LETTER TO THE EDITOR

H2-K1 protects murine *MLL-AF9* leukemia stem cells from natural killer cell-mediated immune surveillance

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H2-K1 protects murine *MLL-AF9* leukemia stem cells from natural killer cell-mediated immune surveillance

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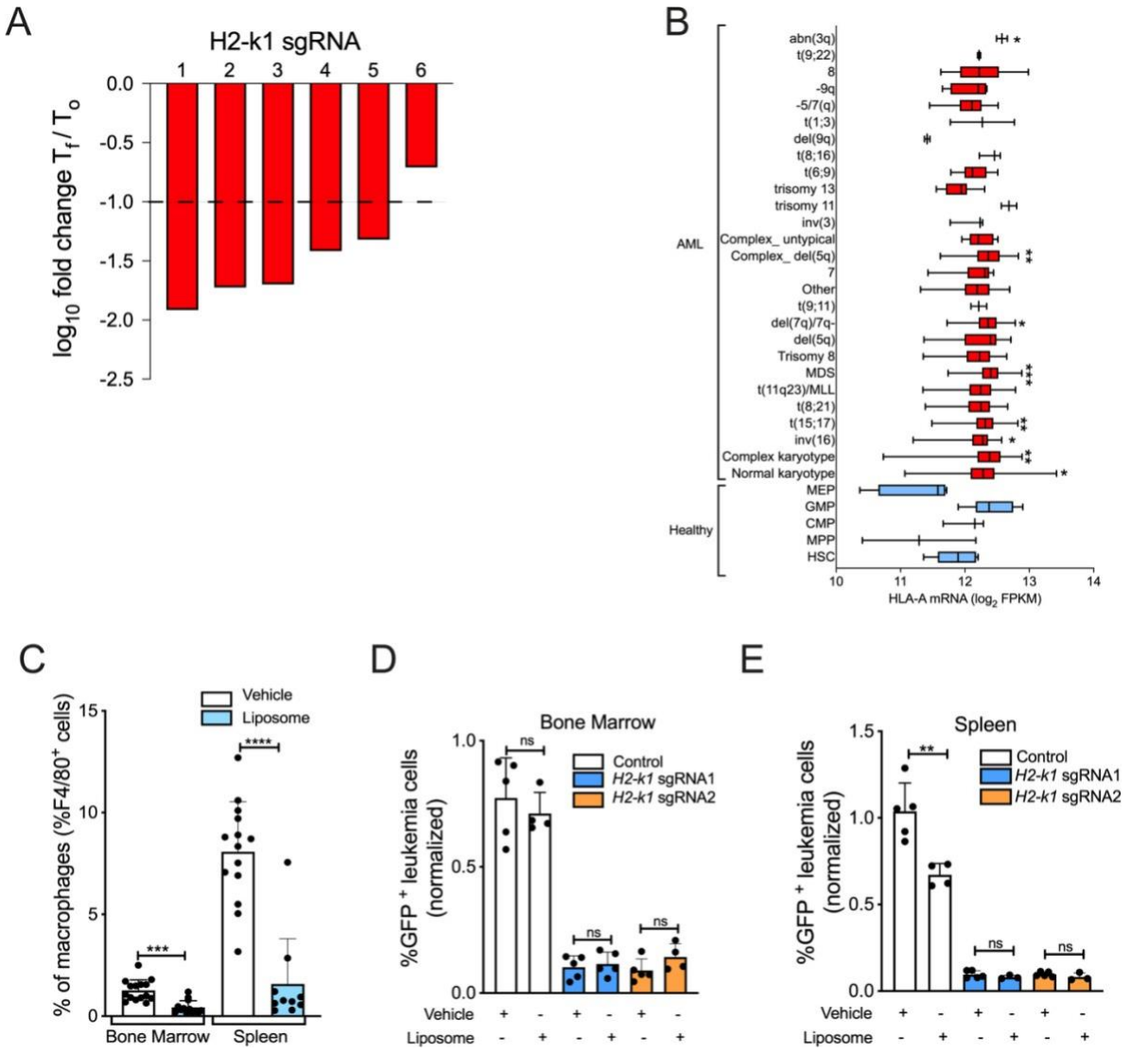

