1.0 mm.

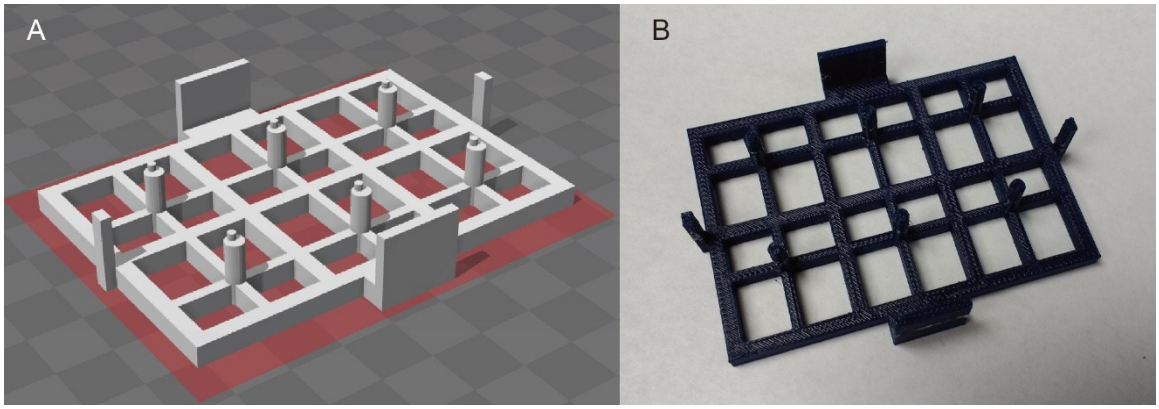

Figure S-5. 3D-printed templates were used to fabricate customized tissue culture interfaces on the µMUX devices. **(A)** 3D CAD rendering of a 6-device template and **(B)** an image of a printed template. By simply changing the design of the 3D CAD file, different templates could be created to give µMUX devices with varying tissue culture region depths (Figure S-4).

