SUPPLEMENTARY MATERIAL

The MATLAB script used for intra-islet synchrony analysis (related to **Fig. 3A-D** and **Supplementary Fig. 1**) is detailed bellow.

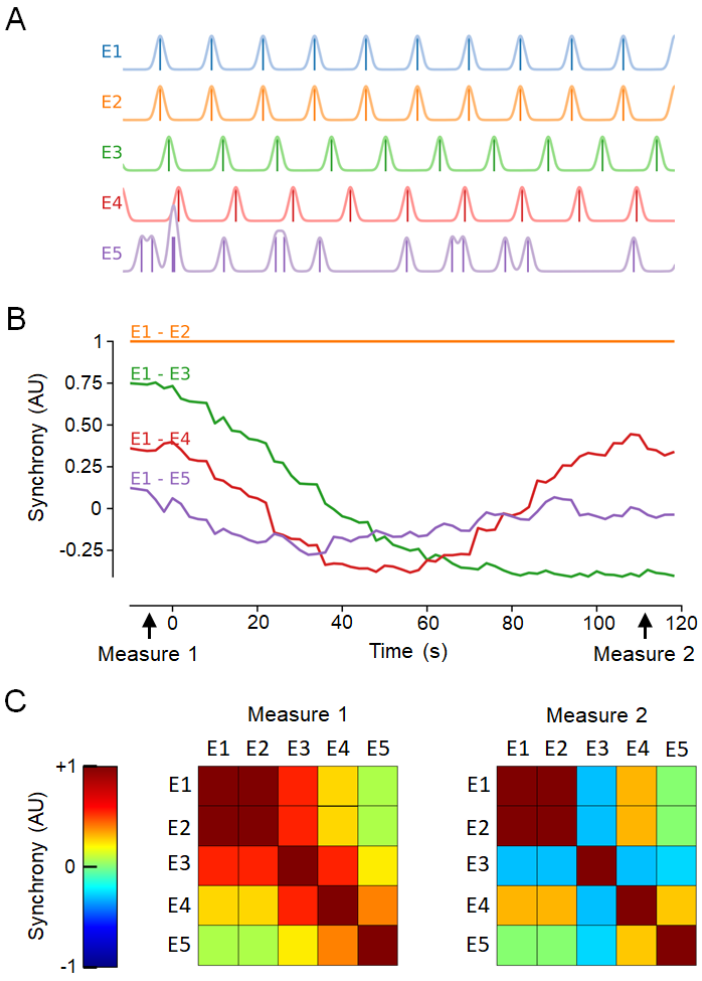
- evt_correlation.m ----------

```
function [correlation_matrix,gauss_sig] =
evt_correlation(timestamps_in,Fs,sigma_s,varargin)
% evt_correlation.m
% - Computes time correlation between events across N channels. Yields an NxN
correlation matrix and a representation of all events and their region of influence.
%
% Outputs
% - correlation_matrix : Correlation matrix (shows correlation index for channels
(i,j))
% - gauss_sig          : Smooth event signal for every channel
%
% Inputs
% - timestamps_in      : Input timestamps. Cell of vectors (one vector of timestamps
per channel). No unit (Nbr of samples).
% - Fs                 : Sampling frequency (Hz)
% - sigma_s            : width of the gaussian (temporal tolerance for event
correlation)
% - varargin           : additional arguments ('nv' makes the function non-verbose)

% Parse additional arguments
if ~isempty(varargin)
    for i=1:length(varargin)
        if isequal(varargin{i},'nv')
                    % Non-verbose flag
            VERBOSE = 0;
        end
    end
end


% Verbose flag check
if ~exist('VERBOSE')
    VERBOSE = 1;
end


% Check all signals for largest timestamp and preallocate memory for event signals
Nsignals = length(timestamps_in);
Lmax = 0;
for i=1:Nsignals
    sp_timestamps = timestamps_in{i};
    sp_timestamps = sort(sp_timestamps(:));
    tmpmax = max(sp_timestamps);
    if ~isempty(tmpmax)
        Lmax = max(Lmax,tmpmax);
```

