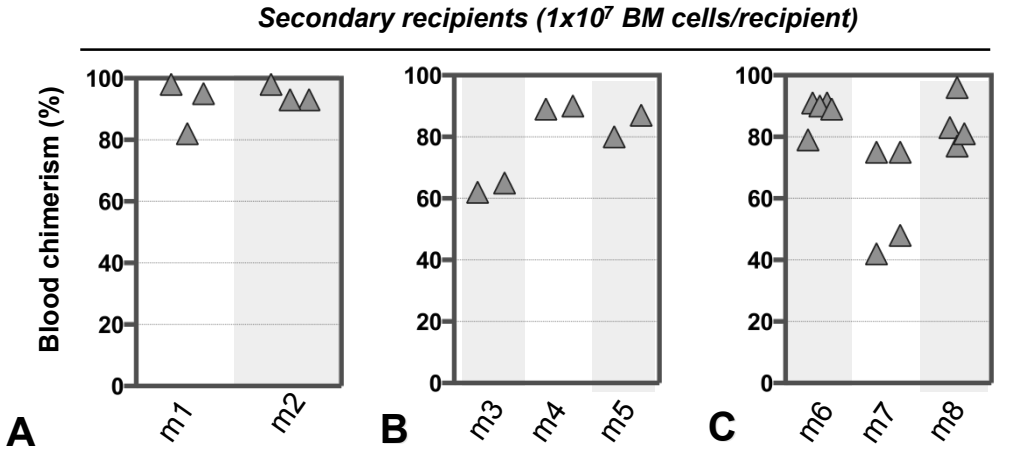

Figure S1. Donor chimerism in peripheral blood of secondary recipients.
Primary recipients which received transplants of fresh and cultured extra-embryonic vessels were analysed by secondary transplantations ($1x10^7$ nucleated bone marrow cells were transplanted per secondary recipient). (A) Uncultured VE-cad$^+$CD45$^+$ cells isolated from UC+VV; (B) UC explants; (C) VV explants. Donor engraftment in peripheral blood of secondary recipients assessed after 12-14 weeks is shown.

