

Supplementary Materials for

Electrogenetic cellular insulin release for real-time glycemic control in type 1 diabetic mice

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Materials and Methods

Key plasmids used in this study

Comprehensive design and construction details for all expression vectors are provided in **Table S7.** Key plasmids include (i) $pCa_v\alpha_1C$ (P_{hCMV} - α_1C -pA), $pCa_v\alpha_2\delta_1$ (P_{hCMV} - α_2/δ_1 -pA) and pCay β_3 (P_{hCMV}- β_3 -pA), which enable constitutive expression of subunits α_1 C, α_2 , δ_1 and β_3 of the L-type voltage-gated channel Ca_V1.2, respectively (ii) pMX57, which encodes a Ca^{2+} -responsive P_{NFAT3}-driven SEAP expression unit (P_{NFAT3}-SEAP-pA) (5), (iii) pKK66, which harbors a Sleeping Beauty (SB) 100X-specific transposon containing a bicistronic unit for P_{hEF1α}-driven expression of the Ca_V1.2 α_1 C subunit and the inwardly rectifying potassium channel K_{ir}2.1, as well as a bicistronic unit for the expression of the blue fluorescent protein (BFP) and the puromycin resistance gene (PuroR) (ITR-P_{hEF1a}-a₁C-P2A-K_{ir}2.1-pA-ITR:P_{RPBSA}-BFP-P2A-PuroR-pA-ITR), (iv) pMX251, which harbors a SB100X-specific transposon containing a constitutive bicistronic unit for the expression of the Ca_V1.2 α_2 , δ_1 and β_3 subunits, as well as a bicistronic unit for constitutive expression of the red fluorescent protein dTomato and the blasticidin resistance gene (BlastR) (ITR- $P_{hEF1\alpha}-\alpha_2/\delta_1$ -P2A- β_3 -pA: P_{RPBSA} -dTomato-P2A-BlastRpA-ITR) (5), and (v) pProinsulin-NanoLuc (PhCMV-Proinsulin-NanoLuc-pA), a lentiviral expression vector containing a modified proinsulin whose C-peptide has been replaced with Oplophorus gracilirostris luciferase (NanoLuc) (33).

Cell culture and transfection

Human embryonic kidney cells (HEK-293T, ATCC: CRL-11268) were cultivated in Dulbecco's modified Eagle's medium (DMEM; cat. no. 52100-039; Thermo Fischer Scientific, Waltham, MA, USA). 1.1E7 cells (cat. no. 10070101-1VL, Sigma-Aldrich, Saint Louis, MO, USA) were cultivated in Roswell Park Memorial Institute 1640 medium (RPMI; cat. no. 72400-021, Thermo Fischer Scientific) supplemented with 10% fetal bovine serum (FBS; cat. no. F7524, lot no. 022M3395, Sigma-Aldrich), 100 U/mL penicillin and 100 μ g/mL streptomycin (penicillin-streptomycin solution 100x; cat. no. L0022, Biowest, Nuaillé, France). Alpha TC-1 cells (alpha TC-1 Clone 9, ATCC: CRL-2350) were cultivated in DMEM supplemented with 10% FBS, 15 mM HEPES, 0.1 mM non-essential amino acids, and 0.02% BSA. Cells were grown at 37°C, in humidified air containing 5% CO₂. For transfection, 35,000 cells were seeded per cm² of the cell culture dish, and incubated for 24 h. Then, they were incubated for another 24 h with a 1:3 DNA:PEI (Polyethylenimine MAX; MW 40,000, cat. no. 24765-2; Polysciences Inc., Warrington, PA, USA) solution containing 1.5 μ g DNA per cm² of transfected cells.

Production of stably transgenic cell lines

Electroβ was produced *via* the following three-step procedure. (i) First, a 1.1E7-derived cell clone deficient in glucose-sensitive insulin secretion was selected. The resulting cell line, INS_{Vesc}, was used for step two. (ii) Second, INS_{Vesc} was transduced with pProinsulin-NanoLuc-derived lentiviral particles, selected in culture medium containing 5 µg/mL blasticidin for 14 days, and cloned by limiting dilution. Monoclonal cell populations were tested for depolarization-triggered NanoLuc secretion. The monoclonal cell line showing the highest induction profile was used for step three. (iii) The proinsulin-NanoLuc-transgenic INS_{Vesc} was cotransfected with the SB100X expression vector pCMV-T7-SB100 (P_{hCMV}-SB100X-pA) and the SB100X-specific transposon pKK66 (ITR-P_{hEF1α}-α₁C-P2A-K_{ir}2.1-pA-ITR:P_{RPBSA}-BFP-P2A-PuroR-pA-ITR) and pMX251 (ITR-P_{hEF1α}-α₂/δ₁-P2A-β₃-pA:P_{RPBSA}-dTomato-P2A-BlastR-pA-ITR) as described above. After seven days, stably transgenic puromycin-resistant cell populations were selected by FACS-

mediated single-cell sorting based on the cell's fluorescence relative to parental 1.1E7 (BFP: 405 nm laser, 450/50 bandpass filter; dTomato: 561 nm laser, 570 nm long-pass filter, 586/15 bandpass filter) using a Becton Dickinson LSRII Fortessa flow cytometer (Becton Dickinson, Allschwil, Switzerland).

Lentiviral particle production

pProinsulin-NanoLuc-derived lentiviral particles were produced by cotransfecting HEK-293T cells with the lentiviral expression vector pProinsulin-NanoLuc (PhCMV-Proinsulin-NanoLuc-pA), as well as the packaging (pSPAX2) and envelope (pMD2.G) vectors at a 4:3:1 ratio, respectively. After 48 h, the lentiviral particles were harvested from the cell culture supernatant and purified by 0.4 μ m filtration.

Electrostimulation

(a) **C-Dish**. Cultured cells were electrostimulated using the C-Dish (Ionoptix, Dublin, Ireland), powered by an HP3245A Universal Source function generator (cat. no. 3245A; Hewlett Packard, Palo Alto, CA, USA) connected to a general-purpose linear amplifier (P200, cat. no. P200; FLC Electronics AB, Molndal, Sweden). C-Dish precisely places pairs of carbon electrodes into each well of a standard 6-well cell culture plate containing 35,000 cells/cm² in 1.4 mL of cell-specific culture medium. (b) **Cell culture chamber**. Cell culture chambers with a 0.4 µm transparent PET membrane supporting the growth of monolayer cultures (Falcon[®] Permeable Support; cat. no 353095, Corning, Corning, NY, USA), fitting individual wells of a standard 24-well plate, were equipped with platinum electrodes (0.5 mm diameter; cat. no. HXA 050, Cooksongold Ltd., Birmingham, United Kingdom) placed above and below the cell-containing membrane and powered by the amplifier-connected HP3245A Universal Source function generator. The PET membrane of the cell culture chamber was seeded with 35,000 cells/cm² cultivated in the cell-specific medium (400 µL inside the cell culture chamber, 1.3 mL inside the 24-well plate).

