SUPPLEMENTAL FIGURE LEGENDS

Figure S1 (refers to Figure 1). Rim4-47A does not exhibit visually different aggregation properties compared to wild-type Rim4.
(A-C) Strains heterozygous for *HTB2-mCherry* and homozygous for either *RIM4-EGFP* (A37489) or *RIM4-47A-EGFP* (A38932) were induced to sporulate at 30°C. Samples were fixed and imaged at 3 and 5 hours in sporulation medium. **(A, B)** Rim4-EGFP signal (vacuole excluded) was quantified in the central z-plane. Neither the mean nor the standard deviation of Rim4 signal is significantly different between the two strains. n = 50 cells per strain per time point. **(C)** Single-plane images of representative cells at 3 and 5 hours are shown. Scale bar = 2 μm

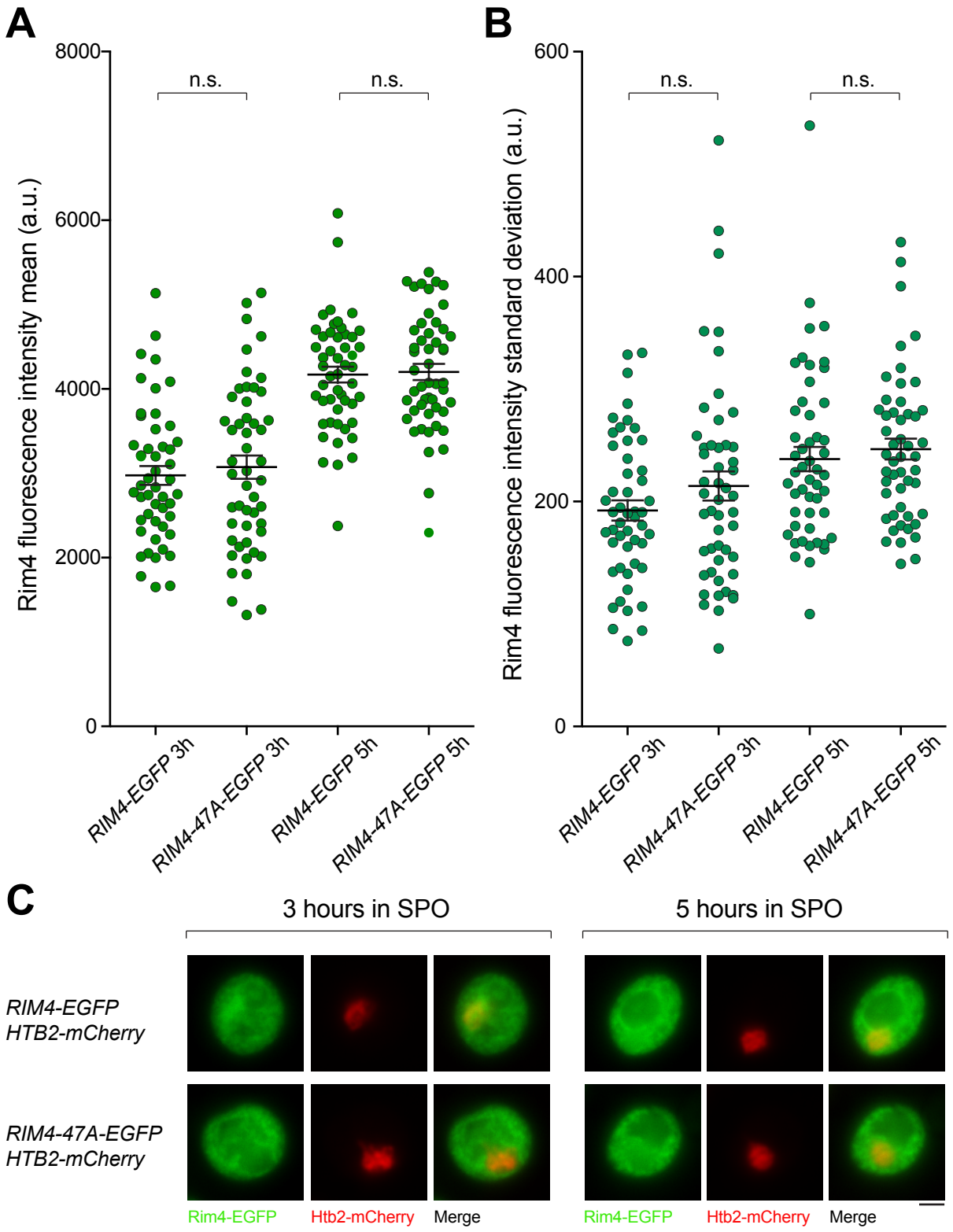
Figure S2 (refers to Figure 2). *RIM4-47A* mutants exhibit meiosis II delays.
(A) Strains harboring fluorescently labelled tubulin (*pTUB1-GFP-TUB1*) and Spc42 (*SPC42-mCherry*) and either *RIM4-3V5* (wild type, B197) or *RIM4-47A-3V5* (B198) were induced to sporulate at 30°C. After 1 hour of growth in batch culture, the cells were loaded onto a microfluidics chip and imaged every 10 minutes. The image series show a representative cell progressing through sporulation. Tubulin is shown in green and the spindle pole bodies (SPBs) in red. The onset of meiosis I was chosen as the 0 time point. Scale bar = 5 μm
(B) Single-cell analysis (n = 25 for each genotype) of time in metaphase I, anaphase I, metaphase II, and anaphase II for live-imaged wild type and *RIM4-47A* cells.

