Electronic supplementary information

Insulin sensor cells for the analysis of insulin secretion responses in single living pancreatic β cells

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This Electronic supplementary information includes:

1. Supplementary Methods

1.1. Vector construction for the expression of NαLY

We genetically connected the two protein probes Nluc-αCT and L1-YPet via the seven-repeat Ser-Ala-Gly-Gly [$(SAGG)_7$] sequence [1] as a linker. The synthesized oligonucleotide encoding $(SAGG)_7$ (5′-AGT GCT GGT GGT AGT GCT GGT GGT AGT GCT GGT GGT AGT GCT GGT GGT AGT GCT GGT GGT AGT GCT GGT GGT AGT GCT GGA TCC GGT GGT-3′; Eurofins Genomics, Brussels, Belgium) was first inserted at the end of the gene of Nluc-αCT with the Ig κ-chain leader sequence as a secretion signal into its expression plasmid [2]. The gene encoding L1-YPet was further inserted, yielding the gene encoding NαLY (Fig. S1) with the secretion signal. The Nluc-αCT-expressing vector had been originally created by utilizing pcDNA3.1/myc-His B (Thermo Fisher Scientific, Waltham, MA, USA); thus, the NαLY-expressing vector had a CMV promoter, enabling expression of a protein probe utilizing an Expi293 Expression System (Thermo Fisher Scientific).

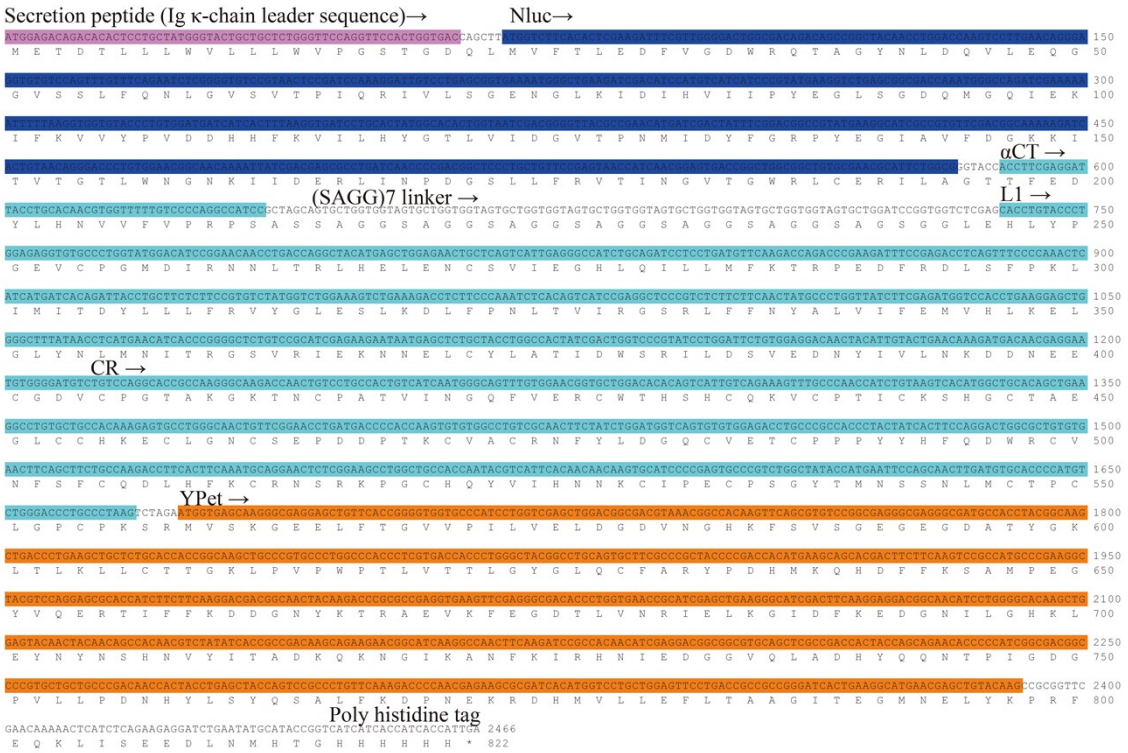

Fig. S1 The gene sequence of NαLY.

