Supporting Information

Hinde et al. 10.1073/pnas.1006731107

SI Materials and Methods

The diffusion propagator is given by Eq. 1:

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

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 δr from the origin is given by:

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

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 δr as a function of the delay time τ is calculated by using the expression:

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

As in normal FCS, the pCF can be calculated analytically only for special cases of the profile of illumination function. In our case, it was assumed that the illumination profile was described by a symmetric 2D Gaussian function.

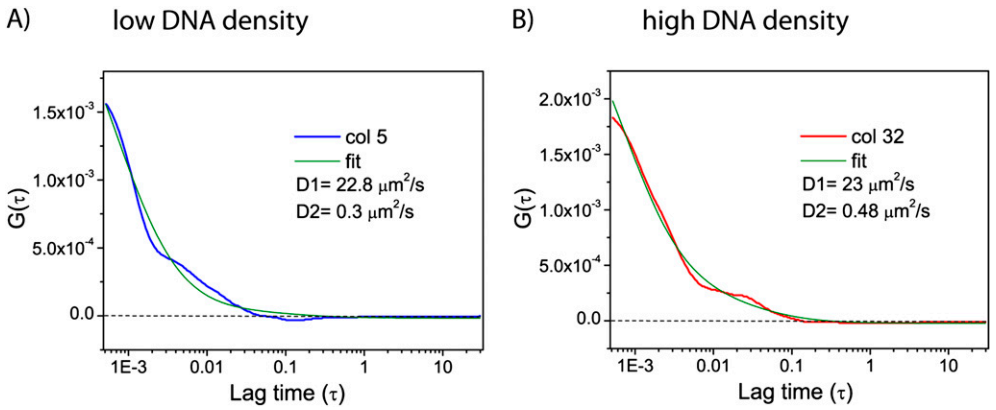

Fig. S1. Fitting of EGFP diffusion in low and high DNA density with a two-component model. (*A*) Fitting of column 5 (which corresponds to low DNA) from the ACF carpet in Fig. 1*I* with a two-species model. (*B*) Fitting of column 32 (which corresponds to high DNA) from the ACF carpet in Fig. 1*I* with a two-species model.

