E08-01-0035 Spector

Supplementary Figure 1. Abnormal localization of full length EYFP-Sp100 in a stable cell line, CpYs55. (A) Whole cell extracts from both parental U2OS cells (wt), double stable cells (CpYs68), and double stable cells (CpYs55) were analyzed by immunoblot. Rabbit anti-Sp100 antibody also shows several Sp100 isoforms. The arrowheads indicate the bands representing EYFP-Sp100 and EYFP-Sp100' fusion proteins. (B) CpYs55 cells were fixed and analyzed using a DeltaVision RT microscope. The projected 3D Z-stacks show the abnormal localization of EYFP-Sp100' fusion proteins in two G1 daughter nuclei. Bar, 10 μm.

Supplementary Figure 2. Summary of PML NB movement types from PML-ECFP tracks and EYFP-Sp100' tracks. The box plot summarizes the distribution of the PML NB population in a cell that exhibiting particular movement types during prophase. The y-axis indicates the percentage of the PML NB population and x-axis indicates the data from two different sets of cells (PML set: N =12 and Sp100 set: N = 20). The PML NBs in set 1 were tracked by the ECFP signal, while set 2 is by the EYFP. The red line indicates the median of the data set. The

box indicates 50% of the data points. The notch of the box indicates the significance of the difference of the mean values in the two data sets. If the notch area overlaps between the two sets, the mean values of the two sets are not significantly different. If there is no overlap, the difference is significant with 95% of confidence. (A) Directed movement. (B) Diffusive movement. (C) Constrained movement. Although there is a low level of cell to cell variation between datasets, the global distribution of each movement type is similar.

Supplementary Figure 3. PML NB dynamics upon treatment with hyper-osmolar medium resulting in induced hypercondensed chromatin (HCC). A double stable cell line, CpYs68, was transiently transfected with an H2A-mCherry construct. Cells were treated with normal live cell medium (A and C) or 570 mOsm live cell medium (B and D) to induce HCC before 4D live cell imaging using the DeltaVision RT microscope (N = 10). A 5 μm Z-stack of 0.5 μm steps was taken in the CFP and mCherry channels every 10 sec for 16 min. The 3D projected still images were used for particle tracking. (A) and (B) show the projected images from T = 0. (C) and (D) show the trajectories after tracking. Hypercondensed chromosomes can be visualized by the H2A-mCherry fluorescent signal (red) in

(B). PML NBs (blue) do not exhibit dynamic movement within the increased interchromatin space, comparing the trajectories in (C) and (D). Bar, 10 μm.

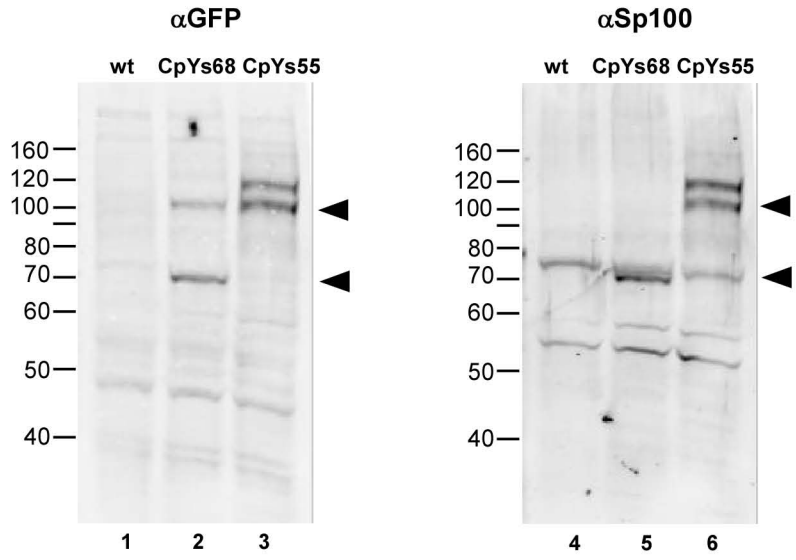
Supplementary Figure 4. Visualization of the correlations between PML NB mobility and chromatin colocalization. Three types of correlations can be typically observed in a prophase cell. The example tracks representing each type of correlation shown in Figure 7 are visualized here. Double stable cell line, CpYs68, stably expressing PML-ECFP and EYFP-Sp100' (green) was transiently transfected with H2A-mCherry (Red). A 7μm Z-stack of 0.5 μm steps were collected every 8 sec for 26 min. Attached: PML NB was attached to chromatin and moved along with chromatin (Blue arrowhead). Detached: a PML NB was released from chromatin and showed increase mobility (Yellow arrowhead). Re-attached: a PML NB was released from chromatin but then reattached to chromatin at a later time point (White arrowhead).