Analytical assays

SEAP (human placental secreted alkaline phosphatase) levels were profiled in cell culture supernatants using a colorimetric assay. 100 µL 2x SEAP assay buffer (20 mM homoarginine, 1 mM MgCl₂, 21% diethanolamine, pH 9.8) was mixed with 80 µL heat-inactivated (30 min at 65°C) cell culture supernatant. After the addition of 20 µL substrate solution (120 mM pnitrophenyl phosphate; cat. no. AC128860100, Thermo Fisher Scientific), the absorbance time course was recorded for 45 min at 405 nm and 37°C using a Tecan Genios PRO plate reader (cat. no. P97084; Tecan Group AG, Maennedorf, Switzerland) and the SEAP levels were determined as described previously (15). Blood SEAP levels were quantified using a chemiluminescencebased assay (cat. no. 11779842001; Roche Diagnostics GmbH, Mannheim, Germany). In brief, serum was isolated from blood samples by centrifugation in Microtainer[®] serum separation tubes (SSTTM, cat. no. 365967; Becton Dickinson) for 5 min at 10,000xg. 50 µL of serum was heatinactivated for 30 min at 65°C, centrifuged for 30 s at 5,000xg, transferred to a well of a 96-well plate containing 50 mL inactivation buffer, and incubated for 10 min at 20°C. 50 µL of substrate reagent was then added to each well and the sample was incubated for 10 min at 20°C. The luminescence was quantified using a Tecan Genios PRO plate reader (Tecan Group AG, Maennedorf, Switzerland).

NanoLuc[®] luciferase was quantified in cell culture supernatants using the Nano-Glo[®] Luciferase Assay System (cat. no. N1110; Promega, Duebendorf, Switzerland). In brief, 7.5 μ L of cell culture supernatant was added per well of a black 384-well plate and mixed with 7.5 μ L

substrate-containing assay buffer. Total luminescence was quantified using a Tecan Genios PRO plate reader (Tecan Group AG). Serum NanoLuc[®] was quantified on an EnVision 2104 Multilabel Reader (PerkinElmer, Waltham, Massachusetts, USA) using the ultrasensitive luminescence program. 5 μ L of serum was diluted in 10 μ L of ddH₂O per well of a black 96-well plate and mixed with 15 μ L of substrate-containing assay buffer. Aliquots of 15 μ L of whole-blood samples were diluted in 5 μ L of 50 mM EDTA and frozen at -20 °C until NanoLuc[®] quantification as described above.

Cell encapsulation

HEK- β cells were encapsulated in coherent alginate-poly-(L-lysine)-alginate beads (400 µm; 500 cells or 1-10 IEQs per capsule) using an Inotech Encapsulator Research Unit IE-50R (EncapBioSystems Inc., Greifensee, Switzerland) set to the following parameters: a 200-µm nozzle with a vibration frequency of 1025 Hz, a 25-mL syringe operated at a flow rate of 410 units, and a voltage of 1.12 kV for bead dispersion.

Cell viability

Cell viability was quantified by incubating the cells for 2 h in working solution containing 60 μ g/mL resazurin (cat. no. R7017, Sigma-Aldrich, Saint Louis, MO, USA), followed by fluorescence measurement of sample supernatants at 560/9 nm excitation and 590/20 nm emission using a Tecan Infinite 200Pro plate reader (Tecan Group AG, Maennedorf, Switzerland).

Bioelectronic implant for wireless electrostimulation in vivo

The casing of the bioelectronic implant containing the cell culture chamber and the electronics was 3D-printed using FDA-licensed polyamide and a Formiga P110 3D printer (EOS GmbH, Krailling, Germany; Fabb-It 3D Druckservice, Loerrach, Germany). To fit this casing, the height of the cell culture chamber (Falcon® Permeable Support; Corning, cat. no 353095 or Corning Transwell®; Corning, cat. no. CLS3462-48EA) was cut to 3 mm. The cell culture chamber's 0.4 µm membrane enables nutrient supply and product delivery via the vascularized fibrous connective tissue of the host while protecting the electrosensitive designer cells from host-cell responses (35, 36). The switchboard, wireless electronics and pulse generator were designed and prototyped by Peter Buchmann, and the final printed circuit board was custommanufactured (ITEAD Intelligent Systems Co. Ltd, Shenzhen, People's Republic of China) (see Supplementary Information). The electrode above the cell culture chamber's membrane containing the electrosensitive designer cells consists of a platinum wire (0.5 mm diameter; cat. no. HXA 050, Cooksongold Ltd.) and the electrode below the chamber's membrane was made of a stainless-steel mesh (cat. no. 165; wire diameter 50 µm, aperture 100 µm; BOPP AG, Zurich, Switzerland) covering the entire membrane to provide a Faraday-cage effect that would protect the host from any electric shock. The bioelectronic implant was assembled by fitting the electronics into the casing, connecting the electrodes to the electronics, covering the electronic switchboard with insulating tape and sealing it with Epo-Tek 301-2 (cat. no. 301-2; Epoxy Technology Inc., Billerica, MA, USA), fitting the cell culture chamber into the casing, and positioning the electrodes. Bioelectronic implants were sterilized by immersion in 70% ethanol followed by rinsing in PBS. Each implant was completely filled with 300 µL of culture medium containing 2 x 10^6 Electro β suspension cells and 3 x 10^6 Electro β cell aggregates, prepared by cultivation in AggreWell[™]400 (cat. no. 34460, STEMCELL Technologies GmbH, Koeln, Germany) via a dedicated hole, then sealed with PDMS-filled pipette tips (Sylgard 184, cat. no. 184.0001; Suter Kunststoffe AG, Fraubrunnen, Switzerland) and subcutaneously implanted into mice after 12 h. To adapt the bioelectronic implant for long-term repetitive in-situ exchange of individual $_{Electro}\beta$ -cell batches (**Fig. S20**), we connected fill-in and exhaust tubes (Venofix® Safety, B. Braun AG, Melsungen, Germany) to the cell culture chamber (**Fig. S20A**). After implantation, 2 x 10⁶ $_{Electro}\beta$ cells were filled into the culture chamber. For cell replacement, a syringe was connected to the fill-in tube and the cell chamber was flushed several times with a physiological salt solution (0.9% NaCl) before the new batches of $_{Electro}\beta$ cells were filled in. Fill-in tubes were closed by thermal welding.

Electronic circuitry of the field generator and the bioelectronic implant

The field generator provides wireless power transmission across living tissues using transmitter coils (L2 and L3) producing an alternating electromagnetic field that is detected by the receiver coil (L1) of the bioelectronic implant and converted into electric current to power and control all electronic components of the implant. The field generator also enables detection and functional monitoring of the bioelectronic implant. The field generator consists of four functional elements: the frequency generator, the resonance unit, the filter and edge shaper and the timer (Fig. S7, S8). (a) Frequency generator (Fig. S7A). A crystal-based oscillating circuit (IC2) connected to a negative impedance converter (T4, T6) drives the output transistor (T5) of the frequency generator. (b) Resonance unit (Fig. S7B). The output transistor controls the main transmitter coil (L3) current, which generates an alternating magnetic field. The secondary transmitter coil (L2) with an adjustable capacitor (C8) in parallel forms an oscillating circuit, which amplifies the resonance of the main transmitter coil (L3). (c) Filter and edge shaper (Fig S7C). The current drawn by the main transmitter coil (L3) generates a proportional voltage drop by passing a resistor (R1). This voltage drop is filtered out by an LC network (L1, C4, C5) and amplified by a class A amplifier (T3). A decoupling capacitor (C1) forms a high-pass filter and drives a positive-negative-positive (PNP) transistor (T1). The high gain factor of the PNP transistor produces digital-like behavior of the data output (Data Out) for each fast change of the main transmitter coil current and enables wireless communication between the field generator and the bioelectronics implant. (d) Timer (Fig. S5D). The data output (Data Out) triggers a timer circuit (IC1) that controls an LED, which enables functional monitoring of the bioelectronic implant in real time.

