Sequential cellular niches control the generation of enucleated erythrocytes from human pluripotent stem cells

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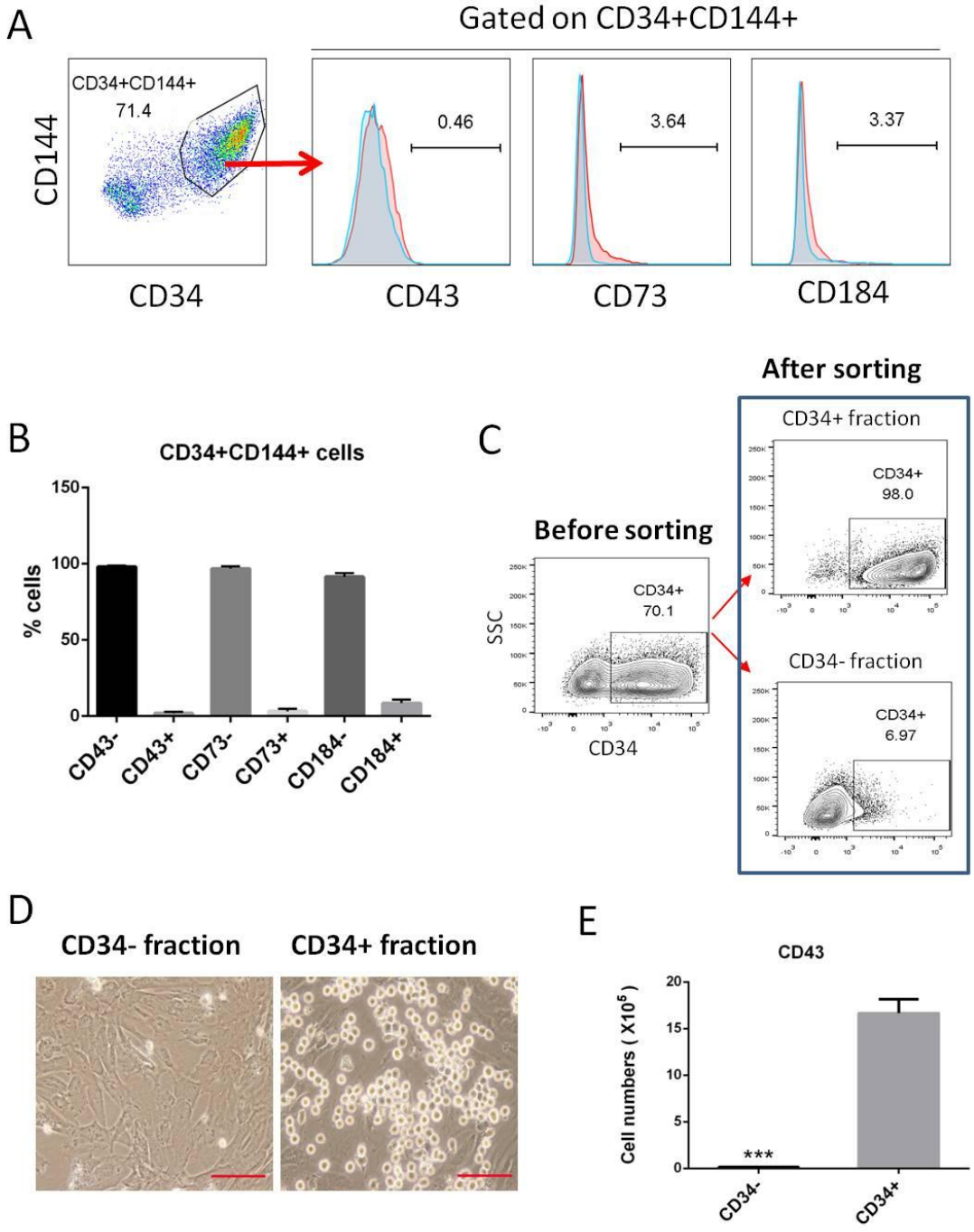
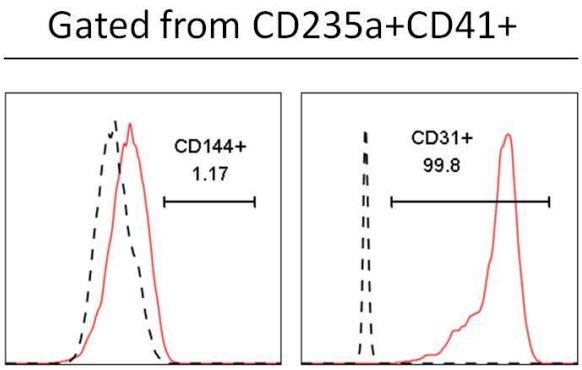
Supplementary Figure S1



Supplementary Figure S1. Characterization of the hematopoietic potential of HE cells in Day 5 cells，Related to Figure 1.

