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Cep57 and Cep57L1 cooperatively maintain centriole engagement during interphase to ensure proper centriole duplication cycle

AUTHORS

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24 **Centrioles duplicate in the interphase only once per cell cycle. Newly**
 25 **formed centrioles remain associated with their mother centrioles. The two**
 26 **centrioles disengage at the end of mitosis, which licenses centriole**
 27 **duplication in the next cell cycle. Therefore, timely centriole**
 28 **disengagement is critical for the proper centriole duplication cycle.**
 29 **However, the mechanisms underlying centriole engagement during**
 30 **interphase are poorly understood. Here, we show that Cep57 and Cep57L1**
 31 **cooperatively maintain centriole engagement during interphase.**
 32 **Co-depletion of Cep57 and Cep57L1 induces precocious centriole**
 33 **disengagement in the interphase without compromising cell cycle**
 34 **progression. The disengaged daughter centrioles convert into**
 35 **centrosomes during interphase in a Plk1-dependent manner. Furthermore,**
 36 **the centrioles reduplicate and the centriole number increases, which**
 37 **results in chromosome segregation errors. Overall, these findings**
 38 **demonstrate that the maintenance of centriole engagement by Cep57 and**
 39 **Cep57L1 during interphase is crucial for the tight control of centriole copy**
 40 **number and thus for proper chromosome segregation.**

41

42 **KEYWORDS**

43 Centriole engagement, Centriole disengagement, Centriole, Centrosome,
 44 Chromosome segregation, Cep57, Cep57L1, Plk1

45

46 **INTRODUCTION**

47 The centrosome is an organelle that serves as a major microtubule
 48 organizing center (MTOC) in animal cells(Conduit et al., 2015). In mitosis, the
 49 two centrosomes migrate to opposite sides of the cell and facilitate the formation
 50 of a bipolar spindle(Petry, 2016). Therefore, the number of centrosomes must be
 51 strictly controlled for proper chromosome segregation(Nigg and Holland, 2018).
 52 Abnormalities in centrosome number cause improper spindle formation,
 53 chromosome instability, and various disorders including cancer and congenital
 54 abnormalities such as microcephaly(Bettencourt-Dias et al., 2011). After cell
 55 division, each daughter cell harbors two centrioles, and a new daughter centriole
 56 forms in proximity to the mother centriole during the S phase(Nigg and Holland,
 57 2018). As the number of centrioles are halved after cell division, the centrioles
 58 are duplicated only once per cell cycle, ensuring that the correct number of
 59 centrioles is maintained(Gönczy and Hatzopoulos, 2019). Defects in the
 60 centriole duplication cycle can lead to aberrations in centrosome number(Nigg
 61 and Holland, 2018).

62 The centrosome is composed of one or two centrioles and the
 63 surrounding pericentriolar material (PCM)(Conduit et al., 2015). The centrioles
 64 are duplicated only once in the S phase(Fu et al., 2015). Each newly formed
 65 daughter centriole grows at the proximity of the mother centriole during centriole
 66 duplication and is orthogonally engaged with the mother centriole until late
 67 mitosis (centriole engagement). After mitotic exit, mother and daughter centrioles
 68 are dissociated (centriole disengagement), and both centrioles are licensed to
 69 duplicate in the next cell cycle(Tsou et al., 2009). When centriole disengagement

occurs precociously in the interphase, centrioles are reduplicated within the same cell cycle(Lončarek et al., 2010; Martino et al., 2015). Such centriole reduplication results in an increase in the number of centrioles in cycling cells and may lead to chromosomal instability and a failure of cell division(Holmes et al., 2010). Thus, the maintenance of centriole engagement is one of the mechanisms limiting centriole duplication to once per cell cycle and controlling proper centrosome cycle progression. However, the molecular mechanisms underlying centriole engagement and disengagement remain largely unknown.

Recently, it has been suggested that the expanded PCM surrounds the pair of centrioles and maintains centriole engagement during mitosis(Seo et al., 2015). In particular, in human cells, pericentrin (PCNT), a major PCM scaffold protein, has been shown to be a critical factor for centriole engagement during mitosis(Lee and Rhee, 2012; Matsuo et al., 2012). PCNT is an elongated molecule that is radially oriented, with its C-terminus region near the centriole and its N-terminus extending outward to the periphery(Lawo et al., 2012; Mennella et al., 2012). A radial array of PCNT acting as a scaffold for PCM facilitates the recruitment of other PCM proteins during PCM expansion, and the depletion of PCNT causes precocious centriole disengagement in early mitosis(Matsuo et al., 2012). Importantly, we recently identified Cep57 (centrosomal protein of 57 kDa) as a binding partner of PCNT(Watanabe et al., 2019). Cep57 localizes at the vicinity of centrioles and binds to the PACT domain, a conserved C-terminus domain, of PCNT. Depletion of Cep57 perturbs the Cep57-PCNT interaction and thereby affects PCM organization in early mitosis, leading to precocious centriole disengagement(Watanabe et al., 2019). For the

centriole disengagement that normally occurs at the end of mitosis, separase-dependent cleavage of PCNT, which presumably occurs around the metaphase-to-anaphase transition, is required for the disassembly of expanded PCM and subsequent centriole disengagement (Lee and Rhee, 2012; Matsuo et al., 2012). Plk1-dependent phosphorylation of PCNT has also been reported as a priming step for the separase-dependent cleavage of PCNT (Kim et al., 2015). In the prolonged G2/M phase induced by treatment with a Cdk1 inhibitor or DNA-damaging reagents, Plk1 and separase are aberrantly activated, which subsequently induces precocious centriole disengagement (Douthwright and Sluder, 2014; Inanç et al., 2010; Prosser et al., 2012). However, in contrast to the recent progress in understanding mechanisms of centriole disengagement in mitosis, very little is known about mechanisms maintaining centriole engagement during interphase.

Centrosomal protein 57 kDa-like protein 1 (Cep57L1) is a paralog of the *Cep57* gene and is conserved in vertebrates. Cep57L1 was originally named for its homology to Cep57. It has been reported that the reduction of Cep57L1 expression level is responsible for the congenital absence of the anterior cruciate ligament (ACL) and the posterior cruciate ligament (PCL) in the knee (Liu et al., 2015). Although we have recently demonstrated that Cep57 localizes at the centrosome and is required for the maintenance of centriole engagement in early mitosis (Watanabe et al., 2019), neither the exact function nor localization of Cep57L1 has been reported so far.

In this study, we reveal that Cep57 and Cep57L1 redundantly regulate centriole engagement in the interphase. Co-depletion of Cep57 and Cep57L1

118 causes precocious centriole disengagement during interphase in human cells.
 119 Such precociously disengaged daughter centrioles acquire PCM and MTOC
 120 activity. The precocious centriole disengagement in interphase is accompanied
 121 by centriole reduplication, and the number of centrioles per cell gradually
 122 increases with each passing cell division, since the amplified centrioles are
 123 inevitably inherited by the daughter cells. Furthermore, the amplified centrioles
 124 cause a higher frequency of chromosome segregation errors. It is therefore most
 125 likely that defects in centriole engagement in interphase are more deleterious
 126 than those in early mitosis. Overall, these findings shed light on the molecules
 127 involved in the maintenance of centriole engagement during interphase and
 128 clarify the effects of the disruption of centriole engagement on the fidelity of
 129 chromosome segregation and cell division.

