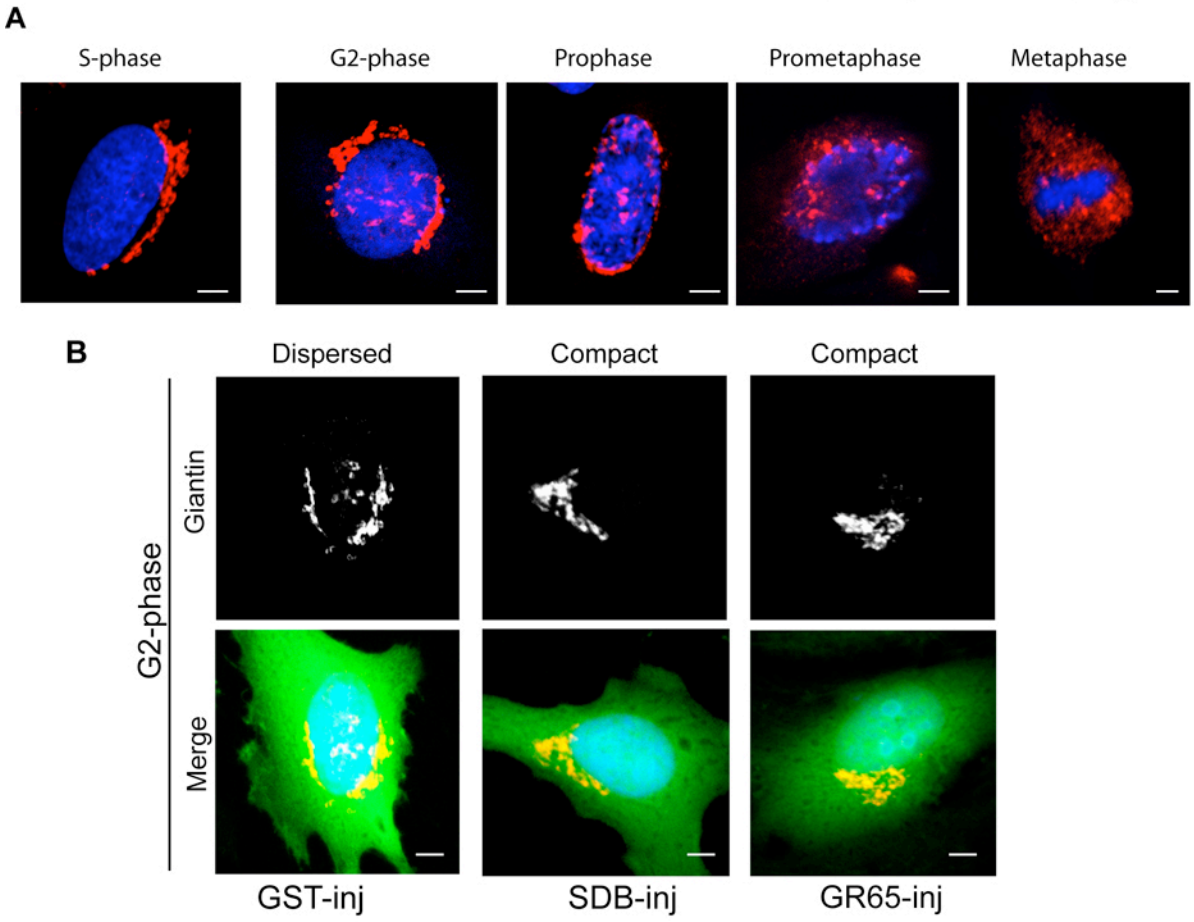
Persico *et al.*, Supplementary Figure 1



C Distribution of Golgi phenotypes:

	GST-inj	SDB-inj	GR65-inj
Dispersed	41	11	13
Compact	19	49	47
Total	60	60	60

Supplementary Figure S1. Morphology of Golgi membranes in HeLa cells through the cell cycle. (A) HeLa cells were seeded on glass coverslips and synchronised for cell-cycle arrest at the G1/S boundary by double-thymidine S-phase block. The cells were then fixed and processed for immunofluorescence either during the S-phase block (S-phase) or 12 h after the S-phase block release. Representative images of cells at the indicated cell-cycle stages labelled with antibodies against giantin (red; Golgi complex) and with Hoechst (blue). (B) One hour after the S-phase block release, the cells were microinjected with fluorescent dextran as injection marker, together with recombinant GST (8 mg/ml), SBD (8-12 mg/ml), or GRASP65 (GR65; 8-10 mg/ml). The cells were fixed at the mitotic peak (12 h after S-phase release) and processed for immunofluorescence. Representative images of non-mitotic injected cells labelled with antibodies against giantin and with Hoechst (blue). The imaging conditions have been set to reveal also less bright Golgi objects. (C) Distribution of Golgi phenotypes as illustrated in (B). Scale bars, 5 μm.

