Phosphorylation of luminal region of the SUN-domain protein Mps3 promotes nuclear envelope localization during meiosis

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Abstract

During meiosis, protein ensembles in the nuclear envelope (NE) containing SUN- and KASH-domain proteins, called linker nucleocytoskeleton and cytoskeleton (LINC) complex, promote the chromosome motion. Yeast SUN-domain protein, Mps3, forms multiple meiosis-specific ensembles on NE, which show dynamic localisation for chromosome motion; however, the mechanism by which these Mps3 ensembles are formed during meiosis remains largely unknown. Here, we showed that the cyclin-dependent protein kinase (CDK) and Dbf4-dependent Cdc7 protein kinase (DDK) regulate meiosis-specific dynamics of Mps3 on NE, particularly by mediating the resolution of Mps3 clusters and telomere clustering. We also found that the luminal region of Mps3 juxtaposed to the inner nuclear membrane is required for meiosis-specific localisation of Mps3 on NE. Negative charges introduced by meiosis-specific phosphorylation in the luminal region of Mps3 alter its interaction with negatively charged lipids by electric repulsion in reconstituted liposomes. Phospho-mimetic substitution in the luminal region suppresses the localisation of Mps3 via the inactivation of CDK or DDK. Our study revealed multi-layered phosphorylation-dependent regulation of the localisation of Mps3 on NE for meiotic chromosome motion and NE remodelling.

Introduction

During meiosis, homologous chromosomes pair with each other. This pairing leads to juxtaposition of the chromosomes along their entire length, resulting in the formation of the synaptonemal complex (SC). SC is a proteinaceous structure comprising two chromosome axes called the axial/lateral element (AE/LE), flanking a central region with a ladder-like structure (Hawley & Gilliland, 2009; Zickler & Kleckner, 1999). AE formation is initiated in the leptotene stage of meiotic prophase I, accompanied by the assembly of the chromosome axis with multiple chromatin loops. In the zygotene stage, some homologous AE pairs form a short SC, while in the pachytene stage, the SC elongates along the entire chromosome. After the pachytene stage, the SC dismantles in the diplotene stage before the onset of meiosis I.

Meiotic cells also exhibit a unique arrangement of chromosomes in the nucleus. In most organisms, telomeres attach to nuclear envelopes (NEs) in early meiotic prophase (or premeiotic phase) and occasionally cluster in one area of NE, particularly near the vicinity of the centrosome (a microtubule organising centre) (Zickler & Kleckner, 1998). Telomere clustering is seen only in the zygotene stage and is known as “telomere/chromosome bouquet.” In the pachytene stage, telomere bouquets are resolved, and telomeres are dispersed over the entire NE. The bouquet configuration of chromosomes is proposed to promote homologous pairing by restricting the homology search process from three dimensions to two dimensions (Zickler & Kleckner, 1998).

The dynamic nature of meiotic chromosomes in various organisms is conserved between yeasts and mammals (Hiraoka & Dernburg, 2009; Koszul & Kleckner, 2009; Burke, 2018; Lee et al., 2020b; Paouneskou & Jantsch, 2019). The oscillating motion of nuclei during meiotic prophase I has been well described in fission yeast (Chikashige et al., 1994). Nuclear movement is driven by cytoplasmic microtubule cables, along which the spindle pole body (SPB), an yeast centrosome equivalent, embedded in NE moves together with the clustered telomeres. This telomere-led movement promotes the pairing of homologous loci on chromosomes. Although less coordinated, chromosome motion throughout prophase I has been reported in budding yeast, nematodes, maize and mouse

(Lee et al., 2015; Penkner et al., 2009; Sheehan & Pawlowski, 2009; Shibuya, Ishiguro, & Watanabe, 2014).

Protein ensembles embedded in NE that connect telomeres in the nucleoplasm with the cytoskeleton in the cytoplasm, which are referred to as the linker of nucleocytoskeleton and cytoskeleton (LINC) complex, promote meiotic chromosome motion (Hiraoka & Dernburg, 2009; Starr & Fridolfsson, 2010) (Jahed, Domkam, Ornowski, Yerima, & Mofrad, 2021; Lee et al., 2020b; Wong, Loo, & Stewart, 2021). The LINC complex necessary for chromosome motion contains an inner nuclear membrane (INM) protein harbouring the SUN (Sad1/UNc-84) domain and an outer nuclear membrane (ONM) protein with the KASH (Klarsicht, ANC-1, SYNE1 Homology) domain. The SUN and KASH domains interact with each other in a space between the INM and ONM, luminal region, or peri-nuclear space. SUN-domain proteins bind to telomeres and/or nucleocytoskeletons in the nucleoplasm, while KASH-domain proteins bind to cytoskeletons. LINC complexes transmit forces generated by the cytoskeleton to NEs for nuclear positioning and migration in somatic cells and telomeres for chromosome motion in meiotic cells (Starr & Fridolfsson 2010).

In *Saccharomyces cerevisiae*, telomeres are tethered to NEs as several clusters during mitosis and move around NEs during meiotic prophase I. These telomeres are transiently clustered during prophase I (Conrad et al., 2008; Koszul, Kim, Prentiss, Kleckner, & Kameoka, 2008; Trelles-Sticken, Dresser, & Scherthan, 2000; Trelles-Sticken, Loidl, & Scherthan, 1999). Telomere movement on NE drives chromosome motion inside the nucleus (Koszul et al., 2008; Scherthan et al., 2007). At least three distinct regulatory steps facilitate chromosome motion during budding yeast meiosis. First, meiosis-specific components of the LINC complex, such as Ndj1 and Csm4, are expressed and incorporated. Ndj1 is a meiosis-specific telomere-binding protein necessary for telomere tethering to NEs (Chua & Roeder, 1997; Conrad, Dominguez, & Dresser, 1997), whereas Csm4 is a meiosis-specific ONM protein (Conrad, Lee, Wilkerson, & Dresser, 2007; Kosaka, Shinohara, & Shinohara, 2008; Wanat et al., 2008), which interacts with a unique class of KASH protein, Mps2 (Chen et al., 2019; Lee et al., 2020a). Second, actin cable formation is specifically induced in the cytoplasm of meiotic cells (Koszul et al., 2008; Taxis et al., 2006). Third, NE dynamics,

including NE remodelling and movement, occur prominently in meiotic prophase I (Conrad *et al.* 2007; this paper). NE remodelling is accompanied by NE localisation of a yeast SUN-domain protein, Mps3/Nep98 (Conrad et al., 2007), and Mps2 (Lee et al., 2020a). During mitosis, Mps3 and Mps2 predominantly localise to SPB as a component of the half-bridge as well as SPB-interacting network (SPIN) (Chen et al., 2019) and play an essential role in SPB duplication (Jaspersen, Giddings, & Winey, 2002; Nishikawa et al., 2003). During meiotic prophase-I, in addition to SPB, Mps3 localises as a distinct protein ensemble on NE as a LINC complex (Conrad et al., 2007). In early meiotic prophase I, Mps3 forms several distinct foci/patches on NE and later shows more dispersed staining around the NE. Mps3 plays an important role in the telomere/chromosome dynamics (Conrad et al., 2007). However, how meiosis-specific Mps3 localisation in NE is induced is poorly understood.

Here, we showed that cyclin-dependent Cdc28 kinase (CDK) and Dbf4-dependent Cdc7 kinase (DDK) are necessary for dynamic telomere movement, particularly for the resolution of telomere clusters. Defects in telomere movement induced by CDK and DDK inactivation are associated with impaired NE localisation of Mps3 and resolution of Mps3 clusters. We also found that the luminal region of Mps3, located near the INM, is important for NE localisation. This region is subject to phosphorylation during prophase I in meiosis. Phospho-defective *mps3* mutant protein showed reduced NE localisation during meiosis. The introduction of negatively charged residues in this region suppresses the defects in the Mps3 resolution in *cdk* and *ddk* mutants. In reconstituted liposomes, an Mps3 peptide containing the luminal region with negatively charged residues showed reduced affinity to the lipid membrane relative to the control wild-type Mps3 peptide. Our results suggest that multiple phosphorylation events, such as CDK and DDK-dependent phosphorylation as well as phosphorylation in the luminal region, control the Mps3 localisation to NE, thereby also controlling the assembly of meiosis-specific canonical LINC complex for chromosome motion and NE remodelling during meiosis.

Results

Force-independent localization of Mps3 on NE during meiosis

We examined the localisation of the Mps3 fusion protein with a green fluorescent protein (GFP), Mps3-GFP (Conrad et al., 2007), in various mutants. Mps3-GFP showed stage-specific distribution and movement in NE during meiotic prophase I (Figure 1A, 1B). At 0 h, Mps3 predominantly localised as a single focus, probably on SPB (Figure 1A) (Jaspersen et al., 2002; Nishikawa et al., 2003) with little movement (Figure 1C; Video 1). During early prophase I, including meiotic S phase (2–3 h), Mps3 formed 2–5 foci (including 1 focus in SPB) on NE with slow movement. After 3 h, the number of Mps3 foci increased to more than 5, and several Mps3 foci fused to form a patch (Figure 1A-C; Videos 2-4), as shown previously (Conrad et al., 2007). During this stage, Mps3 foci/patches moved around the NE with oscillatory motion (Figure 1C, Figure S1A, B). At 3–5 h, some Mps3 foci/patches were localised to one area of NE with clustering. In late prophase I (e.g. at 5 and 6 h), Mps3 often covered most of the NE and was associated with NE deformation and protrusion (Figure 1C; Videos 3, 4). Mps3 localisation was classified into four classes and quantified at each time point (Figure 1B). After 7 h, most Mps3 signals on NE disappeared, leaving two SPB-associated foci (Figure 1A).

Although Mps3 movement is dependent on Cms4 (Conrad et al., 2007; Kosaka et al., 2008; Wanat et al., 2008) and partly on Ndj1 (Chua & Roeder, 1997; Conrad et al., 1997), Mps3 localisation on NE did not depend on Ndj1 and Csm4 (Figure 1D). At late times, such as 5 h, the *ndj1* mutant showed full coverage of Mps3 on NE (Conrad et al., 2007) with NE deformation (distorted nucleus) and motion. The *csm4* mutant formed multiple Mps3 patches/foci on NE (Conrad et al., 2007; Kosaka et al., 2008; Wanat et al., 2008), but did not show full coverage of NE. The *csm4* mutant is also defective in clustering and motion of Mps3 foci/patches, as well as NE deformation with a round nucleus (Figure 1D). Moreover, we examined the effect of an actin polymerisation inhibitor, latrunculin B (LatB), on Mps3 localization (Trelles-Sticken, Adelfalk, Loidl, & Scherthan, 2005). Treatment of meiotic cells with LatB, which disrupted the cytoplasmic actin cables but not the actin patches (Figure S1C), showed normal

183 distribution of Mps3 foci/patches on NE during prophase (Figure 1D). However,
184 as shown previously (Trelles-Sticken et al., 2005), the dynamic movement of
185 Mps3-GFP was largely absent (Figures 1D, S1B, S1D; Video 5) without the
186 deformation of nuclei. These defects were similar to those observed in the *csm4*
187 mutant (Figure S1B, D). One hour after washing out LatB, the motion of Mps3
188 resumed (Figure 1D). These results suggest that Mps3 dynamics during meiosis
189 can be functionally separated into two processes: force-independent NE
190 localisation and force-dependent motion. Force-dependent motion requires actin
191 polymerisation, Csm4, and a party, Ndj1. On the other hand, force-independent
192 NE localisation does not require action polymerisation or Csm4 (Ndj1),
193 suggesting a unique localisation mechanism of Mps3 on NE during meiosis.
194
195 **CDK and DDK promote proper Mps3 localization on NE during meiosis**
196 Because Mps3-dependent telomere movement is initiated in early meiotic
197 prophase (Conrad et al., 2008; Conrad et al., 2007), we analysed the role of two
198 protein kinases that are active during early meiosis: CDK and DDK. Because
199 CDK and DDK are essential for vegetative growth, we used conditional mutant
200 alleles of the catalytic subunits of CDK and DDK (Cdc28 and Cdc7), *cdc28-as1*,
201 and *cdc7-as3*, which are sensitive to specific inhibitors,1 NM-PP1 and PP1,
202 respectively (Benjamin, Zhang, Shokat, & Herskowitz, 2003; Wan, Zhang, Shokat,
203 & Hollingsworth, 2006). Although CDK and DDK are also essential for DNA
204 replication and recombination in meiosis (Benjamin et al., 2003; Sasanuma et al.,
205 2008), telomere movement during meiosis, Mps3 localisation, is independent of
206 replication and recombination (Conrad et al., 2007; Trelles-Sticken et al., 1999).
207 We performed time-lapse analysis of Mps3-GFP in *cdc28-as1* and *cdc7-as3*
208 in the absence or presence of each specific inhibitor (Figure 1E). To inactivate
209 CDK, 1 NM-PP1, which did not affect Mps3-GFP dynamics in wild-type cells or a
210 steady-state level of Mps3-Flag protein in *cdc28-as1* cells (Figure S1E, F), was
211 added to *cdc28-as1* at 0h. In the absence of the inhibitor, the mutant cells showed
212 normal Mps3 localisation during meiosis (Figure 1E). CDK inactivation impaired
213 Mps3 localisation and motion (Figure 1B, E). In the wild type, several Mps3
214 foci/patches are loosely clustered on one area of NE in a transient manner, which
215 reflects telomere bouquet (Conrad et al., 2007). With CDK inhibition, these loose

clusters of Mps3 foci were persistent during prophase I, which largely reduced the spread of Mps3 foci on NE (Figure 1B, G). Tracking the motion of Mps3 foci confirmed persistent clustering with restricted motion of the foci in one region of NE under these conditions (Figure 1F).

We also measured the speed of Mps3 GFP focus/patches on NE (Figure 1H). Previous measurement of the motion of a single chromosome locus showed heterologous nature of chromosome motion (Conrad et al., 2008). To avoid biased results, we simply measured a step-size of a single Mps3-GFP focus or patch in one-second interval through 20 seconds in multiple nuclei, and measured an average velocity a maximum velocity during the measurement (Figure 1H). Wild type Mps3-GFP at 5 h showed 0.19 and 0.25 μm/s in average and maximum speeds, respectively. CDK inactivation slightly decreased the average speed, but not the maximum speed (Figure 1H, Video 6).

Importantly, 2 h after washing the inhibitor, NE localisation and Mps3 motion were recovered. CDK inactivation after meiotic S phase (the addition of the inhibitor at 3 h) interfered with the dispersed localisation of Mps3 throughout NE with loose clustering of Mps3 foci/patches (Figure 1E). Therefore, persistent CDK activity is necessary for the establishment and maintenance of the dispersed localisation of Mps3, and thereby the resolution of clustering. We concluded that CDK plays a role in the resolution of Mps3 focus clustering (probably, not in focus/patch formation *per se*) and a minor role in the motion of Mps3 on NE.

DDK inactivation (*cdc7-as3* + PP1) also induced a defect in Mps3 localisation on NE and motion (Figure 1B, E). PP1 did not affect the localisation of Mps3-GFP in wild-type cells or a steady level of Mps3-Flag protein under DDK inactivation conditions (Figure S1F, G). In its absence, *cdc7-as3* cells showed normal localisation and motion of Mps3 foci/patches (Figure 1B, E). DDK inhibition by PP1 from 0 h reduced the NE localisation of Mps3 with accumulation of the loose cluster of the foci (Figure 1E, F). Removal of the inhibitor restored the NE localisation of Mps3 in *cdc7-as3* cells (Figure 1E). The addition of PP1 at 3 h also induced a Mps3 localisation defect similar to that at 0 h. This shows that persistent DDK activity is critical for the efficient resolution of Mps3 clusters on NE. The resolution defect of Mps3 clusters in reduced DDK activity is similar to that in response to decreased CDK activity, suggesting that CDK and DDK work

249 in the same pathway to control Mps3 dynamics during meiosis. In contrast to
250 reduced CDK, reduced DDK activity clearly decreased the velocity of Mps3 foci
251 on NE (Figure 1H, Video 7). Therefore, DDK appears to play a more critical role
252 in Mps3 motion than CDK.

253 We also analysed the Mps3-GFP localisation in the *cdc7 bob-1-1* double
254 mutant, in which the *bob1-1* mutation in the helicase gene *MCM5* suppresses the
255 lethality of the *cdc7* deletion (Jackson, Pahl, Harrison, Rosamond, & Sclafani,
256 1993; Sasanuma et al., 2008), A small fraction (~20%) of the *cdc7 bob-1* mutant
257 cells showed a few Mps3 foci on NE, whereas most of the cells did not form some
258 Mps3 foci on NE (Figure 1B, E, F). The velocity of Mps3 foci formed in the
259 complete absence of DDK was highly reduced and was similar to that in the *csm4*
260 mutant and in the presence of LatB (Figures 1H, S1D; Video 8). These defects in
261 the *cdc7 bob-1-1* double mutant were more severe than those in the *cdc7-as3*
262 mutant with PP1, confirming the importance of DDK in Mps3 localisation and
263 motion in NE.

264 Whole cell immunostaining of the Mps3-Flag and Ndj1-HA proteins showed
265 that reduced CDK and DDK activities hampered the localisation of Mps3, but not
266 of Ndj1, to NE during meiosis (Figure S1H), suggesting a critical role for the
267 kinases in the coupling of Mps3 to Ndj1 on NE. Double-staining of Mps3 and Ndj1
268 in wild-type meiosis showed distinct Mps3- and Ndj1-staining domains on NE
269 together with colocalized foci/patches of both. The reduced colocalization of Ndj1
270 and Mps3 on NE is clearly seen under CDK and DDK inactivation conditions.
271 Ndj1 was not required for Mps3 localisation on the NE (Figure 1D). These results
272 suggest the presence of an Mps3-independent pathway for Ndj1 localisation on
273 NE or telomeres, probably mediated by Ndj1 binding to Rap1/telomeres. Taken
274 together, these results revealed that CDK and DDK activities are necessary for
275 proper Mps3 localisation and efficient motion in NE during meiosis.
276

277 **CDK and DDK promote telomere dynamics during meiosis**

278 To determine the role of CDK and DDK in telomere movement, we analysed the
279 motion of GFP fusion of a telomere-binding protein, Rap1, as described
280 previously (Trelles-Sticken et al., 2005). During vegetative growth or pre-meiosis,
281 Rap1-GFP exhibited 1–4 foci on NE on a single focal plane with little movement

282 (Figure 2A, B; Video 9) (Trelles-Sticken et al., 2005). Once the cells entered
283 meiosis, Rap1 foci on NE increased in number (up to 10). Rap1-GFP foci moved
284 rapidly and were occasionally clustered in one area of NE (Figure 2A, B; Video
285 10), which corresponds to the telomere bouquet (Trelles-Sticken et al., 2005).
286 This telomere clustering was very transient (half-life less than 5 s) and was
287 predominantly observed after 3–4 h of incubation in sporulation medium (Figure
288 2A, D). Rap1 foci were more dispersed during late meiotic prophase (4–6 h for
289 the pachytene stage) before the onset of meiosis I (MI; Figure 2D). Tracking
290 analysis showed that Rap1 foci move around the meiotic nucleus in an
291 uncoordinated manner (Figure 2B).

