Title

TRAIP plays an essential role in the dissociation of PCNA from chromatin during mitosis, required to precise DNA replication initiation

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Abstract

Loading PCNA onto chromatin is a pivotal step in DNA replication, cell cycle progression, and genome integrity. Conversely, unloading PCNA from chromatin is also a crucial step for maintaining genome stability. Cells defect of the PCNA unloader ATAD5-RFC exhibit a high level of chromatin-bound PCNA, delayed S phase progression, abnormal association/dissociation of chromatin-bound proteins, and aberrant chromosome structures, but exhibit dissociation of PCNA

from chromatin in mitosis. Here, we found that TRAIP, an E3 ubiquitin ligase, plays critical roles in the removal of PCNA from chromatin during mitosis. TRAIP-depleted cells exhibited chromatin-bound PCNA even during mitosis and delayed mitotic progression and exit. Although TRAIP-depleted cells with chromatin-bound PCNA progressed into the next G1 phase, they showed reduced levels of the replication licensing factor Cdt1, is the key regulator of DNA replication licensing, and impaired S phase entry. These results demonstrate that TRAIP functions independently of ATAD5-RFC for the removal of PCNA from chromatin during mitosis, and is required for precise cell cycle progression from mitosis to G1 phase, as well as for pre-RC formation for faithful initiation of DNA replication.

Key words

Replication/cell cycle/ubiquitination/PCNA/ATAD5-RFC/TRAIP

INTRODUCTION

For DNA replication initiation, origins are licensed between the end of mitosis and during G1 phase through the formation of a pre-replication complex (pre-RC). This is conducted by loading the MCM2-7 complex onto ORC-bound origin with the aid of Cdc6 and Cdt1. Cdt1 is the key regulator of replication licensing, because it is rapidly degraded by two independent E3 ubiquitin ligases, CRL1-Skp2 and CRL4-Cdt2, after the onset of replication to avoid re-licensing during S phase. Replication origins are activated by CDK and DDK, resulting in the loading of a DNA helicase complex, called CMG (Cdc45-MCM2-7-GINS) (Nishitani and Lygerou 2004; Masai et al. 2010; Havens and Walter 2011; Costa and Diffley 2022). After origin unwinding, primer is synthesized on both template strands, and then polymerases synthesis the new strands processively and stably by binding to the sliding clamp PCNA. PCNA is loaded onto the primer/template junction by a replication factor C (RFC) complex.

PCNA is required not only for DNA synthesis itself, but also for replication-linked processes, via its ability to recruit and orchestrate crucial players on DNA (Waga and Stillman 1998; Kelch et al. 2012; Burgers and Kunkel 2017). The functions of CRL4-Cdt2 are also controlled by chromatin-bound PCNA.

In addition to an authentic RFC complex, RFC1-RFC, eukaryotic cells possess other PCNA-associating RFC-like complexes, Ctf18-RFC and ATAD5 (Elg1 in yeast)-RFC (Shiomi and Nishitani 2017; Ohashi and Tsurimoto 2017). Previously, we performed RNAi knockdown of the large subunits of these complexes to determine their roles. Studies by our and other groups clearly demonstrated that in contrast to other RFC complexes, ATAD5-RFC plays an important role by regulating PCNA unloading from chromatin (Lee et al. 2013; Kubota et al. 2013; Shiomi and Nishitani 2013). During the course of this study, we noticed that PCNA dissociated from chromatin in cells arrested at mitosis even when they were depleted of ATAD5, suggesting that cells possess additional mechanism for the removal of PCNA from chromatin.

When DNA synthesis during replication is terminated, the key regulated step that induces CMG disassembly from chromatin is ubiquitination of the MCM7 subunit of the MCM2-7 (Maric et al. 2014; Moreno et al. 2014). Poly-ubiquitinated MCM7 is unfolds by the AAA+ ATPase chaperon p97 (also known as VCP or Cdc48) and dissociates the CMG in an irreversible manner (Maric et al. 2017; Mukherjee and Labib 2019; Deegan et al. 2020).

The cullin ligase SCF-Dia2 in *S. cerevisiae* (Maric et al. 2017) and CUL2-LRR1 in *C. elegans* (Merlet et al. 2010; Burger et al. 2013; Sonneville et al. 2017) and *X. laevis* (Dewar et al. 2017; Sonneville et al. 2017) ubiquitinate MCM7 when DNA synthesis terminates during S phase. When LRR-1 was depleted, CMG remained on chromatin after DNA replication termination, but dissociated after entry into mitosis. In those studies, mitotic CMG disassembly required a metazoan-specific RING ubiquitin ligase known as TRUL-1 in *C. elegans* and TRAIP in vertebrates (Sonneville

et al. 2017; Deng et al. 2019; Priego Moreno et al. 2019; Sonneville et al. 2019). TRAIP is required for CMG disassembly from chromatin during mitosis and from stalled replisomes in mitosis prior to MiDAS, and prior to ICL repair or DNA damage sites caused by replication-transcription conflicts in S phase. TRAIP ensures effective DNA damage signaling and repair by interacting with several repair factors associated with the DNA damage response (Chapard et al. 2014; Wallace et al. 2014; Harley et al. 2016; Hoffmann et al. 2016; Soo Lee et al. 2016; Han et al. 2019; Sonneville et al. 2019; Li et al. 2020; Wu, Pellman and Walter 2020; Scaramuzza et al., 2023). PCNA interacting peptide sequence (PIP box) located at its C terminal end is required for the recruitment of TRAIP to sites of DNA damage, but it is not required for CMG disassembly in mitosis during normal cell cycle progression (Villa et al. 2021).

Here, we report that TRAIP plays an essential role in the removal of PCNA from chromatin during mitosis. TRAIP-depleted cells showed retention of PCNA on chromatin throughout mitosis and a delay the mitotic progression and exit. The persistence of chromatin-bound PCNA triggered Cdt1 degradation during next G1 phase and blocked DNA replication initiation.

RESULTS

TRAIP mediated dissociation of PCNA from chromatin during mitosis

Consistent with our previous report (Shiomi and Nishitani 2013), the level of chromatin-bound PCNA, collected after doxycycline (dox) and auxin (IAA) treatment, in HCT116 cells expressing Tet-OsTIR1 and N-terminus mAID-tagged ATAD5 was higher in asynchronous cells than in control cells (Figure 1a). However, PCNA dissociated from chromatin even in ATAD5-depleted cells when they were arrested in mitosis by nocodazole.

We cannot rule out the possibility that TRAIP also associates and ubiquitinates PCNA via

its PIP box during mitosis, because PCNA is retained on chromatin in mitotic-arrested cells after the addition of T2AA, an inhibitor of interactions between PCNA and PIP box-containing proteins (Punchihewa et al. 2012). We monitored the level of proteins in the chromatin-bound fraction during mitosis after depleting TRAIP, resulted in a reduction of chromatin-bound MCM2 when cells were arrested at mitosis, similar to what was observed in control cells, indicating that CMG was dissociated from chromatin by CRL2-LRR1 (Figure 1b). Conversely, PCNA was retained in the chromatin-bound fraction of TRAIP-depleted cells even during mitosis, demonstrating that TRAIP was required for the removal of PCNA from chromatin during mitosis. Since nocodazole inhibits tubulin polymerization, the cells are arrested at early mitosis. To determine whether PCNA was retained on chromatin during early to late mitosis and not in TRAIP-depleted cells, we performed immunofluorescent detection of PCNA in asynchronous growing cells (Figure 1c). PCNA signals disappeared from chromatin in control cells at early mitosis (prometaphase) and at late mitosis (telophase). Conversely, many populations of cells exhibited PCNA signals merging with chromosomes during early to late mitosis in TRAIP-depleted cells.

TRAIP was required for accurate cell cycle progression and correct cell growth

We previously reported that ATAD5-depleted cells exhibit S phase delay due to the high level of chromatin-bound PCNA (Shiomi and Nishitani 2013) (Figure 2a). When we monitored the cell cycle profile of asynchronously growing TRAIP-depleted cells, we observed an increase in the population of cells with 4N DNA content, indicating that TRAIP depletion leads to a delay in mitotic progression. Additionally, depletion of ATAD5 in TRAIP-depleted cells synergistically increased the number of cells in both S phase and mitotic cells. The cell growth defect in TRAIP-depleted cells was not due to activation of DNA replication, the DNA damage checkpoint, or the spindle assembly checkpoint, because activation of Chk1 and γH2AX and chromatin-bound Mad2 were not detected in

TRAIP-depleted cells (Figure S1). A colony formation assay and measurement of the survival rate also showed that TRAIP is critical for correct cell growth (Figure 2b and 2c). Colonies formation and the cell growth of TRAIP-depleted cells was almost half that of control cells. Interestingly, ATAD5 and TRAIP double depleted cells exhibited almost the same growth and survival rate as TRAIP only depleted cells, indicating that TRAIP is more crucial for cell cycle progression than ATAD5-RFC.