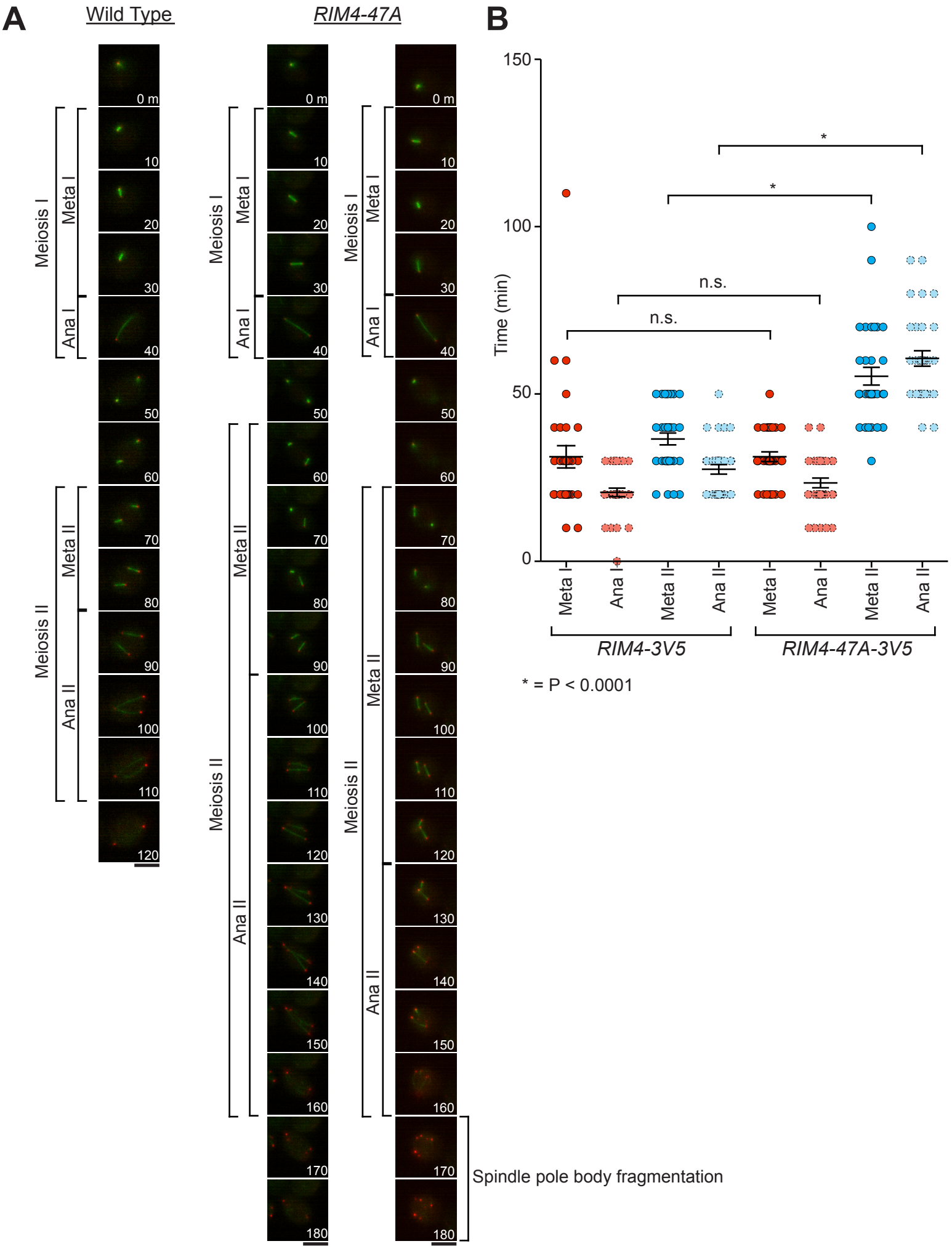
Figure S3 (refers to Figure 3). *RIM4-47A* is a dominant gain-of-function allele.
(A) SDD-AGE and meiotic progression analysis (tubulin IF; n = 100 cells for each time point) corresponding to *RIM4-3V5*/+ experiments shown in **Figure 3**
(B) SDD-AGE and meiotic progression analysis (tubulin IF; n = 100 cells for each time point) corresponding to *RIM4-47A-3V5*/+ experiments shown in **Figure 3**.

