

STEMdiff™ Hematopoietic Kit Reproducibly Generates Functional Hematopoietic Progenitor Cells from Human Pluripotent Stem Cells

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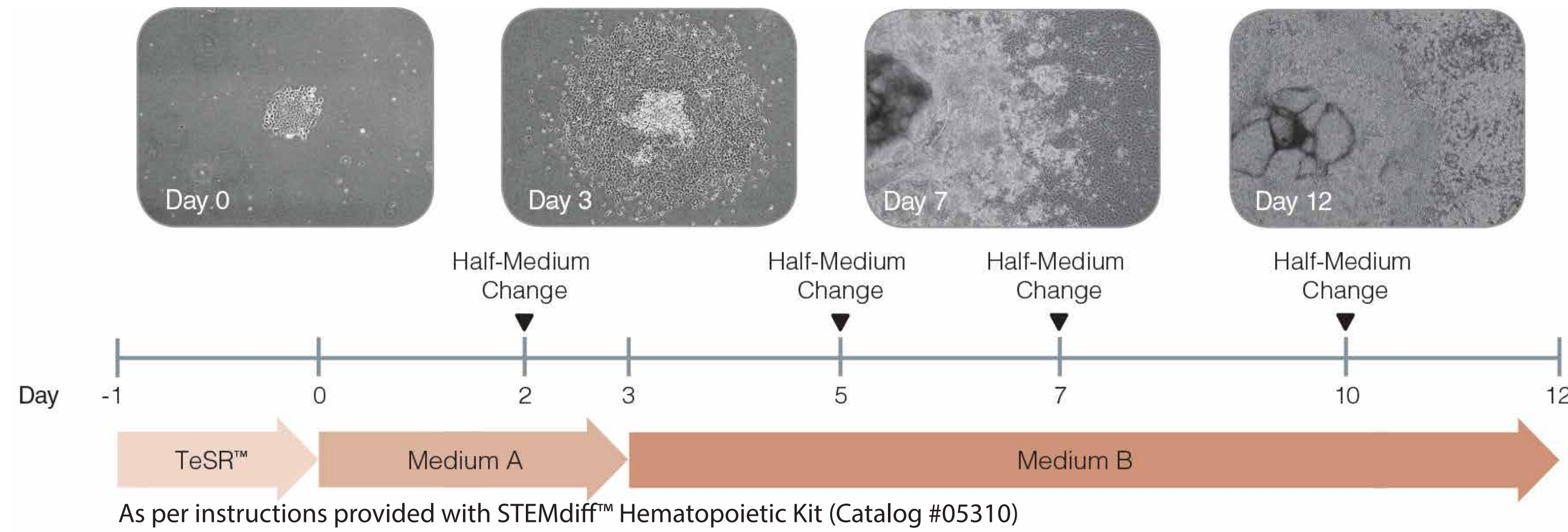
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Introduction

Hematopoietic cells generated from human pluripotent stem cells (hPSCs) can be used to model blood diseases and as an alternate source of blood cells for transplantation. However, most methods to differentiate hPSCs to hematopoietic progenitor cells (HPCs) have been difficult to reproduce between laboratories. The STEMdiff™ Hematopoietic Kit reproducibly generates HPCs expressing key hematopoietic cell surface markers and transcription factors from multiple hPSC lines under both serum- and feeder-free conditions.

