DNA Aptamer-mediated Cell Targeting

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Supporting Information
SI Text

Lipo-DNA Synthesis and Materials. All DNA sequences were synthesized from 3' to 5' using the ABI 3400 synthesizer on 1.0 micromolar scale. DMT-Hexaethyloxy-Glycol (PEG) phosphoramidite was coupled to DNA by extended coupling time (900 seconds) on DNA synthesizer. Each DNA probe was coupled with four PEG phosphoramidite units. Lipid phosphoramidite was synthesized by following a previously published procedure (1,2) and coupled using the DNA synthesizer by extended coupling time (900 seconds). After synthesis, the DNA was cleaved and deprotected from the CPG and purified by reverse phase HPLC using a C4 column (BioBasic-4, 200mm x 4.6mm, Thermo Scientific) with 100 mM triethylamine-acetic acid buffer (TEAA, pH 7.5) and acetonitrile (0-30 min, 10-100%) as an eluent. All purified lipo-DNA probes were stored in DNase/RNase free water.

General Cell Culture Conditions. Jurkat, K562, CCRF-CEM (CCL-119 T-cell, human acute lymphoblastic leukemia) and Ramos cells (CRL-1596, B lymphocyte, human Burkitt's lymphoma)

were obtained from ATCC (American Type Culture Collection) and were cultured in complete RPMI 1640 medium (ATCC) supplemented with 10% fetal bovine serum (FBS) (heat inactivated, GIBCO) and 100 IU/mL penicillin−streptomycin (Cellgro). CMV-specific CD8+ cytotoxic T lymphocyte (CTL) clone was established by immortalizing primary T cells using lentiviral vectors and was cultured in complete RPMI with 20U/mL IL-2, 5ng/mL IL-7 and 20ng/mL IL-15. The washing buffer contained 4.5 g/L glucose and 5 mM MgCl2 in Dulbecco's PBS (Sigma). Binding buffer used for incubation was prepared by adding yeast tRNA (0.1 mg/mL) (Sigma) and BSA (1 mg/mL) (Fisher) into the washing buffer to reduce background binding. Proteinase K was purchased from Fisher Biotech. DNaseI was purchased from BioLabs.

Lipo-DNA insertion study. Cells were incubated with FITC-labeled lipo-Lib probes for different time periods with different probe concentrations. Fluorescent signals from labeled cells were examined by FACS flow cytometry. Data were analyzed using WinMIDI flow software, and mean fluorescent intensity from different incubation conditions was compared. The one that gave the largest fluorescent intensity was selected for subsequent labeling conditions.

Evaluation of Cellular Cytotoxicity of Lipo-DNA. The cytotoxicity of lipid-Lib probe was tested by standard MTS ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell proliferation assay (Promega). K562, CEM and Ramos cells were incubated with lipo-Lib, as previously described, and seeded into a 96-well cell culture plate. After 2 days of incubation, the cell culture medium was replaced by MTS-containing medium. After 4 hours of incubation, the absorbance at 490 nm from each sample was recorded by a microplate spectrophotometer (Molecular Devices). Control samples were cells without lipo-Lib.

Proteinase Treatment of Cells. After washing with 2mL of washing buffer, Ramos cells were incubated with 0.1mg/mL proteinase K in PBS at 37 °C for 20 min. To quench the proteinase digestion, the sample was quickly mixed with 200 μL of PBS and placed on ice. Then the treated cells were washed with 2mL of binding buffer and used for imaging.

Cytosolic Stain. CellTracker Green CMFDA (Invitrogen) and CellTrace Far Red DDAO-SE were first dissolved in DMSO to 10 mM and further diluted to a final working concentration of 1μM and 10μM, respectively, in serum-free medium. Fresh cells were washed twice in PBS buffer and then incubated in stain solution for 15-30 min at 37 °C. Labeled cells were washed twice in PBS buffer and resuspended in cell culture medium for another 30 min at 37 °C before use.

(1) Liu H, Zhu Z, Kang H, Wu Y, Sefah K, Tan W (2010) DNA-based micelles: synthesis, micellar properties and size-dependent cell permeability. *Chemistry* 16:3791-3797.

(2) Gold L, Janjic N, Schmidt P, Vargeese C (2001) Vascular endothelial growth factor (VEGF) nucleic acid ligand complexes. *U.S. patent* 6,168,778.