292 CDK inactivation (*cdc28-as1* + 1 NM-PP1) impaired meiotic Rap1
293 dynamics (Figure 2B, E). With compromised CDK activity from 0 h, cells with
294 clustered Rap1 foci accumulated during prophase I (Figure 2D, E; Video 11),
295 indicating that CDK activity is required for meiotic telomere dynamics, particularly
296 for the resolution of telomere clusters (but not necessarily for clustering *per se*).
297 The 2D tracking confirmed the restricted motion of Rap1 foci in one nuclear area
298 (Figure 2B). The addition of the inhibitor during early prophase I (e.g. at 3 h) also
299 resulted in defective resolution of telomere clustering; washing off the inhibitor
300 restored telomere dynamics (Figure 2E). Treatment of wild-type cells with the
301 inhibitor did not affect Rap1-GFP dynamics, confirming the specificity of the
302 inhibitor (Figure S1I). These results indicate that persistent CDK activity during
303 meiotic prophase I promotes normal telomere dynamics. CDK inactivation of CDK
304 does not affect the formation of cytoplasmic actin cables during meiosis (Figure
305 S1C).

306 Similar to CDK inactivation, Cdc7 inactivation (the treatment of *cdc7-as3*
307 with PP1) at 0 and 3 h impaired Rap1-GFP dynamics, which showed
308 accumulation of Rap1 clusters with restricted motion (Figure 2B-D, F; Video 12);
309 however, treatment of wild-type cells with PP1 did not affect Rap1 dynamics
310 (Figure S1I). This defective resolution of the Rap1 cluster under DDK inhibition
311 was similar to that observed in CDK inhibition. Moreover, the *cdc7* null mutant
312 with *bob1-1* mutation exhibited persistent telomere clustering (Figures 2B, D, F,
313 and S13). These results indicate that persistent Cdc7 kinase activity is also
314 required for meiotic telomere dynamics. Although CDK and DDK inactivation

315 restricted Rap1 foci to one area, inactivation did not affect the velocity of Rap1
316 foci (Figure 2C). Since Mps3 foci are actually formed under CDK and DDK
317 inactivation conditions (Figure 1), the Mps3 complexes on NE are sufficient to
318 ensure some Rap1 motion. Alternatively, the Mps3-independent mechanism may
319 promote motion under reduced CDK or DDK activities, particularly in the *cdc7*
320 null mutant.
321
322 **Mps3 is phosphorylated during meiosis**
323 Previously, it was shown that S70 of SPB-associated Mps3 is phosphorylated in
324 late prophase-I (Li et al., 2017) (Figure 3A). We also found that Mps3 is a
325 phosphoprotein in middle prophase I (Figure 3). On immunoprecipitation (IP)
326 western blots, Mps3-Flag showed a mobility shift, which was induced during
327 meiotic prophase I (4 and 6 h; Figure 3B, left panel). Mps3 mobility in the IP
328 fractions was reduced by treatment with lambda phosphatase (Figure 3C).
329 Moreover, IP fractions of Mps3-Flag from meiotic cells showed that Mps3 cross-
330 reacted with anti-phosphoserine antibody (Figure 3D), whose reactivity was
331 largely diminished by treatment with phosphatase.
332 Previous systematic analyses of CDK substrates *in vitro* identified Mps3
333 as a Cdc28–Clb2 substrate (Ubersax et al., 2003). Mps3 contains six potential
334 CDK sites (S/TPXK/R or S/TP) located in the luminal region, two of which seem
335 to be candidates for CDK/DDK phosphorylation (188–190 <u>TSS</u>PGK and 450–451
336 <u>TS</u>P; Figure 3A, E). Because the 450–451 site is located in the SUN domain,
337 which is not essential for Mps3 localisation on NE (Rao, Shinohara, & Shinohara,
338 2011), we focused on T188-S189-S190 (hereafter, TSS; Figure 3E). We purified
339 a GST fusion protein of an Mps3 fragment (residues 160–260), including TSS. *In*
340 *vitro* Cdc28–Clb5 alone, but not Cdc7–Dbf4 alone, promoted phosphorylation of
341 the Mps3 fragment (approximately 10-fold compared to the background; Figure
342 3F). Co-incubation with CDK and DDK resulted in robust incorporation of ^{32}P in
343 GST-Mps3, indicating that CDK and DDK cooperate in Mps3 phosphorylation *in*
344 *vitro* (~30-fold compared to the background). Triple alanine substitutions (T188A,
345 S189A, S190A; referred to as the *mps3-AAA* mutant; Figure 3E) made the GST-
346 Mps3 fragment a poor substrate by these kinases. These results indicate that
347 TSS is required for efficient CDK/DDK phosphorylation *in vitro*.

348 Consistent with the *in vitro* results, the Mps3-AAA mutant protein showed
349 a reduced band shift (Figure 3B, G) and decreased reactivity to anti-
350 phosphoserine antibody (Figure 3H). Furthermore, we raised an antibody against
351 a Mps3 peptide with 189-phosphoserine and 190-phosphoserine (186-
352 GAT**pSpS**PGKSF-195) and found that this phospho-specific antibody could
353 recognise immunoprecipitated Mps3-Flag specifically between 4 and 8 h in
354 meiosis, but not in mitosis (Figure 3C, left panel). Strong signals were observed
355 during late prophase I (4 h and 6 h). This antibody did not recognise precipitated
356 Mps3-AAA-Flag (Figure 3C, middle panel). These results indicate that 189S and
357 190S are phosphorylated *in vivo*, specifically during meiosis.
358 The Mps3 sequence around the TSS site is similar to that of CDK/DDK-
359 catalysed phosphorylation sites in Mer2 (Henderson, Kee, Maleki, Santini, &
360 Keeney, 2006; Sasanuma et al., 2008; Wan et al., 2008), including the TSSP
361 sequence (Figure 3E). Mer2 is a meiosis-specific protein essential for DSB
362 formation (Cool & Malone, 1992; Engebrecht, Hirsch, & Roeder, 1990). CDK and
363 DDK sequentially phosphorylate the TSS sequence of Mer2, which is essential
364 for meiotic DSB formation (Sasanuma et al., 2008; Wan et al., 2008). We
365 swapped a 10-amino-acid region (GATSSPGKSF) containing the Mps3
366 phosphorylation sites with the corresponding region of Mer2 (TETSSPFRST;
367 Figure 3E). This *MPS3* allele (*MPS3-MER2S*) conferred wild-type spore viability
368 (95%; n=50 tetrads; Figure S2A). Importantly, the Mps3-MER2S protein cross-
369 reacted with an antibody against the Mer2 phospho-S30 antibody (Henderson *et*
370 *al.* 2006) (Figure 3I), but not with the anti-Mps3-phospho antibody (Figure 3B,
371 right panel). *MPS3-MER2S-Flag* exhibited similar telomere (Rap1) clustering to
372 the wild type (Figure 3J, K). The Mps3-MER2S-Flag protein showed meiosis-
373 specific localisation on NE which often co-localized with Ndj1 (Figure 3L). This
374 suggests that residues 26-35 of Mer2 are functionally equivalent to residues 185-
375 194 of Mps3. Rather, the sequence of Mps3, the composition of amino acids,
376 seems to be critical for NE localisation. Similar to Mps3, the region of Mer2 is rich
377 in serine and threonine (Figure 3E).
378
379 **The *mps3-AAA* mutant is defective in Mps3 localization on NE**
380 To investigate the role of the phosphorylated residues of Mps3, we constructed

381 and characterised the phenotypes of the *mps3-AAA-FLAG* mutant (hereafter,
382 *mps3-AAA*; Figures 3E, 4). The *mps3-AAA* mutant showed few defects in mitosis,
383 such as SPB duplication (Figure S2B) seen in other *mps3* mutants (Jaspersen et
384 al., 2002; Nishikawa et al., 2003), suggesting that the TSS residues of Mps3 are
385 not critical for the mitotic function of Mps3. The *mps3-AAA* mutant showed
386 defects in meiosis with a reduced spore viability of 87.5% (Figure 4A), which was
387 slightly higher than that of the *ndj1* mutant (75.8%) (Chua & Roeder, 1997;
388 Conrad et al., 1997). The spore viability of the *ndj1 mps3-AAA* double mutant was
389 72.3%, which is similar to that of the *ndj1* mutant. The *mps3-AAA* mutant delayed
390 entry into meiosis I by ~1 h relative to the wild type (Figure 4A). The *ndj1 mps3-*
391 *AAA* double mutant showed ~2.5 h delayed entry into meiosis I, which is similar
392 to the *ndj1* single mutant, indicating the epistatic relationship of *mps3-AAA* to *ndj1*.
393 The *mps3-AAA* mutant showed normal progression of the meiotic S-phase
394 (Figure S2C). Immunostaining of Zip1, a component of the central region of SC
395 (Sym, Engebrecht, & Roeder, 1993), showed a delay in both the loading of Zip1
396 and the formation of full-length SCs in the *mps3-AAA* mutant (Figure S2D, E),
397 suggesting that Mps3 NE localisation is necessary for timely synapsis.
398 We also analysed chromosome dynamics during meiosis in *mps3-AAA*
399 mutant cells. First, we checked the Rap1-GFP movement in the *mps3-AAA*
400 mutant. In contrast to wild-type cells, the *mps3-AAA* mutant was defective in the
401 localisation and motion of Rap1-GFP (Figure 4B, C). In the mutant, the number
402 of Rap1 foci did not increase (Figure 4B), and little movement of Rap1-GFP foci
403 was observed during meiosis (Figure 4C). Rap1 motion in meiotic prophase I of
404 the *mps3-AAA* mutant is restricted, and the velocity of each Rap1 focus is
405 decreased relative to the wild type (Figure 4C, D; Video 14). Therefore, we
406 concluded that T188, S189, and S190 of Mps3 are important for meiotic telomere
407 dynamics. Indeed, the *mps3-AAA* mutant was defective in chromosome motion,
408 as shown by the analysis of a GFP fusion of Zip1 (Koszul et al., 2008; White,
409 Cowan, Cande, & Kaback, 2004). During prophase I, Zip1-GFP showed dynamic
410 motion (Figure 4E; Videos S15, 16). The *mps3-AAA* mutant reduced the velocity
411 of Zip1 motion by approximately 3-fold relative to that of the wild type (48±15
412 versus 130±31 nm/s, Figure 4F).
413 Next, we examined the localisation of the Mps3-AAA mutant protein in

414 meiosis as a GFP fusion protein (Figure 4G). *mps3-AAA-GFP* cells showed 85%
415 spore viability, which was comparable to the mutant strain without the GFP tag.
416 Similar to the wild-type Mps3-GFP, the Mps3-AAA GFP fusion protein normally
417 resided on the SPB as a single focus during mitosis (Figure 4G). During meiosis,
418 the Mps3-AAA mutant protein showed reduced NE localisation. Upon entry into
419 meiosis, similar to the wild-type Mps3, the Mps3-AAA mutant protein formed a
420 few (usually two or three small) foci on NE, but showed little increase in the
421 number of Mps3 foci. At late time points, unlike wild-type Mps3, the mutant protein
422 did not form an Mps3 patch or cover on NE (Figure 4G, H, I). At 6 h, two or three
423 foci of Mps3 showed limited movement (Figure 4J, K, L; Video 17). These results
424 suggest that T188, S189, and S190 of Mps3 are necessary for meiosis-specific
425 Mps3 localisation and motion on NE, particularly for efficient localisation on NE
426 accompanied by the formation of large complexes. Interestingly, the Mps3-AAA-
427 Flag mutant protein still bound to Ndj1 and Csm4, similar to the wild-type Mps3
428 protein (Figure S2F), which is consistent with the Mps3-AAA protein retaining the
429 N-terminal nucleoplasmic region and SUN domain necessary for Ndj1 and Csm4
430 binding, respectively.
431
432 **The *mps3-AAA* mutant is deficient in NE growth**
433 In addition to localization/movement defects, the *mps3-AAA* mutant showed
434 defects in nuclear morphology. First, the meiotic nucleus of the *mps3-AAA* mutant
435 was smaller than that of the wild type (Figure 4B). The nucleus of the wild type
436 grew when the cell underwent meiosis and exhibited a 4-fold increase (in area; 2
437 folds in length) at 4 h (Figure 4M). Treatment with LatB did not affect the
438 expansion, indicating that meiosis-specific NE remodelling does not require actin
439 polymerisation. The *mps3-AAA* mutant showed defective NE growth in prophase
440 I; the nuclear area in the *mps3-AAA* mutant was similar between 0 and 4 h (Figure
441 4M). These results indicate that the Mps3 luminal region plays a critical role in
442 the growth of the meiotic nucleus, possibly by controlling NE remodelling.
443 Second, a fraction of the *mps3-AAA* mutant cells showed a partial defect
444 in telomere tethering to NE during meiosis. In one or two Rap1-GFP foci, the
445 *mps3-AAA* mutant (40%; n=100) was localised to the nucleoplasm rather than to
446 the nuclear periphery, whereas all Rap1-GFP foci were localised near the NE in

447 the wild type (Figure 4B). The weak telomere tethering defect in the *mps3-AAA*
448 mutant is reminiscent of the defect observed with an *NDJ1* mutant, which also
449 showed a partial tethering defect (Conrad et al., 1997; Trelles-Sticken et al.,
450 2000).
451
452 **Aspartate substitution of TSS in Mps3 overcomes the CDK and DDK defects**
453 We constructed a version of Mps3 with three negative charges on the TSS
454 sequence (T188D, S189D, and S190D), referred to as *MPS3-DDD* (Figure 3E).
455 The *MPS3-DDD* allele did not show any mitotic defects, such as colocalization
456 with tubulin and tubulin elongation (Figure S3A-C). The *MPS3-DDD* cells showed
457 wild-type spore viability (95%) and entered MI similar to wild-type cells (Figure
458 S3D). The *MPS3-DDD* cells also displayed similar Rap1-GFP dynamics to wild-
459 type cells with normal clustering during prophase I (Figure 5A, B). The Mps3-
460 DDD-GFP protein localised to SPB during vegetative growth (Figure S3A) and
461 showed wild-type-like localisation on NE during meiosis (Figures 5C, D, and S3E).
462 Mps3-DDD-GFP displayed movement similar to that of the wild-type protein
463 (Figure 5C, E, F; Video 18). The aspartate substitutions of Mps3 suppressed the
464 resolution defect in Rap1 clustering at late time points of meiosis by CDK and
465 DDK inactivation (Figure 5A, B), although some clustering persisted under these
466 conditions (Figure 5B). Mps3-DDD-GFP showed NE localisation (Figure 5D) and
467 movement during meiosis even under the conditions of either CDK or DDK
468 inactivation (Figure 5E, F; Videos 19-22), which is similar to that in wild-type
469 Mps3-GFP or Mps3-DDD-GFP cells. Clustering of Mps3 induced by CDK/DDK
470 inactivation was clearly ameliorated by the introduction of Mps3-DDD-GFP
471 (Figure 5C, E; Videos 23-25). DDD substitution also suppressed the motion
472 defects of Mps3 by DDK inactivation (Figure 5F). Importantly, NE localisation and
473 dynamics of Mps3-DDD protein still depend on meiosis, suggesting the presence
474 of additional regulation of meiosis-specific localisation of Mps3. In vegetative cells
475 (0 h, Figure S3E), Mps3-DDD showed SPB localisation and normal Rap1
476 localisation (Figures 5G, S3F). Surprisingly, Mps3-DDD, but not wild-type Mps3,
477 showed dispersed staining on NE with SPB in mitotic cells only in the *cdc7 bob-*
478 *1* mutant with, but not in *cdc7as3* with the inhibitor (Figures 5H, S3G). This
479 suggests that, in contrast to meiosis, DDK negatively regulates the NE

480 localisation of Mps3 in vegetative cells.
481
482 **The *mps3-S189A* and *mps3-S190A* mutants phenocopy the *mps3-AAA***
483 **mutant**
484 Although the *MPS3-DDD* mutation suppressed Mps3 localisation defects induced
485 by CDK or DDK inactivation, Mps3 localisation defects in the *mps3-AAA* mutant
486 (reduced localisation) are different from those under CDK/DDK inactivation
487 (persistent clustering). To clarify the relationship between the phosphorylation of
488 the luminal region of Mps3 with CDK and DDK, we created additional mutants in
489 the Mps3 TSS sequence, called *mps3-S189A and -S190A*, which has a single
490 alanine substitution of S189 and S190, respectively (Figure 6A), which are
491 phosphorylated *in vivo* (Figure 3). If CDK could phosphorylate S190 as the CDK
492 site, *mps3-S190A* would phenocopy the CDK defect in Mps3 localisation. And
493 also, if DDK could phosphorylate S189 as the DDK site, *mps3-S189A* would
494 phenocopy the DDK defect in Mps3 localisation. The localisation of GFP fusion
495 of Mps3-S189A and -S190A proteins was analysed as described above (Figure
496 6B). Both Mps3-S189A and -S190A proteins showed reduced NE localisation
497 compared to wild-type Mps3. In the localisation defects, both mutants were
498 weaker than the *mps3-AAA* mutant (Figure 6D). The *mps3-S189A* mutant was
499 more severe than the *mps3-S190A* mutant. Both *mps3* mutants also showed
500 reduced Mps3 motion (Figure 6E, F) and were also partially deficient in meiosis-
501 induced nuclear expansion (Figure 6G). The *mps3-S190A* mutant exhibited
502 weaker defects than the *mps3-S190A* mutant. This suggests that, although
503 weaker, the *mps3-S189A* and *-S190A* mutants shared similar defects to the
504 *mps3-AAA* mutant and were different from *cdk* or *ddk* mutants. Taken together,
505 these results suggest that CDK and DDK phosphorylation sites are not S189 or
506 S190 (see Discussion).
507
508 **Luminal region of Mps3 binds to lipid bilayers *in vitro***
509 To determine the role of putative phosphorylation of the Mps3 luminal region, we
510 analysed the biophysical properties of a 55 amino acid Mps3 peptide (153-208
511 aa) containing both transmembrane (TM) and luminal regions (Figure 3A). The
512 luminal region is referred to as the juxtamembrane (JM) region (Figure 7A). We

513 prepared two peptides, Mps3-wild type (WT) and Mps3-DDD, which contain three
514 aspartates (Figure 7A). Synthesised Mps3 peptides were mixed with liposomes
515 comprising POPC/POPS (10/3)(Sato, Pallavi, Golebiewska, McLaughlin, & Smith,
516 2006) First, we checked the conformation of each peptide in the bilayers by
517 polarised Fourier-transform infrared spectroscopy (FT-IR) and found that both
518 Mps3-WT and Mps3-DDD peptides contained similar contents of α-helices and
519 β-strands (Figure S4A). Dichroic ratio calculated for Mps3-WT and Mps3-DDD
520 were 2.90 and 2.85, suggesting that both transmembrane regions are inserted
521 into lipid bilayers with a similar tilt angle of ~30° relative to the membrane normal
522 (Tamagaki et al., 2014). We cannot assign those peaks from the β-strand to which
523 residues in the JM regions are involved in the β-structure with this FT-IR
524 experiment alone.

