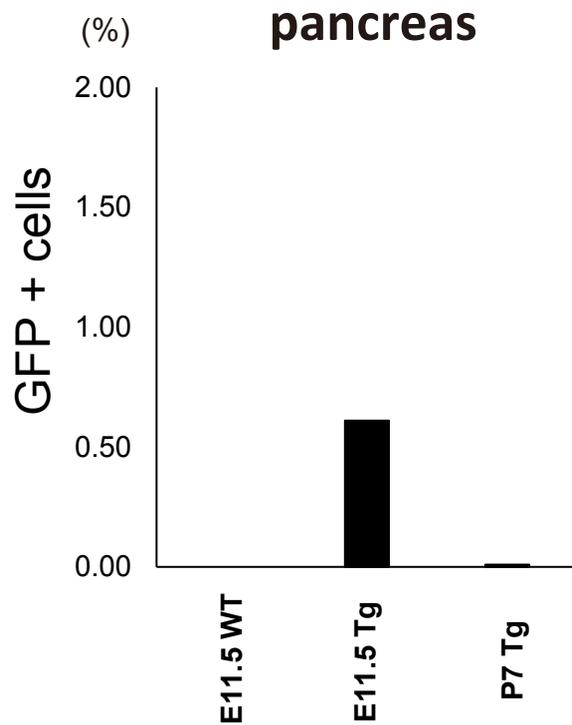
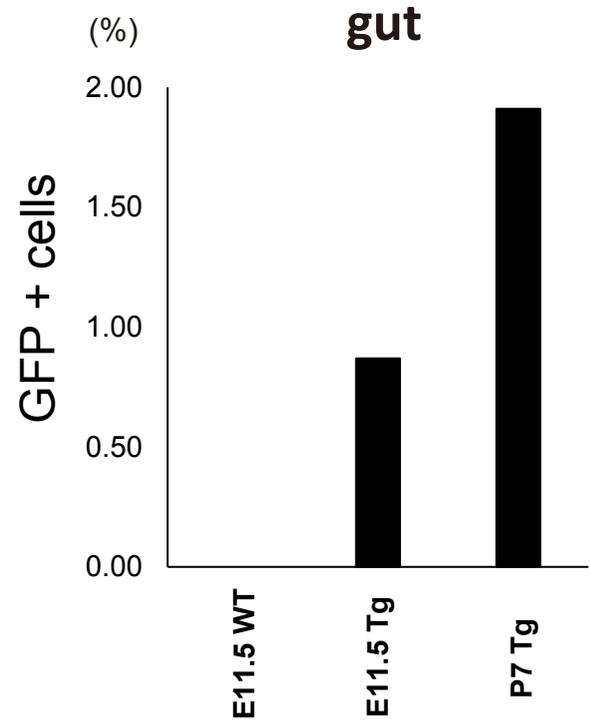
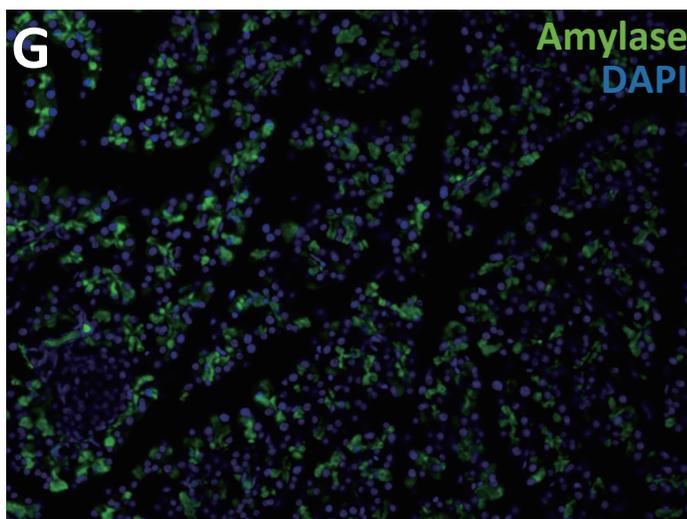
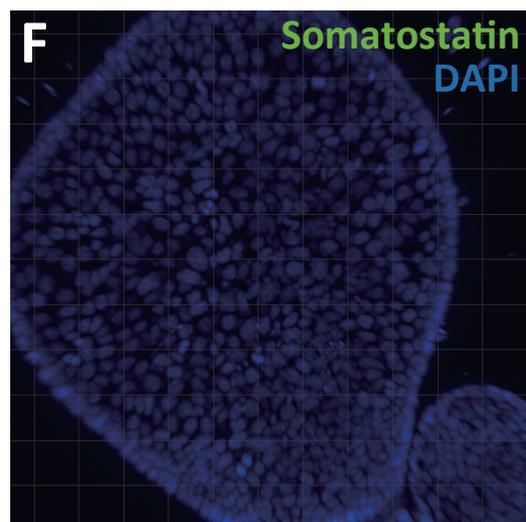
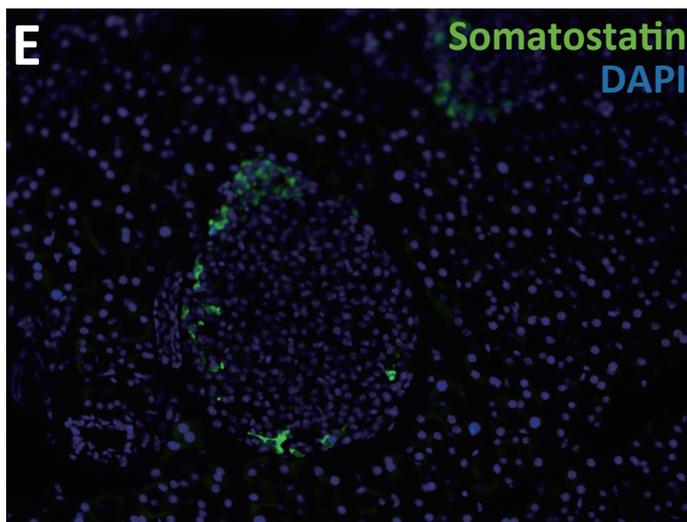
