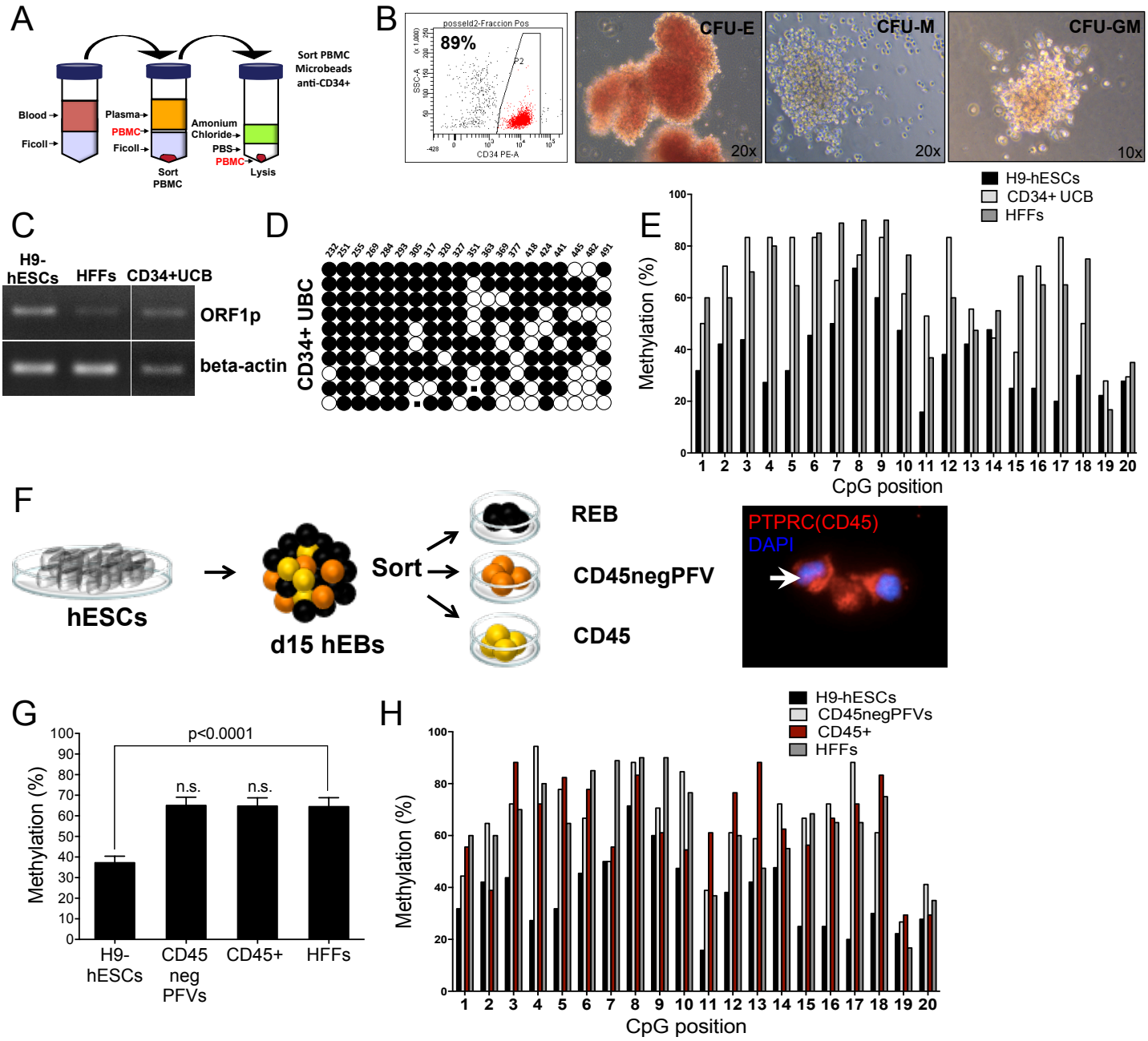


**Supplemental Fig. 5. Endogenous L1 expression and engineered L1 retrotransposition in HSCs.** (**A**) Scheme of the protocol used to isolate CD34-expressing HSCs from UC blood samples. (**B**) FACS analyses of purified CD34+ cells from UC blood. The histogram (SSC vs CD34) also indicates the percentage of cells expressing CD34+ as determined in duplicate. The right panel shows representative images of Colony-Forming Units (CFUs) observed in differentiating HSCs plated on methylcellulose (erythrocyte, E; monocyte, M; granulo-monocyte, GM). (**C**) L1Hs mRNA expression analysis as measured by semi quantitative RT-PCR using a set of primers to the ORF1 sequence of a consensus L1Hs element. The gel shows representative data in the indicated sample and amplification of βeta actin was included as a control of RNA integrity. (**D**) L1Hs promoter methylation analyses in CD34+ HSCs. Each lane corresponds to a sequenced clone (shown are the 10 clones with the highest sequence similarity to L1.3). (**E**) The graph indicates the percentage of methylation for each CpG residue as measured by bisulfite conversion assays. Black bars, H9-hESCs; light grey bars, CD34+ HSCs; dark grey bars, HFFs. In panels d &e, the relative position of each CpG is indicated using the sequence of L1.3 as a reference. (**F**) Scheme of the EB-based method used to isolate CD45negPFV and CD45+ populations (see Methods). In the EB cartoon, the CD45+ and CD45negPFV populations are pictured using yellow and orange ovals within the EB. Black ovals, remaining cells. To the right is shown a merged image of hESC-derived HSCs stained with an antibody against CD45/PTPRC (red); nuclear DNA was stained with DAPI (blue). (**G&H**) DNA-Methylation analyses in CD45+ and CD45negPFV populations isolated using the EB-method. In panel g, the graph indicates the percentage of methylated CpG residues within L1Hs 5´UTR sequences as

measured by bisulfite-PCR assays in the indicated sample. In panel h, the percentage of methylation for each CpG residue is indicated in the graph as measured by bisulfite conversion assays. Black bars, H9-hESCs; light grey bars, CD45negPFVs; dark purple bars, CD45+; dark grey bars, HFFs. In panel G, One-way ANOVA with Tukey was used as a statistical method and the p value is indicated (0.0001 or n.d., not significant).