Persico *et al.*, Supplementary Figure 2

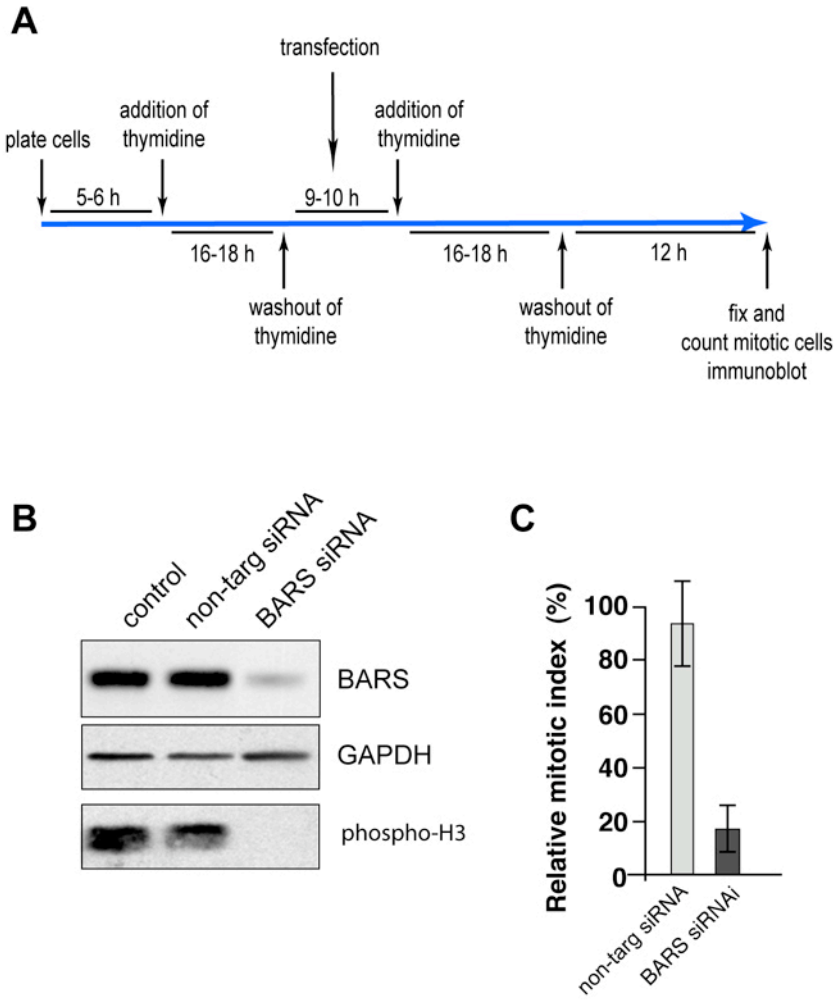


Figure S2. BARS knockdown induces a cell-cycle block in synchronised HeLa cells. (A) Cells were grown on coverslips, and transfected for 36 h with 100 nM non-targeting siRNAs or 100 nM BARS siRNAs, after the first thymidine washout (see Methods). (B) Representative experiment of cells treated as in (A) and processed immediately for immunoblot analysis with antibodies to BARS, GAPDH and phospho-Histone H3 as a marker for mitosis. (C) Quantification of the mitotic index of cells treated as shown schematically in (A), fixed and labelled with Hoechst for staining of the DNA. Quantification data are means (±SD) from two independent experiments, each carried out in duplicate.

