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Supplementary Information for

A single-cell-resolution fate map of endoderm reveals demarcation of pancreatic progenitors by cell cycle

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Supplemental Figures

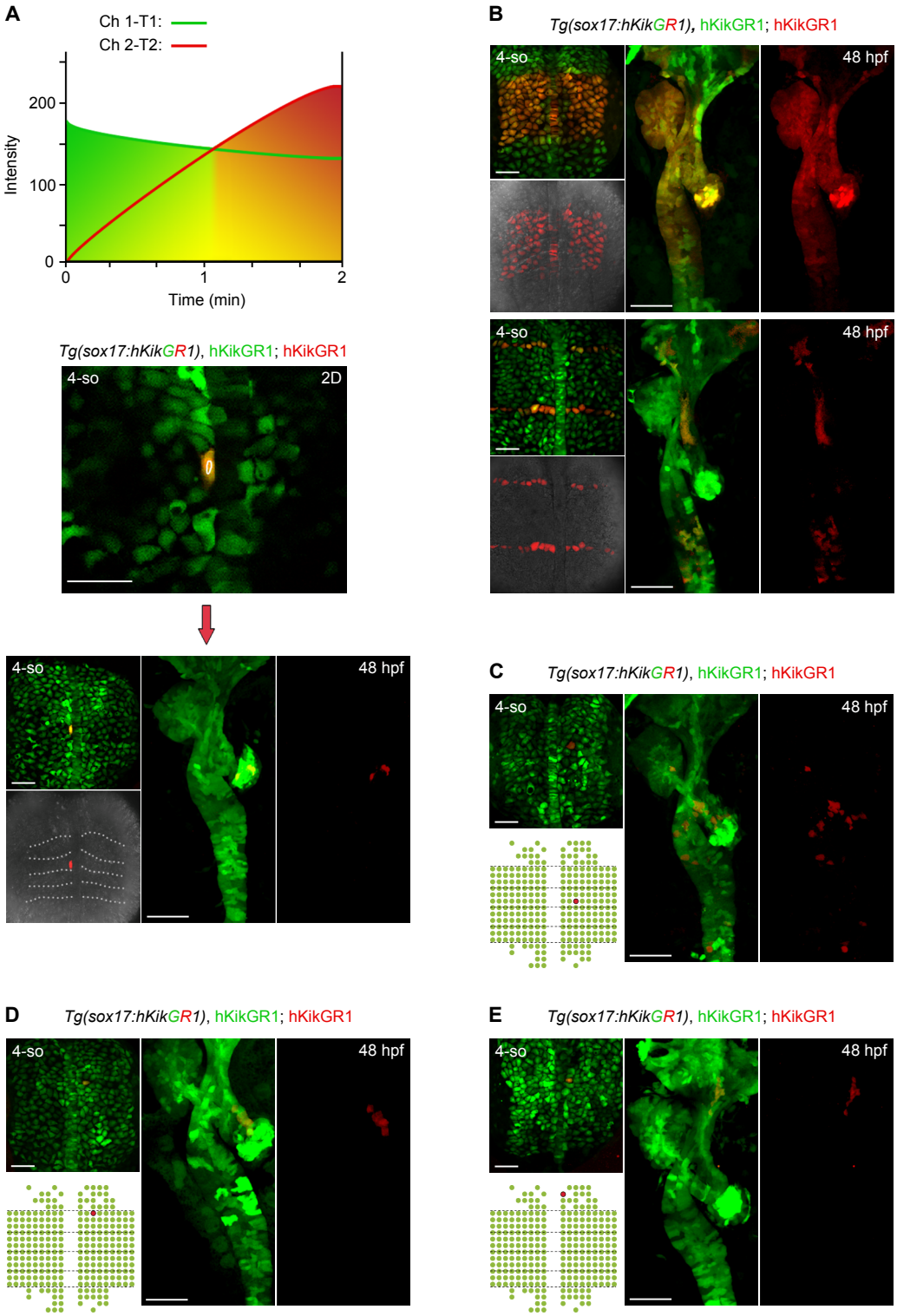

Fig. S1. Labeling by photoconversion and tracing of single and multiple endodermal progenitor cells. (*A*) Single-cell labeling at 4-so and descendant tracing to 48 hpf. Focus and irradiation of 405-nm laser on a single endodermal progenitor cell at 4-so. The irradiated region is marked by the irregular white circle, which was determined by the cell shape for better photoconversion and should be smaller than the cell to avoid mislabeling of neighboring cells. Note that the intensity of red fluorescence of the irradiated cell rapidly increased within 2 minutes. The descendant cells of the photoconverted progenitor were shown to localize in the pancreatic bud at 48 hpf. The