Title; Synchronous chromosome segregation in mouse oocytes is ensured by biphasic securin destruction and cyclin B1-Cdk1

Authors; Christopher Thomas^{1, 2*}, Mark D. Levasseur¹, Rebecca J. Harris¹, Owen R. Davies¹, Suzanne Madgwick^{1*}

¹Cell Division Biology Group, Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, NE2 4HH, UK.

²Present address: Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Gottingen, Germany.

*Corresponding authors; christopher.thomas@mpibpc.mpg.de, suzanne.madgwick@newcastle.ac.uk

Abstract Successful cell division relies on the faithful segregation of chromosomes. If chromosomes segregate prematurely the cell is at risk of aneuploidy. Alternatively, if cell division is attempted in the absence of complete chromosome segregation, non-segregated chromosomes can become trapped within the cleavage furrow and the cell can lose viability. Securin plays a key role in this process, acting as a pseudosubstrate to inhibit the protease separase that functions to cleave the cohesin rings that hold chromosomes together. Consequently, securin must be depleted ahead of anaphase, ensuring chromosome segregation occurs in time with the anaphase trigger. Here we find that MI mouse oocytes contain a large excess of securin over separase and reveal the existence of a novel mechanism that functions to promote the destruction of excess securin in prometaphase. Critically, this mechanism relies on key residues that are only exposed when securin is not bound to separase. We suggest that the majority of non-separase bound securin is removed by this mechanism, allowing for separase activity to be protected until just before anaphase. In addition, we further demonstrate the importance of complementary mechanisms of separase inhibition by directly measuring cleavage activity in live oocytes, confirming that both securin and inhibition by cyclin B1-Cdk1 are independently sufficient to prevent premature separase activation.

35 **Introduction**

36 Successful cell division depends on the precise segregation of chromosomes into two
37 equal sets. In mitosis and meiosis II this involves the segregation of sister chromatids.
38 In meiosis I, pairs of homologous chromosomes are segregated in a reductional
39 division that introduces genetic diversity. If chromosomes missegregate during these
40 divisions, cells are at risk of aneuploidy, a hallmark of cancer and the primary genetic
41 cause of miscarriage and developmental defects in babies¹⁻⁵.

42 Through all cell divisions, the protease separase plays an essential role, cleaving the
43 cohesin complexes that hold both sister chromatids and homologous chromosome
44 pairs together^{6,7}. It is therefore critical that separase remains inactive until all
45 chromosomes are correctly aligned and the cell is prepared for anaphase.

46 Not surprisingly, separase activity is tightly regulated through the cell cycle. This
47 regulation is executed through two distinct inhibitory pathways. In the primary
48 pathway, separase directly interacts with securin, which acts as a pseudosubstrate
49 inhibitor for separase⁸. In addition to this, upon phosphorylation by Cdk1, separase is
50 inhibited by binding Cdk1's activating partner cyclin B1⁹⁻¹². The binding of separase
51 with either securin or cyclin B1 is mutually exclusive and the relative contribution of
52 each pathway varies depending on cell type and developmental state^{10,12}. In female
53 mouse meiosis II and eukaryotic mitosis, securin is largely responsible for separase
54 inhibition, while primordial germ cells and early stage embryos rely primarily on
55 cyclin B1-Cdk1-mediated separase inhibition¹³⁻¹⁵. Interestingly however, while
56 securin binding to separase is the primary inhibitory mechanism in mitotic cells, it is
57 also dispensable, demonstrating the compensatory nature of these two pathways¹⁶⁻¹⁸.
58 Similarly, in fixed MI mouse oocytes Chiang et al. were able to inhibit securin or
59 cyclin B1-Cdk1 mediated separase inhibition individually without observing
60 segregation defects. Chromosome segregation errors were only detected when both
61 inhibitory pathways were removed¹⁹.

62 Importantly, securin and cyclin B1 are not only involved in separase inhibition but
63 their binding also plays a key role in priming separase for activation²⁰⁻²².
64 Furthermore, the degradation of securin and cyclin B1 must be coupled in order to
65 ensure that separase activation and the poleward movement of chromosomes are

66 timed correctly. In situations where these events become disconnected, anaphase is
67 defective^{23–25}.

68 In mitosis, the synchronous loss of cyclin B1 and securin is ensured by the similarity
69 of their destruction mechanisms. Both are ubiquitinated by the Anaphase Promoting
70 Complex/Cyclosome (APC/C) in metaphase^{26,27}. Critically, this ubiquitination relies
71 on both the availability of the APC/C activator protein Cdc20 and on a short linear
72 motif known as the D-box present in the N-terminus of both securin and cyclin B1^{28–}
73 ³⁰. Once all chromosomes are properly attached to spindle microtubules in metaphase,
74 Cdc20 and the APC/C form a bipartite receptor for D-box docking, triggering securin
75 and cyclin B1 destruction by the proteasome^{31,32}. Prior to this, Cdc20 is sequestered
76 by the spindle assembly checkpoint (SAC), a diffusible signal generated at each
77 unattached kinetochore. The SAC functions to prevent docking of D-box motifs to the
78 APC/C and thereby inhibit anaphase until all chromosomes are attached to spindle
79 microtubules^{33,34}.

80 In contrast, securin and cyclin B1 destruction in mouse oocyte meiosis I is initiated in
81 prometaphase, prior to full chromosome alignment^{35,36}. Initially this might seem like a
82 failure to ensure accurate chromosome segregation. However, our recent work
83 demonstrates that degradation of cyclin B1 in prometaphase is in fact a key feature of
84 a mechanism that functions to prevent aneuploidy in mouse oocytes³⁶. At this time
85 point, destruction represents only the loss of non-Cdk1-bound cyclin B1. Here, cyclin
86 B1 is targeted by an additional motif to the D-box, the PM motif. Importantly, the PM
87 motif is only accessible in cyclin B1 that is not bound to Cdk1. By this strategy, an
88 excess of cyclin B1 acts as an APC/C decoy to maintain Cdk1 activity and prolong
89 prometaphase in oocyte meiosis I. This is critical in mouse oocytes since, in the
90 absence of excess cyclin B1, spindle checkpoint activity is insufficient in preventing
91 anaphase for long enough to fully align chromosomes, a process that takes hours
92 rather than the tens of minutes to complete mitosis³⁶.

93 Our discovery of a novel pathway of meiotic cyclin B1 regulation raises significant
94 questions with regard to securin regulation. Since the D-box is insufficient for the
95 correct degradation timing of cyclin B1 in oocytes, it seemed highly likely that
96 additional pathways exist to degrade securin.

97 Here we find that securin exists in large excess to separase in oocyte MI. We identify
98 key residues that function to promote the degradation of non-separase bound securin
99 in prometaphase. Beyond this we show the importance of complementary
100 mechanisms of separase inhibition in live oocytes.

101

102 **Results**

103 **Securin destruction begins 2.5 hours ahead of separase activation in meiosis I in** 104 **mouse oocytes.**

105 In mitosis, securin is destroyed alongside cyclin B1 and only in metaphase once the
106 spindle checkpoint signals that all kinetochores are correctly attached to
107 microtubules^{26,27}. In contrast, while securin and cyclin B1 are also targeted
108 simultaneously in meiosis I of mouse oocytes, their destruction is initiated much
109 earlier, ahead of chromosome alignment and spindle migration in prometaphase (Fig.
110 1a). However, as division errors are rare in mouse oocytes, it seemed unlikely that this
111 early targeting of securin affects separase activity so far ahead of anaphase.