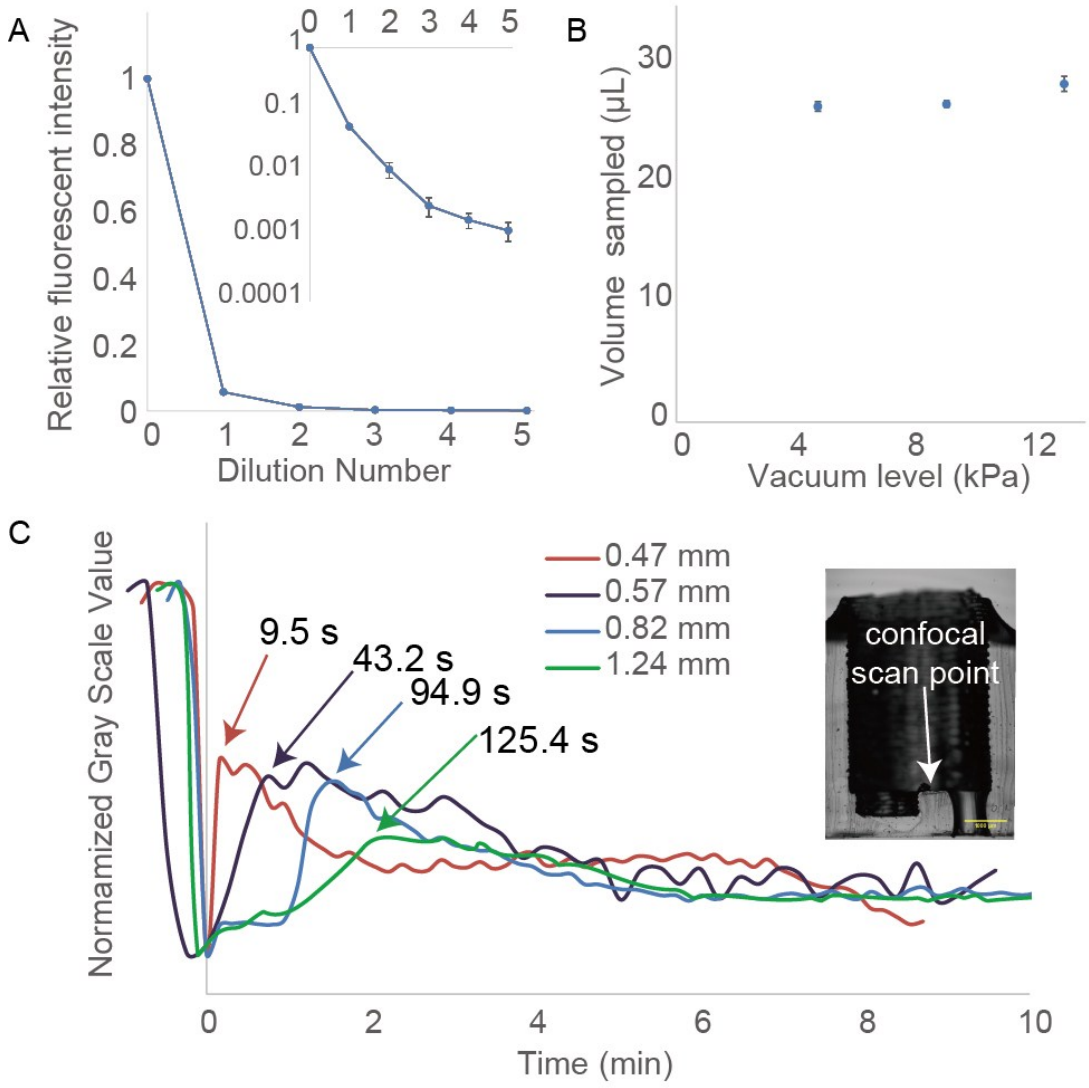

Figure S-6. µMUX device characterization and optimization. **(A)** Carry-over volume measurement by rinsing fluorescein with sequential buffer rinses. The fluorescence intensity of the rinsed buffer decreased to less than 1% after 2 rinse steps. **(B)** Sampling volume consistency study by measuring sample volume at different vacuum levels. Error bars show the standard deviations of 5 samplings. **(C)** Fluorescein diffusion studies via confocal microscopy. The white rectangle in the inset image at the right shows the region of confocal scanning. Upon quickly switching the solution from 200 nM fluorescein to 20 nM fluorescein (from µMUX channels below), the leftover higher concentration fluorescein in the islet culturing was released by diffusion. As tissue culture region depths were increased, the time for the fluorescein diffusion to equilibrate increased. For the chosen optimum depth (0.57 mm), fluorescein required ~40 s to diffuse from the culturing region, an acceptable result for secretion sampling at ~5-minute temporal resolution.

