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Report

Selective Chemical Crosslinking Reveals a Cep57-Cep63-Cep152 Centrosomal Complex

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Summary

The centrosome functions as the main microtubule-organizing center of animal cells and is crucial for several fundamental cellular processes [1]. Abnormalities in centrosome number and composition correlate with tumor progression [2, 3] and other diseases [4–6]. Although proteomic studies have identified many centrosomal proteins, their interactions are incompletely characterized [7, 8]. The lack of information on the precise localization and interaction partners for many centrosomal proteins precludes comprehensive understanding of centrosome biology. Here, we utilize a combination of selective chemical crosslinking and super-resolution microscopy to reveal novel functional interactions among a set of 31 centrosomal proteins. We reveal that Cep57, Cep63, and Cep152 are parts of a ring-like complex localizing around the proximal end of centrioles. Furthermore, we identify that STIL, together with HsSAS-6, resides at the proximal end of the procentriole, where the cartwheel is located. Our studies also reveal that the known interactors Cep152 and Plk4 reside in two separable structures, suggesting that the kinase Plk4 contacts its substrate Cep152 only transiently, at the centrosome or within the cytoplasm. Our findings provide novel insights into protein interactions critical for centrosome biology and establish a toolbox for future studies of centrosomal proteins.

Results and Discussion

The labeling of proteins in cells with synthetic probes is a powerful approach to investigate protein function and localization, which we decided to exploit to study centrosomal proteins. We expressed 31 confirmed or predicted centrosomal proteins as fusions of self-labeling SNAP-tag [9] and CLIP-tag [10] in U2OS cells (see Table S1 available online). Such fusion proteins can be specifically labeled with a large variety of chemical probes suitable for interrogating different aspects of protein function [11]. For an initial characterization, we adapted a triple-labeling procedure [12] for the localization of the 29 SNAP-tag fusions (excluding α- and β-tubulins) relative to C-Nap1 and centrin, two proteins that mark the base of the centriole and the distal ends of both centriole and procentriole, respectively [13, 14]. As shown in Figure S1, we demonstrated that SNAP fusion proteins usually (i.e., in 17 of 23 cases) localize like the corresponding endogenous proteins when this information is known. Importantly, our analysis also uncovered the precise localization on centrosomes of six proteins for which this information was previously not available (see Figure S1). For six other proteins, centrosomal localization could not be detected. In some of these cases, this is likely due to the fact that aggregates precluding detection of centrosomal signal were formed upon overexpression (data not shown), whereas in other cases the presence of the tag may have interfered with localization. Overall, this initial analysis enabled us to localize 23 of 29 fusion proteins with precision at centrosomes.

Next, we set out to systematically identify interactions among the 31 proteins through selective covalent crosslinking (S-CROSS) between SNAP- and CLIP-tagged fusions [10], followed by SDS gel electrophoresis and in-gel fluorescence scanning. S-CROSS utilizes a bifunctional crosslinker that contains the substrates of both tags linked by a chemical fluorophore (Figure 1B); crosslinking efficiency depends critically on the proximity of the two proteins (Figure 1C). The sensitivity of S-CROSS in detecting protein-protein interactions is comparable to affinity purification [15], but the experimental simplicity of the former makes it more suitable for medium-throughput screens. We expressed the 31 centrosomal SNAP-tag and CLIP-tag fusion proteins in a pairwise fashion, as well as SNAP-tag fusions of GFP and mCherry as negative controls, and subjected the resulting 527 combinations to S-CROSS. This allowed us to identify 17 protein-protein interactions (Table S2). Seven of these interactions have been reported previously, testifying to the fact that the S-CROSS methodology can recognize bona fide associations (Table S2). Furthermore, we identified ten novel potential interactions (Table S2). By expressing the same protein as separate CLIP and SNAP-tag fusions, we also identified 20 homotypic interactions (Table S3). Besides previously reported instances of homotypic interactions, including those for HsSAS-6, CPAP, and Plk4 [16–18], our analysis unraveled many hitherto unknown cases of oligomerization. We conclude that a significant fraction of centrosomal proteins (i.e., 20 of 31 tested here) have a propensity to oligomerize, possibly due to the high abundance among centrosomal proteins of coiled-coil motifs that can mediate homotypic interactions [19].

