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Targeted pharmacological therapy restores β-cell function for diabetes remission

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Dedifferentiation of insulin-secreting β cells in the islets of Langerhans has been proposed to be a major mechanism of β-cell dysfunction. Whether dedifferentiated β cells can be targeted by pharmacological intervention for diabetes remission, and ways in which this could be accomplished, are unknown as yet. Here we report the use of streptozotocin-induced diabetes to study β-cell dedifferentiation in mice. Single-cell RNA sequencing (scRNA-seq) of islets identified markers and pathways associated with β-cell dedifferentiation and dysfunction. Single and combinatorial pharmacology further show that insulin treatment triggers insulin receptor pathway activation in β cells and restores maturation and function for diabetes remission. Additional β-cell selective delivery of oestrogen by Glucagon-like peptide-1 (GLP-1-oestrogen conjugate) decreases daily insulin requirements by 60%, triggers oestrogen-specific activation of the endoplasmic-reticulum-associated protein degradation system, and further increases β-cell survival and regeneration. GLP-1-oestrogen also protects human β cells against cytokine-induced dysfunction. This study not only describes mechanisms of β-cell dedifferentiation and regeneration, but also reveals pharmacological entry points to target dedifferentiated β cells for diabetes remission.

The progressive loss or dysfunction of insulin-producing β-cell mass ultimately leads to type 1 diabetes (T1D) or type 2 diabetes (T2D), respectively. Current pharmacological treatments do not stop the decline of β-cell function and number that leads to glucose excursions and, eventually, to devastating micro- and macrovascular complications. Hence, the ideal treatment should be initiated when the first diabetic symptoms appear, to protect or regenerate glucose-sensing and insulin-secreting β cells for optimal blood glucose regulation and to prevent secondary complications. Recently, T1D progression has been halted by anti-CD3 immunotherapy for 2 years, but it will be important to test whether additional β-cell regenerative therapy can further or permanently delay the onset of diabetes. Intensive insulin therapy at disease onset has been shown to partially restore β-cell function, which slows the disease progression in patients with T1D and T2D. Despite both therapies providing similar glycemic control, early intensive insulin treatment, compared with oral anti-diabetic drugs, in patients with T2D better preserves β-cell function, which suggests additional glucose-independent beneficial effects of insulin therapy. Thus, an understanding of the mechanisms of β-cell dysfunction and pharmacological replenishment is urgently required to stop or reverse diabetes progression and to improve the therapeutic options for patients. Dedifferentiation of β cells has been observed in genetic mouse models of T1D and T2D as well as in patients with diabetes, and is characterised by the loss of the expression of key maturation marker genes (for example, Slc2a2 and Ucn3) and by impaired insulin secretion, and thereby contributes to β-cell dysfunction and hyperglycaemia. To investigate whether dysfunctional β cells under hyperglycaemic conditions can be targeted pharmacologically to restore β-cell function, we explored the multiple-low-dose model of streptozotocin-induced diabetes (mSTZ) in mice. STZ specifically ablates β cells, but when STZ is injected in multiple low doses, some residual β cells can survive. Furthermore, the absence of genetic lesions and

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autoimmunity in the mSTZ model permits the investigation of the fate of those remaining β cells and the effect of pharmacological treatment on β-cell protection and regeneration.

**Results**

**Insulin restores β-cell function.** Ten days after the last mSTZ injection, mice were severely hyperglycaemic (Extended Data Fig. 1a) and showed an impaired islet architecture (Extended Data Fig. 1b) with markedly decreased β-cell numbers in comparison to those in control mice (Extended Data Fig. 1bc). Proliferation of remaining β cells was unchanged (Extended Data Fig. 1d), whereas β-cell apoptosis was significantly increased in mSTZ-treated mice compared to control mice (Extended Data Fig. 1e) and was accompanied by a loss of identity and function (Extended Data Fig. 1f–h). Hence, at this time point (diabetes onset), when a fraction of dysfunctional β cells were still remaining, we initiated a permanent drug treatment over 100 d (Fig. 1a). Vehicle-treated mSTZ mice remained diabetic over the length of the study, which suggests that the residual β-cell functionality or endogenous β-cell regeneration is insufficient to maintain or restore sufficient glucose homeostasis (Fig. 1b–f). To correct the insulin deficiency in mSTZ mice, we treated diabetic mice with a long-acting pegylated insulin analogue (PEG–insulin, once daily), which improved glycaemia (Fig. 1b), increased C-peptide levels (Fig. 1c), improved islet structure (Fig. 1d) and increased the number of insulin-positive cells (Fig. 1e,f). This shows functional β-cell recovery upon glycaemia normalisation, which extends findings from insulin treatment in genetic mouse models14,15. However, the risk of hypoglycaemia and unwanted weight gain are undesirable hallmarks of insulin therapy, and thus alternative pharmacological approaches are required to mitigate these possible effects.

**GLP-1–oestrogen and insulin polypharmacotherapy.** Oestrogen and glucagon-like peptide 1 (GLP-1) have been repeatedly implicated in the treatment of diabetes due to insulinotropic and β-cell protective effects in preclinical studies4,17. Chemically optimised GLP-1 analogues profoundly improve glycaemia and body weight management in obese people and individuals with T2D4,18. However, severe gynaecological, oncogenic and mitogenic side effects preclude chronic oestrogen use as a drug for diabetes and, as yet, GLP-1 analogues have failed to preserve β-cell function and mass in obese and diabetic humans3,19.

To circumvent the gynaecological, oncogenic and mitogenic actions of oestrogen, we recently designed and evaluated a stable GLP-1–oestrogen conjugate, which reversed the metabolic syndrome in diet-induced obese male and female mice18. Here, we used the GLP-1–oestrogen conjugate to test whether the specific delivery of oestrogen into the GLP-1 receptor protein (GLP-1R)-expressing β cells could restore β-cell functionality. GLP-1–oestrogen treatment for 100 d was more efficacious in decreasing fasting glucose (Fig. 1b) and increasing fasting C-peptide (Fig. 1c) and insulin levels (Extended Data Fig. 2a) than either of the monoagonists (oestrogen or GLP-1 alone). Moreover, only GLP-1–oestrogen treatment improved pancreatic islet architecture (Fig. 1d) and increased β-cell number, as compared with those in mSTZ-diabetic mice treated with vehicle (Fig. 1e,f). These effects were independent of body weight loss (Extended Data Fig. 2b).

Polypharmacotherapy holds the potential to simultaneously activate redundant or additive pathways to enhance efficacy, and to enable reduced dosing of the individual components and consequently reduce the risk of unwanted side effects20. We tested the combination of insulin and GLP-1–oestrogen to investigate a triple pharmacological approach to enhance the efficacy of both compounds, and particularly to lessen the amount of insulin required. The combination therapy normalised glycaemia (Fig. 1b) and increased C-peptide levels (Fig. 1c), as compared with those in mSTZ-diabetic mice treated with vehicle. Furthermore, the combination therapy displayed a superior effect compared to treatment with insulin alone to limit weight gain (Extended Data Fig. 2b), normalise glucose tolerance (Extended Data Fig. 2c), increase pancreatic insulin content (Extended Data Fig. 2d) and increase β-cell number (Fig. 1e,f). Importantly, we were able to reduce the insulin dose by 60% (10 nmol kg⁻¹) compared with that in the insulin monotherapy (25 nmol kg⁻¹) and still achieve superior therapeutic outcomes, which reduces the risk of hypoglycaemia and unintended weight gain.

To test whether treatment-induced improvements on glucose and islet homeostasis are maintained, we switched a group of mice after 12 weeks of GLP-1–oestrogen treatment to two additional weeks of vehicle injections. Positive effects of GLP-1–oestrogen treatment to
reduce fasting glycaemia (Fig. 2a), increase fasting C-peptide levels (Fig. 2b), and enhance the β-cell maturation state (Fig. 2c,d) were sustained after these two weeks, which supports the notion of preserved islet cell function even after treatment cessation.

**GLP-1–oestrogen targets β cells.** We next wanted to confirm the absence of systemic toxicity that was related to the oestrogen component of the GLP-1–oestrogen conjugate, a pre-requisite for clinical use. To that end, we investigated whether GLP-1–oestrogen (doses up to 10× higher than are generally used in mouse experiments, and at least 1,000× the plasma oestradiol exposure as compared with that in women on hormone replacement therapy (see Methods)) increased uterus weight in ovariectomised (OVX) rats after two weeks of treatment (Extended Data Fig. 3a and Methods).
In contrast to treatment with oestrogen alone, no treatment-related effect was observed with the GLP-1–oestrogen conjugate (Extended Data Fig. 3b), which is consistent with previously reported results in O VX mice.21

To confirm β-cell-specific targeting of the GLP-1–oestrogen conjugate, we used a double knock-in fluorescent reporter mouse model (Flox2-Venus Fusion (FVF) × Pdx1-BFP (blue fluorescent protein) fusion (PBF); FVFPBFΔ(Δx21), which allows α- and β-cell sorting (Extended Data Fig. 3c,d). Male FVFPBFΔ(Δx21) mice develop maturity onset diabetes of the young owing to reduced Pdx1 levels in β-cells and impaired islet architecture at weaning age.21 In this genetic diabetes model, none of the therapies used in this study improved glycated haemoglobin (HbA1c) results show that GLP-1–oestrogen is superior to both of the monoagonists in improving β-cell function, and exceeded the effects with GLP-1–oestrogen increased GSIS, and exceeded the effects with either of the individual components (Fig. 3b). Moreover, only GLP-1–oestrogen treatment increased the total insulin content of cytokine-exposed human micro-islets (Fig. 3c). This was independent of changes in the total ATP content (Extended Data Fig. 4a) and in caspase luciferase activity (Extended Data Fig. 4b), which suggests that the compound treatment improved functionality, but did not improve cell survival of human micro-islets. These results show that GLP-1–oestrogen is superior to both of the monoagonists in improving β-cell function in homoeostasis, and in acting upon cytokine stress in both mice and humans.

β-cell heterogeneity in homoeostatic and healthy mice. To elucidate the molecular mechanisms that underlie β-cell failure in mSTZ diabetes, and β-cell recovery after the different therapeutic approaches, we performed scRNA-seq of isolated islets from mice that responded to treatment (Extended Data Fig. 5).

