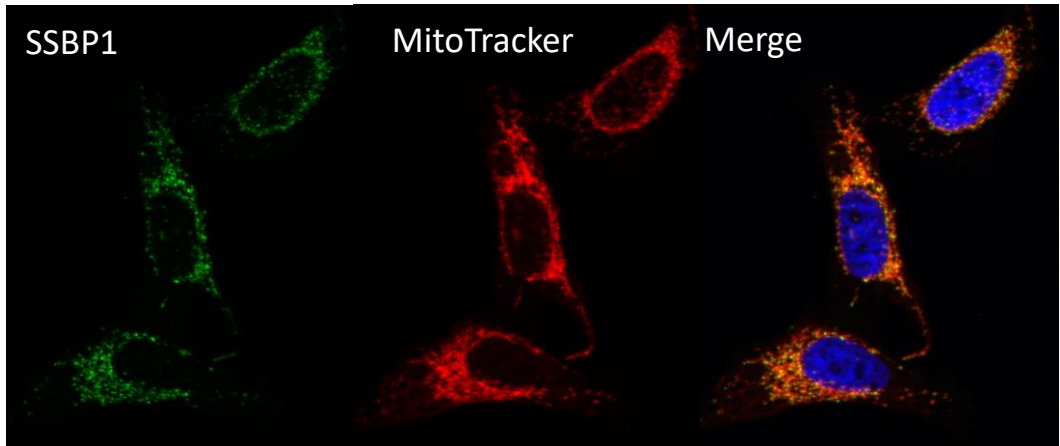
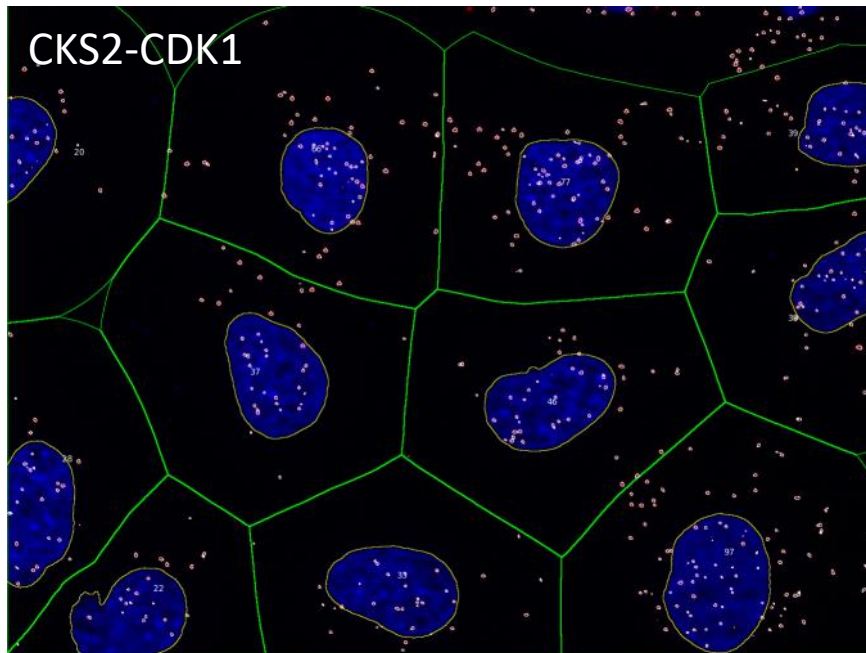


