The midbody interactome reveals unexpected roles for PP1 phosphatases in cytokinesis

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The midbody is an organelle assembled at the intercellular bridge between the two daughter cells at the end of mitosis. It controls the final separation of the daughter cells and has been involved in cell fate, polarity, tissue organization, and cilium and lumen formation. Here, we report the characterization of the intricate midbody protein-protein interaction network (interactome), which identifies many previously unknown interactions and provides an extremely valuable resource for dissecting the multiple roles of the midbody. Initial analysis of this interactome revealed that PP1β-MYPT1 phosphatase regulates microtubule dynamics in late cytokinesis and de-phosphorylates the kinesin component MKLP1/KIF23 of the centralspindlin complex. This de-phosphorylation antagonizes Aurora B kinase to modify the functions and interactions of centralspindlin in late cytokinesis. Our findings expand the repertoire of PP1 functions during mitosis and indicate that spatiotemporal changes in the distribution of kinases and counteracting phosphatases finely tune the activity of cytokinesis proteins.
Growth, development, and reproduction in multicellular organisms depend on the faithful segregation of genomic and cytoplasmic material that occurs during cell division. Errors during this process are responsible for many human diseases, including cancer. In the final step of cell division, the mother cell divides into two daughter cells during the process of cytokinesis. This major cell shape change requires the assembly and coordinated activity of two cytoskeletal structures: the actomyosin contractile ring, which assembles at the equatorial cortex and drives the ingression of the cleavage furrow; and the central spindle, an array of anti-parallel and interdigitating microtubules, which is essential for positioning the cleavage furrow, keeping the dividing genomes apart, and for the final separation, i.e., abscission, of the daughter cells. The contractile ring and the central spindle are composed of several proteins and protein complexes that act as structural and regulatory factors that control the formation, dynamics and stability of these cytoskeletal structures throughout cytokinesis1–5. Like in many other processes during cell division, the functions and interactions of these proteins are often regulated by reversible posttranslational modifications, including phosphorylation/dephosphorylation mostly mediated by serine/threonine kinases and their counteracting phosphatases6. During furrow ingression, the contractile ring compacts the central spindle and, after completion of furrow ingression, the two daughter cells remain connected by an intercellular bridge, which contains at its center an organelle, the midbody, composed of a multitude of proteins that have diverse functions. Some midbody proteins are former components of the contractile ring and central spindle, while others are specifically recruited during the slow midbody maturation process that ultimately leads to the abscission of the two daughter cells4,5. All these proteins are arranged in a very precise and stereotyped spatial pattern along the midbody6, which can be divided in approximately three major regions: the midbody ring, containing mostly former contractile ring components like Anillin and Citron kinase; the midbody central core marked by central spindle proteins such as the centralspindlin complex; and the midbody arms that flank the midbody core and where the chromosomal passenger complex (CPC) and the kinesin KIF20A accumulate4. The proper localization, regulation and interactions of all these proteins are essential for the execution of abscission and for preventing incorrect genome segregation5. Furthermore, recent evidence indicates that the midbody is also involved in many other processes besides cell division, including cell fate, pluripotency, apical-basal polarity, tissue organization, and cilium and lumen formation7,8. Therefore, the characterization of the intricate midbody protein interaction networks (i.e., interactome) and their regulation is essential for understanding how this organelle executes its multiple functions.

In this study, we report the characterization of the midbody interactome identified by affinity purifications coupled with mass spectrometry (AP-MS) of ten key midbody components. This valuable resource provides a molecular blueprint of the intricate connections amongst midbody components that will be pivotal in dissecting the multiple functions of this organelle. In support of this, our initial analysis of the midbody interactome already revealed a plethora of previously unidentified interactions and highlighted a role of the PP1β-MYPT1 phosphatase in regulating the dynamics of central spindle microtubules by antagonizing Aurora B phosphorylation of the centralspindlin component MKLP1 in late cytokinesis.

### Results

**CIT-K interactions increase specifically during cytokinesis.**

Citron kinase (CIT-K) is a contractile ring component that acts as a major midbody organizer by interacting with several midbody components, including the CPC and centralspindlin, and by maintaining their correct localization and orderly arrangement9,10. As a first step toward the characterization of the midbody interactome, we used a human HeLa cell line stably expressing CIT-K tagged with GFP11 to identify the CIT-K interactomes at different cell cycle stages—S phase, metaphase, and telophase—by AP-MS (Fig. 1a, b and Supplementary Data 1). We found that the number of CIT-K interactors consistently increased in telophase in three separate replicates, confirming the important role of this kinase in cytokinesis (Fig. 1b). Notably, only 62 proteins, including the bait CIT-K and two of its known partners, the contractile ring component Anillin and the kinesin KIF14,12,13, were common to all three mitotic stages (Fig. 1b and Supplementary Data 1), indicating that our AP-MS methodology identifies specific interactions and generates little noise.

To assess whether CIT-K was required for recruiting some of these interactors to the midbody, we used SILAC-based quantitative MS to characterize and compare the proteomes of midbodies purified from telophase HeLa cells treated with either CIT-K or control siRNAs (Fig. 1c, Supplementary Fig. 1, and Supplementary Data 2). Only minor differences in the levels of a few midbody proteins were identified, including Filamin B, the kinesin KIFC1, Aurora A kinase and its interactor TPX2 (Fig. 1d, Supplementary Table 1 and Supplementary Data 2). Although some of these differences were significant and validated by western blot (Fig. 1e and Supplementary Table 1), overall our results did not indicate a major role for CIT-K in recruiting midbody proteins and reinforced the evidence that CIT-K has a very specific function in the organization of this organelle.

Finally, it is important to point out that our midbody proteome contains a significant higher number of proteins than a previous study14. This most likely reflects the considerable advancements in MS technology in recent years rather than a difference in the midbody purification protocols.

