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Supplemental Material to:

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Centromere fragmentation is a common mitotic defect of S and G2 checkpoint override

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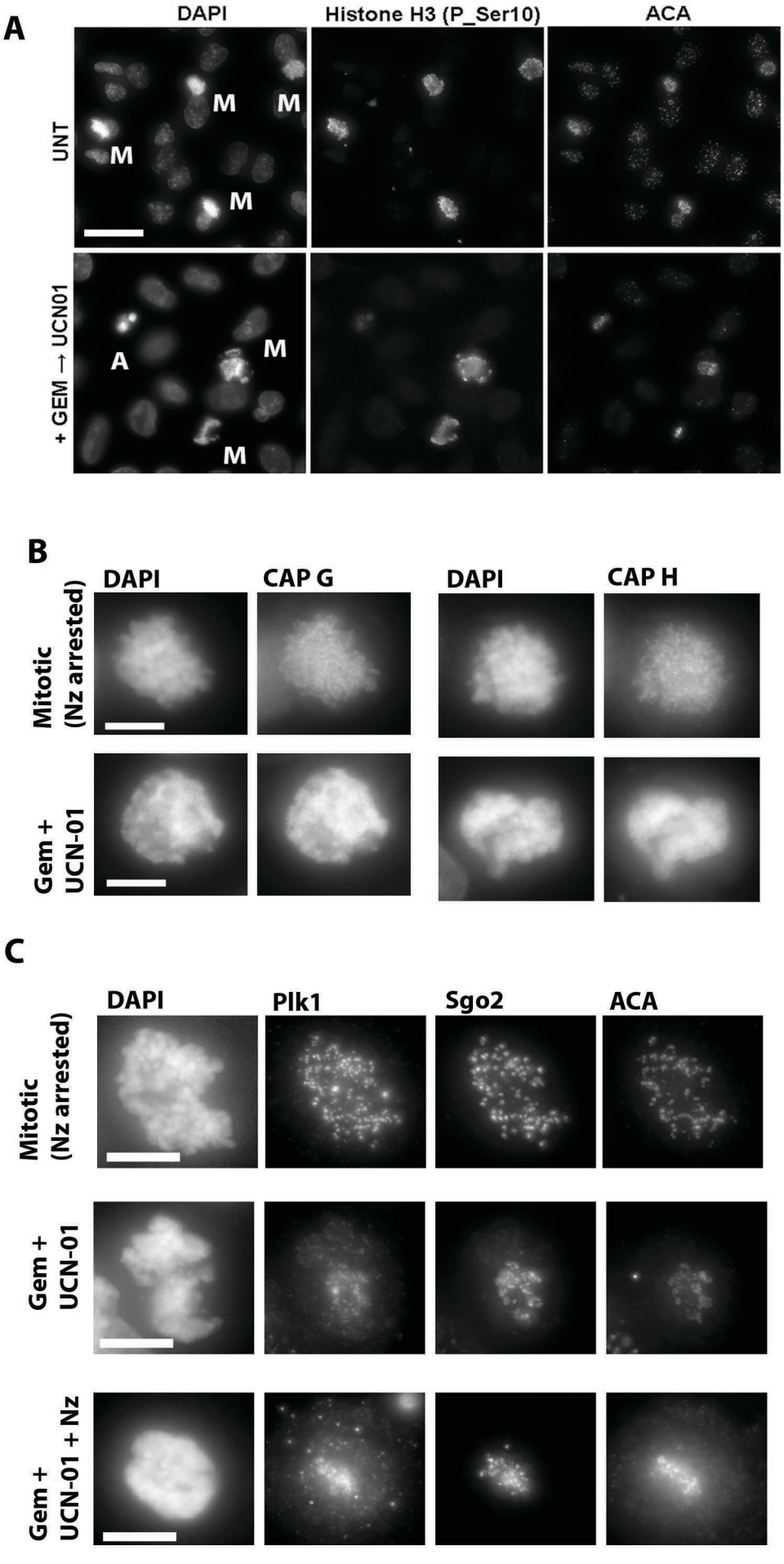
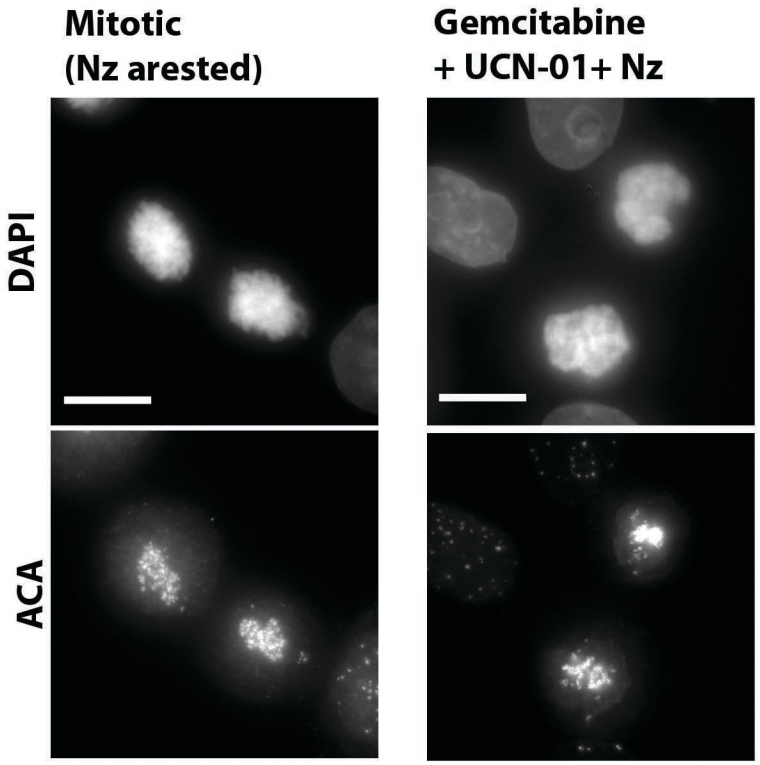
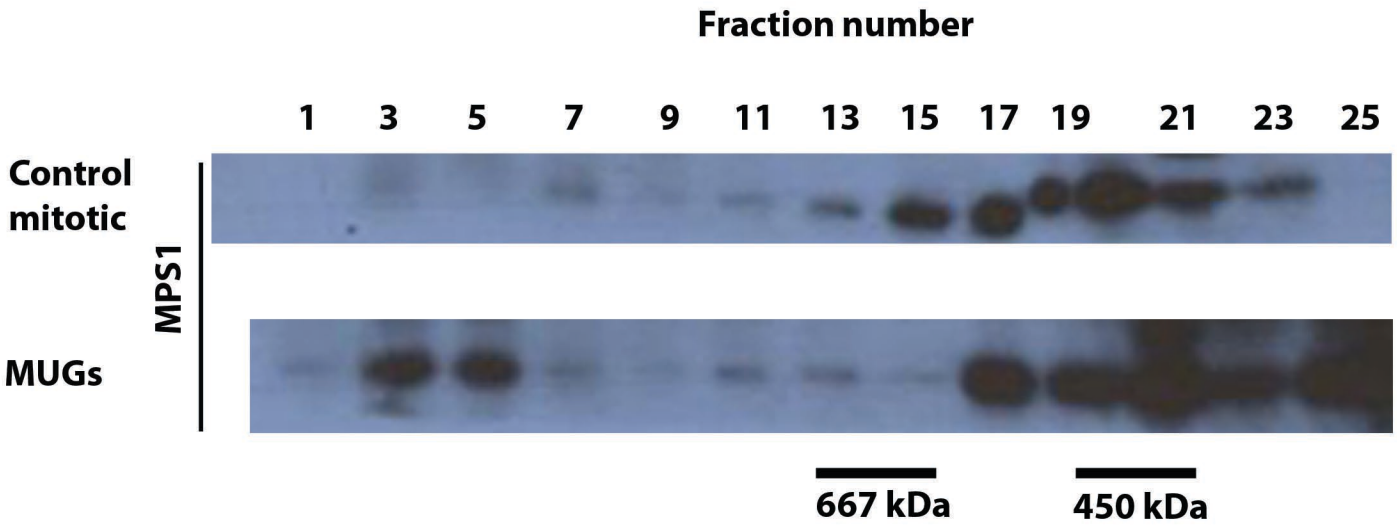
Supplemental figure 1



