Hexameric NuMA:LGN structures promote multivalent interactions required for planar epithelial divisions

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Cortical force generators connect epithelial polarity sites with astral microtubules, allowing dynein movement to orient the mitotic spindle as astral microtubules depolymerize. Complexes of the LGN and NuMA proteins, fundamental components of force generators, are recruited to the cortex by Gαi-subunits of heterotrimeric G-proteins. They associate with dynein/dynactin and activate the motor activity pulling on astral microtubules. The architecture of cortical force generators is unknown. Here we report the crystal structure of NuMA:LGN hetero-hexamers, and unveil their role in promoting the assembly of active cortical dynein/dynactin motors that are required in orchestrating oriented divisions in polarized cells. Our work elucidates the basis for the structural organization of essential spindle orientation motors.

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In multicellular organisms, oriented cell divisions sustain tissue morphogenesis and homeostasis by ensuring the correct positioning of daughter cells after cytokinesis. Accurate execution of oriented divisions relies on the positioning of the mitotic spindle, which is attained in metaphase by the action of molecular motors coordinated with cortical polarity cues.

The major molecular motor responsible for spindle positioning is cytoplasmic dynein 1 (hereon referred to as dynein), a multi-subunit AAA-type ATPase that works with dynactin and moves towards the minus-end of microtubules. The motility of dynein/dynactin is activated by cargo adaptors, including BicD25–3, and Hook3. The most confirmed model assumes that cortical NuMA targets and anchors dynein/dynactin to the plasma membrane so that its retrograde motor activity on dynamic astral microtubules (MTs) results in traction forces pulling the spindle toward the cortex. How the coordinated action of these cortically-localized force generating machines orchestrates spindle placement remains poorly understood.

NuMA is a 250 kDa nuclear protein released in the cytoplasm after nuclear envelope break-down. The first 705 residues of NuMA are sufficient to immuno-precipitate dynein/dynactin from mitotic lysates. Interestingly, light-induced ectopic delivery of this NuMA fragment to the cortex results in dynein/dynactin recruitment but cannot support spindle pulling, implying that during spindle orientation additional functions encoded by the coiled-coil region and the C-terminal cargo binding portion of NuMA are essential for the spindle orientation process.

Overall, the domain structure of NuMA consists of a globular N-terminal domain predicted to fold as a Hook domain, a central 1500-residue long coiled-coil mediating self-assembly, and an unstructured C-terminal portion harboring bindings sites for microtubules, lipids, importin-α, the cortical protein 4.1R, and the spindle orientation protein LGN (Supplementary Fig. 1). How these NuMA binding partners contribute to the cortical localization of the protein has been extensively studied. In metaphase, when spindle positioning takes place, NuMA accumulates at the cortex by association with LGN. LGN in turn is targeted to the plasma membrane by direct interaction with multiple copies of the Gai subunit of heterotrimeric G-proteins inserting a myristoyl group in the lipid bilayer. Depolymerization of astral MTs by low doses of nocodazole does not affect the cortical localization of NuMA in metaphase (unpublished data) in spite of causing spindle misorientation. These observations suggest that NuMA is not transported to the cortex along astral MTs by kinesins, but rather acts upstream of the microtubule motors in the assembly of cortical force generating complexes. The functional role of the MT-binding activity of NuMA is poorly understood. Two discontinuous regions of NuMA associate with MTs: the first one spans residues 1914–1985 and is incompatible with LGN-binding, while the second lies in the C-terminal tail after residue 2001, is compatible with LGN binding, and has recently been implicated in spindle placement. Intriguingly, we previously found that phosphorylation of Ser2047 of NuMA by Aurora-A regulates cortical NuMA recruitment, for reasons that are still unclear. Two positively-charged stretches of NuMA upstream and downstream of the LGN-binding domain associate withPIP2 at the plasma membrane. Being negatively regulated by Cdk1 phosphorylation, this interaction is not involved in spindle placement as in early mitosis. However, PIP2-binding promotes an additional cortical NuMA accumulation occurring in anaphase, upon Cdk1 inactivation, that supports spindle elongation and sister chromatid separation.

The overall three-dimensional organization of active force generators is poorly understood. Ectopic targeting experiments indicate that clustering of NuMA at the cell cortex may be required for efficient pulling on astral microtubules, although the origin of the multivalent interactions required for cluster formation is unknown. Structural studies revealed that the elongated NuMA peptide encompassing residues 1900–1928 lines the inner side of the helical N-terminal TPR scaffold of LGN, engaging in a nanomolar affinity interaction with LGN in a concave super-helical array, in such a way that the first helix A of each TPR faces the inner side of the super-helix, while the second helix B is positioned outside. A peculiar feature of the TPR domain of LGN is the presence of flanking extensions at the N-terminus and C-terminus which are predicted to adopt a helical conformation. The flexible C-terminal portion of LGN associates cooperatively with four Gai molecules. These NuMA/LGN/Gai assemblies constitute core modules connecting dynein/dynactin and MTs plus-TIPs to the cortex.

Here, we report the crystallographic structure of NuMA-LGN hetero-hexamers assembled on a NuMA fragment longer than the minimal binding peptide. We show that these high-order oligomers are required for spindle orientation in an epithelial model. Molecularly, these hexameric complexes in combination with dimeric full-length NuMA can generate extended cortical protein networks that spatially organize dynein/dynactin on astral MTs to position the spindle.

### Results

#### Structural analysis of NuMA/LGN hexamers

To start investigating the architecture of force generators, we reconstituted the NuMA/LGN interaction using human proteins expressed in bacteria, starting from the previously identified binding interfaces. Size-exclusion chromatography (SEC) elution profiles of LGN and LGNTPR in the following) bound to NuMA1900–1928 were consistent with a 1:1 binary interaction, in agreement with the available structural data. However, the longer NuMA fragment encompassing residues 1821–2001 assembles with LGNTPR to form a complex that elutes much earlier than its expected molecular weight. We reasoned that this longer NuMA fragment could form high-order oligomers with the TPR domain of LGN. Static-Light-Scattering (SLS) analysis confirmed that NuMA1821–1928 and LGNTPR form hetero-hexamers, in agreement with the available structural data.

Architecture of NuMA/LGN hexamers. To gain insights into the topology of the NuMA/LGN hetero-hexamers, we determined their crystallographic structure at 4.3 Å resolution. As diffraction from individual crystals was very weak, a multi-crystal approach was adopted. Several datasets were collected from multiple crystals and merged to produce a combined dataset with 99.9% completeness and a I/σ(I) of 1.23 (Table 1). The structure of the NuMA/LGN hetero-hexamer was phased using molecular replacement with NuMA1900–1928/LGN as a search model, and refined to an Rfree of 23.3% and Rwork of 18.2%, with good stereochemistry (Table 1). The final model includes residues 7–367 of LGN, and residues 1864–1928 of NuMA with gapless refinement.