In normal islet homoeostasis, we identified the four main endocrine cell subtypes, α cells, β cells, δ cells and pancreatic polypeptide (PP) cells, by unbiased graph-based clustering. Clusters were annotated on the basis of predominant endocrine hormone expression of glucagon (Gcg), insulin (Ins), somatostatin (Sst) and Pp (Extended Data Fig. 6a,b and Methods). For each of the four endocrine subtypes we identified a specific marker gene signature (Supplementary Table 1 and Methods). Refined clustering of insulin-positive cells revealed the presence of two main β-cell subpopulations, β1 and β2 (Extended Data Fig. 6c and Methods). Single-cell trajectory inference suggests a continuum of transcriptional states, rather than discrete phenotypes within β cells and a transition between β1 and β2 subpopulations (Extended Data Fig. 6d and Methods). We found a progressive increase in expression of β-cell maturation marker genes (for example InSI, InS2 and Ucn3 (ref. 22)), and genes of the secretion machinery (G6pc2, Sylb and Slc2a2), as well as a concomitant decrease in expression of the β-cell immaturity gene MafB23, and pan-endocrine lineage marker genes (Chga and Chgb) along the pseudotime trajectory from β2- to β1-cells (Extended Data Fig. 6d and Supplementary Table 2). The expression of transcription factor genes that are associated with β-cell identity (for example Pdx1,
β-cell dedifferentiation at the single-cell level. To obtain a deeper understanding of the cell autonomous and non-cell autonomous effects that underlie chemical β-cell ablation in the islet cell niche, we performed a scRNA-seq survey of diabetic islets after 100 d of persistent hyperglycaemia. Unsupervised clustering and embedding of the scRNA-seq data revealed altered endocrine subtype composition and cell-intrinsic gene expression profiles that were indicated by a shifted cell cluster location in the Uniform Manifold Approximation and Projection (UMAP) space of mSTZ-diabetic compared to healthy mice (Fig. 4a and Supplementary Table 3). In particular, there was a threefold decrease in the proportion of β cells from mSTZ-treated mice (β-mSTZ), and β-mSTZ formed a cluster that was clearly distinct from healthy β cells (Fig. 4a). In contrast to the results for β cells, few transcriptional changes were detected for α cells, δ cells and PP cells (Extended Data Fig. 7a-f and Supplementary Table 3). We next sought to describe the progression from healthy to dysfunctional β cells and the associated gene expression changes, using single-cell trajectory inference. Cells were ordered on the basis of a cell-to-cell distance metric that was calculated using the concept of diffusion pseudo-time (see Methods). We identified a cellular trajectory in which cells transitioned from mature to immature to β-mSTZ cells (Fig. 4b). Remaining β-mSTZ cells expressed low Ins1 and/or Ins2 messenger RNA and also showed sustained low expression of β-cell identity transcription factor genes, such as Pdx1, Nkx6.2, Nkx6.1, Pax6, Isl1 and NeuroD1 (refs. 20,21) (Fig. 4b). Pathways that are associated with β-cell maturity and functionality included genes with down-regulated expression, whereas genes in ER stress and oxidative phosphorylation pathways were upregulated in the remaining β-mSTZ cells; together these observations indicate an ER stress response and β-cell dysfunction (Fig. 4c and Supplementary Table 3). Along this trajectory, expression of key markers of β-cell maturity and functionality gradually decreased concomitantly with an increase in the very few known markers of β-cell immaturity and dedifferentiation (for example Aldehyde dehydrogenase 1A3 (ref. 22) and gastrin (Fig. 4b,d)). Strikingly, our single-cell analysis uncovered a large number of upregulated genes and pathways in β-mSTZ cells that are not expressed at all or are expressed only subtly in mature, functional murine β cells (Fig. 4e). We confirmed the increased expression of, for example, Cck and Slc5a10 proteins by immunohistochemistry in mSTZ diabetic mice (Fig. 4f). These identified targets may serve as biomarkers for dysfunctional β cells, and may have the potential to be part of druggable pathways to restore β-cell function. Some of these were also identified recently in β cells and pancreata of T1D and T2D human specimens32–35 (Extended Data Fig. 7g,h). There is some debate as to whether β-cell dedifferentiation resembles reversal to a pluripotent (Oct3/4, Nanog, Sox2) or endocrine progenitor state (Neurogenin 3 or Neurog 3, hereafter called Ngn3), whether it is part of normal phenotypic variation described as β-cell heterogeneity, or whether it resembles a glucotoxic-induced reversible state36. Although dedifferentiated β cells had been characterised previously by upregulation of pluripotency or endocrine transcription factors8,9,15, in our study expression levels of Sox9, Pou5f1 (Oct3/4), Myc and Ngn3 genes were unaltered in mSTZ-treated β cells (Extended Data Fig. 7i).

To further characterise the maturation state of mSTZ-derived β cells, we compared our data set to β-cell expression profiles during embryonic (E17.5) to postnatal development (P60)36. We assessed transcriptional similarity of β-cell subpopulations using Partition-based graph abstraction (PAGA) after data integration and computation of a common embedding. The enforcement of integration using only genes associated to β-cell maturation in the reference data allowed us to match maturation states independent of differences in other biological processes between the two data sets (see Methods). PAGA uses a statistical model to measure the relatedness of groups of single cells (see Methods). We found that dedifferentiated β-mSTZ cells were more strongly connected to early time points of the maturation data set, whereas β cells clustered to intermediate time points and β1 cells to late time points (Fig. 5a). Remarkably, the inferred cellular trajectory from β-mSTZ to β1 cells aligned with the trajectory of β-cell maturation from embryonic (E17.5) to mature β cells (P60) of the reference data set (Fig. 5b). An increase in known β-cell maturation and a decrease of immaturity markers along this trajectory further indicated that the cells follow a similar differentiation or dedifferentiation programme (Fig. 5c,d). To validate these findings, we scored each β cell using the gene sets that are characteristic for the start (E17.5/P0) and end

Nkx6.1 and NeuroD1) was unchanged (Extended Data Fig. 6d). The β-cell cluster was characterised by the downregulation of genes that are involved in insulin secretion, oxidative phosphorylation and cell-cycle inhibition, as well as by the upregulation of genes that are involved in cAMP and WNT signalling (Extended Data Fig. 6e), both of which observations are suggestive of a more immature and/or proliferative state for β cells34. We consistently observed upregulation of cell-cycle-associated genes, such as Ki67 and Cdk1 in the immature β2 subpopulation and, in accordance with this result, 16 out of 403 of the β2-cells, but only 2 out of 5,319 of the mature β1-cells, were classified as being involved in cell cycling (Extended Data Fig. 6f (see Methods)). Taken together, these results confirm the co-existence of mature (β1) and immature and/or proliferative β cells (β2) in healthy mouse islets4,25. In addition, we found subpopulations of polyhormonal cells that could be distinguished from doublets and ambient RNA, both of which are common problems with the scRNA-seq technology (Extended Data Fig. 6g,h and Methods).

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**Fig. 3** | GLP-1-oestrogen improves function of human micro-islets. a. Insulin secretion of human micro-islets after acute exposure to 16.7 mM glucose and vehicle, oestrogen, GLP-1 or GLP-1-oestrogen treatment. n = 5–6 micro-islets of n = 3 human donors for each condition. Secretion (mean ± s.e.m.) of donor 1 = 0.36 ± 0.01 ng ml⁻¹, donor 2 = 0.37 ± 0.02 ng ml⁻¹ and donor 3 = 0.29 ± 0.03 ng ml⁻¹ after vehicle exposure. Box plot of all data points. Line indicates the median. Data were analysed by a one-way ANOVA with donor as random effect followed by Tukey post-hoc (Fmedium dose(3, 65) = 14.04; Fhigh dose(3, 64) = 18.59; Fhigh dose(3, 66) = 25.50). *P indicates P value to vehicle. b. Insulin secretion at 16.7 mM glucose of human micro-islets after 7-d exposure to cytokine stress and effect of oestrogen, GLP-1 or GLP-1-oestrogen treatment. n = 6 micro-islets of n = 3 human donors for each condition. Secretion (mean ± s.e.m.) of donor 1 = 0.13 ± 0.02 ng ml⁻¹, donor 2 = 0.56 ± 0.04 ng ml⁻¹ and donor 3 = 0.51 ± 0.06 ng ml⁻¹ after chronic vehicle (no stress) exposure. Box plot of all data points. Line indicates the median. *P indicates P value to vehicle under no stress condition. *P indicates P value to vehicle cytokine exposure. Data from no stress versus cytokine stress were analysed by an unpaired two-sided t-test (t = 4.776; d.f. = 33). Otherwise, data were analysed by a one-way ANOVA with donor as random effect followed by Tukey post-hoc (Flow dose(4, 82) = 6.35; Fmedium dose(4, 81) = 7.65; Fhigh dose(4, 81) = 21.91). c. Total insulin content of human micro-islets after 7-d exposure to cytokine stress and effect of oestrogen, GLP-1 or GLP-1-oestrogen treatment. n = 6 micro-islets of n = 3 human donors for each condition. Insulin content (mean ± s.e.m.) of donor 1 = 41.11 ± 3.73 ng per islet, donor 2 = 30.86 ± 3.36 ng per islet and donor 3 = 82.73 ± 3.99 ng per islet after chronic vehicle (no stress) exposure. Box plot of all data points. Line indicates the median. *P indicates P value to vehicle stress condition. *P indicates P value to vehicle cytokine exposure. Data from no stress versus cytokine stress were analysed by an unpaired two-sided t-test (t = 7.429; d.f. = 32). Otherwise, data were analysed by a one-way ANOVA with donor as random effect followed by Tukey post-hoc (Flow dose(4, 80) = 4.12; Fmedium dose(4, 78) = 3.01; Fhigh dose(4, 79) = 3.31). Additional information is available in Source data.
(P60) point of the developmental trajectory (Supplementary Table 4 and Methods). Healthy mature β cells (β1) scored high for maturity genes, whereas healthy immature cells (β2) and dedifferentiated cells (β-mSTZ) scored higher for the embryonic immaturity gene set (Fig. 5e). Taken together, these results imply that during the transition from healthy β1 to β2 to dedifferentiated β-mSTZ, β cells revert, at least in part, back to a more immature and, further, to an embryonic-like state.

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**Figure Legends**

**a** Acute exposure

**b** Chronic exposure

**c** Chronic exposure

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**Table Legends**

| Compound | 1 nmol | 100 nmol | 1,000 nmol |
|----------|--------|----------|------------|
| Vehicle  |        |          |            |
| Oestrogen|        |          |            |
| GLP-1    |        |          |            |
| GLP-1–oestrogen | |          |            |

*P* values are indicated in the figure.
β-cells (Extended Data Fig. 8a,b). These results suggest that surviving β-cells are dedifferentiated, which shows that mSTZ-induced diabetes is a good model in which to study mechanisms of β-cell dedifferentiation and redifferentiation in the absence of genetic lesions.