525 Next, we synthesised Mps3 TM-JM peptide with Alexa fluorophore-dye
526 (Alexa-568) at the C-terminus of the JM region and analysed the spectrum of the
527 dye in the presence of different concentrations of negatively charged
528 phosphatidyl-inositol 4,5-bisphosphate (PIP_2), which modulates the status of
529 charge on the membrane surface (Sato et al., 2009). Here, we utilised a PIP_2-
530 involving model system to determine whether JM is affected by the membrane,
531 although we are aware of the fact that it is not known whether Msp3 interacts with
532 PIP_2. This simple model system must provide a possible mechanism of how
533 negative charge on the JM region caused by its phosphorylation affects its
534 behaviour against the membrane.

535 For the wild-type peptide, the addition of PIP_2 decreased the peak intensity
536 of the fluorophore at 600 nm up to ~40% (Figure 7B). The triplicates showed
537 similar results. Decreased fluorescence could be due to quenching of the
538 fluorescence from the dye association (self-quenching) by inter-peptide
539 association (Figure 7C), as shown in a previous study on an ErbB2 peptide
540 containing TM and JM regions (Matsushita et al., 2013). These results suggest
541 that the JM region of the Mps3-WT peptide is sensitive to changes in the
542 membrane component and that the JM regions interact with the acidic lipid bilayer.
543 On the other hand, the Mps3-DDD peptide decreased the intensity to ~20% (half
544 of the intensity from the WT peptide). The decrease in the change in the
545 fluorescence intensity was due to electrostatic repulsion between the aspartates

546 and acidic lipids (Figure 7C). This repulsion must place the fluorophores apart,
547 preventing self-quenching. The repulsion can reduce the JM region of the Mps3-
548 DDD peptide binding to the bilayers compared to that of Mps3-WT. These results
549 suggest the different conformations of Mps3-WT and -DDD peptides with respect
550 to the interaction of the JM region of Mps3 with the membrane.

551 To gain more insight into the behaviour of the JM region with the
552 membrane, we utilised a molecular dynamic (MD) simulation technique. Since
553 there is no reported structure on the TM-JM region of Mps3, we artificially
554 constructed the initial structure of the TM-JM sequence as follows: using the
555 software *Chimaera* (Pettersen et al., 2004), R161-M181 is set to be α-helix as
556 the TM region; the rest was set to a random structure. This constructed structure
557 was embedded in a membrane (POPC/POPS/PIP_2=8:2:1 with 80 nm in XY
558 dimension) using *the Membrane Builder* module in *CHARMM-GUI* (Jo, Kim, Iyer,
559 & Im, 2008). The peptide-membrane system was fully hydrated in a 150 mM NaCl
560 solution.

561 First, we performed coarse-grained molecular dynamics simulations of the
562 constructed TM-JM peptide in the membrane. We ran simulations on two
563 sequences, Mps3-WT and Mps3-DDD. The duration of each run was >3 μs, and
564 three sets were performed for each sequence. The results from all the
565 calculations showed that the JM region from Mps3-DDD dissociated from the
566 membrane (Figure S4B). Conversely, that of Mps3-WT remained attached to the
567 membrane (Figure S4B). Second, we performed all-atom simulations on three
568 TM-JM sequence peptides, Mps3-WT, Mps3-DDD, and Mps3-WT
569 phosphorylated at T188-S189-S190. The duration of each calculation was >50
570 ns (Videos 26-28). In Figure 7D, snapshots from trajectories for the three
571 sequences are shown. Results from the simulation, together with mass density
572 profiles (Figure S4C), indicate that JM regions from Mps3-DDD and
573 phosphorylated Mps3 dissociated from the membrane. On the other hand, the
574 JM region from Mps3-WT remained attached to the membrane.
575
576

Discussion

Here, we describe the multi-layered control of the dynamics of an SUN-domain protein, Mps3, during yeast meiosis. In both mitotic and meiotic cells, Mps3 is a major component of a half-bridge as well as the SPB interacting network (SPIN) (Chen et al., 2019; Lee et al., 2020a) (Li et al., 2017). Meiosis induces the localisation and motion of Mps3 on NE, which is accompanied by the formation of large protein ensembles of the LINC complex in NE, detected as foci/patches. The meiosis-specific LINC complex tethers telomeres and interacts with cytoplasmic actin cables (Lee et al., 2020b). In the luminal region between INM and ONM, Mps3 binds to a KASH protein, Mps2, during both mitosis and meiosis, and to a meiosis-specific Mps2-binding protein, Csm4 (Chen et al., 2019; Lee et al., 2020a). Mps2 and Csm4 cooperate to bind to the motor Myo2 in the cytoplasm. Myo2 on actin cables, whose formation is induced in meiotic cells, generates forces for the motion of the LINC complex on NE, which in turn moves chromosomes inside of meiotic cells (rapid prophase movement; RPM) as well as the clustering of telomeres in the zygotene stage (Conrad et al., 2008; Koszul et al., 2008; Trelles-Sticken et al., 2000; Trelles-Sticken et al., 1999). While Mps3 localisation on NE is independent of actin-generated force (and other meiosis-specific factors, such as Ndj1 and Csm4), Mps3 movement on NE is dependent on the force. Meiosis-specific regulation of Mps3 localisation on NE is a key event in the formation of a LINC complex with Mps3 for chromosome pairing/synapsis and chromosome motion.

Our studies revealed that, in addition to its role in chromosome dynamics, Mps3 localisation in NE is important for NE remodelling during meiosis. The characterisation of the *mps3-AAA* and *mps3-S189A* and *-S190A* mutants revealed that Mps3 localisation to NE (NE remodelling) plays a unique role in NE expansion in meiotic cells. NE-bound Mps3 may regulate NE growth by directly promoting lipid synthesis on NE or by importing lipids from the ER (Sosa Ponce, Moradi-Fard, Zaremberg, & Cobb, 2020).

Localisation of Mps3 on NE in meiotic cells is divided into several distinct steps, which are coupled with distinct regulatory mechanisms (Figures 1A and 8A); 1-At early times in prophase I, in addition to the SPB, a few Mps3 foci appear on NE. 2-With increased numbers of foci, patches of Mps3, large cohesive

610 ensembles, probably with multiple foci, are formed on NE, which shows transient
611 clustering in one area of NE. 3-Multiple foci and patches cover the entire NE. 4-
612 Mps3 shows nearly full coverage of NE, sometimes with a hole at the nucleus-
613 vacuole junction (NVJ). 5-During late prophase I or pro-metaphase I, most Mps3
614 on NE disappear from NE, leaving two SPB-associating foci. At each stage, Mps3
615 foci/patches show RPM which is the maximum at mid/late prophase I (Conrad et
616 al., 2008; Koszul et al., 2008; Trelles-Sticken et al., 2000; Trelles-Sticken et al.,
617 1999).

618 In this study, we found that two cell cycle kinases, CDK and DDK, control
619 proper Mps3 localisation on NE during meiosis, particularly by promoting the
620 resolution of clustered Mps3 foci (and, thus, telomere bouquets), suggesting that
621 the phosphorylation of a target protein(s) by these kinases plays a role in the
622 resolution of Mps3 clusters. Moreover, we showed that the JM region of Mps3
623 near the INM, which is located in the lumen of NEs, is critical for meiosis-specific
624 NE localisation of the protein. S179 and S180 of the JM region in Mps3 are
625 subject to meiosis-specific phosphorylation of Mps3 assembly on NEs. The
626 introduction of negative charges in the JM region changes the binding ability of
627 the region to the membrane in reconstituted liposomes. These results suggest
628 that Mps3 localisation in NE is subject to multi-layered regulation governed by
629 distinct phosphorylation.
630

631 **Do CDK and DDK phosphorylate the lumen of Mps3?**

632 During meiosis of *C. elegans*, the N-terminal nucleoplasmic region of Sun-1
633 protein is phosphorylated by Chk-1 and Cdk-1 (Penkner et al., 2009; Zuela &
634 Gruenbaum, 2016). In M phase of human cells, SUN1 protein is phosphorylated
635 at its N-terminal region by CDK and PLK (Patel et al., 2014). Together with results
636 described here, these suggest the importance of the phosphorylation in the
637 regulation of dynamics and/or regulation of SUN-domain proteins.

638 Our *in vitro* study (Figure 3F) showed that CDK and DDK phosphorylate
639 188-TSS-190 sequence of Mps3 in a collaborative manner, suggesting that CDK
640 and DDK collaborate to catalyze the phosphorylation of the JM region of Mps3
641 for its NE localization *in vivo*. However, this is less likely for several reasons. First,
642 it is unlikely that CDK and DDK are responsible for direct phosphorylation reaction

643 in NE lumen. These kinases are not localized to luminal regions of both
644 endoplasmic reticulum (ER) and NE since the catalytic subunits (Cdc28 and
645 Cdc7) and its regulatory subunits (Clb5,6 and Dbf4) lack a leader sequence
646 necessary for the luminal localization. Second, defects in Mps3 localization
647 induced by CDK/DDK inactivation are different from those in the *mps3-AAA*
648 mutant. Third, if CDK is a priming kinase on S190 in SSP sequence of Mps3, a
649 mutation in the CDK site (S190) could phenocopy the defect conferred by CDK
650 defect. But this is not the case (Figure 6). Forth, if DDK is the secondary kinase
651 on S-pS-P primed by CDK, S189 substitution would confer similar (or less) defect
652 to S190 substitution. However, the *mps3-S189A* is more defective than the *mps3-*
653 *S190A*. Rather, S190 and S189 phosphorylation seem to play an overlapping role
654 in Mps3 localization.
655
656 **What is the target for CDK and DDK in Mps3 localization?**
657 If CDK and DDK are unlikely to directly phosphorylate the luminal region of Mps3,
658 what are the target(s) of these kinases? Our results indicate that CDK and DDK
659 activities promote the resolution of Mps3 foci/patches and/or telomeres rather
660 than NE localisation *per se*. Mps3 localisation on NE seems normal, even in the
661 absence of CDK and DDK activities. Phenotypic similarity between *cdk* and *ddk*
662 mutants in Mps3 localisation suggests a common target of these kinases for the
663 resolution of the clusters. Interestingly, telomere-resolution defects under CDK
664 and DDK inactivation are similar to those in the mutant of the *REC8* gene (Challa,
665 Lee, Shinohara, Kim, & Shinohara, 2016; Conrad et al., 2007), which encodes a
666 meiosis-specific kleisin subunit of the cohesin complex (Klein et al., 1999) and
667 cohesin regulator, *RAD61/WPL1* (Challa et al., 2016). It is well known that Rec8
668 is phosphorylated by DDK (and Polo and CK1 kinases), but not by CDK (Katis et
669 al., 2010) and Rad61/Wpl1 is phosphorylated by DDK and Polo-like kinase (PLK)
670 {Challa, 2019 #221}. Thus, there might be another target in cohesin components
671 by the CDK-DDK kinase axis. In mitotic yeast cells, Eco1, which catalyses the
672 acetylation of the Smc3 cohesin subunit, is phosphorylated by CDK and DDK for
673 degradation (Seoane & Morgan, 2017). Interestingly, Eco1 acetylates Mps3 in
674 vegetative cells (Ghosh et al., 2012). Therefore, Eco1 may be a possible target
675 of CDK-DDK for telomere resolution.

676 Telomere clustering during prophase I persisted with CDK or DDK
677 inactivation, suggesting the presence of a constraint on the resolution of the
678 clustering. The phosphorylation, and thus negative charges in the JM region,
679 might overcome this constraint for resolution. This is supported by the fact that
680 *MPS3-DDD* partially rescued the resolution defect of telomere clustering with
681 CDK or DDK inactivation (Figure 4). Moreover, we found that Mps3-DDD, but not
682 wild-type Mps3, showed NE localisation in mitotic cells only in the *cdc7 bob-1*
683 mutant (Figure 5C), suggesting the presence of DDK-dependent constraints on
684 the NE localisation of Mps3 during mitosis (Figure 8A).
685
686 **Phosphorylation in the NE luminal region of Mps3**
687 Protein phosphorylation of the luminal region between INM and ONM is very
688 unusual and has not been reported in the past. However, recent studies have
689 shown that the phosphorylation of secreted proteins and oligosaccharides of
690 membrane proteins is catalysed by an atypical kinase, such as Family with
691 sequence similarity 20, member C (Fam20C), in the lumen of the Golgi apparatus
692 and ER in vertebrate cells (Tagliabracci et al., 2012; Tagliabracci, Pinna, & Dixon,
693 2013; Tagliabracci et al., 2015). Given that the luminal region of NE is consistent
694 with that of ER, where INM/ONM proteins are synthesised, it is possible that a
695 Fam20C family-like kinase might be present in the lumen between INM and ONM,
696 which may catalyse the phosphorylation of the NE luminal region of Mps3.
697 However, there are no reports on the presence of Fam20C orthologues or relative
698 enzymes in lower eukaryotes, such as yeasts. Alternatively, phosphorylation of
699 the luminal region may be mediated by a novel mechanism. Given that
700 membranes are rich in phospho-lipids, the phosphate group in the lipids might
701 act as a donor for transfer to proteins. Further studies are needed to identify the
702 protein responsible for the phosphorylation of the JM region of Mps3.
703
704 **Th role of negative charges in JM region of Mps3**
705 Biophysical analysis of synthetic peptides containing TM and JM regions of Mps3
706 in synthetic liposomes revealed that the JM region of Mps3 has the capability to
707 bind to the lipid bilayers. Importantly, the introduction of three negative charges
708 into the JM region weakens the binding to the membrane, resulting in a change

709 in the geometry of the JM region of Mps3, as an extension, the luminal region of
710 Mps3, including the SUN domain. As a typical LINC complex, the SUN domain of
711 Mps3 in INM binds to the KASH domain of Msp2 in ONM. The connection spans
712 ~20 nm between the INM and ONM (Chen et al., 2019; Lee et al., 2020a).
713 Recently, Jasperson and her colleagues showed "atypical" configuration of Mps3-
714 Mps2 in SPB, in which not only SUN-KASH interaction, but also N-terminal
715 regions of both Mps3 and Mps2 bind to each other in SPIN which surrounds SPB
716 (Chen et al., 2019; Lee et al., 2020a). Typical LINC could accommodate *trans*-
717 membrane configuration in the luminal region, while atypical LINC would have *a*
718 *cis*-membrane configuration (Figure 8B). This suggests that the luminal regions
719 of Mps3 and Mps2 are quite flexible in accommodating two different SUN-KASH
720 configurations. Our results suggest that the switch from *cis*- to *trans*-membrane
721 configurations of the LINC complex might be regulated by the binding of the JM
722 region of Mps3 to the INM. While the interaction between the JM region of Mps3
723 and INM promotes the *cis*-membrane form of the noncanonical LINC complex,
724 negative charges induced by phosphorylation may reduce the interaction
725 between JM and INM, which may accommodate a conformation suitable for the
726 *trans*-membrane form. Alternatively, the negatively charged JM region of Mps3
727 would be a binding site for the other protein which may promote the configuration
728 switch. This idea should be tested in future studies.
729
730 **Meiosis establishes Mps3 localization on NE**
731 As described above, Mps3 localisation in NE is regulated at distinct stages
732 (Figure 8). The *mps3-AAA* mutant retains the ability to form early meiosis-specific
733 Mps3 foci on NE. The Mps3-DDD protein also requires meiosis for NE localisation
734 during meiosis. This suggests that Mps3 localisation on NE is initiated and/or
735 established during very early meiotic prophase I. Given that Mps3 localisation as
736 an ensemble on NE is specific to meiosis, there might be an early meiosis-specific
737 factor or modification that regulates the establishment of Mps3 localisation on NE
738 upon entry into meiosis. We examined two meiosis-specific factors, Ndj1 and
739 Csm4, both of which are involved in chromosome motion and Mps3 dynamics,
740 but it is unlikely that these factors function in the establishment of Mps3
741 localisation. Further studies are necessary to identify the factors necessary for

the initiation of NE remodelling.

Materials and Methods

Strains and plasmids

All strains described here are derivatives of SK1 diploids and are described in Table S1. *MPS3-FLAG* and *MPS3-GFP* diploid cells showed wild-type spore viability, but both showed ~2 h delay in the entry of meiosis I (Rao et al., 2011). *RAP1-GFP* and *NDJ1-HA* diploids showed wild-type spore viability and normal progression of meiosis (Kosaka et al., 2008; Rao et al., 2011; Trelles-Sticken et al., 1999). GST fusion genes of a PCR-amplified Mps3 fragment (160–260 amino acid residues of the Mps3 protein) were constructed on pGEX-2T (Cytiva, 28954653).

Anti-serum and antibodies

Anti-HA antibody (16B12; Babco), anti-Flag (M2, Sigma), anti-tubulin (MCA77G, Bio-Rad/Serotec, Ltd), anti-GFP (3E6; Molecular Probes), and rabbit anti-Dmc1 (M. Shinohara, Gasior, Bishop, & Shinohara, 2000) were used for staining. Anti-Zip1 (rabbit) and anti-Red1 (chicken) were described previously (M. Shinohara, Oh, Hunter, & Shinohara, 2008). Rabbit anti-Rec8 antibody was described previously (Rao et al., 2011). Guiana pig anti-Hop1 serum was described in (Bani Ismail, Shinohara, & Shinohara, 2014). The second antibodies for staining were Alexa-488 (Goat) and -594 (Goat) IgG used at a 1/2000 dilution (Themo Fishers).

