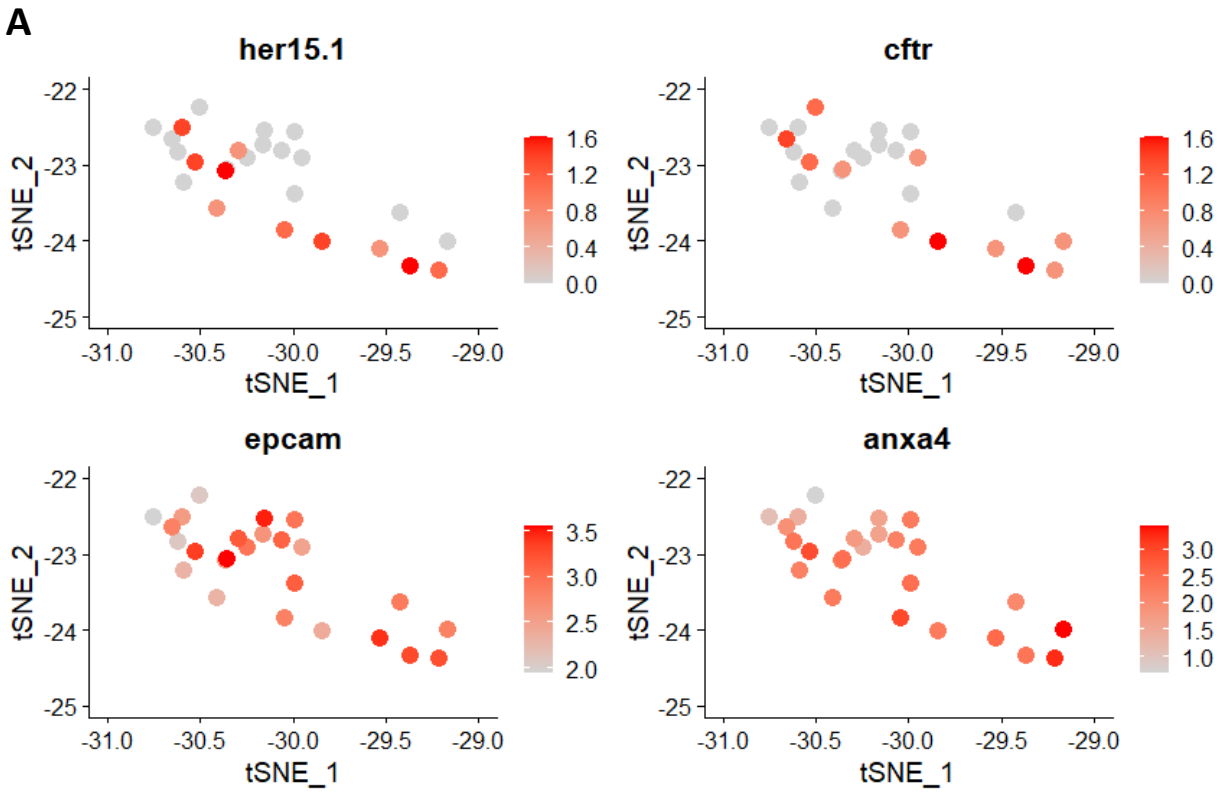


Fig. S1. Markers for duct cells (A). t-SNE plot representation of duct cell population expressing *her15.1, cftr, epcam and anxa4*

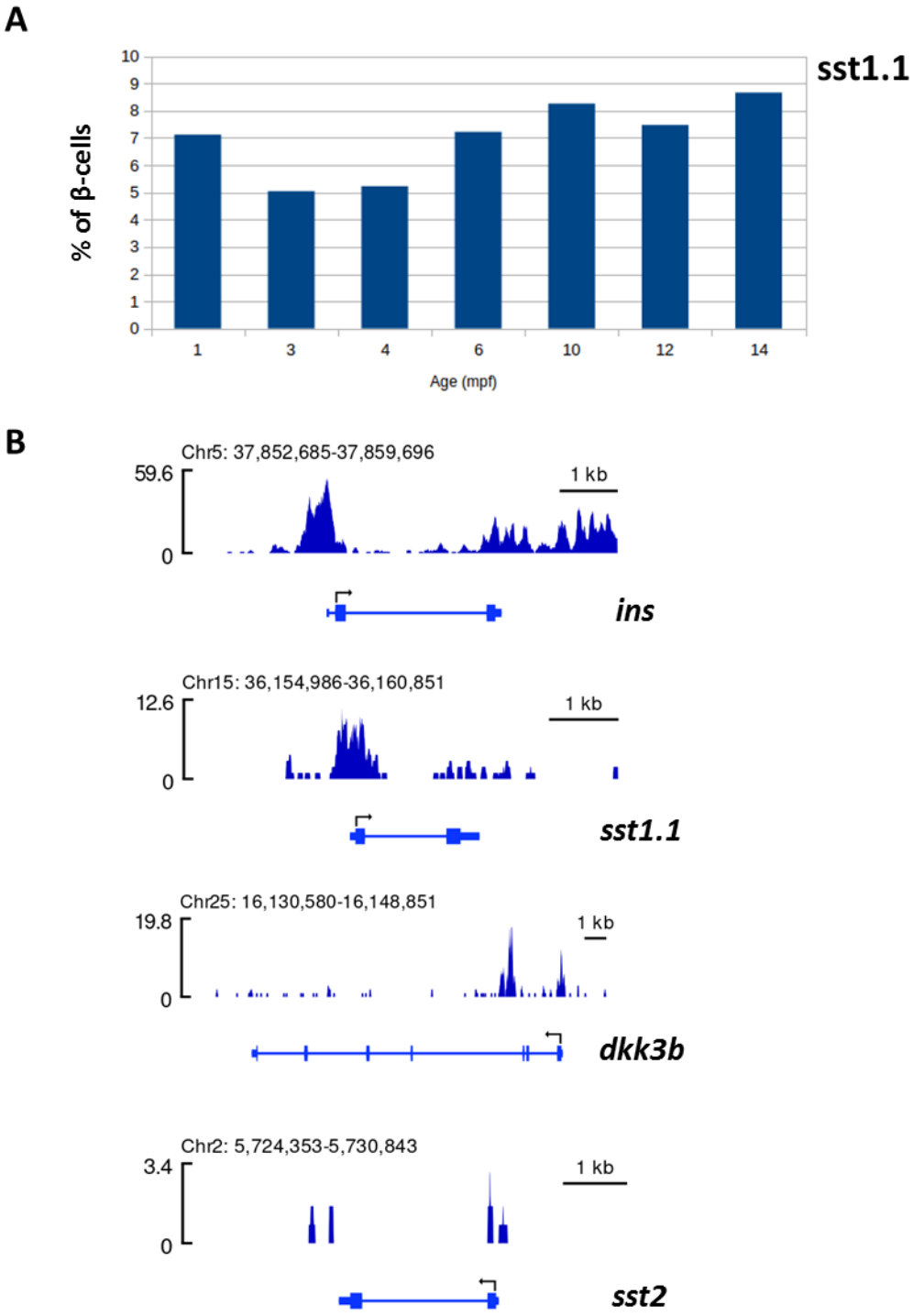


Fig. S2. Persistence of bi-hormonal cells during various developmental stages of zebrafish and ATAC seq analysis of chromatin in β-cells. (A) Boxplot depicting the percentage of β-cells expressing *sst1.1* in a previously published single-cell smartseq2 RNA-Seq. dataset. The dataset contains single-cell RNA-Seq. profiles of β-cells isolated from the *Tg(ins:BB1.0L)* transgenic line using FACS at different stages of the zebrafish juvenile and adult life. **(B)** Plots for selected genes depicting the open genomic regions (in orange) identified using ATAC-Seq. specifically for β-cells. Regions proximal to the promoters of *ins*, *sst1.1* and *dkk3b* genes display open chromatin.

