Supporting Information

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SI Materials and Methods

Flow Cytometry Analysis. Embryonic day (E)7.5–E8.0 *Runx1*$^{LacZ/wt}$ yolk sac cells and E16.5 fetal liver cells were loaded with fluorescein di-β-D-galactopyranoside (FDG) according the recommended procedure (Molecular Probes), and the yolk sac cells were stained with monoclonal antibodies to vascular endothelial (VE)-cadherin (VECD1–phycoerythrin (PE)/Cy7; BioLegend). Cells isolated from the aortic-gonad-mesonephros (AGM) region, fetal liver, thymus, and OP9 stroma cultures were stained with anti-mouse monoclonal antibodies: CD45–allophycocyanin (APC), c-Kit–APC, Ter119–PE, CD-11b (Mac-1)–APC, CD11b–PE, Gr-1–PE, B220–APC, CD19–PE, CD19–APC, CD4–PE, and CD8–PE (BD Biosciences). For some applications, the monoclonal antibodies VECD1 [anti-mouse VE-cadherin (1)] were purified from hybridoma culture by protein G Sepharose columns (Pharmacia) and were labeled with APC by standard methods. For analysis of adult peripheral blood cells, the following monoclonal anti-mouse antibodies were used after red-cell depletion: Ter119–APC, CD11b–PE, CD11b–APC, Gr-1–PE, Gr-1–APC, B220–APC, CD19–PE, CD3–PE, CD4–APC, and CD8–APC (BD Biosciences). Dead cells were generally excluded by propidium iodine staining. Cell fluorescence was analyzed by FACSCalibur (BD Biosciences), and all cell sorting was performed on FACS Aria (BD Biosciences).

Cell and Organ Culture. For analysis of the B-cell potential of early conceptus cells, individual yolk sacs, E7.5 embryo proper parts, and E8.5–E10.5 embryo caudal halves dissected just below the embryo heart were used for separate cocultures on OP9 stroma (2). Dissected conceptus tissues were subjected to one-step or two-step cultures (Fig. S1*C*) in α-minimum essential medium (Gibco BRL) supplemented with 10% FBS, 50 μM 2-mercaptoethanol, 20 ng/mL murine Flt3 ligand (mFlt-3L; R&D Systems), 20 ng/mL IL-7 (PeproTech), and 10 ng/mL stem cell factor (SCF; PeproTech) on confluent OP9 cell layers. After a 2-wk culture, nonadherent hematopoietic cells were analyzed by flow cytometry using the enhanced GFP (eGFP)$^+$ gating.

Yolk sac cells isolated from the late streak–neural plate and head-fold stage conceptuses were sorted by the Runx1 and VE-cadherin expression and cultured as described above, and the hematopoietic colonies were scored on the day 8 of culture with an inverted-phase contrast microscope. Alternatively, the sorted cells were cultured for 2 wk as described above, and the resulting nonadherent hematopoietic cells were analyzed by flow cytometry with gating on eGFP$^+$ cells.

For in vitro reactivation experiments, genotyped E7.5–E10.5 yolk sacs, E7.5 embryo proper parts, and E8.5–E10.5 embryo caudal halves dissected just below the embryo heart were pooled for subsequent culture. Pooled embryonic tissues were subjected to a short-term organ culture in α-minimum essential medium (Gibco BRL) supplemented with 10% FBS and 50 μM 2-mercaptoethanol on confluent OP9 cell layers. No supplemental cytokines were added to the medium. Alternatively, embryonic tissues were trypsinized for few minutes at 37 °C and mechanically dissociated, and the resulting cell suspension was cultured in α-minimum essential medium (Gibco BRL) supplemented with 10% FBS and 50 μM 2-mercaptoethanol on confluent OP9 cell layers in 6-well plates at a density of 1×10^5 cells per well. The concentration of 4-hydroxytamoxifen (4-OHT) was adjusted to 1 μM. After the short-term explant culture, the contents of individual wells were trypsinized and placed onto fresh OP9 cell layers in the same medium. After a 3-d progenitor expansion step (Fig. S1*C*), the nonadherent cells from each well were seeded in two 35-mm dishes with the MethoCult GF M3434 medium (StemCell Technologies). Hematopoietic progenitor assay was performed in the M3434 medium according manufacturer's recommendations in duplicates for at least two different cell densities of each input cell population.

Reconstitution Analysis. *Runx1*$^{LacZ/wt}$:*Rosa26*$^{R26R\text{-}eYFP/R26R\text{-}eYFP}$ parent mice were bred in the C57BL/6 genetic background for at least 10 successive generations. *Runx1*$^{SACRE/wt}$:*Rosa26*$^{wt/wt}$ parents were backcrossed to the C57BL/6 mouse strain to obtain at least F6 generation. *Runx1*$^{SACRE/LacZ}$:*Rosa26*$^{R26R\text{-}eYFP/wt}$ conceptuses along with *Runx1*$^{SACRE/wt}$:*Rosa26*$^{R26R\text{-}eYFP/wt}$ controls were exposed in utero to a single high dose (5–6 mg per dam) of 4-OHT at E7.5 as described previously (3). At E14.5 or E16.5, their fetal livers were dissected and mechanically dissociated to form single-cell suspension, and then live enhanced YFP (eYFP)^{high} cells were sorted by FACS Aria (BD Biosciences). Recipient 8- to 10-wk-old C57BL/6 mice were lethally irradiated with a single dose of 9 Gy, and sorted eYFP^{high} fetal liver cells (2×10^4 cells for E14.5 and 6×10^4 cells for E16.5) were injected along with 2×10^5 cells from C57BL/6 bone marrow into the tail vein of the recipients. Multilineage donor cell engraftment was analyzed in 4-wk intervals by flow cytometry of peripheral blood samples enriched for leukocytes and erythroid blast cells by the standard ammonium chloride lysis.

