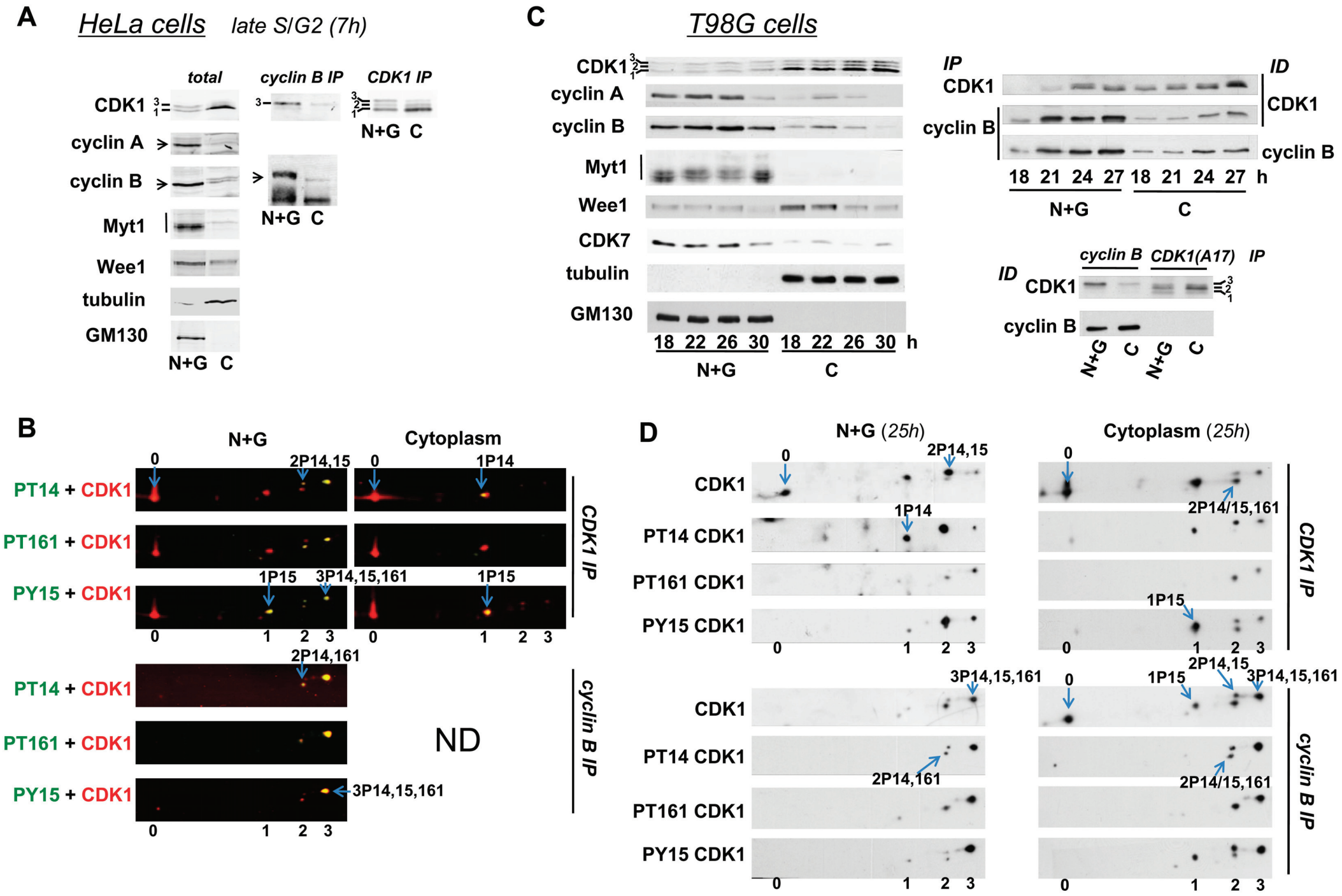
Supplemental Figure S1.