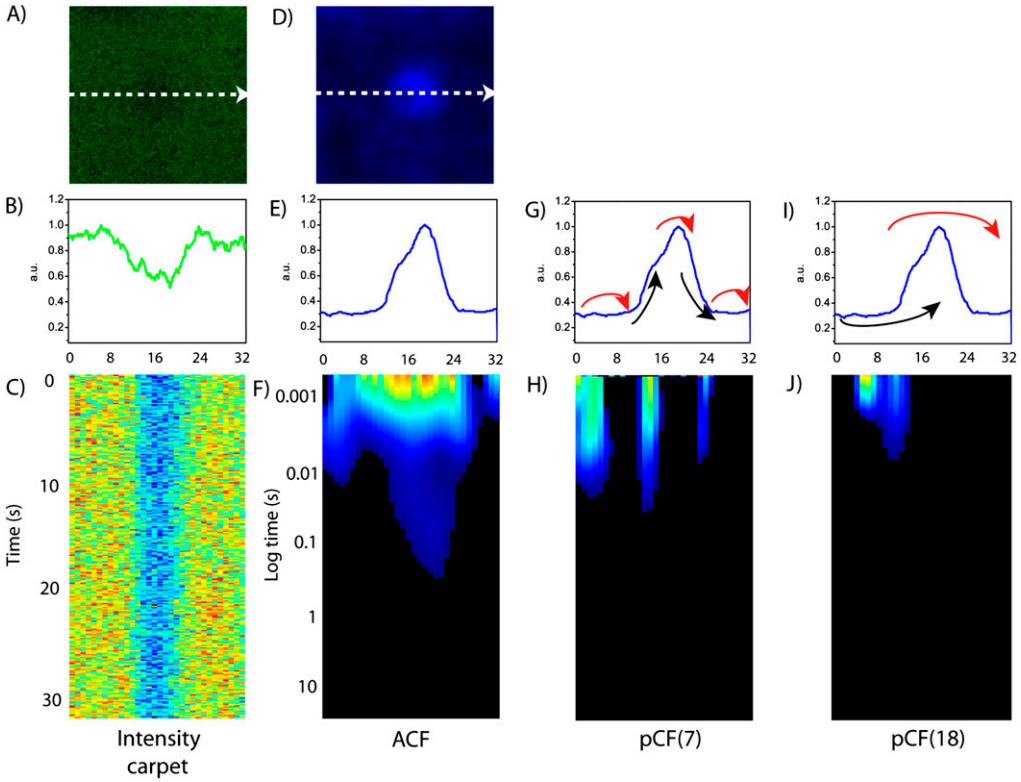

Fig. S2. ACF and pCF carpet analysis of intranuclear diffusion. (*A*) Free EGFP in the plane of the line drawn in the nucleus: 3.3μm. (*B*) Intensity profile of free EGFP across the line drawn. (*C*) Fluorescence intensity carpet of the line drawn across freely diffusing EGFP. (*D*) Hoechst 33342 staining in the plane of the line drawn in the nucleus: 3.3 μm. (*E*) Intensity profile of the Hoechst 33342 stain across the line measured. (*F*) ACF carpet of the line drawn across freely diffusing EGFP (1×10^5 lines analyzed). (*G*) Intensity profile of Hoechst 33342 stain with arrows indicating the pCF calculation at a distance of 7 pixels. (*H*) pCF(7) carpet, which corresponds to cross-correlation of pixels to an adjacent DNA environment. (*I*) Intensity profile of the Hoechst 33342 stain with arrows indicating the pCF calculation at a distance of 18 pixels. (*J*) pCF(18) carpet, which corresponds to cross-correlation of pixels to a distant DNA environment around a barrier.

