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Supplemental Material to:

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Post-slippage multinucleation renders cytotoxic variation in anti-mitotic drugs that target the microtubules or mitotic spindle

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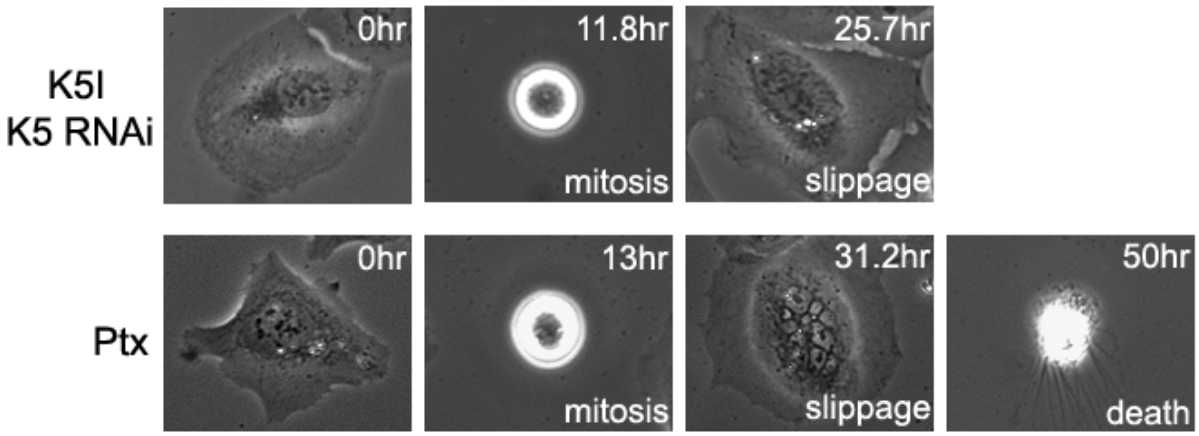
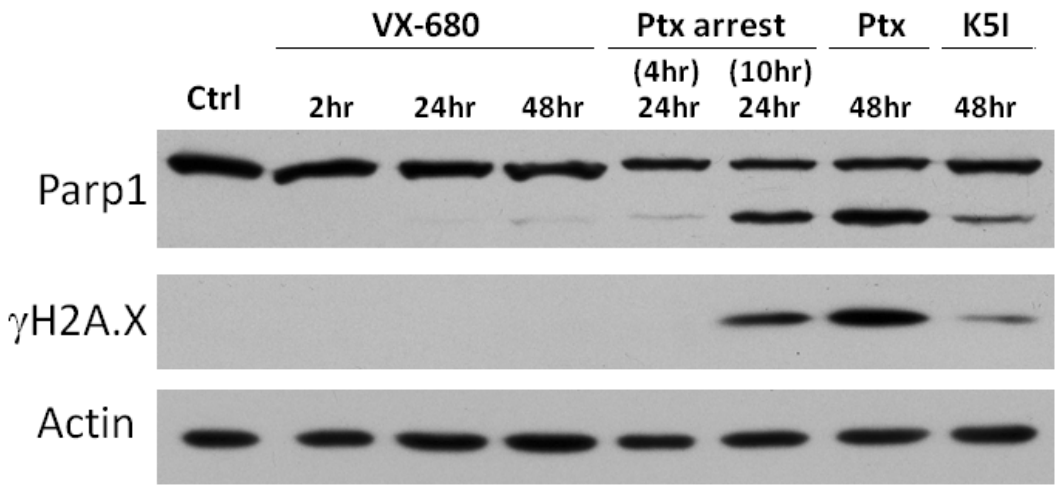
Supplementary Information