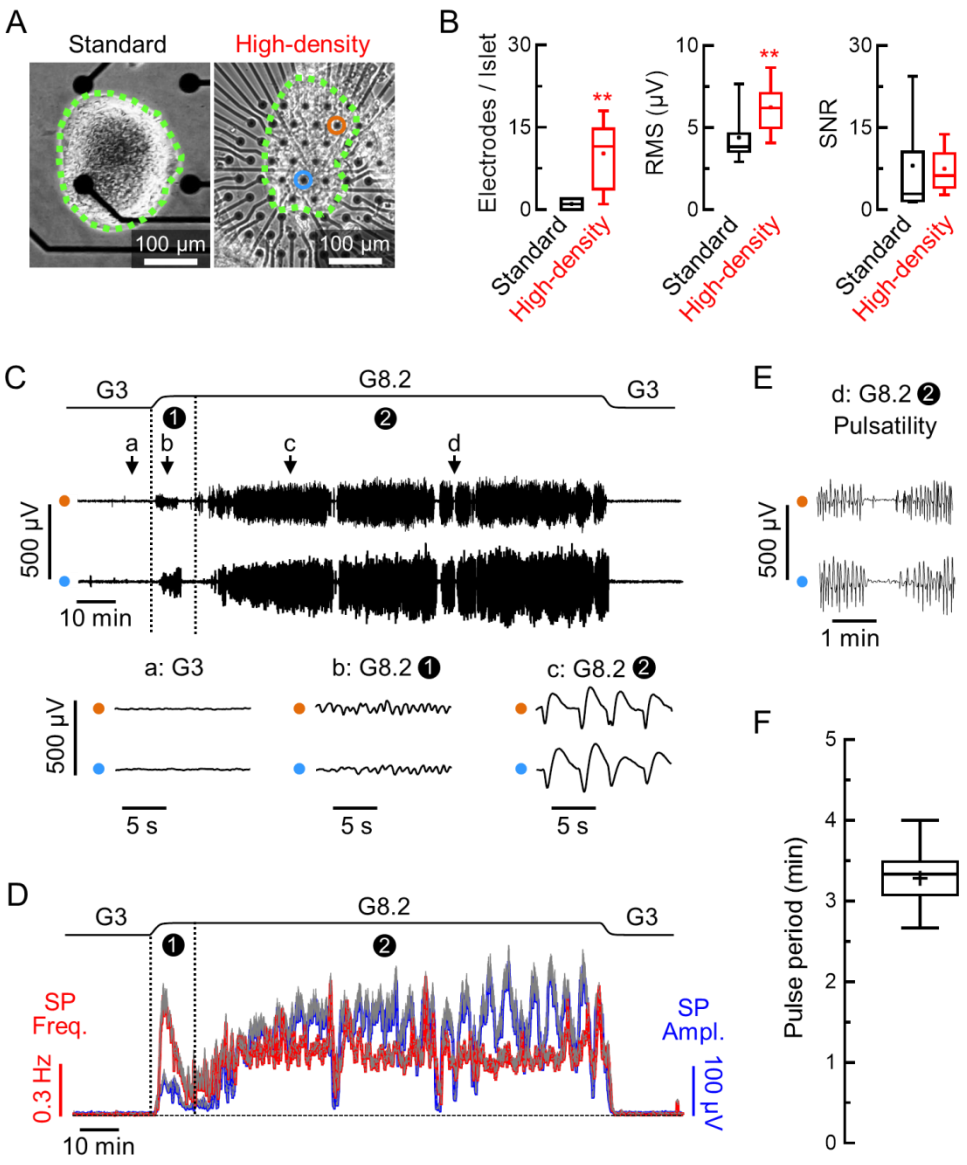
```
    end
end

events = zeros(Nsignals,Lmax);


% Generate gaussian waveform
gauss_width_s = 5*sigma_s;
x = -gauss_width_s:1/Fs:gauss_width_s;
sigma = sigma_s;
H = gaussmf(x,[sigma 0]);


% Generate smooth events (convolve event signal with gaussian)
gauss_sig = zeros(Nsignals,Lmax-1); % Preallocate
for i=1:Nsignals
       % Generate event signal
    sp_timestamps = timestamps_in{i};
    sp_timestamps = sort(sp_timestamps(:));
    events(i,sp_timestamps) = 1;
       % Smooth it out
    gauss_sig(i,:) = fastconv(events(i,:),H,0); % Fast convolution (fft, product,
ifft)
end


% Generate correlation matrix
correlation_matrix = zeros(Nsignals,Nsignals);
if ~isempty(gauss_sig)
    k = 0; % Counter to keep track of how many correlations have been computed
    for i=1:Nsignals
        for j=1:Nsignals
            correlation_matrix(i,j) = corr(gauss_sig(i,:)',gauss_sig(j,:)'); % Compute
correlation index for channel couple (i,j)
            k = k+1;
            if VERBOSE % Print progress every 100 correlations computed
                if mod(k,100) == 0
                    disp(['Event correlation: ' num2str(k) ' out of '
num2str(Nsignals*Nsignals) ' done.'])
                end
            end
        end
    end
    if VERBOSE % Success message
        disp(['Event correlation: all done.'])
    end
    correlation_matrix(isnan(correlation_matrix))=0; % Nullify NaN (Not A Number)
values
end

end

- fastconv.m ------------------------------------------------------------------------
---
function [y]=fastconv(x, h, dim)

Ly=length(x)+length(h)-1;
Ly2=pow2(nextpow2(Ly));

X=fft(x, Ly2); % Fast Fourier transform
H=fft(h, Ly2); % Fast Fourier transform
```

```
if size(X) ~= size(H)
    H=H';
end
Y=X.*H; % Multiply ffts
y=real(ifft(Y, Ly2)); % Inverse fast Fourier transform

if dim==0 % Yield only center part (preserve signal length, time-aligned)
    y=y(round(length(h)/2):1:Ly-round(length(h)/2));
elseif dim==1 % Real time (preserve signal length, delayed)
    y=y(1:1:length(x));
elseif dim==2 % Full size
    y=y(1:1:Ly);
end
```

