



Supplemental Figure 1. NK cell maturation is altered in leukemic mice. (A) NK cells were sorted from the spleens of mice and cultured with FLB1 leukemia cells or with YAC-1 target cells for 4h at the indicated Effector:Target (E:T) ratios. The percentage of target cell death was determined by the expression of caspase-3/7 by flow cytometry. The percentage of lysis mediated by NK cells was determined as follow: (% cell death in the presence of NK cells) - (% cell death in the absence of NK cells) (n=3 mice). (B-F) C57BL/6 mice were injected intravenously with FLB1 cells. Control mice were injected with PBS. (B) The percentage of FLB1 (CD45⁺) cells in relation to total CD45⁺ cells was evaluated by flow cytometry in the bone marrow (BM), spleen and peripheral blood of mice at the indicated days following FLB1 injection (n=2-6 mice/group in 2 independent experiments). (C-F) Freshly isolated cells from the BM and spleen of leukemic or control mice were stained and analyzed by flow cytometry. (C) Percentage of NK cells among total non leukemic CD45.1⁺ cells (n=7-8 mice/group in 2 independent experiments). (D-F) Absolute number of total non leukemic cells (CD45.1⁺) (D), total NK cells (E) or NK cell maturation subsets (F) (n=7-9 mice/group in 5 independent experiments). (G) The percentage of CD27⁺CD11b⁻ immature NK cells among total CD3⁺CD19⁻ NK1.1⁺ NK cells in the BM of control (CTRL) or leukemic mice (MLL-AF9 transgenic mice or C1498 injected mice) (n=3-8 mice/group in 1 to 4 independent experiments). (H) The level of expression of Eomes, as measured by MFI, in Eomes positive NK cells and NK cell maturation subset in the BM of leukemic or control mice (n=8-10 mice/group in two independent experiments). (I-K) Freshly extracted spleens of leukemic or control mice were stained and analyzed by flow cytometry. The graphs represent the percentage of NK cells expressing Eomes (I), the level of expression of Eomes in Eomes positive NK cells (J), and the level of expression of T-bet in total NK cells (K) (n=5 mice/group). The values are presented as the mean +/- SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, NS = Non Significant, as determined by Mann-Whitney test.