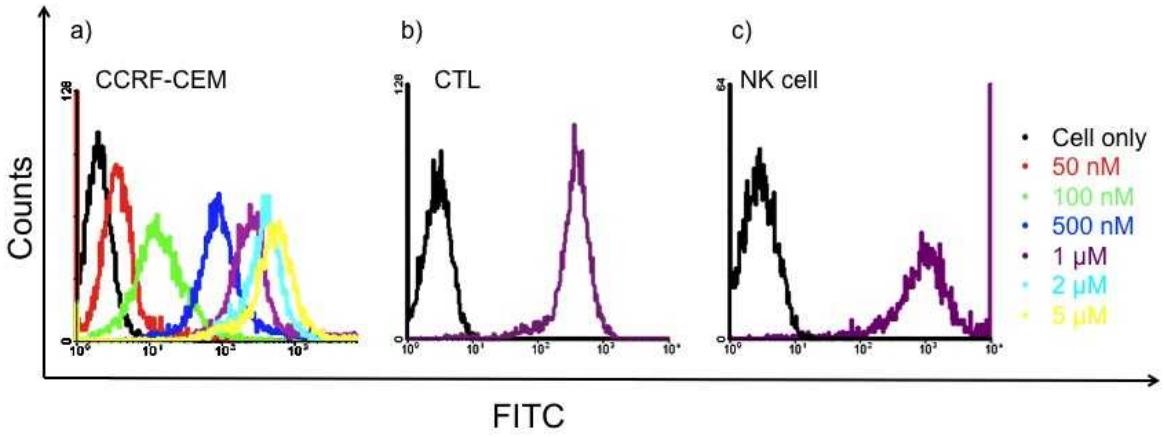

Fig. S1. Lipid-DNA probe can insert into cells. a) FITC signal coming from CEM cells increased with incubation concentration b) CTL and c) NK cells can be modified with lipo-DNA probes.

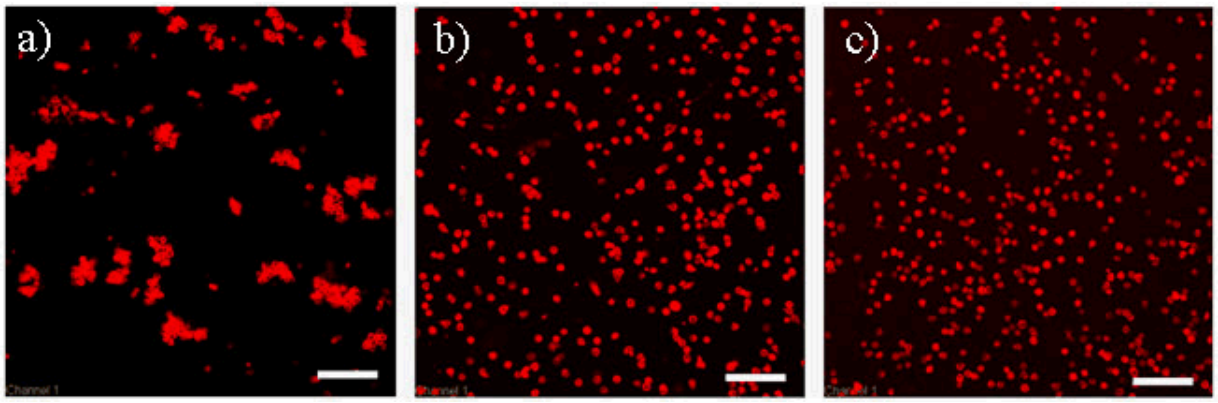

Fig. S2. Aptamer-mediated homotypic assembly of CEM cells. a) Aggregation of CEM cells after treatment with lipo-Sgc8-TMR; b) CEM cells treated with lipo-Lib-TMR; c) Ramos cells treated with lipo-Sgc8-TMR. (Scale bar: 100 μm)

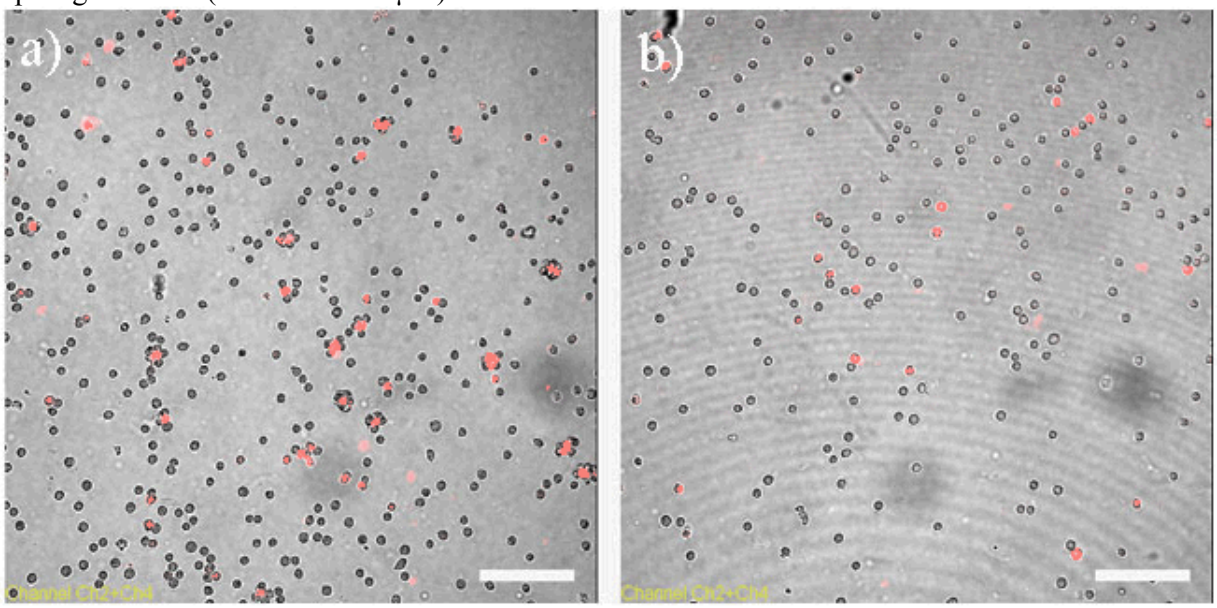

Fig. S3. Sequence-specific heterotypic assemblies between CEM and Ramos. a) 1:10 mixture of lipo-TD05-TMR-modified CEM (red fluorescence) and Ramos (nonfluorescent) cells. b) 1:10 mixture of lipo-Lib-TMR-modified CEM (red fluorescence) and Ramos (nonfluorescent) cells. (Scale bar: 100 μm)

