Supplemental Figures and Figure legends

BubR1 is modified by sumoylation during mitotic progression

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Figure S1. HeLa cells treated with or without taxol and/or caffeine for 18 hours were collected for preparation of cell lysates. Equal amounts of cell lysates were subjected to analysis by SDS-PAGE followed by blotting with antibodies raised in rabbits (#32, #33, and #35) or with an anti-BubR1 antibody purchased from a commercial source (Santa Cruz). Consistently, four independent antibodies all detected the modified form of BubR1 (arrow BubR1-M).

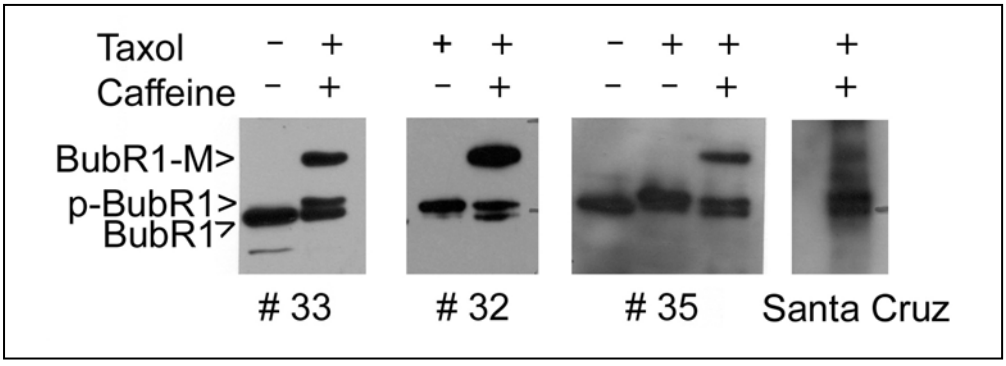
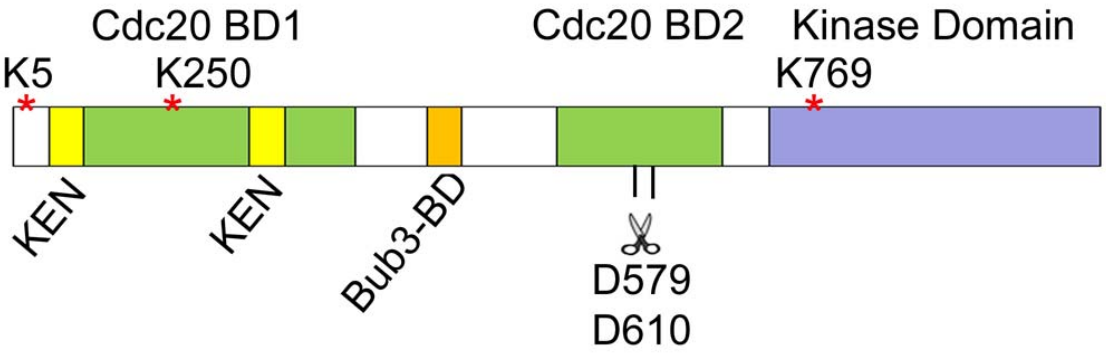


Figure S2. (A) Schematic presentation of BubR1 domain structures. Cdc20 BD1 and Cdc20 BD2 denote Cdc20 binding domain 1 and 2, respectively. Bub3-BD denotes Bub3 binding domain. KEN stands for KEN box. The three candidate lysine residues for sumoylation are highlighted. The cleavage residues (D579 and D610) by caspase 3 are also highlighted.



Domain structures of human BubR1

Figure S3. Ectopic expression of GFP-N-K250R does not induce mitotic slippage. HeLa cells were transfected with a plasmid expressing either GFP-tagged wild-type of N-terminal BubR1 fragment (N-Wt) or its mutant counterpart with K250 replaced with R (N-K250R). GFP expression plasmid was used for transfection as control. After 48 h transfection, HeLa cells were fixed and stained with propidium iodide for flow cytometric analysis. Cell cycle distributions of GFP-positive cells were obtained and summarized. NT denotes the cell population expressing no transfected proteins.