We focused further analysis on the proteins with the three highest-scoring heterotypic interactions: the novel interaction partners Cep63 and Cep57, the recently documented interacting partners CPAP and STIL [20], and Cep152 and Plk4 [21–23]. With the exception of Cep57 (also known as translokin), all of these proteins have been implicated in procentriole formation, a process that is crucial for determining centrosome number. CPAP, STIL, Cep152, and Plk4 are essential for procentriole formation [1, 24], whereas Cep63 appears to contribute to this process by ensuring the centrosomal targeting of Cep152 [25]. In turn, Cep152 is thought to act as a scaffold that binds CPAP and Plk4 and is also phosphorylated by Plk4 [21–23]. Interestingly, mutations in Cep63, Cep152, CPAP, or STIL can result in primary microcephaly (MCPH), and mutations in Cep57 can result in mosaic

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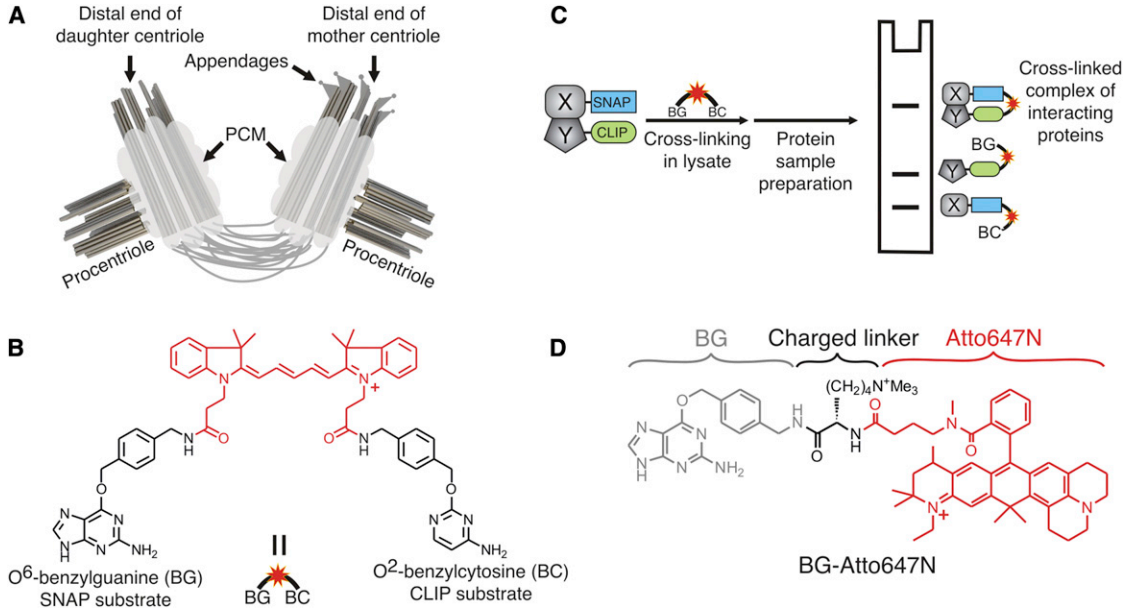

Figure 1. Chemical Tools for Centrosome Characterization

(A) Centrosome organization in the S or G2 cell-cycle phase.

(B) Structure of crosslinker used for selective covalent crosslinking (S-CROSS).

(C) Outline of S-CROSS. Protein complexes of SNAP and CLIP fusions are crosslinked in cell lysates, and the trapped complexes are detected and quantified after SDS gel electrophoresis by in-gel fluorescence scanning.

(D) Structure of SNAP-tag substrate used for stimulated emission depletion (STED) microscopy.

See also Figures S1 and S2 and Tables S1–S3.

variegated aneuploidy (MVA), emphasizing the medical relevance of this set of proteins [4, 6, 25].

We set out to further investigate the interaction between Cep57, Cep63, and Cep152. First, we verified the direct interaction of Cep57 and Cep63 by performing the crosslinking experiments with purified components (Figure S2A). We then conducted small interfering RNA (siRNA) experiments to investigate the functional significance of these interactions, using two different siRNAs to deplete each component. As a control, we first investigated whether depletion of Cep57, Cep63, and Cep152 had an impact on centrosomal γ-tubulin and centrin; with the exception of one siRNA for Cep57 that appeared to affect localization of γ-tubulin for reasons that remain to be determined, all other siRNAs did not alter centrosomal γ-tubulin or centrin distribution (Figures 2A and 2B). Importantly, quantification of the Cep57, Cep63, and Cep152 centrosomal signals in the various siRNA conditions indicated that Cep57 promotes, but is not essential for, Cep63 and Cep152 centrosomal localization, whereas the latter two proteins seem essential for each other's presence at centrosomes (Figures 2A and 2B).

