Article

Developmental Cell

GAS2L1 Is a Centriole-Associated Protein Required for Centrosome Dynamics and Disjunction

Highlights

- GAS2L1 is a proximal end-localizing protein on mature centrioles
- GAS2L1 plays an essential role in centriole motility and centrosome disjunction
- GAS2L1 exerts a centrosome-splitting effect opposing that of the centrosome linker
- GAS2L1 acts on centrosomes through its association with microtubules and actin

Authors

Franco K.C. Au, Yue Jia, Kai Jiang, ..., Shengwang Du, Anna Akhmanova, Robert Z. Qi

Correspondence

qirz@ust.hk

In Brief

Centrosome separation is crucial for bipolar spindle assembly and requires dissolution of the centrosomal linker, as well as actin and microtubule-based forces. Au, Jia et al. now show that GAS2L1, a centriole-localizing protein, links centrosomes to both microtubules and actin, thus promoting centrosome separation upon linker disassembly.

Au et al., 2017, Developmental Cell *40*, 81–94
January 9, 2017
http://dx.doi.org/10.1016/j.devcel.2016.11.019

CellPress

Developmental Cell
Article

GAS2L1 Is a Centriole-Associated Protein Required for Centrosome Dynamics and Disjunction

Franco K.C. Au,[1,4] Yue Jia,[1,4] Kai Jiang,[2] Ilya Grigoriev,[2] Bill K.T. Hau,[1] Yuehong Shen,[1] Shengwang Du,[3] Anna Akhmanova,[2] and Robert Z. Qi[1,5,*]
[1]Division of Life Science and State Key Laboratory of Molecular Neuroscience, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China
[2]Cell Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, the Netherlands
[3]Department of Physics and Division of Biomedical Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China
[4]Co-first author
[5]Lead Contact
*Correspondence: qirz@ust.hk
http://dx.doi.org/10.1016/j.devcel.2016.11.019

SUMMARY

Mitotic spindle formation and chromosome segregation require timely separation of the two duplicated centrosomes, and this process is initiated in late G2 by centrosome disjunction. Here we report that GAS2L1, a microtubule- and actin-binding protein, associates with the proximal end of mature centrioles and participates in centriole dynamics and centrosome disjunction. GAS2L1 attaches microtubules and actin to centrosomes, and the loss of GAS2L1 inhibits centrosome disjunction in G2 and centrosome splitting induced by depletion of the centrosome linker rootletin. Conversely, GAS2L1 overexpression induces premature centrosome separation, and this activity requires GAS2L1 association with actin, microtubules, and the microtubule end-binding proteins. The centrosome-splitting effect of GAS2L1 is counterbalanced by rootletin, reflecting the opposing actions of GAS2L1 and the centrosome linker. Our work reveals a GAS2L1-mediated centriole-tethering mechanism of microtubules and actin, which provide the forces required for centrosome dynamics and separation.

INTRODUCTION

The centrosome is the principal microtubule-organizing center in animal cells, and comprises a pair of centrioles surrounded by the pericentriolar material. In each cell cycle, the centrosome duplicates during S phase to become two centrosomes, which move apart and eventually form spindle poles during mitosis and organize the mitotic spindle (Tanenbaum and Medema, 2010; Nigg and Stearns, 2011; Fırat-Karalar and Stearns, 2014). The centrosome cycle is tightly regulated to ensure the proper organization of mitotic spindles and consequently the equal segregation of sister chromatids into each of the two daughter cells (Kaseda et al., 2012; Silkworth et al., 2012; Nam et al., 2015).

After exit from mitosis, the disengaged centrioles are connected by the centrosomal linker, a fibrous structure that is anchored to the proximal end of the two centrioles (Nigg and Stearns, 2011). A core component of the centrosomal linker is rootletin, which assembles into filaments with other proteins such as Cep68 and LRRC45 and binds to C-Nap1 at the proximal end for centriole attachment (Fry et al., 1998; Mayor et al., 2000; Bahe et al., 2005; Yang et al., 2006; Graser et al., 2007; He et al., 2013). The centrosomal linker is retained after centriole duplication and until the separation of the duplicated centrosomes is initiated in late G2 (Mardin and Schiebel, 2012). The centrosomal linker is dissolved through the following process: First, the NIMA (never in mitosis A)-related kinase Nek2A is activated and recruited to centrosomes by the polo kinase Plk1 and the Hippo-pathway component Mst2/hSav1 (Mardin et al., 2010, 2011). Next, activated Nek2A phosphorylates (at least) rootletin and C-Nap1 in the centrosomal linker and leads to rootletin dissociation from the centrosomes and linker disassembly (Fry et al., 1998; Faragher and Fry, 2003; Bahe et al., 2005; He et al., 2013; Hardy et al., 2014). This step is commonly referred to as centrosome disjunction. Recently, epidermal growth factor (EGF) receptor signaling was also shown to trigger centrosome disjunction through the Mst2/hSav1-Nek2 pathway (Mardin et al., 2013).

Upon entering mitosis, the kinesin Eg5 (kinesin-5) is activated and recruited to centrosomes through a signaling cascade that involves Plk1 and its downstream targets Nek9 and Nek6/Nek7 (Bertran et al., 2011; Mardin et al., 2011; Smith et al., 2011). Activated Eg5 mediates further separation of the centrosomes by sliding apart the antiparallel microtubules (Sawin et al., 1992; Blangy et al., 1995; Smith et al., 2011). In this separation step, the motor proteins Kif15 (kinesin-12) and dynein might also be involved and might complement the function of Eg5 (Tanenbaum et al., 2009; Raaijmakers et al., 2012).

Centrosomes are attached to actin filaments and microtubules, both of which are considered to exert pushing and pulling forces on the centrosomes (Vaughan and Dawe, 2011; Farina et al., 2016). These forces are essential for centrosome positioning, movement, and separation (Uzbekov et al., 2002; Burakov et al., 2003; Wang et al., 2008; Cao et al., 2010; Kimura and Kimura, 2011; Obino et al., 2016). Centrioles have

been hypothesized to provide the physically stable structures on which the forces are exerted to push or pull centrosomes; in the absence of centrioles, centrosomes are fragmented by (at least) microtubule-dependent forces (Abal et al., 2005). Moreover, certain cell types contain motile daughter centrioles, which move separately from the mother centrioles and in a microtubule- and actin-dependent manner (Piel et al., 2000). Although intact microtubule and actin filaments are required even in the early stages of centrosome separation (Schatten et al., 1988; Waters et al., 1993; Whitehead et al., 1996; Uzbekov et al., 2002; Wang et al., 2008; Cao et al., 2010), how these cytoskeletal elements mediate centrosome disjunction remains unclear.

The prototypic members of the microtubule plus-end tracking proteins (+TIPs), the end-binding proteins EB1, EB2, and EB3, bind to all other +TIPs at the plus ends, where these complexes control microtubule dynamics and attachment to various subcellular structures (Akhmanova and Steinmetz, 2008; Jaworski et al., 2008; Komarova et al., 2009). A number of the +TIPs contain the SxIP motif (x = any amino acid) that is responsible for interaction with the EB proteins and consequently for accumulation at the plus ends (Honnappa et al., 2009). Recently, GAS2L1 and GAS2L2 were identified in a systematic search for EB1-binding proteins (Jiang et al., 2012). GAS2L1 and GAS2L2 belong to the GAS2-like family proteins that harbor a calponin homology (CH) domain and a GAS2-related (GAR) domain, which interact with actin filaments and microtubules, respectively (Goriounov et al., 2003). Furthermore, GAS2L1 and GAS2L2 bind to EB1 through the SxIP motif located near their carboxy terminus, which is involved in the regulation of cytoskeletal dynamics (Jiang et al., 2012; Stroud et al., 2014; Gamper et al., 2016).

Here, we report a GAS2L1-mediated mechanism that controls centrosome disjunction and movement. We show that GAS2L1 localizes to the proximal end of mature centrioles, but does not appear on nascent daughter centrioles after duplication. Although the centriolar localization of GAS2L1 is similar to that of C-Nap1, GAS2L1 participates in centrosome disjunction through a mechanism independent from the C-Nap1-rootletin linker: GAS2L1 attaches the proximal ends to the microtubule and actin cytoskeletons that provide the forces required to split the centrosomes. Whereas silencing GAS2L1 expression inhibits centrosome movement and disjunction, GAS2L1 overexpression drives premature separation of the centrioles. Furthermore, the centrosome linker counterbalances the forces exerted by GAS2L1-bound microtubules and actin and thereby maintains centrosome cohesion, and in the absence of the linker in late G2 these forces drive the centrosomes apart.

RESULTS

GAS2L1 Localizes at the Proximal End of Centrioles

To investigate the cellular roles of GAS2L1, we developed antibodies that specifically recognize GAS2L1 and used the antibodies for immunostaining. In RPE-1 cells the staining revealed two prominent spots, which overlapped with sites of γ-tubulin enrichment (Figure 1A). Demonstrating immunostaining specificity, this GAS2L1 pattern was absent in cells in which GAS2L1 was depleted using RNAi (Figure S1A). Furthermore, a commercial GAS2L1 antibody yielded identical labeling. We also obtained this GAS2L1 staining pattern in U2OS and HeLa cells. Next, in a centrosome-isolation experiment, we assessed the distribution of GAS2L1 between the centrosomal and noncentrosomal fractions, and found that >80% of GAS2L1 exists in the noncentrosomal fraction (Figure S1B), which is similar to the distribution measured for the centrosomal proteins γ-tubulin and centrin (Moudjou et al., 1996; Paoletti et al., 1996).

Our immunostaining data suggested that GAS2L1 localizes to a specific region of centrosomes. To examine whether GAS2L1 associates with centrioles, we labeled centrioles with the antibody GT335 and marked the distal lumen of centrioles by ectopically expressing GFP-centrin 2 (Wolff et al., 1992; Paoletti et al., 1996). GAS2L1 staining appeared at the end of the centrioles opposite to the centrin-GFP signal both before and after centriole duplication (Figure 1B). Moreover, the GAS2L1 distribution was highly similar to that of C-Nap1 (Figure 1B), which decorates the proximal end of centrioles (Fry et al., 1998). When we costained for rootletin, a linker between the proximal ends of the two centrioles (Bahe et al., 2005; Yang et al., 2006), GAS2L1 appeared as two discrete spots that marked both ends of the fibrous rootletin pattern (Figure 1B). These staining data indicated the association of GAS2L1 with the proximal region of centrioles. We also imaged GAS2L1 immunofluorescence by using super-resolution microscopy and performed 3D reconstruction of the images; the data corroborated our finding that GAS2L1 localization was confined to the proximal end on the centrioles (Figure 1C and Movie S1). Furthermore, the super-resolution imaging revealed that the GAS2L1 signals extended from the proximal ends toward each other on the two paired centrioles, but did not form a continuous structure between the two centrioles (Figure 1C and Movie S1).

