Cell Reports
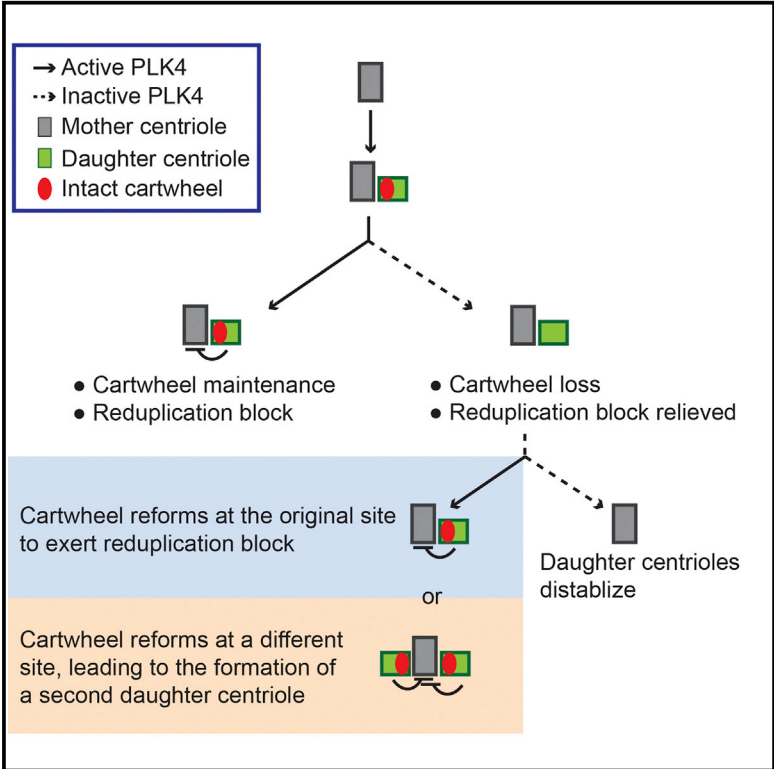
Promotion and Suppression of Centriole Duplication Are Catalytically Coupled through PLK4 to Ensure Centriole Homeostasis

Graphical Abstract

Authors

Minhee Kim, Brian P. O'Rourke,
Rajesh Kumar Soni, Prasad V. Jallepalli,
Ronald C. Hendrickson,
Meng-Fu Bryan Tsou

Correspondence

tsoum@mskcc.org

In Brief

Cellular structures are maintained at constant numbers by balancing the "promotion" and "suppression" of their biogenesis, two processes opposing each other. Through studies on the cell-division organelle, centrioles, Kim et al. found that the two opposing processes are intimately coupled to the same catalytic reaction involving the kinase PLK4, thereby safeguarding centriole homeostasis.

Highlights

- Persistent PLK4 is required to stabilize the cartwheel and newborn centrioles

- Phosphomimetic STIL requires active PLK4 to support centriole duplication

- Phosphomimetic STIL can bypass the requirement of PLK4 for cartwheel maintenance

- Loss of intact cartwheel induces stochastic reduplication of mother centrioles

CellPress

Cell Reports

Report

Promotion and Suppression of Centriole Duplication Are Catalytically Coupled through PLK4 to Ensure Centriole Homeostasis

Minhee Kim,[1,2] Brian P. O'Rourke,[1] Rajesh Kumar Soni,[3] Prasad V. Jallepalli,[4] Ronald C. Hendrickson,[3] and Meng-Fu Bryan Tsou[1,2,*]
[1]Cell Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA
[2]Weill Cornell Graduate School of Medical Sciences, Cornell University, New York, NY 10065, USA
[3]Microchemistry and Proteomics Core Facility, Memorial Sloan-Kettering Cancer Center, New York, NY 10065 USA
[4]Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA
*Correspondence: tsoum@mskcc.org
http://dx.doi.org/10.1016/j.celrep.2016.06.069

SUMMARY

PLK4 is the major kinase driving centriole duplication. Duplication occurs only once per cell cycle, forming one new (or daughter) centriole that is tightly engaged to the preexisting (or mother) centriole. Centriole engagement is known to block the reduplication of mother centrioles, but the molecular identity responsible for the block remains unclear. Here, we show that the centriolar cartwheel, the geometric scaffold for centriole assembly, forms the identity of daughter centrioles essential for the block, ceasing further duplication of the mother centriole to which it is engaged. To ensure a steady block, we found that the cartwheel requires constant maintenance by PLK4 through phosphorylation of the same substrate that drives centriole assembly, revealing a parsimonious control in which "assembly" and "block for new assembly" are linked through the same catalytic reaction to achieve homeostasis. Our results support a recently deduced model that the cartwheel-bound PLK4 directly suppresses centriole reduplication.

INTRODUCTION

Centriole biogenesis in vertebrate cycling cells follows a stereotypical program (Fırat-Karalar and Stearns, 2014; Fu et al., 2015). Cells begin G1 phase with two preexisting centrioles, both of which are capable of recruiting the pericentriolar material (PCM) and thereby functioning as the centrosome or microtubule-organizing center (MTOC). In S phase, the two MTOC-competent centrioles duplicate, each generating one daughter centriole that is incapable of recruiting the PCM (MTOC non-competent) and thus must be tightly attached, or engaged, to its mother for proper segregation in mitosis (Wang et al., 2011). Duplication starts with the formation of the cartwheel, a 9-fold symmetric scaffold forming the base of the daughter centriole.

The newborn, MTOC-non-competent daughter centriole is itself unable to duplicate and can further suppress the duplication potential of the mother centriole to which it is tightly engaged (Loncarek et al., 2008; Tsou and Stearns, 2006; Tsou et al., 2009), strictly limiting centriole duplication to once per cell cycle. At the end of cell cycle, daughter centrioles are converted to centrosomes (MTOC competent) (Wang et al., 2011), disengaged from their mothers (Kuriyama and Borisy, 1981), and removed of the cartwheel (Vorobjev and Chentsov, 1980; Vorobjev and Chentsov YuS, 1982), all of which depend on polo-like kinase 1 (Plk1) (Wang et al., 2011), transforming to mother-like centrioles in the following G1 phase. Centriole-to-centrosome conversion and centriole disengagement, respectively, license daughter and mother centrioles for duplication (Fu et al., 2016; Novak et al., 2014; Wang et al., 2011), but the underlying reason for cartwheel removal is not fully clear.

The newborn (MTOC non-competent) centrioles are "sterile," as they lack the PCM and PCM-associated components such as CEP152 essential for duplication (Cizmecioglu et al., 2010; Dzhindzhev et al., 2010; Fu et al., 2016; Hatch et al., 2010; Novak et al., 2014; Wang et al., 2011). In contrast, it is not understood how mother (MTOC-competent) centrioles also cease to duplicate when they are engaged to newborn centrioles. It has been shown that cells have no arithmetic ability to count the centriole number (Wong and Stearns, 2003) but can somehow differentiate the property intrinsic to duplicated centrioles from that of unduplicated centrioles, allowing "duplication" and "block for re-duplication" to occur simultaneously in the same cytoplasm (Wong and Stearns, 2003). It is possible that the physical property of the engagement, which holds two centrioles closely in short distances (Shukla et al., 2015), blocks reduplication. Alternatively, a specific chemical property intrinsic to newborn centrioles may serve as the "mark" of duplicated centrioles and locally suppress further duplication of the engaged mother. The molecular identity of such a mark, however, is unknown.

