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Brief Report Stem cells Transplantation

Generation of functional hematopoietic stem and progenitor cells (HSPCs) from human pluripotent stem cells (PSCs) has been a long-sought-after goal for use in hematopoietic cell production, disease modeling, and eventually transplantation medicine. Homing of HSPCs from bloodstream to bone marrow (BM) is an important aspect of HSPC biology that has remained unaddressed in efforts to derive functional HSPCs from human PSCs. We have therefore examined the BM homing properties of human induced pluripotent stem cell–derived HSPCs (hiPS-HSPCs). We found that they express molecular effectors of BM extravasation, such as the chemokine receptor CXCR4 and the integrin dimer VLA-4, but lack expression of E-selectin ligands that program HSPC trafficking to BM. To overcome this deficiency, we expressed human fucosyltransferase 6 using modified mRNA. Expression of fucosyltransferase 6 resulted in marked increases in levels of cell surface E-selectin ligands. The glycoengineered cells exhibited enhanced tethering and rolling interactions on E-selectin–bearing endothelium under flow conditions in vitro as well as increased BM trafficking and extravasation when transplanted into mice. However, glycoengineered hiPS-HSPCs did not engraft long-term, indicating that additional functional deficiencies exist in these cells. Our results suggest that strategies toward increasing E-selectin ligand expression could be applicable as part of a multifaceted approach to optimize the production of HSPCs from human PSCs.

# mRNA-mediated glycoengineering ameliorates deficient homing of human stem cell–derived hematopoietic progenitors

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## Introduction

Hematopoietic stem and progenitor cell (HSPC) transplantation is the paradigmatic stem cell therapy, with ~50,000 transplants performed worldwide per year to treat a variety of blood disorders (1). Despite its curative potential, difficulties in obtaining sufficient numbers of HLA-matched HSPCs contribute to poor transplantation outcomes and limit broader applicability. Derivation of large quantities of HSPCs from human pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and/or induced pluripotent stem cells (iPSCs), holds great promise to mitigate many HSPC transplantation–related limitations. However, despite much progress, generation of fully functional and engraftment-competent HSPCs from human pluripotent stem cells ex vivo has remained challenging (2).

"Bone marrow homing" refers to the process by which HSPCs transit from the bloodstream to the bone marrow (BM). This process, which is a prerequisite for functional hematopoiesis, involves two components: (1) trafficking of circulating HSPCs to specialized BM endothelial beds and (2) extravasation of HSPCs at those beds. Homing involves a multistep cascade that begins with the tethering and rolling of transplanted cells on discrete BM sinusoidal vessels that is mediated by interactions between E-selectin on endothelial cells and its ligands on HSPCs. Once cells have migrated to relevant sinusoids, extravasation ensues as integrins (principally VLA-4) become activated via chemokines by binding to their receptors (e.g., SDF-1 [also known as CXCL12] binding to CXCR4) to induce firm adherence of HSPCs to the endothelial wall. Finally, cells undergo transendothelial migration and parenchymal lodgment, processes modulated by chemokine gradients within the BM (3). Although BM homing is a critical aspect of HSPC biology, studies assessing the homing properties of HSPCs from human pluripotent stem cells are lacking (2).

In this study, we examined the expression and function of molecules that mediate HSPC homing to BM and identified a marked deficiency of E-selectin ligands on the surface of PSC-derived HSPCs. We also demonstrate a simple and potent strategy to create functional E-selectin ligands on the surface of iPSC-derived HSPCs using modified mRNA encoding the glycosyltransferase fucosyltransferase 6 (FUT6). The glycoengineered human iPSC-derived HSPCs exhibited markedly enhanced tethering and rolling inter-

**Authorship note:** J. Lee and B. Dykstra contributed equally to this work.

**Conflict of interest:** D.J. Rossi is a founder of Moderna Therapeutics, a Cambridge, Massachusetts, company that is developing modified mRNA therapeutics, and is also a co-founder of Magenta Therapeutics, which is focused on transplantation medicine.