### The midbody interactome has common and specific networks.

To further our knowledge of the interaction networks within the midbody, we expanded our AP-MS experiments to include nine additional baits, all proteins that are well known to play key roles in midbody assembly and cytokinesis and display specific and distinct localization patterns (Table 1). AP-MS analysis of the interactions of these ten baits in telophase revealed a complex midbody interactome comprising almost 3000 proteins (Supplementary Data 3, 4), which included the majority of midbody proteome components and showed a Gene Ontology (GO) enrichment profile very similar to the midbody proteome characterized in our SILAC experiments (Fig. 2 and Supplementary Data 4, 5). The overlap and similarity between the two datasets is highly significant considering that they were obtained using two completely different experimental procedures (see Methods). The midbody interactome contains complex networks shared by several baits as well as networks specific for each bait or for just a few baits (Fig. 3a–d). Interestingly, even proteins strictly related, like the two ESCRT-III paralogs CHMP4B and CHMP4C, showed distinct specific networks (Fig. 3a). Analysis of the interactome networks further confirmed the specificity and selectivity of our AP-MS methodology. For example, the contractile ring component Anillin presents a specific interaction network that includes the vast majority of septin proteins (Fig. 3b), which are known to be recruited by Anillin to midbody15,16. Similarly, the mitotic kinase Polo-like kinase 1 (Plk1) was only identified with the bait PRC1 (Fig. 3d), which directly binds to and recruits Plk1 to the central spindle and midbody in both human and Drosophila cells17,18.
Our midbody interactome revealed a plethora of interactions that can lead to the discovery of important structural and regulatory networks present within this organelle. For example, in the network specifically shared by CIT-K and its partner KIF14, we identified the cyclin-dependent kinase 1 (Cdk1) and the microtubule depolymerizing kinesin KIF2C/MCAK (Fig. 3c), both also found in the midbody proteome (Supplementary Data 2 and 4). KIF2C regulates microtubule dynamics during mitotic spindle assembly, but it has not been implicated in cytokinesis. Cdk1, in complex with cyclin B, is well known to promote mitotic entry and to regulate multiple mitotic events until anaphase, when most of the complex is inactivated through degradation of cyclin B. However, a pool of Cdk1/cyclin B has been described to accumulate at the midbody where it appears to promote abscission. Our data not only indicate a potential role for KIF2C in cytokinesis and confirm the presence and function of Cdk1/cyclin B at the midbody, but also suggest that the...
association with CIT-K and KIF14 might be important for their localization and/or function.

PP1β-MYPT1 controls microtubule dynamics in late cytokinesis. Cell division is regulated by posttranslational modifications, including phosphorylation mostly mediated by serine/threonine kinases and counteracting phosphatases. Although most kinases involved in cytokinesis are known, the identity and function of their opposing phosphatases is just emerging. To address this nescience, we generated a midbody interactome serine/threonine phosphorylation sub-network by extracting from the entire interactome dataset proteins whose full names (Uniprot field: protein names) contain the terms kinase and phosphatase but excluded those containing tyrosine (Supplementary Table 2), and the top scores include the three PP1 cat-

Table 1 Baits used in the AP-MS experiments for the characterization of the midbody interactome

| Name                  | Function                              | Localization                                                                 | Tag and cell line reference |
|-----------------------|---------------------------------------|------------------------------------------------------------------------------|-----------------------------|
| Anillin               | Actomyosin binding protein; contractile ring scaffolding | Cleavage furrow, midbody ring and secondary constriction sites               | GFP48                       |
| Aurora B              | Serine/threonine kinase; CPC component, controls furrow progression, central spindle formation and abscission | Cleavage furrow, central spindle, midbody arms                               | GFP (this study)            |
| CHMP4B                | ESCRT-III protein; required for abscission | Midbody core and abscission site                                             | GFP50                       |
| CHMP4C                | ESCRT-III protein; required for abscission | Central spindle, midbody arms, midbody core and abscission site              | Flag25                      |
| Citron kinase (CIT-K) | Serine/threonine kinase; required for midbody assembly, organization and maturation | Cleavage furrow, central spindle, midbody ring                               | AcGFP11                     |
| Ect2                  | Rho GEF; activates RhoA to promote contractile ring assembly and constriction | AcGFP50                                                                      |
| KIF14                 | Kinesin, CIT-K partner; required for midbody assembly, organization and maturation | Cleavage furrow, central spindle, midbody core                               | GFP51                       |
| KIF20A/MKLP2          | Kinesin; required for central spindle formation and CPC translocation | Central spindle, midbody arms                                                | GFP51                       |
| KIF23/MKLP1           | Kinesin; centralspindlin component, required for furrow progression, central spindle and midbody formation | Central spindle, internal midbody arms and core                              | GFP53                       |
| PRC1                  | Microtubule associated protein, required for central spindle and midbody formation | Central spindle, internal midbody arms and core                              |                            |

PP1β-MYPT1 causes the cytoskeleton to undergo a transition from a network of thin filaments into a thick, rigid cylinder that forms the midbody. MYPT1 depletion leads to a failure in furrow ingression, central spindle and midbody formation. The signals detected by these antibodies are specific because they were strongly reduced after siRNA treatments in both immunofluorescence and western blot analyses (Fig. 4a–d). We then investigated if siRNA-mediated depletion of these phosphatases caused cytokinesis failure. siRNA of PP1β and MYPT1 caused the highest increases in multinucleation (a readout of cytokinesis failure), 4.2- and 7.2-fold, respectively (Fig. 4g, h). PP1α depletion did not result in an increase of multinucleated cells and only a very modest increase (1.6-fold) was observed after PP1γ siRNA (Fig. 4h). However, combined depletion of these two closely related catalytic subunits resulted in a 2.8-fold increase in multinucleated cells (Fig. 4h), suggesting that they could act redundantly and/or synergistically in cytokinesis. In sum, our results indicated that, of all four phosphatases, PP1β and MYPT1 were the two most strongly required for cytokinesis (Fig. 4e, g, h), which is consistent with the evidence that MYPT1 is a known PP1β regulatory subunit. MYPT1 was reported to antagonize Plk1 during mitotic spindle assembly and to be required for cytokinesis, but its exact role in cytokinesis was not investigated, probably assuming that it was required to de-phosphorylate the myosin regulatory light chain (MRLC) at the contractile ring. We found that, indeed, the levels of both mono(pS19)- and di(pT18 pS19)-phosphorylated MRLC levels were elevated in MYPT1 depleted cells (Fig. 5a, b), which had also an abnormal cytoskele-

Notably, after completion of furrow ingression, the midbody was not properly separate, abscission was significant. Furthermore, in MYPT1 depleted cells, the midbody was not properly assembled as many of its components were stretched along the central spindle and lost their precise arrangement: Aurora B kinase spread from the midbody arms into the midbody core (Fig. 6d), the kinesin MKLP1 and the microtubule bundling protein PRC1 failed to localize as two juxtaposed disks (Figs. 6e–g and 7a), and CIT-K assembled into misshapen rings that often collapsed (Fig. 6e, h). Electron microscopy analysis showed that MYPT1 siRNA midbodies contained fewer microtubules and an abnormal midbody matrix compared with control cells (Fig. 6i). These central spindle and midbody defects are not linked to abnormal cortical contractions or adhesion problems because they were also observed in less adherent HeLa S3 cells, which do not form cortical blebs after MYPT1 depletion (Supplementary Fig. 2). Furthermore, very similar results were obtained in immortalized,
The midbody proteome and interactome share many proteins and have similar GO enrichment profiles. 