10 amino acid of Mps3 peptide with 189-phosphoserine and 190-phosphoserine (186-GAT**pSpS**PGKSF-195) was synthesized and used for the immunization of rabbit. From obtained serum, antibody specific phosphor-peptide was affinity-purified using the phosphor-peptide (MBL Co. Ltd. Japan).

Cytology

Time-lapse images of Mps3-GFP, Rap1-GFP and Zip1-GFP were captured using a computer-assisted fluorescence microscope system (DeltaVision; Applied Precision). The objective lens was an oil immersion lens (100×; NA, 1.35). Image deconvolution was performed using an image workstation (SoftWorks; Applied Precision). Time-lapse image acquisition was performed every 0.5 to 1 s at a single focal plane. Meiotic cell cultures (~150μl) were placed on bottom-glass

777 dishes (Matsunami) pre-coated with Concavalin A (10 mg/ml, Sigma).
778 Tracking of Zip1-GFP/Mps3-GFP/Rap1-GFP was analyzed using the
779 ImageJ program (NIH) and plug-in MTrack2 (Nico Shuuman). Tracking and
780 velocity analysis of Mps3-GFP/Rap1-GFP were analyzed using Imaris software
781 (Oxford Instrument). The sectioned area of each nucleus with Rap1-GFP, which
782 showed nuclear and dotty staining, was measured using the Velocity™ program
783 (Applied Precision) with manual assignment of the rim of the sectioned area.
784 Ninety-six nuclei were counted.
785
786 **IP and Western blotting**
787 Yeast cell lysates were prepared by the glass bead disruption method. The
788 lysates were incubated with magnetic beads (Dynal M260; GE Healthcare)
789 coated with anti-Flag antibody (M2, Sigma) for 12 h and washed extensively
790 (Sasanuma et al., 2013). Bound proteins were eluted by adding the SDS sample
791 buffer and were analyzed on an SDS-PAGE gel, transferred to a nylon membrane
792 (Millipore Co. Ltd), and probed with specific antibodies.
793
794 ***In vitro* kinase assay**
795 For *in vitro* phosphorylation, 1 μg of purified GST-Mps3 or GST-Mps3-AAA was
796 incubated with γ-^{32}P-ATP (100 μM; 2.2 MBq) in the presence of CDK (~0.2μg)
797 and/or DDK (~0.2μg) in a 20 μl of buffer [20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$,
798 10 mM β-glycerophosphate]. After 1-h incubation at 30°C, the reaction was
799 stopped by adding 5× SDS-PAGE sample buffer, and the products were analyzed
800 on 10% SDS-PAGE gel. Dried gels were analyzed using a phosphorimager BAS
801 2000 (Fuji Co. Ltd).
802
803 **Synthesis of Mps3 peptides**
804 Peptides with the TM and JM region of Mps3 (residue 153-208) were synthesized
805 by solid-phase method with the following sequence:
806 GEAKKLKWRTYIFYGGLFFVFYFFGSFLMTTVKNNDLESHSSGA**TSS**PGKSFS
807 NL-Cys and
808 GEAKKLKWRTYIFYGGLFFVFYFFGSFLMTTVKNNDLESHSSGA**DDD**PGKSF
809 SNL-Cys (underlines indicate TM region). The synthetic peptides were purified

by reverse-phase HPLC on a C4 column with gradient of 2-propanol over water containing 0.1% trifluoroacetic acid. For fluorescent-labeling, Alexa Fluor 568 C5-maleimide (Molecular probe) was introduced to the sulfide group on cysteine by mixing the peptides and the fluorescent dye in dimethylformamide under basic conditions.

Preparation of lipid bilayer with peptides

The Mps3 peptides were co-solubilized with 1-palmitoyl-2-oleoyl-*sn*-phosphatidycholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-phosphatidyserine (POPS) and octyl-β-glucoside in trifluoroethanol (TFE). The peptide to lipid molar ratio was 1:100. The lipid concentration was 200 μM. The ratio of POPC and POPS was 10:3. The mixture was incubated for 90 min at 37°C. Then TFE was removed under stream of Ar. MES buffer (10 mM MES, 50 mM NaCl, and 5 mM DTT, pH 6.2) was added to the solid from the previous step and mixed at 37 °C for 6 h. The octyl- β -glucoside was removed by dialysis against the MES buffer.

Measurement of FT-IR

Polarized attenuated total reflection FT-IR spectra were obtained on a JASCO FT/IR-4700 spectrometer. Membranes containing Mps3 TM–JM peptides were layered on a germanium internal reflection element using a slow flow of nitrogen gas directed at an oblique angle to the IR plate to form an oriented multilamellar lipid–peptide film.1000 scans were acquired and averaged for each sample at a resolution of 4 cm^{-1}. The absorption of polarized light by the amide I bond yields the dichroic ratio defined as a ratio of absorption intensity for parallel, relative to perpendicular, polarized light. From the dichroic ratio, we estimated the tilt angle of the TM helix relative to the membrane normal based on the method described by Smith and coworkers (Liu, Eilers, Patel, & Smith, 2004; Smith et al., 2002)) using a value of 41.8° for angle α between the helix director and the transition-dipole moment of the amide I vibrational mode. For the FT-IR experiment, the amount of lipid used per experiment was ~4 mg. The film on the ATR plate in our experiment was assumed to be greater than ~10 μm. For calculating the dichroic ratio, the thick film limit is applicable (Bechinger,

Ruysschaert, & Goormaghtigh, 1999). Equations that we used for the calculation of the dichroic ratios were based on this assumption.

Fluorescence spectroscopy

For experiments with PIP_2, TM-JM peptides were inserted into POPC/POPS membrane as explained above. The peptide/lipid ratio was set to 1:1000 for observing the fluorescence from Alexa568. The lipid concentration was 200–250 μM in MOPS buffer (1 mM MOPS, 0.1 M KCl, pH 7.0). Vesicles were formed by extrusion of multilamellar vesicles through 200-nm polycarbonate filters. PIP_2 were added into the membranes by addition of PIP_2 micelles to the vesicle solution. Fluorescence was measured within 1 hour to minimize PIP_2 hydrolysis. The PIP_2 concentration was from 5 μM to 25 μM. Fluorescence measurement was carried out on HITACHI F-2500 fluorescence spectrophotometer at the excitation wavelength of 568 nm.

Coarse-grained MD simulation

All simulations were performed using GROMACS 2016 package (Abraham, 2015) using the MARTINI force field with polarizable water (Marrink, Risselada, Yefimov, Tieleman, & de Vries, 2007). Periodic boundary conditions were applied in all directions, and the time step was 20 fs. The temperature was controlled with the Berendsen temperature coupling scheme (Berendsen, 1984) with a time constant of 1 ps, and the pressure was controlled using the Parrinello–Rahman semi-isotropic barostat (Parrinello, 1981) with a time constant of 12 ps and a compressibility of 3×10^{-4} bar $^{-1}$. All the simulations were carried out in the isothermal–isobaric (NPT) ensemble at a temperature of 303 K and pressure of 1.0 bar. The cut-off of the Van der waals interactions were set to 1.1 nm.

All atom MD simulation

The NAMD software package (Phillips et al., 2005) was used to perform all-atom MD simulations. The CHARMM36m force field (Huang et al., 2017) together with TIP3P water model were used for all simulations. All simulations in NPT dynamics (constant particle number, pressure and temperature) were carried out at 303 K with Langevin dynamics, and at 1 atm maintained by Nosè-Hoover Langevin

piston method. Full electrostatic interactions were treated by the particle mesh Ewald (PME) approach with a grid spacing of less than 1 Å. The cut-off radii of long-range electrostatic and van der Waals interactions were set to be 12 Å, with a smoothing function applied from 10 Å.

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Competing interest

The authors declare no competing financial interest.

Author contributions

H.B.D.P.R., T.S., K.C., M.S. and A.S. designed the experiments. H.B.D.P.R., T.S., K.C., M.S. and A.S. carried out experiments. T.S. performed liposome experiments and MD simulation. M.S. provided materials. H.B.D.P.R., T.S., K.C., Y.F., and A.S. analyzed the data. A.S. wrote the manuscript with inputs from H.B.D.P.R., T.S., K.C. and M.S.

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1240 **Figure legends**

1241

1242 **Figure 1. Meiotic Mps3 localization and movement on NEs**

1243 (A) Mps3-GFP localization was analyzed in wild-type cells (PRY64) at various
1244 time points during meiosis. Representative images at each time are shown.
1245 Bottom color bars indicate classes of Mps3-localization in (B). Bar indicates
1246 2 μm.

1247 (B) Based on Mps3-GFP localization, cells with Mps3-GFP were classified into
1248 four classes (A) and quantified: single Mps3 focus (blue), 2–5 foci (green),
1249 more than 5 foci/patches (orange), and coverage of the Mps3 signal on NE
1250 (red). At each time point, more than 100 nuclei were counted. The graphs are
1251 a representative of two independent time courses.

1252 (C) Time-lapse analysis of Mps3-GFP in the wild-type strain (PRY64) at different
1253 time points in meiosis. A single focal plane of a cell every 3 sec is shown. See
1254 Videos 1-4.

1255 (D) Time-lapse analysis of Mps3-GFP in various strains at different time points in
1256 meiosis. A single focal plane of a cell was analyzed every 3 sec. The inhibitor
1257 LatB (30 μM) was added at 0 h. See supplemental movie S5. While washing,
1258 the inhibitor was washed at 5 h, and the cells were analyzed at 6 h. Wild type,
1259 PRY64; *ndj1*, PRY192; *csm4*, PRY198.

1260 (E) Time-lapse analysis of Mps3-GFP in the *cdc28-as1* mutant (PRY71) treated
1261 with or without the inhibitor 1NM-PP1 (0.5 μM) at 5 h during meiosis. The
1262 inhibitor was added at 0 h (second panels) or 3 h (third panels). While
1263 washing (fourth panels), the inhibitor was added at 0 h and washed at 3 h,
1264 and the cells were analyzed at 5 h. Time-lapse analysis of Mps3-GFP in the
1265 *cdc7-as3* mutant (PRY260) treated with or without the inhibitor PP1 (15 μM).
1266 The *cdc7 bob1-1* mutant (PRY115) was analyzed at 6 h. See Videos 6-8.

1267 (F) Tracking of Mps3-GFP in wild type, *cdc28-as1* (PRY71 with the inhibitor),
1268 *cdc7-as3* (PRY260with the inhibitor), and *cdc7 bob1-1* (PRY115). Tracking
1269 was monitored for all Mps3 foci/patches in a single cell for 20 sec at 4 or 5 h
1270 in SPM. Each line represents tracking of the foci at a single focal plane.

1271 (G) Percentages of cells with different classes of Mps3-GFP were quantified at 5
1272 h under different conditions (triplicates, Error bars show standard deviation;

1273 s.d.); single Mps3 focus (blue), 2–5 foci (green), more than 5 foci/patches
1274 (orange), and coverage of the Mps3 signal on NE (red).
1275 (H) Velocity of Mps3-GFP foci or patches was quantified. Time-lapse images
1276 were taken for every second in 20 seconds. Mps3-GFP foci or patches were
1277 identified as shown in Methods and followed for their tracks. For each track,
1278 an average velocity and maximum velocity were calculated. More than 20
1279 cells were analyzed for the quantification. Red lines show mean with s.d.. *P*-
1280 values were calculated using Man-Whitney's *U*-test.
1281
1282 **Figure 2. Meiotic telomere movement in *cdk* and *ddk* mutants**
1283 (A) Time-lapse analysis of Rap1-GFP in wild type (HKY167) at different time
1284 points during meiosis. An image of a single focal plane of each mutant cell
1285 was taken every 3 sec. Clustering of telomeres is shown by yellow arrows.
1286 Bar indicates 2 μm. See Videos 9, 10.
1287 (B) Tracking of Rap1-GFP in wild type (0 and 4h), *cdc28-as1* (with the inhibitor),
1288 and *cdc7-as3* (with the inhibitor). Tracking was monitored for all Rap1 foci in
1289 a single cell for 20 sec. Each line represents tracking of the center of foci at a
1290 single focal plane.
1291 (C) Velocity of Rap1-GFP foci or patches were quantified. Time-lapse images
1292 were taken for every second in 20 seconds. Rap1-GFP foci or patches were
1293 identified as shown in Methods and followed for their tracks. For each track,
1294 an average velocity and maximum velocity were calculated. More than 20
1295 cells were analyzed for the quantification. Red lines show mean with s.d.. *P*-
1296 values were calculated using Man-Whitney's *U*-test.
1297 (D) Kinetics of telomere clustering during meiosis were quantified. At each time
1298 point, more than 150 nuclei were counted for clustering of telomeres (arrows
1299 in A, E, F). The graphs are a representative of two independent time course.
1300 (left) *cdc28-as1* with 1NM-PP1, blue closed circles; *cdc28-as1* without 1NM-
1301 PP1, blue closed circles; *cdc7-as3* with PP1, red closed circles; *cdc7-as3*
1302 without PP1, red closed circles. (right) wild type, blue closed circles; *cdc7*
1303 *bob1-1*, red closed circles.
1304 (E) Time-lapse analysis of Rap1-GFP in various strains (*cdc28-as1*, PRY68) at
1305 different time points during meiosis. An image of a single focal plane of each

1306 mutant cell was taken every 3 sec. The inhibitors (final 0.5 μM of 1NM-PP1)
1307 were added at 0 h (second panels) or 3 h (third panels). While washing
1308 (bottom panels), the inhibitor was added at 0 h and washed at 3 h, and the
1309 cells were analyzed at 5 h. Clustering of telomeres is shown by yellow arrows.
1310 Bar indicates 2 μm. See Video 11.
1311 (F) Time-lapse analysis of Rap1-GFP in various strains (*cdc7-as3,* PRY79; *cdc7*
1312 *bob1-1* PRY116) at different time points during meiosis. An image of a single
1313 focal plane of each mutant cell was taken every 3 sec. For the *cdc7-as3*
1314 mutant, the inhibitors (final 15 μM PP1, respectively) were added at 0 h
1315 (second panels) or 3 h (third panels). While washing (bottom panels), the
1316 inhibitor was added at 0 h and washed at 3 h, and the cells were analyzed at
1317 5 h. Clustering of telomeres is shown by yellow arrows. Bar indicates 2 μm.
1318 See Videos 12, 13.
1319
1320 **Figure 3. Mps3 is phosphorylated in luminal region**
1321 (A) Schematic representation of the Mps3 protein. The putative transmembrane
1322 (TM) region (dark green) and SUN domain (green) are shown with three
1323 known phosphorylation sites (red circles) in nucleoplasmic region (Lanz et al.,
1324 2021). The position of a CDK consensus site (S/TP) is shown in red lines with
1325 aa sequence on the bottom. All of the six sites are located in a luminal region
1326 of Mps3.
1327 (B) Western blotting of the Mps3-Flag protein during meiosis. Wild-type (left,
1328 HKY404), *mps3-AAA* mutant (middle, PRY163) and *MPS3-MER2S* mutant
1329 proteins (right, PRY514) were analyzed at different time points. Fractions
1330 immunoprecipitated with anti-Flag antibody were probed with anti-Flag
1331 antibody (top) as well as a phospho-specific antibody (bottom) that
1332 recognized T189 and S190 phosphorylation.
1333 (C) *In vivo* phosphorylation was confirmed by a decreased band shift of Mps3-
1334 Flag protein purified from meiotic cell lysates (4 h incubation with SPM) using
1335 the anti-Flag antibody. After immuno-precipitation, the precipitate was
1336 incubated with 1 unit of CIP for one hour either in the presence or absence
1337 of phosphatase inhibitors. Mps3-Flag was detected using anti-Flag (M2)
1338 antibody.

