Amplified centrosomes in dendritic cells promote immune cell effector functions

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Abstract

Centrosomes constitute structural elements organizing the mitotic spindle in animal cells for proper chromosome segregation. Centrosome numbers are tightly controlled and limited to one during interphase and two before a cell enters mitosis. Defects in regulating centrosome numbers lead to the presence of amplified centrosomes, which are a hallmark of malignant cells and sufficient to induce tumorigenesis. By contrast, amplified centrosomes are rarely observed in normal somatic cells and often removed during terminal differentiation. Here, we demonstrate the presence of amplified centrosomes in dendritic cells (DCs) during immune activation. Mature DCs accumulate centrosomes by mitotic defects and show high expression levels of polo-like kinase 2 (PLK2) leading to over-duplication of centrioles. During cell migration, amplified centrosomes tightly cluster and act as functional microtubule-organizing centers, which promote persistent locomotion. Moreover, DCs with amplified centrosomes show enhanced secretion of inflammatory cytokines and optimized T cell responses. Together, these results demonstrate a previously unappreciated role of amplified centrosomes in promoting the ability of leukocytes to enhance immune responses.

Introduction

Centrosomes are highly conserved cell organelles, consisting of two centrioles, which are surrounded by ordered layers of pericentriolar material (PCM)[1,2]. They are present as single copy in interphase and duplicate precisely once before a cell enters mitosis[3]. Centrosome abnormalities are found in virtually all types of human cancers and have been classified as structural or numerical aberrations. Numerical aberrations in cancer cells, such as centrosome amplification (CA), arise due to cell cycle dysregulation, and correlate with karyotype alterations, clinical aggressiveness and lymph node metastasis[4,5]. Mechanistically, amplified centrosomes promote the formation of lagging chromosomes and micronuclei during bipolar cell divisions[6,7]. Chromosomes in micronuclei often undergo defective and asynchronous DNA replication, resulting in DNA damage and extensive fragmentation[7]. These findings establish a causal link between centrosome amplification and chromosomal instability (CIN), which has
recently been confirmed in vivo. Of note, normal somatic cells poorly tolerate amplified centrosomes and lose them in the absence of selection.

Dendritic cells (DCs) are leukocytes, which constitute critical cellular players that integrate innate immune signals and initiate adaptive immunity via antigen-presentation. They reside in peripheral tissues and are characterized by a stellate morphology, high expression of major histocompatibility class (MHC) II, as well as their capacity to sense antigens via Toll-like receptors (TLRs). Upon antigen recognition, DCs enter a maturation program, which triggers antigen uptake and processing and subsequent homing to secondary lymphoid organs. Within lymph nodes, DCs activate antigen-specific T cells, thereby initiating adaptive immune responses. To efficiently navigate through complex three dimensional (3D) environments, DCs choose the path of least resistance and move without tightly adhering to the substrate, while being able to adapt their migration mode according to the environment. Besides DCs, metastasizing cancer cells, which frequently exhibit amplified centrosomes, use similar mechanisms of locomotion to move through interstitial tissues. This motivated us to define the role of centrosomes in DC biology.

Results

DCs show upregulation of centrosome numbers upon maturation

We first analyzed centrosome numbers in peripheral DCs isolated from mouse skin explants. Split ear sheets were floated on culture medium supplemented with the chemokine CCL19, which allows emigration of dermal DCs into the culture medium. After three days of emigration, we collected all non-adhering cells, which expressed high cell-surface levels of classical DC markers such as CD11c and MHC II (Supplementary Fig. 1A) and efficiently migrated towards chemotactic gradients in 3D collagen matrices (Supplementary Fig. 1B). Immunostaining against the PCM component $\gamma$-tubulin and the centriolar marker acetylated (ac)-tubulin revealed that a proportion of dermal DCs displayed four or more centrioles, corresponding to two or more centrosomes, which are all located in close proximity to the nucleus (Fig. 1a,b). To test whether upregulated centrosome numbers in dermal DCs are a
consequence of ongoing cell proliferation, we analyzed 5-ethynyl-2′-deoxyuridine (EdU) incorporation and pospho-Histone3 (pH3) levels as a marker for S-phase and G2-M transition, respectively. Dermal DCs were largely EdU- and pH3-negative, demonstrating that extra centrosomes are not a consequence of ongoing cell proliferation (Fig. 1c). Similar centrosome numbers were also observed in sorted splenic DCs, but not in other subtypes of the myeloid or lymphoid lineage that we analyzed (Supplementary Fig. 1C). These findings indicate that in the DC compartment cells that reside in G1 of the cell cycle carry two or more centrosomes. Due to the absence of proliferation markers, which would explain the presence of two centrosomes, we refer to this phenomenon as CA in DCs.

As DCs constitute a rare population of cells in vivo, accounting for only 1–2% of total cells in most tissues, we used DCs from murine bone marrow-derived stem cells to study the underlying mechanisms of CA. We first analyzed centrosome numbers before and after stimulation with the bacterial cell wall component lipopolysaccharide (LPS). LPS treatment leads to activation of immature DCs into mature cells, which present antigens via MHC I and II complexes. Similar to dermal DCs, mature bone marrow-derived DCs (BMDCs) showed amplified centrosome numbers while staining negative for pH3 or EdU (Fig. 1d,e and Supplementary Fig. 1D,E). In contrast to mature cells, immature DCs stain positive for pH3 and EdU indicating that these cells are actively proliferating. Yet, pH3-negative immature DCs typically showed only one centrosome (Fig. 1e).

We concluded that in primary DCs, centrosomes overamplify when cells become activated by inflammatory stimuli such as LPS.

**DCs acquire amplified centrosomes via two different pathways**

Two major mechanisms are known to contribute to CA in cancer cells: i) over-duplication of centrioles and ii) accumulation of centrosomes due to mitotic defects. During the cell cycle, centrioles duplicate in S phase, using a semi-conservative replication mechanism. A new daughter centriole forms next to a pre-existing mother centriole, that elongates and matures until mitosis. As a result, each cell contains exactly four centrioles at mitosis: two mature
mother and two daughter centrioles (Supplementary Fig. 2A). Consequently, the mother/daughter ratio is equal to one. During centriole over-duplication, multiple daughter centrioles are generated around a single mother centriole leading to a mother/daughter ratio smaller than one. Mitotic defects such as cytokinesis failure yield in centrosome accumulation and simultaneously polyploidization. As centriole duplication occurs normally, the mother/daughter ratio in polyploid cells still equals one. Thus, determination of DNA content, centriole numbers and their maturation state allows to differentiate between the two pathways of centrosome amplification.

