SUPPLEMENTARY DATA

In vitro differentiation of EPCs into INS-producing cells

The EPC aggregates, generated by aggregation culture on a low-binding 96-well plate at a density of 30×10^3 cells/well, were cultured for 10 days in iMEM-B27 with 10 μM ALK5 Inhibitor II (SC-221234A; Santa Cruz Biotechnology), 100 nM LDN193189 hydrochloride (HY-12071A; Medchemexpress) and 1 μM L-3,3′,5-triiodothyronine (64245; Merck Millipore). The medium contained 200 mg/dL glucose (low glucose) or was added glucose (G8769; Sigma) to set the concentration at 540 mg/dL (high glucose). To lower influence of INS, B27 without containing INS (A1895602; Thermo Fisher Scientific) instead of B27, or 100 nM of the synthetic peptide S961 (Phoenix Pharmaceuticals, Burlingame, CA, USA) was added in the medium. S961 at 100 nM was reported to function as a strong INS receptor antagonist (1). Forty-eight aggregates were prepared for each group and the medium renewal was conducted once every 3 or 4 days. After 10 days culture, the propensity to differentiate into $INS^+NKX6.1^+$ cells and INS^+Ki67^+ cells were assessed by flow cytometry (FCM). Three independent experiments using different batches of EPCs were conducted. The gate of FCM data analyzed for $INS^+NKX6.1^+$ cells was set to be under 1.5% at the starting point of 10-day culture in all experiments. The cell number was measured using NC200 (Chemometec, Allerod, Denmark).

Reference

1. Knudsen L, Hansen BF, Jensen P, Pedersen TÅ, Vestergaard K, Schäffer L, Blagoev B, Oleksiewicz MB, Kiselyov VV, De Meyts P. Agonism and antagonism at the insulin receptor. PLoS One. 2012;7(12):e51972.

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SUPPLEMENTARY DATA

Supplementary Table 1. Additional information on EPCs generated *in vitro* from hiPSCs.

Figure	**iPSC line**	***In vitro* differentiation**	
		Period of stage 3 (days)	**Period of stage 5 (days)**
1, 6, S1, S2, S6, and S7	Ff-I14s14	2	3
2 and S4	Ff-I14s14	2	4
3A, 3B and 4A-C	Ff-WJ18	2	3
3C, 3D, and S5	Ff-I14s14	3	3
4D-F	Ff-WJ18	3	3
5 and S3	Ff-WJ18	2	3

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Supplementary Table 2. The information on antibodies used in this study.

Primary antibody	**Species**	**Source**	**Dilution**
SOX2	Rabbit	35795, Cell Signaling Technology	1:100
SOX17	Goat	AF1924, R&D Systems	1:500
FOXA2	Rabbit	07-633, EMD Millipore	1:100
PDX1	Goat	AF2419, R&D Systems	1:200
NKX6.1	Mouse	F55A12, University of Iowa	1:360
CHGA	Rabbit	ab68271, Abcam	1:500
Neurogenin3	Sheep	AF3444, R&D Systems	1:2000
Insulin	Rabbit	C27C9, Cell Signaling Technology	1:200
*Insulin	Rat	GN-ID4, University of Iowa	1:600
Glucagon	Mouse	G2654, Sigma-Aldrich	1:200
Ki67	Mouse	550609, BD Pharmingen	1:100
Ki67	Rabbit	D3B5, Cell Signaling Technology	1:100
CK19	Mouse	M088, Dako	1:200
Trypsinogen	Sheep	AF3848, R&D Systems	1:100
SOX9	Rabbit	ab196450, Abcam	1:100

Fluorescent secondary antibody	**Source**	**Dilution**
Nucleus	Hoechst 33342; H3570, Invitrogen	1:200
Alexa 488 conjugated	For each species; all from Invitrogen	1:500
*Alexa 546 conjugated	For each species; all from Invitrogen	1:500
Alexa 594 conjugated	For each species; all from Invitrogen	1:500

