SUPPLEMENTAL DATA

Regulation of Aurora B kinase by the lipid raft protein Flotillin-1

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Content: 10 Supplemental Figures with Figure Legends

Supplemental Figure S1

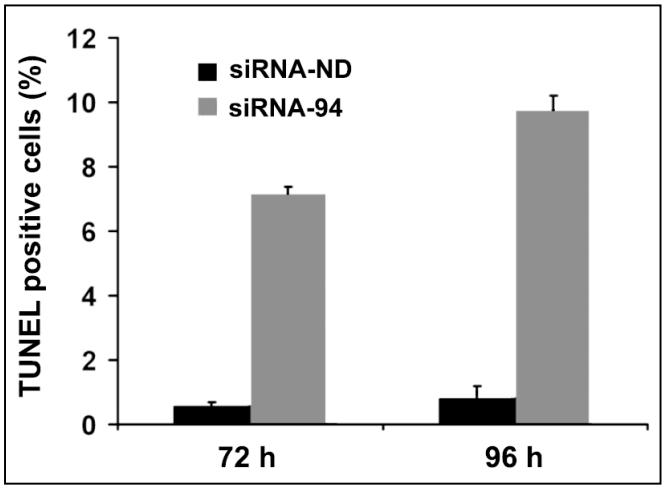


Figure S1. Apoptosis induced by siRNA depletion of Flotillin-1 72 and 96 h after transfection. Shown is the percentage of TUNEL-positive HeLa cells. *, $P < 0.05$ (Student's t-test).

Supplemental Figure S2

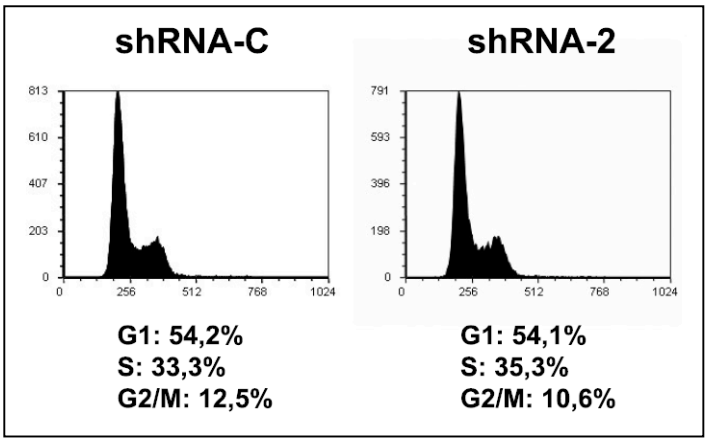


Figure S2. Cell cycle analysis of cells depleted of Flotillin-1 by shRNA-2 and control shRNA-C cells. Control and Flotillin-1 depleted cells (1 x 10^6 cells) were trypsinized, washed, and incubated with propidium iodide (25 μg/ml) and analyzed for DNA content. One representative experiment of two performed in duplicate samples is shown.