Figure S4 (refers to Figure 4). Rim4 clearance is regulated by multi-site phosphorylation.
(A) Immunoblot (Rim4, Clb3, and Pgk1) and Northern blot (*CLB3* and *rRNA*) source data used for quantifications shown in Figure 4 (wild type, *RIM4-52A*, *RIM4-36A*, and *RIM4-17A;* left panel).
(B) Immunoblot (Rim4, Clb3, and Pgk1) and Northern blot (*CLB3* and *rRNA*) source data used for quantifications shown in Figure 4 (wild type, *RIM4-99A*, *RIM4-10A*, and *RIM4-47A*; center panel).
(C) Immunoblot (Rim4, Clb3, and Pgk1) and Northern blot (*CLB3* and *rRNA*) source data used for quantifications shown in Figure 4 (wild type, *RIM4-27A*, *RIM4-25A*, and *RIM4-21A*, right panel).

Figure S5 (refers to Figure 5). Ime2 regulates Rim4 clearance via phosphorylation of the Rim4 C-terminal IDR.
Immunoblot (Rim4, Clb3, and Pgk1) and Northern blot (*CLB3* and *rRNA*) later time points (9 hours -12 hours) for wild type, *RIM4-47A*, *IME2st*, and *RIM4-47A*; *IME2st*. Corresponds to experiment in Figure 5.

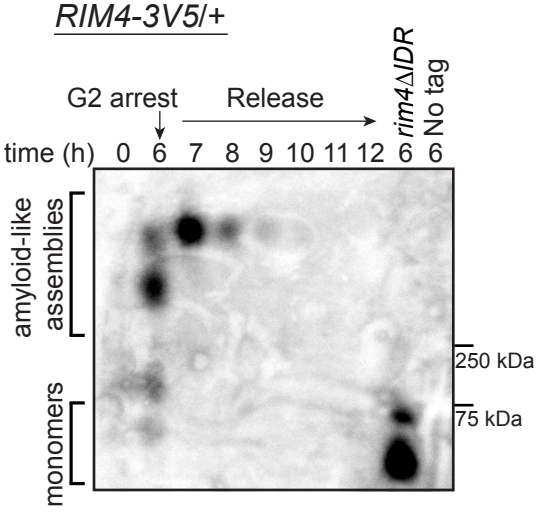
Figure S6 (refers to Figure 6). The proteasome is required for clearance of Rim4 assemblies.
(A, B) Immunoblot (Rim4, Clb3, and Pgk1), Northern blot (*CLB3* and *rRNA*), and meiotic progression analysis (tubulin IF; n = 100 cells for each time point) of wild type, wild type shifted to 37°C at 7.5 hours, *rpn6-1*, and *rpn6-1* shifted to 37°C at 7.5 hours.
(C, D) Immunoblot (Rim4, Clb3, and Pgk1), Northern blot (*CLB3* and *rRNA*), and meiotic progression analysis (tubulin IF; n = 100 cells for each time point) source data used for wild type, *rpn6-1*, *IME2st*, and *rpn6-1*; *IME2st* quantifications shown in Figure 6.