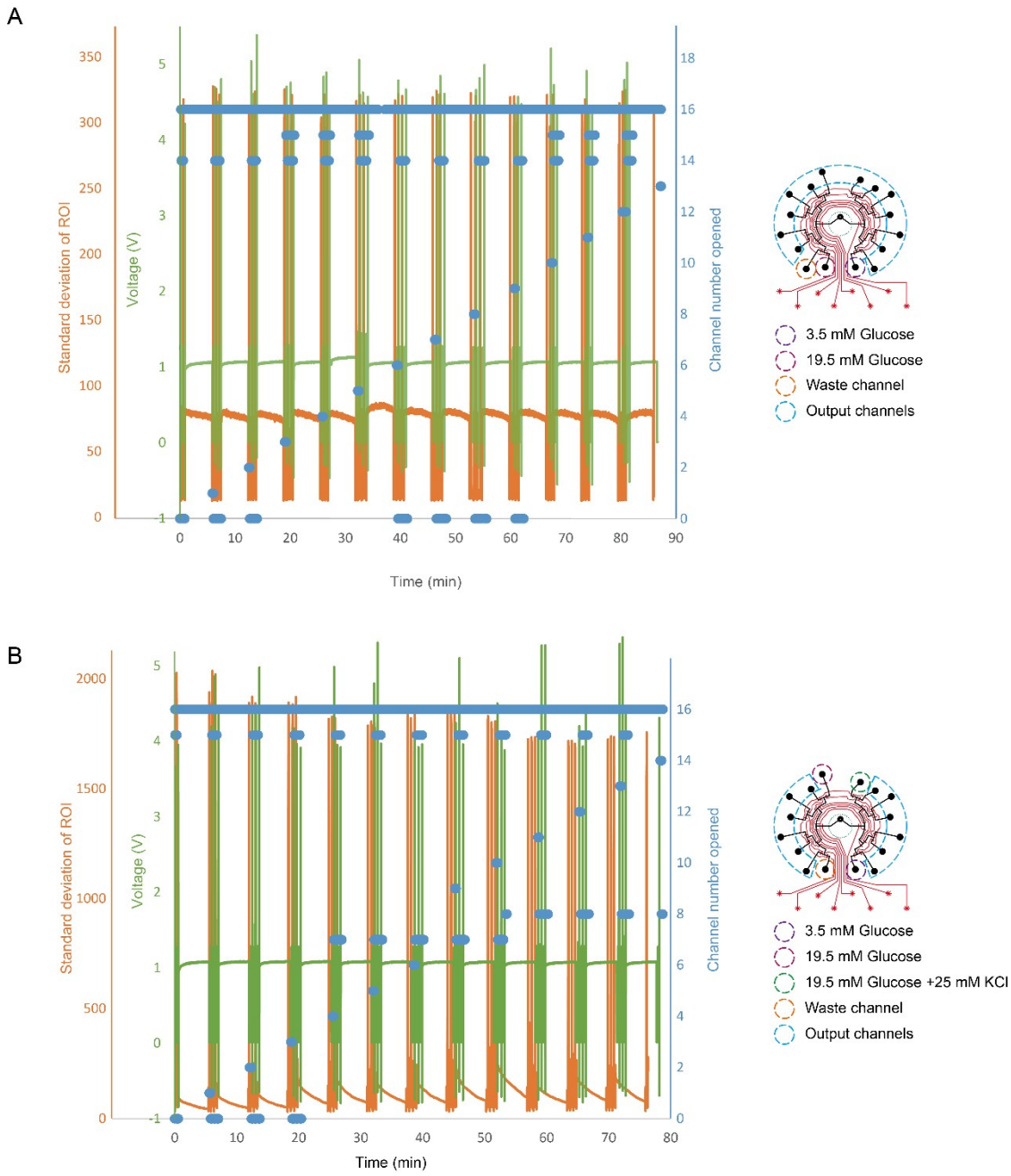

Figure S-7. Full sets of automation data during time programs for dynamic insulin secretion sampling from pancreatic islets (see **Figures 3** and **5** in manuscript text). **(A)** Automation data from high-low glucose square wave treatment and **(B)** from high-low glucose square wave with KCl treatment. Channel assignment map is shown in the rightmost images. Reservoirs were rinsed 3 times between treatments, as indicated by the multiple triggers in the "full" (green" and "empty" (orange) sensor traces. Further details of operation are included in the manuscript text.

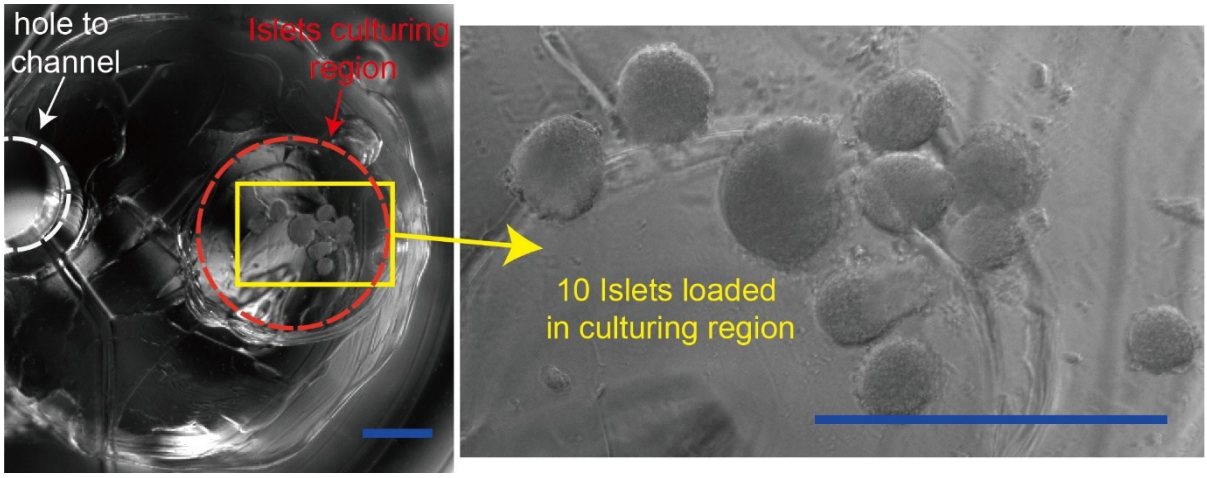

Figure S-8. Images of pancreatic islets on a µMUX device. 10 islets were loaded into the tissue culture region. Scale bar = 500 µm.

