## Supporting Material

Spatiotemporal regulation of Heterochromatin Protein 1- alpha oligomerization and dynamics in live cells

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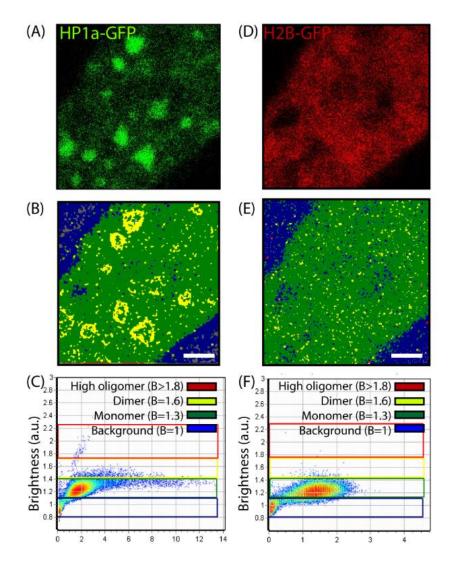


Figure S1: Number and brightness analysis of HP1 alpha and H2B oligomerization in the absence of Hoechst 33342 staining. (A) Intensity image of a NIH3T3 nucleus expressing HP1- $\alpha$ -EGFP. (B) Intensity image in (A) pseudo-colored according to the brightness distribution of HP1 $\alpha$  in (C). As can be seen the HP1 $\alpha$  dimers located around the periphery of the heterochromatin foci remain in the absence of Hoechst 33342 staining. (D) Intensity image of a NIH3T3 nucleus expressing H2B-mCherry. (E) Intensity image in (A) pseudo-colored according to the brightness distribution of H2B in (F). As can be seen H2B remains monomeric throughout the nucleoplasm in the absence of Hoechst 33342 staining.

## Derivation of the pair-correlation function for diffusing particles

The diffusion propagator is given by Equation 1:

$$C(r,t) = \frac{1}{(4\pi Dt)^{3/2}} \exp\left(-\frac{r^2}{4Dt}\right),$$
 (1)

where C(r,t) can be interpreted as being proportional to the probability of finding a particle at position *r* and time *t* if the particle is at position 0 at time t = 0. The fluorescence intensity at any given time and position  $\delta r$  from the origin is given by:

$$F(t,\delta r) = \kappa Q \int W(r) C(r+\delta r,t) dr, \qquad (2)$$

where it is assumed that the fluorescence is proportional to the concentration, quantum yield Q, excitation-emission laser power, filter combination, and the position of the particle in the profile of illumination described by W(r). The pCF for two points at a distance  $\delta r$  as a function of the delay time  $\tau$  is calculated using the expression:

$$G(\tau,\delta r) = \frac{\langle F(t,0) \cdot F(t+\tau,\delta r) \rangle}{\langle F(t,0) \rangle \langle F(t,\delta r) \rangle} - 1 , \qquad (3)$$