SUPPORTING INFORMATION

Supplemental Methods

RNAi knockdown of CD22ΔE12 expression. CD22ΔE12-siRNA has been designed to specifically target the Exon 11 and Exon 13 junction of the CD22ΔE12 mature mRNA produced by aberrant splicing of the CD22ΔE12 pre-mRNA with intronic mutations (Uckun et al., 2010). The specific CD22ΔE12 S-oligos were synthesized and purified with a reverse phase cartridge at Genelink (Hawthorne, NY). The CD22ΔE12-siRNA duplex (MW: 13,492) was custom synthesized by GeneLink as a single strand oligo and annealed after all deprotection and purification steps were completed. Phosphorothioate modification (denoted as "*") was implemented for the first and the last 3 nucleotides of the 5' and 3' ends, respectively, followed by addition of a 3'end TT overhang. The sequence of the CD22ΔE12 antisense S-oligo was 5′-G*A*G*GACUCUGCAUCUC*U*U*UTT-3′ and the sequence of the corresponding sense oligo in the siRNA duplex was 5′- A*A*A*GAGAUGCAGAGUC*C*U*CTT-3′. To document the specificity of the antisense S-oligos we used appropriate scrambled (scr) control S-oligos with the same modification custom-prepared by GeneLink (antisense 5'-A*C*G*UGACACGUUCGGA*G*A*ATT-3'; sense 5'-U*U*C*UCCGAACGUGUCA*C*G*UTT-3'). The transfection of ALL-1 cells and BPL xenograft cells with CD22ΔE12-siRNA and control/scrambled siRNA was accomplished using the Amaxa Cell Line Nucleofector Kit T (Cat. No. VCA-1002, Lonza, Cologne, Germany) and a Nucleofector II device according to a protocol specifically designed for the transfection of human B-lineage lymphoid cells (VCA-1002/C005) following the manufacturer's recommendations. Approximately 2-5x10^6 cells were transfected with 50 nM (unless otherwise indicated) CD22ΔE12-siRNA. Controls included untransfected cells and cells transfected with scr-siRNA. An aliquot of the cells was transferred to a 6-well plate with 1.5 mL pre-warmed RPMI culture medium then kept overnight at 37°C. The

transfection efficiency was confirmed to be >50% by transfecting cells with Green transfection indicator or siGlo^{TM} (Thermo Scientific Dharmacon, D-001630-01-05) and by counting the green fluorescent cells. RNAi knockdown of CD22ΔE12 expression was documented by RT-PCR analysis of RNA samples using the P7 (WT: 182 bp, Mutant: 63 bp) and P10 (213 bp, control) primer pairs after 48 h and Western blot analysis of whole cell lysates after 72 h, as described (Ma et al., 2012).

Preparation and characterization of the liposomal CD22ΔE12 siRNA formulation. In order to achieve RNAi in all leukemic cells in a targeted cell population *in vitro* and for *in vivo* RNAi experiments, we prepared a nanoscale liposomal formulation of the CD22ΔE12 siRNA duplex using a mixture of the cationic lipid 2,3-dioleoyloxypropyltrimethylammonium chloride (DOTAP; Cas Number: 132172-61-3) (3.9 mM) and the neutral lipid 1,2-dioleoyl-sn-glcero-3-phosphoethanolamine (DOPE; Cas Number: 4004-5-1) (3.9 mM) and standard methods (Uckun et al, 2013; Uckun, 2012). Both lipids were purchased from Avanti Polar Lipids (Alabaster, Alabama). siRNA-free (formulation 4B) and scr-siRNA loaded (formulation 4C) control liposomal formulations were also prepared for comparison. The generated liposomal CD22ΔE12-siRNA formulation had a radius of 132.5±4.7 nm, a positive surface charge with a Zeta potential of 54.8 mV in solution consistent with the use of positively charged DOTAP and contained 33±2 μM CD22ΔE12-siRNA. Cyanine dye Cy3 has a maximum excitation at 550 nm and emits maximally in the red end of the spectrum at 570 nm. We used 5' Cy3-labeled CD22ΔE12siRNA to prepare a liposomal CD22ΔE12-siRNA formulation (Radius: 168±4.4 nm) for cellular uptake and trafficking experiments using confocal imaging as well some of the PK experiments. The Cy3-labeled CD22ΔE12-siRNA duplex (reference #93598131) was custom-prepared by Integrated DNA Technologies, Inc. (Coralville, Iowa).

Size measurement by the dynamic light scattering (DLS) technique was performed on a DynaPro Titan Instrument (Wyatt Technology Corp., Santa Barbara, CA) at the USC Nano BioPhysics Laboratory. The Zeta potential measurements were carried out using a Zetasizer Ver 6.12 (Serial # MAL1044603) (Malvern Instruments, UK). The instrument was calibrated with standard polystyrene latex spheres and each sample was analyzed three times. We used the Quant-IT RiboGreen RNA assay (Invitrogen) and a Synergy HT Biotek fluorescence microplate reader to measure the siRNA content of the liposomal formulations in the presence and absence of 1 % TritonX100 for "burst-release" of their siRNA content. The presence of siRNA in the formulations was also confirmed in 2% agarose gels prepared using 0.5 µg/mL of ethidium bromide and 3% SDS that allows staining of the siRNA content with ethidium bromide. A 2% agarose gel was prepared using UltraPure Agarose and 0.5 µg/mL of ethidium bromide (Invitrogen, Grand Island, NY). The 7 x 10 cm gel was immersed in 1X TBE buffer and run in a horizontal electrophoresis system (Bio-Rad, Mini-Sub Cell GT cell) for 1 hour at 60 V. We used the 1kb Plus DNA ladder (Invitrogen) as size markers. Samples were prepared in RNase-Free water (Qiagen, Valencia, CA) and 6X DNA loading buffer (Bioland Scientific, Paramount, CA). After sample migration down the gel, gel images were taken with an UVP digital camera and UV light in an Epi Chemi II Darkroom using the LabWorks Analysis software (UVP, Upland, CA). An analysis of co-variance model was utilized to investigate the effect of a 6-month storage of the CD22ΔE12-siRNA LNF at 4^{o}C using siRNA content measurements determined using the Quant-IT RiboGreen RNA assay (Invitrogen). This statistical model described the overall effect of the 6 month storage (Treatment factor), regression of fluorescence emission at increasing log transformed concentrations of CD22ΔE12-siRNA LNF (Concentration factor as a co-variate), and an interaction term (*Treatment x Concentration) to determine if the slopes of the concentration dependence for control versus 6 month stored sample were significantly different. In serum stability tests, we used the CD22ΔE12-siRNA LNF prepared with the Cy3-labeled

CD22ΔE12siRNA duplex (Integrated DNA Technologies, Inc., Coralville, Iowa). The LNF was incubated for 1 h, 2 h, 4 h, or 24h with either mouse serum (N=3) or human serum (N=3). 10 µL of the LNF were mixed with 100 µL of the serum and then incubated at 37^{o}C for the indicated time periods. The samples were subsequently mixed with 100 µL PBS and transferred to a 96 well plate (NUNC Brand, ordered from ThermoScientific at Waltham, MA) for fluorescence measurements. Cy3-based fluorescence of the samples was measured with an excitation at 530 nm and fluorescence emission intensity at 590 nm using the Synergy HT Biotek fluorescence microplate reader and the Gen5 software (BioTek Instruments Inc., Winooski, VT). Time dependent degradation of the liposomal nanoparticles containing Cy3-labeled CD22ΔE12-siRNA in the presence of serum was monitored and a one-way analysis of variance (ANOVA) model was utilized to determine if there were significant differences across zero, 1, 2, 4 and 24 h. In addition, an analysis of co-variance model was utilized to investigate the effect of 1 h incubation with serum vs. PBS at room temperature for the concentration-dependent fluorescence emission from nanoparticles prepared using Cy3-labeled CD22ΔE12-siRNA. In this experiment, 2 µL of the LNF were mixed with 100 µL of pooled mouse serum and incubated for 1 h at room temperature. The statistical model described the overall effect of serum treatment (Treatment factor), regression of fluorescence emission at increasing concentrations of siRNA nano-particles (Concentration factor as a co-variate), and an interaction term (*Treatment x Concentration) to determine if the slopes of the concentration dependence for control versus 1 h serum treatment were significantly different.

Confocal laser scanning microscopy. Subcellular localization of Cy3-labeled CD22ΔE12-siRNA delivered in a liposomal formulation was examined by immunofluorescence and spinning disk confocal microscopy using previously described procedures for slide preparation and imaging (Uckun et al., 2010; Uckun et al., 2012). Cyanine dye Cy3 has a maximum excitation at

550 nm and emits maximally in the red end of the spectrum at 570 nm. The internalized Cy3-labeled CD22ΔE12-siRNA was detected and localized using the tetramethyl rhodamine (TRITC) filter sets since the excitation and emission spectra are very close to those of TRITC. Slides were imaged using the PerkinElmer Spinning Disc Confocal Microscope and the PerkinElmer UltraView ERS software (Shelton, CT) or the Volocity V5.4 imaging software (PerkinElmer, Shelton, CT). The coverslips were fixed with ice-cold MeOH at -20^{o}C for 10 min. Cells were stained with a mouse monoclonal anti-Tubulin antibody (Sigma Cat# T6199; Sigma-Aldrich, St.Louis, MO) for 1 h at room temperature. Cells were washed with PBS and incubated with green-fluorescent Alexa Fluor 488 dye-labeled goat anti-mouse IgG (secondary Ab) (Cat #: A11001, Invitrogen, Carlsbad, CA) for 1 h. Cells were then washed with PBS and counterstained with the blue fluorescent DNA-specific nuclear dye 4',6-diamidino-2-phenylindole (DAPI). The coverslips were inverted, mounted onto slides in Vectashield (Vector Labs, Burlinghame, CA) to prevent photobleaching, and sealed with nail varnish. UltraCruz Mounting Medium containing 1.5 μg/ml of DAPI was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

RT-PCR analysis of human leukemia cells for CD22ΔE12 mRNA expression Reverse transcription (RT) and polymerase chain reaction (PCR) were used to examine expression levels of wildtype CD22 and CD22ΔE12 transcripts in human leukemia cells, as previously described.[2] Total cellular RNA was extracted from ALL cells using the QIAamp RNA Blood Mini Kit (Catalog No. 52304) (Qiagen, Santa Clarita, CA, USA) following the manufacturer's instructions. Oligonucleotide primers were obtained from Integrated DNA Technologies (IDT, San Diego, CA, USA). The P7 primer set (5'-GCCAGAGCTTCTTTGTGAGG-3' and 5'-GGGAGGTCTCTGCATCTCTG-3') amplifies a 182-bp region of the CD22 cDNA extending from Exon 11 to Exon 13 and deletion of Exon 12 results in a smaller CD22ΔE12-specific PCR product of 63-bp size using this primer set (Ma et a., 2012). The P10 primer set was used as a

positive control to amplify a 213-bp region of the CD22 cDNA in both wildtype CD22 and CD22 with ΔE12 (5'-ATCCAGCTCCCTCCAGAAAT-3' and 5'-CTTCCCATGGTGACTCCACT-3') (Ma et al., 2012). QIAGEN One-Step RT-PCR Kit (Catalog No. 210212) (Qiagen, Santa Clarita, CA, USA) was used following manufacturer's instructions to amplify the target PCR product. The conditions were 1 cycle (30 min 50°C, 15 min 95°C); 35 cycles (45 sec 94°C, 1 min 60°C, 1 min 72°C). PCR products were separated on a 2% agarose gel containing ethidium bromide. Gel images were taken with an UVP digital camera and UV light in an Epi Chemi II Darkroom using the LabWorks Analysis software (UVP, Upland, CA).

Real-time quantitative RT-PCR for detection of CD22ΔE12 mRNA. All assays were conducted in 96-well plates with a 50 µL reaction/sample volume using the Applied Biosystems 7900HT Fast Real-Time PCR System housed in the CHLA Stem Cell Core Facility. In brief, total cellular RNA was extracted from the cells (~$5x10^6$ cells/sample) using the Qiagen RNeasy Mini Kit (Cat# 74104, Qiagen, Valencia, CA) according to the manufacturer's specifications and the RNA concentration in each sample was adjusted to 40 ng/µl using RNAse-free water. The qRT-PCR reaction was carried out using 5-µl (~200-ng) total RNA in a 50-µL reaction volume/sample with a 2-step thermal cycling program, which included (i) reverse transcription reaction at 48^{o}C x 30 min, (ii) initial Taq polymerase activation at 95^{o}C x 10 min followed by 40 cycles of denaturation at 95^{o}C x 30 sec, annealing/extension at 60^{o}C x 1 min with a forward primer (E11-F2) 5'-CAGCGGCCAGAGCTTCTT-3' (100 nM) and a reverse primer (E13-R2) 5'-GCGCTTGTGCAATGCTGAA -3'(100 nM). This primer set amplified a 113-bp fragment from Exon 11 to 13 of the human CD22ΔE12 cDNA. The amplified fragment was then specifically annealed to a pre-mixed oligo DNA probe (5'-TGTGAGGAATAAAAAGAGATGCAGAGTCC-3') conjugated with a 5'FAM reporter and a 3'BHQ Quencher on the junction region between Exon 11 and Exon 13. Quantification was based on the increased intensity of the FAM reporter fluorescence, which was measured and recorded using the sequence detection system of the

real-time qPCR system (SDS2.3) and expressed on the threshold cycle value Ct. To ensure the quality and the quantity of RNA for each patient sample and human leukemia cell lines, we also simultaneously ran a qRT-PCR reaction for β-actin with a forward primer 5'-GGACTTCGAGCAAGAGATGG-3', and a reverse primer 5'- AGCACTGTGTTGGCGTACAG-3'. This primer set amplifies a 234-bp region at the junction between Exon 4 and Exon 5 of the human beta actin gene. These qRT-PCR reactions employed the Power SYBR®Green One-Step PCR Master Mix Kit (Cat #4367659, Invitrogen, Carlsbad, CA) following the manufacturer's recommendations. Each reaction comprised 25 µl 2× SYBR Green PCR Master Mix, forward and reverse primer at 100 nM, ~200 ng total RNA template and sterile water up to a final volume of 50 µl. The quantification was based on the increased reporter fluorescence, which was measured and recorded using the sequence detection system. Results were expressed in terms of the threshold cycle value Ct; the cycle at which the change in fluorescence of the amplicon for the SYBR dye passes a significance threshold automatically determined by the real-time PCR system software (SDS2.3) based on the fluorescence of the fixed threshold no template control sample. In both PCR assays, the Ct value was identified as the intersection of the fluorescent intensity curve and threshold line and used as a relative measurement of the concentration of the CD22ΔE12 cDNA template in the PCR reaction. The Ct-values for the housekeeping gene β-actin were used as a reference to normalize the Ct value of each leukemic sample for comparative analysis.

Western blot analysis of CD22ΔE12 protein expression. Western blot analysis of whole cell lysates for CD22 expression was performed by immunoblotting using the ECL chemiluminescence detection system (Amersham Life Sciences), as described previously (Uckun et al., 2012; Uckun et al., 2013). For immunoblotting, the anti-CD22 mouse monoclonal antibody D-5 from Santa Cruz Biotechnology Inc. (sc-271579) recognizing the N-terminus of

human CD22 and a mouse monoclonal anti-α-Tubulin antibody (Sigma Cat# T6199; Sigma-Aldrich, St.Louis, MO) were used according to the manufacturers' recommendations. HRP-linked goat anti-mouse IgG (Cat# sc-2005, Santra Cruz Biotechnology (Santa Cruz, CA) served as a secondary antibody for these primary mouse antibodies.

