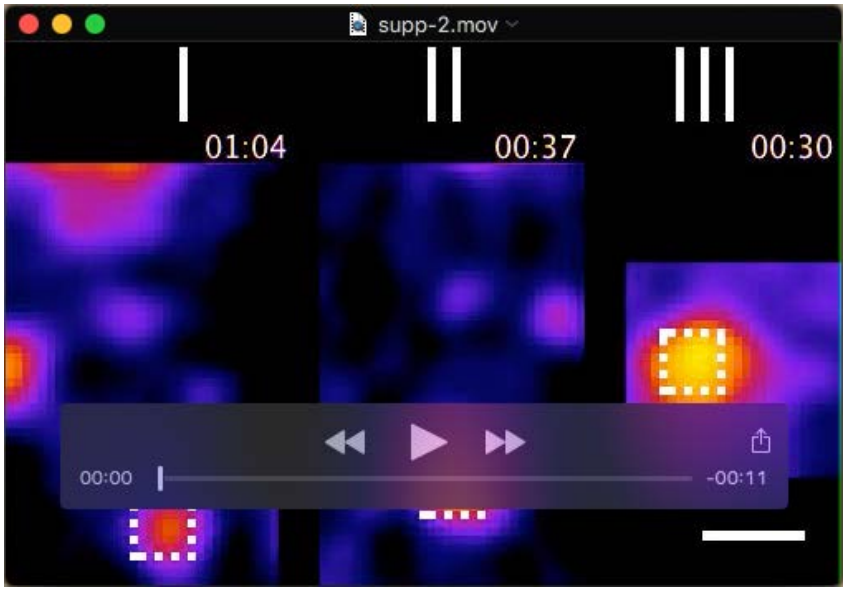
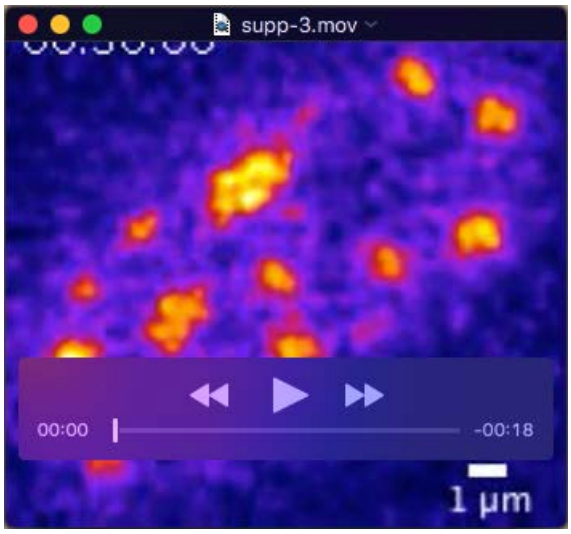
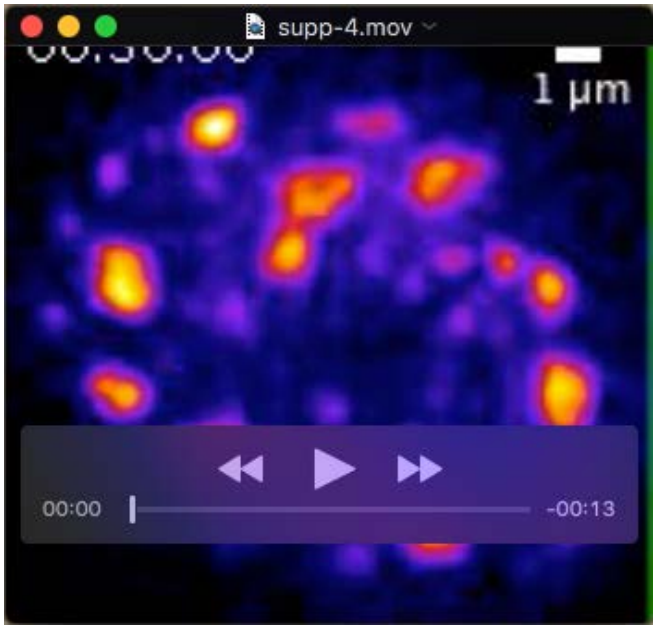
Supplementary Movies



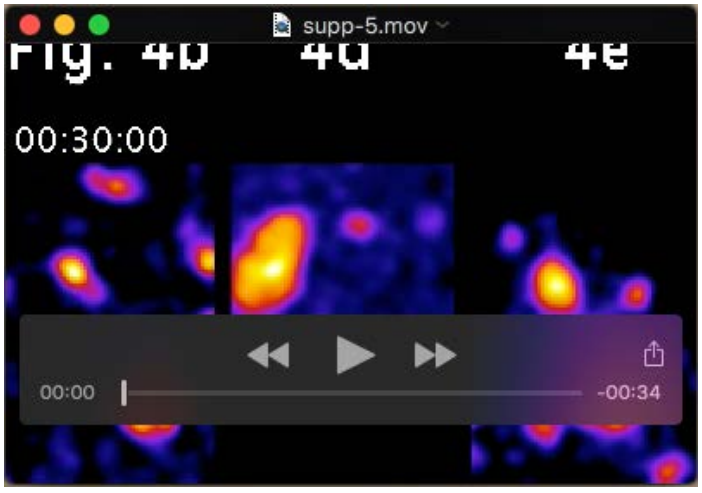
Movie 1. Speckle movements from trajectory types I-III in live CHO cell after DRB treatment.
Corresponds to Fig. 2C. Movie represents maximum intensity 2D projection of 3D image stack for each time point. Time (hr:min) after DRB addition and scale bar(1μm) are stamped on the movie.



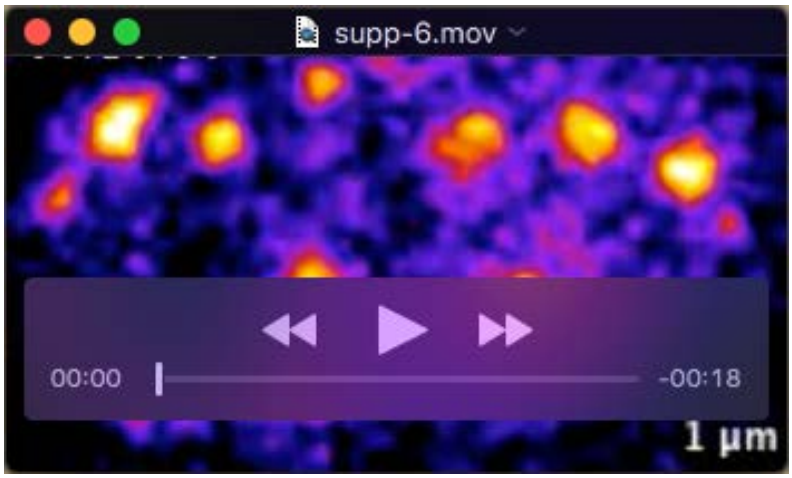
Movie 2. Long-range movement and fusion of nuclear speckles in live CHO cell after DRB treatment.
Corresponds to Fig. 3A. Movie represents maximum intensity 2D projection of 3D image stack for each time point. Time (hr:min:sec) after DRB addition and scale bar are stamped on the movie.