Next, we investigated whether overexpression of either of the aforementioned proteins resulted in increased centrosomal localization of the others. Cells overexpressing SNAP-Cep57, SNAP-Cep63, or SNAP-Cep152 were labeled with a fluorescent SNAP-tag substrate; the presence of the protein of interest at the centrosome was subsequently quantified through immunofluorescence analysis using a different fluorophore, and the resulting centrosomal signal intensities were then subjected to correlation analysis. We found that overexpression of SNAP-Cep57 resulted in increased centrosomal localization of Cep57, and likewise that overexpression of SNAP-Cep63 or SNAP-Cep152 promoted the presence of Cep63 and Cep152, respectively (Figure 2C). Importantly, we found also that overexpression of SNAP-Cep63 resulted in further enrichment of Cep152 at centrosomes. Lower correlations were observed for SNAP-Cep63 and Cep57. Together, our siRNA and overexpression experiments suggest that Cep57, Cep63, and Cep152 are parts of a centrosomal protein complex, with Cep63 playing a critical role in recruiting Cep152.

To further characterize the Cep57-Cep63-Cep152 complex, we analyzed colocalizations among these proteins. Additionally, we included Plk4, STIL, and CPAP in this analysis. In each case, we combined chemical labeling of one SNAP-tag fusion with antibody labeling of the potential interaction partner. Using confocal microscopy, we found that SNAP-Cep63 and Cep57 colocalize to the proximal end of the centriole (Figure 3A), as do SNAP-Cep152 and Cep57 (Figure S3C). By contrast, we found that SNAP-Cep152 and Plk4 reside in two separable foci at the proximal end of the centriole (Figure 3A). As reported previously [26], a second population of Plk4 could be identified near the proximal end of the centriole (Figure 3A). The apparent lack of colocalization of Plk4 and Cep152 suggests that the kinase Plk4 contacts its substrate Cep152 only transiently on the centrosome or within the cytosol. SNAP-STIL and CPAP colocalized to the proximal end of the procentriole (Figure S3C), although CPAP also localized to the proximal end of the centriole [20, 27]. For the latter experiment, we verified that SNAP-STIL colocalized with the endogenous protein (Figure S3B) and was able to rescue the phenotype induced by siRNA-mediated depletion of endogenous STIL (Figure S2B–S2D).

Because the dimensions of centrioles are close to the resolution limit of optical microscopy, we turned to stimulated emission depletion (STED) superresolution microscopy (Figure S3A) [28] to gain further insights into the localization of