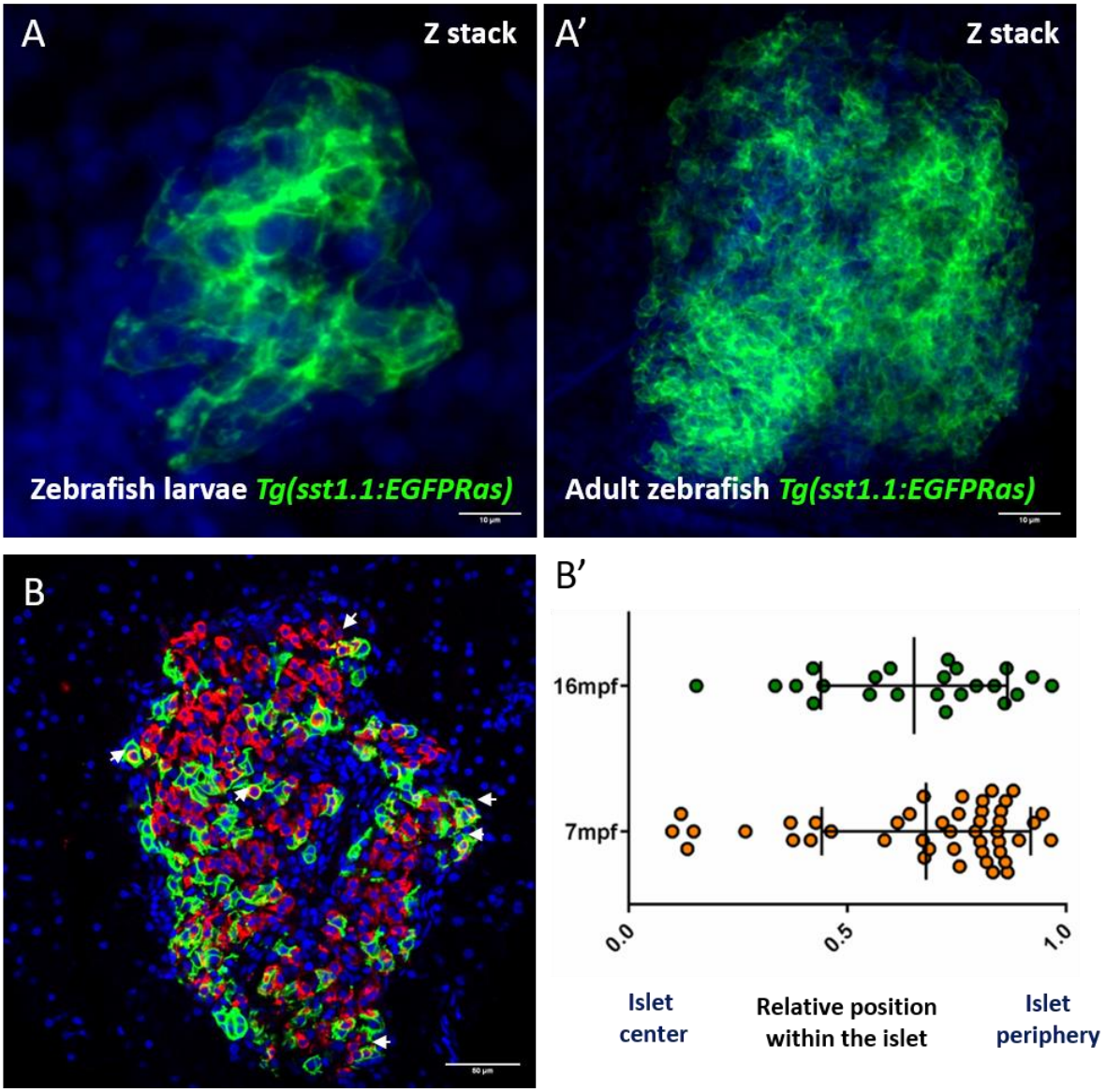


Fig. S3. The distribution of δ1- and β/δ1 bi-hormonal cells in the zebrafish islet. (A-A') Z-projection of the pancreatic islet from 5 dpf zebrafish larvae and adult zebrafish showing the distribution of δ1-cells (EGFP labelled) (DAPI in blue). (B) Single-plane confocal image of the sections from adult zebrafish islets highlighting δ1-cells (green) and β-cells (red). White arrowheads point to the bi-hormonal cells (B'). Scatterplot representing the relative distribution of bi-hormonal cells in the adult zebrafish islet (ay 7 and 16 mpf) (each dot represents a single-cell).

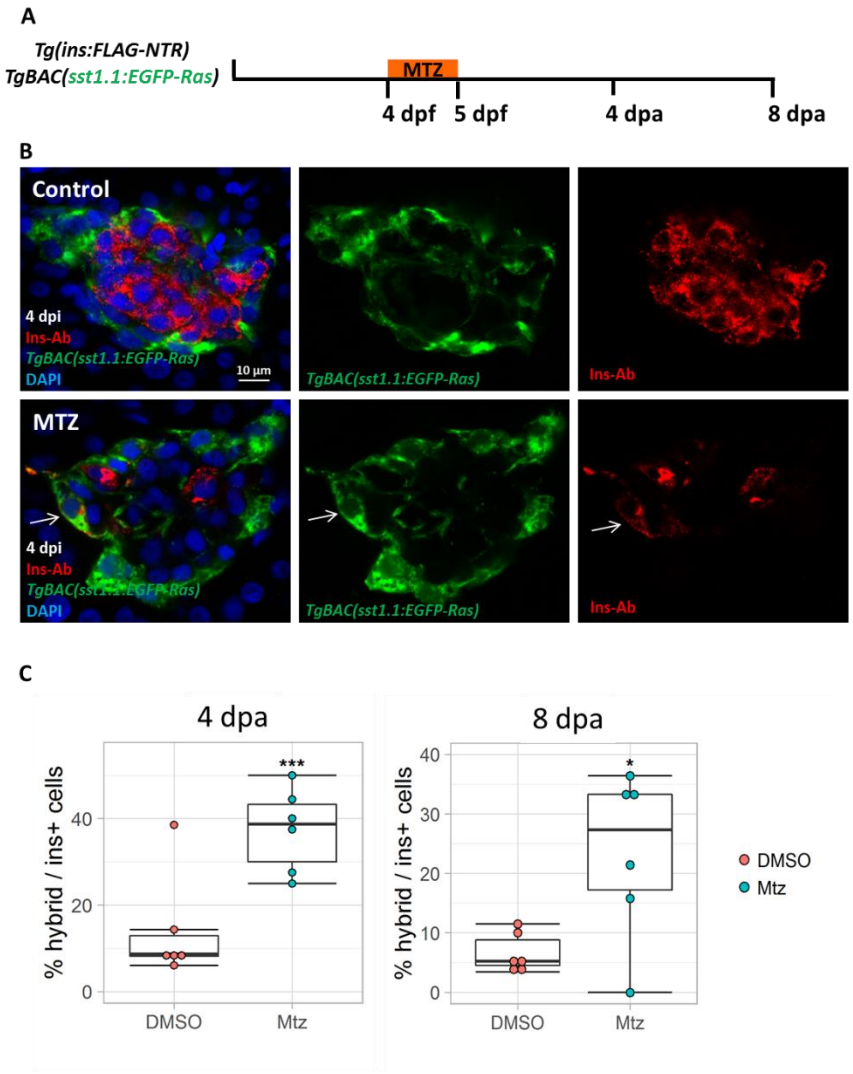


Fig. S4. The proportion of hybrid cells increase during β-cell regeneration. (A) To assess the dynamics of hybrid cells during β-cell regeneration, *Tg(ins: FLAG-NTR)*; *Tg(sst1.1: EGFP-Ras)* double transgenic animals were treated with Mtz at 4 dpf for 24 hours. Following Mtz-based ablation of NTR-expressing β-cells, the samples were collected at 4- and 8-days post-ablation (dpa) for quantification. **(B)** Single confocal stack of islets at 4 dpa. Arrows mark bi-hormonal cells, which express EGFP and are also marked with the Insulin antibody. **(C)** Barplots displaying the percentage of bi-hormonal cells among all insulin+ cells in the islet at 4 and 8 dpa (n = 6 animals for each condition). Comparison based on two-tailed Heteroscedastic t-test. p-value: * < 0.05; *** < 0.001.

