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Transgene-free hematopoietic stem and progenitor cells from human induced pluripotent stem cells.

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Introductory paragraph:

The successful production of Hematopoietic Stem and Progenitor Cells (HSPCs) from human pluripotent sources is conditioned by transgene delivery¹⁻⁵. We describe here a dedicated and tractable one step, GMP-grade, transgene-free and stroma-free protocol to produce HSPCs from human induced pluripotent stem cells (hiPSCs). This procedure, applied to several sources of hiPSCs with equal efficiency, is based on a directed differentiation with morphogens and cytokines to generate a cell population close to nascent HSPCs or their immediate forerunners i.e., hemogenic endothelial cells⁶⁻⁹. Following engraftment into immunocompromised recipients, this cell population was proved capable of a robust myeloid, lymphoid and definitive red blood cell production in sequential recipients for at least 40 weeks. Further identification of the repopulating cells show that they express the G protein–coupled receptor APELIN (APLNR) and the homing receptor CXCR4. This demonstrates that the generation of *bona fide* HSPCs from hiPSCs without transgenes is possible and passes through an early endo-hematopoietic intermediate. This work opens the way to the generation of clinical grade HSPCs for the treatment of hematological diseases and holds promise for the analysis of HSPC development in the human species.

While in standard hiPSC differentiation protocols, CD34⁺CD45⁺ hematopoietic cells appeared from bursting Embryoid Bodies (EBs) at day 10 until day 14^{1,5,10,11}, here D17 EBs remained compact and spherical without burst therefore assessing a dramatic delay in differentiation (Fig. 1a). These culture conditions, reproducibly applied to three individual hiPSC lines, derived using different reprogramming protocols demonstrated the robustness of the method.

To determine the point of hemogenic endothelial cell (EC)/early HSPC commitment, we analyzed EBs by qRT-PCR on D3, 7, 9, 13, 15, 16 and 17 for the expression of 49 endothelial- and hematopoietic-specific genes, with CD34⁺ cord blood (CB) HSPCs as a reference (Supplementary Table 1). Hierarchical clustering (Fig. 1b, and Supplementary Fig. 1) revealed two main groups, one associated with CD34⁺ CB HSPCs and another one with D3 to D17 EBs. The latter displayed two distinct clusters: the early EBs (D3 to D13) and the late EBs (D15 to D17). Further analysis of the qRT-PCR identified D13 as the point of EC commitment based on CD309 (VEGFR2/FLK1) mRNA expression and D16 as hemogenic endothelial commitment/EHT based on RUNX1 mRNA expression (Fig. 1c) in keeping with developmental studies¹². Early HSPC commitment was overt from D17 testified by the expression of ITGA2 (Integrin alpha-2), CEBPA (CCAAT enhancer binding protein alpha), the transcription factors c-MYB and SCL, this latter exhibiting a bimodal curve in keeping with its role on hemangioblast and hematopoietic commitment respectively¹³, the TPO receptor MPL (Fig 1c). Flow cytometry indicated a decrease in CD309 (EC marker) expression from D13 to D17 and an increase of ITGA2, CKIT and MPL (early HSPC markers) expressions (Fig. 1d) in keeping with the qRT-PCR analysis (Fig. 1c).

We next evaluated the endothelial and hematopoietic potential of D15 to D17 EBs using dedicated *ex vivo* functional tests (Fig. 2a and Supplementary Table 2). D15 EBs displayed a

strong endothelial-forming potential since they generate endothelial colony-forming cells (CFCs) (Fig. 2a1), pseudo-microtubules (Fig. 2a2) and endothelial-like cells (Fig. 2a3)¹⁴, but lacked hematopoietic-forming capacity, being unable to generate clonogenic colonies and displaying a low frequency of long term culture-initiating cells (Supplementary Table 2). In contrast D17 EBs lacked endothelial potential but showed an increased hematopoietic capacity (Fig. 2a4, a5), confirming hematopoietic commitment within this period.

D16 cells were probed *in vivo* by subcutaneous transplantation in Matrigel (growth factor reduced) plug with or without human Mesenchymal Stem Cells (hMSCs) into immunocompromised Foxn1^{-/-} (nude) mice (Fig. 2b)¹⁵. Two weeks after transplantation, both human vascular and hematopoietic differentiation was found. Human vascular structures (Fig. 2c, d, e), made of human von Willebrand factor⁺ (Fig. 2d) and CD31⁺ (Fig. 2e) cells were detected in the graft seeded with D16 cells associated with (/) hMSCs. qRT-PCR revealed the expression of hVEGFR2, hENG (ENDOGLIN), hPECAM and hVE-CADHERIN in the D16 cells/hMSCs grafts and, as expected, in the endothelial progenitor cells/hMSCs grafts (Supplementary Fig. 2). Moreover, D16 cells/hMSCs grafts also expressed the CD45 antigen and human beta, gamma and epsilon globin transcripts, while D16 cell grafts alone expressed only human epsilon globin transcript disclosing a block of maturation (Supplementary Fig. 2). D16 EBs thus displayed a balanced endothelial-hematopoietic pattern in keeping with our *ex vivo* results.

Based on the newly-formed hematopoietic capability disclosed at D17, we transplanted 4×10⁵ D17 cells intravenously into 30 sublethally irradiated (3.5 gray) 8-week-old immunocompromised mice for 20 weeks followed by a challenging secondary transplantation in similarly-treated immunocompromised recipients for 20 additional weeks and compared it

systematically to CD34⁺ CB HSPCs (Fig. 3a, Supplementary Fig. 3a). Hematopoietic reconstitution quantified by the surface expression of hCD45, hCD43 and hCD34 (Fig. 3b and Supplementary Fig. 3b-l)¹⁶ was evident in 30/30 primary recipients and quantitatively comparable to that of CD34⁺ CB HSPCs (Fig. 3c and Supplementary Fig. 3b, c-e, j) with 20.3 \pm 2.9 % of hCD45⁺ cells in total mouse BM mononucleated cells, i.e., more than 200 times the threshold of 0.1% usually considered as positive for human hematopoietic engraftment in NSG mice¹⁷, and 12.2 \pm 1.5 % of hCD43⁺ and 7.29 \pm 1.0 % of hCD34⁺. hCD45⁺ BM cells harbor several hematopoietic lineages including B and T lymphoid (hCD19⁺ and hCD3⁺ respectively) and myeloid (hCD14⁺) (Supplementary Fig. 4a-d) whereas hCD45⁻ BM cells harbor hCD235a⁺ erythroid progenitors/precursors (Supplementary Fig. 4a-b, e-f). Sorted hCD45⁺ blood cells displayed the same multilineage pattern (Supplementary Fig. 5a) indicating a peripheralization of the grafted cells comparable to results obtained with CD34⁺ CB HSPCs. Their human origin was confirmed (n=30/30) by qRT-PCR using human-specific primers (Supplementary Fig. 4g.). A human-specific clonogenic hematopoietic assay on BM cells from the first recipient revealed an overall frequency of 17.5 \pm 4.3 clones out of 10⁴ total BM cells (Fig. 3f) distributed into CFU-GEMM, BFU-E and CFU-GM colonies (Fig. 3g1, 2, 3) Cytospin analysis revealed mature macrophages, histiomonocytes, myelocytes and erythroblasts (Fig. 3h1, 2, 3). 7.10⁶ BM cells from the primary recipient were challenged in a secondary (n=30) (Fig 3d, e and Supplementary Fig. 3f-i, k) and eventually a tertiary (n=3) recipient (Supplementary Fig. 3l). Human CD45⁺ cells represented 12.6 \pm 3.9 % of the mononucleated BM cells at 20 weeks (Fig. 3d), indicating a sustained reconstitution capacity. Multilineage engraftment was found in 30/30 secondary mice (Fig. 3e, Supplementary Fig. 6a-e) and was comparable to the pattern obtained on secondary CD34⁺ CB HSPCs transplants (Supplementary Fig. 3f-i, k and Supplementary Fig. 6). The

human CFCs cloning efficiency was 5.5 ± 3.1 % in 10^4 total mouse BM cells, pointing to a robust and prolonged self-renewal potential (Fig. 3f-h). The human origin of the engrafted cells was confirmed as above (Supplementary Fig. 3g).

To ensure the functionality of the grafted cells, we analyzed the ability of the human erythroid precursors from mouse BM to undergo hemoglobin switching *in vivo* and tested the phenotype and the functionality of T cells. Cells from both primary and secondary recipients generated human erythroid progenitors displaying high amounts of β (respectively $39.51 \pm 4.95\%$ and $36.61 \pm 5.86\%$) and γ globin (respectively $57.49 \pm 3.95\%$ and $61.39 \pm 4.86\%$) while ϵ globin was dramatically reduced to respectively $3.0 \pm 1.2\%$ and $2.1 \pm 1.1\%$ of total globin (Fig. 3i), a hallmark of definitive erythrocytes. Peripheral blood $hCD3^+$ T cells displayed high amounts of $TCR\alpha\beta$ (Fig. 3j) and low amounts of $TCR\gamma\delta$ similar to thymic $hCD3^+$ cells (Supplementary Fig. 5b) reminiscent of normal T lymphopoiesis. B and T lymphopoiesis was revealed in spleen $CD45^+$ cells by the expression of $TCR\beta$ and $hCD19$ and were found similar to $CD34^+$ CB HSPCs grafts (Supplementary Fig 5b). Thymus $hCD3^+$ T cells, tested on their ability to expand *ex vivo*, measured by CFSE-labeling, under $hCD3$ and $hCD28$ stimulation (Fig. 3k) displayed a high expansion capacity after 5 days, thereby demonstrating T cells functionality.

To further identify the reconstituting population, we focused on APLNR related to ECs¹⁸ and HCs from human embryonic stem cells¹⁹ and to the homing receptor $CXCR4$ ^{20,21}. Differentiating EBs displayed an enhanced APLNR and $CXCR4$ expression from D7 to D17 with the emergence of a double stained population accounting for 32.1% of the cells at D17 (Fig 4a). Grafting efficiency at 20 weeks was directly proportional to the number of $APLNR^+$ cells (Fig 4b). Moreover, only $APLNR^+$ cells reconstituted hematopoiesis after 20 weeks with 6.6 ± 1.9 % $hCD45^+$ mononucleated cells, 3.4 ± 2.5 % $hCD43^+$ and 1.1 ± 0.4 % $hCD34^+$ in 6/6 grafted

mice (Fig. 4c, Supplementary Fig. 7a) and displayed multilineage reconstitution (Supplementary Fig. 7b-c). Importantly, D17 APLNR⁺ cells did not harbor any CD45⁺ cell indicating that reconstitution was not harbored by hCD45⁺ progenitors (Supplementary Fig. 7d). In contrast, APLNR⁻ cells failed to significantly engraft in 4/4 mice with 0.08+/-0.01 % hCD45⁺ cells in mouse BM (Figure 4c, Supplementary Fig 7a). The APLNR⁺ fraction also exhibited a homogeneous population of ENG⁺/TIE⁺/CKIT⁺ (Fig. 4d) described to enhance definitive hematopoiesis in mice²². We compared the molecular profiles of APLNR⁺ and APLNR⁻ cells to that of D15 and D17 EBs, to hiPSCs and to control CD34⁺ CB HSPCs through the expression of the set of 49 genes (Supplementary Table 3). Principal component analysis (PCA) of the 49 mRNAs as variables and the six cell populations as observations revealed that the first component likely corresponded to the factor “hematopoietic differentiation” (44.9% of the variance) (Fig. 4e). Aiming to reveal the traits involved in the grafting potential, we compared by PCA the APLNR⁺, D17 and CB HSPC populations to the APLNR⁻ and hiPSC populations. The third component that accounted for 19.23% of the variance segregated two groups differing by their grafting potential (Fig. 4f). A statistical SAM test which measures the strength of the relationship between gene expression and a response variable pointed out 8 genes (FDR<10%) significantly up-regulated in the group unable to graft, among them endothelial genes as TEK, PECAM, and KDR (Fig. 4g).

Collectively, we show that the generation of long-term multipotent HSPCs supporting hematopoietic reconstitution and self-renewal *in vivo*, passes through an early differentiated cell expressing APLNR more likely an endothelial cell undergoing EHT or a newly formed HSPC. Since this study has been performed under GMP-grade conditions, it may be envisioned that pluripotent stem cells may become a prioritized source of cells for HSPC transplantation.

Figure Legends

Fig. 1: Phenotypic characterization of hiPSC-derived endo-hematopoietic differentiation *ex vivo*.

- (a) Experimental scheme. HiPSCs were differentiated into EBs over 17 days with the continuous presence of growth factors and cytokines. EBs were characterized at different time points using q-PCR and flow cytometry. Images depicted representative EBs at D13 and 17 respectively.
- (b) Hierarchical clustering summarizing the expression of the set of 49 genes characteristics of the endothelium, hemogenic endothelium and hematopoietic cells with time in D3, D7, D9, D13, D15 and D17 EBs and in CB CD34⁺ HSPCs.
- (c) Q-PCR patterns of genes representative of the EHT balance from D13 to D17 EB differentiation. For each gene, the fold change is the mean +/- SEM of 6 experiments
- (d) Flow cytometry analysis of human CD309, ITGA2, MPL and CKIT expression at D13 and D17 of EB culture.

Fig. 2: Functional endothelio-hematopoietic profiling between D15 and D17.

- (a) *Ex vivo* tests probing the presence of endothelial (1-3) and hematopoietic (4-5) progenitors in EBs with time. Dissociated D15-17 EBs generate (1) CFC-ECs, (2) pseudo-microtubules, (3) EC-like cells capable of several passages, (4) CFC, and (5) LTC-ICs.
- (b) Experimental scheme for *in vivo* tests to probe the endothelial capacity of D16 cells.
- (c) D16 cells/hMSCs plug section. Masson's trichrome staining.
- (d) D16 cells/hMSCs plug section. Human von Willebrand factor⁺ cells (blue) immunostaining.
- (e) D16 cells/hMSCs plug section. Human CD31⁺ immunostaining (red). Cell nuclei are counterstained by DAPI.

Fig. 3: *In vivo* engraftment of D17 EBs in immunocompromised mice.

