Reprogramming human endothelial cells to haematopoietic cells requires vascular induction

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Generating engraftable human haematopoietic cells from autologous tissues is a potential route to new therapies for blood diseases. However, directed differentiation of pluripotent stem cells yields haematopoietic cells that engraft poorly. Here, we have devised a method to phenocopy the vascular–niche microenvironment of haemogenic cells, thereby enabling reprogramming of human endothelial cells into engraftable haematopoietic cells without transition through a pluripotent intermediate. Highly purified non–haemogenic human umbilical vein endothelial cells or adult dermal microvascular endothelial cells were transduced with the transcription factors FOSB, GFI1, RUNX1 and SPI1 (hereafter referred to as FGRS), and then propagated on serum–free instructive vascular niche monolayers to induce outgrowth of haematopoietic colonies containing cells with functional and immunophenotypic features of multipotent progenitor cells (MPPs). These endothelial cells that have been reprogrammed into human MPPs (rEC–hMPPs) acquire colony–forming–cell potential and durably engraft into immune–deficient mice after primary and secondary transplantation, producing long–term rEC–hMPP–derived myeloid (granulocytic/monocytic, erythroid, megakaryocytic) and lymphoid (natural killer and B cell) progenies. Conditional expression of FGRS transgenes, combined with vascular induction, activates endogenous FGRS genes, endowing rEC–hMPPs with a transcriptional and functional profile similar to that of self–renewing MPPs. Our approach underscores the role of instructive cues from the vascular niche in coordinating and sustaining haematopoietic specification and may prove useful for engineering autologous haematopoietic grafts to treat inherited and acquired blood disorders.

Manufacturer of autologous, engraftable haematopoietic stem and progenitor cells (HSPCs) offers tremendous therapeutic potential. Using in vitro cultures, human pluripotent stem cells can be differentiated into haematopoietic progenitors, which often have limited expansion potential and do not engraft myeloablated recipients1–3. Enforced expression of transcription factors has also been used to reprogram somatic cells into haemogenic lineages4–6. Employing cellular fusion, we have shown that direct conversion of somatic cells into fetal HSPCs is also feasible7. However, these previous efforts have been unable to produce human haematopoietic cells capable of long-term multilineage engraftment8–10. We hypothesized that in addition to transcription factor expression, haematopoietic specification and long-term engraftment may require instructive signals from the microenvironment. Indeed, the central instructive role of tissue–specific endothelial cells in supporting organ regeneration9,10, including haematopoietic stem–cell (HSC) self-renewal and reconstitution of multilineage haematopoiesis, has recently come to light11–18.

In mammals, definitive HSCs originate in the vascular microenvironment of the aorta–gonad–mesonephros (AGM)19–24, placenta25 and arterial vessels26. Putative HSCs bud off from haemogenic vascular cells lining the dorsal aorta floor and umbilical arteries, where they are in cellular contact with non–haemogenic endothelial cells27. This ontological endothelial–to–haematopoietic transition (EHT) is mediated in part through expression of the transcription factor RUNX1 (ref. 21), its non–DNA–binding partner core binding factor–β (ref. 28), GFI1 and GFI1b (refs 29, 30). However, the contribution of microenvironmental instructive signals provided by anatomically distinct niches and tissue–specific vascular niches8 within the AGM, fetal liver and placenta remain poorly defined.

We have identified a minimal set of four transcription factors—FOSB, GFI1, RUNX1 and SPI1 (FGRS)—that reprogram full-term human umbilical vein endothelial cells (HUVECs) and human adult dermal microvascular endothelial cells (hDMECs) into haematopoietic cells with long-term MPP activity (rEC–hMPP). The reprogramming was successful only when a unique serum–free vascular niche platform was used. Subsets of rEC–hMPPs were immunophenotypically marked as HSCs and were capable of long-term primary and secondary multilineage engraftment in immunodeficient mice. We demonstrate that constitutive or transient expression of FGRS transcription factors combined with instructive signals from specialized vascular niche cells11,12,13 are essential for efficient conversion of endothelial cells into rEC–hMPPs.

FGRS and vascular induction reprogramming

Primitive HSCs emerge on a vascular bed during development. Thus, we hypothesized that executive functions of the vascular niche could have an important role during reprogramming by inducing and maintaining nascent haematopoietic cells. Since serum impairs vascular function and interferes with expansion of HSCs and MPPs, we devised a vascular niche model in which endothelial cells transduced with the adenoviral E4ORF1 gene (E4ECs, VeraVecs) could be cultured without serum11,12,13. E4ORF1 activates survival pathways in endothelial cells without provoking proliferation or cellular transformation and thereby maintains tissue–specific

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functional and metabolic attributes of endothelial cells. E4ECs derived from HUVECs3,11,12,33 or endothelial cells purified and propagated from haematopoietic organs32,33 balance self-renewal and differentiation of human and mouse long-term HSCs and MPPs by producing physiological levels of Notch ligands, Kit ligand, BMPs, Wnts and other angiocrine factors4,4.

To identify transcription factors that drive EHT, we first identified transcription factors differentially expressed by Lin–CD34+ umbilical cord HSPCs, but not by HUVECs (Extended Data Fig. 1a–d). We then cultivated CD45-CD133-c-Kit+ HUVECs that were devoid of haemogenic potential4 (Fig. 1a) and transduced them with lentiviral vectors expressing various combinations of differentially expressed transcription factor transcripts using GFP as a marker. After 3 days, transduced HUVECs were re-plated onto subconfluent serum-free E4EC monolayers, to force cellular interaction of HUVECs potentially undergoing EHT with inductive vascular niche cells. Within 2 weeks of co-culture with E4ECs, round GFP+CD45+ cells began to bud from transduced HUVECs and form grape-like colonies (Fig. 1b). Systematic one-by-one dropout of candidate transcription factors demonstrated that expression of FGRS transcription factors was necessary and sufficient for haematopoietic reprogramming of HUVECs (Extended Data Fig. 1b, c).

Co-culture of FGRS-transduced endothelial cells with E4EC monolayers augmented the yield and stability of the haematopoietic-like colonies, which displayed morphological features of haematopoietic progenitors (Fig. 1c). Within 4 weeks of co-culture with E4ECs, FGRS-transduced endothelial cells began to proliferate and form GFP+CD45+ colonies (Fig. 1a, c). Serum suppressed colony formation and naive HUVECs could not survive without serum and failed to support the emergence of CD45+ cells (Fig. 1d). FGRS transduction of 5 x 10^5 HUVECs followed by 3 weeks of serum-free co-culture with E4ECs yielded 32.3 ± 10.5 colonies (Fig. 1d) (efficiency of reprogramming is 1.5%; see Methods), occasionally forming multi-colony structures (Extended Data Fig. 2a). Once colonies formed, proliferation of GFP+ cells increased and after 5 weeks of co-culture with E4ECs, up to 20 x 10^5 GFP+CD45+ cells were produced, a ~400-fold expansion of the input FGRS-transduced endothelial cells (Fig. 1d). Since clonal CD45+ cells, but not CD45− cells, form colonies it is unlikely that E4ECs are mistakenly identified as haematopoietic cells (Extended Data Fig. 2b, c). Thus, FGRS-transduced endothelial cells required sustained inductive and supportive signals from the E4EC vascular niche for efficient haematopoietic reprogramming.

Current efforts to differentiate pluripotent stem cells into repopulating haematopoietic cells have had limited success4,5. We hypothesized

Figure 1 | Reprogramming of HUVECs and hES-ECs into haematopoietic cells by FGRS transcription-factor transduction and vascular induction.

a. Schema of reprogramming platform of HUVECs into haematopoietic cells. CD45−CD31−CD133−c-Kit− HUVECs were sorted from freshly purified HUVECs and expanded (days −14 to 0). Sorted cells were transduced with FGRS (GFP marked) (days 1–3) and grown in endothelial cell media. On day 4, transduced cells were re-plated on E4ECs in serum-free haematopoietic media (days 12–40). Distinct GFP+ flat colonies were observed at days 12–16, which by days 21–29 remodelled into three-dimensional grape-like colonies. After a month (days 29–40) GFP+ cells expanded ~400-fold (n = 4). CD144+VEGFR2+ endothelial cells derived from hES-ECs33 were also transduced with FGRS. The process of reprogramming is subdivided into two phases: phase I, specification (day 1–20); phase II, expansion (day 21–40). The expanding cultures were assayed for morphological change, cell number and CD45. Kinetics of reprogramming of HUVECs (green trace) and hES-ECs (black trace) are shown.

b. Emergence of rounded haematopoietic-like GFP+CD45+ cells 2–3 weeks after HUVECs were transduced with FGRS (white arrows). c, Formation of GFP+ haematopoietic-like colonies on the E4ECs 3–4 weeks after FGRS transduction. d, Generation of GFP+CD45+ haematopoietic-like colonies (c) from FGRS-transduced endothelial cells is enhanced by co-culturing with serum-free E4ECs and blocked by the presence of serum (n = 8, P < 0.05). Scale bar, 200 μm. Error bars are average ± s.d.
that FGRS transcription factors could augment haematopoietic differentiation of human embryonic stem (ES) cells. To test this, we first differentiated human ES cells into endothelial cells (hES-EC) and then transduced purified VEGFR2+CD144+hES-ECs with FGRS. Although this approach generated CD45+hCD34+ progeny (Extended Data Fig. 2d), these cells did not form stable haematopoietic-like colonies and did not proliferate (Fig. 1a, black line). Thus, hES-ECs are not as permissive as HUVECs for reprogramming into haematopoietic cells.