112 To assess whether degradation of securin and cyclin B1 in prometaphase caused
113 separase activation, we used a separase activity biosensor generated by Nam et al. that
114 has previously been validated in oocytes³⁷⁻³⁹. The sensor consists of nucleosome-
115 targeted H2B protein fused to eGFP and mCherry fluorophores. Between the two
116 fluorophores, an Scc1 peptide sequence is cleaved by active separase. Scc1 cleavage
117 results in a yellow to red colour shift as the eGFP signal dissociates into the
118 cytoplasm and mCherry remains bound to histones associated with chromosomal
119 DNA. We injected germinal vesicle (GV) stage oocytes with mRNA encoding the
120 biosensor and imaged the live cells through MI. A clear shift in colour was
121 consistently observed just 20-30 minutes ahead of polar body extrusion (Fig. 1c). This
122 timing was confirmed by the quantification of the eGFP/mCherry fluorescence ratio
123 (Fig. 1a). The data indicate that separase is only active from 30 minutes ahead of
124 polar body extrusion, with the majority of substrate cleavage taking place in the final
125 20 minutes. Importantly, separase becomes active more than 2 hours after the
126 initiation of securin destruction.

127 **The D-box is insufficient for wild-type securin destruction in MI**

128 In mouse oocytes, cyclin B1 is destroyed by a two-step mechanism; an initial period
129 of prometaphase destruction requires both a D-box and an additional motif (the PM
130 motif). This is followed by a second period of destruction as the D-box becomes
131 sufficient once the SAC is satisfied in metaphase³⁶. Given that securin and cyclin B1
132 destruction is synchronous in oocytes, we reasoned that securin may also be destroyed
133 by a similar mechanism^{35,36}. To test this, we initially designed and tested two
134 fluorescent reporters; full-length securin (securin FL) and the N-terminal 101 residues
135 (securin N101). Both securin reporters and all that follow were coupled to VFP to
136 give a direct readout of exogenous protein level in the oocyte. Critically both
137 constructs contain the D-box and all neighbouring lysine residues necessary for
138 APC/C recognition and subsequent proteolysis (Fig. 2a).

139 Strikingly, we found that securin FL was consistently targeted for destruction earlier
140 than securin N101 (~80 minutes; Fig. 2b). Furthermore, when we compared the data
141 generated in our cyclin B1 study, we found that securin N101 destruction was
142 restricted to metaphase in time with the cyclin B1 mutant lacking the PM motif. This
143 observation raised the possibility that, similar to cyclin B1 and other prometaphase
144 APC/C substrates, an additional region is necessary to act alongside the D-box and
145 direct wild-type securin degradation in prometaphase I in oocytes.

146 **A discrete region within the C-terminus of securin promotes destruction in** 147 **prometaphase.**

148 To test if a second destruction motif exists within securin to facilitate prometaphase
149 destruction, we replaced a highly conserved C-terminal region located between
150 residues 109-133 (Fig. 2c) with a neutral 25 amino acid TGSGP repeat linker in the
151 full-length securin construct (securin 109-133 mutant). This construct was targeted for
152 destruction in time with securin N101 (Fig. 2d), ~80 minutes after securin FL. This
153 indicated that residues between 109-133 are essential for prometaphase securin
154 destruction in oocyte MI.

155 Following this finding, we divided residues 109-133 into three groups based on
156 sequence conservation, mutating each group to assess their importance in the timing
157 of securin degradation (Supplementary fig. 1a). Based on sequence similarity with the
158 PM motif in cyclin B1, which centres on residues ¹⁷⁰DIY¹⁷² in the mouse ortholog, we
159 predicted that the first mutant, securin DAYPEIE-A, would eliminate prometaphase

targeting. Surprisingly however, securin DAYPEIE-A was instead targeted in time with securin FL (Supplementary fig.1b). In contrast, both FFPFNP-A and DFESFD-A mutations resulted in dramatic shifts in destruction timing (Supplementary fig.1c-d). Securin FFPFNP-A was targeted for destruction approximately 60 minutes after securin FL but still ~20 minutes ahead of securin N101 (Supplementary fig.1c), while securin DFESFD-A destruction mirrored that of securin N101 (Supplementary fig. 1d). This suggests that residues essential for wild-type prometaphase securin degradation lie within both of these regions. Since securin DFESFD-A showed the most striking phenotype, we mutated a pair of highly conserved phenylalanines (F125 and F128) to alanines (securin FxxF-A; Fig. 2e). Here, degradation was delayed by 90 minutes, mimicking securin N101 (Fig. 2f; see supplementary fig. 1e for destruction timings of all mutants). Importantly, mutation of residues F125 and F128 to alanines did not impair securin binding to separase as shown by peptide pull-down (Supplementary fig. 1f). We suggest that these two phenylalanine residues are a crucial component of a novel interaction that mediates the timing of securin loss by permitting prometaphase destruction in MI oocytes. In the absence of this interaction, securin destruction initiates 90 minutes later and only in metaphase. Interestingly, these two residues and their surrounding region have no sequence similarity with the PM motif present in cyclin B1.

To confirm that the difference in degradation timing between securin FL and securin FxxF-A was not simply due to differences in protein expression, oocytes were treated with cycloheximide (CHX) to block protein synthesis (Supplementary fig. 2). Upon addition of CHX, the securin FL protein turnover was evident. Approximately 20% of the total protein was lost before a steep period of destruction began 4 hours after CHX addition. In contrast, securin FxxF-A levels were relatively stable until destruction began ~5.5 hours after CHX addition. Critically, securin FxxF-A destruction begins around 90 minutes after securin FL, consistent with results from non CHX-treated oocytes.

Securin FL destruction in MI mouse oocytes begins in prometaphase at a time when Mad2 staining is still detectable at kinetochores⁴⁰. This suggests that the SAC might have less influence over securin FL than securin FxxF-A. To assess this, we treated oocytes with 150 nM nocodazole. This dose of nocodazole depolymerises microtubules and activates the SAC such that PB1 extrusion is blocked in >98% of

193 oocytes. Under these conditions, the rate of securin FL degradation was dramatically
194 reduced, though it was still almost fully degraded within a 10-hour period. In contrast,
195 securin FxxF-A was almost completely stabilised (Fig. 2g). Together our data suggest
196 that an interaction involving two key phenylalanine residues within securin is able to
197 bypass a SAC signal capable of blocking destruction of substrates where only the D-
198 box is accessible. The D-box is however essential for both phases of securin
199 destruction, since mutation of the D-box perturbs both metaphase and prometaphase
200 securin destruction, and inhibits PB1 extrusion (Supplementary fig. 3c).

201 Our data are consistent with a biphasic securin destruction process in MI oocytes. A
202 first period of destruction 3 hours ahead of PB1 extrusion which requires both the D-
203 box and an additional region with an absolute requirement for residues F125 and
204 F128. This is followed by a second period of destruction in metaphase as the D-box
205 becomes sufficient for degradation 1 hour ahead of PB1 extrusion. Critically, our
206 separase biosensor data indicates that separase is only active during the latter half of
207 the second phase of securin destruction.

208 **Two phenylalanine residues key to prometaphase securin destruction are**
209 **predicted to be masked when securin is bound to separase.**

210 Interestingly, F125 and F128 are positioned within securin's separase interaction
211 segment, in the vicinity of the residues involved in securin's pseudosubstrate binding
212 to the separase active site^{41,42}. We therefore asked how these phenylalanine residues
213 are positioned when securin is in complex with separase. Since a mammalian crystal
214 structure for the securin-separase complex is yet to be solved, we instead used the
215 existing structure of the *S. cerevisiae* complex as a reference⁴¹. Critically, these
216 residues and the surrounding region are largely conserved between mammals and
217 yeast (Fig. 3a). In the structure, residues Y276 and F279, corresponding to F125 and
218 F128 in the human protein, sit deep within a hydrophobic binding pocket on the
219 surface of separase (Fig. 3b). It is therefore highly likely that these two residues
220 would be obscured when securin is in complex with separase. The conservation of
221 these residues throughout eukaryotes (Fig. 3a), coupled with their neighbouring
222 position to the pseudosubstrate region of securin, lead us to propose that these
223 residues would be similarly hidden in other species.

