Supplementary information

CRISPR-guided programmable self-assembly of artificial virus-like nucleocapsids

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SUPPLEMENTARY FIGURES

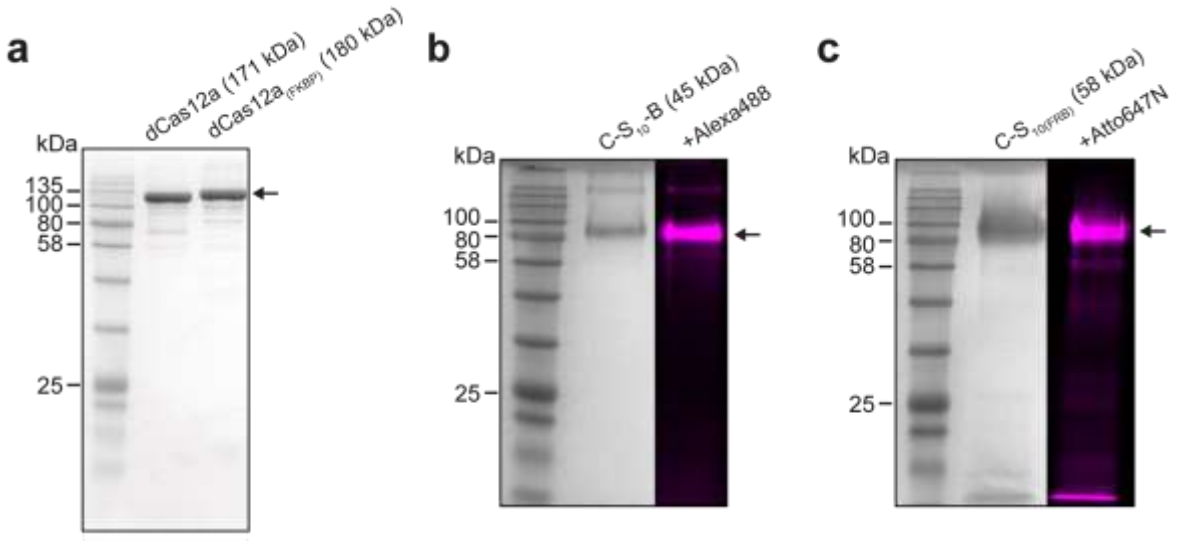

Figure S1: Proteins used in this study. **a**: SDS-PAGE gel with dCas12a (171 kDa) and the fusion protein containing dCas12a and the FKBP domain (dCas12a$_{(FKBP)}$, 180 kDa). **b**: SDS-PAGE gel with Coomassie stained (left) and maleimide-Alexa488 labeled (right) C-S$_{10}$-B (45 kDa). **c**: SDS-PAGE gel with Coomassie-stained (left) and maleimide-Atto647N labeled (right) fusion protein containing C-S$_{10}$ and the FRB domain (C-S$_{10(FRB)}$, 58 kDa). Both C-S$_{10}$-B and C-S$_{10(FRB)}$ show aberrant migration in gel because of the high proline content (22%) in the C domain.

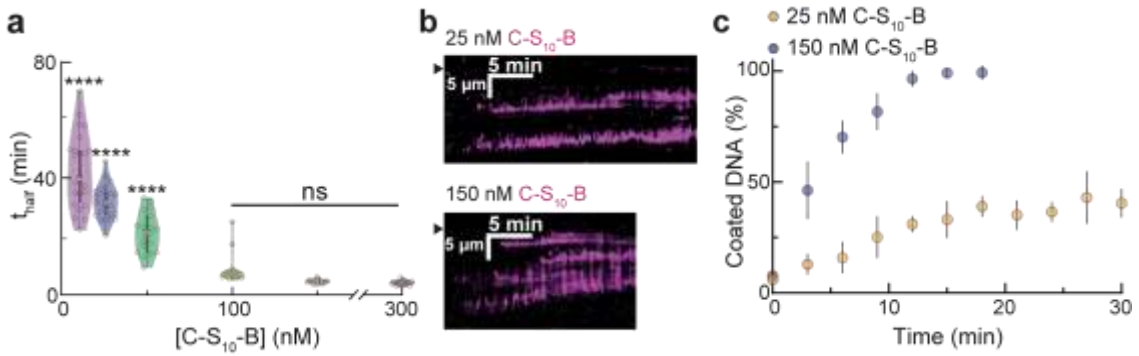

Figure S2: C-S_{10}-B concentration regulates DNA condensation rate and extent of compaction. **a**: The time (t_{half}) required to reach half of maximum condensation for each DNA molecule analyzed in Fig. 1d. We fit condensation profiles (Fig. 1d) to the Hill equation and estimated t_{half} for molecules that did not reach 66.5% encapsidation during the experiment (30 min). (ns) and (****) indicate $p > 0.05$ and $p < 0.0001$, respectively. **b**: Kymographs showing binding of C-S_{10}-B (magenta, Alexa488-labeled) on DNA (unlabeled) at the indicated C-S_{10}-B concentrations. Black arrows indicate the chromium barriers. **c**: Extent of DNA that is coated by fluorescent C-S_{10}-B. Points and error bars indicate the mean and standard deviation, respectively (N = 10 DNA molecules per condition).

