SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Induction of mitotic slippage and p53 activation in HepG2 cells.
(A) Nocodazole-induced mitotic slippage and p53 activation in HepG2 cells. Cells were exposed to nocodazole and harvested at the indicated time points. Cell-free extracts were prepared and the expression of the indicated proteins was detected by immunoblotting. Equal loading of lysates was confirmed by immunoblotting for α-tubulin.
(B) HepG2 cells maintain a 4N DNA content upon prolonged nocodazole treatment. Cells were treated with either control buffer or nocodazole, and harvested for flow cytometry analysis at the indicated time points. The positions of 2N and 4N DNA content are indicated.

Figure S2. Re-replication in cells lacking p53 after prolonged nocodazole exposure.
$p53^{-/-}$ MEFs were exposed to nocodazole in the presence or absence of caspase inhibitor. The cells were harvested at the indicated time points and the DNA content was analyzed with flow cytometry. The positions of 2N, 4N, and 8N DNA contents are indicated.

Figure S3. High level of p53 expression after mitotic slippage.
HepG2 cells were either mock-treated or treated with nocodazole for 24 h or 36 h, before harvested for bivariant analysis of phospho-histone $H3^{Ser10}$ (PE, x-axis) and p53 (FITC, y-axis). The percentage of cells in each panel is indicated. No primary antibody was added in the control reaction. H1299 cells (a p53-null cell line) treated with nocodazole for 24 h was included as another negative control.

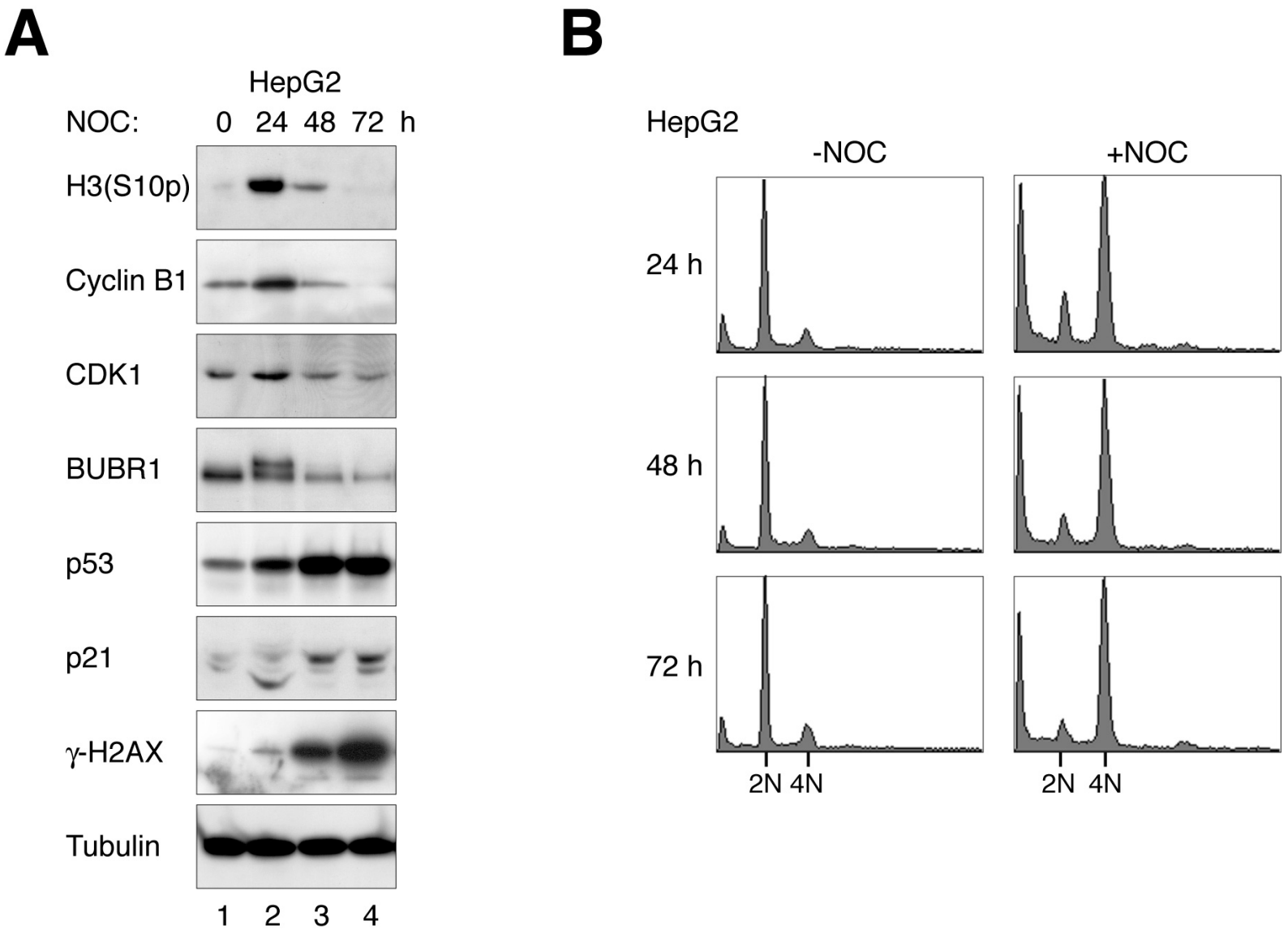
Figure S4. Activation of the p53-p21*CIP1/WAF1* pathway and suppression of S phase entry after release from the spindle-assembly checkpoint.
(A) HeLa cells progress through the cell cycle effectively after release from the spindle-assembly checkpoint. Cells were treated with nocodazole for 20 h before washed extensively and replated in fresh medium. At various time points, the cells were labeled with BrdU for 1 h before harvested for flow cytometry analysis and immunoblotting. Equal loading of lysates was confirmed by immunoblotting for tubulin.
(B) Only a portion of nocodazole-treated HepG2 cells can be released into G_1 phase and entry into S phase is inhibited. HepG2 cells were cultured with nocodazole for 24 h before released into nocodazole-free medium. At various time points, the cells were pulse-labeled with BrdU before harvested for flow cytometry analysis and immunoblotting. The asterisk indicates cells that displayed a 4N DNA content, which contained both G_2 and G_1 tetraploid cells. Tubulin analysis was included to assess protein loading and transfer.