130

131 **RESULTS**

132 **Co-depletion of Cep57 and Cep57L1 causes an increase in the number of** 133 **centrosomes during interphase**

134 We recently reported that the Cep57-PCNT interaction is crucial for the
135 maintenance of centriole engagement during mitosis and that depletion of
136 Cep57 causes precocious centriole disengagement in mitosis, but not in
137 interphase (Mitosis: $42.2 \pm 5.1\%$, Interphase: 0%, N=30 and 50, respectively,
138 from three independent experiments) (Figures 1A and 1B)(Watanabe et al.,
139 2019). While the mechanism of centriole engagement in mitosis is gradually
140 being elucidated, that of interphase remains completely unknown. To address
141 this, we sought to identify the molecules required for centriole engagement
142 during interphase. Given that comprehensive RNAi-based screens have failed to
143 identify such molecules thus far, we assumed that two or more molecules have
144 redundant functions in the maintenance of centriole engagement during
145 interphase. Taking this possibility into account, we considered Cep57 and PCNT
146 as potential targets. We also focused on an uncharacterized paralog of Cep57 in
147 addition to Cep57 and PCNT: Cep57L1. Cep57L1 is a conserved protein in
148 vertebrates and consists of 460 amino acid residues with 39% sequence
149 homology to Cep57 in *Homo sapiens* (Figure S1A). We found that depletion of
150 Cep57L1 did not affect centriole engagement in either interphase or mitosis
151 (Figures 1E and 1F). We accordingly tested co-depletion of two of the three
152 proteins, Cep57, Cep57L1, and PCNT, by treating human cells with two distinct
153 siRNAs, and observed the centrosome and centriole behaviors. Among these
154 combinations, we first found that co-depletion of Cep57 and Cep57L1

(Cep57/Cep57L1) caused an increase in the number of centrosomes, marked by Cep192, in interphase ($46.6 \pm 17.7\%$, N=50, from three independent experiments) (Figures 1C and 1D). Interestingly, we next found by using a centriole maker, CP110, that approximately 20% of the Cep57/Cep57L1-depleted cells exhibited precocious centriole disengagement in the interphase ($16.0 \pm 5.3\%$, N=50, from three independent experiments, we defined precocious centriole disengagement when at least one centriole is more than $0.75 \mu\text{m}$ away from the others) (Figures 1C and 1E) and that approximately 10% of the cells had more than four centrioles ($11.3 \pm 7.6\%$) (Figures 1C and 1F). These phenotypes provoked by the depletion of Cep57/Cep57L1 were also confirmed by using a different siRNA and other human cell lines (Figures S1B and S1C). As expected, this phenotype was rescued by expressing a synthetic RNAi-resistant Cep57 or Cep57L1 construct (siControl: $2.2 \pm 1.9\%$, siCep57/Cep57L1: $33.2 \pm 2.9\%$, siCep57/Cep57L1 + Cep57 expression: $10.0 \pm 3.3\%$, siCep57/Cep57L1 + Cep57L1 expression: $6.7 \pm 3.3\%$, N=30, from three independent experiments) (Figures 1G and 1H). Taking these results together, we conclude that the co-depletion of Cep57 and Cep57L1 causes the aberrations in the number of centrosomes and centrioles during interphase.

173

174 **Cep57 and Cep57L1 redundantly regulate centriole engagement during** 175 **interphase**

176 In general, the presence of four separate centrioles in the interphase can
177 stem from a failure of cytokinesis. To investigate whether the phenotype seen
178 with co-depletion of Cep57 and Cep57L1 is due to a failure of cytokinesis or

precocious centriole disengagement during interphase, we immunostained HeLa cells with an antibody against ODF2, a marker of old mother centrioles (Figures S2A and S2B). If the four separate centrioles were the consequence of a failure of cytokinesis, the number of old mother centrioles in an interphase cell should be two. However, more than 80% of the HeLa cells with four separate centrioles or amplified centrioles possessed only one ODF2 focus ($84.8 \pm 1.7\%$, $N > 50$, from two independent experiments), as was the case in control cells (100%, Figures S2A and S2B). We therefore reasoned that the separate centrioles likely resulted from precocious centriole disengagement in interphase, rather than cytokinesis failure. To confirm this idea, we determined when in the cell cycle the phenotype could be observed in Cep57/Cep57L1-depleted cells using 5-ethynyl-2'-deoxyuridine (EdU, an S phase marker) and an antibody against CENP-F (a G2 phase marker). Immunofluorescence with the cell cycle markers indicated that, as in control cells, there were two centrioles in the G1 phase in Cep57/Cep57L1-depleted cells, (EdU-negative, CENP-F-negative cells), but approximately 20% of S phase cells (EdU-positive, CENP-F-negative cells) and 40% of G2 phase cells (CENP-F-positive cells) exhibited four or more separate centrioles (S phase: $24.9 \pm 2.0\%$, G2 phase: $42.9 \pm 10.1\%$, $N=30$, from three independent experiments) (Figures 2A, 2B and 2C). These data strongly suggest that the presence of the four separate centrioles observed during interphase in Cep57/Cep57L1-depleted cells stem from precocious centriole disengagement in the S and G2 phases. Furthermore, live-imaging analysis using HeLa cells stably expressing GFP-centrin1 (HeLa-GFP-centrin1) confirmed that precocious disengagement occurred during interphase in

203 Cep57/Cep57L1-depleted cells (Figure 2D and Supplementary Movie 1). We
 204 also measured cumulative percentages of the disengagement phenotype based
 205 on live-imaging data and found that approximately 60% of
 206 Cep57/Cep57L1-depleted cells (16 out of 26 cells) exhibited a disengagement
 207 phenotype before cell round-up, that is, in the interphase (Figure 2E, Mean time:
 208 siControl $t=4.20$ h, siCep57/Cep57L1 $t=-2.76$ h, Time zero corresponds to the
 209 cell round-up). Importantly, we also found that centriole disengagement was
 210 occasionally followed by centriole reduplication (Figure 2E), and that around
 211 one-fifth of Cep57/Cep57L1-depleted cells possessed more than four centrioles
 212 in the G2 phase ($22.1 \pm 11.1\%$, $N=30$, from three independent experiments)
 213 (Figure 2C). Taken together, these data suggest that Cep57 and Cep57L1
 214 cooperatively regulate the maintenance of centriole engagement during
 215 interphase and thus suppress centriole reduplication within the same cell cycle.

216 Previous studies have reported that the G2 phase cell-cycle arrest
 217 induced by treatment with a Cdk1 inhibitor or DNA-damaging reagents caused
 218 precocious centriole disengagement (Douthwright and Sluder, 2014; Prosser et
 219 al., 2012). Therefore, to examine whether co-depletion of Cep57 and Cep57L1
 220 induces cell cycle arrest in the interphase, we performed a live-imaging analysis
 221 and measured the duration of the period from anaphase onset to round-up in the
 222 next mitosis. In Cep57/Cep57L1-depleted cells, the duration was not significantly
 223 altered compared with control cells (siControl: 26.8 ± 2.2 h, siCep57/Cep57L1:
 224 26.2 ± 3.6 h, $N=30$) (Figure S2B). In addition, FACS profiling analysis revealed
 225 that the distribution of cell cycle phases was not affected by Cep57/Cep57L1
 226 depletion (Figure S2C). Overall, these findings reveal that the co-depletion of

227 Cep57 and Cep57L1 causes precocious centriole disengagement starting in the
228 S phase without affecting the cell cycle progression.

229

230 **Precociously disengaged daughter centrioles are converted to**
231 **centrosomes in the G2 phase**

232 Given that after depletion of Cep57/Cep57L1 precocious centriole
233 disengagement was apparent starting in the S phase, and that centriole
234 formation proceeds during the S phase, we next examined whether the
235 disengaged daughter centrioles were fully elongated. To address this, we used
236 POC5, which is known to be incorporated at the final stage of new centriole
237 formation(Azimzadeh et al., 2009; Chang et al., 2016). Immunostaining with an
238 antibody against POC5 revealed that approximately half of the disengaged
239 centrioles incorporated POC5 ($57.1\% \pm 4.0\%$, N=30, from three independent
240 experiments), suggesting that upon depletion of Cep57/Cep57L1, a daughter
241 centriole can disengage from its mother centriole during the process of its
242 formation (Figures S3A and S3B).

243 Since fully matured daughter centrioles are converted into centrosomes
244 after mitosis (a process called centriole-to-centrosome conversion)(Wang et al.,
245 2011), we then asked if the precociously disengaged daughter centrioles
246 accomplish the centriole-to-centrosome conversion in the interphase. In normal
247 cells, around mitotic exit, a daughter centriole becomes a functional centrosome
248 and recruits PCM components and centriole duplication factors, such as PCNT
249 and Cep152, respectively. For this conversion, procentriole formation requires
250 the centriolar recruitment of Cep295 and Cep192(Fu et al., 2016; Tsuchiya et al.,

2016). Similar to Cep192, Cep295 was localized at almost all of the precociously disengaged daughter centrioles (Figure S3C). In contrast, the disengaged daughter centrioles did not always acquire PCM components (PCNT), suggesting that PCM proteins were gradually recruited to the disengaged daughter centrioles. Indeed, Cep57/Cep57L1-depleted cells with more than two PCNT-positive centrioles were relatively rare before the G2 phase ($15.0 \pm 2.4\%$, $N=30$ from three independent experiments), but gradually increased during the G2 phase ($51.7 \pm 2.4\%$) and were most frequently observed after the G2 phase was completed (mitosis, $85.0 \pm 11.8\%$) (Figures S4A and S4B). This observation shows that the precociously disengaged daughter centrioles gradually recruited PCM components to their surroundings mainly during the G2 phase and mitosis (Figure S4C). Furthermore, such disengaged daughter centrioles nucleated microtubules after depolymerization of microtubules by cold treatment, indicating that these centrioles had acquired MTOC activity during interphase (Figure S4D). Taken together, these results indicate that upon Cep57/Cep57L1 depletion, the precociously disengaged centrioles can be converted to centrosomes starting in the interphase.