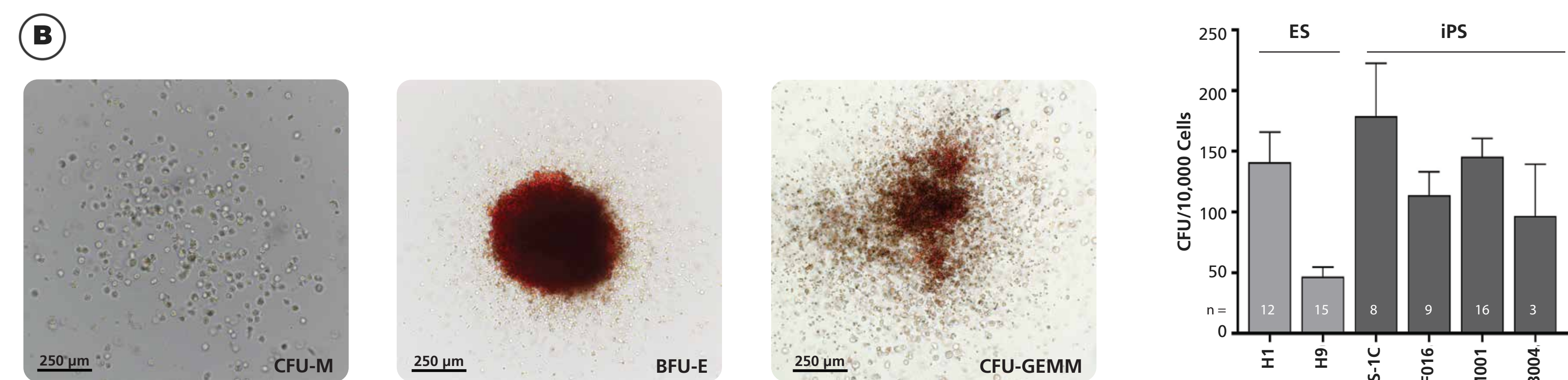
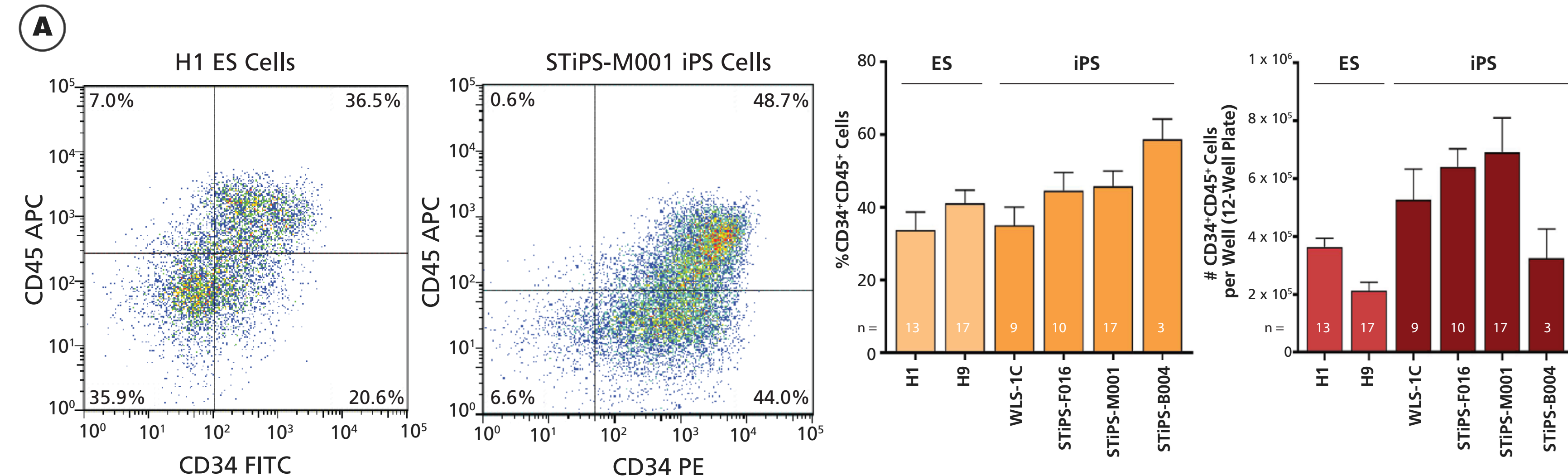
Methods

