Supporting Information

Chung et al. 10.1073/pnas.0910205107

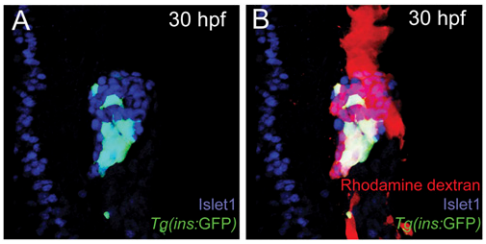

Fig. S1. Control donor cells gave rise to pancreatic endocrine cells in host embryos without displaying an increased preference for an endocrine cell fate. (*A* and *B*) Confocal images of host embryos containing control donor cells (rhodamine dextran, red) at 30 hpf. Control donor cells incorporated into Islet1 (blue)-expressing pancreatic endocrine cells, and some of them differentiated into *Tg(ins:*GFP*)*-expressing pancreatic β-cells (green; triple-labeled cells appear white). For clarity, the red color is excluded in the corresponding *Left* panel (*A*).

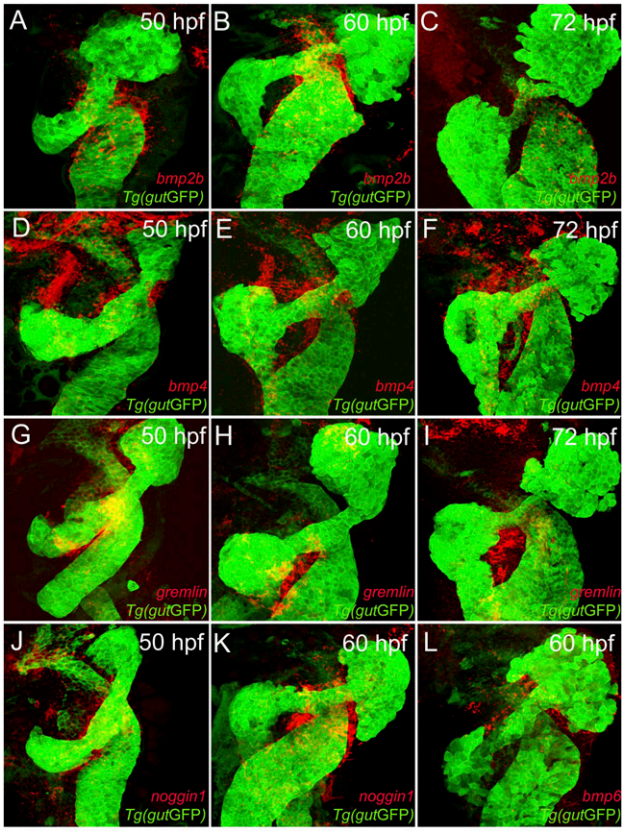

Fig. S2. Genes encoding Bmp ligands and antagonists are expressed in the mesenchymal cells adjacent to the foregut endoderm between 50 and 72 hpf. (*A–L*) Confocal images of *Tg(gut*GFP*)* expression (green) with mRNA expression (red) of *bmp2b* (*A–C*), *bmp4* (*D–F*), *gremlin* (*G–I*), *noggin1* (*J* and *K*), and *bmp6* (*L*) at 50 (*A, D, G,* and *J*), 60 (*B, E, H, K,* and *L*), and 72 (*C, F,* and *I*) hpf.

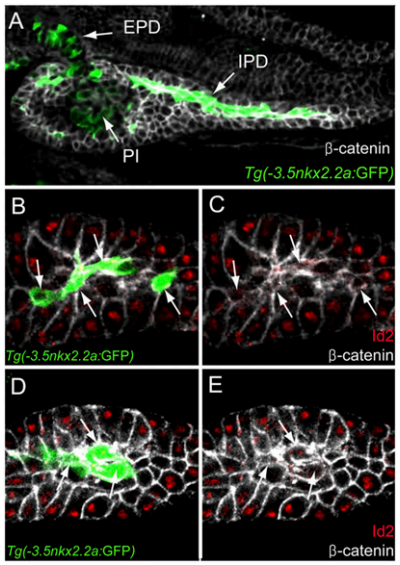

Fig. S3. Expression of Id2 is excluded from the intrapancreatic duct cells. (*A*) Confocal image of *Tg(-3.5nkx2.2a:*GFP*)* expression (green), which strongly marks the intrapancreatic duct (IPD) cells and weakly marks the extrapancreatic duct (EPD) cells and endocrine cells adjacent to the principal islet (PI) at 72 hpf. β-Catenin (white) outlines the general morphology of the pancreas. (*B–E*) Id2 (red) is expressed in acinar cells but appears to be downregulated or excluded from the intrapancreatic duct cells (green; arrows) in distal (*B* and *C*) and proximal (*D* and *E*) regions of the pancreas. For clarity, the green color is excluded in the corresponding *Right* panels (*C* and *E*).