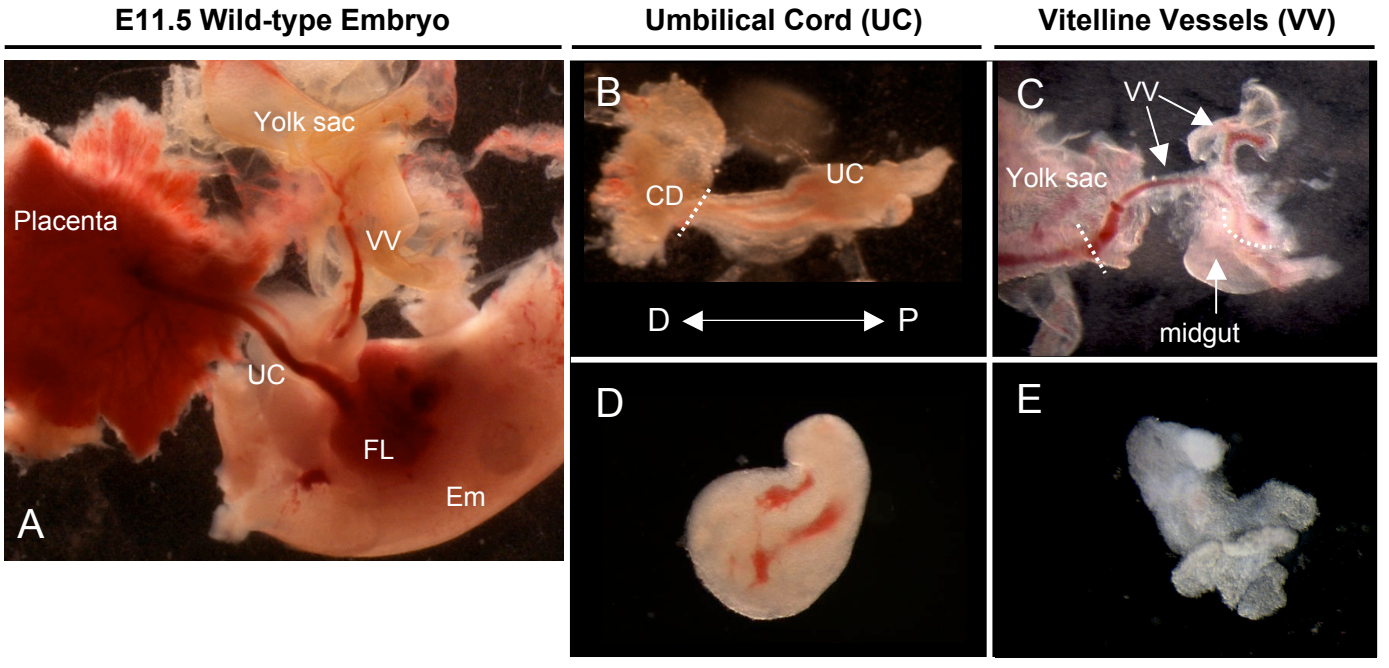

Figure S2. Dissection and culture of extra-embryonic vessels.
(A) E11.5 wild-type embryo: UC; VV; fetal liver (FL); embryo proper (Em); yolk sac and placenta are shown immediately prior to their isolation.
(B, C) UC and VV after dissection, respectively (CD, chorion disk; D, distal; P, proximal).
(D, E) UC and VV after 4 days culture with GF (IL3+SCF+Flt3L).
Original magnification: A, 8x; B,C 32x; D,E 40x. White dotted lines indicated points of dissection to isolate vessels.

