



HAL
open science

Increases in cyclin A/Cdk activity and in PP2A-B55 inhibition by FAM122A are key mitosis-inducing events

Benjamin Lacroix, Suzanne Vigneron, Jean Claude Labbé, Lionel Pintard,
Corinne Lionne, Gilles Labesse, Anna Castro, Thierry Lorca

► To cite this version:

Benjamin Lacroix, Suzanne Vigneron, Jean Claude Labbé, Lionel Pintard, Corinne Lionne, et al..
Increases in cyclin A/Cdk activity and in PP2A-B55 inhibition by FAM122A are key mitosis-inducing
events. *EMBO Journal*, 2024, 43 (6), pp.993-1014. 10.1038/s44318-024-00054-z . hal-04751214

HAL Id: hal-04751214

<https://hal.science/hal-04751214v1>

Submitted on 24 Oct 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Increases in cyclin A/Cdk activity and in PP2A-B55 inhibition by FAM122A are key mitosis-inducing events

Benjamin Lacroix^{1,4*}, Suzanne Vigneron^{1,4*}, Jean Claude Labbé^{1,4}, Lionel Pintard^{2,4}, [Corinne Lionne](#)³, Gilles Labesse³, Anna Castro^{1,4&} and Thierry Lorca^{1,4&}

¹Université de Montpellier, Centre de Recherche en Biologie cellulaire de Montpellier (CRBM), CNRS UMR 5237, 1919 Route de Mende, 34293 Montpellier cedex 5, France.

²Université Paris Cité, Institut Jacques Monod, F-75013 Paris, France.

³Centre de Biochimie Structurale (CBS), CNRS UMR 5048, INSERM 1054, Université de Montpellier, Montpellier, France.

⁴Programme équipes Labellisées Ligue Contre le Cancer

Phone 33 4 34 35 95 56

Fax 33 4 34 35 94 10

*Both authors contributed equally to this work

&Corresponding authors: thierry.lorca@crbm.cnrs.fr / anna.castro@crbm.cnrs.fr

21 **ABSTRACT**

22 Entry into mitosis has been classically attributed to activation of a cyclin B/Cdk1
23 amplification loop via a partial pool of this kinase becoming active at the end of G2 phase.
24 However, how this initial pool is activated is still unknown. Here we discovered a new role of
25 the recently identified PP2A-B55 inhibitor FAM122A in triggering mitotic entry.
26 Accordingly, depletion of the orthologue of FAM122A in *C. elegans* prevents entry into
27 mitosis in germline stem cells. Moreover, data from *Xenopus* egg extracts strongly suggest
28 that FAM122A-dependent inhibition of PP2A-B55 could be the initial event promoting
29 mitotic entry. Inhibition of this phosphatase allows subsequent phosphorylation of early
30 mitotic substrates by cyclin A/Cdk, resulting in full cyclin B/Cdk1 and Greatwall (Gwl)
31 kinase activation. Subsequent to Greatwall activation, Arpp19/ENSA become phosphorylated
32 and now compete with FAM122A, promoting its dissociation from PP2A-B55 and taking
33 over its phosphatase inhibition role until the end of mitosis.

34 Keywords: FAM122A, Arpp19, PP2A-B55, mitosis, Cyclin A.

35

36 **INTRODUCTION**

37 Mitotic entry and progression are induced by massive protein phosphorylation
38 resulting from the fine-tuned balance between the kinase cyclin B/Cdk1 and its counteracting
39 phosphatase PP2A-B55(Mochida *et al*, 2010; Gharbi-Ayachi *et al*, 2010; Mochida *et al*, 2009;
40 Vigneron *et al*, 2009). Cyclin B/Cdk1 activity is maintained low during G2 by the
41 Wee1/Myt1 kinases that phosphorylate Cdk1 on its inhibitory site tyrosine 15. At M phase
42 entry, cyclin B/Cdk1 activity is triggered by a positive feed-back loop(Pomerening *et al*,
43 2003; Sha *et al*, 2003). A partial active pool of this kinase phosphorylates Wee1/Myt1, as well
44 as the Cdc25 phosphatase responsible of Cdk1-tyrosine 15 dephosphorylation, promoting a
45 rapid and complete activation of the cyclin B/Cdk1 complex. How cyclin B/Cdk1 partial
46 activation is firstly triggered at G2-M is a main question yet to be answered. Inhibition of
47 PP2A-B55 was proposed as the putative cause inducing partial cyclin B/Cdk1 pool activation
48 and mitotic entry. PP2A-B55 is regulated by the Gwl-Arpp19/ENSA pathway(Mochida *et al*,
49 2010; Gharbi-Ayachi *et al*, 2010; Burgess *et al*, 2010; Hached *et al*, 2019). During G2, the
50 activity of this phosphatase is high. Then, at mitotic entry, Gwl is activated and
51 phosphorylates its substrates Arpp19/ENSA converting them into high affinity inhibitors of
52 PP2A-B55. Decreased PP2A-B55 activity could then favour a partial Wee1/Myt1/Cdc25
53 phosphorylation and reactivation of cyclin B/Cdk1 triggering the feedback loop. However,

54 Gwl activation itself depends on cyclin B/Cdk1 discarding the modulation of this phosphatase
55 [by the Gwl-Arpp19/ENSA pathway](#) as the first event triggering cyclin B/Cdk1 firing and
56 mitotic entry(Vigneron *et al*, 2011; Blake-Hodek *et al*, 2012). Moreover, although both Gwl
57 and Arpp19/ENSA are essential for mitotic entry in *Xenopus* egg extract model(Gharbi-
58 Ayachi *et al*, 2010; Vigneron *et al*, 2009), the depletion of these proteins do not prevent G2-
59 M transition in human and mouse cells(Hached *et al*, 2019; Alvarez-Fernandez *et al*, 2013).

60 Besides cyclin B/Cdk1, cyclin A/Cdk is also present and is a poor substrate of Myt1
61 kinase during G2(Coulonval *et al*, 2003; Booher *et al*, 1997). This kinase could participate to
62 Wee1/Myt1/Cdc25 phosphorylation during G2-M if the activity of PP2A-B55 is partially
63 inhibit during this transition. In this line, FAM122A has been recently described as a new
64 inhibitor of PP2A-B55 that is present and active during G2.

65 FAM122A was firstly identified as an interactor of the PP2A-B55 complex that
66 inhibits this phosphatase and promotes the degradation of PP2A catalytic subunit.(Fan *et al*,
67 2016) This protein was additionally shown to negatively modulate PP2A-B55 α in the
68 nucleus. Its phosphorylation by Chk1 and its subsequent retention into the cytoplasm is
69 essential for the G2/M checkpoint by preventing the nuclear inhibition of this phosphatase
70 and permitting the dephosphorylation and stabilisation of Wee1(Li *et al*, 2020). By its
71 presence in G2 and its capacity to specifically inhibit PP2A-B55, FAM122A is a good
72 candidate to fulfil the role of PP2A-B55 inhibitor able to potentiate cyclin A/Cdk-dependent
73 phosphorylation at G2 and to trigger M phase. [In this study we investigated the role of
74 FAM122A in mitotic entry and we demonstrated that, by inhibiting PP2A-B55, this protein
75 promotes a first burst of cyclin A/Cdk-dependent phosphorylation that will induce cyclin
76 B/Cdk1 activation and mitotic entry.](#)

77

78 **RESULTS**

79 **PP2A-B55 inhibition by FAM122A does not involve changes in PP2A-C or Wee1 levels**

80 The role of FAM122A as a specific PP2A-B55 inhibitor has been reported by two
81 different laboratories(Fan *et al*, 2016; Li *et al*, 2020). One laboratory reported a role of this
82 protein in the negative modulation of PP2A-B55 via the degradation of the PP2A-B55
83 catalytic subunit C(Fan *et al*, 2016). The second one suggested that the inhibition of PP2A-
84 B55 by FAM122A induces the dephosphorylation and degradation of Wee1. We checked
85 these two premises in interphase *Xenopus* egg extracts (interphase extracts), in which protein
86 phosphorylation mimics the one observed in G2 cells. We, therefore, added either *Xenopus* or

87 human histidine recombinant FAM122A proteins to these extracts and measured the levels of
88 PP2A-C and Wee1 over time. Surprisingly, we could not observe any variation of the amount
89 of these two proteins in the extracts (Figure 1a). We next proceeded by checking whether our
90 recombinant FAM122A forms can inhibit PP2A-B55. To do this, we used interphase egg
91 extracts in which ATP was eliminated and thus endogenous kinases are inactive (called
92 hereafter as kinase-inactivated extracts). Because of the absence of endogenous active
93 kinases, substrate dephosphorylation in these extracts directly reflects phosphatase
94 activity(ies). Kinase-inactivated extracts were supplemented, or not, with the corresponding
95 FAM122A recombinant protein (human/*Xenopus*) and subsequently with recombinant
96 Arpp19 pre-phosphorylated at S113 by PKA known to be a bona fide PP2A-B55
97 substrate(Labbé *et al*, 2021; Lemonnier *et al*, 2019). Dephosphorylation of S113 of Arpp19
98 was then followed by western blot over time. As expected by its capacity to inhibit PP2A-
99 B55, both human and *Xenopus* FAM122A drastically delayed Arpp19 dephosphorylation
100 (Figure 1b). Similar results were observed using phosphorylated-T481 PRC1, another well-
101 established substrate of PP2A-B55 phosphatase(Cundell *et al*, 2013). We next checked the
102 association of His-FAM122A to PP2A-B55 by using histidine-pull down. B55, A and C
103 subunits of PP2A were present in the histidine but not in the control pull-down of *Xenopus*
104 and human FAM122A (Figure 1c). Thus, FAM122A can directly bind and inhibit PP2A-B55
105 without any modification of the levels of the catalytic subunit of this phosphatase.

106

107 **FAM122A triggers mitotic entry by inhibiting PP2A-B55 and permitting cyclin A/Cdk-** 108 **dependent phosphorylation of mitotic substrates**

109 We next checked the impact of FAM112A on mitotic progression by measuring the
110 phosphorylation of mitotic substrates upon the addition of this protein into interphase extracts.
111 FAM122A induced a first phosphorylation of Gwl, the Anaphase Promoting Complex (APC)
112 subunit Cdc27, and of Cdc25 concomitantly with the activation of cyclin B/Cdk1 as reflected
113 by the loss of the phosphate on tyrosine 15 inhibitory site of Cdk1. This was followed by the
114 subsequent degradation of cyclin A and B and the dephosphorylation of the above indicated
115 substrates revealing that FAM122A promotes mitotic entry but is unable to maintain the
116 mitotic state (Figure 2a). We then sought to assess whether, as for Arpp19, FAM122A
117 requires phosphorylation by Gwl to stably bind and inhibit PP2A-B55. Against this
118 hypothesis, our data indicate that Gwl is unable to phosphorylate FAM122A "*in vitro*" (Figure
119 2b) and the depletion of this kinase in interphase extracts did not prevent mitotic entry
120 induced by FAM122A (Figure 2c). Moreover, a FAM122A form in which all serine and

121 threonine residues were mutated into alanine kept its capacity to induce mitosis (S/T-A
122 FAM122A, Figure 2d) and displayed a similar PP2A-B55 binding capacity than the wildtype
123 FAM122A (Figure 2e). **These data suggest that FAM122A phosphorylation is not required for
124 its binding to and the inhibition of PP2A-B55 at least at the used ectopic doses although we
125 cannot exclude other PP2A-B55-independent role of this phosphorylation.** Thus, FAM122A
126 would not require either phosphorylation or Gwl activity to trigger mitotic entry.

127 We then investigated whether mitotic substrate phosphorylation induced by
128 FAM122A requires cyclin B/Cdk1 activation by examining the effect of depleting Cdc25, the
129 phosphatase dephosphorylating tyrosine 15 of Cdk1 and triggering cyclin B/Cdk1 activity. As
130 expected, Cdc25-devoid extracts displayed inactive cyclin B/Cdk1, as stated by the presence
131 of the phospho-tyrosine 15 on Cdk1 (Figure 2f). Moreover, cyclin A became fully
132 proteolyzed whereas cyclin B remained mostly stable although we detected a loss of a small
133 fraction of this protein likely due to the phosphorylation, under these conditions, of Cdc27
134 and the activation of the APC. Besides Cdc27, FAM122A, via PP2A-B55 inhibition, was also
135 able to promote the phosphorylation and activation of Gwl and, most importantly, the
136 phosphorylation and inactivation of the Cdk1 inhibitory kinase Myt1. Since due to Cdc25
137 depletion cyclin B/Cdk1 was inactive in these extracts, we attributed these phosphorylations
138 to cyclin A/Cdk1. In this line, we previously showed that this complex escapes to Wee1-Myt1
139 inhibitory phosphorylation and is fully active in interphase extracts (Vigneron *et al*, 2018). To
140 confirm this hypothesis, we co-depleted Cdc25 and cyclin A in FAM122A-supplemented
141 interphase extracts. Remarkably, the loss of these two proteins prevented substrate
142 phosphorylation resulting from FAM122A-dependent inhibition of PP2A-B55 (Figure 2f).
143 However, this phosphorylation was restored again if 45 minutes upon Cdc25/cyclin A co-
144 depletion and FAM122A addition, cyclin A was supplemented again to these extracts. These
145 data demonstrate that the decrease of the PP2A-B55 activity in interphase extracts induced by
146 FAM122A is sufficient to permit cyclin A-Cdk1-dependent phosphorylation that will then
147 trigger cyclin B/Cdk1 reactivation. Interestingly, this suggests that an increase of the
148 endogenous levels of either FAM122A or cyclin A would be sufficient to trigger mitotic entry
149 and would thus escape to the negative regulation of active PP2A-B55 during interphase.

150

151 **Molecular determinants of FAM122A controlling the association and the inhibition of** 152 **PP2A-B55**

153 AlphaFold artificial intelligence software (Alphabet's/Google's DeepMind) predicts
154 the presence of two α helices in the FAM122A protein, α -helix1: residues 84-93 (human)/71-

155 82 (*Xenopus*) and α -helix2: residues 96-119 (human)/85-110 (*Xenopus*) (Figure 3a). In order
156 to investigate if these structured regions could participate to the association of this protein to
157 PP2A-B55, we constructed a FAM122A *Xenopus* mutant forms deleted of either α -helix1
158 (α H1) or α -helix2 (α H2) and we measured their capacity to induce mitotic entry and to bind
159 to PP2A B55 and C subunits when added to interphase extracts. Neither of these two mutants
160 were able to induce mitosis (Figure 3b) or to bind to PP2A-B55 (Figure 3c) indicating that
161 these two regions are essential for FAM122A inhibitory activity. We next constructed a N-
162 terminal Δ (1-72) and a C-terminal (Δ 110-270) mutant forms in order to determine whether
163 any of these regions could additionally participate to the functionality of this protein. Unlike
164 the two α -helix regions, these two mutants displayed similar properties than the wildtype
165 form (Figure 3d and e). Finally, we proceed with the construction of FAM122A single mutant
166 forms for all conserved residues present in α H1 and α H2 regions. Data on mitotic entry and
167 PP2A-B55 binding capacities of these mutants are shown in Appendix Figure S1a and S1b
168 and summarized in Figure 3f. Residues of α H1, R73, L74, I77 and E80 were essential for
169 FAM122A inhibitory activity. Indeed, α H1 sequence SRLHQIKQEE, is close to the previous
170 reported Short Linear Motif (SLiM) designed for PP2A-B55, with consensus sequence p[ST]-
171 P-x(4,10)-[RK]-V-xx-[VI]-R(Fowle *et al*, 2021) and is highly conserved between species
172 (Appendix Figure S1c). This motif is present in PP2A-B55 substrates and is required for their
173 binding to the phosphatase via their interaction with the B55 subunit. As so, these residues are
174 involved in FAM122A association to B55. For α H2 region, we identified positions E90, H93,
175 E94 and R95 as being essential for FAM122A activity. Thus, we pinpointed these two
176 structured regions of FAM122A essential for its function and we identified the key residues
177 directly mediating PP2A-B55 phosphatase interaction.

178

179 **Gwl activation at mitotic entry promotes the dissociation of FAM122A from PP2A-B55**

180 Data above demonstrate that the addition of FAM122A to interphase extracts
181 promotes mitotic entry but is unable to maintain mitotic substrate phosphorylation. Since the
182 levels of FAM122A remain constant throughout the experiment and that FAM122A activity
183 appears not to be impacted by phosphorylation, we sought to understand why FAM122A is
184 not able to maintain the mitotic state. To this, we first checked the capacity of *Xenopus* and
185 human FAM122A proteins to bind PP2A-B55 when they were supplemented to metaphase II
186 arrested extracts (CSF extracts) which mimics the mitotic state. Interestingly, B55 and C
187 subunit binding to FAM122A is clearly observed during interphase, whereas no association

188 for B55 and a drastic decrease for C catalytic subunit were observed in CSF extracts (Figure
189 4a).

