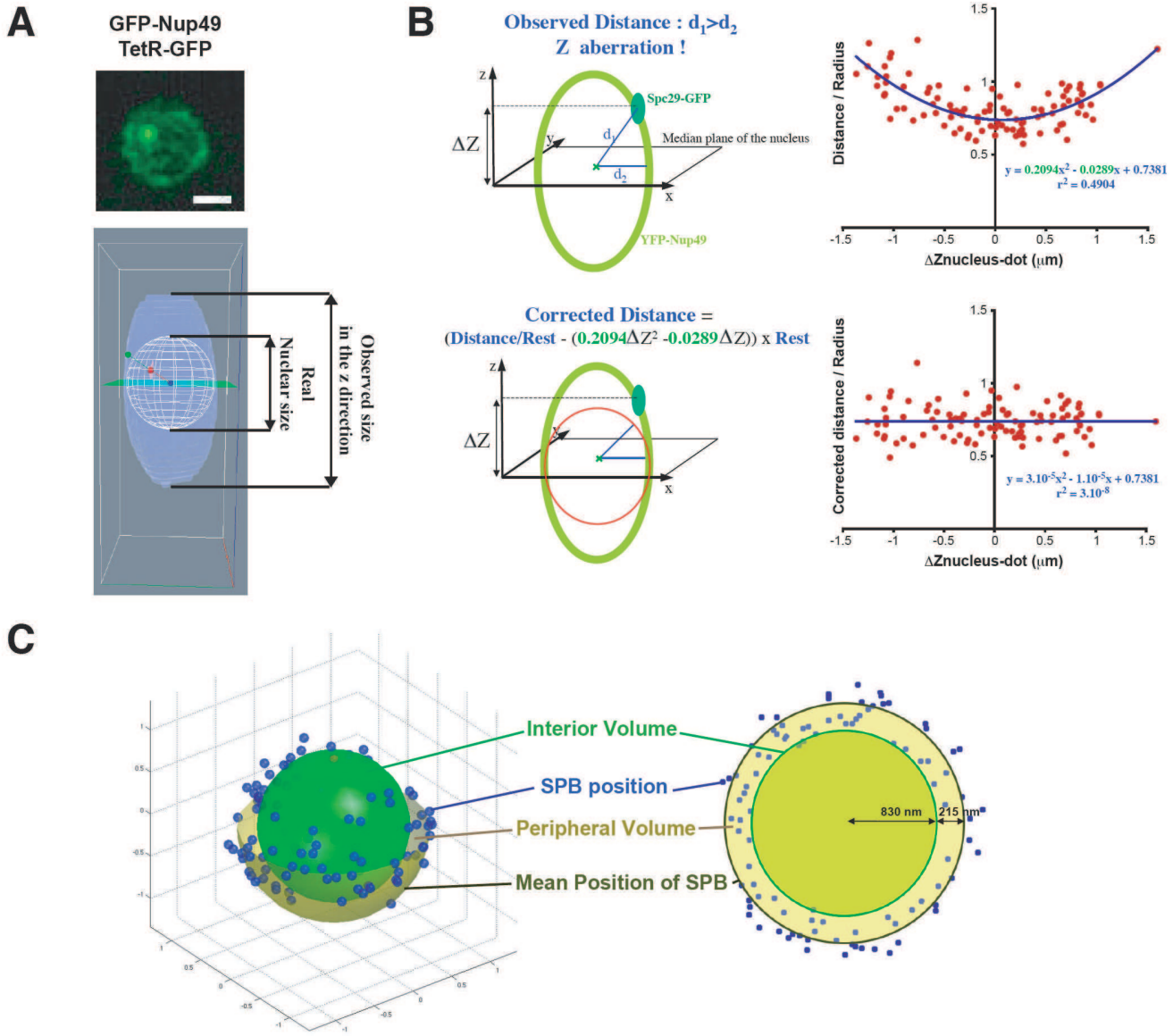
