The fetal liver lymphoid-primed multipotent progenitor provides the prerequisites for the initiation of t(4;11) MLL-AF4 infant leukemia

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Supplementary Material and Methods

Mice
The animal work was done under regulation of the UK Home Office. Males and females from the Mll-AF4 and the VEC-Cre line were mated to obtain Mll-AF4-expressing embryos. The day of the plug was counted as day 0 of embryonic development.

Cell sorting of HSC/MPP, LMPP and LK/CLP populations
The fetal liver was dissected and dissociated in Flow Cytometry Staining Buffer (ThermoFisher Cat# 00-4222-26) using a 21Gx15mm needle attached to a syringe (BD Microlance Cat# 10472204-X, BD Cat# 3000185). Cells were stained using the following antibody mix in Flow Cytometry Staining Buffer: APC anti-mouse CD3ɛ antibody (clone I45-2C11, Biolegend Cat# 100312), APC anti-mouse TER-119 antibody (clone TER119, Biolegend Cat# 116212), APC anti-mouse F4/80 antibody (clone BM8, Biolegend Cat# 123116), APC anti-mouse Nk1.1 antibody (clone PK136, Biolegend Cat# 108709), APC anti-mouse Ly-6G/Ly-6C (Gr-1) antibody (clone RB6-8C5, Biolegend Cat# 108412), PE/Cy7 anti-mouse/human CD45R/B220 antibody (clone RA3-6B2, Biolegend Cat# 103222), PE/Cy7 anti-mouse CD19 antibody (clone 6D5, Biolegend Cat# 115520), APC-eFluor 780 CD117 (ckit) antibody (clone 2B8, ThermoFisher Cat# 47-1171-80), Alexa Fluor® 700 anti-mouse CD45 antibody (clone 30-F11, Biolegend Cat# 103128), Pacific Blue™ anti-mouse Ly-6A/E (Sca-1) antibody (Clone E13-161.7, Biolegend Cat# 122519), PE anti-mouse CD127 antibody (clone A7T34, ThermoFisher Cat# 12-1271-82), biotin anti-mouse CD135 (Flt3) antibody (clone A2F10, ThermoFisher Cat# 13-1351-81). Cells were stained for 20 minutes on ice and washed once with Flow Cytometry Staining Buffer. Cells were then resuspended in diluted Qdot 655 Streptavidin Conjugate (ThermoFisher Cat# Q10123MP) and incubated for 20 minutes on ice. Cells were washed once and resuspended in diluted SYTOX™ Green Nucleic Acid Stain (ThermoFisher Cat# S7020) to exclude dead cells. Sorting was done on a BD FACSARia™ II (BD Biosciences).
Transplantation of CD45.1/2 mice with HSC/MPP, LMPP and LK/CLP

On the day of transplant, recipient mice (CD45.1/2) received two doses of 4.6 Gy at a 3 hours interval. Donor cells (CD45.2/2) were injected through the tail vein along with bone marrow helper cells (CD45.1/1). For HSC/MPP transplants, we used 100 000 helper cells and for LMPP and LK/CLP, we used 20 000 helper cells. Mice were administered antibiotics after transplantation through their drinking water (0.1 mg/mL enrofloxacin, 10% Baytril solution from Bayer). For secondary transplants, the number of bone marrow cells transplanted was adjusted according to the repopulation in primary recipient (85% repopulation in primary recipient, 2 x 10^6 total bone marrow cells injected). Mice were bled on a monthly basis, and blood counts were measured on a Celltac MEK-6500K (Nihon Kohden). Red blood cell lysis was achieved with BD Pharm Lyse™ lysing solution according to the manufacturer's instructions (BD Biosciences Cat# 555899). Cells were stained in Flow Cytometry Staining Buffer using the following antibodies: FITC CD45.2 antibody, (clone 104, ThermoFisher Cat# 11-0454-81), APC-eFluor 780 CD45.1 monoclonal antibody (clone A20, ThermoFisher Cat# 47-0453-80), eFluor450 CD11b monoclonal antibody (clone M1/70, ThermoFisher Cat# 48-0112-80), Alexa Fluor® 700 Ly-6G/Ly-6C (Gr-1) antibody (clone RB6-8C5, Biolegend Cat# 108422), PE/Cy7 CD45R/B220 antibody (clone RA3-6B2, Biolegend, Cat# 103222), Brilliant Violet 605™ CD19 antibody (clone 6D5, Biolegend Cat# 115539), APC mouse IgM monoclonal antibody (clone II/41, ThermoFisher Cat# 17-5790-82), PE CD3e (clone 145-2C11, Biolegend Cat# 100308). For sorting/analysis of hematopoietic stem and progenitor cells in organs and analysis of B cell populations in primary and secondary recipients, we used the following antibodies: the APC lineage cocktail from the sorted E14 FL cells, FITC CD45.2 antibody, (clone 104, ThermoFisher Cat# 11-0454-81), APC-eFluor 780 CD45.1 monoclonal antibody (clone A20, ThermoFisher Cat# 47-0453-80), Brilliant Violet 421™ CD117 (c-Kit) antibody (clone 2B8, Biolegend Cat# 105827), APC-eFluor 780 CD117 antibody (clone 2B8, ThermoFisher 47-1171-82), PE/Cy7 Ly6A/E (Sca-1) antibody (clone E13-161.7, Biolegend Cat#122513), PB Sca1 antibody (clone E13-161.7, Biolegend, Cat# 47-1171-82), PerCP/Cy5.5 CD34 antibody (clone HM34, Biolegend, Cat# 128607), PE CD135 antibody (clone A2F1, Biolegend, Cat# 135306), biotin anti-mouse CD135 (Flt3) antibody (clone A2F10, ThermoFisher Cat# 13-1351-81), Alexa Fluor® 700 CD48 antibody (clone HM48-1, Biolegend, Cat# 103425), PE/Cy7 CD150 antibody (clone TC15-12F12.2, Biolegend, Cat# 115914), Alexa
Fluor® 700 CD45R/B220 antibody (clone RA3-6B2, Biolegend, Cat# 103232), PE/Cy7 CD19 antibody (clone 6D5, Biolegend, Cat# 115520), PerCP CD43 antibody (clone 1B11, Biolegend, Cat# 121222), Brilliant Violet 421™ CD24 antibody (clone M1/69, Biolegend, Cat# 101825), APC CD127 antibody (clone A7R34, Biolegend, Cat# 135011). Cells were incubated on ice for 20 minutes, washed twice with Flow Cytometry Staining Buffer and resuspended in diluted SYTOX AADvanced (ThermoFisher, Cat# S10274) to exclude dead cells. Data was acquired on a BD LSRFortessa™ (BD Biosciences). For end of study analysis, cell types were identified as follows: HSCs (LSK CD34+/- FLT3- CD150+ CD48-), LMPPs (LSK CD34+/- FLT3+), CLP (Lin - ckitlow Sca1low IL7R+ FLT3+), LK (Lin- ckit+ Sca1-), pre-pro-B (CD45.2+ ckit- CD43+ CD24low B220+ CD19-) and pro-B (CD45.2+ ckit+ CD43+ CD24+ B220+ CD19+).

