Direct reprogramming of human fibroblasts into insulin-producing cells using transcription factors

Marta Fontcuberta-PiSunyer1, Ainhoa García-Alamán1,2, Èlia Prades1, Noèlia Téllez2,3,4, Hugo Alves-Figueiredo1,13, Mireia Ramos-Rodríguez5, Carlos Enrich1,6, Rebeca Fernandez-Ruiz1,2, Sara Cervantes1, Laura Clua7, Javier Ramón-Azcón1,8, Christophe Broca9, Anne Wojtusciszyn9,10, Nuria Montserrat7,8,11, Lorenzo Pasquali5, Anna Novials1,2, Joan-Marc Servitja1,2, Josep Vidal1,2,6,12, Ramon Gomis1,2,6 & Rosa Gasa1,2

Direct lineage reprogramming of one somatic cell into another without transitioning through a progenitor stage has emerged as a strategy to generate clinically relevant cell types. One cell type of interest is the pancreatic insulin-producing β cell whose loss and/or dysfunction leads to diabetes. To date it has been possible to create β-like cells from related endodermal cell types by forcing the expression of developmental transcription factors, but not from more distant cell lineages like fibroblasts. In light of the therapeutic benefits of choosing an accessible cell type as the cell of origin, in this study we set out to analyze the feasibility of transforming human skin fibroblasts into β-like cells. We describe how the timed-introduction of five developmental transcription factors (Neurog3, Pdx1, MafA, Pax4, and Nkx2-2) promotes conversion of fibroblasts toward a β-cell fate. Reprogrammed cells exhibit β-cell features including β-cell gene expression and glucose-responsive intracellular calcium mobilization. Moreover, reprogrammed cells display glucose-induced insulin secretion in vitro and in vivo. This work provides proof-of-concept of the capacity to make insulin-producing cells from human fibroblasts via transcription factor-mediated direct reprogramming.

1Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain. 2CIBER de Diabetes y Enfermedades Metabólicas Asociadas, Instituto de Salud Carlos III, Madrid, Spain. 3Faculty of Medicine of University of Vic, Central University of Catalonia (UVic-UCC), Vic, Spain. 4Institute of Health Research and Innovation at Central Catalonia (IRIS-CC), Vic, Spain. 5Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona, Spain. 6Faculty of Medicine and Health Sciences, Universitat de Barcelona, Barcelona, Spain. 7Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Technology (BIST), Barcelona, Spain. 8Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain. 9CHU Montpellier, Laboratory of Cell Therapy for Diabetes (LTCD), Hospital St-Eloi, Montpellier, France. 10Service of Endocrinology, Diabetes and Metabolism, Lausanne University Hospital, Lausanne, Switzerland. 11CIBER de Bioingeniería, Biomateriales y Nanomedicina, Instituto de Salud Carlos III, Madrid, Spain. 12Endocrinology and Nutrition Department, Hospital Clinic of Barcelona, Barcelona, Spain. 13Present address: Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, N.L., México.✉email: rgasa@recerca.clinic.cat
Direct lineage reprogramming entails the direct conversion of one differentiated cell type into another bypassing an intermediate pluripotent stage. This strategy is often based on the forced expression of cocktails of transcription factors that function as potent fate determinants during development of the cell type of interest\(^1\). As the number of cell types produced through direct conversion has rapidly increased in recent years, this strategy has emerged as a possible method for creating cell types with potential for use in therapeutic settings.

Pancreatic beta (\(\beta\)) cells produce insulin, which controls whole body glucose homeostasis. Diabetes is characterized by a relative or total lack of functional \(\beta\) cells, and cell replacement therapy has consequently emerged as a promising therapeutic option to treat and ultimately cure this disease. One of the strategies pursued to produce replacement \(\beta\) cells has been direct lineage reprogramming. A major breakthrough in this area was the discovery that three developmental transcription factors, namely Pdx1, Neurog3, and MafA, promoted the in situ conversion of acinar cells into \(\beta\)-cell-like cells. In this regard, skin fibroblasts have been the preferred cell source for many reprogramming protocols and they have so far been successfully transformed into a variety of somatic cell types including cardiomyocytes\(^12\), chondrocytes\(^13\), neurons\(^14\), oligodendrocyte progenitors\(^15\), hepatocytes\(^16\) or endothelial cells\(^17\). However, research to date suggests that fibroblasts are resistant to being transformed into \(\beta\)-like cells using lineage-specific transcription factors\(^4,9,18\).

In light of the expanding number of cell types produced by direct lineage conversion procedures and the therapeutic interest of insulin-producing cells, we chose to thoroughly consider the viability of using fibroblasts as cells of origin in direct reprogramming protocols to generate \(\beta\)-like cells. Here we present a protocol based on a cocktail of five endocrine transcription factors that induces human fibroblasts to activate the \(\beta\)-cell transcriptional program while downregulating their native fibroblastic transcriptional program, resulting in the generation of cells that produce and secrete insulin in vitro and in vivo. We believe these findings demonstrate the feasibility of this approach and set the basis to further explore this alternative path for generation of \(\beta\)-like cells for disease modeling and cellular therapy.

Results

Exogenous expression of the transcription factors Pdx1, Neurog3, and MafA in human fibroblasts. We first sought to examine whether the transcription factors Neurog3, Pdx1, and MafA could induce expression of the INSULIN (INS) gene in human fibroblasts as readout of the capacity of these cells to be transformed toward a \(\beta\)-cell fate. To deliver these factors we employed a polycistronic adenoviral vector carrying the three transgenes (Ad-NPM hereafter), which had been previously used to promote \(\beta\)-cell reprogramming from pancreatic acinar cells\(^19\). After optimization of adenoviral transduction in fibroblasts (see Methods), abundant (>80%) cells positive for Cherry, which is also encoded by Ad-NPM, were easily observable three and seven days after viral infection (Fig. 1a). Likewise, high levels of transcripts encoding the NPM factors were expressed at both time points (Fig. 1b). Three days after addition of Ad-NPM we detected marginal levels of INS mRNA that were increased >10-fold by day 7 (Fig. 1c). As cell culture formulations can have a major impact on gene expression events and cellular reprogramming, we tested different conditions after Ad-NPM infection. We observed that moving to RPMI-1640 and, to a lesser extent, CMRL-1066 medium and lowering the fetal calf serum concentration to 6% dramatically boosted INS gene activation, reaching values that were 0.12% those of human islets (Fig. 1d and Supplementary Figure 1). Under the same culture conditions, only a very marginal induction of the INS gene occurred when the N + P + M factors were delivered simultaneously via distinct adenoviruses to human fibroblasts (Supplementary Fig. 2). In addition to INS, we discovered that the NPM factors also activated the hormone genes GLUCAGON (GCG) and SOMATOSTATIN (SST), albeit at lower levels than INS as indicated by decreased relative expression values (compared to the housekeeping gene TBP) (Fig. 1e). The NPM factors also induced expression of genes encoding islet differentiation transcription factors including NEUROD1, INSM1, PAX4, NKX2-2, and ARX (Fig. 1f).

