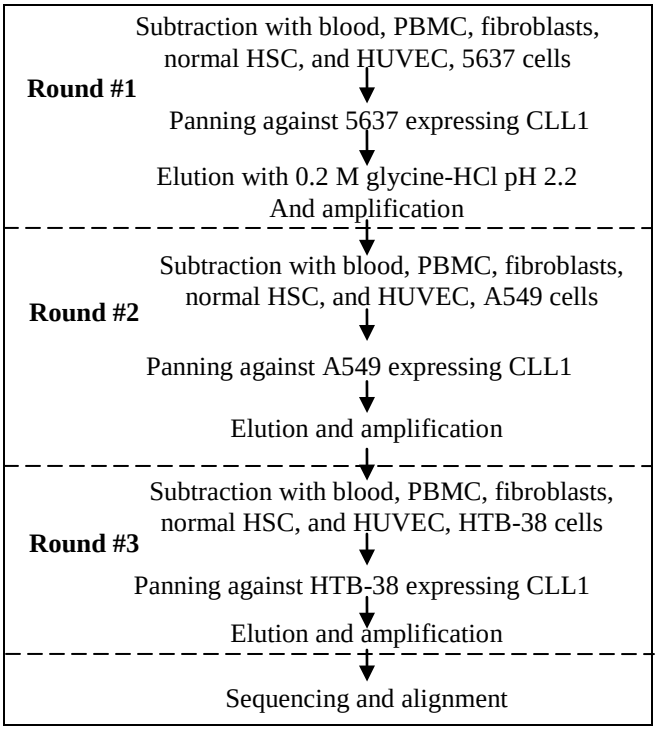
Supplement information 1: Panning strategy and confirmation of phage binding

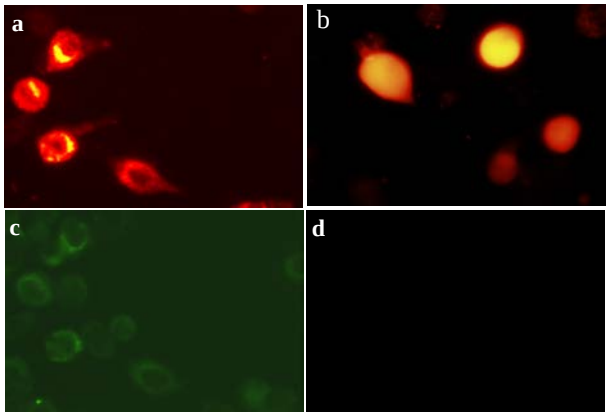
A. Panning strategy

To eliminate any phage/peptides that might bind to any of the confounding cells in vivo, the PhD C7C phage library was subtracted with whole blood, PBMC, fibroblasts, normal hematopoietic stem cells, human umbilical vascular endothelial cells and the corresponding cancer cell lines before each round of panning against the cancer cell line expressing CLL1. After three rounds of panning, 36 phage clones were sequenced and the targeting peptides were aligned and compared.