For secondary transplantations, bone marrow cells of primary recipients were isolated at 12 mo after primary transplantation and stained with APC Mouse Lineage Antibody Mixture (BD Biosciences), and live Lin$^-$eYFP$^+$ cells were purified by cell sorting with FACS Aria (BD Biosciences). Individual lethally irradiated (1×9 Gy) C57BL/6 mice were transplanted via tail vein with 5×10^4 Lin$^-$eYFP$^+$ cells and 10^5 cells of C57BL/6 bone marrow for radioprotection. Three months after transplantation, blood was obtained from the tail veins of recipient mice, subjected to ammonium chloride/potassium bicarbonate red-cell lysis, and analyzed for the presence of eYFP$^+$ cells by flow cytometry.

Whole-Mount in Situ Hybridization. In situ hybridization of mouse embryos was performed according to a standard protocol (4). Samples were fixed in 4% paraformaldehyde overnight followed by dehydration through a graded series of methanol (in PBST) solutions. *Runx1* cDNA probe spanning the coding region between exons 5 and 8 was generated by using RT-PCR and the following primers: 5′-GTTGCCACCTACCATAGAGCCATC-3′ and 5′-GTAGTATAGATGGTAGGAG-3′.

Whole-Mount Immunostaining. Whole-mount immunostaining with diaminobenzidine (DAB) was performed as described previously with slight modification (5). In brief, embryos were treated with H_2O_2 for 15 min to block endogenous peroxidase activity and stained with anti–VE-cadherin monoclonal antibodies VECD1 (6) overnight at 4 °C, followed by incubation with secondary goat anti-rat Ig-HRP. Color was developed by using a DAB Peroxidase Substrate Kit (Vector Laboratories).

RT-PCR and Genotyping. For RT-PCR analysis, total RNA was isolated with the RNeasy Mini Kit (QIAGEN). The RNA was reverse-transcribed with SuperScript II Reverse Transcriptase (Gibco BRL) according to the manufacturer's instructions. Amplification of cDNA fragments was performed with Platinum Taq DNA Polymerase (Invitrogen) using the RT-PCR primer sequences listed below.

i) ex11, 5′-CAATCACACTGAATGCAAACC-3′

ii) ex21, 5′-ATTTCCTTCATATCCACAGTGC-3′
iii) exon 31, 5′-CAATCGGCTTGTTGTGATGC-3′
iv) exon 42, 5′-TCGCTACCTGGTTCTTCATGG-3′
v) exon 43, 5′-GTAGCATTTCTCAGTTCTGC-3′
vi) ex51, 5′-GCTTCACTCTGACCATCACC-3′
vii) ex61, 5′-CTCCTTGAACCACTCCACTG-3′
viii) ex71, 5′-GACCAGCCTCTCTGCAGAAC-3′
ix) ex8d1, 5′-TCATGGCTGACATGCCGATG-3′
x) ex8d2, 5′-TCTCCACCACGTCGCTCTG-3′

To identify the *Runx1*^{Re} allele, the following primers were used: En-1, 5′-TACAACACAC-ACTCCAACC-3′; exo31, 5′-CAAC-TTCTAGGCTGTGAAC-3′; and exo33, 5′-GGCAAGATGAT-CTTCCTCC-3′. Relative positions for the primers are shown in Fig. S2*E*. For embryo and adult genotyping, a small chunk of embryo tissues or an ear punch were lysed in 50 μL of lysis buffer (1× Taq polymerase buffer, 0.1% Triton X-100, and 200 μg/mL of Proteinase K) at 55 °C for 20–60 min with shaking, followed by Proteinase K inactivation at 100 °C for 10 min. DNA fragments were amplified with Platinum Taq DNA Polymerase (Invitrogen).

PCR Analysis of *IgH* Gene Rearrangements in E7.5 Yolk Sac-Derived B Cells. Nonadherent cells were collected from OP9 stromal cultures, concentrated by centrifugation, and lysed as described above. PCR amplification was carried out in two rounds as described (7). The following primers were used to highlight $V(D)J_H$ rearrangements: V_HJ558-1 (5′-GCGAAGCT-TARGCCTGG-GRCTTCAGTGAAG-3′, R = A/G) and V_HJ558-2 (5′-GTGA-RGCCTGGGRC-TTCAGTGAAG-3′). To check $(D)J_H$ rearrangements, we used D_HQ52 primer (5′-ACGTC-GACGCGG-ACGACCACAGTGCAACTG-3′) and D_HFL16 primer (5′-AC-GTCGACTTT-TGTSAAGGGATCTACTACTGT-3′, S = G/C). The downstream primers were: J_H4E (5′-AGGCTCTGA-GATCCCTAGACAG-3′) for the first round of amplification and J_H4A (5′-GGGTCTAGACTCTCAGCCGGCTCCCTCAGGG-3′) for the second round.

Whole-Mount X-Gal Staining and Cryosectioning. Whole embryos from the timed matings were isolated into cold dissection buffer [1× PBS, 5% FCS, and 20 mM Hepes (pH 7.2)] and fixed in excess of X-Gal fix solution (0.2% glutaraldehyde) at 4 °C overnight. Embryos were washed three times for 20 min at room temperature in wash buffer (0.1% sodium deoxycholate and 0.02% Nonidet P-40) and stained for 2–3 h or overnight at 37 °C in 1 mg/mL X-Gal (Promega). Embryos stained with X-Gal were postfixed with 4% paraformaldehyde at 4 °C for 1 h, soaked in 15% sucrose and then in 30% sucrose overnight, and embedded in O.C.T., and frozen sections were cut at a thickness of 10 μm. Sections were counterstained with 0.001% eosin.

