Legends to Supplementary Figures

Supplementary Figure S1. Lack of functional JNK expression in JNK1/2$^{-/-}$ fibroblasts

Wild-type fibroblasts or JNK1/2$^{-/-}$ cells were stimulated for 20 min with anisomycin (10 μg/ml) and total lysates were analysed for presence of phospho-JNK variants using a phospho-specific antiserum against JNK1-3. An immunoblot for tubulin is shown as loading control.

Supplementary Figure S2. SP600125 treatment does not induce major mitotic defects and 4N accumulation in U2OS cells cultured in absence of spindle poisons.

(A) DNA profiles of non-synchronized U2OS cells grown in absence or presence of 10 μM SP600125 for 24h. Figures above the profiles denote the percentage of mitotic cells (MI) as determined by p-Histone H3 positivity. (B) Timelapse analysis of synchronized U2OS cultures progressing through mitosis in absence or presence of 10 μM SP600125. Cells were transfected with Histone 2B-GFP for live imaging of chromosome condensation and movements (*lower panels*).

Supplementary Figure S3. SP600125 treatment results in displacement of BubR1 from kinetochores

U2OS cells were transfected with H2B-GFP to visualize nucleus and chromosomes of fixed cells. Cells were synchronized in S-phase by thymidine treatment and released into normal medium or medium containing taxol or nocodazole, respectively. Cells were then either grown for 15h without further drug addition or were co-treated for the last 3h with

SP600125, a combination of SP600125/MG132, or MG132 alone and immunostained with specific antibodies for BubR1 (A) or Mad1 (B) *upper panels* Confocal images of representative staining patterns obtained upon treatment with the indicated drug combinations. *lower panels* Quantification of BubR1 or Mad1 kinetochore localization of prometaphase cells for the different combinations. An average of two independent experiments +SE is shown. For each combination 36-572 cells were scored.

Supplementary Figure S4. SP600125 treatment does not affect kinetochore localisation of Mps1

(A) U2OS cells were arrested in mitosis by overnight treatment with nocodazole treatment and localization of endogenous Mps1 upon co-treatment with MG132 or MG132/SP600125 (for 3h) was analyzed using an Mps1 specific antibody. (B) Localization of the indicated VSV-tagged Mps1 proteins in mitotic U2OS cells. For visualization of the chromosomes, cells in (A) were co-stained with DAPI. In (B) cells were co-transfected with Histone2B-GFP (green) and localization of the indicated exogenous Mps1 proteins were examined by confocal microscopy using a monoclonal antibody against VSV. Panels show overlays of DAPI-stained (A, blue) or Histone 2B-GFP stained chromosomes (B, green) and Mps1 stainings (red).

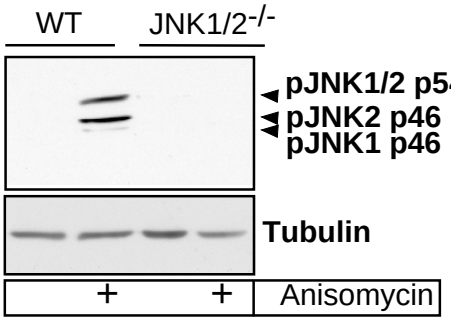
Supplementary Figure S5. RNAi-mediated Mps1 depletion induces a similar phenotype in human cells as SP600125

U2OS cells were co-transfected with spectrin-GFP (A) or Histone 2B-GFP (B) and either empty pRS-puro vector (pRS) or pRS-puro expressing small hairpin RNA (shRNA) against hMps1 (pRS-Mps1), respectively. (A) Cells were grown for 72h without further

treatment and GFP-positive cells were analyzed for pHistone H3 positivity by FACS. In (B) cells were released from a thymidine block into nocodazole (*left*) or taxol (*right*) and fixed 15h after release. Where indicated, MG132 was added for the last 3h prior fixation. Representative BubR1 immunofluorescence stainings of pRS- or pRS-Mps1 transfected prometaphase cells are shown.

