Electronic supplementary information

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

Hajime Shigeto^{a,b,c*}, Takuto Ono^b, Takeshi Ikeda^b, Ryuichi Hirota^b, Takenori Ishida^b, Akio Kuroda^b, Hisakage Funabashi^{a, b*}

aInstitute for Sustainable Sciences and Development, Hiroshima University, Higashihiroshima,

Hiroshima 739-8511, Japan

^bDepartment of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter,

Hiroshima University, Higashihiroshima, Hiroshima 739-8530, Japan

^cHealth Research Institute, National Institute of Advanced Industrial Science and Technology (AIST),

2217-14 Hayashi-cho, Takamatsu, Kagawa 761-0395, Japan

*Corresponding author;

Hajime Shigeto (Email: hajime.shigeto@aist.go.jp)

Hisakage Funabashi (Email: hisafuna@hiroshima-u.ac.jp)

This Electronic supplementary information includes:

1. Supplementary Methods

1.1. Vector construction for the expression of NaLY...Page S3

1.2. Expression and purification of NaLY...Page S5

1.3. Vector construction for fabricating insulin sensor cells...Page S6

2. Supplementary Results

2.1. Specificity analysis of insulin assay using NaLY protein...Page S9

2.2. Imaging analysis of Hepa1-6 cells as insulin sensor cells...Page S11

2.3. Direct analysis of insulin secretion from single living pancreatic β cells with insulin sensor cells...Page S13

References...Page S15

1. Supplementary Methods

1.1. Vector construction for the expression of NaLY

We genetically connected the two protein probes Nluc- α CT and L1-YPet via the sevenrepeat 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 AGT GCT GGT GGT GGT AGT GCT 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- α CT 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 N α LY (Fig. S1) with the secretion signal. The Nluc- α CT-expressing vector had been originally created by utilizing pcDNA3.1/myc-His B (Thermo Fisher Scientific, Waltham, MA, USA); thus, the N α LY-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 NaLY.

1.2. Expression and purification of NaLY

NaLY 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 NaH₂PO₄, 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 CaCl₂, 0.5 mM MgCl₂, 4.5 mM KCl, 120 mM NaCl, 0.7 mM Na₂HPO₄, 1.5 mM NaH₂PO₄, 15 mM NaHCO₃, 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 N α LY were confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis (data not shown).