Overall, the hetero-hexamers arrange in a donut-shaped architecture, with the backbone of the donut formed by the three LGNTPR protomers concatenated in a head-to-tail fashion, and a
central triangular cavity reflecting the threefold symmetry of the assembly (Fig. 2a–c). In such an arrangement, the flexible NuMA chains thread in-between two adjacent LGN subunits, and then line in the internal groove of the TPR domain (Fig. 2c, d). The interface between NuMA1900–1928 and the TPR repeats of LGN in the hexamers is substantially identical to that observed in the crystallographic structure of LGN15–350 in complex with the short NuMA fragment encompassing only residues 1900–1928.19 The circular organization of the NuMA:LGN hetero-hexamers is allowed by the peculiar curvature of the TPR array of LGN induced by the longer helices of the TPR4, that are 10-residue longer than canonical TPR helices (see Supplementary Information in Culurgioni et al.20). Notably, the curvature of the LGNTPR superhelix in the LGNTPR:NuMA1861–1928 hexamers is more pronounced than that of LGNTPR in complex with NuMA1900–1928 because of the more pronounced bending of TPR1–2 induced by the contacts between two adjacent TPR molecules in the donut (Supplementary Fig. 2).

The donut assembly is promoted primarily by the formation of a four-helix bundle containing the TPR8 and the capping helix of one LGNTPR protomer (named here LGN-1 for clarity), and the N-terminal helix preceding the TPR1 of the neighboring LGNTPR molecule (LGN-2), in a sort of molecular grip (Fig. 2b, c). In such an arrangement, the flexible NuMA chains thread in-between two adjacent LGN subunits, and then line in the internal groove of the TPR domain (Fig. 2c, d). The interface between NuMA1900–1928 and the TPR repeats of LGN in the hexamers is substantially identical to that observed in the crystallographic structure of LGN15–350 in complex with the short NuMA fragment encompassing only residues 1900–1928.19 The circular organization of the NuMA:LGN hetero-hexamers is allowed by the peculiar curvature of the TPR array of LGN induced by the longer helices of the TPR4, that are 10-residue longer than canonical TPR helices (see Supplementary Information in Culurgioni et al.20). Notably, the curvature of the LGNTPR superhelix in the LGNTPR:NuMA1861–1928 hexamers is more pronounced than that of LGNTPR in complex with NuMA1900–1928 because of the more pronounced bending of TPR1–2 induced by the contacts between two adjacent TPR molecules in the donut (Supplementary Fig. 2).

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while the N-terminal portion preceding NuMA residues 1900–1928 hooks on the outer surface of the adjacent LGNTPR to secure the toroidal molecular architecture (boxed in Fig. 2d). More specifically, residues 1864–1880 of NuMA-1 start with two helical turns interrupted at Pro1874, where the chain bends in an elongated polypeptide, and packs against helices-A/B of the second TPR motif of the LGN-2 subunit, facing Leu84LGN-2 and Tyr58LGN-2 (Fig. 2f). Since binding of this initial NuMA fragment to the LGNTPR scaffold buries only about 400Å² of an accessible surface area, we asked whether its presence was essential for the hetero-hexamer formation. To this aim, we mutated to Ala the LGNTPR residues engaged in the NuMA1861–1880 interaction, and tested the ability of the LGNTPR-L54A-Y58A double mutant to form oligomers with NuMALGNBD. Analytical SEC experiments

Fig. 2 Structure of the hetero-hexameric LGN:NuMA complex. a–b LGN:NuMA hetero-hexamer with LGN molecules depicted with surface representations in red, orange and yellow, and NuMA molecules as coils in different shades of green. Unstructured regions are represented with dotted lines. Panel b highlights the ‘molecular grip’ between the N-terminal LGN-2 capping helix (yellow N-term) with the LGN-1 TPR8 (red C-term). c–d Cartoon representations of the hetero-hexameric complex. Panel c is in the same orientation of a, whilst panel d highlights the hooking mechanism of NuMALGNBD N-terminal on the adjacent LGNTPR. An electron density map (Fo-Fc omit map calculated omitting NuMALGNBD chains) is displayed at 2.2-σ contour level. e Excerpt of NuMA sequence alignment encompassing the crystallized fragment, colored by percentage of sequence identity (details in Methods section). Residues built in the crystal structure are indicated with overlying yellow and red lines, those for which there is no electron density with overlying green dotted line. Residues of the NuMA hooking peptide contacting LGN are depicted in bold yellow. f Enlarged view of the region highlighted in the box of panel d, with ball-and-stick representation of the residues involved in the hooking interface between NuMA-1 and LGN-2. A 2Fo-Fc electron density map, contoured at 1-σ level, is displayed.
revealed that disrupting the NuMA\textsuperscript{1861–1880}:LGN\textsuperscript{TPR} interface prevents hexamer formation (Supplementary Fig. 2), confirming that hooking of NuMA\textsuperscript{1861–1880} on LGN\textsuperscript{TPR} is essential for oligomerization. No electron density is visible for NuMA residues 1881–1897, it is observable again at the beginning of NuMA\textsuperscript{1900–1928} that binds tightly to the inner concave groove of the TPR scaffold. Given the modest resolution of the data, to confirm the register of our tracing, we decided to generate a NuMA\textsuperscript{GNBD–A1881–1897} construct lacking aa 1881–1897, and analyze by SEC the stoichiometry of the assemblies it formed with LGN\textsuperscript{TPR}. We reasoned that if our map interpretation was correct, LGN\textsuperscript{TPR}:NuMA\textsuperscript{GNBD–A1881–1897} complexes would be hetero-hexamers as the LGN\textsuperscript{TPR}:NuMA\textsuperscript{GNBD} complexes. Conversely, if the visible electron density corresponded to the NuMA residues 1881–1897, we would observe a LGN\textsuperscript{TPR}:NuMA\textsuperscript{GNBD–A1881–1897} complex with a 1:1 stoichiometry, eluting from SEC as the LGN\textsuperscript{TPR}:NuMA\textsuperscript{1877–1928} complex. When loaded on a Superdex-200 column, LGN\textsuperscript{TPR} in complex with NuMA\textsuperscript{GNBD} or with NuMA\textsuperscript{GNBD–A1881–1897} eluted in the same fractions (Supplementary Fig. 2), fully supporting the molecular model that we had built. We conclude that NuMA has a bipartite LGN-binding domain consisting of a nanomolar affinity peptide spanning residues 1900–1928 and a disjunct upstream oligomerizing motif encompassing aa 1861–1880, which is essential for hetero-hexamer formation. Both stretches are evolutionarily conserved (Fig. 2e) and spaced by an intervening sequence of different lengths rich in Gly and Ser, as expected for an unstructured linker domain consisting of a nanomolar affinity peptide spanning residues 1900–1928 and a disjunct upstream oligomerizing motif encompassing aa 1861–1880, which is essential for hetero-hexamer formation. Both stretches are evolutionarily conserved (Fig. 2e) and spaced by an intervening sequence of different lengths rich in Gly and Ser, as expected for an unstructured linker

