A high-throughput electron tomography workflow reveals over-elongated centrioles in relapsed/refractory multiple myeloma

Graphical abstract

Highlights
- High-throughput electron tomography reveals organelle structure in patient material
- The workflow can be adapted to various cell types and organelles
- We characterize 455 centrioles in human bone marrow cells at nanoscale
- Myeloma cells contain over-elongated centrioles with gross structural aberrations

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In brief
Dittrich et al. describe a semi-automated high-throughput electron tomography strategy to study organelle structure in patient-derived material at nanoscale. By applying their methodology to centrosomes, they show that plasma cells from a myeloma patient harbor over-elongated centrioles with gross structural abnormalities as the potential cause of chromosomal aberrations in multiple myeloma.
A high-throughput electron tomography workflow reveals over-elongated centrioles in relapsed/refractory multiple myeloma

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SUMMARY
Electron microscopy is the gold standard to characterize centrosomal ultrastructure. However, production of significant morphometrical data is highly limited by acquisition time. We therefore developed a generalizable, semi-automated high-throughput electron tomography strategy to study centrosome aberrations in sparse patient-derived cancer cells at nanoscale. As proof of principle, we present electron tomography data on 455 centrioles of CD138pos plasma cells from one patient with relapsed/refractory multiple myeloma and CD138neg bone marrow mononuclear cells from three healthy donors as a control. Plasma cells from the myeloma patient displayed 122 over-elongated centrioles (48.8%). Particularly mother centrioles also harbored gross structural abnormalities, including fragmentation and disturbed microtubule cylinder formation, while control centrioles were phenotypically unremarkable. These data demonstrate the feasibility of our scalable high-throughput electron tomography strategy to study structural centrosome aberrations in primary tumor cells. Moreover, our electron tomography workflow and data provide a resource for the characterization of cell organelles beyond centrosomes.

INTRODUCTION
Centrosomes are the major microtubule-organizing centers in mammalian cells and consist of a pair of centrioles embedded in pericentriolar material (Bettencourt-Dias and Glover, 2007; Cosenza et al., 2017; Ganem et al., 2009; Gönzly, 2015). Centrioles are microtubule-based cylinders with a physiological length of up to 500 nm and a diameter of approximately 200 nm (Bettencourt-Dias and Glover, 2007; Kong et al., 2020; Martei et al., 2018; Sharma et al., 2021). They duplicate in S phase, with the formation of one daughter centriole next to each preexisting mother that subsequently elongates and matures until mitosis. Mature centrioles are decorated with two types of appendages, one set of nine distal appendages and...
a variable number of subdistal appendages, which can be located along the entire length of the centriole (Bettencourt-Dias and Glover, 2007; Hall and Hehnly, 2021). During mitosis, the two newly formed centrosomes of a cell migrate to opposite poles, contributing to bipolar spindle formation. To ensure accurate chromosome segregation, centriole number and structure are tightly controlled in non-transformed cells (Nigg and Holland, 2018).

Abnormalities in centrosome structure and number have been identified as drivers of genomic instability in various solid cancer entities and hematological malignancies (Chng et al., 2008; Cosenza et al., 2017; Gönczy, 2015; Krämer et al., 2003; Lopes et al., 2018; Maxwell et al., 2005). Centrosome aberrations have been detected in in situ carcinomas and low-grade tumors already and represent an early event in the evolution of malignant phenotypes in organotypic culture and animal models (Gönczy, 2015; Lopes et al., 2018). However, how centrosome aberrations develop remains unclear, with cancer-associated alterations in centrosomal genes being rare (Gönczy, 2015). Also, as most studies on centrosome aberrations in primary tumor tissues rely on single antigen immunostainings against pericentriolar matrix proteins at low resolution, virtually nothing is known about the detailed composition of centrosome aberrations in primary tumor cells at electron microscopy resolution.

The structural exploration of centrioles and other subcellular structures requires imaging techniques that go beyond the light microscopy diffraction limit of 200–300 nm. Significant advances have been made in super-resolution imaging methods over the last decade. Especially the recently introduced expansion microscopy allows for considerably improved resolution (Bowler et al., 2019; Chong et al., 2020; Gambarotto et al., 2019; Kong et al., 2020; Schemelleh et al., 2019; Vásquez-Limeta et al., 2022), and several centrosome-specific expansion microscopy protocols that allow for the analysis of centriole length (Kong et al., 2020) and structural features (Vásquez-Limeta et al., 2022), including appendages (Bowler et al., 2019), have been developed. These techniques are excellent for studying the function and structural contribution of individual proteins to the assembly of centrioles and centrosomes. Relying on the labeling of molecular components of the centriole, expansion microscopy can reveal protein complexes that are not necessarily visible by electron microscopy (Sahabadu et al., 2019). High-end deconvolution and computation of expansion microscopy images also allow for structural analysis of centrioles, although the resolution is still magnitudes apart from transmission electron microscopy images (Bowler et al., 2019; Gambarotto et al., 2019).