- (a) Experimental design.
- (b) Representative flow cytometry analysis of human vs mouse CD45⁺ cell engraftment in a BM primary recipient.
- (c-d) Engraftment of hCD34⁺ hCD43⁺ and hCD45⁺ HSPCs in primary (c) or secondary (d) mouse BM 20 weeks post graft. Data are the mean +/- SEM.
- (e) Human hematopoietic lineage distribution in representative secondary recipients. Cells were gated on hCD45⁺ expression for hCD33, hCD19 and hCD3 whereas hCD235a was analyzed on whole BM cells. Data are the mean +/- SEM.
- (f) Clonogenic hematopoietic tests on BM cells isolated from primary and secondary recipients. Frequency of CFU-GM, BFU-E and CFU-GEMM colonies.
- (g) Representative colonies of CFU-GEMM (1), BFU-E (2) and CFU-GM (3) from primary and secondary BM cells.
- (h) Cytospins. May Grünwald-Giemsa staining of cells isolated from clonogenic tests performed on primary and secondary recipients. Mature macrophages (1), histiomonocytes (2), myelocytes (2) and erythroblasts (3).
- (i) Human globin expression from CB CD34⁺ HSPCs erythroid culture, from BM primary and secondary recipients and BFU-E from BM primary recipients. Data are mean +/- SEM.
- (j) Maturation of human T cells. Peripheral blood stained with antibodies against hTCR $\alpha\beta$ and hTCR $\gamma\delta$, the cells are first gated on hCD3 and analyzed for TCR expression.
- (k) Functionality of human T cells. The whole thymus population is CFSE labeled at D0 and gated on hCD3⁺ expression (green). At D5, the unstimulated population is red while the stimulated parent population is blue.

Figure 4: Functional and molecular characterization of the APLNR⁺ population.

- (a) Flow cytometry analysis of APLNR and CXCR4 expression from D7 to D17 EB culture.
- (b) Correlation between the percentage of APLNR⁺ cells in the graft to those of hCD45⁺ cells in the NOD-SCID BM primary recipients, 18weeks post-graft.

(c) Engraftment capacities of the APNLR⁺ (n=6, red dots) and APNLR⁻ (n=4, grey triangles) populations. Cells containing the reconstitution potential are within the APLNR⁺ population. Data are expressed as the mean +/- SEM percentages of human engraftment, 18 weeks after transplant.

(d) Combinatorial flow cytometry analysis of the APLNR⁺ population using CD45, TIE, ENG and CKIT anti-human antibodies.

(e) PCA with the set of 49 mRNAs as variables and the six cell populations as observations. PC1 versus PC2 score plot. The PC1 dimension likely corresponds to the trait « hematopoietic differentiation » which accounts for 44.9% of the variance. HiPSCs are segregated from the other populations on the second component which accounts for 24% of the variance.

(f) PCA with the set of 49 mRNAs as variables and the populations endowed or not with grafting potential. The PC3 dimension which accounts for 19.23% of the variance segregates the two groups.

(g) Heat map of the genes permitting the segregation of the two groups in (f).

Materials and Methods:

hiPSC amplification

The study was conducted using three different hiPSC lines: the FD136-25 (skin primary fibroblasts), reprogrammed with retroviral vectors and Thomson's combination (endogenous expression of Oct4, Sox2, Nanog and Lin28); the Pci-1426 and Pci-1432 lines (peripheral blood mononuclear cells-Phenocell) reprogrammed with episomes (Sox2, Oct4, KLF, cMyc). hiPSCs were maintained on CellStart (Invitrogen, Carlsbad, USA) in TESR2 medium (Stem Cell Technologies, Bergisch Gladbach, Germany) and the cells were passaged 1:6 onto freshly coated plates every 5 days using standard clump passaging with TRYple select (Invitrogen).

EB differentiation

EB differentiation was induced as previously described. After 24h, cells were transferred into differentiation medium (Lapillonne et al., 2010) containing 22 ng/mL of SCF, 20 ng/mL of TPO, 300 ng/mL of FLT3, 22 ng/mL of BMP4, 200 ng/mL of VEGF, 50 ng/mL of IL3, 50 ng/mL of IL6, 5 ng/mL of IL1, 100 ng/mL of G-CSF, 50 ng/mL of IGF1 (PeproTech, Neuilly-sur-Seine, France). Medium was changed every other day.

Colony assays

At the indicated times, 1×10^5 dissociated EBs or 3×10^4 cells from xenotransplanted recipient BM were plated into 3 mL of complete methylcellulose medium in the presence of SCF, IL-3, EPO and GM-CSF (PeproTech, Neuilly-sur-Seine, France). As G-CSF also stimulates mouse progenitors, it was replaced by granulocyte-macrophage colony-stimulating factor (GM-CSF).

Aliquots (1 mL) of the mix were distributed into one 30 mm dish twice and maintained in a humidified chamber for 14 days. Colony-forming Cells (CFC) were scored on day 14.

Long-term culture-initiating cell assays

Long-term culture-initiating cell (LTC-IC) assays were performed as described previously (35), 15–100,000 cells/well on day 17 for the EBs and on day 0 for the control CD34+. Absolute LTC-IC counts corresponded to the cell concentrations, yielding 37% negative wells using Poisson statistics.

Pseudo-microtubules and EPC-like cells

For Pseudo-microtubules formation, cells were transferred onto growth factor reduced Matrigel (Corning) and culture in EGM2 medium (Lonza).

For EPC-like cells generation, cells were first plated on gelatin and cultured in EBM2 (Lonza) and split several times, after the first passaged the gelatin was no longer mandatory.

Flow cytometry

Staining of BM cells or dissociated EBs was performed with 2×10^5 cells in 100 μ L staining buffer (PBS containing 2% FBS) with 5:100 dilution of each antibody, for 20 min at room temperature in the dark. Data acquisition was performed on a Becton Dickinson Canto II cytometer.

In vivo analyses of angiogenesis potential

Foxn1^{-/-} nude mice (Charles River, L'Abresle, France) were housed in the IMRB animal care facility. All experiments and procedures were performed in compliance with the French Ministry of Agriculture regulations for animal experimentation and approved by the local ethics committee.

In vivo assessment of the endothelial and hematopoietic potentials were probed on 9 nude mice.

1.750 10⁶ D16 single cells or hEPCs and 1.750 10⁶ hMSCs were mixed with 100µl of Matrigel phenol red free and growth factor reduced (Corning) and subcutaneously injected into the back of nude mice (two different plugs/ mouse). The controls were performed similarly but with 3.5 10⁶ hMSCs or D16 single cells or hEPCs; for each condition n=3. Two weeks later, mice were sacrificed, the matrigel plug dissected out and cut into two parts. One part was processed for paraffin sectioning. Sections were deparaffinized, hydrated and stained whether with Masson's trichrome, a three-color protocol comprising nuclear staining with hematoxylin, cytoplasmic staining with acid fuchsin/xykidine ponceau and collagen staining with Light Green SF (all from VWR); or whether with an anti human Von Willebrand factor antibody (Dako), staining was developed with histogreen substrate (Abcys) and counterstained with Fast nuclear red (DakoCytomation), dehydrated and mounted, or whether with hCD31 (R&D system) as primary antibody and donkey anti-rabbit Cy3 (Jackson ImmunoResearch) as secondary antibody and DAPI and mounted with fluoromount G.

Sorting of APLNR positive cells

Cells were stained with the antibody hAPJ-APC clone 72133 (R&D systems) as described above. Sorting was carried out on a Moflo ASTRIOS Beckman Coulter apparatus and the purity was 98.1% APLNR⁺ cells.

Mouse transplantation

NOD/SCID-LtSz-scid/scid (NOD/SCID) and NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) (Charles River, L'Abresle, France) were housed in the IRSN animal care facility. All experiments and procedures were performed in compliance with the French Ministry of Agriculture regulations for animal experimentation and approved by the local ethics committee.

Mice, 6-8 weeks old and raised under sterile conditions, were sublethally irradiated with 3.5 Grays from a ¹³⁷Cs source (2.115 Gy/min) 24 h before cell injection. To ensure consistency between experiments, only male mice were used. Prior to transplantation, the mice were temporarily sedated with an intraperitoneal injection of ketamine and xylazine. Cells (4 x10⁵ per mouse) were transplanted by retro-orbital injection in a volume of 100 µL using a 28.5 gauge insulin needle. A total of 147 mice were used in this study.

For the engraftment potential of the D17 cells on the three different hiPSC lines:

86 NSG mice were used as followed: 30 primary recipients, 30 secondary recipients and 26 as control.

48 NOD-SCID mice were used as followed: 20 primary recipients and 16 secondary recipients, 3 tertiary recipients and 9 as control.

For the engraftment potential of APLNR⁺ and APLNR⁻ population: 10 NOD-SCID mice were used and 3 NOD-SCID as control.

Assessment of human cell engraftment

Mice were sacrificed at week 12, 18 or 20. Femurs, tibias, liver, spleen and thymus were removed. Single cell suspensions were prepared by standard flushing and aliquots containing 1×10^6 cells were stained in a total volume of 200 μ L staining buffer.

Samples were stained for engraftment assessment with the following markers: hCD45 clone J33, hCD43 clone DFT1, hCD34 clone 581 (Beckman Coulter) and hCD45 clone 5B1, mCD45 clone 30F11 (Miltenyi)

The BM of three mice were pooled to allow hCD45 microbead enrichment (Miltenyi), the multilineage was assessed using the following human markers : hCD3 clone UCHT1, hCD4 clone 13B8.2, hCD8 clone B9.11, hCD14 clone RMO52, hCD15 clone 80H5, hCD19 clone J3-119, hCD20 clone B9E9, hCD43 clone DFT1, hCD34-APC, hCD71 clone YDJ1.2.2 (all from Beckman Coulter antibodies, Brea, USA), CD45 clone 5B1 (Miltenyi), CD235a clone GA-R2 (Becton-Dickinson).

The blood samples of three mice were pooled to allow hCD45 microbead sorting (Miltenyi). The multilineage potential was assessed using the following human markers : hCD3 clone UCHT1, hCD4 clone 13B8.2, hCD8 clone B9.11, hCD14 clone RMO52, hCD15 clone 80H5, hCD11B Bear1, hCD19 clone J3-119, hCD20 clone B9E9, hIGM clone SA-DA4 (all from Beckman Coulter antibodies, Brea, USA).

Non-injected mouse BM was used as a control for non-specific staining.

Compensation was performed by the FMO method with anti-mouse Ig and data were acquired on a BD Canto II cytometer.

T-cell maturity and functionality assay

The presence of TCR $\alpha\beta$ and TCR $\gamma\delta$ in peripheral blood was assessed by flow cytometry using the following human markers : hCD3 clone UCHT1 (positive gating), TCR $\alpha\beta$ clone IP26A and TCR $\gamma\delta$ clone IMMU510 (all from Beckman Coulter antibodies, Brea, USA).

Thymus and spleen cells were isolated, CFSE labelled and seeded in cell culture media complemented or not with hCD3 and hCD28 (Beckman Coulter both 1 μ g/ml). After 5 days, cells were harvested and stained with anti-hCD3 clone UCHT1 and analyzed on a BD Canto II cytometer. FlowJo analysis software was used to gate on CD3⁺ T-cells and generate the overlaid histogram plots.

Assessment of the APLNR cell safety

Three sub-lethally irradiated NOD/SCID mice were subcutaneously injected each with 3 million APLNR positive cells. No teratoma was found after 2 months follow-up according to FDA guidelines.

In addition, no tumor was macroscopically detected in any mouse after analysis of the organs (140/140 mice) or after microscopic analysis of different tissues (brain, lungs, kidneys, BM, liver and gut) (140/140 mice).

Quantitative PCR

Total mRNA was isolated with the RNA minikit (Qiagen, Courtaboeuf, France). mRNA integrity was checked on a Bioanalyzer 2100 (Agilent Technologies, Massy, France). cDNAs were constructed by reverse transcription with Superscript (Life Technologies, Carlsbad, USA). PCR assays were performed using a TaqMan PCR master mix (Life Technologies) and specific primers (Applied BioSystems, Carlsbad, USA) for selected genes (see table below), together

with a sequence detection system (QuantStudio™ 12K Flex Real-Time PCR System, Life Technologies). In each sample the fluorescent PCR signal of each target gene was normalized to the fluorescent signal of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

The human origin of the mRNAs from mouse BM was assessed by measuring hCD45, hCD15, hMPO, hITGA2 and hGAPDH. From CFCs post grafting and globin type expression in the mouse BM, we measured beta, gamma and epsilon globins using Taqman probes.

Controls were cultured erythroblasts generated from cord blood CD34⁺.

Pluripotency genes	Hs01053049_s1	SOX2	Endothelial genes		Hs00945146_m1	TEK
	Hs00153408_m1	MYC			Hs00231079_m1	RUNX1
	Hs00702808_s1	LIN28A			Hs00911700_m1	KDR
	Hs04260366_g1	NANOG			Hs01574659_m1	NOS3
	Hs00358836_m1	KLF4				
	Hs00742896_s1	POU5F1	Human housekeeping genes		Hs00357333_g1	Human beta
Mouse housekeeping genes	Mm99999915_g1	Murine GAPDH			Hs02758991_g1	actin Human GAPDH
Hematopoietic genes	Hs00924296_m1	MPO	Hs01076122_m1	DNTT	Hs00269972_s1	CEBPA
	Hs01106466_s1	FUT4	Hs00172743_m1	RORC	Hs01115556_m1	MITF
	Hs00174029_m1	cKIT	Hs00962186_m1	CD3G	Hs01029175_m1	NFIB
	Hs01116228_m1	ITGA2B	Hs00169777_m1	PECAM	Hs04188695_m1	HOPX
	Hs00766613_m1	APLNR	Hs00231119_m1	GATA2	Hs00171406_m1	HLF
	Hs00161700_m1	STIL	Hs00959427_m1	EPOR	Hs01070488_m1	RBPM5
	Hs00231119_m1	GATA2	Hs00610592_m1	KLF1	Hs00171569_m1	HMGA2
	Hs00995536_m1	BMI1	Hs01085823_m1	GATA1	Hs00223161_m1	PRDM16
	Hs00941830_m1	NCAM1	Hs04186042_m1	RUNX1	Hs01017441_m1	MEIS1
	Hs04189704_m1	PTPRC	Hs00176738_m1	MATK	Hs00414553_g1	NKX2-3
	Hs00174333_m1	CD19	Hs00180489_m1	MPL	Hs00971097_m1	MLLT3
	Hs00162150_m1	SPIB	Hs04334142_m1	FLI1	Hs00925052_m1	GATA3
	Hs00958474_m1	IKZF1	Hs00268388_s1	SOX4	Hs01128710_m1	IRF8
	Hs01851142_s1	RAG2	Hs00193527_m1	C-MYB	Hs00256884_m1	HOXB4
	Hs00266821_m1	HOXA9	Hs01554629_m1	ERG	Hs00610592_m1	KLF1
	Hs00931969_m1	RORA	Hs01547250_m1	LEF1	Hs04334142_m1	FLI1
	Hs00959427_m1	EPOR	Hs00176738_m1	MATK	Hs00180489_m1	MPL

Statistical analysis

All statistics were determined with R Software 3.1.1 (2014-07-10) (R Core Team, 2013), INGENUITY and SAM Software. Data are represented with hierarchical clustering and PCA.

