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Electronic Supplementary Information

Construction of optically controllable CRISPR-Cas9 system using a DNA origami nanostructure

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Experimental Procedure

Materials. All the staple DNAs for the DNA nanoring were purchased from Eurofins Genomics (Tokyo, Japan). Single-stranded p8064 DNA was purchased from Tilibit nanosystems (Garching, Germany). DNA strand containing a cleavable linker was purchased from Japan Bio Services (Saitama, Japan). Cas9 and Streptavidin Magnetic Beads for purification were purchased from New England Biolabs (Ipswich, MA). The gel-filtration column and the Sephacryl S-300 and S-400 were purchased from BioRad Laboratories (Hercules, CA) and GE healthcare (Bucking Hamshire, UK), respectively.

Preparation of DNA nanoring. A solution was prepared with 40 nM p8046 ssDNA (template strand), 4 equivalents of staple strands, 10 mM $MgCl_2$, 1 mM EDTA, and pH was adjusted with 20 mM Tris-HCl (pH 7.6) as a buffer. This solution was incubated at 65 °C for 15 min, then cooled stepwise by 1 °C every 60 min from 60 °C to 40 °C to assemble nanoring. Sequences of staple DNAs used here are listed in Table 1.

Preparation of sgRNA. Single-guide RNA (sgRNA) was prepared by in vitro transcription from a template dsDNA containing T7 promotor sequence. The template dsDNA was prepared by performing overlap extension PCR with an 84 nt and 56 nt template DNA strands (Table S1). The PCR amplification was performed using Go Taq polymerase (Promega) with a forward and reverse primer (Table S1) by following the manufactures protocol. The amplified product was purified using a QIAquick PCR purification Kit (Qiagen).

The in vitro transcription reaction was performed using 30 nM dsDNA template, 40 mM Tris-HCl (pH 8.0), 5 mM DTT, 8 mM $MgCl_2$, 2 mM spermidine, 2 mM NTP and 0.25 uM T7 RNA polymerase (Takara) at 37 °C for 2 h. The resulting sgRNA was purified by RNA purification kit (Qiagen) and the quality was confirmed by running the sgRNA on an 8% denaturing polyacrylamide gel. Sequence of template dsDNA and sgRNA are listed in Table S2.

Introduction of protein. For incorporation of Cas9/sgRNA into the nanoring (Figure S1), 1 μM Cas9 and 500 nM sgRNA were incubated in a buffer containing 20 mM HEPES, 100 mM NaCl, 5 mM

$MgCl_2$ and 0.1 mM EDTA at 25 °C for 10 min. A solution of 250 nM Cas9/sgRNA complex was added to a solution of 10 nM DNA nanorings with PC-linker-containing complementary strand in a buffer (10 mM HEPES, 50 mM NaCl, 5 mM $MgCl_2$, 0.01 mM EDTA), and then incubated at 37 °C for 1 h. Excess staple DNAs were removed by gel filtration using Sephacryl-S400.

Purification of nanoring. For purification of a Cas9-attached nanoring, a single-stranded DNA (ssDNA, 25 nt) was introduced to the nanoring as a capture strand (Figure S2). A 50 µM of biotinylated complementary strand (16 nt) to hybridize with above 25-nt capture ssDNA was added to a mixture of streptavidin-attached magnetic beads (4 mg/mL), and the mixture was kept at rt for 5 min, then the magnetic beads was washed to remove excess biotinylated strand. The Cas9-attached nanoring with 25-nt capture ssDNA was added to the magnetic beads mixture. The mixture was kept at 25 °C for 30 min, then the beads were trapped using a magnet stand, and the remaining supernatant was removed to remove excess Cas9. After three times washing, the Cas9-attached nanoring was separated from the beads using strand displacement with 50 µM complementary ssDNA (25 nt) by incubating at 25 °C for 30 min, and the purified Cas9-attached nanoring was recovered. The sequences of DNA strands used for purification are listed in Table S3.

Confirmation of the Cas-9 activity. For photoirradiation, the purified Cas9-attached nanoring (7 nM) was irradiated with UV light (350 nm) for 10 min using Xenon Light Source (ASAHI SPECTRA MAX-303, 300W). After irradiation, PCR-amplified EGFP-coding dsDNA (850 bp, 10 nM) was added to the solution of photoirradiated Cas9-nanoring, and the mixture was incubated at 37 °C for 60 min. The reaction solution was then heated at 65 °C for 10 min to dissociate Cas9 from the substrate dsDNA, and then the decomposed stapled DNAs were removed by Sephacryl-S300 gel filtration and the reaction substrate was recovered. The cleavage reaction was observed by agarose gel electrophoresis. Sequence of a target dsDNA (EGFP gene) is presented in Table S4

