A 0% Colocalization

Merge X-Y   Merge Y-Z   1x1   1x2   2x2

Colocalization of Spots (%):   100   0   100

B 50% Colocalization

Merge X-Y   Merge Y-Z   1x1   1x2   2x2

Colocalization of Spots (%):   100   50   100

C

Copy 1   Copy 2   Copy 3

☐ Yes   ☐ No

Colocalization/Expression of *CCNA*2 copies (%)

**Copy 1: 25.11%**
Copy 2: 25.78%
Copy 3: 29.37%

Figure S1: Benchmarking of the colocalization metric between DNA FISH and RNA FISH Signals (Related to Figure 1 and 4)

For validation of the colocalization metric, synthetic 3D images of dimension 600x600x31 were generated with 10 randomly positioned spots which mimic the actual RNA FISH and DNA FISH signals. The stack number and pixel size were set to match with those for the actual images from the real experiments. Scale bar: 10 µm. (A) Red spots correspond to synthetic Image 1 and green to Image 2. For this panel, Image 1 has no spots colocalizing with Image 2. The first element in the panel shows X-Y projected image of the merge obtained for Image1 and Image 2 while the second element shows the Y-Z projected image of the same to visualize colocalization in the Z direction. The last panel shows the quantification for colocalization between the spots. Each line in this heat map matrix corresponds to a spot and represents 3D colocalization (yes or no) between spots from two images. 1x1 and 2x2 represent self-colocalization of the spots in Image 1 and Image 2 respectively while 1x2 represent the colocalization between the spots in Image 1 and Image 2. 'Yes' represents that the spots in the two images colocalize and vice versa for 'No'. As is observed the metric captures colocalization with 100% efficiency. More than 400 spots were analysed in total. (B) Here images are generated such that Image 1 and Image 2 have exactly 50% of (that is 5) the spots colocalize. As seen in the second element of the panel, exactly 5 spots between Image 1 and Image 2 colocalize in 3D. Again, the metric quantifying the colocalization captures this with 100% efficiency. More than 400 spots were analysed in total. (C) Quantification for colocalization between DNA FISH and RNA FISH signals for *CCNA2* in experimental images. Each line corresponds to a cell and represents the colocalization (yes or no) between DNA FISH and RNA FISH signals which also measures the expression of a *CCNA2* copy in this case. More than 400 cells were analyzed in total. Copies are numbered such that Copy 1 is closest to the lamina followed by Copy 2 and then Copy 3. Copy 3, which is farthest from the periphery, shows a slightly higher percentage of expression. See Figure 1 for representative cell images.
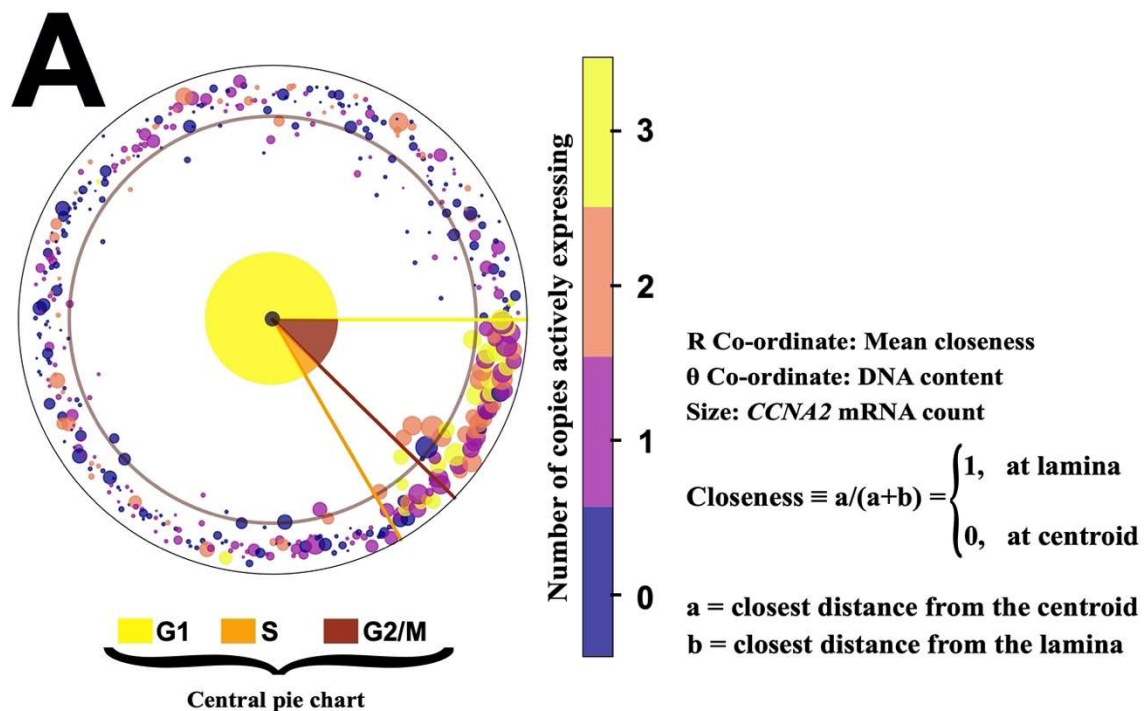
Figure S2: Relation between mRNA expression and the number of actively expressing copies across the cell cycle (Related to Figure 4)
(A) Polar plot relating the total *CCNA2* mRNA expression with the activity of the three copies and the mean of their relative distances from the lamina in a cell. Although the number of *CCNA2* copies actively expressing increases with the increase in the *CCNA2* mRNA count of a cell in G2/M phases, there can be cells with very few mRNAs but more than a single copy expressing.