The appearance of GAS2L1 as two spots before and after centriole duplication (Figure 1B) suggested that GAS2L1 associates only with mature centrioles. To test this idea, we induced centriole overduplication by treating HeLa cells with hydroxyurea. After the overduplication, at the mother centrioles we detected labeling for C-Nap1, a protein that selectively associates with mature centrioles but not nascent daughter centrioles (Wang et al., 2011). In these hydroxyurea-treated cells, GAS2L1 staining was detected on C-Nap1-positive centrioles but not nascent centrioles (Figure 1D), which indicated that GAS2L1 associates specifically with the mother centriole in the pairs of duplicated centrioles. Accordingly, we found that GAS2L1 was stained at only one centriole within the mother and daughter pairs in G2 cells, and GAS2L1 appeared at both centrioles within the pairs as early as in prophase during mitosis (Figure 1E). Collectively, our results indicate that GAS2L1 associates with the proximal end of both centrioles in G1 phase and that after centriole duplication, GAS2L1 associates with both mother centrioles but not with the nascent daughter centrioles until early mitosis.

GAS2L1 Participates in Centrosome Disjunction and Movement

To investigate the function of GAS2L1, we designed two small interfering RNA (siRNA) duplexes to silence its expression, and confirmed that both sequences knocked down GAS2L1 expression effectively (~90% knockdown efficiency; Figure S1A). We

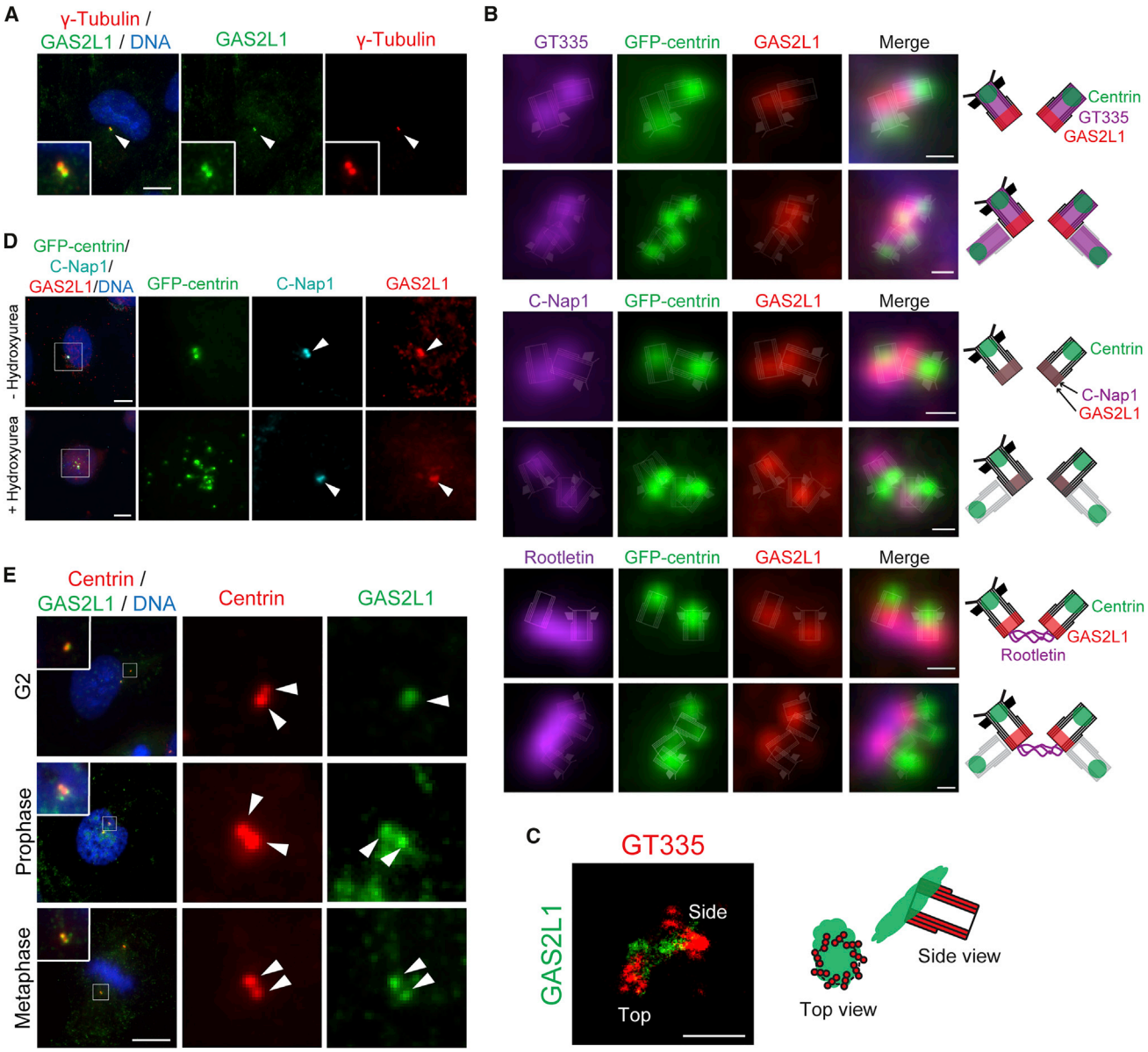

Figure 1. Centrosomal Localization of GAS2L1

(A) RPE-1 cells were costained with anti-GAS2L1 and anti-γ-tubulin antibodies. Arrowheads point to the centrosome. Insets show the enlarged centrosomal region. Scale bar, 10 μm.

(B) GAS2L1 location on centrosomes was defined by costaining for centrioles (GT335), rootletin, or C-Nap1 in RPE-1 cells. The distal region of centrioles was labeled by expressing GFP-centrin 2. Schematic representations of the centriole association of GAS2L1, rootletin, and C-Nap1 are shown. The nascent centrioles formed after duplication are shown in light gray. Scale bars, 1 μm.

(C) Super-resolution images of centrioles (GT335) and GAS2L1 were acquired using RPE-1 cells. Scale bar, 0.5 μm.

(D) HeLa cells stably expressing GFP-centrin 1 were treated with hydroxyurea to induce centriole overduplication. Boxed regions are enlarged, and arrowheads indicate C-Nap1-positive centrioles. Scale bar, 10 μm.

(E) Immunofluorescence images were obtained from RPE-1 cells at various cell-cycle stages. Boxed regions are enlarged and arrowheads indicate centrioles. Scale bar, 10 μm.

See also Figure S1 and Movie S1.

observed identical cellular phenotypes upon using these two siRNAs, and we present herein the results obtained using si-GAS2L1#2. The suppression of GAS2L1 expression did not discernibly affect the centrosomal staining obtained for C-Nap1 and rootletin (Figures S2A and S2B), two proximal end-associated proteins that participate in centrosome cohesion (Fry et al., 1998; Bahe et al., 2005; Yang et al., 2006).

To determine whether GAS2L1 depletion affects centrosome cohesion or disjunction, we measured inter-centrosome distances in interphase cells and identified the G2 cells based on

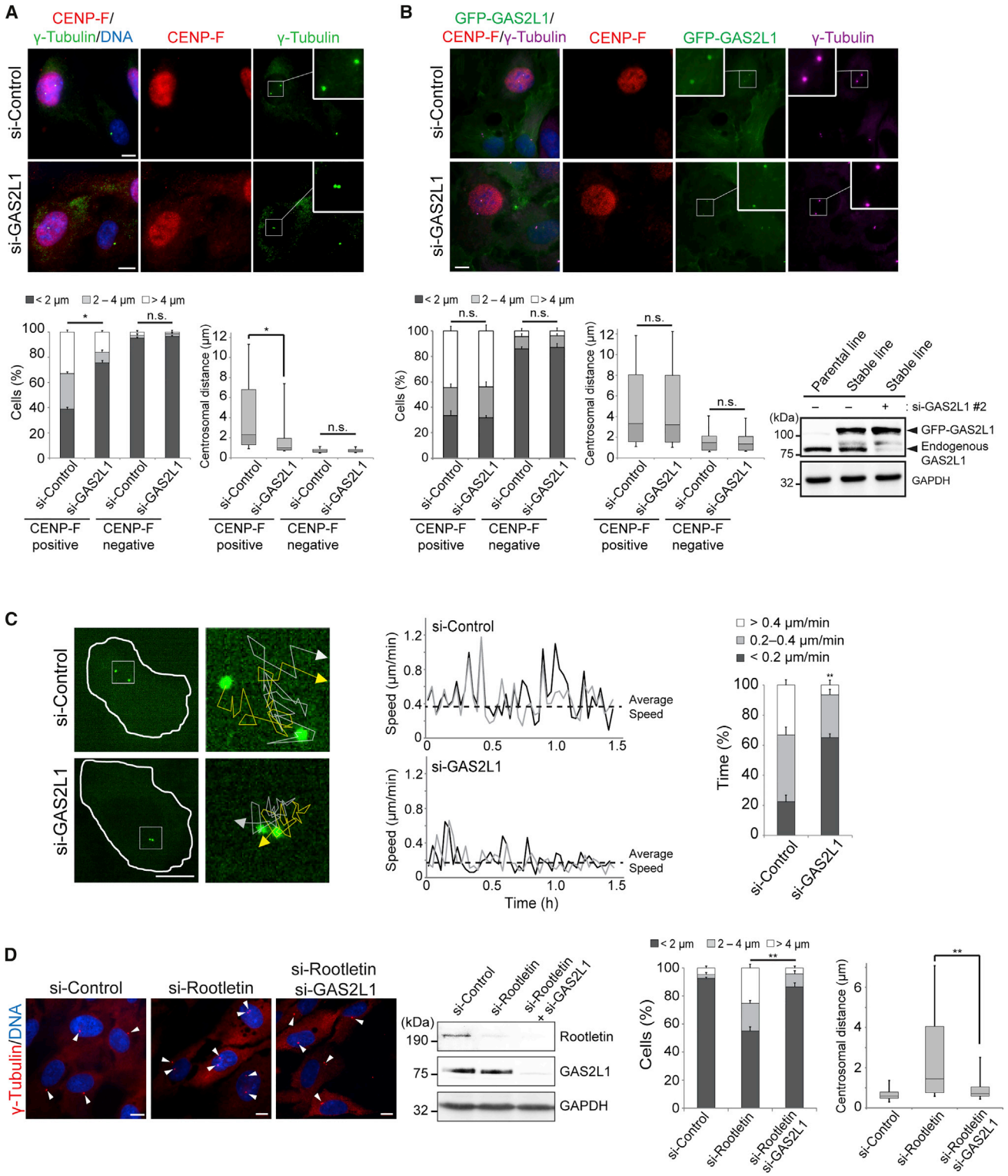

Figure 2. GAS2L1 Is Required for Centrosome Disjunction and Motility

(A) RPE-1 cells were transfected with a *gas2l1*-targeting siRNA (si-GAS2L1) or control siRNA (si-Control). Inter-centrosome distances were measured from CENP-F-positive cells (n = 152 and 194, si-Control and si-GAS2L1, respectively) and CENP-F-negative cells (n = 188 and 186, si-Control and si-GAS2L1) in three independent experiments.

