Supplemental information

The phospho-Drp1-637/Fis1/Mid49 cluster modulates mitochondrial periphery fission signature responses to mitotic arrest

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Figure S1

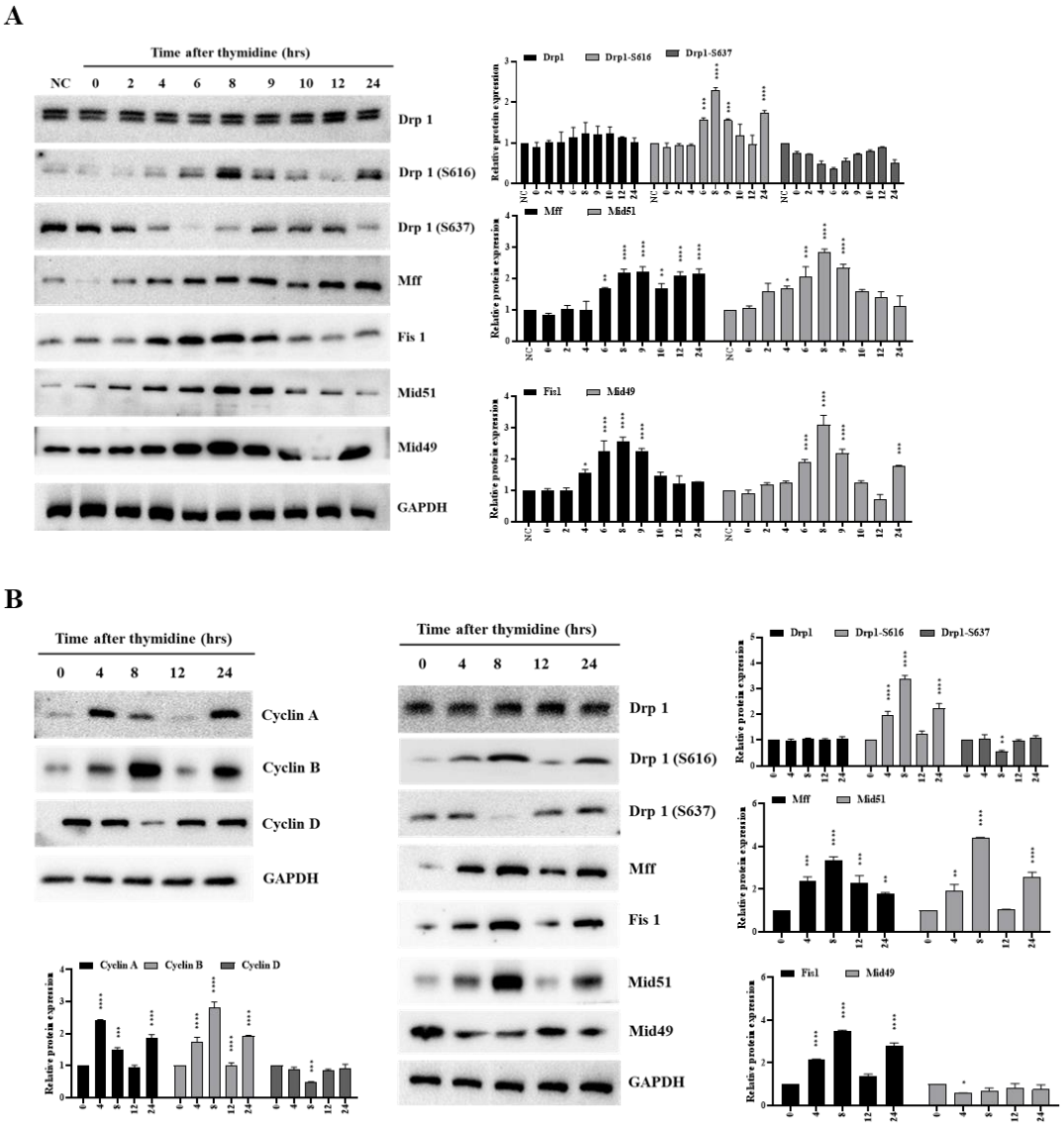
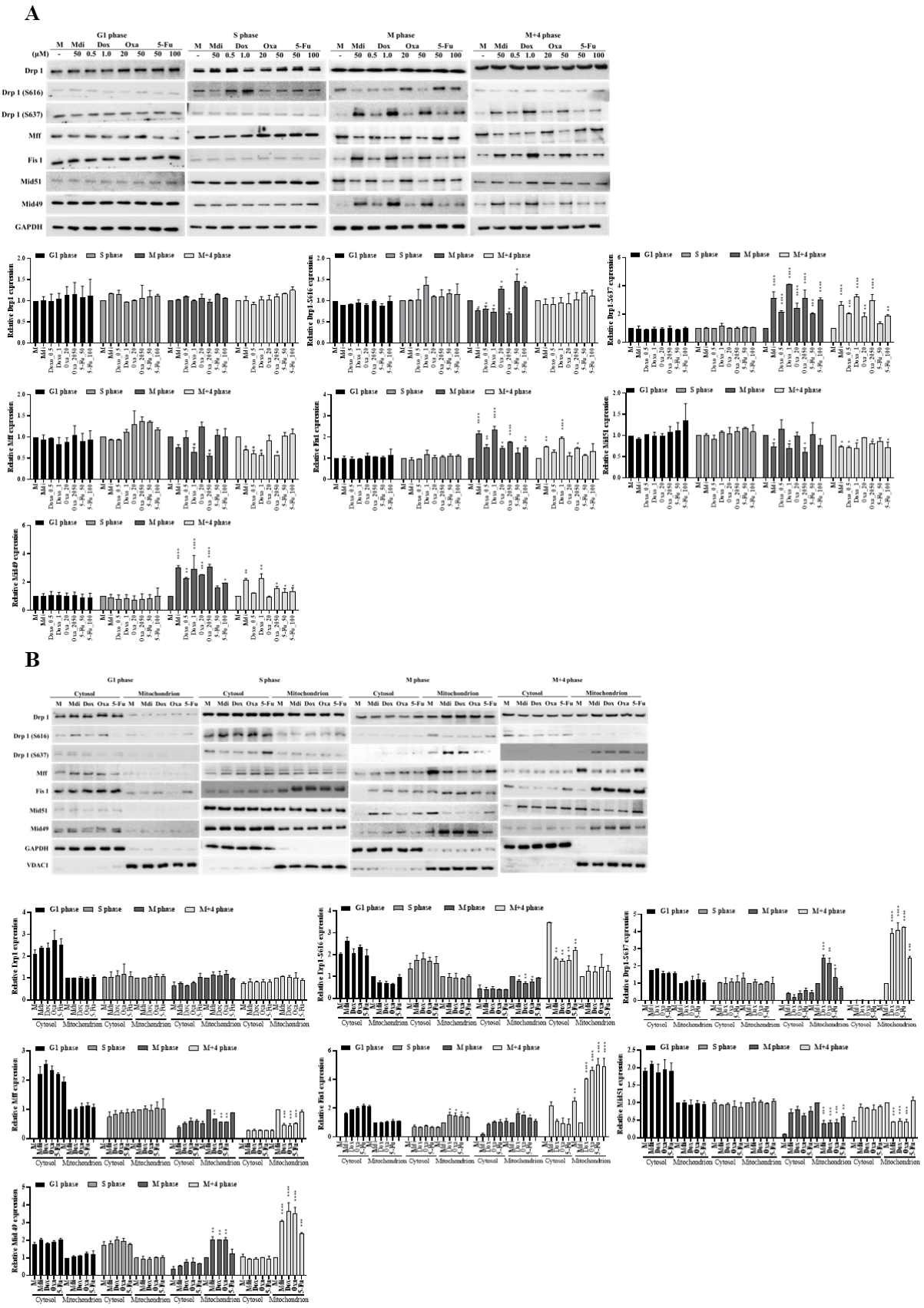