224 **Securin is present in large excess over separase in mouse oocytes.**

225 Given that F125 and F128 are predicted to be masked when securin is bound to
 226 separase, we speculated that destruction in prometaphase must represent that of a non-
 227 separase bound population of securin. In mitosis, securin protein is in excess of
 228 separase^{43,44}. This ratio has been quantified in HeLa cells, where free securin is
 229 reported to be 4-5 fold more abundant than separase-associated securin⁴³. Therefore
 230 we considered that a similar excess of securin in oocytes could provide the basis for a
 231 mechanism able to support an extended period of securin destruction without a release
 232 of separase inhibition. To address this, we compared the separase to securin ratio in
 233 mitosis with that in MI oocytes by western blotting. We find that securin is present at
 234 comparable levels between 96 oocytes and HeLa cell extracts prepared from 2500 and
 235 5000 cells (Fig. 3ci). Yet while separase was readily detectable in the mitotic extracts,
 236 no band was observed in the 96 oocyte lane (Fig. 3cii). By extending the exposure
 237 time from 30 seconds to 10 minutes, a small separase band was detected in the 96
 238 oocyte lane, where all mitotic lanes became strongly overexposed (Fig. 3ciii). We
 239 conclude that in oocytes, securin is present in much greater excess to separase than
 240 the 4-5 fold difference documented in HeLa cells.

241 We therefore suggest a mechanism by which a large pool of excess non-separase
 242 bound securin is preferentially targeted for destruction in late prometaphase by a
 243 mechanism involving both the D-box and a second region where F125 and F128 are
 244 essential. Following this, a smaller pool of separase-bound securin becomes a
 245 destruction target in metaphase.

246 Preferential destruction of non-separase bound securin has previously been
 247 demonstrated in HeLa cells. Hellmuth et al. showed that separase-bound securin is
 248 dephosphorylated by PP2A-B56 phosphatase, whereas free securin exists in a
 249 phosphorylated state and is a preferential APC/C target⁴³. Importantly however, the
 250 destruction of both separase-bound and non-separase bound securin in mitosis takes
 251 place only after SAC satisfaction. It therefore seems unlikely that this same
 252 mechanism is responsible for degradation of non-separase bound securin in
 253 prometaphase in mouse oocytes. To confirm this, we mutated four key
 254 phosphorylation sites in securin to alanines (securin 4A; S31A, T66A, S87A and
 255 S89A) and observed no change in destruction timing compared to securin FL
 256 (Supplementary fig. 4). We therefore suggest that the mechanism of non-separase

bound securin degradation described in this manuscript occurs independently of phosphorylation status.

Two complimentary mechanisms prevent premature separase activation and division errors

We next wanted to investigate how separase activity is affected in meiosis when the large excess of securin is removed. Chiang et al. previously showed that segregation errors in fixed oocytes were only observed when both securin and cyclin B1-Cdk1-mediated separase inhibition were removed¹⁹. However, when these errors arise and how separase is regulated through time remains unclear. To investigate this, we knocked down securin protein expression with a morpholino oligo (MO) such that in prometaphase, MO oocytes contained ~13% of the protein level relative to non-treated control cells (quantified by western blot; Supplementary fig. 5). Despite severe depletion of securin, the cleavage activity of separase was still restricted to the final 30 minutes preceding PB1 extrusion (Fig 4a). This result agrees with observations made in fixed oocytes, suggesting that cyclin B1-Cdk1 levels are sufficient to compensate for the loss of securin. To test whether this was also the case in our live cell assay, we injected oocytes with a previously published separase AA mutant¹⁹. This construct is fully active but lacks Cdk1 phosphorylation sites (S1121A and T1342A) that prevent its inhibition by cyclin B1-Cdk1. Importantly, oocytes expressing separase AA protein produce separase activity profiles identical to control oocytes, suggesting that securin was similarly sufficient to compensate for a loss of cyclin B1-Cdk1-mediated inhibition (Fig. 4a). In contrast, where securin protein levels are restricted in separase AA expressing oocytes (separase AA + securin MO), cleavage activity initiates up to 1 hour ahead of polar body extrusion (Fig. 4a). Furthermore, by the time they extruded a polar body, separase AA + securin MO-injected oocytes had cleaved roughly twice as much sensor compared to control cells (Fig. 4a).

To monitor the impact of premature separase activity in separase AA + securin MO oocytes, we used confocal microscopy to image live oocytes stained with SiR-DNA to track individual chromosome movements⁴⁵. This analysis revealed premature chromosome segregation from 40 minutes ahead of polar body extrusion in separase AA + securin MO oocytes resulting in a non-synchronous anaphase and an increased

289 occurrence of lagging chromosomes (Fig 4bi + supplementary videos 1-4).
 290 Furthermore, in 13% of separase AA + securin MO oocytes, the segregation errors
 291 were so severe that anaphase failed completely (supplementary videos 5 and 6). This
 292 is in stark contrast to the tightly synchronised anaphase observed in untreated control
 293 oocytes ~20 minutes ahead of polar body extrusion (Fig 4bii + supplementary videos
 294 7-9). Quantification of the number of chromosomes segregated in each 10-minute
 295 time interval prior to anaphase (where anaphase was defined as the time point at
 296 which more than 50% of chromosomes had segregated) revealed that the premature
 297 separation of bivalents only took place in separase AA expressing oocytes where
 298 securin levels were restricted. In either separase AA only, or securin MO only
 299 treatment groups, almost all oocytes segregated their chromosomes synchronously
 300 within a single 10-minute time interval (Fig 4c).

301 These data further demonstrate that either inhibitory pathway, an excess of securin or
 302 inhibition by cyclin B1-Cdk1, is sufficient to prevent premature separase activity in
 303 mouse oocytes. When both pathways are diminished, the timing of anaphase and the
 304 timing of separase release are uncoupled. This results in severe chromosome
 305 segregation defects. Interestingly, even when both a securin excess is depleted and
 306 cyclin B1-Cdk1-mediated inhibition is prevented, separase activity still remains
 307 suppressed until the final hour of meiosis. This suggests that even severely restricted
 308 securin levels are sufficient for separase inhibition during the hours leading up to
 309 metaphase in meiosis.

310

311 **Discussion**

312 Similar to sister chromatid segregation in mitosis, the segregation of homologous
 313 chromosome pairs in mouse oocyte meiosis I requires the proteolysis of both securin
 314 and cyclin B1²³. Importantly, in both somatic and germ cells, the degradation of
 315 securin and cyclin B1 is synchronous and both proteins must be depleted in order for
 316 separase activation and Cdk1 inactivation to be temporally coupled²³⁻²⁵. In mouse
 317 meiosis I, a premature loss of Cdk1 activity ahead of securin degradation drives polar
 318 body extrusion, yet chromosomes do not segregate and instead become trapped within
 319 the cleavage furrow⁴⁶.

