Supplementary material

Lidsky et al. 2013. Distinct modes of centromere protein dynamics during cell cycle progression in *Drosophila* S2R+ cells

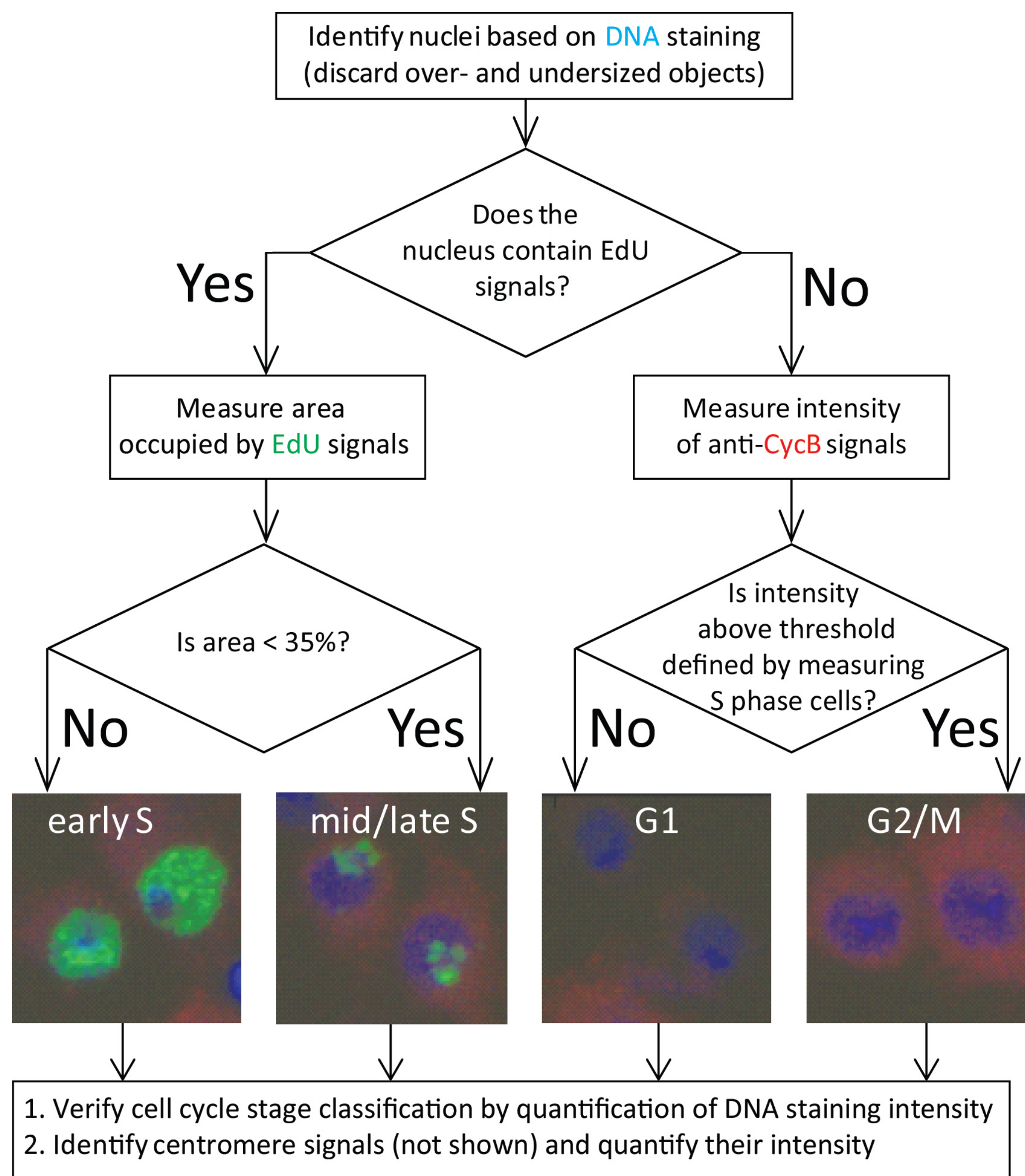
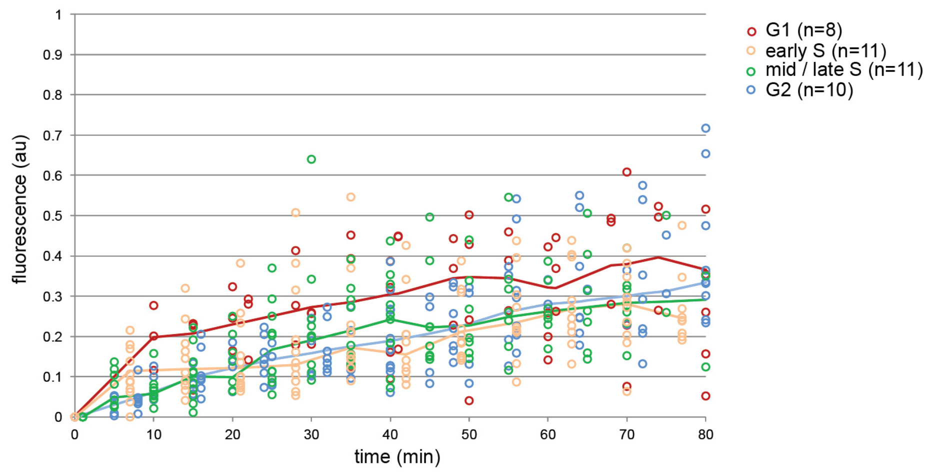
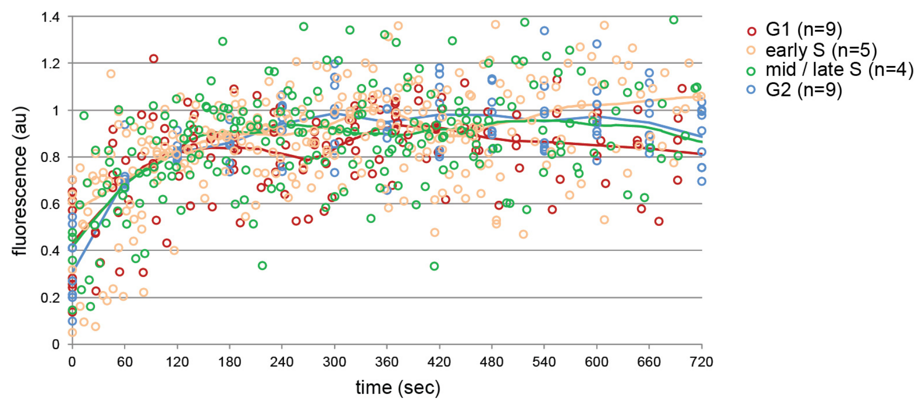