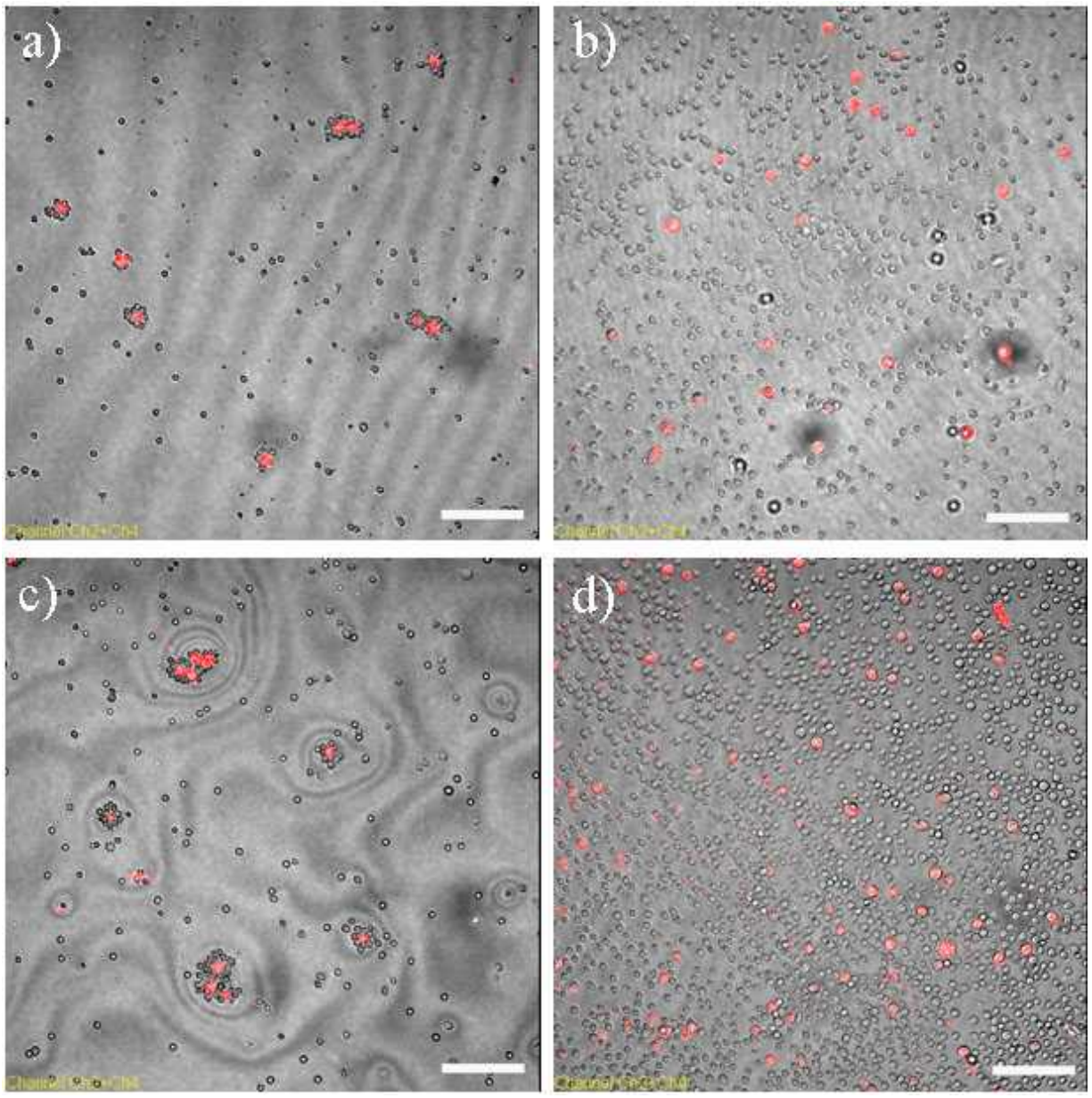

Fig. S4. Sequence-specific heterotypic assemblies between Jurkat and CEM/Ramos cells. a) 1:10 mixture of lipo-Sgc8-TMR- modified Jurkat (red fluorescence) and CEM (nonfluorescent) cells. b) 1:10 mixture of lipo-Lib-TMR-modified Jurkat (red fluorescence) and CEM (nonfluorescent) cells. c) 1:10 mixture of lipo-TD05-TMR-modified Jurkat (red fluorescence) and Ramos (nonfluorescent) cells. d) 1:10 mixture of lipo-Lib-TMR-modified Jurkat (red fluorescence) and Ramos (nonfluorescent) cells. (Scale bar: 100 μm)

