Supplemental Figure Legends

Supplemental Figure 1. Expression pattern of *Sox17*$^{GFPCre/+}$ at E7.5

A) 3D reconstruction of an E7.5 *Sox17*$^{GFPCre/+}$ embryo. Intense GFP fluorescence was observed in the definitive endoderm area (arrow) and weaker expression seen in visceral endoderm area (arrowhead). Anterior (A), posterior (P), proximal (Pr), distal (Di). Scale bar = 100 μm.

Supplemental Figure 2. Cre recombinase efficiency assessment

A-D) β-D-Galactosidase staining of E9.5 *Sox17*$^{GFPCre/+}$;*R26R*$^{LacZ/+}$ embryo sections showed LacZ activity in all endoderm-derived cells such as (A) the first branchial pouch (BP1) (B) the second branchial pouch (BP2) (C) the foregut (Fg), the hepatic primordium (HP) and (D) in the hindgut (Hg). It was also present in endothelia as illustrated for the dorsal aorta endothelium (DA). E-F) β -D-Galactosidase staining was performed on E12.5 *Sox17*$^{GFPCre/+}$;*R26R*$^{LacZ/+}$ embryo sections. The recombination of the *Rosa26* locus was detected in (E) the endocardium (heart, H) as well as in the endothelia, such as in the aorta (A). The enzyme activity was also present in all endoderm-derived organs, such as the liver (Li), the stomach (St), the dorsal and ventral pancreas (DP and VP), the small intestine (It) (F), as well as the colon and the caecum (G). Scale bar = 100 μm (A-D, F-G), 200 μm (E). Magnification insert scale bar = 20 μm.

Supplemental Figure 3. Fate tracing of Sox17-expressing cells at multiple stages

A) Immunolabeling of E9.5 *R26R*^{eYFP};*Sox17*^{GFPCre} embryos revealed YFP and Foxa2 co-localization in the gut tube (GT), ventral pancreas (VP), and liver (Li). However, Foxa2 is not detected in YFP-positive endothelial cells (arrows). Scale bar = 50 μm. B) Immunolabeling of E11.5 and E15.5 *R26R*^{eYFP};*Sox17*^{GFPCre} pancreata revealed YFP co-localization with pancreatic markers, such as Ptf1a, Ngn3, Ins, and the ductal stain, Dolichos biflorus agglutinin (DBA). Scale bar = 50 μm.

Supplemental Figure 4. Fate tracing of Sox17-expressing cells in different organs

A) Immunolabeling of E15.5 *R26R^{eYFP};Sox17^{GFPCre}* embryos revealed YFP and PECAM co-localization in the vein (V), liver (Li), pancreas (P), and hepatic vein (HV). B) Immunolabeling of E9.5 *R26R^{eYFP};Sox17^{GFPCre}* embryos revealed YFP and pre-HSC markers co-localization in the blood island of yolk sac.

Supplemental Figure 5. Analysis of the two Sox17-expressing cell populations

A) EpCAM immunolabeling was used to analyze the number of EpCAM^{+} and EpCAM^{-} cells present in E9.5 *Sox17$^{GFPCre/+}$* whole mouse embryos. GFP/EpCAM co-positive cells represent ventral pancreatic cells (EpCAM^{+}), and GFP-positive/EpCAM-negative cells represent hemogenic endothelial cells (EpCAM^{-}). Percentages indicate the mean percentage of EpCAM^{+} or EpCAM^{-} cells which are GFP-positive (n=4). B) Quantification of EpCAM^{+} and EpCAM^{-} cells analyzed by FACS from all GFP-positive cells identified in E9.5 *Sox17$^{GFPCre/+}$* whole mouse embryos. C) The mid region including ventral pancreas (red box) was dissected from whole embryos to increase sorting efficiency. Immunohistochemistry on E9.5 *Sox17$^{GFPCre/+}$* whole mount embryo cryo-sections showed that GFPCre was expressed in the ventral pancreatic bud (VP) with weaker expression in the dorsal aorta (DA) in P-Sp area. Image was acquired using AZ100 multizoom microscope, Nikon. Zoom = 5.7x. Scale bar = 100 μm.

Supplemental Figure 6. Comparison of coverage plots on mouse Sox17 gene locus

Coverage plot of uniquely mapped sequence reads (red) from EpCAM^{-} and EpCAM^{+} samples following RNA-Seq. Predicted *Sox17* transcripts taken from either the UCSC database (*Sox17* (UCSC)) or RefSeq and EMBL/GenBank databases (*Sox17* (TROMER)) are shown below

mapped reads. Exons 1 – 5 are noted, and a CpG island spanning exons 4 – 5 is indicated. Blue box indicates exon 2 region which contains mapped reads for $EpCAM^-$ sample.