Supporting Information: Chemically programmed cell adhesion with membrane anchored oligonucleotides

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Supplementary Materials and Methods:

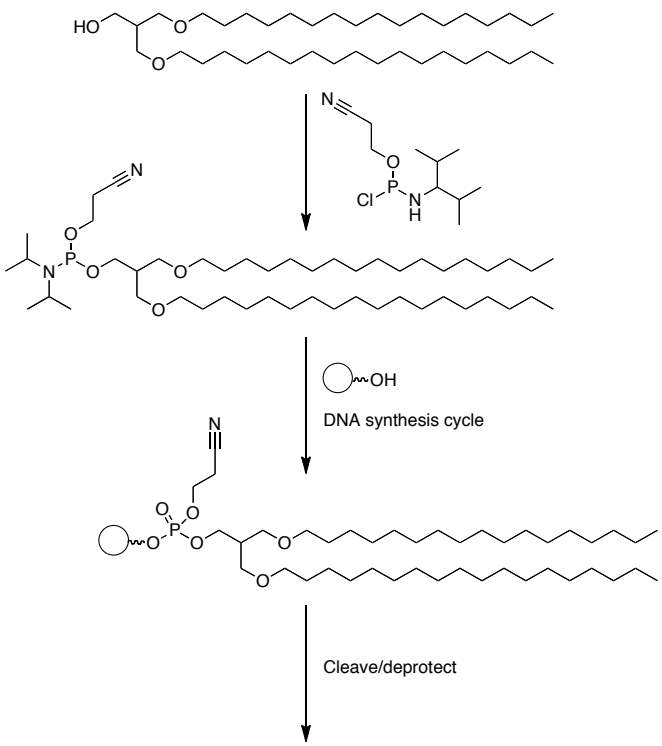
1,2-O-Dihexadecyl-sn-glycerol and 1,2-O-Dioctadecyl-sn-glycerol were obtained from Chem-Impex. *N*,*N*-Diisopropylethylamine (DIPEA), 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite, octadecanoic acid, hexadecanoic acid, *N*-Methyl-2-pyrrolidone (NMP), 5-(Ethylthio)-1*H*-tetrazole (ETT), N,N′-Diisopropylcarbodiimide (DIPC), and succinimidyl-[(N-maleimidopropionamido)-hexaethyleneglycol] ester [SM(PEG)$_6$] were obtained from Sigma-Aldrich. HPLC grade acetonitrile, triethylamine, acetic acid, and tris(2-carboxyethyl)phosphine (TCEP) were obtained from Fisher Scientific. Controlled pore glass (CPG) support, 1-O-Dimethoxytrityl-hexyl-disulfide,1'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (thiol phosphoramidite), and columns were obtained from Glen Research. 6-(4-Monomethoxytritylamino)hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite (amine phosphoramidite), standard phosphoramidites and DNA synthesis reagents were obtained from Azco Biotech. All materials were used as received.

Oligonucleotides were synthesized on an Applied Biosystems Expedite 8909 DNA synthesizer. Thiol and amine modified DNA were synthesized using thiol and amine phosphoramidites (100 mM), respectively, using a standard coupling protocol. Oligonucleotides were purified by reversed-phase high-performance liquid chromatography (HPLC) using an Agilent 1200 Series HPLC System with a diode array detector (DAD) monitoring at 230 and 260 nm. Purifications used 100 mM triethylamine acetate (pH 7) H_2O/acetonitrile mobile phase on a C_{18} (Agilent), C_8 (Agilent) or C_4 (Phenomenex) column for thiol-and amine-, monoalkylamide- and DIFO-, or dialkylphosphoglyceride-modified DNA, respectively, running a gradient between 8% and 95% acetonitrile. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry was performed on a Voyager-DE Pro with a hydroxypicolinic acid/ammonium citrate matrix supplemented with acetone solubilized nitrocellulose. All nuclear magnetic resonance (NMR) were recorded on a Varian Innova 400.

Dialkylglycerol phosphoramidites were synthesized as previously described[1-3]. Briefly, a solution of 1,2-di-O-hexadecyl-glycerol (448 mg, 0.83 mmol) and N,N-diisopropylethylamine (320 µL, 1.8 mmol) in dichloromethane (10 mL) was cooled to 0 °C. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (300 µL, 1.3 mmol) was added dropwise over 1 min. The reaction was then stirred at 0 °C for 1 h then allowed to warm to RT over 3 h. The reaction mixture was washed with sat. NaHCO3 (3 x 20 mL) then dried over MgSO4 and concentrated in vacuo. Silica gel chromatography (90:9:1 hexanes:EtOAc:NEt3) yielded a waxy semisolid (419 mg, 68 % yield estimated at >80% purity). Spectral data was found to agree with previous reports.