1339 (D) Affinity-purified-Mps3-Flag from meiotic cell lysates (4 h incubation with SPM)
1340 using anti-Flag column was treated with CIP as described in (A) and was
1341 probed with anti-Phospho-Serine antibody (Qiagen). During incubation, Mps3
1342 protein was degraded extensively (bottom).
1343 (E) Sequence homology of an Mps3 region with a CDK/DDK phosphorylation site
1344 of the Mer2 protein. Identical amino acids are shown in red. Putative CDK or
1345 DDK phosphorylation sites are shown in orange. Unique amino acid
1346 sequence in Mer2 is shown in pale blue. The substitutions (*mps3-AAA,*
1347 *MPS3-DDD*, and *MPS3-MER2S*) for the putative phosphorylation sites of
1348 Mps3 are shown on the top.
1349 (F) *In vitro* phosphorylation analysis of GST-Mps3 fusion proteins. Purified wild-
1350 type GST-Mps3 and GST-Mps3-AAA fragments (shown on the left by
1351 Coomassie staining) were incubated with different combinations of purified
1352 the Cdc28–Clb5 complex and/or Cdc7–Dbf4 kinase complexes in the
1353 presence of γ-^{32}P-ATP. After 1-h incubation, proteins were fractionated on a
1354 SDS-PAGE gel and analyzed using a phosphorimager for incorporation of ^{32}P
1355 in the Mps3 fragments (right bottom) and Cdc28/Cdc7 protein (right top).
1356 Cdc7 was phosphorylated by CDK. Quantification is shown below the
1357 autoradiogram.
1358 (G) Western blotting of Mps3 and tubulin at various times in meiosis. Cell lysates
1359 from the *MPS3-Flag* (right, HKY404) and *MPS3-AAA-Flag* (left, PRY163)
1360 strains were analyzed by western blotting using anti-Flag and anti-tubulin
1361 antibodies. The wild-type Mps3 protein shows band shift during meiosis for
1362 example at 4 and 6 h.
1363 (H) Reactivity of wild-type Mps3-Flag and Mps3-AAA mutant protein to anti-
1364 phospho-serine antibody was examined by Western blotting. wild type Mps3-
1365 Flag and Mps3-AAA mutant protein at each time point were IPed and probed
1366 with the antibody. While wild type Mps3-Flag shows an increase reactivity to
1367 the antibody, Mps3-AAA-Flag did not increase the reactivity in meiosis (4 h)
1368 compared to mitosis (0 h).
1369 (I) Western blotting of the Mps3-Flag protein during meiosis. Wild-type (left,
1370 HKY404) and *MPS3-MER2S* proteins (right, PRY514) were analyzed at
1371 different time points. Fractions immunoprecipitated with anti-Flag antibody

1372 were probed with anti-Flag and anti-Mer2-phospho-S30 antibodies.
1373 (J) Rap1-GFP dynamics at 4 h of meiosis analyzed in *MPS3-MER2s* (PRY518)
1374 and wild-type (HKY167) strains.
1375 (K) Kinetics of Rap1 clustering in each strain is shown in G. Counting was
1376 performed as described in Figure 2E. Blue, wild type; red, *MPS3-MER2S.*
1377 (L) Localization of Ndj1-HA (green), Mps3-Mer2S-Flag (red), and DAPI (dark
1378 blue) was analyzed by whole cell staining of PRY514. Bar indicates 2 μm.
1379
1380 **Figure 4. The *mps3-AAA* mutant impairs telomere and NE movements**
1381 (A) Meiosis division was analyzed by DAPI staining for various strains. More than
1382 100 cells were counted for divisions. The graphs are a representative of two
1383 independent time course. Spore viability of each strain is shown in
1384 parenthesis. At least 100 asci were dissected for each strain.
1385 (B) Time-lapse analysis of Rap1-GFP in the wild-type (HKY167) and *mps3-AAA*
1386 (PRY138) strains at different time points in meiosis. A single focal plane of a
1387 cell was analyzed every 3 s. A tethering defect was observed in the *mps3-*
1388 *AAA* strains. Bar indicates 2 μm. See supplemental movie S14 for *mps3-AAA*.
1389 (C) Tracking of Rap1-GFP in wild type and *mps3-AAA* for 20 sec. See Methods
1390 in Figure 2B.
1391 (D) Velocity of Rap1-GFP foci or patches were quantified. See Methods in Figure
1392 2C. Red lines show mean with s.d.. *P*-values were calculated using Man-
1393 Whitney's *U*-test.
1394 (E) Time-lapse analysis of Zip1-GFP in wild type (SEY672) and *mps3-AAA*
1395 (PRY332). *mps3-AAA.* See Videos 15, 16.
1396 (F) Tracing of Zip1-GFP shows a step size of each Zip1-GFP line. Step size per
1397 given time is converted into the relative velocity of chromosomes. *n*=35. Blue
1398 bars; wild type; red bars, *mps3-AAA.*
1399 (G) Localization of wild-type Mps3-GFP (PRY64) and Mps3-AAA-GFP (PRY186)
1400 proteins in a cell at different time points in meiosis.
1401 (H) Kinetics of Mps3 distribution. Based on Mps3-GFP morphology, cells with
1402 Mps3-GFP were classified into four classes and quantified: 2–5 foci (green),
1403 more than 5 foci/patches (orange), and coverage on NE (red). See Figure 1B
1404 for quantification.

1405 (I) Percentages of cells with different classes of Mps3-GFP were quantified at 5
1406 h in different conditions (triplicates, Error bars show standard deviation; s.d.);
1407 single Mps3 focus (blue), 2–5 foci (green), more than 5 foci/patches (orange),
1408 and coverage of the Mps3 signal on NE (red).
1409 (J) Time-lapse analysis of wild type and Mps3-AAA-GFP during meiosis. See
1410 Video 17 for Mps3-AAA-GFP.
1411 (K) Tracking of Mps3-GFP in wild type and *mps3-AAA* for 20 sec. See Figure 1F
1412 for quantification.
1413 (L) Velocity of Mps3-GFP foci or patches were quantified. Methods are shown in
1414 Figure 1H. More than 20 cells were analyzed for the quantification. Red lines
1415 show mean with s.d.. *P*-values were calculated using Man-Whitney's *U*-test.
1416 (M) The maximum sectional area of each nucleus was measured using the
1417 Velocity program. Each area of the 96 nuclei is ranked in the figure. Graphs
1418 for wild type and *mps3-AAA* at 0 and 4 h as well as wild type treated with LatB
1419 are shown.
1420
1421 **Figure 5. Acid amino acids in lumen of Mps3 allele can suppress CDK and**
1422 **DDK defects**
1423 (A) Time-lapse analysis of Rap1-GFP in a phosphomimetic allele of *MPS3*,
1424 *MPS3-DDD* (PRY211), with a different combination of *cdc28-as1* (PRY211),
1425 *cdc7-as3* (PRY301), and *cdc7 bob1-1* (PRY272) mutations in meiosis. A
1426 single focal plane of a cell was analyzed at every 3 s. Bar indicates 2 μm. See
1427 Videos 19-22.
1428 (B) Kinetics of clustering of telomere as Rap1-clusters was studied in *cdc7-as3*
1429 (left, PRY309) and *cdc28-as1* (right, PRY303) in the presence of its specific
1430 inhibitors. Wild type (blue, HKY167) and *MPS3-DDD* (PRY236) with the allele
1431 was used.
1432 (C) Time-lapse analysis of Mps3-DDD-GFP with different mutant alleles and in
1433 treatment with a specific inhibitor at different time points in meiosis. See
1434 Videos 18, 23-25.
1435 (D) Kinetics of Mps3. Based on Mps3-GFP morphology, cells with Mps3-GFP
1436 were classified into four classes and quantified: single Mps3 focus (blue), 2–
1437 3 foci (green), 4–5 foci (orange), and more than 5 foci (red). See Figure 2B

1438 for quantification. Graphs for wild type, *cdc28-as1*, and *cdc7-as3* cells are the
1439 same as in Figure 1B.
1440 (E) Tracking of Mps3-GFP in *mps3-DDD*, *cdc28-as1 mps3-DDD* (with 1NM-PP1),
1441 *cdc7-as3 mps3-DDD* (with PP1), and *cdc7 bob1-1mps3-DDD* for 20 sec.
1442 (F) Velocity of Mps3-GFP foci or patches were quantified. Methods are shown in
1443 Figure 1H. More than 20 cells were analyzed for the quantification. Red lines
1444 show mean with s.d.. *P*-values were calculated using Man-Whitney's *U*-test.
1445 (G) Time-lapse analysis of Mps3-DDD-GFP with different mutant alleles during
1446 vegetative growth.
1447
1448 **Figure 6. Biophysical analysis of JM region of Mps3**
1449 (A) Amino acid sequences of Mps3 with amino acid substitution in the *mps3-*
1450 *S189A* and *mps3-S190A* mutants.
1451 (B) Localization of wild-type Mps3-GFP (PRY64), Mps3-S189A-GFP
1452 (KSY407/409) Mps3-S190A-GFP (KSY220/221) and proteins in a cell at
1453 different time points in meiosis.
1454 (C) Kinetics of Mps3 distribution. Based on Mps3-GFP morphology, cells with
1455 Mps3-GFP were classified into four classes and quantified: 2–5 foci (green),
1456 more than 5 foci/patches (orange), and coverage on NE (red). See Figure 1B
1457 for quantification.
1458 (D) Percentage of cells with different classes of Mps3-GFP were quantified at 5 h
1459 in different conditions (triplicates, Error bars show standard deviation; s.d.);
1460 single Mps3 focus (blue), 2–5 foci (green), more than 5 foci/patches (orange),
1461 and coverage of the Mps3 signal on NE (red).
1462 (E) Tracking of Mps3-GFP in wild type (left), *mps3-S189A* (middle) and *mps3-*
1463 *S190A* (right).
1464 (F) Velocity of Mps3-GFP foci or patches were quantified in different *mps3*
1465 mutants. Methods are shown in Figure 1H. More than 20 cells were analyzed
1466 for the quantification. Red lines show mean with s.d.. *P*-values were
1467 calculated using Man-Whitney's *U*-test.
1468 (G) The maximum sectional area of each nucleus was measured using the
1469 Velocity program. Each area of the 96 nuclei is ranked in the figure.
1470

1471 **Figure 7. Biophysical analysis of JM region of Mps3**

1472 (A) Amino acid sequences of Mps3 peptides used. Amino acid sequence in
1473 transmembrane region is shown in green. Phosphorylated residues TSS and
1474 its derivative DDD are shown in blue and red, respectively.

1475 (B) Fluorescence spectra from a synthetic liposome containing Alexa568-labelled
1476 WT-Mps3 (left) peptide and Mps3-DDD peptide (right) were measured in the
1477 presence of various concentrations of PIP_2. The graphs are a representative
1478 of three independent time course.

1479 (C) Schematic illustration of the binding of Mps3-WT peptide (left) and Mps3-DDD
1480 peptide (right) to lipid bilayer with PIP_2 (red). DDD residues may induce
1481 electric repulsion between the peptide and the membrane. Transmembrane
1482 region of the Mps3 peptide is shown by blue a-helix. Peptides with WT JM
1483 region and with DDD substitution are shown blue and red lines, respectively.
1484 Purple and black stars show normal and quenched fluorophores, respectively.

1485 (D) Snapshots from all-atom MD simulation trajectories for Mps3-WT peptide (left),
1486 Mps3-DDD peptide (center) and Mps3-WT peptide with phosphorylation at
1487 TSS sequence (right). See Videos 26-28.

1488

1489 **Figure 8. A model of regulation of Mps3 on NE during meiosis**

1490 (A) Multiple phosphorylation regulates Mps3 localisation and motion in NE during
1491 meiosis. In mitotic cells, Mps3 is mainly located in the SPB. In some strain,
1492 disperse weak Mps3 signal is detected on NE, which might be regulated by
1493 DDK. Upon entry into meiosis, Mps3 forms a few foci on NE in early prophase
1494 I, and phosphorylation of the luminal region of Mps3 and, probably DDK,
1495 promotes localisation of more Mps3 proteins as focus/patch on NE. Mps3
1496 foci/patches transiently form a cluster of some Mps3 foci by an unknown
1497 mechanism, and CDK and DDK and Rec8-cohesin promote dissociation of
1498 Mps3 and Rap1(telomere) clusters. It is likely that positive feedback on Mps3
1499 phosphorylation would increase the localisation of Mps3 on NEs, which
1500 results in full NE coverage of Mps3.

1501 (B) A hypothetical model of the formation of Mps3-containing LINC complex.
1502 During mitosis, Mps3 forms a non-canonical LINC complex with *cis*-
1503 membrane interactions with Mps2 as a component of the half-bridge and

SPIN (left). Upon induction of meiosis, unknown factors promote the formation of Mps3 foci on NE. Then, the JM region of Mps3 is subject to non-canonical phosphorylation, which might in turn weaken the binding of JM to INM, probably by electric repulsion between the JM regions with negative charges and acid lipids. This process promotes the formation of the canonical LINC complex with the *trans*-membrane configuration, in which the Mps3 SUN domain binds to the KASH domain of Mps2 and Csm4. Csm4 may promote structural changes in the luminal region of Mps2. The N-terminal region of Mps3, which is located in the nucleoplasm, binds to a telomere-binding protein, Ndj1. During middle and late prophase I, Mps3 forms a large protein ensemble on the NE, which is seen as a patch.

1516 **Supplemental Figures**

1517

1518 **Figure S1. Meiotic movements of Mps3 and Rap1 in the presence of the**
1519 **inhibitors**

1520 (A) Tracking of Mps3-GFP in wild type. Tracking was monitored for all Mps3
1521 foci/patches in a single cell (PRY64) for 120 sec. Each line with different colors,
1522 representing a single focus/patch, show tracking of the objects at a single
1523 focal plane.

1524 (B) Tracking of Mps3-GFP in wild type. Tracking was monitored for all Mps3
1525 foci/patches in a single cell (PRY64) for 20 sec. See (A).

1526 (C) Cytoplasmic actin cables were visualized by Phalloidin (red) and DAPI
1527 (purple) staining of *cdc28-as1* (PRY68) cells after 5 h of incubation in SPM
1528 both without (left) or with (middle) its inhibitor 1NM-PP1 from 0 h. As a control,
1529 the mutant cells were treated with 30 μM of latrunculin B (LatB) at 4 h and
1530 analysed for actin staining at 5 h (right). Bar indicates 2 μm.

1531 (D) Velocity of Mps3-GFP foci or patches were quantified. Methods are shown in
1532 Figure 1H. More than 20 cells were analyzed for the quantification. Red lines
1533 show mean with s.d.. *P*-values were calculated using Man-Whitney's *U*-test.

1534 (E) Western blotting analysis of Mps3-Flag protein. Cell lysates from the *cdc28-*
1535 *as1* (PRY13) cells at different times in meiosis. The *cdc28-as1* cells treated
1536 with or without 1NM-PP1 from 0 h. Tubulin is a control.

1537 (F) To check the off-target of two inhibitors, 1NM-PP1 and PP1, wild-type diploid
1538 cells (HKY404) with Mps3-GFP were grown in the presence of each inhibitor
1539 (0.5 μM of 1NM-PP1, top panels; 15 μM PP1, bottom panels). The inhibitors
1540 were added at 0 h (second panels) and time-lapse images were examined in
1541 5 h-incubation with SPM. The two inhibitors did not affect rapid movement of
1542 Mps3-GFP foci on NEs. Bar indicates 2 μm.

1543 (G) Western blotting analysis of Mps3-Flag protein. Cell lysates from the *cdc7-*
1544 *as3* (PRY72) cells at 0 and 5 h in meiosis. The *cdc7-as3* cells treated with or
1545 without PP1 from 0 h and collected at 5h. Tubulin is a control.

1546 (H) Whole-cell staining analysis of Mps3-Flag and Ndj1. Various cells were
1547 stained with anti-Flag for Mps3-Flag (red) and anti-HA for Ndj1 (green) at the
1548 nuclear periphery at indicated times. *cdc28-as1* (PRY13) and *cdc7-as3*

1549 (PRY72) cells were treated with their inhibitor from 0 h and examined at the
1550 indicated times. Wild type, HKY404.
1551 (I) To check the off-target of two inhibitors, 1NM-PP1 and PP1, wild-type diploid
1552 cells (HKY167) with Rap1-GFP were grown in the presence of each inhibitor
1553 (0.5 μM of 1NM-PP1, top panels; 15 μM PP1, bottom panels). The inhibitors
1554 were added at 0 h (second panels) and time-lapse images were examined in
1555 5 h-incubation with SPM. The two inhibitors did not affect rapid movement of
1556 Rap1-GFP foci on NEs. Bar indicates 2 μm.
1557
1558 **Figure S2. CDK and DDK are necessary for Mps3 localization on NE**
1559 (A) Image of a part of a tetrad dissection plate of spores from *MPS3-MER2S-Flag*
1560 diploid cells. After the dissection, the plate was incubated at 30C for two days.
1561 (B) Whole cell staining of Mps3-Flag and Tubulin. Wild type (MSY832/833) and
1562 the *mps3-AAA* mutant (PRY122) cells in mitosis were stained with anti-Flag
1563 (red) and anti-tubulin (green). Representative images with short spindles with
1564 two Mps3 foci are shown. Bar indicates 2 μm.
1565 (C) FACS analysis of DNA contents in wild type and the *mps3-AAA* mutant cells
1566 at indicated times. Cells at each time point were fixed and stained with
1567 propidium iodide (PI) and analyzed by FACS after the sonication.
1568 (D) Immunostaining analysis of meiotic chromosome proteins. For SC formation,
1569 Zip1 localization (red) with DAPI (blue) image is also analyzed for wild type
1570 (top) and *mps3-AAA* mutant strains (bottom) at 4 h.
1571 (E) Kinetics of SC formation. SC elongation was analyzed by staining Zip1 protein.
1572 Class I (blue) contains Zip1 dots, Class II (green) contains partial lines of Zip1,
1573 and Class III (red) contains fully elongated Zip1 lines. At each time point, more
1574 than 50 nuclei were counted. The graphs are a representative of two
1575 independent time course.
1576 (F) Interaction of Mps3-Flag with Ndj1-HA and Csm4 in the *mps3-AAA* mutant
1577 was confirmed by immuno-precipitation using anti-Flag antibodies. IPed
1578 fractions of cells with Mps3-Flag (left, HKY404) or Mps3-AAA-Flag (right,
1579 PRY163) at each time point were probed with anti-Flag (top), anti-HA (middle)
1580 and anti-Csm4 antibodies.
1581

1582 **Figure S3. Meiotic phenotypes of *MPS3-DDD* mutants**

1583 (A) Whole cell staining of Mps3-Flag and Tubulin. Wild type (MSY832/833) and
1584 the *mps3-DDD* mutant (PRY122) cells during vegetative growth were stained
1585 with anti-Flag (red) and anti-tubulin (green). Representative images with short
1586 spindles with two Mps3 foci are shown. Bar indicates 2 μm.

1587 (B) Different classes of tubulin staining (red) in vegetative yeast cells.

1588 (C) Cells with different tubulin classes (B) were counted (n=30) and percentages
1589 of each class in wild type and the *MPS3-DDD* strains (PRY201) are shown
1590 (n=3). Error bars show s.d.

1591 (D) Meiosis I division was analyzed by DAPI staining for wild type and the *MPS3-*
1592 *DDD* strains (PRY201). At each time point, more than 100 nuclei were
1593 counted. The graphs are a representative of two independent time course.

1594 (E) Localization of Mps3-DDD-GFP protein in *MPS3-DDD* (PRY201) at different
1595 time points. Bar indicates 2 μm.

1596 (F) Localization of Mps3-DDD-GFP protein in wild type, *cdc28-as1* (with 1NM-
1597 PP1; PRY301) and *cdc7-as3* (with PP1; PRY272) at 0 h. Bar indicates 2 μm.

1598 (G) An image of Rap1-GFP in the *mps3-DDD* strains with a different combination
1599 of *cdc28-as1*, *cdc7-as3* or *cdc7 bob1-1* mutations in mitosis (0 h). A single-
1600 focal plane of a cell was analyzed at every 3 second. Bar indicates 2 μm.

1601

1602 **Figure S4. Structure and dynamics of JM region of Mps3**

1603 (A) Polarized attenuated total reflection FT-IR spectra are shown. Spectra (red)
1604 a synthetic liposome containing Mps3-WT (left) peptide and Mps3-DDD
1605 peptide (right) were obtained with light polarized parallel (top) or
1606 perpendicular (bottom). Deconvoluted spectra into α-helix and β-sheet are
1607 shown in blue.

1608 (B) Snapshots from coarse-grained MD simulation trajectories for Mps3-WT
1609 peptide (left), Mps3-DDD peptide (right).

1610 (C) Mass density profiles relative to the center of the lipid bilayer for residues,
1611 T188, S189 and S190 on Mps3-WT peptide (blue), Mps3-DDD peptide (red)
1612 and Mps3-WT peptide with phosphorylation (green). Density profile for
1613 phosphate groups on lipids are colored with yellow.