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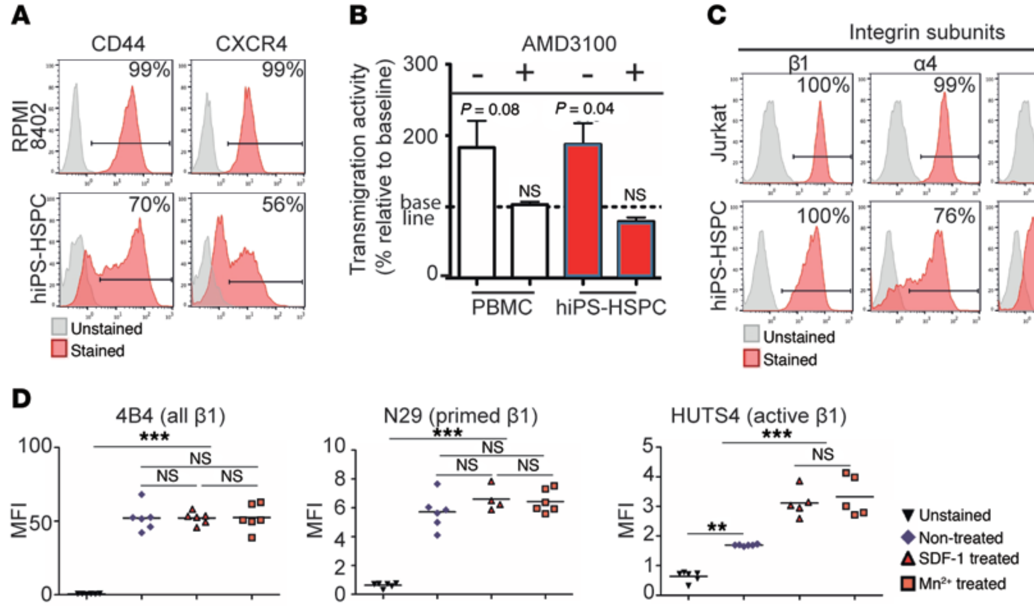



**Figure 1. hiPS-HSPCs possess molecules that mediate extravasation.** (**A**) Representative histograms showing expression of CXCR4 and CD44 on hiPS-HSPCs and control RPMI8402 cells. (**B**) Normalized transmigration activity of hiPS-HSPCs and control human PBMCs in response to SDF-1. CXCR4 antagonist (AMD3100) used to confirm migration was CXCR4-mediated. $n = 3$. (**C**) Representative histograms showing expression of β1, α4, α5 integrin subunits on hiPS-HSPCs and Jurkat control cells. (**D**) β1 integrin activation status of hiPS-HSPCs stimulated with SDF-1 or $MnCl_2$ from multiple experiments. 4B4, all β1 conformations; N29, primed β1 conformation; HUTS4, active β1 conformation. $n = 4–6$; $^{**}P < 0.01$, $^{***}P < 0.001$; NS, not significant by 1-way ANOVA with Tukey's HSD test. Error bars indicate SEM.

actions with endothelial cells under shear stress conditions in vitro and displayed increased homing and extravasation into the calvarial BM of immunocompromised mice in vivo.

## Results and Discussion

Building upon previously reported protocols (4–6), we developed a serum-free, stromal cell–free differentiation protocol capable of generating high percentages of hematopoietic cells from a human iPSC line derived using modified mRNA (Supplemental Figure 1A; supplemental material available online with this article; https://doi.org/10.1172/JCI92030DS1) (7). By day 10 of differentiation, round, refractile, non-adherent hematopoietic cells were observed above the adherent cell layer (Supplemental Figure 1B) and corresponded with the expression of hematopoietic progenitor markers (Supplemental Figure 1, C and D). Hematopoietic differentiation was similarly highly efficient across multiple human PSC lines, including two ESC lines and iPSCs derived from a patient with Pearson's syndrome (Supplemental Figure 1E). Consistent with their acquisition of primitive hematopoietic markers, the human iPSC-derived hematopoietic cells possessed robust progenitor activity (Supplemental Figure 1, F and G), and we termed these cells human iPSC-derived HSPCs (hiPS-HSPCs).

