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Supplemental Figures

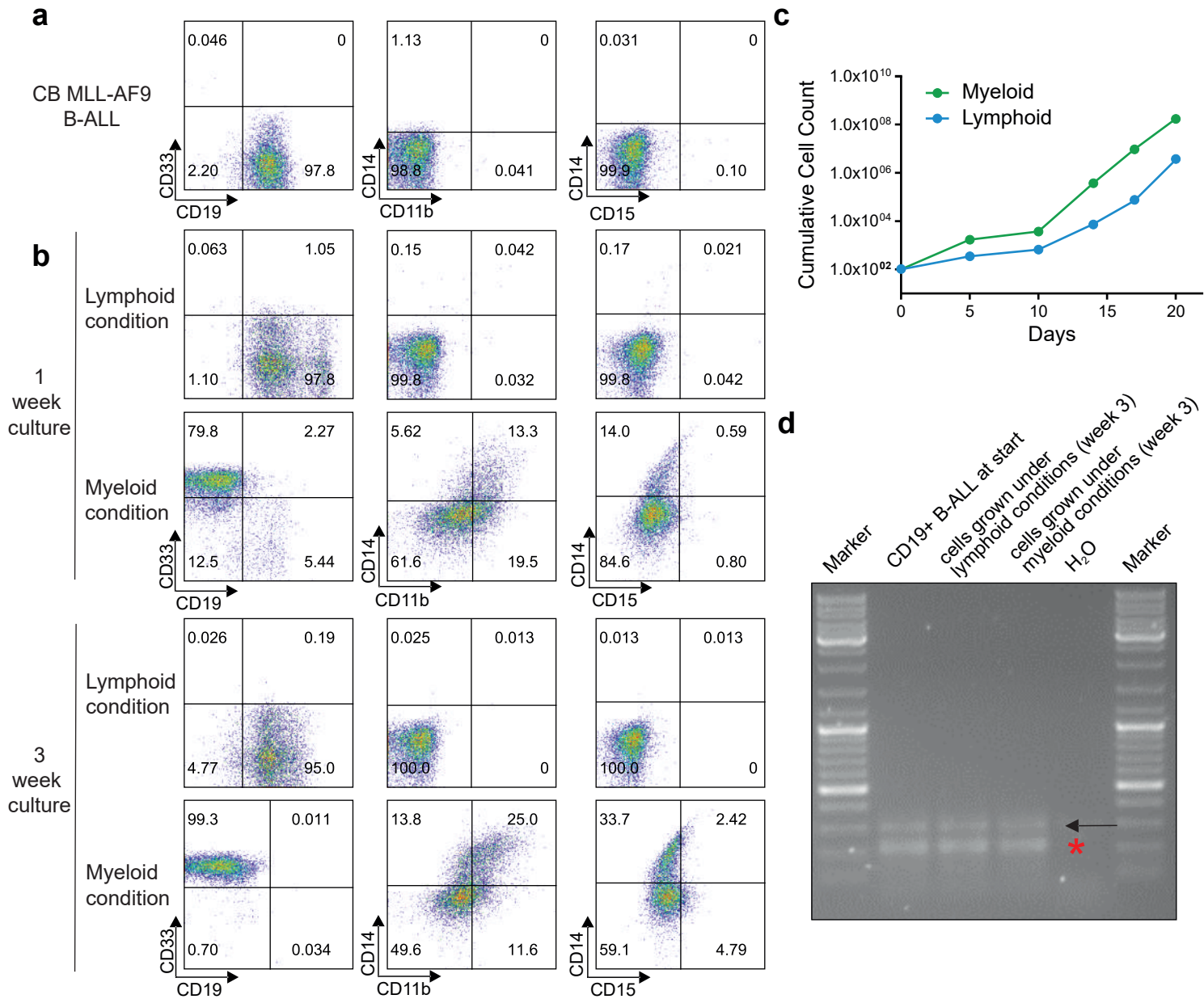
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