Leukemia cells. We used 6 ALL xenograft clones that were derived from spleen specimens of xenografted NOD/SCID mice inoculated with leukemia cells from 3 newly diagnosed (including one patient with Ph^+ BPL) and 3 relapsed pediatric BPL patients. The secondary use of leukemic cells for subsequent laboratory studies did not meet the definition of human subject research per 45 CFR 46.102 (d and f) since it did not include identifiable private information, and it was approved by the IRB (CCI) at the Children's Hospital Los Angeles (CHLA) (CCI-10-00141; CCI Review Date: 7-27-2010; IRB Approval: 7-27-2010). Human Subject Assurance Number: FWA0001914. We also used the ALL-1 (Ph^+ adult BPL) and RAJI (Burkitt's leukemia/B-cell ALL) cell lines. RAJI is a radiation-resistant Burkitt's leukemia/B-ALL cell line. ALL-1 is a chemotherapy-resistant BCR-ABL^+ t(9; 22)/ Ph^+ pre-pre-B ALL cell line. Both cells lines are CD22ΔE12-positive and have homozygous intronic CD22 gene mutations at Rs10413526 (C>G) and Rs4805120 (A>G) that are associated with CD22ΔE12 (Uckun et al., 2010; Ma et al., 2012).

Colony assays. The effects of transfections with CD22ΔE12-siRNA vs. scr-siRNA as well as treatments with liposomal nanoformulations (LNF) of CD22ΔE12-siRNA vs. scr-siRNA or standard chemotherapy drugs on BPL xenograft clones and CD22ΔE12$^+$ leukemia cell lines ALL-1 and RAJI were examined using *in vitro* colony assays as previously described (Uckun et al., 1987). Cells were treated with the respective reagents for 24h at 37^{o}C. Immediately after transfection with siRNA or completion of the 24h treatments with LNF of siRNA, chemotherapy

drugs or combinations thereof, cells ($0.5x10^6$ cells/mL) were suspended in RPMI supplemented with 0.9% methylcellulose, 30% fetal calf serum, and 2 mM L-glutamine. In experiments with ALL-1 and RAJI cells, the LNF of CD22ΔE12-siRNA or scr-siRNA were added directly to the colony assay medium and kept in the cultures throughout the 7d assay period. We used the standard chemotherapy drugs commonly used in BPL therapy, including Vincristine (VCR, Lot #: X067139A; Manufacturer: Hospira Inc Lake Forest, IL), Doxorubicin (DOX, Adriamycin; Lot #: 2005342; Manufacturer: Ben Venue Labs Inc Bedford, OH), PEG-Asparaginase (PEG-ASP, Oncospar; Lot #: 0009A; Manufacturer: Enzon Pharmaceuticals Inc Bridgewater, NJ); as well as Dexamethasone (DEX, Cat. #: D9184-100MG; Sigma Saint Louis, MO) as controls. The chemotherapy drugs were obtained from the Pharmacy of the Children's Hospital Los Angeles. The following concentrations were used in treatments: 49.5 nM CD22ΔE12-siRNA LNF (117 μM lipid), 90.6 μM (a higher content of scr-siRNA was used to confirm the specific action of CD22ΔE12-siRNA with greater confidence) scr-siRNA LNF (117 μM lipid), 11 μM VCR, 10 IU PEG-ASP, 17 μM DOX, 25 μM DEX. Controls included untreated cells and in some experiments cells treated with siRNA-free LNF (0 nM siRNA, 117 μM lipid). Replicate 1 mL samples containing $0.5x10^6$ cells/sample were cultured in 35 mm Petri dishes for 7 d at 37^oC in a humidified 5% CO_2 atmosphere. On day 7, colonies containing ≥20 cells were counted using an inverted Nikon Eclipse TS100 microscope. For colony assays of primary leukemia cells, we substituted RPMI with alpha-MEM and FBS with calf bovine serum. Colony images were taken using a Digital Sight DS-2MBW Nikon camera.

Apoptosis assays. We used standard flow cytometric quantitative apoptosis assays (Uckun et al., 2011; Uckun et al., 2013) to evaluate the ability of CD22ΔE12-siRNA LNF to cause apoptosis in CD22ΔE12-positive leukemia cell lines RAJI and ALL-1. Exposure of phosphatidylserine on the outer leaflet of the target leukemia/lymphoma cell membrane was

measured using FITC-conjugated Annexin-V, the natural ligand of phosphatidylserine. Apoptotic ALL cells were labeled with PI and Annexin-V-FITC using the Annexin V-FITC apoptosis detection kit (Sigma, Catalog # APOAF-50TST) according to the manufacturer's recommendations. The anti-leukemic potency of various treatments was documented by comparing the numbers of residual viable lymphoid cells in the 5-day cultures of untreated control cells vs. cells exposed to the test treatments. Specifically, the numbers of viable cells were determined by first determining in each sample the number of lymphoid cells remaining in the P1 lymphoid window of the FSC/SSC light scatter plots using the formula: N (P1) = 10,000 (i.e. total number of cells analyzed) x % of cells in P1. Then, we determined the % of viable cells in the P1- window as the % of Annexin V-FITC$^-$PI$^-$ cells in the left lower quadrant of the Annexin V-FITC vs. PI fluorescence intensity dot plots for the P1 cell population. The number of viable lymphoid cells (N [viable]) was then determined using the formula: N (P1) x % Viable Cells in P1/100. The percent apoptosis (%A) was calculated using the formula: 100- N [viable] for Test sample] / N [viable for Control sample] x100. Confocal microscopy was used to evaluate the structural damage of leukemia/lymphoma cells caused by CD22ΔE12-siRNA LNF. UltraCruz Mounting Medium containing 1.5 μg/ml of 4′,6-diamidino-2-phenylindole (DAPI) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The mouse monoclonal anti-Tubulin antibody was purchased from Sigma. Green-fluorescent Alexa Fluor 488 dye-labeled secondary antibody Alexa Fluor 488 goat antimouse IgG (A-11001) for confocal microscopy was purchased from Invitrogen (Carlsbad, CA). During confocal imaging, slides were imaged using the PerkinElmer Spinning Disc Confocal Microscope and the PerkinElmer UltraView ERS software (Shelton, CT).

Pharmacokinetic (PK) Studies. The research was conducted according to Institutional Animal Care and Use Committee (IACUC) Protocols that were approved by the IACUC of CHLA. We first used the CD22ΔE12-siRNA LNF prepared using the Cy3-labeled CD22ΔE12-siRNA as a

PK formulation in PK experiments. Five hundred pmols (25 nmol/kg) of the PK formulation (diluted in PBS) were administered to NOD/SCID mice with xenografted human BPL i.v. via tail vein injections in a 100 µL total volume. Groups of 2-4 mice per time point were electively sacrificed at 0 min, 15 min, 30 min, 1 h, 2h, and 4 h after injection of the CD22ΔE12-siRNA LNF by CO_2 inhalation and blood was collected by cardiac puncture after euthanasia. The Cy3-based fluorescence of the plasma samples was measured with an excitation at 530 nm and fluorescence emission intensity at 590 nm using the Synergy HT Biotek fluorescence microplate reader and the Gen5 software (BioTek Instruments Inc., Winooski, VT). For validation employing a different assay platform, we used a quantitative RT-PCR technique and measured CD22ΔE12-specific siRNA levels in plasma samples obtained from healthy C57BL/6 mice at 15 min, 4h and 24h after administration of CD22ΔE12-siRNA LNF prepared with unlabeled CD22ΔE12-siRNA. Plasma samples were treated with 5% Triton-X100 lysis buffer for 10 min at 72^{o}C to burst release the siRNA content of the liposomes. Small RNA species (15-30 nt), including the CD22ΔE12-siRNA, were isolated using the *mir*Vana™ miRNA isolation Kit (Cat#Am1560, Life Technologies). These RNA samples (2 μL/each) were first mixed with a RT stem loop primer (https://www.lifetechnologies.com/ order/custom-genomic-products/tools/smallrna/) that was custom designed specifically to anneal the 3'-end of the sense strand of the target CD22ΔE12-siRNA duplex. (Invitrogen, Cat 4398987) in a total volume of 8 µL and then heat-denatured at 95^{o}C for 5 min prior to RT reactions. The RT reaction was carried out using TaqMan® MicroRNA Reverse Transcription Kit (Cat # 4366596, Life technologies). The qPCR reaction was performed using the TaqMan® Universal PCR Master Mix II, no UNG (Cat# 4440040, Life technologies) in a 20 μL reaction mixture including a) 10 μL TaqMan®Universal PCR Master Mix II, no UNG, b) 8 μL Nuclease-free water, c) 1 μL custom designed specific FAM probe and primer mixture (20x) (Cat# 4398987), d) 1.3μL RT reaction product. Assays were conducted using the Applied Biosystems 7900HT Fast Real-Time PCR

System housed in the CHLA Stem Cell Core Facility in 96-well plates using 2-step thermal cycling conditions, including an initial Taq polymerase activation at 95^{o}C x 10 min followed by 40 cycles of denaturation at 95^{o}C x 30 sec, and annealing/extension at 60^{o}C x 1 min. Quantification was based on the increased intensity of FAM reporter fluorescence, which was measured and recorded using the sequence detection system by the real-time qPCR system (SDS2.3) and expressed on the threshold cycle value Ct. The Ct value was identified as the intersection of the fluorescent intensity curve and threshold line and used as a relative measurement. A standard curve was generated using increasing concentrations of unformulated CD22ΔE12-siRNA duplex as a reference for calculation of the CD22ΔE12-siRNA levels in the tested plasma samples. The standard curve showed a narrow 95% confidence interval band around the line of best fit with a highly significant log-linear relationship was observed between Ct values and siRNA concentration; R^2 = 0.96, P<0.0001). Duplicate measurements of the plasma siRNA concentrations was obtained using the qRT-PCR method at 24h resulted in values of 11 and 11.4 nM and the Ct values for the 24 h time point (19.5 ± 0.1) were significantly above that measured at zero time point (39.2 ± 0.8; Linear Contrast, P < 0.0001). Furthermore, using the 3 parameter exponential fit for the pharmacokinetic parameters using all the plasma values for the qRT-PCR data (0.25, 4 and 24 h) revealed that the 95% confidence interval for the asymptotic parameter ranged from 4.8 to 14.6 nM. Pharmacokinetic modeling and pharmacokinetic parameter estimations were carried out using non-linear fitting of plasma concentrations-time profiles of the combined PK dataset (i.e., fluorescence-based plasma levels and qRT-PCR based plasma levels) to 2 equations (JMP 10 Software, SAS, Cary, NC; 2 parameter exponential, 3 parameter exponential). An appropriate pharmacokinetic model was chosen on the basis of lowest sum of weighted squared residuals, lowest Akaike's Information Criterion value, lowest SE of the fitted parameters, and dispersion of the residuals. The Y-intercept of plasma concentration-time profile determined the C_{max}. Definite analytic integrals

from zero to 24 h were derived to calculate the Area Under the Curve ($AUC_{0\text{-}24h}$) for the exponential form of the equation that best fitted the composite data points for CD22ΔE12-siRNA levels. To determine the Area Under the Moment Curve ($AUMC_{0\text{-}24h}$), definite integrals from zero to 24 h time- point were solved for the first moment exponential curves (CD22ΔE12-siRNA levels x Time versus Time). The Mean Residence time (MRT) was calculated by dividing the AUMC by AUC for each curve. On assumption that the plasma elimination rate is the sum of first order processes, the plasma half life was calculated using the relationship defined by Plasma half life = 0.693 x MRT.

Immunofluorescence Staining and Flow Cytometric Analysis of ALL Xenograft Cells. A panel of commercially available monoclonal antibodies was used for immunophenotyping of leukemic cells from spleen specimens of NOD/SCID mice xenografted with primary human ALL cells by standard immunofluorescent staining and multiparameter flow cytometry as previously reported.[3] The antibodies were obtained from BD Bosciences (SanJose, CA) and included: HLA/DR/DP/DQ FITC: cat. #: 555558, HLA-A,B,C Phycoerythrin: cat. #: 555553, CD10 (APC) BD catalog #: 340923, CD19 (APC-H7) clone: SJ25C1 BD catalog #: 560177, CD34 (Per CP-Cy5.5) BD catalog #: 347203, and CD45 (V450) clone: H130 BD catalog #: 560367. The labeled cells were analyzed on a LSR II flow cytometer (Becton Dickinson, Lakes, NJ). Controls included unstained cells as well as cells that were stained with a cocktail of control mouse IgG labeled with PE, FITC, APC, APC-H7, and PerCP-Cy5.5. The labeled cells were analyzed on a LSR II flow cytometer (Becton Dickinson, Lakes, NJ).

NOD/SCID mouse models of BPL. The anti-leukemic activity of CD22ΔE12-siRNA LNF was studied in a NOD/SCID mouse model of human BPL (Uckun et al., 2013). NOD/SCID mice (NOD.CB17-*Prkdc*^{scid}/J; 4-6 weeks of age at the time of purchase, female) were obtained from

the Jackson Laboratory (Sacramento, CA). The research was conducted according to Institutional Animal Care and Use Committee (IACUC) Protocol #280-12 that was approved by the IACUC of CHLA on 7-10-2012. In some experiments, NOD/SCID mice (6-8 week old, female, same age in all cohorts in each independent experiment) were inoculated intravenously (i.v) with patient-derived BPL xenograft cells via tail vein injection with a 27-gauge needle. Mice were then treated on days 1-3 with the CD22ΔE12-siRNA LNF (Low-dose regimen = 2.5 nmol/kg/d x 3 d, High-dose regimen = 25 nmols/kg/d x 3d), the control LNF of scr-siRNA (25 nmol/kg/d x 3 d, d1-3) or an empty control LNF administered intravenously via tail vein injections. Untreated mice challenged with the same number of leukemia cells were included as controls. Mice were monitored daily and electively euthanized by CO_2 asphyxia when any mouse developed morbidity. At the time of their elective sacrifice, mice were necropsied to confirm leukemia-associated splenomegaly. Spleens of mice were removed, measured, and cell suspensions were prepared for determination of mononuclear cell counts. Multiple organs were preserved in 10% neutral phosphate buffered formalin, and processed for histologic sectioning. For histopathologic studies, formalin fixed tissues were dehydrated and embedded in paraffin by routine methods. Glass slides with affixed 4-5 micron tissue sections were prepared and stained with Hemotoxylin and Eosin (H&E). Brain, liver, kidney, and bone marrow were examined for their leukemic involvement. Images were taken with an EVOS XL Core Light Microscope (AMG Bothel, WA) using 20X and 40X objectives. For the analysis of the NOD/SCID mouse xenograft data on the in vivo potency of CD22ΔE12-siRNA LNF, event-free survival (EFS) times were measured from the day of inoculation of xenograft cells to the day of death or killing for test mice treated with CD22ΔE12-siRNA LNF and untreated control mice or mice treated with control LNF. The probability of survival was determined and the event-free interval curves were generated using the Kaplan-Meier product limit method as in previous studies (Uckun et al., 2013). Pointwise confidence intervals (95% upper and lower intervals for control treatments)