Interestingly, the cartwheel that forms and stabilizes newborn centrioles throughout S/G2 phase (Izquierdo et al., 2014) is removed during mitosis before these centrioles support duplication in the following S phase. The pattern of cartwheel assembly or disassembly correlates nicely with the formation or relief of the
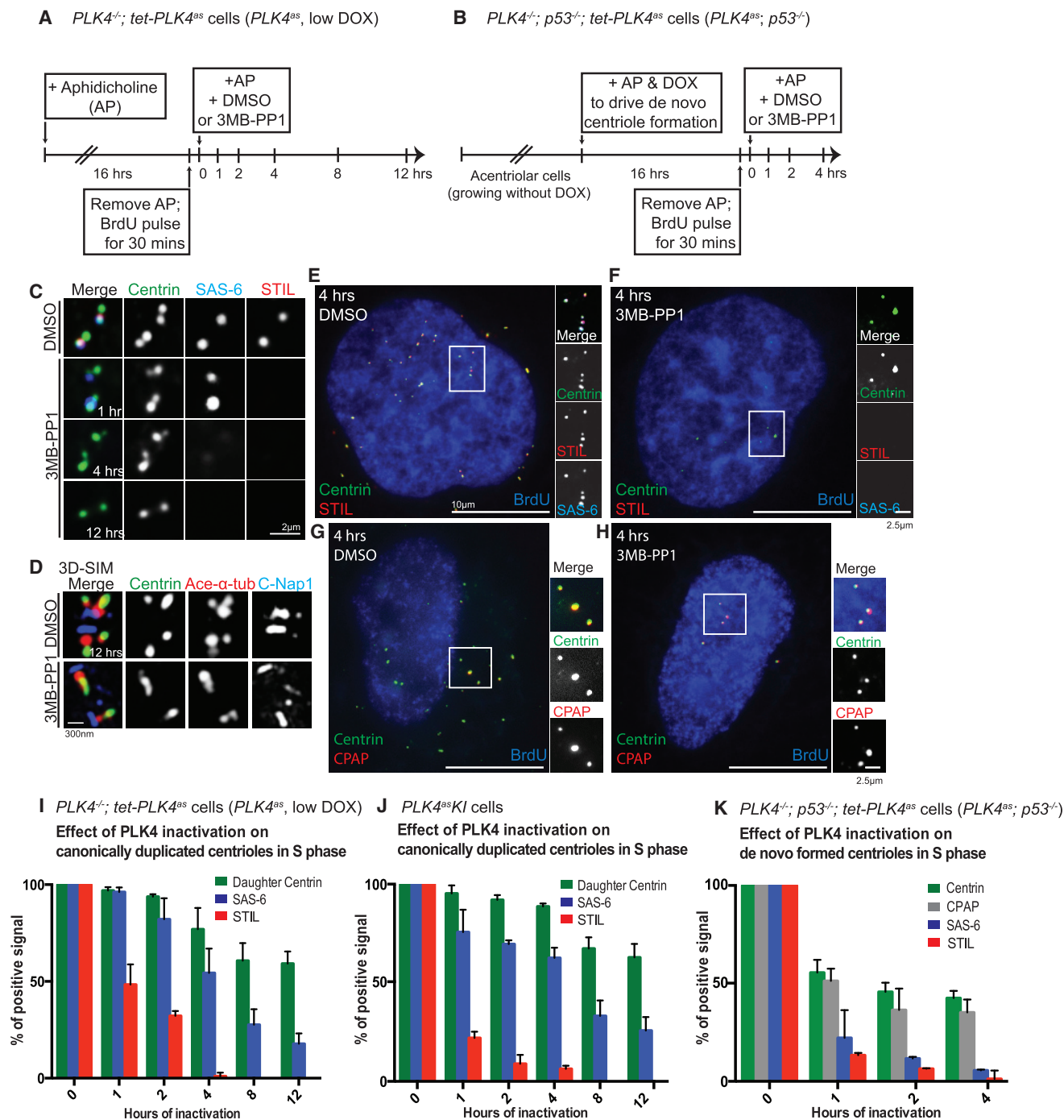
Figure 1. Persistent PLK4 Activity Is Required to Stabilize the Cartwheel and Newborn Centrioles in Either Canonical Duplication or De Novo Synthesis

(A and B) Schematic of the experimental design to inactivate PLK4 in late S phase cells containing either (A) canonically duplicated or (B) de novo centrioles, using the $PLK4^{as}$ or $PLK4^{as}; p53^{-/-}$ cell line, respectively, as indicated. Doxycycline (DOX) was added as indicated to induce PLK4 expression. De novo centrioles were induced to form in acentriolar $PLK4^{as}; p53^{-/-}$ cells in S phase by adding doxycycline.

(C) The serial loss of cartwheel components from canonically duplicated newborn centrioles at indicated time points after PLK4 inactivation was characterized using the indicated antibodies. Top: centrioles from DMSO control treatment. Bottom: three representative images of PLK4 inactivation by 3MB-PP1 at each time point.

(D) Superresolution 3D-SIM image of acetylated tubulin, centrin, and C-Nap1 in S phase centrioles after 12 hr of control treatment (DMSO, top) or PLK4 inactivation (bottom).

(legend continued on next page)

duplication block during the cell cycle, raising a testable model that perhaps the cartwheel is the "mark" of the newborn centriole constituting the block. It has been reported that the centriolar protein SAS-6 and its binding partner STIL form the cartwheel (Cottee et al., 2015; Kitagawa et al., 2011; Qiao et al., 2012; van Breugel et al., 2011) in a process catalyzed by the polo-like kinase PLK4 (Arquint et al., 2015; Dzhindzhev et al., 2014; Kratz et al., 2015; Moyer et al., 2015; Ohta et al., 2014) and that loss of the cartwheel during mitosis depends on Plk1 (Wang et al., 2011) and Cdk1 (Arquint and Nigg, 2014). Here, we aim to disrupt cartwheel dynamics through manipulations of PLK4 and STIL, with a goal to determine whether the cartwheel is involved in the block for centriole reduplication.

RESULTS

Persistent PLK4 Activity Stabilizes the Cartwheel and Newborn Centrioles

To address cartwheel dynamics and maintenance after centriole duplication has occurred, a number of retinal pigment epithelial (RPE1) cell lines in which PLK4 can be conditionally removed or inactivated were made (see Table S1 and Experimental Procedures for details). We generated $PLK4^{flox/neoflox}$ cells in which the endogenous, wild-type $PLK4$ can be conditionally deleted by expression of the Cre recombinase, a derived cell line in which the endogenous Plk4 is deleted, and an analog-sensitive mutant ($PLK4^{as}$) that can be chemically inactivated by bulky ATP analog is expressed under the tetracycline inducible promoter ($PLK4^{-/-}$; tet-$PLK4^{as}$; see Figure S1 and Supplemental Experimental Procedures for details). To ensure proper expression of PLK4as under the endogenous promoter, we also obtained a PLK4as knockin cell line ($PLK4^{as}KI$; unpublished data), similar to the DLD-1 cell line recently reported (Moyer et al., 2015). Moreover, using these cells, we constructed other derived cell lines in which the endogenous p53 and STIL were inactivated by clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene targeting, generating acentriolar cell lines in which centrioles made of different forms of STIL can be subsequently reconstituted (Table S1) (Izquierdo et al., 2014; Wang et al., 2015). For clarity, the brief name of each cell line is used in the main text, and detailed genetic information is listed in Table S1.

We first examined if cartwheel maintenance requires constant PLK4 activity in $PLK4^{as}$ cells ($PLK4^{-/-}$; tet-$PLK4^{as}$) arrested at S phase, when the cartwheel is present in every newborn daughter centriole (Figure 1A). Unlike control cells always carrying two pairs of duplicated centrioles in which the newly formed daughter was labeled with STIL or SAS-6 (Figure 1C, top row), inactivation of PLK4 resulted in serial losses of the cart-

wheel components from daughter centrioles (Figures 1C and 1I). Specifically, 1 hr after PLK4 inactivation, ∼50% of daughter centrioles lost STIL (Figure 1C, second row); by 4 hr, a similar loss of SAS-6 was observed (Figure 1C, third row), consistent with the previous report (Moyer et al., 2015). Moreover, after 12 hr, daughter centrioles started to disappear (Figure 1C, bottom row; Figures 1D and 1I), leaving behind two single mother centrioles each of which was labeled with centrin, C-Nap1, and acetylated alpha tubulin, as revealed by superresolution microscopy (3D-SIM) (Figure 1D). This is consistent with our previous report that the cartwheel is required to stabilize newborn centrioles (Izquierdo et al., 2014). Rapid losses of STIL and SAS-6 from the cartwheel upon PLK4 inactivation were also observed with $PLK4^{as}$ knockin RPE1 cells ($PLK4^{as}KI$) (Figure 1J). Note that in the absence of the cartwheel, daughter centrioles remained engaged to the mother centriole before they eventually lost stability (Figure 1C, second and third row). The requirement of PLK4 in stabilizing the cartwheel was also seen for de novo centrioles (Figures 1E, 1H, and 1K), which were reconstituted in $PLK4^{as}$; $p53^{-/-}$ cells (Figure 1B; Table S1). Interestingly, the loss of SAS-6 and centrin from de novo centrioles upon PLK4 inactivation occurred much faster than that from canonically duplicated centrioles (Figures 1I–1K). After 4 hr of PLK4 inactivation, the number of de novo centrioles, as judged by centrin and CPAP staining, dropped from 59 per cell on average before PLK4 inactivation to 11 per cell. These results together suggest that the cartwheel is a dynamic structure requiring constant maintenance by PLK4 and that cartwheel maintenance is essential for the long-term stability of newborn centrioles.