*: Used only for flow cytometry

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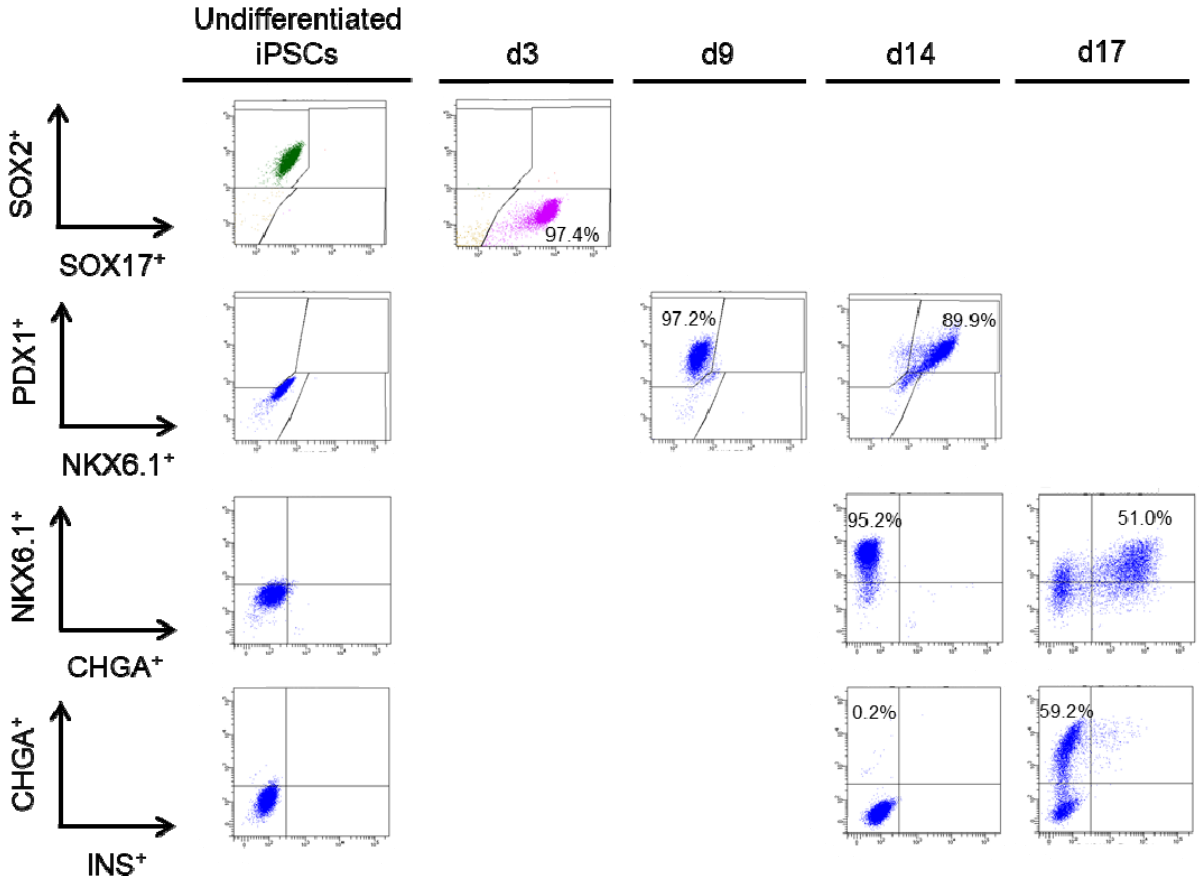
Supplementary Figure 1. EPCs are differentiated from hiPSCs by *in vitro* directed differentiation. Cell populations at individual stages were analyzed by flow cytometry. Representative dot plots indicating high differentiation efficiency of definitive endoderm $SOX17^{+}SOX2^{-}$ cells at d3, posterior foregut $PDX1^{+}NKX6.1^{-}$ cells at d9, pancreatic endoderm $PDX1^{+}NKX6.1^{+}$ cells at d14, and endocrine progenitor $NKX6.1^{+}CHGA^{+}$ and $CHGA^{+}INS^{-}$ cells at d17. Each of these differentiation induction efficiencies is inserted.



