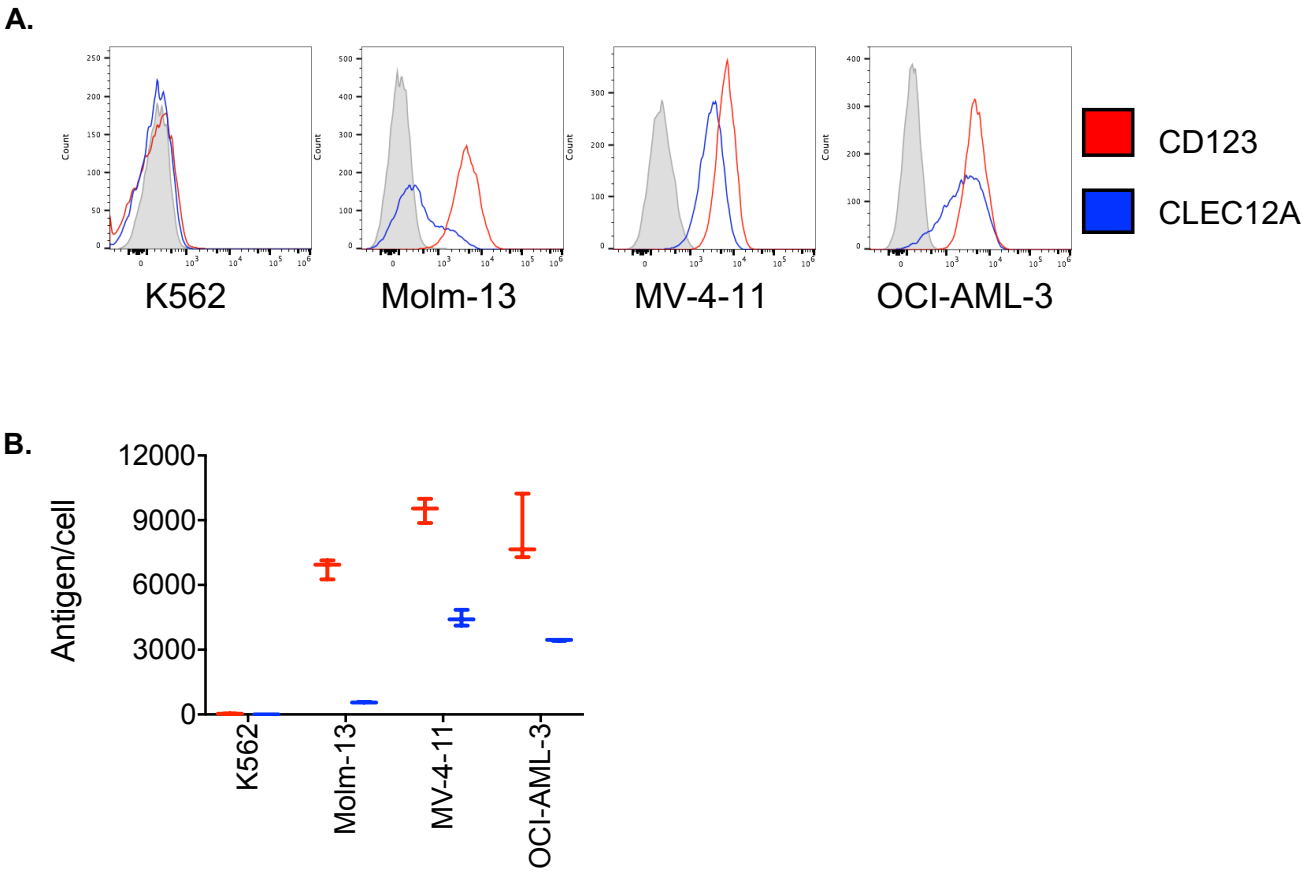
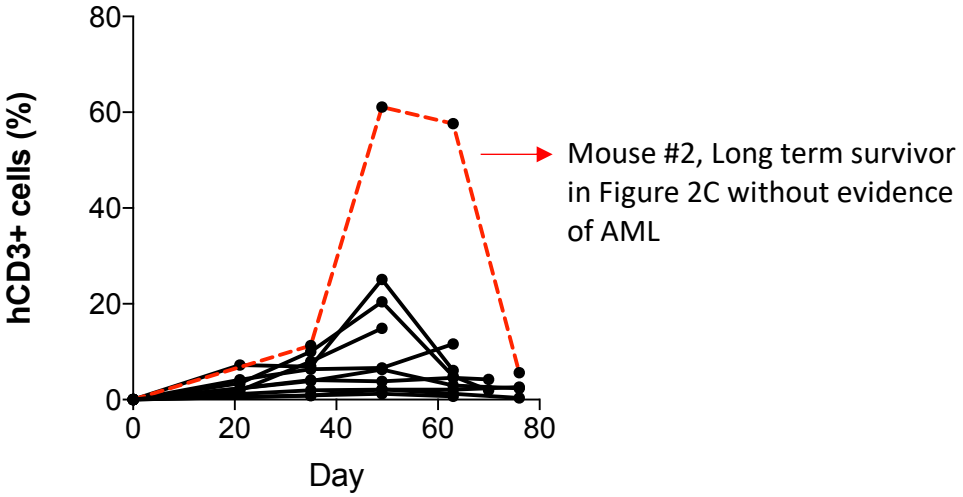
Mutation	Primer Sequence	Endogenous AA sequence	Mutant sequence
Box1 deletion	Forward primer: AAGACCCTGGAACACCTG Reverse primer: GATAGGCTTGATCCGCTTC	269-KPIVWPSLPNHKKTL-283	-KPIKTL-
Y449F	Forward primer: AGAAGAGGCCTTCGTCACCATG Reverse primer: TGATTGGAGCCCAGGCTT	446-EEAYVTM-452	-EEAFVTM-

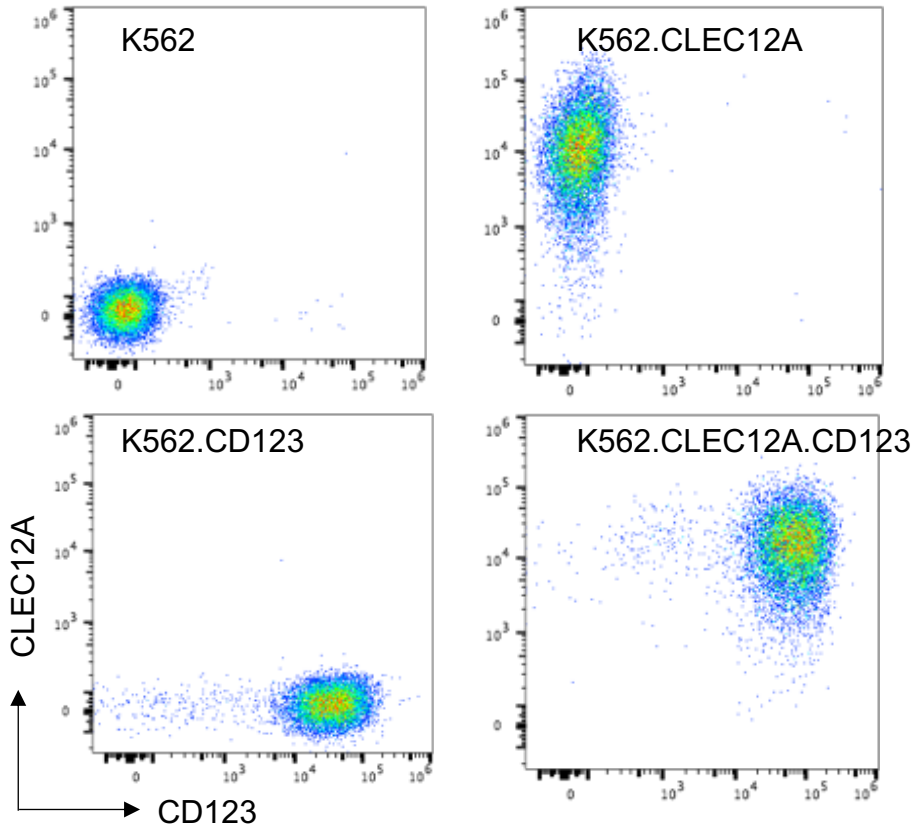