Co-depletion of Cep57 and Cep57L1 induces centriole disengagement in the interphase even without Plk1 kinase activity

To gain insight into the mechanisms by which Cep57/Cep57L1 depletion causes precocious centriole disengagement in the interphase, we tested a requirement of the known factors involved in centriole disengagement at the end of mitosis. Previous studies have reported that canonical centriole

disengagement requires Plk1 activity, and that the inhibition of Plk1 perturbs centriole disengagement at the end of mitosis (Tsou et al., 2009). Precocious centriole disengagement in G2/M phase-arrested cells was also suppressed by Plk1 inhibition (Prosser et al., 2012). To determine whether the precocious centriole disengagement in Cep57/Cep57L1-depleted cells also requires Plk1 activity, we treated G2 phase-arrested cells or Cep57/Cep57L1-depleted cells with a small molecular inhibitor of Plk1 (BI2536). As expected, the precocious centriole disengagement in G2/M-arrested cells was suppressed by BI2536 treatment (Figures 3A and 3B). In contrast, BI2536 treatment did not suppress the precocious centriole disengagement or the increase in the number of centrioles in Cep57/Cep57L1-depleted cells (DMSO: $46.7 \pm 10.0\%$, BI2536: $45.6 \pm 10.1\%$, N=30 from three independent experiments) (Figures 3C and 3D). These data indicate that the precocious centriole disengagement that occurs in Cep57/Cep57L1-depleted cells does not depend on Plk1 activity, unlike canonical centriole disengagement or cell-cycle-arrest-induced precocious centriole disengagement.

291

292 **Plk1 activity is required for PCM recruitment to disengaged daughter** 293 **centrioles, presumably in the G2 phase**

Since Plk1 is necessary not only for centriole disengagement but also for daughter centrioles to acquire PCM in normal cells (Wang et al., 2011), we next asked if Plk1 activity is also required for PCM recruitment at precociously disengaged daughter centrioles in Cep57/Cep57L1-depleted cells. To address this, we treated Cep57/Cep57L1-depleted cells with BI2536 and counted the

299 number of PCM-positive centrioles. Interestingly, in Cep57/Cep57L1-depleted
 300 cells, BI2536 treatment significantly reduced the number of cells with
 301 disengaged centrioles that have acquired PCNT ($13.1 \pm 4.3\%$, N=30, from three
 302 independent experiments), compared to the controls ($51.6 \pm 2.4\%$) (Figures 3E
 303 and 3F), indicating that Plk1 activity is required for PCNT recruitment to the
 304 disengaged daughter centrioles. Similarly, γ -tubulin recruitment to the
 305 disengaged daughter centrioles was also suppressed by BI2536 treatment
 306 (DMSO: $55.1 \pm 7.1\%$, BI2536: $10.0 \pm 9.4\%$, N=30, from three independent
 307 experiments) (Figure 3G). Overall, these findings demonstrate that the
 308 precociously disengaged daughter centrioles acquire PCM during the interphase
 309 in a Plk1-dependent manner (Figure 3H). Given that Plk1 is activated starting
 310 from the late G2 stage preceding mitosis (Schmucker and Sumara, 2014) and is
 311 necessary for PCM recruitment to the disengaged daughter centrioles, it is
 312 reasonable that precociously disengaged daughter centrioles gradually acquire
 313 PCNT in the G2 phase (Figures S4A and S4B).

314

315 **Cep57 and Cep57L1 have distinct properties in spite of their relatively high** 316 **amino acid sequence homology**

317 While Cep57 and Cep57L1 cooperatively regulate centriole engagement
 318 in the interphase, centriole engagement in mitosis is maintained by Cep57 but
 319 not by Cep57L1. This suggests that Cep57 and Cep57L1 share redundant and
 320 distinct functions in centriole engagement. To investigate the similarities and
 321 differences between the functions of Cep57 and Cep57L1 in the regulation of
 322 centrioles, we performed localization and domain analysis of Cep57 and

323 Cep57L1. We first sought to compare the detailed localization pattern of Cep57
 324 and Cep57L1 at the centrosome. To do this, we immunostained HeLa cells with
 325 an antibody against Cep57L1 and found that endogenous Cep57L1 localized at
 326 the centrosome (Figure 4A). We also noticed that the level of Cep57L1 signal at
 327 new mother centrioles gradually increased in the interphase (Figure 4A),
 328 similarly to Cep57 (Watanabe et al., 2019). Moreover, structured illumination
 329 microscopy (SIM) analysis showed that Cep57L1 formed a ring-like structure
 330 around the mother centriole (Figure 4B). The diameter of the Cep57L1 ring was
 331 167.7 ± 35.3 nm, which is close to that of the Cep57 ring (219.9 ± 13.9 nm in
 332 (Watanabe et al., 2019)) (Figure 4B). The specificity of the antibody was
 333 validated using siRNA against Cep57L1 (Figure 4C). Interestingly, recruitment of
 334 Cep57 and Cep57L1 at the centrosome did not depend on each other. Therefore,
 335 these results indicate that Cep57 and Cep57L1 show a similar centriolar
 336 distribution, but independently localize to the centrosome.

337 To further examine the similarities and differences between Cep57 and Cep57L1
 338 function, we next performed domain analysis of Cep57 and Cep57L1. We
 339 previously reported that the conserved PINC (present in N-terminus of Cep57)
 340 domain in the N-terminus of Cep57 is required for its centriolar localization and
 341 the interaction with PCNT, a functional binding partner (Watanabe et al., 2019).
 342 Since Cep57L1 also has a PINC domain, we asked if the role of the PINC motif
 343 in Cep57L1 was similar to its role in Cep57. To address this, we constructed
 344 plasmids expressing the full-length Cep57L1 or a mutant lacking the PINC motif
 345 (52-86 a.a.). Unexpectedly, immunofluorescent (IF) and co-immunoprecipitation
 346 (co-IP) analyses with the mutant revealed that the PINC motif of Cep57L1 was

347 dispensable for the centrosome localization (Figure S5A) and for the binding to
 348 the PCNT C-terminal region, including the PACT domain (Figure S5B). These
 349 data suggest that the PINC motif has different functions between Cep57 and
 350 Cep57L1. In addition to the PINC motif, the Pfam protein family database
 351 predicted the presence of microtubule-binding domains in both Cep57 and
 352 Cep57L1 (Figure S1A). As previously reported, overexpressed Cep57 was
 353 occasionally localized on the microtubule network via its microtubule-binding
 354 domain (Figure S5C) (Momotani et al., 2008). In contrast, overexpressed
 355 Cep57L1 did not show such a localization pattern, but instead aggregated in the
 356 cytoplasm (Figure S5C). This result suggests that the putative
 357 microtubule-binding domain of Cep57L1 does not possess a binding affinity for
 358 microtubules. Furthermore, co-IP assays of Cep57 and Cep57L1 proteins in
 359 HEK293T cells detected self-interaction of Cep57L1, but not of Cep57 (Figure
 360 S5D). Overall, these findings show that Cep57 and Cep57L1 have distinct
 361 properties in spite of their relatively high amino acid sequence homology.

362

363 **Interdependency of centrosome localization between Cep57, Cep57L1,** 364 **Cep63, and Cep152**

365 We next searched for the proteins recruiting Cep57L1 to the centrosome.
 366 Considering the similar distribution patterns of Cep57 and Cep57L1 at
 367 centrosomes, we postulated that the localization of Cep57 and Cep57L1 around
 368 the centriole wall is regulated by common proteins. Cep57 is recruited to the
 369 centrosome dependent on Cep63, Cep152, and NEDD1 (Aziz et al., 2018;
 370 Lukinavičius et al., 2013; Wu et al., 2012). Of these three proteins, Cep63 and

371 Cep152 were demonstrated to form a trimeric complex with Cep57 at the
 372 centrosome (Lukinavičius et al., 2013). Consistent with previous studies (Aziz et
 373 al., 2018; Lukinavičius et al., 2013), we confirmed that Cep63 (Median : 30.6%
 374 compared to siControl, N > 50) or Cep152 depletion (19.3%) significantly
 375 decreased the signal intensity of Cep57 at the centrosome (Figures 4D and S6A).
 376 We then assumed that Cep63 and Cep152 were also responsible for
 377 centrosomal localization of Cep57L1. As expected, depletion of Cep63 (24.8%)
 378 or Cep152 (45.8%) reduced the signal intensity of Cep57L1 at the centrosomes,
 379 indicating that both Cep57 and Cep57L1 localization at the centrosomes was
 380 partially dependent on Cep63 and Cep152 (Figures 4D and S6A). On the other
 381 hand, the signal intensity of Cep63 and Cep152 at the centrosomes was only
 382 slightly affected by the depletion of Cep57 (Cep63 : 81.0%, Cep152 : 80.2%) or
 383 Cep57L1 (Cep63 : 70.6%, Cep152 : 81.0%), while co-depletion of Cep57 and
 384 Cep57L1 reduced the signal intensity of Cep152 at the centrosomes more
 385 drastically (31.1%) (Figures 4D, S6B and S6C). Moreover, as reported in
 386 previous studies (Brown et al., 2013; Kim et al., 2019; Lukinavičius et al., 2013),
 387 the signal intensity of Cep152 was attenuated by Cep63 depletion (25.8%), and
 388 vice versa (66.4%) (Figure 4D). Hence, we propose that Cep57, Cep57L1,
 389 Cep63, and Cep152 mutually influence centrosomal localization (Figure 4E).