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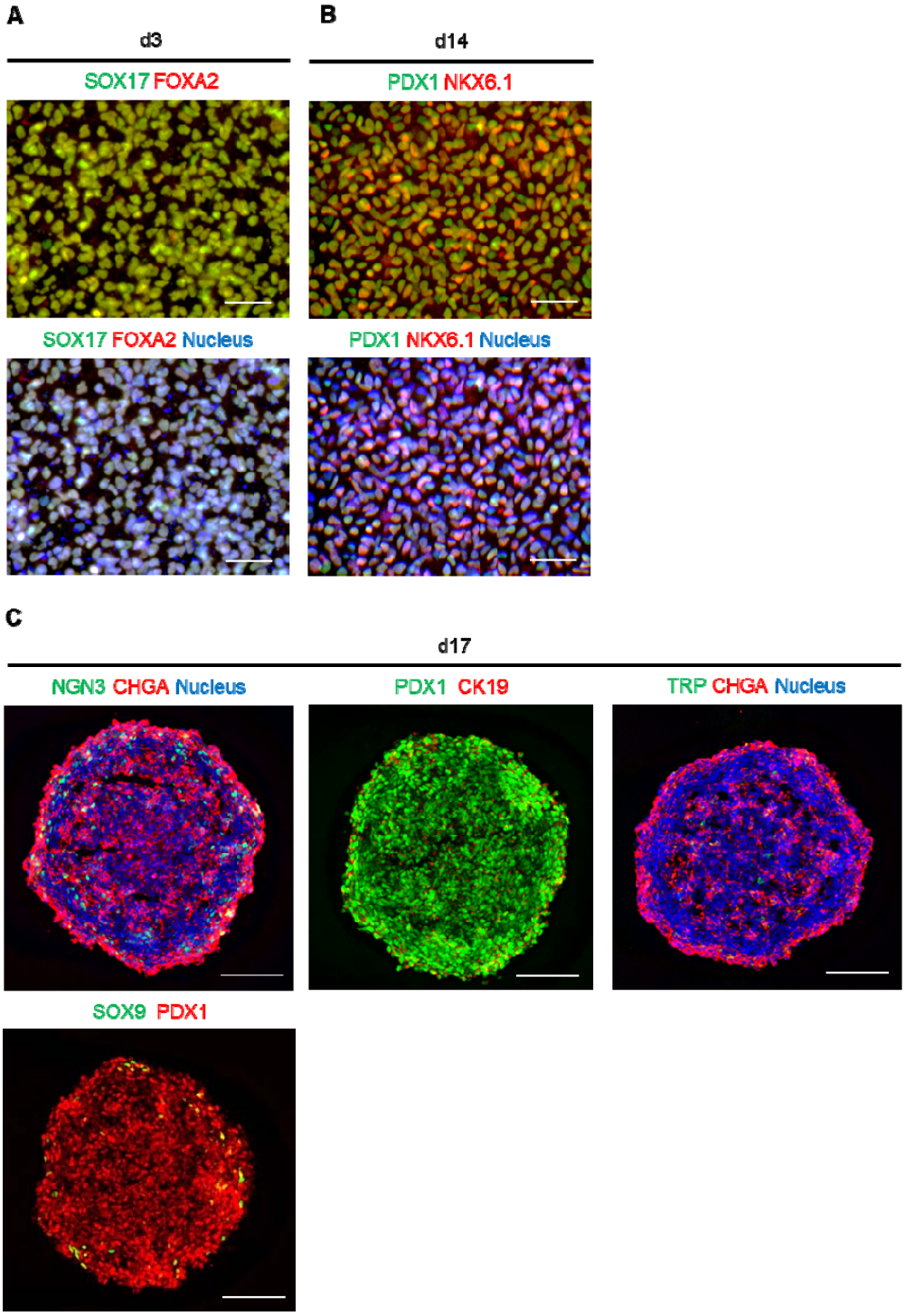
Supplementary Figure 2. Immunostaining of the cell population in EPC aggregates. Representative immunofluorescence images of planar cultured cells stained for (A) SOX17/FOXA2 and (B) PDX1/NKX6.1 at d3 and d14, respectively. (C) Representative cryosectional images of EPC aggregates stained for the indicated markers. Nuclei were stained with DAPI. Scale bars, 50 μm in (A) and (B); 100 μm in (C). TRP, trypsinogen.