To further establish if the NPM factors promoted cell fate conversion and not simply activated their target genes in fibroblasts, we surveyed expression of genes associated with the fibroblastic signature, including several factors involved in maintenance of the fibroblastic transcriptional network such as TWIST2, PRRX1, and LHX9\(^20\). We found that these genes were downregulated as early as three days after NPM introduction. Other fibroblast markers exhibited a more delayed response but, by day 7 post-NPM, all tested genes exhibited significant down-regulation (Fig. 1g). Together, these experiments validate that islet cell fate can be induced in human fibroblasts using a defined set of transcription factors.

Addition of exogenous Pax4 and Nkx2-2 after the NPM reprogramming cocktail in human fibroblasts. The observed induction of the islet hormone genes GCG and SST implied that the NPM factors might not specifically endorse \(\beta\)-cell fate in fibroblasts. Furthermore, we found that these factors did not induce NKX6-1, which encodes a \(\beta\)-cell specific factor required for the formation of pancreatic \(\beta\) cells during development\(^24\) and key for optimal maturation of stem cell \(\beta\) cells in vivo\(^22,23\) (Fig. 2a). These findings indicated that the NPM factors sub-optimally promoted a \(\beta\)-cell state in human fibroblasts. In order to enhance \(\beta\)-cell fate over other islet cell identities, we opted to add new transcription factors to the reprogramming cocktail. Pax4 is activated downstream of Neurog3 during development\(^24\) and has been shown to favor \(\beta\)-over \(\alpha\)-cell specification\(^25,26\), and to contribute to maintenance of the expression of Nkx6.1 in differentiating \(\beta\) cells\(^27\). Despite that the NPM factors induced endogenous PAX4 mRNA, the expression levels attained might not be sufficient to endorse \(\beta\)-over \(\alpha\)-cell fate. Hence, we treated fibroblasts with an adenovirus encoding Pax4 three days after NPM (Fig. 2a). This resulted in the significant enhancement of INS expression as compared to NPM alone but, unexpectedly, GCG expression was also increased (Fig. 2a), indicating that ectopic Pax4 improved islet hormone gene expression without apparent impact on \(\beta\)-versus \(\alpha\)-cell fate conversion in human fibroblasts.

As the NKX6-1 gene remained silent in response to NPM + Pax4 (Fig. 2a), we tried directly adding Nkx6-1 to the NPM reprogramming cocktail. However, exogenous Nkx6-1 resulted in considerable cell death irrespective of level of expression or timing of introduction. As an alternate approach, we added...
exogenous Nkx2-2, which also regulates early β-cell differentiation and is an upstream activator of Nkx6-1 during mouse islet development21. Treatment with an adenovirus encoding Nkx2-2 three days after NPM led to endogenous activation of NKX6-1 expression with no compromise of fibroblast viability (Fig. 2a). Nkx2-2 also induced PAX6, a pan-endocrine gene required to achieve high levels of islet hormone gene expression during mouse pancreas development28,29. Remarkably, ectopic Nkx2-2 reduced NPM-induced GCG gene activation without affecting INS gene expression (Fig. 2a).

During development, Pax4 and Nkx2-2 are found in β-cell precursors at around the same time, and their parallel activities...
INS impact on the minimal
Neither Pax4 nor Nkx2-2, added alone or together, had any
+NPM in the culture were INS
immuno
+NPM fi human
protein was more robust in cells reprogrammed with NPM
Following this protocol, the blockade of
Nkx2.2 sequentially, at day 3 and day 6 post-NPM, respectively.
transcription factor, we treated cells with Ad-Pax4 and Ad-
reprogramming cocktail. To ensure optimal expression of each
test the effects of including both transcription factors in the
are thought to enable the β-cell differentiation program. Hence,
we tested the effects of including both transcription factors in the
reprogramming cocktail. To ensure optimal expression of each
transcription factor, we treated cells with Ad-Pax4 and Ad-
Nkx2.2 sequentially, at day 3 and day 6 post-NPM, respectively.
Following this protocol, the blockade of GCG gene activation and
the induction of the NKX6.1 and PAX6 genes seen with
NPM + Nkx2.2, and the higher INS expression elicited by
NPM + Pax4 relative to NPM alone were all maintained (Fig. 2a).
Neither Pax4 nor Nkx2-2, added alone or together, had any
impact on the minimal INS gene induction shown when the
N + P + M factors were delivered via separate adenoviruses to
human fibroblasts (Supplementary Fig. 2).

Consistent with the gene expression data, staining for insulin protein was more robust in cells reprogrammed with NPM +
Pax4 + Nkx2.2 than in cells reprogrammed with NPM as assessed using two different antibodies, one against human insulin and another against human C-PEP to exclude possible
insulin uptake from the media (Fig. 2b). We quantified the immunofluorescence images and found that 67.9 ± 6.2% of cells
in the culture were INS+ at day 10.

Characterization of cells generated from fibroblasts using the
5TF- reprogramming cocktail. From here on, we used the
sequential introduction of the five transcription factors (5TF
protocol, Fig. 3a) to generate insulin-producing cells from human
fibroblasts (reprogrammed cells will be referred as 5TF cells). At
day 10, 5TF cells displayed an epithelial morphology (Fig. 3b) and
hadn't grown as much as untreated fibroblasts (day 10; 5TF:
44 × 10^3 ± 3 × 10^3 cells/well; control: 238 × 10^3 ± 18 × 10^3 cells/
well, n = 18). This decreased cell number was likely due to
diminished proliferation, which was evident as soon as one day
following Ad-NPM infection (Fig. 3c). The capacity of cells to
reduce the MTT compound, in contrast, was comparable to that of
fibroblasts, indicating that viability was not compromised
(Fig. 3d).

Next we studied expression of selected differentiation tran-
scription factor genes at days 10-11 of the protocol. All genes
tested, except Pax4, were more expressed in 5TF relative to NPM
(NEUROD1, INSM1, HNF1B, MAFB, PDX1, NEUROG3, NKX2.2)
(Fig. 3e). Likewise, several genes (PCSK1, KCNN11, GLP1R,
NCAM1) that are linked to β-cell function were increased in 5TF
cells as compared to NPM cells (Fig. 3f). Remarkably, some genes
were induced de novo by 5TF (ABCC8, GIPR) (Fig. 3f). In line
with a loss of GCG activation, the pro-convertase gene PCSK2,
which is expressed at higher levels in α than in β cells, was
reduced by 5TF as compared to NPM (Fig. 3f). These results
support that sequential introduction of Pax4 and Nkx2-2 after
NPM endorses the β-cell differentiation program in human
fibroblasts. β-cell gene activation was sustained for at least
twenty-one days after initiation of the protocol despite reduced
expression of the reprogramming factor transgenes (Fig. 3g, h).
Furthermore, expression of several of the tested genes increased
with time in culture including NKX6.1, PCSK1, KCNJ11, ABCC8
and CHGB among others (Fig. 3g), suggestive of permanent cell
lineage conversion.