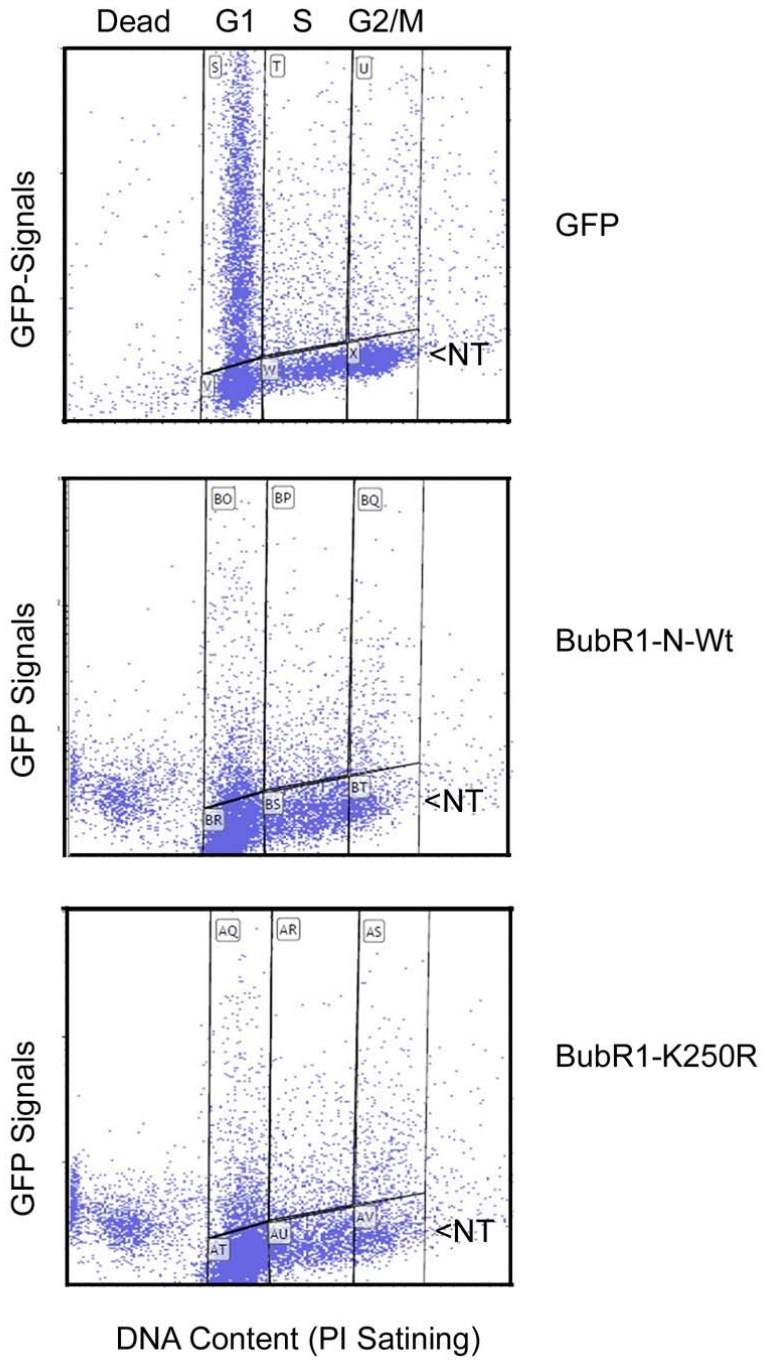


Figure S4. Flow cytometric analysis of cells treated with nocodazole and taxol. HeLa cells treated with nocodazole (40 µg/ml) and taxol (40 nM) for various times as indicated, fixed and stained propidium iodide (50 µg/ml) before analysis by flow cytometry. Cells with sub-G1 DNA content are apoptotic, which increase with prolonged treatment. However, the majority of cells remains arrested at mitosis after treatment for 40 h.