dotted lines mark the borderlines of somites at 4-so. (*B*) Photoconversion of all the early endodermal progenitor cells beneath somite-1 to somite-4 resulted in labeling of more than 99% of foregut digestive organ cells at 48 hpf (n=18). Labeling the first and last rows of endodermal cells showed their contributions to pharyngeal arch/swim bladder and posterior foregut/anterior midgut, respectively. (*C*-*E*) Examples of the early endodermal cells as progenitors of multiple organs (*C*, R-c3s2r2, n=13), and predominantly contributing to the swim bladder (*D*, R-c3s1r1, n=8) and pharynx (*E*, R-A-c1r2, n=5). Scale bar, 50 μm. 4-so, 4-somite stage.

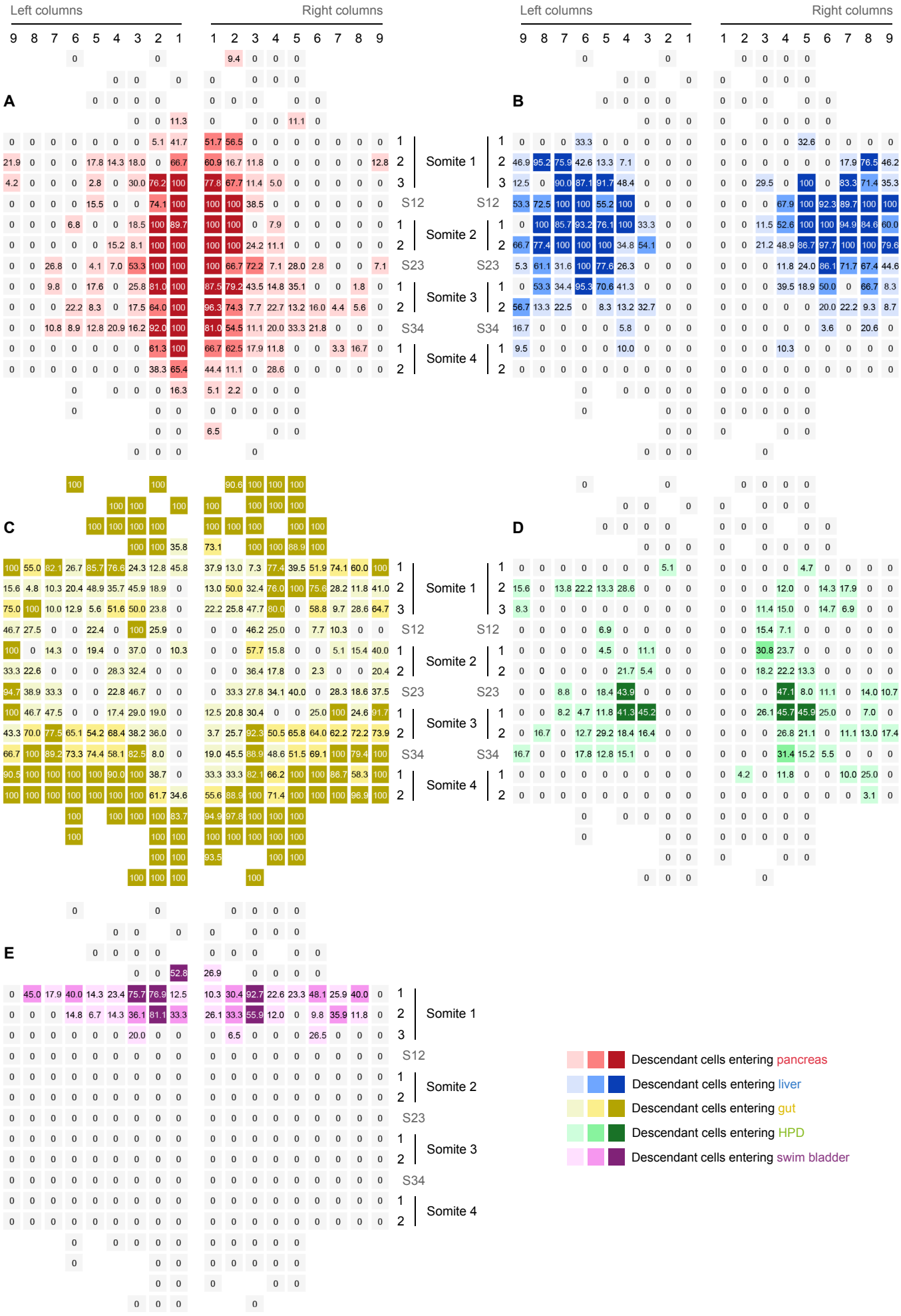
A
B
C
D
E
Left columns
Right columns
9 8 7 6 5 4 3 2 1
1 2 3 4 5 6 7 8 9
Somite 1
Somite 2
Somite 3
Somite 4
S12
S23
S34
Descendant cells entering pancreas
Descendant cells entering liver
Descendant cells entering gut
Descendant cells entering HPD
Descendant cells entering swim bladder

