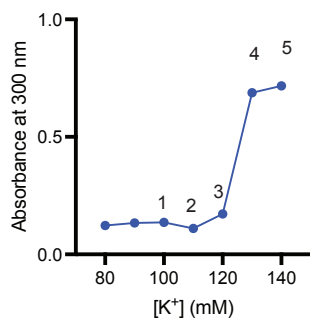


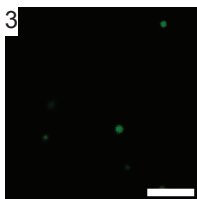
Supplementary Fig. 1



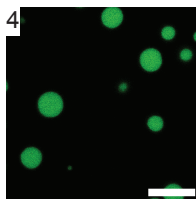
No phase separation  
Baseline Absorbance



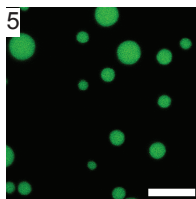
No phase separation  
Baseline Absorbance



Just beyond the threshold  
Increase in Absorbance



Beyond the threshold  
High Absorbance



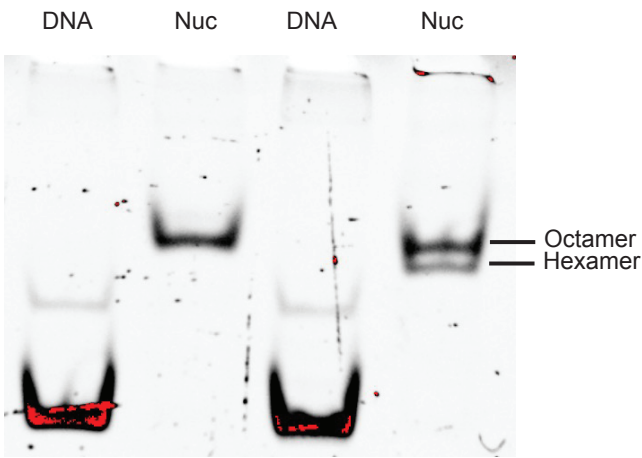
Beyond the threshold  
High Absorbance

**Supplementary Fig. 1. Turbidity assay correlate with microscopy.**

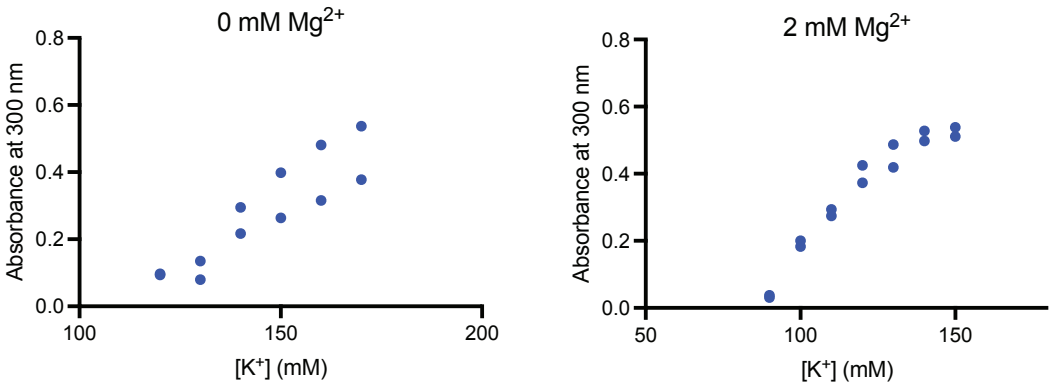
**Top**, Turbidity assay. Each data point represents absorbance measured at 300 nm for the chromatin array in the  $K^+$  concentration indicated. **Bottom**, fluorescence confocal microscopy images of the chromatin array. The numbered data points (1-5) for both the turbidity assay and the microscopy represent the same buffer condition ( $K^+$  concentration). Scale bar: 10  $\mu m$ .

Supplementary Fig. 2

a



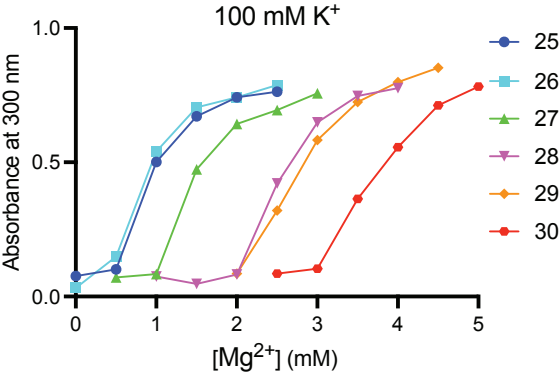
b



**Supplementary Fig. 2. Hexamer-containing 30 bp chromatin arrays have a higher propensity to phase separate.**

**a**, Electrophoretic mobility shift assay (EMSA) of digested DNA or chromatin arrays. Lane 2 shows fully assembled chromatin arrays post digestion to mononucleosomes. Lane 4 shows hexamer containing chromatin array post digestion to mononucleosomes. **b**, Turbidity assay for hexamer containing 30 bp chromatin arrays at 0 mM  $\text{Mg}^{2+}$  (left) or 2 mM  $\text{Mg}^{2+}$  (right) at  $\text{K}^+$  concentrations indicated, with two replicates each.

