SUPPLEMENTARY FIGURES

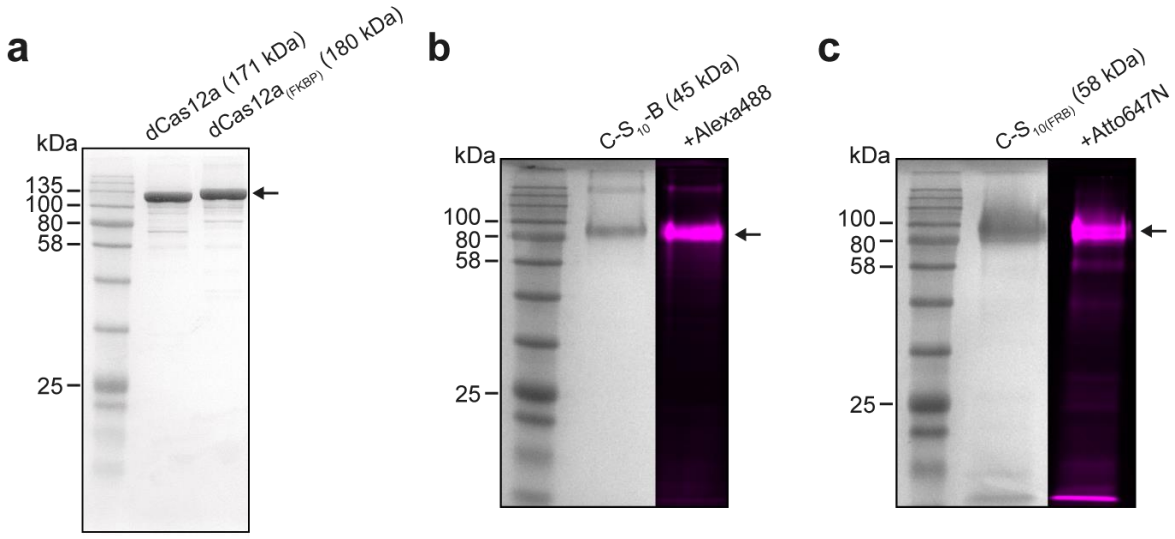


Figure S1: Proteins used in this study. **a**: SDS-PAGE gel with dCas12a (171 kDa) and the fusion protein containing dCas12a and the FKBP domain (dCas12a$_{(FKBP)}$, 180 kDa). **b**: SDS-PAGE gel with Coomassie stained (left) and maleimide-Alexa488 labeled (right) C-S_{10}-B (45 kDa). **c**: SDS-PAGE 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.

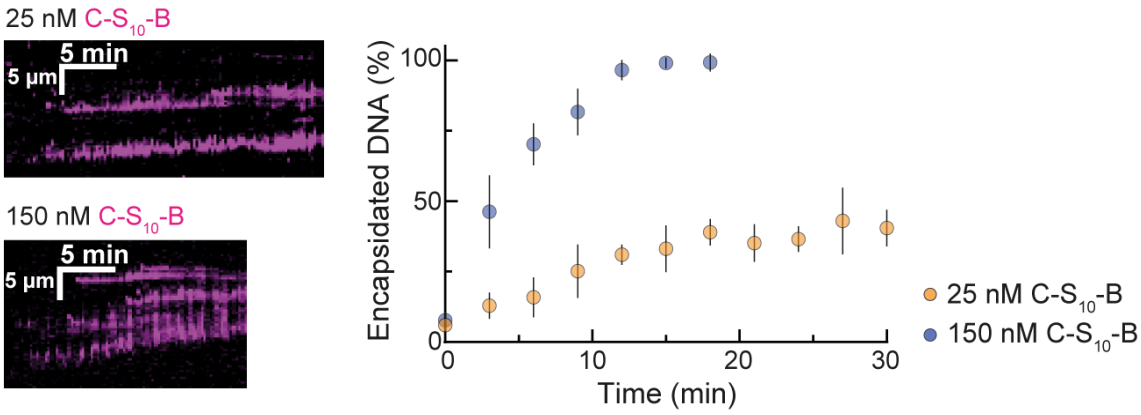


Figure S2: C-S$_{10}$-B concentration determines nucleocapsid nucleation and growth. Kymographs showing binding of C-S$_{10}$-B (magenta) on DNA (dark) at 25 nM and 150 nM polypeptide concentration (left) and percentage of the DNA strand length that is encapsidated by the fluorescent C-S$_{10}$-B (right). Shown are the mean and standard deviation for 10 nucleocapsids per condition.