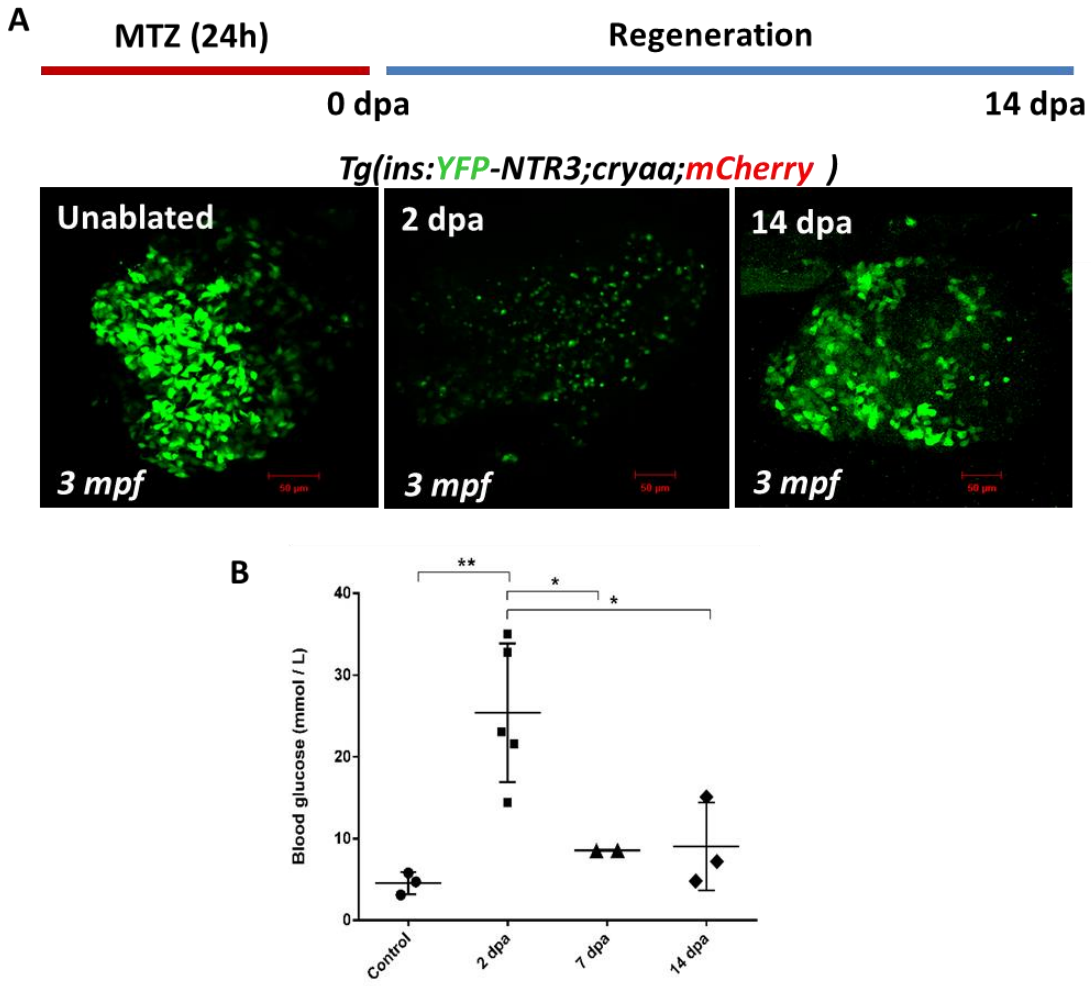


Fig. S5. An efficient NTR system highlighting β-cell ablation and regeneration in the adult zebrafish pancreas. (A) Confocal projection of a pancreatic islet from a 3-month-old *Tg(ins:YFP-NTR3)* transgenic zebrafish showing β-cell destruction and regeneration following Mtz-treatment and wash-out. **(B)** Fasting blood glucose level measurements (mmol/L) show a return to normoglycemic levels by 14-days post β-cell ablation.

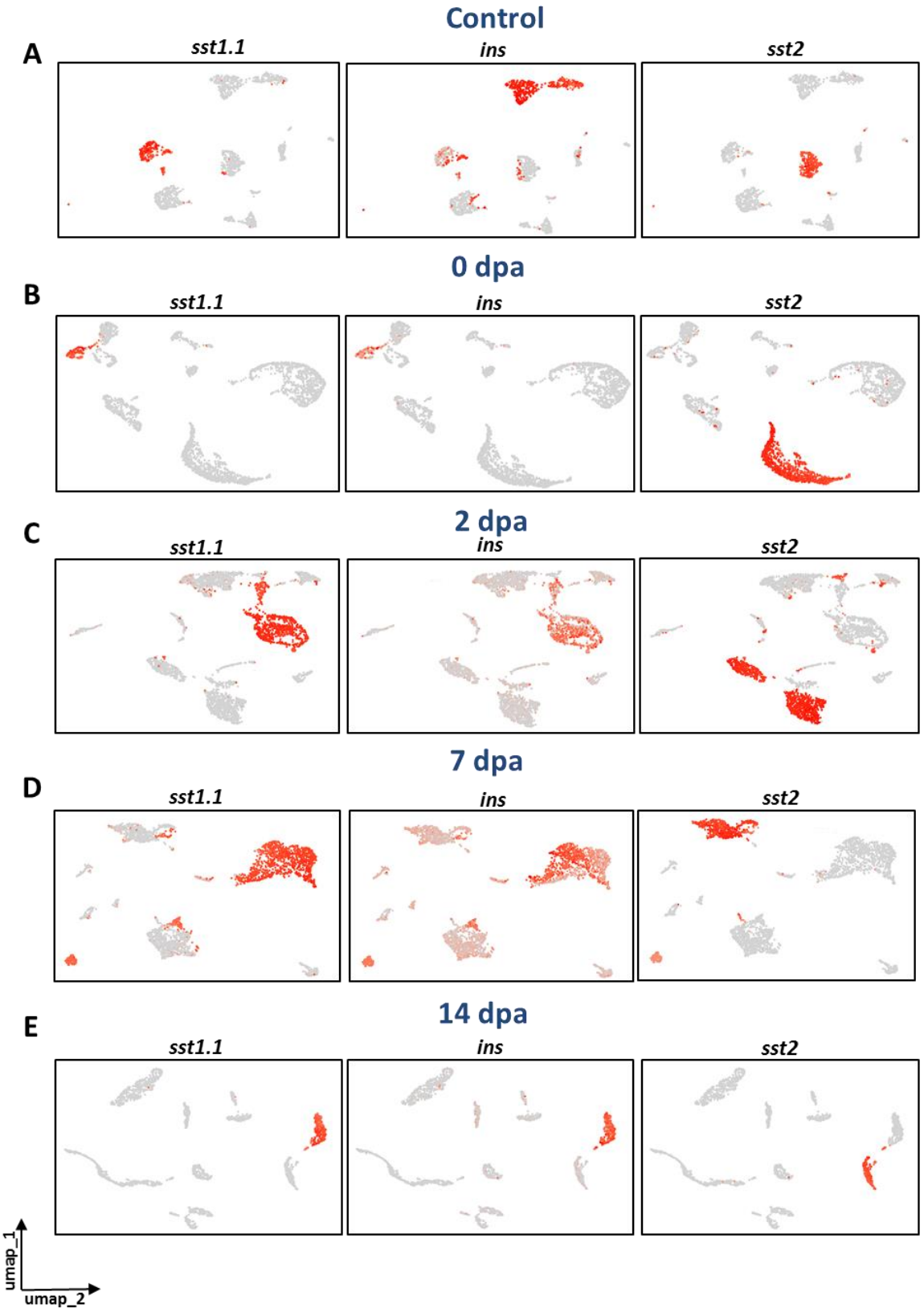
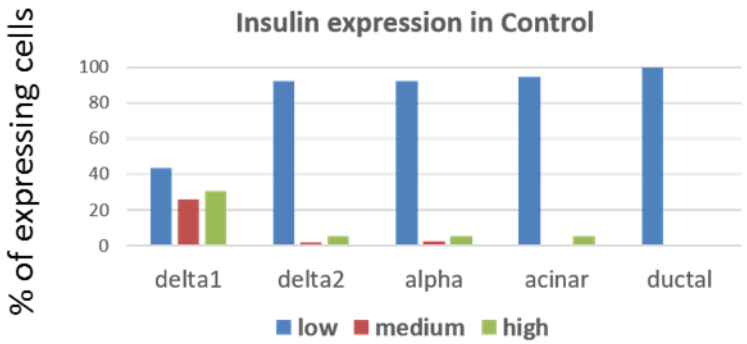


Fig. S6. δ1- but not δ2-cells express insulin upon β-cell loss in adults. (A-E) UMAP plot representation of adult zebrafish pancreatic cells showing expression of *ins*, *sst1.1*, and *sst2* at various β-cell regeneration time-points: Control, 0 dpa, 2 dpa, 7 dpa, and 14 dpa.