Plasma cell disorders comprise a range of increasingly malignant B cell neoplasias, spanning from premalignant monoclonal gammopathies of undetermined significance (MGUS) via asymptomatic smoldering myeloma to overt multiple myeloma and highly aggressive plasma cell leukemia (Manier et al., 2017; Maura et al., 2019). They arise from the malignant transformation of long-lived, terminally differentiated CD138pos bone marrow plasma cells (Halliley et al., 2015). In the absence of disease-defining mutational profiles, chromosome aberrations, including immunoglobulin heavy chain translocations and hyperdiploidy, are considered initiating events in plasma cell disorders, which are prevalent in early disease stages already (Manier et al., 2017; Maura et al., 2019).

Human plasma cells mainly reside within the bone marrow, which is only available by invasive bone marrow aspiration. Moreover, the physiological content of plasma cells in whole-bone marrow aspirates is in the lower single-digit percentage range of mononuclear cells (Nombela-Arrieta and Manz, 2017). To understand the origin and evolution of centrosome aberrations in the spectrum of plasma cell disorders as a paradigm for malignant progression from a precursor lesion to aggressive malignancy, we aimed for the development of a method that allows for targeted imaging of centrioles as one specific subcellular structure at high resolution and high scale in sparse primary material.

Targeted imaging of specific subcellular morphology in human primary tissues by electron microscopy is challenging due to the absence of genetic or antigenic labels that could act as a guide to the feature(s) of interest. In 200-nm-thick sections of human plasma cells, the probability of including a centriole is well below 10% for any given cell, i.e., to find these features in statistically significant numbers, manual inspection of each cell is necessary. We enabled this screening by using computational detection of cell outlines in overview images and employing automated imaging of each cell at a magnification suitable for identifying centrioles by browsing through the generated image stack. This provides target coordinates that can be propagated to neighboring sections, enabling serial electron tomography of the target objects at high resolution (Schorb et al., 2019).

RESULTS

A terabyte-scale electron tomography dataset of centrioles in primary human cells

For ultrastructural analysis, we combined a transmission electron microscopy (TEM) screening procedure with targeted electron tomography (ET) of regions of interest (Figure 1). After magnetic-activated cell sorting (MACS)-based CD138 selection, preparation, and sectioning of both CD138pos and CD138neg fractions of bone marrow mononuclear cells for electron microscopy, a two-dimensional (2D) overview of the central section was obtained at 400× magnification (326.9 nm/px), and cells were semi-automatically detected and labeled for acquisition. Labeled cells were acquired at a magnification suitable for manual screening. For analysis of centrioles, we chose a 3,000× magnification with 42.75 nm/px, values that can be adapted according to the needs of other subcellular target features. The output was generated as an image stack that allowed for the identification of cells containing centrioles. Positions of selected cells containing target centrioles on the central section were then semi-automatically propagated, and the respective cells were re-identified on all adjacent sections to acquire the full length of the centrioles. Subsequently, single-axis ET (15,500× magnification, 1.55 nm/px; tilt range: −60° to 60°; increment: 1°) was performed at all targets on all sections. Next, corresponding tomograms from individual sections were reconstructed and joined to produce a final volume of at least 3.1 × 3.1 × 1 μm per target cell (X × Y × Z). Centrioles were measured and morphometrically analyzed within these volumes. Statistical analysis of centriole
parameters was eventually performed using the exported measured parameters.

Applying this methodology, a total of 6,504 cells were semi-automatically screened by TEM, and 455 completely pictured centrioles in 343 cells were examined, including 250 completely pictured centrioles in 221 bone-marrow-derived CD138pos plasma cells from a patient with relapsed/refractory multiple myeloma and 205 completely pictured centrioles in 138 CD138neg bone marrow mononuclear cells from three healthy donors. An online repository of the complete ET dataset (2.2 TB of raw data and 765 GB of tomography volume data) is publicly available for download and is also viewable in an interactive
Characterization of centriole parameters in CD138neg bone marrow mononuclear cells from healthy individuals

Both length and diameter of centrioles are tightly controlled and well conserved across evolution and usually do not exceed 500 and 250 nm in human cells, respectively (Bettencourt-Dias and Glover, 2007; Kohlmaier et al., 2009; Sharma et al., 2021). To determine length, diameter, and structural features of normal centrioles using the ET workflow described above, and to compare our results with those from previous analyses using conventional electron microscopy (Bettencourt-Dias and Glover, 2007; Chretien et al., 1997; Kong et al., 2020; Marteil et al., 2018; Sharma et al., 2021), we first examined CD138neg bone marrow...
mononuclear cells from three healthy donors. In 138 cells, a total of 265 centrioles, among them 60 partially acquired and 205 completely pictured centrioles, were identified. Further analyses were restricted to complete centrioles. In line with the literature, the median length of centrioles (368 nm, range: 190–753 nm) was normal in these cells, whereas their median diameter (204 nm, range: 182–226 nm) was slightly reduced when compared with human cell lines (Chrétien et al., 1997; Sahabandu et al., 2019; Vásquez-Limeta et al., 2022) (Figures 2A–2D; Table 1). No structurally abnormal centrioles were found in CD138pos bone marrow mononuclear cells from healthy donors, and over-elongation was only detected in a very limited amount of these cells.