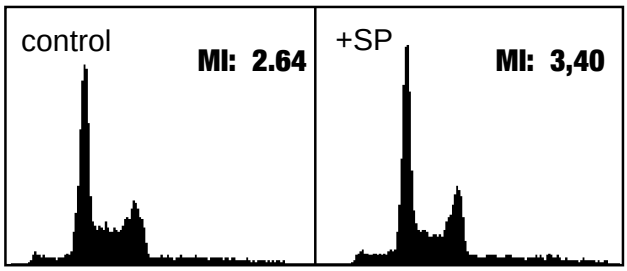
Supplementary Figure S6. Insensitivity of human BJ-tert cells to SP600125-mediated checkpoint override in not due to increased Mps1 expression or a generally lower sensitivity to SP600125

(A) Comparison of endogenous Mps1 protein levels in JNK1/2$^{-/-}$ double-deficient cells, BJ-tert cells and U2OS cells, respectively. As loading control an immunoblot against tubulin is shown. (B) Effect of SP600125 treatment on BubR1 localisation in BJ-tert cells. Cells were released from a single thymidine block into medium containing nocodazole- or taxol alone or together with SP600125 and stained 15h after release using a BubR1 specific antiserum. Chromosomes were visualized by co-staining with DAPI. (C) Comparison of the effective inhibitory concentrations of SP600125 affecting JNK-mediated c-Jun phosphorylation in BJ-tert versus U2OS. Cells were treated as indicated and total lysates were analyzed for c-Jun phosphorylation using a phospho-reactive antiserum against c-Jun. In each case SP600125 was added 15 min prior to anisomycin treatment to allow efficient absorption of the drug. An immunoblot for CDK4 is shown as loading control.



Supplementary Figure S1 Schmidt et al.

