Engineering islets from stem cells for advanced therapies of diabetes

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Abstract | Diabetes mellitus is a metabolic disorder that affects more than 460 million people worldwide. Type 1 diabetes (T1D) is caused by autoimmune destruction of β-cells, whereas type 2 diabetes (T2D) is caused by a hostile metabolic environment that leads to β-cell exhaustion and dysfunction. Currently, first-line medications treat the symptomatic insulin resistance and hyperglycaemia, but do not prevent the progressive decline of β-cell mass and function. Thus, advanced therapies need to be developed that either protect or regenerate endogenous β-cell mass early in disease progression or replace lost β-cells with stem cell-derived β-like cells or engineered islet-like clusters. In this Review, we discuss the state of the art of stem cell differentiation and islet engineering, reflect on current and future challenges in the area and highlight the potential for cell replacement therapies, disease modelling and drug development using these cells. These efforts in stem cell and regenerative medicine will lay the foundations for future biomedical breakthroughs and potentially curative treatments for diabetes.

Currently, more than 460 million people around the globe have diabetes mellitus — a chronic metabolic disorder resulting from progressive loss or dysfunction of the endogenous insulin-producing β-cells that reside in the islets of Langerhans in the pancreas[1] (BOX 1). According to the World Health Organization (WHO), two main types of diabetes can be distinguished. In type 1 diabetes (T1D), organ-specific autoimmune destruction of β-cells causes hyperglycaemia. T1D typically manifests during childhood, is thought to result from a combination of genetic and environmental factors, and is precipitated by defects in the immune system, parts of the exocrine pancreas and/or β-cells[2]. Whereas approximately 10% of patients with diabetes have T1D, the majority (90%) have type 2 diabetes (T2D)[1]. In T2D, hyperglycaemia is a consequence of a hostile metabolic environment, which triggers β-cell hyperresponsiveness, compensation and dedifferentiation. A dedifferentiated β-cell is characterized by the loss of identity, function and maturation features, such as the glucose-sensing and insulin secretion machinery[3–5]. In addition to genetic factors, the risk of developing T2D increases with unhealthy diet, physical inactivity and weight gain[6].

The symptoms of T1D and late-stage T2D can be treated with exogenous insulin. Although the administration of insulin is life-saving and can noticeably improve the quality of patients' lives, the disease is not cured. These patients need lifelong insulin treatment and those who fail to normalize blood glucose within a narrow range may have a reduced life expectancy and severe secondary complications. Furthermore, albeit a rare event, life-threatening hypoglycaemic episodes can occur in patients with diabetes who are treated with insulin. Approaches to protect and regenerate β-cell mass — for example, via immunotherapy[7], intensive insulin therapy[8], bariatric surgery[9] or pharmacological intervention[10,11] — could be curative or prevent disease progression and are being actively pursued. For pharmacological intervention, targeting these therapies to cells within the islet should help minimize side effects from systemic dosing, although this is challenging to achieve[12].

Another possible therapeutic approach for T1D is to replace the insulin-producing β-cells. For T2D, however, this approach is only an option when peripheral tissues are still responsive to insulin and the cardiovascular status of the patient allows transplantation surgery[13]. Currently approved replacement therapies for T1D include the transplantation of either the whole pancreas or isolated islets of Langerhans. The traditional recipients for whole pancreas transplants are patients with T1D and end-stage renal disease, who usually receive a pancreas and a kidney transplant at the same time[14]. Isolated donor islets can be transplanted into the portal vein of the liver following the Edmonton Protocol[15]. Although islet transplantation is among the safest organ transplantations and is minimally invasive, the therapy often requires infusion of islet preparations isolated from multiple donors[16]. Availability of donor islets is limited, and receiving an allogenic graft presupposes

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Box 1 | **Islets of Langerhans — composition and function**

Composition

The human pancreas accommodates about 3.2 million islets, which make up 1–2% of the organ. Both mouse and human islets are very heterogeneous and are diverse in their size, shape, cell type composition, blood supply and innervation[183]. The predominant endocrine cell type of the human pancreatic islets is the insulin-producing β-cell (50–75%), alongside glucagon-secreting α-cells (25–35%), somatostatin-secreting δ-cells (10%), pancreatic polypeptide-secreting cells (PP-cells, <5%) and ghrelin-producing ε-cells (<1%)[106,295]. Islet cell architecture varies substantially between organisms. In humans, for example, β-cells and α-cells are intermingled, whereas in the mouse, islet α-cells are found preferentially at the periphery and β-cells are located closer to the centre[295]. Islets are highly vascularized by microvasculature[296], which enables them to measure nutrient concentrations and release hormones into the bloodstream to regulate systemic glucose levels. The endothelial cells play a crucial role in β-cell function, regeneration and polarization[294]. Furthermore, islets are innervated with sympathetic and parasympathetic neurons, which connect islets to the autonomic nervous system[297]. Surrounding mesenchymal stromal cells play a crucial role in the development of insulin-producing β-cells in mice and humans[142]. The extracellular matrix (ECM) within human pancreatic islets mainly consists of components such as collagen, namely type IV and VI collagen, as well as laminins, and is mostly secreted by endothelial cells[298]. The ECM molecules interact with α/β integrin heterodimers, which are present on islet cells, and thus alters numerous cellular processes. For example, the interaction between the ECM and human and murine β-cells regulates the cytoskeletal state as well as apical–basal and planar cell polarity. These processes regulate endocrine formation, β-cell maturation and function in vivo and in vitro[60,68,69].

Function in glucose homeostasis

The release of the hormones insulin, glucagon and somatostatin is adjusted to maintain blood glucose levels in a defined range. All three hormones influence one another's secretion via intra-islet paracrine signalling, which is crucial for functionality of the human islet[35,299,300]. The endocrine cell types are intermingled in the human islet, and paracrine interactions include signalling via membrane-bound molecules and electrical coupling[301–303]. The released insulin is distributed via the circulation and binds to insulin receptors, which are located on fat, liver, muscle and other cells. This initiates glucose uptake into these peripheral tissues. Insulin also promotes glycogenesis in the liver. During fasting, α-cells maintain blood glucose levels by releasing glucagon and, thus, stimulating hepatic glucose production.

a strong cardiovascular system and requires lifelong immunosuppression in order to avoid graft rejection.

The foundations for a stem cell-based cell replacement approach to cure diabetes were laid by the discovery of a population of insulin-producing cells that spontaneously differentiated from human pluripotent stem cells (hPSCs) in vitro[17,18]. Rebuilding endogenous islets of Langerhans (BOX 1) from hPSCs could be the next step in developing an effective stem cell-derived product to treat both early and late-stage disease. Compared with a stem cell-derived population of β-like cells (SC-β-cells), defined stem cell-derived islet-like clusters (SC-islets) will likely have improved and more predictable functionality in vitro and after transplantation. For research purposes in T1D, SC-islets could be used to model autoimmunity and identify molecular targets and drugs that protect β-cells. Patient-derived SC-islets in combination with immune cells isolated from peripheral blood, for example, are a powerful model system to study alloimmunity and autoimmunity[19]. Combining SC-islet experiments with genome-wide association studies could inform disease mechanisms in both T1D and T2D. For example, SC-islets could be used to investigate the effects of modifying genes that are associated with an increased risk of T1D and T2D in genome-wide association studies[20,21]. Furthermore, SC-islets could be generated from induced pluripotent stem cells (iPS cells) that were themselves reprogrammed from cells isolated from patients with T1D or T2D. These SC-islets could be used to study the diseases, characterize potential disease-causing variants and test novel targets and therapies[22] (FIG. 1).

This Review summarizes advances in the generation and maturation of SC-β-cells and introduces an interdisciplinary approach combining stem cell biology, tissue engineering, single-cell genomics and microfluidics to reproducibly engineer homogeneous SC-islets. Furthermore, the potential to use SC-islets as advanced therapeutic medicinal products for replacement therapy will be discussed. Finally, we describe the uses of SC-islets in drug screening and disease modelling, and thus in developing diabetes treatments that address the underlying causes of the disease.

Engineering islets from hPSCs

hPSCs, such as human embryonic stem cells (hESCs) and human iPS cells, can self-renew indefinitely and differentiate into every cell type. Therefore, hPSCs are a virtually unlimited source to generate human endocrine cells for diabetes therapy and research. Whereas pancreatic differentiation protocols were initially established with hESCs[23–26], recent protocols can use human iPS cells as starting material[27–31]. The advantages of using human iPS cells include fewer ethical concerns, the potential for autologous transplantation and the potential to model disease in a patient-specific manner. However, the application of human iPS cells is limited by mutations that are introduced during reprogramming and expansion, which could increase the risk of tumour formation upon transplantation of a human iPS cell-derived product[32]. Furthermore, the generation, maintenance and adaption of differentiation protocols for patient-specific human iPS cells using current good manufacturing practice (cGMP) standards is cost-intensive and time-consuming. SC-islets derived from T1D human iPS cells might also be susceptible to recurrent autoimmune attacks because the antigens that initiated the disease-causing autoimmune response will still be expressed. Hence, cell biobanks with high-quality hPSC lines that cover most human leukocyte antigens (HLAs) in the population could be generated[33]. To cover 93% of the population of the United Kingdom, as an example, such a bank would consist of approximately 150 human iPS cell lines[34].

The islet of Langerhans is composed of multiple endocrine cell types and supporting cells, which communicate through paracrine signalling to regulate blood glucose levels[35] (BOX 1). hPSC-based diabetes research and therapy should aim to engineer SC-islets that closely resemble the endogenous human counterparts. The rest of this section discusses the generation of stem cell-derived α-like cells (SC-α-cells) and SC-β-cells, the key hormone-producing cell types in the islets of Langerhans that regulate systemic blood glucose levels. Reconstitution following differentiation can produce more complex engineered islets that could be suitable as research tools and therapeutic options. We discuss the shortcomings of existing protocols as well as resulting implications for the generation of SC-islets.

Engineering SC-β-cells

The first insulin-expressing SC-β-cells were generated by spontaneous differentiation of hESCs[17,18]. Since then, stepwise differentiation protocols for guided differentiation of hPSCs towards SC-β-cells were established that mirror pancreas and islet development in vivo[23–25] (FIG. 2).

All published protocols start by inducing mesendoderm and anterior definitive endoderm (ADE), from

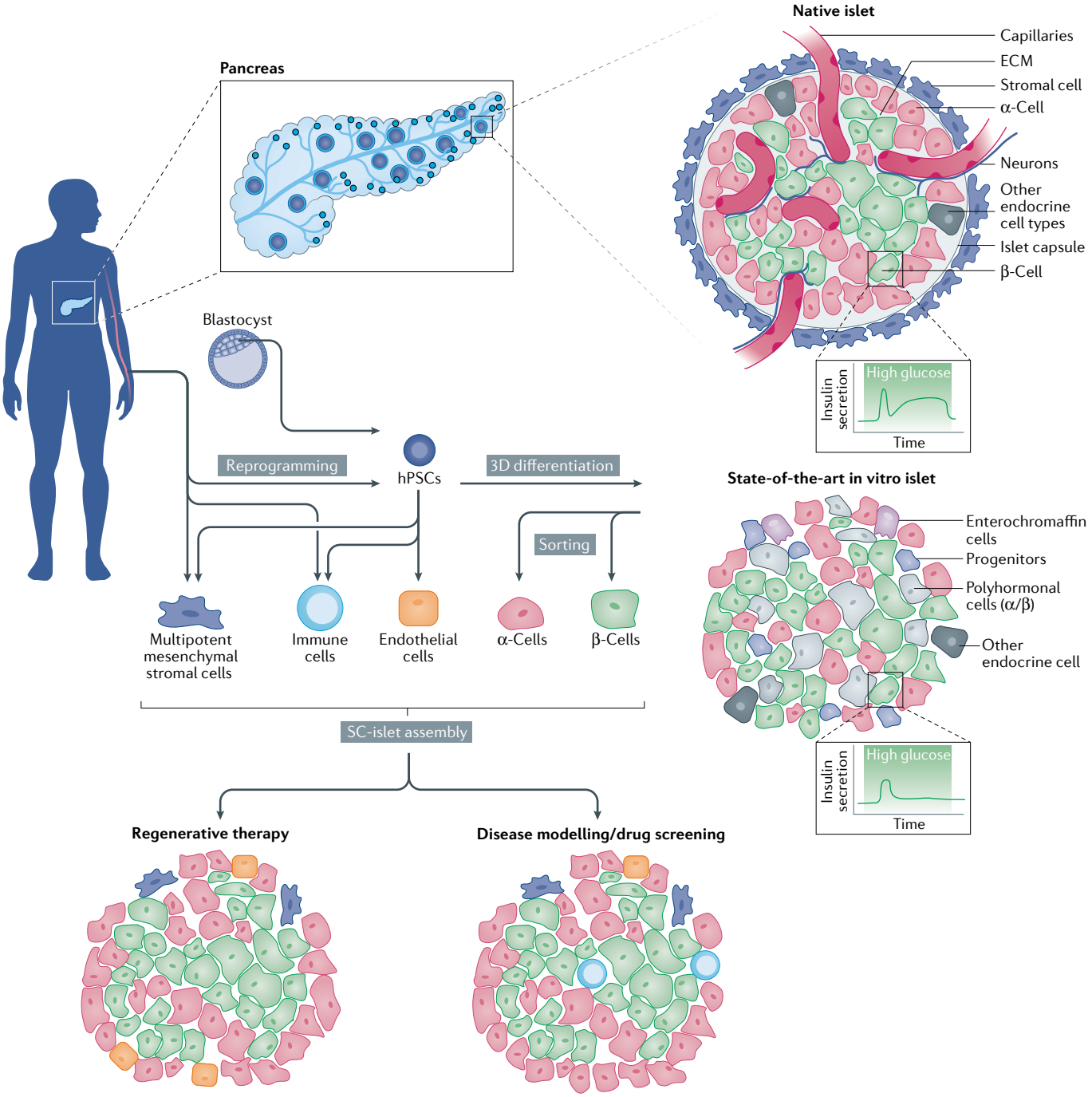
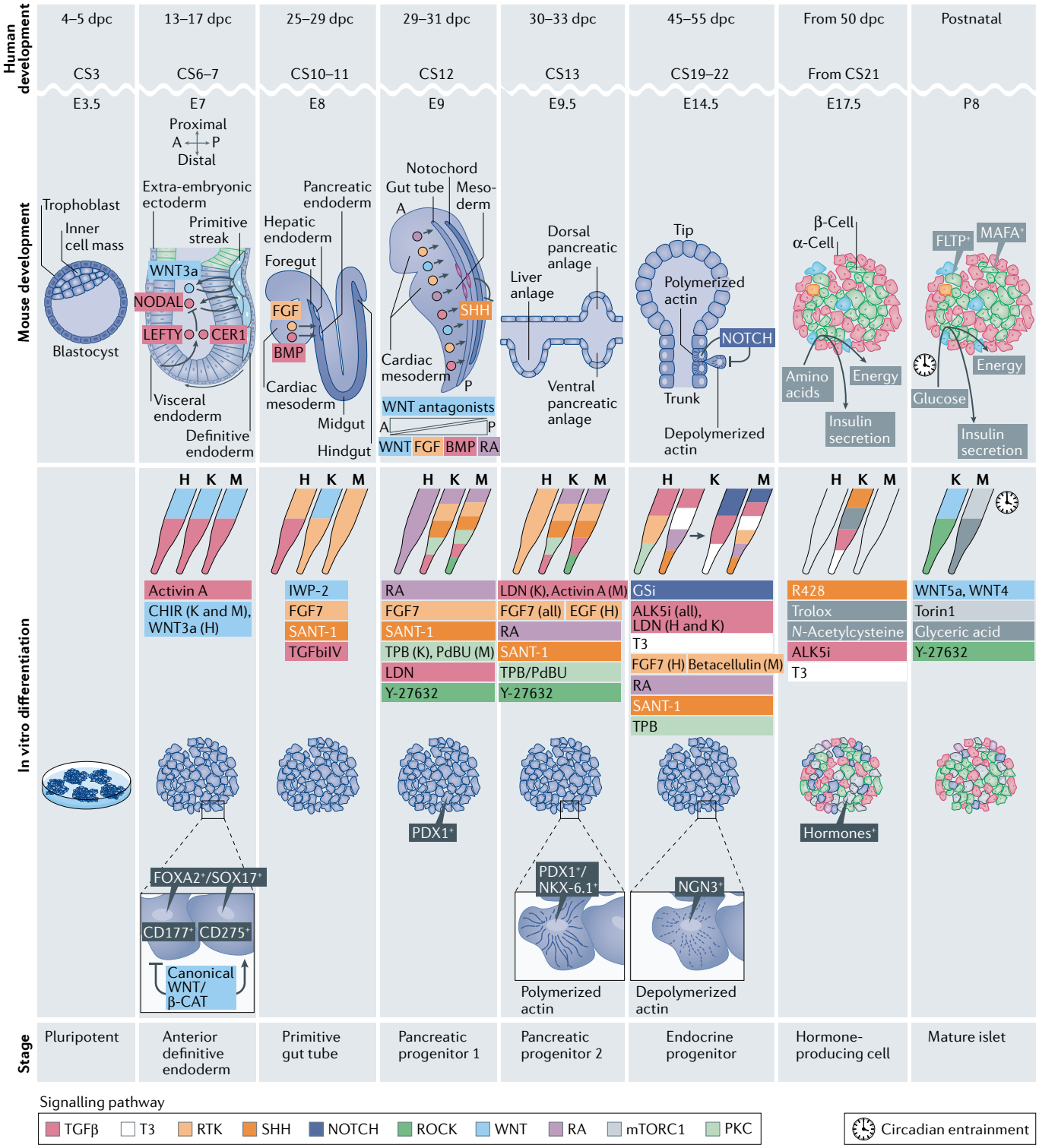


Fig. 1 | **Approaches to generate pancreatic human islets.** A native islet of Langerhans shown for comparison (top right). In addition to the various hormone-producing endocrine cell types, endogenous islets are innervated by neurons, fenestrated with capillaries, embedded within the extracellular matrix (ECM) and surrounded by an islet capsule[294]. Upon glucose stimulation, native β-cells switch on their tightly regulated insulin secretion machinery, and insulin release over time has characteristic dynamics. Existing stem cell-derived products are based on human pluripotent stem cells (hPSCs). In vitro differentiation generates functionally immature stem cell-derived β-like cells (SC-β-cells) and stem cell-derived α-like cells (SC-α-cells) with impaired secretory function. In addition, off-target cell types such as progenitor cells, polyhormonal cells and serotonin-producing enterochromaffin cells are present in islets derived from in vitro protocols[82]. Future stem cell-derived islet engineering approaches could design a mini-organ that closely mimics its native counterpart by integrating additional cell types. Complementing the in vitro derived SC-β-cells and SC-α-cells with other cell types could be useful for cell regenerative therapy, and including immune cells isolated from blood could further increase their relevance for drug screening and disease modelling. SC-islet, stem cell-derived islet-like cluster.



which organs such as the lung, liver and pancreas are derived. Activation of the transforming growth factor-β (TGFβ) and WNT signalling pathways triggers mesendoderm induction in vitro and is thought to recapitulate the events that occur during mouse gastrulation[23,24,36–38]. Currently, C–X–C chemokine receptor type 4 (CXCR4; a pan-endocrine marker) or the expression of the transcription factors Forkhead transcription factor (FOXA2; also known as hepatocyte nuclear factor 3β) and SOX17 is used to measure endoderm induction efficiency, which is usually >90%[30,31].