Supplemental Figure S3

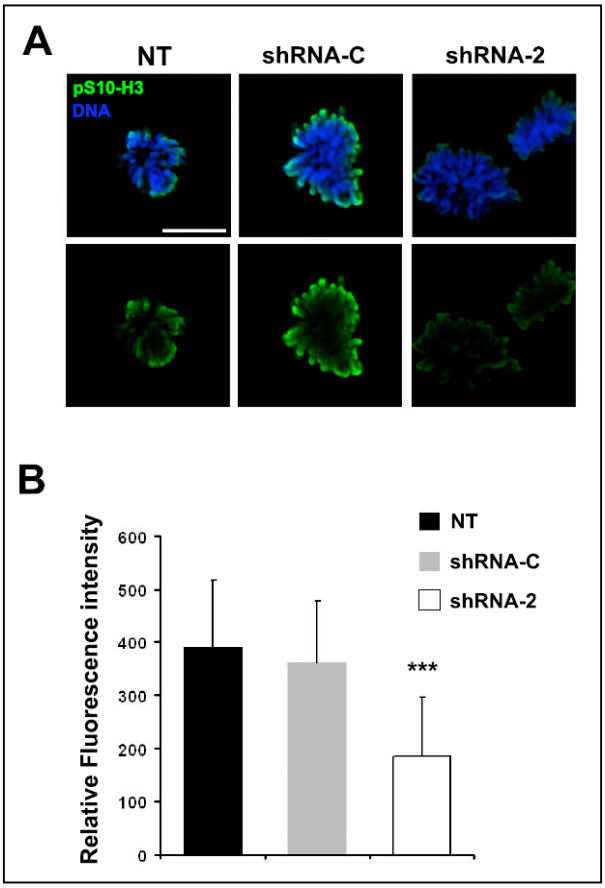


Figure S3. Flotillin-1 depletion causes a decline in total phospho-histone H3 (Ser10). A) Cells transduced with shRNA-2 or control shRNA were stained for pS10-H3 and DNA (Hoechst 33258) and analyzed by confocal microscopy. Scale bar, 10µm. B) 20 to 30 cells for each experimental condition were selected and analyzed individually. Average fluorescence intensity and area values were obtained and integration of both parameters was considered the total fluorescence level per cell. Results are shown as the mean and standard deviation for each group of values. ***, $P < 0.001$.

Supplemental Figure S4

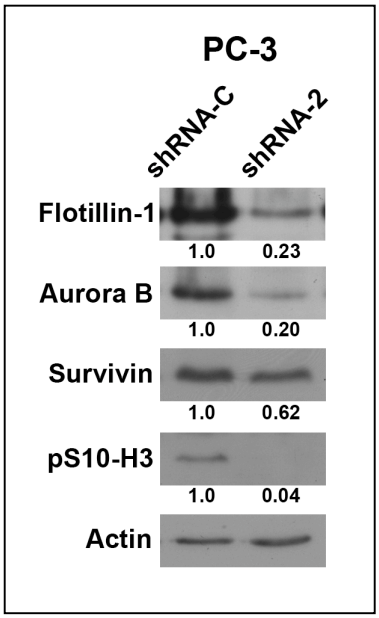


Figure S4. Knockdown of Flotillin-1 in PC-3 prostate cancer cells affects Aurora B protein levels and activity. PC-3 cells were treated with shRNA-2 or control and equal amount of cell lysates (30 μg) were analyzed by Western blotting with the indicated antibodies. Actin is used for normalization of protein levels.

Supplemental Figure S5

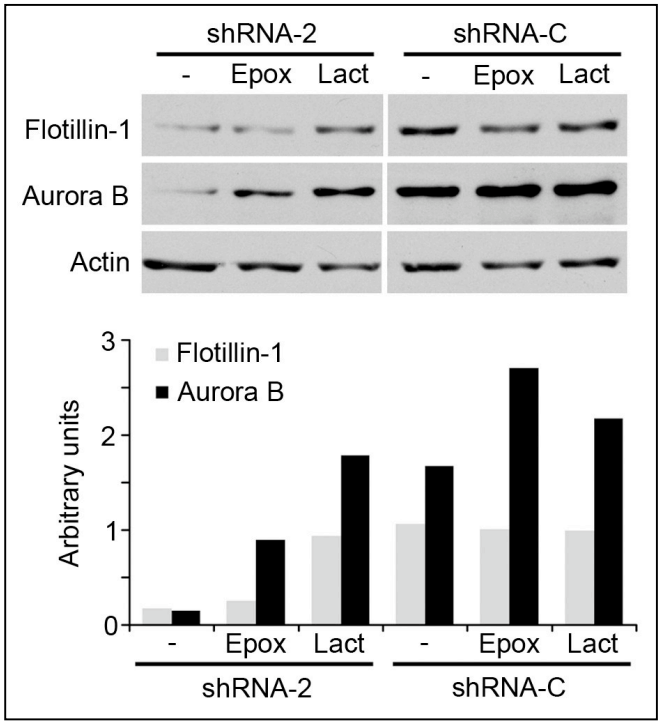


Figure S5. Treatment with proteasome inhibitors (1μM epoxomycin or lactacystin) for 2 h prevents the downregulation of Aurora B caused by Flotillin-1 knockdown. The two panels are from the same original experiment. Bottom panel: quantification of normalized Western blotting signals of Aurora B and Flotillin-1 by scanning densitometry.

