Endoglin expression level discriminates long-term hematopoietic from short-term clonogenic progenitor cells in the aorta

Marion Roques,[1,2] Charles Durand,[1,2] Rodolphe Gautier,[1,2] Pierre-Yves Canto,[1,2] Laurence Petit-Cocault,[1,2,3] Laurent Yvernogeau,[1,2] Dominique Dunon,[1,2] Michèle Souyri[1,2,3] and Thierry Jaffredo[1,2]

[1]CNRS UMR7622, Laboratoire de Biologie du Développement, Paris; [2]UPMC UMR7622, Laboratoire de Biologie du Développement, Paris; and [3]INSERM U602, Hôpital Paul Brousse, Villejuif, France

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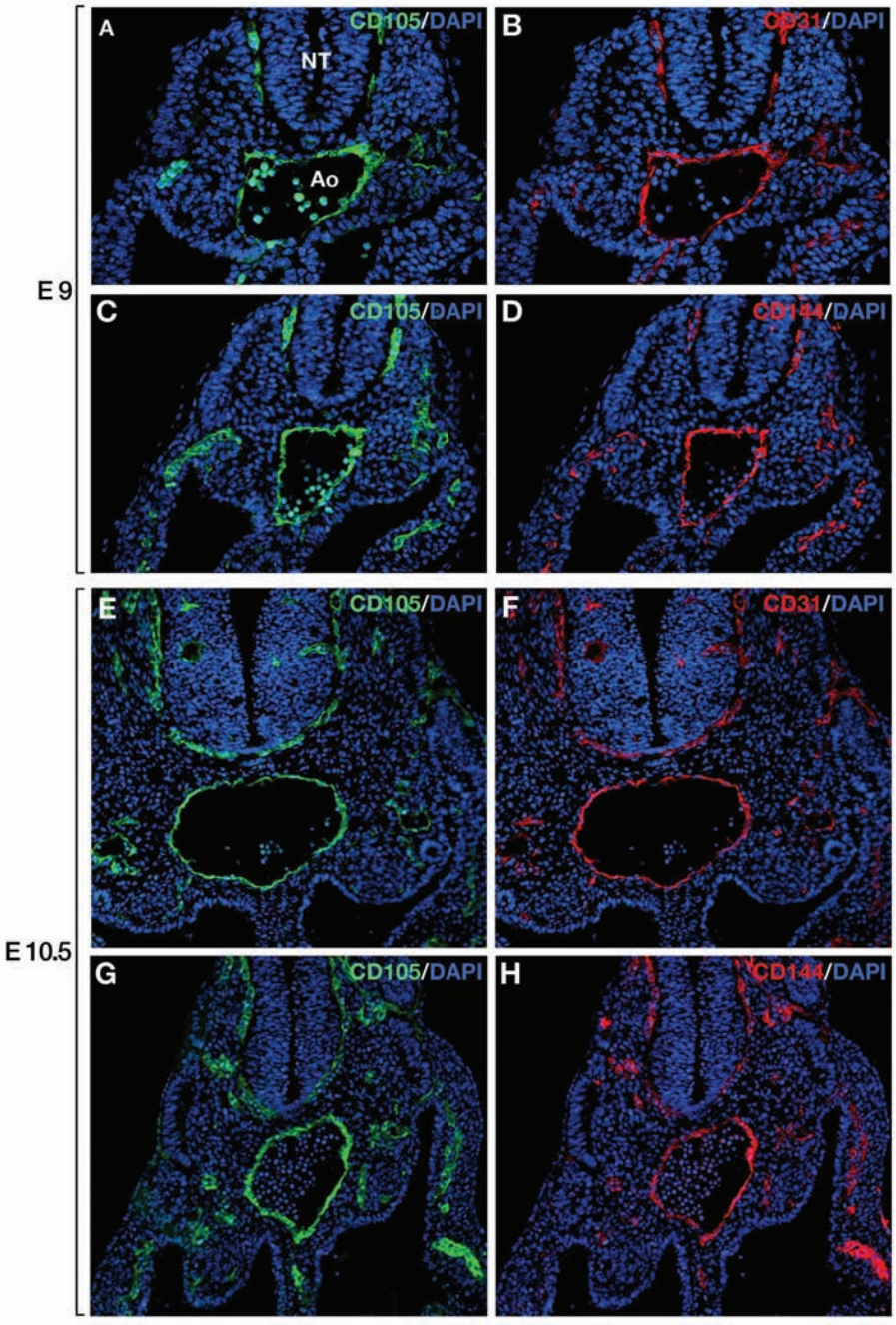
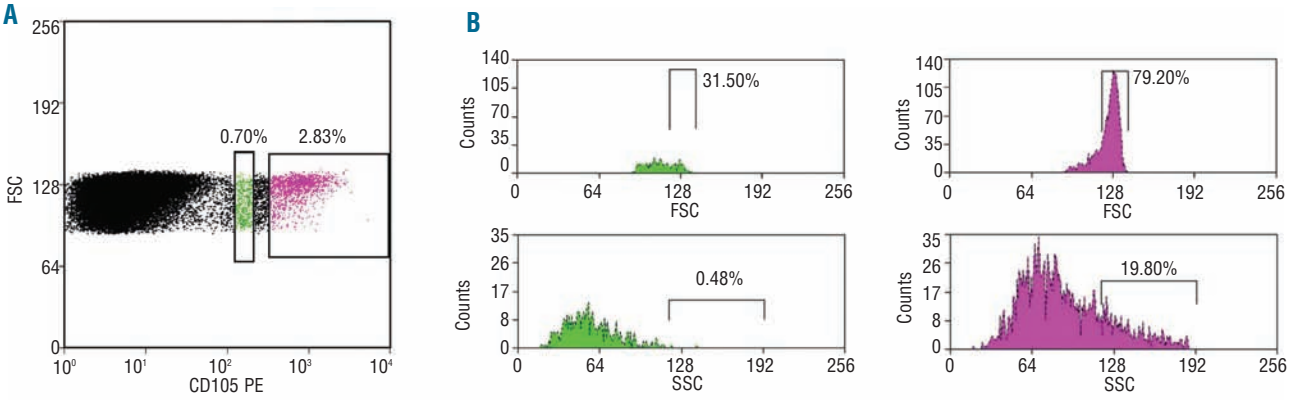
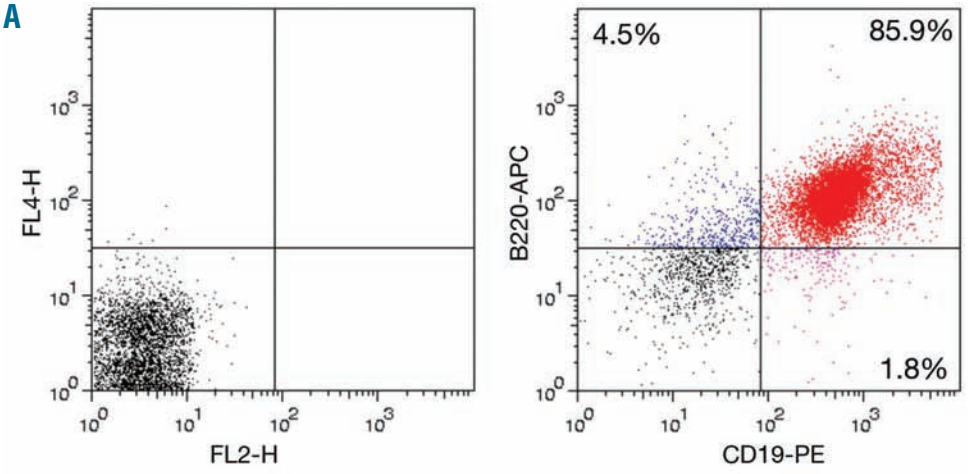
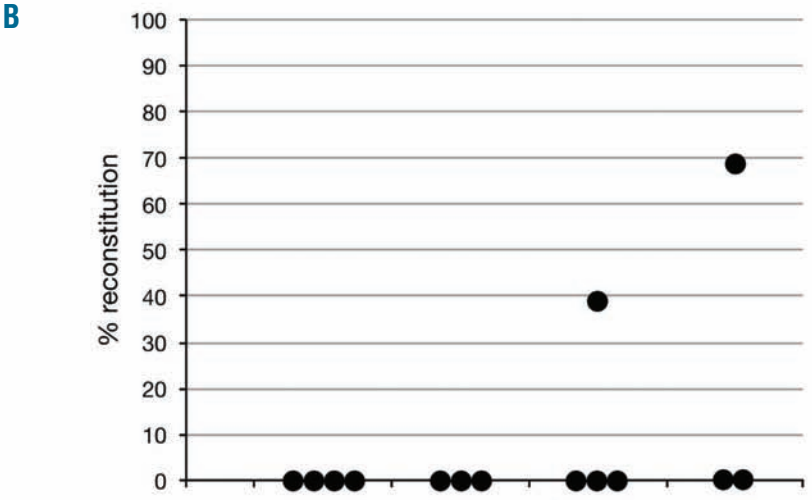