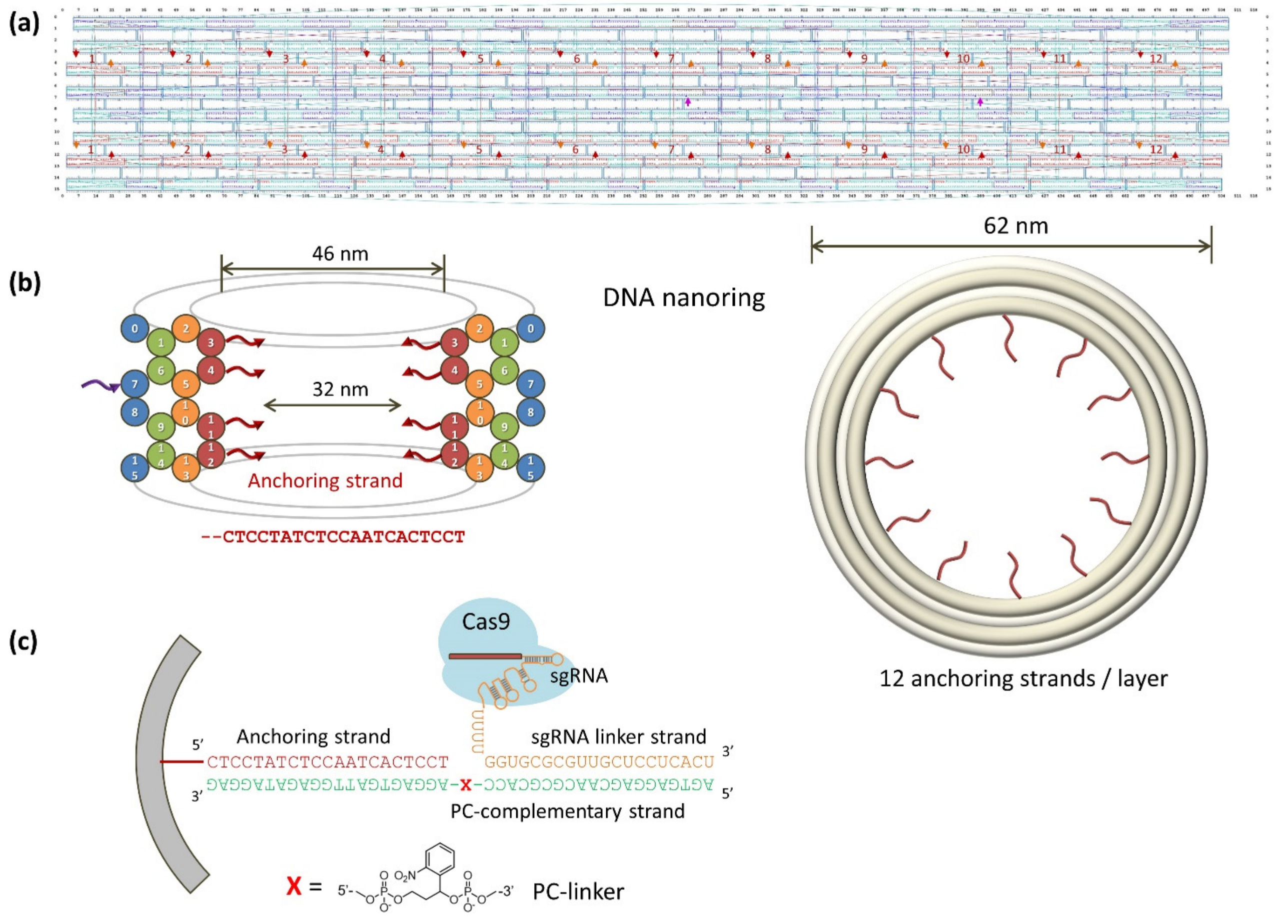

Figure S1. Schematic illustration of introduction of Cas9 into the nanoring. (a) caDNAno design of DNA origami nanoring. (b) Illustration of the nanoring with anchoring strands inside the cavity. Twelve anchoring strands were incorporated for one layer, and total 48 strands were incorporated. (c) Incorporation of Cas9 inside the nanoring. Linker RNA strand was added to 3'-end of sgRNA. Cas9/sgRNA with linker strand was bound to the anchoring DNA strand inside the nanoring with the PC-linker-containing complementary strand.

