Supplementary Information

Accessing and Assessing the Cell-Surface Glycocalyx Using DNA Origami

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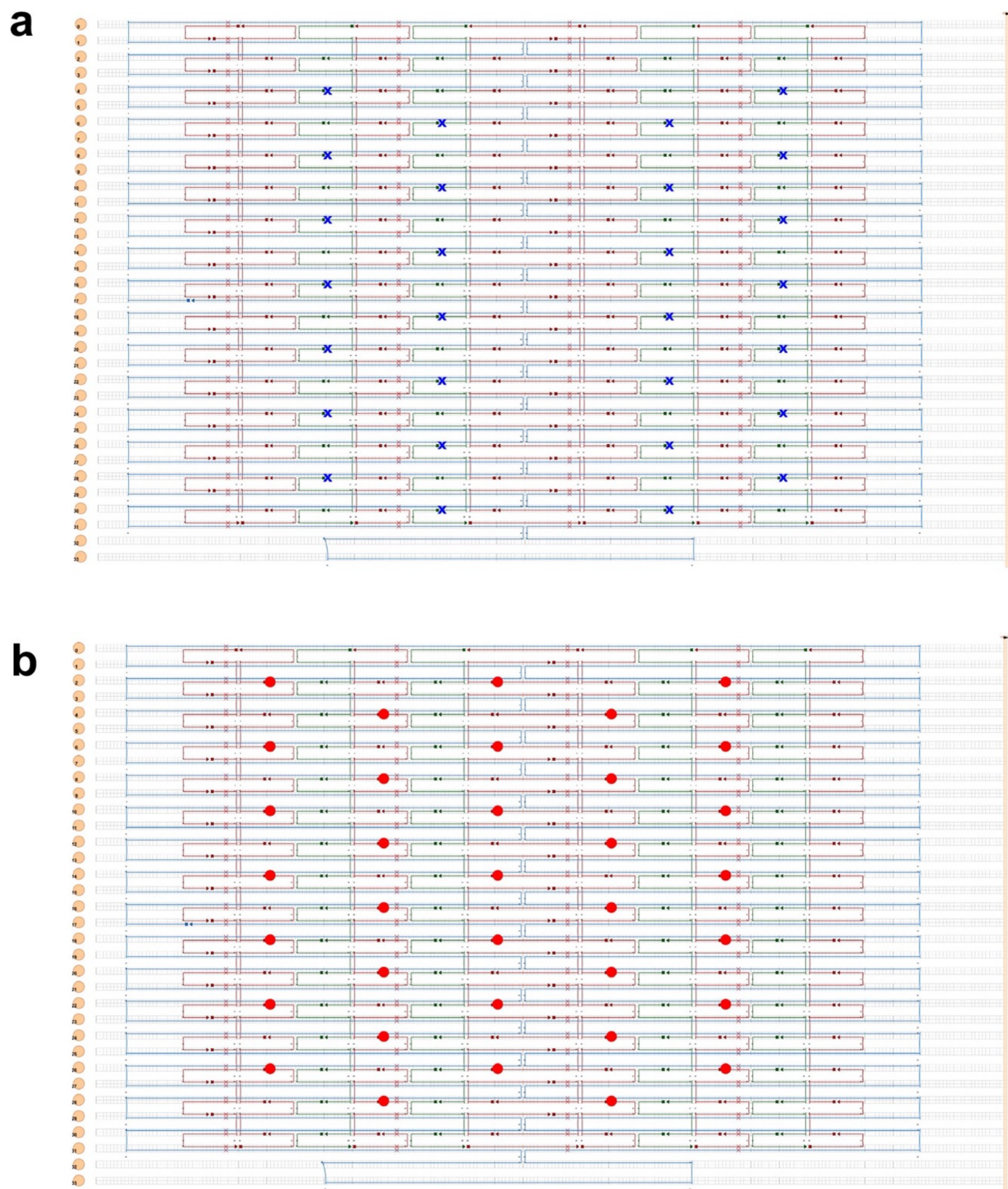
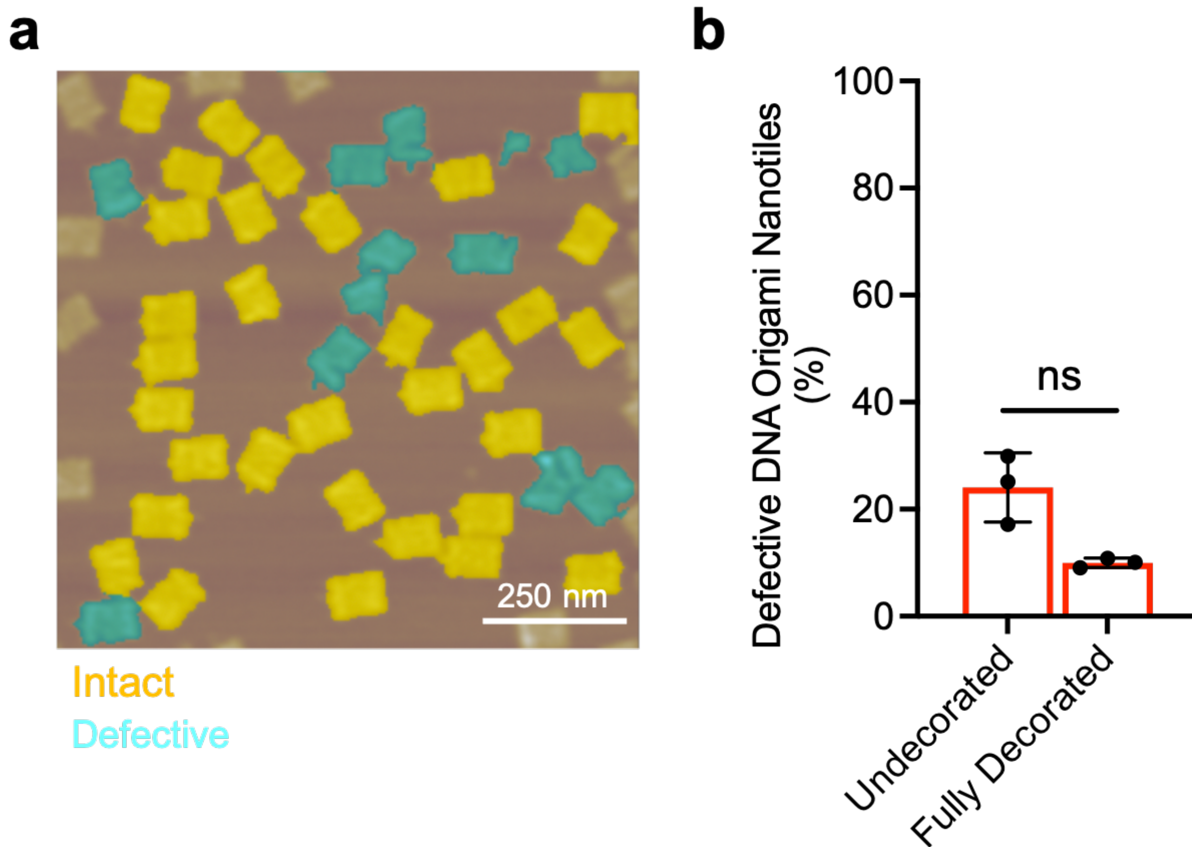
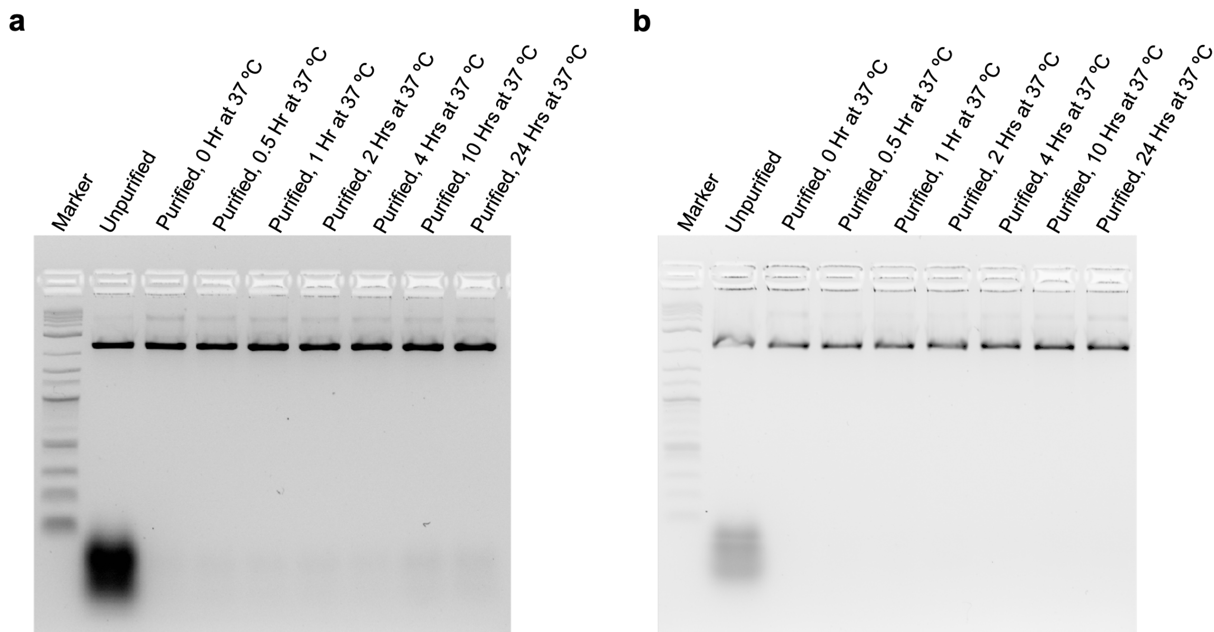
Materials and Methods