Fig. S2. Single-cell-resolution maps indicate ratios of descendant types. (*A*) Ratios of descendant cells localized in the pancreatic bud. (*B*) Ratios of descendant cells localized in the liver bud. (*C*) Ratios of descendant cells localized in the gut primordium. (*D*) Ratios of descendant cells localized in the HPD. (*E*) Ratios of descendant cells localized in the swim bladder. Darker colors represent higher ratios. Each early foregut endodermal progenitor cell was labeled and traced for at least five independent repeats to collect the data.

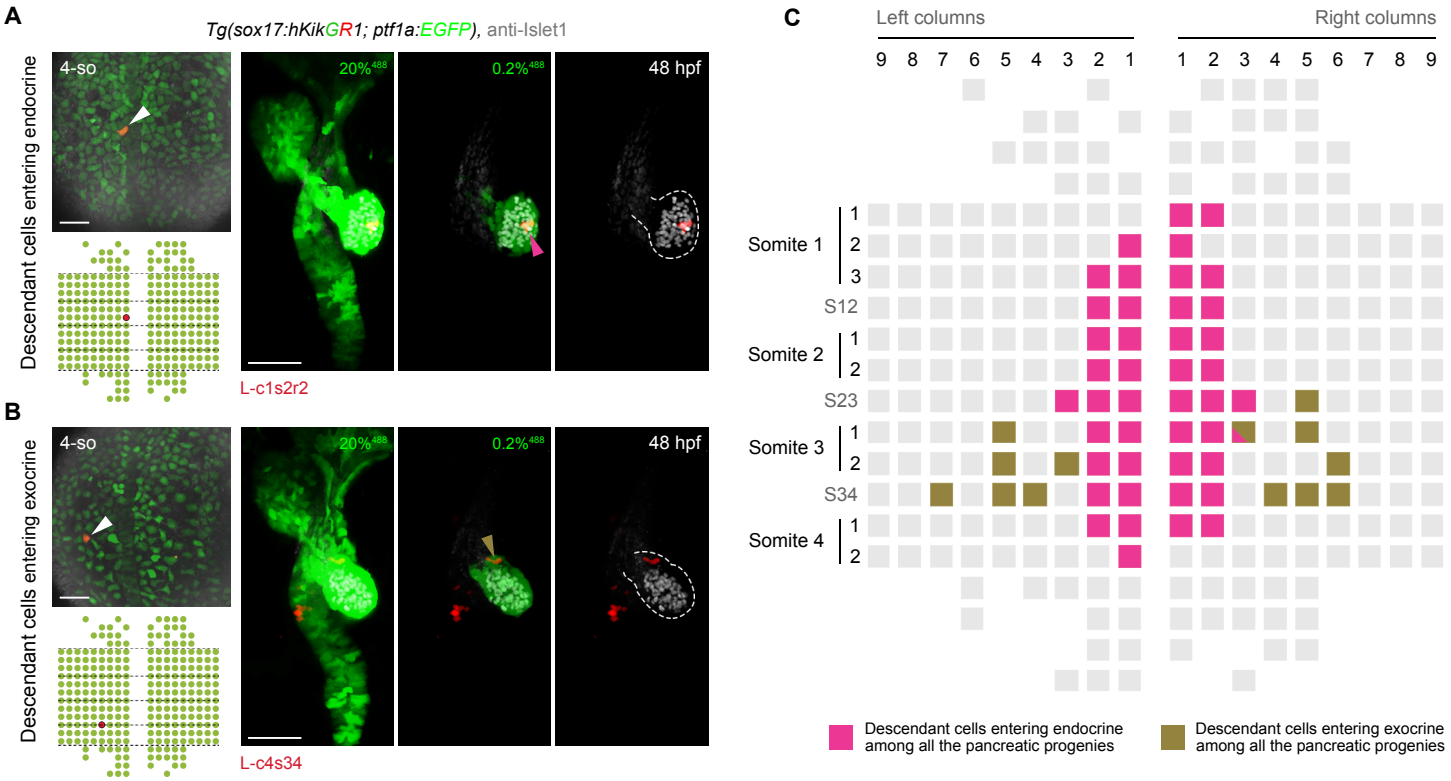

Fig. S3. Pancreatic progenitors close to the dorsal midline give rise to endocrine cells. (*A* and *B*) The descendant cells of the progenitor at L-c1s2r2 contributed to endocrine pancreas (*A*, pink arrowhead, n=3), while the descendant cells of L-c4s34 contributed to exocrine pancreas (*B*, brown arrowhead, n=3). 20% and 0.2% represent the power of 488 nm-laser. The 0.2% laser power could not activate the fluorescence of Kikume-green but could activate EGFP, thus being able to distinguish Kikume-green and EGFP. (*C*) The color-marked progenitors were individually labeled at the 4-somite stage, and the co-localizations of their descendant cells with anti-Islet1 and *ptf1a:EGFP* were analyzed at 48 hpf. At least three independent repeats were collected for each colored position. pink, endocrine; brown, exocrine. Scale bar, 50 μm. 4-so, 4-somite stage.