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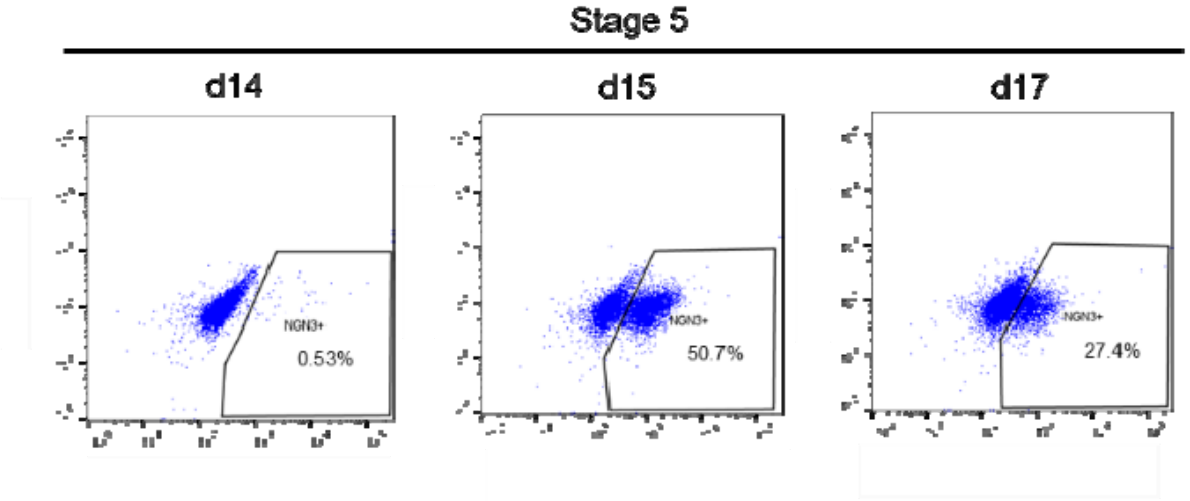
Supplementary Figure 3. The proportion of NGN3^{+} cells is peak at the early phase of stage 5. Representative flow cytometry plots showing the proportion of NGN3^{+} cells at d14, d15 and d17 during stage 5 differentiation *in vitro*.



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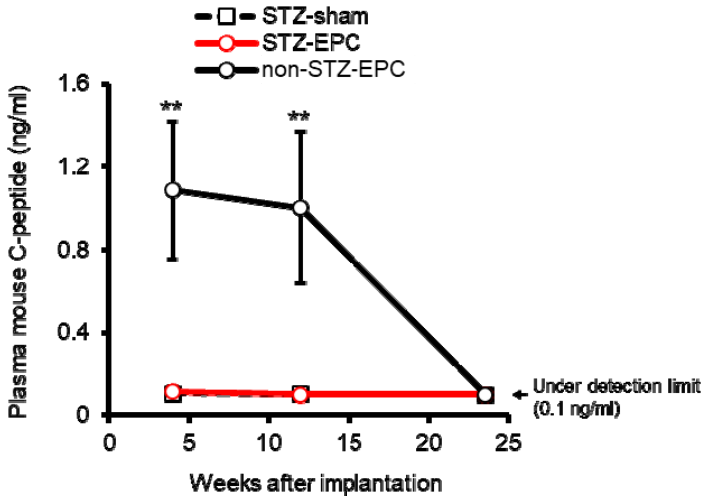
Supplementary Figure 4. Endogenous INS deficiency in diabetic mice is not affected by implantation of EPCs. STZ-induced diabetic NOD/SCID mice and non-diabetic control mice were implanted Ff-I04s04-derived EPCs under the kidney sub-capsule (STZ-EPC and non–STZ-EPC). Sham-operated STZ mice (STZ-sham) were also prepared. Plasma levels of endogenous mouse C-peptide at non-fasting state were measured at the indicated weeks after implantation. Data are presented as the mean ± SD. $^{**}p < 0.01$ non–STZ-EPC vs. STZ-EPC by Bonferroni adjusted Dunnett's test. STZ-sham (N=4), STZ-EPC (N =3-6) and non–STZ-EPC (N=3-6).



