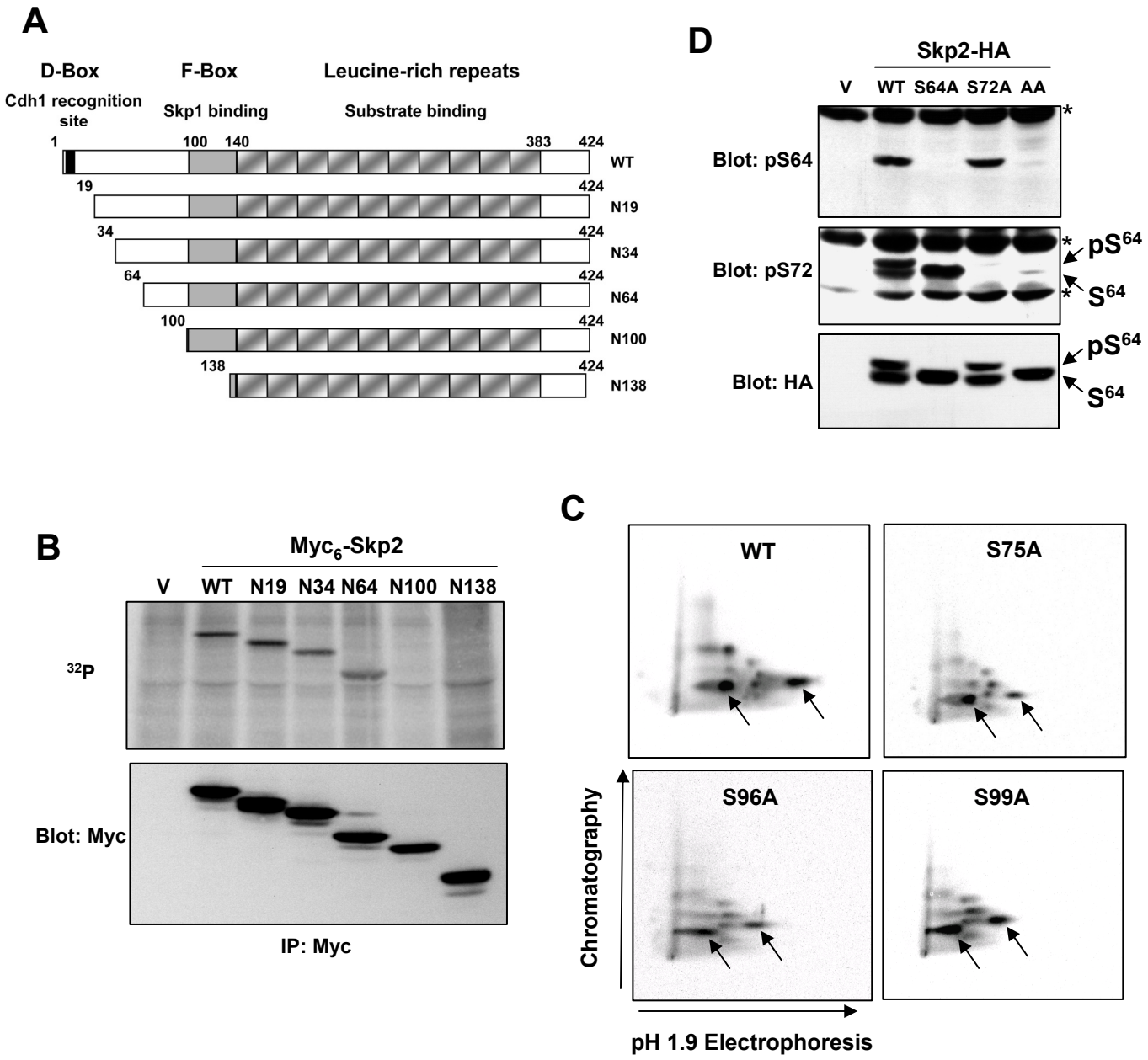

Figure S1. Identification of Skp2 phosphorylation sites

(A) Schematic representation of N-terminal deletion mutants of Skp2 used to characterize Skp2 phosphorylation.
(B) Rat1 cells expressing the indicated Myc_6-Skp2 constructs were labeled with ^{32}P. Ectopic Skp2 was immunoprecipitated with anti-Myc and analyzed for phosphorylation status (upper panel) and expression (lower panel).
(C) Phosphopeptide maps of three of the five Ser-to-Ala mutants spanning the