Supplemental Material

Supplemental figure legends

Fig. S1. The Taxol or IR-induced p53 response is diminished in TTK/hMps1-knockdown cells. (A) Taxol-induced increase in p53 was dampened in U2OS cells transfected with TTK siRNA TK1 or TK3. Sequences targeted by TK3 are 5′-tggttgagtttgttgctca-3′. (B, C) U2OS (B) or LNCaP cells (C) were transfected with control, TTK (TK1), or BubR1 siRNA. Cells treated with IR (8 Gy) were collected at the indicated times and analyzed by Western blotting for the indicated proteins. Note that, in contrast to TTK depletion, BubR1 knockdown did not affect the p53 response.

Fig. S2. Downregulation of TTK/hMps1 by siRNA abrogates nocodazole or DNA damage-induced p53 Thr18 phosphorylation. 293T cells transfected with control, TTK or CHK2 siRNA were treated with nocodazole (Noc, 50 ng/ml) (A), IR (8 Gy) (B) or UV (30 J/m^2) (C). Lysates were collected and analyzed by Western blotting.

Fig. S3. MAD2 facilitates the p53 response in the post-mitotic checkpoint. (A) TTK/hMps1-mediated stabilization of the p53 *N*-terminal domain and Thr18 phosphorylation was decreased by MAD2 downregulation. 293T cells were transfected with HA-tagged p53 N96 (amino acids 1-96) and TTK together with the indicated siRNA. Cell lysates were analyzed by Western blotting using specific antibodies. Actin was used as a loading control. (B) Polyploidy upon mitotic slippage was aggravated in either MAD2 or TTK-depleted HCT116 cells. Cells transfected with indicated siRNA were treated with nocodazole (Noc, 50 ng/ml) and analyzed as in Fig. 6A.

Fig. S4. Impact of TTK/hMps1 on p53ΔC30. (A) WT but not KD TTK stabilized

p53ΔC30. p53ΔC30 was coexpressed with increasing amounts of TTK, either WT or KD, and analyzed by Western blotting as in Fig. 4F. (B) TTK phosphorylates p53 ΔC30 as well as the full-length protein *in vitro*. Kinase assays were conducted by incubating increasing amounts of purified recombinant His-p53, full-length (WT) or ΔC30, with purified GST-TTK, WT or KD in the presence of [γ-^{32}P] ATP. Reactions were analyzed by SDS-PAGE followed by autoradiography (upper panel). Protein inputs and p53 Thr18 phosphorylation was examined by Western blotting (WB, lower panel).