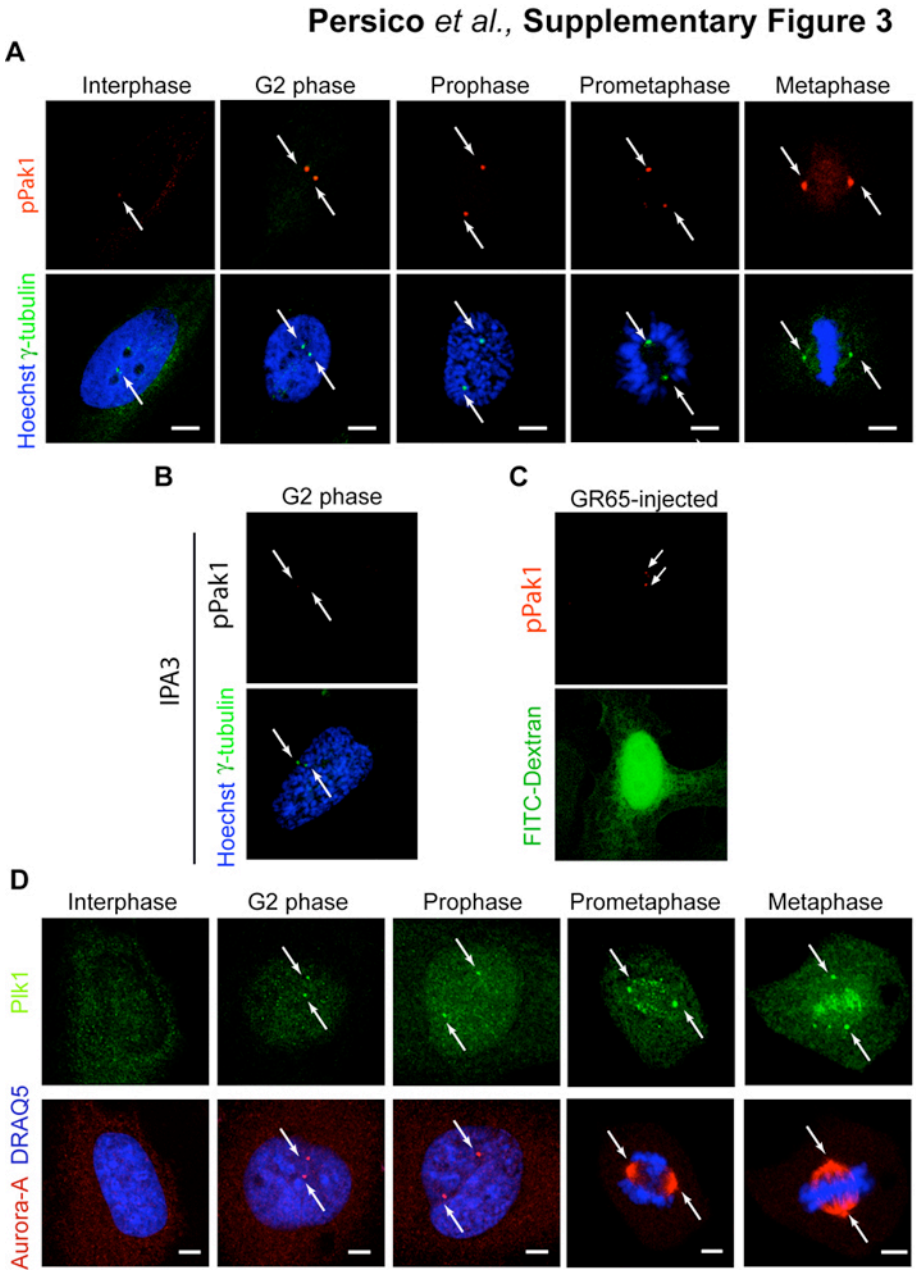


Figure S3. Pak1 and Plk1 are not involved in the mitotic Golgi checkpoint. HeLa cells were grown on coverslips and processed for immunofluorescence after the S-phase block release. (A) Representative images of cells at the indicated cell-cycle stages, labelled with antibodies against γ-tubulin (arrows), and with Hoechst, and labelled with antibodies against Phospho-Pak1/2/3 (T423) (pPak, arrows). (B) Representative images of cells treated with 30 μM IPA3 8 h after S-phase block release, and fixed 4 h later and processed for immunofluorescence with antibodies against T423-phosphorylated Pak (pPak, arrows), γ-tubulin (arrows), and with Hoechst. (C) Double-synchronized cells were microinjected 1 h after thymidine washout, with recombinant GRASP65 (GR65-inj), and with FITC-dextran as microinjection marker. Cells were fixed 12 h after S-phase release labelled with antibodies against T423-phosphorylated Pak (pPak, arrows). (D) Double-synchronized cells were fixed at the mitotic peak. Representative images of cells at the indicated cell-cycle stages labelled with antibodies against Plk1 (arrows), Aurora-A (arrows) and with DRAQ5. Scale bars, 5 μm.

