

Endothelial protein C receptor (EPCR) expression marks human fetal liver hematopoietic stem cells

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Supplemental information

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Supplementary methods

Cells

Umbilical cord blood samples (UCB) were obtained from Skåne University Hospital (Lund and Malmö) and Helsingborg Hospital with informed consent. Normal bone marrow (BM) samples were obtained from Skåne University Hospital in Lund with proper informed consent. Sample collection was approved by the regional ethical committee at Skåne University Hospital. Mononuclear cells from the UCB and BM samples were separated by using Ficoll-Paque (Axis-Shield) density gradient centrifugation. Ficoll-Paque purified week 16 human fetal liver mononuclear cells were obtained from NOVOGENIX, USA. CD34⁺ cells were enriched by using magnetic bead based positive selection (130-046-703, Miltenyi biotec).

Flow cytometry

Following anti-human antibodies were used; CD38-PE (#345806), CD45RA-V450 (#560362), CD90-BV605 (#562685) and CD19-BV605 (#562653) from Becton Dickinson; CD34-FITC (#343604), EPCR-APC (#351906), CD11b-FITC (#367116), CD38-PE-Cy7 (#303516), CD45-APC (#304012) and CD33-PE (#303404) from BioLegend; CD34-eFluor450 (#48-0349-42), CD49f-PE-Cy7 (#25-0495-82), (#48-0349-42) and CD3-PE-Cy7 (#25-0038-42) from ebioscience; CD45RA-FITC (Life technologies, #MHCD45RA01); GPI-80-PE (MBL, D087-5). Stained samples were analyzed in LSRFortessa and FACSCanto (BD). Cell sorting was performed in FACS Aria III (BD).

In-vitro culture

Cells were cultured in serum free expansion medium (Stemcell technologies) supplemented with human stem cell factor (SCF), human Thrombopoietin (TPO) and human FMS-like tyrosine kinase 3 ligand (FLT3L) at 100 ng/mL each (Peprotech).

Transplantation

FACs sorted human CD34⁺ sub-populations were transplanted into the sub-lethally irradiated (300cGy) NSG mice. Bone marrow samples were analyzed for different lineages and HSC markers at week 16 for UCB transplantations and week 20 for fetal liver transplantations. All animal studies were approved by the Lund/Malmö Local Ethical committee.

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Cell cycle analysis

UCB CD34 enriched cells were surface stained and the remaining protocol was performed by using Fixation/Permeabilization Solution Kit (BD biosciences, #554714) according to the manufacturer's protocol. PE Mouse Anti-Human Ki-67 Set (BD Pharmingen; #556027) was used for the intracellular staining.

Statistics

Two groups were analyzed using two-tailed unpaired t-test. Multiple groups were analyzed with one-way ANOVA. Results having a p value of ≤ 0.05 were considered as significant. p values represented in figures, $\leq 0.05 = *$; $\leq 0.01 = **$; $\leq 0.001 = ***$ and $\leq 0.0001 = ****$.

Supplementary figure legends

Supplementary figure 1. A. Representative plots to show the frequency of CD34⁺EPCR⁺ population in FL (Week 16), UCB and BM CD34 enriched cells. B. The numbers from three samples each were represented. Error bars represent \pm SD.

Supplementary figure 2. A. Distribution of EPCR⁺ and EPCR⁻ cells within the CD34⁺CD38⁻ population and CD34⁺CD38⁻CD90⁺ population in FL derived CD34 enriched cells. B. Distribution of EPCR⁺ and EPCR⁻ cells within CD34⁺CD38⁻ population and the MPP, LMPP and HSC populations for UCB derived CD34 enriched cells.