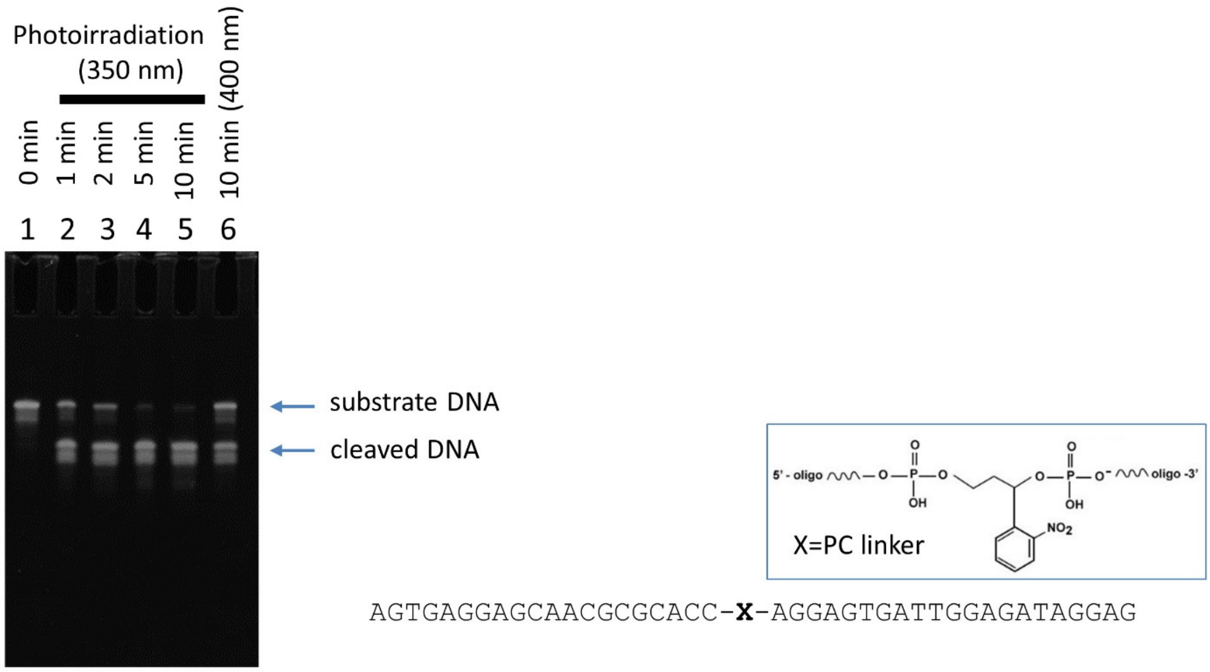

Figure S2. Image of denatured PAGE gel for investigating the efficiency of photo-cleavage reaction. Substrate DNA containing PC-linker without irradiation (lane 1), with 350 nm UV for 1 min (lane 2), 2 min (lane 3), 5 min (lane 4), 10 min (lane 5), and with 400 nm UV for 10 min (lane 6). Electrophoresis conditions: 16% acrylamide, 8 M Urea, TBE buffer, 150 V, 60 min, rt.

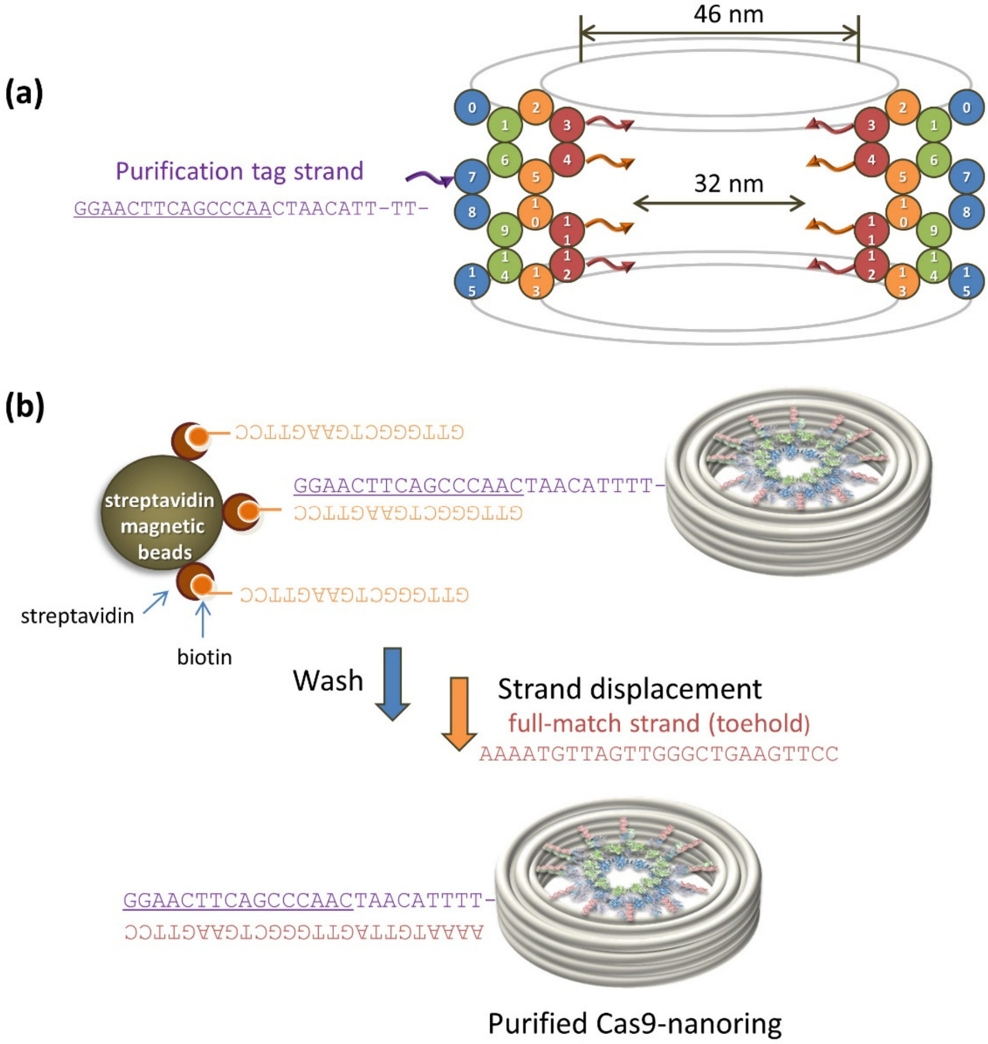

Figure S3. Schematic illustration of the purification. (a) Purification tag strands were incorporate to the outer side of the naoring. (b) Biotinylated DNA strand was immobilized on the streptavidin magnetic beads. Purification-tag attached Cas9-nanoring was attached onto the magnetic beads to wash out excess Cas9, and then separated to recover the purified Cas9-nanoring using strand displacement.