Clearance of PCNA from chromatin by TRAIP during mitosis was required for licensing of DNA replication

Next, we expressed AID2 technology based TRAIP tagged with mAID at its C-terminus in cells to analyze further TRAIP functions. TRAIP-mAID was almost completely degraded at 2 hours after auxin (5-Ph-IAA) addition (Figure S2). The cell cycle profiles up to 24 hours after auxin addition revealed a gradual increase in the mitotic cell population, which is consistent with the result of the RNAi experiment (Figure 3a and 2a). After 72 hours, G1 phase cells represented 44% of the cell population, which was almost the same as that of auxin untreated cells. However, the S phase cell population was much lower than that of the auxin untreated cells. These data indicate that although TRAIP depletion delays mitotic progression, cells eventually exit mitosis and enter G1 phase, but afterwards find it difficult to enter S phase.

To test the TRAIP depletion would lead to a inhibit entry into S phase, TRAIP-mAID expressing cells were released from mitotic arrest with nocodazole, in the absence or presence of auxin (Figure 3b). After release of the arrested cells into the cell cycle, the auxin untreated cells showed a decrease in the mitotic cell population and progressed into G1 phase and then S phase. Conversely, the progression of the auxin treated cells into G1 phase was very slow, and these cells progressed with difficulty into S phase. Next, we checked protein levels, in cells released from mitosis (Figure 3c). In nocodazole-arrested mitotic cells, PCNA was not detected on chromatin in the absence of auxin, but

some PCNA was detected on chromatin in the presence of auxin. Four hours after release from mitosis, the level of chromatin-bound PCNA drastically increased in cells without auxin treatment, indicating that some populations of these cells had entered S phase. However, in cells treated with auxin, the increase in chromatin-bound PCNA was smaller, indicating that TRAIP depletion impairs S phase entry. Expectedly, the levels of Cdt1 and chromatin-bound MCM proteins were reduced in TRAIP-mAID depleted cells. This results were confirmed by immunofluorescence microscopy of Cdt1 in asynchronous growing cells (Figure 3d). In G1 phase cells, Cdt1 was detected in about 76% of cells expressing TRAIP-mAID. Conversely, in TRAIP-mAID depleted cells after the addition of auxin, Cdt1 signals were reduced during G1 phase to about 44%. Next, we were adding the MLN4924, an inhibitor of NEDDylation that inhibits the function of CRL4-Cdt2, after auxin treatment. As evidence of inhibited CRL4-Cdt2 activity, Cdt1 is expressed even in S phase, where it should normally be degraded, and Cdt1 expression was rescued in G1 phase even in the presence of auxin.

DISCUSSION

In this study, we have shown that TRAIP is required for optimal cell cycle progression and cell growth via their ability to remove PCNA from chromatin during mitosis. Especially, we showed that retention of PCNA on chromatin during mitosis in TRAIP-depleted cells triggered the degradation of Cdt1, leading to impaired pre-RC formation and inhibition of DNA replication initiation. Thus, the release of chromatin-bound PCNA by TRAIP is critical for linking replication termination to the next initiation to maintain genome stability, cell cycle progression and faithful cell growth.

A previous report suggested that PCNA is the candidate substrate for TRAIP (Hoffmann et al. 2016), and actually, the regulation of the level of chromatin-bound PCNA by TRAIP has already been reported (Feng et al. 2016). This study, however, was restricted in that it only reported TRAIP-

mediated release of chromatin-bound PCNA during DNA damage-induced replication stress. In our report here, we do not demonstrate the details how the PIP box of TRAIP is involved in the recognition and ubiquitination of PCNA for the removal of PCNA from chromatin during mitosis. Further studies are required to understand the timing of TRAIP interacting with chromatin-bound PCNA, and how TRAIP is activated to ubiquitinate PCNA, but our results demonstrated that TRAIP is indispensable for the removal of PCNA from chromatin in mitosis during normal cell cycle progression.

Cdt1 degradation is regulated by CRL4-Cdt2 in chromatin-bound PCNA dependent manner during S phase. PCNA does not bind to chromatin during G1 phase, but when cells suffer DNA damage, PCNA is loaded onto chromatin for DNA repair, after which Cdt1 is degraded in a CRL4-Cdt2-dependent manner. Thus, PCNA bound to chromatin in TRAIP-depleted cells seems to trigger Cdt1 degradation. Previously, we reported that when mitotic cells were irradiated UV, Cdt1 became resistant to degradation. However, when these cells were released to progress into G1 phase, PCNA was loaded onto chromatin for DNA repair and Cdt1 was degraded by CRL4-Cdt2 in a chromatin-bound PCNA-dependent fashion, and DNA replication licensing was severely inhibited. Such cells had a high frequency of cell cycle arrest during G1 phase. Thus, our data suggested that in addition to the well-known DNA damage checkpoint response, cells have a replication licensing checkpoint that links mitotic DNA damage to cell cycle control (Morino et al. 2015). We also found that TRAIP impairment leads to the retention of PCNA on chromatin during mitosis to G1 phase, and initiation of DNA replication defect. Hence, TRAIP seems to play critical roles as the guardian of the licensing checkpoint during normal cell cycle progression. Indeed, our results demonstrated that depletion of TRAIP severely inhibits progression of the cell cycle from the G1 phase to S phase via destabilizes Cdt1. Although further experiments will be required to further understand the relationships between TRAIP function and DNA replication, our findings provide new insights into replication licensing.

DNA replication occurs once per cell cycle before cell division to maintain genome integrity. DNA replication can be divided into three stages, initiation, elongation, and termination. Recently, studies on DNA replication have focused on the molecular mechanisms of disassembly of the replisome during replication termination (Gambus 2017; Dewar and Walter 2017). In this study, we report a novel pathway for the removal of PCNA from chromatin during mitosis, that is required for precise replication initiation. Thus, the release of chromatin-bound PCNA by TRAIP is critical for linking replication termination to the next initiation to maintain genome stability, integrity, and homeostasis during cell cycle progression and faithful cell growth.

EXPERIMENTAL PROCEDURES

Cell culture

U2OS and HCT116 cell lines were cultured in DMEM (11995-065; Gibco) and in McCoy's 5A medium (16600-082; Gibco), respectively, with 10 % fetal bovine serum and 1 % Penicillin-Streptomycin (P4333; Sigma-Aldrich) at 37°C in a 5 % CO_2 atmosphere. To arrest the cells at mitosis, cells were treated with 75 μg/ml nocodazole for 14 hours. The following siRNA were transfected at 100 μM using HiPerFect (Qiagen): ATAD5 (HSS129125), TRAIP (HSS129125) from Invitrogen. For RNAi, 24 hours after the first transfection, the second transfection was carried out, and the cells were cultured for two more days. T2AA (#21921) were purchased from Cayman chemical. To analyze cell cycle profiles, flow cytometry was performed with FACSMelody and FACSChorus software (Becton Dickinson), and the cell populations with various DNA contents were analyzed with ModFit LT (Verity Software House).

Establishment of HCT116 cell lines expressing mAID-ATAD5 and TRAIP-mAID

A CRISPR-Cas9 plasmid targeting the region adjacent to the start codon of the ATAD5 gene (CACCGGCTGTGGTACCAGGTCACG/agg) was constructed using pX330-U6-Chimeric_BB-CBh-hSpCas9 (Addgene 42230) (Ran et al. 2013). A donor plasmid containing the Hygro-P2A-mAID tag was constructed according to the published protocol (Natsume et al. 2016). Parental HCT116 cells conditionally expressing Tet-OsTIR1 were transfected with CRISPR-Cas9 and donor plasmids. To establish the TRAIP-mAID cell, a CRISPR-Cas9 plasmid targeting the region adjacent to the stop codon of the TRAIP gene (CTCACTGTTCTCACGACCAC/agg) using pX330 and a donor plasmid containing the mAID-Clover-Hygro tag were constructed. These plasmids were transfected into HCT116 cells expressing OsTIR1 (F74G) (Saito and Kanemaki, 2021; Yesbolatova et al., 2020, 2019). After colony isolation, clones harboring bi-allelic insertion at the target locus were verified by genomic PCR.

