Cell Stem Cell, Volume *28*

Supplemental Information

Ductal Ngn3-expressing progenitors contribute to adult β cell neogenesis in the pancreas

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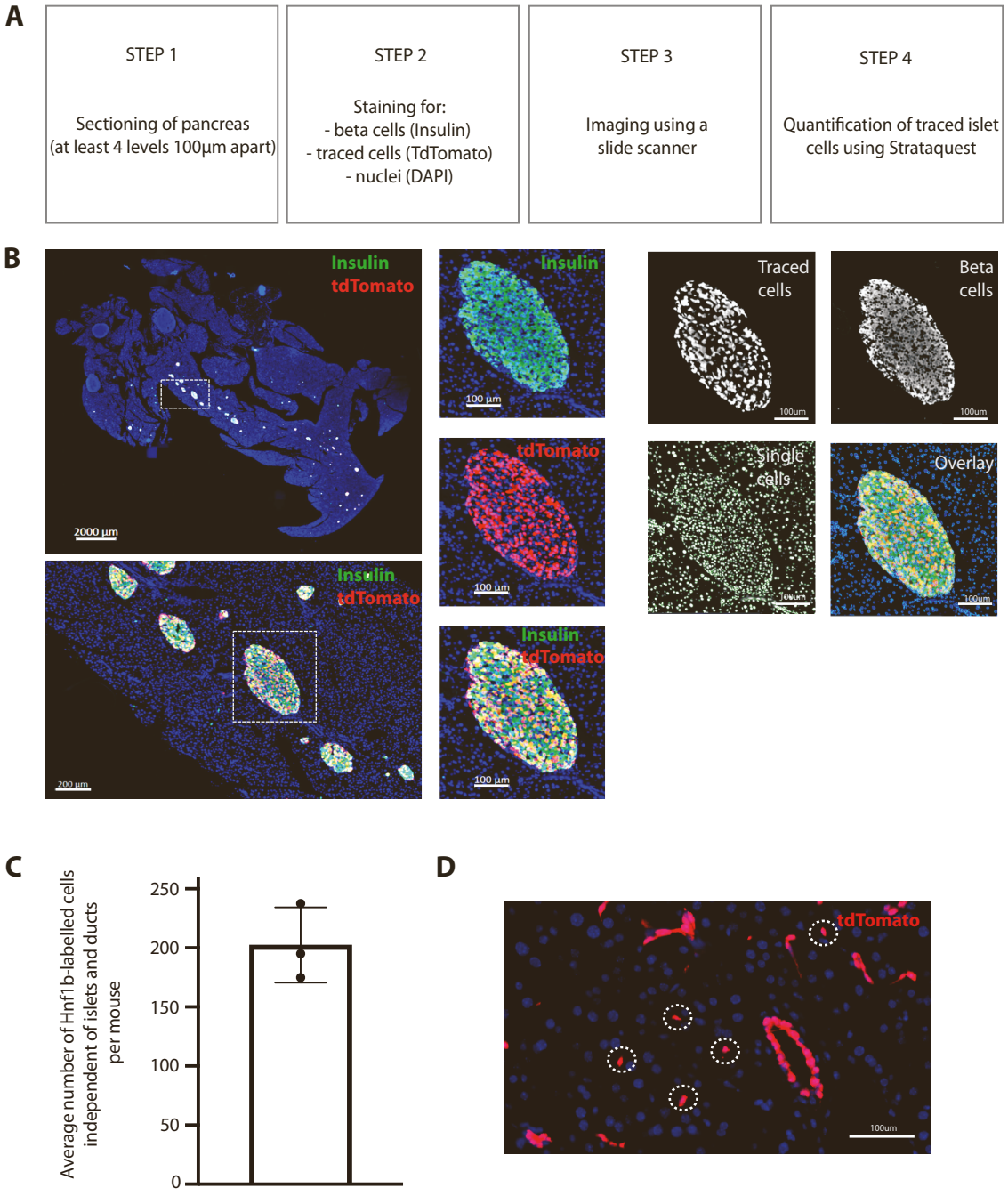

Figure S1: Scheme of lineage tracing analysis and quantification, related to Figure 1

A) Workflow of processing, antibody staining, fluorescent imaging and analysis.
B) Double IF for Insulin and tdTomato. A scan of a whole pancreas section, where multiple images are stitched together is shown (left upper panel) and high magnifications of an islet (middle panel). An example from an analysis is shown (right panels) where traced cells, INS+ cells and DAPI (for nuclei) are overlaid to allow for the percentage of INS+ islet cells which are traced to be determined..
C) Quantification of the number of HNF1b-labelled cells independent of islets and ducts in HNF1b-CreERT; tdTomato mice (n= 3 mice) at 1 week post-tamoxifen injection.
D) tdTomato IF in HNF1b-CreERT; tdTomato mice (n= 3 mice) at 1 week post-tamoxifen injection. Labelled cells independent of ducts and islets are circled with white dashed line.