1614

Supplemental movie Information

Each Video (Videos 1-25) was taken every one second in a single focal plane for 120 s. The speed is 9.1 fold.

Video 1. Mps3-GFP in a wild-type cell at 0 h in SPM.
Video 2. Mps3-GFP in a wild-type cell at 4 h in SPM.
Video 3. Mps3-GFP in a wild-type cell at 5 h in SPM, showing deformation.
Video 4. Mps3-GFP in a wild-type cell at 5 h in SPM, showing protrusion.
Video 5. Mps3-GFP in a wild-type cell treated with LatB (0 h) at 5 h in SPM.
Video 6. Mps3-GFP in *cdc28-as1* cells treated with 1NM-PP1 (0 h) at 5 h in SPM.
Video 7. Mps3-GFP in a *cdc7-as3* cell treated with PP1 (0 h) at 5 h in SPM.
Video 8. Mps3-GFP in a *cdc7 bob1-1* cell at 5 h in SPM.
Video 9. Rap1-GFP in a wild-type cell at 0 h in SPM.
Video 10. Rap1-GFP in a wild-type cells at 4 h in SPM.
Video 11. Rap1-GFP in a *cdc28-as1* cell treated with 1NM-PP1 (0 h) at 5 h in SPM.
Video 12. Rap1-GFP in a *cdc7-as3* cell treated with PP1 (0 h) at 5 h in SPM.
Video 13. Rap1-GFP in a *cdc7 bob1-1* cell at 5 h in SPM.
Video 14. Rap1-GFP in *mps3-AAA* cells at 5 h in SPM.
Video 15. Zip1-GFP in a wild-type cell at 5 h in SPM.
Video 16. Zip1-GFP in *mps3-AAA* cells at 6 h in SPM.
Video 17. Mps3-AAA-GFP at 5 h in SPM.
Video 18. Mps3-DDD-GFP at 5 h in SPM.
Video 19. Rap1-GFP in *MPS3-DDD* cells at 5 h in SPM.
Video 20. Rap1-GFP in a *cdc28-as1 MPS3-DDD* cell treated with 1NM-PP1 (0 h) at 5 h in SPM.
Video 21. Rap1-GFP in a *cdc7-as3 MPS3-DDD* cell treated with PP1 (0 h) at 5 h in SPM.
Video 22. Rap1-GFP in a *cdc7 bob1-1 MPS3-DDD* cell at 5 h in SPM.
Video 23. Mps3-DDD -GFP in a *cdc28-as1 MPS3-DDD* cell treated with 1NM-PP1 (0 h) at 5 h in SPM.
Video 24. Mps3-DDD -GFP in a *cdc7-as3 MPS3-DDD* cell treated with PP1 (0 h) at 5 h in SPM.

1648 Video 25. Mps3-DDD -GFP in a *cdc7 bob1-1 MPS3-DDD* cell at 5 h in SPM.

1649 Video 26. MD simulation of wild-type Mps3 peptide.

1650 Video 27. MD simulation of wild-type Mps3-DDD peptide.

1651 Video 28. MD simulation of wild-type Mps3-phosphorylated peptide.

1652

Figure 1. Rao et. al.

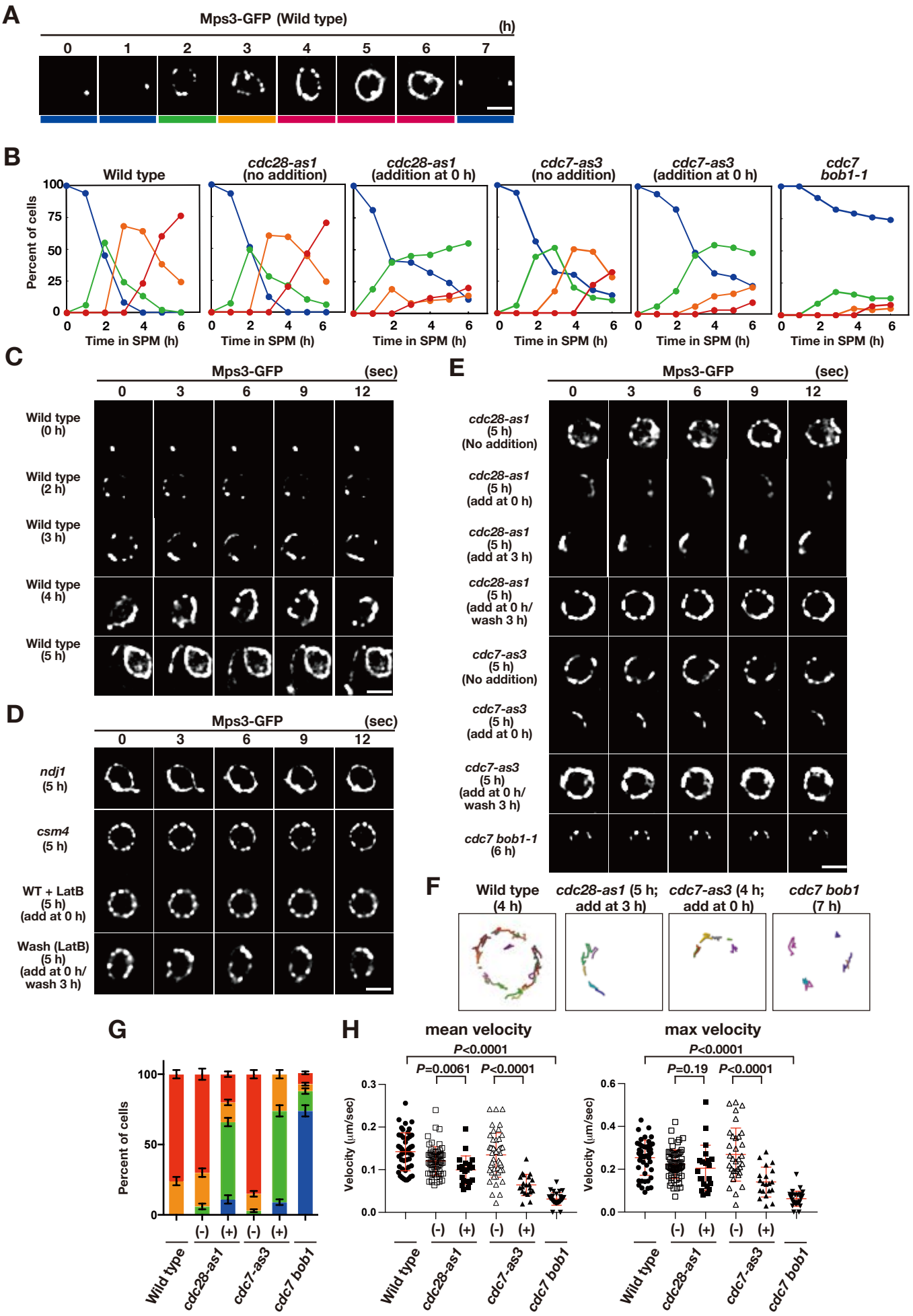


Figure 2. Rao et. al.

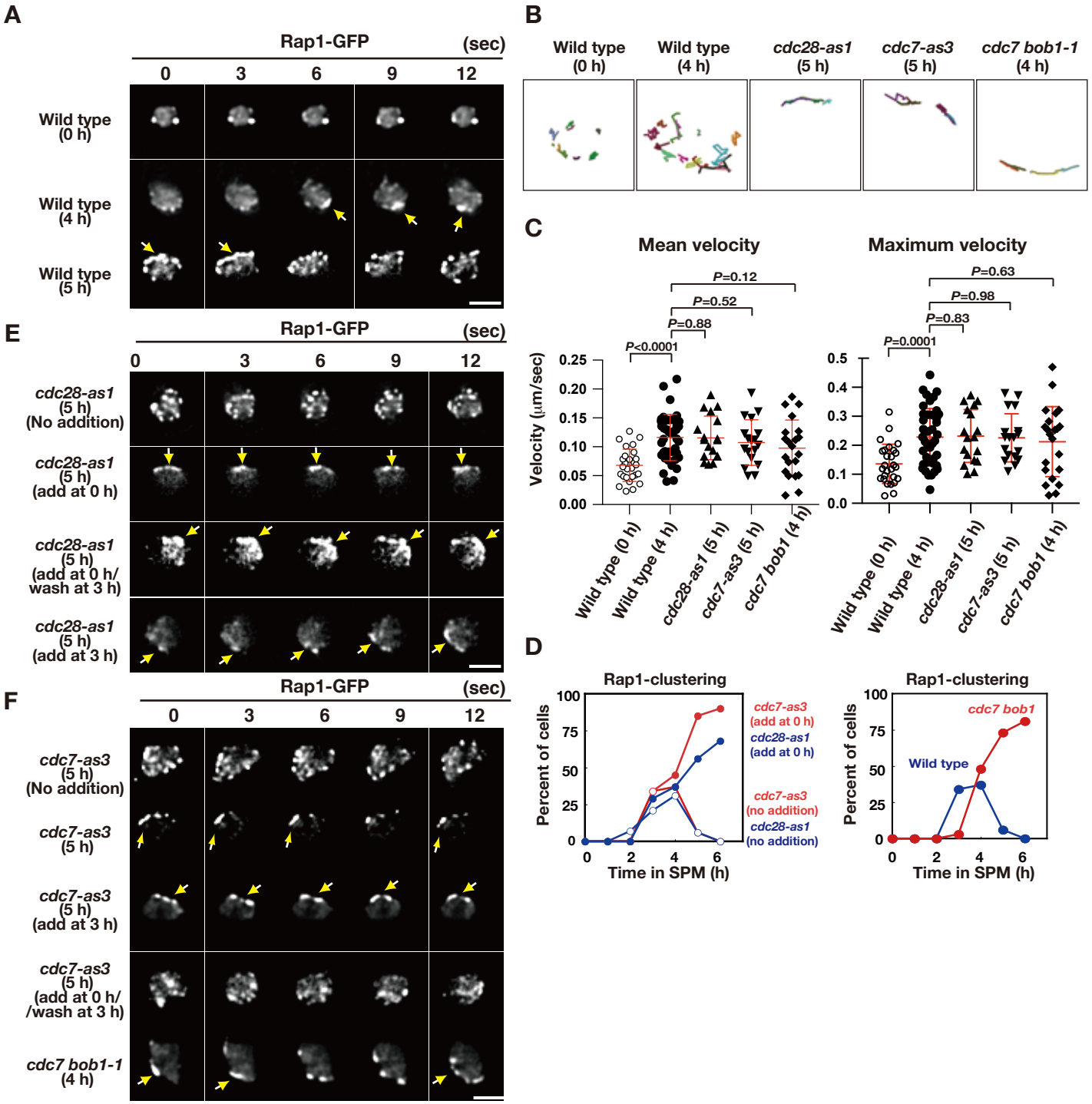


Figure 3 Rao et. al.

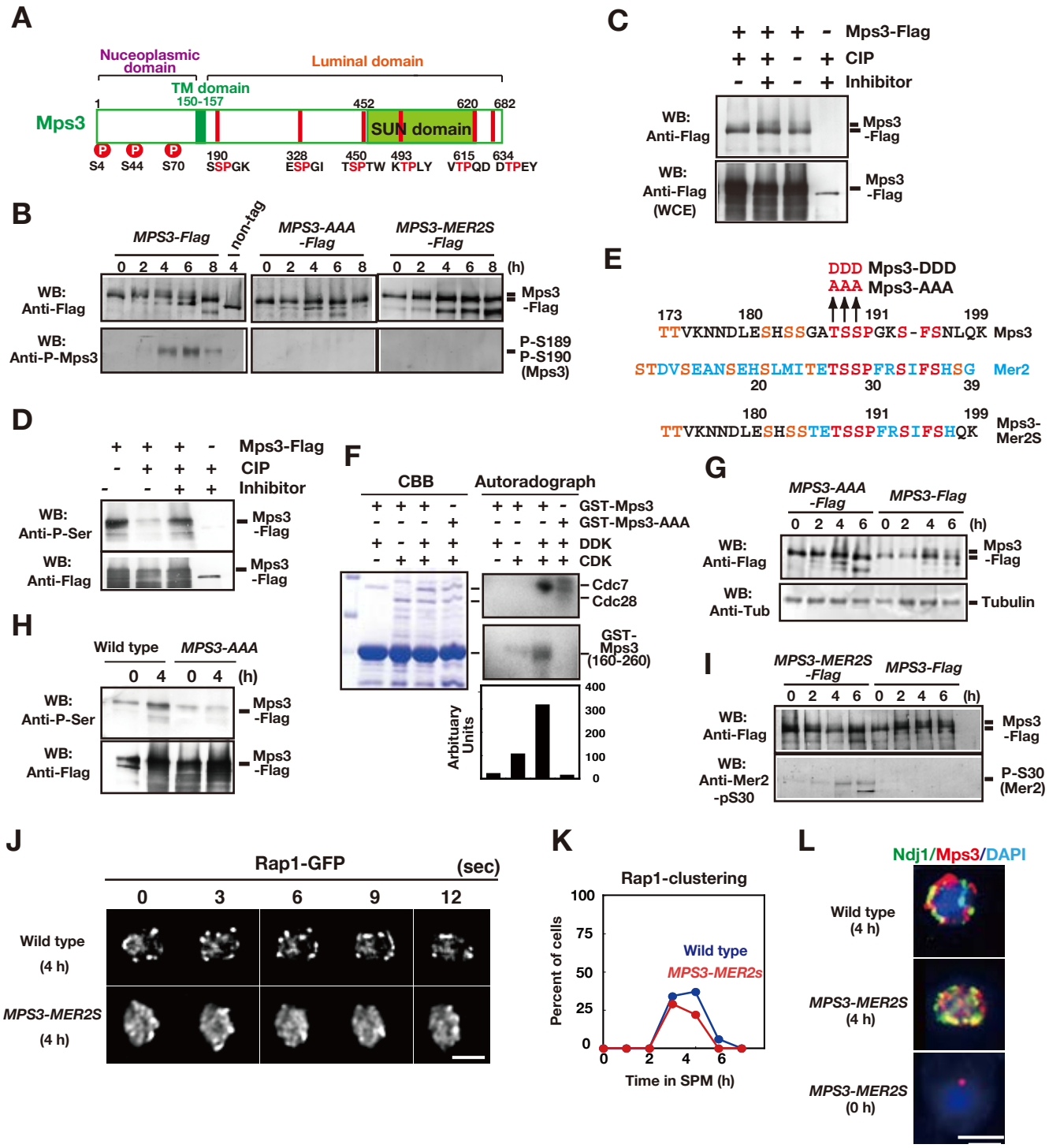


Figure 4 Rao et. al.

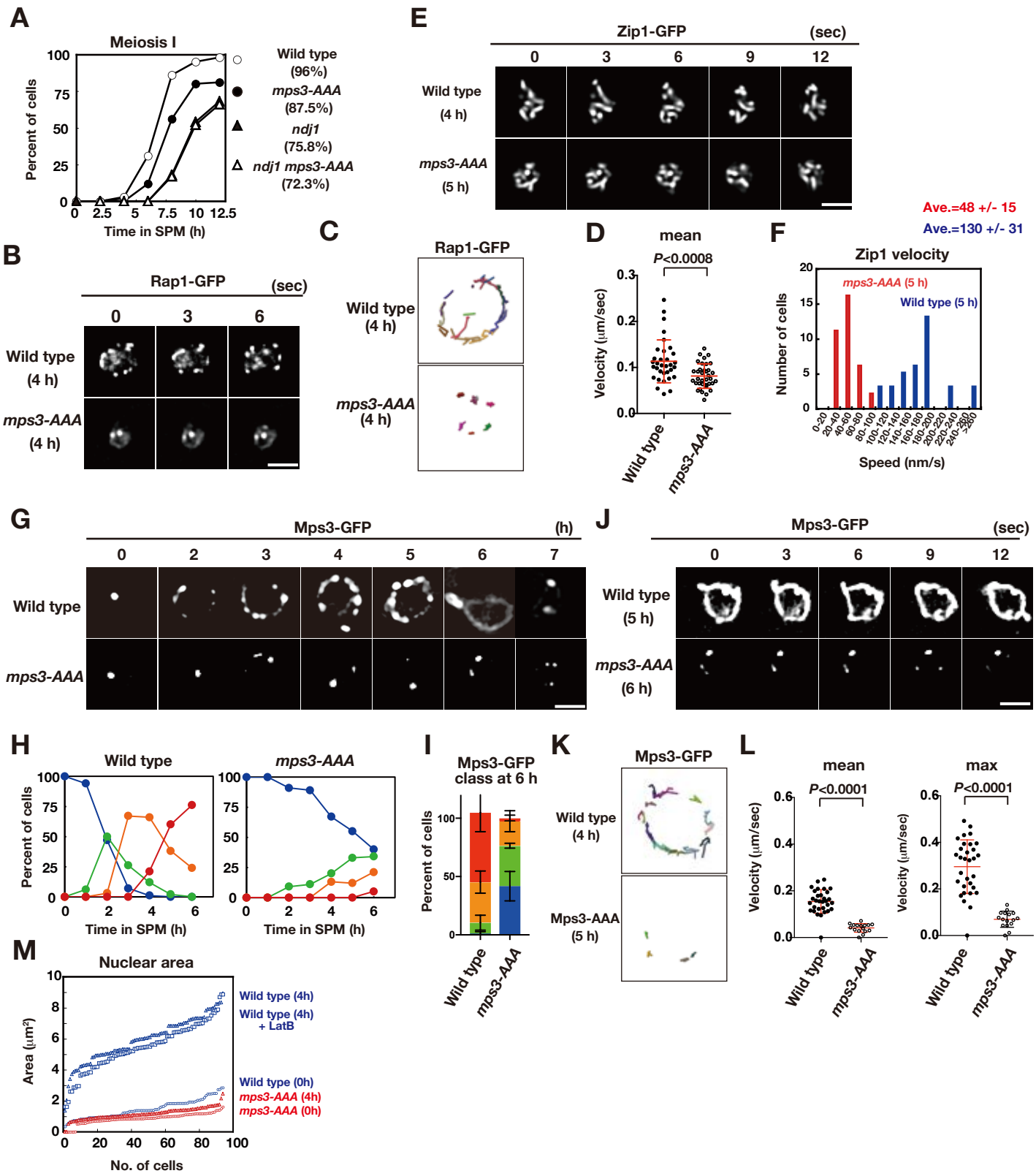


Figure 5 Rao et. al.