Figure S5. Entry into S phase after release from the spindle-assembly checkpoint is inhibited by p53 in Hep3B cells.
(A) Re-introduction of p53 to Hep3B cells inhibits S phase after mitotic release. Hep3B and Hep3B/p53 cells were exposed to nocodazole for 24 h. After extensive washing, the cells were cultured in

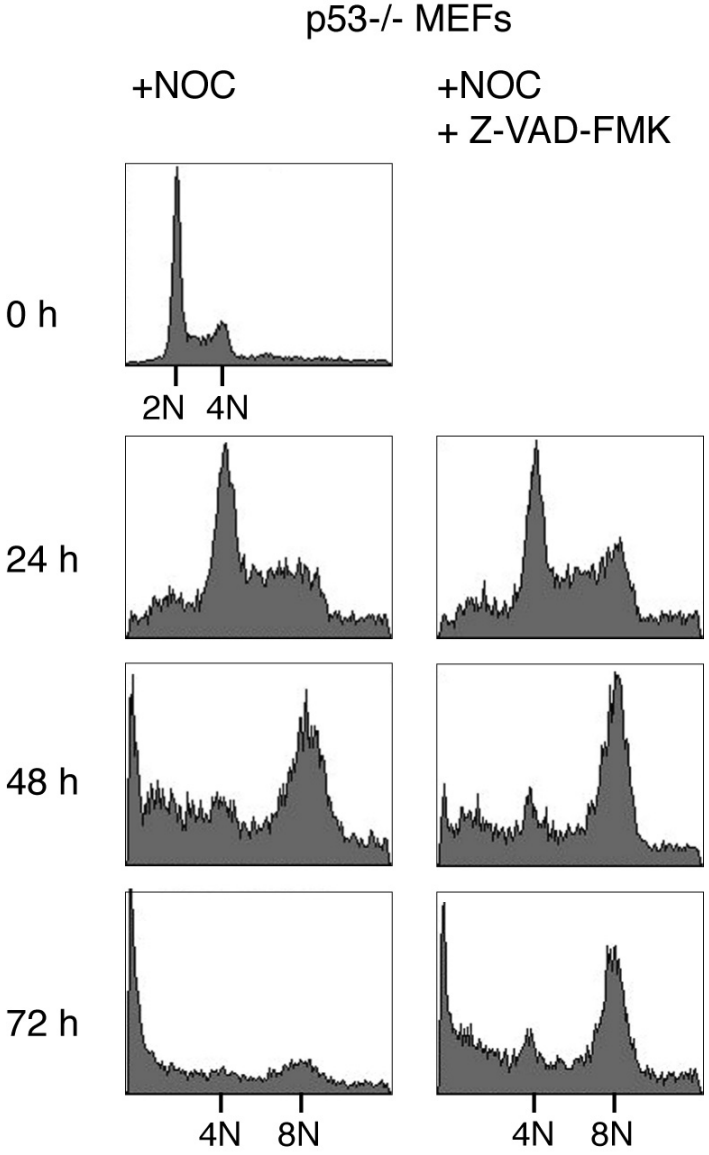
nocodazole-free medium. DNA synthesis at different time points was analyzed by pulse labeling with BrdU followed by flow cytometry.
(B) Hep3B cells were treated with nocodazole for 24 h before released into the cell cycle. Cell-free extracts were prepared at different time points and the indicated proteins were detected by immunoblotting. Extracts of growing Hep3B cells were loaded as a control.
(C) Stabilization of p53 after release from mitotic block in Hep3B/p53 cells. The experiment was performed similarly to panel (B) except that Hep3B/p53 cells were used.