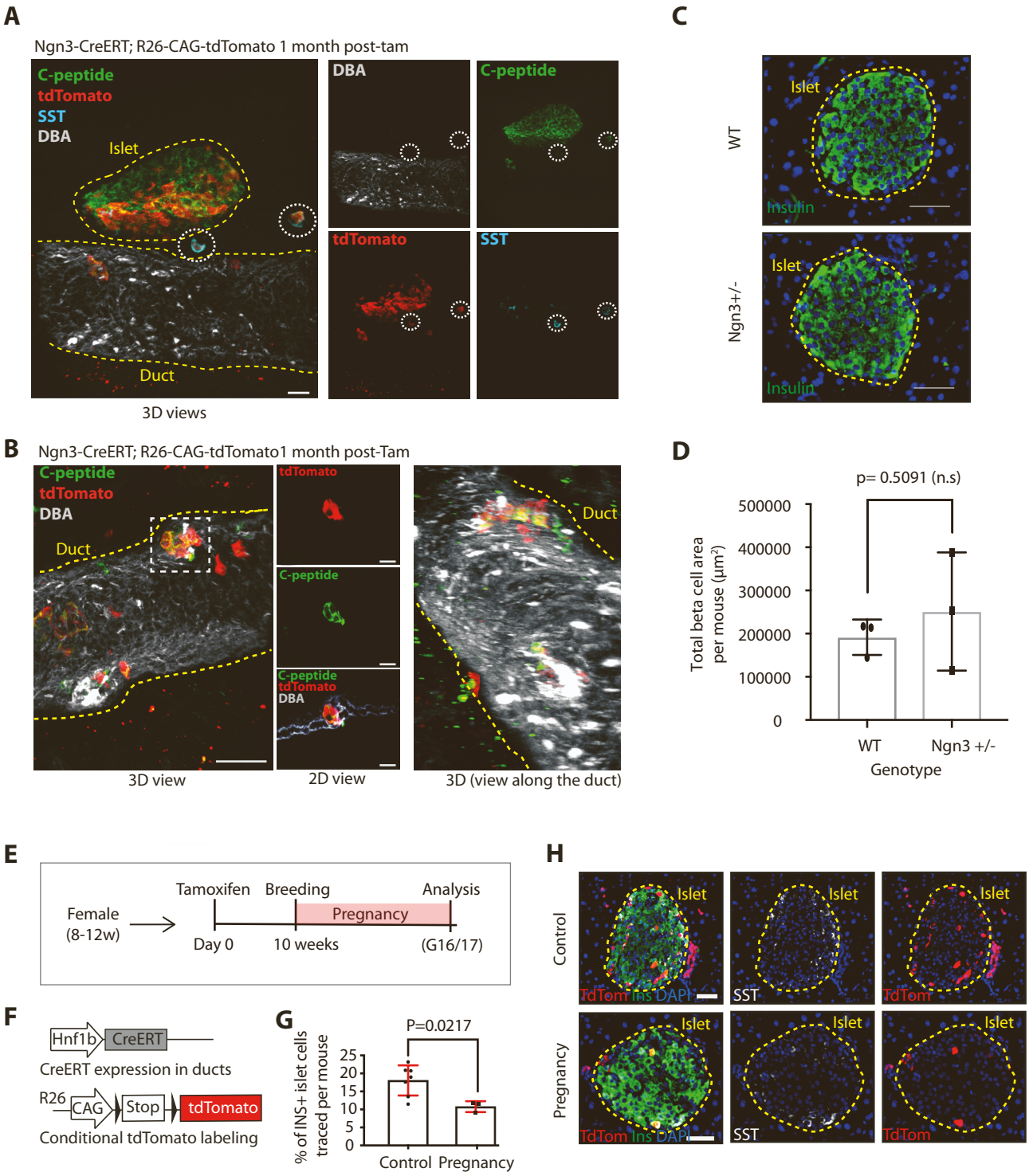

Figure S2: FLASH imaging of potentially migrating cells between duct and islet, related to Figure 2

A) 3D view following FLASH for tdTomato, C-peptide, tdTomato, SST and DBA of a pancreas from a Ngn3-CreERT; R26-CAG-tdTomato mouse at 1 month post-tamoxifen. Triple positive cells in between the duct and islet are circled. Scale bars, 20µm.