320 Here we show that mouse oocytes in meiosis I contain a large excess of securin over
 321 separase (several fold beyond that reported in mitosis). We reveal the existence of a
 322 novel mechanism of targeted degradation that functions to promote the destruction of
 323 non-separase bound securin in prometaphase. Critically this mechanism relies on a
 324 key region of securin which is only exposed when securin is not bound to separase.
 325 We suggest that in oocytes, the majority of non-separase-bound securin is removed by
 326 this mechanism, while a much smaller fraction of inhibitory separase-bound securin is
 327 only targeted in metaphase. By this strategy, the cellular destruction machinery is far
 328 less likely to be overwhelmed by excessive securin molecules during the final stages
 329 of chromosome segregation. This is particularly important given that separase must be
 330 rapidly released simultaneously on all chromosomes, ensuring their synchronous
 331 segregation. Indeed, our recent data demonstrates a similar strategy for the removal of
 332 non-Cdk1-bound cyclin B1 ahead of cyclin B1-Cdk1 activity; essential to permit a
 333 rapid drop in Cdk1 activity and to prevent anaphase from stalling³⁶.

334 We show that securin destruction is biphasic in MI oocytes, consisting of a metaphase
 335 period of D-box only destruction (resembling mitotic destruction), and a
 336 prometaphase period of destruction requiring both the D-box and an additional region
 337 able to bypass spindle checkpoint inhibition. How this additional region functions to
 338 bypass the spindle checkpoint remains unclear. However we suggest that, similar to
 339 other prometaphase APC/C substrates, this region of securin could be involved in
 340 either direct binding to the APC/C or in outcompeting spindle assembly checkpoint
 341 protein inhibition of APC/C-Cdc20⁴⁷⁻⁵⁰. In addition, cyclin B3 has recently been
 342 shown to have an important role in mouse oocyte MI in priming the APC/C for
 343 securin and cyclin B1 destruction^{39,51,52}. Therefore an alternative possibility is that
 344 this region in securin is regulated by cyclin B3 and involved in an oocyte-specific
 345 APC/C activation mechanism. It is worth noting that the regions we describe in
 346 securin and cyclin B1 do not share sequence similarity. It is therefore possible that
 347 while their destruction timings are coupled, the mechanisms by which they are
 348 targeted early in prometaphase may be distinct.

349 Chiang et al. report in fixed oocytes that either securin or cyclin B1-Cdk1 inhibitory
 350 pathways are sufficient to independently prevent chromosome segregation errors in
 351 young mouse oocytes¹⁹. Our results in live oocytes agree with this, and further
 352 demonstrate that separase activity is only released prematurely when both inhibitory

353 pathways are perturbed. It remains to be determined whether the excess of non-
 354 separase-bound securin becomes essential in aged mouse oocytes (where cohesin
 355 levels are significantly reduced^{53,54}), and over the prolonged duration of prometaphase
 356 in human oocytes (14 – 18 hours compared to 4.5 - 6.5 hours in mouse^{55,56}).
 357 Furthermore, it seems likely that an excess of securin in MI, could function to ensure
 358 a threshold level of securin protein in MII. MII securin protein level is particularly
 359 important since unlike MI, cyclin B1-Cdk1 is no longer able to compensate for
 360 depleted securin in this division^{13,19}. Indeed Nabti et al. revealed that oocytes from
 361 aged mice destroy securin more rapidly and to a greater extent than oocytes from
 362 younger mice. As a consequence, separase inhibition in MII is incomplete in these
 363 cells and sister chromatid cohesion is lost prematurely resulting in segregation
 364 defects⁵⁷.

365 Destruction of securin during prometaphase has previously been considered as
 366 precocious, an error resulting from a weakened SAC in oocytes⁵⁸. Our work instead
 367 demonstrates that this period of destruction is deliberate. In prometaphase, an excess
 368 of non-separase bound securin is targeted for destruction through a novel mechanism
 369 in oocytes. By this strategy, separase bound securin is protected, allowing for a
 370 switch-like activation of separase, essential for the fidelity of anaphase in oocytes.

371

372 Main Figure Legends

373 **Figure 1. Securin destruction begins 2.5 hours ahead of separase activation in**
 374 **mouse oocyte meiosis I.** (A) Graph showing the mean destruction profiles of VFP-
 375 tagged securin FL (magenta trace, n = 25) and cyclin B1 FL (blue trace, n = 62)
 376 alongside separase activity as determined by a separase activity biosensor (eGFP/RFP
 377 ratio, n = 20) in MI mouse oocytes relative to PB1 extrusion. Error bars ± SEM.
 378 Representative confocal images show a maturing oocyte expressing Map7-GFP
 379 (microtubules in green) and incubated with SiR-DNA (DNA in red) at times relative
 380 to the x-axis of the graph. Scale bar = 10 μm. (B) Schematic diagram of an H2B-
 381 mCherry-Scc1-eGFP separase activity biosensor. (C) Representative time-lapse
 382 images of an oocyte expressing the separase activity biosensor in Fig. 1B, imaged
 383 every 10 minutes at the times indicated relative to PB1 extrusion. eGFP (green),
 384 mCherry (red) and merged fluorescence + bright field (BF) images are shown. Scale

385 bar = 10 μ m. eGFP and mCherry images correspond to the areas marked by the white
386 dotted lines in the merge + BF images.

387 **Figure 2. A discrete region within the C-terminus of securin promotes**
388 **destruction in prometaphase.** (A) Schematic showing VFP-tagged securin and
389 cyclin B1 truncations and mutations. (B) Average securin FL (magenta trace, n = 25),
390 securin N101 (pink dashed trace, n = 23), cyclin B1 FL (blue trace, n = 32) and cyclin
391 B1 PM mutant (light blue dashed trace, n=34) destruction profiles relative to PB1
392 extrusion. (C) Sequence alignment of residues 109-133 in securin orthologs. (D)
393 Average VFP-tagged securin FL (magenta trace, n = 25), securin N101 (pink dashed
394 trace, n = 23) and securin 109-133 mutant (light blue trace, n = 23) destruction
395 profiles relative to PB1 extrusion. (E) Schematic showing the position of Securin
396 FxxF-A amino acid substitutions. Residues F125 and F128 (shown in green in the
397 wild type protein) were switched to alanines (shown in red in Securin FxxF-A). (F)
398 Average VFP-tagged securin FL (magenta trace, n = 25), securin N101 (pink dashed
399 trace, n = 23) and securin FxxF-A (green trace, n = 20) destruction traces relative to
400 PB1 extrusion. (G) VFP-tagged securin FL (magenta traces, n = 20) and securin
401 FxxF-A (green traces, n = 30) destruction profiles following incubation in 150 nM
402 nocodazole to arrest oocytes in prometaphase. These oocytes do not extrude a polar
403 body and are therefore aligned at GVBD. Error bars $\pm$ SEM.

404 **Figure 3. F125 and F128 are predicted to direct the prometaphase destruction of**
405 **a pool of non-separase-bound excess securin.** (A) Alignment of the region
406 surrounding residues F125 and F128 in securin orthologs. (B) The molecular surface
407 of separase (green) in contact with the region of securin detailed in Fig. 3a (purple).
408 Image generated using the crystal structure of the *Saccharomyces cerevisiae* separase-
409 securin complex. The side chains of securin residues Y276 and F279, which
410 correspond to F125 and F128 in the human protein, are shown as stick models and
411 labelled in purple. The N- and C-termini of the securin segment displayed are also
412 labelled in purple (C) Western blot analysis of lysates prepared from mitotic cells in
413 late prometaphase (U2OS and HeLa cells), and mouse oocytes in late prometaphase
414 (collected at 5.5 hours post GVBD). The numbers of mitotic cells and oocytes loaded
415 into each lane are as indicated. Membranes were used to detect (i) securin, (ii)
416 separase by a short exposure and (iii) separase by a long exposure (iii).