![Fig. 2 Sequential addition of the transcription factors Pax4 and Nkx2-2 enhances β-cell fate in human fibroblasts expressing Neurog3, Pdx1, and MafA.](https://example.com/)
Glucose-induced insulin secretion by β cells is mediated by cellular glucose metabolism, closure of ATP-dependent potassium channels, membrane depolarization and opening of voltage-dependent calcium channels, resulting in an increase in cytosolic Ca\(^{2+}\) that triggers insulin exocytosis. We investigated whether 5TF cells increased intracellular Ca\(^{2+}\) in response to glucose and membrane depolarization elicited by high potassium. We found that 65% of the cells exhibited a response to glucose, high potassium, or both, whilst 35% of cells were unresponsive to either stimulus (Fig. 4a and Supplementary Video 1). Parental fibroblasts not engineered for 5TF expression were unresponsive to these stimuli (Fig. 4b and Supplementary Video 2). Among
responsive cells, approximately half responded to both glucose and high potassium and half responded only to potassium (Fig. 4a). We observed heterogeneity in the amplitude and kinetics of responses among individual cells (Fig. 4c). Next, we performed static incubation assays to study GSIS and found that 5TF cells released similar amounts of human insulin at low (2 mM) and high (20 mM) glucose concentrations (Fig. 4d). Thus, even though 5TF cells increased their intracellular calcium in response to glucose and membrane depolarization, they secreted insulin in a constitutive manner.

**Generation of 5TF cell spheroids and transcriptome-wide analysis.** The differentiation and functionality of many cell types vary dramatically between three-dimensional (3D) and two-dimensional (2D) monolayer cultures, the former being closer to the natural 3D microenvironment of cells in a living organism. Thus, we generated spheroids of 5TF cells (1200–1800 cells/spheroid; average diameter of 128 ± 27 µm) one day after the introduction of Nkx2-2 and maintained them in culture for three additional days (Fig. 5a). At the time of collection, insulin-positive staining was easily identified but glucagon and...
somatostatin staining was undetectable (Fig. 5b and Supplemental Fig. 3). While INS transcript levels were nearly 2-fold higher in 5TF cell spheroids compared to 5TF cells kept in monolayer, other β-cell marker genes, such as the prohormone convertase PCSK1 and the ATP-sensitive potassium channel subunits KCNJ11 and ABCC8, showed a higher response (4 to 5-fold) to 3D culture (Fig. 5c). Thus, cell aggregation during the last stage of reprogramming (note that total length of the protocol was not changed) conferred improved activation of genes associated to β-cell function. Despite increased gene activation, β-cell
gene expression in 5TF cell spheroids remained lower than in human islets, with differences ranging widely among examined genes (Fig. 5c).

To obtain a more comprehensive understanding of the cell identity switch induced by the 5TF-3D reprogramming protocol, we performed RNA-sequencing of 5TF cell spheroids and parental fibroblasts. A total of 2806 genes (1186 upregulated, 1620 downregulated) were differentially expressed between both cell populations (adjusted p-value <0.05 and fold-change (FC) > 2) (Fig. 5d and Supplementary Data 1). Gene set enrichment analysis (GSEA) showed that pancreas/β-cell and peptide hormone metabolism gene sets were enriched in 5TF cells (Fig. 5e). Biological functions associated with gained genes included epithelium development, synaptic signaling, ion transport, calcium sensing and secretion (Fig. 5f). Among the upregulated genes related to stimulus-secretion coupling, there were synaptotagmins (SYT1,2,3,6,13,17), syntaxins (SYN2, SYN3), calcium sensors (SCG2) and SNARE protein complexes (VAMP1). Correlating with our previous results, cell cycle and mitotic function genes were enriched among repressed genes (Fig. 5e, f). Additionally, GSEA demonstrated that 5TF cells had a lower expression of the gene set associated with the epithelial-mesenchymal transition (Fig. 5c). In agreement, functions including cytoskeleton organization and cellular migration were overrepresented among lost genes (Fig. 5g). Interestingly, GSEA also revealed that the β-cell disallowed gene set, which includes genes that are selectively suppressed in β cells and believed to be detrimental for β cell function31–33, was reduced in 5TF cells (Fig. 5g). A total of 23 previously recognized β-cell disallowed were significantly downregulated in 5TF cells (Fig. 5h). By using qPCR, we confirmed the repression of three of these genes – OAT, LDHA, and SMAD3 – which are regarded as part of the core disallowed unit33. Of note, the levels of these genes in 5TF cells matched those of human islets (Fig. 5h). Collectively, these results show that 5TF-3D reprogramming promotes a change in the fibroblast transcriptome, including selective gene activation along with specific gene repression events, enabling a change in cell identity from fibroblast towards a β-cell fate.

Ultrastructure and insulin secretory features of 5TF cell spheroids. Consistent with gene activation events identified in prior gene expression analyses, immunofluorescence staining showed the presence of the mature β-cell markers PCSK1, NCAM1, and KCNJ11 (Kir6.2) in many insulin-positive 5TF cells. PTPRN (IA2) was also expressed albeit more sporadically in insulin-positive 5TF cells (Fig. 6a). Using conventional electron microscopy, we looked for the existence of secretory granules and discovered that most cells contained multiple spherical electron-dense prototypical secretory vesicles (Fig. 6b). These vesicles showed a high degree of morphological heterogeneity, presumably as consequence of their degree of maturation and/or loading. Although they did not have the appearance of typical insulin-containing granules from primary β cells, which are characterized by a clear halo surrounding a dark polygonal dense core34, some of the vesicles exhibited a gray or less electron dense halo and looked like the granules described in immature insulin-positive cells generated in early stem cell differentiation protocols35,36. We next performed static incubation GSIS assays. 5TF cell spheroids exhibited significant insulin secretory response to glucose (fold 20 mM/2 mM: 2.02 ± 0.18) as compared to 2D cultures (fold 20 mM/2 mM: 1.08 ± 0.15) (Fig. 6c, d). To establish the glucose threshold for stimulation of insulin secretion, 5TF cell spheroids were subjected to either 2, 5, 11 or 20 mM glucose. Between 2 mM and 11 mM/20 mM glucose, 5TF spheroids showed a 2.5-fold increase in insulin production on average (Fig. 6e). In contrast, although there was some variability, they did not show a statistically significant increase in insulin secretion between 2 mM and 5 mM glucose (Fig. 6e). These observations indicate that 5TF cell spheroids are stimulated at higher glucose threshold; it is interesting to note that human islets have a glucose threshold at 3 mM and a maximal response at 15 mM37.

