Centriole-independent centrosome assembly in interphase mammalian cells

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Abstract

The major microtubule-organizing center (MTOC) in animal cells, the centrosome, comprises a pair of

centrioles surrounded by pericentriolar material (PCM), which nucleates and anchors microtubules.

Centrosome assembly depends on the interactions of PCM with centrioles, PCM self-association and

dynein-mediated transport. Here, we show that if centrioles are lost due to PLK4 depletion or

inhibition, PCM still forms a single centrally located MTOC when non-centrosomal microtubule minus-

end organization pathways are disabled. Acentriolar MTOC assembly depends on dynein-driven

coalescence of PCM clusters with attached microtubule minus ends and requires γ-tubulin,

pericentrin, CDK5RAP2 and ninein, but not NEDD1, CEP152 or CEP192. PCM self-assembly is inhibited

by AKAP450-dependent PCM recruitment to the Golgi and by CAMSAP2-mediated microtubule minus-

end stabilization. However, if CAMSAP2 is linked to a minus-end-directed motor, a single MTOC

containing PCM components can still form, and its organization depends on the presence of

pericentrin. Our results reveal that the formation of a single central MTOC in interphase mammalian

cells is not strictly centriole dependent but can be driven by self-organization of PCM and microtubule

minus ends.

Introduction

The centrosome is the major microtubule organizing center (MTOC) in animal cells. It consists of two centrioles surrounded by pericentriolar material (PCM) (reviewed in (Conduit et al., 2015; Paz and Luders, 2017)). Major PCM components are microtubule-nucleating and anchoring proteins, which can associate with centrioles and with each other. While for a long time PCM was thought to be amorphous, super-resolution microscopy studies have shown that it has a distinct organization, with some proteins likely attached to the centriole wall and others organized around them (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2014; Mennella et al., 2012). This distinct organization is more obvious in interphase than in mitosis, when the microtubule-organizing capacity of the centrosome increases due to enhanced PCM recruitment. Many PCM components are known to oligomerize and interact with each other, and recent work suggested that phase separation of interacting PCM components can contribute to centrosome assembly (Raff, 2019; Woodruff et al., 2017). This idea is underscored by data showing that various cell-type specific assemblies of PCM components can form clusters which can nucleate and organize microtubules and serve as MTOCs in the absence of centrioles, particularly during the formation of mitotic spindle poles (Balestra et al., 2021; Chinen et al., 2021; Gartenmann et al., 2020; Meitinger et al., 2020; Watanabe et al., 2020; Yeow et al., 2020). Furthermore, an important centrosome component, cytoplasmic dynein, is a motor that can bind to different PCM proteins and transport them to the centrosome-anchored microtubule ends, where these PCM proteins can nucleate and anchor more microtubules, thus generating a positive feedback loop in centrosome assembly (Balczon et al., 1999; Burakov et al., 2008; Purohit et al., 1999; Redwine et al., 2017). Dynein and its mitotic binding partner NuMA also strongly participate in the formation of mitotic and meiotic spindle poles (Chinen et al., 2020; Khodjakov et al., 2000; Kolano et al., 2012). The relative importance of different molecular pathways of MTOC assembly varies between cell systems and phases of the cell cycle.

Many interphase mammalian cell types have a radial microtubule array. In immune cells, which have a sparse microtubule network, the majority of microtubule minus ends are attached to the centrosome (reviewed in (Meiring et al., 2020)). However, in most commonly studied cultured cell lines, such as fibroblasts, epithelial, endothelial or cancer cells, microtubule networks are denser and not all minus ends can be directly attached to the centrosome. In such cells, non-centrosomal microtubule minus ends are often stabilized by the members of CAMSAP family (Jiang et al., 2014; Meng et al., 2008; Tanaka et al., 2012), and the Golgi apparatus serves as a secondary MTOC (Efimov et al., 2007; Rios, 2014; Wu et al., 2016; Zhu and Kaverina, 2013). If centrosomes are lost because centriole duplication is blocked by inhibiting the kinase PLK4 or depleting another essential centriole component, Golgi-dependent microtubule organization becomes predominant (Gavilan et al., 2018;

Martin et al., 2018; Wu et al., 2016). The ability of the Golgi complex to serve as an MTOC critically

depends on the Golgi adaptor AKAP450, which recruits several PCM components that nucleate

microtubules, including the γ-tubulin ring complex (γ-TuRC), CDK5RAP2 and pericentrin. Moreover,

AKAP450 also tethers microtubule minus ends stabilized by CAMSAP2 to the Golgi membranes

(Gavilan et al., 2018; Rivero et al., 2009; Wu et al., 2016). In the absence of AKAP450, the Golgi ribbon

is maintained, but neither PCM components nor CAMSAP-stabilized microtubule minus ends can be

attached to the Golgi membranes (Gavilan et al., 2018; Rivero et al., 2009; Wu et al., 2016). These

data seem to suggest that centrioles and/or Golgi membranes are essential for assembling a single

MTOC in interphase. However, this notion appears to be inaccurate: in our previous study in RPE1

cells, we observed that a single compact acentriolar MTOC (aMTOC) can still form after centriole loss

in AKAP450 knockout cells, which lack the ability to organize microtubules at the Golgi, if the

stabilization of free minus ends in these cells is disabled by knocking out CAMSAP2 (Wu et al., 2016).

Here, we used this observation as a starting point to explore the centriole-independent pathway of

centrosome assembly in interphase mammalian cells. We found that AKAP450/CAMSAP2 knockout

cells formed compact aMTOCs when PLK4 was either inhibited or depleted. aMTOC formation in these

cells required microtubules and depended on dynein, which brought together small PCM clusters with

attached minus ends. The resulting aMTOCs were often cylindrical rather than spherical in shape and

contained a subset of the major centrosome components. γ-tubulin, pericentrin, CDK5RAP2 and

ninein were necessary for the formation of compact aMTOCs, whereas some other major PCM

proteins, namely CEP192, CEP152 and NEDD1, were neither enriched in these structures nor required

for their formation. A single aMTOC could also form in the presence of CAMSAP2, if this protein was

directly linked to a microtubule minus-end-directed motor. The formation of a compact MTOC in such

cells still required pericentrin. Assembly of a single MTOC in interphase mammalian cells can thus be

achieved without centrioles through the concerted action of scaffolds for microtubule nucleation and

stabilization, provided that they are linked to a minus-end-directed motor.

Results

Assembly of microtubule-dependent aMTOCs in AKAP450/CAMSAP2 KO cells lacking PLK4 activity

To induce centrosome loss in RPE1 cells, we treated these cells for 11 days with the PLK4 inhibitor

centrinone B (Wong et al., 2015) (Figure 1A,B). In wild type (WT) cells, PCM (detected with antibodies

against pericentrin) relocalized to the Golgi apparatus, and the microtubule array reorganized around

the Golgi membranes, as described previously (Gavilan et al., 2018; Wu et al., 2016) (Figure 1C,D). In

AKAP450 knockout cells, centriole loss led to the appearance of strongly dispersed PCM clusters,

which could no longer bind to the Golgi, and a highly disorganized microtubule system, consistent with

published work (Gavilan et al., 2018; Wu et al., 2016) (Figure 1D,E). In contrast, in AKAP450/CAMSAP2

double knockout cells, a single compact MTOC with microtubules focused around it was observed

(Figure 1D-F). Formation of a single compact aMTOC was also observed in AKAP450 knockout cells

transiently depleted of CAMSAP2 by siRNA transfection (Figure 1 – figure supplement 1A,B).

Unlike the centrosomes, which always have a spherical shape, compact aMTOCs in

AKAP450/CAMSAP2 knockout cells were cylindrical in ~35% of the cells, whereas in the remaining cells

that lacked centrioles based on staining for centrin, MTOCs had a round shape (~38% of the cells); the

rest of the centrin-negative cells either had dispersed PCM clusters (~7%) or no detectable PCM

clusters (~11%) (Figure 1 – figure supplement 1C,D). In contrast, ~72% of acentriolar AKAP450

knockout cells had dispersed PCM, while compact aMTOCs were very rare (Figure 1E, Figure 1 – figure

supplement 1C,D). Analysis by Stimulated Emission Depletion (STED) microscopy revealed that

cylindrical aMTOCs in AKAP450/CAMSAP2 knockout cells consisted of small clusters of PCM

components, including pericentrin, CDK5RAP2, γ-tubulin, ninein and dynein heavy chain (Figure 1F).

aMTOCs with a clearly elongated shape had an average length of ~11 μm and an average width of ~1.5

μm (Figure 1G).

To study PCM dynamics in aMTOCs, we generated cell lines stably expressing the PCM component

CDK5RAP2 tagged with GFP. In WT cells, GFP-CDK5RAP2 was localized to the centrosome and the Golgi

apparatus as expected (Figure 1 – figure supplement 2A). In centrinone-treated AKAP450/CAMSAP2

knockout cells, it was strongly enriched within aMTOCs and sometimes also present in small motile

clusters around an aMTOC (Figure 1H, Figure 1 – figure supplement 2B). Fluorescence recovery after

photobleaching (FRAP) assays showed that when the whole aMTOC was bleached, the recovery was

very slow and incomplete (Figure 1H, I). If an aMTOC was photobleached partially, the dynamics of

recovery showed cell-to-cell variability. Highly compacted aMTOCs did not undergo rearrangement

and displayed slow redistribution of GFP-CDK5RAP2. In more loosely organized aMTOCs, some

rearrangement of small PCM clusters was observed (Figure 1H,I); however, the recovery was still far

from complete. These data indicate that aMTOCs display variable degrees of compaction and are

composed of PCM clusters that display limited exchange of GFP-CDK5RAP2 with the cytoplasmic pool,

possibly because most of the GFP-CDK5RAP2 is accumulated within the aMTOC.

Next, we investigated whether centriole loss induced by means other than pharmacological PLK4

inhibition could also cause the formation of a single aMTOC in AKAP450/CAMSAP2 knockout cells. To

achieve efficient protein depletion in RPE1 cells, they were transfected with siRNAs twice (on day 0

and day 2), treated with thymidine starting from day 4 to block cell cycle progression and fixed and

stained on day 5 or day 7 (Figure 1 – figure supplement 1E). Depletion of PLK4 using siRNAs caused

the appearance of compact round or cylindrical aMTOCs, similar to those observed after PLK4

inhibition with centrinone, indicating that catalytically inactive PLK4 had no scaffolding role within

these structures (Figure 1 – figure supplement 1E-H). The percentage of cells with compact aMTOCs

increased over time (Figure 1 – figure supplement 1E-H), possibly due to the gradual depletion of PLK4.

In contrast, depletion of an essential centriole biogenesis factor CPAP (Kohlmaier et al., 2009; Schmidt

et al., 2009; Tang et al., 2009), which also led to centriole loss, was much less efficient in inducing

compact aMTOCs, and cylindrical a MTOCs were not observed (Figure 1 – figure supplement 1E-G).

After CPAP depletion, cells in which pericentrin formed dispersed clusters or no visible clusters

predominated (~67%, Figure 1 – figure supplement E-G). Treatment of CPAP-depleted

AKAP450/CAMSAP2 knockout cells with centrinone for 1 day promoted the assembly of compact

round or cylindrical aMTOCs, and the proportion of such cells increased to ~55% after 3 days of

centrinone treatment (Figure 1 – figure supplement 1E-I). We also tested whether the inhibition of

PLK1, a kinase that is known to be a major regulator of PCM self-assembly in mitosis (Haren et al.,

2009; Joukov et al., 2014; Lee and Rhee, 2011), has an effect on the formation of aMTOCs, but found

this not to be the case (Figure 1 – figure supplement 1F). We conclude that PCM can assemble into a

single stable MTOC in the absence of centrioles if PLK4 is either inactivated or depleted and the two

major pathways of microtubule nucleation and minus-end stabilization dependent on AKAP450 at the

Golgi membranes and CAMSAP2 are disabled.

Composition of aMTOCs and their effect on microtubule organization

To investigate the composition of aMTOCs, we stained centrinone-treated AKAP450/CAMSAP2

knockout cells with antibodies against different centrosome and centriole markers and microtubule-

associated proteins (MAPs). As indicated above, abundant PCM components pericentrin, CDK5RAP2,

ninein and γ-tubulin colocalized within aMTOCs (Figure 1F). In contrast, three other major PCM

proteins, CEP152, CEP192 and NEDD1, could not be detected in these structures although they were

present in centrosomes of AKAP450/CAMSAP2 knockout RPE1 cells that were not treated with

centrinone (Figure 2A-C) and were also expressed in centrinone-treated cells (Figure 2 – figure

supplement 1A). We then individually depleted all these proteins in centrinone-treated

AKAP450/CAMSAP2 knockout cells using siRNAs. After the depletion of pericentrin, no clusters of

other PCM components could be detected (Figure 2D, Figure 2 – figure supplement 1B). To confirm

this result, we also attempted to knock out pericentrin in AKAP450/CAMSAP2 knockout cells, but such

cells were not viable, likely because centrosome defects in these cells caused prolonged mitosis and

p53-dependent G1 arrest (Fong et al., 2016; Lambrus et al., 2016; Meitinger et al., 2016). However,

we were able to knock out pericentrin in cells lacking AKAP450, CAMSAP2 and p53 (Figure 2 – figure

supplement 2), confirming that the loss of p53 makes the cells more tolerant to centrosome defects.

Similar to pericentrin-depleted cells, these quadruple knockout cells were unable to form a single

aMTOC when treated with centrinone (Figure 2E). In these acentriolar quadruple knockout cells,

CDK5RAP2, γ-tubulin and cytoplasmic dynein displayed no clustering, while ninein and PCM1, a

centriolar satellite protein that localizes closely around the centrosome in normal cells (Prosser and

Pelletier, 2020), formed small clusters distributed throughout the cytoplasm (Figure 2 – figure

supplement 3).

Depletion of CDK5RAP2, γ-tubulin or ninein in centrinone-treated AKAP450/CAMSAP2 knockout cells

did not prevent the formation of small pericentrin clusters, but these failed to coalesce into a single

aMTOC (Figure 2F-H, J, Figure 2 – figure supplement 1B). In contrast, depletion of CEP152, CEP192 or

NEDD1 had no effect on the formation of aMTOCs (Figure 2A-C, J, Figure 2 – figure supplement 1B), in

agreement with the fact these proteins could not be detected within these structures. aMTOCs

contained several centriole biogenesis factors, including CPAP, CP110 and CEP120, but lacked centrin

and CEP135; however, the depletion of several centriolar proteins did not affect aMTOC formation

(Figure 2J, Figure 2 – figure supplement 1C). We also detected within aMTOCs the component of the

HAUS complex HAUS2, the centrosomal protein CEP170, dynein, dynactin, CLASP1/2, CLIP-115, CLIP-

170, chTOG, KIF2A and KIF1C (Figure 2J, Figure 2 – figure supplement 1C). We tested the importance

of some of these proteins for aMTOC formation by siRNA-mediated depletion (see Figure 2J for an

overview), but among the tested proteins, only cytoplasmic dynein appeared essential for this process.

In dynein-depleted cells, no clusters of pericentrin or other PCM components could be detected after

centrinone treatment (Figure 2I,J). It is important to note, however, that because we used siRNAs to

reduce protein expression, we cannot exclude that the residual levels of some of the investigated

proteins were sufficient to support aMTOC formation. Since we have detected in the aMTOCs several

microtubule plus-end tracking proteins (+TIPs), such as CLIP-170, CLASP1/2 and the large subunit of

dynactin p150Glued, we also tested for the presence of the core components of the +TIP machinery,

EB1 and EB3, but found that they were not enriched within the aMTOCs (Figure 2J, Figure 2 –

supplement 4A). Using the previously described EB1/EB3/CAMSAP2mut cells, which lack EB3,

CAMSAP2 and C-terminal partner-binding half of EB1 (Yang et al., 2017), we generated a knockout cell

line that also lacks AKAP450 and found that aMTOCs could still form in these cells (Figure 2J, Figure 2

– figure supplement 4B,C). We conclude that compact aMTOCs formed in AKAP450/CAMSAP2

knockout cells concentrate many but not all components of conventional centrosomes and require
some of them for their formation.