**Characterization of centriole parameters in CD138pos plasma cells from a patient with relapsed/refractory multiple myeloma**

The CD138pos plasma cells analyzed in this study were derived from the bone marrow of a 73-year-old male patient with relapsed/refractory multiple myeloma. Immunofluorescence microscopy, using antibodies against centrin and pericentrin to label centrioles and pericentriolar material, respectively, revealed numerical centrosome aberrations in only 16 of 552 (2.9%) CD138pos plasma cells of the patient, a frequency within the range found in healthy cells of B lymphatic origin (Krämer et al., 2003) (Figure S1). On the contrary, supernumerary centrioles were frequent (55.7%) in U2OS cells constitutively overexpressing PLK4, the principal kinase regulating centrosome replication (Bettencourt-Dias and Glover, 2005; Habedanck et al., 2005; Cosenza et al., 2017), which were used as a positive control.

By application of the ET workflow described above to six independent sections, which were at least 3 μm apart from each other, we identified 410 individual centrioles in the bone marrow sample from the myeloma patient. As 160 centrioles were only partially acquired, the analysis of centriole dimensions was restricted to the remaining 250 complete centrioles. Manual analysis showed that 120 of these centrioles were decorated with appendages. Accordingly, 120 complete mother centrioles and 130 completely pictured daughter centrioles from the myeloma patient were available for further analysis, which revealed that centrosomal regions in CD138pos plasma cells from the myeloma patient contained a median of two centrioles (range: 1–4), thereby corroborating the immunofluorescence microscopy findings (Table 1). The median centriole diameter was 216 nm (range: 141–256 nm) and not significantly different in mother (216 nm, range: 172–245 nm) versus daughter centrioles (215 nm, range: 141–256 nm; p = 0.54) (Figures 2A and 2B; Table 1). Although significantly larger than in CD138neg bone marrow mononuclear cells from healthy donors, the median centriole diameter of CD138pos myeloma cells was still significantly reduced compared with human cell lines (Chrétien et al., 1997; Sahabandu et al., 2019; Vásquez-Limeta et al., 2022).

The median centriole length was 495 nm (range: 254–1740 nm), with 48.8% of centrioles being over-elongated when a cutoff of 500 nm was applied (Figures 2C and 2D). The median length of mother centrioles (567 nm, range: 319–1740 nm) was significantly longer than the median length of daughter centrioles (436 nm, range: 254–848 nm; p < 0.001), which translated into a higher fraction of over-elongated mother, compared with daughter centrioles (75.0% versus 24.6%). Compared with centrioles in CD138neg bone marrow mononuclear cells from three healthy donors, both mother and daughter centrioles in CD138pos plasma cells from the myeloma patient were significantly over-elongated (Figures 2C and 2D). In addition, subdistal appendages were often not located at the distal ends of the centrioles but frequently in their center (Figures 2E and S2). Also, the number of subdistal appendages increased with the length of centrioles in CD138pos plasma cells from the myeloma patient (Figure 2F).

As additional structural abnormalities, 13 (5.8%), 6 (2.7%), and 18 (8.1%) of mother centrioles were broken, incomplete, and/or asymmetric at their elongated distal ends, respectively (Figure 3A; Table 1). All three phenotypes almost exclusively occurred in plasma cells with over-elongated centrioles, were restricted to mother centrioles, and were completely absent from CD138neg bone marrow mononuclear cells from healthy donors (Figure 3B; Table 1). A 3D visualization of ET data of a representative, structurally aberrant centrosome displaying over-elongation, a broken distal end, and supernumerary appendages is shown in Figures 3C–3E and Video S1.

Although the electron microscopy appearance of the over-elongated structures clearly resembled centrioles, we additionally performed immunostainings to ARL13B to exclude that these threads represent primary cilia rather than bona fide centrioles (Kong et al., 2020; Marteil et al., 2018). In contrast to serum-starved human BJ fibroblasts, which were used as a positive control, neither CD138neg bone marrow mononuclear cells from a healthy donor nor CD138pos plasma cells displaying over-elongated centrioles in electron microscopy contained ARL13Bpos primary cilia (Figure S3).

**DISCUSSION**

**High-throughput TEM as a morphology screening tool for primary tissues**

The methodology we developed allows the screening of tens of thousands of cells from dozens of individual tissue donors. Manual inspection identified the cells to be targeted for ET acquisition. We propagated the target coordinates from the screened central section to the neighboring five consecutive 200-nm-thick sections in a semi-automated fashion.

