

SUPPLEMENTARY DATA

**Supplementary Table 1.** qPCR primers and probes

<b>Gene</b>	<b>Probe / primer sequence</b>
<b>Glucagon</b>	Hs00174967_m1
<b>Insulin</b>	Hs00355773_m1
<b>Arx</b>	Hs00292465_m1
<b>Pax4</b>	Hs00173014_m1
<b>Glucagon</b>	F 5'-CAAGGCAGCTGGCAACGT-3' R 5'-CTGGTGAATGTGCCCTGTGA-3'
<b>Insulin</b>	F 5'-GCAGCCTTTGTGAACCAACA-3' R 5'-TTCCCCGCACACTAGGTAGAGA-3'
<b>MafA</b>	F 5'-CTTCAGCAAGGAGGAGGTCATC-3' R 5'-GCGTAGCCGCGGTTCTT-3'
<b>Pdx1</b>	F 5'-CCATGGATGAAGTCTACCAAAGCT-3' R 5'-CGTGAGATGTACTTGTTGAATAGGAACT-3'

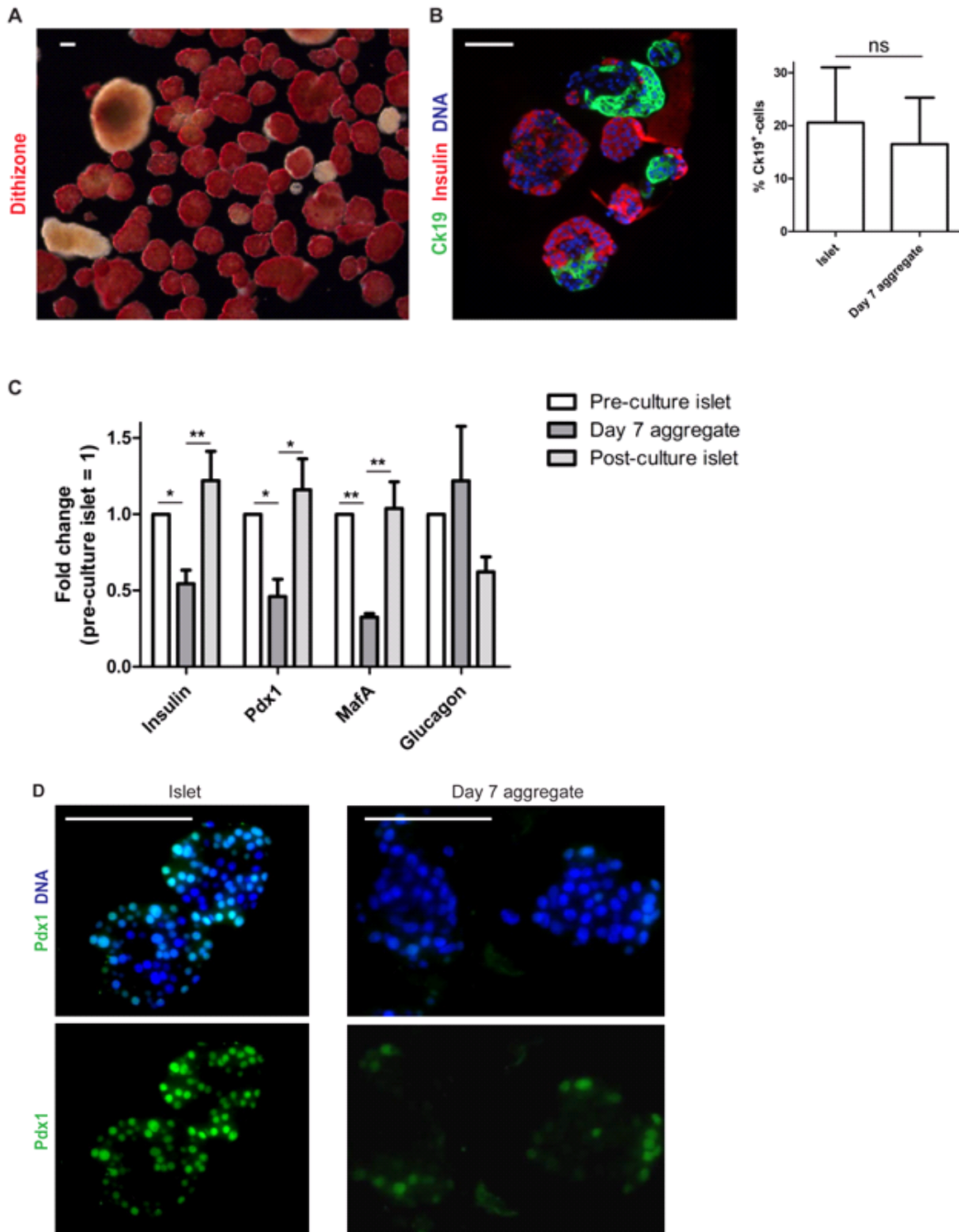
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**Supplementary Table 2.** Donor list

