Expanded View Figures

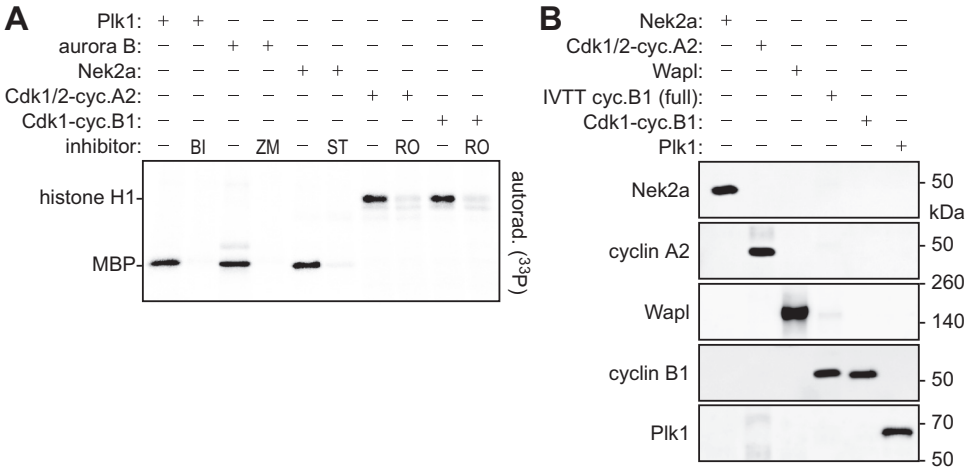

Figure EV1. Characterization of recombinant kinases.

(**A**) Recombinant kinases are active as judged by the phosphorylation of model substrates. Plk1, aurora B-INCENP, Nek2a, Cdk1/2-cyclin A2, or Cdk1-cyclin B1 supplemented with their specific inhibitor or carrier solvent DMSO (−) were incubated with the corresponding model substrate in the presence of [γ^{33}P]-ATP. Reactions were subjected to SDS-PAGE followed by autoradiography. BI BI2536, ZM ZM-447439, ST staurosporine, RO RO-3306, MBP myelin basic protein. (**B**) Recombinant Nek2a, Cdk1/2-cyclin A2, and Wapl are free of cyclin B1 and Plk1. The preparations of Nek2a, Cdk1/2-cyclin A2, Wapl, Cdk1-cyclin B1, and Plk1 used for the release assays were characterized by immunoblotting using the indicated antibodies. In vitro expressed (IVTT) cyclin B1 served as an additional control.

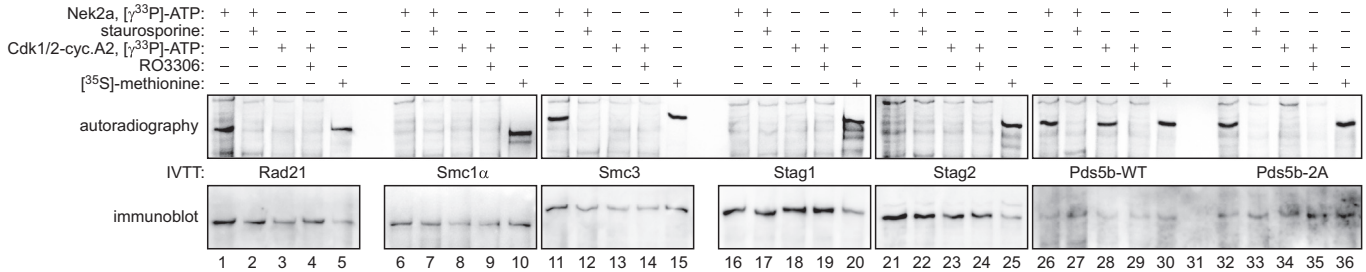

Figure EV2. Nek2a and Cdk1/2-cyclin A2 both phosphorylate in vitro expressed Pds5b.

Cohesin subunits and Pds5b variants were expressed by coupled in vitro transcription-translation (IVTT), incubated with Nek2a, Cdk1/2-cyclin A2, [γ-^{33}P]-ATP, and kinase inhibitors, as indicated, separated by SDS-PAGE and analyzed by autoradiography and immunoblotting. ^{35}S-methionine labeled IVTT products served as indicators of the migration behavior of the respective full-length protein. Note that Pds5b-2A (Ser1161,1166Ala) is resistant to phosphorylation by Cdk1/2-cyclin A2 but not Nek2a.

