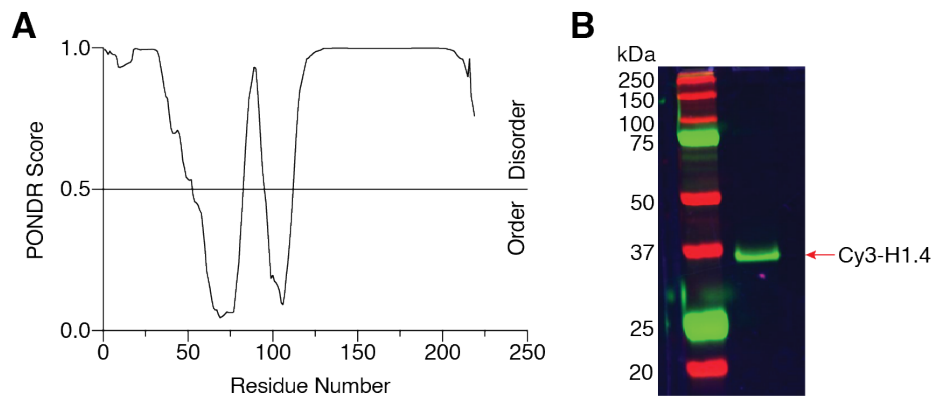
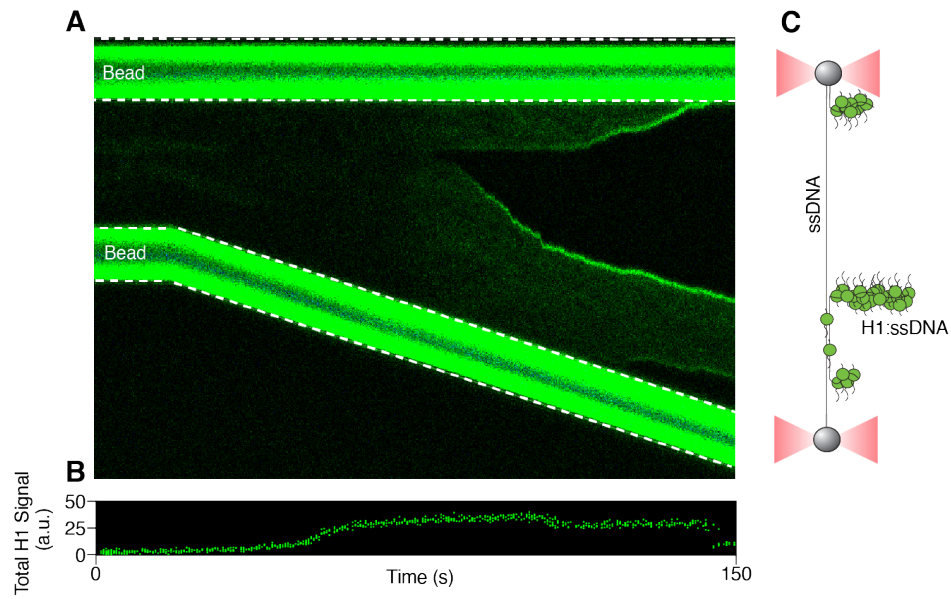


