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Supplemental information

Adult pancreatic islet endocrine cells emerge as fetal hormone-expressing cells

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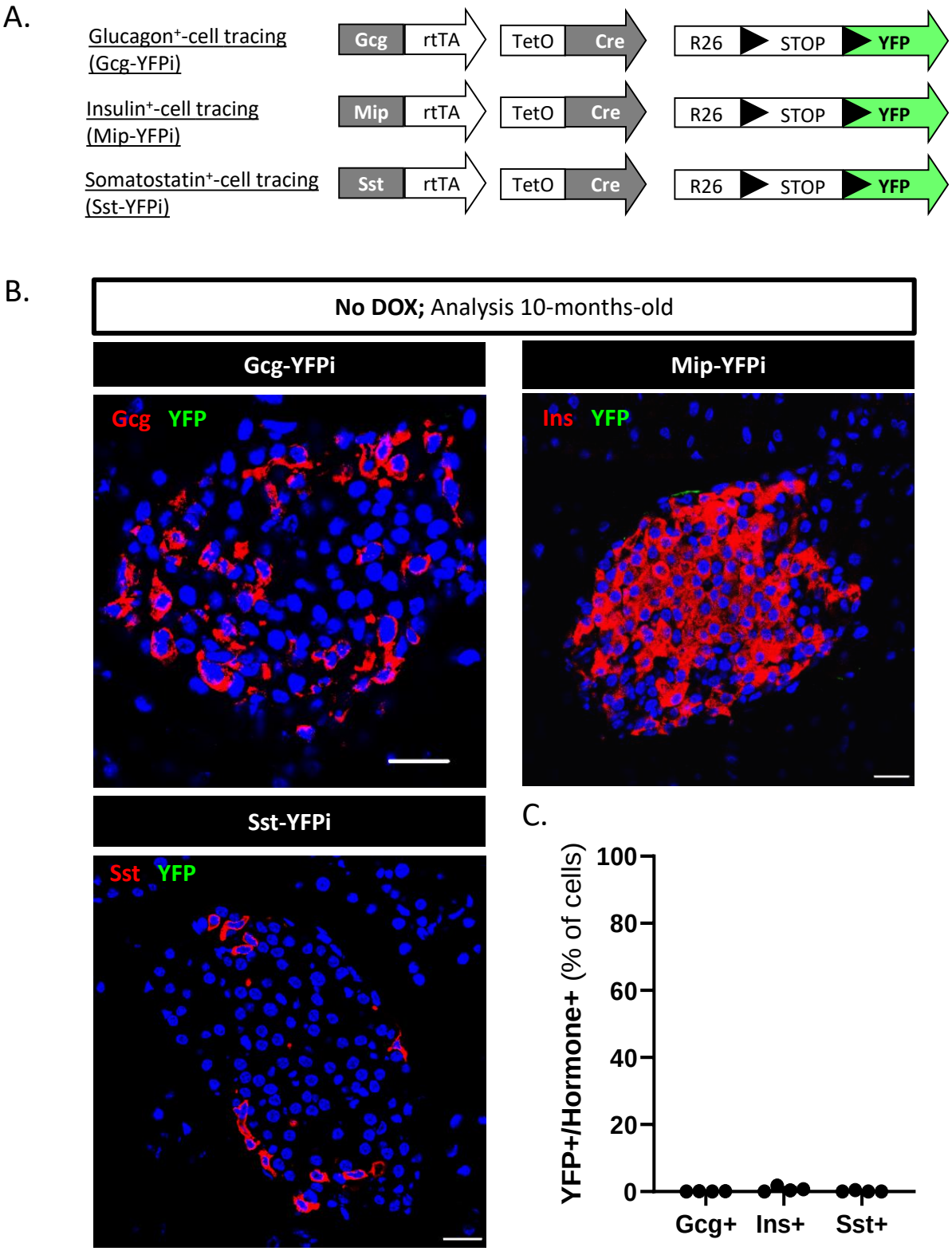
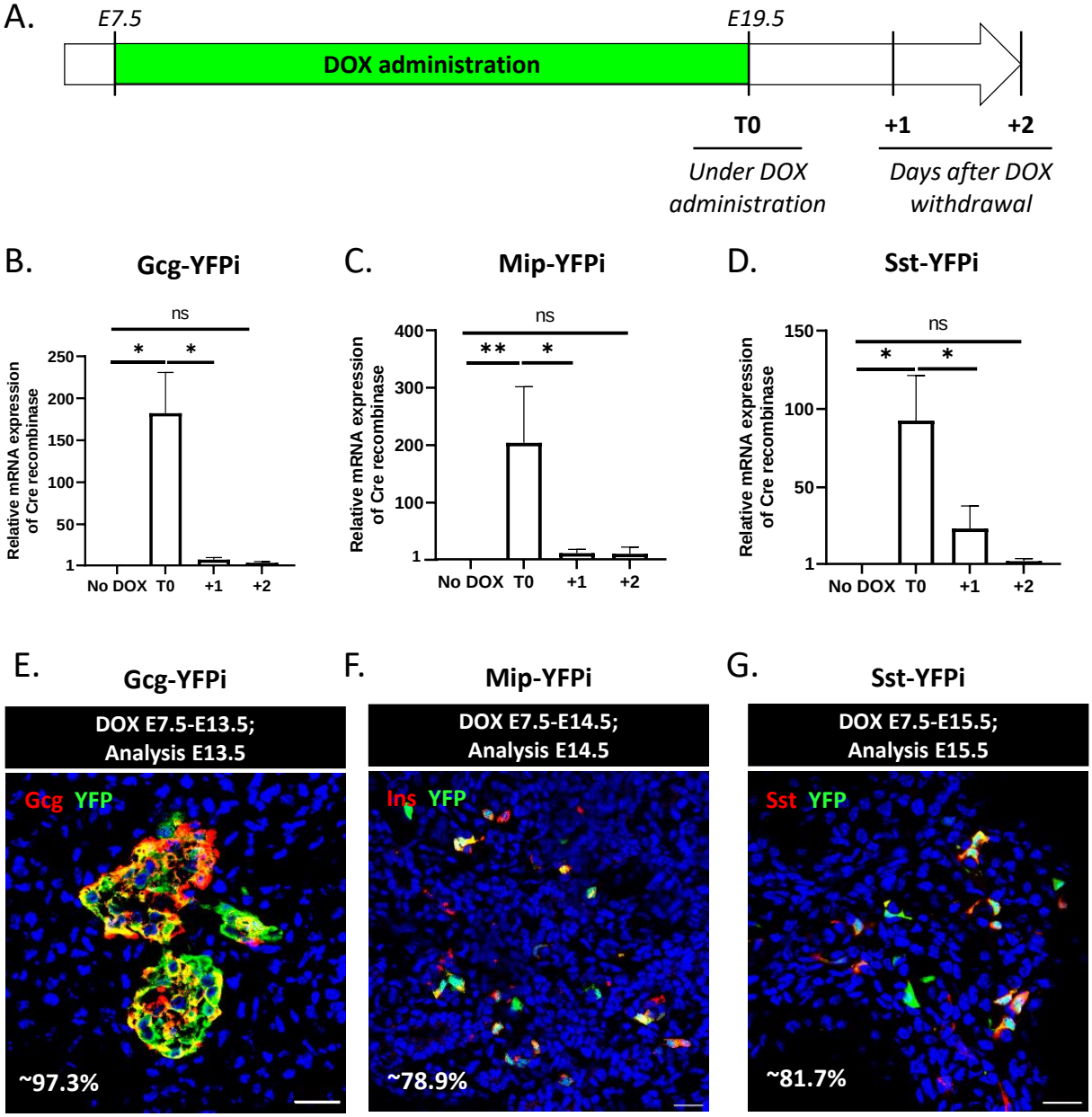
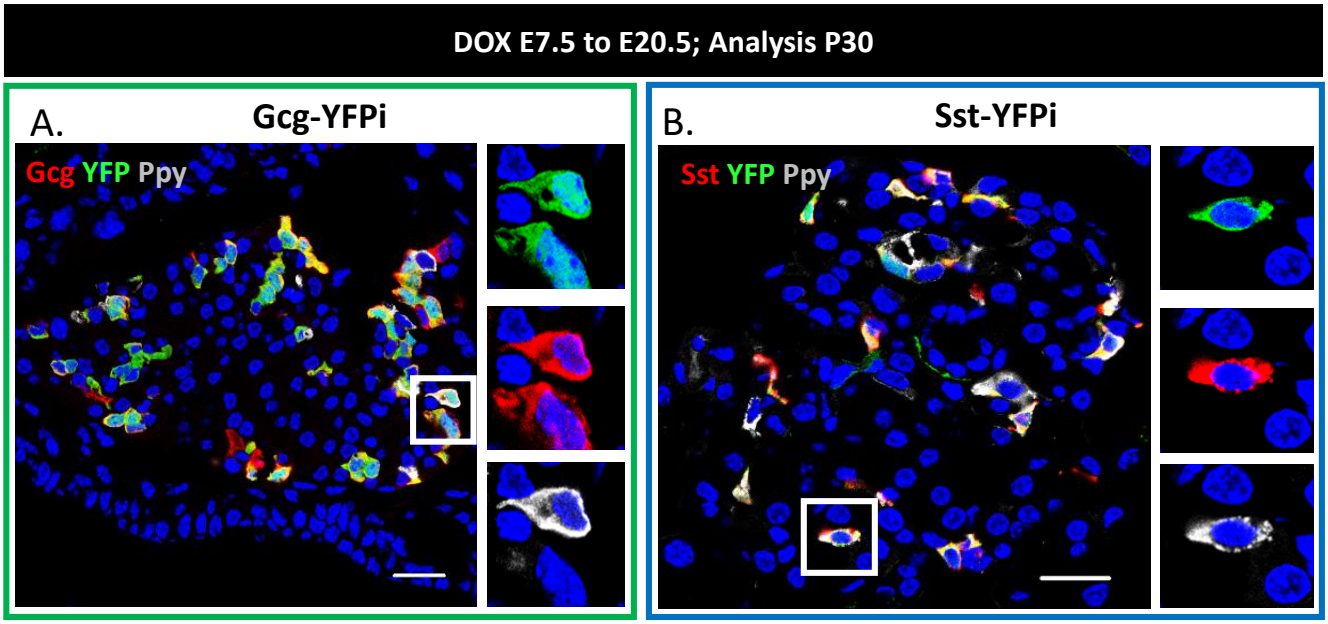