<b>Pancreas number</b>	<b>age</b>	<b>sex</b>	<b>BMI</b>
1	51	F	30
2	58	F	22
3	58	M	28
4	33	F	20
5	49	F	31
6	45	F	23
7	62	F	22
8	71	F	24
9	47	M	36
10	40	F	28
11	61	F	23
12	57	F	21
13	61	F	22
14	63	F	26
15	59	F	29
16	67	F	23
17	47	F	26
18	58	M	34
19	68	M	24
20	55	F	21
21	21	F	21
22	19	M	26
23	42	M	22
24	60	F	28
<b>Average</b>	<b>52</b>		<b>25</b>
<b>LOWEST</b>	19		20
<b>HIGHEST</b>	71		36

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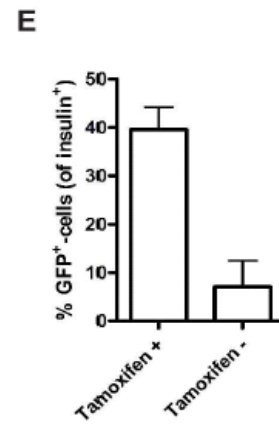
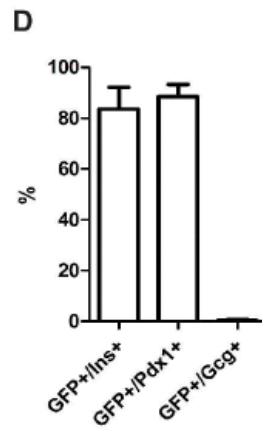
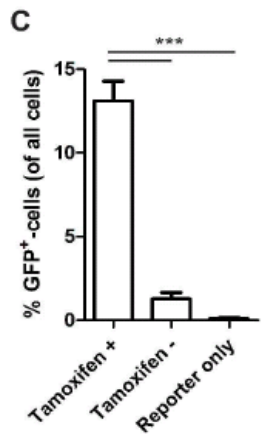
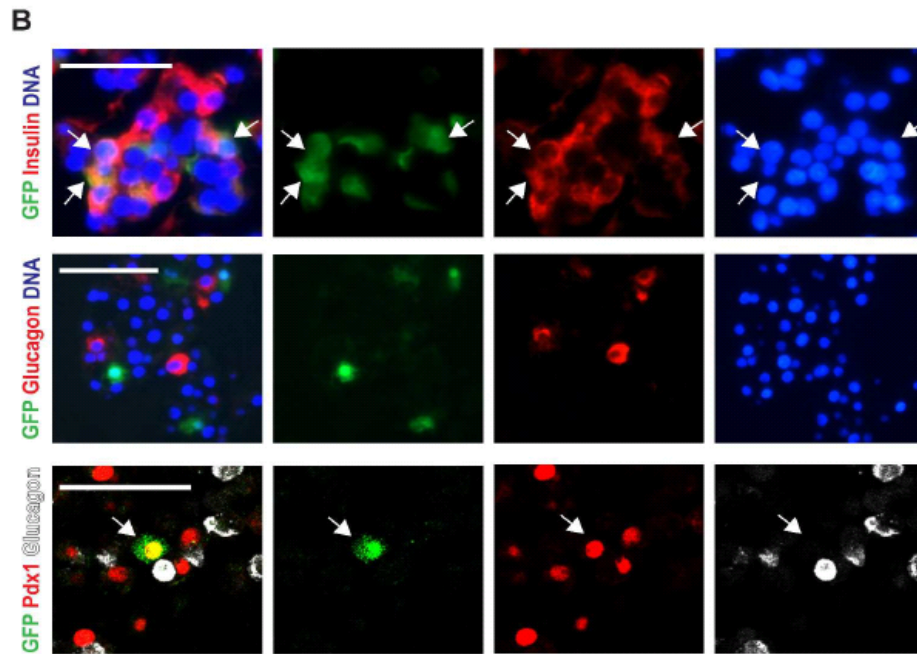
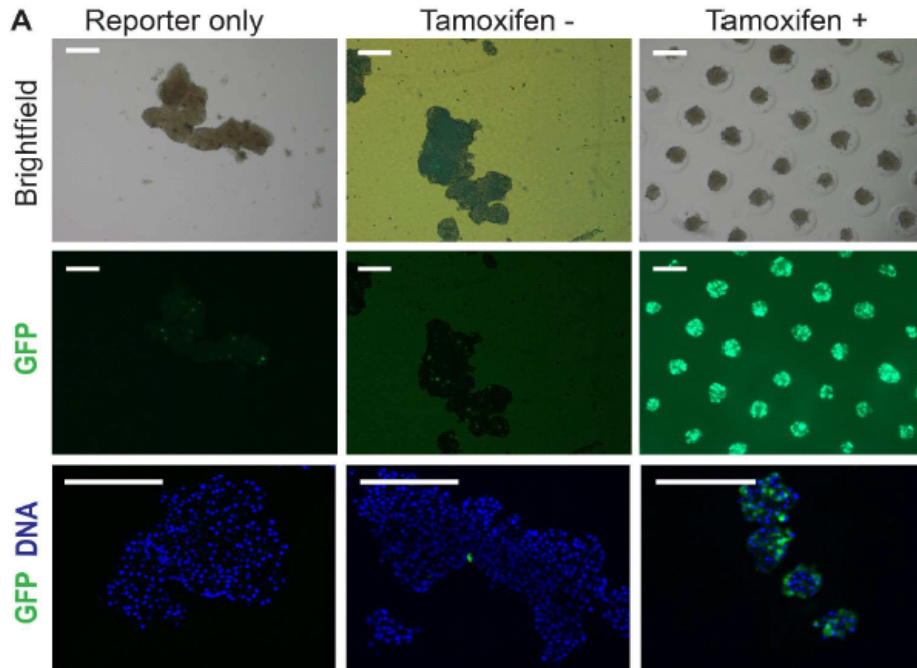
**Supplementary Figure 1.** Decrease in  $\beta$ -cells is accompanied by a decrease in expression of  $\beta$ -cell transcription factors. *A:* Freshly isolated purified human islets are discriminated from non-islet tissue using dithizone staining (red). *B:* Isolated human pancreatic islets stained for Ck19 (green) and insulin (red) and quantified before and after reaggregation (7 days) *C:* qPCR analysis of islet aggregates compared to intact islet before and after 7 days culture (\*  $p < 0,05$ , \*\* $p < 0,01$ ). *D:* Immunostaining for Pdx1 (green) and C-peptide (red) in intact and reaggregated islet cells. Scale bars: 100  $\mu$ m.