We then proceeded to examine on hiPS-HSPCs the molecules known to mediate BM extravasation, and marrow lodgment, of human HSPCs. First, we assessed expression of the SDF-1 receptor CXCR4 and the hyaluronan receptor CD44 (8) and observed that hiPS-HSPCs expressed moderate to high levels of these molecules (Figure 1A). Function of CXCR4 was confirmed by transwell migration assays, where hiPS-HSPCs demonstrated significantly increased transmigration activity toward an SDF-1 gradient, an activity that was completely blocked by the CXCR4 antagonist AMD3100 (Figure 1B). We next assessed expression of the integrin subunits that constitute VLA-4 and VLA-5, as VLA-4 is critical for HSPC extravasation, and both VLA-4 and VLA-5 mediate binding to fibronectin, a key mediator of HSPC lodgment. hiPS-HSPCs expressed robust levels of the VLA-4 integrin subunits α4 and β1, and moderate expression of α5 (Figure 1C), which together with β1, constitutes VLA-5 (9). Integrin dimers are known to exist in three distinct conformations: bent-closed (inactive), extended-closed (primed), and extended-open (active) (10). Activation of integrins, canonically by CXCR4 engagement (SDF-1–induced signaling), is critical to enabling their function as mediators of homing and marrow lodgment (11). To assess the integrin activation status of hiPS-HSPCs at baseline and in response to SDF-1, we used activation-specific antibodies to the integrin β1 subunit, which is common to both VLA-4 and VLA-5 (Figure 1D). This analysis revealed that hiPS-HSPCs natively display β1 integrins in an extended-closed (primed) conformation. Furthermore, the β1 integrins were converted to the extended-open (active) conformation via SDF-1–induced signaling, to levels comparable to that of cells exposed to manganese, a strong signaling-independent integrin activator (Figure 1D). Collectively, these data indicate that hiPS-HSPCs express appreciable levels of CD44, CXCR4, VLA-4, and VLA-5, and that these mediators of extravasation and lodgment function normally in these cells.

Next, we analyzed E-selectin ligand expression using HECA452, an antibody that detects a tetrasaccharide glycan known as sialyl Lewis X ($sLe^x$), the canonical E-selectin ligand binding determinant (12). Strikingly, the hiPS-HSPCs had very low $sLe^x$ expression compared with control peripheral blood