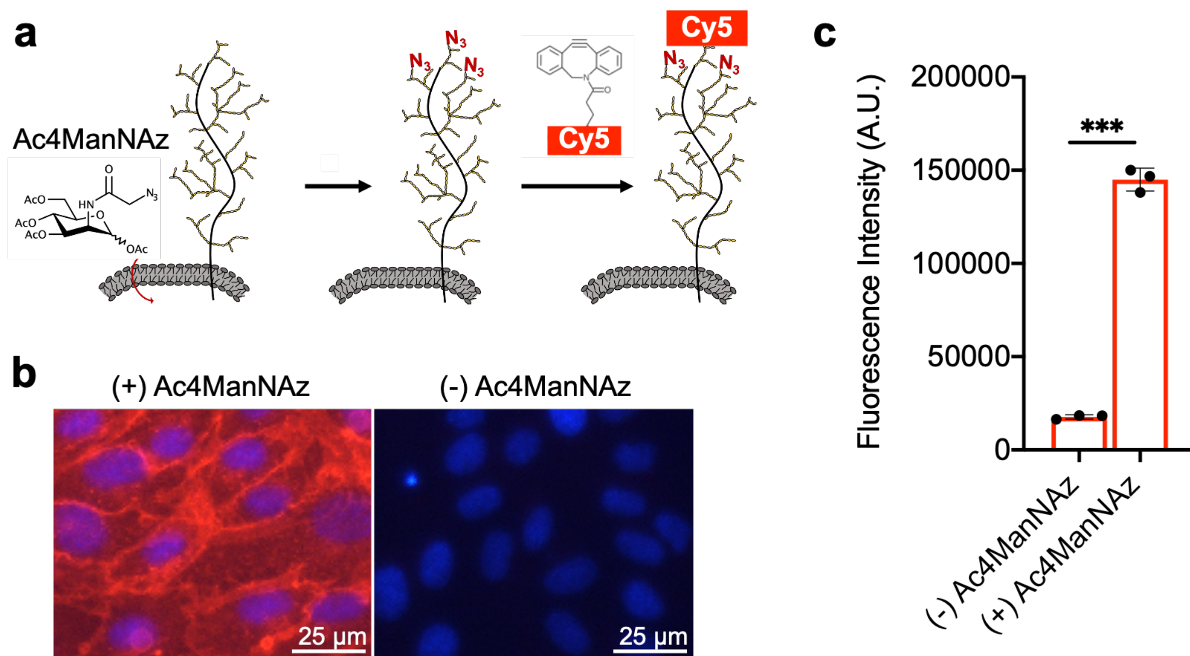
Supplementary Figure 1. Schematics of the cadnano design for the DNA origami nanotile. a, The blue crosses indicate positions of the 28 ssDNA-comp overhangs on the top surface of the fully decorated nanotile. b, The red dots indicate positions of the 35 biotin tags on the bottom surface of the nanotile.



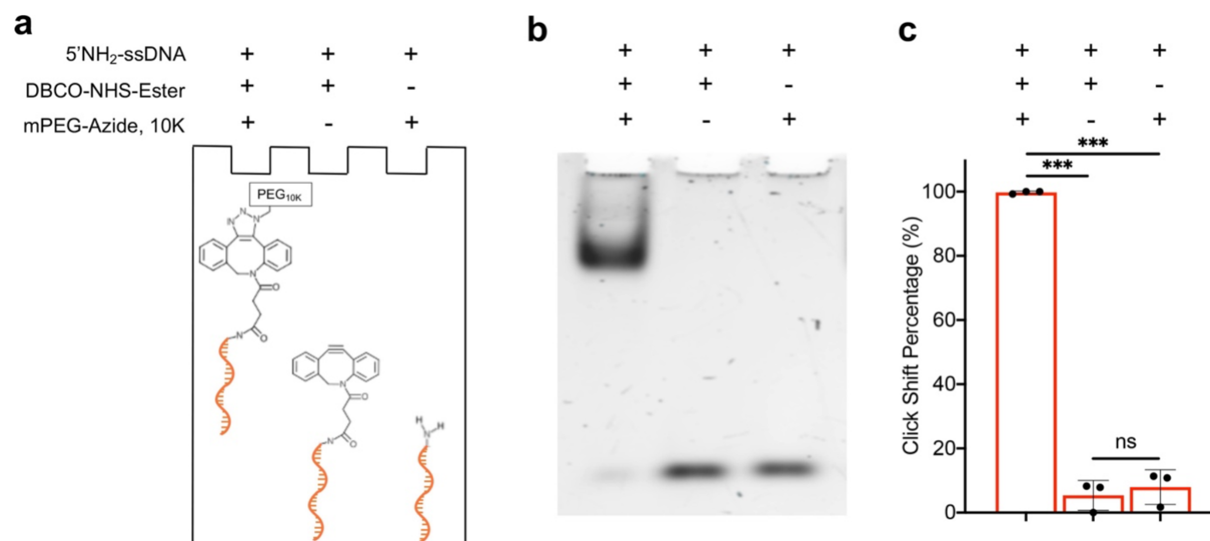
Supplementary Figure 2. Analysis of defective origami nanotile percentage in AFM images. a, intact and defective nanotiles were selected using the Yield Calculator[1] to quantify the yield of intact objects in AFM images. The nanotiles located at the edge of an image and not fully visible were excluded from the analysis. b, Three AFM images of undecorated or fully decorated nanotiles were used to calculate the average defective nanotiles percentage. These quantitative results indicate 24%±6.4 defective percentage in undecorated nanotiles and 10%±0.90 defective percentage in fully decorated nanotiles. Data represent means ± s.d. from three independent replicates.



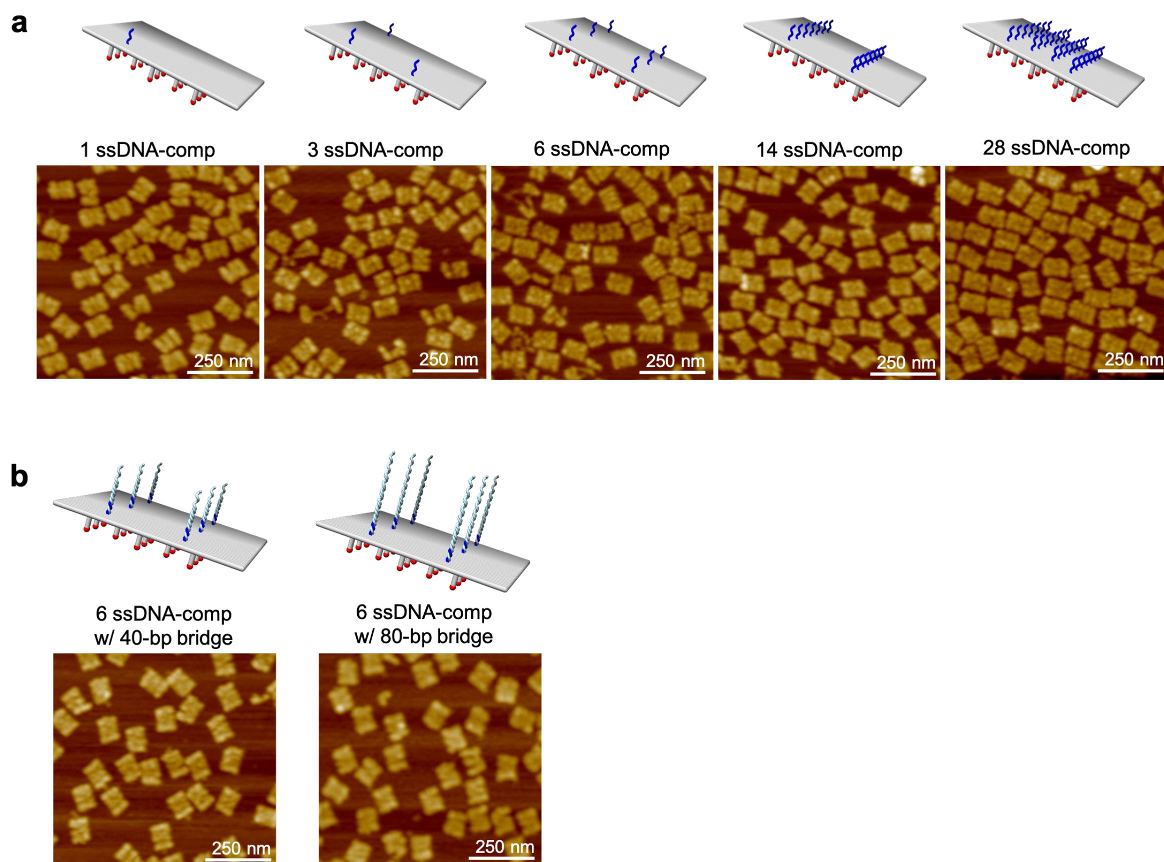
Supplementary Figure 3. Agarose gel electrophoresis analysis of DNA origami nanotiles stability in (a) PBS (with calcium and magnesium) and (b) Endothelial Cell Growth Media (EGM2) over time. Gel analysis of unpurified DNA origami nanotiles with 35 biotin decorations and 6 ssDNA-comp or purified DNA origami nanotiles with 35 biotin decorations and 6 ssDNA-comp, and incubated at 37 ºC for 0, 0.5, 1, 2, 4, 10, and 24 hours (Hrs).



