Supplementary Table 1. Antibodies used in this study

Antibodies	Species	Cat #	Source	Exp. dilutions
Anti-Cep192 N (1–500)	Rabbit		Ref. 3	1:1000 (IB), 1:100 (IF), 5 μg (IP)
Anti-Cep192 C (2240–2538)	Rabbit		This study	1:1000 (IB), 1:100 (IF), 5 μg (IP)
Anti-Cep152 M (491–810)	Rabbit		Ref. 3	1:1000 (IB), 1:100 (IF), 5 μg (IP)
Anti-Plk4 (580–970)	Rabbit		Ref. 3	1:1000 (IB), 1:100 (IF)
Anti-Plk4 p-S305	Rabbit		This study	1:1000 (IB)
Anti-Sas6	Mouse	sc-81431	Santa Cruz	1:100 (IF)
Anti-GFP	Rabbit	sc-8334	Santa Cruz	1:1000 (IB), 2 μg (IP)
Anti-FLAG (clone M2)	Mouse	F1804	Sigma	1:1000 (IB), 2 μg (IP)
Anti-HA (clone 3F10)	Rat	11867423001	Roche	1:1000 (IB), 2 μg (IP)
Anti-Nedd1	Rabbit		Ref. 1	1:1000 (IB)
Anti-Actin	Rabbit	A2066	Sigma	1:1000 (IB)
Anti-Cep63	Rabbit	06-1209	Millipore	1:1000 (IB)

Supplementary Table 2. siRNA sequences used in this study

Target gene	Sequence (nt positions from the start codon)	Source	Type
Luciferase	CGTACGCGGAATACTTCGA	Ref. 8	Synthetic
CEP192	GCTAGTATGTCTGATACTTGG (2407–2427)	Ref. 9	Synthetic
CEP152	GCGGATCCAACTGGAAATCTA (3099–4019)	Ref. 9	Synthetic
CEP63	GGAGCTCATGAAACAGATT	Ref. 10	Synthetic

Supplementary Notes

Plasmid construction

FLAG-tagged constructs containing the full length wild-type (WT) (pKM3445), the full-length K41M kinase-inactive mutant (pKM3488), C (pKM3507), CPB_L (pKM3508), CPB (pKM3674), PB1 (pKM4337), PB2 (pKM4338), or PB3 (pKM3675) of Plk4 were generated by inserting each respective *Pme*I-*Not*I fragment into the pCI-neo-FLAG vector digested by the corresponding enzymes. All the FLAG epitopes used in this study contain three tandem copies. Human influenza hemagglutinin (HA)-tagged Plk4 constructs containing the full-length (pKM3855), C (pKM3671), CPB_L (pKM3862), CPB_{M1} (pKM3886), CPB_{M2} (pKM3885), CPB (pKM3672), PB1 (pKM4339), PB2 (pKM4340), or PB3 (pKM3673) of Plk4 were cloned into the pCI-neo-HA vector in the same way as described above.

To construct full-length FLAG-Plk4 mutants, a respective *Pme*I-*Not*I fragment containing a K608A (pKM4447), K625A (pKM4441), K634A (pKM4442), K681A (pKM4443), R684A (pKM4414), K685A (pKM4448), or K711A (pKM4444) mutation was inserted into the CI-neo-FLAG vector digested by the same enzymes.

The pEGFP-C1-Cep192 (pKM3552) construct expressing the full-length Cep192 (residues 1–2538) and pEGFP-C1-Cep152 (pKM3841) expressing the full-length Cep152 (residues 1–1654) are described previously [3]. To generate pCI-neo-FLAG-Cep192Δ58 (pKM4719) lacking residues 201–258, the N-terminus of pCI-neo-FLAG-Cep192 (pKM4199) construct [3] was replaced with a Cep192Δ58 fragment digested by *Asc*I and *Bcl*I. The pEGFP-C1-Cep152 (E21K) (pKM4100) construct was generated by inserting a *Xho*I-*Sma*I fragment into the pEGFP-C1 vector digested by the corresponding enzymes.

To construct GFP fusion protein of Cep192 (201–280) (pKM3512) or Cep152 (1–217) (pKM3561), a *Sal*I-*Sma*I fragment was inserted into the pEGFP-C1 vector digested by the same enzymes.

To generate serially deleted constructs from pEGFP-C1-Cep152 (1–217) (pKM3561), a *SalI*-*Sma*I fragment containing Cep152 (1–200) (pKM3800), Cep152 (1–175) (pKM3801), Cep152 (1–150) (pKM3802), Cep152 (1–130) (pKM3803), Cep152 (1–110) (pKM3804), Cep152 (1–100) (pKM3835), Cep152 (1–90) (pKM3836), Cep152 (1–80) (pKM3837), Cep152 (1–68) (pKM3838), Cep152 (1–60) (pKM3839), Cep152 (1–50) (pKM3840), Cep152 (10–217) (pKM3805), or Cep152 (37–217) (pKM3806) was cloned into the pEGFP-C1 vector digested by the respective enzymes.

