

Supporting Information

Universal Super-Resolution Multiplexing by DNA Exchange

*Florian Schueder, Maximilian T. Strauss, David Hoerl, Joerg Schnitzbauer,
Thomas Schlichthaerle, Sebastian Strauss, Peng Yin, Hartmann Harz, Heinrich Leonhardt, and
Ralf Jungmann**

anie_201611729_sm_miscellaneous_information.pdf
anie_201611729_sm_movie.avi

Supplementary Information

Materials. Unmodified, dye-labeled and biotinylated DNA oligonucleotides were purchased from MWG Eurofins. Streptavidin was purchased from Invitrogen (catalog number: S-888). Bovine serum albumin (BSA, catalog number: A4503-10G) and BSA-Biotin was obtained from Sigma-Aldrich (catalog number: A8549). Coverslips were purchased from Menzel-Gläser (Cover slips 24 × 60 mm, #1.5, catalog number: BBAD02400600#A*). Flow chambers were purchased from ibidi (Sticky-Slide VI^{0.4} catalog number: 80608). M13mp18 scaffold was obtained from New England BioLabs (catalog number: N4040s). Freeze 'N Squeeze columns were ordered from Bio-Rad (catalog number: 7326165). Monoclonal antibodies against Alpha-tubulin (Thermo Scientific; catalog number: MA1-80017) were purchased from Thermo Scientific. Polyclonal antibodies against LaminB (Santa Cruz; catalog number: sc-6217) and TOM20 (Santa Cruz; catalog number: sc-11415) were ordered from Santa Cruz. The secondary antibodies Anti-Rat (catalog number: 712-005-150), Anti-Rabbit (catalog number: 711-005-152) and Anti-Goat (catalog number: 705-005-147) were purchased from Jackson ImmunoResearch. Cell imaging coverglass (catalog number: 0030 742.036) was purchased from Eppendorf. Formamide (catalog number: F9037-100ML), Protocatechuate 3,4-Dioxygenase from pseudomonas (PCD) (catalog number: P8279), 3,4-Dihydroxybenzoic acid (PCA) (catalog number: 37580-25G-F) and (+)-6-Hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid (Trolox) (catalog number: 238813-5G) were obtained from Sigma. 1M Tris pH 8.0 (catalog number: AM9856) was obtained from Ambion, Beta Mercaptoethanol (Catalog number: 63689-25ml) from Sigma, D+ Glucose(w/vol) (catalog number: 410955000) from Acros, Glucose Oxidase (catalog number: G7141-50KU) from Sigma, Glycerol (catalog number: G5516-25UN) from Sigma, Catalase (catalog number: C3155-50MG) from Sigma and H₂O (catalog number: 10977-035) was ordered from gibco.

The following buffers were used:

- Buffer A: 10 mM Tris-HCl, 100 mM NaCl, 0.05 % Tween 20, pH 7.5
- Buffer B: 5 mM Tris-HCl, 10 mM MgCl₂, 1 mM EDTA, 0.05 % Tween 20, pH 8.0
- Buffer C: 1 × PBS, 500 mM NaCl, pH 8.0
- 1.2 × BME: Tris pH 8.0 50 mM, beta Mercaptoethanol 179 mM, MgCl 50 mM, Glucose 12.5 mM in H₂O
- 6 × GLOX: Glucose Oxidase 2.5 mg, Tris 50 mM, Glycerol 10 mM, Catalase 200 mg/ml in H₂O
- 40 × PCA: PCA solution consists of 154 mg PCA in 10 ml water adjusted to pH 9.0 with NaOH
- 100 × PCD: 9.3 mg PCD, 13.3 ml of buffer (50 % glycerol stock in 50 mM KCl, 1 mM EDTA and 100 mM Tris-HCl pH 8.0)
- 100 × Trolox: 100 mg Trolox, 430 μl 100 % Methanol, 345 μl 1 M NaOH in 3.2 ml H₂O

DNA origami self-assembly. The 6 helix bundle (6HB) DNA origami structures for the dSTORM, STED and SIM experiments, were formed in a one-pot reaction with a 40 μl total volume containing 10 nM scaffold strand (M13mp18), 100 nM folding staples and biotin handles, 1000 nM biotin anti-handles and 500 nM DNA-PAINT docking strands in folding buffer (1× TE buffer with 12 mM MgCl₂).

The solution was annealed using a thermal ramp cooling from 80 °C to 14 °C over the course of 15 h. After self-assembly, monomeric structures were purified by agarose gel electrophoresis (1.5 % agarose, 1× TBE, 10 mM MgCl₂, 1× SybrSafe) at 3 V/cm for 3 h. Gel bands were cut, crushed and filled into a Freeze 'N Squeeze column and spun for 5 min at 1 000× *g* at 4 °C.

Cell culture. Cells were grown in Falcon Tissue Culture Treated Flasks (catalog number: 353136) from Falcon. A mixture of 500 ml Dulbecco's Modified Eagle Medium (catalog number: 31966-021), 50 ml Fetal Bovine Serum (catalog number: 10500-064) and 5 ml Penicillin Streptomycin (catalog number: 15140-122) was used as growing media purchased from gibco. For passaging and washing the cells, 1× PBS pH 7.2 (catalog number: 20012-019) and 0.05 % Trypsin – EDTA (catalog number: 25300-054) were purchased from gibco. For the fixation paraformaldehyde and glutaraldehyde were obtained from Electron Microscopy Sciences. Quenching was done using Sodium Borohydride >97% (catalog number: 4051.1) from Roth. For permeabilization and blocking, Triton X 100 (catalog number: 6683.1) from Roth and Bovine Serum Albumin (catalog number: A4503-10G) were used.

Immunostaining of cells. HeLa cells were cultured with Dulbecco's Modified Eagle Medium supplemented with 10 % (v/v) heat inactivated FBS with 1 % (v/v) penicillin and streptomycin and incubated at 37 °C with 5 % CO₂. At approximately 30 % confluence, cells were seeded into Eppendorf chambered coverglass ~24 h before fixation. Microtubules, mitochondria and Lamin were immunostained using the following procedure: fixation in a mixture of 3 % paraformaldehyde and 0.1 % glutaraldehyde in PBS for 10 min; 3 × washing with PBS for 5 min; reduction with ~1 mg/ml NaBH₄ for 7 min; 3 × washing with PBS for 5 min; blocking and permeabilization with 3 % (w/v) BSA and 0.25 % (v/v) Triton X-100 in 1 × PBS for 2 h; staining overnight at 4 °C with the primary antibodies against alpha-tubulin, TOM20 and LaminB (antibodies were diluted to 10 μg/ml in 5 % BSA); 3 × washing with PBS for 5 min each; and finally were stained for 1 h at RT with preassembled secondary antibody-DNA conjugates^[1] against Rat-antibody, Rabbit-Antibody and Goat-Antibody (conjugates were diluted to 10 μg/ml in 5 % BSA); 3 × washing with PBS for 5 min each; post fixation in a mixture of 3 % paraformaldehyde and 0.1 % glutaraldehyde in PBS for 10 min; and 3 × washing with PBS for 5 min each.

Super-resolution setups. dSTORM. Fluorescence imaging was carried out on an inverted Nikon Eclipse Ti microscope (Nikon Instruments) with the Perfect Focus System, applying an objective-type TIRF configuration with an oil-immersion objective (CFI

Apo TIRF 100×, NA 1.49, Oil). For excitation and dark state transition of Alexa647 fluorophores, a 640 nm laser (150 mW nominal, Toptica iBeam Smart) was used. For facilitation of the transition back from the dark state to the ground state, a 405 nm laser (150 mW nominal, Toptica iBeam Smart) was used. The laser beams were passed through a cleanup filter (ZET405/488/561/640x, Chroma Technology, Bellows Falls, VT) and coupled into the microscope objective using a quad-band beam splitter (ZT405/488/561/640rpc, Chroma Technology). Fluorescence light was spectrally filtered with an emission filter (ZET405/488/561/640m-TRF, Chroma Technology) and imaged on an EMCCD camera (iXon Ultra 897 EMCCD, Andor Technology). Imaging was performed without additional magnification in the detection path and yielding a pixel size of 160 nm.

STED. Stimulated Emission Depletion images were acquired using a 3D STED microscope (Abberior Instruments, Göttingen, Germany). The system was equipped with 594 nm and 640 nm pulsed excitation lasers and a pulsed 775 nm depletion laser. The depletion pattern was generated via phase-modulation by a SLM (Abberior Instruments easy3D STED module). The objectives employed were a UPlanSApo 100x / 1.4 NA oil immersion objective and a UPlanSApo 60x / 1.2 NA water immersion objective (Olympus, Tokyo, Japan). Unless noted otherwise, images were acquired with the 100x objective. Image acquisition was controlled via the software ImSpector (Abberior Instruments and MPI for Biophysical Chemistry, Göttingen, Germany).

SIM. Structured Illumination images were acquired using a commercial Zeiss Elyra PS.1 (Carl Zeiss Microscopy, Germany) system. A 63x Plan-Apochromat 1.40 Oil objective and 37.5 mW @ 642 nm laser excitation power was used to acquire the images onto a PCO Edge sCMOS camera. SIM image acquisition and reconstruction was carried out using Zeiss ZEN software, according to the instructions from the manufacturer.

Sample preparation and image acquisition of DNA origami structures. dSTORM. First, the ibidi flow chamber was cleaned by rinsing 100 µl of isopropanol through the chamber and then washed 3 × with ultra-pure water. Then, 200 µl of biotin-labeled bovine albumin (1 mg/ml, dissolved in buffer A) was flown into the chamber and incubated for 5 min. The chamber was then washed using 100 µl of buffer A. 200 µl of streptavidin (0.5 mg/ml, dissolved in buffer A) was then flown into the chamber and allowed to bind for 5 min. After washing with 100 µl of buffer A and subsequently with 100 µl of buffer B, 50 µl of biotin-labeled DNA structures (~100 pM monomer concentration) in buffer B were finally flown into the chamber and incubated for 45 min. The chamber was washed using 100 µl of buffer B. Next, the sample was incubated with 100 nM Alexa647-modified labeling strands in buffer B for 10 min. Finally, the imaging buffer containing 1× BME and 1× GLOX was flown into the chamber. After image acquisition, the chamber was flushed with 30 % formamide in 1× PBS twice for 2 times ~ 3min. Then washing with 1× PBS was performed to exchange the washing buffer and subsequently buffer B was added. Afterwards the next Alexa647-modified labeling strands were introduced. Acquisition and washing steps were repeated until all targets were imaged. The CCD readout bandwidth was set to 17 MHz at 16 bit and 5.1 pre-amp gain. 100 electron multiplying (EM) gain was used. Imaging was performed using TIR illumination with an excitation intensity of ~1 kW/cm² at 640 nm and ~8 W/cm² at 405 nm.