(B) Inter-centrosome distances were measured from RPE-1 cells stably expressing GFP-GAS2L1. CENP-F-positive cells: n = 104 and 107 for si-Control and si-GAS2L1, respectively; CENP-F-negative cells: n = 102 and 107 for si-Control and si-GAS2L1; the presented data were collected from three

(legend continued on next page)

their nuclear staining of centromere protein F (CENP-F) (Liao et al., 1995). In the majority (~61%) of the control G2 cells (i.e., CENP-F-positive cells), centrosomes were separated by ≥2 μm; by contrast, ≥2-μm centrosome separation occurred in only ~24% of GAS2L1-depleted G2 cells (Figure 2A). The depletion of GAS2L1 did not arrest the cell cycle at G2, because ~14% of both control and GAS2L1-depleted cells were CENP-F-positive cells. Therefore, these data indicate that GAS2L1 is required for the centrosome splitting that occurs in G2. However, GAS2L1 knockdown did not markedly affect the inter-centrosome distance in cells in a stage preceding G2 (Figure 2A; CENP-F-negative cells), which showed that GAS2L1 is not required for centrosome cohesion.

Next, an RPE-1 subline stably expressing GFP-GAS2L1 was generated and used in RNAi complementation experiments. In this subline, GFP-GAS2L1 was expressed at a similar level as the endogenous protein and displayed centrosomal localization, in addition to localizing with F-actin and microtubules (Figure 2B). Moreover, this expression was unaffected by transfection of si-GAS2L1#2 (Figure 2B), which targets the 3′ UTR of *gas2l1*. The expression of GFP-GAS2L1 completely restored centrosome separation in late G2 in the cells that were depleted of the endogenous protein (Figure 2B). These results indicate that the ectopically expressed protein functioned similarly as endogenous GAS2L1, and that the cellular phenotypes observed after transfection of *gas2l1*-targeting siRNAs resulted specifically from the depletion of GAS2L1.

We further examined GAS2L1 function by monitoring centriole movement. In control interphase HeLa cells, the mother and daughter centrioles were separated and moved dynamically (Figure 2C and Movie S2), as observed in previous studies (Piel et al., 2000). However, in GAS2L1-knockdown cells, both centrioles showed diminished motility: GAS2L1 depletion reduced both the moving speeds and the areas of movement of the centrioles (Figure 2C and Movie S2) and, furthermore, the centrioles in the GAS2L1-depleted cells exhibited drastically increased periods of low motility (<0.2 μm/min; Figure 2C). These findings demonstrate the requirement of GAS2L1 for centrosome motility.

Upon mitotic entry, the split centrosomes are further separated through an Eg5-dependent mechanism, which can overcome defects in earlier centrosome disjunction (Mardin et al., 2010; Mardin and Schiebel, 2012; Nam et al., 2015). To verify that GAS2L1 acts in G2, we inhibited the Eg5 motor by using S-trityl-L-cysteine (STLC) (DeBonis et al., 2004; Skoufias et al., 2006). Before STLC treatment, the cells were synchronized using aphidicolin and released to increase the mitotic population. RNAi-mediated depletion of GAS2L1 substantially increased the fraction of STLC-treated cells in which the centrosomes were separated by <2 μm (Figure S3), which demonstrated that the observed function of GAS2L1 is independent from Eg5-mediated separation.

Centrosomes can be separated prematurely by silencing the expression of the centrosome-tethering protein rootletin (Bahe et al., 2005). Transfection of cells with a *rootletin*-targeting siRNA lowered the fraction of cells harboring tightly associated centrosomes (separation <2 μm) from ~93% to ~55% (Figure 2D), which agrees with published findings (Bahe et al., 2005). The transfection did not alter the centrosomal content of GAS2L1 (Figure S2C). Strikingly, concurrent knockdown of GAS2L1 almost eliminated the centrosome-separating effect of rootletin depletion (inter-centrosome distance <2 μm in ~87% of double-knockdown cells; Figure 2D), which indicates that GAS2L1 is indispensable for centrosome disjunction triggered by disassembly of the centrosome linker.

GAS2L1 Overexpression Induces Premature Centrosome Separation

To characterize the cellular properties of GAS2L1, we transiently overexpressed the protein in cell cultures, and the expression levels were significantly higher than the GAS2L1 level of the stable cell line that was used in the RNAi complementation experiments. Transiently expressed GAS2L1, like its endogenous counterpart, localized to centrosomes (Figure 3A). Notably, centrosomes were separated in most of the cells that overexpressed GAS2L1 (inter-centrosome distance ≥2 μm in ~66% of cells) but not control GFP (distance ≥2 μm in ~10% of cells) (Figure 3A). To test the involvement of the Eg5 motor, we treated the cells with STLC, and found that the treatment did not markedly block GAS2L1 overexpression-induced centrosome disjunction (Figure S4). Therefore, this centrosome-splitting activity of GAS2L1 does not require Eg5.

Next, we monitored centrosome movement and inter-centriole distance in RPE-1 cells. To minimize cell-cycle-dependent variations, we synchronized the cells at G1/S. In control untransfected cells, the two centrioles moved in a manner that kept the centrioles close to each other (Figure 3B and Movie S3). By contrast, in GAS2L1-transfected cells the two centrioles exhibited dynamic movement toward and away from each other, and thus the inter-centriole distance changed dynamically within a large range (Figure 3B and Movie S3).

independent experiments. The expression of endogenous and ectopically expressed GAS2L1 is shown in the immunoblots. Stable line, cells stably expressing GFP-GAS2L1.

(C) HeLa cells stably expressing GFP-centrin 1 were transfected with a *gas2l1*-targeting siRNA or control siRNA. Time-lapse images were acquired at 2-min intervals. The images show a representative cell with white lines indicating the cell boundary. Boxed regions are enlarged, and the yellow and white arrows depict the trajectories of the two centrioles. The moving speeds of the two centrioles were plotted and the average speeds were determined (0.37 and 0.18 μm/min, si-Control and si-GAS2L1, respectively). The durations for which the centrioles moved at distinct speeds were derived from 30 each of control and GAS2L1-depleted cells in three independent experiments.

(D) siRNA transfection was used to deplete RPE-1 cells either singly of rootletin or doubly of GAS2L1 and rootletin; arrowheads indicate centrosomes. Inter-centrosome distances were measured from three separate experiments; n = 450 (si-Control), 396 (si-Rootletin), and 564 (si-GAS2L1 + si-Rootletin). Immunoblotting was used to examine the knockdown of rootletin and GAS2L1.

Box-and-whisker plots in (A), (B), and (D): lower and upper edges of the boxes represent lower and upper quartiles (25%–75%), respectively; lines within the boxes represent medians; lower and upper whiskers extend from the lower quartile down to 10% and from the upper quartile up to 90%, respectively.

*$p < 0.01$; **$p < 0.001$; n.s., not significant ($p > 0.5$). Error bars denote SD of three independent experiments. Scale bars, 10 μm. See also Figures S2 and S3; Movie S2.

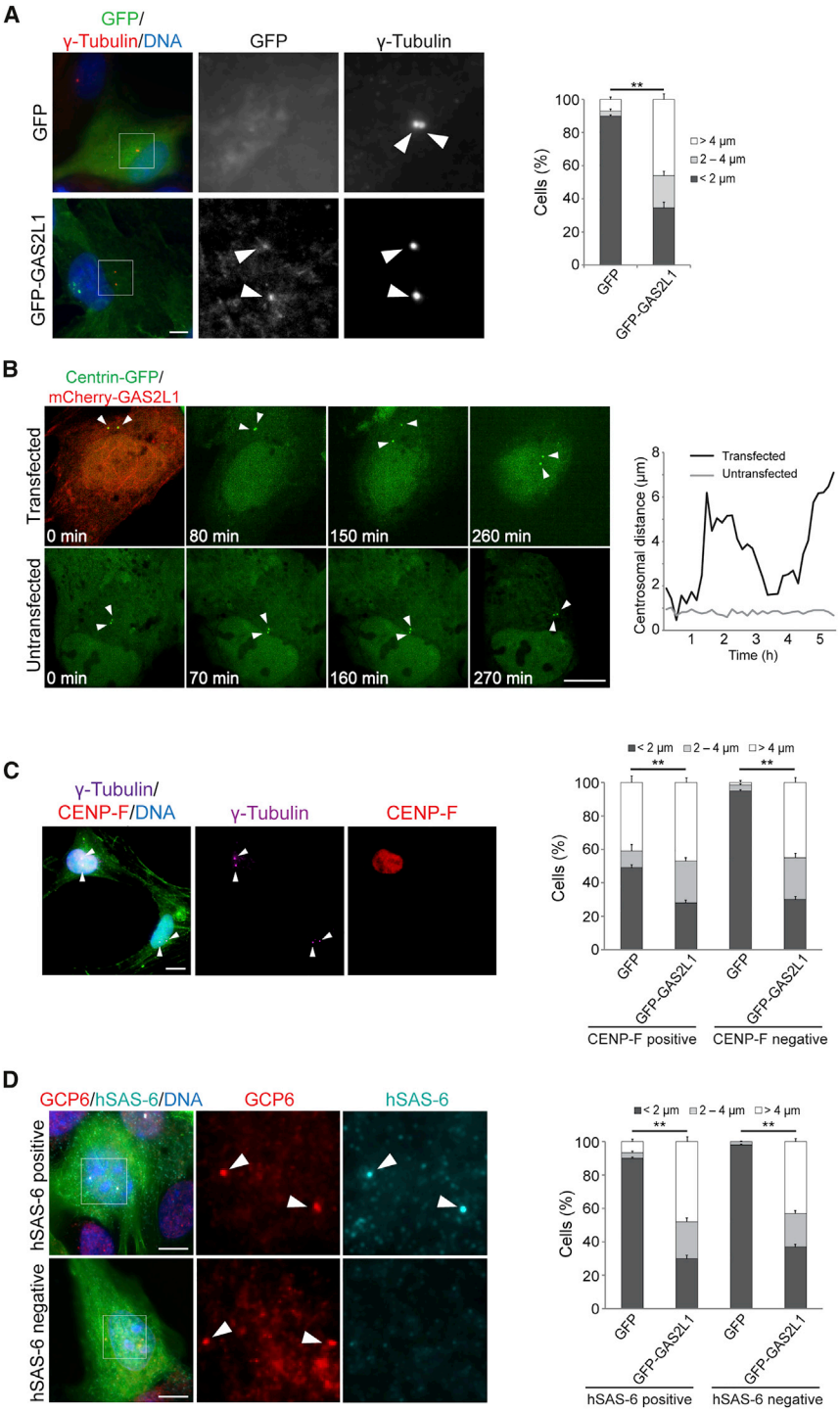

Figure 3. GAS2L1 Overexpression Induces Premature Centrosome Disjunction

(A) RPE-1 cells were transfected with plasmids encoding GFP or GFP-GAS2L1. Boxed areas are enlarged and arrowheads indicate centrosomes. Distance was measured between the two centrosomes in transfected cells from three independent experiments (n = 102 and 161, GFP- and GFP-GAS2L1-transfected cells, respectively).

(B) Time-lapse microscopy was performed on RPE-1 cells stably expressing GFP-centrin 1; the cells were treated with thymidine for G1/S arrest. Representative images are shown with arrowheads indicating GFP-centrin dots, and the inter-centrosome distances are plotted for the mCherry-GAS2L1-transfected and -untransfected cells. The phenotypes were observed in >90% of the 30 cells analyzed for each sample.