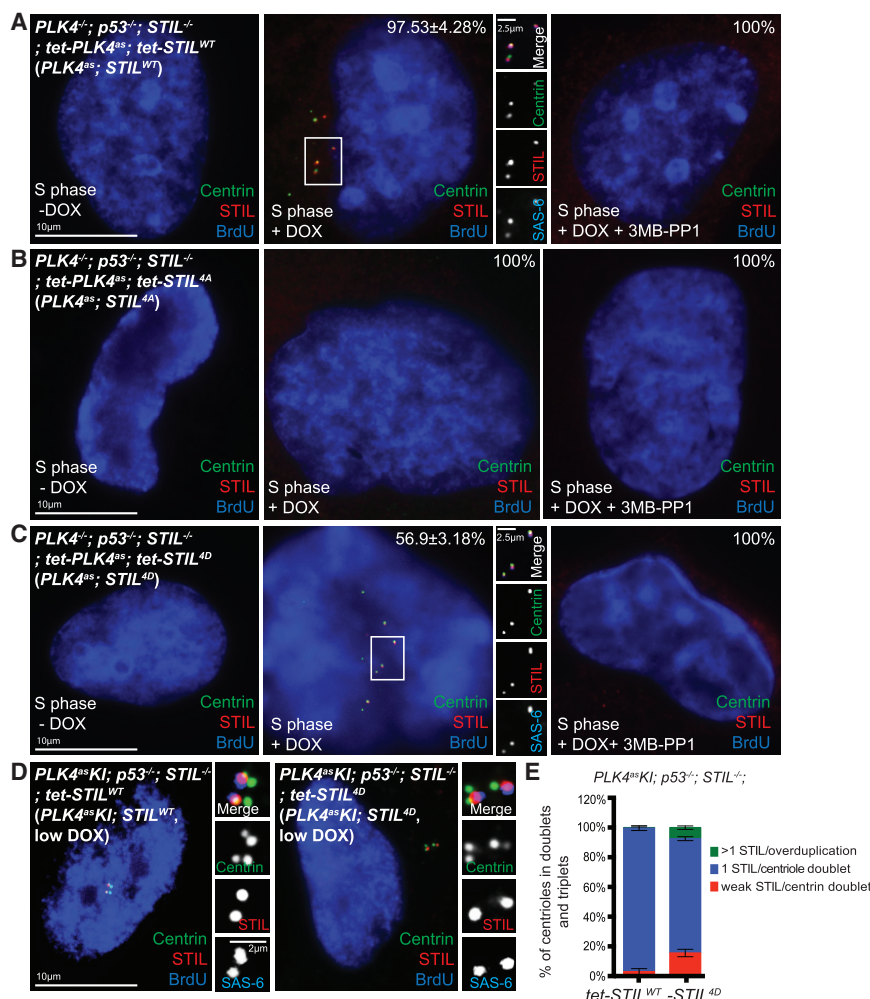### Phosphomimetic STIL Supports Centriole Assembly Only When PLK4 Is Present

Recent studies showed that phosphorylation of STIL by PLK4 drives cartwheel assembly (Dzhindzhev et al., 2014; Kratz et al., 2015; Moyer et al., 2015; Ohta et al., 2014). While the four critical residues phosphorylated by PLK4 (S1061, S1111, S1116, and T1119) are conserved between human and Drosophila (Dzhindzhev et al., 2014), it is unclear if phosphomimetic STIL can bypass the requirement of PLK4 for centriole assembly. To address it, epitope-tagged full-length STIL carrying wild-type ($STIL^{WT}$), phosphonull ($STIL^{4A}$) or phosphomimetic ($STIL^{4D}$) residues were made and expressed in acentriolar, $PLK4^{as}$; $STIL^{-/-}$ cells (Table S1) in the presence or absence of 3MB-PP1. As expected, in the presence of PLK4 activity (no 3MB-PP1), expression of $STIL^{WT}$ or $STIL^{4D}$, but not $STIL^{4A}$, successfully rescued de novo centriole formation, as revealed by centrin, STIL, and SAS-6 staining (Figures 2A–2C, left and center). Conversely, when PLK4 was inactivated, none of the constructs, including $STIL^{4D}$, could drive de novo centriole formation (Figures 2A–2C, right). These results together indicate that while

(E–H) De novo formed centrioles in S phase were treated with DMSO (E and G) or inhibited of PLK4 (F and H) and analyzed by immunofluorescence microscopy using the indicated antibodies. Upon PLK4 inactivation, cartwheel components STIL and SAS-6 (F), but not the core centriolar component CPAP (H), were quickly lost. Note also that the number of de novo centrioles (centrin/CPAP foci) was dramatically reduced after 3MB-PP1 treatments (F and H), faster than that for canonical centrioles (see I–K).

(I) Quantification of (C). Error bars represent SD; n > 50, N = 3.

(J) Similar cartwheel loss was also seen and quantified in $PLK4^{as}KI$ cells. Error bars represent SD; n > 50, N = 3

(K) Quantification of (E)–(H). Error bars represent SD; n > 25, N = 3.

Figure 2. Phosphomimetic STIL Supports Centriole Assembly but Cannot Bypass the Requirement of PLK4

(A–C) Acentriolar $PLK4^{as}$; $STIL^{-/-}$ cells stably carrying exogenous, tetracycline-inducible STIL constructs were induced to express $STIL^{WT}$ (A), $STIL^{4A}$ (B), or $STIL^{4D}$ (C) in the presence or absence of 3MB-PP1, as indicated, and examined for centriole assembly using SAS-6, STIL, and centrin antibodies. Note that $STIL^{WT}$ (A) and $STIL^{4D}$ (C) could drive centriole assembly only when PLK4 was active. $STIL^{4A}$ is not functional in either case (B). Error bars represents SD; n > 25, N = 3.

(D and E) The efficiency of centriole duplication during S phase (BrdU) was examined in stable $PLK4^{as}KI$; $STIL^{WT \text{ or } 4D}$ cell lines in which centriole biogenesis is supported by exogenously expressed $STIL^{WT}$ or $STIL^{4D}$, using indicated antibodies (D). Note that centrioles duplicate normally in most $STIL^{WT}$ and $STIL^{4D}$ cells under low DOX, forming centriole doublets each labeled with 1 STIL dot (1 STIL/Doublet). A minor centriole overduplication is noted in ~7.5% of $STIL^{4D}$ cells (E, green), forming centriole triplets or rosettes each labeled with more than one STIL dots (> 1 STIL/overduplication). Error bars represent SD; n > 50, N = 3.

the $STIL^{4D}$ mutant is functional, it cannot bypass the requirement of PLK4 for centriole assembly, suggesting that PLK4 may need to phosphorylate additional sites on STIL or work on other substrates.

Phosphomimetic STIL Can Stabilize the Cartwheel in the Absence of PLK4 Activity

Next, we asked whether $STIL^{4D}$ is sufficient to stabilize the cartwheel in the absence of PLK4. To test this, we used the CRISPR/Cas9 method to inactivate $STIL$ and $TP53$ genes in the PLK4^as knockin cell line ($PLK4^{as}KI$), generating $PLK4^{as}KI$; $p53^{-/-}$; $STIL^{-/-}$ cells in which centrioles made of different forms of STIL can be subsequently reconstituted under the endogenous level of PLK4 (Table S1). Tetracycline-inducible constructs of $STIL^{WT}$ or $STIL^{4D}$ were reintroduced back to $PLK4^{as}KI$; $p53^{-/-}$; $STIL^{-/-}$ cells. Upon induction, centrioles derived from $STIL^{WT}$ or $STIL^{4D}$ were formed first through de novo assembly, followed by canonical duplication (data not shown). $PLK4^{as}KI$; $STIL^{WT}$ or $PLK4^{as}KI$; $STIL^{4D}$ cell lines stably carrying relatively normal numbers of canonical centrioles were obtained through long-term culture in the presence of low doxycycline (Figures 2D

and 2E; Table S1). We thus examined the effect of PLK4 inactivation in these two cell lines arrested in S phase.

