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Supplemental Information

Serum-free Erythroid Differentiation for Efficient Genetic Modification and High-Level Adult Hemoglobin Production

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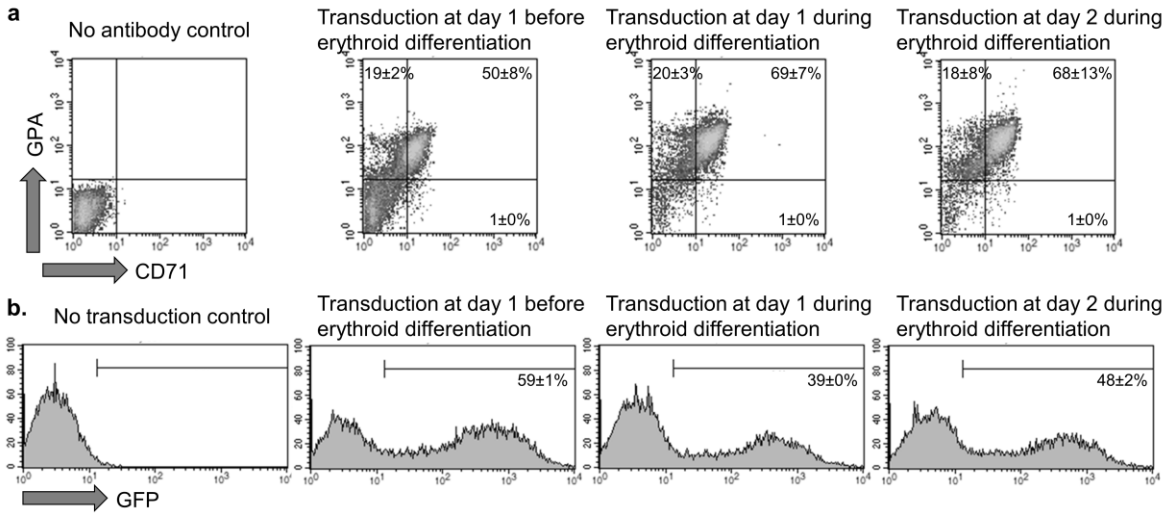
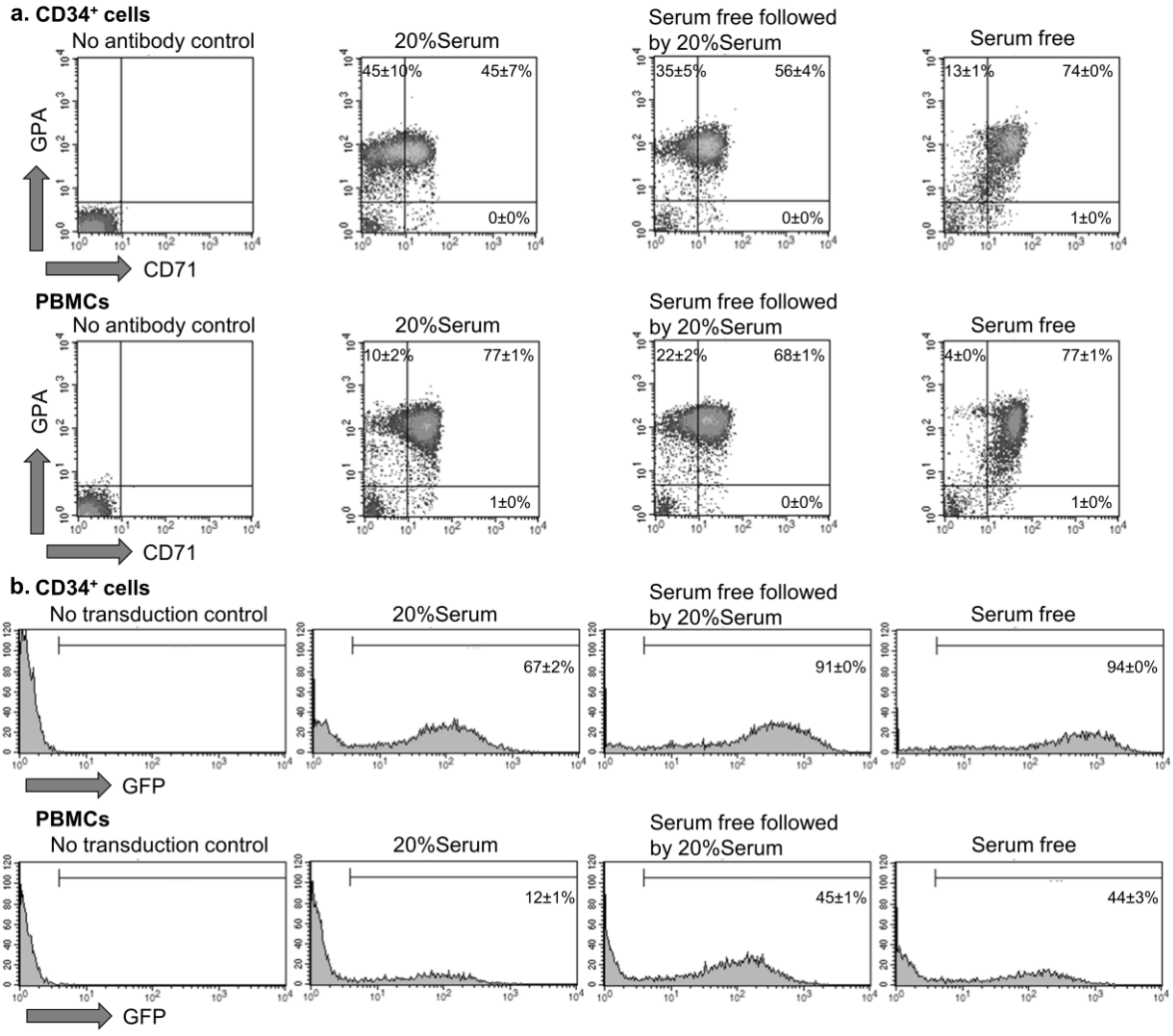
Supplementary figures