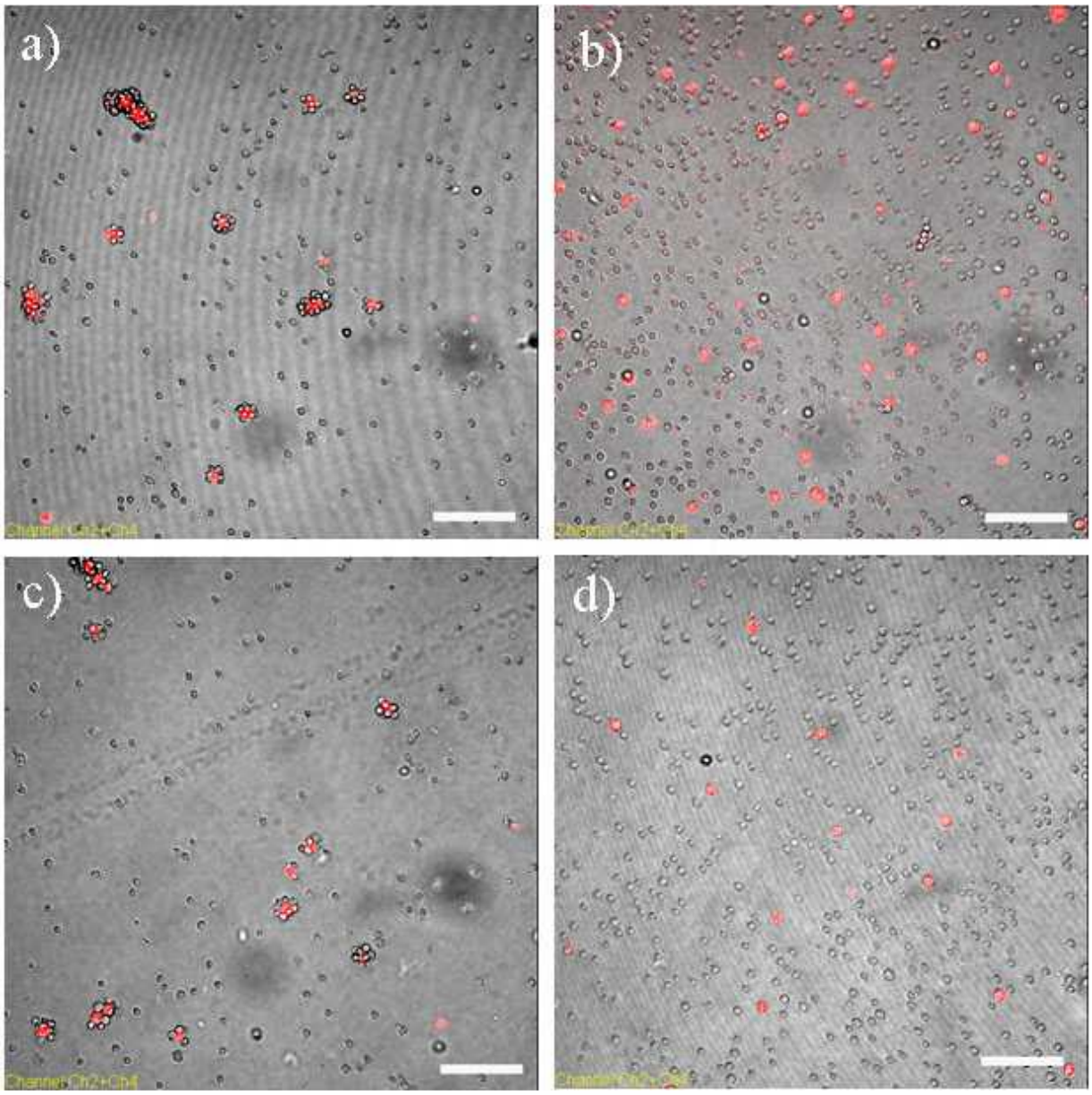

Fig. S5. Sequence-specific heterotypic assemblies between K562 and CEM/Ramos cells. a) 1:10 mixture of lipo-Sgc8-TMR-modified K562 (red fluorescence) and CEM (nonfluorescent) cells. b) 1:10 mixture of lipo-Lib-TM-modified K562 (red fluorescence) and CEM (nonfluorescent) cells. c) 1:10 mixture of lipo-TD05-TMR-modified K562 (red fluorescence) and Ramos (nonfluorescent) cells. d) 1:10 mixture of lipo-Lib-TMR-modified K562 (red fluorescence) and Ramos (nonfluorescent) cells. (Scale bar: 100 μm)