The electronic circuit of the bioelectronic implant consists of three functional elements: The power supply, the time generator and the communication circuit (Fig. S5, Fig. S6). (a) Power supply (Fig. S5A). The receiver coil (L1) and capacitors (C6, C8) connected in parallel form an oscillating circuit that is in resonance with the magnetic field of the transmitter coils of the field generator. To prevent potential impact of the electrical load on the resonance, power is withdrawn from two turns of the receiver coil via current transformation and rectified by two diodes (D4). (b) Time generator (Fig. S5B). Pulses are generated by an integrated time generator. The pulse length and intervals are set by external resistors (R7, R9), a diode (D11) and a capacitor (C14). The timer (IC4) includes an internal voltage divider with three resistors. The potential of the external capacitor and the voltage divider are compared by comparators and used to control the timer output. ON/OFF-states are stored by a flip-flop until the opposite voltage threshold is reached. One output of the flip-flop serves to discharge the capacitor (C14). To reach the required ratio between pulse duration and pulse interval of approximately 1:500, the capacitor is charged through a low-Ohmic resistor (R7) and a diode (D11) and discharged via a high-Ohmic resistor (R9). (c) Communication circuit (Fig. S5C). Upon detection of a negative pulse edge by a capacitor (C12) and a transistor (T3), the oscillating circuit of the power supply is short-circuited for 15-25 µs. This brief overload leads to a breakdown of the resonance in the receiver coil (L1); this results in an abrupt drop of the excitation current in the resonance unit of the field generator and produces a voltage increase. This voltage increase is filtered out by the excitation electronics in the field generator and provides digital information on the presence of the bioelectronic implant and the function of the time generator. All electronic components of the bioelectronic implant are listed in **Table S8**.

Animal experiments

To establish type-1 diabetes, wild-type 6-week old male Swiss mice (weighing 30-32 g RjOrl:SWISS; Janvier Labs, Le Genest-Saint-Isle, France) were fasted 18 h per day for two consecutive days and injected with a single dose of freshly diluted alloxan monohydrate (ALX; Sigma-Aldrich; cat. no. A7413, 195 mg/kg in 300 µL PBS) (5). Persistent fasting hyperglycemia was confirmed after 48 h using a glucometer (Contour®Next, Bayer Healthcare, Leverkusen, Germany). (a) Monitoring of study animals: General well-being of the animals was routinely monitored by animal caretakers by daily visual inspections. Project-specific monitoring was carried out at least five times per week by animal facility staff and animals were euthanized if symptoms of pain and/or distress were observed. Absence of the following applied humane endpoints was defined as asymptomatic disease state: weight loss >20 %, insatiable polydipsia, recurrent dehydration (assessed by skin fold testing), surgical wound complications such as bleedings or inflammation/infection, abnormal breathing pattern, apathy or immobility, closed eyes or self-mutilation, detachment from the group or disinterest to the environment, un-kept appearance e.g. tangled fur and discoloration due to secretions. (b) Bioelectronic implant. At 72 h after alloxan treatment, the animals received inhalational isoflurane anesthesia and the bioelectronic devices were subcutaneously implanted on the back, with the polyester gauzeprotected mesh electrode facing the ventral side. (c) Glucose tolerance test. Treated animals were fasted for 6 hours and then placed for 60 min below a field generator (see Supplementary Information) to power up the bioelectronic implant and wirelessly stimulate insulin release by Electroß cells immediately before the animals were subjected to glucose tolerance tests: blood glucose levels were monitored for indicated period after oral injection of 2 g/kg glucose. Mockstimulated implants produced no insulin. (d) Human pancreatic islets implant. Human pancreatic islets were provided by the Geneva Islet transplantation center through the basic research program of European Consortium for Islet Transplantation (ECIT) supported by the Juvenile Diabetes Research foundation (JDRF; grant no 31-2008-416). At 72 h after alloxan treatment, the animals received anesthesia and the islets-containing chamber (2000 islet equivalents (IEQ) per implant) of a bioelectronic implant was placed as described above. (e) Real-time glycemia. Animals were fasted for 6 hours and glycemia was monitored for the indicated periods of time before and during electrostimulation. (f) In vivo kinetics. Implants were electrostimulated for 30 min and reporter protein levels were quantified in 15 µL blood samples, collected from the tail vein at the indicated time points. (g) Biocompatibility. Mice were subcutaneously implanted on the back with either Electroß-cell-containing implants, cell-free negative control implants, or biocompatible reference implants serving as positive controls according to ISO 10993. The biocompatible reference implants of identical shape were cast from polydimethylsiloxane (Sylgard 184, cat. no. 184.0001; Suter Kunststoffe AG, Fraubrunnen, Switzerland). They were autoclaved for 20 minutes at 121 °C prior to implantation. After three weeks, the animals were sacrificed and the bioelectronic implants as well as tissue samples were fixed in 10% neutral buffered formalin and transferred to AnaPath GmbH (Oberbuchsiten/Liestal, Switzerland), where the biocompatibility assays were performed according to ISO 10993.

Animal experiments were performed according to the directive of the European Community Council (2010/63/EU), approved by the French Republic, and carried out by (i) Ghislaine Charpin-El Hamri (no. 69266309), Marie Daoud-El Baba (no. 69266310) at the Institut

Universitaire de Technologie of the Université Claude Bernard Lyon 1, F-69622, Villeurbanne Cedex, France (project no. APAFIS # 16753 – CEEA-55 DR 2018-40v5), (ii) Marie-Didiée Hussherr and Shuai Xue (license number: 2996/30779) at the ETH Zurich in Basel, Switzerland, and by (iii) Jiawei Shao and Shuai Xue (license number: m20190102) in the laboratory of Haifeng Ye at the Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai, People's Republic of China.

<u>Histology</u>

(a) Limited Systemic Toxicity. Two kidneys and two lobes from the liver of each mouse were processed by paraffin embedding, cut into 2-4 μ m slices, and stained with hematoxylin and eosin (H&E) (Fig. S16). (b) Tissue samples. A small portion of the tissue in contact with the semipermeable membrane of a cell chamber was excised, embedded in paraffin, cut into 2-4 μ m slices, and stained with H&E (Fig. S16). (c) Bioelectronic implant sections. Explanted bioelectronic devices, including surrounding tissue, were embedded in methyl methacrylate resin. The cell chamber of each implant was cut into 400 μ m slices at its central position in the transverse direction using a diamond saw (EXAKT 300 CP System; EXAKT Technologies Inc., Oklahoma City, OK, USA), as shown in Fig. S16. These slices were ground to a thickness of 40-60 using the EXAKT 400 CS System (EXAKT Technologies Inc., Oklahoma City, OK, USA) and stained with Paragon (toluidine blue and basic fuchsin). The same procedure was applied to obtain sections at the switchboard's central position (Fig. S16).