**a** Proportional Venn diagram showing the number of proteins identified in the midbody proteome and interactome. The majority of midbody proteome proteins (62.9%) are contained in the midbody interactome.

**b** Heat map showing the GO annotation enrichment profiles of the midbody proteome and of the midbody interactome. GO enrichment profiles were analyzed using PANTHER under the category GO-slim biological process. Overrepresented GO terms are shown in shades of red while underrepresented GO terms are shown in shades of blue, according to their fold enrichment as indicated in the color scale bar at the right. Only Bonferroni-corrected results for $p < 0.05$ were considered (see Supplementary Data 5).
Fig. 3 The midbody interactome comprises common and specific networks. a Diagram illustrating the entire midbody interactome. Baits are indicated with blue hexagons, while preys are represented as ovals, either in green, if they were also found in the midbody proteome, or in orange. The edges connecting the network nodes are colored according to their Mascot scores as indicated in the color scale bar at the top left. Preys shared by multiple baits are clustered in the center. b Enlargement of the Anillin-specific sub-network shown in the corresponding inset in a. c Enlargement of the baits shared specifically by CIT-K and KIF4 shown in the corresponding inset in a. d Diagram representing the phosphorylation sub-network. All nodes are labeled with their primary gene names according to the UniProt database (https://www.uniprot.org)
non-transformed RPE-1 cells (Supplementary Fig. 3), indicating a general requirement for MYPT1 in cytokinesis in different cell types. Finally, almost identical phenotypes were observed after PP1β siRNA (Fig. 4b and Supplementary Fig. 4), further supporting that MYPT1 is acting as a regulatory subunit for PP1β in late cytokinesis.

**PP1β dephosphorylates the centralspindlin component MKLP1.** Central spindle assembly depends on various microtubule associated proteins (MAPs)\(^2\), including two key protein complexes: centralspindlin, a hetero-tetramer composed of two MKLP1 and two RacGAP1 subunits, and the PRC1-KIF4A complex. These MAPs have been shown to interact and cooperate...
to increase the robustness of the central spindle\(^2\). To understand the molecular mechanisms underpinning the phenotypes observed after MYPT1-PP1\(\beta\) depletion, we investigated whether centralspindlin could be one of the substrates of this phosphatase. Centralspindlin clustering at the central spindle midzone is necessary for its localization and function and requires phosphorylation of the evolutionarily conserved MKLP1 S708 residue by Aurora B\(^2\). MYPT1 depletion caused a significant increase in MKLP1 S708 phosphorylation at the midbody (Fig. 7a, b), reduced the association of this kinase with its RcgA1P1 partner and almost completely abolished its interaction with PRC1 and PP1\(\beta\), but only mildly affected the association with CIT-K (Fig. 7c). MKLP1 contains a highly conserved VQF motif 80 amino acids downstream of S708 (aa 620–858) purified from bacteria was also able to pull down PP1\(\beta\) in vitro (Supplementary Fig. 5b–d), indicating that PP1\(\beta\) directly binds to the MKLP1 C-terminal. MKLP1,620–858 was dephosphorylated at S708 by PP1\(\beta\) in vitro (Fig. 7f, g) and when the VQF residues were mutated to AQA the binding of MKLP1 to PP1\(\beta\) was reduced (Fig. 7e and Supplementary Fig. 5b–d) and MKLP1,620–858 dephosphorylation by PP1\(\beta\) in vitro was less efficient (Fig. 7f, g). To assess the role of MKLP1 dephosphorylation by PP1\(\beta\) in vivo, we generated cell lines stably expressing GFP-tagged versions of either wild type MKLP1 or of the mutant containing the AQA mutation at residues 786–788. Silencing MKLP1 by using an siRNA directed against its 3’UTR that is absent in the GFP-tagged transgenes severely impaired central spindle assembly and cleavage furrow progression (Fig. 7h, top panels). The very few MKLP1 siRNA cells that managed to complete furrowing had very thin actin and microtubules (Fig. 7h, bottom panels), similar again to MYPT1 siRNA cells (Fig. 6). Together, these results indicate that PP1\(\beta\) dephosphorylates MKLP1 at S708 in late cytokinesis via association with the VQF motif and that this dephosphorylation is important for MKLP1 function in late cytokinesis.

### Discussion

Our characterization of the midbody interactome and proteome represents a significant advance in understanding the complex and intricate protein–protein interactions of this organelle. Our interactome is derived from experimental data and provides a much more realistic and accurate picture than a previous bioinformatics study\(^3\). The overlap and highly similar GO enrichment profiles of the interactome and proteome datasets (Fig. 2) strongly support the validity of our approach and methodology. As expected, both datasets are enriched in proteins involved in mitosis and cytokinesis, but they also show a significant enrichment in proteins involved in chromatin assembly and mRNA processing and translation (Fig. 2 and Supplementary Data 4, 5). Although unpredicted, these findings are consistent with the identification of histones at the midbody\(^2\) and the evidence that the RNA-binding protein ATX-2 is involved in posttranscriptional regulation of PAR-5 levels at the midbody\(^3\). They also highlight the possibility that the midbody may function as a translational hub, which could indicate a mechanism by which asymmetric inheritance of the midbody imparts genetic information in cell fate and carcinogenesis\(^3\). The identification of common and specific networks of midbody proteins could serve to disentangle regulatory mechanisms and pathways for midbody function as well as to identify specific roles for each of the many baits used in our study. Together, these specific and common interaction networks will undoubtedly provide an extremely valuable resource for understanding the emerging multifaceted biological roles of this organelle. However, our interactome analysis is limited to one cell type and it is possible that different proteins and protein–protein interactions exist in midbodies of different cell types. Nevertheless, our study provides a molecular blueprint of the interaction networks in the midbody, which can serve to identify major nodes, hubs and pathways that may facilitate the analysis and comparison of midbodies in other cellular and developmental contexts.

