Supporting Information for:

Automated microfluidic droplet sampling with integrated, mix-and-read immunoassays to resolve endocrine tissue secretion dynamics

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Supporting Information (SI) Contents:

Supporting Videos: **Video S-1** of device operation for droplet-based sampling included in ESI files (PumpDrop_sampling_video.avi)

Supplementary experimental procedure

Materials and Reagents. All buffers were prepared with deionized, ultra-filtered water (BDH1168-5G, VWR, Radnor, PA). The following reagents were used as received: Polydimethylsiloxane (PDMS) precursors, Sylgard 184 elastomer base and curing agent (Dow Corning, Midland, MD); SU-82015 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), fluorescein, 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), KCl, NaCl, $MgCl_2$, $CaCl_2 \bullet 2H_2O$, EtOH, MeOH, and DMF were purchased from VWR (West Chester, PA). Minimal Essential Media (MEM) non-essential amino acids solution 100x, collagenase P, collagenase type I, Dulbecco's Modiied Eagle Medium (DMEM), and $MgSO_4 \bullet 7H_2O$ were purchased from Thermo-Fisher Scientific (Grand Island, New York).

Microfluidic Master Wafer Fabrication. Microfluidic devices were made by standard multilayer soft lithography methods[1,2], with 3D-printed templating[3–5] of the tissue culture interfaces. Two master wafers for fluidic channel and pneumatic/control channels were first fabricated by photolithography. The channel layout was designed in Adobe Illustrator (San Jose, CA) and photolithographic masks were printed at 50,800 dpi resolution by Fineline Imaging (Colorado Spring, CO). For the pneumatic control channels (thin lower layer), 20-µm thick negative photoresist (SU-8 2015) was spin-coated onto a H_2SO_4 (1 M) and water washed silicon wafer (Polishing Corporation of America, Santa Clare, CA). The wafers were then baked at 105 °C for 5 min, and UV exposure through the mask was done at ~330 mJ/cm^2 on an in-house built UV LED exposure unit[6]. The wafer was hard baked for another 5 min at 105 °*C* then developed for 5 min in the SU-8 developer solution.

The fluidic channel layer wafers were made by two step photolithography. First, a 60-µm thick microfluidic pattern was made on the silicon wafer by similar procedure for the control wafer with negative photoresist (SU-8 2050). 60-µm thick SU-8 2050 photoresist was spin coated on a silicon wafer (H_2SO_4 and water washed), soft baked at 105 °*C* for 5 min, UV exposed through the mask at ~330 mJ/cm^2, hard baked at 105 °*C* for 5 min, and developed to form the SU-8 micro pattern. Second, 40-µm thick positive photoresist (AZ 40 XT) was spun onto the silicon wafer along with the SU-8 pattern. The wafer was baking at 105 °*C* for 7 min, and the photo mask was carefully aligned onto it under microscope (SE306R-PZ, AmScope). After UV exposure at ~330 mJ/cm^2, hard baked at 105 °C for 5 min, and developed, the wafer was baked at 115 °C for 6 min to anneal the AZ photoresist and round out the cross-section of the AZ fluidic channel template. The silicon wafers were exposed to trimethylsilyl chloride vapor for 30 min before use to enhance PDMS removal. Channels were later characterized by slicing an assembled PDMS device and imaging the channel cross sections (see Figure S-1).