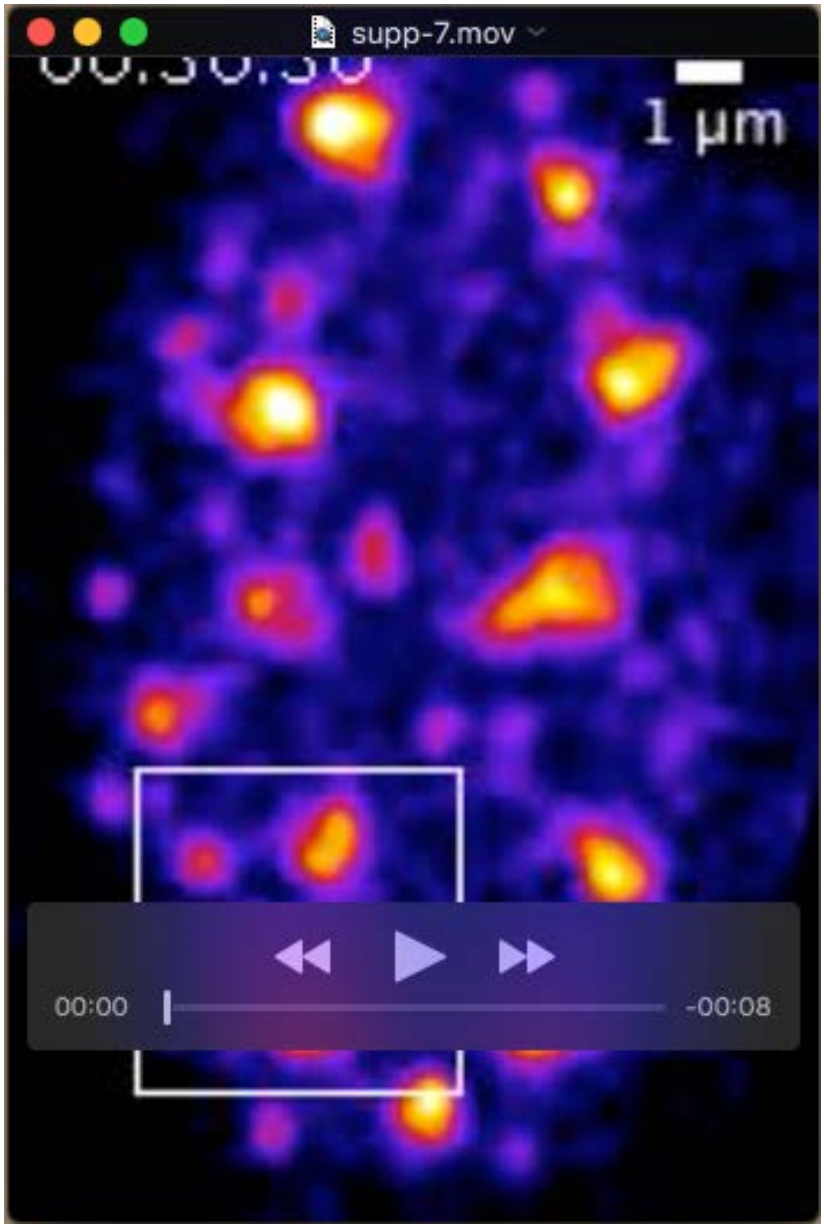
Movie 3. Long-range movement and fusion of nuclear speckles in live CHO cell after DRB treatment.
Corresponds to Fig. 3B. Movie represents maximum intensity 2D projection of 3D image stack for each time point. Time (hr:min:sec) after DRB addition and scale bar are stamped on the movie.



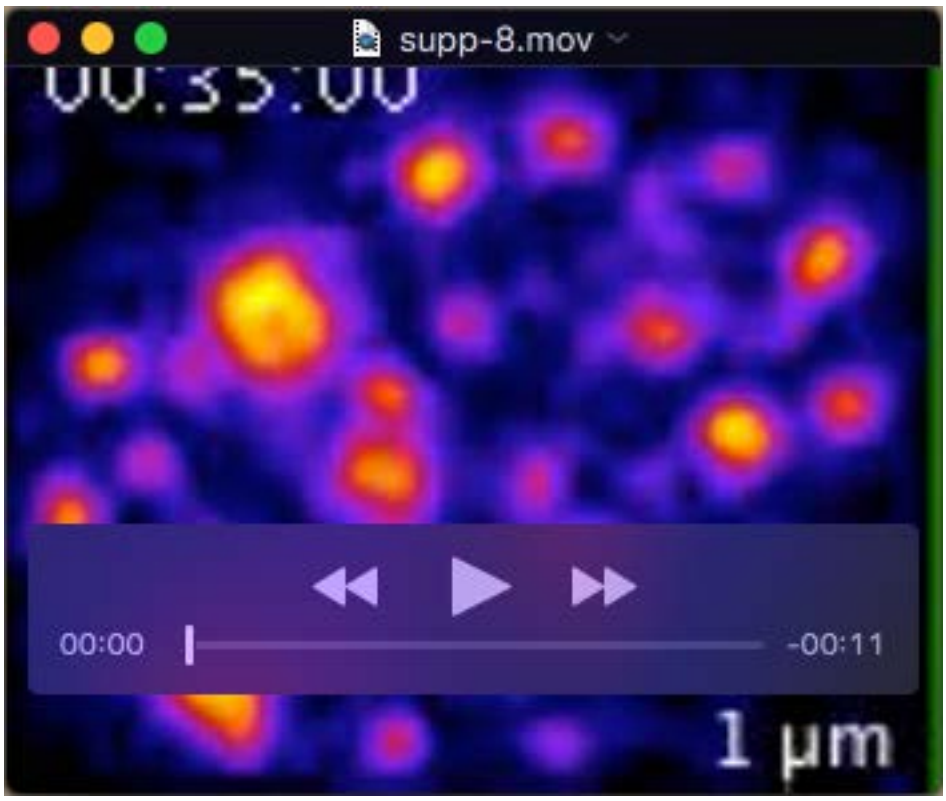
Movie 4. Repeated long-range directional speckle movements after DRB treatment. Corresponds to Fig. 4B, D and E. Each example was found in different cell nucleus. Movie represents maximum intensity 2D projection of 3D image stack for each time point. Time (hr:min:sec) after DRB addition and scale bar(1µm) are stamped on the movie.