390

391 **Precocious centriole disengagement in the interphase results in centriole**
 392 **reduplication and thereby accelerates numerical centrosome**
 393 **abnormalities**

394 We next examined the long-term consequence of precocious centriole
395 disengagement in Cep57/Cep57L1-depleted cells. To this end, we treated HeLa
396 cells with siCep57/Cep57L1 for 96 h and observed the centriole number during
397 mitosis. Immunofluorescence revealed that approximately 80% of the
398 Cep57/Cep57L1-depleted cells had more than four centrioles during mitosis
399 ($80.1 \pm 9.0\%$, N=50, from three independent experiments). In contrast, this was
400 not the case for Cep57-depleted cells, in which centriole disengagement
401 precociously occurred only in mitosis (siControl: $4.7 \pm 3.6\%$, siCep57: $10.7\% \pm$
402 1.3%) (Figures 5A and 5B). This result suggests that the precociously
403 disengaged centrioles upon co-depletion of Cep57 and Cep57L1, were already
404 licensed to reduplicate during the interphase within the same cell cycle. This may
405 have thereby led to the increase in the number of centrioles. To test this idea, we
406 monitored the number of procentrioles using an HsSAS-6 marker. The
407 Cep57/Cep57L1-depleted cells with two pairs of engaged centrioles possessed
408 two HsSAS-6 foci, as in control cells (siControl: $98.9 \pm 1.9\%$, siCep57/Cep57L1:
409 $93.3 \pm 3.3\%$, N=30, from three independent experiments) (Figures 5C and 5D).
410 On the other hand, more than half of the Cep57/Cep57L1-depleted cells with four
411 separate centrioles had no HsSAS-6 foci in the interphase ($60.0 \pm 6.7\%$) (Figures
412 5C and 5D), suggesting that HsSAS-6 disappeared from the centrosomes,
413 presumably just after centriole disengagement. Interestingly, in
414 Cep57/Cep57L1-depleted cells with four HsSAS-6 foci, each HsSAS-6 focus was
415 associated with a pair of centrioles (Figure 5C), which leads us to reason that the
416 precociously disengaged centrioles newly acquired HsSAS-6 and reduplicated
417 within the same cell cycle. To address this further, we performed live-cell imaging

418 using the HeLa-GFP-centrin1 cell line, and revealed that centriole reduplication
 419 occurred after the precocious centriole disengagement in the interphase (Figure
 420 5E). Intriguingly, we noticed that the earlier the precocious centriole
 421 disengagement occurred, the more frequently centriole reduplication could be
 422 observed (Figure 2E). In addition, live-imaging data revealed that the centrioles
 423 amplified by centriole reduplication could be inherited by the two daughter cells
 424 (Figure 5E and Supplementary Movie 2). The inherited amplified centrioles could
 425 normally duplicate in the subsequent cell cycle, which further led to numerical
 426 centrosome abnormalities. Indeed, comparing the number of centrioles between
 427 48 h and 96 h after the siRNA treatment, the numerical abnormalities was more
 428 significant at 96 h post-treatment (Figure 5F). Overall, these findings
 429 demonstrate that precocious centriole disengagement in interphase results in
 430 numerical centrosome abnormalities with each passing cell division due to
 431 continuous centriole reduplication and the inevitable inheritance of amplified
 432 centrioles by daughter cells.

433

434 **Centriole reduplication in Cep57/Cep57L1-depleted cells leads to the high** 435 **frequency of chromosome segregation errors**

436 Since precocious centriole disengagement causes defects in
 437 chromosome segregation (Watanabe et al., 2019), we then explored the fate of
 438 Cep57/Cep57L1-depleted cells in mitosis. We first grouped cells into four
 439 categories based on the timing of centriole disengagement and whether
 440 centriole reduplication occurs: normal (pattern 1), precocious centriole
 441 disengagement in mitosis (pattern 2), precocious centriole disengagement in the

interphase without (pattern 3) and with centriole reduplication (pattern 4). To track centrioles and chromosomes in the mitotic cells, we performed live-imaging using the HeLa-GFP-centrin1 cell line and SiR-DNA, and quantified the frequency of the phenotypes in each siRNA condition. In control cells or Cep57L1-depleted cells, two pairs of centrioles were engaged throughout the interphase and until the end of mitosis (pattern 1) (Figures 6A, 6B and S7A). Single depletion of Cep57 caused precocious centriole disengagement only in mitosis, but not in interphase (pattern 2) (Figures 6A and 6B). As we mentioned above, most Cep57/Cep57L1-depleted cells exhibited precocious centriole disengagement in the interphase (pattern 3), which was frequently followed by centriole reduplication (pattern 4) (Figures 6A and 6B). These phenotypic patterns were consistent with what we had observed in the fixed cells (Figures S7B and S7C). We next quantified the frequency of chromosome segregation errors in each siRNA condition. Cep57- or Cep57/Cep57L1-depleted cells exhibited chromosome segregation errors, which were associated with chromosome misalignment and multipolar spindle formation (siControl; $0.7\% \pm 1.2\%$, siCep57; $15.3 \pm 4.2\%$, siCep57/Cep57L1; $30.0 \pm 2.0\%$, N=30, from three independent experiments) (Figures 6C, 6D and Supplementary Movies 3, 4, 5, 6). The frequency of chromosome segregation errors in Cep57/Cep57L1-depleted cells was about twice that in Cep57-depleted cells. To further investigate what factors most effectively led to the increase of the chromosome segregation errors in Cep57/Cep57L1-depleted cells, we next compared the frequency of chromosome segregation errors according to the phenotypic patterns. We revealed that Cep57/Cep57L1-depleted cells with

466 precocious disengagement and centriole reduplication (pattern 4) showed the
 467 highest frequency of chromosome segregation errors ($57.5 \pm 8.0\%$) (Figures 6C,
 468 6E, S7D and S7E). On the other hand, the frequency of chromosome
 469 segregation errors between the cells with precocious disengagement in mitosis
 470 (pattern 2, in Cep57-depleted cells, $29.0 \pm 8.0\%$) and interphase (pattern 3, in
 471 Cep57/Cep57L1-depleted cells, $34.8 \pm 11.6\%$) were not significantly different
 472 (Figure 6E). These results suggest that, upon Cep57 and Cep57L1 depletion,
 473 the centriole reduplication induced by precocious centriole disengagement in the
 474 interphase is a more direct cause of chromosome segregation errors than the
 475 precocious centriole disengagement itself.
 476

477 **DISCUSSION**

478 In conclusion, our work is the first to identify Cep57 and Cep57L1 as
 479 essential factors maintaining centriole engagement in the interphase. In this
 480 study, we also show that this tight regulation of centriole engagement is critical
 481 for a proper centriole duplication cycle and chromosome segregation (Figure 7).
 482 Surprisingly, depletion of Cep57 and Cep57L1 induced precocious centriole
 483 disengagement in the interphase independent of Plk1 activity or cell-cycle arrest.
 484 Consistent with previous studies (Lončarek et al., 2010), precocious centriole
 485 disengagement in the interphase released mother centrioles from a block to
 486 reduplication, and thereby resulted in an increase in the number of centrioles.
 487 Furthermore, the number of centrioles per cell increased with each cell division
 488 because of the continuous centriole reduplication and inevitable inheritance of
 489 the amplified centrioles by daughter cells. In addition, Cep57/Cep57L1-depleted
 490 cells exhibited a higher frequency of multipolar spindle formation and
 491 chromosome instability mainly because of the amplified centrioles.