Microfluidic Device Fabrication with 3D-printed Templating. 3D-printed templates and devices were designed in SketchUp 3D modeling software, and printed on a MakerBot Replicator 2 (100 µm layer resolution in the z-direction) with polylactic acid filament (HatchBox PLA, 1.75 mm diameter). 36 g of PDMS precursor mixture (5:1 ratio, monomer:curing agent) were well mixed, degassed under vacuum, and poured onto the fluidic master wafer (AZ) wrapped within aluminum foil. The 3D-printed template was carefully aligned over the channel patterns and set directly onto the wafer and into the uncured PDMS. 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 2750 rpm for 45 *s*. Both the assembly of fluid layer PDMS with 3D printed template and control layer PDMS were partially cured at 65 °C for 40 min. Following curing, the template was removed carefully, and the thick fluidic PDMS was peeled from the master, after which it was diced, and access holes and vias were punched. 1.5-mm inner diameter (ID) punches was used for waste outlets, and 2.5 mm ID punches were used for the solution reservoirs (Miltex biopsy punch 33-31A and 33-31B, Miltex, York, PA).0.5-mm ID punches were used for the islet trapping channel in between the 3D-templated reservoir and channels (EMS-Core Sampling Tool 0.5 mm, 69039-05, Electron Microscopy Sciences, Hatfield, PA). Each PDMS replica was washed with methanol, dried with N_2 gas, and cleaned with Scotch tape (3M, St. Paul, MN). The freshly made fluidic PDMS replicas, with access holes punched, were 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 holes for control lines connections were punched with a 0.75 mm ID punch (69039-07, Electron Microscopy Sciences, Hatfield, PA). Finally, the assembled PDMS devices were plasma oxidized and bonded to a glass substrate. The microfluidic devices were thermally aged at 65 °C for 1 week before use to limit uncured PDMS monomer leakage.

Pumping flow rate and dispensing volume measurement. The pumping flow rate and dispensing volume of each pumping cycle were analyzed by measuring the distance traveled by the leading meniscus of the fluid. A microfluidic device was specially made with 0.75 mm ID reference reservoir (instead of 2.5 mm). A 50 cm-long, 100 µm-ID, transparent capillary (1068162095, Molex, Lisle, IL) was connected to the reference reservoir via a tygon tubing (EW-06419-00, Cole-Parmer, Vernon Hills, IL). Red food coloring diluted with PBS buffer (pH 7.4, 0.1% BSA), were loaded into cell culturing reservoir and probe reservoir, and fluorocarbon oil with 1% surfactant was loaded in the oil reservoir. Air trapped in the channel was removed by applying vacuum at the outlet and sequentially opening valves to let fluids from oil, cell culturing, and probe reservoirs to flow through the channel. The capillary was filled with about 1 cm of red buffer. The LabVIEW application was set to chip characterization mode and fluids from each individual reservoirs were continuously pumped at 2 Hz into the reference channel. The distances traveled by the leading meniscus of the fluid after about 5 min were measured with a ruler. The flow rates and the volumes per pump cycle of each fluid were then calculated with the equation $r = \frac{\pi(\frac{d}{2})^2 L}{t}$, and $r = \frac{\pi(\frac{d}{2})^2 L}{c}$, where d is the ID of the capillary (100 µm), L is the distances traveled by the leading meniscus, *t* is the pumping time, and *c* is the cycle number of pumping.