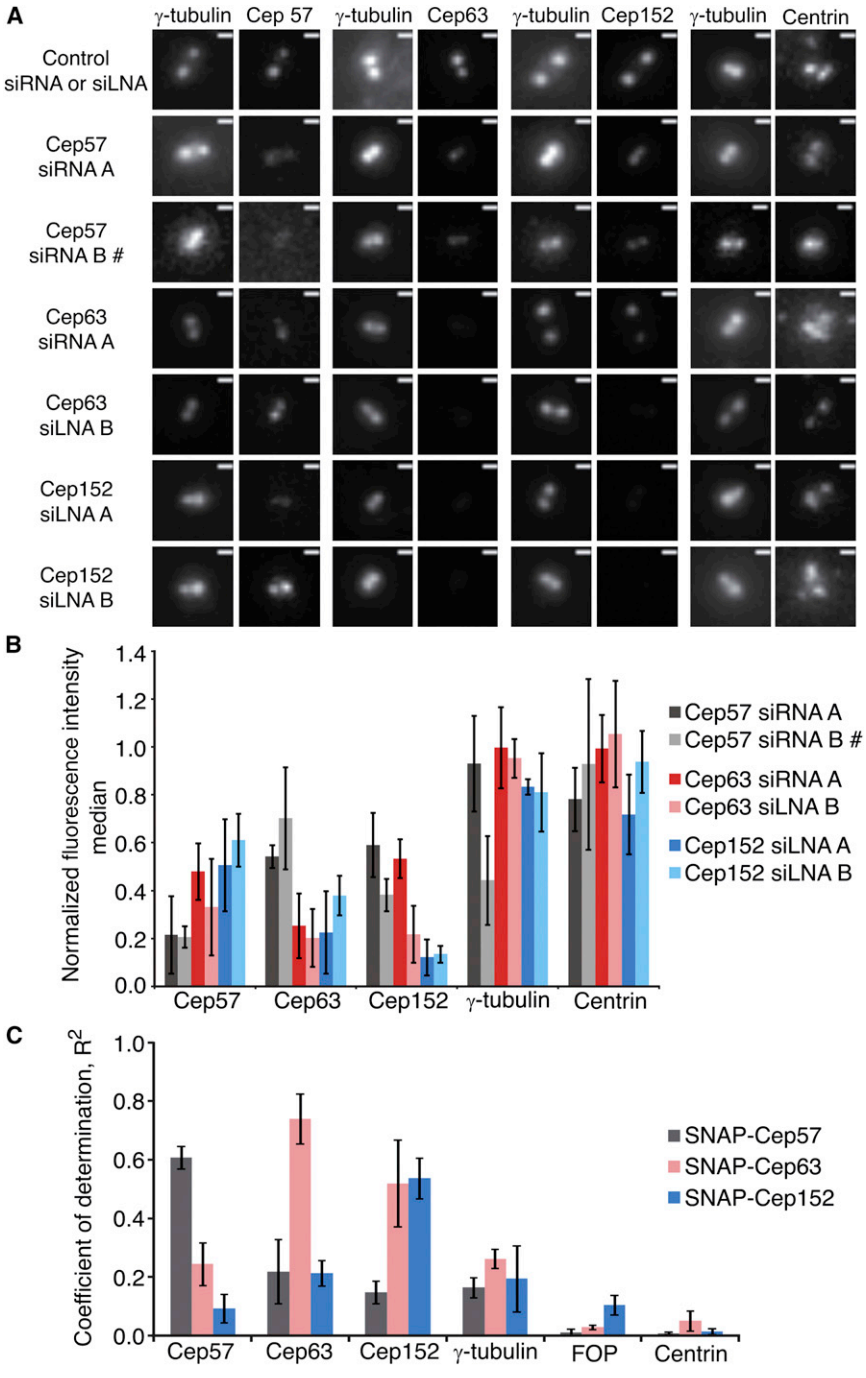

Figure 2. Codependency of Centrosomal Localization of Cep57, Cep63, and Cep152

(A) Representative images of indicated centrosomal proteins in siRNA-treated U2OS cells. Scale bars represent 1 μm.

(B) Medians of fluorescence signal distributions of centrosomal proteins in siRNA-treated U2OS cells. For each protein, the median in the siRNA-treated condition is normalized to that of control siRNA or siLNA (locked nucleic acid-based siRNA; Silencer Select siRNA, Life Technologies). γ-tubulin was used to locate the centrosome. Each column represents mean of duplicate or triplicate experiments with SD error bars. Cells were analyzed after 72 hr, with the exception of cells treated with Cep57 siRNA B (marked #), which were analyzed after 48 hr due to cell death at later time points.

(C) Coefficients of determination reflecting correlation between the measured fluorescence signals of the overexpressed SNAP-tagged protein and the indicated endogenous centrosomal protein. FOP is a negative control that localizes to a different part of the centriole. Centrin was used to locate the centrosome. Each column represents mean of duplicate or triplicate experiments with SD error bars. In (B) and (C), signal intensities were quantified using a CellProfiler pipeline, which automatically identifies centrosomes using γ-tubulin (B) or centrin (C) staining and measures the mean intensity corrected for the background in that region of the other channel.

See also Tables S4 and S5.

the proteins at the heart of our study. For these experiments, we developed a SNAP-tag substrate that permits the specific labeling of SNAP fusion in fixed cells with Atto647N, a fluorophore ideally suited for STED (Figure 1D) [29]. The design of a permanently charged linker that connects BG to Atto647N was crucial in this case, because it prevents unspecific binding of the hydrophobic dye to cellular structures. STED imaging of Cep57, SNAP-Cep63, SNAP-Cep152, and Plk4 each revealed ring-like structures of 200–250 nm in diameter (Figure 3B). This corresponds to the outer diameter of centrioles, suggesting that these proteins reside next to centriolar microtubules. Cep63 and Cep152 have also been shown to associate and form a ring-like structure using structured illumination microscopy [25, 30]. Using two-color STED, we confirmed the colocalization of Cep57 with Cep63 and Cep152, whereas Plk4 and Cep152 were found to localize separately (Figures 3C and S3C). Together with our S-CROSS experiments, these findings indicate that a complex containing Cep57, Cep63, and Cep152 resides near the proximal end of the centriole. STED imaging further revealed that the SNAP-STIL signal is a small disc ~160 nm in diameter (Figures 3B and S3C), suggesting that STIL localizes to the cartwheel within the proximal end of the procentriole [1, 20, 31, 32]. A well-known cartwheel component is HsSAS-6 [33], and we found similarly by STED microscopy that the HsSAS-6 signal is a small disk (Figures S3B and S3C). To test whether STIL and HsSAS-6 reside in the exact same location, we conducted two-color STED of STIL and HsSAS-6, which revealed a high degree of colocalization (Figure S3B and S3C). Two-color STED of SNAP-STIL and CPAP indicated that STIL is encompassed by a broader CPAP signal (Figure 3C). Together, these findings support the hypothesis that STIL is a cartwheel component and suggest that CPAP also resides at least in part within the procentriole.