The most abundant and frequent phosphatases identified in our midbody interactome are members of the PP1 family (Fig. 3d and Supplementary Table 2). This was somewhat unexpected as only PP2A phosphatases had been previously implicated in the regulation of cytokinesis\(^6,7\) and just very recently a role in abscission was described for PP1\(\gamma\) and its targeting co-factor RIF1\(^8\), which was also identified in both our midbody interactome and proteome (Supplementary Data 4). Therefore, our results expand the repertoire of PP1 functions during mitotic exit and indicate that MYPT1-PP1\(\beta\) is required to regulate the pace of cleavage furrow progression and to form strong and stable central spindles and midbodies in late cytokinesis (Fig. 6a–c). Cytokinesis failure after MYPT1 siRNA occurs predominantly at a late stage, after completion of furrow progression (Fig. 6b), highlighting an unanticipated role of MYPT1 in this phase of cell division. Our results indicate that MYPT1-PP1\(\beta\) regulates the dynamics of the two major cytokinetic structures, the actomyosin contractile ring and the central spindle, by de-phosphorylating different

[**Fig. 4**] PP1 phosphatases localize to the midbody and depletion of PP1\(\beta\) and MYPT1 causes cytokinesis failure. a–d HeLa cells were fixed and stained to detect to detect DNA (blue in the merged panels), tubulin, and PP1\(\alpha\) (a), PP1\(\beta\) (b), PP1\(\gamma\) (c), and MYPT1 (d). For RNAi depletions, HeLa cells were treated with siRNAs directed against each of the three PP1 catalytic subunits or MYPT1 and after 48 h were fixed and stained to detect the same epitopes as described above. DNA condensation and shape and thickness of microtubule bundles at the intercellular bridge were used as criteria to stage telophase cells. Insets show a 3× magnification of the midbody. Scale bars, 10 μm. e HeLa Kyoto cells were treated with siRNAs directed against each a random sequence (control) or MKLP1 (right) and after 48 h were proteins were extracted and analyzed by western blot to detect the indicated proteins. The numbers on the left indicate the sizes in kDa of the molecular mass marker. f HeLa cells were treated with siRNAs directed against either a random sequence (control) or MYPT1 and after 48 h were fixed and stained to detect DNA, tubulin, and di-phosphorylated MRLC. Note that MYPT1 siRNA cells show abnormal cell shape and nuclear shape, cortical blebs (arrowheads) and disorganized microtubule and actomyosin cytoskeletal filaments. Scale bars, 10 μm. g HeLa cells were treated with siRNAs directed against either a random sequence (control) or MYPT1 and after 48 h were fixed and stained to detect DNA and tubulin. The arrows indicate multinucleate cells. Scale bars, 10 μm. h Quantification of multinucleate cells obtained after siRNA of the three PP1 catalytic subunits or MYPT1. More than 500 cells were counted in n ≥ 3 independent experiments. Bars indicate standard errors. *p < 0.05, **p < 0.01 (Mann–Whitney U test). Source data for Fig. 4e and h are provided as a Source Data file.
in line with its established role, our data indicate that MYPT1-PP1β dephosphorylates MRLC to control the contractility of the actomyosin ring during furrow ingression (Fig. 5), but also unexpectedly reveal that MYPT1-PP1β controls the dynamics of central spindle microtubules in late cytokinesis (Fig. 6). Our results suggest that the latter could be mediated, at least in part, through dephosphorylation of MKLP1, and most likely of other MAPs, in order to antagonize Aurora B and possibly other mitotic kinases, like Plk1 and CIT-K, in late cytokinesis (Figs. 6 and 7). We surmise that MKLP1 dephosphorylation by PP1β modulates centralspindlin clustering in order to promote different functions of this complex in late cytokinesis, like its close association with other midbody proteins such as PRC1. This, in combination with dephosphorylation of additional midbody components, would contribute to MYPT1-PP1β-mediated regulation of central spindle microtubule dynamics and midbody architecture in late cytokinesis. In sum, our findings indicate that temporal changes in the spatial distribution of kinases and counteracting phosphatases during cytokinesis control the phosphorylation status, and consequently
the activity of cytokinesis proteins as illustrated in Fig. 8. In early telophase MYPT1-PP1β localizes to the cortex of the ingressing furrow (see Fig. 4) where it can de-phosphorylate MRLC to antagonize Rho kinase, but has no or little access to the centralspindlin pool that accumulate at the central spindle midzone, which is instead highly phosphorylated by Aurora B and can therefore form clusters. However, after completion of furrow ingression, MYPT1-PP1β accumulates at the midbody ring whereas Aurora B is slowly degraded and accumulates at the midbody arms. This allows dephosphorylation of MKLP1 at S708, which could strengthen the association of centralspindlin with other midbody proteins, in particular PRC1. This change in the phosphorylation status of MKLP1, and most likely of other midbody components, is important for maintaining a robust central spindle and for establishing proper midbody architecture.

Finally, our data show that MYPT1 is required for the association of MKLP1 and PP1β in telophase cells (Fig. 7c) and that PP1β requires the VQF sub-optimal binding motif of MKLP1 to select its target S708 residue (Fig. 7). However, our current knowledge of PP1 function indicates that PP1β cannot simultaneously interact with both MYPT1 and MKLP1 through their (R) VxF motifs. One possible explanation could be that MYPT1 is
necessary to initially bring PP1β in proximity of MKLP1, but then a pool of PP1β could dissociate from MKT1 to interact with MKLP1 through its less efficient VQF site to de-phosphorylate S708. Future studies can clarify whether such a two-step mechanism of action does indeed exist and how widely it is employed by PP1 catalytic subunits.

Methods

Molecular biology. The coding sequence for PP1β was amplified by PCR using the Addgene plasmid 31677 as a template to create an entry clone in pDONR221 using Gateway technology according to manufacturer’s instruction (ThermoFisher). The plasmid pEGFP-C1::MKLP1 was previously described15. The QuickChange Lightning Site-Directed Mutagenesis Kit (Agilent) was used to generate the PP1β phosphatase dead mutant (harboring the mutations D94N and H124N) and the pEGFP-C1::MKLP1AQ plasmid containing two substitutions in the VQF-binding site for PP1β. All oligonucleotides used in this study are listed in Supplementary Table 3.

Cell culture and treatments. HeLa Kyoto (kind gift from Ina Poser, Max Planck Institute of Molecular Cell Biology and Genetics) were maintained in DMEM (Sigma) containing 10% fetal bovine serum (Sigma) and 1% penicillin/streptomycin (Invitrogen) at 37 °C and 5% CO2. HeLa S3 (ATCC) were maintained in DMEM lacking Arg and Lys (Sigma-Aldrich), and supplemented with 10% (v/v) dialyzed FBS (Sigma-Aldrich), 1% (v/v) penicillin/streptomycin and either unlabeled Arg and Lys or L-[13C5,15N2]Arg and L-[13C5,15N2]lysine (Cambridge Isotope Laboratories) at concentrations of 42 μg ml−1 (Arg) and 72 μg ml−1 (Lys). Trypsin-EDTA was used to split cells as usual. However, as this solution might contain some non-isotopically labeled amino acids, detached cells were pelleted at 250 × g for 5 min, washed once with sterile cold PBS, and either released for 30 min in fresh medium containing 10 μM MG132 (Sigma) to collect cells in metaphase or released in just fresh medium for 5 min to collect cells in telophase. Cells were then harvested by centrifugation and frozen in dry ice.