**Mechanisms of β-cell redifferentiation.** In line with the pharmacological data, single-cell analysis of the different treatments revealed that β-cells of mice treated with vehicle (Extended Data Fig. 9a), oestrogen (Extended Data Fig. 9b) and GLP-1 (Extended Data Fig. 9c) remained dedifferentiated. By contrast, we observed an increased fraction of immature β2-cells from GLP-1–oestrogen-treated mice (Extended Data Fig. 9d). In PEG–insulin-treated mice (Extended Data Fig. 9e) and GLP-1–oestrogen plus PEG–insulin-treated mice (Extended Data Fig. 9f), almost no dedifferentiated β cells remained and most cells clustered with immature β2-cells. To further assess the transcriptional state of β cells from the treated mice, we calculated a cell-to-cell distance so that cells could be ordered along the cellular trajectory from dedifferentiated to healthy β cells (see Methods). On this one-dimensional axis, we observed a continuous transition. TF, transcription factor. Cells are ordered on the basis of a random-walk-based cell-to-cell distance metric. Expression is shown as the running average along the inferred trajectory scaled to the maximum observed level per gene. β1-cells were downsamplled to 1,500 cells for better visualisation. c, Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of significantly up- and downregulated genes in β cells of mSTZ-treated mice compared to β cells of healthy mice (absolute log(fold change) > 0.25, FDR < 0.01; selective pathways are depicted; see also Supplementary Table 3). Cells were pooled from mice treated with no STZ-vehicle (n = 3) and mSTZ-vehicle (n = 3). We used limma-trend to find differentially expressed genes (see Methods). Gene enrichment was done with EnrichR using Fisher’s exact test to identify regulated ontologies or pathways (see Methods). d, Immunohistochemical analysis of Glut2, Ucn3, Aldh1a3 and gastrin in β cells of mSTZ mice and healthy mice at study end. Images are representative of mice treated with no STZ-vehicle (n = 3) and mSTZ-vehicle (n = 3). Scale bars, 50 µm; scale bar zoom-in, 20 µm. e, Gene expression along the trajectory from β1 to β-mSTZ (as in c) of 29 genes specifically expressed in β cells from mSTZ-treated mice (expression in <5% of no-STZ-β cells and in >25% of β-mSTZ cells, see Methods). Cellular locations of associated proteins are indicated. f, Immunohistochemical analysis of Slc3a10 and Cck in β cells of mSTZ and healthy mice at study end. Images are representative of mice treated with no STZ-vehicle (n = 3) and mSTZ-vehicle (n = 3). Scale bars, 50 µm; scale bar zoom-in, 20 µm.

To separate the altered maturation state from other processes induced in dedifferentiated β cells, we compared differentially regulated gene ontologies and pathways between embryonic (E17.5/P0) and mature (P60) β cells and between β-mSTZ cells and healthy control β cells (see Methods). According to the trajectory and PAGA analysis, these β-cell states of the reference data set correspond best to dedifferentiated β-mSTZ cells and mature, healthy β cells, respectively. Embryonic and mSTZ-diabetic β cells shared downregulation of molecular processes connected to β-cell function and maturity (for example, insulin secretion and FoxO signalling) compared to healthy mature β cells (Extended Data Fig. 8a and Supplementary Table 4), whereas genes involved in oxidative phosphorylation and gene and protein transcription were upregulated (Extended Data Fig. 8b and Supplementary Table 4). Specific to embryonic β cells was a downregulation of lipid and carbohydrate metabolism and an upregulation of WNT signalling (Extended Data Fig. 8a,b). This corresponds to known mechanisms of β-cell maturation during embryogenesis. Interestingly, in mSTZ-diabetic β cells, but not the embryonic β cells, we found an upregulation of pathways and ontologies associated with ER stress and a decreased expression of genes involved in insulin and MAPK signalling in comparison to healthy mature β cells (Extended Data Fig. 8a,b). Thus, β-cell dedifferentiation involves partial reversal to an embryonic or immature β-cell programme and upregulation of an ER stress response and altered signalling state. These results suggest that surviving β cells are dedifferentiated, which shows that mSTZ-induced diabetes is a good model in which to study mechanisms of β-cell dedifferentiation and redifferentiation in the absence of genetic lesions.
β cells of PEG–insulin-treated and GLP-1–oestrogen plus PEG–insulin-cotreated mice showed the strongest connection to healthy β cells (Fig. 6d). The observed overall re-establishment of the healthy β-cell expression profiles was substantiated by an increased expression of β-cell maturity markers and decreased expression of immaturity and dedifferentiation markers along the inferred trajectory (Fig. 6e–g). Moreover, Ucn3 expression recovered during the pharmacological treatment (Extended Data Fig. 10a). This shows...
that the maturation state before treatment was different from that achieved after treatment. Hence, upon PEG–insulin or PEG–insulin plus GLP-1–oestrogen treatment, β cells adopt a molecular immature yet functional phenotype that is sufficient for blood glucose normalisation and diabetes remission.

β cells from mice treated with PEG–insulin or the combination of PEG–insulin and GLP-1–oestrogen were grouped in distinct β-cell subpopulations, albeit at a similar maturation state (Fig. 6b). This implies the existence of a compound-specific mechanism-of-action (MOA) that underlies the recovery of β-cell function. To investigate the distinct MOAs of the different treatments, we identified the β-cell-specific transcriptional signature of treated and mSTZ-derived β cells (Supplementary Table 5). An increased expression of genes involved in β-cell functionality and maturation (Fig. 6h) was common to both treatments and may result from improved blood glucose levels and/or stimulation of shared pathways of insulin and GLP-1–oestrogen signalling. GLP-1, oestrogen and insulin receptor activation regulate MAPK and FoxO signalling44, both of which were increased after PEG–insulin, GLP-1–oestrogen and PEG–insulin and GLP-1–oestrogen co-therapy (Fig. 6h). Although we cannot dissect the signalling contribution of each individual receptor, we believe that the polypharmacological approach might potentiate the simultaneous activation of commonly regulated pathways.

Unexpectedly, treatment with PEG–insulin elicited a β-cell-specific stimulation of the insulin signalling cascade as well as stimulation of the recently characterised RNA polymerase II mediated pathway6 (Fig. 6h). Hence, our data suggest that direct effects of insulin on β cells contribute to the improvement of β-cell function and recovery, as was proposed for T2D42,43.

Our goal was to use GLP-1–oestrogen to selectively deliver oestrogen into β-cells. We consistently found β-cell-specific induction of the ER-associated degradation (ERAD) pathway and induction of transfer RNA signalling in PEG–insulin and GLP-1–oestrogen cotreated mice (Fig. 6h). ERAD mitigates ER stress, which, when unresolved, contributes to functional β-cell mass loss in T1D and T2D50. Chemical and genetic disturbances of ERAD impair β-cell function64. We found an increased proinsulin-to-C-peptide ratio in mSTZ-diabetic mice, which is used as an ER-stress surrogate in diabetes65. (Extended Data Fig. 10b). GLP-1–oestrogen, PEG–insulin and GLP-1–oestrogen co-therapy normalised this connection or cell movement from other (non-β) cell populations towards differentiated β cells. We next examined the RNA velocity and potential fate of treated endocrine cells (Fig. 8b). The velocity of some of the immature β cells observed after GLP-1–oestrogen, PEG–insulin and combined treatment pointed towards mature β cells of healthy mice, thus further substantiating β-cell redifferentiation. Moreover, the scRNA-seq data suggested that neogenesis was not increased after 100 d of treatment, as the expression levels of Ngn3 mRNA remained unchanged in endocrine cell subtypes (Fig. 8c). We also found no indication, from Ngn3 immunostaining...
in tissue sections of earlier time points, that overt neogenesis contributed to β-cell regeneration (Fig. 8d). Together, these results suggest that redifferentiation of β cells along dedifferentiation and redifferentiation trajectories is the main mechanism that underlies the re-establishment of functional β cells in the mSTZ model by treatment with GLP-1–oestrogen or PEG–insulin, or by their cotreatment. By using a combination of low dose insulin with GLP-1–oestrogen treatment, we were able to trigger a
β-cell-specific transcriptional response that was characterised by increased β-cell proliferation and enhanced functionality.

**Discussion**

Herein we have established the mSTZ model of diabetes as a model to study β-cell dysfunction and dedifferentiation. Single-cell profiling of remaining β cells identified many markers of β-cell dedifferentiation that were undescribed previously and that code for surface molecules, receptors and secreted proteins. These may be used as biomarkers or may allow the detection, isolation and characterisation of dedifferentiated β cells. This could reveal pathomechanisms of T1D and T2D and may have the potential to identify unique diagnostic markers and therapeutic targets. Using scRNA-seq we were able to delineate a β-cell fate trajectory in which cells transitioned from mature to immature to dedifferentiated β cells, which implies that β cells can be characterised by a continuum of transcriptional states that reflect discrete phenotypes. Inference of cell transitions using the RNA velocity concept further suggested that there
was no ongoing transdifferentiation from other non-β and non-endocrine cells towards dedifferentiated β cells. Upregulation of the endocrine master regulator Ngn3 might depend on the severity of hyperglycaemia; high glucose levels (>33 mM) have been shown to induce Ngn3 expression14,15, whereas lower levels (<25 mM) had no effect14. We show that β-cell dedifferentiation in mSTZ-diabetic mice is independent of induction of Ngn3+ endocrine progenitors and that the transcriptional state of dedifferentiated β cells is more similar to late embryonic or early postnatal β cells.

Recently, the Kushner laboratory has provided evidence that some of the remaining insulin in the blood stream of patients with long-term T1D originates from dedifferentiated β cells and/or from polychormonal non-β cells that function as ‘insulin microsecretors’60. Similarly, the Korsgren laboratory found histological evidence for β-cell dedifferentiation at T1D onset55. Therefore, triggering the redifferentiation of dedifferentiated β cells seems to be an intuitive approach for the treatment of diabetes that does not involve β-cell proliferation or neo-genesis per se61. Preclinical as well as clinical findings from patients with type 1 and 2 diabetes suggest that a transient recovery of β-cell dysfunction occurs upon glycaemia normalisation by intensive insulin treatment by either β-cell rest or re-differentiation16. By using scRNA-seq we can dissect endocrine subtype-specific treatment responses and show that insulin treatment triggers transcriptional changes in β cells that are connected to insulin and/or IRS signalling. This supports the idea that in addition to lowering the glucotoxic stress on β cells, direct insulin- or IGF-signalling improves β-cell health and performance and can re-differentiate β-cell mass in diabetic models62. Importantly, the redifferentiated β cells that were induced by insulin therapy were functional and responded to physiological stimuli, as indicated by increased fasting plasma C-peptide levels.