To simultaneously visualize centrosome numbers and DNA content in DCs, we made use of reporter mice, which constitutively express Centrin-2 (CETN2) fused to green fluorescent protein (GFP) as integral part of the centriole. CETN2-GFP expressing cells generally show two fluorescently labeled centrioles for every organ examined. Similar to wildtype BMDCs and dermal DCs, mature CETN2-GFP expressing BMDCs exhibited amplified centrosomes upon LPS treatment (Fig. 2a). Analysis of DNA content revealed that 17% of mature CETN2-GFP expressing BMDCs showed a DNA profile of tetra- or even higher ploidy (Fig. 2b). Fluorescence-activated cell sorting (FACS) based on DNA content allowed us to separate and enrich diploid (2N) from polyploid (≥4N) cells. The majority of ≥4N cells contained two or more centrosomes but stained negative for pH3, confirming again that amplified centrosomes are not a consequence of ongoing cell proliferation (Fig. 2c and Supplementary Fig. 2B). Moreover, ≥4N cells showed abnormal mitotic figures, such as large chromosome bridges and binucleated cells indicating cytokinesis failure (Fig. 2d). Similar mitotic figures were also observed in dermal DCs (Supplementary Fig. 2C). However, a proportion of 2N cells, which did not experience mitotic defects, also displayed amplified centrosomes (Fig. 2e), indicating that mitotic defects are not the only pathway leading to amplified centrosomes in DCs. To investigate whether over-duplication of centrioles occurs in DCs, we stained CETN2-GFP expressing BMDCs against a marker for maternal centrioles. Proteins such as CEP170 localize to subdistal appendages, which are exclusively present on mature mother centrioles (Fig. 2f). We next characterized CEP170 signals in sorted 2N and ≥4N BMDCs. Most ≥4N cells
revealed two CEP170 signals, leading to a mother/daughter ratio of one (Fig. 2g). Similarly, within the 2N fraction, cells with only one centrosome (2N\textsuperscript{one}) displayed a mother/daughter ratio of one, while cells with amplified centrosomes (2N\textsuperscript{amplified}) showed less mother than daughter centrioles and consequently a ratio smaller than one. These data support the hypothesis that over-duplication of centrioles takes place in DCs and together with mitotic defects, account for amplified centrosome numbers.

**PLK2 expression after LPS stimulations leads to centriole over-duplication in DCs**

Over-duplication of centrioles typically results from increased expression of proteins controlling regular centrosome duplication. Two key players of centriole duplication are the polo-like kinases (PLK) 2 and 4\textsuperscript{27,28}. mRNA levels of Plk2 (and to a lesser extent of Plk4) were highly upregulated in DCs upon LPS stimulation (Fig. 3a). To test whether high expression levels of Plk2 and/or Plk4 account for centriole over-duplication in DCs, we generated CRISPR/Cas9 knock-outs in precursor cell lines\textsuperscript{29} and differentiated them into DCs (Fig. 3b,c). While PLK2-deficient cells differentiated into the DC lineage and upregulated MHC II molecules upon LPS stimulation, PLK4-deficiency diminished cellular growth and differentiation, which is why we excluded these cells from further assays. Of note, Plk2\textsuperscript{−/−} and control cells showed the same extent of polyploidization confirming that PLK2-deficiency does not interfere with cell cycle progression in DCs (Fig. 3c). Analysis of centrosome numbers revealed a significant reduction of amplified centrosomes in Plk2\textsuperscript{−/−} cells compared to wildtype controls(Fig. 3d). These results demonstrate that PLK2 plays a major role in centriole over-duplication in mature DCs.

**Amplified centrosomes promote directional locomotion**

Rapid migration of DCs is a prerequisite for initiating adaptive immune responses. Directional locomotion requires actin-rich protrusions at the cell front and acto-myosin mediated retraction of the cell’s rear. In DCs, the centrosome acts as major microtubule organizing center (MTOC), which determines the path of the cell body. Consequently, loss of microtubule (MT) cytoskeleton integrity leads to impaired persistent locomotion along gradients of chemotactic
cues. To investigate whether amplified centrosomes act as functional MTOCs, we first determined the amount of MT filaments emanating from individual centrosomes in migrating BMDCs by high-resolution microscopy. Cells were injected under a block of agarose and exposed to the chemokine CCL19 to attract mature DCs. Under these 2D conditions, cells form a flat lamellipodium at the front, which allows to monitor intracellular structures. Immunostaining against α-tubulin revealed that all centrosomes nucleated MT filaments along the axis of migration, implying that extra centrosomes act as functional MTOCs. Quantification of individual MT filaments showed that cells with amplified centrosomes contained a larger number of total MT filaments within the cytoplasm (Fig. 4a). Measuring intercentrosomal distances between pairs of centrioles unveiled that all centrosomes were located in close proximity to each other (Fig. 4b). A similar phenomenon of centrosomal clustering was recently reported for mitotic cancer cells in order to avoid spindle multipolarity.

To determine the behavior of individual centrosomes and their impact on cell locomotion, we followed the dynamics of CETN2-GFP expressing BMDCs during 2D migration over time (Supplementary Video 1). Similar to fixed samples, extra centrosomes tightly clustered during DC migration towards chemotactic gradients (Fig. 4c and Supplementary Fig. 3A,B). Single cell tracking allowed us to determine migration parameters such as velocity and directional persistence (ratio of displacement to trajectory length) and directly compare the behavior of cells with one and amplified centrosomes. Persistent locomotion of cells containing amplified centrosomes was significantly increased, while migration velocities were similar (Fig. 4d). Importantly, the same phenomenon was also prominent in dermal DCs where cells with amplified centrosomes changed direction less often than cells with only one centrosome (Supplementary Video 2 and Fig. 4e,f). These findings indicate that cells with amplified centrosomes are able to move straight towards the chemokine source without frequently changing the path of locomotion.