Fig. S1

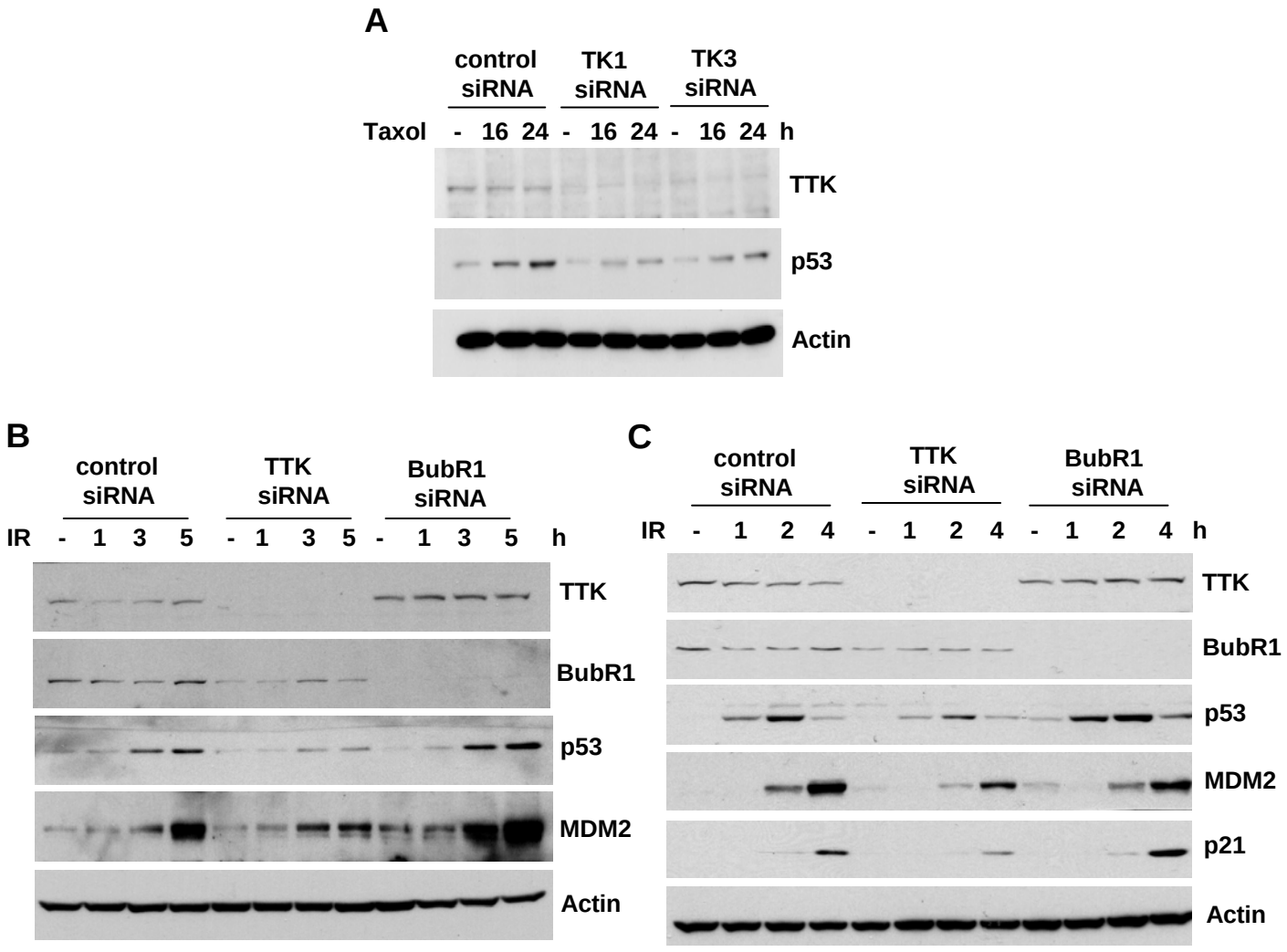

A
control siRNA
TK1 siRNA
TK3 siRNA
Taxol - 16 24 - 16 24 - 16 24 h
TTK
p53
Actin
B
control siRNA
TTK siRNA
BubR1 siRNA
IR - 1 3 5 - 1 3 5 - 1 3 5 h
TTK
BubR1
p53
MDM2
Actin
C
control siRNA
TTK siRNA
BubR1 siRNA
IR - 1 2 4 - 1 2 4 - 1 2 4 h
TTK
BubR1
p53
MDM2
p21
Actin