Supplementary Figure S1. Primer sequences used to generate IL7Rα mutants. Forward and reverse primers used for mutagenesis are displayed. Numbering reflects NCBI Reference Sequence: NP_002176.2 (Interleukin-7 receptor subunit alpha precursor) amino acid sequence



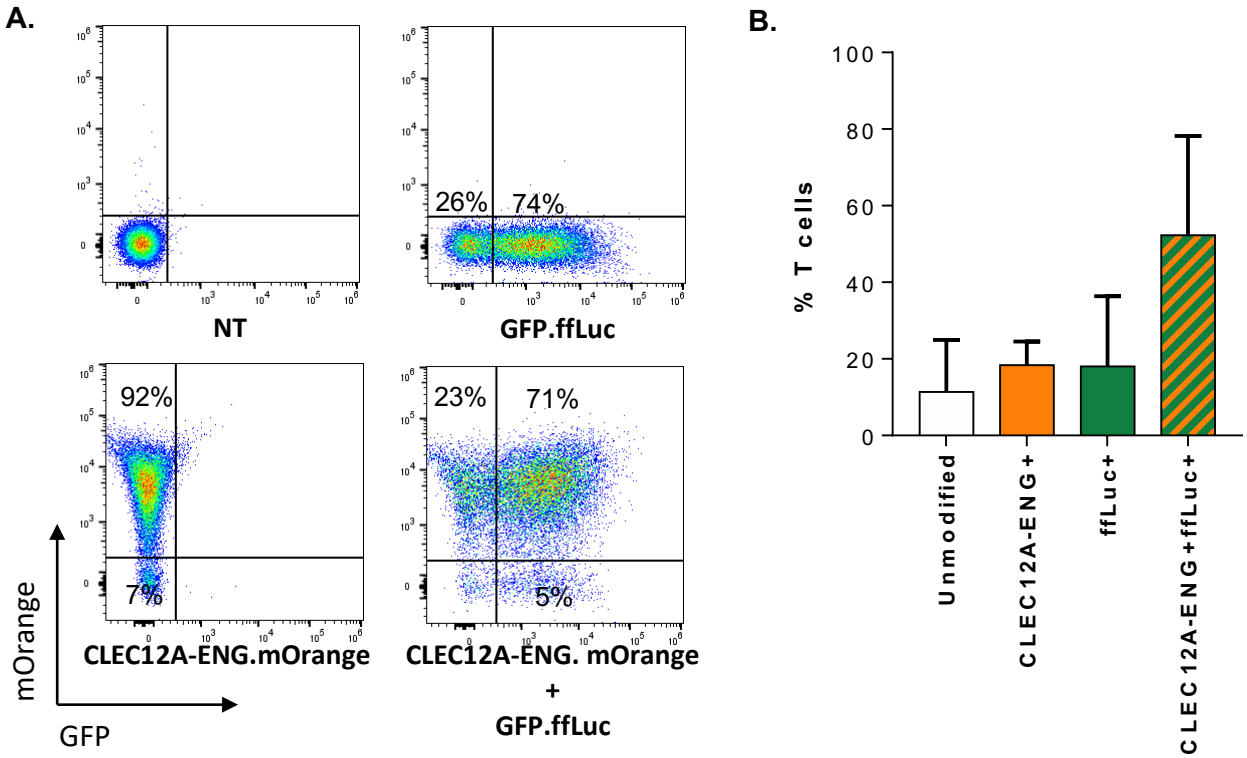
Supplementary Figure S2. CLEC12A and CD123 expression on leukemia cell lines. A. FACS histograms of CLEC12A and CD123 