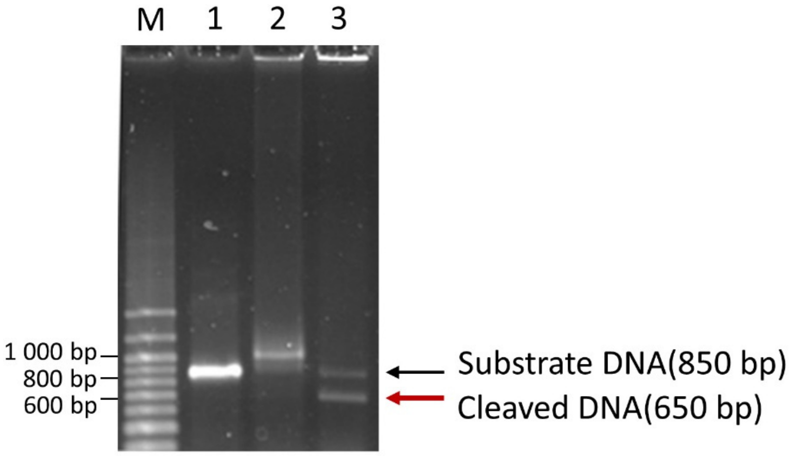

Figure S4. Image of agarose gel for investigating cleavage reaction efficiency of free Cas9. Lane 1: substrate DNA only, lane 2: Substrate DNA incubated with Cas9 at 37 ºC for 1 h (without denaturation to dissociate Cas9 from the substrate DNA), lane 2: Substrate DNA incubated with Cas9 at 37 ºC for 1 h followed by denaturation at 60 ºC for 30 min. Electrophoresis conditions: 1% agarose, TBE buffer, 100 V, 60 min, r.t. In this experiment, Cas9: DNA = 400: 24 (DNA 10 nM) was used.

After the reaction, 74% of the substrate DNA strand were cleaved. Therefore, it is considered that ideally the DNA strand should be cleaved almost completely at the ratio of Cas9: DNA = 400: 1 (DNA 10 nM) as shown in Figure 3c, if Cas9 is used as a free form without being introduced into the nanoring.

Table S1. Staple DNA sequences for ring-shape DNA origami

Staple	sequence
ring 001 h0-1A	TTCACTACTTACCACTCTACC-GGGCGATGTTGGGGTCGAGGTAACGCTCATGTACCGCCAGCCTTGCT
ring 002 h0-2A	TTCACTACTTACCACTCTACC-TAAAGAACCCTTCTGACCTGCCTCATATTCAAACCACCAGTAACAT
ring 003 h0-3A	TTCACTACTTACCACTCTACC-CGAGATAGCAGAAATAAAGAACATATATTTTCAAAGAACAAATGCTG
ring 004 h0-4A	TTCACTACTTACCACTCTACC-GGTGGTTCTTTAGTATCATATTTTTAAATCATCCCGACTCTAAGAAC
ring 005 h0-5A	TTCACTACTTACCACTCTACC-AGCGGTCTTTATCCCAATCCGAGATTGAGGCCAAAGACAATAGAAA
ring 006 h0-6A	TTCACTACTTACCACTCTACC-CAACAGCTCATTAGCAAGGCCACTTCGGAACTATAAACAGGGTCAGT
ring 007 h0-7A	TTCACTACTTACCACTCTACC-TGCGTATTATATAAGTATAGCGGTATATTCGCAACAACCTTGATACC
ring 008 h0-8A	TTCACTACTTACCACTCTACC-TGCCAGCAAAGACTTTTTCACAATTATACCACCTTATGTTAATTTC
ring 009 h0-9A	TTCACTACTTACCACTCTACC-TAATTGCGAATGCAGATACATCAACTAAAGTTGTAGCTCCTTAGAGC
ring 010 h0-10A	TTCACTACTTACCACTCTACC-CATAAAGTTATATTTTCATTTCCAGATTGTAATAATCAGAGCATGTC
ring 011 h0-11A	TTCACTACTTACCACTCTACC-GAAATTGTCTGGCCTTCCTGGCCTTTCTCCCCAGTCCCATCGGCGA
ring 012 h0-12A	TTCACTACTTACCACTCTACC-GGTACCGAAAAATCCCGTAAAGCGTGCACTCGATCCAGCAGCGGTGC
ring 013	CGTGAACCATCCTCACAGTTGAGGATCCCCG
ring 014	CCAACGTCAAAGGGCGATTCTGGACACACGACCAGTAATGCCG
ring 015	TGTTGTTCCAGTTTGGTCAAAATGTTGGAAGGGTTAGAAAAAG
ring 016	GCAAAATCCCTTATACATAATTTATAAATAAGAATAAAATTG
ring 017	TTTGCCCCAGCAGGCGTACAAAAACTTTCCAGAGCCTAAGCGT
ring 018	CTTCACCGCCTGGCCCAAATCACGACCATTTGGGAATTAAAAT
ring 019	GGGTGGTTTTTCTTTATAAGTGCAGGGTTTTGCTCAGTGGAA
ring 020	TGAATCGGCCAACGCGGGCTACAAAAGCATCGGAACGAGCCGG
ring 021	CACTGCCCGCTTTCCATTTAGGATAGTAGAAAGATTCATTGAG
ring 022	TGGGGTGCCTAATGAAATGGTCTCTTGACCATTAGATAAACG
ring 023	TCACAATTCCACACAAACGCCATTGAGCTCATTTTTTAAGGGG
ring 024	TTCGTAATCATGGTCACGGTTGTTGCTCATTTGCCGCCATAGC
ring 025	TAAAGCATTACATTGGCAGGTCTGATACCTATTGCTT
ring 026	CGTAAGAGTTTGGATTATACAATATATTATCATGACGC
ring 027	CGTAGATCGACCGTGTGATTAATGGAATTTCGGACAA
ring 028	TATACAATCTTACCAACGCACAATTTAGTTGAAGACG
ring 029	AAGAAACATTATCACCGTCAATTCATGAAGGTACTTGA
ring 030	ACGTCACCAAGAGAAGGATAGAGGCTATTCTCACCAC
ring 031	AATAGGTCGTCACCCTCAGGCCGCTCTGAGGTTAACG
ring 032	GAAGTTTCTAACGGAACAACCGTTAAAGGACGGATTGT
ring 033	CCAAAAGTTCTGCGAACGAAACAGTTGTCTGTTGTCA
ring 034	CGCGAGCTCGCATTAAATTAATATTCAAATAGACGGG
ring 035	CAGCTTTAGCGGATCAAACTAAGAGATGAAGGCCGGAA
ring 036	GCCGCACTACCGGGGGTTTGCCGTTGTGCTGCGGTCT
ring 037	AGGGAGCCCCATCTAAATCGGAACCCTCGTGCCTGTTCTTCAAAA
ring 038	TATTTTTGAATTATACGTGGCACAGCCAACAGAAAATACCG
ring 039	GATGAATATACACTTTCAGGTTTAACTATTTGCAGCTTTGAA
ring 040	GCCAACGCTCAAATTCTTACCAGTAACTAGAAAATTTAGGC
ring 041	CAAAAATGAAAAGATTTTTTGTTTATAAACAGCGGGAGAAT
ring 042	GCAGCACCGTACTCAATGAAACCATCCAGTAGCATTTCATCG
ring 043	AGGTTTAGTAATGTATCACCGTACTCCGTCGAGACCCTCAT
ring 044	TACGTAATGCCACCATTAAACGGGTGAGGCTTTACTCATCT
ring 045	AAGAGCAACACAAGAATTACGAGGCAATACCACAGCTTTTGC
ring 046	TTCTACTAATATTGAAAAGGTGGCAAATAACCTCAAAATTA