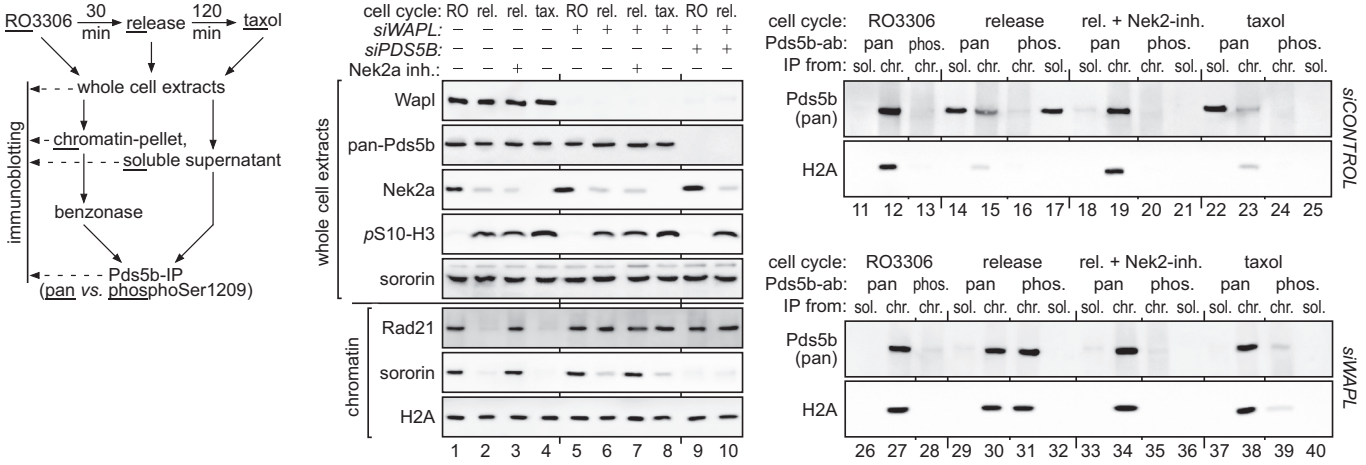

Figure EV3. Pds5b phosphorylated on Ser1209 by Nek2a exhibits Wapl-dependent displacement from early mitotic chromatin.

HeLaK cells transfected to deplete Pds5b and/or Wapl by RNAi were synchronized and harvested in G2-, pro-, and prometaphase, fractionated into chromatin and soluble lysate and subjected to the indicated (IP-) Western analyses. Nek2a inhibitor (NCL 00017509) was added at the time of release from RO-3306 and cells were harvested 30 min thereafter.

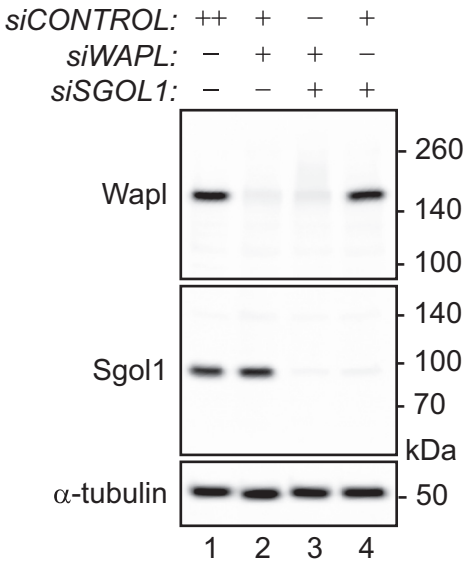

Figure EV4. Wapl and/or Sgo1 are efficiently depleted by RNAi.

HeLaK cells transfected with the indicated siRNAs during a thymidine arrest were released into a G2- and, from there into a prometaphase arrest prior to their analysis by immunoblotting. Aliquots of the cells analyzed in lanes 2 and 3 were subjected to spread-IFs as shown in Fig. 6B (lower row panels).