Figure 4. Both a securin excess and cyclin B1-Cdk1-mediated inhibition are independently sufficient to suppress separase activity and prevent segregation errors in mouse oocyte meiosis I. (A) Graph showing mean MI separase activity profiles as determined by a separase activity biosensor (eGFP/RFP ratio) for control (purple trace, n = 8), separase AA-injected (a fully active separase construct lacking Cdk1 phosphorylation sites S1121A and T1342A; orange trace, n = 7), securin MO-injected (blue trace, n = 8) and separase AA + securin MO-injected (green trace, n = 10) mouse oocytes relative to PB1 extrusion. Error bars $\pm$ SEM. (B) Representative images showing chromosome segregation in the 40 minutes prior to PB1 extrusion in (i) securin MO + separase AA-injected and, (ii) control oocytes. Chromosomes were visualised by incubating oocytes with SiR-DNA (shown in magenta and merged with bright field) at times shown relative to PB1 extrusion. Scale bar = 10 μ m. SiR-DNA images correspond to the areas marked by the white dotted lines in the merged images. (C) Graph showing the mean number of chromosomes segregated per 10 minute time point in the 40 minutes prior to anaphase (defined as the time point in which more than 50% of chromosomes had segregated) for control (purple trace, n = 8), separase AA-injected (orange trace, n = 7), securin MO-injected (blue trace, n = 8) and separase AA + securin MO-injected (green trace, n = 10). Error bars $\pm$ SEM.

Supplementary Figure Legends

Supplementary figure 1. Destruction profiles of securin mutation constructs leading to identification of the F125 and F128 as essential for prometaphase securin destruction. (A) Securin residues 109-133 showing sequence detail relative to the nomenclature of all venus-tagged securin mutations assayed in parts B-D. (B) Average VFP-tagged securin FL (magenta trace, n = 25), securin N101 (dashed pink trace, n = 23) and securin DAYPEIE-A (yellow trace, n = 19) destruction traces. (C) Average VFP tagged securin FL (magenta trace, n = 25), securin N101 (dashed pink trace, n = 23) and securin FFPFNP-A (purple trace, n = 23) destruction traces. (D) Average VFP-tagged securin FL (magenta trace, n = 25), securin N101 (dashed pink trace, n = 23) and securin DFESFD-A (green trace, n = 20) destruction traces. All traces are lined at PB1 extrusion and error bars = $\pm$ SEM throughout. (E) Direct comparison of the timing of destruction of all Venus-tagged securin truncations and

mutants plotted in parts B-D. Schematic representations of securin constructs are shown down the right hand side. To the left, the length of each bar indicates each construct's destruction timing relative to complete destruction (time 0; approximately 0-10 mins post PB1 extrusion). The open, white bars indicate the point at which 75% of the destruction has taken place. The light red extension to this bar indicates the point at which 50% of the destruction has taken place, followed by a dark red extension indicating the point at which 25% of the destruction has taken place. The period over which PB1 extrusions occur is shaded in grey. (F) HeLa cells transfected with securin FL, securin FxxF-A or empty mVenus N1 transfection vector as indicated were synchronised in nocodazole for 16 hours and collected by mitotic shake off. Cells were then lysed and anti-GFP immunoprecipitates (IP) were probed for separase. Input signals are also shown.

Supplementary figure 2. Differences in the timing of securin FL and FxxF-A destruction are not due to a difference in protein expression rate. Average VFP-tagged securin FL (magenta traces, n = 24) and securin FxxF-A (green traces, n = 27) on addition of cycloheximide to inhibit protein synthesis. Traces are aligned to the addition of cycloheximide at 3 hours post GVBD. Fine traces represent destruction profiles from individual oocytes; heavy traces represent the average destruction profile resulting from all injected oocytes of a given construct.

Supplementary figure 3. Meiotic securin destruction is D-box dependent but not KEN box dependent. (A) Alignment of residues 1-69 in securin orthologs containing both KEN box and D-box motifs as indicated. (B) Average VFP-tagged securin FL (magenta traces, n = 25) and securin KEN mutant (purple traces, n = 20) destruction profiles aligned at PB1 extrusion. (C) Average VFP-tagged securin FL (magenta traces, n = 16) and securin D-box mutant (light blue traces, n = 23) destruction profiles aligned at GVBD (oocytes expressing D-box mutant securin arrest their cell cycle in metaphase do not extrude a polar body). (D) Average VFP-tagged securin FL (magenta traces, n = 16) and securin KEN/D-box mutant (red traces, n = 19) destruction profiles aligned at GVBD. Note that though neither securin D-box mutant nor securin KEN/D-box mutant expressing oocytes extrude a polar body, securin destruction is only completely inhibited where both the D- and the KEN-box are

absent. Fine traces represent individual oocyte fluorescence levels and heavy traces represent their average throughout.

Supplementary figure 4. A securin phosphomutant does not affect degradation

timing in meiosis I mouse oocytes. (A) Average VFP-tagged securin FL (magenta trace, n=25) and securin 4A (blue trace, n=17; securin 4A is a phosphomutant containing mutations S31A, T66A, S87A and S89A⁴³) destruction profiles aligned at PB1 extrusion. Error bars = +/- SEM.

Supplementary figure 5. Quantification of securin morpholino knock down.

Western blot of control and securin morpholino (MO) injected oocytes collected 5.5 hours post GVBD (numbers of oocytes loaded per lane are indicated). Quantification of protein bands indicates an 87% knockdown of securin in MO-injected oocytes. The lower blot was probed for vinculin and used as a loading control.

Methods

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to Suzanne Madgwick (suzanne.madgwick@newcastle.ac.uk).

Gamete Collection and Culture

4 to 8-week-old female, outbred, CD1 mice (Charles River) were used. All animals were handled in accordance with ethics approved by the UK Home Office Animals Scientific Procedures Act 1986. GV stage oocytes were collected from ovaries punctured with a sterile needle and stripped of their cumulus cells mechanically using a pipette. For bench handling, microinjections and imaging experiments, oocytes were cultured at 37°C in medium M2 (Sigma) supplemented where necessary with the addition of 30 nM 3-isobutyl-1-methylxanthine (IBMX; Sigma) to arrest oocytes at prophase I. Data was only collected from oocytes that underwent GVBD with normal timings and had a diameter within 95-105% of the population average. To ensure reproducibility, oocyte data sets were gathered from a minimum of 3 independent experiments. For each independent experiment, both control and

513 treatments groups were derived from the same pool of oocytes, collected from a
514 minimum of 2 animals. Oocytes were selected at random for microinjection. Where
515 necessary, and at the times indicated, nocodazole (Sigma) was added to the media at a
516 final concentration of 150 nM. For confocal imaging SiR Hoechst was added to media
517 30 minutes prior to imaging at a final concentration of 250 nM⁴⁵.

518 **Preparation of cRNA constructs for microinjection**

519 Microtubule associated protein 7 (Map7; to visualise microtubules⁵⁹), wild type
520 human securin, securin truncations, separase biosensor (a gift from Jan Van Deursen)
521 and separase AA mutant (a gift from Michael Lampson) sequences were amplified by
522 PCR as previously described⁶⁰. Mutations within securin were generated by primer
523 overhang extension PCR. Following this we performed Sequence and Ligation
524 Independent Cloning (SLIC)⁶¹ using modified pRN3 vectors to generate either a
525 construct with no further tags (the separase biosensor) or constructs coupled to a
526 Venus fluorescent protein (all other sequences)⁶². Resultant plasmids were linearized
527 and cRNA for microinjection was prepared using a T3 mMESSAGE mMACHINE kit
528 according to the manufactures instructions (Ambion Inc.). Maximal stability was
529 conferred on all cRNA constructs by the presence of a 5' globin UTR upstream and
530 both 3'UTR and poly (A)–encoding tracts downstream of the gene. cRNA was
531 dissolved in nuclease-free water to the required micropipette concentration.

