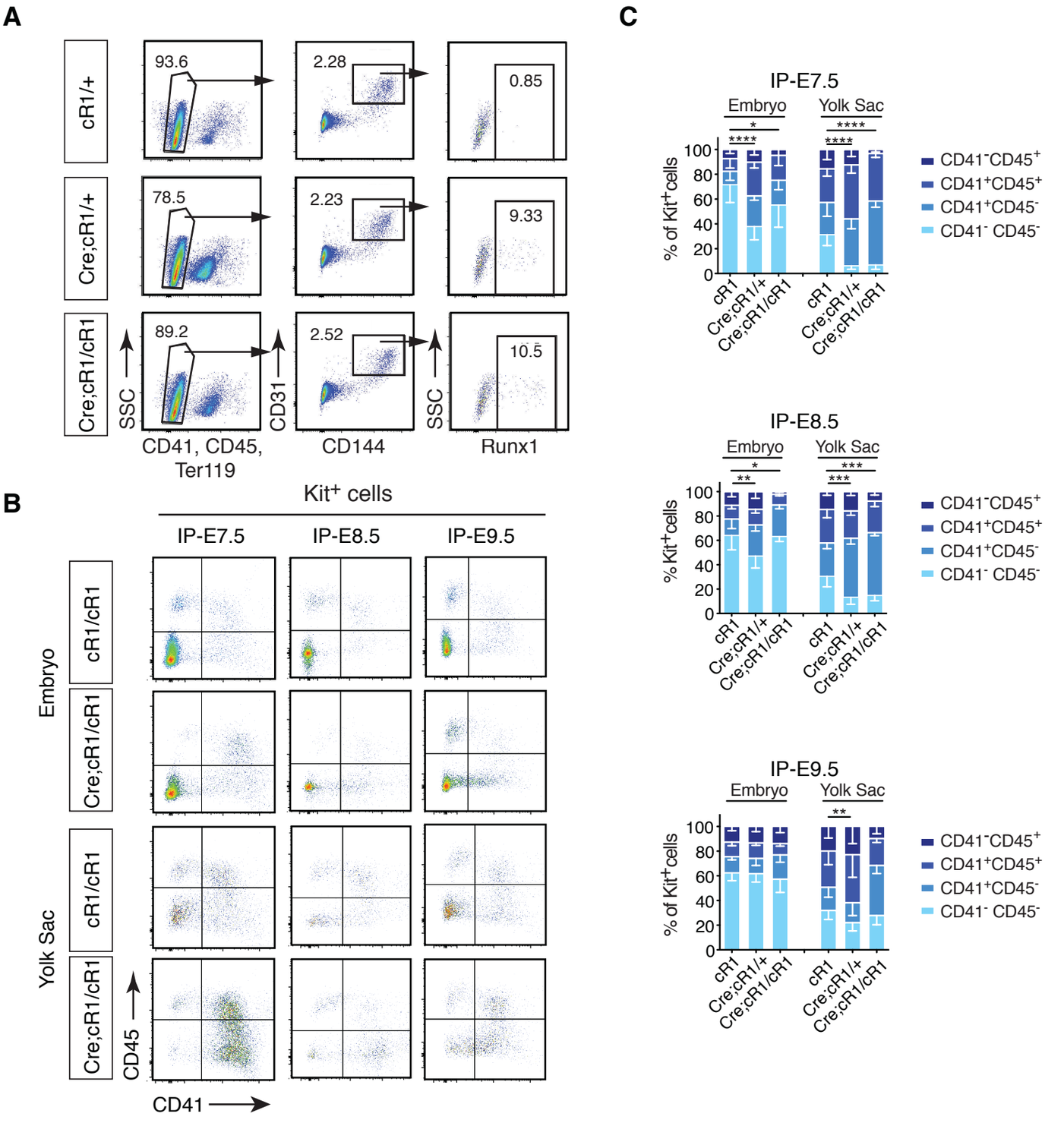
Supplementary Figure 1



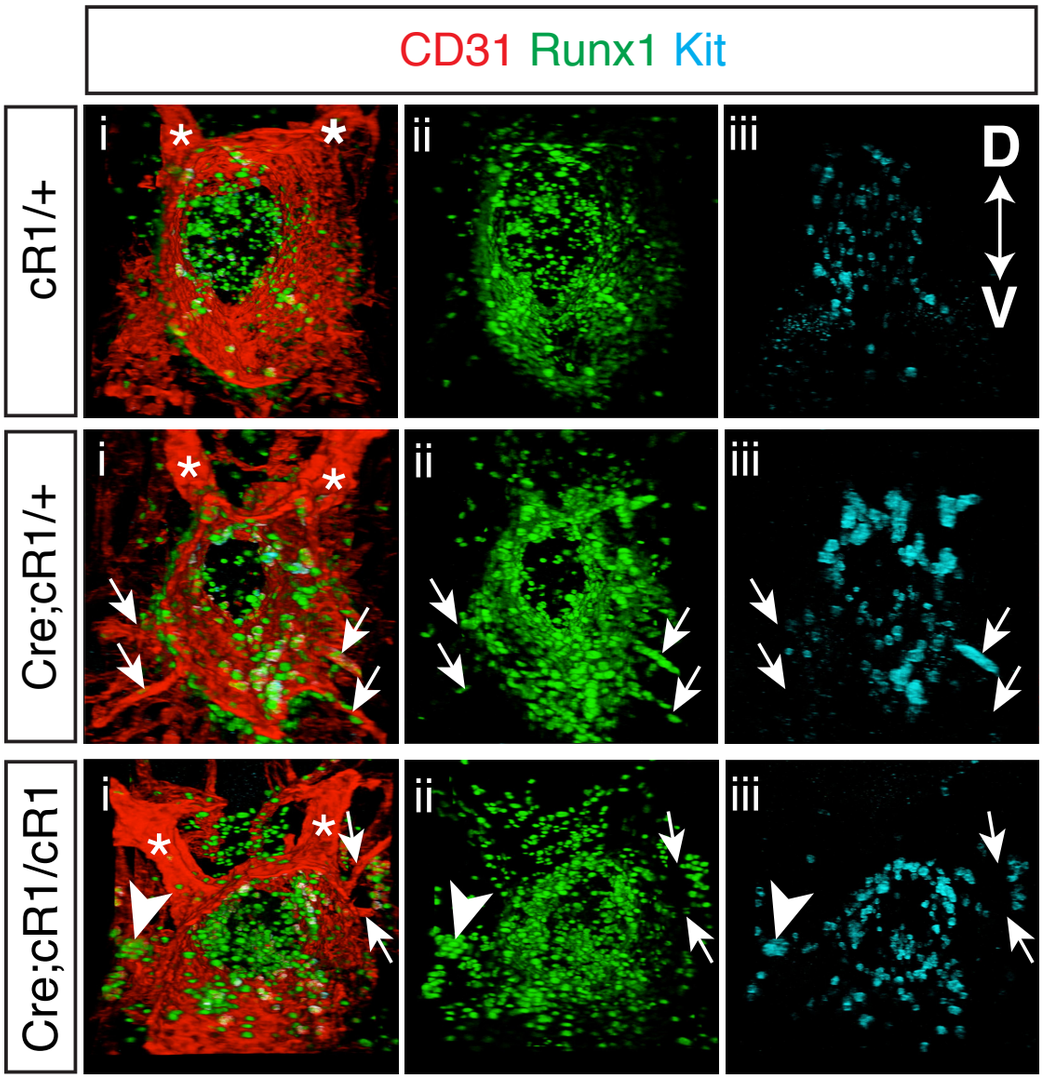
Supplementary Figure 1. Flow cytometric analysis of Runx1, CD41, and CD45 expression following induction of Runx1 in endothelial cells.

A) Representative scatter plots of gating strategy for detecting intra-cellular Runx1 expression in endothelial cells. Lineage (CD41, CD45, Ter119) negative cells were gated based on CD31 and CD144 (vascular endothelial cadherin) expression, and the percentage of $Runx1^+$ cells analyzed in the $CD31^+CD144^+$ population.

B) Representative scatter plots of CD41 and CD45 expression in Kit^+ cells from the E10.5 embryo or yolk sac after the initiation of ectopic Runx1 expression at E7.5, E8.5 or E9.5. Populations are gated through single, live, Kit, and the percentages of CD41 and CD45 cells in the Kit+ population analyzed.