Figure S1. Dynamics of Drp1 Phosphorylations during Cell Cycle Progression. (A) HeLa cell synchronization was achieved using a double-thymidine block, and the expression levels of total Drp1, phospho-Drp1 (Ser616), phospho-Drp1 (Ser637), Mff, Fis1, MiD49, and MiD51 were assessed at indicated time points after release. (B) HeLa cells were synchronized through double thymidine treatment and subsequently released for the specified durations. Expression levels of total Cyclin A, Cyclin B, Cyclin D, Drp1, phospho-Drp1 (Ser616), phospho-Drp1 (Ser637), Mff, Fis1, MiD49, and MiD51 were analyzed at indicated time points after release. GAPDH served as an internal loading control. The term "NC" (normal control) refers to samples without synchronization or double-thymidine treatment.

13 **Figure S2**



14

Figure S2. Effects of Mdi, Doxo, Oxa, and 5-Fu on Drp1 Phosphorylation and Mitochondrial Receptor Proteins. (A) HeLa cells, synchronized through double-thymidine treatment, were treated with various concentrations of Mdi, Doxo, Oxa, and 5-Fu, then released for specified durations. Expression levels of total Drp1, phospho-Drp1 (Ser616/Ser637), Mff, Fis1, MiD49, and MiD51 were analyzed at 1, 4, 8, and 12 hours after release by western blotting. (B) Cytoplasmic and mitochondrial fractions were separated to quantify total Drp1, phospho-Drp1 (Ser-616/Ser-637), Mff, Fis1, Mid49, and Mid51. VDAC1 served as an internal control, and subcellular purity was confirmed with GAPDH. "NC" denotes untreated samples, and "M" represents synchronized/mock-treated samples.