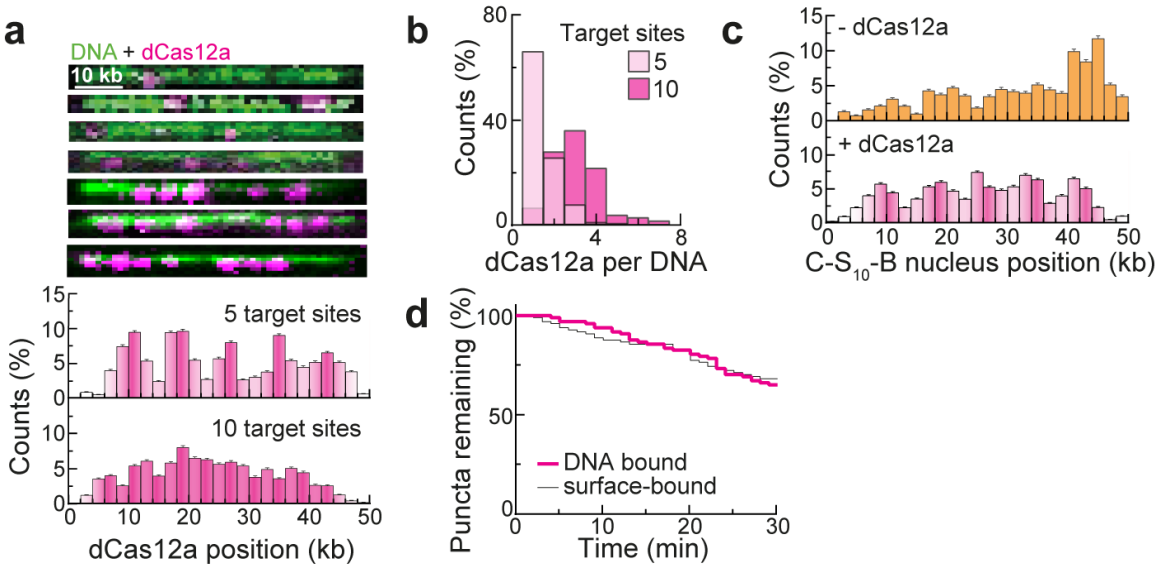
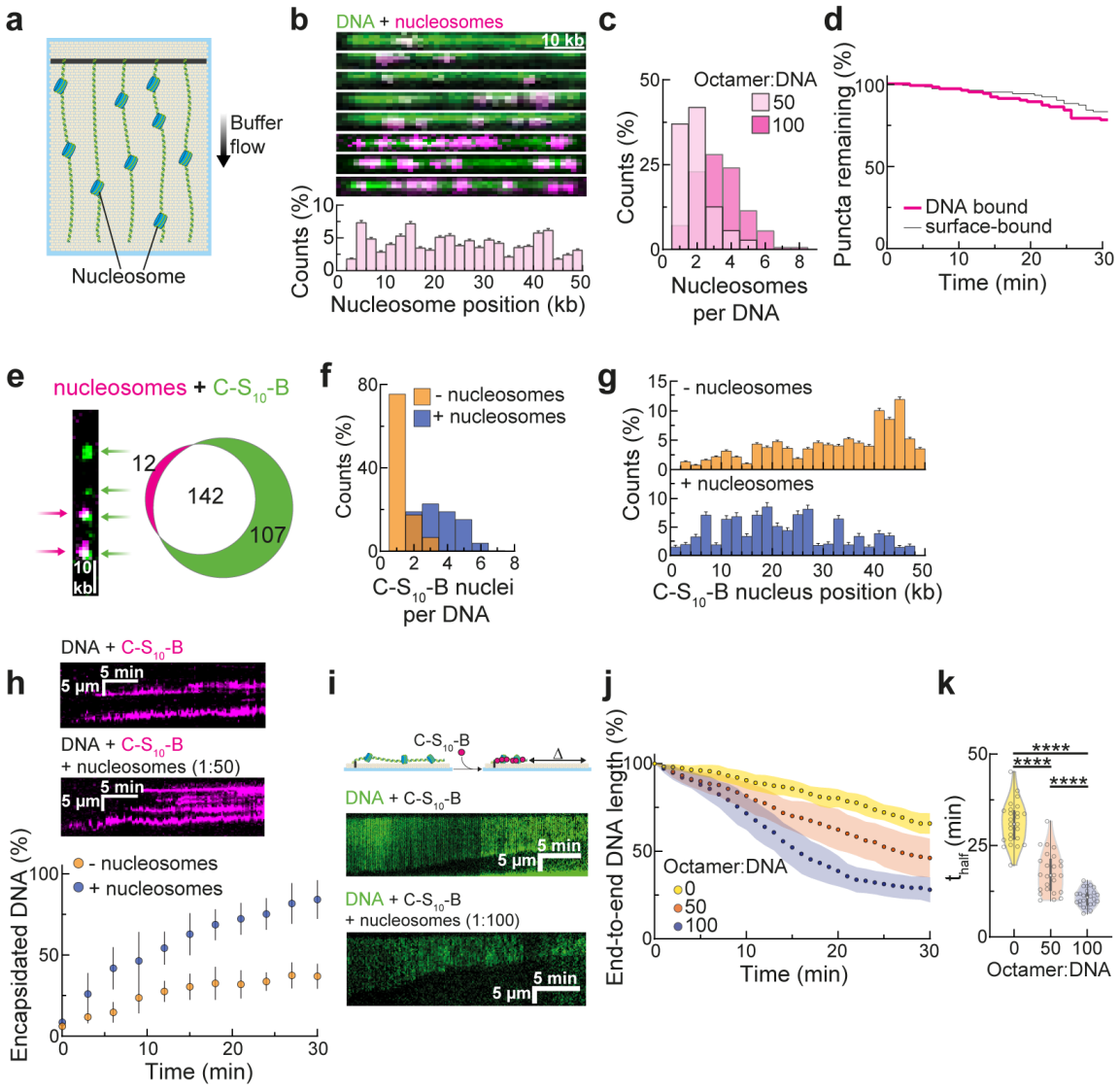