ring 047		AGCGAGTAACACCATCAACATTAAACAAAAATAGGTCACGT
ring 048		GAAGGGTAAAGCAAGGCGGCCTTTAGGTACATCGCGTCGCTG
ring 049	h3-1C	TTCGATTTAGAGCTTGATGCGGCGGCTGCCAGCACGAA-CTCCTATCTCCAATCACTCCT
ring 050	h3-2C	TGGCTATTAGTCTTTAAGCTCAATCATTCACCAGTCAA-CTCCTATCTCCAATCACTCCT
ring 051	h3-3C	ATAGTAACAGTACCTTTAATTCATCTTCTGAATAACA-CTCCTATCTCCAATCACTCCT
ring 052	h3-4C	TGAACAGTAGGGCTTAACCTAAATTAAATAAGGCGTAA-CTCCTATCTCCAATCACTCCT
ring 053	h3-5C	GATAGCAGCCTTTACAGACCCAGCTTAACGAGCGTCGT-CTCCTATCTCCAATCACTCCT
ring 054	h3-6C	TCATCAGTAGCGACAGACGGAAATTCCGACTTGAGTA-CTCCTATCTCCAATCACTCCT
ring 055	h3-7C	GGCCGCCACCCTCAGAAAAGTATTATAGGATTAGCGGG-CTCCTATCTCCAATCACTCCT
ring 056	h3-8C	AACTACGAAGGCACCAAAGTTAAAGCAGCGAAAGACAA-CTCCTATCTCCAATCACTCCT
ring 057	h3-9C	CCTATCATAACCCTCGTAAAATCTAATTATTACAGGT-CTCCTATCTCCAATCACTCCT
ring 058	h3-10C	AAAGTAGTAGCATTAACTTCCATATGTAGATTTAGTAA-CTCCTATCTCCAATCACTCCT
ring 059	h3-11C	AACCCGTCGGATTCTCCTAAACGTTTTTGTTAAATCTG-CTCCTATCTCCAATCACTCCT
ring 060	h3-12C	GTTTAAACGATGCTGATCACGGAAATAAATTTCTGAT-CTCCTATCTCCAATCACTCCT
ring 061		CGAAATGCGCGCCTGTCCGTGAGCCTCACCCAAATCGAAGAAAG
ring 062		AACGACAACTGCGCGAACTGAAATGACAGTATTACAAATCAA
ring 063		TACCAGAGCTACATCGGGAGAAATTAAATTACCTTGAGTGAA
ring 064		AGAGGTTGATTGAGAATCGCGTTCAGACAAAAGGTCCTAATT
ring 065		TAACTTTGCAGAGAATAACATAAGAGCTAATATCCGAACAAA
ring 066		GCATTCAGAATCAAGTTTGCCCCTCATTCATAATACCAGAGC
ring 067		TTTCACCGACCGCCACCCTCCAGACAACTGAGTTTCTGTATG
ring 068		TTGACCAGGCCTAAAACGAACCTGCTACGGAGATAAAGAGGA
ring 069		AAAAGAAGATTACCAGACGAATCCCCACTGGATAGACTATTA
ring 070		AGCAAAACAATCCAATAAATCAACGCAAAACATTAGATTCAA
ring 071		TGGTGAATGGTGGGAACAAACAGCTTTGAGGGGATGGGAAGG
ring 072		GCAGCAGCTTGCCGTTCCGGGCAAGAGGACTTGTCATCAGCG
ring 073		AACAATATGAGAGAAAGGAAGGAAGTTTTGCCCACTA
ring 074		AACAAAGCTCATCGCCATTAAGATAGAACGTGGACT
ring 075		TCGCAAGAAGATTCGCCTGATTCGTAAAAGGTTGAG
ring 076		AGCGAACCAGCGCCAACATGTAAAGCCTGCGAAATCG
ring 077		ACCAGCGGAAGCGCATTAGACCATATTACACGCTGG
ring 078		AGTGCCCGCTACTGTAGCGCGTCCATTACGATTGCC
ring 079	tag1	GGAACTTCAGCCCAACTAACATTTT-GACAATGAGTCTCAGAGCCACCAGGGTTGGGGCGCCA
ring 080		GTGAATTAGATACACTAAAACGAGGACTTGCATTAA
ring 081		TATAATGCACAAATAGCGAGAGTTCAACTTTGCGCT
ring 082	tag2	GGAACTTCAGCCCAACTAACATTTT-CCCGGTTGTAGCAAAGAATTAGGTTTAGCGTAAAGCC
ring 083		ATGTTTAGTCGTAATGGGATAATTCGCGTTATCCGC
ring 084		CATCAGACTGGTTTTTTCGTCTACATAAAGCTCGAA
ring 085	h15-1B	GGTAATATCCACACCCGCCGCGCATCAGTGAGCCAGAATCCTGAGA-TTCCTCTACCACCTACATCAC
ring 086	h15-2B	TATCATTCTGCAACAGTGCCCCGATTAGAGTATCTTTAGGAGCACT-TTCCTCTACCACCTACATCAC
ring 087	h15-3B	ATGCAAATAGAAGATGATGAACTAGCGATAGATTTTCCCTTAGAATC-TTCCTCTACCACCTACATCAC
ring 088	h15-4B	GCGAGGCGATTCTGTCCAGACTCATTAAACCTCTTTCCTTATCATT-TTCCTCTACCACCTACATCAC
ring 089	h15-5B	ATTCATATAACCCACAAGAACAATTAAGACAACGGAATACCCAAAA-TTCCTCTACCACCTACATCAC
ring 090	h15-6B	GCCTTGAGACCGGAACCAGAGGGAAATAAATAGACGATTGGCCTTGA-TTCCTCTACCACCTACATCAC
ring 091	h15-7B	GATAGTTGCAGTACAAACTACGCGGAATTGCGAGTGAGAATAGAAA-TTCCTCTACCACCTACATCAC
ring 092	h15-8B	AACTTTAATCGCCTGATAAAACGACAAGAATGGCTGACCTTCATCA-TTCCTCTACCACCTACATCAC
ring 093	h15-9B	TTAATTGCATACTGCGGAATCTTATCGCGTTTAAGAGGAAGCCCGAA-TTCCTCTACCACCTACATCAC
ring 094	h15-10B	AATCATATTGTAATACTTTTGGATGATAAATATCAATATGATATTC-TTCCTCTACCACCTACATCAC
ring 095	h15-11B	AACGTACAGTATCGGCCTCAACGCGATTAAGCTGGCGAAAGGGGGA-TTCCTCTACCACCTACATCAC