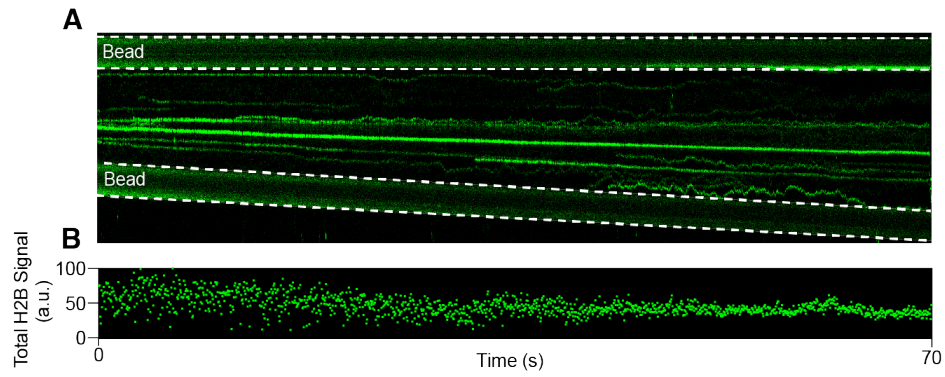
## SUPPLEMENTARY INFORMATION



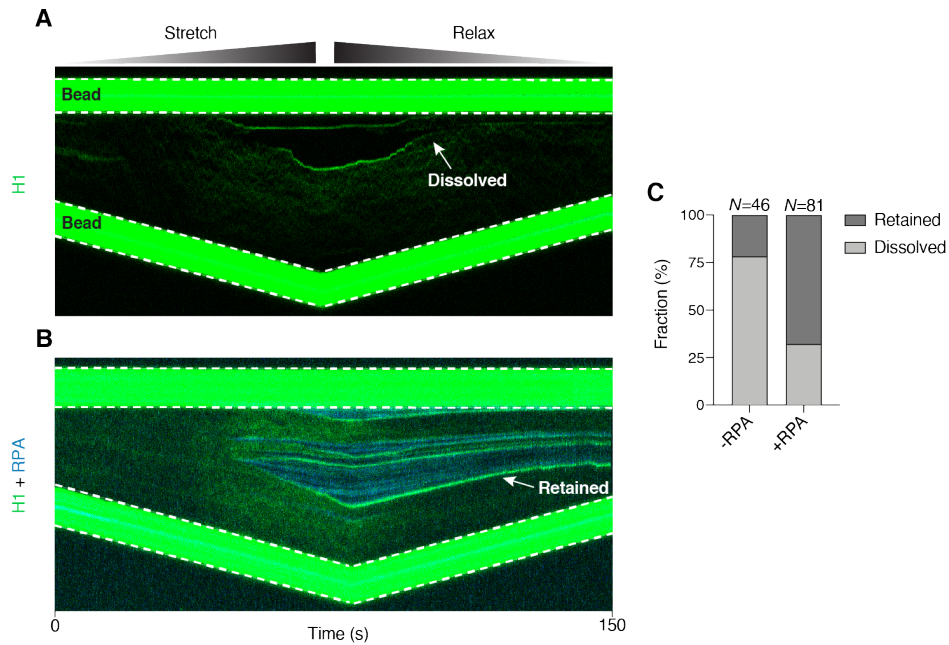
**Supplementary Figure 1. H1 purification and labeling.** (A) Predictor of natural disordered regions (PONDNR) score for the H1.4 amino acid sequence (generated from [www.pondr.com](http://www.pondr.com)). A score of  $>0.5$  is considered intrinsically disordered. (B) SDS-PAGE gel scanned for fluorescence showing purified Cy3-H1.4.