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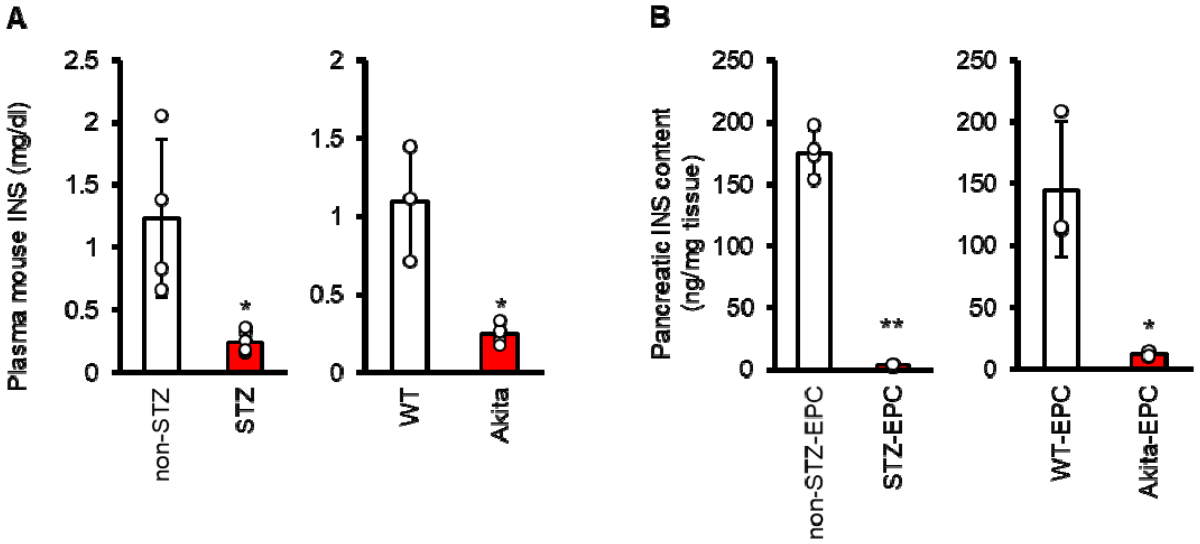
Supplementary Figure 5. Endogenous INS is severely defected in diabetic mouse model used for implantation studies. Levels of (A) plasma mouse INS levels at the day before EPC-implantation and (B) pancreatic INS contents at 18 weeks after the EPC-implantation were measured in STZ-induced diabetic NOD/SCID mice and genetically induced Akita diabetic NOD/SCID mice. These levels in each non-diabetic control mice were measured similarly. Data are presented as the mean ± SD. **p* < 0.05, ***p* < 0.01 STZ vs. non-STZ or Akita vs. WT by Aspin-Welch's t-test. non-STZ (N=4) and STZ (N=6) in (A). non-STZ-EPC (N=4), STZ-EPC (N=5), WT-EPC (N=3) and Akita-EPC (N=3) in (B).