ring 096 h15-12B	CGGTGCCCCAGCGTGGTGCTGGCACTGTTGCGGGCGCGGTTGCGGTA-TTCCTCTACCACCTACATCAC
ring 097	ACTATCGGCCATTGCAAACCACCAGCAGAAGATAACGG
ring 098	AAAGTTTGAGAAGGAGCGAGTTACAAAATCGCGCAGAAA
ring 099	CTATATGTGCGAGAAAACCATTTTCGAGCCAGTAATATT
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ring 101	TCACAATCAAAAGGGCGATTCGGTCATAGCCCCCTTAAC
ring 102	TTTTAACGGTTAATGCCCGGGATAGCAAGCCCAATAGCC
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ring 111	TGAGAGCTTTACAAACAATTTTAGAACAATAG
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ring 119	CACCGGAGTTCCAGTAAGCAGTCTCATTAAA
ring 120	GATGATAGAAACATTGCCTATCAGGCGGTCACCAGTGAGACGGG
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ring 122	TATCAGCCTTGCAGTAACCGATAGCAACCGGGGAGAGGCGGTT
ring 123	GTCGAAATCAGTGAATAAGGAACAAAATATTC
ring 124	AACACCATTGGGAACTGGCTCGTTGAGAGTCGGGAAACCTGTCG
ring 125	TAAATATACAGGTCAGGATCCGGAATTCGAG
ring 126	TTGCTCCGAAGTTTATATGCATTTCGCAGTGAGCTAACTCACAT
ring 127	AGAAGCCTATCAGGTCATTAGAGATGCCGGA
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ring 129	GATCGCACGACGGCCAGTGCCACGACGGTAAC
ring 130	GGAGCCGGATAGCTAGATAGAAGTTGGGTAGCTGTTTCCTGTGT
ring 131	GGTCAGCCATCCCTTACACCTTTGCCGGCTG
ring 132	TCGTTAACGGCCAGAACGGGGAAAGCCGGGCGCGTGCAAA
ring 133 h4-1C	TATGGTTGCTGTCGCTACAGGCGAACGTGGCAAAATGGA-CTCCTATCTCCAATCACTCCT
ring 134 h4-2C	TCTAAAGCATCCCAGCAGCATAGCCCTAAAAGAATCCTGA-CTCCTATCTCCAATCACTCCT
ring 135 h4-3C	CATTTAACAATAAGAAAACAAAACAATAACGGTTTTTGAA-CTCCTATCTCCAATCACTCCT
ring 136 h4-4C	TGCAGAACGCTCACAACATCATATTTAACAAATTTATCC-CTCCTATCTCCAATCACTCCT
ring 137 h4-5C	AAACAATGAAACCCAATAATAAAAACAGGGAGGTAAAGGT-CTCCTATCTCCAATCACTCCT
ring 138 h4-6C	CGCCACCCTCACCACCGCCTCTTTAGCGTCAGATTGAGAC-CTCCTATCTCCAATCACTCCT
ring 139 h4-7C	TCATAGTTAGCTCATTCCAAGAACCGCCACCCGTTTGCG-CTCCTATCTCCAATCACTCCT
ring 140 h4-8C	GTTACTTAGCCTTCCGCGAAGAGGCAAAAGATCTAAAACG-CTCCTATCTCCAATCACTCCT
ring 141 h4-9C	AATGCTTTAAACATCATTGACGATAAAAACCAGGTGATTC-CTCCTATCTCCAATCACTCCT
ring 142 h4-10C	ATAAAAATTTGCTTTATTTCATACAGGCAAGAGTTGTTA-CTCCTATCTCCAATCACTCCT
ring 143 h4-11C	GCACCGCTTCTACTCCAGCCGGCGGATTGACGGCGCAGAA-CTCCTATCTCCAATCACTCCT
ring 144 h4-12C	CAACGGCAGCAAAAGCAACCCAAACGCGGTCCTGTTCACG-CTCCTATCTCCAATCACTCCT