As could be expected, the presence of an aMTOC was a critical determinant of microtubule
organization and density in acentriolar cells: microtubules were focused around aMTOCs if present
and disorganized in cells lacking aMTOCs. The strongest loss of microtubule density was observed in
cells lacking pericentrin, dynein or γ-tubulin, while milder phenotypes were observed in cells lacking
CDK5RAP2 or ninein (Figure 3A,B). To further characterize microtubule organization after the loss of
these proteins, we analyzed the proportion of the radial and non-radial microtubules. Whereas control
cells (AKAP450/CAMSAP2 knockout cells treated with centrinone and control siRNA) formed radial
microtubule networks with ~12% non-radial microtubules, acentriolar cells lacking pericentrin or
cytoplasmic dynein had ~46% non-radial microtubules, and the depletion of CDK5RAP2, ninein and γ-
tubulin led to an intermediate phenotype with 25-30% non-radial microtubules (Figure 3C,D). aMTOC
formed in AKAP450/CAMSAP2 knockout cells upon centrinone treatment can thus organize
microtubules and increase microtubule density (Figure 3E), and the formation and function of such an
aMTOC depend on pericentrin, CDK5RAP2, ninein, γ-tubulin and dynein.

Dynamics of aMTOC assembly and disassembly

To test whether the formation and maintenance of aMTOCs in AKAP450/CAMSAP2 knockout cells
depends on microtubules, we depolymerized them by treating cells with nocodazole at 37°C and found
that aMTOCs fragmented into small clusters during nocodazole treatment and reassembled into a
single structure after nocodazole washout (Figure 4A-C). Because we found that aMTOC formation is
dynein-dependent, we also included in these experiments the dynein inhibitor dynapyrazole A
(Steinman et al., 2017). We confirmed that treatment with dynapyrazole A for 3 hrs had no effect on
dynein expression (Figure 4D) and found that the addition of this drug before nocodazole treatment
prevented the disassembly of aMTOCs, whereas treatment of cells with dynapyrazole A during
nocodazole washout strongly inhibited aMTOC re-assembly (Figure 4A-C). These data indicate that
microtubule-dependent dispersal and coalescence of PCM clusters into aMTOCs are driven by dynein
activity.

We next studied PCM dynamics using stably expressed GFP-CDK5RAP2 as a marker in live cells where
microtubules were labeled SiR-tubulin. GFP-CDK5RAP2 was mostly immobile within aMTOCs before
nocodazole treatment (Figure 1 – figure supplement 2B, Video 1). After a few minutes of nocodazole
treatment, when the microtubule density was significantly reduced, small PCM clusters started to

222 move out of the aMTOC and undergo rapid directional motility with speeds of up to 2 μm/sec, which

223 is within the range characteristic for cytoplasmic dynein (Schlager et al., 2014) (Figure 4E,F, Figure 4 –

224 figure supplement 1A, Video 1). Once microtubules were completely disassembled, the movement of

225 GFP-CDK5RAP2-positive clusters stopped, indicating that it is microtubule-dependent but occurs only

226 when the microtubule network is partly depolymerized. Since cluster dispersal towards the cell

227 periphery could be blocked by a dynein inhibitor, and since cytoplasmic dynein is a minus-end-directed

228 motor, these data indicate that during microtubule disassembly by nocodazole at 37°C, there is a

229 transient stage when PCM clusters interact with only a few microtubules, some of which have their

230 minus-ends facing outwards, and these microtubules serve as tracks for PCM transport. To support

231 this idea, we used motor-PAINT, a technique that employs nanometric tracking of purified kinesin

232 motors on the extracted cytoskeleton of fixed cells to super-resolve microtubules and determine their

233 orientation (Tas et al., 2017). Using this approach, we determined microtubule orientations in

234 centrinone-treated AKAP450/CAMSAP2 knockout cells and in cells that were also treated with

235 nocodazole for 15 min to induce partial microtubule disassembly (Figure 4G, Figure 4 – figure

236 supplement 1B). We found that the cells contained a significant number of minus-end-out

237 microtubules, and their proportion increased during early stages of nocodazole treatment, possibly

238 because minus-end-out microtubules are more stable (Figure 4G, e.g., minus-end-out microtubules

239 constituted ~23% of the total microtubule length determined from kinesin-1 trajectories in the

240 untreated cell and ~46% in the nocodazole-treated cell). These microtubules could serve as tracks for

241 outward movement of PCM, causing the disassembly of aMTOC when the overall microtubule density

242 around the aMTOC was strongly reduced (Figure 4H). These data suggest that the dense network of

243 PCM-anchored microtubule minus-ends keeps aMTOC compacted by dynein-mediated forces, which

244 can, however, pull it apart when the microtubules are disorganized.

245 Next, we investigated in more detail aMTOC reassembly during nocodazole washout. Small PCM

246 clusters positive for pericentrin, CDK5RAP2, γ-tubulin and the centriolar satellite protein PCM1 that

247 co-localized with the ends of microtubules labeled with EB1 could be detected 30 s after nocodazole

248 washout; these nascent microtubules did not colocalize with the Golgi membranes (Figure 5A, Figure

249 5 – figure supplement 1A). Ninein was not detected within the clusters at this early stage of

250 microtubule regrowth but could be found 2 min after nocodazole washout. In contrast, no clusters of

251 CEP192 or NEDD1 were observed even 10 min after nocodazole washout (Figure 5A, Figure 5 –

252 supplement 1A, B). Depletion of pericentrin, CDK5RAP2 and γ-tubulin strongly inhibited microtubule

253 nucleation in these conditions, whereas the depletion of dynein heavy chain or ninein had a milder

254 effect (Figure 5B,C). Live cell imaging with GFP-CDK5RAP2 and SiR-tubulin showed that CDK5RAP2

255 clusters with attached microtubules coalesced by undergoing microtubule-based movements (Figure

5D), and measurements in cells fixed at different time points after nocodazole washout showed that

a partly radial microtubule system emerged already 2 min after nocodazole washout (Figure 5 – figure

supplement 1C). Reassembly of a single aMTOC in the central part of the cell happened within ~15

min after nocodazole washout, though typically it was less compact than in cells that were not treated

with nocodazole (Figure 5D-G). Depletion of pericentrin, γ-tubulin and dynein heavy chain strongly

inhibited the reformation of a radial microtubule network during nocodazole washout, whereas the

effect of depleting CDK5RAP2 and ninein was less strong (Figure 5 – figure supplement 1C-E). Live cell

imaging of acentriolar AKAP450/CAMSAP2 knockout RPE1 cells stably expressing GFP-CDK5RAP2

showed that when pericentrin was depleted, CDK5RAP2 clusters were not detectable and the

microtubule network, both before nocodazole treatment and after nocodazole washout, was

disorganized (Figure 5 – figure supplement 2, Video 2). Taken together, our data show that pericentrin

and γ-tubulin form microtubule-nucleating and anchoring units that can be assembled into larger

structures by dynein-based transport. CDK5RAP2 contributes to microtubule nucleation activity,

whereas ninein appears to act somewhat later and contributes to the formation of a single compact

aMTOC.

The role of CAMSAP2-stabilized minus ends in defining microtubule network geometry

All the components of aMTOCs observed in AKAP450/CAMSAP2 knockout cells were also present in

AKAP450 knockout cells, yet these cells did not form a single aMTOC upon centrinone treatment

because they expressed CAMSAP2. In these cells, CAMSAP2-stabilized microtubule minus ends were

randomly distributed throughout the lamella, and microtubules were largely disorganized, although

many microtubules tended to co-align with the long axis of the lamella or of the whole cell (Figure 6A).

Live imaging of GFP-CDK5RAP2 together with SiR-tubulin in these cells showed that PCM clusters

moved along microtubules and occasionally encountered each other, but the movement directions

were random and PCM clusters did not coalesce into a single structure (Figure 6B, Figure 6 – figure

supplement 1A, Video 3). Treatment with nocodazole and subsequent nocodazole washout confirmed

that the motility of GFP-CDK5RAP2 clusters in centrinone-treated AKAP450 knockout cells is

microtubule-dependent and that these clusters can nucleate microtubules and move together with

their ends, but do not converge into a single compact aMTOC (Figure 6 – figure supplement 1A-C,

Video 3). Treatment with dynapyrazole A strongly inhibited the movements of small PCM clusters

(Figure 6 – figure supplement 1D,E, Video 4), indicating that they are dynein-driven. After the

depletion of pericentrin, GFP-CDK5RAP2 became completely diffuse, and it also did not form any

clusters during nocodazole treatment or washout (Figure 6 – figure supplement 2, Video 5), indicating

that clustering of GFP-CDK5RAP2 in AKAP450 knockout cells is pericentrin-dependent. Based on these data, we conclude that in AKAP450 knockout cells, pericentrin still forms PCM clusters that can nucleate microtubules and can be moved by dynein along other microtubules. However, since CAMSAP2-stabilized microtubule minus-ends in these cells are not attached to the Golgi membranes and do not interact with dynein, they are distributed randomly. This results in a disorganized microtubule network, with the minus ends pointing both in- and outwards, and leads to random motility of PCM clusters that prevents assembly of a single compact aMTOC (Figure 6C).

We next wondered whether we could force aMTOC formation in AKAP450 knockout cells by linking CAMSAP2-stabilized minus ends to a minus-end-directed motor. To avoid potential cell toxicity associated with manipulating cytoplasmic dynein, we used the motor-containing part of a moss kinesin-14, type VI kinesin-14b from the spreading earthmoss *Physcomitrella patens* (termed here ppKin14). The C-terminal motor-containing part of this protein can efficiently induce minus-end-directed motility of different cargoes in mammalian cells when it is tetramerized through a fusion with the leucine zipper domain of GCN4 (GCN4-ppKin14-VIb (861–1321)) and recruited to cargoes using inducible protein heterodimerization (Jonsson et al., 2015; Nijenhuis et al., 2020). Here, we employed a chemical heterodimerization system that is based on inducible binding of two protein domains, FRB and FKBP, upon the addition of a rapamycin analog (rapalog) (Pollock et al., 2000). To ensure that all CAMSAP2-decorated microtubule minus ends are linked to kinesin-14, we rescued centrinone-treated AKAP450/CAMSAP2 knockout cells by expressing CAMSAP2 fused to a tandemly repeated FKBP domain (2FKBP-mCherry-CAMSAP2) (Figure 6D-F). This construct was co-expressed with the FRB-GCN4-tagBFP-ppKin14 fusion, which by itself localized quite diffusely, with only a weak enrichment along microtubules, as described previously (Nijenhuis et al., 2020) (Figure 6D,F). In the absence of rapalog, CAMSAP2-decorated microtubule minus ends were distributed randomly, similar to endogenous CAMSAP2 in AKAP450 knockout cells (Figure 6F). However, upon rapalog addition, ppKin14 was rapidly recruited to CAMSAP2-decorated microtubule ends, and after 2 hrs, more than 90% of cells acquired a radial microtubule organization (Figure 6E-G). In rapalog-treated cells, CAMSAP2-bound microtubule minus ends formed either a tight cluster or a "whirlpool-like" ring in the cell center (Figure 6D-G, Figure 6 – figure supplement 1F). The whirlpool-like arrangement likely comes about when CAMSAP2-stretches are a bit longer and keep sliding against each other, forming a nematic circular bundle. The major aMTOC components, pericentrin, CDK5RAP2, γ-tubulin and ninein were also concentrated within the CAMSAP2 cluster, although they did not form a strongly compacted assembly (Figure 6G, Figure 6 – figure supplement 1F). These data indicate that the positioning of stabilized minus-ends is a very important determinant of the overall microtubule organization in

interphase cells, and that linking a minus-end-directed motor, even a heterologous one, to stable minus ends can induce self-assembly of a radial microtubule array.

The role of PCM in CAMSAP2-driven microtubule organization

Next, we tested whether PCM plays a role in CAMSAP-driven aMTOC formation by inducibly attaching CAMSAP2 to ppKin14 in centrinone-treated cells with both AKAP450 and pericentrin knocked out (AKAP450/CAMSAP2/p53/pericentrin knockout). In the absence of rapalog, CAMSAP2-stabilized minus ends and the whole microtubule network were disorganized, as expected (Figure 7A,D). After rapalog addition, microtubules acquired a radial organization, but their minus ends usually did not converge in a single spot but rather accumulated in a ~30-70 µm-large ring-like structure (Figure 7A,D, Figure 7- figure supplement 1, Video 6). Staining for PCM markers showed that CDK5RAP2 and γ-tubulin were enriched in the vicinity of CAMSAP2-positive microtubule minus ends, whereas ninein appeared rather diffuse (Figure 7A). To determine the nature of the structure "corralled" by the ring of CAMSAP2-decorated minus ends in rapalog-treated cells, we stained for different membrane organelles and found that whereas there was no strong correlation with the nucleus, Golgi membranes or lysosomes, the majority of mitochondria were found within the CAMSAP2 ring, and the endoplasmic reticulum (ER) displayed increased density overlapping with the CAMSAP2 ring (Figure 7B, Figure 7 - figure supplement 1B). It therefore appeared that in the absence of pericentrin, CAMSAP2-decorated minus ends were brought together by ppKin14, but their convergence was impeded by the ER and possibly mitochondria, which were enriched in the central, thicker part of the cell before rapalog addition (see the upper panel of Figure 7 - figure supplement 1B). Transient transfection of centrinone-treated AKAP450/CAMSAP2/p53/pericentrin knockout cells with GFP-pericentrin rescued the formation of a tight CAMSAP2 cluster upon rapalog treatment (Figure 7C). Our data show that pericentrin-containing PCM contributes to the formation of an aMTOC driven by minus-end-directed transport of CAMSAP2-stabilized minus-ends.

To support this notion further, we also generated cells that were knockout for AKAP450, CAMSAP2, CDK5RAP2, myomegalin (MMG, homologue of CDK5RAP2), p53 and pericentrin. To achieve this, we used the previously described RPE1 cell line knockout for AKAP450, CAMSAP2, CDK5RAP2 and MMG (Wu et al., 2016), in which we sequentially knocked out p53 and pericentrin (Figure 7 – figure supplement 2A-G). While it was not possible to induce centriole loss by centrinone treatment in AKAP450/CAMSAP2/CDK5RAP2/MMG knockout cells because the proliferation of these cells was arrested in the absence of centrioles (Wu et al., 2016), centriole removal was successful in AKAP450/CAMSAP2/CDK5RAP2/MMG/p53/pericentrin knockout cells due to the absence of p53 and

led to microtubule disorganization (Figure 7 – figure supplement 2H). Interestingly, when these cells

were co-transfected with FKBP-linked CAMSAP2 and FRB-linked ppKin14 and treated with rapalog

(Figure 6D), we observed that CAMSAP2 clustering was even less efficient than in

AKAP450/CAMSAP2/p53/pericentrin knockout cells (Figure 7D,E). 49% of

AKAP450/CAMSAP2/CDK5RAP2/MMG/p53/pericentrin knockout cells had small bundles of CAMSAP2

stretches dispersed throughout the cytoplasm, and only 37% of these cells formed a ring of CAMSAP2-

decorated minus ends, whereas 80% of AKAP450/CAMSAP2/p53/pericentrin knockout cells did form

such a ring. We examined the ER and mitochondria in these cells and found that in cells that did form

a CAMSAP2 ring, the ER displayed an overlapping ring-like density, although the mitochondria inside

the CAMSAP2 ring were more scattered compared to those of AKAP450/CAMSAP2/p53/pericentrin

knockout cells (Figure 7 – figure supplement 1B). In cells with dispersed CAMSAP2-positive bundles,

no increased ER density or central accumulation of mitochondria were observed (Figure 7 – figure

supplement 1B). These data further support the notion that during the formation an aMTOC, minus-

end-directed transport of CAMSAP2-stabilized minus ends needs to be augmented by PCM function

dependent on pericentrin, CDK5RAP2 and possibly also MMG.