532 **Knockdown of gene expression using morpholinos**

533 A Morpholino antisense oligo designed to recognize the 5' UTR of securin (sequence:
534 GATAAGAGTAGCCATTCTGGATTAC ; MO; Gene Tools) was used to
535 knockdown gene expression. As per the manufacturer's instructions, the oligo was
536 stored at room temperature, heated for 5 minutes at 65°C prior to use and loaded at a
537 micropipette concentration of 1 mM.

538 **Microinjection and imaging**

539 Oocyte microinjection of the MO and construct mRNAs was carried out on the heated
540 stage of an inverted microscope fitted for epifluorescence (Olympus; 1X71). In brief,
541 fabricated micropipettes were inserted into cells using the negative capacitance
542 overcompensation facility on an electrophysiological amplifier (World Precision
543 Instruments). This procedure ensures a high rate of survival (>95%). The final volume

of injection was estimated by the diameter of displaced ooplasm and was typically between 0.1-0.3% of total volume.

To generate destruction profiles, bright field and fluorescence images were captured every 10 minutes throughout meiosis I by an inverted Olympus IX71 microscope (fitted for epifluorescence) and CCD camera (Micromax, Sony Interline chip, Princeton Instruments). Images were then analysed and processed using MetaFluor software (version 7.7.0.0; Molecular Devices). All experiments were performed at 37°C.

Confocal images (including all experiments using the separate biosensor) were captured using a Zeiss LSM-800. Oocytes were imaged at 10 minute intervals through 20+ Z-sections over a 12-hour period from GV stage. All experiments were performed in a temperature-controlled, humidified chamber set at 37°C. Bright-field and fluorescent images were recorded in Zen Blue (Zeiss) and processed in Fiji. By this method, all oocytes extruded polar bodies.

Molecular structure images and multiple sequence alignments

Molecular structure images were generated using the PyMOL Molecular Graphics System, version 1.3 Schrödinger, LLC. Sequence conservation alignments were made by importing protein sequences from Uniprot and aligning in Jalview, version 15.0. All figures were prepared in Adobe Illustrator CC, version 17.1.0.

Mitotic Cell Cultures

Standard laboratory U2OS and HeLa cell lines (a gift from Jonathan Higgins) were used to generate lysates for western blotting. Both cell types were cultured in flasks at 37°C, 5% CO₂ in DMEM (Lonza) with 10% FBS (Life Technologies) and antibiotics. Once cell coverage reached ~90%, flasks were treated with 100 nM nocodazole for an 8-hour incubation period for western blotting and a 16-hour incubation period for immunoprecipitation. Metaphase cells were then collected by mechanical shake off and lysed.

Immunoprecipitation

HeLa cells transfected with securin FL, securin FxxF-A or empty mVenus N1 transfection vector were synchronised and collected as described above. Cells were

574 then lysed in 50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 0.5% NP-40 plus protease
575 inhibitor cocktail (Roche) for 30 min on ice and clarified by a 12,000 g spin for 20
576 min at 4°C. Complexes were immunoprecipitated for 90 minutes at 4°C with GFP-
577 Trap beads (Chromotek). After five washes in lysis buffer, proteins were eluted from
578 beads by incubating for 10 minutes at 95°C in sample buffer. The supernatant was
579 then analysed by immunoblotting.

580 **Immunoblotting**

581 Mitotic U2OS and HeLa cell lysates were prepared using Laemmli buffer following
582 mechanical shake off of metaphase cells. Oocytes were collected 5.5 hours after
583 GVBD $\pm$ 15 min and lysed in Laemmli buffer. SDS-PAGE and immunoblotting were
584 carried out by standard procedures. Immunoblot membrane sections were incubated
585 for 16 hours at 4°C with either anti-securin (Abcam, AB3305) or anti-separase
586 (Abnova, 6H6). Non-fat milk (5%) was used as a blocking solution and anti-mouse
587 IgG (7076P2; Cell Signaling) and ECL Select (RPN2235; GE Healthcare) were used
588 as secondary detection reagents. ECL Select detection reagents were specifically used
589 to produce x-ray signals with broad linear dynamic range. Membranes were exposed
590 to Hyperfilm x-ray film (Amersham Biosciences) and developed using a SRX101 film
591 processor (Konica). Exposure time depended on the strength of the signal.
592 Immunoblots are representative of 2 independent blots.

593 **Quantification and Statistical Analysis**

594 Real-time destruction profiles were recorded in MetaFluor (Molecular Devices) and
595 data was automatically logged in Excel. By taking an average VFP intensity reading
596 from a defined region of interest around the oocyte, fluorescence intensity was plotted
597 over time and oocyte data sets were aligned at PB1 extrusion unless otherwise stated.
598 Average polar body extrusion timings were identical between experimental groups
599 unless otherwise stated. Fluorescence data values are arbitrary. In order to give the
600 destruction profile of each oocyte equal weighting, when generating an average
601 treatment destruction profile, all individual data sets were normalised prior to
602 calculation (taking the maximum point of fluorescence prior to protein destruction as
603 100 a.u.). However, like the examples shown in Figures S2 and S3b-d, we also
604 compared all raw traces, confirming that in each case experiment, the order and

605 pattern of construct destruction remained the same, regardless of data handling
606 method.

607 Average cleavage profiles for separate biosensor experiments were produced in Fiji
608 by creating a clipping mask to the DNA using the far red signal emitted by SiR DNA
609 treatment. The eGFP and mCherry intensity readings from the clipping mask were
610 then plotted over time and aligned at PB1 extrusion. eGFP/mCherry ratios were
611 calculated in Excel.

612 When only two constructs are compared, we have displayed both the average trace
613 and individual traces. Where 3 or more constructs are compared, for clarity, only the
614 average trace is shown with SEM error bars.

615

616 **Acknowledgements**

617 We thank Professor Jonathan MG Higgins for scientific discussion throughout this
618 study and for critical reading of the manuscript along with Dr. Alexandre Webster.

619 We thank F. Davidson for technical assistance. This work was supported by a
620 Wellcome Trust Career Re-entry Fellowship grant to SM [062376]. ORD is a Sir
621 Henry Dale Fellow jointly funded by the Wellcome Trust and Royal Society [Grant
622 Number 104158/Z/14/Z].

623

624 **Author contributions**

625 CT carried out all experiments alongside SM, with critical contribution from BH in
626 western blotting experiments and MDL in microscopy. The project was carried out
627 under the supervision of SM and MDL. CT and SM designed the experiments with
628 assistance from ORD. CT and SM prepared the manuscript with assistance from
629 ORD. The authors declare no competing financial interests.