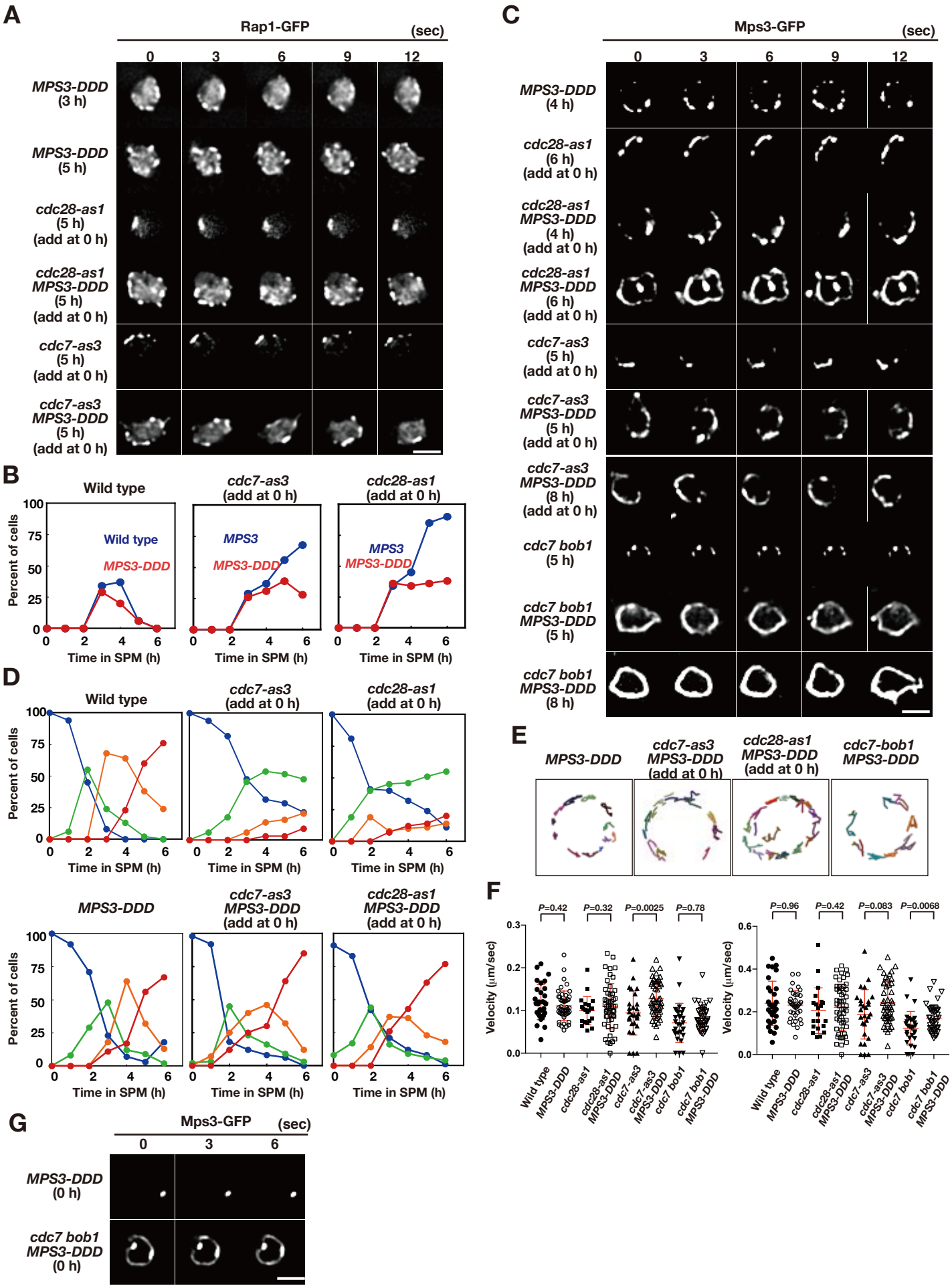


Figure 6 Rao et. al.

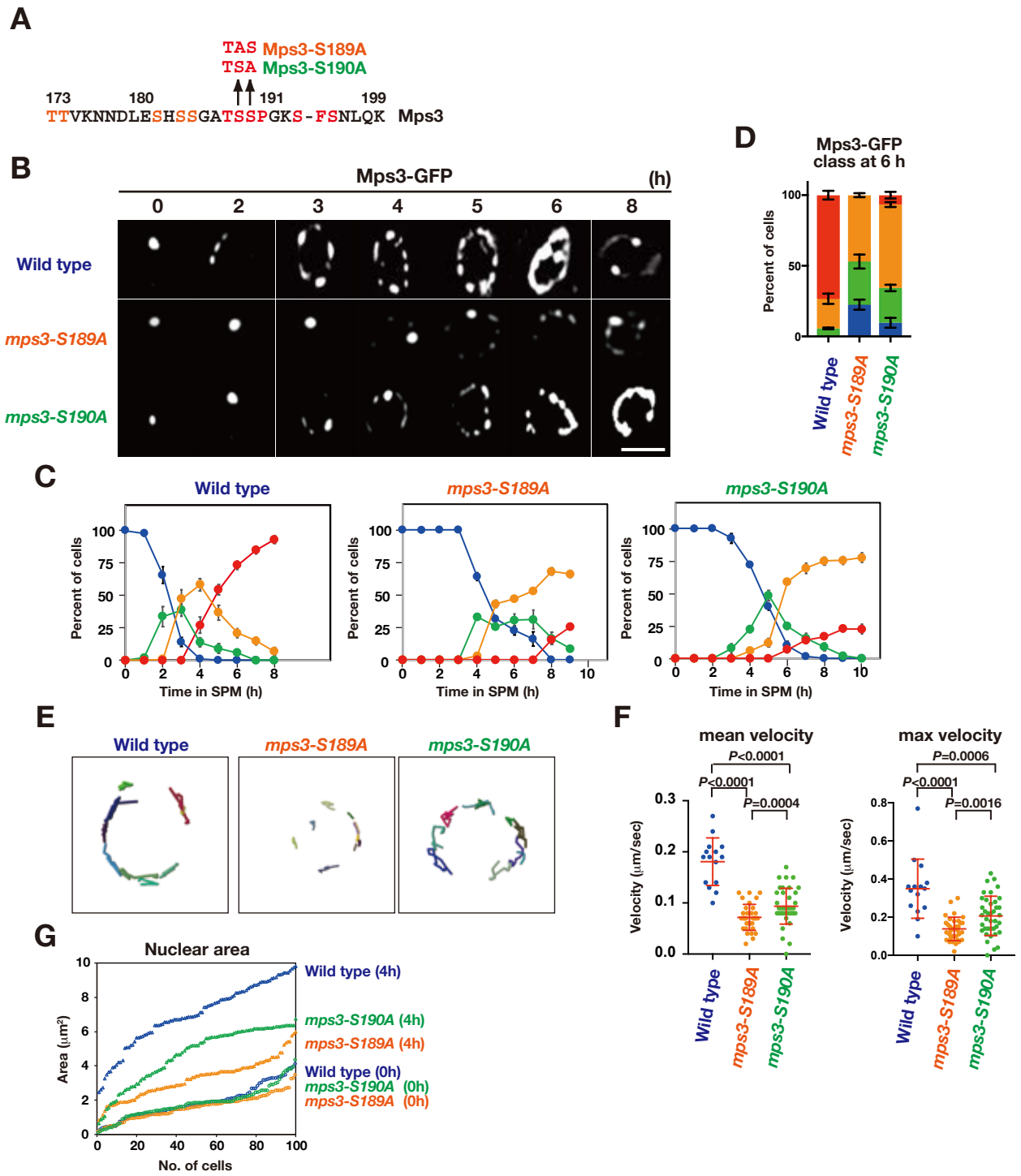


Figure 7. Rao et. al.

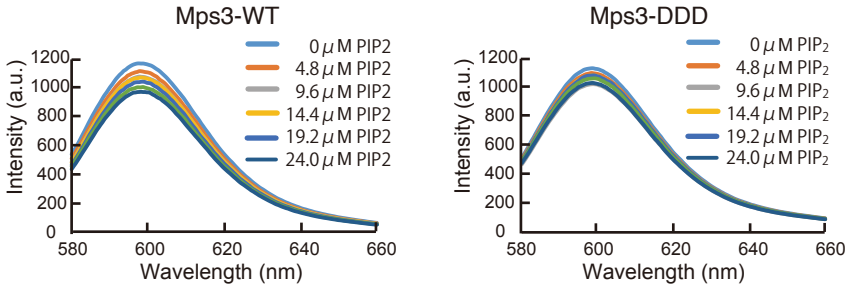
A

Trans-membran domain | Juxtamembrane region

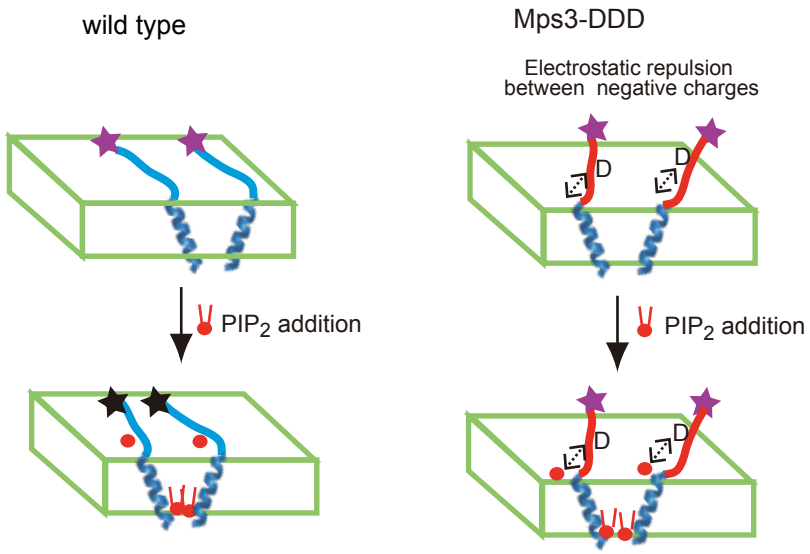
Mps3-WT NH2-GEAKKLKWRTYIFYGGLFFVFFFGSFLMTTVKNNDLESHSSGATSSPGKSFSNL

Mps3-DDD NH2-GEAKKLKWRTYIFYGGLFFVFFFGSFLMTTVKNNDLESHSSGADDDPGKSFSNL

B



C



D

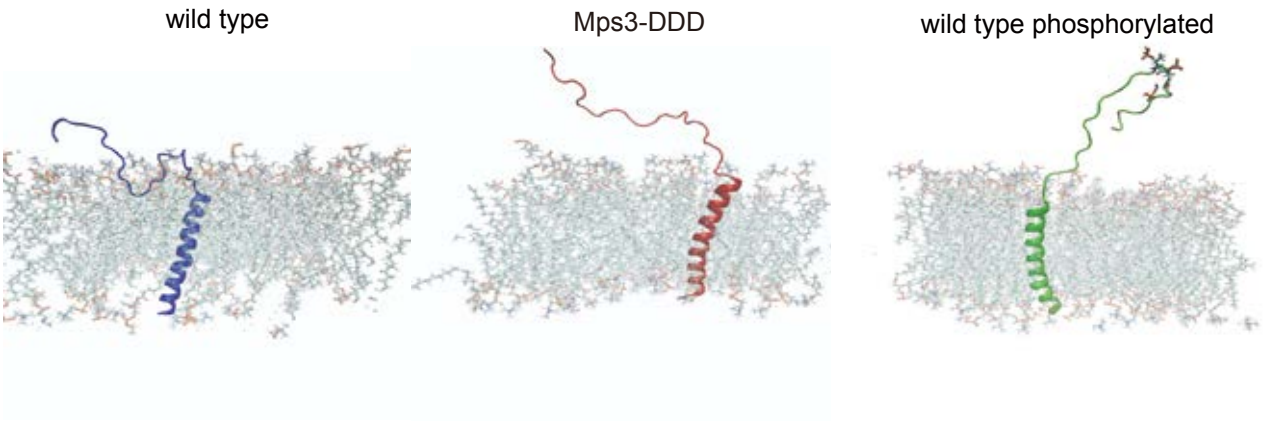


Figure 8. Rao et. al.

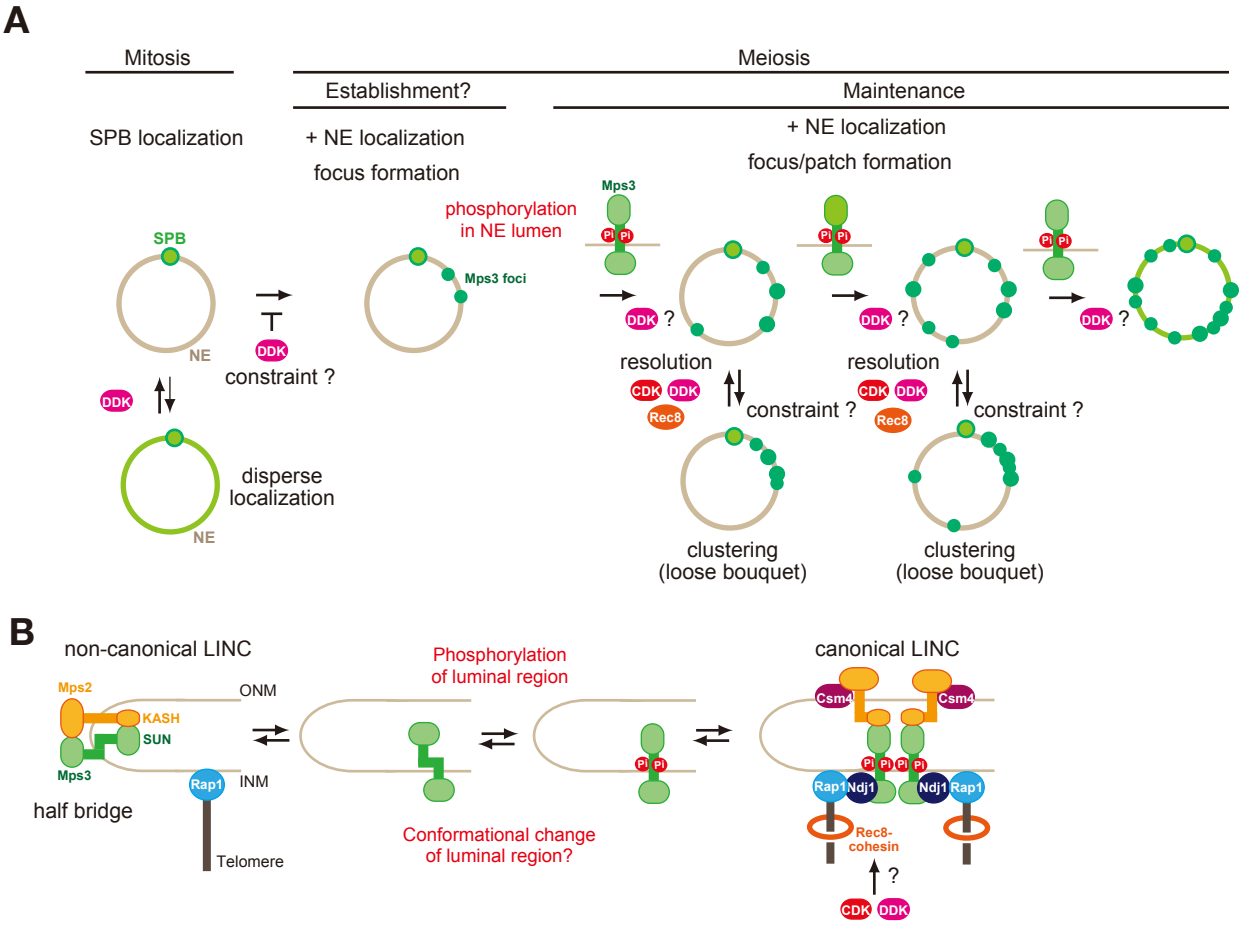


Table S1: Strain list

Strain Name	Genotype
NKY1551	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, his4B-LEU2/his4X-LEU2-URA3, arg4-bgl/arg4-nsp*
MSY832/833	*MAT**a**/α, ho::LYS2, ura3, leu2::hisG, trp1::hisG, lys2*
HKY404	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3,lys2/lys2,MPS3-3FLAG::KanMX6/ MPS3-3FLAG::KanMX6,NDJ1-3HA::KanMX6/NDJ1-3HA::KanMX6*
PRY13	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-3FLAG::KanMX6/ MPS3-3FLAG::KanMX6, NDJ1-3HA::KanMX6/NDJ1-3HA::KanMX6, cdc28-as1/cdc28-as1*
PRY72	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-3FLAG::KanMX6/MPS3-3FLAG::KanMX6, NDJ1-3HA::KanMX6/NDJ1-3HA::KanMX6, cdc7-as3-9MYC/cdc7-as3-9MYC*
PRY 64	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-3GFP::KanMX6/MPS3-3GFP::KanMX6*
PRY71	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3,MPS3-3GFP::KanMX6/MPS3-3GFP::KanMX6, cdc28-as1/cdc28-as1*
PRY260	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-3GFP::KanMX6/MPS3-3GFP::KanMX6, cdc7-as3-9MYC/cdc7-as3-9MYC*