A.



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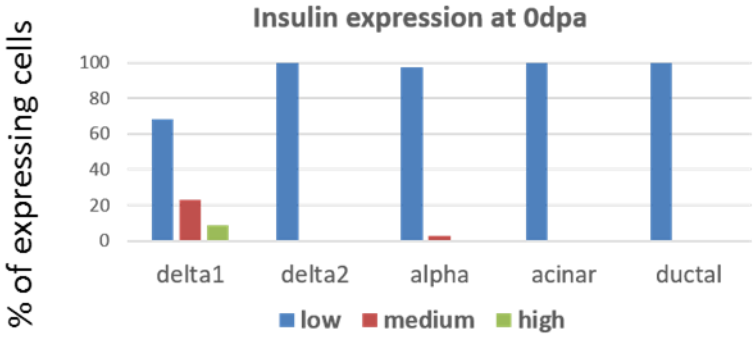

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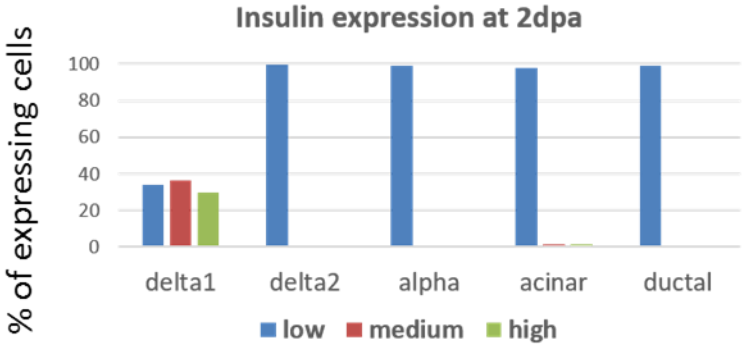
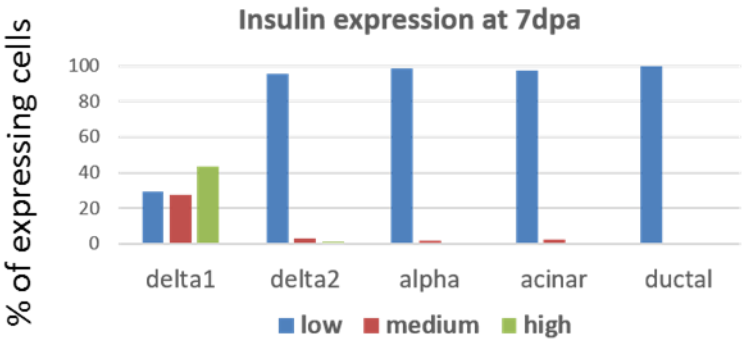


D.



E.

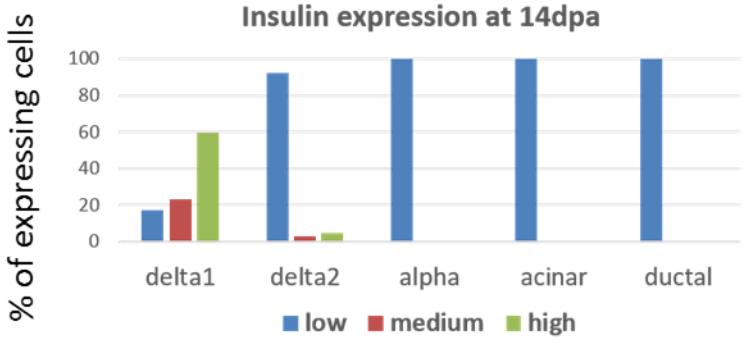


Fig. S7. Time-course of insulin expression in different cell types during β-cell regeneration. (A-E) The 10X regeneration datasets were mined to evaluate the proportions of ins-positive cells in the cell clusters expressing δ1, δ2, α, acinar and ductal markers. Based on the insulin expression level, cells were categorized into low, medium and high ins-expressing cells. The bar-plots highlighting *ins* expression profile across different β-cell regeneration time-points and cell types (control, 0, 2, 7 and 14 dpa respectively). Insulin expression increases (from low to high) in δ1-cells during β-cell regeneration, whereas other cell types retain low ins expression across time.

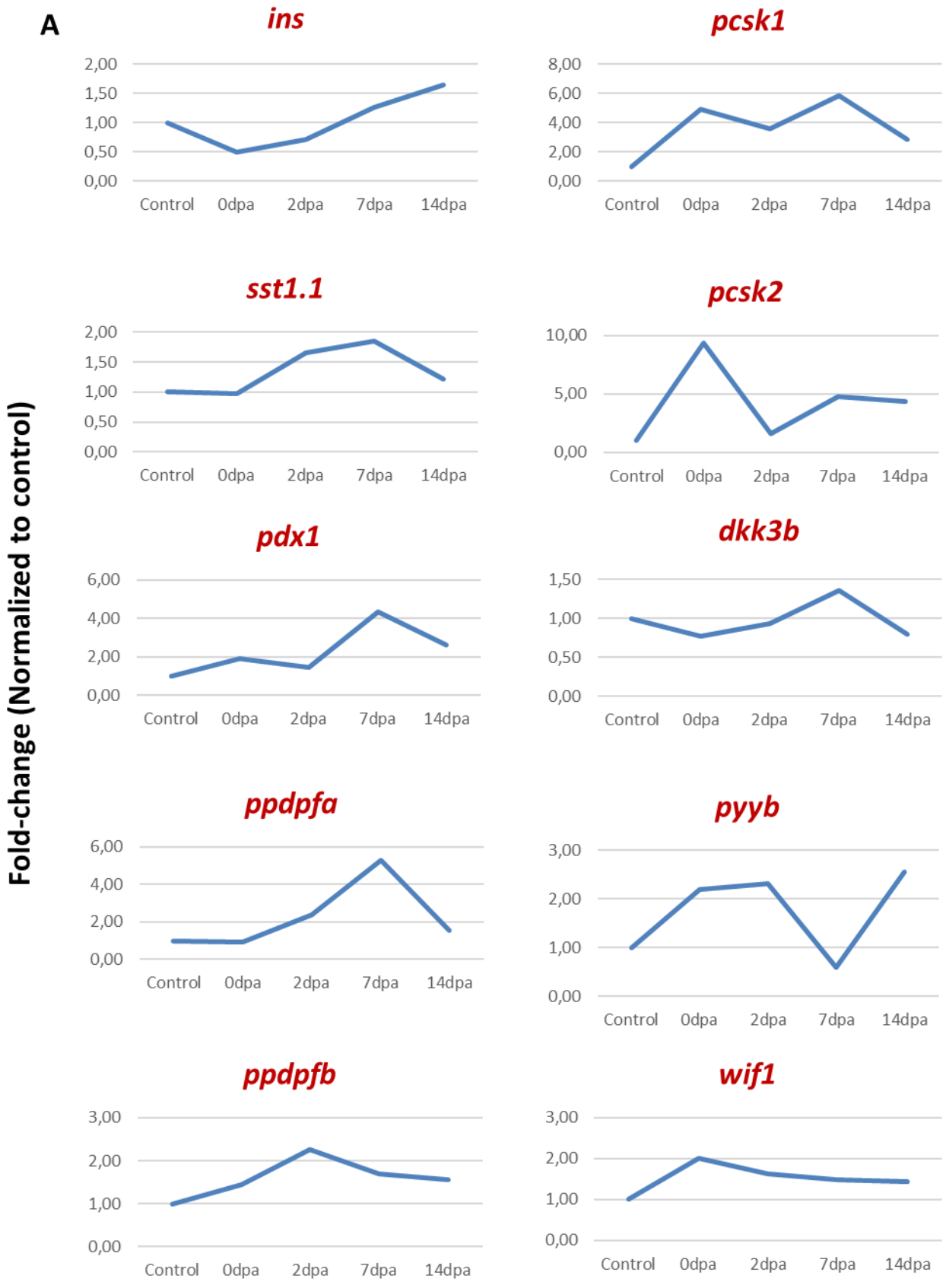


Fig. S8. Average expression profile of genes in the δ1-cluster during β-cell regeneration. (A) The 10X regeneration dataset was mined to determine the average expression profile of various β-cell specific genes and progenitor markers in the δ1-cluster. The graph shows the change in the average expression of specific genes (*ins, sst1.1, pdx1, ppdpfa, ppdpfb, pcsk1, pcsk2, dkk3b, pyyb,* and *wif1*) during β-cell regeneration. The fold-change values on the Y-Axis have been normalized to the average expression profile of the control sample.