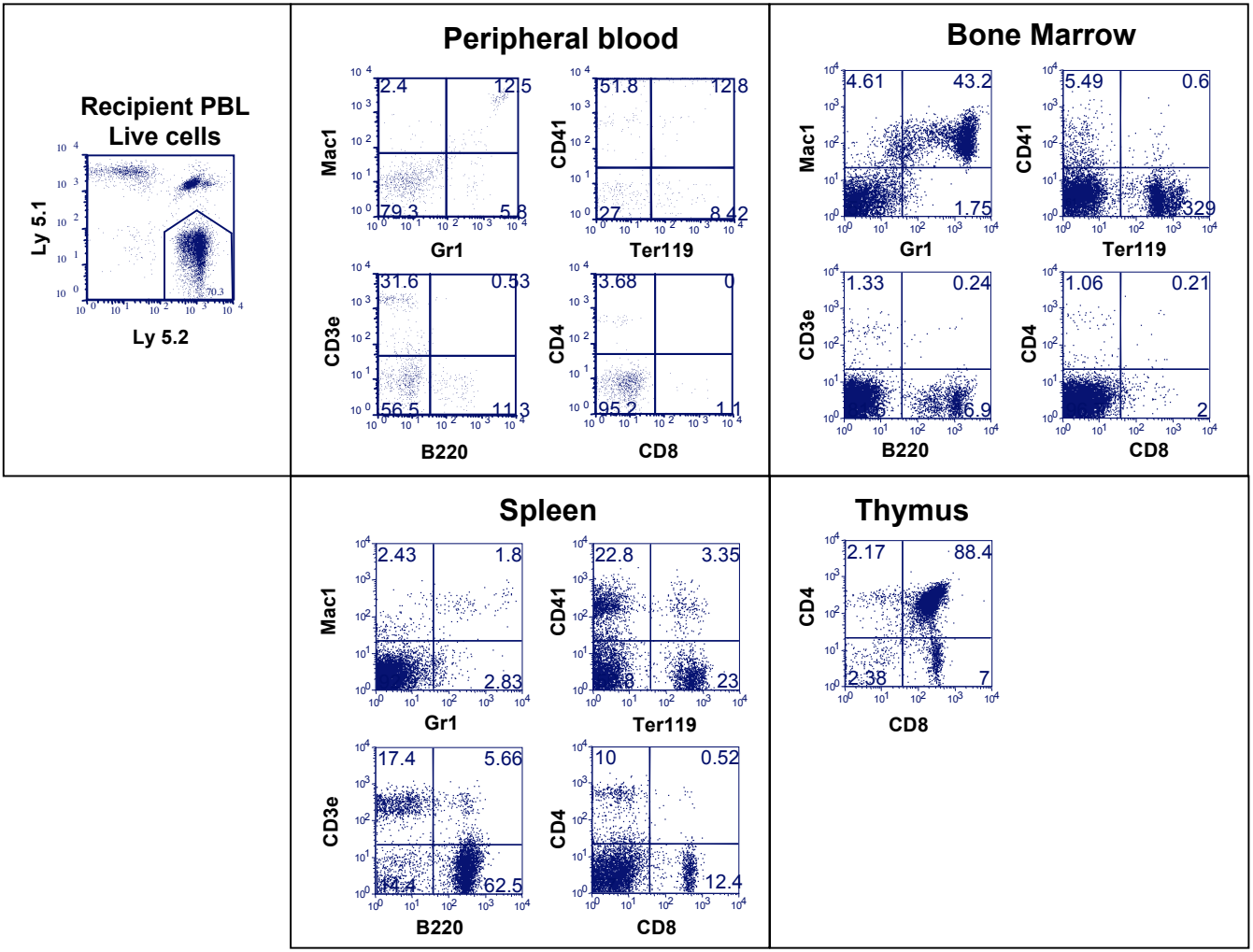

Figure S3. Multilineage analysis of engrafted UC derived HSCs.
Dots plots are representative of donor derived myeloid, erythroid and lymphoid cell populations in peripheral blood, bone marrow, spleen and thymus.