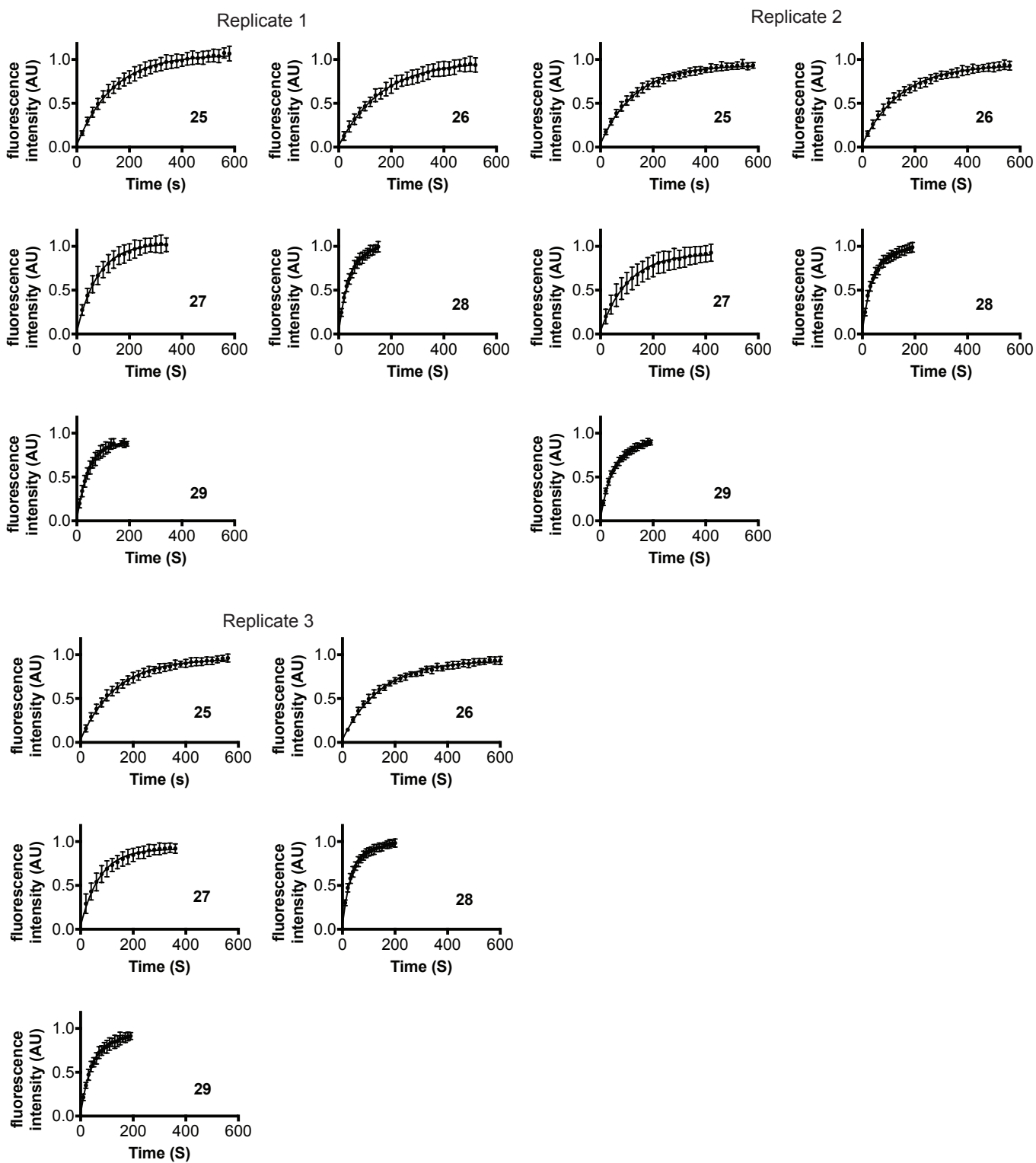
Supplementary Fig. 3



**Supplementary Fig. 3. All tested chromatin arrays phase separate at high  $\text{Mg}^{2+}$  concentrations.**

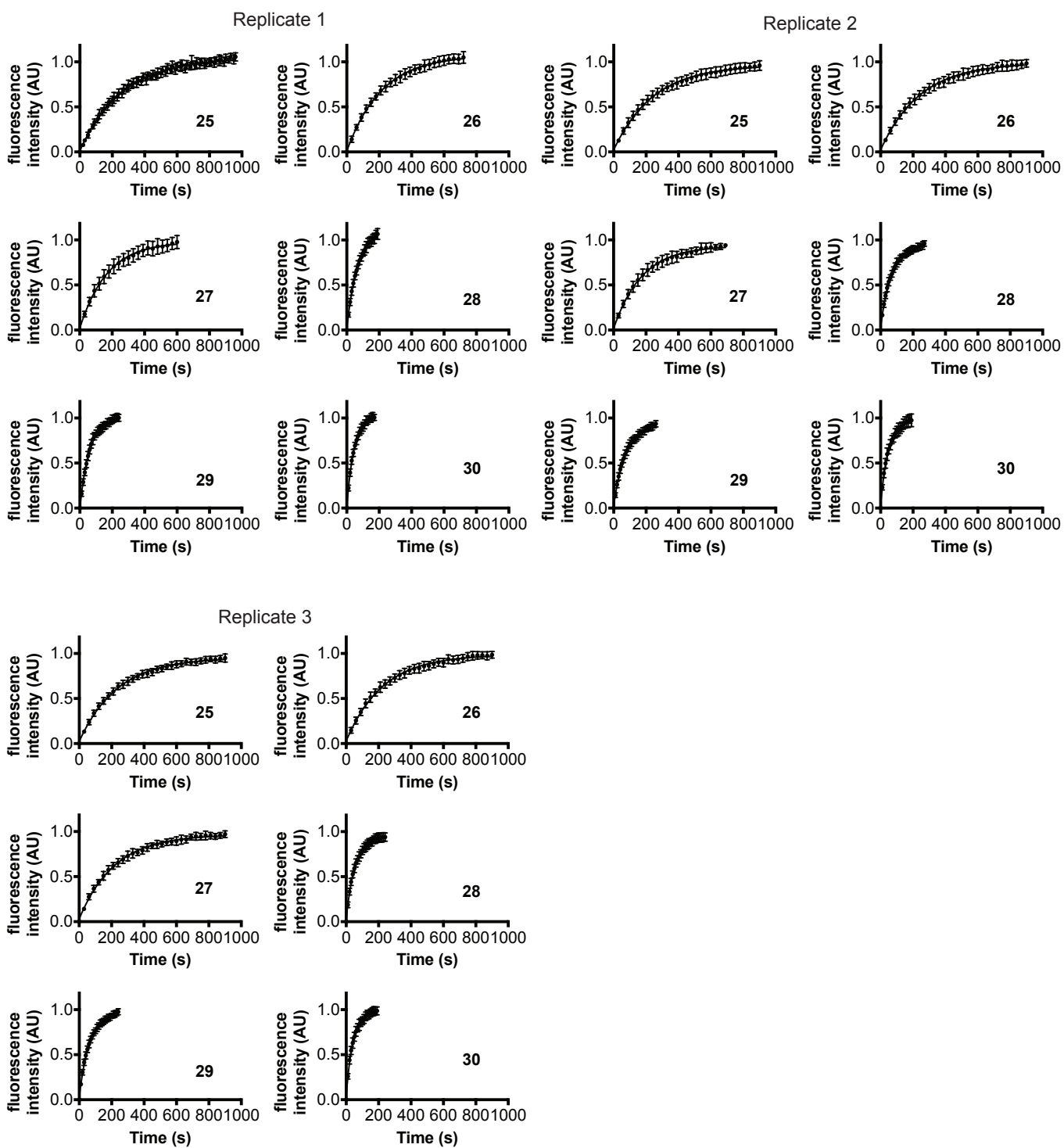
Turbidity assay for arrays with linker DNA lengths from 25 bp to 30 bp. Each data point represents the absorbance measured at 300 nm for arrays in 100 mM  $\text{K}^+$  and  $\text{Mg}^{2+}$  concentration indicated.