Persico *et al.*, Supplementary Figure 4

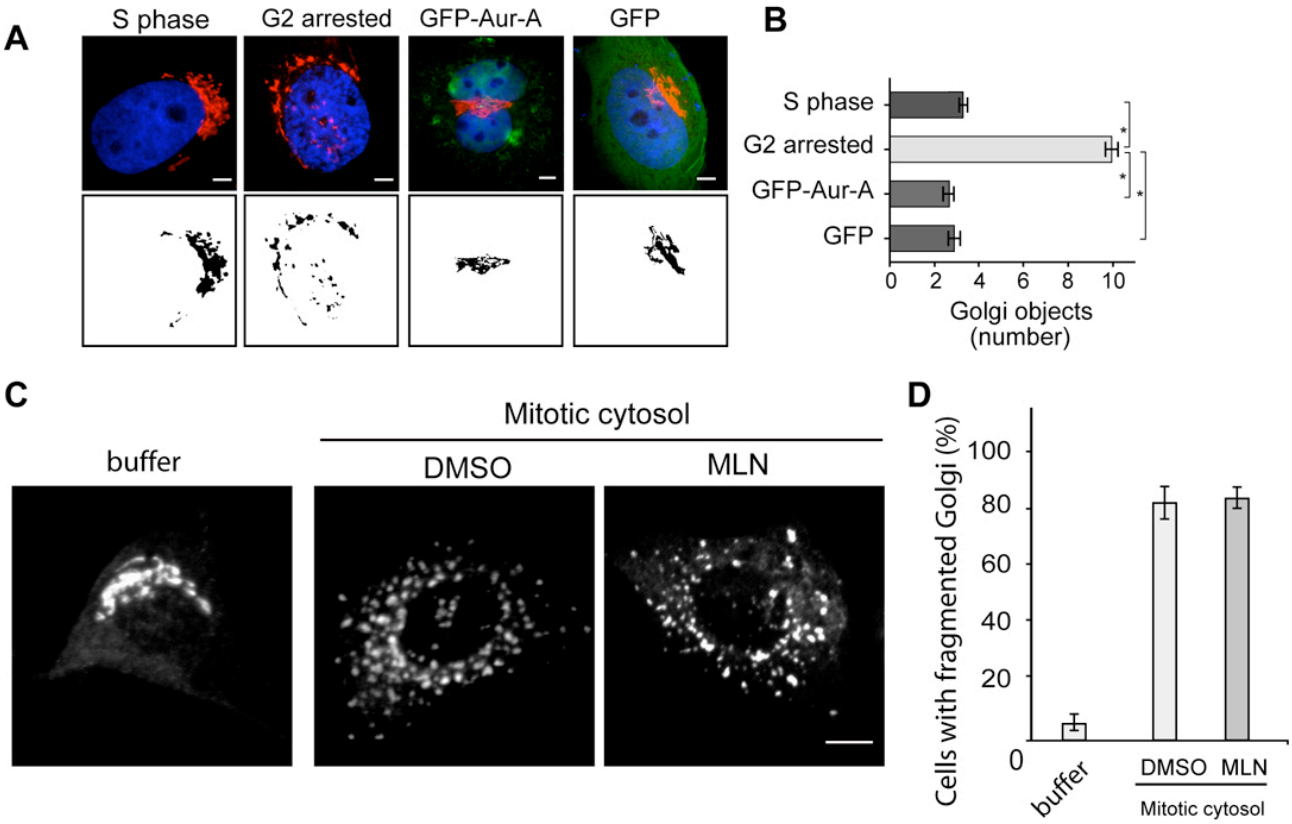


Figure S4. Aurora-A does not induce Golgi fragmentation. (A) HeLa cells were arrested in S-phase using the double-thymidine block were either fixed immediately (S-phase), or treated for 18 h with bisbenzimide (G2 arrested), or trasfected for 24 h with GFP or GFP-Aur-A after the first release from thymidine. Representative images show Hoechst 33342-labeled DNA, the Golgi complex labeled with an antibody against giantin, and trasfected cells detected with an antibody against GFP (upper panel). For qualitative analysis of the phenotype of the Golgi complex (lower panel), the giantin staining was processed with the freeware ImageJ® software package using the Invert function. (B) For quantitative analysis of Golgi integrity in the populations shown in (A), the Golgi-associated fluorescence of randomly chosen cells was acquired. The freeware ImageJ® software package was used to automatically apply a threshold and count above-threshold fluorescent "Golgi objects", using the Analyse Particle function. All images were acquired at maximal resolution, with fixed imaging conditions. Average values (± SEM) from three independent experiments are shown. Two-tailed Student *t*-tests were applied to the data (*: $p < 0.005$). (C,D) NRK cells were grown on coverslips and permeabilzed with digitonin. Permeabilized cells were incubated for 1 h at 32°C with buffer or mitotic cytosol in the presence of vehicle (DMSO) or 1μM of the Aur-A inhibitor MLN8054 (MLN). The cells were fixed and processed for immunofluorescence to label the Golgi complex using an anti-giantin antibody. The structure of the Golgi apparatus was analyzed by immunofluorescence to count the percentage of cells showing a fragmented Golgi apparatus. (C) Representative immunofluorescence images showing permeabilized cells treated with buffer or mitotic cytosol in the presence of vehicle (DMSO) or of MLN (MLN). (D) Quantification of the percentage of cells with fragmented Golgi in digitonin-permeabilized NRK cells, as in (C). The permeabilization procedure and the preparation of mitotic cytosol have been previously described (Acharya *et al.,* Cell, 1998). Data represents means (±SD) from two independent experiments; more than 200 cells per experiment were counted. Scale bar 5 μm.