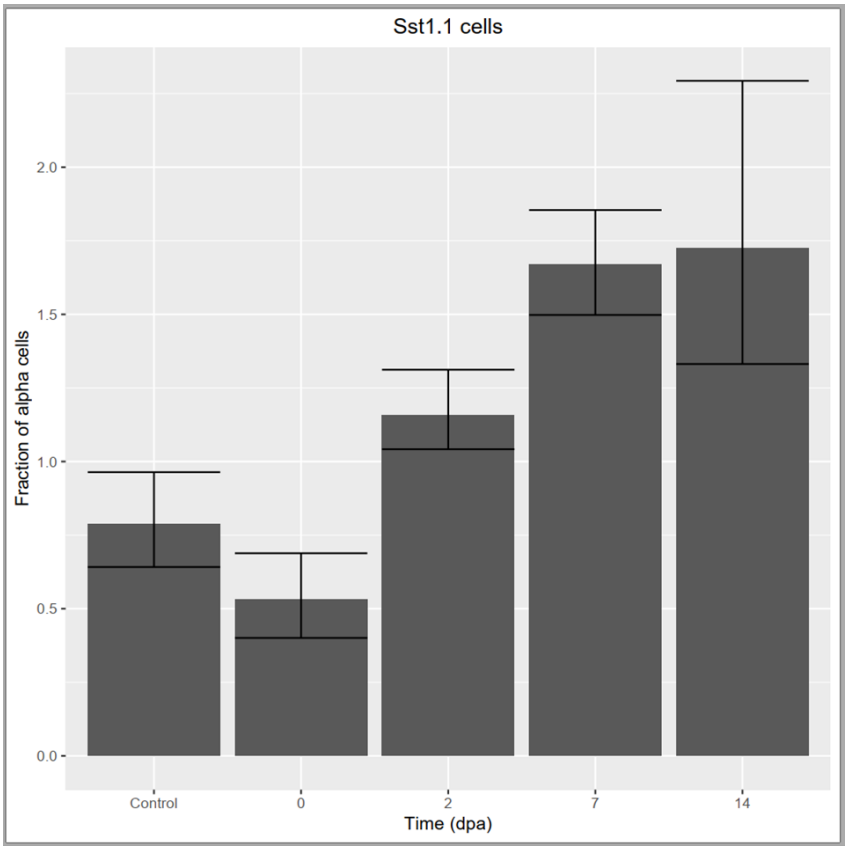


Fig. S9. The δ1-cell population expands following β-cell ablation.

Graph showing the increase of *sst1.1*-expressing cells relative to α-cells after β cell ablation with 99% confidence intervals.

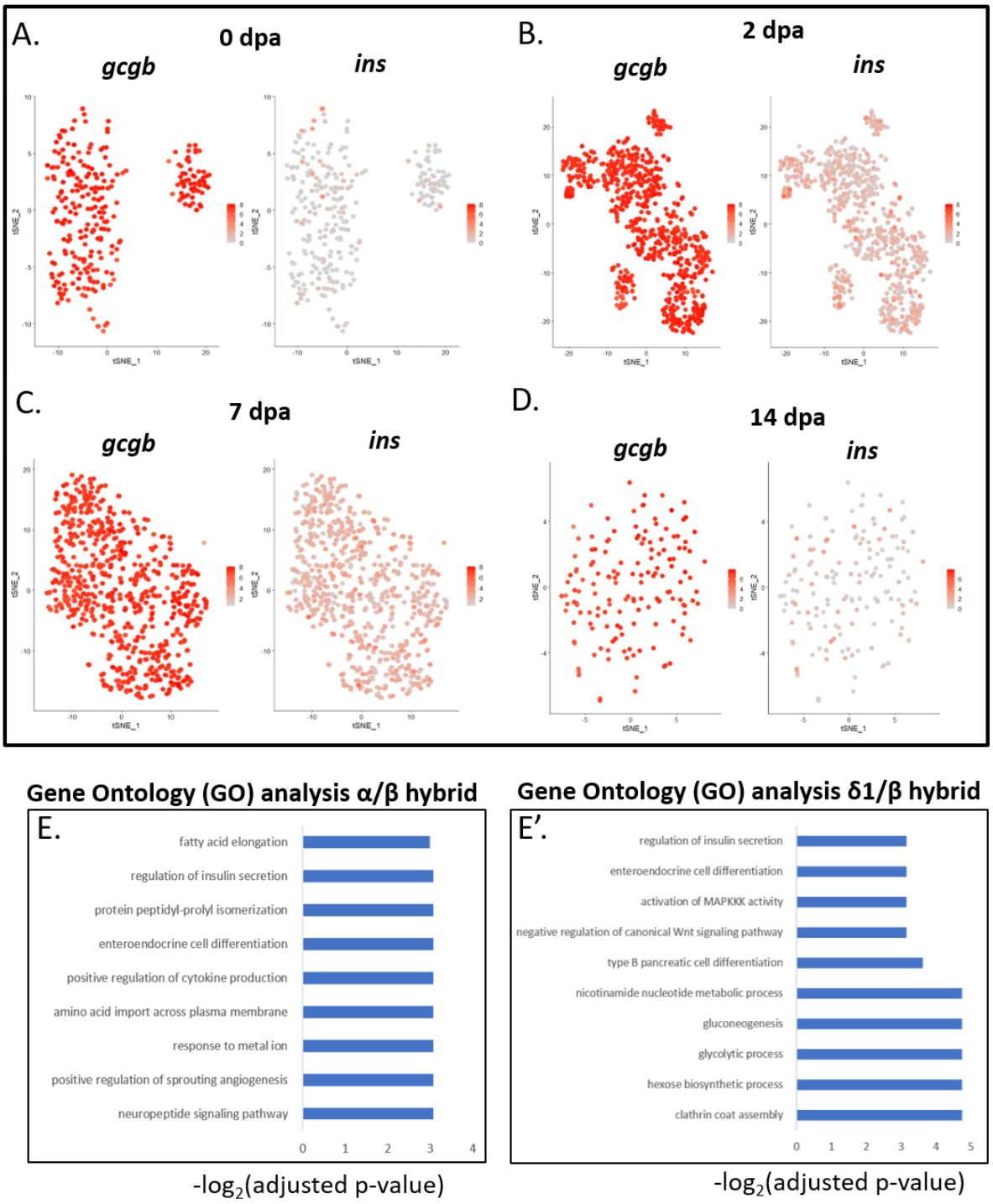


Fig. S10. Insulin expression in α-cells over the course of β-cell regeneration (A-D). t-SNE plot representation of *gcgb* and *ins* expression in the cluster corresponding to α-cells across various β-cell regeneration time-points. **(E-E')** Gene Ontology (GO) analysis using the DEGs from α/β (E) and δ1/β (E') hybrid cells at 7 dpa. GO analysis was performed using the FishEnrichr tool (p-value < 0.05; adjusted p-value (FDR) <0.15). Highly expressed genes (*ins, gcgb* and *sst1.1*) were removed while performing the GO analysis.