References

H. Lapillonne *et al.*, Red blood cell generation from human induced pluripotent stem cells: perspectives for transfusion medicine. *Haematologica*. 95:1651-1659 (2010).

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Author contributions

L.G.H. and L.D. designed the study, analyzed the data, and wrote the manuscript. A.C. designed the study. L.G.H. performed experiments with assistance of B.L., B.B. and N.C.; H.L., C.D. and

L.G. helped to analyze the results; T.J. analyzed the data and wrote the manuscript. M.B. and F.D. helped design the study; A.C. and L.D. supervised the study.

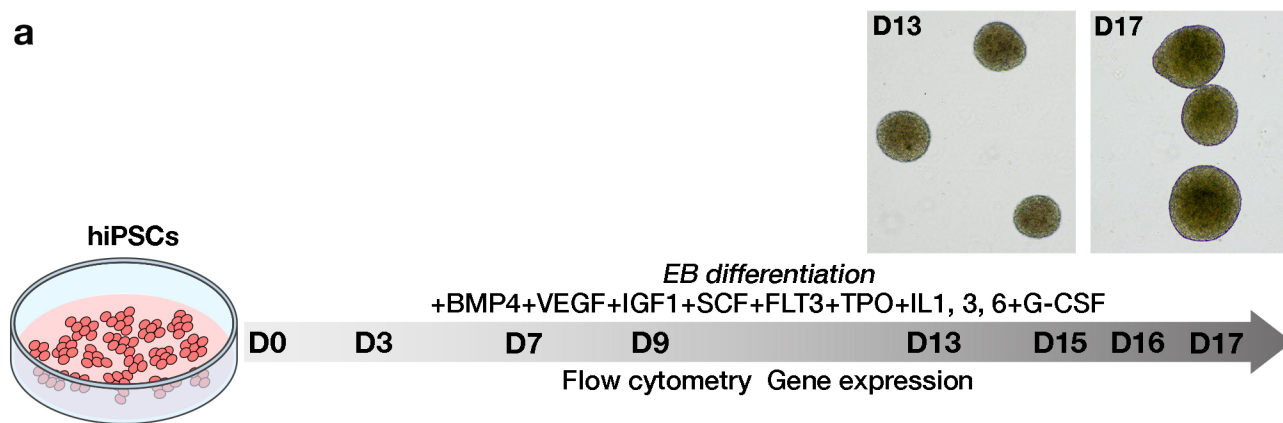
Competing financial interests:

L.G.H., C.D., T.J., L.G., L.D. and A.C. submitted a patent on the use of APLNR+ cell population to improve hematopoietic graft.

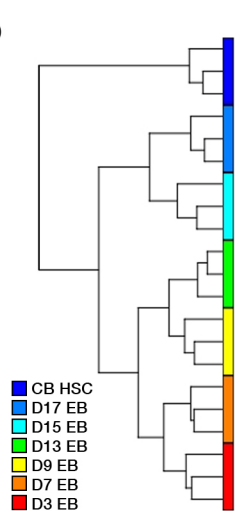
References

1. Doulatov, S., *et al.* Induction of multipotential hematopoietic progenitors from human pluripotent stem cells via respecification of lineage-restricted precursors. *Cell stem cell* **13**, 459-470 (2013).
2. Sandler, V.M., *et al.* Reprogramming human endothelial cells to haematopoietic cells requires vascular induction. *Nature* **511**, 312-318 (2014).
3. Sugimura, R., *et al.* Haematopoietic stem and progenitor cells from human pluripotent stem cells. *Nature* **545**, 432-438 (2017).
4. Lis, R., *et al.* Conversion of adult endothelium to immunocompetent haematopoietic stem cells. *Nature* **545**, 439-445 (2017).
5. Risueno, R.M., *et al.* Inability of human induced pluripotent stem cell-hematopoietic derivatives to downregulate microRNAs in vivo reveals a block in xenograft hematopoietic regeneration. *Stem cells* **30**, 131-139 (2012).
6. Swiers, G., Rode, C., Azzoni, E. & de Bruijn, M.F. A short history of hemogenic endothelium. *Blood cells, molecules & diseases* **51**, 206-212 (2013).
7. Antas, V.I., Al-Drees, M.A., Prudence, A.J., Sugiyama, D. & Fraser, S.T. Hemogenic endothelium: a vessel for blood production. *Int J Biochem Cell Biol* **45**, 692-695 (2013).
8. Sturgeon, C.M., Ditadi, A., Clarke, R.L. & Keller, G. Defining the path to hematopoietic stem cells. *Nature biotechnology* **31**, 416-418 (2013).
9. Slukvin, II. Deciphering the hierarchy of angiohematopoietic progenitors from human pluripotent stem cells. *Cell cycle* **12**, 720-727 (2013).
10. Lengerke, C., *et al.* Hematopoietic development from human induced pluripotent stem cells. *Ann N Y Acad Sci* **1176**, 219-227 (2009).
11. Ditadi, A., *et al.* Human definitive haemogenic endothelium and arterial vascular endothelium represent distinct lineages. *Nature cell biology* **17**, 580-591 (2015).
12. Yzaguirre, A.D., de Bruijn, M.F. & Speck, N.A. The Role of Runx1 in Embryonic Blood Cell Formation. *Advances in experimental medicine and biology* **962**, 47-64 (2017).
13. Van Handel, B., *et al.* Scl represses cardiomyogenesis in prospective hemogenic endothelium and endocardium. *Cell* **150**, 590-605 (2012).
14. Smadja, D.M., Cornet, A., Emmerich, J., Aiach, M. & Gaussem, P. Endothelial progenitor cells: characterization, in vitro expansion, and prospects for autologous cell therapy. *Cell biology and toxicology* **23**, 223-239 (2007).
15. Dutenhoefer, F., *et al.* 3D scaffolds co-seeded with human endothelial progenitor and mesenchymal stem cells: evidence of prevascularisation within 7 days. *European cells & materials* **26**, 49-64; discussion 64-45 (2013).
16. Tourino, C., *et al.* Efficient ex vivo expansion of NOD/SCID-repopulating cells with lympho-myeloid potential in hematopoietic grafts of children with solid tumors. *The hematology journal : the official journal of the European Haematology Association / EHA* **2**, 108-116 (2001).
17. Dick, J.E., Pflumio, F. & Lapidot, T. Mouse models for human hematopoiesis. *Semin Immunol* **3**, 367-378 (1991).
18. Kidoya, H., *et al.* Spatial and temporal role of the apelin/APJ system in the caliber size regulation of blood vessels during angiogenesis. *The EMBO journal* **27**, 522-534 (2008).
19. Yu, Q.C., *et al.* APELIN promotes hematopoiesis from human embryonic stem cells. *Blood* **119**, 6243-6254 (2012).
20. Peled, A., *et al.* Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* **283**, 845-848 (1999).
21. Kollet, O., *et al.* Human CD34(+)CXCR4(-) sorted cells harbor intracellular CXCR4, which can be functionally expressed and provide NOD/SCID repopulation. *Blood* **100**, 2778-2786 (2002).
22. Nasrallah, R., *et al.* Endoglin potentiates nitric oxide synthesis to enhance definitive hematopoiesis. *Biology open* **4**, 819-829 (2015).

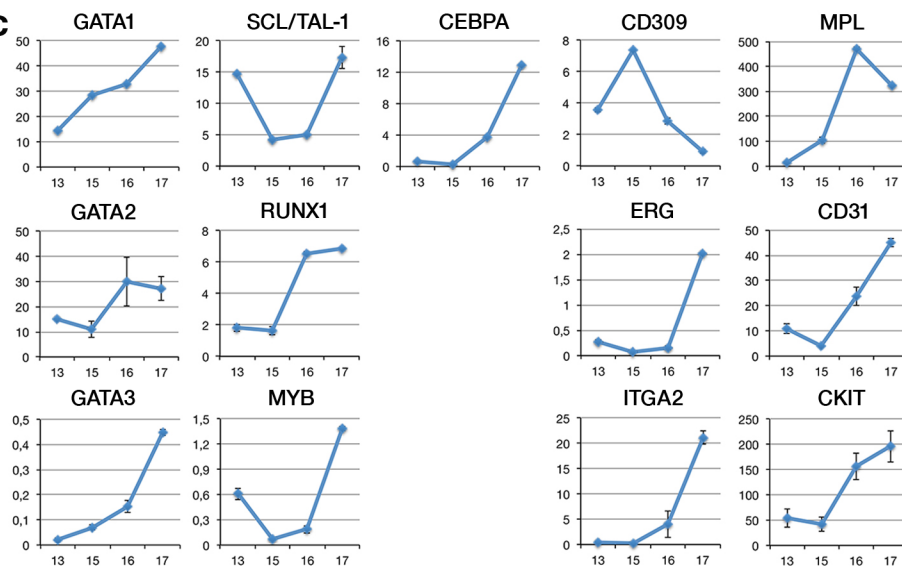
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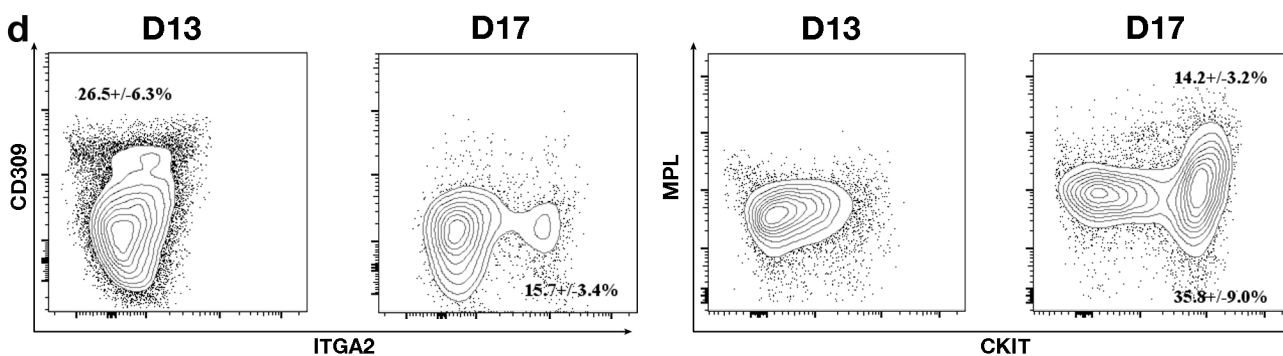
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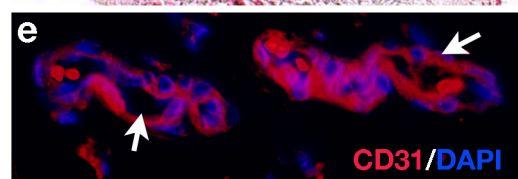
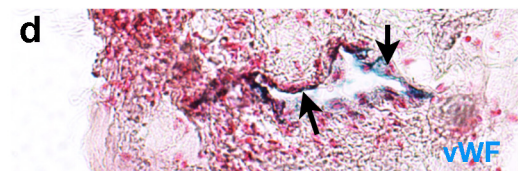
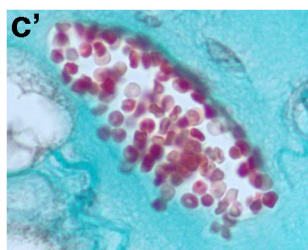
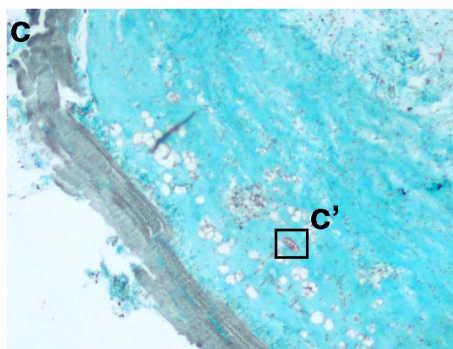
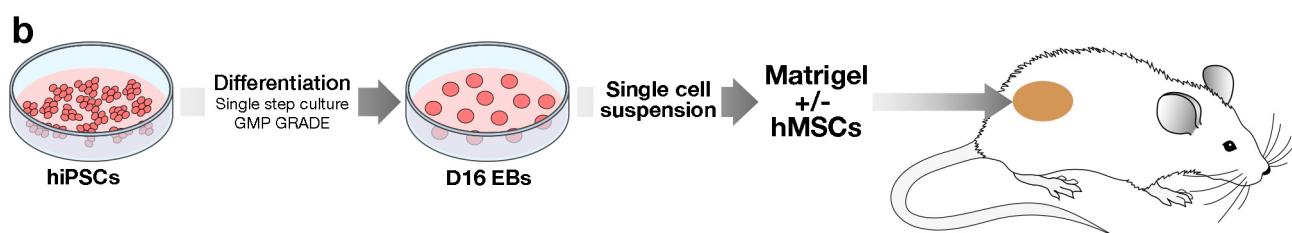
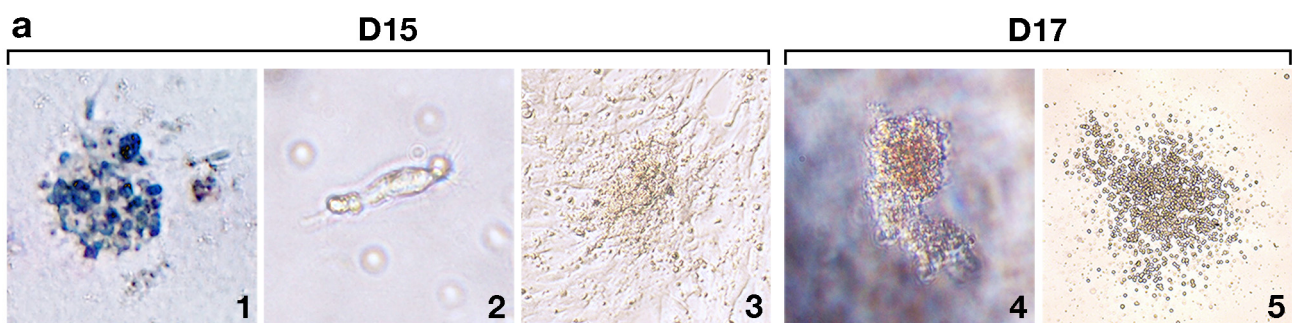