To address whether amplified centrosomes are causally linked to enhanced directional locomotion, we removed extra centrosomes during BMDC migration by laser ablation and measured migration velocity and persistence before and after the ablation process. To this
aim, we first determined the settings required for efficient centriole ablation. Maximal Z-projections of CETN2-GFP signals before and after full centrosomal ablation revealed a loss of fluorescence signal at the irradiated region immediately after laser exposure (Fig. 5a). To test whether laser exposure can efficiently disrupt MTOC function, we stained MT filaments after full centrosomal ablation. Following laser exposure, less MT filaments nucleated from ablated centrioles compared to non-treated cells (Fig. 5b). Overall, MT filaments in ablated cells were shorter and showed a disorganized structure with a more bent configuration compared to control cells (Fig. 5c). From that we concluded that our laser set-up allows for efficient destruction of centrosomes and MT organization. To assess the impact of non-centrosome targeted laser exposure on cell behavior, we exposed random areas in close proximity to centrosomes with identical laser settings. Cells experiencing control, non-centrosome targeted ablations, retained their polarized shape and continued to migrate throughout the imaging period. Migration velocity was slightly decreased, whereas directional locomotion was completely unaffected (Supplementary Video 3 and Fig. 5e). Complete ablation of centrosomes decreased migration velocity and strongly impaired persistent movement compared to control ablated cells. These results demonstrate a critical role of the centrosome for directional DC locomotion (Supplementary Video 4 and Fig. 5e). Similar to complete ablations, also partial ablation of extra centrosomes significantly reduced locomotion persistence (Supplementary Video 5 and Fig. 5d,e) confirming a causal relationship between amplified centrosomes and enhanced directional locomotion. Altogether, our data unequivocally demonstrate that amplified centrosomes enhance persistent migration in DCs.

**Enrichment of diploid cells with amplified centrosomes**

Due to the beneficial role of amplified centrosomes during DC migration, we next sought to address the impact of extra centrosomes on adaptive immune responses. In contrast to our single cell approach for determining migration parameters, T cell activation assays require large amounts of cells. Due to the heterogeneity in centrosome numbers in DCs we aimed for
separating BMDC subpopulations of different centrosomal content to directly compare cells with one and amplified centrosomes. CETN2-expressing BMDCs show a prominent centriole signal, while background fluorescence is low indicating that all CETN2-GFP is either incorporated into centrioles or degraded by the proteasome. This motivated us to separate and enrich cells with different numbers of centrosomes according to CETN2-GFP signal intensities. Staining for DNA content allowed us to separate polyploid (≥4N) from diploid cells (2N) as described for Figure 2. The diploid fraction contains cells with amplified (2NCA) and one (2N2C) centrosome(s). As polyploid cells are generally larger than diploid cells due to their surplus of DNA and show gene-dosage dependent expression of a variety of proteins, we focused exclusively on diploid cells containing either one or amplified centrosome(s). To separate those two populations, we used CETN2 signal intensities of polyploid cells for our gating strategy. We reasoned that due to the surplus of centrioles in polyploid cells, the CETN2-GFP signal should be comparable to the signal of 2NCA cells. By overlaying the intensity distribution of polyploid cells with the distribution of diploid cells we determined the gating areas for 2N2C and 2NCA cells, respectively (Fig. 6a). Re-analysis of sorted BMDC subpopulations indicated that CETN2-GFP signals were shifted to higher values in the 2NCA population, while both populations showed a diploid DNA profile (Fig. 6b). After cell sorting we analyzed centrosome numbers by confocal microscopy to assess the purity of individual subpopulations. We were able to enrich 2NCA cells by a factor of at least 1.5, ranging from 8-42% amplified centrosomes within the 2N2C population and 21-70% amplified centrosomes within the 2NCA population. Both diploid subpopulations expressed the same levels of classical DC markers and co-stimulatory molecules, while macrophage/granulocyte markers were absent (Fig. 6c) confirming that both subpopulations consisted of DCs.

**Cells with amplified centrosomes are more potent in eliciting T cell responses**

To induce T cell immunity, DCs present antigenic peptides by MHC complexes on their cell surface. Immature DCs sequester internalized antigens in lysosomes, process them into small peptides and load them on MHC II molecules for presentation to CD4+ T cells. To test
whether cells with amplified centrosomes are more potent in eliciting T cell responses, we used enriched DC subpopulations, pulsed them with the model antigen ovalbumin (OVA), and incubated the cells with OVA-specific CD4⁺ OT-II T cells. The formation of MHCII-OVA_{323-337} peptide complexes was determined by assessing T cell stimulation via Interleukin-2 (IL-2) cytokine secretion by ELISA. Cells with amplified centrosomes displayed a marked increase in CD4⁺ T cell activation compared to cells with only one centrosome at all OVA concentrations tested (Fig. 7a). To directly address T cell activation, we measured T cell expansion by proliferation-mediated dilution of the fluorescent dye carboxyfluorescein succinimidyl ester (CFSE)³⁵. According to elevated IL-2 levels, T cell proliferation was increased after co-culture of CFSE-labeled OT-II T cells with DCs that exhibit extra centrosomes compared to DCs with only one centrosome (Fig. 7b).

To address whether increased T cell activation is a consequence of enhanced intracellular processing of antigens, we directly loaded sorted DC subpopulations with OVA_{329-337} peptide to bypass the processing step. Similar to OVA protein, we detected marked differences in IL-2 secretion and T cell proliferation (Supplementary Fig. 4A,B), suggesting that enhanced T cell activation is not a consequence of improved intracellular antigen processing. Similarly, we excluded T cell co-stimulation via CD40, CD70 and CD86 as reason for optimized T cell activation as cell-surface levels were indistinguishable on both DC subpopulations (Fig. 7c).