# Supplementary Fig. 4



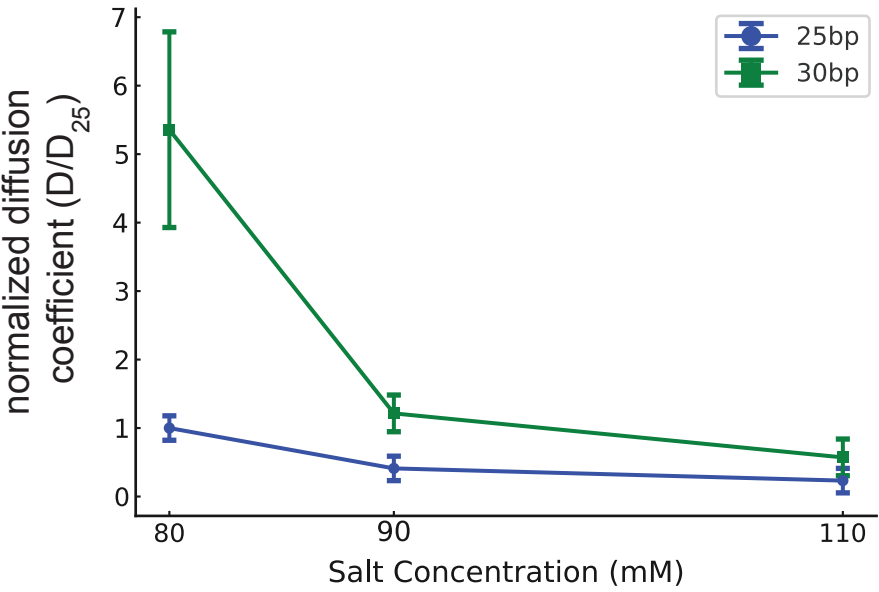
**Supplementary Fig. 4. Fluorescence recovery after photobleaching (FRAP) of chromatin arrays without magnesium in buffer.** Fluorescence recovery over time of a central bleached region in 25 bp to 29 bp chromatin condensates in 180 mM K<sup>+</sup> and 0 mM Mg<sup>2+</sup>. Three sets of replicates are shown. Data are represented as normalized mean fluorescence intensity  $\pm$  s.d.

# Supplementary Fig. 5



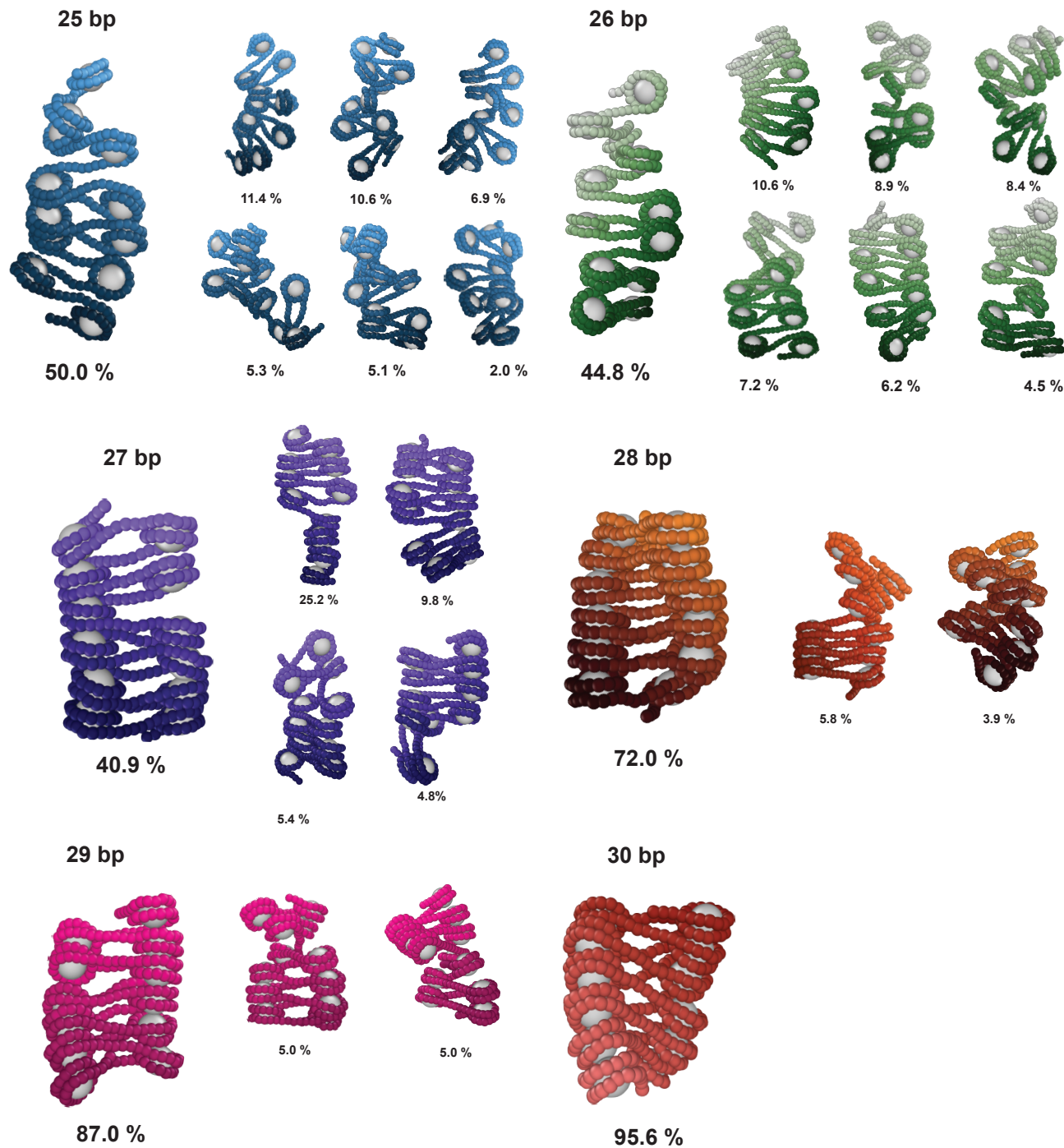
**Supplementary Fig. 5: Fluorescence recovery after photobleaching (FRAP) of chromatin arrays in magnesium buffer.** Fluorescence recovery over time of a central bleached region in 25 bp to 30 bp chromatin condensates in 180 mM K<sup>+</sup> and 3 mM Mg<sup>2+</sup>. Three sets of replicates are shown. Data are represented as normalized mean fluorescence intensity  $\pm$  s.d.