As expected, $STIL^{WT}$-derived centrioles quickly lost cartwheel integrity upon PLK4 inactivation, as indicated by loss of STIL or SAS-6 (Figures 3A and 3B). In contrast, surprisingly, the cartwheel made of $STIL^{4D}$ could stably exist in newborn centrioles in the absence of PLK4 activity, retaining STIL, SAS-6, and other centriolar components (Figures 3A and 3B; data not shown). In addition to canonical centrioles, a similar PLK4-independent stabilization of the cartwheel by $STIL^{4D}$ is also seen in de novo centrioles reconstituted in $PLK4^{as}$; $STIL^{WT \text{ or } 4D}$ cells (Table S1; Figures 3C and 3D). Moreover, to confirm that our observation is not due to a nonspecific effect of the PLK4 analog-sensitive mutation, we inactivated PLK4 with the recently reported small molecule inhibitor centrinone (Wong et al., 2015) in cell lines expressing wild-type PLK4 but carrying centrioles made of exogenous $STIL^{4D}$ or $STIL^{WT}$ as the control (see $STIL^{WT}$ or $STIL^{4D}$ cell lines in Table S1). Consistently, centrinone treatment caused serial losses of the cartwheel components from $STIL^{WT}$ centrioles, but not from centrioles made of $STIL^{4D}$ (Figure 3E). Interestingly, while the $STIL^{4D}$-derived cartwheel is stable in the absence of PLK4, it is still efficiently removed from daughter centrioles during mitosis (Figure 3F), indicating that other mechanisms are involved in normal cartwheel removal (Arquint and Nigg, 2014). Together, our data indicate that phosphomimetic STIL can bypass the requirement of PLK4 for cartwheel maintenance, but not for centriole assembly, suggesting that perhaps PLK4
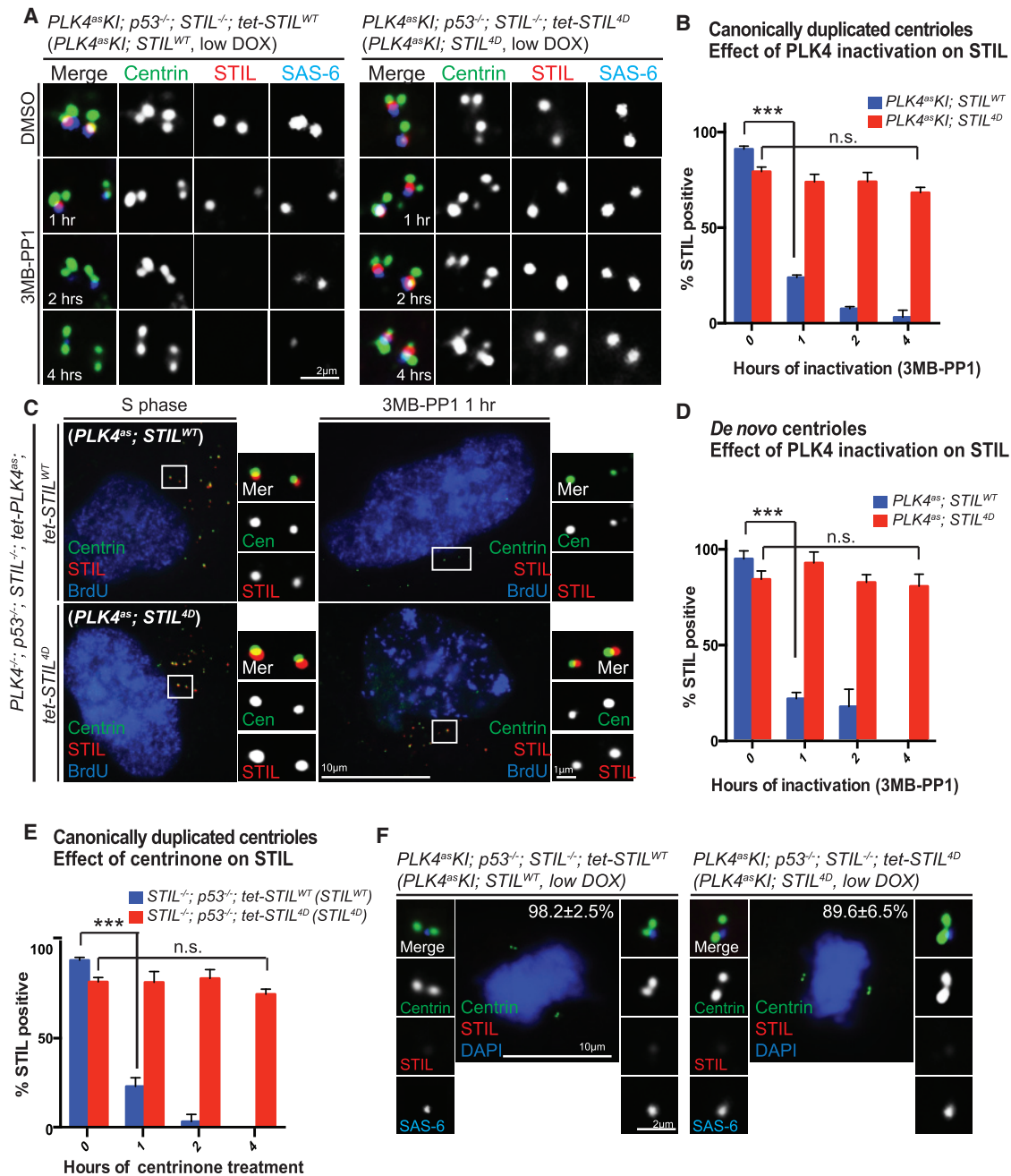
Figure 3. Cartwheels Composed of Phosphomimetic STIL Are Stably Maintained at the Daughter Centrioles Independent of PLK4 Activity

(A) Centrioles in $PLK4^{as}KI; STIL^{WT\ or\ 4D}$ cell lines, as indicated, were stained for STIL and SAS-6 before and after the inhibition of the endogenous PLK4 with 3MB-PP1.

(B) Quantification of STIL loss seen in (A). Error bars represent SD; n > 25, N = 3. ***p < 0.001; n.s., non-significant (two-tailed t test).

(C) De novo centrioles made of STIL^{WT} (top) or STIL^{4D} (bottom) were induced in $PLK4^{as}; STIL^{WT\ or\ 4D}$ cells and analyzed with the indicated antibodies before (left) and after (right) PLK4 inhibition for 1 hr.

(D) Quantification of STIL loss seen in (C). Centrioles made of the exogenous STIL^{WT} or STIL^{4D} were inhibited of PLK4 for indicated amounts of time and analyzed for STIL retention. Error bars represent SD; n > 25, N = 3. ***p < 0.001; n.s., non-significant (two-tailed t test).

(E) Quantification of STIL loss induced by centrinone. Wild-type PLK4 was inhibited with centrinone in $STIL^{WT}$ or $STIL^{4D}$ cell lines for indicated amounts of time. Error bars represent SD; n > 25, N = 3. ***p < 0.001; n.s., non-significant (two-tailed t test).

(F) Loss of the cartwheel from STIL^{4D} newborn centrioles occurs normally in mitosis. Stable $PLK4^{as}KI; STIL^{WT}$ or $PLK4^{as}KI; STIL^{4D}$ cells in metaphase were examined for cartwheel loss with indicated antibodies. Note that by metaphase, both STIL^{WT} and STIL^{4D} were removed from most centrioles.
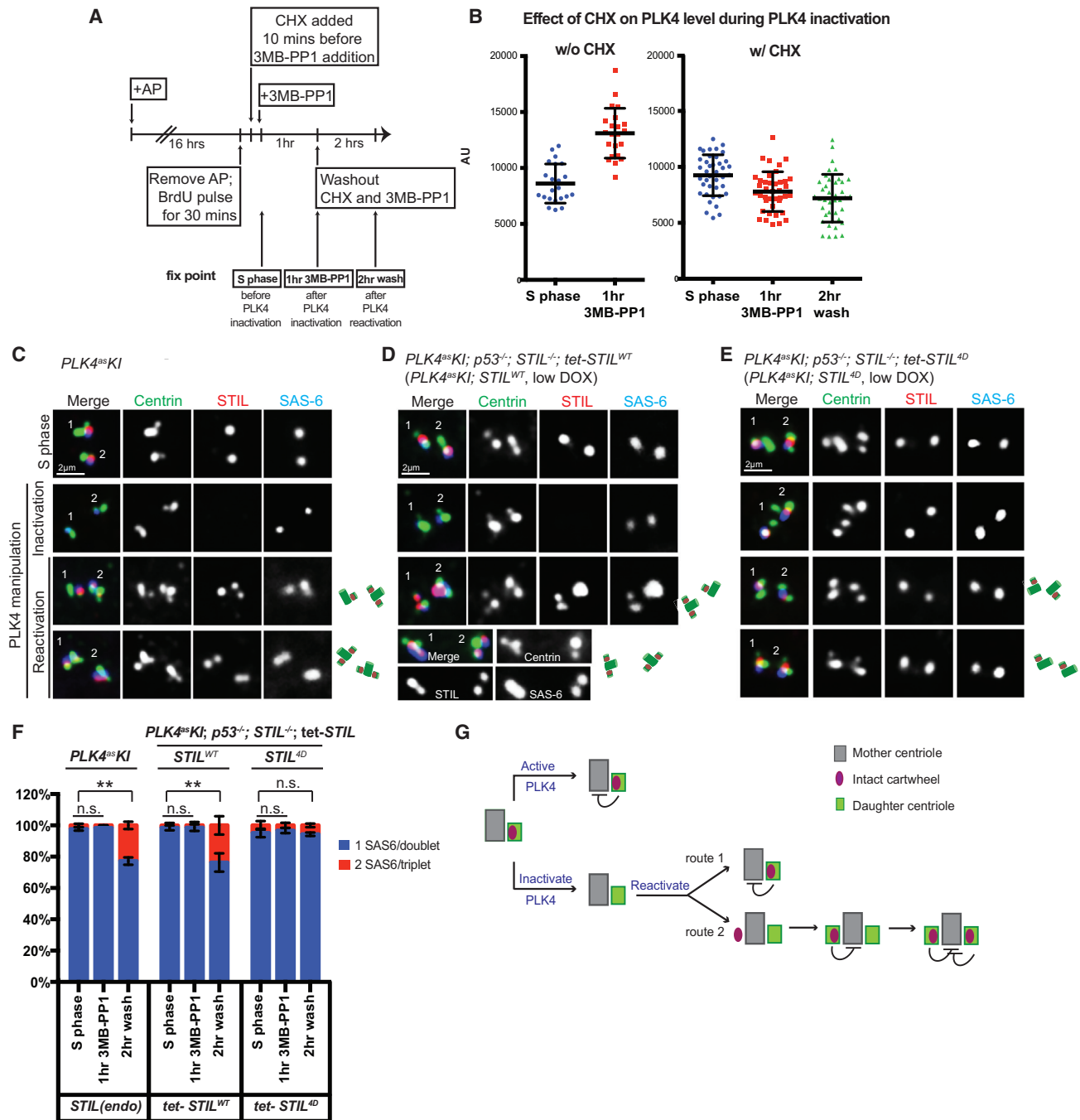
Figure 4. Loss of the Cartwheel from the Daughter Centriole Induces Reduplication of the Engaged Mother Centriole