**Cells with amplified centrosomes show increased cytokine secretion**

Recent data in macrophages suggest an important role of centrosomes for cytokine production in response to inflammatory stimuli³⁶. Moreover, the extra centrosome-associated secretory pathway (ECASp) has been identified as a distinct secretory phenotype in cells with extra centrosomes³⁷. In DCs, cytokines are stored in lysosomes and released via the secretory pathway³⁸. MTs emanating from centrosomes act as main tracks, which orchestrate long-range intracellular vesicle trafficking of cargos to their destination compartment³⁹. As MT numbers were increased in cells with amplified centrosomes (Fig. 4a), we investigated whether cytokine release is altered in cells with distinct centrosome numbers.
To quantify cytokine levels, we collected supernatants of sorted DC subpopulations and monitored cytokines by antibody arrays and ELISA, respectively. Both approaches revealed that cytokine levels were increased in supernatants harvested from cells with amplified centrosomes. In particular, chemokines which attract and activate naïve T cells and neutrophils such as CCL17, CCL5, IL-6 and CXCL1 were elevated (Fig. 7d and Supplementary Fig. 4C). Except for CCL17, which induces T cell chemotaxis rather than stimulating T cells, mRNA levels of CCL5, IL-6 and CXCL1 were indistinguishable in both diploid DC subpopulations (Fig. 7e), pointing out that the production of these cytokines is not affected by the presence of amplified centrosomes. Instead, our data suggest that rather trafficking and secretion of vesicles containing T cell stimulatory molecules account for enhanced activation of T cells. To further elaborate on this concept, we inhibited intracellular protein transport in sorted DC subpopulations using Monensin and Brefeldin A. Intracellular cytokine staining after blocking protein transport revealed increased levels of IL-6 and CCL5 in cells with amplified centrosome numbers (Fig. 7f). As both cytokines are produced at equal amounts in 2N2C and 2NCA cells, these findings support the notion that enhanced cytokine trafficking accounts for improved T cell activation in cells with amplified centrosomes. Altogether, our findings demonstrate that DCs with amplified centrosomes show enhanced secretion of inflammatory cytokines and optimized T cell responses and revise the current view of amplified centrosomes to be present exclusively under pathological conditions.

Discussion

The presence of amplified centrosomes has been demonstrated to cause aneuploidy and constitutes a well appreciated hallmark of malignancy and cancer development. Several studies emphasis the detrimental effects and consequences of misregulating centrosome numbers: artificial induction of centrosome amplification in mice and flies by overexpression of the centriole replication protein PLK4/SAK is sufficient to initiate spontaneous tumorigenesis in certain tissues. Moreover, CA has been described to confer advantageous features such as enhanced invasion to some tumor cells, thereby promoting metastasis and tumor
 Clinically, centrosome amplification is frequently observed in aggressive tumors and associated with lymph node metastasis and poor patient prognosis. Despite the adverse relationship between extra centrosomes and cell transformation, we provide evidence that amplified centrosomes can be part of regular cell physiology in post-mitotic immune cells. DCs serve as first line defense against invading pathogens and represent the most potent antigen-presenting cells of the innate immune system. Our data demonstrate that DCs upregulate centrosome numbers upon exposure to inflammatory stimuli and subsequent maturation. In contrast to immature DCs, mature cells are non-proliferative and reside in G1 of the cell cycle. However, they often exhibit two or more centrosomes, which we refer to as CA. We further identified two pathways being responsible for excess centrosome numbers in DCs: accumulation of centrosomes due to mitotic defects such as cytokinesis failure and over-duplication of centrioles, which is caused by elevated PLK2 levels upon LPS-treatment.

Functionally, amplified centrosomes ameliorate persistent locomotion along gradients of chemotactic cues and enhance the secretion of cytokines which facilitate T cell activation - two fundamental tasks for a properly operating immune system.

In contrast to other terminally differentiated cells, which often show MT nucleation from non-centrosomal MTOCs such as the Golgi, the nucleus or the cell cortex, DCs use the centrosome as major site for MT nucleation. This centrosome-derived MT array is required for executing DC effector functions: In most tissues, DCs reside in an immature state, unable to activate T cells. However, they are well equipped to capture antigens, which trigger full maturation and antigen presentation via MHC complexes. Mature DCs upregulate MHC class II molecules, which bind to peptides that are derived from proteins processed in the endocytic pathway. Fast transport of MHC class II containing vesicles to the cell surface is accomplished along MT filaments. During DC migration, MTs control pathfinding and coordination of multiple protrusions in complex 3D environments as well as maintenance of cell coherence. Upon arrival in lymph nodes, DCs and T lymphocytes engage with each other at a structure termed immunological synapse, which allows transmission of different
types of signals. The DC cytoskeleton undergoes major spatial redistributions during immune synapse formation, whereby the centrosome reorients toward the DC-T cell interface leading to polarized transport of soluble mediators of T cell activation along MT filaments. Due to these multifaceted functional tasks, DCs need to be able to rapidly re-organize their MT array. Amplifying centrosome numbers could be one strategy which allows the cells to adapt to various different environments such as the interstitium or the lymph node and fulfill distinct tasks depending on their location.

One question which arises, is why DCs can tolerate a surplus of centrosomes without transforming into tumor cells. In contrast to other tissues, where cells are actively proliferating, amplified centrosomes might be tolerated in cells, which permanently halt or exit the cell cycle. Several studies addressed the lifetime and cell cycle status of DC subsets: while DC precursor cells retain proliferative capacity, only few tissue-resident cells can be found in S/G2/M phase indicating that these cells are G1 arrested. Given the short lifetime of tissue-resident DCs of three to six days, the likelihood that these cells undergo malignant transformation is very low. Likewise, constitutive PLK4 overexpression in mice led to the formation of B and T cell lymphomas as well as squamous cell carcinomas, which are all cancers originating from tissues with highly proliferative cells.

To our knowledge, this is the first demonstration of a beneficial physiological role of amplified centrosomes. Besides DCs, hepatocytes carry amplified centrosomes as a consequence of cytokinesis failure and liver polyploidization. Interestingly, hepatocytes are able to directly present antigens and activate CD8+ T cells. Future studies have yet to address whether extra centrosomes impact on antigen presentation or T cell activation in hepatocytes. Of note, PLK4 overexpression in B lymphoma cells, which naturally do not show amplified centrosomes, led to an increased antigen presentation capacity suggesting that multiple MTOCs promote antigen presentation.

Altogether, our results challenge the prevailing view of amplified centrosomes to act either as barrier against differentiation or posing a risk by driving tumorigenesis. Instead, we provide
evidence that amplified centrosomes promote the ability of leukocytes to enhance and regulate adaptive immune responses.

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Author contributions
A.W., M.H., S.E. and E.K. performed experiments. E.K. designed and supervised the research. E.M, A.S, W.K. and S.B. gave technical support and conceptual advice. R.H. developed tools for image analysis and T.Q. helped with image analysis. E.K wrote the manuscript. All authors discussed the results and implications and commented on the manuscript at all stages.

Competing financial interests
The authors declare no competing financial interests.

Data availability
Data that support the findings of this study are available within the article and its supplementary information or on request from the corresponding author.