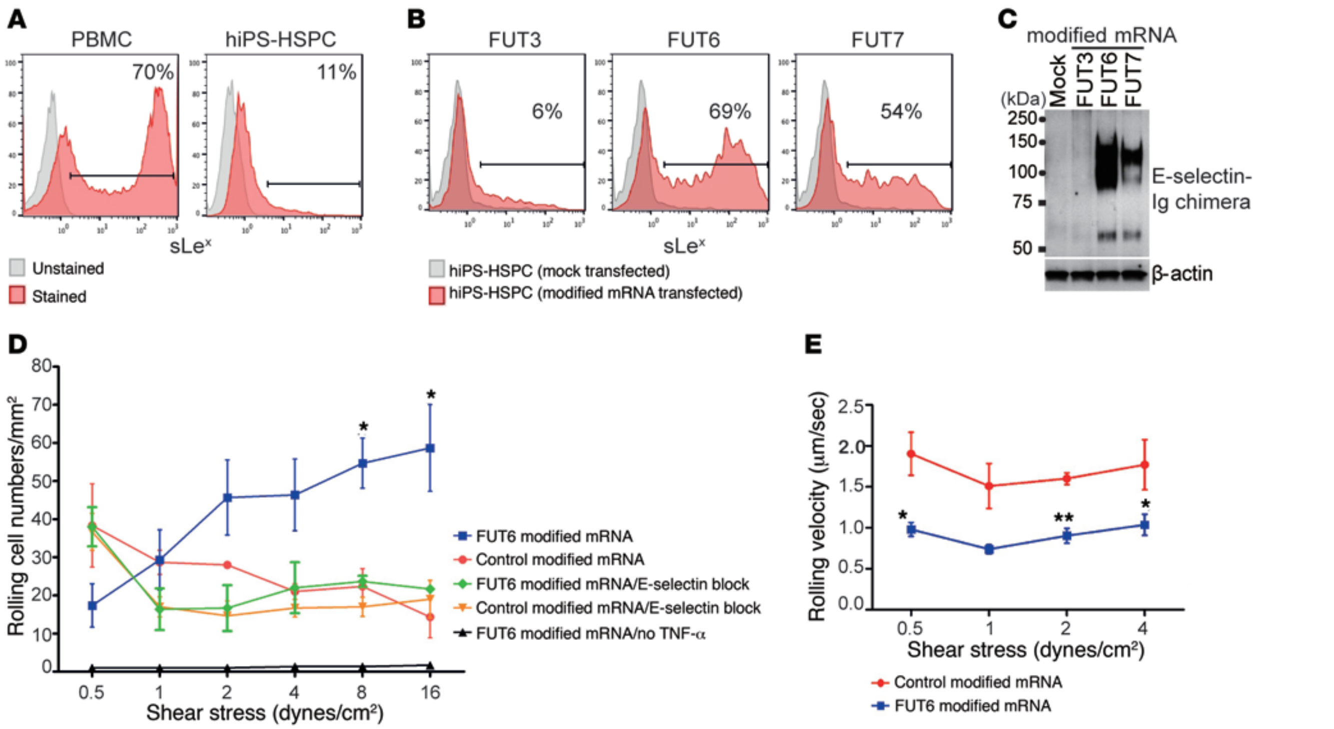
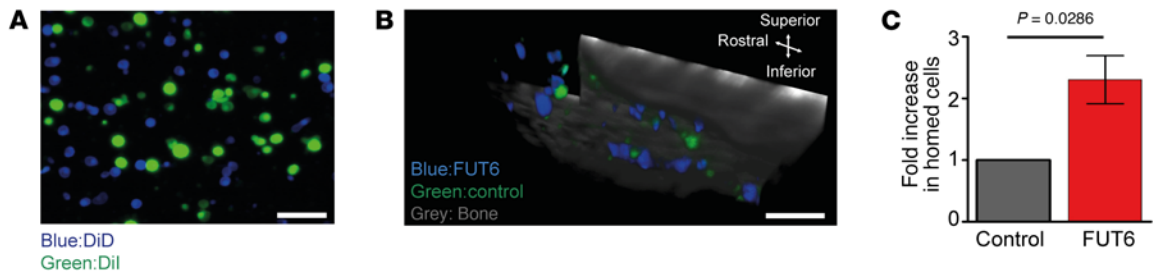



**Figure 2. FUT6 modified mRNA–mediated glycoengineering enhances tethering and rolling of hiPS-HSPCs on endothelial cells under shear conditions.** (**A**) Representative histograms showing expression of sLe$^x$ on hiPS-HSPCs and PBMC control cells measured by HECA452 antibody. (**B**) Representative histograms showing expression of sLe$^x$ on hiPS-HSPCs 24 hours after transfection with modified mRNAs encoding FUT3, FUT6, and FUT7. (**C**) Western blot with an E-selectin–Ig chimera or β-actin (loading control) on lysates of hiPS-HSPCs cells mock transfected or transfected with the indicated modified mRNAs 2 days earlier. (**D**) Quantitation of E-selectin–mediated rolling of control or FUT6 modified mRNA–transfected hiPS-HSPCs on TNF-α–activated HUVECs under increasing shear stress. E-selectin blocking antibody reduces rolling to baseline, while no interaction was observed on HUVECs not activated with TNF-α. $n = 3$. (**E**) FUT6 modified mRNA–transfected hiPS-HSPCs exhibit reduced rolling velocities on TNF-α–activated HUVECs. $n = 3$. Error bars indicate SEM. $^*P < 0.05$, $^{**}P < 0.01$ by Student's $t$ test.