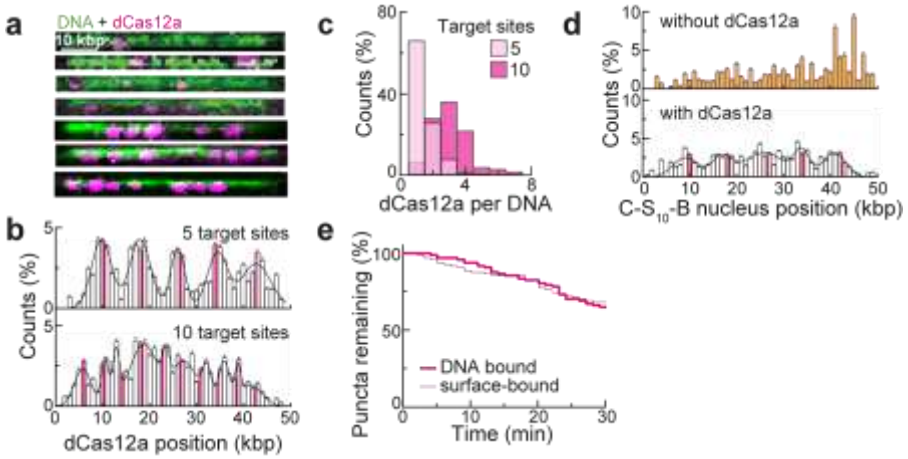

Figure S3: Characterization of dCas12a binding to DNA substrates prior to encapsidation by C-S_{10}-B. **a**: DNA (green, stained with YOYO-1) decorated with dCas12a (magenta, quantum dot-labeled). **b**: Histograms of dCas12a binding positions along the DNA. Apo dCas12a was reconstituted with a mixture of five (top) or ten (bottom) crRNAs. The top and bottom histograms summarize 561 and 473 dCas12a molecules, respectively. The expected binding positions are shown via the magenta lines in the histograms. The black lines are the fit to a sum of five (top) or ten (bottom) Gaussians; see Table S2 for fit parameters. **c**: Number of fluorescent dCas12a proteins per DNA strand after targeting five (N = 561) or ten (N = 473) binding sites. **d**: Distribution of C-S_{10}-B clusters on DNA without any dCas12a (top, N = 246) or pre-bound with dCas12a (bottom, targeting five sites, N = 484). dCas12a target sites are shown via strong magenta lines in the histogram. Black line: fit to a sum of five Gaussians; see Table S2 for fit parameters. **e**: dCas12a lifetime on DNA (with 25 nM C-S_{10}-B) is comparable to that of quantum dots on the flowcell surface (N = 100 each). These results indicate that dCas12a does not dissociate from DNA during the course of the encapsidation experiments.