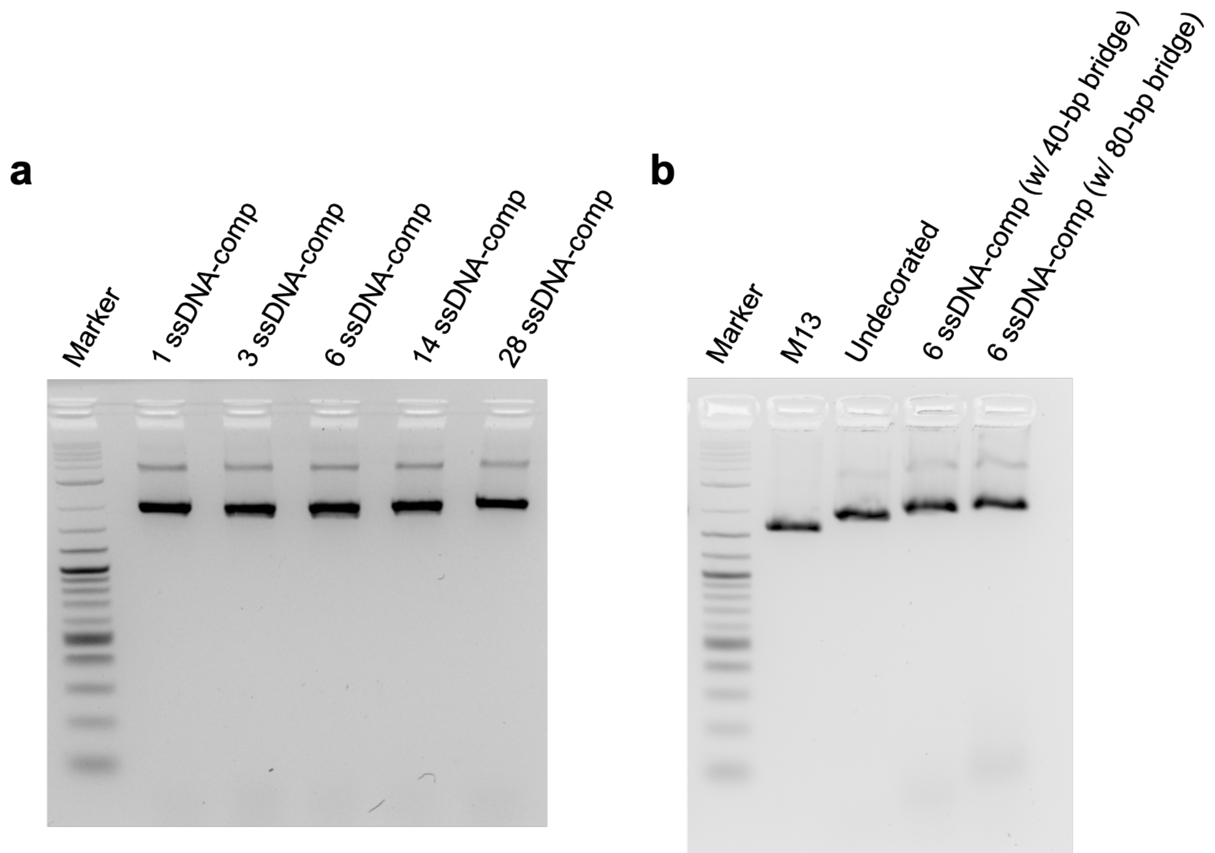
Supplementary Figure 4. Incorporation of azide ligands onto the glycocalyx. a, Azide ligands were incorporated covalently onto the glycocalyx via metabolic glycan labeling using azido monosaccharide, N-azidoacetylmannosamine-tetraacylated (Ac4ManNAz), for 1 day. Cell-surface azide ligands were then incubated with DBCO bearing a Cy5 fluorescence tag (Cy5-DBCO) to allow Cy5 conjugation to potential azide ligands via the DBCO-to-azide click reaction. b,c Fluorescence imaging and quantification indicated that Ac4ManNAz generated robust metabolic azide labeling compared to the control, where no Ac4ManNAz was introduced. Data represent means ± s.d. from three independent replicates. *** $P \leq 0.001$.



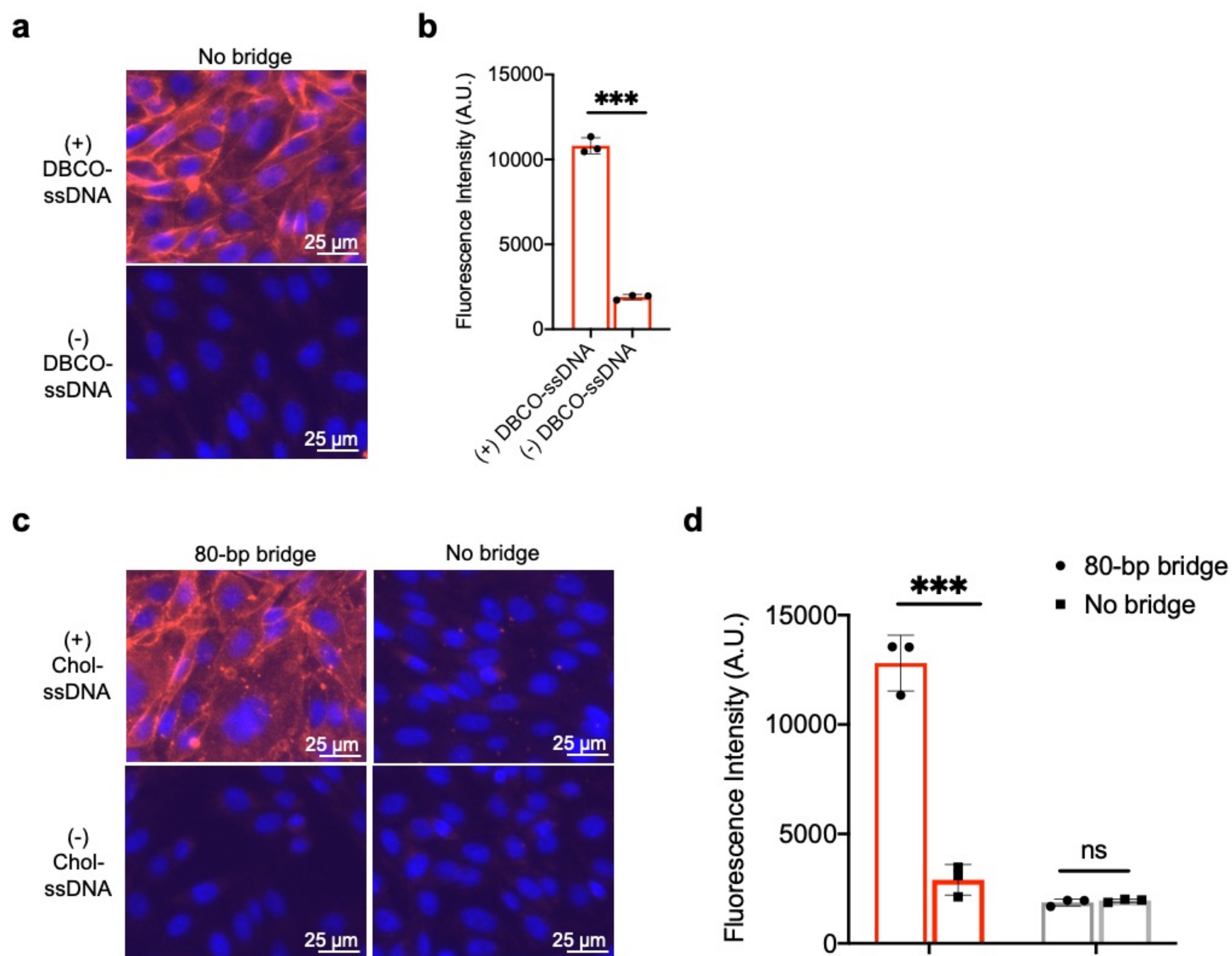
Supplementary Figure 5. Assessment of the synthesized 5’DBCO-ssDNA using a click shift assay and DNA gel electrophoresis. a, In the click shift assay, 5’DBCO-ssDNA was conjugated to PEG-azide (10 kDa) via the DBCO-to-azide click reaction, which is expected to lead to increased molecular weight and reduced mobility in gel electrophoresis. b, 5’DBCO-ssDNA and 5’NH2-ssDNA (control without DBCO) were incubated with PEG-azide (10 kDa) overnight and analyzed by the click shift assay. c, The percentage of click shift was quantified by normalizing the intensity of shifted (high molecular weight) band by the total intensity of the shifted and non-shifted (low molecular weight) bands. Data represent means ± s.d. from three independent replicates. ***$P \leq 0.001$.



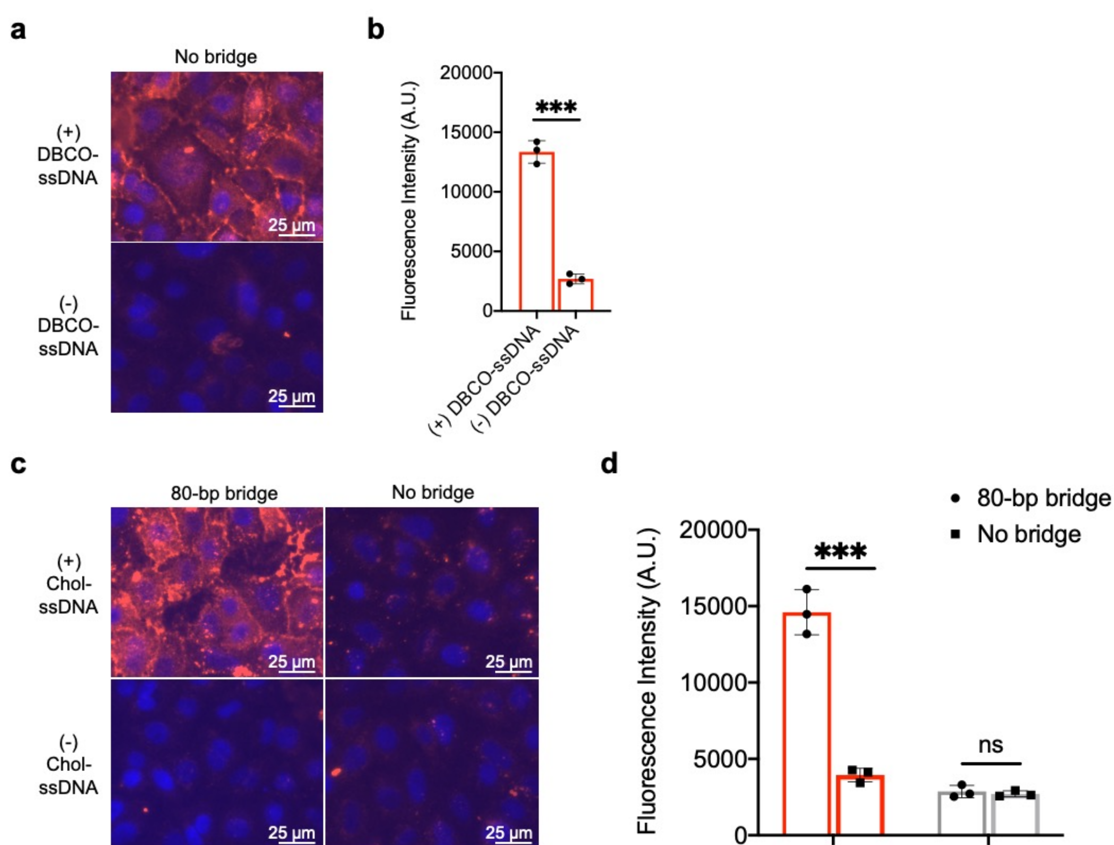
Supplementary Figure 6. AFM characterization of DNA origami nanotiles with different configurations. a, AFM images of nanotiles with 35 biotin decorations and 1, 3, 6, 14, or 28 ssDNA-comp overhangs. b, AFM images of nanotiles with 35 biotin decorations and 6 ssDNA-comp overhangs bearing 40-bp or 80-bp bridges.