region between amino acids 64 and 99 of Skp2. Arrows indicate phosphopeptides containing Ser64 and Ser72.
(D) NIH 3T3 cells were transfected with the indicated Skp2 constructs. Phosphorylation of Ser64 and Ser72, as well as total expression of Skp2 was analyzed by immunoblotting. The position of the Ser64-phosphorylated form of Skp2 is indicated. *, non specific band.

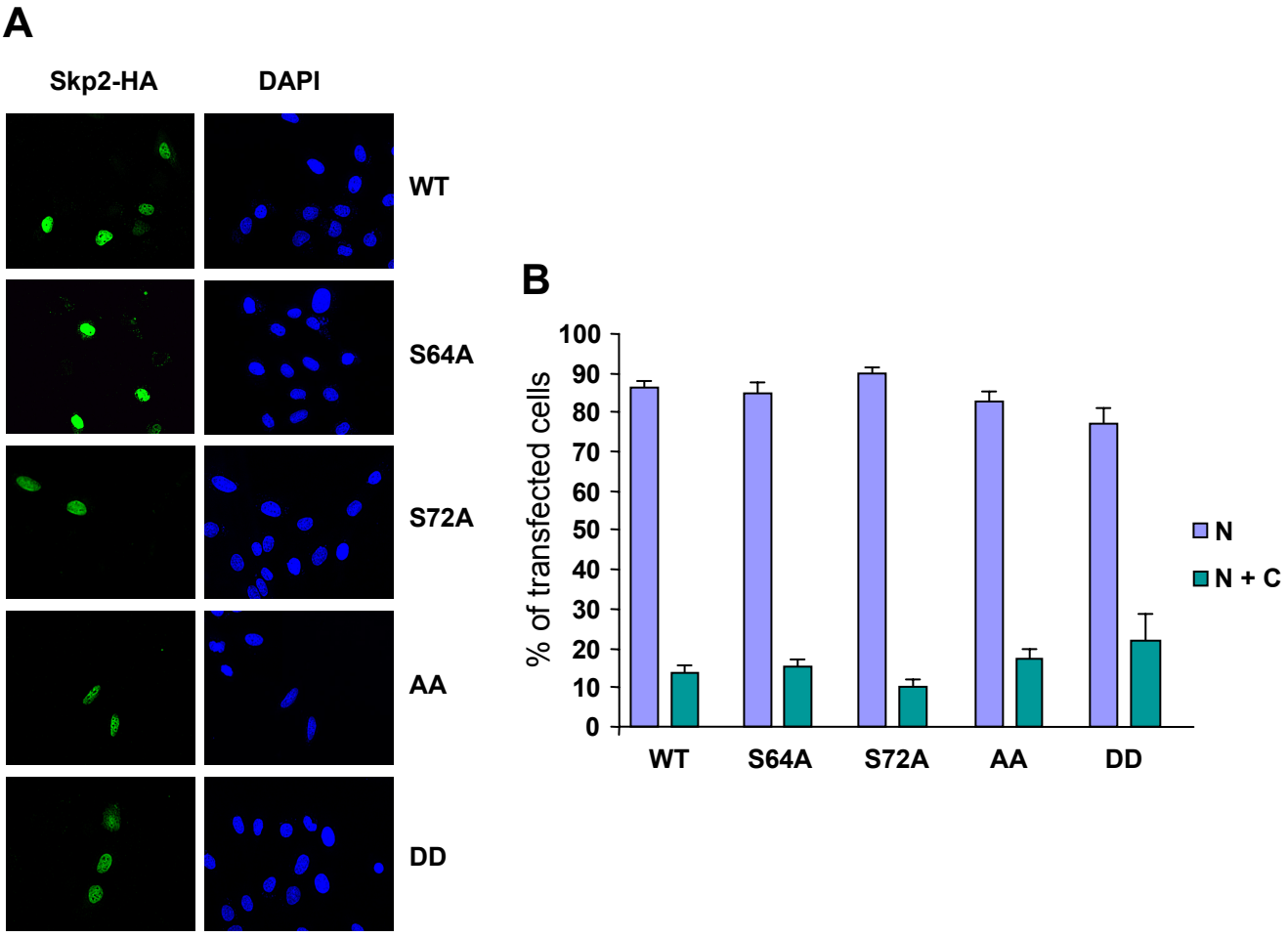

Figure S2. Phosphorylation of Skp2 on Ser64/Ser72 does not influence its subcellular localization