Supplementary Fig. 6



**Supplementary Fig. 6. Diffusion coefficients at different salt concentrations.** Simulated diffusion coefficient, for the indicated linker lengths, at several concentrations of monovalent salt (NaCl). Across the salt regime considered, 25 bp arrays (blue) have a lower diffusion coefficient than the 30 bp arrays (orange). Data are represented as mean  $\pm$  s.d. Error bars were computed using block averaging over the entire simulation trajectory, consisting of  $n = 700$  points.

Supplementary Fig. 7

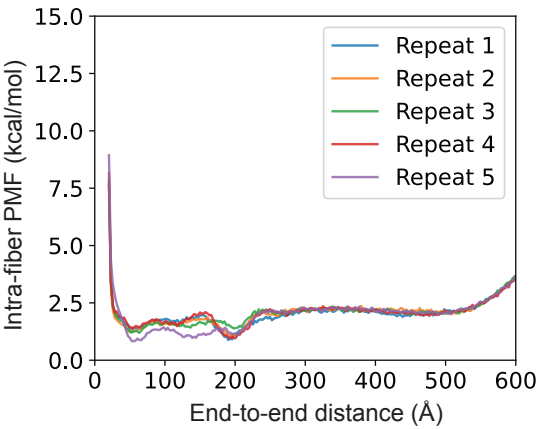


**Supplementary Fig. 7. Extended representative simulation snapshots.**

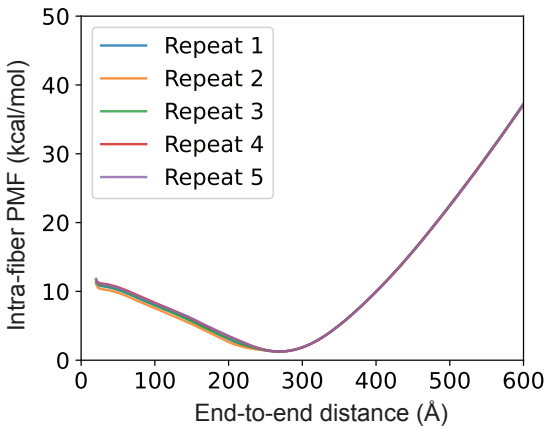
Simulation snapshots of the most common structures found in the bulk of the condensate at 1.2 normalized salt conditions. The distance-RMSD of all fibers in the bulk was computed, in order to then cluster together via k-means the most similar fibers of each linker length. Here, the largest clusters, representing over 90% of the data, are depicted for each linker length: 25 bp (blue), 26 bp (green), 27 bp (purple), 28 bp (orange), 29 bp (pink) and 30 bp (red); with their corresponding percentages.