However, because the liver and pancreas are derived from specific organ progenitors shortly after gastrulation, it is important to identify and isolate distinct endoderm subpopulations using novel surface markers to select for cells of the pancreatic lineage. In this respect, expression of CD177 (also known as NB1) glycoprotein marks ADE cells that will differentiate down

◀ Fig. 2 | **Translation of the in vivo differentiation of islets of Langerhans to a dish.** Islets of Langerhans are derived from the endoderm germ layer that forms from the blastocyst during gastrulation at embryonic day 7 (E7) in mouse, which corresponds to roughly 13–17 days post coitus (dpc) in humans, and is then transformed into the primitive gut tube (E8). Pancreatic buds emerge at E9.5 from the posterior foregut region, specified by signalling factors from the surrounding tissues (E9). Endocrine progenitors leave the pancreatic epithelium in two waves, peaking during the second wave at E14.5. These progenitors form the islets of Langerhans (E17.5), which respond to glucose by postnatal day 8 (P8). Because little is known about human pancreas development, the in vitro differentiation process of human pluripotent stem cells (hPSCs) towards stem cell-derived α-like cells (SC-α-cells) and stem cell-derived β-like cells (SC-β-cells) is based on the events of mouse development. In vitro differentiation protocols aim to recapitulate many of the signals that occur during development, including those that activate the transforming growth factor-β (TGFβ), thyroid hormone triiodothyronine (T3), receptor tyrosine kinase (RTK), Sonic hedgehog (SHH), NOTCH, protein kinase C (PKC), WNT, retinoic acid (RA) and mechanistic target of rapamycin complex 1 (mTORC1) pathways, using small molecules and growth factors. Three commonly used protocols from the Hebrok laboratory (H)[45], Kieffer laboratory (K)[27] and Melton laboratory (M)[28] and corresponding small molecules are depicted. Corresponding signalling pathways involved in mouse development and targeted during in vitro differentiation are indicated with the same colours. Other metabolites or reducing agents that promote in vitro differentiation are shown in dark grey. E17.5 β-cells metabolize predominantly amino acids, which also trigger insulin secretion; glucose is the major energy substrate and provides the signals for insulin release in postnatal β-cells, which are considered functionally mature[163]. Functional maturation in vitro could be enhanced by targeting molecules involved in signalling pathways and metabolic processes to which postnatal islets are exposed as well as by circadian entrainment that mimics the feeding–fasting rhythms[164]. Cells in various stages of maturation can be identified with markers including Forkhead transcription factor (FOXA2; also known as hepatocyte nuclear factor 3β), SOX17, CD177 (also known as NB1), ICOS ligand (ICOSL; also known as CD275), pancreas/duodenum homeobox protein 1 (PDX1), NKX-6.1, neurogenin 3 (NGN3) and hormone secretion. A, anterior; BMP, bone morphogenic protein; CER1, cerberus; CS, Carnegie stage; EGF, epidermal growth factor; FGF, fibroblast growth factor; P, posterior; ROCK, Rho-associated protein kinase.

the pancreatic lineage, whereas ADE cells that express ICOS ligand (ICOSL; also known as CD275) differentiate towards the liver[31]. These populations are also segregated by non-canonical WNT signalling, which favours pancreatic fates, and canonical WNT signalling, which induces liver fates, similar to what is described in the mouse embryo[39] (FIG. 2). Hence, sorting for $CD177^+$ cells increases the efficiency of pancreatic differentiation and functional maturation of SC-β-cells[31].

In vivo, pancreas development continues as morphogenetic movements transform the naive endoderm germ layer into the primitive gut tube. An anterior–posterior gradient along the primitive gut tube is established and specifies regions including the posterior foregut region, which eventually gives rise to the pancreas[40,41]. Interactions with neighbouring cells pattern the posterior foregut and specify stomach, liver, pancreatic and intestinal fate via retinoic acid (RA), fibroblast growth factor (FGF), bone morphogenic protein (BMP) and Sonic hedgehog (SHH) signalling[42].

Those events are recapitulated in vitro by various combinations of growth factors that target the above-mentioned pathways to generate stem cell-derived pancreatic progenitors (SC-PPs) from stem cell-derived ADE cells. Removal of TGFβ agonists that are crucial for ADE formation is sufficient to induce differentiation towards the primitive gut tube fate, and FGFs are added to enhance the induction[24,26]. For differentiation towards posterior foregut and, subsequently, SC-PPs, differentiation protocols include RA[24,43,44] and RA alone is enough to induce the posterior foregut fate[45]. BMP inhibitors present in most of the differentiation protocols prevent differentiation towards intestine and hepatic progenitors[30,44,46]. Most differentiation protocols also include SHH inhibitors[24,27,28], as, in vivo, SHH blocks dorsal pancreatic bud differentiation[47]. However, SHH inhibition is dispensable for SC-PP induction in vitro as not all protocols rely on SHH inhibitors[45,48]. Addition of epidermal growth factor (EGF), nicotinamide[48] and activation of protein kinase C (PKC)[49] also improves the formation of SC-PPs.

To compare the efficiencies for generating SC-PPs in vitro, pancreatic progenitor markers such as pancreas/duodenum homeobox protein 1 (PDX1), which marks posterior foregut cells[50], are analysed. Later in murine development, pancreatic progenitors express homeobox protein NKX-6.1, which specifies cells that differentiate towards an endocrine fate and is required to establish and maintain β-cell identity[51]. NKX-6.1 is commonly used to identify in vitro generated SC-PPs[48,52]. The cell surface marker GP2 can identify differentiated PDX1/NKX-6.1 double-positive SC-PPs and is therefore used for sorting this population to enhance differentiation efficiencies[53,54]. Pancreas transcription factor 1 subunit-α (PTF1A)[55] and SOX9 (REF.[56]), which are restricted to the acinar and ductal lineages, respectively, and are downregulated during endocrine lineage acquisition, are also markers of SC-PPs.

Next, these SC-PPs differentiate into common endocrine progenitors, from which all hormone-producing cells are derived. This process, called endocrine induction, is orchestrated in mice and presumed to be orchestrated in humans by transient expression of the transcription factor neurogenin 3 (NGN3)[57,58]. NGN3 expression is linked to morphological changes during endocrine induction: endocrine specified cells delaminate from the pancreatic epithelium to form peninsular structures that are juxtaposed to the pancreatic epithelium, and these are the progenitors of the islets of Langerhans[59]. This requires F-actin depolymerization, which is associated with inhibition of YAP1 and YAP1-induced NOTCH[60]. Actin depolymerization is orchestrated by loss of the Rho-GTPase CDC42 (REF.[61]) and increased laminin content of the extracellular matrix (ECM), which reduces mechano-signalling via α5 integrin[60]. Reduced NOTCH signalling then induces NGN3 expression[62,63]. NGN3 is degraded following phosphorylation by cyclin-dependent kinase 2 (CDK2) and CDK4/6; therefore, lengthening of the cell cycle is required for its stabilization and transcriptional activity[64,65]. Stable expression of NGN3 upregulates cyclin-dependent kinase inhibitor 1 (also known as p21, encoded by *CDKN1A*), and cells exit the cell cycle[66].

To ensure efficient endocrine induction during in vitro differentiation, NOTCH1 is inhibited by γ-secretase inhibitors and TGFβ inhibitors[27,28,30,45,67]. Loss of cell polarity and actin polymerization is obtained either by culturing cells in three dimensions[27,28,45] or by pharmacologically targeting cytoskeletal components[68]. A chemical inhibitor of YAP was also shown to induce endocrine differentiation and reduce progenitor proliferation[69]. Common markers for hPSC-derived

Box 2 | **Features of mature β-cells — goals for SC-β-cells**

Recent differentiation protocols aim to generate stem cell-derived β-like cells (SC-β-cells) that mimic the features of human β-cells. The following features describe mature human β-cells, and should be used to evaluate SC-β-cells:

1. Insulin expression: the most commonly used β-cell marker is the hormone insulin, the main protein synthesized by β-cells. Alternatively, cells can be stained for C-peptide, which is cleaved from pro-insulin during insulin processing and is absent from the culture medium.
2. Expression of β-cell-specific transcription factors: β-cells express transcription factors that maintain their identity and regulate their function. These include PAX6 (REF.[304]), NEUROD[305], pancreas/duodenum homeobox protein 1 (PDX1)[306], ISLET1 (REF.[307]), NKX-6.1 (REF.[51]), MAFA[132], MAFB[308], SIX2 and SIX3 (REF.[309]).
3. Downregulation of so-called disallowed genes, including those that promote proliferation and anaerobic glycolysis (lactate dehydrogenase A and monocarboxylate transporter 1) or handle oxidative stress[176,310].
4. Insulin granules: the β-cell is characterized by insulin granules, which can be visualized by transmission electron microscopy imaging. The granules contain an electron-dense core, which is surrounded by a halo that separates the core from the granule membrane[203].
5. Insulin content: to evaluate the insulin secretion capacity of a cell, the insulin or C-peptide concentration of the cell lysate can be measured by enzyme-linked immunosorbent assay (ELISA) and normalized to the cell count.
6. Pro-insulin to insulin ratio: in a functional β-cell, pro-insulin is processed to biologically active insulin. If the cell is stressed or immature, pro-insulin is not converted and is present at higher levels than processed insulin[311].
7. Glucose-stimulated insulin secretion (GSIS): the hallmark of a mature β-cell is to secrete insulin upon glucose stimulation. Specifically, mature human β-cells secrete insulin when glucose is present at 3–4 mM or higher and show maximal insulin release upon stimulation with 15 mM glucose[216]. The response is biphasic, characterized by a first peak that results from a readily releasable pool of granules (first phase) and a following smaller peak during which a reserve pool of granules is first transported to the membrane (second phase)[206]. Instead of measuring released insulin, C-peptide, which is more stable than insulin and has an increased half-life after secretion in vivo, can be used as a surrogate marker for insulin[312].
8. Calcium flux: the response to glucose can also be evaluated by measuring the calcium level in the cytoplasm with imaging approaches. Glucose causes calcium influx into the β-cell, leading to the release of insulin granules[206].
9. Oxygen consumption rate: functional β-cells are characterized by an increased rate of mitochondrial oxidative phosphorylation[130]. Dynamic and static mitochondrial respiratory function after glucose treatment can be measured by a Seahorse XF assay[121,197].

endocrine progenitors are chromogranin A (CHGA), NKX-2.2 and neurogenic differentiation factor 1 (NEUROD1), which are permanently expressed after endocrine induction by NGN3 (REFS[70,71]).

SC-β-cell specification and expansion are driven by additional factors identified in mouse studies, such as RA and TGFβ inhibitors. In vivo, RA is required for the specification of β-cells from endocrine progenitors because it regulates expression of WNT pathway components, in fact it inhibits canonical WNT signalling[72,73]. Expression of adenomatous polyposis coli protein (APC), a negative regulator of WNT signalling, distinguishes endocrine from progenitor cells and drives endocrine specification in murine development as well as during in vitro differentiation[74]. TGFβ inhibitors also specify SC-β-cells in vitro[27–30]. In vivo, expression of insulin and genes involved in β-cell function are repressed by TGFβ signalling via SMAD3 (REF.[75]). TGFβ inhibition drives β-cell expansion in mice and humans by repressing expression from the *INK4a/ARF* locus, thus stimulating proliferation[76]. Differentiation media can also include betacellulin, a member of the EGF family[28,29]; in vivo, betacellulin increases expression of PDX1 and insulin[77]. Furthermore, betacellulin promotes endocrine specification by inhibiting atypical PKC (aPKC), thus modifying apicobasal polarity and inhibiting NOTCH1 (REF.[78]). In addition to chemical cues, air–liquid interface culture may promote β-cell specification[27]. Exposure to atmospheric oxygen levels downregulates hypoxia-inducible factor 1α (HIF1α), which is associated with β-cell differentiation[79].

Early specified β-cells are marked in mouse and human by transient expression of PAX4 (REFS[80–82]). Terminally specified SC-β-cells are identified by expression of insulin and C-peptide, a linker peptide that is cleaved in a 1:1 ratio from insulin during its maturation, in combination with β-cell-specific transcription factors such as NKX-6.1 and PDX1 (REFS[48,51,52,83,84]) (BOX 2). Recent protocols produce a heterogeneous cell population, of which approximately 40–60% are C-peptide/NKX-6.1 double-positive cells[27–31,85].

Engineering SC-α-cells

The SC-β-cell differentiation protocols described in the previous section also generate SC-α-cells[82]. In vivo, α-cells produce glucagon during fasting to maintain blood glucose levels by stimulating hepatic glucose production. SC-α-cell progenitors express ARX[86,87], and SC-α-cells express glucagon and α-cell-specific transcription factors such as IRX1, IRX2 (REFS[88,89]) and prohormone convertase 2 (also known as PC2), which is not expressed in β-cells[90].

In vitro differentiation protocols also generate cells that simultaneously express insulin and glucagon. These cells were previously considered immature β-cells[27,45,48,91], but recent studies argue that they are SC-α-cell precursors[74,82]. Similarly, insulin/glucagon double-positive cells were observed from 8 to 21 weeks of fetal age during human pancreas development in vivo[92,93]. The insulin/glucagon double-positive cells expressed α-cell-specific transcription factors such as ARX and lacked β-cell-specific transcription factors, such as PDX1 and NKX-6.1 (REF.[92]). In vitro, insulin/glucagon polyhormonal cells could arise from wrongly timed endocrine induction (before the specification of NKX-6.1$^+$ SC-PPs)[48,52,94–96]. Accordingly, early induction of NGN3 in mice greatly increased the formation of α-cells[97]. Addition of vitamin C to the medium during the early in vitro differentiation stages inhibited NGN3 expression and prevented premature endocrine induction, and could therefore reduce the generation of polyhormonal cells[27]. Similarly, exclusion of BMP inhibitors from the medium for SC-PP specification[45] or shortening the posterior foregut specification stage[48] prevented premature endocrine induction.

Few studies have focused on the enrichment and functional maturation of SC-α-cells. SC-α-cells can be generated from endocrine progenitors by removing noggin from the culture medium, which allows for upregulation of TGFβ signalling, as noggin inhibits BMP[94]. In vivo, TGFβ signalling induced by BMP4 promotes α-cell formation[98]. Furthermore, insulin/glucagon

double-positive cells can be converted to mature SC-α-cells by activation of PKC[99]. SC-α-cells generated with this protocol show glucagon secretion profiles similar to those of endogenous human α-cells (that is, glucagon is secreted when glucose concentrations are ≤7 mM). When transplanted into mice, the animals had reduced fasting-induced hypoglycaemia and increased average blood glucose levels, suggesting that they secreted glucagon[99].

Glucagon secretion from α-cells is dependent on PKC[100], but there is no known role for PKC in α-cell specification in vivo. Novel α-cell and β-cell human iPS cell reporter lines will further clarify the signals and factors that orchestrate fate specification and segregation and will improve current differentiation protocols[101–103]. In addition, a recently generated glucagon knockout mouse model will enable in vivo studies of SC α-cell regulation and function[104].

Limitations of SC-derived islet cells

Studies on human pancreas development are limited, so the in vitro differentiation protocols for SC-α-cells and SC-β-cells are predominantly based on findings from mouse models. However, murine and human development of pancreas and islets differ[105], as illustrated by differences in islet architecture[106] and gene expression[89,107]. Of particular relevance to in vitro differentiation protocols, human endocrine cells appear only during one phase of development and β-cells emerge first[83,108], whereas endocrine induction in mice is biphasic[109] and starts with α-cell formation[97]. Furthermore, expression of differentiation markers such as PDX1, GATA4 and NKX-2.2 occurs later in human development than in mouse development[83]. Most importantly, the generation of endocrine cells during murine development and using in vitro differentiation protocols takes approximately 2 weeks, whereas the process is completed in humans 18 weeks after conception[58]. Those evolutionary and developmental differences currently impede our capacity to understand pancreas and endocrine development in vivo to improve SC-α-cell and β-cell differentiation protocols in vitro[105].

There are three major concerns with current protocols discussed in detail in the following paragraphs. First, the differentiated population is heterogeneous, and the presence of unintended cells could impair the overall function of the transplant. Second, the reproducibility of protocols is low for different hPSC lines, suggesting that there are cell-intrinsic differences that have not been accounted for and that could compromise the utility of the cells. Third, the cells generated incompletely recapitulate the differentiation and maturation status of adult human β-cells. Strategies are being developed to further characterize and address these concerns, as discussed in the next section.

Recent single-cell RNA-sequencing studies characterized the heterogeneous mixture of cells generated with SC-islet cell differentiation protocols, identified cell types and tracked their lineages during the differentiation[67,82]. Three major endocrine populations were identified: SC-α-cells, SC-β-cells and cells resembling enterochromaffin cells (SC-EC-cells).