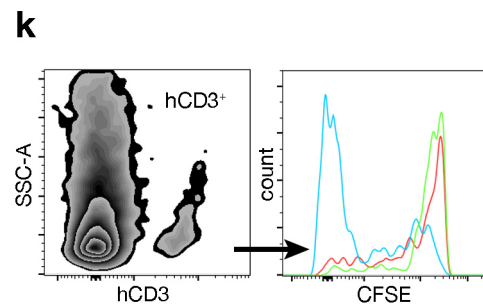
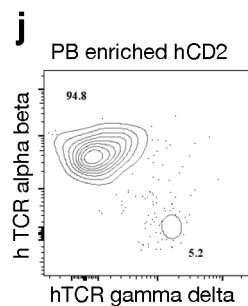
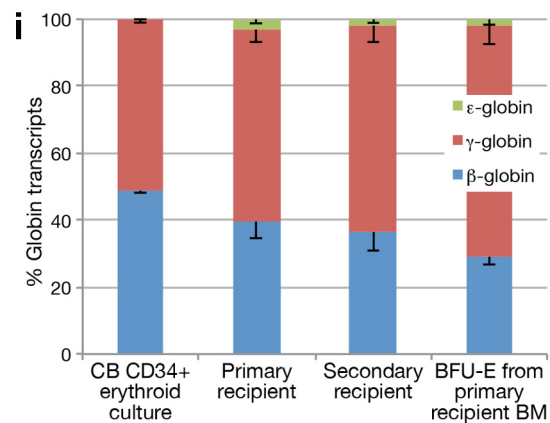
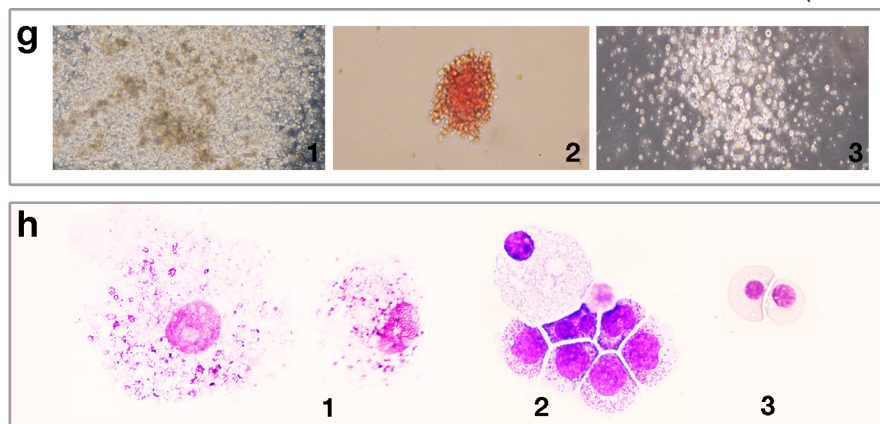
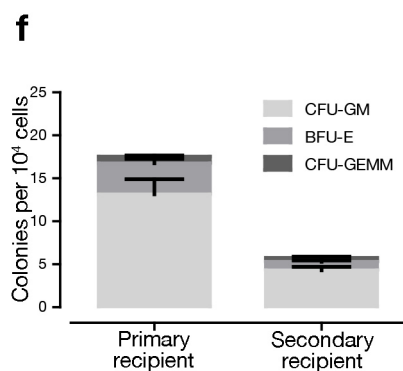
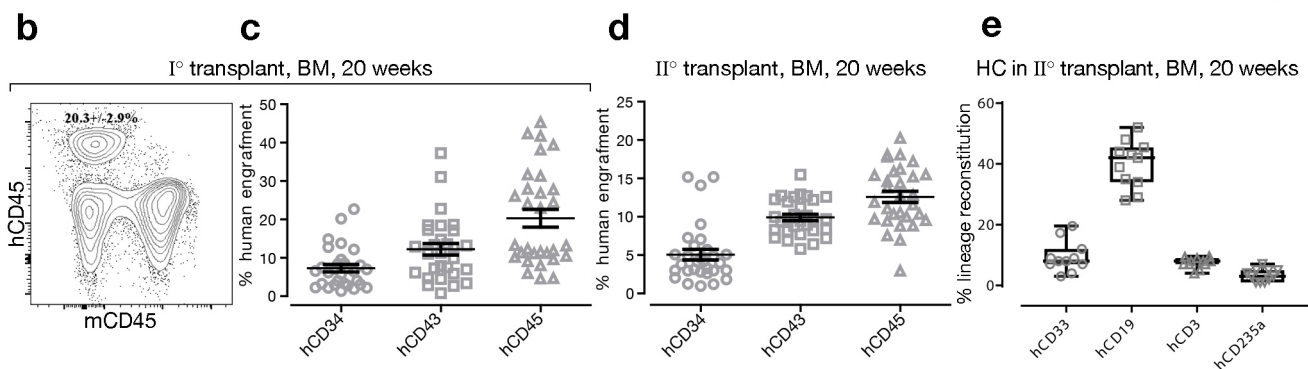
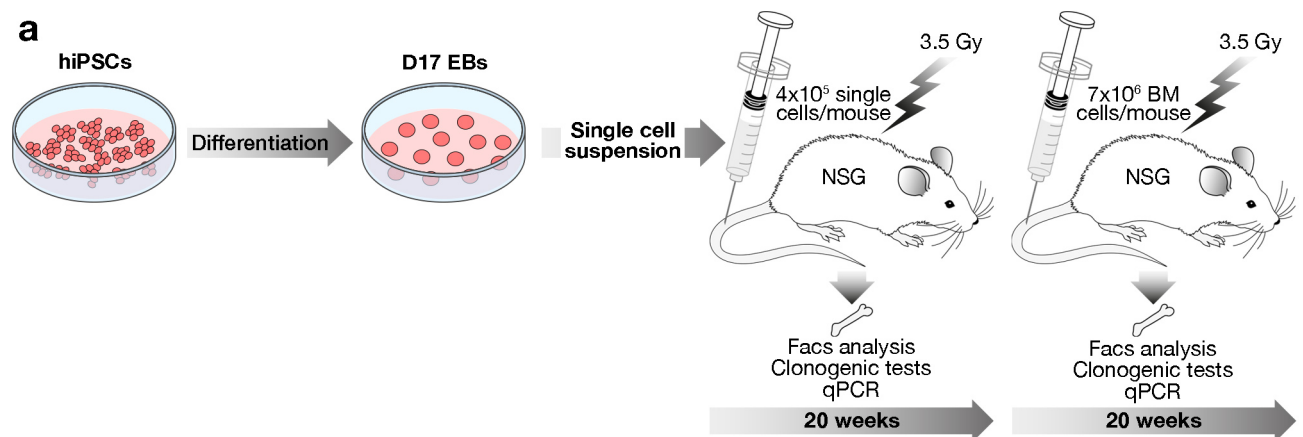
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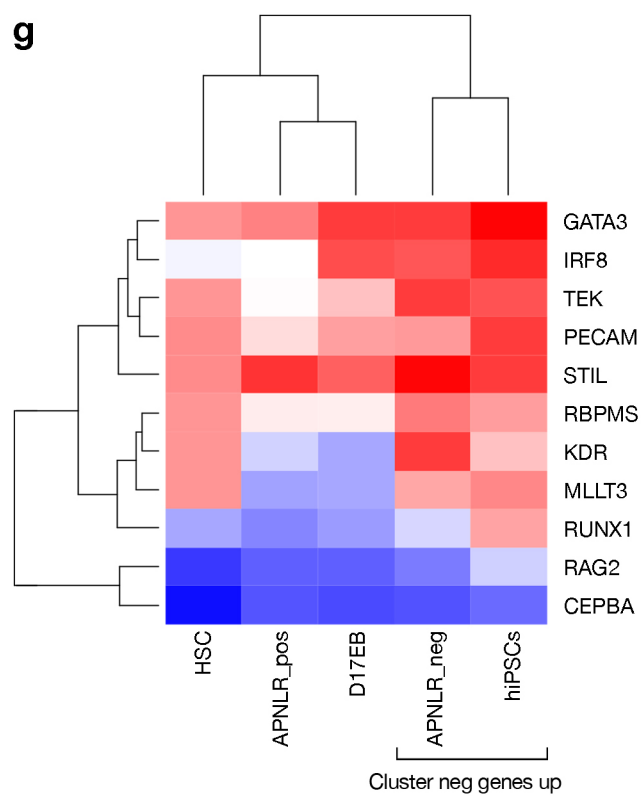
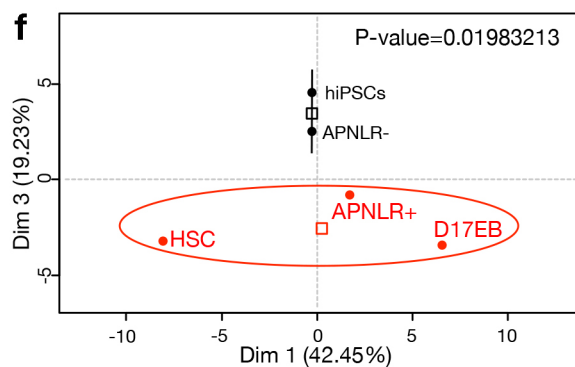
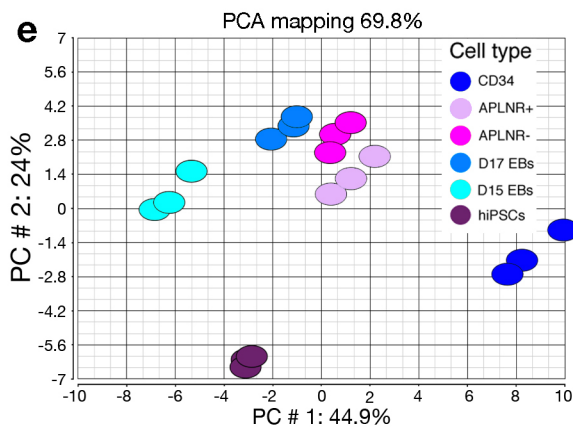
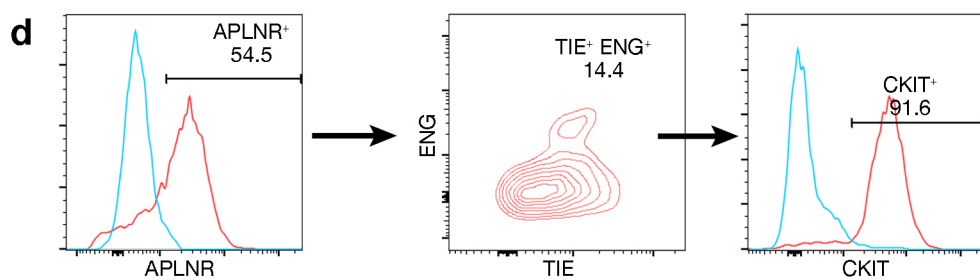
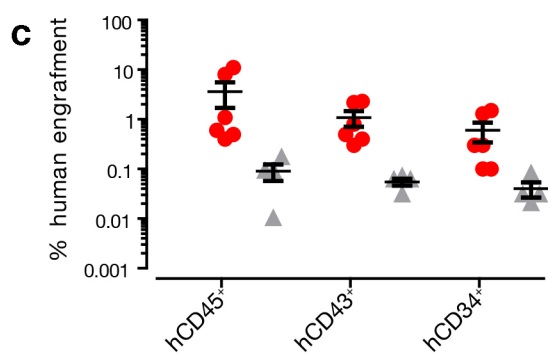
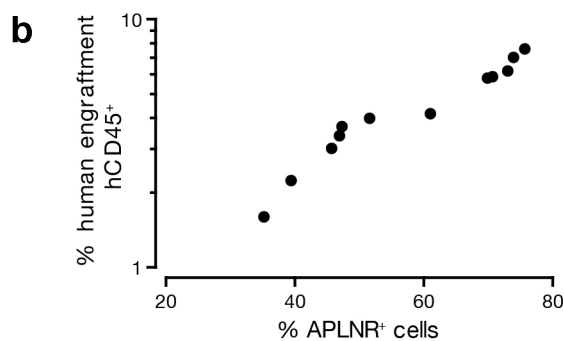
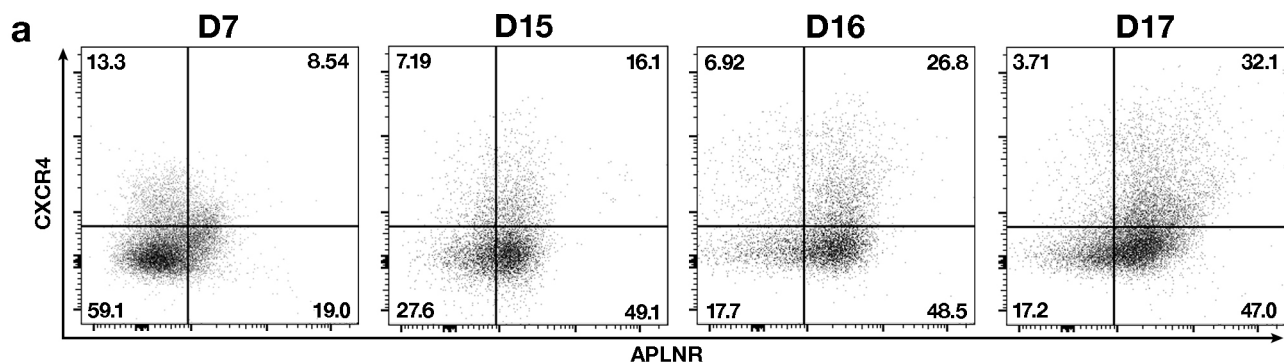


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Supplementary information

Inventory of the supplementary figures and tables

Supplementary figure 1 is related to Figure 1

Shows the heat map of the gene list and the associated hierarchical clustering. It shows that CD34⁺ cells form a first main branch and that the EBs are segregated in a second main branch. D3 to D13 EBs are separated from D15 to D17 EBs.

Supplementary figure 2 is related to Figure 2

Endothelial and hematopoietic potential carried by D16 EBs *in vivo*.

Supplementary figure 3 is related to Figure 3

It gives a complete overview of the analysis of the hematopoietic engraftment with representative examples of multilineage reconstitution.

Supplementary figure 4 is related to Figure 3

Bone marrow analysis of the hematopoietic engraftment, multilineage reconstitution of primary recipients.

Supplementary figure 5 is related to Figure 3

A complete overview of the hematopoietic multilineage engraftment of peripheral blood, thymus and spleen from D17 EB cell and CB CD34⁺ HSPCs.

Supplementary figure 6 is related to Figure 3

Bone marrow analysis of the hematopoietic engraftment, multilineage reconstitution of secondary recipients.

Supplementary figure 7 is related to Figure 4

Characteristics of the APLNR⁺ population in terms of hematopoietic engraftment.

Supplementary table 1 is related to Figure 1

Ex vivo endothelial and hematopoietic potential carried by hiPSC, HSC and D3 to D17 EBs.