To create a FLAG-tagged Cep135 (pKM4419) construct, a *Sma*I-*Xho*I fragment from pcDNA3.1-HA-Cep135 (a gift of by Kunsoo Rhee, Seoul National University, South Korea) was subcloned into the pCI-neo-FLAG vector digested by *Pme*I and *Xho*I.

For the lentiviral constructs expressing siRNA-insensitive *CEP152-sil* WT (pKM4325), *CEP152-sil (V8A)* (pKM4329), or *CEP152-sil (E21K)* (pKM4330), a *Xho*I (end-filled)-*Sma*I fragment containing *CEP152-sil* WT or the corresponding mutant was inserted into a pHR′.J-CMV-SV-puro vector (pKM2994) digested by *Sma*I.

To generate bacterial expression constructs, an *Nde*I-*Xho*I fragment containing Plk4 CPB (pKM3677), Cep192-58mer (201–258) (pKM4371), Cep152-60mer (1–60) (pKM4276), Cep192 (201–280) (pKM3544), or Cep152 (1–217) (pKM4268) was cloned into the $pHis_6$-MBP-TEV vector [11] digested by the corresponding enzymes. To generate $pHis_6$-MBP-TEV-Cep192 full-length (pKM4595) and $pHis_6$-MBP-TEV-Cep152 full-length (pKM4596), an *Nde*I-*Sal*I fragment of Cep192 and an *Nde*I-*Xho*I fragment of Cep152, respectively, were cloned into a $pHis_6$-MBP-TEV vector digested by *Nde*I and *Xho*I. To express MBP_L (a longer form of MBP with a 30 residue insertion)-fused Cep192-58mer, a *EcoR*I-*Sal*I fragment containing the MBP C-terminal 30 residue-TEV-Cep192-58mer from pKM4371 was cloned into the pMAL-C2 vector (New England Biolabs) digested by the same enzymes. MBP_L-fused Cep192-58mer migrates markedly slower than MBP-fused Cep152-60mer, thus allowing us to differentiate these two proteins in SDS-PAGE. pGEX-4T3-Plk4 C (580–970) (pKM3584) was cloned by inserting a *Sal*I-*Not*I fragment into the pGEX-4T3 vector (Amersham Pharmacia) digested by the same enzymes. pET21b-Cep192 (201–310) (pKM3619) was generated by inserting a *Not*I-*Sal*I fragment into the pET21b vector (Novagen) digested by the corresponding enzymes. To generate bacterial constructs expressing either His_6-Cep192 (2240–2538) (pKM4251) or GST-Cep192 (2240–2538) (pKM3944), a *Sal*I-*Not*I fragment containing the corresponding Cep192 coding sequence was cloned into the pET21b or the pGEX-4T3 (Pharmacia) vector digested by the same enzymes, respectively.

Supplementary References

1 Johmura, Y. *et al.* Regulation of microtubule-based microtubule nucleation by mammalian polo-like kinase 1. *Proc. Natl. Acad. Sci. USA*. **108**, 11446-11451. (2011).

2 Leung, G. C. *et al.* The Sak polo-box comprises a structural domain sufficient for mitotic subcellular localization. *Nat. Struct. Biol.* **9**, 719-724. (2002).

3 Kim, T.-S. *et al.* Hierarchical recruitment of Plk4 and regulation of centriole biogenesis by two centrosomal scaffolds, Cep192 and Cep152. *Proc. Natl. Acad. Sci. USA*. 110:E4849-4857 (2013).

4 Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126-2132. (2004).

5 Larkin, M. A. *et al.* Clustal W and Clustal X version 2.0. *Bioinformatics.* **23**, 2947-2948. (2007).

6 Yun, S. M. *et al.* Structural and functional analyses of minimal phosphopeptides targeting the polo-box domain of polo-like kinase 1. *Nat. Struct. Mol. Biol.* **16**, 876-882. (2009).

7 Sillibourne, J. E. *et al.* Autophosphorylation of polo-like kinase 4 and its role in centriole duplication. *Mol. Biol. Cell* **21**, 547-561. (2010).

8 Elbashir, S. M. *et al.* Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **411**, 494-498. (2001).

9 Cizmecioglu, O. *et al.* Cep152 acts as a scaffold for recruitment of Plk4 and CPAP to the centrosome. *J. Cell Biol.* **191**, 731-739. (2010).

10 Zhao, H. *et al.* The Cep63 paralogue Deup1 enables massive de novo centriole biogenesis for vertebrate multiciliogenesis. *Nat Cell Biol.* **15**, 1434-1444. (2013).

11 Elia, A. E. *et al.* The molecular basis for phospho-dependent substrate targeting and regulation of Plks by the polo-box domain. *Cell* **115**, 83-95. (2003).