Erratum to: Stem cell factor and erythropoietin-independent production of cultured reticulocytes

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The original article entitled "Stem cell factor and erythropoietin-independent production of cultured reticulocytes" published in this issue of Haematologica[1] contains an old version of two figures (Figure 1 and 5) that had not yet been modified based on the reviewers' suggestions. We apologize for this. The final version of the two figures is shown below.

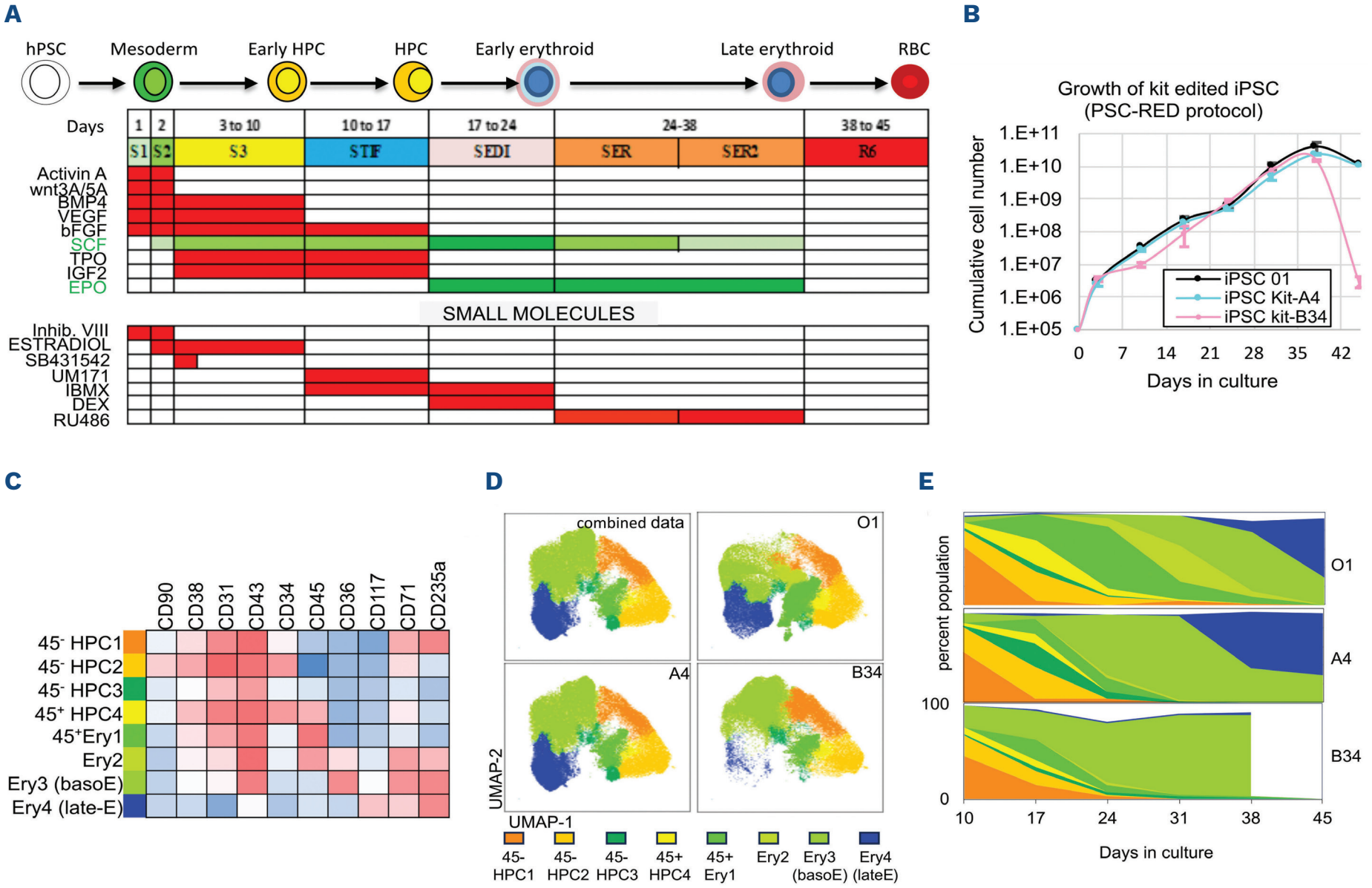

Figure 1. Differentiation of *kit*D816V induced pluripotent stem cells. (A) Diagram and table illustrating the long chemically-defined pluripotent stem cell robust erythroid differentiation (PSC-RED) protocol to differentiate induced pluripotent stem cells (iPSC) into erythroid cells. STIF stands for S: stem cell factor (SCF); T: Tpo; I: IGF-2; F: bFGF. SEDI stands for S: SFF; E: erythropoietin (Epo); D: dexamethasone (Dex); I: 3-isobutyl-1-methylxanthine (IBMX). SER and SER2 stand for S:. SCF; E: Epo; R: RU486. Concentrations of all components are provided in the *Online Supplementary Method Section*. The short version of the protocol is similar except that the expansion in STIF between day 10 and 17 is omitted. The short version yields red blood cells (RBC) that express more embryonic globins than the long protocol. (B) Growth curve of iPSC hemizygous (line A4) or homozygous (line B34)