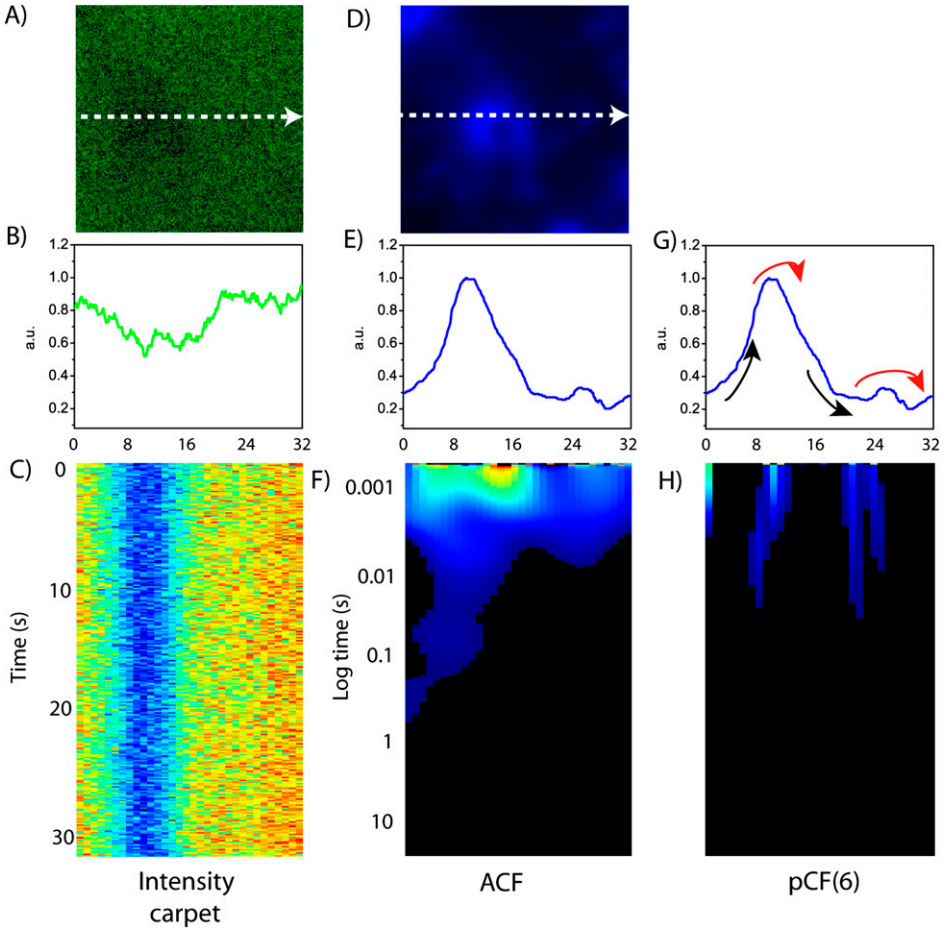

Fig. S3. ACF and pCF analysis of intranuclear diffusion. (*A*) Free EGFP in the plane of the line drawn in the nucleus: 3.3 μm. (*B*) Intensity profile of free EGFP across the line drawn. (*C*) Fluorescence intensity carpet of the line drawn across freely diffusing EGFP. (*D*) Hoechst 33342 staining in the plane of the line drawn in the nucleus: 3.3 μm. (*E*) Intensity profile of the Hoechst 33342 stain across the line measured. (*F*) ACF carpet of the line drawn across freely diffusing EGFP (1×10^5 lines analyzed). (*G*) Intensity profile of the Hoechst stain 33342 stain with arrows indicating pCF calculation at the distance of 6 pixels. (*H*) pCF(6) carpet, which corresponds to cross-correlation of pixels to an adjacent DNA environment.

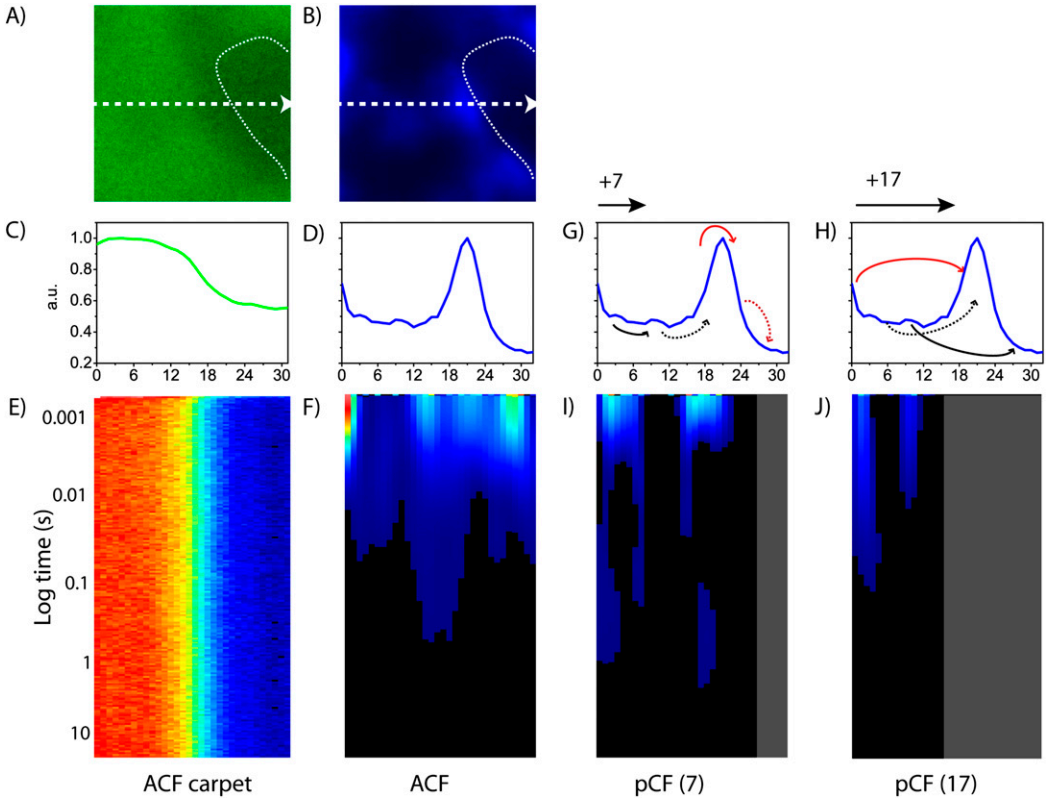

Fig. S4. pCF analysis of diffusion across the nucleolus. (*A*) Free EGFP across a line drawn in the nucleus that traverses the nucleolus: 3.3 μm. (*B*) Hoechst 33342 staining. (*C*) Intensity profile of freely diffusing EGFP across the line measured. (*D*) Intensity profile of the Hoechst 33342 stain across the line measured. (*E*) Fluorescence intensity carpet of the line drawn across freely diffusing EGFP. (*F*) ACF carpet of the line drawn across freely diffusing EGFP. (*G* and *H*) Intensity profile of Hoechst 33342 stain across the line measured. (*I*) pCF(7) carpet, which corresponds to cross-correlation of pixels to adjacent pixels in the same density DNA environments. (*J*) pCF(17) carpet, which corresponds to cross-correlation of pixels in low-low DNA around a high DNA density environment or pixels in high-high DNA around a low DNA density environment.