Antibodies, Immunoblotting and Fluorescent microscopy

For immunoblotting, whole cell extract and chromatin fraction were carried out as described previously (Shiomi and Nishitani 2013). Protein levels were calculated by examining band densities with ImageJ software. For immuno-fluorescent microscopy, the cells were treated as described previously (Shiomi and Nishitani 2013). To observed mitotic cells, the cells were simultaneous fixation and permeabilization using PTEMF buffer (Bhowmick et al., 2016). The following primary antibodies were used: TRAIP (AG0491), H3 (AG10644), MCM5 (11703-1-AP), MCM6 (13347-2-AP) from Proteintech, PCNA (sc-56; Santa Cruz), phospho-histone H3 (Ser10)(06-570; Sigma-Aldrich). ATAD5 antibodies were raised in rabbits against a synthetic peptide containing aa 183 to aa 210 of human ATAD5. Antibodies for Cdt1, Cdt2 and MCM2 have been described previously (Nishitani et al. 2001; Nishitani et al. 2014). The following secondary antibodies were used: anti-mouse IgG (NA0310V) and anti-rabbit IgG (NA9340V) from GE Healthcare for immunoblotting,

and: anti-mouse antibody (A11032) or anti-rabbit antibody (A11037) from Molecular Probes for immuno-fluorescence. To visualize DNA, hoechst 33342 (346-07951; Dojindo) was used. To identify S phase cells, cells treated with 10mM of EdU for 10min and incorporated EdU was detected using Click-IT chemistry, according to the instructions (C10337; Invitrogen).

Acknowledgments

We thank Y. Inoue and A. Kusaka (Univ. of Hyogo) for initial experiment of this work. We also thank T. Natsume (NIG), A. Tadokoro, Y. Shimizu and K. Matsubara (Univ. of Hyogo) for experimental support. This work was supported by JSPS KAKENHI (JP16K07257) to YaS, JSPS KAKENHI (JP21H0419 and JP22H04703) and JST CREST (JPMJCR21E6) to MTK, JSPS KAKENHI (JP20K06547) to HN.

Author Contributions

YaS performed the all experiments described in this report. YuS and MTK performed AID strain construction. YaS conceived the study, designed the experiments and wrote the manuscript. HN supervising the work.

Conflict of interest

We declare that we have no conflict of interest.

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Yesbolatova, A., Saito, Y., Kitamoto, N., Makino-Itou, H., Ajima, R., Nakano, R., Nakaoka, H., Fukui, K., Gamo, K., Tominari, Y., Takeuchi, H., Saga, Y., Hayashi, K.-I., Kanemaki, M.T., 2020. The auxin-inducible degron 2 technology provides sharp degradation control in yeast, mammalian cells, and mice. Nat. Commun. 11, 5701. https://doi.org/10.1038/s41467-020-19532-z

Figure legends

Figure 1. PCNA was removed from chromatin by TRAIP and p97 during mitosis

(a) Asynchronous (phase; A) and mitosis-arrested (phase; M) HCT116 cells expressing mAID-ATAD5 were lysed and separated into whole cell extract (W) fractions and chromatin fractions (C), and the fractions were immunoblotted using the indicated antibodies. To deplete mAID-ATAD5, cells were cultured in McCoy's medium with 2 μg/ml doxycycline and 500μM IAA. T2AA was added to inhibit the interaction between PCNA and PIP-containing proteins.

(b) Asynchronous (A) and mitosis-arrested (M) HCT116 cells with and without TRAIP depletion were lysed and separated into W and C fractions, and the fractions were immunoblotted with the indicated antibodies. The graph is representative of the results of four independent experiments.

(c) Endogenous PCNA immunofluorescence in asynchronously growing U2OS cells. The percentages of the cells with at least one PCNA signal (cyan) on mitotic chromatin are indicated at the bottom of each image. The bar indicates 10 μm.

For RNAi, 24 hours after the first transfection, the second transfection was carried out, and the cells were cultured for two more days (b and c). The levels of chromatin-bound PCNA (a and b) (normalized to the level of each protein in the W) are indicated under the immunoblot bands (a) and by vertical bars with errors (b), relative to those in asynchronous cells set as 1.0.

Figure 2. TRAIP is required for accurate cell cycle progression and appropriate cell growth

(a) Cell cycle profiles of asynchronously growing cells after depletion of the indicated proteins by RNAi. The ratios of cell populations at each cell cycle phase are indicated.

(b) Colony formation assays of U2OS cells with depletion of the indicated proteins. The cells were cultured for indicated weeks.

(c) Cell growth assays of U2OS cells with depletion of the indicated proteins. The cells were cultured for indicated days.

Figure 3. TRAIP is required for precise mitotic progression and exit, and faithful S phase entry

(a) TRAIP-mAID expressing cells were collected at the indicated times after the addition of 1μM auxin (5-Ph-IAA). The graph shows the ratios of cell cycle phases of asynchronously growing cells after the addition of auxin, based on the cell cycle profiles indicated at the right.

(b) The ratios of cell cycle phases of cells arrested in mitosis and released in the absence or presence of auxin, based on the cell cycle profiles indicated at the right. The graph is representative of the results of four independent experiments and the averages of each ratio are shown.

(c) The whole cell extract (W) and chromatin fraction (C) of the same cell samples collected in (A) were immunoblotted with the indicated antibodies.

(d) Asynchronous TRAIP-mAID expressing cells grown in the absence or presence of auxin were immunostained with anti-Cdt1 antibody (cyan). To test the expression levels of Cdt1 during G1 phase, we excluded S phase cells based on EdU incorporation (green) and mitotic cells based on their chromosomal structure (blue). Additionally, among EdU unincorporated cells, we also excluded G2 phase cells based on their large nucleus size. After treating the cells with auxin for 24 hours, the cells were cultured for an additional 1 hour with the addition of MLN4924. The percentages of Cdt1 signals

detected in G1, S or G2 phases, and mitosis are as follows. Non-treated cells, G1: 75.5% (n=253), S: 0% (n=335), G2: 13.8% (n=174), mitosis: 66.7% (n=9). Auxin treated cells, G1: 43.8% (n=217), S: 0% (n=338), G2: 19.6% (n=270), mitosis: 42.1% (n=19). Auxin and MLN4924 treated cells, G1: 93.1% (n=203), S: 98.9% (n=178), G2: 86.3% (n=146), mitosis: 100% (n=4). The bar indicates 5 μm.

SUPPORTING INFORMATION

Figure S1.