were calculated to compare differences in survival between control and CD22ΔE12-siRNA-treated mice at specific time points, whereby survival proportion in CD22ΔE12-siRNA-treated mice that greater than the upper confidence interval for control mice were deemed significantly different. Log-rank tests were performed to compare differences in median survival estimates between all groups and pairwise comparison of pooled controls vs. CD22ΔE12-siRNA-treated mice. In other experiments, NOD/SCID mice were challenged with leukemia xenograft cells (cell density: $2x10^6$ cells/ml) isolated from spleens of xenografted mice challenged with an IV inoculum of primary leukemic cells from 2 relapsed pediatric BPL patients. These BPL xenograft cells were either left untreated or treated x 24 h with CD22ΔE12-siRNA LNF (660 nM), unformulated CD22ΔE12-siRNA (660 nM) or siRNA-free control LNF formulation (same lipid as in CD22ΔE12-siRNA LNF but no siRNA) and scr-siRNA LNF (1.2 μM scr-siRNA) at 37^{o}C and then reinjected (Pretreatment cell number of inoculum samples: $1x10^6$ cells/mouse) into NOD/SCID mice in order to evaluate the importance of CD22ΔE12 for the engraftment and leukemia-initiating ability of the human leukemic stem cell fractions of the ALL xenograft samples derived from BPL patients. Mice injected with xenograft cells were monitored and processed in the same fashion as described above for mice injected with xenograft cells and subjected to various systemic treatment protocols. All mice were electively euthanized by CO_2 asphyxia when any control mouse developed morbidity. For the analysis of the *in vitro* potency of CD22ΔE12-siRNA LNF against leukemic stem cells in xenograft specimens, two-tailed T-tests with correction for unequal variance (Microsoft, Excel) were performed comparing the mean spleen size and cellularity for the various treatments. In addition, 2-Tailed Fisher's Exact test was used to compare the extent of leukemic multi-organ involvement in mice challenged with control xenograft cells vs. xenograft cells treated with CD22ΔE12-siRNA LNF. P-values of less than 0.05 were deemed significant and not corrected for multiple comparisons as the false discovery rate was less than 5% for the planned limited number of comparisons that were

performed. Similar experiments were performed using CD22ΔE12-Tg mouse BPL cells. NOD/SCID mice were inoculated via. i.v. tail vein injections with $1x10^6$ cells of a CD22ΔE12-Tg BPL clone. Mice were then treated on days 1-3 with the CD22ΔE12-siRNA LNF (25 nmols/kg/d x 3d) or the control LNF of scr-siRNA (25 nmol/kg/d x 3 d, d1-3) administered intravenously via tail vein injections. Untreated mice challenged with the same number of leukemia cells were included as controls. Mice were monitored daily and electively euthanized by CO_2 asphyxia when any mouse developed morbidity. At the time of their elective sacrifice, mice were necropsied to confirm leukemia-associated splenomegaly. Spleens of mice were removed, measured, and cell suspensions were prepared for determination of mononuclear cell counts. The event-free survival (EFS) times were measured from the day of inoculation of CD22ΔE12-Tg BPL cells to the day of death or killing for test mice treated with CD22ΔE12-siRNA LNF and untreated control mice or mice treated with scr-siRNA-LNF. The probability of survival was determined and the event-free interval curves were generated using the Kaplan-Meier product limit method as in previous studies (Uckun et al., 2013). Log-rank tests were performed to compare differences in median survival estimates between groups. In other experiments, NOD/SCID mice were challenged with CD22ΔE12-Tg BPL cells ($1x10^6$ cells/mouse) that were either left untreated or treated x 24 h at 37^oC with 200 nM CD22ΔE12-siRNA LNF or 200 nM scr-siRNA-LNF. Mice were then monitored and processed in the same fashion as described above for mice injected with human BPL xenograft cells. Two-tailed T-tests with correction for unequal variance (Microsoft, Excel) were performed comparing the mean spleen size and cellularity for the various treatments.

CD22ΔE12 Transgenic Mice. We established a CD22ΔE12-transgenic mouse model using a novel transgenic expression vector designated 'pEμ-SR-CD22ΔE12' (Accession # LM652705). The pEμ-SR backbone plasmid containing the mouse IgH enhancer (Eμ) and the potent SRα

mammalian promoter along with SV40 polyA termination sequences was kindly provided by Dr. Jerry Adams, WEHI, Melbourne, Australia). Pronuclear microinjection of the human CD22ΔE12 transgene, founder generation, and genotyping analysis of tail DNA were performed under a service contract at the UC Davis Mouse Biology Program using standard methods. The 5.3-kb [promoter – CD22ΔE12 cDNA - poly A] fragment of the pEμ-SR-CD22ΔE12 transgene construct was microinjected into the pronuclei of freshly fertilized oocytes from C57BL/6J mice. Injected oocytes were transferred to day 0.5 postcoitus (dpc) pseudopregnant CD-1/Crl females to generate CD22ΔE12-Tg mice. Founders were mated to C57BL/6 J mice obtained from Jackson Laboratories; CD-1/Crl mice were obtained from Charles River laboratories. All mouse procedures were carried out in accordance with the Institutional Animal Care and Use Committee at the University of California, Davis (IACUC #15723). Tg founder mice were identified by PCR analysis of genomic tail DNA. Tg male mice were crossed to age-matched wildtype female C57BL/6 mice to produce transgenic lines and pups were screened for the presence of the transgene by PCR analysis of tail-extracted DNA. We also performed RT-PCR to confirm the expression of the CD22ΔE12 transgene transcript in splenocytes from pre-leukemic CD22ΔE12-Tg mice as well as BPL cells isolated from leukemic CD22ΔE12-Tg mice by using a 5' CD22ΔE12 E10 primer (E10-F) and a 3' vector backbone primer (CD22ΔE12Tg-R2) to amplify a segment of the transgene message spanning CD22ΔE12 exons 10, 11, 13, and 14. Total RNA was extracted from splenocytes of wild type and CD22ΔE12-Tg mice using the RNeasy Plus Mini Kit (Cat# 74134, Qiagen, CA). A semi-quantitative RT-PCR reaction was carried out where the first-strand cDNA was synthesized using SuperScript III (Cat#18080-051, Invitrogen CA) and, the second PCR reaction was performed using the Phusion High-Fidelity PCR Master Mix Kit (New England Biolabs) and the following thermal cycling conditions: initial denaturation 98^{o}C x 30 s, 35 cycles of denaturation 98^{o}C x 10 s, annealing 60^{o}C x 10s and extension 72 C x 30 s. The primers that specifically detect the 3' end of the CD22ΔE12

transgene were: Fwd: E10-F: ATCCTCATCCTGGCAATCTG and Rev: CD22ΔE12Tg-R2: 5'-CACCACCTTCTGATAGGCAGCC-3' (Estimated amplicon size: 436-bp). To ensure the quality and quantity of the 1st strand cDNA, a fragment of the mouse β-Actin cDNA was also amplified yielding a 206-bp product with primers Fwd 5'-CCTCTATGCCAACACAGTGC-3', Rev 5'-CCTGCTTGCTGATCCACATC-3'.

Construction of pEμ-SR-R3Y yeast cloning vector and pEμ-SR-CD22ΔE12 transgenic expression vector. We established a CD22ΔE12 transgenic mouse model by using a novel yeast-cloning vector ("pEμSR-R3Y") for preparation of the transgenic expression vector through a service contract with the UC Davis Mouse Biology Program. The plasmid constructs described herein were created using a yeast homologous recombination cloning method as follows: The pEμ-SR plasmid (kindly provided by Dr. Jerry Adams, WEHI, Melbourne, Australia) containing the mouse IgH enhancer (Eμ) and the potent SRα mammalian promoter along with SV40 polyA termination sequences was linearized within the plasmid backbone using the restriction enzyme *AatII*. The R3R4 yeast plasmid containing a yeast plasmid 2-micron origin of replication and TRP1 selection marker was digested with enzymes *AscI* and *NdeI* (**Fig.S3A**). The restriction digested plasmids contained sufficient ends of homologous sequence to facilitate recombination within yeast, resulting in the insertion of the yeast plasmid origin of replication / selectable marker cassette (4.4-kb R3R4 yeast fragment) into the pEμ-SR plasmid backbone. Tryptophan producing yeast colonies from the transformation were pooled, and then plasmid DNA was isolated from the yeast mixture and transformed into *E. coli*. The *E. coli* transformation was plated onto Ampicillin media, and 8 colonies were subsequently selected for restriction verification of the anticipated homologous recombination event (**Fig.S3B**). The human CD22ΔE12 cDNA was inserted into the pEμ-SR-R3Y vector by using yeast recombineering to generate the plasmid pEμ-SR-CD22ΔE12 (**Fig.S3C1**) as follows: The

CD22ΔE12 cDNA fragment was PCR amplified from a pBluescript cDNA clone using oligonucleotides CD22-SR-5'ARM (TCTAGGCCTGTACGGAAGTGTTACTTCTGCTCTAAAAGCTGCTGCAGCCAatgcatctcctcggcc cctggctcctgctcctggttctagaatact) and CD22-SR-3'ARM (TGATGAGACAGCACAACAACCAGCACGTTGCCCAGGAGTCGACAATCGATtcaatgtttgaggatc acatagtcca); the nucleotide sequences indicated in uppercase are homologous to the Eμ-SR promoter and SV40-polyA terminator, respectively; lowercase nucleotide sequences are specific for the CD22ΔE12 cDNA PCR amplification. The 2525-bp PCR fragment had terminal sequences directing homologous recombination of the mutant cDNA fragment into the pEmu-SR-R3Y vector between the Eμ-SRα promoter and SV40 poly A terminator. The pEμ-SR-R3Y vector was linearized between the promoter and terminator sequences using restriction enzyme *SacI*, and subsequently co-transformed into yeast with the mutant CD22ΔE12 cDNA PCR fragment. Recombinant plasmids were purified from yeast and moved to *E. coli* as described above. Fifteen *E. coli* colonies were screened by PCR using primers flanking the expected recombination junctions. The primer sequences are shown in **Table S1**. Eight PCR positive colonies were subsequently restriction verified for the mutant CD22ΔE12 cDNA insertion (**Fig.S3 C2&C3**). The inserts of the clones were sequenced with primers spanning the CD22ΔE12 cDNA and promoter / terminator recombination junctions to verify that the PCR amplification of the cDNA insert did not introduce any unwanted errors within the final transgenic construct. One clone that was verified to contain a cDNA insertion with no PCR introduced errors was selected as the final construct, designated 'pEμ-SR-CD22ΔE12' (Accession # LM652705). All restriction enzymes used for construct development and analyses were obtained from New England Biolabs (Ipswich, MA) and used per the manufacturer's instructions. PCR fragments used in cloning procedures were amplified using 'Sequalprep Taq polymerase', obtained from Invitrogen (Carlsbad, CA), as per the manufacturer's instructions. PCR screening

of *E. coli* colonies was performed using 'Chromataq' taq polymerase, obtained from Denville Scientific (Metuchen, NJ), according to the manufacturer's specifications. Nucleotide sequencing of plasmid constructs was outsourced to Davis Sequencing (Davis, CA). Nucleotide sequence analysis was performed using Sequencher 4.9 (Genecodes, Ann Arbor, MI). Chromatogram sequences were aligned in contigs with expected vector map sequences, and inspected visually for any unintended PCR introduced polymorphisms. Genbank formatted vector sequence maps were generated using Vector NTI (Invitrogen, Carlsbad, CA). Plasmids used for cloning procedures were all isolated from *E. coli* and / or Yeast using a plasmid mini-prep kit obtained from Qiagen (Hilden, Germany). Agarose gel purification of restriction-digested plasmids was perfomed using a Qiagen QIAquick gel extraction kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. Transformations of *E. coli* DH10B (Invitrogen, Carlsbad, CA) were all performed using a BioRad '*E. coli* Genepulser', according to the manufacturers instructions (Biorad, Hercules, CA). Transformations of yeast strain RFY-206 (Origene Technologies, Rockville, MD) were all performed using a Lithium Acetate PEG procedure adapted from the manufacturer's protocol. Yeast transformations were plated onto minimal media lacking tryptophan.

Purification of the transgene for pronuclear microinjection. The pEμ-SR-CD22ΔE12 plasmid was digested with *NotI* and *EcoRV* in order to release the 5.3-kb 'promoter – CD22ΔE12 cDNA - poly A' fragment from the vector backbone (**Fig.S3 D1**). The 5.3-kb *NotI* fragment was purified from a 0.8 % agarose gel (**Fig.S3 D1**). The purified fragment was verified by agarose gel electrophoresis and subsequently purified further on an Elutip column (Whatman) according to the manufacturer's specifications (**Fig.S3 D2**). Following ethanol purification of the fragment, the purified DNA was resuspended into microinjection buffer (5mM Tris-HCl, 0.1mM EDTA, pH 7.4, sterile filtered). Elutip purified DNA was run on a 0.8 %

agarose gel to verify its integrity. Prior to pronuclear microinjection, the purified DNA was diluted to a concentration of 1 ng/µl in microinjection buffer.

Details of generation and molecular characterization of CD22ΔE12-transgenic (Tg) mice (Fig.S4-Fig.S9). Pronuclear microinjection of the human CD22ΔE12 transgene, founder generation, and genotyping analysis of tail DNA were performed under a service contract at the UC Davis Mouse Biology Program using standard methods. The 5.3-kb [promoter – CD22ΔE12 cDNA - poly A] fragment of the pEµ-SR-CD22ΔE12 transgene construct was microinjected into the pronuclei of freshly fertilized oocytes from C57BL/6J mice. Injected oocytes were transferred to day 0.5 postcoitus (dpc) pseudopregnant CD-1/Crl females to generate CD22ΔE12-Tg mice. Founders were mated to C57BL/6 J mice obtained from Jackson Laboratories; CD-1/Crl mice were obtained from Charles River laboratories. All mouse procedures were carried out in accordance with the Institutional Animal Care and Use Committee at the University of California, Davis (IACUC #15723). Tg founder mice were identified by PCR analysis of genomic tail DNA. Tg male mice were crossed to age-matched wildtype female C57BL/6 mice to produce transgenic lines and pups were screened for the presence of the transgene by PCR analysis of tail-extracted DNA. DNA was extracted from approximately 3 mm tail snips using the Qiagen DNEasy blood and tissue kit according to the manufacturer's protocol. DNA was then amplified with a forward primer in the promoter (gaccctgcttgctcaactctacg) and a reverse primer in the cDNA (ggtcaccatctagggctctgtagg) for an expected transgenic amplicon of 391-bp. 25µl reactions included 0.4µM of each primer, 1X PCR buffer, 1.7mM MgCl2, 0.2mM each dNTPs, 1 Unit Amplitaq polymerase (Applied Biosystem), and 1.3M Betaine, 1.3% DMSO (Sigma) with approximately 50ng of template DNA. Thermal cycling included an initial denaturing at 94^{o}C for 5 min; 10 cycles of 94^{o}C for 15 s, 65^{o}C to 55^{o}C for 30 s (↓1^{o}C/cycle), 72^{o}C for 40 s; 30 cycles of 94^{o}C for 15 s, 55^{o}C for 30 s, 72^{o}C for 40 s; final extension of 72^{o}C for 5 min and maintained at

4°C. PCR reactions included a non-template control (NTC), negative wildtype control (B6), and approximately 10pg plasmid/2µg genomic DNA as a positive control. Amplicons were sized by high-resolution multicapillary electrophoresis using a QIAxcel® DNA high-resolution gel cartridge (Qiagen) on a QIAxcel system (Qiagen) and the results were analyzed using the BioCalculator analysis software according to published procedures (Thomas et al., 2010). PCR products from representative mice were also analyzed using standard gel electrophoresis on a 1.5% Agarose gel matrix at 120 volts for 90 minutes and analyzed using a Kodak Gel Logic 200 imaging system. Large transgenic inserts are generally known to be susceptible to intragenic and 3' deletions. Initial experiments performed using tail DNA and the Phusion High-Fidelity PCR kit gave inconsistent results in the genomic PCR reactions with a 3'-CD22ΔE12 primer set indicating that 3' deletions within the transgene have occurred in at least some of the CD22ΔE12 transgene inserts. Therefore, we performed additional PCR assays to confirm the presence of intact copies of the CD22ΔE12 transgene in the genome of the transgenic mice. Genomic DNA isolated from splenocytes of CD22ΔE12-Tg mice and control wildtype C57BL/6 mice using the Qiagen DNeasy Blood & Tissue kit (Catalog No. 69504, Qiagen, Valencia, CA) was subjected to PCR assays using the HotstarTaq Master Mix Kit (Qiagen, Cat No 203445) in a 50-µL reaction volume to confirm both the 5'- and 3'-integrity of the CD22ΔE12 transgene. We first performed a stepwise PCR amplification of specific segments of the transgene using a common 5' forward primer, which specifically anneals to the SRα mammalian promoter region of the transgene, and a set of reverse primers that specifically anneal to exons 3 to 9. These specific primer sets and the corresponding thermal cycling conditions for individual PCR amplification reactions are detailed in **Fig.S6**. In addition, two separate PCR reactions were performed to confirm that the 3' portion of the transgene encompassing CD22 exons 11, 13 and 14 was not separated from the remainder of the transgene due to intragenic deletions (**Fig.S8**). We first amplified the entire CD22ΔE12 cDNA insert with the following primer sets:

CD22ΔE12Tg-F: 5'-GACCCTGCTTGCTCAACTCTACG-3' and CD22ΔE12Tg-R1: 5' – CCTTCCGAGTGAGAGACACA-3' that span across the cDNA insert from the SRα promoter region to a region distal to the CD22ΔE12 cDNA insert and proximal to the SV40 poly A segment of the vector backbone using the Phusion High-Fidelity PCR kit (Cat#E0553L, New England Biolabs) in a 50 µL reaction volume with the thermal cycle conditions 98^{o}C x 30 s initial denaturation followed by 30 cycles of 98^{o}C x 10s denaturation, 60^{o}C x 30 s annealing and 72^{o}C x 90 s extension. Then, a second PCR reaction was performed using 3 µL of the 1st PCR reaction solution as a template with 2 sets of primers: 1) Primer Set 1: CD22ΔE12Tg-F 5'-GACCCTGCTTGCTCAACTCTACG-3', E3-R: 5'-GGTCACCATCTAGGGCTCTGTAGG-3' to confirm the 5' integrity of the transgene (Amplicon size: 391-bp encompassing a segment extending from the SRα promoter to CD22ΔE12 exon 3), and 2) Primer Set 2: E11-F: 5'-GCCAGAGCTTCTTTGTGAGG-3' and CD22ΔE12Tg-R2: 5'-CACCACCTTCTGATAGGCAGC-3' to confirm the 3' integrity of the transgene (Amplicon size: 348-bp; encompasses CD22ΔE12 exons 11, 13, 14) in a 50 µL reaction volume of HotstarTaq Master Mix (Qiagen, Cat No 203445). The PCR thermal cycle conditions are: 95^{o}C x 15 min initial activation followed by 35 cycles of 94^{o}C x 1 min denaturation, 58^{o}C x 1 min annealing and 72 C x 1 min. PCR products were separated on 1% agarose gels and sized using the 1-Kb Plus DNA ladder from Invitrogen (Cat. No.10787-018). The PCR product obtained using Primer Set 2 was cleaned using the Qiagen QIAquick PCR Purification Kit (Cat No. 28104) and sequenced at GENEWIZ, Inc (South Plainfield, NJ) using the corresponding reverse primer and the Applied Biosystems' dye-based (BigDye V3.1TM) DNA sequencing method. In this procedure, 3'-fluorescent-labeled dideoxynucleotides (dye terminators) were incorporated into DNA extension products (cycle sequencing). DNA sequencing was performed on an ABI 3730 DNA Analyzer using a long read protocol. The sequence obtained from each genomic PCR product was analyzed and aligned using the SeqMan II contiguous alignment software (LaserGene suite from DNASTAR, Inc) to

confirm the unique sequence of CD22ΔE12, characterized by deletion of CD22 Exon 12. The DNA sequence results were analyzed by BioEdit v7.2.0 (http://www.mbio.ncsu.edu/bioedit).

We also performed RT-PCR to confirm the expression of the CD22ΔE12 transgene transcript in splenocytes from pre-leukemic CD22ΔE12-Tg mice as well as BPL cells isolated from leukemic CD22ΔE12-Tg mice by using a 5' CD22ΔE12 E10 primer (E10-F) and a 3' vector backbone primer (CD22ΔE12Tg-R2) to amplify a segment of the transgene message spanning CD22ΔE12 exons 10, 11, 13, and 14. Total RNA was extracted from splenocytes of wild type and CD22ΔE12-Tg mice using the RNeasy Plus Mini Kit (Cat# 74134, Qiagen, CA) (**Fig.S8**). A semi-quantitatve RT-PCR reaction was carried out where the first-strand cDNA was synthesized using SuperScript III (Cat#18080-051, Invitrogen CA) and, the second PCR reaction was performed using the Phusion High-Fidelity PCR Master Mix Kit (New England Biolabs) and the following thermal cycling conditions: initial denaturation 98^{o}C x 30 s, 35 cycles of denaturation 98^{o}C x 10 s, annealing 60^{o}C x 10s and extension 72 C x 30 s. The primers that specifically detect the 3' end of CD22ΔE12 transgene were: Fwd: E10-F: ATCCTCATCCTGGCAATCTG and Rev: CD22ΔE12Tg-R2: 5'-CACCACCTTCTGATAGGCAGCC-3' (Estimated amplicon size: 436-bp). To ensure the quality and quantity of the 1st strand cDNA, a fragment of the mouse β-Actin cDNA was also amplified yielding a 206-bp product with primers Fwd 5'-CCTCTATGCCAACACAGTGC-3', Rev 5'-CCTGCTTGCTGATCCACATC-3'.

Chromosomal localization of the human CD22ΔE12-transgene in CD22ΔE12-Tg mouse BPL cells. Fluorescent *in situ* hybridization (FISH) and spectral karyotyping **(**SKY) analyses were performed at the Van Andel Institute (Grand Rapids, Michigan) under a service contract. Specifically, FISH was used first to visualize the transgene signal on metaphase spreads and to identify the approximate band location of integration. After taking at least 40 FISH images and

recording the X and Y vernier scale coordinates, SKY analysis was performed on the same slide to identify the chromosome(s) with transgene integration and determine the karyotype of the CD22ΔE12-Tg BPL cells.

Two separate FISH probes were used for FISH, including the pEmu-SR-delta-E12 transgenic vector described herein above (**Fig.S3**) and a pCL6-2AEGwo lentiviral vector containing human CD22 cDNA for exons 3-11 (**Fig.S7**). For the preparation of the latter, a cDNA fragment encoding the truncated human CD22 exons 3-11 (CD22ΔE12-14) was generated from full-length human CD22 cDNA (Clontech) by PCR amplification using the Phusion High Fidelity PCR Kit (New England Biolabs, Cat#E0553L) and the following primer sets: Fwd.: 5'-CTTGGTGCTAGCATGCATCTCCTCGGC-3'/Rev.: 5'CCGGTCTCGAGCCTTTTTATTCCTCAC-3'. PCR amplified 2213-bp CD22ΔE12-14 cDNA was ligated into the 8497-bp lentiviral vector pCL6-2AEGwo containing both a fragment encoding the 2A-like peptide APVKQTLNFDLLKLAGDVESNPGP and an in-frame eGFP fluorescent coding sequence downstream of a multiple cloning site to mark transduced cells (kindly provided by Dr. Zanxian Xia, School of Biological Science and Technology, Central South University, Changsha, Hunan 410078, China). The correct PCR fragments were ligated into the pCL6-2AEGwo lentiviral backbone through the NheI and XhoI restriction sites (underlined) using the Quick Ligase (New England Biolabs Cat# M2200L) following the manufacturer's instructions. Subcloning of the CD22ΔE12-14 cDNA fragments into the lentiviral backbone vector was confirmed through restriction enzyme digestion. The rescued plasmids containing the CD22ΔE12-14 cDNA insert were further characterized by sequencing the backbone-insert junctions on both 5' and 3' ends with the forward primer 5-CAGCCTGCTTCTCGCTTCTGTT-3' and the reverse primer 5'-CTCCTGCCAACTTGAGAAGGTC-3'. DNA sequencing was performed by GENEWIZ, Inc (South Plainfield, NJ) using Applied Biosystems BigDye version 3.1. In this procedure, 3'-fluorescent-labeled dideoxynucleotides (dye terminators) were incorporated into DNA extension

products (cycle sequencing). The reactions were then run on an Applied Biosystem 3730xl DNA Analyzer with the forward primer 5-CAGCCTGCTTCTCGCTTCTGTT-3' and the reverse primer 5'-CTCCTGCCAACTTGAGAAGGTC-3', that were derived from the pCL6-2AEGwo vector sequence 81-bp upstream and 75-bp downstream from the junctions. The resultant DNA sequences were analyzed by BioEdit v7.2.0 (http://www.mbio.ncsu.edu/bioedit). The plasmid with the correct insert was used to transform and propagate in chemically competent DH5α bacterial cells. Milligram quantities of plasmid DNA were extracted and purified from approximately 100 mL of the bacterial culture using an Invitrogen PureLink® HiPure Plasmid Filter Maxiprep Kit (Cat. No. K2100-17) following the manufacture's instructions. Plasmid DNA quality and quantity were determined using a Thermo Scientific NanoDrop 2000 spectrophotometer. Plasmid DNA from both vectors was labeled with Green-dUTP (Abbott Molecular Inc., Abbott Park, IL) by nick translation. CD22ΔE12-Tg leukemia cells obtained from markedly enlarged spleens of NOD/SCID mice that were inoculated with primary leukemia cells from CD22ΔE12-Tg mice were cultured for 4 hours, harvested according to standard cytogenetic procedures, and metaphase slides were made. The metaphase slides were pretreated with 2X saline/sodium citrate (SSC) at 37 °C for 10 min, 0.005% pepsin/0.01M HCl at 37 °C for 4 min, and 1X PBS for 5 min. Slides were then placed in 1% formaldehyde/1X PBS for 10 min at room temperature, washed with 1X PBS for 5 min, and dehydrated in an ethanol series (70%, 85%, and 95%) for 2 min each. Slides were denatured in 70% formamide/2X SSC at 74 °C for 3.5 min, washed in a cold ethanol series (70%, 85%, 95%) for 2 min each, and air-dried. The FISH probes were denatured at 75°C for 5 min and held at 37 °C for 10-30 min until 8 µl of the respective FISH probe were applied to each slide. The slides were coverslipped and hybridized overnight at 37 °C in the ThermoBrite hybridization system (Abbott Molecular Inc.). The post-hybridization wash was with 2X SSC at 73 °C for 2 min followed by a brief water rinse. Slides were air-dried and counterstained with VECTASHIELD mounting medium with 4'-6-diamidino-2-phenylindole (DAPI) (Vector Laboratories Inc., Burlingame, CA). Image acquisition

was performed at 1000x system magnification with a COOL-1300 SpectraCube camera (Applied Spectral Imaging-ASI, Vista, CA) mounted on an Olympus BX43 microscope. Images were analyzed using FISHView EXPO v6.0 software (ASI). After the FISH images were captured and the coordinates were recorded, the coverslip and anti-fade DAPI reagent were removed from the slides and spectral karyotyping (SKY) was preformed according to the standard supplied protocol (ASI). Seven microliters of denatured SkyPaint probe (ASI) were added to each denatured metaphase slide, after which a coverslip was applied, and the slides were incubated overnight at 37 °C in the ThermoBrite (Abbott Molecular Inc.). The next morning the slides were washed according to the supplied protocol (ASI), air-dried, and counterstained with anti-fade DAPI (Vector Laboratories Inc.). At least 20 metaphases were captured at 1000x system magnification and analyzed using the HiSKY v6.0 software (ASI).

PCR-based clonality assays. Genomic DNA of CD22ΔE12-Tg murine leukemia cells (~10^7 cells/sample) was extracted using the Qiagen DNeasy Blood & Tissue kit (Catalog No. 69504, Qiagen, Valencia, CA) following the manufacturer's specifications. Contaminating RNA in the samples was enzymatically eliminated through RNase treatment (0.02 ng/μL for 30 min at 37^{o}C). RNase-treated DNA was washed and recovered using a Qiagen QIAquick PCR Purification Kit (Catalog No 28106, Valencia, CA), and the final DNA concentration in the samples was adjusted to 50 ng/μL. PCR assays were performed using New England Biolabs Phusion high fidelity PCR kit (Cat No. E0553L, Ipswich, MA) with a 5' VH primer recognizing the mouse VH7183 family (5' TGCGAGGTCGACCTGGTGGAGTCTGGG 3') and a reverse primer (5' CTCCACCAGACCTCTCTAGACA 3') that is complementary to a region within the murine JH4 intronic gene segment. The PCR amplification was initiated by 98^{o}C x 30 s followed by 35 cycles of 98^{o}C x 10 s, 56^{o}C x 30 s and 72^{o}C x 30 s. The final PCR product was electrophoresed on a 1% agarose gel. For sequencing of the PCR product, the 930-bp specific amplicon was ligated to the pCR blunt-II-TOPO vector (K2800-2, Invitrogen CA) following the manufacturer's

recommendations. Two separate clones were sequenced at Genewiz (Berkeley, CA) using the M13 forward and reverse primers; Fwd.: 5'-CAGGAAACAGCTATGAC-3'/Rev.: 5'-GTAAAACGACGGCCAG-3'. A basic local alignment search tool (BLAST) search was performed by query of the NCBI Nucleotide BLASTN Mus musculus database.

Immunofluorescence staining and flow cytometric analysis of nucleated mouse spleen and bone marrow cells from CD22ΔE12-Tg mice. A panel of commercially available monoclonal antibodies was used for immunophenotyping of nucleated cells from spleen and bone marrow specimens of wildtype and transgenic mice by multicolor immunofluorescent staining and multiparameter flow cytometry as previously reported (Uckun et al., 2012; Uckun et al., 2013). The antibodies were obtained from BD Biosciences or eBioscience (Place) and included the following: (1) Several FITC labeled lineage-specific antibodies (LIN), including TER-119 (cat. #561032/BD; reacts with erythroid cells), HL3 (cat # 553801/BD; reacts with CD11c on myeloid cells/macrophages), R6-60.2 (cat# 553408/BD; reacts with IgM on B-cells), 17A2 (cat#555274/BD; reacts with CD3 on T-cells) and PK136 (cat# 553164/BD; reacts with NK1.1 on NK T-cells); (2) PerCP-Cy5.5 labeled RA3-6B2 (cat#552771/BD; reacts with CD45R/B220), (3) BV421 labeled SB/199 (Cat#562959/BD; reacts with CD127/IL7R), (4) Alexa-Fluor 700-labeled 1D3 (cat#557958/BD; reacts with CD19, (5) PE-Cy7 labeled D7 antibody (cat #558162/BD; reacts with Ly-6A/E), (6) APC labeled M1/69 (cat#562349/BD; reacts with CD24/HSA), (7) APC-H7 labeled 2B8 (Cat#560185/BD; reacts with CD117/KIT, and (8) BV421 labeled A2F10 antibody (Cat#562898/BD; reacts with CD135/FLT3). The labeled cells were analyzed on a LSR II flow cytometer (Becton Dickinson, Lakes, NJ).

Histopathology and frozen bone marrow section immunofluorescence staining. Mice were monitored daily and electively euthanized at the indicated time points by CO_2 asphyxia. At the time of their death or elective sacrifice, mice were necropsied to confirm leukemia-

associated marked splenomegaly. Spleens of mice were removed, sized, and cell suspensions were prepared for determination of mononuclear cell counts and immunophenotype. Brain, liver, kidney, lymph nodes, and bone marrow were subjected to standard histopathologic examinations to assess their leukemic involvement. Organs were preserved in 10% neutral phosphate buffered formalin, and processed for histologic sectioning. For histopathologic studies, formalin fixed tissues were dehydrated and embedded in paraffin by routine methods. Glass slides with affixed 4-5 micron tissue sections were prepared and stained with Hemotoxylin and Eosin (H&E). For in situ immunofluorescence staining of bone marrows, decalcified crystat-cut 7 µm-thick fresh frozen sections were prepared using published procedures (Lo Celso et al. 2007).