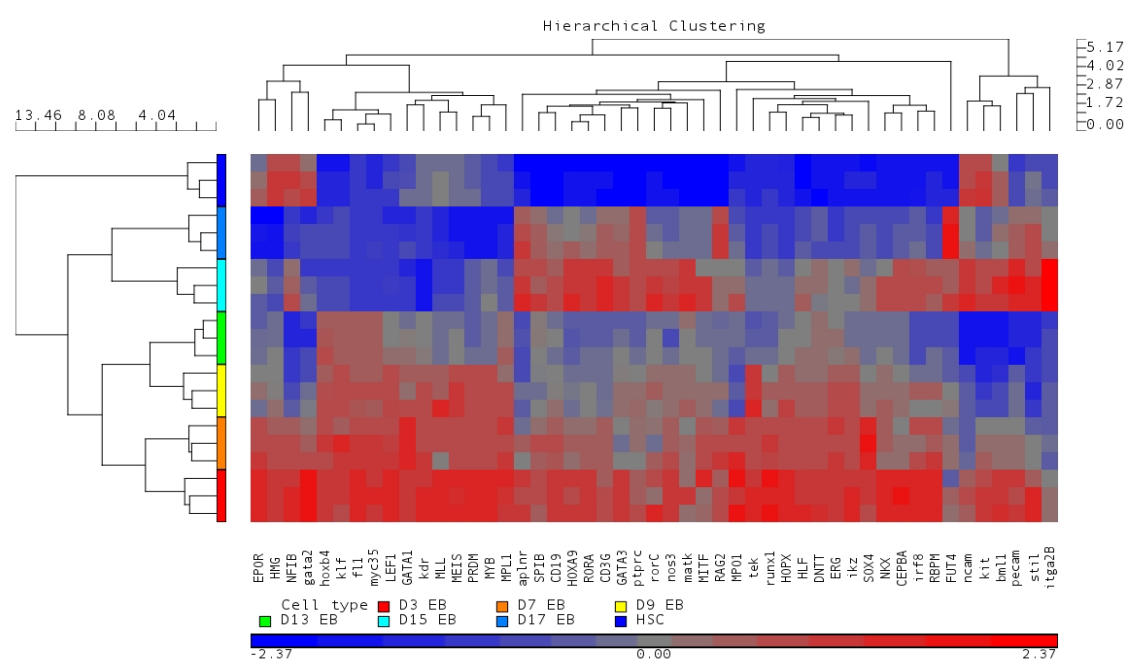
Supplementary table 2 is related to Figure 2

Ex vivo endothelial and hematopoietic potential of EBs from D15 to D17.

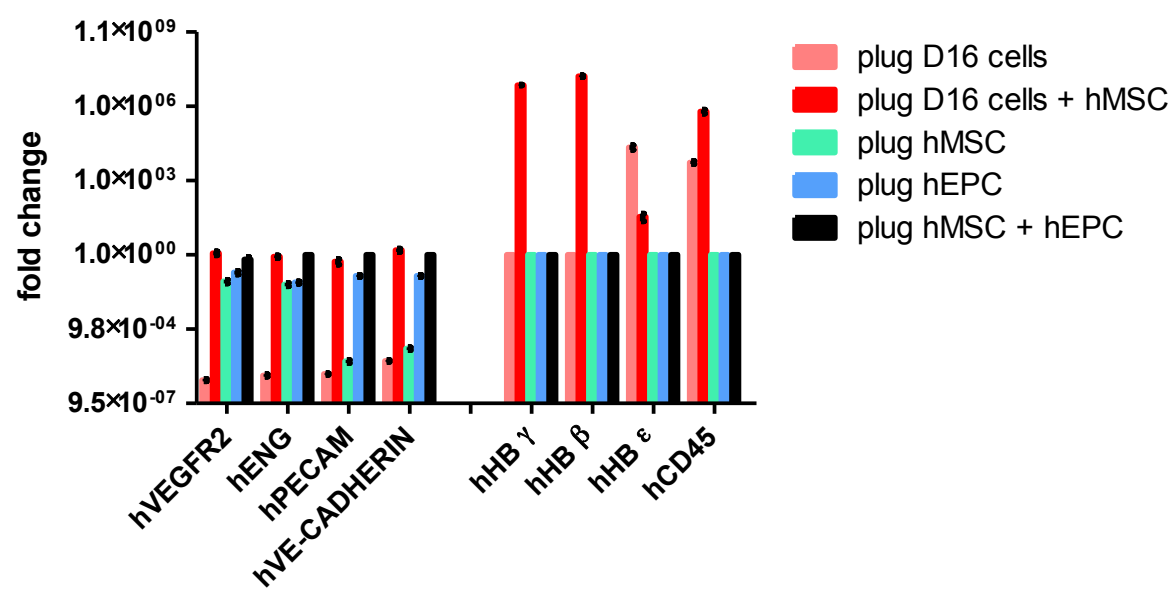
Supplementary table 3 is related to Figure 4

Ex vivo endothelial and hematopoietic potential carried by hiPSC, CD34⁺ CB HSPC and APLNR⁺, APLNR⁻ and D17 EBs.

Supplementary Figure 1

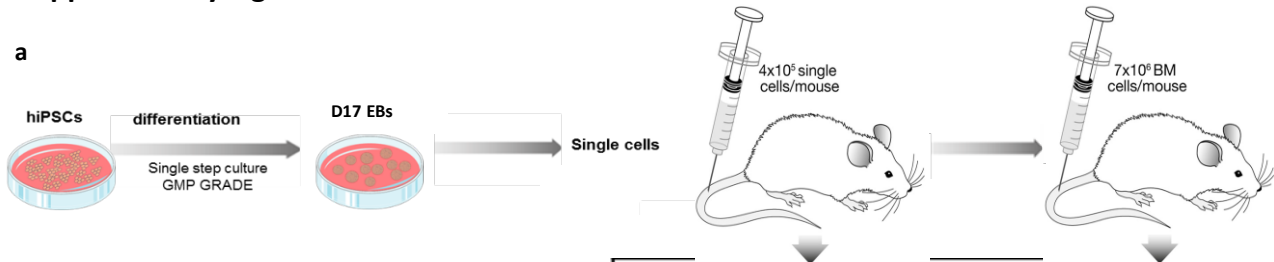


Supplementary Figure 2.

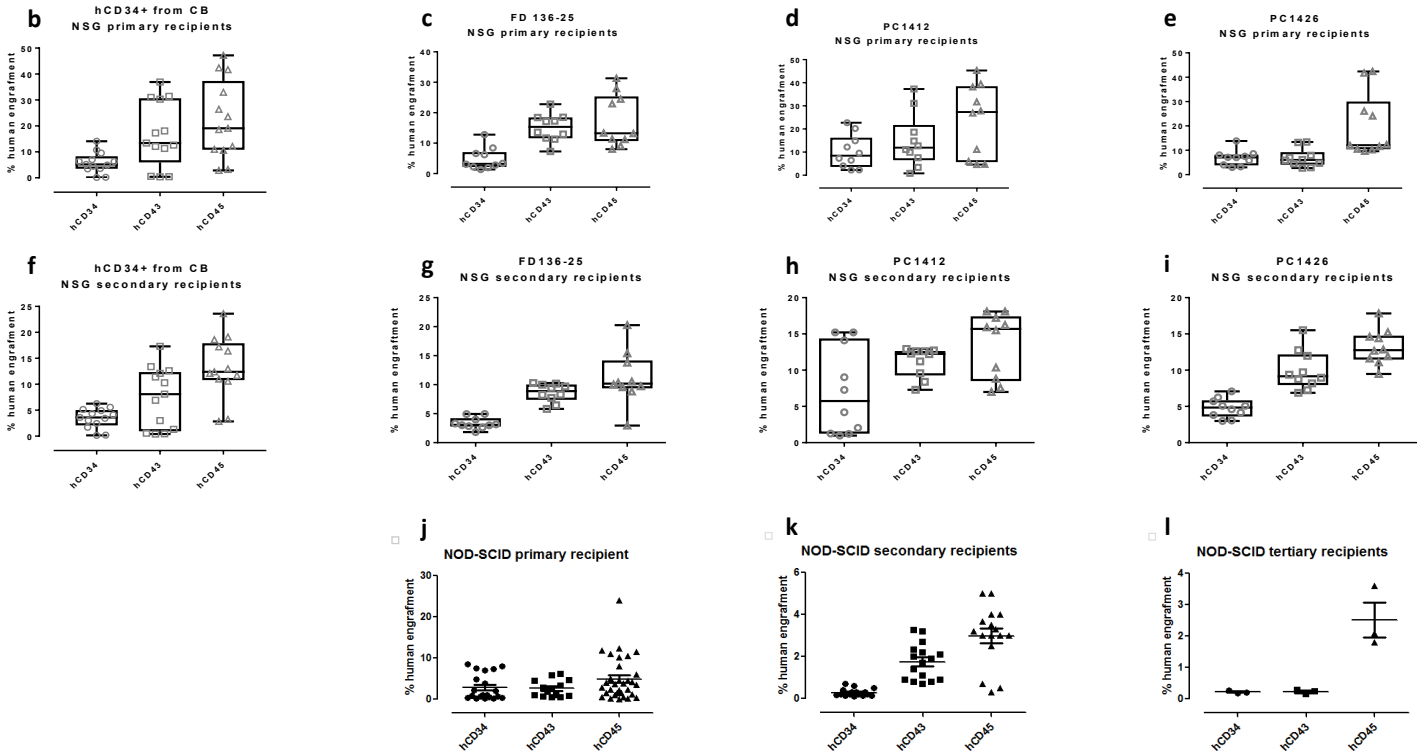


Supplementary Figure 3

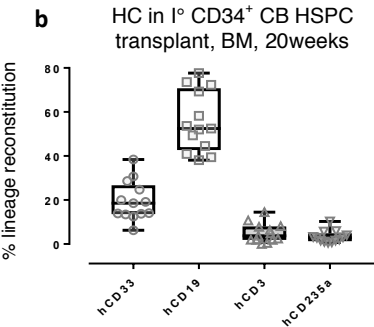
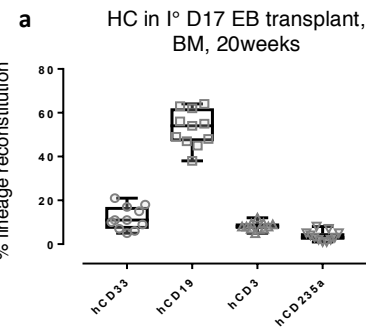
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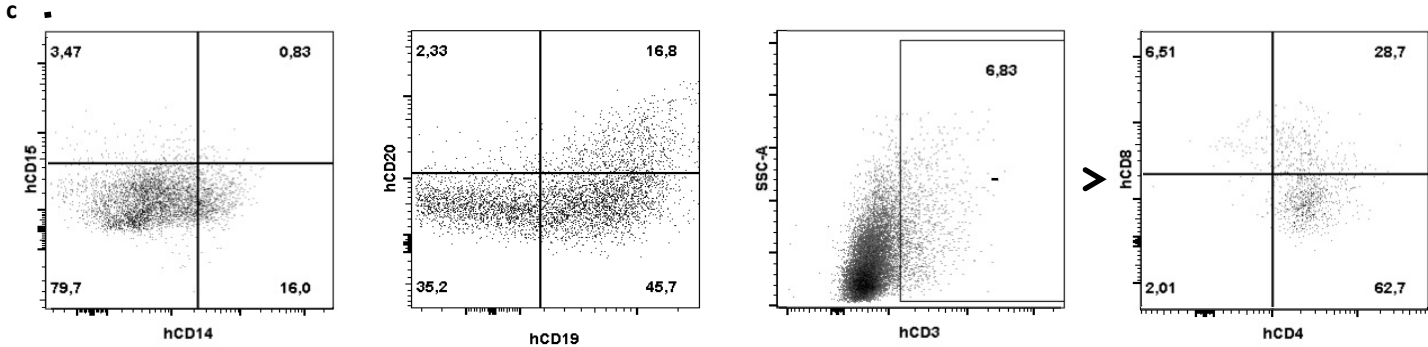
Graft assessment	Total bone marrow	culture	Facs analysis	qPCR human genes
			hCD45/mCD45 hCDd45/hCD43/hCD34	GAPDH
Multilineage assessment	hCD45 bone marrow sorting		hCD19 / hCD20 hCD14 / hCD15 hCD3 / hCD4 / hCD8	CD45 CD15 MPO ITGA2 GAPDH
	hCD45 blood sorting		hCD19 / hIgM hCD14 / hCD11B hCD3 / hCD4 / hCD8	
Stemness assessment	hCD45 bone marrow sorting	Colony assays		Beta globin Gamma globin Epsilon globin GAPDH
Definitive hematopoiesis assessment	Total bone marrow		hCD235a / hCD71	Beta globin Gamma globin Epsilon globin GAPDH
B and T cells functionality assessment	hCD45 blood sorting		TCR αβ ,TCR γδ	
	Thymus cells	CFSE labeling Stimulation with hCD3 and hCD28	hCD3/hCD4/hCD8	



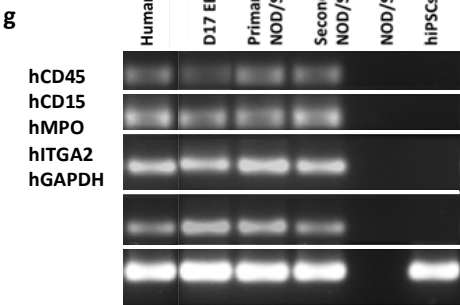
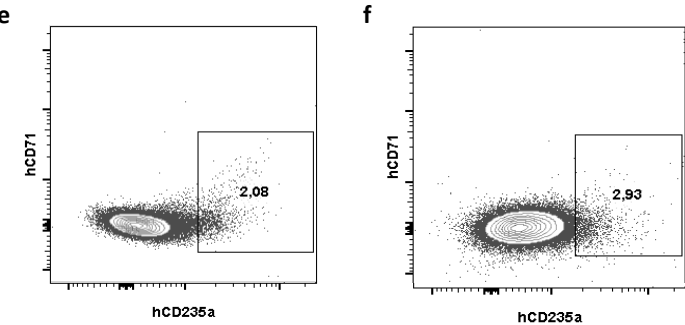
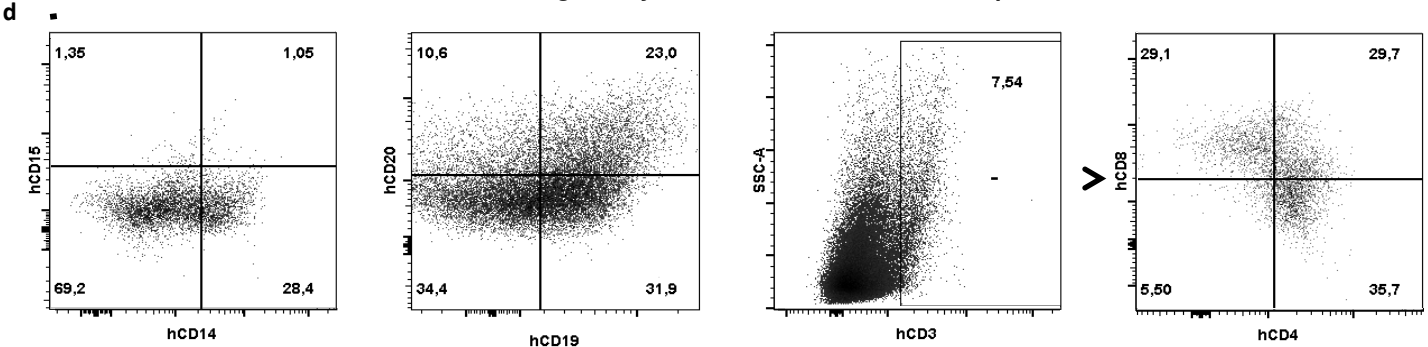
Supplementary Figure 4