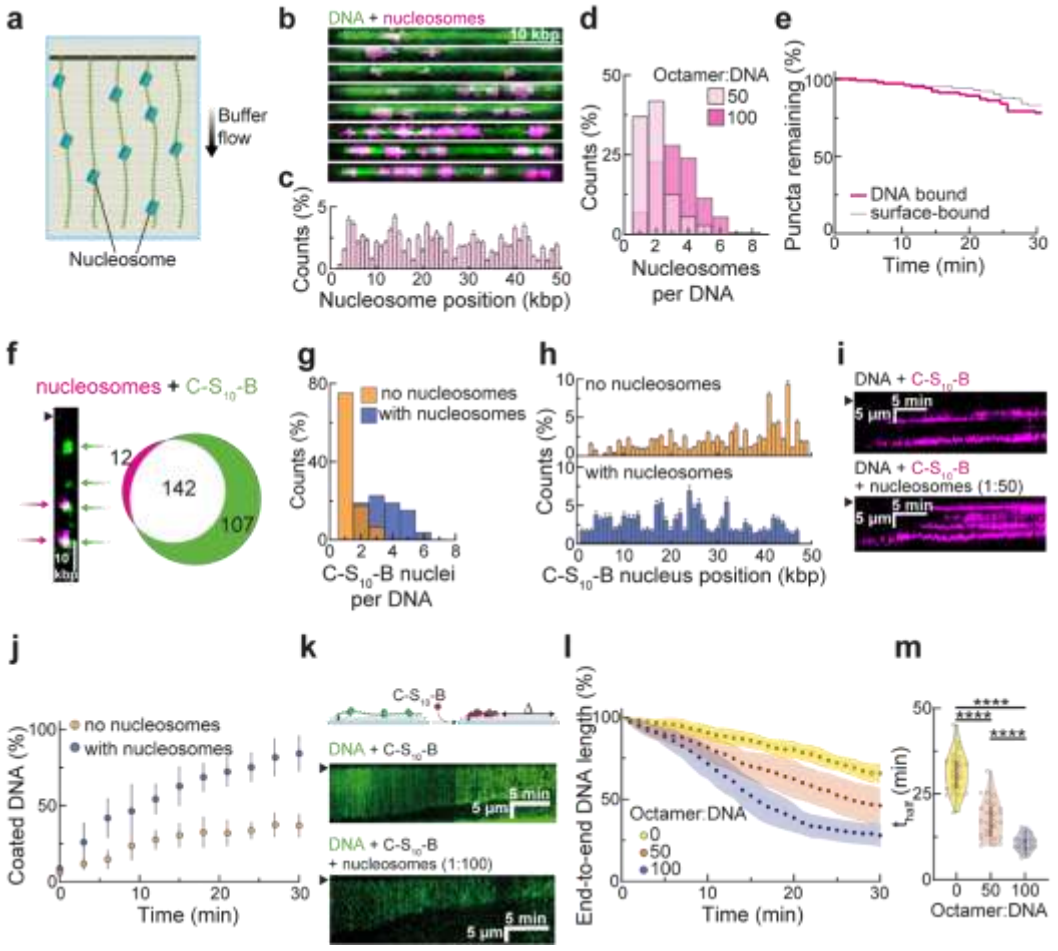

Figure S4: Nucleosomes accelerate DNA encapsidation by C-S$_{10}$-B. **a**: Illustration of nucleosome-decorated DNA. The nucleosomes were assembled via salt dialysis of histone octamers onto the DNA substrate. **b**: DNA (green, stained with YOYO-1) decorated with fluorescent nucleosomes (magenta, quantum dot-labeled). **c**: Histograms of nucleosome binding positions along the DNA at the 50:1 ratio (N = 279 nucleosomes). **d**: Histograms of the number of fluorescent nucleosomes per DNA strand at the 50:1 (N = 279) and 100:1 (N = 832) octamer:DNA molar ratios. **e**: Nucleosomes do not dissociate from DNA during encapsidation with 25 nM C-S$_{10}$-B. Surface-immobilized quantum dots are included as a comparison (N = 100 for each condition). **f**: Double labeling experiments show that 92% of nucleosomes (magenta) co-localized with 57% of fluorescent C-S$_{10}$-B clusters (green).

Barrier position is indicated with a black arrow. **g**: Histograms of the number of C-S_{10}-B clusters on DNA (N = 246 clusters) and nucleosomal DNA (N = 362 clusters; 50:1 octamer:DNA molar ratio). **h**: Distribution of C-S_{10}-B clusters along the DNA with (N = 362) or without (N = 246) nucleosomes deposited at a 50:1 octamer:DNA molar ratio. **i**: Kymographs showing binding of C-S_{10}-B (magenta, Alexa488-labeled) on non-decorated (top) or nucleosome-decorated (bottom) DNA at 25 nM C-S_{10}-B. Black arrows denote the barrier position. **j**: Coating of DNA by fluorescent C-S_{10}-B is faster after nucleosome deposition. Points and error bars indicate the mean and standard deviation, respectively (N = 10 DNA molecules per condition). **k**: Representative kymographs showing faster encapsidation by C-S_{10}-B for nucleosomal DNA (green) reconstituted at the 100:1 histone octamer:DNA molar ratio. Black arrows denote the barrier position. **l**: Condensation profiles at 25 nM C-S_{10}-B for DNA, and nucleosomal DNA reconstituted at the indicated histone octamer:DNA molar ratios. Shown are the mean and standard deviation for 25 DNA molecules per condition. **m**: Violin plots showing the time (t_{half}) required to reach half of maximum condensation for each DNA strand analyzed in (l). Extrapolation after curve fitting to the Hill equation was used to estimate t_{half} for molecules that did not reach 66.5% encapsidation during the experiment (30 min). (****) indicates $p < 0.0001$.

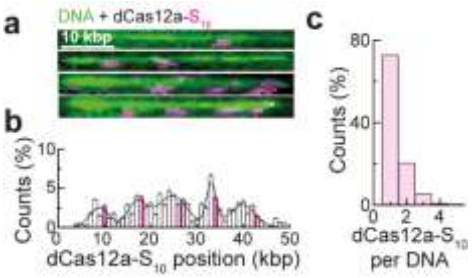

Figure S5: Decoration of DNA with the previously dimerized complex dCas12a-S_{10}. **a**: Representative DNA molecules (green, stained with YOYO-1) decorated with dCas12a-S_{10} (magenta, Atto647N-labeled). **b**: Distribution of dCas12a-S_{10} when binding was targeted to five sites along the DNA (N = 397 dCas12a-S_{10}). The expected binding positions of dCas12a-S_{10} are shown via the magenta lines in the histogram. Black line: the fit to a sum of five Gaussians; see Table S2 for fit parameters. **c**: Histogram of the number of Atto647N-labeled dCas12a-S_{10} per DNA strand (N = 397 dCas12a-S_{10} molecules) along the DNA.

