Name	Length (nt/bp)	GC%	Sequence
ssDNA ₁₆	16	50	TGT ACT TCT AGA GGC C
ssDNA ₃₀	30	50	GCA TGT ATT GAA GTA CTT CTA GAG GCC GCC
dsDNA ₃₀	30	50	same as above
RNA ₁₆	16	50	rUrGrU rArCrU rUrCrU rArGrA rGrGrC rC
RNA ₃₀	30	50	rGrCrA rUrGrU rArUrU rGrArA rGrUrA rCrUrU rCrUrA rGrArG rGrCrC rGrCrC
RNA ₆₀	60	50	rGrCrA rUrGrU rArUrU rGrArA rGrUrA rCrUrU rCrUrA rGrArG rGrCrC rGrCrC rGrCrA rUrGrU rArUrU rGrArA rGrUrA rCrUrU rCrUrA rGrArG rGrCrC rGrCrC
ssDNA ₇₀	70	50	GCA TGT ATT GAA GTA CTT CTA GAG GCC GCC GCA TGT ATT GAA GTA CTT CTA GAG GCC GCC TGC CTC TAG A
dsDNA ₇₀	70	50	same as above
ssDNA ₇₅	75	33	CCA AAA CTA ATC TTT ATA TAA ATG GGA GAC TCA CTA TCA TGG CTA CTA CGA CTA CTA AAC GAG GTT AAA ATG AAT
dsDNA ₇₅	75	33	same as above
ssDNA ₁₄₇	147	56	CT GGA GAA TCC CGG TGC CGA GGC CGC TCA ATT GGT CGT AGA CAG CTC TAG CAC CGC TTA AAC GCA CGT ACG CGC TGT CCC CCG CGT TTT AAC CGC CAA GGG GAT TAC TCC CTA GTC TCC AGG CAC GTG TCA GAT ATA TAC ATC CTG T

Supplementary Table 1 | Oligonucleotides used in this study.



Supplementary Fig. 1 | Additional results from bulk phase separation assays. a,

Representative brightfield images of H1 mixed with 10 μ M ssDNA₇₅ (left), H1 alone (middle), and H1 mixed with and 10 μ M ssDNA₇₅ in the absence of PEG (right) (among 3 independent experiments). **b**, Solution turbidity (A₃₅₀) measurements for H1 mixed with 10 μ M ssDNA₇₀ and H1 alone. Significance calculated using an unpaired t-test (***p<0.001). **c**, A₃₅₀ measurements for H1 mixed with ss/ds DNA₃₀ or ss/ds DNA₇₀. Significance calculated using a one-way ANOVA with Dunnett's test for multiple comparisons (**p<0.01, ***p<0.001). **d**, A₃₅₀ measurements for H1 mixed with ssDNA₁₄₇ (5 μ M), mononucleosomes (5 μ M), or 12-nucleosome arrays (600 nM). Significance calculated using a one-way ANOVA with Dunnett's test for multiple comparisons (**p<0.001). All experiments were performed with 2.5 μ M H1. All A₃₅₀ experiments performed with n=3 independent measurements. All data in bar charts presented as mean values ± SD.



Supplementary Fig. 2 | Force profiles from droplet fusion experiments. **a**, Raw data between the normalized force values of 0.3 and 0.8 for H1:ssDNA₃₀ (Left) and H1:dsDNA₃₀ (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 Fig. 3 | eGFP-H1:ssDNA droplet formation and fusion *in vitro*. a, A representative image of droplets (among 3 independent experiments) formed with 2.5 μ M recombinant eGFP-H1 and 10 μ M ssDNA₇₅ visualized by GFP fluorescence. b, Representative snapshots of two eGFP-H1:ssDNA₇₅ droplets pre- and post-fusion (among 8 independent fusion events).



Supplementary Fig. 4 | Nuclear distributions of eGFP-H1 and H2B-mCherry.

Representative confocal images of HEK293T cells co-transfected with H2B-mCherry and either eGFP-H1 or eGFP-H1 Δ C and treated with either mock or 2 mM HU + 20 μ M AZD6738 for 18h. eGFP-H1, mock (n=16 independent nuclei); eGFP-H1 Δ C, mock (n=13); eGFP-H1, treated (n=20); eGFP-H1 Δ C, treated (n=12).