Supplemental Figure S6

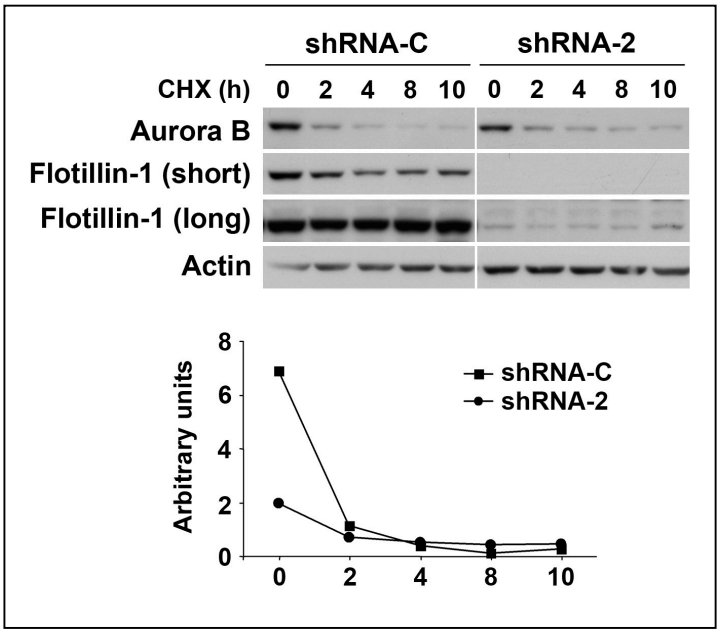
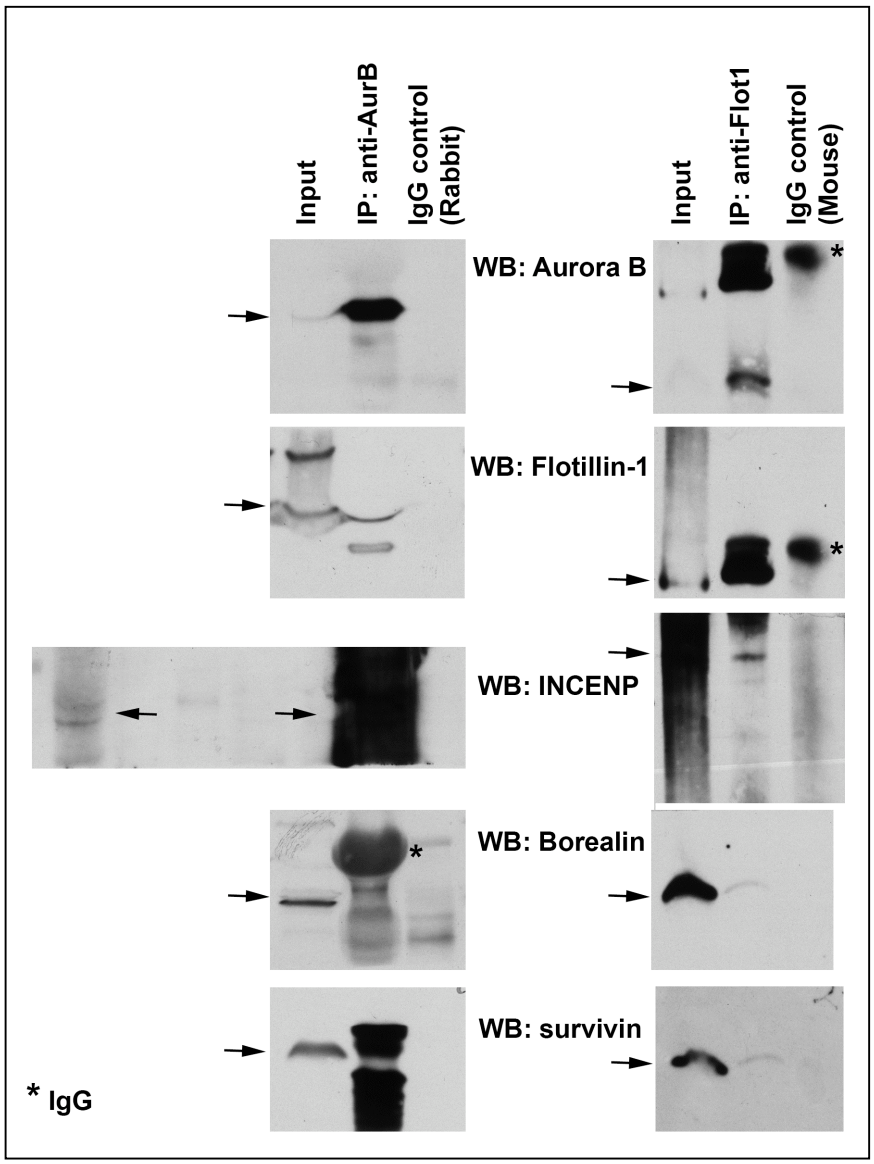