B) 3D view following FLASH for tdTomato, C-peptide, tdTomato and DBA of a pancreas from a Ngn3-CreERT; R26-CAG-tdTomato mouse at 1 month post-tamoxifen. Endocrine cell cluster within the duct is indicated by the white dash line. Scale bars, 20µm.

C) Immunofluorescence staining for Insulin in WT and Ngn3+/- mice pancreas. Scale bars, 50µm.

D) Quantification of the beta cell area in WT and Ngn3+/- mice pancreas. N=3 for WT and Ngn3+/-.

E) Schematic of experimental strategy to analyse the contribution of ductal cells to islets during pregnancy.

F) Schematic of the lineage tracing strategy.

G) Quantification of the percentage of INS+ islet cells traced per mouse in either mice at gestational day 16-17 of pregnancy or non-pregnant mice. Control N=7 mice, Pregnant N=3 mice.

H) Triple IF for Insulin (Ins), Somatostatin (Sst) and tdTomato (tdTom) in representative islets from control or pregnant mice. Scale bars, 50µm.

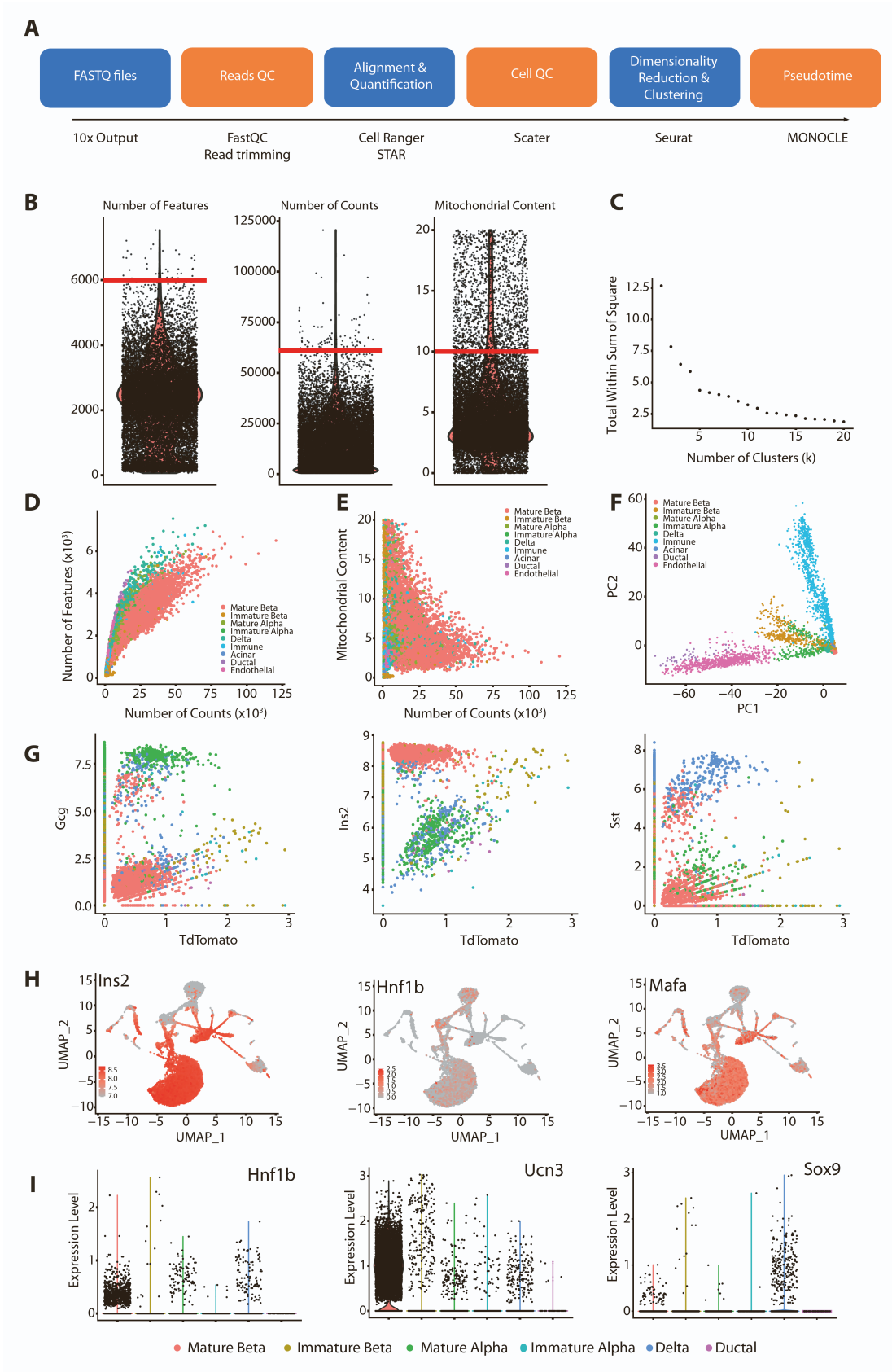

Figure S3: Analysis and validation of single-cell RNAseq data, related to Figure 4

A) Schematic of workflow for scRNAseq data analysis.
B) Cell quality control assessing number of features, count number and mitochondrial content. Red line indicate cutoff values.
C) Validation of unsupervised clustering by scree plot (k=10).
D) Number of detected features plotted against number of counts.
E) Mitochondrial content plotted against number of counts.
F) Principal component analysis of cells passing QC metrics.
G) Feature plots of traced cells against non-traced equivalents for Gcg, Ins2 and Sst expression.
H) UMAP plots of Ins2, Hnf1b and MafA across scRNAseq data set.
I) Violin plots showing Hnf1b, Ucn3 and Sox9 expression per cluster

