Supplementary Information

Plastic CD34 and CD38 expression in adult B-precursor acute lymphoblastic leukemia explains ambiguity of leukemia-initiating stem cell populations

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Supplementary Methods

Supplementary Figures S1-S3

Supplementary Movies S1-S3

Supplementary Methods

Cell culture

PDLTC-PH cells were cultivated as described [1]. All the experiments were performed with cells from an early passage of PDLTC-PH, unless indicated otherwise.

FACS analysis and cell sorting

Surface marker expression was analyzed using anti-CD34-APC (clone 8G12, BD, Heidelberg, Germany) and CD38-PE (clone HB7, eBioscience, Frankfurt, Germany) on a FACS Canto II or LSR Fortessa (both BD). Viability was determined using a Fixable Viability Dye (eBioscience) for live/dead cell exclusion. Fcγ receptor block and isotype controls were routinely used. Cell sorting was performed on a FACS Aria I or III (BD). Single cells were sorted in 96 well U-bottom plates, containing 100 µl of IMDM based medium. Subpopulations were subsequently cultured and expanded according to the above mentioned culturing protocol, after microscopic confirmation of single cell plating.

Single cell imaging and tracking

Sorted PDLTC-PH cells were seeded in 24-well plates equipped with silicon culture inserts (IBIDI, Martinsried, Germany) and time-lapse microscopy and tracking were performed as described [2, 3]. CD34-APC and CD38-PE antibodies were added to the culture at a dilution of 1 to 5000 before the start of the time-lapse-microscopy to allow real time surface marker expression tracking [3]. Plates were gas-tight sealed with adhesive tape after 5% CO_2 saturation. Microscopy was performed using a CellObserver (Zeiss, Goettingen, Germany) at 37°C. Phase contrast images were acquired every 2-3 min using a 10x phase contrast objective (Zeiss), and an AxioCamHRm camera (at 1388x1040 pixel resolution) with a self-written VBA module remote controlling Zeiss AxioVision 4.8 software. Fluorescence was detected every 2h with HXP illumination (Osram) and the filter sets for Cy3 and Cy5 (F46-004 and F46-006, AHF Analysetechnik, Tübingen, Germany).

Cell tracking was performed using a self-written computer program (TTT) as described [2, 3]. All cell tracking was done by scientists; the current analysis does not rely on data generated by an unsupervised computer algorithm for automated tracking.

Xenotransplantation

Sub-lethally irradiated (2.5 Gy) NSG mice at 6–14 weeks of age were intravenously (tail vein) inoculated with $5x10^6$ cells (bulk and subclones) or $1x10^6$ cells (sorted subpopulations). Peripheral blood was analyzed every 2-4 weeks after transplantation by flow cytometry (anti human CD45-V450, BD) and mice were sacrificed when showing adverse symptoms caused by their leukemia burden according to the animal welfare guidelines. All mice were bred and maintained under specific pathogen-free conditions. Animal experiments were performed in accordance with the German animal welfare legislation and were approved by the relevant authorities (Regierungspräsidium Darmstadt, Germany).

References

[1] B. A. Nijmeijer, K. Szuhai, H. M. Goselink, M. L. J. van Schie, M. van der Burg, D. de Jong, E. W. Marijt, O. G. Ottmann, R. Willemze, and J. H. F. Falkenburg, “Long-term culture of primary human lymphoblastic leukemia cells in the absence of serum or hematopoietic growth factors,” *Exp. Hematol.*, vol. 37, no. 3, pp. 376–385, 2009.

[2] N. Haetscher, Y. Feuermann, S. Wingert, M. Rehage, F. B. Thalheimer, C. Weiser, H. Bohnenberger, K. Jung, T. Schroeder, H. Serve, T. Oellerich, L. Hennighausen, and M. A. Rieger, “STAT5-regulated microRNA-193b controls haematopoietic stem and progenitor cell expansion by modulating cytokine receptor signalling.,” *Nat. Commun.*, vol. 6, p. 8928, 2015.

[3] F. B. Thalheimer, S. Wingert, P. De Giacomo, N. Haetscher, M. Rehage, B. Brill, F. J. Theis, L. Hennighausen, T. Schroeder, and M. A. Rieger, “Cytokine-regulated GADD45G induces differentiation and lineage selection in hematopoietic stem cells,” *Stem Cell Reports*, vol. 3, no. 1, pp. 34–43, 2014.