(A) Rat1 cells were transfected with the indicated Skp2 constructs and the localization of the ectopic proteins was analyzed by immunofluorescence with anti-HA antibody (left panels). Nuclei were stained with DAPI (right panels). Wild type and phosphorylation mutants of Skp2 are found mainly in the nucleus.
(B) Quantification of Skp2 subcellular localization. Cells showing either exclusive nuclear (N) or both nuclear and cytoplasmic (N + C) staining were counted. Results are expressed as the mean of 3 experiments with more than 100 transfected cells scored for each coverslip.

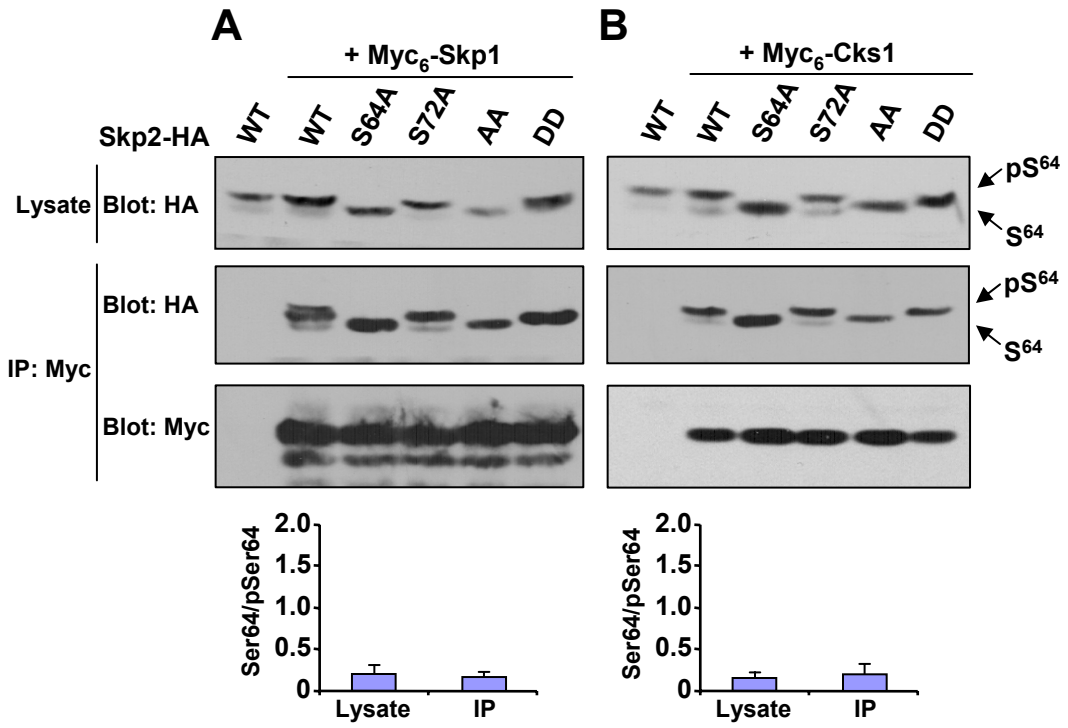

Figure S3. Interaction of Skp2 with Skp1 and Cks1 is not affected by phosphorylation of Ser64/Ser72

293 cells were co-transfected with wild type Skp2-HA or indicated mutants together with Myc_6-Skp1 (A) or Myc_6-Cks1 (B). After 48 hr, the cells were lysed and Myc immunoprecipitates were analyzed by immunoblotting. The ratio of unphosphorylated Ser64 to phospho-Ser64 was quantified for wild type Skp2 protein in the lysate and Myc-immunoprecipitate from three independent experiments.

