Long-range migration of centrioles to the apical surface of the olfactory epithelium

Kaitlin Ching^{1,2,*}, Jennifer T. Wang^{1,*}, Tim Stearns^{1,3,*}

¹Department of Biology, Stanford University, Stanford, CA 94305, USA

²Department of Molecular, Cell, and Developmental Biology, University of California, Los Angeles, CA 90095, USA

³Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA

*Correspondence: stearns@stanford.edu

[†]Authors contributed equally to this work

Abstract

Olfactory sensory neurons (OSNs) in vertebrates detect odorants using multiple cilia, which protrude from the end of the dendrite and require centrioles for their formation. In mouse olfactory epithelium, the centrioles originate in progenitor cells near the basal lamina, often 50 to 100 μm from the apical surface. It is unknown how centrioles traverse this distance or mature to form cilia. Using high-resolution expansion microscopy, we found that centrioles migrate together, with multiple centrioles per group and multiple groups per OSN, during dendrite outgrowth. Centrioles were found by live imaging to migrate slowly, with a maximum rate of 0.18 $\mu\text{m}/\text{min}$. Centrioles in migrating groups were associated with microtubule nucleation factors, but acquired rootletin and appendages only in mature OSNs. The parental centriole had preexisting appendages, formed a single cilium prior to other centrioles, and retained its unique appendage configuration in the mature OSN. We developed an air-liquid interface explant culture system for OSNs and used it to show that centriole migration can be perturbed *ex vivo* by stabilizing microtubules. We consider these results in the context of a comprehensive model for centriole formation, migration, and maturation in this important sensory cell type.

keywords: centriole, centrosome, cilium, dendrite, expansion microscopy, migration, olfaction, OSN, sensory neuron

Introduction

Chemosensation in many animals is mediated by ciliated neurons that have a sensory cilium at the end of a dendrite, positioned to sense the external environment. In vertebrates, olfactory sensory neurons (OSNs), found within the olfactory epithelium, have multiple cilia in a dendritic knob exposed to odorants (Cuschieri & Bannister, 1975, Menco 1997, McEwen et al., 2008, Oberland & Neuhaus, 2014). The structure and function of these cilia are critical to olfaction.

They provide a specialized membrane area for signaling via G protein coupled olfactory receptors and disruption of cilium structure and function can result in anosmia (Jenkins et al., 2009, Kulaga et al., 2004, McEwen et al., 2007). The microtubule structure of each cilium is templated by a centriole at its base, which is also referred to as a basal body. Centrioles in many cell types also recruit pericentriolar material (PCM) to form the centrosome, the major microtubule organizing center.

To create the requisite number of olfactory cilia, cells must amplify centrioles from an initial pair. We previously demonstrated that centriole amplification occurs in mitotically active progenitor cells located near the basal lamina of the olfactory epithelium in mice (Ching & Stearns, 2020). This is unique among cells with multiple cilia, as epithelial cells with multiple motile cilia in the trachea, oviduct, and brain ependyma (multiple motile cilia cells, MMCCs) amplify centrioles only after mitosis is complete.

Progenitor cells generate OSNs during embryonic development and throughout adult life, continuing to generate new ciliated olfactory neurons long after the epithelium has been established (Moulton & Beidler, 1967; Mulvaney & Heist, 1971). As a daughter cell from the progenitor division differentiates into an immature OSN, it extends projections in opposite directions: a dendrite toward the apical surface to detect odorants, and an axon through the basal lamina toward the olfactory bulb. *In vivo* fate mapping in mouse has shown that this process begins one day after the progenitor's last cell cycle (Rodriguez-Gil et al., 2015). During neurite extension, the cell body of the neuron remains close to the basal lamina (**Fig 1A**) (Rodriguez-Gil et al., 2015).

Centrioles are amplified in the cell body and must migrate to the apical surface, traversing a distance of 50 to 100 μm or more (**Fig 1A**). Using transmission electron microscopy, migrating centrioles have been previously observed in mammalian olfactory sensory neurons (Heist & Mulvaney, 1968; Mulvaney & Heist, 1971). Within the thin, 1- to 2- μm -wide dendrites, centrioles were found to be grouped, with more groups being found in the most apical portion of the epithelium (Mulvaney & Heist, 1971). Six days after the progenitor cell's last cell cycle, immature OSNs differentiate into mature OSNs and express markers of maturation, such as olfactory marker protein (OMP) (Rodriguez-Gil et al., 2015). Upon maturation, they form multiple cilia at the apical end of the dendrite, and their nuclei migrate towards the apical surface in the days to weeks that follow (Rodriguez-Gil et al., 2015).

For OSNs, centrioles must be moved to the apical surface of the olfactory epithelium to assemble sensory cilia where they will have access to odorants. Many cell types across a variety of organisms are known to relocate or reorient their centrioles, including ciliating vertebrate cells (Dawe et al., 2007), *Drosophila* nurse cells in the germarium (Mahowald & Strassheim, 1970; Bolivar et al., 2001), and macrociliary cells in the comb jelly *Beroë* (Tamm & Tamm, 1988). Perhaps the most relevant example is the *C. elegans* PQR neuron, in which a single centriole migrates approximately 5 μ m along the dendrite by the activity of the minus-end directed microtubule motor, dynein (Li et al., 2017). In contrast to the PQR neuron, vertebrate OSNs each contain multiple centrioles, move those centrioles over a 10-fold greater distance, and develop throughout the life of the animal, which frequently requires centrioles to migrate through an established epithelium. Despite these biologically interesting challenges, mechanisms of movement and the molecular composition of migrating OSN centrioles remain unclear.