190 To corroborate these observations, we tested whether FAM122A would be dissociated
191 from B55 subunit in extracts blocked in mitosis. In order to obtain mitotic blocked extracts,
192 we prevented cyclin B degradation in interphase extracts by the depletion of the APC subunit
193 Cdc27 and then we added FAM122A. FAM122A addition promoted mitotic entry but,
194 because of the incapacity to proteolyze cyclin B, extracts remained blocked in this phase of
195 the cell cycle. Under these conditions, we measured the temporal pattern of protein
196 phosphorylation and association of FAM122A to PP2A-B55. As expected, addition of
197 FAM122A to control interphase extracts promoted mitotic entry and exit, while mitosis was
198 maintained throughout the experiment when Cdc27 was depleted (Figure 4b). In control
199 extracts, FAM122A rapidly associated to B55, A and C subunits from its addition, then
200 dissociated concomitantly with mitotic entry and re-associated again upon exit of mitosis by
201 cyclin B degradation (Figure 4b). Conversely, in Cdc27-depleted interphase extracts,
202 FAM122A also displayed a first association to these proteins but then, definitively lost this
203 interaction from the establishment of the mitotic state. Taken together, these observations
204 suggest that FAM122A-B55 binding is prevented during mitosis.

205 Besides, cyclin B/Cdk1, the Gwl kinase is also required to maintain the mitotic state.
206 Although our data shown above demonstrate that Gwl cannot phosphorylate FAM122A to
207 promote mitotic entry, we tested whether the PP2A-B55 inhibitory activity of FAM122A
208 could be indirectly modulated by this kinase once the mitotic state is achieved. To this, we
209 repeated the previous experiment except that extracts were immunodepleted of both, Cdc27
210 and the Gwl kinase. As expected by the depletion of Cdc27, all extracts entered into mitosis
211 and kept the mitotic state as shown by the stability of cyclin B, the continuous
212 phosphorylation of Cdc25 and the loss of phospho-tyrosine 15 Cdk1 signal throughout the
213 experiment (Figure 4c). Interestingly, unlike Cdc27/control-co-depleted extracts (Figure 4c,
214 lane 1, His Pull Down) and despite the presence of a fully active cyclin B/Cdk1 complex,
215 FAM122A was able to bind to B55 and to increase its association to PP2A C subunit in
216 Cdc27/Gwl-co-depleted extracts (Figure 4c, lane 2, His Pull Down). However, FAM122A
217 dissociated again from B55 when a Gwl hyperactive form was further supplemented (Figure
218 4c, lane 4, His Pull Down). Thus, FAM122A dissociation from PP2A during mitosis is
219 modulated by the Gwl kinase.

220

221 **Arpp19 competes and dissociates FAM122A from PP2A-B55 during mitosis**

222 We next sought to decipher how Gwl could regulate FAM122A association to PP2A-
223 B55. Gwl phosphorylates Arpp19 during mitosis, turning it into a high affinity interactor of
224 PP2A-B55 (Mochida *et al*, 2010; Gharbi-Ayachi *et al*, 2010; Williams *et al*, 2014). We thus,
225 asked whether during mitosis, phosphorylated Arpp19 competes with FAM122A for PP2A-
226 B55 binding. To test this hypothesis, we supplemented interphase extracts with FAM122A
227 and 40 minutes later, once the extract returned to an interphase state and FAM122A binds
228 again PP2A-B55 (Figure 2a and 4b), it was either (1) used to perform a histidine-FAM122A
229 pulldown and subsequently supplemented with thio-S71-phosphorylated Arpp19 or the other
230 way around, (2) first supplemented with a thio-S71-phosphorylated Arpp19 and subsequently
231 submitted to histidine-FAM122A pulldown (Figure 5a, scheme). Finally, FAM122A
232 association to B55 was measured. Strikingly, the presence of a thio-S71-phosphorylated form
233 of Arpp19 (Thio-S71-Arpp19) that cannot be dephosphorylated, induced the dissociation of
234 FAM122A from PP2A-B55 in both cases confirming our hypothesis that Arpp19 displaces
235 FAM122A from PP2A-B55 by competing for this phosphatase.

236 We also performed the reverse experiment and tested whether GST-FAM122A could
237 dissociate a p-S71-Arpp19 complexed to PP2A-B55 phosphatase. Thus, CSF extracts were
238 supplemented with His-Arpp19 protein. Because of the activated Gwl kinase present in these
239 extracts, exogenous Arpp19 is immediately phosphorylated and tightly binds PP2A-B55. p-
240 S71-Arpp19-PP2A-B55 complex was then recovered by histidine-pulldown and
241 supplemented with high doses of GST-FAM122A. Then the level of B55 bound to pArpp19
242 was measured by western blot. Interestingly, the levels of B55 bound to pArpp19 did not
243 change upon FAM122A addition indicating that FAM122A was unable to dissociate this
244 protein from PP2A-B55 complex (Figure 5b). These data indicate that p-S71-Arpp19 has a
245 much higher affinity for PP2A-B55 than FAM122A and induces its dissociation. Supporting
246 this hypothesis, our data above (Figure 4 and Figure 5b) demonstrate that endogenous Arpp19
247 is able to dissociate ectopic His-FAM122A protein from PP2A-B55 despite we estimated the
248 molarity of the former in these experiments being 215 times lower than the latter (0.1 μ M
249 endogenous Arpp19 versus 21.5 μ M ectopic His-FAM122A, Appendix Figure S2a and S2b).

250 To deeper address the different binding affinities of pArpp19 and FAM122A for
251 PP2A-B55, we performed kinetic studies directed to identify the inhibitor constants (K_i) of
252 these two proteins by measuring dephosphorylation of p-S113-Arpp19 in kinase inactivated
253 extracts supplemented with increasing doses of Thio-S71-Arpp19 or FAM122A. Inhibition of
254 PP2A-B55 by FAM122A and Thio-S71-Arpp19 was characterized by varying both the

255 concentration of the substrate p-S113-Arpp19 (from 120 to 2000 nM) and that of the
256 inhibitors FAM122A (from 0 to 250 nM) or Thio-S71-Arpp19 (from 0 to 3 nM). Of the four
257 inhibition modes tested, the competitive mode was the one fitting the experimental data with
258 the lowest reduced χ^2 value which reflects the fitting quality (Appendix Figure S3 and S4).
259 The values of the K_i were 28 ± 4.6 nM for FAM122A and 0.22 ± 0.04 nM for Thio-S-71-
260 Arpp19 with a consistent K_m for p-S113-Arpp19 of around 146 ± 23.9 nM in both series of
261 experiments (Figure 5c and Appendix Figures S3 and S4). The K_i for FAM122A was 127-fold
262 higher than the one observed for Thio-S71-Arpp19, an affinity value very close to the one that
263 we obtained by measuring p-S71-Arpp19-dependent dissociation of FAM122A in pulldown
264 assays (215-fold higher). Thus, these data confirm the higher affinity of p-S71-Arpp19 for
265 PP2A-B55 and its capacity to dissociate FAM122A of this phosphatase.

266 We next checked whether the temporal patterns of (1) association and dissociation of
267 FAM122A/PP2A-B55, (2) activation of Gwl and (3) Gwl-dependent phosphorylation of
268 Arpp19 in S71 conferring its PP2A-B55 inhibitory activity, were in line with the differential
269 K_i constants obtained for FAM122A and Thio-S71-Arpp19. Accordingly, the addition of
270 FAM122A to interphase extracts promoted the immediate presence of B55 in FAM122A
271 pulldowns (10 minutes) and its subsequent dissociation concomitantly with Gwl activation
272 (10 and 20 minutes) and Arpp19 phosphorylation (20 and 30 minutes) (Figure 5d). However,
273 this protein was re-associated again once cyclin B was degraded, Gwl was inactivated and
274 Arpp19 was dephosphorylated at mitotic exit (40 minutes). These data support that Arpp19
275 phosphorylation during mitosis promotes the dissociation of FAM122A from PP2A-B55 and
276 takes over the inhibition of this phosphatase. However, to fully confirm the physiological role
277 of FAM122A in mitotic entry, it is essential to show that ectopic FAM122A doses
278 supplemented to these extracts are close to the endogenous concentration of this inhibitor. We
279 thus tried to measure the endogenous concentration of FAM122A in *Xenopus* extracts.
280 However, unfortunately, although our antibodies recognize the ectopic protein, they were
281 unable to detect FAM122A in the extracts. Since this protein is nuclear (Li *et al*, 2020), we
282 reasoned that it was lost during the purification of oocyte cytoplasmic fraction. As an
283 alternative, we decided to compare the minimal dose of FAM122A used in the extracts to the
284 concentration of endogenous FAM122A in human cells. By comparing western blot signals of
285 recombinant His-FAM122A and RPE1 cell lysates we estimated endogenous FAM122A
286 concentration to be 59.6 ± 6.37 nM (Appendix Figure S5). We next performed dose-response
287 assays to identify the minimal dose of FAM122A inducing mitotic entry when supplemented

288 to interphase extracts using the wildtype and the phosphorylation S/T-A mutant FAM122A
289 forms. We could establish that a dose between 3 and 15 nM of ectopic wildtype FAM122A
290 was sufficient to promote mitotic entry (Figure 5e). Conversely, the S/T-A FAM122A mutant
291 minimal dose inducing mitotic entry was fixed between 30 and 75 nM suggesting that the
292 phosphorylation of this protein exerts a positive role, but is not essential for PP2A-B55
293 inhibition. Moreover, although higher than the one obtained for the wildtype protein, S/T-A
294 FAM122A mutant minimal concentration is very close to the one estimated for endogenous
295 FAM122A in human cells (30 to 75 vs 59.6 nM). All together these data are in line with a
296 first role of FAM122A in PP2A-B55 inhibition at mitotic entry that would be subsequently
297 taken over by Arpp19 once phosphorylated by active Gwl.

298

299 **The different binding of Arpp19 and FAM122A to PP2A-B55 could explain the ability of**
300 **the first inhibitor to dissociate the second from phosphatase**

301 In order to understand how Arpp19 can dissociate FAM122A from PP2A-B55, we
302 modeled the quaternary complex of PP2A with Arpp19 from three distant species (human,
303 *Xenopus* and *C. elegans*) using Alphafold_multimer (preprint: Evans *et al*, 2021) with default
304 settings and no relaxation. The resulting models looks globally similar. The molecular
305 organization of the PP2A subunits reproduces well the known crystal structure (PDB3DW8)
306 with little unfolded regions. Arpp19 (for the human and *Xenopus* complexes) and the unique
307 orthologue for Arpp19/ENSA in *C. elegans* called ENSA are mostly unfolded (Figure 6a, left
308 panel, orange) and share three common helical stretches, labelled hereafter as $\alpha 1$, $\alpha 2$ and $\alpha 3$.
309 A first helix, $\alpha 0$, in human Arpp19 and *Xenopus* Arpp19, that is not predicted in worm
310 ENSA, seems to form no interaction with PP2A and will not be further considered. On the
311 contrary, the three conserved helices ($\alpha 1$: residues 24-35, $\alpha 2$:45-54 and $\alpha 3$:60-73 in human
312 Arpp19) are predicted to interact with the phosphatase. The first two contact the B55 subunit
313 (Figure 6a, left panel, blue) while the third one points toward the catalytic site of the C
314 subunit (Figure 6a, left panel, green). No contact is detected between the Arpp19 chains with
315 the scaffolding subunit (Figure 6a, left panel, violet). The interaction with B55 mainly relies
316 on the helix $\alpha 1$ that displays tight contacts along the helix from Arpp19. The interactions
317 conserved among the three complexes includes two salt bridges (E25 and R35 from Arpp19
318 with R330 and E338 from B55 in the human complex) and hydrophobic interactions
319 involving L32 from Arpp19 and F280, F281 and C334 from B55 subunit in human. In
320 addition, two N-capping and one C-capping (involving E28, E29 and K26 in human and

321 Arpp19, respectively) are predicted. The second helix of Arpp19, α_2 , shows more loose
322 interactions and it seems to mainly bridge the helix α_1 with the third helix. Helix α_3 partially
323 overlaps with the QKYFDSGDY motif conserved in all Arpp19 and ENSA sequences and
324 containing the serine phosphorylated by Gwl. This phosphorylation is essential for strong
325 inhibition of PP2A. We modeled the phosphoserine using a dedicated server
326 (<https://isptm2.cbs.cnrs.fr> described in the "Methods" section) and also added the two
327 manganese atoms within the catalytic center of the C subunit of PP2A for further analysis.
328 Although in the human and *Xenopus* complexes, the serine is not predicted to directly interact
329 with the catalytic manganese (distance PO4-Mn: ~ 8 Å), such interactions appeared plausible
330 in the worm complex (distance PO4-Mn: ~ 5 Å). This direct contact would explain the strong
331 interaction of phosphorylated Arpp19 with PP2A-B55, **although it is worth highlighting that**
332 **the Mn^{2+} ion present in the structure obtained with recombinant PP2A-B55 enzyme does not**
333 **correspond to the $Zn^{2+}:Fe^{2+}$ divalent cation pair known to be present in the native PP2A-B55**
334 **enzyme (Brautigam & Shenolikar, 2018).** However, the inhibitory activity has been shown to
335 be not only characterized by the high affinity of Arpp19 towards PP2A-B55, but also by the
336 slow dephosphorylation of its Gwl site (Labbé *et al*, 2021). The present modeling does not
337 permit to fully understand the absence of catalytic activity on the phosphorylated serine.
338 However, this model agrees with the previously reported mutational scanning of the
339 QKYFDSGDY motif (Labbé *et al*, 2021) to suggest that a precise and peculiar orientation of
340 the phosphorylated sidechain would be required to maintain a tight but unreactive interaction.
341 Indeed, mutations to alanine of close phosphoserine neighbor residues in this sequence
342 dramatically accelerate Arpp19 dephosphorylation by PP2A-B55 while lowering binding at
343 the same time (Labbé *et al*, 2021; Williams *et al*, 2014). Additional interactions are predicted
344 at this interface in the worm complex (and partially reproduced in the human and *Xenopus*
345 assemblies) (see Figure 6b). These interactions include two salt-bridges involving the Gwl
346 site phosphoserine (S62) and the preceding aspartate residue (D61) from human Arpp19 and
347 two arginines of human PP2A C subunit (R89 and R214) lying at the entrance of the catalytic
348 site. Another salt bridge may occur between another aspartate from Arpp19 (D64 in human
349 Arpp19) and a lysine (K88) in the human B55 subunit. In addition, in the worm complex, we
350 observed the nearby and conserved phenylalanine F65 (F60 in human Arpp19) stacked in
351 between the subunits B55 and C. This residue seems to make hydrophobic contact with a
352 leucine L126 of B55 (L87 in human B55). Other contacts are not systematically predicted in
353 the three complexes but they may enhance the overall interaction between Arpp19 and the

354 PP2A phosphatase. Noteworthy, the three conserved residues surrounding the phosphorylated
355 serine, F60, D61 and D64 in human Arpp19, seems important for precise positioning of the
356 serine into the catalytic site of PP2A. This nicely explained the impact of their mutation to
357 alanine, that produce variants that are rapidly dephosphorylated by PP2A-B55 and that lose
358 their affinity for the phosphatase (Labbé *et al*, 2021).

359 Interestingly, no complexes could be predicted for the full-length FAM122A
360 inhibitor with PP2A-B55 using Alphafold 2.2. On the contrary, short segments of FAM122A
361 (from the three species used above) led to prediction of interactions between FAM122A and
362 PP2A-B55. The interface partially overlaps with that observed for PP2A-B55-Arpp19
363 complexes supporting the fact that the two inhibitors compete for PP2A-B55 binding.
364 Nevertheless, the precise interactions differ significantly. First, FAM122A does not seem to
365 point into the catalytic site as Arpp19 seems to do, although there are also two predicted salt
366 bridges between two glutamate residues from FAM122A (E100 and E104 in human
367 FAM122A) with the same arginines contacting D61 and S62 of Arpp19 of the catalytic
368 subunit of PP2A (R89 and R214 in human PP2A) (Figure 6b). These two interactions are
369 crucial since, as shown above (Figure 3f), the corresponding E90A and E94A *Xenopus*
370 FAM122A mutants are unable to bind the phosphatase. Finally, as expected, the second helix
371 of FAM122A containing the putative conserved SLiM motif, interacts with the B55 subunit
372 and this interaction takes place in a region of B55 close to the one binding $\alpha 2$ helix of
373 Arpp19.

374 In conclusion, our modeling data obtained for Arpp19 and FAM122A interaction
375 with PP2A-B55 using Alphafold multimer suggest that the two inhibitors would produce
376 analogous but distinct complexes with their common target. The size of these interfaces
377 suggests that Arpp19 would be a stronger inhibitor than FAM122A and could thus promote
378 the dissociation of the latter. This assumption is supported by newly predicted contacts with
379 PP2A-B55 likely playing a major role in the strong inhibition of this enzyme by Arpp19 and
380 that include the phosphorylation of the central serine that would tightly interact with the
381 catalytic manganese ions.