SUPPLEMENTARY FIGURES & LEGENDS

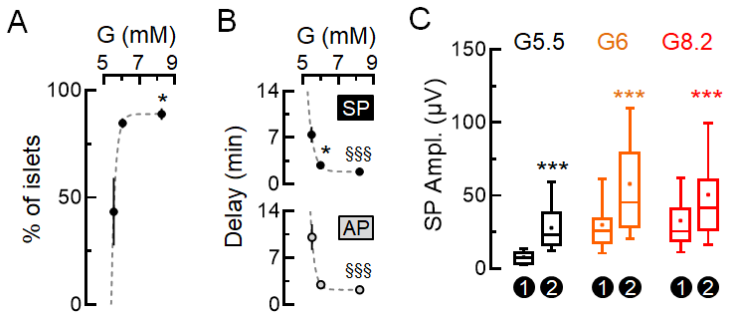


Supplementary Fig. 1. Simulations in MATLAB illustrating the method used to compute temporal event correlations and their matrix representation. To quantify the degree of SP synchrony between electrodes during the biphasic activation, a dynamic code was developed, inspired from works in neurons by Schreiber and colleagues (33). (**A**) Temporal detection of events (vertical lines) and continuous signals constructed by correlating events with Gaussian curves on 5 electrodes (E1 to E5). The time axis is as in (**B**). E1 and E2 are identical trains of regular events. E3 is a regular train of events, at 95% of the speed of E1. E4 is a regular train of events, at 90% of the speed of E1. E5 is a random train of events. (**B**) Time-dependent correlation measurements (synchrony; AU, arbitrary units) between E1 and E2-E5. Colors indicate with which electrode E1 is compared, following the color indicated in (**A**). (**C**) Matrix representations of correlation at the two time points indicated in (**B**). Each square represents the degree of synchrony (color code on the left), between -1 and 1, of the couple of electrodes given by its coordinates (hence the unitary diagonal). The degree of synchrony varies between -1 and +1: +1 representing SPs perfectly synchronized, 0 representing the absence of SP or SPs without any synchrony, and -1 representing SPs in opposition of phases.