Here, we develop new techniques for imaging and manipulating OSNs and use these to explore the mechanisms by which multiple centrioles migrate and become competent to form the multiple cilia that are important for the sensory function of OSNs. Our findings inform our understanding of OSN cell biology, as well as long-range organelle movement more broadly.

Results

Identification of migrating and mature centrioles in the olfactory epithelium.

The olfactory epithelium is composed of multiple cell types, including olfactory sensory neurons (OSNs) and non-neuronal cell types known as sustentacular cells (**Fig 1A**). To investigate the migration of OSN centrioles, we observed their localization in fixed olfactory epithelium taken from mice expressing eGFP-centrin2, a component of centrioles (Bangs et al., 2015). OSNs are generated from progenitors throughout development and adult life, and we chose to use adult mice in these experiments to enable observation of centriole migration across the full depth of the mature olfactory epithelium. *En face* imaging of the apical surface allowed us to identify dendritic knobs ensheathed in sustentacular cells with borders rich in filamentous actin (**Fig 1B, 1C**) (Liang, 2018). In side views of the olfactory epithelium, migrating centrioles were identified

as elongated clusters of puncta, in accordance with previous reports (**Fig 1D**) (Heist & Mulvaney, 1968; Klimenkov et al., 2018; Mulvaney & Heist, 1971, Ying et al., 2014).

To better visualize centriole migration in OSNs at higher resolution we applied expansion microscopy (Gambarotto et al., 2019, Sahabandu et al., 2019) to sections of adult olfactory epithelium. We were able to reproducibly expand these samples approximately 4-fold, and could resolve olfactory cilia in mature OSNs, as well as migrating centrioles in immature OSNs (**Fig 1E, 1F**) by staining for acetylated tubulin, which marks centrioles, cilia, and, more faintly, the microtubules of the dendrite. *En face* imaging of the dendritic knob using expansion microscopy identified groups of centrioles at the apical surface (**Fig 1E**). Side views of the olfactory epithelium identified groups of migrating centrioles positioned below the apical surface (**Fig 1F**).

With the increased resolution afforded by expansion microscopy, we counted centriole number in progenitor cells and mature OSNs by staining for acetylated tubulin and using a semi-automated volume segmentation and counting pipeline (Fig 1 - Supplementary Fig 1A, B). We found that basally-positioned progenitor cells with amplified centrioles harbored, on average, 26.40 centrioles (SEM = 2.234; standard deviation = 9.992; n = 20 cells from 2 mice, Fig 1 - Supplementary Fig 1C). In mature OSNs that had completed centriole migration with centrioles docked at the dendritic knob, each cell had, on average, 36.46 centrioles (SEM = 1.665; standard deviation = 8.156, n = 24 dendritic knobs from 2 mice, Fig 1 - Supplementary Fig 1C). These results suggest that progenitor cells form the majority of centrioles present in the dendritic knob of mature OSNs, in keeping with our previously published work (Ching & Stearns 2020).

To determine whether all of the centrioles at mature dendritic knobs nucleate cilia, we counted the number of cilia found in each dendritic knob (Figure 1 - Supplementary Fig 1B, 1D). We found that, on average, 85% of centrioles at a dendritic knob were associated with cilia (SEM=2.47; standard deviation = 12.10), with an average of 30.54 cilia per knob (SEM=1.455; standard deviation = 7.126). It is unclear whether the 15% of centrioles not associated with a cilium previously had a cilium, will eventually make a cilium, or whether they are excess to an independent process that determines cilium number.

Groups of OSN centrioles migrate from the basal lamina to the apical surface in tandem with dendrite outgrowth.

The dendrites of mature OSNs extend from the cell body in the basal region of the olfactory epithelium to the ciliated dendritic knobs at the apical surface. In our expansion microscopy images, we noticed that centrioles within dendrites were often grouped together (**Fig 1F**). We first tested whether multiple groups of centrioles could be present within a single dendrite, as suggested by previous TEM results (Heist & Mulvaney, 1968; Mulvaney & Heist, 1971). We imaged immature OSNs using expansion microscopy, through the entire thickness of the dendrite. We found that centrioles were positioned in multiple groups within the same dendrite (**Fig 2A**).

We next asked whether the dendrite completely extends prior to centriole migration, or whether migration occurs in tandem with dendrite extension. We imaged growth-associated protein 43 (GAP43), a marker of immature OSNs and some progenitor cells, to observe the extent of dendrites during outgrowth (McIntyre et al., 2010). We observed centrioles in partially elongated dendrites with growth cones, indicating that the dendrite does not fully extend prior to centriole migration (**Fig 2B**). By probing for β -tubulin III (TUBB3), a tubulin isotype expressed primarily in neurons, we observed that microtubules were present both apical to and basal to the migrating centrioles (**Fig 2B**). Together, these data indicate that the leading group of centrioles migrates in tandem with dendrite extension and can be followed by lagging groups within the dendrite.

To begin to assess the mechanisms by which centrioles migrate, we established an *ex vivo* live imaging system for dissected olfactory epithelium. To measure rates of centriole movement in the final stage of migration we used time-lapse confocal microscopy to image migrating eGFP-centrin2-labeled centrioles. Flattened olfactory epithelium was mounted on agarose pads and imaged *en face*, and z stacks were acquired to track the movement of centriole groups in the apical 15 to 25 μ m of the epithelium. We found that the cells in the excised olfactory epithelium remained intact with few dying or delaminating cells over the time course of imaging.