Online Supplementary Figure S1. Immunofluorescence analysis of CD105, CD31 and CD144 expression in the mouse at E9 (A-D) and E10.5 (E-H). Double labelings. For clear-cut identification of the signals, the green and red channels are presented independently. Although displaying some differences in the fluorescence intensity, CD31 and CD144 are coexpressed with CD105 in the vascular system. Ao: aorta; NT: neural tube.



Online Supplementary Figure S2. Morphology of CD105^{int} and CD105^{hi} cells analyzed by flow cytometry. (A) FACS analysis of the CD105-expressing cells in the E11 mouse AGM. A CD105^{int} (green) and a CD105^{hi} (pink) population are clearly visible. (B) Size (FSC, Forward Scatter) and granulosity (SSC, Side Scatter) of CD105^{int} and CD105^{hi} gated cells. Representative of 3 independent experiments.



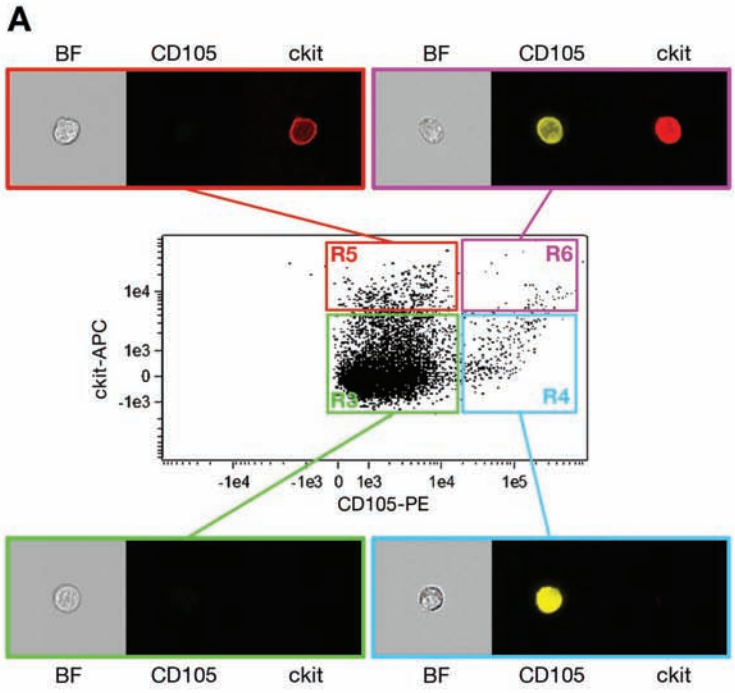


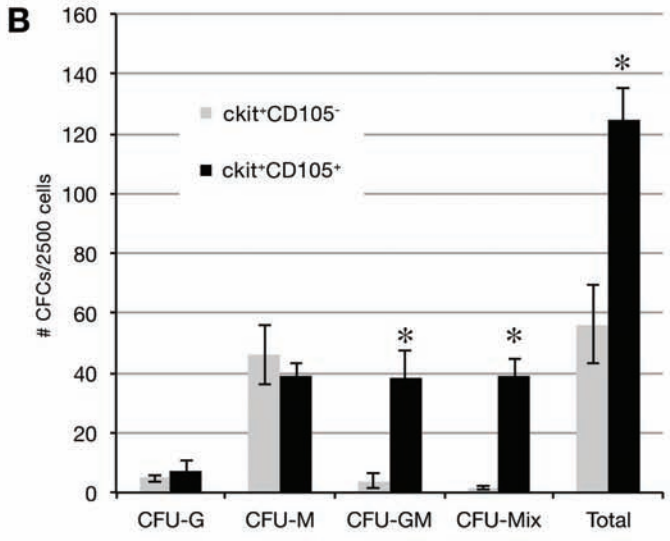
Cells injected	10 000 CD105$^-$	100 000 CD105$^-$	10 000 CD105$^+$	10 000 CD105^{hi}
N. mice injected	4	3	4	3
N. mice reconstituted	0	0	1	1
% reconstituted mice	0	0	25	33.3