Discussion

In this study, we explored the mechanisms driving MTOC assembly in acentriolar cells and showed that centrioles are not strictly needed to form a central compact MTOC and a radial microtubule array in interphase mammalian cells. Our data indicate that in the absence of the pathways that promote formation of non-centrosomal microtubules, cytoplasmic dynein can assemble a single aMTOC by bringing together microtubule minus ends that are nucleated and stabilized by PCM. However, the formation of MTOC in acentriolar cells is slow and less robust than in centriole-containing cells. Similar to the formation of spindle poles in mitosis (Meraldi, 2016), centrioles can thus be regarded as catalysts of interphase centrosome assembly.

We found that self-assembly of aMTOCs is driven by dynein-dependent transport of PCM components that include pericentrin, CDK5RAP2, γ-TuRC and ninein. In acentriolar cells that we studied, the major scaffold for interphase PCM assembly is pericentrin, which recruits CDK5RAP2 and ninein, in agreement with previous work (Chen et al., 2014; Delaval and Doxsey, 2010; Kim and Rhee, 2014; Lawo et al., 2012). Moreover, it has been established previously that CDK5RAP2 binds and activates γ-TuRC (Choi et al., 2010; Fong et al., 2008), and the same may be true for pericentrin (Takahashi et al., 2002) and possibly also for ninein (Delgehyr et al., 2005; Mogensen et al., 2000). Pericentrin and ninein can both directly interact with dynein (Purohit et al., 1999; Redwine et al., 2017), and an interaction between CDK5RAP2 and dynein has also been reported (Jia et al., 2013; Lee and Rhee, 2010). All these interactions likely contribute to the formation of aMTOCs.

Pericentrin-dependent MTOC assembly has also been observed in acentriolar mitotic cells (Chinen et al., 2021; Watanabe et al., 2020), but the interphase pathway displays some interesting differences. It seems that while oligomerization and clustering of pericentrin molecules is important for MTOC formation both in interphase and in mitosis, during mitotic entry, pericentrin may form condensates (Jiang et al., 2020), while in interphase, dynein-mediated motility dominates the behavior of pericentrin clusters. The compact aMTOC structure we observed in interphase was not simply a result of small pericentrin- and CDK5RAP2-positive PCM clusters coming into proximity of each other and sticking together, but also depended on active transport. In AKAP450 knockout cells, we also frequently observed CDK5RAP2-containing PCM clusters encountering each other, yet they did not coalesce into a single MTOC because they kept moving in random directions on CAMSAP2-stabilized microtubules. When most CAMSAP2-decorated minus ends were brought together by a minus-end-directed motor, pericentrin accumulated in the vicinity of such an artificially formed MTOC and contributed to its formation, but did not form a single solid body, likely because it kept moving along the sides of CAMSAP2-decorated microtubules but was not attached to their outermost minus ends.

A strongly compacted aMTOC only formed when most microtubule minus ends were anchored by PCM clusters. The shape of the MTOC was cylindrical rather than spherical in a significant proportion of such cells, suggesting that it was optimized for increasing the surface area. A larger surface area allows for the attachment of more microtubules and fits with the idea that aMTOC formation is primarily driven by the coalescence of minus-end-attached PCM clusters rather than free oligomers of PCM proteins. Altogether, it appears that active, dynein-mediated transport of pericentrin-based PCM clusters rather than spontaneous formation of PCM condensates is the major driver of interphase aMTOC assembly. This explains why aMTOCs are exquisitely sensitive to microtubule disassembly by nocodazole: during intermediate stages of microtubule loss, PCM complexes that are no longer attached to microtubule minus ends start to move on the remaining microtubules, and therefore, a few surviving minus-end-out microtubules can serve as rails to drive the dispersal of the aMTOC (Figure 4H). Furthermore, PCM dynamics within aMTOCs show no hallmarks of liquid droplet-like behavior: PCM components do not appear to be in equilibrium with the soluble cytoplasmic pool, and GFP-CDK5RAP2 displays very slow redistribution within the aMTOC. As CDK5RAP2 is recruited to the aMTOC by pericentrin, these data suggest that pericentrin is also quite immobile within aMTOCs.

Although pericentrin can directly bind to γ-TuRC, CDK5RAP2 and ninein are also needed for aMTOC formation. Analysis of different stages of microtubule recovery after nocodazole washout suggested that CDK5RAP2 is important for efficient microtubule nucleation, consistent with its role as an activator of γ-TuRC (Choi et al., 2010). Ninein can be detected within nascent aMTOC structures with some delay after their formation and might be important for stabilizing these structures or promoting minus-end anchoring, as proposed by many previous studies (Abal et al., 2002; Chong et al., 2020; Delgehyr et al., 2005; Goldspink et al., 2017; Lechler and Fuchs, 2007; Mogensen et al., 2000; Shinohara et al., 2013; Zheng et al., 2020). The importance of ninein for the formation of aMTOCs illustrates its function in minus-end organization within the PCM that is independent of centriolar appendages, one of the sites of ninein localization at the centrosome (Chong et al., 2020; Delgehyr et al., 2005; Sonnen et al., 2012).

A major difference between interphase and mitotic MTOC assembly is the role of other major PCM components, CEP192 and the γ-TURC-binding protein NEDD1. In our experiments, CEP192 and its binding partners CEP152 and NEDD1 (Gomez-Ferreria et al., 2012; Joukov et al., 2014; Kim et al., 2013; Sonnen et al., 2013) were not enriched at aMTOCs, and their depletion appeared to have no impact on aMTOC formation. This is surprising, because during mitosis, CEP192 is essential (Gomez-Ferreria et al., 2007; Joukov et al., 2014; Yang and Feldman, 2015; Zhu et al., 2008), and in acentriolar cells that rely on pericentrin and CDK5RAP2 for spindle pole formation, CEP192 is recruited to pericentrin clusters (Chinen et al., 2021; Watanabe et al., 2020). Moreover, although NEDD1 is

targeted to centrosomes by CEP192, pericentrin can also contribute to the centrosomal targeting of NEDD1 independently of CEP192 (Chi et al., 2021). Therefore, the lack of enrichment of CEP192, CEP152 and NEDD1 in interphase aMTOC is unexpected, particularly because these three proteins are present within interphase PCM at the centrosome, and multiple other centrosomal and centriolar proteins and MAPs are recruited to aMTOCs. Among the proteins enriched at aMTOCs, we did not identify any additional factors that would be crucial for aMTOC formation, but it is of course possible that this process requires some proteins that we have not tested. It is also important to note that because siRNA-mediated protein depletion may be incomplete, we cannot exclude that low amounts of some of the tested centrosome components or MAPs are sufficient to support aMTOC assembly.

An interesting question that remains unanswered by our work is the inhibitory role of PLK4 in interphase aMTOC formation. We did observe aMTOCs in cells depleted of PLK4, indicating that, unlike cells lacking TRIM37, which form PCM clusters containing catalytically inactive PLK4 (Meitinger et al., 2020; Yeow et al., 2020), interphase cells studied here do not rely on enzymatically inactive PLK4 for PCM assembly. PLK4 is known to phosphorylate NEDD1 (Chi et al., 2021), and it is possible that the lack of phosphorylation prevents this γ-TuRC activator and its partners, such as CEP192, from participating in interphase aMTOC assembly. It is of course also possible that PLK4 phosphorylation inhibits the interactions or activities of some of the players that are driving aMTOC formation.

An important conclusion of our study is the key role of CAMSAP2-stabilized minus ends in determining microtubule organization in the absence of the centrosome. Unlike the PCM components, CAMSAP2 does not interact with dynein, and if CAMSAP2-decorated minus ends are not attached to the Golgi membranes, which are themselves subject to dynein-based transport, the microtubule system becomes strongly disorganized. However, linking a minus-end-directed motor to CAMSAP2 can restore a radial microtubule array with a single MTOC, provided that the major active PCM components are also transported to microtubule minus ends due to the presence of pericentrin. Surprisingly, in the absence of pericentrin, minus-end-directed convergence of CAMSAP2-bound minus ends drives "corralling" of ER and mitochondria. Since there are currently no indications that CAMSAP2 specifically interacts with ER or mitochondria, we favor the idea that these organelles, which are normally enriched in the central, thicker part of the cell, form a physical obstacle for CAMSAP2-ppKin14-driven minus-end clustering, and that dynein-mediated transport of pericentrin-anchored minus ends helps to overcome this obstacle and form a compact aMTOC. Removal of CDK5RAP2 and its homologue MMG in these conditions further inhibits minus-end organization and aMTOC formation. This indicates that minus-end-directed transport of CAMSAP2-stabilized microtubule minus ends is by itself not sufficient to form a radial microtubule network but needs to synergize with PCM-dependent microtubule organization.

These data have implications for understanding how microtubules are reorganized in differentiated cells. During cell differentiation, the centrosome often loses its activity, and PCM is redeployed to other cellular locations (Martin and Akhmanova, 2018; Muroyama and Lechler, 2017; Sallee and Feldman, 2021). Our study shows that interphase PCM has the potential to self-assemble, but this ability is inhibited by PLK4, and the regulation of this kinase may thus influence PCM assembly and activity at different cellular locations. Further, we show that CAMSAP-dependent minus-end organization has a very dominant effect on the overall microtubule network geometry but can be affected by the presence of PCM components. This suggests that during microtubule reorganization in differentiated cells, PCM-dependent and CAMSAP-dependent pathways are likely to be co-regulated. This idea is strongly supported by studies of microtubule organization at the Golgi membranes (Gavilan et al., 2018; Wu et al., 2016) and the apical membranes of polarized epithelial cells, where components of both pathways colocalize and can display some redundancy (Goldspink et al., 2017; Khanal et al., 2016; Noordstra et al., 2016; Toya et al., 2016; Wang et al., 2015). In muscle cells, CAMSAPs are not expressed, and the organization of microtubule minus-ends is driven by the Golgi adaptor AKAP450 and some PCM proteins that are localized to the nuclear envelope and the Golgi (Gimpel et al., 2017; Oddoux et al., 2013; Vergarajauregui et al., 2020). In neurons, minus-ends can be organized by PCM, CAMSAPs, the Golgi membranes and endosomes (Fu et al., 2019; Garbrecht et al., 2021; Liang et al., 2020; Magescas et al., 2021; Pongrakhananon et al., 2018; Yau et al., 2014), though the cross-talk between different pathways is insufficiently understood. One common feature between these different systems and the data described here is that the function of PCM components, redeployed to different locations in differentiated cells, is dominated by two structurally related adaptors, AKAP450 and pericentrin, as well as CDK5RAP2 and ninein. In contrast, CEP192 seems to have a much more important role in mitotic rather than interphase cells. In line with this idea, recent work demonstrated that certain types of worm neurons contain an aMTOC that is formed by the functional counterparts of CDK5RAP2 (SPD-5), pericentrin (PCMD-1) and γ-tubulin, but lacks CEP192/SPD-2 (Garbrecht et al., 2021; Magescas et al., 2021). Future work will show whether this distinction is generally applicable. It would also be important to know to which extent PCM transport along microtubules contributes to microtubule organization in different cell types, and to determine how the biochemical and functional coupling between different pathways of microtubule nucleation and minus-end stabilization defines various geometries of microtubule networks.

 Materials and Methods

 DNA constructs and protein purification

To generate the lentiviral vector pLVX-GFP-CDK5RAP2-IRES-Puro, pLVX-IRES-Puro plasmid (Clontech)

was digested with AgeI and NotI (FastDigest, Thermo Fisher), and then Gibson Assembly (NEB) was

performed with gel-purified PCR product of GFP-CDK5RAP2 (Wu et al., 2016). To generate pB80-FRB-

TagBFP-GCN4-ppKin14 and pB80-FRB-HA-GCN4-ppKin14, pB80-FRB-GFP-GCN4-ppKin14-VIb was

digested with XbaI and BsrGI (FastDigest, Thermo Fisher), and then TagBFP and HA-tag encoding DNA

fragments were subcloned into the linearized vector by Gibson Assembly.

To generate the PX459 with single guide RNA (sgRNA) sequences, pSpCas9(BB)-2A-Puro (PX459) V2.0

(Ran et al., 2013)(purchased from Addgene) was digested with FastDigest BbsI (Thermo Fisher), and

the annealing product of single-strand sgRNA-encoding oligonucleotides was inserted into the linear

PX459 linear vector by T4 ligation (Thermo Fisher). The sgRNA sequences that were used in this study

are: sgRNA targeting AKAP450 5'- gAGGGTTACCTATGGGACTGA -3'; sgRNA targeting CAMSAP2

encoding gene 5'-gCATGATCGATACCCTCATGA-3'; sgRNA targeting p53-encoding gene exon 2 #1 5'-

gCGTCGAGCCCCCTCTGAGTC-3'; sgRNA targeting p53 exon 4 #2 5'-gTCCATTGCTTGGGACGGCAA-3';

sgRNA targeting p53 exon 4 #3 5'-gCCATTGTTCAATATCGTCCG-3'; sgRNA targeting PCNT exon 5-1 #1

5'-gAGACGGCATTGACGGAGCTG-3'; sgRNA targeting pericentrin-encoding gene exon 5-2 #2 5'-

GCTCAACAGCCGGCGTGCCC-3'.

To generate the GST-DmKHC(1-421)-mNeonGreen construct used for protein purification for motor-

PAINT, the fragment containing amino acids 1 to 421 of the Drosophila melanogaster Kinesin Heavy

Chain (DmKHC) was amplified from donor construct DmKHC(1-421)-GFP-6x-His with a C-terminal

mNeonGreen tag by PCR and then cloned into a pGEX vector. The plasmid was transformed into *E.*

coli BL21 cells for purification. Bacteria were cultured until OD600 $\approx$0.7 and cultures were cooled

prior to inducing protein expression with 0.15 mM IPTG at 18°C overnight. Cells were then pelleted by

centrifugation, snap frozen in liquid nitrogen, and stored at -80°C until use. Cells were rapidly thawed

at 37°C before being resuspended in chilled lysis buffer (phosphate buffered saline (PBS)

supplemented with 5 mM MgCl$_2$, 0.5 mM ATP, 0.1% Tween 20, 250 mM NaCl, and 1x complete

protease inhibitor; pH 7.4). Bacteria were lysed by sonication (5 rounds of 30 s) and supplemented

with 5 mM DTT and 2mg/mL lysozyme and then incubated on ice for 45 min. The lysate was clarified

by centrifuging at 26000 xg for 30 minutes before being incubated with equilibrated Glutathione

Sepharose 4B resin for 1.75 hrs. Beads were then pelleted, resuspended in wash buffer (PBS

supplemented with 5 mM MgCl$_2$, 0.1% Tween 20, 250 mM NaCl, 1 mM DTT, and 0.5 mM ATP; pH 7.4),

and transferred to a BioRad column. Once settled, the resin was washed with 2 x 10 column volumes

(CV) wash buffer, followed by 1 x 10CV PreScission buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 100mM NaCl,

1 mM DTT, 0.5 mM ATP; pH 8.0). The resin was then incubated overnight in 4CV PreScission buffer

with 80U PreScission protease to cleave off the GST tag. The following morning, after allowing the

resin to settle, the eluent was collected, concentrated by spinning through a 3000kDa MWCO filter,

supplemented with an additional 0.1mM ATP, 1mM DTT, and 20% w/v sucrose before flash freezing

in liquid nitrogen, and finally stored at -80°C. Concentration was determined using a Nanodrop. All

steps from lysis onwards were performed at 4°C.