Figure S6. Cells depleted for Flotillin-1 and controls were treated with cycloheximide (100 μg/ml) for indicated periods and equal amounts of protein extracts (30 μg) were analyzed by Western blotting. Bottom panel: quantification of normalized Western blotting signals for Aurora B and Flotillin-1 by scanning densitometry. The estimated Aurora B half-life was 1.7 h in control cells and 1.75 h in Flotillin-1-depleted cells .

Supplemental Figure S7



Supplemental Figure S8

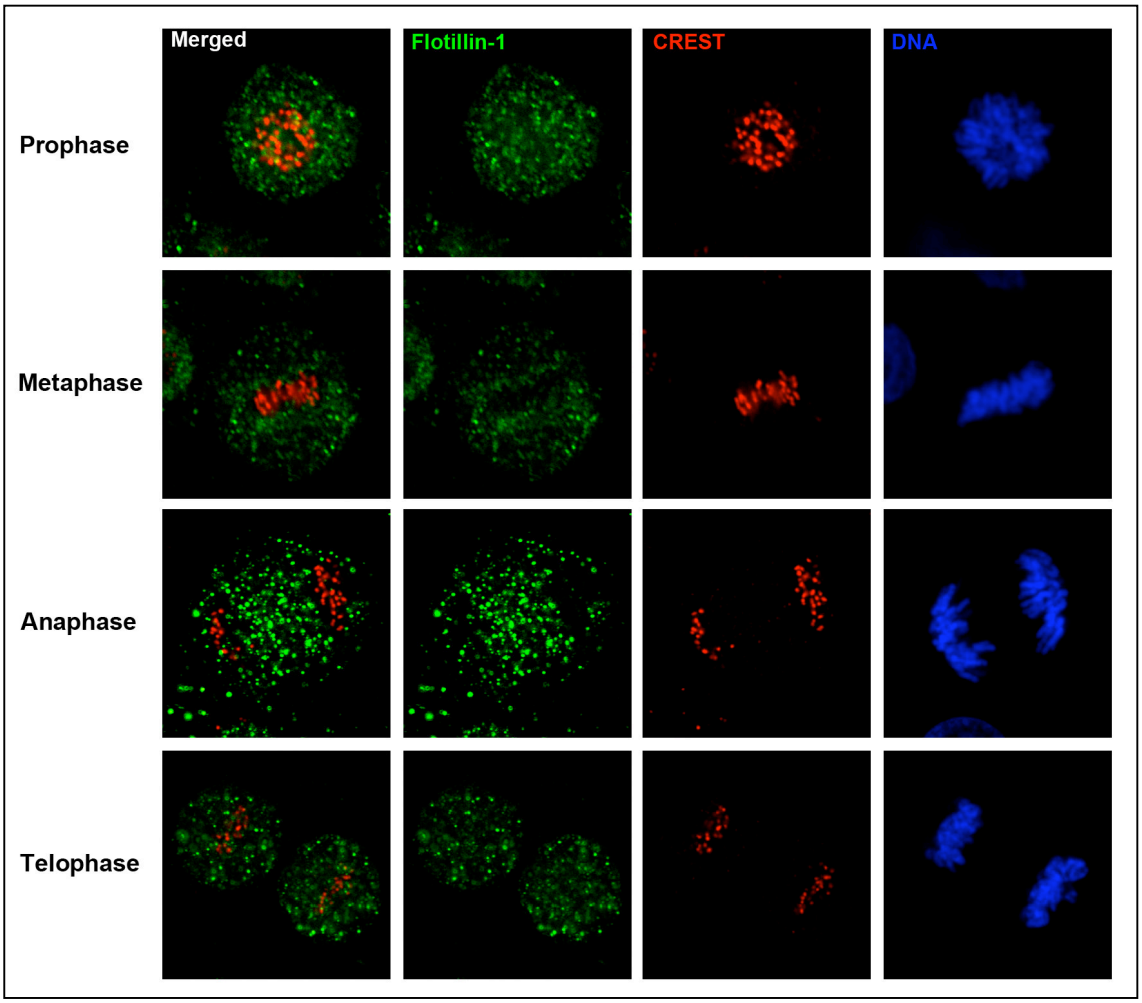


Figure S8. Localization of Flotillin-1 in mitotic HeLa cells. Cells were stained with antibody to Flotillin-1 (green) and with serum from a patient with CREST to identify kinetochores (red). DNA was stained with Hoechst 33258 and images captured by confocal microscopy. Shown are examples of stained cells in different phases of mitosis.