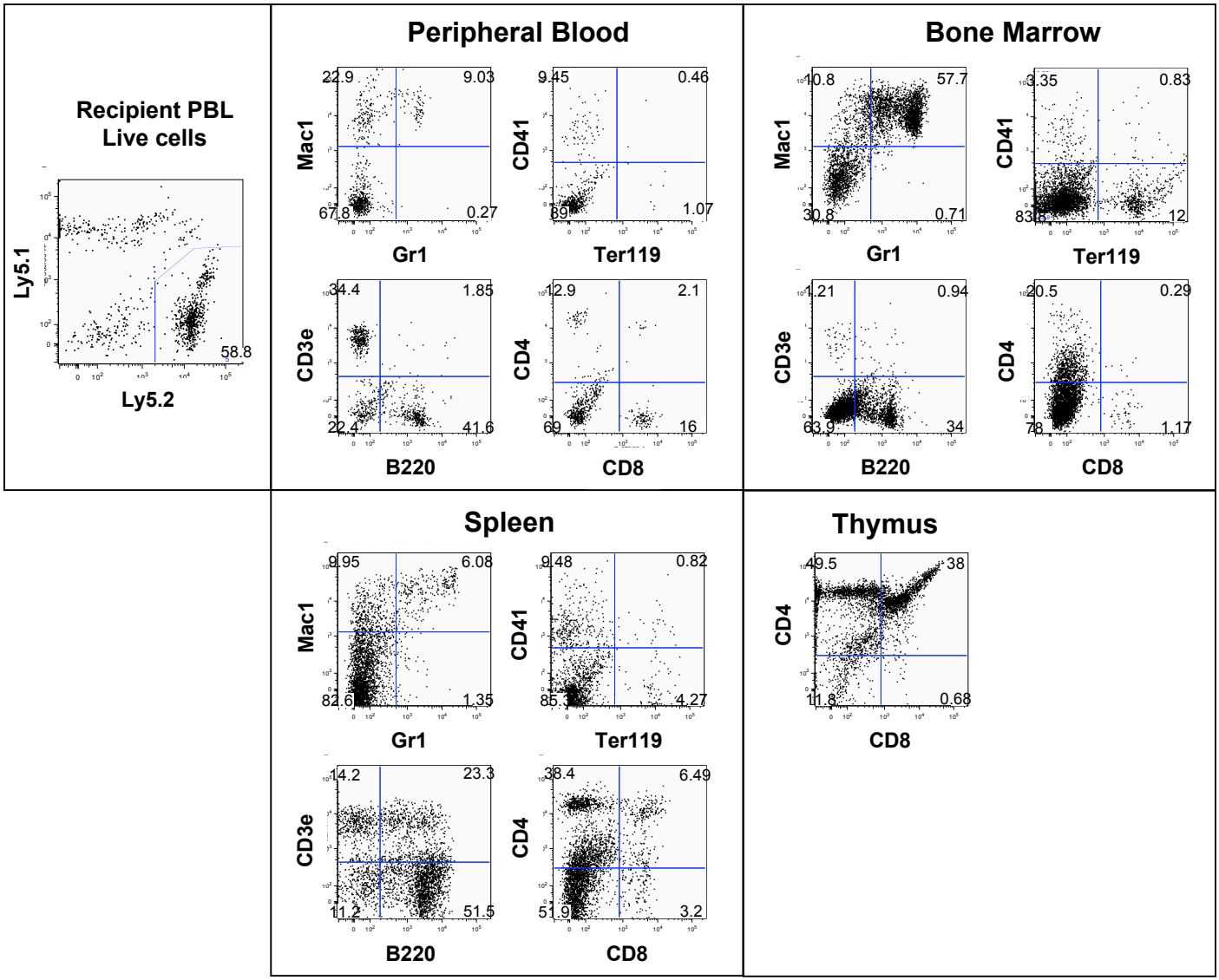

Figure S4. Multilineage analysis of engrafted VV derived HSCs.
Dots plots are representative of donor derived myeloid, erythroid and lymphoid cell populations in peripheral blood, bone marrow, spleen and thymus.

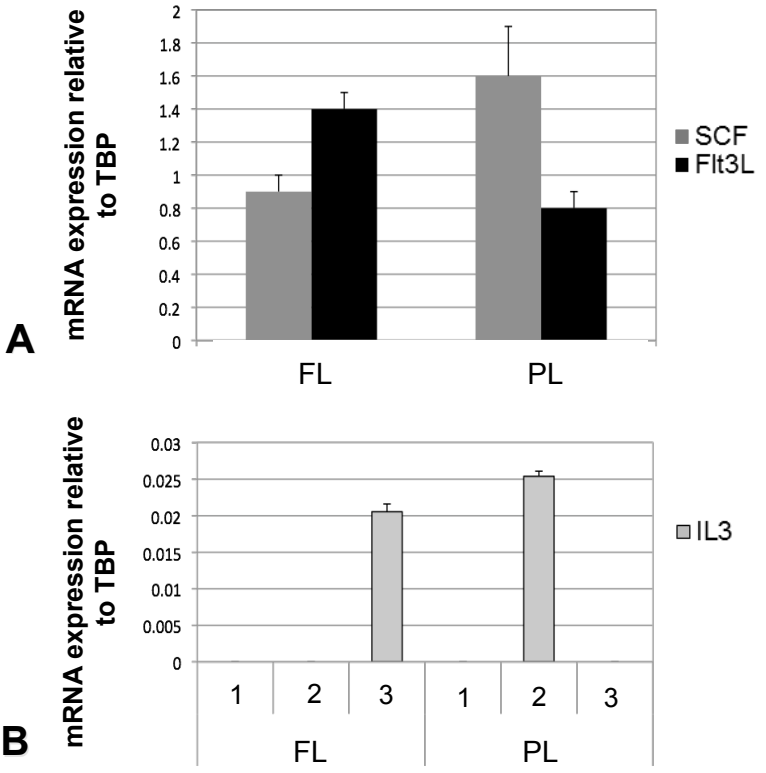

Figure S5. Expression of growth factors in freshly isolated E11.5 tissues.
(A) Expression of SCF and Flt3L mRNA in freshly dissected E11.5 foetal livers (FL) or placentas (PL), relative to TBP housekeeping gene. Standard deviations of 3 independent samples are shown.
(B) Expression of IL3 mRNA in several freshly dissected FL or PL, relative to TBP housekeeping gene. 3 independent samples were analysed seperately (labeled 1, 2, 3).
FL, foetal liver; PL, placenta.