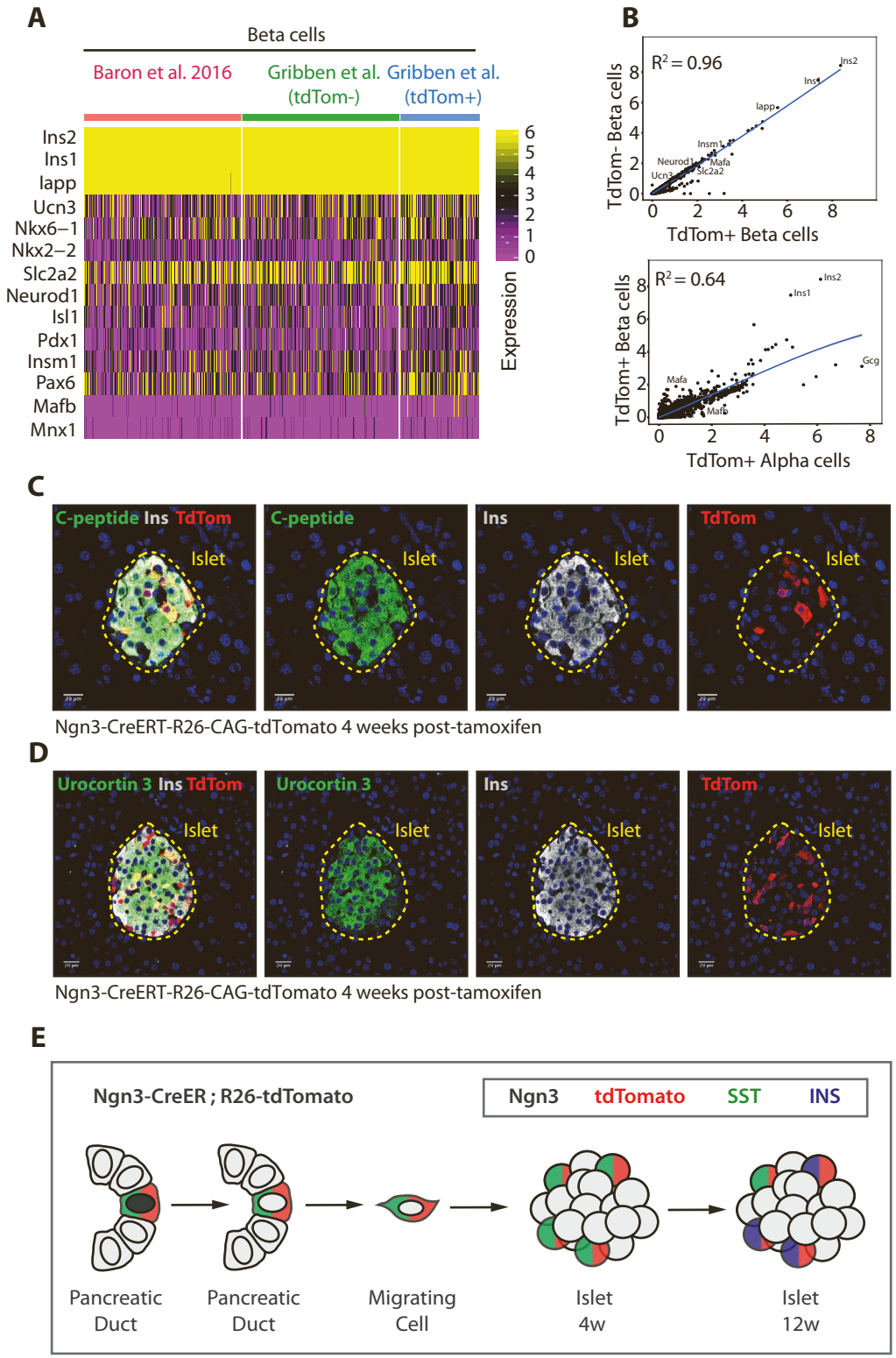

Figure S4: Analysis of tdTomato+ beta cells in Ngn3CreERT; R26-CAG-tdTomato mice, related to Figure 4.

(A) Heatmap showing equivalent expression of beta cell markers across Baron dataset and non-traced and traced beta cells from our study.
(B) Correlation analysis of tdTomato- and tdTomato+ (top) beta cells and tdTomato+ Alpha and tdTomato+ Beta cells
(C) Triple IF for Insulin, C-peptide and tdTomato in Ngn3CreERT; R26-CAG-tdTomato mice at 4 weeks post-tamoxifen. A representative islet is shown.
(D) Triple IF for Insulin, C-peptide and tdTomato in Ngn3CreERT; R26-CAG-tdTomato mice at 4 weeks post-tamoxifen. A representative islet is shown.
(E) Proposed model for Ngn3+ ductal cell to beta cell differentiation.

Supplementary table 1

InsCreERT experiment

Time post-tamoxifen	Male/Female	Age when injected
1m	Male	20 weeks
1m	Male	26 weeks
1m	Female	17 weeks
1m	Female	17 weeks
6m	Male	21 weeks
6m	Female	10 weeks
6m	Male	18 weeks
6m	Male	18 weeks

Hnf1bCreERT experiment

Time post-tamoxifen	Male/Female	Age when injected
1 week	Male	12 weeks
1 week	Male	8 weeks
1 week	Male	8 weeks
1 week	Male	8 weeks
5 weeks	Male	16 weeks
5 weeks	Female	16 weeks
5 weeks	Male	16 weeks
5 weeks	Female	16 weeks
12 weeks	Male	10 weeks
12 weeks	Female	10 weeks