**rEC-hMPPs have features of multilineage progenitors**

During reprogramming, GFP+ FGRS-transduced endothelial cells and vascular-induced haematopoietic-like colonies lost CD31 expression but gained the expression of human haematopoietic markers hCD45, hCD43, hCD90 (also called Thy-1) and hCD14 (Fig. 2a and Extended Data Fig. 2e). In contrast, the GFP− E4ECs remained CD31+CD34+ CD45−. Importantly, a subset of GFP+ hCD45+ FGRS-transduced endothelial cell progeny manifested the immunophenotype of human HSCs (hCD45+Lin− hCD45RA−hCD38−hCD90−hCD34+) and MPPs (hCD45+Lin− hCD45RA−hCD38+hCD90−hCD34+). CD34+/hCD34+ rEC-hMPPs and seeded them in colony-forming cell (CFC) assays to enumerate progenitor cells. The rEC-hMPPs gave rise to GFP+hCD45+ colonies with CFC-GEMM (granulocytic/erythroid/megakaryocytic/monoctyc), CFC-GM (granulocytic/macrophage) and haemoglobinized burst-forming unit-erythroid BFU-E morphologies (Fig. 2c). Flow cytometry and cytospin analysis documented the presence of cells with morphological (Fig. 2d) and immunophenotypic features of granulocyte/macrophage (CD11b+, CD14+), erythroid (CD235a+), and megakaryocyte (CD41a+) progenies (Extended Data Fig. 2f). The function of rEC-hMPP-derived macrophages was corroborated using a phagocytosis assay (Extended Data Fig. 2g). Thus, rEC-hMPPs contain functional multilineage progenitor cells.

**rEC-hMPPs engraft long-term into primary recipients**

To assess the engraftment potential of rEC-hMPPs, we transplanted 1.5 × 106 GFP+hCD45+rEC-hMPPs into adult sublethally irradiated (275 rad) immunocompromised NSG mice (Fig. 3a). We detected circulating human CD45+ cells in the peripheral blood of recipient engrafted mice from 2 to 44 weeks (Fig. 3b) and found hCD45−CD34+ erythroid cells 16 weeks post-transplantation (Fig. 3c). We then sorted human CD45+ (hCD45+) cells from bone marrow of recipient mice 22–24 weeks after transplantation and cultured them for 24 h. The hCD45+hCD34+ cells were resorted and seeded into CFC assays. They formed CFC-GM, HUVECs, and E4ECs remained CD31−.

**Figure 2 | rEC-hMPPs phenotypically and functionally resemble multilineage HSPCs.** a, FACS analysis of co-cultured GFP+E4EC vascular niche along with GFP+ FGRS-transduced HUVECs 4 weeks after transduction (n = 9). b, Immunophenotypic analysis of reprogrammed HUVECs (red and blue; n = 3). c, Four weeks after FGRS transduction and E4EC induction, human GFP+hCD45+hCD34+ cells were sorted and seeded for CFC assay (n = 3). Haematopoietic colonies arose in the CFC assay (original magnification, ×4); wide field (upper row) and corresponding fluorescent images (bottom row) are shown. Left to right: granulocytic-erythroid-monoctyc-megakaryocytic (GEMM; scale bar, 400 μm), erythroid/myeloid, and granulocytic-macrophage (GM) colonies (scale bar, 1,000 μm), and haemoglobinized colonies (original magnification × 4). Graph shows CFC assay quantification. d, Wright–Giemsa stain of cells obtained from the CFC assay colonies. Original magnification, ×60. Error bars are average ± s.d.
At 16 weeks after transplantation of hCD45
b through right-hand colony; scale bar, 1,000 μm).

CFC-GEMM and BFU-E haematopoietic colonies with typical myeloid progeny morphologies (Fig. 3d). Hence, rEC-hMPPs are capable of robust multilineage engraftment.

**rEC–hMPPs arise from non–haemogenic endothelium**

To rule out the possibility that rEC-hMPPs are derived from rare contaminating haemogenic cells, we tested whether naive endothelial cells or highly purified, mature CD45<sup>−</sup>CD144<sup>−</sup>CD31<sup>−</sup>CD62E<sup>+</sup> (E-selectin<sup>+</sup>) endothelial cells, could form haematopoietic cells when cultured in optimal pro-haematopoietic media. Neither serum withdrawal nor addition of haematopoietic cytokines induced formation of CD45<sup>+</sup>CD34<sup>+</sup> cells from naive HUVECs (Extended Data Fig. 3a, b). However, FGRS transduction and E4EC induction of the clonal or oligo-clonal CD45<sup>−</sup>CD144<sup>−</sup>CD31<sup>−</sup>CD62E<sup>+</sup> mature endothelial cells (Extended Data Figs 3c–e and 4a–c). Thus, rEC-hMPPs are not derived from a scarce population of spontaneously differentiating endothelial cells with pre-existing haemogenic potential.

The bone marrow of robustly engrafted recipient NSG mice contained a small population of hCD45<sup>+</sup> cells with Lin<sup>−</sup>CD45RA<sup>−</sup>CD38<sup>−</sup>CD90<sup>−</sup>CD34<sup>−</sup> immunophenotype of human MPPs (Fig. 3e). To ensure that engrafted cells were derived from FGRS-transduced endothelial cells, we purified hCD45<sup>+</sup> cells from recipient bone marrow (Fig. 3f) and seeded single cells into 96-well plates for whole-genome amplification (WGA) and detection of viral vector integration. All hCD45<sup>+</sup> cells had been transduced by lentiviral vectors, and 19 of 21 cells showed integration of all four FGRS transcription factors (Fig. 3f and Extended Data Fig. 5). To verify that these cells were the progeny of rEC-hMPPs, we seeded hCD45<sup>+</sup> cells for CFC assays to examine viral integration in individual colonies (Fig. 3g). We demonstrated that all tested colonies were derived from cells that had integrated the lentiviral vectors expressing FGRS (Fig. 3g). Therefore, engrafted human haematopoietic cells were derived from transplanted rEC-hMPPs.

To test whether FGRS-induced reprogramming triggered expression of endogenous human FGRS genes, we expressed genetically distinct murine transcription factors (mFGRS) using inducible lentiviral vectors to reprogram HUVECs into rEC-hMPPs and then assessed expression of the endogenous human FGRS genes. Transient expression of mFGRS with E4EC co-culture for 3 weeks induced a 100-fold greater expression of endogenous genes than that of switched-off mFGRS transcripts (Extended Data Fig. 6a–c). Therefore, rEC-hMPPs do not require continuous expression of exogenous FGRS transcription factors to sustain their haematopoietic cell fates.

Furthermore, we speculated that enforced SPI1 expression might prevent rEC-hMPPs from differentiating into T cells (Fig. 4a). After 4 weeks, GFP<sup>−</sup> hCD45<sup>−</sup> hCD34<sup>−</sup> MPPs were sorted for CFC assay. The rEC-hMPPs yielded cells with morphological features of human MPPs (Fig. 3e). To ensure that their haematopoietic cell fates.

**Reprogramming adult endothelial cells to rEC–hMPPs**

To test whether our approach could reprogram adult human endothelial cells, we transduced human DMECs (hDMECs) with FGRS transcription factors and propagated them on serum-free E4EC monolayers (Fig. 4a). After 4 weeks, GFP<sup>−</sup> CD45<sup>−</sup> CD34<sup>−</sup> CD38<sup>−</sup> CD90<sup>−</sup> CD34<sup>−</sup> MPPs were derived from transplanted hDMECs. They were derived from human DMECs with high efficiency (Extended Data Figs 3c–e and 4a–c). Therefore, rEC-hMPPS do not require continuous expression of specific transcription factors.
**Figure 4** Functional and transcriptional analysis of adult hDMEC-derived rEC-hMPPs. a, Schematic representation of in vitro and in vivo functional tests of hDMEC-derived rEC-hMPPs. b, Left: haematopoietic colony assays of hDMEC CD45+CD34+ cells transduced with inducible Tet-On murine FGRS (hDMEC CD45+CD34+ colonies (from hDMECs) by transduction with inducible Tet-On murine FGRS (FGRS (m)) or (hDMEC CD45+CD34+). c, Broad comparison of the global gene transcription profiles of: primary HUVECs and hDMECs; rEC-hMPPs reprogrammed from HUVECs MMRN2 6 weeks after secondary engraftment with hDMEC-reprogrammed rEC-hMPPs (hDMEC CD45+CD34+ in vivo); engrafted human CD45+CD34+ cells purified from the bone marrow of NSG mice 22 weeks after primary transplantation with HUVEC-reprogrammed rEC-hMPPs (HUVEC CD45+CD34+ in vivo); engrafted human CD45+CD34+ cells purified from the bone marrow of NSG mice 15 weeks after secondary engraftment with hDMEC-reprogrammed rEC-hMPPs (hDMEC CD45+CD34+ in vivo); and naive purified Lin−CD34− cells from cord blood (CB). d, Comparison of expression of prototypical pluripotency genes shown in c. e, Hematopoietic niche induction assay of adult mature endothelial cells (lower plots). f, Schematic representation of steps of reprogramming of endothelial cells into rEC-hMPPs by FGRS transcription factors and E4EC vascular niche induction.