Image acquisition parameters: 50 ms integration time, 10 000 frames per exchange round. RAW data was processed using a custom software package called “Picasso”^[2] (www.jungmannlab.org, <https://github.com/jungmannlab/picasso>), employing single-molecule spot detection and standard maximum likelihood fitting routines^[3]. For the *in vitro* DNA origami data, we detected on average 3 475 photons per localization and achieved an average NeNA^[4] localization precision of ~9.8 nm, yielding a FWHM resolution of ~23 nm.

STED and SIM. First, the ibidi flow chamber was cleaned by rinsing 100 µl of isopropanol through the chamber and then washed 3 × with ultra-pure water. Then, 200 µl of biotin-labeled bovine albumin (1 mg/ml, dissolved in buffer A) was flown into the chamber and incubated for 5 min. The chamber was then washed using 100 µl of buffer A. 200 µl of streptavidin (0.5 mg/ml, dissolved in buffer A) was then flown through the chamber and allowed to bind for 5 min. After washing with 100 µl of buffer A and subsequently with 100 µl of buffer B, 50 µl of biotin-labeled DNA structures (~100 pM monomer concentration) in buffer B were finally flown into the chamber and incubated for 45 min. The chamber was washed using 100 µl of buffer B. The chamber was washed using 100 µl of buffer B. Then the sample was incubated with 100 nM Atto647N-modified labeling strand in buffer B for 10 min. After three-times 3 min of washing, the imaging buffer was introduced. Finally, the imaging buffer containing 1× Trolox, 1× PCA and 1× PCD (diluted in buffer B) was flown into the chamber. After image acquisition, the chamber was flushed with 30 % formamide in 1× PBS twice for ~3 min. Then washing with 1× PBS was performed to exchange the washing buffer and subsequently Buffer B was added. Afterwards the next Atto647N-modified labeling strand was introduced. Acquisition and washing steps were repeated until all targets were imaged. Images were acquired with 20 nm pixel steps and 10 µs pixel dwell time and 10-fold line accumulation. The average laser powers in the back focal plane were set to ~13 µW (640 nm excitation) and ~195 mW (775 nm depletion). The size of the confocal pinhole was set to 1 A.U. For the *in vitro* DNA origami data, we achieved an average localization precision of ~30 nm, yielding a FWHM STED resolution of ~68 nm.

Sample preparation and image acquisition of cell samples. dSTORM. An Eppendorf chamber was adapted for fluid exchange. Images were acquired with an EMCCD readout bandwidth of 17 MHz at 14 bit, 5.1 pre-amp gain and 100 EM gain. Imaging was performed using oblique illumination. Sequential labeling, imaging and washing was performed analogous to the DNA origami *in vitro* experiments. The laser power densities at 640 nm were ~2 kW/cm² and 405 nm at ~6 W/cm².

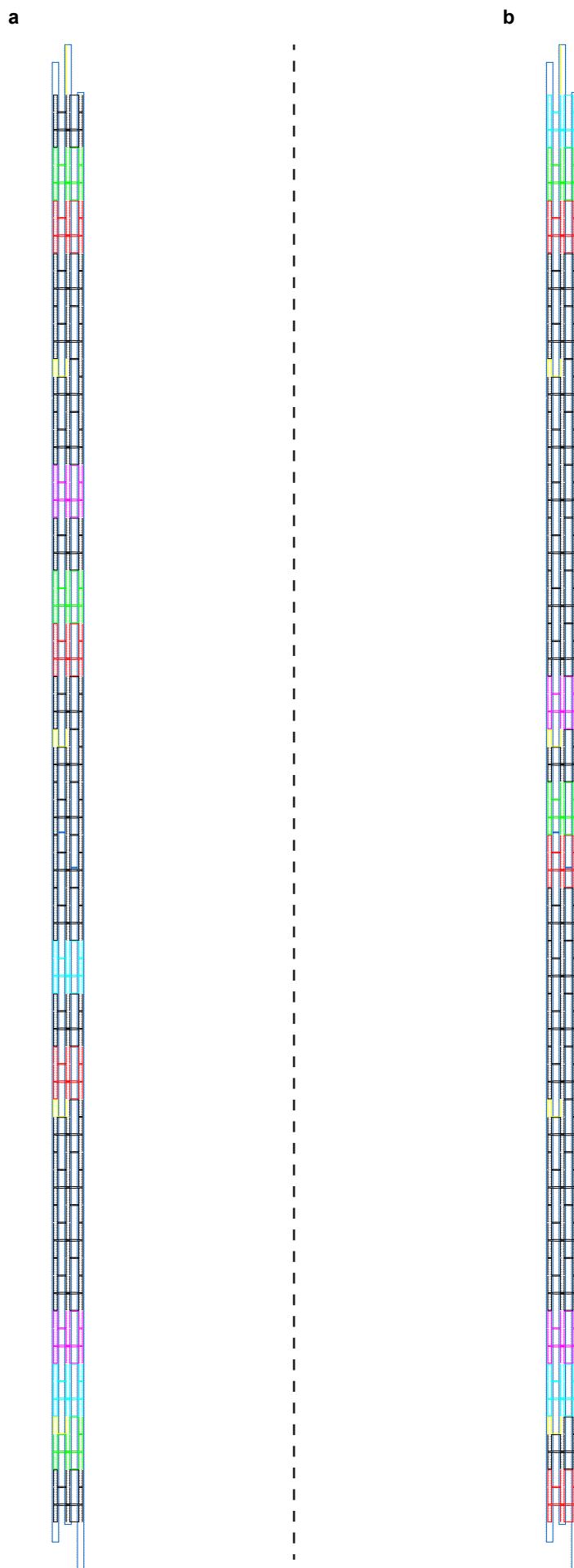
Image acquisition parameters: 50 ms integration time, 50 000 frames per exchange round. RAW data was processed using a custom software package called “Picasso”^[2] (www.jungmannlab.org, <https://github.com/jungmannlab/picasso>), employing single-molecule spot detection and standard maximum likelihood fitting routines^[3]. For the *in situ* cell data, we detected on

average 5 400 photons per localization and achieved an average NeNA^[4] localization precision of ~19 nm, yielding a FWHM resolution of ~44 nm.

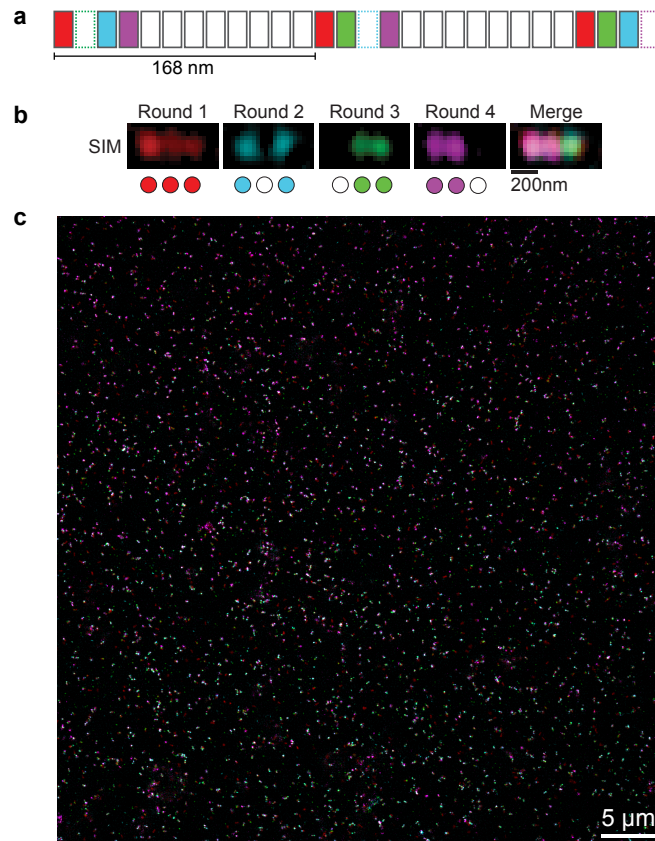
STED. Images were acquired with 20 nm pixel steps and 10 μ s pixel dwell time and 3-fold line accumulation. The average laser powers in the back focal plane were set to ~13 μ W (640 nm excitation) and ~190 mW (775 nm depletion). The size of the confocal pinhole was set to 1 A.U.

The STED-stack (Supplementary Video 1) was acquired with the 60x water immersion objective. The pixel steps were 40 nm in x and y and 400 nm in z, with a pixel dwell time of 15 μ s and 2-fold line accumulation. The average laser powers in the back focal plane were set to ~13 μ W (640 nm excitation) and ~195 mW (775 nm depletion). The size of the confocal pinhole was set to 1 A.U. 33 % of the depletion laser power was subjected to a circular phase mask to achieve axial resolution improvement in addition to the lateral super-resolution.