Detection of serotonin-producing enterochromaffin cells is surprising because in vivo they reside in the intestine. If a cell product is transplanted into a patient with diabetes, the presence of SC-EC-cells is concerning because serotonin influences digestion and other functions[110]. SC-EC-cells presumably arise in vitro during endocrine induction from primitive gut tube progenitors that were specified for intestinal instead of pancreatic fate[44,111]. Accordingly, the ratio of SC-EC-cells to pancreatic endocrine cells varies when the signalling factors for the primitive gut tube and the pancreatic progenitor specification stage are modified[82]. However, SC-β-cells and SC-EC-cells seem to emerge from a common $NGN3^+$ intermediate[67,82]. Further studies are required to refine protocols so that they more precisely specify pancreatic, rather than intestinal, progenitors.

Cultures differentiated using current protocols also include non-endocrine cells, which eventually differentiate into other pancreatic lineages such as acinar-like, ductal-like and mesenchymal-like cells[67,82]. Pancreatic progenitors are proliferative compared with differentiated endocrine cells, which have exited the cell cycle, increasing the risk of uncontrolled proliferation upon transplantation.

Different hPSC lines behave differently during differentiation into SC-β-cells. For example, the hESC line HUES8 differentiates more efficiently into pancreatic cells upon spontaneous and induced differentiation than the HUES6 line[112]. Similarly, generation of SC-PPs can vary from approximately 0 to 80% between different human iPS cell and hESC lines[44]. The differentiation potential of 12 nuclear transfer hESC lines ranged from 8 to 75%, as measured by C-peptide-positive cells[113]. These variations are thought to result from differences in gene expression and DNA methylation profiles[114,115], which predict the differentiation success of individual hPSC lines[116–118]. Recent protocols efficiently induce differentiation in multiple lines, but still require time-intensive optimizations, such as strict control of the seeding density[29,68].

Sorting for cell types of interest and reassembling them into SC-islets reduces the presence of unwanted cell types in the final differentiated population and reduces the problems caused by variable differentiation efficiencies. Simply dissociating and reaggregating cells after the endocrine induction step enriches for endocrine cells, for example[82]. However, SC-EC-cells and a small fraction of proliferative non-endocrine cells still remain.

Genetically engineered reporter hPSC lines can help sort for SC-α-cells and SC-β-cells. These cells express either a fluorescent marker protein under a cell type-specific promoter or a cell type-specific protein fused to a fluorescent protein. Fluorescent cells are then sorted by flow cytometry. Recently, reporter hPSC lines were generated using CRISPR–Cas9 genome engineering to insert the transgene at a target site and express the protein at endogenous levels[119]. Existing reporter lines for SC-β-cells include the heterozygous $INS^{GFP/w}$ hESC line, in which one insulin allele is replaced by GFP[120,121]. Loss of one insulin allele is suboptimal, but newer insulin human iPS cell reporter lines avoid this

problem[102,103]. For pre-α-cell and α-cell analysis, we recently generated a homozygous human iPS cell line in which a nuclear-localized histone 2B-cyan fluorescent protein (nCFP) is fused to ARX[101]. These genetically engineered lines are useful tools for research purposes, but their use in therapeutic approaches is limited by the risk of chromosomal abnormalities or mutations.

To circumvent using genetically modified human iPS cell or hESC lines, SC-α-cells and SC-β-cells can be identified with cell type-specific surface markers. The markers can be targeted on living cells with specific fluorophore-labelled antibodies and, subsequently, sorted by flow cytometry or magnetic cell sorting. SC-β-cells express α1 integrin (CD49a). Magnetic sorting for CD49a and reaggregation of the sorted cells generated aggregates with up to 80% SC-β-cells[82,122]. To sort SC-α-cells, the CD49a$^-$ population was sorted for dipeptidyl peptidase 4 (DPP4; also known as CD26), resulting in a population of 50% SC-α-cells[19,122]. In addition, CD9 was used as a cell surface marker for negative selection of glucose-responsive SC-β-cells (which express the transcription factor MAFA, NKX-6.1 and C-peptide)[123]. Additional cell surface markers have been identified for human endocrine cells[124,125], such as the human β-cell marker ectonucleoside triphosphate diphosphohydrolase 3 (NTPDase-3). However, surface markers that identify human adult β-cells might not be suitable to identify immature SC-β-cells.

Future directions: SC-islet protocols

Current research focuses on the generation of SC-β-cells by following stepwise in vitro differentiation protocols that mimic mouse development. To engineer SC-islets, the most relevant cell types for glucose control — SC-α-cells and SC-β-cells — should be included. Both cell types can be generated simultaneously with current in vitro differentiation protocols, reducing the complexity of manufacturing SC-islets. An ideal differentiation protocol would generate aggregates that are highly enriched for SC-α-cells and SC-β-cells and are shaped like islets. However, current protocols also produce substantial numbers of other endocrine cell types, particularly SC-EC-cells or proliferative SC-PPs. Therefore, sorting SC-α-cells and SC-β-cells will likely generate a better biomimetic islet. The sorted cells can be reassembled into SC-islets either through spontaneous aggregation in suspension culture or by culturing them in micromoulds to generate aggregates with defined cell type composition, size and cell numbers[126,127]. However, as many cells are lost during sorting and reaggregation, this approach might not be suitable for applications that require large-scale production, and thus, current differentiation protocols should be improved to reduce the presence of unwanted cell types.

Maturation of SC-islets

In vitro generated SC-islets composed of SC-β-cells and SC-α-cells form the core insulin-producing unit for transplantation and research purposes. However, in vitro generated SC-β-cells are functionally immature, as indicated by impaired glucose-stimulated insulin secretion (GSIS), so their therapeutic applications are limited. Although SC-β-cells have the capacity to mature in an in vivo environment after transplantation, this process takes several weeks and therefore comes with a delay in functionality[25,26–28,52,95,128,129]. In addition, assays for research purposes often require in vitro functionality of SC-β-cells and introducing an in vivo maturation step is not always feasible. This section gives an overview of the cues that can induce functional maturation of SC-β-cells, the resulting implications for in vitro differentiation protocol modifications, and SC-islet assembly.

In addition to the capacity for GSIS, the phenotype of a functionally mature adult β-cell (BOX 2) includes a switch from glycolysis to oxidative phosphorylation[130] and expression of urocortin 3 (UCN3) and MAFA[131,132]. However, recent studies show inter-species differences in their expression profiles, which questions the usefulness of these markers. In particular, UCN3 is expressed in both β-cells and α-cells in humans[89,133] and levels of MAFA were high in adult, but not juvenile, β-cells[134].

In vivo, islets functionally mature postnatally. Immediately after birth, β-cells of murine pups have impaired GSIS because the glucose threshold for insulin secretion is lower, so these cells are considered immature[131,135]. Similarly, human β-cells of newborns show a weak response to glucose, but increased insulin secretion upon amino acid stimulation[136,137]. SC-β-cells functionally resemble fetal β-cells[131] and, accordingly, share similar gene expression profiles[138], impaired GSIS and lower glucose thresholds, so these cells secrete high levels of insulin even under unstimulated conditions[27,28].

Recent approaches have substantially advanced the capacity to induce the functional maturation of SC-β-cells. Transplanting SC-β-cells into mice can reliably and efficiently trigger functional maturation. Alternatively, functional maturation can be induced in vitro by recapitulating the pancreatic niche and modelling postnatal β-cell maturation. Those approaches generated SC-β-cells with GSIS profiles that are similar to human islets and should therefore be considered when engineering SC-islets[29,68,85].

Transplantation and in vivo maturation

Functionally impaired SC-β-cells mature after transplantation into functional β-cells, which can reverse diabetes in mice within 40 days after transplantation[27,28,128]. Even SC-PPs will differentiate into functional β-cells after transplantation, although this process takes up to 4 months in murine models[25,26,52,95]. However, the timing and efficiency of this in vivo maturation in humans is currently unclear.

In vivo maturation is accompanied by changes in energy metabolism[139] and redox balance[129,139], mediated by HNF1A and HNF4A[139,140]. Furthermore, increased signalling through the FOS–JUN pathway was observed in in vivo matured SC-β-cells[129]. The upstream factors of this in vivo effect most likely come from the transplantation niche, for example through vascularization[141], but remain largely unknown. Therefore, one research approach to mature SC-islets is to closely mimic the in vivo microenvironment.

Recapitulating the pancreatic niche

The endogenous pancreatic islets are embedded within a multicellular environment built up by non-endocrine cells including mesenchymal cells, neural cells, immune cells, endothelial cells and the ECM components as well as paracrine factors, which these cells produce[142].

Multipotent mesenchymal stromal cells. Integrating factors with known roles in pancreas development into the directed differentiation protocols can greatly improve the in vitro development of SC-β-cells. For example, treating SC-β-cells with the glycoprotein SLIT3, which is naturally present in the human pancreatic mesenchyme, resulted in a higher fraction of cells that express C-peptide and NKX-6.1 as well as increased GSIS[142].

Similarly, co-culturing human multipotent mesenchymal stromal cells (MSCs) isolated from bone marrow, skeletal muscle, adipose tissue or umbilical cord with mouse and human-derived primary islets improved insulin secretion in vitro and prolonged normoglycaemia in mice after transplantation in vivo[143–145]. In addition, MSCs could reverse β-cell dedifferentiation associated with T2D diabetes and improve glycaemic control in two distinct mouse models[146]. MSC co-culture improves islet function through direct contact, which is mediated via N-cadherins[144] and has been associated with mitochondria transfer[143]. Furthermore, the beneficial effect of the co-culture could be attributed to ECM components secreted by MSCs[145]; pre-co-culturing islets with MSCs was sufficient to improve islet functionality in vitro[147].

Neural cells. During in vivo development, signals from the neural crest regulate the β-cell mass via a non-cell-autonomous feedback loop between the neural progenitor and pancreatic endocrine transcription factors paired mesoderm homeobox protein 2B (PHOX2B) and NKX-2.2, respectively[148].

Co-transplantation of neural crest stem cells and murine pancreatic islets improved insulin release from the transplant in normoglycaemic mice and β-cells seemed to be more proliferative[149]. Transplanted mouse islets that were surface coated with neural crest stem cells showed a better response after glucose challenge and improved graft innervation as well as vascularization in diabetic mice. Neural crest stem cells on the surface partially migrated into the islet tissues and terminally differentiated into glial and neural cells[150].

Endothelial cells. Endothelial cells, derived via directed differentiation from hPSCs[151,152] or from primary material such as human umbilical vein endothelial cells, could further complement SC-islets. Stem cell-derived human endothelial cells and pericytes self-assemble into capillary networks[152]. Including such a capillary network in SC-islets would therefore allow for vascularization of the transplant and prevent hypoxia-induced cell death.

In addition to improving oxygenation, paracrine factors secreted from endothelial cells have a beneficial effect on β-cell functionality. The presence of endothelial cells during pancreatic differentiation of hPSCs enhanced the expression of SC-β-cell maturation markers, such as UCN3, and increased the insulin secretion capacity in vitro via activation of BMP signalling[153]. Furthermore, SC-PPs differentiated towards endocrine cells when they were cultured in close proximity to endothelial cells in vitro without added differentiation-promoting chemical cues[154]. Upon transplantation into diabetic mice, SC-β-cells co-cultured with endothelial cells had increased secretion of C-peptide and significantly reduced hyperglycaemia[153]. Co-culturing the human β-cell line EndoC-βH3 and human umbilical vein endothelial cells in a two-layered pseudo-islet suggested that co-culture with endothelial cells promotes GSIS[155]. In addition, co-culture of rat islets with endothelium-conditioned medium improved GSIS and insulin content within the islets via upregulation of glucokinase and the mitochondrial glycerol-3-phosphate dehydrogenase 2 (encoded by *GPD2*), most likely because of laminins produced by the endothelial cells[156].

Extracellular matrix. The ECM could also contribute to β-cell differentiation and function. Adding decellularized ECM obtained from rat β-cells to hESC cultures that were differentiating into SC-PPs increased SC-β-cell functionality, as demonstrated by improved GSIS and restoration of normoglycaemia when transplanted into mice with diabetes induced by streptozotocin treatment[157]. Studies combining human iPS cells and decellularized rat pancreatic ECM identified that collagen V regulates endocrine lineage commitment and improves insulin and glucagon secretion after glucose challenge[158]. In addition, the decellularized rat pancreatic ECM promoted self-assembly of differentiating human iPS cells into structures resembling native human islets[158]. However, further studies need to elucidate which chemical and mechanical properties of the ECM contribute to improved SC-β-cell commitment and would, hence, be beneficial for SC-islets.

Future directions: islet niche. Taken together, these studies demonstrate that non-endocrine cell types that occur naturally in the islet niche likely impact endocrine differentiation and function. To generate more physiological SC-islets, the minimal functional complexity of the multicellular SC-islet that can recapitulate endogenous human islet function needs to be defined. These other cell types can be primary cells or cells generated by hPSC differentiation. However, generation of a defined SC-islet requires standardized differentiation protocols for every cell type; the fewer the better, to have the least complicated production for therapeutic purposes.

Mimicking postnatal β-cell maturation

To induce expression of the β-cell maturation marker MAFA, protocols for SC-β-cell differentiation in vitro use the thyroid hormone triiodothyronine (T3), the reducing agents vitamin E and *N*-acetylcysteine as well as an inhibitor of the tyrosine kinase receptor AXL[27,28]. In vivo, treatment with growth arrest-specific protein 6 (GAS6), an AXL agonist, decreased MAFA expression and impaired GSIS[159]. Reduction of oxidative stress via overexpression of glutathione peroxidase preserved nuclear MAFA in a mouse model[160]. T3 induced

MAFA expression in rats, leading to enhanced insulin expression and β-cell function[161]. However, it was also suggested that removing both T3 and TGFβ inhibitors during the final step of the protocol promotes β-cell maturation[29]. SC-β-cells generated with this protocol are characterized by GSIS with a biphasic response to glucose (which is characteristic of human primary islets), indicating their advanced maturation status. Both T3 and TGFβ inhibitors are present in the maturation stages of other differentiation protocols that produce cells with similar GSIS profiles[31,85] and underlying molecular mechanisms need to be identified.

In vivo, the metabolic environment of the β-cell drastically changes after birth, as β-cells are exposed to intermittent feeding–fasting cycles and nutritional input gradually changes from predominantly amino acids to glucose[137,162,163]. These changes contribute to the functional maturation of β-cells and can be mimicked in vitro to promote the maturation of SC-β-cells. Accordingly, exposing SC-β-cells to circadian fasting and feeding rhythms[164] and reducing the concentration of amino acids in the culture medium of the final differentiation stage[29,163] improved GSIS.

Mechanistic target of rapamycin complex 1 (mTORC1) is the master regulator of the response to metabolites, and thus provides a link between the nutritional environment and maturation status of β-cells. Mouse studies revealed that, after birth, mTORC1 is activated and promotes β-cell maturity by upregulating maturation markers, such as PAX6, UCN3, GLUT2 and MAFA[165,166], and by increasing the insulin content of β-cells[167,168]. mTORC1 is also required for postnatal β-cell mass expansion by increasing the β-cell size[166,167,169], decreasing β-cell apoptosis[166,167] and boosting β-cell proliferation[167,169].

However, sustained hyperactivation of mTORC1 in older mice is associated with β-cell apoptosis[169,170] and loss of maturity[171] due to endoplasmic reticulum stress, inhibition of autophagy[170] and downregulated IRS–AKT–FOXO1 signalling[169]. The change in mTORC1 activity after birth has been linked to changes in nutrition[163]. The mTORC1 signalling pathway in fetal β-cells was sensitive to amino acids, but not to glucose, and was therefore constitutively active as fetal β-cells are constantly exposed to high levels of amino acids. In adult β-cells, mTORC1 activity was downregulated and dynamically activated by glucose. SC-β-cells were similar to fetal β-cells and showed high basal activity of mTORC1. Consequently, inhibiting mTORC1 in SC-β-cells could enhance GSIS[163].

Secretion of insulin relies on increasing the ATP to ADP ratio, meaning that oxidative phosphorylation is crucial for GSIS, which can be further potentiated with metabolites from the tricarboxylic acid (TCA) cycle[172]. In mature β-cells, a high proportion of glucose that enters glycolysis proceeds to the TCA cycle[173]. Postnatal β-cells are less reliant on oxidative phosphorylation. Accordingly, comparison of the proteome[174] and the transcriptome[130,175] of young and adult mouse β-cells revealed an age-dependent increase in metabolic enzymes involved in ATP biosynthesis and oxidative phosphorylation. By contrast, the genes encoding lactate dehydrogenase A and monocarboxylate transporter 1, enzymes involved in anaerobic glycolysis that, in other tissues, are considered housekeeping genes, are downregulated during postnatal functional maturation[176]. This ensures effective oxidation of glucose and prevents exercise-induced hyperinsulinaemia.

It was suggested that the enhanced oxidative phosphorylation that leads to metabolic maturation of β-cells could be coordinated by the orphan nuclear receptor oestrogen-related receptor-γ (ERRγ)[130]. mTORC1 activation also enables the postnatal increase in oxidative phosphorylation by promoting mitochondrial biogenesis[168]. With the transition from maternal milk to a carbohydrate-rich diet during weaning, 5′-AMP-dependent kinase (AMPK) replaces mTORC1 as the metabolic master regulator, which also promotes oxidative phosphorylation[171]. Further studies confirmed the link between weaning and the switch to oxidative phosphorylation[177] and showed that this switch is accompanied by changes in islet microRNA levels[178].

SC-β-cells show impaired oxidative phosphorylation, which limits their maturation status[121]. Hence, increased mitochondrial oxidative phosphorylation by overexpression of ERRγ improved the GSIS of SC-β-cells[130]. Furthermore, genetic disruption of SIX2, a positive regulator of mitochondrial respiration and calcium signalling, impaired the functionality of SC-β-cells[179]. The impaired GSIS of SC-β-cells is associated with reduced metabolic flux through the mitochondrial TCA cycle. In particular, these cells had a bottleneck in glycolysis, which could be bypassed by treating the cells with cell-permeable intermediates to increase the function of SC-β-cells[180].