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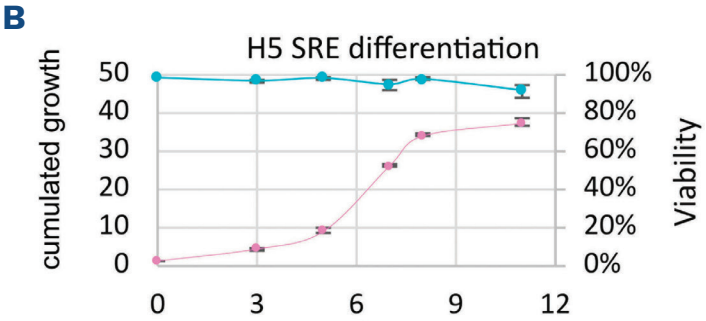
for the D816V mutation. The control O1 cells were differentiated according to the PSC-RED protocol including SCF. The A4 and B34 lines were differentiated according to the same protocol but SCF was omitted at all steps. (C) A 15-color flow cytometry assay was used to examine the antigen expression profiles of differentiating cells that we collected weekly between day 10 and 45. Dimensionality reduction analysis using flowSOM and UMAP revealed that dividing the cells into 8 major populations according to expression of 10 markers provided a useful summary of the evolution of the cells undergoing the PSC-RED protocol. Heatmap summarizes the relative expression of the 10 markers used to define the 8 populations. Populations expressing CD34 were labeled HPC1 to 4 while populations negative for this marker but positive for erythroid markers were labeled Ery1 to 4. (D) UMAP analysis. Data from the 15-color flow cytometry analysis of control A4 and B34 cells assessed weekly from day 10 to 45 was concatenated and analyzed using UMAP generating the pattern illustrated in (D). The concatenated data was then analyzed with flowSOM which segmented the data into 12 populations. Four populations representing each less than 1% of the cells and were eliminated. The UMAP graph was then colored with the 8 remaining major populations which represented more than 96% of all cells. (E) Chart summarizes the evolution of the 8 populations defined by FlowSOM during erythroid differentiation. At day 10, the phenotype of the A4 and B34 cells is similar to the control cells with populations of 43^{+}; 34^{+}; 45^{-} HPC1 and HPC2 (which differed by expression of CD235a) dominating the culture. At days 17 and 24, the control cells differentiated progressively into populations of 45^{+} HPC4 and 45^{+} Ery1 cells. At day 31, Ery2 cells (34^{low}; 45^{+}; 36^{low}; 71^{+}; $235a^{+}$) resembling pro-erythroblasts and Ery3 cells (34^{-}; 45^{-}; 36^{+}; 71^{+}; $235a^{+}$) resembling basophilic erythroblasts became prominent and matured into Ery4 late erythrocytes (34^{-}; 45^{-}; 36^{-}; 71^{low}; $235a^{+}$) by day 38. In the kit-mutated clones, the HPC2 and Ery1 cells did not amplify to the same degree as in the control cells, and the Ery2 cells were barely detectable. Instead, an HPC3 population with a phenotype intermediate between HPC1 and 2 briefly expanded and, most notably, the Ery3 population became prominent much earlier, particularly in the homozygous B4 cells. The Ery3 cells from the A4 cells eventually differentiated into Ery4 cells, but those from the B34 clone did not, resulting, at days 31 and 38, in cultures composed almost exclusively of Ery3 cells. Because of massive cell death of the B34 cells during the last week of differentiation, a fluorescence-activated cell sorting analysis was not performed at day 45 for these cells. hPSC: human pluripotent stem cells.