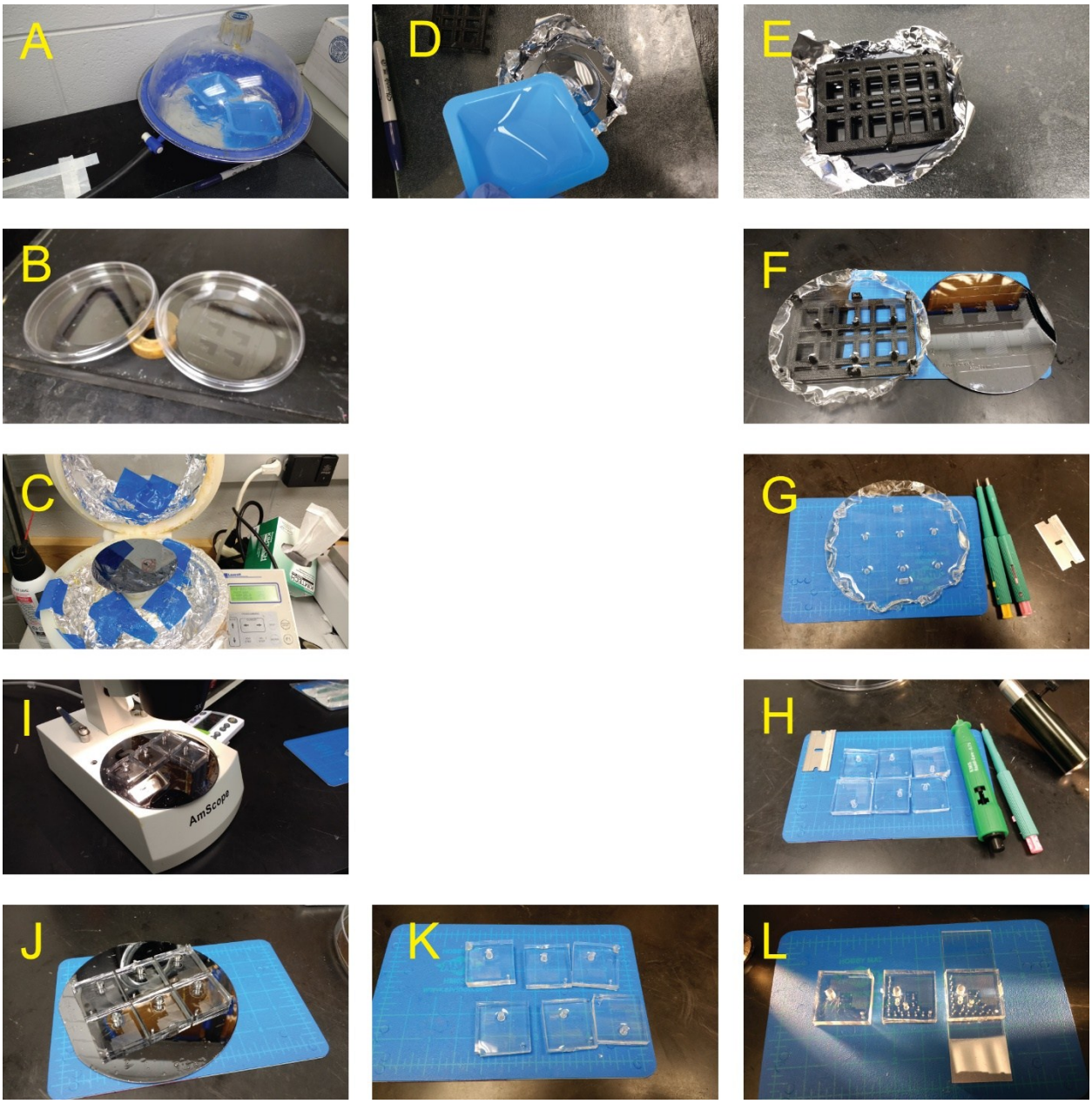

Figure S-1. *Fabrication procedure of microfluidic devices.*
(A) 36 g of PDMS in 5:1 ratio and 10.5 g of PDMS in a 20:1 ratio were mixed and degassed under vacuum. (B) Both of the control wafer and fluidic wafer was treated with trimethylsilyl chloride vapor for 30 min. (C) PDMS (20:1) was spin-coated over the control wafer. (D) PDMS (5:1) was poured onto the fluidic channel master in an aluminum foil boat. (E) 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 60 °C undisturbed for 40 min. (F, G, H) The cured PDMS with template was peeled from the wafer, after the 3D-printed template was removed, Devices were diced, and holes were punched to prepare for bonding with the control channel PDMS layer. (I) After careful alignment (in step I) under microscope, the fluidic layer PDMS and partially cured control layer were permanently bonded together (J) by placing in the oven at 65 °C for at least 2 hours. (K) Completed PDMS devices were diced, peeled from the wafer, and holes were punched for control channel pressure lines. (L) PDMS devices were finally plasma oxidized and bonded onto glass substrate (left: before hole-punch, middle: after hole-punch, and right: fully assembled device).