Moreover, we showed that targeted delivery of oestrogen using GLP-1 as a peptide carrier and intensive insulin co-therapy by a dis-therapy were functional and responded to physiological stimuli, as dysfunctions occur upon glycaemia normalisation by intensive insulin therapy by either

Methods

mSTZ treatment. mSTZ (Sigma–Aldrich cat. no. S0130) was injected intraperitoneally in 8-week-old male C57BL/6 mice (n = 125) at 50 mg kg−1 for five consecutive days following the mSTZ model to induce diabetes. A subset of age-matched male mice was injected with ice-cold citrate buffer (pH 4.5) as a control animals (n = 20). C57BL/6 mice were obtained from Janvier Labs. Ten days after the last mSTZ injection, fasting blood glucose was taken, as well as fasting plasma, to determine fasting insulin and C-peptide levels. We included hyperglycaemic mice with fasting blood glucose levels of >190 mg dl−1 (n = 116). We estimated β-cell function and mass of mSTZ-treated mice by combining fasting blood glucose levels, the homeostatic model assessment (HOMA)-β-score and the ratio of fasting C-peptide to blood glucose levels. Among mSTZ-treated mice, animals with fasting glucose levels of <25th percentile, a HOMA-β-score of ≥25th percentile and a C-peptide to blood glucose ratio of <25th percentile were excluded from the study (n = 9).

Pharmacological study in mSTZ mice. mSTZ diabetic mice were randomised and evenly distributed to different treatments according to fasting blood glucose levels. Ten days after the last STZ injection, mice were allocated to different treatments of daily subcutaneous injection with vehicle (PBS; n = 17, mSTZ-treated), a GLP-1 analogue (n = 16), oestrogen (n = 14), GLP-1–oestrogen (n = 28, of which n = 11 mice were switched to vehicle (PBS) treatment after 12 weeks of GLP-1–oestrogen treatment), PEG–insulin (n = 13), GLP-1–oestrogen and PEG–insulin (n = 16) at the doses indicated in Fig. 1a for 100 d. Mice were housed up to four per cage on a 12:12-h light-dark cycle at 22 °C and given free access to a normal chow diet (Altromin 1314) and water. Compounds were administered in a vehicle of PBS (Gibco) and were given by daily subcutaneous injections at the indicated doses at a volume of 5 μl g−1 body weight for the indicated durations as indicated in the figure legends. The investigators were not blinded to group allocation during the in vivo experiments or to the assessment of longitudinal treatment outcomes. All rodent studies were approved and performed according to the guidelines of the Animal Use and Care Committee of Bavaria, Germany.

Study in FVFPBF– mice. Male 8-week-old FVFPBF mice with fasting blood glucose > 250 mg dl−1 were randomised to vehicle (n = 7), oestrogen (n = 5), GLP-1 (n = 9) or GLP-1–oestrogen (n = 11) treatment according to their fasting blood glucose levels. FVFPBF mice were obtained from breeding. Mice were treated daily for four weeks with subcutaneous injections. Fasting blood glucose was measured after a 6-h fast. We single- or group-housed the mice on a 12-h light,
12-h dark cycle at 22°C and provided free access to food and water. This study was approved by and performed according to the guidelines of the Animal Use and Care Committee of Bavaria, Germany.

**Uterotrophic assessment in OVX rats.** The study was designed in accordance with the Endocrine Disruptor Screening Programme Test Guidelines OPPTS 890.1600: Uterotrophic Assay, a standardised in vivo screening test intended to evaluate the ability of a chemical to elicit biological activities consistent with agonists of natural oestrogens (for example, 17ß-oestradiol). The test measures the increase in uterine weight or uterotrophic response in comparison with non-treated controls. The study was further designed according to accepted pharmacological principles and followed Good Laboratory Practice (conducted by Envigo). A total of 44 OVX female Sprague–Dawley rats (Charles River) were supplied for the study, of which 40 animals were allocated to treatment groups (randomised by body weight to ensure equal group mean starting body weight) and the remaining 4 animals were allocated as spares. On the day of dosing (following 14–22 d of acclimatisation), rats were approximately 9–12 weeks of age, and weighed 217 g to 348 g. Four groups of 8 rats each were treated for 14 consecutive days with
once-daily subcutaneous administration of GLP-1–oestrogen at doses of 400 μg kg⁻¹, 2,200 μg kg⁻¹ and 4,000 μg kg⁻¹ per day or with volume-matched vehicle (PBS). An additional group of animals received once-daily subcutaneous injections of vehicle only. The first 10 days followed by once-daily injection of 17 α-ethyl-α-methyltrityl-l-lysine (Sigma-Aldrich) and a tenfold excess of ethyl 2-bromoacetate (Sigma) performed on a Waters instrument with a Kinetex C8 column and a gradient of 10%–80% eluent B. Eluent A is water with 0.05% TFA and eluent C is acetonitrile. Molecular weights and character by liquid chromatography–mass spectrometry on a Agilent 1260 Infinity/6120 Quadrupole instrument with a Kinetex C8 column. Trifluoroacetic acid (TFA, VWR Chemicals), triisopropylsilane (TIS, Sigma-Aldrich) and dichloromethane (DMF, VWR Chemicals) for coupling and 20% piperidine in DMF for deprotection of N-terminal amines. Peptides were synthesised by standard Fmoc methods using diisopropylcarbodiimide/1-hydroxybenzotriazole chloride (DIC/HOBT-Cl, both from AAPPTec) in dichloromethane (DMF, VWR Chemicals) for coupling and 20% piperidine in DMF for deprotection of N-terminal amines. Peptides were synthesised by standard Fmoc methods using diisopropylcarbodiimide/1-hydroxybenzotriazole chloride (DIC/ HOBT-Cl, both from AAPPTec) in dichloromethane (DMF, VWR Chemicals) for coupling and 20% piperidine in DMF for deprotection of N-terminal amine. Aldehydes were oxidised for 20 h with 0.05% TFA and eluent C is acetonitrile. Molecular weights and character by liquid chromatography–mass spectrometry on an Agilent 1260 Infinity/6120 Quadrupole instrument with a Kinetex C8 column and a gradient of 10%–80% eluent B. Eluent A is water with 0.05% TFA and eluent C is acetonitrile. The crude extract was purified by reversed-phase HPLC. The derivatised oestrogen was covalently added to the side-chain amine of the C-terminal lysine amide residue. We used a C-terminal N-methyltryptyl-l-lysine (Lys(Mtt)-OH) residue, whose side chain was orthogonally deprotected with four 10-min treatments with 1.5% TFA, 2% TIS and 1% anisole in DMF. The oestrogen was attached through this side-chain amine of the C-terminal lysine after treatment with a threefold excess of the purified derivatised oestrogen and DIC/HOBt/Cl in N-methyl-2-pyrrolidone. After TFA cleavage as described above, the crude extract was resuspended in aqueous buffer containing 20% ACN and 0.1 M NH₄HCO₃. The conjugate was purified as described above.  

**PIG–insulin.** Pegylated insulin (PEG–insulin) was prepared by insulin N-terminal amide formation with 20 K methoxy PEG propionaldehyde in brief, human insulin was dissolved in 50 mM sodium acetate buffer (pH 5.0) and 50% ACN. A 30-fold excess of sodium cyanoborohydride and a 1.5-fold excess of methoxy PEG propionaldehyde (M-ALD-20K, JenKem Technology) was added to the buffer containing insulin for 3 h at room temperature with stirring. Purification by reverse phase chromatography on a C-8 column in 0.1% TFA acetonitrile solvent yielded the final product at greater than 95% purity. Oestrogen (17β-oestradiol, Sigma-Aldrich) was dissolved in 100% ethanol (Sigma-Aldrich) at a concentration of 1 mg ml⁻¹ and was diluted with PBS to the required concentration.  

**Blood parameters.** Blood was collected from tail veins after a 4-h fast, using EDTA-coated microvette tubes (Sarstedt). Blood was immediately chilled on ice. Plasma was separated by centrifugation at 5,000g for 4°C for 10 min using a micro centrifuge and was stored at −20°C until further usage. Plasma insulin and C-peptide solution and sections were incubated overnight at 4°C. Thereafter, sections were rinsed 3× and washed 3× with 1× PBS. All secondary antibodies were used at a 1:800 dilution prepared in blocking buffer. We used the following secondary antibodies: donkey anti-goat IgG (H+L) secondary antibody (Alexa Fluor 633, Invitrogen A-2108), donkey anti-rabbit IgG (H+L) secondary antibody (Alexa Fluor 555, Invitrogen A-31572); donkey anti-rabbit IgG (H+L) secondary antibody (Alexa Fluor 488, Invitrogen A-21206); donkey anti-guinea pig IgG (H+L) secondary antibody (1:100; Novo Nordisk), rabbit anti-guinea pig IgG (H+L) secondary antibody (DYLight 488, Invitrogen A-21206); donkey anti-rabbit IgG (H+L) secondary antibody (Cy3, Invitrogen 71270–165–153); donkey anti-guinea pig (H+L) secondary antibody (Alexa Fluor 488, Invitrogen A-21206). Sections were incubated with DAPI (1:500 in 1× PBS) for 20 min, rinsed and washed 3× with 1× PBS, and subsequently mounted. All images were acquired with a Leica SP8 confocal microscope and analyzed using the software LAS AF (Leica). Images were analysed using the LAS AF and/or Image software programme.  

### Automatic tissue analysis. Stained tissue sections were scanned with an Axiocam 4.1 digital slide scanner (Zeiss) equipped with a ×20 magnification objective. We scanned three sections per animal. Images were evaluated using the commercially available image analysis software Definiens Developer XD 2 (Definiens). First, regions of interest were annotated manually to select islets of Langerhans for analysis. A specific rule set was then defined to detect and quantify the cells within each defined region on the basis of the fluorescence intensity of DAPI, morphology, size and neighbourhood. The Ins-, Gcg- or Sst-expressing cells were classified automatically using the fluorescence intensity of each hormone. EdU detection protocol. EdU staining was carried out according to the EdU imaging kit manual (Life Technologies) after staining with the secondary antibody. DAPI staining and mounting was performed as described above.  

### Pancreatic insulin content. Pancreatic insulin content was determined by an acid extraction method. The pancreas was dissected, washed in 1× PBS and homogenised in an acid–ethanol solution (5 mL 1.5% HCl in 70% ethanol) followed by incubation at −20°C for 24 h. After 2 rounds of acid–ethanol precipitation, the tissue was centrifuged (2000 rpm, 15 min, 4°C) and the supernatant was neutralised with 1 M Tris pH 7.5. Insulin was measured using a mouse insulin ELISA (Crystal Chem) and was normalised over the protein concentration that was determined by BCA protein assay.  