Considering net movement over the course of the sequences, centriole groups moved slowly, with the fastest moving at approximately 0.18 μ m per minute ($n = 22$ centriole groups, $N = 3$ animals, **Fig 2C**, Fig 2 - Supp Vid 1). Most centriole groups exhibited net movement in the apical direction (**Fig 2C**, Fig 2 - Supp Vid 1, $n = 14$ out of 22 groups observed), although approximately one third had either net movement in the basal direction or no movement during the imaging period (**Fig 2D**, Fig 2 - Supp Vid 2, $n=8$ out of 22 groups observed).

Our results show that centrioles often migrate in groups, moving relatively slowly towards the apical end of the dendrite. How might these groups of centrioles be held together during migration? Centrioles in dividing cells are kept in close proximity by two forms of linkage (Breslow & Holland 2019; Nigg & Stearns 2011). Newly formed centrioles are attached to their associated mother centriole by an engagement linker, which is broken by passage through mitosis, and disengaged centrioles are linked by cohesion fibers. Our inability to resolve centriole-centriole relationships in the migrating groups due to tight packing within the narrow dendrite prevents us from being able to determine whether the engagement link is responsible for their grouping; however, we consider it unlikely that all centrioles are held together this way, based on our previous results showing that centriole amplification is initiated prior to mitotic divisions in progenitors (Ching & Stearns, 2020).

Given that centriole engagement is unlikely to be the major determinant of grouping, we tested whether grouping is mediated by centriole cohesion. Cohesion fibers contain rootletin (CROCC) (Vlijm et al., 2018), which is also a component of the striated rootlet that anchors centrioles bearing cilia (Yang et al., 2002). We performed expansion microscopy to detect rootletin on centrioles. Strikingly, migrating centrioles lacked rootletin signal (**Fig 3A**), indicating that these groups are not held together by rootletin-based cohesion fibers. We also observed that migrating centrioles not only move in multiple groups, but that some centrioles are not grouped at all, localizing singly between migrating groups in immature OSNs (**Fig 3A**). The rootletin antibody did detect rootlets associated with centrioles in mature OSNs (**Fig 3A**) (Menco et al., 1978; McClintock et al., 2008). Thus, rootletin is not present on migrating centrioles and therefore centriole cohesion is unlikely to play a role in grouping centrioles together during migration.

Centriole composition shifts toward maturation during OSN development.

In the typical animal cell cycle, centrioles require two cell cycles after their formation to become competent to form cilia (Breslow & Holland 2019; Nigg & Stearns 2011). During this maturation process they lose proteins associated with the early steps of centriole formation, including components of the cartwheel, and acquire proteins associated with microtubule nucleation, as well as distal and subdistal appendages. To understand the progression of centriole maturation

in the olfactory epithelium, we examined proteins associated with centriole formation and microtubule nucleation in progenitors, immature OSNs and mature OSNs.

First, we examined the procentriole markers STIL (SCL/Tal1 interrupting locus protein) and SASS6 (spindle assembly abnormal protein 6) using expansion microscopy. In cycling mammalian cells grown in culture, these proteins are present during procentriole formation in S-phase and are lost from centrioles in mitosis following centriole disengagement (Arquint and Nigg 2014; Strnad et al., 2007). In the olfactory epithelium, both STIL and SASS6 were present on amplifying centrioles in progenitors and localized to the proximal end of newly formed centrioles, supporting the model that these proteins are involved in centriole amplification from rosettes (**Fig 3B, 3C**). Remarkably, STIL and SASS6 are retained on at least some of the migrating centrioles in immature OSNs and are associated with the presumptive proximal end of these centrioles (**Fig 3B, 3C**). In mature OSNs, STIL and SASS6 are lost from centrioles (**Fig 3B, 3C**). Together, these results suggest that immature procentrioles are capable of migrating, while the dendritic knob is primarily formed of mature centrioles that lack STIL and SASS6.

In many differentiated neurons, centrioles lose the microtubule nucleating capability that allows them to function as centrosomes. Pericentriolar material (PCM) proteins involved in microtubule organization, including gamma-tubulin, CDK5RAP2 (CDK5 Regulatory Subunit Associated Protein 2) and pericentrin (PCNT), are reduced at centrosomes in these neurons and relocated to non-centrosomal sites (Leask et al., 1997; Steiss et al., 2010; Yonezawa et al., 2015; Sanchez-Huertas et al., 2016; Wilkes & Moore, 2020). We were interested in assessing the association of PCM proteins with centrioles in OSNs. We first probed for the microtubule nucleator gamma-tubulin using expansion microscopy. In progenitor cells with amplifying centrioles, gamma-tubulin is present surrounding centrioles (**Fig 3D**). In immature OSNs, gamma-tubulin localizes to a diffuse cloud around centriole barrels. In mature, fully ciliated OSNs, gamma-tubulin was also present around centrioles. Next, we determined the localization of CDK5RAP2 and pericentrin, PCM proteins involved in recruiting and activating gamma-tubulin (Fong et al., 2008, Choi et al., 2010, Delaval & Doxsey, 2010) (**Fig 3E, 3F**). Both CDK5RAP2 and pericentrin are present in progenitor cells with amplifying centrioles, as well as migrating centrioles in immature OSNs. In dendritic knobs, CDK5RAP2 was present, while pericentrin signal decreased. Taken together, our data show that groups of migrating OSN centrioles remain associated with microtubule nucleation proteins and pericentriolar material, in contrast to the centrioles of many other types of mammalian neurons. This is similar to

observations in MMCCs, where amplified centrioles were also reported to have associated PCM (Jurczyk et al., 2004; Vladar et al., 2012; Zhao et al., 2021).