Supplemental Figure S9

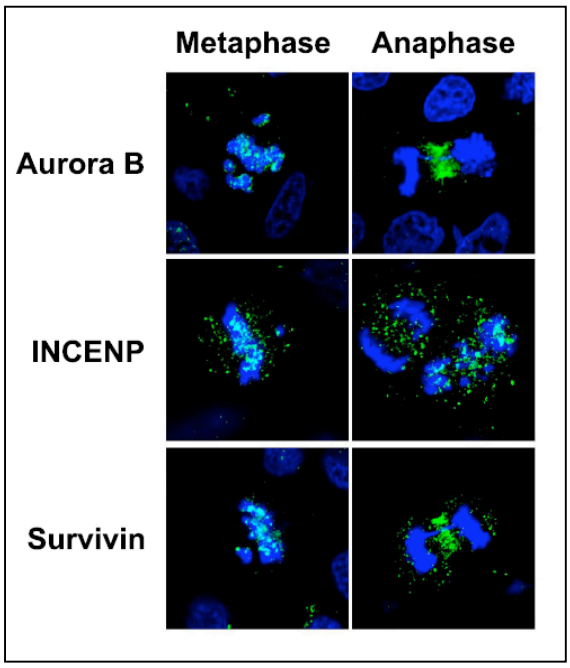


Figure S9. Localization of Aurora B, INCENP and survivin in mitotic HeLa cells depleted of Flotillin-1. DNA was stained with Hoechst 33258 (blue) and images captured by confocal microscopy. Images from cells in metaphase and anaphase show that localization of CPC proteins is as expected, in spite of the aberrant chromosome segregation.

Supplemental Figure S10

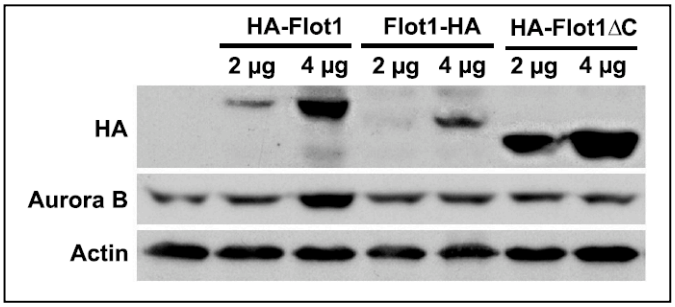


Figure S10. Overexpression of forms of Flotillin-1 that retain their capability of nuclear localization (HA-Flot1), but not of forms defective in nuclear localization (Flot1-HA and HA-Flot1ΔC), increased Aurora B protein levels. HeLa cells transfected with the indicated plasmids were analyzed by Western blotting with indicated antibodies. Signal for actin was used as control for protein loadings.