Persico *et al.*, Supplementary Figure 5

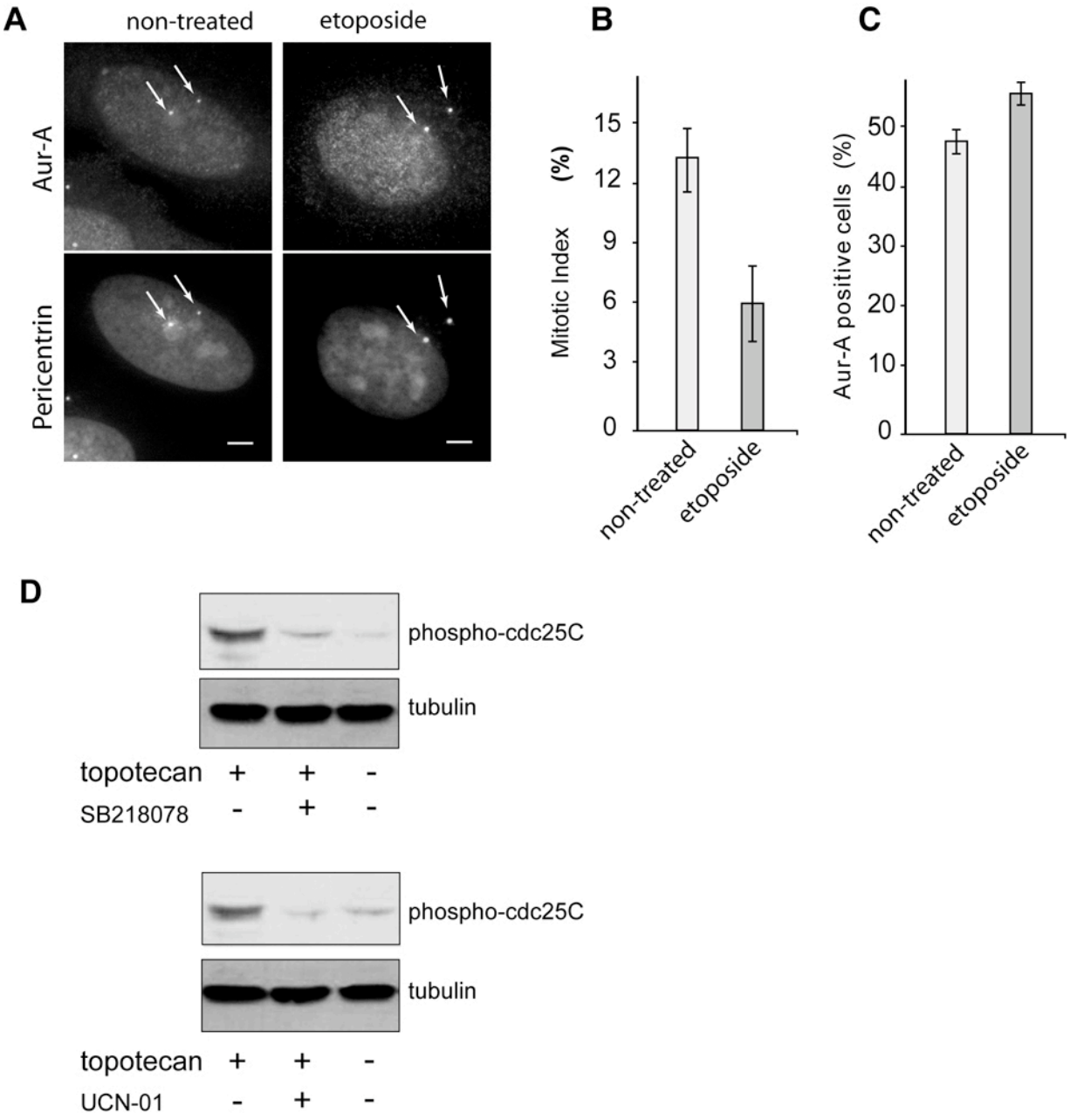


Figure S5. The DNA damage checkpoint is not involved in the Golgi checkpoint. (A) HeLa cells were grown on coverslips and arrested in S phase using the double-thymidine block. (A) Ten hours after S-phase block release, the cells were either left in growth medium (non-treated) or treated with 5 μM etoposide (to induce DNA damage) and fixed and processed for immunofluorescence using antibodies against Aur-A and pericentrin. Cells were also labelled with Hoechst 33342 to determine the mitotic indeces (not shown). (B) Quantification of the mitotic indeces of cells treated as in (A). (C) Quantification of cells as in (A), with percentages of Aur-A positive cells calculated as percentages of cells with Aur-A at the centrosome. Quantification data are means (±SD) from two independent experiments, each carried out in duplicate. More than 200 cells were counted for each condition. (D) HeLa cells were treated for 3 h with 5 μM SB218078 or 300 nM UCN-01 to inhibit Chk1 kinase in the presence of 50 nM topotecan during the last 2 h of incubation to induce DNA damage. At the end of the incubations, the cells were harvested and processed for immunoblot analysis with antibodies against S216-phosphorylated cdc25C (phospho-cdc25C) to monitor Chk1 activity and β-tubulin as a loading marker. Scale bar, 5 μm.

