Fig. S1. Viability of paclitaxel-treated MCF10A cell lines. The Hygro and D1K2 CL1 cell lines were treated with the indicated concentration of paclitaxel for 72 hours, after which the number of cells remaining was counted using a hemocytometer. A parallel set of cells were incubated a further 48 hours after removal of paclitaxel before being counted. Cell numbers were normalized to the number of cells present upon starting paclitaxel treatment (T_0).

Fig. S2. Effects of paclitaxel and CVT313 on MCF10A cell lines. (A) [^{3}H]Thymidine incorporation of the indicated cell lines treated with 1 μM paclitaxel and/or 5 μM CVT313 for 24 hours. Indicated values represent the average of three replicate samples. Error bars represent the s.d. of three replicates. (B) Immunoblot analysis of the indicated cell lines treated with or without 5 μM CVT313 for 24 hours. Actin serves as a loading control.

Fig. S3. Analysis of Mad2 in the spindle assembly checkpoint. (A) Immunoblot analysis of the indicated cell lines showing Mad2 expression levels before and after knockdown. (B) Immunoblot analysis of p-MPM2 levels in the indicated cell lines after treatment with DMSO or 1 μM paclitaxel for 24 hours.

Fig. S4. Centrosome amplification in MCF10A cell lines in the presence of paclitaxel and CVT313. Rate of centrosome amplification (>2 centrosomes/cell) in the MCF10A D1K2 CL1 cell line treated with vehicle control, 1 μM paclitaxel, or 1 μM paclitaxel along with 5 μM CVT313. Number of centrosomes were scored visually by immunofluorescence using an antibody directed toward pericentrin. Indicated values represent the average of three replicate samples. Error bars represent the s.d. of three replicates. *** denotes $P<0.001$ using the unpaired *t*-test.

Fig. S5. Analysis of p53 knockdown efficiency. Immunoblots of the indicated cell lines showing p53 expression. p53 was induced in all cell lines by treatment with 0.5 μg/ml doxorubicin for 8 hours. Actin serves as a loading control.