NuMA:LGN oligomerization is needed for planar divisions. To promote spindle orientation, in metaphase NuMA is recruited to the cortex by direct interaction with LGN. To understand whether the multimeric nature of the LGN:NuMA assemblies is important for the activation of force generators, we set out to rescue spindle orientation defects caused by the loss of LGN by ectopic expression of an LGN construct unable to oligomerize, referred to as LGN\textsuperscript{ΔOLIGO} in the following paragraphs. Based on the molecular information provided by the structural analyses, LGN\textsuperscript{ΔOLIGO} lacks residues 1–12 (corresponding to the N-terminal helix) and 350–366 (corresponding to the capping helix), but retains its ability to bind NuMA with the TPR scaffold in a 1:1 stoichiometry (Fig. 3a). We first addressed the relevance of the NuMA:LGN oligomerization for the morphogenesis of Caco-2 cysts that grow as monolayered single-lumen spheres by oriented planar divisions, with the spindle aligned within the monolayer. To this aim, we generated Caco-2 cell lines ablated for LGN, and stably expressing LGN wild-type or LGN\textsuperscript{ΔOLIGO} at levels comparable with the endogenous protein (Supplementary Fig. 3). When plated in Matrigel, wild-type Caco-2 cells form single-lumen cysts by oriented division (Fig. 3b, top-left panel, Fig. 3c, d, and Supplementary Fig. 3). In contrast, Caco-2 cells lacking LGN undergo misoriented divisions and fail to organize a single lumen (Fig. 3b, top right, Fig. 3c, d). Ectopic expression of wild-type LGN in Caco-2 cells fully rescues the misorientation and multi-lumen phenotypes, while expression of LGN\textsuperscript{ΔOLIGO} does not (Fig. 3b bottom panels, and Fig. 3c–d, and Supplementary Fig. 3). Importantly, LGN loss does not impair apico-basal polarity\textsuperscript{22}; thus, the defective cystogenesis observed upon LGN ablation can be directly ascribed to misoriented mitoses. We conclude that NuMA:LGN oligomerization is essential for oriented planar division and correct cystogenesis.

We next set out to dissect the molecular mechanism underlying the misorientation phenotype observed in Caco-2 cysts using HeLa cells, that are more amenable to imaging, and when plated on fibronectin-coated coverslips, divide with the spindle parallel to the substratum\textsuperscript{23}. Similarly to what we had done for Caco-2 cells, we first generated HeLa cell lines stably interfered for LGN and expressing LGN wild-type or the oligomerization-deficient mutant (Supplementary Fig. 3), and confirmed that also in this cellular system, the misorientation caused by LGN ablation is rescued by wild-type LGN but not by the oligomerization-deficient LGN\textsuperscript{ΔOLIGO} (Fig. 3e, f). To explain why LGN\textsuperscript{ΔOLIGO} cannot support spindle orientation, we first reasoned that NuMA:LGN oligomerization could favor cortical clustering of NuMA molecules, and hence of dynein/dynactin motors, this way effectively activating pulling forces on astral MTs. To test this hypothesis, we evaluated cortical levels of LGN, NuMA, and dynactin in the mitotic HeLa cell lines generated above. The current model for force generator assembly posits that GTP moieties anchored at the plasma membrane recruit LGN, which in turn targets NuMA to the cortex to assemble dynein/dynactin\textsuperscript{2}. Consistent with this model, quantification of LGN at the cortex showed that both LGN and LGN\textsuperscript{ΔOLIGO} enrich at the cortex to the same extent of the endogenous protein (Fig. 3g, h) because they all contain a proficient C-terminal GoLoco region. Cortical NuMA is lost upon LGN depletion (Fig. 3i, j, second left panel) but accumulates back in equal amounts upon re-expression of LGN wild-type or LGN\textsuperscript{ΔOLIGO} (Fig. 3i, j, last two right panels), indicating that NuMA is recruited to the cortex by the binary interaction with LGN without the need of oligomerizing. According to the notion that NuMA is the driver for force generator assembly, in the four analyzed HeLa cell lines, the distribution of the p150 subunit of dynactin and the light-intermediate chain 1 of dynein (LIC1) mirrors the behavior of NuMA (Fig. 3k, l, and Supplementary Fig. 3), because they are lost in LGN-ablated cells and present in LGN wild-type and LGN\textsuperscript{ΔOLIGO} HeLa cells. The LGN\textsuperscript{ΔOLIGO} construct cannot form high-order oligomers with NuMA, but is still able to bind NuMA in a 1:1 stoichiometry. To assess whether excess of LGN can overcome the requirement of oligomerization in generating cortical MT-pulling forces, we performed a spindle-rocking experiment\textsuperscript{7} in which we overexpressed LGN wild-type or LGN\textsuperscript{ΔOLIGO} in HeLa cells stably expressing H2B-GFP, and filmed the metaphase plate oscillations (Supplementary Fig. 3). This analysis showed that large excess of LGN\textsuperscript{ΔOLIGO} triggers spindle rocking to the same extent of that observed upon overexpression of LGN-WT. This result is not unexpected, considering that the LGN constructs are massively overexpressed in transiently transfected HeLa cells, as compared to the endogenous protein (Supplementary Fig. 3), likely bypassing the regulatory mechanisms governing spindle positioning under physiological conditions. Whether NuMA:LGN oligomerization becomes dispensable upon cell treatments accumulating aberrantly LGN at the cortex\textsuperscript{24} remains an open issue.