Subcellular compartmentalization of CDK1 complexes and kinases

Lysates of synchronized HeLa cells (A,B) in late S/G2 phase 7 h after release from double thymidine block, or T98G cells (C,D) stimulated with FBS for the indicated times, were fractionated as described in "*Material and Methods*".

(A,C) Crude nuclei + Golgi (*N+G*) and cytoplasmic (*C*) fractions of HeLa and T98G cells were either directly immunoblotted with the indicated antibodies (*total*), or were first precipitated (*IP*) with a cyclin B1 or CDK1 (C19 or A17 clone) antibody and then analyzed by immunoblotting (*ID*). CDK1 separated in three bands (*1,2,3*). The arrows point cyclin A or cyclin B. The mobility shift of Myt1 is highlighted.

(B,D) Those N+G and cytoplasmic (*C*) fractions of HeLa and T98G cells were immunoprecipitated either with an anti-CDK1 antibody (*CDK1 IP*) or with an anti-cyclin B1 (*cyclin B IP*), separated by 2D-gel electrophoresis and immunoblotted for detection of CDK1 and PT14, PT161 or PY15 CDK1. The most characteristic forms of CDK1 in each fraction are pointed. In HeLa cells (B), cyclin B1-bound CDK1 was insufficiently abundant in the cytoplasmic fraction, which precluded its reliable analysis by immunofluorescence scanning after 2D-gel separation (*ND*). In T98G cells (D), detection by enhanced chemiluminescence of cytoplasmic cyclin B1-bound CDK1 required a three-time higher amount of cell extract than in the other conditions. *0, 1, 2, 3* stands for the number of phosphorylation(s) of CDK1.


A
HeLa cells late S/G2 (7h)
total
cyclin B IP
CDK1 IP
CDK1
cyclin A
cyclin B
Myt1
Wee1
tubulin
GM130
N+G C
C
T98G cells
CDK1
cyclin A
cyclin B
Myt1
Wee1
CDK7
tubulin
GM130
18 22 26 30 18 22 26 30 h
N+G C
IP
ID
CDK1
cyclin B
18 21 24 27 18 21 24 27 h
N+G C
cyclin B
CDK1(A17)
IP
ID
CDK1
cyclin B
N+G C N+G C
B
N+G
Cytoplasm
PT14 + CDK1
PT161 + CDK1
PY15 + CDK1
0
2P14,15
1P14
1P15
3P14,15,161
0 1 2 3
CDK1 IP
2P14,161
ND
cyclin B IP
D
N+G (25h)
Cytoplasm (25h)
CDK1
PT14 CDK1
PT161 CDK1
PY15 CDK1
0
2P14,15
1P14
2P14/15,161
1P15
3P14,15,161
2P14,161
0 1 2 3
CDK1 IP
cyclin B IP

Supplemental Figure S2.