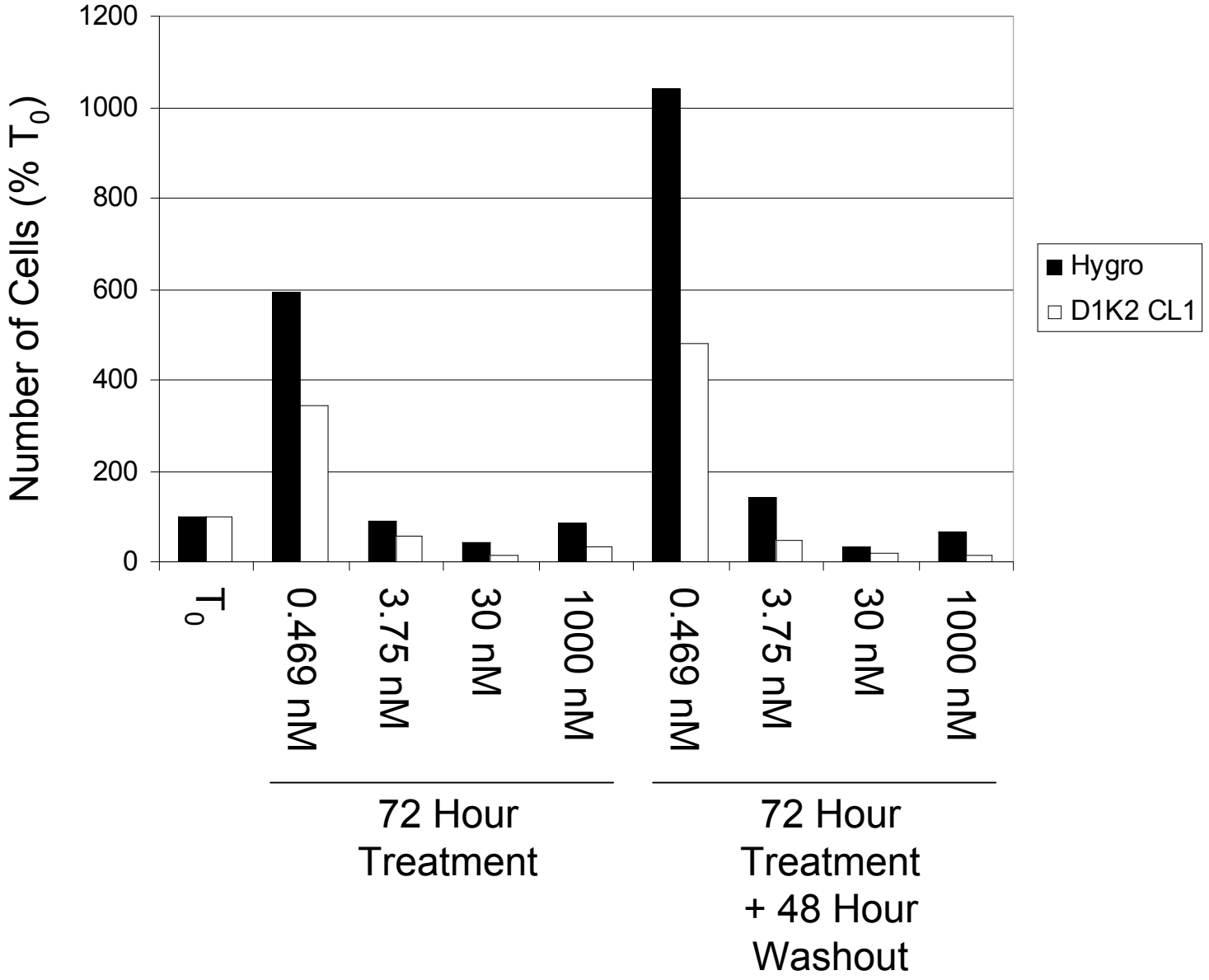
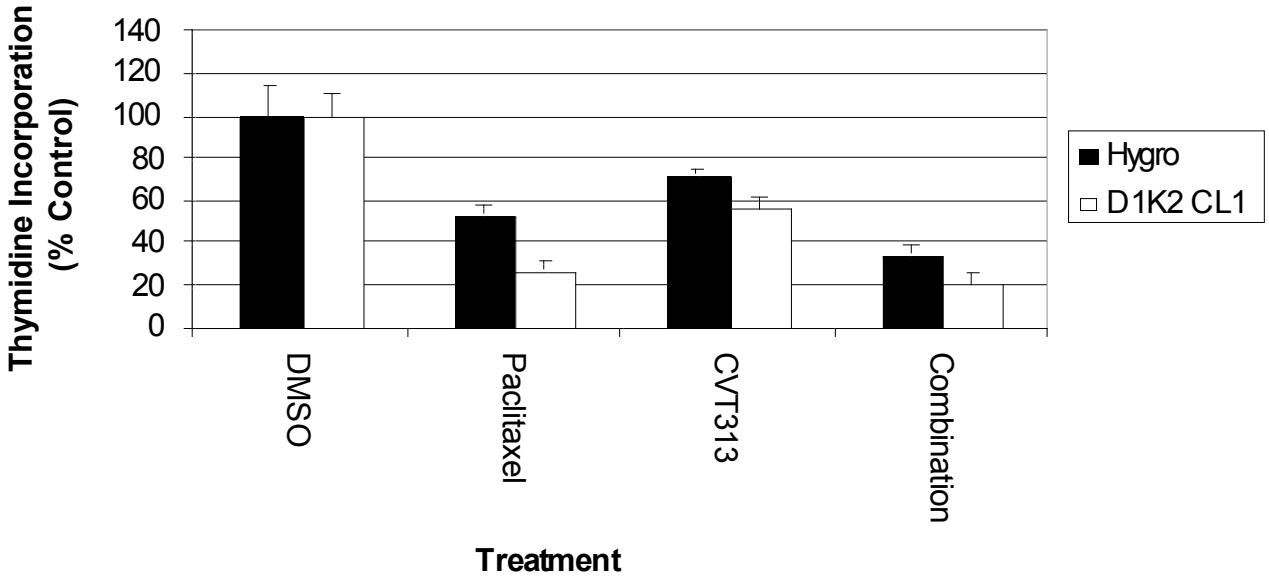