To corroborate these results obtained with LGN\textsuperscript{ΔOLIGO}, we exploited the information provided by the crystallographic structure of NuMA:LGN donuts to engineer also a NuMA mutant lacking residues 1861–1900 that still associates with LGN but does not form oligomers, that we refer to as NuMA\textsuperscript{ΔOLIGO} (Supplementary Fig. 4). We then used a HeLa cell line in which NuMA was stably ablated\textsuperscript{8}, and measured the spindle orientation angles of the cells plated on fibronectin-coated coverslips upon transfection of NuMA rescue constructs. Under these conditions, unperturbed cells aligned the spindle parallel to the substratum,
while cells lacking NuMA divide with a randomized orientation (Supplementary Fig. 4). Transient transfection of wild-type NuMA rescues misorientation defects, whereas transfection of NuMA-ΔOLIGO does not, similarly to what was observed with a NuMA mutant lacking the entire LGN-binding domain (Supplementary Fig. 4). Although these results are consistent with the ones obtained with LGN-ΔOLIGO, they cannot be fully ascribed to the cortical activities of NuMA in complex with LGN, because the LGN-binding domain of NuMA overlaps with a site (spanning residues 1944–2003 of human NuMA) involved in...
spindle pole activities. In line with these considerations, quantifications of the amounts of NuMA constructs at the spindle poles revealed that NuMA-ΔOLIGO and NuMA-ΔGNBD accumulate significantly less at the poles than wild-type NuMA (Supplementary Fig. 4), implying that the inability of these constructs to rescue misorientation might be not only due to impaired oligomerization with LGN but also due to spindle assembly defects.

In summary, from the analyses in Caco-2 cysts and HeLa cells, we conclude that NuMA:LGN hetero-hexamers are fundamental for spindle positioning and epithelial morphogenesis promoted by planar cell divisions, although they are not required for targeting dynein/dynactin at the cortex.

**NuMA/LGN oligomers assemble in a multicellular protein network.** We next set out to understand how NuMA:LGN hetero-hexamers promote spindle orientation. As a first step, we started testing whether the NuMA:LGN hexamers could form in cells. To assess the assembly of the NuMA molecules independently from the oligomerization driven by the dimerizing NuMA coiled-coil region, we generated a HEK293T cell line stably depleted of endogenous NuMA and expressing a C-terminal portion of NuMA encompassing the LGN-binding domain but not the coiled-coil (i.e., NuMA1821–2115). We then co-transfected these cells with either GFP-LGN-WT and LGN-ΔOLIGO-FLAG, or tested whether in mitotic lysates the GFP-tagged version of LGN could immunoprecipitate the FLAG-tagged version of LGN. Our experiment revealed that only LGN wild-type proteins immunoprecipitate each other together with NuMA1821–2115 while LGN-ΔOLIGO cannot (Fig. 4a). This evidence supports the notion that LGN and NuMA can assemble higher-order oligomers in mitotic cells, independently of NuMA self-assembly, and that the same mutations impairing oligomerization in vitro disrupt LGN:NuMA oligomer formation in cells, indicating that the NuMA:LGN hetero-hexamers are key for multivalent mitotic NuMA:LGN interactions.

We next tested the oligomeric state of NuMA. First, we designed a NuMA construct encompassing residues 1592–1694, which are predicted to be the C-terminal portion of the NuMA coiled coil. Measurement of the molecular weight of purified NuMA1592–1694 by static-light scattering showed that it forms homodimers (Fig. 4c). Importantly, co-immunoprecipitation of GFP-tagged and FLAG-tagged NuMA constructs from mitotic lysates confirmed that full-length NuMA proteins self-assemble in cells (Supplementary Fig. 4).

We then reasoned that the combination of full-length NuMA dimers with the 3:3 stoichiometry of the NuMA:LGN interaction would result in the formation of multimeric assemblies, in which the NuMA dimers are physically linked to different donuts. To test this idea, we assessed NuMA:LGN protein network formation using recombinant proteins. While the NuMA fragment 1592–2001 was insoluble, a chimeric NuMA construct in which the two functional stretches, i.e., the dimerizing coiled-coil (aa. 1592–1694) and the LGN-binding fragment (aa. 1821–2001), were connected by an artificial linker of eight Thr-Gly-Ser repeats (NuMA chimera henceforth, Fig. 4b), was soluble and could be purified to homogeneity. Upon incubation with LGNTPR, NuMA chimera formed high-order oligomers eluting from a size-exclusion Superose-6 column at high molecular weight but well clear of the void volume (Fig. 4d). Static-light-scattering analyses confirmed that the NuMA-chimera:LGNTPR sample is a polydisperse population of assemblies with a molecular weight up to 4.5 MDa (Fig. 4e). This result is fully consistent with our network hypothesis, predicting that a minimum of three NuMA dimers are needed to satisfy the 3:3 stoichiometry of the NuMA:LGN hetero-hexamers, but that larger multimeric clusters can form if the chains of each NuMA dimers engage with different donuts (Fig. 4f). When analogous SEC and SLS analyses were repeated with a sample assembled with NuMA chimera and LGNTPR–ΔOLIGO, a significant shift toward a lower molecular weight was observed in the SEC elution profile (Fig. 4d), which was accompanied by a decrease in the molecular weight measured by SLS to about 136 kDa, corresponding to NuMA:LGN 2:2 complexes formed by the interaction of two LGNTPR–ΔOLIGO with the two copies of NuMA present in the NuMA-chimera dimer (Fig. 4g). Taken together, this evidence confirms that in vitro binding of dimeric NuMA moieties to LGNTPR results in high-order multivalent protein assemblies in which hetero-hexameric NuMA(LGNBD)2:LGNTPR donuts are connected to elongated coiled-coil regions of NuMA by flexible linkers.

**Cortical NuMA clusters dynein/dynactin with LGN.** We reasoned that if NuMA and LGN enable the formation of a multivalent protein network, it might be that it is precisely this supra-molecular organization of the microtubule motors to be key in sustaining the activation of pulling forces orienting the spindle. To test this idea, we designed an experimental setting capable of decoupling the cortical recruitment of NuMA from the assembly of oligomeric NuMA:LGN protein networks. To this aim, we took advantage of the notion that treatment of HeLa cells with the Aurora-A inhibitor MLN8237 causes massive accumulation of endogenous NuMA at the spindle poles accompanied by a misorientation phenotype, which can be bypassed by expression of a NuMA-LGN-GoLoco fusion protein (hereon NuMA-GoLoco) ectopically localizing...
at the cortex by direct binding to Gai5. In this setting, we engineered an oligomerization-deficient GFP-NuMA-ΔOLIGO-GoLoco construct that localizes at the cortex but is unable to form high-stoichiometry NuMA:LGN assemblies (Fig. 5a). When probed in HeLa cells treated with MLN8237, GFP-NuMA-ΔOLIGO-GoLoco did not rescue spindle misorientation in spite of accumulating at the cortex at the same levels of the orientation-proficient NuMA-GoLoco (Fig. 5b, c). This result indicates that it is not the presence, but the molecular organization of cortical NuMA in complex
with LGN that is required for the onset of productive pulling forces.