Figure S3: Decoration of DNA with multiple dCas12a prior to DNA encapsidation by C-S_{10}-B. a: YOYO-1 stained DNA molecules (green) decorated with quantum dot-labeled dCas12a (magenta) (top) and distribution of dCas12a along the DNA (bottom) when binding was targeted to 5 (N = 561 dCas12a molecules) or 10 sites (N = 473 dCas12a molecules). Binding sites are shown with stronger magenta in the histograms. **b**: Number of quantum dot-labeled dCas12a proteins per DNA strand after targeting 5 (N = 561) or 10 (N = 473) binding sites. **c**: Distribution of C-S_{10}-B clusters along the DNA with (N = 484) or without (N = 246) decoration with 5 dCas12a. dCas12a binding sites are shown with stronger magenta in the histograms. **d**: Fluorescent quantum dots remaining in the field of view, either bound on the DNA (via dCas12a) or on the lipid surface (N = 100 each) throughout encapsidation with 25 nM C-S_{10}-B.



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29 **Figure S4: Prior decoration with nucleosomes improves DNA encapsidation by C-S10-**

30 **B. a**: Human histone octamers were incubated with the DNA at molar ratios 50:1 and 100:1

31 prior to tethering in the flowcell. **b**: YOYO-1 stained DNA molecules (green) decorated

32 with quantum dot-labeled nucleosomes (magenta) (top) and distribution of nucleosomes on

33 the DNA (bottom) at the 50:1 ratio (N = 279). **c**: Number of quantum dot-labeled

34 nucleosomes per DNA strand at the 50:1 (N = 279) and 100:1 (N = 832) molar ratios. **d**:

35 Fluorescent quantum dots remaining in the field of view, either bound on the DNA (via

36 nucleosomes) or on the lipid surface (N = 100 each) throughout encapsidation with 25 nM

C-S_{10}-B. **e**: Double labeling experiment showing co-localization of QD-tagged nucleosomes (magenta, 92%) with fluorescent C-S_{10}-B clusters (green, 57%). **f**: Number of C-S_{10}-B clusters on naked DNA (N = 246 clusters) versus DNA decorated with nucleosomes at the 50:1 molar ratio (N = 362 clusters). **g**: Distribution of C-S_{10}-B clusters along the DNA with (N = 362) or without (N = 246) decoration with nucleosomes at the 50:1 molar ratio. **h**: Kymographs showing C-S_{10}-B (magenta) binding on naked or nucleosomes-decorated DNA and percentage of the DNA strand length that is encapsidated by the fluorescent C-S_{10}-B. Shown are the mean and standard deviation for 10 nucleocapsids per condition. **i**: Representative kymographs showing faster encapsidation by C-S_{10}-B after the DNA (green) is decorated with nucleosomes. **j**: Condensation profiles at 25 nM C-S_{10}-B for naked DNA, and DNA decorated with nucleosomes at the 50:1 and 100:1 molar ratios. Shown are the mean and standard deviation for 25 DNA molecules per condition. **k**: Violin plots showing the time (t_{half}) required to reach half of maximum condensation for each DNA strand analyzed in (j). Sigmoid curve fitting and extrapolation was used to estimate t_{half} for molecules that did not reach 65.5% encapsidation during the experiment (30 min).

