SUPPLEMENTAL FIGURE LEGENDS

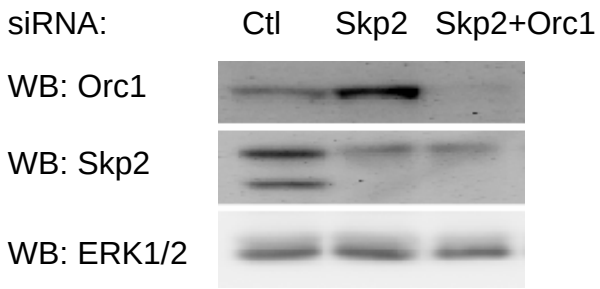
Supplemental Figure 1. Depletion of Orc1 does not block Skp2-induced G2 arrest. WM115 cells were transfected with control siRNA, Skp2 #13 siRNA alone or Skp2 plus Orc1 siRNA (GAACAGGAAUUCCAAGACAUU) for 6 days. (A) Cells were lysed and lysates analyzed by Western blotting for Skp2, Orc1 and ERK1/2 (loading). (B) Cells were stained with propidium iodide and analyzed by FACS. (C) The average percent and s.d. of cells in G0/G1, S, 4N and polyploid (8N) phases of the cell cycle from three independent experiments.

Supplemental Figure 2. Expression of 3A-cyclin B1 blocks Skp2 knockdown-induced G2/M arrest.
WM115-TR-WT-cyclin B1 and WM115-TR-3A-cyclin B1 cells were transfected with control or Skp2 #13 siRNA for 6 days in the presence or absence of 0.1 μg/ml doxycycline. Cells were stained with propidium iodide and analyzed by FACS. Representative propidium iodide profiles are shown.

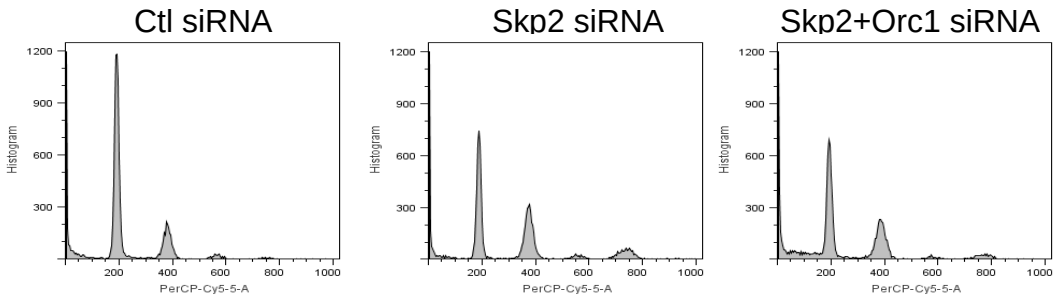
Supplemental Figure 3. Use of a second p53 siRNA to reverse effects of Skp2 depletion on G2/M arrest.
WM115 cells were transfected with control siRNA, Skp2 #13 siRNA alone, p53 siRNA (duplex #6, CAGUCUACCUCCCGCCAUAUU) alone, or Skp2 plus p53 siRNA for 6 days. (A) Cells were lysed and lysates analyzed by Western blotting for p53, $p27^{Kip1}$, and ERK1/2. (B) Cells were stained with propidium iodide and

analyzed by FACS. Representative profiles are shown and the average percent of 4N cells from three independent experiments is indicated.