492 Although Plk1 is critical for centriole disengagement during mitosis in
 493 normal cells (Kim et al., 2015; Tsou et al., 2009), precocious centriole
 494 disengagement induced by co-depletion of Cep57/Cep57L1 did not depend on
 495 Plk1 activity (Figures 3C and 3D). In addition to Plk1, APC/C and separase are
 496 also required for canonical centriole disengagement (Tsou et al., 2009).
 497 Separase is a protease activated by APC/C at anaphase onset, and separase
 498 cleaves PCNT, a major PCM component, to induce PCM disassembly and
 499 centriole disengagement (Lee and Rhee, 2012; Matsuo et al., 2012). Since the
 500 artificial activation of APC/C or separase causes centriole disengagement, even

501 in the interphase (Hatano and Sluder, 2012; Prosser et al., 2012), it is possible
 502 that co-depletion of Cep57 and Cep57L1 aberrantly activated APC/C or
 503 separase in the interphase and thereby caused precocious centriole
 504 disengagement. However, even precocious centriole disengagement induced by
 505 overexpression of APC/C or separase is suppressed by BI2536 (Prosser et al.,
 506 2012). Considering our result showing that Plk1 inhibition did not suppress the
 507 phenotype induced by Cep57/Cep57L1 depletion (Figures 3C and 3D), we
 508 speculate that co-depletion of Cep57 and Cep57L1 induces precocious centriole
 509 disengagement without activating the canonical pathway involving Plk1, APC/C,
 510 and separase.

511 One interesting issue raised in this study is the difference in the timing of
 512 precocious centriole disengagement: co-depletion of Cep57/Cep57L1 causes
 513 the phenotype in the interphase whereas a single depletion of Cep57 causes the
 514 phenotype only in mitosis (Figure 1) (Watanabe et al., 2019). Similar to Cep57,
 515 PCNT is involved in the maintenance of centriole engagement only in mitosis,
 516 but the depletion of PCNT does not disrupt centriole engagement in the
 517 interphase (Figure 1). These results imply that the mode of centriole
 518 engagement somehow changes from interphase to mitosis. Importantly, the
 519 surrounding PCM expands dynamically and functionally matures toward mitosis
 520 (Khodjakov and Rieder, 1999), which is likely a critical event for switching the
 521 status of centriole engagement. This PCM maturation at the G2/M transition is
 522 known to be a Plk1-dependent process, which includes dynamic reorganization
 523 of the PCM from interphase (highly ordered state) to mitosis (amorphous state)
 524 (Lawo et al., 2012). In addition, a recent study showed that the activity of Plk1 is

needed for extending the distance between mother and daughter centrioles from 50 nm to 80 nm in early mitosis (Shukla et al., 2015). Given these observations, we speculate that the Plk1-dependent dynamic rearrangement of PCM components couples the PCM expansion with changes in the status of centriole engagement. In line with this idea, the precocious centriole disengagement was induced only in mitosis by Cep57 depletion, and this could be suppressed by treatment with a Plk1 inhibitor (BI2536) (Watanabe et al., 2019; Wilhelm et al., 2019). Meanwhile, the precocious centriole disengagement that occurred in the interphase after co-depletion of Cep57 and Cep57L1 was not suppressed by Plk1 inhibition. It has also been reported that Plk1-dependent phosphorylation of PCNT is required for PCM expansion (Lee and Rhee, 2011). However, how Plk1 and PCNT cooperatively regulate PCM expansion and the shift of the centriole engagement mode in early mitosis remains elusive.

According to the findings in this study, the timing of centriole disengagement in the cell cycle is also critical for the occurrence of centriole reduplication. Given that precocious centriole disengagement in Cep57-depleted cells was not accompanied by centriole reduplication, the reloading of centriole duplication factors must be tightly restricted in mitosis so as not to increase the centrosome number. In contrast, in Cep57/Cep57L1-depleted cells, precocious centriole disengagement occurs in interphase, which secures sufficient centriole components and enough time for centriole duplication to occur. Such centriole reduplication thereby results in the drastic increase of centrosome numbers, which then more frequently leads to chromosome segregation errors. Thus, on the basis of these observations, it is conceivable that the tight control of

549 maintenance of centriole engagement is more important in interphase than in
550 mitosis.

551

552 **Figure legends**

553 **Figure 1. Co-depletion of Cep57 and Cep57L1 causes the increase of**
 554 **centrosome number during interphase**

555 **(A)** Depletion of Cep57 induced precocious centriole disengagement in mitosis,
 556 but not in interphase. HeLa cells were treated with siControl or siCep57, and
 557 immunostained with antibodies against CP110 (green) and Cep192 (red). **(B)**
 558 Histograms represent frequency of cells in interphase and mitosis with the
 559 indicated phenotypes observed in (A). Values are mean percentages $\pm$ s.d. from
 560 three independent experiments (n = 50 for interphase, 30 for mitosis for each
 561 experiment). **(C)** Co-depletion of Cep57 and Cep57L1 (Cep57/Cep57L1)
 562 induced precocious centriole disengagement and increase of centrosome
 563 number in interphase. HeLa cells were treated with siControl or
 564 siCep57/Cep57L1, and immunostained with antibodies against CP110 (green)
 565 and Cep192 (red). **(D-F)** Histograms represent frequency of the interphase cells
 566 with >2 Cep192 foci (in (D)), centriole disengagement (in (E)) or >4 centrioles (in
 567 (F)), respectively. Values are mean percentages $\pm$ s.d. from three independent
 568 experiments. (n = 50 for each experiment). **(G)** Precocious centriole
 569 disengagement in interphase induced by Cep57/Cep57L1 co-depletion was
 570 rescued by exogenous expression of Cep57 or Cep57L1. HeLa cells were
 571 treated with siControl or siCep57/Cep57L1, followed by the transfection with
 572 FLAG empty (control), FLAG-Cep57 (RNAi-resistant) or FLAG-Cep57L1
 573 (RNAi-resistant). The cells were immunostained with antibodies against FLAG
 574 (green), CENP-F (red) and Cep192 (cyan). **(H)** Histograms represent frequency
 575 of cells in the G2 phase with >2 Cep192 foci in each condition observed in (G).

576 Values are percentages from three independent experiments (n = 30 for each
 577 experiment). All scale bars, 5 μ m in the low-magnified view, 1 μ m in the inset.
 578 Two-tailed, unpaired Welch's t-test was used in (A) to obtain p value. Dunnett's
 579 multiple comparisons test was used in (D), (E), (F) and (H) to obtain p value. ***p
 580 < 0.001; *p < 0.05.
 581

Figure 2. Cep57 and Cep57L1 redundantly regulate centriole engagement during interphase

(A) Cep57/Cep57L1 co-depletion induced four separated centrioles in the G2 phase. HeLa cells were treated with siControl or siCep57/Cep57L1 for 36 h in the presence of 5-ethynyl-2'-deoxyuridine (EdU, S phase marker, cyan) for the last 30 min before fixation, and immunostained with antibodies against CP110 (red) and CENP-F (green). Scale bars, 5 μ m in the low-magnified view, 1 μ m in the inset. **(B)** Histograms represent the number of centrioles in the G1, S and G2 phase treated with the indicated siRNAs in (A). **(C)** Histograms represent frequency of cells in the S and G2 phase treated with the indicated siRNAs exhibiting the indicated phenotype. Values are mean percentages $\pm$ s.d. from three independent experiments (n = 30 for each experiment) in (B) and (C). **(D)** Time-lapse observation of cells upon Cep57/Cep57L1 depletion. HeLa cells stably expressing GFP-centrin1 (HeLa-GFP-centrin1) were treated with siControl or siCep57/Cep57L1 in the presence of SiR-DNA (200 nM). Scale bar, 5 μ m. **(E)** Cumulative scatter plot indicates the duration from cell round-up to centriole disengagement observed in (D). Orange open circles indicate precocious centriole disengagement accompanied by centriole reduplication. Two-tailed, unpaired Welch's t-test was used in (E) to obtain p value. ***p < 0.001.

Figure 3. Inhibition of Plk1 prevents disengaged daughter centrioles from acquiring PCM, but does not suppress precocious centriole disengagement.