1.2. Expression and purification of NαLY

NαLY was expressed using an Expi293 Expression System (Thermo Fisher Scientific) following the manufacturer's protocol. Seven days after transfection with the corresponding protein expression vector, the supernatants of culture medium were collected. The supernatants were then dialyzed against a His-tag affinity chromatography buffer (20 mM NaH_2PO_4, 500 mM NaCl, 5 mM imidazole, pH 7.4). The dialyzed sample was applied on a His-Trap FF column (GE Healthcare, Chicago, IL, USA), and the target protein was eluted using an imidazole gradient. The eluted protein was dialyzed against KREBS-Ringer buffer (1.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 4.5 mM KCl, 120 mM NaCl, 0.7 mM Na_2HPO_4, 1.5 mM NaH_2PO_4, 15 mM $NaHCO_3$, pH 7.4). The protein was stored at 4°C with 1 mg/mL bovine serum albumin (BSA, F-V; Nacalai Tesque, Kyoto, Japan), after determination of protein concentrations using a Micro BCA Kit (Thermo Fisher Scientific). During each step, the expression and the purification of NαLY were confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis (data not shown).

1.3. Vector construction for fabricating insulin sensor cells

To fabricate insulin sensor cells, we genetically engineered Hepa1-6 cells to express NαLY on the extracellular membrane. A protein harboring both a secretion signal and a transmembrane region was immobilized on the extracellular membrane as depicted in Fig. S2. Therefore, we genetically added the transmembrane region of platelet-derived growth factor receptor (PDGFR) [3–5] at the C-terminus of NαLY in the expression vector created in Section 1.1, harboring the Ig κ-chain leader sequence as a secretion signal at the N-terminus (Fig. S3). The synthesized oligonucleotide encoding PDGFR (5′-GCT GTG GGC CAG GAC ACG CAG GAG GTC ATC GTG GTG CCA CAC TCC TTG CCC TTT AAG GTG GTG GTG ATC TCA GCC ATC CTG GCC CTG GTG GTG CTC ACC ATC ATC TCC CTT ATC ATC CTC ATC ATG CTT TGG CAG AAG AAG CCA CGT-3′; Eurofins Genomics) was inserted into the end of the NαLY gene in the expression vector (Fig. S3).

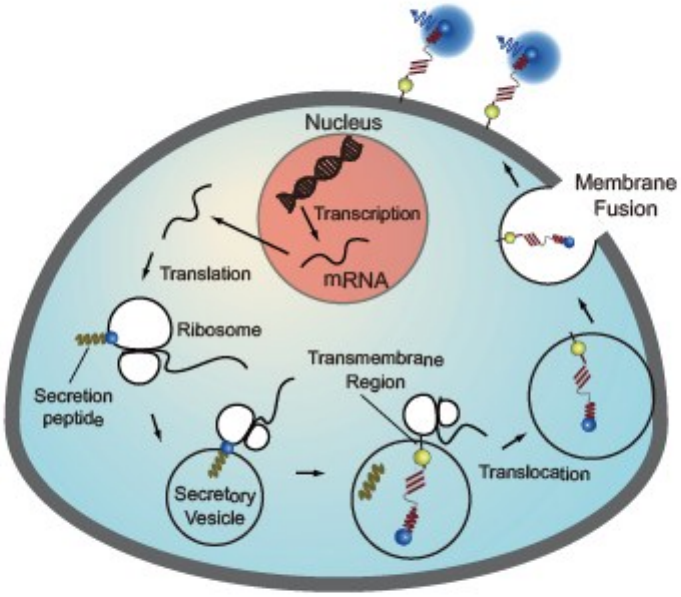

Fig. S2 The mechanism of immobilization of the protein probe on the extracellular membrane.