(C) Cells transfected as in (A) were stained with anti-γ-tubulin and anti-CENP-F antibodies. Inter-centrosome distances were quantified in three independent experiments; n = 181 (GFP, CENP-F negative), 109 (GFP-GAS2L1, CENP-F negative), 81 (GFP, CENP-F positive), and 78 (GFP-GAS2L1, CENP-F positive).

(D) Cells transfected as in (A) were costained for GCP6 and hSAS-6. Inter-centrosome distances were measured from hSAS-6-negative cells (n = 106 and 69, GFP- and GFP-GAS2L1-transfected cells, respectively) and hSAS-6-positive cells (n = 211 and 110, GFP- and GFP-GAS2L1-transfected cells). Data are from three separate experiments.

**p < 0.001. Error bars denote SD of three independent experiments. Scale bars, 10 μm. See also Figures S4 and S5; Movie S3.

The GAS2L1-induced relative movement between the mother and daughter centrioles observed in the G1/S-synchronized cells suggested that GAS2L1 overexpression triggers premature centrosome separation. We characterized this centrosome-splitting effect of GAS2L1 expression further by examining interphase cells identified to be in G2 or in a stage earlier than G2 based on their CENP-F and nuclear-DNA distribution patterns. Even in the earlier-than-G2 cells, GAS2L1 overexpression potently induced centrosome separation: the fraction of cells presenting an inter-centrosome distance of <2 μm was reduced from ~95% (control) to ~30% (Figure 3C). However, GAS2L1 overexpression did not delocalize the centrosome linker proteins rootletin and C-Nap1 (Figure S5) and did not alter the cell fraction in G2. Moreover, we examined the centriole-duplication status by immunostaining for hSAS-6, a marker of newly formed centrioles (Leidel et al., 2005). GAS2L1 overexpression induced centrosome separation independently of centriole duplication (inter-centrosome distance ≥2 μm: ~69% versus ~63% of centrosomal hSAS-6-positive cells versus hSAS-6-negative cells; Figure 3D), and, specifically, GAS2L1 expression triggered the separation even before centriole duplication.

GAS2L1 Function Requires Its Centrosomal Localization

Because GAS2L1 exhibits centrosomal and noncentrosomal distributions, we tested whether GAS2L1 centrosomal localization is required for its centrosome-splitting function. We

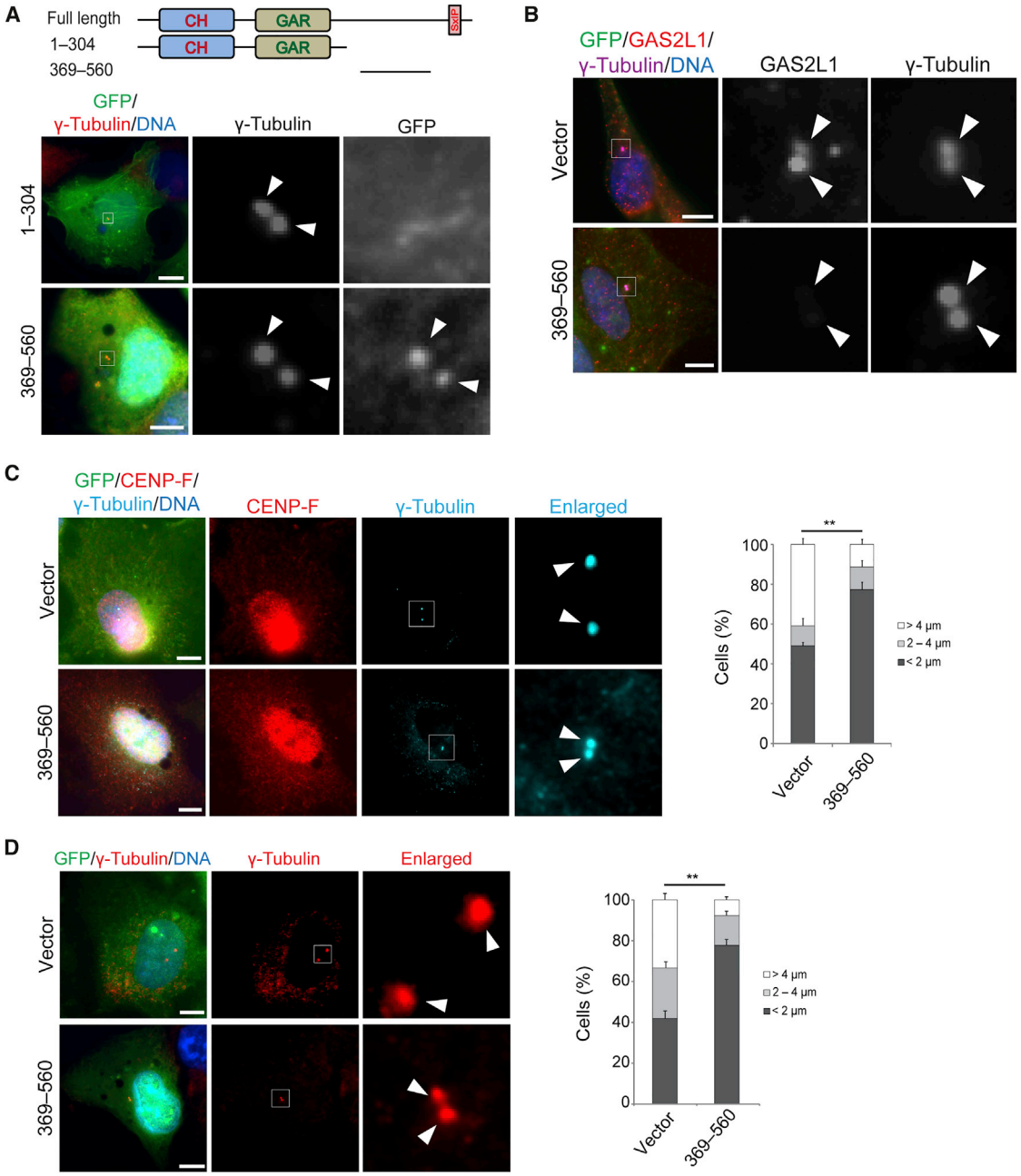

Figure 4. Centrosome-Separating Function of GAS2L1 Requires Its Centrosomal Localization

(A) GFP-tagged GAS2L1 fragments were ectopically expressed in RPE-1 cells.

(B) Cells were transfected with GFP-tagged GAS2L1 fragment 369–681 and stained with a GAS2L1 antibody that recognizes 1–207 (and thus only detects endogenous GAS2L1).

(C) Cells were transfected with GFP-tagged 369–560 or GFP (vector) and inter-centrosome distances were measured in CENP-F-positive cells; n = 110 and 108, GFP and GFP-tagged 369–560, respectively, from three independent experiments.

(D) Following transfection with the GAS2L1 fragment or GFP vector, rootletin expression was suppressed using RNAi and then inter-centrosome distances were measured from three independent experiments; n = 105 and 110, GFP and GFP-tagged 369–560, respectively.

Boxed areas are enlarged, and arrowheads indicate centrosomes. **$p < 0.001$. Error bars denote SD of three independent experiments. Scale bars, 10 μm.

constructed various GAS2L1 fragments to map the centrosome-localizing domain, and when expressed in cells 369–560 was the only GAS2L1 region that showed centrosomal localization (Figure 4A). Moreover, overexpression of 369–560 drastically reduced the centrosomal content of endogenous GAS2L1 (Figure 4B). Collectively, these results identified 369–560 as the centrosome-localizing domain of GAS2L1. Next, we measured the inter-centrosome distance in cells transfected with

369–560 or its vector control, and found that overexpression of this GAS2L1 region hindered centrosome separation not only in G2 cells (Figure 4C), but also in cells in which centrosome separation was induced through rootletin depletion (Figure 4D). Therefore, the displacement of GAS2L1 from centrosomes eliminates its ability to function in centrosome disjunction.

GAS2L1 Attaches Microtubules and Actin to Centrosomes

Given that GAS2L1 is a microtubule- and actin-binding protein (Goriounov et al., 2003; Stroud et al., 2014), we examined whether GAS2L1 attaches microtubules and actin to centrosomes. We altered GAS2L1 expression through knockdown or overexpression and then stained for microtubule and actin filaments. Because the microtubule-anchoring capacities of the mother and daughter centrioles differ, we separated the two centrioles by knocking down rootletin to observe the cytoskeletal attachment to each centriole. The mother centrioles, which were positive for ODF2, were associated with more microtubules than the daughter centrioles (Figure 5A), as reported previously (Piel et al., 2000; Ibi et al., 2011), and RNAi-mediated suppression of GAS2L1 expression reduced microtubule attachment to both mother and daughter centrioles, by ~29% and ~17%, respectively (Figure 5A). By contrast, GAS2L1 overexpression enhanced microtubule attachment to both mother and daughter centrioles, by ~38% and ~32%, respectively (Figure 5B). Next, we stained for centrosome-associated actin by using a method modified from a recent report (Farina et al., 2016). Whereas RNAi-induced GAS2L1 depletion reduced the actin intensity surrounding the centrosomes by ~47%, GAS2L1 overexpression increased the actin intensity by ~43% (Figures 5C and 5D). Thus, GAS2L1 clearly functions at centrosomes in the attachment of both microtubules and actin.

Microtubule and Actin Binding Is Required for GAS2L1 to Function in Centrosome Disjunction

Microtubule and actin cytoskeletons are considered to exert forces on centrosomes and thus play pivotal roles in centrosome positioning and separation (Uzbekov et al., 2002; Burakov et al., 2003). We tested the involvement of microtubules and actin filaments in GAS2L1-mediated centrosome disjunction by using pharmacological compounds that disrupt microtubule and actin cytoskeletons or block their dynamics. In these assays, GAS2L1 was overexpressed in cells from an inducible system in the absence and presence of the pharmacological compounds. GAS2L1 overexpression-induced centrosome separation was inhibited following treatment with either nocodazole (microtubule-depolymerizing agent) or taxol (microtubule-stabilizing agent) (Figure 6A), or latrunculin B (F-actin-depolymerizing agent) or jasplakinolide (F-actin-stabilizing agent) (Figure 6A). Thus, we conclude that intact microtubule and actin organization and cytoskeletal dynamics are essential for GAS2L1-induced centrosome disjunction.

GAS2L1 harbors a CH domain and a GAR domain, and these domains bind to actin filaments and microtubules, respectively (Goriounov et al., 2003). By creating and testing GAS2L1 mutants lacking the CH or GAR domain, we determined that both of these domains were indispensable for GAS2L1 function in centrosome separation (Figure 6B). These results indicate that the centrosome-separating function of GAS2L1 requires its association with microtubules and actin filaments at centrosomes.