Supplementary Figure 7. Agarose gel electrophoresis analysis of DNA origami nanotiles with different configurations. a, Gel electrophoresis analysis of nanotiles with 35 biotin decorations and 1, 3, 6, 14, or 28 ssDNA-comp overhangs. b, Gel analysis of M13 scaffold, undecorated DNA origami nanotile, and nanotiles with 35 biotin decorations and 6 ssDNA-comp overhangs bearing 40-bp or 80-bp bridges. Marker is 2-log DNA ladder.

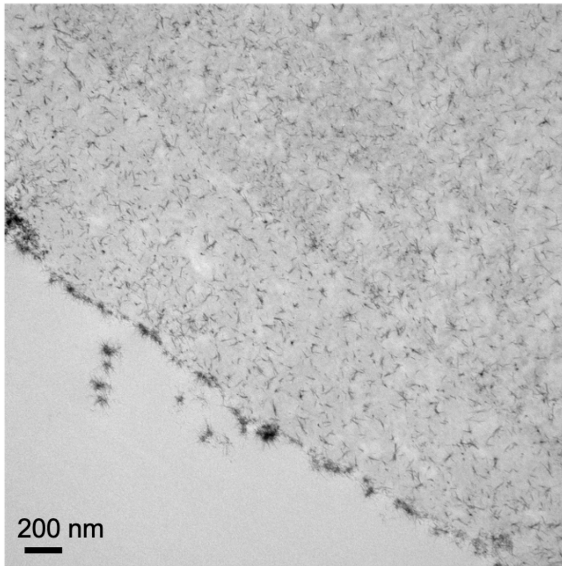


Supplementary Figure 8. Targeting DNA nanotiles to CHO cells. a,b, DBCO-ssDNA initiators were anchored onto the glycocalyx via biorthogonal glycocalyx labeling and copper-free click chemistry. The DNA nanotiles were then targeted to glycocalyx-anchored ssDNA initiators without the DNA duplex bridge. c,d, 5'Chol-ssDNA initiators were anchored onto the PLB via hydrophobic interaction and used to target DNA nanotiles bearing ssDNA-comp overhangs with 80-bp bridge or no bridge. Targeted nanotile immobilization, via glycocalyx or PLB anchored ssDNA initiators, was quantified through fluorescence intensity of biotin staining using fluorophore-conjugated streptavidin. In b and d, data represent means ± s.d. from three independent replicates.

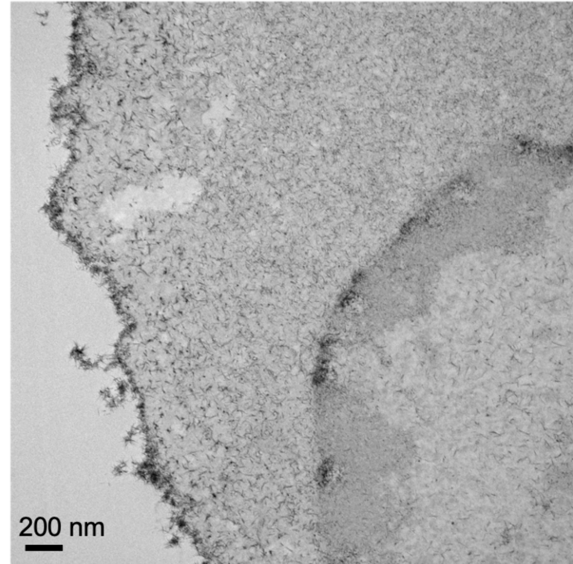


Supplementary Figure 9. Targeting DNA nanotiles to A549 cells. a,b, The DNA nanotiles were then targeted to the epithelial cell glycocalyx via glycocalyx-anchored DBCO-ssDNA initiators without the DNA duplex bridge. c,d, DNA nanotiles with 80-bp bridge or no bridge were targeted to the PLB via PLB-anchored 5'Chol-ssDNA initiators. Cell surface nanotile immobilization was quantified through fluorescence intensity of biotin staining using fluorophore-conjugated streptavidin. In b and d, data represent means ± s.d. from three independent replicates.