Cell culture and drug treatment

hTERT immortalized RPE-1 (RPE1) cell lines were grown in an 1:1 mix of DMEM and F-10 (Lonza) and

Human Embryonic Kidney (HEK) 293T cells line were cultured in DMEM, both supplemented with 10%

fetal bovine serum (FBS, GE Healthcare) and 1% penicillin and streptomycin (Sigma-Aldrich). All cells

were grown in tissue culture polystyrene flasks (Corning) and were maintained in a humidified

incubator at 37°C with 5% CO_2. Mycoplasma contamination was routinely checked with LT07-518

Mycoalert assay (Lonza).

FuGENE 6 (Promega) was used to transfect RPE1 cells with plasmids for generating CRISPR/Cas9

knockouts, immunofluorescence staining and live cell imaging; RNAiMAX (Thermo Fisher Scientific)

was used to transfect RPE1 cells with siRNAs at 20 nM; MaxPEI was used to transfect HEK293T cells

for lentivirus packaging. Transfections were performed according to the manufacturer's instructions

within the recommended reagent/DNA or reagent/siRNA ratio range.

We used the following drugs: centrinone B (Tocris Bioscience), nocodazole (Sigma), rapalog (A/C

Heterodimerizer, Takara), dynapyrazole A (Sigma-Aldrich) and BI2536 (Selleckchem).

To remove centrioles, RPE1 cells were treated with 125 nM centrinone B containing complete medium

for ~10 days, and drug-containing medium was refreshed every 24 hrs; cell confluence was maintained

around ~50-80% during the treatment.

For the microtubule disassembly and regrowth assay, the acentriolar RPE1 cells were seeded onto

coverslips in 24-well plates and incubated for 24 hrs, then cells were treated with 10 μM nocodazole

for 1 hr in an incubator (37°C, 5% CO_2) and followed by another 1 hr treatment at 4°C to achieve

complete disassembly of stable microtubule fragments. Nocodazole washout was then carried out by

at least six washes on ice with ice-cold complete medium; subsequently, plates were moved to a 37°C

water bath and pre-warmed medium was added to each well to allow microtubule regrowth.

For the inducible ppKin14-CAMSAP2 heterodimerization experiment, acentriolar cells were seeded onto coverslips in 24-well plates, cultured with centrinone B containing medium and co-transfected with 2FKBP-mCherry-CAMSAP2 and FRB-TagBFP-GCN4-ppKin14 vectors. 24 hrs after transfection, rapalog was added into the medium at a final concentration of 50 nM and incubated overnight for preparation of fixed cells. For live imaging, rapalog was used at 100 nM.

Lentivirus packaging and generation of transgenic stable cell lines

Lentiviruses were produced by MaxPEI-based co-transfection of HEK293T cells with the transfer vectors together with the packaging vector psPAX2 and envelope vector pMD2.G (psPAX2 and pMD2.G were a gift from Didier Trono, Addgene plasmid #12259 and #12260; RRID:Addgene_12259 and RRID:Addgene_12260). Supernatant of packaging cells was harvested 48-72 hrs after transfection, filtered through a 0.45 µm filter, incubated with a polyethylene glycol (PEG)-6000-based precipitation solution overnight at 4°C and centrifuged for 30 minutes at 1500 rpm to concentrate the virus. Lentiviral pellet was resuspended in PBS.

Wild type, AKAP450 and AKAP450/CAMSAP2 knockout RPE1 cells were infected with lentivirus and incubated in complete medium supplemented with 8 µg/ml polybrene (Sigma-Aldrich). After 24 hrs, the cell medium was replaced with fresh medium. Starting 72 hrs after viral transduction, cells were subjected to selection with puromycin at a concentration of 25 µg/ml for wild-type, 20 µg/ml for AKAP450 knockout and 15 µg/ml for AKAP450/CASMAP2 knockout for up to 3 days (until most of the untransduced control cells, treated with the same concentration of antibiotic, were dead). After selection, cells were grown in normal medium for 3 days and individual colonies expressing GFP were isolated into 96-well plates by fluorescence-activated cell sorting (FACS). Sorted single transgenic stable cell lines were further confirmed by immunofluorescence staining to check the expression level of GFP-CDK5RAP2 and its colocalization with other centrosomal proteins.

Generation of CRIPSR/Cas9 knockout cell lines

The CRISPR/Cas9-mediated knockout of p53-, pericentrin-, AKAP450- and CAMSAP2-encoding genes was performed as described previously (Ran et al., 2013). In brief, AKAP450/CAMSAP2 knockout RPE1 cells (Wu et al., 2016) were transfected with the vectors bearing the appropriate targeting sequences using FuGENE 6. One day after transfection, the transfected AKAP450/CAMSAP2 knockout RPE1 cells were subjected to selection with 15 µg/ml puromycin for up to 3 days. After selection, cells were allowed to recover in normal medium for ~7 days, and knockout efficiency was checked by

immunofluorescence staining. Depending on the efficiency, 50–500 individual clones were isolated

and confirmed by immunofluorescence staining, and the resulted single colonies were characterized

by Western blotting, immunostaining and genome sequencing. AKAP450/CAMSAP2/p53 and

AKAP450/CAMSAP2/MMG/CDK5RAP2/p53 knockout cell lines were generated first and subsequently,

each of them was used to knock out the gene encoding pericentrin. The mutated portions of the p53-

and pericentrin-encoding genes were sequenced using gel-purified PCR products obtained with

primers located in the vicinity of the corresponding sgRNA targeting sites.

Antibodies, immunofluorescence staining and Western blotting

Antibodies used for immunostaining and Western blotting are listed in the Key Reagent or Resource

table. For immunofluorescence cell staining, cultured cells were fixed with −20°C methanol for 5 min

or with 4% paraformaldehyde (PFA) for 12 min at room temperature, rinsed in PBS for 5 min,

permeabilized with 0.15% Triton X-100 in PBS for 2 min, washed 3 times for 5 min with 0.05% Tween-

20 in PBS, sequentially incubated for 20 min in the blocking buffer (2% BSA and 0.05% Tween-20 in

PBS), 1 hr with primary antibodies in the blocking buffer, washed 3 times for 5 min with 0.05% Tween-

20 in PBS, then for 1 hr in secondary antibodies in the blocking buffer, washed 3 times for 5 min with

0.05% Tween-20 in PBS, and air-dried after a quick wash in 96% ethanol. Cells were mounted in

Vectashield mounting medium with or without DAPI (Vector laboratories, Burlingame, CA). Alexa Fluor

-405, -488, −594 and −647 conjugated goat antibodies against rabbit, rat and mouse IgG were used as

secondary antibodies (Molecular Probes, Eugene, OR).

For Western blotting, cells were harvested from six-well plates or 10 cm dishes at 90% confluence and

protein extracts were prepared using the lysis buffer containing 20 mM Tris-Cl, pH 7.5, 150 mM NaCl,

0.5 mM EDTA, 1 mM DTT, 1% Triton X-100 or RIPA buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM

NaCl, 1% Triton X-100, 0.5% Sodium Deoxycholate supplemented with protease inhibitor and

phosphatase inhibitors (Roche). Samples were run on polyacrylamide gels, followed by transfer on

0.45 μm nitrocellulose membrane (Sigma-Aldrich). Blocking was performed in 2% BSA in PBS for 30

min at room temperature. The membrane was first incubated with the primary antibodies overnight

at 4°C and washed with 0.05% Tween-20 in PBS 3 times and subsequently incubated with secondary

antibodies for 1 hr at room temperature and washed 3 times with 0.05% Tween-20 in PBS. IRDye

800CW/680 LT Goat anti-rabbit and anti-mouse were used as secondary antibodies (Li-Cor

Biosciences, Lincoln, LE) and membranes were imaged on Odyssey CLx infrared imaging system (Image

Studio version 5.2.5, Li-Cor Biosciences).

Imaging and analysis of fixed cells

Images of fixed cells were collected with a Nikon Eclipse Ni upright fluorescence microscope equipped with a DS-Qi2 CMOS camera (Nikon), an Intensilight C-HGFI epi-fluorescence illuminator (Nikon), Plan Apo Lambda 100× NA 1.45 or Plan Apo Lambda 60x N.A. 1.40 oil objectives (Nikon) and driven by NIS-Elements Br software (Nikon).

Gated STED imaging was performed with Leica TCS SP8 STED 3X microscope driven by LAS X software using HC PL APO 100x/1.4 oil STED WHITE objective, white laser (633 nm) for excitation and 775 nm pulsed lased for depletion. Images were acquired in 2D STED mode with vortex phase mask. Depletion laser power was equal to 90% of maximum power and an internal Leica HyD hybrid detector with a time gate of $1 \leqslant tg \leqslant 8$ ns was used.

ImageJ was used for adjustments of intensity levels and contrast, quantification of the immunofluorescence signal intensity and maximum intensity projections. To analyze PCM clustering after nocodazole washout in AKAP450/CAMSAP2 knockout RPE1 cells, images were separated into concentric circular areas using Concentric Circles plugin of ImageJ. The biggest PCM cluster (which normally also had the highest fluorescence intensity) was selected as the center, around which 20 circles with 2 µm inner radius and 20 µm outer radius were drawn. Fluorescence intensity of PCM clusters in these concentric circles was measured automatically and normalized by the sum of the total PCM intensity in each cell per condition. To quantify the areas occupied by PCM clusters, immunofluorescence images of fixed cells and time lapse images of live cells were analyzed by drawing the smallest circle that covered visible PCM clusters to indicate the area occupied by the PCM clusters, and the diameters of the circles were used for the quantification.

Measurements of microtubule radiality

To analyze microtubule radiality, images of fluorescently labeled microtubules were separated into radial and non-radial components using a customized ImageJ macro (https://github.com/ekatrukha/radialitymap). First, a local orientation angle map was calculated for each pixel using the OrientationJ plugin. We used 'cubic spline gradient' method and tensor sigma parameter of 6 pixels (0.4 µm). The new origin of coordinates was specified by selecting the centrosome position in the corresponding channel, or the brightest spot in case of centrinone treatment. Radial local orientation angle was calculated as a difference between the local orientation angle and the angle of the vector drawn from the new origin of coordinates to the current pixel

position. A radial map image was calculated as an absolute value of the cosine of the radial local

orientation angle at each pixel providing values between zero and one. A non-radial map image was

calculated as one minus the radial map. Both maps were multiplied with the original image to account

for different signal intensities; the two maps illustrate separated radial and non-radial image

components.

Live cell imaging and analysis

Live fluorescent imaging was performed with spinning disk confocal microscopy on inverted research

microscope Nikon EclipseTi-E (Nikon), equipped with the Perfect Focus System (Nikon), Nikon Plan

Apo VC 60x NA 1.4 and Nikon Plan Apo VC 100x N.A. 1.40 oil objectives (Nikon) and spinning-disc

confocal scanner unit (CSU-X1-A1, Yokogawa). The system was also equipped with ASI motorized stage

with the piezo top plate MS-2000-XYZ (ASI), Photometrics Evolve 512 EMCCD camera (Photometrics)

and controlled by the MetaMorph 7.8 software (Molecular Devices). Vortran Stradus lasers (405 nm

100 mW, 488 nm 150 mW and 642 nm 165 mW) and Cobolt Jive 561 nm 110 mW laser were used as

the light sources. System was equipped with ET-DAPI (49000), ET-GFP (49002), ET-mCherry (49008)

and ET-Cy5 (49006) filter sets (Chroma). 16-bit images were projected onto the EMCCD chip with the

intermediate lens 2.0X (Edmund Optics) at a magnification of 110 nm per pixel (60x objective) and 67

nm per pixel (100x objective). To keep cells at 37°C and 5% CO_2 we used stage top incubator (INUBG2E-

ZILCS, Tokai Hit). Cells were plated on round 25 mm coverslips, which were mounted in Attofluor Cell

Chamber (Thermo fisher). Cells were imaged with a 2 s interval and 200 ms exposure for 1-3 hrs at

10% laser power.

For live imaging of nocodazole treatment and washout experiments, cells were incubated with the

medium containing 100 nM SiR-tubulin (Tebu-bio) overnight to image the microtubule network.

Centrinone B-treated cells were imaged for a desired period of time prior to the nocodazole

treatment, and then nocodazole was added into the medium at a final concentration of 10 µM while

imaging simultaneously. Culture medium was carefully removed when microtubules were completely

depolymerized and washed with prewarmed medium six times to let microtubules regrow. GFP-

CDK5RAP2 and SiR-tubulin imaging was performed with a 2 s interval with 200 ms exposure for 1-3

hrs in total, and maximum intensity projections, contrast adjustment and further processing was

performed using ImageJ.

FRAP

FRAP experiments were performed on the spinning disc microscope describe above, equipped with

iLas platform and using Targeted Laser Action options of iLas and controlled with iLas software (Roper

Scientific, now Gataca Systems). Photobleaching in the GFP channel was performed with the 488 nm

laser. For the FRAP analysis, Polygon ROIs were set in photobleached and non-bleached regions as

well as in the background. The average fluorescence intensity was measured using ImageJ for each

frame, the background intensity was subtracted from the bleached and non-bleached areas and

normalized to the average of the frames acquired prior to the bleach. The mean fluorescence

intensities of the images before photobleaching were set as 100%, and the subsequent relative

recovery percentages were calculated. Time lapse acquisitions were corrected for drift with the

ImageJ plugins Template Matching.

motor-PAINT and analysis

For motor-PAINT, a protocol published previously (Tas et al., 2017) was used, with minor adjustments.

Cells were incubated with 50 nM SiR-tubulin and 500 nM verapamil overnight to allow fields of view

suitable for imaging to be located before the addition of purified GST-DmKHC(1-421)-mNeonGreen.

For nocodazole-treated samples, cells were first incubated with 10 µM nocodazole for 15 minutes at

37°C. A single nocodazole-treated or control sample was then transferred to an imaging chamber, and

cells were subjected to extraction for 1 minute in extraction buffer (BRB80: 80 mM K-Pipes, 1 mM

MgCl$_2$, 1 mM EGTA; pH 6.8, supplemented with 1M sucrose and 0.15% TritonX-100) pre-warmed to

37°C. Pre-warmed fixation buffer (BRB80 supplemented with 2% PFA) was added to this (i.e. final PFA

concentration of 1%) and the solutions were mixed by gentle pipetting for 1 minute. This buffer was

removed and the chamber was washed for 4 times for 1 minute in pre-warmed wash buffer (BRB80

supplemented with 1 µM Taxol) before adding imaging buffer (BRB80 supplemented with 583 µg/mL

catalase, 42 µg/mL glucose oxidase, 1.7% w/v glucose, 1 mM DTT, 1 µM Taxol, and 5mM ATP). An

aliquot of GST-DmKHC(1-421)-mNeonGreen motors was warmed, spun in the Airfuge at 20 psi for 5

minutes in a pre-chilled rotor to remove any aggregates, and then transferred to a clean tube prior to

use. Motors were kept on ice and added locally to cells in 0.3 µl increments.

Imaging was performed immediately after sample preparation at room temperature on a Nikon Ti-E

microscope equipped with a 100x Apo TIRF oil immersion objective (NA. 1.49) and Perfect Focus

System 3 (Nikon). Excitation was achieved with a Lighthub-6 laser combiner (Omicron) containing a

647 nm laser (LuxX 140 mW, Omicron), a 488 nm laser (LuxX 200 mW, Omicron), and optics allowing

for a tunable angle of incidence. Illumination was adjusted for (pseudo-) total internal reflection

fluorescence (TIRF) microscopy. Emission light was separated from excitation light using a quad-band

polychroic mirror (ZT405/488/561/640rpc, Chroma), a quad-band emission filter
(ZET405/488/561/640m, Chroma), and an additional single-band emission filter (ET525/50m for
mNeonGreen emission, Chroma). Detection was achieved using a Hamamatsu Flash 4.0v2 sCMOS
camera. Image stacks were acquired with a 60 ms exposure time, 7% laser power, and 15000-22000
images per field of view. Components were controlled using MicroManager (Edelstein et al., 2014).