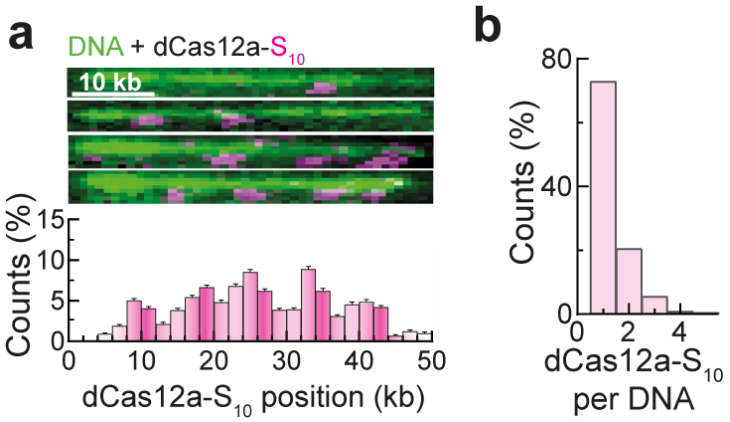

Figure S5: Decoration of DNA with the previously dimerized complex dCas12a-S_{10}. a: YOYO-1 stained DNA molecules (green) decorated with Atto647N-labeled dCas12a-S_{10} (magenta) (top) and distribution of dCas12a-S_{10} along the DNA (bottom) when binding was targeted to 5 sites (N = 397 dCas12a-S_{10} molecules) along the DNA. Binding sites are shown with stronger magenta in the histograms. **b**: Number of Atto647N-labeled dCas12a-S_{10} per DNA strand after targeting 5 sites (N = 397 dCas12a-S_{10} molecules) along the DNA.

