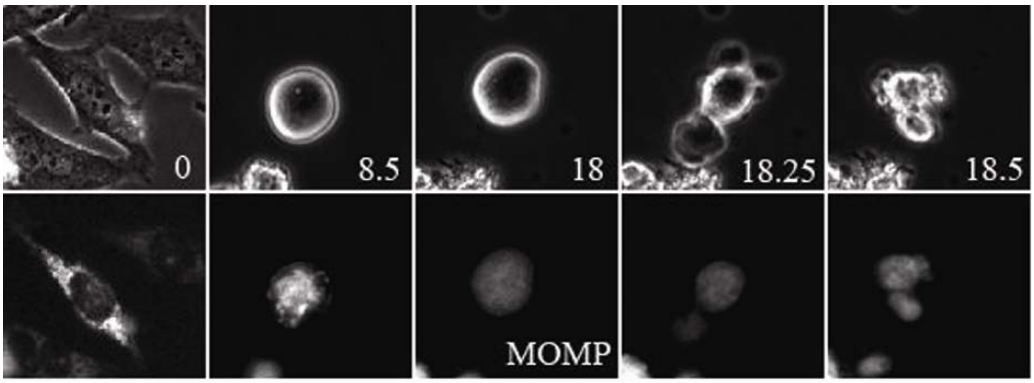
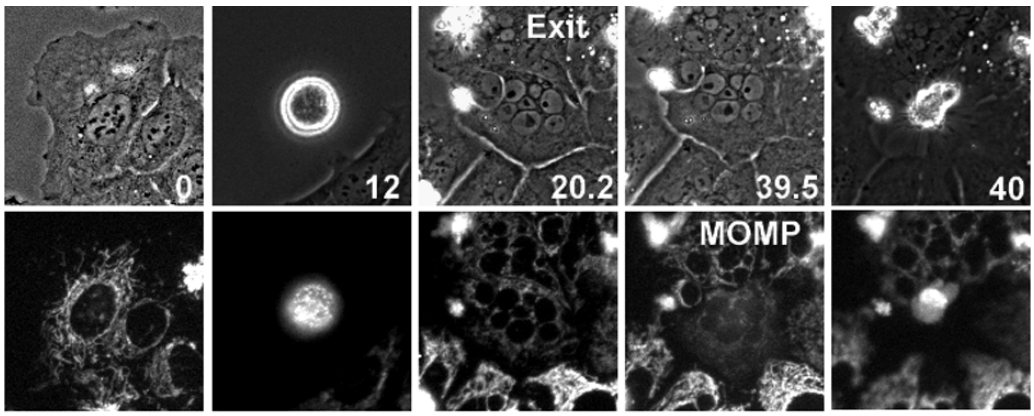
Supplementary figures