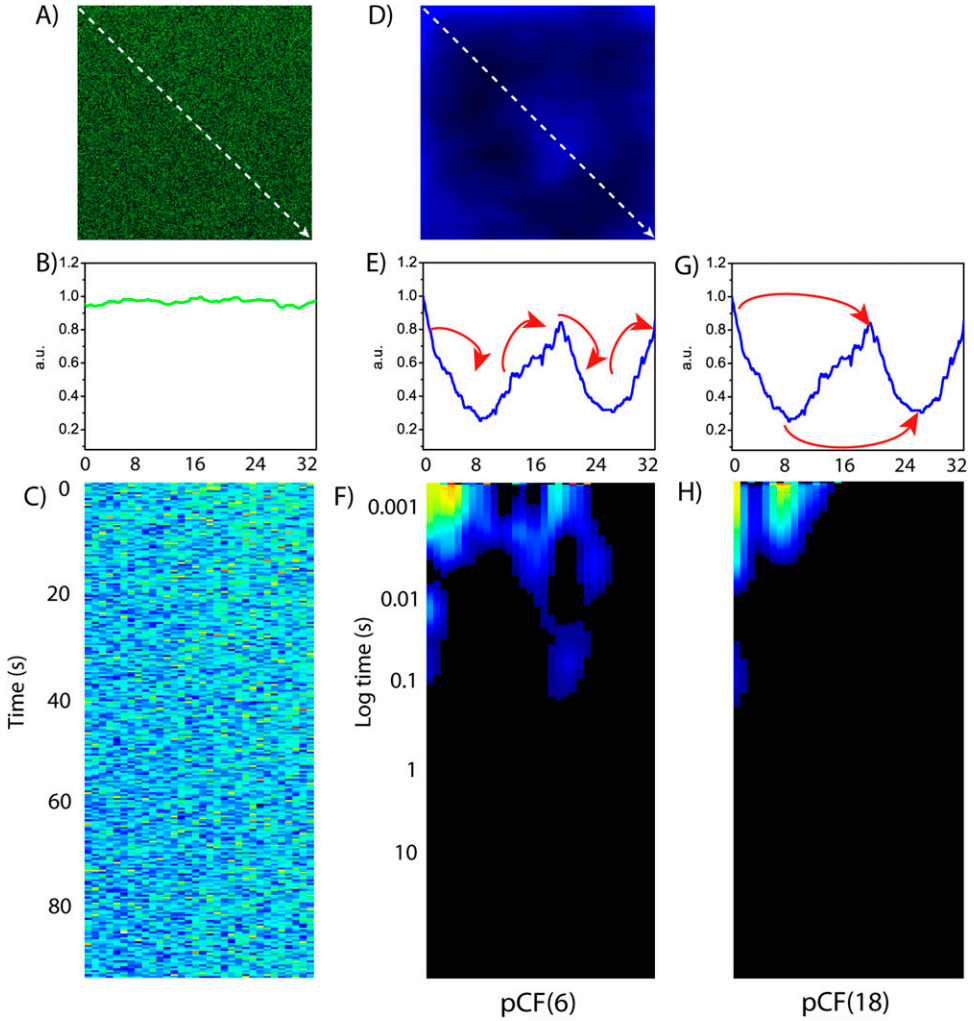

Fig. S5. ACF and pCF analysis of intranuclear diffusion of fluorescein. (*A*) Fluorescein in the plane of the line drawn in the nucleus: 4.8 μm. (*B*) Intensity profile of fluorescein across the line drawn. (*C*) Fluorescence intensity carpet of the line drawn across freely diffusing fluorescein (*D*) Hoechst 33342 staining in the plane of the line drawn in the nucleus: 4.8 μm. (*E*) Intensity profile of the Hoechst 33342 stain across the line with arrows indicating pCF calculation at a distance of 6 pixels. (*F*) pCF(6) carpet, which corresponds to cross correlation of pixels from low-to-high and high-to-low DNA density regions. (*G*) Intensity profile of the Hoechst 33342 stain across the line with arrows indicating pCF calculation at a distance of 18 pixels. (*H*) pCF(18) carpet, which corresponds to cross correlation of pixels from high-to-high and low-to-low DNA density.