Based on the current knowledge, spindle orientation is attained by traction forces exerted on astral microtubules by dynein/dynactin motors targeted to specialized cortical areas via NuMA: LGN proteins. Intriguingly, the C-terminal region of NuMA spanning residues 2002–2115 contains a MT-binding domain compatible with concomitant binding to LGN, which is important for NuMA’s ability to support pulling forces when ectopically localized at the cell cortex. To start investigating the MT-binding activities of NuMA, we performed co-sedimentation assays with a purified C-terminal fragment encompassing the MT-binding domain (aa 2002–2115). NuMA 2002–2115 co-sediments with taxol-stabilized MTs, both in the presence and
in the absence of tubulin tails (Supplementary Fig. 5), indicating that NuMA recognizes directly the MT lattice. We next tested the ability of NuMA to interact with tubulin dimers. SEC experiments confirmed that NuMAcannot revert the misorientation phenotype of HeLa cells lacking endogenous NuMA (Fig. 5d, e). This result can be ascribed to the evidence that NuMA cannot enrich at the cortex (Supplementary Fig. 5). However, we suspected that binding of NuMA to MT could also be implicated in spindle pole organization, this way indirectly contributing to correct spindle orientation. To test this possibility, we compared the amounts of mCherry-NuMA wild-type and NuMA−ΔMT that accumulate at the spindle poles in mitotic HeLa cells. For these experiments, we used HeLa cells depleted of the endogenous protein in order to prevent homodimerization. As expected, mCherry-NuMA−ΔMT cannot decorate spindle MTs emanating from the poles, but is retained only on centrosomes, and overall accumulates at the poles at lower levels than the wild-type counterpart (Supplementary Fig. 5). We conclude that the microtubule-binding domain of NuMA plays essential roles at the cortex and at the spindle poles, and that these activities are essential to grant proper spindle orientation.

Discussion
Spatial organization of traction forces pulling on astral microtubules to orient the spindle is achieved by recruitment of cytoplasmic dynein/dynactin at the cortex. Here, we show that the dynein adaptor NuMA and membrane-localized LGN assemble in oligomers that can form multivalent interactions key to sustain effective pulling on astral MTs. Biochemical and structural studies revealed that these NuMA:LGN networks are organized on a hetero-hexameric modules in which three TPR domains of LGN and a NuMA:TGNBD form hetero-hexameric rings whose determinants are the LGN helices preceding and following the eight TPR repeats, and a NuMA motif spanning residues 1861–1899 preceding the high-affinity LGN-binding peptide. The toroidal architecture of the hexamers is allowed by the characteristic TPR array of LGN featuring a long TPR4 that confers a pronounced curvature to the scaffold. In addition, hooking of the N-terminal helix of one LGN molecule onto the last TPR repeat of the subsequent one in the donut generates a molecular tension, resulting in an increased curvature of the TPR scaffold (Supplementary Fig. 2). Interestingly, we observed that the TPR domain of LGN reveals a great conformational versatility, which allows the assembly of oligomers of different geometry with the diverse LGN-binding partners, as visible in the LGN:InsC assembly, where the LGN:TPR domains engage in a head-to-head tetrameric interaction with InsC26.

In spite of the overall conservation of the NuMA:LGN pathway throughout species, it is not trivial to predict from sequence comparison if all orthologs of human LGN and NuMA can form hexamers with a similar architecture. For instance, Drosophila Pins have a 40-residue long N-terminal sequence before the TPR domain with poor propensity to adopt a helical conformation, and the Pins-binding domain of Mud (the counterpart of NuMA in flies) is not sufficiently well-defined to allow an accurate prediction of the oligomerizing properties of Mud.

An important implication of the current NuMA:LGN structural characterization is that the combination of NuMA homodimers with NuMA:LGN hetero-hexamers may foster the assembly of a subcortical protein network that clusters dynein/dynactin. The evidence that in HeLa cells, oligomerization-deficient LGN and NuMA constructs can recruit correct amounts of dynein/dynactin at the cortex but cannot sustain spindle orientation, supports the notion that the multimeric nature of the NuMA:LGN protein network is key in activating the motor activity of cortical dynein. This is an unexpected result, highlighting how force generators rely on the self-organization of large localized protein assemblies that are ultimately instructed by restricted GaI-GDP pools triggering LGN cortical recruitment. The fact that LGN binds four GaI-GDP subunits in a cooperative manner confers to the pathway the ability of responding quickly to an initial cortical GaI-GDP cue that ignites the formation of NuMA:LGN complexes. In some respect, the overall activation of cortical force generators is reminiscent of the activation of the TNF receptor Fas, that upon extracellular ligand binding, oligomerizes triggering the assembly of an intracellular Fas-FADD proteineaceous platform that is essential for apoptotic signaling27. In the case of the GaI-GDP, it will be interesting to explore if in vertebrate cells, specialized GPCRs are responsible for creating an initial pool of GaI-GDP starting force generators’ activation, as it was demonstrated for Drosophila neuroblasts28.
Elegant optogenetic reconstitution of dynein/NuMA/LGN pathway in HeLa cells revealed that targeting of dynein at the cortex is not sufficient to ensue pulling forces positioning the spindle, while cortical recruitment of dynein by ectopic targeting of NuMA results in the formation of dotted patterns that are required to promote effective spindle pulling3. These clusters seem to depend on a conserved and hydrophobic motif of NuMA positioned at residues 1768–1777, which belong to the linker region between the coiled-coil and the LGNBD, and we know makes the recombinant protein unstable in vitro. Although it is possible that these NuMA cortical clusters are affected by the ectopic targeting system, their appearance is consistent with the requirement of a supramolecular organization of force generators. Our result demonstrates that cortical multivalent interactions mediated by NuMA:LGN hetero-hexamers are key in triggering the formation of a protein network that sustains spindle placement. Whether the clustering reported by Okumura and colleagues relies on a mechanisms that complements the NuMA:LGN hetero-hexamers of the current study, and can therefore synergize with them to support a robust spindle pulling, will need to be explored further.