Acquired stacks were pre-processed using the Faster Temporal Median ImageJ plugin
(https://github.com/HohlbeinLab/FTM2; (Jabermoradi et al., 2021)) with a window size of 100 frames.
These stacks were then analyzed using Detection of Molecules (DoM) plugin v.1.2.1 for ImageJ
(https://github.com/ekatrukha/DoM_Utrecht), as has been described previously (Chazeau et al.,
2016; Tas et al., 2017). Each image in an acquired stack is convoluted with a two-dimensional Mexican
hat kernel. The resulting intensity histogram is used to create a thresholded mask based on a cut-off
of three standard deviations above the mean. This mask is then subject to rounds of dilution and
erosion to create a filtered mask used to calculate the centroids on the original image. These centroids
are used as initial values to perform unweighted nonlinear least squares fitting with a Levenberg-
Marquardt algorithm to an asymmetric two-dimensional Gaussian point spread function (PSF),
allowing for the sub-pixel localization of particles.

Images were drift-corrected using DoM. The normalized cross-correlation between intermediate
reconstructions of consecutive sub-stacks is used to calculate the drift in x and y between sub-stacks,
which is then linearly interpolated to adjust each individual frame in the stack.

Detected particles were linked into tracks again using DoM, which performs a quicker variant of a
nearest neighbor search, with a maximum distance of 5 pixels (~320nm) between consecutive frames
and no permitted frame gap. Tracks were later filtered to remove those shorter than 4 frames or
longer than 200 frames, those in which an angle between parts of the trajectory exceeded 90 degrees,
and those in which the speed of the motor was less than 100nm/s or more than 1500nm/s.

The particle table was then split into four particle tables corresponding to the four quadrants of the
image with tracks sorted based on their net displacement (i.e., $\Delta x>0 \wedge \Delta y>0$; $\Delta x>0 \wedge \Delta y<0$; Δ
$x<0 \wedge \Delta y>0$; $\Delta x<0 \wedge \Delta y<0$), as described previously (Tas et al., 2017). These directionality-filtered
particle tables were reconstructed using DoM, creating four super-resolved images of microtubule
segments pointing in a similar direction. These were merged with the reconstructed image of all
localizations to determine the direction of each microtubule segment. Each microtubule was manually
assessed to assign it as being plus-end-in or plus-end-out. Microtubules were manually traced with
lines 4 pixels (80 nm) wide, assigned a color based on their orientation, flattened onto the image,

filtered with a Gaussian Blur of radius 2, and finally merged with the reconstructed image of all
localizations.

To quantify the percentage of minus-end-out microtubule length to total microtubule length before
and after nocodazole treatment, the length of each microtubule (determined from kinesin-1
trajectories) in the cell was measured by calculating the Euclidean distance between all subsequent
pairs of points along the microtubule and summed. The ratio was calculated as the total minus-end-
out microtubule length divided by the total microtubule length.

Analysis of PCM cluster dynamics

To represent the motion of PCM clusters during nocodazole treatment, ImageJ plugin
KymoResliceWide v.0.4 (https://github.com/ekatrukha/KymoResliceWide) was used for generating
kymographs from the time lapse images. The velocity of PCM clusters was measured manually using
kymographs starting from the time point when a small PCM cluster moved out of an aMTOC.
Microtubule density around each PCM cluster was determined by measuring the mean fluorescence
intensity of SiR-tubulin in a circular area with a 2 μm radius centered on the PCM cluster and
normalizing it to the mean fluorescence intensity of 20 images prior to nocodazole addition (set as
100%). The moment when a PCM cluster started to move out of the aMTOC was set as the initial time
point (0 min), and the subsequent PCM cluster motion velocity and the relative local microtubule
density at 43 time points were calculated and averaged.

The movement trajectories of PCM clusters were generated using ImageJ plugin TrackMate (version
is 6.0.2). The parameters and the settings used were as following: LoG (Laplacian of Gaussian) detector
with estimated blob diameter: 14.9 μm; thresholding value 12.25; sub-pixel localization was selected.
HyperStack Displayer was selected to overlay the spots and tracks on the current hyperstack window.
Simple LAP tracker was selected to track the distance and time with the linking max distance: 32.0 μm,
gap-closing max distance: 55.0 μm and gap-closing max frame gap: 2. All other parameters and
settings were used as the default.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 9. Statistical details for each experiment
can be found in the corresponding figure legends and supporting files.

Data and software availability

All mentioned ImageJ plugins have source code available and are licensed under open-source GNU
GPL v3 license. The source data for the original Western blots are available within the paper.

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Competing financial interests

The authors declare no competing financial interests.

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 Figure legends.

 Figure 1. Formation and characterization of aMTOCs in AKAP450/CAMSAP2 knockout cells

(A) Immunofluorescence images of control or centrinone B-treated wild type (WT) RPE1 cells stained

for centrioles (CEP135, red; centrin, green). The zooms of the boxed area show the centrioles stained

with the indicated markers.

(B) Quantification shows the percentage of cells with centrioles before and after the centrinone

treatment. 350 cells (n=7 fields of view) analyzed for each measurement in three independent

experiments. The statistical significance was determined by unpaired two-tailed Mann-Whitney test

in Prism 9.1 (***p<0.001). Values represent mean±SD.

(C) Immunofluorescence images of centrinone-treated WT RPE1 cells stained for pericentrin (PCNT,

green) and the Golgi marker GM130 (red). Inset shows the merged image of the boxed area.

(D) Diagrams of the microtubule organization in WT and knockout (KO) cells used.

(E) Immunofluorescence images of centrinone-treated WT and knockout RPE1 cell lines stained for

pericentrin (green) and microtubules (α-tubulin, red). Enlargements of the boxed areas are shown in

the bottom row.

(F) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells

stained for different PCM components as indicated and imaged by STED microscopy.

(G) Quantification of the length and width of cylindrical PCM clusters. n=65 cells analyzed in three

independent experiments. Values represent mean±SD.

(H) (Top left) Two frames of time-lapse images of centrinone-treated AKAP450/CAMSAP2 knockout

RPE1 cells stably expressing GFP-CDK5RAP2 prior to FRAP experiments. (Top right) Schemes show

regions of aMTOC where photobleaching was performed. (Middle) Kymographs illustrating

fluorescence of unbleached aMTOC (No FRAP), fully photobleached aMTOC (Whole FRAP) and

partially photobleached MTOC (Partial FRAP). (Bottom) Time-lapse images illustrating partial FRAP of

a compact aMTOC. Time is min:sec.

(I) Normalized fluorescence intensity as a function of time. The blue line shows averaged intensity

traces of unbleached MTOCs (No FRAP), the black line shows averaged intensity traces of fully

photobleached MTOCs (Whole FRAP), the red line shows averaged intensity traces of whole MTOC

that were partially photobleached (whole MTOC intensity, Partial FRAP) and the green line shows

averaged intensity traces of the photobleached region of the partially photobleached MTOC (FRAP

region intensity, Partial FRAP). n=3 for No FRAP, 3 for Whole FRAP, 5 for Partial FRAP (whole MTOC

intensity) and 5 for Partial FRAP (FRAP region intensity); time-lapse images of ~1600 timepoints with

2 s interval were analyzed for each measurement. Values are mean±SD.

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Figure 2. Molecular composition of aMTOCs in AKAP450/CAMSAP2 knockout cells

(A-C) Immunofluorescence images of control or centrinone-treated AKAP450/CAMSAP2 knockout

RPE1 cells stained for and depleted of the indicated proteins.

(D, F-I) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells

stained for and depleted of the indicated proteins.

(E) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2/p53 knockout and

AKAP450/CAMSAP2/p53/pericentrin knockout RPE1 cells stained as indicated.

In panels A-I, insets show enlargements of the merged channels of the boxed areas, and dashed lines

indicate cell edges in panels where these are poorly visible.

(J) Summarizing table of PCM localization and the depletion effects on aMTOC formation in

AKAP450/CAMSAP2 knockout RPE1 cells. NT, not tested.

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Figure 3. Microtubule organization in acentriolar cells missing different aMTOC components

(A) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells

depleted of the indicated proteins and stained for microtubules (α-tubulin, red) and different PCM

proteins (green). Insets show enlargements of the merged channels of the boxed areas and dashed

lines show cell boundaries.

(B) Quantification of the normalized overall microtubule intensity for the indicated conditions. The

number of cells analyzed in three independent experiments: n=56 (siLuci), 45 (siPCNT), 33

(siCDK5RAP2), 36 (siNinein), 43 (siγ-tubulin) and 28 (siDHC). The statistical significance was

determined by unpaired two-tailed Mann-Whitney test in Prism 9.1 (***$p<0.001$). Values represent

mean±SD.

(C) Microtubule images were split into a radial and non-radial components (heat maps) based on

microtubule orientation in relation to the PCM clusters or the brightest point, as described in

Methods.

(D) Quantification of the proportion of the non-radial microtubules shown in panel C (see Methods

for details). The number of cells analyzed for each measurement in three independent experiments:

n=25 (siLuci), 43 (siPCNT), 32 (siCDK5RAP2), 34 (siNinein), 37 (siγ-tubulin) and 25 (siDHC). The

statistical significance was determined by unpaired two-tailed Mann-Whitney test in Prism 9.1

(***p<0.001). Values represent mean±SD.

(E) Diagram illustrating the distribution of PCM clusters and microtubule organization upon the

depletion of the indicated proteins in centrinone-treated AKAP450/CAMSAP2 knockout cells.

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Figure 4. Microtubule- and dynein-dependent disassembly of aMTOCs

(A) Diagram illustrating different order of cell treatments with centrinone and/or dynapyrazole A (5

µM) and the time points when the cells were fixed.

(B) Immunofluorescence staining of centrinone-treated AKAP450/CAMSAP2 knockout cells treated as

shown in panel A. Dashed red circles represent the areas occupied by PCM clusters in each condition.

(C) Quantification of the area occupied by PCM clusters in each condition, as shown in panels A and B.

n=35-53 cells analyzed for each measurement in three independent experiments. The statistical

significance was determined by unpaired two-tailed Mann-Whitney test in Prism 9.1 (not significant

(NS), *P*<0.12; **P*<0.033; ***P<0.001). Values are represented as mean±SD.

(D) Western blot showing that 3 hr treatment with dynaprazole A does not affect the expression of

the endogenous dynein heavy chain and the dynactin large subunit p150Glued.

(E) Time-lapse images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells stably

expressing GFP-CDK5RAP2. Microtubules were visualized by treating cells with 100 nM SiR-tubulin

overnight. Red arrows show the immobilized PCM clusters at indicated timepoints. Time is min:sec.

Time-lapse images of the same cell prior to the nocodazole treatment were shown in Figure 1 - figure

supplement 2B.

(F) (Top) Kymograph illustrating the motility of PCM clusters during microtubule disassembly with

nocodazole. (Bottom) Measurements of the normalized microtubule (SiR-tubulin) fluorescence

intensity (red plot, left Y-axis) and the instantaneous velocity of GFP-CDK5RAP2 clusters (green plot,

right Y-axis) during the movement of GFP-CDK5RAP2 clusters away from aMTOC. Microtubule density

around each PCM cluster was determined by measuring mean fluorescence intensity of SiR-tubulin in

a circular area with a 2 µm radius centered on the PCM cluster and normalizing it to the mean

fluorescence intensity of 20 images prior to nocodazole addition (set as 100%). The moment when a

PCM cluster started to move out of the aMTOC was set as the initial time point (0 min) for this cluster, and the subsequent PCM cluster motion velocity and the relative local microtubule density of 43 time points were calculated and averaged. n=12 clusters were analyzed in each condition. Values are represented as mean±SD.

(G) Motor-PAINT images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells before and after nocodazole treatment. Plus-end-out microtubules are shown in white whereas minus-end-out microtubules are shown in magenta. Asterisks represent the putative position of aMTOC.

(H) Summarizing diagram illustrating microtubule organization and the motility of GFP-CDK5RAP2-positive PCM clusters during nocodazole treatment and dynapyrazole A (treat first) and nocodazole co-treatment.

Figure 5. Dynamics of microtubule nucleation and MTOC re-assembly in acentriolar cells

(A) Immunofluorescence images of microtubule regrowth after nocodazole washout at the indicated timepoints in centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells stained for PCM components (green) and newly nucleated microtubules (EB1, red). A Golgi marker, GM130 (blue), is included in the left row, and zooms of the boxed regions (numbered 1 and 2) show that microtubules nucleate from PCM clusters but not from the Golgi membranes. Dashed lines show cell boundaries.

(B) Immunofluorescence images of microtubule regrowth experiments after depletion of the indicated proteins in centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells stained for the indicated PCM markers (green) and EB1 as a marker of nascent microtubules (red). Cell outlines are indicated with dashed lines and enlargements of the merged channels of the boxed areas are shown on the right.

(C) Quantification of normalized microtubule intensity at 30 s after nocodazole washout in control cells and cells depleted of the indicated PCM proteins. n=40 (siLuci, siPCNT), 57 (siCDK5RAP2), 48 (siNIN), 45 (siγ-tubulin) and 50 (siDHC) cells analyzed for each measurement in three independent experiments. The statistical significance was determined by unpaired two-tailed Mann-Whitney test in Prism 9.1 ($**P<0.002$; $***P<0.001$). Values are represented as mean±SD.

(D) Time-lapse images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells stably expressing GFP-CDK5RAP2 before and after nocodazole washout. Dispersed GFP-CDK5RAP2-positive PCM clusters (GFP, green) serve as microtubule nucleation sites (SiR-tubulin, red) and coalesce into a big cluster after nocodazole washout. Time is min:sec.

(E) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells stained for pericentrin (green) and microtubules (α-tubulin, red) at the indicated timepoints after nocodazole washout.

(F) Measurements of normalized fluorescence intensity of PCM clusters at the indicated distances in relation to the brightest point, as described in Methods. The biggest PCM cluster (which normally also had the highest fluorescence intensity) was selected as the center, around which 10 concentric circles with 2 μm width were drawn. Fluorescence intensity of PCM clusters in these concentric circles was measured automatically and normalized by the sum of the total PCM intensity in each cell per condition. n=12 cells per plot per timepoint. Values represent mean±SD.

(G) Summarizing diagram illustrating microtubule organization and motility of GFP-CDK5RAP2-positive PCM clusters during nocodazole washout.

Figure 6. CAMSAP2-driven microtubule organization in acentriolar cells

(A) Immunofluorescence images of control or centrinone treated AKAP450 knockout RPE1 cells stained for CAMSAP2 (green), PCM protein (γ-tubulin, cyan) and microtubules (α-tubulin, red). Enlargements show the boxed regions of the merged images.

(B) Time lapse images of centrinone treated AKAP450 knockout RPE1 cells stably expressing GFP-CDK5RAP2 (green). Microtubules were labeled with 100 nM SiR-tubulin overnight (red, top row). The maximum intensity projection includes 200 frames, 200 ms/frame. Red arrows show the motion directions of GFP-CDK5RAP2-positive PCM clusters. Time is min:sec.

(C) A diagram of microtubule organization and PCM motility in AKAP450 knockout cells.

(D, E) Diagram of the inducible heterodimerization assay with ppKin14 and CAMSAP2. (D) CAMSAP2 was tagged with mCherry and fused to a tandemly repeated FKBP domain; tetramerized ppKin14 was tagged with TagBFP and fused to FRB. Rapalog induces the binding of CAMSAP2 and ppKin14 by linking FKBP to FRB. (E) Rapalog treatment induces the binding of CAMSAP2 (red) and ppKin14 (blue) and the formation of radial microtubule network. In this scheme, PCM-anchored microtubules are not shown.

(F) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells co-transfected with 2FKBP-mCherry-CAMSAP2 and FRB-TagBFP-GCN4-ppKin14 and stained for the indicated proteins in cells treated with DMSO or rapalog. Zooms show the magnifications of boxed areas.