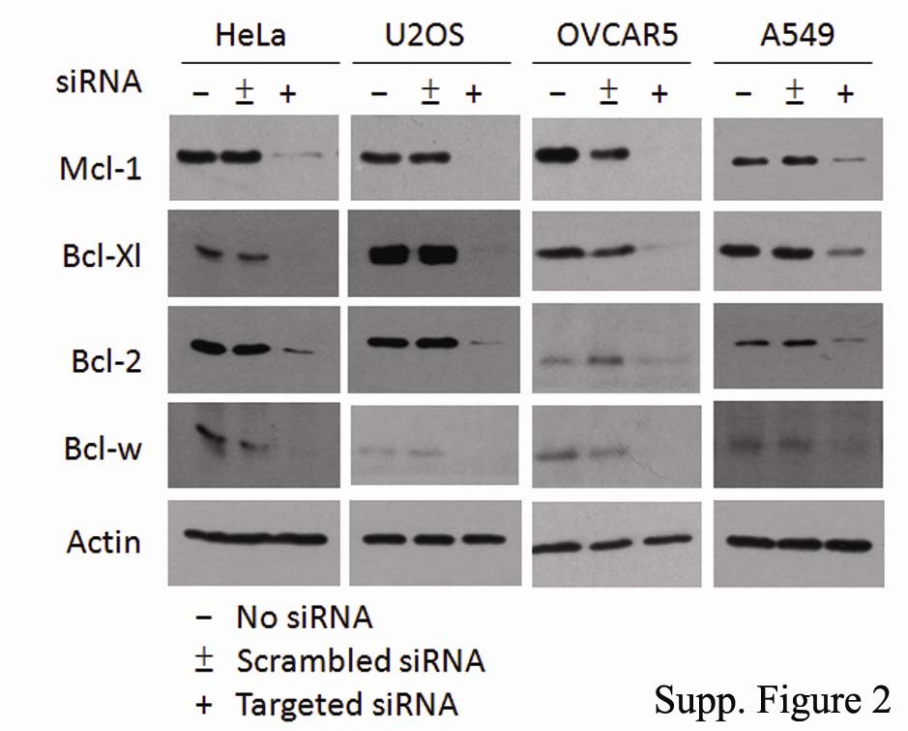
Supp. Figure 1a

Supp. Figure 1a MOMP kinetics of HeLa treated with 150nM paclitaxel. Still frames were obtained from live imaging of HeLa cells expressing MOMP reporter, IMS-RP, which consists of monomeric red fluorescent protein targeted to the inter-membrane space of mitochondria by fusion to the leader peptide of SMAC. The upper row are phase-contrast images, and the lower row are IMS-RP fluorescence. Paclitaxel was added at time 0 (timing is indicated in unit of hour at the lower corner of the phase-contrast image). Cell entered mitosis after 8.5 hr of paclitaxel treatment. MOMP then occurred after 9.5 hours in mitotic arrest, indicated by a change from punctuate to smooth distribution in fluorescence. Cell started to bleb 15 minutes after MOMP and then lysed.



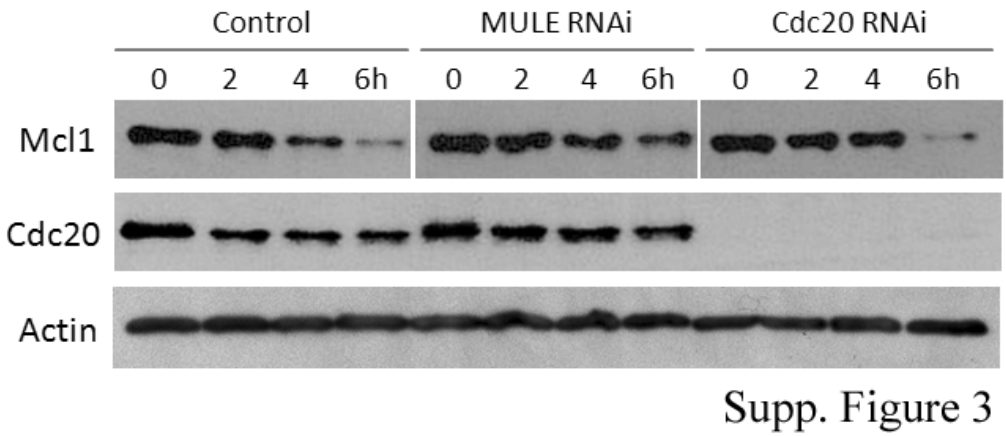
Supp. Figure 1b

Supp. Figure 1b MOMP kinetics of U2OS cell treated with 150 nM paclitaxel. Cell entered mitosis 12 hr after paclitaxel treatment, and then exited mitosis after 8.2 hr of mitotic arrest. MOMP then occurred 19.3 hr after exit. Cell started to bleb 30 minutes after MOMP and then lysed.