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Supplemental Table and Figures

Table S1. Primers used for PCR screening and sequence verification of the CD22ΔE12-cDNA insertion

Primer designation	Sequence
SR-cd22-jxn-F	cgtccgccgtctaggtaagt
SV40pA-cd22-jxn-R	ccttccgagtgagagacaca
cd22-cDNA-F1	gagccctagatggtgacctg
cd22-cDNA-R1	ttcccatcctttgtgctttc
cd22-cDNA-R2	gggagtgaccttgatctcca
cd22-cDNA-F2	cctcgctgaagaagcagaat
cd22-cDNA-R3	aggcgatggttgtgttgtc
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cd22-cDNA-R4	ctgctggctctgtgtcctct

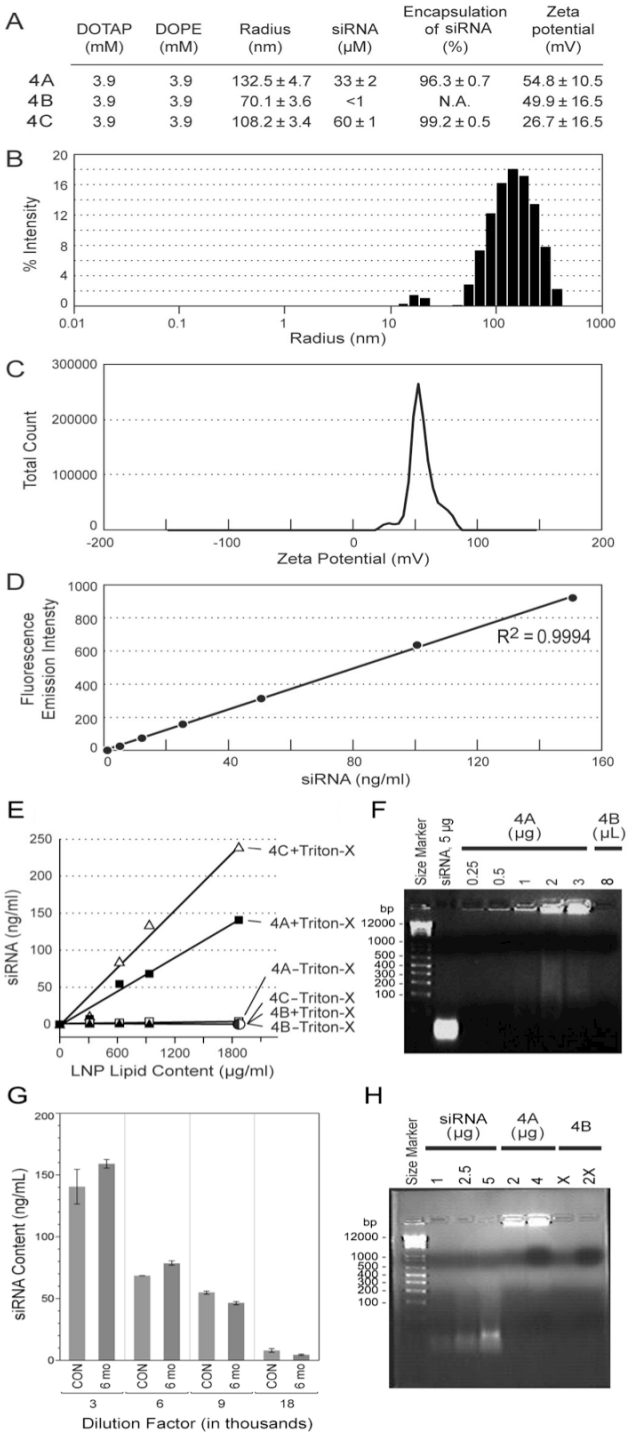


Figure S1. CD22ΔE12 siRNA Liposomal Nanoformulation (LNF) [A] Characteristics of the CD22ΔE12-siRNA LNF 4A and the control LNF 4B (no siRNA content) and scr-siRNA loaded 4C were determined using published procedures (Supporting Information). Depicted are the

average hydrodynamic size (radius), liposome composition and siRNA encapsulation efficiency. **[B]** The size of the NP was determined by DLS (Dynamic Light Scattering) on a DynaPro Titan Instrument. **[C]** Zeta potentials were determined using a Zetasizer Ver 6.12. **[D]:** We used the Quant-iT RiboGreen RNA assay (Invitrogen) and a Synergy HT Biotek fluorescence microplate reader to measure the siRNA content of the NP. Depicted is the standard curve obtained with increasing concentrations of unformulated siRNA. **[E]:** 96.3±0.7% (N=3) of the siRNA content was encapsulated within the liposomes as only 3.7±0.7% of the siRNA was detected in the formulation samples unless the samples were first treated with 1 % TritonX100 for "burst-release" of their siRNA content. **[F]:** The presence of siRNA in siRNA in increasing amounts (in µg based on the siRNA payload) of 4A was also confirmed in 2% agarose gels prepared using 0.5 µg/mL of ethidium bromide and 3% SDS that allows staining of the encapsulated siRNA (at top of the gel) with ethidium bromide. 4B: LNF with no siRNA load. siRNA (5.4 µg): unencapsulated siRNA control. We used the 1Kb Plus DNA ladder (Invitrogen) for sizing. The depicted gel image was taken with an UVP digital camera and UV light in an Epi Chemi II Darkroom using the LabWorks Analysis software (UVP, Upland, CA). **[G]** We measured the siRNA concentrations of the CD22ΔE12-siRNA LNF 4A at 1:3,000, 1:6:000, 1:9,000 and 1:18,000 dilutions in PBS as in E. Depicted are bar graphs comparing the initial siRNA content of 4A and the siRNA content of 4A after 6 months of storage at 4^{o}C. A highly significant log linear relationship existed between the siRNA concentration and the dilutions ($F_{1,16}$ = 365, P < 0.0001). No difference was found between 0 months and 6 months of storage at any of the dilutions ($F_{1, 16}$ = 0.60, P = 0.45). **[H]** The presence of siRNA in different amounts (in µg based on the siRNA payload) of 4A that has been in storage for 6 months was also confirmed in 2% agarose gels prepared using 0.5 µg/mL of ethidium bromide and 3% SDS that allows staining of the encapsulated siRNA (at top of the gel) with ethidium bromide. 4B: LNF with no siRNA load used at two different lipid concentrations corresponding to the lipid content of 4A at the tested concentrations. siRNA: unencapsulated siRNA control. We used the 1Kb Plus DNA ladder

(Invitrogen) for sizing. The depicted gel image was taken with an UVP digital camera and UV light in an Epi Chemi II Darkroom using the LabWorks Analysis software (UVP, Upland, CA).

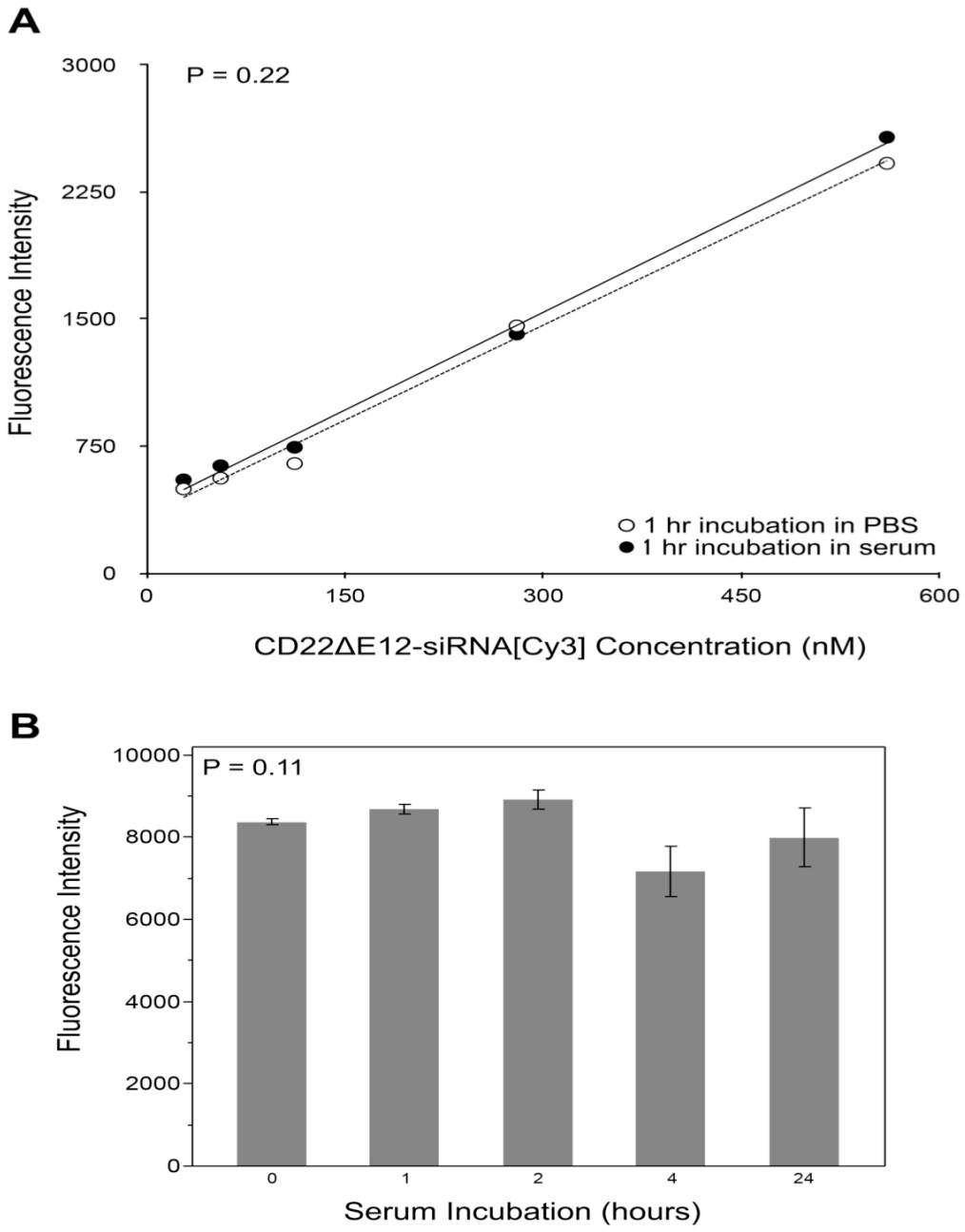


Figure S2. Serum stability of CD22ΔE12-siRNA LNF [A] Depicted are the concentration vs. fluorescence intensity plots for CD22ΔE12-siRNA LNF after 1 h incubation at room temperature with pooled mouse serum vs. PBS. The LNF was prepared using Cy3-labeled CD22ΔE12-siRNA. There was a significant linear relationship between concentration and fluorescence ($P<0.0001$) and there was no difference between the slopes of the concentration vs.

fluorescence lines ($F_{1, 6}$ = 0.2, P=0.7). Serum enzymes did not degrade the LNF or its siRNA payload to cause a drop in fluorescence intensity as a measure of the Cy3-labeled siRNA content after incubation with serum. **[B]** The CD22ΔE12-siRNA LNF in A was incubated for 1 h, 2 h, 4 h, or 24h at 37^{o}C with either mouse serum (N=3) or human serum (N=3). Depicted are bar graphs of the Cy3 fluorescence intensity initially (0 hours, N=3) and after 1-24 h (N=6 for each time point) of incubation with mouse or human serum using the combined dataset. No significant differences in Cy3-fluorescence as a measure of the Cy3 labeled siRNA payload of the LNF were observed across the different time points ($F_{4;22}$ = 2.15, P=0.1). Hence, serum enzymes did not cause degradation of the LNF or its siRNA content after 1-24 h incubation at 37^{o}C.

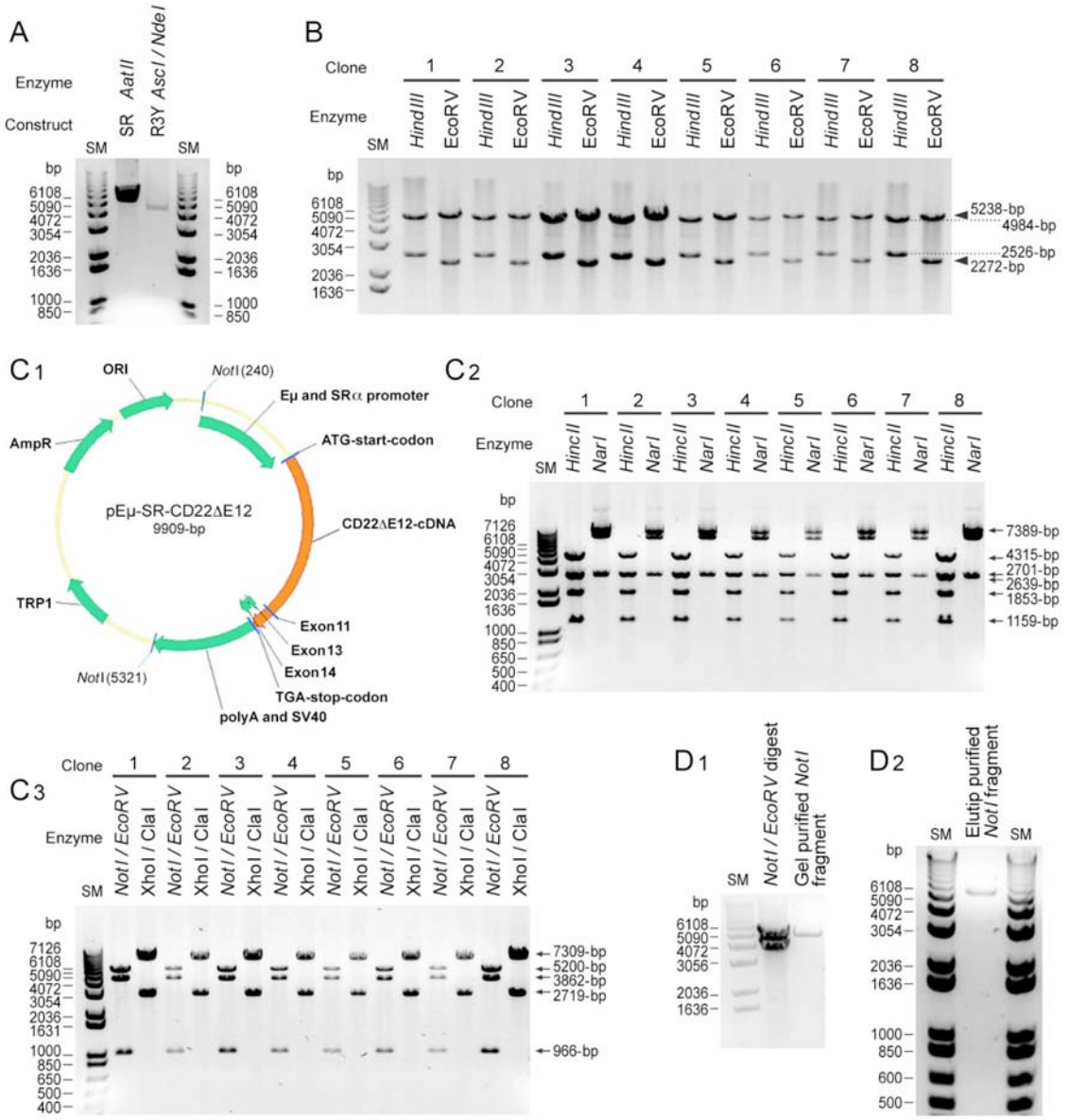


Figure S.3 Preparation of the pEμ-SR-CD22ΔE12 vector for microinjection. [A] Depicted in this gel image are the restriction enzyme digested fragments used to generate the construct pEμ-SR-R3Y. The construct annotated as SR is the original pEμ-SRα plasmid containing the mouse IgH enhancer Eμ and the potent SRα promoter (kindly provided by Dr. Jerry Adams (Walter & Eliza Hall Institute of Medical Research, Victoria, Australia), the construct annotated as R3Y is the R3R4 yeast plasmid vector. **[B]** Depicted in this gel image are the expected restriction patterns for the pEμ-SR-R3Y plasmid construct, indicating that the E. coli / yeast

shuttle vector was successfully constructed. **[C.1]** The human CD22ΔE12-cDNA was inserted into the pEμ-SR-R3Y vector by using a yeast homologous recombination cloning method to generate the Tg vector pEμ-SR-CD22ΔE12. Depicted is the Genbank formatted plasmid map.