Dialkylglycerol phosphoramidites were coupled to 5'-OH oligonucleotides on controlled pore glass. We found that low support loading was critical for efficient coupling. 500 µl of C_{16} or C_{18} dialkylglycerol phosphoramidite (200 mM) and 1 ml of ETT (300 mM) in dichloromethane were loaded into syringes and hand coupled to 200 nmol of oligonucleotide on solid support.

Supplementary Scheme 1



Fatty acid acylated DNA was synthesized by adding octadecanoic acid or hexadecanoic acid (200 mM), DIPC (200 mM), and DIPEA (400 mM) in NMP to 200 nmol of amine-modified oligonucleotide on solid support shaking overnight at room temperature.

NHS-DNA was synthesized as previously described with slight modifications[4]. Thiol-modified DNA was resuspended in 10% distilled water, 40% 10 mM TCEP, and 50% 1x TE buffer (10 mM Tris with 1mM EDTA, pH 7.5) to a final DNA concentration of 775 μM before being aliquoted and stored at -20 °C until use. Thiol-modified DNA (50 μl) was desalted using an aqueous equlibrated Centri-Spin 10 column (Princeton Separations) prior to adding 20 μl of $SM(PEG)_6$ (5mg/ml in DMSO) for 10 minutes at room temperature. The reaction was passed through a PBS equilibrated Centri-Spin 10 column prior to DNA concentration verification by UV-vis spectroscopy and application to cells.

DIFO-DNA was synthesized as previously described[5].

All oligonucleotides were cleaved from solid support with a 1:1 mixture of ammonium hydroxide/methylamine (AMA) for 15 minutes at 65°C followed by evaporation of AMA with a speedvac system. Oligonucleotides were filtered through 0.2-μm filters and purified by reversed-phase HPLC as described above. Lipid-modified oligonucleotides were resuspended in distilled water and lyophilized repeatedly to remove residual HPLC buffer salts prior to use. The masses of representative samples were confirmed by MALDI-MS. Purified lipid-modified oligonucleotides were resuspended in water and concentrations were determined by measuring their absorbance at 260 nm.

Molecule	**Actual MW**	**Measured (M+H)**			**Average**	**StDev**
		1	2	3		
20mer C16 Dialkyl	6719	6723	6724	6739	6,729	9
20mer C18 Dialkyl	6776	6779	6778	6777	6,778	1
30mer C16 monoalkyl	9614	9627	9624	9644	9,632	11
30mer C18 monoalkyl	9642	9641	9695	9668	9,668	27
100mer C16 dialkyl actg	31055	31040	30983	30909	30,977	66
100mer C16 dialkyl cagt	31055	30140	30219	30201	30,187	41
20mer unmod	6117	6143	6132	6159	6,145	14

DNA Labeling of Cells and Quantification of Cell Surface Oligonucleotides:

Lipid DNA molecules: adherent cells were lifted by incubating with 0.25% trypsin at 37°C for 5 minutes followed quenching with 10% FBS containing media. In order to maintain intact proteins on the cell surface, cells labeled with NHS-DNA and DIFO-DNA were lifted by incubating at 37°C in 0.04% EDTA in PBS for 20-30 minutes followed by a 30 second 0.05% trypsin pulse. Trypsin was quenched by addition of soybean trypsin inhibitor (1 mg/ml) and 10% FBS containing media. Lifted cells were washed with calcium and magnesium free PBS three times, and (10^6) cells were resuspended in 49 μl of PBS and labeled by addition of 1 μl of 250 μM lipid-DNA in water making a final DNA concentration of 5 μM. Cells were gently agitated by slow vortexing for 5 minutes at room temperature.

NHS-DNA and DIFO-DNA: was prepared as previously described and added to cells (10^6) in 50 μl of 175 μM NHS-DNA or DIFO-DNA and mixed for 30 minutes at room temperature or 37°C, respectively. All cells were washed in ice-cold PBS three times to remove residual, unreacted DNA before incubating in 50 μl of 20mer complementary 6-FAM modified oligonucleotide (1 μg/ml, Operon) for 30 minutes at 4°C protected from light. Cells were washed one time before resuspending in LIVE/DEAD® Fixable Dead Cell Stain (Invitrogen) for 15 minutes at 4°C protected from light. Cells were washed one last time before flow cytometry analysis. For kinetic stability time course experiments, cells were incubated at 37°C for designated amount of time before probing with fluorescent, complementary oligonucleotide. All reported values are the average of 3 independent measurements. Median fold fluorescence increase (MFI) was calculated as the fold increase of fluorescence levels over unmodified cells.

Programmed Cell Assembly: CellTracker Green CMFDA and CellTrace Far Red DDAO-SE (Invitrogen) stocks were prepared at a concenration of 10 mM in anhydrous DMSO. Cells were resuspended in 10 μM stain in serum-free media for 15 minutes at 37°C before going through labeling manipulations described above. After washing away unreacted DNA, cells were resuspended at $1x10^6$ cells/ml. Cell type of interest that had been labeled green was mixed with far red Jurkats at a ratio of 1:100 in 200 μl and mixed at 150 rpm for 10 minutes in an Ultra-Low Attachment 24-well plate (Corning) before analyzing via flow cytometry.