Figure S6. Entry into S phase after release from the spindle-assembly checkpoint is inhibited by p53 in MEFs.
Disruption of p53 permits S phase entry after release from mitotic blockade. E1A-immortalized wild type and $p53^{-/-}$ MEFs were exposed to nocodazole for 24 h. After extensive washing, the cells were cultured in nocodazole-free medium. DNA synthesis at different time points was analyzed by pulse labeling with BrdU followed by flow cytometry.

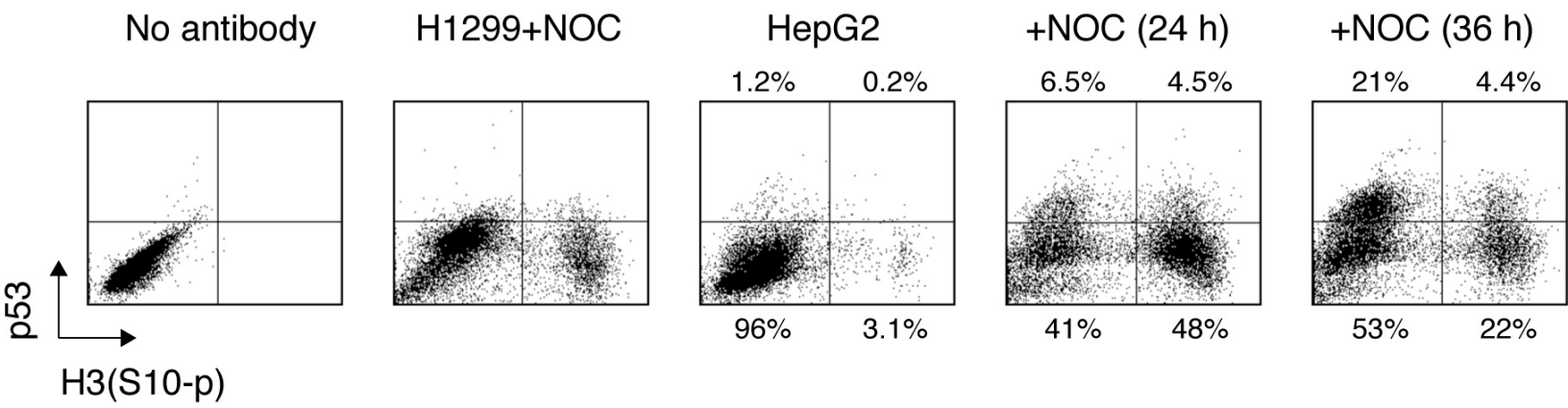
Figure S7. MAD2L1BP disrupts the KIF11 siRNA-induced spindle-assembly checkpoint and postmitotic checkpoint.
(A) MAD2L1BP promotes re-replication after knockdown of KIF11. Parental U2OS and U2OS/MAD2L1BP cells were transfected with siRNA targeting KIF11. MAD2L1BP expression was induced with doxycycline for 48 h. The cells were then harvested and processed for flow cytometry analysis.
(B) p53 and $p21^{CIP1/WAF1}$ are activated during KIF11 siRNA-induced re-replication. Parental U2OS and U2OS/MAD2L1BP cells were treated as in panel (A). Cell-free extracts were prepared and were subjected to immunoblotting for the indicated proteins. Uniform loading of lysates was confirmed by actin.



