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Supplemental Information

Tracing the Origin of the HSC Hierarchy Reveals an SCF-Dependent, IL-3-Independent CD43^{-} Embryonic Precursor

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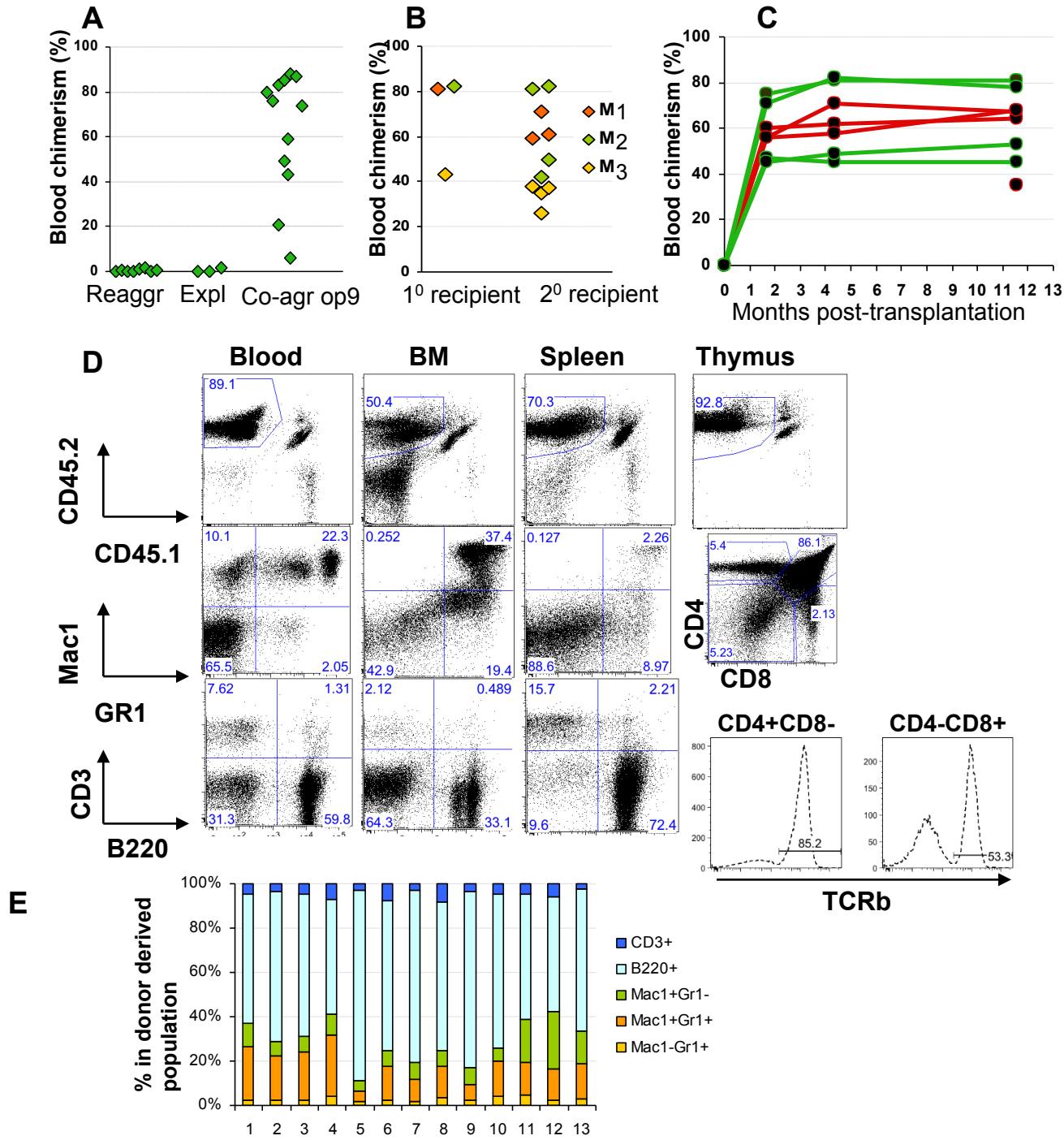


Suppl. Fig. 1 Long-term multi-lineage haematopoietic repopulation with definitive HSCs matured from pro-HSCs.