To boost oxidative phosphorylation in SC-β-cell differentiation protocols, upstream mechanisms and pathways need to be identified. Studies in primary mouse and human islets as well as SC-islets showed that acquisition of 3D architecture and activation of non-canonical WNT signalling promotes oxidative phosphorylation[85,181]. In particular, WNT–planar cell polarity signalling via JUN induces β-cell specification[182]. Furthermore, WNT4 enhanced the metabolic maturation of SC-β-cells by inducing ERRγ[85]. In line with this finding, the WNT–planar cell polarity reporter flattop marks a mature β-cell population[181,183]. Furthermore, a chemical screen identified that a Rho-associated protein kinase 2 (ROCK2) inhibitor promotes SC-β-cell maturation[184]. In vivo, ROCK signalling in β-cells is associated with impaired maturation status; ROCK inhibits GSIS through actin polymerization and inhibition of RhoA[185]. Although another study could not confirm the involvement of ROCK2 inhibitors in SC-β-cell maturation[67], the majority of studies indicate that 3D architecture induces metabolic maturation[183].

Future directions: SC-islet maturation

In conclusion, SC-β-cells resemble fetal β-cells in their maturation status and thus have impaired functionality, but have the potential to mature after transplantation in vivo. Current SC-β-cell differentiation protocols aim at recapitulating this maturation entirely in vitro by refining the final differentiation steps. An attractive

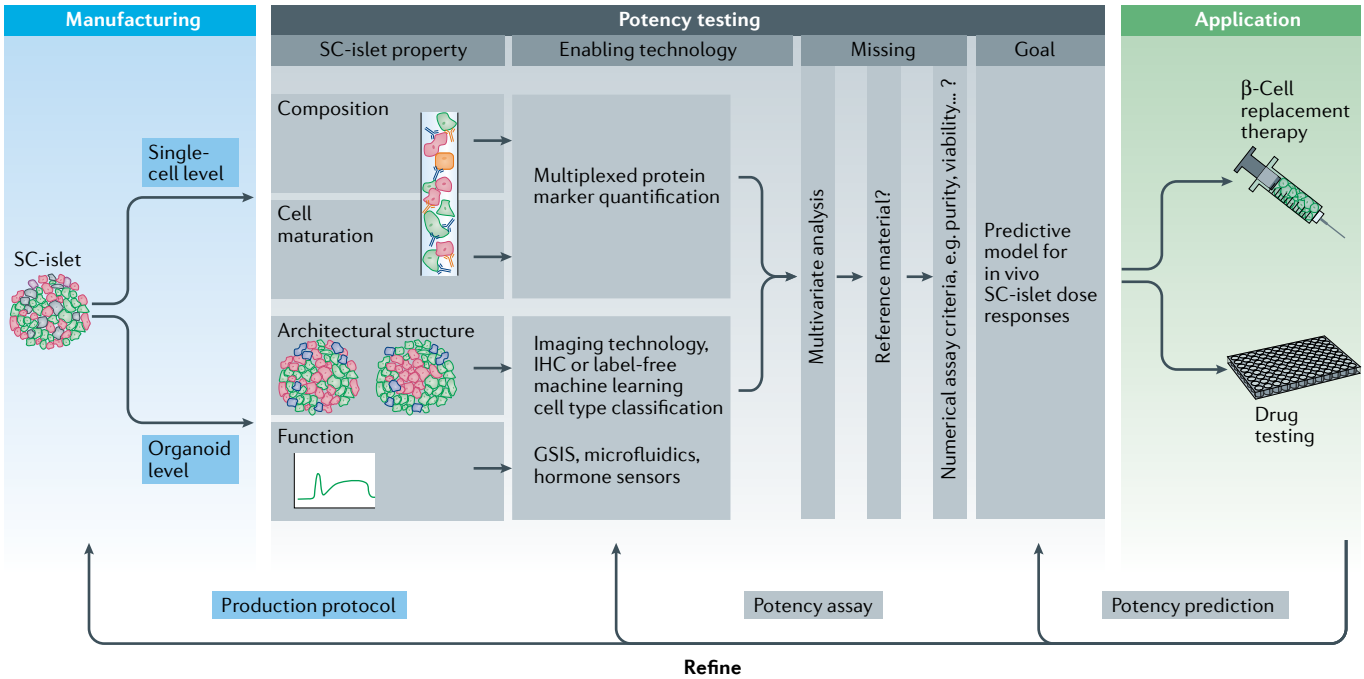


Fig. 3 | **Potency assay for SC-islets to predict function after transplantation.** Stem cell-derived islet-like clusters (SC-islets) must be subjected to a series of potency assays to ensure cell type composition, viability, structure and function before they could be used for transplantation. For this, current islet potency assays must be translated to the single SC-β-cells and whole SC-islet levels or new sets of these assays need to be developed. A central future research question will be to define iterative standards, metrics for tests, the components of multivariate analysis and criteria to score SC-islet potency together with clinical trial data. When designed in a scalable fashion, the potency assay will be concomitantly applicable for quality control during the manufacturing processes and/or drug screening applications. GSIS, glucose-stimulated insulin secretion; IHC, immunohistochemistry.

approach could be to recreate the pancreatic β-cell niche, which is crucial for maturation of endogenous β-cells, by, for example, including cell types such as endothelial cells into a SC-islet or supplying factors secreted by cells of the pancreatic niche. Improving functional maturation in vitro can furthermore be tackled by mimicking the postnatal in vivo maturation of β-cells governed by metabolic and morphological factors. Hence, modifying the 3D architecture of SC-islets or available nutrients in the culture medium could be promising approaches to enhance the SC-β-cell maturation status.

Potency analysis of SC-islets

Replacement therapy with SC-islets will require a new set of potency tests to ensure manufacturing quality and efficacy of the cell product after transplantation (FIG. 3). In analogy to the potency of a drug compound, the potency of a cell product can be defined as the therapeutic activity of the cells as indicated by appropriate in vitro and/or in vivo assays, including clinical data. The first set of potency assays for islets has been previously defined for donor material used in transplantation therapies[186]. Procedures and protocols implemented for human donor islets are described under the Clinical Islet Transplantation protocol CIT-7 (REF.[187]) and further FDA regulations including good clinical practice guidelines[188,189]. However, the high variability of donor material and graft failure have made it difficult to establish quantitative correlations between in vitro characterization data of the starting material and the capacity of transplanted islets to provide glucose homeostasis in humans[190]. Animal studies are of limited use because the time frame of potency testing is a few weeks or a month. Furthermore, the mouse — the main host for preclinical islet studies — is too metabolically distant from humans to predict efficacy[106,191]. Therefore, new in vitro potency assays are urgently needed for SC-derived islets to assess islet cell type composition, maturation and structure, and islet function[190]. Potency assays for traditional cell characterization parameters could be based on protocols established for donor islets, but assays with criteria for maturation state of the cells, in particular, structure and function, need to be established. These new assays need to be reproducible and specific, and need to be performed within a few hours with single islets or even with single-cell-level resolution.

Viability, composition and structure

The reference method to evaluate islet viability is to stain disaggregated islet cells with fluorescein diacetate, propidium iodide or ethidium bromide to identify dead cells[192]. This method is usually performed by islet isolation centres to characterize and validate islet preparations before transplantation and could be applied in the same way to SC-islets[193]. The difference from donor material validation is that cell viability is a somewhat secondary factor because cells come from a defined manufacturing process and not from a patient. The metabolic

state can also be used to validate cell viability. Indeed, mitochondrial activity[194], ADP to ATP ratios[195] and the presence of reactive oxygen species[196] have all been linked to insulin secretion function of β-cells. Static and glucose-stimulated oxygen consumption rates of donor islets have previously been used to predict the transplantation efficacy[197] (BOX 2). Other secreted biomarkers, including microRNAs[198] or apoptosis markers[199], which are used for donor islets, are less useful for SC-islets because these biomarkers are released during the harsh human islet preparation procedure. Staining of whole-mount islets with chelating zinc dyes such as dithiozone is used to quantify the β-cell content[193]; zinc ions are present at up to millimolar concentrations in insulin granules, which are the storage organelles for insulin in β-cells. Concomitantly, from cross-sectional light microscopy images of dithiozone-stained islets, the fraction of islets (by volume) is determined, with the assumption that islets exhibit a spheroidal shape. By convention, one islet equivalence (IEQ) is defined as the volume of spheroidal islets with a diameter of 150 µm. IEQ parameters are central to establishing a dose–response correlation between the number of SC IEQs and the in vivo contribution of these islets to regulating glucose homeostasis. Particle light-scattering techniques with low sample consumption rates are an attractive rapid alternative method to determine IEQs[200].

For determining the cell type composition of SC-islets, cell marker profiles are measured on the single-cell level. Immunohistology and fluorescence-activated cell sorting methods are widely used and could be readily applied to clinical workflows. The expression of insulin and glucagon identifies β-cells and α-cells, respectively, and transcription factors can also be used. Quantitative approaches for probing the α-cell and β-cell content of intact SC-islet clusters are possible with cell type-specific fluorescent dyes and imaging. To further distinguish between mature and immature SC-β-cells, the ratio of pro-insulin to insulin can be assessed (BOX 2). Maturation markers for α-cells and β-cells can also serve as targets for potency testing. Broadly available imaging techniques such as light microscopy can be utilized to classify SC-β-cells. For more detailed analysis, state-of-the-art multiplexed cell markers could be used and protocols could be modified as more cell subtypes are identified. Unfortunately, there are currently no unique cell surface markers with corresponding analytical antibodies for β-cells and α-cells; however, cell type-specific fluorescent dyes are being developed[201,202].

Imaging techniques are used to characterize structural SC-islet properties. The insulin granules exhibit a high electron density within β-cells, and thus can be visualized by transmission electron microscopy imaging[203]. The scattering electron density maps from electron microscopy can distinguish mature from immature insulin granules[121]. Equally, the maturity of glucagon-containing granules in SC-α-cells could be assessed upon morphological comparison with glucagon granules of human adult α-cells[99,204]. Electron microscopy cannot currently be integrated into standard procedures because of the long sample preparation time and the need for specialist equipment: however, with rapid improvements of the technology, such analysis will become increasingly relevant.

SC-islet function

The central parameter for predicting donor and SC-islet therapeutic potency is GSIS. In a GSIS assay, a bulk islet sample is first exposed to a low glucose concentration and then stimulated with a high glucose concentration. Secreted insulin in the respective glucose stimulation phases is quantified by enzyme-linked immunosorbent assay (ELISA). Under static glucose stimulation conditions, the ratio of islet-secreted insulin at low and high glucose is the stimulation index, which is unfortunately a poor predictor for transplantation outcome[205]. Reasons for these consistent findings are multilayered but stressing donor material may lead to extensive degranulation and/or insulin leakage, which influences the GSIS quantifications. After transplantation the donor material can recover, which is not reflected in the in vitro potency analysis.

A more accurate way to assess β-cell function is to measure the dynamic insulin secretion capacity. Insulin secretion from human and mouse islets is characterized by a two-phase profile upon high and constant glucose stimulation for a defined period of time (known as a square pulse). In the first phase, insulin secretion peaks after 5–10 min, whereas insulin secretion is sustained in the second phase but at a lower level, and persists under high glucose stimulation[206]. Insulin secretion stops when the glucose concentration is lowered to the fasting level. Newer differentiation protocols generate SC-β-cells that more accurately recapitulate the biphasic insulin secretion profile of human islets upon high glucose stimulation and shut down insulin secretion at low glucose concentrations[29,68]. If the SC-islets contain α-cells, glucagon secretion (which counteracts insulin) can be quantified in parallel. Similar to primary human α-cells, SC-α-cells secreted increased levels of glucagon in an in vitro test under low glucose conditions[94,99].

Currently, dynamic GSIS assays of donor islets and SC-islets do not serve as a quantitative metric to predict potency because they are too variable and lack a generally accepted standard. For example, the glucose concentrations used to recapitulate the resting and high glucose states are 1–6 mM and 10–28 mM, respectively[189,207]. Furthermore, insulin secretion is sensitive to local oxygen tension, which is not accounted for in existing protocols.

Human islet donor material has become the gold standard for referencing SC-islet GSIS profiles. However, variability in patient material, islet preparation and stability and the low availability of donor material mean human islets are not an ideal standard to assess SC-islets. In addition, technological limitations and challenges, including low sample handling, limited time resolution of the hormone secretion profiles and missing heterogeneity information among islets, result in high variability in dynamic GSIS experiments. Standardized dynamic GSIS assays are also required for drug screening. For example, the functional potency or dose–responses of secretagogues[208] can be determined with dynamic GSIS profiles in the presence of the drug[209], amino acids[163] or

ions such as potassium chloride to decouple the cellular membrane potential. Notably, functional potency assays based on GSIS measurements are only applicable for advanced therapeutic medicinal products based on SC-β-cells and not on SC-PPs, as SC-PPs acquire insulin secretion function upon transplantation.

One of the major bottlenecks in this respect is ELISA technology, which was not designed for massive parallel time-resolved measurements. For establishing one dynamic GSIS profile of a SC-β-cell batch with a time resolution of 1 min and technical repeats, the ELISA costs are more than €200. Alternative assays for pancreas hormones have been developed, using affinity reagents including aptamers[210,211], fluorescent engineered hormone-binding proteins[212], fluorescent engineered reporters or sensor cell lines[213]. These affinity-based sensors have usually been coupled to fluorescent signal read-out technologies to obtain real-time information[214].

Instead of profiling hormone secretion, SC-islet function can be determined by monitoring the islet electrical membrane potential. Alterations in ion currents regulate insulin release from β-cells[215]. Glucose stimulation of β-cells leads to oscillatory electrical activity, and ion currents can thus provide information about the functional state of SC-β-cells[216,217]. Although β-cells from murine islets[218] and human islets[219] have been investigated using the patch clamp technique, the technique has not yet been used for SC-β-cells. By contrast, patch clamp experiments provided evidence that SC-α-cells had physiological characteristics similar to those of primary human α-cells[99]. In the future, the combination of patch clamp analysis and single-cell sequencing should be able to validate the maturity of the physiological functions of SC-islets, as has been done for donor β-cells[220]. For standardized potency assays, however, a technologically simpler and more cost-effective approach is required to obtain electrophysiological signals: for example, measuring membrane potentials by calcium imaging[221,222].

The main challenges for the development of a functional islet potency assay are the need for low-cost assays that can be automated and analyse multiple samples in parallel. Miniaturizing the assays on microfluidic chip platforms would be an enabling step. Indeed, numerous chip platforms have been developed to integrate human and rodent islets and subject them to insulin secretion profiling[223,224]. These chips have reduced the volumes of reagents and cells needed for potency assays, increased the homogeneity of glucose stimulation profiles and increased the resolution down to single islets[225,226]. The majority of chip platforms for SC-β-cells are still focused on optimizing cell differentiation protocols rather than improving functional testing[227]. To leverage the chip technology to test islet potency, sensitive and specific hormone sensors are required. Currently available hormone sensors either lack the specificity for hormones diluted in cell culture media instead of an appropriate buffer system or lack the sensitivity to accurately detect hormones at low concentrations in biological fluids. The most attractive strategies for developing future secreted hormone assays could include DNA barcoding strategies[228] for parallelization of the assays or platforms that compartmentalize samples in droplets[229,230] or microwells[231].

Applications for SC-islets

After passing quality controls, SC-islets could be used in β-cell replacement therapy, screens for antidiabetic drugs and to investigate human β-cell physiology and pathological mechanisms in T1D and T2D. To date, their application is hypothetical, but studies performed with human SC-PPs and SC-β-cells inform about the enormous potential for future studies.

β-Cell replacement therapy

Engrafted allogeneic human islets can efficiently regulate blood glucose levels in patients with T1D, as demonstrated by islet transplantation according to the Edmonton Protocol[15]. In this protocol, 12,000 IEQs per kilogram of patient body weight were infused into the portal vein of patients with T1D and were functional after 10 years[232,233]. The Clinical Islet Transplantation Consortium was then founded. It defined guidelines for islet preparation and quality controls to ensure transplantation of a defined cell product and then initiated a phase III clinical trial[234,235]. In this trial, 87.5% of the graft recipients met their treatment goals, including glycaemic control and reduced severe hypoglycaemia, during the first year after transplantation, a result that no other treatment strategy has achieved in the clinic[16,236]. Rapid progress is also being made in the development of closed-loop insulin systems, which automatically deliver an algorithm-computed amount of insulin using an insulin pump. Those systems effectively improve glycaemic control in patients with T1D compared with standard therapies[237].

SC-islet transplantation. The first successful hPSC-derived transplant to mice was composed of SC-PPs that matured into functional MAFA$^+$, insulin monohormonal SC-β-cells[25]. Improved in vitro SC-PP differentiation protocols produced SC-PPs that could revert diabetes in mice and rats[26,52,95,238]. SC-PPs were chosen over SC-β-cells because correctly specified SC-β-cells could not be reliably generated at that time. In addition, the hypoxia-induced stress resistance of SC-PPs is greater than that of SC-β-cells, so the transplant would be more likely to survive the initial phase, when the transplant is not yet vascularized[239]. SC-PPs are furthermore characterized by low HLA expression compared with endocrine cells, which should minimize the anti-transplant immune response[240]. In 2014, Viacyte launched clinical trials to test the transplantation of SC-PPs in patients with T1D (NCT02239354, NCT02939118, NCT03162926, NCT03163511 and NCT04678557). Early clinical evaluation of the transplant demonstrated survival of the graft 24 months after transplantation as well as the formation of SC-α-cells and SC-β-cells[241]. The first clinical studies investigating graft efficacy will be completed in summer 2021.

However, differentiation of SC-PPs into functional SC-β-cells in animal studies varies with alterations in the in vivo environment, including nutritional conditions[242],

gender[243] and the concentration of thyroid hormones[244]. Furthermore, in vivo maturation takes up to 4 months, which requires bridging with exogenous insulin after transplantation. Transplantation of properly specified SC-β-cells generated with recent, improved protocols circumvents those concerns. After transplantation of those cells into diabetic mice, human C-peptide was detected 2 weeks after transplantation and glycaemic control was achieved within 40 days[27,28]. Further advanced protocols generated SC-β-cells, which led to glucose control similar to human islets upon transplantation into mice[68]. Based on these findings, Vertex Pharmaceuticals launched a phase I/II clinical trial in patients with T1D to test VX-880, an investigational SC-islet therapy (NCT04786262).

