Supplemental Methods

NK cell culture media

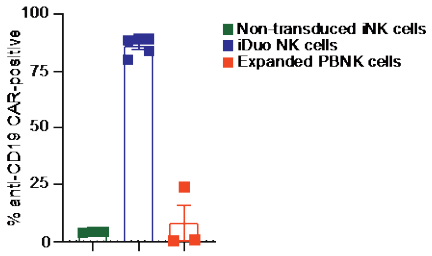
All iNK cells and peripheral blood NK cells were cultured in B0 media consisting of DMEM plus Ham's F-12 medium (Gibco) mixed at a 2:1 ratio supplemented with 10% heat-inactivated human AB sera, 1% penicillin-streptomycin, 25 μM β-mercaptoethanol, 20 μg/ml ascorbic acid, and 0.05 μg/ml sodium selenite. IL-15 (10 ng/ml; National Cancer Institute) as added for the culture and expansion of peripheral blood NK cells and iNK cells lacking IL-15RF. Flow cytometry data was acquired on an LSR II instrument (BD Biosciences).

Flow cytometric analysis

For flow cytometry, cell surface staining was performed with fluorochrome-conjugated antibodies against the surface epitopes CD56 (HNCD56; BioLegend), CD3 (OKT3; BioLegend), IL-15Rα (JM7A4; BioLegend), CD16 (3G8; BioLegend), CD19 (HIB19; BioLegend), CD20 (2H7; BioLegend), CD5 (UHCT2; BioLegend), CD107a (H4A3; BioLegend), NKp46 (9E2; BioLegend), NKp44 (P44-8; BioLegend), LFA-1 (m24; BioLegend), NKG2D (1D11; BioLegend), DNAM-1 (11A8; BioLegend), 2B4 (C1.7; BioLegend), NKp30 (P30-15; BioLegend), mouse CD45 (30-F11; BD Biosciences), and human CD45 (HI30; BD Biosciences). Intracellular cytokine staining was performed using a fluorochrome-conjugated antibody against IFN-γ (B27; BD Biosciences). Cells were surface stained with a dead cell stain (Thermo Fisher Scientific) in FACS buffer (PBS supplemented with 2% FBS and 2 mM EDTA) and fixed in 2%

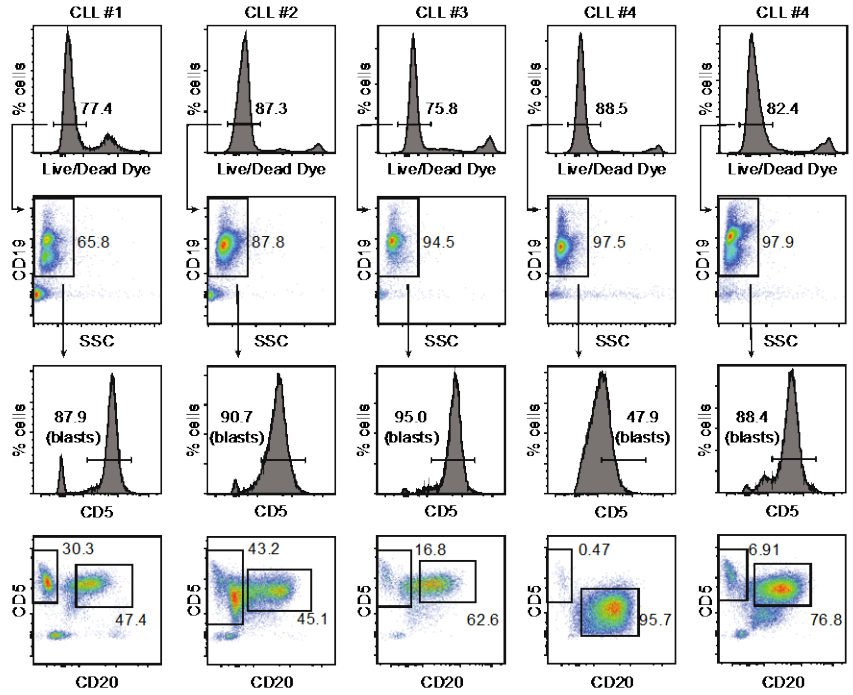
formaldehyde. Anti-CD19 CAR staining was performed using a biotin-conjugated goat anti-mouse IgG cross-adsorbed secondary antibody, biotin (Invitrogen) at a 1:200 dilution followed by PE Streptavidin (BioLegend) staining at a 1:100 dilution.

Supplemental Figure 1



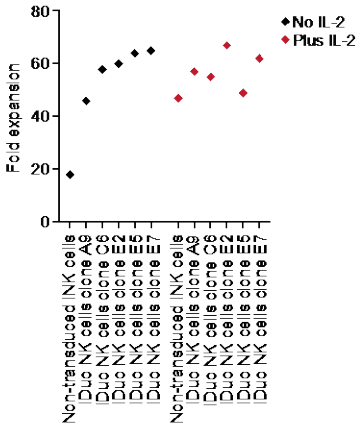
Supplemental Figure 1. Comparison of anti-CD19 CAR staining on iNK cells and PBNK cells. Non-transduced iNK cells (n = 3), iDuo NK cells (n = 5) and expanded PBNK cells (n =3) were stained for surface expression of the anti-CD19 CAR and analyzed by flow cytometry. Shown is cumulative data of the frequency of cells positive for anti-CD19 CAR expression.

Supplemental Figure 2



Supplemental Figure 2. Phenotypes of primary CLL cells used for in vitro function assays with iDuo NK cells. CLL samples from 5 patients were stained with live/dead cell dye and fluorescently labeled antibodies against CD19, CD20, and CD5. Shown is the flow cytometry gating strategy to determine the percentages of viable $CD19^+CD5^+$ leukemia blasts and the frequencies of $CD20^+$ populations.

Supplemental Figure 3



Supplemental Figure 3. iDuo NK cells expand in vitro in the absence of exogenous cytokines. Non-transduced iNK cells and iDuo NK cells were expanded for two weeks with or without IL-2 on K562 feeder cells expressing mbIL-21 and 4-1BBL. Shown are fold expansion values for non-transduced iNK cells and 5 different iDuo NK cell clones.