Movie 5. Long-range movement and fusion of nuclear speckles after latrunculin A and DRB treatment.
Corresponds to Fig. 5. Movie represents maximum intensity 2D projection of 3D image stack for each time point. Time(hr:min:sec) after DRB addition and scale bar are stamped on the movies.



Movie 6. Repeated speckle movements along similar path and fusions with same target speckle after DRB treatment.
Corresponds to Fig. 6B. The local region of interest is marked with box in movie. Correlative STED and SIM images of SON and DNA (DAPI) are shown in Fig. 6B following fixation and staining. Movie represents maximum intensity 2D projection of 3D image stack for each time point. Time(hr:min:sec) after DRB addition and scale bar are stamped on the movies.



Movie 7. Repeated speckle motions along similar path and fusions with same target speckle after latrunculin A and DRB treatment.
Corresponds to Fig. 6C. The local region of interest is in the center of the nucleus. Correlative STED and SIM images of SON and DNA (DAPI) are shown in Fig. 6C following fixation and staining. Movie represents maximum intensity 2D projection of 3D image stack for each time point. Time(hr:min:sec) after DRB addition and scale bar are stamped on the movies.

Movie 8. Speckles fuse after chromatin barrier is depleted.

Corresponds to nucleus I in Fig. 7. SiR-Hoechst staining (red) with GFP-SON (green) after DRB treatment. Movie is a single optical section for each time point. Time(hr:min:sec) after DRB addition and scale bar are stamped on the movies.

Movie 9. Speckles fuse after chromatin barrier is depleted.

Corresponds to nucleus II in Fig. 7. SiR-Hoechst staining (red) with GFP-SON (green) after DRB treatment. Movie is a single optical section for each time point. Time(hr:min:sec) after DRB addition and scale bar are stamped on the movies.

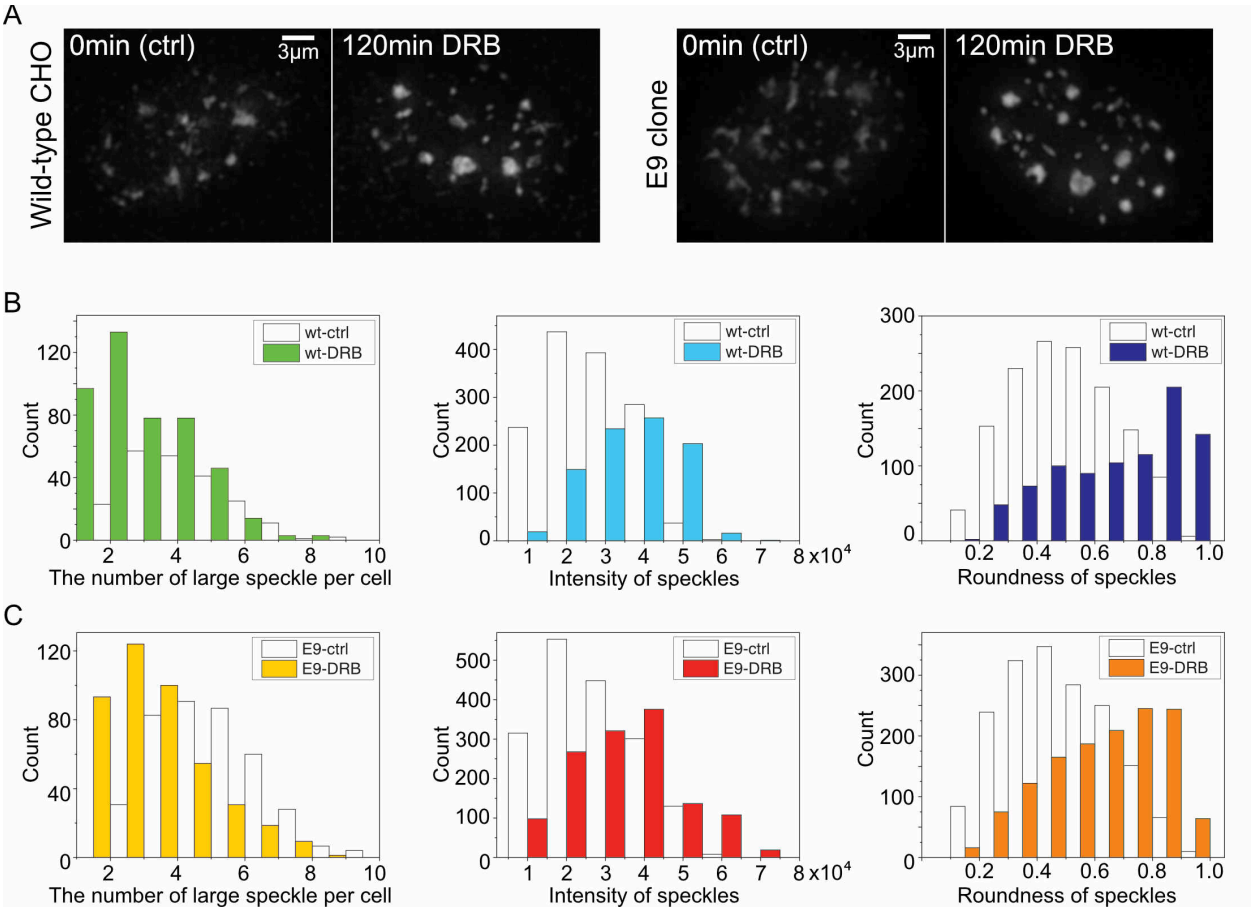


Fig. S1. Speckle morphology before and after DRB treatment in wild-type (wt) CHO cells is comparable to that observed in EGFP-SON expressing clone (E9) cells. (A) Changes in speckle morphology, visualized by immunostaining against SON, before and after DRB addition in wt CHO (left) and E9 clone (right) cells. Scale bar: 3μm. (B) Measurement of speckle number (>1 um in diameter) (left), intensity (middle), and roundness (right) before and after 2 hrs DRB treatment. Large speckles reduce in number (green), become brighter (~1.5-fold, light blue), and rounder (1.5-fold increase in roundness, navy) after DRB addition. (C) Same but for E9 clone. Large speckles reduce in number (yellow), become brighter (~1.5 fold, red), and rounder (1.5-fold increase in roundness, orange) after DRB addition.