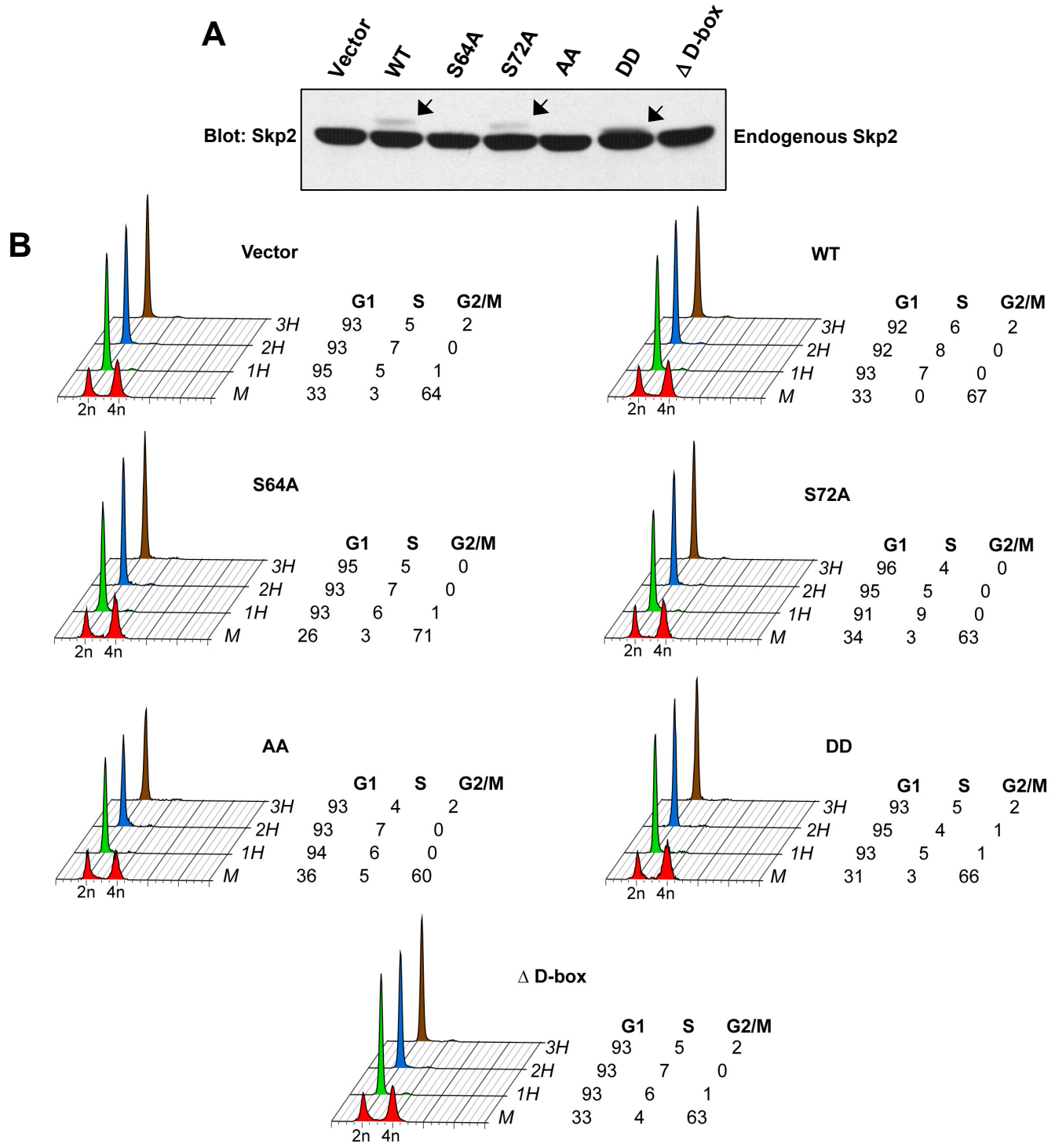

Figure S4. Low ectopic expression of Skp2 does not affect the kinetics of mitosis exit in HeLa cells

(A) HeLa cells were transfected with the indicated Skp2 constructs. Lysates from exponentially growing cells were analyzed by immunoblotting. Small arrows indicate the position of ectopic Skp2. Note that Skp2 S64A, AA and ΔD-box co-migrate with the endogenous protein. (B) HeLa cells were transfected as in A and synchronized at the G1/S border by a double thymidine block. Around 8 hr after release from the block, round mitotic cells were harvested by mitotic shake-off (M) and plated onto new dishes. Adherent cells were then trypsinized at the indicated times and processed for flow cytometry. Transfected cells were identified by co-transfection of eYFP. Note that in these experimental conditions (without nocodazole), cells obtained by shake-off are a mixture of mitotic (4n) and post-mitotic (2n) cells.

