

Tracking Ca²⁺ dynamics in NOD mouse islets during spontaneous diabetes development

Short running title: Tracking pancreatic α - and β -cells functions in NOD mice

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Supplemental Methods and Materials:

Pancreas slice preparation:

Upon CO₂ euthanasia, the mouse abdominal cavity was assessed via laparotomy. 1.9% low-melting-point agarose (Lonza, USA) dissolved in extracellular solution (ECS, (in mM): 125 NaCl, 26 NaHCO₃, 6 glucose, 6 lactic acid, 3 myo-inositol, 2.5 KCl, 2 Na-pyruvate, 2 CaCl₂, 1.25 NaH₂PO₄, 1 MgCl₂, 0.25 ascorbic acid) at 40°C was infused into the pancreas via the distally clamped common bile duct. The pancreas was then cooled, excised, and sliced using a vibratome (VT1000S, Leica Microsystems, Germany). The sliced pancreas (140 µm) were maintained in HEPES-ECS (in mM: 125 NaCl, 10 NaHCO₃, 10 HEPES, 6 glucose, 6 lactic acid, 3 myo-inositol, 2.5 KCl, 2 Na-pyruvate, 2 CaCl₂, 1.25 NaH₂PO₄, 1 MgCl₂, 0.25 ascorbic acid; titrated to pH 7.4 using 1 M NaOH) or loaded with fluorescent Ca²⁺ indicator in HEPES-ECS (6 µM Calbryte 520-AM (AAT Bioquest, Montluçon, France), 0.03% Pluronic F-127 (w/v), and 0.12% dimethylsulphoxide (v/v)). All chemicals were obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

Supplementary Figure Legends

Figure S1. Quantification and analysis of [Ca²⁺]_i dynamics.

A normal-like islet is used to demonstrate the method of quantifying and analyzing [Ca²⁺]_i dynamics recorded in islet cells. **A:** Regions of interest (ROIs) obtained by segmentation algorithm. The numbers indicate representative ROIs whose filtered [Ca²⁺]_i dynamics traces, representing intracellular fluorescence changes over time, are presented in **D**. A heatmap below provides color coding of the number of events detected in the ROI traces, upon a high-pass

filtering at 0.2 Hz. In the bottom histogram, ROIs with number of events below the threshold (red dashed line in the histogram in the bottom) are discarded. **B**: Halfwidth duration of $[Ca^{2+}]_c$ events through time. Note the ranges of halfwidth duration occurring. Color indicates the statistical significance in terms of z -score (heatmap on the right). Only events with a z -score higher than 3 were included. **C**: A schematic of a transient event to describe the features of $[Ca^{2+}]_c$ events from background subtraction of the raw data, to height and halfwidth duration. Peak-point is the time of highest amplitude of an event. **D**: *top*: time courses from ROIs indicated in **A**, exposed to a stimulation protocol of 4, 6, 8, 6mM glucose, sharing the abscissa with **B**. and are rebinned to 2 Hz (recorded 20 Hz). *Bottom*: The left panel is a closeup of the trace above to highlight the long and short $[Ca^{2+}]_c$ events and the right panel highlights short $[Ca^{2+}]_c$ events (in circle). **E**: in a z -score vs. halfwidth density plot. The events are clearly separated into three groups, which we named ultra-short, short, and long events. Short and long events were further analyzed since dominant pattern of glucose sensitivity was observed. **F**: Normalized Gaussian fit through the logarithmic distribution of halfwidth duration during 8mM glucose condition.

Figure S2. Categorization of NOD mice islets.

Each square corresponds to the position of islets shown in **Figure 6**. The categorization assigned to each islet is depicted within the square and color-coded. Note the progressive deepening of color of the squares along the severity of diseased progression. Note also from Mouse 4 onward, each mouse harboured islets that crossed at least two islet destruction phases.

Table S1. Statistical analyses of α - and β -cells $[Ca^{2+}]_c$ events across all islet groups.

Data are expressed as mean of events/minute/ROI \pm Standard Error of the Mean (SEM).

68 Red font indicates outcomes of statistical analyses comparing mean values derived at different
69 glucose concentration within the same islet groups. * denotes $p\text{-value}<0.05$; **denotes $p\text{-}$
70 $value<0.01$. Blue font indicates outcomes of statistical analyses comparing mean values of the
71 normal islet group with the other islet groups within the same glucose concentration. † denotes $p\text{-}$
72 $value<0.05$; ††† denotes $p\text{-value}<0.001$.

73 **Table S2. Animal characteristics, *in vivo* parameters and islets analyzed.**