Online Supplementary Figure S3. Lymphoid potential of the CD105^{hi} (A) and multilineage reconstitution potential of the CD105$^+$ and CD105^{hi} populations (B). (A) B220-CD19 cytometer analysis 10 days after lymphoid switch (right panel). Left panel: negative controle. About 86% of the cells expressed both markers indicating their commitment into the B-cell lineage and revealing their multilineage potential. (B) Plot representing the number of reconstituted mice according to the CD105 status and the number of cells injected. Only the CD105$^+$ or CD105^{hi} fractions allow reconstitution of lethally irradiated mice. CD105$^+$, CD105^{hi} or CD105$^-$ sorted cells were injected intravenously to lethally irradiated Ly5.1 mice (9.5 Gy). Peripheral blood was collected 16 to 20 weeks after injection and analyzed by flow cytometry (FACSCalibur cytometer, BD Biosciences) for the presence of donor-derived (Ly5.2+) cells as previously described (Petit-Cocault *et al.*).[1] The percentage of reconstitution was determined by the following formula:

$$\frac{\%CD45.2}{\%CD45.1+\%CD45.2} \times 100$$

A recipient mouse was considered positive when the percentage of reconstitution was above 5%.





Online Supplementary Figure S4. Ckit$^+$CD105$^+$ cells are highly enriched in hematopoietic progenitors. (A) ImageStream analysis of AGM E11 cells stained with ckit-APC and CD105-PE antibodies. Each panel represents an example of the brightfield, CD105 and ckit staining for the gated cell populations (i.e. ckit$^-$CD105$^-$, ckit$^-$CD105$^+$, ckit$^+$CD105$^-$ and ckit$^+$CD105$^+$). (B) Colony Forming Cell potential of AGM ckit$^+$CD105$^-$ and ckit$^+$CD105$^+$ cells. Cells were sorted by flow cytometry and cultured in semi-solid (methylcellulose) conditions. Colonies were scored at day 10. Data represent the mean±SEM from 3 independent experiments. *P=0.001.

References

1. Petit-Cocault L, Volle-Challier C, Fleury M, Peault B, Souyri M. Dual role of Mpl receptor during the establishment of definitive hematopoiesis. Development. 2007;134 (16):3031-40.

Online Supplementary Table S1. Specific primers used for cDNA synthesis.

	Forward Reverse	Size Product (bp)	
Alk-1	cttggggagcttcagaagggg	tgcctgttcagatgccttcag	501
Alk-3	gctccatggcactggtatg	cacaaccagccatcggatg	382
Alk-5	cagatggcagagctgtg	cacagcagctccctaagg	437
Alk-6	gccagctggttccgagag	agccaagcccaggtctgc	347
TgfbRII	gctggaatgctgtgggag	ggtcgtgatcccagcac	196
BmpRII	gtgccagctggccaggca	ggcgccaccgcttaagag	468
Tgfb1	cgtcactggagttgtacgg	tggttgtagagggcaaggac	439
Tgfb2	cacctcccctccgaaaatgccat	acccaggttcctgtctttgtggt	328
Tgfb3	gtcactgacactgtgcgcga	ctggcctcagctgcacttac	636
Smad1	gtgctggttccgaagcac	ggcagtggaggcgccatc	326
Smad2	gctgctcttctggctcag	cggaggagctgttacagc	267
Smad4	gcggcagtgtcaccggca	gctgtgggtccgcaatgg	328
Gapdh	ccgtattgggcgcctggt	gccttgactgtgccgttg	149