Figure 1. Protocol Schematic for STEMdiff™ Hematopoietic Kit



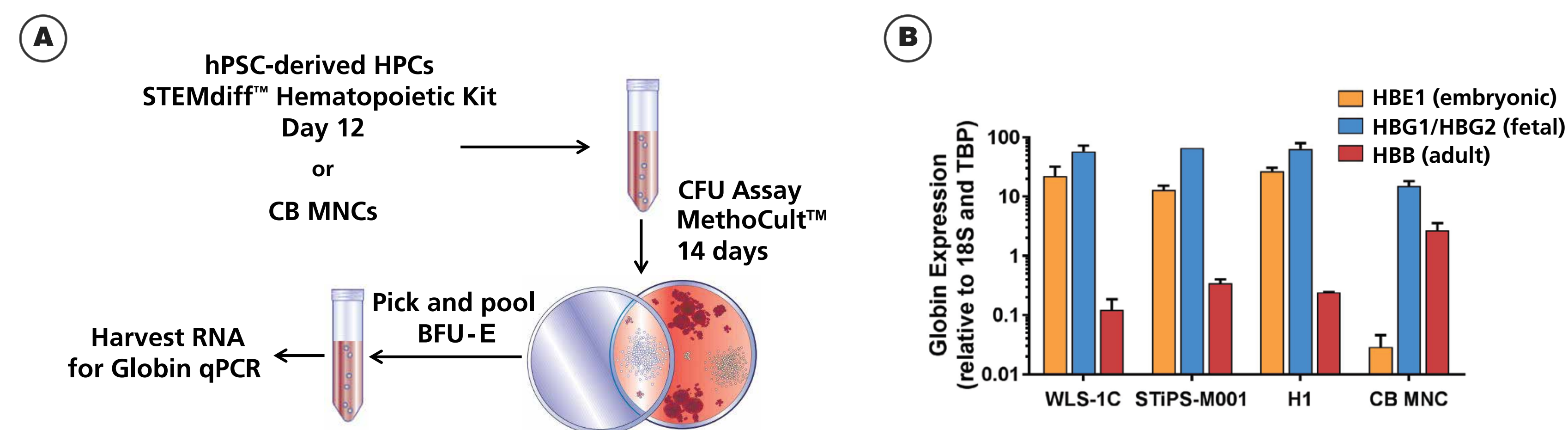
Results

Figure 2. Efficient Generation of Hematopoietic Progenitors from Multiple Human ES and iPS Cell Lines