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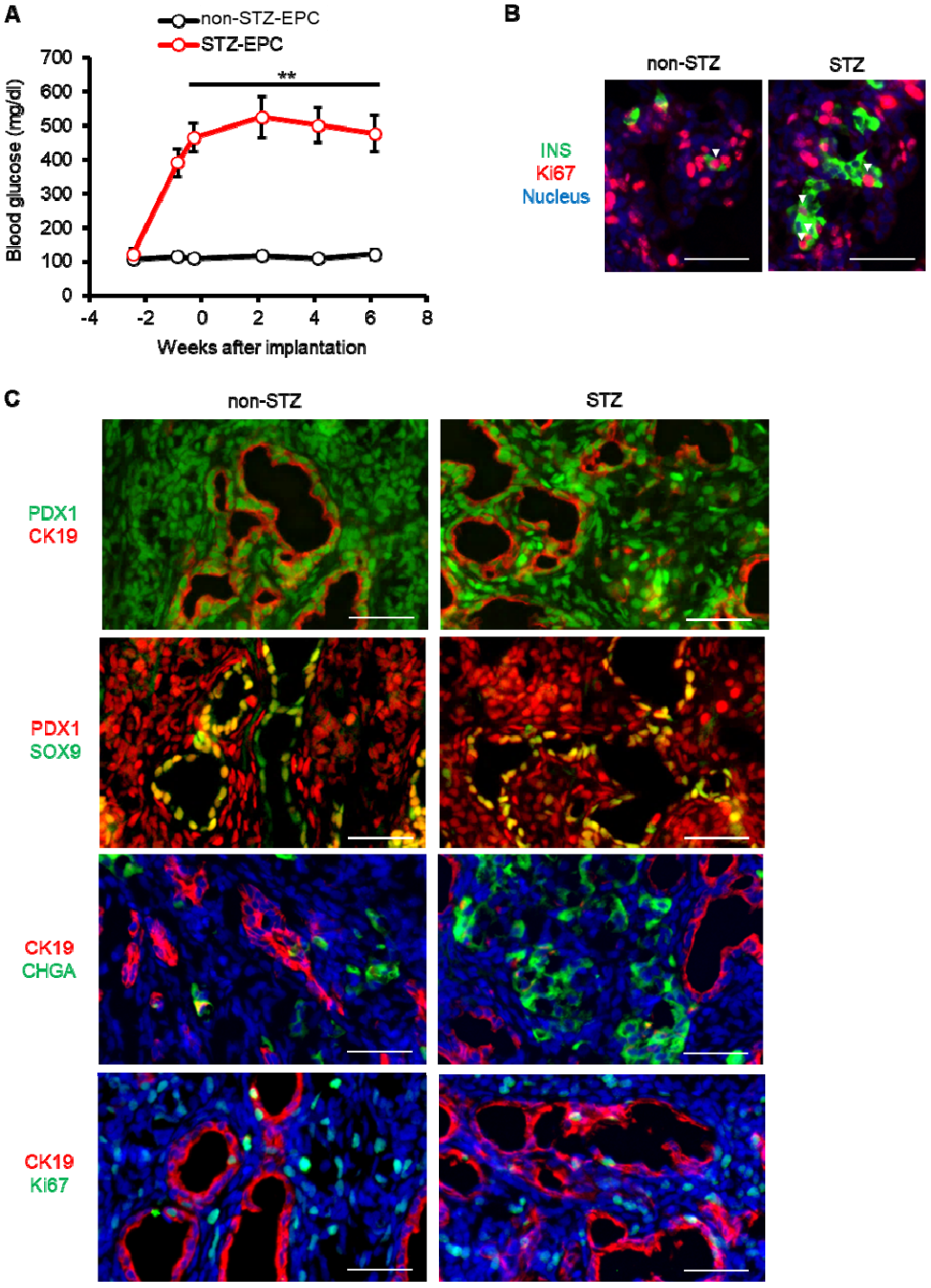
Supplementary Figure 6. Supplementary information referred to Figure 6. (A) Levels of blood glucose in STZ-induced diabetic and non-diabetic NOD/SCID mice before and after EPC implantation. Data are presented as the mean ± SD. STZ-EPC (N=8) and non–STZ-EPC (N=6). Representative immunofluorescence images of the explanted grafts stained for (B) INS/Ki67 and (C) PDX1/CK19, PDX1/SOX9, CK19/CHGA and CK19/Ki67. Nuclei were stained with DAPI. Scale bars, 50 μm.