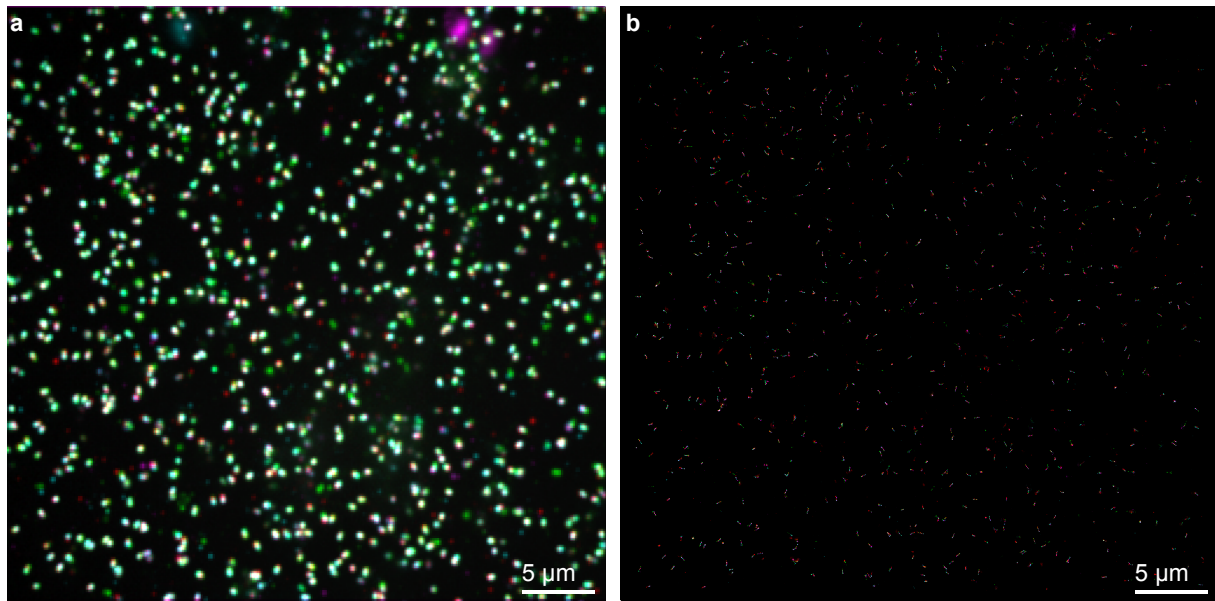
Supplementary Figures



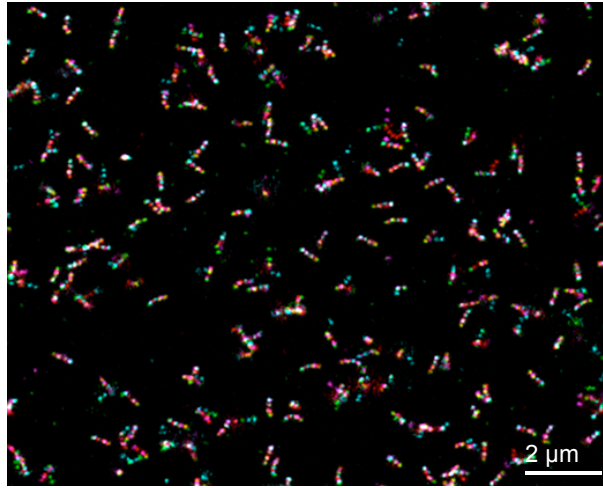
Supplementary Figure 1 | DNA origami designs. (a) 6HB origami for dSTORM and STED study. (b) 6HB origami for SIM study.



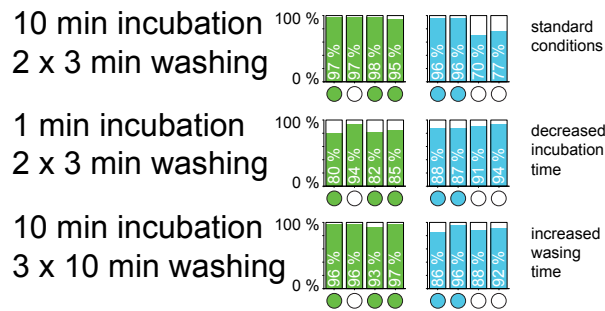
Supplementary Figure 2 | Exchange-SIM. (a) Schematic representation of 6HB DNA origami structure for Exchange-SIM demonstration. 3 spots are designed that can display up to 4 orthogonal DNA sequences for Exchange imaging. Spots are spaced ~ 168 nm apart. (b) Representative DNA origami structures for 4-round Exchange-SIM experiment. (c) 4-round Exchange-SIM overview.



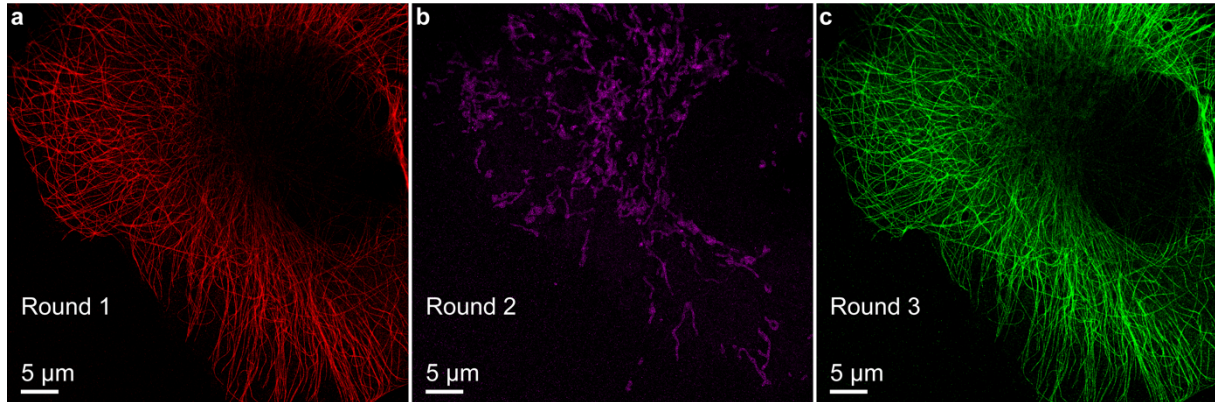
Supplementary Figure 3 | Exchange-dSTORM overview. (a) Diffraction-limited overview for 4-round Exchange-dSTORM. (b) Corresponding dSTORM super-resolution micrograph.



Supplementary Figure 4 | Exchange-STED overview. 4-color Exchange-STED micrograph.



Supplementary Figure 5 | Washing and hybridization assay. Results from different hybridization and washing times.



Supplementary Figure 6 | Exchange-dSTORM relabeling experiment. (a) Alpha-tubulin imaging in round 1. (b) TOM20 proteins are imaged in round 2 after probe exchange. Note that no microtubule structures are visible anymore. (c) Docking strands on anti-alpha-tubulin antibodies are relabeled and reimaged in round 3, highlighting the high labeling, erasing, and relabeling efficiency in our Exchange-dSTORM experiment.

Supplementary Table 1 | Strand sequences for 6HB DNA origami structure used for Exchange-STED and Exchange-dSTORM

Positon	Sequence	Color	Spot Number / Mod
0 [293] 4 [280]	TGCTGAACCTCAAATAAAGCCAGAATGGGGAAGGTAAATATT		
0 [335] 4 [322]	CGCTGAGAGCCAGCAGTAAGCGTCATACAGCGCCAAAGACAA		
0 [377] 4 [364]	GAGGTGAGGCGGTTCGTAATAAGTTTTAGTTTATTTTGTAC		
0 [419] 4 [406]	CCATTAAAAATACCCCGTATAAACAGTTGGTGGCAACATATA		
0 [461] 4 [448]	CTATTAGTCTTTAATATTCTGAAACATGATTACGCAGTATGT		
0 [503] 4 [490]	GAAAGCGTAAGAATGAGAAGGATTAGGAATAACGGAATACCC		
0 [545] 4 [532]	AAAGGGACATTCTGGATAAGTGCCGTCGAGCCGAACAAAGTT		
0 [587] 4 [574]	TTATTTACATTGGCTAGGTGTATCACCGTAGCTATCTTACCG		
0 [797] 4 [784]	AGGCCACCGAGTAAAAGTTTTGTCTCTATTTTAAAGAACTGG		
0 [839] 4 [826]	AACGGTACGCCAGATATGGGATTTTGCTTGGTTAATTTCAA		
0 [881] 4 [868]	CGGGAGCTAAACAGGAGAATAGAAAGGACTGACGAGAAACAC		
0 [923] 4 [910]	TTGACGAGCACGTATTTTCACGTTGAAAATCAACGTAACAAA		
0 [965] 4 [952]	CCGCGCTTAATGCGCCTTTAATGTATCATCAAGAGTAATCT		
0 [1007] 4 [994]	CGAAAACCGTCTATTCTTAAACAGCTTATGAACGGTGTACA		
0 [1049] 4 [1036]	AAGAGTCCACTATTACAACCATCGCCCAAATCATAAGGGAAC		
0 [1091] 4 [1078]	GAATAGCCCAGATGCTTGCAGGGAGTTACCTGCTCCATGTT		
1 [280] 3 [293]	AAAGAAATTGCGTATAATTTAGGCAGAGCCGACTTGCGGGAG		
1 [322] 3 [335]	TACAGTAACAGTACAGGGCTTAATTGAGGCTATTTTGCACCC		
1 [364] 3 [377]	TTGCGCTGATTGCTGTTATACAAAATTCTACGCTAACGAGCGT		
1 [406] 3 [419]	AGGCGAATTATTCAGGAATCATAATTACAAATAAACAGCCAT		
1 [448] 3 [461]	TGAAACAAACATCACCGACCGTGTGATAGATTTTTTGTTTAA		
1 [490] 3 [503]	TTTCATTTGAATTAAGTTAATTTTCATCTAGAGAGAATAACAT		
1 [532] 3 [545]	CAATATATGTGAGTCGCAAGACAAAGAAAATTAACGTAACAC		
1 [574] 3 [587]	TATTAATTAATTTTTTGGGTATATAACGCTAATATCAGAGA		
1 [784] 3 [797]	GCTTCTGGTGCCGTTGTTAAAATTCGCCTTAAACAGTTCA		
1 [826] 3 [839]	AGGCTGCGCAACTGAAGATTGTATAAGCTCAGGTCTTTACCC		
1 [868] 3 [881]	TCGCTATTACGCCAATATGTACCCCGGTTTGCATCAAAAAGA		
1 [910] 3 [923]	CGATTAAGTTGGGTAGAGAAATCGATGAAATCGCGTTTTAATT		
1 [952] 3 [965]	TGTAAAACGACGGCACAAAGGCTATCAGCAAACCTCCAACAGG		
1 [994] 3 [1007]	GACTCTAGAGGATCGATAAATTAATGCCCTTTTGATAAGAG		
1 [1036] 3 [1049]	ATGGTCATAGCTGTACAGTCAAATCACCATTGCTGAATATAA		
1 [1078] 3 [1091]	AATTCCACACAACATAATGTGTAGGTAACAACATAAAGTACGG		
2 [307] 0 [294]	ACAACGCCAACATGGATTTTCAGGTTTACTAAAGCATCACCT		
2 [349] 0 [336]	CAACGCTCAACAGTCTTTTACATCGGGACTGCAACAGTGCCA		
2 [391] 0 [378]	TTAGTATCATATGCTTGAATACCAAGTTCAGAAGATAAAAACA		
2 [475] 0 [462]	AATGGTTTGAATAAAGAAAACAAAATTAATATTTTTGAATGG		
2 [517] 0 [504]	TTCAAATATATTTTCTTTTAAATGGAAACCTTCTGACCT		
2 [559] 0 [546]	GATGCAAATCCAATGAATAACCTTGCTTACGACCAGTAATA		
2 [601] 0 [588]	ACCTCCGGCTTAGGCCCTTAGAATCCTTTTCGCTGAAATGGA		
2 [811] 0 [798]	TAAACGTTAATATTAACCAGGCAAAGCTTTTATAATCAGTG		