To treat diabetes by islet replacement therapy, 5,000–13,000 IEQs are required per kilogram of body weight according to the Edmonton Protocol[15]. Assuming a similar number of IEQs are needed for SC-islet transplantation, a minimum of 375,000 SC-islets would be required for a patient weighing 75 kg. If approximately 50% of the cells of one differentiation batch become endocrine cells and can be assembled into SC-islets of approximately 1,000–2,000 cells, 1×10^9 starting cells are required per patient. Large numbers of cells can be cultured in two dimensions in multiple stack cell factories, but large-scale manufacturing is typically done in 3D cultures[245]. Upscaling the differentiation is feasible; in one experiment, 3.3×10^9 SC-PPs were generated in one differentiation round using cGMP standards[246].

Differentiating large numbers of hPSCs towards SC-islets under cGMP conditions is, however, cost-intensive. Establishing patient-specific human iPS cell lines would further increase the cost of generating SC-islets. Differentiation costs — including cytokines, growth factors and small molecules — could be reduced by better understanding the in vivo development and honing the differentiation steps. Sorting correctly specified endoderm progenitors by targeting CD177 (REF.[31]) or sorting SC-PPs by targeting GP2 (REFS[53,54]) might enable the in vitro expansion of stem cell-derived ADE or SC-PPs that still have proliferative capacity. This might reduce the differentiation costs because protocols that expand these intermediate cells could start with fewer hPSCs and therefore use fewer cytokines during the early differentiation stages. Indeed, SC-PPs can be expanded in 3D culture using chemically defined conditions that include R-Spondin-1 and EGF[247], or FGF2, a ROCK inhibitor and B27 (REF.[107]). Other studies have expanded endoderm cells by co-culturing them with feeder cells[248] or mesenchymal cells[249], which is difficult to adapt to cGMP standards. Cryopreservation of progenitor cells derived from a set of HLA-matched hPSC lines[34] in working cell banks would not reduce the production costs but could accelerate the process of SC-islet generation as it would allow for the advance generation of stocks, which can be thawed when required. Few protocols are established for cryopreserving SC-PPs[107,247] and endocrine precursors[250], demonstrating the feasibility of the process. However, most studies rely on the well-established expansion of hPSCs, which divide rapidly.

SC-islet encapsulation. Grafts can be encapsulated before transplantation, which protects the graft from the host's immune response and protects the host from potential tumours arising from the graft. Two encapsulation strategies can be used. During microencapsulation, single cells or islets are typically encapsulated with alginate, whereas macroencapsulation devices contain more cells or islets and allow for easy graft retrieval[251]. Encapsulated as well as non-encapsulated SC-β-cells and SC-PPs have been studied in immunocompromised and immunocompetent rat and mouse models (TABLE 1). In 2014, Viacyte launched clinical trials to test the safety and potential of encapsulated SC-PPs with two different devices.

The macroencapsulation device Encaptra (PEC-Encap, VC-01) that Viacyte used for the first phase I/II clinical trial (NCT02239354) is derived from the Theracyte device. The device, which is half the size of a business card, is transplanted subcutaneously and is characterized by a porous polytetrafluorethylene membrane that allows oxygen, nutrients and hormones to pass through but prevents contact with immune cells[252]. SC-PPs residing in this device matured into SC-β-cells after transplantation in multiple animal studies[253–258]. However, functional maturation, as measured by comprehensive glycaemic control and diabetes reversal, required up to 1 year of maturation after transplantation into mice[254]. Recent reports from phase I/II clinical trials confirm the safety of the device[241], but its efficiency for glycaemic control has not been demonstrated so far. In a second clinical phase I/II trial, Viacyte is currently testing an open device that allows blood vessels to centre the device but requires immunosuppression to avoid graft rejection (NCT03163511; PEC-Direct, VC-02). Other macroencapsulation devices, which could be used to deliver SC-islets to patients, are currently being tested in clinical trials with human primary islets[14,259]. For example, the Sernova cell pouch (NCT01652911)[260] and the ßAir device (NCT02064309)[261] both aim to improve graft oxygenation, which is beneficial for SC-β-cell survival after engraftment[141]. The Sernova cell pouch is transplanted subcutaneously, where it is allowed to vascularize before being filled with graft cells[262]. The ßAir device is composed of the graft, encapsulated in an alginate hydrogel and an external gas chamber, which is filled with oxygen daily[263]. However, very low levels of circulating C-peptide were observed in the clinical trials with human islets, indicating the need for further optimization[260,261].

The initial clinical studies with encapsulated cells indicate that the design of the devices needs further optimization and transplanting unencapsulated SC-islets after thoroughly confirming their safety might be a viable alternative.

Regulating the immune response. Transplanted SC-islets are the targets of an alloimmune reaction, which occurs when HLAs on the graft cells are recognized by the host T cells, which leads to graft rejection[14,264]. In patients with T1D, the presence of islet autoantigen-specific T cells multiplies this immune attack. Therefore, drugs that broadly suppress the immune system, such as sirolimus,

Table 1 | **Strategies for SC-islet delivery for β-cell replacement therapy**

Encapsulation technology	Transplantation site	Differentiation stage of transplanted cells	Tested model system	Outcome in diabetes model	Immunosuppression required?	Currently tested in clinics?	Refs
No encapsulation							
None	Kidney capsule	SC-β-cells	Mouse	Glycaemic control after ~40 days of maturation	Yes	No	27–29,68
None	Epididymal fat pad, subcutaneously, or kidney capsule	SC-PPs	Mouse, rat	Glycaemic control after ~4 months of maturation	Yes	No	25,26,95,238
Microencapsulation							
Alginate plus CXCL12	Intraperitoneal	SC-β-cells	Mouse	Prolonged graft survival compared with encapsulation with alginate only; glycaemic control	No	No	275
Triazole-thiomorpholine dioxide alginate	Intraperitoneal	SC-β-cells	Mouse	Long-term glycaemic control, mitigated immune response	No	No	128
Conformal coating							
Thin film encapsulated	Gonadal fat pad	SC-β-cells	Mouse	Insulin secretion similar to non-encapsulated cells	Yes	No	250
Macroencapsulation							
Viacyte Encaptra	Subcutaneous	SC-PPs	Mouse	Functional maturation of the graft within 1 year, sufficient functional β-cell mass for glycaemic control	Yes	Yes, device met safety standards	241,253–258
Viacyte Direct	Subcutaneous	SC-PPs	NA	NA	Yes	Yes, ongoing	
ßAir	Subcutaneous	SC-PPs	Rat	No glycaemic control	No	No, but the device has been clinically tested for human islets	259
Sernova cell pouch	Subcutaneous	Not specified	NA	NA	Yes	Yes, planned	292
Nanoporous thin film membranes	Liver	SC-β-cells	Mouse	No glycaemic control	No	No	293
Pre-vascularization							
None	Subcutaneous	SC-PPs	Mouse	Glycaemic control after ~100 days of maturation; no graft failure	Yes	No, but the technique has been tested for human islets	141

CXCL12, C–X–C motif chemokine 12; NA, not available; SC-β-cell, stem cell-derived β-like cell; SC-islet, stem cell-derived islet-like cluster; SC-PP, stem cell-derived pancreatic progenitor.

tacrolimus and daclizumab, are administered after islet transplantation according to the Edmonton Protocol. However, treatment with these drugs increases the risks of infections or cancer[232]. Novel approaches aim to modulate the immune response; for example, using regulatory T cells (T_{reg} cells), which can suppress an immune response. Graft-specific T_{reg} cells were isolated from patients, trained, modified and expanded ex vivo, and these cells reduced the graft-specific immune response when readministered to the patients[265]. Another approach is to specifically block effector T cells, but not T_{reg} cells, by targeting cell surface molecules linked to intrinsic immunoinhibitory pathways[264,266]. As one example, the development of T1D in individuals with increased T1D risk could be delayed by the anti-CD3 monoclonal antibody teplizumab, which inhibits effector T cells[7].

Modifying the composition of SC-islets could modulate the immune response. In particular, inclusion of immunomodulatory cells could dampen the resulting alloimmune reaction. In animal studies, co-transplantation of mesenchymal stem cells and human islets into mice reduced the immune response to human islets by regulating the balance between T helper 1 cells (T_H1 cells) and T_H2 cells[267]. Furthermore, co-transplanted T_{reg} cells decreased the autoimmune response[265,268].

The immunogenic properties of the hPSC-derived cells of the SC-islet can also be altered. hPSCs can be genetically engineered by CRISPR–Cas9 to generate

hypoimmunogenic endocrine cells. Deleting HLA-A, HLA-B, HLA-C and HLA class II genes and/or overexpressing immunomodulatory genes diminishes the immune responses mediated by both T cells and natural killer cells[269,270]. Moreover, the transplanted cells could be engineered to withstand the immune attack, for example by deleting the endoplasmic reticulum stress mediator renalase[271] or by overexpressing a high-affinity variant of a fusion protein containing the extracellular domain of CTLA4 and a modified Fc portion of human immunoglobulin (CTLA4–Ig), a T cell co-stimulation inhibitor[272]. Accordingly, overexpression of the immunoinhibitory cell surface protein PDL1 or induction of endogenous PDL1 expression by IFNγ enabled SC-islet survival in immunocompetent mice and immunodeficient mice engrafted with human immune cells[85].

Finally, the encapsulation material — the barrier between the graft cells and the immune cells — should be minimally immunogenic[251]. In animal studies, modifying the porosity or nano-topography of the material influenced the immune response by polarizing macrophages[273]. Immunosuppressive factors such as the cytokines C–X–C motif chemokine 12 (CXCL12; also known as SDF1) or TGFβ increase graft survival when embedded into the encapsulation material[274,275]. Therefore, designing a rational encapsulation strategy for SC-islets before their transplantation will define their success as an advanced therapeutic medicinal product.

Studying human β-cell physiology

SC-islets can be utilized to examine whether pathological mechanisms identified in mice are relevant to humans. SC-islet engineering also opens new directions for in vitro modelling of different stages of disease progression.

Monogenic diabetes can be modelled by SC-islets derived from hPSCs that carry disease-related mutations and are generated either from patients or by CRISPR–Cas9 genome engineering[119]. Genome editing can reduce the variabilities caused by using different genetic backgrounds[119]. For example, point mutations in the transactivation domain of PDX1 found in patients at increased risk of diabetes impaired the formation of SC-PPs and reduced the functionality of SC-β-cells[276]. In vitro models of monogenic diabetes variants affecting β-cell specification and functionality have also led to insights into the underlying biology, such as the role of increased endoplasmic reticulum stress[277]. Endoplasmic reticulum stress was upregulated in models of Wolfram syndrome[278,279] and diabetes caused by either mutations in the insulin gene[280] or loss of *MANF*[281].

Genome-wide association studies have discovered risk-associated genes for T1D and T2D[20,21]; for example, alterations in *KCNJ11*, *KCNQ1* and *CDKAL1* confer an increased risk of developing T2D. Disrupting these genes in SC-β-cells increased glucolipotoxicity and decreased insulin secretion[282,283].

Interestingly, SC-β-cells that were differentiated from human iPS cells derived from patients with T1D have not generally shown a conclusively diabetes-like phenotype[22,113]. However, one study using SC-β-cells derived from a donor with fulminant T1D demonstrated increased apoptosis of SC-β-cells after treatment with inflammatory cytokines[284]. SC-islets that incorporate other aspects of T1D, such as immune cells, could reveal novel T1D pathomechanisms[19]. Modelling T1D with SC-islets could be further improved by considering the different stages of disease progression. By the time of clinical diagnosis of T1D, the pancreas has already undergone architectural and functional changes, including a loss in overall weight. Some of these changes have been identified in human islets from donors with short-term (<2 years), mid-term (5–11 years) and long-term (>21 years) T1D disease using imaging mass cytometry[285]. In vitro models that recapitulate some of these stages should be developed.

Screening chemical compounds

The failure rate of compounds in clinical trials is more than 90%[286] and has not improved over the past 50 years[287], despite improvements in target identification and compound manufacturing. Animal models have been indispensable for drug discovery, but often do not accurately predict efficiency and toxicity in clinical studies because there are substantial inter-species differences. Hence, SC-islets, which are of human origin and can be customized for the assay, are a valuable tool for screening compound libraries to identify antidiabetic drugs. This approach could improve success rates in clinical trials or could be used to test the toxicity and efficacy of previously identified drug candidates.

Multiple compound screens have identified factors that improve the differentiation of hPSCs into SC-β-cells[49,184]. For example, compounds that induce differentiation towards the endodermal lineage — such as the TGFβ activators IDE1 and IDE2 (REF.[288]), the negative regulator of MYC stauprimide[289] and the ROCK inhibitor Fasudil[290] — were discovered by high-throughput chemical screens. Other screens performed in 2D cultures have identified novel drugs and targets for diabetes therapy by investigating the death and expansion of SC-β-cells. For example, screens identified an activator of TGFβ signalling for the treatment of diabetes associated with mutations in *GLIS3* (REF.[291]) and a compound that inhibits the FOS–JUN pathway rescued a diabetic phenotype induced by an alteration in the diabetes-associated gene *CDKAL1* (REF.[282]).

Microfluidic platforms have been developed that can be used for the functional assessment of β-cells by monitoring insulin secretion, oxygen consumption and calcium concentrations[190]. Advantages of microfluidic platforms are that they require small amounts of material, allow for automation and real-time read-outs, and can take measurements from single islets. Combining SC-islet cultures that are able to recapitulate the insulin secretion profile of human islets with microfluidic chip technology will make it possible to investigate drugs that improve GSIS.

Outlook

Although great progress in diabetes research has been made, the number of patients with diabetes is estimated to rise to 700 million by 2045. As insulin injections have side effects and only treat the symptoms, and the

availability of cadaveric islets is limited, new therapeutic approaches are needed to meet the rising future demands.

In an ideal future scenario, defined SC-islets could be manufactured and used to treat patients with brittle T1D and late-stage T2D diabetes. The first clinical trials with SC-PPs and SC-β-cells as well as the Edmonton Protocol demonstrate that transplantation is feasible and that those therapies can improve the quality of patients' lives. However, hurdles — such as the need to improve endocrine differentiation protocols and determine which cell types in which ratios can generate a safe and functional SC-islet — need to be overcome. A better understanding of human islet development will facilitate the engineering of physiologically relevant SC-islets.

In addition, protocols for manufacturing SC-islets need to be designed. This includes strategies to maximize yields by expanding progenitor cells and to cryopreserve cells in banks. Furthermore, reliable and valid potency assays and definitions of thresholds for transplantable SC-islets need to be established.

Reducing the immunogenicity of transplants will also improve their efficacy. For example, genetically engineering immune-evasive hPSCs or developing and optimizing a protective device for the transplant could be important strategies. Finally, the improved production of functional and mature SC-islets in vitro will allow T1D and T2D to be modelled. Such models could improve our understanding of the pathomechanisms of these diseases and be used to perform drug screens to identify and validate new molecular targets for improved therapy.