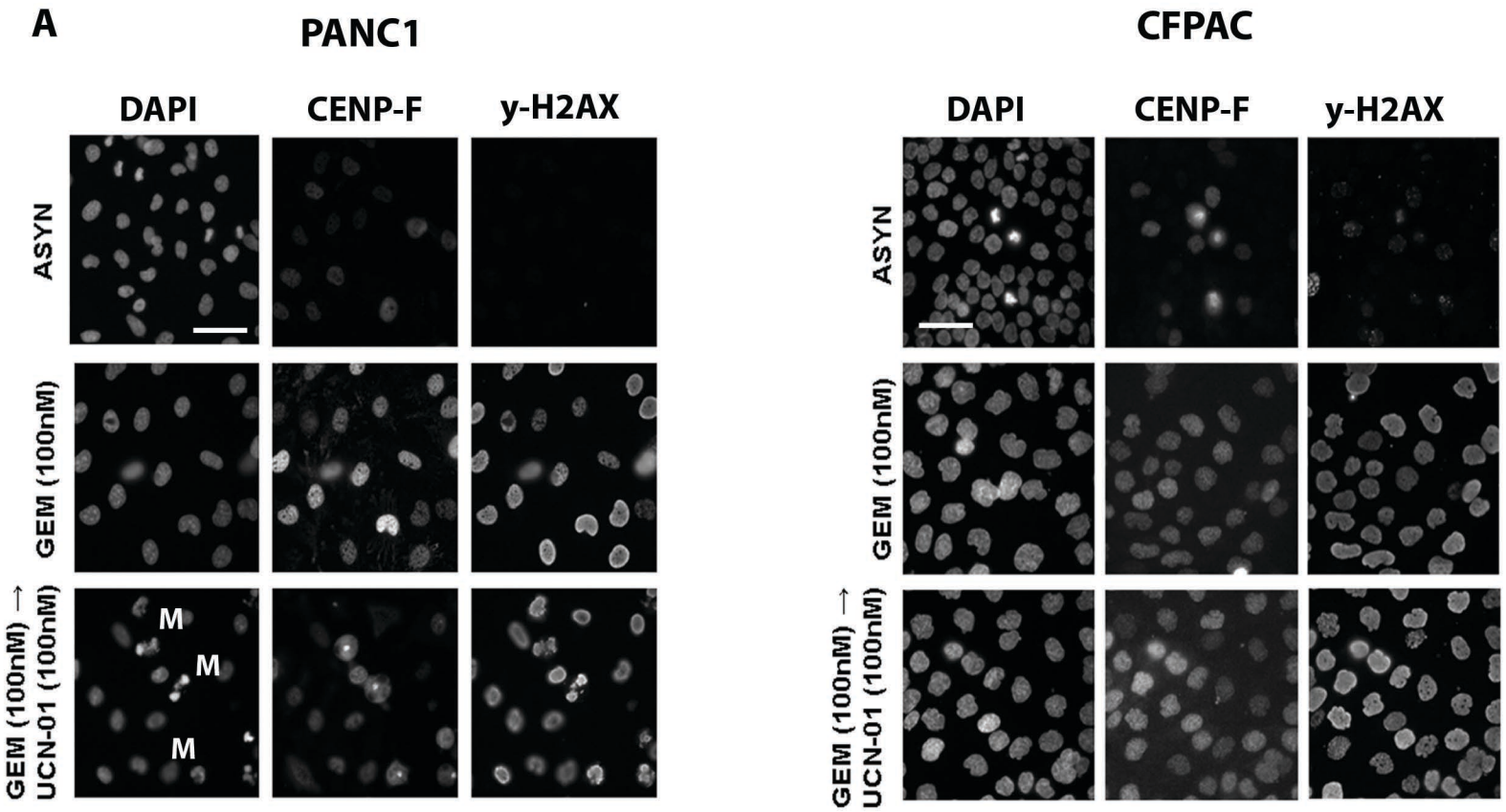
Supplemental figure 2

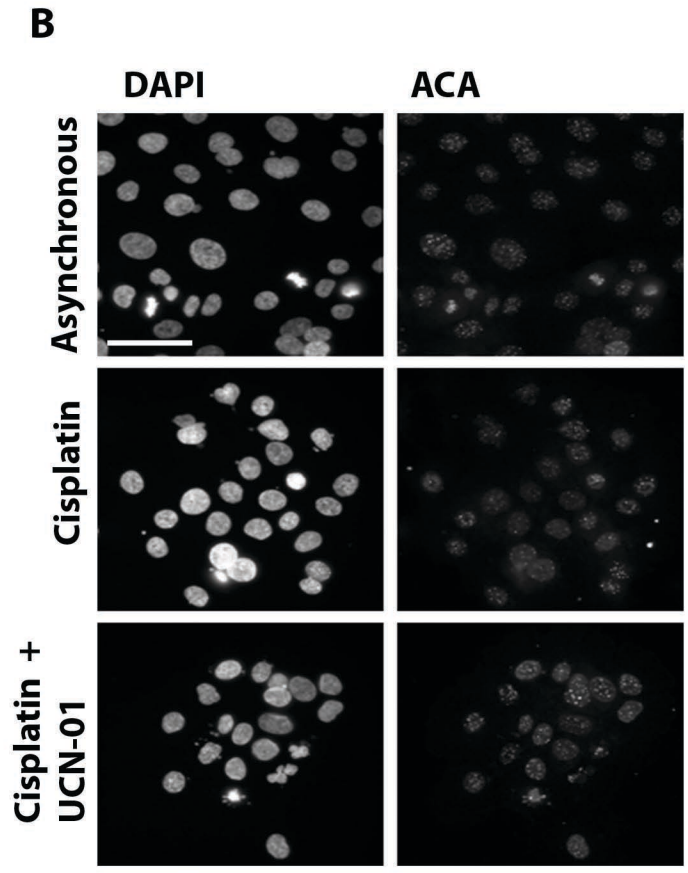


Supplemental figure 3

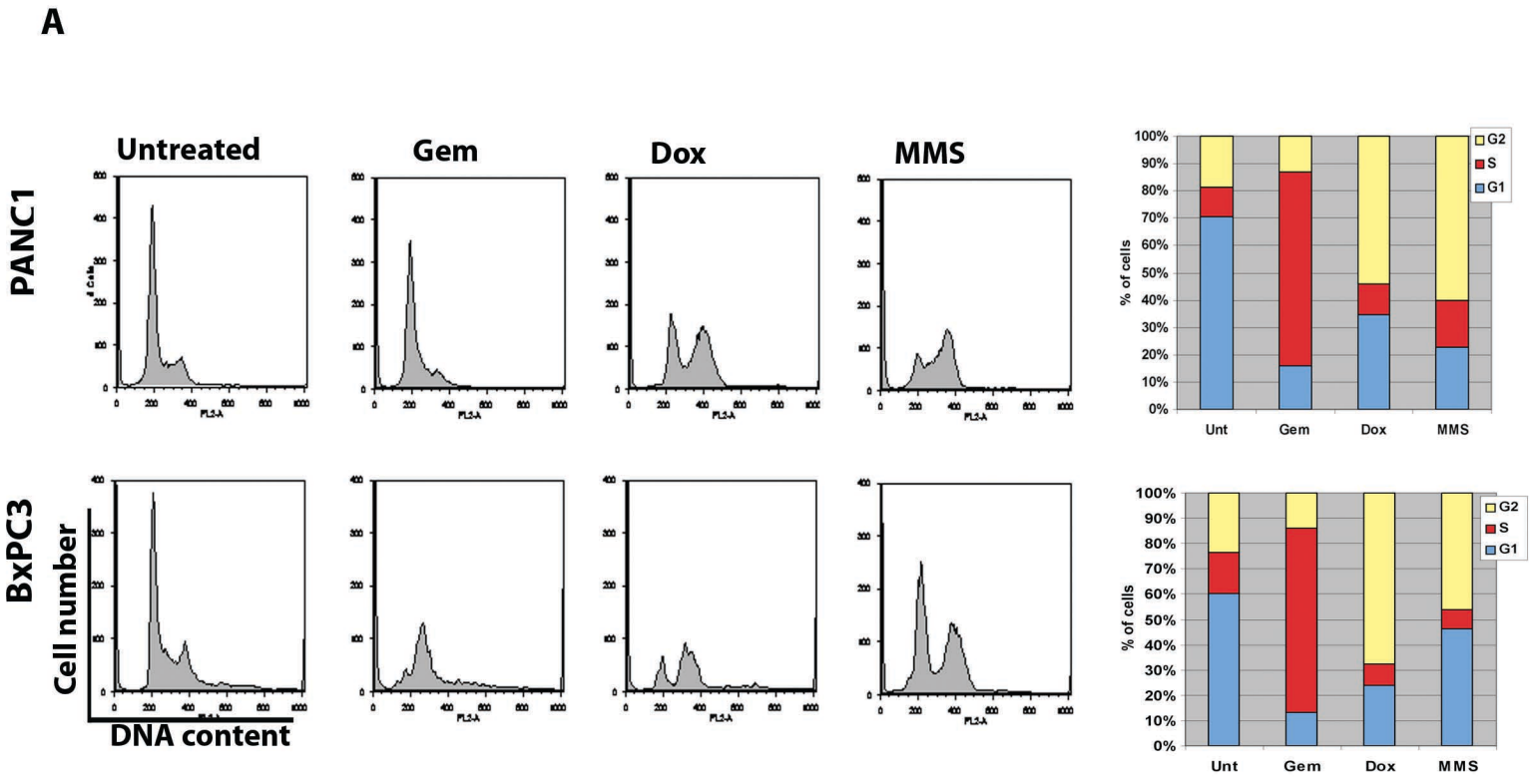


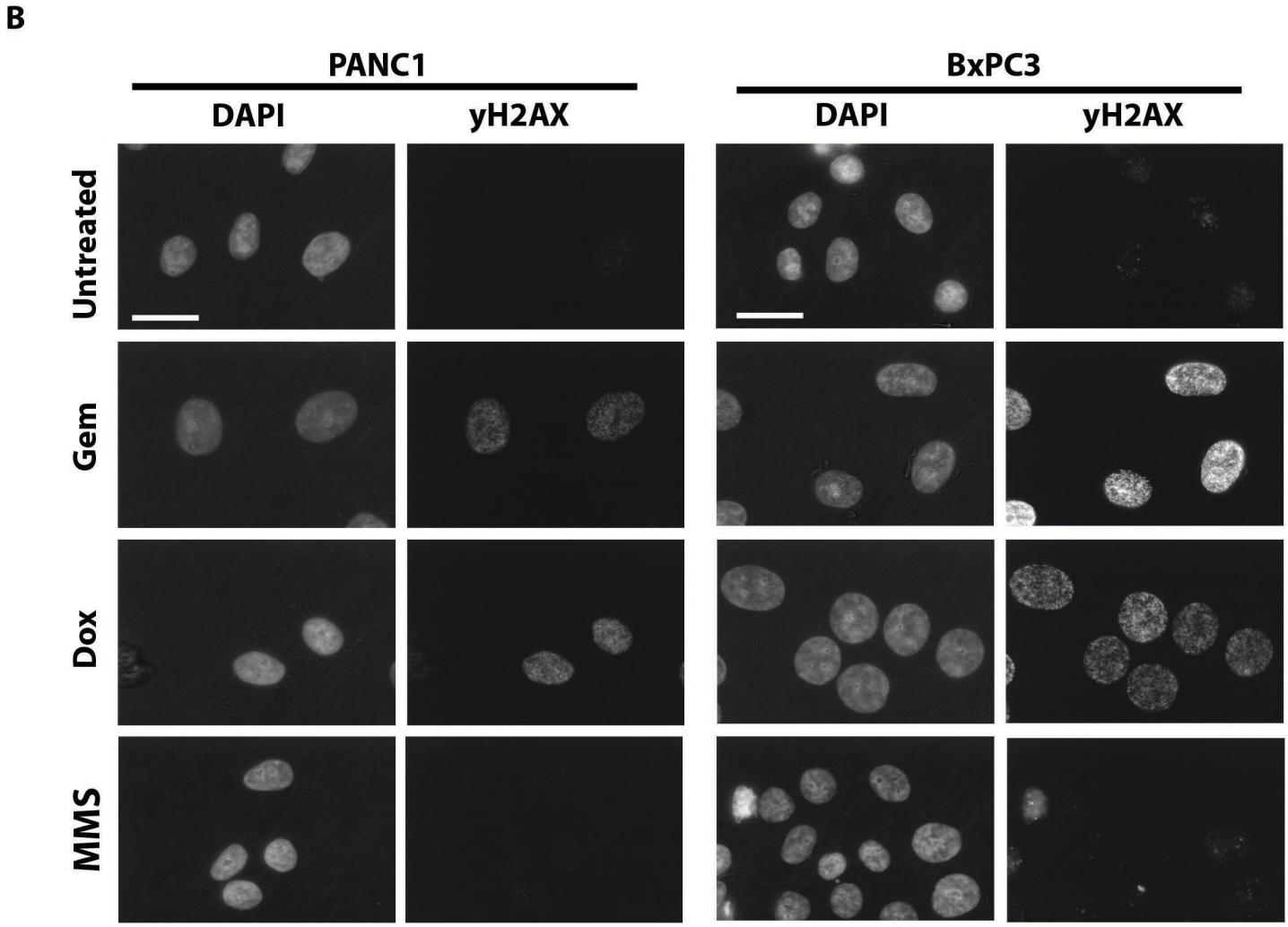
Supplemental figure 4





Supplemental figure 5





Supplemental figure legends

Supplemental Figure 1.

A. Entry into mitosis was verified by immunostaining for phospho histone H3. Immunofluorescence analysis of DAPI, Phospho- Histone $H3^{SER10}$ and ACA of asynchronous cells (upper panel) and cells forced to inappropriately enter mitosis (lower panel). 'M' denotes mitotic cells, while (A) shows an apoptotic nuclei. Scale bar is 25μm.