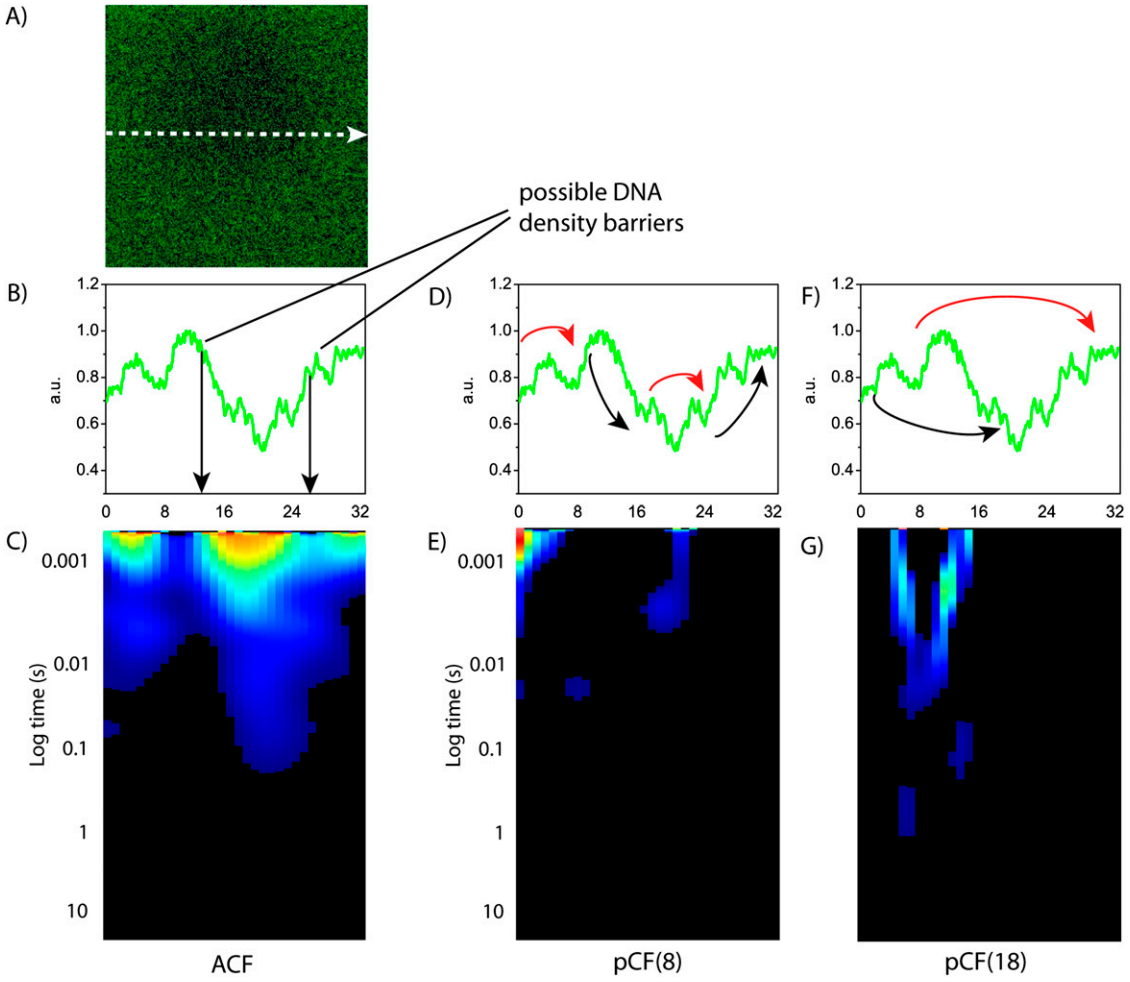

Fig. S6. ACF and pCF carpet analysis of intranuclear diffusion of EGFP in an unstained cell (unstained with Hoechst 33342). (*A*) Free EGFP in the plane of the line drawn in the nucleus: 3.3 μm. (*B*) Intensity profile of free EGFP across the line drawn: the presence of a possible barrier to diffusion (high DNA density area) is indicated in black. (C) ACF carpet of the line drawn across freely diffusing EGFP (1×10^5 lines analyzed). (*D*) Intensity profile of EGFP with arrows indicating pCF calculation at the distance of 8 pixels. (*E*) Intensity profile of the Hoechst 33342 stain across the line measured. (*E*) pCF(6) carpet, which corresponds to cross-correlation of pixels to an adjacent DNA environment. (*F*) Intensity profile of EGFP with arrows indicating pCF calculation at the distance of 18 pixels. (*G*) pCF(18) carpet, which corresponds to cross-correlation of pixels to a distant DNA environment around a barrier.

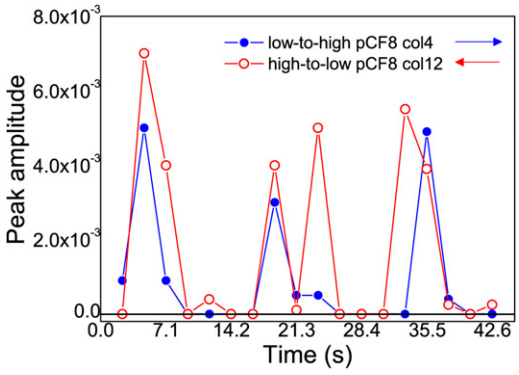

Fig. S7. Decomposition of pCF carpet analysis in two directions. The left-to-right pCF(8) analysis of column 4 (reported in Fig. 3 and here, blue line) is compared with the right-to-left pCF(8) analysis of column 12 (red curve). The whole acquisition is decomposed in short time fragments of 5×10^3 lines, corresponding to ≈2.36 s. The maximum amplitude of correlation detected for each fragment of columns 4 and 12 against the time of acquisition is plotted. This analysis shows that the observed bursts of EGFP diffusion are bidirectional with respect to the DNA density discontinuity. The slightly higher amplitudes reported in the pCF(8) analysis of column 12 (corresponding to EGFP diffusion from high to low DNA density) correlate with EGFP localization with respect to DNA.