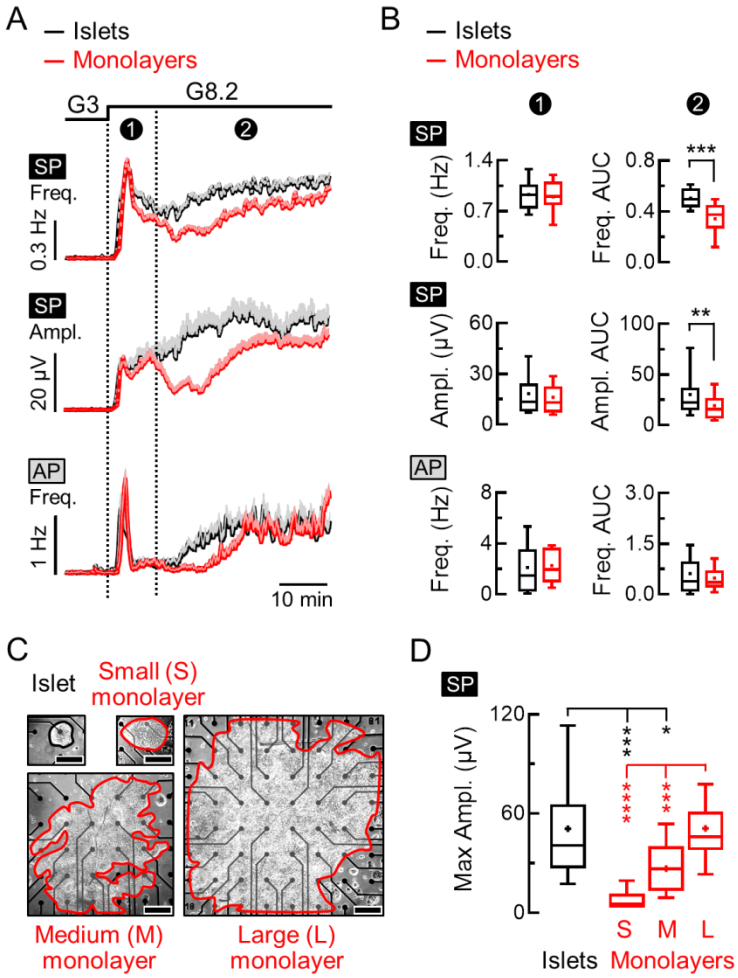


Supplementary Fig. 2. Monitoring of SPs in different regions of an islet with high-density (HD) MEAs. (**A**) Comparison of a standard MEA (4/59 electrodes shown) and a HD-MEA (59/59 electrodes shown). An islet is delimited by green dots. Note the difference between the size of the electrodes (images at identical scales): ø 30 µm for standard MEAs and 10 µm for HD-MEAs. Data from encircled electrodes (orange and light blue) are shown as examples in (**C**) and (**E**) (**B**) HD-MEAs increased the number of electrodes per islets (left panel; N=3-4 MEAs, n=8-49 islets). The smaller electrode diameter increased the noise level (middle panel; root mean square - RMS - noise level, N=3 MEAs, n=12 uncovered electrodes) without affecting the signal-to-noise ratio (SNR) of the very robust SPs (right panel, N=3 MEAs, n=26-30). The RMS noise level was measured with Spike2 on unfiltered traces with a time constant of 5 s and the SNR was taken as the ratio of RMS noise levels between covered and uncovered electrodes when islets were stimulated by glucose ≥8.2 mM for >20 min. ** 2p<0.01, *** 2p<0.001. (**C**) Top traces:

