## Supplemental Materials and Methods

### β-galactosidase staining

The Tie2Cre strain was verified using the GT(ROSA)26Sor$^{tm1Sor}$ Cre reporter strain (JAX 003309). Embryos and yolk sacs were dissected between embryonic day (E) 8.5 and E9.5, fixed in 0.2% glutaraldehyde/PBS, washed in detergent rinse (phosphate buffer, 2 mM $MgCl_2$, 0.01% sodium deoxycholate, 0.02% NP40), and stained overnight at 37°C (0.1M phosphate buffer, 5mM potassium ferrocyanide, 5mM potassium ferricyanide, 0.02% NP-40, 2mM $MgCl_2$, 0.01% sodium deoxycholate and 1mg/ml X-gal).

### Whole mount and histological analyses

Tissues were fixed in 4% paraformaldehyde, paraffin embedded, and sectioned at 5-6μm. Sections were either stained with hematoxylin and eosin (H&E) or immunostained with anti-myc (9E10, Developmental Studies Hybridoma Bank, Univ. Iowa) and PECAM-1. For whole mount immunostaining, samples were fixed in 4% paraformaldehyde overnight at 4°C, dehydrated, and bleached in 3% $H_2O_2$ in methanol. Samples were rehydrated and placed in PBSMT solution (3% nonfat milk/0.1% Triton X-100/PBS). Specimens were incubated with anti-mouse PECAM (1:100; rat MEC 13.3. BD Pharmingen) or anti-SMA (1.200; mouse clone 1A4, Sigma) in PBSMT overnight at 4°C. For whole mount PECAM staining, biotinylated secondary antibodies (1:400, Sigma) and the avidin/streptavidin-based detection system (Vector Laboratories) were used, with 3,30 diaminobenzidinetetrahydrochloride (DAB) as the substrate. For whole mount SMA and Myc immunohistochemistry. Alex-flour-488 conjugated goat anti-mouse secondary antibody (1:400, Molecular Probes) was used.

**Cell culture**

Three dimensional tubulogenesis assays were performed using human umbilical vein endothelial cells (HUVEC) by characterized methods [12] For analysis of hematopoiesis, tissues were treated with 0.25% collagenase/IMDM/20% plasma-derived serum (PDS) at 37ºC for 1h with trituration. Cells were plated in triplicate at 0.2-2x$10^5$ cells/ml in 60% methylcellulose supplemented with 10% PDS (Antech, Tyler TX), 5% protein-free hybridoma medium (Gibco/BRL, Grand Island, NY), GM-CSF (2.5ng/ml), SCF (60ng/ml), IL-6 (20ng/ml), IL-3 (20ng/ml), glutamine (2mM) at 37°C. BFU-E, CFU-E, macrophage, mast cell, and granulocyte/macrophage colonies were counted after 7-10 days.

**Gene expression analysis by RT-PCR.**

At 9.5 whole embryos, yolk sac and a dissected pooled heart were collected and RNA was isolated using Tri Reagent (sigma). cDNA synthesis and PCR was carried out as described previously (12). For expression analysis of P27, P15, P53, VEGFR1-R3, Ang1-2, TBX2, TBX5, BMP2, BMP10, Gata1, Gata6, PEG1, FLK1, cKit, SCL, Lmo2, RNX-1, globins and cyclophilin. Specific primers were used to amplify fragments using equal amounts of cDNA.

| Gene | Primer 1 | Primer 2 |
|---|---|---|
| TBX5 | ATGGAGGGAGAGCAGAGACA | AGACTGAGCCCGACATCCTA |
| TBX2 | GGCTTCAGCGGTCAGAATAG | GCATCCTCCACACCAGTTTC |
| BMP2 | AAGTCAGTGGGAGAGCTTCG | GCTGTTTGTGTTTGGCTTGA |
| BMP10 | ACCAAGCTGAGGACACCGGAAGG | CTTCGTGGGCACACAGCAGGCTTT |
| PEG-1 | CCAAAAGCTCCTCAAAGACG | AATGGGGATGGACACAGAAG |
| GATA6 | ATGGCGTAGAAATGCTGAGG | TGAGGTGGTCGCTTGTGTAG |
| cKit | CATCCATCCAGCACAATCAG | AAGGCCAACCAGGAAAAGTT |
| SCL | GGAGGTGGACAAGAGTGGAA | CGGCTGTTCAAATGGATTCT |
| LMO2 | GGTGGGTGAGGCATTTCTTA | CCATGAACACCAAGGTCACA |
| Runx | AAACAAAACTGACCCGCAAG | TCAAAGTCAAATGCCCAACA |
| β-globin | GAAGGCAGCTATCACAAGCA | GGTCTCCTTGAGGTTGTCCA |

| | | |
|---|---|---|
| Major | TTGGACCCAGCGGTACTTT | ACGATCATATTGCCCAGGAG |
| p27 | CGCTTTTGTTCGGTTTTGTT | TCCAGGGGCTTATGATTCTG |

All other primer sequences were taken from Thijssen et al. 2004, Exp Cell Research 299: 286-293.

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For flow cytometry, collagenase-disrupted tissues were fixed in 70% EtOH and washed, resuspended in 0.2ml 0.1% BSA/PBS, incubated at 4$^o$C with rat IgG (1μg) (Jackson Immunoresearch Westgrove, PA), and stained with PE-Ter119 (1μg) and FITC-CD71 (1μg) APC-conjugated cKit. (BD Biosciences San Jose, CA). Cells were analyzed in a FACS Calibur (BD Bioscience).