Assay for Cell Attachment and Dynamic Membrane Observations:

Preparation of DNA arrays: 5'-amino-modified DNA was prepared in a buffer of 450 mM NaCl, 50 mM dibasic sodium citrate, 50 mM dibasic sodium phosphate, 5% trehalose, and 1% fresh $NaCNBH_3$. DNA was patterned onto aldehyde-silanized glass (Schott) using either a micropipette for large-scale patterns or a BioForce Nano eNabler for small-scale patterns and incubated 12 hours in a humidified chamber. Slides were reduced with $NaBH_4$ and passivated with both SigmaCote (Sigma) and Pluronic F108 before use.

Cells incorporating lipid-DNA were allowed to settle onto patterned glass within a PDMS-based flow cell for 30 minutes. Only cells hybridized to the surface via DNA were retained when the surface was washed with PBS. Patterned cells were imaged at 37 ˚C on a Zeiss Axiovert 200M at 200x or 400x magnification using phase contrast settings.

Cells were incubated with 10 ng/mL phorbol 12-myristate 13-acetate (PMA) and 1.3 uM ionomycin 4 hours prior to lipid incorporation.

Image Analysis. Using ImageJ, cells were picked automatically from 20x fields of view and subsequently binarized. For each time point, scanning down the y-axis, the horizontal slice with the greatest width was identified. For each cell, the coefficient of variation was calculated for this set of widths, and this statistic was used to characterize each cell's membrane motility for binning. Error bars signify standard error. The ImageJ script is available upon request.

Resazurin Cell Viability Assay: Cells were labeled with DNA and washed as described above. For each cell type, optimal cell density was determined for each well of a 96-well plate. Resazurin (10 μl) was added to culture for 24 hours before fluorescence was read with excitation and emission wavelengths at 530 nm and 590 nm, respectively, using Molecular Devices SpectraMax M5 on three consecutive days. The change in fluorescence intensity over time was used as a proxy for cell growth rates. Fluorescence was log transformed and plotted against time for lipid-modified and untreated cells. Slopes and intercepts of the growth rates for the two cell treatments were compared using ANCOVA (GraphPad Prism) to determine if doubling time was inhibited by DNA treatment.

Oligonucleotide Sequences:

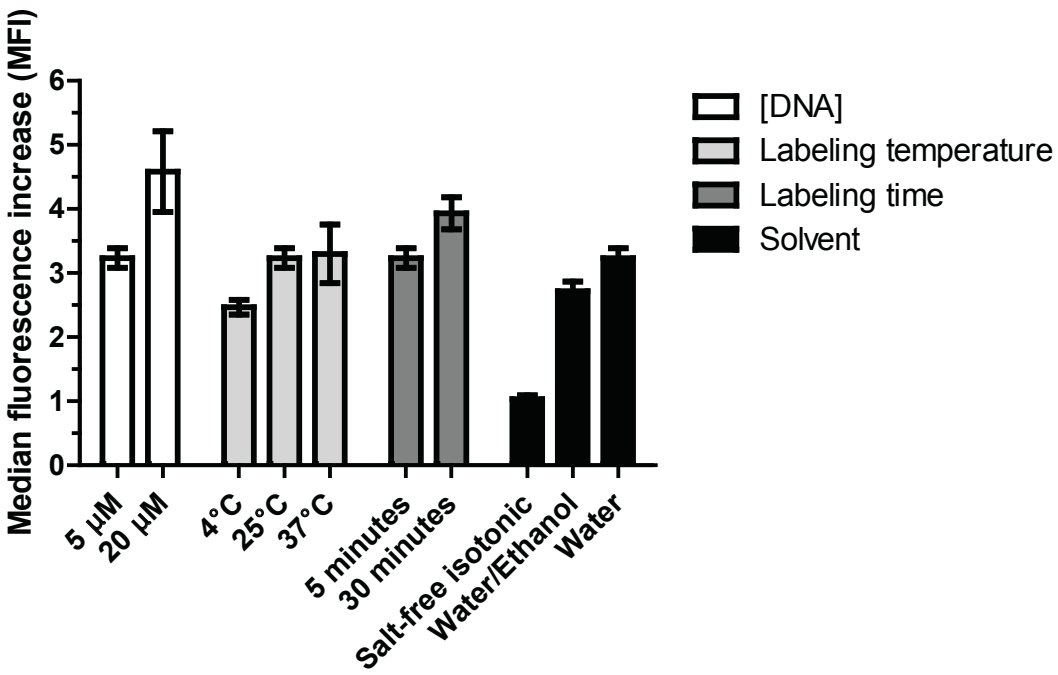
Y-5'-$T_x(CAGT)_5$-3' x=0, 20, 40, 60, or 80, Y= C_{16} dialkylphosphoglyceride
Y-5'-$T_x(ACTG)_5$-3' x=0, 20, 40, 60, or 80, Y= C_{16} dialkylphosphoglyceride