Results

Cell lines stably expressing MKLP1 or MKLP1AQ constructs were generated by transfecting HEK 293 cells (ATCC) with the mammalian expression vector pCDNA3.1 containing the coding sequence of human MKLP1, with pcDNA3-1 being re-seeded in fresh medium.

Cell lines stably expressing MKLP1 or MKLP1AQ constructs were generated by transfecting 2 × 106 HEK 293 cells with 10 μg of plasmid DNA using the Neon transfection system (ThermoFisher) using manufacturer’s instructions. After 48 h, cells in 100 mm culture dish were selected in complete selective medium containing 400 μg ml−1 G418 for ~2 weeks until colonies became visible. Individual colonies were picked, cultured under resistance and tested for expression of the construct by western blot and immunofluorescence. To generate the cell line expressing Aurora A::GFP, HEK 293 cells (originally from ATCC) were transfected with the pCAGGS-N2A vector containing the coding sequence of human Aurora A, terminally fused to GFP, using FuGENE transfection reagent according to the manufacturer’s instructions. Twenty-four hours posttransfection G418 was added to the medium (500 μg ml−1), and cells incubated for a further 7 days. The population was then expanded and FACS sorted on the GFP signal, and maintained under G418 selection.

HeLa cell lines were synchronized in S phase by double thymidine block. Cells were first arrested in S phase by the addition of 2 μM thymidine (Sigma- Aldrich) for 19 h, washed twice with phosphate-buffered saline (PBS) and released for 5 h in fresh complete medium. After release, cells were incubated again for 19 h in complete medium containing 2 μM thymidine, washed twice with PBS, released in fresh medium for 10 min, harvested by centrifugation at 1000 × g for 3 min, washed in PBS, frozen immediately in dry ice and stored at −80 °C. To synchronize HeLa cells in metaphase and telophase, we used a thymidine-nocodazole block and release procedure. Cells were first arrested in S phase by a single thymidine treatment as described above, washed twice with phosphate-buffered saline (PBS) and released in fresh complete medium. Cells were then cultured for additional 13 h in fresh complete medium containing 50 mg ml−1 nocodazole (Sigma Aldrich) and then harvested by mitotic shake-off. Mitotic cells were washed three times with PBS, and either released for 30 min in fresh medium containing 10 μM MG132 (Sigma) to collect cells in metaphase or released in just fresh medium for 90 min to collect cells in telophase. Cells were then harvested by centrifugation and frozen in dry ice.
Affinity purification (AP). For large-scale AP of GFP-tagged proteins and associated partners, cells were plated at 1/6 confluence in either six 175 cm² flasks or in two three-layer 525 cm² tissue culture flasks (BD Biosciences) and after 24 h synchronized at different stages of the cell cycle as described in the previous section, collected, washed in PBS and cell pellets stored at −80 °C. Each cell pellet was resuspended in 5 ml of lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 2 mM MgCl₂, 1 mM EGTA, 0.1% [v/v] NP-40, 1 mM DTT, 5% [v/v] glycerol and Roche Complete Protease Inhibitors) and homogenized using a high-performance disperser (Fisher). The homogenate was clarified by centrifugation at 750 × g for 15 min at 4 °C and the supernatant was incubated with 200 μl of GFP-Trap_MA magnetic beads (ChromoTek) for 4 h on a rotating wheel at 4 °C. Beads were then washed four times using a magnetic stand in 10 ml of lysis buffer for 5 min on a rotating wheel at 4 °C, transferred to a new tube and washed one more time in 10 ml of PBS. After removing as much liquid as possible, beads were stored at...
Fig. 7 PP1β dephosphorylates MKLP1 at S708 in cytokinesis. a HeLa Kyoto cells were treated with control or MYPT1 siRNA for 48 h and stained to detect the indicated epitopes. Cells were staged as in Fig. 4. Insets indicate 3× magnification of the midbody. Scale bars, 10 µm. b Quantification of total and pS708 MKLP1 in control and MYPT1 siRNA cells. The boxes indicate the first quartile to the third quartile, the horizontal lines the median and the whiskers the minimum or maximum. AU, arbitrary unit; n = 32 independent control cells and n = 35 MYPT1 siRNA independent cells for MKLP1 stained cells; n = 38 independent control cells and n = 42 MYPT1 siRNA independent cells for MKLP1-pS708 stained cells; p values from student’s T-test. c HeLa stably expressing GFP-MKLP1 were treated with control or MYPT1 siRNA, synchronized in telophase and GFP pull-down protein extracts analyzed by western blot. The numbers indicate the size of the molecular mass marker. d Schematic diagram of MKLP1 protein. The Aurora B phosphorylation site and the VQF PP1-binding site are indicated. e MBP-tagged MKLP1, MKLP1ΔC or MBP alone were co-expressed in yeast and used for MBP pull-down assay. Extracts and pull downs were analyzed by western blot to detect GST and MBP. Numbers indicate the size of the protein ladder. f HeLa Kyoto cells stably expressing GFP-MKLP1, GFP-MKLP1ΔC or no transgene were treated with either control or MYPT1 3'UTR siRNA were stained to detect the indicated epitopes. Scale bars, 10 µm. i Quantification of multinucleate cells from the experiments shown in h. More than 500 independent cells were counted in n ≥ 3 independent experiments. Bars indicate standard errors. *p < 0.05, ** p < 0.01, ***p < 0.001 (Mann–Whitney U test). Source data for Fig. 1b, c, e-g and i are provided as a Source Data file.

For the identification of proteins from AP experiments, raw MS/MS data were analyzed using the Mascot search engine (Matrix Science). Peptides were searched against the Uniprot human sequence database and the following search parameters were employed: enzyme specificity was set to trypsin, a maximum of two missed cleavages were allowed, carbamylation (Cys) was set as a fixed modification, whereas oxidation (Met), phosphorylation (Ser, Thr and Tyr), and ubiquitylation (Lys) were considered as variable modifications. Peptide and MS/MS tolerances were set to 25 parts per million (ppm) and 0.8 daltons (Da). Peptides with Mascot Score exceeding the threshold value corresponding to < 5% false positive rate, calculated by Mascot procedure, and with the Mascot score above 30 were considered to be positive.