382

383 **FAM122A is required "*in vivo*" for mitotic entry in the *C. elegans* germline**

384 Our data above using ectopic FAM122A demonstrates that the addition of this protein
385 to interphase *Xenopus* egg extracts promote mitotic entry. We thus sought to assess the impact
386 of the depletion of the endogenous FAM122A from these extracts in mitotic entry. However,
387 unfortunately, [probably because we lost FAM122A during cytoplasmic extract purification](#),

388 we were unable to immunoprecipitate endogenous FAM122A. We thus used the nematode
389 *Caenorhabditis elegans* as a model to investigate the role of FAM122A in PP2A-B55
390 inhibition and mitotic entry. The gene *F46H5.2* in *C. elegans* has been previously suggested
391 as the putative orthologue of FAM122A protein(Kim *et al*, 2018). To confirm that it does
392 indeed correspond to *C. elegans* FAM-122A (Ce FAM-122A), we produced and purified this
393 protein and we checked whether it was able to inhibit PP2A-B55 and to promote mitotic entry
394 in interphase extracts. As shown in Figure 7a, dephosphorylation of S113 of Arpp19 and of
395 T481 of PRC1 by PP2A-B55 was strongly delayed by the addition of this protein. Moreover,
396 when supplemented to interphase extracts, Ce FAM-122A induced mitotic entry and exit
397 (Figure 7b). Thus, the product of the gene *F46H5.2* does correspond to the FAM122A
398 orthologue.

399 We then investigated the capacity of endogenous Ce FAM-122A to inhibit PP2A-B55
400 "*in vivo*" in the worm. To this, we first took advantage of a multivulva phenotype induced by
401 the mutant *let-60(n1046gf)*. This mutant, corresponding to a gain of function of the human
402 orthologue Ras, promotes the formation of several vulvas in the worm (Figure 7c). It has been
403 shown that the multivulva phenotype induced by *let-60(n1046gf)*(Sieburth *et al*, 1999) can be
404 attenuated by negatively modulating PP2A-B55 activity as this protein promotes Ras
405 signalling during vulva development. Accordingly, we observed a suppression of the
406 multivulva phenotype after depletion of SUR-6 (SUpressor of Ras-6, the orthologue of human
407 B55 subunit of PP2A) by RNAi. In order to assess whether endogenous Ce FAM-122A acts
408 as an inhibitor of PP2A-B55, we examined the effect of the depletion of this protein by RNAi
409 on the multivulva phenotype induced by the *let-60(n1046gf)* mutant. If this protein is a PP2A-
410 B55 inhibitor "*in vivo*", its depletion should promote the reactivation of this phosphatase and
411 thus significantly increase the presence of multivulvas in the worms. Confirming this
412 hypothesis, the depletion of Ce FAM-122A by RNAi dramatically increased this phenotype
413 (Figure 7c). Thus, Ce FAM-122A does act as an inhibitor of PP2A-B55 "*in vivo*".

414 We further explored the role of this protein in *C. elegans* in mitosis "*in vivo*", in
415 *F46H5.2^{FAM122A}(RNAi)* treated worms. Interestingly these worms exhibit a decreased
416 fecundity (Figure 7d) with a significant increased percentage of embryonic lethality at 25°C
417 (median: 8.4% in RNAi-treated vs 0.30% in controls; p<0.0001) and a double number of
418 unfertilized eggs than controls (median 26.18 % in RNAi-treated vs 12.10% in controls;
419 p<0.014) (Figure 7e). Even wildtype *C. elegans* can produce unfertilized eggs when they age
420 however, *F46H5.2^{FAM122A}(RNAi)* worms display this phenotype on their first day of
421 adulthood. These results suggest a fertility defect and led us to explore the function of the

422 reproductive system. We thus investigated cell division within the *C. elegans* germline using
423 nematode strains expressing histone and gamma-tubulin tagged with GFP under a germline
424 and embryonic promoter. We focused on mitotic division of the germline stem cells of the
425 distal gonad. Using chromatin and centrosome as proxy of mitotic progression (Gerhold *et al*,
426 2015), we counted the number of stem cells in interphase and in mitosis (Appendix Figure
427 S6). We observed a significant reduction of interphase cells and an increase in mitotic cells in
428 RNAi treated worms (Figure 8a). Interestingly, the number of cells in prophase (assessed by
429 the presence of two separating centrosomes and a circular intact nuclei), was also dramatically
430 increased, although some cells could progress into mitosis, probably due to a partial depletion
431 of FAM-122A. However, these cells displayed a perturbed mitotic progression since we
432 observed a drop of the number of anaphases (Figure 8b). These data suggest that depletion of
433 *F46H5.2* gene product decreases the capacity of germline stem cells to enter into mitosis and
434 perturbs their progression through this phase of the cell cycle. To further investigate this
435 hypothesis, we used a cell line with GFP-tagged gamma-tubulin and with RFP-tagged histone
436 in which we followed by time lapse microscopy the duration of mitosis. We considered
437 mitosis duration as the time from first centrosome separation movement starts to anaphase
438 onset. As depicted in Figure 8c, stem cells from control worms displayed a rapid mitosis, with
439 a timing around 21 minutes. Conversely, **most** Ce FAM-122A depleted stem cells, stayed with
440 separated centrosome throughout the time of the experiment (**40 to 50 minutes**) indicating that
441 these cells were arrested or highly delayed in prophase. **We observed fewer cells entering**
442 **mitosis in the FAM-122A depleted worms (Figure 8c, upper graph)**. In addition, although
443 they did not display differences in the timing from nuclear envelope breakdown to metaphase
444 (congression), they showed a significant increase of the time required for anaphase onset
445 (**Figure 8c, lower graph**). This delay is in accord with the drop of the number of anaphase in
446 RNAi-treated germline stem cells and suggests that FAM122A-dependent inhibition of PP2A-
447 B55 could not be only required for mitotic entry, but could also be essential again during
448 mitotic exit to promote anaphase onset when Gwl and cyclin B/Cdk1 become inactivated by
449 cyclin B proteolysis.

450

451 **Endogenous FAM122A proteins accumulates during G2 in human cells**

452 How cyclin B/Cdk1 is triggered at G2-M is a main question yet to be answered. To
453 activate cyclin B/Cdk1, Wee1/Myt1 and Cdc25 have to be phosphorylated. To that, an
454 increase of the activity of cyclin A/Cdk, as well as a drop of PP2A-B55 are essential. The fact
455 that FAM122A does not require phosphorylation to inhibit PP2A-B55 at the endogenous

456 concentration and that cyclin A/Cdk is a poor substrate of Myt1 kinase during G2 (Coulonval
457 *et al*, 2003; Booher *et al*, 1997) make of these two factors good candidates for triggering
458 mitosis. However, to promote G2-M transition, it is essential that these two proteins
459 accumulate during G2 to reach sufficient levels to revert cyclin B/Cdk1 inhibition.
460 Accumulation of cyclin A has been largely demonstrated in the bibliography. Much less is
461 known about the levels of FAM122A during G2. We thus measured the levels of FAM122A
462 throughout the cell cycle in RPE1 cells. Interestingly, as depicted in Figure 8d, FAM122A
463 protein accumulates during G2 reaching maximal level at G2-M transition concomitantly with
464 cyclin A accumulation. Moreover, the amount of these two proteins rapidly decrease during
465 mitotic progression supporting the hypothesis of a putative role of these two proteins in
466 triggering cyclin B/Cdk1 activation and mitotic entry

467

468 **DISCUSSION**

469 Protein phosphorylation plays a major role in the control of cell cycle progression.
470 This phosphorylation results from a fine-tuned balance between the activities of cyclin/Cdk
471 complexes and phosphatases. The modulation of the phosphatase PP2A-B55 is key for S and
472 M phases. During DNA replication, PP2A-B55 has to be inhibited to maintain the
473 phosphorylation of the replication factor Treslin responsible of origin firing, while at G2-M,
474 the negative modulation of this phosphatase is essential for cyclin B/Cdk1 activation.

475 To enter into mitosis, a partial pool of cyclin B/Cdk1 has to be turned on to induce
476 the amplification **loop that triggers** cyclin B/Cdk1 full activation via Wee1/Myt1/Cdc25
477 phosphorylation. However, Wee1/Myt1/Cdc25 phosphorylation can be also achieved if
478 PP2A-B55 is at least partially inhibited. This inhibition depends on the phosphorylation of
479 Arpp19/ENSA and thus, requires the activity of its upstream kinase Gwl. However, at M
480 entry, both Gwl and cyclin B/Cdk1 are inactive and their activation depends on each other.
481 Thus, none of them can be the initial event triggering mitosis.

482 In this study we investigated the role of FAM122A, a new identified regulator of
483 PP2A-B55, in triggering mitotic entry. Using *Xenopus* egg extracts we demonstrate that the
484 negative regulation of PP2A-B55 by FAM122A does not induce Wee1 or PP2A catalytic
485 subunit proteolysis as previously proposed (Fan *et al*, 2016; Li *et al*, 2020). Conversely, it
486 promotes mitotic entry by directly binding and inhibiting the activity of this phosphatase.
487 PP2A-B55 inhibition by FAM122A precedes Gwl and cyclin B/Cdk1 activation since **its**
488 **phosphorylation is not required** to negatively modulate the phosphatase, **although we cannot**
489 **exclude an additional positive modulation of its activity by this posttranslational modification.**

490 However, FAM122A-dependent induction of mitosis depends on cyclin A/Cdk, indicating
491 that FAM122A promotes M-Phase entry by promoting cyclin A/Cdk-dependent
492 phosphorylation. We, thus, hypothesize that the initial event triggering mitotic entry
493 corresponds to the accumulation of FAM122A and cyclin A/Cdk1 that would result in a first
494 burst of FAM122A-dependent inhibition of PP2A-B55 and the subsequent cyclin A/Cdk1-
495 dependent phosphorylation of Cdc25 and Myt1. This will in turn result in the full activation of
496 cyclin B/Cdk1 and mitotic entry (Figure 9). Accordingly, our data show that, as for cyclin
497 A/Cdk, FAM122A protein accumulates during G2, reaching a maximum level at G2-M
498 transition. We additionally show that FAM122A plays a key role in PP2A-B55 inhibition and
499 mitotic entry "in vivo" in the *C. elegans* model in which, FAM122A knockdown modulates
500 phosphatase activity during vulval development and prevents mitotic entry in germ line stem
501 cells.

502 Remarkably, a very low dose of FAM122A (15 nM) is sufficient to promote mitotic
503 entry when added to interphase extracts. This dose would correspond to about 15% of the
504 total concentration of PP2A-B55 present in these extracts (100-300 nM (Mochida *et al*,
505 2009)) suggesting that, the inhibition of a very small proportion of this phosphatase, would be
506 enough to reverse the balance towards mitotic substrate phosphorylation. Alternatively,
507 FAM122A localization could also play an essential role by locally inhibiting the phosphatase
508 at the vicinity of cyclin A/Cdk kinase permitting the phosphorylation of its substrates.

509 However, strikingly, although FAM122A clearly binds PP2A-B55 during
510 interphase, we discovered that this protein rapidly dissociates from the phosphatase as soon as
511 the extract entered into mitosis and re-associates again upon mitotic exit. Our data show that
512 this dissociation is induced by the phosphorylation of Arpp19 by Gwl. Accordingly, the
513 addition of phospho-S71-Arpp19 to interphase extracts or to FAM122A-PP2A-B55 pull-
514 downs promotes the exchange of FAM122A by phospho-S71-Arpp19 in the PP2A-B55
515 complex. These data indicate that, when phosphorylated by Gwl, Arpp19 competes with
516 FAM122A and promotes its dissociation from the phosphatase. Indeed, our data demonstrate
517 that phospho-S71-Arpp19 has a 100-fold lower K_i than FAM122A indicating that p-S71-
518 Arpp19 displays much higher affinity, inhibitory activity and binding to PP2A-B55 than
519 FAM112A. Accordingly, in our analysis, endogenous phospho-S71-Arpp19 is able to
520 dissociate ectopic His-FAM122A from PP2A-B55 even when the latter is present in the
521 extract at a molar concentration 215 times higher than the former. These properties were
522 supported by the structure predictions of FAM122A- and Arpp19-PP2A-B55 complexes
523 obtained by AlphaFold2.2. Interestingly, structures predicted for FAM122-PP2A-B55 and

524 phospho-Arpp19-PP2A-B55 complexes superposed from three different species (human,
525 *Xenopus* and *C. elegans*) revealed that FAM122A and phospho-S71-Arpp19 **inhibit the**
526 **phosphatase by competing with the substrate through directly blocking its access, a**
527 **hypothesis that is also supported by our kinetics experiments that confirm the competitive**
528 **model as the best fitting our data.** However, FAM122A and phospho-S71-Arpp19 display
529 different interactions with the PP2A-B55 heterocomplex. FAM122A weakly interacts with
530 the C subunit via two glutamic acids forming salt bridges with two arginine residues of the C
531 subunit of PP2A. These interactions locate the second alpha helix of this protein on the
532 surface of the catalytic site occluding, in this way, substrate entry. Conversely, phospho-S71-
533 Arpp19 tightly binds PP2A C mostly because of the presence of the phospho-serine residue
534 that would directly interact with manganese ions of the catalytic site, an interaction that would
535 be stabilized by two supplementary salt bridges formed with two arginine of the C subunit.
536 This tight interaction can be only dissociated upon Arpp19 dephosphorylation, however, as
537 previously reported, dephosphorylation of the Arpp19 Gwl-site is very slow compared to
538 regular substrates of this phosphatase making of this phosphoprotein a potent inhibitor of
539 PP2A-B55 (Labbé *et al*, 2021; Williams *et al*, 2014). **These data nicely explain the different**
540 **affinities for P22A-B55 of FAM122A and p-S71-Arpp19, however, it is worth highlighting**
541 **that, the 2 Mn²⁺ divalent cations present in the bacterial purified PP2A-B55 complex used for**
542 **structural studies, do not reflect the native PP2A-B55 enzyme that contains a Zn²⁺: Fe²⁺ ion**
543 **pair (Brautigam & Shenolikar, 2018), a feature that could modify the interaction of p-S71-**
544 **Arpp19 to the phosphatase. However, during the review process of this manuscript, new cryo-**
545 **EM structures of the complex of FAM122A or Arpp19 with purified PP2A-B55 from human**
546 **cells were published (Padi *et al*, 2024). Interestingly, these structural results confirm our**
547 **modelling predictions and kinetic data and reinforce the idea that FAM122A and Arpp19**
548 **interphases with PP2A-B55 partially overlap. Moreover, according with our model, data also**
549 **support a role of the phosphorylation of Arpp19 central serine in tightly interacting with the**
550 **native ions of the catalytic site of the C subunit providing a stronger phosphatase inhibitory**
551 **activity to this protein compared to FAM122A. However, besides cryo-EM results, authors**
552 **also presented NMR data suggesting a simultaneously binding of a C-terminal truncated**
553 **FAM122A and Arpp19 with a loopless B55 form, a B55 variant unable to bind PP2A A**
554 **subunit. However, whether this trimer can be also observed with the wildtype Arpp19 and**
555 **FAM122A and the functional PP2A-B55 heterocomplex is not clear. Indeed, these would be**
556 **very surprising since, as supported by the authors themselves, these two inhibitors interact**
557 **with the same or very close residues of B55 and C subunits of the phosphatase making highly**

558 unlikely the presence of a Arpp19/FAM122A/PP2A-B55 trimer. In this line, our data clearly
559 establish a dissociation of FAM122A from PP2A-B55 when both proteins are incubated with
560 the phosphatase both in *in vitro* pulldown experiments and *in extracto*.

561 Importantly, our data additionally show that FAM122A is dissociated from PP2A-
562 B55 by phospho-Arpp19 (S71) during mitosis. Although we do not know what could be the
563 role of this dissociation, we predict that it could be physiological relevant and required to
564 induce a correct mitotic progression. In this line, it is possible that a higher PP2A-B55
565 inhibitory activity is required during mitotic progression to permit the late mitotic substrate
566 phosphorylation. In agreement with this hypothesis, previous data demonstrate that cyclin
567 B1/Cdk1 levels required to enter mitosis are lower than the amount of cyclin B1–Cdk1
568 needed for mitotic progression (Lindqvist *et al*, 2007). Moreover, besides the high inhibitory
569 activity of phospho-S71-Arpp19, the fact that its dissociation from PP2A-B55 is gradually
570 induced by dephosphorylation could be also essential to establish the temporal pattern of
571 mitotic substrate dephosphorylation required for the correct order of mitotic events during
572 mitotic exit.