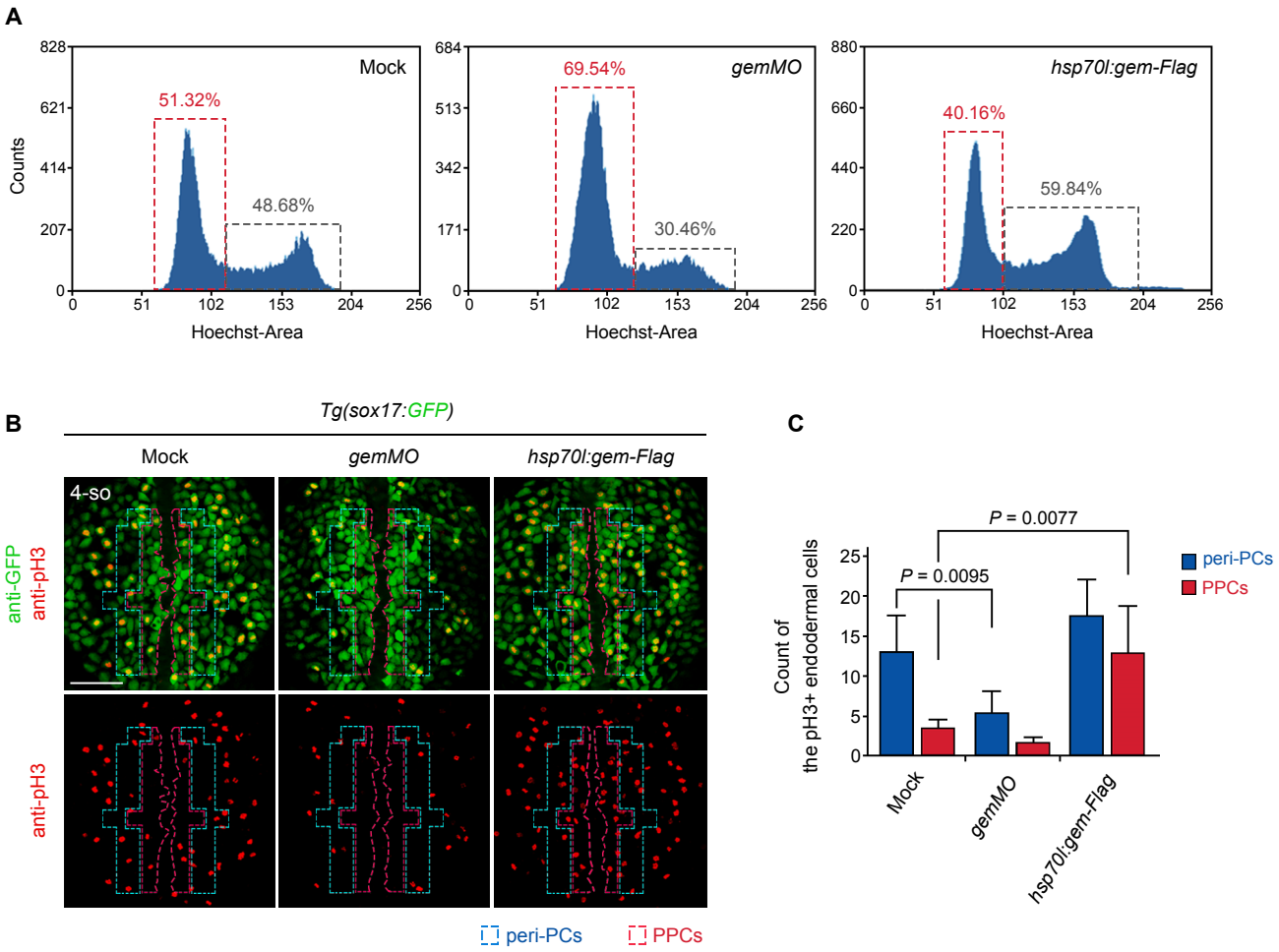

Fig. S4. Modulations of the cell cycle as indicated by alterations of G1 fraction and the number of pH3+ cells. (*A*) The cell cycle analyses by Hoechst vital staining and FACS showed cell fractions in different cell cycle phases. (*B* and *C*) Double labeling with anti-pH3 and anti-GFP antibodies in the *Tg(sox17:GFP)* embryos at 4-so (*B*). The statistics show the number of endodermal cells double positive for pH3 and GFP in the peri-PC and PPC regions (*C*, n=5). Note that *gemMO* increased the G1 fraction and reduced the number of pH3+ cells in the peri-PC region, while overexpression of geminin-Flag reduced the G1 fraction and increased the number of pH3+ cells in the PPC region. Scale bar, 50 μm. 4-so, 4-somite stage. Data are expressed as mean±SD; *P*-value is calculated using Student's *t*-test.