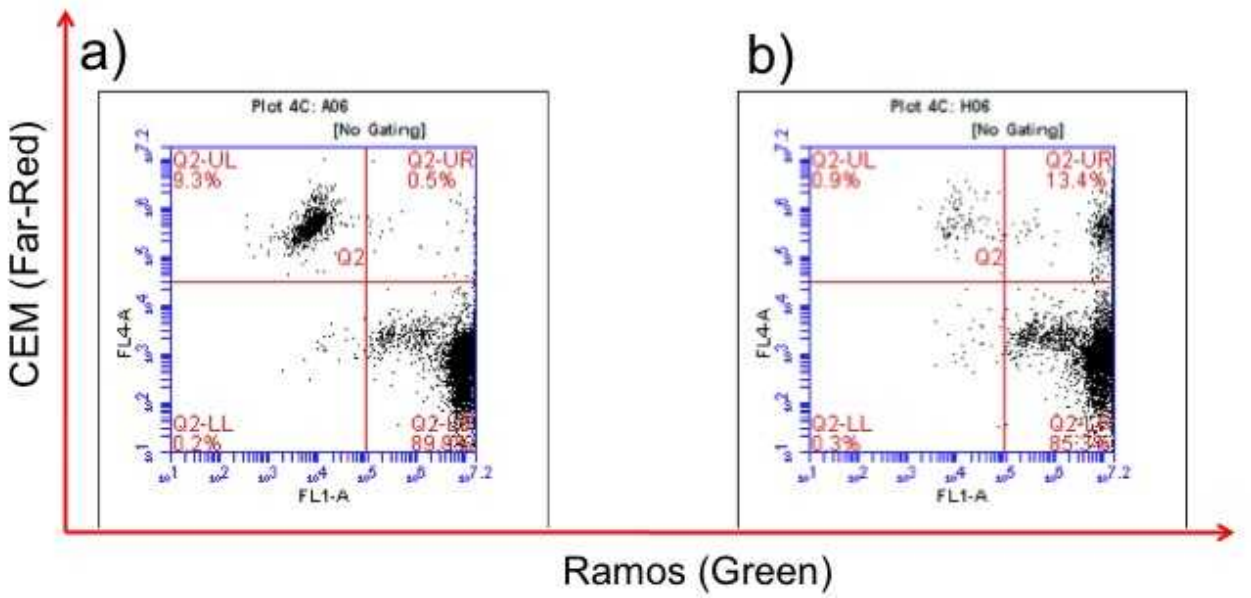

Fig. S6. Sample flow cytometry data for quantification of CEM aggregation. CEM (far red, FL4) incubated with 1μM lipo-Lib (a) or lipo-TD05 (b) and incubated with 10 equivalent Ramos (green, FL1) cells. The percentage of aggregation was counted as the number of cells in the upper right region over total upper region.

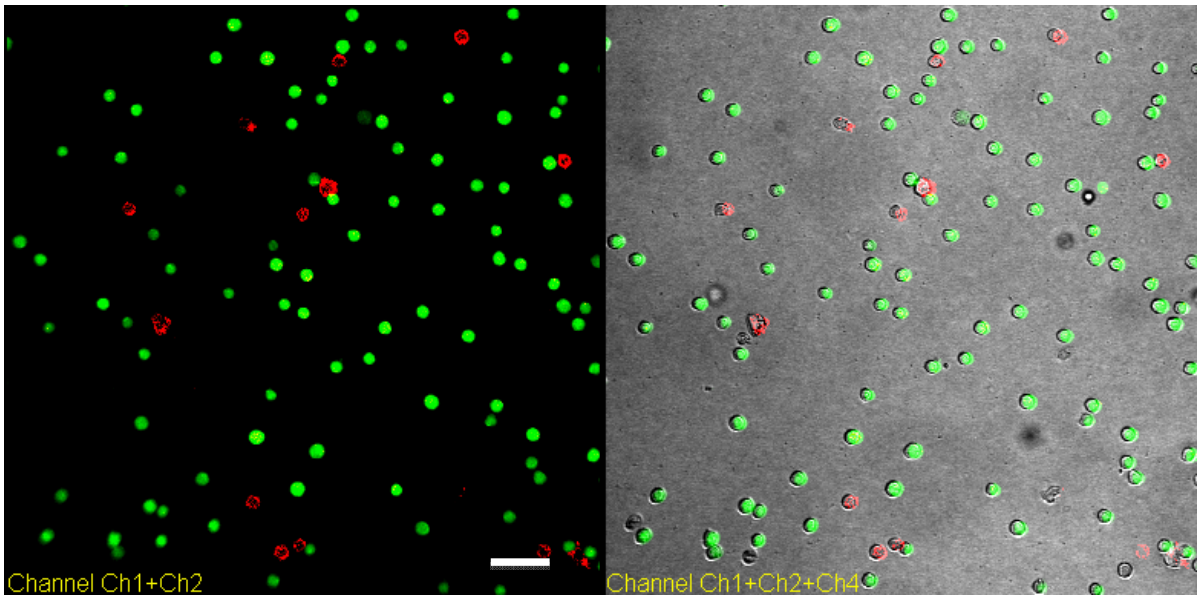

Fig. S7 1:5 mixture of lipo-TD05-TMR-modified CEM (red) cells and Ramos (green) cells imaged right after mixing. CEM and Ramos cells remained apart, and only a few small aggregates were observed. Scale bar: 100 μm.

