

## Supplementary Figure Legends

Figure S1. **Characterization of co-replicative labeling of early-replicating RDs for correlative confocal-STORM imaging.** **(A)** Example of an NRK cell with a single CT labeled with ATTO633-dUTP (magenta). The transmission image, along with an outline of the nuclear envelope is shown (grey image and dashed white line). The spatial distribution of RDs within the nuclear volume was captured with a z-stack. **(B)** Estimation of the number of RDs/CT ( $n = 71$  cells). **(C)** Examples of  $\lambda$ DNA fragments used in DNA combing experiments to calibrate the extent of DNA stretching as a function of DNA kb. Left panels: 4.36 kb; middle panels: 9.41 kb; right panels: 23.13 kb. Scale bar, 5  $\mu\text{m}$ . **(D)** Calibration of the DNA length as a function of DNA size in kb, from measurements performed on stretched  $\lambda$ DNA fragments (sizes as described in C). **(E)** DNA combing performed on DNA content from NRK cells subjected to replicative labeling with ATTO633.dUTP (magenta). The DNA fiber was counterstained with Picogreen (green). Scale bar, 30  $\mu\text{m}$ . **(F)** Size of co-replicating regions estimated from the size of the ATTO633-dUTP labeled DNA backbone stretch.

Figure S2. **Dual-color live-cell confocal imaging measures a weak mechanical coupling of neighboring RDs.** Averaged mean correlation angle  $\langle\alpha\rangle$  is extracted as a function of distance between pairs of RDs labeled with ATTO633 and ATTO565. The individual plots show the angles corresponding to unpooled data from  $\Delta t = 15$  min ( $n = 3144$  pairs, 26 cells), 30 min ( $n = 8256$  pairs, 27 cells), 45 min ( $n = 5126$  pairs, 21 cells), 60 min ( $n = 5306$  pairs, 15 cells), 90 min ( $n = 4103$  pairs, 22 cells), and 120 min ( $n = 4329$  pairs, 22 cells).

Table S1. **Fluorophores tested for optimized replicative labeling of RDs using aminoallyl-dUTP derivatives in correlative confocal-superresolution imaging applications.**

The performance was qualitatively assessed by visual inspection of confocal/STORM images of the fluorophores, and ranked as excellent (+++), very good (++), acceptable (+), or not suitable (-).