2 [853] 0 [840]	GCCCCAAAAACAGGTTGGGAAGGGCGATGGATTTTAGACAGG	
2 [895] 0 [882]	CTAGCATGTCAATCGCTGGCGAAAGGGCGTTAGAATCAGAG	
2 [937] 0 [924]	GTCTGGAGCAAACAAACGCCAGGGTTTTGTACTATGGTTGCT	
2 [979] 0 [966]	TTTTTGAGAGATCTCAGTGCCAAGCTTGAACCACCACACCCG	
2 [1063] 0 [1050]	GAGAAAGGCCGGAGTTCCTGTGTGAAATTTCCAGTTTGGAAC	
2 [1105] 0 [1092]	ATGCAATGCCTGAGTACGAGCCGGAAGCCTTATAAATCAAAA	
3 [294] 5 [307]	GTTTTGAAGCCTTAACCGATTGAGGGAGAAAGCGCAGTCTCT	
3 [336] 5 [349]	AGCTACAATTTTATTTCATATGGTTTACCATGGCTTTTGATGA	
3 [378] 5 [391]	CTTTCCAGAGCCTAACACCACGGAATAAACGGGGTCAGTGCC	
3 [420] 5 [433]	ATTATTTATCCCAAATACATACATAAAAATGCCCCCTGCCT	
3 [462] 5 [475]	CGTCAAAAAATGAAAGATTAAGACTCCTTAAAGTATTAAGAGG	
3 [504] 5 [517]	AAAAACAGGGAAGCGAGGAAACGCAATATTAGCGGGGTTTTG	
3 [546] 5 [559]	CCTGAACAAAGTCAAAAAGTAAGCAGATAGAGGGTTGATATA	
3 [588] 5 [601]	GATAACCCACAAGACAATGAAATAGCAATACTCAGGAGGTTT	
3 [798] 5 [811]	GAAAACGAGAATGAAATTACCTTATGCGTTCCAGACGTTAGT	
3 [840] 5 [853]	TGACTATTATAGTCAATTGGGCTTGAGAAAACAACTTTCAAC	
3 [882] 5 [895]	TTAAGAGGAAGCCCGAATAAGGCTTGCCACAACATAAGGAAT	
3 [924] 5 [937]	CGAGCTTCAAAGCGTATTCATTACCCAAATCTCAAAAAAAAA	
3 [966] 5 [979]	TCAGGATTAGAGAGGCTGGCTGACCTTCGGTTTATCAGCTTG	
3 [1008] 5 [1021]	GTCATTTTTGCGGATTGAAAGAGGACAGGATACCGATAGTTG	
3 [1050] 5 [1063]	TGCTGTAGCTCAACAGGCGCAGACGGTCCGCATAACCGATAT	
3 [1092] 5 [1105]	TGTCTGGAAGTTTCTGTGCGAAATCCGCGAAAGCCGCTTTTTG	
4 [279] 2 [266]	GACGGAATTTATTCTTTTAGCGAACCTCGCATTTTCGAGCCA	
4 [321] 2 [308]	AAGGGCGACATTCAAATCAAGATTAGTTAATCGCCATATTTA	
4 [363] 2 [350]	AATCAATAGAAAACTCTGAATCTTACCATACCAGTATAAAGC	
4 [405] 2 [392]	AAAGAAACGCAAAGATTTGCCAGTTACATAGAAAAAGCCTGT	
4 [447] 2 [434]	TAGCAAACGTAGAATCCAAATAAGAAACAATAAGGCGTTAAA	
4 [489] 2 [476]	AAAAGAACTGGCATATAGCAGCCTTACTCTGACCTAAATTT	
4 [531] 2 [518]	ACCAGAAGGAAACCGCATTAGACGGGAGCGCGAGAAAACTTT	
4 [573] 2 [560]	AAGCCCTTTTTAAGGAGGGTAATTGAGCTATATGTAAATGCT	
4 [783] 2 [770]	CTCATTATACCAGTATCCCCCTCAAATGATTAAATTTTTGTT	
4 [825] 2 [812]	CTTTAATCATGTGCCATAAATCAAAAAAAAAATTTAAATTG	
4 [867] 2 [854]	CAGAACGAGTAGTAAGAAGCAAAGCGGATGATAATCAGAAAA	
4 [909] 2 [896]	GCTGCTCATTCAGTGAAAGACTTCAAATCGGTAATCGTAAAA	
4 [951] 2 [938]	TGACAAGAACCGGAAACCGACCGGAAGGTCATTGCCTGAGA	
4 [993] 2 [980]	GACCAGGCGCATAGTACCTTTAATTGCTGGAGAGGGTAGCTA	
4 [1035] 2 [1022]	CGAACTGACCAACTTGGCTTAGAGCTTAATCAATATGATATT	
4 [1077] 2 [1064]	ACTTAGCCGGAACGATGTTTTAAATATGAGATTCAAAAGGGT	
5 [266] 1 [279]	AAATAAATCCTCATTATCAAACCTCAACGTAAAACAGAAAT	
5 [308] 1 [321]	GAATTTACCGTTCCAGCAAATGAAAAATACGTCAGATGAATA	
5 [350] 1 [363]	TACAGGAGTGTACTAGTATTAACACCGCGAAACAATAACGGA	
5 [392] 1 [405]	TTGAGTAACAGTGCGAACGAACCACCAGACAAAATCGCGCAG	
5 [434] 1 [447]	ATTTCCGGAACCTATTGCGGAACTGATAGCAAAAAGAAGATGA	

5 [476] 1 [489]	CTGAGACTCCTCAAACGTGGCACAGACAATTACATTTAACAA		
5 [518] 1 [531]	CTCAGTACCAGGCGGCCAACAGAGATAGAACAGTACATAAAT		
5 [560] 1 [573]	AGTATAGCCCCGAAAGATTACCAGTCACTGTAAATCGTCGC		
5 [770] 1 [783]	AGCGTAACGATCTAAAGAGTCTGTCCATAGCTTTCCGGCACC		
5 [812] 1 [825]	AAATGAATTTTCTGATCCTGAGAAGTGTGCCATTCGCCATTC		
5 [854] 1 [867]	AGTTTCAGCGGAGTGAGGCCGATTAAAGCGGTGCGGGCCTCT		
5 [896] 1 [909]	TGCGAATAATAATTTAACGTGCTTTCCTGATGTGCTGCAAGG		
5 [938] 1 [951]	GGCTCCAAAAGGAGCCGCTACAGGGCGCCCCAGTCACGACGT		
5 [980] 1 [993]	CTTTCGAGGTGAATTTCAACGCTGCGCGTCATGCCTGCAGGTC		
5 [1022] 1 [1035]	CGCCGACAATGACAAAAGAACGTGGACTTCGAATTCGTAATC		
5 [1064] 1 [1077]	ATTCCGGTCGCTGAGAGGGTTGAGTGTGTGTATCCGCTCAC		
0 [251] 4 [238]	GTTGGCAAATCAACCAGACGATTGGCCTATCACCGTCACCGA		1
0 [755] 4 [742]	GTTGTAGCAATACTTAGCATTCCACAGAGAAAAATCTACGTT		2
1 [238] 3 [251]	AGAACCTACCATATTAAGTACCGACAATTATCCGGTATTCT		1
1 [742] 3 [755]	TCGGCCTCAGGAAGTTTAACCAATAGGACTGCGGAATCGTCA		2
2 [265] 0 [252]	GTAATAAGAGAATACAAAATTAATTTGCATCAATATCTGGTCA		1
2 [769] 0 [756]	AAATCAGCTCATTTATCGCACTCCAGCCCACGCAAATTAACC		2
3 [252] 5 [265]	AAGAACGCGAGGCGATTAAAGGTGAATTTGATATTCACAAAC		1
3 [756] 5 [769]	TAAATATTCATTGACAGGACGTTGGGAACAGCCCTCATAGTT		2
4 [237] 2 [224]	CTTGAGCCATTTGGCAGATATAGAAGGCAAGGTAAAGTAATT		1
4 [741] 2 [728]	AATAAAACGAACTAGATAGCGTCCAATAACGCCATCAAAAAT		2
5 [224] 1 [237]	AGGTTGAGGCAGGTAGTTGAAAGGAATTATAATGGAAGGGTT		1
5 [728] 1 [741]	AACTACAACGCTGTCTTTGATTAGTAAGGACGACGACAGTA		2
0 [167] 4 [154]	TCAATAGATAAATACCACCCTCAGAGCCAGTCACCAATGAAAC		1
0 [671] 4 [658]	AGAACAATATTACCCCTCATTTTCAGGGCATTCAACTAATGC		2
0 [1175] 4 [1162]	GCGGTCCACGCTGGACAGAGGCTTTGAGTGACCCCCAGCGAT		3
1 [154] 3 [167]	ATCATATTCCTGATATCAACAATAGATATCATCGAGAACAAG		1
1 [658] 3 [671]	TTGACCGTAATGGGAACAACCCGTCGGAACCAAAATAGCGA		2
1 [1162] 3 [1175]	GCGCTCACTGCCGAATACTTTTGCGGGTAGATACATTTTCGC		3
2 [685] 0 [672]	AAATGTGAGCGAGTATAGGTCACGTTGGTGCTGGTAATATCC		2
2 [1189] 0 [1176]	CATTATGACCCTGTCTTTCAGTCGGGAGAGAGTTGCAGCAA		3
3 [168] 5 [181]	CAAGCCGTTTTTATGCAAGGCCGAAACCCACCCTCAGAGCC		1
3 [672] 5 [685]	GAGGCTTTTGCAAAATTTAGGAATACCAATAGCAAGCCCAAT		2
3 [1176] 5 [1189]	AAATGGTCAATAACAAAACACTCATCTTGACTAAAGACTTTT		3
4 [153] 2 [140]	CATCGATAGCAGCAACCAAGTACCGCACAGTCTGAACAAGA		1
4 [657] 2 [644]	AGATACATAACGCCAGACGACGATAAAATTCCTCGTGGGATA		2
4 [1161] 2 [1148]	TATACCAAGCGGATTTAGTTTGACCATAGAAGCCTTTATTT		3
5 [140] 1 [153]	ACCCTCAGAACCGCATTTGAGGATTTAGGGAGCGGAATTATC		1
5 [644] 1 [657]	CTCAGAGCCACCACGCCAGCCATTGCAAAAACAAACGGCGGA		2
5 [1148] 1 [1161]	GGGTAGCAACGGCTTTTGCCCCAGCAGGACATTAATTGCGTT		3
0 [209] 4 [196]	AAAATATCTTTAGGCACCAGAGCCGCCGAAAATCACCAGTAG		1
0 [713] 4 [700]	TGAGTAGAAGAACTCGTAACACTGAGTTTTACAGGTAGAAAG		2
0 [1217] 4 [1204]	CTGATTGCCCTTCACCATTAACCGGTATAAAACGAAAGAGG		3