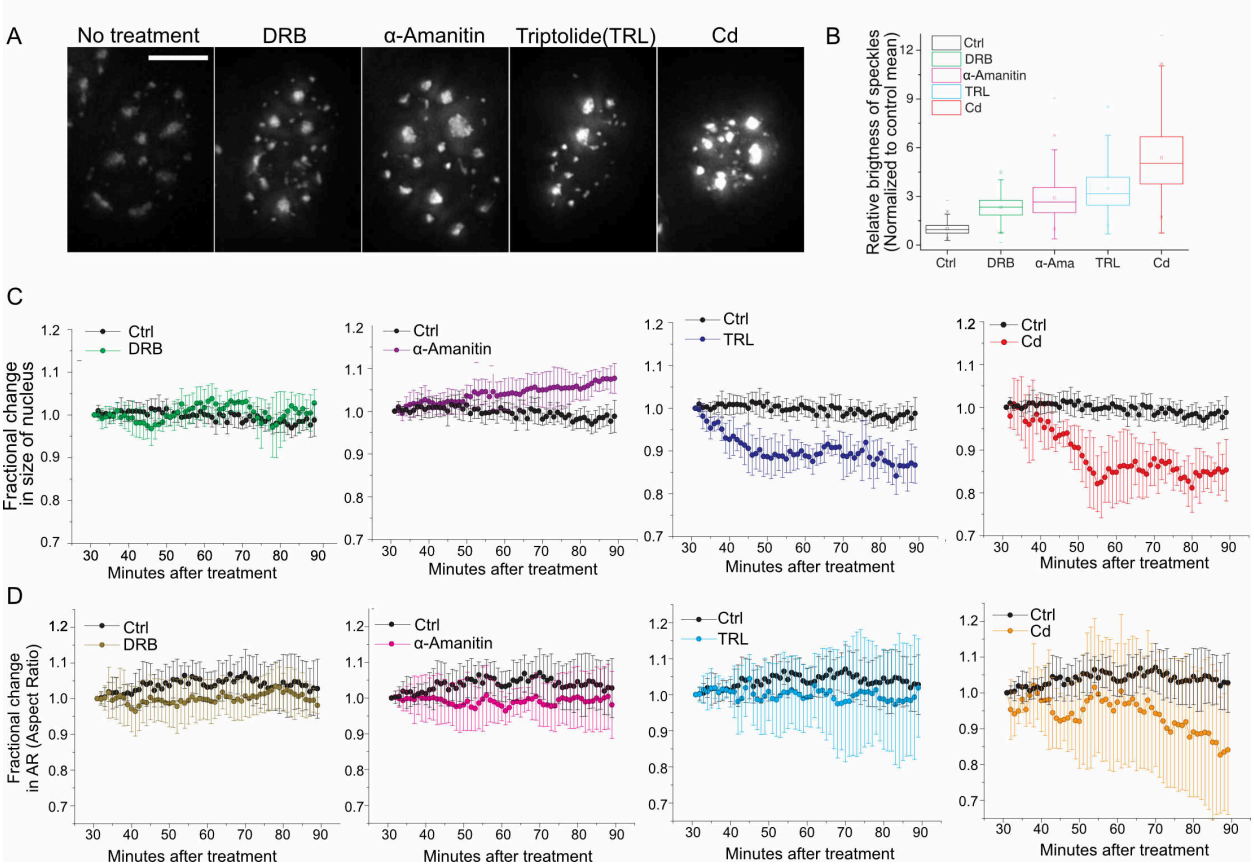


Fig. S2. Morphological changes of nuclear speckles and nuclei after treatment with different transcription inhibitors or cadmium (Cd). (A) Fixed cell images after 2 hr treatment with the indicated chemical. Scale bar: 5um. (B) Box plots summarizing statistical distribution of relative brightness of individual speckles after treatment with indicated chemical normalized to the mean of speckles in control cells. (C) Fractional change in the size of nucleus during 1 hr live cell imaging of control (Ctrl, black) or treated cells (Chemical indicated, color). Triptolide (TRL). Error bars represent standard deviations between cell nuclei. (D) Fractional change in aspect ratio of the nuclear major axis to minor axis during 1 hr live cell imaging. Error bars represent standard deviations between cell nuclei.