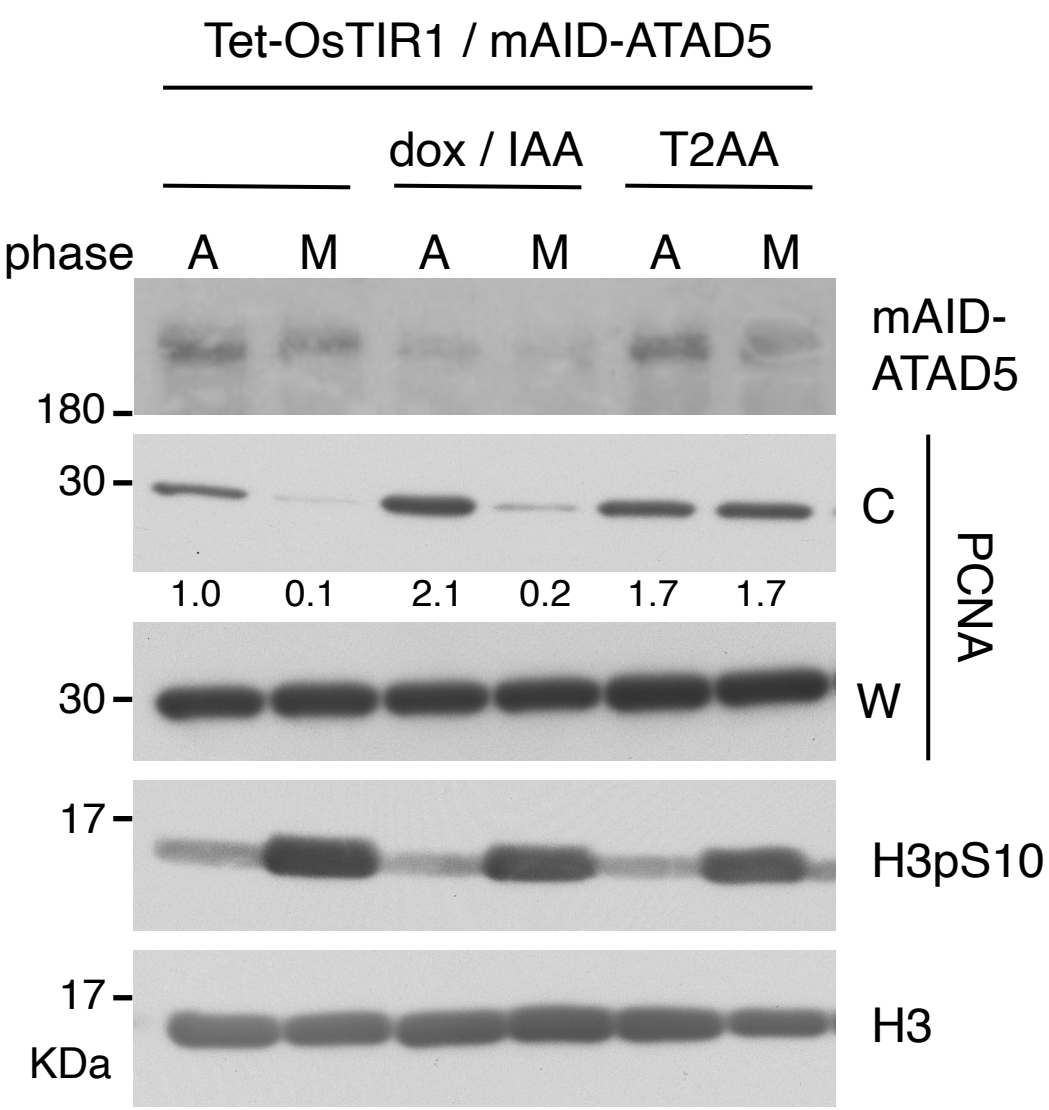
Asynchronous (A) and mitosis-arrested (M) U2OS cells, depleted in the indicated proteins by RNAi or treated with the indicated drugs (APH; aphidicolin, HU; hydroxy urea, and Zeocin), were collected and divided into whole cell extract (W) and chromatin fraction (C), and immunoblotted with the indicated antibodies. The following antibodies were used as primary antibodies: Chk1 (2345), phospho-Chk1 (Ser 296) (2349), H2A (3636) from Cell Signaling, and γH2AX (05-636; Millipore).

Figure S2.

TRAIP-mAID expressing cells were collected at the indicated times after the addition of 1μM auxin (5-Ph-IAA). The whole cell extract (W) and chromatin fraction (C) were prepared and immunoblotted with the indicated antibodies.

Figure 1

(a)


Tet-OsTIR1 / mAID-ATAD5
dox / IAA
T2AA
phase A M A M A M
mAID-ATAD5
180
30
C
PCNA
1.0 0.1 2.1 0.2 1.7 1.7
30
W
17
H3pS10
17
KDa
H3

(b)

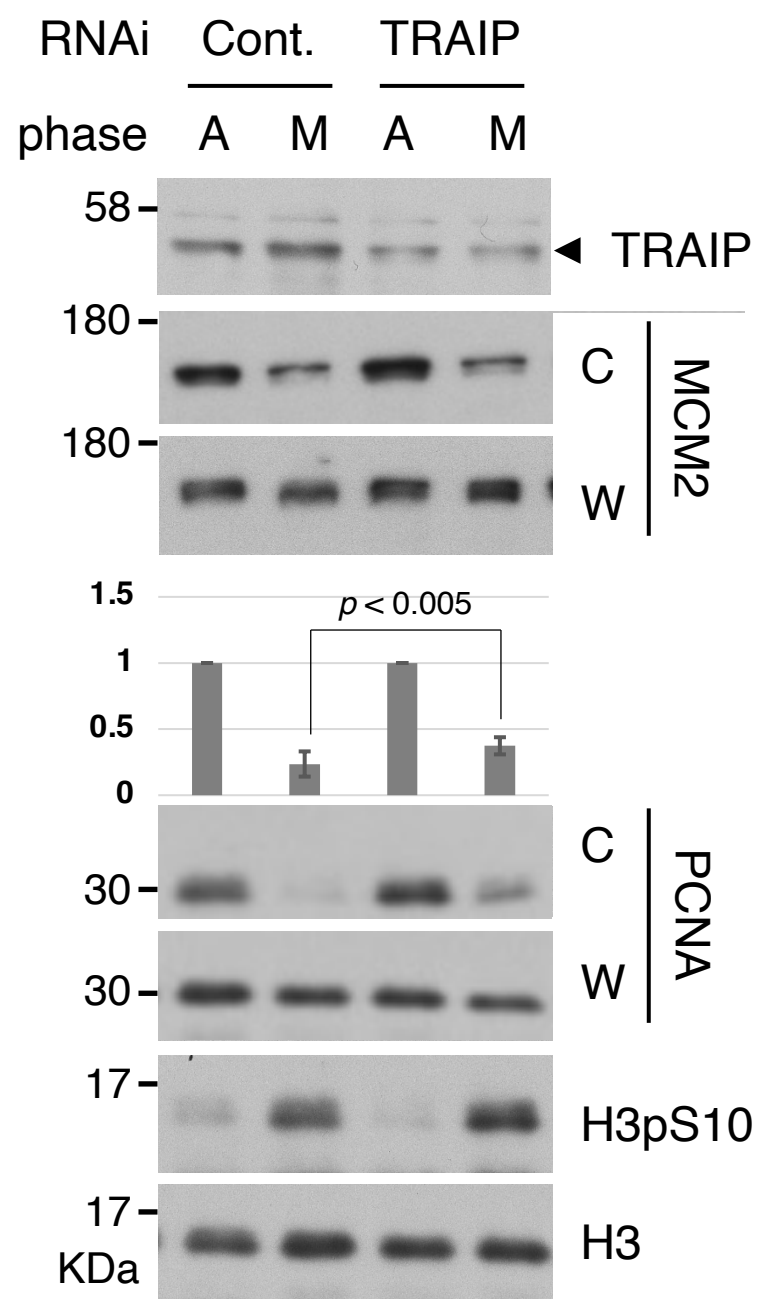
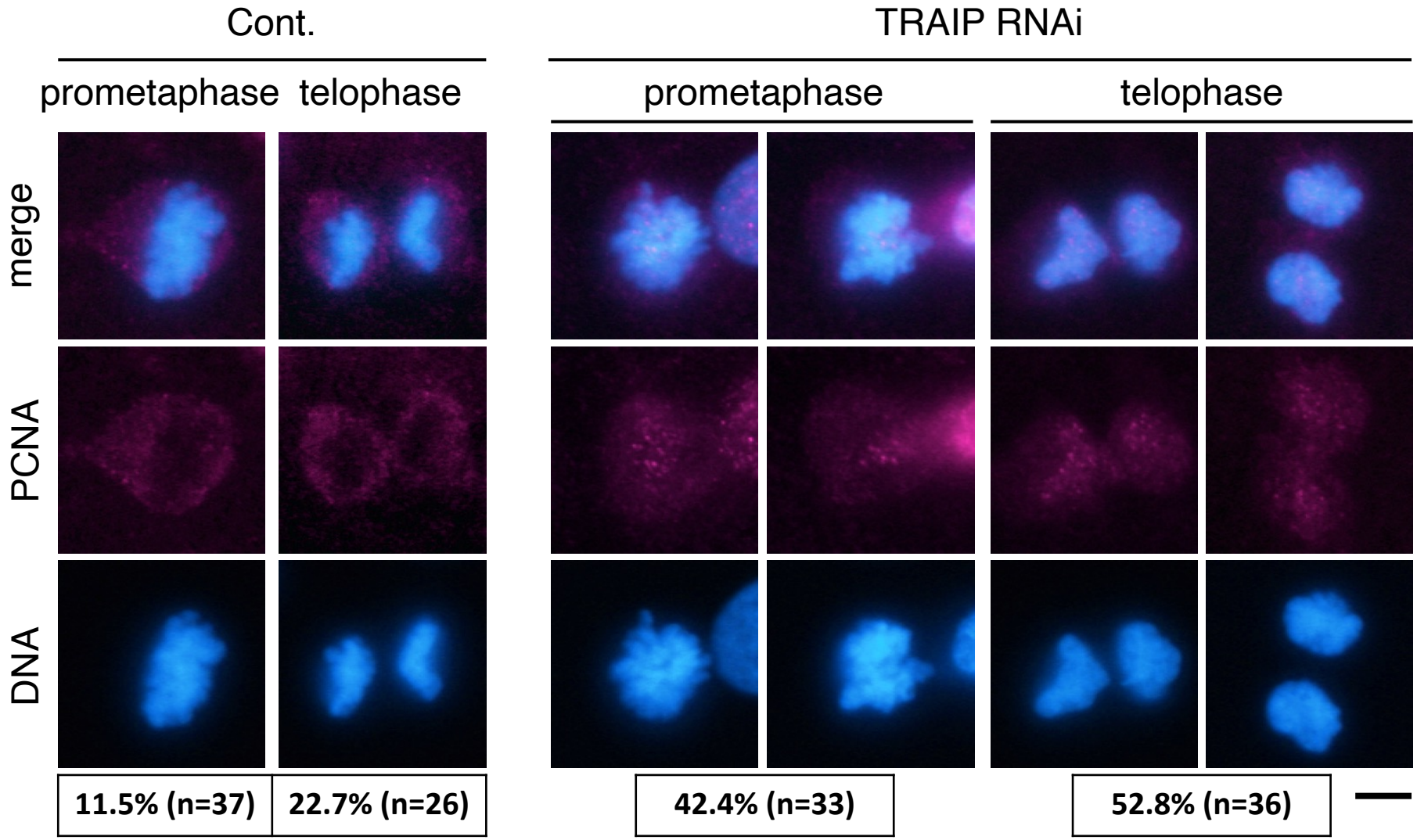