Z-5'-$(CAGT)_5$-3' Z=DIFO, NHS, C_{18} dialkylphosphoglyceride, or $C_{16/18}$ monoalkylamide
Z-5'-$(ACTG)_5$-3' Z=DIFO, NHS, C_{18} dialkylphosphoglyceride, or $C_{16/18}$ monoalkylamide

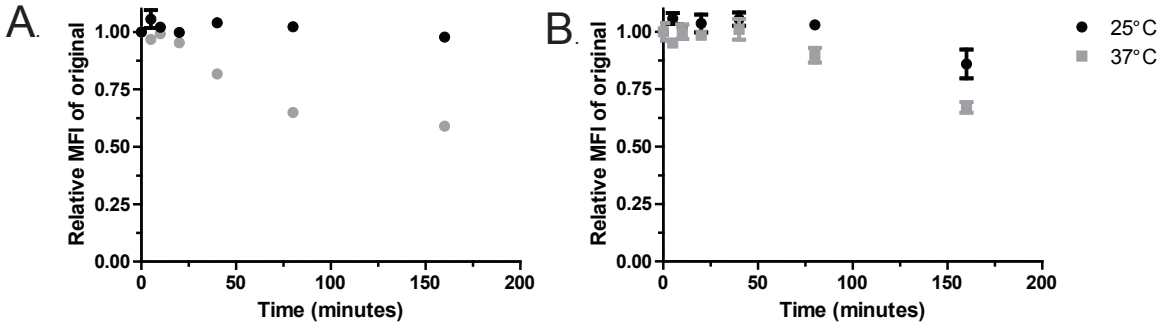
FAM-5'-$(CAGT)_5$-3'
FAM-5'-$(ACTG)_5$-3'

(1) Brush, C. K.; Pharmacia P-L Biochemicals Inc.: US Patent 5,420,330, 1990.
(2) Chan, Y. H.; van Lengerich, B.; Boxer, S. G. *Biointerphases* **2008**, *3*, FA17.
(3) Mackellar, C.; Graham, D.; Will, D. W.; Burgess, S.; Brown, T. *Nucleic Acids Research* **1992**, *20*, 3411-3417.
(4) Hsiao, S. C.; Shum, B. J.; Onoe, H.; Douglas, E. S.; Gartner, Z. J.; Mathies, R. A.; Bertozzi, C. R.; Francis, M. B. *Langmuir* **2009**, *25*, 6985-91.
(5) Gartner, Z. J.; Bertozzi, C. R. *Proc Natl Acad Sci U S A* **2009**, *106*, 4606-10.

Supplementary Figure 1: The effect of concentration, temperature, reaction time, and cosolvent on membrane insertion of 5' C_{18} phosphoglyceride-labeled oligonucleotides.



Supplementary Figure 3: Fraction of initial cell-surface oligonucleotides introduced by (a) **3** and (b) **4** as a function of time. (c) Tabulated data from (a) and (b).

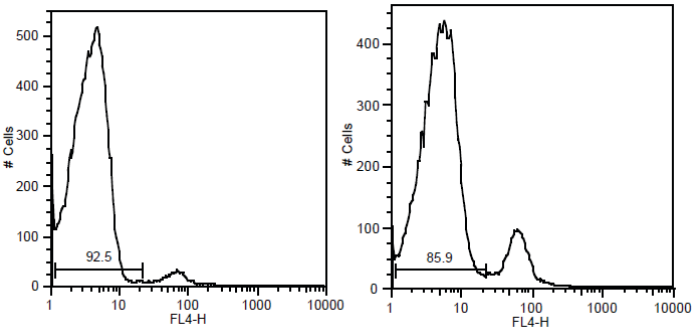


C.

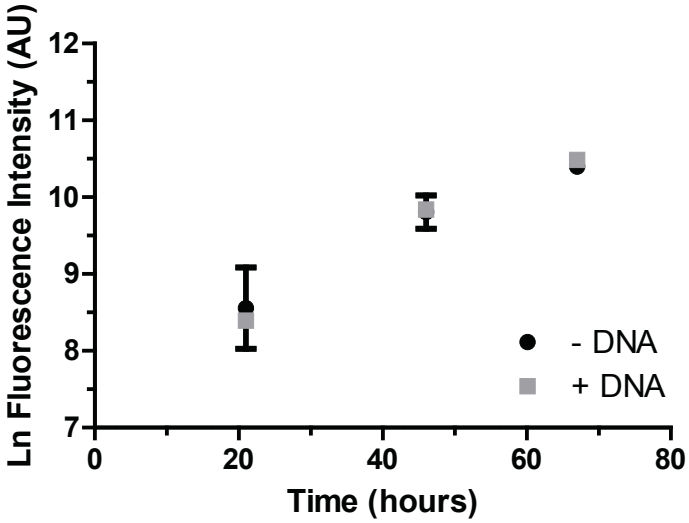
	25°C	37°C
NHS-DNA	98%	59%
Lipid-DNA	86%	67%

Supplementary Figure 4: Viability and proliferation of lipid-DNA modified cells. (a) Flow cytometry viability analysis of unlabeled (left) and **4**-labeled (right) populations of Jurkat cells stained with Invitrogen Far Red Fixable Dead Cell Stain. Live cell population is indicated by the gate at the bottom of the plot. (b) Representative growth curve for Jurkat cells. (c) Tabulated growth rates (slope) from (b) for all analyzed cell types.