### Islet isolation. Islet isolation was performed by collagenase P (Roche) digestion of the adult pancreas. In brief, 3 mL of collagenase P (1 mg ml⁻¹) was injected into the bile duct and the perfused pancreas was consequently dissected and placed into another 3 mL of collagenase P for 15 min at 37°C. Then, 10 mL of G-solution (HBSS (Lonza) +1% BSA (Sigma–Aldrich)) was added to the samples, and this was followed by centrifugation at 1600 rpm for 4°C. After another washing step with G-solution, the cells were re-suspended in 5 mL of gradient per sample. 100 μL of G-solution (Lonza) +3 mL of 40% Optiprep (Sigma–Aldrich) per sample, and placed on top of 2.5 mL of the same solution. To form a 3-layer gradient, 6 mL of G-solution was added on the top. Samples were then incubated for 10 min at room temperature before centrifugation at 1,700 rpm. Finally, the interface between the upper and the middle layers of the gradient was harvested and was filtered through a 70 μm Nylon filter then washed with G-solution. Islets were hand picked under the microscope.  

### Single-cell suspension. To achieve a single-cell suspension of islets, islets were hand picked into a 1.5 mL Eppendorf tube, pelleted (800 rpm, 1 min) washed with PBS minus Mg or Ca, Gibco) and digested with 0.25% trypsin with EDTA (Gibco) at 37°C for 5 min. Mechanical dissociation every 2–3 min. The digestive reaction was then stopped and cells were pelleted (1200 rpm, 5 min).  

### Single-cell sequencing. Single-cell libraries were generated using the Chromium Single-cell 3 library and gel bead kit v2 (PN 120237) from 10x Genomics. In brief, to reach a target cell number of 10,000 cells per sample 16,000 cells per sample
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were loaded onto a channel of the 10x chip to produce Gel Bead-in-Eмуasions (GEMs). This underwent reverse transcription to barcode RNA before cleanup and cDNA amplification followed by enzymatic fragmentation and 5’ adapter and sample barcoding. Libraries were sequenced on the HiSeq4000 (Illumina) with 150 bp paired-end sequencing of read2.

FACS sorting. FACS sorting of endocrine cells was performed using the FACS-Aria III (BD Biosciences). Single cells were gated according to their FSC-A (forward scatter area) and SSC-A (side scatter area). Single cells were gated dependent on the FSC-W (forward scatter width) and FSC-H (forward scatter height) and cells were excluded using the marker 7AAD (eBioscience). The FVF endocrine populations were discriminated according to their Venus fluorescence emission at 488 nm and the β- and α-lineages were discriminated according to their GFP emission at 405 nm (positive and negative respectively). To enrich for β cells the distinct SSC-A high populations were selected. FACS sorted cells were sorted directly into QiaZen (Qiagen) for RNA isolation.

RNA isolation and cDNA preparation. The mRNA isolation was performed using the mirNeasy Micro Kit (Qiagen) according to the manufacturer’s instructions. On-column DNase I treatment was applied to degrade DNA. For cDNA preparation the SuperScript Vilo cDNA Synthesis Kit (Thermo Fisher Scientific) and the GoScript Reverse Transcript System Kit (Promega) were used. The cDNA synthesis was carried out according to both manufacturers’ instructions.

Quantitative PCR. Quantitative PCR (qPCR) was carried out using the Viia7 Real Time PCR System (Thermo Fisher Scientific) and the following TaqMan probes (Life Technologies): Ins 1 (Mm01950294_s1), Ins 2 (Mm00731595_Gh), Prss2 (Mm01269955_m1), Sst (Mm00436671_m1), Ppy (Mm01250509_g1), Gcg (Mm00445450_m1), Pecam1 (Mm01242584_m1), Gapdh (Mm004680_17_g1) and 18S (Mm03928990_g1). Each reaction contained 25 ng of cDNA. For analysis, the Cₜ-values were transformed to the linear expression values and were normalised to the reference genes (Gapdh and 18S) and to the control samples.

Reaggregated human micro-islets. All primary human islets were obtained from Proredo Laboratories with no information on the identity of the donor for ethical and privacy reasons (donor 1: male, BMI 32.38, age 48, HbA1c 5.6%; donor 2: male, BMI 33.2, age 46, HbA1c 5.4%; donor 3: male, BMI 28.65, age 34, HbA1c 5.2%). For all donors, consent was obtained from next of kin. For each preparation of InSphero 3D InSight human islet microtissues, 10,000–20,000 islet equivalents (5.2%). For all donors, consent was obtained from next of kin. For each preparation of InSphero 3D InSight human islet microtissues, 10,000–20,000 islet equivalents were dispersed in dissociation solution (1x TrypLE Express solution (Thermo Fisher Scientific) containing 40 μg/ml DNase I (Sigma-Aldrich 10140159001)) by gentle pipetting at 37°C. Remaining cell clumps were removed by filtering the cell suspension through a cell strainer (70μm pore size). Islet microtissues were produced by hanging-drop-based scaffold-free reaggregation of 2,500 cells in each well of the InSphero 96-well Hanging Drop System for 5 d. The primary aggregates were then transferred to the Akura 96 well-plate to further mature for at least another 8 d before the start of the experiments. All experiments were performed within 30 d after the start of the aggregation. Islet microtissues were maintained in 3D InSight Human Islet Maintenance Medium (InSphero).

Compound, cytokine treatments and GSIS with human micro-islets. Dilution series of compounds were used to show basal expression of the hormone genes (40% or more); (2) expressed more than 7,000 genes; or (3) had more than 100,000 UMI counts. Cell-by-gene count matrices of all samples from mitochondrial genes (40% or more); (2) expressed more than 7,000 genes; or (3) had more than 100,000 UMI counts. Cell-by-gene count matrices of all samples were then concatenated to a single matrix. To account for differences in sequencing depth, UMI counts of each cell were normalised by total counts of that cell (pp. normalise_per_cell with mean = TRUE) and values were log-transformed. Highly variable genes (n = 1,625) were selected on the basis of normalised dispersion using the setting the lower cutoffs for the mean to 0.0125 and for the dispersion to 0.5. This matrix was used as input for all further analyses unless otherwise indicated.

Embedding, clustering and cell type annotation. Clustering was performed on the full data set to reduce batch effects such as batch effects as was recommended in ref. 14. A single-cell neighbourhood graph (kNN-graph) was computed on the 50 principal component axes between 15 neighbours. To minimise condition effects and to facilitate clustering we recomputed the kNN-graph using the first 15 diffusion components of the PCA-based graph as suggested in ref. 16. For clustering and cell type annotation, Louvain-based clustering was used as implemented in louvain-igraph (v0.6.1 https://github.com/vtraag/louvain-igraph) and adopted by scply (illouvain). The resolution parameter was varied in different parts of the data manifold to account for strong changes in resolution (for details, see Data availability). Clusters were annotated on the basis of the mRNA expression of the four main hormone genes Ins1 and Ins2, Gcg, Sst and Ppy (endocrine cells) and other known marker genes (non-endocrine) and were filtered if they reflected heterogeneity only in a cell type outside the focus of this study.

Ducal cells (that express Ktc19), acinar cells (that express Prss2), endothelial cells (that express Pfpw), stellate cells (that express Coll1a2) and small clusters of potential doublet-like cells that co-express endocrine and non-endocrine markers were excluded from further consideration. Of the immune cells that co-express Cdt9 (that express Cdt9a and Cdt9b) and T cells (that express Cdt8a and Cdt8b). The hormone genes Ins1 and Ins2, Gcg, Sst and Ppy were expressed at very high levels and showed background expression in subtypes and non-endocrine cell types. Such background expression is a common phenomenon in droplet-based scRNA-seq data. It is commonly said to be due to free-floating mRNA in the single-cell solution that comes from lysed cells and that is incorporated into all droplets. For annotation, only hormone expression that was well above the background level in non-endocrine cells, such as ductal, immune and endothelial cells, was considered.

For the identification of β-cell substates a new KNn-graph on the first 50 principal component axes was calculated and put into Louvain-based clustering of both Ins monohormonal and the connected Ins-P cell clusters. Similarly, Ins-Sst cell clusters were subclustered from Ins-Sst-PP cells after recalculating the kNN-graph on the first 15 components. For the classification of Ins-Gcg-Sst cells were assigned using a manual threshold for all three hormones that was well above ambient levels (threshold = normalised data).

For visualisation, UMAP was run as recommended in ref. 17. For each UMAP plot the UMAP was newly calculated by recomputing the kNN-graph on the represented cell subset using the first 50 principal components.

Identification of polyhormonal singlets and doublet-like endocrine cell clusters. Polyhormonal cells have previously been reported to exist in pancreatic islets19. However, the expression of multiple hormones in the same droplet can also be an indication of a doublet. It can therefore be difficult to distinguish polyhormonal singlets from doublets. A doublet rate of 10% was empirically found in experiments with the same concentration of cells using the 10x Genomics technology. This rate includes doublets with contributions from two different cell types (here, polyhormonal doublets) and from the same cell type (here, monohormonal doublets). The monohormonal doublets resemble monohormonal singlets and do not affect subsequent analyses17. We calculated the expected doublet frequency

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of polynormal doublets for a doublet rate of 10% using the frequency of mononormal cell types that contribute to the doublet (doublet contributors) and assuming that doublets are generated by sampling singlet cells uniformly at random. In every sample the proportion of observed polynormal cells clearly exceeded the expected polynormal doublet frequency. Therefore, in our data set it is unlikely that all detected polynormal cells are doublets. Application of doublet detection tools Scrublet (v0.1.1, https://github.com/AllonKleinLab/scrublet) and DoubletDetection (https://github.com/JonathanShor/DoubletDetection) failed to resolve which clusters represent doublets and which clusters represent polynormal singlets. Predictions of the tools disagreed with each other and the doublet rate was consistently overestimated. We therefore used the following criteria to evaluate polynormal cell clusters and to distinguish between singlets and doublets:

1. Doublets should not express unique genes. All genes should also be expressed in at least one doublet contributor.

2. Doublets have expression of the updated marker genes or lineage-determining transcription factors of the doublet contributors. Downregulation of these genes indicates singlet populations.

3. Previous reporting of polynormal singlet cells in the literature.

4. Clusters of polynormal cells with a higher frequency than expected by our doublet simulation indicate polynormal singlet clusters (Extended Data Fig. 6h).