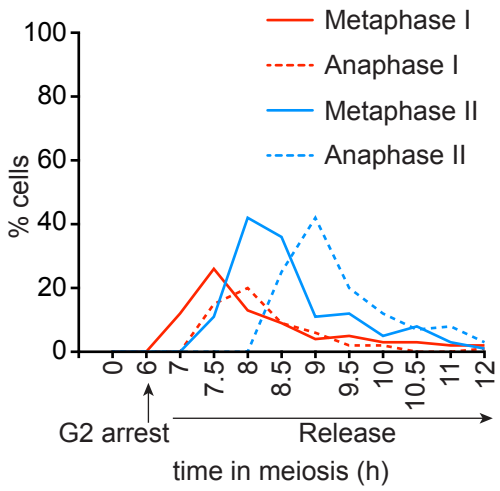

A
B
C
Rim4 fluorescence intensity mean (a.u.)
Rim4 fluorescence intensity standard deviation (a.u.)
n.s.
n.s.
RIM4-EGFP 3h
RIM4-47A-EGFP 3h
RIM4-EGFP 5h
RIM4-47A-EGFP 5h
3 hours in SPO
5 hours in SPO
RIM4-EGFP
HTB2-mCherry
RIM4-47A-EGFP
HTB2-mCherry
Rim4-EGFP
Htb2-mCherry
Merge


A
Wild Type
RIM4-47A
Meiosis I
Meta I
Ana I
Meiosis II
Meta II
Ana II
0 m
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
Spindle pole body fragmentation
B
Time (min)
150
100
50
0
n.s.
n.s.
*
*
Meta I
Ana I
Meta II
Ana II
RIM4-3V5
RIM4-47A-3V5
* = P < 0.0001