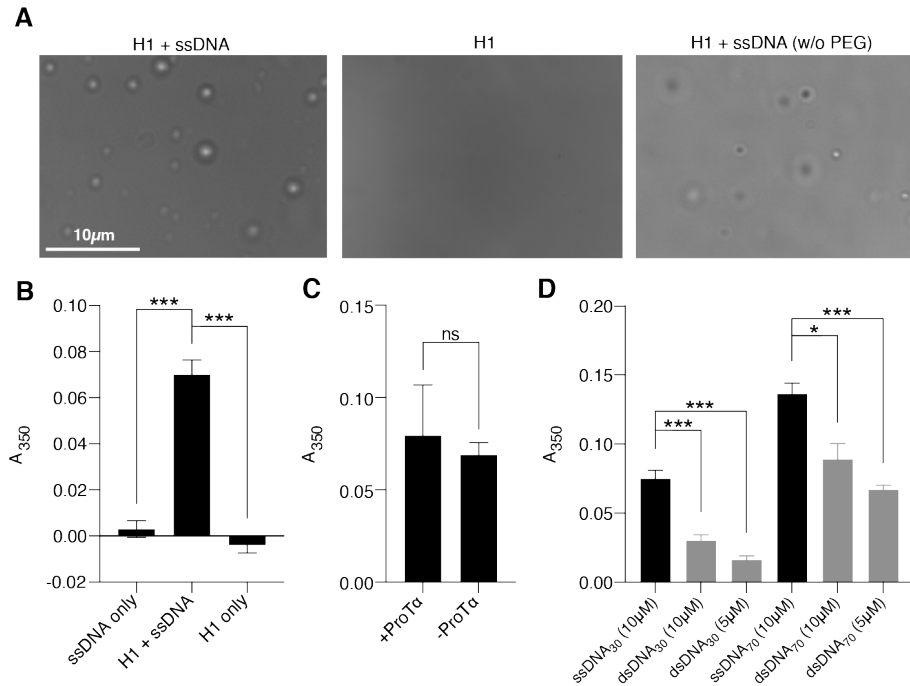
**Supplementary Figure 2. Additional kymograph of H1-DNA interaction.** (A) A kymograph showing Cy3-H1 binding to  $\lambda$ -DNA over time as the inter-bead distance was increased. (B) Total Cy3 intensity across the DNA tether over time for the kymograph shown in (A). (C) Schematic of the final H1 binding configuration for the example shown in (A).



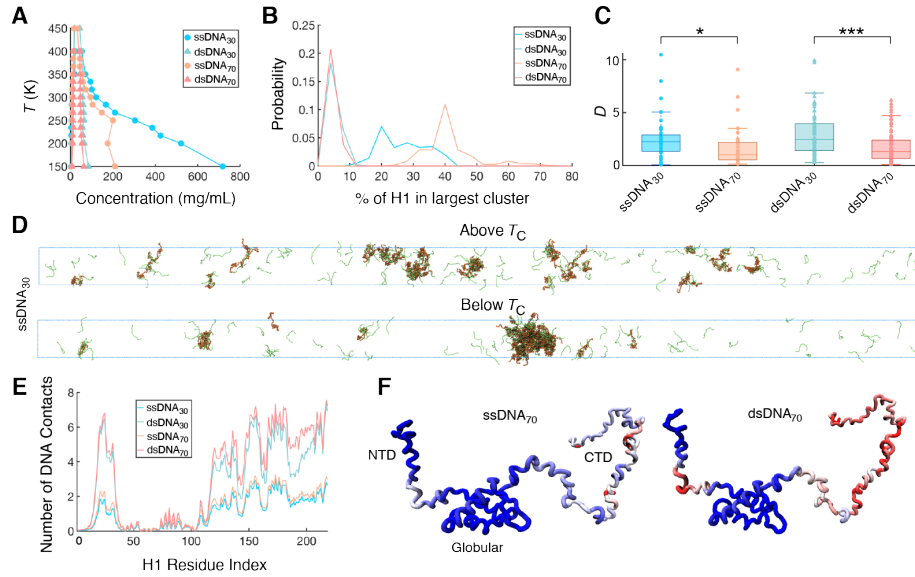
**Supplementary Figure 3. Example kymograph of H2B-DNA interaction. (A)** A representative kymograph showing Cy3-H2B binding to  $\lambda$ -DNA over time as the inter-bead distance was increased. **(B)** Total Cy3 intensity across the DNA tether over time for the kymograph shown in (A).