1. Matsuyoshi N, et al. (1997) In vivo evidence of the critical role of cadherin-5 in murine vascular integrity. *Proc Assoc Am Physicians* 109:362–371.
2. Kanatsu M, Nishikawa S-I (1996) In vitro analysis of epiblast tissue potency for hematopoietic cell differentiation. *Development* 122:823–830.
3. Samokhvalov IM, Samokhvalova NI, Nishikawa S-I (2007) Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. *Nature* 446:1056–1061.
4. Wilkinson DG (1992) *In Situ Hybridization: A Practical Approach* (Oxford Univ Press, London).
5. Takakura N, et al. (1997) PDGFRα expression during mouse embryogenesis: Immunolocalization analyzed by whole-mount immunohistostaining using the monoclonal anti-mouse PDGFRα antibody APA5. *J Histochem Cytochem* 45:883–893.
6. Perry ACF, et al. (1999) Mammalian transgenesis by intracytoplasmic sperm injection. *Science* 284:1180–1183.
7. Ehlich A, Martin V, Müller W, Rajewsky K (1994) Analysis of the B-cell progenitor compartment at the level of single cells. *Curr Biol* 4:573–583.

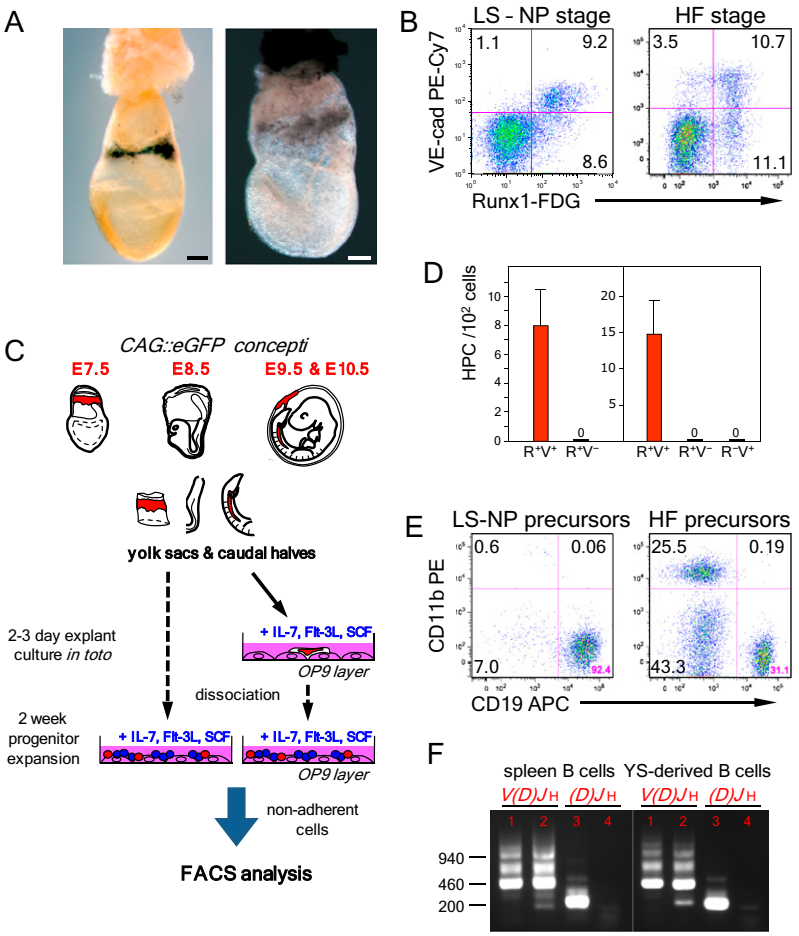
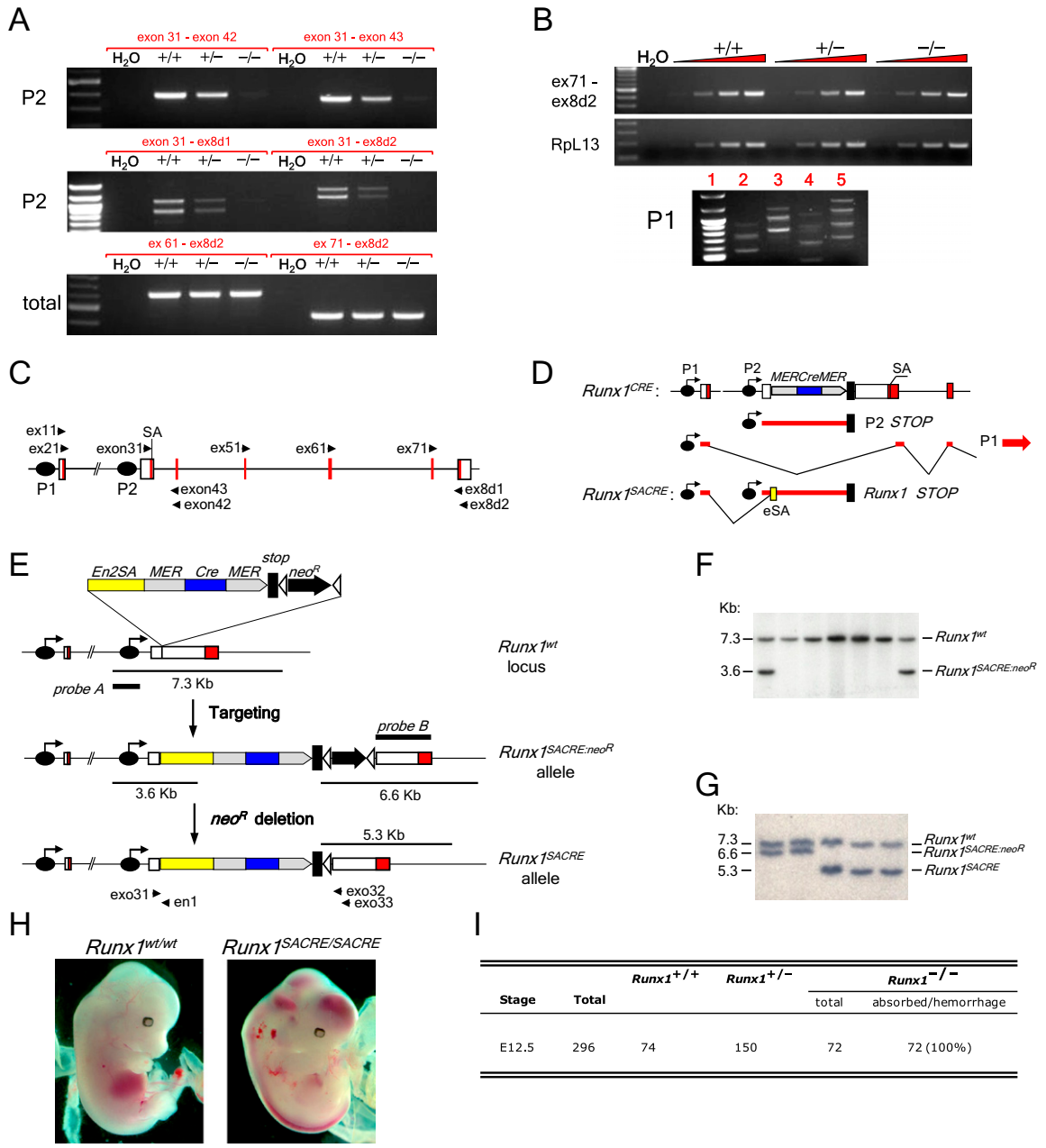