Number of Cells (% T_0)
1200
1000
800
600
400
200
0
Hygro
D1K2 CL1
T_0
0.469 nM
3.75 nM
30 nM
1000 nM
0.469 nM
3.75 nM
30 nM
1000 nM
72 Hour Treatment
72 Hour Treatment + 48 Hour Washout

Fig. S1

A.


Thymidine Incorporation (% Control)
140
120
100
80
60
40
20
0
DMSO
Paclitaxel
CVT313
Combination
Treatment
Hygro
D1K2 CL1

B.

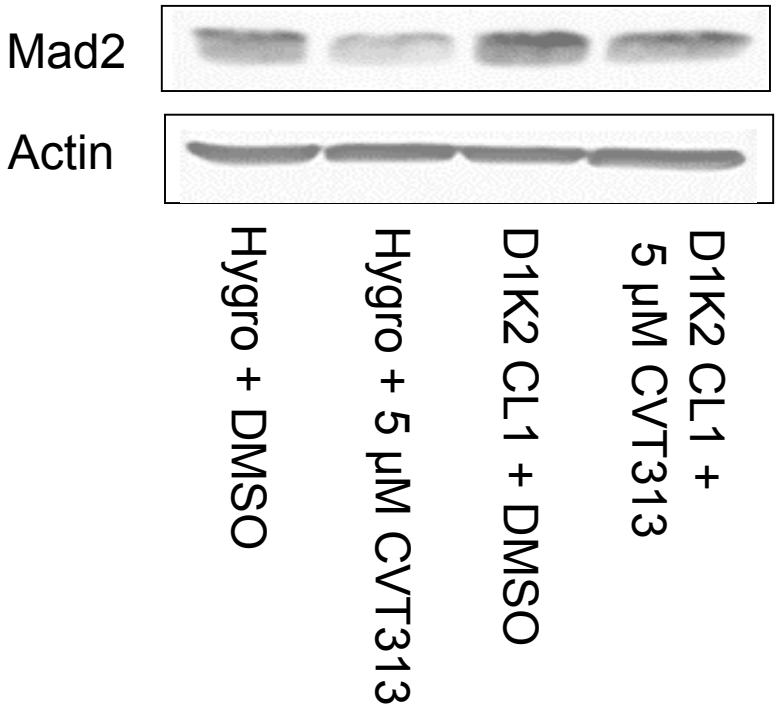

Mad2
Actin
Hygro + DMSO
Hygro + 5 µM CVT313
D1K2 CL1 + DMSO
D1K2 CL1 + 5 µM CVT313