This study systematically generates ET data of centrioles in human primary cells. Using the presented approach, it is possible to generate a statistically significant (n > 30) number of volume datasets depicting the subcellular target morphology of an individual tissue donor from bedside to data storage within 7 working days. As many of the acquisition steps are fully automated, data processing and analysis of already imaged data can be done in parallel. This enables a systematic characterization of organelles using volume electron microscopy with reasonable resource investment and within a decent experimental time frame. Due to the numbers of observations, a statistical correlation with clinical data is possible. A sample containing as little as 100,000 cells is sufficiently large for such an experiment. 150,000 cells even enable additional immunofluorescence imaging.

In principle, our workflow can be further improved and expedited by automated identification of regions of interest using
Of note, our data demonstrate an unexpected variability in human centriole length. Apart from the large fraction of machine-learning approaches. Automated analysis and recognition of different centriole morphologies, on the other hand, would require a significant amount of training data for machine learning.

Sharing the data through public repositories and providing means of on-the-fly visualization without the necessity of accessing or downloading huge datasets enables easy sharing, exploring, studying, and annotating volume electron microscopy data of primary (tumor) cells by a global research community.

### Over-elongation and structural aberrations of centrioles in multiple myeloma

Recent data suggest a contribution of structural centriole/centrosome aberrations to several aspects of tumorigenesis including chromosomal instability (CIN) (Kohlmaier et al., 2009; Kong et al., 2020; Marteil et al., 2018). A recent screen identified centriole length deregulation as a recurrent structural aberration type in the NCI-60 panel of human cancer cell lines (Marteil et al., 2018). Accordingly, our findings suggest that centriole over-elongation can occur in non-proliferating, quiescent cells, whereas numerical centrosome aberrations are subject to negative selection as they require cell-cycle progression for their development.

We also observed subdistal appendages to be often not located at the distal ends of the centrioles but frequently in their center. It has already been reported that aberrantly positioned subdistal appendages can occur on over-elongated centrioles in aortic endothelial cells (Bystrevskaya et al., 1992) and on over-elongated centrioles after CPAP overexpression (Kohlmaier et al., 2009).
Centriole length (nm)

A: Images of centrioles with various phenotypes.
B: Box plot showing centriole length for different phenotypes: normal, abnormal, broken, and incomplete asymmetric.

Legend:
- Normal
- Abnormal
- Broken
- Incomplete asymmetric

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over-elongated centrioles in multiple myelomas, the mixture of CD138<sup>neg</sup> bone marrow mononuclear cell types from healthy donors revealed a fraction of centrioles that is shorter than the reported length of canonical human centrioles. Further analysis is warranted to evaluate the possibility that this variability in centriole length is specific for the bone marrow compartment.

**Conclusion**
Due to their small size, structural aberrations of centrosomes have been notoriously difficult to analyze by immunofluorescence microscopy. Using a semi-automated high-throughput workflow with cutting-edge ET, which allows for a resolution that is several magnitudes higher than the current limit of super-resolution microscopy (Chong et al., 2020; Gambarotto et al., 2019; Kong et al., 2020; Schermelleh et al., 2019; Vasquez-Limeta et al., 2022), we were able to determine the centriolar phenotype of healthy and rare, malignant bone marrow cells from patient material at the ultrastructural level on a high scale.

By assembling EM data requiring a total beam time of 273 h, of which more than 235 h were automated acquisition, we have analyzed the centrosomal phenotype of a primary cancer at the ultrastructural level on a high scale and describe over-elongated centrioles in multiple myeloma, a plasma cell malignancy that is characterized by a multitude of both numerical and structural chromosome aberrations.

Given the recent progress on the functional contribution of structural centriole aberrations in vitro, in-depth ultrastructural characterization of centriole phenotypes in primary cancer samples will be important to unravel the contribution of this aberration type to cancer development and evolution.

**Limitations of study**
The method we employ in this pilot study is generally applicable to various kinds of biological source material as long as a specimen for preparation compatible with conventional TEM is available (Cortese et al., 2020; Gomes Pereira et al., 2021). In our hands, a sample containing as little as 100,000 cells was sufficiently large to be handled for ET. Adaption of sample preparation to other types of specimens could allow for even smaller sample sizes. Our method is also applicable for targeting various different subcellular structures as long as they can be contrasted for and visualized by TEM. The use of open-source software facilitates the adaption and extension of our workflow to various experimental settings, which is an important prerequisite for further utilization and development of this imaging strategy by the scientific community.

Importantly, this workflow will always visualize only a fraction of all centrioles present in a given cell population and also potentially not all centrioles within single cells. For this reason, our EM methodology is not applicable for quantification of numeric centriole/centrosome aberrations at the cell population or single-cell level. Several immunofluorescence microscopy techniques have been developed within the past years to specifically address quantification of numerical centriole/centrosome aberrations. These techniques can be combined with the workflow described here to evaluate numeric and structural centriole/centrosome aberrations in parallel.