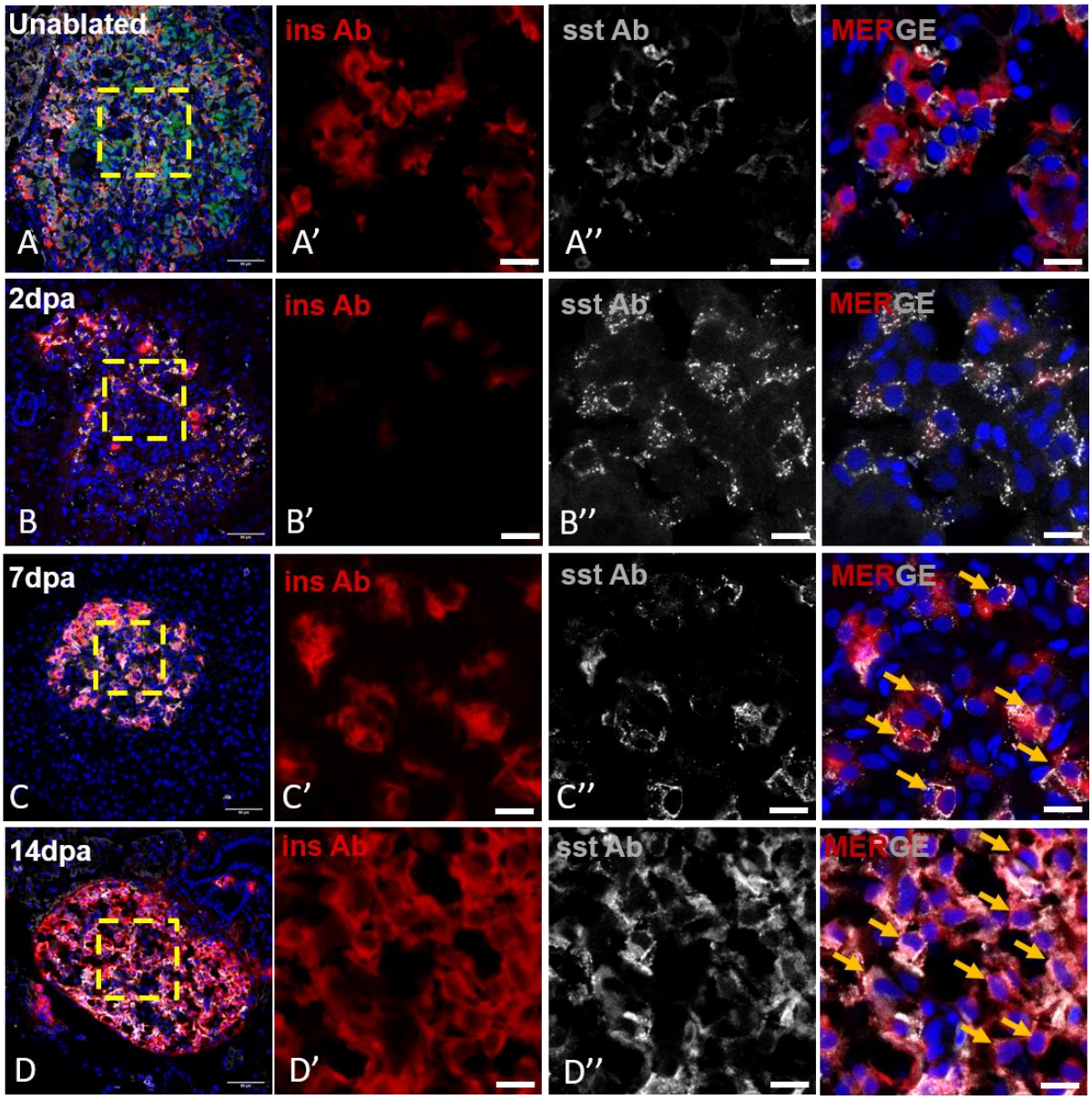


Fig. S11. Insulin and somatostatin co-expressing cells in the primary islets during the course of β-cell regeneration. (A-D) Single-plane confocal images of the primary islet from adult zebrafish pancreas over the course of β-cell regeneration: unablated (A), 2 dpa (B), 7 dpa (C) and 14 dpa (D). (Red: insulin antibody; Gray: somatostatin antibody). Yellow arrows point to ins/sst bi-hormonal cells. Scale bars = 50μm (lower magnification); Scale bars = 10μm (higher magnification) images.

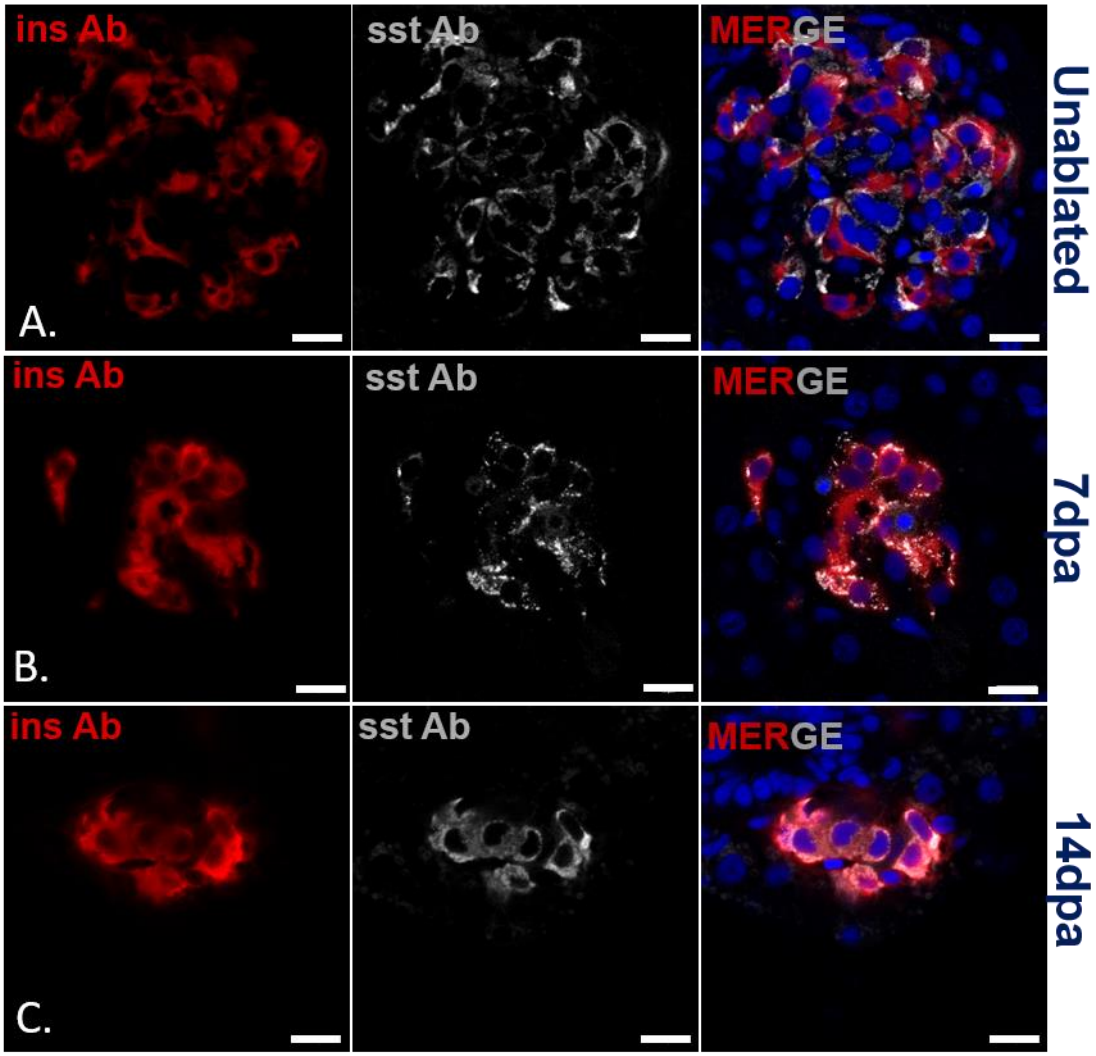


Fig. S12. Insulin and somatostatin co-expressing cells in the secondary islets during the course of β-cell regeneration. (A-C) Single-plane confocal images of secondary islets from adult zebrafish pancreas over the course of β-cell regeneration**:** unablated (A), 7 dpa (B), and 14 dpa (C). (Red = insulin antibody; grey = somatostatin antibody). Scale bars = 10μm.