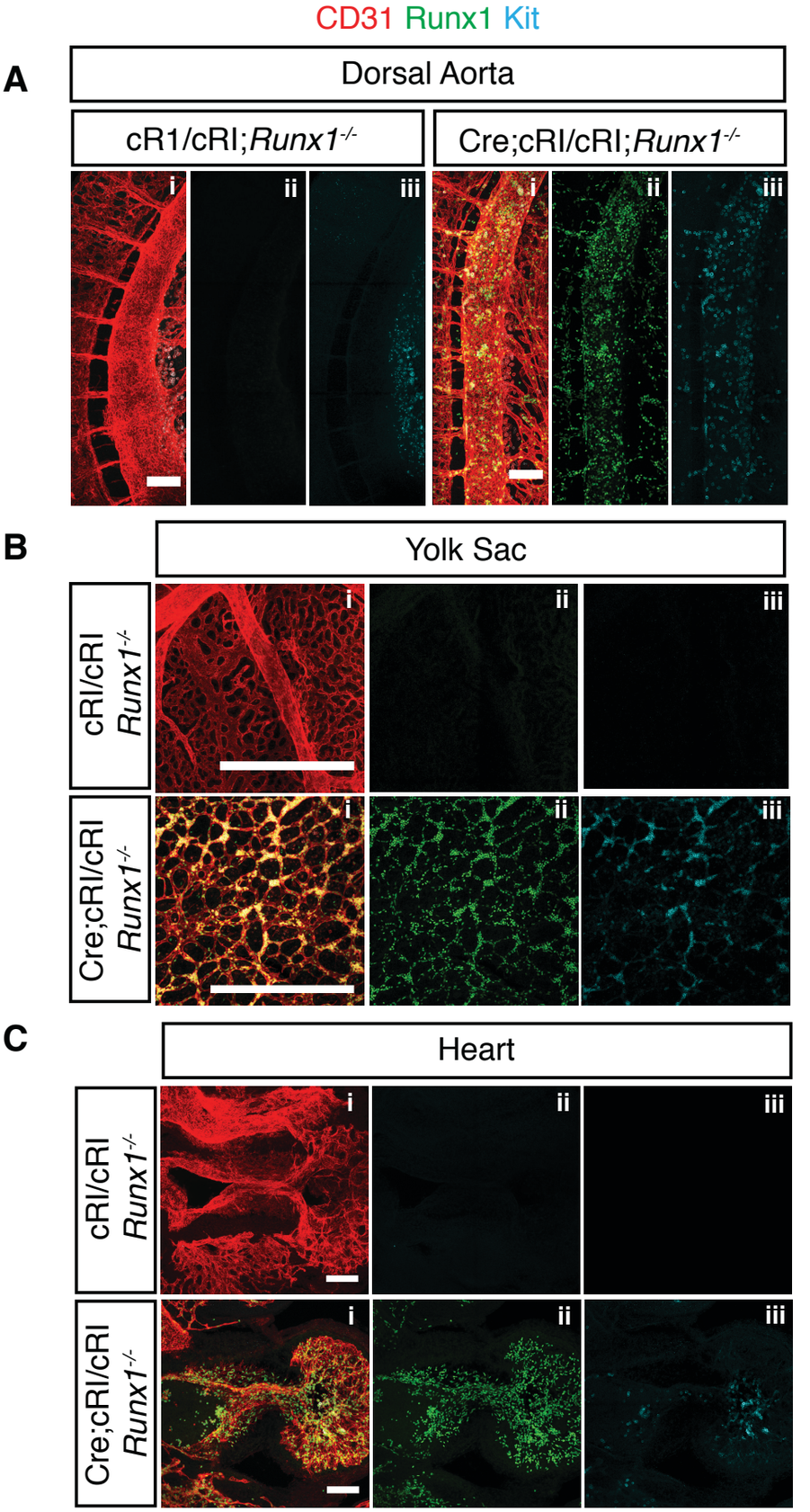
C) Stacked bar graphs representing the percent of Kit^+ cells that are $CD41^-CD45^-$, $CD41^+CD45^-$, $CD41^+CD45^+$ or $CD41^-CD45^+$. Differences in percentages of cells that were $CD41^+$ and/or $CD45^+$ were analyzed using one-way ANOVA and Tukey's test. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; **** $P \leq 0.0001$.

Supplementary Figure 2



Supplementary Figure 2. Ectopic angiogenic sprouts from the dorsal aorta after the initiation of ectopic Runx1 expression at E7.5. Transverse confocal Z-projections of the dorsal aortas of E10.5 embryos. Samples were immunostained for CD31 (i), Runx1 (i,ii) and Kit (i,iii). Asterisks indicate inter-somitic vessels, arrows indicate ectopic angiogenic sprouts, and arrowhead points to an ectopic extravascular blood island.