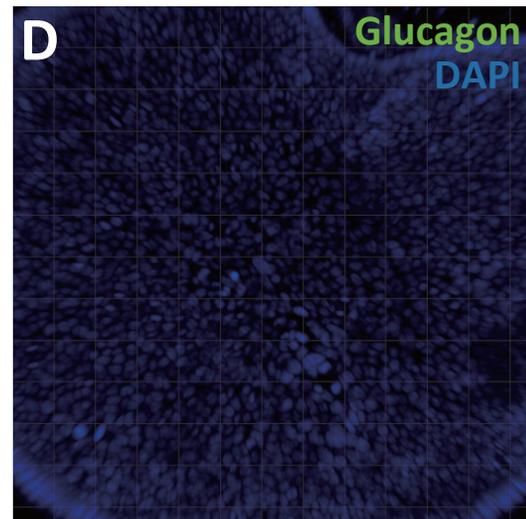
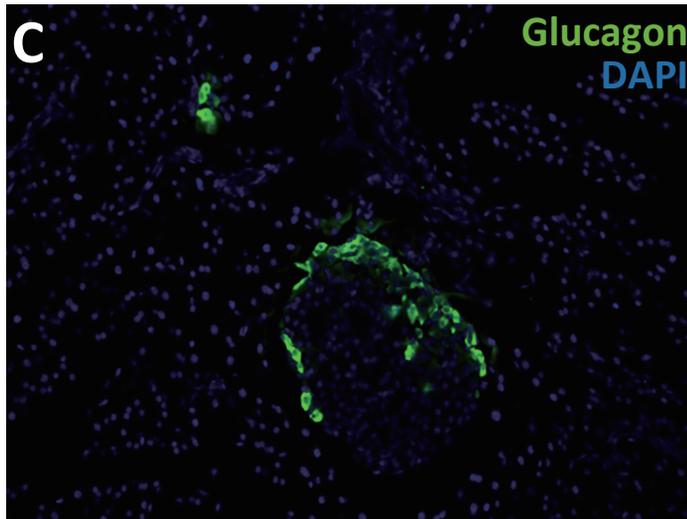
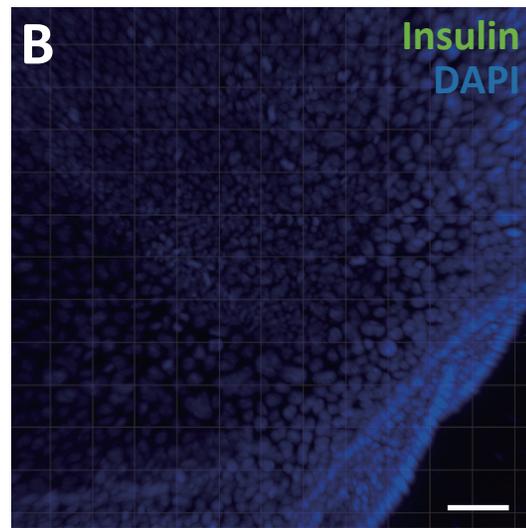
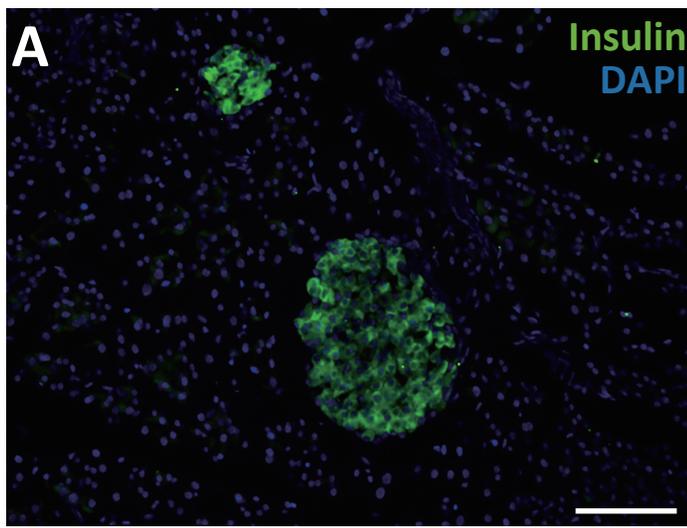