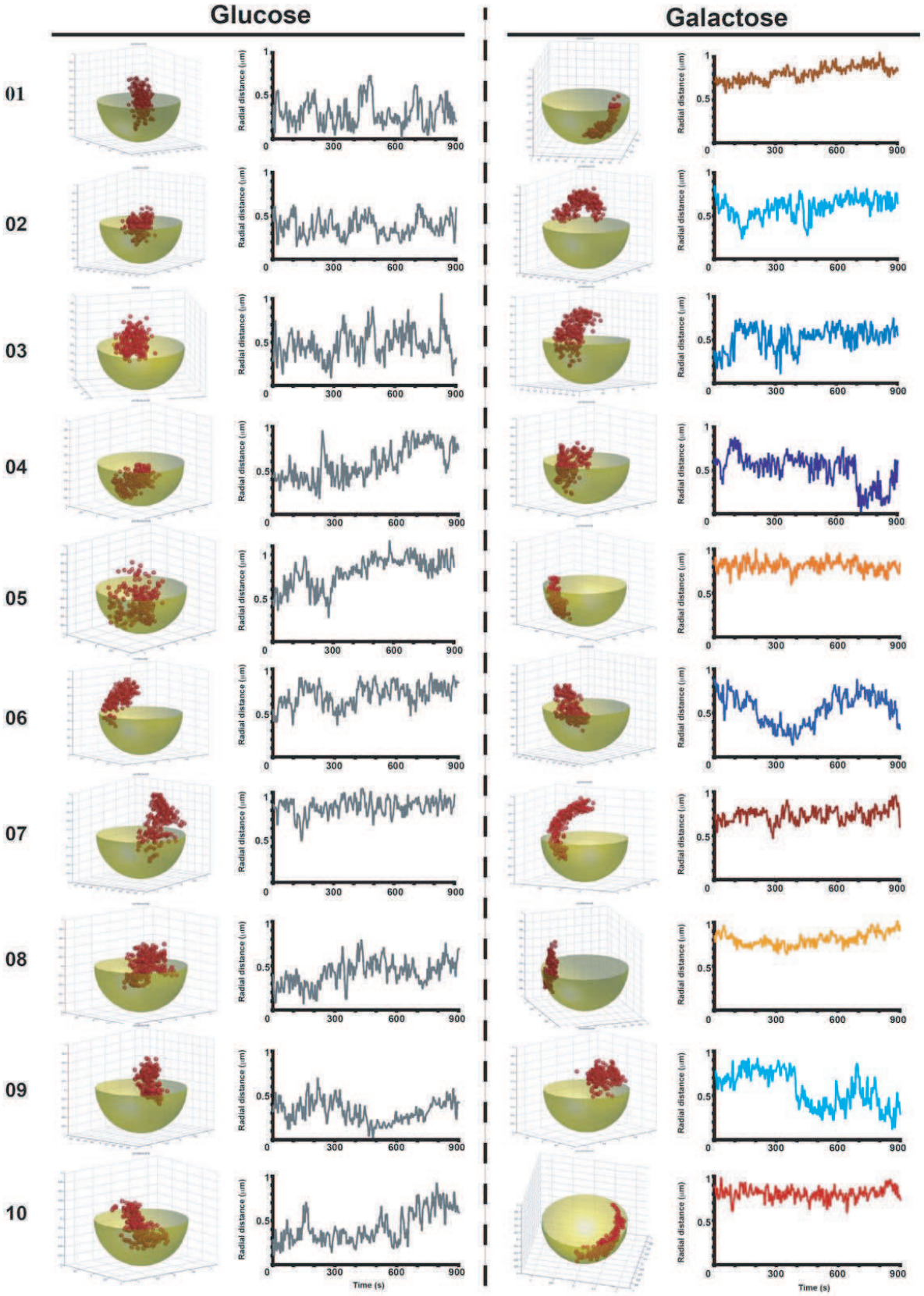


