

Supplementary Information for:

Ca²⁺ oscillations, waves, and networks in islets from human donors with and without type 2 diabetes

Marko Gosak^{1,2,*}, Richard Yan-Do^{3,4,*}, Haopeng Lin⁴, Patrick E. MacDonald^{4,‡}, Andraž Stožer^{1,‡}

¹ Institute of Physiology, Faculty of Medicine, University of Maribor, Taborska ulica 8, 2000 Maribor

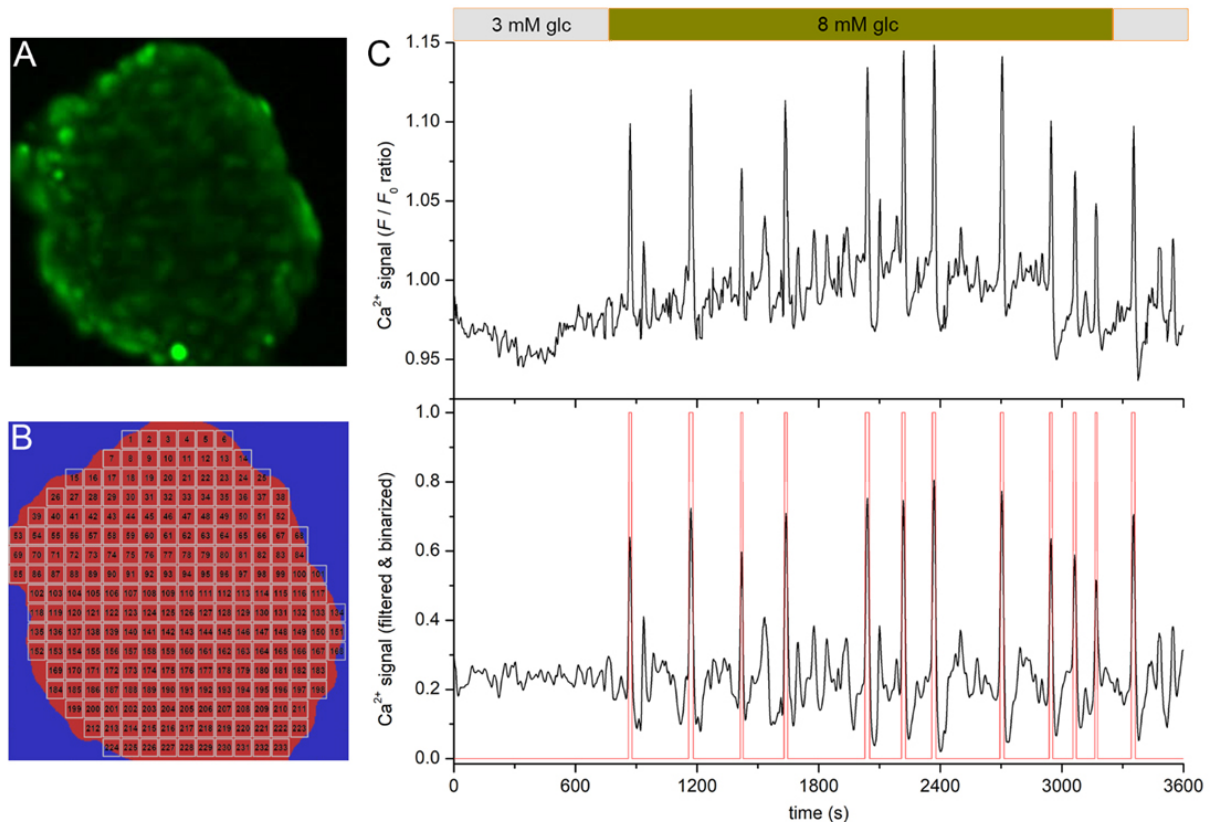
² Department of Physics, Faculty of Natural Sciences and Mathematics, University of Maribor, Koroška cesta 160, 2000 Maribor

³ Hong Kong Centre for Cerebro-Cardiovascular Health Engineering (COCHE), Hong Kong Science Park, Department of Biomedical Engineering, City University of Hong Kong, Kowloon, Hong Kong