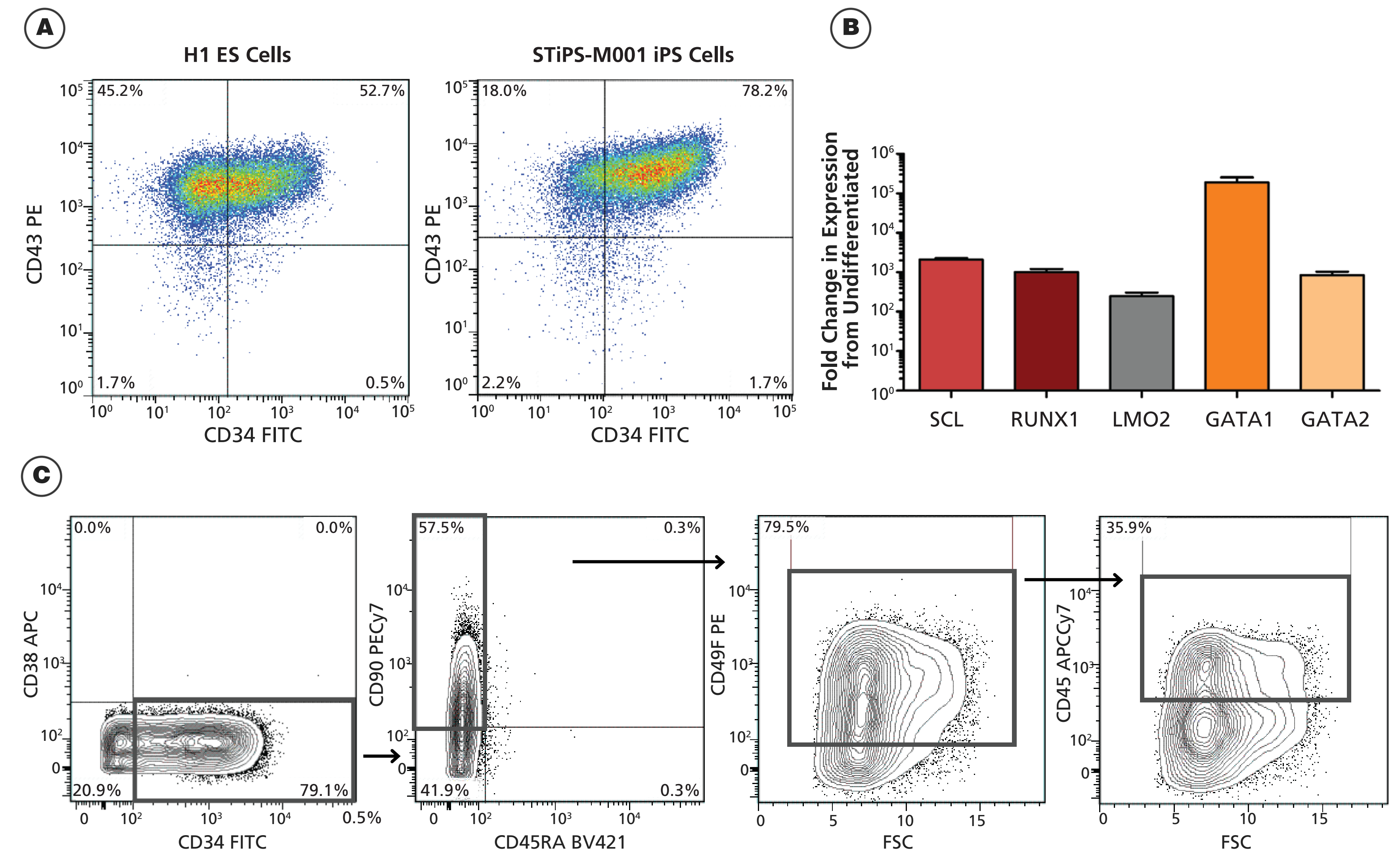
At day 12 of differentiation using the STEMdiff™ Hematopoietic Kit, cells were harvested from the supernatant and assessed by **A**) flow cytometry and **B**) colony-forming unit (CFU) assay in MethoCult™ H4435 Enriched medium (Catalog #04435). **A**) A high proportion of differentiated cells are CD34⁺CD45⁺ HPCs. Representative flow cytometry plots are shown and bar graphs summarize the percentage and yield of HPCs across 6 hPSC lines (mean ± SEM). **B**) Multiple colony types are generated in the CFU assay. Representative images are shown and bar graph summarizes the frequency of CFU per 10,000 differentiated cells (mean ± SEM).

Figure 3. hPSC-Derived Erythroid Colonies Express Primarily Embryonic and Fetal Globins