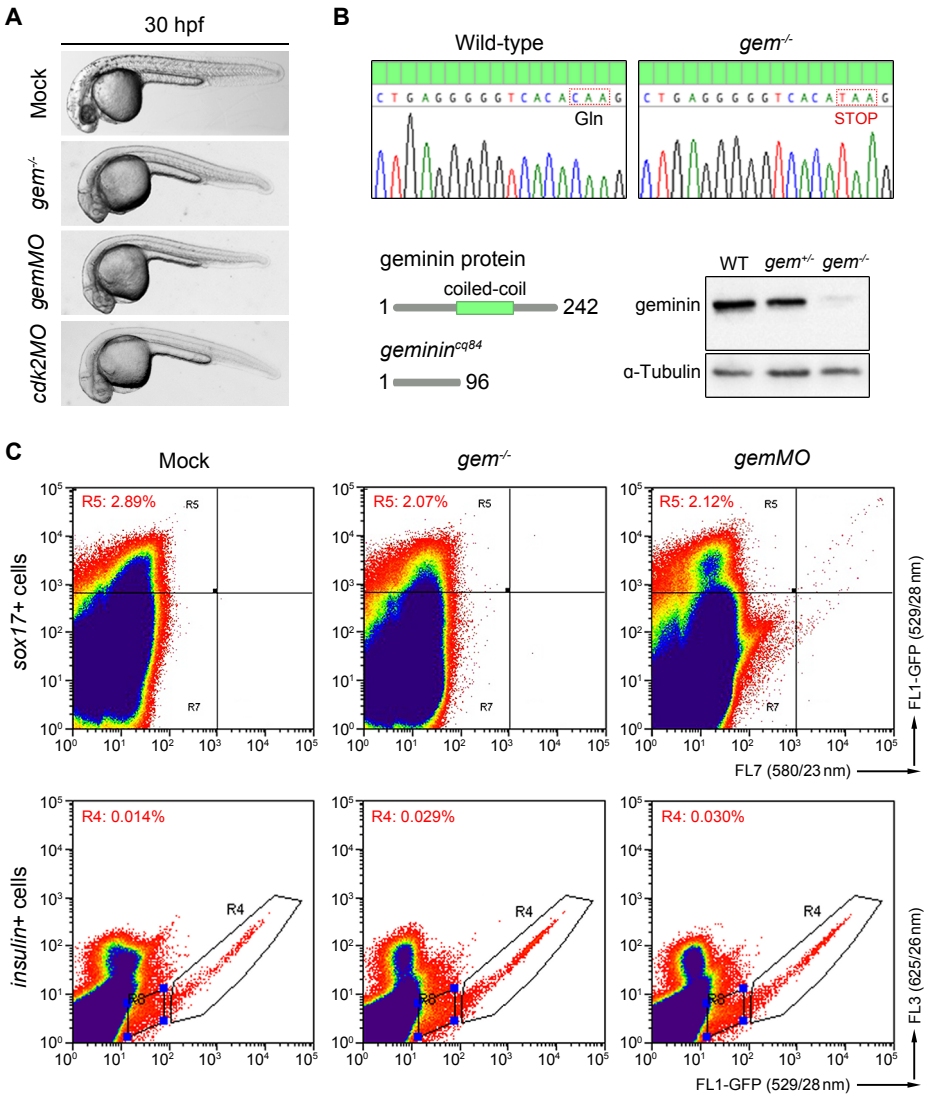

Fig. S5. Loss of geminin leads to increased pancreatic endocrine cells. (*A*) The overall phenotypes of developmental delay were comparable in the *geminin* morphant, *geminin* mutant, and *cdk2* morphant. (*B*) Generation of the *geminin*^{cq84} mutant using CRISPR/Cas9. (*C*) FACS analyses showed that in the *geminin* morphant or mutant, the ratios of *sox17*+ cells (gate R5) became reduced, whereas the ratios of *insulin*+ cells (gate R4) were increased. The *Tg(sox17:GFP)* and *Tg(insulin:GFP)* transgenic background were used for analyses.