A.



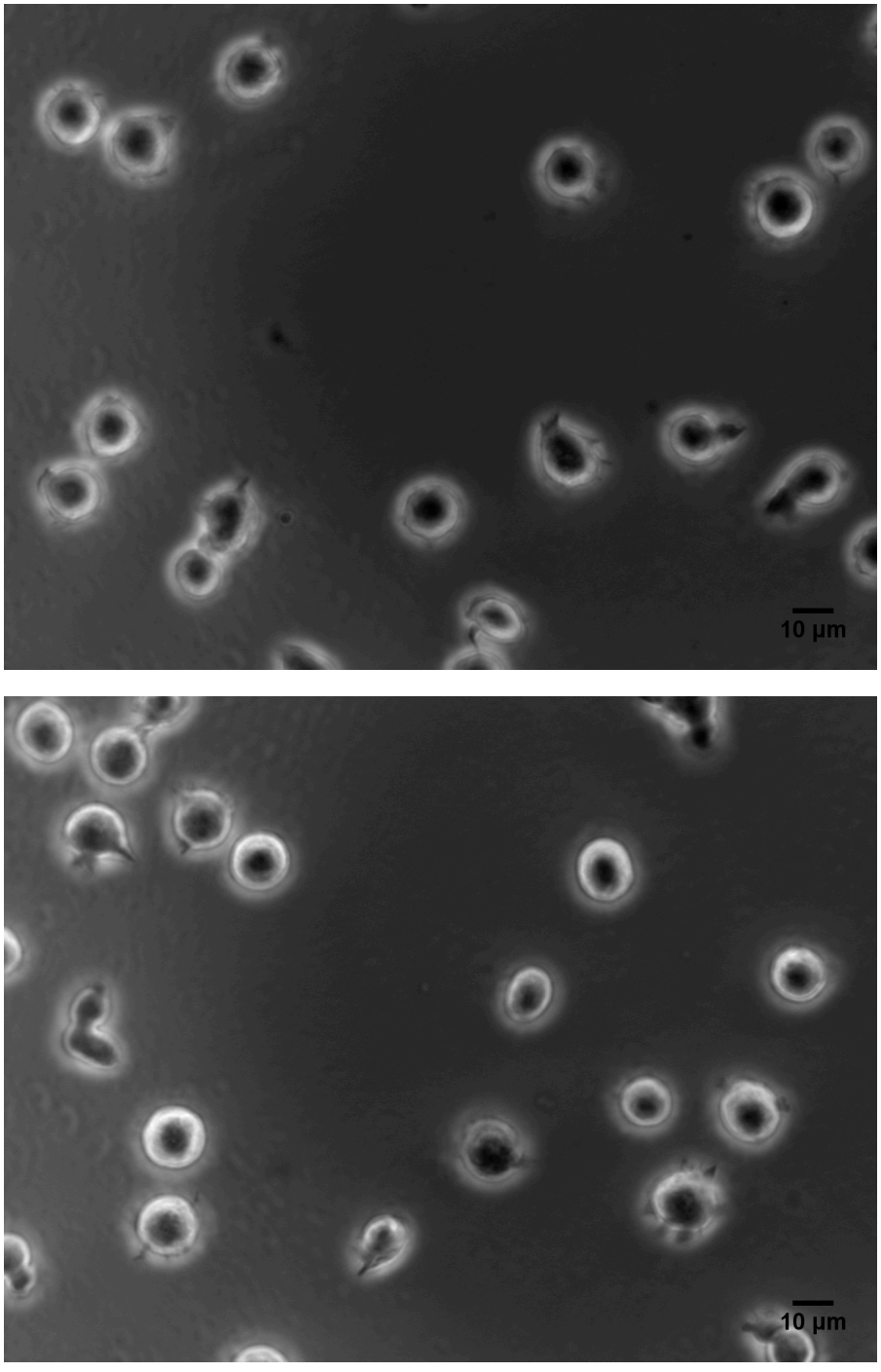
B.