Fig. S1. Lymphopoietic potential in the E7.5–E8.0 yolk sac is confined to VE-cadherin$^+$Runx1$^+$ cells. (*A*) Whole-mount Runx1-LacZ staining (*Left*) and VE-cadherin immunostaining (*Right*) at the neural-plate stage. (*B*) Typical flow cytometry profiling of Runx1 and VE-cadherin expression in the yolk sac of the late streak–neural plate (LS-NP) and the head-fold (HF) stage *Runx1*$^{LacZ/wt}$ conceptuses. Numbers here and elsewhere in FACS data represent percentages of respective populations. (*C*) Scheme of the B-cell potential assay. All conceptuses in the analysis bore a CAG-eGFP transgene (1), whereas their mothers were eGFP$^-$, allowing for the exclusion of maternal blood contamination. Embryo caudal halves or yolk sacs isolated from hemizygous CAG-eGFP conceptuses were either directly cell-dissociated and cultured on OP9 stroma (one-step culture) or first cultured as tissue explants on OP9 cell layers for 2–3 d followed by cell dissociation and the progenitor expansion step (two-step culture). The cell-culture medium was supplemented by cytokines as shown. The resulting non-adherent cell fraction was analyzed by flow cytometry with gating for eGFP$^+$ live cells. (*D*) Hematopoietic progenitor colony (HPC) formation analysis of cell fractions sorted from LS-NP (*Left*) and HF (*Right*) yolk sacs at day 8 of culture (progenitor expansion step). Data are mean ± SD, $n = 3$. (*E*) Representative B-cell phenotyping of progeny generated in vitro by Runx1$^+$VE-cadherin$^+$ precursor cells sorted from the LS-NP and HF yolk sacs. (*F*) PCR analysis of *IgH* rearrangements in B cells derived from E7.5 Runx1$^+$VE-cadherin$^+$ yolk sac precursors. Genomic DNA isolated from unfractionated adult spleen cells was used as a control. The sizes (in bp) of most prominent PCR products representing various configurations of *IgH* loci are shown.

1. Perry ACF, et al. (1999) Mammalian transgenesis by intracytoplasmic sperm injection. *Science* 284:1180–1183.



Stage	Total	Runx1+/+	Runx1+/–	Runx1–/–	
				total	absorbed/hemorrhage
E12.5	296	74	150	72	72 (100%)

Fig. S2. The hypomorphic *Runx1*^{Cre} allele and inactivation of *Runx1* locus by insertion of a splice acceptor site sequence in front of the *MER-Cre-MER–STOP* cassette. (*A*) RT-PCR analysis of transcription initiated from the proximal promoter (P2). mRNAs from E17.5 fetal liver of three genotypes—*Runx1*$^{wt/wt}$ (+/+), *Runx1*$^{wt/Cre}$ (+/–), and *Runx1*$^{Cre/Cre}$ (–/–)—were isolated, reverse-transcribed, and amplified with four P2-transcription–specific primer pairs (*Top* and *Middle*) and with two primer pairs for total gene expression analysis (*Bottom*). Primer designations are in red. (*B*) (*Top* and *Middle*) Semiquantitative RT-PCR analysis of total *Runx1* expression (with primers ex71 and ex8d2) in the fetal liver of the three genotypes. RpL13, expression of the housekeeping gene for large subunit ribosome protein L13. (*Bottom*) Analysis of transcription initiated from the distal promoter (P1) in *Runx1*$^{Cre/Cre}$ homozygotes. 1, 100-bp DNA ladder (New England Biolabs); 2, RT-PCR with primers ex11-ex8d1; 3, RT-PCR with primers ex11-ex8d2; 4, RT-PCR with primers ex21-ex8d1; 5, RT-PCR with primers ex21-ex8d2. (*C*) Schematic location and orientation of all primers (arrowheads) used in the RT-PCR analysis. *Runx1* exons are shown as open boxes or vertical bars, coding sequences are in red, and promoters are designated as black ovals. SA, endogenous alternative splice acceptor site. (*D*) Scheme of a putative splicing mechanism that renders the *Runx1*^{Cre} allele hypomorphic. Black rectangle, transcription stop cassette (*STOP*); black ovals, *Runx1* promoters. In the *Runx1*^{Cre} allele, the P2-transcription is blocked by the *STOP* signal. Splicing out of the *STOP* cassette in the P1 transcripts unblocks their expression. Insertion of the exogenous splice acceptor site (eSA, yellow rectangle) prevents the *STOP* cassette from being spliced out and suppresses P1-transcription of the *Runx1*^{SACRE} allele. (*E*) Knock-in schematics and removal of the neomycin-resistance gene cassette in properly targeted ES cell clones. Sizes and locations of genomic BamHI restriction fragments hybridizing with external and internal probes are shown. Arrowheads indicate relative positions and orientations of the PCR primers used for identification of the reactivated allele *Runx1*^{Re}. Designations are as in *C* and *D*. (*F*) Southern blotting of genomic DNA from ES cell clones after the homologous recombination with the targeting vector (BamHI digest, external probe A). (*G*) Deletion of the neomycin-resistance gene cassette (Southern blot, BamHI digest, internal probe B). (*H*) Typical lethal phenotype of E12.5 *Runx1*$^{SACRE/SACRE}$ homozygotes. (*I*) Statistics of the Runx1-null phenotype; *Runx1*$^{+/+}$, *Runx1*$^{wt/wt}$; *Runx1*$^{+/–}$, *Runx1*$^{wt/LacZ}$ or *Runx1*$^{wt/SACRE}$; and *Runx1*$^{–/–}$, *Runx1*$^{SACRE/SACRE}$ or *Runx1*$^{LacZ/SACRE}$.