Specificity of the SPSS1 antibody



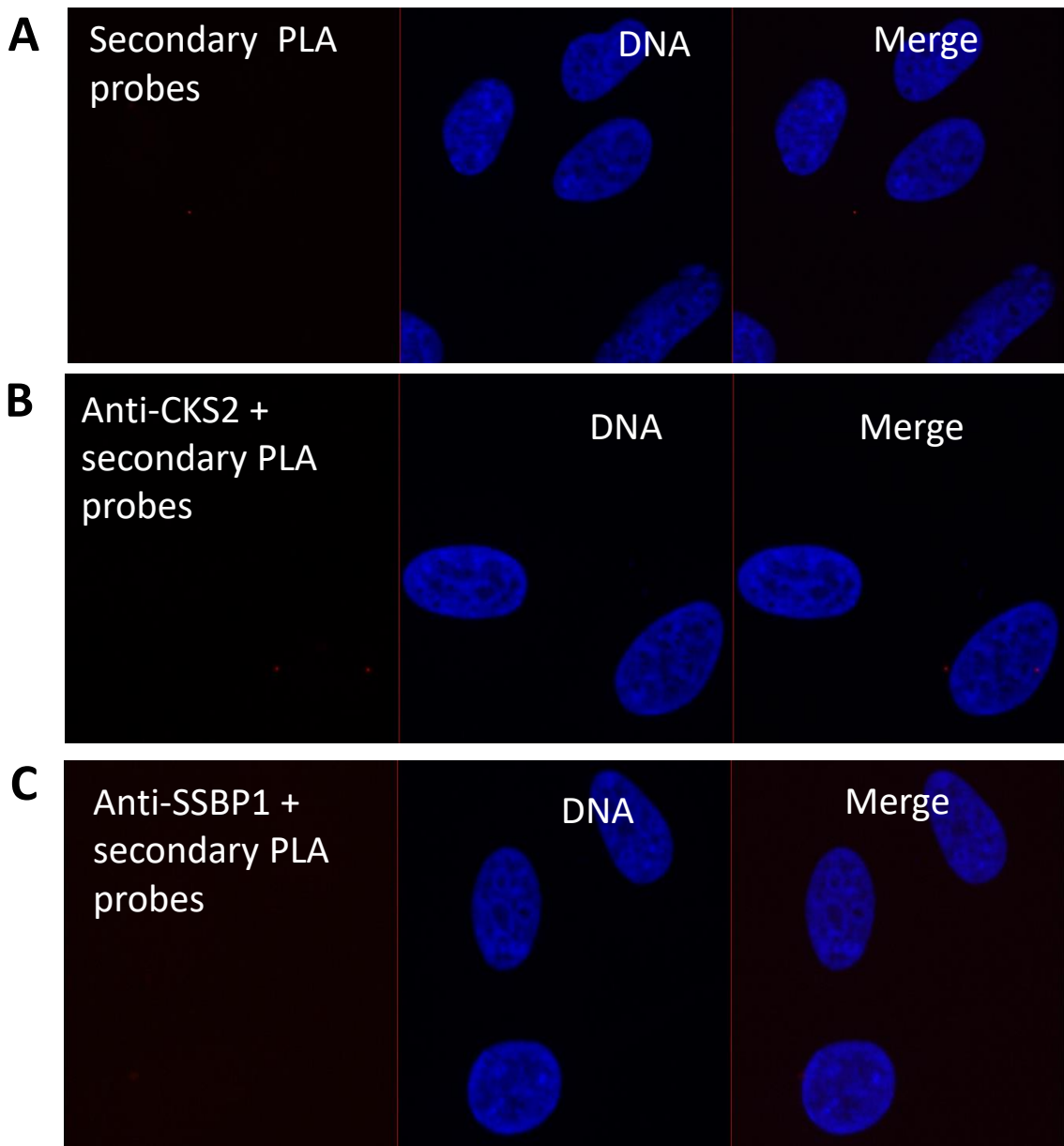
Supplementary Figure S1. SSBP1 (green) was expressed only in the cytoplasm of HeLa cells, and was visible as green foci often close to the cell nucleus. The SSBP1 signal co-localized perfectly with a MitoTracker probe (red), demonstrating high specificity of the antibody. Staining was performed by seeding cells on cover slips overnight before treating them with MitoTracker Deep Red FM (300 nM; Invitrogen) for 45 minutes at 37°C in the dark. Cells were then rinsed with warm PBS and fixed with 10% neutral buffered formalin in 10 minutes at room temperature and ice-cold acetone (-20°C) in 5 minutes, protected from light, washed three times with PBS and treated according to the immunofluorescence protocol.