1 [196] 3 [209]	ATCCTGATTGTTTGACAATAAACAAACATTTCATTACCGCGCCC		1
1 [700] 3 [713]	TCGTAACCGTGCATCTTCCTGTAGCCAGAGGGGTAATAGTA		2
1 [1204] 3 [1217]	GCTGCATTAATGAACATAAAGCTAAATCTTTCATTTGGGGCG		3
2 [223] 0 [210]	CTGTCCAGACGACGGATTATACTTCTGAGAGGAAGGTTATCT		1
2 [1231] 0 [1218]	ATAAAGCCTCAGAGTCGGCCAACGCGGGAGACGGGCAACAG		3
3 [210] 5 [223]	AATAGCAAGCAAATGAATTAGAGCCAGCCCAGCATTGACAGG		1
3 [714] 5 [727]	AAATGTTTAGACTGACGGAACAACATTATCGTCACCAGTACA		2
3 [1218] 5 [1231]	CGAGCTGAAAAGGTCGAAGGCACCAACCAAATACGTAATGCC		3
4 [195] 2 [182]	CACCATTACCATTATTTTCATCGTAGGAAGTTCAGCTAATGCA		1
4 [699] 2 [686]	ATTCATCAGTTGAGAGAAGTTTTCGCCAGCTTTCATCAACATT		2
4 [1203] 2 [1190]	CAAAAGAATACACTCTGTTTAGCTATATGGTGTACCAAAAA		3
5 [182] 1 [195]	GCCACCAGAACCACAGCACTAACAACTAAATTCATCAATATA		1
5 [686] 1 [699]	AGGAACCCATGTACCAAACCTATCGGCCTGTAGATGGGCGCA		2
5 [1190] 1 [1203]	TCATGAGGAAGTTTCCGCCTGGCCCTGAAACCTGTCTGTGCCA		3
0 [125] 4 [112]	ACAAACAATTCGACCACCGGAACCGCCTGACAGAATCAAGTT		1
0 [629] 4 [616]	ATGGAAATACCTACCAGAACCGCCACCCGGCATAGTAAGATA		2
0 [1133] 4 [1120]	GATGGTGGTCCGATCAGCAGCGAAAGAGAGATTTGTATCAT		3
1 [112] 3 [125]	ATTTTGCAGCAACCTAATTTTACGAGTCTTATCATTCCA		1
1 [616] 3 [629]	AGCTTAGATTAAGAATCATAGGCTGAGAAGCAACACTATCA		2
1 [1120] 3 [1133]	CTGGGGTGCCTAATAAATTTTGTAGAACCTTGATTCCCAATTC		3
2 [139] 0 [126]	AAAATAATATCCCAAGAAACCACCAGAAAAGTATTAGACTTT		1
2 [643] 0 [630]	GTGAATTTATCAAACGCTGAGAAGAGTCCAGGAAAACGCTC		2
2 [1147] 0 [1134]	CAACGCAAGGATAAGAGTGAGCTAACTCCGAAAATCCTGTTT		3
3 [126] 5 [139]	AGAACGGGTATTAACCGTAATCAGTAGCCCCCTCAGAGCCGCC		1
3 [630] 5 [643]	TAACCCCTCGTTTACAAAAGGAATTACGATCAGAACCGCCACC		2
3 [1134] 5 [1147]	TGCGAACGAGTAGAAAACAAAGTACAACGCAGCATCGGAACGA		3
4 [111] 2 [98]	TGCCTTTAGCGTCATAATCGGCTGTCTTCATGTAGAAACCAA		1
4 [615] 2 [602]	ATAAGAGCAAGAAAATTGAGTTAAGCCCAGACTACCTTTTTTA		2
4 [1119] 2 [1106]	CGCCTGATAAATTGATTCCATATAACAGCTCATATATTTTAA		3
5 [98] 1 [111]	GGAACCAGAGCCACAACCTCGTATTAAATTGAGTAACATTATC		1
5 [602] 1 [615]	AGTACCGCCACCCTATTTTGACGCTCAAGAAAACATAGCGAT		2
5 [1106] 1 [1119]	CGGGATCGTCACCCAATCGGCAAAATCCATAAAGTGTAAGC		3
2 [181] 0 [168]	GAACGCGCCTGTTTTATCAGATGATGGCATAGATTAGAGCCG		Biotin
2 [433] 0 [420]	TAAGAATAAACACCTTTCAATTACCTGAGCCCTAAAACATCG		Biotin
2 [727] 0 [714]	AATTCGCGTCTGGCCTGCCAGTTTGTAGGTAACATCACTTGCC		Biotin
2 [1021] 0 [1008]	CAACCGTTCAGCTCCCGGGTACCGAGCCCAACGTCAAAGGG		Biotin
2 [1271] 2 [1232]	AAATCATAACAGGCAAGGCAAGAATTAGCAAAATTAAGCA		Biotin

Supplementary Table 2 | Strand sequences for 6HB DNA origami structure used for Exchange-SIM

Position	Sequence	Color	Spot Number / Mod
0 [293] 4 [280]	TGCTGAACCTCAAATAAAGCCAGAATGGGGAAGGTAAATATT		
0 [335] 4 [322]	CGCTGAGAGCCAGCAGTAAGCGTCATACAGCGCCAAAGACAA		
0 [377] 4 [364]	GAGGTGAGGCGGTCCGTAATAAGTTTTAGTTTATTTTGTAC		
0 [419] 4 [406]	CCATTAAAAATACCCCGTATAAACAGTTGGTGGCAACATATA		
0 [629] 4 [616]	ATGGAAATACCTACCAGAACCGCCACCCGGCATAGTAAGATA		
0 [671] 4 [658]	AGAACAATATTACCCCTCATTTTCAGGGCATTCAACTAATGC		
0 [713] 4 [700]	TGAGTAGAAGAACTCGTAACACTGAGTTTTACAGGTAGAAAG		
0 [755] 4 [742]	GTTGTAGCAATACTTAGCATTCCACAGAGAAAAATCTACGTT		
0 [965] 4 [952]	CCGCGCTTAATGCGCCTTTAATTGTATCATCAAGAGTAATCT		
0 [1007] 4 [994]	CGAAAAACCGTCTATTCTTAAACAGCTTATGAACGGTGTACA		
0 [1049] 4 [1036]	AAGAGTCCACTATTACAACCATCGCCCAAATCATAAGGGAAC		
0 [1091] 4 [1078]	GAATAGCCCGAGATGCTTGCAGGGAGTTACCTGCTCCATGTT		
1 [280] 3 [293]	AAAGAAATTGCGTATAATTTAGGCAGAGCCGACTTGCGGGAG		
1 [322] 3 [335]	TACAGTAACAGTACAGGGCTTAATTGAGGCTATTTTGCACCC		
1 [364] 3 [377]	TTCGCCTGATTGCTGTTATACAAATCTACGCTAACGAGCGT		
1 [406] 3 [419]	AGGCGAATTAATCAGGAATCATAATTACAAATAAACAGCCAT		
1 [616] 3 [629]	AGCTTAGATTAAGAATCATAGGTCTGAGAAGCAACACTATCA		
1 [658] 3 [671]	TTGACCGTAATGGGAACAACCCGTCGGAAACCAAATAGCGA		
1 [700] 3 [713]	TCGTAACCGTGCATCTTCTGTAGCCAGAGGGGTAATAGTA		
1 [742] 3 [755]	TCGGCCTCAGGAAGTTTAACCAATAGGACTGCGGAATCGTCA		
1 [952] 3 [965]	TGTAAAACGACGGCACAAAGGCTATCAGCAAACCTCCAACAGG		
1 [994] 3 [1007]	GACTCTAGAGGATCGATAAAATTAATGCCCTTTTGATAAGAG		
1 [1036] 3 [1049]	ATGGTCATAGCTGTACAGTCAAATCACCATTTGCTGAATATAA		
1 [1078] 3 [1091]	AATTCACACAACATAATGTGTAGGTAACAACATAAAGTACGG		
2 [307] 0 [294]	ACAACGCCAACATGGATTTTCAGGTTTACTAAAGCATCACCT		
2 [349] 0 [336]	CAACGCTCAACAGTCTTTTACATCGGGACTGCAACAGTGCCA		
2 [391] 0 [378]	TTAGTATCATATGCTTGAATACCAAGTTCAGAAGATAAAACA		
2 [643] 0 [630]	GTGAATTTATCAAACGCTGAGAAGAGTCCAGGAAAAACGCTC		
2 [685] 0 [672]	AAATGTGAGCGAGTATAGGTCACGTTGGTGTGGTAATATCC		
2 [769] 0 [756]	AAATCAGCTCATTTATCGCACTCCAGCCCACGCAAATTAACC		
2 [979] 0 [966]	TTTTTGAGAGATCTCAGTGCCAAGCTTGAACCACCACACCCG		
2 [1063] 0 [1050]	GAGAAAGCCGGAGTTCCGTGTGAAATTTCCAGTTTGGAAC		
2 [1105] 0 [1092]	ATGCAATGCCTGAGTACGAGCCGGAAGCCTTATAAATCAAAA		
3 [294] 5 [307]	GTTTTGAAGCCTTAACCGATTGAGGGAGAAAAGCGCAGTCTCT		
3 [336] 5 [349]	AGCTACAATTTTATTCATATGGTTTACCATTGGCTTTTGTATGA		
3 [378] 5 [391]	CTTTCAGAGCCTAACACCACGGAATAAACGGGGTCAGTGCC		
3 [420] 5 [433]	ATTATTTATCCCAAATACATACATAAAAATGCCCCCTGCCT		
3 [630] 5 [643]	TAACCCTCGTTTACAAAAGGAATTACGATCAGAACCGCCACC		
3 [672] 5 [685]	GAGGCTTTTGCAAAATTTAGGAATACCAATAGCAAGCCCAAT		
3 [714] 5 [727]	AAATGTTTAGACTGACGGAACAACATTATCGTCACCAGTACA		
3 [756] 5 [769]	TAAATATTCATTGACAGGACGTTGGGAACAGCCCTCATAGTT		
3 [966] 5 [979]	TCAGGATTAGAGAGGCTGGCTGACCTTCGGTTTATCAGCTTG		
3 [1008] 5 [1021]	GTCATTTTTGCGGATTGAAAGAGGACAGGATACCGATAGTTG		