RNAi Cont. TRAIP
phase A M A M
58
TRAIP
180
C
MCM2
180
W
1.5
p < 0.005
1
0.5
0
30
C
PCNA
30
W
17
H3pS10
17
KDa
H3

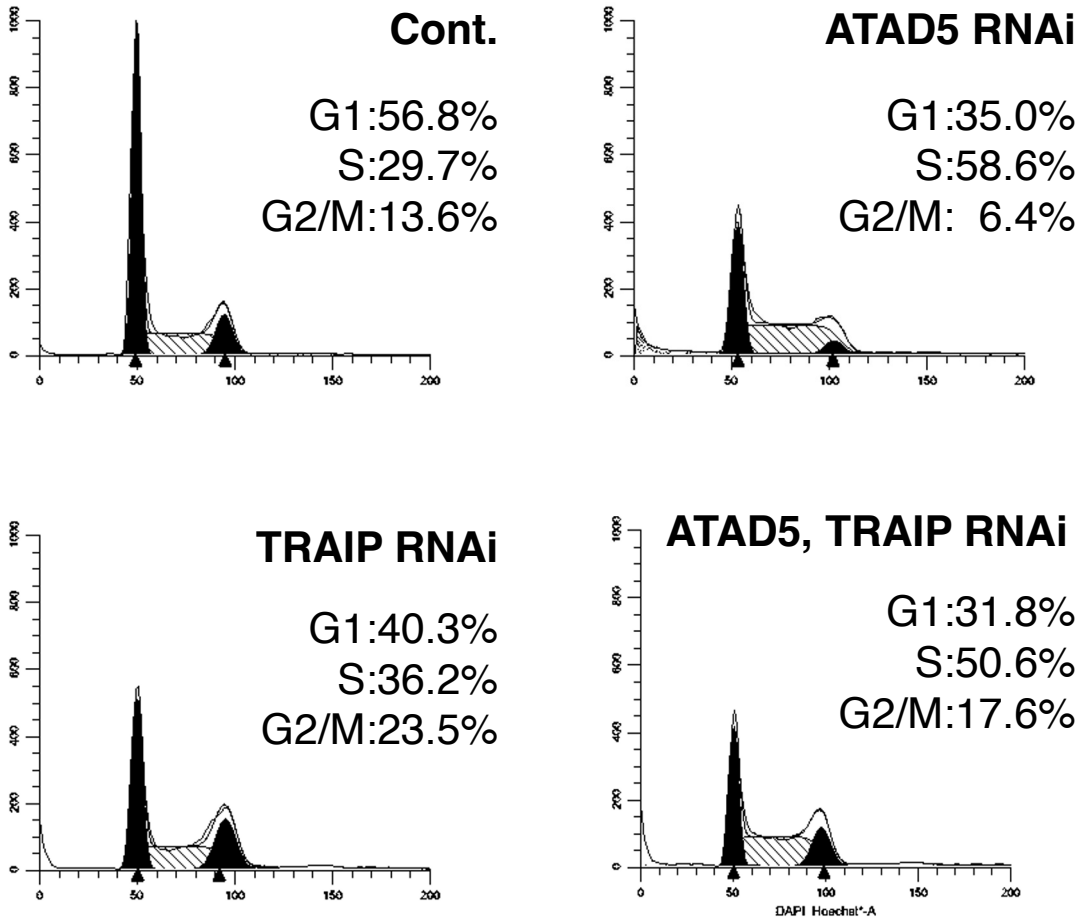
Figure 1

(c)

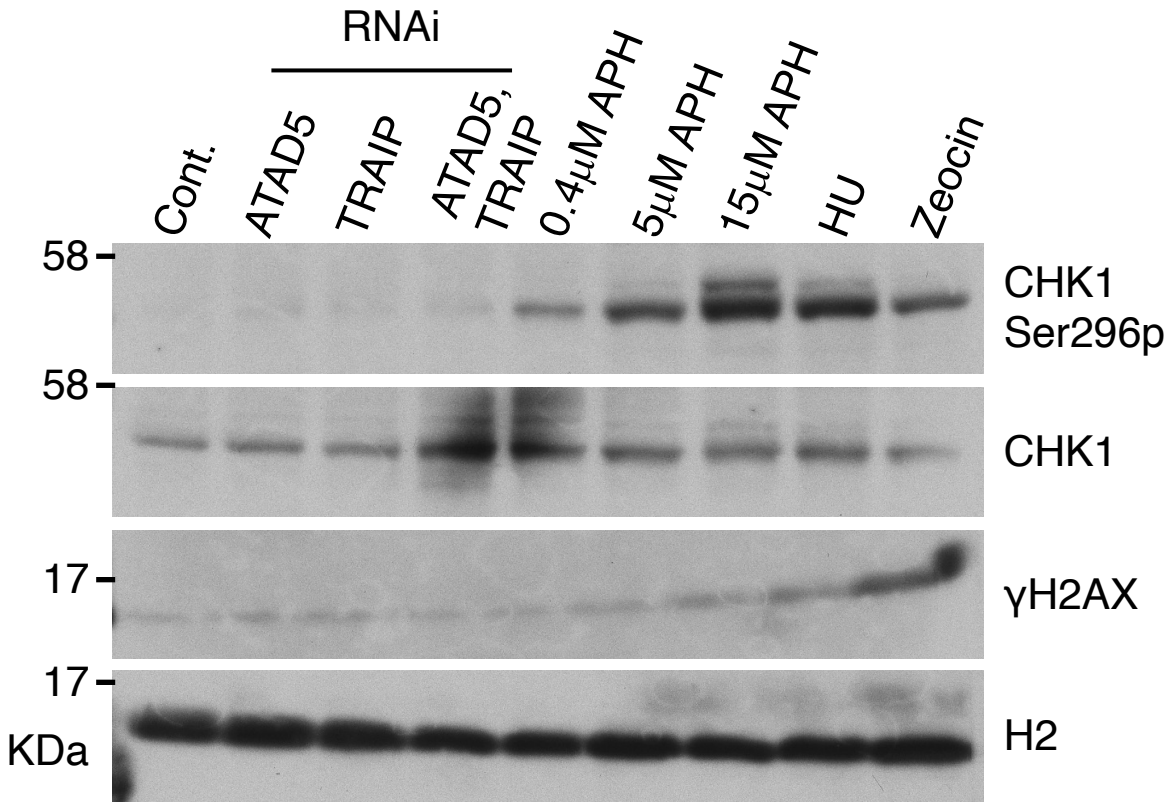

Cont.
TRAIP RNAi
prometaphase
telophase
prometaphase
telophase
merge
PCNA
DNA
11.5% (n=37)
22.7% (n=26)
42.4% (n=33)
52.8% (n=36)