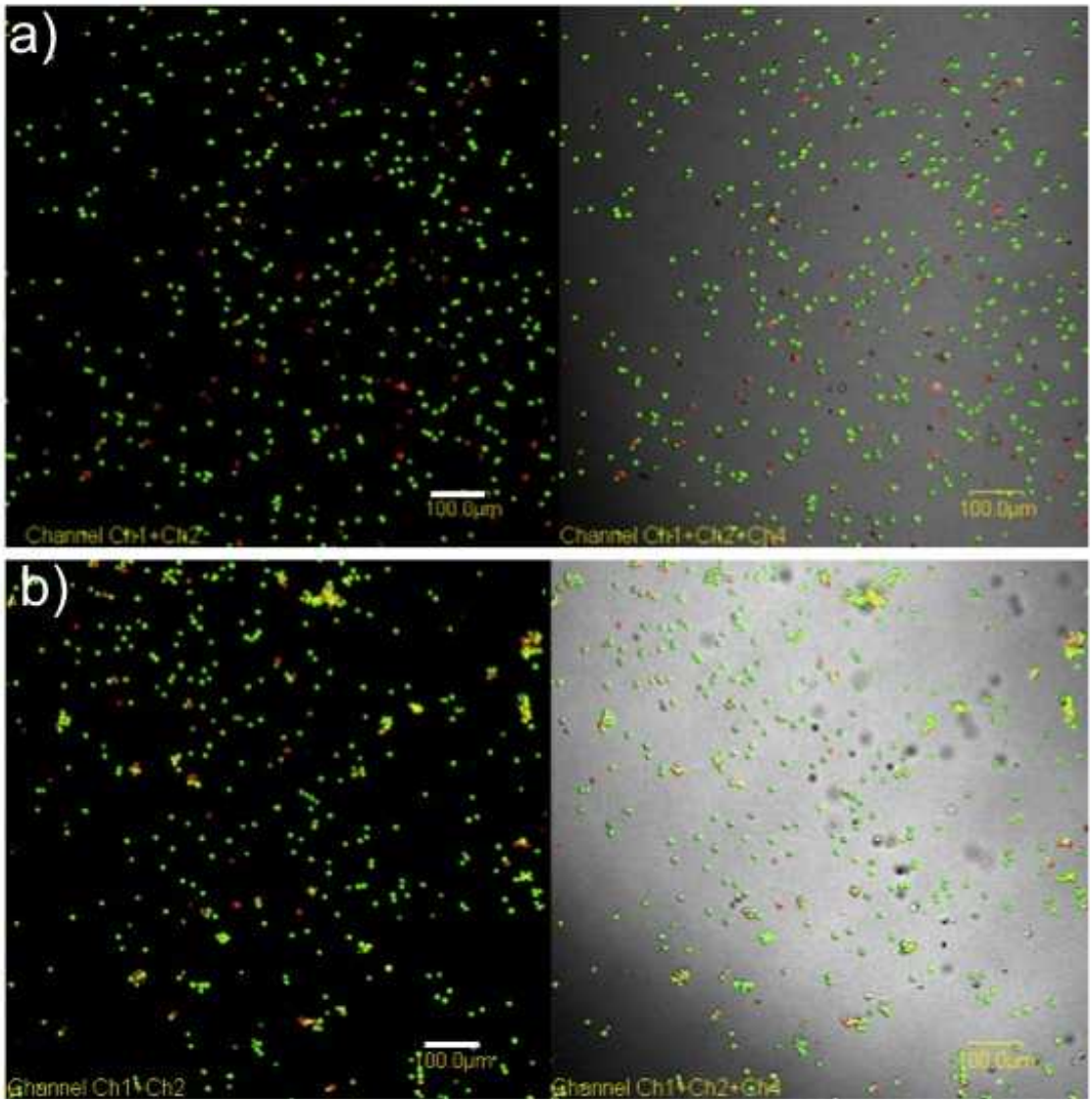

Fig. S8. 1:5 mixture of a) lipo-Lib-TMR- or b) lipo-TD05-TMR-modified CEM (red) cells and Ramos (green) cells after 25 min incubation. Scale bar: 100 µm.