mononuclear cells (PBMCs) (Figure 2A). To determine whether this low sLe$^x$ expression was differentiation protocol– or cell line–specific, we assessed sLe$^x$ expression on HSPCS derived from iPSCs according to two alternative published protocols (6, 13), as well as HSPCs derived from two different ESC lines. Gating on putative HSPCs as described in the respective published protocols (Supplemental Figure 2A), we observed similarly low sLe$^x$ levels of the populations examined (Supplemental Figure 2, B and C). The lack of E-selectin ligand expression led us to hypothesize that increasing E-selectin ligand expression might result in increased homing of these cells upon transplantation. Based on earlier experiments in other cell types (14), we predicted that hiPS-HSPCs could express sLe$^x$ upon fucosylation with α(1,3)-fucosyltransferase. We therefore generated modified mRNAs (7) encoding 3 human α(1,3) fucosyltransferases (hereafter referred to as FUT3, FUT6, and FUT7 modified mRNAs; Supplemental Figure 2D) and transfected them into hiPS-HSPCs (Supplemental Figure 3). Total cell surface sLe$^x$ expression was markedly increased in hiPS-HSPCs transfected with modified mRNAs encoding FUT6 and FUT7, but not FUT3 (Figure 2B). To specifically visualize E-selectin binding glycoproteins, we performed Western blot analysis using an E-selectin–Ig chimera as a probe (Figure 2C). FUT6 modified mRNA–transfected hiPS-HSPCs had higher levels of E-selectin binding glycoproteins than FUT3 modified mRNA–, FUT7 modified mRNA–, or mock-transfected hiPS-HSPCs (Figure 2C). Since sLe$^x$-bearing glycoproteins are specifically known to play a critical role in cellular trafficking, FUT6 modified mRNA was utilized for all further experiments. FUT6 modified mRNA transfection consistently and robustly increased sLe$^x$ expression of hiPS-HSPCs in multiple independent experiments (Supplemental Figure 2E). Time course analysis of FUT6 modified mRNA–transfected hiPS-HSPCs showed that sLe$^x$ expression peaked between 24 and 72 hours after transfection and decreased thereafter (Supplemental Figure 2F). No detrimental effects on hematopoietic differentiation or colony-forming activity were observed (Supplemental Figure 2, G–I). Collectively, these data indicate that FUT6 modified mRNA transfection is an effective strategy for generating E-selectin ligands on hiPS-HSPCs.

To determine whether the increased sLe$^x$ and E-selectin–Ig reactivity corresponds to functional E-selectin binding activity, we tested the ability of FUT6 modified mRNA–transfected hiPS-HSPCs to tether and roll under fluid shear conditions on TNF-α–activated HUVEC monolayers using a parallel plate flow chamber. FUT6 modified mRNA–transfected hiPS-HSPCs showed increasingly higher numbers of rolling cells with increasing shear stress, in contrast to control cells, which maintained uniformly low levels of rolling cells (Figure 2D and Supplemental Video 1). Furthermore, rolling velocities were significantly lower in FUT6 modified mRNA–transfected hiPS-HSPCs, indicative of increased E-selectin ligand binding (Figure 2E). Blocking E-selectin or