5. Clusters with Scrublet doublet score distributions that are comparable to mononormal singlet clusters indicate polynormal singlets (Extended Data Fig. 6h).

Cell-cycle classification. To classify cells into cycling and non-cycling cells, first, a score was assigned to each cell for a set of 5 and 2/M phase genes as proposed, and second, cells were assigned to different phases of cell cycling. The threshold was chosen on the basis of the score distribution. The score for a given gene set was computed as described and implemented in scannpy (tl. gene cycle).

Marker genes of the main endocrine cell types. For the characterisation of the four endocrine cell types, specific marker genes were identified by comparing the gene expression profile of each cell type against all of the other three cell types using a test with overestimated variance as implemented in scannpy (tl.rank_genes_groups). All genes that ranked within the top 500 genes, had a test score of >8 and were unique markers for one cell type were considered as marker genes.

Differential expression testing to describe subpopulations and treatment responses. Differential expression testing between treatments and for the characterisation of immature β cells and polynormal subpopulations was performed on quantile-normalised (quantile threshold = 0.95) and log-transformed data to account for extremely highly expressed genes (for example, the main hormones in endocrine cells) that may incorrectly alleviate the expression of other genes in a cell when total count normalisation is applied. By quantile normalisation, each cell is normalised by the total UMI count in the cell, of genes that account for less than 5% of the total UMI counts across all cells. Thus, very highly expressed genes were not normalised. As an example of a gene expression variation we first considered only the 2,000 top-ranked highly variable genes (hp.highly_variable_genes) of the reference data set that were expressed in fewer than 15 cells in each data set. In addition, cycling cells (Mki67>1, 33 reference genes and 30 of the cells from this study) were excluded, as their expression profile is dominated by the expression of cell-cycle genes and these cells therefore formed a separate cluster which was not part of the linear maturation trajectory. To ensure that β-cell maturation dominates the gene expression variation we first considered only the 2,000 top-ranked highly variable genes (hp.highly_variable_genes) of the reference data set that were expressed in our data, and, second, we reduced the contribution of heterogeneity within the β and β-mSTZ cluster to gene expression variation by randomly subsetting both clusters of 300 cells. We then scaled and zero centred each data set separately (pp. scale) and concatenated the two data sets, which resulted in a 1,788 cells by 1,654 genes count matrix. We computed a common kNN graph on the first ten principal components using the sce.pp.bknn function with default parameters and k = 5 within batch neighbours. As the data sets did not show a strong batch effect even without integration, assessed by visual inspection of the first principal components and diffusion components, and thus transitions between cells from the two data sets also showed a high probability, we were able to use diffusion pseudotime (tl.dpt) to infer the maturation trajectory with the common kNN graph as input. The trajectory was calculated by selecting a random root cell from the embryonic cells that were sampled at E17.5. The choice of root cell did not affect the ordering strongly except for one data set. For normalisation of the cells was largely consistent with the ordering obtained prior to integration, assessed by the distribution along the trajectory of the time points or β-cell subgroups. To quantify the cluster similarity, PAGA was applied to the common kNN graph (tl.paga).

To compute an embryonic or immaturity cell score and a maturity cell score we extracted the gene signatures from the reference data set. The reference data was subset to the 2,000 top ranked highly variable genes and 1,654 based clustering was performed on the common kNN graph that was computed on the first 50 principal components with 15 neighbours. The cluster that consisted of cells that were sampled at E17.5 and P0 was annotated as ‘embryonic or neonatal’, whereas the cluster consisting mainly of cells that were sampled at P60 was annotated as ‘mature’. Genes differentially expressed between these two clusters were considered for scoring. Genes that were upregulated in the mature cluster or in the embryonic or neonatal cluster were used as a gene set for maturity or for embryonic or immaturity score, respectively. For differential expression testing the t-test with overestimated variance implemented in the tl.rank_genes_groups function of scannpy was used. The top 300 ranked genes with a log(foldchange) > 0.25 and an adjusted p value < 0.01 were considered. Cell scores were computed using the tl.score_genes function in scannpy.

Inference of cluster-to-cluster distances, lineage relations and cell movement. PAGA was performed to infer cluster and lineage relations using the tl.paga function, which identifies a spanning tree with edge significance of 0.05. The spanning tree graph, paths represent cluster connections or relations that indicate potential routes of cellular transitions. Edge weights represent the confidence of a connection calculated on the basis of a measure of cluster connectivity.

To infer the direction of possible transitions and cell movements we estimated RNA velocity using a stochastic version implemented in the scVelo python package.
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Author contributions
S.S. performed in vivo and ex vivo rodent experiments, pancreas histology, analysed and interpreted all data, interpreted scRNA-seq data, and wrote the manuscript. A.B.P. performed ex vivo rodent experiments, pancreas histology, analysed and interpreted data, and cowrote the manuscript. S.T. analysed and interpreted scRNA-seq data and cowrote the manuscript. M.B. performed ex vivo experiments and helped to draft the manuscript. A.B. performed ex vivo experiments and helped to prepare the single-cell suspensions for scRNA-seq. M.A.S.-G. performed in vivo experiments. M.T-M. performed ex vivo experiments. M.K., F.F., S.F., and A.H. performed in vivo experiments and helped to interpret data. E.B. and S.R. performed ex vivo experiments and helped to interpret data. S.H. helped to interpret data. A.F. conducted and analysed automatic pancreatic histology. B.Y. and A.N. performed, analysed, and interpreted human micro-islet experiments. C.B.J. designed, analysed, interpreted and supervised the rat study, interpreted in vivo data and helped to write the manuscript. M.C. designed and oversaw human micro-islet experiments and helped to interpret data. B.Y. synthesised and characterised compounds. B.E. designed the in vivo rodent experiment, synthesised and characterised compounds, interpreted the data, and helped to write the manuscript. R.M. performed H.M.T conceptualised and interpreted all studies and helped to write the manuscript. F.J.T. conceptualised, supervised, and interpreted the scRNA-seq analysis and helped to write the manuscript. S.M.H., T.D.M., and H.L. conceptualised, designed, supervised, and interpreted all studies and wrote the manuscript.

Competing interests
C.R.J., M.C., B.Y., B.F., and R.D.D. are current employees of Novo Nordisk. Novo Nordisk has licensed from Indiana University intellectual property pertaining to this report. M.H.T. serves as a scientific advisory board member of ERX Pharmaceuticals, Inc., Cambridge, MA. F.I.T. reports receiving consulting fees from Roche Diagnostics GmbH and Cellarity Inc., and ownership interest in Cellarity, Inc. and Dermagnostx. S.T. reports receiving consulting fees from Cellarity, Inc. The Institute for Diabetes and Obesity receives research support from Novo Nordisk. All other authors declare no conflict of interest.

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Extended Data Fig. 1 | Remaining β cells lose cell identity 10 days after last STZ injection. Effects of either vehicle or the mSTZ treatment on a, fasting blood glucose (No STZ: n = 20, mSTZ: n = 107; unpaired two-sided t-test; t = 14.64, df = 125), b, pancreatic islets histology (No STZ: 179 islets of n = 3 mice, mSTZ: 182, n = 3; unpaired two-sided t-test; β: t = 11.44, df = 358; α: t = 10.98, df = 356; δ: t = 4.27, df = 338; images are representative from no STZ n = 3 and mSTZ n = 3 mice), c, the insulin positive area within pancreatic sections (No STZ: 27 sections of n = 3 mice; STZ: 27, n = 3; unpaired two-sided t-test; t = 3.646, df = 52), d, the proliferation (No STZ: 58 islets of n = 3 mice; STZ: 69, n = 3) and e, apoptosis rate in β cells (No STZ: 46 islets of n = 3 mice; STZ: 42, n = 3; unpaired two-sided t-test, t = 3.955, df = 86), f, the expression of β-cell functional marker Ucn3 and Glut2 (images are representative of dataset plotted in b from no STZ n = 3 and mSTZ n = 3 mice), g, the homeostatic model assessment of β-cell function (HOMA-β) (No STZ: n = 20, STZ: n = 107; unpaired two-sided t-test; t = 20.65, df = 124) and h, the ratio of fasting C-peptide to fasting blood glucose (No STZ: n = 20, STZ: n = 106; unpaired two-sided t-test; t = 14.03, df = 122). Boxplots covering all data points are depicted. Line indicates the median. Scale bar, 50 μm. Scale bar zoom-in, 20μm.
Extended Data Fig. 2 | Benefits of polypharmacotherapy to ameliorate mSTZ diabetes in mice. Effect of treatment with indicated compounds and doses on a, fasting plasma insulin levels at week 12 of treatment (mSTZ-vehicle, n = 12; oestrogen, n = 10; GLP-1, n = 11; GLP-1/oestrogen, n = 11; one-way ANOVA with Tukey post-hoc; F (3, 39) = 10.66) and b, body weight in the end of the study (no STZ-vehicle, n = 12; mSTZ-vehicle, n = 13; GLP-1, n = 11; oestrogen, n = 11; GLP-1/oestrogen, n = 11; PEG-insulin, n = 9; GLP-1/oestrogen and PEG-insulin, n = 10; unpaired two-sided t-test; t = 2.436, df = 17). (c, d) Comparison of PEG-insulin and GLP-1/oestrogen plus PEG-insulin co-treated mice. c, Blood glucose after intraperitoneal glucose (0.5 g/kg) at week 12 (no STZ-vehicle, n = 12; PEG-insulin, n = 9; GLP-1/oestrogen and PEG-insulin, n = 10; one-way ANOVA with Tukey post-hoc (F (2, 27) = 24.71)). d, Pancreatic insulin content in the end of the study (no STZ-vehicle, n = 4; PEG-insulin, n = 4; GLP-1/oestrogen and PEG-insulin, n = 4; unpaired two-sided t-test; t = 4.534, df = 6). All data are mean ± SEM.
Extended Data Fig. 3 | Tissue specificity and β-cell selectivity of the GLP-1/oestrogen conjugate. (a, b) Treatment of female OVX Sprague-Dawley rats. (a) Study scheme. (b) Dry uterus weight. Data are mean ± SEM. N = 8 female rats per group. One-way ANOVA with Tukey post-hoc (F (4, 34) = 44.89). (c-g) Treatment of male FVFPBF<sup>Hom</sup> mice. (c) FACS gating strategy of dispersed endocrine cells based on granularity (Side Scatter Cell (SSC)) and PBF (405 nm) intensity. (d) qPCR analysis confirmed sorting strategy of endocrine cells. Data are values of sorted cells from n = 2 mice. (e) Study scheme; FVFPBF<sup>Hom</sup> male mice were treated with vehicle (n = 7), oestrogen (n = 5), GLP-1 (n = 9), or GLP-1/oestrogen (n = 11) at the indicated doses for four weeks. (f) Fasting blood glucose. Data are mean ± SEM. (g) Sorted endocrine cell populations after treatment (vehicle (n = 4, cells of n = 2 mice each were pooled), oestrogen (n = 4, cells of n = 2 mice each were pooled), GLP-1 (n = 5, cells of n = 2 and n = 3 mice were pooled), or GLP-1/oestrogen (n = 6, islets of n = 3 mice each were pooled)). Data are mean ± SD.
Extended Data Fig. 4 | Viability and cell death of human micro-islets. Measurement of human micro-islet viability and cell death with and without cytokine exposure and in the present of different compounds at the indicated doses. (a) ATP content and (b) Caspase 3/7 activity of human micro-islets. 

a, N = 6 micro-islets of n = 3 human donors for each condition. Boxplot of all data points. Line indicates the median. *P indicates P-value to vehicle no stress condition. #P indicates P-value to vehicle cytokine exposure. No stress versus cytokines stress by unpaired two-sided t-test (t = 1.756, df = 33).

