Supporting Information

Pascreau et al. 10.1073/pnas.0914874107

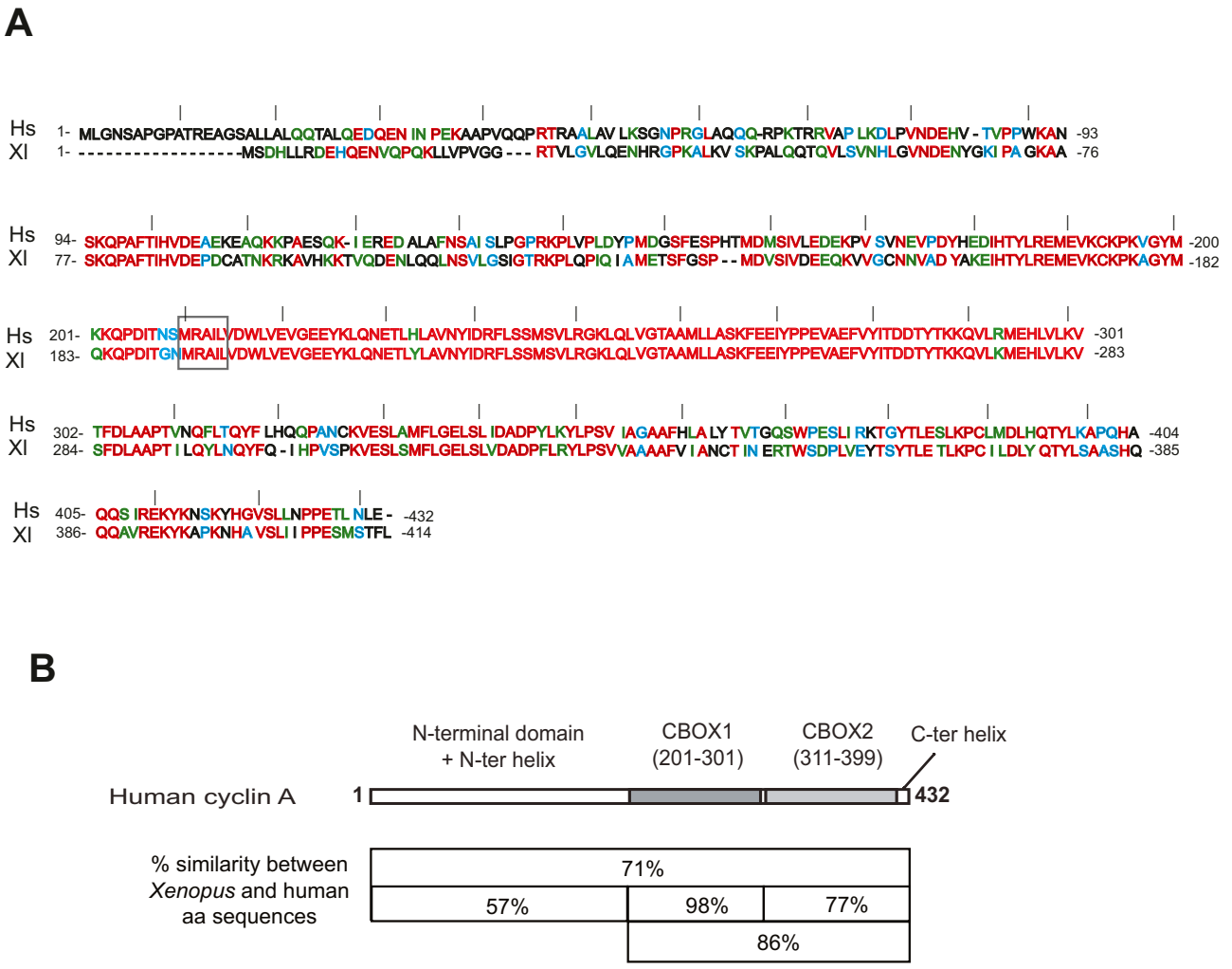


Fig. S1. High sequence similarity of human and *Xenopus* cyclin A. (*A*) Amino acid sequence alignment between human and *Xenopus* cyclin A. Identical residues are shown in red, strongly similar residues are shown in green, and weakly similar residues are shown in blue. Residues shown in black are not conserved. The MRAIL motif, also called the hydrophobic patch, is boxed in gray. The regions containing the cyclin box folds (CBOX 1 and 2) are well conserved, particularly CBOX1 (amino acids 201–301 in human). This alignment was obtained using CLUSTALW Alignment software (NPS@ server: Network Protein Sequence Analysis) at http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_clustalw.html. (*B*) Diagram representing the percentage similarity between the different domains of human and *Xenopus* cyclin A.