**Figure 5** Adult human hDMEC-derived rEC-hMPPs are capable of in vivo primary and secondary multilineage engraftment. a, Analysis of peripheral blood of mice at 4, 6 and 12 weeks after primary transplantation (n = 6). b, Analysis of peripheral blood of mice at 3 weeks, 8 weeks (for all time points; n = 6), 15 weeks (n = 4) and 23 weeks (n = 4) after secondary transplantation (n = 6). FACS plots on the right show representative analysis of rEC-hMPP secondary engraftment. Mouse Ter119+ and human CD235a+ erythrocytes were excluded to obtain an accurate estimation of hCD45+ and mcCD45+ cells. c, Clonal CFC assay of bone marrow hCD45+CD34− cells (n = 3; left plot). Emerging colonies were counted and classified (middle table). CFC colonies derived from single-plated hCD45+CD34− cells comprise mixed-lineage erythroid and myeloid progenies (right plots). d, Reprogrammed cells isolated from the host retained their multilineage potential in vitro, secondary CFC assay. e, Schematic representation of steps of reprogramming of endothelial cells into rEC-hMPPs by FGRS transcription factors and E4EC vascular niche induction.

rEC–hMPPs engraf primary and secondary recipients

To assess the engraftment potential of hDMEC-derived rEC-hMPPs, we transplanted 1 × 10⁶ CD45+ GFP+ rEC-hMPPs into sublethally irradiated (100 rad) 2-week-old neonatal NSG mice (Fig. 5a). Circulating hCD45+ cells were detected in the peripheral blood of recipient animals 4 weeks (2.09 ± 1.27%), 6 weeks (4.46 ± 3.66%) and 12 weeks (4.05 ± 3.50%) after transplantation (Fig. 5a). Fourteen weeks post-transplantation, human haematopoietic cells were found in peripheral blood, bone marrow and spleen (Fig. 5a and Extended Data Fig. 7b–d). Notably, these recipient animals harboured myeloid and lymphoid populations, including hCD19+ B cells (10.13 ± 4.98%), hCD56+ natural killer cells (1.62 ± 0.67%), hCD11b+ monocyte/macrophages (27.66 ± 8.92%) and hCD41a+ megakaryocytes (4.90 ± 1.51%) in their spleens (Fig. 5a and Extended Data Fig. 7b–d). Hence, rEC-hMPPs are capable of prolonged multilineage haematopoietic engraftment.

The bone marrow of primary recipient mice (weeks 12–14) contained populations with the immunophenotype of human HSCs (hCD45− Lin−hCD45RA+ hCD38− hCD90+hCD34+, 10.37 ± 2.55%) and MPPs (hCD45− Lin−hCD45RA− hCD38+hCD90−hCD34+, 13.83 ± 2.14%) (Extended Data Fig. 7d)⁷⁻⁸. Because these populations can self-renew, we tested whether bone marrow cells of mice engrafted by primary rEC-hMPPs (12 weeks post-transplant) could engraft secondary NSG recipient mice. Indeed, the peripheral blood of secondary recipients was engrafted by human myeloid and lymphoid progenies 3 weeks (14.61 ± 15.7%), 5 weeks (2.01 ± 1.5%), 8 weeks (17.78 ± 16.23%), 15 weeks (7.99 ± 7.36%)
and 23 weeks (26.3 ± 25.7%) post-transplantation (Fig. 5b). Thus, subpopulations of rEC-hMPPs can self-renew and are capable of durable myeloid and lymphoid engraftment in NSG mice—characteristics similar to true hMPPs.

To examine whether individual rEC-hMPPs retained clonal multilineage potential, we isolated hCD45+ hCD34+ cells from the bone marrow of a secondary robustly engrafted mouse 15 weeks post-transplantation and then assessed the multilineage CFC activity in clonal (1 cell per well), oligo-clonal (2 and 5 cells per well) and bulk (1,000 cells per well) sorted cells (Fig. 5c, d). All single-cell-derived colonies displayed multilineage differentiation, including hCD33+ hCD14+ hCD11b+ myeloid, hCD41+ megakaryocytic and CD235a erythroid progenies (Fig. 5c, d), indicating that engrafted rEC-hMPPs from secondary transplants retained their MPP potential. Thus, individual cells within the rEC-hMPPs have the immunophenotypic and functional attributes of HSPC-like/self-renewing hMPPs (Fig. 5e).

Notably, rEC-hMPPs isolated from primary and secondary engrafted mice showed no evidence of malignant transformation (Extended Data Figs 8, 9, 10a) or genetic abnormalities (Extended Data Fig. 10b).

Discussion

The availability of engraftable autologous human cells offers the potential to cure a wide spectrum of benign and malignant haematological disorders. Previous efforts using pluripotent stem cell lines have been handicapped by low efficiency and poor engraftment2,3,41,42. Here, we have taken advantage of an ontological link between endothelial and haematopoietic cells to efficiently reprogram mature, fetal and adult endothelial cells into engraftable self-renewing hMPPs without transitioning through a potentially destabilizing pluripotent intermediate. Just as support from non-haemogenic vascular cells is important for EHT during development, we found that the instructive contribution of the vascular niche was central to reprogramming endothelial cells to haematopoietic cells.

Differentiating pluripotent stem cells or expanding AGM-derived cells to engraftable haematopoietic progenitors has been inefficient when stromal cells have been used for niche-like support3,4,11. This could be due to: (1) poor inductive function of stromal cells in serum-free culture; and/or (2) distinguishing features of endothelial cells that resemble the hematopoietic niche cells that support EHT11–15. For example, E4ECs produce the proper physiological levels of inductive angiocrine factors, including Notch and c-Kit pathways14 that are important for EHT4,43. Thus, adult organ-specific pro-haematopoietic vascular niches, such as HUVECs11–18, bone marrow11–18,32,33, hepatic and splenic sinusoids8 may support CD34+ cells from the bone marrow or whole bone marrow of the primary engrafted mice were transplanted into secondary recipients. After 3 months of primary and 6 months of the secondary transplantation, engrafted hCD45+ cells in bone marrow, spleen and peripheral blood of mice was FACS sorted and processed for: (1) multivariate immunophenotypic analyses; (2) clonal and oligo-clonal CFC assay; and (3) molecular profiling. Tissues of the engrafted mice were processed for histological examination to rule out malignant transformation.

Online Content

Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Raffi, S. et al. Human ESC-derived hemogenic endothelial cells undergo distinct waves of endothelial to hematopoietic transition. Blood 121, 770–780 (2013).
2. Sturgeon, C. M., Ditadi, A., Clarke, R. L. & Keller, G. Defining the path to hematopoietic stem cells. Nature Biotechnol. 31, 416–418 (2013).
3. Choi, K. D. et al. Identification of the hemogenic endothelial progenitor and its direct precursor in human pluripotent stem cell differentiation cultures. Cell Rep. 2, 553–567 (2012).
4. Pereira, C. F. et al. Induction of a hemogenic program in mouse fibroblasts. Cell Stem Cell 13, 205–218 (2013).
5. Szabo, E. et al. Direct conversion of human fibroblasts to multilineage blood progenitors. Nature 468, 521–526 (2010).
6. Xie, H., Ye, M., Feng, R. & Grai, T. Stepwise reprogramming of B cells into macrophages. Cell 117, 663–676 (2004).
7. Sandler, V. M., Lailier, N. & Bouhassira, E. E. Reprogramming of embryonic human fibroblasts into fetal hematopoietic progenitors by fusion with human fetal liver CD34+ cells. PLoS ONE 6, e18265 (2011).
8. Nolan, D. J. et al. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. Dev. Cell 26, 204–219 (2013).
9. Ding, B. S. et al. Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. Nature 468, 310–315 (2010).
10. Ding, B. S. et al. Endothelial-derived angiocrine signals induce and sustain regenerative lung alveolarization. Cell 147, 539–553 (2011).
11. Butler, J. M. et al. Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. Cell Stem Cell 6, 251–264 (2010).
12. Butler, J. M. et al. Development of a vascular niche platform for expansion of repopulating human cord blood stem and progenitor cells. Blood 120, 1344–1347 (2012).
13. Hooper, A. T. et al. Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated repopulation of sinusoidal endothelial cells. Cell Stem Cell 4, 263–274 (2009).
14. Kobayashi, H. et al. Angiocrine factors from Akt-activated endothelial cells balance self-renewal and differentiation of hematopoietic stem cells. Nature Cell Biol. 12, 1046–1056 (2010).
15. Poulos, M. G. et al. Endothelial Jagged-1 is necessary for homeostatic and regenerative hematopoiesis. Cell Rep. 4, 1022–1034 (2013).
16. Aveicila, S. T. et al. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. *Nature Med.* **10**, 64–71 (2004).

17. Ding, L., Saunders, T. L., Enikolopov, G. & Morrison, S. L. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature* **481**, 457–462 (2012).

18. Doan, P. L. et al. Tie2+ bone marrow endothelial cells regulate hematopoietic stem cell regeneration following radiation injury. *Stem Cells* **31**, 327–337 (2013).

19. Orkin, S. H. & Zon, L. I. Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* **132**, 631–644 (2008).

20. Medvinsky, A. & Dzierzak, E. Definitive hematopoiesis is autonomously initiated by the AGM region. *Cell* **86**, 597–906 (1996).

21. North, T. E. et al. RUNXL1 expression marks long-term repopulating hematopoietic stem cells in the megadigestation mouse embryo. *Immunity* **16**, 661–672 (2002).

22. Yoshimoto, M., Porayette, P. & Yoder, M. C. Overcoming obstacles in the search for endothelial progenitor cells isolated from human umbilical cord blood. *Blood* **86**, (1994).

23. Eiken, H. M., Nishikawa, S. & Schroder, T. Continuous single-cell imaging of blood generation from haemogenic endothelium. *Nature* **457**, 896–900 (2009).

24. Swiers, G. et al. Early dynamic fate changes in haemogenic endothelium characterized at the single-cell level. *Nature Commun.* **4**, 2924 (2013).

25. Rhodes, K. E. et al. The emergence of hematopoietic stem cells is initiated in the placental vasculature in the absence of circulation. *Cell Stem Cell* **2**, 252–263 (2008).