Supplementary Figure 1. New approach for *in vivo* detection and localization of a single chromosomal locus in 3D

(A) A single chromosomal locus was tagged by insertion of one array of 112 repeats of the bacterial TetO operator. This array labelled by the green fluorescent protein coupled to the TetR repressor (TetR-GFP) and the nuclear envelope labelled with the nuclear pore protein Nup49 fused to GFP were visualized simultaneously using a Nipkow-disk confocal microscope. A single confocal plane is shown as an example. Scale bar : 1μm. Following z-stacks images acquisition (Voxel size = 65.8 x 65.8 x 200nm), detection and localization of the centroid of the nucleus (blue mark), GFP-tagged chromosomal locus (red mark) and its projection on the nuclear edge (green mark) are performed automatically in three-dimension. 3D reconstruction allows one to visualize the Z-distortions.

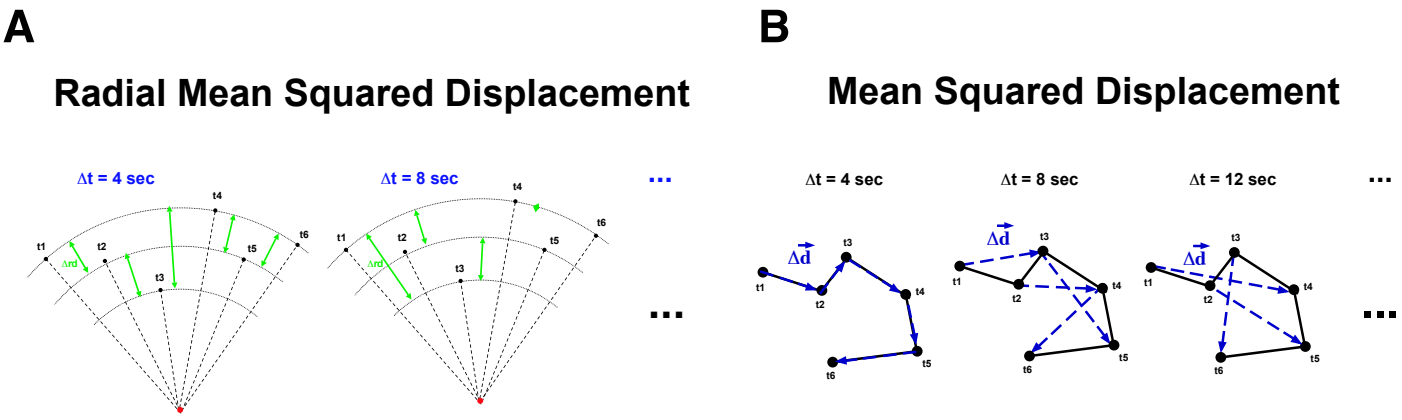
(B) In order to quantify the z-distortions, a single nuclear envelope complex (the Spindle Pole Body (SPB)) was labelled by expressing the Spc29 protein coupled to GFP in a cell expressing the nuclear pore protein Nup49 labelled with yellow fluorescent protein. Observed distances between the nucleus centroid and the SPB in 100 nuclei (strain yGC83) were plotted as a function of the difference between the z-level of these two points. The observed distance between the nucleus centroid and the SPB is not constant as would be expected for a spherical nucleus but increases with the distance of the SPB from the median plane of the nucleus. The observed distribution which is best fitted by a second order curve was corrected by removing the z contribution (see Supplementary Methods).

(C) The Relative 3D positions (R3Dp) of the SPB (blue spheres or dots) in 100 nuclei are plotted both in 3D and in radial projection. The edge of the nucleus is defined as the mean position of the SPB (black circle). The nuclear volume is divided in two equal volumes named Peripheral (yellow) and Interior (green) volumes. The SPB is found in the Peripheral volume in 99% of the analyzed nuclei.