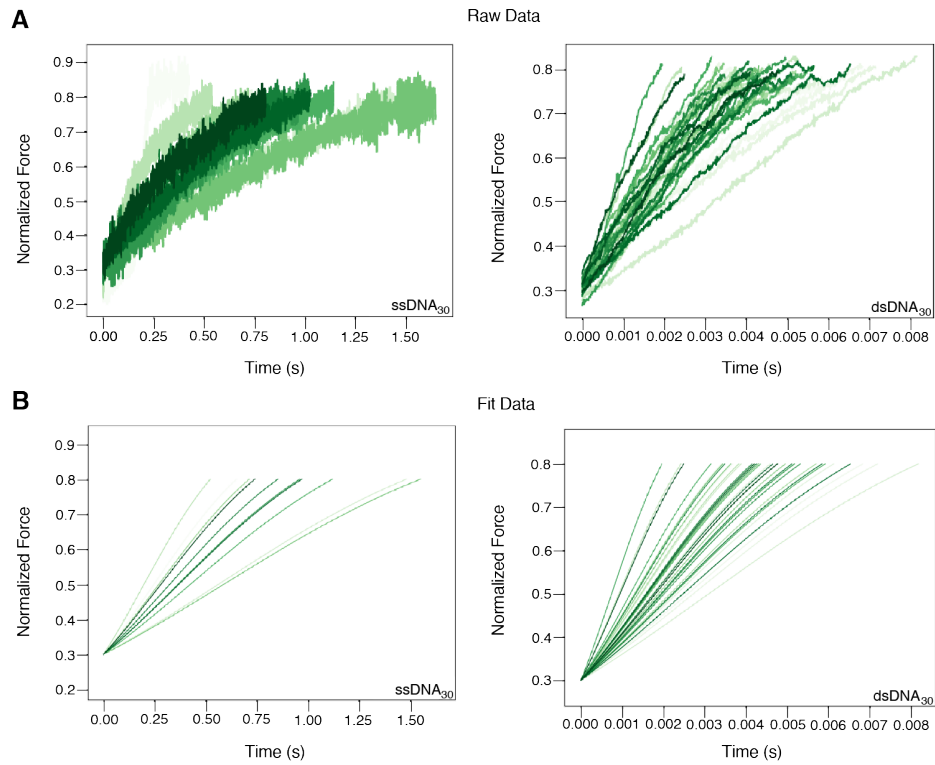
**Supplementary Figure 4. Reversibility of H1:ssDNA foci.** (A) A representative kymograph showing reversible formation and dissolution of Cy3-H1 foci during DNA tether stretching and relaxing. (B) A representative kymograph showing the persistence of Cy3-H1 foci after tether relaxing in the presence of AlexaFluor488-RPA. (C) Fraction of H1 foci dissolved or retained in the absence and presence of RPA.



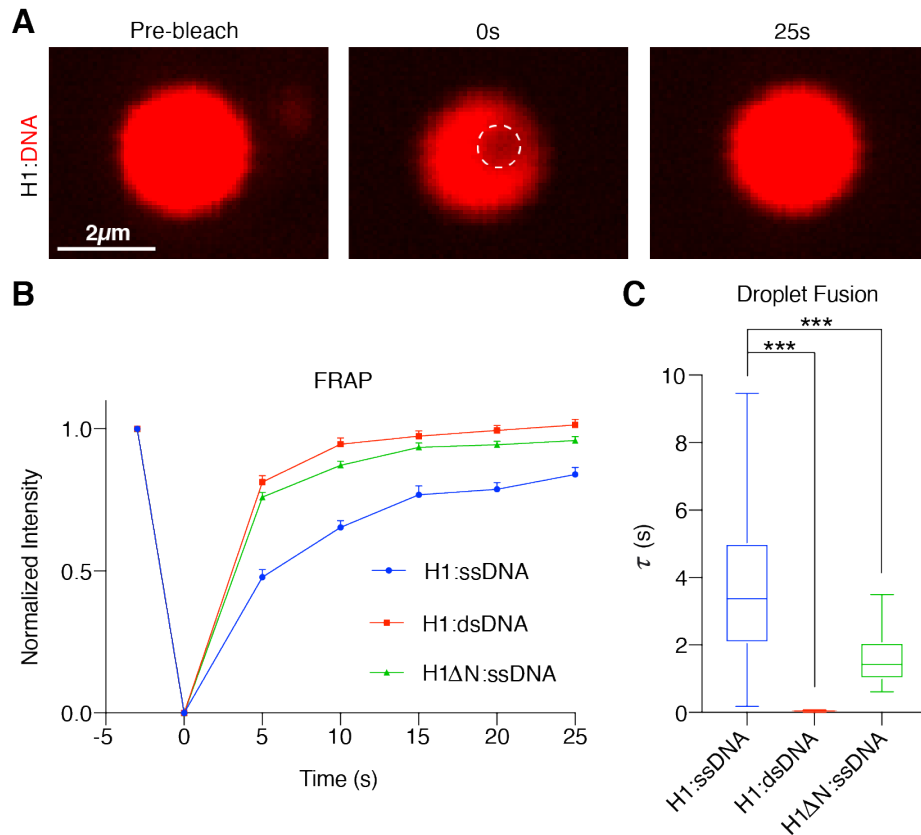
**Supplementary Figure 5. Additional results from bulk phase-separation assays.** (A) Brightfield images of 2.5  $\mu$ M H1 mixed with 10  $\mu$ M ssDNA<sub>75</sub> (left), 2.5  $\mu$ M H1 alone (middle), and 10  $\mu$ M H1 mixed with and 10  $\mu$ M ssDNA<sub>75</sub> in the absence of PEG (right). (B) Solution turbidity ( $A_{350}$ ) measurements for 10  $\mu$ M ssDNA<sub>75</sub> alone, 2.5  $\mu$ M H1 mixed with 10  $\mu$ M ssDNA<sub>75</sub>, and 2.5  $\mu$ M H1 alone. (C)  $A_{350}$  measurements for 2.5  $\mu$ M H1 mixed with 10  $\mu$ M ssDNA<sub>75</sub> in the presence or absence of the H1 chaperone ProT $\alpha$  (2.5  $\mu$ M). (D)  $A_{350}$  measurements for 2.5  $\mu$ M H1 mixed with 10  $\mu$ M ssDNA, 10  $\mu$ M dsDNA, or 5  $\mu$ M dsDNA (to have the same number of nucleotides as 10  $\mu$ M ssDNA) of 30 or 70 nt/bp in length. Data are mean  $\pm$  SEM of at least three independent measurements.