Immunohistochemistry (IHC)

IHC was performed using BOND-III Fully Automated IHC and ISH Stainer (Leica Biosystems, Wetzlar, Germany). Rabbit monoclonal anti-CD11b (Ab133357; dilution 1:5000, lot no. GR3209213-2; Abcam, Cambridge, United Kingdom) and rabbit monoclonal anti-CD68 (Ab125212; dilution 1:1000, lot no. GR300618-28; Abcam, Cambridge, United Kingdom) were used. Prior to staining, the samples were incubated in Bon Epitope Retrieval Solution 1 (citrate-based buffer, pH: 5.9-6.1; Leica Biosystems, Wetzlar, Germany) at 100 °C for 20 min.

Histopathology

Histopathology of the implantation site was analysed at AnaPath GmbH using a scoring system according to ISO 10993-6:2016(E). Representative images were taken with an Olympus UC30 camera.

Image Analysis

Quantitative analyses of immunohistochemistry sections were performed using the image analysis software QuPath (https://qupath.github.io).

Cytokine Profiling

Serum samples of each treatment group were mixed and analyzed with a Proteome Profiler Array, Mouse Cytokine Array Panel A (cat. no. ARY 006; R&D Systems, Inc., Minneapolis, USA), according to the manufacturer's instructions. Chemiluminescence was analysed using an ImageQuant LAS 4000 mini (GE Healthcare Life Sciences, Chicago, Illinois, USA).

Hematology

Hematological analysis was performed using the scil Vet abc Plus+ analyzer (scil animal care company GmbH, Viernheim, Germany).

Sample size determination and statistics

Data are presented as mean values. Error bars show the standard error of the mean. The "n" number refers to biological replicates. Sample size for in vivo experiments was chosen to provide statistical power $(1-\beta) \ge 0.8$ and type I error rate (α) ≤ 0.05 for a 30% change of the mean in treated groups, assuming 25% standard deviation. The p-value was calculated by performing the two-tailed t-test. Outliers were considered by applying the ROUT method (59).



Fig. S1 Validation of system components.

HEK293T cells were co-transfected with plasmids encoding the indicated channel subunits (+): pCav1.2 (P_{hCMV}- α_1 C-pA), pCav $\alpha_2\delta_1$ (P_{hCMV}- α_2/δ_1 -pA) and pCav β_3 (P_{hCMV}- β_3 -pA), pKir2.1 (P_{hCMV}-Kir2.1-pA) or mock DNA (-), as well as P_{NFAT3}-driven SEAP expression plasmid (pMX57). Cell membrane was depolarized with 40 mM KCl for one hour (red bars) and after 24 hours SEAP production was quantified in supernatant samples. Blue bars represent negative control samples. ns – not significant, ***p<0.001.



Fig. S2 Impact of salt concentration on the electrogenetic circuit.

(A) ElectroHEK cells were cultivated for 24 hours in medium supplemented with various concentrations of sodium chloride (NaCl), sodium phosphate (Na₂PO₄), magnesium sulphate (MgSO₄), calcium chloride (CaCl₂), or potassium chloride (KCl), and then SEAP was quantified in the culture supernatants. (B) Electroβ cells were cultivated for 10 min in medium containing various concentrations of NaCl, Na₂PO₄, MgSO₄, CaCl₂ or KCl, and then NanoLuc was quantified in the culture supernatants. Data points represent mean ± SEM. Statistical significance was calculated versus time 0. ns – not significant, * p<0.05, **p<0.01, ***p<0.001.



Fig. S3 Electrically inducible gene expression system.

(A-D) Toxicity of electrical stimulation. HEK293T cells were co-transfected with plasmids encoding the L-type voltage-gated calcium channel Cav1.2: pCav1.2, (P_{hCMV} - α_1 C-pA), pCav $\alpha_2\delta_1$

 $(P_{hCMV}-\alpha_2/\delta_1-pA)$, $pCav\beta_3$ ($P_{hCMV}-\beta_3-pA$), Kir2.1: pKir2.1 (P_{hCMV} -Kir2.1-pA). Electrical stimulation (orange bars) was applied at the indicated voltage (**A**,**C**), or for the indicated period of time (**B**,**D**), and 24 hours later cell viability was assessed. (**E**) Electrical stimulation of cells lacking a voltage-gated calcium channel. HEK293T cells were co-transfected with pCDNA3.1(+) and P_{NFAT3}-driven SEAP expression plasmid (pMX57). Electrical stimulation (50 V, 1 Hz, 1 hour and pulse length as indicated below the graph; orange bars) was applied and 24 hours later SEAP was quantified in supernatant samples. Depolarization with 40 mM KCl for 1 hour was used as a positive control (red bar). Blue bars represent unstimulated control. Bars represent mean ± SEM. n = 3. ns – not significant, * p<0.05, **p<0.01.





HEK293T cells were co-transfected with plasmids encoding pCa_V1.2 (P_{hCMV}- α_1 C-pA), pCa_V $\alpha_2\delta_1$ (P_{hCMV}- α_2/δ_1 -pA) and pCa_V β_3 (P_{hCMV}- β_3 -pA), pKir2.1 (P_{hCMV}-Kir2.1-pA) and P_{NFAT3}-driven SEAP and mINS expression plasmid (pKK137). (A) SEAP production kinetics. Cells were electrostimulated with 50 V, 2 ms pulses at 1 Hz frequency for one hour (red line). SEAP concentration was quantified in supernatant samples every one hour. Blue line represents control samples (not stimulated). (B) Reversibility assay. Cells were electrostimulated at the 0 and 24 h time points (blue line), or at the 12 h time point (red line). The culture medium was exchanged every 12 hours and the electrostimulation status was changed (from ON to OFF or from OFF to ON). SEAP was quantified in supernatant samples. Data points represent mean \pm SEM. n = 3. Statistical significance was calculated between on (red dots) and off (blue dots) states for a given time point. * p<0.05, **p<0.01, ***p<0.001.



Fig. S5 Electronic circuit of the bioelectronic implant.

The three main functional elements are marked with dashed frames: the power supply (A), the time generator (B) and the communication circuit (C). (A) Power supply. The coil (L1) and capacitors (C6, C8) connected in parallel form an oscillating circuit that is in resonance with the magnetic field of the transmitter coil. To prevent potential impact of the electrical load on the resonance, power is withdrawn from two turns of the receiver coil via current transformation and rectified by two diodes (D4). (B) Time generator. Pulses are generated by an integrated time generator. Pulse length and intervals are set by external resistors (R7, R9), a diode (D11) and a capacitor (C14). The timer (IC4) includes an internal voltage divider with three resistors. The potential of the external capacitor and the voltage divider are compared by comparators and used to control the timer output. ON/OFF-states are stored by a flip-flop until the opposite voltage threshold is reached. One output of the flip-flop serves to discharge the external capacitor (C14). To reach the required ratio between pulse duration and pulse interval of approximately 1:500, the capacitor is charged through a low-Ohmic resistor (R7) and a diode (D11) and discharged via a high-Ohmic resistor (R9). (C) Communication circuit. Upon detection of a negative pulse edge by a capacitor (C12) and a transistor (T3), the oscillating circuit of the power supply is short-circuited for 15-25 µs. This brief overload leads to a breakdown of the resonance in the receiver coil (L1) and results in an abrupt drop of the excitation current in the transmitter coil and produces a voltage increase.