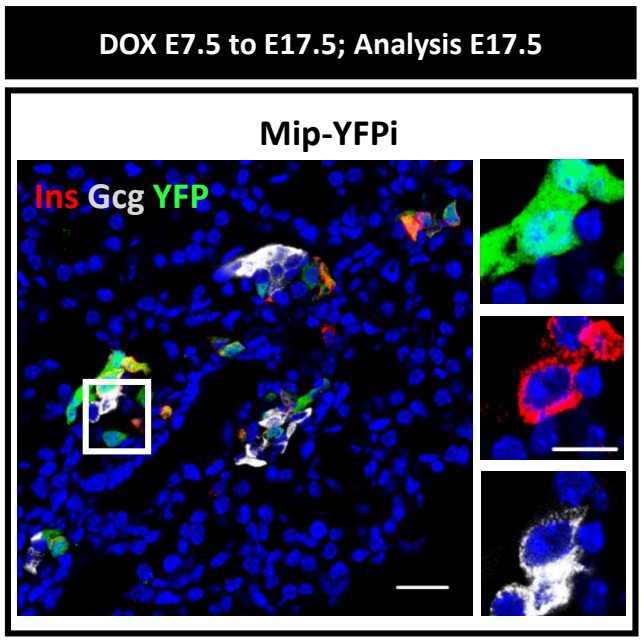
Supplementary Figure 1. Long-term inducible islet cell lineage-tracing systems, related to Figure 1. a, Transgenes required for tracing the lineages of Gcg-expressing α-cells, Ins-expressing β-cells and Sst-expressing δ-cells. **b,** No YFP labelling is observed in untreated ten-month-old mice. Immunofluorescence: Gcg-YFPi (Gcg, red; YFP, green), Mip-YFPi (Ins, red; YFP, green) and Sst-YFPi (Sst, red; YFP, green). Scale bars: 20μm. **c,** Percentage of Gcg-, Ins- and Sst-expressing cells labelled with YFP without DOX treatment (Gcg-YFPi, n=4; Mip-YFPi n=4; Sst-YFPi, n=4). One representative biological replicate of an experiment is presented in the micrographs. Experiments were performed two or more times independently under identical or similar conditions.Quantification details are provided as Source Data file (table b).