Relying on the labeling of molecular components of the centriole, expansion microscopy can reveal protein complexes that are not necessarily visible in electron microscopy (Sahabandu et al., 2019). Yet, even though expansion microscopy is performing at a throughput that surpasses electron microscopy even with our approach, it fails so far to reach the resolution of TEM tomography that gives access to subtriplet resolution. Only ultrastructure expansion microscopy (Gambarotto et al., 2019) is getting close but has only been achieved on purified centrioles so far and requires high-end deconvolution and computation. Our workflow therefore provides a valuable resource for high-throughput in situ characterization of subcellular structures in primary patient material.

**STAR★METHODS**
Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability

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**Figure 3. Over-elongated centrioles show additional structural abnormalities**

(A) Segmented electron tomography data showing representative over-elongated centrioles with the phenotypes “broken” (top, red), “incomplete” (middle, green), and “asymmetric” (bottom, blue). The respective key feature of each phenotype is marked with a black triangle: breakage point with diverging triplets at the distal end (broken) in the top panel, multiple subdistal appendages on a longitudinal structure consisting of less than nine microtubule triplets (incomplete) in the middle panel, and an asymmetrically elongated distal end (asymmetric) in the bottom panel. Scale bars, 500 nm.

(B) Violin plots and integrated box plots showing the length distribution of normal versus abnormal centrioles in CD138<sup>neg</sup> plasma cells from a patient with relapsed/refractory MM. The abnormal group is subdivided and color coded into broken, incomplete, and asymmetric. The respective key feature of each phenotype is marked with a black triangle: breakage point with diverging triplets at the distal end (broken) in the top panel, multiple subdistal appendages on a longitudinal structure consisting of less than nine microtubule triplets (incomplete) in the middle panel, and an asymmetrically elongated distal end (asymmetric) in the bottom panel. Scale bars, 500 nm.

(C–E) Different views of electron tomography data, reconstructed and joined from five consecutive sections, of one representative structurally aberrant mother centriole and two adjacent structurally normal daughter centrioles.

(F) Different z-planes of the aberrant mother centriole as displayed in the original reconstructed electron tomography volume. Scale bar, 200 nm.

(G) Model view of the centrioles containing data used for measurements. For the mother centriole, length measurements for both parts of the broken centriole are displayed as two distinct vectors along the longitudinal axis. Appendages are marked with triangles. Scale bar, 200 nm.

(H) 3D visualization using Amira software. The mother centriole (orange) shows over-elongation (total length: 1692 nm) and a broken distal end (dark orange and light orange). Supernumerary subdistal appendages are marked in yellow. Two structurally normal daughter centrioles (lengths: 358 and 443 nm) are displayed in green and blue, respectively.

See also Figure S3.
SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.crmeth.2022.100322.

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AUTHOR CONTRIBUTIONS

A.K. conceived the project. A.K., Y.S., T.D., and S.K. designed the experiments. H.G., M.S.R., C.M.-T., U.H., and S.O.S. collected the samples. T.D., S.K., M.B., M.S., G.P., and I.H. performed experiments. A.K., Y.S., T.D., M.S., and S.K. analyzed data. A.K., Y.S., M.S., and S.K. wrote the paper. All co-authors approved the final manuscript.

DECLARATION OF INTERESTS

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Mouse anti-centrin  | Merck Millipore | Cat.#04-1624, RRID:AB_10563501 |
| Rabbit anti-pericentrin | Abcam | Cat.#ab4448, RRID:AB_304461 |
| Rabbit anti-ARL13B | Proteintech | Cat.#17711-1-AP, RRID:AB_2060867 |
| Mouse anti-polyglutamylated Tubulin | AdipoGen | Cat.#AG-20B-0020B, RRID:AB_2490211 |
| Rabbit anti-MUM-1 (MRQ-43) | Cellmarque | Cat.#358R-74 |
| Mouse anti-Ki67 (MIB-1) | Agilent Dako | Cat.#GA626, RRID:AB_2687921 |
| Alexa Fluor 488 goat anti-mouse IgG | Molecular Probes Invitrogen | Cat.#A11017, RRID:AB_143160 |
| Alexa Fluor 488 goat anti-mouse IgG | Molecular Probes Invitrogen | Cat.#A11029, RRID:AB_2534088 |
| Alexa Fluor 568 goat anti-rabbit IgG | Molecular Probes Invitrogen | Cat.#A11036, RRID:AB_10563566 |
| Alexa Fluor 568 goat anti-rabbit IgG | Molecular Probes Invitrogen | Cat.#A11011, RRID:AB_143157 |
| **Biological samples** |        |            |
| Human bone marrow | This paper | N/A |
| Human peripheral blood | This paper | N/A |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| Vectashield mounting medium | Vector Laboratories | Cat.#H-1000 |
| Vectashield mounting medium with DAPI | Vector Laboratories | Cat.#H-1200 |
| Puromycin | Life Technologies | Cat.#2600023 |
| Hygromycin | Life Technologies | Cat.#10687010 |
| Tetracyclin | SigmaAldrich | Cat.#T7660-5G |
| FicoLite-H separation medium | Linaris | Cat.#GTF1511KYA |
| Triton-X-100 | ThermoFisher Scientific | Cat.#HFH10 |
| Hoechst 33342 nucleic acid stain | Invitrogen | Cat.#H3570 |
| TRIzol RNA extraction reagent | Invitrogen | Cat.#15596026 |
| **Critical commercial assays** |        |            |
| RNeasy Mini Kit | Qiagen | Cat.#74104 |
| SV Total RNA Isolation System | Promega | Cat.#23101 |
| Small Sample Labeling Protocol | Affymetrix | N/A |
| GeneChip™ Human Genome U133 Plus 2.0 Array | Applied Biosystems | Cat.#900466 |
| **Deposited data** |        |            |
| Raw and analyzed data | This paper | EMPIAR DOI: 10.6019/EMPIAR-11243 |
| Code and exported workflows | This paper | Mendeley https://doi.org/10.17632/kf7nbbxp.1; Github: https://github.com/mobie/centriole-tomo-examples |
| Additional supplemental data | This paper | Mendeley, https://doi.org/10.17632/kf7nbbxp.1 |
| Gene expression profiling data | This paper; Weinhold et al. (2021); Seckinger et al. (2012) | ArrayExpress: E-MTAB-81/E-GEOD-2658 |
| **Experimental models: Cell lines** |        |            |
| U-2 OS | ATCC | Cat.#HTB-96, RRID:CVCL_0042 |
| U2OS-PLK4 | Konotop et al. (2016) | N/A |
| BJ | ATCC | Cat.#CRL-2522, RRID:CVCL_3653 |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources, reagents, and scripts should be directed to and will be fulfilled by the lead contact, Alwin Krämer (a.kraemer@dkfz-heidelberg.de).