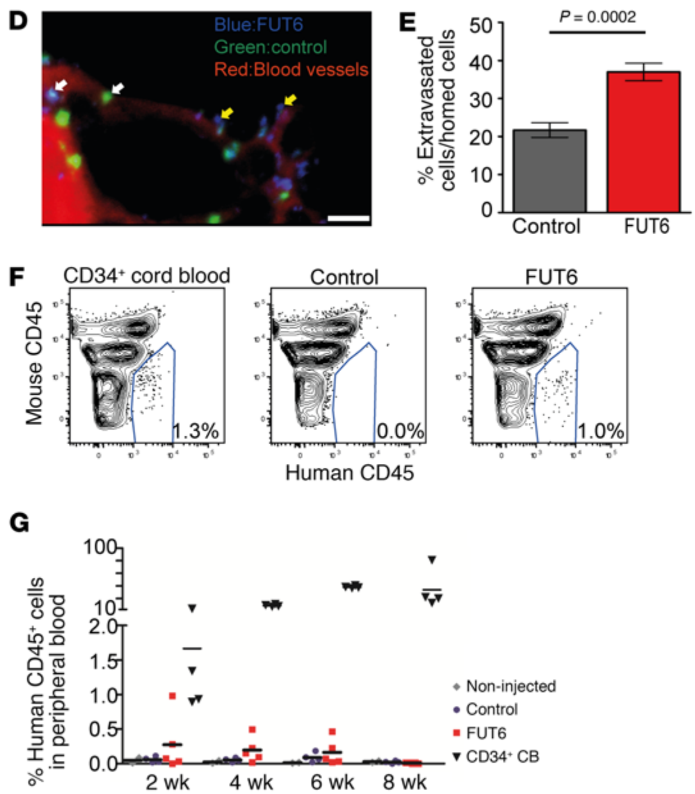




**Figure 3. FUT6 modified mRNA enhances BM homing and extravasation of xenotransplanted hiPS-HSPCs.** (**A**) Dye-labeled FUT6- (DiD, blue) or control modified mRNA–transfected (DiI, green) hiPS-HSPCs mixed at a 1:1 ratio and imaged before transplant. (**B**) 3D reconstruction of a calvarium section of an NSG mouse transplanted with DiD- and DiI-labeled hiPS-HSPCs. Image represents view of sagittal plane, with a portion of bone digitally removed to optimize visualization. Imaging depth extends to approximately 150 μm beneath the bone. (**C**) FUT6 modified mRNA–transfected hiPS-HSPCs showed increased homing at 2 hours after transplant compared with control modified mRNA–transfected cells. $n = 3$ independent experiments (6 mice). (**D**) Image taken 24 hours after transplant showing DiD- (blue) and DiI-labeled (green) hiPS-HSPCs and AngioSense-labeled blood vessels (red). Extravasated hiPS-HSPCs were defined as those completely distinct from the blood vessel. White arrows indicate non-extravasated cells; yellow arrows indicate extravasated cells. (**E**) FUT6 modified mRNA transfection increases the percentage of homed hiPS-HSPCs that extravasated from the calvarial blood vessels. $n = 3$ independent experiments (5 mice). (**F**) Representative FACS plots showing human chimerism at 2 weeks after transplant. (**G**) Time course of peripheral blood chimerism in NSG mice receiving transplant of human CD34⁺ cord blood (CB) cells, and control or FUT6 modified mRNA–transfected hiPS-HPCs. Scale bars: 50 μm. Error bars in **C** and **E** indicate SEM. *P* values in **C** and **E** were calculated by Student's *t* test.

withholding TNF-α activation decreased the numbers of rolling FUT6 modified mRNA–transfected cells, confirming that their altered rolling properties are mediated exclusively by increased E-selectin ligand activity (Figure 2D and Supplemental Video 2). These data show that FUT6 modified mRNA glycoengineering generates functional E-selectin ligands on hiPS-HSPCs, and mediates increased tethering and rolling interactions on activated endothelium.