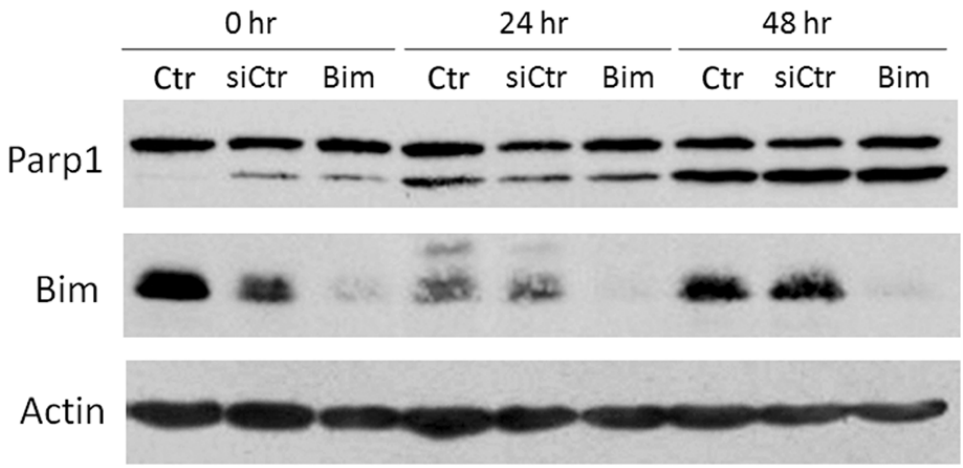
Supp. Figure 2

Supp. Figure 2 Knockdown level of Bcl-2 proteins by RNAi in the four chosen cell lines.



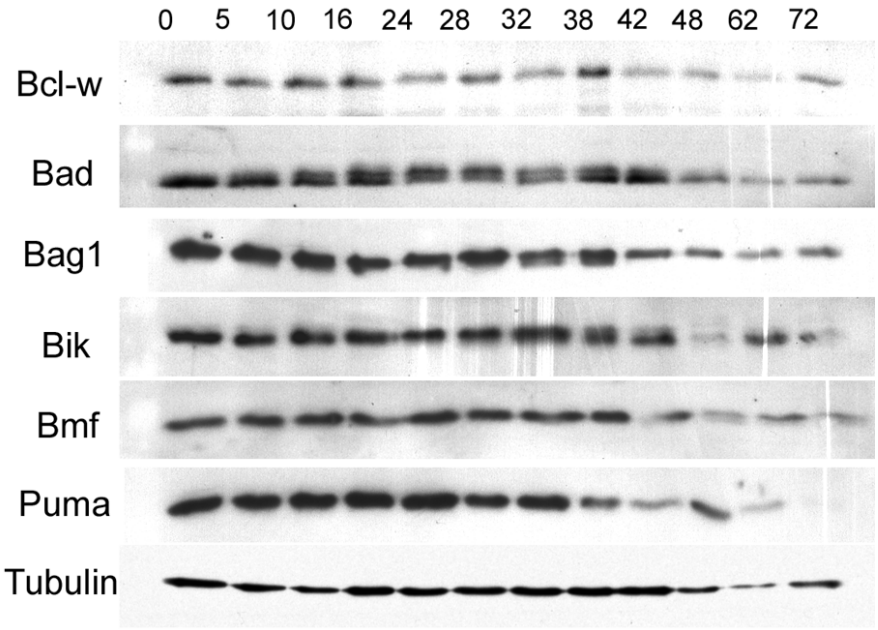
Supp. Figure 3

Supp. Figure 3 Comparison of kinetics and degree of Mcl-1 degradation at 4 time points during mitotic arrest in HeLa cells, HeLa cells pre-treated with MULE/HUWE RNAi and HeLa cells pretreated with Cdc20 RNAi. Actin served as a loading control. Pure mitotic HeLa cells for the comparison were obtained by mitotic shake-off as discussed in material and method. We were not able to determine the knockdown efficiency of MULE1/HUWE by western blotting due to the lack of decent antibody. However, the knockdown efficiency of the specific siRNA oligo that we used had been quantified before by RT-PCR and showed nearly complete depletion of MULE/HUWE1 mRNA [25].