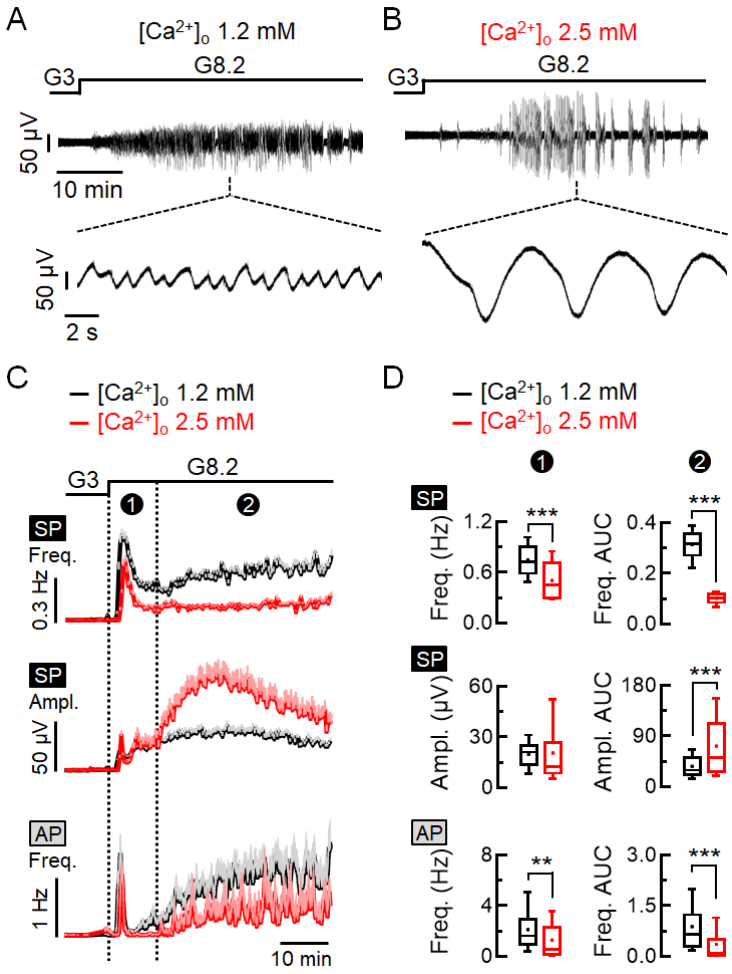
representative recordings of SPs from the 2 electrodes indicated in orange and light blue in (**A**) during the 1st (❶) and the 2nd (❷) phases induced by G8.2 and during the decrease of glucose level to G3. See methods for the optical determination of the kinetics of changes in glucose concentrations (black line at the top). Bottom traces: portions of top traces with higher temporal resolution at the timestamps indicated by a, b and c. (**D**) Kinetics of SP frequency (red) and amplitude (blue) measured in a mouse islet on a HD-MEA (means +SEM, n=13 electrodes) during the 1st (❶) and the 2nd (❷) phases induced by G8.2 and during the decrease of glucose level to G3. (**E**) Portions of top traces shown in (**C**) with higher temporal resolution at the timestamp d showing the appearance of SP pulsatility during the 2nd phase. (**D**) Period of the pulsatility (number of pulses per min) measured after 40 min at G8.2 and during 40 min (N=7 islets).



Supplementary Fig. 3. SP and AP analysis in response to glucose. Concentration-dependencies to glucose (G) of the electrical responses generated by mouse islets were analyzed with polymer-coated MEA-electrodes. (**A**) Proportion of islets (means ±SEM) responding electrically to G5.5, G6 and G8.2. * $p<0.01$ for G8.2 vs. G5.5. (**B**) Delays (means ±SEM) between the change in concentration and the first SPs (top) and APs (bottom). * $p<0.05$ for G5.5 vs. G6 and §§§ $p<0.001$ for G8.2 vs. G5.5 and G6. Gray dotted lines are the best fitting curves. (**C**) Statistics comparing maximal amplitude of SPs (means of the 10th biggest SPs for each electrode) during the 1st (❶) vs. the 2nd (❷) phase for each glucose concentration. *** $2p<0.001$. (N=3-6, n=29-114).