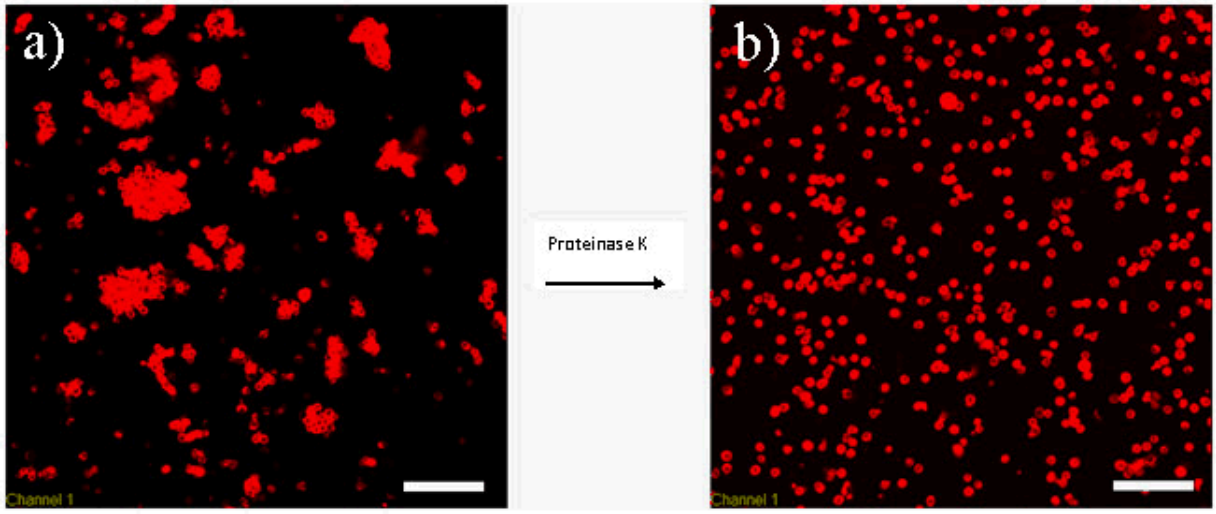

Fig. S9. Aggregates of Ramos cells disappear after treatment with proteinase K. a) homotypic aggregates of Ramos cells after modification with lipo-TD05-TMR; b) the same assembled cells after incubation at 37°C in the presence of Proteinase K. (Scale bar: 100 μm)

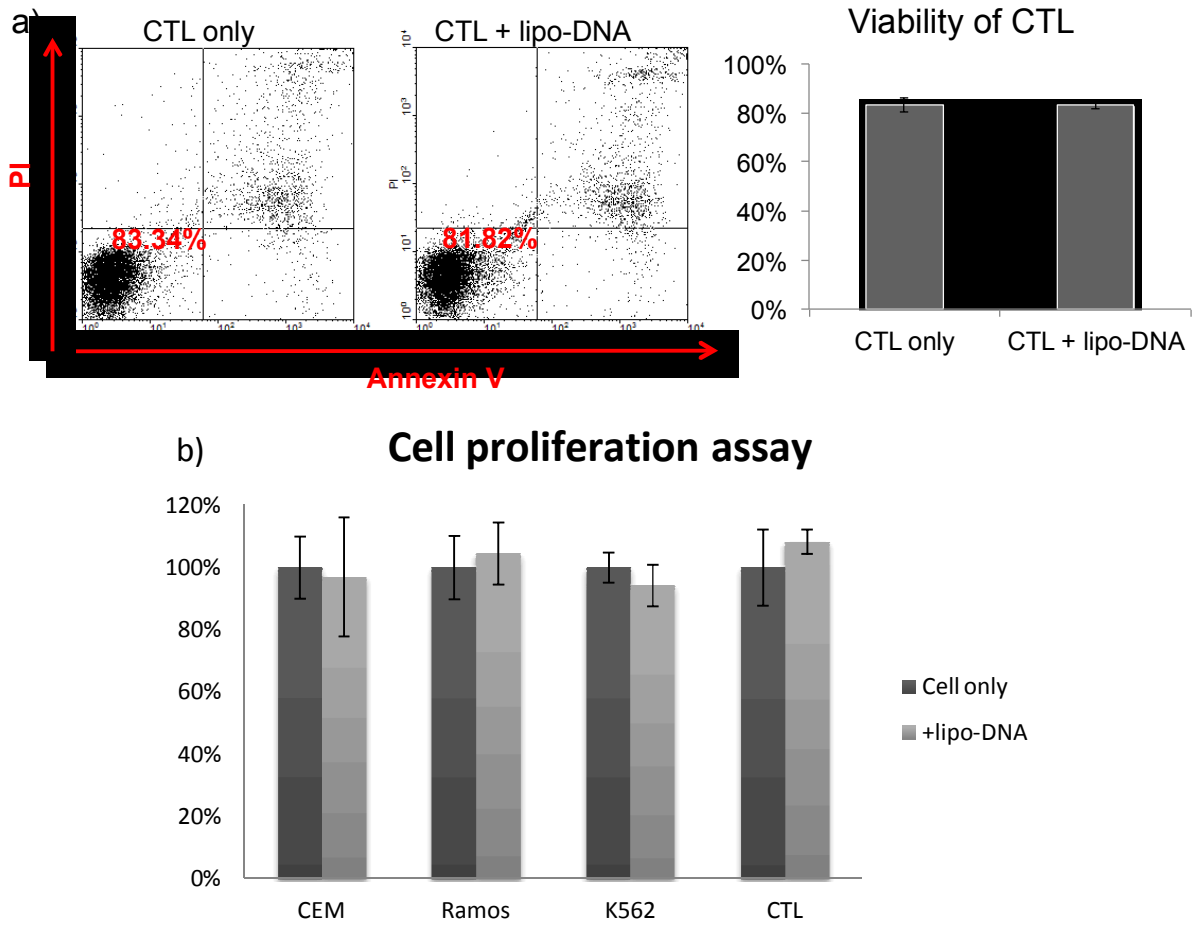

Fig. S10 Cytotoxicity of the lipo-DNA probe. a) Apoptosis and cell death staining of unmodified and lipo-DNA modified CTL. The lower left square represented healthy cell population.
b) Cell proliferation assay. Cells labeled with lipid-DNA (grey bars) showed no significant difference in proliferation rate compared with cell-only control (black bars), indicating that lipid-DNA is not toxic to cells at 1 μM concentration. Data are means of three measurements. Bars are standard deviations.