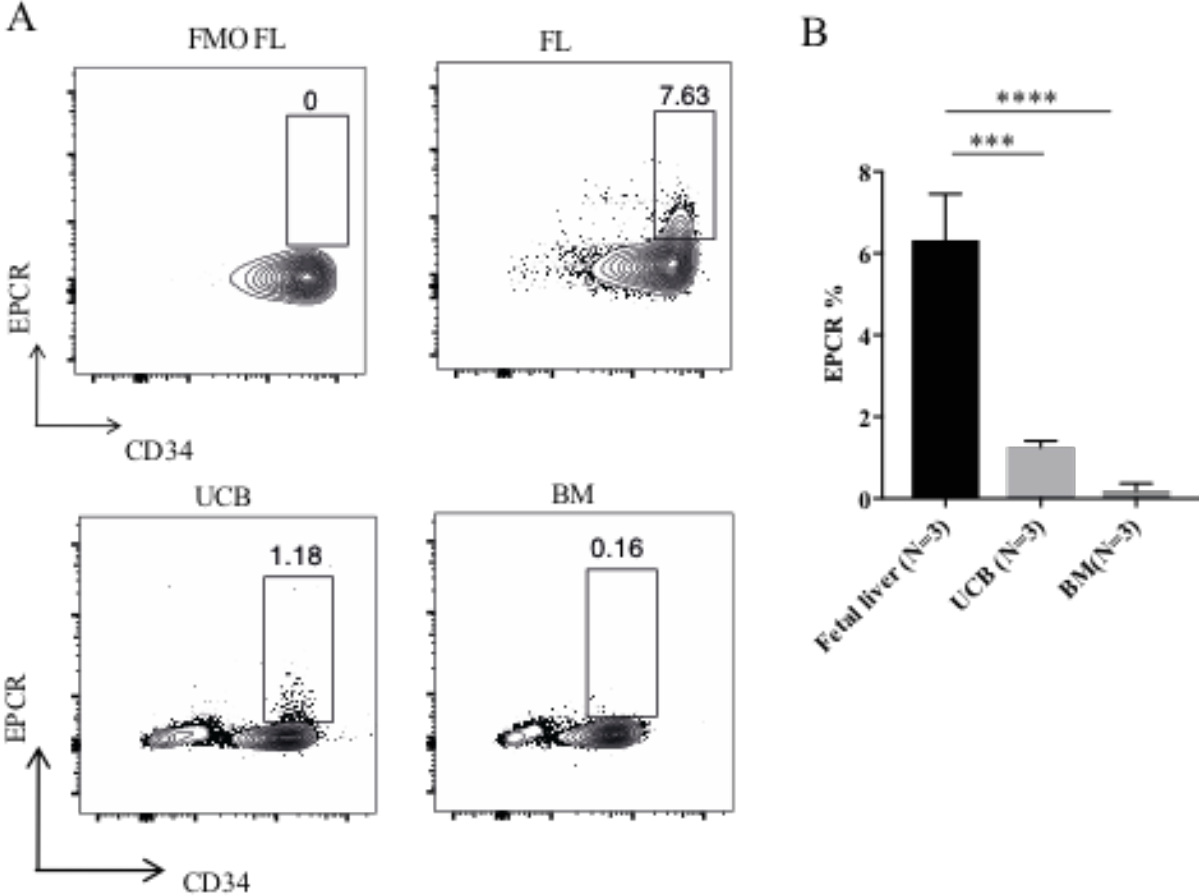
Supplementary figure 3. A. Frequencies of human CD19 and CD33 populations in the mice transplanted with FL derived EPCR⁺ population. Lineage data is presented for mice with $>0.1\%$ hCD45 engraftment. B. Frequency of HSPCs (CD34⁺CD38^{low} cells) within the hCD45 population for FL EPCR⁺ cells transplanted mice.

Supplementary figure 4. Post sorting purity analysis for the UCB derived EPCR⁺ and 49f⁺EPCR⁻ populations. B. Lineage analysis and C. frequency of CD34⁺CD38^{low} HSPCs within the hCD45 population in mice transplanted with UCB derived EPCR⁺ and 49f⁺EPCR⁻ populations.

Supplementary figure 5. Frequency of CD34⁺CD38^{low} HSPCs (A) and lineage analysis (B) within the hCD45 population in mice transplanted with EPCR⁺ cells (4000/mice) and EPCR⁻ cells (4000/mice in one group and 40000/mice in the second group) derived from in vitro cultured UCB CD34⁺CD90⁺ cells. The representative plots show the frequency of the CD34⁺CD38^{low} population and lineage distribution for each condition.