For the sequencing of proteins from AP experiments, raw MS/MS data were analyzed using the Mascot search engine (Matrix Science). Peptides were searched against the Uniprot human sequence database and the following search parameters were employed: enzyme specificity was set to trypsin, a maximum of two missed cleavages were allowed, carbamylation (Cys) was set as a fixed modification, whereas oxidation (Met), phosphorylation (Ser, Thr and Tyr), and ubiquitylation (Lys) were considered as variable modifications. Peptide and MS/MS tolerances were set to 25 parts per million (ppm) and 0.8 daltons (Da). Peptides with Mascot Score exceeding the threshold value corresponding to < 5% false positive rate, calculated by Mascot procedure, and with the Mascot score above 30 were considered to be positive.

Computational and statistical analyses. We used in-house written Perl scripts to combine the Mascot data from the replicates of AP-MS experiments for each bait and to compare them with datasets obtained from AP-MS experiments using HeLa cells expressing GFP alone at the same cell cycle stage (5 phase, metaphase, or telophase) in order to eliminate non-specific hits. Prey hits absent from these GFP negative controls were classed as being specific. Additional common contaminants, such as keratins and hemoglobin, were eliminated manually. The filtered data (Supplementary Data 3) were then analyzed and visualized using Cytoscape (version 3.7.0).

To generate the serine/threonine phosphorylation sub-network, we searched the interactome dataset for proteins whose Uniprot protein names field contained the terms kinase and phosphorylate but not tyrosine via grep in the Unix command line. This generated a dataset of 190 proteins that was subsequently manually curated to eliminate proteins that were not directly involved in phosphorylation/ dephosphorylation, such as kinase-associated proteins. The final list of 136 proteins was entered into a raw tab-delimited file text and then imported into Cytoscape to generate the network shown in Fig. 3d.

GO enrichment analysis was performed using PANTHER41, Prisym (GraphPad) and Excel (Microsoft) were used for statistical analyses and to prepare graphs.

Time-lapse imaging. For time-lapse experiments, HeLa Kyoto cells expressing GFP-tubulin and H2B:mCherry were plated on an open µ-Slide with 8 wells (Ibidi, 80826) 30 h after RNAi treatment. Imaging was performed on a Leica DMi8 CS AFC Motorized Research Inverted Digital Microscope. Images were collected with a 40 × 1.30 NA HC Plan APO CS2 - OIL DIC 240 µm objective and excitation Lasers of Argo (65 mw, 488 nm) and of DPSS (20 mw, 561 nm). We used the Application Suite X software (LAS-X; Leica) for multidimensional image acquisition. Specimens were maintained at 37 °C and 5% CO2 via a chamber, and z-series of 14, 1 µm sections were captured at 2 min intervals. All images were processed using Fiji41 to generate maximum intensity projections, to adjust for brightness and contrast, and to create the final movies.

Fluorescence microscopy. HeLa cells were grown on microscope glass coverslips (Menzel-Gläser) and fixed in either PHEM buffer (60 mM Pipes, 25 mM Heps pH 7, 10 mM EGTA, 4 mM MgCl₂, 3.7% [v/v] formaldehyde) for 12 min at room temperature or in ice-cold methanol for 10 min at −20 °C. They were then washed three times for 10 min with PBS and incubated in blocking buffer (PBS, 0.5% [v/v] Triton X-100 and 5% [w/v] BSA) for 1 h at room temperature. Coverslips were incubated overnight at 4 °C with the primary antibodies indicated in the figure legends, diluted in PBT (PBS, 0.1% [v/v] Triton X-100 and 1% [w/v] BSA). The day after, coverslips were washed twice for 5 min in PBT, incubated with secondary antibodies diluted in PBT for 2 h at RT and then washed twice with PBT and once with PBS. Coverslips were mounted on SuperFrost Microscope Slides (VWR) using VECTASHIELD Mounting Medium containing DAPI (Vector Laboratories).

Phenotypes were blindly scored by at least two people independently. Images were...
acquired using a Zeiss Axiovert epifluorescence microscope equipped with MetaMorph software. Fiji was used to generate maximum intensity projections, which were adjusted for contrast and brightness and assembled using Photoshop. Fluorescence intensity values in Fig. 7b were measured from identically sized areas at the midbody (I_M), in the nucleus (I_N), and in the background (I_B) using Fiji and then normalized values were calculated using the following formula: 

\[ \frac{(I_M - I_B) - (I_N - I_B)}{I_N - I_B} = \frac{I_M - I_N}{I_N - I_B}. \]

Antibodies. The following antibodies and dilutions for western blot (WB) and immunofluorescence (IF) were used in this study: mouse monoclonal anti-α-tubulin (clone DM1A, Sigma, T9026 dilutions for WB 1:20,000, for IF 1:2000), rabbit polyclonal anti-β-tubulin (Abcam, ab6046 dilutions for WB 1:5000, for IF 1:400), mouse monoclonal anti-cyclin B1 (clone GNS1, Santa Cruz, sc-245 dilution for WB 1:1000), mouse monoclonal anti-PP1α (clone G-4, Santa Cruz, sc-271762 dilutions for WB 1:1000, for IF 1:50), mouse monoclonal anti-PP1β (clone A-6, 

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Fig. 8 Model of regulation of centralspindlin by Aurora B and MYPT1-PP1β during cytokinesis. During furrowing (top panel) MYPT1-PP1β accumulates at the cortex with no or very limited access to the centralspindlin pool that localizes to the central spindle midzone, which is highly phosphorylated by Aurora B and therefore can form clusters. After completion of furrow ingression, MYPT1/PP1β accumulates at the midbody ring whereas Aurora B localizes to the midbody arms (bottom panel). This allows PP1β to de-phosphorylate MKLP1 at S708, which could strengthen the association of centralspindlin with PRC1. See text for more details.
Santa Cruz, sc-36587 dilutions for WB 1:10,000, for IF 1:50), mouse monoclonal anti-PPT1 (clone A-4, Santa Cruz, sc-519493 dilutions for WB 1:2000, for IF 1:50), mouse monoclonal anti-CIT-K (BD Transduction Laboratories, 611377 dilutions for WB 1:1500, for IF 1:250), mouse monoclonal anti-MYPT1 (clone C-6, Santa Cruz, sc-614261 dilutions for WB 1:1500, for IF 1:50), rabbit polyclonal anti-Aurora A (Abcam, ab21387 dilution for WB 1:4000), rabbit polyclonal anti-TPX2 (Novus, NB110-179 dilution for WB 1:5000), rabbit polyclonal anti-Aurora B (clone AB-6, Abcam), 1:500, KIF14 (Bethyl Laboratories), A300-233A dilution for WB 1:2000) rabbit polyclonal anti-MKLP1 (clone N9, Santa Cruz Biotechnology, sc-867, dilutions for WB 1:3000, for IF 1:500), rabbit polyclonal anti-phospho-MKLP1 pS70289 (dilutions for WB 1:2000, for IF 1:200), rabbit polyclonal anti-tri-phospho CHMP4C pS210 pS212 (dilution for WB 1:1000), mouse monoclonal anti-Aurora A (Abcam, ab76623 dilution for WB 1:12,000), rabbit polyclonal anti-phospho-histone H3 pS10 (Merck, 06-570 dilution for WB 1:10,000), rabbit polyclonal anti-mono-phospho MRLC pS19 (Cell Signaling Technology, 3671), dilutions for WB 1:1000, for IF 1:50), rabbit polyclonal anti-di-phospho MRLC pT18 pS19 (Cell Signaling Technology, 3674, dilutions for WB 1:1000, for IF 1:100), goat polyclonal anti-RacGAP1 (Abcam, ab22270 dilution for WB 1:1000), mouse monoclonal anti-GST (clone A-4, Santa Cruz, sc-5113, dilutions for WB 1:2000, anti-MBP (NEB, E0302 dilution for WB 1:10,000). Peroxidase and Alexa-fluor conjugated secondary antibodies were purchased from Jackson Laboratories and Thermolabo, respectively.