A**B****Supplementary fig. 1**

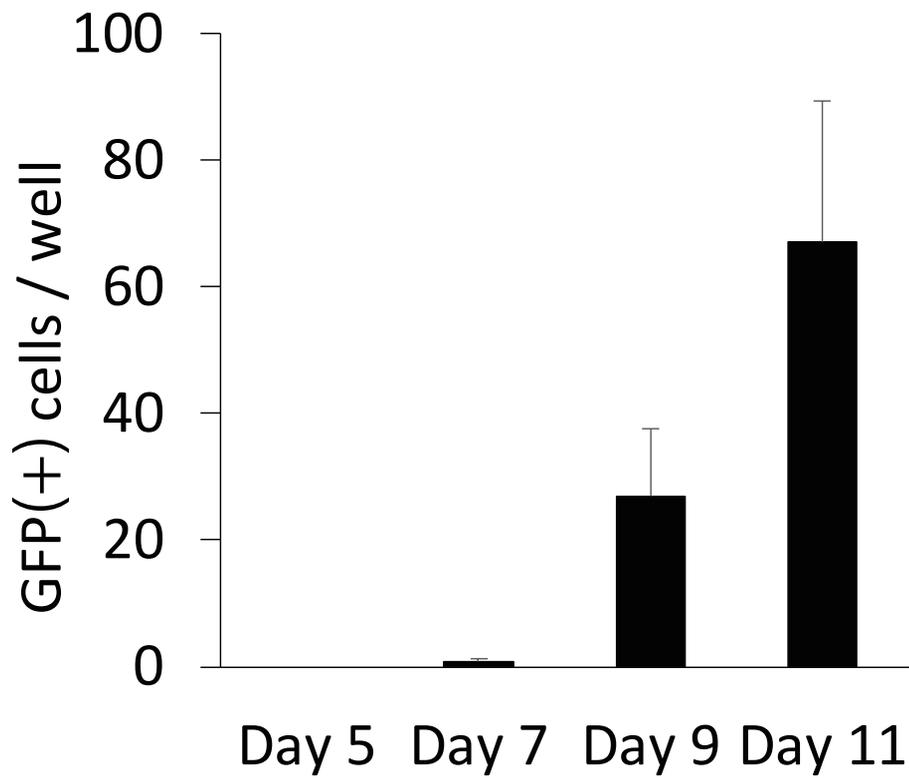
Ngn3-positive cells appear transiently during pancreas development. Pancreas (A) and gut (B) cells dissected from Ngn3-GFP-transgenic (Tg) mouse and GFP-marked cells were counted by fluorescence activated cell sorting (FACS) system at embryonic day 11.5 (E11.5) and post-natal day 7 (P7) and the ratio of GFP-positive cells is shown by the percentage (%). GFP-positive cells from Ngn3-GFP-Tg mouse appear in the E11.5 pancreas but will almost disappear at P7. In contrast, GFP-positive cells continuously appear in the gut of P7 Ngn3-GFP-Tg mouse. E11.5 wild-type (Wt) mice were used as a negative control.