Figure S1. H2-K1 disruption depletes *MLL-AF9* leukemia cells in a macrophage-independent manner and HLA-A is upregulated in AML cells from patients compared to normal hematopoietic stem cells. (A) Waterfall plot showing the normalized fold change representation of *H2-k1* sgRNAs in the CRISPR screen. A fold-change threshold of 10-fold was used to define depleted sgRNAs. (**B**) HLA-A expression in a cohort of $CD34^+$ hematopoietic stem and myeloid progenitor cells from healthy donors highlighted in blue (n=6; GSE42519) and AML subtypes from TCGA; GSE13159; GSE15434; GSE61804; and GSE14468 (n=1824). Significance measured by one-way ANOVA, AML subtypes versus normal HSC. **(C)** Percentages of macrophages ($F4/80^+$) in the bone marrow and spleen of the mice treated with clodronate liposomes or vehicle (PBS) determined at the endpoint of the experiment. Percentage of GFP^+ cells within leukemia cells in **(D)** bone marrow and **(E)** spleen of vehicle or clodronate liposome-injected mice prior to transplantation of leukemia cells transduced with *H2-k1* sgRNAs or control. Data are represented as mean ± SD with n=5. Significance was measured by non-parametric students t-test with the following significance thresholds: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns: non-significant. SD: standard deviation. T_0, initial time point (day2); T_f, final time point (day 5); FPKM, fragments per kilobase of transcript per million mapped reads; The Cancer Genome Atlas, TCGA; ALL; acute lymphoblastic leukemia; HSC, hematopoietic stem cell; MPP, multipotent progenitor; CMP, common myeloid progenitor; MEP, megakaryocytic-erythroid progenitor; GMP, granulocyte-monocyte progenitor.

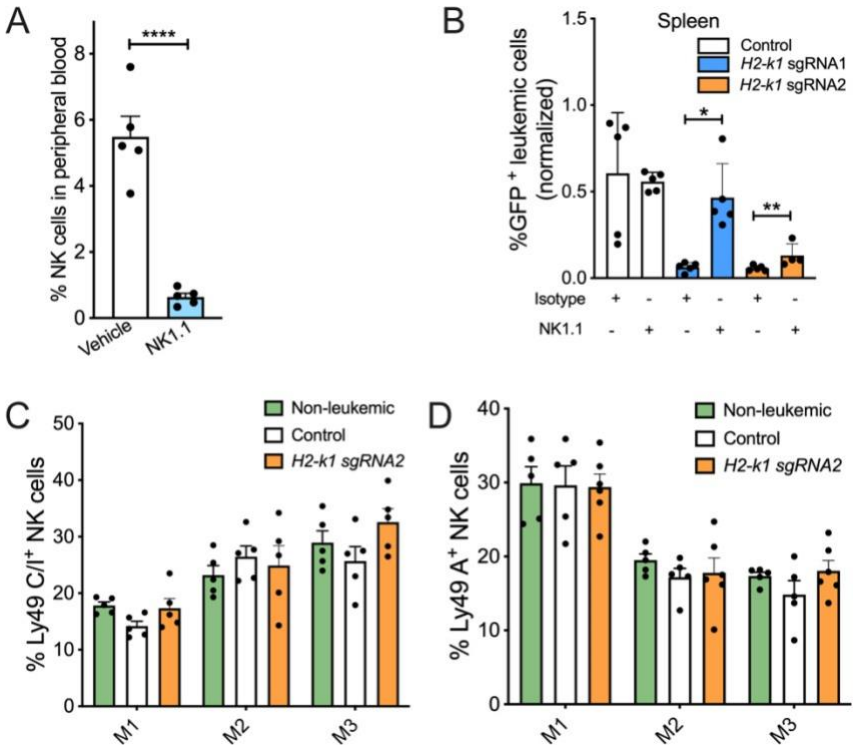

Figure S2. Depletion of NK cells neutralizes the anti-leukemic effect of *H2-k1* ablation in the spleen. (A) Percentage of NK cells (NK1.1$^+$) in peripheral blood of mice 21 days post initiation of injection of either the isotype or NK1.1 antibodies. **(B)** Percentage of GFP$^+$ cells within *MLL-AF9* leukemia cells in the spleen following isotype or NK1.1 antibody treatment. Mice were transplanted with *H2-k1* sgRNAs or control transduced leukemic cells (n=5). Percentages of **(C)** Ly49 C/I or **(D)** Ly49A expressing cells within M1-M3 subpopulations of mature NK cells in the BM of mice transplanted with sorted *H2-k1* sgRNA or control transduced leukemic cells (n=5 mice per group). Mice not receiving leukemia cells, referred to as non-leukemic, were included as an additional control. Data are represented as mean ± SD. Significance was measured by non-parametric students t-test with the following significance thresholds: *p<0.05; **p<0.01; *** p<0.001; **** p<0.0001. SD: Standard Deviation.