Supplementary figure 6. Cell cycle analysis in UCB CD34⁺CD38⁺, CD34⁺CD38⁻EPCR⁻ and CD34⁺CD38⁻EPCR⁺ populations. For the CD34⁺CD38⁻EPCR⁻ and CD34⁺CD38⁻EPCR⁺ populations data from 5 independent samples are shown in the bar chart.

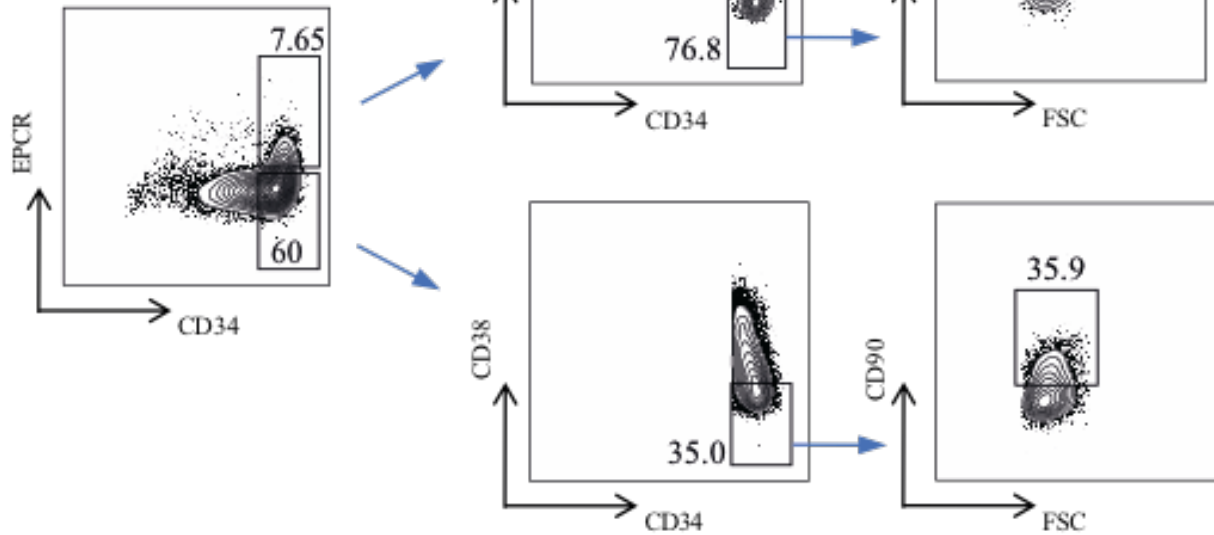
Supplementary figures



Supplementary figure 1