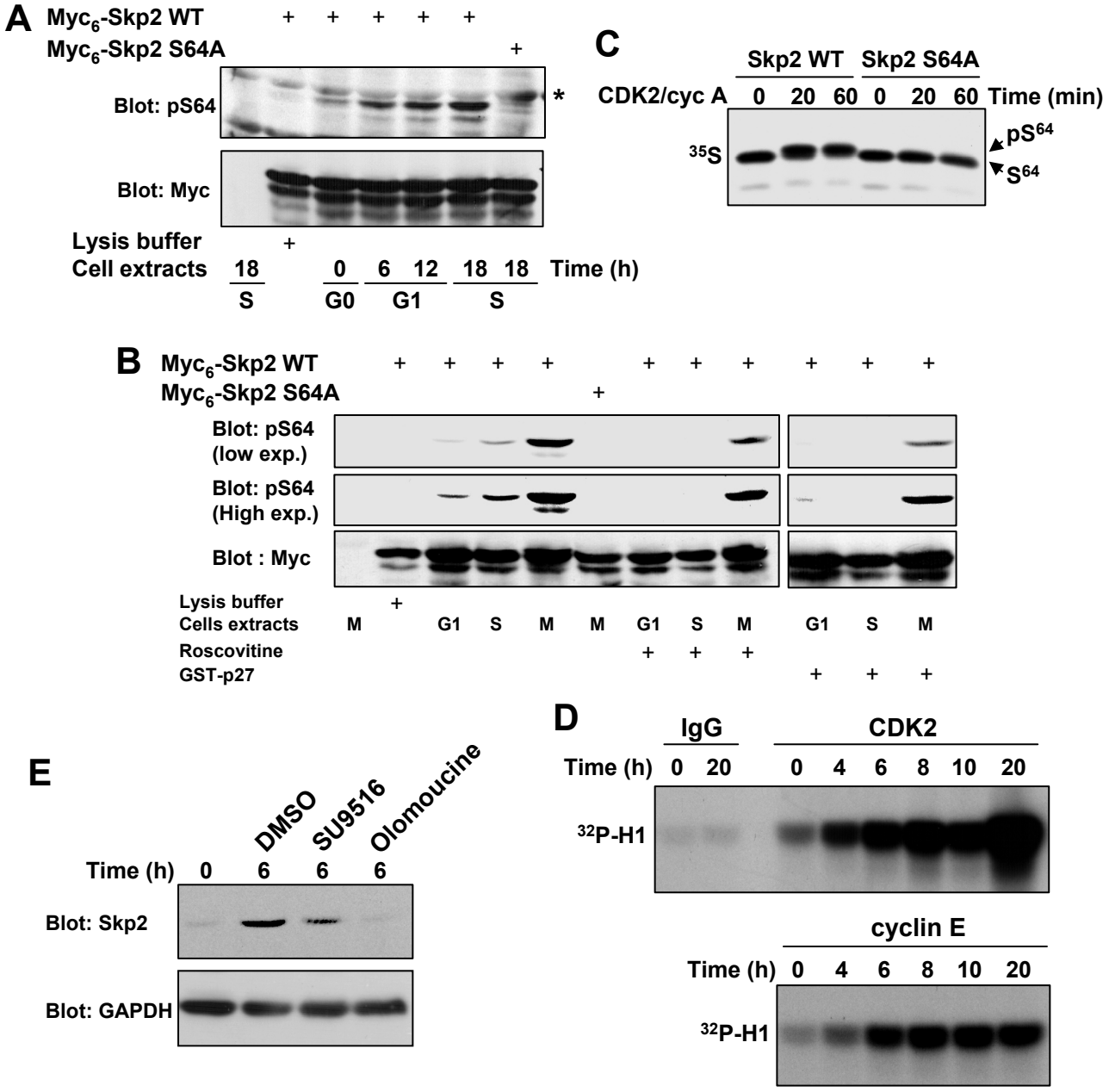

Figure S5. CDK2 phosphorylates Skp2 on Ser64 leading to its stabilization

(A) *In vitro* translated Myc_6-tagged wild type Skp2 or S64A mutant was incubated in kinase buffer in the presence of extracts from Rat1 cells synchronized in G0 by contact inhibition and released for the times indicated. The phosphorylation of Skp2 was monitored by immunoblotting with phospho-Ser64 specific antibody.
(B) *In vitro* translated wild type Myc_6-Skp2 or S64A mutant was incubated with extracts from HeLa cells synchronized in G1, S or M phase. When indicated, roscovitine (150 μM) or GST-p27 (80 nM) was added to the kinase assay. Phosphorylation of Skp2 was analyzed as in A.
(C) ^{35}S-labeled *in vitro* translated wild type Skp2 or S64A mutant was incubated in kinase buffer with active recombinant CDK2/cyclin A. The reaction mixture was analyzed by autoradiography. Note the shift associated with Ser64 phosphorylation.
(D) Contact-inhibited Rat1 cells were released into G1 by replating the cells at low density for the indicated times. Histone H1 kinase activity associated with CDK2, cyclin E or control IgG was measured by immune complex kinase assay.
(E) Rat1 cells synchronized as in D were released into G1 in the presence of vehicle (0.1% DMSO) or of the CDK2 inhibitors SU9516 (5 μM) or olomoucine (200 μM) for 6 hr. The expression of Skp2 was analyzed by immunoblotting.