1.3. Vector construction for fabricating insulin sensor cells



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

Se	cr	eti	on	pe	pti	de	(Is	g K	-C	ha	in	lea	ad	er	se	qu	let	100	:)-	+				N	llu	IC-	÷																	-	_	_					
ATC	E R	T	D	T	L	L	L	TGG	GTA V	L	L	DCT L	CTG	1007	FIC	CAG P	G	5	T	GGT	D	CAG	L	м	v	7	т	L	2	D	P	v	G	D	N	1	0	т	A	G	Y	8	L	D	0	v	L	1	0	G	150
				~~												~	2				<u></u>					_									-			2				1.00	6	_	1	_	_	_	-		100
G	v	s	s	L	7	۹	8	L	G	۷	s	v	7			I	۹	R	I	۷	L	5	G	2	H	G	L	ĸ	I	D	I	н	v	I	I	P	Y		G	L	4	G	۵	Q		G	9	I	н	ĸ	100
	-	-			T ac	-	5 3	gur	gur			-	-	33	9		19		taut 1	22		et e	gt i		- 20	-99	997	-	gee	3 88	ewk		-	-	tte	35*	regg		-	9.00	ggr		900	313	-	9-1	-		ومد	at o	450
I		ĸ	v	v	¥	P	۷	D	D	8	8	7	3		1	I	L	8	¥	G	т	L	v	I	D	G	v	Т	P	N	м	I	D	Y	P	G		P	Y		G	I	*	v		-	aĈ'	T-T	*	I	150
7	-	7	6	7	L		10	6	12	r	T	T				2	I.	T	12	7		G		L	L		-		T	T		G	v	Ŧ	G	N N	1	L	5	-	- 97	T	L	-	6	ACC	ACC	TTO	GAGC	D	600
_	_	_	_	_	_			_		_	-	_		6	<u>s</u> A	G	G)	7	lin	ke	r -	+	_	_	_	_		_	_	_	_	_		_	_	_	_	_	_	_	_	_	_		_]	LÌ	\rightarrow	•	_	_
Y	L	8		v	v	7	v	P		P	s	Å	s	-		A	G	G	S	A	G	6	s	A	G	G	S	A	6	6	S	A	G	G	S	A	G	G	S	A	G	s	G	G	L	E	8	L	Y	P	250
66	ugau		700		1 Parts	ATG	GA G	ATC	096		30.0	007	GAC	CM	200	TAC	AT	2,1,2	CTG	GAG	AAC	TGO	TO	GT	AL	rgav		CON	TOT	RCA	GAT I	CT.	OCTO	ATG	TTC	AAG	ACC	AGA		GAA	GAT	TTO	CGA	AGA C		3.97	TIC		aur	ere	900
G		۷	c	P	G		D	I	R	5	55	L	3			L	8		L		H	c	-	۷	I	2	G	н	L	6	I	L	L	м	P	ĸ	т	а.	P	2	D	P	R	D	L	s		P	ĸ	L	300
AT	AT	ATO	3.0	LGUN T	TAO	-		ere	-	-	NoT O	CTA	790	TC	ngg		C.			GAR	CTC.	TTO			TOT 1	CACT.	AGT	CAT		AGG	ere	-	rere	TTC	TTC	-	TAI	GCC	CTG	GTT	ATC	TTO	GAG	ATC	GTC.	CAC	cas		GAG	eng	1050
1	2	•			•	-	-	-	1			•				•	•	-	^		-		1	-	-			•	-		^	-	-			1	1	^	-		1		1	-		-	-	^	1	-	150
G	L	Y	BR I	L	H	B B	I	T		G	s	v		1	100	E	x	8	8	E	L	C	TA	L	A	T	I	D	CTG N	a	E.	I	L	D	5	v	E	D	AAC B	Y	I	V	L	8	x	D	D	8	EAGO	E	400
The		-	De T	CI	R -	+	ACO	PCC			-	and a	-	-	TC	-				ATC	AAT		-					PILE	-	-	ACA	Aut	-	THE	CAG		GTT	THE		acc	ATT	THE		TC		1990	THE		PCT		1350
c	G	۵	۷	c	P	G	т	٨	ĸ	G	ĸ	т	5		2	P	٨	т	۷	I	H	G	8	P	۷	2	8.	c	N	т	H	\$	н	c	8	ĸ	٧	c	P	т	I	c	ĸ	8	8	G	c	T	*	2	450
990	CT	med	TOO		-	G.N.9	190	CTG	660	944	-Toy	770	994		-76	ATG	a.c	000	ACC		Tur	GTO	AGC 1	Tur		CRAC	TT	CTA	TCT	GGA	TOO	TCA.	Turt	GTG	GRG	ACC	Tue		cca	cee	TAC	TAT	CAC	TTO	CAG	GAR	TGG	090	Terre	9TG	1500
	r	c	c	-	ĸ		c	r			c					•	•	*	Ŧ	x	c	×	~	c				1	L	B	6	6	c	v		т	c		*		T	T	-	,	6	•		R	c	v	500
8	77	5	77	C	0	B 10	L	B	770	x	C	CAG R	GJU S			Carcola Et	x	3	6	C	E AC	0	TA	V	AT	ICAC II	BAA B	CRA B	EAA	C C	I	7	E	C	P	5	G	TAT	T	M	BAT B	a a	AGC S	3000	L	ATC	C	ACC T	P	C	1650
-		_	-		-			YP	'et		-	-	-	COLUMN 1	LD.S		-			-	-		-	-		-	-	-	-		D D D D D D D D D D D D D D D D D D D	1221	1000	TRAC .	220		-	THE OWNER	Ter	THE	100	-	100.0	100	THE OWNER	1000	-	-	-	110	1900
L	G	P	c	P	ĸ	s	R		v	5	ĸ	G	-			L	7	т	G	٧	v	P	I	L	v	E	L	D	G	D	۷	H	G	н	ĸ	F	5	٧	5	G	E	G		G	D	٨	т	¥	G	ĸ	600
	a.co			erre	erc	193	ACG		666		ern	900	097	1900		660		NCC		976	ACC	acc	OT	-	TR	1966		903	ste	ott			TRO		GRAC	CAC	ATC	aag	cas	cac	GRAD	TTO	TTO	2000	TOO		a and	000	GULU	99C	1950
L	T	r	ĸ	L	r	c	T	T	G	ĸ	L	3			•		3	т	L	v	т	т	L	G	Y	G	L	6	C	P	A		Y	P	D	ж	м	ĸ	6	H	D	P	,	ĸ	8	*		P		G	650
TAC Y	1010	0	1000	8000	7	I	770	770	x	D	D	566 G	200	CTI I		AGA K	7	890	200	2	or o	AAC	TT		G	D	T	L	GGT			I	GAG	L	AAG	G	ATC	D	F	AAC	CALC E	D	GOO	333.0	I	L	GGGG	CAG	X	L L	2100
				-	-			_			-		-										-	_	-							-			_	-	_		-	-	-	-							-	-	
I	Y	58	Y	58	s	8	58	v	Y	I	T				κ.	9	ĸ	58	G	I	ĸ	A		F	ĸ	I	8	H	N	I	E	D	G	G	v	6	L	A	D	H	Y	6	9	51	T	3	I	G	D	G	750
000	0970	1000	erro	2000	064/0		exe	TAC	erre		TRAC .	-	are o	000		TGt	10.0		GAC	000	aac	GRAG	-	acrea	GRA	rca/	TRO	GOT	COT	GOT	GGRU	at the	Detto	acc			666	arc	ACT	gas	GGC	ATC	a.k.	GANG	cro	TAC		000	0.000	TTC	2400
č.	Ň	vc	en	nite	ne	8	-	¥	L	8	Y	0	-		•	L F	'n	Ğ	FR	tr	an	sn	nei	mb	ra	ne	те	gic	n	-	2	P	L	т	A	A	G	I	т		G	м	51		L	¥	ĸ	P	R	7	800
ŝ	CU	ŝ	ch	TAC	fou		GAG	GAT	CTG		TAT	PCA	TAC	000	ATA	AT				-			-					i in		ACR	-			-					ATC.	-		-	-		-			ere	a.co	NTC 7	2550
-	4	^	-	-	-	-	-	-	-	-	1	-				-	^				-	-	*	-		•		1	1	-	1	-			-				-	-	~	•	-	-	-			-	-	•	
I	6	L	I	I	L	I	A DO	1	M	0	x	x				D	I		26	8																															