1280 (G) Quantification of the proportion of cells with a radial, whirlpool-like or non-radial microtubule
1281 organization with and without rapalog treatment. Numbers on the histogram show the percentages.
1282 414 cells treated with DMSO (-Rapa) and 385 cells treated with rapalog (+Rapa) analyzed for each
1283 measurement in three independent experiments (n=3). Values represent mean±SD.

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Figure 7. The role of the PCM in CAMSAP2-driven formation of aMTOCs

(A,B) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2/p53/pericentrin
knockout RPE1 cells transfected with 2FKBP-mCherry-CAMSAP2 and FRB-TagBFP-GCN4-ppKi14 and
stained for the indicated components before (top) or after an overnight rapalog treatment. Zooms
show magnifications of boxed areas. Black dashed lines show the position of the nucleus.

(C) Cells treated as described for panel A were co-transfected with GFP-pericentrin and stained for
mitochondria (cytochrome C, red) and CAMSAP2 (red) in same channel overnight after rapalog
addition. Zooms show magnifications of boxed areas.

(D) Quantification of the proportion of cells with different types of microtubule minus end
organization before and after overnight rapalog treatment. Numbers on the histogram show the
percentages. 334 (-Rapa), 424(+Rapa) cells of AKAP450/CAMSAP2/p53/pericentrin knockout RPE1
cells, 206(-Rapa) and 239(+Rapa) of AKAP450/CAMSAP2/CDK5RAP2/MMG/p53/pericentrin knockout
RPE1 cells analyzed for each measurement in three independent experiments (n=3). Values represent
mean±SD.

(E) Immunofluorescence images of centrinone-treated
AKAP450/CAMSAP2/CDK5RAP2/MMG/p53/pericentrin knockout RPE1 cells transfected with 2FKBP-
mCherry-CAMSAP2 and FRB-TagBFP-GCN4-ppKi14 and stained for microtubules (α-tubulin, green)
after an overnight rapalog treatment. Zooms show magnifications of boxed areas.

Legends to Supplemental Figures

Figure 1 – figure supplement 1. Characterization of aMTOCs in AKAP450/CAMSAP2 knockout cells

(A) Immunofluorescence images of centrinone B treated AKAP450 knockout RPE1 cells stained for CAMSAP2 (green) and γ-tubulin (red), transfected either with control (Luciferase) or CAMSAP2 siRNAs. Insets show enlargements of the merged images of the boxed areas.

(B) Quantification of CAMSAP2 depletion and aMTOC formation related to panel A. For normalized CAMSAP2 intensity, n=76 (siLuciferase) and 62 (siCAMSAP2) cells from three independent experiment were analyzed; to calculate the percentage of cells showing dispersed or clustered PCM, n=300 (siLuciferase) and 299 (siCAMSAP2) cells analyzed for each measurement in three independent experiments. The statistical significance was determined by unpaired two-tailed Mann-Whitney test in Prism 9.1 (***p<0.001). Values represent mean±SD.

(C) Immunofluorescence images of AKAP450/CAMSAP2 knockout treated with DMSO or centrinone B and stained for pericentrin and centrin show the main PCM organization types (centrin-negative cells with cylindrical or round PCM cluster, dispersed PCM or no cluster, and centrin-positive centrosomes) in each condition. Enlargements of the merged channels of the boxed areas are shown on the right.

(D) Quantification of the main PCM organization types in centrinone-treated AKAP450 knockout and AKAP450/CAMSAP2 knockout RPE1 cells. Numbers on the histogram show the percentages. 465 (AKAP450 KO) and 495 (AKAP450/CAMSAP2 KO) cells analyzed for each measurement in three independent experiments (n=3). Values represent mean±SD.

(E) Diagram showing the generation of CPAP or PLK4 depleted AKAP450/CAMSAP2 knockout RPE1 cells with different drug treatments. Cells were transfected with siRNAs to depleted CPAP and PLK4 respectively. After 2 days, the transfection was performed again to increase the depletion efficiency. After 2 more days, cells were treated with thymidine to block cell proliferation or with a combination of thymidine and centrinone B, or thymidine, centrinone B and the PLK1 inhibitor BI 2536 for one or three days. Cells were fixed for the first time (Fix1) after a 24 hr drug treatment, and for the second time (Fix2) after a 72 hr drug treatment.

(F) Quantification of the main PCM organization types, as described for panel D, for cells prepared as described in panel E. Numbers on the histogram show the percentages and numbers in brackets show cells analyzed for each measurement in three independent experiments (n=3; 247-612 cells analyzed per condition). Values represent mean±SD.

1333 (G-I) Immunofluorescence images of Fix2 (as described in panel E) showing control (transfected with

1334 siRNA against Luciferase), or depleted of PLK4 or CPAP and treated as indicated. Cells were stained for

1335 centrioles (CEP135, centrin), PCM proteins (PCNT) and microtubules (α-tubulin). Images illustrate the

1336 PCM organization types in each condition. Insets show enlargements of the merged channels for the

1337 boxed areas.

1338

Figure 1 – figure supplement 2. PCM dynamics visualized with GFP-CDK5RAP2

(A) Immunofluorescence images of control and centrinone B treated WT RPE1 cell stably expressing

GFP-CDK5RAP2 (green) stained for α-tubulin (red) and pericentrin (PCNT, blue). Zooms show

enlargements of the boxed regions.

(B) Time-lapse images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells stably

expressing GFP-CDK5RAP2. Microtubules are visualized by treating the cells with 100 nM SiR-tubulin

overnight. PCM labeled with GFP-CDK5RAP2 (green) forms a stable cluster that functions as the MTOC.

Zooms show the enlarged GFP channel of the boxed regions. Time is min:sec.

1347

Figure 2 – figure supplement 1. Characterization of PCM components localizing to aMTOCs in

AKAP450/CAMSAP2 knockout cells

(A) Western blot showing that endogenous NEDD1 and CEP192 are present in centrinone-treated

AKAP450/CAMSAP2 knockout cells.

(B) Western blot results showing the depletion of the indicated PCM proteins.

(C) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells

showing the localization and the effects of depletion of the indicated proteins on the aMTOCs. siRNA

against luciferase was used as a control. Zooms show enlargements of the merged channels of the

boxed areas.

1357

1358 **Figure 2 – figure supplement 2. Generation of the AKAP450/CAMSAP2/p53/pericentrin knockout**

1359 **RPE1 cell line**

1360 (A) Diagram illustrating two sgRNA sites targeting p53 exon 2 and exon 4. The green and red boxes

1361 indicate the position of the PAM sites, and the predicted Cas9 cut sites are indicated by scissors.

(B,C) Genotyping results of the genomic mutation using gel-purified PCR product which covers exon 2 and exon 4 of the p53-encoding gene in AKAP450/CAMSAP2/p53 knockout cell line and a diagram illustrating the induced inversion.

(D) Diagram illustrating the binding sites of p53 antibodies and the position of the mutation induced in p53-encoding gene.

(E,F) Immunofluorescence and Western blot images showing p53 expression in control and AKAP450/CAMSAP2/p53 knockout cells.

(G-H) Genotyping results of the genomic mutation using gel-purified PCR product which covers the two sgRNAs targeting sites within pericentrin-encoding gene in AKAP450/CAMSAP2/p53/pericentrin knockout cell line and a diagram illustrating the induced genomic mutations within exon 5 of the pericentrin-encoding gene. The green and red boxes indicate the position of the PAM sites, and the predicted Cas9 cut sites are indicated by scissors.

(I) Diagram illustrating the binding domain of the pericentrin antibody and the positions of the mutations induced downstream of the Cas9 cutting sites.

(J) Immunofluorescence images confirming the loss of pericentrin in AKAP450/CAMSAP2/p53/pericentrin knockout cells.

(K) Western blot confirming the loss of p53 and pericentrin in AKAP450/CAMSAP2/p53/pericentrin knockout cells.

Figure 2 – figure supplement 3. Characterization of PCM organization in AKAP450/CAMSAP2/p53/ pericentrin knockout cells

Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2/p53 knockout (Ctl) and AKAP450/CAMSAP2/p53/pericentrin knockout (PCNT KO) RPE1 cells showing staining for the indicated proteins. Zooms of the boxed areas show merged channels.

Figure 2 – figure supplement 4. Effects of the depletion or knockout of EB1 and EB3 on aMTOC formation in AKAP450/CAMSAP2 knockout cells

(A) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2 knockout cells stained for EB1 or EB3 and pericentrin showing effects of EB1 or EB3 depletion on aMTOCs.

1391 (B) Western blot confirming the loss of AKAP450 and CAMSAP2 in AKAP450/CAMSAP2/EB1/EB3

1392 mutant cells.

1393 (C) Immunofluorescence images of control or centrinone-treated AKAP450/CASMSAP2/EB1/EB3

1394 mutant RPE1 cells stained for the indicated proteins. Zooms of the boxed areas show merged

1395 channels.

1396

Figure 4 – Figure supplement 1. PCM dynamics during nocodazole treatment in centrinone-treated

AKAP450/CAMSAP2 knockout cells

(A) Time-lapse images illustrating the dynamics of GFP-CDK5RAP2 clusters (green, white arrows) and

microtubules (visualized with an overnight treatment with 100 nM SiR-tubulin, red) during nocodazole

treatment. A single frame of the whole cell is on the bottom left, with the boxed area indicating the

location of the enlarged images. Time is min:sec.

(B) Motor-PAINT images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells before and

after treatment with 10 µM nocodazole for 15 min (the same cells, colorized differently, are displayed

in Figure 4G). Microtubule segments were assigned a color based on their absolute orientation, with

their plus-ends pointing in the directions indicated with the compass. Asterisks indicate approximate

assumed positions of aMTOCs.

1408

Figure 5 – figure supplement 1. PCM dynamics and microtubule regrowth during nocodazole

washout in AKAP450/CAMSAP2 knockout cells

(A) Immunofluorescence images of acentriolar AKAP450/CAMSAP2 knockout RPE1 cells fixed at 30 s

and 10 min after nocodazole washout and stained for the indicated markers. Zooms show the

enlargements of boxed areas. Dashed lines show the cell boundaries.

(B) Summarizing table showing the presence of PCM components in clusters (including centrosome

and aMTOC) before and after nocodazole washout in indicated conditions.

(C) Immunofluorescence images of control acentriolar AKAP450/CAMSAP2 knockout RPE1 cells and

cells lacking the indicated PCM proteins fixed 2 min after nocodazole washout and stained for EB1 (a

marker of growing microtubules, red) and different PCM proteins (γ-tubulin, NIN and DHC, green;

CDK5RAP2 and PCNT, blue). Insets show the enlargements of the boxed areas and zooms show the

radial and non-radial part of growing microtubules. Dashed lines show the cell boundaries.

(D) Quantification of the proportion of non-radial microtubules 2 min after nocodazole washout. The radial and the non-radial microtubule intensities were measured and the non-radial microtubule intensity was normalized by dividing it by the sum of the radial and the non-radial microtubule intensities for each group. n=20-25 cells analyzed for each measurement in three independent experiments. The statistical significance was determined by unpaired two-tailed Mann-Whitney test in Prism 9.1 (***$p < 0.001$). Values represent mean±SD.

(E) Diagram showing microtubule regrowth and PCM assembly in control acentriolar AKAP450/CAMSAP2 knockout RPE1 cells and cells depleted of the indicated PCM proteins at 30 s and 2 min after nocodazole washout.

Figure 5 – figure supplement 2. Pericentrin is required for PCM clustering in acentriolar AKAP450/CAMSAP2 knockout RPE1 cells

Time-lapse images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells stably expressing GFP-CDK5RAP2 (green), which is diffusely distributed and does not form any clear clusters. Microtubule regrowth experiments show random nucleation of microtubules (labeled with 100nM SiR-tubulin overnight) that remain disorganized. Summarizing diagram shows PCM clusters and microtubule network in the indicated conditions.

Figure 6 – figure supplement 1. PCM dynamics and the effects of CAMSAP2 clustering on PCM organization in centrinone-treated RPE1 cells lacking AKAP450

(A) Time-lapse images illustrating the dynamics of PCM clusters (green, white arrows) and microtubules (SiR-tubulin, red) after nocodazole washout in centrinone-treated AKAP450 knockout RPE1 cells stably expressing GFP-CDK5RAP2. A single frame of the whole cell with numbered boxed areas indicating the location of enlarged time-lapse images is shown on the left. White arrows indicate moving PCM clusters after microtubules re-grow. Time is min:sec.

 (B) Time-lapse images of centrinone-treated AKAP450 knockout RPE1 cells stably expressing GFP-CDK5RAP2. GFP-CDK5RAP2 forms small clusters that are dynamic before microtubules (SiR-tubulin, red) are disassembled by nocodazole treatment. Red arrows show that these clusters become immobile when microtubules are completely depolymerized (enlargements of the boxed areas shown in the bottom rows). GFP-CDK5RAP2-positive clusters are sites of microtubule nucleation after

nocodazole washout and become dynamic again once microtubules re-grow. The maximum intensity projections include 100 or 400 frames, 200 ms/frame. Time is min:sec.

(C) Summarizing diagram illustrating microtubule organization and the motility of PCM clusters in acentriolar AKAP450 knockout RPE1 cells.

(D) Movement trajectories of PCM clusters in acentriolar AKAP450 knockout cells before and after 1 hr treatment with 5µM dynapyrazole A. 350 frames with a 2 s interval from the same cell were analyzed. Shaded areas represent the cell body.

(E) Quantification of the length of the trajectories of moving PCM clusters. n=132 (without dynapyrazole A, from 5 cells) and 111 (after 1 hr dynapyrazole A treatment, from 4 cells) trajectories were analyzed for each condition. The statistical significance was determined by unpaired two-tailed Mann-Whitney test in Prism 9.1 (***$p<0.001$). Values represent mean±SD.

(F) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2 knockout RPE1 cells transfected with 2FKBP-mCherry-CAMSAP2 and FRB-TagBFP-GCN4-ppKin14 and stained for the indicated proteins after overnight rapalog treatment. Zooms show the magnification of boxed area.

Figure 6 – figure supplement 2. Pericentrin is required for PCM clustering in acentriolar AKAP450 knockout RPE1 cells

Time-lapse images of centrinone-treated AKAP450 knockout RPE1 cells stably expressing GFP-CDK5RAP2 (green) depleted of pericentrin. GFP-CDK5RAP2 is diffusely distributed and does not form any clear clusters. Microtubule regrowth experiments show random nucleation of microtubules (labeled with 100nM SiR-tubulin overnight) that remain disorganized. Summarizing diagram shows PCM clusters and microtubule network in the indicated conditions.

Figure 7 – figure supplement 1. Inducible CAMSAP2-driven radial microtubule rearrangement in AKAP450/CAMSAP2/p53/pericentrin knockout cell

(A) Time-lapse images of a centrinone-treated AKAP450/CAMSAP2/p53/pericentrin knockout cell transiently expressing 2FKBP-mCherry-CAMSAP2 and FRB-GFP-GCN4-ppKin14 imaged for 10 min (100 frames, 6 s interval) prior to treatment with 100 nM rapalog and ~90 min after. Time is hr: min: sec.

(B) Immunofluorescence images of centrinone-treated AKAP450/CAMSAP2/p53/pericentrin knockout and AKAP450/CAMSAP2/MMG/CDK5RAP2/p53/pericentrin knockout RPE1 cells, stained for the

1481　indicated markers. In the upper row, the cells were neither transfected nor treated with rapalog,

1482　whereas in the other panels, cells were transfected with 2FKBP-mCherry-CAMSAP2 and FRB-HA-

1483　GCN4-ppKin14 and treated with rapalog overnight. Zooms show the magnification of boxed areas.

1484

Figure 7 – figure supplement 2. Generation of the AKAP450/CAMSAP2/CDK5RAP2/MMG/

p53/pericentrin knockout RPE1 cell line

(A) Diagram illustrating two sgRNA sites targeting p53 exon 4. The green and red boxes indicate the

position of the PAM sites, and the predicted Cas9 cut sites are indicated by scissors. Genotyping results

of the genomic mutation using gel-purified PCR product which covers exon 4 of the p53-encoding gene

in AKAP450/CAMSAP2/MMG/CDK5RAP2/p53 knockout cell line are shown below.