**Supplementary Figure 6. Computational examination of DNA-driven H1 phase separation.** (A) Phase behavior of the four simulated systems as a function of temperature. (B) Probability distribution of the percentage of H1 molecules found in the largest cluster at a temperature of 300 K. (C) Diffusion coefficient ( $D$ ) of H1 in each system at 300 K. All values were normalized by the median  $D$  for ssDNA<sub>70</sub>. (D) Representative configurations for the ssDNA<sub>30</sub> system above and below  $T_C$ . (E) Average number of DNA residues in contact with any given H1 residue, as a function of the H1 residue index. (F) Average number of DNA residues in contact with any given H1 residue projected onto a structure of H1. Data ranges from most contacts (red) to least contacts (blue).



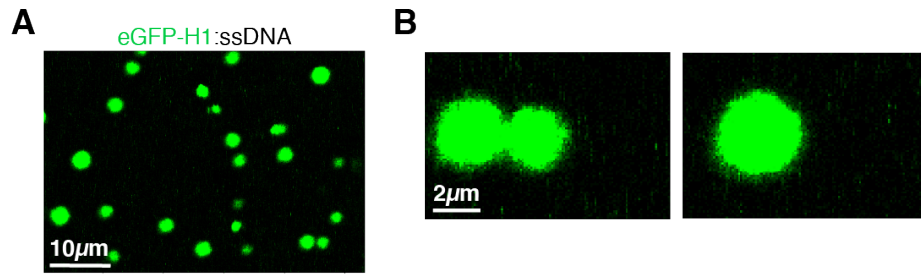
**Supplementary Figure 7. Force profiles from droplet fusion experiments. (A)** Raw data between the normalized force values of 0.3 and 0.8 for H1:ssDNA<sub>30</sub> (left) and H1:dsDNA<sub>30</sub> (right) droplets. Each trace represents a fusion event between two optically trapped droplets. **(B)** Corresponding sigmoidal fits of the force-time traces shown in (A).