ring 145 h11-1C	CCTTGACGAGCACGTATAAAGGTTTTGGTGTGTTCAAC-CTCCTATCTCCAATCACTCCT
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ring 147 h11-3C	TCTTCATTTGAATTACCTTGAGGATCGACAACTCGTA-CTCCTATCTCCAATCACTCCT
ring 148 h11-4C	TTGCCTGTTTATCAACAAAGAGTCAGAGAGACTACCAA-CTCCTATCTCCAATCACTCCT
ring 149 h11-5C	ATAGCAATAGCTATCTTAACAAGCAATTACCGCGCCAG-CTCCTATCTCCAATCACTCCT
ring 150 h11-6C	TAGAACCGCCACCCTCATAGCAAACCATATAAAAGGC-CTCCTATCTCCAATCACTCCT
ring 151 h11-7C	GGCGTAACGATCTAAAGGAAAGCGCGTCATACATGGCC-CTCCTATCTCCAATCACTCCT
ring 152 h11-8C	AGGAACGAGGCGCAGACTTGAAAATTTTAATTGTATAT-CTCCTATCTCCAATCACTCCT
ring 153 h11-9C	TCCAGTTCAGAAAACGAATCAACGTCTTGCCCTGACA-CTCCTATCTCCAATCACTCCT
ring 154 h11-10C	AGTTAGAACCCTCATATGAACCAGATAGAGAGTACCGG-CTCCTATCTCCAATCACTCCT
ring 155 h11-11C	AGGTGCCGGAAACCAGGTATTTTTGGCCTGAGAGTCCG-CTCCTATCTCCAATCACTCCT
ring 156 h11-12C	TACCGTCGGTGGTGCCATTCCCAGTCAAGCTTTCAGC-CTCCTATCTCCAATCACTCCT
ring 157	TAAAAGATTTTATACTGAGTAGAAGAGTAATAATA
ring 158	ATAATACACTAATACGAACGTTATTATCATTATTA
16HB ring 159	GACGCTGAAAACATTAGGTTGGGTTACGCTTTTTT
16HB ring 160	CTCATCGAACGGGTAGATATAGAAGGCGCACAATA
16HB ring 161	GCAGTATTGGCATGCGGAATAAGTTTTTGAAAACG
16HB ring 162	GCCAGAACACAAACAGTGTACTGGTAACGCCTTTT
16HB ring 163	TTTTTCAAACTAAATTTCGAGGTGAACACCCGGTT
16HB ring 164	ATTACCCTAATCTTGAGTAGTAAATTAGCTCGAGA
16HB ring 165	CTTCAAATTCAAATGATAAGAGGTCACAAATTTAA
16HB ring 166	GAGGGTATTCTAGCATCGATGAACGGTTTCTGGAG
16HB ring 167	GCCAGGGCTGCAAGGGGAACGGATAACCATGAGGT
16HB ring 168	GGCCGAGCCGGGTCATCAGATGCCGGCACGCTGCGCAAACAGGA
16HB ring 169 h12-1C	GTAATGGGTAACGTGCTTTCCTCGTTAGAATCCCAATTAA-CTCCTATCTCCAATCACTCCT
16HB ring 170 h12-2C	CAGTTGTGTAATATCAAACCCTCAATCAATATGTGTATTAG-CTCCTATCTCCAATCACTCCT
16HB ring 171 h12-3C	TAACCCAATTTTTTTAATGGAAACAGTACATATAAATTTA-CTCCTATCTCCAATCACTCCT
16HB ring 172 h12-4C	TACGATGAGATAGATAAGTCCTGAACAAGAAAGTTTTTAT-CTCCTATCTCCAATCACTCCT
16HB ring 173 h12-5C	GTTACAGAGACCGAAGCCCTTTTTAAGAAAAGCTAATACAT-CTCCTATCTCCAATCACTCCT
16HB ring 174 h12-6C	CGCCGACGTGAGCCACCACCCTCAGAGCCGCCTCTGAATT-CTCCTATCTCCAATCACTCCT
16HB ring 175 h12-7C	GGATTTTTGTTTTGTCGTCTTTCCAGACGTTAATAAAAAA-CTCCTATCTCCAATCACTCCT
16HB ring 176 h12-8C	CAGATACCGGGTCAATCATAAGGGAACCGAACGGGCTGCTC-CTCCTATCTCCAATCACTCCT
16HB ring 177 h12-9C	TAGTCAGAAGAATGACCATAAATCAAAAATCAAAGCAAAC-CTCCTATCTCCAATCACTCCT
16HB ring 178 h12-10C	AAGGGACGCATTTTAAATGCAATGCCTGAGTAATCTACAA-CTCCTATCTCCAATCACTCCT
16HB ring 179 h12-11C	GCGATCGGCCAAAGCGCCATTCGCCATTCAGGTGGTTGTAA-CTCCTATCTCCAATCACTCCT
16HB ring 180 h12-12C	GGGTCTGTTTCCCACGCAACCAGCTTACGGCTTGTCGTCA-CTCCTATCTCCAATCACTCCT
16HB ring 181	GGGAGCTGTAACCACCTGGCAAGTGTAGCGGTGTTAC
16HB ring 182	CAGTTGGACACCGCTTGAACAGAGGTGAGGCGACTCAA
16HB ring 183	ATATATGGAGCAAACCGGCGAATTATTCATTATTTA
16HB ring 184	TATCCCATAAAGTATTAGAGAATATAAAGTACTATAA
16HB ring 185	AGATAGCAGAGAGATGGCAGAGGGTAATTGAGCTTATC
16HB ring 186	AACCACCCAAAATCTATTAGCGTTTGCCATCATTTTG
16HB ring 187	TGAATTTTCGTCACCGGAACCCATGTACCGTAATAAG
16HB ring 188	AACTTTGTTGTATCATCGCGCGAAACAAAGTATTTCTT
16HB ring 189	TTACCCTGCGTCCATGAATAGTAAAATGTTTGGGCTT
16HB ring 190	TAGGTAAATGACCCGTGCTAAATCGGTTGTACTTTTT
16HB ring 191	CAACTGTCGACGACAGCCCGTGCATCTGCCAGTAATCG
16HB ring 192	TGTCCAGAGAACGTCCTCATAACGGAACGTGCCTCAC
16HB ring 193	AGTGAAAGGAATTGAGGAAGGTTATCTAAAACCCTGGT