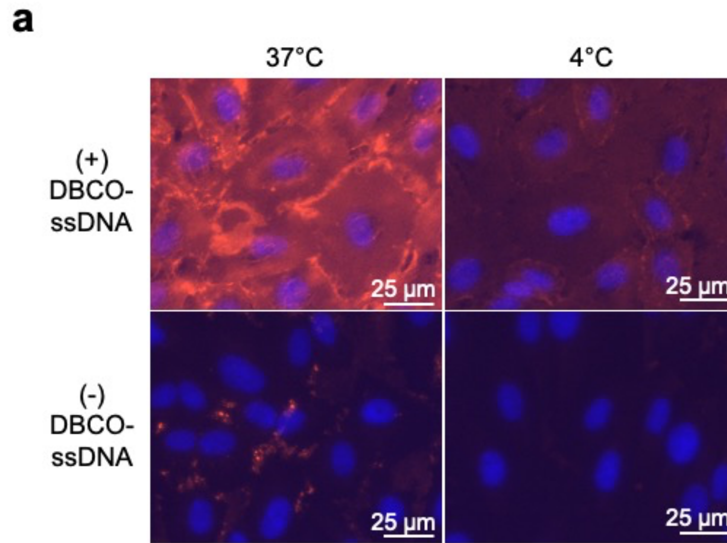
a

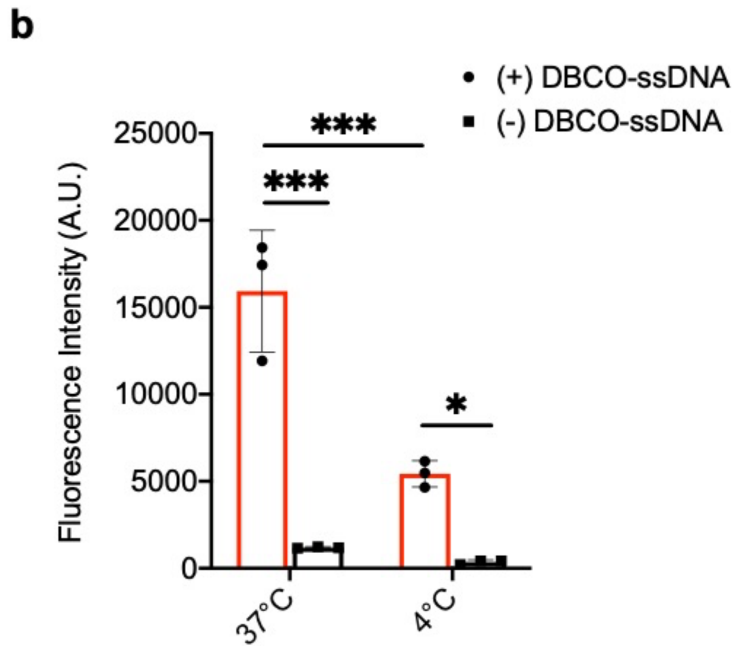


b

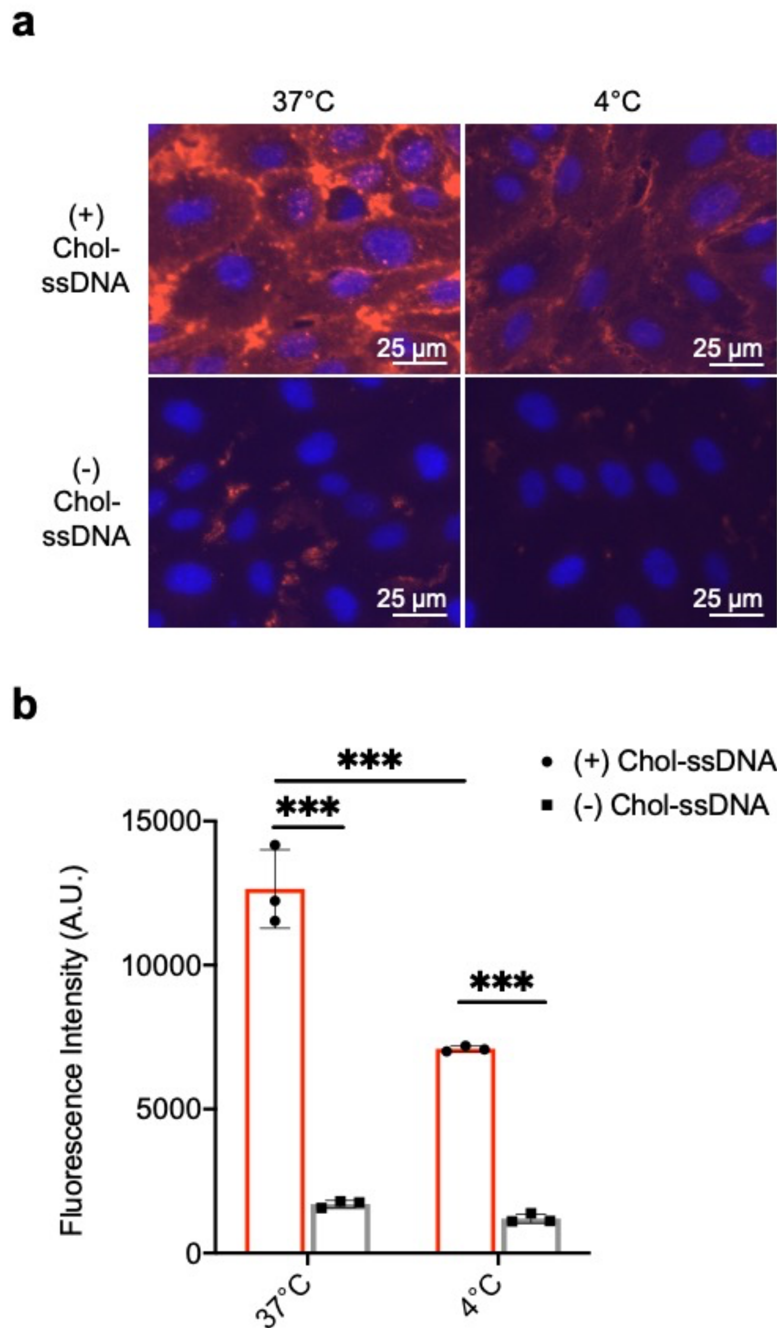


Supplementary Figure 10. TEM images of neuraminidase-treated HUVECs. a, HUVECs pre-treated with 1 U/mL neuraminidase for 1 hour. b, HUVECs not pre-treated with neuraminidase were used as control (b). Data represent means ± s.d. from three independent replicates. * $P \leq 0.05$, ** $P \leq 0.01$.





Supplementary Fig. 11. Targeting DNA nanotiles with 80-bp bridge to glycocalyx-anchored ssDNA initiators at different temperatures. DNA nanotiles with 80-bp bridge were targeted to HUVECs via glycocalyx-anchored ssDNA initiators at 37 °C and 4 °C. Cell surface nanotile immobilization was quantified through fluorescence intensity of fluorophore-labeled streptavidin that is conjugated to biotin in the nanotiles. In b, data represent means ± s.d. from three independent replicates.



Supplementary Fig. 12. Targeting DNA nanotiles with 80-bp bridge to PLB-anchored ssDNA initiators at different temperatures. DNA nanotiles with 80-bp bridge were targeted to HUVECs via PLB-anchored ssDNA initiators at 37 °C and 4 °C. Cell surface nanotile immobilization was quantified through fluorescence intensity of fluorophore-labeled streptavidin that is conjugated to biotin in the nanotiles. In b, data represent means ± s.d. from three independent replicates.

Supplementary Table 1. Reagents

Name	Source	Catalog No.
Acetic acid	Fisher Chemical	A491-212
AFM tips	NanoAndMore	HQ:NSC15/AL BS
Agarose, LE	GenMate	E-3120-500
Amicon Ultra Centrifugal Filter Units	Millipore	UFC5003BK
Boric Acid	Fisher Chemical	A73-500
Bovine Serum Albumin	Fisher BioReagents	BP9706-100
DBCO-Cy5	Sigma-Aldrich	777374-5MG
DBCO-Sulfo-NHS-Ester	Sigma-Aldrich	762040
Dulbecco's Phosphate-Buffered Saline (DPBS)	Corning	21-031-CV
DPBS with calcium and magnesium	Corning	21-030-CV
EDTA	VWR	0105-1KG
Endothelial Cell Growth Medium-2 BulletKit	Lonza	CC-3162
Gelatin	Sigma-Aldrich	G1890-100GA
Hoechst 33258	ThermoFisher Scientific	H3569
Human Umbilical Vein Endothelial Cells	Lonza	C2519A
Hyclone FetalClone 1 Serum (U.S)	GE Healthcare	SH30080.03
Magnesium Chloride	VWR	J364-1KG
Mini-PROTEAN TBE Precast Gels	Bio-Rad	4565013
Neuraminidase, Purified	Worthington Biochemical Company	LS004759

Paraformaldehyde	Sigma-Aldrich	P6148-500G
PEG 8000	Sigma-Aldrich	89510-250G-F
Quick-Load 1 kb Plus DNA Ladder	New England Biolabs	N0469S
RPMI1640	Corning	10-040-CV
Sodium Chloride	Sigma-Aldrich	S3014-500G
Streptavidin, Alexa Fluor 488 Conjugate	Invitrogen	S32354
Streptavidin, Alexa Fluor 647 conjugate	Invitrogen	S32357
SYBR Safe DNA gel stain	Invitrogen	S33102
TRIS	VWR	0826-1KG
Trypsin-EDTA (0.25%)	Gibco	25200056
10X TBE	Fisher BioReagents	BP13334

Supplementary Table 2. Equipment

Name	Source	Catalog No.
ChemiDoc Imaging System	Bio-Rad	12003154
EVOS FL Auto 2 Imaging System	Thermo Fisher Scientific	AMAFD2000
MiniAmp Plus Thermal Cycle	Applied Biosystems	A37029
MFP-3D-BIO AFM	Asylum Research Oxford Instruments	MFP-3D-BIO
SpectraMax	Molecular Devices	i3X
Thermal Cycler	Bio-Rad	C1000 Touch

Supplementary Table 3. List of DNA sequences for cell-surface targeting

(Synthesized by Integrated DNA Technologies)

Name	Sequence
5'NH_2-ssDNA	5'-/5AmMC6/ACTG ACTG ACTG ACTG ACTG/-3'
5'NH_2-ssDNA(bridge)	5'-/5AmMC6/TTGT CTCG TCGT CTAC CGCA AT/-3'
5'NH_2-ssDNA-comp (complementary to ssDNA)	5'-/5AmMC6/CAGT CAGT CAGT CAGT CAGT/-3'
5'FAM-ssDNA-comp	5'-/6FAM/CAGT CAGT CAGT CAGT CAGT/-3'
5'Chol-ssDNA	5'-/Chol-TEG/ACTG ACTG ACTG ACTG ACTG/-3'
5'Chol-ssDNA(bridge)	5'-/Chol-TEG/TTGT CTCG TCGT CTAC CGCA AT/-3'
5'Chol-ssDNA-comp	5'-/Chol-TEG /CAGT CAGT CAGT CAGT CAGT/-3'
40-bp bridge, with both ends complementary to ssDNA and ssDNA(bridge), respectively	Annealed from the following two oligos: 5'-/GTCC TTAC ACAG AGGC TTGA ATTG CGGT AGAC GACG AGAC/-3' 5'-/TCAA GCCT CTGT GTAA GGAC CAGT CAGT CAGT CAGT CAGT/-3'
80-bp bridge, with both ends complementary to ssDNA and ssDNA(bridge), respectively	Annealed from the following two oligos: 5'-/CAGG AATG TGTC TCCG AACT GGTG CTAG TACA ACCT GCCT GAGG TGAA CGAA TATC ACTG ATTG CGGT AGAC GACG AGAC/-3' 5'-/CAGT GATA TTCG TTCA CCTC AGGC AGGT TGTA CTAG CACC AGTT CGGA GACA CATT CCTG CAGT CAGT CAGT CAGT CAGT/-3'

Materials and Methods

Detailed information regarding all materials used in this study was described in Supplementary Tables: reagents (Supplementary Table 1), equipment (Supplementary Table 2), DNA sequences for cell-surface targeting (Supplementary Table 3), and DNA sequences for assembly of origami nanotiles (Supplementary Table 4).

Self-assembly of DNA origami nanotiles

DNA nanotiles were folded from M13mp18 scaffold together with 192 staple strands. For annealing, 10 nM of the scaffold and 50 nM of the staples were mixed in 1x TAE buffer with 12.5 mM MgCl2. The biotin-labeled strands were added pre-annealing at a final concentration of 3 μM. The mixture was heated to 95°C and gradually cooled down to 4°C within 4 hours. The detailed annealing ramp was as the following: 90ºC, 5 minutes; 90ºC-70ºC, 1ºC per minute; 70ºC-45ºC, 0.2 ºC per minute; 45ºC-30ºC, 1ºC per minute; and 30ºC-4ºC, 2ºC per minute.

PEG purification of DNA nanotiles

Following annealing, DNA nanotiles were precipitated in PBS with 7.5% polyethylene glycol (PEG), 12.5 mM MgCl2, 255 mM NaCl, 22.5 mM Tris, 10 mM acetic acid, and 1 mM EDTA. The supernatant was removed, and the pellet was resuspended in DPBS with 12.5 mM MgCl2 to a final concentration of 50 ng/μL.

Agarose gel electrophoreses

Agarose gel electrophoreses of DNA nanotiles were performed with 2% agarose gel in TBE buffer with 12.5 mM MgCl2 at 100 V and room temperature (RT) for 2 hours. 20 μL of 20 nM

samples were loaded into each well. Following electrophoresis, the gel was stained with SYBR Safe DNA gel stain and imaged with the ChemiDoc Imaging System.