Figures 2

(a)


Cont.
G1:56.8%
S:29.7%
G2/M:13.6%
ATAD5 RNAi
G1:35.0%
S:58.6%
G2/M: 6.4%
TRAIP RNAi
G1:40.3%
S:36.2%
G2/M:23.5%
ATAD5, TRAIP RNAi
G1:31.8%
S:50.6%
G2/M:17.6%
DAPI Hoechst*-A

Figures S1


RNAi
Cont.
ATAD5
TRAIP
ATAD5, TRAIP
0.4μM APH
5μM APH
15μM APH
HU
Zeocin
58
58
17
17
KDa
CHK1 Ser296p
CHK1
γH2AX
H2

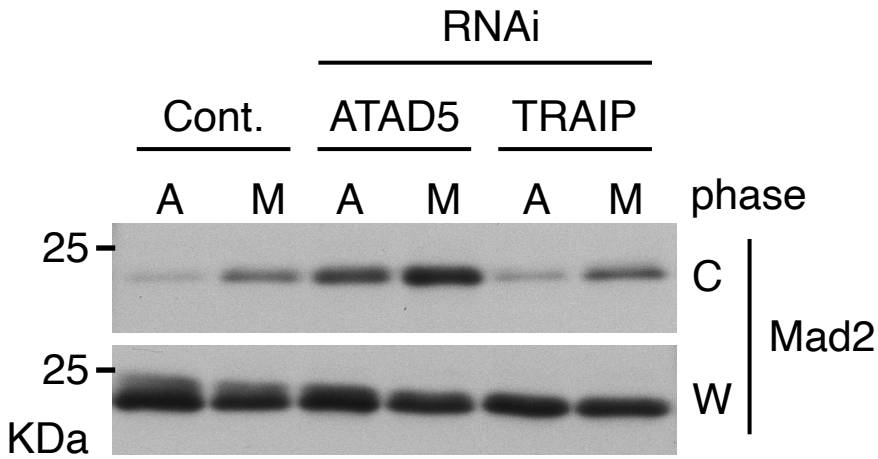

RNAi
Cont.
ATAD5
TRAIP
A
M
A
M
A
M
phase
25
25
KDa
C
W
Mad2

Figure 2

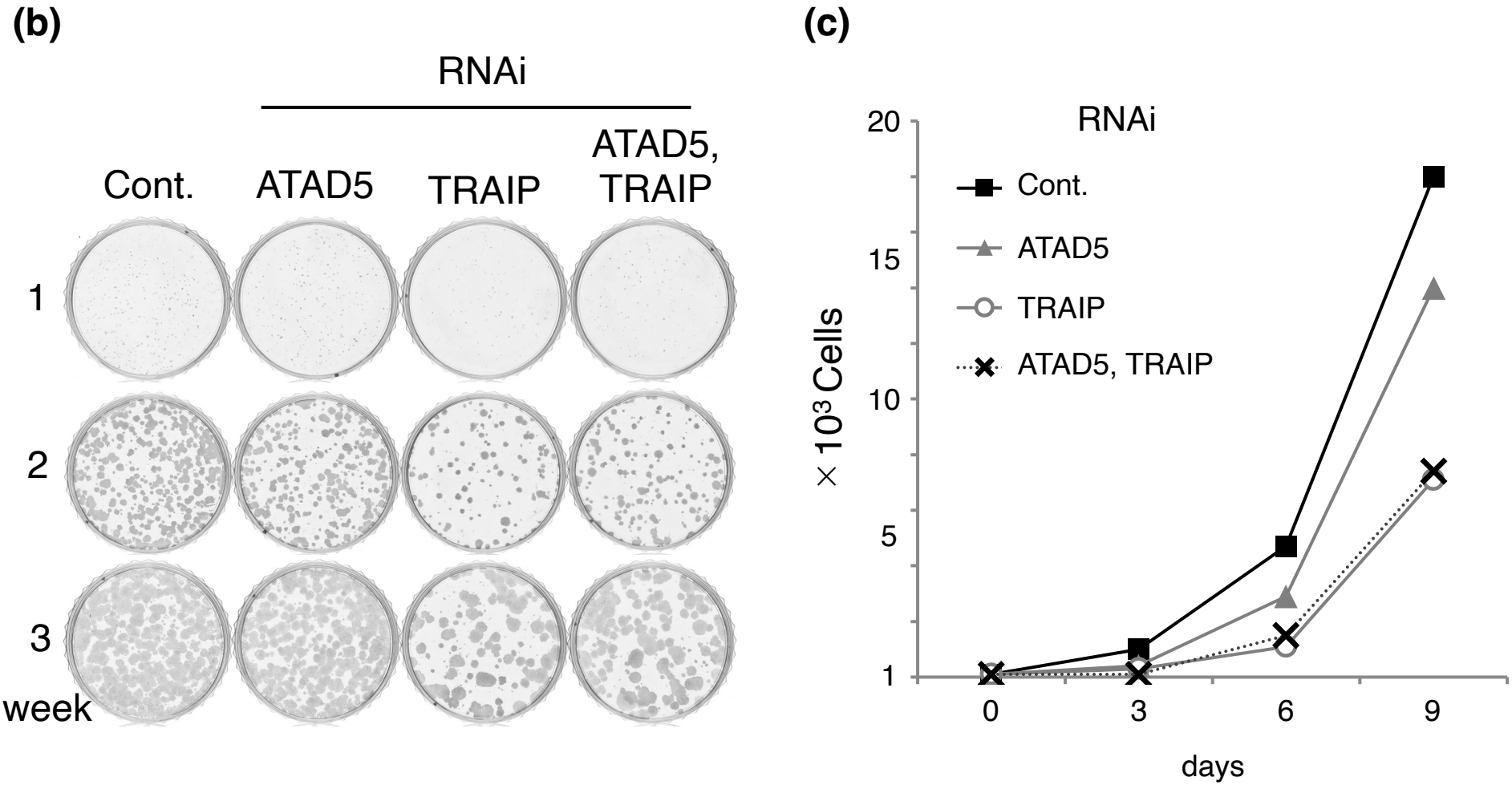

(b)
RNAi
Cont.
ATAD5
TRAIP
ATAD5, TRAIP
1
2
3
week
(c)
RNAi
Cont.
ATAD5
TRAIP
ATAD5, TRAIP
× 10³ Cells
20
15
10
5
1
0
3
6
9
days

Figure S2

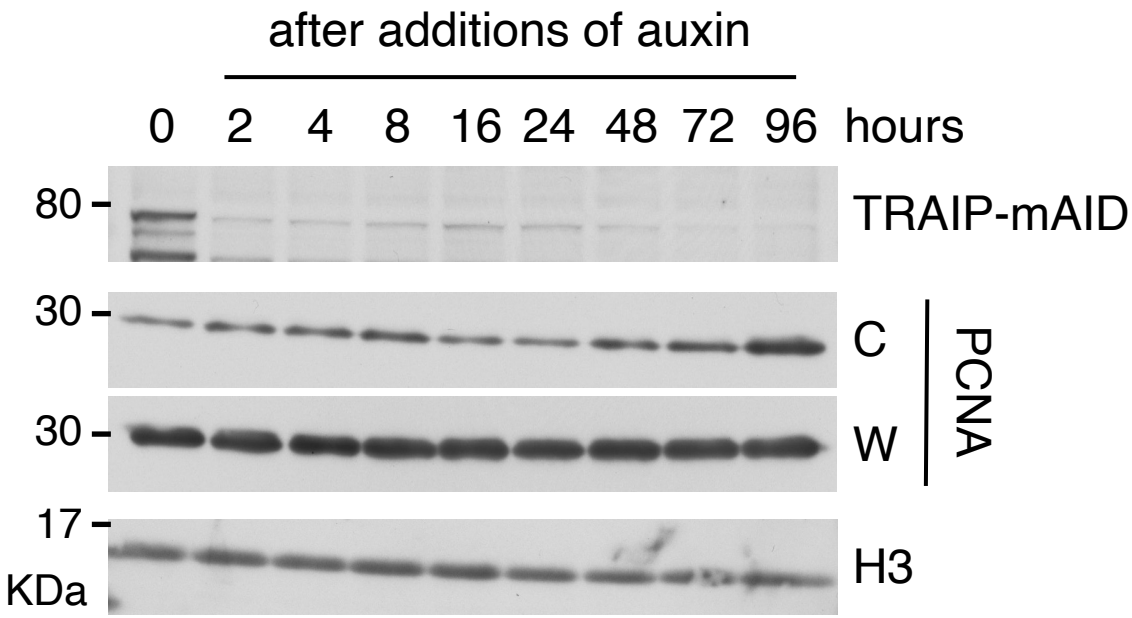

after additions of auxin
0 2 4 8 16 24 48 72 96 hours
80
TRAIP-mAID
30
C
PCNA
30
W
17
KDa
H3

