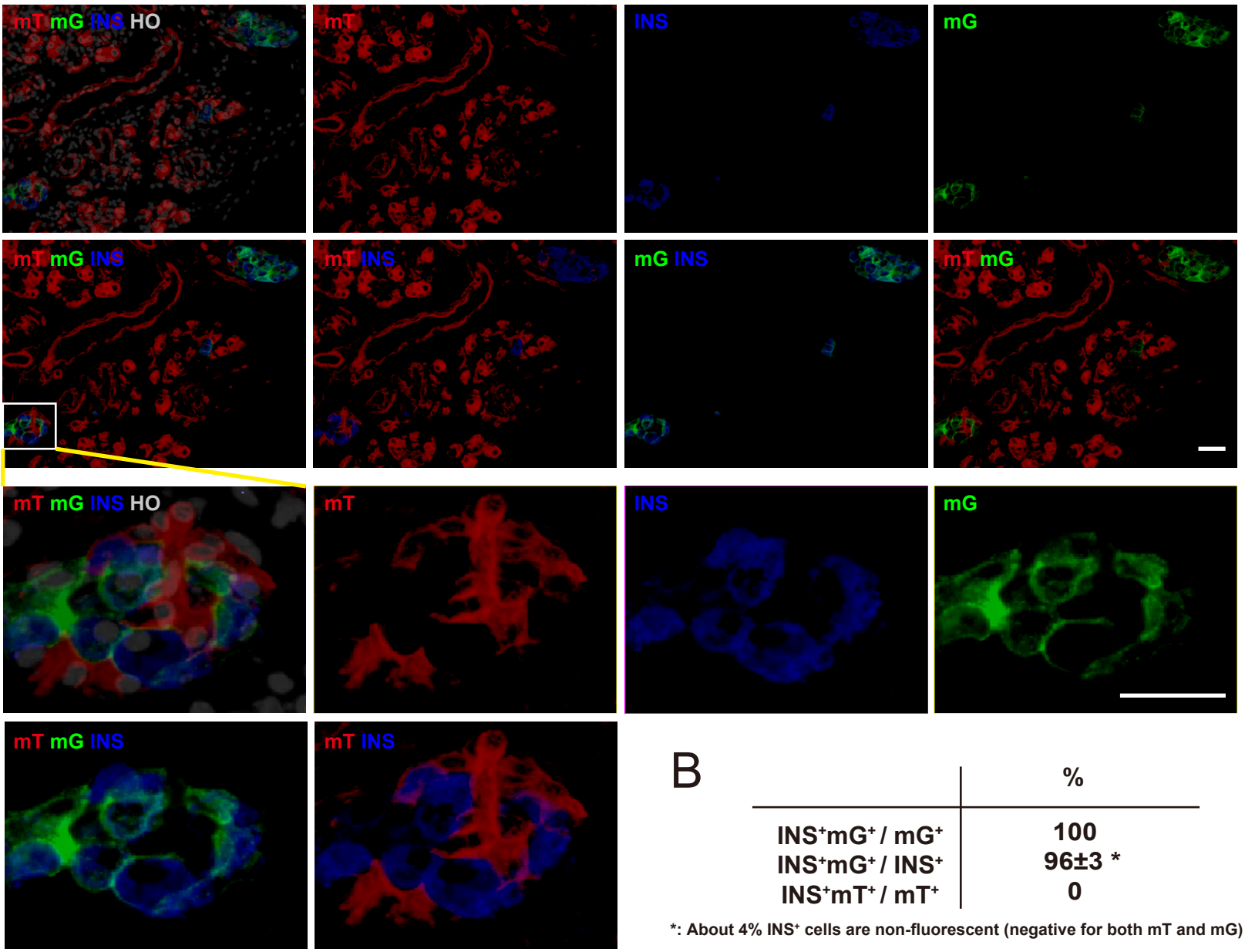
A

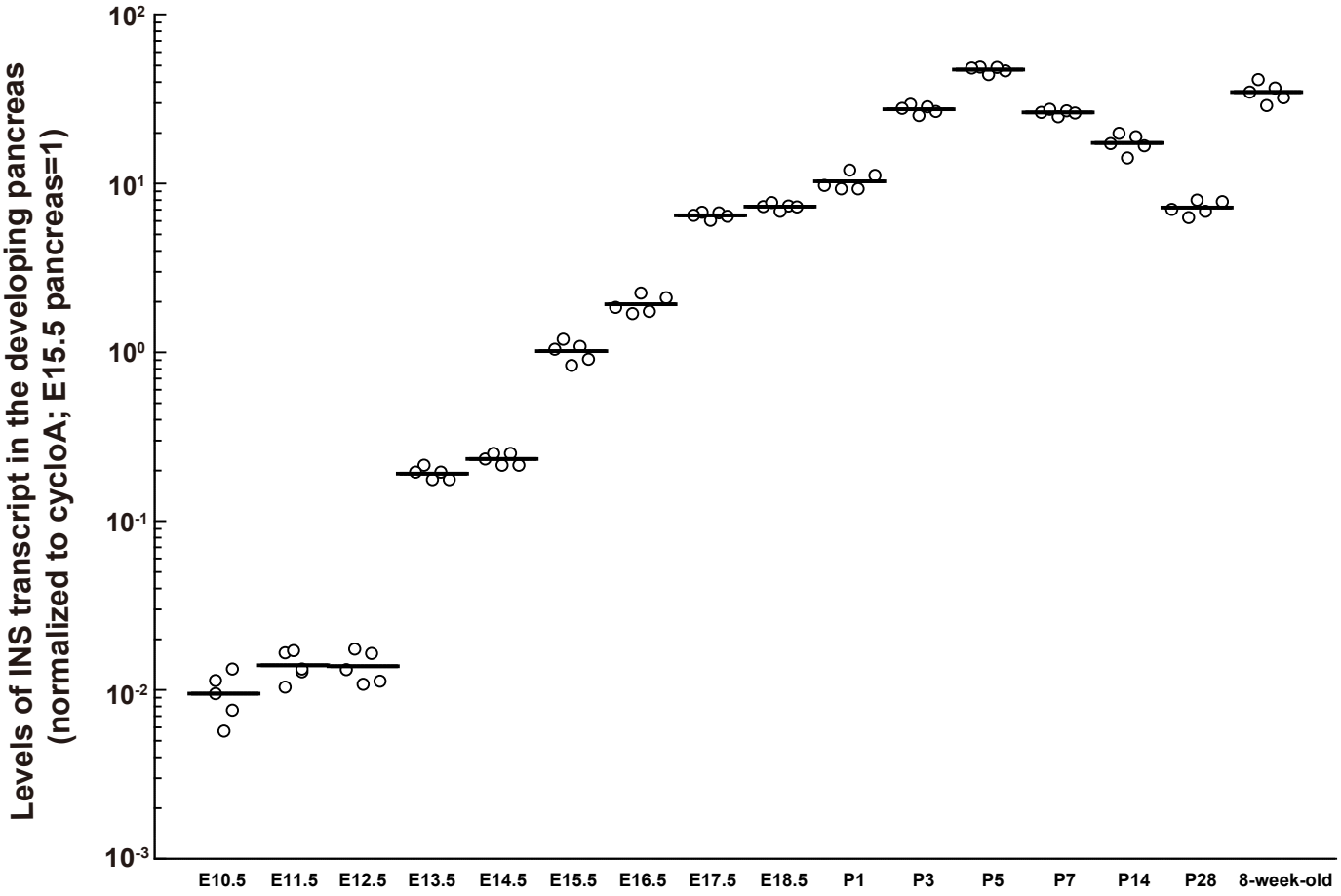


B

	%
INS^+mG^+ / mG^+	100
INS^+mG^+ / INS^+	96±3 *
INS^+mT^+ / mT^+	0

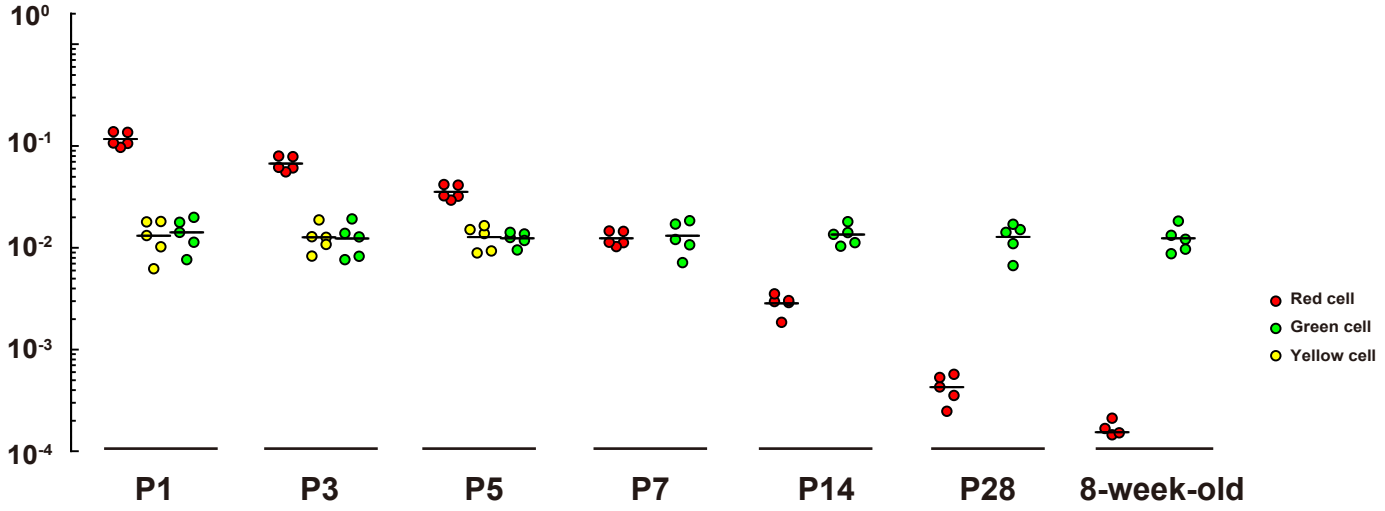
*: About 4% INS^+ cells are non-fluorescent (negative for both mT and mG)

Supplementary figure 1: Validation of INS^{cre}mTmG model. (A) Pancreas sections from 8-week-old INS^{cre}mTmG mice were stained for insulin, and a representative picture, with high magnification of the boxed islet (bottom panels), is shown with various combinations of different channels. Scale bar is 10 µm. (B) Quantification was done showing that all mG^+ cells are also positive for insulin. 96±3% of INS^+ cells are positive for mG. The very few (4%) mG^- INS^+ cells are non-fluorescent, rather than positive for mT, probably due to random loss or inactivation of the mTmG cassette. Notably, in INS^+ cell populations, no cells positive for mT were detected.