Fig. S6 Bioelectronic implant switchboard.

(A) Top view of the bioelectronic implant switchboard. Dashed frames correspond to functional elements described in Figure S5: power supply (yellow), time generator (white) and communication circuit (red). (B) Schematic view of the bioelectronics implant. Conductive paths are marked with lighter green. Components are marked with white frames. Orange rectangles represent connectors. Components are described in Table S8.



Fig. S7 Electronic circuit of the field generator.

Four main functional elements are marked with dashed frames: the frequency generator (A), the resonance coils (B), the filter and edge shaper (C), and the timer (D). (A) Frequency generator. A crystal-based oscillating circuit (IC2) connected to a negative impedance converter (T4, T6) drives the output transistor (T5) of the frequency generator. (B) Resonance coils. The output transistor controls the coil (L3) current, which generates an alternating magnetic field. The second coil (L2) with an adjustable capacitor (C8) in parallel forms an oscillating circuit, which amplifies resonance of the main coil. (C) Filter and edge shaper. The current drawn by the resonance coil generates a proportional voltage drop by passing a resistor (R1). This voltage drop is filtered out by an LC network (L1, C4, C5) and amplified by a class A amplifier (T3). A decoupling capacitor (C1) forms a high-pass filter and drives a PNP transistor (T1). The high gain factor of the PNP transistor produces digital-like behavior of the data output (Data Out) for each fast change of the coil current and enables wireless communication from the implant to the field generator. (D) Timer. The data output triggers a timer circuit (IC1) that drives an LED, which enables function monitoring of the bioelectronics implant.





Fig. S8 Field generator switchboard.

(A) Schematic view of the field generator. Conductive paths are marked with lighter green. Components are marked with white frames. Orange rectangles represent connectors. (B) Enlarged view of the part enclosed with a black frame in (A). Dashed frames correspond to the functional elements described in Figure S7. frequency generator (yellow), the resonance coils (purple), the filter and edge shaper (red), and the timer (white).



Fig. S9 Position-dependent coupling intensity between the field generator and the bioelectronic device.

(A) Schematic representation of the experimental design. The Y-axis indicates the vertical position of the implant. The X-axis indicates the horizontal position of the implant measured from the middle point. (B) Dependence of the impulse voltage reached in the bioelectronic implant upon the horizontal (0 - 8 cm) and vertical (0 - 4 cm) positions.





 2×10^5 Electro β cells were either electro-stimulated for 20 minutes (orange bars) using 2 ms pulses at 5 Hz and 10 V or not electro-stimulated (blue bars) on day 1 and after a continuous cultivation period of 90 days. NanoLuc levels were quantified in the culture supernatants. Bars represent mean \pm SEM. n = 3. ns – not significant, **p<0.01, ***p<0.001.



Fig. S11 Comparative insulin-release kinetics.

Comparative insulin-release kinetics of $_{Electro}\beta$ (constitutive expression, vesicular secretion) and $_{Electro}HEK$, $_{Opto}HEK$ and HEK- β (P_{NFAT}-driven expression, secretory pathway). (A) $_{Electro}HEK$ (4 hours electrostimulation), (B) $_{Opto}HEK$ (24 hours blue-light illumination) and (C) HEK- β (40 mM glucose over 24 hours) were stimulated (green line) or non-stimulated (blue line) and insulin secretion was profiled in the culture supernatant. Likewise, (D) $_{Electro}\beta$ were (15 minutes electrostimulation) stimulated (green line) or non-stimulated (blue line) and the immediate insulin release was monitored in the culture supernatant.



Fig. S12 Electrically inducible fast insulin secretion system.

(A, B) Voltage dependence. Electroß cells were stimulated with 2 ms pulses at 1 Hz frequency and at the indicated voltage. NanoLuc was quantified from supernatant samples before (blue bars) and after electrical stimulation (orange bars) from above (A) and below (B) the cell layer. (C, D) Pulse length dependence. Electroß cells were stimulated with 7.5 V pulses at 5 Hz frequency with the indicated pulse length. NanoLuc was quantified from supernatant samples before (blue bars) and after electrical stimulation (orange bars) from above (C) and below (D) the cell layer. The statistical significance of differences between the negative control and each of the stimulation conditions was calculated. Bars represent mean \pm SEM. n = 3. ***p<0.001, ****p<0.0001.



Fig. S13 Electro-stimulated glucagon secretion by pancreatic α-cells.

 2×10^5 pancreatic α -cells (α -TC1) cells were seeded into cell-culture inserts and electrostimulated with 2 ms pulses at 5 Hz and 10 V for 20 minutes (red bar). Then, glucagon levels were quantified in the culture supernatant. Bars represent mean \pm SEM. n = 3. *p<0.05.



Fig. S14 In vivo kinetics of protein secretion.

(A) HEK- β cell line secretion kinetics *in vivo*. HEK- β cells were encapsulated into alginate beads and injected into hyperglycemic mice. Insulin was quantified from serum samples after 4, 24 and 48 hours. Data points represent mean \pm SEM. n = 7. (B) _{Electro} β secretion kinetics *in vivo*. _{Electro} β cells inside the bioelectronic implant were electrostimulated for 30 minutes. Insulin levels in whole-blood samples were quantified before the stimulation, and after 30, 60, 120 minutes and 24 hours.



Fig. S15 Impact of materials used in the biomedical device on cell viability.

The viability of parental HEK-293T cells was measured after incubation for 24 hours with implant materials: solidified PDMS (biocompatible reference material), stainless steel (electrode material) and Epo-Tek 301-2 (implant material). Bars represent mean \pm SEM. n = 3. Statistical significance was calculated between cells only (negative control) and each of the implant materials. ns – not significant, *p<0.05.



Fig. S16 Biocompatibility.

Histological sections of tissue-surrounded bioelectronic implant. Devices containing $_{Electro\beta}\beta$ cells were surgically placed on the dorsal side of mice for 21 days. Fibrotic capsule formation was examined by Paragon staining. (A) Cross section of the cell-containing chamber. Green arrow, porous membrane; red arrows, 3D-printed frame; orange arrows,

electronic switchboard; blue arrows, cell insert frame; fibrotic tissue, yellow arrows (please note that, compared to the structural part of the biocompatible implant material of the bioelectronic device (**Fig. S15**), the fibrotic tissue formation at the porous membrane where the $_{Electro}\beta$ cells interface with the host tissue, is less prominent. (**B**) Enlarged image of the fibrotic tissue formed at the implant material of the bioelectronic device. Red arrows - blood vessels, green arrows - fibroblasts, blue arrows - fibrocytes, yellow arrows - granulocytes, orange arrows - lymphocytes.





Fig. S17 Local immune response.

(A) Local effects on the immune response after implantation were scored according to ISO 10993. A higher score indicates greater local effects and lower biocompatibility. (**B**,**C**) Immunohistochemistry. Fragments of tissue adherent to the porous membrane of a bioelectronic implant were excised and immunostained. Graphs represent percentage of positively stained cells in experimental groups. (**B**) Anti-CD11b antibodies. CD11b is expressed on macrophages, neutrophils and natural killer cells. (**C**) Anti-CD68 antibodies. CD68 is expressed on activated macrophages. ns – not significant.