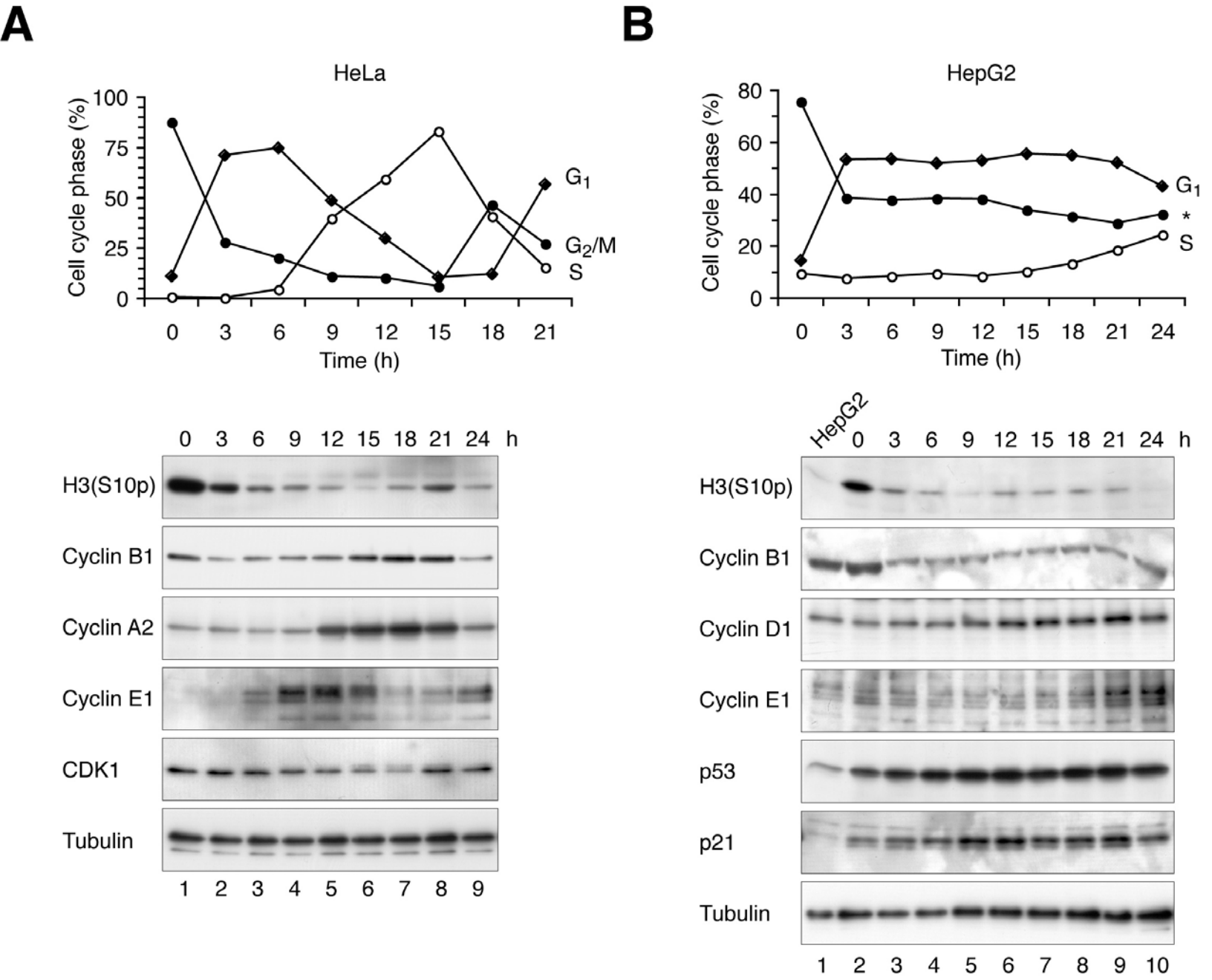
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