(A) Experimental schematic inactivating PLK4 in S phase-arrested cells without causing PLK4 accumulation at the centrosome. Following S phase arrest and BrdU labeling, cycloheximide was added to the cells to block protein synthesis 10 min before PLK4 inactivation. PLK4 was then inactivated with 3MB-PP1 for 1 hr, followed by restoring of PLK4 activity and protein translation for 2 hr. At each fixation time point, PLK4 intensity at centrosomes was quantified using immunofluorescence.

(B) Scatterplot quantification of centrosomal PLK4 intensity at each fix point. Error bars represent SD; n > 20.

(C–E) Representative images of each fix point in $PLK4^{as}KI$ (C, endogenous STIL), $PLK4^{as}KI;\ STIL^{WT}$ (D, exogenous STIL^{WT}), and $PLK4^{as}KI;\ STIL^{4D}$ cells (E, exogenous STIL^{4D}). Top row: fix point 1 (S phase). Second row: fix point 2 (after 1 hr of PLK4 inhibition). Third and fourth row: fix point 3 (after 2 hr of washout; images from two independent cells are shown). Centriole reduplication was indicated by two colocalizing STIL/SAS-6 foci per centin triplet.

(legend continued on next page)

has additional substrates involved in other steps of centriole biogenesis.

Loss of the Cartwheel from Daughter Centrioles Induces Reduplication of the Mother Centriole

Inactivation of PLK4 induces rapid loss of the cartwheel components from daughter centrioles (Figure 1J). To test if constant maintenance of an intact cartwheel is required for the block of reduplication, PLK4 activity was resumed after cartwheel loss. A caveat of this procedure is that inactivation of PLK4 also results in gradual accumulation of PLK4 at centrosomes (Cunha-Ferreira et al., 2013; Guderian et al., 2010; Klebba et al., 2013), leading to centriole over-duplication upon PLK4 reactivation. To inactivate PLK4 without causing its accumulation, cells were treated with cycloheximide (CHX) to block protein synthesis, shortly before and during PLK4 inhibition (Figure 4A). As expected, in the absence of new translation, PLK4 levels at the centrosome after PLK4 inhibition stayed the same (Figure 4B), unlike cells untreated with CHX. Note that PLK4 and protein synthesis were inhibited for 1 hr only, followed by PLK4 reactivation (Figure 4A), as long-term inhibition of protein synthesis caused non-specific adverse effects on cells (data not shown).

Intriguingly, 2 hr after restoring PLK4 activity and protein synthesis, all preexisting daughter centrioles, which were still engaged to the mother centriole (Figure 4C, second row), regained intact cartwheels (100%), as revealed by STIL and SAS-6 staining. Moreover, we consistently saw that ~20% of mother centrioles reduplicated, carrying two engaged daughter centrioles, one preexisting and one newly formed, both equipped with the cartwheel containing STIL and SAS-6 (Figures 4C, third and fourth row, and 4F, left). To further test if the reduplication of mother centrioles depends on loss of STIL from the daughter, we repeated the same assay in cells where daughter centrioles were made of the exogenous STIL[4D] or STIL[WT] as the control (Figures 4D and 4E). Strikingly, whereas a significant fraction of mother centrioles engaged to STIL[WT] daughter centrioles reduplicated after a transient PLK4 inactivation and reactivation (Figure 4D; Figure 4F, center), very few mother centrioles engaged to STIL[4D] daughter centrioles reduplicated (Figure 4E; Figure 4F, right). Importantly, the stochastic nature of the reduplication (i.e., not all mother centrioles losing the associated cartwheel could reduplicate) is consistent with the principle of the duplication control (see Figure 4G and Discussion). Collectively, our results suggest that loss of intact cartwheel, or STIL, from daughter centrioles can induce stochastic reduplication of the engaged mother centriole in the same S phase, leading to overproduction of centrioles. Thus, the intact cartwheel carried by the daughter centriole constitutes a critical part of the duplication block, which is specifically tethered to mother centrioles through the engagement to locally suppress reduplication.

DISCUSSION

Phosphorylation of STIL by PLK4 drives cartwheel assembly for centriole duplication (Dzhindzhev et al., 2014; Kratz et al., 2015; Moyer et al., 2015; Ohta et al., 2014), which occurs only once per cell cycle. Our results here showed that cartwheel maintenance at the newborn daughter centriole is essential for the duplication block, requiring persistent PLK4 activity. Inactivation of PLK4 after duplication causes rapid loss of cartwheel components from daughter centrioles. Resuming PLK4 activity, intriguingly, induces cartwheel assembly not only back to the preexisting daughter centriole but also, stochastically, at a new site around the mother, where the second daughter forms. Conversely, the cartwheel made of phosphomimetic STIL can exist stably, continuously exerting duplication block independent of PLK4. Thus, PLK4 not only promotes centriole duplication but also preserves the "mark" of duplicated centrioles, the cartwheel, to prevent reduplication, revealing a counterintuitive control in which "assembly" and "block for new assembly" are both driven by the same machinery to ensure homeostasis.

We reason that the reduplication process induced by loss of cartwheel integrity occurs stochastically through one of the following two routes (Figure 4G): First, the intact cartwheel is rebuilt in the preexisting daughter centriole, where it exerts a block for reduplication, leading to stabilization of only one daughter centriole. In the second route, the cartwheel is randomly formed at a new site around the mother and quickly establishes a block for reduplication, but it has no effect on the recruitment of cartwheel components back to the preexisting daughter, leading to stabilization of two daughter centrioles. It is likely that the first route is preferred, as the preexisting daughter still carries some cartwheel components, such as PLK4 or SAS-6, which may provide high-affinity binding sites for efficient reassembly. The stochastic nature of the process is consistent with the basic principle of the "once-only" control for centriole duplication and, more importantly, is supported by our observations that only a fraction of mother centrioles losing the cartwheel from the associated daughter could reduplicate to form the second daughter.

How does the cartwheel locally block the duplication potential of the mother centrioles to which it is engaged? A recent model proposes that STIL-bound PLK4 at the cartwheel may specifically target the PCM-bound (or CEP152-bound) PLK4 around the mother centriole for degradation and thereby suppress centriole reduplication (Arquint et al., 2015; Ohta et al., 2014).

(F) Quantification of normally duplicated (blue) and reduplicated (red) centrioles in (C)–(E). Error bars represent SD; n > 50, N = 3. Centriole overproduction was detected in a small population of *PLK4*[asKI]; *STIL*[4D] cells (exogenously expressed STIL[4D]) regardless of the treatment (see Figure 2E). **p < 0.01; n.s., non-significant (two-tailed t test).

(G) Stochastic nature of centriole reduplication. In normal S phase (top), the cartwheel is actively maintained at the daughter centriole by PLK4 and exerts a block for reduplication. When PLK4 is inactivated (bottom) the cartwheel fails to remain and the mother centriole is relieved from the reduplication block. Once PLK4 activity is resumed, an intact cartwheel can be rebuilt in the preexisting daughter centriole and exert an immediate duplication block (route 1). Alternatively, the cartwheel can randomly form at a new site around the mother, resulting in the formation of an additional daughter centriole and establishing a block for reduplication (route 2). However, the recruitment of the cartwheel component back to the preexisting daughter centriole is unaffected, leading to stabilization of the two daughter centrioles.