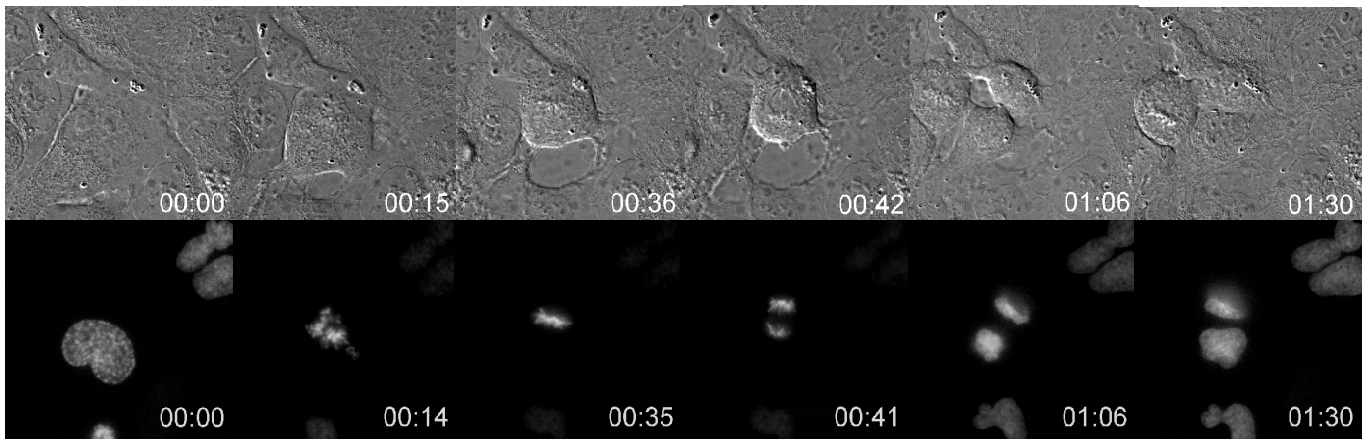
A



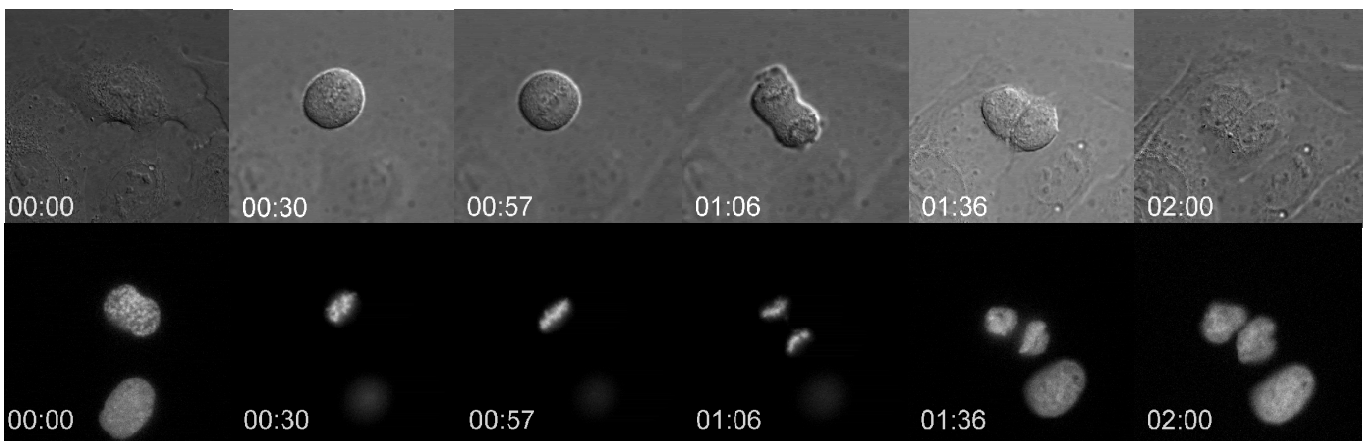
U2OS

B

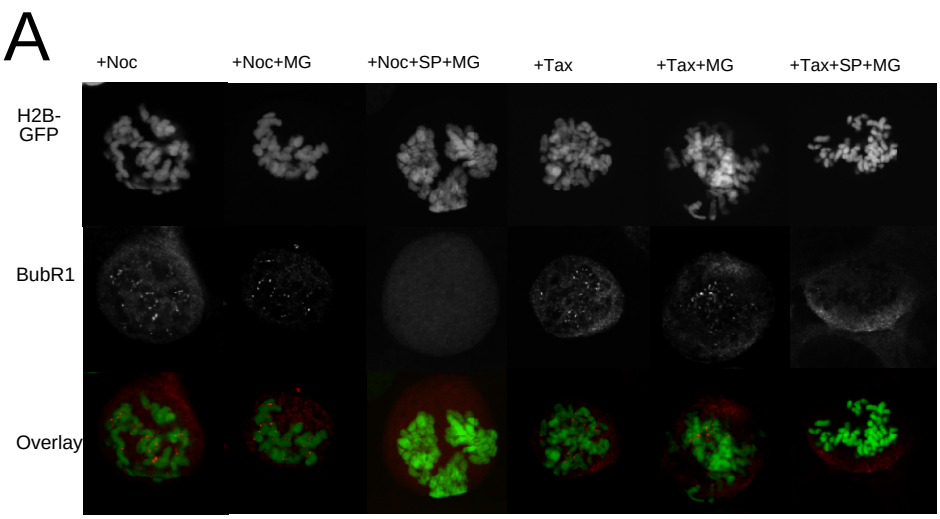
+SP600125

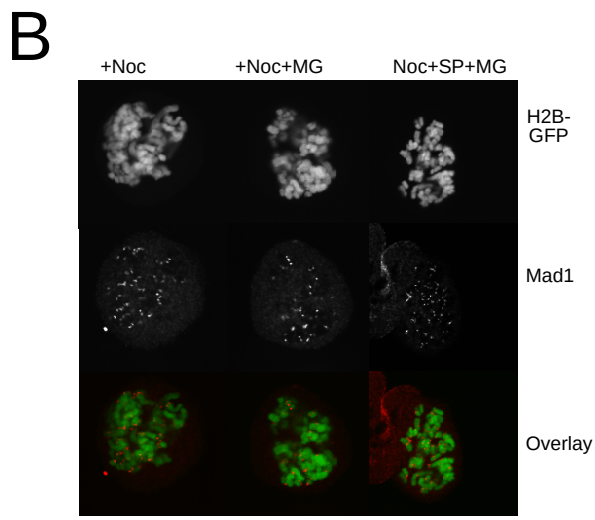


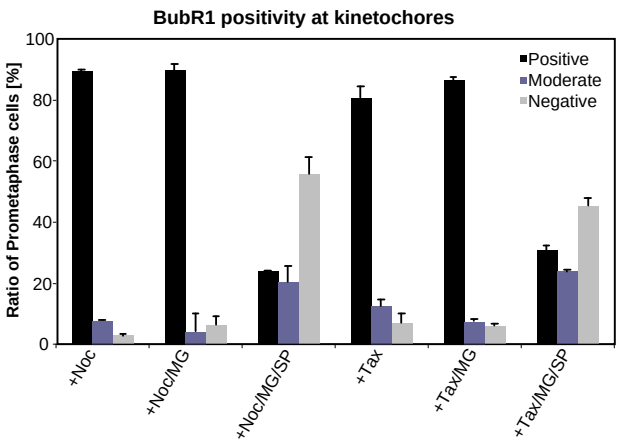
control

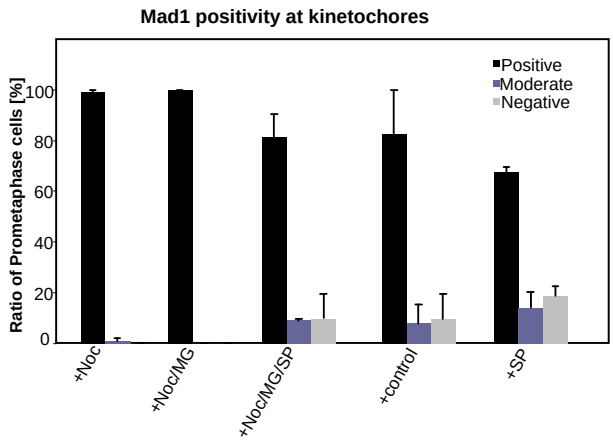


Supplementary Figure S2 Schmidt et al.



