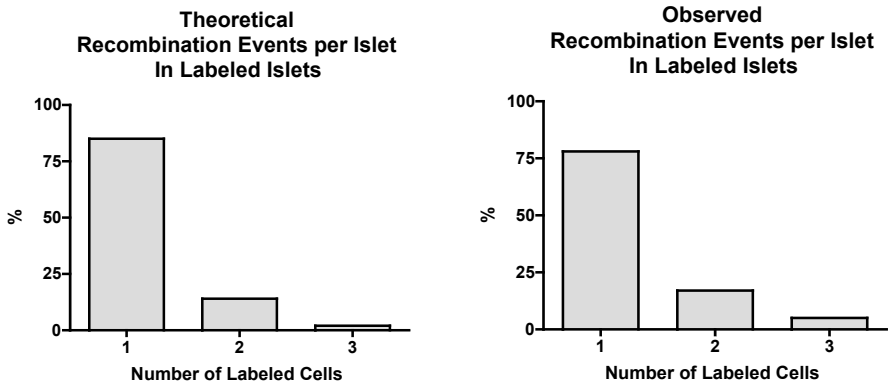
Statistical appendix
Assessment of recombination frequency

In *Ngn3-GR/RG* mice, islet cell labeling results from an induced chromosomal translocation (or 'recombination') that occurs within a reduced period of a mouse lifetime, namely the second half of intrauterine development until birth (between E10.5 and P0). This event happens randomly, at very low frequency, and so it follows the Poisson law. In this situation, the mean number of recombination events per islet (i.e. of labeled cells per islet) is λ; this number determines the probability of having labeled cells at birth. A fraction $e^{-\lambda}$ of islets have no recombination (i.e. no labeled cells); $\lambda.e^{-\lambda}$ islets have one single labeled cell, and, in general, a fraction $\lambda^n.e^{-\lambda}/n!$ of islets bear *n* recombinations.

According to the Poisson law, $\lambda<10=\sigma^2$, where σ^2 is the variance of the distribution. P (0) is $e^{-\lambda}$, i.e. the probability for a given islet of having no labeled cells. In adult *Ngn3-GR/RG* mice, P (0)=0.72, as 72% of islets had no labeled cells (see Fig. 2D). Therefore, $\lambda=-\ln(P(0))=0.32$. The variance is $\sigma^2=(0.55)^2=0.30$, then $\lambda \sim \sigma^2$. Since islet cell recombination in *Ngn3-GR/RG* mice follows the Poisson law, the Poisson equation ($\lambda^n.e^{-\lambda}/n!$) allows us to estimate the number of recombination events occurring per islet at birth:



Expected (theoretical, left) and observed (right) frequencies of labeled cells found in newborn islets are alike (χ^2 test; *P*=0.3922).

At birth, nearly 80% of labeled islets only contained one single labeled cell, thus suggesting that fetal Ngn3-expressing cells differentiate into mature islet endocrine cells without proliferation. In adult pancreas, the clonal progeny of these early isolated labeled cells formed small groups (clusters). In islets that contained more than one labeled cell at birth (20% of labeled islets), the probability that the clusters derived from two or more independent labeled cells are in fact composed of different cell types is defined by the following equation:

P (heterogeneous cluster)=P (>1 recombination event/islet) × P (different hormone)

Thus, P (het. β)=(1–0.72) × (1–0.75)=7%; P (het. α)=(1–0.72) × (1–0.15)=24%, and P (het. δ)=(1–0.72) × (1–0.05)=27%, where 0.75, 0.15 and 0.05 are the relative proportions of the different endocrine cell types per islet (for β-, α- and δ-cells, respectively). Stated otherwise, this means that the probabilities of having homogeneous clusters for each cell type are:

Homogeneous Clusters	β-cell	α-cell	δ-cell
Expected (%)	93	76	73

These expected values were not different from values observed in *Ngn3-GR/RG* mice:

Homogeneous Clusters	β-cell	α-cell	δ-cell
Expected (%)	93	76	73
Observed (%)	84	57	64
Fisher's exact test (P value)	0.16 (NS)	0.17 (NS)	0.50 (NS)

In all cases, $P>0.05$, indicating that expected and observed frequencies are not different (NS).

At 2 months of age, 50% of the clusters in *Ngn3-GR/RG* mice were made of two cells (median; see Fig. 3E). The probability that two cells randomly chosen in an islet are of the same type is: two β-cells, $0.75 \times 0.75=0.5625$; two α-cells, $0.15 \times 0.15=0.0225$; and two δ-cells, $0.05 \times 0.05=0.0025$. This indicates that the odds of picking two β-cells are 56%, 2.25% for two α-cells and 0.25% for two δ-cells. These proportions are different from those observed (84% of β-cell clusters, 57% of α-cell clusters and 64% of δ-cell clusters were homogenous; $P<0.001$).

In conclusion, mathematical evidence suggests that heterogeneous clusters observed in aged islets are in fact fused homogeneous clusters.

Estimation of islet volume and number

Islet volume was determined using the following formula: $V=4/3\ \pi\ 125\ N\ \sqrt{N}$, where we considered that islets are roughly spherical and islet cells have an average diameter of 10 µm, and where N is the number of cells per islet section. Total pancreatic volume was estimated by dividing the total pancreatic weight by its density [1 mg/mm^3 (Finegood et al., 1995)]. The sampled pancreatic surface was multiplied by the thickness (5 µm) of the section ($=V_{sampled}$). The total number of islets was then estimated by multiplying the number of islets scored in $V_{sampled}$ by the ratio $V_{total}/V_{sampled}$.

Estimation of the proliferation rate for Ngn3$^+$ cells

In MADM mice, a quiescent Cre-expressing cell becomes one DL cell; this is the 1:1 ratio. A proliferating cell gives rise to either two SL daughters, of which one is EGFP$^+$ and the other RFP$^+$, or, with the same probability, two daughters of which one is DL (i.e. EGFP$^+$ RFP$^+$) and one unlabeled. This is the 1:2 ratio (see Fig. S1).

Between birth and 2 months of age in *Ngn3-GR/RG* mice, SL and DL cells undergo one division (cluster size is two cells at 2 months), and 10% of the total number of labeled cells is EGFP$^+$ only. Therefore, in a population of 100 labeled islet cells, 10 cells are EGFP$^+$: they represent one-quarter of the daughters originated from proliferating Ngn3$^+$ cells; the other three-quarters are RFP$^+$, DL and unlabeled cells. Then, although only 100 cells are labeled, the actual population of Cre-recombined cells is 110, because 10 cells are the unlabeled one-quarter progeny of proliferating Ngn3$^+$ cells. Out of these 110 labeled islet cells, 40 (10×4) are the progeny of 20 proliferating Ngn3$^+$ cells. Thus, the initial population of recombined Ngn3$^+$ cells was composed of these 20 proliferating cells and 110–40=70 quiescent cells, i.e. 20+70=90 recombined Ngn3$^+$ cells in total. The proliferation rate of Cre-expressing Ngn3$^+$ cells is thus 20/90=22%.

Reference

Finegood, D. T., Scaglia, L. and Bonner-Weir, S. (1995). Dynamics of beta-cell mass in the growing rat pancreas. Estimation with a simple mathematical model. *Diabetes* **44**, 249-256.