In addition to the CH and GAR domains, GAS2L1 contains an SxIP motif that is responsible for binding to EB1 and its homologs (Jiang et al., 2012). We substituted Ala for the crucial Ile-Pro residues in the SxIP motif (Figure 6C), and determined that whereas wild-type GAS2L1 robustly coimmunoprecipitated EB1, the I647A/P648A mutant failed to do so (Figure 6C). Next, we expressed the mutant and wild-type GAS2L1 in RPE-1 cells to test their centrosome-splitting activities, and found that the mutant protein poorly induced centrosome separation compared with wild-type GAS2L1: the inter-centrosome distance was ≥ 2 μm in ~66% and ~38% of the cells transfected with wild-type and mutant GAS2L1, respectively (Figure 6D). Furthermore, RNAi-mediated depletion of both EB1 and EB3, two related proteins that bind to the SxIP motif (Akhmanova and Steinmetz, 2008; Honnappa et al., 2009), impaired the centrosome-splitting effect produced by overexpressing GAS2L1 (Figure 6D). However, neither the double mutation in GAS2L1 nor the depletion of EB1/3 affected the centrosomal localization of GAS2L1 (Figure S6). These results reveal that GAS2L1-dependent centrosome separation requires the interaction of GAS2L1 with EB1/3.

The attachment of microtubules and actin to centrosomes by GAS2L1 suggests a model in which the centrosomes are separated by the forces exerted by the microtubules and actin bound to centrosomal GAS2L1. Our model predicts that strengthening the centrosome linker might counteract the centrosome-splitting effect of overexpressed GAS2L1. To test this we overexpressed rootletin, and found that the overexpression markedly increased the intensity of the rootletin filaments that linked the two centrosomes (Figure 7A); this observation agrees with previous findings (Bahe et al., 2005). Notably, rootletin coexpression with GAS2L1 blocked GAS2L1-induced centrosome disjunction (Figure 7B). These results confirm that GAS2L1 and rootletin exert opposite effects on centrosome cohesion and thereby govern the distance between the centrosomes.

DISCUSSION

In this study, we identified a previously unrecognized function of GAS2L1: GAS2L1 mediates centrosome disjunction by attaching microtubules and actin filaments to the proximal end of mother centrioles in the two centrosomes, and this attachment is essential for centrosome dynamics and disjunction. Centrosome separation involves a sequence of events governed by mechanisms that remain poorly understood. Based on our findings, we provide a model that unifies the actions of the microtubule and actin cytoskeletons and the centrosome linker, the two prominent factors that control centrosome cohesion and disjunction (Figure 7C). Furthermore, our results indicate that GAS2L1 regulates centriole motility through the attachment of microtubules and actin. Therefore, GAS2L1 serves as a spatial organizer on centrioles for the cytoskeletal elements that function in centrosome movement and disjunction.

We have shown that GAS2L1 is a centrosomal protein that associates specifically with the proximal end of mature centrioles. This centrosomal localization is unaffected by the depolymerization of microtubules or actin filaments, which implies that

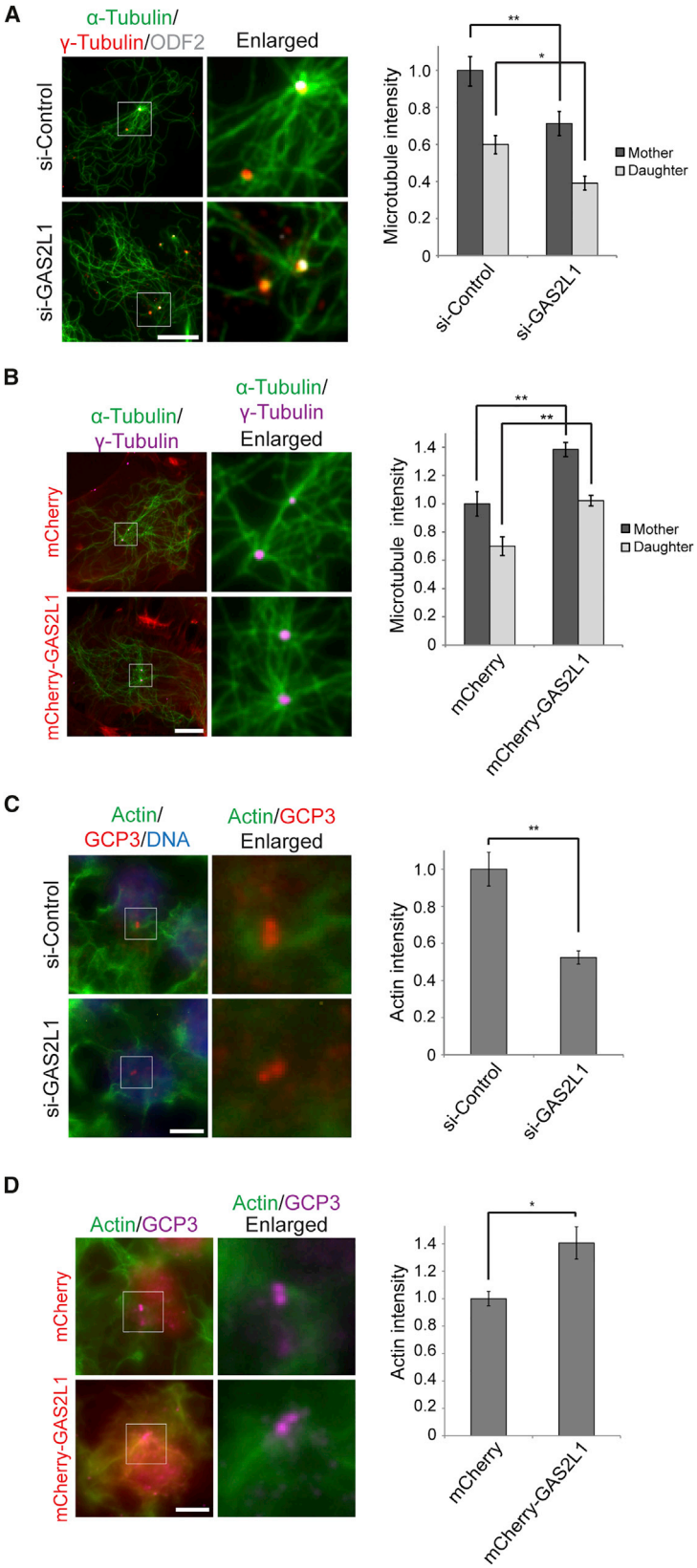

Figure 5. GAS2L1 Attaches Microtubules and Actin to Centrosomes

(A) RPE-1 cells were depleted of rootletin through RNAi to separate the centrosomes. The cells were also transfected with a control or *gas2l1*-targeting siRNA. Before immunostaining, the cells were treated with 5 μM nocodazole for 20 min. Boxed areas are magnified. Microtubules attached to the mother and daughter centrioles were quantified; n = 61 and 67, si-Control and si-GAS2L1, respectively, from three independent experiments.

(B) mCherry and mCherry-GAS2L1 were transiently expressed in cells depleted of rootletin through RNAi. Centriole-attached microtubules were quantified; n = 88 and 85, mCherry and mCherry-GAS2L1, respectively, from three independent experiments.

(C) HEK293T cells were transfected with a control or *gas2l1*-targeting siRNA and then labeled with phalloidin and anti-GCP3. Boxed centrosomal areas are enlarged. Actin intensity of the centrosomal areas was quantified from three independent experiments; n = 60 for both si-Control and si-GAS2L1 transfections.

(D) Centrosomal actin intensity was quantified from cells expressing GFP or GFP-GAS2L1; n = 60 for both GFP and GFP-GAS2L1, from three independent experiments.

*p < 0.01; **p < 0.001. Error bars denote SD of three independent experiments. Scale bars, 10 μm.

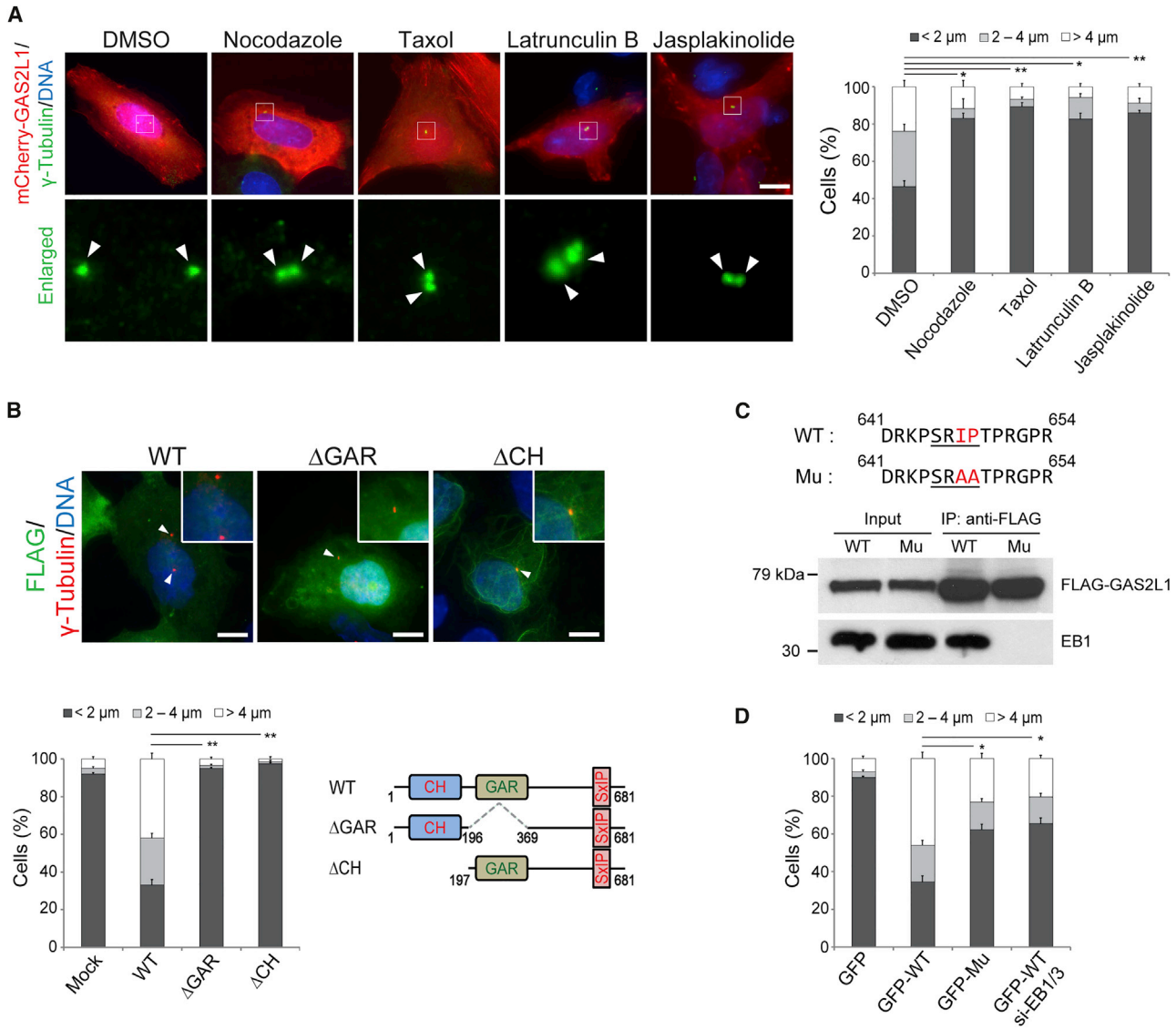

Figure 6. Centrosome-Separating Function of GAS2L1 Requires Its Binding of Microtubules, Actin, and EB Proteins

(A) Upon induction of expression from pLVX-tight-puro-mCherry-GAS2L1, the RPE-1 cells were treated as indicated. Boxed areas are enlarged with arrowheads pointing to centrosomes. Inter-centrosome distances were determined in three independent experiments; n = 71 (DMSO), 77 (nocodazole), 75 (taxol), 72 (latrunculin B), and 77 (jasplakinolide).