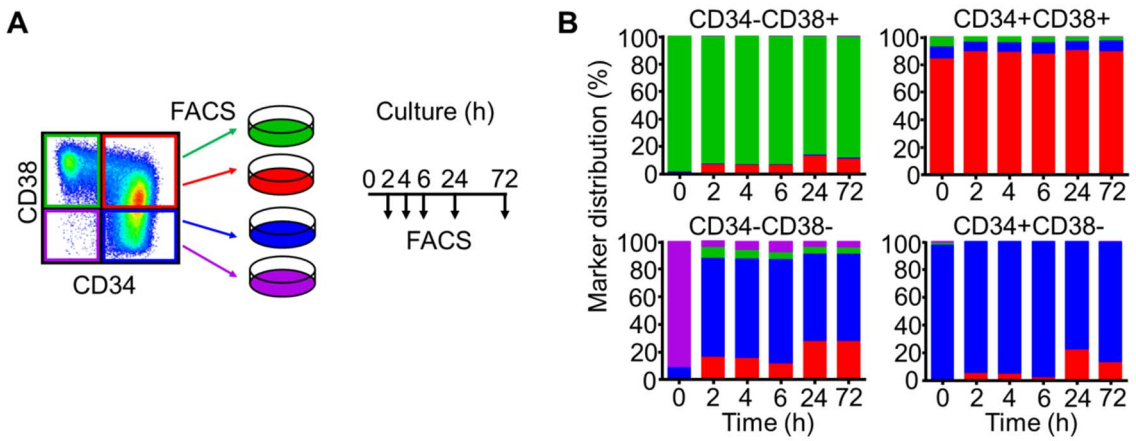

Fig. S1. Plasticity in CD34 and CD38 expression of prospectively isolated PDLTC-PH subpopulations within hours. A) Experimental scheme and B) FACS results of marker expression within the first 72 h after prospective FACS-based enrichment of subpopulations.