(A) Representative flow cytometric analysis of the frequency of HE cells after 5 days of H1 hESC differentiation. HE cells were characterized as $CD34^{+}CD144^{+}CD43^{-}CD73^{-}CD184^{-}$ cells. n=3. (B) Flow cytometric analysis of $CD34^{+}CD144^{+}$ population from D5 cells. The majority of $CD34^{+}CD144^{+}$ cells had the HE phenotype of $CD34^{+}CD144^{+}CD43^{-}CD73^{-}CD184^{-}$. n = 3. (C-E) Hematopoietic potential of $CD34^{+}$ cells. $CD34^{+}$ cells isolated from Day 5 cells by MACS using CD34 magnetic beads, and the purity of sorted cells were analyzed by flow cytometry (B). $CD34^{+}$ cells and $CD34^{-}$ cells were subjected for EHT assay by plating them on fibronectin-coated plates in hematopoietic differentiation medium containing SCF, TPO, FLT3-L, IGF-1, IL-3, IL-6, VEGF, and bFGF. Hematopoietic cells were analyzed after 5 days by microscopy (C) and flow cytometry for $CD43^{+}$ hematopoietic cells (D). Scale bars, 100 μm. n=3. Experiments were performed on H1 hESCs. Data are shown as means ±SEM. Statistical analysis was done by two-tailed Student's t test. ***P<.001.

Supplementary Figure S2

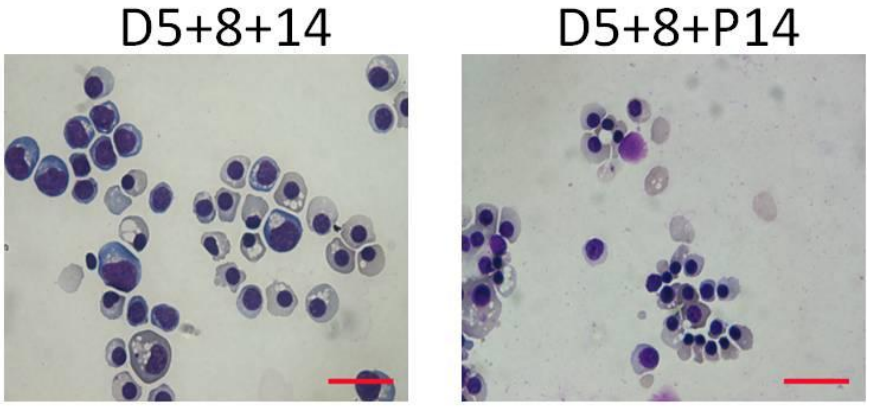


Supplementary Figure S2. Characterization of the phenotype of CD235a^{+}CD41^{+} cells, Related to Figure 1.

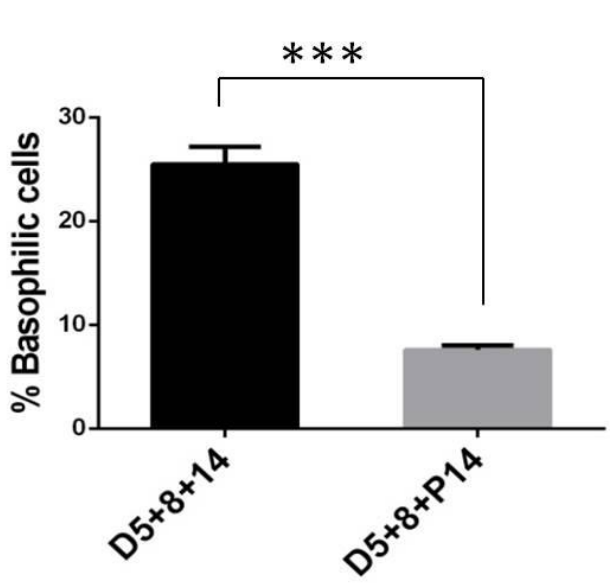
Flow cytometric analysis of the expression of CD144 and CD31 in CD235a^{+}CD41^{+} cells of D5+2. Flow data were representative from three experiments (n=3). Experiments were performed on H1 hESCs.