(B) Distance was measured between the two centrosomes in cells transfected with FLAG-GAS2L1 or its mutants. WT, wild-type; ΔCH, construct lacking the CH domain (1–196); ΔGAR, construct lacking the GAR domain (198–368). Arrowheads point to centrosomes, and insets show the enlarged centrosomal regions. Data shown are from three independent experiments (mock, n = 131; WT, n = 268; ΔCH, n = 162; ΔGAR, n = 162).

(C) GAS2L1 contains the SxIP motif that binds to EB1. Wild-type (WT) and mutant constructs of GAS2L1 were ectopically expressed in HEK293T cells, and following immunoprecipitation, lysate inputs and immunoprecipitates were analyzed through anti-FLAG and anti-EB1 immunoblotting. Mu denotes the GAS2L1 I647A/P648A mutant.

(D) Distance between the two centrosomes was measured in RPE-1 cells transiently transfected with vector (GFP), GAS2L1 (WT), or I647A/P648A mutant (Mu). WT + si-EB1/3 denotes GAS2L1 expressed in cells in which both EB1 and EB3 were depleted using RNAi. The inter-centrosome distances presented here are from three independent experiments (GFP, n = 128; WT, n = 148; Mu, n = 167; WT + si-EB1/3, n = 188).

*$p < 0.01$; **$p < 0.001$. Error bars denote SD of three independent experiments. Scale bars, 10 μm. See also Figure S6.

GAS2L1 is stably localized on centrosomes. Although both GAS2L1 and C-Nap1 are proximal end-associated proteins, they localize at centrosomes independently: RNAi-mediated suppression of GAS2L1 expression did not affect the centrosomal localization of C-Nap1 and vice versa (Figure S2B and data not shown). At present, how GAS2L1 localizes to centrioles is unclear. The centrosomal localization was mapped to a GAS2L1 region (i.e., 369–560) that does not show clear homology to known centrosomal proteins. To elucidate the centrosome-targeting mechanism one could search for centrosomal proteins that interact with this GAS2L1 region, and the search can be performed using various methods, such as proximity-dependent

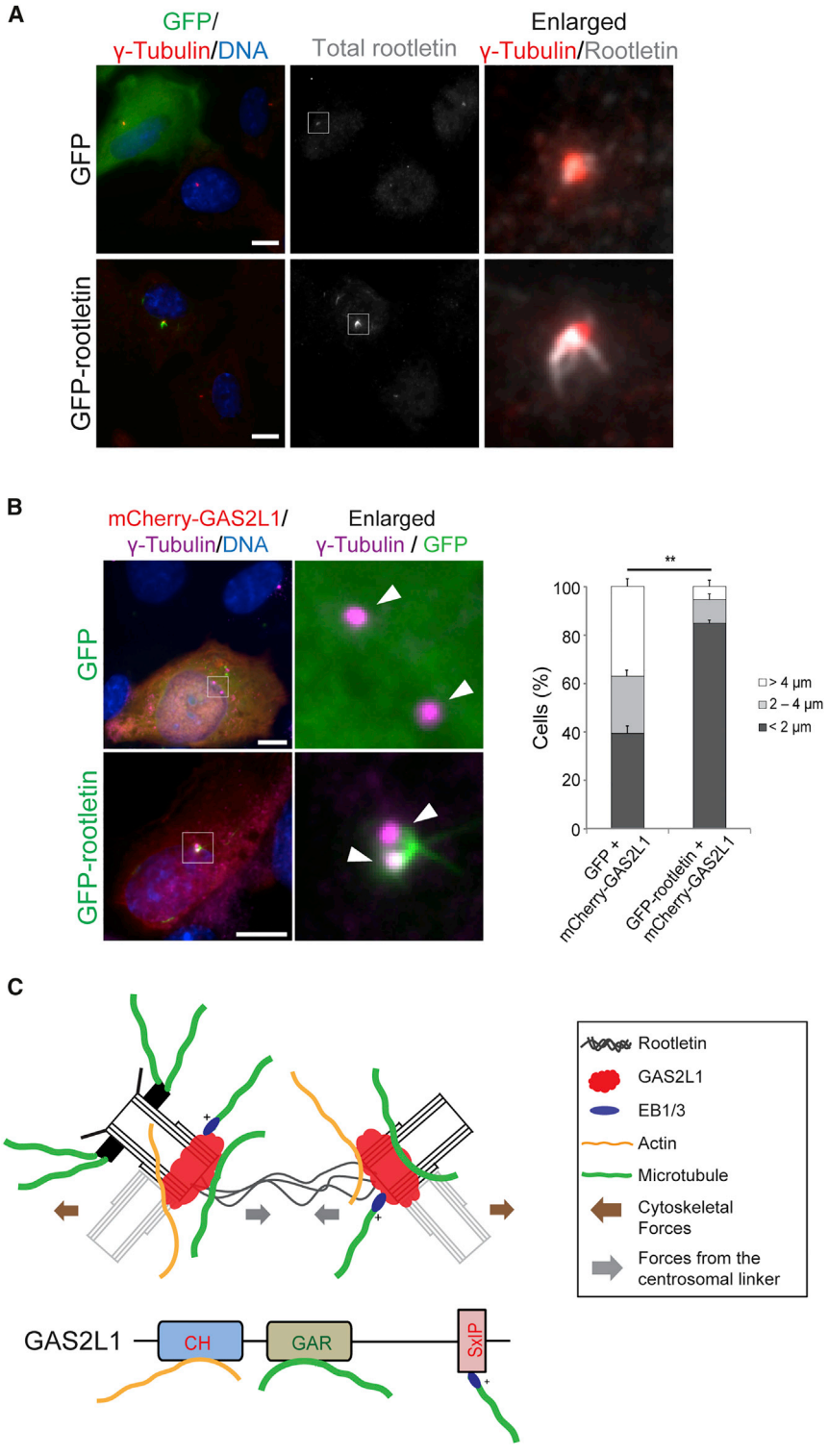

Figure 7. Rootletin Overexpression Inhibits GAS2L1-Induced Centrosome Separation

(A) RPE-1 cells were transfected with plasmids expressing GFP or GFP-rootletin and stained for total rootletin and γ-tubulin. Scale bars, 10 μm.

(B) mCherry-GAS2L1 was coexpressed with GFP or GFP-rootletin. Boxed areas are enlarged with arrowheads pointing to centrosomes. Inter-centrosome distances were measured from three independent experiments (n = 89 for mCherry-GAS2L1 + GFP and n = 92 for mCherry-GAS2L1 + GFP-rootletin). **p < 0.001. Error bars denote SD of three independent experiments. Scale bars, 10 μm.

(C) Model depicting the opposing roles of the GAS2L1-attached cytoskeletons and the centriole linker in the regulation of inter-centrosomal distance. The newly synthesized procentrioles are depicted in light gray. The binding of the cytoskeletons to the GAS2L1 domains is shown below. After disassembly of the centriole linker in late G2, the forces exerted by the GAS2L1-attached cytoskeletons drive centrosome separation.

biotin identification, a method that has recently been applied for analyzing proximity interactors on centrosomes (Roux et al., 2012; Firat-Karalar et al., 2014). Moreover, this centrosome-targeting sequence is not found in other GAS2-like family members, which suggests that GAS2L1 is a unique GAS2-like protein that possesses the aforementioned centrosomal function.

Our data have shown that GAS2L1 attaches microtubules and actin to centrosomes and that centrosome disjunction requires the centrosomal function of GAS2L1. Centrosomes experience strong forces exerted at least by microtubules; the forces are likely exerted on centrioles because nullifying the centrioles renders the centrosomes susceptible to fragmentation by microtubule-derived forces (Abal et al., 2005). GAS2L1 serves as the attachment site for microtubules and actin filaments at the proximal end of the mother centrioles in the two centriole pairs and thus directs the forces to the two centrioles. Such forces are required at least for the dynamic movement of centrioles and for centrosome separation: disruption of GAS2L1 function inhibited centriole motility and centrosome disjunction (Figure 2). Therefore, the cytoskeletons attached to centrosomal GAS2L1 play key roles in centriole movement and centrosome positioning and separation.

Both microtubules and actin filaments participate in separating the duplicated centrosomes before nuclear-envelope breakdown (Schatten et al., 1988; Waters et al., 1993; Whitehead et al., 1996; Uzbekov et al., 2002; Wang et al., 2008; Cao et al., 2010). Our results indicate that the GAS2L1-attached cytoskeletons and the rootletin-dependent centrosome linker exert opposing effects on centrioles, and a balance appears to be maintained in interphase between the two opposing actions until late G2, during which disjunction occurs (Figure 7C). An imbalance caused by either overexpressing GAS2L1 or disrupting rootletin function leads to premature

centrosome separation (Figures 2D and 3A), and rootletin coexpression blocks GAS2L1-induced centrosome separation presumably by restoring the balance between the opposing actions of the GAS2L1-attached cytoskeletons and the centrosome linker (Figure 7B). In late G2, the forces exerted on the centrosomes increase drastically (Abal et al., 2005), and this increase would facilitate centrosome separation triggered by the Nek2A-mediated dissolution of the centrosome linker. Notably, GAS2L1 is absent from the nascent daughter centrioles in the two centriole pairs (Figures 1B and 1D), and this localization of GAS2L1 might ensure that the forces do not segregate the mother and daughter centrioles at this stage in the cell cycle.

Intriguingly, the centrosome-splitting function of GAS2L1 strongly depends on its interaction with EB1/3 (Figure 6D), although the interaction is not required for localizing GAS2L1 to centrosomes (Figure S6). Apparently the EB1/3-GAS2L1 complex could attach microtubule plus ends to the centrioles (Figure 7C). A recent study revealed that GAS2L1 requires its binding with EB proteins to mediate the crosstalk between microtubules and actin filaments and to regulate microtubule dynamics and stability (Stroud et al., 2014). Accordingly, we propose that GAS2L1 and its bound EB1/3 potentially regulate the microtubule-actin crosstalk and microtubule dynamics on centrosomes, and that these functions are involved in centrosome dynamics and disjunction. EB1 and EB3 have long been recognized to associate with centrosomes (Rehberg and Gräf, 2002; Pedersen et al., 2003; Louie et al., 2004; Schrøder et al., 2011), and our findings now further reveal the functional involvement of EB1/3 in centrosome separation.