Figure S3

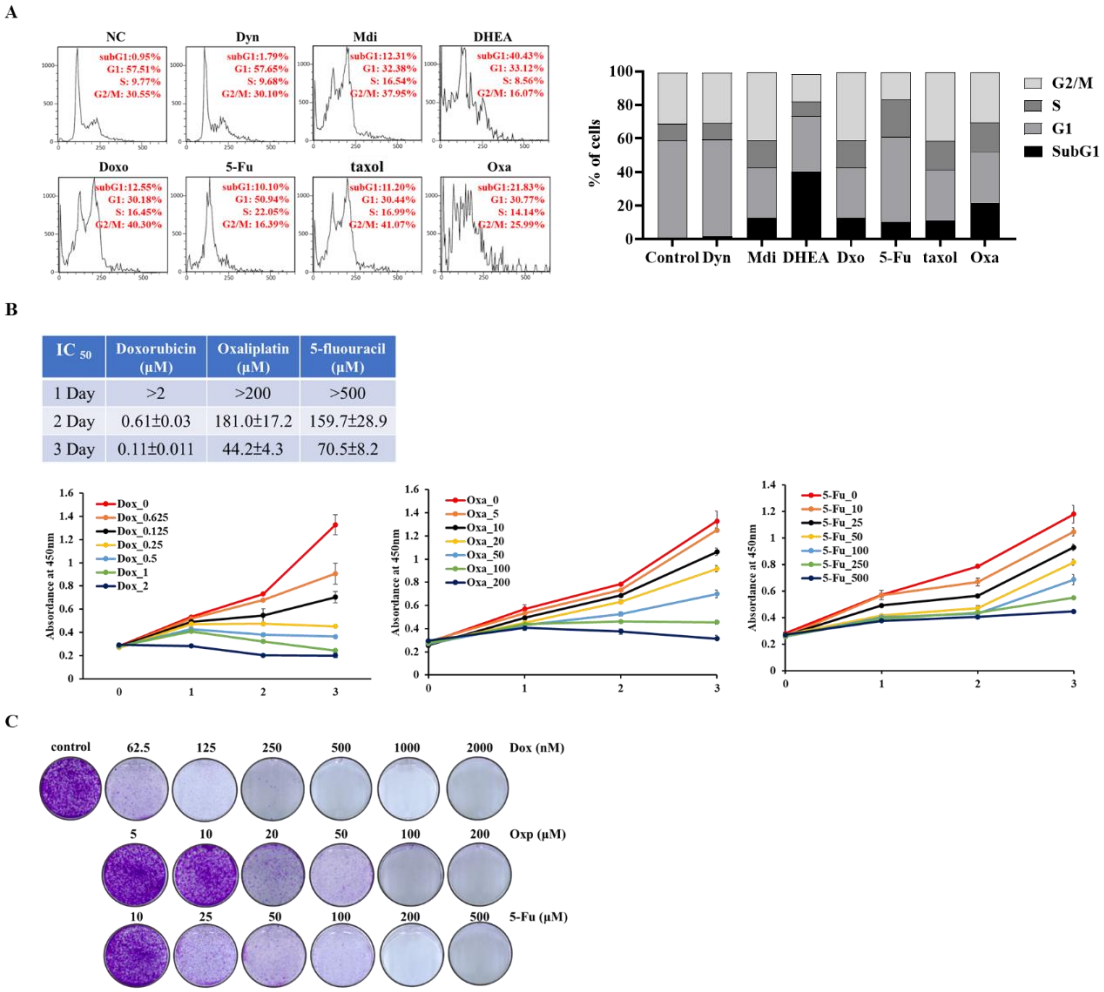
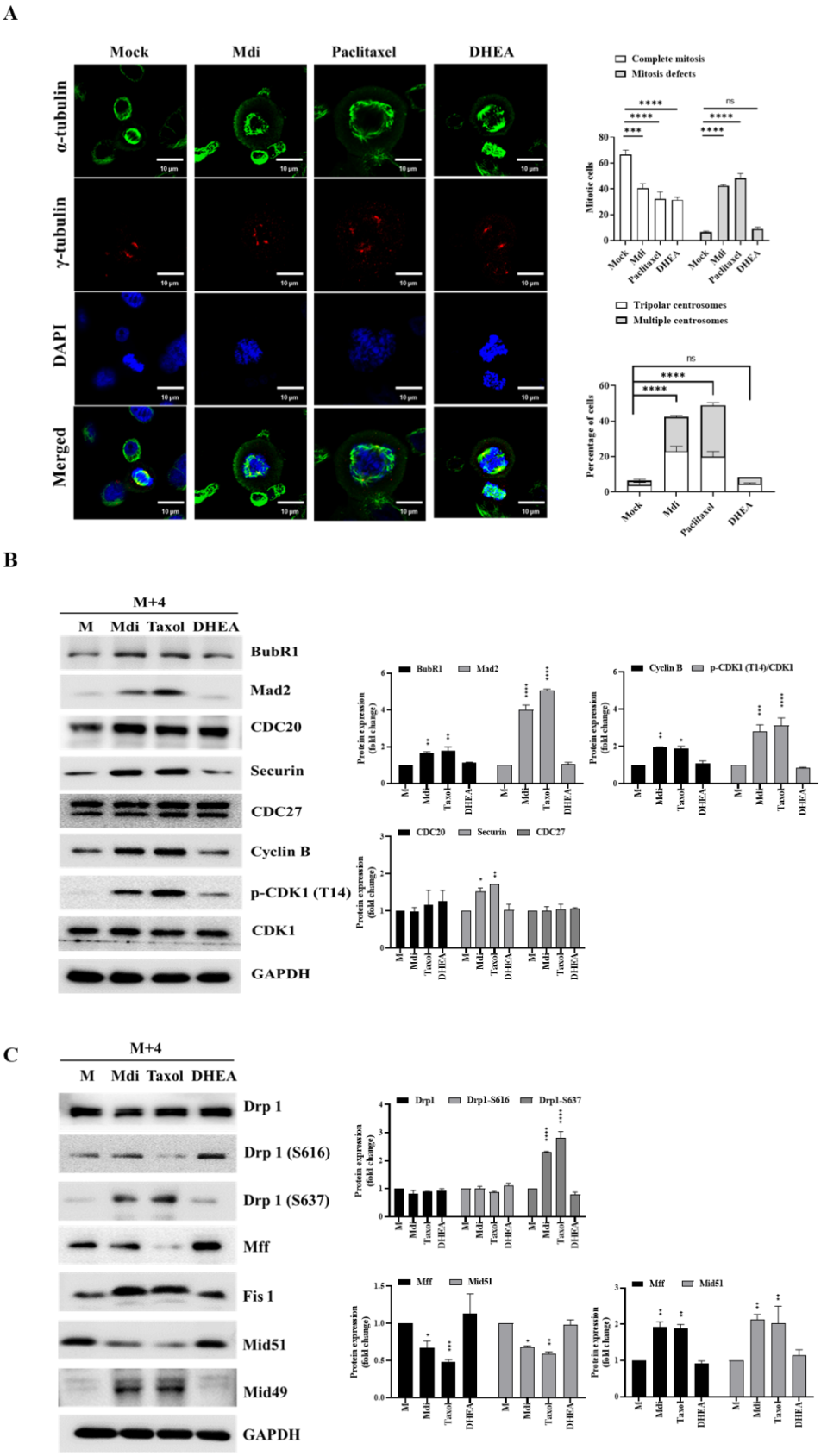