Supplementary Figure 2. Analysis of the *GAL* genes recorded tracks in glucose and galactose grown cells.

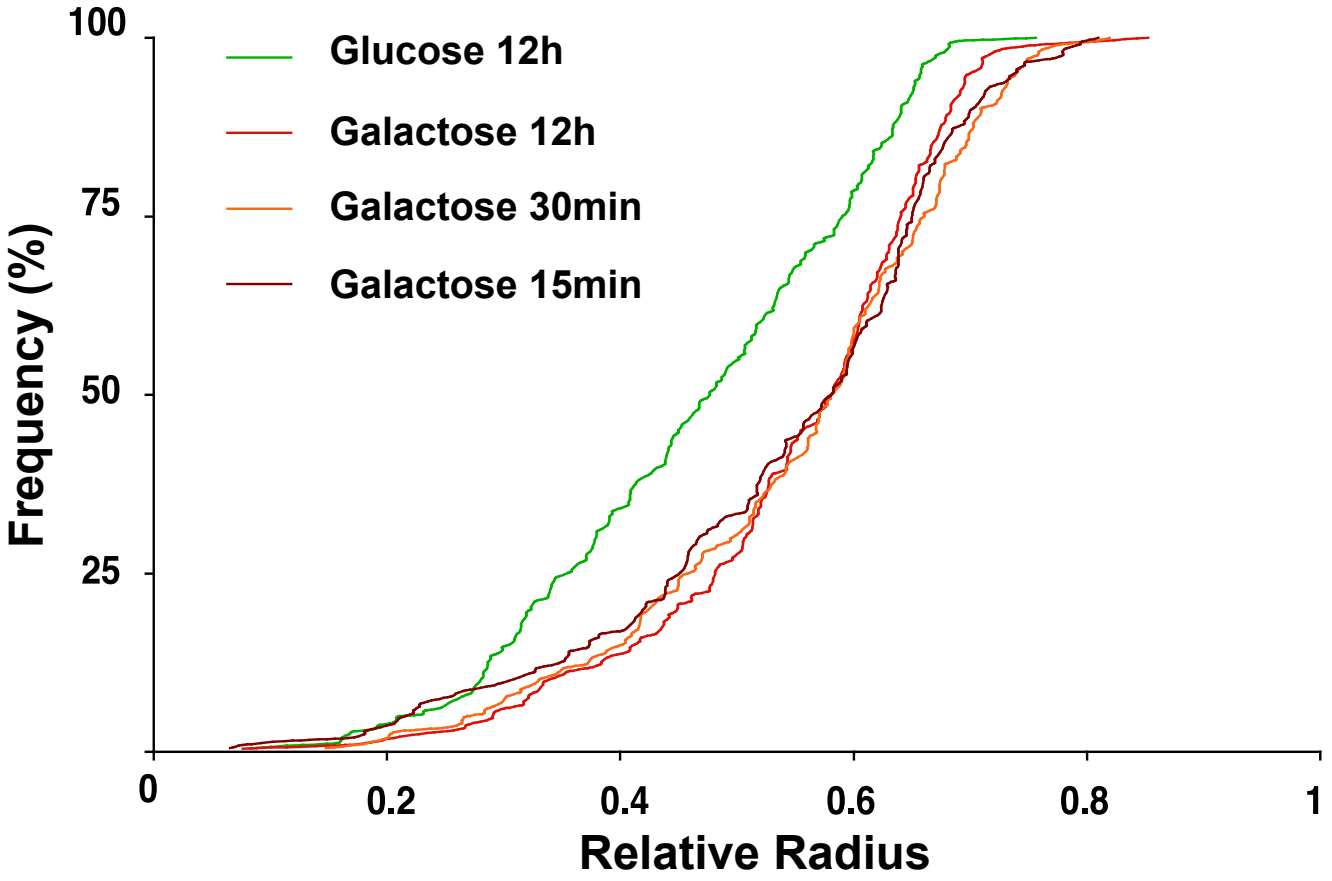
Time-lapse confocal microscopy in 3D was performed to track the *GAL* genes in 10 cells (strain yGC105) grown in glucose and galactose containing media. A 25 images z-stack (z-step : 250 nm) was taken every 4 seconds during 15 minutes (226 times points). The 3D plot of the tracking sequence of *GAL* genes is shown for each cells. The distance (µm) between the centroid of the nucleus and the *GAL* genes is plotted as a function of the time (s) for each tracking sequence.