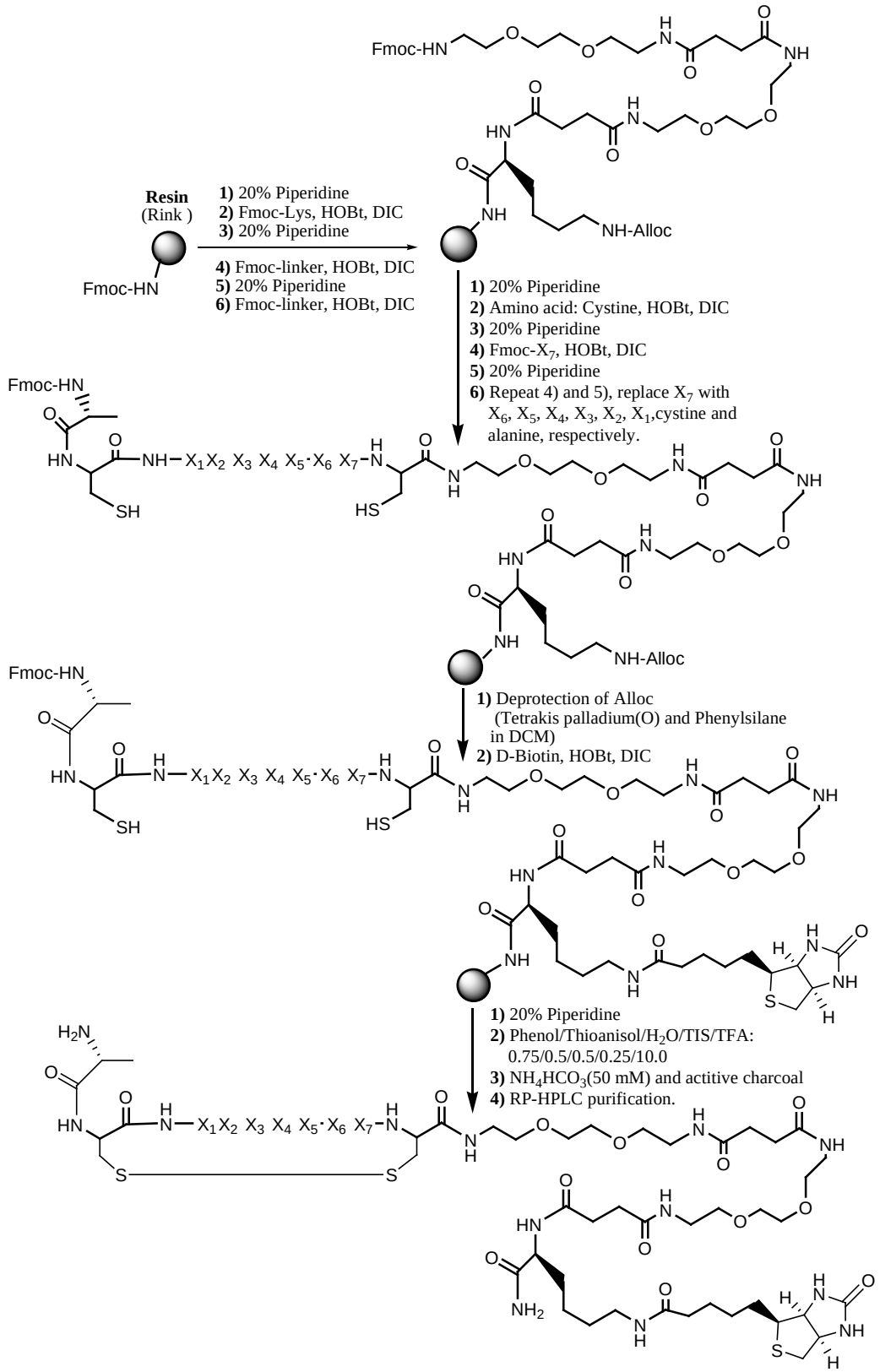
B. Confirmation of phage binding

To confirm that these ligands indeed bind to CLL1, A549 cells were transfected with a CCL1-RFP expression vector (Panel a) or RFP (Panel b, the control). Expression of CLL1-RFP or RFP could be visualized by the appearance of red fluorescence. A549 cells expressing CCL1-RFP or RFP alone were incubated with phage displaying the CDLRSAAVC peptide, and probed with anti-M13 monoclonal antibody-FITC conjugate. Phage expressing the CDLRSAAVC peptide were detected on the surface of A549 cells expressing CLL1-RFP (Panel c), but not on cells expressing RFP (Panel d). This peptide is named CLL1-L1 and was used for all subsequent experiments.

