# Online Supporting Information

## Online Supporting Table

**Table SI.** Quantitative analysis of the nuclear distribution of NLS-SAv-Cy5.

| Nuclear compartment | Mean Fluorescence (CLSM) [$] | Relative Particle Density (SMT) [§] |
|---|---|---|
| Nucleoplasm | 1 | 1 |
| Pericentric Heterochromatin | 0.96 ± 0.09 | 0.83 ± 0.16 |
| Nucleolus | 0.42 ± 0.05 | 0.58 ± 0.12 |

[$] Mean intensity of the Cy5 fluorescence was determined in MeCP2-GFP labeled pericentric heterochromatin, the nucleoli and remaining nucleoplasm from confocal microscopy (CLSM) images (see Fig. S1).

[§] In the single molecule tracking data (SMT) the number of particles per total compartment area was calculated. The particle number was extrapolated from the number of jumps.

## Online Supporting Figures

**Figure S1.** Steady state distribution of NLS-SAv-Cy5 complexes in nuclear subcompartments.

(A) A C2C12 cell expressing MeCP2-GFP was microinjected into the cytoplasm with NLS-SAv-Cy5 complexes, and imaged 90 min later by confocal laser scanning microscopy. (B) The complexes distributed homogeneously in the cytoplasm and nucleus but showed a much lower concentration in the nucleoli, (C) which were identified according to the phase contrast (PC) image. (D) For the quantitative analysis of steady state distributions (see Table SI) the boundaries of the nuclei were determined in the green fluorescence (MeCP2-GFP) or phase contrast image. A border region near the nuclear envelope (width 0.95 µm, orange) was excluded from the analysis. The pericentric heterochromatin was identified by the bright MeCP2-GFP signal (yellow). Nucleoli (blue) were marked according to the phase contrast image. Scale bar, 5 µm.

**Figure S2.** NLS-SAv-Cy5 complexes do not form aggregates and are completely mobile within the nucleus and cytoplasm.
Fluorescence photobleaching experiments were performed in C2C12 mouse cells expressing MeCP2-GFP (green, pericentric heterochromatin), which had been microinjected into the cytoplasm with NLS-SAv-Cy5 (red) 90 min before. (A) For FLIP, the Cy5 fluorescence was bleached within a small spot (BA) in the nucleus (N) with 100% laser power at 633 nm followed by acquisition of an image. This cycle was repeated 200 times and numbers above the NLS-SAv-Cy5 fluorescence image indicate the imaging/bleaching cycle. At the end of the FLIP experiment the nuclear and cytoplasmic Cy5 fluorescence was completely lost. The FLIP curve in (B) shows the loss of Cy5 fluorescence (as mean fluorescence intensity, FI) in the nucleus (red curve) averaged for 10 cells. A fast loss of the nuclear fluorescence indicated a very high mobility and no binding or aggregation of the NLS-SAv-Cy5 complexes.

**Figure S3.** Temporal stability of trapping sites in the nucleoplasm.
The existence of structural traps would be supported, if trapping could occur repeatedly in the same spatial intranuclear regions. The observation of two different molecules at exactly the same site by pure chance is, however, exceptionally improbable considering the extremely low concentration used in our experiments. However, we did observe such an event. The first molecule entered into the confined region at frame 421 and remained there for 41 frames. The same region was revisited at frame 522 and finally at frame 883. Revisiting events were rare, but still they show the temporal stability of nuclear structures. Scale bar, 5 µm.

**Figure S4.** Streptavidin can cross compartment borders without hindrance.

Examples for tracks of molecules diffusing rapidly between the nucleoplasm and the heterochromatin (dark green, A and B) and to the nucleolus (bright green, C). (D) Jump distance distributions of particles crossing compartment borders. The relative frequency of short jumps is significantly decreased compared to the individual compartments (see Fig. 4), since crossing of the

border is more probable when a particle performs a large jump than for an almost immobile particle. Scale bar, 1 μm.

**Movie 1.** Probing mammalian nuclear compartments with single streptavidin molecules.
The movie shows NLS-SAv-Cy5 molecules after microinjection into the cytoplasm. An overlay of the heterochromatin compartment staining (green) and the NLS-SAv-Cy5 moving through the cell (red) is shown. The data was recorded at 191 Hz, and the display rate is 20 Hz, which corresponds to about 10-fold slower motion. On the right hand side raw single molecule data are displayed; on left hand side the SMT raw data were processed using a 2-20 pixel band pass filter. One pixel corresponds to 96 nm and the whole object field shown is 12.2 μm x 12.2 μm. NLS-SAv-Cy5 is concentrated around the nuclear border, while single molecules can be distinguished inside the nucleus.