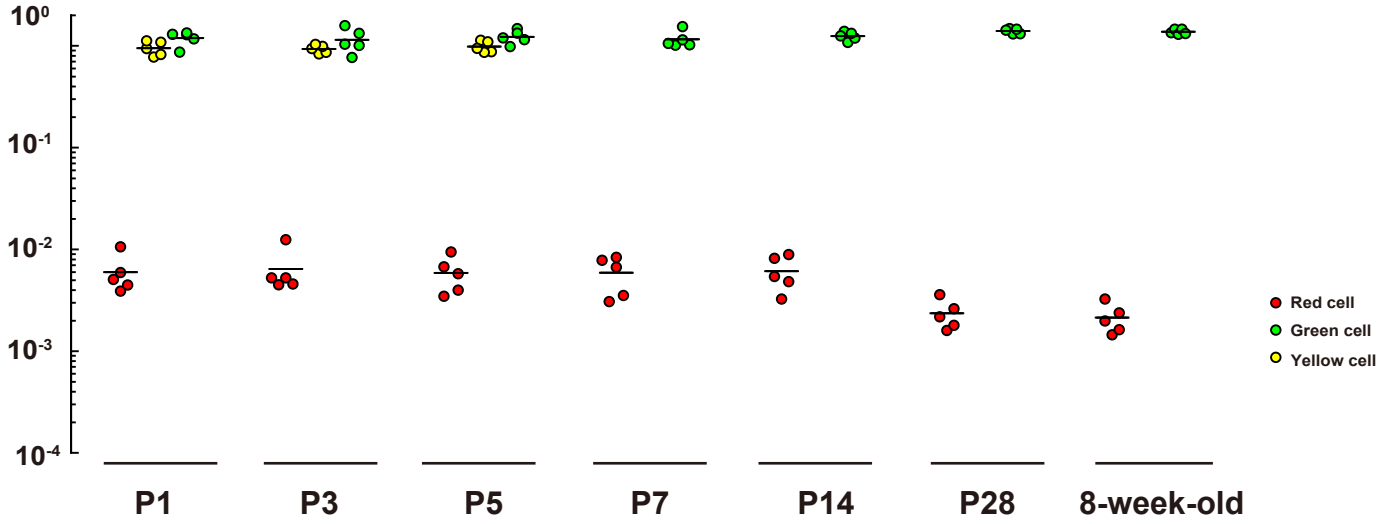


Supplementary figure 2: levels of insulin transcript in the developing pancreas. RNA was extracted from whole pancreas and Q-PCR performed for insulin and cycloA. Insulin levels were normalized to cycloA. The relative levels of insulin were presented as the percentage of the insulin level at E15.5.

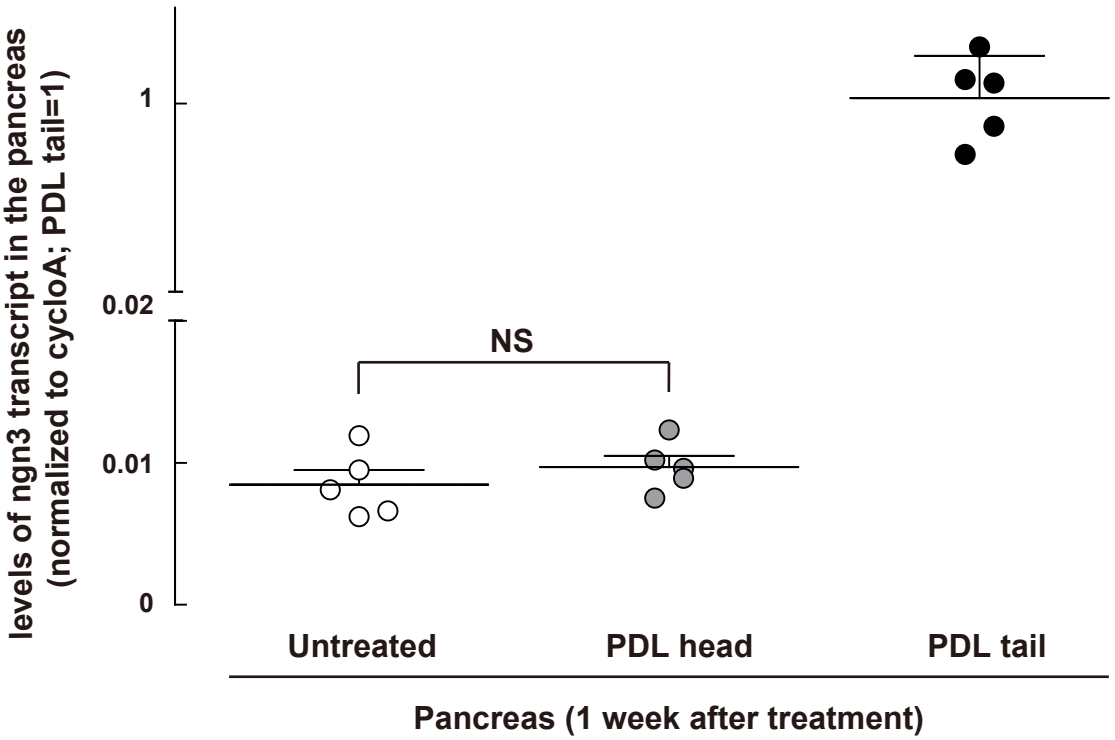
A **Ngn3 trancript enrichment in sorted cells (normalized to cycloA; red cells from E15.5=1)**



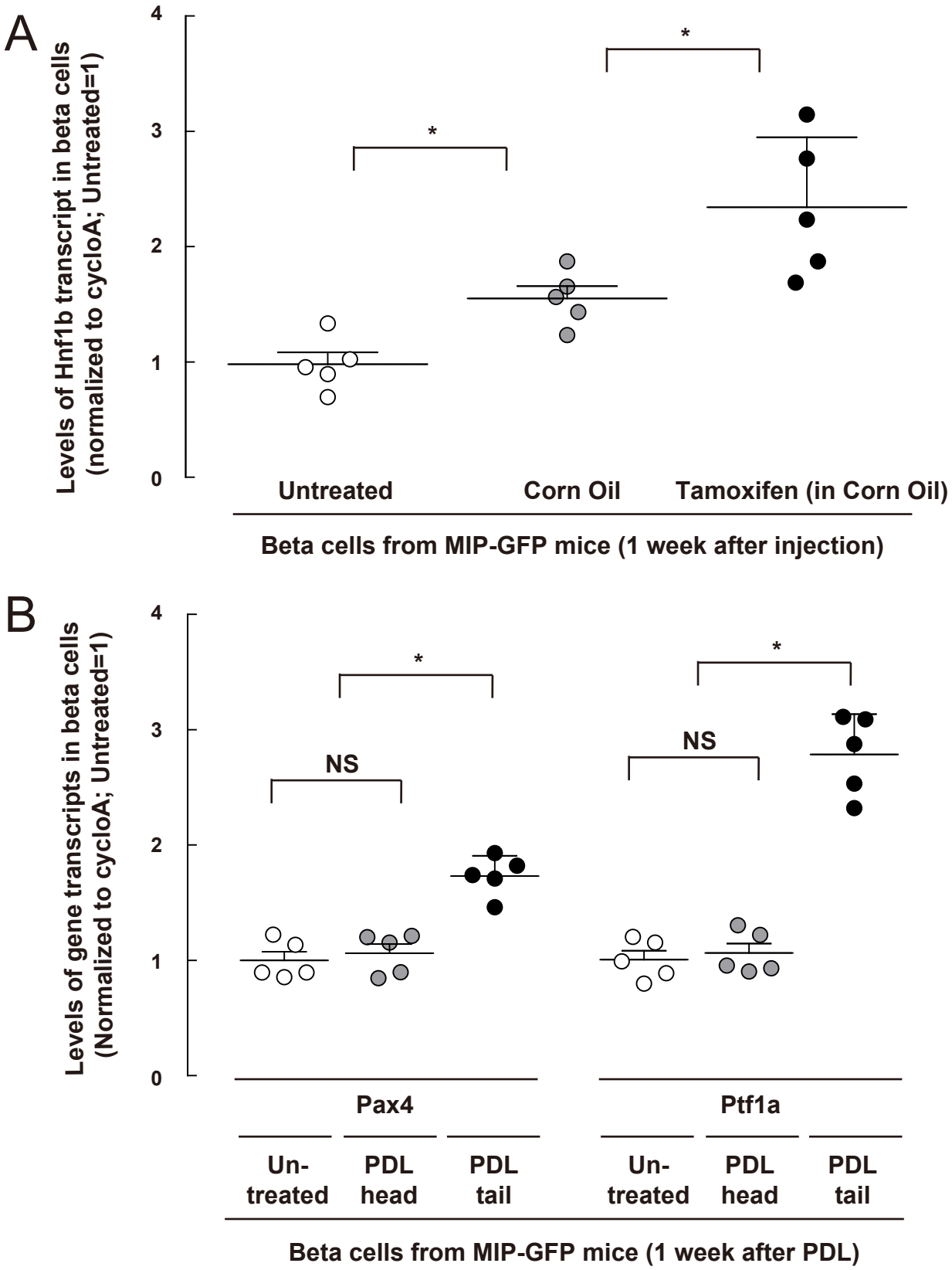
B **INS trancript enrichment in sorted cells (normalized to cycloA; green cells from E15.5=1)**



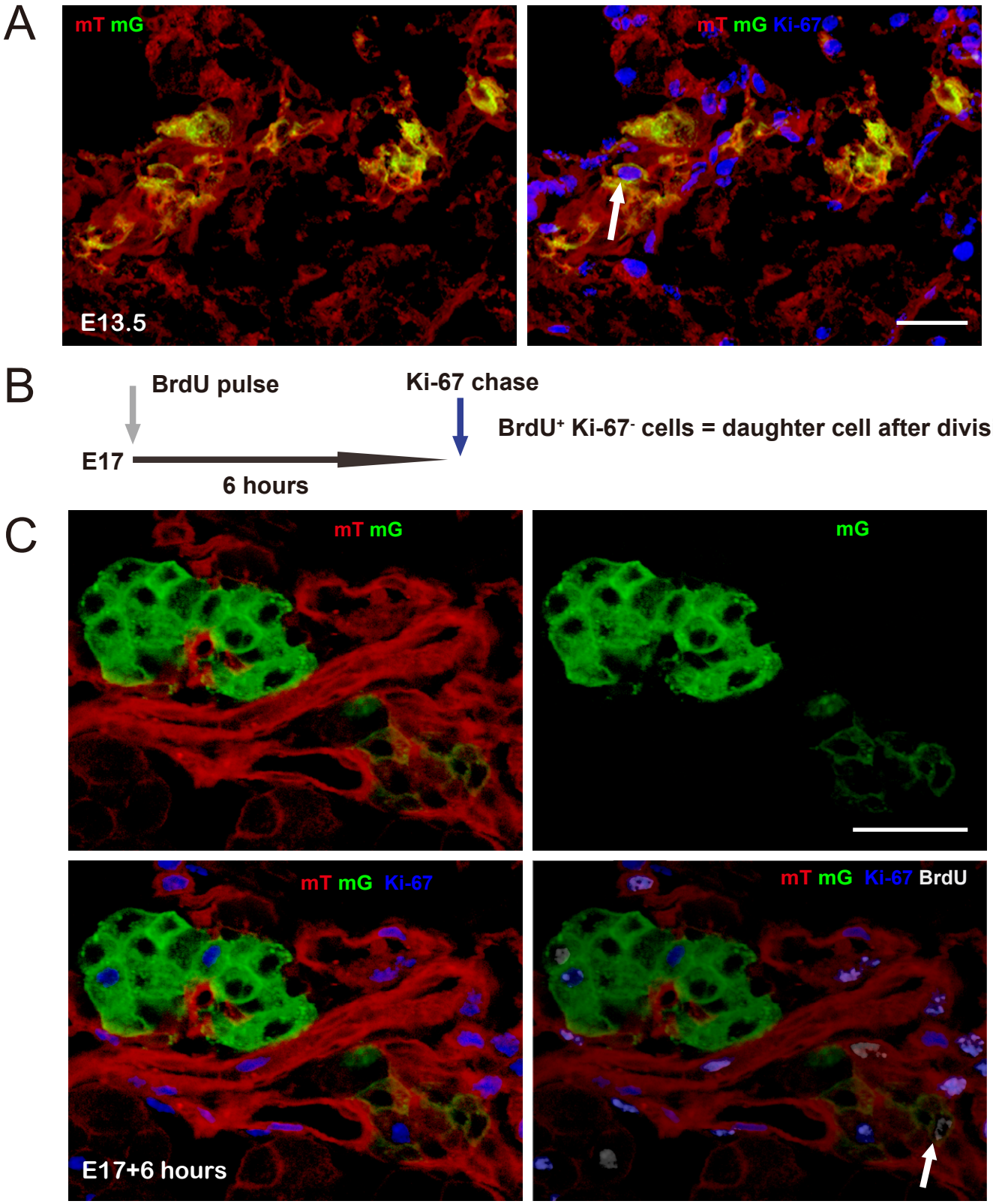
Supplementary figure 3: Beta cell neogenesis is not detected in the pancreas after P5 post-natally. (A-B) INS^{cre}mTmG pancreas at