expression (K562, Molm-13, MV-4-11, OCI-AML-3) on human leukemia cell lines. **B.** Quantification of antigen density performed using QuantiBrite beads (BD Biosciences). Data expressed as median(range), n=3. CD123 - K562: 32.4 (0.2-42.3), Molm-13: 6937.8(6255.4-7144.6), MV-4-11: 9545.7(8869.4-9991.1), OCI-AML-3: 7653.9(7292.4-10235.1); CLEC12A - K562: 0.0(0.0-0.0), Molm-13: 552.8(552.8-578.1), MV-4-11: 4412.1(4113.7-4848.9), OCI-AML-3: 3454.2(3410.8-3462.3)



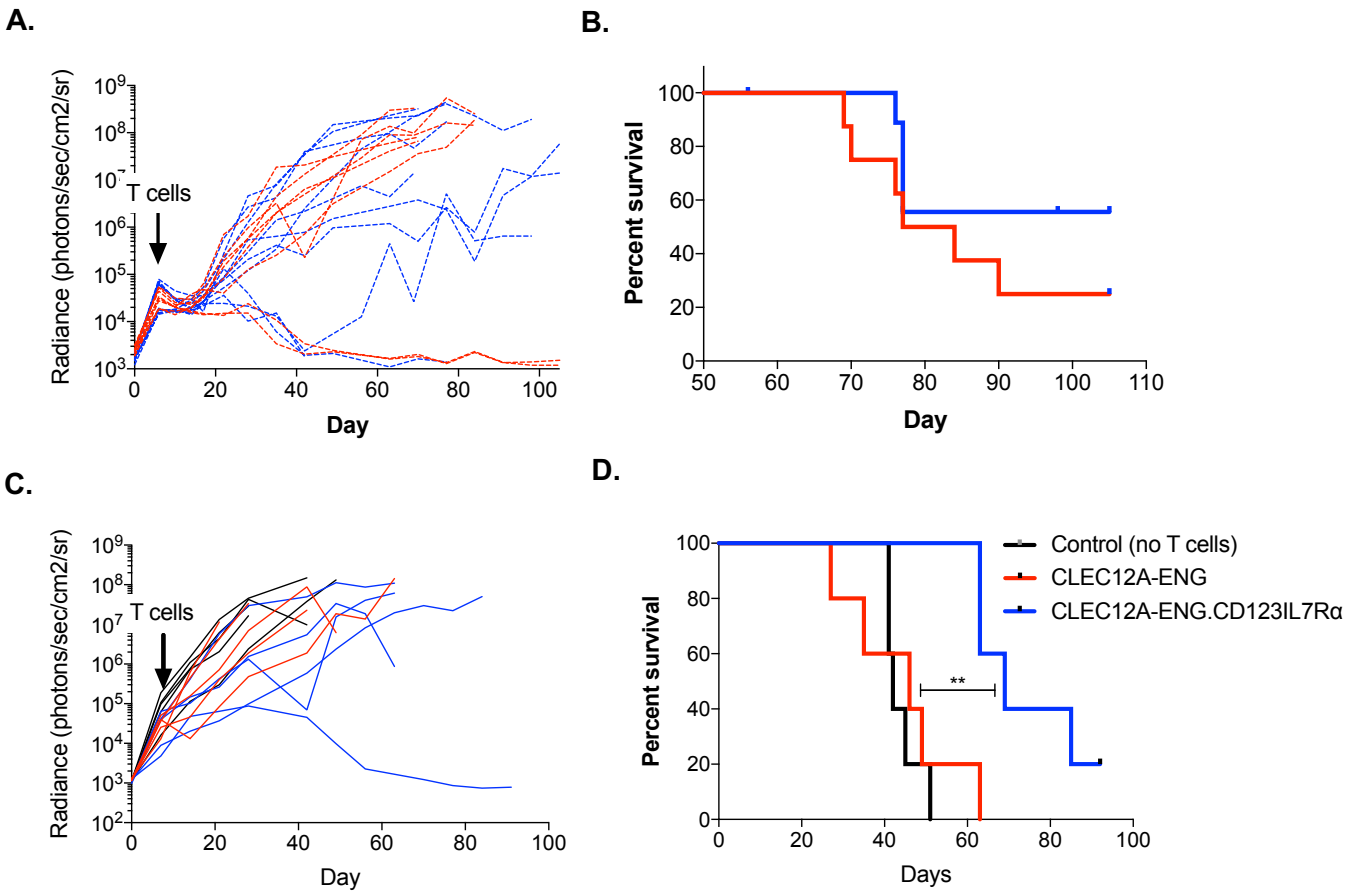
Supplementary Figure S3. CLEC12A-ENG T cell expansion in MV-4-11.ffLuc engrafted mice. Murine peripheral blood was collected, RBCs lysed, and remaining cells stained with hCD3 antibody. Data analyzed by FACS. Mouse #2 in cohort 1 of MV-4-11.ffLuc xenograft studies exhibited significantly enhanced human T cell expansion between D49 and 63.



