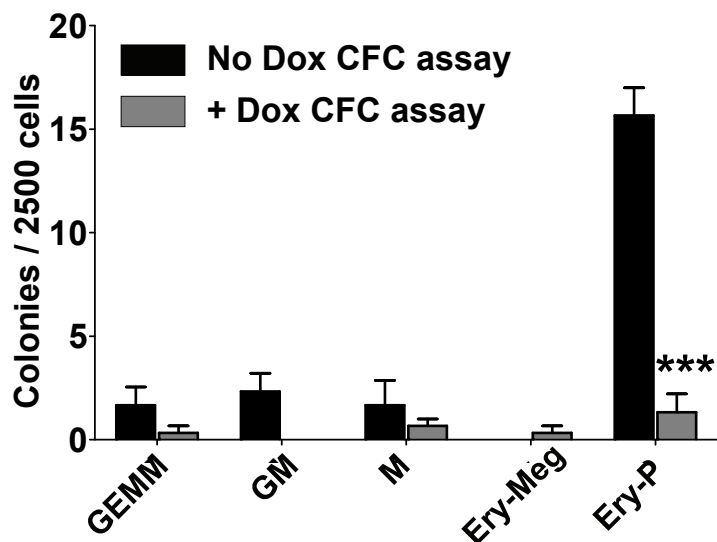


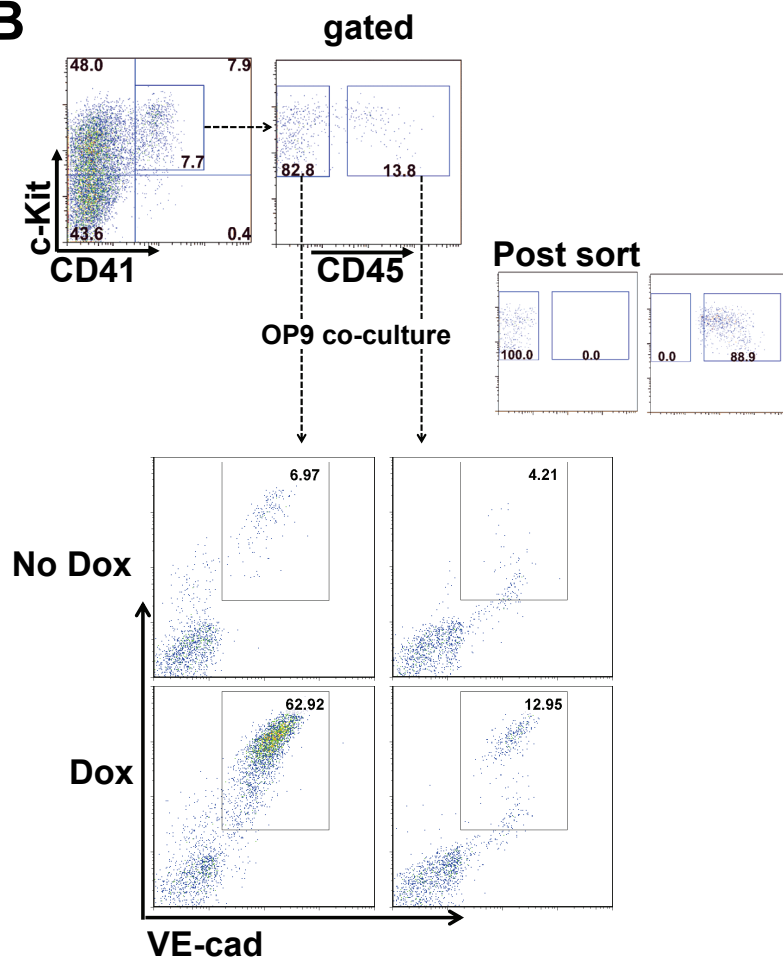
Supplementary Figure 1. *In situ* hybridizations of whole mounts and cross sections of mouse embryos. HoxA3 (A C E G I) and Runx1 (B D F H J). (A,B) At E7.25 HoxA3 is expressed only in the embryo proper, while Runx1 is expressed in the yolk sac. (C-F) Between E8.25 and E8.5 HoxA3 is expressed in the neural tube, the mesenchyme, and the endothelium of dorsal aorta, while Runx1 is only expressed in endothelial cells of omphalomesenteric artery (not shown). Scale bar in blowups is 5 μ m. (G, I) At E9.5 and E10.5 HoxA3 expression is diminished and lost in the endothelium of the AGM. (H, J) Runx1, in contrast, now appears in the posterior dorsal aorta. (a, aorta; nt, neural tube; ys yolk sac). Scale bar indicates 100 μ m except blowups in C,D (5 μ m), and left panels of E,F (1 mm).