We next set out to measure Förster resonance energy transfer (FRET) between the proteins of interest at centrosomes of fixed cells (Figures 4A and 4B). Efficient FRET between interacting fluorophores requires distances below

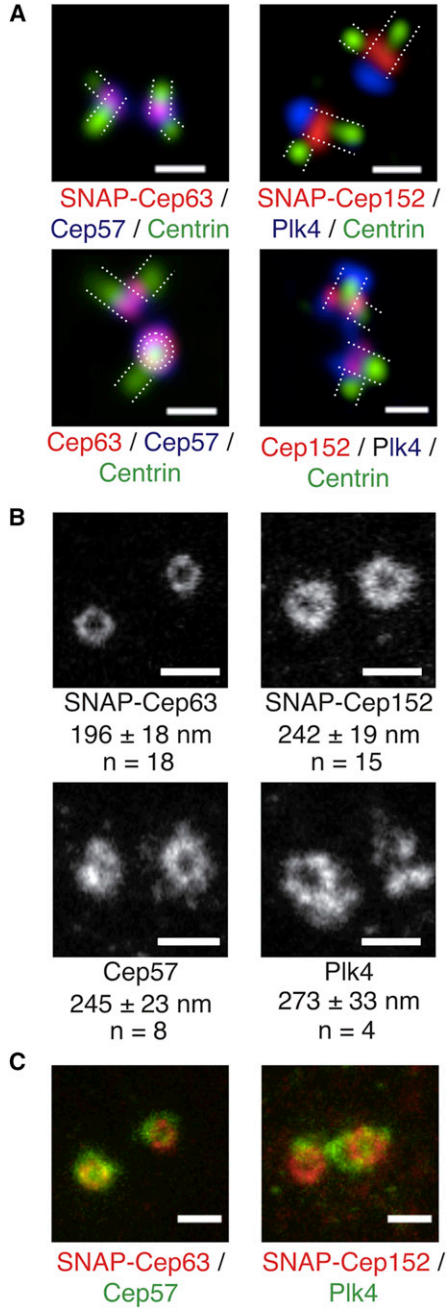

Figure 3. Confocal Microscopy and Superresolution STED Microscopy of Interacting Centrosomal Proteins

Images of endogenous and/or SNAP fusions are presented as indicated. Centrin was used as a marker of the distal end of both centriole and procentriole. Scale bars represent 500 nm.

(A) Confocal images of indicated centrosomal proteins.

(B) STED images of the indicated centrosomal proteins (see also Figure S3 for a comparison between confocal and STED microscopy). Dimensions of the structures are given below the images; data are presented as mean ± SD. Measurements were taken on single optical sections, examples of which are presented. The diameters of the structures were measured as the distance between intensity peaks on the line profiles (rings) or by taking the full width at half maximum of the structure on the line profile of the images (disks). n equals the number of centrioles measured.

(C) Two-color STED images of interacting protein pairs identified by S-CROSS screen. For the estimated extent of fluorescent signal overlap, see Figure S3B.

See also Figures S1 and S2.

10 nm [34]; consequently, FRET measurements offer an even greater spatial resolution than colocalizations measured through dual-color STED. We expressed Cep57, Cep63, and Cep152 as SNAP-tagged fusions and labeled them with CP-Atto565 as the potential FRET donor. Cells were then fixed and the protein of interest was labeled with antibodies conjugated to Atto647N as the potential FRET acceptor. FRET was then revealed through acceptor photobleaching, which resulted in an increased fluorescence intensity of the FRET donor. These experiments revealed close spatial proximities of SNAP-Cep57 and Cep63, SNAP-Cep63 and Cep57, SNAP-Cep57 and Cep152, as well as SNAP-Cep152 and Cep63 (Figure 4C). In contrast, no significant FRET was measured between SNAP-Cep57 and C-Nap1 or between SNAP-Cep152 and C-Nap1 (Figure 4C). These observations provide further evidence that Cep57, Cep63, and Cep152 are parts of a centrosomal protein complex.

We also performed coimmunoprecipitation experiments to systematically monitor the interactions between Cep57, Cep63, and Cep152 in the cytoplasm. Interestingly, these experiments failed to detect an interaction between these components (data not shown), which we interpret to reflect the fact that they either do not interact in the cytosol or interact only transiently. By contrast, we propose that Cep63, Cep57, and Cep152 form a stable complex at centrosomes where these proteins are present at high local concentrations, a proposal fully supported by both our FRET data and the fact that they exhibit the same ring-like structure by STED microscopy.