Fig. S2

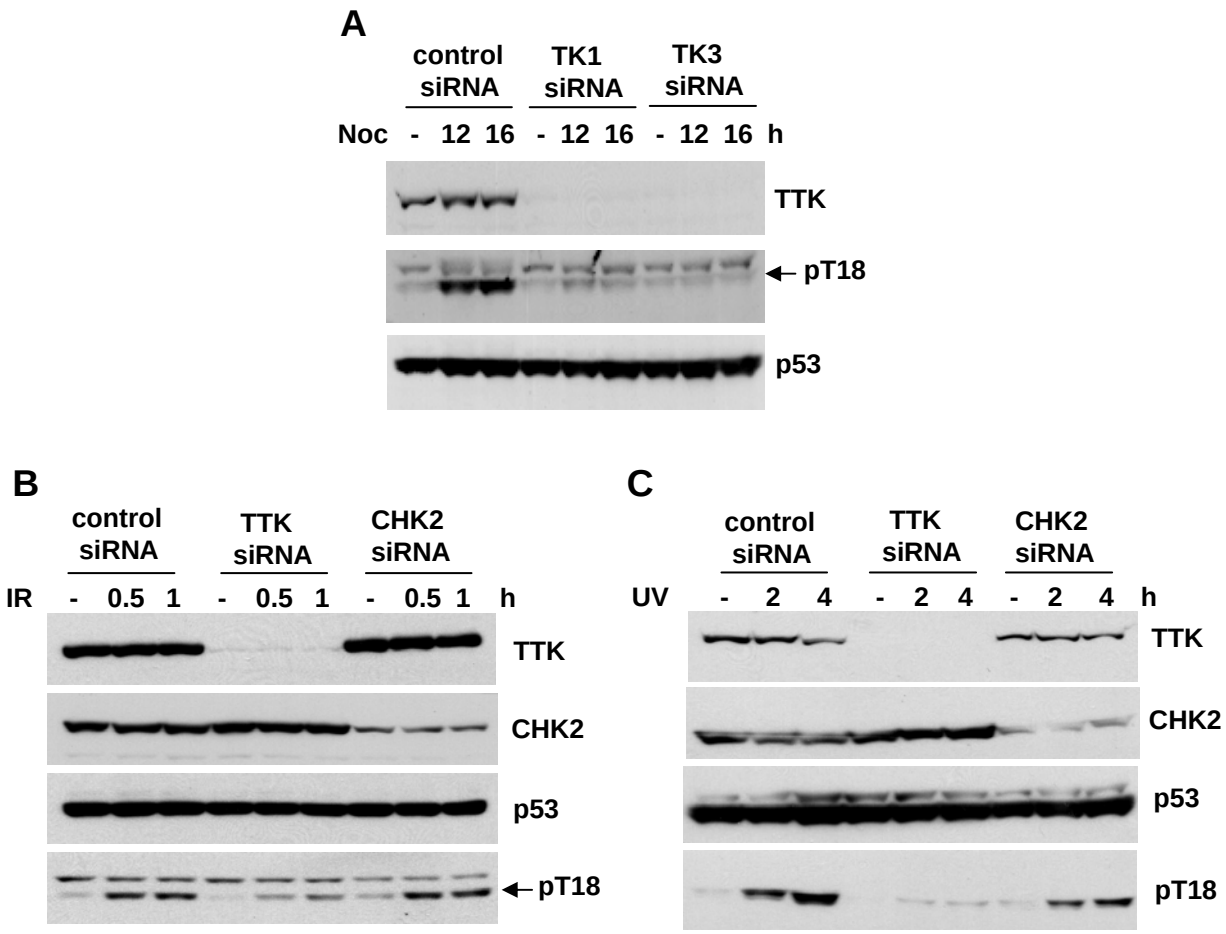

A
control siRNA
TK1 siRNA
TK3 siRNA
Noc - 12 16 - 12 16 - 12 16 h
TTK
pT18
p53
B
control siRNA
TTK siRNA
CHK2 siRNA
IR - 0.5 1 - 0.5 1 - 0.5 1 h
TTK
CHK2
p53
pT18
C
control siRNA
TTK siRNA
CHK2 siRNA
UV - 2 4 - 2 4 - 2 4 h
TTK
CHK2
p53
pT18