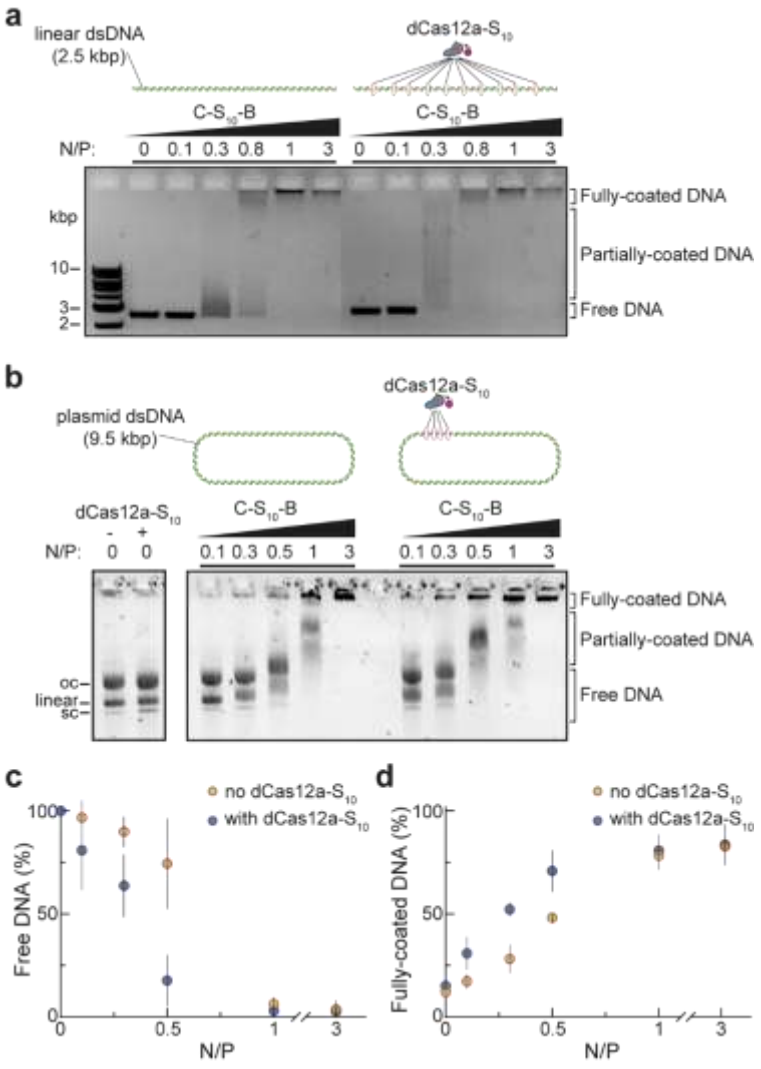

Figure S6: Positioning multiple dCas12a-S_{10} on DNA improves encapsidation at sub-saturating C-S_{10}-B concentrations. C-S_{10}-B binding is assessed via electrophoretic mobility shift assays. **a**: A linear 2.5 kbp dsDNA fragment was decorated with ten dCas12a-S_{10} uniformly distributed along the template via a pooled crRNA library. **b**: dCas12a-S_{10} was directed to four identical sites (via a single crRNA) on a 9.5 kbp pPIC9 plasmid (in supercoiled, linear, and nicked open-circular conformations). **c**: Intensity of the bands corresponding to the free plasmid observed in (b). Shown are the mean and standard deviation for three replicates. **d**: Intensity of the band corresponding to the fully-coated plasmid observed in (b). Shown are the mean and standard deviation for three replicates. N/P:

stoichiometric ratio between C-S_{10}-B molecules and the number of available DNA binding sites (assuming a 6 bp footprint per C-S_{10}-B monomer).

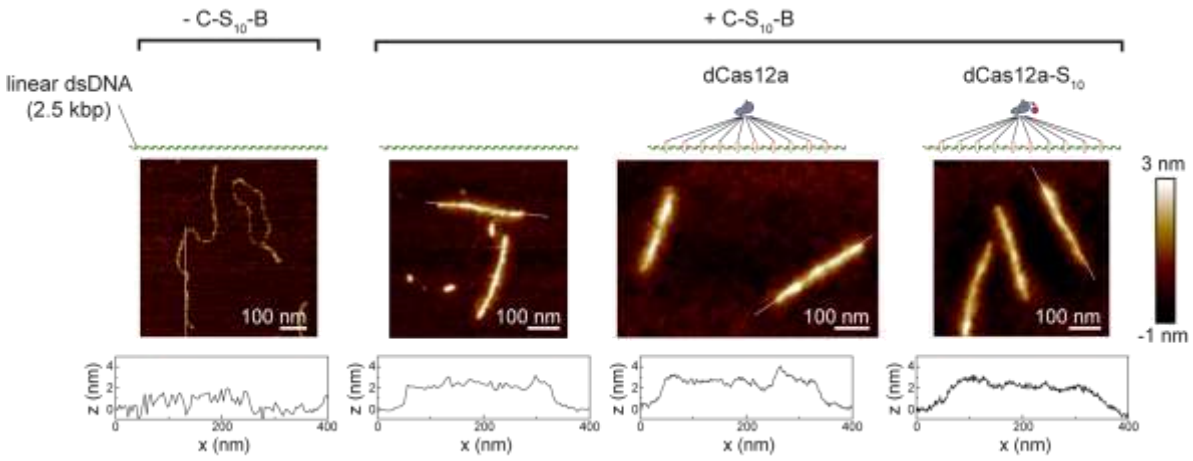

Figure S7: C-S_{10}-B packages DNA and DNA decorated with dCas12a or dCas12a-S_{10} into artificial nucleocapsids. Shown are the 2.5 kbp (850 nm) DNA fragment, and particles obtained with DNA or decorated DNA after an overnight incubation with C-S_{10}-B. The height profiles of the particles are shown below.

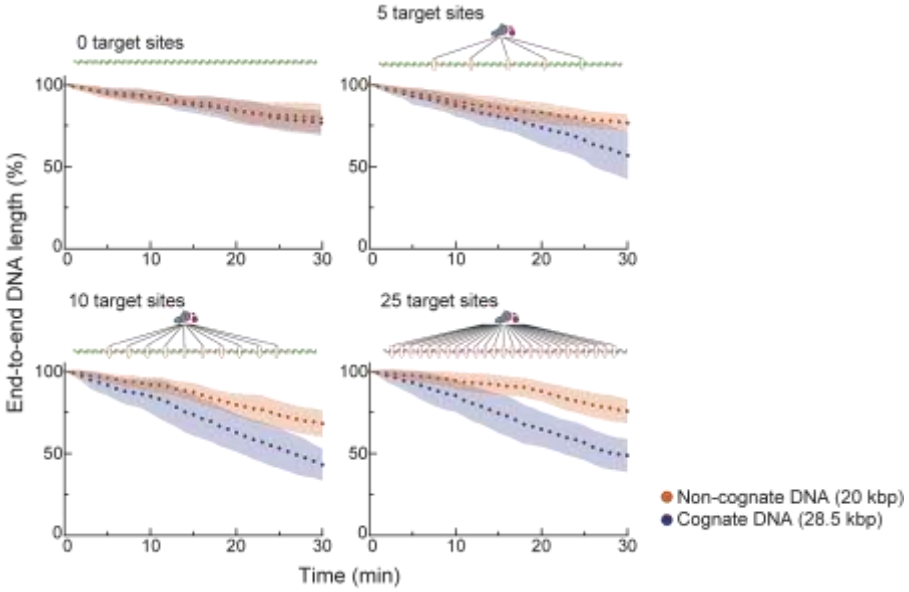

Figure S8: Encapsidation of cognate and non-cognate DNA templates after selective DNA decoration with dCas12a-S_{10}. Condensation profiles at 10 nM C-S_{10}-B for the non-cognate (20 kbp) and cognate (28.5 kbp) DNA strands after dCas12a-S_{10} binding at zero, five, ten or twenty-five sites along the cognate DNA. Circles and shaded areas indicate the mean and standard deviation, respectively (N = 25 DNA molecules per condition).