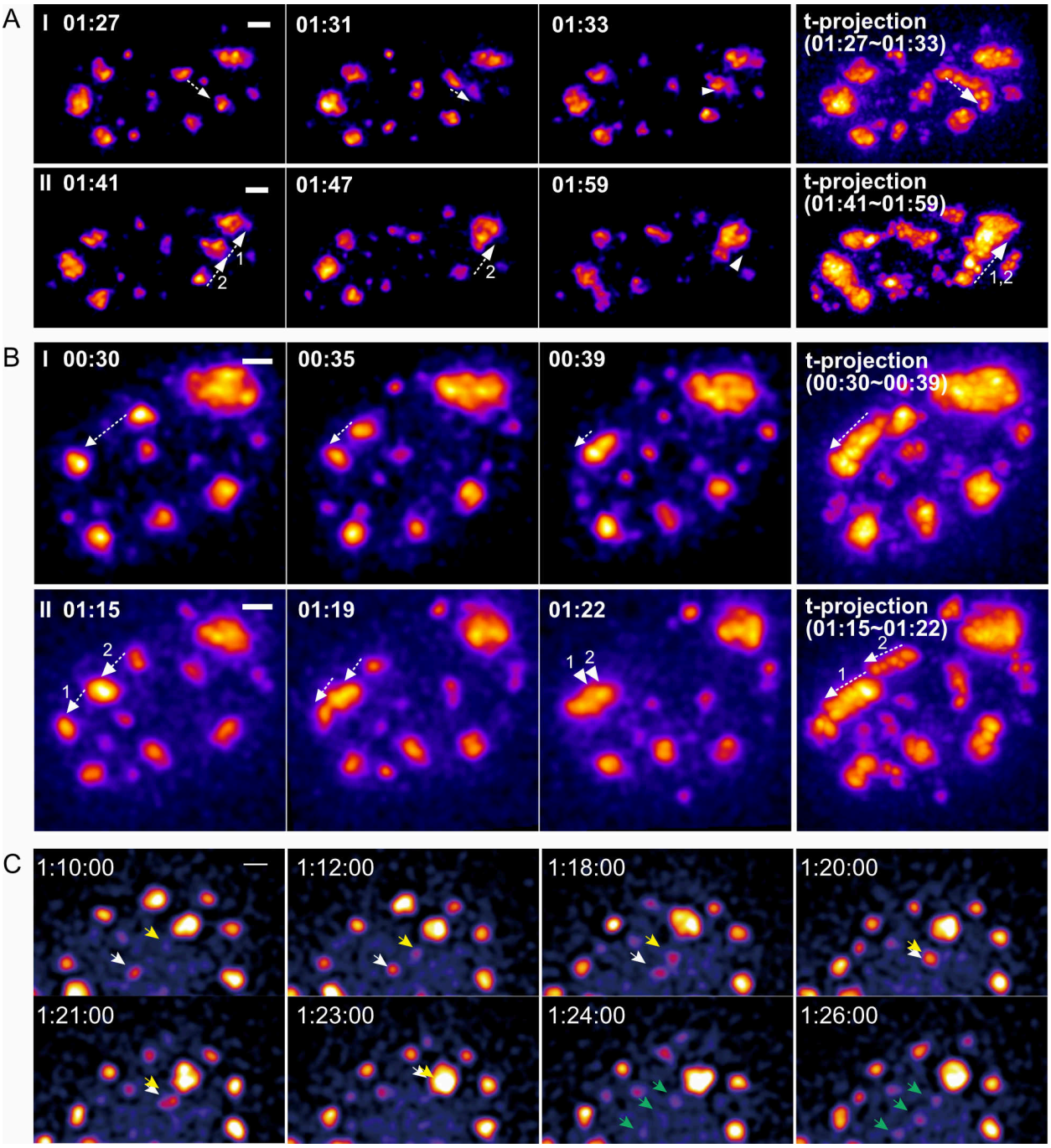


Fig. S3. Linear path of long-range (>1µm) directional speckle movements and nucleation of speckles along a path followed by a nuclear speckle. Scale bars: 1 µm. (A-B) GFP-SON images represent maximum intensity 2D projections (left) of 3D image stacks or maximum intensity projections over time (t-projection) of these 2D projections (right). Time (hr:min) is after DRB treatment. (A) (I,II) Examples of long-range directional speckle movements within one nucleus. (Left) White arrows: direction of each speckle movement. (B) (I,II) Additional examples from second nucleus. (C) GFP-SON images represent maximum

intensity 2D projections of 3D image stacks. Time (hr:min:sec) is after DRB treatment. As a first speckle (white arrowhead) moves towards a large, target speckle, a second, small speckle (yellow arrowhead) appears to nucleate at a position on linear path connecting the first and target speckles. The first speckle moves along this linear path, fuses with the second speckle, and then the fused speckle continues along this linear path and fuses with the large target speckle. One minute later, three new small, collinear speckles (green arrowheads) form along this same path.

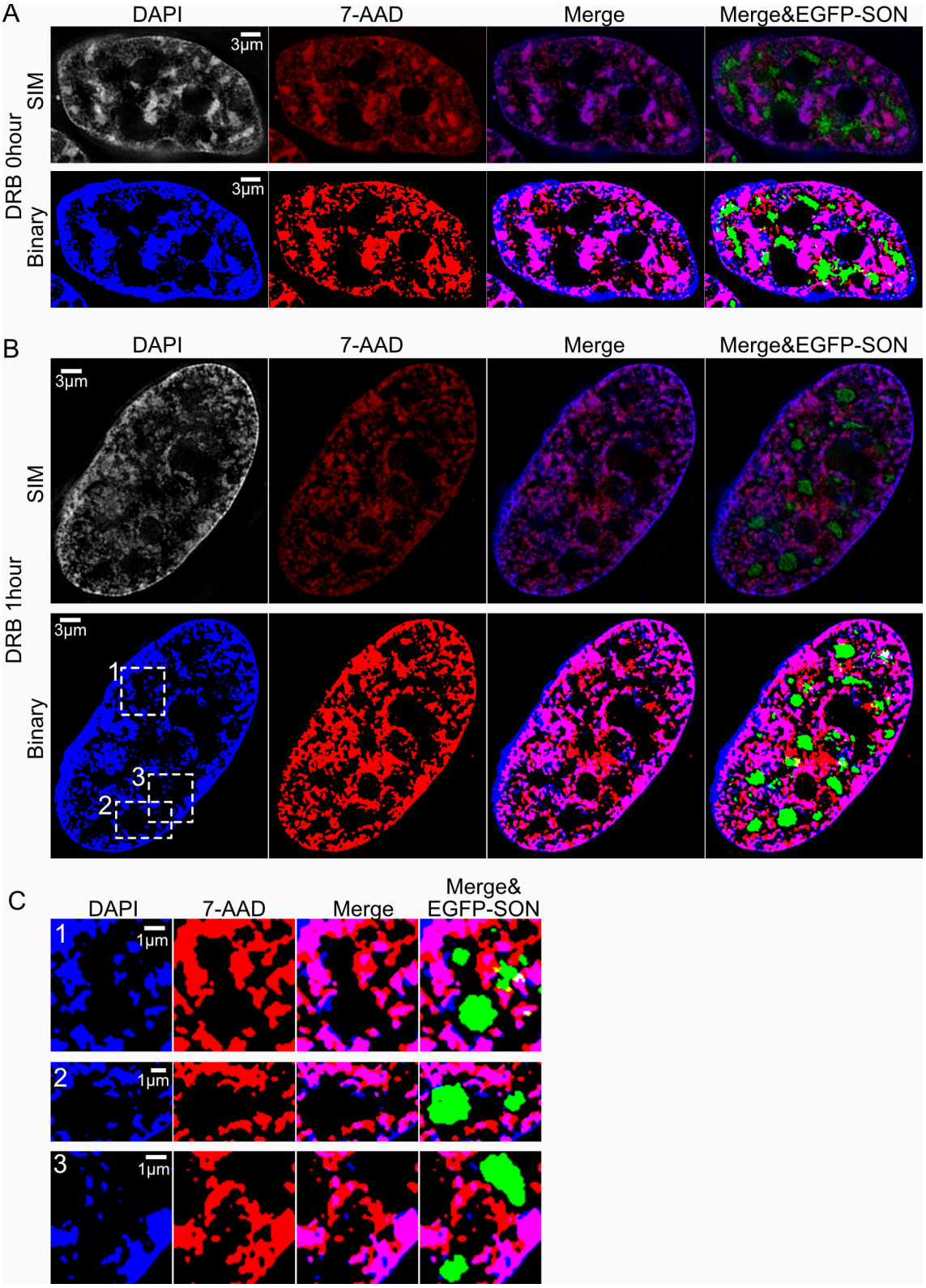
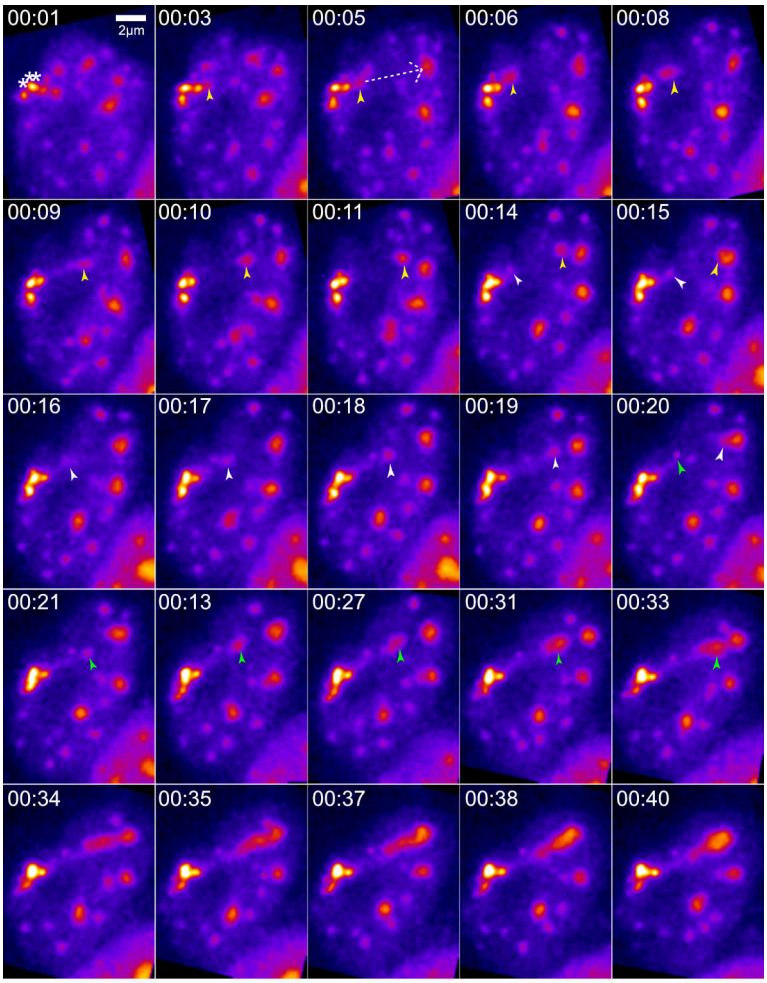