Immature OSNs form a single cilium from the parental centriole.

We next used expansion microscopy to examine the events associated with arrival of the centrioles at the dendritic knob and formation of cilia. We observed that in some OSN dendrite tips with multiple centrioles, near the apical surface, a single cilium was present (**Fig 4A, 4A'**). We hypothesized that this reflected an inherent asymmetry in centriole states, such that only one centriole is competent to form a cilium at this stage prior to formation of the full complement of cilia. Ciliogenesis is a multi-step process that, in many organisms, requires a mature centriole to bear distal and subdistal appendages. To assess distal appendage acquisition, we used expansion microscopy to image CEP164 (Centrosomal Protein 164), a distal appendage protein required for ciliogenesis (Tanos et al., 2013, Cajanek & Nigg, 2014). In OSNs with a single cilium, distal appendages were present on the centriole at the base of the cilium and not at other centrioles (**Fig 4A, 4A'**), confirming that an inherent asymmetry in centriole states exists. By contrast, in OSNs with multiple cilia, many centrioles acquired distal appendages, indicating that centriole maturation and remodeling occurred at the apical surface (**Fig 4B**).

How might this centriole asymmetry arise? Based on our previous results regarding centriole amplification (Ching & Stearns, 2020), we hypothesized that in progenitor cells, a single, mature, parental centriole segregates to each daughter cell whereas the newly amplified centrioles remain immature and do not acquire appendages. In support of this hypothesis, we found that both in migrating centrioles (**Fig 4C**, Fig 4-Supp Fig 1A, Fig 4-Supp Fig 1B) and in centrioles in the process of amplification (Fig 4-Supp Fig 1C), only a single centriole was found to have distal and subdistal appendages. These data suggest that the centriole modifications required to make cilia occur after centriole migration during OSN differentiation, and that the already-modified parental centriole is able to make a cilium before all other centrioles. The temporal progression of centriole maturation is consistent with the presence of markers of immature centrioles on some migrating centrioles (**Fig 3B, 3C**).

In tracheal MMCCs, the parental centriole retains its unique structural characteristics even in mature cells after all cilia form (Liu et al., 2020). In particular, the parental centriole has a radial array of subdistal appendages, in contrast to all other centrioles, which bear a single basal foot. Radial subdistal appendages and basal feet share molecular components, but can be

distinguished by the arrangement of these components around centrioles. To test whether the parental centriole could be distinguished from the other centrioles in mature OSNs, we stained for the shared component centriolin, using expansion microscopy. In migrating centrioles, centriolin was present on only one centriole, surrounding that centriole in a subdistal appendage-like pattern (Fig 4-Supp Fig 1B). In cells in which this centriole formed a single cilium, centriolin retained this subdistal appendage-like pattern around the base of the cilium, while other centrioles had acquired single puncta of centriolin (**Fig 4D**). In mature OSNs with multiple cilia, one centriole was surrounded by centriolin whereas the other centrioles still had only single foci of centriolin, consistent with basal feet as observed in multiciliated epithelial cells (**Fig 4E**, Fig4-Supp Fig 1D). We conclude that in OSNs, as in multiciliated epithelial cells, the cilium nucleated by the parental centriole has unique structural characteristics compared to the other cilia at the mature dendritic knob.

Microtubule dynamics are necessary for centriole migration in OSNs.

Microtubules are a critical component of the neuron cytoskeleton, involved in neurite structure and transport. Neurons contain many stable microtubules as well as a population of dynamic microtubules (Baas et al., 2016). We sought to determine whether perturbation of the dynamic microtubules would affect centriole migration to the dendrite tip. To facilitate these experiments, we developed an explant culture system that retained the architecture of the epithelium *in vivo*. Olfactory epithelium dissected from the septa of mice expressing eGFP-centrin2 and Arl13b-mCherry was plated on transwell filters. To mimic the environment that the olfactory epithelium experiences *in vivo*, the bottom compartment was filled with medium to create an air-liquid interface at the filter (**Fig 5A**).

First, we tested whether explant cultures were viable over the required time course and whether cells retained the ability to progress through the cell cycle. We found that EdU, which labels newly-replicated DNA, incorporated into nuclei near the basal lamina in the explant, indicating that cells were able to replicate DNA (**Fig 5B, 5C**). In addition, many of the EdU-positive cells appeared to have amplified centrioles, in accordance with our previous observations (Ching & Stearns, 2020). We treated explants with EdU for two timepoints, one and six hours post-dissection, and found that the number of EdU-positive nuclei increased from 0.63 nuclei per 100 μ m basal length of epithelium to 0.96 nuclei per 100 μ m of epithelium (total lengths quantitated = 634.84 μ m and 621.52 μ m for one and six hours, respectively). Treatment during the time course did not affect overall morphology of the olfactory epithelium. These results demonstrate

that cells in the explant cultures can synthesize DNA and amplify centrioles, and that the culture retains its characteristic architecture during the time course.