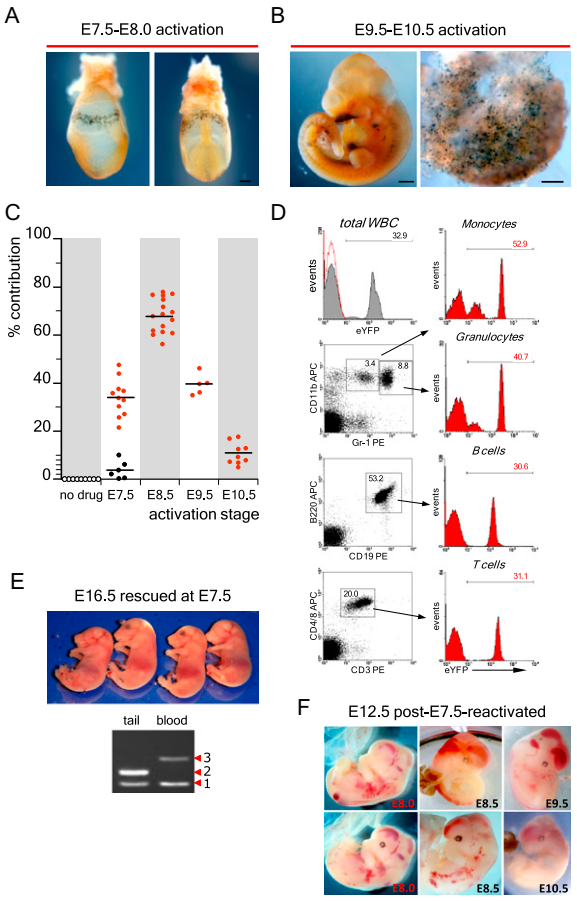


Fig. S3. Characterization of the Runx1-dependent embryo-rescue system. (*A*) Representative whole-mount LacZ staining of the E7.5–E8.0 *Runx1*$^{SACRE/wt}$:*Rosa26*$^{R26R/wt}$ conceptuses (4-h induction). (*B*) Similar analysis of Cre-recombination expression pattern in E10.5 embryo proper and placenta after 24-h induction. (*C*) Relative efficiency of ligand-dependent Cre recombination in embryonic hematopoietic lineage. Contribution of eYFP^{+} cells was measured in peripheral blood leukocytes of 1- to 3-mo-old mice exposed to single high (5–6 mg; red dots) or low (1–2 mg; black dots) doses of 4-OHT at different developmental stages or to no drug (open dots). (*D*) Adult multilineage contribution of *Runx1*$^{SACRE/wt}$:*Rosa26*$^{R26R\text{-}eYFP/wt}$ conceptus cells labeled at E7.5 by a high-dose induction. *Top Left* shows the total contribution of the eYFP-labeled cells in adult white blood cells (WBC). Red line in the histogram denotes white blood cell fluorescence from the control, nonactivated mice. Representative multilineage cell labeling is shown for a peripheral blood sample obtained from a single mouse. Numbers represent percentages of respective populations. (*E*) (*Upper*) E16.5 *Runx1*$^{SACRE/LacZ}$ embryos rescued by the gene reactivation at E7.5. Eye pigmentation varies because of the mixed genetic background of embryos. (*Lower*) Genomic PCR shows that blood cells underwent full recombination; PCR fragments correspond to alleles: 1, *Runx1*^{SACRE}, 238 bp; 2, *Runx1*^{LacZ}, 285 bp; and 3, *Runx1*^{Re}, 350 bp. (*F*) Gene reactivations at E8.0, E8.5, E9.5, and E10.5 ("post-E7.5") failed to improve survival of *Runx1*$^{SACRE/LacZ}$ embryos. Shown are the typical phenotypes of E12.5 Runx1-null embryos, which were exposed to the high dose of 4-OHT at post-E7.5 stages as labeled.

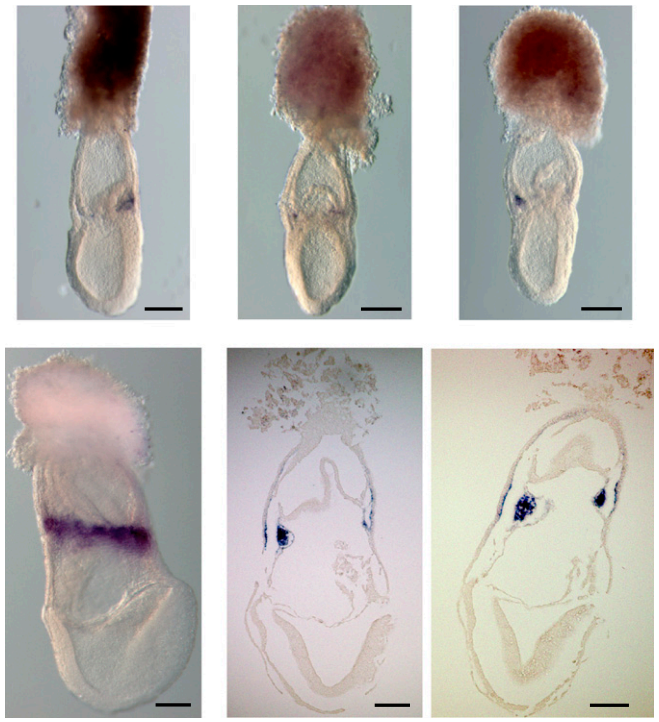


Fig. S4. *Runx1* expression during specification of the blood lineage. Whole-mount in situ hybridization analysis of the gene expression at E6.5–E7.0 (*Upper*) and E7.5–E8.0 (a whole mount and two sagittal sections; *Lower*). (Scale bars: 100 μm.)