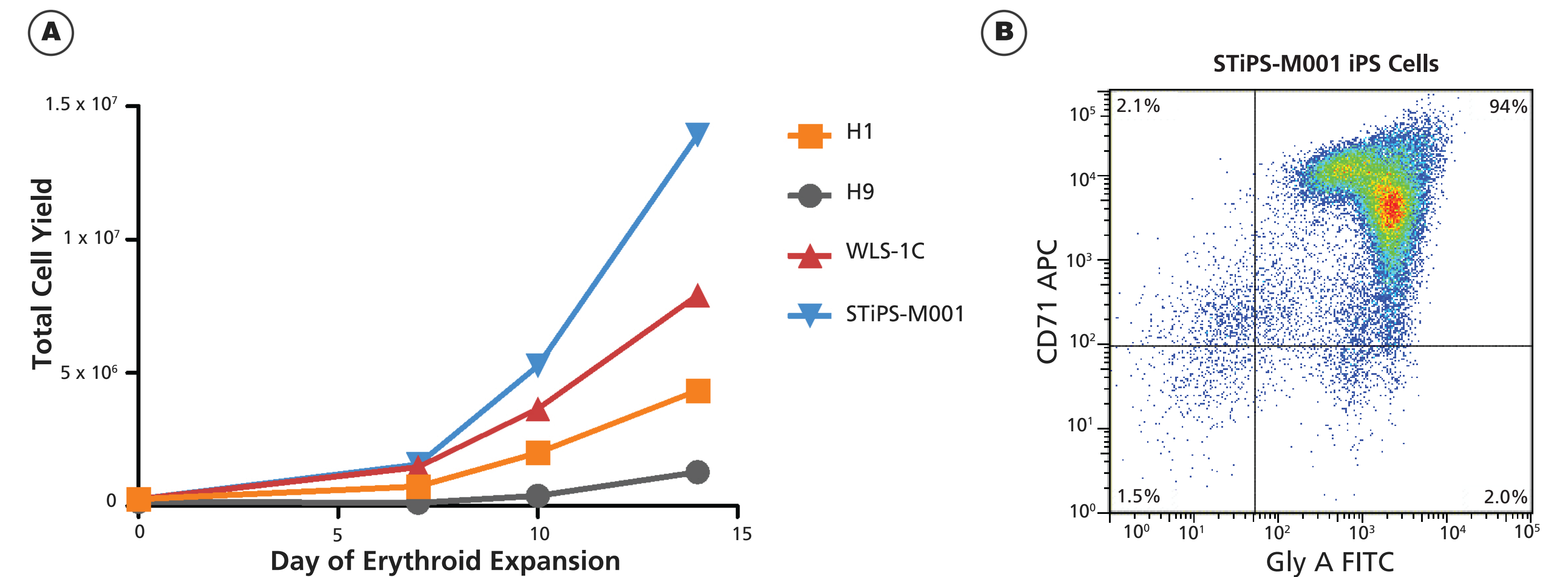
A) Schematic showing how samples were prepared for globin expression analyses by qPCR. **B**) Globin expression was normalized to expression of housekeeping genes 18S and TBP. hPSC-derived erythroid colonies from 1 ES (H1) and 2 iPS (WLS-1C, STiPS-M001) cell lines expressed primarily embryonic (HBE1) and fetal (HBG1/HBG2) globins. In contrast, erythroid colonies generated from cord blood mononuclear cells (CB MNC) expressed primarily fetal and adult (HBB) globins (mean ± SD, n = 2).

Figure 4. hPSC-Derived HPCs Express Key Hematopoietic Cell Surface Markers and Transcription Factors



A) The embryonic pan-hematopoietic marker CD43 is expressed on 96.4% ± 0.5 of cells harvested from the supernatant at day 12 of differentiation (mean ± SEM, n = 8). Representative flow cytometry plots are shown. **B**) Transcription factors involved in developmental hematopoiesis are highly upregulated during differentiation. Gene expression was analyzed by qPCR and normalized to housekeeping genes 18S and TBP, and day 12 samples were compared to undifferentiated controls to calculate the fold change in expression (mean ± SEM, n = 8). **C**) The hematopoietic stem cell phenotype CD34⁺CD38⁺CD45RA⁺CD90⁺CD49F⁺CD45⁺ was assessed by flow cytometry. Expression was variable with 0.8 to 17% of total cells expressing this phenotype (n = 3). An example set of plots are shown; all gates are set on fluorescence minus one (FMO) controls.

Figure 5. hPSC-Derived HPCs Further Differentiate Into Erythroid Progenitor Cells



Cells were harvested from the day 12 supernatant of the STEMdiff™ Hematopoietic Kit protocol, seeded at 2.5 × 10⁵ cells/mL into StemSpan™ SFEM II + Erythroid Expansion Supplement (Catalog #09605 and #02692), and cultured for 14 days. **A**) Cumulative cell yield over 14 days is shown for 4 hPSC lines, with expansion of between 8- and 56-fold. **B**) After 14 days of culture in erythroid expansion conditions, 79 - 95% of cells were CD71⁺GlyA⁺ as assessed by flow cytometry. A representative plot from STiPS-M001 cells is shown.

Summary

STEMdiff™ Hematopoietic Kit provides:

- A defined, serum-free and feeder-free formulation for the generation of high yields of HPCs from human pluripotent stem cells in 12 days
- hPSC-derived HPCs that are functional and capable of downstream multi-lineage differentiation
- hPSC-derived HPCs that express key cell surface markers and transcription factors
- Reproducibility across multiple human ES and iPS cell lines