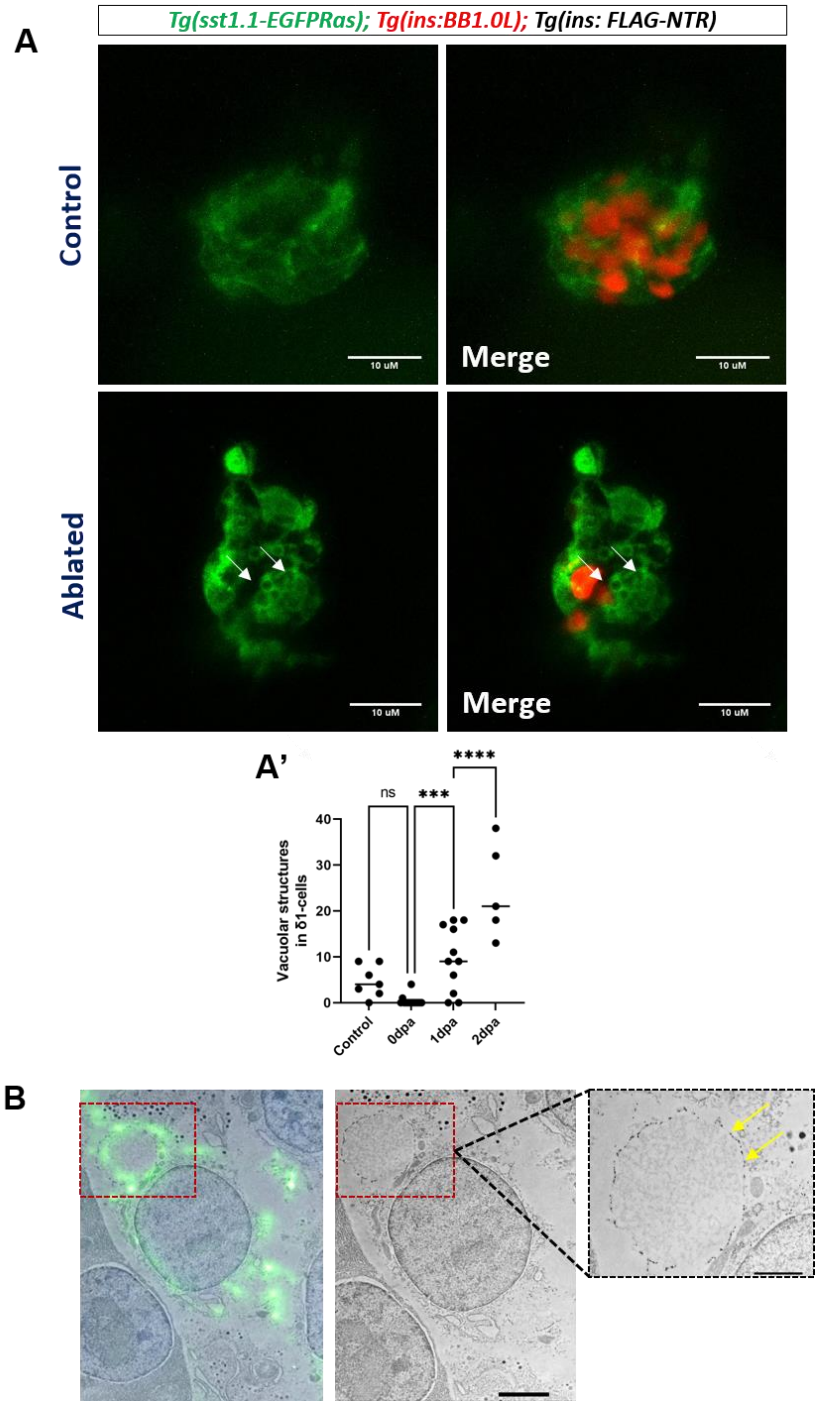


Fig. S13. Accumulation of vacuolar structures in the δ1-cells upon β-cell loss. (A) 3D-confocal projection of control and β-cell-ablated samples in *Tg(ins: FLAG-NTR)*; *Tg(sst1.1: EGFP-Ras), Tg(ins: BB1.0L)*-triple transgenic animals. Upon β-cell ablation, there is emergence of prominent vacuolar structures

in the GFP-positive cells. **(A')** Scatter-plot showing the total number of vacuolar structures in the δ1-cells post β-cell loss in the zebrafish larvae. **(B)** CLEM (Correlative Light and Electron Microscopy) section obtained from the pancreatic islet larvae following β-cell ablation. The animals are *Tg(ins: FLAG-NTR)*; *Tg(sst1.1:EGFP-Ras), Tg(ins:BB1.0L)*-triple transgenic. The animals were treated with vehicle or Mtz from 2.5 to 3 dpf and were fixed at 5 dpf. During the CLEM protocol, the sections were labelled with immuno-gold particles labelling the GFP protein (indicated by the yellow arrow). The section highlights the appearance of vacuolar structures (highlighted with the red-box) in δ1-cells at 2-days post β-cell ablation.