Table S1: Variable sequence of crRNAs used for positioning dCas12a/dCas12a-S_{10} on the DNA. All crRNAs consist of a 5' constant region (UAAUUUCUACUCUUGUAGAU, 20 nt) followed by the 3' variable region (24 nt) shown in the table.

Full λ DNA	
5 sites	AUGAUGUUCUGCUGGAUAUGCACU
	CCUGACACCGGACGGAAAGCUGAC
	AAUGUCGGCUAAUCGAUUUGGCCA
	GCUAGCAAUUAAUGUGCAUCGAUU
	AUGAACGCAAUAUUCACAAGCAAU
10 sites	CGUGAGAGCUAUCCCUUCACCACG
	AUGAUGUUCUGCUGGAUAUGCACU
	CGUAUGUCGCCGGAAGACUGGCUG
	CCUGACACCGGACGGAAAGCUGAC
	UGAUAUGCCGCAGAAACGUUGUAU
	AAUGUCGGCUAAUCGAUUUGGCCA
	AUGUUCAUCGUUCCUUAAAGACGC
	GCUAGCAAUUAAUGUGCAUCGAUU
	CCGGACAGGAGCGUAAUGUGGCAG
	AUGAACGCAAUAUUCACAAGCAAU
28 kbp cognate strand	
5 sites	UGGCCAAAUCGAUUAGCCGACAUU
	GCGUCUUUAAGGAACGAUGAACAU
	AAUCGAUGCACAUUAAUUGCUAGC
	CUGCCACAUUACGCUCCUGUCCGG
	AUUGCUUGUGAAUAUUGCGUUCAU
10 sites	UGGCCAAAUCGAUUAGCCGACAUU
	GUCAGAGGCUUGUGUUUGUGUCCU
	GCGUCUUUAAGGAACGAUGAACAU
	ACUGCGCAUCGCUGGCAUCACCUU
	AAUCGAUGCACAUUAAUUGCUAGC
	GCUUAAUGACAUUCCUUUCCCGAU
	CUGCCACAUUACGCUCCUGUCCGG
	CAGGAACGCAACCGCAGCUUAGAC
	AUUGCUUGUGAAUAUUGCGUUCAU
	AUACCGGAAGCAGAACCGGAUCAC
25 sites	UUGCUUCUCUUGACCGUAGGACUU
	CAGUAUUAUGUAGUCUGUUUUUUA
	UAAACUCCUUGCAAUGUAUGUCGU
	UGGCCUCGAAACCACCGAGCCGGA
	CAUCAUUCCAGUCGAACUCACACA

	UCACCGCAGAUGGUUAUCUGUAUG
	UCUGGCGAUUGAAGGGCUAAAUUC
	CGGGGUGGAUCUAUGAAAAACAUC
	AGAAGGAAGAUAUCCUCGCAUGGU
	CCAACCAAAUGUAUAUCGAUACCG
	ACCCUCAGAGAGAGGCUGAUCACU
	ACUUAAUAGUAUUGGUUGCGUAAC
	UGAUAUGCCGCAGAAACGUUGUAU
	AAUGUCGGCUAAUCGAUUUGGCCA
	AUGUUCAUCGUUCCUUAAAGACGC
	GCUAGCAAUUAAUGUGCAUCGAUU
	CCGGACAGGAGCGUAAUGUGGCAG
	AUGAACGCAAUAUUCACAAGCAAU
	AGGCCACCGCAUCUCGUGCUGAAG
	UUGAAGCAAAUCUGAAACCUAUUA
	AGGACACAAACACAAGCCUCUGAC
	AAGGUGAUGCCAGCGAUGCGCAGU
	AUCGGGAAAGGAAUGUCAUUAAGC
	GUCUAAGCUGCGGUUGCGUUCCUG
	GUGAUCCGGUUCUGCUUCCGGUAU
Electrophoretic mobility shift assay	
Linear dsDNA (10 sites)	UAGAGCAUAAGCAGCGCAACACCC
	AUGAUGAUAUUGAACAGGAAGGCU
	UCUCUGCGAGCAUAAUGCCUGCGU
	GCCUCCCACGUCUCACCGAGCGUG
	UUGAUGGCCUCAUCCACACGCAGC
	UAACCGCUUCACACUGACGCCGGA
	UUGUUGGUUGCUGCACCAUCCUCU
	UGUAUGAAAACGCCCACCAUUCCC
	CGGCUCAGUCAUCGCCCAAGCUGG
	CGGACACAGUUCCGGAUGGUCAGC
Plasmid DNA (4 repeats of 1 site)	CCUUGUUCACCUGGUUGACCAGGG

Table S2: Parameters of multiple Gaussian fitting. Binding of dCas12a, dCas12a-S_{10} and C-S_{10}-B clusters was fit to the sum of five or ten Gaussians in Figs. S3b, S3d and S5b. For each Gaussian we report the mean ± standard deviation, and the offset with respect to the expected position in parenthesis (in kbp).