[C2 & C3] Depicted are the gel images and expected band sizes for restriction enzyme verification of the pEμ-SR-CD22ΔE12 construct. The *NarI* enzyme only partially digested the construct as is evidenced by the larger size band in the gel; some *NarI* sites have been reported to demonstrate slower cleavage rates than others by the manufacturer (http://www.neb.com/nebecomm/products/productr0191.asp). **[D]** Depicted in D1 is the gel image for the pEmuSR-R3yeast-CD22 gel excised fragment (NotI – Eμ-SR promoter-human CD22ΔE12-cDNA – SV40 polyA – NotI) used for microinjection. The image shows the migration patterns of the gel purified *NotI* fragment (Lane 2) adjacent to the *NotI* / *EcoRV* digest used for the gel extraction (Lane 1). Depicted in D2 is the gel image of the *NotI* gel purified fragment following elutip purification.

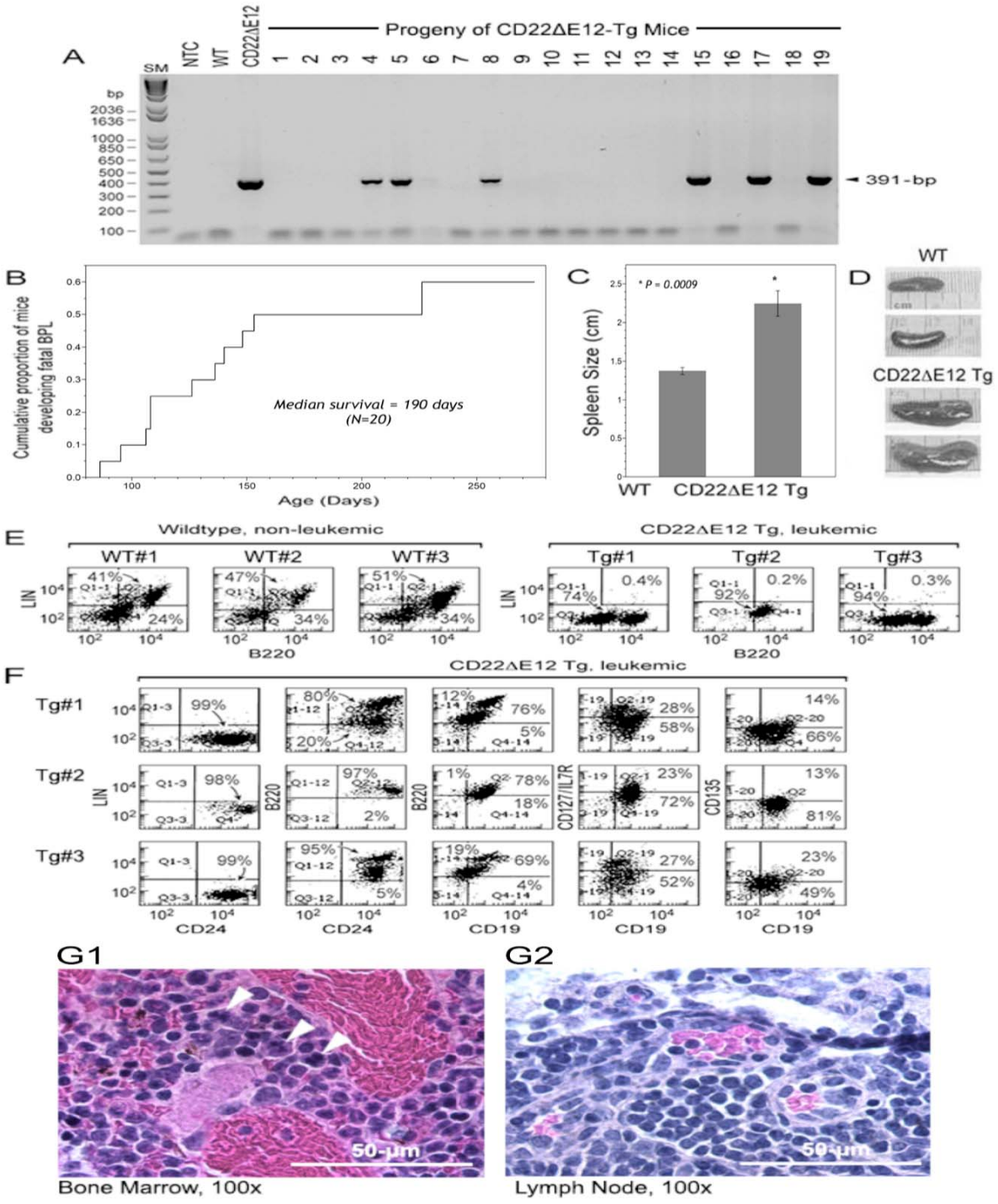


Figure S4. CD22ΔE12-Transgenic (Tg) Mice Develop Fatal B-precursor Leukemia (BPL).

[A] We established a CD22ΔE12-Tg mouse model with consistently high Tg transmission by using a novel Tg vector designated as pEμSR-R3Y. The human CD22ΔE12 cDNA was inserted

into the pEμ-SR-R3Y vector by using a yeast homologous recombination cloning method to generate the Tg vector pEμ-SR-CD22ΔE12 (see Figure S3). The 5.3-kb [promoter – CD22ΔE12 cDNA - poly A] fragment of this construct was microinjected into the pronuclei of freshly fertilized oocytes from C57BL/6J mice. Injected oocytes were transferred to day 0.5 postcoitus (dpc) pseudopregnant CD-1/Crl females to generate CD22**Δ**E12-Tg mice. Tg male founders were crossed to age-matched wildtype female C57BL/6 mice to produce Tg lines and pups were screened for the presence of the CD22ΔE12-transgene by PCR analysis of DNA extracted from approximately 3 mm tail snips using a Qiagen DNEasy blood and tissue kit. DNA was then amplified with a forward primer in the promoter (GACCCTGCTTGCTCAACTCTACG) and a reverse primer in the CD22ΔE12-cDNA (GGTCACCATCTAGGGCTCTGTAGG) for an expected Tg amplicon of 391-bp. Routine PCR reactions included a non-template control (NTC), negative wildtype control, and approximately 2 μg genomic DNA from a CD22ΔE12-Tg mouse as a positive control. Amplified PCR products were analyzed using agarose gel electrophoresis on a 1.5% agarose gel matrix. Depicted is a gel image from a representative genotyping experiment in which multiple Tg mice were identified among the progeny of CD22ΔE12-Tg founders. **[B]** Depicted is a graphic representation of the cumulative proportion of CD22ΔE12-Tg mice developing fatal BPL over time. **[C & D]** Tg mice with BPL have an enlarged spleen. **[E]** Leukemic cells of CD22ΔE12-Tg mice were B220/CD45R^{+} but negative for several mature lineage markers, including the surface IgM recognized by a lineage (LIN) antibody cocktail (TER-119 anti-erythroid cells, HL3/CD11c anti-myeloid cells/macrophages, R6-60.2 anti-IgM on B-cells, 17A2 anti-CD3 on T-cells, PK136 anti-NK1.1 on NK T-cells). **[F]** Depicted are two-color dot profiles from multicolor FACS analyses of leukemic spleen specimens from representative CD22ΔE12-Tg mice that developed fatal BPL. The composite immunophenotype was CD19^{+}B220^{+}CD24^{+}LIN^{-}CD127/IL7-R^{+} consistent with BPL. **[G]** The depicted histopathology images of H&E stained tissue slides illustrates that bone marrow (depicted in G1) and lymph

nodes (a representative lymph node depicted in G2) were diffusely infiltrated by leukemic cells with a high mitotic rate. Arrowheads in G1 point to mitotic cells in the perivascular leukemic cell clusters.

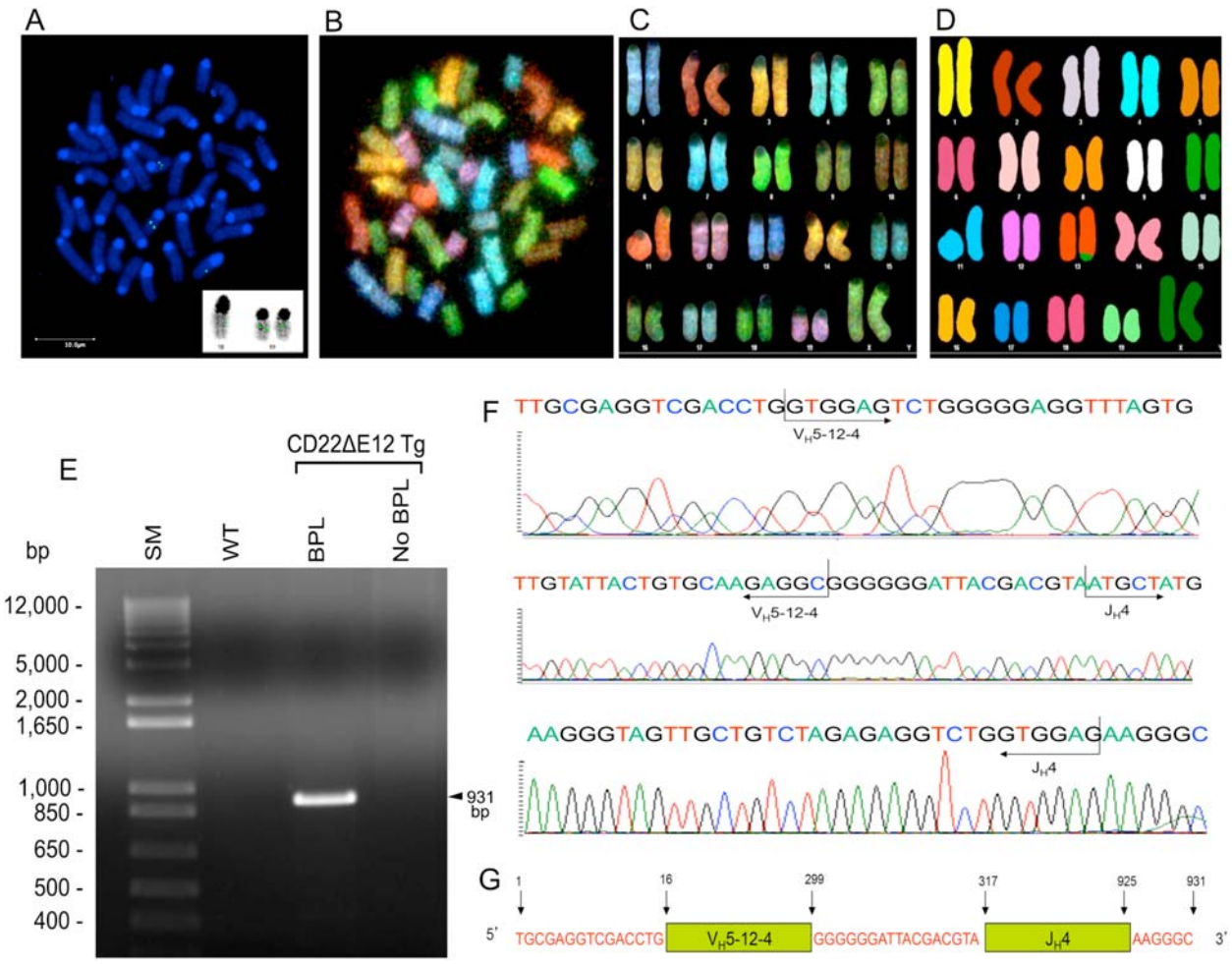
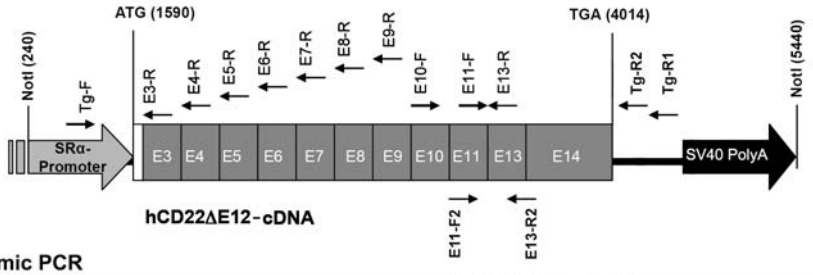


Figure S5. FISH, SKY and PCR Analyses of CD22ΔE12-Tg Leukemia Cells. [A] Depicted is a FISH image of metaphase chromosomes of CD22ΔE12-Tg mouse leukemia cells showing human CD22ΔE12 transgene integration on one Chr 18 homolog and on both Chr 19 homologs [FISH probe pEmu-SR-delta-E12 transgenic vector (green), DAPI (blue), and inset image (reverse DAPI)]. Image acquisition was performed at 1000x system magnification with a COOL-1300 SpectraCube camera (Applied Spectral Imaging-ASI, Vista, CA) mounted on an Olympus BX43 microscope. Images were analyzed using FISHView EXPO v6.0 software (ASI). After the FISH images were captured and the coordinates were recorded, the coverslip and anti-fade DAPI reagent were removed from the slides and spectral karyotyping (SKY) was preformed

according to the standard supplied protocol (ASI). **[B]** Depicted is a SKY image of the same metaphase spread shown in A. **[C]** SKY spectral color karyogram of metaphase B spread with a karyotype of 40,XX, Der(13)T(10D2;13D2). **[D]** SKY karyogram in classified colors. **[E]** Depicted is the image of a 1% agarose gel showing a single 931-bp PCR product obtained with a 5' V_H primer for the V_H7183 family and 3' J_H4 primer a V_H 7183 gene family using as templates the genomic DNA sample of CD22ΔE12-Tg leukemia cells. The presence of a single PCR product in CD22ΔE12-Tg BPL cells is consistent with a clonal origin of BPL in CD22ΔE12-Tg mice. This PCR product was not seen in genomic DNA samples of splenocytes from wildtype or non-leukemic CD22ΔE12-Tg mice. **[F]** The partial DNA sequence data for the PCR product depicted in E is shown as a chromatogram. **[G]** The IgH locus of their immunoglobulin genes was aberrantly rearranged with a noncanonical incomplete $V_H(D)J_H$ recombination. This was evidenced by a direct V_H to J_H joining with intervening intronic segments between the identified VH5-12-4 and JH4 sequences in the 930-bp specific amplicon instead of a joining of the V_H segments to rearranged DJ_H segments that is characteristic for the productive canonical immunoglobulin heavy chain gene rearrangement process. Schematic representation of the sequence data from F to illustrate the direct V_H to J_H joining with intervening intronic segments between the identified V_H5-12-4 and J_H4 sequences in the 931-bp specific amplicon.