Supplementary fig. 2

Immunohistochemical analysis comparing the expression of hormones between the pancreas and day 4 organoids prepared from pancreatic ducts.

Insulin, glucagon, and somatostatin were detected within islets using specific antibodies against each hormone (A, C, E). Amylase-specific antibodies marked most acinar cells in the pancreas (G), whereas amylase expression was absent from day 4 pancreatic organoids (Fig. 2G in the main manuscript). We did not detect hormone expression in day 4 pancreatic organoids (B, D, and F). (bars: 100 μm).



Supplementary fig. 3

Changes in the number of Ngn3-GFP-positive cells within pancreatic organoids generated from Ngn3-GFP mouse. GFP-positive cells per well were counted on days 5, 7, 9, and 11 of organoid culture (n = 9). GFP-positive cells were first identified on day 7, and the number of GFP-positive cells increased towards day 11.

Table S1 Primer sequences in RT-PCR

Gene name	Forward (5'-3')	Reverse (3'-5')
<i>Ngn3</i>	GAG TCG GGA GAA CTA GGA TG	CAG TCA CCC ACT TCT GCT TC
<i>NeuroD1</i>	GTC CCA GCC CAC TAC CAA TT	CGG CAC CGG AAG AGA AGA TT
<i>Isl1</i>	CTG CTT TTC AGC AAC TGG TCA	AGG ACT GGC TAC CAT GCT GT
<i>Gcg</i>	CCA CTC ACA GGG CAC ATT CA	GTC CCT GGT GGC AAG ATT AT
<i>Sst</i>	TCC GTC CAG TTT CTG CAG AAG	ACA GGA TGT GAA TGT CTT CC
<i>Ghrl</i>	GAC AAG CAG AAG AGA CAG AG	TCT TCC CAG AGG ATA TCC TG
<i>Chga</i>	CTG TCA GCC CTG AGT GTC TG	ATG GAA GTG GGA ACT GGA TG
<i>Trpm5</i>	AGT GTG ACA TGG TGG CCA TC	AGA GAA CAG TTC ACA CGA GC
<i>Gnat3</i>	CCA GGA ATC CAA GCC TGC TT	ACC ATG TCA TAG GCA CTT AGC
<i>Pou2f3</i>	GCA TCG AGA CCA ACA TAC GC	GAG ACG AAG GCC TGC TGT TG
<i>G3PDH</i>	ACC ACA GTC CAT GCC ATC AC	TCC ACC ACC CTG TTG CTG TA