A

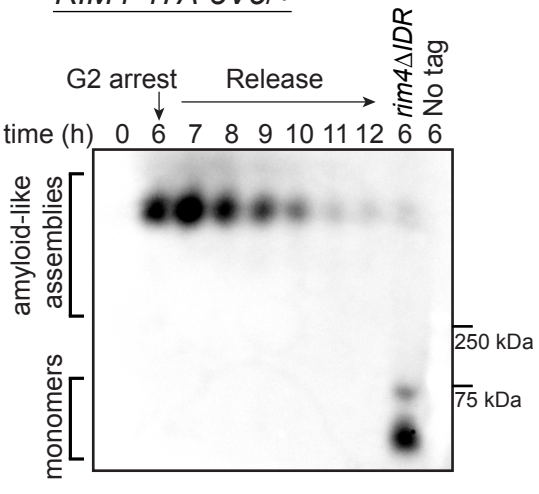
RIM4-3V5/+

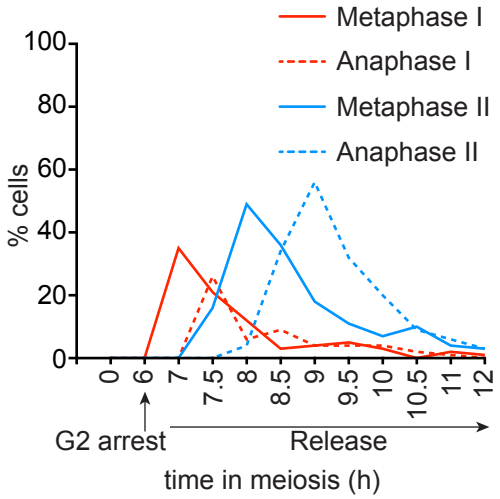


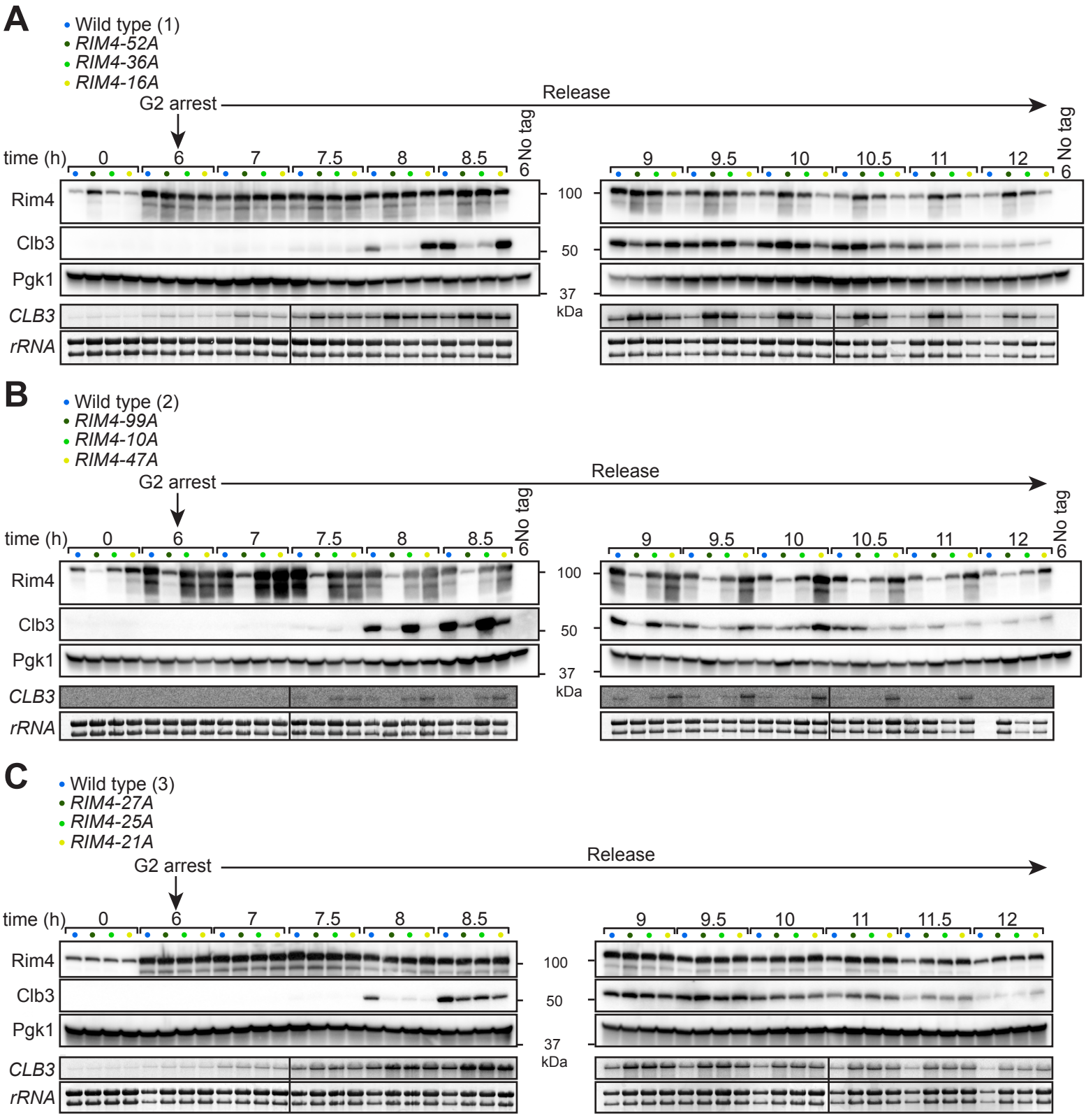


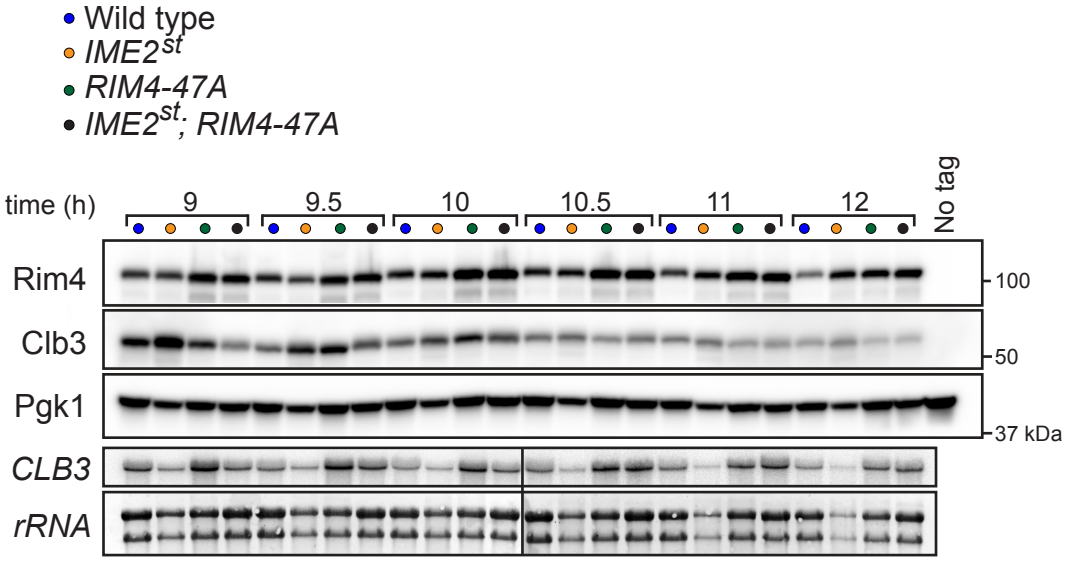
B

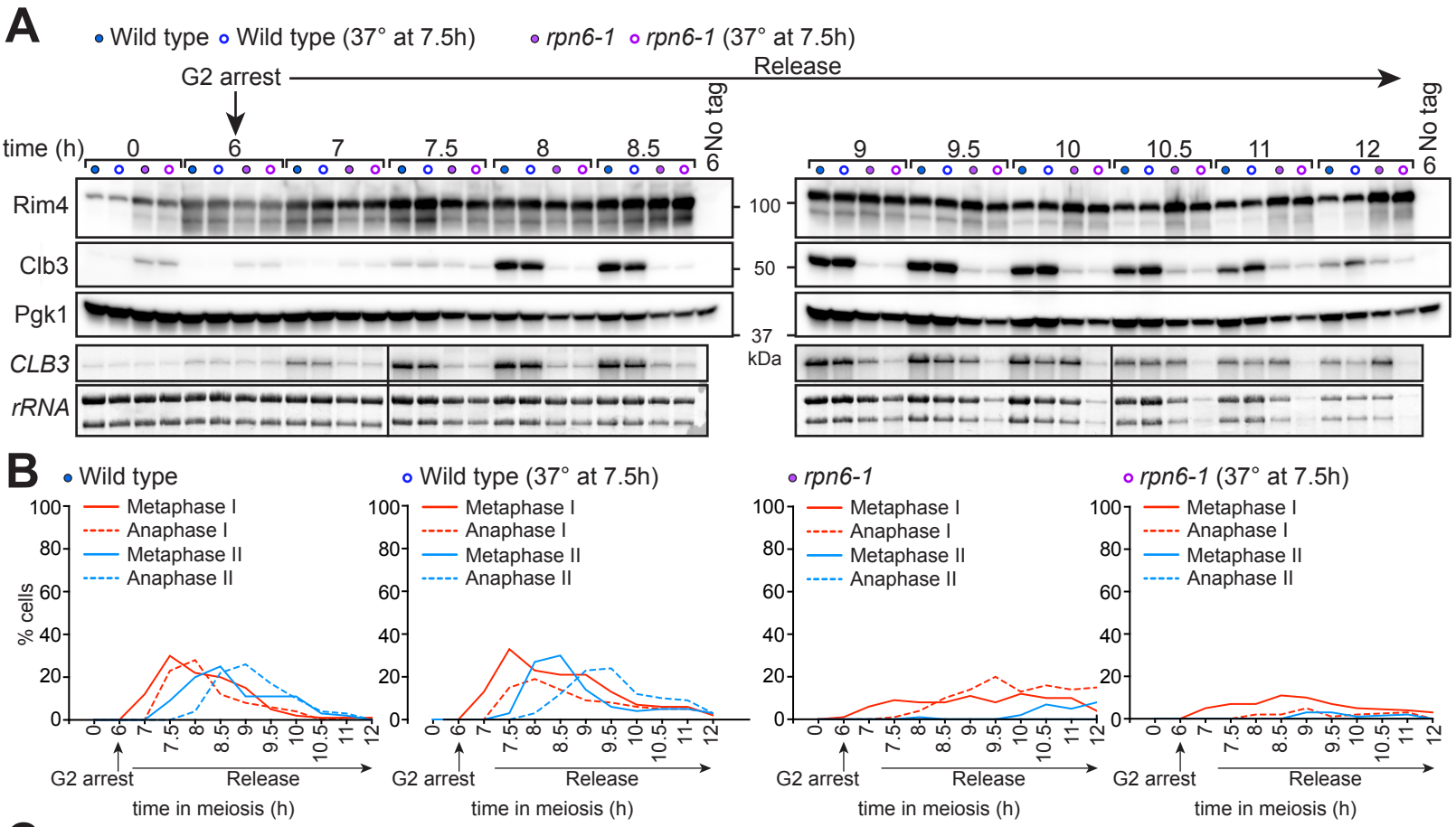
RIM4-47A-3V5/+



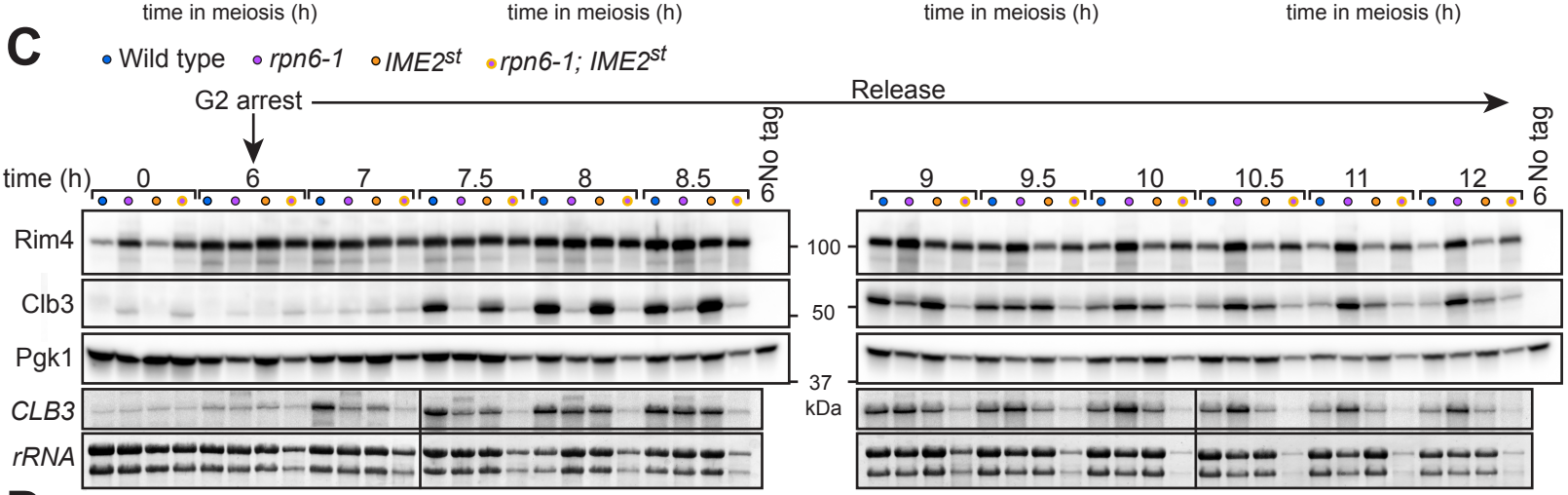


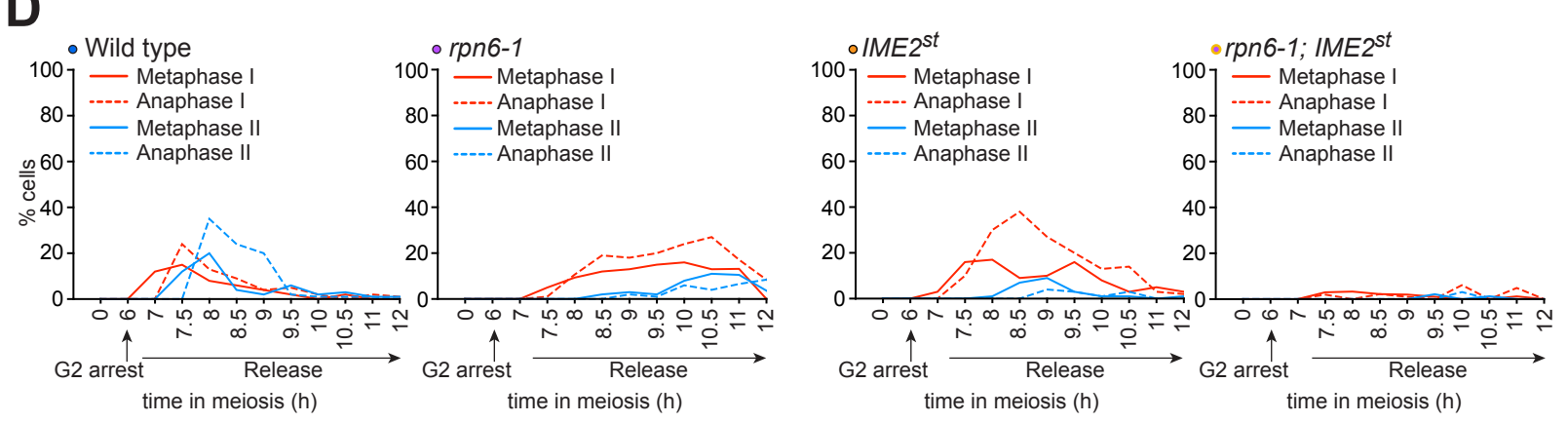

A
Wild type (1)
RIM4-52A
RIM4-36A
RIM4-16A
G2 arrest
Release