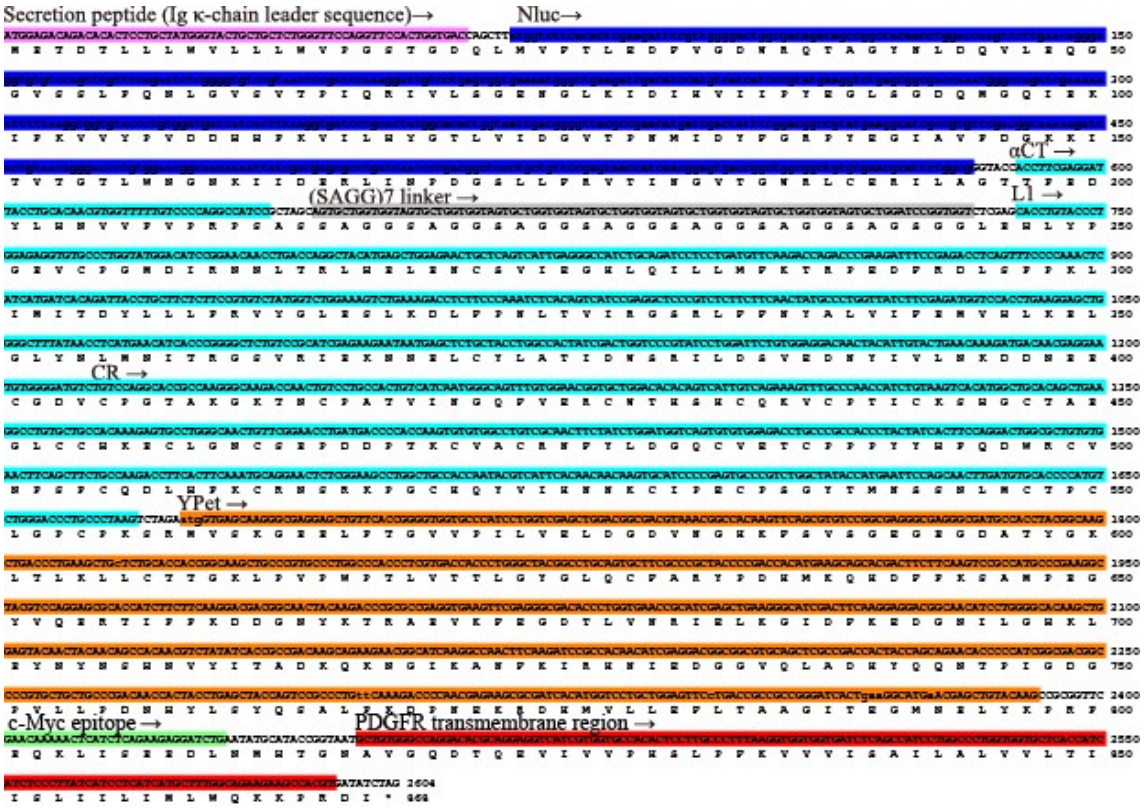

Fig. S3 The gene sequence for the expression of NαLY on the extracellular membrane used to establish insulin sensor cells.

2. Supplementary Results

2.1. The specificity analysis of insulin detection using NαLY protein.

We evaluated the specificity of insulin assay using NαLY protein. C-peptide is a peptide cleaved from pro-insulin in a secretary vesicle and secreted together with matured insulin and insulin like growth factor-I (IGF-I) is known to activate the insulin receptor. The assay was carried according to the described method in Experimental section of main manuscript but adding the IGF-I and c-peptide instead of insulin. NαLY produced a BRET signal in response to insulin but not to c-peptide or IGF-I (Fig. S4). Therefore, we concluded that insulin sensor cells also be able to detect insulin septicity because sensor cells expressing the same protein, NαLY, in their cellular membrane.

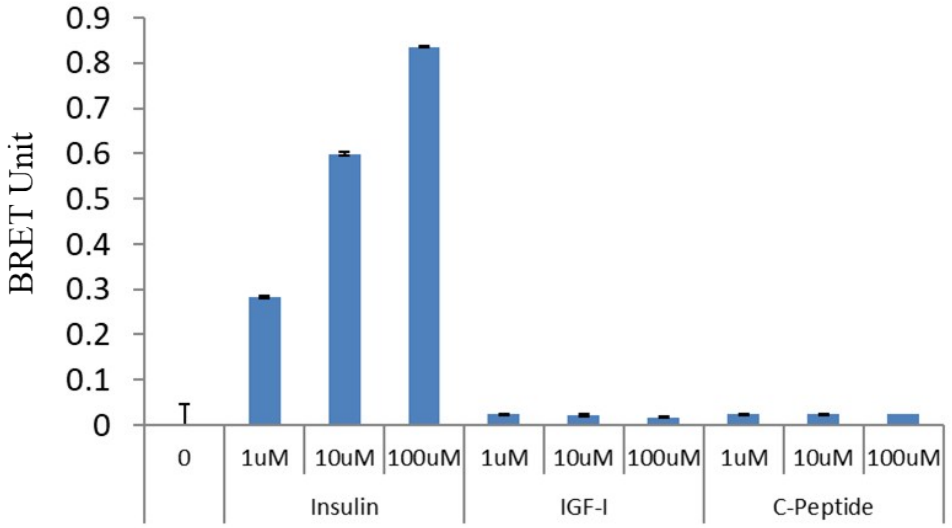

Fig. S4 The specificity analysis of insulin assay using NαLY protein. The BRET signals measured with 0, 1, 10, 100 μM insulin, IGF-I, and C-Peptide using NαLY protein. The results in the figures are shown as means ± standard deviation of three replicates.