Atomic force microscopy (AFM)

10 μL of 2 nM origami structures in DPBS with 12.5 mM $MgCl_2$ was deposited onto a freshly cleaved mica surface, incubated at RT in a humid chamber for 5 minutes, and blow-dried with nitrogen. The sample was washed twice with 20 μL DI water and thoroughly blow-dried with nitrogen before imaging. AFM scans of nanotiles were performed using MFP-3D-BIO AFM with a 5-nm AFM tip in the tapping mode. The quality and integrity of nanotiles were assessed using their AFM images and the Yield Calculator.[1]

Synthesis of 5’DBCO-modified oligos

The 5’NH2-ssDNA, 5’NH2-ssDNA-comp, and 5’NH2-ssDNA(bridge) oligos were incubated overnight in DPBS with DBCO-sulfo-n-hydroxysuccinimidyl ester (DBCO-Sulfo-NHS-Ester) at a 1:10 molar ratio under agitation at RT. This allowed NH2-to-NHS conjugation, giving rise to 5’DBCO-ssDNA, 5’DBCO-ssDNA-comp, and 5’DBCO-ssDNA(bridge). Following overnight incubation, the reaction mixture was dialyzed five times against DPBS using Amicon Ultra Centrifugal filters (molecular weight cut-off, 3 kDa) to remove unconjugated DBCO-Sulfo-NHS-Ester. The above-described conjugation and purification procedures were repeated once again to increase the conjugation efficiency. The final concentration of 5’DBCO-modified oligos was adjusted to 500 μM with DPBS.

Click-shift assay of 5’DBCO-ssDNA

The 5'DBCO-ssDNA was diluted to 10 μM in DPBS and reacted with 100 μM PEG-azide (molecular weight, 10 kDa) overnight at RT. The resulting reaction mixture was analyzed via DNA gel electrophoresis along with controls of 5'NH2-ssDNA that has only been incubated with DBCO-Sulfo-NHS-Ester or PEG-azide. The gel was stained with SYBR Safe DNA gel stain and imaged with the ChemiDoc Imaging System.

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were purchased from the Lonza and have been tested negative for mycoplasma contamination. Cells were cultured in Endothelial Cell Growth Medium-2 (EGM-2) at 37°C with 5% CO_2. HUVECs of passage 3-5 were plated into 96-well culture plates at a density of 40,000 cells per well, one day prior to the experiment.

Metabolic labeling of the cell surface with azide ligands

Azido monosaccharide, N-azidoacetylmannosamine-tetraacylated (Ac4ManNAz, stock in DMSO) was diluted in culture medium to a final concentration of 50 μM and administered to cells for two days. DMSO (0.1%) diluted in culture medium was administered to control cell groups. Cell surface azide incorporation was visualized by its conjugation with azide-reactive, fluorescent Cy5-DBCO, and was imaged using the SpectraMax i3X Multi-Mode Microplate Reader and EVOS FL Auto 2 Cell Imaging System.

Anchorage of ssDNA onto the glycocalyx

The cells, metabolically labeled with azide ligands, were first stained with Hoechst 33258 (1 μg/mL in culture medium for 30 minutes) to label cell nuclei. Next, cells were incubated with 5'DBCO-ssDNA at 50 μM in DPBS (with Ca^{2+} and Mg^{2+}) for 1 hour at 37°C to allow DBCO-

to-azide click conjugation. Cells incubated in DPBS (with Ca2+ and Mg2+), but without 5'DBCO-ssDNA, were used as a control. To examine ssDNA immobilization on the glycocalyx, cells were further incubated with complementary ssDNA oligos with a 5'FAM fluorescent tag (5'FAM-ssDNA-comp) at 1 μM in DPBS (with Ca2+ and Mg2+) for 30 minutes. Cells were imaged using the EVOS System.

Targeting DNA nanotiles to ssDNA initiators anchored on the glycocalyx

Cells with ssDNA immobilized on their glycocalyx were incubated with DNA nanotiles bearing biotin tags and complementary ssDNA-comp overhangs, diluted to 5nM in DPBS (with Ca2+ and Mg2+), for 30 minutes at 37°C. Following washing off the unbound nanotiles, the cells were fixed with 4% paraformaldehyde (PFA) for 10 minutes. Cell-surface nanotiles were visualized by staining with Alexa Fluor 647-conjugate streptavidin in 1% BSA in DPBS (with Ca2+ and Mg2+) for 30 minutes at RT and imaging with the EVOS System.

Anchorage of ssDNA to the phospholipid bilayer (PLB)

First, the cell nuclei were stained with Hoechst 33258. The 5'Chol-ssDNA was diluted to 0.5 μM in DPBS (with Ca^{2+} and Mg^{2+}) and administered to cells for 1 hour at 37°C. Cells incubated in DPBS (with Ca^{2+} and Mg^{2+}) without 5'Chol-ssDNA were used as a control. ssDNA immobilized on the PLB were visualized by further incubating the cells with the complementary 5'FAM-ssDNA-comp at 1 μM in DPBS (with Ca2+ and Mg2+) for 30 minutes and imaging using the EVOS System.

Targeting DNA nanotiles to ssDNA initiators anchored on the PLB

ssDNA immobilized on the PLB were incubated with DNA nanotiles bearing ssDNA-comp overhangs with and without the further extension by the 40-bp or 80-bp bridges, diluted to 5 nM in DPBS (with Ca2+ and Mg2+), for 1 hour at 37°C. The cells were fixed and the surface nanotiles were visualized via fluorescent streptavidin as described in the previous section.

Enzymatic digestion of glycocalyx proteins

Cells pre-stained with Hoechst 33258 were washed twice with 1 mM EDTA in DPBS and incubated with 2.5% trypsin for 30 minutes or 0.05% trypsin for 5 min. The trypsinized cells were neutralized and washed with DPBS (with Ca2+ and Mg2+) prior to the sequential binding to 5'Chol-ssDNA and DNA nanotiles bearing complementary ssDNA-comp overhangs. Following nanotile binding, the cells were transferred to a 48-well plate for fluorescence imaging using the EVOS System.

Neuraminidase treatment of HUVECs

HUVECs were incubated with neuraminidase (dissolved in PBS) at 1 U/mL for 1 hour in Endothelial Cell Basal Medium-2 (EBM-2)[2]. The control HUVEC groups were incubated in EBM-2 (with respective volume of PBS added) alone without neuraminidase. The cells were then washed three times with PBS (with calcium and magnesium) before introduction of ssDNA initiators and DNA nanotiles.

Transmission electron microscopy

The cells were fixed with 2% glutaraldehyde, 2% sucrose, and 2% lanthanum nitrate in 0.1 mol/L sodium cacodylate buffer (pH 7.3) for 2 hours at room temperature and prepared for electron microscopy imaging according to the reported protocol[3].

DNA-guided multicellular assembly via cell-surface initiators

HUVECs were trypsinized into single-cell suspension. The two participating cell groups were fluorescently labeled with two different dyes: CellTracker Green CMFDA Dye (10 μM in DPBS, supplemented with 1mM EDTA, for 30 minutes at 4°C) and CellTracker Deep Red Dye (1 μM in DPBS, supplemented with 1mM EDTA, for 30 minutes at 4°C).

For assembly mediated by PLB-anchored initiators, the PLB of the red cell group was decorated with 5'Chol-ssDNA. The green cell group was further divided into two sub-groups. For direct assembly without the dsDNA bridge, the PLB of the green cells was decorated with 5'Chol-ssDNA-comp. For assembly with the dsDNA bridge, the PLB of the green cells was first decorated with 5'Chol-ssDNA(bridge) and then hybridized with an 80-bp bridge that carries ssDNA-comp at one end and ssDNA(bridge)-comp at the other end.

For assembly mediated by glycocalyx-anchored initiators, the glycocalyx of the red cell group was decorated with 5'DBCO-ssDNA. The green cell group was further divided into two sub-groups. For direct assembly without the dsDNA bridge, the PLB of the green cells was decorated with 5'DBCO-ssDNA-comp. For assembly with the dsDNA bridge, the PLB of the green cells was first decorated with 5'DBCO-ssDNA(bridge) and then hybridized with an 80-bp bridge that carries ssDNA-comp at one end and ssDNA(bridge)-comp at the other end.

For cell-to-cell assembly, red and green cells were mixed in a 0.2-mL microcentrifuge tube at a green-to-red cell ratio of 1:100 (10,000 cells/mL : 1,000,000 cells/mL), centrifuged at 10 g for 2 minutes and incubated on ice for an additional 10 minutes. Following assembly, cells were transferred into a 24-well plate for fluorescence imaging using the EVOS System.

Comparing cellular uptake of DNA nanotiles anchored on the glycocalyx versus the PLB

The ssDNA was immobilized onto the glycocalyx or PLB of Hoechst 33258 stained HUVECs. The cells were incubated with DNA nanotiles carrying ssDNA-comp overhangs with the 80-bp bridge for 30 minutes, 1 hour, or 2 hours. Cells were then fixed with PFA and stained with the Alexa Fluor 647-conjugated streptavidin for visualization of cell-surface nanotiles. Cells were then permeabilized with 0.5% Triton-X for 15 minutes at RT. The uptaken nanotiles were then visualized via incubation of the permeabilized cells with the Alexa Fluor 488-conjugated streptavidin in 1% BSA in DPBS (with Ca^{2+} and Mg^{2+}) for 30 minutes at RT.

Quantitative image analysis of the uptaken DNA nanotiles

Image analysis for the quantification of cellular uptake of DNA nanotiles was performed using custom MATLAB codes. Images were manually selected in the Cy5 and GFP channels from all groups. For the GFP channel, uptaken signals were identified by constructing structuring elements and performing morphological transformations[50-51]. The images were then binarized using adaptive thresholding to further subtract the background noise[4-7]. The intensities of post-processed images were then calculated by measuring the intensity of all pixels. For the Cy5 channel, the intensity of each image was directly computed without post-processing.