Following centrosome disjunction and mitotic entry of cells, the kinesin Eg5 plays a crucial role in further moving the centrosomes to the two opposite ends of the cell (Mardin et al., 2010; Smith et al., 2011). We have shown that GAS2L1-mediated disjunction occurs in G2 and is independent of the Eg5 motor. Previous work has shown that after defective disjunction, Eg5 can still push the unseparated centrosomes apart to generate a bipolar spindle (Mardin et al., 2010; Smith et al., 2011) and, in accord, knockdown of Nek2, Mst2, or hSav1—proteins that participate in centrosome disjunction—did not block the formation of bipolar spindles (Mardin et al., 2010). Similarly, we found that bipolar spindles still formed in cells after RNAi-mediated depletion of GAS2L1 (data not shown).

Centrosome disjunction and subsequent Eg5-driven poleward movement represent a well-coordinated process that allows proper spindle formation and accurate chromosome segregation. After defective centrosome disjunction, bipolar spindles are assembled through the motor-dependent pathway, which is prone to developing mitotic errors, such as spindle geometry defects, kinetochore misattachments, and chromosome missegregation (Silkworth et al., 2012; Zhang et al., 2012; Nam and van Deursen, 2014). By contrast, centrosome separation before nuclear-envelope breakdown or even in the S phase facilitates mitotic progression and reduces the error rates of chromosome segregation (Kaseda et al., 2012; Mardin et al., 2013). *gas2l1* has been reported to be one of the genes whose expression is silenced in acute myeloid leukemia (Desmond et al., 2007), a disorder frequently associated with genome instability. Therefore, GAS2L1 is likely to play a role in maintaining genome stability by ensuring the proper timing of centrosome separation.

EXPERIMENTAL PROCEDURES

The DNA constructs and siRNAs used in this study are described in detail in Supplemental Experimental Procedures.

Antibodies

The following primary antibodies were obtained from commercial sources: rabbit anti-GAS2L1 (Abcam), mouse anti-γ-tubulin (Sigma-Aldrich), goat anti-γ-tubulin (Santa Cruz Biotechnology), mouse anti-C-Nap1 (BD Biosciences), mouse and goat anti-rootletin (Santa Cruz), mouse anti-polyglutamylated tubulin (GT335, Adipogen), rabbit anti-CENP-F (Santa Cruz), mouse anti-SAS-6 (Santa Cruz), rabbit anti-ODF2 (Abcam), mouse anti-GAPDH (Ambion), mouse anti-centrin (20H5, Millipore), mouse anti-α-tubulin (Sigma-Aldrich), and anti-FLAG (M2 and polyclonal, Sigma-Aldrich). The following polyclonal antibodies were generated by immunizing rabbits with His6-tagged proteins purified from bacterial expression systems: anti-GAS2L1, using 1–207 or 494–681 of GAS2L1; anti-EB1, using the full-length sequence of mouse EB1; and anti-GFP, using the entire protein. To purify the antibodies, antigens were expressed as glutathione S-transferase (GST) fusions in bacteria, purified, and immobilized on CNBr-activated Sepharose (GE Healthcare), then the antisera were adsorbed using the respective GST proteins for affinity isolation. The purified antibodies were tested through immunoblotting and immunostaining. The presented GAS2L1 staining data were obtained using the antibody that recognizes 494–681, unless indicated otherwise. Antibodies against GCP3 and GCP6 were generated and used as described by Choi et al. (2010).

Cell Culture

HEK293T, HeLa, and U2OS cells were cultured on poly-D-lysine-coated dishes or coverslips with DMEM plus 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen). hTERT RPE-1 cells were maintained in DMEM/F12 (1:1) supplemented with 10% fetal bovine serum, 0.01 mg/mL hygromycin B (Sigma-Aldrich), and 1% penicillin/streptomycin. The percentage of G2 cells (i.e., CENP-F-positive cells) harboring separated centrosomes (inter-centrosome distance ≥ 2 μm) measured by different research groups could potentially vary, possibly due to differences in cell-culture reagents (e.g., EGF content in the serum used). RPE-1 and HeLa cells stably expressing GFP-centrin 1 were provided by Dr. Alexey Khodjakov of the Wadsworth Center. Details of transfection, stable cell line generation, and other treatments are described in Supplemental Experimental Procedures.

Cell Imaging

Fluorescence images of fixed cells were acquired using an inverted microscope (Axio Observer ZI, Carl Zeiss). Super-resolution imaging was performed using stochastic optical reconstruction microscopy, as described previously (Zhao et al., 2015). Live-cell imaging was performed on a spinning-disk microscope (Axio Observer ZI, Carl Zeiss; Laser-based Spinning Disk System, Andor). Imaging details are provided in Supplemental Experimental Procedures.

Immunoprecipitation

HEK293T cells were extracted in a lysis buffer (25 mM Tris-HCl [pH 7.4], 0.5% IGEPAL CA-630, 100 mM NaCl, 5 mM $MgCl_2$, 5 mM NaF, 1 mM DTT, Roche Complete Protease Inhibitor Cocktail) and the extracts were clarified through centrifugation (16,000 × *g*, 15 min) at 4°C. Anti-FLAG immunoprecipitation was performed with ANTI-FLAG M2 Affinity Gel (Sigma-Aldrich) for 2 hr at 4°C (with rotation), and the obtained immunoprecipitates and lysate inputs were immunoblotted.

Statistical Analysis

All quantified datasets were collected from at least three independent experiments, and were analyzed using the unpaired Student's t test.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.devcel.2016.11.019.

AUTHOR CONTRIBUTIONS

F.K.C.A., Y.J., A.A., and R.Z.Q. designed the study. F.K.C.A. and Y.J. performed the experiments. K.J. and I.G. contributed tools for live-cell imaging. B.K.T.H. and Y.S. contributed to antibody generation. S.D. contributed tools for super-resolution microscopy. F.K.C.A., Y.J., A.A., and R.Z.Q. wrote the paper with input from all authors.

ACKNOWLEDGMENTS

We thank Daiying Xu (NanoBioImaging Ltd., Hong Kong), Teng Zhao, and Pik Ki Lau (The Hong Kong University of Science and Technology, Hong Kong) for help with STORM imaging; NanoBioImaging Ltd. for developing the software for STORM imaging and analysis; and Jingchao Wu (Utrecht University, the Netherlands) for technical assistance. This work was supported by grants from the Research Grants Council (General Research Fund, Theme-based Research Scheme, and Collaborative Research Fund) of Hong Kong, the National Key Basic Research Program of China (2013CB530900), the University Grants Committee (Area of Excellence Scheme) of Hong Kong, the Innovation and Technology Commission (ITCPD/17-9) of Hong Kong, and the TUYF Charitable Trust. S.D. acknowledges support from the Offices of the Provost, VPRG, and Dean of Science, HKUST (VPRGO12SC02), and Hong Kong Research Grants Council (HKUST12/CRF/13G, C6030-14E). F.K.C.A. gratefully acknowledges support from the Hong Kong Ph.D. Fellowship Scheme.

Received: May 29, 2016
Revised: October 17, 2016
Accepted: November 21, 2016
Published: December 22, 2016

REFERENCES

Abal, M., Keryer, G., and Bornens, M. (2005). Centrioles resist forces applied on centrosomes during G2/M transition. Biol. Cell *97*, 425–434.

Akhmanova, A., and Steinmetz, M.O. (2008). Tracking the ends: a dynamic protein network controls the fate of microtubule tips. Nat. Rev. Mol. Cell Biol. *9*, 309–322.

Bahe, S., Stierhof, Y.-D., Wilkinson, C.J., Leiss, F., and Nigg, E.A. (2005). Rootletin forms centriole-associated filaments and functions in centrosome cohesion. J. Cell Biol. *171*, 27–33.

Bertran, M.T., Sdelci, S., Regué, L., Avruch, J., Caelles, C., and Roig, J. (2011). Nek9 is a Plk1-activated kinase that controls early centrosome separation through Nek6/7 and Eg5. EMBO J. *30*, 2634–2647.

Blangy, A., Lane, H.A., d'Hérin, P., Harper, M., Kress, M., and Nigg, E.A. (1995). Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. Cell *83*, 1159–1169.

Burakov, A., Nadezhdina, E., Slepchenko, B., and Rodionov, V. (2003). Centrosome positioning in interphase cells. J. Cell Biol. *162*, 963–969.

Cao, J., Crest, J., Fasulo, B., and Sullivan, W. (2010). Cortical actin dynamics facilitate early stage centrosome separation. Curr. Biol. *20*, 770–776.

Choi, Y.-K., Liu, P., Sze, S.K., Dai, C., and Qi, R.Z. (2010). CDK5RAP2 stimulates microtubule nucleation by the γ-tubulin ring complex. J. Cell Biol. *191*, 1089–1095.

DeBonis, S., Skoufias, D.A., Lebeau, L., Lopez, R., Robin, G., Margolis, R.L., Wade, R.H., and Kozielski, F. (2004). In vitro screening for inhibitors of the human mitotic kinesin Eg5 with antimitotic and antitumor activities. Mol. Cancer Ther. *3*, 1079–1090.

Desmond, J.C., Raynaud, S., Tung, E., Hofmann, W.-K., Haferlach, T., and Koeffler, H.P. (2007). Discovery of epigenetically silenced genes in acute myeloid leukemias. Leukemia *21*, 1026–1034.

Faragher, A.J., and Fry, A.M. (2003). Nek2A kinase stimulates centrosome disjunction and is required for formation of bipolar mitotic spindles. Mol. Biol. Cell *14*, 2876–2889.

Farina, F., Gaillard, J., Guérin, C., Couté, Y., Sillibourne, J., Blanchoin, L., and Théry, M. (2016). The centrosome is an actin-organizing centre. Nat. Cell Biol. *18*, 65–75.

Fırat-Karalar, E.N., and Stearns, T. (2014). The centriole duplication cycle. Philos. Trans. R. Soc. Lond. B Biol. Sci. *369*, 20130460.

Firat-Karalar, E.N., Rauniyar, N., Yates, J.R., and Stearns, T. (2014). Proximity interactions among centrosome components identify regulators of centriole duplication. Curr. Biol. *24*, 664–670.

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.

Gamper, I., Fleck, D., Barlin, M., Spehr, M., El Sayad, S., Kleine, H., Maxeiner, S., Schalla, C., Aydin, G., Hoss, M., et al. (2016). GAR22β regulates cell migration, sperm motility, and axoneme structure. Mol. Biol. Cell *27*, 277–294.

Goriounov, D., Leung, C.L., and Liem, R.K.H. (2003). Protein products of human Gas2-related genes on chromosomes 17 and 22 (hGAR17 and hGAR22) associate with both microfilaments and microtubules. J. Cell Sci. *116*, 1045–1058.

Graser, S., Stierhof, Y.-D., and Nigg, E.A. (2007). Cep68 and Cep215 (Cdk5rap2) are required for centrosome cohesion. J. Cell Sci. *120*, 4321–4331.

Hardy, T., Lee, M., Hames, R.S., Prosser, S.L., Cheary, D.-M., Samant, M.D., Schultz, F., Baxter, J.E., Rhee, K., and Fry, A.M. (2014). Multisite phosphorylation of C-Nap1 releases it from Cep135 to trigger centrosome disjunction. J. Cell Sci. *127*, 2493–2506.

He, R., Huang, N., Bao, Y., Zhou, H., Teng, J., and Chen, J. (2013). LRRC45 is a centrosome linker component required for centrosome cohesion. Cell Rep. *4*, 1100–1107.