(B) Diagram illustrating the binding sites of p53 antibodies and the position of the mutation induced

in p53-encoding gene.

(C)　Immunofluorescence　images　showing　p53　staining　in　control　and

AKAP450/CAMSAP2/MMG/CDK5RAP2/p53 knockout cells.

(D) Diagram illustrating two sgRNA sites targeting pericentrin exon 5. The green and red boxes indicate

the position of the PAM sites, and the predicted Cas9 cut sites are indicated by scissors. Genotyping

results of the genomic mutation using gel-purified PCR product which covers exon 5 of the pericentrin-

encoding gene in AKAP450/CAMSAP2/MMG/CDK5RAP2/p53/pericentrin knockout cell line are shown

below.

(E) Diagram illustrating the binding domain of the pericentrin antibody and the positions of the

mutations induced downstream of the Cas9 cut sites.

(F) Immunofluorescence images showing the confirmation of pericentrin loss in control and

AKAP450/CAMSAP2/MMG/CDK5RAP2/p53/pericentrin knockout cells.

(G)　Western　blot　illustrating　the　p53　and　pericentrin　loss　in　control　and

AKAP450/CAMSAP2/MMG/CDK5RAP2/p53/pericentrin knockout cells.

(H)　Immunofluorescence images of control and centrinone-treated AKAP450/CAMSAP2/

CDK5RAP2/MMG/p53/pericentrin knockout cells staining microtubule (α-tubulin, red) and

centrosome (CEP192, green). Zooms show magnification of the boxed area.

1509

Legends to Supplemental Videos

Video 1. aMTOC disassembly during nocodazole treatment of acentriolar AKAP450/CAMSAP2
knockout cells

PCM dynamics visualized by stable expression of GFP-CDK5RAP2 (green) in centrinone-treated
AKAP450/CAMSAP2 knockout RPE1 cells. Microtubules were labeled overnight with 100 nM SiR-
tubulin (red). The cell was imaged for ~3.5min (100 frames, 2 s interval) prior to the addition of 10 μM
nocodazole. Time is min: sec.

Video 2. Depletion of pericentrin inhibits PCM clustering in acentriolar AKAP450/CAMSAP2
knockout cells

A pericentrin-depleted acentriolar AKAP450/CAMSAP2 knockout cell stably expressing GFP-CDK5RAP2
(green) and labeled overnight with 100 nM SiR-tubulin (red) was imaged for ~4.5 min (140 frames, 2 s
time interval) prior to treatment with 10 μM nocodazole. Nocodazole was washed out at ~20 min
(frame 591), when all microtubules were depolymerized. Time is hr: min: sec.

Video 3. PCM dynamics during nocodazole treatment and washout in acentriolar AKAP450 knockout
cells

An acentriolar AKAP450 knockout RPE1 cell stably expressing GFP-CDK5RAP2 (green) and labeled
overnight with 100 nM SiR-tubulin (red) was imaged for ~7min (200 frames, 2 s interval) prior to
treatment with 10 μM nocodazole. Nocodazole was washed out at ~27 min (frame 801) when all
microtubules were depolymerized. Time is min: sec.

Video 4. PCM dynamics in acentriolar AKAP450 knockout cells are inhibited by dynapyrazole

An acentriolar AKAP450 knockout RPE1 cell stably expressing GFP-CDK5RAP2 (green) and labeled
overnight with 100 nM SiR-tubulin (red) was imaged for ~12 min (350 frames, 2 s interval) prior to the
dynapyrazole treatment, treated with 5 μM Dynapyrazole A for 1h, and then the same cell was imaged
for ~12 min. Time is min: sec.

Video 5. Depletion of pericentrin inhibits PCM clustering in acentriolar AKAP450 knockout cells

A pericentrin-depleted acentriolar AKAP450 knockout cell stably expressing GFP-CDK5RAP2 (green)
and labeled overnight with 100 nM SiR-tubulin (red) was imaged for ~3 min (90 frames, 2 s time
interval) prior to treatment with 10 μM nocodazole. Nocodazole was washed out at ~23 min (frame
701) when all microtubules were depolymerized. Time is hr: min: sec.

Video 6. Inducible CAMSAP2-driven radial microtubule rearrangement in an AKAP450/CAMSAP2/p53/pericentrin knockout cell

An acentriolar AKAP450/CAMSAP2/p53/pericentrin knockout cell transiently expressing 2FKBP-mCherry-CAMSAP2 (red) and FRB-GFP-GCN4-ppKin14 (green) was imaged for 10 min (100 frames, 6 s interval) prior to treatment with 100 nM rapalog. Subsequently, the cell was imaged for ~1 hr and 35 min after rapalog addition. Time is hr: min: sec.

Source data 1

Uncropped Western blots shown in this manuscript

(A,B) Western blots showing that NEDD1 and CEP192 are present in centrinone-treated AKAP450/CAMSAP2 knockout cells shown in Figure 2 – figure supplement 1A.

(C-J) Western blots showing the depletion of indicated proteins in centrinone-treated AKAP450/CAMSAP2 knockout cells shown in Figure 2 – figure supplement 1B.

 (K) Western blot showing that 3 hr treatment with dynaprazole A does not affect the expression of the endogenous dynein heavy chain and the dynactin large subunit p150Glued in centrinone-treated AKAP450/CAMSAP2 knockout cells shown in Figure 4D.

Source data 2

Uncropped Western blots shown in this manuscript

(A) Western blots showing the knockout of p53 from AKAP450/CAMSAP2 knockout cell line shown in Figure 2 – figure supplement 2F.

(B) Western blots showing the knockout of pericentrin from AKAP450/CAMSAP2/p53 knockout cell line shown in Figure 2 – figure supplement 2K.

(C) Western blots showing the knockout of AKAP450 and CAMSAP2 from EB1/EB3 mutant RPE1 cell line shown in Figure 2 – figure supplement 4B.

(D) Western blots showing the knockout of pericentrin in AKAP450/CAMSAP2/CDK5RAP2/MMG/p53/ knockout cell line shown in Figure 7 – figure supplement 2G.

1566   *Key reagent or resource table*

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Antibody	anti-Pericentrin (mouse monoclonal)	Abcam	Abcam Cat# ab28144, RRID:AB_2160664	(1:500) for IF
Antibody	anti-Pericentrin (rabbit polyclonal)	Abcam	Abcam Cat# ab4448, RRID:AB_304461	(1:500) for IF; (1:1000) for WB
Antibody	anti-CDK5RAP2 (rabbit polyclonal)	Bethyl Laboratories	Bethyl Cat# A300-554A, RRID:AB_477974	(1:300) for IF; (1:1000) for WB
Antibody	anti-γ-tubulin (mouse monoclonal)	Sigma-Aldrich	Sigma-Aldrich: T6557; RRID:AB_477584	(1:300) for IF; (1:2000) for WB
Antibody	anti-γ-tubulin (rabbit polyclonal)	Sigma-Aldrich	Sigma-Aldrich:T3559, RRID:AB_477575	(1:300) for IF
Antibody	anti-NEDD1 (mouse monoclonal)	Abnova	Abnova Corporation Cat# H00121441-M05, RRID:AB_534956	(1:300) for IF
Antibody	anti-NEDD1 (rabbit polyclonal)	Rockland	Rockland Cat# 109-401-C38S, RRID:AB_10893219	(1:1000) for WB
Antibody	anti-Ninein (rabbit polyclonal)	BETHYL	Bethyl Cat# A301-504A, RRID:AB_999627	(1:300) for IF; (1:2000) for WB
Antibody	anti-Ninein (mouse monoclonal)	Santa Cruz Biotechnology	Santa Cruz Biotechnology Cat# sc-376420, RRID:AB_11151570	(1:300) for IF
Antibody	anti-Dynein HC (rabbit polyclonal)	Santa Cruz Biotechnology	Santa Cruz Biotechnology Cat# sc-9115, RRID:AB_2093483	(1:300) for IF; (1:500) for WB
Antibody	anti-p150Glued (mouse monoclonal)	BD Biosciences	BD Biosciences Cat# 610473, RRID:AB_397845	(1:100) for IF; (1:500) for WB
Antibody	anti-PCM1 (mouse monoclonal)	Santa Cruz Biotechnology	Santa Cruz Biotechnology Cat# sc-398365, RRID:AB_2827155	(1:300) for IF
Antibody	anti-PCM1 (rabbit polyclonal)	Bethyl Laboratories	Bethyl Cat# A301-150A, RRID:AB_873100	(1:300) for IF
Antibody	anti-AKAP450 (mouse monoclonal)	BD Biosciences	BD Biosciences Cat# 611518, RRID:AB_398978	(1:500) for WB
Antibody	anti-CAMSAP2 (rabbit polyclonal)	Novus	Novus:NBP1-21402; RRID:AB_1659977	(1:200) for IF; (1:1000) for WB
Antibody	anti-p53 (mouse monoclonal)	Santa Cruz Biotechnology	Santa Cruz Biotechnology Cat# sc-126, RRID:AB_628082	(1:300) for IF; (1:1000) for WB
Antibody	anti-p53 (rabbit polyclonal)	BETHYL	Bethyl Cat# A300-248A, RRID:AB_263349	(1:300) for IF
Antibody	anti-EB1 (mouse monoclonal)	BD Biosciences	BD Biosciences:610535; RRID:AB_397892	(1:400) for IF

Antibody	anti-EB3 (rabbit polyclonal)	Martin, et al., 2018;		(1:300) for IF
Antibody	anti-Centrin (mouse monoclonal)	Millipore	Millipore Cat# 04-1624, RRID:AB_10563501	(1:500) for IF
Antibody	anti-CEP120 (rabbit polyclonal)	Thermo Fisher Scientific	Thermo Fisher Scientific Cat# PA5-55985, RRID:AB_2639665	(1:300) for IF
Antibody	anti-CEP135 (rabbit polyclonal)	Sigma-Aldrich	Sigma-Aldrich:SAB4503685; RRID:AB_10746232	(1:300) for IF
Antibody	anti-CEP152 (rabbit polyclonal)	Abcam	Abcam, Cat # ab183911	(1:300) for IF; (1:1000) for WB
Antibody	anti-CEP170 (mouse monoclonal)	Thermo Fisher Scientific	Thermo Fisher Scientific Cat# 41-3200, RRID:AB_2533502	(1:200) for IF
Antibody	anti-CEP192 (rabbit polyclonal)	Bethyl Laboratories	Bethyl Cat# A302-324A, RRID:AB_1850234	(1:300) for IF; (1:1000) for WB
Antibody	anti-GM130 (mouse monoclonal)	BD Biosciences	BD Biosciences:610823; RRID:AB_398142	(1:300) for IF; (1:2000) for WB
Antibody	anti-α-tubulin YL1/2 (rat monoclonal)	Pierce	Pierce: MA1-80017; RRID:AB_2210201	(1:300) for IF
Antibody	anti-α-tubulin (mouse monoclonal)	Sigma-Aldrich	Sigma-Aldrich:T5168; RRID:AB_477579	(1:400) for IF
Antibody	anti-α-tubulin (rabbit monoclonal antibody)	Abcam	Abcam Cat# ab52866, RRID:AB_869989	(1:800) for IF
Antibody	Anti-β-tubulin (mouse monoclonal)	Sigma-Aldrich	Sigma-Aldrich Cat# T8660, RRID:AB_477590	(1:2000) for WB
Antibody	anti-CLASP1 (rabbit polyclonal)	(Akhmanova et al., 2001)		(1:400) for IF
Antibody	anti-CLASP2 (rabbit polyclonal)	(Akhmanova et al., 2001)		(1:400) for IF
Antibody	anti-CLIP-115 #2238 (rabbit polyclonal)	(Akhmanova et al., 2001)		(1:300) for IF
Antibody	anti-CLIP-170 #2360 (rabbit polyclonal)	(Akhmanova et al., 2001)		(1:300) for IF
Antibody	anti-ch-TOG (rabbit polyclonal)	(Charrasse et al., 1998)	Dr. Lynne Cassimeris (Lehigh University, USA)	(1:200) for IF
Antibody	anti-CPAP (rabbit polyclonal)	(Kohlmaier et al., 2009)	Dr. Pierre Gönczy (EPFL, Switzerland)	(1:200) for IF
Antibody	anti-CP110 (rabbit monoclonal)	Proteintech	Proteintech Cat# 12780-1-AP, RRID:AB_10638480	(1:300) for IF
Antibody	anti-KIF1C (rabbit polyclonal)	Cytoskeleton	Cytoskeleton Cat# AKIN11-A, RRID:AB_10708792	(1:300) for IF
Antibody	anti-KIF2A (rabbit polyclonal)	(Ganem and Compton, 2004)	Dr. Duane Compton (Geisel School of Medicine at Dartmouth, USA)	(1:300) for IF

Antibody	anti-HAUS2 (rabbit polyclonal)	(Lawo et al., 2009)	Dr. Laurence Pelletier (Lunenfeld-Tanenbaum Research Institute, Canada)	(1:200) for IF
Antibody	anti-BICD2 (rabbit polyclonal)	(Hoogenraad et al., 2003)		(1:2500) for WB
Antibody	anti-Actin (mouse monoclonal)	Millipore	Millipore Cat# MAB1501, RRID:AB_2223041	(1:4000) for WB
Antibody	anti-Ku80 (mouse monoclonal)	BD Biosciences	BD Biosciences Cat# 611360, RRID:AB_398882	(1:2000) for WB
Antibody	anti-LaminA/C (mouse monoclonal)	BD Biosciences	BD Biosciences Cat# 612162, RRID:AB_399533	(1:400) for IF
Antibody	anti-Cytochrome C (mouse monoclonal)	BD Biosciences	BD Biosciences Cat# 556432, RRID:AB_396416	(1:300) for IF
Antibody	anti-Calnexin (rabbit polyclonal)	Abcam	Abcam Cat# ab22595, RRID:AB_2069006	(1:300) for IF
Antibody	Anti-Lamtor4 (rabbit monoclonal)	Cell Signaling (CST)/Bioke	Cell Signaling Technology Cat# 12284, RRID:AB_2797870	(1:800) for IF
	Anti-Tom20 (mouse monoclonal)	BD Biosciences	BD Biosciences Cat# 612278, RRID:AB_399595	(1:200) for IF
Antibody	IRDye 800CW/680LT secondaries	Li-Cor Biosciences	LI-COR Biosciences Cat# 926-32219, RRID:AB_1850025' LI-COR Biosciences Cat# 926-68020, RRID:AB_10706161; LI-COR Biosciences Cat# 926-32211, RRID:AB_621843; LI-COR Biosciences Cat# 926-68021, RRID:AB_10706309	(1:5000) for WB
Antibody	Alexa Fluor 405–, 488–, and 594–secondaries	Molecular Probes/ Thermo Fisher Scientific	Molecular Probes Cat# A-11007, RRID:AB_141374; Cat# A-11034, RRID:AB_2576217; Cat# A32723, RRID:AB_2633275; Cat# A-31553, RRID:AB_221604; Cat# A-11029, RRID:AB_138404; Cat# A-11032, RRID:AB_2534091; Cat# A-11006, RRID:AB_141373; Thermo Fisher Scientific Cat# A-11012, RRID:AB_2534079	(1:500) for IF
Sequence-based reagent	siRNA against PCNT #1	(Gavilan et al., 2018)	5'-AAAAGCUCUGAUUUAUCAAAAGAAG-3'	
Sequence-based reagent	siRNA against PCNT #2	(Gavilan et al., 2018)	5'-UGAUUGGACGUCAUCCAAUGAGAAA-3'	