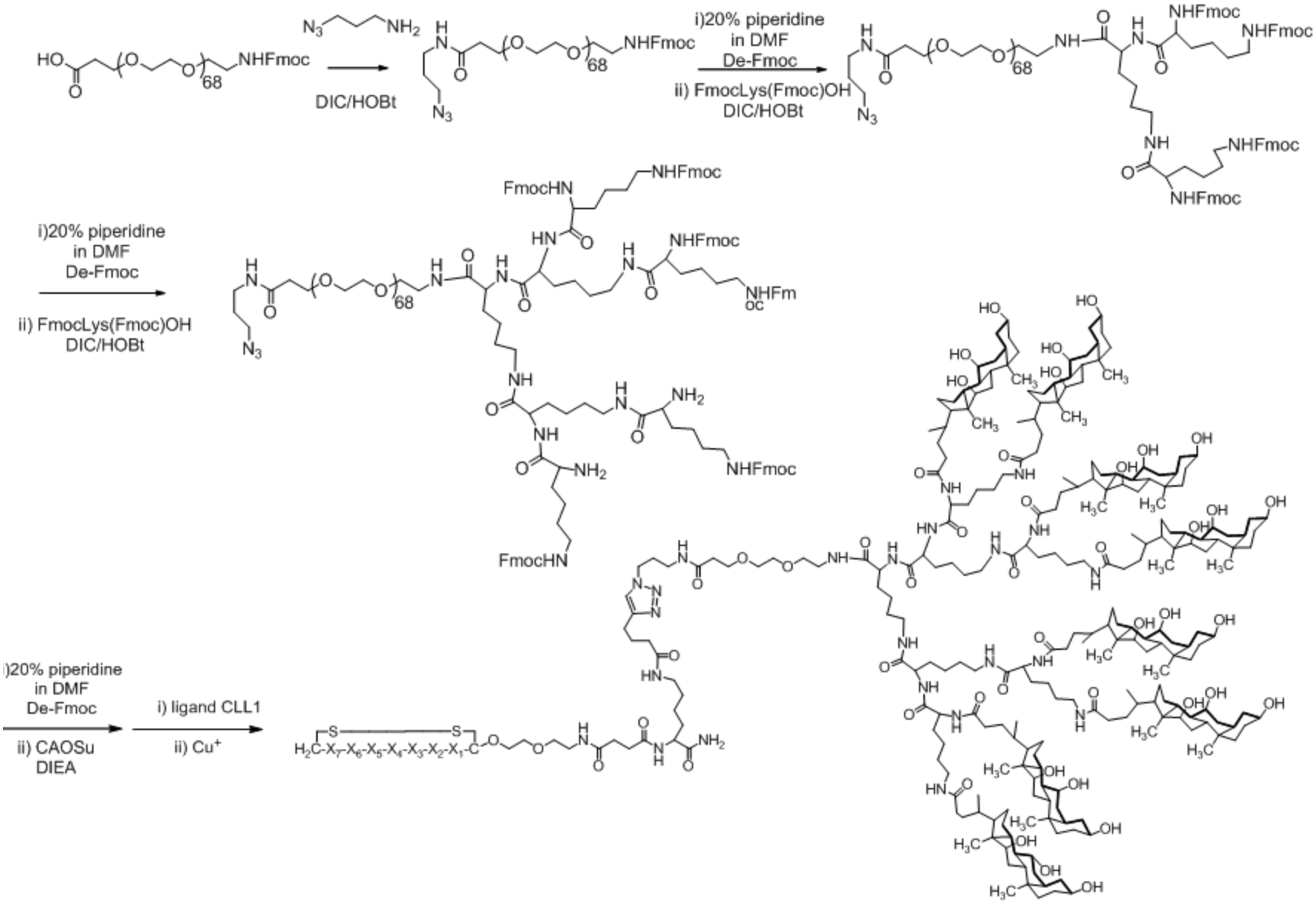


Supplement 2. Synthesis of biotinylated peptide

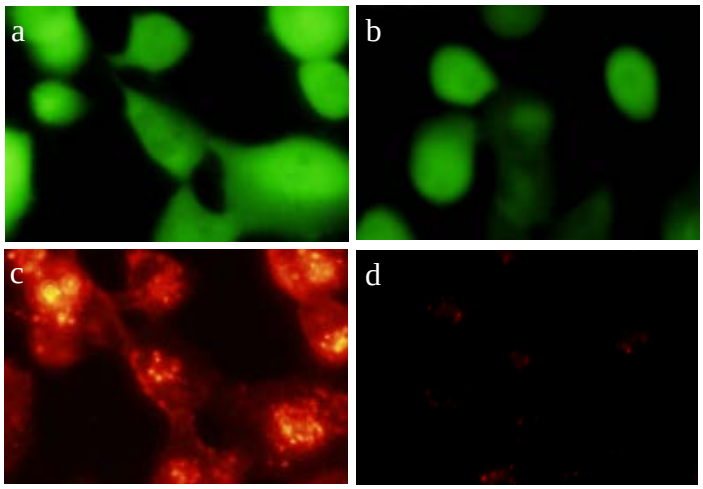
Rink amide resin beads (0.4g, loading 0.59 mmol/g) were swollen in DMF for 3 hours, and de-protected with 20% piperidine in DMF twice (5min, 10min). The beads were washed sequentially with DMF, MeOH, DCM, MeOH and DMF, three times each. A mixture of Fmoc-Lys(Alloc)-OH (3 equiv.), HOBt (3 equiv.) and DIC ((3 equiv.) in DMF was added to the beads. The coupling was carried out at room temperature. A Kaiser test was performed after coupling to confirm the complete coupling. The beads were washed with DMF five times, followed by Fmoc deprotection. The beads were then successively tethered with two Fmoc-linker-OH using HOBt and DIC coupling. After washing and Fmoc deprotection, the beads were subjected to stepwise assembly of L-Cys, L-Asp, L-Leu, L-Arg, L-Ser, L-Ala, L-Ala, L-Val, L-Cys. Finally, the Alloc of lysine was removed by using $Pd(PPh_3)_4$ (0.24 equiv.) and $PhSiH_3$ (20 equiv.) in DCM, with agitation for 30min, twice. After washing, a solution of biotin (3 equiv.), HOBt (3 equiv.), and DIC (3 equiv.) in DMF was added to the beads. The reaction was proceeded at room temperature until a Kaiser test was negative. After deprotection of the Fmoc group, the beads were washed sequentially with DMF, MeOH, and