26. Gordon-Keylock, S., Sobiesiak, M., Rybtsov, S., Moore, K. & Medvinsky, A. Mouse extraembryonic arterial vessels harbor precursors capable of maturing into definitive HSCs. *Blood* **122**, 2338–2345 (2013).

27. Zou, G. M., Chen, J. J., Yoder, M. C., Wu, W. & Rowley, J. D. Knockdown of Pu.1 by small interfering RNA in CD34+ embryoid body cells derived from mouse ES cells turns cell fate determination to pro-B cells. *Proc. Natl Acad. Sci. USA* **102**, 13236–13241 (2005).

28. Notta, F. et al. Isolation and characterization of human bone marrow microvascular endothelial cells by ETS factors and TGFβ inhibition is Id1 dependent. *Nature Biotechnol.* **26**, 218–221 (2008).

29. Doan, P. L. et al. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. *Nature Med.* **10**, 64–71 (2004).

30. Hock, H. R. et al. Fate tracing reveals the endothelial origin of hematopoietic stem cells. *Cell Stem Cell* **3**, 625–636 (2008).

31. Seandel, M. et al. Generation of a functional and durable vascular niche by the adenoviral E4ORF1 gene. *Proc. Natl Acad. Sci. USA* **105**, 19288–19293 (2008).

32. Rafii, S. et al. Isolation and characterization of human bone marrow microvascular endothelial cells: hematopoietic progenitor cell adhesion. *Blood* **84**, 10–19 (1994).

33. Rafii, S. et al. Human bone marrow microvascular endothelial cells support long-term proliferation and differentiation of myeloid and megakaryocytic progenitors. *Blood* **86**, 3353–3363 (1995).

34. Wu, X., Lensch, M. W., Wylie-Sease, J., Daley, G. Q. & Bischoff, J. Hemogenic endothelial cells derived from human umbilical cord blood. *Stem Cells* **25**, 2770–2776 (2007).

35. James, D. et al. Expansion and maintenance of human embryonic stem cell-derived endothelial cells by TGF-β inhibition is Id1 dependent. *Nature Biotechnol.* **26**, 161–166 (2010).

36. Ginsberg, M. et al. Efficient direct reprogramming of mature amniotic cells into endothelial cells by TGFβ factors and TGFβ1 suppression. *Cell* **151**, 559–575 (2012).

37. Chao, M. P., Seita, J. & Weissman, I. L. Establishment of a normal hematopoietic and leukemia stem cell hierarchy. *Cold Spring Harb. Symp. Quant. Biol.* **73**, 439–449 (2008).

38. Notta, F. et al. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science* **333**, 218–221 (2011).

39. Zou, G. M., Chen, J. J., Yoder, M. C., Wu, W. & Rowley, J. D. Knockdown of Pu.1 by small interfering RNA in CD34+ embryoid body cells derived from mouse ES cells turns cell fate determination to pro-B cells. *Proc. Natl Acad. Sci. USA* **102**, 13236–13241 (2005).

40. Ledran, M. H. et al. Efficient hematopoietic differentiation of human embryonic stem cells on stromal cells derived from hematopoietic niches. *Cell Stem Cell* **3**, 85–98 (2008).

41. Doulatov, S. et al. Induction of multipotential hematopoietic progenitors from human pluripotent stem cells via reprogramming of lineage-restricted precursors. *Cell Stem Cell* **13**, 459–470 (2013).

42. Marcelo, K. L. et al. Hemogenic endothelial cell specification requires c-Kit, Notch signaling, and p27-mediated cell-cycle control. *Dev. Cell* **27**, 504–515 (2013).

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Author Contributions V.M.S. and S.R. conceived and designed the project, performed experiments, analysed the data and wrote the manuscript. D.J., O.E. and A.K. performed the experiments and interpreted and analysed data. J.M.S. interpreted, analysed data and wrote the manuscript. D.J., O.E. and A.K. performed experiments, interpreted and analysed data. J.M.S. conceived and designed the project, performed the experiments, interpreted and analysed data. J.M.S. is supported by grants from NCI (CA159175 and CA163167), NHLBI (U01HL099997), NIDDK (R01DK095039), NCI (U54CA163167), Qatar National Priorities Research Foundation grant NPRP08-663-3-140 and the Qatar Foundation Biomedical Research Program. J.M.S. is supported by grants from NCI (CA159175 and CA163167), NHLBI (HL119872 and HL055748), Starr Foundation and a Leukemia & Lymphoma Society Scholar award. J.M.B. is supported by an American Society of Hematology Scholar Award. NHLBI U01-HL099997 and Angiocrine Bioscience and ASCI. We acknowledge the contribution of J. Z. Xiang and Agnes J. Viale for enabling and executing molecular profiling, and E. Gars for technical support. We appreciate W. Schachtterle for recommendations and edits of the manuscript.

Author Information Data have been deposited in the GEO database under accession number GSE57662. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.R. (sralfi@med.cornell.edu).
METHODS

Fetal and adult endothelial cells used for reprogramming. Full-term human umbilical vein endothelial cells (HUVECs) were obtained as previously described1−3. Multiple purified populations of CD45^−CD133^− c-kit^+ CD31^+ HUVECs were isolated from separate umbilical cords (n = 8) and were cultured in endothelial growth media (EM); Medium 199 (Thermo Scientific: FB-01), 20% fetal bovine serum (Omega Scientific), 20 ng ml^−1^ endothelial cell supplement (Biomedical Technologies: BT-203), 1X Pen/Strep, and 20 units ml^−1^ heparin (Sigma: H3149-100KU). Multiple batches (n = 3) of adult primary human dermal microvascular endothelial cells (hDMECs) were purchased from ScienCell Research Laboratories (catalogue 2020). In addition, cultured HUVECs were passaged for 3–5 times and then CD45^+ CD31^+ CD62E^+ HUVECs were sorted for GFP expression to enrich 10^6 endothelial cells. Analyses to rule out contamination with pre-existing haemogenic endothelial cells.

For reprogramming experiments, transfused HUVECs or hDMECs were co-cultured with E4ECs in serum-free haematopoietic media (HM) formulated as StemSpan SFEM (StemCell Technologies), 10% KnockOut Serum Replacement (Invitrogen), 5 ng ml^−1^ bFGF (FGF-2), 10 ng ml^−1^ EGF, 20 ng ml^−1^ SCF (soluble Kit-ligand), 20 ng ml^−1^ FLT3, 20 ng ml^−1^ TPO, 20 ng ml^−1^ IFG-1, 10 ng ml^−1^ IFG-2, 10 ng ml^−1^ IL-3, 10 ng ml^−1^ IL-6 (all from Invitrogen, eBioscience, or Peprotech).

Manufacture of vascular niche platform. To establish the vascular niche monolayers, HUVECs were purified and transduced with lentiviral vector carrying a cassette of E4ORF1 gene (E4ECs) as previously described4 or obtained as VeraECs from Angiocrine Bioscience, New York, NY. E4ECs proliferate in serum-free and xenobiotic-free conditions only supplemented with minimal angiogenic factors. All naive endothelial cells that are non-transduced with E4ORF1 are depleted during passing in serum-free conditions. Confluent monolayers of E4ECs are contact inhibited, non-transformed and propagate as homogenous monolayers providing an ideal instructive niche for reprogramming and sustaining FGRS-transduced endothelial cells into rEC-hMPPs.

Reprogramming of endothelial cells into MPPs (rEC-hMPPs). Endothelial cells were reprogrammed into haematopoietic cells by transfection with transcription factors and vascular niche induction. Purified populations of CD45^−CD133^− c-kit^+ CD31^+ and clonal CD45^+ CD144^+ CD31^+ CD62E^+ full-term HUVECs and adult primary hDMECs were cultured in the endothelial cell growth medium (EM). Then, HUVECs or hDMECs were transduced with lentiviral vectors expressing GFP and a combination of transcription factors—FOSB, GFI1, RUNX1 and SPI1 (FGRS)—and maintained in EM. After 3 days, GFP^+ FGRS transduced endothelial cells were plated in co-culture with 30% to 50% subconfluent E4EC monolayers supplemented with serum-free haematopoietic media (HM) composed of StemSpan SFEM, 10% KnockOut Serum Replacement, 5 ng ml^−1^ FGF-2, 10 ng ml^−1^ EGF, 20 ng ml^−1^ SCF, 20 ng ml^−1^ FLT3, 20 ng ml^−1^ TPO, 20 ng ml^−1^ IFG-1, 10 ng ml^−1^ IFG-2, 10 ng ml^−1^ IL-3, 10 ng ml^−1^ IL-6. After 3–4 weeks of co-culture the outgrown GFP^+ rEC-hMPPs were commonly identified as liver-like endothelial colonies. At the end of 4 weeks, human CD45^+ rEC-hMPPs were FACS sorted for: (1) immunophenotypic analyses; (2) methylcellulose-CFC assay (five thousand to ten thousand cells per well); (3) molecular profiling; (4) comparative genomic hybridization; and (5) transplanted retro-orbitally into primary subcutaneously irradiated (275 rad) 6-week-old NSG mice or subcutaneously irradiated (100 rad) 2-week-old mice. Four weeks after transplantation, formed colonies were isolated and transplanted into secondary recipients. After 3 months of primary and 6 months of the secondary transplantation, reprogrammed hCD45^+ cells were bone marrow, spleen and peripheral blood of mice were FACS sorted and processed for: (1) multivariate immunophenotypic analyses; (2) multi-cell and clonal methylcellulose CFC assay; and (3) molecular profiling. Tissues of the engrafted mice were processed for histological examination to rule out malignant transformation.