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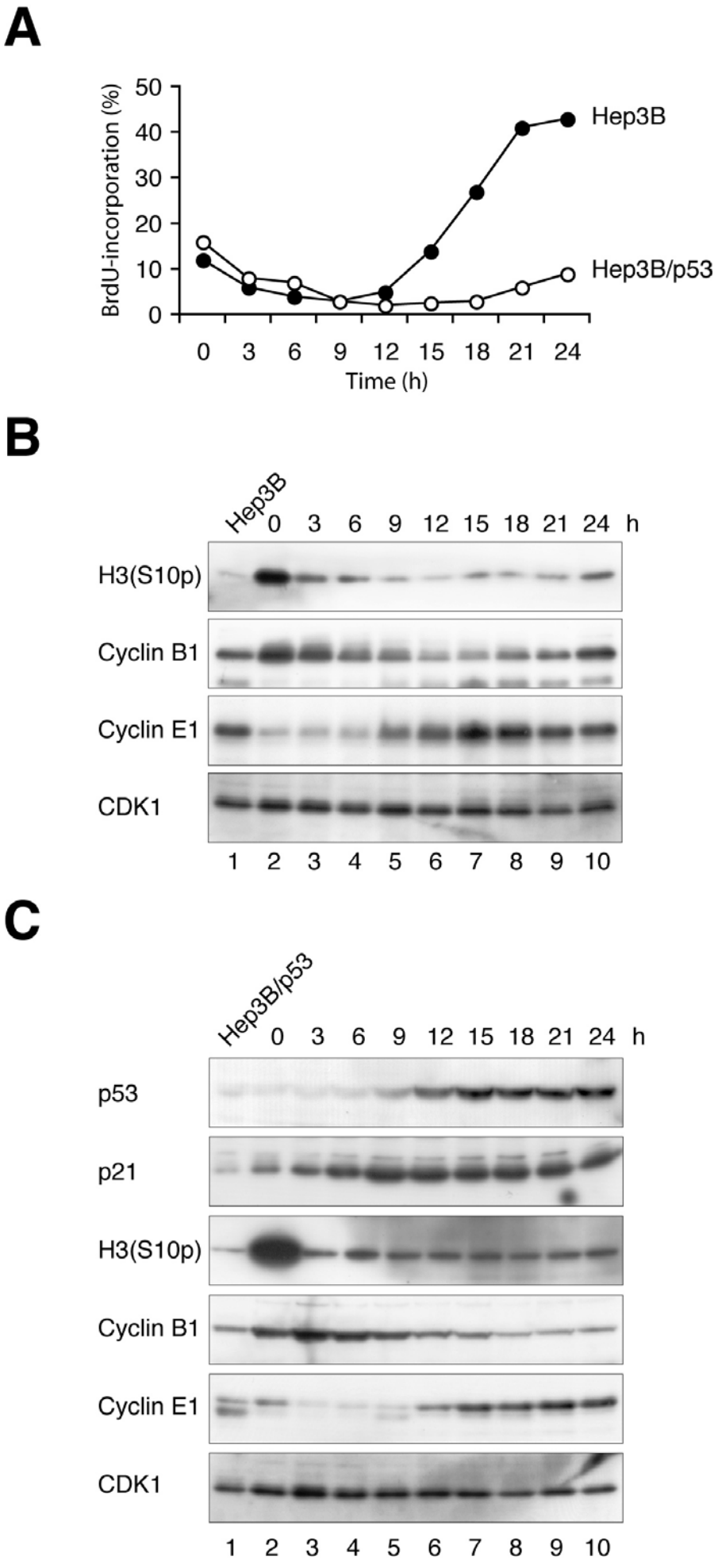

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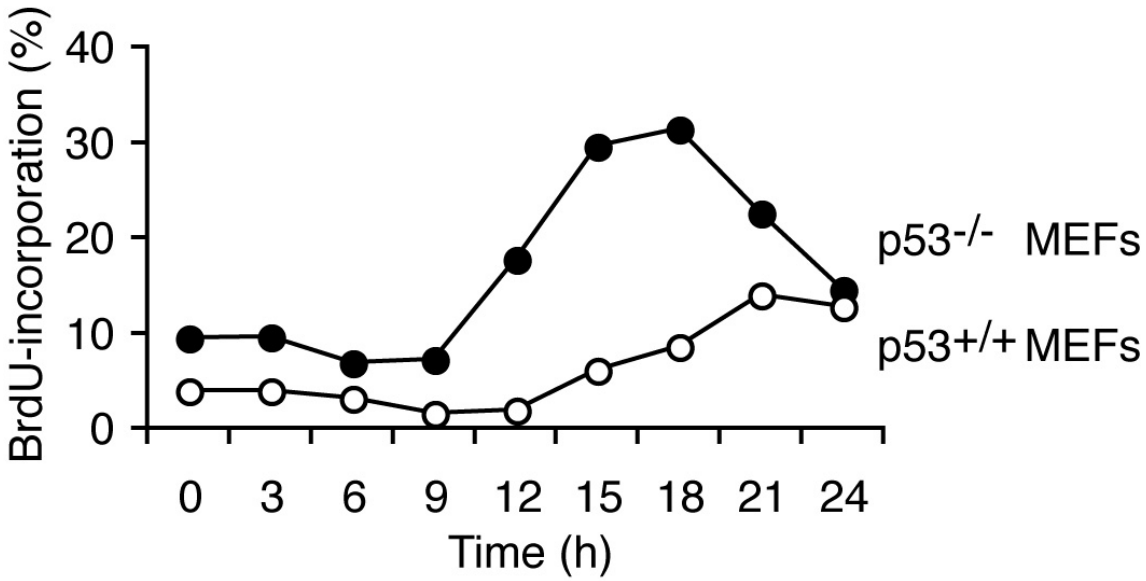