Statistics

Quantitative data were displayed as means ± s.d. Statistical significances were determined using one-way analysis of variance (ANOVA) with post-hoc Tukey's Test and unpaired t-test. Statistical analyses were performed using GraphPad.

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Supplementary Table 4. List of DNA sequences for assembly of origami nanotiles

Name	Sequence	Note
seam01	TCAGGAGGTTTAGTACCGCCACCCTCAGAAC	
seam02	TTTGCTAAGTAAATGAATTTTCTGAGTGCCTT	
seam03 S1	GAGTAACAGTTTTAACGGGGTCTATGGGATTTGAGAGCAGACCTGGAACTCG	
seam04	TAACCGATATGACAACAACCATCGCACCACCC	
seam05	TCAGAGCCGCCACCCTCAGAGCCCCACGCA	
seam06	CACTAAAATAAAACGAAAGAGGCAACGTCACC	
seam07 S1	AATGAAATTAGCAAGGCCGGAAAAAGAATATTGAGAGCAGACCTGGAACTCG	
seam08	TGACCTTCTACAGACCAGGCGCATACCACGGA	
seam09	ATAAGTTAAGAAACGCAAAGACAGGCTGGC	
seam10	AAAATCTAATACCAGTCAGGACGTGCAAGAAA	
seam11 S1	CAATGAAAGCCCAATAATAAGATGGGAAGATTGAGAGCAGACCTGGAACTCG	
seam12	GGGGTAATTTTGCAAAAGAAGTTTAAATAAAC	
seam13	AGCCATAAATTTGCCAGTTACATGCCAGAG	
seam14	TTCGAGCTAAGACTTCAAATATCGATCGTAGG	
seam15 S1	AATCATTGCCGTTTTTATTTTCCGTTTTAATTGAGAGCAGACCTGGAACTCG	
seam16	AGTAGATTAGTTGATTCCCAATTCTTCTGTCC	
seam17	AGACGACCAAAAGGTAAAGTAATGCGAACG	
seam18	CCCTGTAATCGGTTGTACCAAAAATAAGGCGT	
seam19 S1	TAAATAACGACCGTGTGATAAACATTATGATTGAGAGCAGACCTGGAACTCG	
seam20	AGGCTATCTAGCTATTTTTGAGAGAAGACGCT	
seam21	GAGAAGAGCGATAGCTTAGATTATCTACAA	
seam22	ACGCCATCCAGCTCATTTTTTAACAGGCGAAT	
seam23 S1	TATTCATTACAAAATCGCGCAGCAATAGGATTGAGAGCAGACCTGGAACTCG	
seam24	AGCTTTCCCCTCAGGAAGATCGCACATCAATA	
seam25	TAATCCTCAGATGATGGCAATTCTCCAGCC	
seam26	GGTCGACTCAGTGCCAAGCTTGCAGGAGCACT	
seam27 S1	AACAACTTCTAAAATATCTTTATGCCTGCATTGAGAGCAGACCTGGAACTCG	
seam28	TTAATGAAGGGAAACCTGTCGTGCATTAAAAA	
seam29	TACCGAACCCTAAAACATCGCCCAGCTGCA	
seam30	CCCGAGATATCCCTTATAAATCAAAAACGCTC	
seam31	ATGGAAACCATTGCAACAGGAAAAGAATAG	
seam32	GAGCTTGACGGGGAAAAAAGGGATTTTAGAC	
X01Y01	TAGGAACCCATGTACACAACGCC	
X01Y02	TGTAGCATAATTTTTTCACGTTCCTTTAAT	
X01Y03	TGTATCGAGCGAAAGACAGCATTGAGGACT	
X01Y04	AAAGACTCATCGCCTGATAAATTTAGCCGG	
X01Y05	AACGAGGATTCAGTGAATAAGGTAAATTGG	
X01Y06	GCTTGAGTAGGAATACCACATTTTACGAGG	
X01Y07	CATAGTACCCCTCAAATGCTTTCAAAAATC	
X01Y08	AGGTCTTTCCTTTTGATAAGAGCTGAATAT	
X01Y09	AATGCTGGGGCGCGAGCTGAAATTAACATC	
X01Y10	CAATAAATTTAAATGCAATGCCGAGAAAGG	
X01Y11	CCGGAGAATGTCAATCATATGTCAGGAAGA	
X01Y12	TTGTATAACAACCCGTCGGATTTGGGATAG	
X01Y13	GTCACGTTGGGAAGGGCGATCGGAAAGGGG	
X01Y14	GATGTGCAATTGTTATCCGCTCAAAGTGTA	
X01Y15	AAGCCTGAGTGAGACGGGCAACGAGTTGCA	
X01Y16	GCAAGCGCAAAGGGCGAAAAACCATCACCCAAATCAA	
X02Y01	GCGAATAATTCCACAGACAGCCCTGGGATAGCAAGCCCAA	
X02Y02 S1	CCCTCAGCGTTTATCAGCTTGCTTAAGGAATTTTGAGAGCAGACCTGGAACTCG	
X02Y03	ATTTGTATTTTTCATGAGGAAGTTGATCGTCA	
X02Y04 S1	AGCTGCTCCGCAGACGGTCAATCACAACGGAGTTGAGAGCAGACCTGGAACTCG	
X02Y05	TTGAGATTATGGTTTAATTTCAACCGTAACAA	
X02Y06 S1	ATTGAATCAGAGCAACACTATCATTTCATCAGTTGAGAGCAGACCTGGAACTCG	
X02Y07	TTAATTGCTACCCTGACTATTATAAAATATTC	
X02Y08 S1	TTCATTTGTAGCTCAACATGTTTTGAGTACCTTTGAGAGCAGACCTGGAACTCG	
X02Y09	TCATATATTCATACAGGCAAGGCAGCTATATT	

X02Y10 S1	AAACTAGCCAGTCAAATCACCATCTAGAACCCTTGAGAGCAGACCTGGAACTCG	
X02Y11	AGCGAGTAAGCAAATATTTAAATTTAATCGTA	
X02Y12 S1	GCAACTGTTGGTGTAGATGGGCGCTAAATGTGTTGAGAGCAGACCTGGAACTCG	
X02Y13	CTGTGTGATGCAAGGCGATTAAGTCAGGCTGC	
X02Y14 S1	TTTTCACCGGGTGCCTAATGAGTGGCTGTTTCTTGAGAGCAGACCTGGAACTCG	
X02Y15	TCCAACGTGTCCACGCTGGTTTGCGGTTTTTC	
X02Y16	GTTTTTTGGGGTCGAGACGTGGAC	
X03Y01	ACCACCCTCATTTTCACATAGTTAGCGTAACGTAGAAAGG	
X03Y02	AACAACTATCGAGGTGAATTTCTTAAGGCCGC	S2 stalk / bridge (via S3 stalk)
X03Y03	TTTTGCGGTCCATTAAACGGGTAAAGCGCGAA	
X03Y04	ACAAAGTATAAGGGAACCGAACTGTCATTACC	S2 stalk
X03Y05	CAAATCAATTTAATCATTGTGAATATTACAGG	
X03Y06	TAGAAAGAAACCCTCGTTTACCAGACTGCGGA	S2 stalk
X03Y07	ATCGTCATGTCAGAAGCAAAGCGGACAGGTCA	
X03Y08	GGATTAGAAAATATGCAACTAAAGGTCAATAA	S2 stalk / bridge (via S3 stalk)
X03Y09	CCTGTTTAAAGAATTAGCAAAATTCAAGGATA	
X03Y10	AAAATTTTAATATGATATTCAACCGAGAATCG	S2 stalk
X03Y11	ATGAACGGGTAAACGTTAATATTTCAGCTTTC	
X03Y12	ATCAACATATCGTAACCGTGCATCAGCGCCAT	S2 stalk
X03Y13	TCGCCATTTGGGTAACGCCAGGGTCGTAATCA	
X03Y14	TGGTCATAAGCTAACTCACATTAATATTGGGC	S2 stalk / bridge (via S3 stalk)
X03Y15	GCCAGGGTCCCAGCAGGCGAAAATAGTCCACT	
X03Y16	ATTAAAGAGTGCCGTAAAGCACTA	
X04Y01	AGTGAGAAATCTAAAGTTTTGTCGCCACCCTCAGAGCC	
X04Y02	GGGAGTTAAAACAGCTTGATACCTCAGCGG	
X04Y03 S1	TTATACCAAATACGTAATGCCACGCTTGCATTGAGAGCAGACCTGGAACTCG	
X04Y04	CCGGATATACCAACTTTGAAAGACCAGCGA	
X04Y05 S1	ACAACATTTACCTTATGCGATTTACAAGAATTGAGAGCAGACCTGGAACTCG	
X04Y06	CGTCCAATACGACGATAAAAACCTAACGGA	
X04Y07 S1	AAACTCCAATTGCATCAAAAAGATGGATAGTTGAGAGCAGACCTGGAACTCG	
X04Y08	CGCAAATGTACGGTGTCTGGAAGCGGAAGC	
X04Y09 S1	TTTCAACGAAGCAATAAAGCCTCTACATTTTTGAGAGCAGACCTGGAACTCG	
X04Y10	GCAAACAAGTTCTAGCTGATAAAGCCTTTA	
X04Y11 S1	CCTGTAGCTGTTAAAATTCGCATGTCTGGATTGAGAGCAGACCTGGAACTCG	
X04Y12	CCAGGCAATGCCAGTTTGAGGGGTGGCCTT	
X04Y13 S1	CTCGAATTTTTCCCAGTCACGACCCGGAAATTGAGAGCAGACCTGGAACTCG	
X04Y14	GGTTTGCGTTGCGTTGCGCTCACTACCGAG	
X04Y15 S1	GGAACAAGCCTGTTTGATGGTGGGAGAGGCTTGAGAGCAGACCTGGAACTCG	
X04Y16	AATCGGAACCCTAAACCAGTTT	
X05Y01	CGCCACCCTCAGAACCGTCTTTCCAGACGTTAACAACTTT	
X05Y02	CAACAGTTGATAGTTGCGCCGACAATATTCGG	
X05Y03	TCGCTGAGTACGAAGGCACCAACCCACTCATC	S2 stalk
X05Y04	TTTGACCCGGACAGATGAACGGTGATCAAGAG	
X05Y05	TAATCTTGTAAGAACTGGCTCATTCGTTAATA	S2 stalk
X05Y06	AAACGAACAAAATAGCGAGAGGCTAGTAAAAT	
X05Y07	GTTTAGACTTAAGAGGAAGCCCGATCAAAGCG	S2 stalk
X05Y08	AACCAGACTTTCATTCCATATAACTAGTTTGA	
X05Y09	CCATTAGAAGAGCATAAAGCTAAATACTTTTG	S2 stalk
X05Y10	CGGGAGAATTAATGCCGGAGAGGGAGGTCATT	
X05Y11	GCCTGAGATAAATTTTTGTTAAATAAAAATAA	S2 stalk
X05Y12	TTCGCGTCACGACGACAGTATCGGGGCACCGC	
X05Y13	TTCTGGTGGTTGTAAAACGACGGCCTAGAGGA	S2 stalk
X05Y14	TCCCCGGGTGCCCGCTTTCCAGTCTCGGCCAA	
X05Y15	CGCGCGGGTTCCGAAATCGGCAAAAGGGTTGA	S2 stalk
X05Y16	GTGTTGTTGGGAGCCCCCGATTTA	
X06Y01	TGGTAATAAGTGCCCGTATAAACAAGGTGTATCACCGTAC	
X06Y02	CTCAGAACCGCCACCAGAACCACCGAGTGTAC	
X06Y03 S1	CATTACCACCATCGATAGCAGCACCCGCCACCTTGAGAGCAGACCTGGAACTCG	
X06Y04	ACATATAATATTTTGTCACAATCAAGTAGCAC	
X06Y05 S1	TTGAGTTAATAGCAATAGCTATCTAGGTGGCATTGAGAGCAGACCTGGAACTCG	

