14 12 MEFs-WT MEFs-KO 4 4

6

12

18

Nocodazole exposure time (h)

24

30

36

2 -| 0 + 0

В

Α



**Supplemental Figure S1. A.** Quantification of mitotic index in MEFs-WT/KO cells. The cells were treated with 50 ng/ml nocodazole for different times, then collected and fixed at the indicated time, the percentage of pH3-positive cells were determined by flow cytometry. **B.** The expression levels of DAB2IP, cyclin B1, and HSP70 were analyzed by immunoblot analysis.





**Supplemental Figure S2. A.** Quantification of BubR1 levels (normalized by CREST) on prometaphase kinetochores in DU145 siCon/siD2 cells. (n=34 cells) (\*\*p < 0.01 as compared with control cells). **B.** DU145 siCon/siD2 cells were treated with nocodazole (50 ng/ml) for 16 hours. Mitotic cells and untreated cells were analyzed by immunoblotting with anti-BubR1, anti-CyclinB1, anti-DAB2IP and anti-Actin antibodies.

Α



**Supplemental Figure S3.** The siRNA-resistant DAB2IP expression plasmid rescued the Plk1' and BubR1' phosphorylation during mitosis progression.



**Supplemental Figure S4. A.** HeLa siCon/siD2 cells were treated with paclitaxel and collected at 24 hours. Proportion of pH3-positive cells were analyzed by FC. **B and C.** HeLa siCon/siD2 cells were exposed to different concentrations of the indicated chemotherapeutic drugs for 48 hours and MTT assay was performed. Results obtained from three independent experiments (means  $\pm$  SD). (\*\*p < 0.01 and \*p < 0.05 as compared with control cells). **D.** Cells were lysed 24 hours after exposure to indicated concentration of paclitaxel and subjected to IB with anti-PAPR, anti-DAB2IP and anti-Actin antibodies.

## **Supplementary Figure 5**





**4C** 



5E





## **Supplementary Figure 5**

p-Plk1

Plk1

Actin

(Thr<sup>210</sup>)





Cdc25C loading



6F

His-Plk1

His-Plk1-

PBD







50

Plk1

**6B** 

75

50

75

50

37

Asy

Noc

Childre Di Neo Di

## **Supplementary Figure 5**



**Supplemental Figure S5.** The full-length images of all Western blots in the Figure 1, 4, 5, 6 and 7 (including the size makers).

Supplementary Movies 1-3. 1. Normal mitotic progression to Figure 1G siCon. 2 and3. Missegragation (lagging) or misaliged chromosomes to Figure 1G siD2.