Supplementary Figure Legends

Supplementary Figure S1. Validation of the anti-H3K27me3 antibody used for ChIP-qPCR and ChIP-Seq by Western blot.
Validation of the anti-H3K27me3 antibody by western blot using an adult liver extract. The marker lane (left) is from a different portion of the same gel.

Supplementary Figure S2. FACS gating to sort Pdx1-GFP+ cells and Ngn3-GFP+ cells from embryos.
(A) FACS gating to sort Pdx1-GFP+ cells from E10.5 embryos. (B) FACS gating to sort Ngn3-GFP+ cells from E14.5 pancreas. Liv2 isolates liver cells, whereas Pdx1-GFP (PDX1) isolates pancreas progenitors. The negative control are cells from the embryo tail, which expresses neither Liv2 nor Pdx1-GFP.

Supplementary Figure S3. DNA size analysis of H3K27me3 ChIP-Seq libraries.
The DNA sizes of the amplified ChIP-Seq libraries, as assessed by a BioAnalyzer, are consistent between samples and about 200-500 bp. Input and ChIP-Seq products for H3K27me3 are shown.

Supplementary Figure S4. Cell proliferation rate is not affected in Ezh2 KO pancreas.
FACS analysis of the percentage (%) of BrdU+ cells in total E14.5 pancreas cells of the designated genotypes. N = number of embryos assayed.
Supplementary Figure S5. NGN3+ cells locate in Sox9+ truck areas, but not in the Amylase+ tip areas of the E14.5 Ezh2 KO pancreas.

(A) Immunohistochemistry for Amylase (brown), an acinar marker expressed in the developing tips of the branching pancreatic epithelium, and NGN3 (blue) show there is no overlap of acinar and endocrine progenitor cells in Ezh2 KO pancreas (n=3) and WT counterpart (n=3), as affirmed by single-stain studies of NGN3 (not shown). (B) Sox9 (brown), a duct marker expressed in the truck of the branching pancreatic epithelium, and NGN3 (blue) show that endocrine progenitor cells locate in truck areas in Ezh2 KO pancreas (n=3) and WT counterpart (n=3).

Supplementary Figure S6. Beta cell mass expansion in Ezh2 knockout pancreas.

(A) Immunohistochemistry for insulin (brown) shows beta cell area increases in P9 Ezh2 homozygous KO pancreas compared to WT counterparts. (B) Statistics of the percentage of beta cell area to total pancreas area. Intermittent sections in 100 µM intervals were analyzed for each pancreas and 8 sections were measured for their beta cell area over total pancreas area; n = number of animals.

Supplementary Figure S7. Ezh2 methyltransferase inhibitors improve pancreatic progenitor specification.

(A) Experimental flow chart (right) and representative images of 2-7S half embryos before and after 48 hr treatment with 0.1 µM DZNep or 0.5 µM GSK-126, showing no overt inhibition of morphogenesis. (B) FACS analysis of the % of GFP+ pancreatic
progenitor cells to total embryonic cells in cultured half-embryo explants, expressed as mean ± SEM. Both Ezh2 inhibitors increase the number of Pdx1-GFP+ cells.

**Supplementary Figure S8.** Ezh2 methyltransferase inhibitors lead to a decrease of the H3K27me3 mark on the NGN3 promoter in human endodermal progenitor cells.

EP cells were incubated for 48 hours with 0.1 μM DZNep or 2 μM GSK-126 and H3K27me3 occupancy at the NGN3 promoter was analyzed by ChIP-qPCR.

**Supplementary Dataset S1**

Tab 1: Gene targets where “TRUE” = positive, “FALSE” = negative for H3K27me3 at the developmental stage shown.

Tab 2: Gene Ontology data.

Tabs 3-9: Genes within each developmental progression category (e.g., TFF = TRUE FALSE FALSE = H3K27me3 bound in endoderm and not in pancreatic or endocrine progenitors.