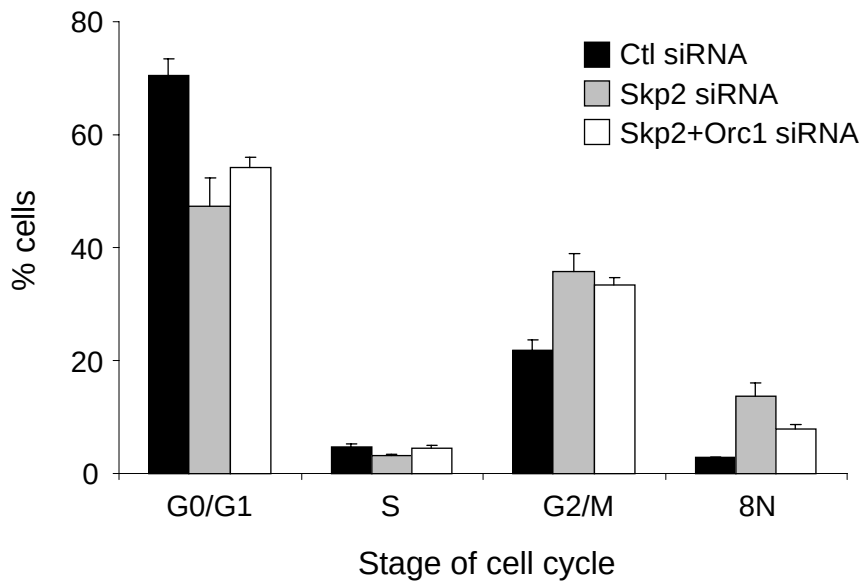
A

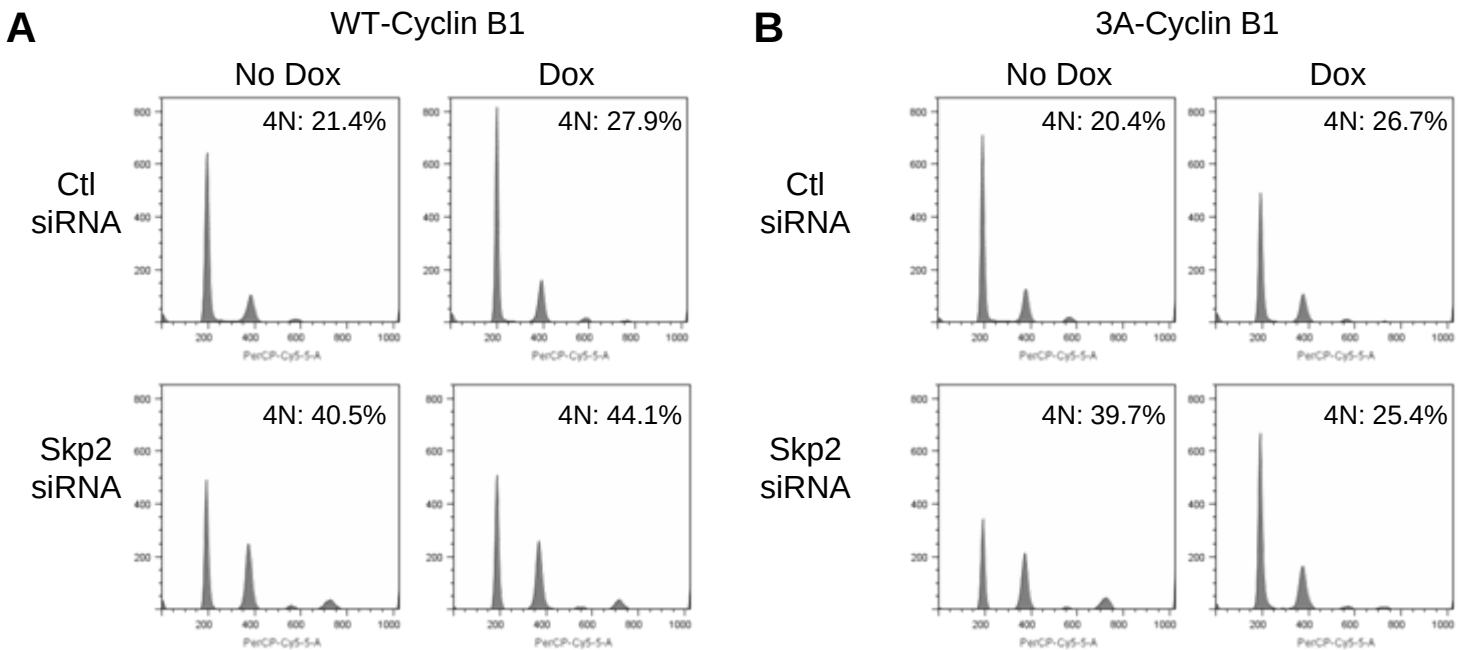

siRNA:
Ctl
Skp2
Skp2+Orc1
WB: Orc1
WB: Skp2
WB: ERK1/2

B

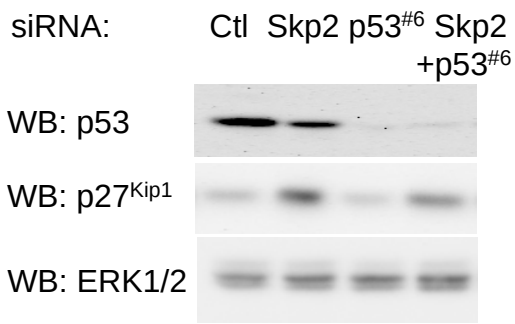

Ctl siRNA
Skp2 siRNA
Skp2+Orc1 siRNA
Histogram
PerCP-Cy5-5-A

C

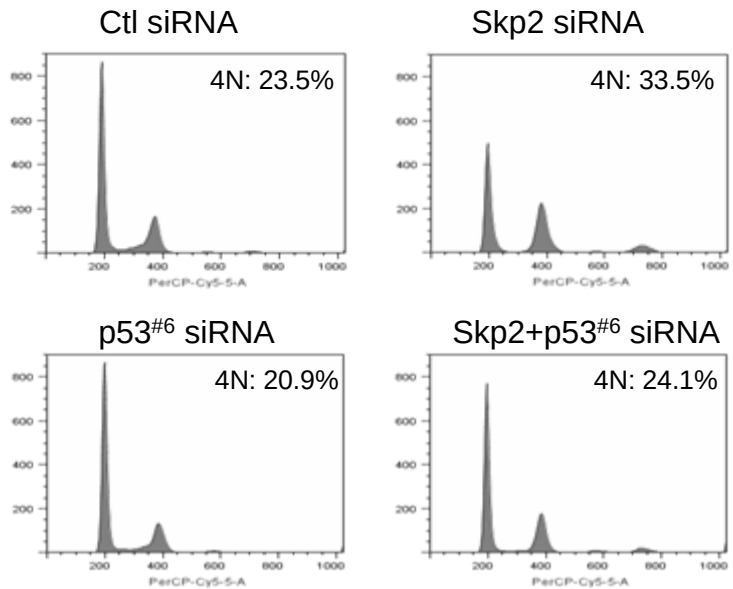

Ctl siRNA
Skp2 siRNA
Skp2+Orc1 siRNA
% cells
G0/G1
S
G2/M
8N
Stage of cell cycle


A
WT-Cyclin B1
No Dox
Dox
Ctl siRNA
Skp2 siRNA
4N: 21.4%
4N: 27.9%
4N: 40.5%
4N: 44.1%
B
3A-Cyclin B1
No Dox
Dox
Ctl siRNA
Skp2 siRNA
4N: 20.4%
4N: 26.7%
4N: 39.7%
4N: 25.4%
PerCP-Cy5-5-A

A


siRNA:
Ctl
Skp2
p53#6
Skp2 +p53#6
WB: p53
WB: p27Kip1
WB: ERK1/2

B


Ctl siRNA
4N: 23.5%
Skp2 siRNA
4N: 33.5%
p53#6 siRNA
4N: 20.9%
Skp2+p53#6 siRNA
4N: 24.1%
PerCP-Cy5-5-A