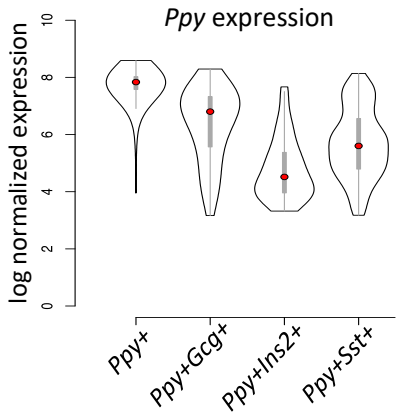
Supplementary Figure 2. A short DOX pulse during pancreas development efficiently labels differentiating hormone+ cells, related to Figure 2. a, Experimental design. DOX administration from E7.5 to E19.5 to pregnant females (T0: under DOX exposure, +1: one day after DOX removal, +2: 2 days after DOX removal). **b-d,** Relative mRNA expression of *Cre* recombinase normalized on hormone expression at different DOX administration timings. Gcg-YFPi: No DOX, n=4; T0, n=4; +1, n=4; +2, n=4 (**b**). Mip-YFPi: No DOX, n=5; T0, n=4; +1, n=4; +2, n=4 (**c**). Sst-YFPi: No DOX, n=4; T0, n=5; +1, n=5; +2, n=6 (**d**). Data are shown as fold change of normalized ct values relative to No DOX (No DOX = 1). Data are presented as mean values ± s.e.m. Two-tailed Mann-Whitney test, P values for Gcg-YFPi: No DOX versus T0: *P* value = 0.0286, T0 versus +1: *P* value = 0.0286, No DOX versus +2: *P value* = 0.20; P values for Mip-YFPi: No DOX versus T0: *P* value = 0.0048, T0 versus +1: *P* value = 0.0286, No DOX versus +2: *P value* = 0.20; P values for Sst-YFPi: No DOX versus T0: *P* value = 0.0357, T0 versus +1: *P* value = 0.0317, No DOX versus +2: *P value* = 0.5357. **e-g.** Labelling efficiency in embryos at E13.5, E14.5 or E15.5 for the Gcg-YFPi (**e;** red, Gcg; green, YFP), Mip-YFPi (**f;** red, Ins; green, YFP) and Sst-YFPi (**g;** red, Sst; green, YFP), respectively. Scale bars: 20μm. Region of the pancreas: whole pancreas. One representative biological replicate of an experiment is presented in the micrographs. Experiments were performed two or more times independently under identical or similar conditions. Quantification details are provided as Source Data file (tables d, e).



C.



D.



Cell Type	Mean *Ppy* expr	P-adj
Ppy+	7,74	-
Ppy+Gcg+	6,35*	2,30E-126
Ppy+Ins2+	4,81*	2,24E-37
Ppy+Sst+	5,68*	2,14E-114

* Significantly lower as compared to monohormonal *Ppy*+ cells

Supplementary Figure 3. YFP-traced bihormonal cells in embryonic and adult pancreatic islets, related to Figure 4. a-b, Immunofluorescence on pancreatic sections from one-month-old Gcg-YFPi (**a**) and Sst-YFPi (**b**) mice. Bihormonal Ppy-Gcg (**a;** Gcg, Red; YFP, green; Ppy, grey) and Ppy-Sst (**b;** Sst, Red; YFP, green; Ppy, grey) cells are detected. Scale bars: 20µm or 10µm (insets 1, 2, 3). Region of the pancreas: Ventral. **c,** Immunofluorescence on E17.5 pancreatic sections from Mip-YFPi mice. One bihormonal Gcg-Ins cell is shown (inset). Ins, Red; YFP, green; Gcg, grey. Scale bars: 20µm or 10µm (insets). One representative biological replicate of an experiment is presented in the micrographs. **d,** Adult bihormonal PPY+ cells display lower *Ppy* mRNA levels than monohormonal PPY+ cells. Log normalized expression of *Ppy* gene in monohormonal PPY+ cells versus bihormonal Ppy+Gcg+, Ppy+Ins2+ and Ppy+Sst+ cells. Experiments were performed two or more times independently under identical or similar conditions.