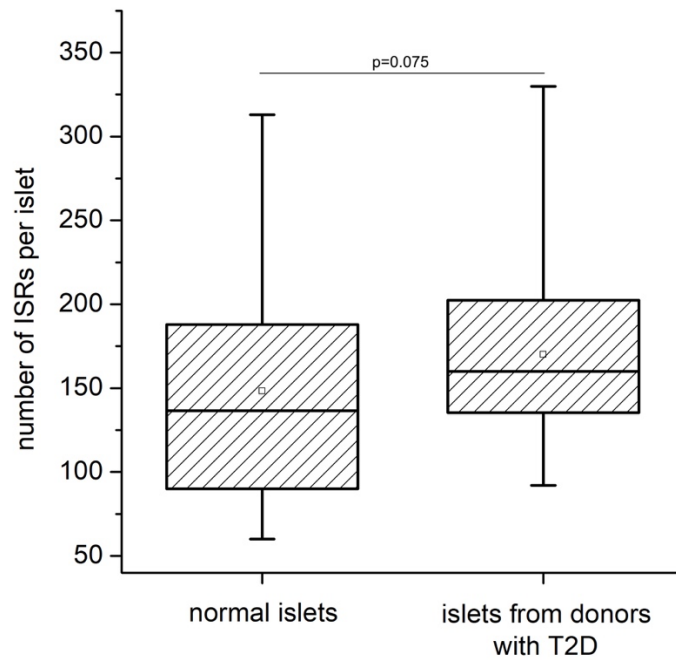
⁴ Department of Pharmacology and Alberta Diabetes Institute, University of Alberta, 6-126 LKS Centre, Edmonton