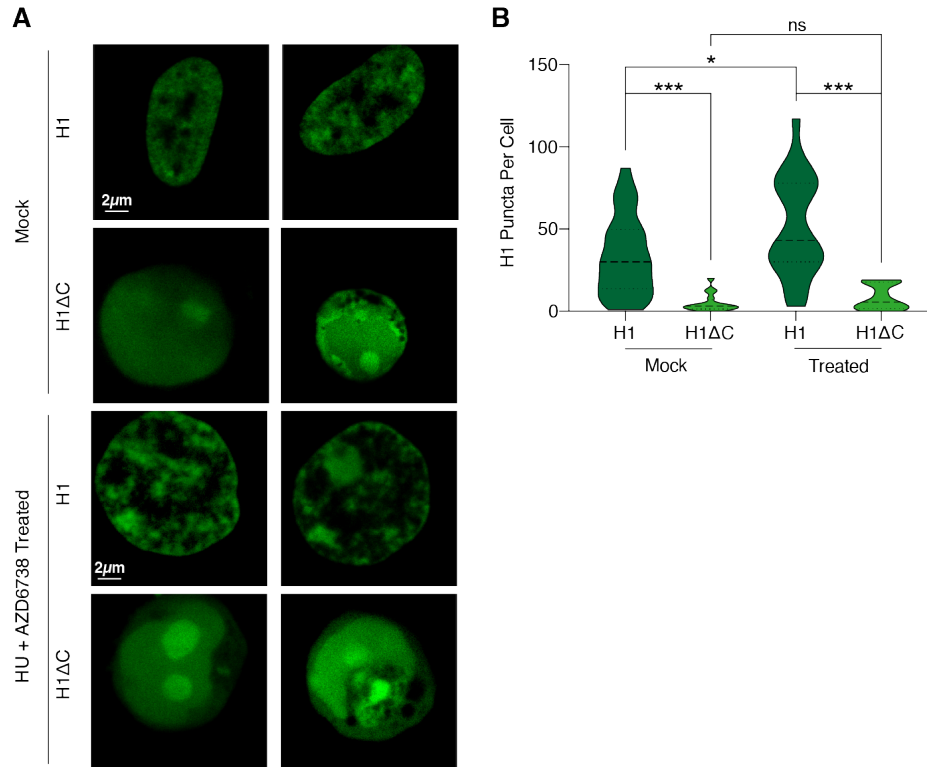
**Supplementary Figure 8. Additional analyses of the biophysical properties of H1:DNA droplets.**

(A) A representative series of images during the photobleaching and fluorescence recovery of an H1:cy5-ssDNA<sub>75</sub> droplet. (B) Kinetics of fluorescence recovery for H1:cy5-ssDNA<sub>75</sub> (blue), H1:cy5-dsDNA<sub>75</sub> (red), and H1 $\Delta$ N:ssDNA<sub>75</sub> (green) droplets. At least 15 droplets were used for FRAP analysis for each condition. (C) Droplet fusion time ( $\tau$ ) for H1:cy5-ssDNA<sub>75</sub>, H1:cy5-dsDNA<sub>75</sub>, and H1 $\Delta$ N:ssDNA<sub>75</sub> droplets. At least 12 fusion events were analyzed for each condition. All experiments were performed with 2.5  $\mu$ M H1 and 10  $\mu$ M DNA (10% labeled). Data are mean  $\pm$  SEM.





**Supplementary Figure 9. eGFP-H1:ssDNA droplet formation and fusion *in vitro*.** (A) A representative image of droplets formed with 2.5 μM recombinant eGFP-H1 and 10 μM ssDNA<sub>75</sub> visualized by GFP fluorescence. (B) Snapshots of two eGFP-H1:ssDNA<sub>75</sub> droplets pre- and post-fusion.



**Supplementary Figure 10. Additional live-cell imaging results.** (A) Representative confocal fluorescence images of HEK293T cells transfected with either eGFP-H1 or eGFP-H1ΔC and treated with either mock or 2 mM HU + 20 μM AZD6738 for 12 h. (B) Violin plot showing the distribution of number of H1 puncta per cell for eGFP-H1 and eGFP-H1ΔC cells after mock or HU + AZD6738 treatment.