Increasing efficiency of reprogramming. To increase efficiency of the reprogramming, we developed a strategy to select those subsets of endothelial cells that were most likely transduced with the proper stoichiometry of all four FGRS transcription factors. We initially focused on generating endothelial cells transduced with GFI1, SPI1 and FOSB transcription factors because their expression in endothelial cells is negligible (Extended Data Fig. 1). To accomplish this, we transduced 5 × 10^6 endothelial cells with FGRS lentiviral ‘cocktail’ marked by puromycin resistance (SPI1) or GFP (FOSB and GFI1). We then applied puromycin selection for 2 days to enrich SPI1-expressing cells and sorted them for GFP expression to enrich for SPI1^+ GFP^+ (FOSB/GFI1) endothelial cells. Subsequently, we transduced these GFP^+ puromycin-resistant cells with RUNX1, seeded into 12-well plates, and expanded them for 2 days (10^4 cells per well, n = 3). We then transduced these GFP^+ puromycin-resistant cells on serum-free E4EC vascular niche layer in haematopoietic media and quantified the number of haematopoietic colonies that emerge after ~20 days of co-culture. We found that GFP^+ puromycin-resistant cells yielded 1560 ± 2.36 (n = 3) haematopoietic-like colonies per 10^4 re-plated cells, suggesting that the efficiency of reprogramming was at least 1.5% (156 of 10^4). This calculation assumes that each colony originates from a single reprogrammed cell. The efficiency is probably much higher in cells expressing the appropriate stoichiometric quantities of each factor.

Identification of viral integration on a single-cell and single-colony level. To identify the presence of viral integration on a single-cell level, we sorted human CD45^+ cells from the marrow of reEC-hMPP engrafted mice into a 96-well plate (1 cell per well) containing a lysis buffer for the Phi29 (multiple displacement amplification) applied whole-genome amplification (WGA). We used a commercially available kit, REPLI-g (Qagen, catalogue no. 150343). Each WGA reaction product was followed by a set of PCR reaction with primers specific to the CMV promoter and a transgene (FOSB, GFI1, RUNX1, SPI1). All PCR reactions were conducted separately. We used empty wells (no cells sorted) as controls for nonspecific amplification. WGA products of the control wells were used for PCR reactions with primers specific to the CMV promoter and a transgene.

To identify the presence of viral integration on a single-cell level, we captured expanding colonies from the plates for CFC assay. Fourteen days after the start of CFC assay 3 distinct cell aggregations/colonies were detected and analysed. Four PCR reactions were performed for each amplified colony using their genomic DNA as template. Cells from the colonies were re-suspended and washed twice in excess amounts of PBS (10 ml) and transferred into the lysis buffer for the WGA. All following procedures were the same as those for the single-cell viral integration identification.

CMV primer, 5′-GCGAAATTTGCGAGCCGCTTGC-3′; FOsb primer, 5′-GC TCTGCTTTTCCTTCCACAAC-3′; GFI1 primer, 5′-CCAGGGCCCACACG TGGCGTAGTCG-3′; RUNX1 primer, 5′-TTGGCGTTTGTGTGGTAAGAC-3′; SPI1 primer, 5′-CGGATCTTCCCTGTGCGCTGTC-3′.

Clonal reprogramming of HUVECs to rEC-hMPPs. HUVECs were isolated from umbilical cord and grown in endothelial cell growth medium. After 2–3 passages, CD45^−CD144^+ CD31^+ CD62E^− (E-selectin) CD45^− CD133^+ cells were sorted into 96-well plates at 1, 2, 5 and 10 cells per well densities for clonal expansion. We used CD62E^− (E-selectin) surface marker to sort mature activated endothelial cells. Passing of HUVECs results in upregulation of E-selectin in 40–60% of the HUVECs. Expanding clonal populations of selected cells were subsequently transduced with the FGRS transcription factors followed by re-plating onto the E4EC monolayers to reprogram them into rEC-hMPPs. Haematopoietic activity of clonally derived CD45^+ CD34^+ rEC-hMPPs was assessed using standard methylcellulose-CFC assay.

RNA-seq processing and analysis. Total RNA was prepared using the Applied Biosystems Arcturus PicoPure RNA isolation kit. The quality of the extracted RNA was checked on an Agilent Technologies 2100 Bioanalyzer. The extracted RNA was used for sequencing using Illumina HiSeq2000. The sequencing output was checked for quality using Illumina pipeline. PE 51x2 and SE 51 reads were mapped to the human genome (hg18) using TopHat (http://tophat.cbcb.umd.edu/) default parameters. RefSeq transcript levels, identified as fragments per kilobase of transcripts per million reads (FPKM), were then quantified using CuffLinks (http://cufflinks.cbcb.umd.edu/) with upper-quartile normalization and sequence-specific bias correction. For heat-map visualization we determined the maximum FPKM of each transcript across the samples shown. FPKMs were then divided by this number to produce scaled expression values. Heat maps of gene expression and gene expression clustering were built using GENE-E matrix visualization and analysis platform (http://www.broadinstitute.org/cancer/software/GENE-E/). Clustering of gene expression in the heat maps was conducted using one minus Pearson correlation as dissimilarity measure between transcription profiles. GEO accession number GSE57662.

Comparative genomic hybridization (CGH). Genomic DNA was extracted from HUVECs, FACS sorted CD45^− rEC-hMPPs and hCD45^− hD45^+ rEC-hMPPs sorted from the bone marrow of NSG mice. Before DNA extraction, hCD45^− hD45^+ rEC-hMPPs were incubated with 10 ml PBS (10 ml) and transferred into the lysis buffer for the WGA. All following procedures were the same as previously described. One day before plating hES to begin differentiation, MEF conditioned medium was replaced with hES culture medium without FGF-2 and supplemented with 2 ng ml^−1^ BMP4. The next day, hES cells were plated directly onto E4EC monolayers in hES culture medium (without FGF-2, plus 2 ng ml^−1^ BMP4) and left undisturbed.
for 48 h. This point of culture was considered as differentiation day zero. Cells were sequentially stimulated with recombinant cytokines in the following order: day 0 to 7, supplemented with 10 ng ml\(^{-1}\) BMP4; day 2 to 14, supplemented with 10 ng ml\(^{-1}\) VEGF-A; day 2 to 14, supplemented with 5 ng ml\(^{-1}\) FGF-2; day 7 to 14, supplemented with 10 \(\mu\)M SB-431542. At day 14 of culture, FACS sorting was used to purify the fraction of hES-derived endothelial cells co-expressing the vascular-specific CD144 (VE-cadherin) reporter and CD31. These cells were transduced with the FGRS cocktail and 2–3 days later plated on a layer of serum-free E4EC monolayers. The extent of reprogramming was assessed by flow cytometry. To accurately detect the expression of CD144 in the hES-ECs being reprogrammed into putative eEC-hMPPs, we used fluorescent monoclonal antibodies to human CD144.

**Phagocytosis assay.** The rEC-hMPPs generated from 3 to 4 weeks were cultured in the presence of M-CSF (10 ng ml\(^{-1}\)), SCF (10 ng ml\(^{-1}\)), Flt-3 (10 ng ml\(^{-1}\)), TPO (10 ng ml\(^{-1}\)) and 10% FBS for an additional 2 weeks with E4EC vascular niche layer. We observed an increase in size and granularity of the cultured cells (data not shown). The culture was washed with PBS twice to remove non-adherent cells. Growth media mixed with green fluorescent beads (GFB) at a low concentration of 1 \(\mu\)l ml\(^{-1}\) was applied to the attached cells for one hour at 37 °C. After the incubation, the cells were washed twice with PBS and live cells were stained with the monocytic CD14 antibody. Cells were fixed and stained with DAPI for nuclear visualization. We visualized GFB inside CD14\(^+\) cells, but not in CD144 (VE-cadherin)\(^+\) endothelial cells (Extended Data Fig. 2g).

**Purification of human cord blood stem and progenitor cells (HSPCs).** Human umbilical cord blood was obtained under the IRB protocol ‘stage specific differentiation of hematopoietic stem cells into functional hemangiogenic tissue’ (Well Cornell Medical College IRB 0906010445). Cord blood mononuclear cells were purified by density gradient using Ficoll-Paque (GE) and enriched for CD34\(^+\) HSPCs using magnetic separation using anti-CD34 microbeads (Miltenyi) or FACS sorting. Further purification was achieved by negative selection of Lin\(^-\) cells using the human progenitor cell enrichment kit (StemCell Technologies) or FACS sorting. RNA from FACS sorted Lin\(^-\) CD34\(^+\) CD45\(^-\) cells was isolated by using Arcturus PicoPure RNA isolation kit (Applied Biosystems; this kit was used for all RNA extraction procedures).

**Lentiviral vectors.** Candidate transcription factors used for screening were subcloned into pLVX-IREs-ZaGreen1 lentivector (Clontech), pLOC lentivector (OpenBiosystems), or LV105 and LV151 lentivectors (Genecopoeia), pLOC lentivector contained CMV-MCS-IREs-GFP (MCS, multicloning site where a cDNA of interest such as FOSB or GFI1 was subcloned). Human FGRS were subcloned as follows: FOSB and GFI1 were each subcloned into pLOC lentivector containing IRES-GFP cassette, SP11 was subcloned into LV105 lentivector containing puromycin selection marker, and RUNX1 was subcloned into LV151 lentivector containing neomycin selection marker. Tet-On 3G inducible lentivectors (Clontech) were used for inducible expression of either mouse FGRS (mFGRS) or human FGRS (hFGRS) factors. Expression of all FGRS transgenes was driven by the CMV promoter. Lentiviral particles were packaged as previously described. In short, human embryonic kidney 293FT (HEK293FT) cells were co-transfected with a lentiviral vector and two helper plasmids, pSPAX2 and pMDLG (Viron Lab through Addgene), in an equal molar ratio. Supernatant was collected 48–52 h after transfection, filtered and concentrated using Lenti-X concentrator (Clontech). Viral titres were determined in limiting dilution experiments using HUVECs as target cells. We used either the number of GFP\(^+\) cells or the number of formed colonies in the presence of selection antibiotics (puromycin) as a read-out for the number of infectious viral particles per volume. We used an average multiplicity of infection (MOI) of 5 to 10 for infection of endothelial cells.