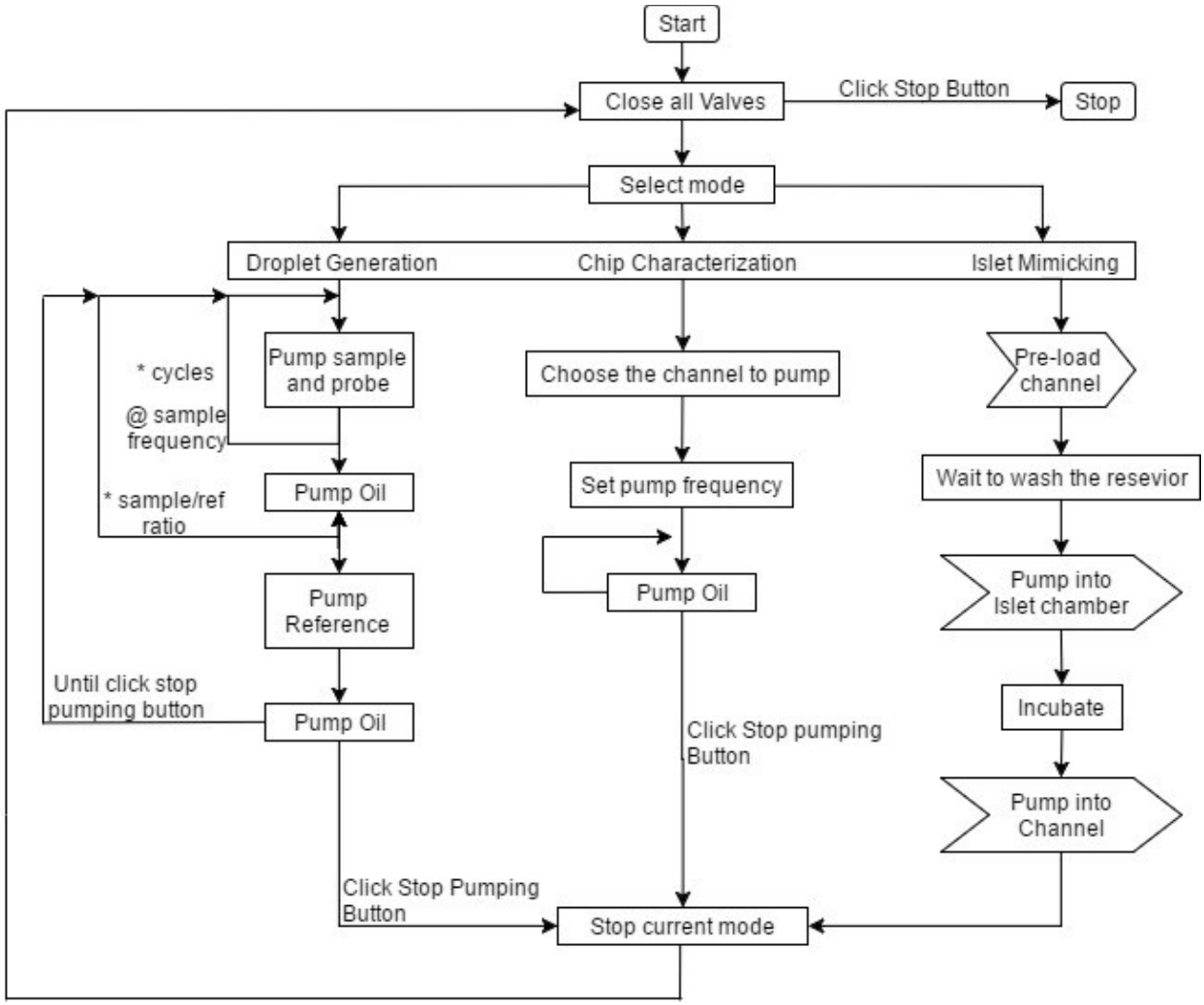

***Figure S-2**. Programmatic flow chart of LabVIEW application for microfluidic chip automation.*

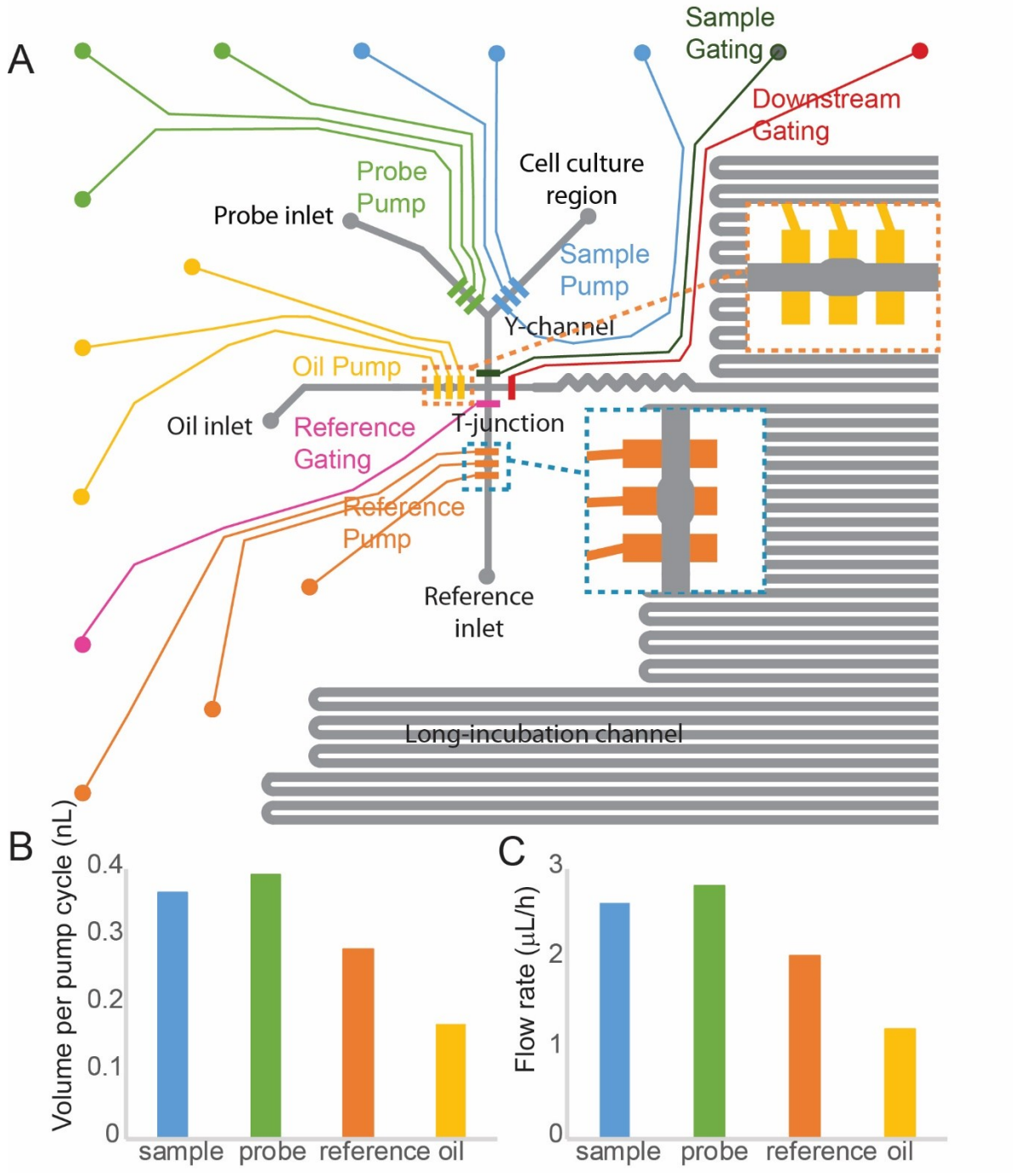

***Figure S-3**. Microfluidic control channel description and color-coded pumping test.*
(A) Enlarged microfluidic channel layout with control channel description in corresponding color and fluidic channel in black. The two insets indicate the enlarged center valve on oil (yellow) and reference (orange) channels. (B) Measured dispensing volume per pump cycle at 2 Hz pumping frequency and 30 psi pressure. (C) Measured flow rate of each pump at 2 Hz pumping frequency and 30 psi pressure.

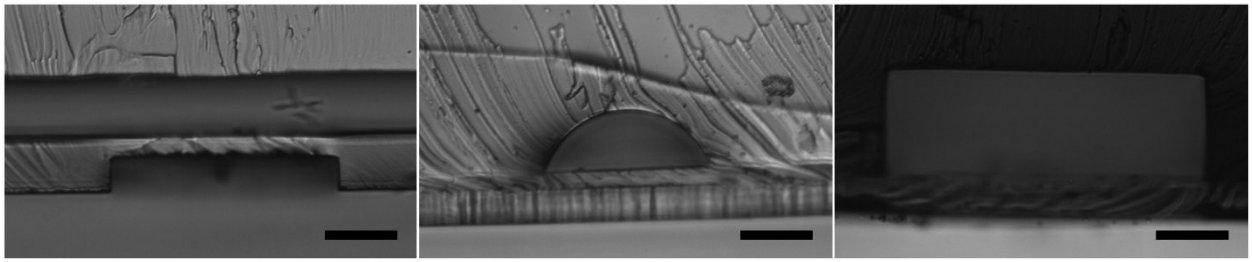

Figure S-4. *The cross sections of microfluidic channels. Control valve under flow channel (left), rounded flow channel (middle), and squared-off droplet storage channel (right). Scale bars = 50 µm.*