Fig. S3

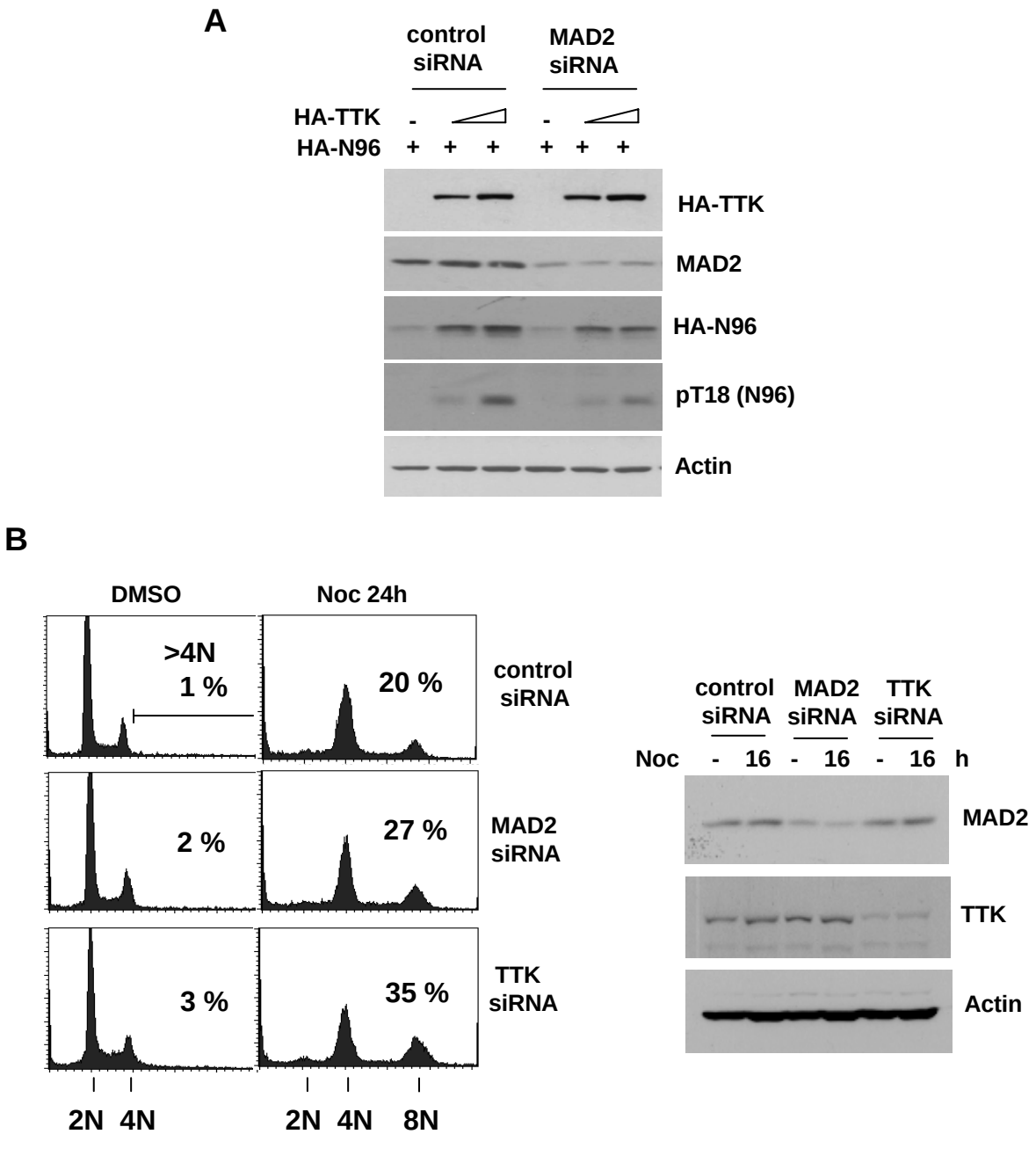
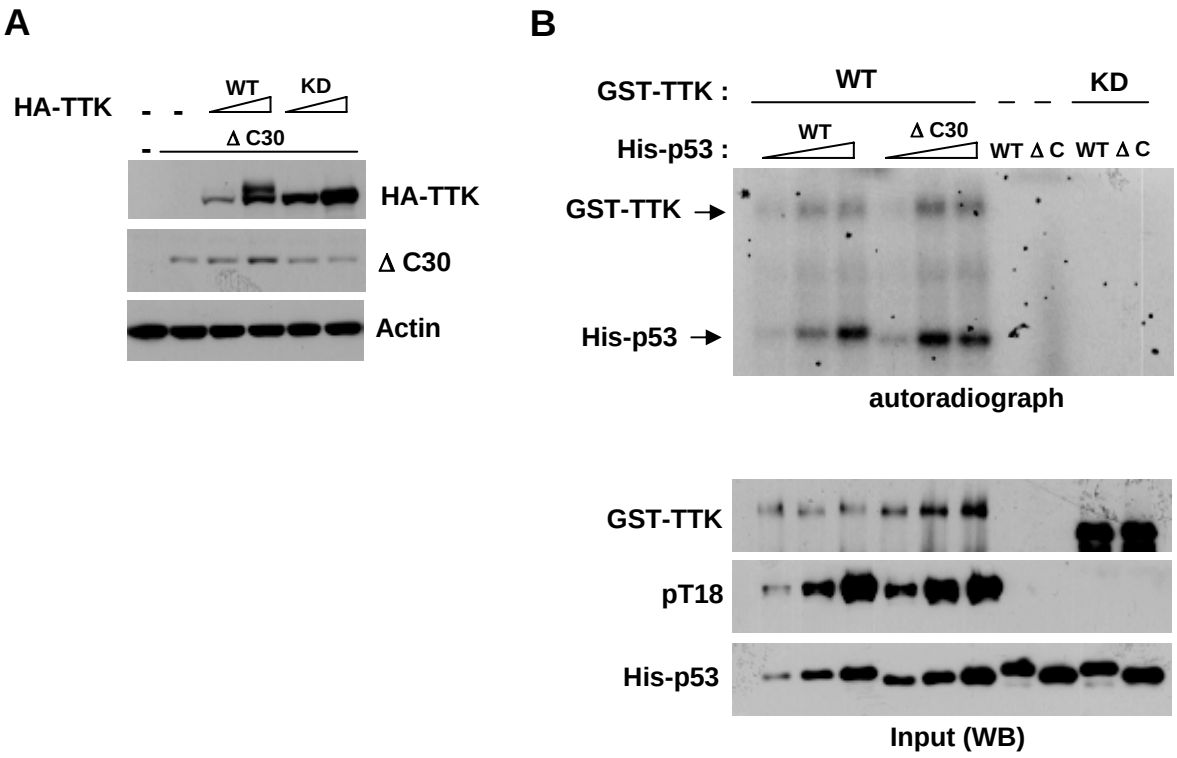

A
control siRNA
MAD2 siRNA
HA-TTK - - -
HA-N96 + + + + + +
HA-TTK
MAD2
HA-N96
pT18 (N96)
Actin
B
DMSO
Noc 24h
>4N
1 %
20 %
control siRNA
2 %
27 %
MAD2 siRNA
3 %
35 %
TTK siRNA
2N 4N
2N 4N 8N
control siRNA
MAD2 siRNA
TTK siRNA
Noc - 16 - 16 - 16 h
MAD2
TTK
Actin

Fig. S4


A
HA-TTK
- -
WT
KD
-
Δ C30
HA-TTK
Δ C30
Actin
B
GST-TTK :
WT
KD
His-p53 :
WT
Δ C30
WT Δ C
WT Δ C
GST-TTK
His-p53
autoradiograph
GST-TTK
pT18
His-p53
Input (WB)