The 5TF-3D protocol was repeated on an additional HFF line and produced results that were comparable (Supplementary Fig. 4) proving the reproducibility of the reprogramming protocol.

Transplantation of 5TF cell spheroids. Finally, we studied the stability of reprogramming in vivo. With this aim, we transplanted 300 5TF cell spheroids (1000–1200 cells/spheroid) into the anterior chamber of the eye (ACE) of non-diabetic immune-deficient NOD scid gamma (NSG) mice (Fig. 7a). The ACE allows fast engraftment38 and in vivo imaging39. Ten days following transplantation, we used two-photon microscopy to evaluate in vivo graft re-vascularization and confirmed the presence of functioning vessels in the grafts (Fig. 7b). Additionally, by observing the long-term tracer CFDA’s fluorescence, we confirmed that the transplanted cells were alive (Fig. 7b). To assess the maintenance of insulin expression in vivo, we harvested the eye grafts at day 10 for RNA extraction and immunostaining. Human INS mRNA was readily detectable and levels, calculated relative to human TBP, were comparable to those in 5TF cell clusters prior to transplantation (Fig. 7c). In agreement, abundant HLA + (human cell marker) cells that stained for insulin were detected in the eye grafts by immunofluorescence staining (43.5 ± 2.8% INS+HLA+/total HLA+, n = 5) (Fig. 7d, e and Supplementary Fig. 5). We observed positive staining for the reprogramming transcription factors in 20–30% of the INS + cells (Supplementary Figure 6). Although we were unable to discriminate between the two, high transgene expression found by qPCR analysis in eye grafts (Supplementary Fig. 6) indicated that the staining represented virally encoded exogenous protein rather than endogenous protein. Since adenoviral vectors do not normally integrate into the host DNA, we speculate that the cessation of cell division induced by reprogramming may explain persistent transgene expression in 5TF cells. In fact, similar findings were reported in reprogrammed human duct-derived insulin-producing cells8. We were able to identify INS + cells in 4 (of 5) grafts harvested one month after transplantation even though their number was reduced relative to day 10 grafts (Supplementary Fig. 7). The proportion of INS + HLA + cells in 30-day grafts was more heterogeneous than in 10-day grafts, and in 3 (of 5) grafts, it was comparable or even higher than that of 10-day grafts, demonstrating the maintenance of reprogramming (Supplementary Fig. 7).

To study if 5TF cells secreted insulin in vivo, we first measured the presence of human insulin by ELISA in the aqueous humor of the transplanted eyes. Human insulin was readily detectable in eyes carrying 5TF cell grafts (17 of 17, ranging from 76 to 1103 pmol/L) whilst no insulin was detected in eyes transplanted with parental fibroblast clusters or in non-transplanted mice (Fig. 7f). For comparison, eyes containing 300 5TF spheroids showed on average approximately 20-fold lower levels of human insulin than eyes containing 150–200 human islets (Fig. 7f). Due to space limitations in the ACE, we transplanted a larger number of spheroids (3500–5000) into the omentum of normoglycemic NSG mice in order to detect circulating human insulin in host animals. We measured low amounts of human insulin in the plasma of most transplanted mice, and these levels increased in 6 (of 10) mice after receiving an intraperitoneal glucose injection on day 30 post-transplantation (3.6 ± 0.9 vs 13.9 ± 3.7 pmol/L, p = 0.014).
Transplants were repeated in other locations yielding similar results (Supplementary Table 2). As observed in the ACE grafts, a low number of INS+ cells were identified in omentum grafts harvested at 30 days post-transplantation (Supplementary Fig. 8). These findings show that, despite restricted survival, reprogramming is maintained and 5TF cells maintain the capacity to release insulin in an in vivo setting.

Discussion
This study describes a direct reprogramming protocol based on the sequential introduction of five lineage-determining
transcription factors that induces β-cell fate in human fibroblasts. Reprogrammed fibroblasts exhibit the concomitant activation of β-cell genes and the repression of fibroblastic and β-cell disallowed genes. Significantly, reprogrammed cells display functional features of β cells, including the ability to mobilize calcium and secrete insulin upon glucose stimulation. To the best of our knowledge, this is the first instance where it has been shown that skin fibroblasts can serve as cells of origin for β-cell derivation using transcription factor-based direct conversion methodologies.

The N + P + M cocktail was initially described for the conversion of pancreatic exocrine cells into β cells in the mouse pancreas in situ. Following studies demonstrated that these factors were also able to reprogram other endoderm-derived gastrointestinal tract cell lineages towards β-like cells. Contrarily, information in the literature suggested that these transcription factors were ineffective in promoting β-cell fate from mesoderm-derived mouse and human fibroblasts. However, in our view, the available studies did not fully analyze this option.

In the present work, we examined a range of simultaneous and sequential transcription factor combinations, and were able to show that the N + P + M cocktail also works in human fibroblasts. Successful reprogramming is known to depend on a number of variables in addition to the selection of the proper reprogramming cocktail. We found that reprogramming was only possible when the three transcription factors were supplied by a single polycistronic adenovirus rather than multiple adenoviruses that expressed them separately, which is line with earlier research employing this same vector in acinar cells. This may be owing to the random nature of viral co-infection, which makes it impossible to ensure that every infected cell receives every transgene when employing distinct viruses. Two other crucial parameters in reprogramming are the stoichiometry and the expression level of the reprogramming factors. Regarding the former, a polycistronic expression system instead of using...
several vectors offers a more homogenous TF stoichiometry across the infected cells. We speculate that successful reprogramming in our study was made possible by the use of this polyclinastic construct and the high levels of transgene expression achieved in fibroblasts through a minor modification of the infection protocol. It is interesting that the NPM factors, identified as a β-cell promoting reprogramming cocktail in acinar cells, induced expression of the GCG and SST genes in human fibroblasts. Despite the fact that INS was the most activated by difference, we used other factors (Pax4, Nkx2-2) to confer enhanced β-cell specificity in fibroblasts. These observations highlight the need to customize conversion transcription factor cocktails to the selected cell source.

The 5TF-3D protocol used in our study promoted transcriptome alterations consistent with a change in cell identity from fibroblast toward a β-like cell. It is noteworthy that nearly 60% of the detected transcriptional changes are gene repression events. In addition to the downregulation of fibroblast-specific genes, we found that 5TF cells had selectively suppressed the group of β-cell disallowed genes. This finding suggests that, in a cellular environment of a different lineage, lineage-specific transcription factors can promote the precise suppression of potentially damaging genes for their lineage. Intriguingly, SLC16A1 (MCT1), one of the founding members of the β-cell disallowed gene set, was not repressed in 5TF cells. Developmentally, β-cell disallowed genes are marked for repression by Polycomb Group (PcG) proteins in pancreatic progenitors during pancreas organogenesis. However, the mechanisms that support their silencing in adult cells are still poorly understood and might vary amongst genes. These results spur future experiments into the way developmental transcription factors repress undesirable genes during the reprogramming process.