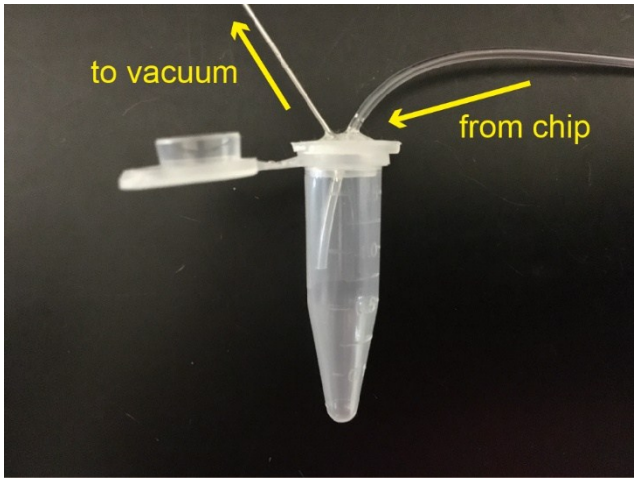

Figure S-9. Buffer bottle for collecting islet secretion samples. Caps of 1.7-mL microcentrifuge tubes were drilled with two holes. Tygon tubing (0.01 inch ID) and hollow steel tubing taken from 22-gauge blunt syringe needles were feed into the two holes and sealed with epoxy glue (Gorilla Glue Co., Cincinnati, OH). The Tygon tubing was deeper than the steel tubing. When used for secretion sampling, the other end of the Tygon tubing was inserted into the corresponding output channel interface on the µMUX device, and the steel tubing was connected to vacuum applied by a syringe through Tygon tubing (0.01 inch ID).

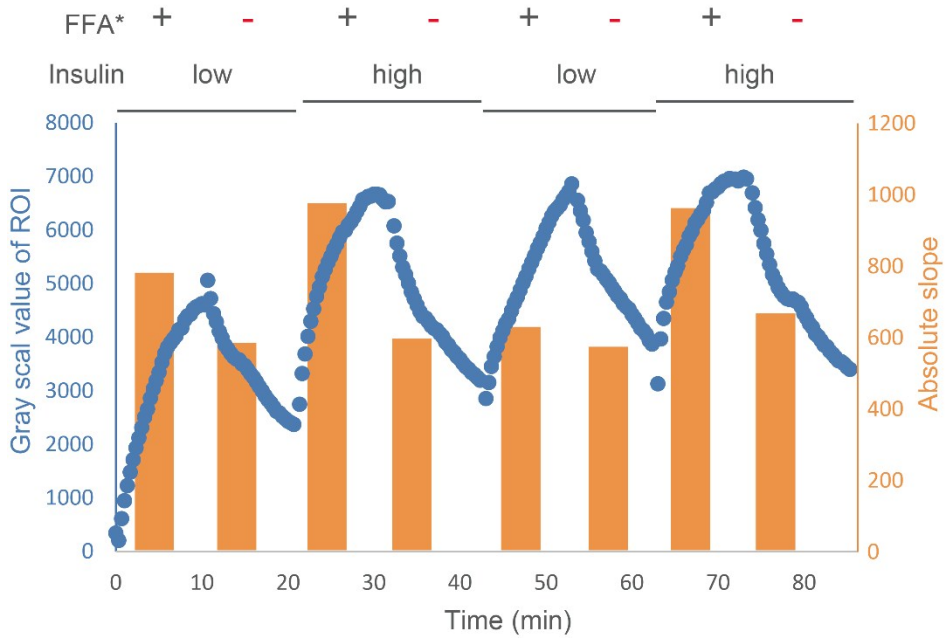

Figure S-10. Dynamic fatty acid uptake with 2 treatments (4-input channels from µMUX). The adipose tissue explant was treated with a square wave of low and high insulin/glucose buffer while alternating bodipy-laurate (FFA*) or unlabeled laurate. The explant was exposed to each solution for 10 min before switching to the next solution. The fluorescent microscopic images was taken in real time, and the background corrected gray scale value of the image is shown as blue dots. The initial rate of the fatty acid uptake is proportional to the absolute slop of gray scale value of ROI at the beginning of each treatment, which is shown in orange. An insulin dependence of fatty acid uptake was observed in this experiment, in agreement with data shown in **Figure 6** in the manuscript text.