Supplementary Figure 3. Mean squared displacement computing methods.

(A) For each tracking sequence of a single locus Radial MSD (radMSD) was performed by computing the mean squared change in radial distance (<Δrd2> = <(rd(t)-rd(t+Δt))2>, Δrd in μm) as a function of the time interval (Δt in second). Overlapping distances were used because of small sample size (i.e. 226 time points). The distances were calculated using the X, Y and Zcorrected coordinates given by the detection software. Here are shown as example the determination of the Δrd for the two first Δt on the six first time positions. This was repeated for 226 Δt and with 226 positions at each Δt.

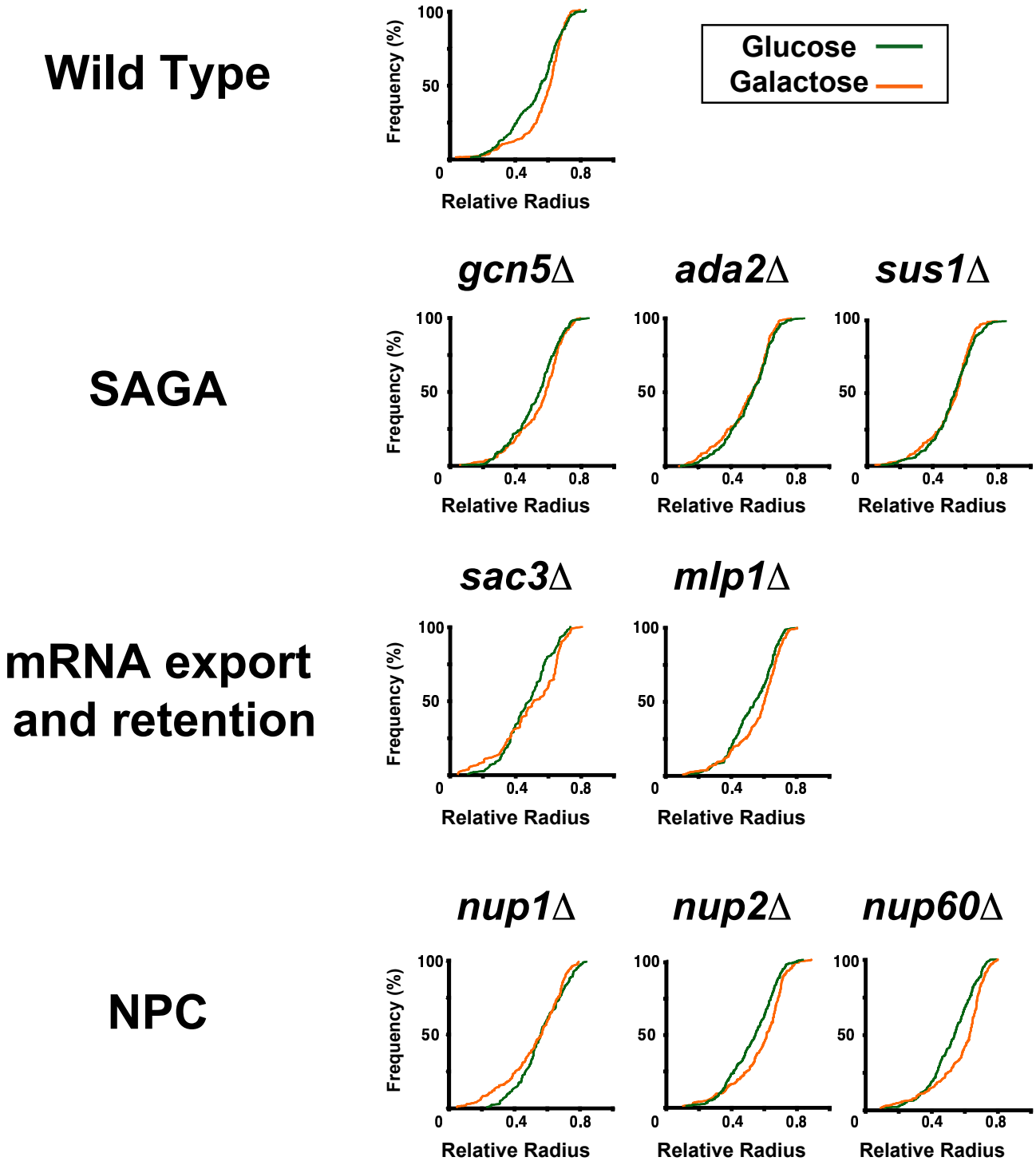
(B) Mean Squared Displacement (MSD) analysis was also performed by computing the mean squared change in distance (<Δd2> = <(X(t)-X(t+Δt))2>+<(Y(t)-Y(t+Δt))2>+<(Zcorrected(t)- Zcorrected (t+Δt))2>, Δd in μm) as a function of the time interval (Δt in second). The calculation is the same as for radMSD but vectors rather than radial distances are taken into account.