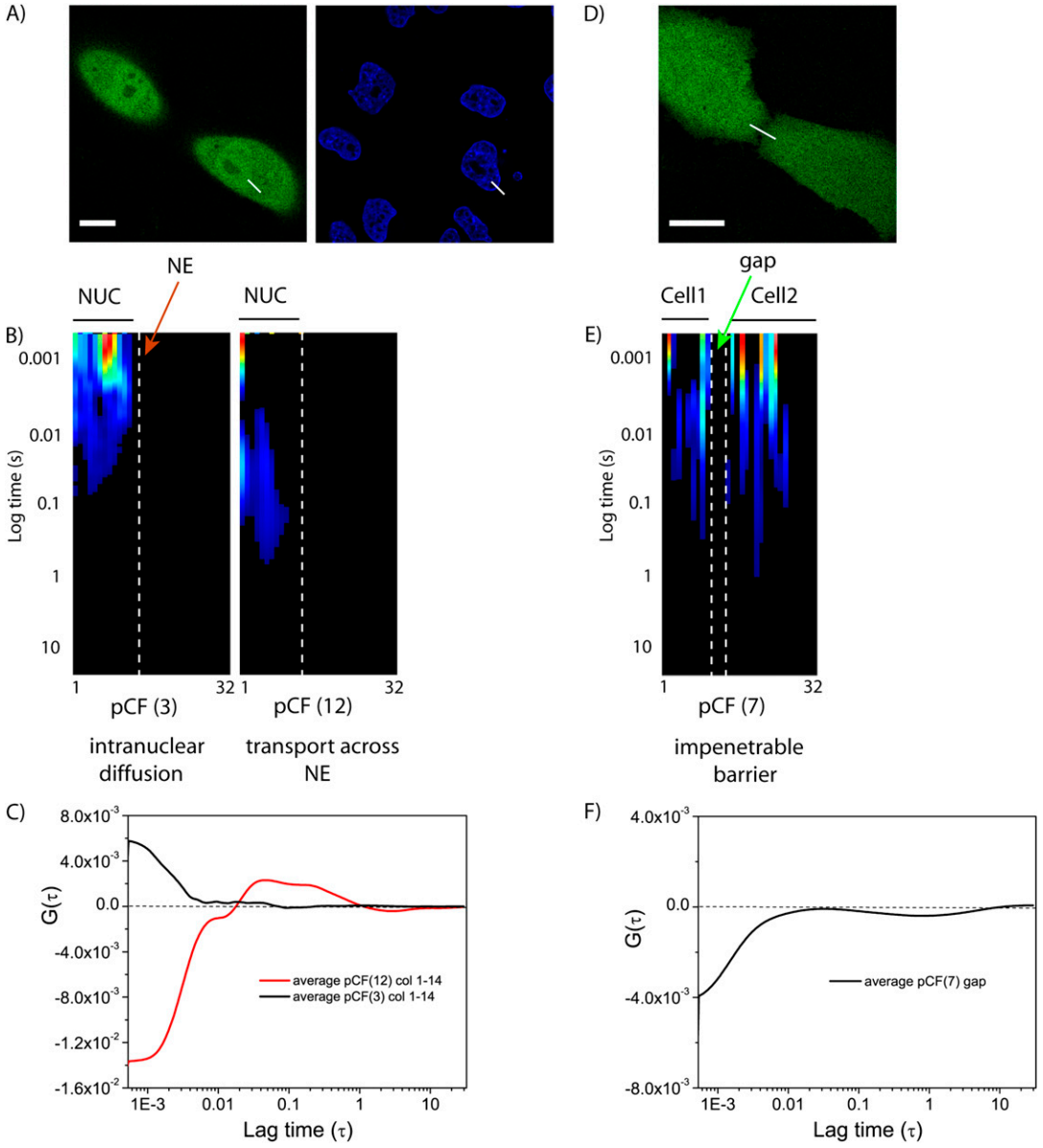

Fig. S8. ACF and pCF carpet analysis of intracellular EGFP diffusion across obvious barriers. (*A*) Image of free EGFP and Hoechst 33342 stain in two neighbor CHOK1 cells in the plane of the nucleus. The line scanned across the nuclear envelope is depicted in white. (Scale bar: 5 μm.) (*B*) pCF(3) analysis depicts intranuclear diffusion, as can be seen a barrier to diffusion exists where the nuclear envelope is positioned, and pCF(12) analysis depicts transport across the nuclear envelope. (*C*) A plot of the average cross-correlation profile across the columns measured for pCF(3) and pCF(12) analysis. As can be seen intranuclear diffusion occurs on the microsecond timescale and transport across the nuclear enveloped occurs on the millisecond timescale. (*D*) Image of free EGFP in two neighbor CHOK1 cells in the plane of where the two cell boundaries are in contact. The line scanned across the two neighbor cell boundaries is depicted in white. (Scale bar: 5 μm.) (*E*) pCF(7) analysis depicts the impenetrable gap between the two cells. (*F*) A plot of the average cross-correlation profile across the columns within the gap for pCF(7) analysis. As can be seen there is only anticorrelation and, therefore, no diffusion from pixels in one cell to the other.

Table S1. Diffusion coefficients derived by ACF analysis

	Low DNA	High DNA
EGFP	$D_1 = 23 \pm 1$ $D_2 = 0.2 \pm 0.1$	$D_1 = 22 \pm 2$ $D_2 = 0.4 \pm 0.1$

Average diffusion coefficients ("D", $\mu m^2/s$) calculated separately in regions of high and low DNA density across five sets of lines in the nuclei of five observed cells. High and low DNA density regions were best fitted to a two-component model, yielding two characteristic diffusion coefficients (D1 and D2). For each measurement, one representative column corresponding to either low or high DNA density was fitted to the 3D equations of diffusion. Single-column D values were then averaged to obtain the cumulative values displayed here (mean ± SD).