Fig. S4. Structure illumination microscopy (SIM) images of chromatin structures stained by DAPI and 7-aminoactinomycin D (7-AAD). (A) Chromatin structure in

a control cell. (Top) SIM images of DAPI (gray), 7-AAD (red), merged image and EGFP-SON-merged image. (Bottom) Binary images of top SIM images. (B) Same as (A) but for a 1hour-DRB treated cell. The regions of interest (ROI) 1~3 are marked by dashed lines, and shown enlarged in (C). (C) Enlarged binary images marked in (B). ROI 1 and 2 show colocalization between DAPI-poor regions and 7-AAD-poor regions surrounding two speckles. ROI 3 shows 7-AAD-stained chromatin in DAPI-poor region between two speckles separated by a large distance.



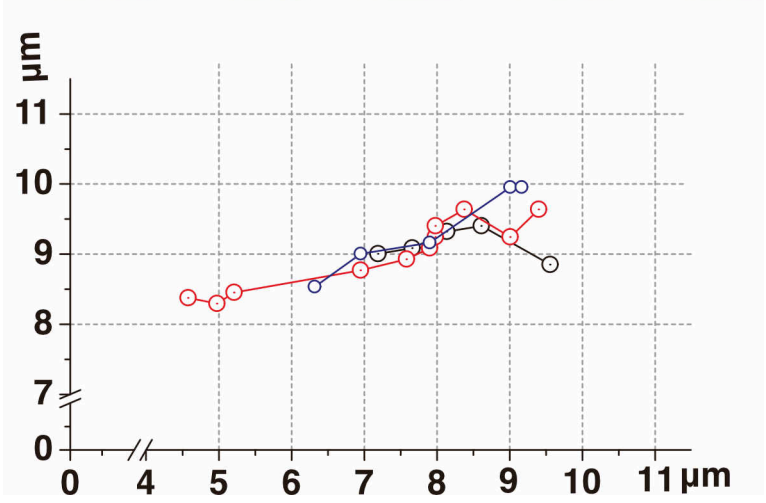


Fig. S5. Repeated cycles of speckle formation, translocation and fusion. Top: Pseudo-colored GFP images showing GFP-SON (speckles) and GFP-lac repressor (Hsp70 plasmid transgene array) (Khanna et al. 2014) represent maximum intensity 2D projections of 3D image stacks. Time (hr:min) is after heat-shock induction of Hsp70 transgene array. Scale bar: 2

μm. Nuclear speckles nucleate adjacent to Hsp70 transgene array (brightest spot, marked by asterisks 1min), move along linear path (white dashed arrow, 5 min) to fuse with large target nuclear speckle. Three cycles of speckle nucleation, long-range movement, and fusion to same target speckle are seen. Arrowheads (yellow, white, and green- in order of speckle formation) mark moving speckles. Bottom: Trajectories (x,y) of each long range directional speckle movement.