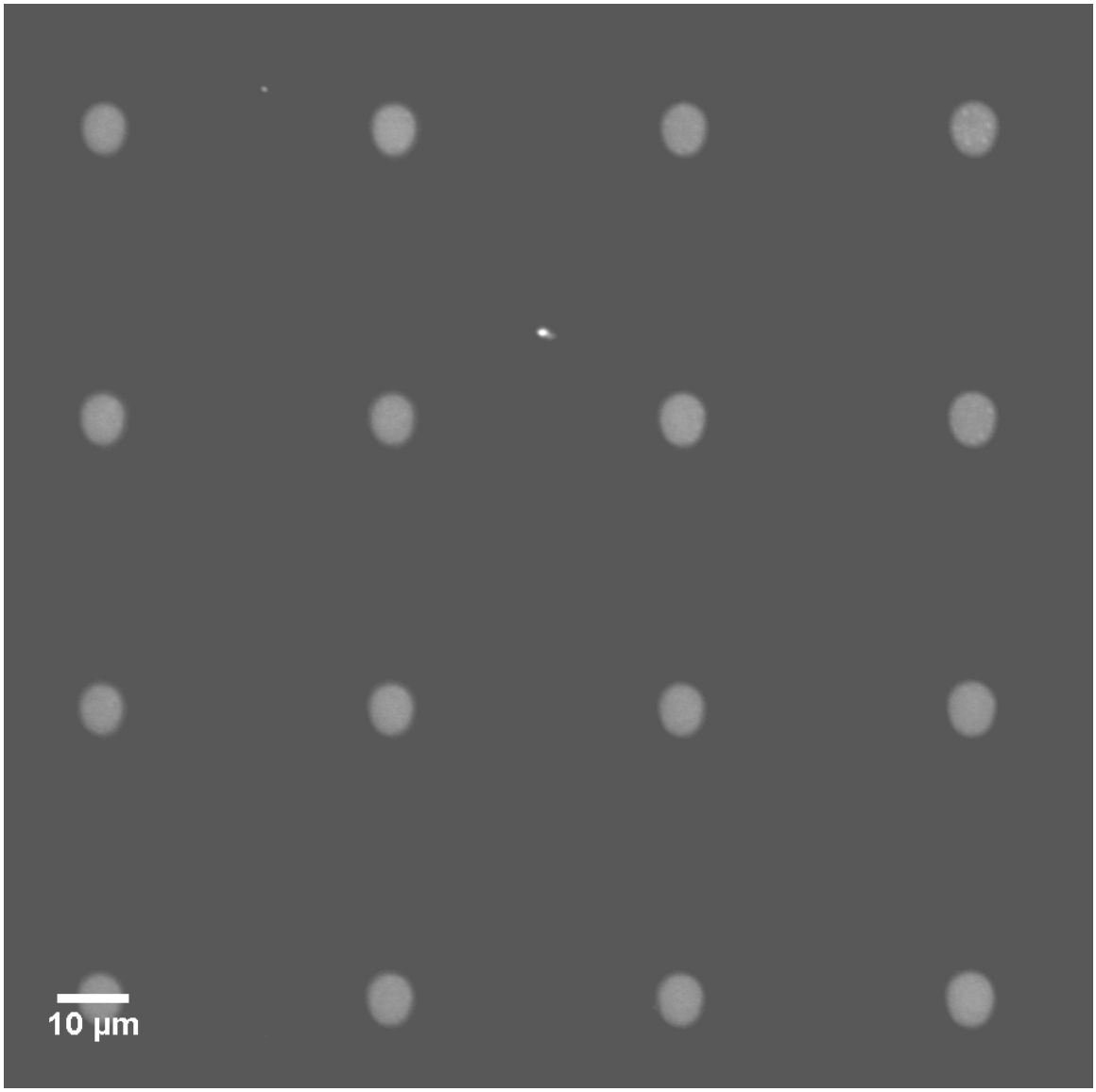
C.

	- DNA	+ DNA	p-value
Jurkat	0.049 ± 0.0041	0.054 ± 0.0033	0.33
MCF-10A	0.042 ± 0.011	0.038 ± 0.0072	0.79
HeLa	0.040 ± 0.0059	0.046 ± 0.0029	0.43
MEF	0.060 ± 0.0086	0.086 ± 0.012	0.11