Cell Cycle
Sorted HSC/MPP, LMPP and LK/CLP cells were collected in Flow Cytometry Staining Buffer and an equivalent volume of 5 μg/mL DAPI 1% IGEPAL (Sigma-Aldrich D9542 and CA-630) solution was added. Cells were incubated at room temperature for 1 minute, in the dark. Data was acquired on a BD LSRFortessa™ (BD Biosciences).

RNA extraction, reverse transcription and quantitative PCR
RNA extraction and reverse transcription were performed using the RNeasy Micro Kit (QIAGEN Cat# 74004) and iScript Ready-to-Use cDNA Supermix (Bio-Rad Laboratories Ltd Cat# 1708841) according to the manufacturer’s instructions. Primer sequences are: Flt3.F/R (gccagttcagcgcgctca/agattccctcggactggtgc), Meis1.F/R (attcacactgtggagacgc/cgtcgctacctttgcgccatc), Cdk6.F/R (ccacagctgtggtgtt/ggcgggctcggacatttat), Mcl1.F/R (agaggctgggatggtttgt/ccctattgcactcacaaggc), Twist1.F/R (gccggagacctagatgtcatt/ccacgccccgtattctgtgta), Runx1.F/R (tttgccagcgcgttgaagaa/tgctgltctgaagccatc_tt), Hoxa9.F/R (ccacgcttgacactcact/cagccgggtattttggatc), Hmga2.F/R (gcctgtcagagactgaag/ggaagtagaaagccgtgga), Bcl2.F/R (ggagggctggatctttgt/acttgtggcaggtatgc), Lmo2.F/R (ctggacccgctgtggaac/gacacccacagaggtcag), Ikaros.F/R (ccctggacccgtttccatcagc/acgcctttctttcatcac), Il7r.F/R
E2a.F/R
(aagaggacaagaaggacctgaa/ttattggccatacgcttctc),  Pax5.F/R
(accatcaggacagcagcatgg/gcggactacatctgggagtg),  MII-AF4  F/R
(agtgggcatgtaggggatc/atggctcagctgtactaggc) and beta-actin  F/R
(tctgtctctactgtcaa/gtccgcctagaagcacttgc). Quantitative PCR was carried out with
Brilliant III Ultra-Fast SYBR QPCR (Agilent Technologies Cat#600883) according to
the manufacturer’s instructions. Data was acquired on a QuantStudio™ 7 Flex Real-
Time PCR System (ThermoFisher).

**Data analysis, statistics and graphs**
Analysis of flow cytometry data was performed with FlowJo (version 10) and graphs
were generated with GraphPad Prism (version 6). Statistical analysis was performed
with GraphPad using a non-parametric t test (Mann-Whitney) with a bi-lateral p-value.
Data are presented as Mean ± SEM.
References for the supplementary

Figure S1. Sorting strategy and cell cycle analysis of E14FL HSC/MPP, LMPP and LK/CLP cells (A) Sorting strategy of E14 FL HSC/MPP (Lin- B220- CD19- CD45+ ckit+ Sca1high IL7R- Flt3-), LMPP (Lin- B220- CD19- CD45+ ckit+ Sca1high Flt3+) and LK/CLP (Lin- B220- CD19- CD45+ ckit+ Sca1low/). (B,C) Cell cycle analysis (n = 5-7). HSC – hematopoietic stem cell, MPP – multipotent progenitor, LMPP - lymphoid-primed multipotent progenitor, LK – Lin- ckit+, CLP – common lymphoid progenitor. (D) Repopulation of Mll-AF4+ VEC-Cre+ E14 FL HSC/MPPs in primary and secondary recipients.