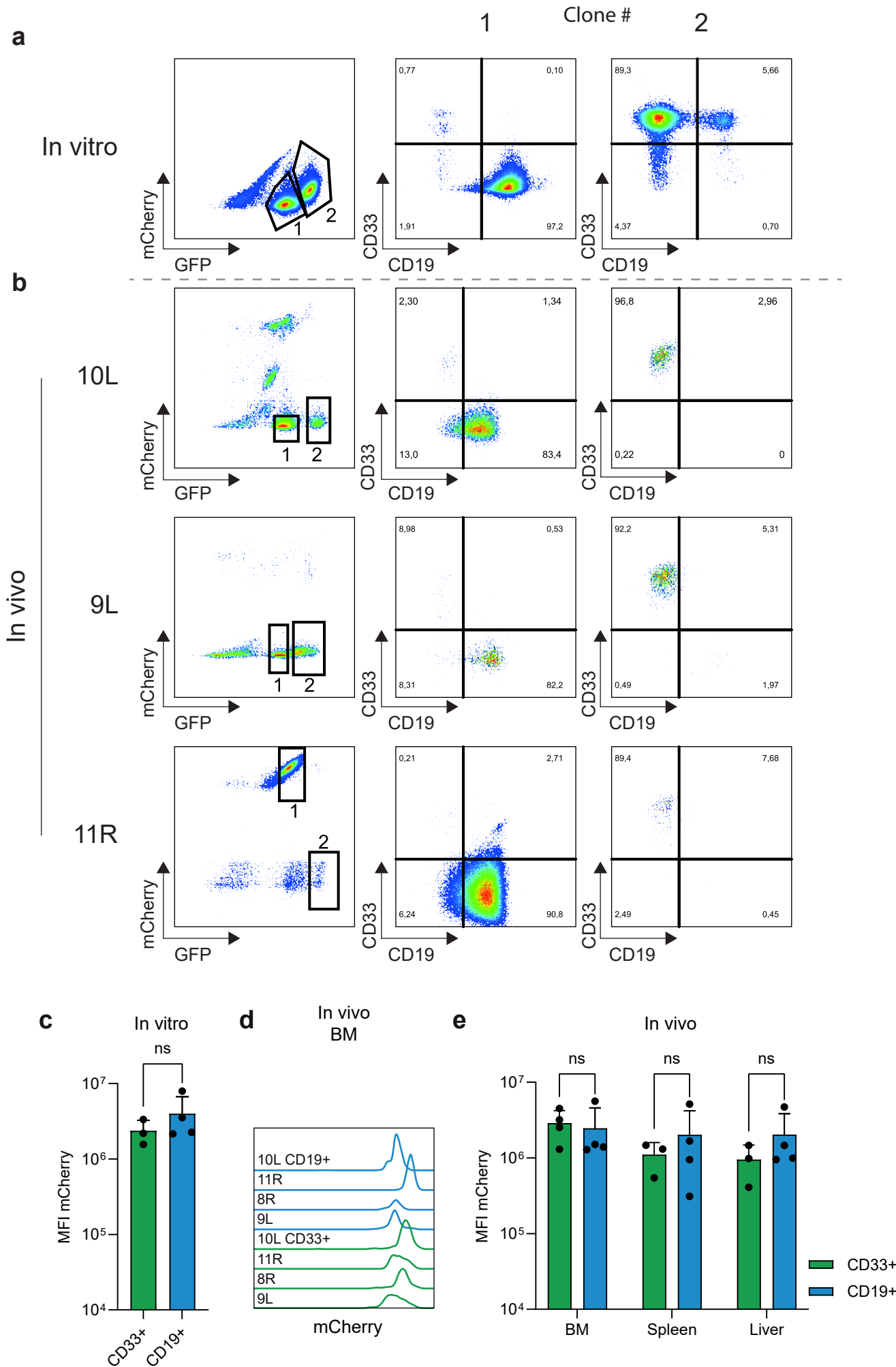
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Supplemental Figure 1