573 In summary, we identified a new role of FAM122A in promoting G2-M transition
574 through the inhibition of PP2A-B55 and we provided data indicating that cyclin A/Cdk,
575 together with FAM122A-dependent inhibition of PP2A-B55 promote Gwl and cyclin B/Cdk1
576 activation and trigger mitotic entry. Finally, we showed that, upon mitotic entry, FAM122A is
577 dissociated from PP2A-B55 by phospho-Arpp19 until the time when Gwl inactivation and
578 Arpp19 becomes fully dephosphorylated permitting to FAM122A to re-associate again to the
579 phosphatase.

580

581 MATERIALS & METHODS

582 “*In vitro*” phosphorylation

583 Phosphorylation of Arpp19 on S71 or of *Xenopus* FAM122A by Gwl was induced by using
584 GST-K72M hyperactive mutant form of Gwl purified from SF9 cells. For “*in vitro*”
585 phosphorylation reaction, *Xenopus* His-Arpp19 protein or His-FAM122A and GST-K72M
586 Gwl kinase were mixed at a final concentration of 133 and 67 μM for the two first proteins
587 and 0.34 μM for the last one in a reaction buffer containing 7×10^{-2} μM ATP $\gamma^{33}\text{P}$ at a specific
588 activity of 3000 Ci/ mmol, 200 μM ATP, 2 mM MgCl_2 and Hepes 50 mM, pH 7,5.

589 For phosphorylation of Arpp19 on S113 by PKA, a final concentration of 33 μM of Arpp19
590 and 1.25 μM of His-PKA catalytic subunit purified from His-tag column were mixed to the

591 reaction buffer containing 8×10^{-3} μM $\text{ATP}\gamma^{33}\text{P}$ at a specific activity of 3000 Ci/ mmol, 200
592 μM ATP, 2 mM MgCl_2 , 100 mM NaCl and 50 mM Tris pH 7.5.

593 For phosphorylation of GST-PRC1 on T481, Cdk1 activity was obtained by
594 immunoprecipitation. In brief, His-human cyclin A was mixed with CSF extracts at a final
595 concentration of 0.2 μM for 30 minutes and subsequently supplemented with 50 μl of protein
596 G magnetic Dynabeads pre-linked with 10 μg of *Xenopus* Cdk1 C-terminus antibodies. After
597 45 minute-incubation the beads were washed 3 times with 500 mM NaCl, 50 mM Tris pH 7.5,
598 twice with 100 mM NaCl, 50 mM Tris pH 7.5 and finally resuspended with 100 μl of reaction
599 buffer containing 1mM ATP, 2 mM MgCl_2 , 100 mM NaCl and 50 mM Tris pH 7.5. GST-
600 PRC1 was then added to the beads at a final concentration of 21 μM .

601 For thio-phosphorylation of Arpp19 on S71, His-tag of His-Arpp19 protein was removed
602 using TEV protease. Non-tagged Arpp19 was then used for phosphorylation at a final
603 concentration of 66 μM in presence of 1mM $\text{ATP}\gamma\text{S}$, 2 mM MgCl_2 , 100 mM NaCl, 50 mM
604 Tris pH 7.5 and 0.34 μM of GST-K72M Gwl kinase.

605 All “*in vitro*” phosphorylation reactions were incubated for 1 h, aliquoted and frozen at -70°C
606 until use.

607

608 **Dephosphorylation reactions in kinase inactivated extracts**

609 When the impact of *Xenopus*, human, or *C. elegans* FAM122A proteins on either S113
610 Arpp19 or T481 PRC1 dephosphorylation was checked in ATP-devoid interphase extracts, 1
611 μl of the corresponding “*in vitro*” phosphorylation reaction was mixed to 1 μl His-
612 FAM122A to a final concentration of 14.3 μM , diluted with 8 μl of Tris 50 mM-10 mM
613 EDTA buffer and supplemented with 10 μl of ATP-devoid interphase extracts adjusted to 500
614 mM NaCl with a solution of 5M NaCl and a sample of 2 μl was recovered at the indicated
615 time-points.

616

617 **Immunoprecipitation/Immunodepletion**

618 Immunodepletions were performed using 20 μl of extracts, 20 μl of G-magnetic Dynabeads
619 (Life Technologies), and 2 μg of each antibody except for cyclin A for which we used 3.3 μl .
620 Antibody-linked beads were washed 2 times with XB buffer, 2 times with Tris 50 mM, pH
621 7.5 and incubated for 15 min at room temperature (RT) with 20 μl of *Xenopus* egg extracts.
622 The supernatant was recovered and used for subsequent experiments. To fully deplete
623 endogenous proteins, two rounds of immunodepletion were performed.

624 For Gwl rescue experiments, a final concentration of 19.2 nM of K72M GWL was added to
625 depleted egg extracts and a sample of 2 µl was recovered and used for western blot.

626 For histidine pulldown experiments, 20 µl of interphase or CSF extracts containing His-
627 *Xenopus* or His-human FAM122A proteins, were supplemented with 20 µl of
628 HisPurTMNiNTA Magnetic beads (Life Technologies) at a final concentration of 7.15 µM and
629 incubated for 10 minutes of continuous mixing at 21°C. Upon centrifugation, beads are
630 washed twice with XB buffer and supplemented with Laemmli sample buffer for western blot
631 use.

632

633 **Protein purification**

634 His-*Xenopus* Arpp19, His-*Xenopus* wildtype and mutant of FAM122A in which all serine and
635 threonine residues were mutated into alanine, as well as, His-human FAM122A, His-*C*
636 *elegans* FAM122A, His-human PRC1, and His-Rat Catalytic Subunit of PKA were produced
637 in *Escherichia coli* and purified using TALON Superflow Metal Affinity Resin. GST-
638 *Xenopus* FAM122A protein was produced in *Escherichia coli* and purified using a glutathione
639 column.

640

641 **CSF, interphase and kinase-inactivated egg extracts**

642 Frogs were obtained from « TEFOR Paris-Saclay,
643 CNRS UMS2010 / INRAE UMS1451, Université Paris-Saclay», France and kept in a
644 *Xenopus* research facility at the CRBM (Facility Centre approved by the French Government.
645 Approval n° C3417239). Females were injected of 500 U Chorulon (Human Chorionic
646 Gonadotrophin) and 18 h later laid oocytes were used for experiments. Adult females were
647 exclusively used to obtain eggs. All procedures were approved by the Direction Generale de
648 la Recherche et Innovation, Ministère de L'Enseignement Supérieur de la l'Innovation of
649 France (Approval n° APAFIS#40182-202301031124273v4).

650 Metaphase II-arrested egg extracts (CSF extracts) were obtained by crushing metaphase II-
651 arrested oocytes in the presence of EGTA at a final concentration of 5 mM(Lorca *et al*, 2010).

652 Interphase *Xenopus* egg extracts were obtained from metaphase II-arrested oocytes 35
653 minutes after Ca²⁺ Ionophore (final concentration 2 µg/ml) treatment.

654 To measure the capacity of FAM122A to promote mitotic entry, 20 µl of CSF or Interphase
655 extracts were supplemented with a final concentration of 7.15 µM of *Xenopus*, human or *C*
656 *elegans* FAM122A proteins.

657 For dephosphorylation assays kinase inactivated interphase extracts were mixed with Arpp19
658 or PRC1 proteins "*in vitro*" phosphorylated by PKA or by cyclin A/Cdk1 respectively at a
659 final concentration of 1.65 μ M together with *Xenopus* or human FAM122A at a final
660 concentration of 14.3 μ M.

661 Kinase-inactivated egg extracts were obtained from dejellied unfertilised eggs transferred into
662 MMR solution (25 mM NaCl, 0.5 mM KCl, 0.25 MgCl₂, 0.025 mM NaEGTA, 1.25 mM
663 HEPES-NaOH pH7.7), washed twice with XB Buffer (50 mM sucrose, 0.1 mM CaCl₂, 1 mM
664 MgCl₂, 100 mM KCl, HEPES pH 7.8) and centrifuged twice for 20 minutes at 10 000 g.
665 Cytoplasmic fractions were then recovered, supplemented with RNase (10 μ g/ml final
666 concentration) and dialyzed versus a solution of 50 mM Tris pH 7.7, 100 mM NaCl overnight
667 to eliminate ATP. Upon dialysis, extracts were ultracentrifuged for 50' at 300 000 g and
668 supernatant recovered for use.

669

670 **Calculation the K_i values for FAM122A and p-S71-Arpp19 inhibitors**

671 Arpp19 phosphorylated on S113 by PKA was mixed with kinase inactivated interphase
672 extracts at final concentrations of 120, 180, 300, 600, 1200 and 2000 nM. Since at lower
673 concentrations of p-S113-Arpp19, the substrate is more rapidly dephosphorylated, a dilution
674 of the PP2A-B55 phosphatase was required to record sufficient dephosphorylation
675 experimental points on these initial linear phases. We thus diluted the extracts from 1/50 to
676 1/200 when the concentration of p-S113-Arpp19 varied from 120 to 2000 nM. Upon substrate
677 addition to the extracts, an aliquot was taken every one or two minutes until 7 or 14 minutes
678 respectively, depending on the substrate concentration. The reaction was stopped by adding
679 Laemmli blue buffer and heating for 5 minutes at 90°C.

680 Dephosphorylation was measured by western blot using anti-pS113-Arpp19 antibody, a
681 secondary goat anti-rabbit DyLight 800 conjugated antibody and quantified by Li-cor Odyssey
682 M System. Inhibition constants (K_i) were determined by repeating these experiments at
683 different concentrations of FAM122A (50, 125 and 250 nM) or Thio-S71-Arpp19 (1, 2 and 3
684 nM). Values of the steady state rate constants, k_{ss} , were determined using GraFit 7.0.3
685 software (Erithacus software) from the slope of the initial linear phases of the reaction time
686 courses. The actual sampling times were normalized by dividing by the enzyme dilution
687 factor, so that all experiments could be combined for global fitting procedures. Global fittings
688 were performed on the raw data with GraFit 7.0.3 software using four different inhibition
689 modes: competitive, non-competitive, mixed and uncompetitive. Times courses and

690 secondary curves and the corresponding Lineweaver-Burk representations are shown as
691 Appendix Materials (Appendix Figures S3 and S4).

692

693 **Plasmids**

694 *Xenopus* wildtype and the $\Delta(1-73)$ and serine/threonine-to-alanine mutant form (accession
695 number NP_001085566.1) cDNAs, as well as human (accession number NP_612206.5), and
696 *C. elegans* (accession number NP_001024675.1) cDNAs were synthesized by GeneCust
697 (France) and subcloned into the HindIII-XhoI site of pET14b for human FAM122A, into the
698 NdeI-BamHI site of pET14b for *C. elegans*, into the XhoI-BamHI site of pET14b for *Xenopus*
699 wildtype and mutant forms and into the BamHI-XhoI site of pGEX4T1 for wildtype *Xenopus*
700 FAM122A.

701

702 **Antibodies**

703 *Xenopus*, human and *C. elegans* FAM122A protein was detected using anti-histidine
704 antibodies. Antibodies used in this study are detailed in Appendix Table S1.

705

706 **Mutagenesis**

707 Deletions and single-point mutations of *Xenopus* FAM122A were performed using Pfu ultra
708 II fusion DNA polymerase. Oligonucleotides were purchased from Eurogentec and are
709 detailed in the Appendix Table S2.

710

711 ***C. elegans* culture and RNAi mediated depletion.**

712 *C. elegans* worm strains N2 (wildtype ancestral, Bristol) and MT2124 (*let-60(gf)*) were
713 obtained from the CGC (<https://cgc.umn.edu>) and maintained at 20 °C on NGM plate using
714 standard procedures(Brenner, 1974) except that worms were fed with HT115 bacteria to
715 standardize their growth condition with the RNAi mediated depletion. RNAi feeding was
716 performed as described previously (Kamath *et al*, 2001). HT115 thermocompetent *E. coli*
717 were transformed with L4440 empty vector (control) or containing a sequence targeting,
718 SUR-6^{PP2AB} or F46H5.2^{FAM122A}. The L4440 vector targeting F46H5.2^{FAM122A} was generated
719 using a fragment amplified from *C. elegans* cDNA corresponding to 715 bp of the coding
720 sequence (nucleotides 398 to 1112) which was subsequently inserted between XhoI and NotI
721 restriction enzyme sites in L4440 vector. Control, SUR-6^{PP2AB} vector come from the
722 Arhinger's library. In a 14 ml culture tubes, 2 ml of LB medium supplemented with 100
723 $\mu\text{g/mL}$ of ampicillin were inoculated with a colony of HT115 bacteria transformed with

724 respective L4440 vectors and incubated at 37 °C under agitation. After 7 h, 200 µl of this
725 bacterial culture was transferred on a 60 mm diameter NGM plate containing 0.2 mM IPTG
726 and 50 µg/ml of carbenicillin. Plates were allowed to dry overnight at room temperature,
727 stored at 4 °C and used within 48 h. Worms were synchronized using the alkaline bleach
728 method (1.2% NaOCl, 250 mM KOH in water (Stiernagle, 2006)). Eggs obtained from the
729 alkaline bleach were allowed to hatch overnight at 16 °C in M9 buffer. To score FAM122A
730 effect on Ras (*let-60(gf)*) induced multivulva phenotype, around 200 synchronized larvae
731 were placed on a single feeding plate and incubated 66-72 h at 20 °C until control worms
732 reach adulthood. Phenotypes were scored at the time where the first eggs laid by control
733 worms started to hatch to ensure that all worms fully develop to the adult stage. Vulval defect
734 phenotypes was scored under a dissecting scope by counting the total number of worms and
735 the number of worms exhibiting multiple vulva. The counting was repeated once for each
736 plate to minimize scoring errors. Representative images of vulval phenotypes were acquired
737 with a sCMOS ZYLA 4.2 M camera on a Zeiss Axioimager Z2 and a 20X Plan Apochromat
738 0.8 NA using worms immobilized with a solution of 10 mM sodium azide and mounted on an
739 3 % agarose pad in between a microscopy slide and a cover glass.

740

741 **Live imaging of *C. elegans***

742 For "*in vivo*" imaging, we used nematode strains expressing GFP-gamma-tubulin and GFP-
743 histone (TH32: *unc-119(ed3)*, *ruIs32[pAZ132; pie-1/GFP::histone H2B]* III; *ddIs6[GFP::tbg-*
744 *1; unc-119(+)]* V or GFP-tubulin and histone-mCherry (JDU19: *ijmSi7 [pJD348; Pmex-*
745 *5_gfp::tbb-2; mCherry::his-11; cb-unc-119(+)]* I; *unc-119(ed3)* III) and JDU233: *ijmSi63*
746 *[pJD520; mosII_5'mex-5_GFP::tba-2; mCherry::his-11; cb-unc-119(+)]* II; *unc-119(ed3)*
747 III) kindly provided by Julien Dumont (Institut Jacques Monod, Paris). Worms were cultured
748 at 25 °C and bacterial feeding was performed similarly to the multivulva experiment, except
749 that worms were not synchronized by alkaline bleach. Instead, 6 worms were allowed to lay
750 eggs on a 60 mm diameter NGM feeding plate. After 2 h at 25 °C adults were removed and
751 their progeny was allowed to develop on the feeding plate and at 25 °C for 44-52 h until the
752 first adults start to lay eggs (young adults). Worms were anesthetized in 0.02 % tetramisole in
753 M9 buffer for 10 min before being transferred onto a 4 % agarose pad (Laband *et al*, 2018) .
754 The pad was then covered with 22x22 mm coverslip and sealed with VaLaP (vaseline and
755 paraffin wax and lanolin, 1:1:1). The chamber was filled with M9 to prevent drying and to
756 dilute tetramisole. Immobilized worms were imaged for a maximal time of 50 min and were
757 not maintained more than one hour after being removed from their feeding plate. Germline

758 stem cell division imaging was performed according to Gerhold et al.(Gerhold *et al*, 2015).
759 To minimize toxicity, we used minimal laser intensity and exposure using a spinning disk
760 confocal Yokogawa W1 on an Olympus inverted microscope coupled to a sCMOS Fusion BT
761 Hamamatsu camera. Long-term live imaging (up to 50 min) was performed with a 30x
762 UPLSAPO 1.05 NA DT 0.73 mm silicone objective by taking a full z-stack of the entire
763 worms (~40 μm) with 2 μm steps every 20 s or 30 s. For higher resolution images and to
764 score mitotic phases using centrosome and chromatin markers, a single z-stack was performed
765 on each worm with a 1 μm z-section and using a 60x UPLSAPO 1.3 NA DT 0.3 mm silicone
766 objective.

