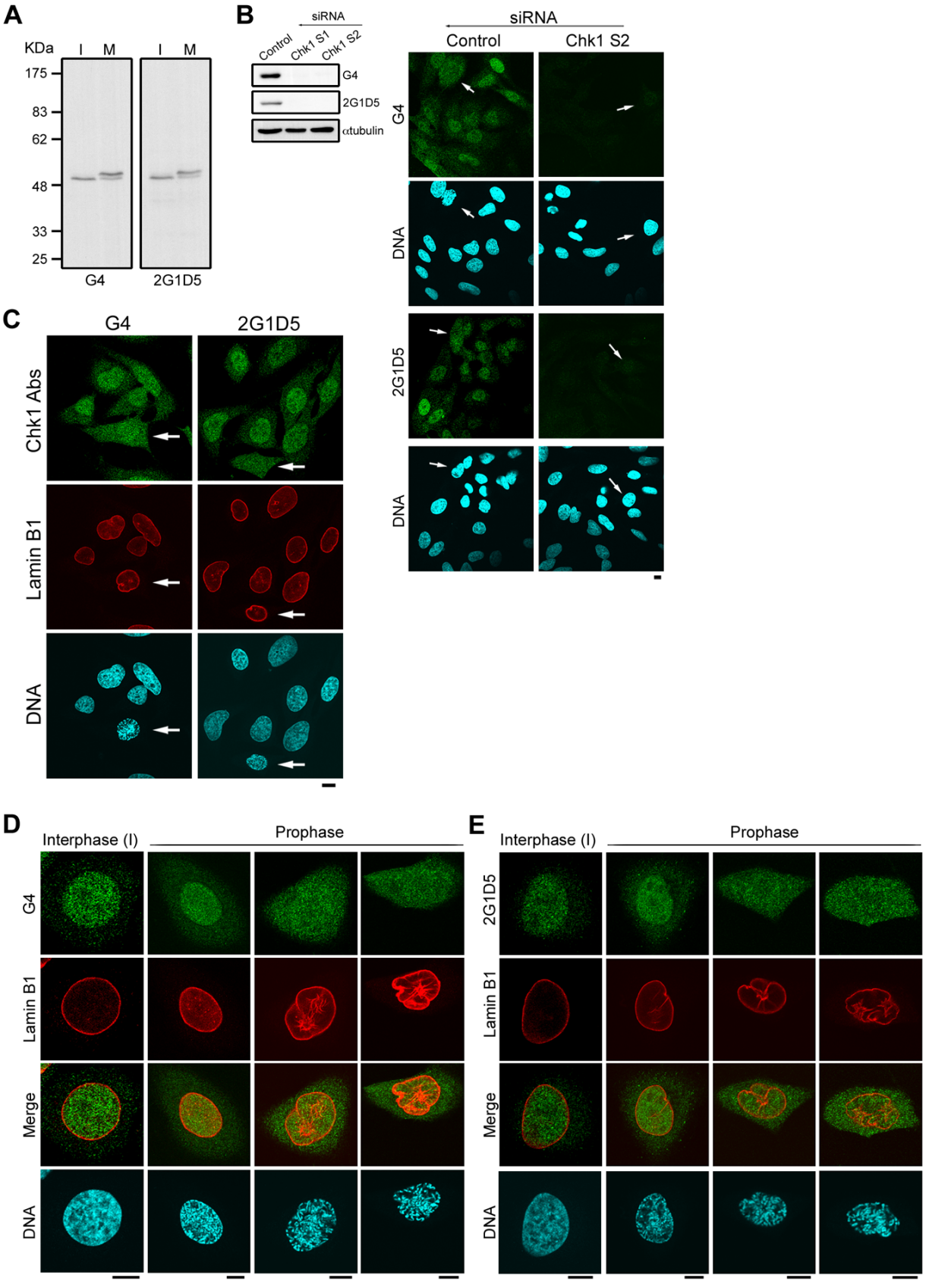
SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. Chk1 moves from the nucleus to the cytoplasm in prophase. *A*. Interphase and mitotic HeLa cell lysates were subjected to the immunoblotting with 2 additional mouse monoclones (G4 and 2G1D5) of anti-Chk1 antibodies. *B*. At 48 h after transfection with each siRNA, cells were subjected to immunoblotting or immunocytochemistry. Similar diminishment of G4 and 2G1D5 signals in the immunocytochemistry was observed in cells transfected with Chk1 S1 siRNA (data not shown). The scale bar represents 10 μm. *C-E*. Confocal microscopic images of HeLa cells stained with G4 or 2G1D5 (Alexa488-conjugated anti-mouse, green). Nuclear membranes and DNA were simultaneously stained with anti-Lamin B1 (Alexa546-conjugated anti-rabbit, red) and DAPI (blue), respectively. The scale bars represent 10 μm.