Quantification of PLA foci in immunofluorescence images of adherent cells on cover slips



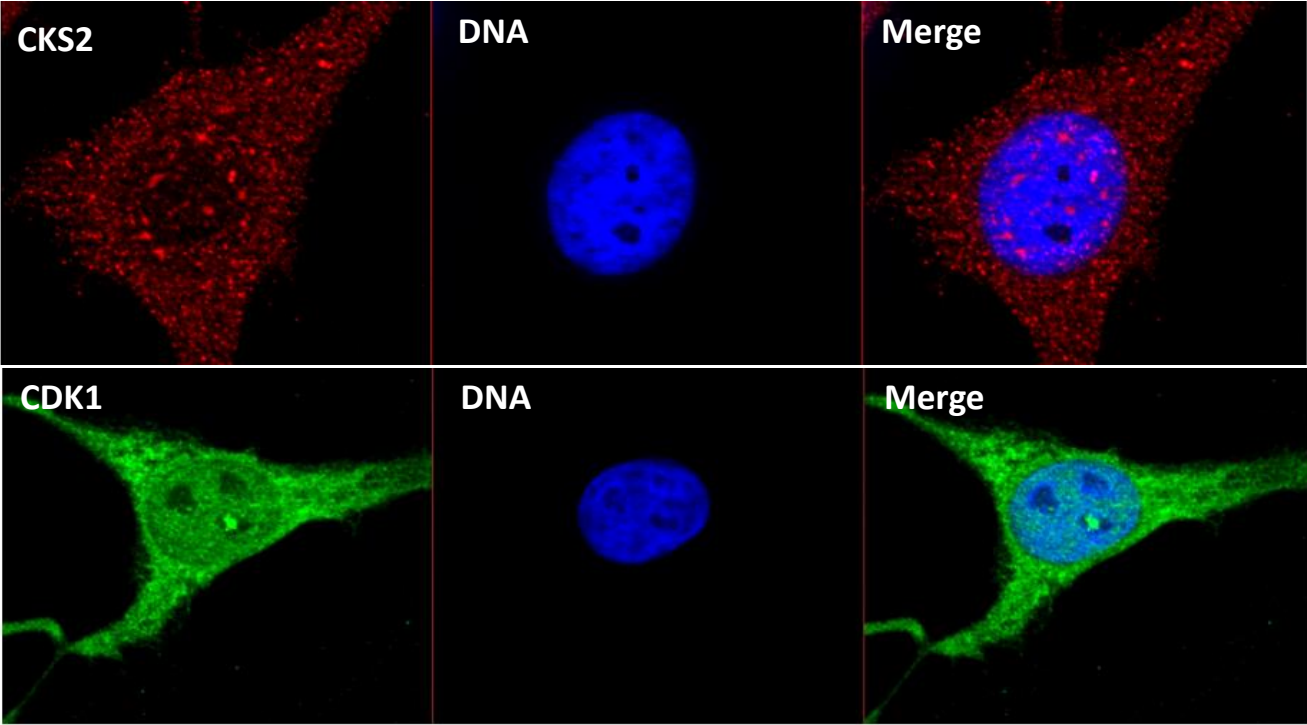
Supplementary Figure S2. (A), Identification of cells and corresponding PLA foci with the single cell analysis algorithm in BlobFinder software in a typical image of CKS2-CDK1 foci. Cells and nuclei (blue) are outlined in green and yellow, respectively. PLA foci are indicated as orange spots.