767

768 **Analysis of germline stem cells divisions.**

769 Images and movies were visualized using ImageJ or CellSens visualization software
770 (OlyVIA, Olympus). Analysis of germline stem cells divisions was made following a
771 previous article from Gerhold et al. (Gerhold *et al*, 2015). Accordingly, timing of prophase
772 corresponds to the initiation of centrosome separation (GFP-tubulin) to the beginning of
773 chromosome congression monitored using the mCherry-histone. As ovulation generates
774 movement within the gonad arm, cells can move in 3D, therefore tracking of individual cell
775 progression was performed by navigating throughout z-stack over time. Representative
776 images and montages in the Figure 8 correspond to a maximal intensity projection of 4-5 z-
777 sections (8-10 μm). To quantify the number of cells in each respective cell cycle phases, we
778 used GFP-histone and GFP-gamma-tubulin signal to monitor centrosome separation, nuclear
779 envelope breakdown, chromosome congression and separation. The number of stem cells in
780 the mitotic zone of the distal gonad was estimated by counting the number of nuclei based on
781 histone signal. Here, cells were considered in prophase when having 2 separating or opposed
782 centrosomes (spots) closed to nuclei or condensed chromosomes. Metaphase and anaphase
783 correspond respectively to a single plate or two separated plates of tightly condensed
784 chromosomes.

785

786 **Protein structure modelling**

787 Complexes of PP2A-B55 with Arpp19/ENSA or FAM122A were produced using
788 AlphaFold_Multimer version 2.2 (preprint: Evans *et al*, 2021) with the protein sequences from
789 three species (*C. elegans*, *X. laevis* and *H. sapiens*). Manganese ions were added to the
790 resulting models by superposition and extraction from the crystal structure of PP2A
791 (PDB3DW8). The phosphoserine was modelled by superposing a phosphoserine onto the

792 corresponding serine in each complex of interest and searching for the best orientation using
793 our recent webserver ISPTM2 (<https://isptm2.cbs.cnrs.fr>). Because full-length FAM122A
794 sequences led to no interactions as predicted by Alphafold 2.2, truncated version of
795 FAM122A were used instead. For the human, *Xenopus*, and worm sequences, the shortened
796 versions comprised residues 84 to 117, 61 to 120 and 70 to 149, respectively.

797

798 **Cell culture and synchronization.**

799 RPE1 cells were maintained adherent using standard cell culture procedures in DMEM-F12
800 medium supplemented with 10 mM HEPES pH 7.2, 10% of fetal bovine serum and antibiotics
801 (penicillin, streptomycin). Synchronization of cells at the G1/S boundary was achieved by a
802 double thymidine block. In brief, cells were plated at 14,000 cells per cm² in 6 cm diameter
803 petri dishes and, 24 h later, incubated for 18 h in medium containing 2 mM thymidine. Cells
804 were then washed twice with 1xPBS to remove excess of thymidine and fresh medium
805 containing 25 μM of 2'-deoxycytidine was added for 8-9 h. Cells were washed twice with
806 1xPBS and a second block was performed by adding medium containing 2 mM of thymidine
807 for 18h. Release of G1/S arrested cells was performed by washing twice with 1xPBS and
808 exchanging the medium for fresh medium containing 25 μM of 2'-deoxycytidine. Cells were
809 collected at 0, 4, 6, 7, 8 and 9 h after release. Cells were collected and analysed by western
810 blot analysis and flow cytometry (FACS). For western blot analysis, 40 μg of total protein
811 were loaded and separated by SDS-PAGE using 10% acrylamide gels. Proteins were then
812 transferred onto a PVDF membrane compatible with fluorescent analysis. Protein amounts
813 were measured by western blot using polyclonal anti-FAM122A (1/1000) and anti-cyclin A
814 (1/2000) and monoclonal anti-βTubulin (1/2000) primary antibodies and with goat anti-rabbit
815 Dylight 680 conjugated and goat anti-mouse Dylight 800 conjugated fluorescent secondary
816 antibodies and quantified by Li-cor Odyssey M System. For cell sorting analysis, after ice-cold
817 70% ethanol fixation at -20°C, cells were resuspended and incubated for 5 min in 500 μL of
818 0.4% TritonTX100, 3% BSA in 1xPBS for cell permeabilization. DNA staining was then
819 performed by adding 300 μL of 1xPBS containing 3% BSA, 0,01 μg/mL of 7-AAD and 0.01
820 mg/mL RNase A. Cells were incubated in the dark for one hour at room temperature, washed
821 2 times with 1xPBS supplemented with 3% BSA by centrifugation (3 min at 400xg) and
822 resuspend in 130 μL prior to cell sorting using Novocyte flow cytometer system.

823

824 **Data Availability**

825 Live imaging data sets used in this manuscript are available on BioImage Archive with the
826 accession number: S-BIAD984. [https://www.ebi.ac.uk/biostudies/bioimages/studies/S-](https://www.ebi.ac.uk/biostudies/bioimages/studies/S-BIAD984)
827 [BIAD984](https://www.ebi.ac.uk/biostudies/bioimages/studies/S-BIAD984).

828

829 **ACKNOWLEDGMENTS**

830 We are grateful to Marc Plays and Phillipe Richard for animal and antibody production and to
831 MRI for microscopy facility. We thank Julien Dumont (Institut Jacques Monod, Paris) for
832 kindly providing GFP-gamma-tubulin and GFP-histone nematode strains and Lucie Van
833 Hove, Sylvain Roque, Morgane Robert, Lucie Van Hove (LP lab) and Celia Benchoug for
834 their technical help. This work was supported by the Agence National de la Recherche
835 (REPLIGREAT, ANR-18-CE13-0018-01, MITODISSECT, ANR-22-0022 and MTDiSco,
836 ANR-20-CE13-0033), La Ligue Nationale Contre le Cancer (Equipe Labellisée, EL2019
837 CASTRO and EL2018 PINTARD), Ligue Nationale Contre le Cancer (Comité Département
838 66/ LACROIX), the French Infrastructure for Integrated Structural Biology (FRISBI, ANR-
839 10-INSB-005 and the infrastructure ChemBioFrance. Some nematode strains were provided
840 by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40
841 OD010440).

842

843 **Disclosure and competing interests statement**

844 The authors declare no competing interests.

845

846

847 **REFERENCES**

848 Alvarez-Fernandez M, Sanchez-Martinez R, Sanz-Castillo B, Gan PP, Sanz-Flores M,
849 Trakala M, Ruiz-Torres M, Lorca T, Castro A & Malumbres M (2013) Greatwall is essential
850 to prevent mitotic collapse after nuclear envelope breakdown in mammals. *Proceedings of the*
851 *National Academy of Sciences of the United States of America* 110: 17374–9

852 Blake-Hodek KA, Williams BC, Zhao Y, Castilho PV, Chen W, Mao Y, Yamamoto TM &
853 Goldberg ML (2012) Determinants for activation of the atypical AGC kinase Greatwall
854 during M phase entry. *Molecular and cellular biology* 32: 1337–53

855 Booher RN, Holman PS & Fattaey A (1997) Human Myt1 Is a Cell Cycle-regulated Kinase
856 That Inhibits Cdc2 but Not Cdk2 Activity*. *Journal of Biological Chemistry* 272: 22300–
857 22306

858 Brautigan DL & Shenolikar S (2018) Protein Serine/Threonine Phosphatases: Keys to

859 Unlocking Regulators and Substrates. *Annual Review of Biochemistry* 87: 921–964
860 Brenner S (1974) The genetics of *Caenorhabditis elegans*. *Genetics* 77: 71–94
861 Burgess A, Vigneron S, Brioudes E, Labbe JC, Lorca T & Castro A (2010) Loss of human
862 Greatwall results in G2 arrest and multiple mitotic defects due to deregulation of the cyclin B-
863 Cdc2/PP2A balance. *Proceedings of the National Academy of Sciences of the United States of*
864 *America* 107: 12564–9
865 Coulonval K, Bockstaele L, Paternot S & Roger PP (2003) Phosphorylations of cyclin-
866 dependent kinase 2 revisited using two-dimensional gel electrophoresis. *J Biol Chem* 278:
867 52052–52060
868 Cundell MJ, Bastos RN, Zhang T, Holder J, Gruneberg U, Novak B & Barr FA (2013) The
869 BEG (PP2A-B55/ENSA/Greatwall) Pathway Ensures Cytokinesis follows Chromosome
870 Separation. *Molecular Cell* 52: 393–405
871 Evans R, O’Neill M, Pritzel A, Antropova N, Senior A, Green T, Žídek A, Bates R, Blackwell
872 S, Yim J, *et al* (2021) Protein complex prediction with AlphaFold-Multimer.
873 2021.10.04.463034 doi:10.1101/2021.10.04.463034 [PREPRINT]
874 Fan L, Liu M-H, Guo M, Hu C-X, Yan Z-W, Chen J, Chen G-Q & Huang Y (2016)
875 FAM122A, a new endogenous inhibitor of protein phosphatase 2A. *Oncotarget* 7: 63887–
876 63900
877 Fowle H, Zhao Z, Xu Q, Wasserman JS, Wang X, Adeyemi M, Feiser F, Kurimchak AN,
878 Atar D, McEwan BC, *et al* (2021) PP2A/B55 α substrate recruitment as defined by the
879 retinoblastoma-related protein p107. *eLife* 10: e63181
880 Gerhold AR, Ryan J, Vallée-Trudeau J-N, Dorn JF, Labbé J-C & Maddox PS (2015)
881 Investigating the Regulation of Stem and Progenitor Cell Mitotic Progression by In Situ
882 Imaging. *Current Biology* 25: 1123–1134
883 Gharbi-Ayachi A, Labbé J-C, Burgess A, Vigneron S, Strub J-M, Brioudes E, Van-Dorselaer
884 A, Castro A & Lorca T (2010) The Substrate of Greatwall Kinase, Arpp19, Controls Mitosis
885 by Inhibiting Protein Phosphatase 2A. *Science* 330: 1673–1677
886 Hached K, Goguet P, Charrasse S, Vigneron S, Sacristan MP, Lorca T & Castro A (2019)
887 ENSA and ARPP19 differentially control cell cycle progression and development. *The*
888 *Journal of Cell Biology* 218: 541–558
889 Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG & Ahringer J (2001) Effectiveness
890 of specific RNA-mediated interference through ingested double-stranded RNA in
891 *Caenorhabditis elegans*. *Genome Biol* 2: RESEARCH0002
892 Kim W, Underwood RS, Greenwald I & Shaye DD (2018) OrthoList 2: A New Comparative

893 Genomic Analysis of Human and *Caenorhabditis elegans* Genes. *Genetics* 210: 445–461
894 Laband K, Lacroix B, Edwards F, Canman JC & Dumont J (2018) Live imaging of *C. elegans*
895 oocytes and early embryos. *Methods Cell Biol* 145: 217–236
896 Labbé JC, Vigneron S, Méchali F, Robert P, Roque S, Genoud C, Goguuet-Rubio P, Barthe P,
897 Labesse G, Cohen-Gonsaud M, *et al* (2021) The study of the determinants controlling Arpp19
898 phosphatase-inhibitory activity reveals an Arpp19/PP2A-B55 feedback loop. *Nat Commun*
899 12: 3565
900 Lemonnier T, Daldello EM, Poulhe R, Le T, Miot M, Jesus C & Dupré A (2019) The M-
901 phase regulatory phosphatase PP2A-B55 δ opposes protein kinase A on Arpp19 to initiate
902 meiotic division *Cell Biology*
903 Li F, Kozono D, Deraska P, Branigan T, Dunn C, Zheng X-F, Parmar K, Nguyen H,
904 DeCaprio J, Shapiro GI, *et al* (2020) CHK1 Inhibitor Blocks Phosphorylation of FAM122A
905 and Promotes Replication Stress. *Mol Cell* 80: 410-422.e6
906 Lindqvist A, Zon W van, Rosenthal CK & Wolthuis RMF (2007) Cyclin B1–Cdk1 Activation
907 Continues after Centrosome Separation to Control Mitotic Progression. *PLOS Biology* 5: e123
908 Lorca T, Bernis C, Vigneron S, Burgess A, Brioude E, Labbe JC & Castro A (2010)
909 Constant regulation of both the MPF amplification loop and the Greatwall-PP2A pathway is
910 required for metaphase II arrest and correct entry into the first embryonic cell cycle. *Journal*
911 *of cell science* 123: 2281–91
912 Mochida S, Ieko S, Gannon J & Hunt T (2009) Regulated activity of PP2A-B55 delta is
913 crucial for controlling entry into and exit from mitosis in *Xenopus* egg extracts. *The EMBO*
914 *journal* 28: 2777–85
915 Mochida S, Maslen SL, Skehel M & Hunt T (2010) Greatwall Phosphorylates an Inhibitor of
916 Protein Phosphatase 2A That Is Essential for Mitosis. *Science* 330: 1670–1673
917 Padi SKR, Vos MR, Godek RJ, Fuller JR, Kruse T, Hein JB, Nilsson J, Kelker MS, Page R &
918 Peti W (2024) Cryo-EM structures of PP2A:B55–FAM122A and PP2A:B55–ARPP19.
919 *Nature* 625: 195–203
920 Pomerening JR, Sontag ED & Ferrell JE (2003) Building a cell cycle oscillator: hysteresis and
921 bistability in the activation of Cdc2. *Nat Cell Biol* 5: 346–351
922 Sha W, Moore J, Chen K, Lassaletta AD, Yi C-S, Tyson JJ & Sible JC (2003) Hysteresis
923 drives cell-cycle transitions in *Xenopus laevis* egg extracts. *Proceedings of the National*
924 *Academy of Sciences* 100: 975–980
925 Sieburth DS, Sundaram M, Howard RM & Han M (1999) A PP2A regulatory subunit
926 positively regulates Ras-mediated signaling during *Caenorhabditis elegans* vulval induction.

927 *Genes Dev* 13: 2562–2569
928 Stiernagle T (2006) Maintenance of *C. elegans*. *WormBook*: 1–11
929 Vigneron S, Brioude E, Burgess A, Labbe JC, Lorca T & Castro A (2009) Greatwall
930 maintains mitosis through regulation of PP2A. *EMBO J* 28: 2786–93
931 Vigneron S, Gharbi-Ayachi A, Raymond A-A, Burgess A, Labbe J-C, Labesse G, Monsarrat
932 B, Lorca T & Castro A (2011) Characterization of the Mechanisms Controlling Greatwall
933 Activity. *Molecular and Cellular Biology* 31: 2262–2275
934 Vigneron S, Sundermann L, Labbé J-C, Pintard L, Radulescu O, Castro A & Lorca T (2018)
935 Cyclin A-cdk1-Dependent Phosphorylation of Bora Is the Triggering Factor Promoting
936 Mitotic Entry. *Dev Cell* 45: 637-650.e7
937 Williams BC, Filter JJ, Blake-Hodek KA, Wadzinski BE, Fuda NJ, Shalloway D & Goldberg
938 ML (2014) Greatwall-phosphorylated Endosulfine is both an inhibitor and a substrate of
939 PP2A-B55 heterotrimers. *eLife* 3: e01695

940

941 **FIGURE LEGENDS**

942 **Figure 1. PP2A-B55 inhibition by FAM122A does not involve changes in PP2A-C or**
943 **Wee1 levels.** (a) 20 µl of interphase extracts were supplemented with a final concentration of
944 7.15 µM of *Xenopus* or of human FAM122A and 0,8 µl of the mix was recovered at different
945 time points to measure the indicated proteins by western blot. (b) Arpp19 or PRC1 proteins
946 "in vitro" phosphorylated by PKA or by cyclin A/Cdk1 respectively were supplemented at a
947 final concentration of 1.65 µM together with *Xenopus* or human FAM122A (final
948 concentration of 14.3 µM) to kinase inactivated interphase extracts and the dephosphorylation
949 rate of S113 of Arpp19 and T481 of PRC1 as well as the levels of *Xenopus* and human
950 FAM122A were analysed by western blot. (c) Interphase extracts were supplemented
951 (Xe/HuFAM-PD) or not (CT-PD) with either *Xenopus* or human FAM122A as in (a) and used
952 for histidine pull-down. The levels of PP2A B55, A and C subunits as well as the amount of
953 FAM122A were checked by western blot.

954

955 **Figure 2. FAM122A triggers mitotic entry by inhibiting PP2A-B55 and permitting cyclin**
956 **A/Cdk-dependent phosphorylation of mitotic substrates.** (a) Extracts were treated as for
957 Figure 1a and the levels of the indicated proteins as well as the phosphorylation of Cdk1 on
958 tyrosine 15 were checked by western blot. (b) His-wildtype Arpp19, the Gwl phosphorylation
959 site mutant (Serine 71 -to-Alanine) of this protein, His-human FAM122A and His-*Xenopus*

960 FAM122A were phosphorylated "*in vitro*" by GST-human GwlK72M hyperactive mutant in a
961 final phosphorylation reaction mixture of 10 μ l. 5 μ l were then used for western blot using
962 anti-histidine antibodies to detect Arpp19 and human and *Xenopus* FAM122A levels or anti-
963 Gwl antibodies to detect Gwl amount. The rest was used to detect γ^{33} P by autoradiography.
964 (c) Interphase extracts were depleted with a control or anti-Gwl antibodies and supplemented
965 with Xe FAM122A. Samples were then analysed over time for the levels and phosphorylation
966 of the indicated proteins. (d) Interphase extracts were supplemented with the *Xenopus*
967 FAM122A protein in which all serine/threonine residues were mutated into alanine. Samples
968 were recovered at the indicated time points and used for western blot. (e) Interphase extracts
969 supplemented with a wildtype or a *Xenopus* FAM122A protein in which all serine/threonine
970 residues were mutated into alanine and used for histidine pulldown and western blot to
971 measure their association with B55, A and C subunits of PP2A. (f) Interphase extracts were
972 depleted using control or anti-Cdc25 antibodies, or submitted to two consecutive depletions
973 using firstly anti-Cdc25 and secondly anti-cyclin A antibodies. Depleted supernatants were
974 then supplemented with Xe FAM122A protein. When indicated, purified cyclin A was added
975 to the mix to a final concentration of 100 nM. Samples were recovered over time and used for
976 western blot. Depletion of cyclin A and Cdc25 in the extracts was confirmed by western blot.