Materials availability
This study did not generate any new unique reagents. Epoxy resin blocks for electron microscopy experiments of the patient analyzed in this study were archived and are available upon request from the lead contact.

Data and code availability
Data
Electron tomography data generated during this study have been deposited at EMPIAR (Iudin et al., 2016) and are publicly available using the accession code EMPIAR-11243 (https://doi.org/10.6019/EMPIAR-11243). Immunofluorescence and immunohistochemical data generated during this study will be shared by the lead contact upon request.

Code
All original code has been deposited at Github and Mendeley and is publicly available as of the date of publication. DOIs are listed in the key resources table.
Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plasma cell disorder patients and healthy donors
The investigated patient (73 year old male) and the healthy donors (Control 1: 62 year old female, control 2: 55 year old male, control 3: 56 year old female) gave written informed consent. The study was performed in accordance with the principles of the Declaration of Helsinki. Both the Ethics Committee of the University of Heidelberg as well as the European Molecular Biology Laboratory (EMBL) review board approved the study (Ethics Committee of the University of Heidelberg approval reference number: S-206/2011; EMBL BIAC application number: 2019-005).

Cell lines
U2OS (ATCC HTB-96, RRID:CVCL_0042) were obtained from ATCC and were kept in a humidified incubator with 37°C temperature and 5% CO₂. The generation of U2OS-PLK4 cells was already described in (Konotop et al., 2016). U2OS cells were cultured in DMEM Glutamax medium (Life Technologies, Cat. No. 31966047) supplemented with 10% fetal bovine serum (FBS) (Clontech, Cat. No.
METHOD DETAILS

Plasma cell disorder and healthy donor samples
The analyzed bone marrow aspirate was acquired according to clinical standard operating procedures at the Department of Internal Medicine V, University of Heidelberg. Bone marrow mononuclear cells were isolated using Ficoll gradient centrifugation. Plasma cells were enriched by magnetic-activated cell sorting (MACS) for CD138 according to the manufacturer’s instructions, leading to a mean plasma cell purity of 88.9 ± 9.6% as checked by fluorescence-activated cell sorting (FACS).

Sample splitting
For comprehensive analysis, the sample was asymmetrically split: Up to 4 × 10^5 cells (depending on sample size) were used for immunofluorescence imaging, the remainder of the cells was fixed for electron microscopy and tomography.

Light microscopy and immunofluorescence imaging
After washing in PBS, approximately 10^5 cells were spun onto slides and fixed in 100% methanol at −20°C for 10 min.

Methanol-fixed cells were blocked with blocking buffer containing 10% goat serum in PBS at room temperature for 20 min and incubated with primary antibodies in blocking buffer at room temperature for additional 60 min. Samples were then washed three times with PBS and incubated with secondary antibodies in blocking buffer for 30 min at room temperature. After washing in PBS again, nuclei were stained using Hoechst (Invitrogen, Cat. No. H3570) in PBS for 5 min at room temperature. Samples were washed with PBS and de-mineralized purified water, incubated with 100% ethanol for 30 s, and then mounted onto coverslides using Vectorshield mounting medium (Vector Laboratories, Cat. No. H-1000). Semi-automated immunofluorescence imaging with pre-defined regions of interest (ROI) was performed on the same or the following day by using ZEN blue 2.6 software (Carl Zeiss Microscopy) on a Zeiss Cell Observer equipped with a 40 × 1.3 Plan Apochromat objective.