A



B. Genomic PCR

	Primer Set	Primer sequence (5'→3')	Amplicon size (bp)	Thermal Protocol	PCR Kit
1	CD22ΔE12Tg-F E3-R	GACCCTGCTTGCTCAACTCTACG GGTCACCATCTAGGGCTCTGTAGG	391	A	HotStar Taq Master Mix
2	CD22ΔE12Tg-F E4 R	GACCCTGCTTGCTCAACTCTACG CTTCCCATGGTGACTCCACT	879	A	HotStar Taq Master Mix
3	CD22ΔE12Tg-F E5-R	GACCCTGCTTGCTCAACTCTACG TCATTGGAGACCTGACAGCA	1175	B	HotStar Taq Master Mix
4	CD22ΔE12Tg-F E6-R	GACCCTGCTTGCTCAACTCTACG TGTTTTCTGCCACACAGGAA	1435	B	HotStar Taq Master Mix
5	CD22ΔE12Tg-F E7-R	GACCCTGCTTGCTCAACTCTACG AGGCGATGGTTGTGTTGTC	1684	B	HotStar Taq Master Mix
6	CD22ΔE12Tg-F E8-R	GACCCTGCTTGCTCAACTCTACG TGGACGCTGTCTGTCCTATG	1981	B	HotStar Taq Master Mix
7	CD22ΔE12Tg-F E9-R	GACCCTGCTTGCTCAACTCTACG AGTAGACGGTGAGGGTGCTG	2269	B	HotStar Taq Master Mix
9	E11-F (P7-F) CD22ΔE12Tg-R2	GCCAGAGCTTCTTTGTGAGG CACCACCTTCTGATAGGCAGC	348	C	HotStar Taq Master Mix
10	CD22ΔE12Tg-F CD22ΔE12Tg-R1	GACCCTGCTTGCTCAACTCTACG CCTTCCGAGTGAGAGACACA	2932	D	Phusion High-Fidelity PCR

C. RT-PCR

E10-F CD22ΔE12Tg-R2	ATCCTCATCCTGGCAATCTG CACCACCTTCTGATAGGCAGC	436	D (See Text)	Phusion High-Fidelity PCR
mouse β-actin-F mouse β-actin-R	CCTCTATGCCAACACAGTGC CCTGCTTGCTGATCCACATC	206	D	Phusion High-Fidelity PCR

D. Thermal Protocols

Protocol No	Thermal Cycling Conditions
A	Initial Activation: 95 C x 15 min Denaturation: 94 C x 1 min Annealing: 56 C x 45 sec Extension: 72 C x 1 min Cycles: 35 Final extension 10 min
B	Initial Activation: 95 C x 15 min Denaturation: 94 C x 1 min Annealing: 60 C x 45 sec Extension: 72 C x 2 min Cycles: 35 Final extension 10 min
C	Initial Activation: 95 C x 15 min Denaturation: 94 C x 1 min Annealing: 58 C x 1 min Extension: 72 C x 1 min Cycles: 35 Final extension 10 min
D	Initial Denaturation: 98 C x 30 s Denaturation: 98 C x 10 s Annealing: 60 C x 30 s Extension: 72 C x 1 min 30 cycles Final extension 10 min

Figure S6. Primer Sets and Assay Conditions for Genomic PCR and RT-PCR.

[A] Schematic representation of the various PCR primers in relation to the specific CD22ΔE12 exons they allign to. [B] Genomic PCR Primers and amplicon sizes **[C]** RT-PCR primers and amplicon sizes **[D]** PCR protocols.

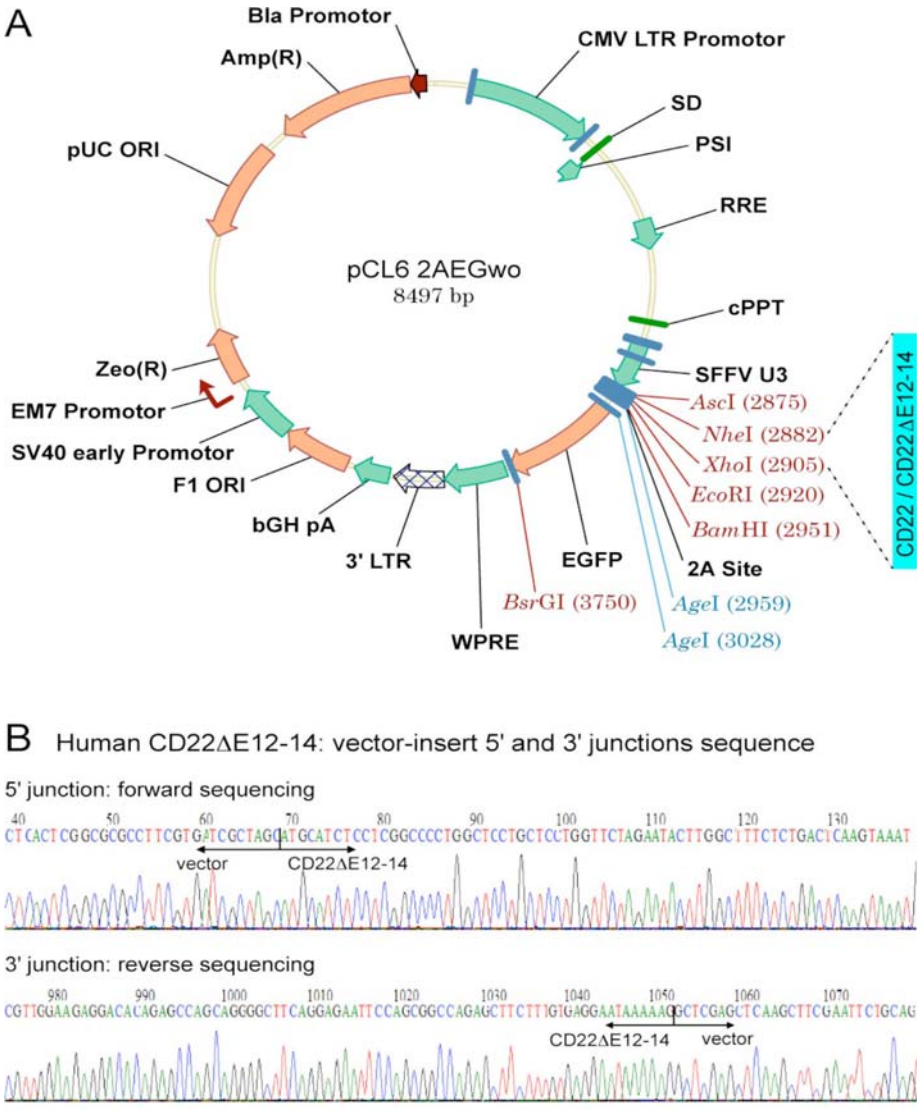


Figure S7. Lentiviral Construct with CD22ΔE12-14 Insert. [A] Depicted is a plasmid map of the lentiviral vector (pCL6-2AEGwo) that was used to clone human CD22ΔE12-14 to generate a FISH probe for the CD22ΔE12-transgene. This vector contains a 2A site (APVKQTLNFDLLKLAGDVESNPGP) and an in-frame eGFP-coding fragment downstream of a multiple cloning site. **[B]** Shown are the sequencing chromatograms depicting the sequence of the CD22ΔE12-14 insert and vector junctions at both the 5' and 3' ends of the inserts. The Sanger sequencing method was used by GENEWIZ, Inc. (South Plainfield, NJ) using Applied Biosystems BigDye version 3.1, and the reactions were run on an Applied

Biosystem's 3730xl DNA Analyzer with the forward primer 5-CAGCCTGCTTCTCGCTTCTGTT-3' and the reverse primer 5'-CTCCTGCCAACTTGAGAAGGTC-3'. The obtained DNA sequences were analyzed by BioEdit v7.2.0 (http://www.mbio.ncsu.edu/bioedit).

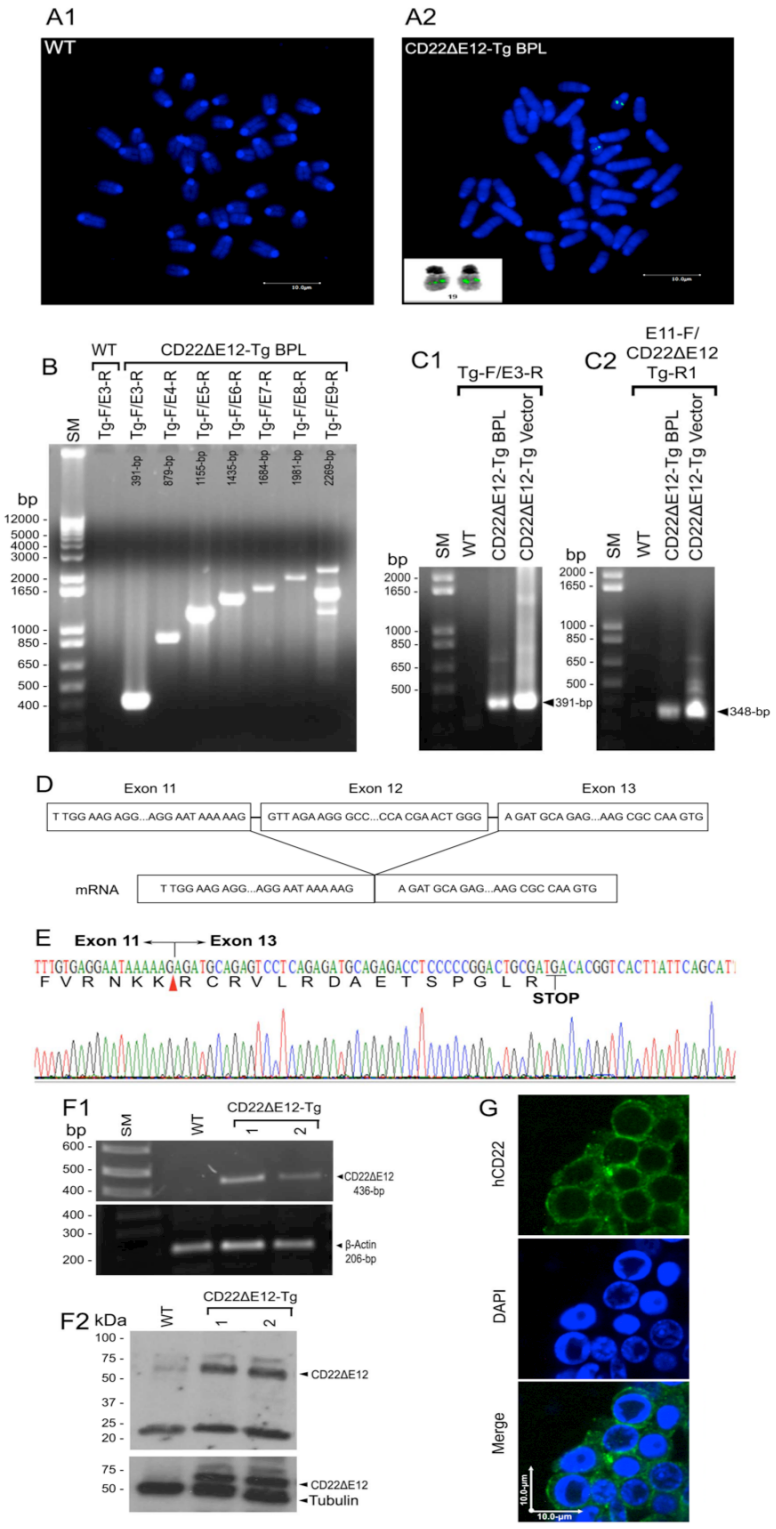


Figure S8. Characterization of CD22ΔE12-Tg Leukemia Cells. [A1 & A2] Depicted are FISH images of metaphase chromosomes of splenocytes from wildtype mice and CD22ΔE12-Tg

mouse leukemia cells showing human CD22ΔE12 transgene integration on both Chr 19 homologs in transgenic leukemia cells [FISH probe: pCL6-2AEGwo lentiviral vector containing human CD22 cDNA for exons 1-11 (green), DAPI (blue)]. The inset image depicts the Chr 19 homologs identified by reverse DAPI karyotyping. System magnification: 1,000X. **[B]** Depicted is the image of a 1% agarose gel showing a single 391-bp PCR product obtained with the 5' CD22ΔE12 primer set: Tg-F/E3-R using the genomic DNA sample of CD22ΔE12-Tg leukemia cells (but not the genomic DNA sample of wildtype splenocytes). The PCR products obtained using other primer sets were also separated on this gel and confirmed the integrity of the E3-E9 segment of the CD22ΔE12- transgenic insert. **[C.1]** Depicted is the image of a 1% agarose gel showing a single 391-bp PCR product obtained with the 5' CD22ΔE12 primer set: Tg-F/E3-R using the genomic DNA sample of CD22ΔE12-Tg leukemia cells or the pEmu-SR-delta-E12 transgenic vector (but not the genomic DNA sample of wildtype splenocytes). **[C2].** Depicted is the image of a 1% agarose gel showing a single 349-bp PCR product obtained with the 3' CD22ΔE12 primer set: E11-F/CD22ΔE12Tg-JtR using the genomic DNA sample of CD22ΔE12-Tg leukemia cells or the pEmu-SR-delta-E12 transgenic vector (but not the genomic DNA sample of wildtype splenocytes). **[D]** CD22ΔE12 cDNA is characterized by deletion of Exon 12. The bottom box depicts the cDNA sequence of CD22ΔE12 at the E11-E13 junction. **[E]** Depicted is the sequence chromatogram for the 348-bp amplicon from the 2-step genomic PCR reactions using Primer Set 2 CD22E11-F 5' GCCAGAGCTTCTTTGTGAGG 3' and CD22ΔE12TgJt-R 5' CACCACCTTCTGATAGGCAGCC 3' to confirm the 3' integrity of the transgene. This amplicon encompasses CD22ΔE12 exons 11, 13, 14 and it contains the correct sequence for the E11-E13 junction. The PCR product was sequenced at GENEWIZ, Inc using the corresponding reverse primer and the Applied Biosystems' BigDye V3.1TM) DNA sequencing method. **[F1]** RT-PCR was used to confirm the expression of the CD22ΔE12 transgene transcript in splenocytes from pre-leukemic CD22ΔE12-Tg mice as well as BPL cells

isolated from leukemic CD22ΔE12-Tg mice by using a 5' CD22ΔE12 E10 primer (E10-F) and a 3' vector backbone primer (CD22ΔE12Tg-R2) to amplify a segment of the transgene message spanning CD22ΔE12 exons 10, 11, 13, and 14. Depicted is the image of a 1% agarose gel showing a single 436-bp RT-PCR product obtained with a two-step RT-PCR reaction using the 5' CACCACCTTCTGATAGGCAGCC 3' Fwd/Rev primer set on the first strand cDNA as the template. To ensure the quality and quantity of the 1st strand cDNA, a fragment of the mouse β-Actin cDNA was also amplified yielding a 206-bp product with primers Fwd 5' CCTCTATGCCAACACAGTGC 3', Rev 5' CCTGCTTGCTGATCCACATC 3'. **[F2]** Depicted is a representative Western blot analysis of CD22ΔE12-Tg BPL cells and control splenocytes from wildtype mice using D-5 MoAb (Santa Cruz) recognizing the N-terminus of human CD22. Depicted in the lower portion of the panel is the Western blot of the samples with a combination of D5 and a mouse monoclonal anti-Tubulin antibody. **[G]** For *in situ* immunofluorescence staining of bone marrows from CD22ΔE12-Tg mice, decalcified crystat-cut 7 µm-thick fresh frozen sections were prepared. Cells were stained with D5 MoAb for 1 hour at room temperature, washed with PBS and subsequently incubated with green-fluorescent Alexa Fluor 488 dye-labeled goat anti-mouse IgG (secondary Ab) (Cat #: A11001, Invitrogen) for 1 h. Slides were then washed with PBS and counterstained with the blue fluorescent DNA-specific nuclear dye 4',6-diamidino-2-phenylindole (DAPI). Slides were imaged using the PerkinElmer Spinning Disc Confocal Microscope and the PerkinElmer UltraView ERS software (Shelton, CT) or the Volocity V5.4 imaging software (PerkinElmer, Shelton, CT). Depicted is a representative confocal image of a frozen bone marrow section stained with the D-5 MoAb (Santa Cruz) recognizing the N-terminus of human CD22, which shows the expression of the human CD22ΔE12 protein in the cytoplasm and on the surface of mouse BPL cells from CD22ΔE12-Tg mice.