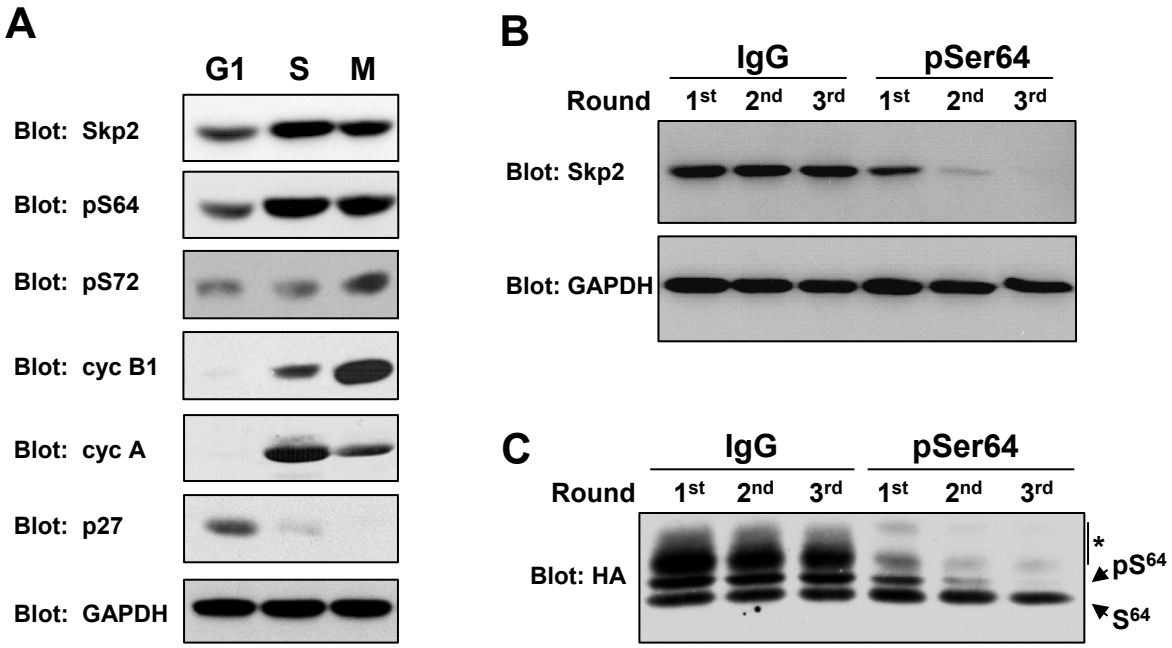

Figure S6. Skp2 is stoichiometrically phosphorylated on Ser64 in cells

(A) HeLa cells were synchronized in G1, S and M phase. Cell lysates were analyzed by immunoblotting with the indicated antibodies.
(B) Cellular extracts from exponentially proliferating HeLa cells were subjected to three consecutive rounds of immunodepletion with control IgG or phospho-Ser64 antibody. The resulting lysates were analyzed for Skp2 and GAPDH expression. As a control of specificity, the phospho-Ser64 antibody did not immunodeplete the non phospho-Ser64 form of Skp2-HA expressed in NIH 3T3 cells (C). *, IgG leakage from protein A-beads.