* Contributed equally

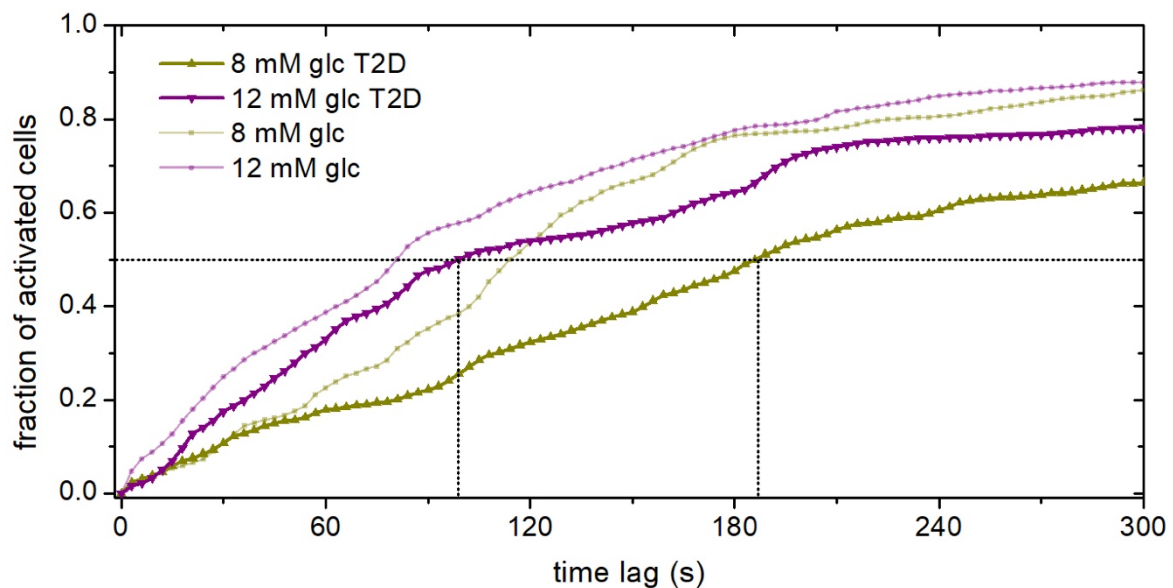
‡ Corresponding authors



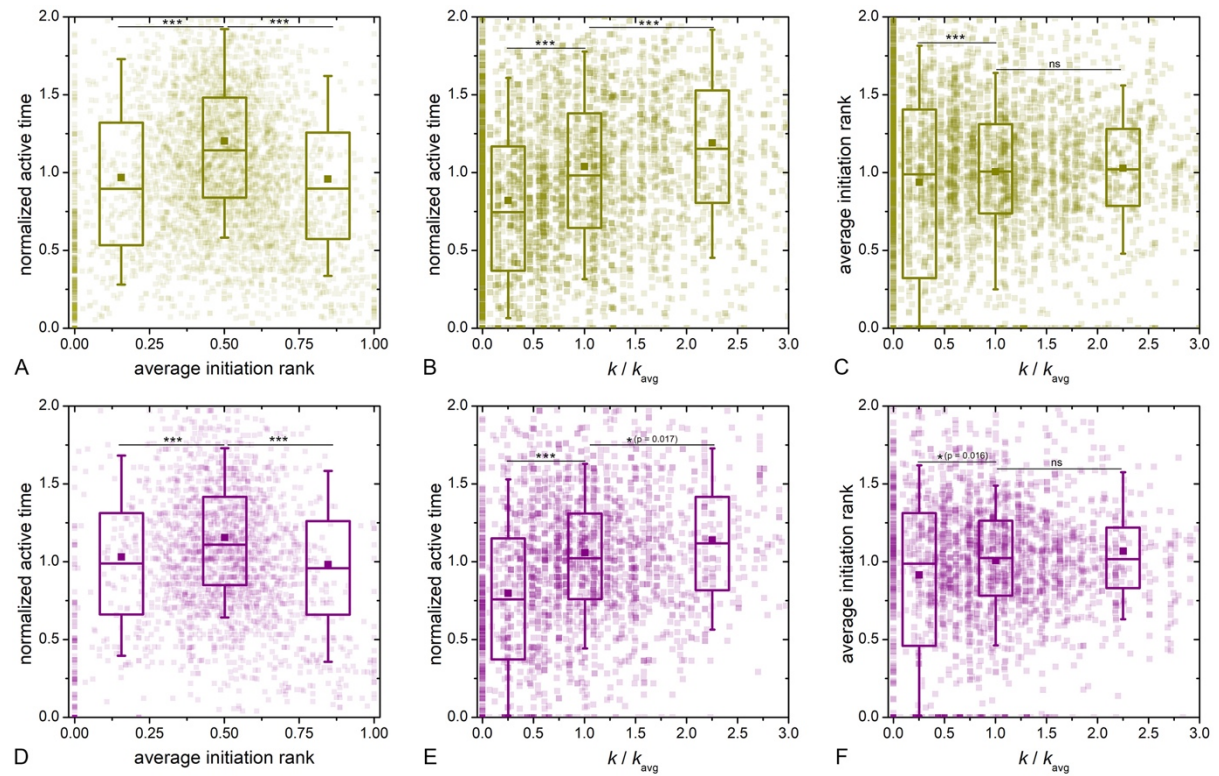
Supplementary figure S1. Methodology to extract $[Ca^{2+}]_i$ dynamics in isolated human islets. An image of an isolated islet loaded with Fluo-4 (A). Binarized image (red area on blue background) of the islet that is divided into square islet subregions (ISRs) with a linear size of $15\ \mu\text{m}$ (white squares), from which the $[Ca^{2+}]_i$ signals were extracted (B). Unprocessed $[Ca^{2+}]_i$ signal from one ISR (upper row) and the corresponding high-pass filtered $[Ca^{2+}]_i$ trace (black line) along with the binarized activity (red line) (C). A cut-off frequency 0.02-0.04 Hz of the high-pass filter was used, and these values were determined empirically to ensure the best binarization. The latter was determined on the basis of the computed standard deviation (SD) of the filtered $[Ca^{2+}]_i$ traces, so that all values above $x \cdot \text{SD}$ were set to 1, whilst 0 otherwise. Factor x designates the degree to which the $[Ca^{2+}]_i$ oscillations exceed the estimated noise and was determined empirically (values typically spanning between 1.25 and 2). The binarized signals were finally smoothed and refined to remove artefacts, so that narrow gaps (1 frame) within the active phases were set to 1 and narrow active phases (1 frame) within the silent phases were set to 0. The binarized data was used to calculate the parameters of cellular responses: the average frequency, the average duration, and the average relative active time (duty cycle). The latter was determined as the fraction of 1 (i.e., “on” states) in the binarized signals, reflecting thereby the average proportion of time that cells spend in an active state with increased $[Ca^{2+}]_i$. The binarized signals were also used to extract individual Ca^{2+} waves by means of a space-time cluster algorithm, as described previously (40). In brief, we traced the course of the intercellular wave from the first activated ISR to the last. If proximate ISRs (less than $35\ \mu\text{m}$ apart) became activated within a short time period (3 frames), the given activation sequence ISRs was considered as one Ca^{2+} wave with a given size. Accordingly, this size encompasses the information about the number of ISRs involved and the duration of the wave. Very small waves (size < 30 s; size is the number of activated ISRs multiplied by the time each ISR spend in the active phase) were discharged, as they represent very short elevations in one (or a few) ISRs due to noise (and not intercellular events).