NuMA is the dynein/dynactin adaptor that in mitosis assists spindle assembly and orientation processes27,28. We recently discovered that the last hundred residues of NuMA code for a MT-binding region that associate directly to MTs, whose function is to date poorly understood29. We discovered that in vitro NuMA2002–2115 co-sediments with taxol-stabilized MTs regardless of the presence of the negatively-charged tails, suggesting that it recognizes the MT lattice. Interestingly, the same fragment enters a 1:1 complex with tubulin dimers, indicating that NuMA also interacts with depolymerized tubulin (Supplementary Fig. 5). Collectively, this evidence is consistent with a role of NuMA in regulating astral microtubule plus ends dynamics while the dynein/dynactin/NuMA complex slides towards the spindle pole, possibly conferring processivity to the motor. The findings that in HeLa cells a NuMA truncation mutant lacking the MT-binding region cannot rescue misorientation defects fully supports this notion, although more data are needed to uncouple the spindle orientation and spindle assembly activity of dynein/dynactin/NuMA.

In conclusion, our studies uncovered the existence of NuMAΔGND-LGTTP hetero-hexamers, which can form multimeric networks of LGN/NuMA complexes at the cortex, whose assembly is likely triggered by localized pool of Gai-GDP molecules. Such protein complexes favor dynein/dynactin cortical clusters, and are essential for the spatial organization of dynein-dependent pulling forces positioning the spindle in HeLa cells and in polarized epithelial cysts. How the Gai GDP/GTP cycle, and the numerous NuMA interactors and post-translational modifications affect the stoichiometry and the spatial arrangement of the NuMA/LGN complexes remains to be addressed in vitro and in vivo.

Methods

Protein expression and purification. GST-LGN3–350, GST-LGN3–409, GST-LGN1861–1928 (NuMAΔGND in the text), GST-LGN3–367, and GST-NuMA1861–1928 (NuMAΔGND in the text) were cloned into pGEX-6P1 vector (GE Healthcare), and expressed in BL21 Rosetta E. coli cells (Novagen) as indicated in Carminati et al.30. NuMAΔΔ1881–1897 was generated by substitution of residues 1881–1897 of NuMA with a Thr-Gly-Ser triplet on the GST-NuMA1861–1928 vector using the QuickChange mutagenesis kit (Agilent). Cells were lysed in 0.1 M Tris-HCl pH 8.0, 0.3 M NaCl, 10% glycerol, 0.5 mM EDTA, and 1 mM DTT, and cleared for 1 h at 100,000 g. Proteins were first affinity purified on glutathione beads (GSH), and then incubated with PreScission protease (GE Healthcare) overnight at 4 °C to remove the GST-tag. Cleaved LGN constructs were eluted from the GSH beads in a 50 μM glycine-HCl buffer, whose function is to remove the GSH tag. Cleaved LGN constructs were eluted from the GSH beads in a desalting buffer consisting of 20 mM Tris-HCl pH 8.0, 0.4 M NaCl, 5% glycerol, 1 mM DTT, and loaded on a 4-ml Resource-Q ion exchange column. Bound proteins were eluted by a salt gradient from 40 mM to 450 mM NaCl over 20 column volumes. To remove chaperone contaminants from LGN3–367, after the ion-exchange column the protein was incubated on ice for 1 h with 1.5 mM ATP supplemented with 1.5 mM MgCl2, and further polished on a Superose-20 column in a buffer containing 20 mM Tris-HCl pH 8.0, 0.1 M NaCl, and 0.1 M DTT. NuMAΔGND was gel filtered in the same buffer on Superdex-200 right after PreScission tag removal. For crystallization experiments, LGN3–367 and NuMAΔGND were combined in a 1:1.3 molar ratio, and the resulting complex was purified on a Superdex-200 column equilibrated in 10 mM Tris-HCl pH 8.0, 0.15 M NaCl, and 1 mM DTT. Peak fractions were analyzed by SDS-PAGE analysis, pooled and concentrated to 14 mg/ml prior freezing at –80 °C.

The NuMA C-terminal fragment spanning residues 2002–2115 used in the MT co-sedimentation assays and SEC analysis of Supplementary Fig. 5, and LGN3–367 produced for SLS analysis, were cloned into a pETM14 vector (Novagen), expressed in BL21 E. coli cells by overnight induction with 0.2 mM IPTG at 20 °C, and purified by affinity and cation exchange chromatography as previously described31. For the MT co-sedimentation assay of Supplementary Fig. 5, NuMAΔGND was purified as reported by Ciferri et al.32.

The chimeric construct of NuMA (NuMA-chimera in the text) was generated as follows: NuMA residues 1592–1861 were inserted into the MT-binding region of LGTTP, and the resulting NuMA-LGNTPR hetero-hexamers, which can form multi-hexamers, cannot rescue misorientation defects fully supports this notion, because Pendin et al.33 reported that the MT-binding region of NuMA-LGNTPR hetero-hexamers cannot rescue misorientation defects fully supports this notion. Our result demonstrates that cortical multivalent interactions mediated by NuMA:LGN hetero-hexamers of the current study, and can therefore synergize with them to support a robust spindle pulling, will need to be explored further.

Analytical size exclusion chromatography (SEC). For SEC analyses of Fig. 1 and Supplementary Fig. 2, LGN and NuMA variants were mixed in equimolar amounts (20 μl), loaded on a Superdex-200 Increase 3.2/300 column (GE Healthcare) and eluted in 20 mM Tris-HCl pH 8.0, 0.15 M NaCl, 5% glycerol, 1 mM DTT, while incubating with PreScission protease (GE Healthcare) to remove the histidine-tag. The protein was then injected onto a Resource-Q anion exchange column, and eluted with a gradient of 40 mM—0.35 M NaCl in 20 column volumes. NuMA-chimera was further purified on a Superose-6 column equilibrated in 20 mM Tris-HCl pH 8.0, 0.15 M NaCl, 5% glycerol, and 1 mM DTT. Eluted fractions were pooled, concentrated, and frozen to –80 °C. To isolate samples of oligomeric NuMA:LGN complexes suitable for Static-Light-Scattering analysis, LGNTTP or LGNTTPΔΔ1694 were combined with NuMA-chimera in a 1:1.2 molar ratio and separated on a Superose-6 column.

Static-Light-Scattering measurements. Static-Light-Scattering (SLS) analyses of Fig. 1c–e and g–j were performed on a Visco tek GPCmax/TDA instrument equipped with two TSKgel G3000PWXL columns (Tosoh Biosciences). Typically, 75 μl of purified samples concentrated at about 1.5–2 mg/ml were loaded on the columns.