977
978 **Figure 3. Molecular determinants controlling FAM122A inhibitory activity. (a)**

979 AlphaFold Monomer v2.0 predicts two structured α helices (α Helix 1 and α Helix 2) in the
980 human FAM122A protein and human. *Xenopus laevis* amino acid sequence alignment.
981 α Helix 1 and α Helix 2 are indicated. (b) Interphase extracts were supplemented with the
982 wildtype or the α H1 or α H2 mutants of Xe FAM122A and the phosphorylation and levels of
983 the indicated proteins measured at the indicated times. (c) Interphase extracts supplemented
984 with wildtype and α H1 or α H2 mutants were used for His-pulldown to measure the
985 association of these proteins to B55 and C subunits of PP2A-B55. (d) The wildtype and the
986 indicated mutant forms of Xe FAM122A were supplemented to interphase extracts and used
987 for western blot. (e) His-pulldown was performed in extracts treated as in (d) to check the
988 binding of the different forms of Xe FAM122A form to B55 and C. (f) Table indicating the
989 capacity of the different single mutants of Xe FAM122A to induce mitotic entry and to bind
990 to PP2A-B55. Mutants that have lost their inhibitory activity are depicted in red. The
991 sequence of the α H1, the SLiM consensus sequence and the sequence of α H2 are shown.
992 Residues essential to keep the inhibitory activity of Xe FAM122A are highlighted in red.

993

994 **Figure 4. Gwl activation at mitotic entry promotes the dissociation of FAM122A from**
995 **PP2A-B55. (a)** Interphase and CSF extracts were supplemented with Xe FAM122A and Hu
996 FAM122A and used for His-pulldown to measure the association of this protein with B55 and
997 C subunits of PP2A-B55. The levels of His-Xe/HuFAM122A recovered in the pull down are
998 also shown. Inter PD: Interphase Pull Down. CSF PD: CSF pull down. **(b)** Interphase extracts
999 were depleted with control or with anti-Cdc27 antibodies and subsequently supplemented
1000 with Xe FAM122A and used for western blot or for His-pulldown to assess the binding of Xe
1001 FAM122A to PP2A-B55. **(c)** Interphase extracts were depleted of Cdc27, submitted to a
1002 second immunodepletion using control (lanes 1 & 3) or anti-Gwl antibodies (lanes 2 & 4) and
1003 supplemented with Xe FAM122A. 20 minutes later Gwl activity was restored (lanes 3 & 4) or
1004 not (lanes 1 & 2) by adding a hyperactive form of Gwl (GwlK72M). Samples were used for
1005 western blot and His-pulldown.

1006

1007 **Figure 5. Arpp19 dissociates FAM122A from PP2A-B55 during mitosis. (a)** Interphase
1008 extracts were supplemented with a final concentration of 7.15 μM of Xe FAM122A and 40
1009 minutes later divided into two samples. One of these samples was used to perform a His-
1010 pulldown that was then supplemented with a final concentration of 2.7 μM of untagged Thio-
1011 Arpp19 protein and used to measure bound B55 protein. The second sample was firstly
1012 supplemented with untagged 69.3 pmol of Thio-Arpp19 and subsequently used for His-
1013 pulldown to measure His-Xe FAM122A bound B55 subunit of PP2A. A sample of the His-Xe
1014 FAM122A pull downs supplemented (PD+Arp) or not (PD-Arp) with untagged Thio-Arpp19
1015 is shown. **(b)** CSF extracts were supplemented with a final concentration of 3,6 μM His-
1016 Arpp19 and, 40 minutes later, used for His-pulldown. Arpp19-pull downs were then
1017 supplemented with a final concentration of 10 μM GST-Xe FAM122A and the levels of B55
1018 remaining were measured. A sample of the His-Arpp19 pull downs supplemented (PD+FAM)
1019 or not (PD-FAM) is shown. **(c)** Table indicating the kinetic parameters of the
1020 dephosphorylation of the PP2A-B55 substrate p-S113-Arpp19 and the inhibitory constant of
1021 FAM122A and Thio-S71-Arpp19 in kinase inactivated interphase extracts. Conditions used in
1022 these assays are detailed in Methods. **(d)** Interphase extracts were supplemented with His-Xe
1023 FAM122A and used for either western blot to measure the levels and the phosphorylation of
1024 the indicated proteins and or for His-pulldown determine Xe FAM122A binding to PP2A
1025 B55, A and C subunits at the indicated time points. **(e)** Interphase extracts were supplemented
1026 with His-FAM122A to the indicated final concentrations and mitotic entry was assessed by

1027 the phosphorylation of Gwl, dephosphorylation of tyrosine 15 of Cdk1 and the degradation of
1028 cyclin B2.

1029

1030 **Figure 6. Arpp19 and FAM122A differently bind PP2A-B55.** (a) Superposition of the
1031 models of Arpp19/ENSA and FAM122A in complex with ternary PP2A-B55 as predicted by
1032 Alphafold_multimer v 2.2 for three species (human, *Xenopus* and *C. elegans*). Arpp19/ENSA
1033 and truncated FAM122A are in orange ribbon while PP2A-B55 is in blue (B55), dark green
1034 (catalytic C subunit) and pink (the scaffold A subunit). (b) Zoom onto the entrance of the
1035 catalytic site of PP2A in complex with human Arpp19 and FAM122A. Manganese ions are
1036 shown as black spheres and sidechains are drawn as sticks with carbon colours as the
1037 corresponding polypeptide chains. Figure was drawn using Pymol (<http://www.pymol.org>).

1038

1039 **Figure 7. FAM122A is required to correctly enter into mitosis in *C. elegans*.** (a) Arpp19 or
1040 PRC1 "*in vitro*" phosphorylated by PKA or Cdk1 respectively were supplemented together
1041 with Ce FAM122A to interphase extracts and the levels and dephosphorylation rate of S113
1042 of Arpp19 or T481 of PRC1, as well as the amount of Ce FAM122A, were analysed by
1043 western blot. (b) Ce FAM122A was added to interphase extracts and the levels and
1044 phosphorylation of the indicated proteins measured by western blot. (c) Scheme representing
1045 the genetic dependences of the gain of function *let-60(gf)* mutant and *sur-6* (B55 orthologue)
1046 mutant on multivulva phenotype in *C. elegans*. *sur-6* mutant (diminishes PP2A-B55 activity)
1047 on a *let-60(gf)* mutant background decreases the multivulva phenotype. Conversely, the
1048 depletion of *F46H5.2* (FAM122A orthologue) in a *let-60(gf)* mutant background significantly
1049 increases this phenotype suggesting that, as expected, *F46H5.2* protein would act as an
1050 inhibitor of PP2A-B55 in *C. elegans*. Big red arrows: increased multivulva phenotype. Shown
1051 are two representative images of a wildtype worm displaying one vulva or a mutant worm
1052 with a multivulva phenotype. The percentage of worms displaying multivulva phenotype in
1053 each mutant is represented as a mean \pm standard deviation. Significant differences calculated
1054 by non-parametric two tailed Mann-Whitney test are shown. (d) The number of larvae, dead
1055 embryos and unfertilized eggs were counted for control and *F46H5.2(RNAi)* worms and
1056 represented as mean values \pm standard deviation. (e) The percentage of dead embryos and
1057 unfertilized eggs were counted in worms at first day of adulthood and represented as for (d).

1058

1059 **Figure 8. FAM122A is required "*in vivo*" in *C. elegans* to permit mitotic entry and
1060 progression in germ stem cells.** (a) Nematode strains expressing histone and gamma-tubulin

1061 tagged with GFP were immobilised and immediately imaged by confocal microscopy for the
1062 quantification of interphase and mitotic cells as reported in the scheme of Appendix Figure
1063 S6. Data are represented as mean \pm standard deviation. **(b)** Prophase, metaphase and anaphase
1064 germ stem cells in gonads of worms were counted as in (a) and represented as mean \pm
1065 standard deviation. **(c)** A GFP-tagged tubulin and with RFP-tagged histone nematode strain
1066 was followed by time lapse confocal microscopy and the timing of mitotic progression was
1067 determined in germ stem cells. Shown are representative images of stem cells performing
1068 mitotic division over time in control and RNAi treated worms. **The number of cells**
1069 **performing nuclear envelope breakdown (NEBD) per minute and per gonad were counted and**
1070 **represented as the mean values \pm standard deviation.** Timing of cells to perform prophase
1071 (from first centrosome movement to nuclear envelope breakdown), congression (from nuclear
1072 envelope breakdown to metaphase plate) or anaphase onset were also recorded and
1073 represented as mean values \pm standard deviation. Significant differences calculated by non-
1074 parametric two tailed Mann-Whitney test are shown. Arrowheads: centrosomes. P:prophase.
1075 Sp: Spermatozoids. PM: prometaphase. A: anaphase. Scale bar: 10 μ m. * $p<0.01$; ***
1076 $p<0.001$; ***** $p<0.0001$. $n\geq 10$. **(d)** RPE1 cells were (Thymidine Block) or not
1077 (Asynchronous) synchronized with thymidine treatment and released at the indicated
1078 timepoints. Released cells were used for western blot to measure FAM122A, cyclin A and
1079 tubulin levels at the different timepoints. Western blot signals were measured and FAM122A
1080 and cyclin A amounts were normalized and represented. $n=2$.

1081

1082 **Figure 9. Hypothetic model representing the network of activation/inactivation of cyclin**
1083 **B/Cdk1 and mitotic entry/exit by the sequential inhibition of PP2A-B55 by FAM122A**
1084 **and Arpp19.** PP2A-B55 activity is maintained high during G2 preventing the
1085 phosphorylation and activation of Cdc25 by cyclin A/Cdk. A first phosphorylation and
1086 activation of Cdc25 phosphatase by cyclin A/Cdk is achieved when a critical FAM122A-
1087 dependent inhibition of PP2A-B55 is reached. Cdc25 will then dephosphorylate and activate
1088 the cyclin B/Cdk1 feedback loop resulting in the activation of Gwl, phosphorylation of
1089 Arpp19 and mitotic entry. Phospho-Arpp19 will then induce FAM122A dissociation of
1090 PP2A-B55 taking over the inhibition of this phosphatase and promoting the correct temporal
1091 dephosphorylation of mitotic substrates. Active and inactive pathways are represented as solid
1092 thick arrows and dashed thin arrows respectively. Activatory phosphorylation sites are
1093 represented in red whereas inhibitory phosphorylation of Cdk1 is shown in blue.