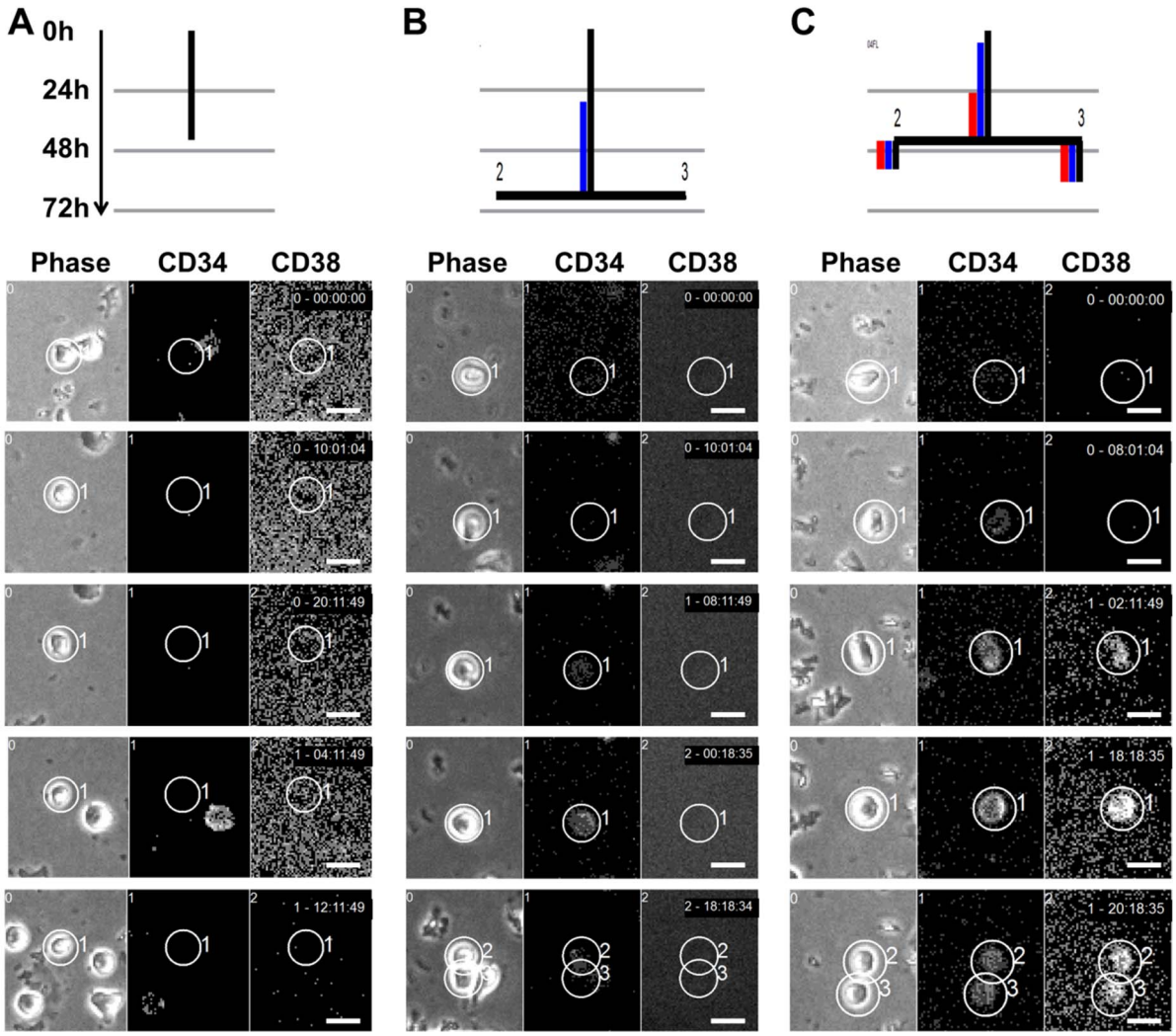

Fig. S2. Surface marker expression changes in realtime at the single cell level. Time-lapse microscopy and continuous single cell tracking reveal plasticity of CD34 and CD38 surface expression on single cells. Prospectively isolated $CD34^{-}CD38^{-}$ PDLTC-PH cells and their progeny were continuously imaged and CD34 and CD38 expression were determined at 2 h intervals for up to three days. Fluorescent anti-CD34-APC and anti-CD38-PE antibodies were added to the medium at low concentrations to allow non-invasive detection. Some cells did not re-express CD34 or CD38 (A), some cells expressed only one marker (B) and others sequentially turn on both markers (C). The respective cell pedigree and selected images of the supplementary movies S1 to S3 are displayed. Expression of CD34 is illustrated by a blue line, CD38 expression by a red line. Track label 1 indicates the tracked mother cell, label 2 and 3 are the daughter cells. Time scale in days-hours:minutes:seconds. Scale bar indicates 40 µm.

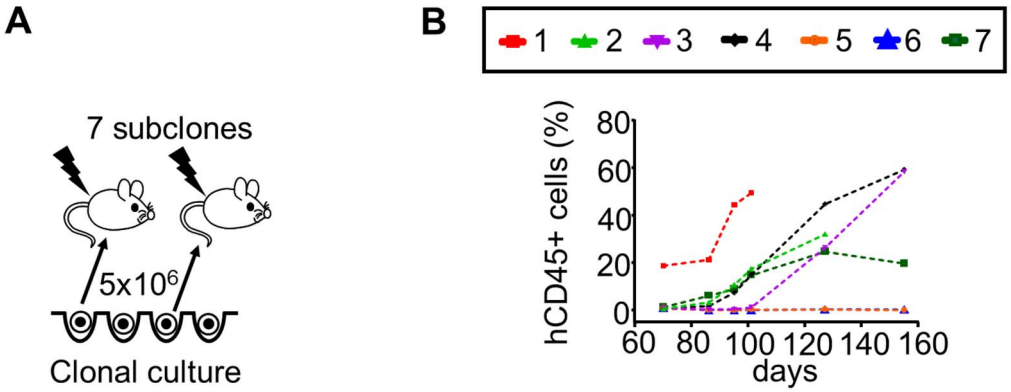

Fig. S3. Single cell-derived clonal isogenic subpopulations of PDLTC-PH show various leukemic engraftment and progression potential. A) Experimental scheme and leukemic peripheral blood engraftment of NSG mice receiving individual subclones. Only clone 1 and 2 resulted in early leukemic death of recipients, all other mice survived the observation period of 155 days as shown in Figure 2D.

Supplementary Movie S1. Prospectively isolated CD34$^-$CD38$^-$ PDLTC-PH cell does not express CD34 or CD38 within 36h. Time-lapse microscopy-based cell tracking, tracks are indicated with numbers. Track label 1 indicates the tracked mother cell. Phase contrast images (channel 0) were acquired every 2 min, fluorescent images for CD34 (channel 1) and CD38 (channel 2) were acquired every 2 h. Only fluorescent images and corresponding phase contrast images were used to assemble the movie. Corresponding pedigree is displayed in Fig. S1A. Time scale in days-hours:minutes:seconds.

Supplementary Movie S2. Prospectively isolated CD34$^-$CD38$^-$ PDLTC-PH cell will express CD34, but not CD38 within one cell generation. Time-lapse microscopy-based cell tracking, tracks are indicated with numbers. Track label 1 indicates the tracked mother cell, label 2 and 3 are the daughter cells. Phase contrast images (channel 0) were acquired every 2 min, fluorescent images for CD34 (channel 1) and CD38 (channel 2) were acquired every 2 h. Only fluorescent images and corresponding phase contrast images were used to assemble the movie. Corresponding pedigree is displayed in Fig. S1B. Time scale in days-hours:minutes:seconds.

Supplementary Movie S3. Prospectively isolated CD34$^-$CD38$^-$ PDLTC-PH cell will express sequentially CD34 and CD38 within one cell generation. Time-lapse microscopy-based cell tracking, tracks are indicated with numbers. Track label 1 indicates the tracked mother cell, label 2 and 3 are the daughter cells. Phase contrast images (channel 0) were acquired every 2 min, fluorescent images for CD34 (channel 1) and CD38 (channel 2) were acquired every 2 h. Only fluorescent images and corresponding phase contrast images were used to assemble the movie. Corresponding pedigree is displayed in Fig. S1C. Time scale in days-hours:minutes:seconds.