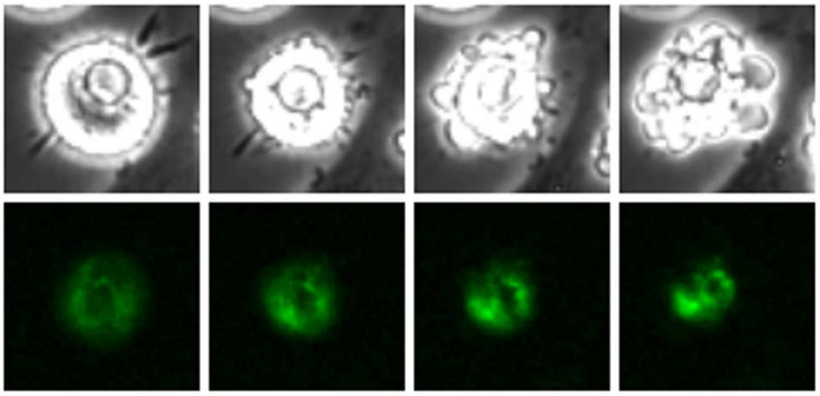
Supp. Figure 4

Supp. Figure 4 Western blot analysis of effect of Bim knockdown on apoptosis induction of HeLa cells treated with 1μM K5I. Lysates from cells with Bim siRNA were compared on the same gel with those from control cells (no siRNA) and cells with control siRNA at three time points, i.e. 0 hr, 24hrs and 48hrs after drug addition.



Supp. Figure 5

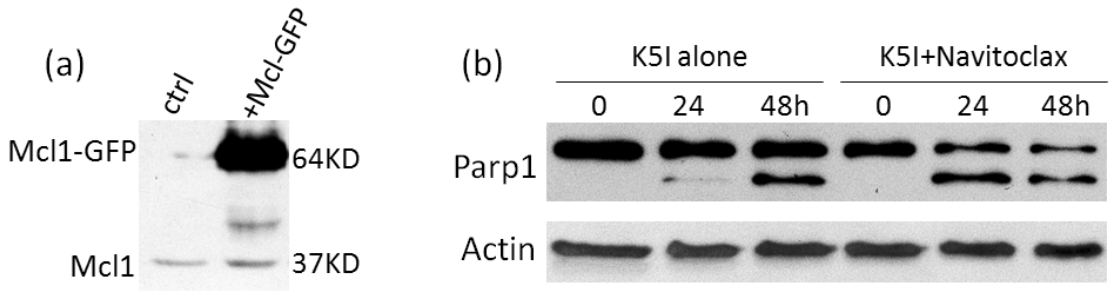
Supp. Figure 5 Western blot analysis of selective BH-3 proteins of HeLa cells in response to 1μM K5I. Refer to [8] for western blot results of Bcl-2, Bcl-xl, Bid and Bax.



Supp. Figure 6

Supp. Figure 6 Bak oligomerization in U2OS cells treated with 1μM K5I + 1μM Navitoclax. The four still frames were obtained from live imaging of U2OS cells expressing Bak-EGFP and were spaced by 10 minutes. The upper row are phase-contrast images, and the lower row are the corresponding Bak fluorescence. Preceding

morphological cell death, U2OS cells treated with anti-mitotics + Navitoclax exhibited rapid Bak oliogermization, indicated by the fluorescence signal change from diffused to punctuate.



Supp. Figure 7

Supp. Figure 7 Western blot analysis of Mcl-1-GFP over-expression and its effect on apoptotic response in U2OS cells. (a) Endogenous Mcl-1 and the over-expressed Mcl-1-GFP appeared at molecular weight of 37KD and 64KD, respectively. (b) U2OS cells over-expressing Mcl-1-GFP were treated with either 1μM K5I or 1μM K5I + 1μM Navitoclax, and the degree of apoptosis was compared by Parp1 cleavage at three selected time points, i.e. 0hr, 24hr and 48hr after drug addition.