Fig. S2. Assessment of each Tet-On HeLa cell line. *A*. HeLa cells were transfected with the mixture of LipofectAMINE Plus^{TM} (Invitrogen, Carlsbad, CA) and pIRES-puro3 (BD Clontech, San Diego, CA) vector carrying each type of Myc-Chk1. Control indicates the empty vector. At 48 h after the transfection, HeLa cells were collected for the immunoblotting. Each Tet-ON HeLa cell line was treated with (+) or without (-) doxycycline (Dox) for 48 h. WT cells treated with 3 mM hydroxyurea (HU) for 16 h were indicated as a positive control. After the treatment, each sample was subjected to the immunoblotting with pS345 (to detect Chk1 phosphorylation at Ser345) or anti-Myc. *B & C*. Each Tet-ON HeLa cell line was treated with (+) or without (-) doxycycline for 16 h. After the treatment, each sample was subjected to the immunoblotting with anti-Chk1 or anti-α-tubulin. The position of endogenous (Chk1) or exogenous (Myc-Chk1) Chk1 was also indicated. *D*. Schema showing experimental procedures to evaluate the effect of exogenous Myc-Chk1 protein on the timing of mitotic entry. Before Myc-Chk1 induction, each Tet-ON HeLa cell line was synchronized at G1/S boundary by the method of double thymidine block. At the release of the second thymidine block, 1 μg/ml doxycycline (Dox) was added in the growing media. In most experiments (except for Fig. 3, *A & B*), 50 ng/ml nocodazole was simultaneously added not to pass through mitosis. *E*. Data were obtained as described in Fig. 3B except for using nocodazole after the release. *F*: After the release of the

second thymidine block, experiments were performed with or without Dox in the presence of nocodazole, as described in Fig. S2*C*. Graph data of cumulative mitotic index at each time point indicate the mean ± S.E.M. values for 200 cells from three independent experiments (A and C). *G*. The effect of a potent Chk1 kinase inhibitor UCN-01 on the timing of mitotic entry. HeLa cells were synchronized at G1/S boundary by the method of double thymidine block. At 8 h after the release, cells were treated with 300 nM UCN-01 or the equal volume of DMSO in the presence of 50 ng/ml nocodazole. Graph data of cumulative mitotic index at each time point represent as described in Fig. S2F. One asterisk indicates $0.01<P<0.05$.