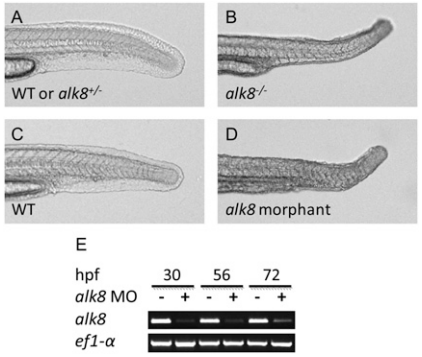

Fig. S4. Verification of the *alk8* splice-blocking morpholino by comparing *alk8* mutants with *alk8* morphants, and RT-PCR. (*A–D*) Morphology of the tail at 30 hpf. (*A* and *C*) Wild-type and *alk8* heterozygote embryos display normal tail development. (*B*) *alk8* mutants display a dorsalized phenotype resulting in a lack of the ventral tail fin. (*D*) All embryos injected with the *alk8* morpholino exhibit ventral tail fin defects (75% of the embryos lacked the whole ventral fin and 25% had a partial reduction of the fin). (*E*) RT-PCR showing reduced amount of spliced *alk8* mRNA in embryos injected with *alk8* morpholino at 30, 56, and 72 hpf. Twenty embryos from each condition and stage were used for cDNA synthesis. The *alk8* morpholino blocks the splice donor site of the second coding exon, leading to a decreased PCR product because the primers used anneal to the second (5′-ctgctagtcatgtggtagaatgctg-3′) and fifth (5′-cacttcaccgtaccgtcctt-3′) coding exons. Equal amounts of cDNA template is indicated by amplification of elongation factor 1-α, using the primers (5′-tcaccctgggagtgaaacagc-3′) and (5′-acttgcaggcgatgtgagcag-3′).

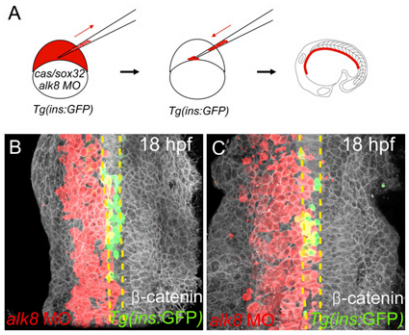

Fig. S5. *alk8* splice-blocking morpholino-injected donor cells behave like control cells and can give rise to dorsal bud-derived endocrine cells. (*A*) Schematic diagram of the cell transplantation protocol. *Tg(ins:GFP)* donors were injected with *cas/sox32* mRNA and *alk8* MO along with rhodamine dextran, and the cells were transplanted into *Tg(ins:GFP)* hosts. (*B* and *C*) Ventral confocal images of *Tg(ins:*GFP*)* (green), β-catenin (white), and rhodamine dextran (red) at 18 hpf (the notochord is outlined by yellow dashed lines). The donor cells (red) did not give rise to ectopic *Tg(ins:*GFP*)*-expressing cells, because maternally derived Alk8 is present during early stages.

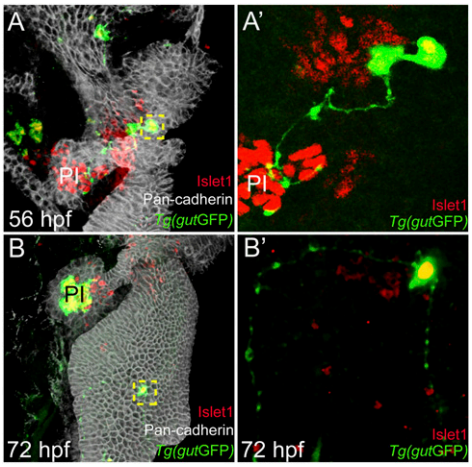

Fig. S6. Some ectopic endocrine cells induced by mosaic knockdown of *alk8* send out long protrusions. (*A*–*B*′) Confocal projections of hosts containing *alk8* morphant donor cells (*Tg*(*gut*GFP*)*; green) stained for Pan-cadherin (white) and Islet1 (red) at 56 (*A* and *A*′) and 72 (*B* and *B*′) hpf. *alk8* morphant donor cells gave rise to Islet1-expressing endocrine cells in nonpancreatic tissues, such as the pharyngeal endoderm and intestine (yellow dashed squares). These ectopic endocrine cells appear to send out long protrusions, possibly in an attempt to connect with the principal islet (PI). For clarity, *A*′ and *B*′ show a magnified view of the cells marked by the yellow dashed squares in *A* and *B*, respectively. Also, the white color is excluded in the corresponding *Right* panels.