Next, we used this explant culture system to determine whether microtubule dynamics are required for centriole migration. Explant cultures were treated for one hour or six hours with paclitaxel (taxol), a microtubule stabilizing drug, or DMSO as a vehicle control. Treatment with DMSO or paclitaxel for one hour or six hours did not affect overall tissue morphology, including the actin-rich apical domain (**Fig 5D, 5E**). We then scored the number of migrating centriole groups in distinct regions of the olfactory epithelium: the subapical region, and the middle and basal region (regions marked in **Fig 1A**). In all conditions, more centriole groups were found in the subapical compartment than in the middle and basal region, consistent with previous reports (Mulvaney & Heist, 1971). Upon treatment with paclitaxel, more centrioles were found in the subapical compartment (**Fig 5F**). After one hour of paclitaxel treatment, the subapical compartment had 1.38-fold more centrioles than control (total basal lengths quantitated = 905.37 μm and 988.17 μm for paclitaxel and DMSO, respectively). Strikingly, after six hours of paclitaxel treatment, the subapical compartment had 2.58-fold more centrioles compared to control (total basal lengths quantitated = 1019.76 μm and 1126.44 μm for paclitaxel and DMSO, respectively, **Fig 5F**). The subapical accumulation of centrioles with paclitaxel treatment suggests that microtubule dynamicity is required for centriole groups to complete their progression to the apical membrane.

Discussion

Olfactory sensory neurons (OSNs) in vertebrates have a morphology specialized for their role, with an array of sensory cilia at the tip of a dendrite. We showed previously that the centrioles required to form these cilia are produced in progenitor cells that undergo centriole amplification and divide before becoming OSNs (Ching & Stearns, 2020). Here we have examined centriole migration and maturation in OSNs, with a focus on the unique biology of OSNs. We established new techniques for manipulating and studying the olfactory epithelium, applying expansion microscopy, live imaging, and an explant system. These will be useful for future experiments on the cells of this tissue, important for their sensory role and as a point of entry for respiratory infection.

Our results are summarized in **Figure 6**. Progenitor cells, located near the basal lamina of the olfactory epithelium, amplify centrioles from an initial pair. The progenitor cell divides and the daughter cell differentiates into an immature OSN, which extends a dendrite towards the apical surface. Centrioles migrate in multiple groups within a single dendrite in immature OSNs. The leading group of centrioles moves in tandem with dendrite extension and can be followed by lagging groups. Centriole groups move relatively slowly towards the apical end of the dendrite and during migration most of the centrioles retain markers associated with immature centrioles and lack the appendages required to make cilia. The exception is a single migrating centriole, presumably an original parental centriole, which has appendages and is able to form a single cilium in the dendritic knob, prior to the others. Finally, most centrioles acquire distal and subdistal appendages and form the dozens of olfactory cilia found in the mature OSN. The parental centriole retains structural characteristics that differentiate it from the other centrioles within the same cell.

We previously showed that centrioles in OSNs are amplified, at least in part, by formation of centriole rosettes in progenitor cells, without deuterosomes like those seen in MMCCs (Ching & Stearns, 2020). Here we exploited the resolution afforded by expansion microscopy to precisely count centrioles, finding that the number of centrioles during migration and in mature OSNs was even higher than previously reported, with a mean of approximately 36 centrioles in the latter. The number of centrioles was similar for both states, suggesting that all centriole amplification takes place prior to the initiation of migration and dendrite extension. This is a higher number of centrioles than reported by Uytingco et al. (2019) and Ukhanov et al. (2022); this could be due to differences in microscopy and counting methods, to regional differences in the mouse olfactory epithelium (Challis et al., 2015), or to variation between animals. Based on the new evidence we present here, a parsimonious explanation for how OSNs achieve their final centriole number is that amplification is initiated by rosette formation around the two parental centrioles in progenitor cells, followed by a single mitotic division and centriole disengagement from the rosettes, and further amplification in post-mitotic cells that become OSNs.

OSNs are, to our knowledge, unique in that they undergo a mitotic division with amplified centrioles, which is then followed by further amplification (Ching & Stearns, 2020). Thus, an immature OSN potentially has centrioles of three different ages: the parental centriole, amplified centrioles formed prior to mitosis, and those that may have formed after mitosis. We found that some of the amplified centrioles in immature OSNs have nucleation-associated proteins of the

PCM, including gamma-tubulin, CDK5RAP2, and pericentrin, consistent with a portion of the amplified centrioles having undergone the centriole-to-centrosome conversion as described in cultured mammalian cells (Wang et al., 2011; Izquierdo et al., 2014). Similarly, some of the centrioles in immature OSNs have the cartwheel components SASS6 and STIL, as might be expected for those centrioles formed after the mitotic division, whereas all centrioles lack these components in mature OSNs.

Centrioles in mature OSNs have the proteins associated with rootlets, distal appendages and subdistal appendages/basal feet, typical of centrioles underlying cilia in other multiciliated cells (Anderson & Brenner 1971; Menco et al., 1978; McClintock et al., 2008; Sorokin 1968). It is interesting that the final modification of the non-parental centrioles with these components in OSNs appears to be controlled spatially, by their arrival in the forming dendritic knob, as they were absent on migrating centrioles in immature OSNs. This might occur, for example, by localized translation or accumulation of the protein components of these structures or the regulators of their assembly on the centriole. In cycling mammalian cells grown in culture, centriole maturation is driven by cell cycle progression and the activity of PLK1 kinase (Kong et al., 2014, Shukla et al., 2015). In MMCCs, low-level activation of mitotic kinases is important for centriole amplification (Al Jord et al., 2017). In OSNs, it is possible that centriole maturation is also controlled by PLK1 and other mitotic regulators, with the added element of spatial control. Interestingly, it has also been shown that loss of centrin2 results in fewer cilia on OSNs as well as more OSNs with centrioles below the apical surface (Ying et al., 2014). This would be consistent with a defect in acquisition of appendages, although the mechanism by which centrin2 affects centriole docking is unclear.