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1. IDF. *IDF Diabetes Atlas* 9th edn. (International Diabetes Federation, 2019).
2. Bender, C., Rodriguez-Calvo, T., Amirian, N., Coppieters, K. T. & von Herrath, M. G. The healthy exocrine pancreas contains preproinsulin-specific CD8 T cells that attack islets in type 1 diabetes. *Sci. Adv.* **6**, 5586–5602 (2020).
3. Talchai, C., Xuan, S., Lin, H. V., Sussel, L. & Accili, D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell* **150**, 1223–1234 (2012).
4. Wang, Z., York, N. W., Nichols, C. G. & Remedi, M. S. Pancreatic β cell dedifferentiation in diabetes and redifferentiation following insulin therapy. *Cell Metab.* **19**, 872–882 (2014).
5. Cinti, F. et al. Evidence of β-cell dedifferentiation in human type 2 diabetes. *J. Clin. Endocrinol. Metab.* **101**, 1044–1054 (2016).
6. Schnurr, T. M. et al. Obesity, unfavourable lifestyle and genetic risk of type 2 diabetes: a case–cohort study. *Diabetologia* **63**, 1324–1332 (2020).
7. Herold, K. C. et al. An Anti-CD3 antibody, teplizumab, in relatives at risk for type 1. *Diabetes N. Engl. J. Med.* **381**, 603–613 (2019).
8. Harrison, L. B., Adams-Huet, B., Raskin, P. & Lingvay, I. β-cell function preservation after 3.5 years of intensive diabetes therapy. *Diabetes Care* **35**, 1406–1412 (2012).
9. Schauer, P. R. et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N. Engl. J. Med.* **366**, 1567–1576 (2012).
10. Wang, P. et al. Combined inhibition of DYRK1A, SMAD, and trithorax pathways synergizes to induce robust replication in adult human beta cells. *Cell Metab.* **29**, 638–652 (2019).
11. Thompson, P. J. et al. Targeted elimination of senescent beta cells prevents type 1 diabetes. *Cell Metab.* **29**, 1045–1060 (2019).
12. Sachs, S. et al. Targeted pharmacological therapy restores β-cell function for diabetes remission. *Nat. Metab.* **2**, 192–209 (2020).
13. Kandaswamy, R. et al. OPTN/SRTR 2016 Annual Data Report: pancreas. *Am. J. Transplant.* **18**, 114–171 (2018).
14. Sneddon, J. B. et al. Stem cell therapies for treating diabetes: progress and remaining challenges. *Cell Stem Cell* **22**, 810–823 (2018).
15. Shapiro, A. M. J. et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* **343**, 230–238 (2000).
16. Latres, E., Finan, D. A., Greenstein, J. L., Kowalski, A. & Kieffer, T. J. Navigating two roads to glucose normalization in diabetes: automated insulin delivery devices and cell therapy. *Cell Metab.* **29**, 545–563 (2019).
17. Lumelsky, N. et al. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* **292**, 1389–1394 (2001).
18. Assady, S. et al. Insulin production by human embryonic stem cells. *Diabetes* **50**, 1691–1697 (2001).
19. Leite, N. C. et al. Modeling type 1 diabetes in vitro using human pluripotent stem cells. *Cell Rep.* **32**, 107894 (2020).
20. Krentz, N. A. J. & Gloyn, A. L. Insights into pancreatic islet cell dysfunction from type 2 diabetes mellitus genetics. *Nat. Rev. Endocrinol.* **16**, 202–212 (2020).
21. Chiou, J. et al. Large-scale genetic association and single cell accessible chromatin mapping defines cell type-specific mechanisms of type 1 diabetes risk 2 3. Preprint at *bioRxiv* https://doi.org/10.1101/2021.01.13.426472 (2021).
22. Millman, J. R. et al. Generation of stem cell-derived β-cells from patients with type 1 diabetes. *Nat. Commun.* **7**, 11463 (2016).
23. D'Amour, K. A. et al. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat. Biotechnol.* **23**, 1534–1541 (2005).
24. D'Amour, K. A. et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat. Biotechnol.* **24**, 1392–1401 (2006).
25. Kroon, E. et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat. Biotechnol.* **26**, 443–452 (2008).
26. Rezania, A. et al. Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. *Diabetes* **61**, 2016–2029 (2012).
27. Rezania, A. et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat. Biotechnol.* **32**, 1121–1133 (2014).
28. Pagliuca, F. W. et al. Generation of functional human pancreatic β cells in vitro. *Cell* **159**, 428–439 (2014).
29. Velazco-Cruz, L. et al. Acquisition of dynamic function in human stem cell-derived β cells. *Stem Cell Rep.* **12**, 351–365 (2019).
30. Nostro, M. C. et al. Stage-specific signaling through TGFβ family members and WNT regulates patterning and pancreatic specification of human pluripotent stem cells. *Development* **138**, 861–871 (2011).
31. Mahaddalkar, P. U. et al. Generation of pancreatic β cells from CD177$^+$ anterior definitive endoderm. *Nat. Biotechnol.* **38**, 1061–1072 (2020).
32. Yamanaka, S. Pluripotent stem cell-based cell therapy-promise and challenges. *Stem Cell* **27**, 523–531 (2020).
33. Huang, C. Y. et al. Human iPSC banking: barriers and opportunities. *J. Biomed. Sci.* **26**, 87 (2019).
34. Taylor, C. J., Peacock, S., Chaudhry, A. N., Bradley, J. A. & Bolton, E. M. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. *Cell Stem Cell* **11**, 147–152 (2012).
35. Noguchi, G. M. & Huising, M. O. Integrating the inputs that shape pancreatic islet hormone release. *Nat. Metab.* **1**, 1189–1201 (2019).
36. Brennan, J. et al. Nodal signalling in the epiblast patterns the early mouse embryo. *Nature* **411**, 965–969 (2001).
37. Engert, S. et al. Wnt/β-catenin signalling regulates Sox17 expression and is essential for organizer and endoderm formation in the mouse. *Development* **140**, 3128–3138 (2013).
38. Gadue, P., Huber, T. L., Paddison, P. J. & Keller, G. M. Wnt and TGF-β signaling are required for the induction of an in vitro model of primitive streak formation using embryonic stem cells. *Proc. Natl Acad. Sci. USA* **103**, 16806–16811 (2006).
39. Rodríguez-Seguel, E. et al. Mutually exclusive signaling signatures define the hepatic and pancreatic progenitor cell lineage divergence. *Genes Dev.* **27**, 1932–1946 (2013).
40. Dessimoz, J., Opoka, R., Kordich, J. J., Grapin-Botton, A. & Wells, J. M. FGF signaling is necessary for establishing gut tube domains along the anterior–posterior axis in vivo. *Mech. Dev.* **123**, 42–55 (2006).
41. Wells, J. M. & Melton, D. A. Early mouse endoderm is patterned by soluble factors from adjacent germ layers. *Development* **127**, 1563–1572 (2000).
42. Bastidas-Ponce, A., Scheibner, K., Lickert, H. & Bakhti, M. Cellular and molecular mechanisms coordinating pancreas development. *Development* **144**, 2873–2888 (2017).
43. Johannesson, M. et al. FGF4 and retinoic acid direct differentiation of hESCs into PDX1-expressing foregut endoderm in a time- and concentration-dependent manner. *PLoS ONE* **4**, e4794 (2009).
44. Wesolowska-Andersen, A. et al. Analysis of differentiation protocols defines a common pancreatic progenitor molecular signature and guides refinement of endocrine differentiation. *Stem Cell Rep.* **14**, 138–153 (2020).
45. Russ, H. A. et al. Controlled induction of human pancreatic progenitors produces functional β-like cells in vitro. *EMBO J.* **34**, 1759–1772 (2015).
46. Shahjalal, H. M. et al. Generation of insulin-producing β-like cells from human iPS cells in a defined and completely xeno-free culture system. *J. Mol. Cell Biol.* **6**, 394–408 (2014).
47. Hebrok, M., Kim, S. K. & Melton, D. A. Notochord repression of endodermal Sonic hedgehog permits pancreas development. *Genes Dev.* **12**, 1705–1713 (1998).
48. Nostro, M. C. et al. Efficient generation of NKX6-1$^+$ pancreatic progenitors from multiple human pluripotent stem cell lines. *Stem Cell Rep.* **4**, 591–604 (2015).
49. Chen, S. et al. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat. Chem. Biol.* **5**, 258–265 (2009).
50. Shih, H. P. et al. A gene regulatory network cooperatively controlled by Pdx1 and Sox9 governs lineage allocation of foregut progenitor cells. *Cell Rep.* **13**, 326–336 (2015).
51. Taylor, B. L., Liu, F. F. & Sander, M. Nkx6.1 is essential for maintaining the functional state of pancreatic β cells. *Cell Rep.* **4**, 1262–1275 (2013).
52. Rezania, A. et al. Enrichment of human embryonic stem cell-derived NKX6.1-expressing pancreatic progenitor cells accelerates the maturation of insulin-secreting cells in vivo. *Stem Cell* **31**, 2432–2442 (2013).
53. Ameri, J. et al. Efficient generation of glucose-responsive β cells from isolated GP2$^+$ human pancreatic progenitors. *Cell Rep.* **19**, 36–49 (2017).
54. Cogger, K. F. et al. Glycoprotein 2 is a specific cell surface marker of human pancreatic progenitors. *Nat. Commun.* **8**, 1–13 (2017).

55. Schaffer, A. E., Freude, K. K., Nelson, S. B. & Sander, M. Nkx6 transcription factors and Ptf1a function as antagonistic lineage determinants in multipotent pancreatic progenitors. *Dev. Cell* **18**, 1022–1029 (2010).
56. Seymour, P. A. et al. SOX9 is required for maintenance of the pancreatic progenitor cell pool. *Proc. Natl Acad. Sci. USA* **104**, 1865–1870 (2007).
57. Gu, G., Dubauskaite, J. & Melton, D. A. Direct evidence for the pancreatic lineage: NGN3$^+$ cells are islet progenitors and are distinct from duct progenitors. *Development* **129**, 2447–2457 (2002).
58. Salisbury, R. J. et al. The window period of NEUROGENIN3 during human gestation. *Islets* **6**, e954436 (2014).
59. Sharon, N. et al. A peninsular structure coordinates asynchronous differentiation with morphogenesis to generate pancreatic islets. *Cell* **176**, 790–804 (2019).
60. Mamidi, A. et al. Mechanosignalling via integrins directs fate decisions of pancreatic progenitors. *Nature* **564**, 114–118 (2018).
61. Kesavan, G. et al. Cdc42/N-WASP signaling links actin dynamics to pancreatic β cell delamination and differentiation. *Development* **141**, 685–696 (2014).
62. Apelqvist, Å. et al. Notch signalling controls pancreatic cell differentiation. *Nature* **400**, 877–881 (1999).
63. Lee, J. C. et al. Regulation of the pancreatic pro-endocrine gene neurogenin3. *Diabetes* **50**, 928–936 (2001).
64. Krentz, N. A. J. et al. Phosphorylation of NEUROG3 links endocrine differentiation to the cell cycle in pancreatic progenitors. *Dev. Cell* **41**, 129–142 (2017).
65. Azzarelli, R. et al. Multi-site neurogenin3 phosphorylation controls pancreatic endocrine differentiation article multi-site neurogenin3 phosphorylation controls pancreatic endocrine differentiation. *Dev. Cell* **41**, 274–286 (2017).
66. Miyatsuka, T., Kosaka, Y., Kim, H. & German, M. S. Neurogenin3 inhibits proliferation in endocrine progenitors by inducing Cdkn1a. *Proc. Natl Acad. Sci. USA* **108**, 185–190 (2011).
67. Weng, C. et al. Single-cell lineage analysis reveals extensive multimodal transcriptional control during directed β-cell differentiation. *Nat. Metab.* **2**, 1443–1458 (2020).
68. Hogrebe, N. J., Augsornworawat, P., Maxwell, K. G., Velazco-Cruz, L. & Millman, J. R. Targeting the cytoskeleton to direct pancreatic differentiation of human pluripotent stem cells. *Nat. Biotechnol.* **38**, 460–470 (2020).
69. Rosado-Olivieri, E. A., Anderson, K., Kenty, J. H. & Melton, D. A. YAP inhibition enhances the differentiation of functional stem cell-derived insulin-producing β cells. *Nat. Commun.* **10**, 1464 (2019).
70. Sussel, L. et al. Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic β cells. *Development* **125**, 2213–2221 (1998).
71. Huang, H.-P. et al. Regulation of the Pancreatic Islet-Specific Gene BETA2 (neuroD) by Neurogenin 3. *Mol. Cell. Biol.* **20**, 3292–3307 (2000).
72. Östrom, M. et al. Retinoic acid promotes the generation of pancreatic endocrine progenitor cells and their further differentiation into β-cells. *PLoS ONE* **3**, e2841 (2008).
73. Lorberbaum, D. S. et al. Retinoic acid signaling within pancreatic endocrine progenitors regulates mouse and human β cell specification. *Development* **147**, dev189977 (2020).
74. Sharon, N. et al. Wnt signaling separates the progenitor and endocrine compartments during pancreas development. *Cell Rep.* **27**, 2281–2291 (2019).
75. Lin, H. M. et al. Transforming growth factor-β/Smad3 signaling regulates insulin gene transcription and pancreatic islet β-cell function. *J. Biol. Chem.* **284**, 12246–12257 (2009).
76. Dhawan, S., Dirice, E., Kulkarni, R. N. & Bhushan, A. Inhibition of TGF-β signaling promotes human pancreatic β-cell replication. *Diabetes* **65**, 1208–1218 (2016).
77. Thowfeequ, S., Ralphs, K. L., Yu, W. Y., Slack, J. M. W. & Tosh, D. Betacellulin inhibits amylase and glucagon production and promotes β cell differentiation in mouse embryonic pancreas. *Diabetologia* **50**, 1688–1697 (2007).
78. Löf-Öhlin, Z. M. et al. EGFR signalling controls cellular fate and pancreatic organogenesis by regulating apicobasal polarity. *Nat. Cell Biol.* **19**, 1313–1325 (2017).
79. Heinis, M. et al. Oxygen tension regulates pancreatic β-cell differentiation through hypoxia-inducible factor 1β. *Diabetes* **59**, 662–669 (2010).
80. Sosa-Pineda, B., Chowdhury, K., Torres, M., Oliver, G. & Gruss, P. The Pax4 gene is essential for differentiation of insulin-producing β cells in the mammalian pancreas. *Nature* **386**, 399–402 (1997).
81. Collombat, P. et al. The ectopic expression of pax4 in the mouse pancreas converts progenitor cells into α and subsequently β cells. *Cell* **138**, 449–462 (2009).
82. Veres, A. et al. Charting cellular identity during human in vitro β-cell differentiation. *Nature* **569**, 368–373 (2019).
83. Jennings, R. E. et al. Development of the human pancreas from foregut to endocrine commitment. *Diabetes* **62**, 3514–3522 (2013).
84. Gao, X. et al. PEDF and PEDF-derived peptide 44mer protect cardiomyocytes against hypoxia-induced apoptosis and necroptosis via anti-oxidative effect. *Sci. Rep.* **4**, 5637 (2014).
85. Yoshihara, E. et al. Immune-evasive human islet-like organoids ameliorate diabetes. *Nature* **586**, 606–611 (2020).
86. Collombat, P. et al. Embryonic endocrine pancreas and mature β cells acquire α and PP cell phenotypes upon Arx misexpression. *J. Clin. Invest.* **117**, 961–970 (2007).
87. Collombat, P. et al. Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes Dev.* **17**, 2591–2603 (2003).
88. Petri, A. et al. The effect of neurogenin3 deficiency on pancreatic gene expression in embryonic mice. *J. Mol. Endocrinol.* **37**, 301–316 (2006).
89. Baron, M. et al. A single-cell transcriptomic map of the human and mouse pancreas reveals inter- and intra-cell population structure. *Cell Syst.* **3**, 346–360.e4 (2016).
90. Ramzy, A., Asadi, A. & Kieffer, T. J. Revisiting proinsulin processing: Evidence that human β-cells process proinsulin with prohormone convertase (pc) 1/3 but not pc2. *Diabetes* **69**, 1451–1462 (2020).
91. Bruin, J. E. et al. Characterization of polyhormonal insulin-producing cells derived in vitro from human embryonic stem cells. *Stem Cell Res.* **12**, 194–208 (2014).
92. Riedel, M. J. et al. Immunohistochemical characterisation of cells co-producing insulin and glucagon in the developing human pancreas. *Diabetologia* **55**, 372–381 (2012).
93. Riopel, M., Li, J., Fellows, G. F., Goodyer, C. G. & Wang, R. Ultrastructural and immunohistochemical analysis of the 8–20 week human fetal pancreas. *Islets* **6**, e982949 (2014).
94. Rezania, A. et al. Production of functional glucagon-secreting α-cells from human embryonic stem cells. *Diabetes* **60**, 239–247 (2011).
95. Kelly, O. G. et al. Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells. *Nat. Biotechnol.* **29**, 750–756 (2011).
96. Petersen, M. B. K. et al. Single-Cell Gene Expression Analysis of a Human ESC Model of Pancreatic Endocrine Development Reveals Different Paths to β-Cell Differentiation. *Stem Cell Rep.* **9**, 1246–1261 (2017).
97. Johansson, K. A. et al. Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. *Dev. Cell* **12**, 457–465 (2007).
98. Hua, H. & Sarvetnick, N. Expression of Id1 in adult, regenerating and developing pancreas. *Endocrine* **32**, 280–286 (2007).
99. Peterson, Q. P. et al. A method for the generation of human stem cell-derived α cells. *Nat. Commun.* **11**, 1–14 (2020).
100. De Marinis, Y. Z. et al. Enhancement of glucagon secretion in mouse and human pancreatic α cells by protein kinase C (PKC) involves intracellular trafficking of PKCα and PKCδ. *Diabetologia* **53**, 717–729 (2010).
101. Moya, N. et al. Generation of a homozygous ARX nuclear CFP (ARXnCFP/nCFP) reporter human iPSC line (HMGUi001-A-4). *Stem Cell Res.* **46**, 101874 (2020).
102. Siehler, J. et al. Generation of a heterozygous C-peptide–mCherry reporter human iPSC line (HMGUi001-A-8). *Stem Cell Res.* **50**, 1873–5061 (2021).
103. Blöchinger, A. K. et al. Generation of an INSULIN-H2B-Cherry reporter human iPSC line. *Stem Cell Res.* **45**, 101797 (2020).
104. Tellez, K. et al. In vivo studies of glucagon secretion by human islets transplanted in mice. *Nature Metab.* **2**, 547–557 (2020).
105. Nair, G. & Hebrok, M. Islet formation in mice and men: lessons for the generation of functional insulin-producing β-cells from human pluripotent stem cells. *Curr. Opin. Genet. Dev.* **32**, 171–180 (2015).
106. Steiner, D. J., Kim, A., Miller, K. & Hara, M. Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. *Islets* **2**, 135–145 (2010).
107. Gonçalves, C. A. et al. A 3D system to model human pancreas development and its reference single-cell transcriptome atlas identify signaling pathways required for progenitor expansion. *Nat. Commun.* **12**, 3144 (2021).
108. Piper, K. et al. β cell differentiation during early human pancreas development. *J. Endocrinol.* **181**, 11–23 (2004).
109. Villasenor, A., Chong, D. C. & Cleaver, O. Biphasic Ngn3 expression in the developing pancreas. *Dev. Dyn.* **237**, 3270–3279 (2008).
110. Berger, M., Gray, J. A. & Roth, B. L. The expanded biology of serotonin. *Annu. Rev. Med.* **60**, 355–366 (2009).
111. Theis, F. J. & Lickert, H. A map of β-cell differentiation pathways supports cell therapies for diabetes. *Nature* **569**, 342–343 (2019).
112. Osafune, K. et al. Marked differences in differentiation propensity among human embryonic stem cell lines. *Nat. Biotechnol.* **26**, 313–315 (2008).
113. Sui, L. et al. β-Cell replacement in mice using human type 1 diabetes nuclear transfer embryonic stem cells. *Diabetes* **67**, 26–35 (2018).
114. Nishizawa, M. et al. Epigenetic variation between human induced pluripotent stem cell lines is an indicator of differentiation capacity. *Cell Stem Cell* **19**, 341–354 (2016).
115. Kim, K. et al. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat. Biotechnol.* **29**, 1117–1119 (2011).
116. Cuomo, A. S. E. et al. Single-cell RNA-sequencing of differentiating iPS cells reveals dynamic genetic effects on gene expression. *Nat. Commun.* **11**, 1–14 (2020).
117. Tsankov, A. M. et al. A qPCR ScoreCard quantifies the differentiation potential of human pluripotent stem cells. *Nat. Biotechnol.* **33**, 1182–1192 (2015).
118. Müller, F. J. et al. A bioinformatic assay for pluripotency in human cells. *Nat. Methods* **8**, 315–317 (2011).
119. Balboa, D., Prasad, R. B., Groop, L. & Otonkoski, T. Genome editing of human pancreatic β cell models: problems, possibilities and outlook. *Diabetologia* **62**, 1329–1336 (2019).
120. Micallef, S. J. et al. INSGFP/w human embryonic stem cells facilitate isolation of in vitro derived insulin-producing cells. *Diabetologia* **55**, 694–706 (2012).
121. Nair, G. G. et al. Recapitulating endocrine cell clustering in culture promotes maturation of human stem-cell-derived β cells. *Nat. Cell Biol.* **21**, 263–274 (2019).
122. Molakandov, K. et al. Methods for differentiating and purifying pancreatic endocrine cells. US Patent Application 15/780,153 (2018).
123. Li, X. et al. Single-cell RNA-seq reveals that CD9 is a negative marker of glucose-responsive pancreatic β-like cells derived from human pluripotent stem cells. *Stem Cell Rep.* **15**, 1111–1126 (2020).
124. Dorrell, C. et al. Human islets contain four distinct subtypes of β cells. *Nat. Commun.* **7**, 1–9 (2016).
125. Saunders, D. C. et al. Ectonucleoside triphosphate diphosphohydrolase-3 antibody targets adult human pancreatic β cells for in vitro and in vivo analysis. *Cell Metab.* **29**, 745–754 (2019).
126. Yu, Y. et al. Bioengineered human pseudoislets form efficiently from donated tissue, compare favourably with native islets in vitro and restore normoglycaemia in mice. *Diabetologia* **61**, 2016–2029 (2018).
127. Hilderink, J. et al. Controlled aggregation of primary human pancreatic islet cells leads to glucose-responsive pseudoislets comparable to native islets. *J. Cell. Mol. Med.* **19**, 1836–1846 (2015).
128. Vegas, A. J. et al. Long-term glycemic control using polymer-encapsulated human stem cell–derived beta cells in immune-competent mice. *Nat. Med.* **22**, 306–311 (2016).
129. Augsornworawat, P., Maxwell, K. G., Velazco-Cruz, L. & Millman, J. R. Single-cell transcriptome profiling reveals β cell maturation in stem cell-derived islets after transplantation. *Cell Rep.* **32**, 108067 (2020).
130. Yoshihara, E. et al. ERRγ is required for the metabolic maturation of therapeutically functional glucose-responsive β cells. *Cell Metab.* **23**, 622–634 (2016).