2.2. Imaging analysis of Hepa1-6 cells as insulin sensor cells

We evaluated the ability of the potential sensor cells to detect insulin. The sensor cells were incubated KREBS-Ringer buffer containing each concentration of insulin at 37°C for 5 min, and the buffer was then replaced with KREBS-Ringer buffer containing the same concentration of insulin and 1 μL Nano-Glo Luciferase Assay Substrate (Promega) in a total volume of 100 μL. As shown in Fig. 3B and S5A, when the cells were incubated with insulin, the BRET signals were increased. The Fig. S5B indicates the average BRET signals calculated at each cell. The BRET signals were insulin concentration-dependently increased. The lower detection limit using sensor cells was 1 μM.

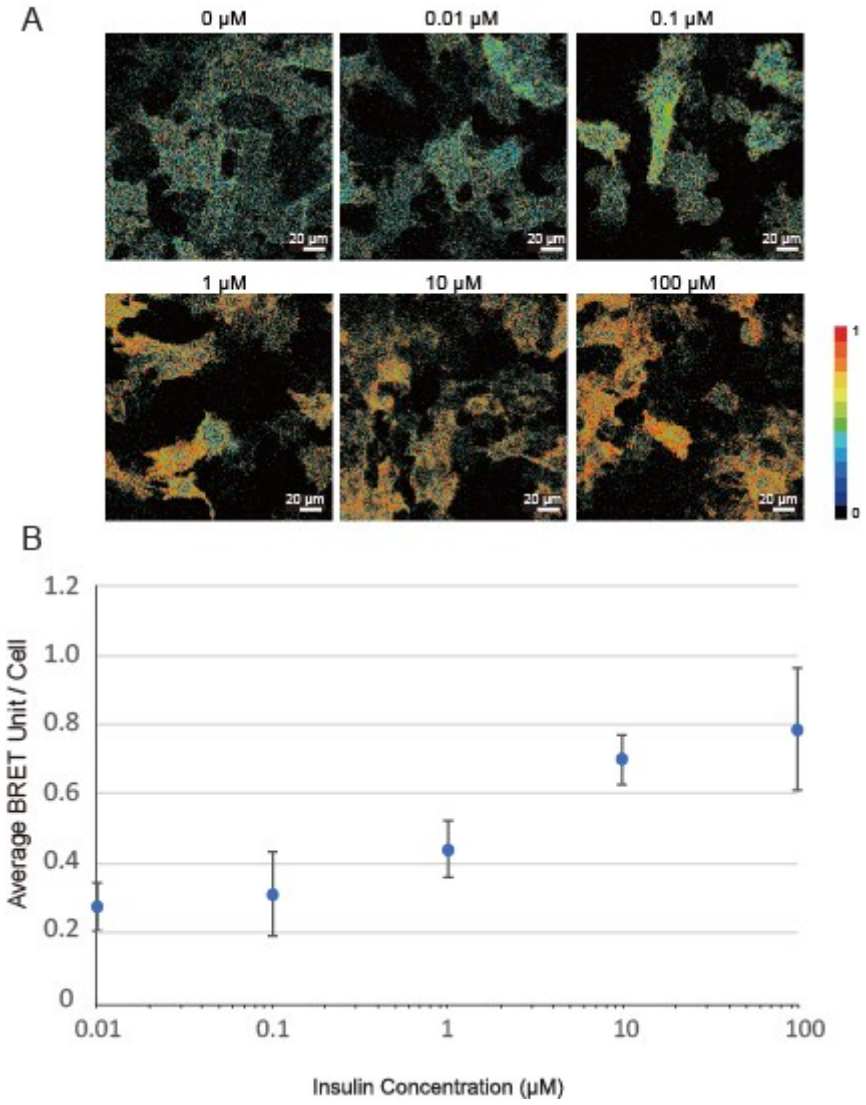

Fig. S5 Imaging analysis of insulin concentration dependent BRET signal response of Hepa1-6 cells as insulin sensor cells. (A) BRET images of insulin sensor cells incubated in each concentration of insulin. Images were obtained after a 5-min incubation with insulin. (B) Concentration-dependent responses of the BRET unit / cell area from (A). The results are shown as means ± standard deviations at least three replicates.