1094

1095

1096

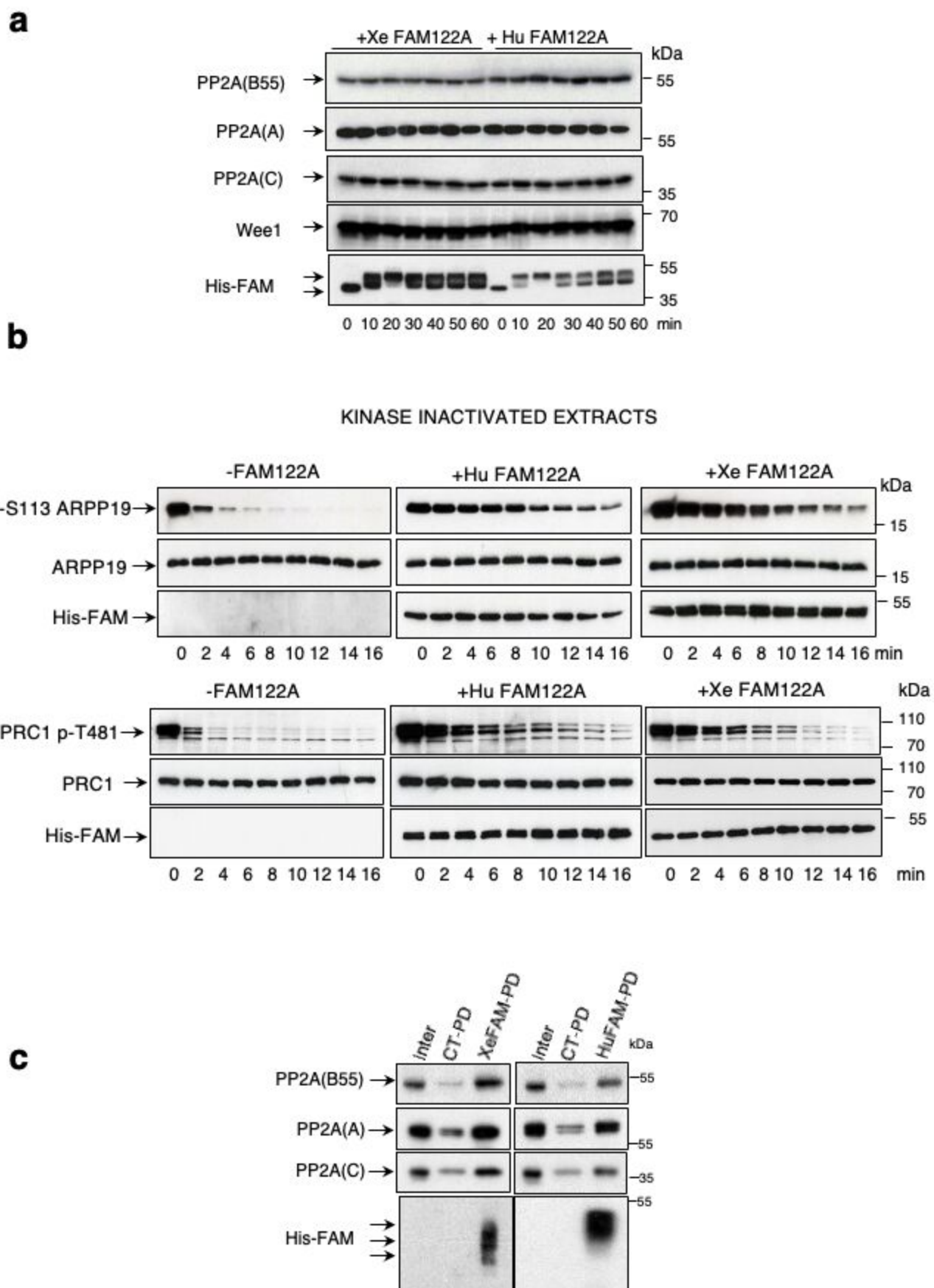
Figure 1

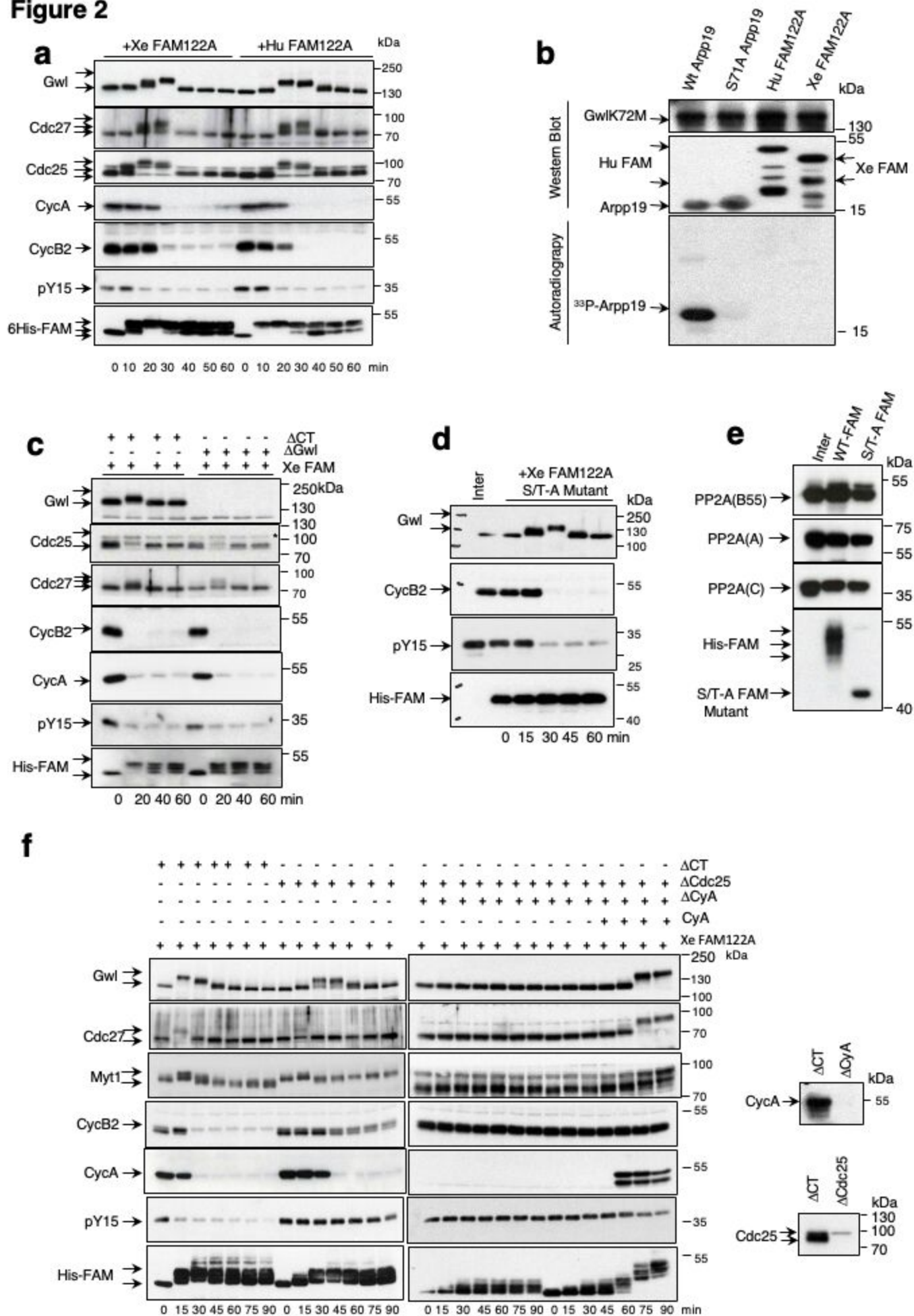
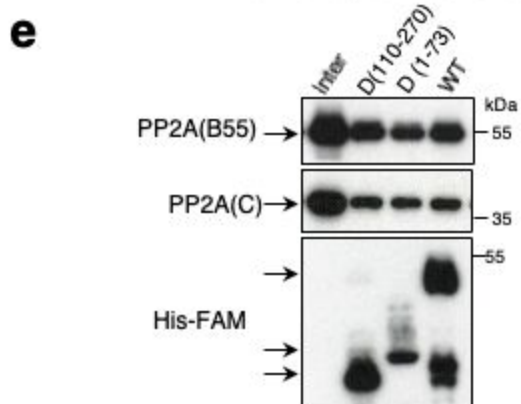
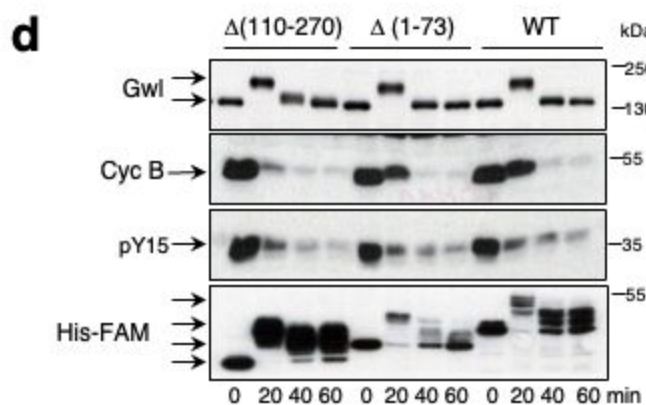
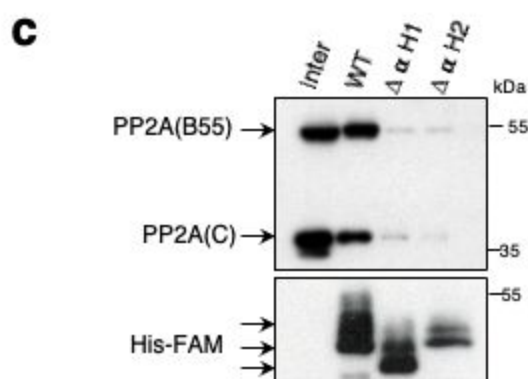
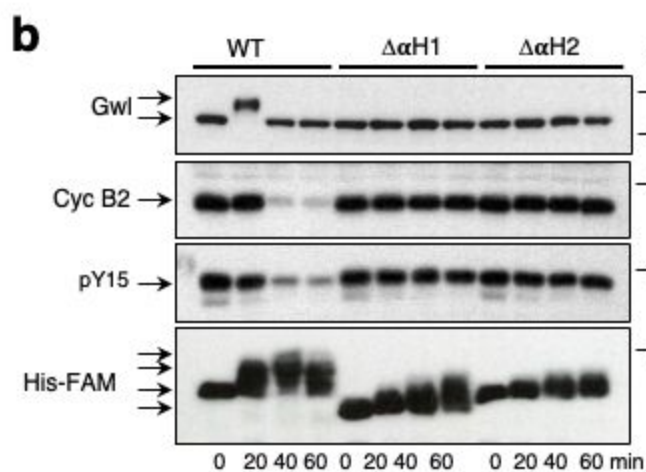
Figure 2

Figure 3

a ALPHA_FOLD FAM122A



α Helix 1 (H1): 84-93 (Human)/ 71-82 (Xenopus)
 α Helix 2 (H2): 96-119 (Human)/ 85-110 (Xenopus)



f

	Mutants Xenopus	Mitotic Entry	PP2A Binding
H1	R73A	-	-
	L74N	-	-
	I77N	-	-
	K78R	+	+
	E80A	-	-
H2	L85N	+	+
	I87N	+	+
	E90A	-	-
	H93A	-	-
	E94A	-	-
	R95A	-	-
	S104A	+	+
	E108A	+	+
E109A	+	+	

α H1: SRLHQIKQEE SLIM Consensus: [RK]-V-xx-[VI]-R

α H2: DLMINRETAHEREVQVAMQMSQSWSEE

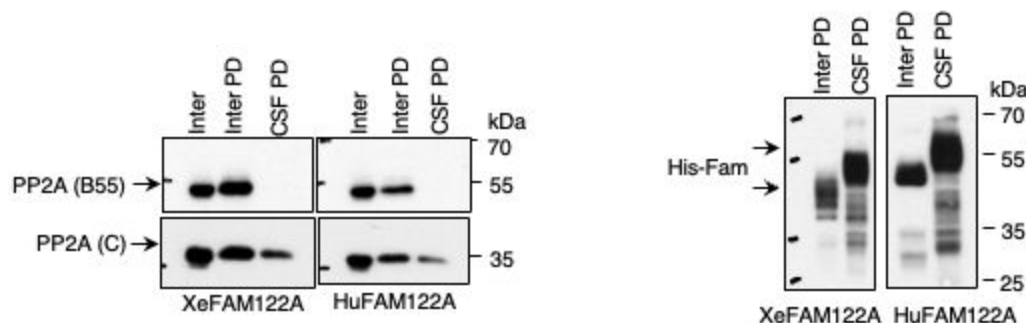
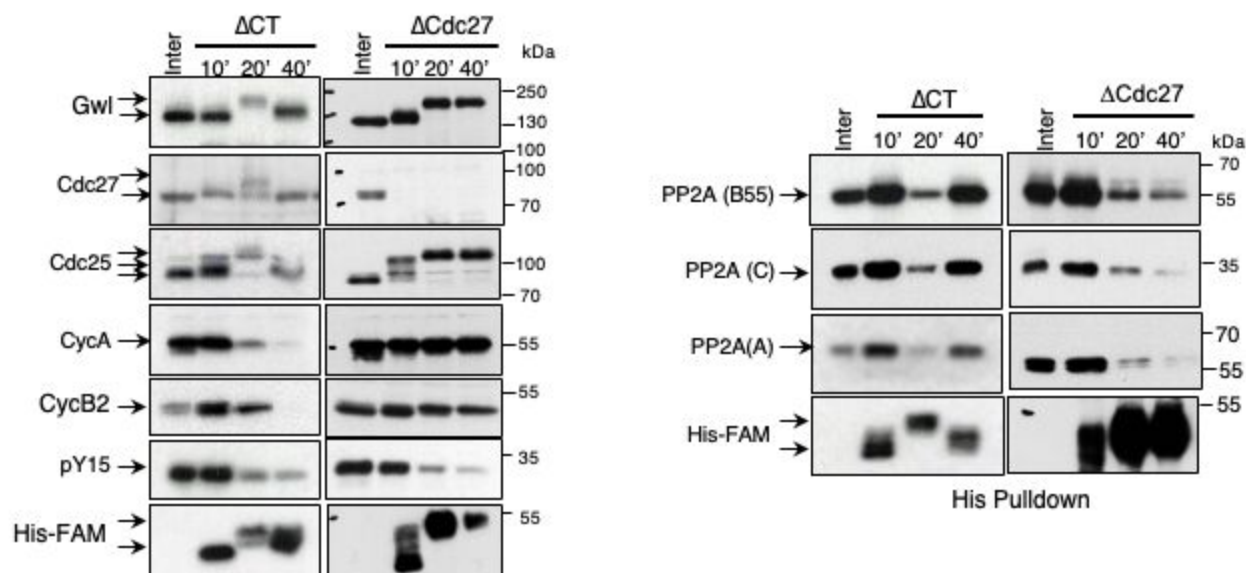
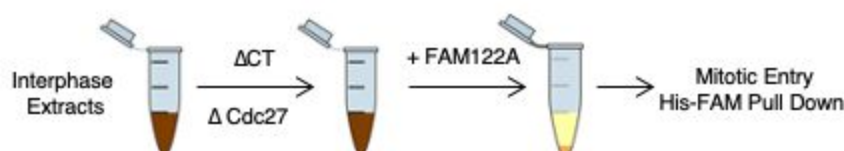
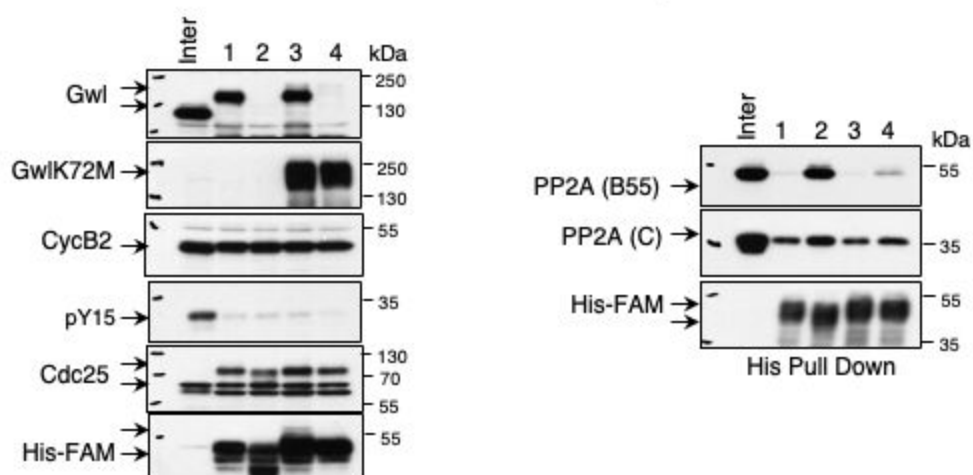
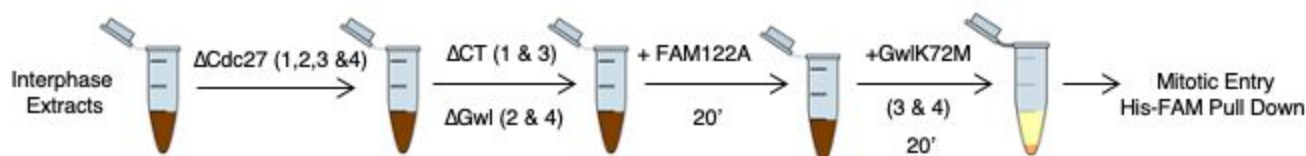
Figure 4**a****b****c**

Figure 5

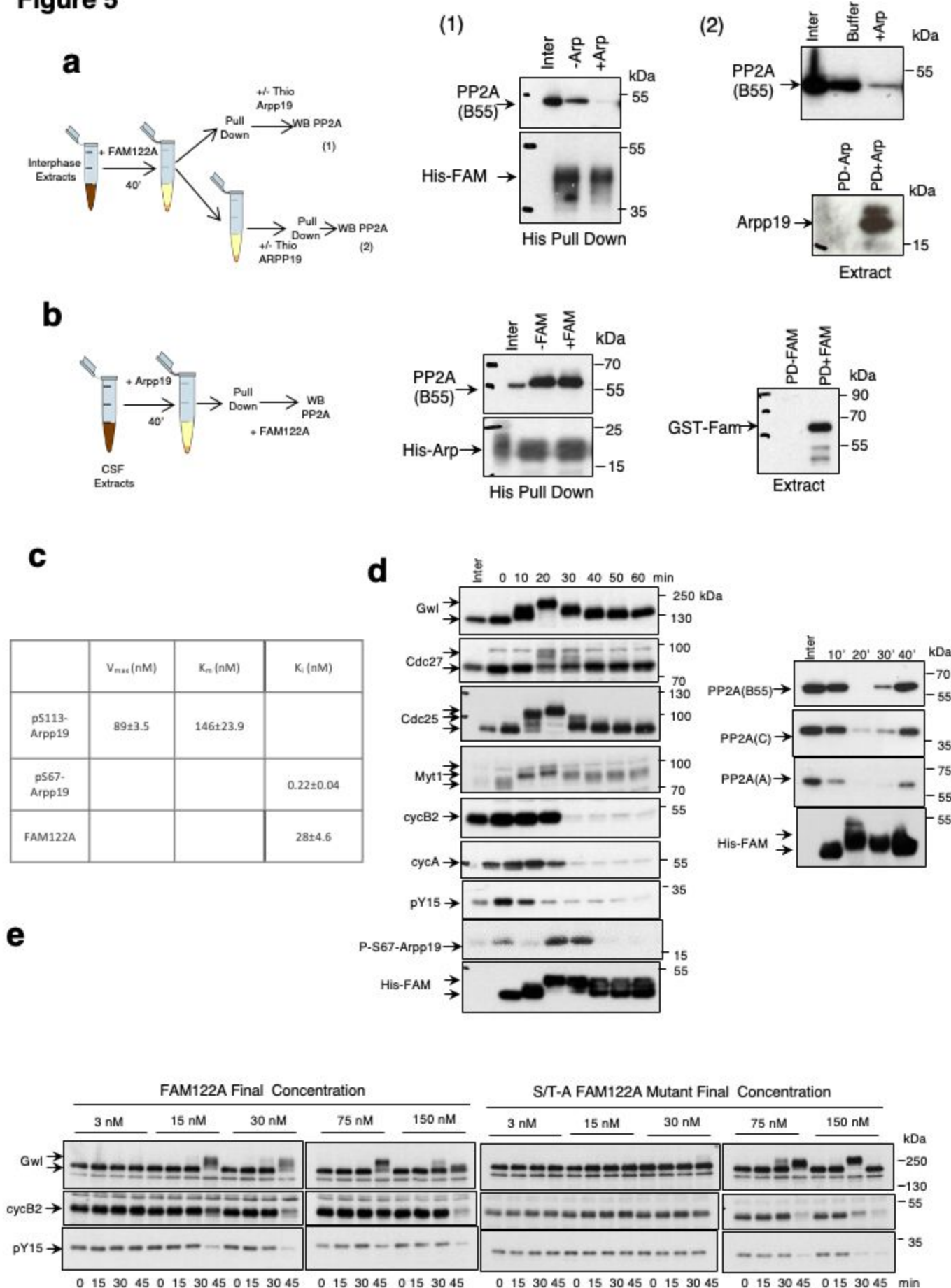
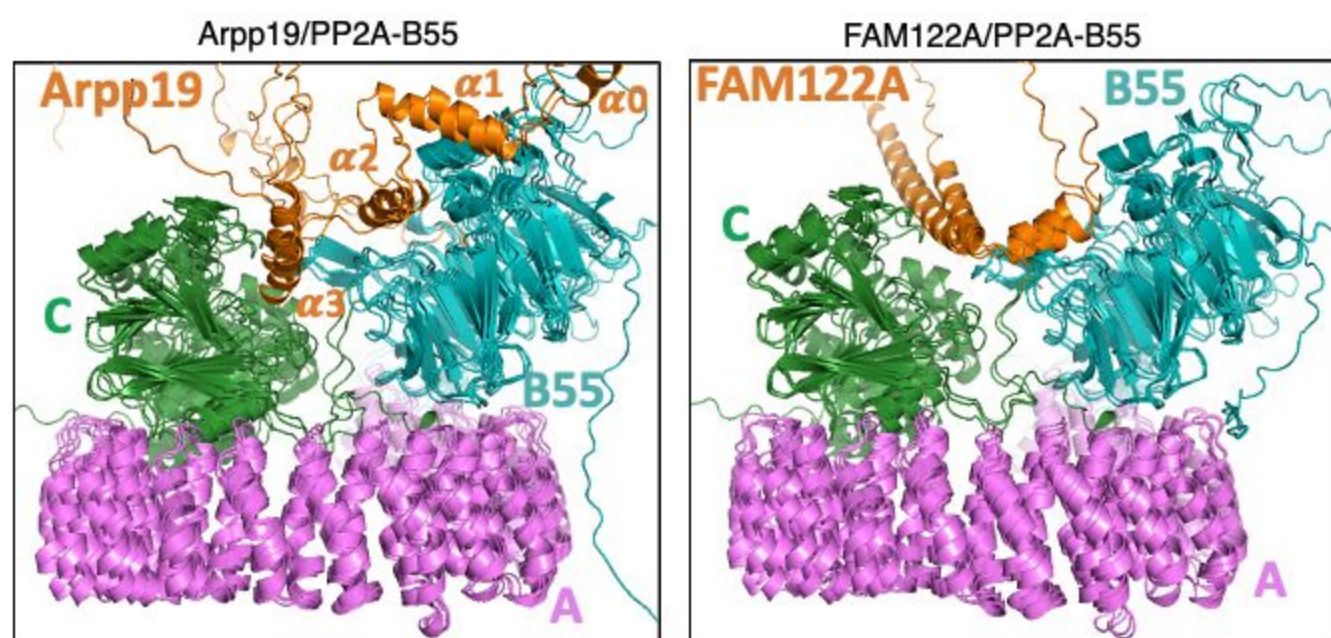