131. Blum, B. et al. Functional β-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. *Nat. Biotechnol.* **30**, 261–264 (2012).
132. Hang, Y. et al. The MafA transcription factor becomes essential to islet β-cells soon after birth. *Diabetes* **63**, 1994–2005 (2014).
133. van der Meulen, T. et al. Urocortin 3 marks mature human primary and embryonic stem cell-derived pancreatic α and β cells. *PLoS ONE* **7**, e52181 (2012).
134. Arda, H. E. et al. Age-dependent pancreatic gene regulation reveals mechanisms governing human β cell function. *Cell Metab.* **23**, 909–920 (2016).
135. Aguayo-Mazzucato, C., Sanchez-Soto, C., Godinez-Puig, V., Gutiérrez-Ospina, G. & Hiriart, M. Restructuring of pancreatic islets and insulin secretion in a postnatal critical window. *PLoS ONE* **1**, e35 (2006).
136. Henquin, J. C. & Nenquin, M. Immaturity of insulin secretion by pancreatic islets isolated from one human neonate. *J. Diabetes Investig.* **9**, 270–273 (2018).
137. Grasso, S., Messina, A., Saporito, N. & Reitano, G. Serum-insulin response to glucose and aminoacids in the premature infant. *Lancet* **2**, 755–756 (1968).
138. Hrvatin, S. et al. Differentiated human stem cells resemble fetal, not adult, β cells. *Proc. Natl Acad. Sci. USA* **111**, 3038–3043 (2014).
139. Legøy, T. A. et al. In vivo environment swiftly restricts human pancreatic progenitors toward mono-hormonal identity via a HNF1A/HNF4A mechanism. *Front. Cell Dev. Biol.* **8**, 109 (2020).
140. Cardenas-Diaz, F. L. et al. Modeling monogenic diabetes using human ESCs reveals developmental and metabolic deficiencies caused by mutations in HNF1A. *Cell Stem Cell* **25**, 273–289 (2019).
141. Pepper, A. R. et al. Transplantation of human pancreatic endoderm cells reverses diabetes post transplantation in a prevascularized subcutaneous site. *Stem Cell Rep.* **8**, 1689–1700 (2017).
142. Cozzitorto, C. et al. A specialized niche in the pancreatic microenvironment promotes endocrine differentiation. *Dev. Cell* **55**, 150–162 (2020).
143. Rackham, C. L. et al. Optimizing beta cell function through mesenchymal stromal cell-mediated mitochondria transfer. *Stem Cells* **38**, 574–584 (2020).
144. Montanari, E. et al. Multipotent mesenchymal stromal cells enhance insulin secretion from human islets via N-cadherin interaction and prolong function of transplanted encapsulated islets in mice. *Stem Cell Res. Ther.* **8**, 199 (2017).
145. Arzouni, A. A. et al. Mesenchymal stromal cells improve human islet function through released products and extracellular matrix. *Clin. Sci.* **131**, 2835–2845 (2017).
146. Wang, L. et al. Mesenchymal stem cells ameliorate β cell dysfunction of human type 2 diabetic islets by reversing β cell dedifferentiation. *EBioMedicine* **51**, 102615 (2020).
147. Arzouni, A. A. et al. Characterization of the effects of mesenchymal stromal cells on mouse and human islet function. *Stem Cells Transl. Med.* **8**, 935–944 (2019).
148. Nekrep, N., Wang, J., Miyatsuka, T. & German, M. S. Signals from the neural crest regulate beta-cell mass in the pancreas. *Development* **135**, 2151–2160 (2008).
149. Olerud, J. et al. Neural crest stem cells increase beta cell proliferation and improve islet function in co-transplanted murine pancreatic islets. *Diabetologia* **52**, 2594–2601 (2009).
150. Lau, J., Vasylovska, S., Kozlova, E. N. & Carlsson, P. O. Surface coating of pancreatic islets with neural crest stem cells improves engraftment and function after intraportal transplantation. *Cell Transplant.* **24**, 2263–2272 (2015).
151. Olmer, R. et al. Differentiation of human pluripotent stem cells into functional endothelial cells in scalable suspension culture. *Stem Cell Rep.* **10**, 1657–1672 (2018).
152. Wimmer, R. A. et al. Human blood vessel organoids as a model of diabetic vasculopathy. *Nature* **565**, 505–510 (2019).
153. Talavera-Adame, D. et al. Effective endothelial cell and human pluripotent stem cell interactions generate functional insulin-producing β cells. *Diabetologia* **59**, 2378–2386 (2016).
154. Jaramillo, M., Mathew, S., Mamiya, H., Goh, S. K. & Banerjee, I. Endothelial cells mediate islet-specific maturation of human embryonic stem cell-derived pancreatic progenitor cells. *Tissue Eng. Part A* **21**, 14–25 (2015).
155. Urbanczyk, M., Zbinden, A., Layland, S. L., Duffy, G. & Schenke-Layland, K. Controlled heterotypic pseudo-islet assembly of human β-cells and human umbilical vein endothelial cells using magnetic levitation. *Tissue Eng. Part A* **26**, 387–399 (2020).
156. Johansson, Å. et al. Endothelial cell signalling supports pancreatic β cell function in the rat. *Diabetologia* **52**, 2385–2394 (2009).
157. Narayanan, K. et al. Extracellular matrix-mediated differentiation of human embryonic stem cells: differentiation to insulin-secreting β cells. *Tissue Eng. Part A* **20**, 424–433 (2014).
158. Bi, H., Ye, K. & Jin, S. Proteomic analysis of decellularized pancreatic matrix identifies collagen V as a critical regulator for islet organogenesis from human pluripotent stem cells. *Biomaterials* **233**, 119673 (2020).
159. Haase, T. N. et al. Growth arrest specific protein (GAS) 6: a role in the regulation of proliferation and functional capacity of the perinatal rat β cell. *Diabetologia* **56**, 763–773 (2013).
160. Harmon, J. S. et al. β-Cell-specific overexpression of glutathione peroxidase preserves intranuclear MafA and reverses diabetes in db/db mice. *Endocrinology* **150**, 4855–4862 (2009).
161. Aguayo-Mazzucato, C. et al. Thyroid hormone promotes postnatal rat pancreatic β-cell development and glucose-responsive insulin secretion through MAFA. *Diabetes* **62**, 1569–1580 (2013).
162. Salinno, C. et al. β-Cell maturation and identity in health and disease. *Int. J. Mol. Sci.* **20**, 1–20 (2019).
163. Helman, A. et al. A nutrient-sensing transition at birth triggers glucose-responsive insulin secretion. *Cell Metab.* **31**, 1004–1016 (2020).
164. Alvarez-Dominguez, J. R. et al. Circadian entrainment triggers maturation of human in vitro islets. *Cell Stem Cell* **26**, 108–122 (2020).
165. Sinagoga, K. L. et al. Distinct roles for the mTOR pathway in postnatal morphogenesis, maturation and function of pancreatic islets. *Development* **144**, 2402–2414 (2017).
166. Ni, Q. et al. Raptor regulates functional maturation of murine β cells. *Nat. Commun.* **8**, 15755 (2017).
167. Blandino-Rosano, M. et al. Loss of mTORC1 signalling impairs β-cell homeostasis and insulin processing. *Nat. Commun.* **8**, 1–15 (2017).
168. Koyanagi, M. et al. Ablation of TSC2 enhances insulin secretion by increasing the number of mitochondria through activation of mTORC1. *PLoS ONE* **6**, e23238 (2011).
169. Elghazi, L. et al. Decreased IRS signaling impairs β-cell cycle progression and survival in transgenic mice overexpressing S6K in β-cells. *Diabetes* **59**, 2390–2399 (2010).
170. Bartolomé, A. et al. Pancreatic β-cell failure mediated by mTORC1 hyperactivity and autophagic impairment. *Diabetes* **63**, 2996–3008 (2014).
171. Jaafar, R. et al. MTORC1-to-AMPK switching underlies β-cell metabolic plasticity during maturation and diabetes. *J. Clin. Invest.* **129**, 4124–4137 (2019).
172. Rorsman, P. & Ashcroft, F. M. Pancreatic β-cell electrical activity and insulin secretion: of mice and men. *Physiol. Rev.* **98**, 117–214 (2018).
173. Schuit, F. et al. Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in β cells. *J. Biol. Chem.* **272**, 18572–18579 (1997).
174. Wortham, M. et al. Integrated in vivo quantitative proteomics and nutrient tracing reveals age-related metabolic rewiring of pancreatic β cell function. *Cell Rep.* **25**, 2904–2918 (2018).
175. Jermendy, A. et al. Rat neonatal β cells lack the specialised metabolic phenotype of mature β cells. *Diabetologia* **54**, 594–604 (2011).
176. Pullen, T. J. et al. Identification of genes selectively disallowed in the pancreatic islet. *Islets* **2**, 89–95 (2010).
177. Stolovich-Rain, M. et al. Weaning triggers a maturation step of pancreatic β cells. *Dev. Cell* **32**, 535–545 (2015).
178. Jacovetti, C., Matkovich, S. J., Rodriguez-Trejo, A., Guay, C. & Regazzi, R. Postnatal β-cell maturation is associated with islet-specific microRNA changes induced by nutrient shifts at weaning. *Nat. Commun.* **6**, 1–14 (2015).
179. Velazco-Cruz, L. et al. SIX2 regulates human β cell differentiation from stem cells and functional maturation in vitro. *Cell Rep.* **31**, 107687 (2020).
180. Davis, J. C. et al. Glucose response by stem cell-derived β cells in vitro is inhibited by a bottleneck in glycolysis. *Cell Rep.* **31**, 107623 (2020).
181. Bader, E. et al. Identification of proliferative and mature β-cells in the islets of Langerhans. *Nature* **535**, 430–434 (2016).
182. Cortijo, C., Gouzi, M., Tissir, F. & Grapin-Botton, A. Planar cell polarity controls pancreatic β cell differentiation and glucose homeostasis. *Cell Rep.* **2**, 1593–1606 (2012).
183. Roscioni, S. S., Migliorini, A., Gegg, M. & Lickert, H. Impact of islet architecture on β-cell heterogeneity, plasticity and function. *Nat. Rev. Endocrinol.* **12**, 695–709 (2016).
184. Ghazizadeh, Z. et al. ROCKII inhibition promotes the maturation of human pancreatic β-like cells. *Nat. Commun.* **8**, 1–12 (2017).
185. Hammar, E., Tomas, A., Bosco, D. & Halban, P. A. Role of the Rho–ROCK (Rho-associated kinase) signaling pathway in the regulation of pancreatic β-cell function. *Endocrinology* **150**, 2072–2079 (2009).
186. Weber, D. J. FDA regulation of allogeneic islets as a biological product. *Cell Biochem. Biophys.* **40**, 19–22 (2004).
187. Hering, B. et al. Clinical islet transplantation (CIT) protocol CIT-07. Islet Transplantation in Type 1 diabetes. *IsletStudy* https://www.isletstudy.org/CITDocs/CIT-07%20Protocol%20ver%202.0F%20_11%20Oct%202007_.pdf (2007).
188. Yamamoto, T. et al. Quality control for clinical islet transplantation: organ procurement and preservation, the islet processing facility, isolation, and potency tests. *J. Hepatobiliary. Pancreat. Surg.* **16**, 131–136 (2009).
189. Kayton, N. S. et al. Human islet preparations distributed for research exhibit a variety of insulin-secretory profiles. *Am. J. Physiol. Endocrinol. Metab.* **308**, E592–E602 (2015).
190. Glieberman, A. L., Pope, B. D., Melton, D. A. & Parker, K. K. Building biomimetic potency tests for islet transplantation. *Diabetes* **70**, 347–363 (2021).
191. MacDonald, M. J. et al. Differences between Human and Rodent Pancreatic Islets. *J. Biol. Chem.* **286**, 18383–18396 (2011).
192. Bank, H. L. Assessment of islet cell viability using fluorescent dyes. *Diabetologia* **30**, (1987).
193. Colton, C. K. et al. Characterization of Islet Preparations. *Cell. Transplant.* https://doi.org/10.1016/B978-012369415-7/50007-7 (2007).
194. Hanson, M. S., Steffen, A., Danobeitia, J. S., Ludwig, B. & Fernandez, L. A. Flow cytometric quantification of glucose-stimulated β-cell metabolic flux can reveal impaired islet functional potency. *Cell Transplant.* **17**, 1337–1347 (2008).
195. Goto, M., Holgersson, J., Kumagai-Braesch, M. & Korsgren, O. The ADP/ATP ratio: a novel predictive assay for quality assessment of isolated pancreatic islets. *Am. J. Transplant.* **6**, 2483–2487 (2006).
196. Armann, B., Hanson, M. S., Hatch, E., Steffen, A. & Fernandez, L. A. Quantification of basal and stimulated ROS levels as predictors of islet potency and function. *Am. J. Transplant.* **7**, 38–47 (2007).
197. Pepper, A. R. et al. The islet size to oxygen consumption ratio reliably predicts reversal of diabetes posttransplant. *Cell Transplant.* **21**, 2797–2804 (2012).
198. Saravanan, P. B. et al. Islet damage during isolation as assessed by miRNAs and the correlation of miRNA levels with posttransplantation outcome in islet autotransplantation. *Am. J. Transplant.* **18**, 982–989 (2018).
199. Todorov, I. et al. Quantitative assessment of β-cell apoptosis and cell composition of isolated, undisrupted human islets by laser scanning cytometry. *Transplantation* **90**, 836–842 (2010).
200. Ilegems, E. et al. Light scattering as an intrinsic indicator for pancreatic islet cell mass and secretion. *Sci. Rep.* **5**, 10740 (2015).
201. Agrawalla, B. K. et al. Glucagon-secreting α cell selective two-photon fluorescent probe TP-α: for live pancreatic islet imaging. *J. Am. Chem. Soc.* **137**, 5355–5362 (2015).
202. Kang, N. Y. et al. Multimodal imaging probe development for pancreatic β cells: from fluorescence to pet. *J. Am. Chem. Soc.* **142**, 3430–3439 (2020).
203. MacDonald, P. E. & Rorsman, P. Oscillations, intercellular coupling, and insulin secretion in pancreatic β cells. *PLoS Biol.* **4**, e49 (2006).
204. Pfeifer, C. R. et al. Quantitative analysis of mouse pancreatic islet architecture by serial block-face SEM. *J. Struct. Biol.* **189**, 44–52 (2015).
205. Street, C. N. et al. Islet graft assessment in the Edmonton Protocol: implications for predicting long-term clinical outcome. *Diabetes* **53**, 3107–3114 (2004).
206. Seino, S., Shibasaki, T. & Minami, K. Dynamics of insulin secretion and the clinical implications for obesity and diabetes. *J. Clin. Investig.* **121**, 2118–2125 (2011).