Otherwise one-way ANOVA with donor as random effect followed by Tukey post-hoc (F_{low dose}(4, 81) = 6.68; F_{medium dose}(4, 82) = 10.68; F_{high dose}(4, 81) = 6.30).

b, N = 3–4 micro-islets of n = 3 human donors for each condition. Boxplot of all data points. Line indicates the median. *P indicates P-value to vehicle no stress condition. #P indicates P-value to vehicle cytokine exposure. No stress versus cytokines stress by unpaired two-sided t-test (t = 2.567, df = 22).

Otherwise one-way ANOVA with donor as random effect followed by Tukey post-hoc (F_{low dose}(4, 52) = 5.58; F_{medium dose}(4, 52) = 4.44; F_{high dose}(4, 53) = 4.23).
Extended Data Fig. 5 | Physiological characteristics of mice used for scRNA-seq. Representative mice (n = 3) of each treatment were used for scRNA-seq. 

a, Fasting glucose levels. One-way ANOVA with Tukey post-hoc test among mSTZ, oestrogen, GLP-1 and GLP-1/oestrogen treated mice (F (3, 8) = 21.23). One-way ANOVA with Tukey post-hoc among no STZ, GLP-1/oestrogen, PEG-insulin, and co-treated mice (F (3, 8) = 94.06).

b, Fasting C-peptide levels. One-way ANOVA with Tukey post-hoc test among STZ, oestrogen, GLP-1 and GLP-1/oestrogen treated mice (F (3, 8) = 9.073). All data are mean ± SEM.
Extended Data Fig. 6 | β-cell heterogeneity in healthy mice. a, Endocrine cell annotation is based on the hormone expression of insulin (Ins), glucagon (Gcg), somatostatin (Sst), and pancreatic polypeptide (PP). b, UMAP plot showing all endocrine cells (7578 cells in total) from healthy mice. The cell number and proportion of each endocrine cluster is indicated. Redefined clustering of the Ins+ β cells revealed two main β-cell subpopulations. c, d, Expression changes of genes from selected pathways along a pseudotime trajectory from β2- to β1 cells. β1-cells were downsampled to 1000 cells for better visualisation. e, GO term and KEGG pathway enrichment analysis of up-(log(fold change) > 0.25) and downregulated (log(fold change) < -0.25) genes in β2- (278 cells) compared to β1 cells (5380 cells). Cells were pooled from n = 3 mice. Representative terms from Supplementary table 2 are depicted. We used limma-trend to find differentially expressed genes (M&M). Gene enrichment was done with EnrichR using Fisher’s exact test to identify regulated ontologies/pathways (M&M). f, Violin plots showing the distribution of the expression of proliferation and β-cell maturation genes suggesting an immature phenotype of cycling β-cells. Accordingly, 16/403 of the β2 cells, whereas only 2/5319 of the mature β1 cells were classified as cycling (M&M). Cells were pooled from n = 3 mice. Violin shows the distribution as a kernel density estimate fit. Points in violin interior show individual data points. Boxplot in violin interior shows median, quartile and whisker values. g, Measured proportion and expected doublet frequency of polyhormonal cell clusters. h, Boxplot displaying the doublet score distribution of mono- and polyhormonal cell clusters. A high score indicates a high doublet probability. Cells were pooled from n = 3 mice. Boxplot shows the quartile values and extreme values. Whiskers extend to 1.5 IQRs of the lower and upper quartile. Outliers are displayed individually.
Extended Data Fig. 7 | β-cell dedifferentiation in mSTZ-diabetic mice. (a–d). Volcano plots showing differential expression and its significance (-log10(adjusted p-value), limma-trend) for each gene in (a) β-, (b) α-, (c) PP-, and (d) δ-cells from mSTZ treated versus healthy mice. Red line indicates thresholds used on significance level and gene expression change. Significantly regulated genes are highlighted in black. Genes significantly regulated in only one cell type but not the others are highlighted in blue. p-values were correct for multiple testing using BH. Cells were pooled from no STZ (n = 3) and mSTZ-vehicle (n = 3) treated mice. (e, f) GO term and KEGG pathway enrichment analysis of up- (log(fold change) > 0.25) and downregulated (log(fold change) < -0.25) genes in (e) α- and (f) δ-cells in mSTZ treated versus healthy mice. We used limma-trend to find differentially expressed genes (M&M). Gene enrichment was done with EnrichR using Fisher’s exact test to identify regulated ontologies/pathways (M&M). Cells were pooled from no STZ (n = 3) and mSTZ-vehicle (n = 3) treated mice. (g, h) Comparison between dysregulated genes in mSTZ-β-cells in mice with (g) data from RNA-seq of human T2D pancreata and (h) from scRNA-seq of human T1D β cells. Gene names of overlapping genes and identified dedifferentiation markers in Fig. 4e) are listed. i, Violin plots showing the distribution of the expression of endocrine developmental genes in beta cells of mSTZ treated and healthy mice. Violin shows the distribution as a kernel density estimate fit. Points in violin interior show individual data points. Boxplot in violin interior shows median, quartile and whisker values. Cells were pooled from no STZ (n = 3) and mSTZ-vehicle (n = 3) treated mice.
Extended Data Fig. 8 | Common and distinct pathways of embryonic and dedifferentiated β-cells. Gene ontologies (P-value < 0.0001) and KEGG pathways (P-value < 0.05) that are commonly and specifically (a) down- and (b) upregulated in embryonic and mSTZ-derived β-cells. Representative terms from Supplementary Table 4 are depicted. Gene enrichment was done with EnrichR using Fisher’s exact test to identify regulated ontologies/pathways (M&M). Cells were pooled from mSTZ-vehicle (n = 3) treated mice.
Extended Data Fig. 9 | Effects on endocrine cells of different treatments. UMAP plot of all endocrine cells after 100 days of treatment showing endocrine cell distribution in each individual treatment. Total cell number for (a) mSTZ diabetic mice 5001, for (b) oestrogen treated mice 4889, for (c) GLP-1 treated mice 3874, for (d) GLP-1/oestrogen treated mice 5201, for (e) PEG-insulin treated mice 3217, and for (f) GLP-1/oestrogen (GLP-1/E) and PEG-insulin (PEG-ins) co-treated mice 3276. Values indicate the proportions of each cell cluster. Cells of n = 3 mice for each treatment were pooled.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | β-cell maturation after compound treatment. a, Immunohistochemical analysis of Ucn3 expression during the course of the study. Scale bar, 50 μm. Day 0: Images are representative of no STZ-vehicle (n = 3) and mSTZ-vehicle (n = 3) treated mice. Day 25: Images are representative of no STZ-vehicle (n = 3), mSTZ-vehicle (n = 3), GLP-1/oestrogen (n = 3), PEG-insulin (n = 3), and GLP-1/oestrogen and PEG-insulin (n = 3) co-treated mice. Day 100: Images are representative of no STZ-vehicle (n = 3), mSTZ-vehicle (n = 3), GLP-1/oestrogen (n = 2), PEG-insulin (n = 2), and GLP-1/oestrogen and PEG-insulin (n = 3) co-treated mice. b, Plasma proinsulin/C-peptide ration in the end of the study (no STZ-vehicle, n = 8; mSTZ-vehicle, n = 8; oestrogen, n = 6; GLP-1, n = 5; GLP-1/oestrogen, n = 6; PEG-insulin, n = 6; GLP-1/oestrogen and PEG-insulin, n = 6; one-way ANOVA with Tukey post-hoc: F(6, 36) = 8.12). Data are mean ± SEM. c, Representative staining for insulin and Sel1l after 25 days of treatment. Arrow indicates Sel1l+ insulin+ cells, which were especially found in GLP-1/oestrogen and PEG-insulin co-treated mice. Sel1l+ insulin–cells (arrow head) were more common in mSTZ-diabetic and PEG-insulin treated mice. Images are representative of no STZ-vehicle (n = 3), mSTZ-vehicle (n = 3), PEG-insulin (n = 3), and GLP-1/oestrogen + PEG-insulin (n = 3) co-treated mice. Scale bar, 20μm. d, Expression of selected ER stress and ERAD-associated genes by scRNA-seq at study end.
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Data collection

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oPCR was carried out using the Viia7 Real Time PCR System (Thermo Fisher Scientific).
All histological images were obtained with a Leica microscope of the type DMI 6000 using the LAS AF software. Images were analyzed using the LAS AF and/or ImageJ software program.
Automatic image analyses were done using the commercially available image analysis software Definiens Developer XD 2 (Definiens AG, Munich, Germany).

Data analysis

Prism software 8 (Graphpad) http://www.graphpad.com/scientific-software/ N/A prism/
All histological images were obtained with a Leica microscope of the type DMI 6000 using the LAS AF software. Images were analyzed using the LAS AF and/or ImageJ software program.
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R version 3.5.2 (2018-12-20)
scRNAseq data was analyzed using python 3.5 and 3.6. The raw reads were processed using the CellRanger analysis pipeline [v2.0.0] provided by 10X Genomics. Subsequent analyses were carried out using custom scripts, Scanpy [https://github.com/theislab/scanpy, v1.0.4+92.g9a754bb], scvelo [v0.1.16.dev134+c1af6d4a, https://github.com/theislab/scvelo with scanpy v1.3.2], veclocyto [v0.17.7, http://veclocyto.org], Scrublet [v0.1, https://github.com/AlonKieferLab/scrublet], limma [http://bioinf.wehi.edu.au/limma/, v3.28.10] via an rpy2 interface [v2.9.1] and Enrichr [amp.pharm.mssm.edu/Enrichr/].
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Life sciences study design
All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample size for in vivo analysis (mSTZ study) was calculated based on $\alpha$-error = 0.05, power = 0.8, and effect size = 1.58. We obtained a sample size of n=8/treatment group for physiological end points in the end of the treatment [e.g. blood glucose, C-peptide]. For single-cell RNA-seq a minimum expected requirement of ~3000 cells per experiment was used and by far exceeded in most cases for confident identification of rare cell subpopulations (~0.05% of cells). No statistical test or power analyzes were used to pre-determine sample size. Otherwise, we chose the sample size of individual experiments based on past experience on detecting differences with a given method.