Experimental data showing that intracellular calcium concentration and insulin release were stimulated by glucose suggest that fibroblast-derived 5TF cells are progressing toward a β-cell at the functional level. The levels of INS gene activation reached in 5TF cells were higher than those of reprogrammed human hepatocytes and comparable to those reported for reprogrammed human pancreatic duct cells. They were, nonetheless, less than those of human islets. 5TF cells share traits with first generation pluripotent-derived β-like cells, such as decreased insulin production, low β-cell gene expression, and the absence of mature insulin granules. Hence, we acknowledge that there is room for advancement and that optimization of our current reprogramming procedure is required in order to generate cells that are more similar to a primary β cell. A potential strategy is to modify the cell culture settings. Here, for instance, we demonstrated how switching to a different culture media after the addition of the NPM factors noticeably improved INS gene activation. We also showed how switching 5TF cells from a 2D to a 3D culture system increased expression of key β-cell genes and enabled converted cells to develop the ability to release insulin in response to glucose. Thus, additional adjustments to culture conditions, like extended culture times or agitation, may be helpful to improve quality of the generated cells. In this same line, inclusion of soluble signaling molecules during or after introduction of the conversion transcription factors could be employed, as it has been shown in direct reprogramming examples towards neurons. In recent years, stem cell research has developed a significant body of knowledge on how to improve the maturity of pluripotent cell-derived insulin-secreting cells created in vitro. We anticipate that this information can be very helpful in the effort to optimize direct reprogramming approaches from somatic cell types toward the β-cell lineage.

Poor long-term survival of 5TF cells after transplantation precluded physiological studies in mouse models. Although the causes of cell loss remain to be elucidated, limited graft survival is a prevalent concern in transplantation of cadaveric donor islets and in vitro created islet tissue. In our case, it is possible that additional endocrine and/or non-endocrine cell types normally found in islets such as endothelial cells are required for better engraftment and prolonged survival. Additionally, our observation of sustained expression of exogenous reprogramming factors in 5TF cells raises the question of what effects this might have, especially the continued presence of Neurog3 and Pax4 given that these factors are not present in mature β cells. Future effort will be required to develop reprogramming strategies to guarantee that the conversion factors are turned off if necessary.

The generation of substitute β cells from a cell source that can be replenished has been a long-standing major goal in diabetes research. The most advanced approach to date involves the guided differentiation of pluripotent stem cells to islet cells. The first clinical studies involving actual patients have been made possible by the enormous advancements made in this field over the past fifteen years. Recent publication of the first midterm results of one of these trials shows positive outcomes as well as the need to continue improving current differentiation protocols and transplantation techniques. On the other hand, the idea of direct cellular reprogramming to produce therapeutically relevant cell types, as an alternative to their derivation from stem cells, regained momentum with the discovery of iPSCs. Since then, there has been a notable increase in the amount of somatic cells produced from readily accessible cell types, such as fibroblasts. In the β-cell field, however, available evidence indicated that human fibroblasts were not keen to change identity toward a β cell via direct reprogramming. Our results challenge this view and show that this is possible if the appropriate combination of conversion factors and conditions are found. The value of our approach is related to the major benefit of using a cell source that is readily available, such as the skin fibroblast, in terms of translational potential. Two further assets that should be taken into account from a clinical standpoint are the possibility of auto-transplantation and the avoidance of tumor-related complications linked to pluripotent cell states. Finally, the relative simplicity of direct reprogramming methodology compared to pluripotent stem cell derivation supports the ongoing interest in developing this kind of approaches.

In conclusion, here we demonstrate that human fibroblasts can be directly converted toward a β-cell fate using a defined set of developmental transcription factors. Further research should refine this strategy so that generated insulin-producing cells more closely resemble primary β cells. These findings provide a promising starting point for future investigation into an alternative pathway to produce β-like cells for therapeutic and modeling purposes.

Methods: Fibroblasts. Human fibroblasts were obtained from a child foreskin biopsy after signed informed consent and approval of the institutional Review Board of the Center of Regenerative Medicine in Barcelona. In brief, skin biopsy was collected in sterile saline solution, divided into small pieces, and allowed to attach to cell culture dishes before adding Iscove’s modified Dulbecco’s medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% human serum (Sigma, St. Louis, MO, USA) and penicillin/streptomycin (0.5X) (Invitrogen). After 10 days of culture at 37 °C, 5% CO2, fibroblast outgrowths were dissociated and split 1:4 using a recombinant trypsin-like Enzyme (TrypLE Select, Invitrogen). Preparation was negative for hematopoietic markers, including CD34. This fibroblast line (HFF1) was used to design the reprogramming protocol. Once established, the protocol was validated in another HFF preparation (HFF2) that was purchased from a commercial source (SCRC1041TM, ATCC, Manassas, VA, USA).
Human islets. Human islets were prepared by collagenase digestion followed by density gradient purification at the Laboratory of Cell Therapy for Diabetes (Hopital Saint-Eloi, Montpellier, France), as previously described. After reception in Barcelona, human islets were maintained in culture at 37 °C, 5% CO2; for 1–3 days in RPMI-1640 with 5.5 mM glucose, 10% fetal bovine serum (FBS) and antibiotics, before performing the experiments. Experiments were performed in agreement with the local ethic committee (CHU, Montpellier) and the institutional ethic committee of the Biomedecine (DC Nos. 2014-2477 and 2016-2716). Informed consent was obtained for all donors.

Recombinant adenoviruses. The adenoviral expression vector pAd/CMV/V5-DEST carrying mouse Neurog3, Pdx1, MaA and 2A-Cherry under the CMV promoter and separated by self-cleaving 2A peptides was kindly provided by Dr. Q. Zhou, Cornell University. The recombinant adenovirus (hereafter termed Ad-Nkx2-2) was used at 50 moi (2 mM) to infect P19 cells. The recombinant adenovirus encoding Pdx1 was kindly provided by the Beta Cell Biology Consortium. The recombinant adenovirus encoding MaA was purchased from Vector Biolabs (Chicago, IL, USA). All other recombinant adenoviruses encoding single transcription factors (Neurog3, NKX2-2, Nkx6.1, and Pax4) were described previously.32,35. Crude virus lysates were used for infection of fibroblasts.