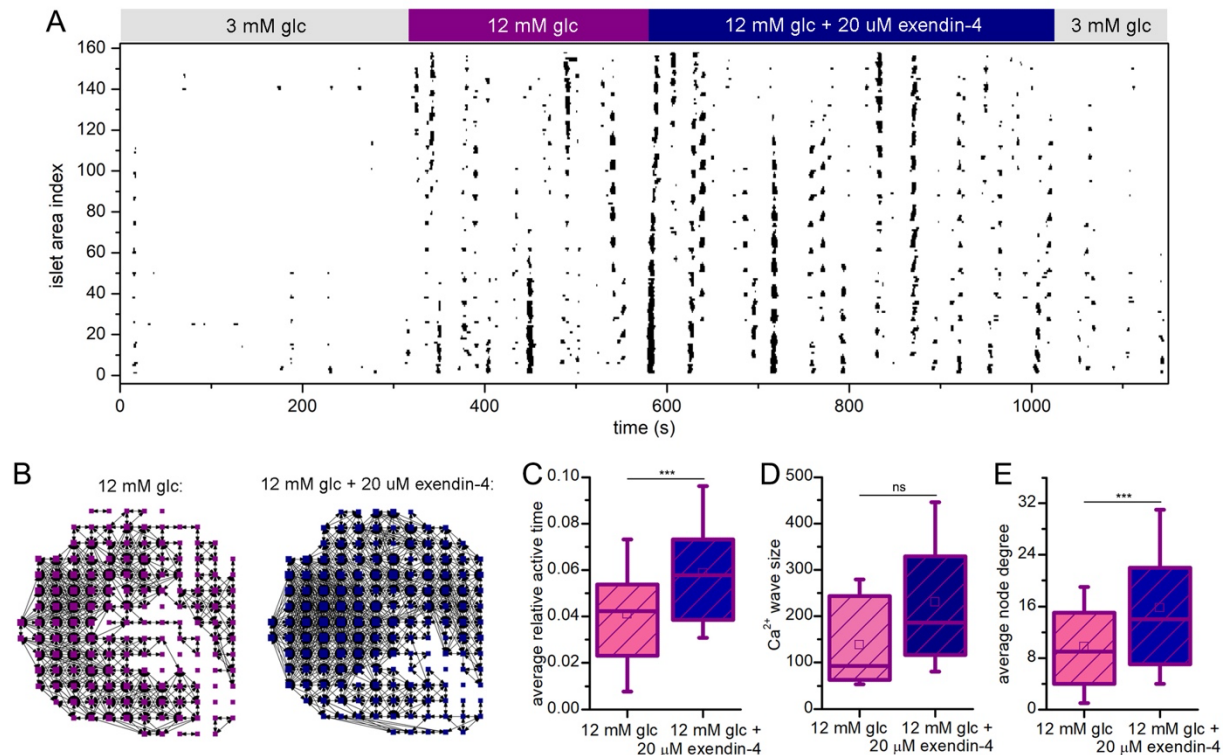
Supplementary figure S2. Size comparison of islets from healthy donors (41 islets) and from donors with type 2 diabetes (38 islets). One ISR corresponds to a square with a side length of 15 μm .