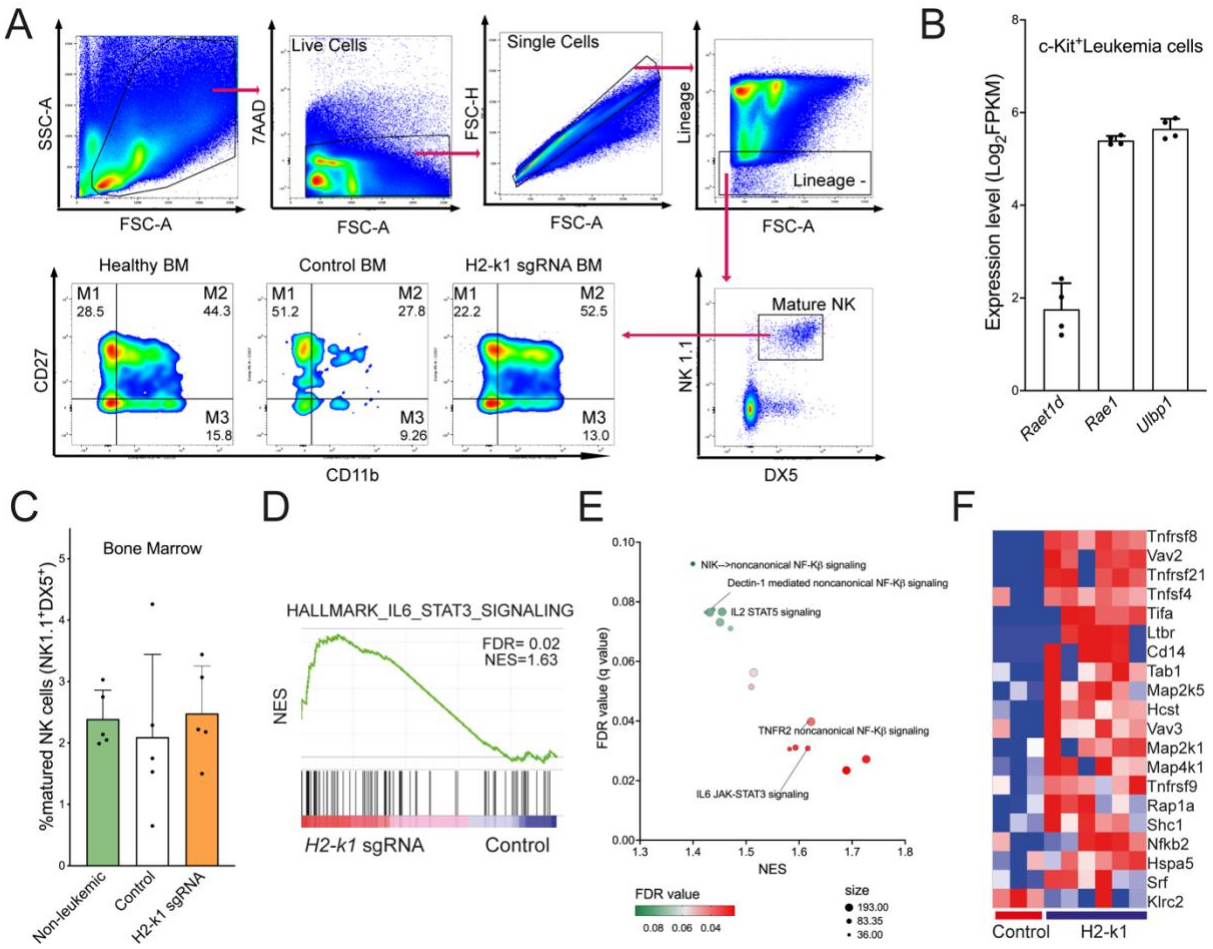

Figure S3. Leukemia development affects NK cell maturation. (A) Gating strategy for flow cytometric analysis of the NK cell population from the bone marrow of healthy or leukemic mice with or without *H2-k1* disrupted *MLL-AF9* leukemia cells. BM, bone marrow. **(B)** mRNA expression of NKG2D ligands *Raet1d, Rae1* and *Ulbp1* in c-Kit^{+} *MLL-AF9* leukemic cells. Gene expression shown as Log_2FPKM values. (**C)** Percentages of mature NK cells (NK1.1^{+}DX5^{+}) within the Lineage^{-} BM cells of mice transplanted with sorted *H2-k1* sgRNA or control transduced leukemic cells (n=5 mice per group). Mice not receiving leukemia cells, referred to as non-leukemic, were included as an additional control. **(D)** Gene set enrichment analysis (GSEA) of the transcriptional signature in NK cells from healthy mice co-cultured with *H2-k1* sgRNA versus control transduced leukemic cells (n=4). **(E)** Gene set enrichment analysis (GSEA) identifies significantly upregulated pathways in NK cells co-cultured with leukemic cells. Pathways from the Hallmark gene set database are shown. **(F)** Heatmap of leading-edge upregulated genes within NF-kb signaling gene sets. FPKM, Fragments Per Kilobase of transcript per Million mapped reads; NES, normalized enrichment score; FDR, false discovery rate; circle size, gene set size (number of genes in each gene set).