Supplementary Figure 5. Visualization of PML NB formation in G1 cells with high temporal resolution. (A) Double stable cell line, CpYs68, stably expressing PML-ECFP (red) and EYFP-Sp100' (green). A 23 μm Z-stack of 1.5 μm steps

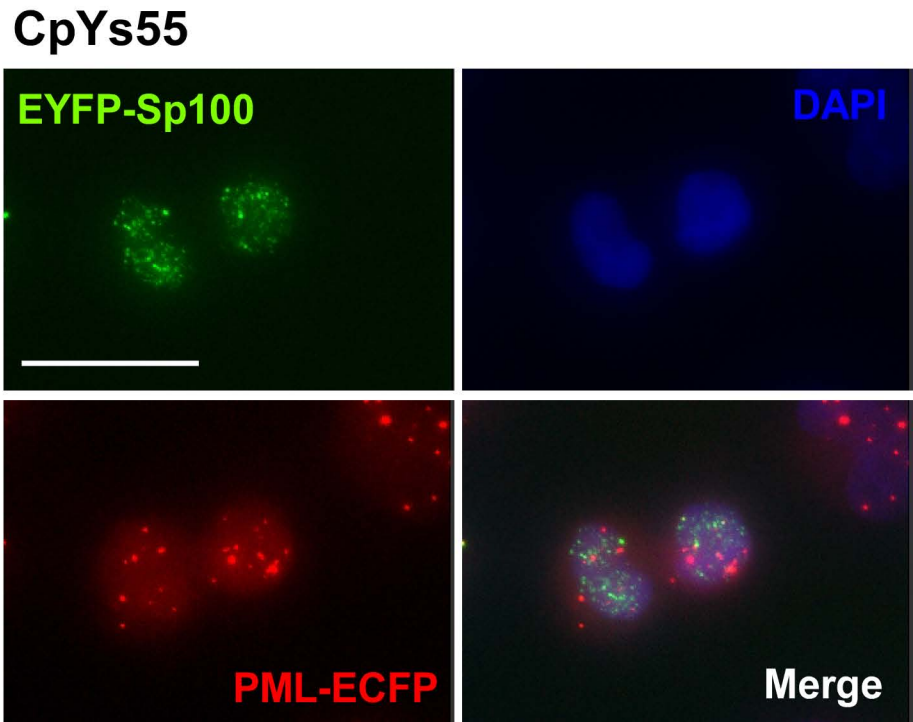
was taken every 1 min for 200 min in 2 channels. Selected 3D-projected images show that Sp100' protein (green) enters daughter nuclei prior to PML protein (red). However, the recruitment of Sp100' to PML NBs (yellow) occurs only after PML NBs reform (white arrowhead). (B) Single stable cell line, Cp89, expressing PML-ECFP (red) was transiently transfected with EYFP-Daxx (green). A 21.5 μm Z-stack images of 1.5 μm steps was taken every 3 min for 100 min in 2 channels. Selected 3D-projected images show that Daxx protein enters daughter nuclei first before its recruitment into newly formed PML NBs (white arrowhead). Bar, 10 μm.