Fig. S18 Dot-blot-based cytokine profiling of treated mice.

Reference inflammatory cytokines CXCL-10, CXCL-13 and C5a were profiled in the serum of mice 3 weeks after the introduction of bioelectronic implants with (A) and without (B) cells, or biocompatible reference implants (C).



Fig. S19 Impact of bioelectronic implants on the body weight of mice.

The body weight of mice was profiled over three weeks after the introduction of bioelectronic implants with and without $_{Electro}$ HEKs or biocompatible control implants. The weight of the implant (2 g) was subtracted from the body weight after implantation. Data points represent mean \pm SEM. n = 8. There were no significant differences between the groups after implantation.





Fig. S20 Bioelectronic implants modified for long-term glycemic control in type-1diabetic mice.

(A) Picture of bioelectronic implants containing fill-in and exhaust tubes to allow repetitive in-situ exchange of $_{Electro}\beta$ -cell batches. (B) Validation of implant functionality over 3 weeks in type-1-diabetic mice. Bioelectronic devices shown in (A) were implanted into type-1 diabetic mice (T1DM), allowing weekly exchange of the $_{Electro}\beta$ -cell batches.

A

Insulin levels were quantified once a week. Data represent mean \pm SEM, n = 5 mice. Statistical significance of blood-insulin levels was calculated against blood-insulin levels before implantation and against homeostatic insulin levels of wild-type mice. (C) Electrostimulated insulin secretion kinetics of bioelectronic implants in treated type-1-diabetic mice after three weeks. Electro β -cell-containing bioelectronic implants were electrostimulated for 30 minutes and resulting blood-insulin levels were profiled. Data represent mean \pm SEM, n = 5 mice per group. (D) Picture of representative bioelectronic devices explanted from mice after termination of the experiment described in (B).

Supply Voltage [V]	25°C	30°C	35°C	40°C	45°C	50°C
3	2.32	2.32	2.32	2.32	2.32	2.31
4	2.39	2.37	2.38	2.38	2.38	2.38
5	2.46	2.44	2.45	2.44	2.45	2.45
6	2.51	2.49	2.5	2.49	2.48	2.48
7	2.55	2.53	2.52	2.52	2.51	2.51
8	2.58	2.55	2.55	2.54	2.52	2.52
9	2.62	2.6	2.59	2.58	2.57	2.56
10	2.66	2.62	2.62	2.61	2.59	2.57
11	2.69	2.63	2.63	2.61	2.6	2.58
12	2.7	2.63	2.64	2.62	2.6	2.58

Table S1. Temperature dependence of implant functionality

The implant was equipped with a 1 kOhm load at the impulse output, placed on a hot plate and covered with a 3 cm layer of polystyrene. The circuit was powered with an adjustable power supply. The output voltage was measured with a digital oscilloscope, LeCroy Wavesurfer, at the indicated temperature.

Table S2.	Quality	control	of bioe	lectronic	devices
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Water immersion test ¹	
Batch size (no. of items)	50
Implants containing functional electronics after 24 h of water immersion	48
% Functional	96
Cell leakage test (mechanical integrity) ²	
Number of devices	5
Number of devices with cell leakage detected	0
Electronic functionality in vivo ³	
Number of devices	10
Functionality at week 1 after implantation	10
Functionality at week 2 after implantation	10
Functionality at week 3 after implantation	10

¹A batch of 50 implants was placed in 20 cm deep water for 24 hours. Electronic functionality of the implants was tested after 24 hours.

²Bioelectronic devices were filled with 2 x 10^5 cells and incubated in 60 mm cell culture dishes. After 12 hours, the cell-containing bioelectronic devices were transferred to fresh cell culture dishes, incubated for 5 days and carefully examined to determine whether or not cells were present outside the bioelectronic device.

³Functionality of bioelectronic devices used for experiment of Fig. S20B measured by a resonance-indicating diode. Please refer to Fig. 4 and Fig. S7 for details.

 Table S3. Systemic toxicity

Group	Animal Number	Organ	Findings
	1	Kidney	Pyelitis, chronic, focal, unilateral, minimal
	1	Liver	Hemopoietic cell foci, minimal
	5	Kidney	Pyelitis, chronic, focal, unilateral, minimal
	5	Liver	No findings
Biocompatible	1.4	Kidney	No findings
reference item	14	Liver	Inflammatory cell foci, minimal
	10	Kidney	Mononuclear cell foci, unilateral, minimal
	18	Liver	Hemopoietic cell foci, minimal
	10	Kidney	Pyelitis, chronic, focal, biilateral, minimal
	19	Liver	No findings
	2	Kidney	Mononuclear cell foci, unilateral, minimal
	3	Liver	No findings
	1	Kidney	Mononuclear cell foci, unilateral, minimal
	4	Liver	Inflammatory cell foci, minimal
Implant without	12	Kidney	Mononuclear cell foci, unilateral, minimal Pyelitis, chronic, focal, unilateral, minimal Cast, hyaline, focal, unilateral, minimal
cells		Liver	Hemopoietic cell foci, minimal
	12	Kidney	Pyelitis, chronic, focal, unilateral, minimal
	15	Liver	Hemopoietic cell foci, minimal
	16	Kidney	Cyst, cortical, present Urothelial hyperplasia, unilateral, minimal Pyelitis, chronic, focal, unilateral, minimal
		Liver	No findings

Group	Animal Number	Organ	Findings
	8	Kidney	No findings
	0	Liver	Inflammatory cell foci, Slight
		Kidney	Mononuclear cell foci, unilateral, minimal
	9	Liver	Hemopoietic cell foci, minimal
			Inflammatory cell foci, minimal
.		Kidney	Pyelitis, chronic, focal, unilateral, minimal
Implant with cells	15		Cast, hyaline, focal, unilateral, minimal
		Liver	Hemopoietic cell foci, minimal
	21	Kidney	Pyelitis, chronic, focal, unilateral, minimal
	21	Liver	Hemopoietic cell foci, minimal
		Kidney	Mononuclear cell foci, unilateral, minimal
		Liver	Hemopoietic cell foci, minimal

	Biocompatible control implant			Implant with cells	Implant without cells		
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
White blood cells $[10^3/\text{mm}^3]$	11.08	3.33	13.18	4.17	13.90	3.76	
Lymphocytes [10 ³ /mm ³]	6.70	2.25	7.80	2.46	8.19	2.03	
Monocytes [10 ³ /mm ³]	0.55	0.18	0.73	0.32	0.71	0.22	
Granulocytes [10 ³ /mm ³]	3.83	0.99	4.65	1.82	5.00	2.04	
Eosinophils [10 ³ /mm ³]	1.43	0.65	1.09	0.64	1.06	0.78	
Erythrocytes [10 ⁶ /mm ³]	7.94	0.60	7.17	0.52	7.28	0.73	

Blood samples were taken from mice 21 days after implantation of one of the three items. White blood cells, lymphocytes, monocytes, granulocytes, eosinophils and erythrocytes were counted. n = 6 for the biocompatible control implant group and n = 8 for the other two groups.