X06Y06	CAGAGCCTTTATTTATCCCAATCCCACAAGAA	
X06Y07 S1	ACAAGCAAACCGCGCCCAATAGCACGTCTTTCTTGAGAGCAGACCTGGAACTCG	
X06Y08	AGTACCGAGACAATAAACAACATGCATCGAGA	
X06Y09 S1	TGAAATACGAATAAACACCGGAATGAATATAATTGAGAGCAGACCTGGAACTCG	
X06Y10	AAAACATAGTCAATAGTGAATTTATAATGGTT	
X06Y11 S1	TACCAAGTTTCAATTACCTGAGCAAATCCTTGTTGAGAGCAGACCTGGAACTCG	
X06Y12	CTGATTATGATTGTTTGGATTATAGCTTTGAA	
X06Y13 S1	GAAGGTTAAATAGATTAGAGCCGTTCATATTCTTGAGAGCAGACCTGGAACTCG	
X06Y14	ACTGATAGCGAACCACCAGCAGAAGAATTGAG	
X06Y15 S1	ACCGCCAGTACCTACATTTTGACGATGCGCGATTGAGAGCAGACCTGGAACTCG	
X06Y16	AGGAACGGTACGCCAGACAATATT	
X07Y01	AAGTATAGCCCGGAATGTTAATGCCCCCTGCCGCTTTTGA	
X07Y02	TGATACAGACCAGAGCCGCCGCCAACCGCCTC	
X07Y03	CCTCAGAGCGTAATCAGTAGCGACAGCCAGCA	S2 stalk
X07Y04	AAATCACCATAGAAAATTCATATGGAAAATAC	
X07Y05	ATACATAATACCGAAGCCCTTTTTTATCAGAG	S2 stalk
X07Y06	AGATAACCAAATAAGAAACGATTTTACCAACG	
X07Y07	CTAACGAGAGCAAATCAGATATAGAACCAAGT	S2 stalk
X07Y08	ACCGCACTTTCAGCTAATGCAGAACGAGCCAG	
X07Y09	TAATAAGACATAATTACTAGAAAATCTTCTGA	S2 stalk
X07Y10	CCTAAATTTCAAAATCATAGGTCTATTAATTT	
X07Y11	TCCCTTAGAAAGAAGATGATGAAAACGGATTC	S2 stalk
X07Y12	GCCTGATTCTTCTGAATAATGGAAAGGAGCGG	
X07Y13	AATTATCACAATAGATAATACATTAATCAACA	S2 stalk
X07Y14	GTTGAAAGGATAAAACAGAGGTGATGGCTATT	
X07Y15	AGTCTTTACTCAATCGTCTGAAATGCTGGTAA	S2 stalk
X07Y16	TATCCAGAAATCCTGAGAAGTGTT	
X08Y01	CATACATGTATTTCGGAACCTATCGAGAGGGTTGATAT	
X08Y02 S1	CCACCGGAGCATTGACAGGAGGTTAAGCGTTTGAGAGCAGACCTGGAACTCG	
X08Y03	GGAATTAGAGAATCAAGTTTGCCAGAGCCA	
X08Y04 S1	CAAACGTAGTTTACCAGCGCCAACCATTTGTTGAGAGCAGACCTGGAACTCG	
X08Y05	AGCGCTAAAAGAAAAGTAAGCAGATGTTAG	
X08Y06 S1	CTGAATCTTTTGTTTAACGTCAAGTAATTGTTGAGAGCAGACCTGGAACTCG	
X08Y07	GGGTATTAAAGGCTTATCCGGTATTTTATC	
X08Y08 S1	GGCATTTTCGCGCCTGTTTATCACAAGAACTTGAGAGCAGACCTGGAACTCG	
X08Y09	TAATTTCAAGCCTGTTTAGTATCAGGCAGA	
X08Y10 S1	CGCTATTAGAGAGACTACCTTTTTTTTAGTTTGAGAGCAGACCTGGAACTCG	
X08Y11	AAACAATACAAACATCAAGAAAAAAATCGT	
X08Y12 S1	CCACCAGAGGGTTAGAACCTACCTCGGGAGTTGAGAGCAGACCTGGAACTCG	
X08Y13	AGTTGGCATGAGGATTTAGAAGTAAAGAAA	
X08Y14 S1	TTTTTGAAGGCGGTCAGTATTAATCTGGTCTTGAGAGCAGACCTGGAACTCG	
X08Y15	TCGGCCTTGGATTATTTACATTGGACAATA	
X08Y16	TTTATAATCAGTGAGCAAACTA	
X09Y01	GGCGGATAAGTGCCGTTATTCTGAAACATGAAGAATTTAC	
X09Y02	CGTTCCAGTGAGGCAGGTCAGACGCAAAATCA	S2 stalk / bridge (via S3 stalk)
X09Y03	CCGGAACCTTTAGCGTCAGACTGTCCGTCACC	
X09Y04	GACTTGAGAGACAAAAGGGCGACACTCCTTAT	S2 stalk
X09Y05	TACGCAGTATAGCCGAACAAAGTTTGAACAAA	
X09Y06	GTCAGAGGAAATGAAAATAGCAGCTTGCACCC	S2 stalk
X09Y07	AGCTACAATTCTAAGAACGCGAGGTCTTTCCT	
X09Y08	TATCATTCACAATAGATAAGTCCTCGCCAACA	S2 stalk / bridge (via S3 stalk)
X09Y09	TGTAATTTATATGCGTTATACAAAAACTTTTT	
X09Y10	CAAATATATAACCTCCGGCTTAGGATAACCTT	S2 stalk
X09Y11	GCTTCTGTCAAAATTAATTACATTAACAGTAC	
X09Y12	CTTTTACAATATCAAAATTATTTGTATCATTT	S2 stalk
X09Y13	TGCGGAACATTAGACTTTACAAACAAACCCTC	
X09Y14	AATCAATACACCGCCTGCAACAGTAAGAATAC	S2 stalk / bridge (via S3 stalk)
X09Y15	GTGGCACAGCAGATTCACCAGTCACCTGAGTA	
X09Y16	GAAGAACTGCCACCGAGTAAAAGA	
X10Y01	CAGTCTCTAGTATTAAGAGGCTGAGTTTTGCTCAGTACCA	

X10Y02	TTCATAATATTGGCCTTGATATTCGGAAAGCG	
X10Y03	AATTATCAAGCGCGTTTTCATCGGGCCATCTT	
X10Y04	GATTAAGATTCAACCGATTGAGGGTAAAGGTG	
X10Y05	GAACACCCACCAGAAGGAAACCGAACTGGCAT	
X10Y06	TTGCTATTCTTTACAGAGAGAATAAATTAACT	
X10Y07	ATCGGCTGCGTTTTAGCGAACCTCAAGATTAG	
X10Y08	TTTAACAAGAACAAGAAAAATAATAATCAATA	
X10Y09	CGCGAGAATTCTTACCAGTATAAATCGCCATA	
X10Y10	GTGAGTGATTGGGTTATATAACTAACAAAGAA	
X10Y11	TATACAGTTAACAATTTCATTTGACAATATAT	
X10Y12	AGTAACATCACGTAAAACAGAAATCAGATGAA	
X10Y13	CAAATATCAATTCGACAACTCGTAAAAGTTTG	
X10Y14	GAAAGCGTGCCACGCTGAGAGCCACTGAACCT	
X10Y15	ATCACTTGCACGACCAGTAATAAATCTGACCT	
X10Y16	GTCTGTCCATCACGCAGTAATAAC	
S1-complementary-biotin	biotin-CGAGTTCCAGGTCTGCTCTC	
S2 stalk	staples - TTCAGTCAGTCAGTCAGTCAGT	
S3 stalk	staples - TTGTCTCGTCGTCTACCGCAAT	