**Flow cytometry.** Flow cytometry analysis was performed on a Becton Dickinson LSRII SORP, and FACS was performed on an Aria II SORP. Antibodies used were raised against human CD45, CD34, CD14, CD31, CD43, CD90, CD41a, CD33, CD19, CD3, CD4, CD8, CD235A, CD45RA, CD38, C11b, CD38, Lin cocktail, CD117, CD133, CD144 (BD Pharmingen, ebioscience) or mouse CD45 (ebioscience). Voltage adjustments and compensation was performed with CompBeads (BD Pharmingen), and gating was performed on fluorophore minus one (FMO) controls and unstained controls.

The list of antibodies used in our experiments is given below. Anti-human antibodies obtained from Ebioscience: CD45 catalogue no. 47-0459-42; clone HI30, CD34 catalogue no. 25-0249-42; clone 4F11, CD33 catalogue no. 48-0337-42; clone p67.6; CD19 catalogue no. 12-0199-41; clone HIB19, CD3 catalogue no. 93-0037-42; clone OKT3, CD4 catalogue no. 17-0048-41; clone OKT4, CD8 catalogue no. 8048-0087-02; clone SK1, CD43 catalogue no. 17-0439-73; clone eBio84-3C1, CD83 catalogue no. 25-0839-41; clone HB1S, CD11b catalogue no. 12-0118-41; clone ICRF44, CD14 catalogue no. 22-7778-72, CD31 catalogue no. 11-0319-42; clone WM59, CD31 catalogue no. 48-0319-42; clone WM59. Anti-human antibodies obtained from BD Pharmingen: CD90 catalogue no. 561971; clone 5E10, CD3 catalogue no. 557851; clone SK7, CD14 catalogue no. 557742; CD14 catalogue no. 555399, C235A catalogue no. 340947, CD45RA catalogue no. 347723, CD41a catalogue no. 555466, CD38 catalogue no. 646851, CD117 catalogue no. 333944, CD33 catalogue no. 333946, CD144 catalogue no. 560410, clone 55-7H1, FLK1 (VEGF-R2) catalogue no. 560871, clone 89106. Anti-human antibodies obtained from BioLegend: Lin catalogue no. 348805. Anti-mouse antibodies obtained from Ebioscience CD45 catalogue no. 25-0451-82; clone 30-011.

**Statistics and animals.** All statistics are presented as average ± standard deviation. To identify statistical significance all groups of data were compared using a paired student t-test. Experiments were repeated for at least three times. Number of repeats is demonstrated in all figure legends. Animal experiments contain at least three animals per group. The number of animals is described in all figure legends and the text of the paper. We included all tested animals for quantification. Representative images and flow cytometry plots are shown in the figures. Age- and sex-matched animals were allocated in all corresponding experimental groups. All NSG (NOD.Cg-PkdckesldIl2rgtm1Vwp/Sjcl, Jackson laboratory) animals for transplantation experiments were female. All ages are specified in the text. Animals were chosen according to their age and their sex (females only). A description of every experiment states the age of the animals used in the experiment. Transplanted animals were not individually labelled. Hence, subgroups of transplanted animals for organ engraftment were chosen blindly, without previous knowledge of the level of engraftment. Animal experiments were performed under the guidelines set by the Institutional Animal Care and Use Committee (IACUC).
| ORF   | HUVEC | Lin^−CD34^− (CB) |
|-------|-------|-----------------|
| ZFP36 | 4.81  | 12.40           |
| FOS   | 3.82  | 12.36           |
| JUNB  | 6.26  | 12.17           |
| GMFG  | 5.33  | 10.30           |
| KLF1  | 7.70  | 10.28           |
| FOSB  | 1.51  | 10.28           |
| NFE2  | 0.00  | 9.45            |
| KLF1  | 0.00  | 9.29            |
| LYL1  | 8.55  | 9.03            |
| LMO2  | 7.24  | 8.95            |
| TAL1  | 6.20  | 8.31            |
| GATA1 | 0.00  | 8.18            |
| SPI1  | 0.00  | 8.04            |
| IKZF1 | 0.00  | 7.83            |
| GFI1B | 0.00  | 7.70            |
| VAV1  | 0.00  | 7.67            |
| MEIS1 | 3.23  | 6.75            |
| MYB   | 0.23  | 6.47            |
| MLLT3 | 4.21  | 6.40            |
| RUNX1 | 0.21  | 6.23            |
| GFI1  | 0.00  | 5.54            |
| HLF   | 0.00  | 4.67            |
| BEX1  | 2.52  | 4.47            |
| PBX1  | 4.24  | 4.88            |
| BEX2  | 0.03  | 3.95            |

**Absent TFs**

- BEX2
- PBX1
- BEX1
- HLF
- GFI1
- RUNX1
- MLT3
- MYB
- MEIS1
- VAV1
- GFI1B
- IKZF1
- SPI1
- GATA1
- TAL1
- LMO2
- LYL1
- KLF4
- KLF1
- NFE2
- FOSB
- KLF2
- GMFG
- JUNB
- FOS
- ZFP36
- None
- Not Transduced

**Number of Colonies**

**gfi1**

**RUNX1**

**SPI1**

**FOSB**

**All**

**Not Transduced**

---

**d**

1) Transduce

- **FOSB**
- **GFI1**
- **RUNX1**
- **SPI1**

*(GFP as marker)*

2) Replate on Vascular Niche

- Hematopoietic Cells

- In vitro

- CFC assay

- Differentiation

- E4EC Vascular Niche Induction

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(HUVEC) Human umbilical vein endothelial cells

(hDMEC) Adult human dermal microvascular endothelial cells

E4EC: E4ORF1 expressing endothelial cells (VeraVec)
Extended Data Figure 1 | Screening strategy for identification of a minimal set of transcription factors for reprogramming endothelial cells into haematopoietic cells. 

**a**. Candidate genes tested for reprogramming of HUVECs into haematopoietic colonies. To identify transcription factors that drive EHT transition, we performed RNA-seq of HUVECs and Lin^−^CD34^+^ umbilical cord HSPCs to select transcription factors differentially expressed by HSPCs, but not by HUVECs. We then screened various combinations of differentially expressed transcription factors to identify a minimal set capable of reprogramming endothelial cells to haematopoietic cells. Levels of expression (log2[RNA-seq value]) in HUVECs and freshly purified Lin^−^CD34^+^ cord blood cells are shown. 

**b**. One-by-one elimination of transcription factor experiment revealed a minimal set of transcription factors—FOSB, GFI1, RUNX1 and SPI1 (FGRS)—capable of generating haematopoietic colonies in the HUVEC culture. A pooled set of 26 transcription factors minus one transcription factor was evaluated for the ability to evoke formation of haematopoietic clusters (day 21–25; n = 3). Asterisks show statistically significant (P < 0.05) reduction of the number of haematopoietic clusters in the transduced HUVECs compared to the full set of transcription factors. Control represents non-transduced HUVECs. Transduced cells were cultured on a layer of serum-free E4EC monolayers. 

**c**. One-by-one elimination of the FGRS factors shows that all four FGRS transcription factors are necessary and sufficient for generation of haematopoietic colonies (day 21–25; n = 3). 'All' in b and c indicates that all transcription factors are present. 'Not transduced' in b and c indicates that all transcription factors are absent. b, c, Error bars are average ± s.d.

**d**. Schema for reprogramming of endothelial cells into human multipotent progenitor cells (rEC-hMPPs). Clonal or bulk populations of HUVECs or hDMECs were transduced with the FGRS and after 3 days were replated on subconfluent monolayers of E4EC endothelial cells (VeraVecs). The emerging colonies of haematopoietic cells were subjected to (1) multivariate immunophenotypic analyses; (2) clonal and oligo-clonal CFC assay; and (3) molecular profiling (RNA-seq). Tissues of the engrafted mice were processed for histological examination to rule out any malignant transformation.
Extended Data Figure 2 | FGRS transduction and vascular induction reprogram HUVECs, but not hES-ECs, to proliferating functional rEC-hMPPs. a, Multi-colony niche-like structure that physically separates developing haematopoietic colonies from surrounding E4EC vascular niche. The emerging multi-colony sinusoidal-like structures create a unique cellular interface between E4EC monolayers and transduced endothelial cells giving rise to haematopoietic clusters ($n = 4$, scale bar is 1,000 μm).

b, Expansion potential of reprogrammed hCD45$^+$ haematopoietic cells. hCD45$^+$ ($12\times 10^3$) and hCD45$^-$ ($60\times 10^3$) cells were sorted into separate wells and expanded for 2 days. We observed fivefold expansion of hCD45$^-$ cells ($56.6\times 10^3 \pm 7.9\times 10^3$; $n = 3$) and marked reduction of hCD45$^+$ cell number ($4.6\times 10^3 \pm 1.0\times 10^3$; $n = 3$). c, Clonal expansion of hCD45$^+$ cells. hCD45$^+$ cells were FACS sorted into 96-well plates at the density of 1 and 2 cells per well. After 7 days of culture, we observed hCD45$^+$ cell expansion in $6.3 \pm 2.1$ wells ($93.1 \pm 14.5$ cells per well) of 1-cell sort and $29.0 \pm 4.3$ wells ($112.1 \pm 21.2$ cells per well) of the 2-cell sort ($n = 3$). The difference between cell number in 1- and 2-cell sort was statistically not significant ($P = 0.78$), suggesting that the difference in the number of wells with detected cell expansion was due to survival of sorted cells rather than a reflection of the number of cells sorted into a well.