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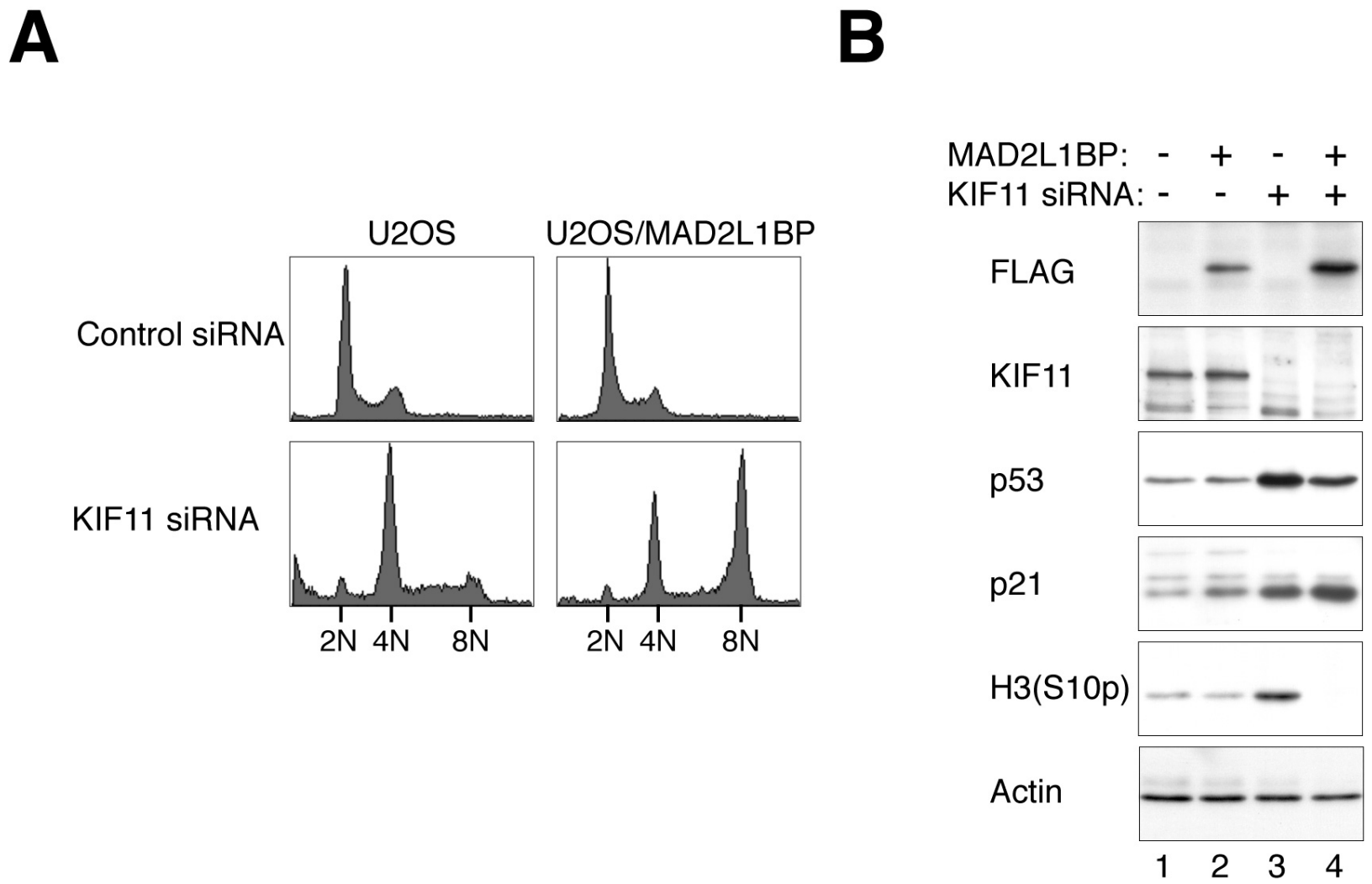

Supplemental Figure 7