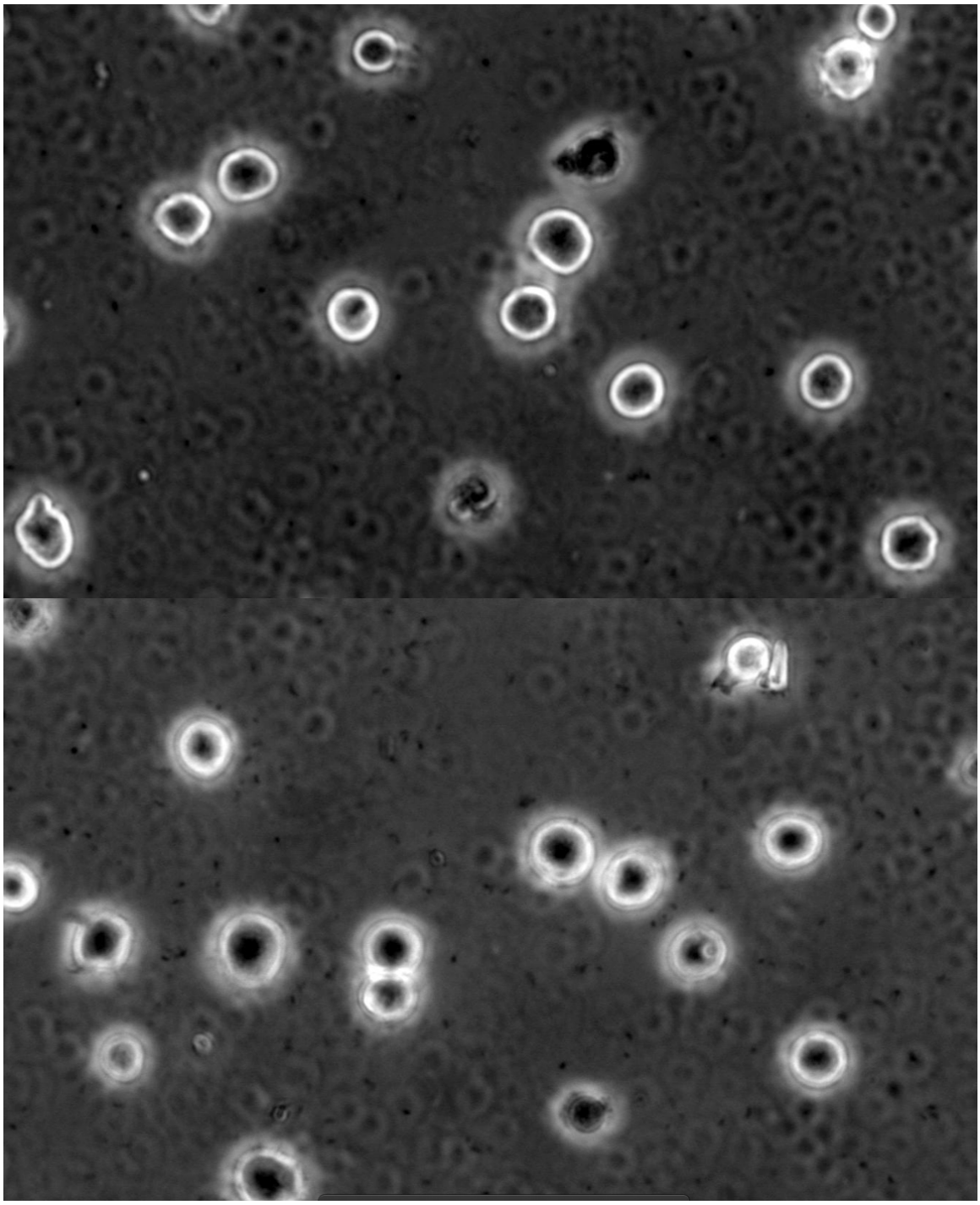
Supplementary movie 5: unmodified (single frame, top) and 4-modified (single frame, bottom) cells on naked glass surfaces.