207. Lyon, J. et al. Research-focused isolation of human islets from donors with and without diabetes at the Alberta Diabetes Institute IsletCore. *Endocrinology* **57**, 560–569 (2016).
208. Song, W. J., Mondal, P., Li, Y., Lee, S. E. & Hussain, M. A. Pancreatic β-cell response to increased metabolic demand and to pharmacologic secretagogues requires epac2a. *Diabetes* **62**, 2796–2807 (2013).
209. Hallakou-Bozec, S., Kergoat, M., Fouqueray, P., Bolze, S. & Moller, D. E. Imeglimin amplifies glucose-stimulated insulin release from diabetic islets via a distinct mechanism of action. *PLoS ONE* **16**, (2021).
210. Yoshida, W. et al. Selection of DNA aptamers against insulin and construction of an aptameric enzyme subunit for insulin sensing. *Biosens. Bioelectron.* **24**, 1116–1120 (2009).
211. Rafati, A., Zarrabi, A., Abediankenari, S., Aarabi, M. & Gill, P. Sensitive colorimetric assay using insulin G-quadruplex aptamer arrays on DNA nanotubes coupled with magnetic nanoparticles. *R. Soc. Open Sci.* **5**, 171835 (2018).
212. Dishinger, J. F. & Kennedy, R. T. Serial immunoassays in parallel on a microfluidic chip for monitoring hormone secretion from living cells. *Anal. Chem.* **79**, 947–954 (2007).
213. Shigeto, H. et al. Insulin sensor cells for the analysis of insulin secretion responses in single living pancreatic β cells. *Analyst* **144**, 3765–3772 (2019).
214. Poudineh, M. et al. A fluorescence sandwich immunoassay for the real-time continuous detection of glucose and insulin in live animals. *Nat. Biomed. Eng.* **5**, 53–63 (2021).
215. Eliasson, L., Renström, E., Ding, W. G., Proks, P. & Rorsman, P. Rapid ATP-dependent priming of secretory granules precedes Ca^{2+}-induced exocytosis in mouse pancreatic B-cells. *J. Physiol.* **503**, 399–412 (1997).
216. Henquin, J. C., Dufrane, D. & Nenquin, M. Nutrient control of insulin secretion in isolated normal human islets. *Diabetes* **55**, 3470–3477 (2006).
217. Tarasov, A., Dusonchet, J. & Ashcroft, F. Metabolic regulation of the pancreatic beta-cell ATP-sensitive K+ channel: A pas de deux. *Diabetes* **53**, S113–S122 (2004).
218. Göpel, S., Kanno, T., Barg, S., Galvanovskis, J. & Rorsman, P. Voltage-gated and resting membrane currents recorded from B-cells in intact mouse pancreatic islets. *J. Physiol.* **521**, 717–728 (1999).
219. Braun, M. et al. Voltage-gated ion channels in human pancreatic β-cells: electrophysiological characterization and role in insulin secretion. *Diabetes* **57**, 1618–1628 (2008).
220. Camunas-Soler, J. et al. Patch-seq links single-cell transcriptomes to human islet dysfunction in diabetes. *Cell Metab.* **31**, 1017-1031.e4 (2020).
221. Speier, S. & Rupnik, M. A novel approach to in situ characterization of pancreatic β-cells. *Pflug. Arch. Eur. J. Physiol.* **446**, 553–558 (2003).
222. Marciniak, A. et al. Using pancreas tissue slices for in situ studies of islet of Langerhans and acinar cell biology. *Nat. Protoc.* **9**, 2809–2822 (2014).
223. Mohammed, J. S., Wang, Y., Harvat, T. A., Oberholzer, J. & Eddington, D. T. Microfluidic device for multimodal characterization of pancreatic islets. *Lab. Chip* **9**, 97–106 (2009).
224. Jun, Y. et al. In vivo–mimicking microfluidic perfusion culture of pancreatic islet spheroids. *Sci. Adv.* **5**, eaax4520 (2019).
225. Glieberman, A. L. et al. Synchronized stimulation and continuous insulin sensing in a microfluidic human Islet on a Chip designed for scalable manufacturing. *Lab. Chip* **19**, 2993–3010 (2019).
226. Misun, P. M. et al. In vitro platform for studying human insulin release dynamics of single pancreatic islet microtissues at high resolution. *Adv. Biosyst.* **4**, 1900291 (2020).
227. Tao, T. et al. Engineering human islet organoids from iPSCs using an organ-on-chip platform. *Lab Chip* **19**, 948–958 (2019).
228. Lundberg, M., Eriksson, A., Tran, B., Assarsson, E. & Fredriksson, S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. *Nucleic Acids Res.* **39**, e102–e102 (2011).
229. Li, X., Hu, J. & Easley, C. J. Automated microfluidic droplet sampling with integrated, mix-and-read immunoassays to resolve endocrine tissue secretion dynamics. *Lab. Chip* **18**, 2926–2935 (2018).
230. Byrnes, S. A. et al. Wash-Free, digital immunoassay in polydisperse droplets. *Anal. Chem.* **92**, 3535–3543 (2020).
231. Chen, Z. et al. Multiplexed, sequential secretion analysis of the same single cells reveals distinct effector response dynamics dependent on the initial basal state. *Adv. Sci.* **6**, 1801361 (2019).
232. Shapiro, A. M. J. et al. International trial of the Edmonton Protocol for islet transplantation. *N. Engl. J. Med.* **355**, 1318–1330 (2006).
233. Brennan, D. C. et al. Long-term follow-up of the Edmonton Protocol of islet transplantation in the United States. *Am. J. Transplant.* **16**, 509–517 (2016).
234. Ricordi, C. et al. National Institutes of Health-sponsored clinical islet transplantation consortium phase 3 trial: manufacture of a complex cellular product at eight processing facilities. *Diabetes* **65**, 3418–3428 (2016).
235. Hering, B. J. et al. Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia. *Diabetes Care* **39**, 1230–1240 (2016).
236. Foster, E. D. et al. Improved health-related quality of life in a phase 3 islet transplantation trial in type 1 diabetes complicated by severe hypoglycemia. *Diabetes Care* **41**, dc171779 (2018).
237. Fuchs, J. & Hovorka, R. Closed-loop control in insulin pumps for type-1 diabetes mellitus: safety and efficacy. *Expert Rev. Med. Devices* **17**, 707–720 (2020).
238. Bruin, J. E. et al. Accelerated maturation of human stem cell-derived pancreatic progenitor cells into insulin-secreting cells in immunodeficient rats relative to mice. *Stem Cell Rep.* **5**, 1081–1096 (2015).
239. Schulz, T. C. Concise review: manufacturing of pancreatic endoderm cells for clinical trials in type 1 diabetes. *Stem Cell Transl. Med.* **4**, 927–931 (2015).
240. van der Torren, C. R. et al. Immunogenicity of human embryonic stem cell-derived β cells. *Diabetologia* **60**, 126–133 (2017).
241. Henry, R. R. et al. Initial clinical evaluation of VC-01TM combination product — a stem cell-derived islet replacement for type 1 diabetes (T1D). *Diabetes* **67**, 138-OR (2018).
242. Bruin, J. E. et al. Treating diet-induced diabetes and obesity with human embryonic stem cell-derived pancreatic progenitor cells and antidiabetic drugs. *Stem Cell Rep.* **4**, 605–620 (2015).
243. Saber, N. et al. Sex differences in maturation of human embryonic stem cell-derived β cells in mice. *Endocrinology* **159**, 1827–1841 (2018).
244. Bruin, J. E. et al. Hypothyroidism impairs human stem cell-derived pancreatic progenitor cell maturation in mice. *Diabetes* **65**, 1297–1309 (2016).
245. Nair, G. G., Tzanakakis, E. S. & Hebrok, M. Emerging routes to the generation of functional β-cells for diabetes mellitus cell therapy. *Nat. Rev. Endocrinol.* **16**, 506–518 (2020).
246. Schulz, T. C. et al. A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. *PLoS ONE* **7**, 37004 (2012).
247. Konagaya, S. & Iwata, H. Chemically defined conditions for long-term maintenance of pancreatic progenitors derived from human induced pluripotent stem cells. *Sci. Rep.* **9**, 1–10 (2019).
248. Cheng, X. et al. Self-renewing endodermal progenitor lines generated from human pluripotent stem cells. *Cell Stem Cell* **10**, 371–384 (2012).
249. Sneddon, J. B., Borowiak, M. & Melton, D. A. Self-renewal of embryonic-stem-cell-derived progenitors by organ-matched mesenchyme. *Nature* **491**, 765–768 (2012).
250. Stock, A. A. et al. Conformal coating of stem cell-derived islets for β cell replacement in type 1 diabetes. *Stem Cell Rep.* **14**, 91–104 (2020).
251. Desai, T. & Shea, L. D. Advances in islet encapsulation technologies. *Nat. Rev. Drug Discov.* **16**, 338–350 (2017).
252. Rafael, E., Wernerson, A., Arner, P., Wu, G. S. & Tibell, A. In vivo evaluation of glucose permeability of an immunoisolation device intended for islet transplantation: a novel application of the microdialysis technique. *Cell Transplant.* **8**, 317–326 (1999).
253. Motté, E. et al. Composition and function of macroencapsulated human embryonic stem cell-derived implants: comparison with clinical human islet cell grafts. *Am. J. Physiol. Metab.* **307**, E838–E846 (2014).
254. Robert, T. et al. Functional β cell mass from device-encapsulated hESC-derived pancreatic endoderm achieving metabolic control. *Stem Cell Rep.* **10**, 739–750 (2018).
255. Kirk, K., Hao, E., Lahmy, R. & Itkin-Ansari, P. Human embryonic stem cell derived islet progenitors mature inside an encapsulation device without evidence of increased biomass or cell escape. *Stem Cell Res.* **12**, 807–814 (2014).
256. Bruin, J. E. et al. Maturation and function of human embryonic stem cell-derived pancreatic progenitors in macroencapsulation devices following transplant into mice. *Diabetologia* **56**, 1987–1998 (2013).
257. Haller, C. et al. Macroencapsulated human iPSC-derived pancreatic progenitors protect against STZ-induced hyperglycemia in mice. *Stem Cell Rep.* **12**, 787–800 (2019).
258. Lee, S.-H. et al. Human β-cell precursors mature into functional insulin-producing cells in an immunoisolation device: implications for diabetes cell therapies. *Transplantation* **87**, 983–991 (2009).
259. Bukys, M. A. Xeno-Transplantation of macro-encapsulated islets and pluripotent stem cell-derived pancreatic progenitors without Immunosuppression. *J. Stem Cell Transplant. Biol.* **02**, 1–19 (2017).
260. Gala-Lopez, B. L. et al. Subcutaneous clinical islet transplantation in a prevascularized subcutaneous pouch — preliminary experience. *CellR4* **18**, e2132 (2016).
261. Carlsson, P. O. et al. Transplantation of macroencapsulated human islets within the bioartificial pancreas βAir to patients with type 1 diabetes mellitus. *Am. J. Transplant.* **18**, 1735–1744 (2018).
262. Pepper, A. R. et al. A prevascularized subcutaneous device-less site for islet and cellular transplantation. *Nat. Biotechnol.* **33**, 518–523 (2015).
263. Evron, Y. et al. Long-term viability and function of transplanted islets macroencapsulated at high density are achieved by enhanced oxygen supply. *Sci. Rep.* **8**, 6508 (2018).
264. Bluestone, J. A. & Tang, Q. Solving the puzzle of immune tolerance for β-cell replacement therapy for type 1 diabetes. *Cell Stem Cell* **27**, 505–507 (2020).
265. Ferreira, L. M. R., Muller, Y. D., Bluestone, J. A. & Tang, Q. Next-generation regulatory T cell therapy. *Nat. Rev. Drug Discov.* **18**, 749–769 (2019).
266. Casey, S. C. et al. MYC regulates the antitumor immune response through CD47 and PD-L1. *Sci* **352**, 227–231 (2016).
267. Ezquer, F. et al. The antidiabetic effect of mesenchymal stem cells is unrelated to their transdifferentiation potential but to their capability to restore T_H1/T_H2 balance and to modify the pancreatic microenvironment. *Stem Cell* **30**, 1664–1674 (2012).
268. Graham, J. G. et al. PLG scaffold delivered antigen-specific regulatory T cells induce systemic tolerance in autoimmune diabetes. *Tissue Eng. Part. A* **19**, 1465–1475 (2013).
269. Deuse, T. et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat. Biotechnol.* **37**, 252–258 (2019).
270. Han, X. et al. Generation of hypoimmunogenic human pluripotent stem cells. *Proc. Natl Acad. Sci. USA* **116**, 10441–10446 (2019).
271. Cai, E. P. et al. Genome-scale in vivo CRISPR screen identifies RNLS as a target for β cell protection in type 1 diabetes. *Nat. Metab.* **2**, 934–945 (2020).
272. Wolf-Van Buerck, L. et al. LEA29Y expression in transgenic neonatal porcine islet-like cluster promotes long-lasting xenograft survival in humanized mice without immunosuppressive therapy. *Sci. Rep.* **7**, 1–9 (2017).
273. Lee, S. et al. Analysis on migration and activation of live macrophages on transparent flat and nanostructured titanium. *Acta Biomater.* **7**, 2337–2344 (2011).
274. Liu, J. M. H. et al. Transforming growth factor-β1 delivery from microporous scaffolds decreases inflammation post-implant and enhances function of transplanted islets. *Biomaterials* **80**, 11–19 (2016).
275. Alagpulinsa, D. A. et al. Alginate-microencapsulation of human stem cell–derived β cells with CXCL12 prolongs their survival and function in immunocompetent mice without systemic immunosuppression. *Am. J. Transplant.* **19**, 1930–1940 (2019).
276. Wang, X. et al. Point mutations in the PDX1 transactivation domain impair human β-cell development and function. *Mol. Metab.* **24**, 80–97 (2019).
277. Bilekova, S., Sachs, S. & Lickert, H. Pharmacological targeting of endoplasmic reticulum stress in pancreatic β cells. *Trends Pharmacol. Sci.* **42**, 85–95 (2021).
278. Shang, L. et al. β-cell dysfunction due to increased ER stress in a stem cell model of Wolfram syndrome. *Diabetes* **63**, 923–933 (2014).

279. Maxwell, K. G. et al. Gene-edited human stem cell-derived β cells from a patient with monogenic diabetes reverse preexisting diabetes in mice. *Sci. Transl Med.* **12**, 9106 (2020).
280. Balboa, D. et al. Insulin mutations impair beta-cell development in a patient-derived iPSC model of neonatal diabetes. *Elife* **7**, (2018).
281. Montaser, H. et al. Loss of MANF causes childhood onset syndromic diabetes due to increased endoplasmic reticulum stress. *Diabetes* **70**, 1006–1018 (2021).
282. Zeng, H. et al. An isogenic human ESC platform for functional evaluation of genome-wide-association-study-identified diabetes genes and drug discovery. *Cell Stem Cell* **19**, 326–340 (2016).
283. Guo, M. et al. Using hESCs to probe the interaction of the diabetes-associated genes CDKAL1 and MT1E. *Cell Rep.* **19**, 1512–1521 (2017).
284. Hosokawa, Y. et al. Insulin-producing cells derived from 'induced pluripotent stem cells' of patients with fulminant type 1 diabetes: vulnerability to cytokine insults and increased expression of apoptosis-related genes. *J. Diabetes Investig.* **9**, 481–493 (2018).
285. Wang, Y. J. et al. Multiplexed in situ imaging mass cytometry analysis of the human endocrine pancreas and immune system in type 1 diabetes. *Cell Metab.* **29**, 769-783.e4 (2019).
286. Hay, M., Thomas, D. W., Craighead, J. L., Economides, C. & Rosenthal, J. Clinical development success rates for investigational drugs. *Nat. Biotechnol.* **32**, 40–51 (2014).
287. DiMasi, J. A., Feldman, L., Seckler, A. & Wilson, A. Trends in risks associated with new drug development: success rates for investigational drugs. *Clin. Pharmacol. Ther.* **87**, 272–277 (2010).
288. Borowiak, M. et al. Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. *Cell Stem Cell* **4**, 348–358 (2009).
289. Zhu, S. et al. A small molecule primes embryonic stem cells for differentiation. *Cell Stem Cell* **4**, 416–426 (2009).
290. Korostylev, A. et al. A high-content small molecule screen identifies novel inducers of definitive endoderm. *Mol. Metab.* **6**, 640–650 (2017).
291. Amin, S. et al. Discovery of a drug candidate for GLIS3-associated diabetes. *Nat. Commun.* **9**, 1–12 (2018).
292. Hasilo, C. et al. Methods of treating diabetes using devices for cellular transplantation. US Patent Application 14/993,416 (2016).
293. Chang, R. et al. Nanoporous immunoprotective device for stem-cell-derived β-cell replacement therapy. *ACS Nano* **11**, 7747–7757 (2017).
294. Lammert, E. & Thorn, P. The role of the islet niche on β cell structure and function. *J. Mol. Biol.* **432**, 1407–1418 (2020).
295. Brissova, M. et al. Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J. Histochem. Cytochem.* **53**, 1087–1097 (2005).
296. Bonner-Weir, S. & Orci, L. New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* **31**, 883–889 (1982).
297. Rodriguez-Diaz, R. & Caicedo, A. Neural control of the endocrine pancreas. *Best. Pract. Res. Clin. Endocrinol. Metab.* **28**, 745–756 (2014).
298. Llacua, L. A., Faas, M. M. & de Vos, P. Extracellular matrix molecules and their potential contribution to the function of transplanted pancreatic islets. *Diabetologia* **61**, 1261–1272 (2018).
299. Brunicardi, F. C. et al. Immunoneutralization of somatostatin, insulin, and glucagon causes alterations in islet cell secretion in the isolated perfused human pancreas. *Pancreas* **23**, 302–308 (2001).
300. Rodriguez-Diaz, R. et al. Paracrine interactions within the pancreatic islet determine the glycemic set point. *Cell Metab.* **27**, 549–558 (2018).
301. Meda, P., Kohen, E., Kohen, C., Rabinovitch, A. & Orci, L. Direct communication of homologous and heterologous endocrine islet cells in culture. *J. Cell Biol.* **92**, 221–226 (1982).
302. Konstantinova, I. et al. EphA–Ephrin-A-mediated β cell communication regulates insulin secretion from pancreatic islets. *Cell* **129**, 359–370 (2007).
303. Briant, L. J. B. et al. δ-Cells and β-cells are electrically coupled and regulate α-cell activity via somatostatin. *J. Physiol.* **596**, 197–215 (2018).
304. Swisa, A. et al. PAX6 maintains β cell identity by repressing genes of alternative islet cell types. *J. Clin. Invest.* **127**, 230–243 (2017).
305. Gu, C. et al. Pancreatic β cells require NeuroD to achieve and maintain functional maturity. *Cell Metab.* **11**, 298–310 (2010).
306. Gao, T. et al. Pdx1 maintains β cell identity and function by repressing an α cell program. *Cell Metab.* **19**, 259–271 (2014).
307. Du, A. et al. Islet-1 is required for the maturation, proliferation, and survival of the endocrine pancreas. *Diabetes* **58**, 2059–2069 (2009).
308. Russell, R. et al. Loss of the transcription factor MAFB limits β-cell derivation from human PSCs. *Nat. Commun.* **11**, 1–15 (2020).
309. Bevacqua, R. J. et al. SIX2 and SIX3 coordinately regulate functional maturity and fate of human pancreatic β cells. *Genes Dev.* **35**, 234–249 (2021).
310. Pullen, T. J. & Rutter, G. A. When less is more: the forbidden fruits of gene repression in the adult β-cell. *Diabetes Obes. Metab.* **15**, 503–512 (2013).
311. Lee, A. H., Heidtman, K., Hotamisligil, G. S. & Glimcher, L. H. Dual and opposing roles of the unfolded protein response regulated by IRE1α and XBP1 in proinsulin processing and insulin secretion. *Proc. Natl Acad. Sci. USA* **108**, 8885–8890 (2011).
312. Leighton, E., Sainsbury, C. A. & Jones, G. C. A practical review of C-peptide testing in diabetes. *Diabetes Ther.* **8**, 475–487 (2017).

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