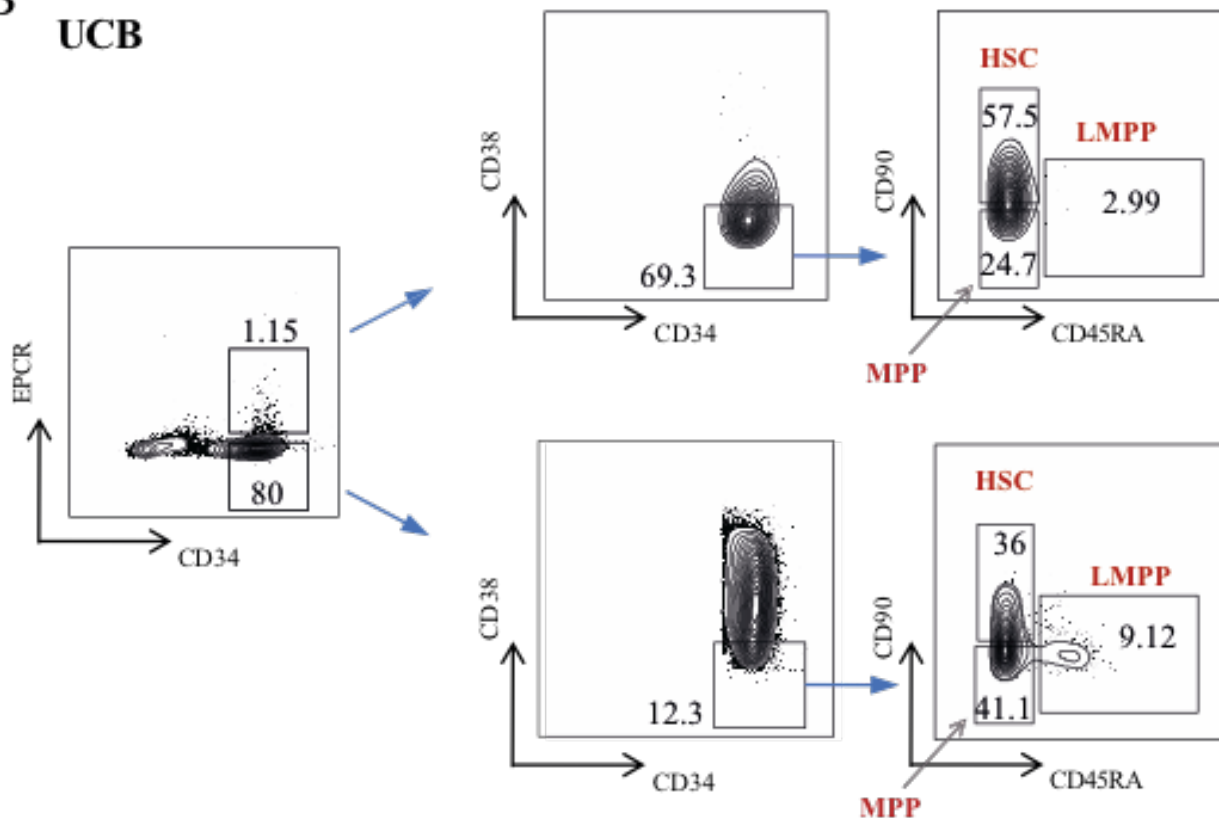
A

FL

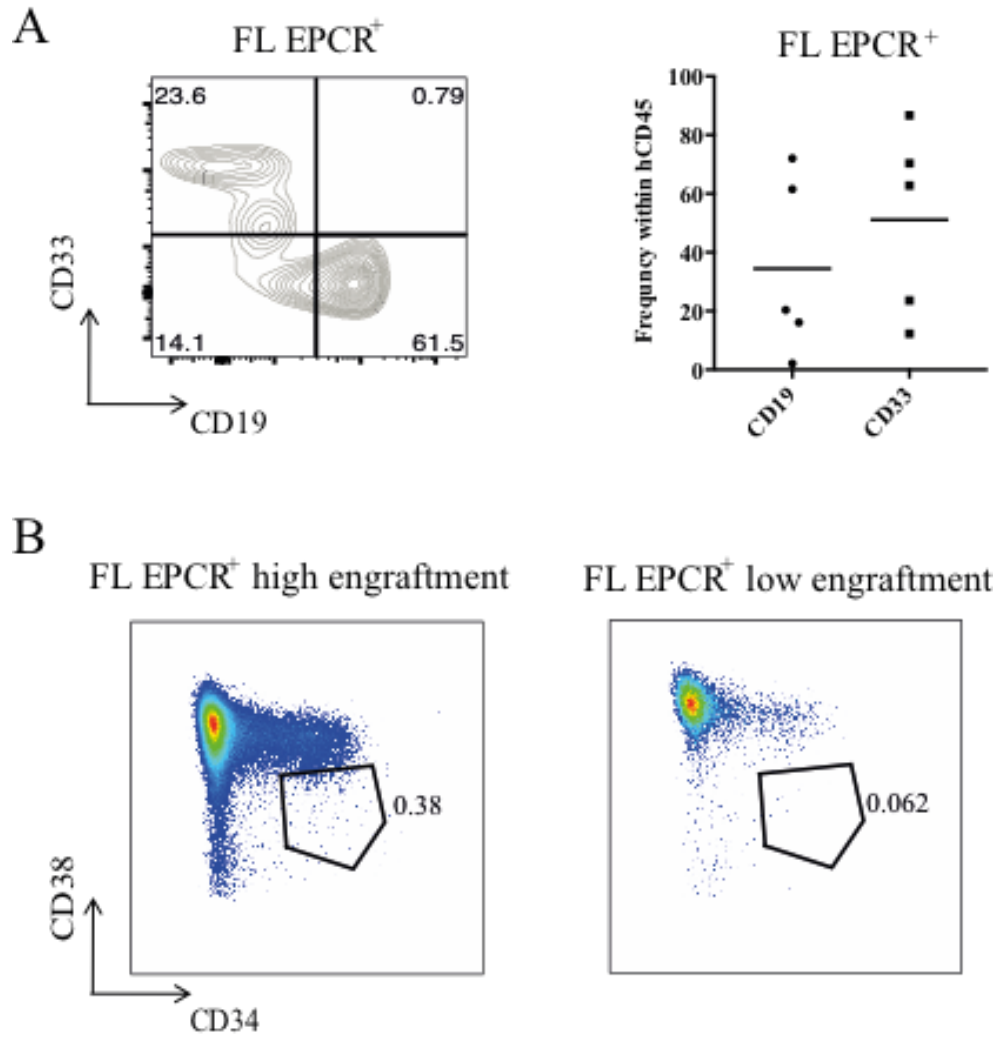


B

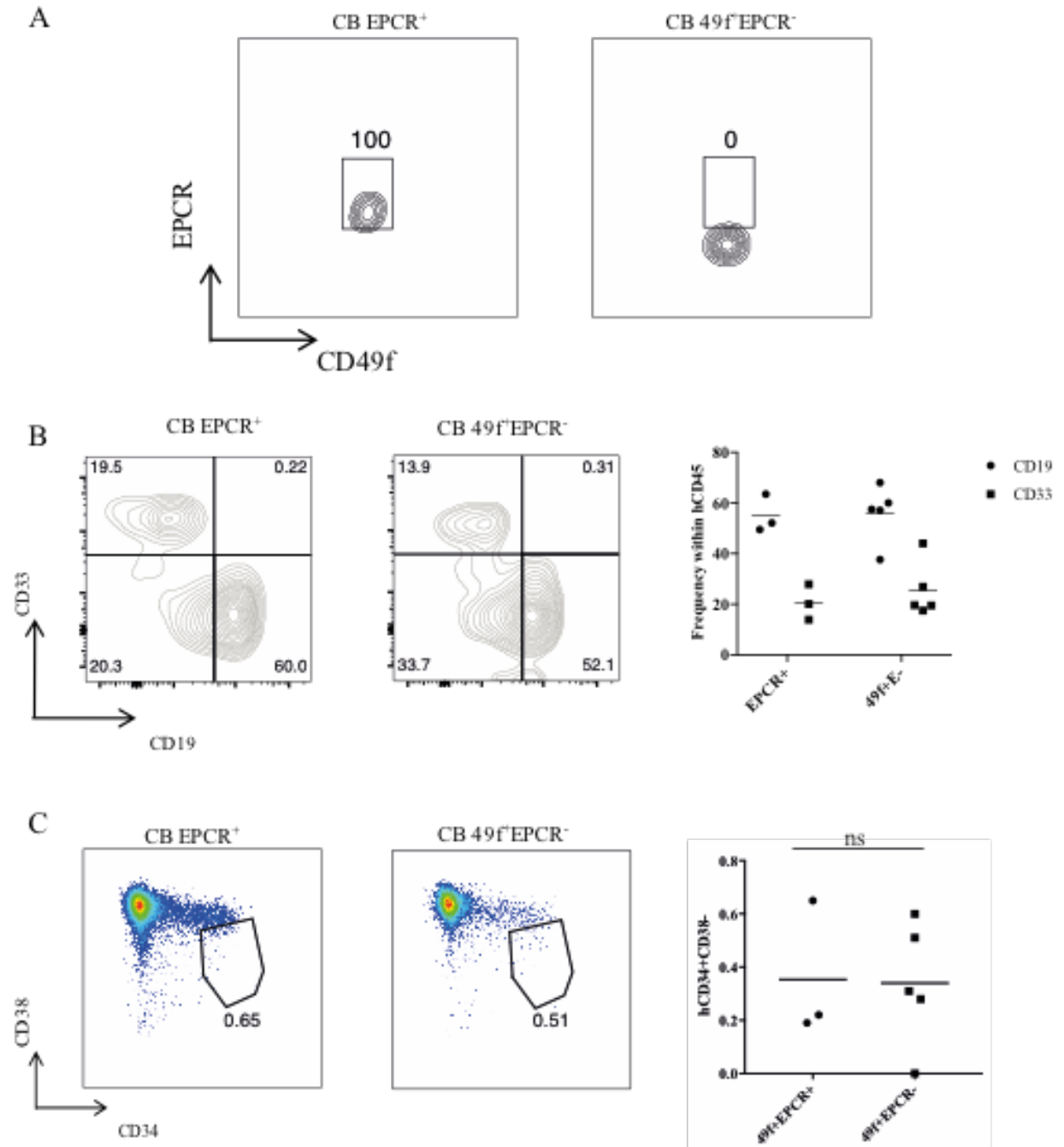
UCB



Supplementary figure 2

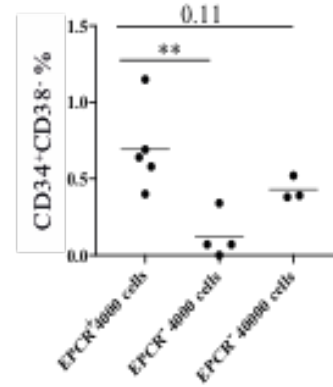