Supplementary figure 1. Flow cytometry panels for lentiviral transduction of human CD34⁺ cells before and after initiating erythroid differentiation. (a) Dot plot panels for co-expression of GPA and CD71 in CD34⁺ cell-derived erythroid cells with lentiviral transduction 12 days after differentiation. (b) Histograms of GFP expression in CD34⁺ cell-derived erythroid cells with lentiviral transduction 12 days after differentiation. Values: mean ± SEM. All experiments were performed in triplicate.

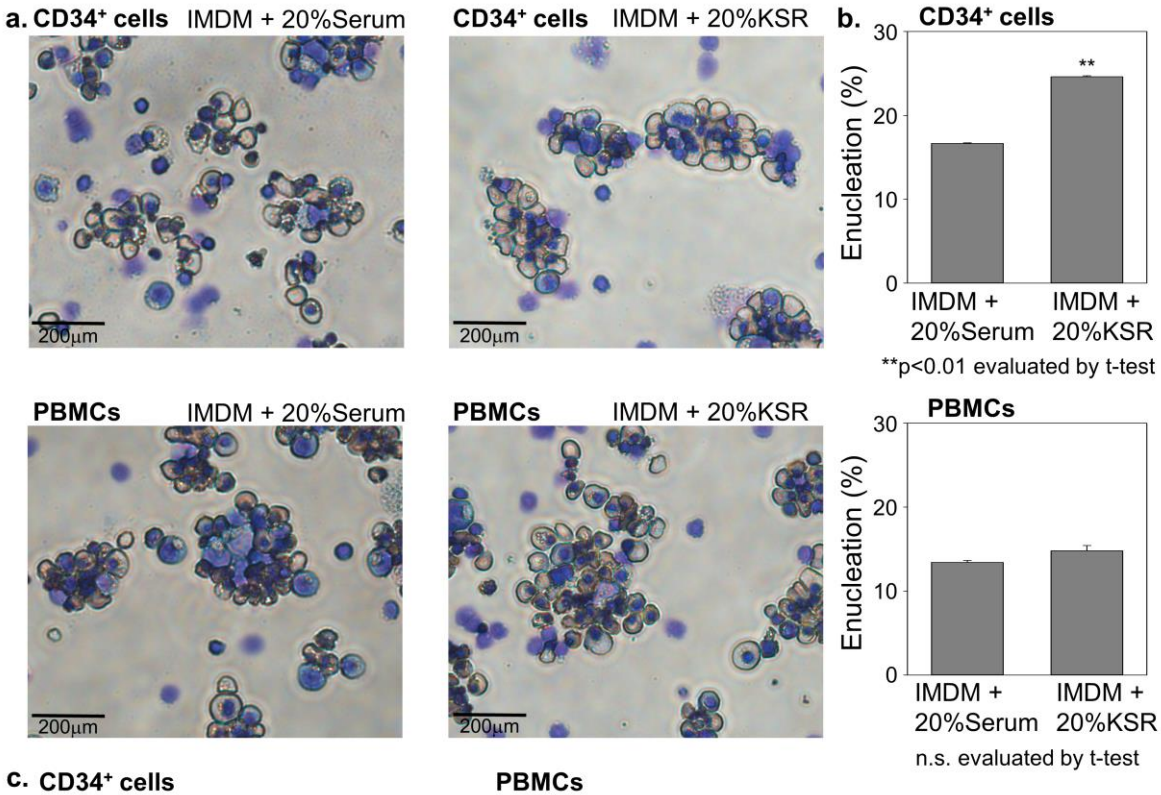