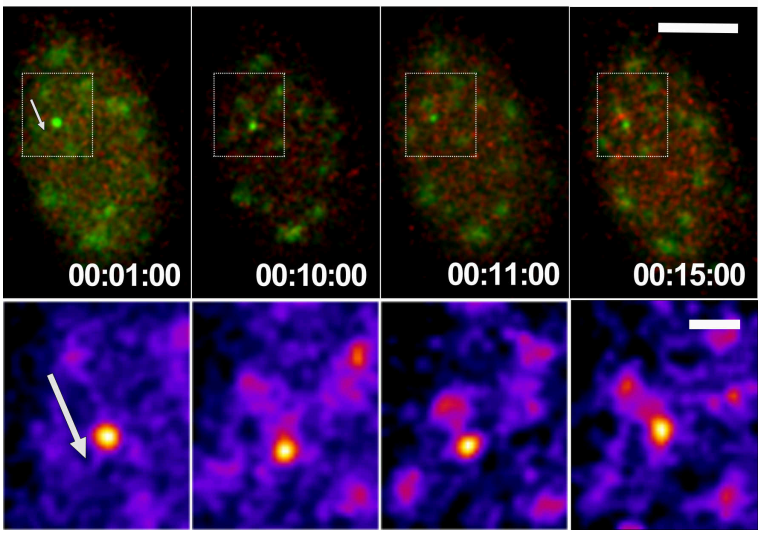


Fig. S6. Nuclear speckle movement towards heat-shock, transcriptionally activated Hsp70BAC transgene occurs along path marked by local GFP-SON accumulation. Top: Speckle movement (white arrow) occurs along a linear path. Images of GFP-SON (speckles, light green) and GFP-lac repressor (Hsp70 transgene, brighter green) and mCherry-MS2-binding protein (red) represent 2D maximum intensity projections of 3D image stacks. Time (hr:min:sec) is after heat-shock induction of Hsp70 transgene. The mCherry-MS2-binding protein stains MS2-tagged transcripts of the Hsp70 transgene. Scale bar: 5µm. Bottom: Enlarged views of boxed region from top images. Subsequent speckle movement occurs along linear path (white arrow) marked by GFP-SON accumulation at 1:00 min after heat-shock. Pseudo-color display of GFP intensities provides improved dynamic range for visualization. Images were coded with 'Fire' LUT in ImageJ. The brightest spot (yellow) marks the Hsp70 transgene; purple regions are GFP-SON. Scale bar: 1 µm.

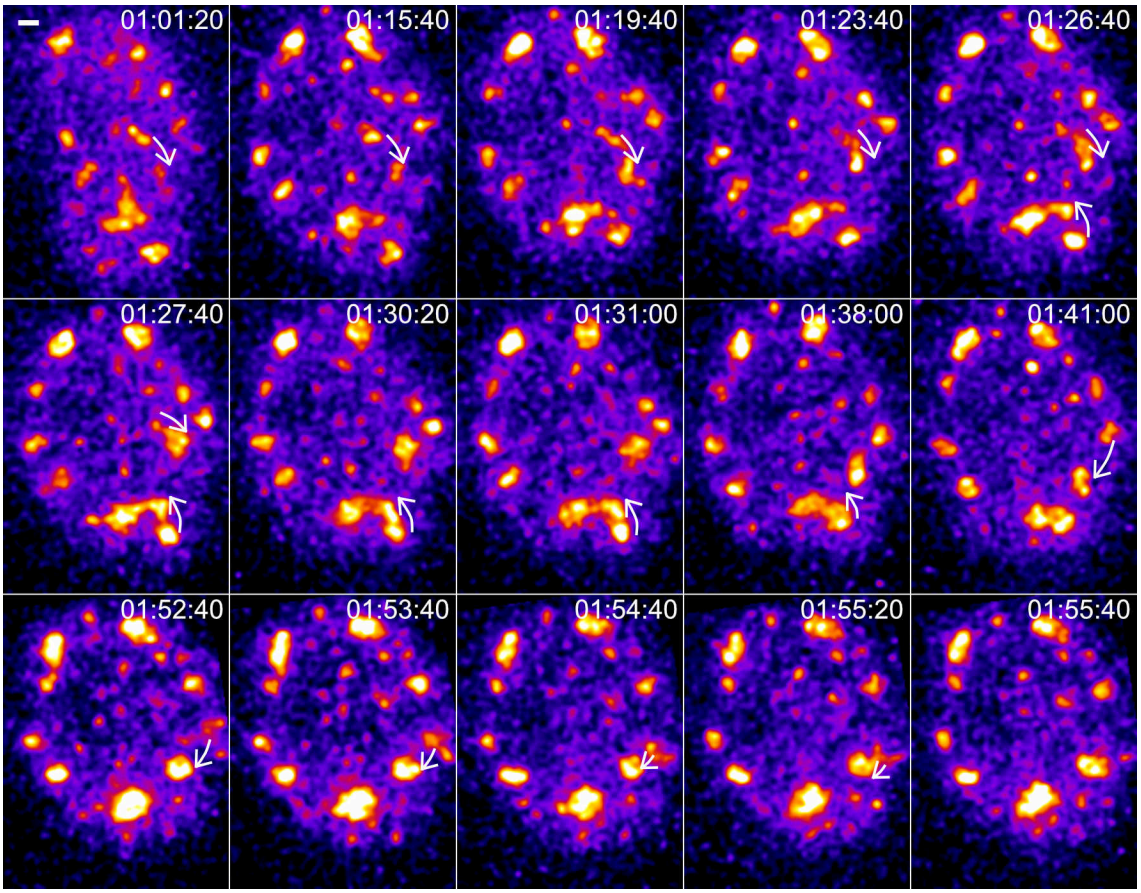
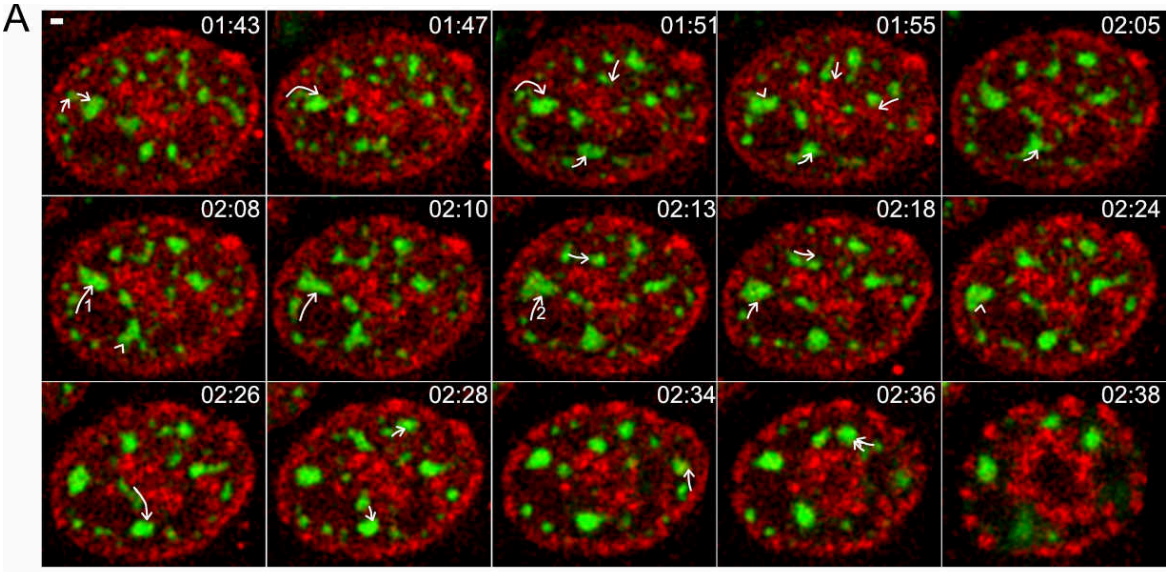
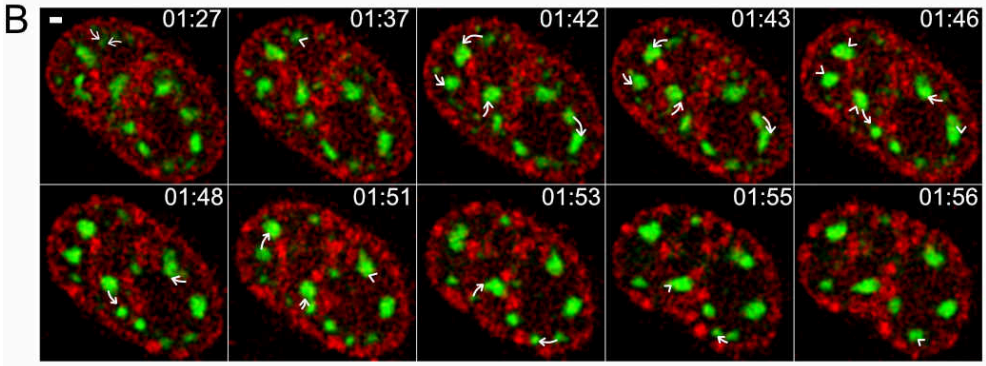