Honnappa, S., Gouveia, S.M., Weisbrich, A., Damberger, F.F., Bhavesh, N.S., Jawhari, H., Grigoriev, I., van Rijssel, F.J.A., Buey, R.M., Lawera, A., et al. (2009). An EB1-binding motif acts as a microtubule tip localization signal. Cell *138*, 366–376.

Ibi, M., Zou, P., Inoko, A., Shiromizu, T., Matsuyama, M., Hayashi, Y., Enomoto, M., Mori, D., Hirotsune, S., Kiyono, T., et al. (2011). Trichoplein controls microtubule anchoring at the centrosome by binding to Odf2 and ninein. J. Cell Sci. *124*, 857–864.

Jaworski, J., Hoogenraad, C.C., and Akhmanova, A. (2008). Microtubule plus-end tracking proteins in differentiated mammalian cells. Int. J. Biochem. Cell Biol. *40*, 619–637.

Jiang, K., Toedt, G., Montenegro Gouveia, S., Davey, N.E., Hua, S., van der Vaart, B., Grigoriev, I., Larsen, J., Pedersen, L.B., Bezstarosti, K., et al. (2012). A proteome-wide screen for mammalian SxIP motif-containing microtubule plus-end tracking proteins. Curr. Biol. *22*, 1800–1807.

Kaseda, K., McAinsh, A.D., and Cross, R.A. (2012). Dual pathway spindle assembly increases both the speed and the fidelity of mitosis. Biol. Open *1*, 12–18.

Kimura, K., and Kimura, A. (2011). A novel mechanism of microtubule length-dependent force to pull centrosomes toward the cell center. Bioarchitecture *1*, 74–79.

Komarova, Y., De Groot, C.O., Grigoriev, I., Gouveia, S.M., Munteanu, E.L., Schober, J.M., Honnappa, S., Buey, R.M., Hoogenraad, C.C., Dogterom, M., et al. (2009). Mammalian end binding proteins control persistent microtubule growth. J. Cell Biol. *184*, 691–706.

Leidel, S., Delattre, M., Cerutti, L., Baumer, K., and Gönczy, P. (2005). SAS-6 defines a protein family required for centrosome duplication in *C. elegans* and in human cells. Nat. Cell Biol. *7*, 115–125.

Liao, H., Winkfein, R.J., Mack, G., Rattner, J.B., and Yen, T.J. (1995). CENP-F is a protein of the nuclear matrix that assembles onto kinetochores at late G2 and is rapidly degraded after mitosis. J. Cell Biol. *130*, 507–518.

Louie, R.K., Bahmanyar, S., Siemers, K.A., Votin, V., Chang, P., Stearns, T., Nelson, W.J., and Barth, A.I.M. (2004). Adenomatous polyposis coli and EB1 localize in close proximity of the mother centriole and EB1 is a functional component of centrosomes. J. Cell Sci. *117*, 1117–1128.

Mardin, B.R., and Schiebel, E. (2012). Breaking the ties that bind: new advances in centrosome biology. J. Cell Biol. *197*, 11–18.

Mardin, B.R., Lange, C., Baxter, J.E., Hardy, T., Scholz, S.R., Fry, A.M., and Schiebel, E. (2010). Components of the Hippo pathway cooperate with Nek2 kinase to regulate centrosome disjunction. Nat. Cell Biol. *12*, 1166–1176.

Mardin, B.R., Agircan, F.G., Lange, C., and Schiebel, E. (2011). Plk1 controls the Nek2A-PP1γ antagonism in centrosome disjunction. Curr. Biol. *21*, 1145–1151.

Mardin, B.R., Isokane, M., Cosenza, M.R., Krämer, A., Ellenberg, J., Fry, A.M., and Schiebel, E. (2013). EGF-induced centrosome separation promotes mitotic progression and cell survival. Dev. Cell *25*, 229–240.

Mayor, T., Stierhof, Y.D., Tanaka, K., Fry, A.M., and Nigg, E.A. (2000). The centrosomal protein C-Nap1 is required for cell cycle-regulated centrosome cohesion. J. Cell Biol. *151*, 837–846.

Moudjou, M., Bordes, N., Paintrand, M., and Bornens, M. (1996). gamma-Tubulin in mammalian cells: the centrosomal and the cytosolic forms. J. Cell Sci. *109*, 875–887.

Nam, H.J., and van Deursen, J.M. (2014). Cyclin B2 and p53 control proper timing of centrosome separation. Nat. Cell Biol. *16*, 538–549.

Nam, H.-J., Naylor, R.M., and van Deursen, J.M. (2015). Centrosome dynamics as a source of chromosomal instability. Trends Cell Biol. *25*, 65–73.

Nigg, E.A., and Stearns, T. (2011). The centrosome cycle: centriole biogenesis, duplication and inherent asymmetries. Nat. Cell Biol. *13*, 1154–1160.

Obino, D., Farina, F., Malbec, O., Sáez, P.J., Maurin, M., Gaillard, J., Dingli, F., Loew, D., Gautreau, A., Yuseff, M.-I., et al. (2016). Actin nucleation at the centrosome controls lymphocyte polarity. Nat. Commun. *7*, 10969.

Paoletti, A., Moudjou, M., Paintrand, M., Salisbury, J.L., and Bornens, M. (1996). Most of centrin in animal cells is not centrosome-associated and centrosomal centrin is confined to the distal lumen of centrioles. J. Cell Sci. *109*, 3089–3102.

Pedersen, L.B., Geimer, S., Sloboda, R.D., and Rosenbaum, J.L. (2003). The microtubule plus end-tracking protein EB1 is localized to the flagellar tip and basal bodies in *Chlamydomonas reinhardtii*. Curr. Biol. *13*, 1969–1974.

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.

Raaijmakers, J.A., van Heesbeen, R.G.H.P., Meaders, J.L., Geers, E.F., Fernandez-Garcia, B., Medema, R.H., and Tanenbaum, M.E. (2012). Nuclear envelope-associated dynein drives prophase centrosome separation and enables Eg5-independent bipolar spindle formation. EMBO J. *31*, 4179–4190.

Rehberg, M., and Gräf, R. (2002). Dictyostelium EB1 is a genuine centrosomal component required for proper spindle formation. Mol. Biol. Cell *13*, 2301–2310.

Roux, K.J., Kim, D.I., Raida, M., and Burke, B. (2012). A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. J. Cell Biol. *196*, 801–810.

Sawin, K.E., LeGuellec, K., Philippe, M., and Mitchison, T.J. (1992). Mitotic spindle organization by a plus-end-directed microtubule motor. Nature *359*, 540–543.

Schatten, H., Walter, M., Biessmann, H., and Schatten, G. (1988). Microtubules are required for centrosome expansion and positioning while microfilaments are required for centrosome separation in sea urchin eggs during fertilization and mitosis. Cell Motil. Cytoskeleton *11*, 248–259.

Schrøder, J.M., Larsen, J., Komarova, Y., Akhmanova, A., Thorsteinsson, R.I., Grigoriev, I., Manguso, R., Christensen, S.T., Pedersen, S.F., Geimer, S., et al. (2011). EB1 and EB3 promote cilia biogenesis by several centrosome-related mechanisms. J. Cell Sci. *124*, 2539–2551.

Silkworth, W.T., Nardi, I.K., Paul, R., Mogilner, A., and Cimini, D. (2012). Timing of centrosome separation is important for accurate chromosome segregation. Mol. Biol. Cell *23*, 401–411.

Skoufias, D.A., DeBonis, S., Saoudi, Y., Lebeau, L., Crevel, I., Cross, R., Wade, R.H., Hackney, D., and Kozielski, F. (2006). S-Trityl-L-cysteine is a reversible, tight binding inhibitor of the human kinesin Eg5 that specifically blocks mitotic progression. J. Biol. Chem. *281*, 17559–17569.

Smith, E., Hégarat, N., Vesely, C., Roseboom, I., Larch, C., Streicher, H., Straatman, K., Flynn, H., Skehel, M., Hirota, T., et al. (2011). Differential control of eg5-dependent centrosome separation by Plk1 and Cdk1. EMBO J. *30*, 2233–2245.

Stroud, M.J., Nazgiewicz, A., McKenzie, E.A., Wang, Y., Kammerer, R.A., and Ballestrem, C. (2014). GAS2-like proteins mediate communication between microtubules and actin through interactions with end-binding proteins. J. Cell Sci. *127*, 2672–2682.

Tanenbaum, M.E., and Medema, R.H. (2010). Mechanisms of centrosome separation and bipolar spindle assembly. Dev. Cell *19*, 797–806.

Tanenbaum, M.E., Macůrek, L., Janssen, A., Geers, E.F., Alvarez-Fernández, M., and Medema, R.H. (2009). Kif15 cooperates with eg5 to promote bipolar spindle assembly. Curr. Biol. *19*, 1703–1711.

Uzbekov, R., Kireyev, I., and Prigent, C. (2002). Centrosome separation: respective role of microtubules and actin filaments. Biol. Cell *94*, 275–288.

Vaughan, S., and Dawe, H.R. (2011). Common themes in centriole and centrosome movements. Trends Cell Biol. *21*, 57–66.

Wang, W., Chen, L., Ding, Y., Jin, J., and Liao, K. (2008). Centrosome separation driven by actin-microfilaments during mitosis is mediated by centrosome-associated tyrosine-phosphorylated cortactin. J. Cell Sci. *121*, 1334–1343.

Wang, W.-J., Soni, R.K., Uryu, K., and Tsou, M.-F.B. (2011). The conversion of centrioles to centrosomes: essential coupling of duplication with segregation. J. Cell Biol. *193*, 727–739.

Waters, J.C., Cole, R.W., and Rieder, C.L. (1993). The force-producing mechanism for centrosome separation during spindle formation in vertebrates is intrinsic to each aster. J. Cell Biol. *122*, 361–372.

Whitehead, C.M., Winkfein, R.J., and Rattner, J.B. (1996). The relationship of HsEg5 and the actin cytoskeleton to centrosome separation. Cell Motil. Cytoskeleton *35*, 298–308.

Wolff, A., de Néchaud, B., Chillet, D., Mazarguil, H., Desbruyères, E., Audebert, S., Eddé, B., Gros, F., and Denoulet, P. (1992). Distribution of glutamylated alpha and beta-tubulin in mouse tissues using a specific monoclonal antibody, GT335. Eur. J. Cell Biol. *59*, 425–432.

Yang, J., Adamian, M., and Li, T. (2006). Rootletin interacts with C-Nap1 and may function as a physical linker between the pair of centrioles/basal bodies in cells. Mol. Biol. Cell *17*, 1033–1040.

Zhang, Y., Foreman, O., Wigle, D.A., Kosari, F., Vasmatzis, G., Salisbury, J.L., van Deursen, J., and Galardy, P.J. (2012). USP44 regulates centrosome positioning to prevent aneuploidy and suppress tumorigenesis. J. Clin. Invest. *122*, 4362–4374.

Zhao, T., Wang, Y., Zhai, Y., Qu, X., Cheng, A., Du, S., and Loy, M.M.T. (2015). A user-friendly two-color super-resolution localization microscope. Opt. Express *23*, 1879–1887.