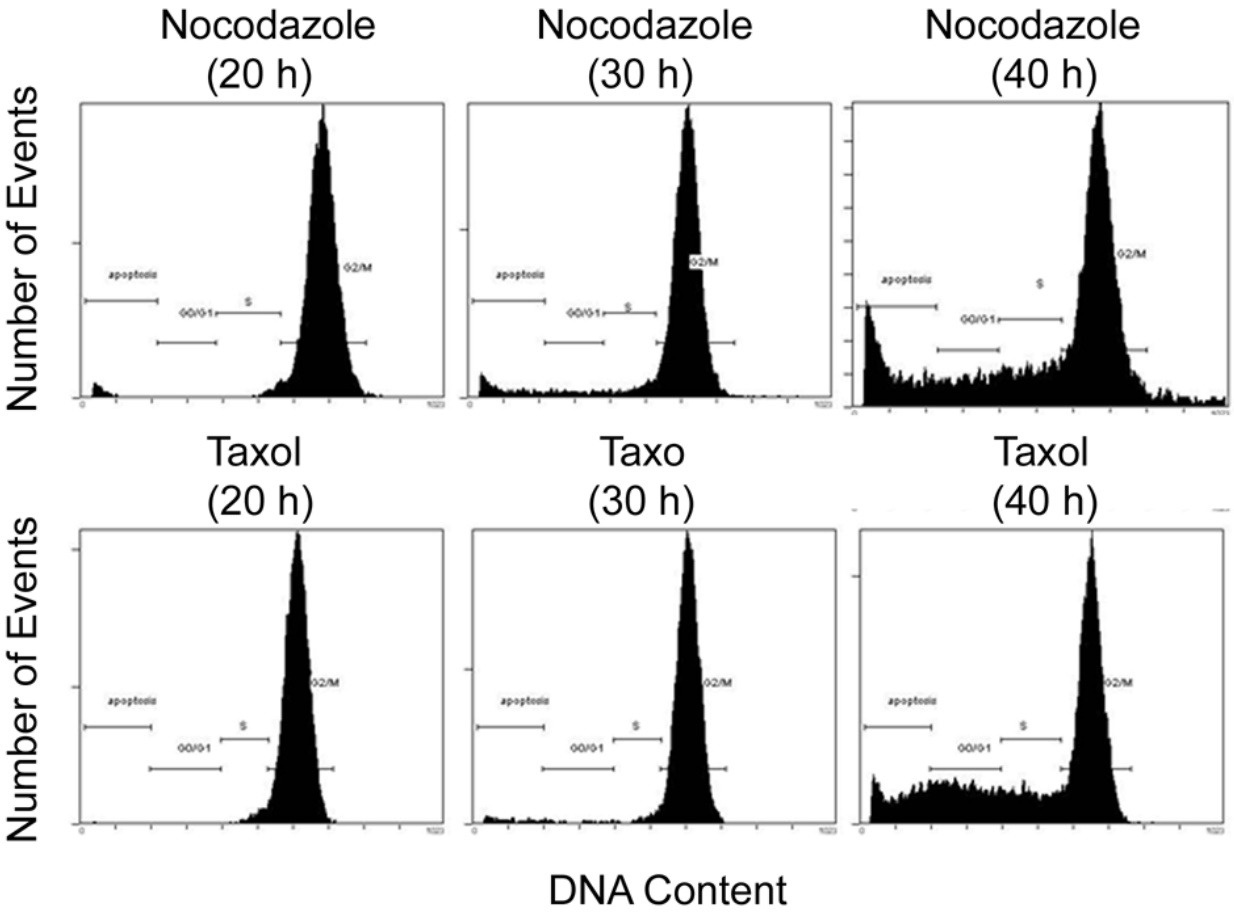


Table 1. Ectopic expression of sumoylation-deficient mutant N-K250R does not induce mitotic slippage. HeLa cells were transfected with a plasmid expressing either GFP-N-Wt or GFP-N-K250R. GFP expression plasmid was used for transfection as control. After 48 h transfection, HeLa cells were fixed and stained with propidium iodide for flow cytometric analysis. Cell cycle distributions of GFP-positive cells were obtained and summarized.

	GFP	GFP-N-Wt	GFP-N-K250R
Apoptotic (%)	1.0±0.4	8.0±1.9	6.0±2.4
G1 (%)	76.0±0.1	55.0±10	51.0±17
S (%)	14.0±0.8	30.0±14	29.0±21
G2/M (%)	9.0±0.1	14.0±4.0	20.0±4.0

Movie 1

HeLa cells transfected with plasmids expressing GFP-tagged BubR1 N-Wt and BubR1 N-K250R for 24 h (or vector) were subjected to time-lapse confocal microscopy. Only the GFP channel (ectopically expressed BubR1 or its mutant) from representative mitotic cells is shown. Please note prolonged localization of BubR1 N-K250R (left panel) at the kinetochores as compared with BubR1 N-Wt (right panel).