Supplemental Figure 1: CB MLL-AF9 B ALL model shows lineage plasticity *in vitro*. **a.** CB CD34⁺ cells were transduced with UMG LV6 MLL-AF9 lentiviral vectors¹ and cells were transformed along the lymphoid lineage on MS5 bone marrow stromal cells for a period of three weeks as described in Horton et al.² Flow cytometry was performed for CD33 (WM53), CD19 (HIB19), CD14 (HCD14), CD15 (W6D3) and CD11b (ICRF44) markers (all from Biolegend) confirming a B-ALL phenotype. **b.** B-ALL transformed MLL-AF9 cells were plated *in vitro* under myeloid restricted conditions that block lymphoid commitment (MS5 co-culture in α MEM, 12.5% heat-inactivated (HI) fetal calf serum (FCS), 12.5% HI horse serum (HS), PenStrep, 2mM glutamine, 57.2 μ M β -mercaptoethanol and 1 mM hydrocortisone (HC), supplemented with 20 ng/ml IL-3, SCF, FLT3-L, GCSF and TPO) or under lymphoid permissive conditions that allow outgrowth of both B-lymphoid and myeloid cells² (similar as above but without HC, HS, and IL-3 and with the addition of 10 ng/ml IL-7 and 50 μ g/ml vitamin C). **c.** Cumulative cell growth for both myeloid and lymphoid conditions as described under b. **d.** LM-PCR (as previously described)^{3,4} was performed on CD19⁺ B-ALL cells at start, on cells grown under lymphoid conditions, and on cells grown under myeloid conditions as described under b-c, showing that the same clone with the identical integration site expanded under all conditions (arrow: specific PCR product, star: internal control).