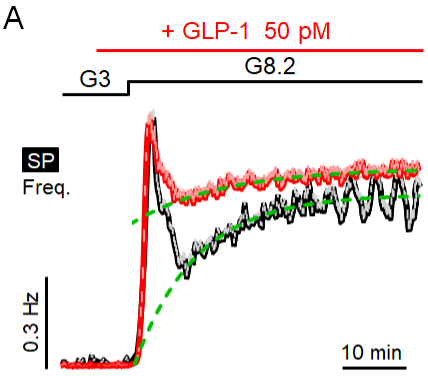


Supplementary Fig. 4. SP and AP analysis of islets versus islet cell monolayers on polymer-coated MEA-electrodes. (**A** and **B**) Comparison of glucose-induced electrical responses of native islets and islet cell monolayers. (**A**) Mouse entire islets (black) or mouse islet cells in monolayers (red) cultured on PEDOT-MEAs were stimulated by an increase in glucose from G3 to G8.2. Kinetics of SP frequency and amplitude as well as AP frequency during the two phases were evaluated (means +SEM). (**B**) Statistics on data in (**A**) (N=3, n=40-80). Left: peak frequency and mean amplitude of SPs and peak frequency of APs during the 1st phase were determined for each electrode. Right: AUCs of SP frequency, SP amplitude and AP frequency during the 2nd phase normalized over time. (**C**) Influence of the size of islet cell monolayers on the maximal amplitude of SPs. Left: representative images (scale bars 200 µm) of an islet (surrounded in black) and a small (<0.1 mm^2), a medium (0.1-0.5 mm^2) and a large (>0.5 mm^2) islet-cell monolayer (surrounded in red). Right: maximal amplitudes of SPs (means of the 10th biggest SPs during the 2nd phase) of islets, small (S), medium (M) and large (L) islet-cell monolayers (n=6-46). * 2p<0.05, ** 2p<0.01, *** 2p<0.001, **** 2p<0.0001.



Supplementary Fig. 5. Supraphysiological extracellular Ca^{2+} levels considerably alter SP and AP dynamics. (**A** and **B**) Top traces: representative recordings of the electrical activity of the same mouse islet stimulated by an increase in glucose from G3 to G8.2 in the presence of two different concentrations of extracellular Ca^{2+} ($[Ca^{2+}]_o$) as indicated (PEDOT-MEA; Bandpass: 0.1-700 Hz). Bottom traces: portions of top traces at higher temporal resolution (representative of n=26 recordings from N=3 independent biological preparations). (**C**) Kinetics of SP frequency and amplitude as well as AP frequency during the two phases in the two Ca^{2+} conditions (means +SEM). Similar results were obtained regardless of the concentration of Ca^{2+} tested first (N=3). (**D**) Statistics (N=3; n=26 for SPs and n=20 for APs). Left: peak frequency and mean amplitude of SPs and peak frequency of APs during the 1st phase were determined for each electrode. Right: AUCs of SP frequency, SP amplitude and AP frequency during the 2nd phase normalized over time. ** 2p<0.01, *** 2p<0.001.