Chen et al. Supp. Fig 1

A

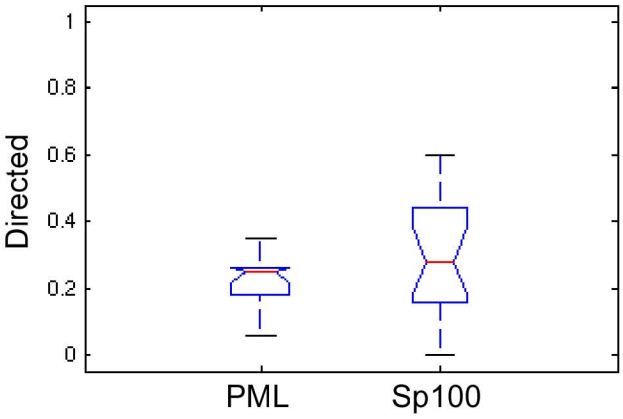


B

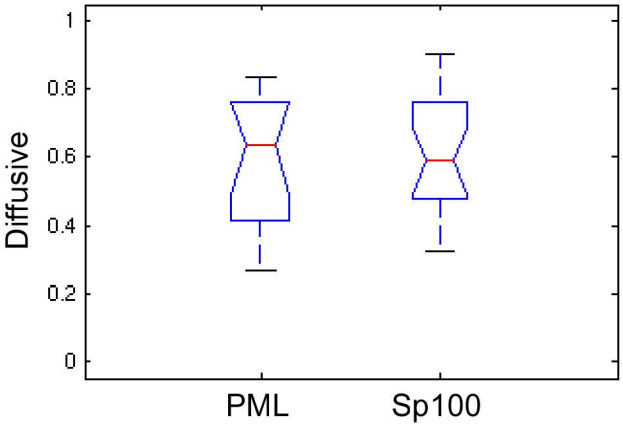


Chen et al. Supp. Fig 2

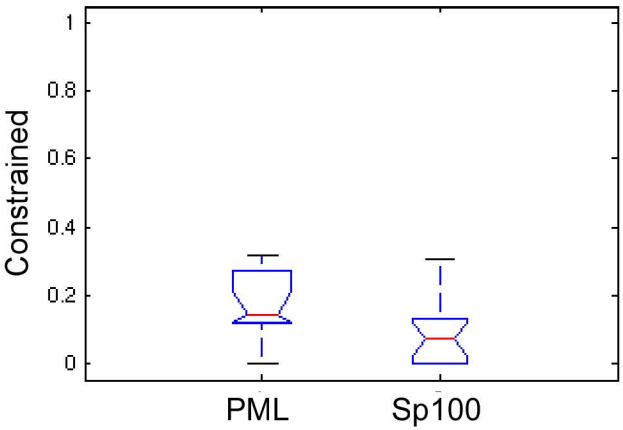