Supplementary Figure 2

A



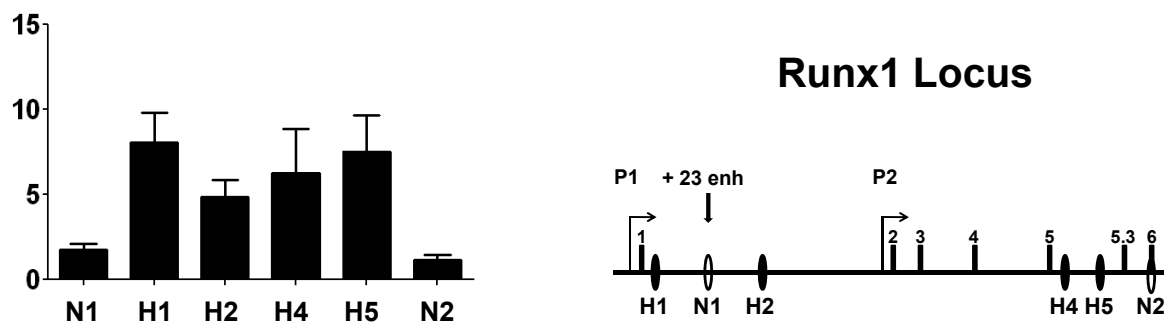
B



Supplementary Figure 2. (A) 2500 c-Kit⁺/CD41⁺ cells (in triplicates) from undifferentiated EBs, were plated in methylcellulose colony assays either supplemented or not supplemented with 1 mg/mL of doxycycline. Colonies: GEMM (granulocyte/erythrocyte/macrophage/megakaryocyte), GM (granulocyte/macrophage), M (macrophage), Ery-P (primitive erythrocyte) $p=0.0004$, Ery-Meg (erythrocyte/megakaryocyte). $n=3$ Black bar: no dox treatment, gray bar: dox treatment. (B) Kit⁺/CD41⁺ HoxA3 (top left) undifferentiated cells from day 6 EBs were subfractionated according to their expression of CD45 and cultured for 5 days on OP9. Both CD45⁺ and CD45⁻ fractions were able to generate Flk1⁺/VE-cadherin⁺ cells upon dox induction.

Supplementary Figure 3

A



B

H2 conservation

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Xt2  CTTCTCTAATAACTCTCTTCTTCTTCCGGCCCCAATTATCAGATTTTGTGTATGACTTTA
Gg3  CTTTATTAGTAAGGTTCTTCTCCTGCCTGACCCAATTATCAGATTTTGCATTTAAGTTTA
Hs18 CTTTATTGCTCAGTTTCTTCT---GCCTGACCCAATTATCAGATTTTGCATTTAAGTTTA
Mm9  CTTTGCTGTGCAGTTTCTTCTCCTGCCTGACCCAATTATCAGATTCTGCATTTAAGTTTG
    *** * * ***** ** * ***** ** * * * *
Xt2  AGACAGCAGATCAAACAGCGAGCGCCACTTTGTACTCGTGCCAGCGGAACATATGATATC
Gg3  AGACAGTAGATCAAACAACAAGAGCCACTTTGTACTTTTGCCAGGAGACCATATGATATC
Hs18 AGACAGTAGATCAAACAGCGAGAGGCACTTTGTACTTCTGCCAGGAGACCATATGATATC
Mm9  AGACAGAAGATCAAACAGCAAGAGGCACTTTGTACTTCTGCCAGGAGACCATATGATATC
    ***** ***** * ** * ***** ***** ** *****
Xt2  TCTCAGCAAGGCCGCTGCAAGCCAAGGA
Gg3  TCTCAGTGA CTCTCCTGCATGCCAGGAA
Hs18 TCTCAGTGA CTCTCCTGCATGCCAGGAA
Mm9  TCTCAGCGACTCTCCTGCATGCCAGGAA
    ***** * * ***** ***** * *
  
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Supplementary Figure 3. (A) HoxA3 chromatin immunoprecipitation in EBs induced to express HoxA3 from day 4-6. At right is the Runx1 locus indicating sites of enriched (H1, H2 and H5) and non-enriched (N1 and N2) DNA sequences. (B) Alignment of Runx1 genomic sequence containing the conserved H2 site from *Xenopus laevis* (Xt2), *Gallus gallus* (Gg3), *Homo sapiens* (Hs18), and *Mus musculus* (Mm9), identifies a conserved region with a putative HoxA3 binding site (indicated in red).