Persico *et al.*, Supplementary Figure 6

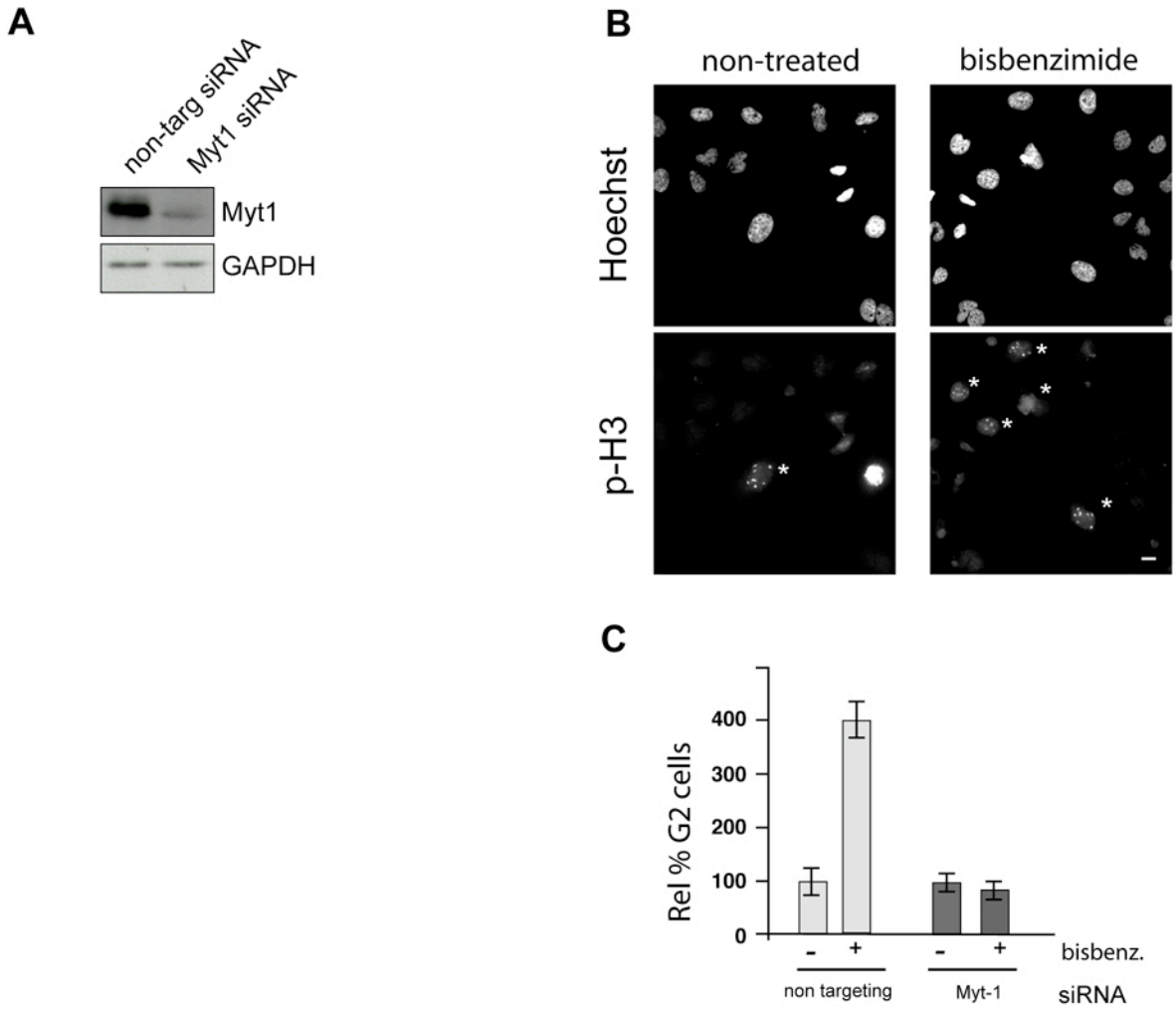


Figure S6. Knockdown of Myt1 overrides a bisbenzimide-induced G2 cell cycle arrest. HeLa cells were grown on coverslips and maintained in growth medium plus 2 mM thymidine for 16 h, and then rinsed and maintained in growth medium for 8 h. The cells were then maintained in thymidine for an additional 16 h. Then, they were rinsed and incubated in medium with bisbenzimide (1 μg/ml Hoechst 33342) for 18 h before fixation. The cells were also transfected for 48 h with 100 nM non-targeting siRNAs or 100 nM Myt1 siRNAs. (A) Representative experiment of cells processed for immunoblotting with antibodies against Myt1 and GAPDH as a loading control. (B) Representative images of cells left non-treated or treated with bisbenzimide. Cells were stained with Hoechst to visualize DNA organization, and with anti-phosphorylated Histone H3 antibody (pH3) to identify cells in the G2 phase of cell cycle (*). (C) Quantification of the relative percentages of G2 positive cells calculated according to the non-treated cells. All images were acquired with fixed imaging conditions. Quantification data are means (±SD) from two independent experiments, each carried out in duplicate. Scale bar, 20 μm.