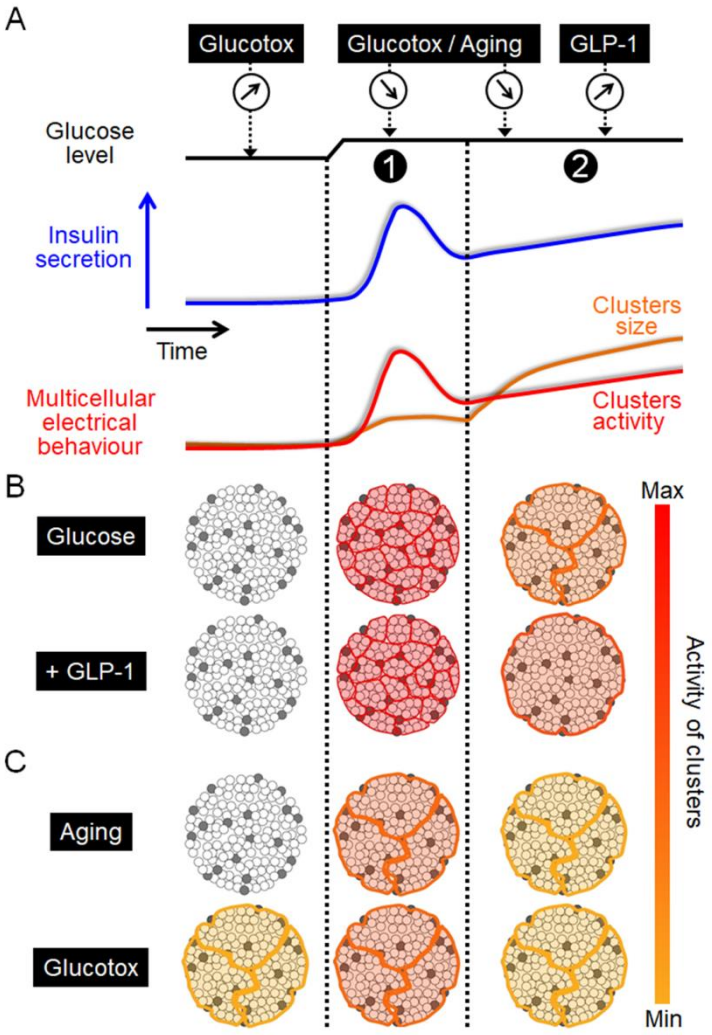
A



B

One-phase decay	Ctr	GLP-1
Y0 (s)	-544.52	-6
Plateau (Hz)	0.468	0.6202
K	0.002291	0.001191
Half Life (s)	302.5	582.1
Tau (s)	436.4	839.8

Supplementary Fig. 6. Physiological (picomolar) levels of GLP-1 increase and promote the passage from the 1st to the 2nd phase. (**A**) SP frequency kinetics and best fits (green) of the 2nd phase obtained on mouse islets stimulated by increasing glucose from G3 to G8.2 in the absence (black) and in the presence of GLP-1. GLP-1 was applied 5 min before changing glucose concentrations and was present during the stimulation by G8.2 (means +SEM; N=4-6, n=64). (**B**) Details of the fitting curve parameters for the stimulation with G8.2 alone (Ctr) and with G8.2 and GLP-1.



Supplementary Fig. 7. Proposed functional model. (**A**) Biphasic insulin secretion induced by glucose and corresponding multicellular electrical behavior of islets in terms of activity (i.e. SP frequency) and of cluster sizes (i.e. SP amplitude). Modulations of phases in physiological and pathophysiological conditions are indicated on top. (**B** and **C**) Representation of the multicellular behavior of an islet during glucose-induced phases in different physiological and pathophysiological conditions. Unfilled and filled circles represent β and non-β-cells, respectively. Intra-islet clusters are represented by colored areas. Relative sizes of clusters are represented, as well as their level of activity with the color code on the right. (**B**) Model in physiological conditions: upon glucose stimulation, the 1st phase originates from a multitude of highly active small β-cell clusters, but poorly coordinated together, whereas during the subsequent 2nd phase, clusters enlarge and contain less active but highly synchronized β-cell clusters. Physiological levels of GLP-1 promote only the 2nd phase by enhancing multicellular signals (size and activity of clusters). (**C**) Model in aging and glucotoxicity (glucotox). Aging reduces the reactivity, but not the size, of β-cell clusters, similarly to glucotoxicity for which in addition an increased basal activity is observed.