References
1. Bornens, M. The Centrosome in Cells and Organisms. Science 335, 422–426 (2012).
2. Mennella, V., Agard, D. A., Huang, B. & Pelletier, L. Amorphous no more: subdiffraction view of the pericentriolar material architecture. *Trends in Cell Biology* **24**, 188–197 (2014).

3. Banterle, N. & Gönczy, P. Centriole Biogenesis: From Identifying the Characters to Understanding the Plot. *Annu. Rev. Cell Dev. Biol.* **33**, 23–49 (2017).

4. Chan, J. Y. A clinical overview of centrosome amplification in human cancers. *Int. J. Biol. Sci.* **7**, 1122–1144 (2011).

5. Nigg, E. A. & Holland, A. J. Once and only once: mechanisms of centriole duplication and their deregulation in disease. *Nat. Rev. Mol. Cell Biol.* **19**, 1–16 (2018).

6. Ganem, N. J., Godinho, S. A. & Pellman, D. A mechanism linking extra centrosomes to chromosomal instability. *Nature* **460**, 278–282 (2009).

7. Crasta, K. *et al.* DNA breaks and chromosome pulverization from errors in mitosis. *Nature* **482**, 53–58 (2012).

8. Levine, M. S. *et al.* Centrosome Amplification Is Sufficient to Promote Spontaneous Tumorigenesis in Mammals. *Developmental Cell* **40**, 313–322.e5 (2017).

9. Banchereau, J. & Steinman, R. M. Dendritic cells and the control of immunity. *Nature* **392**, 245–252 (1998).

10. Banchereau, J. *et al.* Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **18**, 767–811 (2000).

11. Lämmermann, T. *et al.* Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature* **453**, 51–55 (2008).

12. Renkawitz, J. *et al.* Adaptive force transmission in amoeboid cell migration. *Nature Cell Biology* **11**, 1438–1443 (2009).

13. Renkawitz, J. *et al.* Nuclear positioning facilitates amoeboid migration along the path of least resistance. *Nature* **42**, 7 (2019).
14. Madsen, C. D. & Sahai, E. Cancer Dissemination—Lessons from Leukocytes. *Developmental Cell* **19**, 13–26 (2010).

15. Stoitzner, P., Romani, N., McLellan, A. D., Tripp, C. H. & Ebner, S. in *Antigen Processing* **595**, 235–248 (Humana Press, 2009).

16. Piperno, G., biology, M. F. T. J. O. C. 1985. Monoclonal antibodies specific for an acetylated form of alpha-tubulin recognize the antigen in cilia and flagella from a variety of organisms. *rupress.org* **101**, 2085–2094 (1985).

17. PURE, E. *et al.* Antigen Processing by Epidermal Langerhans Cells Correlates with the Level of Biosynthesis of Major Histocompatibility Complex Class-Ii Molecules and Expression of Invariant Chain. *J Exp Med* **172**, 1459–1469 (1990).

18. Cella, M., Engering, A., Pinet, V., Pieters, J. & Lanzavecchia, A. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature* **388**, 782–787 (1997).

19. Nigg, E. A. Centrosome aberrations: Cause or consequence of cancer progression? *Nat Rev Cancer* **2**, 815–825 (2002).

20. Duensing, S. A tentative classification of centrosome abnormalities in cancer. *Cell Biology International* **29**, 352–359 (2005).

21. Robbins, E., Jentzsch, G. & Micali, A. The centriole cycle in synchronized HeLa cells. *The Journal of Cell Biology* **36**, 329–339 (1968).

22. Vorobjev, I. A. & Chentsov YuS. Centrioles in the cell cycle. I. Epithelial cells. *The Journal of Cell Biology* **93**, 938–949 (1982).

23. Cosenza, M. R. & Krämer, A. Centrosome amplification, chromosomal instability and cancer: mechanistic, clinical and therapeutic issues. *Chromosome Res* **24**, 105–126 (2015).

24. Duensing, A., Chin, A., Wang, L., Kuan, S.-F. & Duensing, S. Analysis of centrosome overduplication in correlation to cell division errors in high-risk
human papillomavirus (HPV)-associated anal neoplasms. *Virology* **372**, 157–164 (2008).

25. Higginbotham, H., Bielas, S., Tanaka, T. & Gleeson, J. G. Transgenic mouse line with green-fluorescent protein-labeled Centrin 2 allows visualization of the centrosome in living cells. *Transgenic Res.* **13**, 155–164 (2004).

26. Guarguaglini, G. *et al.* The forkhead-associated domain protein Cep170 interacts with Polo-like kinase 1 and serves as a marker for mature centrioles. *Molecular Biology of the Cell* **16**, 1095–1107 (2005).

27. Cizmecioglu, O., Warnke, S., Arnold, M., Duensing, S. & Hoffmann, I. Plk2 regulated centriole duplication is dependent on its localization to the centrosome and a functional polo-box domain. *Cell Cycle* **7**, 3548–3555 (2014).

28. Bettencourt-Dias, M. *et al.* SAK/PLK4 Is Required for Centriole Duplication and Flagella Development. *Current Biology* **15**, 2199–2207 (2005).

29. Leithner, A. *et al.* Fast and efficient genetic engineering of hematopoietic precursor cells for the study of dendritic cell migration. *Eur. J. Immunol.* **392**, 245–4 (2018).

30. Kopf, A. *et al.* Microtubules control cellular shape and coherence in amoeboid migrating cells. *The Journal of Cell Biology* **219**, 193–24 (2020).

31. Quintyne, N. J., Reing, J. E., Hoffelder, D. R., Gollin, S. M. & Saunders, W. S. Spindle multipolarity is prevented by centrosomal clustering. *Science* **307**, 127–129 (2005).

32. Øvrebø, J. I. & Edgar, B. A. Polyploidy in tissue homeostasis and regeneration. *Development* **145**, dev156034–16 (2018).

33. Wubbolts, R. Direct vesicular transport of MHC class II molecules from lysosomal structures to the cell surface. *The Journal of Cell Biology* **135**, 611–622 (1996).