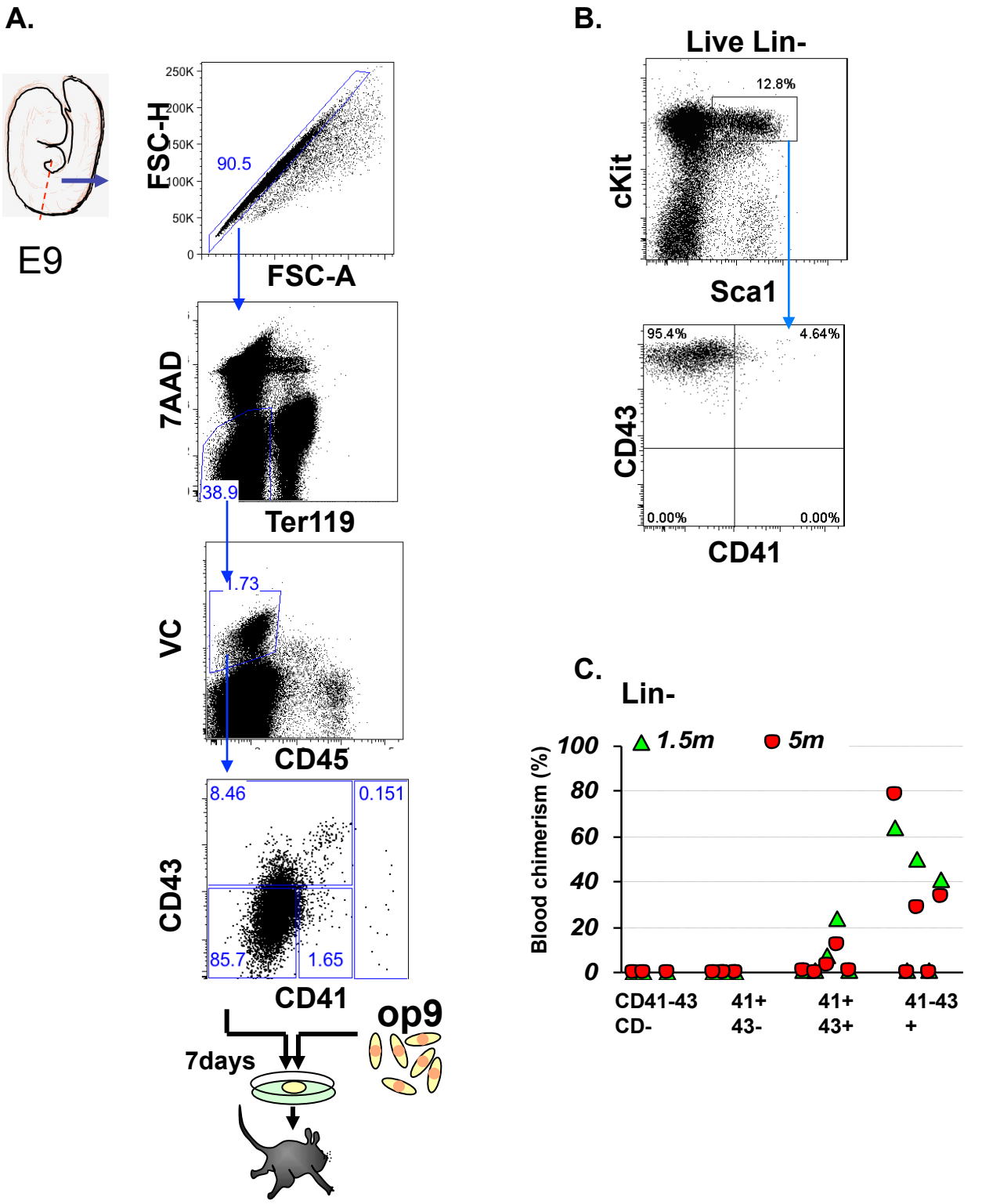
A. Maturation of pro-HSCs requires co-culture with OP9. Note that reaggregation of the caudal part on itself or explant cultures have not generated HSCs (All transplantations were performed with 1 e.e./recipient).