	Biod	Biocompatible reference Implant without cells							Implant with cells											
	R	eplica S	of Cl Section	hamb n	er		C S	hamb Sectio	er n			Cham	ıber S	ectio	n	Switchboard Section				
Animal:	1	5	14	18	19	3	4	12	13	16	8 F	9	15	21	22	8	9	15	21	22
Polymorphonuclear	2	2	3	2	1	2	2	1	2	3	2	3	2	2	2	1	2	1	2	2
Lymphocytes	1	2	2	2	2	1	2	2	1	1	2	2	2	1	1	2	1	2	1	1
Plasma cells	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrophages	2	2	2	2	1	2	2	2	1	1	2	1	1	1	2	2	1	1	1	1
Giant cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Subtotal (x2)	10	12	14	12	10	12	12	10	8	10	12	12	10	8	10	10	8	8	8	8
Neovascularisation	2	2	3	2	2	1	1	1	1	1	2	1	2	2	1	1	1	1	1	2
Fibrosis	3	3	3	3	3	2	3	3	3	2	3	3	3	2	3	2	2	2	2	3
Fatty infiltrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Detritus within fibrous capsule	1	1	1	2	0	2	1	0	0	1	2	1	1	1	1	1	1	0	1	1
Subtotal	6	6	7	7	5	5	5	4	4	4	7	5	6	5	5	4	4	3	4	6
Total	16	18	21	19	15	17	17	14	12	14	19	17	16	13	15	14	12	11	12	14

 Table S5. Biocompatibility - single animal findings according to adapted ISO 10993-6:2016(E) scoring system

	Peak current	Average current	Predicted	
Description		[mA]	theoretical battery	Source
	[[lifetime [h]	
Near infrared optogenetic implant	28	14	143	Folcher et al. (10)
Near infrared optogenetic implant	350*	350	57	Shao <i>et al</i> (6)
"HydroLED"	550	550	5.7	
Electrogenetic implant	13	0.027	75000	This work

Table S6. Energy consumption of optogenetic and electrogenetic implants

Average current was calculated assuming that no current is drawn between pulses. Predicted theoretical battery lifetime was calculated for a typical battery used for implantable cardiac pacemakers, which is 2 Ah (52). The electrogenetic implant used in this work generates **peak current** of around 13 mA in 2 ms pulses at 1 Hz frequency. The optogenetic implant published by Folcher *et al.* (10) generates **peak current** of up to 28 mA for 30 s followed by 30 s of resting time.

*Authors did not measure the output current. The calculation was based on typical LED current value provided by the manufacturer.

Plasmid	Description and cloning strategy	Reference
pCa _v a ₁ C	Constitutive $\alpha_1 C$ expression vector (P _{hCMV} - $\alpha_1 C$ -pA). (Addgene no. 26572).	(60)
$pCa_v\alpha_1D_{\Delta 42}$	Constitutive $\alpha_1 D_{\Delta 42}$ expression vector (P _{hCMV} - $\alpha_1 D_{\Delta 42}$ -pA). (Addgene no. 49332).	(61)
$pCa_v\alpha_1D_{42A}$	Constitutive $\alpha_1 D_{42A}$ expression vector (P_{hCMV} - $\alpha_1 D_{42A}$ -pA). (Addgene no. 49333).	(61)
$pCa_v\alpha_2\delta_1$	Constitutive α_2/δ_1 expression vector (P _{hCMV} - α_2/δ_1 -pA). (Addgene no. 26575).	(62)
pCa _v β ₃	Constitutive β_3 expression vector (P _{hCMV} - β_3 -pA). (Addgene no. 26574).	(63)
pcDNA3.1	Constitutive mammalian expression vector containing a NeoR resistance gene (PhCMV-MCS-pA)	Thermo
(+)		Fisher
		Scientific,
		CA
pcDNA3.1/	Constitutive mammalian expression vector containing a HygroR resistance gene (PhCMV-MCS-pA).	Thermo
Hygro(+)		Fisher
		Scientific,
		CA
pCMV-T7-	Constitutive SB100X expression vector (PhCMV-SB100X-pA). (Addgene no. 34879).	(64)
SB100		
pEGFP-N1	Constitutive EGFP expression vector (PhCMV-EGFP-pA).	Clontech,
		CA

Plasmid	Description and cloning strategy	Reference
pGEM-T	Bacterial expression vector.	Promega,
Easy		WI
pGEMTEZ	pGEM-T Easy containing K _{ir} 2.1. (Addgene no. 32641).	(65)
-K _{ir} 2.1		
pMD2.G	Constitutive VSV-G expression vector (PhCMV-VSV-G-pA). (Addgene no. 12259).	(66)
pMS2-P65-	Lentiviral vector containing a $P_{hEF1}\alpha$ -driven expression unit. (Addgene no. 61426).	(67)
HSF1_Hyg		
ro		
pMX57	P _{NFAT3} -driven SEAP expression vector (P _{NFAT3} -SEAP-pA).	(5)
pMX251	SB100X-specific transposon containing a constitutive dTomato and BlastR expression unit and a constitutive	(5)
	expression unit for α_2/δ_1 and β_3 (ITR-P _{hEF1a} - α_2/δ_1 -P2A- β_3 -pA:P _{RPBSA} -dTomato-P2A-BlastR-pA-ITR).	
pProinsulin	Lentiviral vector for constitutive expression of Proinsulin-NanoLuc (PhCMV-Proinsulin-NanoLuc-pA).	(45)
-NanoLuc	(Addgene no. 62057).	
pSBbi-BP	SB100X-specific transposon containing a constitutive BFP and PuroR expression unit (ITR-PhEF1 α -MCS-	(68)
	pA:P _{RPBSA} -BFP-P2A-PuroR-pA-ITR). (Addgene no. 60512)	
pSEAP2-	Constitutive mammalian SEAP expression vector (P _{SV40} -SEAP-pA)	Clontech,
Control		CA
psPAX2	Lentiviral packaging vector. (Addgene no. 12260)	(69)
pKK5	Constitutive Kir2.1 expression vector (PhCMV-Kir2.1-pA). Kir2.1 was excised from pGEMTEZ-Kir2.1 using	This work