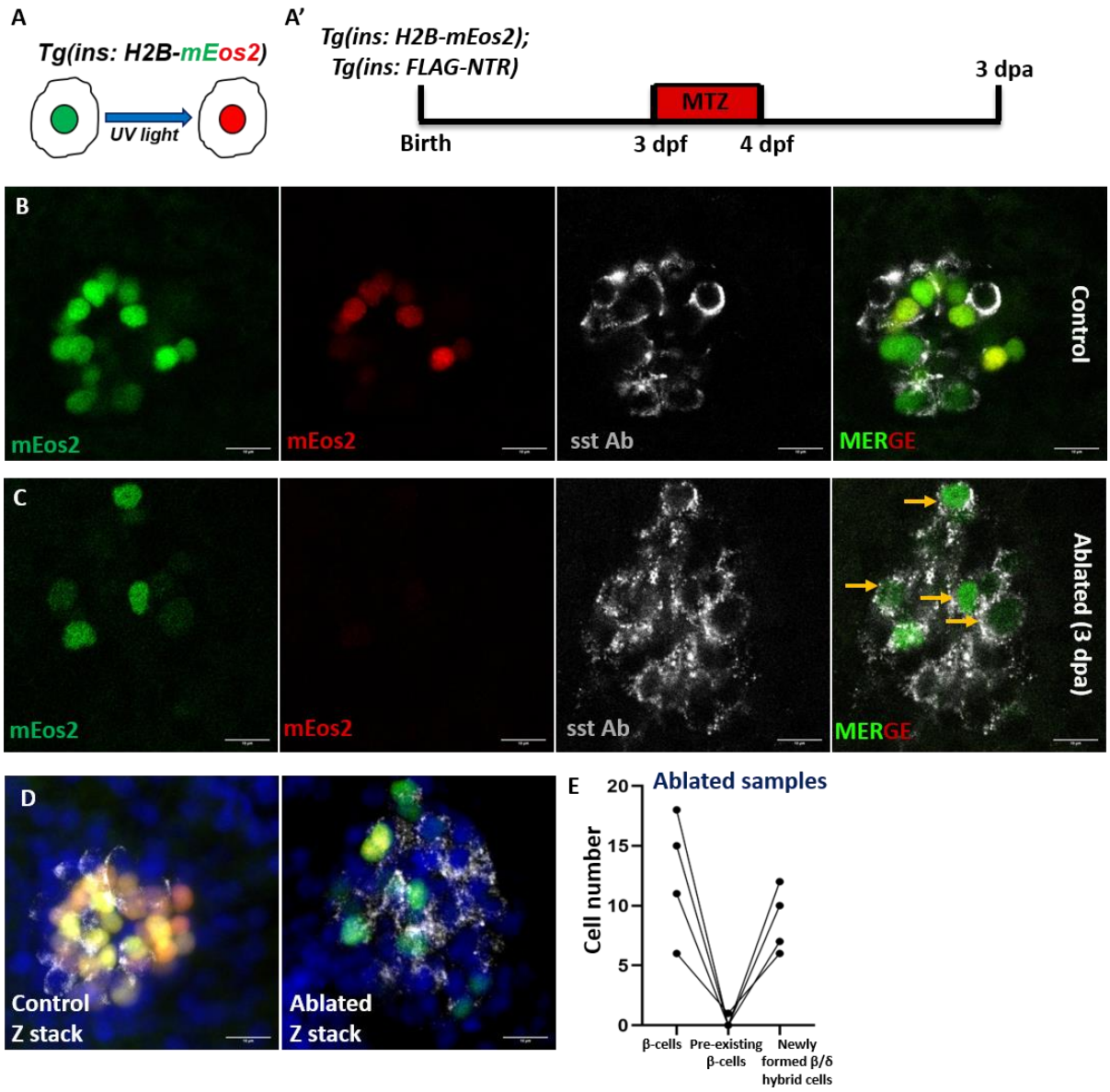


Fig. S14. Bi-hormonal cells do not arise from pre-existing β-cells. **(A)** Schematic of the *Tg(ins: H2B-mEos2)* reporter line, where a green-to-red photoconvertible protein mEos2 (fused to histone H2B) is expressed under the insulin promoter. The nuclei of pre-existing β-cells are photo labeled in red upon exposure to blue light. **(A')** Schematic of the experimental setup for performing β-cell tracing in zebrafish larvae. Double transgenic *Tg(ins: H2B-mEos2);Tg(ins: FLAG-NTR)* larvae were exposed to blue light at 3 dpf, followed by Mtz treatment. Control larvae were incubated in E3 medium. The samples were evaluated at 3 dpa. **(B-C)** Single-plane confocal images of the larval zebrafish pancreas from control (unablated) and β-cell

ablated samples (3 dpa). Somatostatin immunohistochemistry is shown in grey. Yellow arrowheads in the ablated samples point to newly formed cells that express un-photoconverted mEOS (green) under the *insulin* promoter and somatostatin (grey). **(D)** Z-stack projection of the zebrafish islets from control and ablated samples. Nuclei are sown in blue (DAPI). **(E)** Dot-plot showing the regeneration of β-cells in the zebrafish larvae at 3 dpa (β-cells = total number of β-cells at 3 dpa; pre-existing β-cells = number of β-cells with the red nuclear label; newly formed β/δ hybrid cells = number of β-cells with green nuclear label and somatostatin expression). The lines connect the data points from each individual sample. Scale bars = 10μm.

Table S1. List of marker genes used for annotating the clusters from 2 mpf zebrafish dataset.

Click here to download Table S1

Table S2. List of differentially expressed genes (DEGs) for δ1- and δ2-cells from 2 mpf zebrafish dataset.

Click here to download Table S2

Table S3. List of marker genes used for annotating the clusters in the β-cell regeneration integrated dataset.

Click here to download Table S3

Table S4. List of DEGs for α/β and δ1/β hybrid cells from 7 dpa. The genes were used for performing GO analysis.

Click here to download Table S4

Table S5. List of co-expressed genes for zebrafish δ1 and δ2 cells on comparison with human δ and γ cells.

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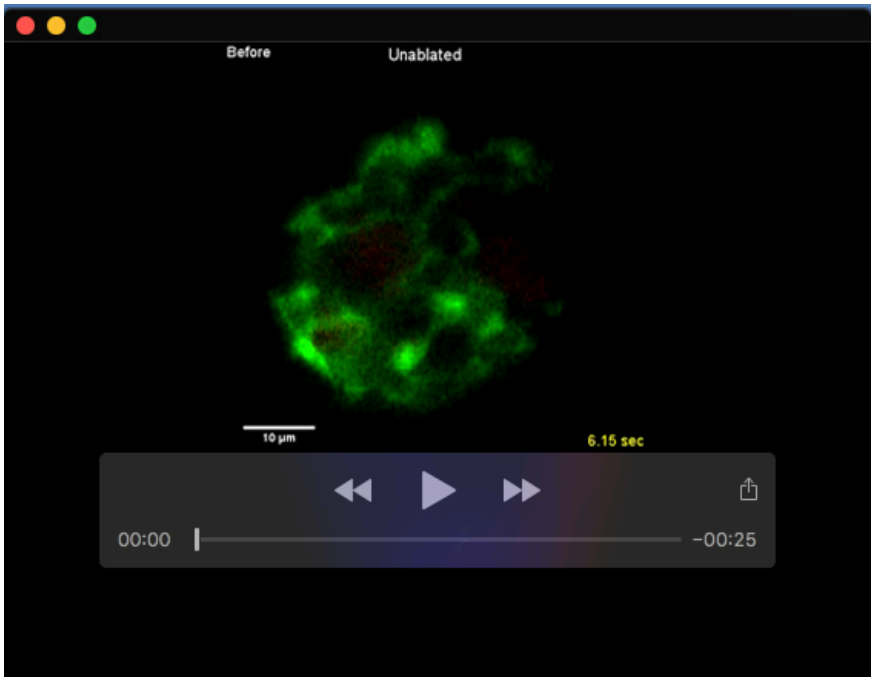
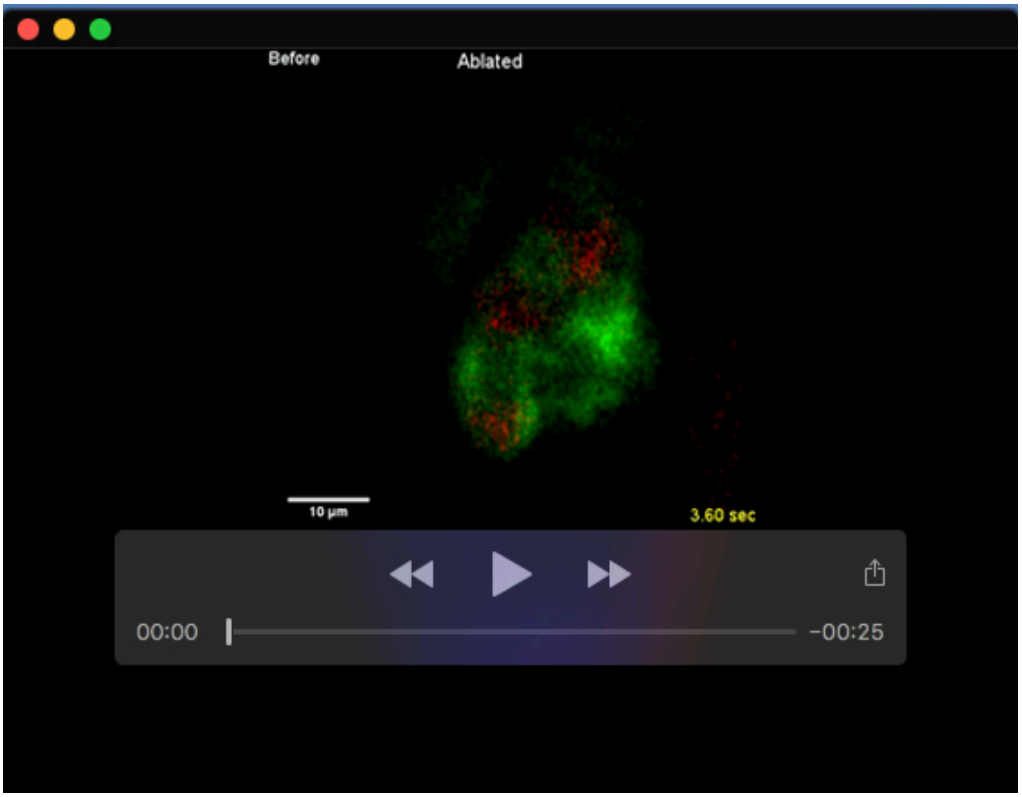


Movie 1. *In vivo* live imaging of glucose stimulated calcium influx in the pancreas of zebrafish larvae (control larvae); β-cells (red), δ1-cells (green)



Movie 2. *In vivo* live imaging of glucose stimulated calcium influx in the pancreas of zebrafish larvae at 4 days post β-cell ablation; β-cells (red), δ1-cells (green)