Overall, our experiments demonstrate the utility of the specific labeling of centrosomal proteins with synthetic probes. We exploited the potential of this approach for the identification of novel protein-protein interactions through selective crosslinking and for their characterization through multicolor confocal imaging, superresolution microscopy, and FRET microscopy. These methods and reagents establish a powerful toolbox for studying centrosomal proteins. One of the many future applications includes the use of fluorophores suitable for chromophore-assisted laser inactivation to inactivate proteins of interest with high spatiotemporal resolution [35]. In addition, our studies provide novel insights into centriole organization and procentriole formation (Figure 4D). Of note, we report that STIL localizes to the region of the cartwheel in the proximal end of the procentriole, as has also been reported recently [30]. Moreover, together with the findings of others [25], our results suggest that Cep57, Cep63, and Cep152 form a complex at the proximal end of centrioles. Finally, the mutual effect of Cep57, Cep63, and Cep152 on their centriolar localization raises the possibility that Cep63 and Cep152 mutations could also result in MVA, and reciprocally that Cep57 mutations could also result in MCPH.

Supplemental Information

Supplemental Information includes three figures, five tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.12.030.

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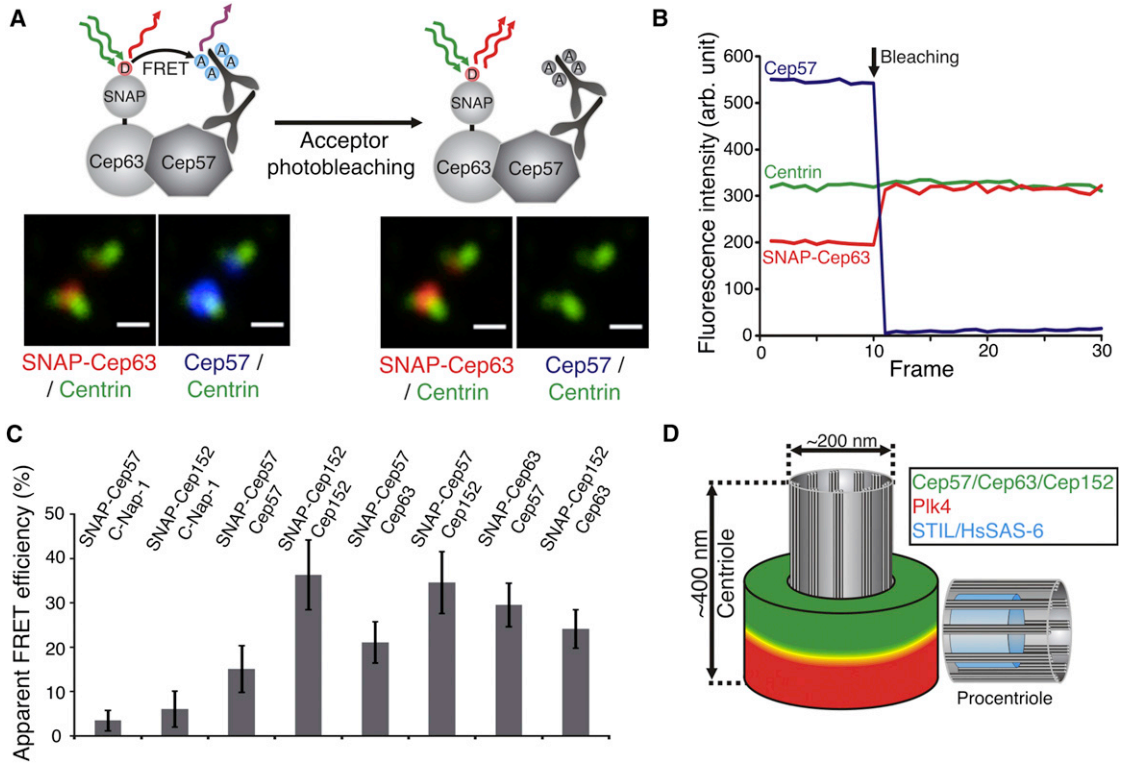

Figure 4. Confirmation of Colocalization of Centrosomal Proteins by FRET Measurements

(A) Schematic representation of Förster resonance energy transfer (FRET) detection by the acceptor photobleaching experiment. SNAP-Cep63 (labeled with the donor fluorophore) and Cep57 (stained via the antibodies with the acceptor fluorophore) are used as an example of interacting protein pair. Images of donor and acceptor fluorophores before and after acceptor photobleaching are presented below. Scale bars represent 500 nm.

(B) Representative example of the acceptor photobleaching experiment. Upon photobleaching, the fluorescence intensity of the acceptor decreases and the fluorescence intensity of the donor increases, indicating spatial proximity of proteins to which fluorophores are conjugated (i.e., Cep57 and SNAP-Cep63). The fluorescence intensity of the control protein (centrin) remains unchanged. Experiment duration is ~70 s and photobleaching duration is ~16 s with maximal microscope scanning speed.

(C) Apparent FRET efficiency for the selected protein pairs. The first two columns serve as negative controls, with differently localizing proteins pairs. Data are shown as mean ± SD ($n \geq 5$).