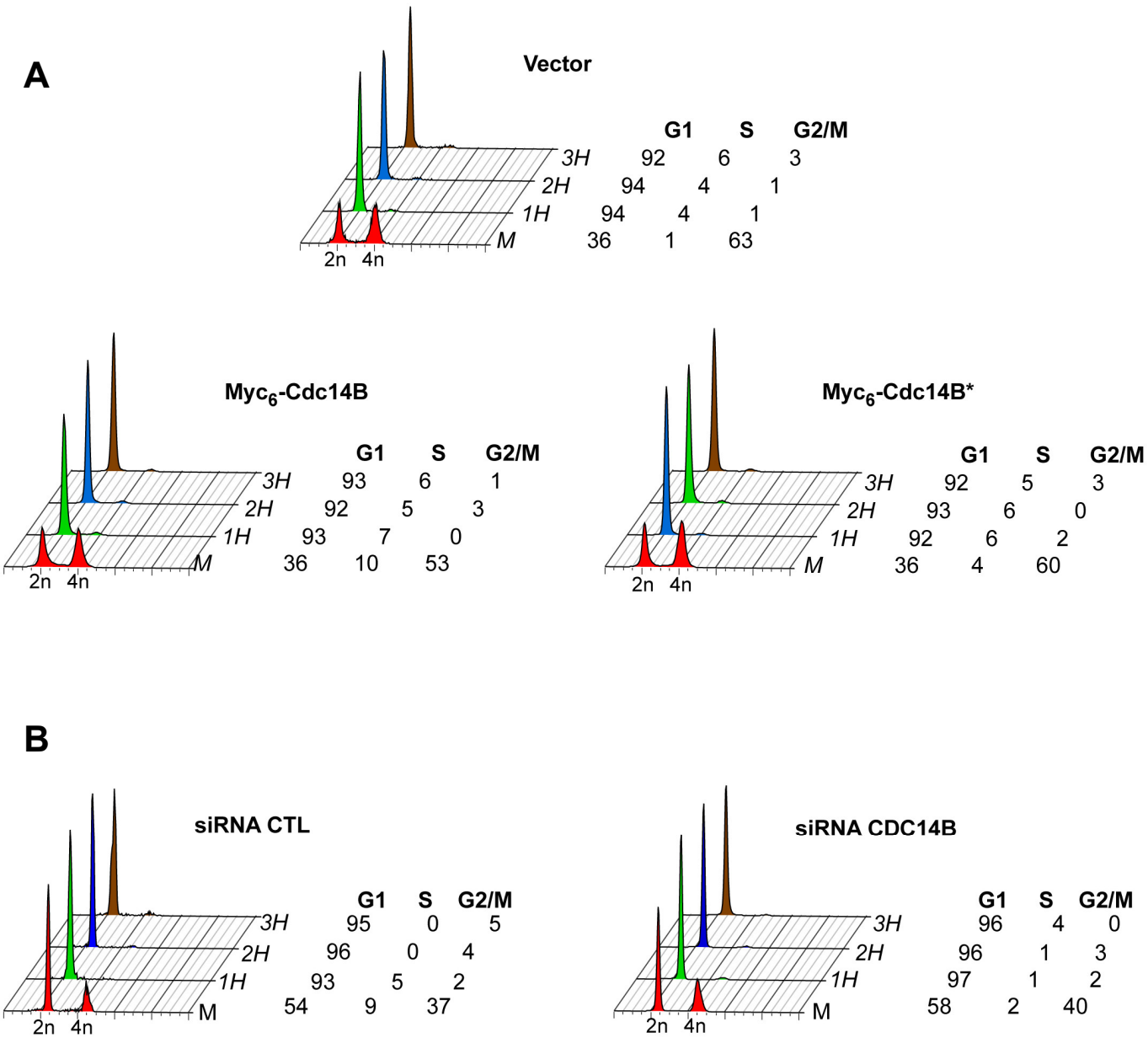

Figure S7. Modulating Cdc14B levels does not perturb mitosis exit in HeLa cells

HeLa cells were transfected with the indicated constructs (A) or siRNAs (B) and synchronized at the M/G1 transition as described in Figure S4. Mitosis exit was analyzed by flow cytometry.

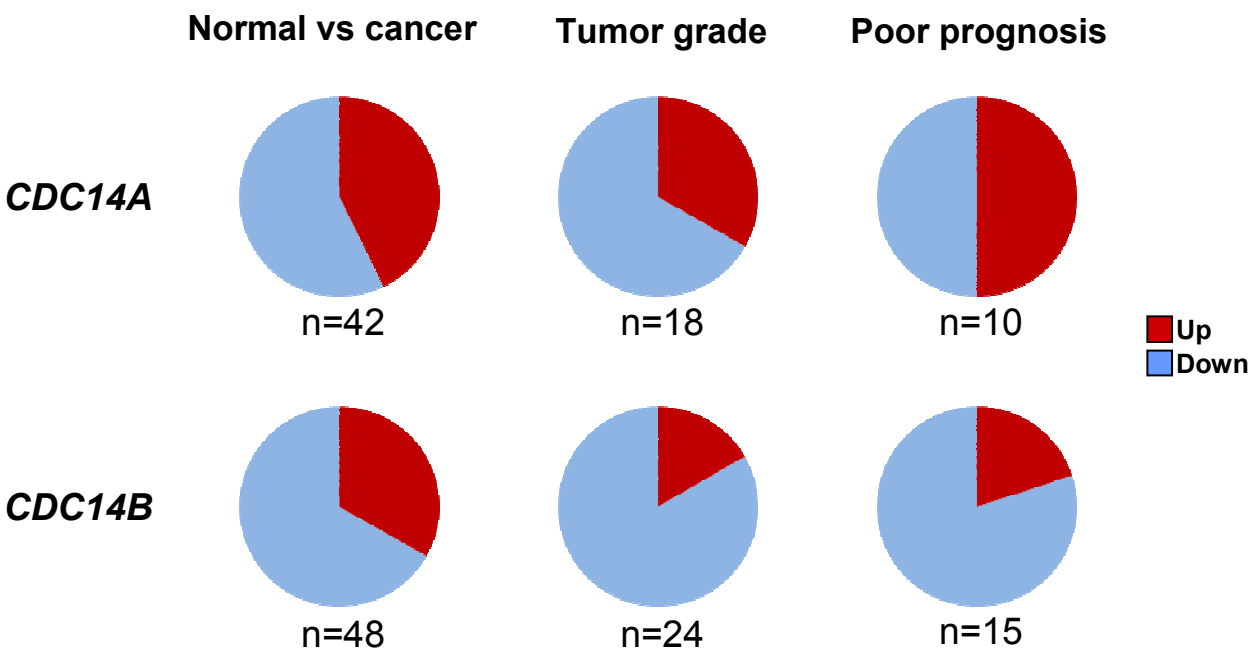

Figure S8. Downregulation of *CDC14B* is associated with tumorigenesis, cancer progression and poor prognosis

Differential expression of *CDC14A* and *CDC14B* genes in human cancer tissues analyzed with microarrays and compiled in the Oncomine database (www.oncomine.org). Illustrated is the proportion of studies showing significant ($p<0.05$) differences in the expression of *CDC14A* and *CDC14B* mRNAs (up- or down-regulation) in normal versus cancer tissues (left panels), in association with tumor grade (middle panels) or associated to poor prognosis (right panels). The number of analysis with statistical significance is indicated under the graph. See Oncomine website for references to primary studies.