3 [1050] 5 [1063]	TGCTGTAGCTCAACAGGCGCAGACGGTCCGCATAACCGATAT		
3 [1092] 5 [1105]	TGTCTGGAAGTTTCTGTGCGAAATCCGCGAAAGGCCGCTTTTG		
4 [279] 2 [266]	GACGGAAATTATTCTTTTAGCGAACCTCGCATTTTCGAGCCA		
4 [321] 2 [308]	AAGGGCGACATTCAAATCAAGATTAGTTAATCGCCATATTTA		
4 [363] 2 [350]	AATCAATAGAAAATCCTGAATCTTACCATACCAGTATAAAGC		
4 [405] 2 [392]	AAAGAAACGCAAAGATTTGCCAGTTACATAGAAAAGCCTGT		
4 [615] 2 [602]	ATAAGAGCAAGAAAATTGAGTTAAGCCCAGACTACCTTTTTA		
4 [657] 2 [644]	AGATACATAACGCCAGACGACGATAAAATCTCCGTGGGATA		
4 [699] 2 [686]	ATTCATCAGTTGAGAGAAGTTTTGCCAGCTTTCATCAACATT		
4 [741] 2 [728]	AATAAAACGAACTAGATAGCGTCCAATAACGCCATCAAAAAT		
4 [951] 2 [938]	TGACAAGAACCAGAAACCAGACCGGAAGGTCATTGCCTGAGA		
4 [993] 2 [980]	GACCAGGCGCATAGTACCTTTAATTGCTGGAGAGGGTAGCTA		
4 [1035] 2 [1022]	CGAACTGACCAACTTGGCTTAGAGCTTAATCAATATGATATT		
4 [1077] 2 [1064]	ACTTAGCCGGAACGATGTTTTAAATATGAGATTCAAAAGGGT		
5 [266] 1 [279]	AAATAAATCCTCATTATCAAACCCTCAACGTAAAACAGAAAT		
5 [308] 1 [321]	GAATTTACCGTTCCAGCAAATGAAAAATACGTCAGATGAATA		
5 [350] 1 [363]	TACAGGAGTGTACTAGTATTAACACCGCGAAACAATAACGGA		
5 [392] 1 [405]	TTGAGTAACAGTGCGAACGAACCACCAGACAAAATCGCGCAG		
5 [602] 1 [615]	AGTACCGCCACCCTATTTGACGCTCAAGAAAACATAGCGAT		
5 [644] 1 [657]	CTCAGAGCCACCACGCCAGCCATTGCAAAAACAAACGGCGGA		
5 [686] 1 [699]	AGGAACCCATGTACCAAATATCGGCCTTGTAGATGGGCGCA		
5 [728] 1 [741]	AACTACAACGCCTGTCTTTGATTAGTAAGGACGACGACAGTA		
5 [938] 1 [951]	GGCTCCAAAAGGAGCCGCTACAGGGCGCCCAGTCACGACGT		
5 [980] 1 [993]	CTTTCGAGGTGAATTCACGCTGCGGTCATGCCTGCAGGTC		
5 [1022] 1 [1035]	CGCCGACAAATGACAAAAGAACGTGGACTTCGAATTCGTAATC		
5 [1064] 1 [1077]	ATTCCGGTCGCTGAGAGGGTTGAGTGTGTGTATCCGCTCAC		
0 [251] 4 [238]	GTTGGCAAATCAACCAGACGATTGGCCTATCACCGTCACCGA		1
0 [587] 4 [574]	TTATTTACATTGGCTAGGTGTATCACCGTAGCTATCTTACCG		2
0 [923] 4 [910]	TTGACGAGCACGTATTTTCACGTTGAAAATCAACGTAACAAA		3
1 [238] 3 [251]	AGAACCTACCATATTAAGTACCGACAATTATCCGGTATTCT		1
1 [574] 3 [587]	TATTAATTAATTTTTTGGGTTATATAACGCTAATATCAGAGA		2
1 [910] 3 [923]	CGATTAAGTTGGGTAGAGAATCGATGAAATCGCGTTTTAATT		3
2 [265] 0 [252]	GTAATAAGAGAATACAAAATTAATTTGCATCAATATCTGGTCA		1
2 [601] 0 [588]	ACCTCCGGCTTAGGCCCTTAGAATCCTTTTCGCTGAAATGGA		2
2 [937] 0 [924]	GTCTGGAGCAAACAACGCCAGGGTTTTGTAATGTTGCT		3
3 [252] 5 [265]	AAGAACGCGAGGCGATTAAAGGTGAATTTGATATTCACAAAC		1
3 [588] 5 [601]	GATAACCCACAAGACAATGAAATAGCAATACTCAGGAGGTTT		2
3 [924] 5 [937]	CGAGCTCAAAGCGTATTCAATACCCAAATCTCCAAAAA		3
4 [237] 2 [224]	CTTGAGCCATTTGGCAGATATAGAAGCAAGGTAAAGTAATT		1
4 [573] 2 [560]	AAGCCCTTTTAAGGAGGGTAATTGAGCTATATGTAAATGCT		2
4 [909] 2 [896]	GCTGCTCATTCAGTGAAGACTTCAAATCGGTAATCGTAAAA		3
5 [224] 1 [237]	AGGTTGAGGCAGGTAGTTGAAAGGAATTATAATGGAAGGGTT		1
5 [560] 1 [573]	AGTATAGCCCGAAAGATTCACCAGTCACTGTAAATCGTCGC		2
5 [896] 1 [909]	TGCGAATAATAATTTAACGTGCTTTTCTGATGTGCTGCAAGG		3
0 [167] 4 [154]	TCAATAGATAATACCACCCTCAGAGCCAGTCACCAATGAAAC		1

0 [503] 4 [490]	GAAAGCGTAAGAATGAGAAGGATTAGGAATAACGGAATACCC		2
0 [839] 4 [826]	AACGGTACGCCAGATATGGGATTTTGCTTGGTTAATTTCAA		3
0 [1175] 4 [1162]	GCGGTCCACGCTGGACAGAGGCTTTGAGTGACCCCCAGCGAT		4
1 [154] 3 [167]	ATCATATTCCTGATATCAACAATAGATATCATCGAGAACAAG		1
1 [490] 3 [503]	TTTCATTTGAATTAAGTTAATTTTCATCTAGAGAGAATAACAT		2
1 [826] 3 [839]	AGGCTGCGCAACTGAAGATTGTATAAGCTCAGGTCTTTACCC		3
1 [1162] 3 [1175]	GCGCTCACTGCCCCGAATACTTTTGCGGGTAGATACATTTTCGC		4
2 [517] 0 [504]	TTCAAATATATTTTTCCCTTTTAAATGGAAACCCCTTCTGACCT		2
2 [853] 0 [840]	GCCCCAAAAACAGGTTGGGAAGGGCGATGGATTTTAGACAGG		3
2 [1189] 0 [1176]	CATTATGACCCCTGTCTTTCCAGTCGGGAGAGAGTTGCAGCAA		4
3 [168] 5 [181]	CAAGCCGTTTTTATGCAAGCCGGAACCCACCCCTCAGAGCC		1
3 [504] 5 [517]	AAAAACAGGGAAGCGAGGAAACGCAATATTAGCGGGGTTTTG		2
3 [840] 5 [853]	TGACTATTATAGTCAATTGGGCTTGAGAAAACAACTTTCAAC		3
3 [1176] 5 [1189]	AAATGGTCAATAACAAAACACTCATCTTGACTAAAGACTTTT		4
4 [153] 2 [140]	CATCGATAGCAGCAACCAAGTACCGCACAGTCTGAACAAGA		1
4 [489] 2 [476]	AAAAGAACTGGCATATAGCAGCCTTTACTCTGACCTAAATTT		2
4 [825] 2 [812]	CTTTAATCATTTGTGCCATAAATCAAAAAAATATTTAAATTG		3
4 [1161] 2 [1148]	TATACCAAGCGCGATTTAGTTTGACCATAGAAGCCTTTATTT		4
5 [140] 1 [153]	ACCCTCAGAACCGCATTTGAGGATTTAGGGAGCGGAATTATC		1
5 [476] 1 [489]	CTGAGACTCCTCAAACGTGGCACAGACAATTACATTTAACAA		2
5 [812] 1 [825]	AAATGAATTTTCTGATCCTGAGAAGTGTGCCATTCGCCATTC		3
5 [1148] 1 [1161]	GGGTAGCAACGGCTTTTGCCCCAGCAGGACATTAATTGCGTT		4
0 [209] 4 [196]	AAAATATCTTTAGGCACCAGAGCCGCCGAAAATCACCAGTAG		1
0 [545] 4 [532]	AAAGGGACATTCTGGATAAGTGCCGTCGAGCCGAACAAAGTT		2
0 [881] 4 [868]	CGGGAGCTAAACAGGAGAATAGAAAGGACTGACGAGAAACAC		3
0 [1217] 4 [1204]	CTGATTGCCCTTACCATTAAACGGGTATAAAACGAAAGAGG		4
1 [196] 3 [209]	ATCCTGATTGTTTGACAATAAACAAACATTCAATTACCGCGCCC		1
1 [532] 3 [545]	CAATATATGTGAGTCGCAAGACAAAGAAAATTAAGTGAACAC		2
1 [868] 3 [881]	TCGCTATTACGCCAATATGTACCCCGGTTGCATCAAAAAGA		3
1 [1204] 3 [1217]	GCTGCATTAATGAACATAAAGCTAAATCTTTCATTTGGGGCG		4
2 [223] 0 [210]	CTGTCCAGACGACGGATTATACTTCTGAGAGGAAGGTTATCT		1
2 [559] 0 [546]	GATGCAAATCCAATGAATAACCTTGCTTCACGACCAGTAATA		2
2 [895] 0 [882]	CTAGCATGTCAATCGCTGGCGAAAGGGGCGTTAGAATCAGAG		3
2 [1231] 0 [1218]	ATAAAGCCTCAGAGTCGGCCAACGCGCGGAGACGGGCAACAG		4
3 [210] 5 [223]	AATAGCAAGCAAATGAATTAGAGCCAGCCAGCATTGACAGG		1
3 [546] 5 [559]	CCTGAACAAAGTCAAAAAGTAAGCAGATAGAGGGTTGATATA		2
3 [882] 5 [895]	TTAAGAGGAAGCCCGAATAAGGCTTGCCACAACCTAAAGGAAT		3
3 [1218] 5 [1231]	CGAGCTGAAAAGGTGCAAGGCACCAACCAAAATACGTAATGCC		4
4 [195] 2 [182]	CACCATTACCATTATTTTCATCGTAGGAAGTTCAGCTAATGCA		1
4 [531] 2 [518]	ACCAGAAGGAAACCGCATTAGACGGGAGCGCGAGAAAACCTTT		2
4 [867] 2 [854]	CAGAACGAGTAGTAAGAAGCAAAGCGGATGATAATCAGAAAA		3
4 [1203] 2 [1190]	CAAAAGAATACACTCTGTTTAGCTATATGGTTGTACCAAAAA		4
5 [182] 1 [195]	GCCACCAGAACCACAGCACTAACAACCTAAATTCATCAATATA		1
5 [518] 1 [531]	CTCAGTACCAGGCGGCCAACAGAGATAGAACAGTACATAAAT		2
5 [854] 1 [867]	AGTTTCAGCGGAGTGAGGCCGATTAAAGCGGTGCGGGCCTCT		3