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

2. Supplementary Results

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

We evaluated the specificity of insulin assay using N α LY 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. N α LY 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, N α LY, in their cellular membrane.



Fig. S4 The specificity analysis of insulin assay using N α LY protein. The BRET signals measured with 0, 1, 10, 100 μ M insulin, IGF-I, and C-Peptide using N α LY protein. The results in the figures are shown as means \pm 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

- Komatsu, N.; Aoki, K.; Yamada, M.; Yukinaga, H.; Fujita, Y.; Kamioka, Y.; Matsuda, M. *Mol. Biol. Cell* **2011**, *22* (23), 4647–4656.
- (2) Shigeto, H.; Ikeda, T.; Kuroda, A.; Funabashi, H. *Anal. Chem.* **2015**, *87* (5), 2764–2770.
- (3) Claesson-Welsh, L.; Eriksson, A.; Morén, A.; Severinsson, L.; Ek, B.; Ostman,
 A.; Betsholtz, C.; Heldin, C. H. *Mol. Cell. Biol.* 1988, *8* (8), 3476–3486.
- (4) Claesson-Welsh, L.; Eriksson, A.; Westermark, B.; Heldin, C. H. Proc. Natl. Acad. Sci. 1989, 86 (13), 4917–4921.
- (5) Chen, P. H.; Unger, V.; He, X. J. Mol. Biol. 2015, 427 (24), 3921–3934.