**Electronic Supplementary Material**

**Methods**

**Islet culture Microfluidic Chip Fabrication.** A microfluidic chip was designed to perform single-islet culture (Figure 1), and fabricated according to standard photolithographic techniques 1. Briefly, the silicon substrate was spin-coated with the negative photoresist SU8-2100 (MicroChem) to obtain a 300-µm thickness of the resist layer. After soft baking, UV exposure, post-exposure baking, and development in a SU-8 Developer (MicroChem), the mold was hard baked up to 160°C and then cooled to room temperature. Polydimethylsiloxane (PDMS) (Sylgard, Dow Corning) was used for replica molding in the ratio 10:1 (base:cure agent). The mold was treated with hexamethyldisilazane (Sigma-Aldrich) vapor for 30 min before casting. The input-output holes on the PDMS chip were made using a 20G needle punch (Small Parts, USA). The PDMS chip was bonded by plasma treatment (Harrick Plasma) onto a borosilicate glass cover slip (Menzel Glaser). A cut 200-µL pipette tip was inserted into the inlet hole to ease islet insertion and then removed.

**Multi-inlet Microfluidic Chip Fabrication.** A microfluidic chip containing the multi-inlet system was produced as previously reported (Zambon et al 2014). Briefly, it required two molds for its fabrication to obtain a two-layer PDMS chip including the pneumatic controls 3. The former had 25-µm round channels (round mold), obtained from SPR 220-7 (Dow Corning) after reflow during the hard bake time. The latter had 25-µm square channels (square mold) obtained from SU-8 2025 (MicroChem). PDMS was spun on the square mold to obtain a 50-µm thickness layer using a spin coater (Laurell Technologies), and poured on the round mold to make a thick layer (4-5 mm). The thicker PDMS chip was bonded and aligned under a microscope onto the thin one, at the end of the curing phase. The final two-layer chip was bound on a microscope borosilicate glass slide (Menzel Glaser).

**Animals and Pancreatic Islet Extraction and Preparation.** The protocol followed the Guide for the care and use of laboratory animals, Eighth edition (2011) (http://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) and was compliant with local and national laws. For murine islet isolation, we optimized a protocol previously described 4. We used 8-12 weeks old C57Bl/6J mice (Jackson Laboratory), fed a standard diet containing 10% of energy from fat. Transgenic mice that express green fluorescent protein (GFP) under the control of the mouse insulin I gene promoter (MIP) were purchased from Jackson Laboratories and used as a control to detect beta-cells. MIP-GFP mice have normal islet architecture with co-expression of insulin and GFP in beta-cells of all islets 5. Briefly, mice were anesthetized, and the pancreatic duct was perfused with collagenase (Vitacyte, 001-2030)/protease (Vitacyte, 003-1000) mixture. The pancreas was then removed and digested for 14 min at 37°C. Islets were subsequently picked by hand, transferred to fresh medium, and maintained at 37°C. The islets were cultured in RPMI 1640 supplemented with 10% bovine serum, 11 mM glucose, 100 units/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, pH 7.4. The pancreatic islets were plated on a low-adhesion dish and kept at 37°C and 5% CO2 within a biological incubator for 1-2 days.

**Microfluidic System Running Conditions.** The multi-inlet microfluidic system was connected to medium vials of different compositions. The basal solution was composed of Krebs-Ringer-HEPES (KRH) buffer, whose composition (in mM) is as follows: NaCl 136, KCl 4.7, CaCl2 1.3, MgCl2 1.25, and HEPES 10, with pH adjusted to 7.4 by NaOH. The other vials contained KRH buffer added with different concentrations of glucose (J.T.Baker), simvastatin (Sigma-Aldrich), tolbutamide (Sigma-Aldrich), and FluoZin-3 (ThermoFisher). First, the islet culture microfluidic chip was filled with KRH buffer and pressurized for bubble removal. Then, a single islet per channel was inserted, placing it with a pipette inside the tip at the inlet of a microfluidic channel, where it entered by gravity after few minutes. The microfluidic multi-inlet system was placed upstream of the islet culture chip and connected to it by a 5-cm long PEEK tube, having an inner diameter of 65 µm (Idex, USA). Each flow channel of the multi-inlet system was connected to a pressurized vial containing solutions of different composition. This system allowed switching between up to 9 different medium compositions during an experiment by controlling valve opening and closing via LabView software (National Instruments). At the outlet of the islet culture chip, a syringe pump (Harvard Apparatus) working in aspiration, equipped with a glass 100-µL syringe (Hamilton), was connected to precisely control flow rate at 2 µL/min. The complete system is displayed in Figure 1.