Plasmid	Description and cloning strategy					
	<i>Eco</i> RI and cloned into the corresponding site (<i>Eco</i> RI) of pcDNA3.1(+).					
pKK55	Constitutive α_1 C and Kir2.1 expression vector ($P_{hEF1\alpha}$ - α_1 C-P2A- K_{ir} 2.1-pA).					
	The vector backbone of pEGFP-N1 was PCR-amplified with OKK119 (5'-CTTA					
	AGGCGAGAATCGGAGATATGATAAAGCGGCCGCGACTCTAG-3') and OKK108 (5'-GCCCACT					
	$GACGGGCACTAATAACTAATGCATGGCGGTAATACGGTTATC-3'), P_{hEF1\alpha} was PCR-amplified$					
from pMS2-P65-HSF1_Hygro with OKK107 (5'-GATAACCGTATTACCGCCATGCATTAGTTATTA						
	GTGCCCGTCAGTGGGC-3') and OKK110 (5'-GGTGGCGAATTCAAGCTTGCTAGCGTCACGACA					
	CCTGAAATGGAAG-3'), $\alpha_1 C$ was PCR-amplified from $pCa_v\alpha_1 C$ with OKK109 (5'-					
	GTGTCGTGACGCTAGCAAGC TTGAATTCGCCACCATGGTCAATGAAAACACGAGGATGTAC-3')					
	and OKK112 (5'-CTCCTCCA CGTCTCCAGCCTGCTTCAGCAGGCTGAAGTTAGTAGCTCCGC					
	TTCCCAGGTTGCTGACGTAGGACC-3') and Kir2.1 was PCR-amplified from pKK5 with OKK111 (5'-					
	CTTCAGCCTGCTGAAG CAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGGCAGTGTG					
	AGAACCAAC-3') and OKK120 (5' CTAGAGTCGCGGCCGCTTTATCATATCTCCGATTCTCGCCT					
	TAAG 3') and all fragments were assembled by Gibson cloning.					
pKK56	Constitutive α_2/δ_1 and β_3 expression vector ($P_{hEF1\alpha}-\alpha_2/\delta_1-P2A-\beta_3-pA$). This					
	The vector backbone of pcDNA3.1/Hygro(+) was PCR-amplified with OKK130 (5'-					
	GACAGCTACTGATGAGCGGCCGCTCGAGTCGATCAGCCTCGACTGTG-3') and OKK126 (5'-					
	GCCCACTGACGGGCACTAATCAACGCGTATATCTGGCCCGTAC-3'), $P_{hEF1\alpha}$ was PCR-amplification of the provided of the second statement of the provided statemen					
	from pMS2-P65-HSF1_Hygro with OKK125					

Plasmid	Description and cloning strategy				
	(5'-GTACGGGCCAGATATACGCGTTGATTAGTGCCCGTCAGTGGGC-3') and OKK122 (5'-				
	CAGGCAGCCAGCAGCCATGGTGGCGAATTCAAGCTTGCTAGCGTCACGACACCTGAAATGGAA				
	G -3'), $\alpha_2\delta_1$ was PCR-amplified from $pCa_V\alpha_2\delta_1$ with OKK121 (5'-				
	CTTCCATTTCAGGTGTCGTGACGCTAGCAA GCTTGAATTCGCCACCATGGCTGCTGGCTGCCTG-				
	3') and OKK116 (5'-CTACGTCCCCGGCCT				
	GTTTGAGAAGGCTGAAGTTGGTCGCTCCGCTCCCCATAGATAG				
	was PCR-amplified from $pCa_V\beta_3$ with OKK115 (5'-CAACTTCAGCCTTCTCAAACAGGCCGGGGAC				
	GTAGAGGAGAACCCCGGGCCGATGTATGACGACTCCTACGTGC-3') and OKK124 (5'-				
	GACTCGAGCGGCCGCTCATCAGTAGCTGTCCTTAGGCCAAG-3') and all fragments were assembled				
	by Gibson cloning.				
pKK66	SB100X-specific transposon containing a constitutive BFP and PuroR expression unit and a constitutive				
	expression unit for $\alpha_1 C$ and $K_{ir}2.1$ (ITR-P _{hEF1a} - $\alpha_1 C$ -P2A- $K_{ir}2.1$ -pA:R _{PBSA} -BFP-P2A-PuroR-pA-ITR). $\alpha_1 C$ -				
	P2A-K _{ir} 2.1 was PCR-amplified from pKK55 using OKK141 (5'-attaggcctctgaggccA				
	TGGTCAATGAAAAACACGAGGATGTACGTTC-3') and OKK142 (5'-attaggcctgacaggccTCATAT				
	CTCCGATTCTCGCCTTAAGGGC-3'), restricted with SfiI and cloned into corresponding site (SfiI) of				
	pSBbi-BP.				
pKK137	P _{NFAT3} -driven SEAP and mINS expression vector (P _{NFAT3} - SEAP-P2A-mINS-pA). SEAP-P2A-mINS was	This work			
	excised from pMX256 using <i>Eco</i> RI/SalI, and cloned into corresponding sites of pMX57.				

Oligonucleotides: Restriction endonuclease-specific sites are shown in italics and annealing sequences are shown in capital letters.

Abbreviations: $\alpha_1 C$, $\alpha_1 C$ subunit of the murine L-type voltage-gated calcium channel Cav1.2; $\alpha_1 D_{42A}$, $\alpha_1 D_{42A}$ subunit of the murine L-type voltage-gated calcium channel Cav1.3; α_2/δ_1 , α_2 and δ_1 subunits of the murine L-type voltage-gated calcium channel Cav1.2; β_3 , β_3 subunit of the murine L-type voltage-gated calcium channel Cav1.2; **BFP**, blue fluorescent protein; **BlastR**, blasticidin resistance gene; Cav1.2, member 2 of the Cav1 family of L-type voltage-gated calcium channels; Cav1.3, member 3 of the Cav1 family of L-type voltage-gated calcium channels; **Cav1.3**, member 3 of the Cav1 family of L-type voltage-gated calcium channel; **HygroR**, hygromycin resistance gene; **ITR**, inverted terminal repeats of SB100X; Kir2.1, murine inwardly rectifying potassium channel; **MCS**, multiple cloning site; **mINS**, modified insulin variant for optimal expression in HEK-293 cells; **NanoLuc**, *Oplophorus gracilirostris* luciferase; *NeoR*, neomycin resistance gene; **PCR**, polymerase chain reaction; **P**_{hCMV}, human cytomegalovirus immediate early promoter; **P**_{hEF1a}, human elongation factor 1 alpha promoter; **P**_{NFAT3}, synthetic mammalian promoter containing five tandem repeats of a human IL-4 NFAT-binding site; **Proinsulin-NanoLuc**, modified proinsulin with its C-peptide replaced by NanoLuc; **P**_{RPBSA}, constitutive synthetic mammalian promoter; **P**_{SV40}, simian virus 40 promoter; **PuroR**, puromycin resistance gene; **SB100X**, optimized Sleeping Beauty transposase; **SEAP**, human placental secreted alkaline phosphatase; **VSV-G**, vesicular stomatitis virus protein G; **ZeoR**, zeocin resistance gene.

Designator	Description	Manufacturer	Manufacturer part number
BAS28	High-speed double diode	NXP	BAS28,215
BC856	PNP Epitaxial Silicon Transistor	NXP	BC856B
ZD15	Voltage regulator diodes BZX84	NXP	BZX84/C15
BAT54C	Schottky barrier (double) diode	NXP	BAT54C
LMC555	CMOS Timer	Texas Instruments	LMC555CMM/NOPB
10 k	Resistor 10 k	Vishay	CRCW080510K0FKEA
1.5 k	Resistor 1.5 k	Vishay	CRCW08051K50FKEA
1.6 M	Resistor 1.6 M	Vishay	CRCW08051M60FKEA
100 pF	Capacitor 100 pF	Murata	GRM2165C1H101JA01D
68 pF	Capacitor 68 pF	RND Electronics	RND1500805N680J500
1 uF	Capacitor 1 uF	RND Electronics	RND1500805B105K250
6.5-30 pF	Trimmer capacitor	Murata	TZC3P300A310R00

Table S8. Electronic components of the bioelectronic implant

Manufacturers: NXP Semiconductors, Eindhoven, Netherlands; Texas Instruments, Dallas, TX, USA; Vishay, Malvern, PA, USA; RND Electronics, Nänikon, Switzerland; Murata Manufacturing Co., Nagaokakyō, Japan.

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