Supplementary Fig. 8

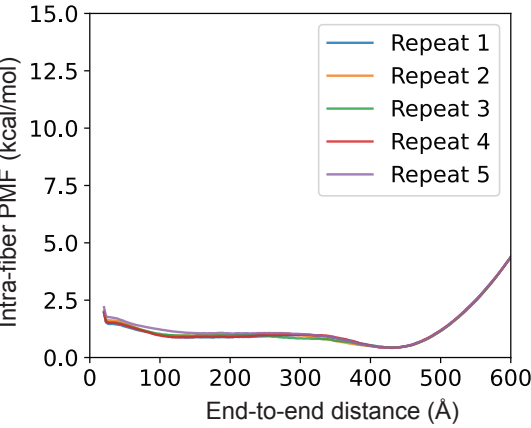
15 bp



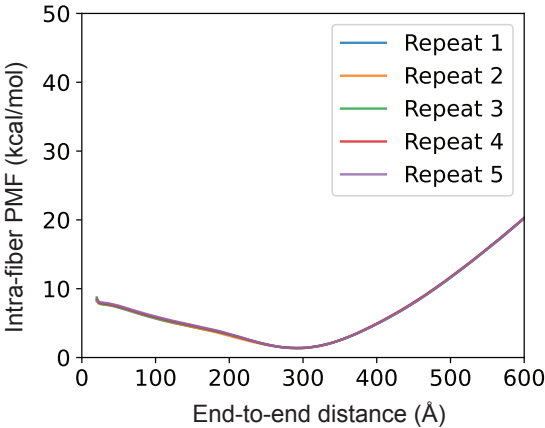
20 bp



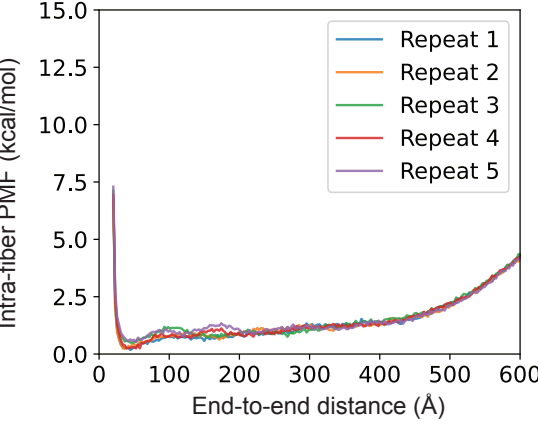
25 bp



30 bp



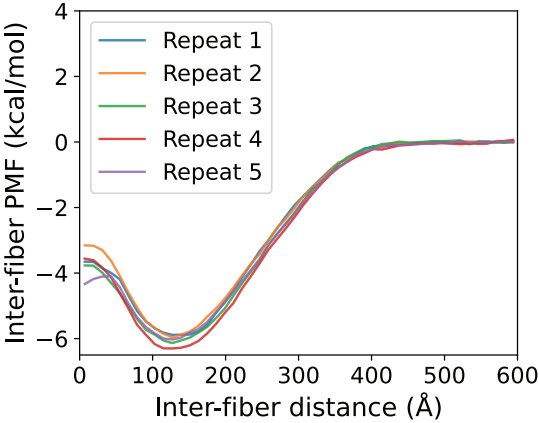
35 bp



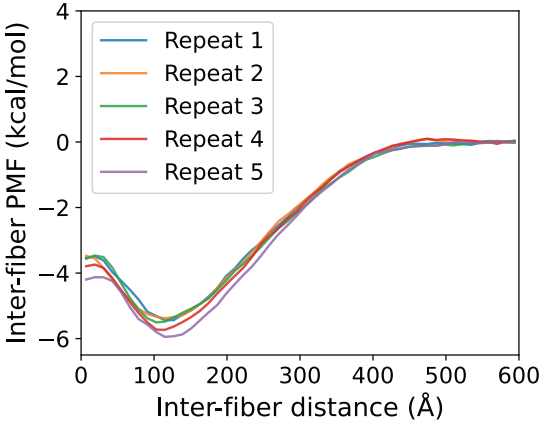
**Supplementary Fig. 8. End-to-end extension of individual chromatin fibers.** Related to Fig. 3d, where each replicate of end-to-end extension of arrays of indicated linker length is shown.