2.3. Direct analysis of insulin secretion from single living pancreatic β cells with insulin sensor cells

We validated the feasibility of direct analysis of insulin secretion from single living pancreatic β cells with the insulin sensor cells. When cells in a co-cultured format were stimulated with glucose, insulin sensor cells nearby pancreatic β cells showed the spike-shaped BRET signal response, whereas the insulin sensor cell ($ID\#_{sens}$ 4) close to one MIN6 cell ($ID\#_{MIN}$ 4) did not exhibit such a response as described in the main text. To confirm if this "no-response" was reflecting the "no-secretion of insulin" from MIN6 cell $ID\#_{MIN}$ 4, the BRET responses of two more insulin sensor cells (Fig. S6A; $ID\#_{sens}$ 5 and $ID\#_{sens}$ 6) surrounding MIN6 cell $ID\#_{MIN}$ 4 were analyzed. As depicted in Fig. S6B, both of the insulin sensor cells $ID\#_{sens}$ 5 and $ID\#_{sens}$ 6 did not show the spike-shaped response but indicated a gradual increase. Therefore, we believe that the adjacent MIN6 cell ($ID\#_{MIN}$ 4) appeared to not secrete insulin in response to glucose stimulation in this assay.

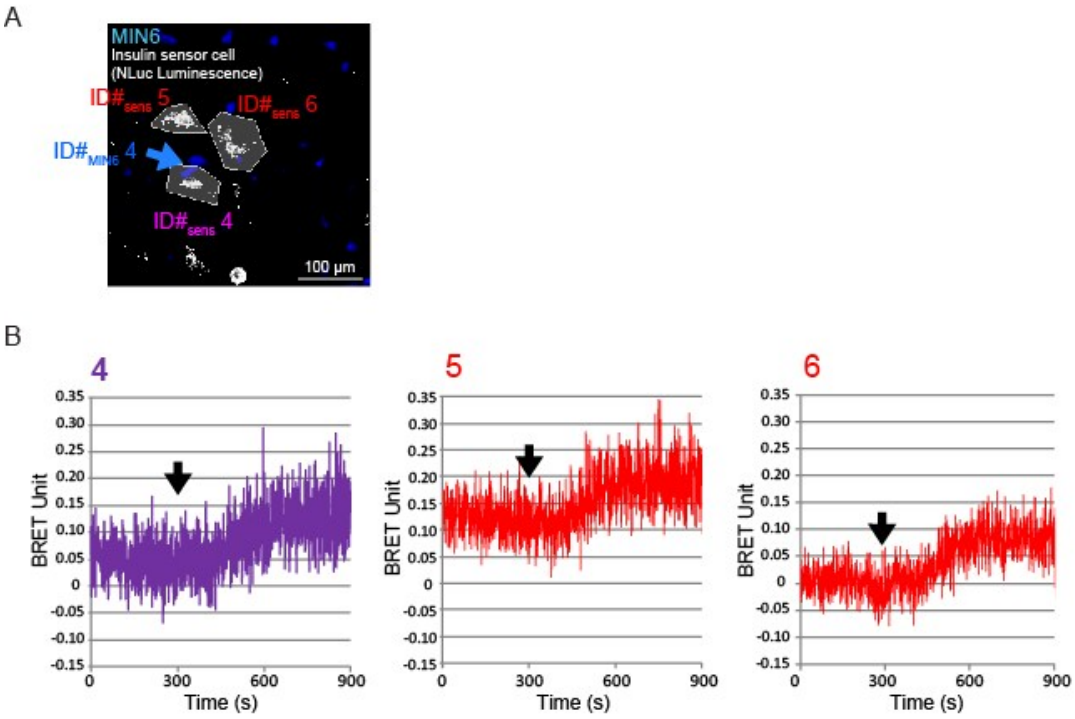

Fig. S6 Direct analysis of insulin secretion from single living pancreatic β cells with insulin sensor cells. (A) The merged image of MIN6 cells (blue) and insulin sensor cells (grey) before stimulation. Two insulin sensor cells (ID#$_{sens}$ 5 and ID#$_{sens}$ 6) surrounding MIN6 cell (ID#$_{MIN}$ 4) were further selected and analyzed their BRET responses. (B) Time course of average BRET units calculated from the surrounded area identified in the merged image indicated in (A). Glucose was added at the 300 s as indicated with black arrows.

References

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