Fig. S2

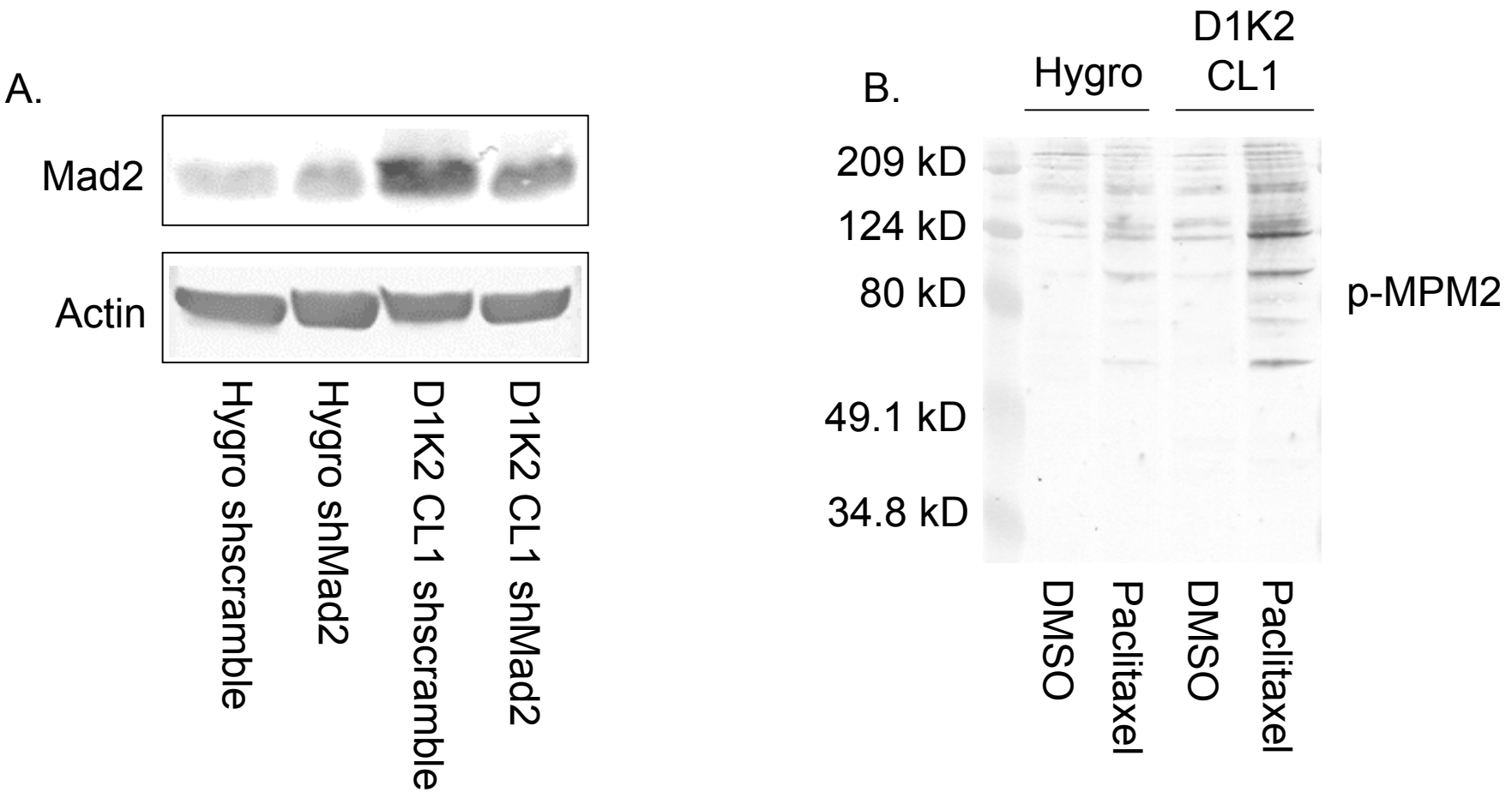

A.
Mad2
Actin
Hygro shscramble
Hygro shMad2
D1K2 CL1 shscramble
D1K2 CL1 shMad2
B.
Hygro
D1K2 CL1
209 kD
124 kD
80 kD
49.1 kD
34.8 kD
p-MPM2
DMSO
Paclitaxel
DMSO
Paclitaxel