Sequence-based reagent	siRNA against PCNT #3	(Tibelius et al., 2009)	5'-GCAGCUGAGCUGAAGGAGA-3'	
Sequence-based reagent	siRNA against CDK5RAP2	(Fong et al., 2008)	5'-UGGAAGAUCUCCUAACUAA-3'	
Sequence-based reagent	siRNA against γ-tubulin #1	(Luders et al., 2006)	5'-GGAGGACAUGUUCAAGGAA-3'	
Sequence-based reagent	siRNA against γ-tubulin #2	(Vinopal et al., 2012)	5'-CGCAUCUCUUUCUCAUAU-3'	
Sequence-based reagent	siRNA against Ninein	(Goldspink et al., 2017)	5'-CGGUACAAUGAGUGUAGAAUU-3'	
Sequence-based reagent	siRNA against PCM1	(Wang et al., 2013)	5'-UCAGCUUCGUGAUUCUCAG-3'	
Sequence-based reagent	siRNA against CEP152	(Cizmecioglu et al., 2010; Komarova et al., 2005)	5'-GCGGAUCCAACUGGAAAUCUA-3'	
Sequence-based reagent	siRNA against CEP120	(Ganem et al., 2005; Lin et al., 2013)	5'-AAUAUAUCUUCUUGCAUCUCCUUCC-3'	
Sequence-based reagent	siRNA against CEP192	(Sonnen et al., 2013)	5'-CAGAGGAAUCAAUAAUAAA -3'	
Sequence-based reagent	siRNA against NEDD1 #1	(Luders et al., 2006)	5'-GCAGACAUGUGUCAAUUUA-3'	
Sequence-based reagent	siRNA against NEDD1 #2	(Haren et al., 2006)	5'-GGGCAAAAGCAGACAUGUG-3'	
Sequence-based reagent	siRNA against DHC #1	(Splinter et al., 2010)	5'-CGUACUCCCGUGAUUGAUG-3'	
Sequence-based reagent	siRNA against DHC #2	(Splinter et al., 2010)	5'-GCCAAAAGUUACAGACUUU-3'	
Sequence-based reagent	siRNA against CAMSAP2	(Jiang et al., 2014)	5'-GUACUGGAUAAAUAAGGUA-3'	
Sequence-based reagent	siRNA against CEP170	(Stolz et al., 2015)	5'-GAAGGAAUCCUCCAAGUCA-3'	
Sequence-based reagent	siRNA against CPAP	(Tang et al., 2009)	5'-AGAAUUAGCUCGAAUAGAA-3'	
Sequence-based reagent	siRNA against CLIP170 #1	(Lansbergen et al., 2004; Mimori-Kiyosue et al., 2005)	5'-GGAGAAGCAGCAGCACAUU-3'	
Sequence-based reagent	siRNA against CLIP170 #2	(Lansbergen et al., 2004; Mimori-Kiyosue et al., 2005)	5'-UGAAGAUGUCAGGAGAUAA-3'	

Sequence-based reagent	siRNA against CLIP115 #1	(Lansbergen et al., 2004)	5'-GGCACAGCAUGAGCAGUAU-3'	
Sequence-based reagent	siRNA against CLIP115 #2	(Lansbergen et al., 2004)	5'-CUGGAAAUCCAAGCUGGAC-3'	
Sequence-based reagent	siRNA against ch-TOG:	(Cassimeris and Morabito, 2004; Lansbergen et al., 2004)	5'-GAGCCCAGAGUGGUCCAAA-3'	
Sequence-based reagent	siRNA against EB1	(Grigoriev et al., 2008; Lansbergen et al., 2004)	5'-AUUCCAAGCUAAGCUAGAA-3'	
Sequence-based reagent	siRNA against EB3	(Cassimeris and Morabito, 2004; Komarova et al., 2005)	5'-CUAUGAUGGAAAGGAUUAC-3'	
Sequence-based reagent	siRNA against KIF2A	(Ganem et al., 2005; Grigoriev et al., 2008)	5'-GGCAAAGAGAUUGACCUGG-3'	
Sequence-based reagent	siRNA against CP110	(Cizmecioglu et al., 2010; Spektor et al., 2007)	5'-AAGCAGCAUGAGUAUGCCAGU-3'	
Sequence-based reagent	siRNA against Luciferase	(Lansbergen et al., 2004; Lin et al., 2013)	5'-CGUACGCGGAAUACUUCGA-3'	
Sequence-based reagent	sgRNA target CAMSAP2	(Lansbergen et al., 2004; Wu et al., 2016)	5'-gCATGATCGATACCCTCATGA-3	
Sequence-based reagent	sgRNA target p53 e2 #1	This study	5'-gCGTCGAGCCCCCTCTGAGTC-3';	
Sequence-based reagent	sgRNA target p53 e4 #2	This study	5'-gCCATTGTTCAATATCGTCCG-3';	
Sequence-based reagent	sgRNA target p53 e4 #3	This study	5'-gTCCATTGCTTGGGACGGCAA-3';	
Sequence-based reagent	sgRNA target PCNT e5-1 #1	This study	5'-gAGACGGCATTGACGGAGCTG-3';	
Sequence-based reagent	sgRNA target PCNT e5-2 #2	This study	5'-GCTCAACAGCCGGCGTGCCC-3';	

Sequence-based reagent	p53 KO sequencing primer F	This study	5′-TCAGACACTGGCATGGTGTT-3′;	
Sequence-based reagent	p53 KO sequencing primer R	This study	5′-AGAAATGCAGGGGGATACGG-3′;	
Sequence-based reagent	PCNT KO sequencing primer F	This study	5′-ATACAGCGAGGGAATTCGGG-3′;	
Sequence-based reagent	PCNT KO sequencing primer R	This study	5′-TAGAATGCCCACACCGAGC-3′;	
Chemical compound	Centrinone B	Tocris Bioscience	Tocris Bioscience Cat # 5690	125nM
Chemical compound	Nocodazole	Sigma-Aldrich	Sigma-Aldrich, Cat # M1404-10MG	10µM
Chemical compound	Rapalog (A/C Heterodimerizer)	Takara	Takara, Cat # 635056	50nM (fixation), 100nM (live imaging).
Chemical compound	Dynapyrazole A	Sigma-Aldrich	Sigma-Aldrich, Cat # SML2127-25MG	5µM
Chemical compound	BI2536	Selleckchem	Selleckchem, Cat # S1109	500nM
Chemical compound	SiR-tubulin	Tebu-bio	Tebu-bio, Cat # SC002	100nM
Software, algorithm	ImageJ radiality plugin	https://github.com/ekatrukha/radialitymap	Katrukha, 2017. radialitymap. Github. https://github.com/ekatrukha/radialitymap cf1e78f	
Recombinant DNA reagent	pLVX-IRES-puro (plasmid)	Clontech		
Recombinant DNA reagent	pB80-FRB-TagBFP-GCN4-ppKin14 (plasmid)	This work		
Recombinant DNA reagent	pB80-FRB-GFP-GCN4-ppKin14 (plasmid)	This work		
Recombinant DNA reagent	pB80-FRB-HA-GCN4-ppKin14 (plasmid)	This work		
Recombinant DNA reagent	2FKBP-mCherry-CAMSAP2 (plasmid)	This work		

Recombinant DNA reagent	GFP-PCNT (plasmid)	This work	
Recombinant DNA reagent	GST-DmKHC(1-421)-mNeonGreen (plasmid)	This work	
Cell line (Homo sapiens)	hTERT-RPE-1	ATCC	CRL-4000
Cell line (Homo sapiens)	hTERT-RPE-1 AKAP450 knockout	(Wu et al., 2016)	
Cell line (Homo sapiens)	hTERT-RPE-1 AKAP450/CAMSAP2 knockout	(Wu et al., 2016)	
Cell line (Homo sapiens)	hTERT-RPE-1 AKAP450/CAMSAP2/p53 knockout	This work	
Cell line (Homo sapiens)	hTERT-RPE-1 AKAP450/CAMSAP2/p53/Pericentrin knockout	This work	
Cell line (Homo sapiens)	hTERT-RPE-1 AKAP450/CAMSAP2/EB1/EB3 mutant	This work	
Cell line (Homo sapiens)	hTERT-RPE-1 AKAP450/CAMSAP2/CDK5RAP2/MMG/p53/Pericentrin knockout	This work	
Cell line (Homo sapiens)	HEK 293T	ATCC	CRL-11268

1567

Figure 1

Figure 2

J — AKAP450/CAMSAP2 KO

		Present in aMTOC	Loss affects aMTOC
γ-TuRC binding	PCNT	+	+
	CDK5RAP2	+	+
	NEDD1	-	-
	γ-tubulin	+	+
	HAUS2	+	NT
Pericentriolar matrix	CEP170	+	NT
	CEP152	-	-
	Ninein	+	+
	CEP192	-	-
Centriole	CPAP	+	-
	Centrin	-	NT
	CP110	+	-
	CEP135	-	NT
	CEP120	+	-
Dynein/ Dynactin	DHC	+	+
	p150Glued	+	NT
Microtubule associated	CLASP1	+	NT
	CLASP2	+	NT
	CLIP115	+	-
	CLIP170	+	-
	chTOG	+	-
	KIF2A	+	-
	KIF1C	+	NT
	EB1	-	-
	EB3	-	-
Centriolar satelites	PCM1	+	-

NT=not tested

Figure 3

Figure 4

Figure 5

A AKAP450/CAMSAP2 KO + CentB, Noco washout

B AKAP450/CAMSAP2 KO + CentB, Noco washout 30 s

C Microtubule regrowth Noco washout 30 s

D AKAP450/CAMSAP2 KO stable GFP-CDK5RAP2 + CentB

E AKAP450/CAMSAP2 KO + CentB, Noco washout

F PCM clustering

G AKAP450/CAMSAP2 KO +CentB +Noco

Figure 6

Figure 7

Figure 1 - figure supplement 1

Figure 1 - figure supplement 2

A WT stable GFP-CDK5RAP2

α-tubulin GFP-CDK5RAP2 PCNT Merge

-CentB

+CentB

5µm 5µm

B AKAP450/CAMSAP2 KO stable GFP-CDK5RAP2 + CentB

GFP SiR-tub

00:00 00:40 01:10 01:50 02:30 03:10 03:40 04:20 5µm

GFP

5µm

Figure 2- figure supplement 1

Figure 2 - figure supplement 2

A p53 sgRNA targets AKAP450/CAMSAP2 KO

B AKAP450/CAMSAP2/p53 KO

C AKAP450/CAMSAP2/p53 KO

D Mouse anti p53 (N) (target 11-25aa), Rabbit anti p53(C) (target 325-375aa)

E p53 (N), p53(C), DAPI, Merge

F

G PCNT sgRNA targets AKAP450/CAMSAP2/p53 KO

H AKAP450/CAMSAP2/p53 KO, AKAP450/CAMSAP2/p53/PCNT KO

I Rabbit anti PCNT (target 100-600aa)

J AKAP450/CAMSAP2/p53 KO, AKAP450/CAMSAP2/p53/PCNT KO

K

Figure 2 - figure supplement 3

Ctl=AKAP450/CAMSAP2/p53 KO + CentB

PCNT KO=AKAP450/CAMSAP2/p53/PCNT KO + CentB

Figure 2 - figure supplement 4

Figure 4 - figure supplement 1

A

AKAP450/CAMSAP2 KO stable GFP-CDK5RAP2 + CentB + Noco

B

Figure 5 - figure supplement 1

B Presence of PCM proteins in clusters before/after Noco washout

CentB	-	+	+	+	+	+
Nocodazole	-	-	+	+	+	+
Washout			30 s	2 min	5 min	10 min
PCNT	+	+	+	+	+	+
CDK5RAP2	+	+	+	+	+	+
γ-tubulin	+	+	+	+	+	+
NEDD1	+	-	-	-	-	-
Ninein	+	+	-	+	+	+
CEP192	+	-	-	-	-	-
PCM1	+	+	+	+	+	+

A AKAP450/CAMSAP2 KO + CentB, Noco washout

C AKAP450/CAMSAP2 KO + CentB, Noco washout 2 min

D Nocodazole washout 2 min non-radial part (%)

E AKAP450/CAMSAP2 KO CentB

Figure 5 - figure supplement 2

Figure 6 - figure supplement 1

Figure 6 - figure supplement 2

Figure 7 - figure supplement 1

A AKAP450/CAMSAP2/p53/PCNT KO, +CentB

Rapalog

FKBP-CAMSAP2 · FRB-ppKin14 · Merge

00:00 · 10:00 · 13:00 · 17:24 · 27:48 · 50:18 · 01:39:06

10µm

B AKAP450/CAMSAP2/p53/PCNT KO, +CentB

Untransfected, -Rapa

α-tubulin · Calnexin · Merge · Merge · α-tubulin · Calnexin

+Rapa

α-tubulin · Calnexin · FKBP-CAMSAP2 · Merge · Merge · FKBP-CAMSAP2 · Calnexin

AKAP450/CAMSAP2/MMG/CDK5RAP2/p53/PCNT KO, +CentB

α-tubulin · Calnexin · FKBP-CAMSAP2 · Merge · Merge · FKBP-CAMSAP2 · Calnexin

Ring-like

Bundled

+Rapa

α-tubulin · Tom20 · FKBP-CAMSAP2 · Merge · Merge · FKBP-CAMSAP2 · Tom20

Ring-like

Bundled

5µm · 5µm

Figure 7- figure supplement 2

A AKAP450/CAMSAP2/MMG/CDK5RAP2 KO

p53 sgRNA targets

sg 1   sg 2

exon 4

PAM1   PAM2

...CCCATCTACAGTCC**CCC**TTGCCGTCCCAAGCAATGGATGATTTGATGCTGT**CCC**CGGACGATATTGAACAATGGTTCACTGAAGA...

37 bp

allele 1+2

TGCTCTTTTCACCCATCTACAGTCC **CCC** TTG ACGATATTGAACAATGGTTCACTGAAGACCC

B Mouse anti p53 (N)   Rabbit anti p53(C)
(target 11-25aa)   (target 325-375aa)

p53 protein (393aa)

sg1 sg2

* =mutated from 36th aa
* =1st stop codon at 110th aa
— =sg1 covers the 35-41 aa
— =sg2 covers the 47-53 aa

C

	p53 (N)	p53(C)	DAPI	Merge

AKAP450/CAMSAP2/MMG/CDK5RAP2 KO

AKAP450/CAMSAP2/MMG/CDK5RAP2/p53 KO

10μm

D AKAP450/CAMSAP2/MMG/CDK5RAP2/p53 KO

PCNT sgRNA targets

sg e5-1   sg e5-2

exon 5

PAM1   PAM2

...GGAGAAGGAGACGGCATTGACGGAGCTG**CGG**GAGATGCTCAACAGCCGGCGTGCCC**AGG**AGCTGGCCCTGCTACA...

28 bp

AAGGAGAAGGAGACGGCATTGACGGAGCCCGG **AGG** CAACTGGCCCTGCTACCGACC

28 bp deletion CCC **AGG** AGCTGGCCCTGCTACAGAGCAGGCA

E

Rabbit anti PCNT
(target 100-600aa)

PCNT protein(3336aa)

* =mutated from 280th aa
* =1st stop codon at 319th aa

F

	NIN	PCNT	Merge

AKAP450/CAMSAP2/CDK5RAP2/MMG/p53 KO

AKAP450/CAMSAP2/CDK5RAP2/MMG/PCNT KO

10μm

G

kDa

AKAP450/CAMSAP2 KO

AKAP450/CAMSAP2/p53/PCNT KO

AKAP450/CAMSAP2/MMG/CDK5RAP2/p53/PCNT KO

PCNT

250-

p53

50-

Ku80

100-

H AKAP450/CAMSAP2/CDK5RAP2/MMG/p53/PCNT KO

	α-tubulin	CEP192	Merge	CEP192	α-tubulin

-CentB

+CentB

5μm   5μm

Source data 1
Uncropped Western blots shown in this manuscript

Source data 2
Uncropped Western blots shown in this manuscript