B. Mitotically arrested PANC1 cells and MUGs were immunostained with the chromosome CAP proteins CAP-G and CAP-H. Representative figures are shown. Scale bar is 5μm. **C.** Mitotically arrested cells (+ nocodazole), MUGs, or MUGs arrested (+nocodazole) were immunostained for DNA (DAPI), the mitotic proteins Plk1, Sgo2 and the centromere protein ACA. Scale bar is 5μm.

Supplemental Figure 2.

Mitotic figures and MUGs were generated in the absence of microtubules by the treatment of nocodazole. Following treatments, PANC1 cells were fixed and stained with ACA (centromere) and DAPI (DNA). Representative images are shown. Scale bar is 5μm.

Supplemental Figure 3.

Normal mitotic and MUGs lysates were separated by sucrose gradient fractionation and fractions were subjected to western blot analysis with MIS12 antibody. The molecular weight markers for thyroglobulin (667kDa) and the MCC (450 kDa) are shown.

Supplemental Figure 4.

A. PANC1 and CFPAC cells were synchronized by thymidine (2mM) arrest and release. Cells were treated in G1 with gemcitabine (100nM) for an additional 24h. UCN-01 (100nM) was then added for a further 9h before cells were fixed and immunostained. Cells were stained with DAPI (DNA), CENP-F (kinetochore) and

yH2AX (DNA damage). ‘M’ denotes the presence of MUGs. Images are representative of those observed. Scale bar is 50 μm. **B.** BxPC3 cells were synchronized as stated above and treated in G1 with cisplatin for a further 24h. UCN-01 (100nM) was added to cells for a further 9h before cells were fixed and stained with DAPI (DNA) and ACA (centromere). Representative images are shown. Scale bar is 50 μm.

Supplemental Figure 5.

PANC1 and BxPC3 cells were synchronized by thymidine (2mM) arrest and release. Cells were treated in G1 with gemcitabine (100nM), doxorubicin (250nM) or MMS (200μM) for an additional 24h. **A.** Cells were then harvested and fixed for DNA content analysis. Cell cycle profiles were determined using FACScan flow cytometer. **B.** Cells were fixed and stained with γ-H2AX. Representative images are shown. Scale bar shown is 20μm.