(D) Model of centrosomal localizations of Cep57, Cep63, Cep152 (all green), Plk4 (red), as well as STIL and HsSAS-6 (cyan). The yellow color indicates a possible overlap in the localizations of Plk4 with Cep57, Cep63, and Cep152. The localization of the second population of Plk4 at the distal end of the centriole is omitted for clarity.

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References

1. Gönczy, P. (2012). Towards a molecular architecture of centriole assembly. Nat. Rev. Mol. Cell Biol. *13*, 425–435.
2. Duensing, A., and Duensing, S. (2010). Centrosomes, polyploidy and cancer. Adv. Exp. Med. Biol. *676*, 93–103.
3. Nigg, E.A. (2002). Centrosome aberrations: cause or consequence of cancer progression? Nat. Rev. Cancer *2*, 815–825.
4. Mahmood, S., Ahmad, W., and Hassan, M.J. (2011). Autosomal Recessive Primary Microcephaly (MCPH): clinical manifestations, genetic heterogeneity and mutation continuum. Orphanet J. Rare Dis. *6*, 39.
5. Bettencourt-Dias, M., Hildebrandt, F., Pellman, D., Woods, G., and Godinho, S.A. (2011). Centrosomes and cilia in human disease. Trends Genet. *27*, 307–315.
6. Snape, K., Hanks, S., Ruark, E., Barros-Núñez, P., Elliott, A., Murray, A., Lane, A.H., Shannon, N., Callier, P., Chitayat, D., et al. (2011). Mutations in CEP57 cause mosaic variegated aneuploidy syndrome. Nat. Genet. *43*, 527–529.
7. Andersen, J.S., Wilkinson, C.J., Mayor, T., Mortensen, P., Nigg, E.A., and Mann, M. (2003). Proteomic characterization of the human centrosome by protein correlation profiling. Nature *426*, 570–574.
8. Jakobsen, L., Vanselow, K., Skogs, M., Toyoda, Y., Lundberg, E., Poser, I., Falkenby, L.G., Bennetzen, M., Westendorf, J., Nigg, E.A., et al. (2011). Novel asymmetrically localizing components of human centrosomes identified by complementary proteomics methods. EMBO J. *30*, 1520–1535.
9. Keppler, A., Gendreizig, S., Gronemeyer, T., Pick, H., Vogel, H., and Johnsson, K. (2003). A general method for the covalent labeling of fusion proteins with small molecules in vivo. Nat. Biotechnol. *21*, 86–89.
10. Gautier, A., Juillerat, A., Heinis, C., Corrêa, I.R., Jr., Kindermann, M., Beaufils, F., and Johnsson, K. (2008). An engineered protein tag for multiprotein labeling in living cells. Chem. Biol. *15*, 128–136.
11. Hinner, M.J., and Johnsson, K. (2010). How to obtain labeled proteins and what to do with them. Curr. Opin. Biotechnol. *21*, 766–776.
12. Strnad, P., Leidel, S., Vinogradova, T., Euteneuer, U., Khodjakov, A., and Gönczy, P. (2007). Regulated HsSAS-6 levels ensure formation of

a single procentriole per centriole during the centrosome duplication cycle. Dev. Cell *13*, 203–213.

