Supplemental Figure 1 -

Generation and characterization of human myeloid precursor (MP) cells. Human $CD34^+$ cells were incubated with human G-CSF and SCF. Hematoxylin and Eosin (H&E) staining of expanded cells indicated that the majority of cytokine-expanded cells contained a granular cytoplasm with a round to oval nucleus that was flattened or indented which is typical of MP cells (data not shown). The cells doubled approximately every 24 hours resulting in a 50-100 fold expansion by day 10 (Fig S1A). While the frequency of CFU-GM and CFU-GEMM remained unchanged to at least day 8 post-expansion, the frequency of BFU-E declined from day 0 to day 8 post-expansion. (Fig S1B). To follow the emergence of the myeloid population, a hematopoietic-specific panel of antibodies was utilized. By 7 days in culture, flow cytometric analysis indicated that 99% of the cells were $CD33^+$ and ~50% of these cells were $CD34^+CD33^+$ myeloid progenitors (Fig S1C) which correlated with maintenance of CFU-GM and CFU-GEMM in CFU assays (Fig S1B). During the highly proliferative phase, 94% of the cells expressed the transferrin receptor (CD71), a cell-surface receptor commonly associated with proliferating cells. By days 10-12 post-expansion, the percentage of $CD71^+$ cells began to decrease although proliferation rates were still maintained. Additionally, 90% of the expanded cells were myeloperoxidase (MPO) positive at days 10-12 (Fig S1C) providing further confirmation that all cells were of the myeloid lineage. Flow cytometric analyses indicated that cell-surface markers indicative of myeloid cells (CD33, CD13, CD15, CD64, CD11b) were expressed on the majority of the expanded MP cells (Fig S1C). The expanded cells did not express CD14, a marker of mature monocytic cells, indicating that the expanding cells had not undergone terminal monocyte maturation at

the time of harvest. Additionally, no increases in $CD19^+$ and $CD3^+$ cells were observed indicating a lack of B- and T-lymphocytes in the culture.

Figure Legend:

Supplemental Figure 1. Characterization of expanded primary human myeloid precursor (MP) cells. (A) Isolated cord blood $CD34^+$ cells were expanded in G-CSF and SCF and cell expansion was enumerated over time by counting cells on a hemacytometer in the presence of trypan blue. (B) Cells were analyzed by colony-forming unit assay prior to expansion (D0) and at day 8 (D8) post-expansion. (C) Expanded cells were phenotyped on days 7 and 12 with a panel of hematopoietic-specific antibodies: monocyte (mono); macrophage (mac); hematopoietic stem & progenitor cell (HSPC); endothelial cell (endo); granulocyte (gran); progenitor cell (PC); dendritic cell (DC); dendritic cell (DC); natural killer cell (NK); antigen-presenting cell (APC); activated T lymphocyte (act T); myeloperoxidase (MPO). Similar phenotypes and clonogenic frequencies were seen when the cells populations were analyzed at days 7-8 post-expansion. In addition, once rapid cell expansion commenced, the population continued to differentiate and phenotypes as assessed by flow cytometric analyses were similar at days 10-12 post-expansion.