DCM, and then dried in vacuum. The dried beads were cleaved with a TFA-based cocktail containing 82.5%TFA, 5% phenol, 5% thioanisole, 5% H_2O and 2.5% triisopropylsilane (TIS), at room temperature for 3 hours. The cleavage solution was collected, concentrated, and precipitated from cold diethyl ether. After air drying, the crude peptide was diluted in 100 ml of 50 mM NH_4HCO_3 buffer, and 100 mg of activated charcoal was added into the solution, followed by agitation at room temperature until the Ellman test was negative. The charcoal was filtered off and the filtrate was lyophilized. The powder was finally purified by a preparative reverse-phase high performance liquid chromatography (RP-HPLC). The desired peptide was confirmed by ESI MS.


Resin
(Rink)
Fmoc-HN
1) 20% Piperidine
2) Fmoc-Lys, HOBt, DIC
3) 20% Piperidine
4) Fmoc-linker, HOBt, DIC
5) 20% Piperidine
6) Fmoc-linker, HOBt, DIC
NH-Alloc
1) 20% Piperidine
2) Amino acid: Cystine, HOBt, DIC
3) 20% Piperidine
4) Fmoc-X7, HOBt, DIC
5) 20% Piperidine
6) Repeat 4) and 5), replace X7 with X6, X5, X4, X3, X2, X1,cystine and alanine, respectively.
1) Deprotection of Alloc (Tetrakis palladium(O) and Phenylsilane in DCM)
2) D-Biotin, HOBt, DIC
1) 20% Piperidine
2) Phenol/Thioanisol/H2O/TIS/TFA: 0.75/0.5/0.5/0.25/10.0
3) NH4HCO3(50 mM) and actitive charcoal
4) RP-HPLC purification.