**Supplementary Fig. 9**

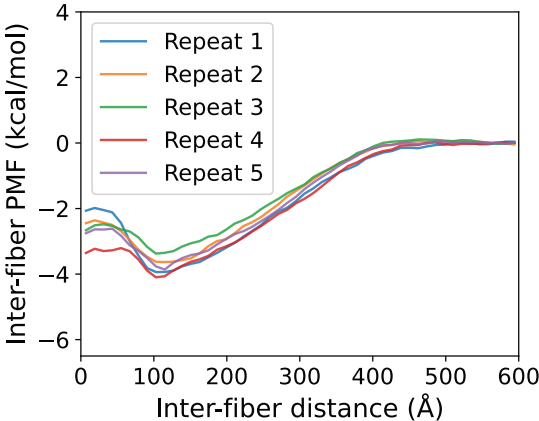
**25 bp**



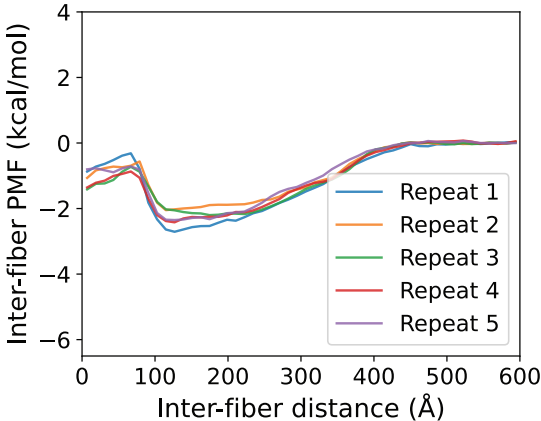
**26 bp**



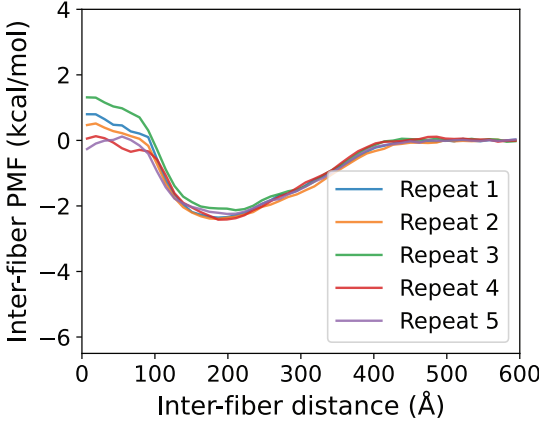
**27 bp**



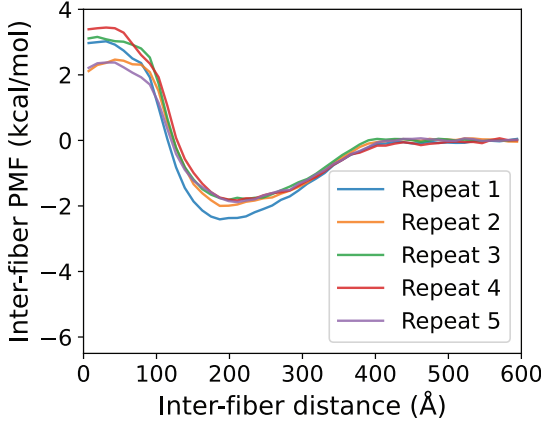
**28 bp**



**29 bp**

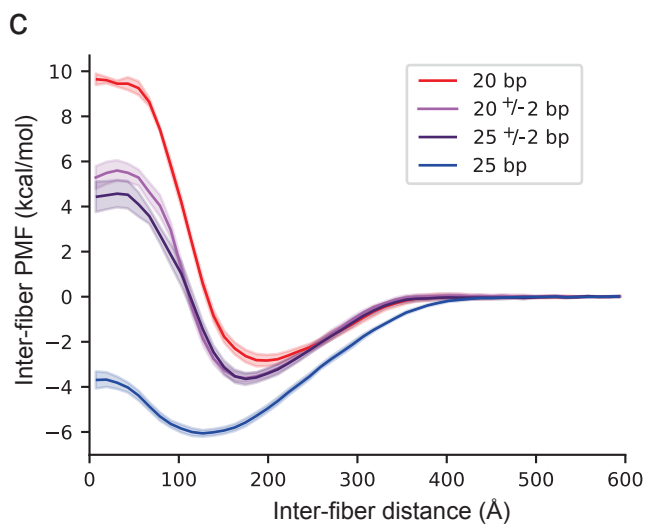
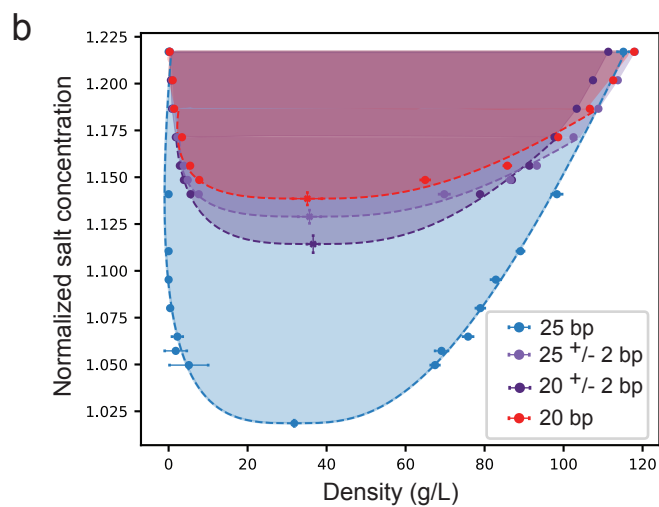
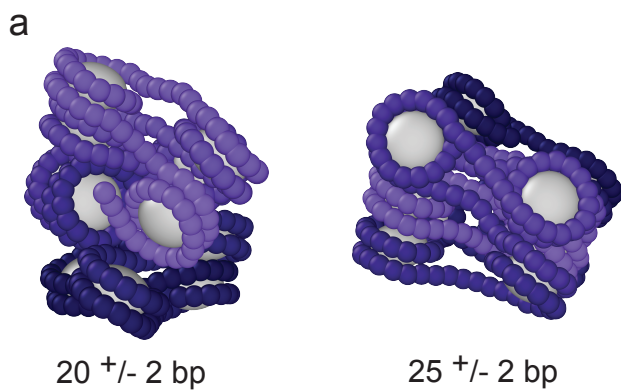


**30 bp**



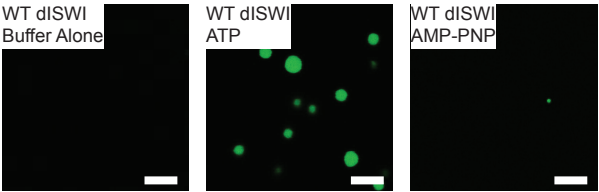
**Supplementary Fig. 9. PMFs of pairs of chromatin fibers.** Related to Fig. 4c, where each replicate of PMFs of pairs of chromatin fibers of indicated linker length is shown.