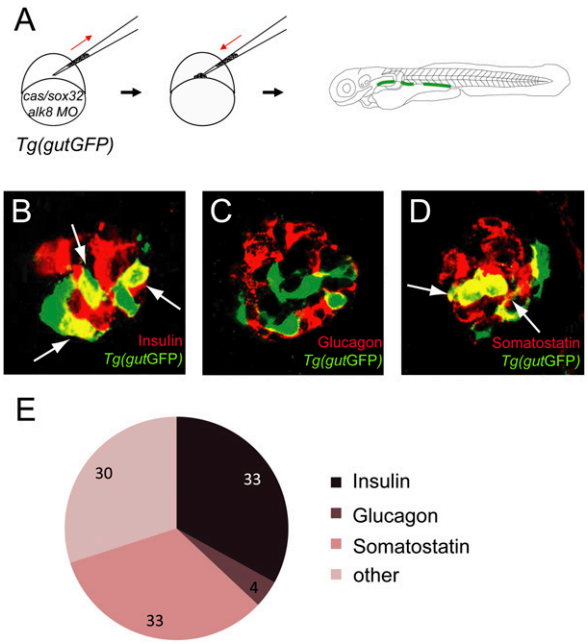

Fig. S7. Mosaic knockdown of *alk8* preferentially generates additional Insulin and Somatostatin-expressing cells. (*A*) Schematic diagram of the cell transplantation protocol. *Tg(gutGFP)* donor embryos were injected with *cas/sox32* mRNA and *alk8* splice-blocking MO before transplantation into wild-type hosts. (*B–D*) Confocal images of hosts containing *Tg(gut*GFP*)* donor cells (green) and stained for Insulin (*B*), Glucagon (*C*), or Somatostatin (*D*) (red) at 72 hpf. (*E*) Proportional distribution of hormone content of *alk8* morphant cells located in the islet. The percentages were calculated by counting the number of donor-derived hormone-expressing cells and dividing it by the total number of the donor cells in the islet (total number of cells counted = 306). Host embryos were stained for Insulin or Glucagon/Somatostatin; because the sum of the percentages of Insulin, Glucagon, and Somatostatin-expressing cells did not reach 100%, the data indicate that some donor cells differentiated into other endocrine cell types (e.g., ε- or γ-cells), or nonendocrine cells.

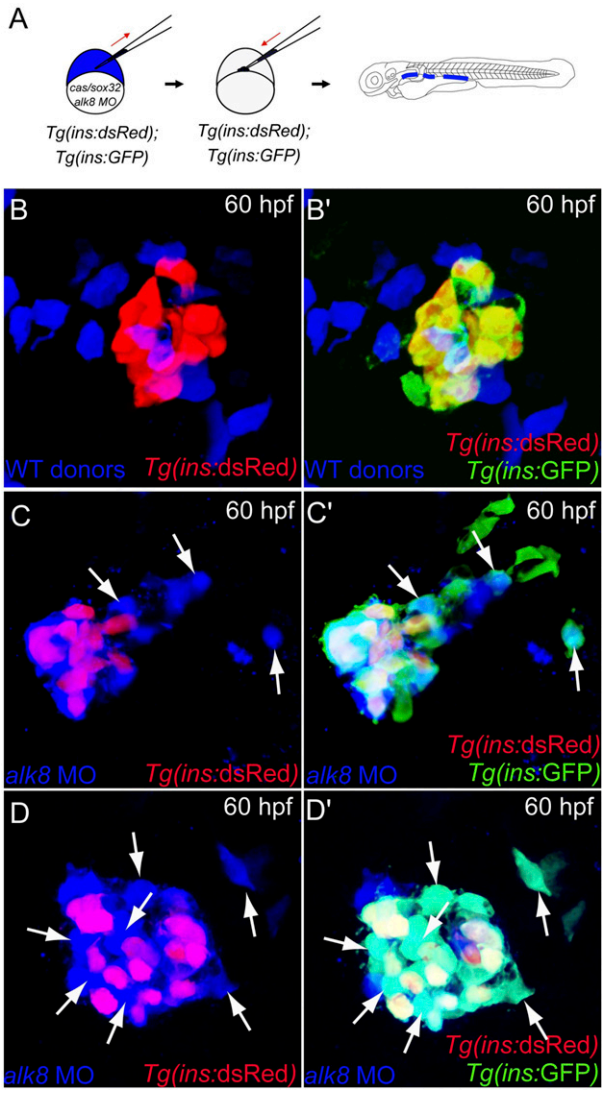

Fig. S8. Mosaic knockdown of *alk8* cell-autonomously generates ventral bud-derived β-cells. (*A*) Schematic diagram of the cell transplantation protocol. *Tg (ins:GFP);Tg(ins:dsRed)* donors were injected with *cas/sox32* mRNA and *alk8* splice-blocking MO along with Alexa Fluor 647 dextran (blue), and the donor cells were transplanted into *Tg(ins:GFP);Tg(ins:dsRed)* hosts. (*B*–*C*′) Confocal projections of *Tg(ins:*GFP*)*- and *Tg(ins:*dsRed*)*-expressing islets containing control (*B* and *B*′) and *alk8* morphant (*C*–*D*′) donor cells (blue) at 60 hpf. Control donor cells incorporated into *Tg(ins:*GFP*)*- and/or *Tg(ins:*dsRed*)*-expressing β-cells without showing any preference. In contrast, *alk8* morphant donor cells (blue) gave rise to many GFP-only–positive β-cells (arrows). For clarity, the green color is excluded in the corresponding *Left* panels (*B*–*D*).