Supplemental Figure 1

(A) Numbers of nucleated erythroid cells present in yolk sac cells from wild type, $Evil^{+/-}$ or $Evil^{-/-}$ embryos at E9.5.
(B) Yolk sac hematopoietic progenitors in E9.5 embryos. Hematopoietic colony assays from total yolk sac cells of Evi1−/− embryos and wild-type littermates at E9.5. Numbers of hematopoietic colonies were scored after 7 days of culture of yolk sac cells. Total numbers of GM colonies (a) or erythroid colonies (b) per one yolk sac were obtained from five independent experiments. Columns represent mean values ± SD (n=5). Methylcellulose culture was performed in the presence of IL-3 (200 U/mL), SCF (50 ng/mL), and Epo (2 U/mL).
Supplemental Figure 2 Differentiation marker analysis of cultured P-Sp region on OP9 cells

Each separated P-Sp region was cultured on OP9 to differentiate with SCF, IL-6, IL-7 and EPO for 7 days. Separated and floating cells were harvested, stained by CD11b (macrophage and monocytes), Gr-1 (granulocytes), CD3 (lymphocytes), B220 (B cells), Ter119 (erythrocytes) or CD41 (hematopoietic progenitor cells) and sorted by cell-sorter. Percentages of each fraction are indicated in the upper right quadrant.
Supplemental Figure 3

In vivo hematopoietic reconstitution by transplanted cells. Cells from E9.5 yolk sac were injected into the conditioned newborn recipients. Transplanted yolk sac cells from *Evi1−/−* mice did not contribute to the hematopoiesis, compared with WT (wild type) and *Evi1+/−* mice at 8-12 weeks after the transplant.
Supplemental Figure 4

(A) Expression of Evi1, GATA-2 and GFP in P-Sp cultures with enforced Evi1 expression by RT-PCR at 4 days after infection. Enforced expression of Evi1 into P-Sp culture was used mock EGFP retrovirus or Evi1 retrovirus (Evi1-IRES-EGFP). To confirm the retroviral infection into cells, we determined the expression of GFP in the P-SP culture by RT-PCR along with Evi1, GATA-2 and GAPDH.
(B) GATA-2 or Evi1 rescues defective expansion of CD45+ c-Kit+CD34+ HSCs in E9.5 Evi1−/− P-Sp cultures. P-Sp explants on OP9 stromal cells were infected with a GATA-2 (+GATA-2), an Evi1 (+Evi1) or a mock EGFP (+GFP) retrovirus as a control. Cells from P-Sp cultures were harvested at 7 days in culture and adherent cells (non-hematopoietic cells) were removed with Sephadex G-10. CD45+ GFP+ cells were gated and examined for expression of CD34 and c-Kit (percentages of each fraction by FACS analysis are indicated in the lower right quadrant).
Supplemental Figure 5

(A) Schematic representation of the promoter region of the GATA-2 IS exon (IS) and potential DNA-binding sites of Evi1. Four putative binding sites (a, b, c and d) and four unique restriction sites (KpnI, XhoI, XbaI and NotI) are indicated.

(B) Detection of Evi1 binding to the GATA-2 promoter by EMSA. Binding of the GST-fused DNA-binding domain 1 (Evi1-D1) was analyzed of each potential binding site (a, b, c and d). An oligonucleotide containing a consensus-binding site for DNA-binding domain 1 (D1-CONS) and an equal amount of random oligonucleotides (NSP) were used as controls in a competition assay. Each labeled oligonucleotide (a, b, c, or d) was incubated with control GST protein or GST-fused Evi1-D1.
(C) GST-fused Evi1-D1 bound the DNA-binding sequence b with the same affinity as D1-CONS. Labeled D1-CONS or b oligonucleotides were incubated with GST alone or GST-fused Evi1-D1. The legends below the autoradiographs indicate the competitor used: D1-CONS (D1-CONS), site b (b) and random oligonucleotides (NSP).

(D) Schematic representation of the promoter region of the GATA-2 IS exon (IS) and possible DNA binding sites of Evi1. The top line shows localization of eight possible binding sites (e, f, g, h, i, j, k and l) for DNA binding domain 2 with unique restriction sites (KpnI, XhoI, XbaI and NotI) indicated.
(E) Detection of binding of *Evi1* to the *GATA*-2 promoter by EMSA. Binding of GST-fused DNA binding domain 2 (Evi1-D2) was analyzed on each potential binding site (e to l) by EMSA. Each labeled oligonucleotide (e to l) was incubated with control GST protein or GST-fused Evi1-D2.

(F) Detection of DNA binding affinity of *Evi1* to the possible DNA binding sites by EMSA. The labeled DNA binding oligonucleotides for DNA binding domain 2 (D2-CONS) was incubated with GST-D2 along with indicated excess competitor oligonucleotide. D2-CONS and random oligonucleotides (NSP) were used as a positive and negative control for the competition assay, respectively. The complex consisting of D2-CONS and GST-D2 was completely competed with five oligonucleotides (f, g, h, j and l).
Supplemental Figure 6

(A) Schematic representation of the 5’ region of the GATA-2 gene with the location of amplified genomic fragments by chromatin immunoprecipitation (ChIP). Dark boxes with indicated name present the location of the amplified genomic fragments by ChIP. Vertical numbers at each dark box present the nucleotide numbers from GATA-2 promoter (IS).
(B) Analysis of mRNA expression of genes related to hematopoiesis or angiogenesis in EML C1 cells with or without transfection of Evi1. EML C1 cells were transfected by mock (Mock) or Evi1 expression vector (Evi1). mRNA expression of each gene was assayed by TaqMan real-time PCR (PE Applied Biosystems) 2 months after selection by G418, and was normalized to that of GAPDH mRNA and calibrated to the gene/GAPDH ratio (ΔCT) in EML C1 cells with control expression vector (Mock). Relative expression rate in each gene are presented as the mean and standard deviation of $2^{\Delta\Delta CT}$ in quadruplicate assays. *$P < 0.001$, relative to controls.