**Supplementary Fig. 10**



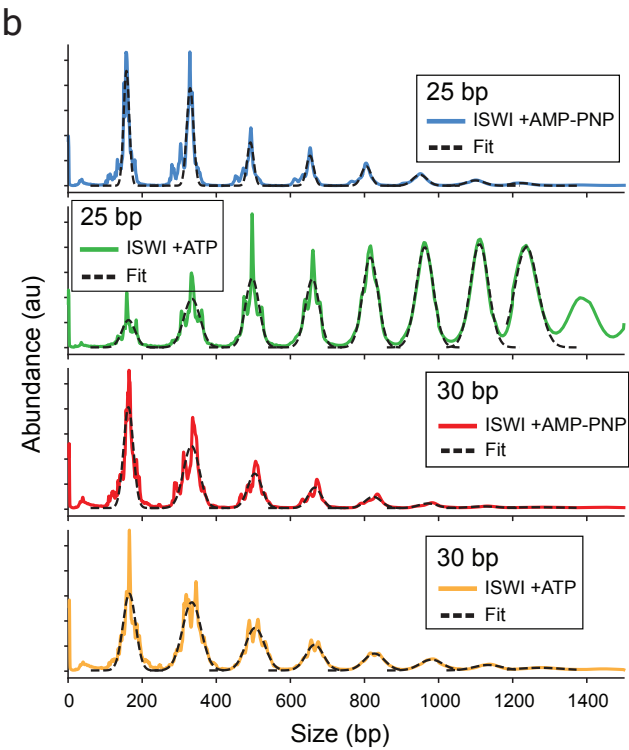
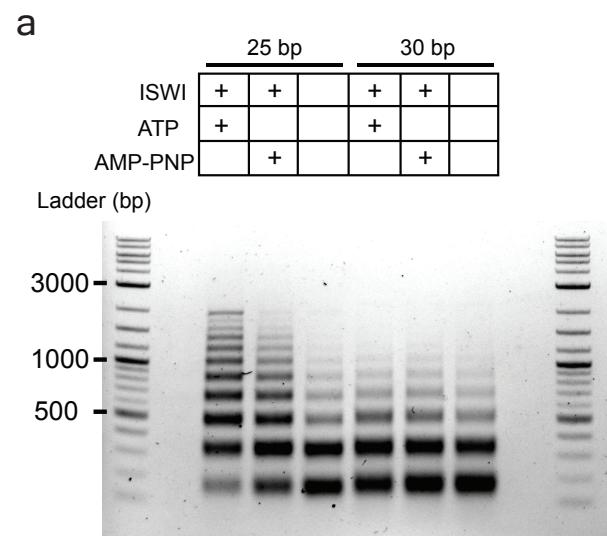
**Supplementary Fig. 10. Simulated phase behavior of chromatin arrays with irregular linker DNA lengths.** **a**, Representative structures of  $20 \pm 2$  bp arrays (left) and  $25 \pm 2$  bp arrays (right). **b**, Simulation coexistence curves for arrays with indicated regular and irregular linker lengths. Circular points represent chromatin density in the dilute phase (left branch) and dense phase (right branch) at a given monovalent salt concentration. Data are represented as mean  $\pm$  s.d. (error bars are smaller than the symbols in most cases). Critical points are calculated by fitting the data to equations (7) and (8), and the error bars are  $\pm$  error from the least-squares fitting procedure. The dashed lines represent coexistence curves and are obtained by fitting the data to equations (7) and (8). **c**, PMF calculated for interactions between pairs of chromatin arrays with the indicated linker length at 150 mM monovalent salt. Distance indicates inter-fiber distance between the geometric centers of the fibers. Computed using umbrella sampling.  $n = 5$  independent repeats. Solid curves represent the mean, and the shading represents the standard deviation.

Supplementary Fig. 11



**Supplementary Fig. 11. WT ISWI induces 30 bp LLPS.** Remodeling 30 bp nucleosome arrays to 35 bp arrays by WT ISWI. Representative images of remodeling reactions with conditions indicated after a 6-hour incubation.  $n = 3$  independent replicates. Scale bar: 10  $\mu\text{m}$ .

Supplementary Fig. 12



**Supplementary Fig. 12. ISWI increases linker lengths.** **a**, Agarose gel of MNase digested nucleosome arrays (25 bp and 30 bp) with indicated reaction conditions (ISWI-2RA, ATP, AMP-PNP). **b**, Bioanalyzer result traces for MNase digested nucleosome arrays (25 bp and 30 bp) with indicated reaction conditions with overlays for peak fitting.