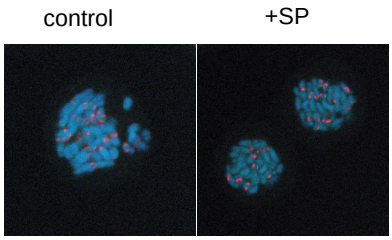






Supplementary Figure S3 Schmidt et al.

A



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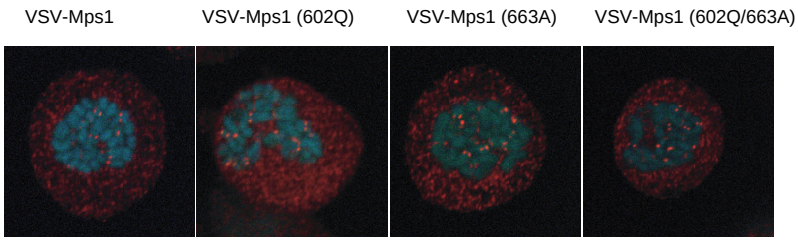
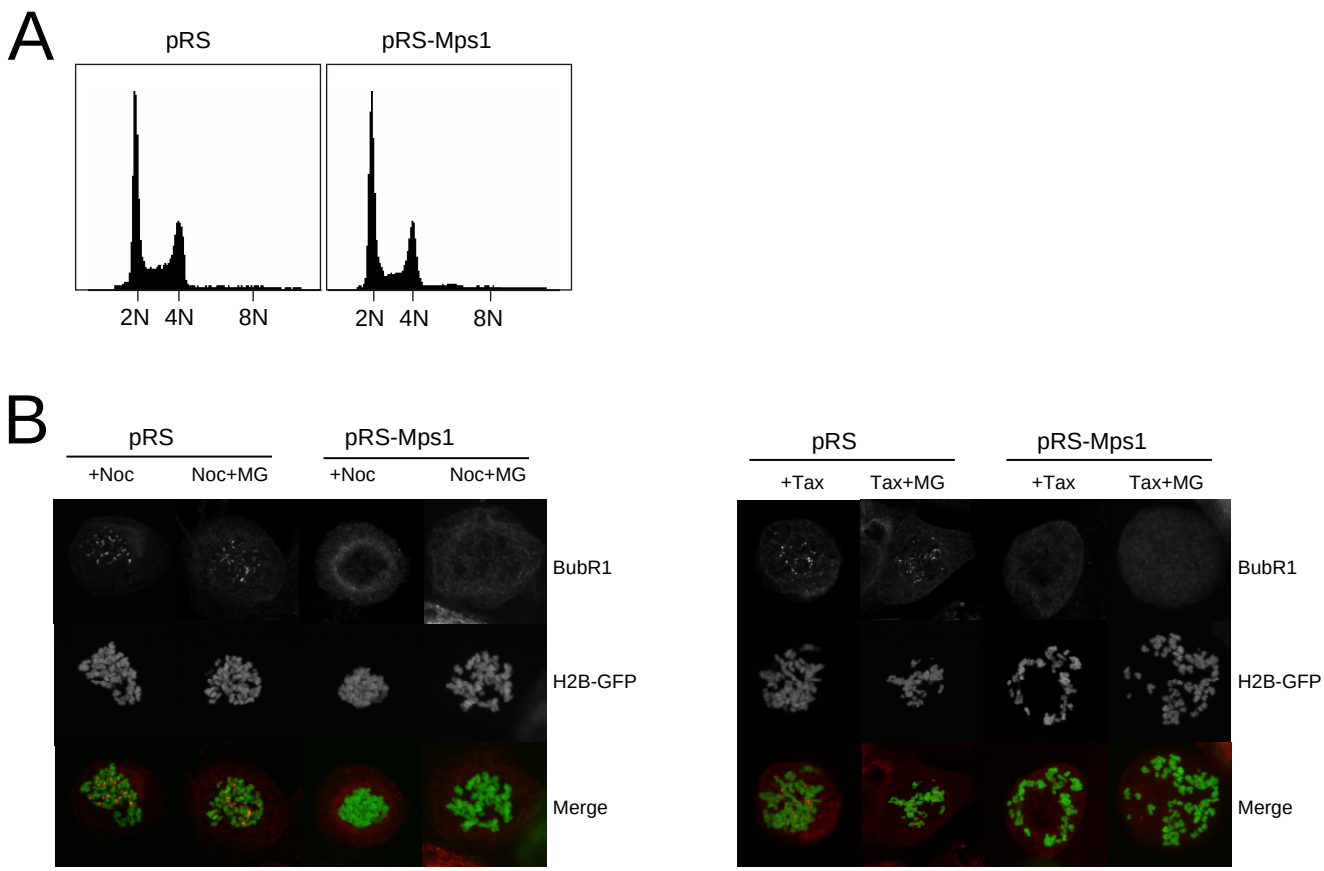