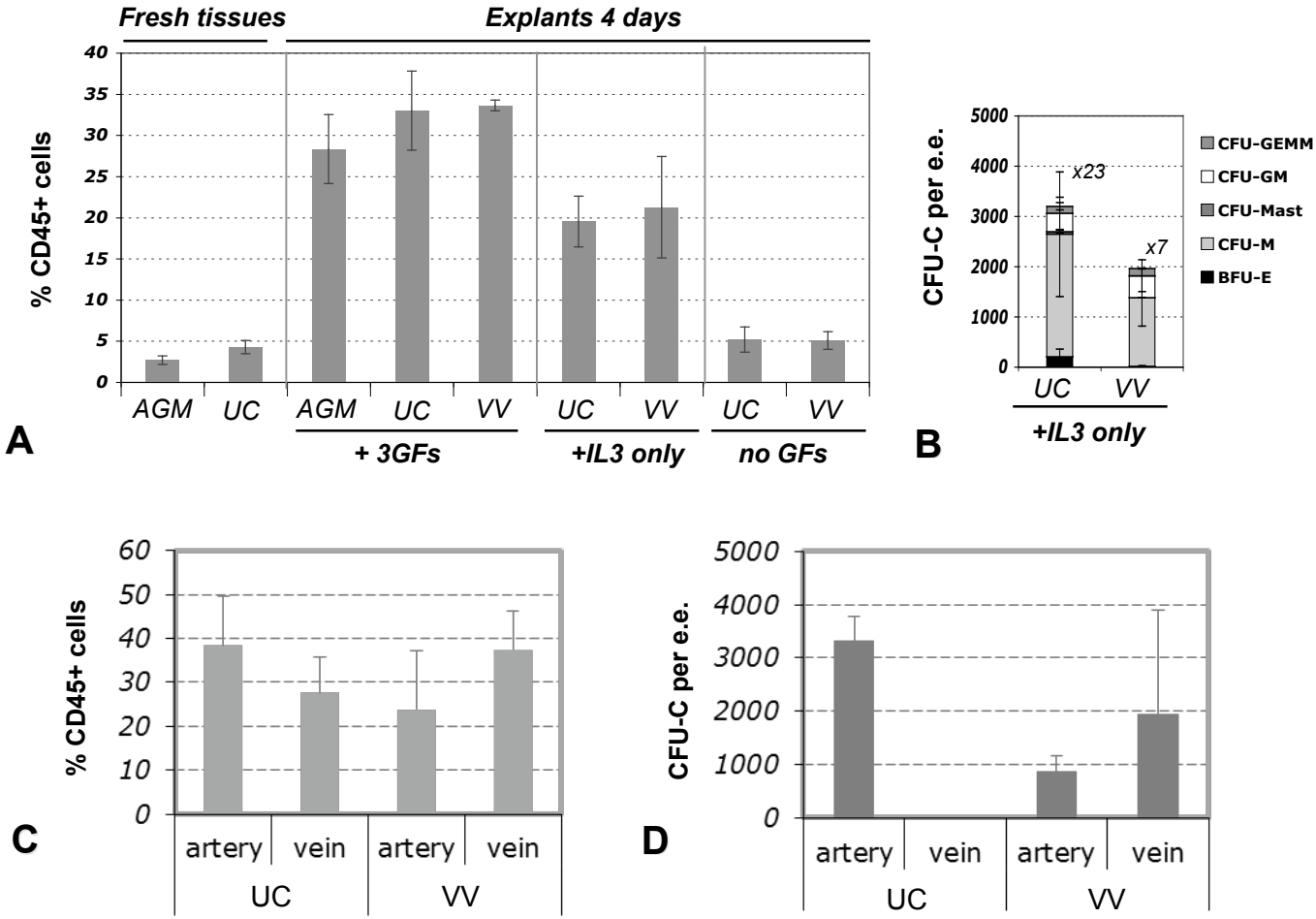

Figure S6. Expansion of CD45$^+$ cells and CFU-C progenitors in explants of extra-embryonic vessels.

(A) Percentage of CD45$^+$ cells in fresh tissues or explants cultured for 4 days in the presence of 3 growth factors (SCF, IL3, Flt3L 100ng/ml), with IL3 only (200ng/ml), or without growth factors. Standard errors are shown; (n=2-6 independent experiments).

(B) Number of CFU-C (per embryo equivalent) after 4 days explant culture with IL3 alone. Shown is the fold increase in CFU-C in explants relative to their fresh uncultured counterparts.

(C) Percentage of CD45$^+$ cells in explants of veins or arteries isolated from UC or VV, after 4 days culture with 3 growth factors (SCF, Flt3L, IL3 100ng/ml); (n=5-6 independent experiments).

(D) CFU-C per embryo equivalent in explants of veins or arteries isolated from UC or VV, after 4 days culture with 3 growth factors (SCF, Flt3L, IL3 100ng/ml); (n=2-3 independent experiments).