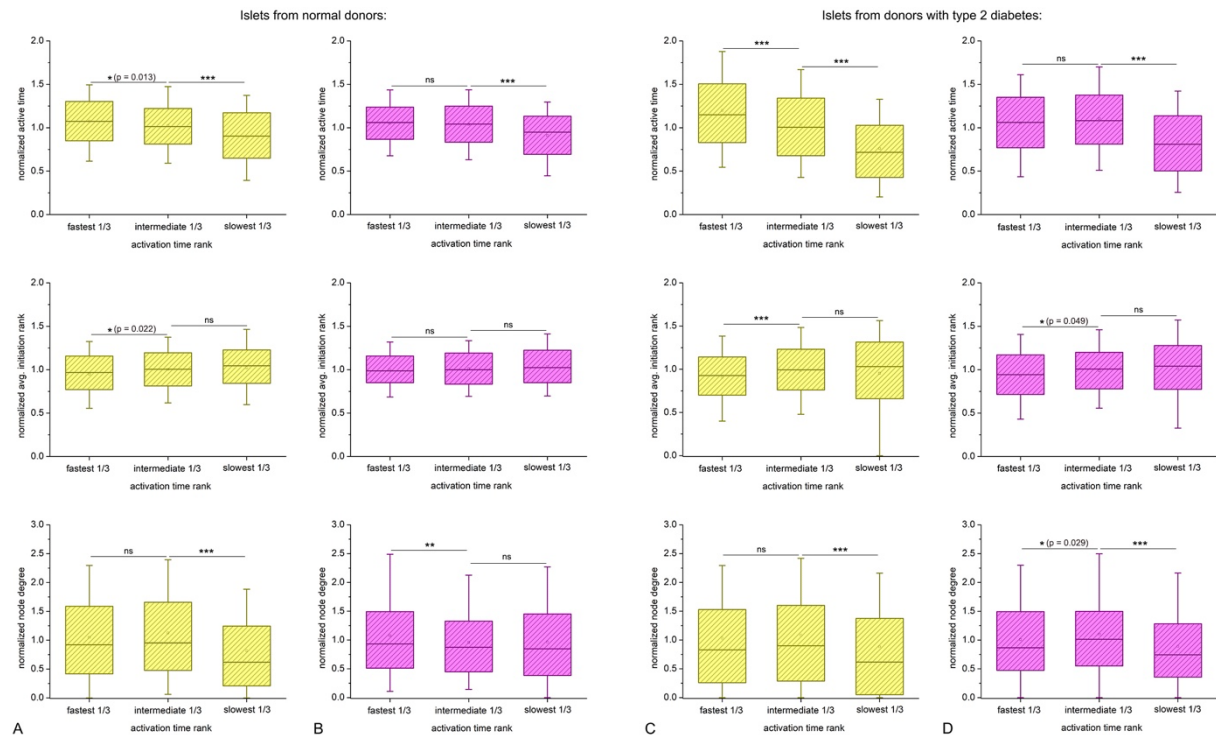
Supplementary figure S3. Activation is faster in higher glucose and delayed in islets from donors with type 2 diabetes. Cumulative activation of ISRs after the rise in glucose concentration from substimulatory 3 mM to stimulatory 8 mM or 12 mM for islets from control donors and donors with type 2 diabetes. An ISR was considered activated the first time a $[\text{Ca}^{2+}]_i$ oscillation occurred after rise in glucose stimulation. Both thick lines denote the average over all islets from donors with type 2 diabetes, whereas the thin light lines indicate the average behavior from non-diabetic donors, for easier comparison. Dotted lines indicate t_{50} .



Supplementary figure S4. The relationships between active time, average initiation rank, and relative node degree for all islets from donors with type 2 diabetes. Transparent dots indicate individual ISRs, whereas the box-plots denote the lowest, the intermediate, and the highest one third of initiation rank parameter values (A and D) or the least connected cells ($k/k_{avg} \leq 0.5$), intermediately connected cells ($0.5 < k/k_{avg} \leq 1.5$), and the most connected cells ($k/k_{avg} > 1.5$) (B, C, E and F). Box charts are defined as in Figures 1-4. The results were obtained on the basis of 3971 and 2256 ISRs for 8 mM and 12 mM glucose, respectively.



Supplementary figure S5. GLP-1 induced changes in $[\text{Ca}^{2+}]_i$ activity in a normal human islet. A) Binarized activity of all ISRs as a function of time with the indicated stimulation protocol: 3 mM glucose \rightarrow 12 mM glucose \rightarrow 12 mM glucose + 20 μ M exendin-4 \rightarrow 3 mM glucose. B) Functional islet networks extracted from the interval with 12 mM glucose stimulation only (purple) and from the interval with added exendin-4 (blue). Panels (C-E) present the relative active times, Ca^{2+} wave sizes, and node degrees of all ISRs, separately for the 12 mM glucose and 12 mM glucose + exendin-4 intervals. Apparently, exendin-4 evoked an increase in overall activity as well as an increase in the number of functional connections, particularly by increasing the degrees of the most connected ISR.



Supplementary figure S6. Functional characterization of first-responder ISRs. Box plots show the fastest 1/3th, the intermediate 2/3rd, and the slowest 1/3th of activated ISRs (characterized by their activation time ranks) and the corresponding $[Ca^{2+}]_i$ signaling parameters: the relative active times, initiation ranks, and node degrees, for normal islets stimulated with 8 mM (A) and 12 mM (B) glucose, as well as for islets from diabetic donors stimulated with 8 mM (C) and 12 mM (D) glucose. All panels show the pooled data from all recordings in the given group. Boxes determinate the interval between the 25th and the 75th percentile, whiskers denote the 10th and the 90th percentile, lines within the boxes indicate the median, and the small square the average value of a given parameter. The results in all groups indicate a tendency of ISRs which responded faster to stimulation being more active, which is more pronounced in 8 mM glucose than in 12 mM glucose (in both normal islets and islets from diabetic donors). Moreover, in all four groups there is a weak relationship between the response time to stimulation and the initiation ranks. Apparently, ISRs that are activated last are not only the least active but also seldom act as wave initiators. Finally, there is overall no relationship between the node degrees and the response time, which is in accordance with previous reports (30,32,48).