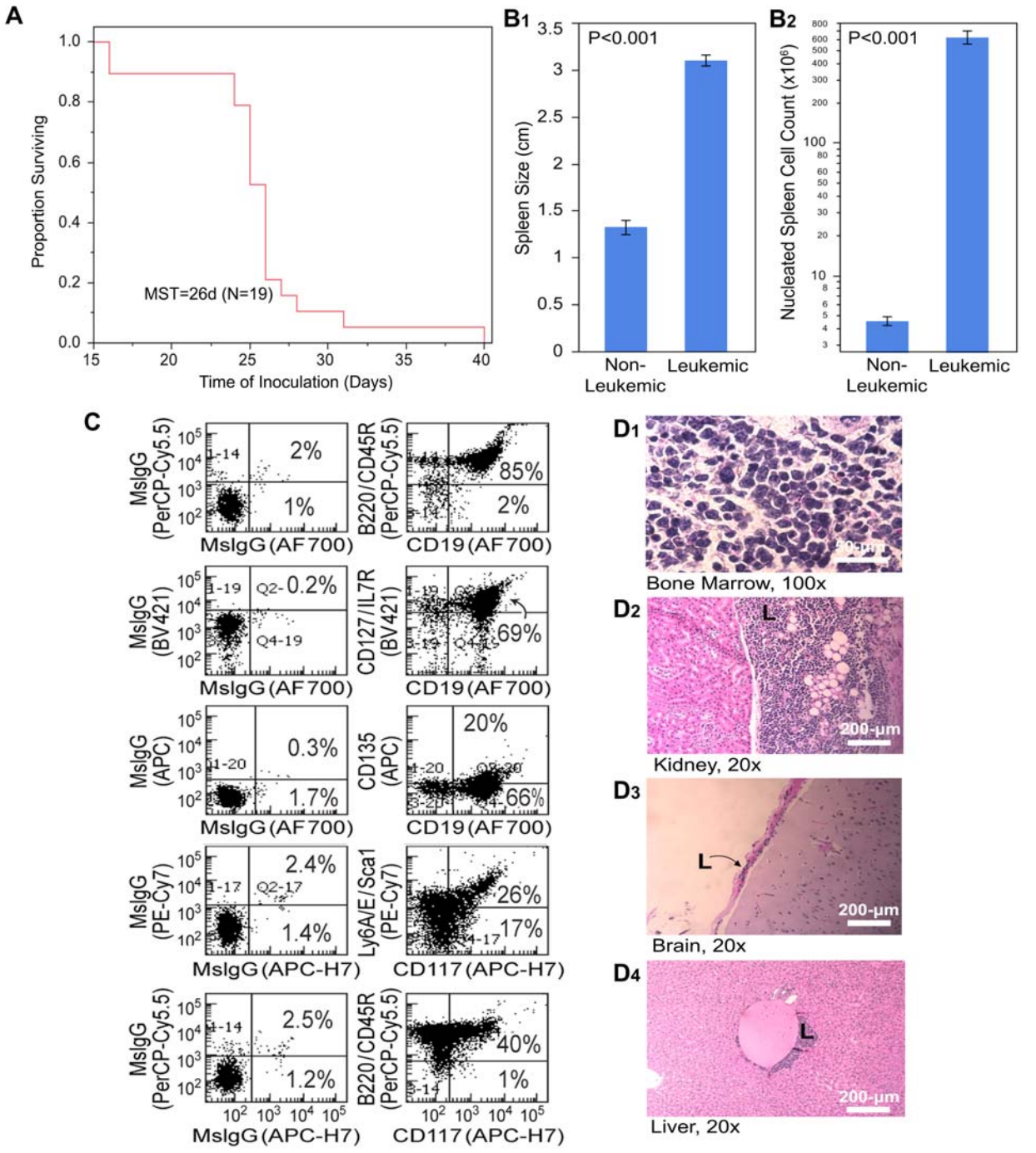
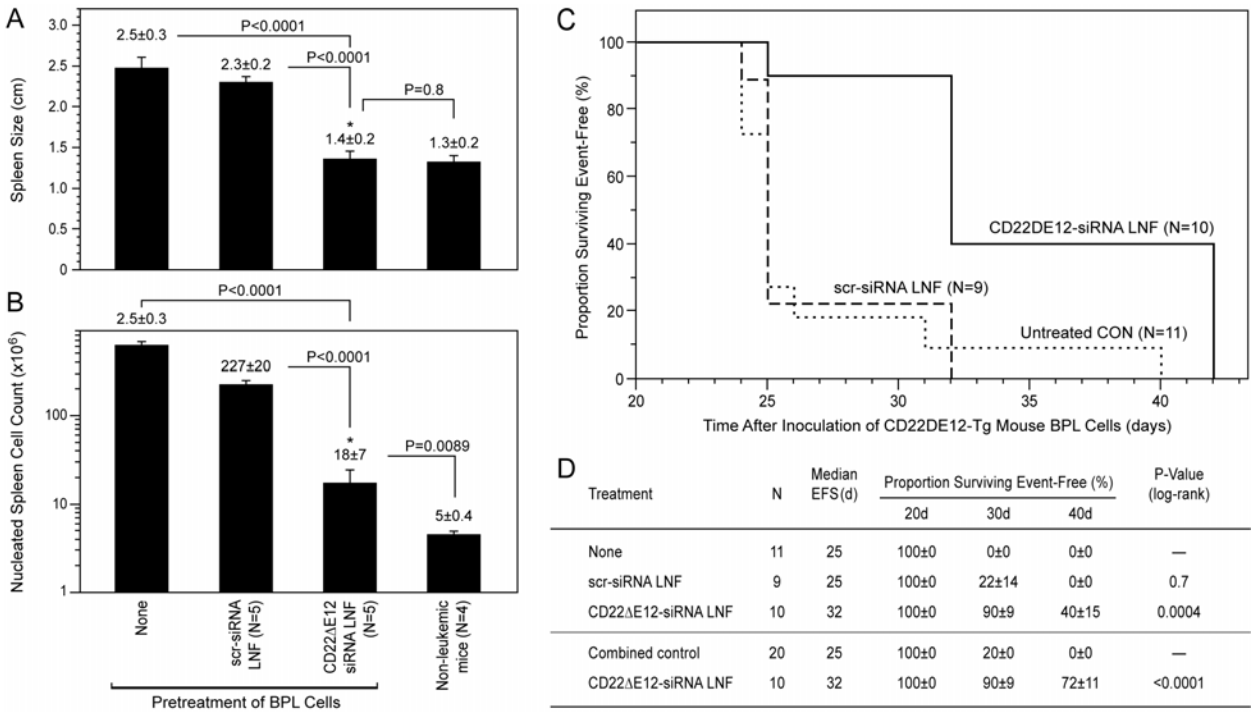


Figure S9. CD22ΔE12-Tg BPL cells cause fatal leukemia in NOD/SCID mice. [A] Depicted is the event-free survival (EFS) curve of NOD/SCID mice that were challenged with an iv inoculum of $2x10^6$ CD22ΔE12-Tg mouse BPL cells. All mice rapidly developed fatal leukemia with a median EFS of only 26 days. **[B]** Depicted are bar graphs of spleen size and nucleated spleen cell count for non-leukemic control NOD/SCID mice that were not inoculated with any

leukemia cells vs. leukemic test mice injected with CD22ΔE12-Tg BPL cells. Leukemic mice were characterized by a massive splenomegaly with very high nucleated cell counts reflecting their leukemia load. **[C]** Splenocytes from leukemic mice were immunophenotyped by multicolor flow cytometry. Depicted are two-color fluorescence intensity plots showing a composite immunophenotype ($CD19^{+}CD45R/B220^{+}CD127/IL7\text{-}R^{+}CD117/cKIT^{+}$) consistent with BPL. **[D]** Depicted are histopathology images of H&E stained sections of bone marrow, kidney, brain and liver confirming the leukemic infiltration of these organs.



D

Treatment	N	Median EFS(d)	Proportion Surviving Event-Free (%)			P-Value (log-rank)
			20d	30d	40d	
None	11	25	100±0	0±0	0±0	—
scr-siRNA LNF	9	25	100±0	22±14	0±0	0.7
CD22ΔE12-siRNA LNF	10	32	100±0	90±9	40±15	0.0004
Combined control	20	25	100±0	20±0	0±0	—
CD22ΔE12-siRNA LNF	10	32	100±0	90±9	72±11	<0.0001

Figure S10. Anti-leukemic activity of CD22ΔE12-siRNA LNF against murine BPL cells from CD22ΔE12-transgenic mice. [A&B] CD22ΔE12-siRNA loaded nanoscale liposomal formulation 4 A (200 nM x 24 h) abrogates the ability of in vivo clonogenic BPL xenograft cells to engraft and initiate leukemia in NOD/SCID mice. We observed massive splenomegaly in NOD/SCID mice that invariably developed disseminated leukemia after iv injection of $1x10^6$ CD22ΔE12-Tg BPL cells that were either untreated or treated for 24h at 37^oC with scr-siRNA LNF (200 nM scr-siRNA). Test mice were inoculated with CD22ΔE12-Tg BPL cells that were treated with the CD22ΔE12-siRNA LNF 4A (200 nM x 24h). The cumulative data on spleen size are shown in **A**. The cumulative data on the nucleated spleen cell count are shown in **B**. These findings provide direct experimental evidence that CD22ΔE12-directed RNAi in vivo initiated by a 24h in vitro exposure to the CD22ΔE12-siRNA formulation 4A severely damages the in vivo clonogenic fraction in BPL cells derived from CD22ΔE12-Tg mice and reduces their ability to engraft and initiate leukemia in NOD/SCID mice. **[C&D]** CD22ΔE12-siRNA LNF exhibits in vivo anti-leukemic activity against CD22ΔE12-transgenic BPL cells. Depicted in **C** are the EFS

curves of NOD/SCID mice that were inoculated i.v. with mouse BPL cells ($1x10^6$ cells/mouse) derived from a CD22ΔE12-Tg mouse that developed rapidly progressive fatal BPL. Twenty control mice were either left untreated (N=11) or treated with the liposomal control nanoformulation of scr-siRNA (2.5 nmol/kg/d x 3 d, d1-3) (N=9). Test mice were treated with the LNF of CD22ΔE12-siRNA LNF (2.5 nmol/kg/d x 3 d, d1-3, N=10). CD22ΔE12-siRNA LNF improved the median EFS. All mice developed fatal leukemia. At the time of death or elective sacrifice, the nucleated spleen counts (in millions) were 660±118 for untreated control mice (N=11), 588±78 for scr-siRNA LNF treated control mice (N=9) and 523±83 for CD22ΔE12-siRNA LNF treated test mice (N=10). By comparison, the nucleated spleen count of non-leukemic control NOD/SCID mice (N=4) was only 4.6±0.4. **[D]** shows the life table statistics for the EFS curves shown in [C].

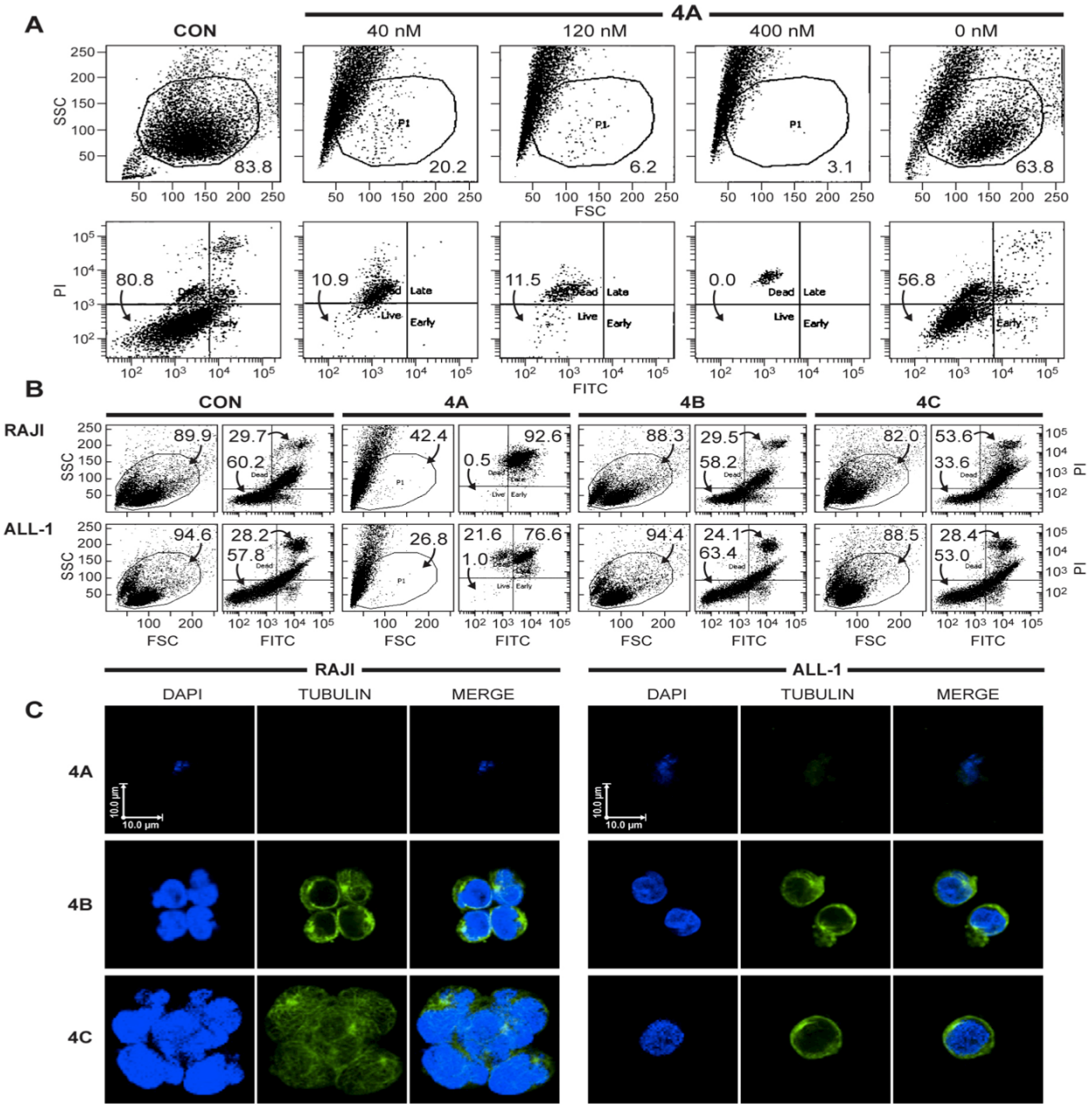


Figure S11. CD22ΔE12-siRNA LNF causes apoptotic destruction of CD22ΔE12^{+} human leukemia cells. [A & B] Cells were treated for 96 h at 37^{o}C with CD22ΔE12-siRNA LNF (4A, 40 nM – 400 nM in [A], 40 nM in [B]), siRNA-free control LNF (4B, 0 nM siRNA), or scr-siRNA LNF (4C, 40 nM). Controls included sham-treated cells (CON) cultured x 96 h. Panel A depicts the results for the ALL-1 cell line, whereas Panel B shows data for both ALL-1 and RAJI. Cells were analyzed for apoptosis using the standard quantitative flow cytometric apoptosis assay with the

Annexin V-FITC Apoptosis Detection Kit (Sigma, Catalog # APOAF-50TST). The labeled cells were analyzed on a LSR II flow cytometer. The anti-leukemic potency of CD22ΔE12-siRNA LNF is evidenced by the significantly lower percentages of Annexin V-FITC$^-$PI$^-$ live cells located in the left lower quadrant of the corresponding two-color fluorescence dot plots within the P1 lymphoid window as well as a marked shrinkage and altered SSC as well as decreasing numbers of remaining cells in the P1 lymphoid window in the corresponding FSC/SSC light scatter plot from the 10,000 cells analyzed. [C] Confocal images of RAJI and ALL-1 cells analyzed in [B] showing nuclear (Blue) destruction and loss of tubulin (Green) cytoskeleton after treatment with the CD22ΔE12-siRNA LNF, 4A.