Persico *et al.*, Supplementary Figure 7

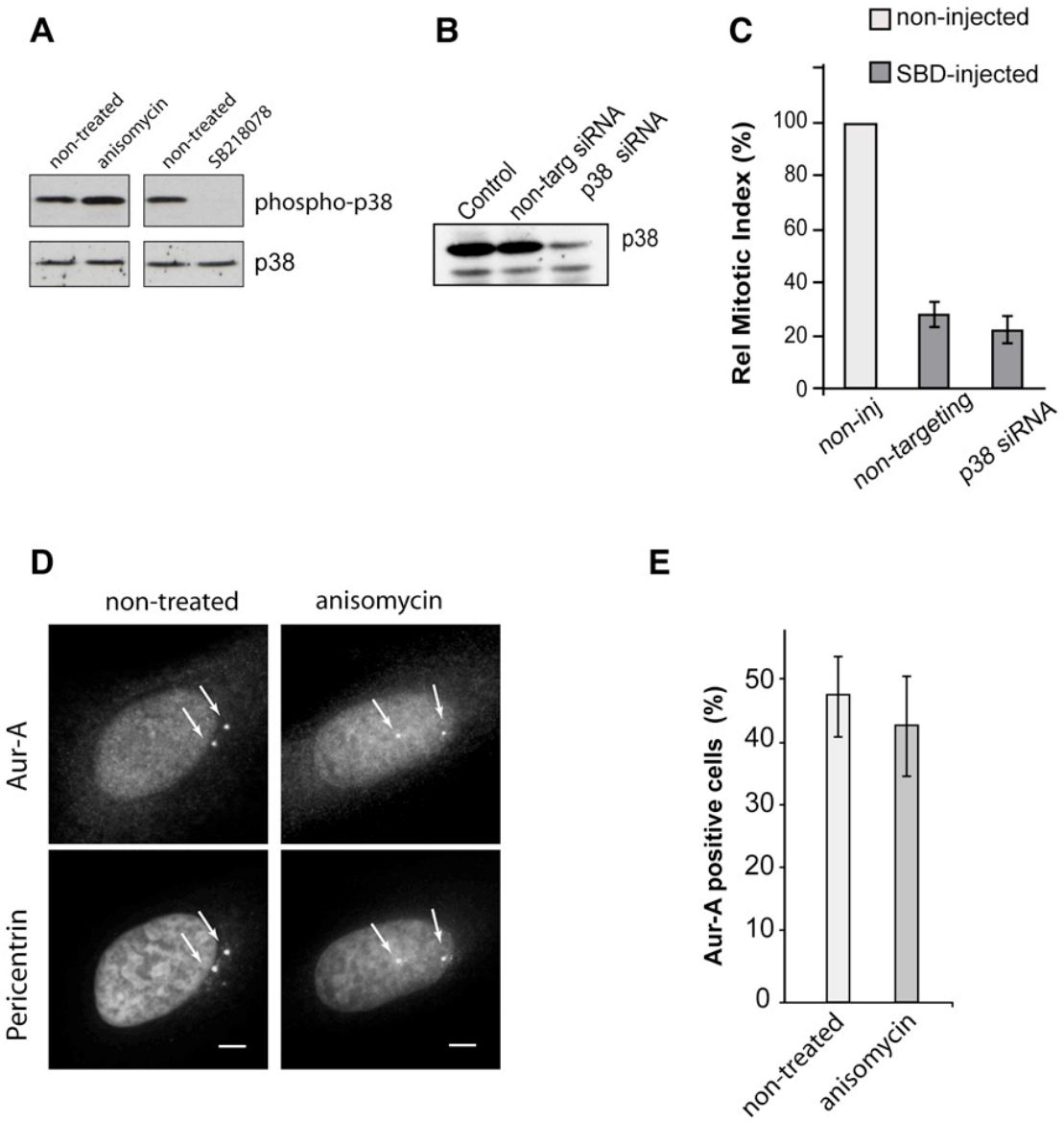


Fig S7. p38 MAP kinase is not involved in the Golgi-dependent block of the cell cycle. (A) HeLa cells were left non-treated or incubated with 75 nM anysomicin to activate p38 MAP kinase or with 5 μM SB218078 and processed for immunoblot analysis with antibodies to phospho-p38 MAP kinase (T180/182) (phospho-p38) and to p38 MAP kinase (p38). (B) HeLa cells were transfected with 30 nM non-targeting siRNAs or 30 nM p38 MAP Kinase siRNAs for 48 h and processed for immunoblot analysis with antibodies against p38. (C) The cells were transfected as in (B) during the first thymidine-block of the cell synchronization protocol. After 1 h without thymidine, cells were microinjected with recombinant GST-SBD and with FITC-conjugated dextran as microinjection marker. Cells were fixed at the mitotic peak and labelled with Hoechst 33342 to monitor cell-cycle phase. The quantification of the relative mitotic index of non-targeting and p38 MAP kinase siRNA transfected cells was calculated after measuring the percentages of microinjected cells in mitosis normalized to non-microinjected cells on the same coverslip. Means (±SD) from three independent experiments. (D) HeLa cells were incubated with 75 nM anisomycin, fixed at the mitotic peak and processed for immunofluorescence microscopy using antibodies against Aur-A and pericentrin. (E) Quantification of the percentage of control (non-treated) and anisomycin-treated cells positive for Aur-A (±SD) from two independent experiments. Scale bar, 5 μm.