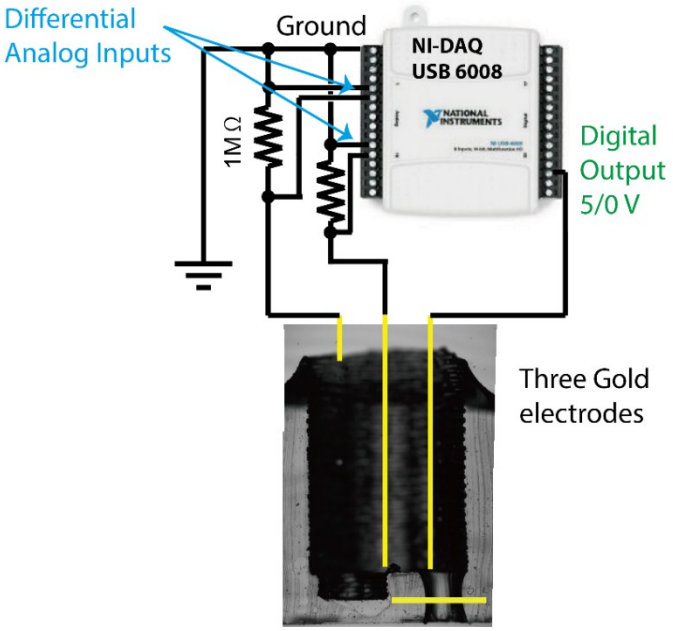

Figure S-11. Circuitry of the initial three-electrode system for conductivity sensing of both the "full" and "empty" states, used in the first generation sensor design. The source electrode was wired to a 5-V digital output of the NI-DAQ. The "full" sensing electrode was placed at the opening of the reservoir, and the "empty" sensing electrode was placed just above the bottom of the reservoir. Both of the sensing electrodes were connected to 1 MΩ resistors across differential analog inputs for voltage readouts. The "empty" signal from this tri-electrode sensor was not reproducible in cell culture media (with BSA) due to inconsistent electrode wetting problems. Scale bar = 2 mm. A video of the μMUX device operation with these sensors is shown in Video S-1.

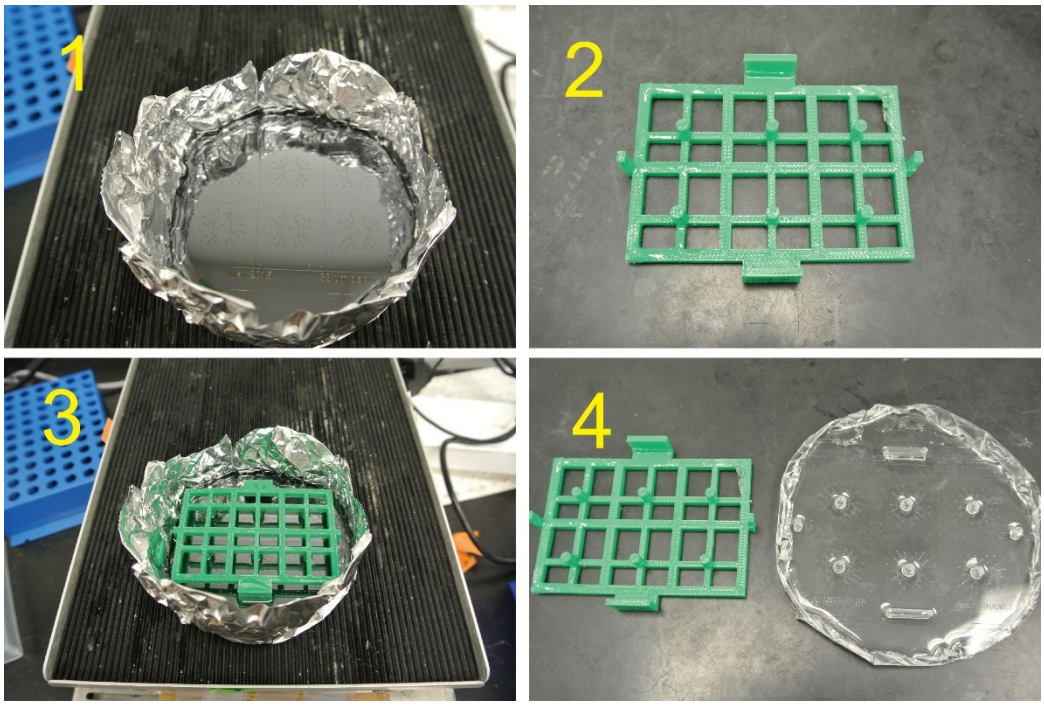

Figure S-12. Enlarged view of 3D-templating process for fabricating tissue culture reservoirs into the PDMS devices. (1) PDMS (5:1) was poured onto the fluidic channel master in an aluminum foil boat. (2) 3D-printed template for sculpting tissue culture reservoirs. (3) 3D-printed insert was carefully aligned around the channel and set directly onto the wafer into the layer of uncured PDMS. The entire assembly was baked in the oven at 50 °C undisturbed for 4 h. (4) The cured PDMS with template was peeled from the wafer, and the 3D-printed template was removed. Following this process (previously reported[4]; also included as reference 11 from main text), tissue culture reservoirs were sculpted into the PDMS above the sets of fluidic channels in the µMUX device.

References:

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