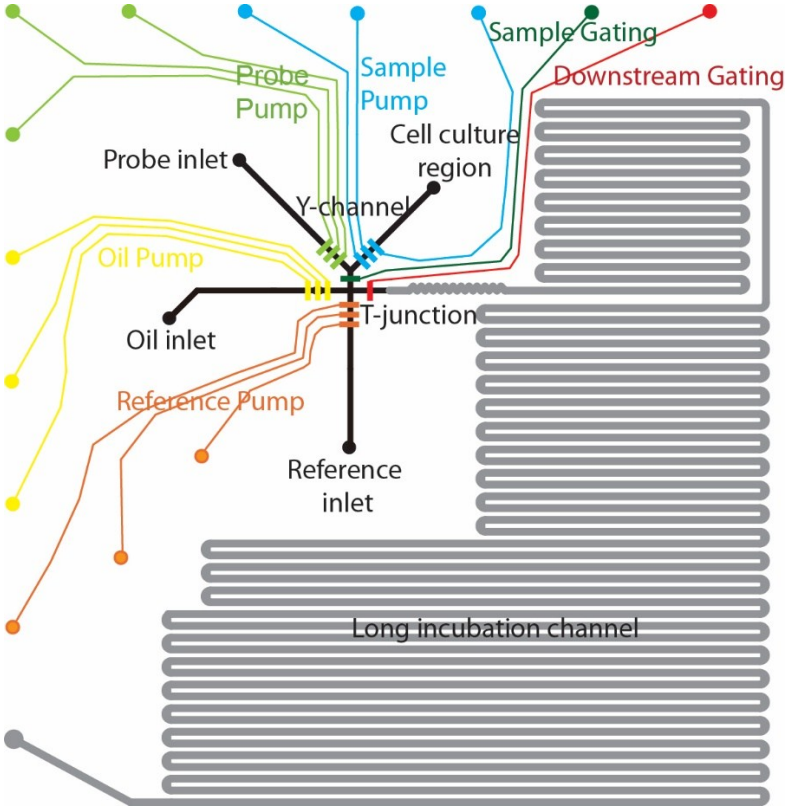

Figure S-5. *Previous version of microfluidic device channel layout. Compared to the current version, there are no gating valves for the reference channel, and the Y-channel is closer to the T-junction.*