A

Cells	Self-renewing conditions	Day 0 - 5	Day 5 – 11
KitD816V (A4)	Dex Epo, IBMX	R5 medium Epo 4U/mL (day 0) RU486, 5% human plasma	Pure RPMI
Kit (H5/H11, G19/H12)	Dex IBMX		

B



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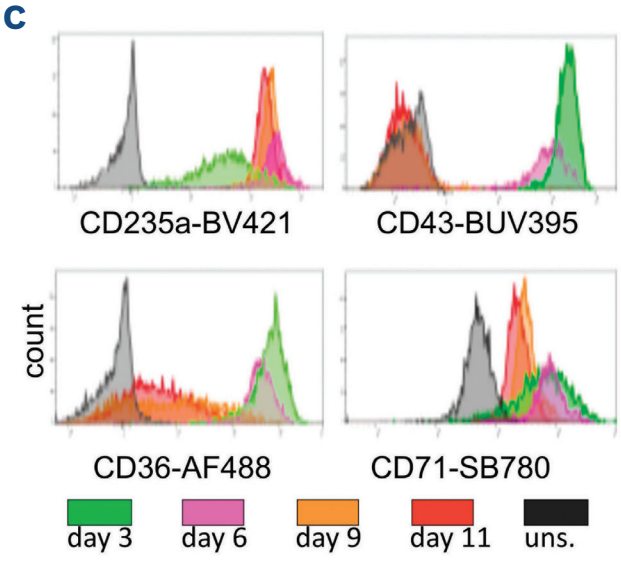

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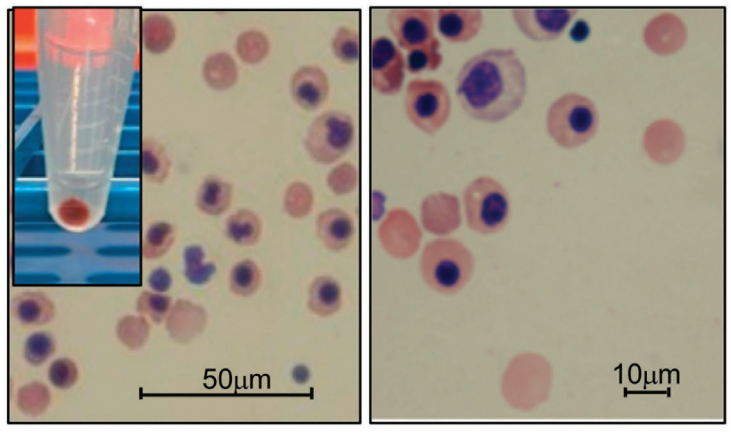

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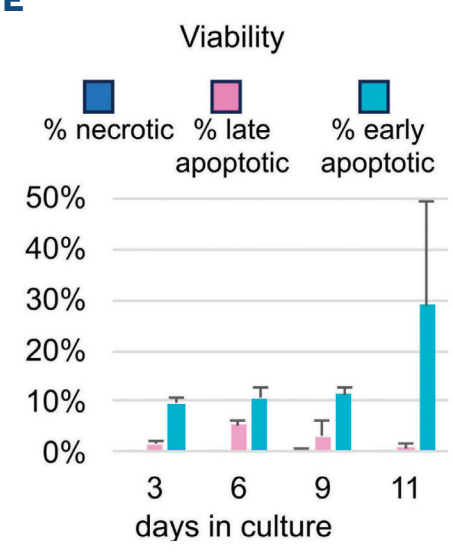

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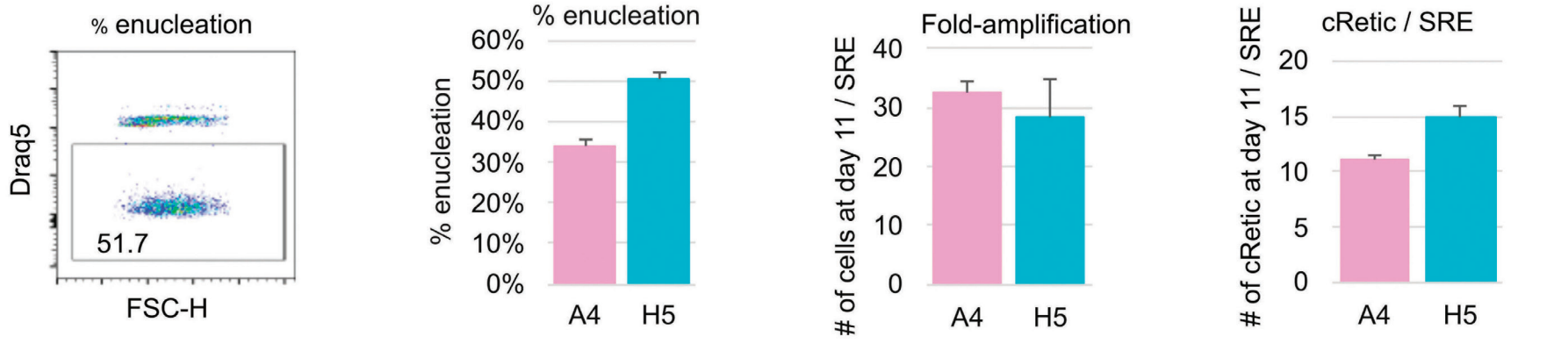