To determine whether the increased E-selectin ligand expression engendered by FUT6 modified mRNA is functionally relevant in vivo, we assessed BM homing of hiPS-HSPCs in vivo using calvarial BM imaging (Figure 3). Control or FUT6 modified mRNA–transfected hiPS-HSPCs stained with DiI or DiD were coinjected into NSG mice, and calvaria were imaged using intravital microscopy to measure BM homing (Figure 3, A–C). Imaging and enumeration of labeled cells 2 hours after transplant showed that the FUT6 modified mRNA–transfected hiPS-HSPCs exhibited a significant increase in homing compared with control-transfected cells (Figure 3C). Extravasation was assessed by labeling blood vessels with a vascular imaging dye and quantifying the number of hiPS-HSPCs that had migrated out of the blood vessel 24 hours after transplant (Figure 3D). These studies revealed that FUT6 modified mRNA–transfected hiPS-HSPCs had significantly increased extravasation frequency compared with cotransplanted control-treated cells (Figure 3E). To establish the absolute homing efficiency of FUT6 modified mRNA–transfected hiPS-HSPCs, we performed short-term BM homing experiments, comparing FUT6 modified mRNA–transfected hiPS-HSPCs with CD34⁺ cells from mobilized peripheral blood (CD34⁺ mPB cells) (Supplemental Figure 4, A and B). Despite the improved homing and transmigration engendered by the creation of functional E-selectin ligands, FUT6 modified mRNA–transfected hiPS-HSPCs still showed 5-fold lower BM homing efficiency compared with CD34⁺ mPB cells (Supplemental Figure 4, A and B). To determine whether the increased BM homing of the modified cells was sufficient to enable hematopoietic engraftment, we injected control or FUT6 modified mRNA–transfected hiPS-HSPCs into NSG mice. Mice transplanted with control-transfected hiPS-HSPCs did not show detectable human engraftment (Figure 3, F and G). In contrast, a subset of mice receiving transplant of FUT6 modified mRNA–transfected hiPS-HSPCs exhibited low human chimerism in the peripheral blood at 2 weeks after transplant (Figure 3, F and

G). However, human chimerism decreased over time and was no longer detectable 8 weeks after transplant (Figure 3, F and G).

In the present study, we focused on BM homing, a previously unaddressed aspect for deriving engraftment-competent hiPS-HSPCs. We report that molecular determinants of extravasation are intact in hiPS-HSPCs, but hiPS-HSPCs are deficient in expression of E-selectin ligands. To overcome this deficiency, we developed a simple modified mRNA–based approach to temporarily increase the E-selectin ligands on these cells, resulting in increased tethering/rolling in vitro, and improved calvarial homing and extravasation in vivo. We anticipate that strategies to increase E-selectin ligand expression could be applicable as part of a multifaceted approach to optimize the production of HSPCs from human PSCs. As an example of the power of glycoengineering to enforce E-selectin ligand expression, fucosylation of human cord blood using recombinant fucosyltransferase has recently been reported to accelerate engraftment in xenotransplant models (15, 16) and has also shown promising results in early-stage clinical trials (17).

It should be noted that in this study, increasing homing and extravasation by FUT6 modified mRNA was not sufficient to generate long-term engraftable hiPS-HSPCs. Additional functional deficiencies (unrelated to homing) likely exist and must be overcome before fully engraftable HSPCs from human PSCs can be generated. One promising strategy that we are pursuing is the use of modified mRNA to transiently increase expression of relevant transcription factors. Since several modified mRNAs encoding different proteins can be introduced simultaneously (7), a combinatorial modified mRNA transfection approach could be a powerful strategy to generate fully functional human PSC–derived HSPCs. Importantly, since modified mRNA is non-permanent and non-genome-integrative, it is particularly amenable to clinical use. In conclusion, we have identified E-selectin ligand deficiency as an important factor limiting the BM homing ability of hiPS-HSPCs and provide evidence that a modified mRNA–based glycoengineering strategy can overcome this deficiency. Importantly, our findings suggest that glycoengineering to enforce E-selectin ligand expression could be considered within the context of future efforts focused on generating functional HSPCs from human PSCs.

## Methods

Details about experimental procedures are provided in Supplemental Methods.