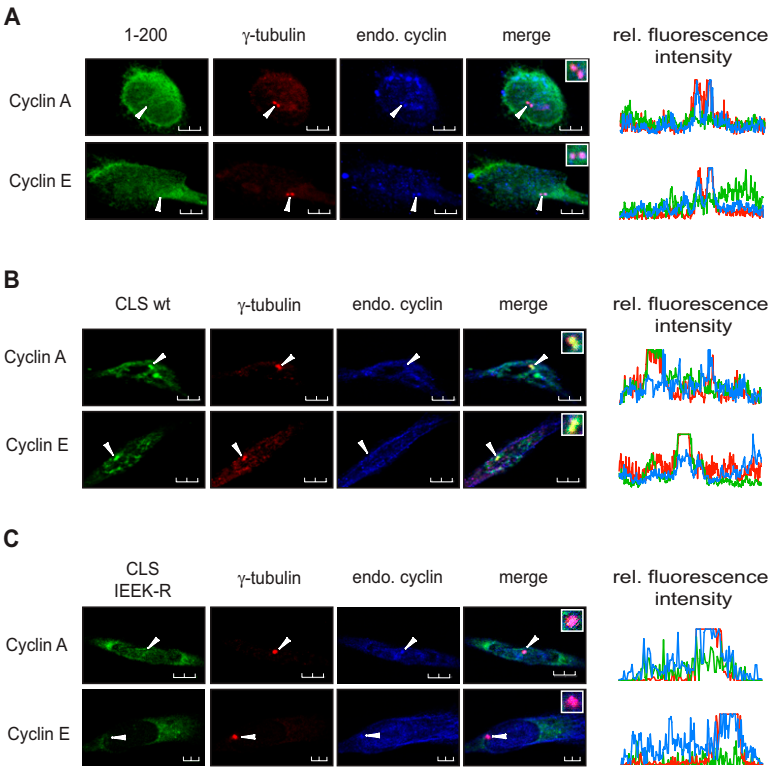


Fig. S2. Overexpression of the cyclin A CLS displaces endogenous cyclins A and E from centrosomes. (*A*) CHO-K1 cells were transfected with a plasmid encoding cyclin A 1–200 EGFP. Cells were methanol-fixed and immunostained with antibodies to γ-tubulin and cyclin A2 (*Upper*) or cyclin E (*Lower*). Expression of cyclin A 1–200 EGFP was analyzed by direct fluorescence (green). Localization of endogenous cyclin A and E (endo. cyclin) was monitored by confocal microscopy (blue) and compared with the centrosomal localization of γ-tubulin (red). (*B*) The same experiment was performed with the cyclin A CLS (201–255) fused to EGFP. (*C*) The same experiment was performed with the quadruple mutant form of the cyclin A CLS (201–255 IEEK-R) fused to EGFP. Arrows indicate the position of centrosomes. (*Insets*) Magnification of the centrosomal region in the merged image. Line scans measuring centrosome-associated relative fluorescence intensity (rel. fluorescence intensity) are displayed on the right, with the green, blue, and red lines representing GFP, the endogenous cyclins, and the γ-tubulin fluorescence, respectively. (Scale bars, 10 μm.)