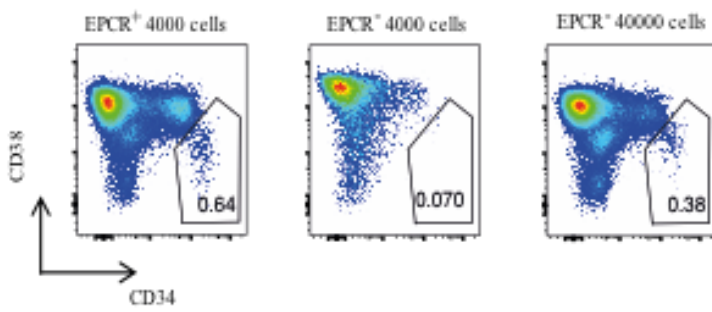


Supplementary figure 3

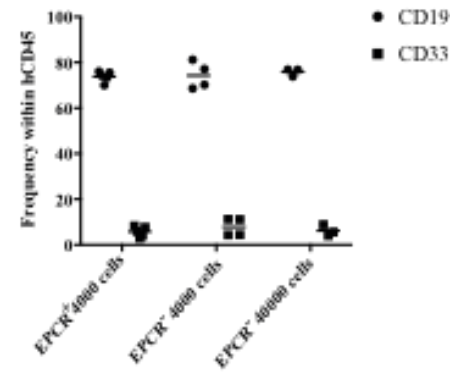
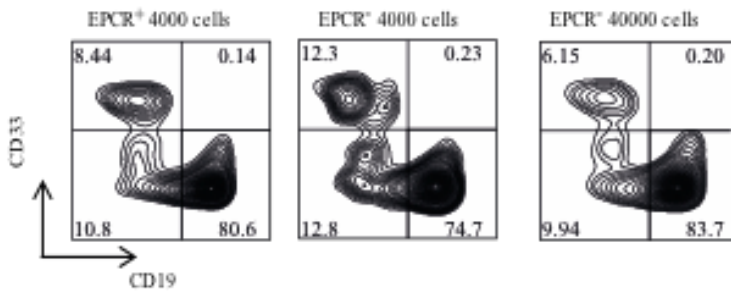