Expected positions	**dCas12a, 5 target sites (Fig. S3b top)**	**dCas12a, 10 target sites (Fig. S3b bottom)**	**C-S_{10}-B clusters, 5 target sites (Fig. S3d)**	**dCas12a-S_{10}, 5 target sites (Fig. S5b)**
6.2	-	5.9 ± 3.6 (0.4)	-	-
10.3	9.7 ± 2.1 (0.5)	10.5 ± 1.4 (0.3)	9.0 ± 2.8 (1.3)	8.0 ± 2.1 (1.3)
14.3	-	12.9 ± 1.3 (1.4)	-	-
18.4	18.0 ± 2.2 (0.4)	18.8 ± 6.0 (0.4)	16.7 ± 2.0 (1.7)	17.1 ± 2.0 (1.3)
22.6	-	23.5 ± 1.3 (0.9)	-	-
26.6	26.0 ± 1.5 (0.6)	27.5 ± 5.3 (0.9)	25.8 ± 4.5 (0.9)	24.7 ± 3.3 (2.0)
30.7	-	32.2 ± 1.8 (1.5)	-	-
34.7	34.5 ± 2.1 (0.2)	35.9 ± 2.3 (1.2)	33.8 ± 1.6 (0.9)	33.1 ± 1.1 (1.6)
38.7	-	38.8 ± 1.1 (0.1)	-	-
42.8	42.7 ± 3.1 (0.1)	41.6 ± 4.7 (1.2)	40.7 ± 2.4 (2.1)	39.8 ± 2.8 (3.1)
R^2	0.6978	0.86921	0.5582	0.6511

VIDEO CAPTIONS

Video 1: DNA coating at 25 nM C-S_{10}-B. Binding of fluorescent C-S_{10}-B (magenta, at 25 nM) on a single DNA molecule (unlabeled). Only two clusters of C-S_{10}-B are observed, a larger one (top, and first to form) and a smaller one at the free end of the DNA. The video is displayed at a rate 150X (10 frames per second) from that at which it was recorded (1 frame/15 seconds). The scale (10 kbp) is shown with a white vertical line.

Video 2: DNA coating at 150 nM C-S_{10}-B. Binding of fluorescent C-S_{10}-B (magenta, at 150 nM) on a single DNA molecule (unlabeled). Almost total coating of the DNA occurs halfway through the experiment. The video is displayed at a rate 150X (10 frames per second) from that at which it was recorded (1 frame/15 seconds). The scale (10 kbp) is shown with a white vertical line.

EXPERIMENTAL SECTION

Plasmids, protein expression and purification

The FK506 Binding Protein (FKBP) and FKBP-Rapamycin Binding domain (FRB) sequences were extracted from pGEX-2T plasmids (kindly provided by Tom Wandless, Stanford University) and inserted at the C-terminus of nuclease inactivated version of AsCas12a (D908A) and C-S_{10}, respectively.

The pET19 plasmids with dCas12a and dCas12a$_{(FKBP)}$ (both with an N-terminus 6XHis-SUMO tag) were used to transform BL21 (DE3) *Escherichia coli*. Colonies were grown on Luria Bertani plates with 100 µg/mL ampicillin and used to inoculate Terrific Broth liquid culture in Fernbach flasks. Cultures were grown at 37°C until OD_{600} ~0.8 and then transferred to 12°C for 1 h before induction with 1 mM IPTG. Cultures were grown for 24 h at 12°C and the pellets were collected by centrifugation and stored at -70°C.

The pellets were resuspended in buffer A (20 mM Tris-HCl pH 8.0, 250 mM NaCl, 10 mM imidazole) supplemented with 1 mM PMSF and sonicated on ice. The lysate was clarified by centrifugation at 30 000 g for 45 min and the supernatant containing the protein of interest was filtered through 0.2 µm syringe filters. The supernatant was then loaded into a 5 mL HisTrap (GE Healthcare) column, washed with buffer B (20 mM Tris-HCl pH 8.0, 1 M NaCl, 25 mM imidazole) and eluted with buffer C (20 mM Tris-HCl pH 8.0, 1 M NaCl, 250 mM imidazole). The eluted fraction was loaded into a dialysis membrane with 2.4 µM SUMO protease and dialyzed overnight at 4°C in buffer D for dCas12a (50 mM phosphate buffer pH 6.0, 100 mM KCl, 5 mM $MgCl_2$, 10% glycerol, 2 mM DTT) and buffer E for dCas12a$_{(FKBP)}$ (20 mM HEPES-KOH pH 7.2, 100 mM KCl, 5 mM $MgCl_2$, 10% glycerol, 2 mM DTT). The 0.2 µm filtered cleaved product was injected in a 5 mL HiTrap

SP HP (GE Healthcare) cationic exchange column and eluted with a gradient to buffer F (20 mM HEPES-KOH pH 7.5, 2 M KCl). The fractions containing the protein of interest were pooled, concentrated with 100 kDa Amicon centrifugal filter units and injected into a size exclusion HiLoad 16/600 Superdex 200 pg column (GE Healthcare) equilibrated with storage buffer G (20 mM HEPES-KOH pH 7.5, 500 mM KCl, 10% glycerol). Purified proteins were concentrated to 10-12 μM, flash frozen in liquid nitrogen and stored at -70°C until use. Protein identity and purity were assessed with western blots and SDS-PAGE, respectively.

pPIC9 plasmids with C-S_{10}-B and C-$S_{10(FRB)}$ were linearized with SacI and electroporated into histidine auxotrophic *Pichia pastoris* GS115. Mut^+ colonies expressing the protein were used to inoculate 300 mL of MGY and grown for 24 h until OD_{600} ~6.0. Cells were pelleted and resuspended in MM medium for protein expression for 72 h, with 1% methanol addition every 24 h. Culture supernatant was collected by centrifugation, the medium pH was adjusted to 8.0 with NaOH and 1 mM PMSF, 5 mM EDTA was added to inhibit proteolysis followed by 0.2 μm filtration.