Primary antibodies used for this study were: Centrosome staining: Mouse anti-centrin (Merck Millipore, 04–1624, RRID:AB_10563501) and rabbit anti-pericentrin (Abcam, Cat. No. ab4448, RRID:AB_304461). Alexa Fluor 488 (Molecular Probes Invitrogen, Cat. No. A11029, RRID:AB_2534088) and 568 (Molecular Probes Invitrogen, Cat. No. A11036, RRID:AB_10563566) conjugated with fluorescent dye were used as secondary antibodies.

Sample preparation for electron microscopy and tomography
A workflow scheme is depicted in Figure 1A. CD138\textsuperscript{pos} plasma cells were fixed by adding freshly prepared electron microscopy (EM) fixative at 4°C (composition of fixative: 2.5% EM-grade glutaraldehyde and 2% EM-grade paraformaldehyde in 0.1 M Na-cacodylate buffer (pH 7.4)). After incubation in EM fixative at room temperature for 5 min, fixative was renewed, and samples were incubated in the fridge at 4°C overnight. After removal of fixative, cells were stained by incubation with Evans Blue (1 mg/mL in 0.1 M cacodylate buffer, pH 7.4) for 20 min at room temperature, followed by three washing steps with 0.1 M cacodylate buffer. For pre-embedding, cells were resuspended in 2% low-melting agarose and centrifuged (1000 g) for 10 min at 37°C to form a cell pellet. All subsequent embedding steps were performed using a temperature-controlled microwave at 24°C. For post-fixation and staining, cells were incubated in 1% osmium tetroxide in dH\textsubscript{2}O for 20 min. Cells were washed in dH\textsubscript{2}O for 1 min four times and stained with 1% uranyl acetate in dH\textsubscript{2}O for 14 min. Afterward, cells were rinsed in dH\textsubscript{2}O four times for 1 min each. Dehydration with an acetone series (50%, 70%, 90%, 2 × 100%) was performed for 45 s per step in the microwave. Cells were infiltrated with EPON epoxy resin 812 (hard formula) using increasing resin concentrations in 100% acetone (10%, 30%, 50%, 70%, 90%, 3 × 100% EPON in acetone). All infiltration steps were performed in the microwave for 3 min each at 24°C. Cell pellets in 100% resin were transferred into an embedding mould, incubated at room temperature overnight, and subsequently polymerized at 60°C for 2 days. Blocks were trimmed and serial sections (200 nm thickness each) were obtained using a Leica UC7 conventional ultramicrotome with a diamond knife (Diatome). At least five consecutive sections were collected on formvar-coated slot grids. Grids were post-stained with 2% uranyl acetate and lead citrate to enhance imaging contrast.

Electron microscopy and tomography
A high-throughput transmission electron microscopy workflow on a JEM 2100Plus electron microscope (JEOL Ltd., Akishima, Japan) equipped with a JEOL Matataki sCMOS camera was used to screen for centrosome-containing cells within the respective sections, as described previously (Schorb et al., 2019). After assessment of overall grid quality at 80× magnification, a 400× magnification montage image of the central section of the grid was obtained. Utilizing a KNIME (Konstanz Information Miner) (Berthold et al., 2007)- and pyEM-based software workflow, each cell on the grid was labeled and a virtual map of the cell at 1000× magnification was created by extrapolating data from the 400× magnification image. Using SerialEM’s advanced navigator functionality, we automatically acquired actual map images of each cell on the section at 1000× and 3000× magnification, respectively (Figure 1B). Output at 3000× magnification was generated as a gallery of TIFF images and manually evaluated for cells exposing centrosomal structures (Figure 1C).
After transferring the grid to a Tecnai F30 electron microscope (Thermo Fisher Scientific, Waltham, USA) equipped with a Gatan OneView camera (Gatan Inc., Pleasanton, USA), we semi-automatically targeted these cells on the remaining sections of the grid (Figure 1D). Map images were acquired at 2300× magnification for all cells of interest on all sections. Centrosome-containing regions were marked as points in SerialEM and labelled according to their location (i.e., cell and section). Single axis electron tomography was performed automatically at each point at 155.00× magnification (1.55 nm/px; tilt range: −60° to +60°; increment: 1°) using a custom SerialEM script.

Reconstruction of the acquired tilt series was achieved using an automated workflow based on the batch tomogram reconstruction feature of the IMOD software package (Kremer et al., 1996) on a high-performance computer cluster. The reconstructed tomograms of all sections were joined manually with etomo within the IMOD software package (Mastronarde, 2005; Mastronarde and Held, 2017) to produce full 3D volumes of each acquired centriole. The XY-dimensions for the final acquired 3D volumes are approximately 3.1 × 3.1 μm with at least 1 μm in Z (Figure 1E). Selected tomograms were manually segmented in Amira-Avizo software platform version 2020.1 (ThermoFisher Scientific), using the threshold-based brush segmentation tool. 3D visualizations of representative electron tomography data were generated using IMOD and Amira-Avizo, respectively (Figure 3; Video S1). Volume rendering and animations were computed and created in Amira-Avizo.