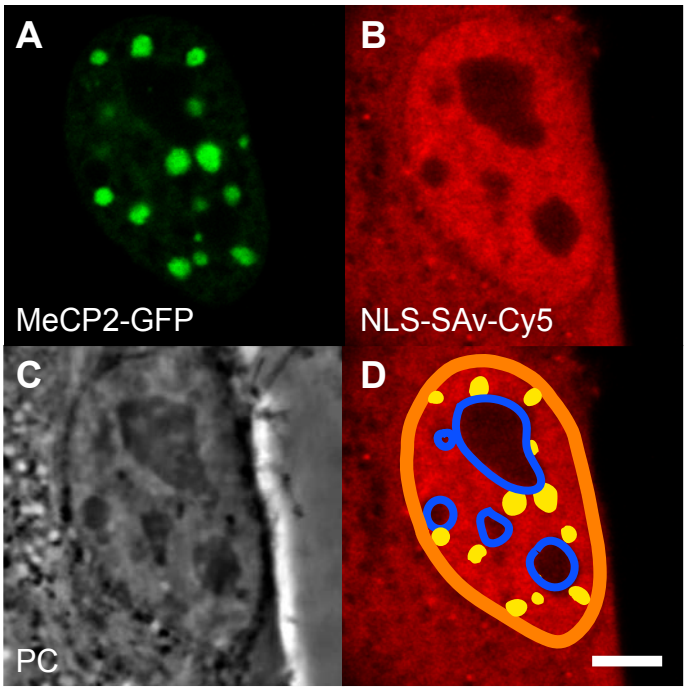


Fig. S1 - Grünwald et al.