34. Turley, S. J. *et al.* Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* **288**, 522–527 (2000).
35. Quah, B. J. C., Warren, H. S. & Parish, C. R. Monitoring lymphocyte proliferation in vitro and in vivo with the intracellular fluorescent dye carboxyfluorescein diacetate succinimidyl ester. Nat Protoc 2, 2049–2056 (2007).
36. Vertii, A. et al. The Centrosome Undergoes Plk1-Independent Interphase Maturation during Inflammation and Mediates Cytokine Release. Developmental Cell 37, 377–386 (2016).
37. Amandis, T. et al. Oxidative Stress in Cells with Extra Centrosomes Drives Non-Cell-Autonomous Invasion. Developmental Cell 47, 409–424.e9 (2018).
38. Blott, E. J. & Griffiths, G. M. Secretory lysosomes. Nat. Rev. Mol. Cell Biol. 3, 122–131 (2002).
39. Fourriere, L., Jimenez, A. J., Perez, F. & Boncompain, G. The role of microtubules in secretory protein transport. Journal of Cell Science 133, jcs237016–10 (2020).
40. Ritzman, A. M. et al. The chemokine receptor CXCR2 ligand KC (CXCL1) mediates neutrophil recruitment and is critical for development of experimental Lyme arthritis and carditis. IAI 78, 4593–4600 (2010).
41. Fülle, L. et al. RNA Aptamers Recognizing Murine CCL17 Inhibit T Cell Chemotaxis and Reduce Contact Hypersensitivity In Vivo. Molecular Therapy 1–10 (2017). doi:10.1016/j.ymthe.2017.10.005
42. Griffith, J. W., Sokol, C. L. & Luster, A. D. Chemokines and Chemokine Receptors: Positioning Cells for Host Defense and Immunity. Annu. Rev. Immunol. 32, 659–702 (2014).
43. Dienz, O. & Rincon, M. The effects of IL-6 on CD4 T cell responses. Clinical Immunology 130, 27–33 (2009).
44. Imai, T. et al. Molecular Cloning of a Novel T Cell-directed CC Chemokine Expressed in Thymus by Signal Sequence Trap Using Epstein-Barr Virus Vector. J. Biol. Chem. 271, 21514–21521 (1996).
45. Basto, R. et al. Centrosome Amplification Can Initiate Tumorigenesis in Flies. Cell 133, 1032–1042 (2008).
46. Godinho, S. A. et al. Oncogene-like induction of cellular invasion from centrosome amplification. Nature 510, 167–171 (2014).
47. Muroyama, A. & Lechler, T. Microtubule organization, dynamics and functions in differentiated cells. Development 144, 3012–3021 (2017).
48. Neefjes, J., Jongsma, M. L. M., Paul, P. & Bakke, O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol 11, 823–836 (2011).
49. Wubbolts, R. et al. Opposing motor activities of dynein and kinesin determine retention and transport of MHC class II-containing compartments. Journal of Cell Science 112 (Pt 6), 785–795 (1999).
50. Pulecio, J. et al. Cdc42-mediated MTOC polarization in dendritic cells controls targeted delivery of cytokines at the immune synapse. J Exp Med 207, 2719–2732 (2010).
51. Cabeza-Cabreroiz, M. et al. Tissue clonality of dendritic cell subsets and emergency DCpoiesis revealed by multicolor fate mapping of DC progenitors. Sci Immunol 4, eaaw1941 (2019).
52. Liu, K. et al. In vivo analysis of dendritic cell development and homeostasis. Science 324, 392–397 (2009).
53. Gentric, G., Desdouets, C. & Celton-Morizur, S. Hepatocytes Polyploidization and Cell Cycle Control in Liver Physiopathology. International Journal of Hepatology 2012, 1–8 (2012).
54. Grakoui, A. & Crispe, I. N. Presentation of hepatocellular antigens. Cell. Mol. Immunol. 13, 293–300 (2016).
55. Yuseff, M.-I. et al. Polarized Secretion of Lysosomes at the B Cell Synapse Couples Antigen Extraction to Processing and Presentation. Immunity 35, 361–374 (2011).
Figure 1

(a) Immature BMDCs

(b) Mature BMDCs

(d) Immature BMDCs

(e) Mature BMDCs

Legend:
- **ac-tubulin**
- **γ-tubulin**
- **pH3**
- **EdU**
- **Hoechst**
- **DAPI**

Graphs show the percentage of cell number (% of dermal DCs) vs. centrosome number for immature and mature BMDCs.
**Figure 1** DCs amplify centrosome numbers upon activation with inflammatory stimuli. (a) Immunostaining of dermal DCs isolated from wildtype ear explants. Merged and individual channels of ac-tubulin (red)/DAPI (blue) and γ-tubulin (green) are shown. White arrows point to amplified centrosomes. Scale bar, 5 µm. (b) Quantification of centrosome numbers in dermal DCs isolated from split ears. Graph displays mean values ± s.d. of 5 independent experiments. 

\( N = 96/90/109/131/108 \) cells, which were pooled from three different mice for each experiment.

(c) EdU incorporation and pH3 staining in dermal DCs isolated from split ears. Left: immunostaining of dermal DCs against pH3 (red) and EdU (green)- incorporation. Scale bar, 10 µm. Right: quantification of EdU-positive (EdU⁺) and pH3-positive (pH3⁺) dermal DCs. Graphs display mean values ± s.d. of 6 independent experiments. 

\( N = 84/119/316/181/191/183 \) cells, which were pooled from three different mice for each experiment. (d) Immunostaining of wildtype mature (upper panel) and immature (lower panel) BMDCs. Merged and individual channels of ac-tubulin (red)/DAPI (blue) and γ-tubulin (green) are shown. White arrows point to amplified centrosomes. Scale bars, 5 µm. (e) Quantification of centrosome numbers in immature and mature BMDCs. Left: immunostaining of immature and mature BMDCs against ac-tubulin (red) and pH3 (cyan). White arrows point to amplified centrosomes. Scale bars, 5 µm. Right: quantification of centrosome numbers in pH3-negative BMDCs. ****, \( P < 0.0001 \); **, \( P = 0.002 \) (two-tailed, unpaired Student’s \( t \)-test). Graph displays mean values ± s.d. of 7 independent experiments. 