For C-S_{10}-B, the culture supernatant was saturated to 50% with ammonium sulfate and incubated overnight at 4°C. The protein precipitate was resuspended in Milli-Q water at 65°C and the precipitation step was repeated once. The precipitate was resuspended in Milli-Q water at 65°C before 50 mM NaCl and 40% acetone addition. The sample was centrifuged to discard precipitates and the concentration of acetone in the supernatant was increased to 80% to selectively precipitate C-S_{10}-B. The precipitate was air-dried for 15 min, resuspended, and dialyzed overnight against Milli-Q water at 4°C, followed by flash

freeze and lyophilization. Before use, the lyophilized protein was dissolved in Milli-Q water at 0.1 mg/mL and incubated at 70°C for 15 min.

For C-$S_{10(FRB)}$, the culture supernatant was concentrated with 30 kDa Amicon stirred cells and proteins were precipitated with 80% saturation ammonium sulfate at 4°C overnight. The protein precipitate was resuspended in PBS pH 7.5 and the precipitation step was repeated once. The precipitate was resuspended in PBS pH 7.5 and 40% acetone was added followed by centrifugation. The concentration of acetone in the supernatant was increased to 80% to selectively precipitate C-$S_{10(FRB)}$. The precipitate was resuspended to ~80 μM in PBS pH 7.5, dialyzed overnight against PBS pH 7.5 at 4°C and flash frozen. Protein identity and purity of C-S_{10}-B and C-$S_{10(FRB)}$ were assessed with mass spectrometry (MALDI-TOF) and SDS-PAGE, respectively.

crRNA pools

The 24 nt forward oligo with the promoter for T7 RNA polymerase (`GAAATTAATACGACTCACTATAGG`), and the 68 nt reverse oligos with the 24 nt sequence complementary to the forward oligo plus the 44 nt crRNA sequence (see Table S1) were purchased from IDT. The T7 promoter forward oligo was mixed with pools of reverse oligos (1.5:1.0 molar ratio) and hybridized by incubation at 75°C for 5 min and then cooling to 25°C over 25 min in annealing buffer (10 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA). The pool of partially double stranded DNA templates was used for *in vitro* transcription with HiScribe™ T7 Quick High Yield RNA Synthesis Kit (New England BioLabs) and the crRNA was purified with TRizol (Ambion). crRNA integrity and purity were assessed with 15% acrylamide Urea-PAGE.

Preparation of DNA substrates

To prepare the DNA template for curtain assays, λDNA (125 μg, NEB) was incubated with 2 μM biotinylated oligo complementary to one of the two 12 nt cohesive ends in λDNA in T4 DNA ligase reaction buffer (NEB) and hybridized at 70°C for 15 min followed by cooling to 15°C over 2 h. T4 DNA ligase (2000 units, NEB) was added and the mixture was incubated at room temperature overnight. The ligase was inactivated with 2 M NaCl and the sample was injected into a Sephacryl S-1000 size exclusion column (GE Healthcare) to remove excess oligos and DNA ligase.

For experiments with nucleosomal DNA, purified biotinylated DNA was precipitated with sodium acetate (pH 5.5) and 0.3 M isopropanol to 1:1 v/v on ice, and centrifuged at 15 000 g for 30 min. The precipitate was resuspended to 0.8 nM and incubated with human histone octamers (3xHA H2A and H2B, H3, H4; Histone Source) at molar ratios of 50:1 and 100:1 (histone octamer to λDNA) in 2 M TE buffer (10 mM Tris-HCl pH 8, 1 mM EDTA, 2 M NaCl) with 1 mM DTT. The 100 μL mixture was loaded in dialysis buttons (10 kDa MWCO, BioRad) and dialyzed against dialysis buffer (10 mM Tri-HCl pH 7.6, 1 mM EDTA, 1 mM DTT and NaCl). The first 1 h dialysis step was done against 1.5 M NaCl, followed by 2 h dialysis steps against 1, 0.8, 0.6 and 0.4 mM with a final overnight step against 0.2 M NaCl at 4°C.

For experiments with two DNA strands, λDNA was ligated with biotinylated oligos complementary to both cohesive ends. Hybridization and ligation were performed as described above except that CutSmart buffer (NEB) supplemented with 1 mM rATP was used instead of T4 ligase buffer. The mixture was heated at 65°C for 10 min to inactivate the ligase, and NgoMIV was added for DNA cleavage at 37°C for 3 h. Lastly, 2 M NaCl

was added to the solution prior to size exclusion chromatography. Complete cleavage by NgoMIV was assessed with gel electrophoresis. All DNA substrates were stored at 4°C until use.

Single-tethered DNA curtain assay

A solution containing 20 µL of a liposome stock solution (97.7% DOPC, 2.0% DOPE-mPEG2k and 0.3% DOPE-biotin) and 980 µL of lipid buffer (10 mM Tris-HCL pH 8, 100 mM NaCl) was injected into the assembled flowcell and incubated for 30 min at room temperature. The flowcell was washed with BSA buffer (40 mM Tris-HCl pH 8, 2 mM $MgCl_2$, 0.2 mg/mL BSA) and incubated for 10 min before injection of 0.1 mg/mL streptavidin in BSA buffer followed by 10 min incubation. The biotinylated λDNA was diluted in BSA buffer and injected into the flowcell for tethering, excess DNA was removed with buffer. Imaging buffer consisted of BSA buffer supplemented with 100 mM NaCl, 5 mM MgCl2, 2 mM DTT and gloxy solution (500 units of catalase, 70 units of glucose oxidase and 1% glucose w/v). YOYO-1 was added to the imaging buffer when DNA staining was required.