(A) Precocious centriole disengagement caused by G2/M phase arrest was suppressed by BI2536 (Plk1 inhibitor, 100 nM). HeLa cells were synchronized in the G1/S phase by Aphidicolin (1.2 µg/mL) for 17 h, then released into fresh medium for 4 h. Next, the cells were treated with DMSO (control), RO3306 (Cdk1 inhibitor, 10 µM), or both of RO3306 and BI2536 (100 nM) for 24 h. HeLa cells were immunostained with antibodies against centrin2 (green) and Cep192 (red). Scale bar, 5 µm. **(B)** Histograms represent frequency of the interphase cells with the indicated phenotype observed in (A). Values are mean percentages ± s.d. from three experiments (n = 50 for each experiment). **(C)** Precocious centriole disengagement in Cep57/Cep57L1-depleted cells was not suppressed by BI2536. HeLa cells were treated with siControl or siCep57/Cep57L1 for 24 h, followed by treatment of DMSO (control) or BI2536 (100 nM) for additional 24 h. The cells were immunostained with antibodies against centrin2 (green) and CENP-F (red). **(D)** Histograms represent frequency of cells in the G2 phase with the indicated phenotype observed in (C). Values are mean percentages ± s.d. from three experiments (n = 30 for each experiment). **(E)** BI2536 suppressed recruitment of PCNT at disengaged daughter centrioles. HeLa-GFP-centrin1 cells were treated as in (C), and immunostained with antibodies against GFP (green), CENP-F (red) and PCNT (cyan). White/black arrowheads indicate PCNT positive/negative centrioles, respectively. **(F, G)** Histograms represent frequency of cells with >2 PCNT or γ-tubulin foci among

627 the G2 phase cells with disengaged or amplified centrioles. Values are mean
628 percentages $\pm$ s.d. from three experiments (n = 30 for each experiment). **(H)**
629 Schematic illustration of the results in Figure 3. The activity of Plk1 is required for
630 the recruitment of PCM components at the disengaged daughter centrioles, but
631 not for precocious centriole disengagement itself. All scale bars, 5 μ m. Tukey's
632 multiple comparisons test was used in (D) against total value of centriole
633 disengagement and >4 centrioles to obtain p value. Two-tailed, unpaired
634 Student's t-test was used in (F) and (G) to obtain p value. **p < 0.01; *p < 0.05;
635 NS not significantly different (p > 0.05).
636

Figure 4. Cep57L1, a paralog of Cep57 conserved in vertebrates, shows similar centriolar distribution to Cep57

(A) Centriolar distribution of Cep57L1 at different cell cycle stages. HeLa cells were immunostained with antibodies against Cep57L1 (red) and centrin2 (green). Scale bar, 1 μ m. **(B)** SIM images representing top view of Cep57L1 (red) and PCNT (green) at mother centrioles. Scale bar, 200 nm. The graph shows radial profiles from the center of the Cep57L1 and PCNT rings. Values are mean normalized intensity $\pm$ s.d. (n = 5). **(C)** The signals of Cep57L1 at the centrosome were attenuated by siCep57L1 or siCep57/Cep57L1, but not by siCep57. HeLa cells were treated with siControl, siCep57, siCep57L1 or both of siCep57/Cep57L1, and immunostained with antibodies against Cep57 (green), Cep57L1 (red) and Cep192 (cyan). Scale bars, 5 μ m in the low-magnified view, 1 μ m in the inset. **(D)** Beeswarm plots piled on boxplots represent the normalized signal intensity of Cep57, Cep57L1, Cep63 and Cep152 at the old mother centrioles upon the indicated siRNAs (n = 50). **(E)** Schematic of the dependency of the centrosome localization among Cep57, Cep57L1, Cep63 and Cep152. The wide arrows indicate strong dependency defined as more than 50% reduction of the signal intensity, and narrow arrows indicate weak dependency defined as 25-50% reduction of the signal intensity. Tukey's multiple comparisons test was used in (D) to obtain p value. *p < 0.05; ***p < 0.001; NS not significantly different (p > 0.05).

Figure 5. Precocious centriole disengagement results in an increase of the number of centrioles

(A) Long-term Cep57/Cep57L1 co-depletion increased the number of centrioles. HeLa cells were treated with siCep57/Cep57L1 for 96 h, and immunostained with antibodies against centrin2 (green) and Cep192 (red). **(B)** Histograms represent frequency of mitotic cells with the indicated number of centrioles observed in (A). Values are mean percentages $\pm$ s.d. from three independent experiments (n = 30 for each experiment). Note that depletion of Cep57L1 for 96 h slightly reduced the number of centrioles. **(C)** HsSAS-6 was absent from disengaged centrioles and was re-acquired. HeLa cells were treated with siControl or siCep57/Cep57L1, and immunostained with antibodies against HsSAS-6 (green) and CP110 (red). **(D)** Histograms represent frequency of the number of HsSAS-6 foci on the two pairs of centrioles or four centrosomes in (C). Values are mean percentages $\pm$ s.d. from three independent experiments (n = 30 for each experiment). **(E)** Precocious centriole disengagement and centriole reduplication in interphase, and inevitable inheritance of amplified centrioles to daughter cells observed in Cep57/Cep57L1-depleted cells. HeLa-GFP-centrin1 cells were treated with siCep57/Cep57L1 in the presence of SiR-DNA (200 nM). White arrowheads indicate centrosomes. **(F)** Histograms represent frequency of mitotic cells with the indicated number of centrioles 48 h or 96 h after Cep57/Cep57L1 co-depletion observed in (A) and Figure S7A. Values are mean percentages $\pm$ s.d. from three independent experiments (n = 30 for each experiment). All scale bars, 5 μ m in the low-magnified view, 1 μ m in the inset. Dunnett's multiple comparisons test was used in (B) to obtain p value. ***p <

683 **0.001.**

684

685

686

Figure 6. Amplified centrioles caused by co-depletion of Cep57 and Cep57L1 result in high frequency of chromosome segregation errors

(A) Phenotypic patterns were categorized into four groups: normal (pattern 1), precocious centriole disengagement in mitosis (pattern 2), precocious centriole disengagement in the interphase without (pattern 3) and with centriole reduplication (pattern 4). HeLa-GFP-centrin1 cells were treated with siControl, siCep57 or siCep57/Cep57L1 in the presence of SiR-DNA (200 nM). (B) Histograms represent frequency of mitotic cells with the indicated phenotypes in (A). Values are mean percentages $\pm$ s.d. from three independent experiments (n = 50 for each experiment). (C) Chromosome segregation errors observed in Cep57/Cep57L1-depleted cells with the indicated phenotype. HeLa-GFP-centrin1 cells were treated with siCep57/Cep57L1 in the presence of SiR-DNA (200 nM). (D, E) Histograms represent frequency of the mitotic cells with the indicated chromosome segregation errors observed in (C). Values are mean percentages $\pm$ s.d. from three independent experiments (n = 50 for each experiment in (D))(Normal (Pattern 1, siControl): n = 48, 47, 49, Disengagement in mitosis (Pattern 2, siCep57): n = 25, 18, 18, Disengagement in interphase (Pattern 3, siCep57/Cep57L1): n = 14, 14, 10, Disengagement in interphase with reduplication (Pattern 4, siCep57/Cep57L1): n = 21, 16, 12 in (E)). All scale bars, 5 μ m. Tukey's multiple comparisons test was used in (D) and (E) against total value of chromosome misalignment and multipolar to obtain p value. ***p < 0.001; *p < 0.05; NS not significantly different (p > 0.05).

710 **Figure 7. A speculative model for the mode shift of centriole engagement**

711 Centriole engagement in interphase is maintained cooperatively by Cep57 and
 712 Cep57L1, and Plk1 changes the engagement mode to Cep57- and
 713 PCNT-dependent one at the mitotic entry. Cep57/Cep57L1 co-depletion induces
 714 precocious centriole disengagement during interphase, and such disengaged
 715 daughter centrioles are converted into centrosomes and can reduplicate before
 716 entering mitosis. Consequently, the centriole number increases, which results in
 717 the high frequency of chromosome segregation errors.

718

719 **Supplementary Figure 1.**

720 **(A)** Alignments of full-length *H. sapiens* Cep57L1 and Cep57. Asterisks indicate
 721 the residues identical in aligned sequence; colons: conserved substitutions;
 722 periods: semi-conserved substitutions. The position of PINC motif and predicted
 723 microtubule binding domain are indicated in pink and green boxes. **(B)** The
 724 phenotype induced by Cep57/Cep57L1 co-depletion was confirmed by using
 725 another siRNA. HeLa cells were treated with siCep57 and siCep57L1#2, and
 726 immunostained with antibodies against centrin2 (green) and Cep192 (red).
 727 siCep57L1#2 targets a different sequence in open reading frame from
 728 siCep57L1#1 which is used in main figures. **(C)** U2OS cells also exhibited
 729 precocious centriole disengagement and amplified centrioles upon
 730 Cep57/Cep57L1 co-depletion. U2OS cells were treated with siControl or
 731 siCep57/Cep57L1, and immunostained with antibodies against centrin2 (green)
 732 and Cep192 (red). All scale bars, 5 μ m in the low-magnified view, 1 μ m in the
 733 inset.