5[1190]1[1203]	TCATGAGGAAGTTTCCGCCTGGCCCTGAAACCTGTCGTGCCA		4
0[125]4[112]	ACAAACAATTCGACCACCGGAACCGCCTGACAGAATCAAGTT		1
0[461]4[448]	CTATTAGTCTTTAATATTCTGAAACATGATTACGCAGTATGT		2
0[797]4[784]	AGGCCACCGAGTAAAAGTTTGTGCTCTATTTTAAGAAGTGG		3
0[1133]4[1120]	GATGGTGGTTCCGATCAGCAGCGAAAGAGAGATTTGTATCAT		4
1[112]3[125]	ATTTTGCAGCAACAATCCTAATTTACGAGTCCTTATCATTTCA		1
1[448]3[461]	TGAAACAAACATCACCACCGTGTGATAGATTTTTTGTTTAA		2
1[784]3[797]	GCTTCTGGTGCCGTTGTAAAATTCGCCTTTAAACAGTTCA		3
1[1120]3[1133]	CTGGGGTGCCATAATAATTTTGTAGAACCTTGATTTCCCAATTC		4
2[139]0[126]	AAAATAATATCCCAAGAAACCACCAGAAAAGTATTAGACTTT		1
2[475]0[462]	AATGGTTTGAATAAGAAAACAAAATTAATTTTTTGAATGG		2
2[811]0[798]	TAAACGTTAATATTAACCAGGCAAAGCTTTTATAATCAGTG		3
2[1147]0[1134]	CAACGCAAGGATAAGAGTGAGCTAACTCCGAAAATCCTGTTT		4
3[126]5[139]	AGAACGGGTATTAACCGTAATCAGTAGCCCTCAGAGCCGCC		1
3[462]5[475]	CGTCAAAAATGAAAGATTAAGACTCCTTAAAGTATTAAGAGG		2
3[798]5[811]	GAAAACGAGAATGAAATTACCTTATGCGTTCCAGACGTTAGT		3
3[1134]5[1147]	TGCGAACGAGTAGAAACAAAGTACAACGCAGCATCGGAACGA		4
4[111]2[98]	TGCCTTTAGCGTCATAATCGGCTGTCTTCATGTAGAAACCAA		1
4[447]2[434]	TAGCAAACGTAGAATCCAAATAAGAAACAATAAGGCGTTAAA		2
4[783]2[770]	CTCATTATACCAGTATCCCCCTCAAATGATTAAATTTTTGTT		3
4[1119]2[1106]	CGCCTGATAAATTGATTCCATATAACAGCTCATATATTTTAA		4
5[98]1[111]	GGAACCAGAGCCACAACCTCGTATTAATTTGAGTAACATTATC		1
5[434]1[447]	ATTTTCGGAACCTATTGCGGAACTGATAGCAAAGAAGATGA		2
5[770]1[783]	AGCGTAACGATCTAAAGAGTCTGTCCATAGCTTTCCGGCACC		3
5[1106]1[1119]	CGGGATCGTCAACCAATCGGCAAAATCCATAAAGTGTAAGC		4
2[181]0[168]	GAACGCGCTGTTTTATCAGATGATGGCATAGATTAGAGCCG		Biotin
2[433]0[420]	TAAGAATAAACACCTTTCAATTACCTGAGCCCTAAAACATCG		Biotin
2[727]0[714]	AATTCGCGTCTGGCCTGCCAGTTTGTAGGTAACATCACTTGCC		Biotin
2[1021]0[1008]	CAACCGTTCTAGCTCCCGGGTACCGAGCCCAACGTCAAAGGG		Biotin
2[1271]2[1232]	AAATCATACAGGCAAGGCAAAGAATTAGCAAAATTAAGCA		Biotin

Supplementary Table 3 | DNA Exchange extensions and labeling strand sequences

Name	Sequence	Corresponding "color" or antibody
S1 anti-handle	5' -GTGGTAGAGGAA-dye-3'	
S2 anti-handle	5' -GTTAGGAATGTTA-dye-3'	
S3 anti-handle	5' -TGGTGAGGGATT-dye-3'	
S4 anti-handle	5' -AGGTGGTAAGTT-dye-3'	
Biotin anti-handle	5' -GAATCGGTCACAGTACAACCG	
S1 handle	5' -TTCCTCTACCAC-3'	Anti-rat (alpha-tubulin)
S2 handle	5' -TAACATTCCTAAC-3'	Anti-goat (LaminB)
S3 handle	5' -AATCCCTCACCA-3'	Anti-rabbit (TOM20)
S4 handle	5' -AACTTACCACCT-3'	
Biotin handle	5' -Staple-TTCGGTTGTACTGTGACCGATTTC-3'	