Total internal reflection fluorescence images were acquired with an inverted Nikon Ti-E microscope. Excitation (488 nM) and emission signals were split with a 638 nm dichroic beam splitter (Chroma) and captured by two EM-CCD cameras (Andor iXon DU897).

dCas12a or $dCas12a_{(FKBP)}$ ribonucleoprotein complexes were prepared by mixing the protein with crRNA pools at 1:10 molar ratio in reaction buffer (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$, 2% glycerol, 2 mM DTT) followed by incubation at 37°C for

30 min. The complexes were diluted to 10 nM in imaging buffer, injected in the flowcell and incubated at room temperature for 30 min.

Labeling of dCas12a and nucleosomes was carried out *in situ* by injection into the flowcell of anti-FLAG or anti-HA coupled quantum dots (QD_{705}), respectively. C-S_{10}-B and C-$S_{10(FRB)}$ were labeled via maleimide reaction with Alexa488 and Atto647N, respectively. Both C-S_{10}-B and C-$S_{10(FRB)}$ contain an N-terminal cysteine residue and C-$S_{10(FRB)}$ has an additional cysteine in the FRB moiety.

Images were processed with FIJI software. DNA condensation kinetics were obtained by measuring the DNA length at each time step with rate 1 frame/min. DNA coating by fluorescent C-S_{10}-B was obtained by measuring the number of fluorescent pixels on the vertical path along the DNA. For this experiment, 100% indicates a DNA that has been fully coated with C-S_{10}-B.

Dynamic light scattering

Dimerization of dCas12a$_{(FKBP)}$ and C-$S_{10(FRB)}$ via rapamycin was assessed by dynamic light scattering with a Zetasizer μV (Malvern). Both proteins were diluted to 2 μM in reaction buffer (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$, 2% glycerol, 1 mM DTT) for a total volume of 20 μL and loaded in the cell. Measurements were taken before and immediately after (~30 s) addition of 5 μM rapamycin.

Electrophoretic mobility shift assay

The crRNA-loaded dCas12a-S_{10} complex was incubated with 100 ng DNA (linear with 10 different target sites or plasmid with 4 identical target sites) at stoichiometry 10:1 ([dCas12a]:[target site]) for 1 h before C-S_{10}-B addition at 0 to 3 N/P ratios ([C-S_{10}-

B]:[DNA binding sites of 6 bp]) for 3 h (linear DNA) or overnight incubation (plasmid). Encapsidation was assessed on agarose gel electrophoresis at 4°C and DNA bands were stained with SYBR Green.

Gel images were processed with FIJI. To quantify the EMSAs we calculated the % of the DNA that remained free and the % of the DNA that was stalled at the well (for each lane). This quantification renders how much of the total DNA (per lane) is either in its free form or completely encapsidated.

Atomic force microscopy

crRNA-loaded dCas12a and dCas12a-S_{10} were mixed with a 2.5 kbp linear dsDNA fragment (10 target sites) at stoichiometry 10:1 (([dCas12a]:[target site]) for 1 h. C-S_{10}-B was added at N/P = 3 and incubated overnight. The sample with 5 nM DNA was deposited on the mica and incubated for 10 min before rinsing with 0.5 mL Milli-Q water and air-drying. Images were acquired on a Digital Instruments NanoScope V (Bruker) in ScanAssyst^{TM} mode and processed with NanoScope Analysis 1.20.

Statistics and data analysis

All results are given as mean ± standard deviation except for Fig. 1e (mean ± 95% confidence intervals). Non-parametric statistical tests for two (Mann-Whitney) and multiple (Kruskal-Wallis) means were performed on GraphPad Prism 8. Multiple Gaussian fitting was performed with OriginPro 8.5. Errors bars for binding distributions of dCas12a, nucleosomes, C-S_{10}-B clusters and dCas12a-S_{10} were generated by bootstrap analysis.

To calculate t_{half} values, the curves with DNA compaction versus time were fit to the Hill equation (Equation 1). Here, $DNA\ length_0$ and $DNA\ length_f$ are the lengths of the fully

extended DNA (100%) and the fully compacted DNA (33%), respectively. *Hill* defines the steepness of the curve and t_{half} is the time required to achieve the midpoint between 100% and 33% (= 66.5%).

$$\text{DNA length} = \text{DNA length}_f + \left(\frac{\text{DNA length}_0 - \text{DNA length}_f}{1 + (t_{half}/\text{time})^{\text{Hill}}}\right) \qquad (1)$$

Compaction rate versus C-S_{10}-B concentration (Fig. 1e) was also fit to the Hill equation (Equation 2). Here, $rate_0$ and $rate_f$ stand for the condensation rates at the lowest and highest C-S_{10}-B concentrations, respectively. *Hill* defines the steepness of the curve and $[C\text{-}S_{10}\text{-}B]_{50}$ is the C-S_{10}-B concentration at which the midpoint between $rate_0$ and $rate_f$ was achieved.

$$\text{rate} = \text{rate}_0 + \left(\frac{\text{rate}_f - \text{rate}_0}{1 + ([\text{C-S}_{10}\text{-B}]_{50}/[\text{C-S}_{10}\text{-B}])^{\text{Hill}}}\right) \qquad (2)$$