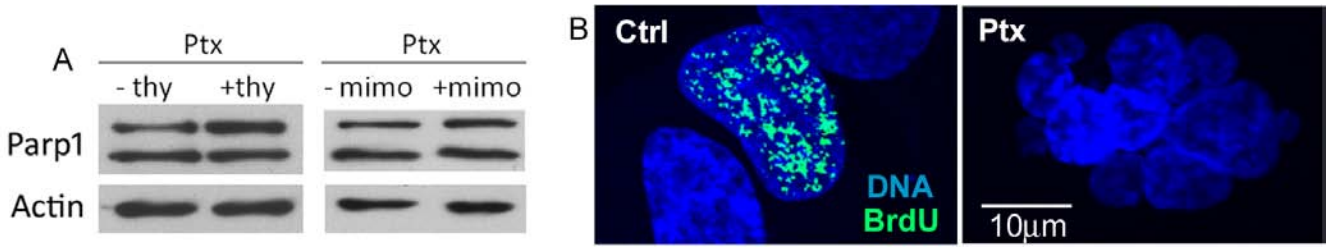
This supplementary information file includes supplementary figures S1, S2, S3, S4 and their figure legends, as well as the legends for the four supplementary videos, S1-S4.



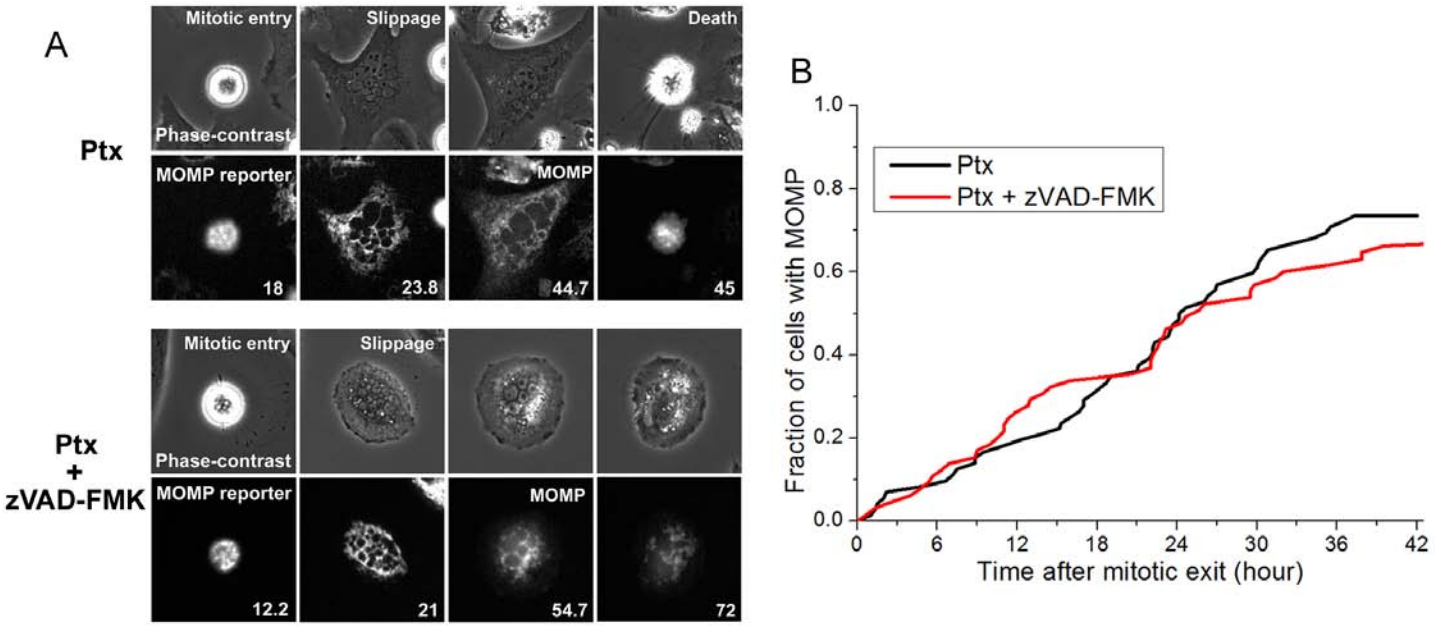
Supplementary Figure S1. Dynamics of U-2 OS cells in response to K5I/K5 RNAi (upper panel) or paclitaxel (lower panel). The still frames were acquired from time-lapse imaging. Timing is indicated in unit of hour at the upper corner of the phase-contrast images. Note that the time 0 for K5I and paclitaxel treatment is the time when drug was added, while the time 0 for K5 RNAi is 36 hours after transfection with K5 siRNA, as it takes 30-36 hours to sufficiently knock down the K5 protein. Based on the phase-contrast images, we scored by morphological tracking: interphase (by flat morphology), entry into mitosis (by cell rounding), mitotic slippage (by re-spreading without cytokinesis) and cell death (by blebbing followed by cell lysis). We then quantified the time of the respective cellular changes for further statistical analysis.