ID	Age	Sex	Donation type	CIT (h)	BMI	HbA1c	T2D duration (y)	T2D treatment	Culture (d)	Medium 1	Medium 2
R234	50	F	NDD	16.3	31.7	5.7	n.a.	n.a.	2	CMRL	DMEM, 5.5 mM
R235	53	F	NDD	15	24.5	5.7	n.a.	n.a.	3	CMRL	DMEM, 5.5 mM
H2169	26	M	NDD	n.a.	22.1	5.2	n.a.	n.a.	n.a.	CMRL	DMEM, 5.5 mM
H2171	54	M	NDD	n.a.	33.4	6.3	n.a.	n.a.	n.a.	CMRL	DMEM, 5.5 mM
R272	56	M	NDD	13.5	26.9	5.6	n.a.	n.a.	2	CMRL	DMEM, 5.5 mM
R280	17	F	NDD	10	26.3	5.2	n.a.	n.a.	3	CMRL	DMEM, 5.5 mM
R236	51	M	DCD	23	35.3	8.6	unk.	none	3	CMRL	DMEM, 5.5 mM
R240	55	F	NDD	13	30.9	6.9	2	diet	1	CMRL	DMEM, 5.5 mM
R244	48	F	NDD	11.5	30.4	7.5	3	metformin	1	CMRL	DMEM, 5.5 mM
R262	54	F	NDD	3	22.4	5.6	unk.	metformin	5	CMRL	DMEM, 5.5 mM
R263	71	M	NDD	20	38.3	7.6	9	metformin	2	CMRL	DMEM, 5.5 mM
R307	43	M	NDD	6.5	25.4	6.5	2	metformin	2	CMRL	DMEM, 5.5 mM
R276*	54	F	NDD	21	24.4	7.2	10	insulin	2	CMRL	RPMI 7.5 mM

Supplementary table S1. Basic characteristics of donors with and without type 2 diabetes.

The sample was balanced by the inclusion in our analyzes of islets from 3 female control and diabetic donors, as well as 3 male control and diabetic donors. Within both control and diabetic donors, female and male donors did not significantly differ with respect to age, BMI, or HbA1c. Moreover, they did not differ in terms of insulin secretion at 1 mM and 10 mM glucose. For all the other analyses, data were pooled together for female and male donors. Moreover, control and diabetic donors did not differ in age or BMI, but as expected, donors with T2DM had significantly higher values of HbA1c.

*Used for preliminary analyzes presented in Figure 5 upon request of one of the reviewers. Medium 1- The medium used after islet isolation and before transfer to the main lab. Medium 2- the medium used upon transfer to the main lab and before calcium recordings. NDD-neurological. DCD-Cardio-circulatory. N.a.-not applicable.

ID	1 mM glucose (pg/ml)			AVG	10 mM glucose (pg/ml)			AVG	Content (ng/ml)			AVG
R234	483	883	321	562	4585	9379	4818	6261	552	705	699	652
R235	1273	2130	809	1404	5307	2712	5446	4488	876	1021	2017	1305
H2169	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
H2171	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
R272	2076	524	1987	1529	3236	2870	3741	3282	1459	1157	1555	1390
R280	364	614	394	457	4938	6868	5010	5605	558	439	278	425
R236	119	8	9	45	116	47	132	98	9	3	0	4
R240	1472	1935	383	1263	5587	5297	3022	4635	1271	1094	666	1010
R244	573	819	1233	875	3103	2368	4706	3392	568	669	1381	873
R262	318	120	197	212	1394	2342	3880	2539	1538	1538	824	1300
R263	1217	2358	1505	1693	5655	6575	7752	6661	1080	2086	1935	1700
R307	550	631	561	581	1812	2067	24633	9504	568	1039	549	719
R276*	336	621	486	481	1696	1734	808	1413	767	950	1158	958

Supplementary table S2. islet insulin secretion and content in donors with and without type 2 diabetes.

Supplementary video S1. Recording of a human islet and the corresponding animation of binarized spatiotemporal $[Ca^{2+}]_i$ activity in a representative islet stimulated with the 3-12-3 mM glucose protocol.