Supplementary figure 4

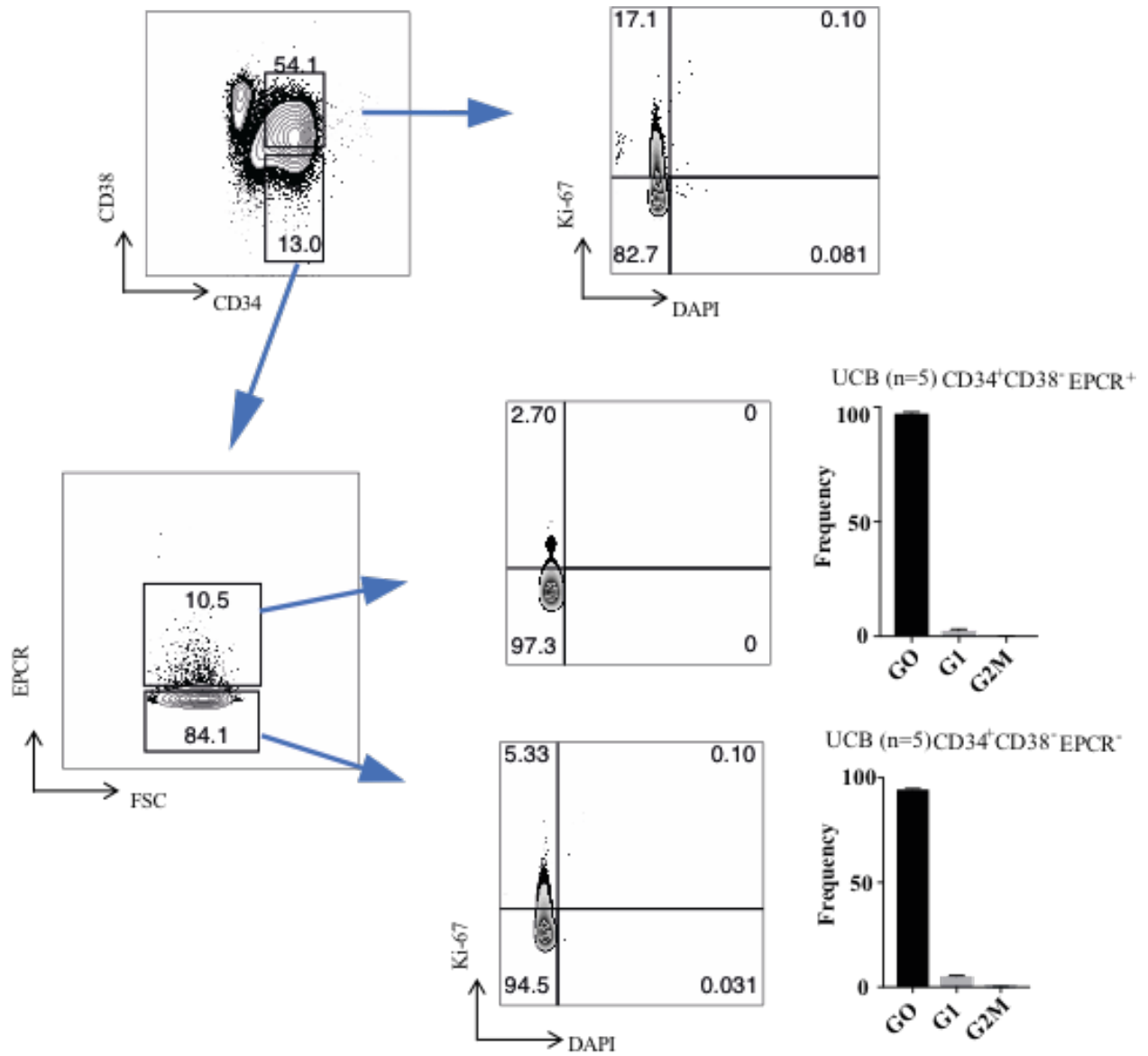
A



B



Supplementary figure 5



Supplementary figure 6