Negative PLA controls in HeLa cells



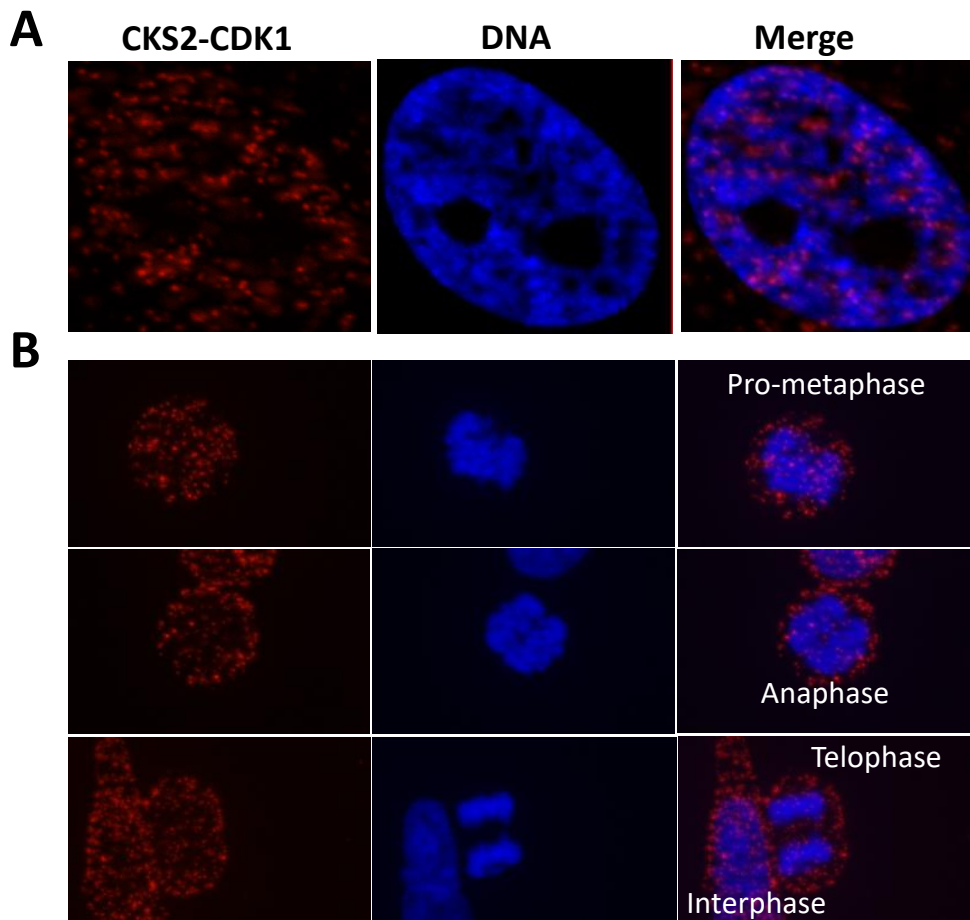
Supplementary Figure S3. (A), secondary PLA probes without primary antibodies. (B), primary CKS2 (anti-CKS2) antibody and both secondary PLA probes. (C), primary SSBP1 (anti-SSBP1) antibody and both secondary PLA probes. (A-C), No PLA signal was detected, demonstrating the need of two primary and two secondary antibodies for foci detection. The images were acquired using the same gain as in Figure 2.

Expression pattern of CKS2 and CDK1 protein in HeLa cells



Supplementary Figure S4. Typical immunofluorescence images of CKS2 (red, upper), CDK1 (green, lower), DNA (blue) and the images merged.

CKS2-CDK1 PLA foci in HeLa cells



Supplementary Figure S5. (A), Nuclear CKS2-CDK1 PLA foci in interphase cells. (B), Nuclear and cytoplasmic CKS2-CDK1 PLA foci in interphase and mitotic cells. (A, B), Typical immunofluorescence images of CKS2-CDK1 (red), DNA (blue) and the two images merged are shown.