d, Reprogramming of hES-derived endothelial cells (hES-ECs) into haematopoietic cells. Representative experiment demonstrating that transduction of hES-ECs with FGRS (F and G lentivector constructs containing IRES-GFP cassette) and E4EC vascular induction generated significantly higher numbers of GFP$^+$ hCD45$^+$hCD144$^-$ cells (four panels on the right) compared to control non-transduced hES-ECs (three panels on the left). To accurately detect the expression of CD144 in the hES-ECs being reprogrammed into putative rEC-hMPPs, we used fluorescent monoclonal antibodies to human CD144.

e, Lineage-specific surface marker analysis of the hGFP$^+$ CD45$^+$ population of rEC-hMPPs. hGFP$^+$ CD45$^+$ population showed that some of these cells expressed lineage-specific surface markers, such as hCD43$^+$ ($8.96 \pm 2.3%$; $n = 3$), hCD90$^+$ (Thy-1$^+$) ($6.15 \pm 1.13%$; $n = 3$) and hCD14$^+$ ($40.0 \pm 4.95%$; $n = 3$) (representative flow cytometry measurements; top four panels, statistics for all experiments is in the bottom bar graph, $n = 3$).

f, Immunophenotypic analysis of CFC colonies grown in the CFC assay performed in Fig. 2c, d. g, Macrophages differentiated from rECC-hMPPs are functionally capable of phagocytosis. The images (upper row and lower left) show groups of firmly plastic-adherent hCD14$^+$ cells (red staining) with clearly visible phagocytosed green fluorescent beads (GFB; green). Endothelial CD144$^+$ (VE-cadherin) cells (white staining) were not co-localized with beads. Most ($85.1 \pm 15.1%$) GFBs were localized inside hCD14$^+$ cells (bottom-right graph, 1). A smaller population of GFBs was distributed outside hCD14$^+$ and CD144 (VE-cadherin)$^+$ cells ($14.8 \pm 7.43%$; bottom-right graph, 2). The percentage of GFBs co-localized with endothelial cells was negligible ($4.8 \pm 0.83%$; bottom-right graph, 3), $n = 9$. Scale bars are 25 μm. Error bars are average ± s.d.
a) HUVEC Cell Number x10^4

- SF+CK+SB: P<0.0001
- SF+CK: P<0.01
- SF: HUVEC

b) % hCD34+

- SF+CK+SB: p<0.005
- SF+CK: p<0.04
- SF: HUVEC

Clonal Expansion of HUVECs

c) hCD31, hCD62E, hCD45, hCD144

d) Colony number

- 1 cell clone 1
- 1 cell clone 2
- 2 cells
- 5 cells
- 10 cells

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Extended Data Figure 3 | Naive HUVECs are devoid of haemogenic potential capable of spontaneous differentiation into MPPs. We performed two sets of experiments to exclude the possibility that rEC-hMPPs were derived from spontaneously differentiating HUVECs with haemogenic or haemangioblastic potential. a, b, In optimal pro-haematopoietic cultures, naive non-transduced endothelial cells fail to spontaneously differentiate into rEC-hMPPs. a, We grew non-FGRS-transduced HUVECs in the serum-free media used for reprogramming. Neither serum withdrawal nor addition of haematopoietic cytokines induced formation of hCD45−hCD34− cells and HUVECs sustained their vascular identity. Indeed, serum withdrawal increases the number of CD34+ HUVECs. CK, cytokine cocktail (see Methods); SB, TGF-β inhibitor SB431542; SF, serum free. b, Serum withdrawal suppresses HUVEC proliferation. Inhibition of TGF-β signalling (SB) combined with cytokine cocktail (see Methods) restores proliferative potential of HUVECs in serum free media. The difference between proliferation of HUVECs in serum free media and all other conditions is statistically significant (asterisk; \( P < 0.005 \)). Statistical significance between pairs of different conditions is shown with blue arrows and \( P \) values, where \( P < 0.005 \) is statistically significant. Therefore, human rEC-hMPPs originate from reprogrammed endothelial cells, but not cytokine-mediated outgrowth of contaminating pre-existing haemogenic endothelial cells. c–e, Clonal reprogramming of non-haemogenic HUVECs into rEC-hMPPs using FGRS transduction and vascular induction. We performed endothelial cell clonal reprogramming experiments to exclude the possibility that rEC-hMPPs were derived from spontaneously differentiating HUVECs with pre-existing haemogenic or haemangioblastic potential. c, Because E-selectin is only expressed in activated endothelial cells, we generated clonal cultures of CD45−CD144+CD31+CD62E (E-selectin)+ endothelial cells. To this end, CD144+CD31+CD62E−CD45− HUVECs were sorted into 96-well plates at 1, 2, 5 and 10 cells per well densities for clonal expansion. Proliferating clones were transduced with FGRS and induced with serum-free E4EC monolayers. d, These clonal cultures yielded rEC-hMPPs comparable to bulk HUVEC cultures. The numbers of haematopoietic-like colonies emerging from 1-cell, 2-cell, 5-cell and 10-cell clones are not statistically different (\( P > 0.05 \)). e, An example of a haematopoietic-like colony derived from a 1-cell clone number 2. It is unlikely that rEC-hMPPs are derived through spontaneous differentiation of pre-existing endothelial cells with haemogenic or haemangioblastic potential. Error bars are average ± s.d. Scale bars, 400 μm.
Extended Data Figure 4 | Clonal reprogramming of non-haemogenic HUVECs into immunophenotypic and functional rEC-hMPPs using FGRS transduction and vascular induction. a–c, CFC assay of reprogrammed hCD45^+ hCD34^+ rEC-hMPPs generated from clonally selected CD45^+ CD144^− CD31^+ CD62E^+ mature HUVECs, as shown in Extended Data Fig. 3d, e. CD45^+ CD144^− CD31^+ CD62E^+ endothelial cells were sorted as 1 cell per well, 2 cells per well, and 5–10 cells per well. Expanding clones of the endothelial cells were transduced with FGRS and then induced with vascular niche. After 3 to 4 weeks, emerging hCD45^+ hCD34^+ rEC-hMPPs were sorted out (red gate in FACS plots; upper left) and plated for CFC assay. Typical haematopoietic colonies arose in the assay (middle column, microphotographs: original magnification, ×4). FACS plots on the right show immunophenotypic analysis of the cells that arose in the CFC assay, demonstrating differentiation into human CD45^+ CD235^− erythroid, CD11b^+ macrophage CD14^+ monocyctic, CD41a^+ megakaryocytic and CD83^+ dendritic progenies. The graph in the left lower corner shows quantification of the CFC assay (n = 3). Identical panels are shown for two 2-cell clones and one 5-cell clone. A total of three independent clones is shown. Thus, given the high efficiency of clonal reprogramming of mature authentic endothelial cells into rEC-hMPPs, it is unlikely that rEC-hMPPs are spontaneously derived from a very rare population of a pre-existing haemogenic or haemangioblastic HUVECs. Scale bars, 400 μm.
Extended Data Figure 5 | Single-cell analysis of lentiviral integration into engrafted rEC-hMPPs. Engrafted hCD45
rEC-hMPPs purified from the bone marrow of primary NSG recipient mouse (Fig. 3e, f) were sorted into a
96-well plate (1 cell per well), lysed in corresponding well for whole genome amplification (WGA) using Phi29 enzyme (see Methods). Amplified DNA is shown for all 21 cells in the top two gels. Amplified DNA was used as a template for PCR reactions with a forward primer specific for the CMV promoter and reverse primer specific for the coding sequence of a reprogramming factor. t-test PCR with a lentiviral vector. EW indicates empty well (no template DNA). Red asterisks show failed PCR amplification of viral integration. PCR products are visible as low molecular mass bands labelled as 1, FOSB; 2, GFI1; 3, RUNX1; 4, SPI1.
Extended Data Figure 6 | Conditional expression of FGRS is sufficient for optimal generation of rEC-hMPPs with multilineage potential, including T-cell lymphoid cells. a–c, Conditional expression of mouse inducible FGRS (mFGRS) factors activates endogenous human FGRS in HUVECs sustaining functional haematopoietic cell fate of rEC-hMPPs. a, To test whether FGRS-induced reprogramming triggered expression of endogenous FGRS genes24, HUVECs were transduced with lentiviral vectors expressing mFGRS-Tet-On and a trans-activator, and grown on E4EC vascular niche for 18–22 days (n = 4) in the presence of doxycycline. Doxycycline was removed from the culture medium after 18–22 days to shut off the expression of mouse FGRS and cells were cultured for an additional 7–10 days. Human CD45^+ CD34^- cells were FACS isolated for CFC assay and whole-transcriptome deep sequencing (RNA-seq). CFC assay revealed emergence of haematopoietic colonies with cells expressing human CD235, CD11b, CD83 and CD14. b, Comparison of transcriptional gene profiles of human FGRS in: primary HUVECs; rEC-hMPPs reprogrammed from hDMECs by transduction with inducible Tet-On mouse FGRS (mFGRS) and vascular induction for 3–4 weeks (tet-CD45^+ hDMEC-rEC-hMPPs); engrafted human CD45^-CD34^- cells purified from the bone marrow of NSG mice 22 weeks post-primary transplantation with HUVEC-reprogrammed rEC-hMPPs (HUVEC CD45^-CD34^- in vivo); engrafted human CD45^-CD34^- cells purified from the bone marrow of NSG mice 15 weeks post-secondary engraftment with hDMEC-reprogrammed rEC-hMPPs (hDMEC CD45^-CD34^- in vivo); and naïve purified Lin^-CD34^- cells from cord blood (CB). c, Analysis of whole-transcriptome RNA-seq of rEC-hMPPs derived using inducible mouse FGRS (n = 3). All RNA-seq reads were aligned against human and mouse FGRS sequences. 'Map to human' indicates RNA-seq reads that align to human FGRS sequences; 'Map to mouse' indicates RNA-seq reads that align to mouse FGRS sequences; and 'Map to mouse only' indicates RNA-seq reads that align to mouse FGRS sequences without a possibility to align to human sequences. d, e, Optimizing differentiation of rEC-hMPPs into lymphoid progeny. d, The number of T-lymphoid progeny of engrafted rEC-hMPPs was negligibly small, raising the possibility that constitutive SPI1 expression prevents rEC-hMPPs from differentiating into T cells39,40. To test this, HUVECs were transduced with lentiviral vectors expressing GFP and that constitutively express FGR transcription factors with a Tet-inducible SPI1 (FGR+SPI1-Tet-On construct) for 3 days followed by re-plating for E4EC induction. After 27 days of FGR and doxycycline-induced SPI1 expression on E4ECs, GFP^+ hCD45^- haematopoietic-like colonies emerged. Then, doxycycline was withdrawn and the reprogrammed cells were cultured serum-free haematopoietic media (SFHM) with Delta-like-4 expressing OP9-stroma (OP9-DLL4) supplemented with IL-7, IL-11 and IL-2. There is an increase of the number of GFP^+ cells emerging during reprogramming of HUVECs by FGR+SPI1-Tet-On construct and E4EC induction. e, rEC-hMPPs differentiate into CD3^-CD19^- and CD14^- haematopoietic cells in the absence of exogenous expression of SPI1. After 3 weeks, the numbers of myeloid and lymphoid cells were quantified by flow cytometry. We were able to reliably detect a small fraction of CD3^- cells (0.16 ± 0.01%; n = 3), a larger number of CD19^- (1.17 ± 0.13%; n = 3) and CD14^- (16.46 ± 1.02%; n = 3) cells. Thus, generation of lymphoid cells from rEC-hMPPs could be optimized by transient expression of transcription factors. Error bars are average ± s.d.
Extended Data Figure 7 | Adult human hDMEC-derived rEC-hMPPs are capable of in vivo primary multilineage engraftment.  
a. Immunophenotypic analysis of cells grown in the CFC assay (from Fig. 4b). These panels show quantification of surface marker expression in the cells isolated from colonies in the CFC assay (n = 3). hDMECs differentiated into hCD45^+CD235^− erythroid, CD11b^+CD14^− monocyte/macrophage and CD83^+ dendritic cell progenies. Minimal CD144 (VE-cadherin) was detected.
b. Analysis of peripheral blood (PB) of mice at 4, 6 and 12 weeks after primary transplantation (Fig. 5a) revealed circulating hCD45^+ and their hCD33^−, hCD14^− myeloid and hCD45^+CD235^− erythroid progenies (n = 3). Mouse CD45 (mCD45^+) cells were excluded from analyses. Mouse cells, blue; human cells, red.
c. Analysis of spleen of mice at 14 weeks after primary transplantation (Fig. 5a) revealed the presence of hCD45^+ (red gate) and their lymphoid (hCD19^+, hCD56^−) and myeloid (hCD11b^+, hCD41a^−) progenies (n = 3). Mouse CD45 (mCD45^+) cells (blue populations) is shown. Lin^-CD45RA^- cells (blue gate) were analysed for CD38 and CD90 expression (green and red gates) and subsequently examined for human CD45 and CD34 expression. This analysis revealed the presence of hCD34^+ cells with small populations of both Lin^-CD45RA^-CD38^-CD90^-CD34^- and Lin^-CD45RA^-CD38^-CD90^-CD34^- cells satisfying phenotypic definition of human HSCs and MPP, respectively (n = 3).
**Extended Data Figure 8 | Analysis of bone marrow and liver of primary transplanted mice for signs of malignant transformation.** Analysis of bone marrow (a) and liver (b) of mice 10 months after primary transplantation (from Fig. 3b) of HUVEC-derived rEC-hMPPs for signs of malignant transformation. The level of fibrosis was determined using Masson and PicroSirius staining. The architectonic geometry of the bone marrow was determined by sequential multi-cross-sectional Wright–Giemsa and haematoxylin and eosin (H&E) staining and compared to age-controlled, non-transplanted NSG mice. We did not observe any evidence of fibrosis or alteration of the geometry of the bone marrow or liver of the transplanted mice. Furthermore, no recipient mouse manifested any anatomic or symptomatic evidence of leukaemias, lymphomas or myeloproliferative neoplasms (MPN) (that is, lymphadenopathy, organomegaly, illness or haemorrhage). Circulating hCD45^+^ cells in peripheral blood displayed no evidence of lympho/myeloproliferation or dysplasia. Furthermore, microscopic architecture of bone marrow and liver was normal and without fibrotic remodelling or aberrant deposition of collagen or desmin. All images were acquired using a colour CCD camera. The scale bar is 200 μm for low-resolution images in the left columns and 50 μm for high-resolution images in the right columns. Upper-left image (Giemsa, control) shows a white square in the centre that corresponds to the portion of the image shown at high resolution on the right (the same Giemsa control sample). This rule applies to all images shown.
Extended Data Figure 9 | Analysis of spleen of primary transplanted mice and bone marrow, spleen and liver of secondary transplanted mice for signs of malignant transformation. 