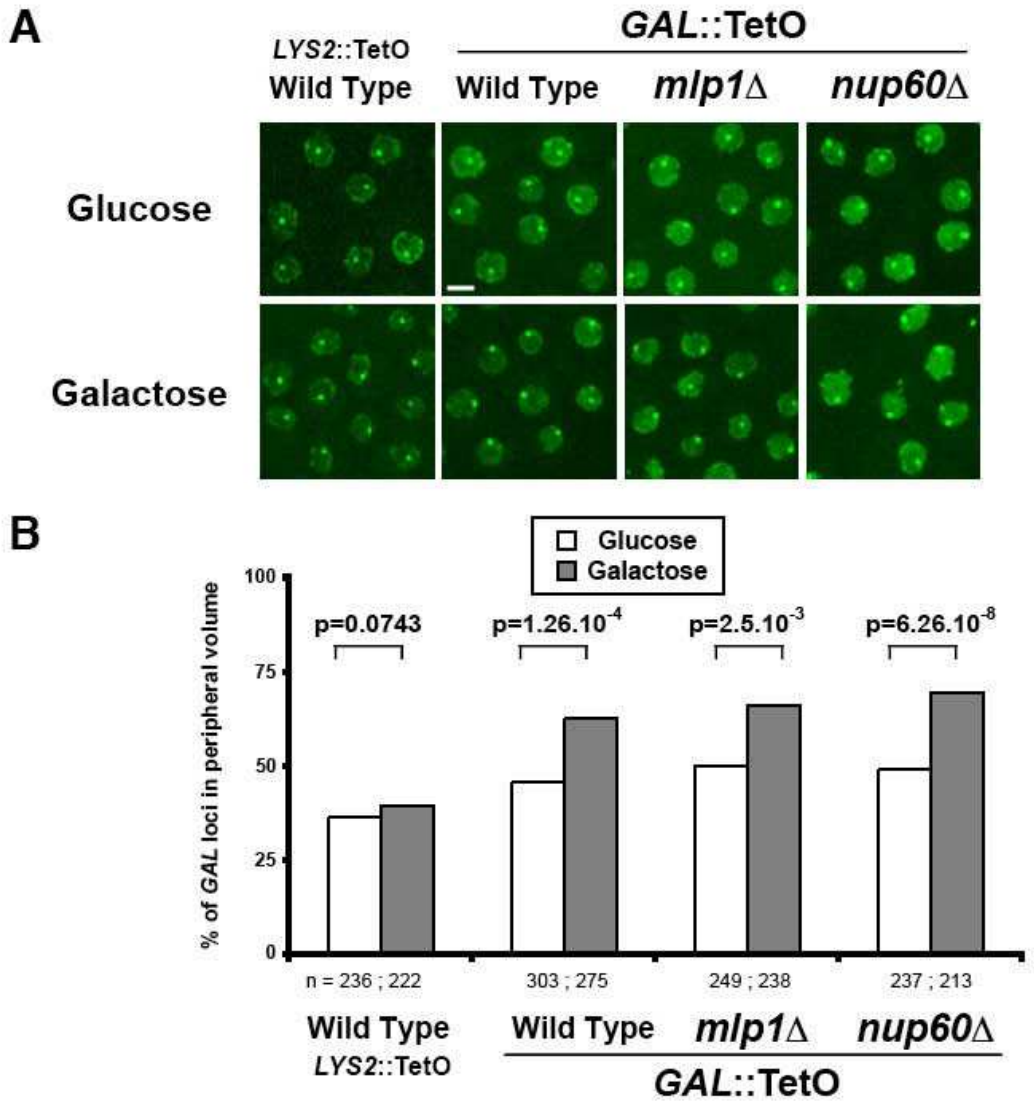
P value of KS test	Glucose 12h (N=246)	Galactose 12h (N=213)	Galactose 30' (N=204)
Galactose 12h (N=213)	2.9145E-8		
Galactose 30' (N=204)	2.3842E-8	0.2302	
Galactose 15' (N=206)	1.99E-6	0.3488	0.6763