**Multimodal big image sharing and exploration (MoBIE)**

All acquired tomography data of this project can be visualized using the ImageJ (Schneider et al., 2012) plugin MoBIE (Vergara et al., 2021). We converted the reconstructed volumes into BDV-N5 format and determined the coordinate transformation to display the centrioles along their longitudinal axis from the model coordinates to produce insightful views to visualize the data.

General information on how to use and install MoBIE is accessible under the following link: https://github.com/mobie/mobie-viewer-fiji.

Visualizing the project data requires opening them as a MoBIE project. For this, open MoBIE in Fiji, and choose the plugin click ‘MoBIE - > Open MoBIE Project. Then enter the link to the dataset (https://github.com/mobie/centriole-tomo-examples). In the upcoming window, electron tomography data are listed in the ‘Tomograms’ dropdown menu (labeled according to their respective entity and patient number), where they can be visualized as reconstructed volumes. Alternatively, it is possible to display each centriole as a crop view along its longitudinal axis by selecting it from the ‘Centrioles’ dropdown menu in the same window.

All applicable metadata as well as KNIME, R, and, respectively, python scripts used to generate, visualize, and/or analyze the data of this project are made publicly available as well and can be found in the same Git repository and on Mendeley. DOIs are listed in the key resources table.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

For all experiments, results with p values not larger than 5% were considered statistically significant. Performed statistical analyses of each experiment are described in the respective paragraph below and sample sizes are given in the in the figure legends.

**Quantification and evaluation of numerical centrosome aberrations by immuno-fluorescence microscopy**

After immunostaining, cells were evaluated manually in ZEN 2.6 blue edition (Carl Zeiss Microscopy, Germany) and classified as normal or amplified. Classification criteria were: normal, if four or less distinct signal maxima in the centrin channel, else amplified; normal, if two or less distinct signals in the pericentrin channel, else amplified. Cells were excluded from the evaluation if they (i) were not fully featured in 20 Z-stacks (out of focus), (ii) overlapped with other cells and thus, centrosomes could not be attributed to one or the other cell, or (iii) showed no maxima in the assumed centrosomal region in one of the two channels. Cells with visible amplification in at least one of both pericentrin and centrin signals were deemed amplified. At least 100 evaluable cells were analysed per sample.

**Morphometrical analysis of electron tomography data**

Using IMOD’s model feature, one electron microscopy expert marked the longitudinal axis, diameters (proximal, distal, and central), and appendages of each centriole. Structurally aberrant centriole phenotypes were tagged as well. A centriole was deemed (i) asymmetric, if it showed the typical, cylindrical 9 × 3 configuration on its one end, but only consisted of less than nine triplets on its other end; (ii) broken, if its 9 × 3 architecture was preserved, but showed clear breakage points (e.g. diverging triplets at the distal end); and (iii) incomplete, if it was clearly consisting of centriolar structures (e.g. microtubule triplets with or without appendages), but never displayed the typical 9 × 3 cylinder shape of a normally configured centriole along its longitudinal axis. Sample images of phenotypically aberrant centrioles are depicted in Figure 3A. Centrioles carrying appendages and/or subjacent appendages were termed ‘mother’, else ‘daughter’.

Measurements of centriole dimensions were performed with the 3dmod tool within the IMOD software package. Using the “Slicer” window, a longitudinal slice through the center of the centriole at the largest possible longitudinal axis was generated, paying particular attention to the correct slicing angle to avoid incorrectness of diameter measures due to oblique cutting of the longitudinal axis. We measured centriole diameters at three different locations along the longitudinal axis: close to the proximal end, close to the distal end, and at the center. Branching microtubule blades as well as appendages were not included into the diameter measures. To measure diameters of fragmenting over-elongated centrioles, we only used the parts of the centriole that clearly showed no
fragmentation. Fragmented areas which showed no $9 \times 3$ or $9 \times 2$ cylinder-shape were labeled, but neither used for measurements of length nor diameters.

**Data extraction from IMOD-files and statistical analysis**

Statistical analysis was done with R statistical environment 3.5.3 (R Core Team, 2016) on a x86_64-w64-mingw32/x64 (64-bit) platform, together with 'survival' package (version 3.1-12) (Therneau et al., 2021; Therneau and Grambsch, 2000). A table containing coordinates and lengths for the longitudinal axis and the diameter marks as well as coordinates of individual appendages and phenotype tags was semi-automatically generated from the model files using a KNIME workflow (provided in the online resources). The Shapiro–Wilk test was used to test for normality and parametric or non-parametric statistics were applied for the analysis of centriole measures where applicable. Continuous data were described with median and range. If not stated otherwise, the Wilcoxon rank-sum test was used to test differences in continuous variables of two groups, the Kruskal-Wallis rank sum test was used to test differences in continuous variables of three or more groups, and Fisher exact test was used to test differences in categorical variables between groups. All statistical tests were two-sided.