B. Donor-derived engraftment in secondary recipients after 6 months (each secondary recipient received 10^7 nucleated bone marrow cells from primary recipients);

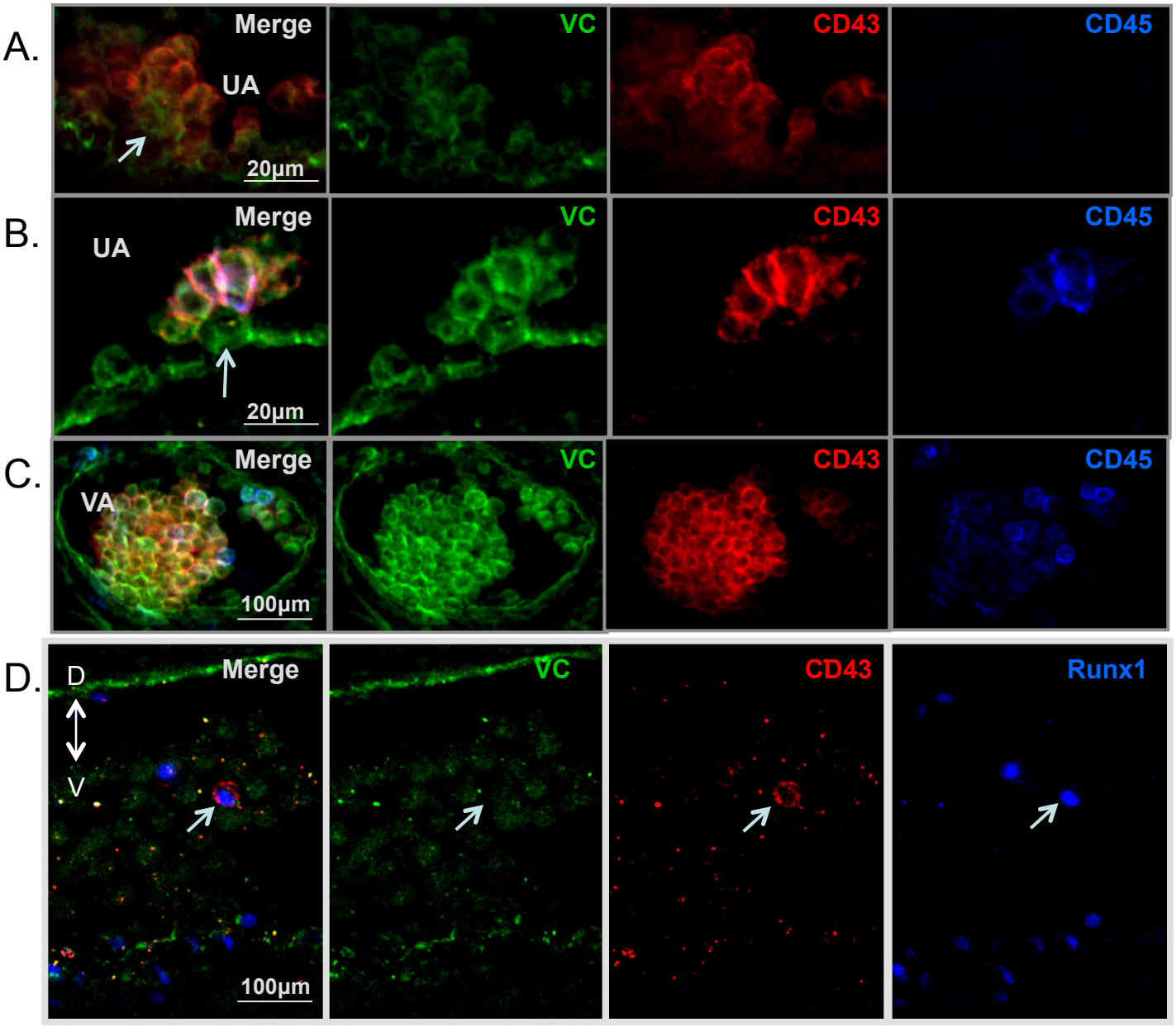
C. Stable donor-derived haematopoietic repopulation of secondary recipients observed over 12 months' period.