The presumptive parental centriole differed from all others in OSNs in having subdistal and distal appendage proteins prior to its migration to the dendrite tip. This was apparent during migration of centrioles and in the early stage of cilium formation when only this centriole was associated with a cilium. The presence of a single cilium before formation of the full complement of multiple cilia might enable signaling important for OSN differentiation, as has been proposed for the primary cilium of MMCCs that precedes multiciliated differentiation (Jain et al., 2010), although we note that Schwarzenbacher, et al. (2005) found that the earliest OSN cilia formed lack olfactory receptors. The parental centriole retained a radial array of the subdistal appendage component centriolin even in the mature OSN, whereas all other centrioles had a single focus of centriolin, consistent with them having basal feet. This is remarkably similar to

the situation in MMCCs in which a single parental centriole also differed from other centrioles and was associated with what was termed a “hybrid cilium” (Liu et al., 2020). It is unclear what prevents the parental centriole from forming a cilium prior to reaching the dendritic knob, although it might be due to the same spatial regulation that we postulated to control centriole maturation.

How do centrioles migrate from the cell body to the tip of the dendrite? Our work rules out several possibilities. First, centrioles within a single cell often travel in multiple groups separated by relatively long distances along the dendrite. This excludes the possibility that forces act upon only a single centriole, for instance the parental centriole. Second, our data also exclude the possibility that centrioles might migrate by docking to membranes, as only the presumptive parental centriole bears the distal appendages required for membrane association during migration. Third, migrating centrioles lack rootletin, ruling out models in which centrioles are moved by attachment of centriolar rootlets to actin filaments, as observed during macrociliary cell development in the comb jelly *Beroë* (Tamm & Tamm, 1988).

Our data support a model in which the microtubule cytoskeleton is critical for centriole migration. Using a novel explant system, we found that inhibiting microtubule dynamics disrupts centriole migration to the apical surface. In this respect, OSNs are similar to MMCCs of the oviduct, in which paclitaxel-mediated stabilization of microtubules does not affect centriole biogenesis, but can inhibit centriole migration (Lemullos et al., 1988; Boisvieux-Ulrich et al., 1989). Because of the very different cell and tissue architectures in MMCCs compared to OSNs it is unclear whether the mechanism of inhibition by microtubule stabilization is the same in these cell types. Our establishment of an explant system will be useful for future work in distinguishing the exact mechanisms by which cytoskeletal elements influence centriole migration in OSNs.

How do forces act upon migrating centrioles? One possibility is that microtubule motors are directly involved in centriole transport. In the *C. elegans* ciliated PQR neuron, SAS-5, an orthologue of STIL, directly interacts with a dynein light chain (DLC-1/LC8), suggesting that centrioles may be cargos of cytoplasmic dynein in the dendrite (Li et al., 2017). In addition, the rate of migration for the single centriole that migrates in *C. elegans* neurons is similar to that of mouse OSN centrioles (in OSNs: maximum of 0.18 $\mu\text{m}/\text{min}$; in *C. elegans*: 0.076 $\mu\text{m}/\text{min}$, Li et al., 2017), suggesting that similar mechanisms may drive centriole migration in both contexts. However, dynein motors acting processively exhibit velocities much higher than those observed

in OSNs or the PQR neuron, approximately 60 μm per minute in cells and *in vitro* (Presley et al., 1997; King & Schroer, 1999). Thus, dynein might act non-processively and/or interact with centrioles transiently in OSNs. This is reminiscent of slow axonal transport, which drives the anterograde transport of cytoskeletal elements within neuronal axons (Baas & Buster, 2004; Roy, 2020). Recent work has indicated that cargos undergoing slow axonal transport interact transiently or intermittently with molecular motors (Roy, 2020), and it is possible that similarly transient interactions are responsible for centriole movement in OSN dendrites.

Our finding that microtubule nucleation proteins are present on migrating centrioles suggests another, non-mutually exclusive hypothesis: that migrating centrioles are microtubule organizing centers, with self-nucleated microtubules involved in their motility. Force could be derived from microtubule polymerization itself, or by interaction of nucleated microtubules with an existing dendritic microtubule array. In other mammalian neurons, centrioles remain near the nucleus and lose microtubule nucleating proteins as the cell differentiates (Leask et al., 1997; Steiss et al., 2010; Yonezawa et al., 2015; Sanchez-Huertas et al., 2016; Wilkes & Moore, 2020). In cycling mammalian cells, PCM nucleates microtubule asters that center and position the centrosome. This occurs through pushing forces driven by microtubule nucleation, and pushing and pulling forces driven by molecular motors (reviewed in Burakov & Nadezhdina, 2020). Similarly, microtubule nucleation may help orient and position centrioles toward the apical surface in OSNs. This would be consistent with a model in which forces are acting upon multiple centrioles, perhaps even individual centrioles, within the same dendrite.