a, b, Analysis of spleen of mice 10 months after primary transplantation (from Fig. 3b) of HUVEC-derived rEC-hMPPs as well as bone marrow (n = 2), spleen and liver (n = 2, also Extended Data Fig. 10a) of mice that were engrafted with secondary transplanted hDMEC-derived rEC-hMPP cells 15 weeks after transplantation (from Fig. 5b) for signs of malignant transformation. The level of fibrosis was determined using Masson and PicroSirius stainings. The architecture of the bone marrow was determined by sequential multi-cross-sectional Wright–Giemsa and haematoxylin and eosin (H&E) staining and compared to age-controlled non-transplanted NSG mice. We did not observe any evidence of fibrosis or alteration of the geometry of the bone marrow, spleen or liver of the transplanted mice. Furthermore, no recipient mouse manifested any anatomical or symptomatic evidence of leukaemias, lymphomas or myeloproliferative neoplasm (MPN) (that is, lymphadenopathy, splenomegaly/organomegaly, illness or haemorrhage). Circulating hCD45+ cells in peripheral blood displayed no evidence of lympho/myeloproliferation or dysplasia. Furthermore, microscopic architecture of bone marrow, spleen and liver was normal and without fibrotic remodelling or aberrant deposition of collagen or desmin. All images were acquired using a colour CCD camera. In primary transplants the scale bar is 200 μm for low-resolution images in the left columns and 50 μm for high-resolution images in the right columns. The upper-left image (Giemsa, control) shows a white square in the centre that corresponds to the portion of the image shown at high resolution on the right (the same Giemsa control sample). This rule applies to all images shown (primary transplant). All images in secondary transplant are acquired at an original magnification of ×60. All images are acquired at original magnification of ×60. The top rows of images for each organ are secondary transplants; bottom rows of images for each organ are controls.
Extended Data Figure 10 | Analysis of liver and spleen of secondary transplanted mice for signs of malignant transformation and analyses of rEC-hMPPs for genetic stability. a, Analysis of liver and spleen of secondary transplanted mice for signs of malignant transformation. Repeat analysis of spleen and liver of mice that were engrafted with secondary transplanted hDMEC-derived rEC-hMPP cells 15 weeks post-transplantation for signs of malignant transformation (from Fig. 5b). The level of fibrosis was determined by Masson and PicroSirus stainings. The architecture of the bone marrow was determined by sequential multi-cross-sectional haematoxylin and eosin (H&E) staining and compared to age-controlled non-transplanted NSG mice. We did not observe any evidence of fibrosis or alteration of the geometry of the spleen or liver of the transplanted mice. Furthermore, no recipient mouse manifested any anatomical or symptomatic evidence of leukaemias, lymphomas or myeloproliferative neoplasm (MPN) (that is, lymphadenopathy, splenomegaly/organomegaly, illness or haemorrhage). Circulating hCD45^+ cells in peripheral blood displayed no evidence of lympho/myeloproliferation or dysplasia. Furthermore, microscopic architecture of bone marrow, spleen and liver was normal and without fibrotic remodelling or aberrant deposition of collagen or desmin. All images are acquired at original magnification of ×60. Top rows of images for each organ are secondary transplants; bottom rows of images for each organ are controls. b, Comparative genomic hybridization analysis (CGH) shows that rEC-hMPPs are genetically stable both in vitro and in vivo. Genomic DNA was extracted from HUVECs, CD45^+ rEC-hMPPs (35 days post-transduction) or in CD45^+CD34^+ rEC-hMPPs sorted from the engrafted NSG bone marrow (24 weeks post-transplantation) and expanded for 72 h in vitro. A human tumour sample was used as positive control of chromosome rearrangement. Extracted DNA was digested, labelled by random priming and hybridized to the Agilent 1M CGH arrays. The arrays were scanned in an Agilent DNA microarray scanner and obtained data were visualized using Feature Extraction software (version 10.7; Agilent). No genomic abnormalities were identified in CD45^+ rEC-hMPPs (or in CD45^+CD34^+ rEC-hMPPs) engrafted in NSG bone marrow. Hence, rEC-hMPPs remain genetically stable in vitro and in vivo and are not transformed.