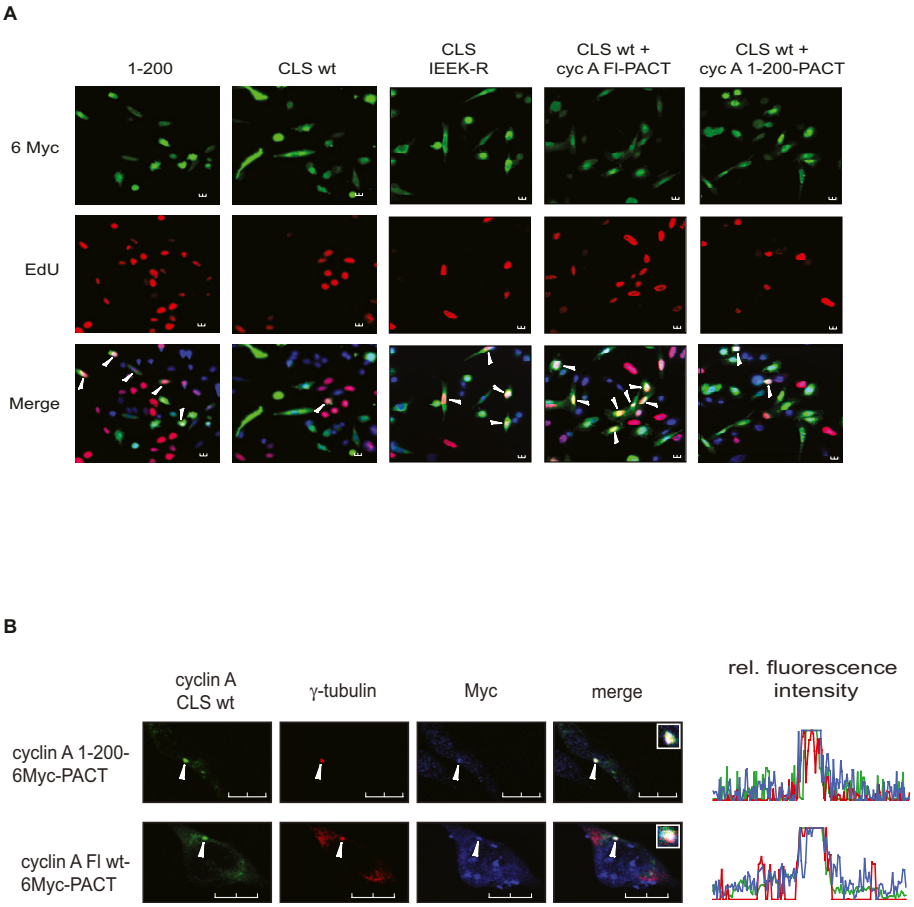
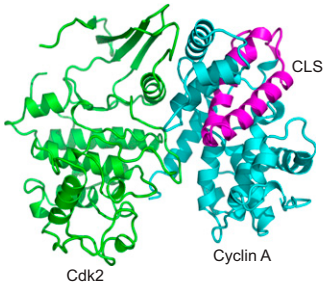


Fig. S3. Overexpression of the cyclin A CLS inhibits DNA replication. (*A*) Unsynchronized CHO-K1 cells were transfected with the indicated cyclin A-6myc constructs and incubated with EdU to follow DNA replication. Cells were methanol-fixed and stained for EdU (red) and DAPI (blue). Cyclin A-6Myc constructs were observed by indirect immunofluorescence (green). Arrows indicate the position of transfected cells incorporating EdU. (Scale bars, 10 μm.) (*B*) The PACT domain localizes cyclin A constructs on centrosomes independently of the cyclin A CLS. Plasmids encoding the human cyclin A 1–200-6Myc (*Upper*) or cyclin A FI wild type (wt)-6Myc fused to the PACT domain were transfected into CHO-K1 cells expressing the cyclin A CLS. Cells were methanol-fixed and immunostained with antibodies to γ-tubulin (red) and Myc (green). Arrows indicate the position of centrosomes. (*Insets*) Magnification of the centrosomes from the merged image. (Scale bars, 10 μm.)



Cyclin A-Cdk2

Fig. S4. The cyclin A CLS region is not involved in Cdk binding. Ribbon diagram (adapted from ref. 1) representing the crystal structure of the cyclin A-Cdk2 complex (2) and showing that the cyclin A CLS (magenta) has no residues in contact with Cdk2 (green).

1. Honda R, et al. (2005) The structure of cyclin E1/CDK2: Implications for CDK2 activation and CDK2-independent roles. *EMBO J* 24:452–463.
2. Jeffrey PD, et al. (1995) Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. *Nature* 376:313–320.

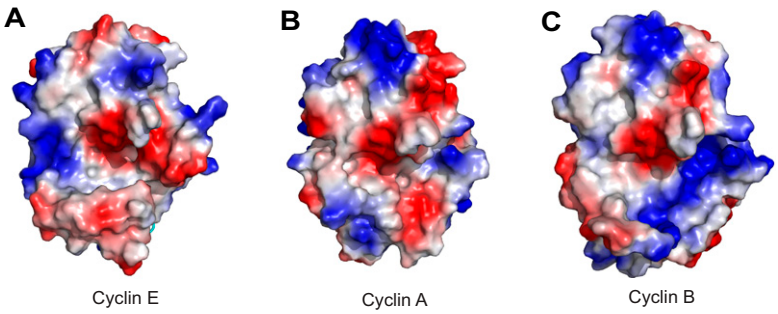


Fig. S5. Surface charge may alter the "cyclin A CLS-like" motif functionality in cyclin E. (*A*–*C*) Space-filling models of cyclins E, A, and B, respectively, showing the surface charge on the molecules. In cyclin A (*B*) the region surrounding the CLS is largely negatively charged (red), whereas the same region in cyclin E (*A*) is mostly neutral (gray) or even positively charged (blue), which may explain why this "cyclin A CLS-like" sequence is not a functional CLS in cyclin E. Cyclin B (*C*) has a charge distribution similar to that of cyclin A in the region of the cyclin A CLS.