In addition to migration within dendrites, the PCM of OSN centrioles might also influence dendrite microtubule organization itself. OSN dendrites have a polarized microtubule cytoskeleton, in which the microtubule minus ends are oriented out, toward the dendritic tips (Burton, 1985). This polarity, observed in bullfrog OSNs, is in contrast to dendrites of many neurons, which have a mixed polarity. An attractive possibility is that the centrioles positioned at the tip of the dendrite function as a centrosome, nucleating microtubules, with their minus ends out. Supporting this hypothesis, we observed that PCM proteins are present at centrioles in both immature and mature OSNs. An interesting parallel is that microtubule polarity in dendrites of non-ciliated *C. elegans* PVD neurons is established by a non-centrosomal microtubule organizing center that is present at the dendritic tip (Liang et al., 2020), suggesting that this may be a conserved mechanism of establishing and maintaining microtubule polarity in dendrites.

Finally, we have focused here on microtubule-based mechanisms, but acknowledge that other mechanisms, for example based on the actin cytoskeleton, might also be important for centriole migration and docking in OSN dendrites. We expect that the new techniques for manipulating and studying the olfactory epithelium that we describe will facilitate further research into these and other questions about how multiple centrioles and cilia are formed in OSNs and participate in their structure and function.

Methods

Ethics Statement

This study uses samples from mice. All animal procedures in this study were approved by the Stanford University Administrative Panel for Laboratory Animal Care (SUAPLAC protocol 11659) and carried out according to SUAPLAC guidelines.

Mice

Olfactory epithelium samples were taken from both male and female mice, euthanized by CO₂ inhalation, for all experiments. Mice were between 1 and 6 months old. (See each section for specific ages.) Mice constitutively overexpress eGFP-centrin2 to mark centrioles, and Arl13b-mCherry to mark primary cilia (line generated by Bangs et al., 2015). In no instance did we observe Arl13b-mCherry in olfactory cilia, but Arl13b-mCherry was present in primary cilia of olfactory epithelium progenitor cells. Native fluorescence signal from eGFP and mCherry was not preserved in expansion microscopy.

Microscopy

All confocal microscopy images were acquired as single planes or Z-stacks collected at 0.27-μm intervals using a Zeiss Axio Observer microscope (Carl Zeiss) with a PlanApoChromat 63×/1.4 NA objective, a Yokogawa CSU-W1 head, and a Photometrics Prime BSI express CMOS

camera. Slidebook software (Intelligent Imaging Innovations, 3i) was used to control the microscope system.

Antibodies

Primary antibodies used for immunofluorescent staining are listed in Table 1. AlexaFluor-conjugated secondary antibodies (Thermo-Fisher) were diluted 1:1000 for standard immunofluorescence and 1:500 or 1:1000 for expansion microscopy.

Table 1. Antibodies used in this study.

target	source	dilution
acetylated tubulin	Sigma-Aldrich, clone 6-11B-1, RRID:AB_477585	1:1000
CDK5RAP2	Millipore, 06-1398 rabbit polyclonal, RRID:AB_11203651	1:200
centrin	EMD Millipore, clone 20H5, RRID:AB_10563501	1:1000
CEP164	rabbit polyclonal previously described in Lee et al 2014	1:500
GFP	rabbit antibody previously described in Hatch et al., 2010	1:2000
GAP43	Abcam, clone EP890Y, RRID:AB_1310252	1:750 (requires overnight, 4-°C incubation)
Pericentrin	BD Biosciencies, clone 30, RRID:AB_399294	1:500
Centriolin	Santa Cruz Biotech, clone C-9, RRID:AB_10851483	1:50
STIL	Abcam, rabbit polyclonal, RRID:AB_2197878	1:1000

SASS6	Santa Cruz Biotech, RRID:AB_1128357	1:200
ODF2	Novus Biologicals, Mouse IgG2a, RRID:AB_1146453	1:200
rootletin (CROCC)	Santa Cruz Biotech, clone C-2, RRID:AB_10918081	1:100-200
β -tubulin III	BioLegend, clone TuJ1, RRID:AB_2313773	1:1000-2000
gamma-tubulin	Sigma-Aldrich, clone GTU88, RRID:AB_532292	1:1000

514

515

516 *Immunofluorescent staining of cryosections*

517 Olfactory epithelia were dissected mechanically from mice that were 1 to 4 months old. All
518 stainings were performed at least twice, in olfactory epithelium taken from both a male and a
519 female mouse. After initial snout removal, fine dissection was performed in a dish of cold
520 Tyrode's solution (140mM NaCl, 5mM KCl, 10mM HEPES, 1mM CaCl₂, 1mM MgCl₂, 1mM
521 sodium pyruvate, 10mM glucose in ddH₂O), as in other reports (Oberland and Neuhaus, 2014)
522 and at github.com/katieching/Protocols. Whole olfactory epithelia, turbinate epithelia, or septum
523 epithelia were fixed immediately in 4% PFA in PBS at 4°C for 3 to 24 hours. Samples were then
524 washed in phosphate buffered saline (PBS) and stored at 4°C. Before mounting, samples were
525 equilibrated in 1 to 5 mL of 30% sucrose solution in water for a minimum of 12 hours at 4°C.
526 Samples were embedded in OCT compound (Sakura Tissue-Tek) on dry ice and stored at -
527 80°C. Embedded samples were sectioned at 14 μ m on a Leica cryostat and adhered to charged
528 slides by drying at room temperature for approximately 1 hour. Slides were stored with drying
529 pearls (Thermo Fisher) at -80°C and thawed under desiccation no more than twice. Sections
530 were outlined with a hydrophobic pen. Residual free aldehydes were quenched while samples
531 were rehydrated in PBS with 0.3 M glycine, 1% calf serum, and 0.1% Triton-x 100 for 0.5 hours
532 at room temperature. Samples were blocked and permeabilized for an additional 0.5 to 4 hours