Figure 6

a



b

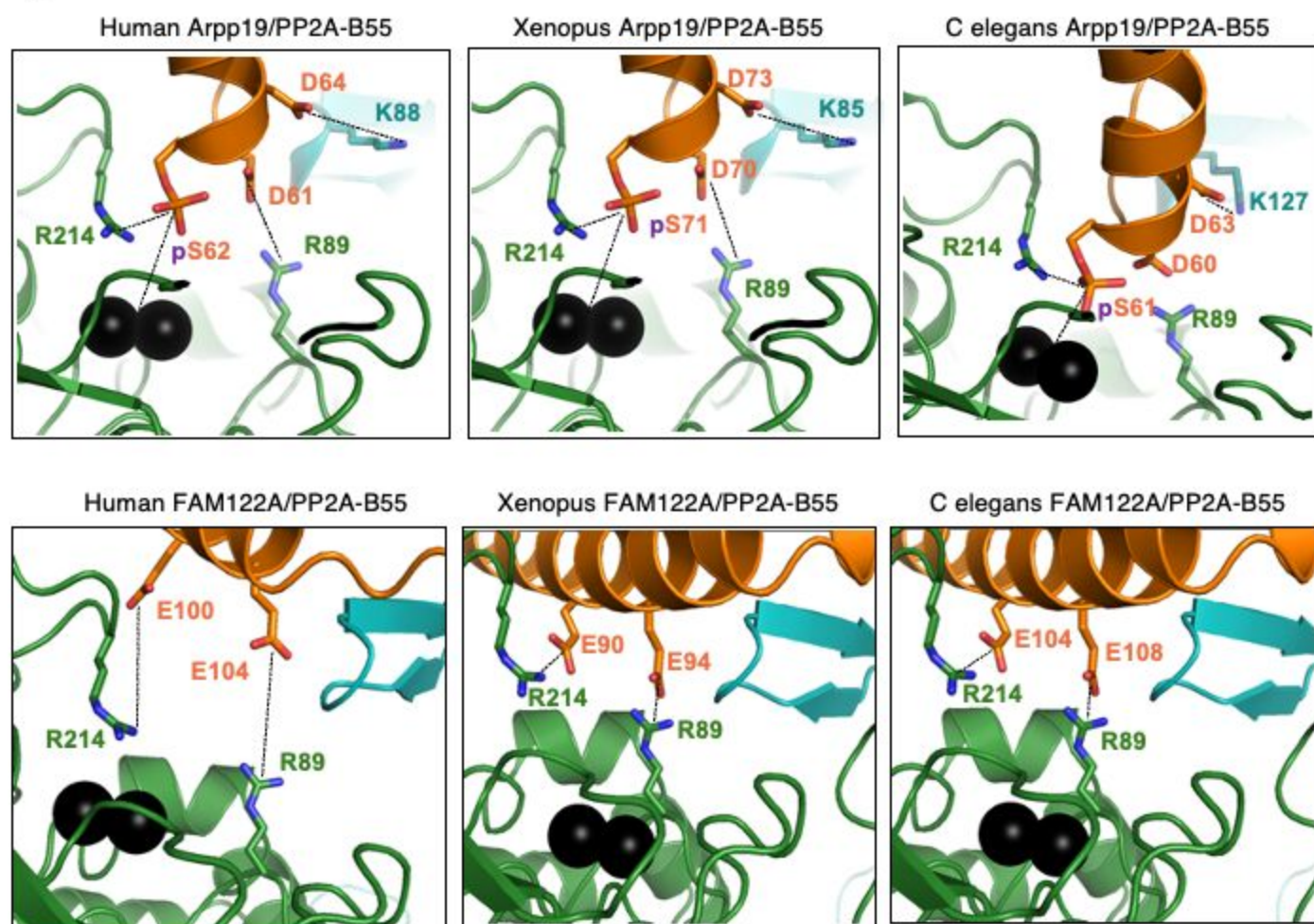


Figure 7

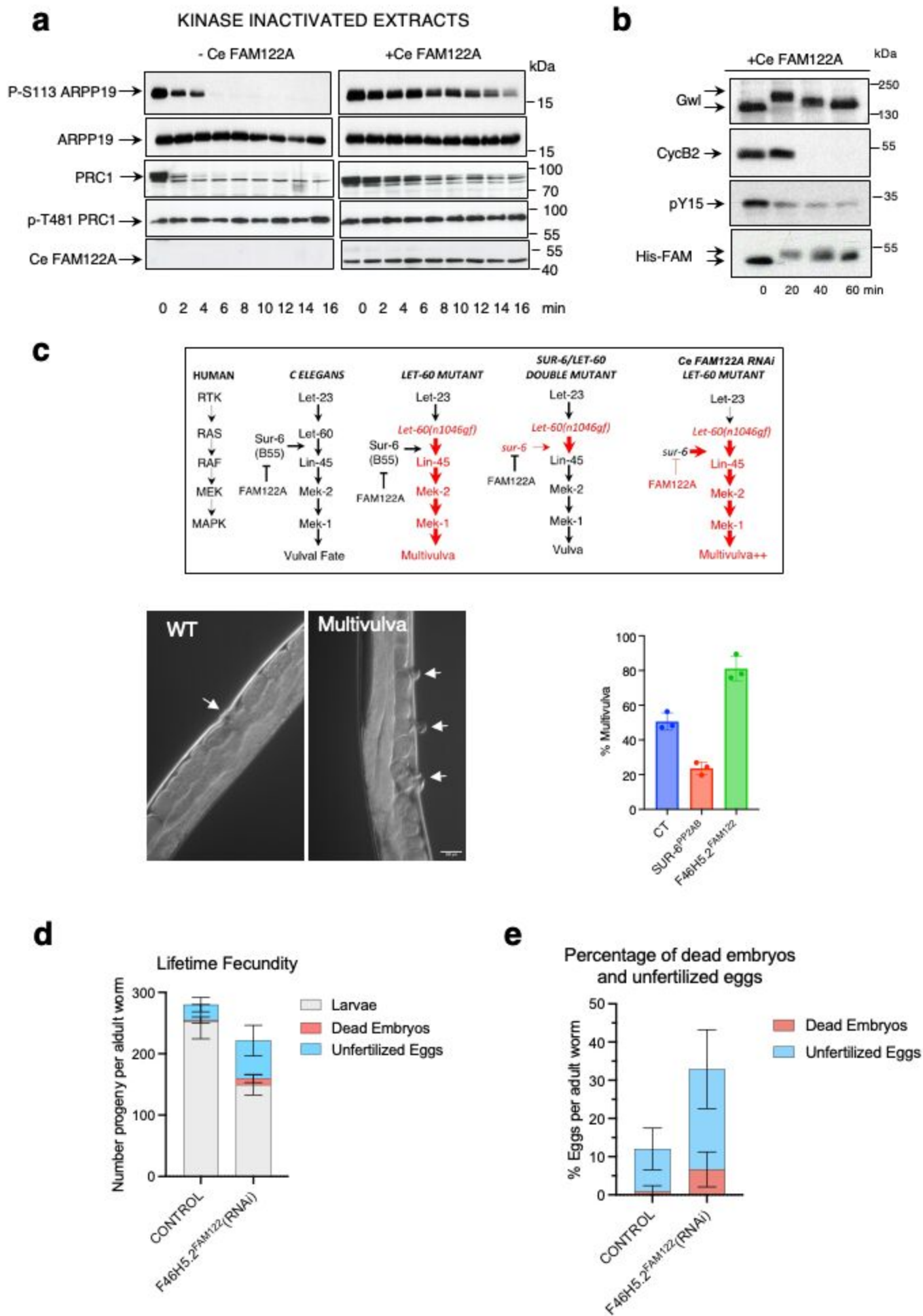
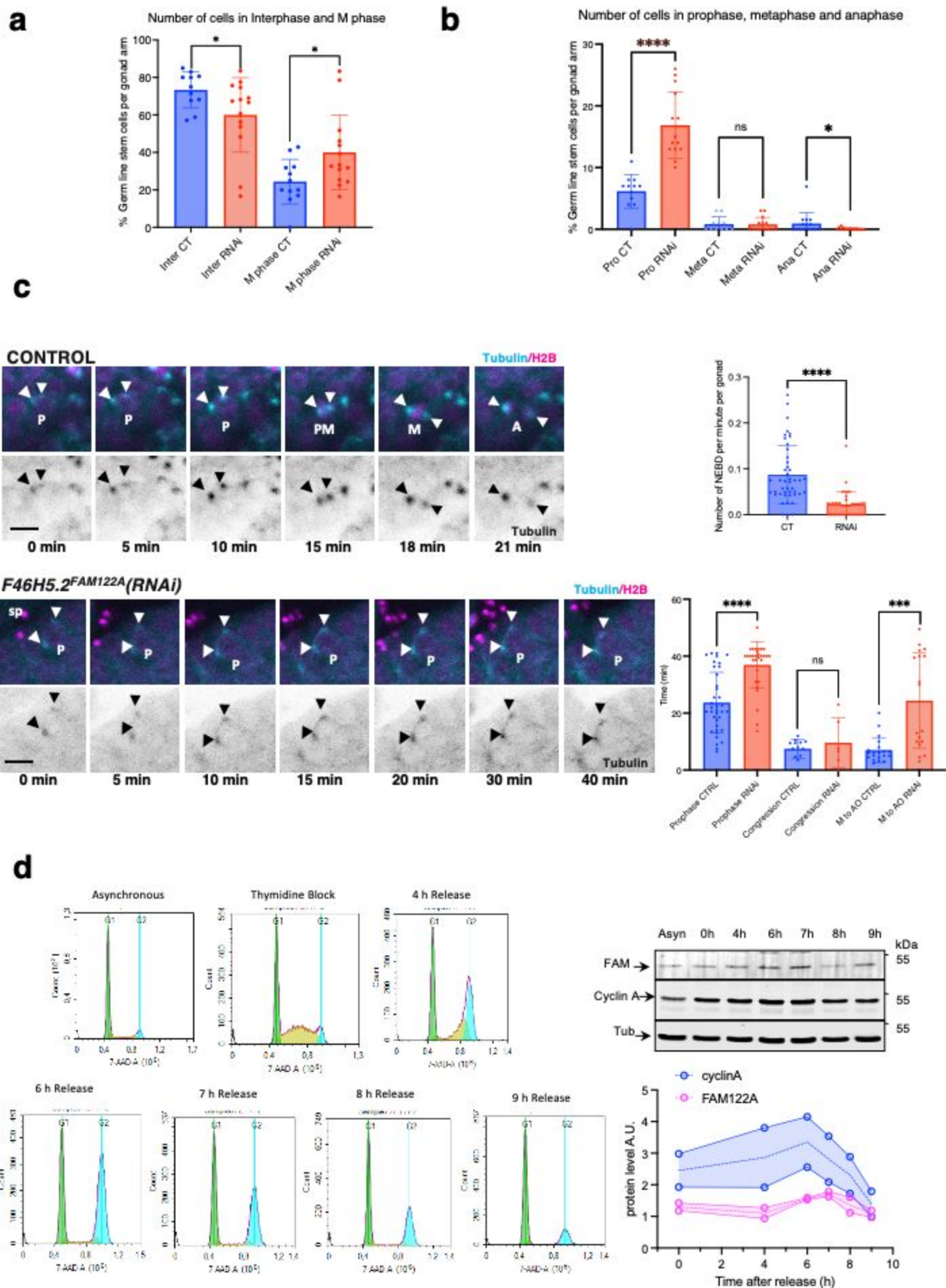
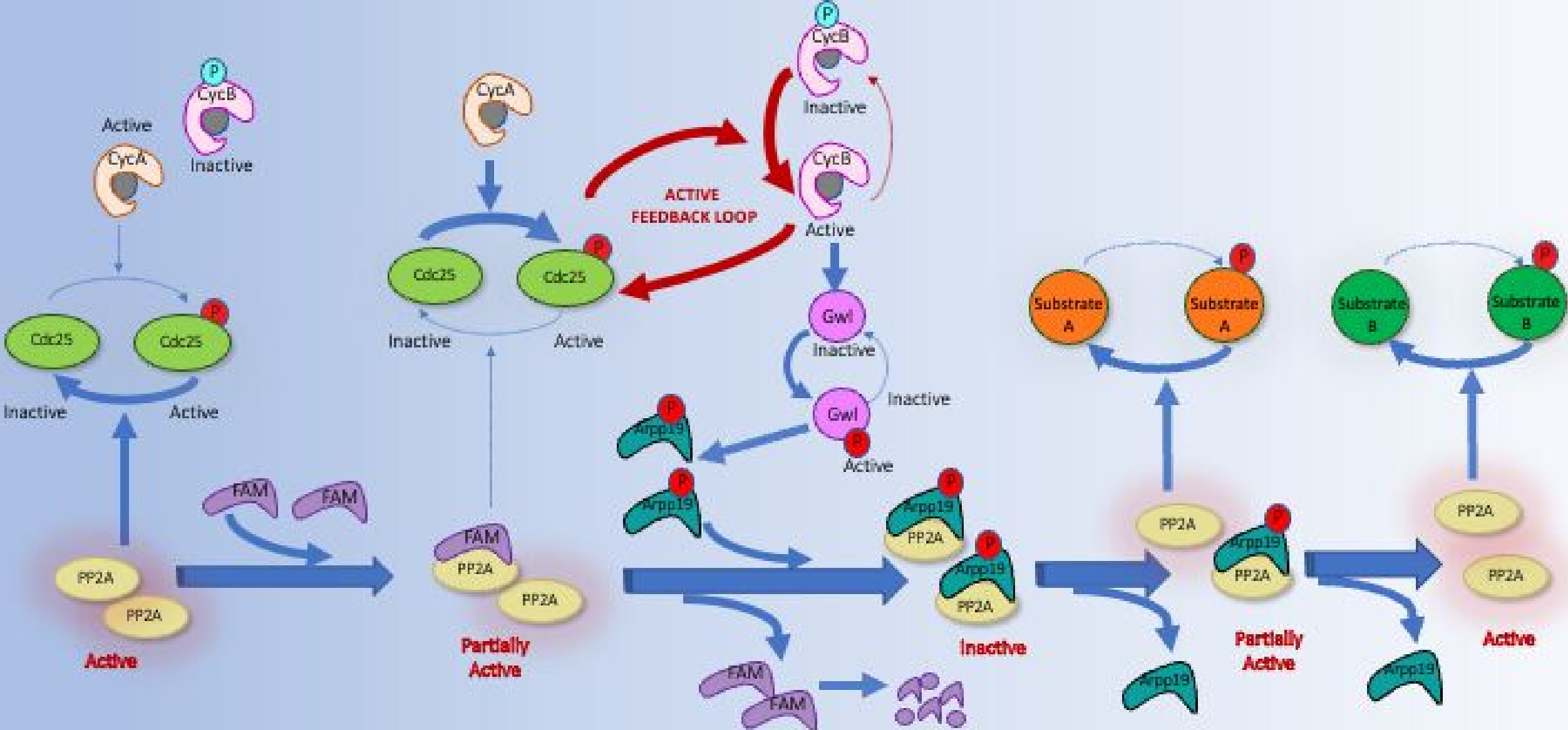


Figure 8



G2

Mitotic Entry

Mitotic Exit