This model was deduced from observations that PLK4 can induce self-destruction through trans-autophosphorylation (Cunha-Ferreira et al., 2013; Guderian et al., 2010; Holland et al., 2010; Klebba et al., 2013), that the kinase activity of PLK4 is greatly enhanced by binding to STIL (Moyer et al., 2015), and that the binding of PLK4 to STIL prevents PLK4 from self-destruction (Arquint et al., 2015; Ohta et al., 2014). Consistent with this model, overexpression of PLK4 or STIL should and is indeed known to alleviate the duplication block, allowing multiple daughters to be born by the same mother. However, in this case, the model would also predict that when more daughter centrioles are born, more cartwheel-associated inhibitory activities would arise to counteract PLK4 at the mother centriole, which in turn would require even higher levels of PLK4 to drive the formation of more daughter centrioles, explaining why a higher level of PLK4, rather than a longer period of time in S phase, is the key to amplify daughter centrioles. Our studies here provide us an angle at which the model can be tested, and the results are largely consistent. More efforts are required to engineer specific reagents and tools to fill in the remaining gap.

EXPERIMENTAL PROCEDURES

Details of experimental procedures regarding cell culture, drug treatment, antibodies, microscopy, immunoblots, and statistical analysis are provided in Supplemental Experimental Procedures.

Transgenic Cell Lines and Plasmid Constructs

To generate PLK4 conditional knockout cell lines, exon 3 and 4 of PLK4 were targeted by adeno-associated virus (AAV)-mediated homologous recombination as described previously (Berdougo et al., 2009; Tsou et al., 2009). A PLK4[as] knockin cell line (PLK4[as]KI) in which centrosomes biogenesis are supported by PLK4[as] expressed under the endogenous promoter was obtained from the Andrew J. Holland lab (unpublished data), similar to the PLK4[as] DLD-1 cell line the Andrew J. Holland lab recently reported (Moyer et al., 2015). CRISPR/Cas9-mediated gene targeting was used to inactivate p53, STIL, or both in various cell lines (see Table S1), as described previously (Izquierdo et al., 2014). For further details, see Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, one figure, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.06.069.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS

REFERENCES

Arquint, C., and Nigg, E.A. (2014). STIL microcephaly mutations interfere with APC/C-mediated degradation and cause centriole amplification. Curr. Biol. 24, 351–360.

Arquint, C., Gabryjonczyk, A.M., Imseng, S., Böhm, R., Sauer, E., Hiller, S., Nigg, E.A., and Maier, T. (2015). STIL binding to Polo-box 3 of PLK4 regulates centriole duplication. eLife 4, e07888.

Berdougo, E., Terret, M.E., and Jallepalli, P.V. (2009). Functional dissection of mitotic regulators through gene targeting in human somatic cells. Methods Mol. Biol. 545, 21–37.

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.

Cottee, M.A., Muschalik, N., Johnson, S., Leveson, J., Raff, J.W., and Lea, S.M. (2015). The homo-oligomerisation of both Sas-6 and Ana2 is required for efficient centriole assembly in flies. eLife 4, e07236.

Cunha-Ferreira, I., Bento, I., Pimenta-Marques, A., Jana, S.C., Lince-Faria, M., Duarte, P., Borrego-Pinto, J., Gilberto, S., Amado, T., Brito, D., et al. (2013). Regulation of autophosphorylation controls PLK4 self-destruction and centriole number. Curr. Biol. 23, 2245–2254.

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.

Dzhindzhev, N.S., Tzolovsky, G., Lipinszki, Z., Schneider, S., Lattao, R., Fu, J., Debski, J., Dadlez, M., and Glover, D.M. (2014). Plk4 phosphorylates Ana2 to trigger Sas6 recruitment and procentriole formation. Curr. Biol. 24, 2526–2532.

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

Fu, J., Hagan, I.M., and Glover, D.M. (2015). The centrosome and its duplication cycle. Cold Spring Harb. Perspect. Biol. 7, a015800.

Fu, J., Lipinszki, Z., Rangone, H., Min, M., Mykura, C., Chao-Chu, J., Schneider, S., Dzhindzhev, N.S., Gottardo, M., Riparbelli, M.G., et al. (2016). Conserved molecular interactions in centriole-to-centrosome conversion. Nat. Cell Biol. 18, 87–99.

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.

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.

Holland, A.J., Lan, W., Niessen, S., Hoover, H., and Cleveland, D.W. (2010). Polo-like kinase 4 kinase activity limits centrosome overduplication by autoregulating its own stability. J. Cell Biol. 188, 191–198.

Izquierdo, D., Wang, W.J., Uryu, K., and Tsou, M.F. (2014). Stabilization of cartwheel-less centrioles for duplication requires CEP295-mediated centriole-to-centrosome conversion. Cell Rep. 8, 957–965.

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.

Klebba, J.E., Buster, D.W., Nguyen, A.L., Swatkoski, S., Gucek, M., Rusan, N.M., and Rogers, G.C. (2013). Polo-like kinase 4 autodestructs by generating its Slimb-binding phosphodegron. Curr. Biol. 23, 2255–2261.

Kratz, A.S., Bärenz, F., Richter, K.T., and Hoffmann, I. (2015). Plk4-dependent phosphorylation of STIL is required for centriole duplication. Biol. Open 4, 370–377.

Kuriyama, R., and Borisy, G.G. (1981). Microtubule-nucleating activity of centrosomes in Chinese hamster ovary cells is independent of the centriole cycle but coupled to the mitotic cycle. J. Cell Biol. 91, 822–826.

Loncarek, J., Hergert, P., Magidson, V., and Khodjakov, A. (2008). Control of daughter centriole formation by the pericentriolar material. Nat. Cell Biol. 10, 322–328.

Moyer, T.C., Clutario, K.M., Lambrus, B.G., Daggubati, V., and Holland, A.J. (2015). Binding of STIL to Plk4 activates kinase activity to promote centriole assembly. J. Cell Biol. 209, 863–878.

Novak, Z.A., Conduit, P.T., Wainman, A., and Raff, J.W. (2014). Asterless licenses daughter centrioles to duplicate for the first time in Drosophila embryos. Curr. Biol. 24, 1276–1282.

Ohta, M., Ashikawa, T., Nozaki, Y., Kozuka-Hata, H., Goto, H., Inagaki, M., Oyama, M., and Kitagawa, D. (2014). Direct interaction of Plk4 with STIL ensures formation of a single procentriole per parental centriole. Nat. Commun. 5, 5267.

Qiao, R., Cabral, G., Lettman, M.M., Dammermann, A., and Dong, G. (2012). SAS-6 coiled-coil structure and interaction with SAS-5 suggest a regulatory mechanism in C. elegans centriole assembly. EMBO J. 31, 4334–4347.

Shukla, A., Kong, D., Sharma, M., Magidson, V., and Loncarek, J. (2015). Plk1 relieves centriole block to reduplication by promoting daughter centriole maturation. Nat. Commun. 6, 8077.

Tsou, M.F., and Stearns, T. (2006). Mechanism limiting centrosome duplication to once per cell cycle. Nature 442, 947–951.

Tsou, M.F., Wang, W.J., George, K.A., Uryu, K., Stearns, T., and Jallepalli, P.V. (2009). Polo kinase and separase regulate the mitotic licensing of centriole duplication in human cells. Dev. Cell 17, 344–354.

van Breugel, M., Hirono, M., Andreeva, A., Yanagisawa, H.A., Yamaguchi, S., Nakazawa, Y., Morgner, N., Petrovich, M., Ebong, I.O., Robinson, C.V., et al. (2011). Structures of SAS-6 suggest its organization in centrioles. Science 331, 1196–1199.

Vorobjev, I.A., and Chentsov, Y.S. (1980). The ultrastructure of centriole in mammalian tissue culture cells. Cell Biol. Int. Rep. 4, 1037–1044.

Vorobjev, I.A., and Chentsov YuS. (1982). Centrioles in the cell cycle. I. Epithelial cells. J. Cell Biol. 93, 938–949.

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

Wang, W.J., Acehan, D., Kao, C.H., Jane, W.N., Uryu, K., and Tsou, M.F. (2015). De novo centriole formation in human cells is error-prone and does not require SAS-6 self-assembly. eLife 4, 10586.

Wong, C., and Stearns, T. (2003). Centrosome number is controlled by a centrosome-intrinsic block to reduplication. Nat. Cell Biol. 5, 539–544.

Wong, Y.L., Anzola, J.V., Davis, R.L., Yoon, M., Motamedi, A., Kroll, A., Seo, C.P., Hsia, J.E., Kim, S.K., Mitchell, J.W., et al. (2015). Cell biology. Reversible centriole depletion with an inhibitor of Polo-like kinase 4. Science 348, 1155–1160.