Figure 3

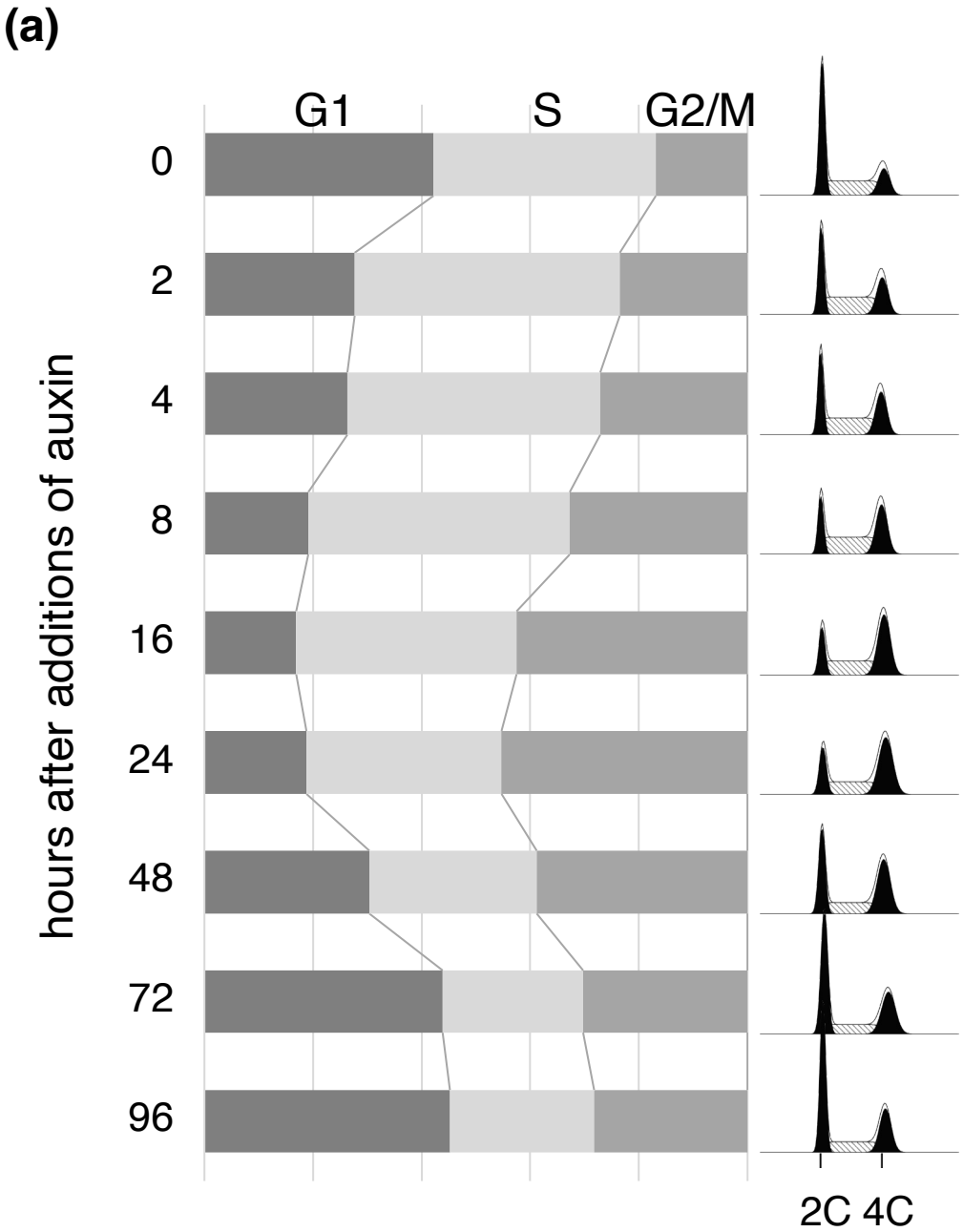

(a)
G1
S
G2/M
hours after additions of auxin
0
2
4
8
16
24
48
72
96
2C
4C

Figure 3

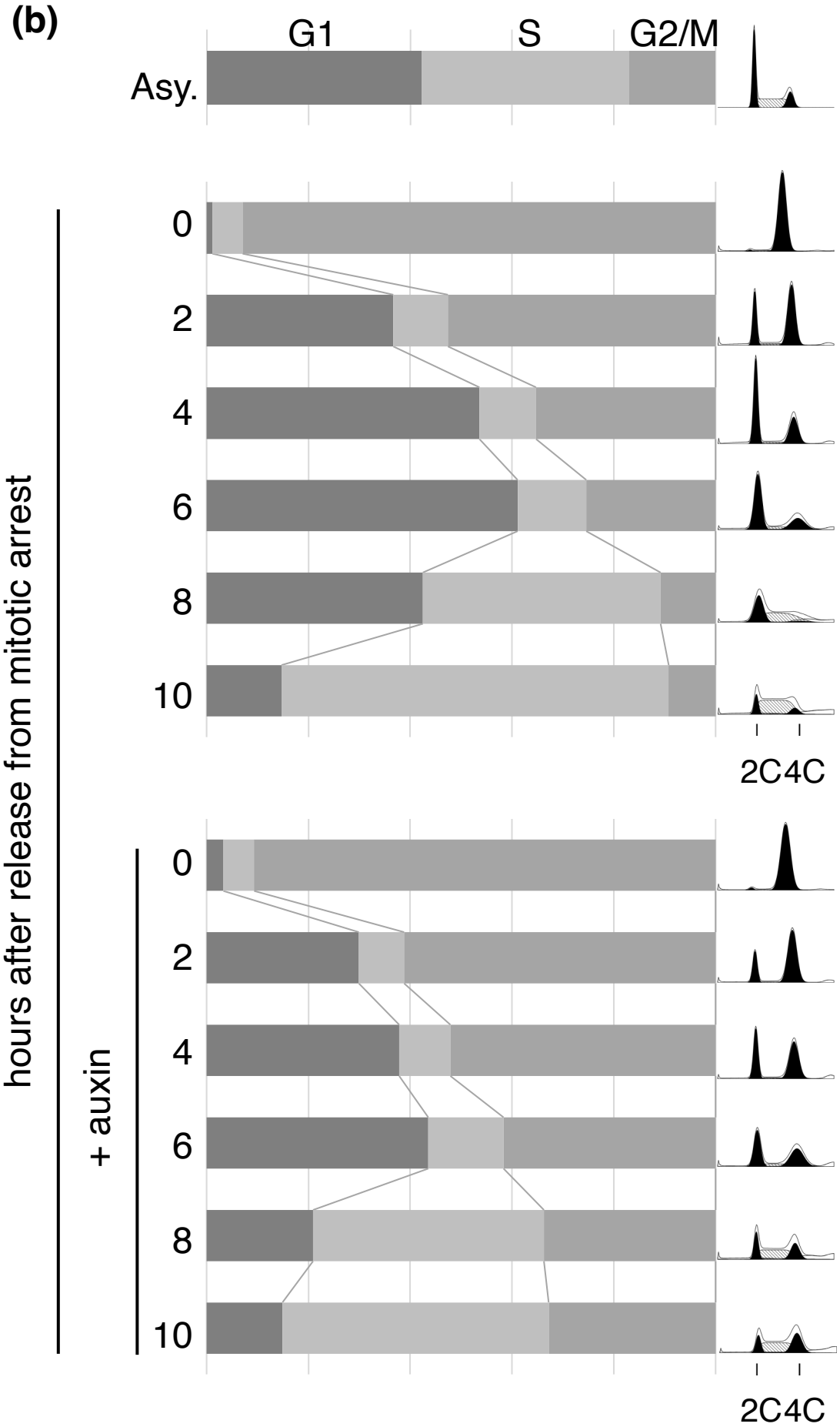

(b)
G1
S
G2/M
Asy.
0
2
4
6
8
10
hours after release from mitotic arrest
2C4C
+ auxin
0
2
4
6
8
10
2C4C