Reprogramming protocol. Fibroblasts were grown in DMEM-F12 media supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, and 1% Glutamax. They were plated onto 96-well plates (9500 cells per well) for MTT and BrdU assays, onto 12-well plates (1.25 × 105 cells/well) for gene expression, insulin secretion, immunofluorescence and caspase assays and onto 10 cm2 dishes for neotrka and T-75 flask (3000 cells) for cell cultivation experiments. Reprogramming was initiated when fibroblasts reached 80% confluence, normally 1–2 days post seeding. Cells were sequentially incubated with 15 µmol (multiplicity of infection) of Ad-NPM (day 1), 50 µol of Ad-Pax4 (day 4), and 50 µol of Ad-Nkx2-2 (day 7). As fibroblasts show limited infection by adenoviral vectors,35,36 we added a DNA transfection reagent to the virus incubation conditions to improve the transgene expression. This small change significantly increased viral transduction efficiency and allowed human fibroblasts to express significant amounts of the reprogramming factors (Supplementary Fig. 9). This reagent was Superfect (Qiagen) or NPM (NextSeq2000 Illumina, Inc.) in paired-end mode with a read length of 2 x 50 base pair. More than 60 million paired-end reads were generated for each sample/condition. Quality of sequenced reads was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). To better discern between reads coming from mouse transgenes and from human endogenous genes, we generated a custom transcriptome sequence database using the Genome Reference Consortium (GRC) mouse transcriptome from the transgenes Pdx1, Pax4, Neurog3 and MaA to the human GENCODE release 39 human transcripts. We assigned reads to this custom transcriptome using Salmon v.1.3.057 with parameters贪婪1 -a -validateMappings). We obtained > 40 million aligned reads per sample. Next, we summarised the transcript counts to gene counts using the program featureCounts from Subreads. Then, reads coming from mouse transgenes and human endogenous genes were assigned to the human GENCODE transcriptome using the “zlog” function from DESeq2. Gene Ontology enrichment analysis was performed using the goseq R package v.1.48.063 and the resulting p-values were adjusted for multiple testing using the FDR method. KEGG enrichment analysis was produced using the ClusterProfiler R package v.4.4.163. Gene Set Enrichment Analysis (GSEA) was conducted using the feature from the gseaq R package v.1.22.0 with default parameters, except in the case of “Beta cell disallowed genes” in which we specified the expected direction of enrichment with the argument “scoretype = 'neg'”. In all the above analyses, terms were considered statistically significant when adjusted p-values < 0.05.

Electron microscopy. Cell spheroids were collected, washed with PBS and fixed with 4% formaldehyde/0.1% glutaraldehyde (Sigma) mixture in PBS. Cells were then pelleted by gentle centrifugation at 4 °C for 5 min. Cell pellets were transferred to fresh fixation solution and maintained at 4 °C until secondarily fixed with 1% uranyl acetate and 1% osmium tetroxide. Cells were then dehydrated, embedded in Spurr resin and sectioned using Leica ultramicrotome (Leica Microsystems). Conventional transmission electron microscopy (TEM) images were acquired from thin sections using a JEOL-1010 electron microscope equipped with an SC1000 ORIUS-CCD digital camera (Gatan).

Mice studies. Adult (8–20 week old) normoglycemic male NOD scid gamma (NSG™) mice (catalog no 005557, Jackson Laboratories) were used as transplantation recipients. Approximately 300 cell spheroids (reprogrammed cells or parental fibroblasts) or 150–200 human islets were transplanted into the anterior chamber of the eye (ACE)38. In brief, an incision was made in the cornea near the corneoscleral junction, and a cannula (0.4 mm internal diameter) loaded with spheroids/islets, connected to a 500 µl syringe, was introduced into the incision. Cells were carefully injected without damaging the iris. The omentum, subcutaneous space and kidney were used for transplantation of higher number of cell spheroids (between 3500 and 5000). For transplantation in the omentum and subcutaneous space, cell spheroids were preloaded in collagen/Matrigel scaffolds. Briefly, the cell-laden collagen/Matrigel hydrogel was prepared by first mixing 110 µl of a 4 mg/ml rat tail type I collagen (Corning, NY, USA) solution with 40 µl of Matrigel (Corning) and then adding 50 µl of 20 µg/ml EGTA (pH 7.4) to the mix to 5T cells (5–2x10^5 in total). The cell-spheroid mixture was then poured onto a cylindrical 8 mm diameter x 1 mm thick PDMS mold (Dow Corning Sylgard 184 Silicone Elastomer). The hydrogel was polymerized for 20 min at 37 °C.
detached from the mold and maintained in tissue culture dishes with warm RPMI-1640 medium until transplant (usually 2–3 h). Constructs were placed on the omentum close to the native stomach junction. Alternatively, constructs were introduced in the abdominal subcutaneous space through a small (5 mm) incision. Transplantation in the kidney was performed following standard procedures.79

NSG mice transplanted with fibroblast spheroids or non-transplanted NSG mice were used as controls in transplantation experiments.

To assess vascularization and cell viability in ACE implants, STF cell spheroids were labeled with the long-term tracer for viable cells Vybrant CFDA SE (Invitrogen) before transplantation. At day 10 post-transplantation, mice received an intravenous injection of RITC-dextran and in vivo imaging was used to assess functional vascularization and cell viability.26 For the determination of glucose-induced insulin secretion, mice were fasted for 5–6 h and then injected intraperitoneally with glucose (3 g/kg). Tail blood was collected before and after (20 min) the glucose challenge. Aqueous humor from mice with ACE implants was obtained at time of sacrifice and kept frozen until human insulin determination.

Human insulin in plasma and in aqueous humor was determined using an ultrasensitive Human Insulin ELISA (Chrysal Chem).

The Animal Research Committee of the University of Barcelona approved all animal procedures. European and local guidelines (Generalitat de Catalunya) on accommodation and care of laboratory animal were followed.

**Immunofluorescence and morphometric measurements.** Cells grown in 2D were fixed with 4% (v/v) paraformaldehyde (PFA) for 15 min and incubated with blocking solution (0.25% (v/v) Triton, 6% (v/v) donkey serum, 5% (w/v) BSA in PBS for 1 h at room temperature. Slides were then incubated with primary antibodies diluted in PBS-triton 0.1% (v/v) containing 1% donkey serum overnight at 4 °C. STF cells grown in spheroids were fixed with 4% (v/v) paraformaldehyde (PFA) for 15 min at 4 °C, permeabilized with 0.5% (v/v) Triton in PBS for 20 min and blocked with 0.5% (v/v) Triton/ FBS 10% (v/v) in PBS during 1 h at room temperature. Slides were then incubated with primary antibodies diluted in blocking solution overnight at 4 °C. Eyes and omentum implants were fixed overnight in 2 and 4% (v/v) PFA, respectively, dehydrated with ethanol gradient, cleared with xylene and paraffin-embedded. 3 µm thick eye sections were used for standard immunofluorescence staining protocol.