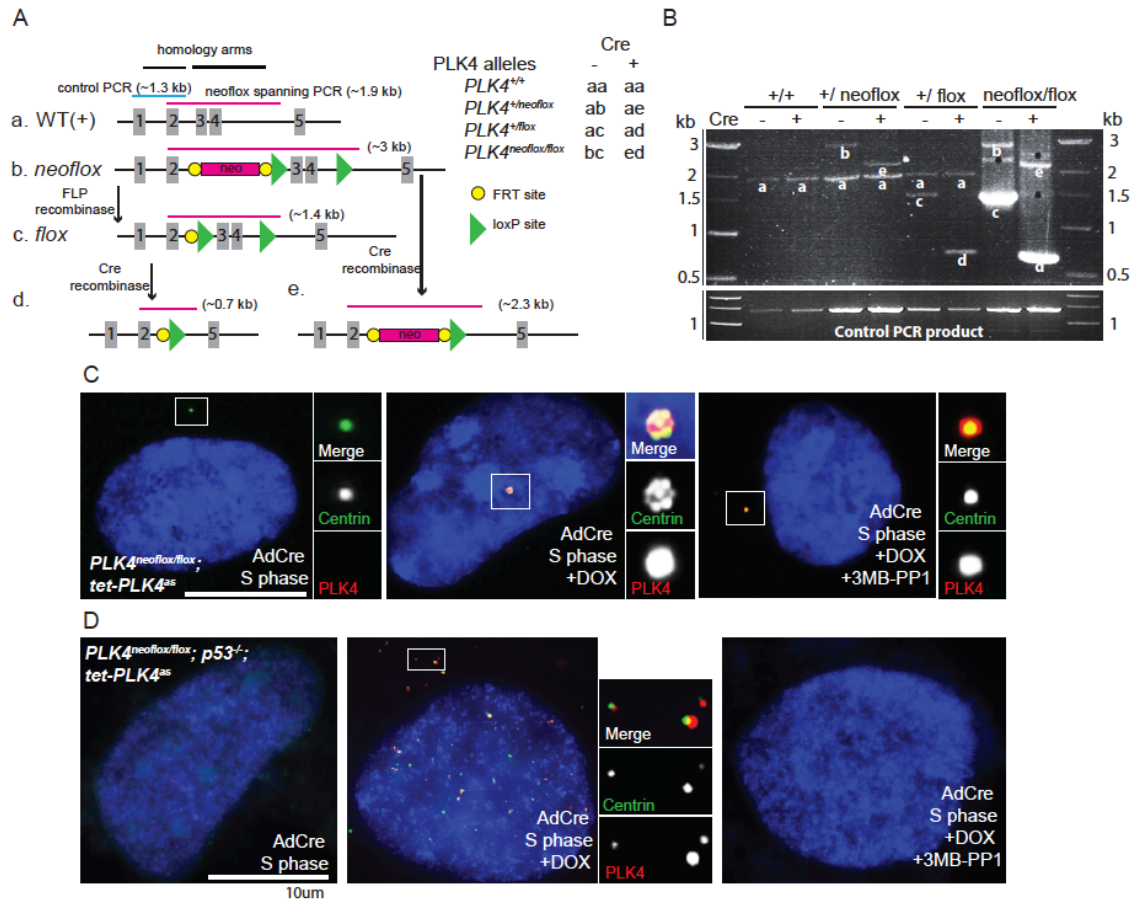**Supplemental Information**

Promotion and Suppression of Centriole

Duplication Are Catalytically Coupled

through PLK4 to Ensure Centriole Homeostasis

Minhee Kim, Brian P. O'Rourke, Rajesh Kumar Soni, Prasad V. Jallepalli, Ronald C. Hendrickson, and Meng-Fu Bryan Tsou

Supplemental Figure 1.

Figure S1 (Related to Figure 1). A chemical genetic system for manipulating PLK4 activity in human cells

(A) Diagram of *PLK4* locus targetd to create *PLK4$^{flox/neoflox}$* alleles. *loxP* sites and *FRT* sites are indicated by triangles and circles, respectively. Wild-type, *neoflox* and *flox* alleles are detected as PCR products a, b, and c, respectively. Expected PCR products to confirm genetic manipulation are shown in magenta. Control PCR products are blue. Deletion of exon 3 and 4 from *Flox* and *neoflox* alleles by AdeCre infection give rise to PCR product d and e, respectively.

(B) PCR analysis confirming gene replacement and Cre-induced recombination in indicated *PLK4* alleles. PCR products corresponding to each alleles are indicated. Non-specific bands are noted as *.

(C) Functional test of *PLK4$^{flox/neoflox}$*; *tet-PLK4^{as}* cell. AdCre infected cells were cultured for about 2 days (left), then were supplied with a high concentration of doxycycline (center), or high doxycycline with 3MB-PP1(right). Centrioles are stained with the indicated antibodies.

(D) Functional test of *PLK4$^{flox/neoflox}$*; *tet-PLK4^{as}*; *p53$^{-/-}$* cell. AdCre infected cells were cultured for more than a week (left), then were supplied with a high concentration of doxycycline (center), or high doxycycline with 3MB-PP1(right). Centrioles are stained with the indicated antibodies.

Supplemental Table 1. Transgenic cell lines generated in this study

Name	Genetic Background	Centrosome Status	Relevant Figures
$PLK4^{as}$	$PLK4^{-/-}$; tet-$PLK4^{as}$	-$PLK4^{as}$ inducible cell line propagating under low doxycycline (DOX) and carrying nearly normal numbers of centrioles/centrosomes	Figure 1 (A, C, D, and I)
$PLK4^{as}$; $p53^{-/-}$	$PLK4^{-/-}$; $p53^{-/-}$; tet-$PLK4^{as}$	-$PLK4^{as}$ inducible cell line capable of losing centrioles -Acentriolar cell line in which *de novo* centriole assembly can be triggered by DOX addition	Figure 1 (B, E-H, and K)
$PLK4^{as}$; $STIL^{-/-}$	$PLK4^{-/-}$; $p53^{-/-}$; tet-$PLK4^{as}$; $STIL^{-/-}$	Acentriolar cell line used to reconstitute centrioles made of different forms of STILs	
$PLK4^{as}$; $STIL^{WT}$	$PLK4^{-/-}$; $p53^{-/-}$; $STIL^{-/-}$; tet-$PLK4^{as}$; tet-$STIL^{WT}$	-Acentriolar, $PLK4^{as}$ cell line in which *de novo* centrioles made of $STIL^{WT}$ can be reconstituted by DOX addition.	Figure 2 (A) and 3 (C & D)
$PLK4^{as}$; $STIL^{4A}$	$PLK4^{-/-}$; $p53^{-/-}$; $STIL^{-/-}$; tet-$PLK4^{as}$; tet-$STIL^{4A}$	-Acentriolar cell line with or without DOX; $STIL^{4A}$ is not functional for centriole assembly	Figure 2 (B)
$PLK4^{as}$; $STIL^{4D}$	$PLK4^{-/-}$; $p53^{-/-}$; $STIL^{-/-}$; tet-$PLK4^{as}$; tet-$STIL^{4D}$	-Acentriolar, $PLK4^{as}$ cell line in which *de novo* centrioles made of $STIL^{4D}$ can be reconstituted by DOX addition.	Figure 2 (C) and 3 (C & D)
$STIL^{WT}$	$STIL^{-/-}$; $p53^{-/-}$; tet-$STIL^{WT}$	-Acentriolar in the absence of DOX -Under low DOX, cell lines with nearly normal numbers of $STIL^{WT}$-derived centrioles were obtained through long-term culture.	Figure 3 (E)
$STIL^{4D}$	$STIL^{-/-}$; $p53^{-/-}$; tet-$STIL^{4D}$	-Acentriolar in the absence of DOX. -Under low DOX, cell lines with nearly normal numbers of $STIL^{4D}$-derived centrioles were obtained through long-term culture.	Figure 3 (E)
$PLK4^{as}$KI; $STIL^{WT}$	$PLK4^{as}$KI; $p53^{-/-}$; $STIL^{-/-}$; tet-$STIL^{WT}$	-Acentriolar in the absence of DOX. -$PLK4^{as}$ knock-in cell lines carrying nearly normal numbers of $STIL^{WT}$-derived centrioles were obtained through long-term culture with low DOX.	Figure 2 (D & E), Figure 3 (A, B, and F), Figure 4 (D & F)
$PLK4^{as}$KI; $STIL^{4D}$	$PLK4^{as}$KI; $p53^{-/-}$; $STIL^{-/-}$; tet-$STIL^{4D}$	-Acentriolar in the absence of DOX. -$PLK4^{as}$ knock-in cell lines carrying nearly normal numbers of $STIL^{4D}$-derived centrioles were obtained through long-term culture with low DOX.	Figure 2 (D & E), Figure 3 (A, B, and F), Figure 4 (E & F)

Supplemental Experimental Procedures

Transgenic cell lines and plasmid constructs

PLK4$^{flox/neoflox}$ cells were generated by Adeno-associated virus (AAV)-mediated homologous recombination as described previously (Berdougo et al., 2009; Tsou et al., 2009). Template genomic DNA was from RPCI-11 human BAC clone, 398H1 (Invitrogen). Primers used in the targeting process are listed below.