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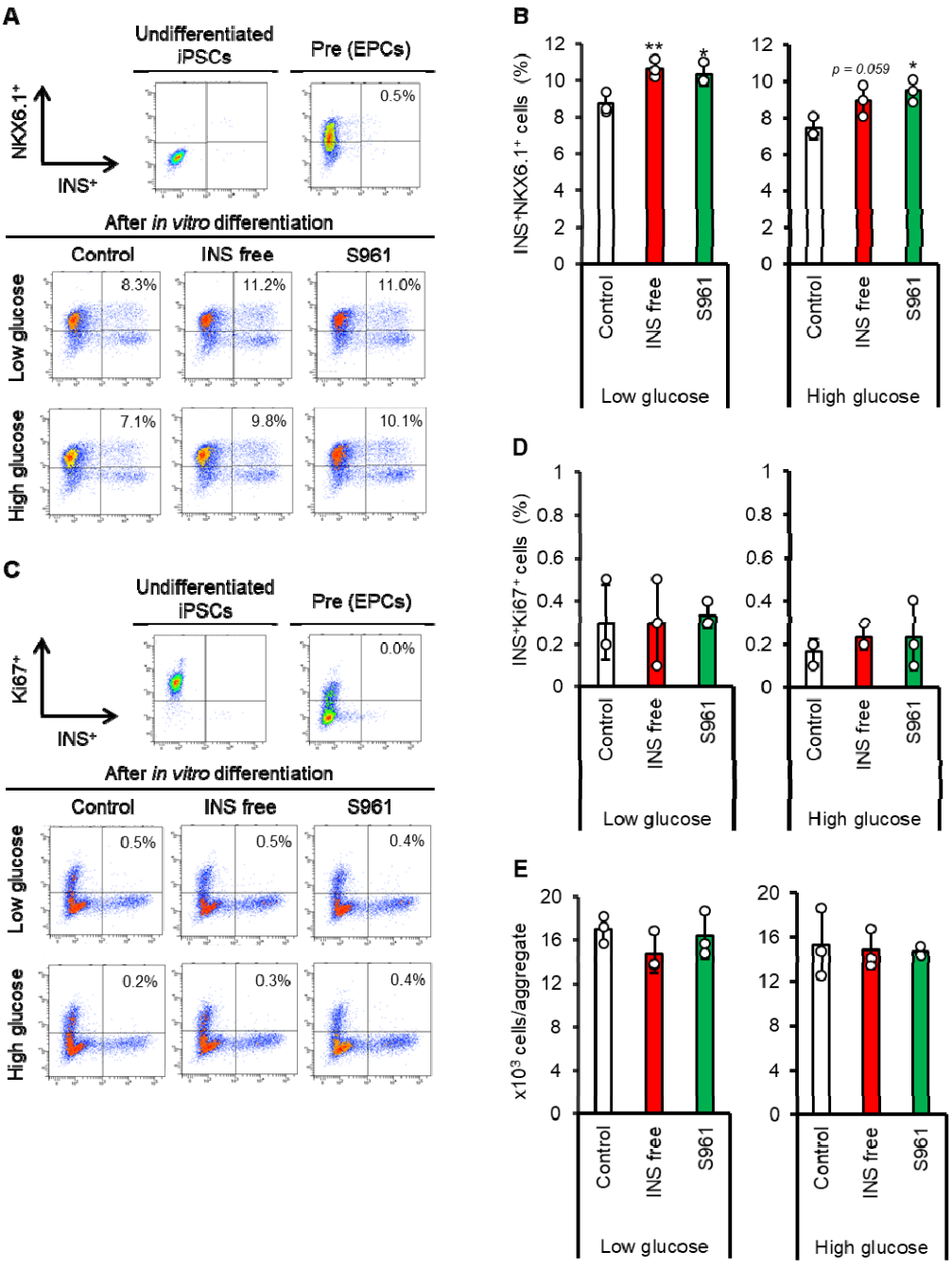
Supplementary Figure 7. Lowering INS action directly promotes the differentiation of EPCs into INS$^+$NKX6.1$^+$ cells *in vitro*. The EPC aggregates were cultured for 10 days with previous report-based medium to induce the differentiation into INS-producing cells. The medium contained glucose at 200 mg/dL (low) or 540 mg/dL (high). Under the both glucose concentration, medium without containing INS (INS free) or added an INS receptor antagonist S961 (100 nM) was used. (A) Representative flow cytometry plots showing the proportion of INS$^+$NKX6.1$^+$ cells and (B) the average of three independent experiments. (C) Representative flow cytometry plots showing the proportion of INS$^+$Ki67$^+$ cells and (D) the average of three independent experiments. (E) Cell number of the aggregates after the 10-day culture. *$p < 0.05$, **$p < 0.01$ vs. control by Dunnett's test.



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