Figure 3

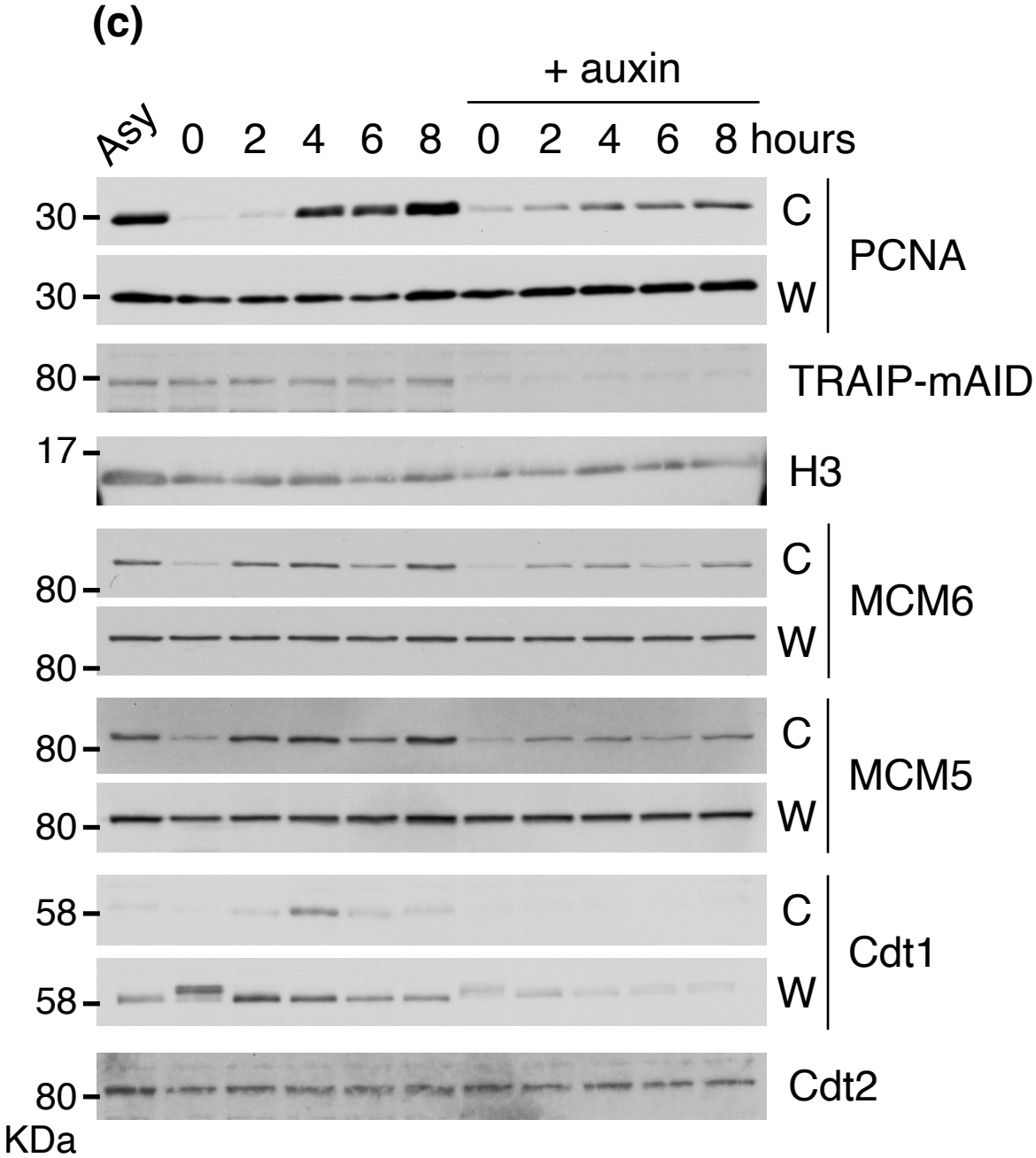
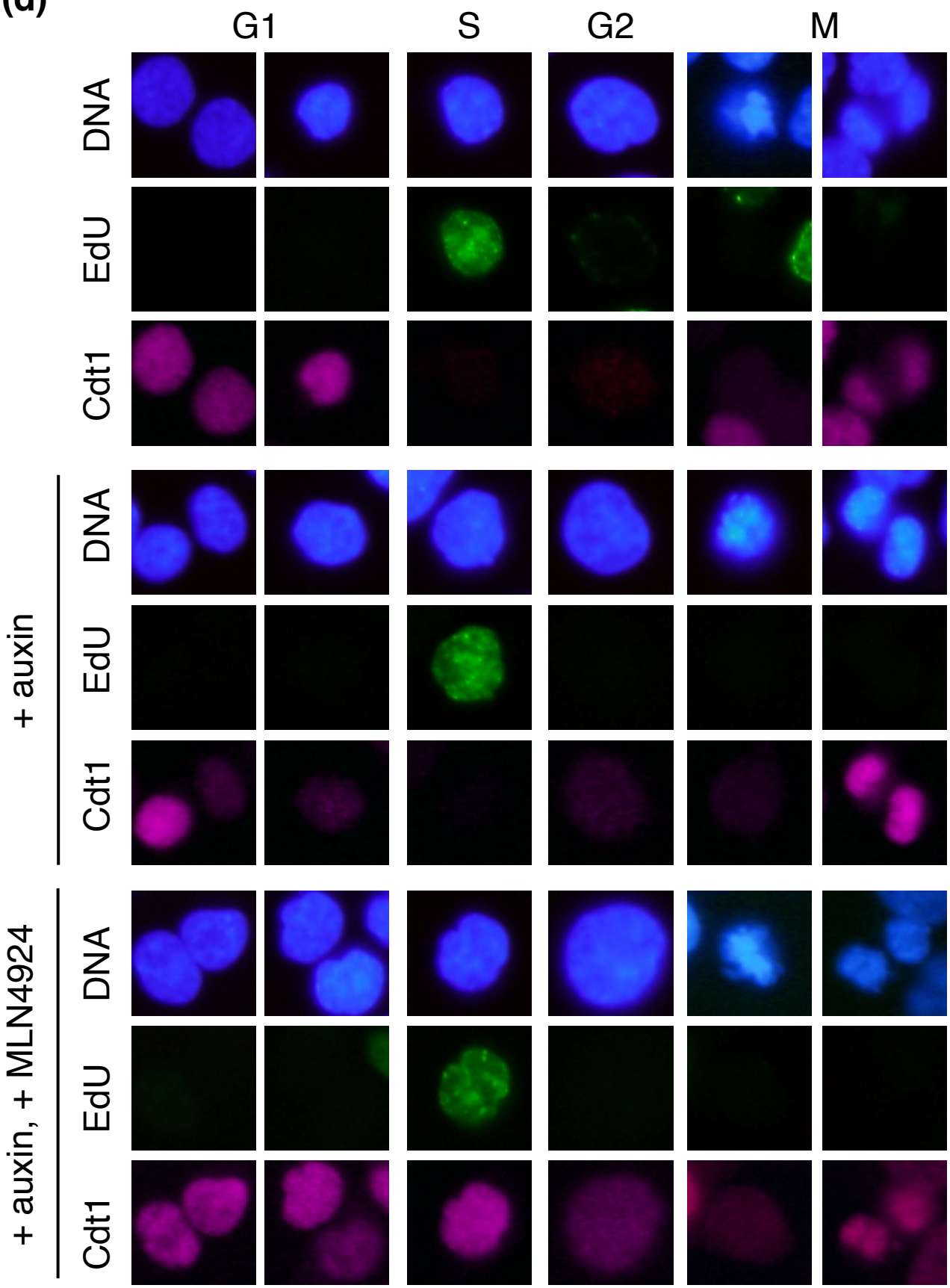

(c)
+ auxin
Asy
0 2 4 6 8 0 2 4 6 8 hours
30
30
80
17
80
80
80
80
58
58
80
KDa
C
W
PCNA
TRAIP-mAID
H3
C
W
MCM6
C
W
MCM5
C
W
Cdt1
Cdt2

Figure 3

(d)


G1
S
G2
M
DNA
EdU
Cdt1
+ auxin
DNA
EdU
Cdt1
+ auxin, + MLN4924
DNA
EdU
Cdt1