Supplementary Figure 4. Distribution of the *GAL* genes in different growth conditions.

The position of *GAL* genes in time course experiments was analyzed. Starting from media containing raffinose as unique carbon source, cells were diluted either in glucose or galactose containing media, and the position of the *GAL* genes was recorded at different times after changing the media. CDF are plotted for each time and media. Pair-wise comparisons of the distributions of Relative 3D Position (R3Dp) (n=100) computed were performed using Kolmogorov-Smirnov tests and presented in the table. The null hypothesis for this test is that the two populations have the same continuous distributions. For each growth conditions the number of cells, the average and standard deviation of the R3Dp distribution are as follow: Glucose 12h (246; 0.465; 0.14), Galactose 15 minutes (206; 0.54; 0.155) Galactose 30 minutes (204; 0.55, 0.142), Galactose 12h (213; 0.549; 0.129).



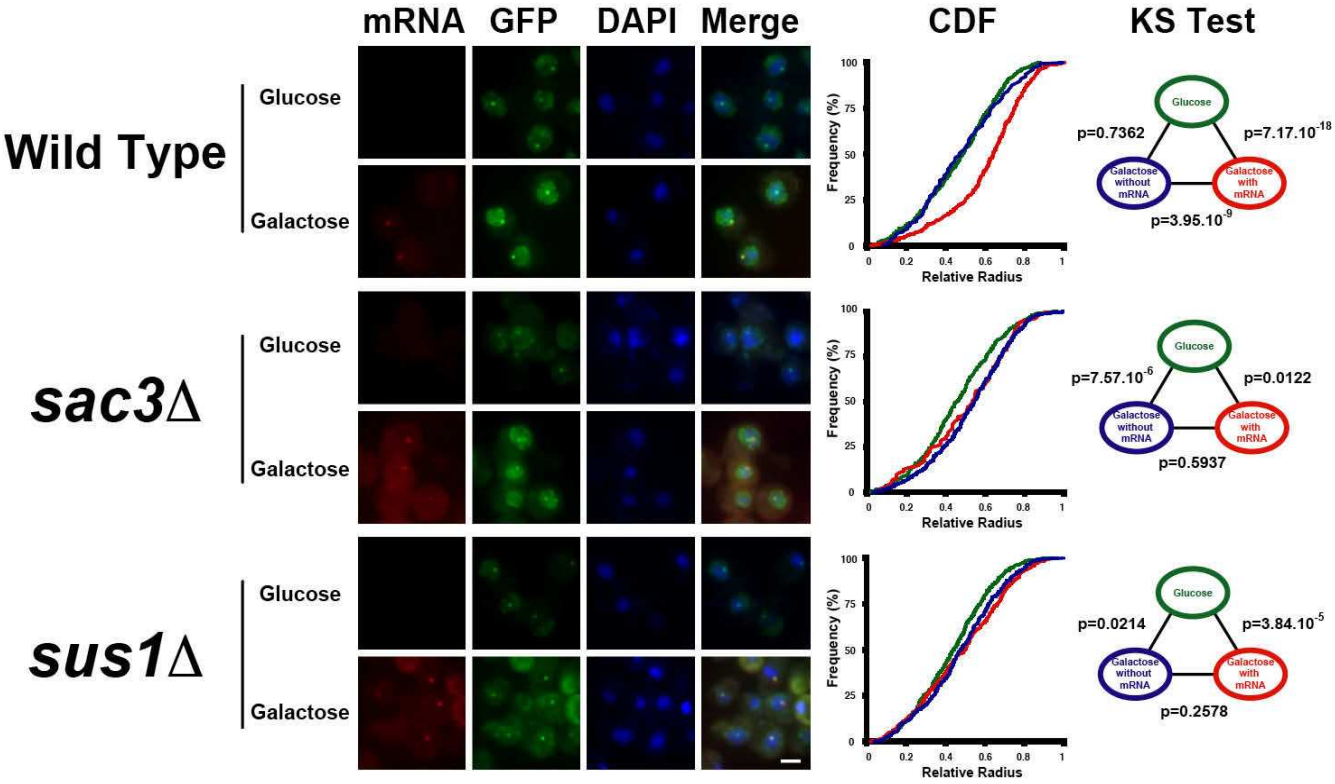
Supplementary Figure 5. Cumilative Distribution Function.
CDF (see Methods) of the Relative 3D Position (R3Dp) distribution in glucose (green curve) and galactose (orange curve) populations are plotted for wild type and each indicated mutant of SAGA complex, mRNA export machinery, mRNA retention and nuclear pore complex.



