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Supplementary Information for Isolation of Human Umbilical Cord Blood Hematopoietic Stem Cells and Directed Differentiation Into Megakaryocytes

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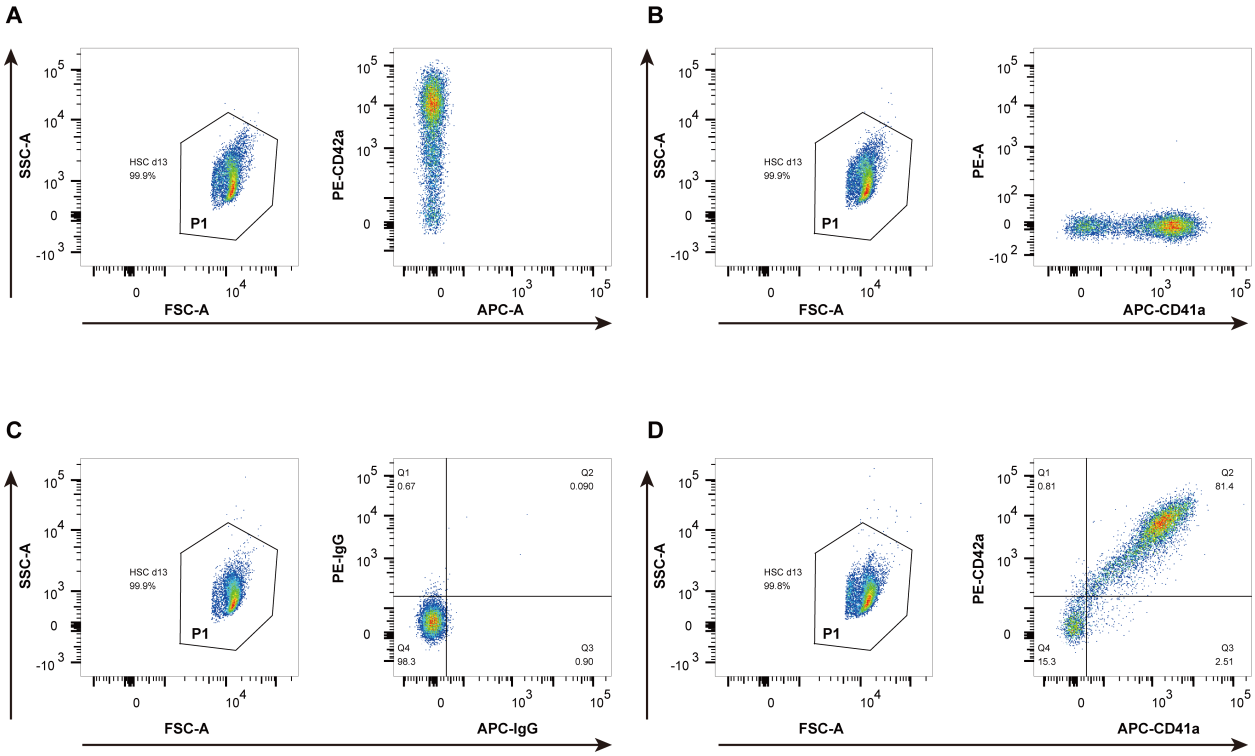

Supplementary Figure 1. Representative flow cytometry gating strategy for CD41a and CD42a detection. (A) Single-stained tube for PE-CD42a. (B) Single-stained tube for APC-CD41a. These two single-color controls were acquired once to establish a fixed compensation template, which was applied for all subsequent experimental batches. (C) Isotype-matched IgG control (APC-IgG/PE-IgG): gate P1 encompasses all acquired cellular events without pre-exclusion of debris. Quadrant boundaries were set within P1 using this isotype control to define the threshold for positive signals. The quadrants were adjusted so that ≤1% of events appear in the positive quadrants of the isotype control. (D) Fully stained experimental sample (APC-CD41a vs. PE-CD42a). The identical quadrant boundaries defined in panel C were applied to this sample. Q2 corresponds to the $CD41a^{+}CD42a^{+}$ double-positive mature megakaryocyte population.