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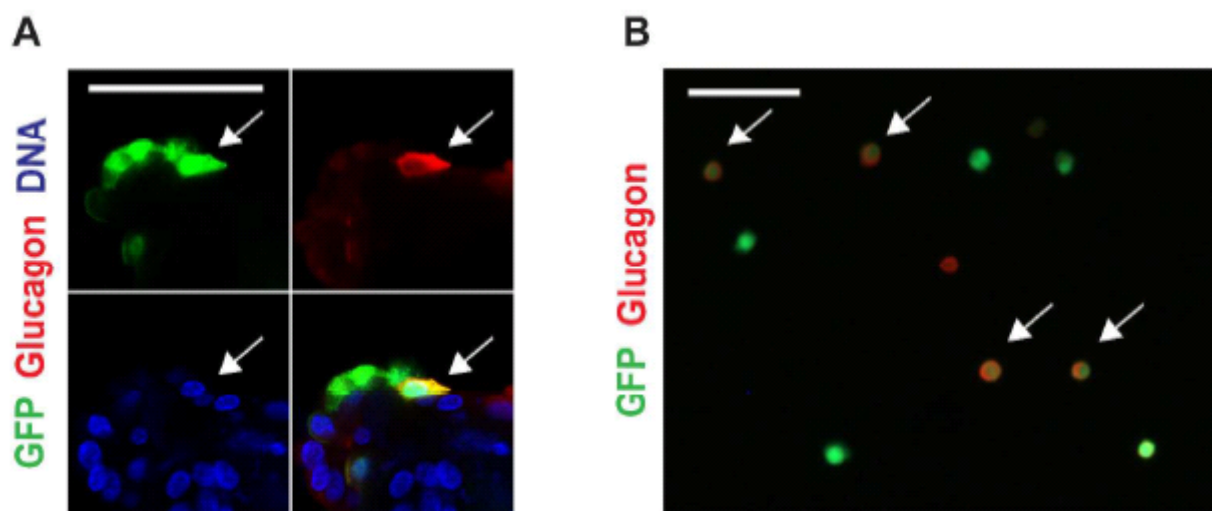
**Supplementary Figure 2.**  $\beta$ -cell lineage tracing specificity and confirmation of conversion. *A*: Live imaging and immunostaining shows few GFP<sup>+</sup>-cells in aggregates transduced with the reporter construct only (CMV-stop-GFP) or without tamoxifen compared to with tamoxifen administration. *B*: Immunostainings that combine GFP with either insulin, Pdx1 or glucagon were performed directly following tamoxifen-induced GFP expression (24-48 hours following dispersion) to assess specificity of the lineage tracing. *C*: Quantification of the percentage of GFP<sup>+</sup>-cells out of all cells. *D* Quantification of the percentage of GFP<sup>+</sup>-cells co-expressing either insulin (83.6 $\pm$ 8.6%), Pdx1 (88.5 $\pm$ 4.9%) or glucagon (<1%), showing the specificity of lineage tracing (arrows indicate double positive cells). *E*: Quantification of the percentage of insulin<sup>+</sup>-cells that express GFP showing the efficiency of the lineage tracing. Data are shown as mean  $\pm$ S.E.M., \*\*\*p<0,001, n=2-4 donors. Scale bars *A*: 200  $\mu$ m and *B*: 50  $\mu$ m.