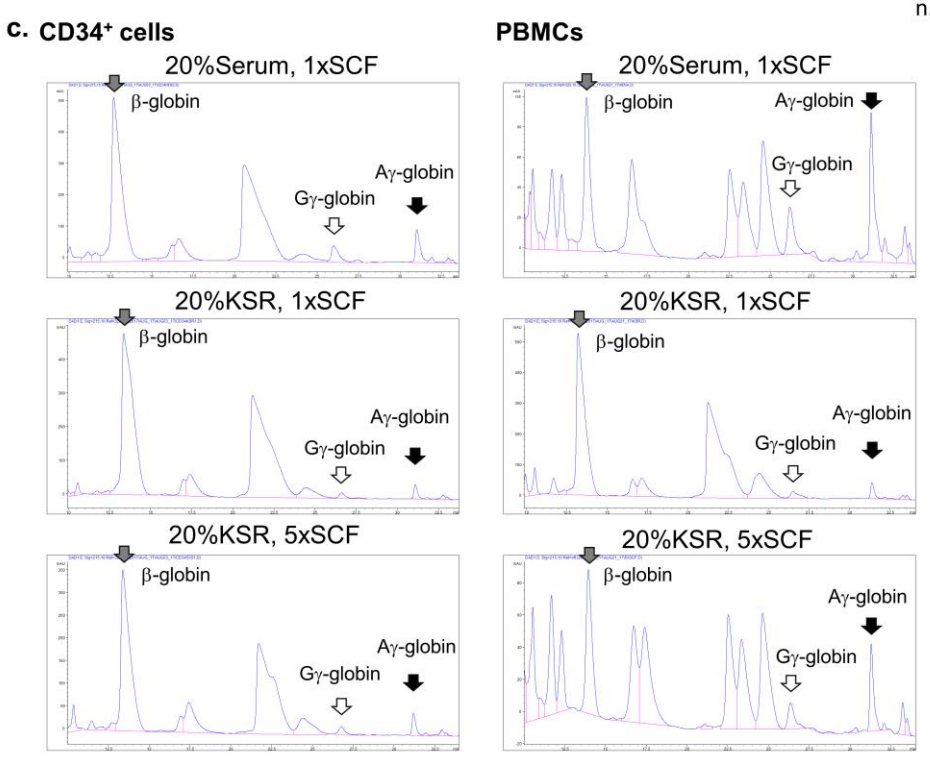
Supplementary figure 2. Flow cytometry panels for transduction in serum-free erythroid differentiation for human CD34$^+$ cells and PBMCs. (a) Dot plot panels for co-expression of GPA and CD71 in CD34$^+$ cell and PBMC-derived erythroid cells with lentiviral transduction 11 days after differentiation. (b) Histograms of GFP expression in CD34$^+$ cell and PBMC-derived erythroid cells with lentiviral transduction 11 days after differentiation. Values: mean ± SEM. All experiments were performed in triplicate.