Supplementary Figure S4 Schmidt et al.



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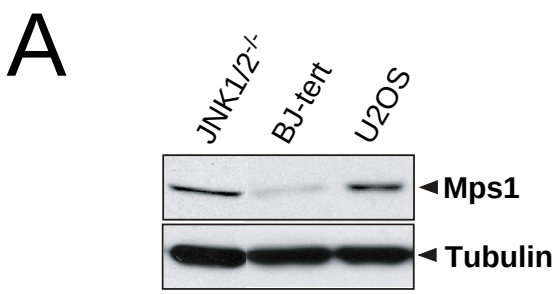

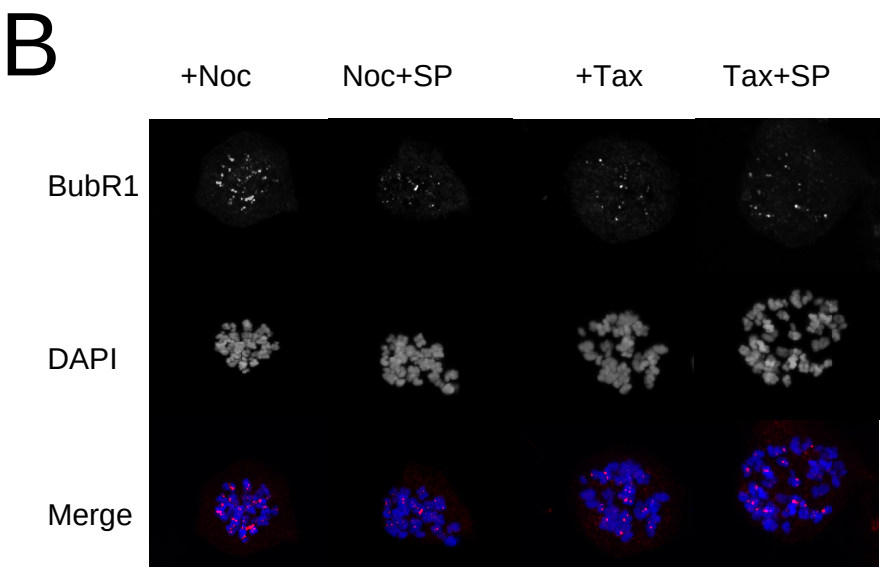

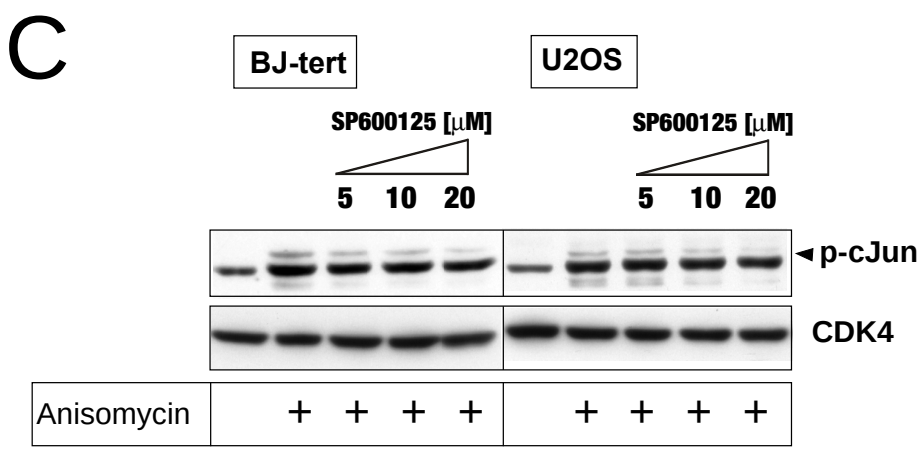

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