Supplementary Table 4 | Scaffold strand sequence M13mp18

TGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACCTGGAAACAACACTCAACCCTATCTCGGGC
TATTTCTTTGATTATAAGGGATTATTTGCCGATTTCCGGAACACCACCTCAACACAGGATTTTTCCGCCTGTCTGGGGCAAACACGCGTGGACCGCTTGTCTG
AATCTCTCAGGGCCAGGCGGTGAAGGCAATCAGCTGTTGCCCGTCTACTGGTAAAAAGAAAACACCCTGGCGCCCAATACGCAACCGCTCT
CTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCAGCAGGTTTTCCCGACTGGAAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAG
CTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGC
TATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTGCACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTTACAACG
TCGTGACTGGGAAAACCTGGCGTTACCCAACCTAATCGCCTTGACGACATCCCCCTTTCCGACGCTGGCGTAATAGCGAAGAGGCCCGCACCGA
TCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTTCCGGCACCAGAAGCGGTGCCGAAAAGCTGGCTGGAGTGCGA
TCTTCCGTAGGCGCTACTGTCGCTGCCCTCCCTCAAACCTGGCAGATGCACGGTTACGATCGGCCCATCTACACCAACGTCGACCTATCCGATTTACGGT
CAATCCGTGGTTTTGTTCCAGCTCGCGAGAATCCGACGGTTGTTACTCGCTCACATTTAATGTGTGATGAAAGCTACAGGAATGCCAGACCGCAAT
TATTTTTGATGGCGTTTCTATTGGTTAAAAAATGAGCTGATTTAACAATAATTTAATGCGAATTTTAACAATAATTAACGTTTACAATTTAATA
TTTGCTTATAACAATCTTCTGTTTTTGGGGCTTTTCTGATTATCAACCGGGGTACATATGATTGACATGCTAGTTTTACGATTACCGTTTATCGAT
TCTCTGTTTTGCTCCAGACTCTCAGGCAATGACCTGATAGCTTTGTAGATCTCTCAAAAAATAGCTACCCTCTCGGCATTAATTTATCAGCTAGA
ACGGTTGAATATCATATTGATGGTGAATTTGACTGTCTCCGGCCTTTCTCACCTTTTGAATCTTTACCTACACATTACTCAGGCATTGCATTTAAA
ATATATGAGGGTCTAAAAAATTTTATCCTTGCGTTGAAATAAAGGCTTCTCCCGAAAAGTATTACAGGGTCATAATGTTTTTGGTACAACCGAT
TTAGCTTTATGCTCTGAGGCTTATTGCTTAATTTGCTAATTTGCTTTCCTTGCTGCTGATGATTTATTGGATGTTAATGCTACTACTATTAGTAGA
ATTGATGCCACCTTTTCCAGCTCGCGCCCAAATGAAAATATAGCTAAACAGGTTATTGACCATTTGCGAAAATGATCTAATGGTCAAACATAAATCT
ACTCGTTCCGAGAATTGGGAATCAACTGTTATATGGAATGAAACTTCCAGACACCGTACTTTAGTTGCATATTTAAAAACATGTTGAGCTACAGCAT
TATATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAGGAGCAATTAAGGTTACTCTTAATCCTGACCTGTTGGAGTTT
GCTTCCGGTCTGGTTCGCTTTGAAGCTCGAATTAACCGCGATATTTGAAGTCTTTCCGGCTTCCCTCTTAATCTTTTTGATGCAATCCGCTTTGCT
TCTGACTATAATAGTCAGGGTAAAGACCTGATTTTTGATTTATGGTCAATCTCGTTTTTCTGAACTGTTTTAAAGCATTTGAGGGGGATTCAATGAAT
ATTTATGACGATTCGCGAGTATTGGACGCTATCCAGCTAAACATTTACTATTACCCCTCTGGCAAACTTCTTTTTGCAAAAGCCTCTCGCTAT
TTTTGTTTTATTCGCTCGTCTGGTAAACGAGGGTTATGATAGTGTGTTGCTTACTACTGCTGTAATTTCCCTTTTGGCCTTGTATCTGCATGATTT
GAATGTGGTATTCCCTAATCTCAACTGATGAATCTTTCTACCTGTAATAATGTTGTTCCGTTAGTTCGTTTTTATAACGTAGATTTTTCTCCCAA
CGTCTGACTGGTATAATGAGCCAGTCTTAAAAATCGCATAAGGTAATTCACAATGATTAAGTGAATTAACCATCTCAAGCCCAATTTACTA
CTCGTTCTGGTGTCTTCTGTCAGGGCAAGCCTTATTCAGTGAATGAGCAGCTTTGTTACGTTGATTTGGGTAATGAATATCCGGTTCTTGTCAAGA
TTACTCTTGATGAAGGTCAGCCAGCCTATGCGCCTGGTCTGTACACCGTTCATCTGTCCCTTTCAAAGTTGGTCAGTTCGTTCCCTTATGATTTG
ACCGTCTGCGCTCGTTCCGGTAAAGTAAACATGAGGACAGGTCGCGGATTTTCGACACAATTTATCAGGCGATGATACAAAATCTCCGTTGTACTTTGT
TTCCGCTTTGGTATAACTCGTGGGGTCAAAGATGAGTGTTTTTAGTGTATCTTTTTGCCTCTTTTCTGTTTTAGGTTGGTGGCTCTGCTAGTGGCATT
CGTATTTTACCGTTTTAATGGAAAATCTCCATGAAAAAGTCTTTAGCTCTCAAAGCCTCTGAGCCGTTGCTACCCTCGTCCGATGCTGCTCTTT
CGCTGCTGAGGGTGACGATCCCGCAAAAGCGGCCTTTAACCCTGCAAGCCTCAGCGACCGAATATATCGGTTATGCGTGGCGATGTTGTTGT
CATTTGTCGGCGCAACTATCGGTATCAAGCTGTTAAGAAATTCACCTCGAAAGCAAGCTGATAAACCGATAACAATTAAGGCTCCTTTTGGAGCCT
TTTTTTTGGAGATTTTCAACGTGAAAAAATTAATTATTCGAATTCCTTTAGTGTTCCTTCTATTCTCACFCGCTGAAACGTTTGAAGTGT
TAGCAAAATCCCATACAGAAAATTCATTTACTAACGCTGGAAGAGCAGCAAAAATTTAGATCGTTACGCTAACTATGAGGGCTGTCTGTGGAATG
CTACAGGCGTTGTAGTTTGTACTGGTGACGAAAATCAGTGTACGCTACATGGGTCCCTATTGGGCTTGCATCCCTGAAAATGAGGGTGGTGGCT
CTGAGGGTGGCGTCTGAGGGTGGCGTCTGAGGGTGGCGTACTAAACCTCTGAGTACGCTGATACACCTATTCCGGCTATACTATATATCA
ACCCCTCTCGACGGCACTTATCCGCTGTACTGAGCAAAAACCCCGCTAATCTAATCTTCTTGTAGGGATTTGCTGGCTTAATFCCCAATGCTGCT
TTCAGAAATAATAGGTTCCGAAAATAGGCAGGGGGCATTAACTGTTTTATACGGGCACTGTTACTCAAGGCACTGACCCCGTTAAAACTTATTACCAGT
ACACTCTGTATCATCAAAGCCATGTATGACGCTTACTGGAACGGTAAATTCAGAGACTGCGCTTCCATTTCTGGCTTTAATGAGGATTTATTTG
TTTTGTGAATATCAAGGCAATCGTCTGACCTGCCTCAACCTCCTGTCAATGCTGGCGGGCCTCTGGTGGTGGTCTGGTGGCGGCTCTGAGGGTG
GTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGGCGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGG
CAAACGCTAATAAGGGGCTATGACCGAAAATGCCGATGAAAACCGCTACAGCTGACGCTAAAGGCAAACTGATTCTGCTCGCTACTGATTACG
GTGCTGTATCGATGGTTCATTGGTGACGTTTCCGGCTTGTCTAATGGTAAATGGTGTACTGGTGAATTTGCTGGCTTAATFCCCAATGCTGCT
AAGTCCGTGACGGTGATAATTCACCTTAAATGAATAATTTCCGTCATAATTTTACCTTCCCTCCCTCAATCGGTTGAATGTCCGCCCTTTTGTCTTTG
GCGCTGGTAAACCATATGAATTTTCTATTGATTGTGACAAAAATAACTTATTCGTTGGTGTCTTTGCGTTTCTTTTATATGTTGCCACCTTTATGT
ATGATTTTCTACGTTTGTCTAACATATGCGTAATAAGGAGTCTTAATCATGCCAGTCTTTTGGGTATTCGTTATTATTGCGTTTCCCTCGGTTT
CCTTCTGGTAACTTTGTTCCGGCTATCTGCTTACTTTTTCTAAAAAGGGCTTCGGTAAGATAGCTATTGCTAATTTTATTGTTTCTTGCTCTTATTA
TGGGCTTAACTCAATTTCTTGTGGTTATCTCTGATATTAGCGCTCAATTTACCCTTGACTTTGTTTTCAGGGTGTTCAGTAAATTTCCCGCTCAA
TGCGTTCCCTGTTTTTATGTTATTCTCTGTAAGGCTGCTAATTTTACTTTTTGAGCTTAAACAAAAAATCGTTTTCTATTGATGGGATAA
ATAATATGGCTGTTTTATTGTTAACTGGCAAAATAGGCTCTGAAAAGACGCTCGTTAGCTTGGTAAAGATTCAGGATAAAAATGTAGCTGGGTGCA
AAATAGCAACTAATCTTGATTTAAGGCTTCAAACCTCCCGCAAGTCCGGAGGTTGCTAAAACGCTCGGTTCTTAGAATAACCGGATAAGCCTT
CTATATCTGATTTGCTTGCTATTGGGCGGGTAATGATTCCTACGATGAAAATAAAAACGGCTTGCTTGTCTCGATGAGTGCGGTACTTGGTTA
ATACCGGTTCTTGAATGATAAGGAAAGACAGCCGATTTATTGATTGGTTTCTACATGCTCGTAAATTAGGATGGGATATTATTTTCTTGTTCAAG
ACTTATCTATTGTTGATAAACAGGGCGGTTCTGCATTAGCTGAACATGTTGTTTATTGTCGCTCGTCTGGACAGAATTTACTTTTGTCCGGTA
CTTTATATTCTCTATTACTGGCTCGAAAATGCCTCGCTAAATTTACATGTTGGCGTTGTTAAATATGGCGATTCTCAATTAAGCCCTACTGTTG
AGCGTTGGCTTTTATACTGTAAGAATTTGTATAACGCTATGATACTAAACAGGCTTTTTCTAGTAATTTAGTATTCGGGTGTTATTCTTATTAA
CGCCTTATTATCACACGGTCGGTATTTCAAACATTAATTTAGGTCAGAAGATGAAATTAACAAAAATATATTGAAAAGTTTTCTCGCGTTC
TTTTGCTTGGGATTGGATTGCATCAGCATTTACATATAGTTATATAACCCAACTAAGCCGGAGGTTAAAAAGGTAGTCTCTCAGACCTATGATT
TTGATAAATTCACTATTGACTCTTCTCAGCGTCTTAATCTAAGCTATCGCTATGTTTTCAAGGATTTCTAAGGAAAATTAATTAATAGCGACGATT
TACAGAAAGCAAGGTTATCTACTACATATATTGATTTATGTAAGTCTTTCCATTAAAAAAGGTAATTCAAATGAAATTTGTTAATGTAATTAATTTT
GTTTTCTTGATTTGTTTTCATCTCTTCTTTGCTCAGGTAATGAAATGAATAATTCGCCCTTGCGCGATTTTGTAACTTGGTATTCAAAGCAA
TCAGGCAATCCGTTATTGTTTCTCCGATGAAAAGTACTGTTACTGATATTATTCATCTGACGTTAAACCTGAAAATCTACGCAATTTCTTTAT
TCTGTTTTACGTGCAAAATTTTGTATGTTAGGTTCTAACCTTCCATTTATTCAGAAGTATAATCCAAACATCAGGATTTCCGGTGTATTGATGAATG
CCATCATCTGATAATCAGGAATATGATGATAATTCGGCTCCTTCTGGTGGTTCCTTTGTTCCGCAAAAATGATAATGTTACTCAAACCTTTAAAAAT
AATAACGTTCCGGCAAAGGATTTAATACGAGTTGTCGAATTTGTTGTAAGTCTAATACTTCTAAATCCTCAAATGTATTATCTATTGACGGCTCT
AATCTATTAGTTGTTAGTCTCCTAAAGATATTTTAGATAACCTTCCCTCAATTCCTTTCACTGTTGATTTGCCAACTGACCAGATATTGATTGAG
GGTTTGATATTTGAGGTTTCAGCAAGGTTGATGCTTTAGATTTTTTCAATTTGCTGCTGGCTCTCAGCGTGGCACTGTTGCAGGCGGTGTTAATACTGAC
CGCCTCACCTATGTTTATCTTCTGCTGGTGGTTCGTTCCGTTATTTTTAATGGCGATGTTTTAGGGCTATCAGTTCCGCGCATTAAGACTAATAGC
CATTCAAAAATTTGTTGCTGCCAGTATTTCTACGTTTCCAGTTCAGGTCAGAAGGTTCTACTGTTGGCCAGAATTCCTTTTTATTACTGGTCTGT
GTGACTGGTGAATCTGCCAATGTAATAATCCATTTACAGCATTGAGCGTCAAAAATGTAGGTTATTTCCATGAGCGTTTTTCTGTGGCAATGGCT
GGCGGTAATATTGTTCTGGATATTACCAGCAAGGCCGATAGTTTGGATTCTTACTCAGGCAAGTGTATTACTAATCAAAGAAGTATTGCT


```
ACAACGGTTAATTTGCGTGATGGACAGACTCTTTTACTCGGTGGCCTCACTGATTATAAAAAACACTTCTCAGGATTCTGGCGTACCGTTCCTGTCT  
AAAATCCCTTTAATCGGCCTCCTGTTTAGCTCCCGCTCGATTCTAACGAGGAAAGCACGTTATACGTGCTCGTCAAAGCAACCATAGTACGCGCC  
CTGTAGCGGCGCATTAAAGCGCGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTT  
CCCTTCCTTTCTCGCCACGTTTCGCCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCGATTTAGTGCTTTACGGCACCTCGA  
CCCCAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCC
```

Supplementary References

- [1] R. Jungmann, M. S. Avendano, M. Dai, J. B. Woehrstein, S. S. Agasti, Z. Feiger, A. Rodal, P. Yin, *Nat Methods* **2016**, *13*, 439-442.
- [2] J. Schnitzbauer, M. T. Strauss, T. Schlichthaerle, F. Schueder, R. Jungmann, *Nat Protoc* **in review**.
- [3] C. S. Smith, N. Joseph, B. Rieger, K. A. Lidke, *Nature Methods* **2010**, *7*, 373-U352.
- [4] U. Endesfelder, S. Malkusch, F. Fricke, M. Heilemann, *Histochem Cell Biol* **2014**, *141*, 629-638.