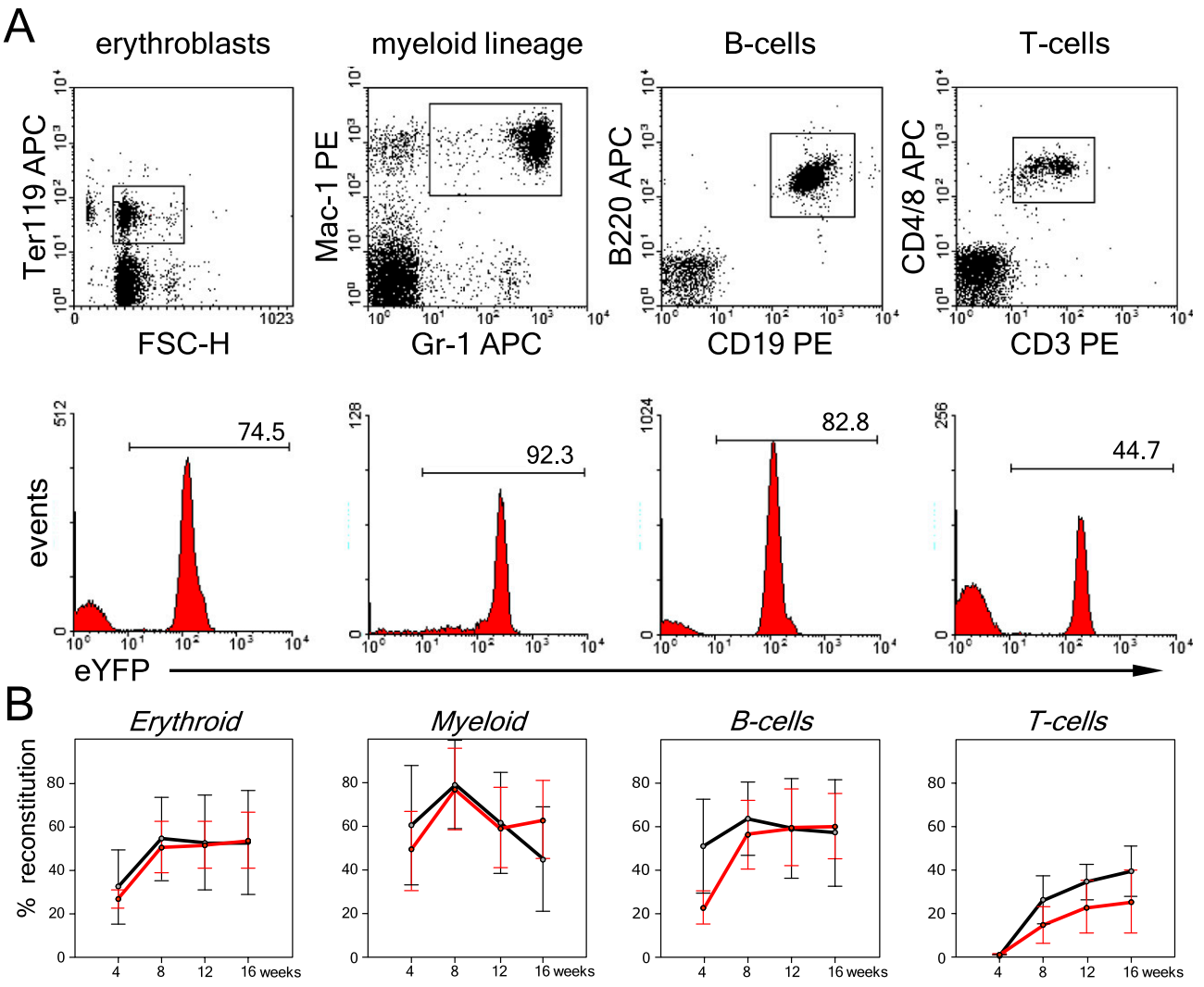


Fig. S5. Competitive long-term repopulation assay of the E7.5-rescued fetal liver hematopoietic stem cells (HSCs). (*A*) (*Upper*) Lineage gating in total peripheral blood leukocytes used for the repopulation analysis. (*Lower*) Histograms display correspondingly the maximum levels of reconstitution in the major blood-cell lineages achieved by the rescued E16.5 HSCs at 16 wk after transplantation. (*B*) The reconstitution levels by the E7.5-rescued E14.5 fetal liver HSCs measured in 4-wk intervals. All recipients were repopulated. Red lines, mean levels of reconstitution by the rescued donor cells (n = 4); black lines, levels of reconstitution by the control heterozygous donor cells (n = 4). (Error bars: SD.)