12 weeks	Female	8 weeks
12 weeks	Male	12 weeks
12 weeks	Female	10 weeks
12 weeks	Female	10 weeks

Ngn3CreERT experiment

Time post-tamoxifen	Male/Female	Age when injected
1 week	Male	43 weeks
1 week	Female	23 weeks
1 week	Female	22 weeks
4 weeks	Female	12 weeks
4 weeks	Female	16 weeks
4 weeks	Male	12 weeks

Ngn3CreERT experiment single cell RNAseq

Time post-tamoxifen	Male/Female	Age when injected
10 days	Female	10 weeks
10 days	Female	10 weeks
10 days	Male	12 weeks
10 days	Male	12 weeks
10 days	Female	12 weeks
10 days	Female	12 weeks

Ngn3CreERT Akita ducts quantification

Cohort	Male/Female	Age when injected
Akita tam injected	Male	13 weeks
Akita tam injected	Male	13 weeks
Akita tam injected	Female	34 weeks
WT	Male	43 weeks
WT	Female	23 weeks
WT	Female	22 weeks
Akita no tamoxifen control	Male	15 weeks
Akita no tamoxifen control	Male	15 weeks
Akita no tamoxifen control	Male	15 weeks
Akita no tamoxifen control	Male	15 weeks

Supplementary table 1. Details of mice included in lineage tracing experiments, related to Figures 1-4.

Supplementary table 2

Lineage tracing experiment	Average number of islet cells analysed per section
Hnf1bCreERT	1960
Ngn3CreERT	2130
InsCreERT	2854

Supplementary table 2. Details of the number of islets counted per lineage tracing experiment, related to Figures 1 and 2.