ages P1, P3, P5, P7, P14, P28 and 8-week-old was digested and sorted for red, green, and yellow cell (if present) fractions. While a few yellow cells were detected at P1, P3 and P5, no yellow cells were detected at P7, P14, P28 and 8-week-old. Gene expression was then analyzed by Q-PCR. (A) Ngn3 mRNA in the red cells went down with age and was nearly undetectable after P5. Of note, the levels of ngn3 mRNA in both yellow and green cell populations from all time points are consistently about 1% of the ngn3 mRNA levels in red cells from E15.5 pancreas (B) INS mRNA was quite stable across the green and yellow cell fractions in the developing pancreas.



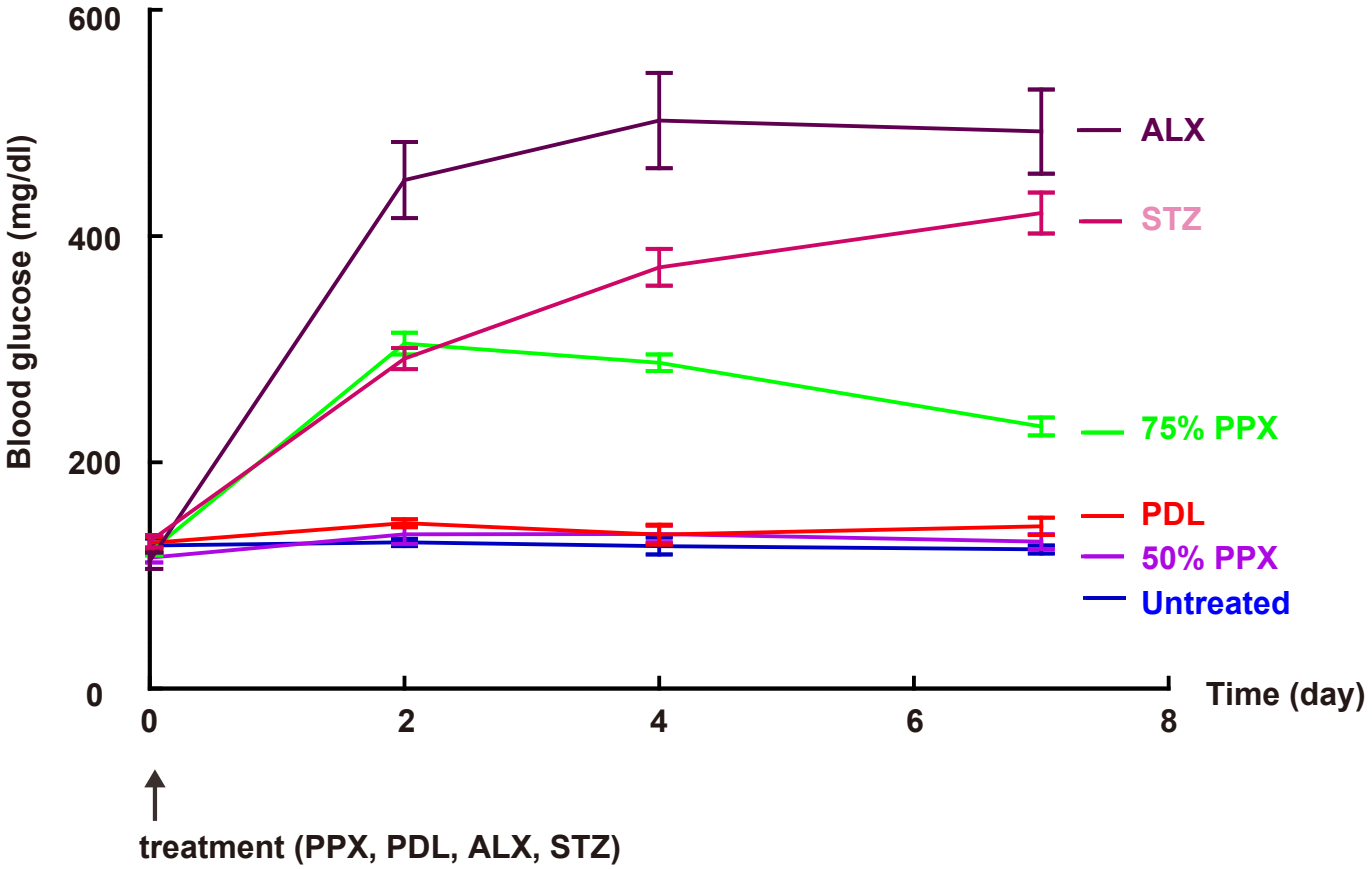