Multilineage analysis in 1° D17 EB transplant

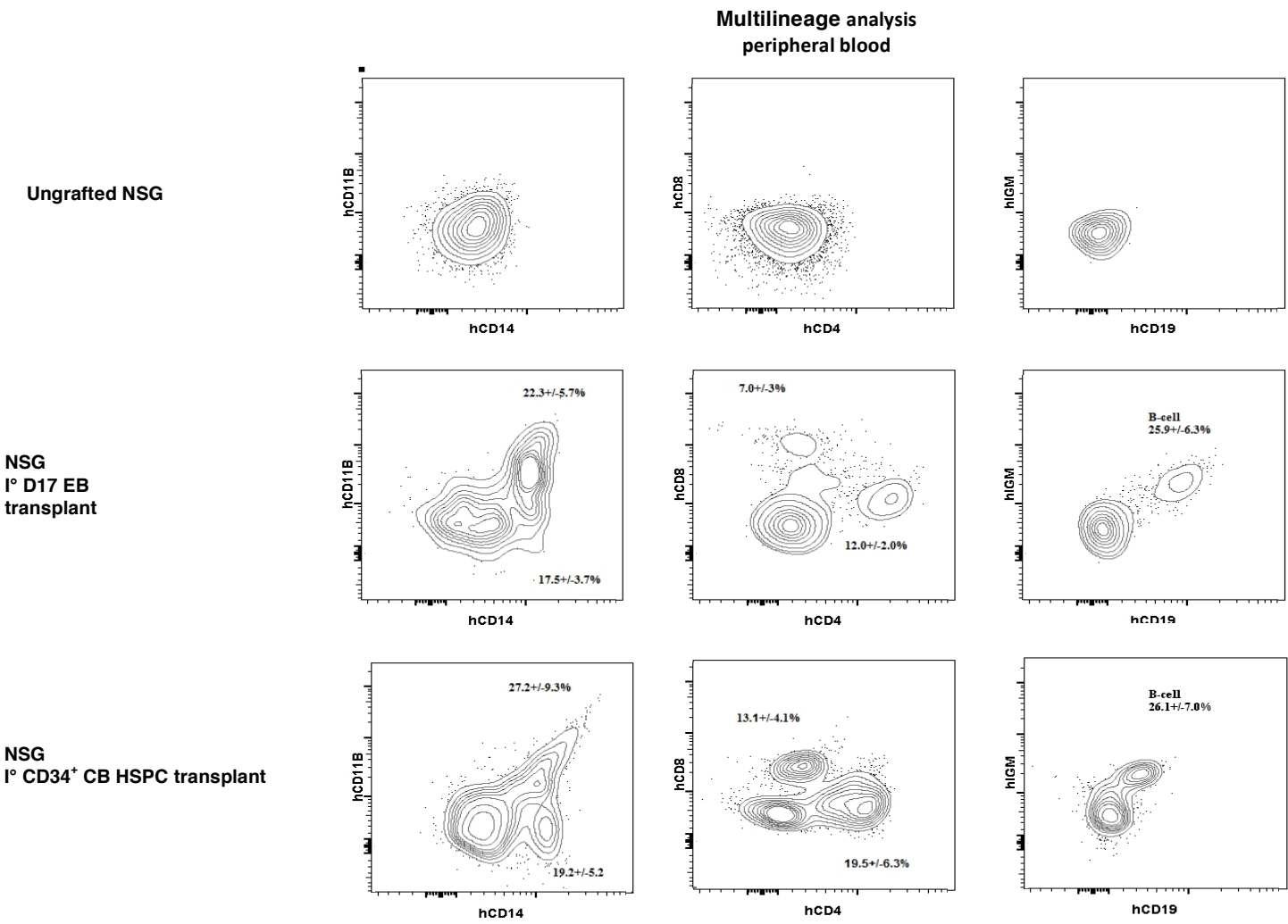


Multilineage analysis in 1° CD34+ CB HSPC transplant

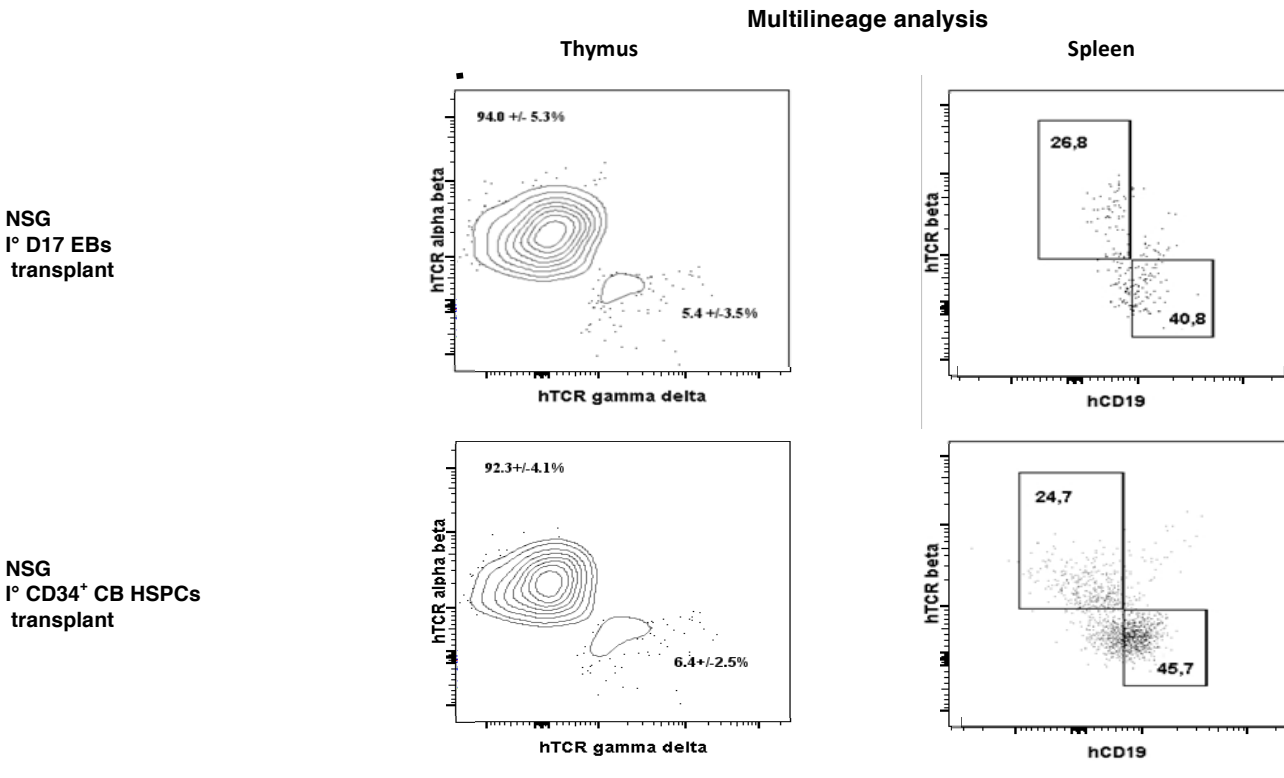


Supplementary Figure 5

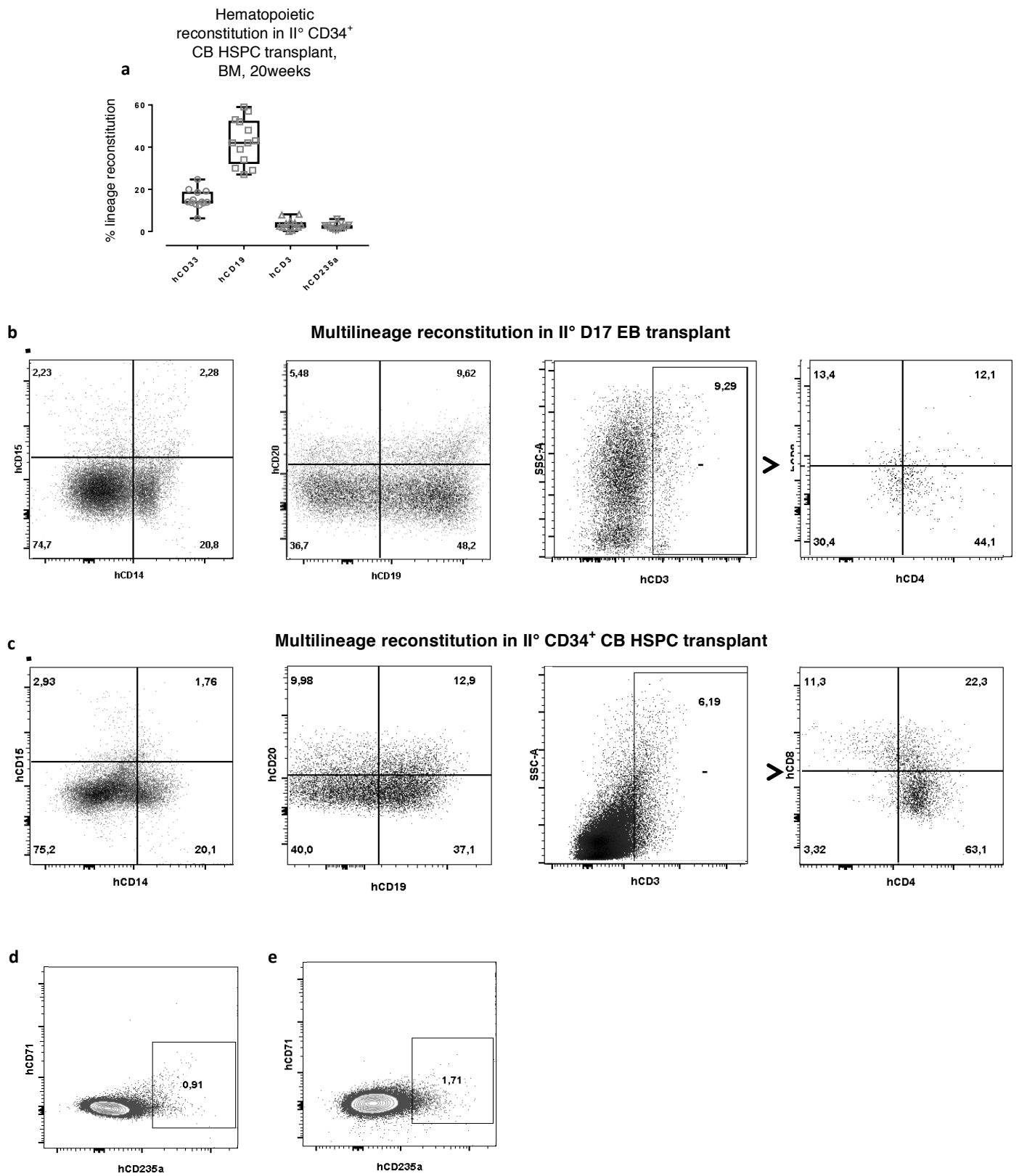
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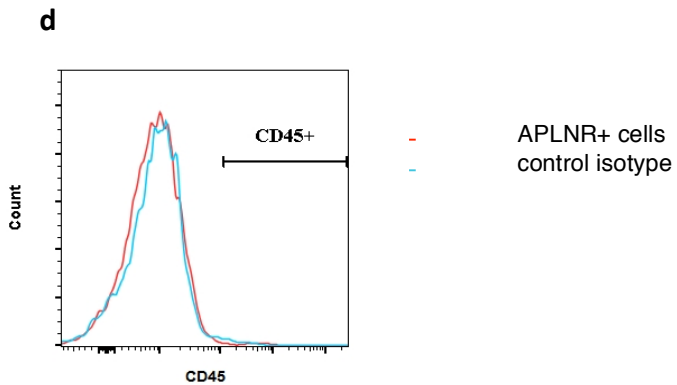
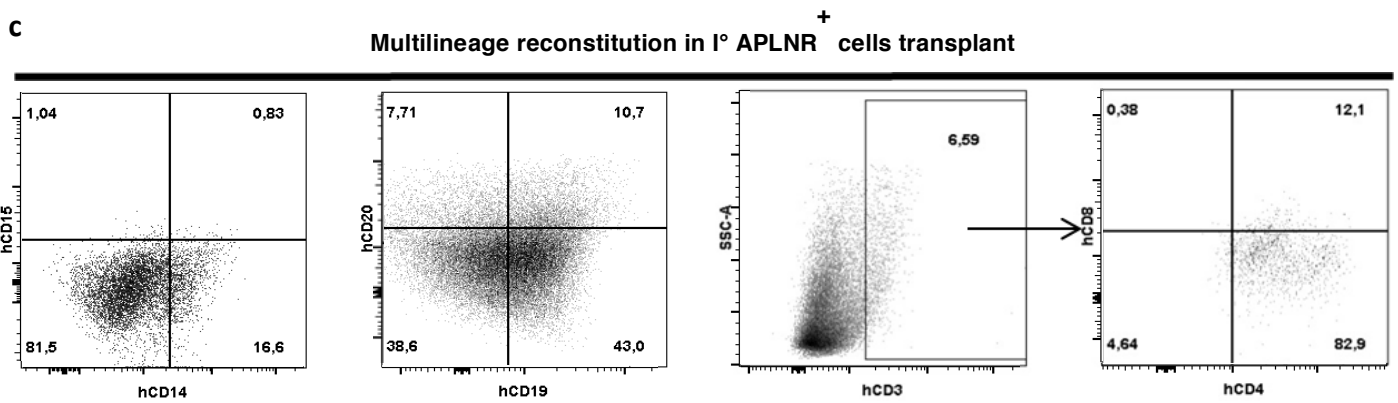
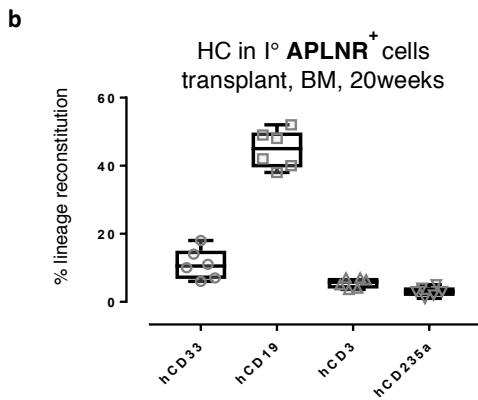
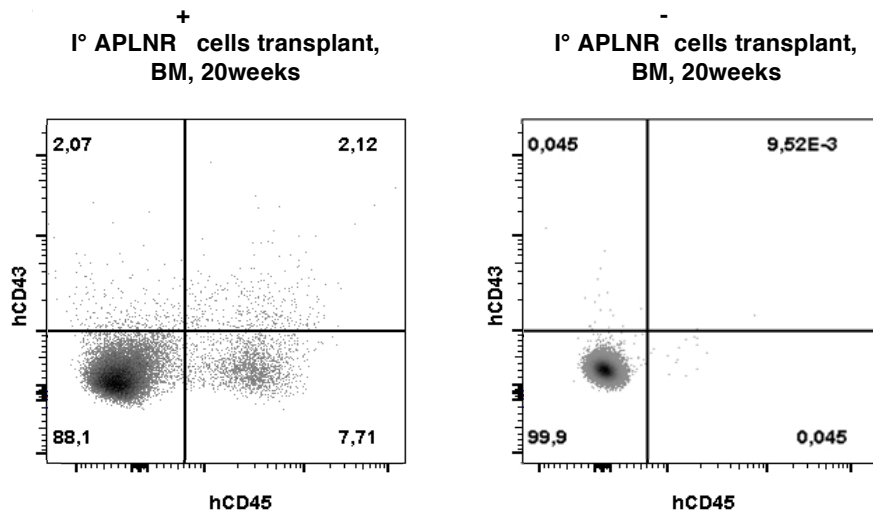
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Supplementary Figure 6