time (h)
0
6
7
7.5
8
8.5
No tag
6
9
9.5
10
10.5
11
12
No tag
6
Rim4
Clb3
Pgk1
CLB3
rRNA
100
50
37
kDa
B
Wild type (2)
RIM4-99A
RIM4-10A
RIM4-47A
G2 arrest
Release
time (h)
0
6
7
7.5
8
8.5
No tag
6
9
9.5
10
10.5
11
12
No tag
6
Rim4
Clb3
Pgk1
CLB3
rRNA
100
50
37
kDa
C
Wild type (3)
RIM4-27A
RIM4-25A
RIM4-21A
G2 arrest
Release
time (h)
0
6
7
7.5
8
8.5
9
9.5
10
11
11.5
12
Rim4
Clb3
Pgk1
CLB3
rRNA
100
50
37
kDa


Wild type
IME2st
RIM4-47A
IME2st; RIM4-47A
time (h)
9
9.5
10
10.5
11
12
No tag
Rim4
Clb3
Pgk1
CLB3
rRNA
100
50
37 kDa


A
Wild type
Wild type (37° at 7.5h)
rpn6-1
rpn6-1 (37° at 7.5h)
G2 arrest
Release
time (h)
0
6
7
7.5
8
8.5
No tag
6
9
9.5
10
10.5
11
12
No tag
6
Rim4
Clb3
Pgk1
CLB3
rRNA
100
50
37
kDa

B
Wild type
Wild type (37° at 7.5h)
rpn6-1
rpn6-1 (37° at 7.5h)
Metaphase I
Anaphase I
Metaphase II
Anaphase II
% cells
G2 arrest
Release
time in meiosis (h)


C
Wild type
rpn6-1
IME2st
rpn6-1; IME2st
G2 arrest
Release
time (h)
0
6
7
7.5
8
8.5
No tag
6
9
9.5
10
10.5
11
12
No tag
6
Rim4
Clb3
Pgk1
CLB3
rRNA
100
50
37
kDa


D
Wild type
rpn6-1
IME2st
rpn6-1; IME2st
Metaphase I
Anaphase I
Metaphase II
Anaphase II
% cells
G2 arrest
Release
time in meiosis (h)