Fig. S7. Long-range nuclear speckle movements after heavy metal (Cd) treatment. GFP-SON images represent maximum intensity 2D projections of 3D image stacks. Time (hr:min:sec) is after Cd addition. Scale bar: 1 um. Long-range speckle movements, marked by white arrows, are similar to those observed after transcriptional inhibition or heat-shock.





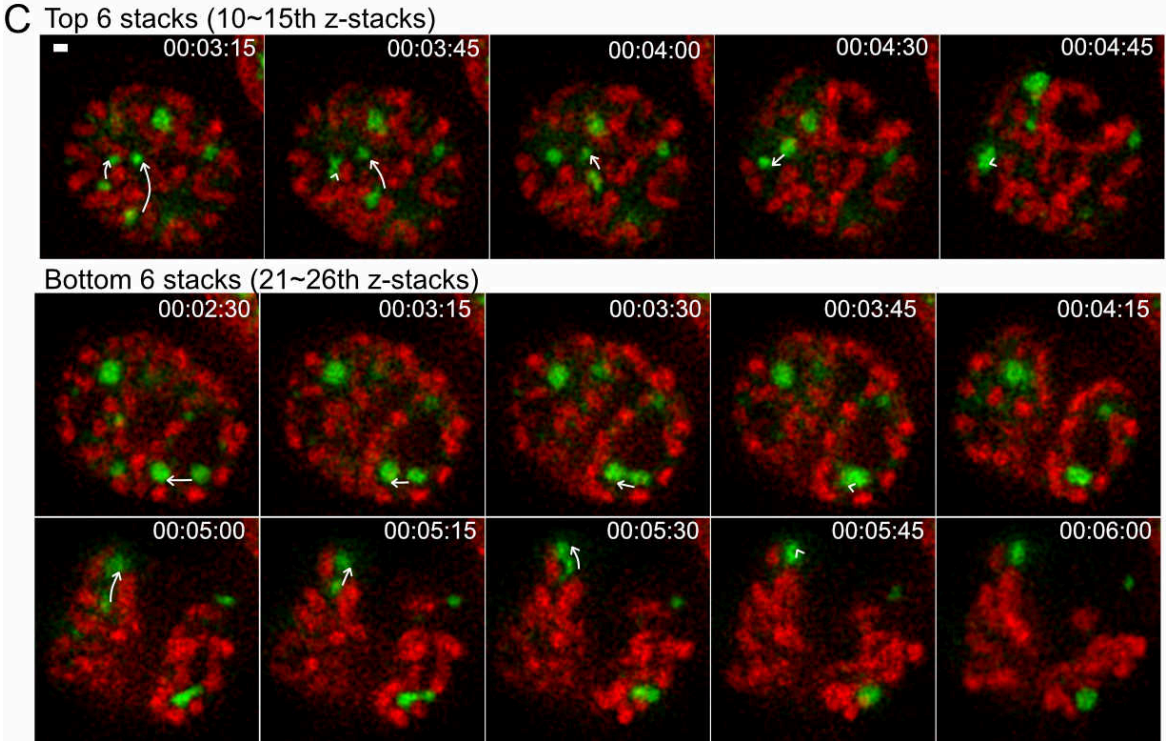


Fig. S8. Long-range speckle movements during late G2 and prophase. Live cell imaging shows nuclear speckle (GFP-SON, green) movements relative to chromatin staining (SiR-Hoescht, red). All images are 2D maximum intensity projections over several optical sections (6 sections, panels a&c, 3 sections, panel b, z-spacing=300nm). Time (hr:min or hr:min:sec) is after start of imaging. Scale bars= 1 μm. Long-range nuclear speckle movements (white arrows) and fusions between speckles during late G2 into early prophase (A&B) and during prophase and pro-metaphase (C) are similar to those observed after transcriptional inhibition, heat-shock, or cadmium treatment in interphase nuclei. These long-range speckle movements occur within interchromatin regions, as previously observed after transcriptional inhibition, with Increased speckle mobility temporally correlated with the increase in chromatin condensation observed during the late G2 to prophase transition.

	Number of cells	Average number of long-range movements per cell	Average distance(μm)
Control	16	9.8	1.53
LatA	18	2.7	1.50
WT actin(T)	17	13.4	1.48
WT actin (U)	17	12.8	1.48
G13R actin(T)	20	12.3	1.28
G13R actin(U)	15	12.2	1.28
S14C actin(T)	17	14	1.38
S14C actin(U)	22	13.5	1.34

Table S1. Numbers of long-range speckle movements (>1 μm) per nucleus in cells treated with latA or expressing an actin mutant after DRB addition. "T" stands for transfected cells with indicated actin construct, "U" stands for non-transfected control cells in same cell dish. LatA was added 30 mins prior to DRB addition. DRB was added at time 0, and time-lapse measurements were made from 30-90 mins after DRB treatment.