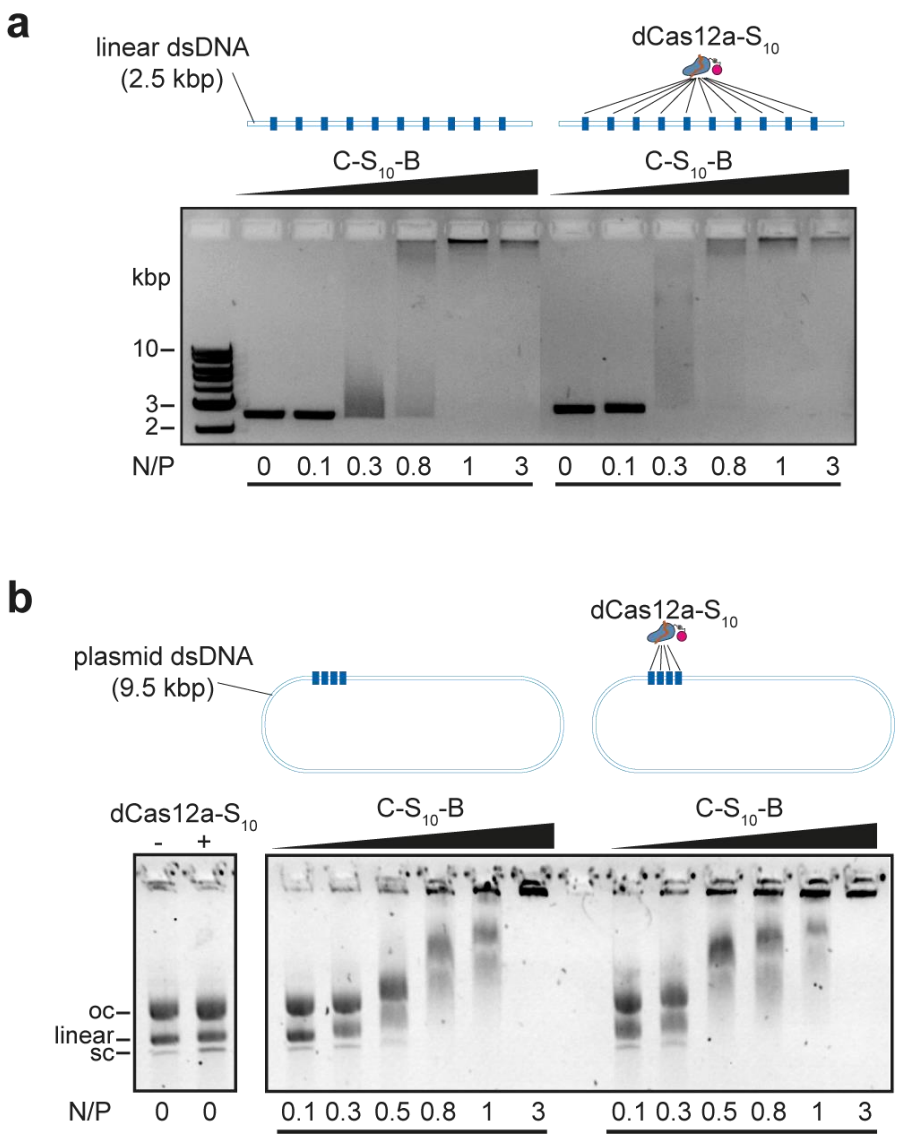

Figure S6: Positioning multiple dCas12a-S$_{10}$ complexes on DNA substrates improves encapsidation by C-S$_{10}$-B. C-S$_{10}$-B binding is assessed via electrophoretic mobility shift assays. **a**: A linear 2.5 kbp dsDNA fragment was decorated with ten dCas12a-S$_{10}$ uniformly distributed along the template. Incubation time with C-S$_{10}$-B was 3 h. **b**: dCas12a-S$_{10}$ was directed to four sites on a 9.5 kbp pPIC9 plasmid (in supercoiled, linear and nicked open-circular conformations). Incubation time with C-S$_{10}$-B was 15 h. N/P stands for the stoichiometric ratio between C-S$_{10}$-B and available DNA binding sites (6 bp).

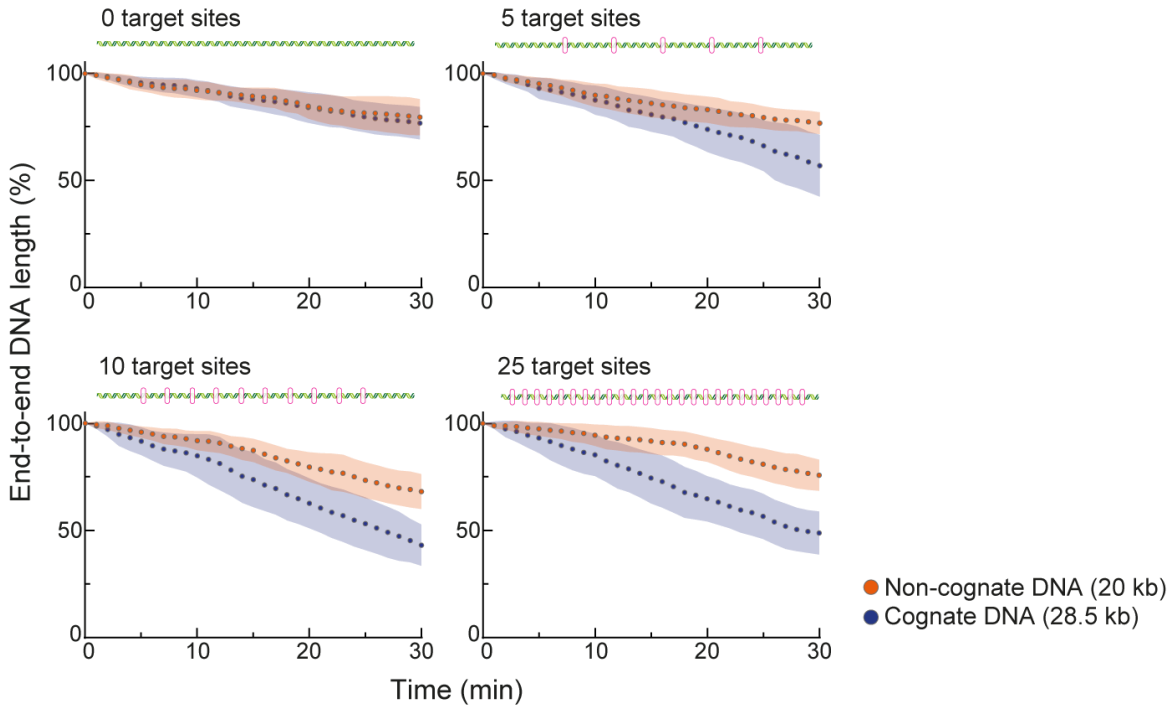

Figure S7: Encapsidation of cognate and non-cognate DNA templates after selective DNA decoration with dCas12a-S_{10}. Condensation profiles at 10 nM C-S_{10}-B for the non-cognate (20 kbp) and cognate (28.5 kbp) DNA strands after dCas12a-S_{10} binding at 0, 5, 10 or 25 sites along the cognate DNA. Circles and shaded areas are the mean and standard deviation for 25 DNA molecules per condition.