PLK4 targeting primers
5'-TTTTCTCGAGCGGAAGGTGTCAGGGAGAACT-3'
5'-CCCCGATATCTGACAAATAATTCTTAATTTGCTTATGTTTAT-3'
5'-CCCCGGATCCCATTTATCTAAACCACTTAGAAAAAGAACTGG-3'
5'-TTTTGTCGACATAACTTCGTATAGCATACATTATACGAAGTTATCCTATAAAATAGCATATTCCTCAAAACTACAC-3'
5'-TTTTGGATCCTGGCGTCGACATATAGTAAAACTGTTTGAATTATTACTTTAAGC-3'

5'-CCCCGCTAGCATTATGAGAAATTGAGGAAGTCTGAAGC-3'

Primers to confirm gene replacement
5'-GATTTTAAAGTTGGAAATCTGCT-3'
5'-AGCAGCTTGGGATGTAGGAAATC-'3

Control PCR primers
5'-CGGAGCGATCCATCTCGTTAC-3'
5'-TGACAAATAATTCTTAATTTGCTTATGTTTAT-3'

After generating the $PLK4^{flox/neoflox}$ cells, we utilized the lentiviral pLVX-Tight-Puro vector system (Clonetech) to transduce a tetracycline inducible construct expressing the analog sensitive mutant form of PLK4 (tet-PLK4^{as}). $PLK4^{flox/neoflox}$; tet-PLK4^{as} cells were then infected with adenovirus expressing Cre recombinase (AdeCre) to deplete endogenous $PLK4$, plated in 96 well plates, and cultured in the media containing 5 ng/ml of doxycycline to generate stable $PLK4^{-/-}$; tet-PLK4^{as} cell lines that carry normal number of centrosomes.

Various $STIL^{-/-}$; $p53^{-/-}$ cells inducibly expressing exogenous STIL$^{WT \text{ or } 4D}$ were generated through lentiviral based transduction as described above, and isolated clonally. The targeting sequences of gRNAs used for CRISPR are as follows: p53 (5'-GGGCAGCTACGGTTTCCGTCTGG-3') and STIL (5'-GTGTGGAATTTGACTTGCATTGG-3').

$PLK4^{-/-}$; tet-PLK4^{as}; $p53^{-/-}$; $STIL^{-/-}$; tet-STIL$^{WT/4A/4D}$ cell lines were generated in the following order: p53 and STIL were targeted sequentially by CRISPR/Cas9 in $PLK4^{flox/neoflox}$; tet-PLK4^{as} cells. $PLK4^{flox/neoflox}$; tet-PLK4^{as}; $p53^{-/-}$; $STIL^{-/-}$ cells were infected with lentivirus carrying doxycycline-inducible STIL$^{WT, 4A, \text{ and } 4D}$, followed by deletion of the endogenous PLK4 with AdeCre. Wild type STIL construct (Openbiosystems) and PLK4 construct (Origene RC206015) were used for subcloning into pLVX-Tight-Puro vector (Clonetech). STIL$^{4D/4A}$ (S1061D/A, S1111D/A, S1116D/A, and T1119D/A) or PLK4^{as} (L89G) mutant constructs were created with site-directed mutagenesis (Stratagene).

Cell culture
RPE1 cells were cultured in DME/F12 (1:1) medium supplemented with 10% FBS and 1% penicillin-streptomycin. $PLK4^{as}$ cells ($PLK4^{-/-}$; tet-PLK4^{as}) carrying centrosomes were grown under constant supply of 5 ng/ml of doxycycline. $STIL^{4D}$ and $PLK4^{as}KI$; $STIL^{4D}$ cell lines were grown under constant supply of 20 ng/ml of doxycycline. $STIL^{WT}$ and $PLK4^{as}KI$; $STIL^{WT}$ cell lines were grown under 5 ng/ml of doxycycline. For S-phase arrest, cells were treated with 2 μg/ml aphidicolin for 12-18 hours. S-phase cells were labeled by BrdU for 30 minutes.

Assay and drug treatment
To inhibit PLK4^{as} in $PLK4^{as}$ cells, excess amounts of 3MB-PP1 (20 μM, EMD Millipore) was used; to inactivate PLK4^{as} followed by reactivation, 0.2 μM of 3MB-PP1 was used to ensure proper drug washout. To inactivate endogenous PLK4, 0.2 μM of centrinone was used (Wong et al., 2015). To inhibit protein translation, 1 μg/ml of CHX was used. For reduplication assay, $PLK4^{as}KI$ cells pretreated with 1 μg/ml CHX for 10 minutes were additionally treated with 0.2 μM of 3MB-PP1 for 1 hour. Cells were then thoroughly washed 4 times with buffers in the absence of drugs, followed by incubation in the regular medium for 2 hours.

Antibodies
Mouse monoclonal antibodies used in this study are anti-centrin2 (clone 20H5; 04-1624, Millipore), anti-SAS-6 (sc-81431, Santa Cruz Biotechnology), anti-acetylated alpha tubulin (clone 6-11B-1; T7451, Sigma-Aldrich), anti-alpha tubulin (clone DM1A, Sigma-Aldrich). A rabbit polyclonal antibody against the human C-Nap1 was produced as previously described (Tsou and Stearns, 2006). Other rabbit polyclonal antibodies used include anti-CPAP (Proteintech), anti-STIL (Bethyl Laboratories), and anti-PLK4 (kind gift from Andrew Holland at Johns Hopkins University). PLK4 and STIL antibodies were pre-adsorbed by incubating with excess amounts of methanol fixed $PLK4^{-/-}$; $p53^{-/-}$ and $STIL^{-/-}$; $p53^{-/-}$ cells, respectively to

3

remove non-specific materials. Rat-anti-BrdU was from AbD Serotec. Secondary antibodies Alexa-Fluor 405, 488, 594, 680 were from Molecular Probes.

Immunofluorescence and microscopy
Cells were washed once in phosphate-buffered saline (PBS) and extracted in PTEM (20 mM PIPES pH 6.8, 0.2% Triton X-100, 10 mM EGTA, 1 mM $MgCl_2$) for 2 minutes, before being fixed in methanol at -20°C for at least 10 minutes. Slides were blocked with 3% bovine serum albumin (w/v) with 0.1% Triton X-100 in PBS before incubating with primary antibodies. DNA was visualized using 4',6-diamidino-2-phenylindole (DAPI). Fluorescent images were acquired on an upright microscope (Axioimager; Carl Zeiss) equipped with 100x oil objectives, NA of 1.4, and a camera (ORCA ER; Hamamatsu Photonics). Captured images were processed with Axiovision (Carl Zeiss) and Photoshop CS6 (Adobe). For PLK4 quantification at centrosomes, all cells were treated the same during the process of immunocytochemistry and image acquisition. The images were analyzed using ImageJ software (National Institutes of Health) as described previously (Wang et al., 2011).

Super-resolution microscopy
DeltaVision OMX was employed as previously described (Fong et al., 2014).

Immunoblots
Cells were lysed in RIPA buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate) supplemented with complete protease inhibitor (Roche) at 4°C for 15 minutes. Lysates were cleared by centrifugation and resolved by SDS-PAGE.

Statistical analysis
All statistical analyses were performed using Graph Pad Prism software (version 6.0). Comparisons among groups were performed by two-tailed t-test. Statistical significance was assigned for $p < 0.05$.

Supplemental References

Berdougo, E., Terret, M.E., and Jallepalli, P.V. (2009). Functional dissection of mitotic regulators through gene targeting in human somatic cells. Methods in molecular biology *545*, 21-37.
Fong, C.S., Kim, M., Yang, T.T., Liao, J.C., and Tsou, M.F. (2014). SAS-6 assembly templated by the lumen of cartwheel-less centrioles precedes centriole duplication. Developmental cell *30*, 238-245.
Tsou, M.F., and Stearns, T. (2006). Mechanism limiting centrosome duplication to once per cell cycle. Nature *442*, 947-951.
Tsou, M.F., Wang, W.J., George, K.A., Uryu, K., Stearns, T., and Jallepalli, P.V. (2009). Polo kinase and separase regulate the mitotic licensing of centriole duplication in human cells. Developmental cell *17*, 344-354.
Wang, W.J., Soni, R.K., Uryu, K., and Tsou, M.F. (2011). The conversion of centrioles to centrosomes: essential coupling of duplication with segregation. The Journal of cell biology *193*, 727-739.
Wong, Y.L., Anzola, J.V., Davis, R.L., Yoon, M., Motamedi, A., Kroll, A., Seo, C.P., Hsia, J.E., Kim, S.K., Mitchell, J.W.*, et al.* (2015). Cell biology. Reversible centriole depletion with an inhibitor of Polo-like kinase 4. Science *348*, 1155-1160.