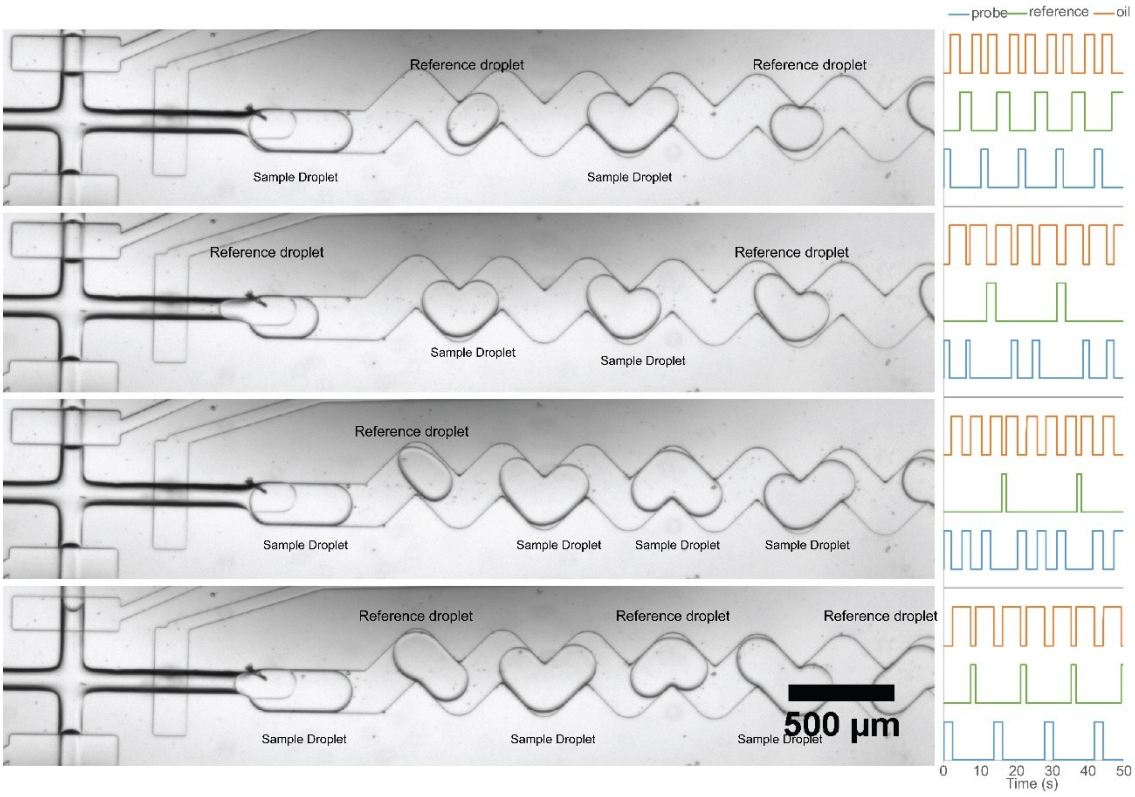

Figure S-6. *Droplet formation with various parameters.*
The left side shows the representative images of droplet formation with various pumping cycles, sample/reference ratios, and oil pumping cycles. The right side is the corresponding pumping codes. Up indicates pumping and down represents closing, and each pump cycle was 500 ms.

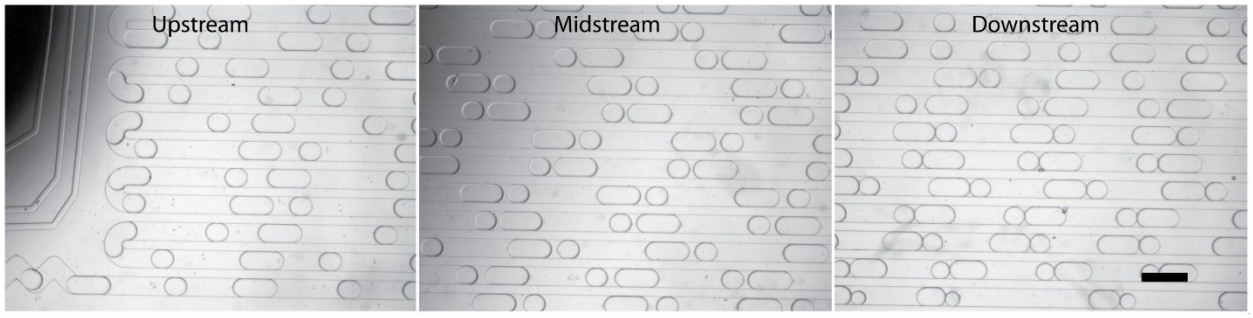

Figure S-7. *Droplets packed up within the channel as they moved downstream in the long incubation channel. Scale bar = 500 µm.*

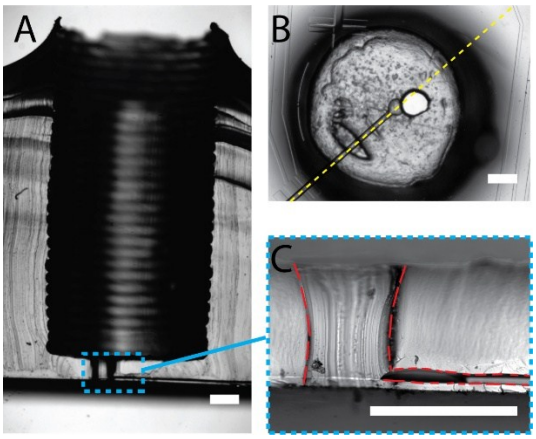

Figure S-8. *Cross-sections of the islet trapping region.*
(A) cross-section of the big reservoir and the cell culturing region. (B) bottom view of the reservoir with a line indicating the cutting line of the cross-section in (A and C). (C) the enlarged image of the islet trapping region with red dash line for the chamber and the microchannel. Scale bar = 500 μm.