Figure S1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I
M
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48
33
25
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2G1D5
B
siRNA
Control
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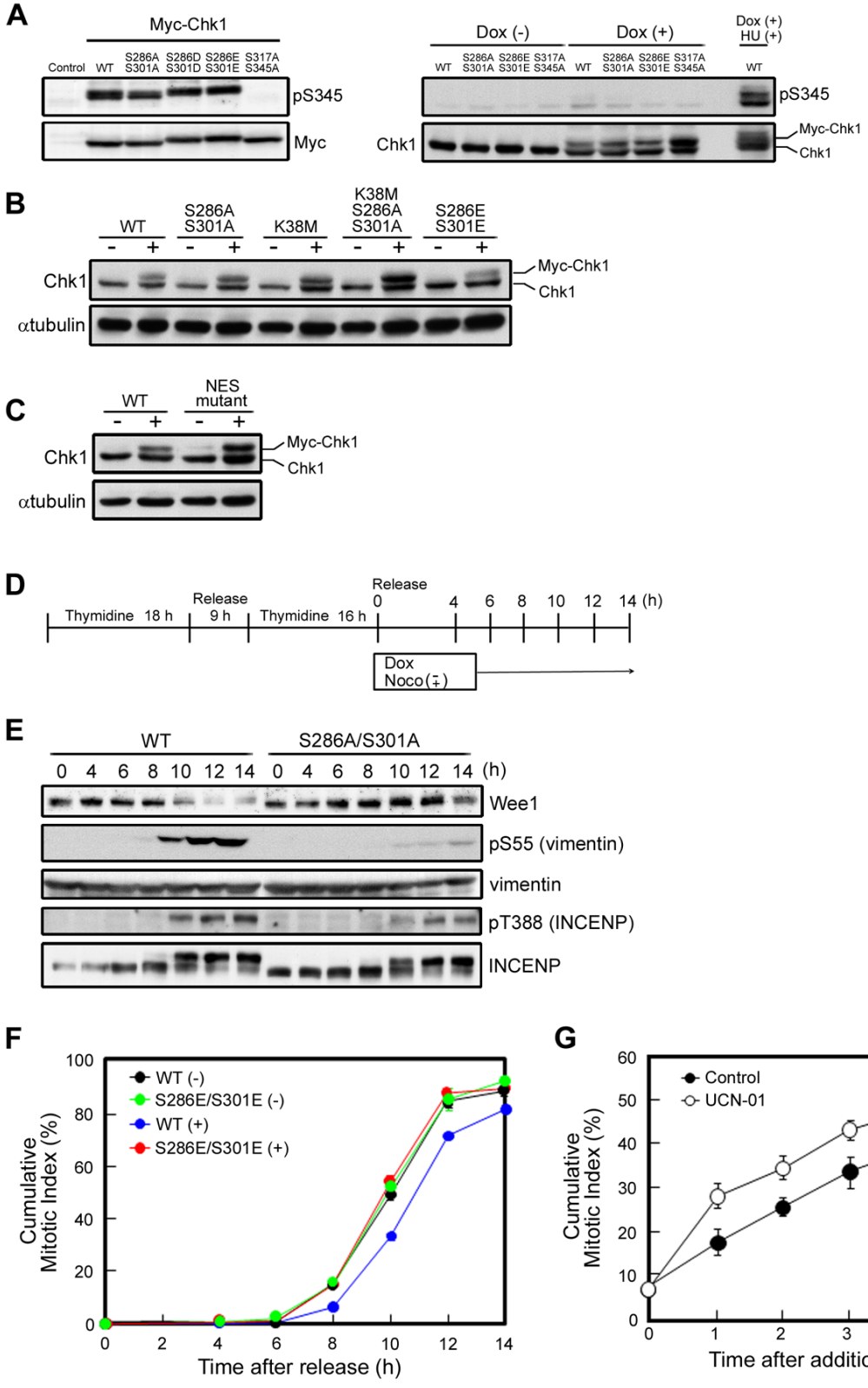
Chk1 S2
G4
2G1D5
αtubulin
siRNA
Control
Chk1 S2
G4
DNA
2G1D5
DNA
C
G4
2G1D5
Chk1 Abs
Lamin B1
DNA
D
Interphase (I)
Prophase
G4
Lamin B1
Merge
DNA
E
Interphase (I)
Prophase
2G1D5
Lamin B1
Merge
DNA

Figure S2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S286E S301E
S317A S345A
pS345
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Dox (-)
Dox (+)
Dox (+) HU (+)
Chk1
Myc-Chk1
Chk1
B
WT
S286A S301A
K38M
K38M S286A S301A
S286E S301E
Chk1
αtubulin
Myc-Chk1
Chk1
C
WT
NES mutant
Chk1
αtubulin
Myc-Chk1
Chk1
D
Thymidine 18 h
Release 9 h
Thymidine 16 h
Release 0
4 6 8 10 12 14 (h)
Dox
Noco(∓)
E
WT
S286A/S301A
0 4 6 8 10 12 14 0 4 6 8 10 12 14 (h)
Wee1
pS55 (vimentin)
vimentin
pT388 (INCENP)
INCENP
F
WT (-)
S286E/S301E (-)
WT (+)
S286E/S301E (+)
Cumulative Mitotic Index (%)
Time after release (h)
G
Control
UCN-01
*
Cumulative Mitotic Index (%)
Time after addition of drug (h)