630

631

632

633 Bibliography

- 634 1. Sansregret, L. & Swanton, C. The role of aneuploidy in cancer evolution. *Cold*
635 *Spring Harb. Perspect. Med.* **7**, 1–18 (2017).
- 636 2. Gordon, D. J., Resio, B. & Pellman, D. Causes and consequences of
637 aneuploidy in cancer. *Nat. Rev. Genet.* **13**, 189–203 (2012).
- 638 3. Nagaoka, S. I., Hassold, T. J. & Hunt, P. A. Human aneuploidy: mechanisms
639 and new insights into an age-old problem. *Nat Rev Genet* **13**, 493–504 (2012).
- 640 4. Herbert, M., Kalleas, D., Cooney, D., Lamb, M. & Lister, L. Meiosis and
641 Maternal Aging: Insights from Aneuploid Oocytes and Trisomy Births. *Cold*
642 *Spring Harb Perspect Biol.* **7**, a017970 (2015).
- 643 5. Webster, A. & Schuh, M. Mechanisms of Aneuploidy in Human Eggs. *Trends*
644 *Cell Biol.* **27**, 55–68 (2017).
- 645 6. Uhlmann, F., Wernic, D., Poupart, M.-A., Koonin, E. V & Nasmyth, K.
646 Cleavage of Cohesin by the CD Clan Protease Separin Triggers Anaphase in
647 Yeast. *Cell* **103**, 375–386 (2000).
- 648 7. Hauf, S., Waizenegger, I. C. & Peters, J. M. Cohesin cleavage by separase
649 required for anaphase and cytokinesis in human cells. *Science (80-.).* **293**,
650 1320–1323 (2001).
- 651 8. Waizenegger, I. C., Giménez-Abián, J. F., Wernic, D. & Peters, J. M.
652 Regulation of human separase by securin binding and autocleavage. *Curr. Biol.*
653 **12**, 1368–1378 (2002).
- 654 9. Stemmann, O., Zou, H., Gerber, S. A., Gygi, S. P. & Kirschner, M. W. Dual
655 inhibition of sister chromatid separation at metaphase. *Cell* **107**, 715–726
656 (2001).
- 657 10. Gorr, I. H., Boos, D. & Stemmann, O. Mutual inhibition of separase and Cdk1
658 by two-step complex formation. *Mol. Cell* **19**, 135–141 (2005).
- 659 11. Stemmann, O., Gorr, I. H. & Boos, D. Anaphase topsy-turvy: Cdk1 a securin,
660 separase a CKI. *Cell Cycle* **5**, 11–13 (2006).

- 661 12. Holland, A. J. & Taylor, S. S. Cyclin-B1-mediated inhibition of excess
662 separase is required for timely chromosome disjunction. *J. Cell Sci.* **119**, 3325–
663 3336 (2006).
- 664 13. Nabti, I., Reis, A., Levasseur, M., Stemmann, O. & Jones, K. T. Securin and
665 not CDK1/cyclin B1 regulates sister chromatid disjunction during meiosis II in
666 mouse eggs. *Dev. Biol.* **321**, 379–386 (2008).
- 667 14. Huang, X. *et al.* Preimplantation Mouse Embryos Depend on Inhibitory
668 Phosphorylation of Separase To Prevent Chromosome Missegregation. *Mol.*
669 *Cell. Biol.* **29**, 1498–1505 (2009).
- 670 15. Kamenz, J. & Hauf, S. Time To Split Up: Dynamics of Chromosome
671 Separation. *Trends Cell Biol.* **27**, 42–54 (2017).
- 672 16. Mei, J., Huang, X. & Zhang, P. Securin is not required for cellular viability, but
673 is required for normal growth of mouse embryonic fibroblasts. *Curr. Biol.* **11**,
674 1197–1201 (2001).
- 675 17. Pflieger, K., Heubes, S., Cox, J., Stemmann, O. & Speicher, M. R. Securin is
676 not required for chromosomal stability in human cells. *PLoS Biol.* **3**, 1–8
677 (2005).
- 678 18. Wirth, K. G. *et al.* Separase: A universal trigger for sister chromatid
679 disjunction but not chromosome cycle progression. *J. Cell Biol.* **172**, 847–860
680 (2006).
- 681 19. Chiang, T., Schultz, R. M. & Lampson, M. a. Age-Dependent Susceptibility of
682 Chromosome Cohesion to Premature Separase Activation in Mouse Oocytes.
683 *Biol. Reprod.* **85**, 1279–1283 (2011).
- 684 20. Hornig, N. C. D., Knowles, P. P., McDonald, N. Q. & Uhlmann, F. The dual
685 mechanism of separase regulation by securin. *Current Biology* **12**, 973–982
686 (2002).
- 687 21. Holland, A. J. & Taylor, S. S. Many faces of separase regulation. *SEB Exp.*
688 *Biol. Ser.* **59**, 99–112 (2008).
- 689 22. Hellmuth, S. *et al.* Positive and negative regulation of vertebrate separase by

- 690 Cdk1-cyclin B1 may explain why securin is dispensable. *J. Biol. Chem.* **290**,
691 8002–8010 (2015).
- 692 23. Herbert, M. *et al.* Homologue disjunction in mouse oocytes requires proteolysis
693 of securin and cyclin B1. *Nat. Cell Biol.* **5**, 1023–1025 (2003).
- 694 24. Kamenz, J., Mihaljev, T., Kubis, A., Legewie, S. & Hauf, S. Robust Ordering
695 of Anaphase Events by Adaptive Thresholds and Competing Degradation
696 Pathways. *Mol. Cell* **60**, 446–459 (2015).
- 697 25. Rattani, A. *et al.* Dependency of the spindle assembly checkpoint on Cdk1
698 renders the anaphase transition irreversible. *Curr. Biol.* **24**, 630–637 (2014).
- 699 26. Yamano, H. *et al.* The role of the destruction box and its neighbouring lysine
700 residues in cyclin B for anaphase ubiquitin- dependent proteolysis in ssion
701 yeast: de ning the D-box receptor. **17**, 5670–5678 (1998).
- 702 27. Hagting, A. *et al.* Human securin proteolysis is controlled by the spindle
703 checkpoint and reveals when the APC/C switches from activation by Cdc20 to
704 Cdh1. *J. Cell Biol.* **157**, 1125–1137 (2002).
- 705 28. Glotzer, M., Murray, A. W. & Kirschner, M. W. Cyclin is degraded by the
706 ubiquitin pathway. *Nature* **349**, 132–138 (1991).
- 707 29. Pflieger, C. M., Lee, E. & Kirschner, M. W. Substrate recognition by the Cdc20
708 and Cdh1 components of the anaphase-promoting complex. *Genes Dev.* **15**,
709 2396–2407 (2001).
- 710 30. Zur, A. & Brandeis, M. Securin degradation is mediated by fzy and fzr, and is
711 required for complete chromatid separation but not for cytokinesis. *EMBO J.*
712 **20**, 792–801 (2001).
- 713 31. Chao, W. C. H., Kulkarni, K., Zhang, Z., Kong, E. H. & Barford, D. Structure
714 of the mitotic checkpoint complex. *Nature* **484**, 208–213 (2012).
- 715 32. He, J. *et al.* Insights into degron recognition by APC/C coactivators from the
716 structure of an Acm1-Cdh1 complex. *Mol. Cell* **50**, 649–660 (2013).
- 717 33. Foley, E. A. & Kapoor, T. M. Microtubule attachment and spindle assembly
718 checkpoint signalling at the kinetochore. *Nat. Rev. Mol. Cell Biol.* **14**, 25–37