Transmission electron microscopy. For electron microscopy analyses, HeLa Kyoto cells were plated on microscope glass coverslips (Menzel-Gläser). Cells were fixed overnight at 4 °C in 2.5% [v/v] glutaraldehyde in PBS, post fixed for 1 h in 1% [v/v] OsO4 in PBS, dehydrated in a graded series of alcohols, embedded in Epon-Araldite resin, and polymerized for 2 days at 60 °C. Glass slides were sectioned from the resin after a short immersion in liquid nitrogen. Sections were selected with a LKB ultratome, stained with uranyl acetate and lead citrate, and observed with a Tecnai G2 Spirit transmission electron microscope operating at an accelerating voltage of 100 kV and equipped with a Morada CCD camera (Olympus).

Protein expression time course and GFP pull-down assay. 1.4 × 10⁶ HeLa Kyoto cells were plated in large—157 cm²—flasks and transfected with siRNAs (800 pmol) directed against either a scrambled sequence (control) or MYPT1. After 24 h, cells were synchronized by thymidine/nocodazole block, released in fresh medium and divided into four 75 cm² flasks, and collected after 9, 45, 90, and 120 min as indicated in Fig. 7. Proteins were then extracted, separated on a SDS-PAGE gel, transferred onto PVDF membrane, and probed to detect the antigens indicated in Fig. 7. Uncropped versions of all blots can be found in the Source Data file.

For the phosphatase assay, 10 μg of purified GST-MKP1-Δfl-AQA proteins (wild-type and AQA mutant) bound to beads were phosphorylated in vitro with 2 μg of recombinant His-tagged Aurora B (ThermoFisher) in 40 μl final volume of kinase buffer (20 mM HEPES pH 7.5, 5 mM MgCl₂, 1 mM DTT, 0.1 mM cold ATP) at 30 °C for 30 min and then washed three times with 500 μl of wash buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.2% NP-40), and a cocktail of Roche Complete Protease Inhibitors) for 60 min at 4 °C on a rotating wheel and then washed five times with 500 μl of wash buffer (50 mM Tris-HCl pH 7.4, 250 mM NaCl, 5 mM EDTA, 0.2% NP-40, and a cocktail of Roche Complete Protease Inhibitors), followed by centrifugation at 500 × g for 1 min. Beads were resuspended in 25 μl of Laemmli SDS-PAGE sample buffer and typically 1/5 was loaded on a 4–20% Tris-Glycine gel for western blot analysis. Uncropped versions of all blots can be found in the Source Data file.

For the GST pull downs, MBP::PP1 purified from yeast and eluted from beads (see previous section) was mixed with 25 μl of Glutathione Sepharose beads containing purified GST or GST-MKP1-Δfl-AQA proteins (wild type and AQA mutant). Samples were incubated in 300 μl of NET-N + buffer (50 mM Tris-HCl, pH 7.4, 130 mM NaCl, 5 mM EDTA, 0.2% NP-40, and a cocktail of Roche Complete Protease Inhibitors) for 60 min at 4 °C on a rotating wheel and then washed five times with 500 μl of wash buffer (50 mM Tris-HCl pH 7.4, 250 mM NaCl, 5 mM MgCl₂, 0.2% NP-40, and a cocktail of Roche Complete Protease Inhibitors), followed by centrifugation at 500 × g for 1 min. Beads were then resuspended in 100 μl of phosphate buffer, divided in 10 aliquots, and each aliquot was incubated with 200 ng of either MBP::PP1 or MBP::PP1dead enzymes purified from yeast (see previous section) at 30 °C with gentle agitation. Samples were collected at 10, 20, 40, and 60 min and reactions were immediately stopped with the addition of 2× Laemmli sample buffer. Proteins were separated by SDS-PAGE and analyzed by western blot with anti-phospho MKLP1 pS708 and anti-GST antibodies. Uncropped versions of all blots can be found in the Source Data file.