Primary antibodies used were: Insulin (DAKO, 1:400, Fig. 2b); Insulin/C-PEP (Hybrida Bank; 1:40, used in all figures); HLA (Abcam, 1:100), PCSK1 (Gene Tex; 1:100); KCNJ11, PTPRN, and NCAM1 (Santa Cruz, 1:50). The antigen–primary antibody immune complex was visualized with secondary antibodies 90 Fluorescent staining and morphometric measurements.

**Statistics and reproducibility.** Data are presented as mean ± standard error of the mean (SEM) from at least three independent reprogramming experiments, with one to four biological replicates per experiment. Significant differences between the means were analyzed by the two-tailed unpaired Student’s t-test, one sample t-test or one-way ANOVA followed by Tukey’s or Dunnnett’s multiple comparison tests as indicated in the figure legends. Statistical analysis was performed with GraphPad Prism 8.00 and Microsoft Office Excel 2007 and differences were considered significant at P < 0.05. No methods were used to determine whether the data met assumptions of the statistical approach (e.g., test for normal distribution).

**Reporting summary.** Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

**Data availability**

RNA sequencing data are deposited in the Gene Expression Omnibus database under accession code GSE21075. Source data for main and supplementary figures are provided in Supplementary Data 2. All other data are available from the corresponding author upon reasonable request.

Received: 3 December 2021; Accepted: 24 February 2023; Published online: 24 March 2023

**References**

1. Graif, T. Historical origins of transdifferentiation and reprogramming. Cell Stem Cell 9, 504–516 (2011).

2. Vierbuchen, T. & Wernig, M. Molecular roadblocks for cellular reprogramming. Mol. Cell 47, 827–838 (2012).

3. Xu, J., Du, Y. & Deng, H. Direct lineage reprogramming: strategies, mechanisms, and applications. Cell Stem Cell 16, 119–134 (2015).

4. Minh, Q., Brown, J., Pavecek, A., Rajapala, J. & Meister, D. A. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 455, 627–632 (2008).

5. Li, W. et al. Long-term persistence and development of induced pancreatic beta cells generated by lineage conversion of acinar cells. Nat. Biotechnol. 32, 1223–1232 (2014).

6. Arijach et al. C. et al. Reprogrammed tissue as a sustainable source for functional beta cells for blood glucose regulation. Cell Stem Cell 18, 410–421 (2016).

7. Chen, Y. J. et al. De novo formation of insulin-producing “neo-beta cell islets” from intestinal crypts. Cell Rep. 6, 1046–1058 (2014).

8. Furuyama, K. et al. Diabetes relief in mice by glucose-sensing insulin-secreting human alpha-cells. Nature 587, 43–48 (2019).

9. Lee, J. et al. Expansion and conversion of human pancreatic ductal cells into insulin-secreting endocrine cells. Elife 2, e00940 (2013).

10. Galivo, F. et al. Reprogramming human gallbladder cells into insulin-producing β-like cells. PLoS One 12, e0181812 (2017).

11. Yechoor, V. S. et al. Neurogenin3 is sufficient for transdifferentiation of hepatic progenitor cells into neo-islets in vivo but not transdifferentiation of hepatocytes. Dev. Cell 16, 358–373 (2009).

12. Ieda, M. et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell 142, 375–386 (2010).

13. Hiramatsu, K. et al. Generation of hyaline cartilaginous tissue from mouse adult dermal fibroblasts culture by defined factors. J. Clin. Invest. 121, 640–657 (2011).

14. Vierbuchen, T. et al. Direct conversion of fibroblasts to functional lineages by defined factors. Nature 463, 1035–1041 (2010).

15. Najm, F. J. et al. Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. Nat. Biotechnol. 31, 426–433 (2013).

16. Huang, P. et al. Direct reprogramming of human fibroblasts to functional, expandable hepatocytes. Cell Stem Cell 14, 370–384 (2014).

17. Han, J.-K. et al. Direct conversion of adult skin fibroblasts to endothelial cells by defined factors. Circulation 130, 1168–1178 (2014).

18. Akinci, E. et al. Reprogramming of various cell types to a beta-like state by Pdx1, Ngn3, and MaA. PLoS One 8, e82424 (2013).

19. Li, W. et al. In vivo reprogramming of pancreatic acinar cells to three islet endocrine subtypes. Elife 3, e01846 (2014).

20. Tomaru, Y. et al. A transient disruption of fibroblastic transcriptional regulatory network facilitates trans-differentiation. Nucleic Acids Res. 42, 3960–3973 (2014).

21. Sander, M. et al. Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of beta-cell formation in the pancreas. Development 127, 5533–5540 (2000).

22. 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 Cells 31, 2432–2442 (2013).

23. 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).

24. Smith, S. B. et al. Neurogenin3 and hepatic nuclear factor 1 cooperate in activating pancreatic expression of Pax4. J. Biol. Chem. 278, 38254–38259 (2003).

25. Collombat, P. et al. The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the alpha- and beta-cell lineages in the mouse endocrine pancreas. Development 132, 2969–2980 (2005).

26. Sosa-Pineda, R., Chowdhury, K., Torres, M., Oliver, G. & Gruss, P. The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. Nature 386, 399–402 (1997).

27. Wang, J. et al. The concerted activities of Pax4 and Nkx2.2 are essential to initiate pancreatic b-cell differentiation. Dev. Biol. 266, 178–189 (2004).

28. Sander, M. et al. Genetic analysis reveals that PAX4 is required for normal transcription of pancreatic hormone genes and islet development. Genes Dev. 11, 1662–1673 (1997).

29. St-Onge, L., Sosa-Pineda, B., Chowdhury, K., Mansouri, A. & Gruss, P. Pax4 is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. Nature 387, 406–409 (1997).