Data exclusions
For the single-cell RNAseq data cells were filtered using previously described standards for quality control. For each experiment the count matrix was filtered as follows: genes with expression in less than 10 cells were removed; cells with a fraction of UMI counts from mitochondrialy encoded genes of 40% or more, with more than 1000000 UMI counts or with more than 7000 genes expressed were excluded. These criteria were based on the cell quality within this study. Otherwise, no data were excluded.

Replication
All attempts of in vivo replication were successful. For single-cell RNAseq experiments cells from three independently treated mice that showed similar responses in assessed physiological parameters were pooled to account for inter-individual variation.

Randomization
Diabetic mice were randomized to different treatment groups by 4h-fasting blood glucose levels. Rats were randomized by body weight.

Blinding
The investigators were not blinded to group allocation during in vivo experiments to be able to assess the health status of diabetic mice.

Reporting for specific materials, systems and methods
We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | Involved in the study |
| - Antibodies | - ChIP-seq |
| - Eukaryotic cell lines | - Flow cytometry |
| - Paleontology | - MRI-based neuroimaging |
| - Animals and other organisms | |
| - Human research participants | |
| - Clinical data | |

Antibodies

| Antibodies used |
|-----------------|
| Guinea pig polyclonal anti-insulin, Thermo Scientific PA1-26938, 1:300 |
| Goat polyclonal anti-Glut2 Abcam ab111117; 1:500 |
| Goat polyclonal anti-NK6.1 R&D systems AF5857; 1:200 |
| Goat polyclonal anti-somatostatin Santa Cruz sc-7819; 1:500 |
Rat monoclonal anti-somatostatin Invitrogen MAS-16987; 1:300
Rabbit anti-Ngn3 Donated by H. Edlund N/A; 1:800
Rabbit polyclonal anti-urocortin 3 Phoenix Pharmaceuticals H-019-29; 1:300
Rabbit monoclonal anti-insulin Cell Signaling 3014; 1:1000
Guinea pig polyclonal anti-glucagon Takara M182; 1:500
Guinea pig polyclonal anti-insulin ABD Serotec 5330-0104G; 1:300
Rabbit polyclonal cleaved caspase-3 (Ass 175) Cell Signaling 9661; 1:300
Rabbit polyclonal anti-Aldh1a3 Novus NBP2-15339; 1:300
Rabbit monoclonal anti-GLP-1R Novo Nordisk N/A; 1µg/ml
Rabbit polyclonal anti-gastrin Abcam Ab16035; 1:100
Rabbit polyclonal anti-cholecytokinin ENZO Life Sciences BML-CA1124; 1:100
Goat polyclonal anti-Sell1 Novus Biologicals NB100-93463; 1:300
Donkey anti-Goat IgG (H+L) secondary antibody, Alexa Fluor 633 Invitrogen A-21082; 1:800
Donkey anti-Rabbit IgG (H+L) secondary antibody, Alexa Fluor 555 Invitrogen A-31572; 1:800
Donkey anti-Rabbit IgG (H+L) secondary antibody, Alexa Fluor 488 Invitrogen A-21206; 1:800
Donkey anti-Guinea pig IgG (H+L) secondary antibody, Dylight 649 Dianaov 706-495-148; 1:800
Donkey anti-Rat IgG (H+L) secondary antibody, Dylight 647 Dianaov 711 605 152; 1:800
Donkey anti-Rat IgG (H+L) secondary antibody, Cy3 Dianaov 712-165-153; 1:800
Donkey anti-Guinea pig IgG (H+L) secondary antibody, Alexa Fluor 488 Dianaov 706-545-148; 1:800

Validation
Guinea pig polyclonal anti-insulin, https://www.thermofisher.com/antibody/product/Insulin-Antibody-Polyclonal/PA1-26938
Goat polyclonal anti-Glut2 https://www.abcam.com/glucose-transporter-glut2-antibody-ab111117.html
Goat polyclonal anti-Nkx6.1 https://www.rnkdynamics.com/products/human-mouse-nkx61-antibody_a5f857
Goat polyclonal anti-somatostatin https://www.scbt.com/scbt/de/product/somatostatin-antibody-6-20
Rat monoclonal anti-somatostatin https://www.thermofisher.com/antibody/product/Somatostatin-Antibody-clone-YC7-Monoclonal/MAS-16987
Rabbit anti-Ngn3 We used embryonic mouse pancreas to ensure binding of Ngn3 included in this study. We used no 2nd as control stainings to show specificity.
Rabbit polyclonal anti-urocortin 3 https://www.phoenixpeptide.com/products/view/Antibodies/H-019-29
Rabbit monoclonal anti-insulin https://www.cellsline.de/products/primary-antibodies-insulin-c77c9-rabbit-mab/3014
Guinea pig polyclonal anti-glucagon https://www.tekilibio.com/products/antibodies-and-elisa-primary-antibodies-and-elisa-by-research-areas/metabolic-diseases/glucagon/Katalog/M182
Guinea pig polyclonal anti-insulin https://www.bio-rad-antibodies.com/polyclonal/pig-porcine-insulin-antibody-5330-0054.html?refpurified
Rabbit polyclonal cleaved caspase-3 (Ass 175) https://www.cellsline.de/products/primary-antibodies/cleaved-caspase-3-ass175-antibody/9661
Goat polyclonal anti-Sell1 https://www.novobio.com/products/anti-1-antibody_nb100-93463
Rabbit polyclonal anti-Aldh1a3 https://www.novusbio.com/products/aldh1a3-antibody_nbp2-15339
Rabbit polyclonal anti-GLP-1R This is a antibody produced and checked for specificity by Novo Nordisk. We demonstrate binding of this antibody exclusively to beta cells in the islet of Langerhans by using a GLP-1R knock out mouse.
Rabbit polyclonal anti-gastrin https://www.abcam.com/gastrin-antibody-ab16035.html
Rabbit polyclonal anti-cholecytokinin http://www.enzolifesciences.com/BML-CA1124/cholecytokinin-39-polycyonal-antibody/
Donkey anti-Goat IgG (H+L) secondary antibody, Alexa Fluor 633 https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21082
Donkey anti-Rabbit IgG (H+L) secondary antibody, Alexa Fluor 555 https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31572
Donkey anti-Rabbit IgG (H+L) secondary antibody, Alexa Fluor 488 https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206
Donkey anti-Guinea pig IgG (H+L) secondary antibody, Dylight 649 https://www.dianova.com/produkte/706 605 148 esel-igg anti-meerschweinchen-igg-anti-meerschweinchen-igg-hl-alexan-fluor-647-mink-bckgphshohumsrtrsh-500-ug/
Donkey anti-Rat IgG (H+L) secondary antibody, Dylight 647 https://www.dianova.com/produkte/711 605 152 esel-igg anti-kaninchen igg-hl-alexan fluor 647 mink-bckgphshohumsrtrsh-500-ug/
Donkey anti-Rat IgG (H+L) secondary antibody, Cy3 https://www.dianova.com/produkte/712-165-153 esel-igganti-ratte-igg-hl-cy3-mink-bckgphshohumsrtrsh-500-ug/
Donkey anti-Guinea pig (H+L) secondary antibody, Alexa Fluor 488 https://www.dianova.com/produkte/706 545 148 esel-igg anti-meerschweinchen-igg-anti-meerschweinchen-igg-hl-alexan-fluor-488-mink-bckgphshohumsrtrsh-500-ug/
ETHICS OVERSIGHT

Rat study was further designed according to accepted pharmacological principles and followed Good Laboratory Practice conducted by Envigo CRS Limited, UK.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

FLOW CYTOMETRY

PLOTS

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

METHODOLOGY

SAMPLE PREPARATION

We used isolated islets from FVB/NJ mice for FACS analysis. Islet isolation was performed by collagenase P (Roche) digestion of the adult pancreas. Briefly, 3 ml of collagenase P (1 mg/mL) was injected into the bile duct and the perfused pancreas was consequently dissected and placed into another 3 ml collagenase P for 15 min at 37 °C. 10 ml of G solution (HBSS (Lonza) + 1% BSA (Sigma)) was added to the samples followed by centrifugation at 1600 rpm at 4 °C. After another washing step with G-solution, the pellets were re-suspended in 5.5 ml of gradient preparation (5 ml 10% RPMI (Lonza) + 3 ml 40% Optiprep (Sigma)/ per sample), and placed on top of 2.5 ml of the same solution. To form a 3-layers gradient, 6 ml of G-solution was added on the top. Samples were then incubated for 10 min at RT before subjecting to centrifugation at 1700 rpm. Finally, the interphase between the upper and the middle layers of the gradient was harvested and filtered through a 70 μm Nylon filter and washed with G-solution. Islets were handpicked under the microscope. In order to achieve a single cell suspension of islets, islets were handpicked in an 1.5 ml Eppendorf tube, pelleted (800 rpm, 1 min) washed with PBS (-Mg/Ca, Gibco) and digested with 0.25% Trypsin with EDTA (Gibco) at 37°C for 8 min. Mechanical disaggregation every 2-3 min was required. After, the digestive reaction was stopped and cells were pelleted (1200 rpm, 5 min).

INSTRUMENT

Single cells were analyzed by FACS-Aria III (BD).

SOFTWARE

Data were analyzed by FlowJo.

CELL POPULATION ABUNDANCE

Endocrine cell populations are reported in this manuscript.

GATING STRATEGY

Single cells were gated according to their FSC-A (forward scatter area) and SSC-A (side scatter area). Singlets were gated dependent on the FSC-W (forward scatter width) and SSC-W (forward scatter height) and dead cells were excluded using the marker 7AAD (eBioscience). The EVF endocrine populations were discriminated upon their Venus fluorescence emission at 488 nm and the β- and α-lineages according to their BFP emission at 405 nm (positive and negative respectively). To enrich for β-cells the distinct SSC-A high populations were gated.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.