734

735

736 **Supplementary Figure 2.**

737 **(A)** The number of old mother centriole was one irrespective of the phenotypes
 738 in Cep57/Cep57L1-depleted cells. HeLa cells were treated with siControl or
 739 siCep57/Cep57L1, and immunostained with antibodies against centrin2 (green)
 740 and ODF2 (red). Scale bars, 5 μ m in the low-magnified view, 1 μ m in the inset.
 741 **(B)** Histograms represent frequency of the interphase cells with the indicated
 742 number of ODF2 foci observed in (A). Values are mean percentages $\pm$ s.d. from
 743 two independent experiments (n = 50 for each experiment). **(C)** Quantification of
 744 the duration from anaphase onset to next mitotic entry. HeLa-GFP-centrin1 cells
 745 were treated with siControl or siCep57/Cep57L1, and visualized for live imaging
 746 for 48 h (n = 25). **(D)** Cep57/Cep57L1 co-depletion did not affect cell cycle
 747 progression. HeLa cells were treated with the indicated siRNAs, and followed by
 748 flow cytometry analysis. Two-tailed, unpaired Welch's t-test was used in (C) to
 749 obtain p value. NS not significantly different (p > 0.05).

750

751

752 **Supplementary Figure 3.**

753 **(A)** POC5 was recruited to a portion of disengaged centrioles. HeLa cells were
 754 treated with siControl or siCep57/Cep57L1, and immunostained with antibodies
 755 against centrin2 (green) and POC5 (red). **(B)** Histograms represent frequency of
 756 cells with the indicated number of POC5 positive centrioles among cells with two
 757 pairs of centrioles or disengaged four centrioles observed in (A). Values are
 758 mean percentages $\pm$ s.d. from three independent experiments (n = 30 for each
 759 experiment). **(C)** Cep295 was recruited to the disengaged centrioles. HeLa cells
 760 were treated with siControl or siCep57/Cep57L1, and immunostained with
 761 antibodies against centrin2 (green) and Cep295 (red). Scale bars, 5 μ m in the
 762 low-magnified view, 1 μ m in the inset.

763

764 **Supplementary Figure 4.**

765 **(A)** PCNT was recruited to disengaged daughter centrioles mainly in the G2
766 phase and mitosis. HeLa-GFP-centrin1 cells were treated with
767 siCep57/Cep57L1, and immunostained with antibodies against GFP (green),
768 PCNT (red) and CENP-F (cyan). White/black arrowheads indicate PCNT
769 positive/negative centrioles, respectively. Scale bar, 5 μ m. **(B)** Histograms
770 represent frequency of cells with >2 PCNT positive centrioles among cells with
771 disengaged four centrioles before, in and after the G2 phase observed in (A).
772 Values are mean percentages $\pm$ s.d. from three independent experiments (n = 30
773 for each experiment). **(C)** Schematic illustration of the result in (A) and (B).
774 PCNT was recruited onto disengaged daughter centrioles mainly in the G2
775 phase and mitosis. **(D)** Ectopic MTOC activity of precociously-disengaged
776 daughter centrioles in Cep57/Cep57L1-depleted cells. HeLa cells were treated
777 with siControl or siCep57/Cep57L1 and followed by nocodazole treatment (10
778 μ M) for 3 h. After nocodazole treatment, the cells were cold-treated for 1 h,
779 followed by 1 min incubation at 37°C and immunostaining with antibodies
780 against EB1 (green) and CP110 (red). White arrowheads indicate centrosomes.
781 Scale bar, 5 μ m.

782

783

784 **Supplementary Figure 5.**

785 **(A)** The Cep57L1 PINC motif was not required for the centrosome localization.
786 HeLa cells expressing FLAG-Cep57L1 or FLAG-Cep57L1 mutant lacking the
787 PINC motif (Δ 52-86 a.a.) were immunostained with antibodies against FLAG
788 (green) and Cep192 (red). **(B)** The Cep57L1 PINC motif was not required for
789 binding to the C-terminal region of PCNT containing the conserved PACT
790 domain. HEK293T cells co-expressing FLAG empty (control), FLAG-Cep57,
791 FLAG-Cep57L1 or FLAG-Cep57L1 mutant lacking the PINC motif and
792 GFP-PCNT (3132-3336 a.a.) were immunoprecipitated with FLAG antibodies
793 and immunoblotted with the indicated antibodies. **(C)** Overexpressed Cep57 was
794 accumulated on the microtubules, but overexpressed Cep57L1 aggregated in
795 the cytoplasm. HeLa cells expressing HA-Cep57 or HA-Cep57L1 were
796 immunostained with antibodies against HA (red) and α -tubulin (green). **(D)**
797 Cep57L1 formed homodimer. HEK293T cells co-expressing FLAG empty
798 (control), FLAG-Cep57 or FLAG-Cep57L1 and HA-Cep57 or HA-Cep57L1 were
799 immunoprecipitated with FLAG antibodies and immunoblotted with the indicated
800 antibodies. All scale bars, 5 μ m in the low-magnified view, 1 μ m in the inset.

801

802

803 **Supplementary Figure 6.**

804 **(A)** The centrosome localization of Cep57 and Cep57L1 was dependent on
 805 Cep63 and Cep152. HeLa cells were treated with siControl, siCep63 or
 806 siCep152, and immunostained with antibodies against Cep57 (green), Cep57L1
 807 (red) and Cep192 (cyan). **(B)** The centrosome localization of Cep63 was partially
 808 dependent on Cep57 and Cep57L1. HeLa cells were treated with siControl or
 809 siCep57/Cep57L1, and immunostained with antibodies against ODF2 (green),
 810 Cep63 (red) and GT335 (cyan). **(C)** The centrosome localization of Cep152 was
 811 partially dependent on Cep57 and Cep57L1. HeLa cells were treated with
 812 siControl or siCep57/Cep5L1, and immunostained with antibodies against ODF2
 813 (green), Cep152 (red) and GT335 (cyan). All scale bars, 5 μ m in the
 814 low-magnified view, 1 μ m in the inset.

815

816 **Supplementary Figure 7.**

817 **(A)** Normal bipolar spindle formation (pattern 1) was observed in
 818 Cep57L1-depleted cells. HeLa-GFP-centrin1 cells were treated with siCep57L1
 819 in the presence of SiR-DNA (200 nM). Scale bar, 5 μ m. **(B)** Phenotypic patterns
 820 observed in fixed cells are consistent with live-imaging analysis. HeLa cells were
 821 treated with siControl, siCep57, siCep57L1 or siCep57/Cep57L1, and
 822 immunostained with antibodies against centrin2 (green) and Cep192 (red). Scale
 823 bars, 5 μ m in the low-magnified view, 1 μ m in the inset. **(C)** Histograms represent
 824 frequency of mitotic cells with the indicated phenotypes observed in (B). Values
 825 are mean percentages $\pm$ s.d. from three independent experiments (n = 30 for
 826 each experiment). **(D)** Normal bipolar spindle formation observed in control and
 827 Cep57L1-depleted cells. HeLa-GFP-centrin1 cells were treated with siControl or
 828 siCep57L1 in the presence of SiR-DNA (200 nM). Scale bar, 5 μ m. **(E)**
 829 Chromosome segregation errors observed in Cep57-depleted cells.
 830 HeLa-GFP-centrin1 cells were treated with siCep57 in the presence of SiR-DNA
 831 (200 nM). Scale bar, 5 μ m.

832

833 **MATERIALS AND METHODS**

834 **Cell culture and transfection**

835 HeLa, U2OS and HEK293T cells were obtained from the ECACC (European
836 collection of cell cultures). HeLa, U2OS and HEK293T cells were cultured in
837 Dulbecco's modified Eagle's medium (DMEM) supplemented 10% fetal bovine
838 serum (FBS) and 100 µg/ml penicillin-streptomycin at 37°C in a 5% CO₂
839 atmosphere. Transfection of siRNA or DNA constructs into HeLa and HEK293T
840 cells was conducted using Lipofectamine RNAiMAX (Life Technologies) or
841 Lipofectamine 2000 (Life Technologies), respectively. Unless otherwise noted,
842 the transfected cells were analyzed 48 h after transfection with siRNA and 24h
843 after transfection with DNA constructs. When the cells were analyzed 96 h after
844 transfection with siRNA, additional siRNA was transfected 48 h after the first
845 transfection.