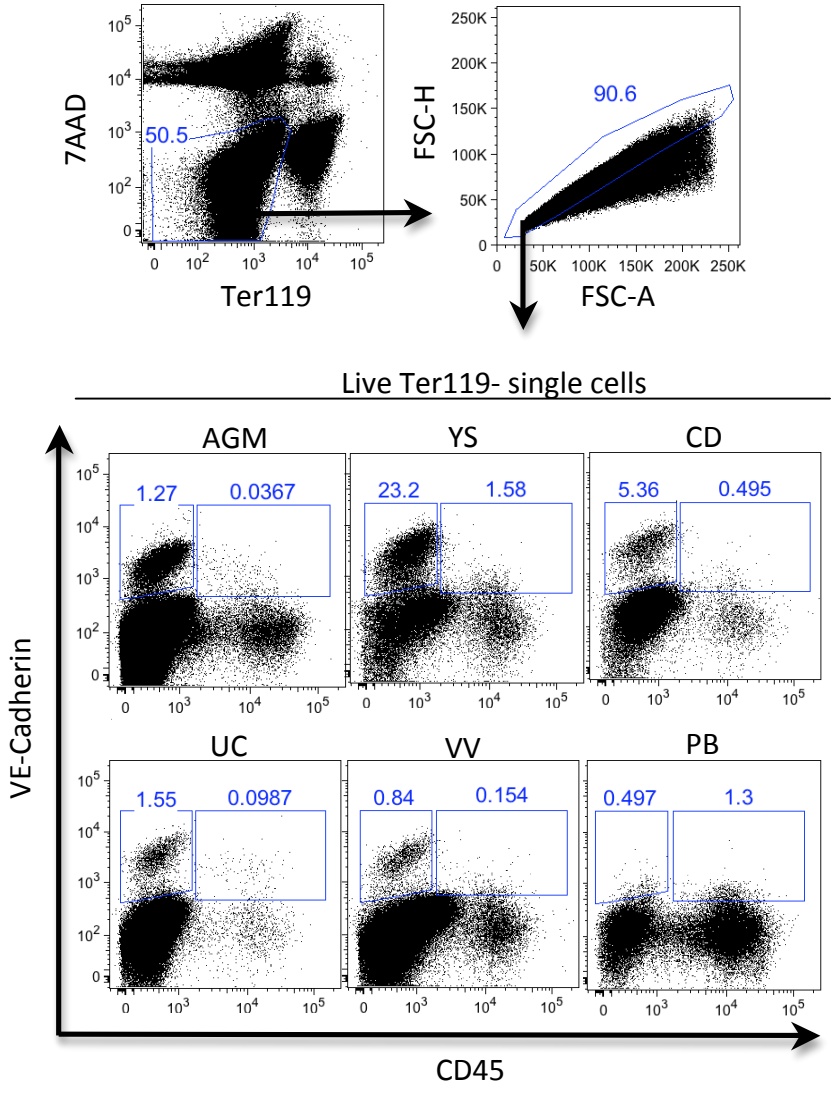

Figure S7. Sorting VE-cadherin^{+}CD45^{-} cells from E11.5 tissues.
E11.5 tissues were dissected, dissociated and stained before isolation of live Ter119^{-} VE-cadherin^{+}CD45^{-}cells by FACS. Shown are gating strategies for AGM, yolk sac (YS), chorion disk (CD), umbilical cord (UC), vitelline vessels (VV) and peripheral blood (PB).

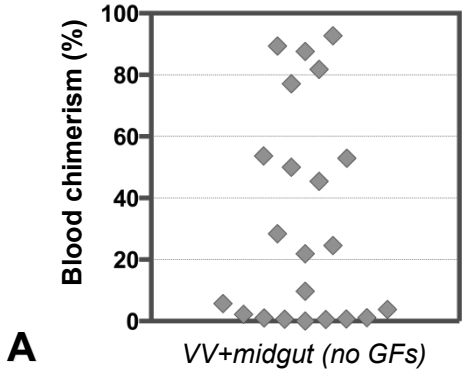

Figure S8. HSC activity in vitelline vessel explants is maintained in the absence of growth factors when cultured together with associated midgut.
E11.5 VV, including the associated midgut region, were cultured for 4 days in the absence of growth factors and transplanted into irradiated adult recipients (1ee/ mouse). The levels of donor chimerism in the peripheral blood was assessed 12-14 weeks post-transplantation; (n=4 independent experiments).