Supplementary Figure 3



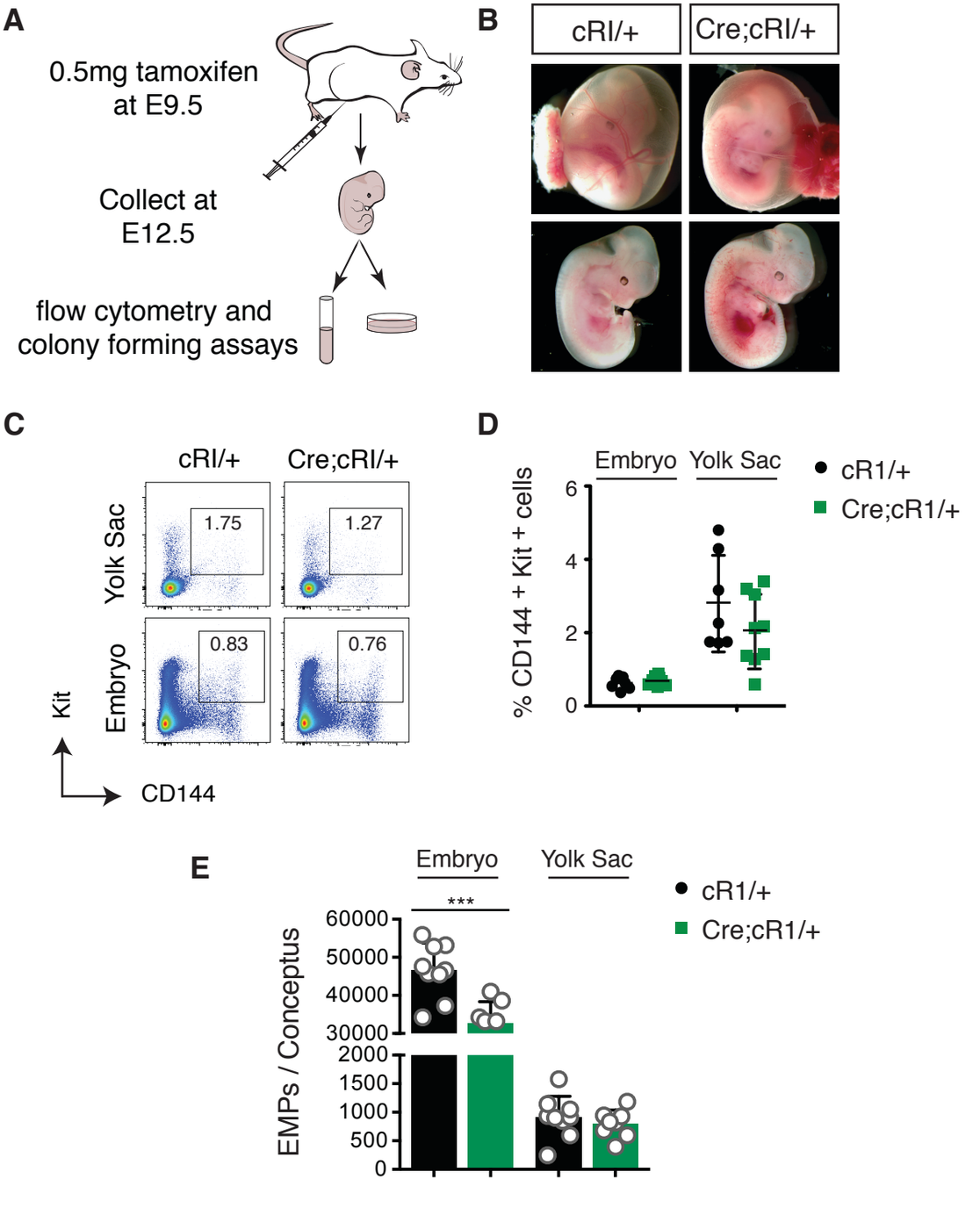
Supplementary Figure 3. Endogenous Runx1 expression is not required for hematopoietic cluster formation in ectopic sites.

A) Confocal Z-projections of dorsal aortas with z-intervals of 2 μm from E10.5 cR1/cR1;*Runx1*$^{-/-}$ and Cre;cR1/cR1;*Runx1*$^{-/-}$ embryos in which Runx1 expression was induced at E7.5. The dorsal side of the embryo is on the left. Scale bar = 100μm. Samples were immunostained for CD31(i), Runx1 (i,ii) and Kit (i,iii). Runx1^{-} Kit^{+} cells ventral to the dorsal aorta (e.g. in iii) are primordial germ cells.