Supplementary Figure 7



Supplementary Legends

Supplementary Figure. 1. Hierarchical clustering and principal component analysis of the set of 49 genes representative of endothelial and hematopoietic commitment.

Heat map of the gene expression and the associated hierarchical clustering. CD34⁺ CB HSPCs are segregated from the EBs. D3 to D13 EBs are separated from D15 to D17 EBs.

Supplementary Figure 2. Endothelial and hematopoietic potential carried by D16 EBs *in vivo*.

Levels of expression of hVEGFR2, ENDOGLIN, PECAM-1, VE-CADHERIN, γ -globin, β -globin, ϵ -globin and CD45 in the matrigel plugs. For each gene, the fold change is the mean \pm SEM of 3 independent experiments.

Supplementary Figure 3. *In vivo* engraftment of D17 human EBs in immunocompromised mice.

(a) Experimental design. The table summarizes the different tests and analyses performed on the hematopoietic populations.

(b-l) *In vivo* engraftment capacity in immunocompromised mice. (b) CD34⁺ CB HSPCs in NSG primary recipient (n=13) at 20 weeks post-graft after inoculation of 2×10^5 CD34⁺ cells. results are in % of CD34⁺, CD43⁺ and CD45⁺ human cells. (c-e) D17 EBs from the three different hiPSCs lines (FD 136-25, PC1412 and PC1425) in NSG primary recipient (n=30) at 20 weeks post-graft after inoculation of 4×10^5 D17 EBs. Results are in % of CD34⁺, CD43⁺ and

CD45⁺ human cells. (f-i) Follow up of the secondary recipients (f) NSG secondary recipient at 20 weeks post-graft after inoculation of 7×10^6 total BM cells from b. (g-i) NSG secondary recipient at 20 weeks post-graft after inoculation of 7×10^6 total BM cells from respectively c, d, e. (j-l) Follow up of D17 EBs from the FD136-25 hiPSC line grafted in primary (e, 4×10^5 cells, n=20), secondary (f, 7×10^6 total BM cells, n=16) and tertiary (g, 7×10^6 total BM cells, n=3,) NOD-SCID recipients. Results are in % of human CD34⁺ CD43⁺ CD45⁺ cells in mouse BM at 20 (e), 20 (f) and 12 (g) weeks after transplant. Data are mean +/- SEM.

Supplementary Figure 4. Flow cytometry and qRT-PCR analysis of hematopoietic engraftment in primary BM recipients.

(a-b) Human hematopoietic cell lineages in primary recipients: (a) grafted with D17 EBs (n=30) and (b) grafted with CD34⁺ CB HSPCs (n=13). Cells were gated on hCD45⁺ expression for hCD33, hCD19 and hCD3 whereas hCD235a was analysed on whole BM cells. Data are mean +/- SEM.

(c-d) Flow cytometry analysis for myeloid (hCD14/hCD15) and lymphoid (hCD19/hCD20, SSC-A/hCD3 and hCD4/hCD8) lineages from a representative NSG primary recipient grafted with D17 EB cells (c) or with CD34⁺ CB HSPCs (d). Analyses were performed on hCD45⁺ gated BM cells.

(e-f) Flow cytometry analysis of the erythroid lineage from a representative NSG primary recipient grafted with D17 EBs (e) or with CD34⁺ CB HSPCs (f). Analyses were performed on whole BM cells.

(g) qRT-PCR analysis for hCD45, hCD15, hMPO, hITGA2 and hGAPDH expression in human BM cells (positive control), D17 EBs, NOD/SCID BM primary recipient grafted with D17 EBs, NOD/SCID BM secondary recipient, NOD/SCID BM ungrafted control, FD136-25 hiPSCs. PCR samples were run on several agarose gels. For presentation purpose, bands were assembled one above the other (white lines).

Supplementary Figure 5. Flow cytometry analysis of human hematopoietic lineages from representative primary recipients peripheral blood, thymus and spleen.

(a) Myeloid (hCD11B/hCD14) and lymphoid (hCD19/hIGM and hCD4/hCD8) lineages on peripheral blood of ungrafted recipient (1st line), of D17 EBs grafted recipient (2nd line) and of CD34⁺ CB HSPCs grafted recipient (3rd line). The analyses were performed on hCD45⁺ gated cells, aside the ungrafted recipient.

(b) Representative flow cytometry analysis of thymus and spleen cells. Thymus hCD3 gated cells exhibited a large amount of TCR alpha beta cells very close to that obtained using CD34⁺ CB HSPCs. hCD45⁺ gated spleen cells exhibited B or T lymphoid phenotype with ratios similar to those obtained with CD34⁺ CB HSPCs.

Supplementary Figure 6. Flow cytometry analysis of hematopoietic engraftment in secondary BM recipients.

(a) Human hematopoietic lineage distribution in secondary recipients grafted with 7.10^6 whole bone marrow from CD34⁺ CB HSPCs-grafted primary recipient (n=13). Cells were

gated on hCD45⁺ expression for hCD33, hCD19 and hCD3 whereas hCD235a was analysed on whole BM cells. Data are mean +/- SEM.

(b-c) Flow cytometry analysis of the myeloid (hCD14/hCD15) and lymphoid (hCD19/hCD20, SSC-A/hCD3 and hCD4/hCD8) lineages. (b) From a representative NSG secondary recipient grafted with 7.10^6 whole BM from D17 EB primary recipient. Analyses were performed on hCD45⁺ gated BM cells. (c) From a representative NSG secondary recipient grafted with 7.10^6 whole BM from CD34⁺ CB HSPCs-grafted primary recipient.

(d-e) Representative flow cytometry analysis of the erythroid lineage. Analyses performed on whole BM cells. : (d) Representative NSG secondary recipient grafted with 7.10^6 whole BM of D17 EBs primary recipient and (e) Representative NSG secondary recipient grafted with 7.10^6 whole BM CD34⁺ CB HSPCs-grafted primary recipient.

Supplementary Figure 7. Characterization of the APLNR⁺ and APLNR⁻ populations.

(a) Representative profile of hCD45 and hCD43 expression in mice BM grafted with either the D17 APLNR⁺ (left) or ⁻ (right) cell fractions.

(b) Human hematopoietic lineage distribution in primary BM recipient grafted with APLNR⁺ cells (n=6). Data are mean +/- SEM.

(c) Representative flow cytometry analysis of the myeloid (hCD14/hCD15) and lymphoid (hCD19/hCD20, SSC-A/hCD3 and hCD4/hCD8) lineages present in a primary BM recipient grafted with APLNR⁺ cells.

(d) Representative flow cytometry analysis showing the absence of CD45 expression on APLNR⁺ cells

Supplementary Tables

Supplementary Table 1. Ex vivo endothelial and hematopoietic potential carried by hiPSC, CD34⁺ CB HSPC and D3 to D17 EBs.

Refer to the attached supptable1.xlsx file.

		Day 15 EBs (n=3)	Day 16 EBs (n=3)	Day 17 EBs (n=4)
Endothelial capacity	CFC-EC	+	-	-
	Pseudo-microtubules	+++	+	-
	EC's culture	++	+++	nd
EBs hematopoietic capacity versus CD34 ⁺ CD45 ⁺ hematopoietic capacity	CFC	0	1/200	1/40
	CFC per LTC-IC	<1/600	1/15	1/4



Supplementary Table 2. Endothelial and hematopoietic potential carried by D15 to D17 EBs *ex vivo*.

EBs were probed for their capacities to give rise to either EC or HC colonies based on recognized functional tests. The highest endothelial potential was found at D15, markedly decreased at D16 and was absent at D17. In contrast, the hematopoietic potential was first detected at D16 and was high at D17.

Legend: EB: embryonic bodies; LTC-IC: Long Term Culture-Initiating Cells; CFC: Colony-Forming Cell.

Supplementary Table 3. Ex *vivo* endothelial and hematopoietic potential carried by hiPSC, CD34⁺ CB HSPC and APLNR⁺, APLNR⁻ and D17 EBs.

Refer to the attached supptable3.xlsx file.

	hiPSC_1		hiPSC_2	hiPSC_3	D3	EBs_1	D3	EBs_2	D3	EBs_3	D7	EBs_1
D7	EBs_2	D7	EBs_3	D9	EBs_1	D9	EBs_2	D9	EBs_3	D13	EBs_1	D13
EBs_2	D13	EBs_3		D15	EBs_1		D15	EBs_2		D15	EBs_2	D15
EBs_3	D17	EBs_1		D17	EBs_2		D17	EBs_3		D17	EBs_3	CD34
HSPCs_1	CD34	HSPCs_2		CD34	HSPCs_3							
HOPX	7.3714	7.0732		6.6233	13.3164		13.2554	13.6364		10.5444		
9.9853	9.7502	9.3740		8.8180	10.8621		6.7338	6.3784		7.1156		
6.2102	6.0942	5.3418		2.9681	2.6392		2.2135	1.0406		0.7402		
0.2861												
RBPM	4.0737	3.7816		3.4865	12.0818		12.0361	12.4018		10.0581		
10.4622	11.1729	9.6973		7.8417	8.5548		7.9122	6.8338		6.8549		
10.4894	10.0099	9.8023		6.7166	6.5380		5.9919	4.6664		4.6541		
3.9029												
PRDM	6.2481	6.4327		5.7977	11.0850		11.0518	11.4050		9.0673		
8.5028	9.2207	8.9212		8.3635	9.0746		4.7363	4.3735		4.4036		
3.8602	4.1314	3.5279		-0.7339	-0.4540		-1.2654	4.4435		4.6541		
3.8168												
NKX	8.9189	9.0755		8.4508	18.8239		18.6945	18.6562		14.5958		
14.0516	13.6140	13.7424		13.2024	14.0354		12.1006	11.7649		11.8710		
15.9205	16.2438	12.0193		9.4827	9.5973		8.8511	4.5224		4.6541		
3.8908												
GATA3	10.2627	10.3681		9.8175	19.5774		19.9357	19.3531		13.6532		
14.2512	14.8428	15.8103		15.1308	15.7498		14.8763	12.4039		13.5076		
19.5109	20.1576	17.0843		16.3061	16.6025		15.8884	4.6664		4.7392		
4.1292												
TEK	6.6877	6.9123		6.2624	13.1168		13.9377	14.3273		12.1146		
11.6596	12.3660	13.7268		13.2512	13.9444		8.4572	7.1540		7.4739		
8.6071	8.7656	7.9550		5.5029	5.7497		5.0712	4.6664		4.6541		
4.1491												
NOS3	11.3284	11.7808		11.0486	19.1094		18.9764	18.6215		16.5221		
15.9851	17.3272	15.5785		15.0454	15.2645		13.2662	12.9348		11.2083		
18.3889	19.2071	18.2240		12.3298	12.8229		11.9933	4.6664		5.0028		
4.3299												
MATK	5.4948	5.6179		5.0033	9.0663		9.6657	12.8194		9.7967		
8.7035	9.7284	9.5438		9.3911	10.2241		8.3670	9.5993		8.7393		
12.9506	13.3972	12.0185		7.9681	8.0673		7.4200	0.6980		0.8962		
0.1638												
MITF	8.1788	8.4535		7.7597	19.5774		16.0575	16.0192		17.8103		
16.8891	17.4936	15.8103		15.1308	15.9637		14.8763	14.4037		14.5437		
18.4583	18.4342	14.8675		14.6030	14.4630		14.0920	8.4066		8.5481		
7.8257												
HLF	10.0255	10.5624		9.7133	19.5774		19.9357	19.5477		17.8103		
17.1308	17.8171	15.8103		15.1308	15.8171		14.8763	14.4037		14.3970		
14.8994	16.1316	14.0049		11.1736	11.8465		11.1662	5.0447		5.5719		
4.7440												
HMG	-1.3810	-1.6508		-1.9730	4.9250		4.9682	5.2449		3.5230		
2.9381	3.6764	1.7828		1.1989	1.9362		1.3622	0.9870				
1.0296	-0.0661	-0.8822		-1.2771	-4.2257		-4.6088	-4.9812		4.6664		
4.6541	4.3034											
MEIS	4.9645	5.4892		4.7154	11.0528		11.0199	10.6818		8.9966		
8.4318	8.6233	9.4280		8.8722	9.4895		5.4137	5.0535		5.4066		
1.4695	2.0969	1.1000		-0.9310	-0.3504		-1.2367	4.6664		5.1286		
4.3607												
MLL	2.5769	3.4352		2.2761	9.8497		10.5028	10.1927		7.2192		