a. CD34+ cells
No antibody control
20%Serum
Serum free followed by 20%Serum
Serum free
GPA
CD71
45±10%
45±7%
0±0%
35±5%
56±4%
0±0%
13±1%
74±0%
1±0%
PBMCs
No antibody control
20%Serum
Serum free followed by 20%Serum
Serum free
10±2%
77±1%
1±0%
22±2%
68±1%
0±0%
4±0%
77±1%
1±0%
b. CD34+ cells
No transduction control
20%Serum
Serum free followed by 20%Serum
Serum free
GFP
67±2%
91±0%
94±0%
PBMCs
No transduction control
20%Serum
Serum free followed by 20%Serum
Serum free
GFP
12±1%
45±1%
44±3%

Supplementary figure 3. Cell morphology and RP-HPLC panels for β-globin production with IMDM-based serum-free erythroid differentiation media with KSR supplementation.

(a) Two weeks after serum-containing or serum-free erythroid differentiation, cell morphology was evaluated by cytospin with Wright-Giemsa stain in $CD34^+$ cell- and PBMC-derived erythroid cells. (b) Enucleation was analyzed by flow cytometry with Hoechst 33342 stain. (c) RP-HPLC peaks of β-globin and γ-globin production from differentiated erythroid cells with 20% serum (1xSCF), 20% KSR (1xSCF), and 20% KSR (5xSCF). Bars: mean ± SEM. Gray arrow: β-globin, white arrow: Gγ-globin, black arrow: Aγ-globin.


a. CD34+ cells IMDM + 20%Serum
CD34+ cells IMDM + 20%KSR
200μm
b.
CD34+ cells
Enucleation (%)
30
20
10
0
**
IMDM + 20%Serum
IMDM + 20%KSR
**p<0.01 evaluated by t-test
PBMCs IMDM + 20%Serum
PBMCs IMDM + 20%KSR
PBMCs
n.s. evaluated by t-test


c. CD34+ cells
PBMCs
20%Serum, 1xSCF
20%KSR, 1xSCF
20%KSR, 5xSCF
β-globin
Aγ-globin
Gγ-globin

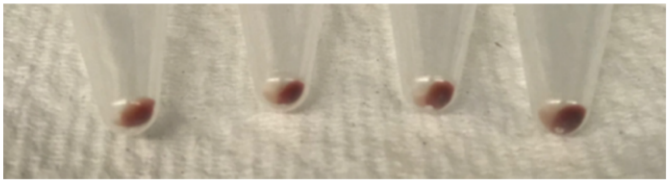
Supplementary figure 4. Vector construct, cell pellets, and RP-HPLC panels for adult Hb production with β-globin lentiviral transduction in CD34$^+$ cells and PBMCs from SCD. (a) Schematic construct of β-globin-expressing lentiviral vectors (with or without T87Q). (b) Red cell pellets 2 weeks after erythroid differentiation to asses for hemoglobinization. (c) RP-HPLC peaks of βT87Q-globin, β-globin, βS-globin, and γ-globin production from differentiated erythroid cells with lentiviral transduction encoding βT87Q-globin, β-globin, or GFP. LTR: long terminal repeat, ψ: packaging signal, 3'UTR: 3' untranslated region, P: β-globin promoter, LTR: locus control region, light gray arrow: βT87Q-globin or β-globin, gray arrow: βS-globin, white arrow: Gγ-globin, black arrow: Aγ-globin.

a.

b. CD34⁺ cells

IMDM +20%KSR

βT87Q -globin | β-globin | GFP | No vector



c. CD34⁺ cells **PBMCs**