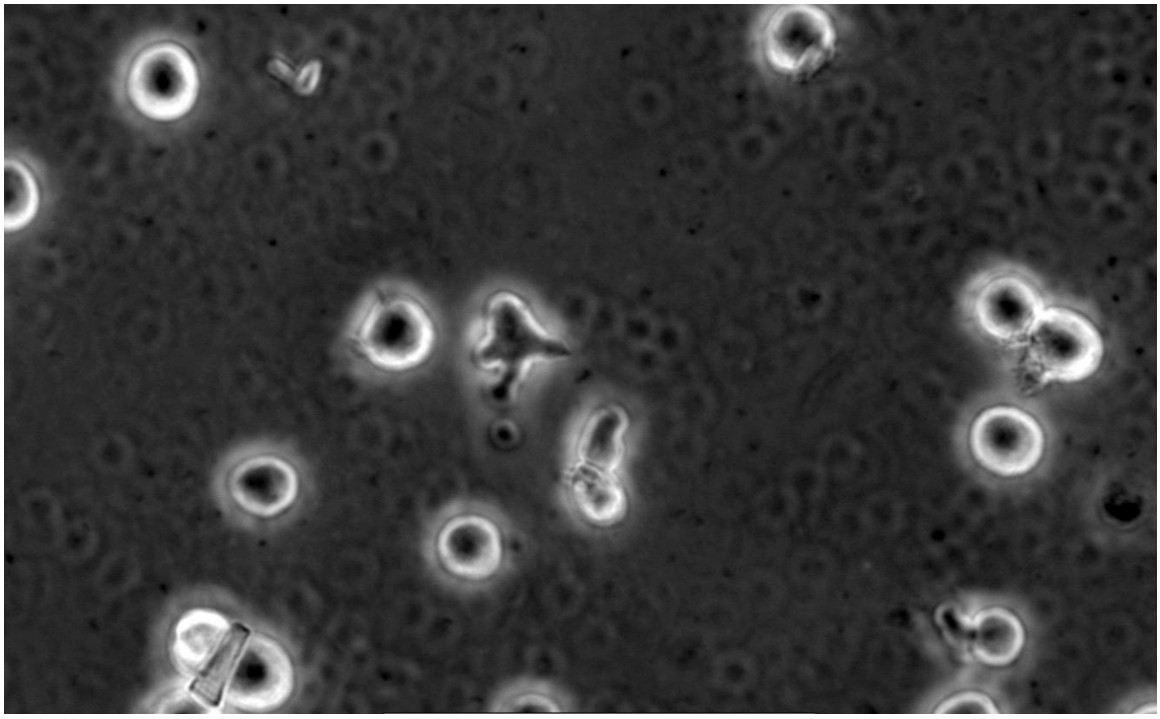


Supplementary Figure 6: Representative DNA spots on a passivated glass surface.

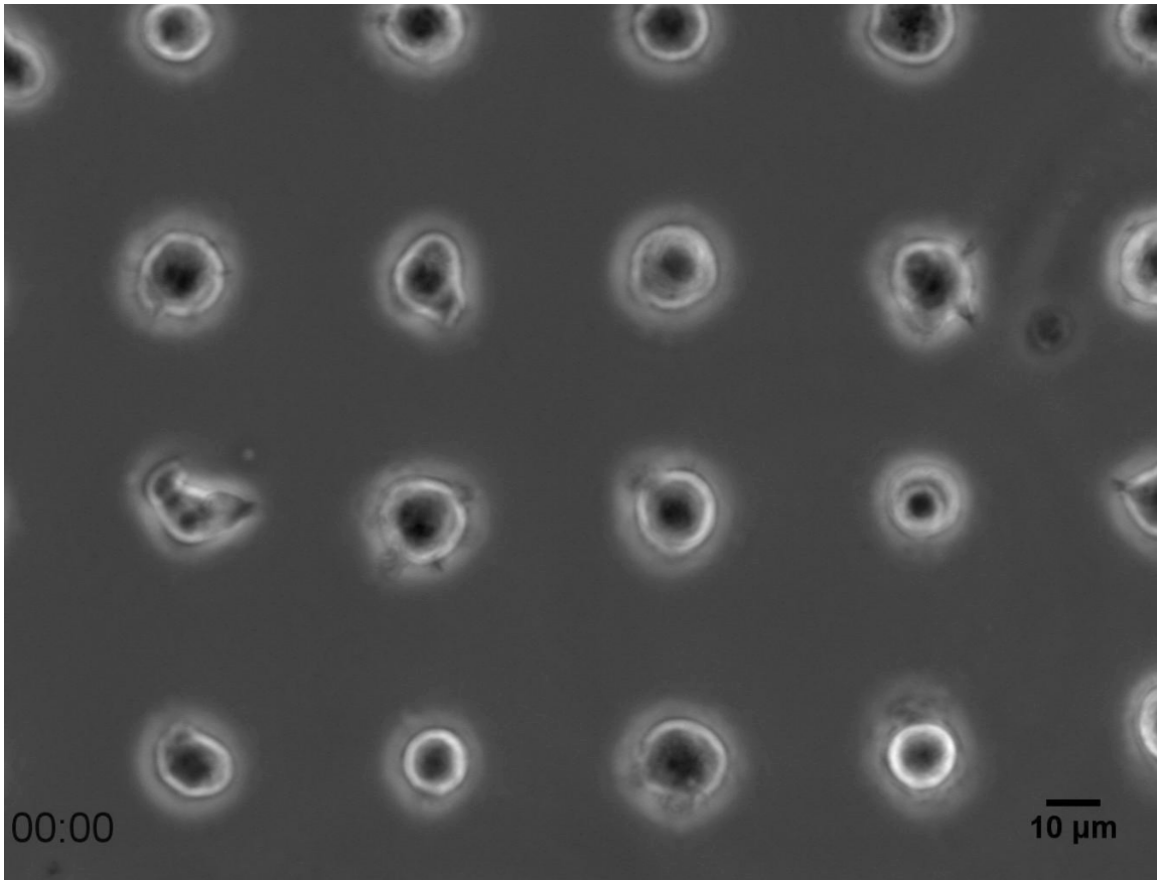


Supplementary movies 7: Single frames of DNA-labeled jurkat cells over bare glass, polylysine coated glass, and passivated glass.





Supplementary Movie 8: Single frame from a 30 minute 400x timelapse video of DNA-labeled Jurkat cells immobilized on a passivated surface with grid of 5-7 μm DNA spots.



Supplementary Movie S9: Single frame from a 30 minute 400x timelapse video of PMA/ionomycin activated DNA-labeled Jurkat cells immobilized on a passivated surface with grid of 5-7 μm DNA spots.