Crystallography and structure determination. The LGN3–367:NuMAΔGND complex at 14 mg/ml was supplemented with 20 mM TCEP and screened for crystallization using commercially available screen kits in a 1:1 volume ratio. Crystallization experiments were conducted in 200 nl vapor diffusion sitting drop trials using an Art Robbins automated plate changer (Genomic Solutions) in three square-well CrystalQuick Greiner plates. Diffraction-quality crystals were obtained using the Molecular Dimensions Ltd PACT screen at 20 °C at half concentration, with a reservoir containing 10% PEG3350, 0.05 M Bis-Tris propane pH 7.5, and 0.1 M Na-formate or 0.1 M Na-acetate trihydrate. For data collection, crystals were transferred to buffer-supplement containing 0.1 M Na-acetate trihydrate and flash-frozen in liquid nitrogen. X-ray diffraction data were collected to 4.2–5.0 Å resolution at 104 and 104–1 beamlines at Diamond Light Source, Didcot, United Kingdom.
Kingdom (visits n5966 and n5967, respectively), exploiting the kappa-goniometer reorientation in order to optimize data acquisition along the c axis. All data were initially processed with MOSFLM implemented in xia2. A crystallographic space group, unit cell and data collection statistics. Thirteen datasets were selected for merging according to their data quality (higher resolution limits, completeness, and lower CC1/2) and to the degree of crystal isomorphism. Combination and data merging were carried out according to the help of the BLEND3D computer program. After the first run in analysis mode only eleven datasets were selected to ctf files that were combined into a single and final dataset resolution limit of 4.3 Å (combination mode). Indeed, the removal of two datasets caused a significant improvement in crystal isomorphism (Linear Cell Variation drop from 392.15 to a 1.61 Å), while additional removal of another individual diffraction Nunum showed a substantial elimination of intensities affected by radiation damage. The merged dataset was used for molecular replacement using a search model of LTPPP1:NuMA1900-1928 obtained by aligning and combining chain A (LTPPP1) and chain B (NuMA1900–1928) of pB entries 3SF4 and 3RO2, respectively. Molecular replacement was performed with Phaser34, which automatically found eight copies of LTPPP1:NuMA1900–1928 dimer. After placing some helices manually in the clearest electron densities, the position of the remaining four LTPPP1:NuMA1900–1928 dimers became evident enough to place them manually into the densities. The model was progressively optimized by iterative cycles of low resolution jelly body refinement in Refmac35 and manual model building in Coot36. Additional steps of re-dimer. After placing some helices manually in the clearest electron densities, the final model was refined to Rfree/Rtotal values of 0.182/0.233, and contains 4 copies of LTPPP1 and of NuMA1GNBD hetero-hexamers in the asymmetric unit. PyMOL was used to generate all the illustrations of the structure (http://www.pymol.org).

**Sequence alignment.** NuMA sequences from Homo sapiens (Uniport entry Q41569), Homo sapiens (Uniport entry E67559), Homo sapiens (Uniport entry F7ELR7), Rhinoceros typus (NCBI entry XP_020991007), Danio rerio (NCBI entry NP_001316910), Galleria gallus (NCBI entry NP_001177854), Gekko japonicus (NCBI entry XP_015270477), Xenopus laevis (NCBI entry XP_018103292), and Halosaccus leucosceopus (NCBI entry XP_010574983) were aligned with MUSCLE39 and colored by percentage of identity in Jalview40.

**MT co-sedimentation assays.** Cytoskeleton Inc.) was polymerized into microtubules according to the producer's instructions. Microtubule co-sedimentation assays were performed as previously described in Griend et al.41. Briefly, microtubules were diluted to a final concentration of 9 μM in general tubulin (GT) buffer (80 mM PIPES pH 6.8, 1 mM MgCl2, 1 mM EGTA) supplemented with 1 mM GTP, 50 μM Paclitaxel and 60 mM NaCl. In order to remove the C-terminal tubulin tails, microtubules were treated with 200 μg/ml subtilisin A (Sigma-Aldrich) for 30 min at 30 °C. Proteolysis was stopped after the addition of 10 mM PMSF. Microtubules with and without tails were incubated for 10 min at RT with 5 μM NuMA1900-2115 or 1 μM Ndc80Bonsai31 in a final volume of 50 μl. Reactions were transferred onto 100 μl of cushion buffer (80 mM PIPES pH 6.8, 1 mM MgCl2, 1 mM EGTA, 50 μM Paclitaxel, 50% glycerol) and ultraacentrifuged for 15 min at 400,000 × g at 25 °C in a Beckman TL1A rotor. Pellets and supernatants were analyzed by SDS-PAGE and visualized by Coomassie staining.

**Cell culture.** HeLa cells (ATCC, CCL-2) and HEK293T (ATCC, CRL-11268) were cultured at 37 °C in a 5% CO2 atmosphere, in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 1% L-glutamine and antibiotics. For all the experiments, HeLa cells were plated on fibronectin-coated coverslips (5 μg/ml, Roche) and pre-synchronized with a single thymidine block/release. After 5 days, cells were fixed with 4% paraformaldehyde for 30 min at room temperature, followed by permeabilization with 0.3% Triton X-100 in PBS for 1 h. For all conditions, background was blocked with 3%BSA for 1 h at room temperature. Cells were stained with mouse anti-LGN (1:5, monoclonal, Mapelli lab), mouse anti-NuMA (1:3000, monoclonal, Mapelli lab), mouse anti-α-tubulin (1:200, Sigma-Aldrich #T5168), rabbit anti-a-tubulin (1:50, Abcam #ab4074), mouse anti-a-tubulin (1:200, Sigma-Aldrich #T1568), mouse-anti-FLAG (1:200, Sigma-Aldrich #F3165) or Cy3 conjugated anti-y-tubulin (1:200, Sigma-Aldrich #C7604) in 3% BSA + 0.05% Tween-20, followed incubation with anti-mouse or anti-rabbit AlexaFluor 488 or anti-mouse AlexaFluor 488 (1:300, Jackson ImmunoResearch #715-605-152, #715-545-150).

For Caco-2 cyst staining, cells were fixed with 4% paraformaldehyde for 30 min at room temperature, followed by permeabilization with 0.3% Triton X-100 in PBS for 1 h. For all conditions, background was blocked with 3%BSA for 1 h at room temperature. Cells were stained with mouse anti-LGN (1:5, monoclonal, Mapelli lab), mouse anti-NuMA (1:3000, monoclonal, Mapelli lab), mouse anti-α-tubulin (1:200, Sigma-Aldrich #T5168), rabbit anti-a-tubulin (1:50, Abcam #ab4074), mouse anti-a-tubulin (1:200, Sigma-Aldrich #T1568), mouse-anti-FLAG (1:200, Sigma-Aldrich #F3165) or Cy3 conjugated anti-y-tubulin (1:200, Sigma-Aldrich #C7604) in 3% BSA + 0.05% Tween-20, followed incubation with anti-mouse or anti-rabbit AlexaFluor 488 or anti-mouse AlexaFluor 488 (1:300, Jackson ImmunoResearch #715-605-152, #715-545-150).