Figure S3. Different drugs induce cell death and G2/M cell cycle arrest in HeLa cells. (A) Cell cycle analysis of HeLa cells following treatment with various drugs. The histogram illustrates the percentages of cells in different cell cycle phases. The bar chart below shows the distribution of cells across different cell cycle phases. (B) Assessment of the effects of the different drugs on HeLa cell viability. HeLa cell viabilities were evaluated after treating with Dox, Oxa, and 5-Fu at indicated concentrations from 24 hours to 72 hours. The right panel represents % cell viability versus the logarithm of the concentration, and IC_{50} values were obtained for Dox, Oxa, and 5-Fu. (C) The proliferation of Hela cells was suppressed by Dox, Oxa, and 5-FU. The ability of HeLa cells to form colonies was assessed following treatment with Dox, Oxa, and 5-Fu.

Figure S4


A
Mock
Mdi
Paclitaxel
DHEA
α-tubulin
γ-tubulin
DAPI
Merged
Complete mitosis
Mitosis defects
Mitotic cells
Tripolar centrosomes
Multiple centrosomes
Percentage of cells
B
M+4
M
Mdi
Taxol
DHEA
BubR1
Mad2
CDC20
Securin
CDC27
Cyclin B
p-CDK1 (T14)
CDK1
GAPDH
Protein expression (fold change)
C
Drp 1
Drp 1 (S616)
Drp 1 (S637)
Mff
Fis 1
Mid51
Mid49
GAPDH

Appendix Figure S4. Induced in nocodazole-treated mitotic HeLa cells are multiple spindle foci by taxol and DHEA. (A) Confocal images depict microtubules (green), centrosomes (red), and DNA (blue) in HeLa cells after a 16-hour nocodazole treatment. (B) Impact of Mdi, Taxol and DHEA on the SAC during the M phase. (C) Dynamic changes in Drp1 phosphorylation and mitochondrial adaptor protein levels during treatment with Mdi, Taxol, and DHEA in nocodazole-treated mitotic HeLa cells. The scale bar is 10 μm, and the cells were stained with anti-α-tubulin and anti-γ-tubulin, with DNA stained using DAPI. Inhibition of multipolar spindle formation in HeLa cells by drug treatment is shown in panels C and F on the right. The percentage of cells exhibiting bipolar spindles and multiple spindle foci is presented. The data represent the mean ± SE from three independent experiments, with approximately 300–350 mitotic cells analyzed per experiment.