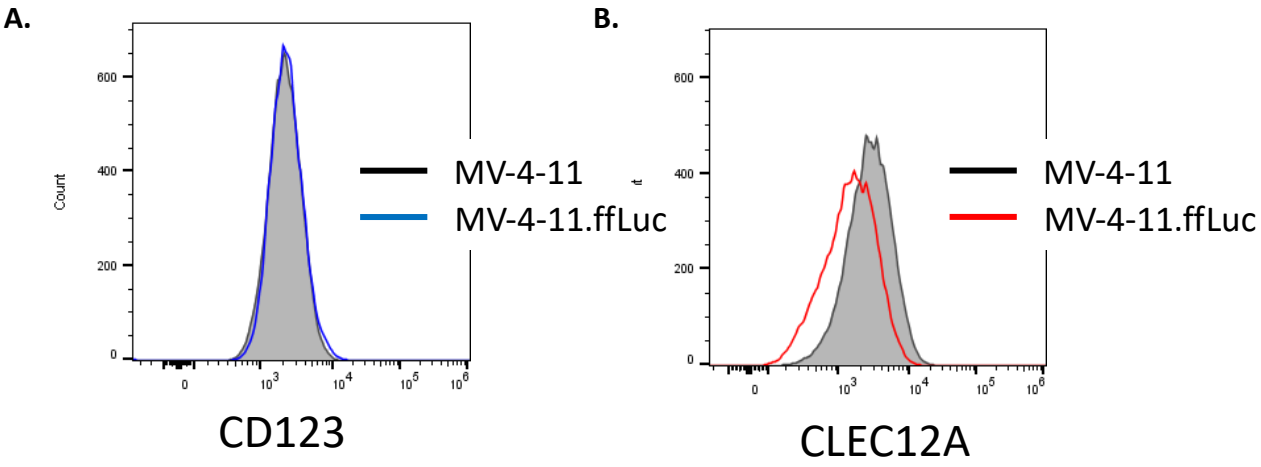
Supplementary Figure S4. K562 can be effectively modified to express CLEC12A and CD123. Lentiviral targeting vectors were designed to encode either CLEC12A or CD123. The K562 cell line was transduced with lentiviral vector, sorted for antigen expression, and expression verified with FACS analysis.



Supplementary Figure S5. Co-expression of GFP.ffLuc and CLEC12A-ENG.mO in primary T cells. **A.** Representative FACS analysis of T cells transduced with retroviral vectors encoding a GFP.ffLuc fusion protein and/or CLEC12A-ENG.IRES.mOrange. **B.** Percentages of each T cell population in culture (n=2 independent T cell donors).



Supplementary Figure S6. CD123.IL7Rα expressing T cells have improved *in vivo* anti-leukemia control. **A.** Quantitation of sequential bioluminescent imaging of mice (n=8-10 mice each group) engrafted with 1e6 MV-4-11.ffLuc on D0 and treated with 10e6 T cells i.v. on D7. **B.** Kaplan-Meier survival analysis, Median survival: CLEC12A-ENG – 80.5 days, CLEC12A-ENG.CD123.IL7Rα – 91 days. Of note, two mice in CLEC12A-ENG.CD123IL7Rα group were censored from analysis, D56 and D77. One was cannibalized by cage mates and the other died of GVHD. Neither had evidence of bioluminescent positive disease at the time of death. All mice dead in CLEC12A-ENG group died with high leukemic burden. **C.** Serial bioluminescent imaging data of alternate CLEC12A+/CD123+ xenograft model. Mice were injected via tail vein with 1e6 OCI-AML-3.ffLuc cells on D0 and treated with indicated T cells on D7. Untreated mice served as a control. (n=5 mice each group) **D.** Kaplan-Meier survival analysis, Median survival: No T cells - 42 days, CLEC12A-ENG – 46 days, CLEC12A-ENG.CD123.IL7Rα – 69 days.



Supplementary Figure S7. MV-4-11 and MV-4-11.ffLuc have different levels of CLEC12A expression. A. MV-4-11 and MV-4-11.ffLuc stained with CD123-PE antibody (BD Biosciences, Clone 7G3). **B.** MV-4-11 and MV-4-11.ffLuc stained with CLEC12A-Alexa647 antibody (BD Biosciences, Clone 50C1).