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References
1. D’Avino, P. P., Giancanti, M. G. & Petronczki, M. Cytokinesis in animal cells. Cold Spring Harb. Perspect. Biol. 7, a015834 (2015).
2. Douglas, M. E. & Mishima, M. Still entangled: assembly of the central spindle by multiple microtubule modulators. Semin Cell Dev. Biol. 21, 899–908 (2010).
3. NASA, I. & Kettenbach, A. N. Coordination of protein kinase and phosphoprotein phosphatase activities in mitosis. *Front Cell Dev. Biol.* 6, 30 (2018).
4. D’Avino, P. P. & Capalbo, L. Regulation of midbody disassembly and function by mitotic kinases. *Semin Cell Dev Biol.* 53, 57–63 (2016).
5. Mierzwa, B. & Gerlich, D. W. Cytokeletal abscission: molecular mechanisms and temporal control. *Dev. Cell* 31, 525–538 (2014).
6. Hu, C. K., Coughlin, M. & Mitchison, T. J. Midbody assembly and its regulation during cytokinesis. *Mol. Biol. Cell* 23, 1024–1034 (2012).
7. Dionne, L. K., Wang, X. J. & Prekèris, R. M. Bidouille: from cellular junk to regulator of cell polarity and cell fate. *Curr. Opin. Cell Biol.* 35, 51–58 (2015).
8. Pohl, C. The midbody and its remnants in cell polarization and asymmetric cell division. *Results Protoc. Cell Diff.* 61, 165–182 (2017).
9. Bassi, Z. I., Audusseau, M., Gari, F. & Heald, R. Dissection of the mitotic spindle midzone. *Nat. Protoc.* 45, 1537–1553 (2010).
10. McKenzie, C. et al. Cross-regulation between Aurora B and Citron kinase controls midbody architecture in cytokinesis. *Open Biol.* 6, 160019 (2016).
11. McKenzie, C. & D’Avino, P. P. Investigating cytokinesis failure as a strategy in cancer therapy. *Onco-targete* 7, 8732–8741 (2016).
12. Gai, M. et al. Citron kinase controls abscission through RhOa and anillin. *Mol. Biol. Cell* 22, 3786–3787 (2011).
13. Grameneb, U. et al. Fli1 and citron kinase act together to promote efficient cytokinesis. *J. Cell Biol.* 172, 363–372 (2006).
14. Skop, A. R., Liu, H., Yates, J. 3rd, Meyer, B. J. & Heald, R. Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. *Science* 305, 61–66 (2004).
15. Field, C. M., Coughlin, M., Doberstein, S., Marty, T. & Sullivan, W. Characterization of anillin mutants reveals essential roles in septin localization and plasma membrane integrity. *Development* 133, 2849–2860 (2005).
16. Renshaw, M. J., Liu, J., Lavoie, B. D. & Wilde, A. Anillin-dependent organization of septin filaments promotes intercellular bridge elongation and 1Dmp4-targeting to the abscission site. *Open Biol.* 4, 130190 (2014).
17. D’Avino, P. P. et al. Recruitment of Polo kinase to the spindle midzone during cytokinesis requires the Feo/Klp3A complex. *Dev. Cell* 26, 250–263 (2013).
18. Wurzenberger, C. & Gerlich, D. W. Phosphatases: providing safe passage through mitotic exit. *Nat. Rev. Mol. Cell Biol.* 12, 469–482 (2011).
19. Trinkle-Mulchay, L. et al. Time-lapse imaging reveals dynamic relocalization of PP1gamma throughout the mammalian cell cycle. *Mol. Biol. Cell* 14, 107–117 (2003).
20. Kiss, A., Erdodi, F. & Lontay, B. Myosin phosphatase: unexpected functions of a long-known enzyme. *Biochim Biophys. Acta Mol. Cell Res.* 1866, 2–15 (2019).
21. Yamashiro, S. et al. Myosin phosphatase-targeting subunit 1 regulates mitosis by antagonizing polo-like kinase 1. *Dev. Cell* 14, 787–797 (2008).
22. Jiang, W. et al. PRC1: a human mitotic spindle-associate CKD substrate protein required for cytokinesis. *Mol. Cell* 2, 877–885 (1998).
23. Capalbo, L. et al. Coordinated regulation of the ESCRT-III component CHMP4C by the chromosomal passenger complex and centralspindlin during cytokinesis. *Open Biol.* 6, https://doi.org/10.1098/rsob.160248 (2016).
24. Capalbo, L. & Doberstein, S. The chromosomal passenger complex controls the function of endosomal sorting complex required for transport-III Snf7 proteins during cytokinesis. *Open Biol.* 2, 120070 (2012).
25. Lee, K. Y., Esmaeili, B., Zealley, B. & Mishima, M. Direct interaction between centralspindlin and PRC1 reinforces mechanical resilience of the centrosome. *Nat. Commun.* 6, 7290 (2015).
26. Geymonat, M., Spanos, A. & Sedgwick, S. Production of mitotic regulators using an autoselection system for protein expression in budding yeast. *Methods Mol. Biol.* 545, 63–80 (2009).
27. Rinaldo, C. et al. HIPK2 controls cytokinesis and prevents tetraploidization by phosphorylating histone H2B at the midbody. *Mol. Cell* 47, 87–98 (2012).
28. Guazzu, M. M. et al. The RNA-binding protein ATX-2 regulates cytokinesis through PAR-5 and ZEN-4. *Mol. Cell Biol.* 27, 3052–3064 (2016).
29. Ettinger, A. W. et al. Profilin-related differentiation and stem cells exhibit distinct midbody-release behaviour. *Nat. Commun.* 2, 503 (2011).
30. Kuo, T. C. et al. Midbody accumulation through evasion of autophagy controls transition to cellular reprogramming and tumorigenicity. *Nat. Cell Biol.* 13, 1214–1223 (2011).
31. Bastos, R. N., Cundell, M. J. & Barr, F. A. KIF1A and PAR-5 form a spatially restricted feedback loop opposing Aurora B at the anaphase central spindle. *J. Cell Biol.* 207, 683–693 (2014).
32. Cundell, M. J. et al. The BGG (PAR-5/ENSA/Greatwall) pathway ensures cytokinesis follows chromosome separation. *Mol. Cell* 52, 393–405 (2013).
33. Bhomnick, R. et al. The RIF1-PPI axis controls abscission timing in human cells. *Curr. Biol.* 29, 1232–1242 (2019).
34. Neumann, B. et al. Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes. *Nature* 464, 721–727 (2010).
35. Lehmann, A., Kiewer, A., Martens, J. C., Nagel, F. & Schulz, S. Carboxyl-terminal receptor domains control the differential dephosphorylation of somatostatin receptors by protein phosphatase 1 isoforms. *PLoS One* 9, e91526 (2014).
36. Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein detection. *Nat. Biotechnol.* 26, 1367–1372 (2008).
37. Cox, J. et al. A practical guide to the MaxQuant computational platform for SILAC-based quantitative proteomics. *Nat. Protoc.* 4, 698–705 (2009).
38. Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. Genetic applications of yeast transformation with linear and gapped plasmids. *Methods Enzymol.* 101, 228–245 (1983).
39. Zanin, E. et al. Conserved RhoGAP limits M phase contractility and coordinates with microtubule asters to confine RhoA during cytokinesis. *Dev. Cell* 26, 496–510 (2013).
40. Guizetti, J. et al. Cortical constriction during abscission involves helices of ESCRT-III-dependent filaments. *Science* 331, 1616–1620 (2011).
41. Su, K. C., Takaki, T. & Petronczki, M. Targeting of the RhoGEC Fct2 to the equatorial membrane controls cleavage furrow formation during cytokinesis. *Cell* 21, 1104–1115 (2011).
42. Maliga, Z. et al. A genomic toolkit to investigate kinesin and myosin motor function in cells. *Nat. Cell Biol.* 15, 325–334 (2013).
43. Hutterer, A., Glotzer, M. & Mishima, M. Clustering of centralspindlin is essential for its accumulation to the central spindle and the midbody. *Curr. Biol.* 19, 2043–2049 (2009).
44. Poser, I. et al. BAC TransgeneOmic: a high-throughput method for exploration of protein function in mammals. *Nat. Methods* 5, 409–415 (2008).

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**Competing interests**

The authors declare no competing interests.