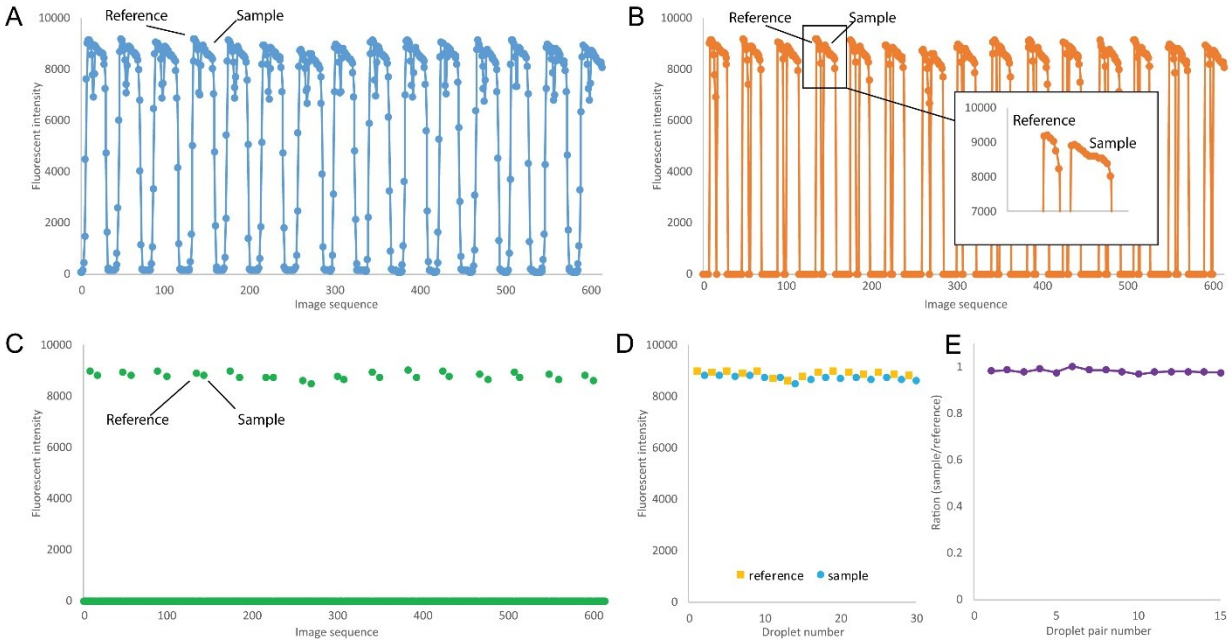

Figure S-9. *Example of droplet-sampled insulin secretion and assay data analysis.*
(A) The raw data was determined directly from the average intensity of fluorescence images by ImageJ. (B) A standard deviation filter and intensity filter were applied to select the data points of droplets (other data points were set to 0). (C) Mean intensity of each individual droplet. (D) Mean intensities of droplets were sorted into "sample" and "reference" data categories. (D) Fluorescent ratio of sample droplets and corresponding reference droplets was calculated.

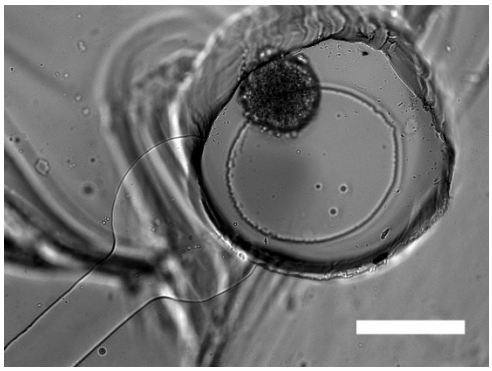

Figure S-10. *Example of bottom view of single islet in the trapping chamber.*

Reference

(1) Unger, M. A.; Chou, H. P.; Thorsen, T.; Scherer, A.; Quake, S. R. *Science (80-.).* **2000**, *288* (5463), 113–116.

(2) Thorsen, T.; Maerkl, S. J.; Quake, S. R. *Science (80-.).* **2002**, *298* (5593), 580.

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(6) Erickstad, M.; Gutierrez, E.; Groisman, A. *Lab Chip* **2015**, *15* (1), 57–61.