13. Fry, A.M., Mayor, T., Meraldi, P., Stierhof, Y.D., Tanaka, K., and Nigg, E.A. (1998). C-Nap1, a novel centrosomal coiled-coil protein and candidate substrate of the cell cycle-regulated protein kinase Nek2. J. Cell Biol. *141*, 1563–1574.
14. Piel, M., Meyer, P., Khodjakov, A., Rieder, C.L., and Bornens, M. (2000). The respective contributions of the mother and daughter centrioles to centrosome activity and behavior in vertebrate cells. J. Cell Biol. *149*, 317–330.
15. Gautier, A., Nakata, E., Lukinavicius, G., Tan, K.T., and Johnsson, K. (2009). Selective cross-linking of interacting proteins using self-labeling tags. J. Am. Chem. Soc. *131*, 17954–17962.
16. Kitagawa, D., Kohlmaier, G., Keller, D., Strnad, P., Balestra, F.R., Flückiger, I., and Gönczy, P. (2011). Spindle positioning in human cells relies on proper centriole formation and on the microcephaly proteins CPAP and STIL. J. Cell Sci. *124*, 3884–3893.
17. Zhao, L., Jin, C., Chu, Y., Varghese, C., Hua, S., Yan, F., Miao, Y., Liu, J., Mann, D., Ding, X., et al. (2010). Dimerization of CPAP orchestrates centrosome cohesion plasticity. J. Biol. Chem. *285*, 2488–2497.
18. Guderian, G., Westendorf, J., Uldschmid, A., and Nigg, E.A. (2010). Plk4 trans-autophosphorylation regulates centriole number by controlling betaTrCP-mediated degradation. J. Cell Sci. *123*, 2163–2169.
19. Rose, A., and Meier, I. (2004). Scaffolds, levers, rods and springs: diverse cellular functions of long coiled-coil proteins. Cell. Mol. Life Sci. *61*, 1996–2009.
20. Tang, C.-J.C., Lin, S.-Y., Hsu, W.-B., Lin, Y.-N., Wu, C.-T., Lin, Y.-C., Chang, C.-W., Wu, K.-S., and Tang, T.K. (2011). The human microcephaly protein STIL interacts with CPAP and is required for procentriole formation. EMBO J. *30*, 4790–4804.
21. Hatch, E.M., Kulukian, A., Holland, A.J., Cleveland, D.W., and Stearns, T. (2010). Cep152 interacts with Plk4 and is required for centriole duplication. J. Cell Biol. *191*, 721–729.
22. Dzhindzhev, N.S., Yu, Q.D., Weiskopf, K., Tzolovsky, G., Cunha-Ferreira, I., Riparbelli, M., Rodrigues-Martins, A., Bettencourt-Dias, M., Callaini, G., and Glover, D.M. (2010). Asterless is a scaffold for the onset of centriole assembly. Nature *467*, 714–718.
23. Cizmecioglu, O., Arnold, M., Bahtz, R., Settele, F., Ehret, L., Haselmann-Weiss, U., Antony, C., and Hoffmann, I. (2010). Cep152 acts as a scaffold for recruitment of Plk4 and CPAP to the centrosome. J. Cell Biol. *191*, 731–739.
24. Kitagawa, D., Flückiger, I., Polanowska, J., Keller, D., Reboul, J., and Gönczy, P. (2011). PP2A phosphatase acts upon SAS-5 to ensure centriole formation in C. elegans embryos. Dev. Cell *20*, 550–562.
25. Sir, J.H., Barr, A.R., Nicholas, A.K., Carvalho, O.P., Khurshid, M., Sossick, A., Reichelt, S., D'Santos, C., Woods, C.G., and Gergely, F. (2011). A primary microcephaly protein complex forms a ring around parental centrioles. Nat. Genet. *43*, 1147–1153.
26. Sillibourne, J.E., Tack, F., Vloemans, N., Boeckx, A., Thambirajah, S., Bonnet, P., Ramaekers, F.C., Bornens, M., and Grand-Perret, T. (2010). Autophosphorylation of polo-like kinase 4 and its role in centriole duplication. Mol. Biol. Cell *21*, 547–561.
27. Kohlmaier, G., Loncarek, J., Meng, X., McEwen, B.F., Mogensen, M.M., Spektor, A., Dynlacht, B.D., Khodjakov, A., and Gönczy, P. (2009). Overly long centrioles and defective cell division upon excess of the SAS-4-related protein CPAP. Curr. Biol. *19*, 1012–1018.
28. Hell, S.W., and Wichmann, J. (1994). Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. Opt. Lett. *19*, 780–782.
29. Westphal, V., Rizzoli, S.O., Lauterbach, M.A., Kamin, D., Jahn, R., and Hell, S.W. (2008). Video-rate far-field optical nanoscopy dissects synaptic vesicle movement. Science *320*, 246–249.
30. Sonnen, K.F., Schermelleh, L., Leonhardt, H., and Nigg, E.A. (2012). 3D-structured illumination microscopy provides novel insight into architecture of human centrosomes. Biol. Open *1*, 965–976.
31. Stevens, N.R., Roque, H., and Raff, J.W. (2010). DSas-6 and Ana2 coassemble into tubules to promote centriole duplication and engagement. Dev. Cell *19*, 913–919.
32. Stevens, N.R., Dobbelaere, J., Brunk, K., Franz, A., and Raff, J.W. (2010). Drosophila Ana2 is a conserved centriole duplication factor. J. Cell Biol. *188*, 313–323.
33. Kitagawa, D., Vakonakis, I., Olieric, N., Hilbert, M., Keller, D., Olieric, V., Bortfeld, M., Erat, M.C., Flückiger, I., Gönczy, P., and Steinmetz, M.O. (2011). Structural basis of the 9-fold symmetry of centrioles. Cell *144*, 364–375.
34. Wallrabe, H., and Periasamy, A. (2005). Imaging protein molecules using FRET and FLIM microscopy. Curr. Opin. Biotechnol. *16*, 19–27.
35. Keppler, A., and Ellenberg, J. (2009). Chromophore-assisted laser inactivation of alpha- and gamma-tubulin SNAP-tag fusion proteins inside living cells. ACS Chem. Biol. *4*, 127–138.