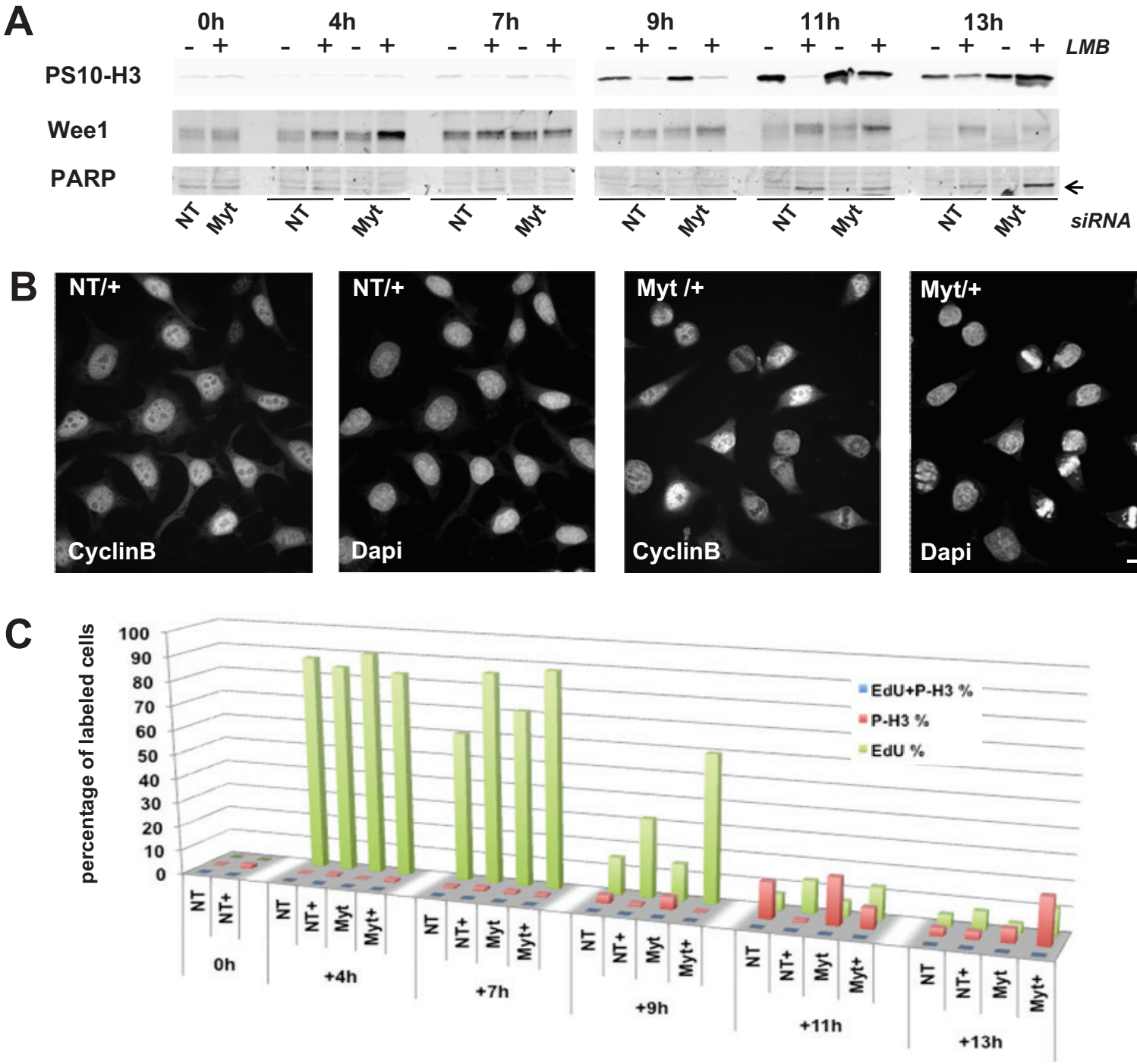
Impact of leptomycin B (LMB) and Myt1 knockdown on cell cycle progression.

Synchronized HeLa cells were treated with the indicated siRNA pools ((*NT*, control non-targeting siRNA pool)) as in Fig. 6. At time 0 (second release), cells were either left untreated (-) or incubated with 30 nM LMB (+).

(A) Whole cell extracts were analyzed by SDS-PAGE followed by immunoblotting with the indicated antibodies. The arrow points the cleaved fragment of PARP.

(B) HeLa cells transfected with *NT* or *Myt1* siRNA pools, treated (+) with LMB, and fixed 13 h after release from the last thymidine block. Cells were stained with anti-cyclin B1 antibody and Dapi. Bar, 10 μm.

(C) Percentages of DNA replicating cells (EdU incorporation), phosphohistone H3 (*P-H3*)–positive cells, and cells positively stained for both phosphohistone H3 and EdU incorporation. More than 400 cells were counted in each condition.


A
0h
4h
7h
9h
11h
13h
LMB
PS10-H3
Wee1
PARP
siRNA
NT
Myt
B
NT/+
CyclinB
Dapi
Myt /+
Myt/+
C
percentage of labeled cells
EdU+P-H3 %
P-H3 %
EdU %
+4h
+7h
+9h
+11h
+13h