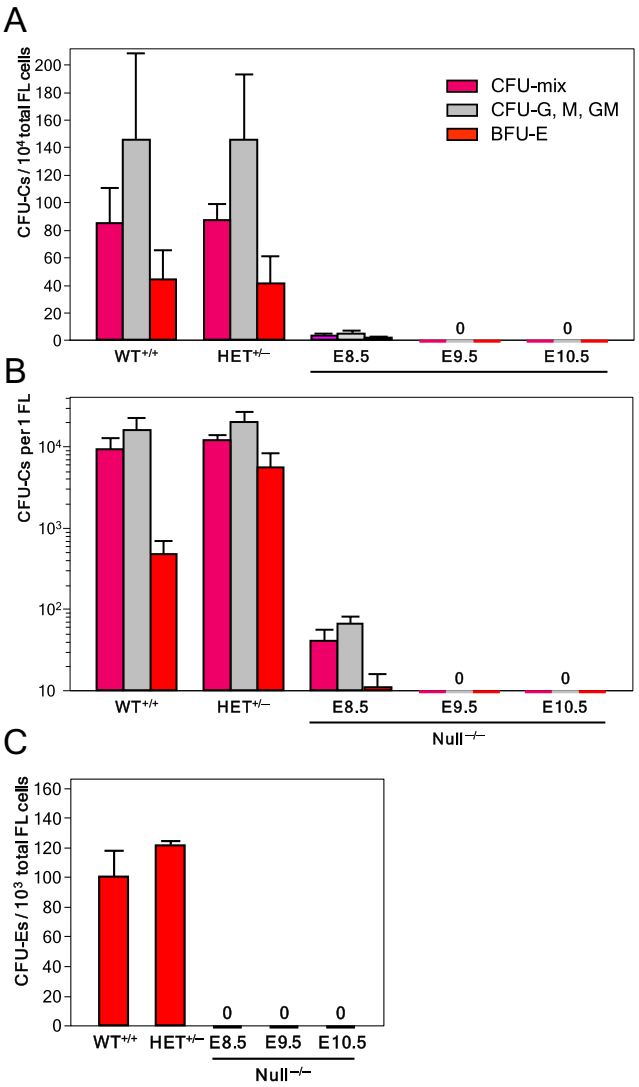


Fig. S6. Definitive hematopoietic progenitors in E11.5–E12.0 fetal liver of nonrescued post-E7.5 reactivated embryos. $WT^{+/+}$, $Runx1^{wt/wt}$; $HET^{+/-}$, $Runx1^{SACRE/wt}$ or $Runx1^{LacZ/wt}$; $Null^{-/-}$, $Runx1^{SACRE/LacZ}$. Stages of injections are indicated. Data are mean ± SD, $n = 3$ for each genotype. Zero denotes complete absence of hematopoietic colonies or cell clusters. (*A*) Colony forming cell (CFC) counts per 1×10^4 total fetal liver cells. (*B*) Same counts recalculated per one fetal liver. The *y* axis is logarithmic. (*C*) Numbers of committed erythroid CFU (CFU-E) progenitors per 1×10^3 of total fetal liver cells of the three genotypes.