Supplementary Figure S2. Comparison of cell death (measured by Parp1 cleavage) and DNA damage (measured by γH2A.X) induction under the indicated drug treatment. From left to right: Ctrl (no drug treatment); VX-680 (cells treated with 150 nmol/L VX-680 for 2 hours, 24 hours and 48 hours, respectively); Ptx arrest (cells were in paclitaxel-induced mitotic arrest for 4 hours or 10 hours and then induced to slip out of the arrest by VX-680. Cell lysates were collected 24 hours after the induced slippage); Ptx (cells treated with 150 nmol/L paclitaxel for 48 hours); K5I (cells treated with 1 μmol/L K5I for 48 hours). VX-680 treatment alone did not appear to trigger observable cell death or DNA damage if the treatment time was short, i.e. less than 24 hours.



Supplementary Figure S3. (A) Comparison of Parp1 cleavage in U-2 OS cells that were synchronized in paclitaxel-induced mitotic arrest and then treated with the indicated conditions for 40 hours: 150 nmol/L paclitaxel vs. paclitaxel + 2 mmol/L thymidine; 150 nmol/L paclitaxel vs. paclitaxel + 300 µmol/L mimosine. (B) Confocal images of control interphase U-2 OS cells vs. cells that slip out of paclitaxel-induced mitotic arrest. Blue: DNA; Green: bromodeoxyuridine (BrdU) (from Invitrogen).



Supplementary Figure S4. (A) Still images from time-lapse movies that showed MOMP kinetics of U-2 OS treated with 150 nmol/L paclitaxel or paclitaxel + 100 μmol/L zVAD-FMK. The MOMP fluorescent reporter, IMS-RP, consists of monomeric red fluorescent protein targeted to the inter-membrane space of mitochondria by fusion to the leader peptide of SMAC.[9] The upper row are phase-contrast images, and the lower row are IMS-RP fluorescence. MOMP was scored by an abrupt change from punctate to smooth distribution in fluorescence in the cytoplasm. Elapse time is indicated in unit of hour at the lower corner of the IMS-RP image. (B). Comparison of MOMP kinetics in post-slippage U-2 OS cells treated with paclitaxel alone or paclitaxel + zVAD-FMK.

Supplementary Video Legends

Supplementary Video S1 Time-lapse movie of the MDC1-EGFP fluorescence of a representative U-2 OS cell treated with 150 nmol/L paclitaxel. Note that when we crop still images from this movie file to make Figure 3B, we removed fluorescence background from debris of other cells that flowed into the field of view, as they did not belong to the selected U-2 OS cell.

Supplementary Video S2 Time-lapse movie of the p53-Venus fluorescence of a representative U-2 OS cell treated with 150 nmol/L paclitaxel.

Supplementary Video S3 Time-lapse movie of the MDC1-EGFP fluorescence of a representative U-2 OS cell treated with 1 μmol/L K5I. The accumulative signal of MDC1-EGFP under K5I after mitotic slippage is significantly less than that observed under paclitaxel.

Supplementary Video S4 Time-lapse movie of the MDC1-EGFP fluorescence of a representative U-2 OS cell treated with 150 nmol/L paclitaxel plus 1 μmol/L K5I. The kinetics and extent of MDC1-EGFP accumulation under the two drug treatment is similar to those observed under paclitaxel alone.