D. Multi-lineage long-term repopulation in blood, bone marrow, spleen and thymus of primary recipients (6 months post-transplantation). Donor-derived TCRβ+ cells are detectable in the thymus.

E. Donor derived myeloid and lymphoid contribution in blood of individual recipients (shown by individual bars). Each experiment was repeated at lease twice.



Suppl Fig. 2. Gating strategy for sorting pro-HSCs and phenoptype of adult HSC.
A. Gating strategy for isolation of pro-HSCs from E9.5 embryos followed by co-culture with OP9 cells and transplantation into adult irradiated recipients (cell duplets, erythroid cells and dead cells excluded).
B. High expression levels of CD43 is observed in the adult bone marrow LSK population.
C. Adult bone marrow HSCs reside mainly within single CD41-CD43+Lin- fraction. Some HSC with low level repopulation potential reside within the CD41+CD43+ fraction. Other cell fractions are devoid of HSC activity. Green triangles represent short-term repopulation; red circles represent long-term repopulation; m, months (two independent experiments).



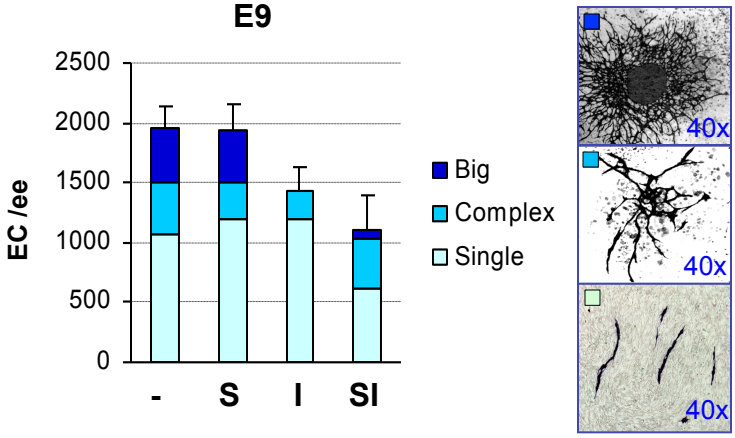
Suppl. Fig. 3. Confocal analysis of markers in cells clusters inside the the vitelline artery (VA) and umbilical cord (UC); (whole mount preparations).

A. VE-cad+CD43+CD45- haematopoietic cluster inside a E10.5 umbilical artery.

B. Small haematopoietic cluster in E10.5 umbilical artery suggests gradual upregulation of CD43 followed by CD45 expression. Note that all cells are VE-cad+, of which many more apically located are CD43+, of which some express CD45+ (follow images from left to right).

C. Large haematopoietic cluster adhered to the luminal surface of the E10.5 vitelline artery (transverse section, Z-stuck derived).

D. Solitary CD43+RUNX1+ haematopoietic cells not expressing VE-cadherin can be observed in circulation of the E9.5 dorsal aorta.



Suppl. Fig. 4. CFU- En (Colony-Forming Unit- Endothelial) assay.
While SCF (**S**) does not affect numbers and complexity of endothelial colonies, IL3 (**I**) inhibits formation and complexity of colonies (colony types are colour coded). (Three independent experiments).