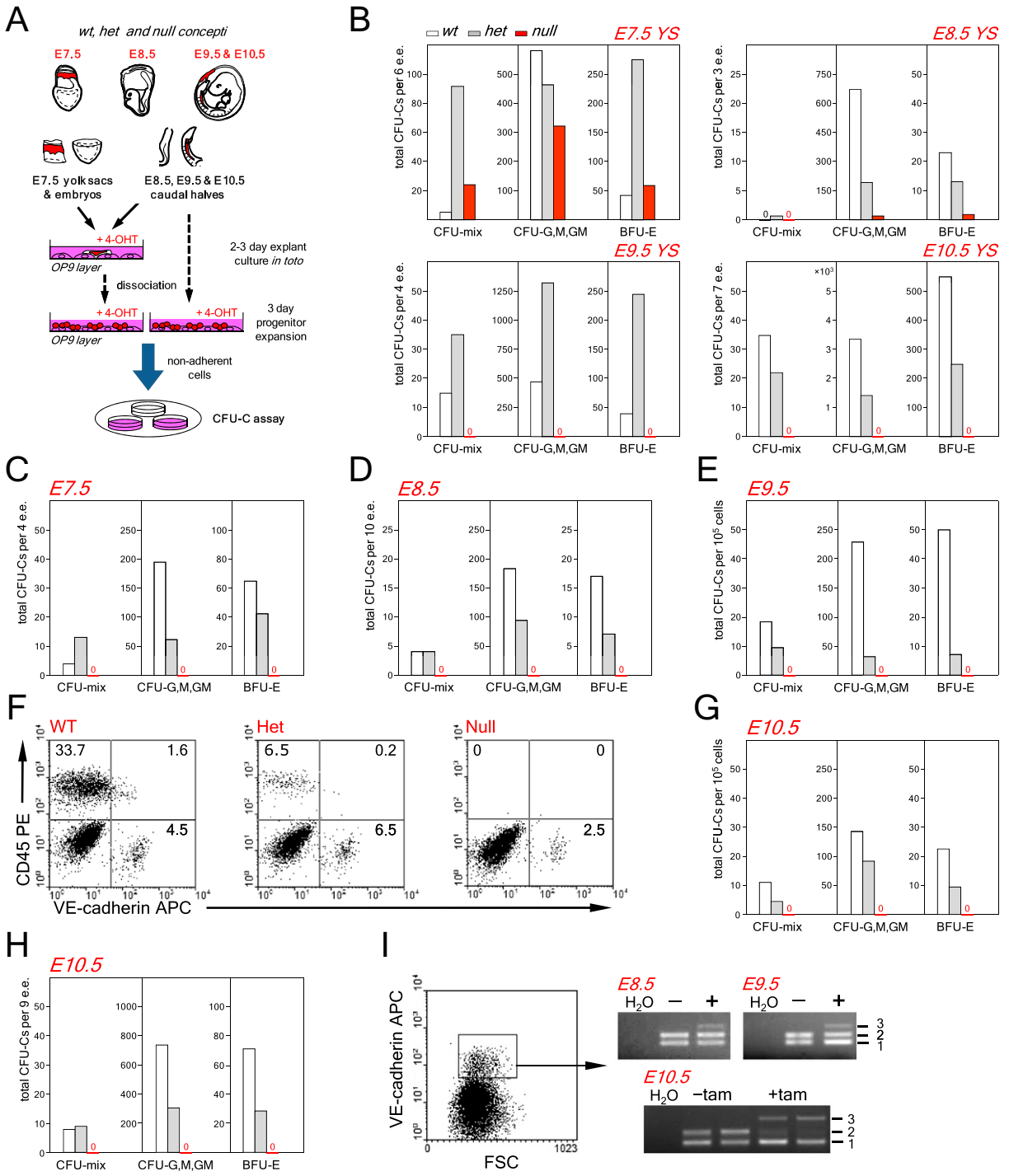


Fig. S7. In vitro *Runx1* reactivation. Conceptuses of the three genotypes—wt, *Runx1*$^{wt/wt}$; het, *Runx1*$^{SACRE/wt}$ or *Runx1*$^{LacZ/wt}$; and null, *Runx1*$^{SACRE/LacZ}$—were used in the analysis. The *y* axis indicates CFC numbers either per total number of pooled embryo equivalents (e.e.) or 10^5 initial embryonic cells. Zero denotes complete absence of hematopoietic colonies or cell clusters. (*A*) Design of complementary in vitro *Runx1* reactivation assay. E7.5–E10.5 yolk sacs, whole-embryo proper parts of E7.5 conceptuses, and embryo caudal halves from E8.5–E10.5 *Runx1*$^{SACRE/LacZ}$ conceptuses were exposed to 4-OHT in a one-step or two-step OP9 coculture system as indicated. Nonadherent cells from the stromal cultures were analyzed for the presence of hematopoietic progenitors by the standard colony-formation assay. (*B*) Rescuing hematopoietic progenitors in two-step culture of E7.5–E10.5 yolk sac explants. (*C*) CFC development in two-step culture of E7.5 embryo proper explants. (*D*) Progenitor analysis after two-step culture of the E8.5 embryo caudal halves. (*E*) Progenitor analysis after one-step culture of caudal halves of E9.5 embryos. (*F*) FACS analysis of total (stromal and nonadherent) viable cells after culturing E9.5 caudal half cells for 6 d. (*G*) One-step OP9 culture of E10.5 caudal halves. (*H*) Two-step OP9 culture of E10.5 caudal halves. (*I*) Cre recombination in VE-cadherin$^+$ fractions of total viable cells in the rescue-negative cultures. PCR fragment designations are the same as in Fig. S3*E*.

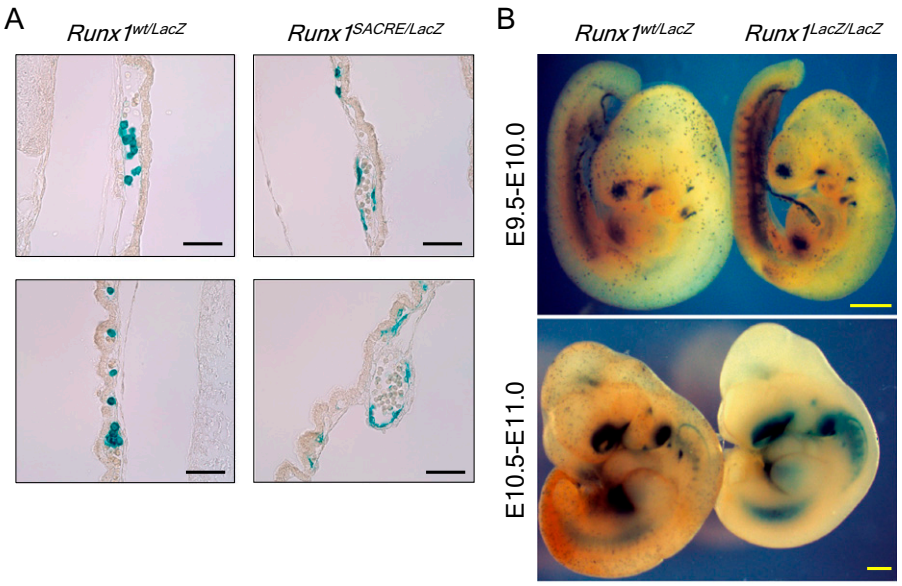


Fig. S8. Runx1-dependent definitive hematopoietic precursor cells differentiate into endothelial cells in absence of the transcription factor. (*A*) Sectioned views of whole-mount LacZ staining of E9.5 *Runx1*$^{wt/LacZ}$ and *Runx1*$^{LacZ/LacZ}$ yolk sacs. Note that the Runx1^{+} blast-like cells in heterozygotes are visibly larger than the LacZ/Runx1^{-} cells of the primitive hematopoietic wave, which lost their Runx1 expression at around E8.5. (Scale bar: 100 μm.) (*B*) E9.5–E10.0 versus E10.5–E11.0 whole-mount LacZ embryo staining. Runx1-null embryos have some Runx1^{+} cells in peripheral blood; these cells disappear by E11.0–E11.5. (Scale bar: 500 μm.)

Table S1. B-cell potential of early conceptus cells in OP9 stroma coculture

Stage	Somites	Tested tissues	Method	CD19^{+}CD11b^{-} B cells
E7.5	Presomite stage	YS	One-step culture	19(4^{high})/23
		Caudal half		0/23
		YS	Two-step culture	4(4^{high})/17
		Caudal half		13(6^{high})/17
E8.0–E8.25	Early somite stage	YS	One-step culture	6(0^{high})/9
		Caudal half		0/9
		YS	Two-step culture	5(3^{high})/11
		Caudal half		9(5^{high})/11
E8.25–E8.75	6–13	YS	One-step culture	1(0^{high})/15
		Caudal half		2(2^{high})/15
		YS	Two-step culture	3(2^{high})/9
		Caudal half		1(1^{high})/9
E9.5	20–25	YS	One-step culture	7(1^{high})/8
		Caudal half		0/8
		YS	Two-step culture	3(3^{high})/5
		Caudal half		0/5

For the flow cytometry analysis the gating was set for GFP+ cells to avoid maternal blood contamination. Two used methods of culture are explained schematically in Fig. S1C. Cultures containing more that 0.1% of CD19^{+}CD11b^{-} cells were regarded as B-cell–positive. If the CD19^{+}CD11b^{-} fraction exceeded 10%, the cultures were designated as highly positive and labeled correspondingly. YS, visceral yolk sac.