Supplement 3. Synthesis of nanoparticle with CLL1-L1 ligand



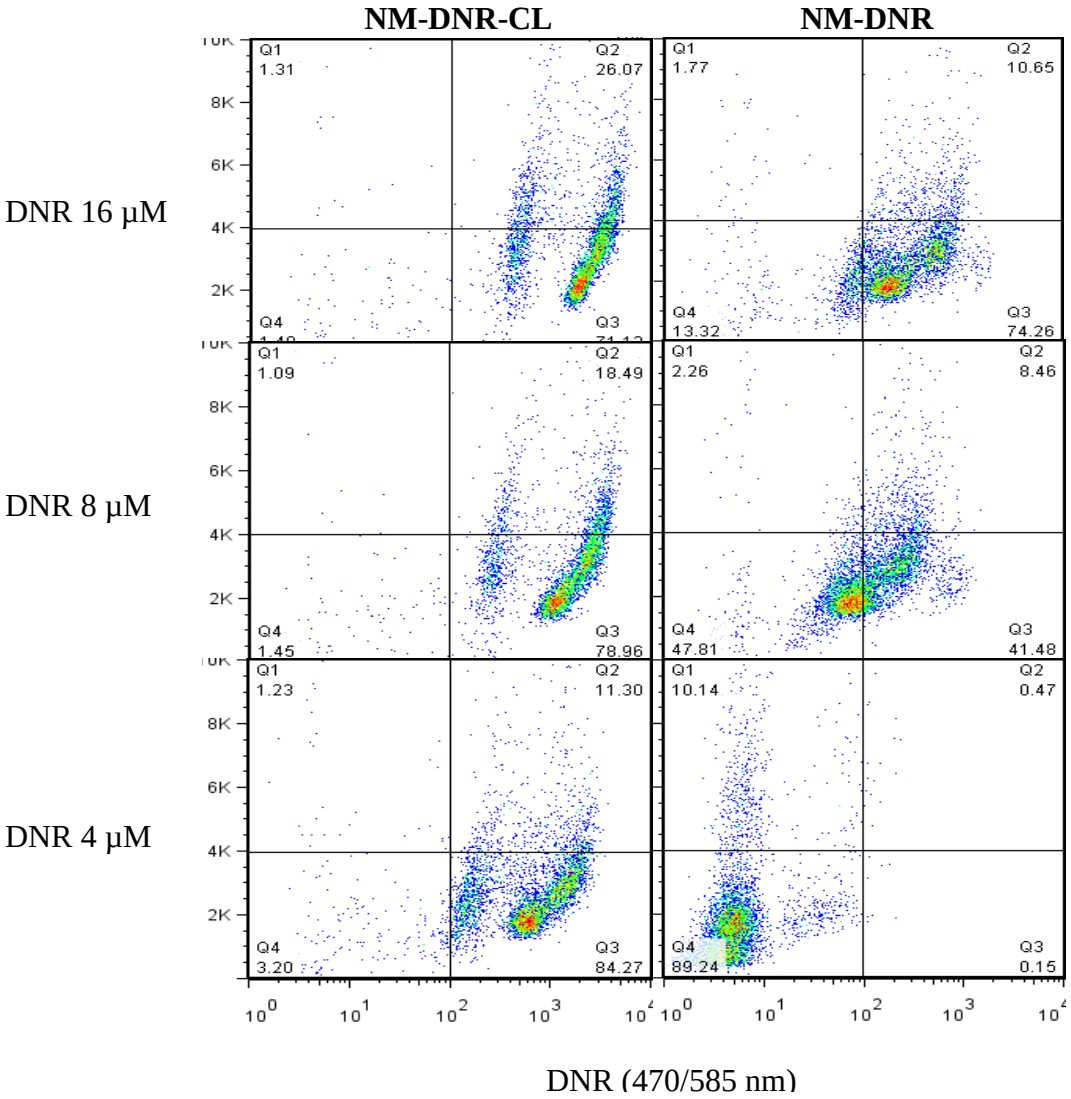
Supplement 4. Targeted delivery of targeting nanomicelles to cells expressing CLL1
Adherent A549 cells transfected with GFP-CLL1 (left upper panel) or GFP (right upper panel, control) were cultured overnight. On the second day, these cells were incubated with nanomicelles loaded with DiI (red) for 30 minutes before washing to remove unbound nanomicelles. The cells were then examined under the fluorescence microscope to determine the nanomicelle uptake. Significant more DiI-loaded nanomicelles were taken up by A549 cells expressing GFP-CLL1 (left lower panel) than by the A549 control cells expressing GFP (right lower panel).



Supplement 5

Dose-dependent targeted delivery of targeting micelles to leukemia cells

A. Dose-dependent drug delivery against CD34(+) clinical leukemic cells. CD34(+) clinical leukemic cells were incubated with targeting (left panels) or non-targeting (right panels) nanomicelles, both loaded with DNR, at different concentration for 30 minutes before flow cytometry analysis (excitation 470 nm/emission 585 nm).



B. Dose-dependent delivery against CD34(+) clinical leukemic cells. CD34(+) clinical leukemic cells were incubated with non-targeting (left panels, NM-DiI) or targeting (right panels) nanomicelles, both loaded with DiI, at different concentration for 30 minutes before flow cytometry analysis. Control: cells were treated with nanomicelles not loaded with DiI. The concentrations shown at the left of each row were DiI concentration.