\( N = 134/98/158/93/124/344/274 \) cells (immature) and \( N = 141/203/158/127/128/200/175 \) cells (mature).
Figure 2 Mitotic defects and over-duplication of centrioles account for amplified centrosomes in mature DCs. (a) Quantification of centrosome numbers in mature CETN2-GFP expressing BMDCs. Left: immunostaining against γ-tubulin (red) to confirm functional MTOCs. Graph shows mean values ± s.d. of 8 independent experiments with \( N = 261/248/305/298/180/150/258/152 \) cells analyzed per experiment. Scale bar, 5 \( \mu \)m. (b) Left: representative histogram of DNA content distribution of CETN2-GFP expressing BMDCs. Right: quantification of percentage of polyploid BMDCs according to DNA content. Graph displays mean values ± s.d. of 22 independent experiments. \( N = 10,000 \) cells per experiment. (c) Enrichment of polyploid BMDCs. Left panel: representative picture of enriched polyploid (≥4N) CETN2-GFP expressing BMDCs. Scale bar, 5 \( \mu \)m. Right: quantification of centrosome numbers in sorted ≥4N CETN2-GFP expressing BMDCs. Graph displays mean values ± s.d. \( N = 20 \) with at least 141 cells analyzed per experiment. (d) Abnormal mitotic figures of enriched ≥4N CETN2-GFP expressing BMDCs. Left: bi-nucleated cell (upper picture) and cell with large chromosome bridges during anaphase (lower picture). Dotted lines indicate cell outline. Scale bars, 5 \( \mu \)m. Right: quantification of percentage of polyploid cells showing one nucleus (mono), two (bi-) nuclei and chromosome bridges. Graph displays mean values ± s.d. of 8 independent experiments. \( N = 204/91/120/87/77/91/107 \) cells. (e) Enrichment of diploid (2N) BMDCs. Left: representative picture of enriched 2N CETN2-GFP expressing BMDCs. Scale bar, 5 \( \mu \)m. Right: quantification of centrosome numbers in sorted 2N CETN2-GFP expressing BMDCs. Graph displays mean values ± s.d. \( N = 17 \) with at least 144 cells analyzed per experiment. (f) Visualization of mother and daughter centrioles in unsorted BMDCs. Immunostaining of CEP170 (red) in CETN2-GFP expressing BMDCs. Scale bars, 2 \( \mu \)m. (g) Quantification of mother/daughter ratio in enriched ≥4N and 2N CETN2-GFP expressing BMDCs. A mother/daughter ratio <1 in diploid cells with amplified centrosomes (\( 2N^{\text{amplified}} \)) indicates over-duplication of centrioles. Graph displays mean values ± s.d. of two to three independent experiments. \( N = 61/52/54 \) (4N), 50/57 \( 2N^{\text{amplified}} \), 62/71 \( 2N^{\text{one}} \).
Figure 3

(a) Relative gene expression over time after LPS stimulation.

(b) CETN2-GFP HOXB8-derived DCs measured for CETN2- and Plk2-mediated centrosome number.

(c) CETN2-GFP HOXB8-derived DCs measured for CETN2- and Plk2-mediated centrosome number.

(d) CETN2-GFP HOXB8-derived DCs measured for CETN2- and Plk2-mediated centrosome number.
**Figure 3** PLK2 induction after LPS-stimulation leads to over-duplication of centrioles. (a) mRNA expression levels of *Plk2* (magenta) and *Plk4* (grey) after LPS stimulation of immature wildtype BMDCs. mRNA levels were normalized to the expression of TATA-box binding protein. Graph displays mean values ± s.d. of three independent experiments. (b) PLK2 depletion in CETN2-GFP HOXB8-derived DCs. Immunoblotting against PLK2 in control (sc., scrambled) and *Plk2*<sup>−/−</sup> (sg_1 and sg_2) DCs. Note that only single guide 1 (sg_1) and not sg_2 yields to efficient knock-out of PLK2. (c) Differentiation and maturation of HOXB8-derived *Plk2*<sup>−/−</sup> and control DCs. Mature DCs were identified as MHC II<sup>+</sup>/CD11c<sup>+</sup> cells and further analyzed for DNA content (lower panels) and DC-specific cell-surface marker (CD135, CD86, CCR7; right panels). Unstained samples served as control and were included as light grey filled line. Staining for DC marker has been conducted in parallel with PE-conjugated antibodies. Representative histograms of one out of three independent experiments are shown. N = 10,000 cells per experiment. (d) Quantification of centrosome numbers in *Plk2*<sup>−/−</sup> and control CETN2-GFP-expressing HOXB8-derived DCs. **, *P* = 0.0095 (two-tailed, unpaired Student’s *t*-test). Graph displays mean values ± s.d. of three independent experiments. N = 315/263/155 cells (scramble) and 295/268/130 cells (*Plk2*<sup>−/−</sup>).
Figure 4

(a) 

(b) 

(c) Maximal projection

(d) 

(e) Maximal projection

(f)
**Figure 4** Extra centrosomes cluster during migration and promote directional locomotion. (a) Mature BMDCs migrating in 2D under agarose. Left: CETN2-GFP expressing BMDCs were fixed during migration and immunostained against MT filaments (α-tubulin, black) and γ-tubulin (cyan). Centrioles are pseudo-color coded in red. Inset: magnification of MT-nucleating centrosomes. Arrows indicate pairs of centrioles. Scale bar, 10 μm. Right: quantification of MT filaments emanating from centrosomes in cells with one (blue) or amplified (red) centrosomes. Images were post-treated by deconvolution. Graph displays mean values ± s.d. N = 52 cells (one centrosome) and 53 cells (amplified centrosomes) pooled from two independent experiments. ****, P < 0.0001 (two-tailed, unpaired Student’s t-test). (b) Left: schematic illustration of measuring intra- and intercentrosomal distances in cells with amplified centrosomes. Right: quantification of intra- and intercentrosomal distances in fixed CETN2-GFP expressing BMDCs with amplified centrosomes. Graph displays mean values ± s.d. N = 50 cells pooled from two independent experiments. (c) Left: maximal Z-projection of a migrating CETN2-GFP expressing cell with amplified centrosomes. Scale bar, 10 μm. Right: quantification of intra- and intercentrosomal distances of amplified centrosomes during migration in one representative cell over time. (d) Quantification of migration velocity (left) and persistence (right) of CETN2-GFP expressing BMDCs migrating under agarose. Graphs display mean values ± s.d. N = 32 cells (one centrosome) and 45 cells (amplified centrosomes) pooled from 5 independent experiments. *, P = 0.033 (Mann-Whitney test). n.s., non-significant. (e) Maximal Z-projection of migrating CETN2-GFP expressing dermal DCs with one (left) and amplified (right) centrosomes. Scale bar, 10 μm. (f) Quantification of migration velocity (left) and persistence (right) of dermal CETN2-GFP expressing DCs migrating under agarose. Graphs display mean values ± s.d. N = 30 cells (one centrosome) and 22 cells (amplified centrosomes) pooled from 4 independent experiments. **, P = 0.0014, n.s., non-significant (two-tailed, unpaired Student’s t-test (velocity) and two-tailed, unpaired Student’s t-test with Welch’s correction (persistence)).
Figure 5