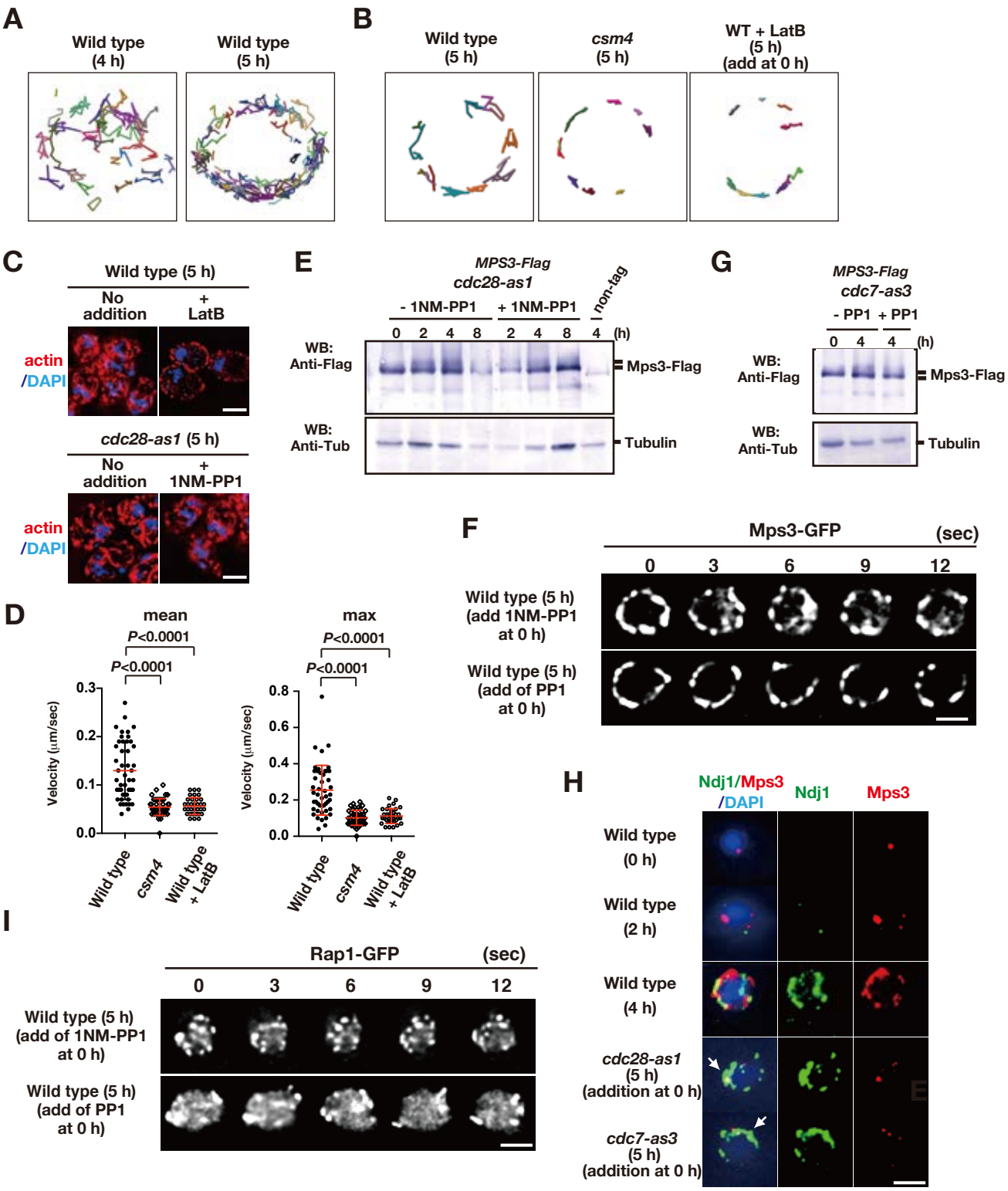
PRY115	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-3GFP::KanMX6/MPS3-3GFP::KanMX6, cdc7::KanMX6/cdc7::KanMX6, bob1-1/bob1-1*
PRY122	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, mps3-AAA(T188A S189A S190A-PSTI)/ mps3-AAA (T188A S189A S190A-PSTI)*
PRY186	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, mps3-AAA(T188A S189A S190A-PSTI)-3GFP::KanMX6/mps3-AAA (T188A S189A S190A-PSTI) -3GFP::KanMX6*
PRY163	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, mps3-3A(T188A S189A S190A-PSTI)-3FLAG::KanMX6/ mps3-3A(T188A S189A S190A-PSTI)-3FLAG::KanMX6 ,NDJ1-3HA ::KanMX6/ NDJ1-3HA::KanMX6*
PRY138	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, mps3-AAA(T188A S189A S190A-PSTI)/ mps3-AAA (T188A S189A S190A-PSTI), Rap1-GFP::LEU2/Rap1-GFP::LEU2*
PRY201	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-DDD(T188D S189D S190D–BSPEI)/MPS3-DDD(T188D S189D S190D–BSPEI)*
PRY211	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6/ MPS3-DDD(T188D S189D S190D–BSPEI)-*

	3GFP::KanMX6
PRY219	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-DDD(T188D S189D S190D–BSPEI)-3FLAG::KanMX6/ MPS3-DDD(1 T188D S189D S190D–BSPEI)-3FLAG::KanMX6, NDJ1-3HA ::KanMX6/NDJ1-3HA::KanMX6*
PRY236	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-DDD(T188D S189D S190D–BSPEI)/MPS3-DDD(T188D S189D S190D–BSPEI), Rap1-GFP::LEU2/Rap1-GFP::LEU2*
PRY301	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6/ MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6, cdc28-as1/cdc28-as1*
PRY272	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6/ MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6, cdc7-as3::9MYC/cdc7-as3::9MYC*
HKY167	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, RAP1-GFP::LEU2/RAP1-GFP::LEU2*
PRY68	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, RAP1-GFP::LEU2/RAP1-GFP::LEU2, cdc28-as1/cdc28-as1*
PRY79	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG,*

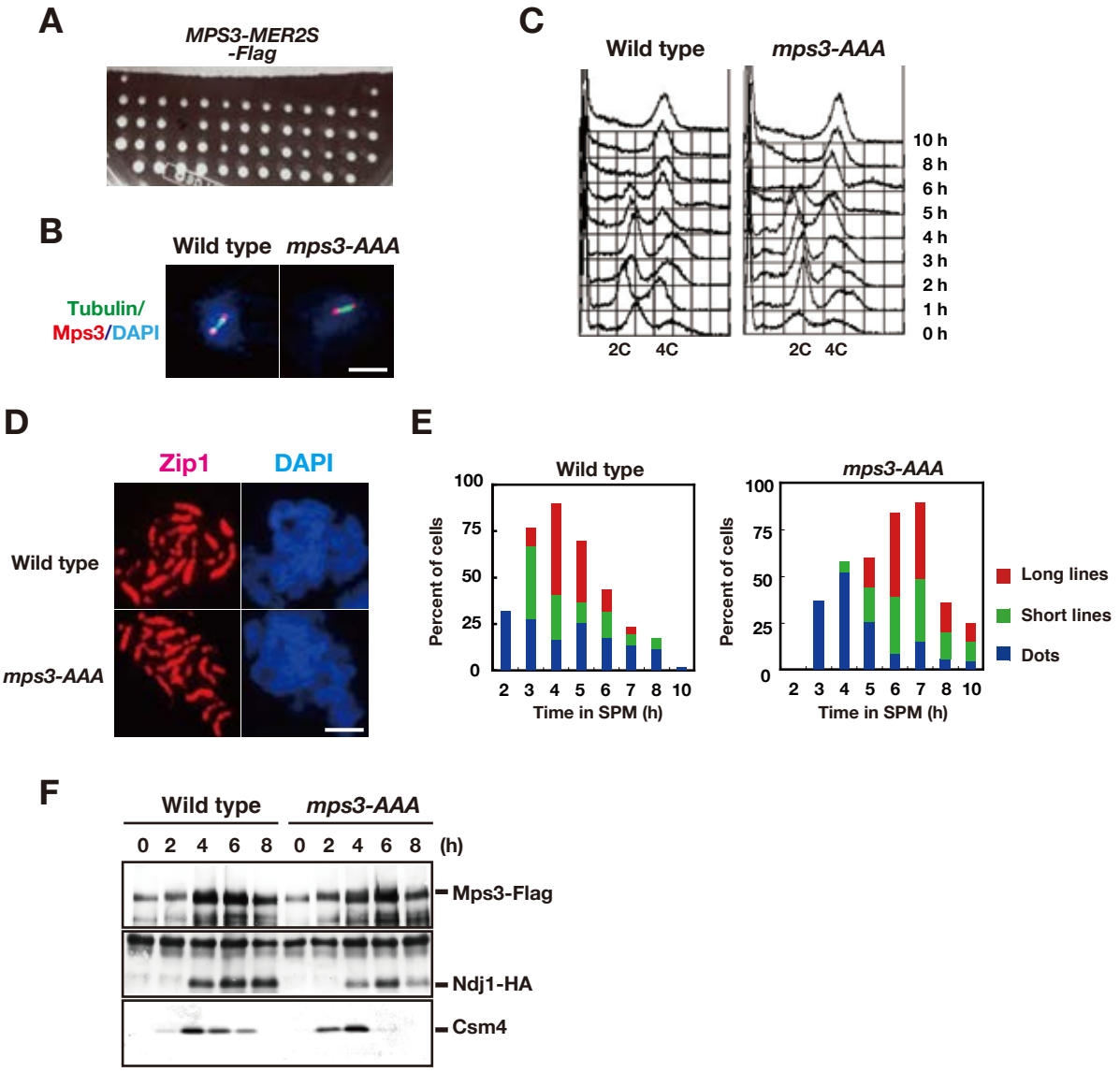
	ura3/ura3, RAP1-GFP::LEU2/RAP1-GFP::LEU2, cdc7-as3-9MYC/cdc7-as3-9MYC
PRY116	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, RAP1-GFP::LEU2/RAP1-GFP::LEU2, cdc7::KanMX6/ cdc7::KanMX6, bob1-1/bob1-1*
PRY303	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6/ MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6, Rap1-GFP::LEU2/Rap1-GFP::LEU2, cdc28-as1/cdc28-as1*
PRY309	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6/ MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6, Rap1-GFP::LEU2/Rap1-GFP::LEU2, cdc7-as3::9MYC/cdc7-as3::9MYC*
PRY322	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6/ MPS3-DDD(T188D S189D S190D–BSPEI)-3GFP::KanMX6, Rap1-GFP::LEU2/Rap1-GFP::LEU2, cdc7::KanMX6/ cdc7::KanMX6, bob1-1/bob1-1*
PRY192	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-3GFP::KanMX6/MPS3-3GFP::KanMX6 ndj1::KanMX6/ndj1::KanMX6*
PRY198	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, MPS3-3GFP::KanMX6/MPS3-3GFP::KanMX6,*

	csm4::KanMX6/csm4::KanMX6
PRY514	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, Mps3-MER2S (swap)-SacI-Flag/ Mps3-MER2S (swap)-SacI-Flag, Rap1-GFP::LEU2/Rap1-GFP::LEU2, NDJ1-HA/NDJ1-HA*
PRY518	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, Mps3-MER2S (swap)-SacI-Flag/ Mps3-MER2S (swap)-SacI-Flag, Rap1-GFP::LEU2/Rap1-GFP::LEU2, Ndj1-HA/ Ndj1-HA*
KSY220/221	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, mps3-S190A (-PSTI)-3GFP::KanMX6/mps3-S190A(-PSTI)-3GFP::KanMX6*
KSY407/409	*MAT**a**/α, ho::LYS2/ho::LYS2, lys2/lys2, leu2::hisG/leu2::hisG, ura3/ura3, mps3-S189A-(BSPEI)-3GFP::KanMX6/mps3-S189A-(BSPEI)-3GFP::KanMX6*

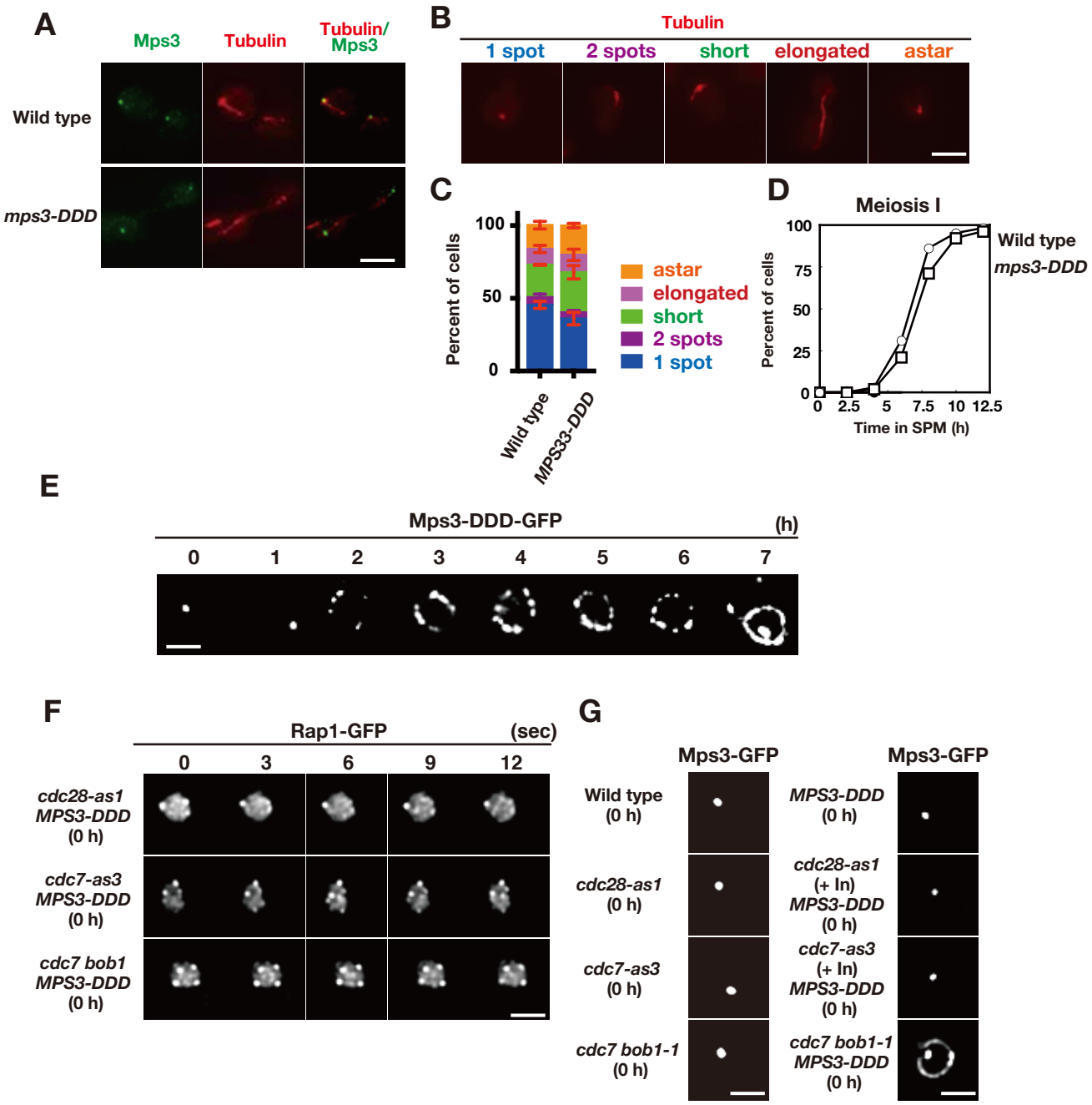
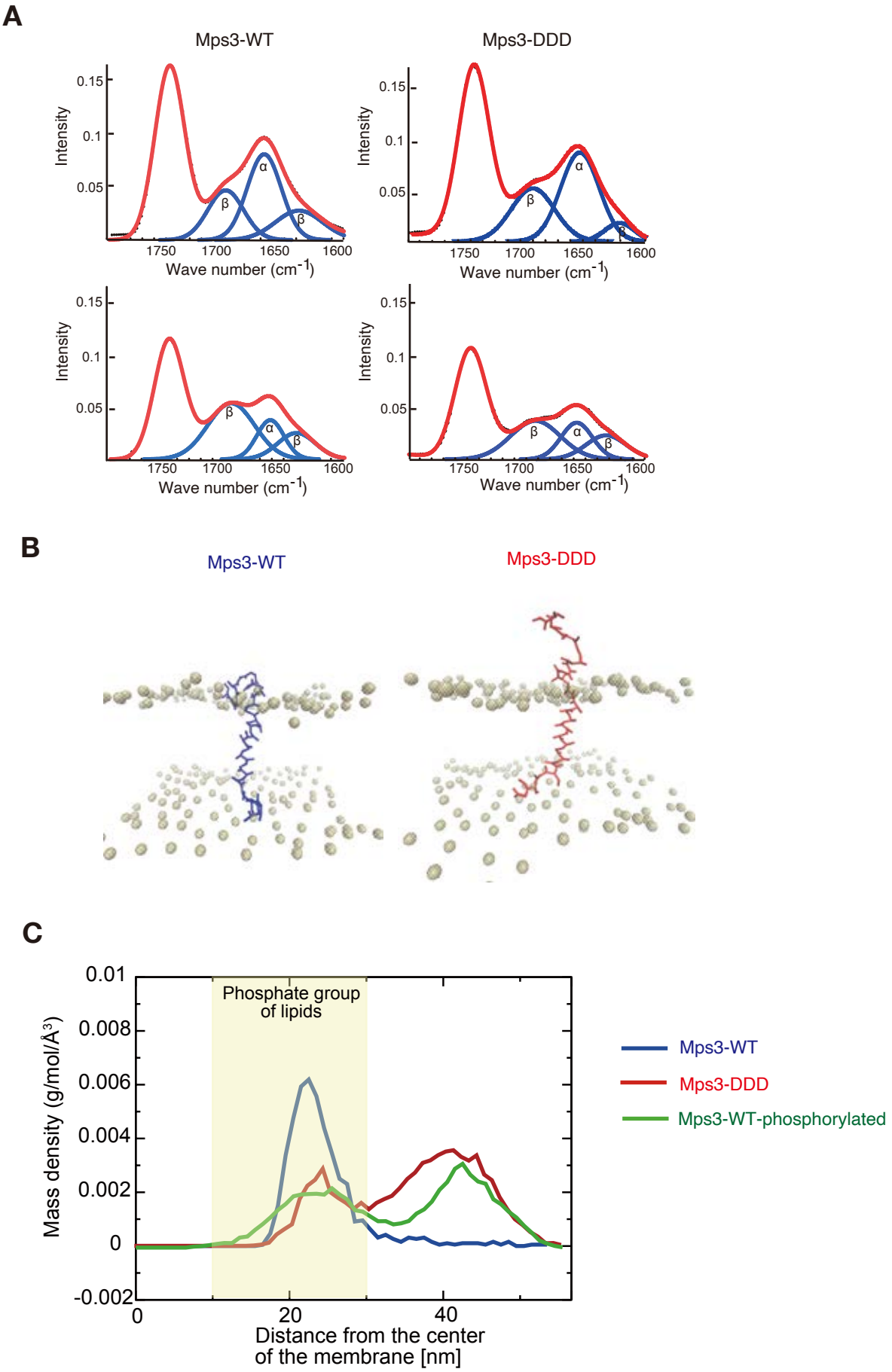
Supplemental Figure S1 Rao et. al.



Supplemental Figure S2 Rao et. al.



Supplemental Figure S3 Rao et. al.



Supplemental Figure S4 Rao et. al.