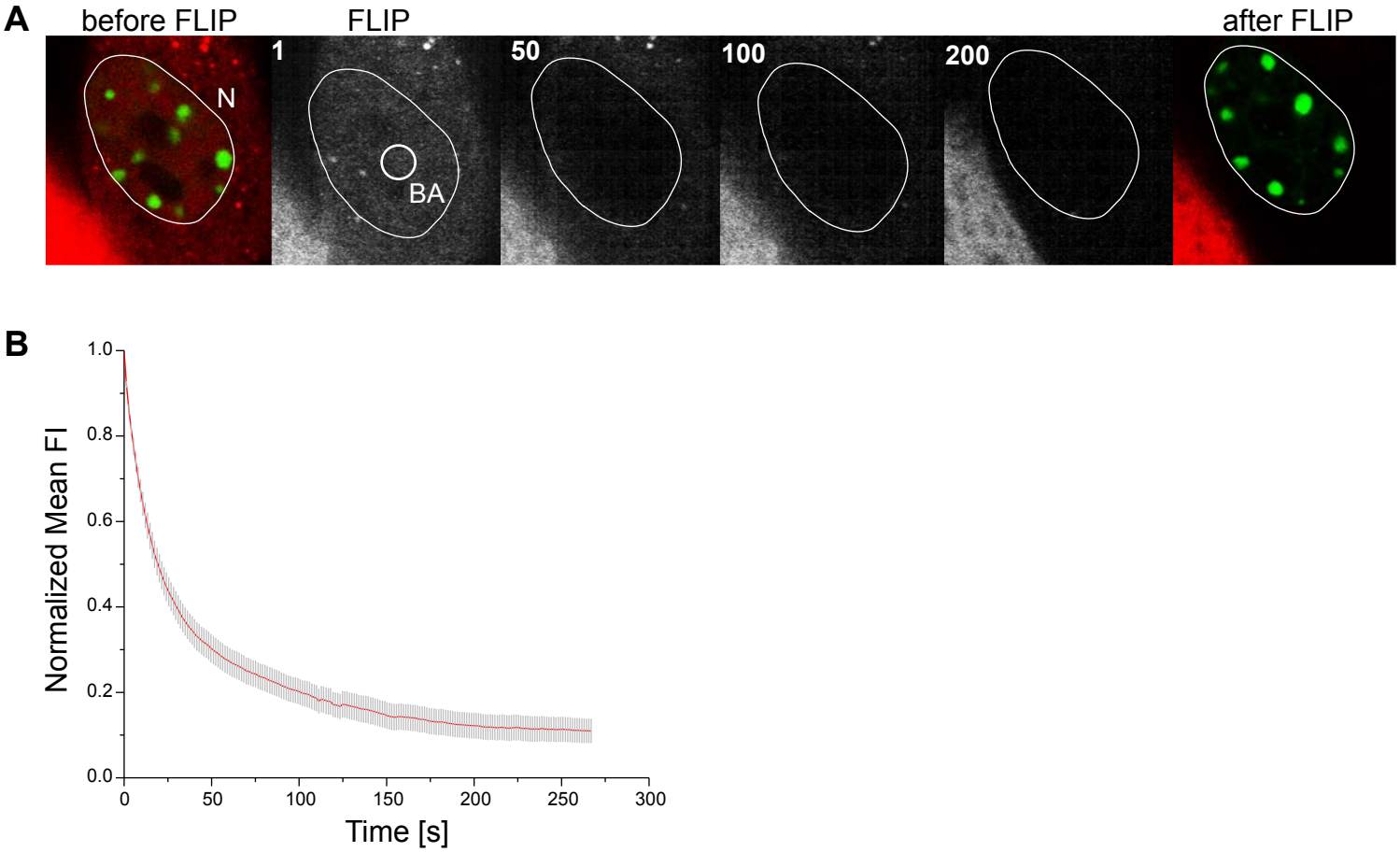


Fig. S2 - Grünwald et al.

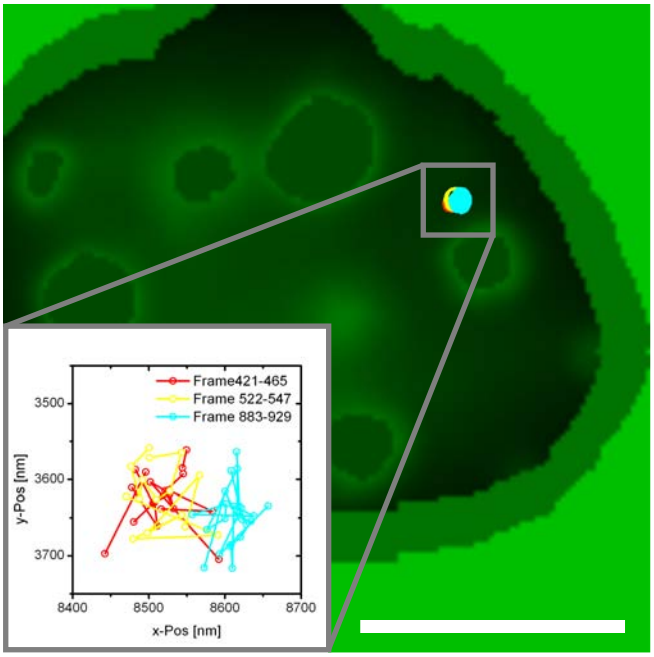


Figure S3 - Grünwald et al.

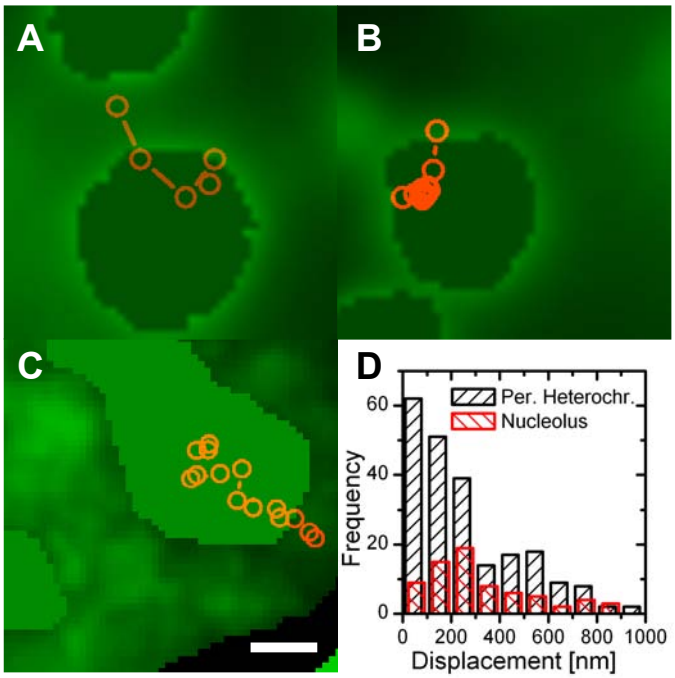


Fig. S4 - Grünwald et al.