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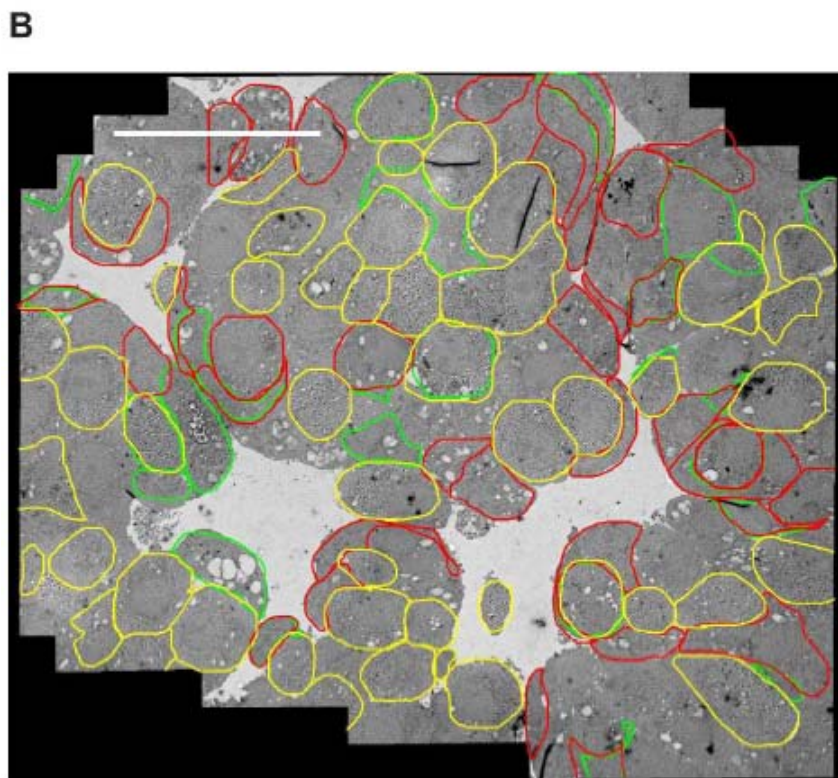
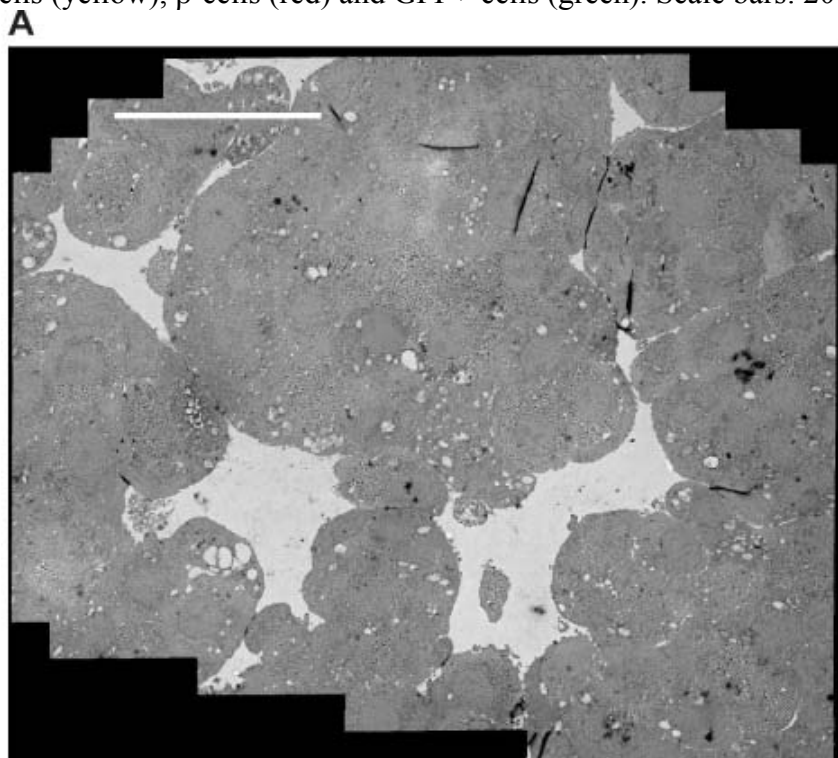
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**Supplementary Figure 3.** *A:* Confocal image shows an optical section of epifluorescent GFP (green) and glucagon (red). *B:* FACSsorted GFP<sup>+</sup>-cells immunostained for glucagon on cytospin. Scale bars: 50  $\mu$ m.



SUPPLEMENTARY DATA

**Supplementary Figure 4.** Quantification of electron microscopy analysis using digital stitching. *A*: Approximately 2500 electron microscopic photographs were stitched together to image a complete islet cell aggregate tissue section (150-250 cells per stitch) *B*: Annotations of a stitched image showing the localization of  $\alpha$ -cells (yellow),  $\beta$ -cells (red) and GFP+-cells (green). Scale bars: 20  $\mu$ m.



## SUPPLEMENTARY DATA

**Supplementary Figure 5.** Double immunogold labeling for glucagon and c-peptide. Double immunogold labeling was performed for glucagon (15 nm gold labels) and c-peptide (10 nm gold labels) after 4 days reaggregation. Borders between the cells are marked manually by a broken line on the left panel. Right panel shows higher magnification of the marked area. Scale bars: 500 nm