A



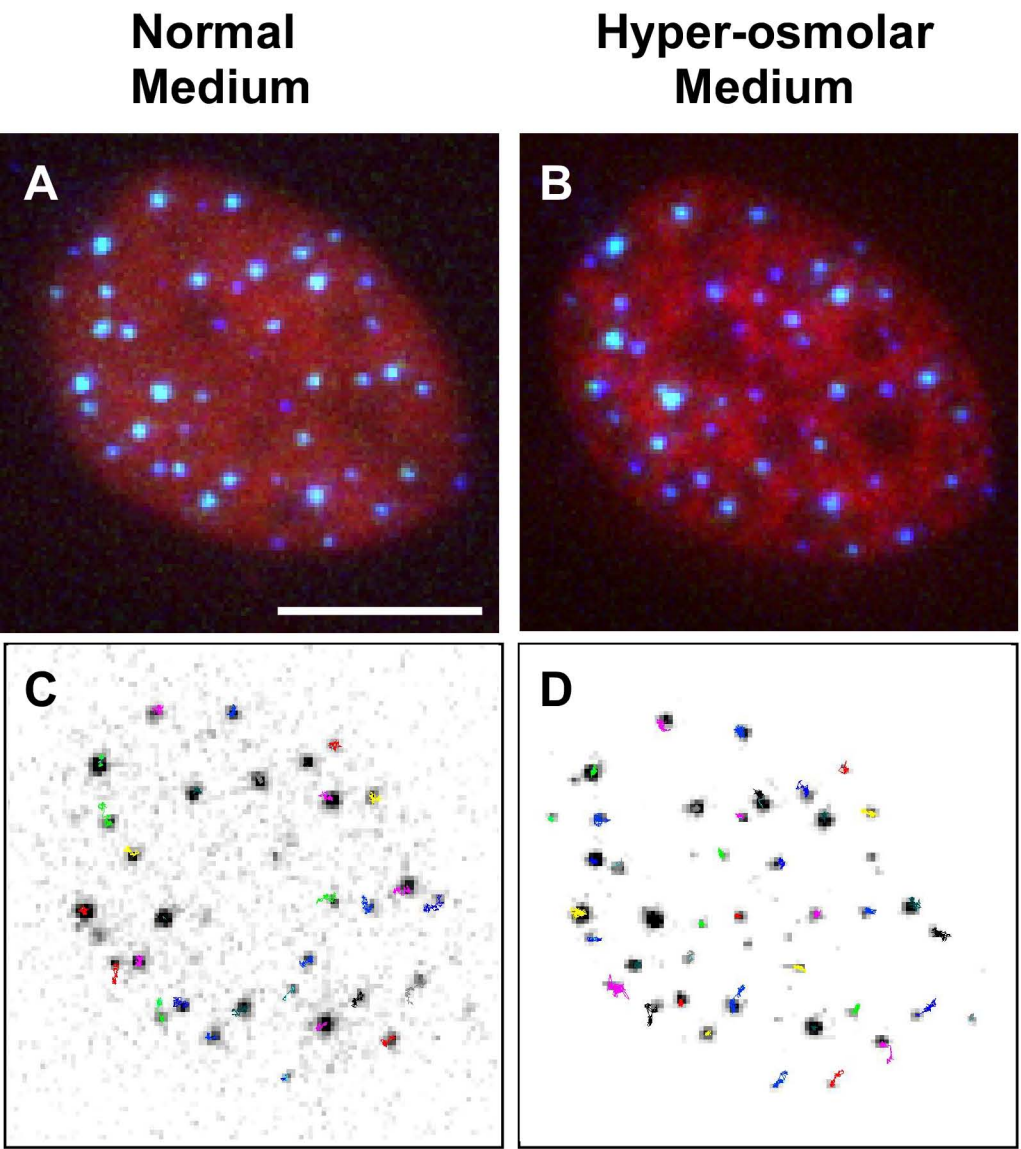
B



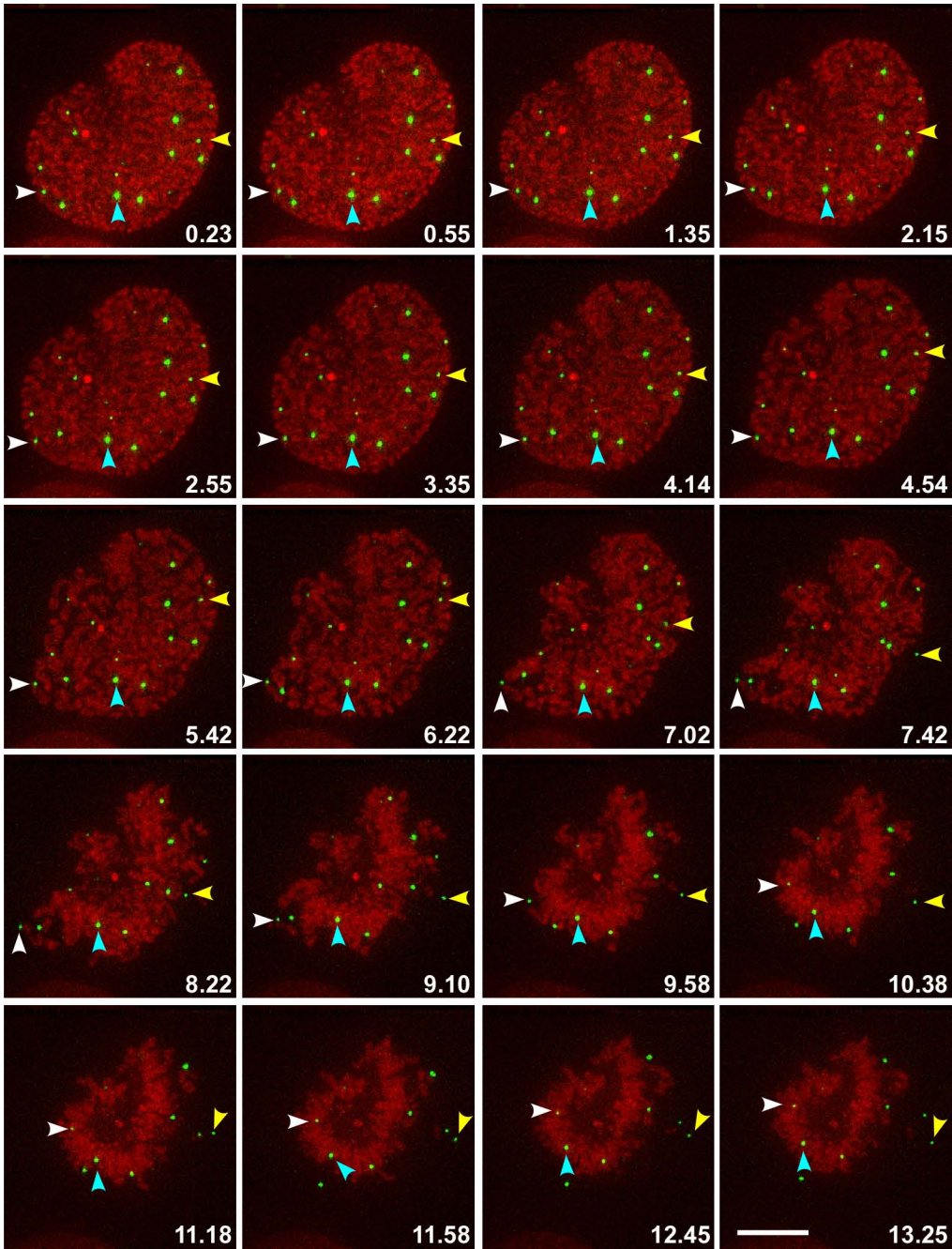
C



Chen et al. Supp. Fig 3



Chen et al. Supp. Fig 4



Chen et al. Supp. Fig 5