Figure S1. Classification of cell cycle stages. S2R+ cells expressing SNAPf-tagged Cid were pulse labeled with EdU before TMR-Star addition. After fixation cells were stained with anti-Cyclin B (CycB) and a DNA stain. The CellProfiler pipeline used for identification of cells in G1, early S, mid/late S and G2/M is illustrated.

A Cenp-C-EGFP



B Cal1-EGFP



C Cal1-EGFP

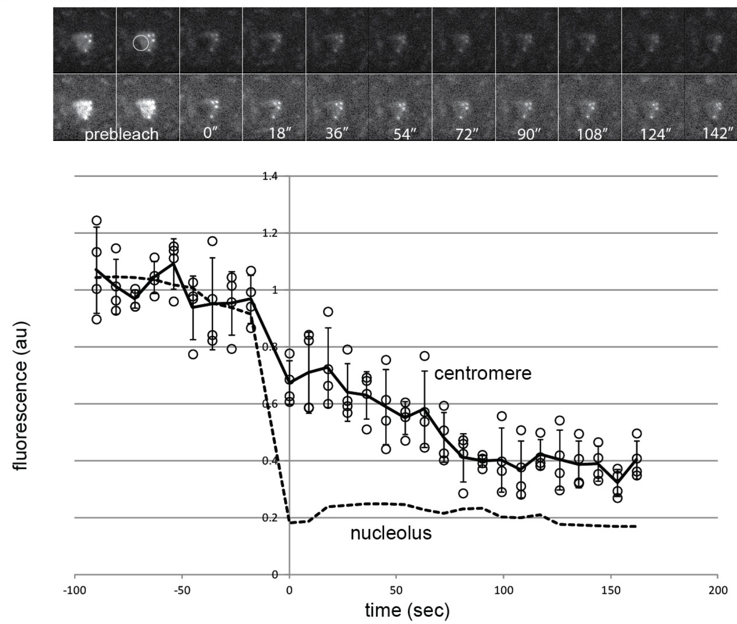
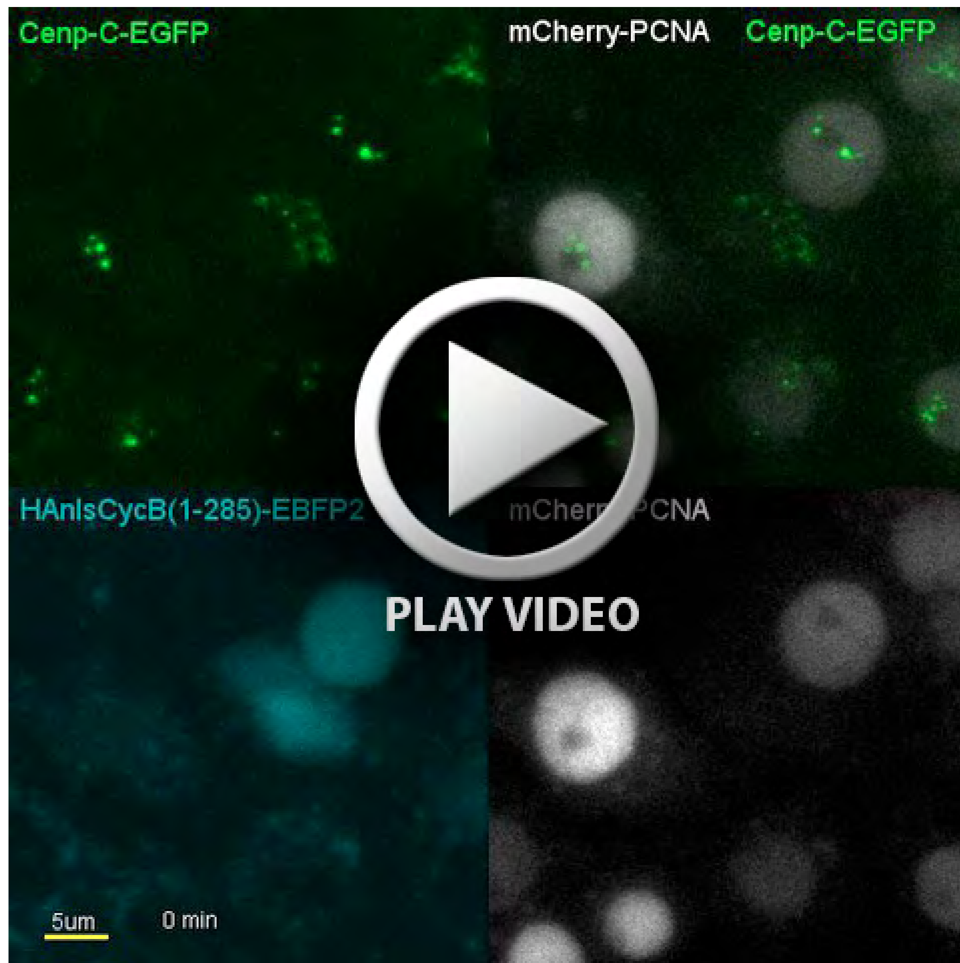


Figure S2. Mobility of Cenp-C and Cal1 during the cell cycle. (A,B) Centromere clusters in S2R+ cells expressing Cenp-C-EGFP (A) or Cal1-EGFP (B) in combination with mCherry-PCNA and HAnlsCycB$^{1\text{-}285}$-EBFP2 were photobleached in either G1, early S, mid/late S or G2 followed by analysis of recovery of centromeric EGFP signals over time. (C) A nucleolar region (white circle in upper panel) in S2R+ cells expressing Cal1-EGFP in combination with mCherry-PCNA and HAnlsCycB$^{1\text{-}285}$-EBFP2 was exposed to a strong bleach pulse. EGFP signals in individual centromere cluster (circles) and the sum of nucleolus and centromere clusters were quantified over time, followed by correction for additional bleaching after the photobleach pulse and calculation of nucleolar (dashed line) and average centromere cluster signals (unbroken line). The displayed data is from a representative experiment with a cell in late S. Frames at the indicated time points are displayed above the curves. To display nucleolar signals more clearly, contrast was increased in the lower row. Six additional experiments with cells in different subphases of interphase also revealed that nucleolar bleaching results in a slightly delayed decrease in centromeric Cal1-EGFP signals, indicating rapid exchange between centromeric and nucleolar Cal1-EGFP pools.



Movie 1. S2R+ cells expressing Cenp-C-EGFP and the cell cycle marker proteins mCherry-PCNA and HAnlsCycB$^{1\text{-}285}$-EBFP2 were analyzed by time lapse *in vivo* imaging using a laser scanning confocal microscope. Stacks with 20 focal planes spaced by 0.5 nm were acquired every 25 minutes. Maximum projections were prepared and are displayed in the movie. Time in minutes is given in the lower left panel.