**Immunofluorescence.** For immunofluorescence, HeLa cells were plated on 13 mm coverslips coated with 5 μg/ml fibronectin. To visualize NuMA and p150Juxted at the cortex, cells were fixed with methanol at −20 °C for 10 min. For detection LGN, α-tubulin and γ-tubulin, cells were fixed with 4% paraformaldehyde for 10 min at room temperature, followed by permeabilization with 0.3% Triton X-100 in PBS for 1 h. For all conditions, background was blocked with 3%BSA for 1 h at room temperature. Cells were stained with mouse anti-LGN (1:5, monoclonal, Mapelli lab), mouse anti-NuMA (1:3000, monoclonal, Mapelli lab), mouse anti-α-tubulin (1:200, Sigma-Aldrich #T5168), rabbit anti-a-tubulin (1:50, Abcam #ab4074), mouse anti-a-tubulin (1:200, Sigma-Aldrich #T1568), mouse-anti-FLAG (1:200, Sigma-Aldrich #F3165) or Cy3 conjugated anti-y-tubulin (1:200, Sigma-Aldrich #C7604) in 3% BSA + 0.05% Tween-20, followed incubation with anti-mouse or anti-rabbit AlexaFluor 488 or anti-mouse AlexaFluor 488 (1:300, Jackson ImmunoResearch #715-605-152, #715-545-150).

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**Immunoblotting.** For western blot analysis, HeLa and Caco-2 cells were synchronized with a single thymidine block as described above, and collected 8 h after the release. Cells were then lysed in lysis buffer containing 57 mM Hepes pH 7.5, 1.5 mM EGTA, 1.5 mM MgCl2, 150 mM KCl, 0.1% NP40 and 15% Glycerol and protein extracts were separated by SDS-PAGE and visualized by SDS-electrophoresis and transferred on a nitrocellulose membrane. Blocking was performed in TBS containing 0.1% Tween-20 and 5% low fat milk. Primary...
antibody incubation was performed at room temperature for 2 h with the following dilutions: anti-LGN (1:500, Mapelli lab), anti-NuMA (1:200, Mapelli lab), anti-Vinculin (1:30000 in-house IEO), anti-α-tubulin (1:600, Abcam #ab6074), anti-GFP (1:1000, in-house EIO) and anti-FLAG (1:8000, Sigma-Aldrich #F7425).

Microscopy. Confocal images shown in Figs. 3, 5d, and Supplementary Fig. 4, 5 were acquired on a Leica SP2 AOPS confocal microscope controlled by Leica confocal software. For HeLa cells analysis, a ×63 oil-immersion objective lens (HCX Plan-Apochromat ×63 NA 1.4 Ldb Bl) was used. For Caco-2 cyst multilumen experiments, a ×20 objective lens (HC PL FLUOTAR ×20 0.5 DRY) was used. For Caco-2 spindle angle analysis, a ×40 objective lenses (HC PL Apochromat 40X NA 1.30 CS2) was used. Images shown in Fig. 5b and Supplementary Fig. 3 were acquired on a Leica SP8 confocal microscope controlled by Leica confocal software. For Caco-2 cyst multilumen objective lens (HC PL Apochromat ×63 NA 1.4 CS2) was used. All images were processed using the software Fiji[40].

Spindle orientation analysis. Mitotic spindle orientation was monitored on HeLa and Caco-2 cells synchronized in metaphase. HeLa cells were plated on fibronectin-coated coverslips and stained with γ-tubulin to visualize poles and DAPI to visualize DNA. Cells were imaged in x-z optical sections passing through the spindle poles. To determine the orientation of metaphase spindle, the angle formed by a line passing through the spindle poles and the substratum was measured exploiting the angle tool of the software Fiji. Spindle orientation analysis in Caco-2 cysts were conducted as described in Jaffe, JCB 2008[41]. Briefly, three x-y confocal sections of the equatorial region of the cyst were acquired and then merged, in order to visualize both the spindle poles. To analyze the spindle angle orientation, the angle formed by a line passing through the spindle poles and the centroid of the cyst marked by Phalloidin was determined using the software Fiji. Statistical analysis of angle distributions was performed in Prism with the Kruskal-Wallis test.

Quantification of cortical and polar fluorescence intensity. To quantify LGN, NuMA and p150fil signal at the cell cortex, confocal sections of metaphase cells were analyzed as follow. Using the software Fiji, a 30-pixel-wide line was drawn from the spindle poles to the cell cortex, to obtain the intensity profile along the line. Using the software Matlab, the amount of protein at the cortex was calculated by integrating the profile of a 10 pixel-wide area of the peak, while the amount of protein in the cytoplasm was calculated by integrating a 10 pixel-wide area, 5-pixel distant from the peak. In Fig. 3b, line 1 cortex/cytoplasm ratio is shown.

To quantify the fluorescence intensity of mCherry-NuMA at the spindle poles, confocal section of metaphase cell stained with α-tubulin were analyzed with the software Fiji. In details, per each cell imaged, the α-tubulin signal of one pole in focus was used to build a tubulin mask, and the mCherry signal inside the mask was integrated. A tubulin mask of the same dimension was positioned in the cytoplasm to obtain the mCherry intensity in the cytoplasm. In Supplementary Figs. 4f and 5g the pole/cytoplasm ratio of mCherry-NuMA wild-type or NuMA-cytoplasm to obtain the mCherry intensity in the cytoplasm. In Supplementary Fig. 3b, line 1 cortex/cytoplasm ratio is shown.

To visualize dynactin recruitment at the cortex, human dynein light-intermediate chain 1 (LIC1) was cloned in a pCDH lentivector in frame with a 3xFLAG tag, and transfected in HeLa cells ablated of endogenous LGN and stably expressing LGN-WT or LGN-AOLIGO. Transfected cells were analyzed with the software Fiji and defined as cortical LIC when a crescent of LIC was visible at the cortex.
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Author contributions

L.P. and C.G. conducted the cell biology experiments; M.C., A.A., S.M., V.C., and F.R. performed the biochemical experiments; S.C. and J.F. have processed, combined, and merged individual diffraction datasets to produce the final complete dataset; S.C. has crystallized the protein and solved the structure; S.P. refined and illustrated the structure; M.M. supervised the project and wrote the manuscript.

Additional information

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