(a) Pre-ablation vs. post-ablation

(b) Control vs. full ablation

(c) Integrated fluorescence density [AU]

(d) Maximal Z-projection

(e) Change in velocity [%] vs. centrosomal ablation

Change in persistence [%] vs. centrosomal ablation
**Figure 5** Laser ablation of centrosomes in CETN2-GFP expressing BMDCs. (a) Left: maximal Z-projection of two representative cells before and after laser ablation. Right: quantification of integrated CETN2-GFP signal densities in defined regions of interest (ROIs) drawn around centrosomes. Graph displays pairs of cells before (pre-) and after (post-) ablation. Cell pairs with one centrosome are depicted in grey, cells with amplified centrosomes in orange. $N = 5/5$ cells (one/amplified centrosomes). ****, $P < 0.0001$. (b) Left: immunostaining of MTs in cells migrating under agarose after complete centrosome and control ablation. Cells have been fixed immediately after the ablation process. Individual channels of $\alpha$-tubulin (white)/DAPI (blue) and CETN2-GFP (green) are shown. Images were post-treated by deconvolution. Red circles indicate ablated areas. White dotted lines depict cell outline. Scale bars, 10 $\mu$m. Right: Quantification of MT filament numbers nucleating in non- and fully ablated cells with one (grey) and amplified (orange) centrosomes. Graph displays mean values. Each data point represents one cell. (c) Quantification of MT length and straightness (end-to-end distance/total length of MT filament) in non- and fully ablated cells. Graphs show mean values ± s.d. $N = 413/205$ filaments traced from 9 or 6 different cells (non/full ablation). ****, $P < 0.0001$ (Mann-Whitney test). (d) Maximal Z-projection of one representative cell before and after partial laser ablation. Inset: magnification of centrosome tracks. Red arrow indicates time point of extra centrosome ablation. Scale bars, 10 $\mu$m. (e) Quantification of migration velocity (upper panel) and persistence (lower panel) before and after laser ablation. Graphs display changes as mean values ± s.d. $N = 14/19/9$ cells (non/complete/partial ablation) pooled from 6 independent experiments. *, $P = 0.015$ and **, $P = 0.009$ (Kruskal-Wallis with Dunn’s test). n.s., non-significant.
Figure 6 Enrichment of diploid cells with amplified centrosomes. (a) Sorting strategy of CETN2-GFP expressing BMDCs to separate polyploid cells (≥4N), diploid cells with amplified centrosomes (2NCA) and diploid cells with only one centrosome (2N2C). CD11c+/MHC II+ cells were first analyzed for DNA content and distinguished between ≥4N and 2N cells. CETN2-GFP signal intensities of 2N (green) and ≥4N (grey) cells were combined to identify 2NCA (red) and 2N2C (blue) cells. (b) Left: sorted 2N2C and 2NCA cells were re-analyzed for CETN2-GFP signal intensities and DNA content. Centrosome numbers in 2N2C and 2NCA cells were determined by confocal microscopy. Right: graph displays the ratio of sorted cells with amplified centrosomes (2NCA) vs. cells with one centrosome (2N2C) for each experiment performed. (c) Cell-surface expression of DC, monocyte, macrophage and granulocyte markers on enriched 2N2C and 2NCA subpopulations. Left panel: MHC II+/CD11c+ cells were analyzed for myeloid markers. Unstained controls were included in the histograms as grey filled lines. Representative histograms of one out of two independent experiments are shown. Graph below shows quantification of cell-surface markers indicated as mean fluorescence intensity (MFI). Graphs display mean values ± s.d. from at least two independent experiments.
Figure 7 Cells with amplified centrosomes show optimized T cell activation and enhanced secretion of cytokines. (a) Quantification of IL-2 levels after co-culture of OVA-pulsed diploid DC subpopulations with OT-II CD4+ T cells. Graph displays mean values ± s.d. of one representative out of four experiments. Data points represent technical replicates. 2N2C, cells enriched for one centrosome (blue); 2NCA, cells enriched for amplified centrosomes (red). (b) Representative histogram of T cell proliferation of co-cultured OT-II CD4+ T cells with enriched BMDC subpopulations pulsed with OVA. Unprimed T cells (-OVA) served as negative control and are displayed as grey filled line. Graph below shows quantification of the percentage of OT-II CD4+ T cells that divided after co-culture with OVA-pulsed enriched DC subpopulations and displays mean values ± s.d. of one representative out of four experiments. Data points represent technical replicates. (c) Quantification of cell-surface expression levels of co-stimulatory molecules on DC subpopulations. Graph below displays mean values of mean fluorescence intensities (MFI) ± s.d. Each data point represents one independent experiment. *, P = 0.027 (all two-tailed, paired Student’s t-test), n.s. non-significant. (d) Levels of indicated cytokines in conditioned medium harvested from sorted DC subpopulations were quantified by ELISA. Graph shows mean values ± s.d. of at least 9 independent experiments. **, P = 0.002 (CCL17) (Wilcoxon test); ***, P = 0.0005 (IL-6); ****, P < 0.0001 (CCL5) and **, P = 0.0057 (CXCL1) (all two-tailed, paired Student’s t-test). (e) mRNA levels of indicated cytokines in DC subpopulations. Graph displays mean values ± s.d. of at least 3 independent experiments. *, P = 0.012 (two-tailed, paired Student’s t-test), n.s., non-significant. (f) Intracellular staining of cytokines upon blocking protein transport. After cell sorting, DC subpopulations were treated with Monensin and Brefeldin A to inhibit cytokine secretion and intracellular levels of IL-6 and CCL5 determined by flow cytometry. Graph displays mean values of mean fluorescence intensities (MFI) ± s.d. Each data point represents one independent experiment. ****, P < 0.0001 (CCL5), **, P = 0.002 (IL-6) (both two-tailed, paired Student’s t-test).