Fig. S3

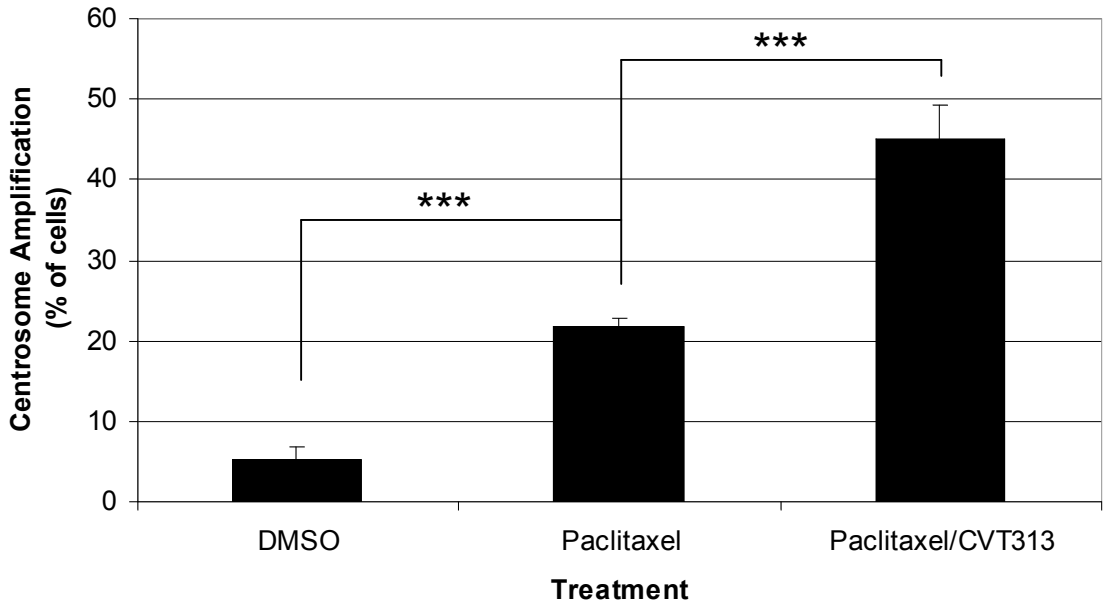

60
50
40
30
20
10
0
Centrosome Amplification
(% of cells)
***
***
DMSO
Paclitaxel
Paclitaxel/CVT313
Treatment

Fig. S4

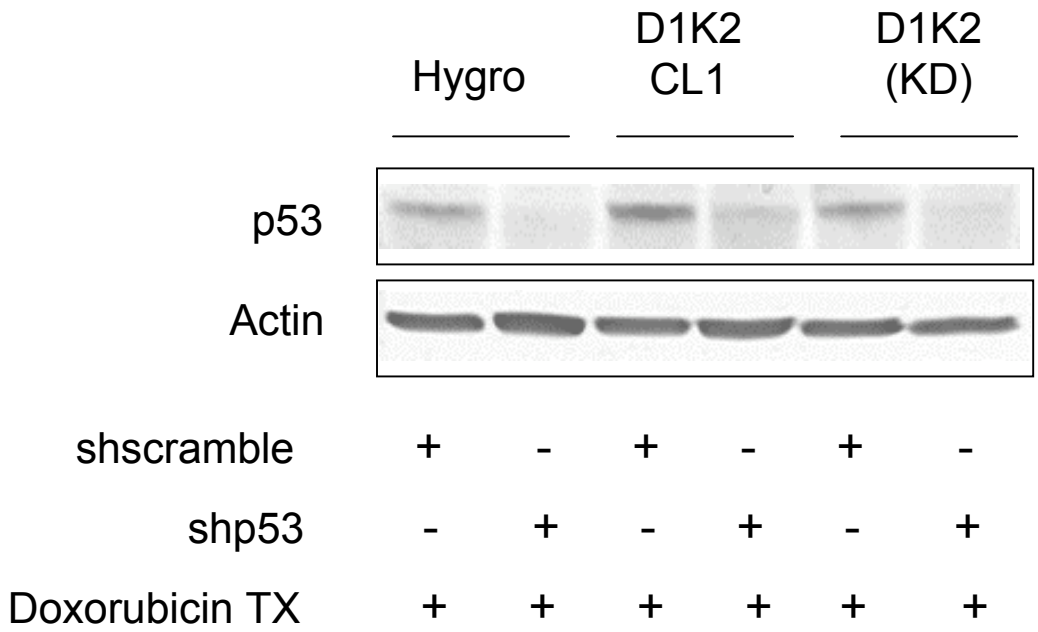

Hygro
D1K2 CL1
D1K2 (KD)
p53
Actin
shscramble + - + - + -
shp53 - + - + - +
Doxorubicin TX + + + + + +

Fig. S5