B) Confocal Z-projection of E10.5 yolk sacs. Scale bar = 500μm.

C) Confocal Z-projection of E10.5 hearts. Scale bar = 100μm.

Supplementary Figure 4



Supplementary Figure 4. Prolonging ectopic endothelial-specific expression of Runx1 from E9.5 to E12.5 *in vivo* does not increase HSPCs.

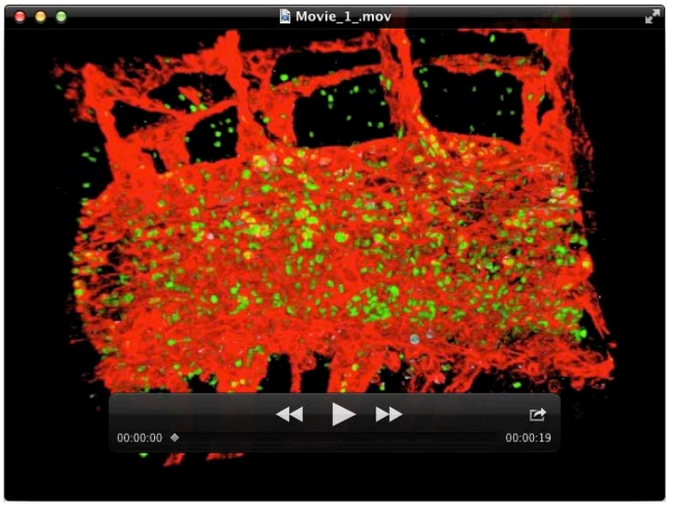
A) Scheme of experiment for initiation of ectopic Runx1 expression *in utero* at E9.5 and analysis at E12.5.

B) Gross images of E12.5 conceptuses.

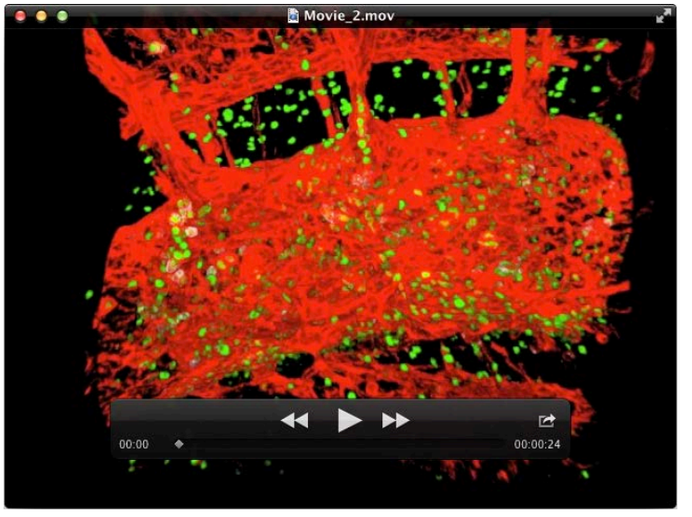
C) Representative scatter plots of $CD144^+$ Kit^+ cells in the yolk sac and embryo.

D) Percentage of $CD144^+$ Kit^+ cells in the embryo and yolk. Data are from two independent experiments and two litters (mean ± SD). Unpaired 2-tailed Student's t-test, $P \leq 0.44$ for embryos and $P \leq 0.21$ for yolk sacs.

E) Total number of EMPs in the embryo and yolk sac (mean ± SD). Data are from three independent experiments. Unpaired 2-tailed Student's t-test, *** $P \leq 0.001$.



Movie 1. 3-dimensional reconstruction of the dorsal aorta of an E10.5 Cre:cR1;cR1 embryo after initiation of ectopic Runx1 expression at E7.5. Sample is immunostained for CD31 (red), Runx1 (green) and Kit (cyan). Arrow points to an ectopic angiogenic sprout associated with hematopoietic cells.



Movie 2. 3-dimensional reconstruction of the dorsal aorta of an E10.5 Cre:cR1;cR1 embryo after initiation of ectopic Runx1 expression at E7.5. Sample is immunostained for CD31 (red), Runx1 (green) and Kit (cyan). Arrow points to an ectopic extravascular island near the dorsal aorta.