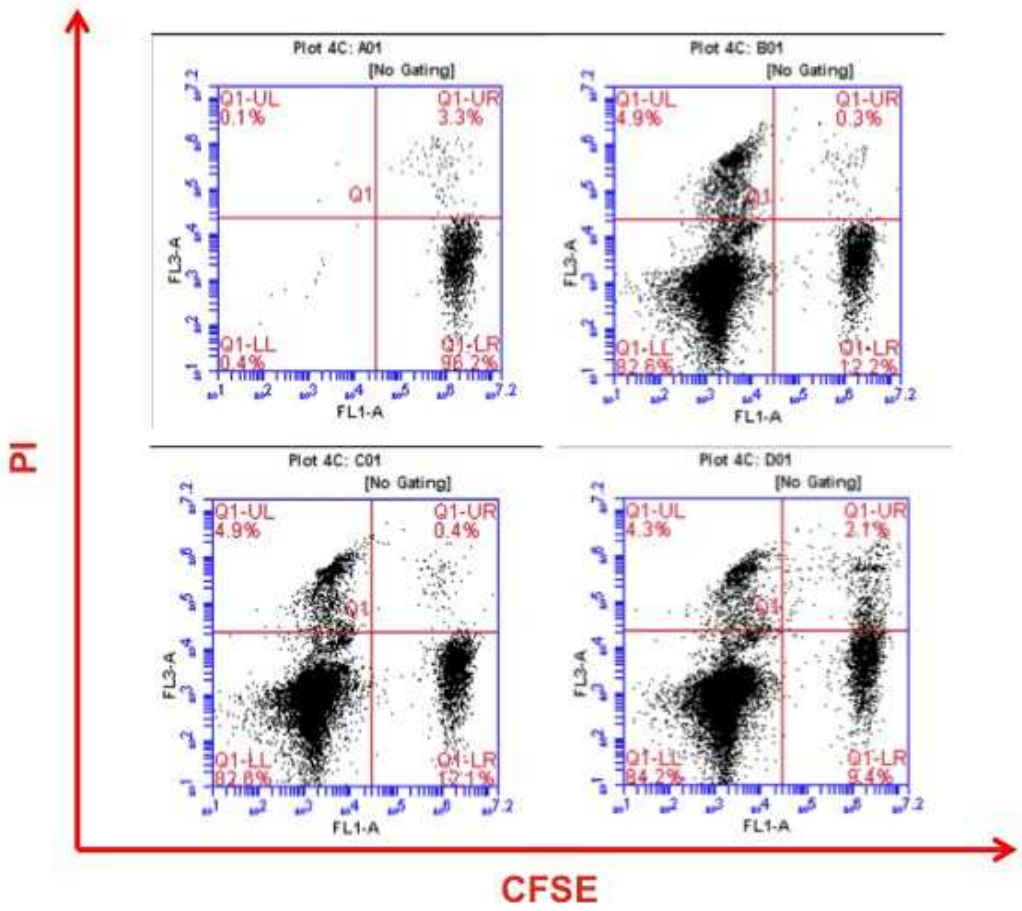

Fig S11. Sample flow cytometry data of CTL-Ramos killing assay. CFSE- and PI-positive cells were dead Ramos cells. A01: Ramos only; B01: unmodified CTL and Ramos; C01: lipo-Lib-modified CTL and Ramos; D01: lipo-TD05-modified CTL and Ramos. The percentage of dead Ramos was calculated as the number of CFSE- and PI-positive cells over the number of CFSE-positive cells.

Table S1: Aggregation percentage of CEM cells

Lipid-DNA concentration		500nM			1μM			2μM			5μM		
CEM to Ramos Ratio		1:1	1:5	1:10	1:1	1:5	1:10	1:1	1:5	1:10	1:1	1:5	1:10
Lipo-Lib	Sample 1	4.51	6.84	5.47	4.33	5.11	5.39	3.32	4.78	5.67	5.24	6.11	6.40
	Sample 2	7.04	5.15	6.56	3.40	4.47	4.30	4.38	5.39	4.28	3.96	6.90	6.69
	Sample 3	3.63	5.81	5.47	3.57	7.77	5.52	3.55	4.72	4.92	3.45	6.70	7.19
	Mean	5.06	5.93	5.83	3.77	5.78	5.07	3.75	4.96	4.96	4.22	6.57	6.76
	SD	1.77	0.85	0.63	0.50	1.75	0.67	0.56	0.37	0.70	0.92	0.41	0.40
Lipo-TD05	Sample 1	49.98	68.75	65.15	79.49	91.64	93.73	86.25	96.38	93.82	78.57	97.31	97.68
	Sample 2	31.62	60.56	65.92	77.12	84.54	91.18	81.19	96.29	94.31	86.16	95.14	95.22
	Sample 3	33.57	63.00	69.72	80.04	91.18	93.46	83.95	94.89	95.71	85.34	94.71	95.85
	Mean	38.39	64.10	66.93	78.88	89.12	92.79	83.80	95.85	94.61	83.36	95.72	96.25
	SD	10.08	4.21	2.45	1.55	3.97	1.40	2.53	0.84	0.98	4.17	1.39	1.28