7.1551	5.0987	6.9688	6.4897	10.6278	4.2281	3.5527	4.1243
0.1483	1.4081	0.4149	-0.7987	0.1009	-1.1079	4.6664	4.6541
4.2021							
IRF8	9.1168	10.0666	9.0463	19.2218	19.9357	19.3586	13.9610
17.1308	16.6983	15.6409	11.1636	12.7477	9.3879	8.4117	8.8355
16.0534	17.0795	15.7855	10.7760	11.7956	10.6941	3.0656	4.2763
2.8577							
RUNX1	4.5033	4.8527	4.0825	10.1772	10.1867	9.2439	7.7288
7.2491	7.4296	6.0467	4.7498	5.4420	3.9484	3.2351	3.3319
3.7874	4.0487	3.6351	1.8341	2.0904	1.3214	0.5525	0.9204
0.0752							
KDR	0.3740	0.7412	0.0466	10.2469	11.5504	11.5122	9.3294
9.3478	10.1808	8.5188	7.9954	8.8284	5.7831	5.3568	
5.4968	-2.5139	-2.4042	-2.5584	0.4948	0.8634	0.1555	4.6664
4.6541	4.2470						
FLI1	5.2316	5.4024	4.7735	19.5774	19.9357	19.6188	17.8103
17.1308	17.7204	15.8103	15.1308	15.5266	14.8763	14.4037	14.5437
5.2316	5.4024	4.7735	5.2316	5.4024	4.7735	5.2316	5.4024
4.7735							
MYC	1.2180	1.7649	0.9357	7.7928	7.6512	8.0825	5.8577
5.3499	6.5120	3.7102	2.8433	3.3566	2.9711	2.3159	
2.8709	-7.9068	-8.1330	-8.7345	-7.9401	-7.4231	-8.2119	-10.0795
-9.5781	-10.3890						
H0XB4	6.0216	6.1387	5.5368	8.6522	8.5261	8.2073	7.8336
6.8078	7.1817	5.8433	5.5142	6.8133	5.9547	5.0267	
5.7937	-1.1602	-0.8976	-2.6499	-1.5792	-1.4416	-2.0085	-5.0263
-4.9096	-5.5225						
NFIB	4.5185	4.4276	3.9068	8.2167	8.8587	8.5744	7.0850
6.8654	7.6433	3.9541	2.8493	4.2543	2.8547	1.8207	2.1265
6.4076	7.5474	6.0778	3.3816	3.1509	2.6873	8.0706	7.3624
7.1720							
RAG2	3.2504	3.3148	2.7597	1.7425	2.2910	3.0163	2.6533
2.0198	3.5713	0.5403	-0.6392	-0.4012	-0.5334	-1.1539	-0.8860
0.4973	0.4935	-0.0518	3.3718	3.2147			
2.7047	-7.0681	-7.4822	-7.5066				
SPIB	-2.2027	-2.1636	-2.7222	14.1956	13.4104	13.7933	13.8401
12.1497	13.3049	9.9993	10.1805	10.8849	10.7524	11.1396	11.1450
13.5194	16.2929	13.8033	12.6414	12.6288			
12.0653	-0.8492	-0.2420	-0.9642				
S0X4	10.4954	10.1914	9.1848	15.7257	15.6348	16.0457	17.8103
16.7660	17.4537	13.4747	12.9338	13.6281	12.2670	11.9319	11.9343
13.7376	13.2264	12.3050	11.4967	11.2336	10.1767	8.2218	7.7004
6.8193							
MYB	5.7862	5.7702	5.1635	15.2467	15.1617	15.1235	12.9687
12.4185	13.2515	12.9298	12.3869	13.2198	9.0614	8.7145	8.8545
9.4659	9.6166	9.4207	3.2386	3.2335	2.5982	7.4350	7.3877
6.7525							
H0XA9	12.6539	13.6803	12.1869	19.5774	19.9357	19.0150	17.8103
17.1308	16.8642	15.8103	15.1308	15.4163	14.8763	14.4037	14.0700
20.1234	21.5431	19.3669	16.4051	17.4507	15.9109	9.3995	10.2691
8.8499							
ERG	5.8874	6.2409	5.4849	14.4151	15.5593	15.5210	13.0553
14.5256	15.3585	12.6419	11.9463	12.7792	9.9676	9.9155	10.0555
9.3980	9.8957	9.4470	4.6106	5.1559	4.2727	0.0409	

0.3615	-0.3499						
RORA	12.6885	13.2743	11.6565	19.5774	19.9357	19.6007	17.8103
17.1308	17.8171	15.8103	15.1308	15.8171	14.8763	14.4037	14.3970
20.2953	20.4542	19.5193	16.4397	17.0447	15.4431	8.8263	9.4026
7.7562							
LEF1	3.3233	3.0408	2.4735	10.2337	10.2111	10.5537	8.3351
7.7679	8.4885	8.1420	7.5815	8.2954	4.7258	4.3630	
4.3932	-0.0990	-0.1473	-1.1553	-1.1492	-1.4735	-2.0176	
0.4274	-0.1945	-0.5986					
CEPBA	3.6455	3.4011	3.0264	8.2969	8.2983	8.8644	2.7402
2.1524	2.8936	2.8535	2.2736	2.8620	1.1825	0.8066	0.9023
5.1752	5.1549	5.1464					
0.0303	-0.2396	-0.5983	-4.7972	-5.0360	-5.4256		
EPOR	6.5140	6.0491	5.6933	11.4750	11.1677	11.5333	10.0831
9.5224	10.3553	8.4187	8.0356	7.5322	7.4411	7.0232	7.3219
7.9699	6.9438	7.6577	4.4829	3.8647	3.5703	8.5875	9.1469
7.5455							
GATA1	8.7722	10.4808	8.0736	16.3199	16.2091	16.0201	17.8103
17.1308	17.4407	12.4171	13.2872	14.4484	12.2203	10.4666	11.9413
7.0414	7.0511	5.2609	6.2853	7.6878	5.1171	8.9147	10.8537
8.2660							
IKZ	6.6242	6.7153	6.1183	12.3638	12.8589	13.6536	11.1133
10.8750	11.0953	10.8082	9.8302	10.6631	8.8854	7.1990	7.3390
7.6197	8.3593	9.0394	5.9941	5.4902	5.3246	2.9345	1.9589
1.8899							
KLF	11.4937	12.1131	11.1870	16.7194	16.1611	16.8892	17.8103
14.4879	16.9322	15.8103	15.1308	15.4761	14.8763	14.4037	14.5437
9.8417	10.3439	9.0712	9.8299	10.1432	9.3468	6.6165	6.6930
6.0229							
MPL1	12.9788	13.8778	12.1867	18.3643	17.9191	18.0651	16.3234
17.1308	17.8566	15.8103	14.1328	15.7409	14.8763	14.4037	13.9442
11.0722	11.7014	10.2042	6.9502	7.7831	6.1766	9.8195	9.9893
8.5215							
NCAM	12.8672	13.5782	12.6086	6.5597	6.7878	7.1229	4.0498
3.4408	4.1262	3.7648	2.5405	3.5555	-0.0298	-0.1206	-0.4468
8.8082	9.7399	8.6319	5.1943	5.6921	4.9720	7.8237	8.5147
7.5085							
PECAM	17.0334	17.0891	16.3635	13.0888	12.9874	12.3399	10.7956
10.3021	11.0177	9.6331	8.7304	9.5323	5.6790	5.7804	6.1118
14.9788	14.7874	14.3060	11.5790	11.5794	10.8030	7.8595	7.9270
7.1646							
PTPRC	9.1821	9.2906	8.7038	7.4861	8.3684	6.8817	6.7439
6.1671	5.9121	6.6060	5.9549	6.7878	4.6872	4.0338	4.1738
8.2761	8.4459	7.8949	8.4380	8.4207			
8.1129	-1.1682	-1.0555	-1.6517				
RORC	15.5461	15.8810	14.6577	16.7992	19.1554	15.6776	14.3420
15.0846	16.2194	15.8103	15.1308	15.0568	14.8763	14.4037	14.5437
18.2071	18.6152	17.2936	14.1428	14.6540	13.1596	9.2457	9.6241
8.3088							
FUT4	4.5133	5.3964	4.3866	-0.0675	2.2475	2.5189	1.9334
1.0635	2.4434	1.7881	0.4176	1.8154	-0.0623	-0.5931	-0.5400
3.0826	4.1432	2.8111	6.4065	6.5130			
5.7509	-3.4111	-2.5714	-3.6297				
APLNR	17.0324	17.5544	16.1144	16.0328	15.8920	15.5785	14.7484

13.2436	13.8934	13.2144	11.7176	12.7121	12.1808	11.8383	12.2783
16.5968	17.1793	15.4520	15.5791	16.1511	14.6163	9.8259	10.3607
8.8922							
BMI1	17.0457	16.8117	16.0815	8.2926	8.8724	8.6128	6.7697
6.2378	7.0344	4.6903	3.8838	5.1331	3.2223	2.5242	2.7022
8.9816	8.6204	8.1051	6.6656	6.5258	5.7374	7.5189	7.2786
6.5403							
ITGA2-B	11.0373	11.6504	10.8464	6.6302	7.1970	7.4826	5.9472
5.3028	5.1924	5.1226	4.0247	5.0200	4.1307	3.6185	3.9329
13.1184	13.4129	13.0793	6.6071	7.1950	6.4328	4.1531	4.7657
3.9557							
MP01	12.5047	13.5649	12.1909	15.3298	15.2683	13.8288	12.7473
13.2782	14.2574	7.9865	7.1271	7.6882	8.6489	8.9153	8.9926
10.7283	12.2185	10.1319	8.0890	9.2884	7.6140	4.9503	5.9813
4.6180							
STIL	16.3427	16.1914	15.1624	8.7871	10.3516	10.3236	7.8353
7.0348	7.6268	4.7592	5.7768	5.6189	5.5032	5.0863	5.0633
10.6395	10.7712	9.1835	9.3565	9.2100	8.2109	7.5237	7.3526
6.3377							
KIT	13.6601	13.9053	13.2368	6.0285	6.3778	6.3449	4.9774
4.5186	5.4457	2.7680	2.0804	3.0100	1.6865	1.3571	1.4477
6.1602	6.6672	5.9113	3.8607	4.2344	3.5268	6.6609	6.9222
6.2251							
GATA2	10.4935	10.8050	9.6199	9.8459	10.1980	10.1597	7.9024
7.1197	7.9526	6.5856	5.4597	6.6480	5.4874	5.0709	4.3838
6.1647	6.3061	5.0153	5.2388	5.4945	4.3228	8.2535	8.4898
7.2164							
CD19	15.1001	15.2333	13.5740	19.5774	19.9357	19.7970	17.8103
17.1308	17.2897	13.6412	13.2544	15.0616	10.5852	11.9274	13.2127
17.7611	17.9675	16.2099	13.6967	14.0062			
12.0759	-2.3351	-2.2501	-4.0246				
CD3G	16.0572	15.2338	14.0264	14.6337	15.0801	15.0419	13.6808
13.6161	14.4490	11.3041	10.5380	11.3710	10.8488	9.3107	9.4507
18.7183	17.9679	16.6178	14.6039	13.8305	12.5283	3.0172	2.1854
0.9678							
DNTT	15.1001	13.8089	15.1624	19.5774	19.9357	19.7770	17.8103
17.1308	17.4201	15.8103	15.1308	15.8063	14.8763	14.4037	14.5437
14.6277	13.0667	14.6228	10.7456	9.1485	10.6705	4.1302	2.1332
3.6867							

	hiPSCs	APNLR+	APNLR-	D17 EBs	CD34 HSPCs
CMYC	6.23	5.04	4.58	4.21	6.66
HOXB4	8.52	6.52	5.58	4.57	6.66
LIN28	-1.89	1.54	2.04	-2.89	-7.25
NANOG	-2.38	1.71	2.77	0.68	-9.33
POU5F	-6.44	2.96	1.48	3.42	-8.11
SOX2	-3.02	0.37	0.76	2.71	-8.13
FUT4	-2.85	2.94	4.19	0.24	-4.59
APNLR	9.80	10.43	12.16	15.19	7.00
BMI1	9.80	7.46	6.32	6.23	7.00
ITGA2B	3.54	6.48	4.70	6.66	1.54
MP01	5.23	4.81	5.92	7.41	7.00
STIL	9.80	10.15	11.72	8.54	7.00
KIT	7.91	4.40	3.01	4.02	7.00
GATA2	9.80	7.12	6.10	7.38	5.51
CD19	9.80	10.43	12.16	17.00	-0.90
CD3G	9.80	10.43	12.16	14.13	7.00
NCAM	5.42	4.55	2.60	4.50	7.00
PECAM	9.80	5.11	6.54	6.36	7.00
PTPRC	1.43	8.82	8.08	5.42	-2.89
RORC	9.80	10.43	12.16	17.02	7.00
ERG	9.31	6.26	9.52	10.62	3.18
GATA3	11.81	7.40	9.77	9.78	6.66
HOXA9	9.31	7.84	9.77	10.66	4.61
IRF8	10.45	4.50	8.88	9.27	4.33
KDR	5.58	3.71	9.77	2.90	6.66
MITF	6.74	7.41	8.55	5.44	6.66
MLLT3	7.16	2.57	6.06	2.93	6.66
RBPM5	6.41	4.79	7.63	4.78	6.66
RUNX1	6.17	0.36	3.81	2.01	2.98
TEK	9.05	4.52	9.77	5.59	6.66
HMG	0.10	-0.16	-0.29	8.69	6.66
HOPX	7.72	4.97	4.49	12.11	2.71
LEF1	6.21	4.57	5.98	9.50	4.61
MEIS	6.72	4.10	3.71	8.09	6.66
MYB	6.21	7.03	6.71	12.30	4.61
NKX	8.61	7.73	6.71	13.66	6.66
PRDM	7.28	2.69	2.04	7.66	6.66
NOS3	9.73	7.73	5.46	9.55	6.66
SOX4	5.90	7.73	5.46	6.98	3.61
CEPBA	-1.83	-3.41	-3.45	-4.20	-8.86
HLF	9.10	7.73	5.46	10.47	6.66
RORA	5.90	7.73	5.46	10.47	4.61
EPOR	6.64	6.64	3.22	6.86	3.05
GATA1	12.99	7.96	6.79	10.16	7.06
IKZ	8.58	6.35	5.98	8.66	6.19
KLF1	12.17	13.84	5.12	13.17	4.57
MATK	10.83	8.93	5.93	9.28	2.80
MPL1	16.29	11.74	6.79	13.12	6.08
NFIB	4.84	3.32	2.21	3.43	4.61
RAG2	3.64	-2.64	-0.40	-2.60	-5.56
SPIB	-1.82	11.27	6.79	11.31	-3.65
HLF	9.10	7.73	5.46	10.47	6.66