30. Huczy, A. K. & 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).
31. Schult, F. et al. B-cell-specific gene repression: a mechanism to protect against inappropriate or maladjusted insulin secretion? Diabetes 61, 969–973 (2012).
32. Pullen, T. J. et al. Identification of genes selectively disallowed in the pancreatic islet. Islets 2, 89–95 (2010).
33. Pullen, T. J., Huising, M. O. & Rutter, G. A. Analysis of purified pancreatic islet beta and alpha cell transcriptomes reveals 11β-hydroxysteroid dehydrogenase (Hsd11b1) as a novel disallowed gene. Front. Genet. 8, 41 (2017).
34. Brereton, M. F., Vergari, E., Zhang, Q. & Clark, A. Alpha-, delta- and PP-cells: are they the architectural cornerstones of islet structure and co-ordination? J. Histochim. Cytochem 63, 575–591 (2015).
35. D’Amour, K. A. et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat. Biotechnol. 24, 1392–1401 (2006).
36. Pagliuca, F. W. et al. Generation of functional human pancreatic beta cells in vitro. Cell 159, 428–439 (2014).
37. Henquin, J., Dufrane, D., Kerr-Conje, J. & Nenquin, M. Dynamics of glucose-induced insulin secretion in normal human islets. Am. J. Physiol. Endocrinol. Metab. 309, E640–E650 (2015).
38. Adeghate, E. & Donath, T. Morphological findings in long-term pancreatic tissue transplants in the anterior eye chamber of rats. Pancreas 5, 298–305 (1990).
39. Leibiger, I. B. & Berggren, P. O. Intracellular in vivo imaging of pancreatic islet cell physiology/pathology. Mol. Med. 6, 1002–1009 (2007).
40. Carey, B. W. et al. Reprogramming factor stoichiometry influences the epigenetic state and biological properties of induced pluripotent stem cells. Cell Stem Cell 9, 598–609 (2011).
41. Brouwer, M., Zhou, H. & Kast, N. N. Choices for Induction of Pluripotency: Recent Developments in Human Induced Pluripotent Stem Cell Reprogramming Strategies. Stem Cell Rev. Rep. 12, 54–72 (2016).
42. van Arensbergen, J. et al. Derepression of Polycomb targets during pancreatic organogenesis allows insulin-producing b-cells to adopt a neural gene activity program. Genes Dev. 20, 722–732 (2010).
43. van Arensbergen, J. et al. Ring1b bookmarks genes in pancreatic embryonic progenitors for repression in adult b cells. Genes Dev. 27, 52–63 (2013).
44. Sapir, T. et al. Cell-replacement therapy for diabetes: Generating functional insulin-producing tissue from adult human liver cells. Proc. Natl Acad. Sci. USA 102, 7964–7969 (2005).
45. 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).
46. Balboa, D. et al. Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells. Nat. Biotechnol. 40, 1042–1055 (2022).
47. Fontcuberta-PiSunyer, M. et al. Modulation of the endocrine transcriptional program by targeting histone modifiers of the H3K27me3 mark. Biochem. Biophys. Acta 1861, 473–480 (2018).
48. Liu, M. L. et al. Small molecules enable neurogenin 2 to efficiently convert human fibroblasts into cholinergic neurons. Nat. Commun. 4, 2183 (2013).
49. Zhu, S. et al. Reprogramming of human primary saccular cells by OCT4 and transcriptional network of neurogenin3 reveals an islet cell precursor population in the pancreas. Development 127, 3533–3542 (2000).
50. Maehr, R. et al. Generation of pluripotent stem cells from patients with type 1 diabetes. Proc. Natl Acad. Sci. USA 106, 15768–15773 (2009).
51. 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).
52. Ramzy, A. et al. Implanted pluripotent stem-cell-derived pancreaticendoderm cells secrete glucose-responsive C-peptide in patients with type 1 diabetes. Cell Stem Cell 28, 2047–2061 (2021).
53. Shapiro, A. M. et al. Insulin expression and C-peptide in type 1 diabetes subjects implanted with stem cell-derived pancreaticendoderm cells in an encapsulation device. Cell Rep. Med. 2, 100466 (2021).
54. Takahashi, K. et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872 (2007).
55. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676 (2006).
56. Wang, J., Yang, Y. H., Liu, J. S. & L. Q. Direct cell reprogramming: approaches, mechanisms, and progress. Nat. Rev. Mol. Cell Biol. 22, 410–424 (2021).
57. Bolci, R., Masserottodi, G. & Götz, M. Direct neuronal reprogramming: fast forward from new concepts toward therapeutic approaches. Neuron 110, 366–393 (2022).
58. Srivastava, D. & DeWitt, N. In vivo cellular reprogramming: the next generation. Cell 166, 1386–1396 (2016).
59. Wu, J. et al. Prothymosin alpha function mediates high glucose-induced apoptosis in rodent beta cells and human islets. PLoS One 9, e92066 (2014).
60. Gasç, R. et al. Identification of a novel component of the embryonic pancreatic transcriptional network. PLoS One 3, e2430 (2008).
61. Granoio, O. et al. Improved adenosine type 5 vector-mediated transduction of resistant cells by piggybacking on coxsackie B adenovirus receptor-pseudotyped baculovirus. J. Virol. 83, 6048–6066 (2009).
62. Tsoh, M. L. et al. Enhancement of adenosine-mediated gene delivery to rheumatoid arthritis synovocytes and synovium by fibrin modifications role of arginine-glycine-aspartic acid (RGD)- and non-RGD-binding integrins. J. Immunol. 175, 7687–7698 (2005).
63. Patro, R., Duggal, G., Love, M. I., Irizarry, R. A. & Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods 14, 417–419 (2017).
64. Love, M. L., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550–571 (2014).
65. Young, M. D., Wakefield, M. J., Smyth, G. K. & Oshlack, A. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol. 11, R14 (2010).
66. Wip, T. et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation 2, 100141 (2021).
67. Marin-Cañas, S. et al. Pancreatic ductal cells may have a negative effect on human islet transplantation. PLoS One 14, e0220064 (2019).

Acknowledgements
We are indebted to Lidia Sanchez, Yaiza Esteban (IDIBAPS, CIBERDEM), Adriana Viladegu and Nailwe Dogheche (IDIBAPS) for their help in specific experiments, to Anna Soler (Hospital Clinic) for karyotyping the reprogrammed cells, and to Chris J.M.S., L.P., M.F., C.E., J.V., R.Go, R.Ga. Wrote the manuscript: M.F. and R.Ga. Conceived and designed the experiments: M.F. and R.Ga. Performed the experiments: M.F., B.F., C.P., D.P., E.A., E.C., E.G., F.M., F.S., F.M., J.V., J.V., L.P., M.F., R.D., R.F., R.G., R.G., R.G., R.G., R.G., R.G. Data analysis and interpretation: M.F., B.F., C.P., D.P., E.A., E.C., E.G., F.M., F.S., F.M., J.V., J.V., L.P., M.F., R.D., R.F., R.G.a. Wrote the manuscript: M.F. and R.Ga.

Author contributions
Conceived and designed the experiments: M.F. and R.Ga. Performed the experiments: M.F., A.G., E.P., N.T., H.F., R.F.R., S.C., and M.R.R. Provided human islets: C.B. and A.W. Provided materials: L.C., J.R., N.M., and A.A. Analyzed and discussed the data: J.M.S., L.P., M.F., C.E., J.V., R.Go, R.Ga. Wrote the manuscript: M.F. and R.Ga.
**Competing interests**
The authors declare no competing interests.

**Additional information**

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s42003-023-04627-2.

Correspondence and requests for materials should be addressed to Rosa Gasa.

Peer review information Communications Biology thanks Nidheesh Dadheech and Qiao Zhou for their contribution to the peer review of this work. Primary Handling Editor: Eve Rogers. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.