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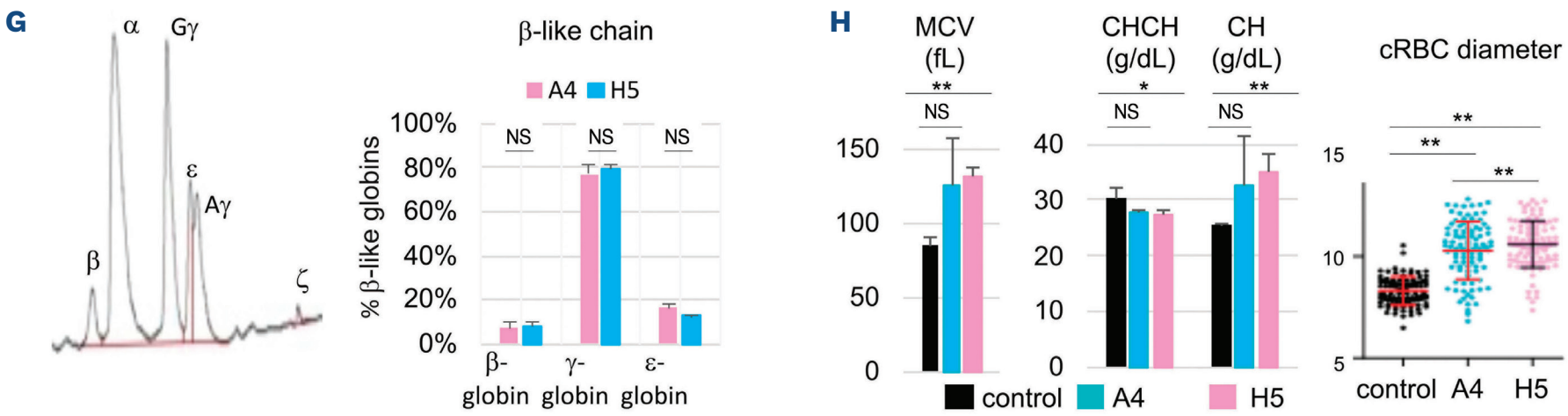

Figure 5. Differentiation of the self-renewing erythroblasts. (A) Differentiation conditions. (B) Growth curve illustrating the average cell growth and viability ± standard deviation (SD); (N=3) during H5 self-renewing erythroblast (SRE) differentiation. (C) Dotplots illustrating the expression of CD235a, CD43, CD36 and CD71 during H5 *kit*Jak2 SRE differentiation. Expression of CD235a increases overtime, while CD43, CD36 and CD71 are sequentially silenced. Uns.: unstained. (D) Micrographs illustrating Romanovsky staining of cells generated from the H5 SRE after 10 days of differentiation. Most cells are reticulocytes or orthochromatic erythroblasts. Inset: cell pellet obtained at day 9 illustrates the strong hemoglobinization of the cells. (E) Differentiating H5 SRE were analyzed by fluorescence-activated cell sorting (FACS) after staining with DAPI and Annexin V-FITC. Bar graph illustrates the average percentage ± SD; (N=3) of necrotic, late and early apoptotic cells at days 3, 6, 9 and 11. (F, left) Dotplots illustrating a flow cytometry analysis of H5 cells stained with Draq5 at day 10 of differentiation. Right: the 3 bar graphs respectively illustrate the percentage enucleation, the cumulated fold-amplification at day 10 or 11 (calculated as the product of the fold-amplification observed after each feeding between days 0 and 10 or 11), and the number of cultured reticolocytes (cRetic)/SRE (calculated by multiplying the rate of enucleation by the cumulated fold-amplification at day 10 or 11). The averages (± SD) of 5 experiments are plotted. (G, right) Chromatogram illustrating a reverse phase high pressure liquid chromatograhpy (HPLC) analysis of globin chain expression of cRetic obtained by differentiation of A4 and H5 SRE. Bar graphs illustrate the averages (± SD) of 2 experiments. (H, left) Red blood cell (RBC) indices of cells generated from the A4 and H5 lines obtained using an Advia blood count analyzer; (N=3), CHCM: cellular hemoglobin concentration mean of intact RBC (optically measured MCHC); CH: mean optical hemoglobin content of intact RBC. (F, right) cRetic diameter assessed by microscopy; 100 cells/cell type were analyzed.

References

1. Olivier E, Zhang S, Yan Z, Bouhassira EE. Stem cell factor and erythropoietin-independent production of cultured reticulocytes. Haematologica. 2024;109(11)3705-3720.