16HB ring 194	AATTGCTTCTGTAAATCGTCGCTATTAATTACTAATCA
16HB ring 195	CTGCATGTAGAAACCAATCAATAATCGGCTGAAAATAA
16HB ring 196	CCACAGAAGGAAACCGAGGAAACGCAATAATTCTAAGC
16HB ring 197	GACCAGCATTGACAGGAGGTTGAGGCAGGTCCCACCAG
16HB ring 198	TATTGCTAAACAACTTTCAACAGTTTCAGCGGAGTAAA
16HB ring 199	GGAGAACGGTGTACAGACCAGGCGCATAGGCCCTGACC
16HB ring 200	AGAGAAGCAAAGCGGATTGCATCAAAAAGATTTGGTCT
16HB ring 201	AGTGAGAAAGGCCGGAGACAGTCAAATCACCTAATGTG
16HB ring 202	AACCGGTGCGGGCCTCTTCGCTATTACGCCAGTCTGCG
16HB ring 203	TGATTGCAGGCGCTTTCGCACTCAATCCGCCCCGGAGG
16HB ring 204	TGATTAAAGGGATTTTAGACAGGAACGGTACGGAGAGC
complementary strand A	GGTAGAGTGGTAAGTAGTGAA
complementary strand B	GTGATGTAGGTGGTAGAGGAA
complementary strand C	AGGAGTGATTGGAGATAGGAG

Table S2. Sequences of sgRNA and temple dsDNA.

	sequence
sgRNA + connector	GGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGA GUCGGUGCUUUUGGUGCGCGUUGCUCCUCACU
T7 promoter + sgRNA template + connector top 84 nt	TTCTAATACGACTCACTATA- GGGCACGGGCAGCUUGCCGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTT
T7 promoter + sgRNA template + connector bottom 80 nt	AGTGAGGAGCAACGCGCACC-AAAA- GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
Forward primer for template	TTCTAATACGACTCACTATAGG
Reverse Primer for template	AGTGAGGAGCAACGCGCACCAA

Table S3. DNA sequences of biotinylated strand and fully complementary strand for purification.

Staple	sequence
biotinylated strand	GTTGGGCTGAAGTTCC[BIOTEG]
complementary strand	AAAATGTTAGTTGGGCTGAAGTTCC

Table S4. Substrate sequence (EGFP complementary strand, 714 bp)

GTACAGCTCGTCCATGCCGAGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTCTTTG
CTCAGGGCGGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGGGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGG
CGAGCTGCACGCTGCCGTCCTCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGTCGGCCATGATATAGAC
GTTGTGGCTGTTGTAGTTGTACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTC
ACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGC
CTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGT
CACGAGGGTGGGCCA**GGGCACGGGCAGCTTGCCGGTGG**TGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCC
TCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCT
TGCTCACCAT

The underline presents the target sequence.

Reference

1. P. Ketterer, A. N. Ananth, D. S. Laman Trip, A. Mishra, E. Bertosin, M. Ganji, J. van der Torre, P. Onck, H. Dietz and C. Dekker, *Nature Communications*, **2018**, 9, 902.