Supplementary figure 4: Activation of ngn3 in PDL tail. RNA was extracted from total pancreas (unsorted), and analyzed for the levels of ngn3 trancript. More than 100-fold increase in ngn3 mRNA transcripts was detected in the ligated tail of the pancreas (PDL tail), compared with the non-ligated head pancreas (PDL head) or untreated pancreas. NS: no significance.



Supplementary figure 5: Expression of some genes in beta cells can be altered by tamoxifen treatment or by PDL. (A-B) Beta cells from MIP-GFP mice were sorted by flow cytometry one week after various treatments. (A) Beta cells from the mice that received a single i.p. injection of 100 µl corn oil or 1mg tamoxifen in 100µl corn oil significantly altered transcripts of some genes (HNF1b is shown here. (B) Beta cells in the PDL tail significantly altered transcripts of some genes (Pax4 and Ptf1a are shown here).



Supplementary figure 6: Proliferating/proliferated yellow cells can be readily detected in INS^{cre}mTmG embryos. (A) Representative image of Ki-67 staining of the E13.5 pancreas. White arrow points to a Ki-67^{+} (blue nucleus) membrane yellow beta cell. 2.2±0.3% of the yellow cells were labeled with Ki-67^{+} at E13.5. Since there were still no green cells 24 hours later at E14.5, it seems unlikely that the dividing Ki-67^{+} yellow cells at E13.5 diluted out the red color by cell division (see also Figure 2A). (B) BrdU was given at E17, when fetal beta cell proliferation is high. The embryonic pancreas was harvested 6 hours later and stained for BrdU (in white) and Ki-67 (in blue). (C) Representative images for BrdU (in white) and Ki-67 (in blue) are shown with direct fluorescence from mT and mG. 16.5±1.3% of yellow cells were labeled with BrdU, suggesting that proliferating yellow cells can be readily detected in our model. Moreover, detection of BrdU^{+}Ki-67^{-} yellow beta cells (white arrow, 6.4±0.5% of all BrdU^{+} yellow cells) suggest that the daughters of yellow cells (cells become Ki-67^{-} upon entering G_0 , and therefore recently divided cells here will be BrdU^{+}Ki-67^{-}) remain yellow despite dilution of the membrance-bound red fluorescence that may occur due to cell division. Scale bar is 20 μm.



Supplementary figure 7: Control of the fasting blood glucose in the mice following various treatments before harvesting at one week. 50% PPX and PDL had no effect on blood glucose, 75% PPX slightly increased blood glucose, and ALX and STZ treatment induced hyperglycemia.