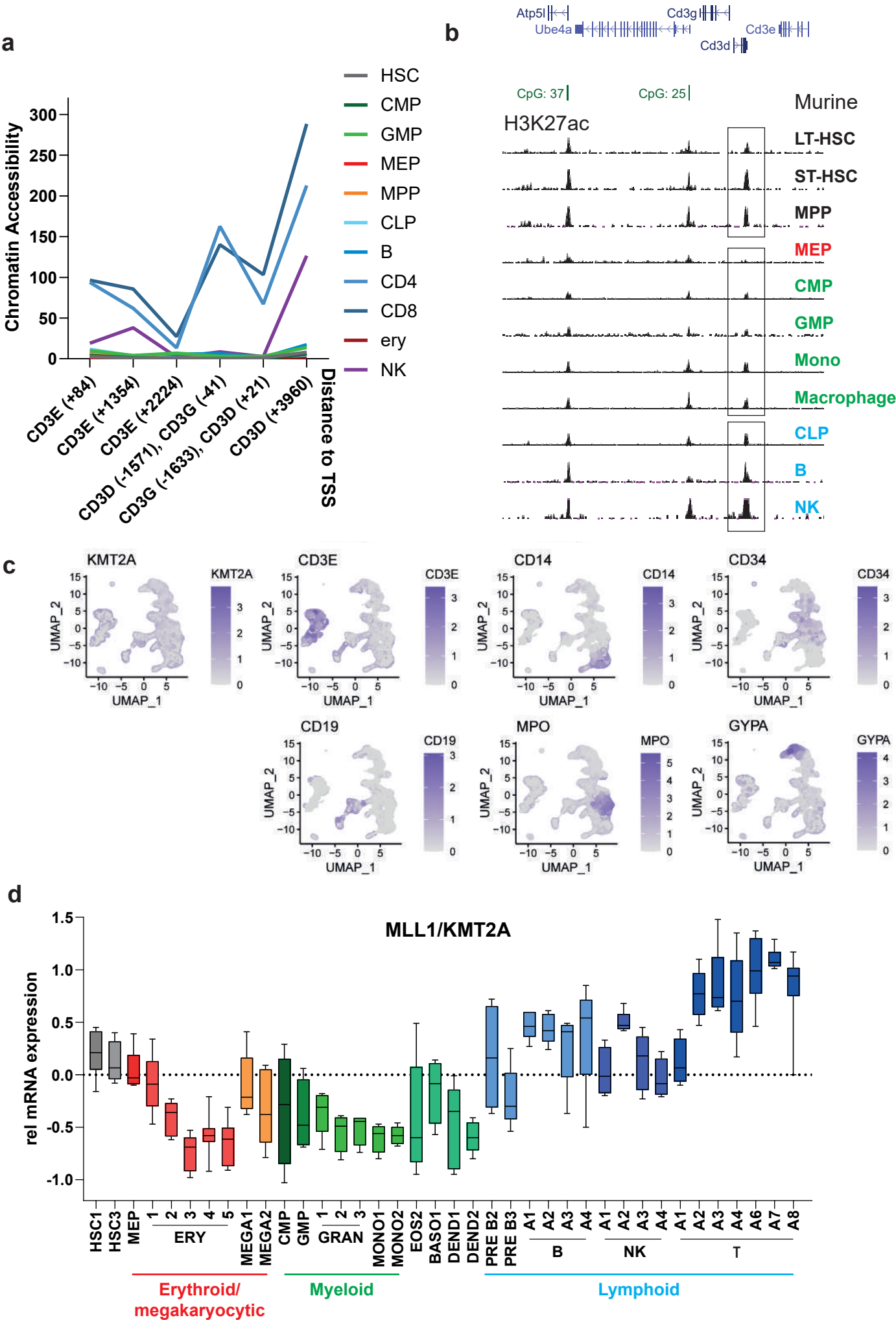
Supplemental Figure 2



Supplemental Figure 2: Sorting strategy of clones for qPCR analysis.

a. Sorting strategy on day 48 of in vitro CB MLL-AF9 model under lymphoid permissive conditions (MS5 co-culture in IMDM medium supplemented with 20% FCS, PenStrep, 1% Insulin-Transferrin-Selenium-Ethanolamine, 50µg/mL vitamin C, and cytokines IL3, IL7, SCF and FLT3-L all at 10ng/mL). **b.** CB CD34⁺ cells were transduced with MLL-AF9 and tail vein-injected into sub-lethally irradiated (1Gy) MISTRG mice, expressing human alleles for M-CSF, GM-CSF, IL3, TPO and SIRP α .⁵ At sacrifice, clones derived from liver (10L) and spleen (9L and 11R) were sorted based on gates 1 and 2 as indicated. Clones #1 and #2 represent GFP-low and GFP-high cells, respectively, distinguishing CD33 (myeloid) from CD19 (lymphoid) cells. **c-e.** Co-transduced empty vector mCherry-expressing cells (pRRL-SFFV_EV-mCherry)⁶ did not show differences in MFI levels between myeloid and lymphoid clones, in vitro (c, week 10) or in vivo upon transplantation in MISTRG mice (d, histograms of BM; e, MFI quantification from BM, spleen and liver). T-test (c), two-way analysis of variance (ANOVA) (e), not significant (ns).

Supplemental Figure 3



Supplemental Figure 3: The epigenetic landscape of the CD3 locus has similarities with that of MLL1. **a.** ATACseq data showing increased chromatin accessibility in human lymphoid cell lineages at the *CD3D/E/G* loci (GSE96772).⁷ **b.** H3K27ac deposition at the *CD3D* locus. ChIP-seq analysis of murine (C57BL/6) hematopoiesis has a similar pattern as the H3K27ac at the MLL1 locus (see Figure 3) (GSE59636).⁸ **c.** Healthy bone marrow single cell RNA sequencing UMAP projection (own unpublished data, Pereira-Martins et al.) of KMT2A and lineage defining genes: Lymphoid (CD3E, CD19), Myeloid (CD14, MPO), Erythroid (GYPA) and hematopoietic stem cells (CD34), projection based on AGX Zeng et al.⁹ **d.** MLL1 mRNA levels in human erythroid and myeloid cells/progenitors are lower than in lymphoid counterparts (GSE24759).¹⁰

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