Supplementary Figure S3

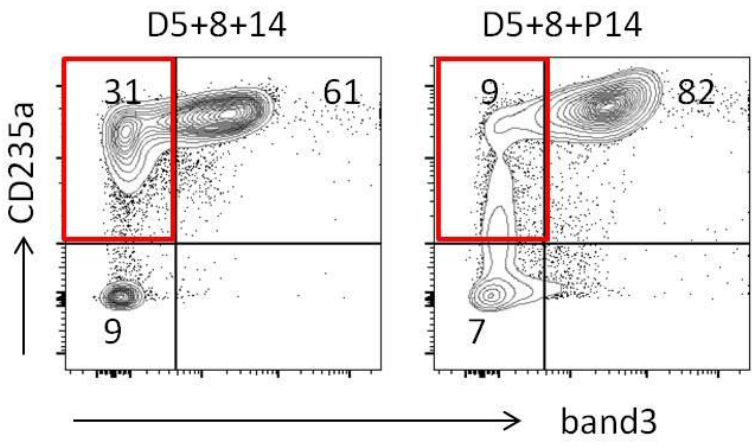
A



B



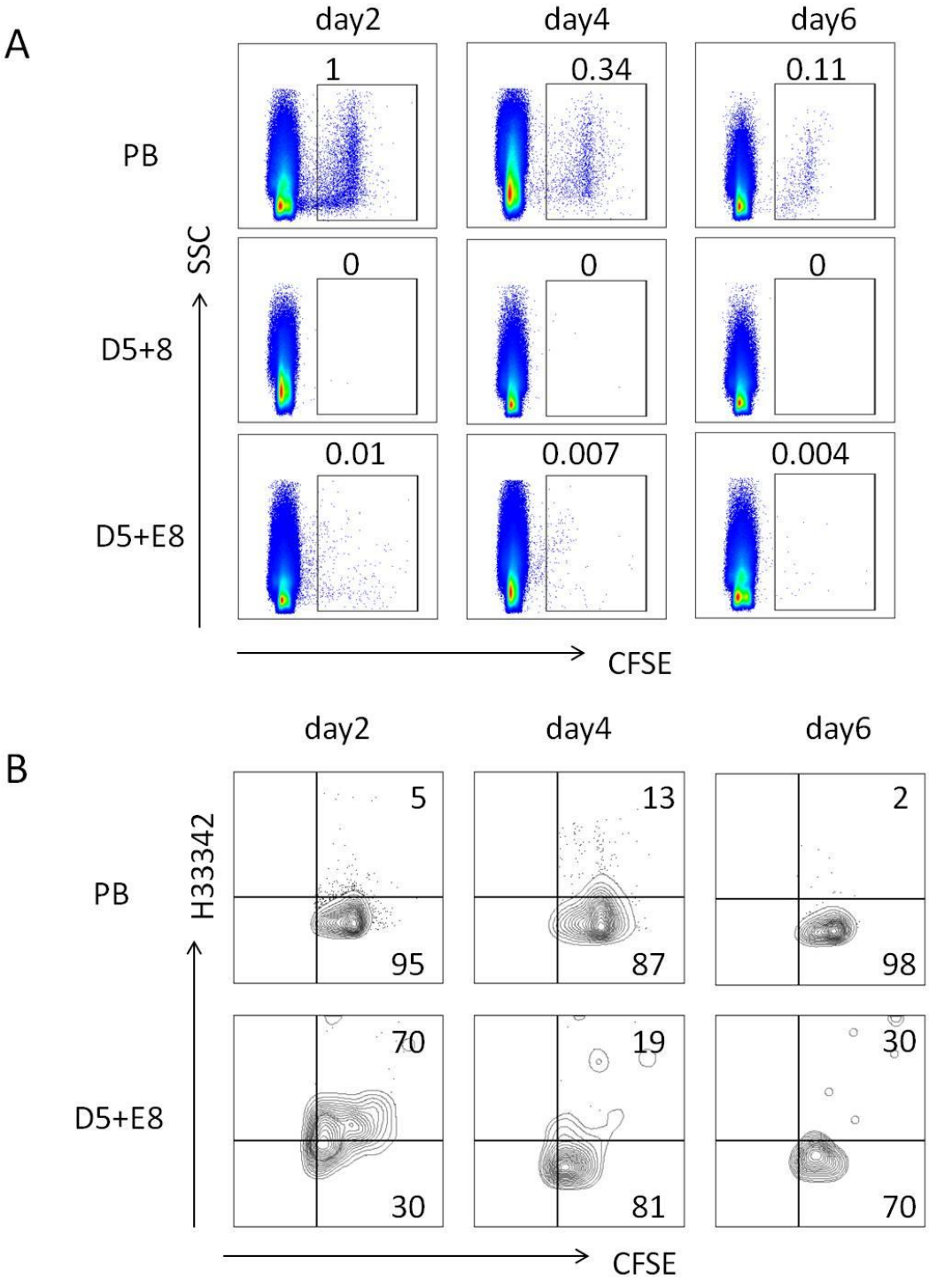
C



Supplementary Figure S3. Enhancing the maturation of erythroblasts by providing OP9 cellular niche, Related to Figure 2.

(A) Wright-Giemsa staining of D5+8 erythroid cells following 14 days of co-culture with OP9 cells (D5+8+P14) or without OP9 cells (D5+8+14). Scale bars, 20μm. (B) Percentage of remaining basophilic cells in erythroid cells of D5+8+P14 and D5+8+14. n=3. (C) Representative flow cytometric analysis of the expression of band3 in erythroid cells of D5+8+14 and D5+8+P14. n=3. Experiments were performed on H1 hESCs. Data are shown as means ±SEM. Statistical analysis was done by two-tailed Student's t test. ***P<.001.