*Statistics.* $P$ values were calculated using a 1-way ANOVA with Tukey's honest significant difference (HSD) or Student's $t$ test as indicated in the figure legends. Student's $t$ test was 2-tailed. A $P$ value less than 0.05 considered significant, and all data are reported as mean ± SEM.

*Study approval.* All animal use was in accordance with the guidelines of the Animal Care and Use Committee of the University of Massachusetts Medical School and The Jackson Laboratory. Human cord blood and PBMCs from peripheral blood draws were collected in heparin from healthy volunteers under signed informed consent in accordance with the Declaration of Helsinki and with approval from the Institutional Review Board of University of Massachusetts Medical School or Brigham and Women's Hospital, respectively.

## Author contributions

JML, BD, JAS, RS, and DJR designed experiments; JML, BD, JAS, and LLK performed experiments and analyzed data; and JML, BD, JAS, LLK, DLG, LDS, MAB, CPL, RS, and DJR wrote the manuscript.

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1. Gratwohl A, et al. Hematopoietic stem cell transplantation: a global perspective. *JAMA*. 2010;303(16):1617–1624.
2. Lee J, Dykstra B, Sackstein R, Rossi DJ. Progress and obstacles towards generating hematopoietic stem cells from pluripotent stem cells. *Curr Opin Hematol*. 2015;22(4):317–323.
3. Sackstein R. The lymphocyte homing receptors: gatekeepers of the multistep paradigm. *Curr Opin Hematol*. 2005;12(6):444–450.
4. Ledran MH, et al. Efficient hematopoietic differentiation of human embryonic stem cells on stromal cells derived from hematopoietic niches. *Cell Stem Cell*. 2008;3(1):85–98.
5. Nakajima-Takagi Y, et al. Role of SOX17 in hematopoietic development from human embryonic stem cells. *Blood*. 2013;121(3):447–458.
6. Sturgeon CM, Ditadi A, Awong G, Kennedy M, Keller G. Wnt signaling controls the specification of definitive and primitive hematopoiesis from human pluripotent stem cells. *Nat Biotechnol*. 2014;32(6):554–561.
7. Mandal PK, Rossi DJ. Reprogramming human fibroblasts to pluripotency using modified mRNA. *Nat Protoc*. 2013;8(3):568–582.
8. Avigdor A, et al. CD44 and hyaluronic acid cooperate with SDF-1 in the trafficking of human CD34+ stem/progenitor cells to bone marrow. *Blood*. 2004;103(8):2981–2989.
9. Lapidot T, Dar A, Kollet O. How do stem cells find their way home? *Blood*. 2005;106(6):1901–1910.
10. Su Y, et al. Relating conformation to function in integrin α5β1. *Proc Natl Acad Sci USA*. 2016;113(27):E3872–E3881.
11. Peled A, et al. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood*. 2000;95(11):3289–3296.
12. Sackstein R. Glycosyltransferase-programmed stereosubstitution (GPS) to create HCELL: engineering a roadmap for cell migration. *Immunol Rev*. 2009;230(1):51–74.
13. Gori JL, et al. Vascular niche promotes hematopoietic multipotent progenitor formation from pluripotent stem cells. *J Clin Invest*. 2015;125(3):1243–1254.
14. Dykstra B, et al. Glycoengineering of E-selectin ligands by intracellular versus extracellular fucosylation differentially affects osteotropism of human mesenchymal stem cells. *Stem Cells*. 2016;34(10):2501–2511.
15. Robinson SN, et al. Fucosylation with fucosyltransferase VI or fucosyltransferase VII improves cord blood engraftment. *Cytotherapy*. 2014;16(1):84–89.
16. Wan X, et al. Fucosyltransferase VII improves the function of selectin ligands on cord blood hematopoietic stem cells. *Glycobiology*. 2013;23(10):1184–1191.
17. Popat U, et al. Enforced fucosylation of cord blood hematopoietic cells accelerates neutrophil and platelet engraftment after transplantation. *Blood*. 2015;125(19):2885–2892.