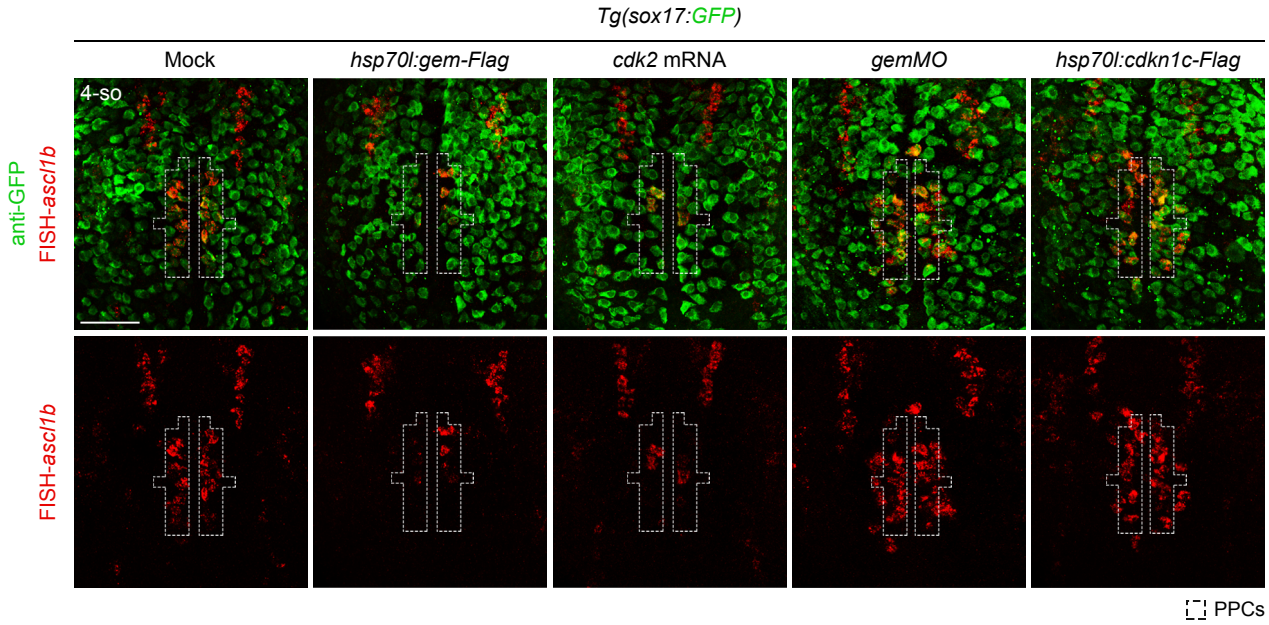

Fig. S6. Manipulations of the cell cycle modulate the expression of *ascl1b* in the early endoderm. At the 4-somite stage, *ascl1b* is expressed in the pancreatic endocrine progenitors in the early endoderm (n=30/30), which was reduced by the overexpression of geminin-Flag (n=28/35) or Cdk2 (n=26/36), and expanded by *gemMO* (n=25/33) or overexpression of Cdkn1c-Flag (n=28/38). White dashed frames indicate PPC regions. The top bilateral expression of *ascl1b* is outside the endoderm and resistant to cell cycle manipulations. Scale bar, 50 μm. 4-so, 4-somite stage.

Supplemental Dataset Legends

Dataset S1. Single-cell labeling and descendant tracing for all the 216 positions of early foregut endoderm underneath the levels between somite-1 and somite-4. In the *Tg(sox17:hKikGR1)* transgenic embryos at the 4-somite stage, each single endodermal progenitor cell beneath somite-1 to somite-4 was labeled by green-to-red photoconversion at the marked position (e.g. L-c1s1r1). Localizations of its descendant cells at 48 hpf were detected by red fluorescence. Scale bar, 50 μm.

Dataset S2. Single-cell labeling and descendant tracing for the 57 positions of early endoderm outside the levels between somite-1 and somite-4. In the *Tg(sox17:hKikGR1)* transgenic embryos at the 4-somite stage, single endodermal progenitor cell outside somite-1 to somite-4 was labeled by green-to-red photoconversion at the marked position (e.g. L-A-c1r4). Localizations of its descendants at 48 hpf were detected by red fluorescence. Scale bar, 50 μm.