Supplementary Figure 6. *LYS2* and *GAL* genes localization.

(A) *In vivo* 3D localizations of labelled *LYS2* or *GAL* genes were performed for wild type, *MLP1* or *NUP60* deleted cells (respectively strain yGC185, yGC105, yGC211 and yGC194). For each mutant, example of 3D raw data are shown Z-projected both in glucose and galactose (scale bar : 2μm),

(B) Bar graph indicates proportion of loci found in peripheral volume (as defined in Supplementary Fig. 1). Pair-wise comparisons of the distributions of R3Dp computed for each strain and growth conditions were performed using Kolmogorov-Smirnov tests. The p-value is indicated for each test and n is the number of cells analysed.



Supplementary Figure 7. Localization and expression of *GAL* genes upon repression or activation in *sac3*Δ and *sus1*Δ mutants.

RNA fluorescent in situ hybridization on wild type, *sac3*Δ and *sus1*Δ strains (yGC157, yGC159 and yGC160) containing a galactose-dependent lacZ gene and the TetO array inserted at the GAL locus. lacZ mRNA (red), TetR-GFP and GFP-Nup49 (green), and DNA (blue) are shown. Scale bar : 2 μm. Cumulative distribution function of GAL locus position in glucose, in galactose when mRNA is not detectable and in galactose when mRNA is visible are plotted. Pair-wise comparisons of these distributions were computed using Kolmogorov-Smirnov tests and p-values are indicated.