- 719 (2012).
- 720 34. Lara-Gonzalez, P., Westhorpe, F. G. & Taylor, S. S. The spindle assembly
721 checkpoint. *Curr. Biol.* **22**, R966–R980 (2012).
- 722 35. Homer, H. A., McDougall, A., Levasseur, M., Murdoch, A. P. & Herbert, M.
723 Mad2 is required for inhibiting securin and cyclin B degradation following
724 spindle depolymerisation in meiosis I mouse oocytes. *Reproduction* **130**, 829–
725 843 (2005).
- 726 36. Levasseur, M. D., Thomas, C., Davies, O. R., Higgins, J. M. G. & Madgwick,
727 S. Aneuploidy in Oocytes Is Prevented by Sustained CDK1 Activity through
728 Degron Masking in Cyclin B1. *Dev. Cell* **48**, 672–684.e5 (2019).
- 729 37. Nam, H.-J. & van Deursen, J. M. Cyclin B2 and p53 control proper timing of
730 centrosome separation. *Nat. Cell Biol.* **16**, 538–549 (2014).
- 731 38. Nikalayevich, E., Bouftas, N. & Wassmann, K. Detection of Separase Activity
732 Using a Cleavage Sensor in Live Mouse Oocytes. in *Mouse Oocyte*
733 *Development: Methods and Protocols* (eds. Verlhac, M.-H. & Terret, M.-E.)
734 99–112 (Springer New York, 2018). doi:10.1007/978-1-4939-8603-3_11
- 735 39. Karasu, M. E., Bouftas, N., Keeney, S. & Wassmann, K. Cyclin B3 promotes
736 anaphase i onset in oocyte meiosis. *J. Cell Biol.* **218**, 1265–1281 (2019).
- 737 40. Lane, S. I. R., Yun, Y. & Jones, K. T. Timing of anaphase-promoting complex
738 activation in mouse oocytes is predicted by microtubule-kinetochore
739 attachment but not by bivalent alignment or tension. *Development* **139**, 1947–
740 55 (2012).
- 741 41. Luo, S. & Tong, L. Molecular mechanism for the regulation of yeast separase
742 by securin. *Nature* **542**, 255–259 (2017).
- 743 42. Luo, S. & Tong, L. Structural biology of the separase–securin complex with
744 crucial roles in chromosome segregation. *Curr. Opin. Struct. Biol.* **49**, 114–122
745 (2018).
- 746 43. Hellmuth, S., Böttger, F., Pan, C., Mann, M. & Stemmann, O. PP2A delays
747 APC/C-dependent degradation of separase-associated but not free securin.

- 748 *EMBO J.* **33**, 1134–1147 (2014).
- 749 44. Heinrich, S. *et al.* Determinants of robustness in spindle assembly checkpoint
750 signalling. *Nat. Cell Biol.* **15**, 1328–1339 (2013).
- 751 45. Lukinavičius, G. *et al.* SiR–Hoechst is a far-red DNA stain for live-cell
752 nanoscopy. *Nat. Commun.* **6**, 8497 (2015).
- 753 46. Wei, Z., Greaney, J., Zhou, C. & A. Homer, H. Cdk1 inactivation induces post-
754 anaphase-onset spindle migration and membrane protrusion required for
755 extreme asymmetry in mouse oocytes. *Nat. Commun.* **9**, (2018).
- 756 47. Hayes, M. J. *et al.* Early mitotic degradation of Nek2A depends on Cdc20-
757 independent interaction with the APC/C. *Nat. Cell Biol.* **8**, 607–614 (2006).
- 758 48. Zon, W. van & Wolthuis, R. M. F. Cyclin A and Nek2A: APC/C–Cdc20
759 substrates invisible to the mitotic spindle checkpoint. *Biochem. Soc. Trans.* **38**,
760 72–77 (2010).
- 761 49. Di Fiore, B. & Pines, J. How cyclin a destruction escapes the spindle assembly
762 checkpoint. *J. Cell Biol.* **190**, 501–509 (2010).
- 763 50. DiFiore, B. *et al.* The ABBA Motif binds APC/C activators and is shared by
764 APC/C substrates and regulators. *Dev. Cell* **32**, 358–372 (2015).
- 765 51. Li, Y. *et al.* Cyclin b3 is required for metaphase to anaphase transition in
766 oocyte meiosis I. *J. Cell Biol.* **218**, 1553–1563 (2019).
- 767 52. Bouftas, N. & Wassmann, K. Cycling through mammalian meiosis: B-type
768 cyclins in oocytes. *Cell Cycle* **18**, 1537–1548 (2019).
- 769 53. Tachibana-Konwalski, K. *et al.* Rec8-containing cohesin maintains bivalents
770 without turnover during the growing phase of mouse oocytes. *Genes Dev.* **24**,
771 2505–2516 (2010).
- 772 54. Lister, L. M. *et al.* Age-related meiotic segregation errors in mammalian
773 oocytes are preceded by depletion of cohesin and Sgo2. *Curr. Biol.* **20**, 1511–
774 1521 (2010).
- 775 55. Schuh, M. & Ellenberg, J. Self-Organization of MTOCs Replaces Centrosome

776 Function during Acentrosomal Spindle Assembly in Live Mouse Oocytes. *Cell*
777 **130**, 484–498 (2007).

778 56. Holubcova, Z., Blayney, M., Elder, K. & Schuh, M. Error-prone chromosome-
779 mediated spindle assembly favors chromosome segregation defects in human
780 oocytes. *Science* (80-.). **348**, 1143–1147 (2015).

781 57. Nabti, I., Grimes, R., Sarna, H., Marangos, P. & Carroll, J. Maternal age-
782 dependent APC/C-mediated decrease in securin causes premature sister
783 chromatid separation in meiosis II. *Nat. Commun.* **8**, 15346 (2017).

784 58. Kyogoku, H. & Kitajima, T. S. Large Cytoplasm Is Linked to the Error-Prone
785 Nature of Oocytes. *Dev. Cell* **41**, 287–298.e4 (2017).

786 59. Prodon, F. *et al.* Dual mechanism controls asymmetric spindle position in
787 ascidian germ cell precursors. *Development* **137**, 2011–2021 (2010).

788 60. Madgwick, S. *et al.* Maintenance of sister chromatid attachment in mouse eggs
789 through maturation-promoting factor activity. *Dev. Biol.* **275**, 68–81 (2004).

790 61. Jeong, J. Y. *et al.* One-step sequence-and ligation-independent cloning as a
791 rapid and versatile cloning method for functional genomics Studies. *Appl.*
792 *Environ. Microbiol.* **78**, 5440–5443 (2012).

793 62. Levasseur, M. Making cRNA for Microinjection and Expression of
794 Fluorescently Tagged Proteins for Live-Cell Imaging in Oocytes. in
795 *Mammalian Oocyte Regulation: Methods and Protocols* (ed. Homer, H. A.)
796 121–134 (Humana Press, 2013). doi:10.1007/978-1-62703-191-2_8

797

Figure 1

A.

B.

C.

Figure 2

bioRxiv preprint doi: <https://doi.org/10.1101/824763>; this version posted October 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

A.

Securin (human)	117	F	F	P	F	N	P	L	D	F	E	S	F	D	L	P	E	E	H	Q	135
Securin (mouse)	114	F	F	P	F	N	P	L	D	F	E	S	F	D	L	P	E	E	H	Q	132
Securin (barn owl)	111	M	F	P	Y	D	P	R	D	F	E	S	F	D	L	P	E	E	H	K	129
Securin (frog)	107	F	V	P	Y	N	P	L	D	F	E	S	F	D	V	P	E	D	H	K	125
Securin (yeast)	268	P	L	P	Y	V	P	E	G	Y	S	P	F	Q	Q	D	D	I	E	K	286
										*		*									

B.

C.

Figure 4

bioRxiv preprint doi: <https://doi.org/10.1101/824763>; this version posted October 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.

Supplementary Figure 1

A.

Securin FL	109	D A Y P E I E K F F P F N P L D F E S F D L P E E	133
Securin DAYPEIE-A	109	A A A A A A A K F F P F N P L D F E S F D L P E E	133
Securin FFPFNP-A	109	D A Y P E I E K A A A A A A L D F E S F D L P E E	133
Securin DFESFD-A	109	D A Y P E I E K F F P F N P L A A A A A A L P E E	133
Securin FxxF-A	109	D A Y P E I E K F F P F N P L D A E S A D L P E E	133

B.

C.

Supplementary Figure 1

D.

E.

F.

Supplementary figure 2

Supplementary figure 3

A.

B.

Supplementary figure 3

C.

D.

Supplementary figure 4

A.

Supplementary figure 5

A.