Dataset S3. The number and distribution of descendant cells of each individual foregut progenitor. According to the single-cell labeling and descendant tracing strategy, the number and loci of descendant cells at 48 hpf were recorded for each progenitor cell in the early endoderm. n=x means the progenitor at indicated position in the standard map was observed for x independent repeats. For each position, results of every observation and in total are displayed. The left and right columns are displayed in different sub-tables. panc, pancreas; swim-b, swim bladder.

Dataset S4. The number and distribution of pancreatic endocrine and exocrine descendant cells. According to the single-cell labeling and descendant tracing strategy in combination with anti-Islet1 and *ptf1a:EGFP*, all the pancreatic progenitors close to the dorsal midline as well as some endodermal progenitors distant from dorsal midline were analyzed. n=x means the endodermal progenitor at indicated position was observed for x independent repeats. For each position, results of every observation are displayed. Note that all the progenitors close to the dorsal midline do not generate pancreatic exocrine cells. The progenitors that could produce pancreatic descendant cells but were not analyzed were marked with “not analyzed”. endo, endocrine cells; exo, exocrine cells.

Dataset S5. Average number of descendant cells produced by each foregut endodermal cell. The average number of descendant cells produced by each endodermal progenitor cell within the 36-hour-time window from the 4-somite stage to 48 hpf was calculated. The thresholds of darker fillstyle are $\geqslant$5 and $\geqslant$8.