Supplementary Fig. 5 | Additional examples of eGFP-H1 and H2B-mCherry co-transfected cells. Representative confocal images of HEK293T cells co-transfected with H2B-mCherry and eGFP-H1 and treated with either mock or 2 mM HU + 20 μ M AZD6738 for 18h. eGFP-H1, mock (n=16 independent nuclei); eGFP-H1, treated (n=20).

a	2μm		
		t= 0 s	t= 74 s
		t= 149 s	t= 223 s
		t= 298 s	t= 372 s
		t= 446 s	t= 521 s



С			 	
	2 <u>µ</u> m			
		t= 0 s	t= 91.1 s	t= 182 s

t= 365 s





t= 547 s

t= 456 s

d 2 <u>µ</u> m		
	t= 0 s	t= 95 s
	t= 191 s	t= 286 s
	t= 381 s	t= 476 s



Supplementary Fig. 6 | Timepoints from continuous Z-stack monitoring for merging eGFP-H1 puncta. Slices from continuous Z-stack imaging over 10 minutes of HEK293T cells transfected with eGFP-H1 or eGFP-H1 Δ C and treated with either mock or 2 mM HU + 20 μ M AZD6738 for 18h. At each timepoint, top left= x plane, top right= y plane, bottom left= z plane. **a-b**, Representative slices (among 6 imaging acquisitions on independent nuclei) of eGFP-H1 treated with 2 mM HU + 20 μ M AZD6738. Red arrows highlight merging puncta. **c**, eGFP-H1 Δ C treated with 2 mM HU + 20 μ M AZD6738. **d**, eGFP-H1 with mock treatment. **e**, eGFP-H1 Δ C with mock treatment.



Supplementary Fig. 7 | Nuclear distributions of eGFP-H1 and RFP-PCNA. Representative confocal images and their corresponding brightfield images of HEK293T cells co-transfected with RFP-PCNA and either eGFP-H1 or eGFP-H1 Δ C and treated with either mock or 2 mM HU + 20 μ M AZD6738 for 18h. eGFP-H1, mock (n=12 independent nuclei); eGFP-H1 Δ C, mock (n=9); eGFP-H1, treated (n=14); eGFP-H1 Δ C, treated (n=12).



Supplementary Fig. 8 | Additional examples of eGFP-H1 and RFP-PCNA co-transfected cells. Representative confocal and brightfield images of HEK293T cells co-transfected with RFP-PCNA and eGFP-H1 and treated with either mock or 2 mM HU + 20 μ M AZD6738 for 18h. eGFP-H1, mock (n=12 independent nuclei); eGFP-H1, treated (n=14).





Scatter plot showing the distribution of Mander's tM1 auto-thresholded correlation coefficients for eGFP-H1 (n=11 independent nuclei) or eGFP-H1 Δ C (n=9) (channel 1) colocalizing with RFP-PCNA (channel 2). Each point represents the coefficient for one nucleus. Significance calculated using Welch's t-test (*p<0.05).

Supplementary Data

A350			
ssDNA ₇₀	H1 only		
0.12133333	0.00266667		
0.12866667	0.001		
0.11733333	-0.0013333		

Supplementary Fig. 1b

A350					
ssDNA ₃₀	dsDNA ₃₀	dsDNA ₃₀	ssDNA ₇₀	dsDNA ₇₀	dsDNA ₇₀
(10µM)	(10µM)	(5µM)	(10µM)	(10µM)	(5µM)
0.05833333	0.048	0.04	0.12133333	0.109	0.101
0.05966667	0.052	0.04533333	0.12866667	0.09133333	0.10333333
0.07	0.05533333	0.04433333	0.11733333	0.11333333	0.09233333

Supplementary Fig. 1c

A350				
ssDNA ₁₄₇	Mononucleosome	12-nucleosome array		
0.18266667	0.062	0.054		
0.22633333	0.076	0.063		
0.20366667	0.078	0.059		

Supplementary Fig. 1d

Correlation Coefficient (Tm1)			
GFP-H1 FL	GFP-H1DC		
0.864	0.1		
0.654	0.302		
0.552	0.545		
0.605	0.478		
0.659	0.138		
0.444	0.427		
0.592	0.304		
0.81	0.155		
0.266	0.224		
0.127			
0			
0.114			
0.802			

Supplementary Fig. 9