Supplementary Figure S4

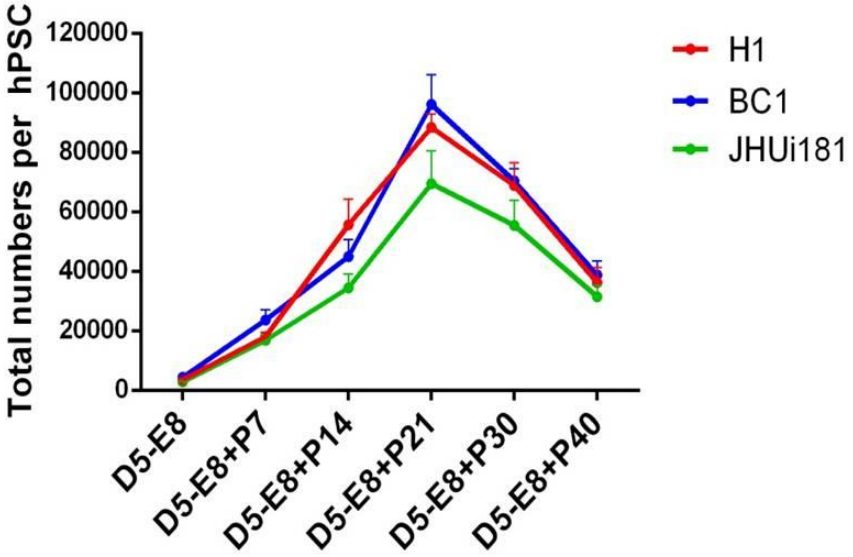


Supplementary Figure S4. Enucleation potential of erythroblasts *in vivo*, Related to Figure 2.

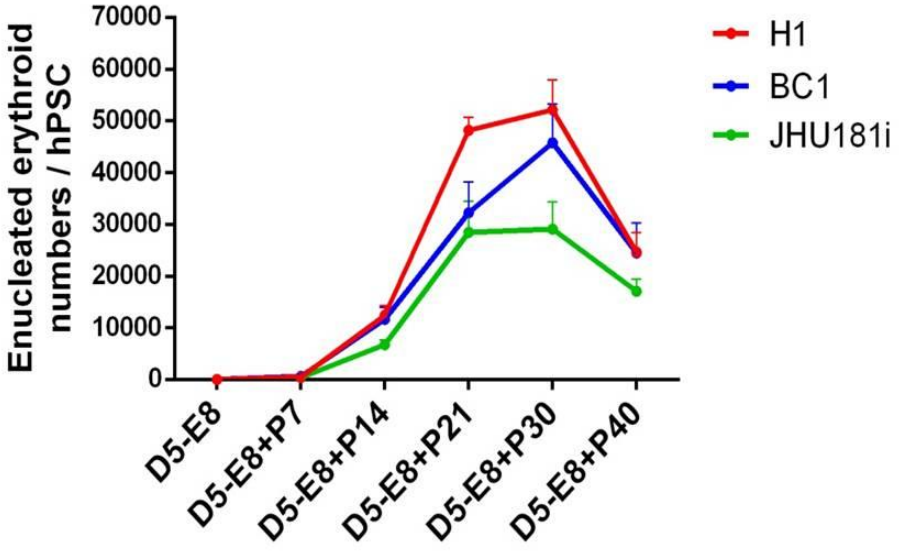
The CFSE-labeled H1 hESC-derived erythroblasts ($1x10^8$) generated with EC co-culture (D5+E8) or without EC-co-culture (D5+8) were infused into 8-week old sublethally irradiated NOG mice. Macrophages in NOG mice were depleted by intraperitoneally injection of Clodronate Liposomes prior to infusion of erythroblasts. Control mice received human peripheral blood under the same conditions. Three animals were used at each time point. (A) Representative flow cytometric analysis of $CFSE^+$ cells in the peripheral blood of NOG mice on the indicated days. n=3. (B) Representative flow cytometric analysis of $CFSE^+H33342^-$ enucleated cells in the peripheral blood of NOG mice on the indicated days. n=3.

Supplementary Figure S5

A



B



Supplementary Figure S5. Generation of enucleated erythroid cells from hiPSCs by sequential endothelium and OP9 cellular niche induction, Related to Figure 3.

(A) and (B) The yields of total hematopoietic cells and enucleated erythrocytes. The yields were calculated based on the cells generated from a single hiPSC. BC1-hiPSCs and JHU181i-hiPSCs were used and H1-hESCs were used as control. n=3. Data are shown as means ±SEM.