Table S1.

Probes Sequence (from 5' to 3')

CCNA2 smFISH probes		
1 gtctgctgcaatgctagcag	20 ttagtgatgtctggctgttt	39 gaaaggcagctccagcaata
2 tttttccgggttgatattctc	21 cacgaggatagctctcatac	40 gtgactgtgtagagtgctaa
3 aagatccttaaggggtgcaa	22 tctcctacttcaactaacca	41 aatgattcaggccagctttg
4 ggtgacatgctcatcattta	23 gggtctcattctgtagttta	42 ggtatatccagtctttcgta
5 tgtttgctttccaaggagga	24 atcaatgtagttcacagcca	43 gacaaggcttaagactttcc
6 aatggtgaacgcaggctgtt	25 gcactgacatggaagacagg	44 tttgaggtaggtctggtgaa
7 tctttttctgcttcatccac	26 acaagctgaagttttcctct	45 tgactgttgtgcatgctgtg
8 ttcagctggcttcttctgag	27 tgaggctaacagcatagcag	46 gaatttttgtacttttctct
9 cttcacgctctattttttga	28 tggggggtatatttcttcaa	47 gttgaggagagaaacaccat
10 gctgaattaaaagccagggc	29 tgtacacaaactctgctact	48 acagatttagtgtctctggt
11 tctgggtccaggtaaactaa	30 ttggtgtaggtatcatctgt	
12 aatcaagagggaccaatggt	31 agatgctccattctcagaac	
13 tcaaaactaccatccattgg	32 gtcaaaagtaaggactttca	
14 catgtccatagtatgtggtg	33 tgatttactgttggagcagc	
15 cactggctttttcatcttcta	34 gctgctgatgcagaaagtat	
16 ggtagtctggtacttcatta	35 ctttcaactttgcagtttgc	
17 aggtatgtgtgaatatcctc	36 attctcccaaaaacattgct	
18 acatttaacctccatttccc	37 gggtcagcatctatcaaact	
19 tcatgtaacccactttaggt	38 tgatggcaaatacttgaggt	
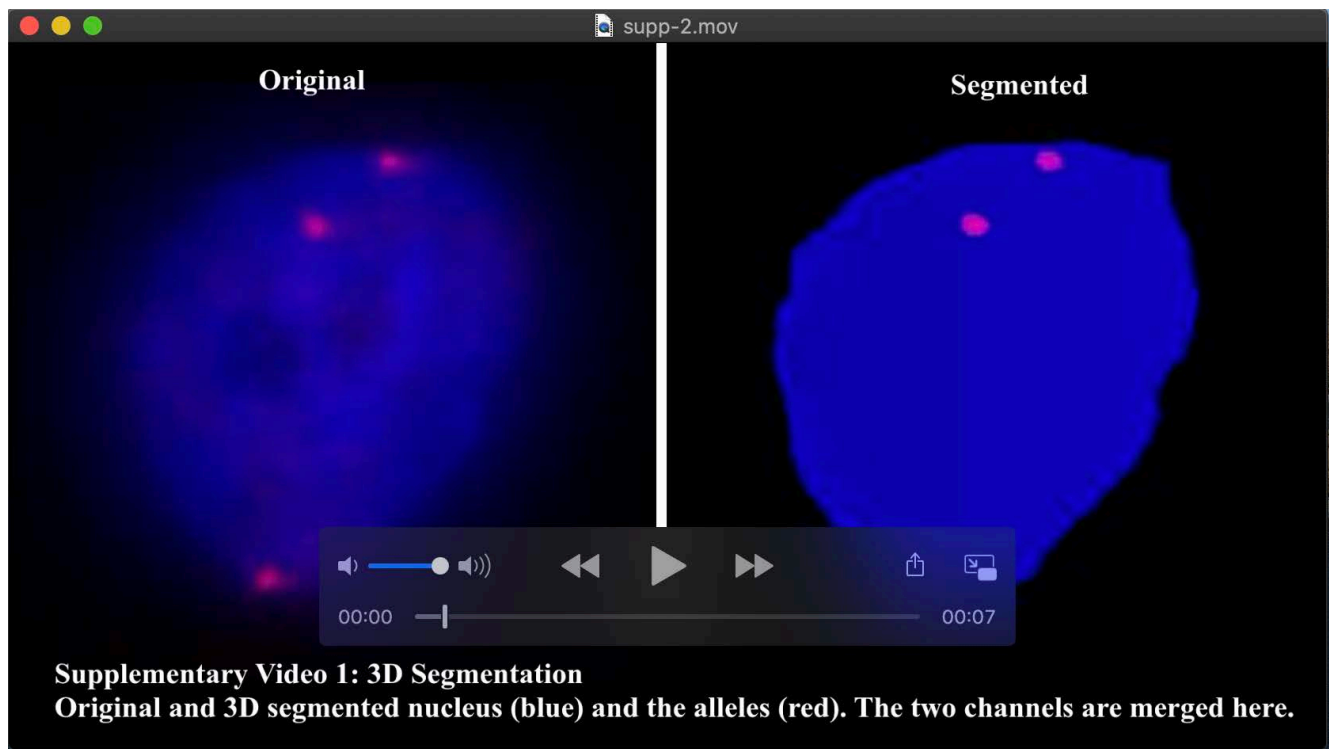
Supplementary Video 1: 3D Segmentation
Original and 3D segmented nucleus (blue) and the alleles (red). The two channels are merged here.

Movie 1. 3D Segmentation

Original and 3d segmented nuclcus (blue) and the alleles (red). The two channels are meged here.