846

847 **RNA interference**

848 The following siRNAs were used: Silencer Select siRNA (Life Technologies)
849 against Cep57 (s18692), Cep57L1#1 (s226224), Cep57L1#2 (s226223), Cep63
850 (s37123), Cep152 (s225921) and negative control (4390843). Unless otherwise
851 noted, Cep57L1 #1 were used in this study.

852

853

854 **Plasmids**

855 Complementary DNA (cDNA) encoding Cep57L1 isoform 1 (NCBI
856 NP_001258781.1) was amplified from cDNA library of A549 cells. The Cep57L1

cDNA was subcloned into pCMV5-HA (Addgene) and pCMV5-FLAG (Addgene). pCMV5 constructs encoding full-length Cep57 and pTB701 constructs encoding PCNT were described previously (Watanabe et al., 2019; Takahashi et al., 2002). The Cep57L1 deletion mutant constructs were created using PrimeSTAR mutagenesis basal kit (TaKaRa) and In-Fusion HD cloning kit (Clontech) according to manufacturer's protocol.

863

864 **Antibodies**

The following primary antibodies were used in this study: rabbit antibodies against Cep57L1 (Proteintech, 24957-1-AP, IF 1:500), Cep63 (Proteintech, 16268-1-AP, IF 1:1000), PCNT (Abcam, ab4448, IF 1:2000), Cep192 (Bethyl Laboratories, A302-324A, IF 1:1000), Cep152 (Bethyl Laboratories, A302-480A, IF 1:1000), CP110 (Proteintech, 12780-1-AP, IF 1:500), ODF-2 (Abcam, ab43840, IF 1:1000), GFP (MBL, 598, WB 1:1000), Cep295 (Merck, HPA038596, IF 1:500), CENP-F (Abcam, ab108483, IF 1:500), POC5 (Bethyl Laboratories, A303-341A, IF 1:1000), FLAG-tag (Merck, F7425, IF 1:1000, WB 1:1000) and HA-tag (Abcam, ab9110, IF 1:1000, WB 1:1000); mouse antibodies against Cep57 (Abcam, ab169301, IF1:1000), PCNA (Santa Cruz Biotechnology, sc-56, IF 1:1,000), PCNT (Abcam, ab28144, IF 1:1000), centrin2 (Merck, 20H5, IF 1:500), EB1 (BD Transduction Laboratories, 610534, IF 1:1000), HsSAS-6 (Santa Cruz Biotechnology, sc-81431, IF 1:300), Polyglutamylation Modification (GT335) (AdipoGen, AG-20-B0020-C100, IF 1:2000), γ -tubulin (GTU88) (Sigma-Aldrich, T5192, IF 1:1000), FLAG-tag (Merck, F3165, IF 1:1000, WB 1:1000) and α -tubulin (Merck, DM1A, IF1:1000, WB 1:1000) ; goat antibody

881 against GFP (Abcam, ab6662, IF 1:500, conjugated to FITC); Alexa 488-labelled
882 ODF-2 (Abcam, ab43840, IF 1:500) and Alexa 647-labelled Cep192 (Bethyl
883 Laboratories, A302–324A, IF 1:500) were generated with Alexa Fluor labelling
884 kits (Life Technologies). The following secondary antibodies were used: Alexa
885 Fluor 488 goat anti-mouse IgG (H+L) (Molecular Probes, A-11001, 1:1000),
886 Alexa Fluor 647 goat anti-mouse IgG (H+L) (Abcam, ab150115, 1:1000), Alexa
887 Fluor 568 goat anti-rabbit IgG (H+L) (Molecular Probes, A-11011, 1:1000) for IF;
888 Goat polyclonal antibodies-horseradish peroxidase against mouse IgG
889 (Promega, W402B, 1:10000) and rabbit IgG (Promega, W401B, 1:10000) for
890 WB.

891

892 **Chemicals**

893 The following chemicals were used in this study: nocodazole (Wako,
894 31430-18-9), RO3306 (SIGMA, SML0569), BI2536 (AdooQ, A10134) and
895 SiR-DNA (Spirochrome, CY-SC007).

896

897 **Microscopy**

898 For immunofluorescence analysis, the cells cultured on coverslips (Matsunami,
899 18 mm, thickness No. 1_0.12-0.17 mm for widefield microscope) were fixed
900 using -20°C methanol for 7 min and washed with PBS. The cells were
901 permeabilized after fixation with PBS/0.05% Triton X-100 (PBSX) for 5 min
902 twice, and incubated for blocking in 1% BSA in PBSX for 30 min at room
903 temperature (RT). The cells were incubated with primary antibodies for 24 h at
904 4°C or 2 h at RT, washed with PBSX three times, and incubated with

secondary antibodies for 1 h at RT. The cells were thereafter washed with PBSX twice, stained with 0.2 µg/ml Hoechst 33258 (DOJINDO) in PBS for 5 min at RT, washed again with PBSX and mounted onto glass slides.

Taking the images and counting the number of immunofluorescence signals were performed using an Axioplan2 fluorescence microscope (Carl Zeiss) with 63 × or 100 × /1.4 NA plan-APOCHROMAT objectives and DeltaVision Personal DV-SoftWoRx system (Applied Precision) equipped with a CoolSNAP CH350 CCD camera.

SIM images were taken by Elyra 7 (Carl Zeiss Microscopy GmbH, Jena, Germany) equipped with a x63 ZEISS alpha Plan-Apochromat N.A. 1.46 objective and PCO edge sCMOS camera.

Live imaging

Cell Voyager CQ1 (Yokogawa Electric Corp) equipped with a 40 × dry objective lens was used for live cell imaging. HeLa cells stably expressing GFP-centrin1 were cultured on 24-well glass bottom plate (Greiner-bio-one, #662892) at 37 °C in a 5% CO₂ atmosphere. Before imaging, cells were treated with siRNAs for 24 h or 72h and with 200 nM of SiR-DNA for 6 h. Images were taken by sCMOS camera. After 24 or 72 h from transfection, the cells were visualized every 10 min over 24 h or 48 h. The images were collected at 1.2 µm z steps. Maximum projections were generated using ImageJ (National Institutes of Health).

928 **Immunoprecipitation and western blotting**

929 For preparation of human cell lysates for western blotting, HEK293T cells were
 930 collected 24 h after transfection, lysed on ice in lysis buffer (20 mM Tris/HCl
 931 pH7.5, 50 mM NaCl, 1% TritonX-100, 5 mM EGTA, 1 mM DTT, 2 mM MgCl₂
 932 and 1/1000 protease inhibitor cocktail (Nakarai tesque)). Insoluble material was
 933 removed after centrifugation for 10 min. For IP of FLAG-tagged proteins, whole
 934 cell lysates were incubated with FLAG antibody-conjugated M2 agarose gel
 935 (Merck) for 2 h at 4 °C. The beads were washed at least three times with lysis
 936 buffer and resuspended in SDS-sample buffer before loading onto 8%
 937 polyacrylamide gels, followed by transfer on Immobilon-P membrane (Merk).
 938 The membrane was probed with the primary antibodies, followed by incubation
 939 with their respective horseradish peroxidase-conjugated secondary antibodies
 940 (Promega). The membrane was soaked with Amersham ECL Prime (GE
 941 Healthcare) or Chemi-Lumi One Ultra (Nakarai tesque). Washes were
 942 performed in PBS containing 0.02% Tween-20 (PBST). The signal was detected
 943 with Chemi Doc XRS+ (BIO RAD). Unless otherwise specified, the experiments
 944 of western blotting were repeated at least three times. The antibody against
 945 α-tubulin was used as a loading control.

946

947 **Data availability**

948 The data that support the findings of this study are available from the
 949 corresponding author upon request.

950

951 **Contribution**

952 K.K.I., K.W. and D.K. designed the study; K.K.I., K.W., T.C., S.H. and D.K.
 953 designed experiments; K.K.I., K.W., H.I. and K.M. performed experiments; K.K.I.
 954 and H.I analyzed data; K.K.I., K.W. and D.K. wrote the manuscript, which was
 955 commented on by all authors.

956

957

958 **Acknowledgements**

959 We gratefully acknowledge A. Sekigawa for technical support in SIM
 960 microscopy; and the members of the Kitagawa laboratory for technical support
 961 and discussion. This work was supported by a Grant-in-Aid for Scientific
 962 Research (S) and Research Activity start-up from the Ministry of Education,
 963 Science, Sports and Culture of Japan, by the Takeda Science Foundation, by
 964 the Japan Science Society (The Sasagawa Scientific Research Grant), by the
 965 Daiichi Sankyo Foundation of Life Science.

966

967 **Competing interests: The authors declare no competing interests**

968

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 1084

Figure 1

Figure 2

A

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Figure 3

Figure 4

Figure 5

Figure 6

Figure 7