in PBS with 1% calf serum and 0.1% Triton-x 100. Antibodies were also diluted in PBS with 1% calf serum and 0.1% Triton-x 100 (see Table 1). Slides were incubated with primary antibodies for approximately 3 hours at room temperature or overnight at 4°C, washed in PBS, incubated with secondary antibodies for approximately 1 hour at room temperature, washed in PBS, incubated in DAPI diluted 1:1000 for 5 minutes, washed in PBS, and mounted in MOWIOL. Where phalloidin conjugated to Alexa Fluor 568 was used, it was included at the secondary antibody step at a 1:1000 dilution (Molecular Probes). Where EdU was used, incorporation was visualized using the Click-iT Alexa Fluor 594 kit according to the manufacturer's instructions (Molecular Probes). Slides were imaged by spinning disk confocal microscopy, and images were acquired with the SlideBook software by Intelligent Imaging Innovations (3i). Images were processed in FIJI (Schindelin et al., 2012). Images are rotated to orient the apical surface toward the top of the page. For images with high background, contrast in the representative images was adjusted uniformly across the image such that the area outside of cells was black and areas of high signal were just below saturation.

Expansion microscopy - olfactory epithelium

Details about expansion microscopy for olfactory epithelium can be found at github.com/katieching/Protocols and were based on work by Gambarotto et al., 2019 and Sahabandu et al., 2019. In brief, samples from 1- to 4-month-old mice were dissected mechanically in a dish of cold Tyrode's solution (140mM NaCl, 5mM KCl, 10mM HEPES, 1mM CaCl₂, 1mM MgCl₂, 1mM sodium pyruvate, 10mM glucose in ddH₂O), fixed, washed, and cryoprotected by the same methods used for unexpanded immunofluorescent staining. All stainings were performed at least twice, in olfactory epithelium taken from both a male and a female mouse. Samples were embedded in OCT (Sakura Tissue-Tek) on dry ice and stored at -80°C. Embedded samples were sectioned at 14 µm thickness on a Leica cryostat, placed on charged glass slides within a border pre-drawn with hydrophobic pen, and allowed to thaw for 1

minute. Sections were then washed three times in PBS for approximately 5 minutes per wash. PBS was removed, and sections were incubated in a monomer fixative solution (0.7% formaldehyde and 1% w/v acrylamide in water) for 2 hours at room temperature. Sections were washed once in monomer solution (19% w/v sodium acrylate, 10% w/v acrylamide, and 0.1% BIS in PBS). Solution was removed, and slides containing sections were inverted onto droplets of cold gelation solution (monomer solution with TEMED and ammonium persulfate added to a final concentration of 0.5% each). Stacks of approximately five coverslips were used as spacers to achieve the desired gel thickness. Gels were set for 5 minutes on ice and then overnight at room temperature in a sealed chamber with wet paper towels. Slides with gels attached were incubated at 95°C in a large volume of denaturation buffer (200 mM SDS, 200 mM NaCl, and 50 mM Tris in water) for approximately 4 to 6 hours. Gels were washed and then pre-expanded in water overnight. Gels were then incubated in PBS for at least 30 minutes. Gels were incubated in primary antibody solution in PBS with or without 3% bovine serum albumin and 0.1% Triton-x100 (see Table 1) overnight on a nutator at 4°C. Gels were washed three times in PBS for 10 to 30 minutes per wash and then incubated in a solution of secondary antibodies conjugated to Alexa Fluors plus DAPI, diluted 1:500 or 1:1000, overnight with gentle agitation at 4°C. Gels were washed in PBS for at least 30 minutes, then in water three times for 30 minutes per wash. Gels were then allowed to fully expand in water for at least an hour before mounting in a glass-bottom imaging dish, and imaging by spinning disk confocal microscopy. Native fluorescence signal from eGFP and mCherry was not preserved. As needed, gels were imaged in a poly-lysine-coated dish to reduce sample movement during acquisition. Centriole counts were semi-automated: an initial volume segmentation was performed in Imaris, followed by manual inspection. Graphs for Fig 1 - Supp 1 were made using GraphPad Prism.

Live imaging of olfactory epithelium

Septal or flattened turbinate olfactory epithelium was mechanically dissected from 3.5- to 6-month-old mice in the same manner as for explant preparation. Imaging was performed in samples taken from three different animals and included both male and female mice. The procedure for mounting was partly based on work from Williams et al. (2014). For this study, dissected samples were pulled onto a coverslip containing an agarose pad made of 1% low-melt agarose (Lonza #50100) dissolved in Tyrode's buffer. The coverslip with the sample was then inverted into the center of an imaging dish containing 150 μ L of imaging buffer (Tyrode's with 5% cosmic calf serum and 5 μ g/mL Hoechst 34580 (Invitrogen #H21486), mixed by pipette and vortexed) and allowed to sit for at least 5 minutes before imaging. Coverslips were sealed into place with melted Vaseline as needed. Samples were imaged *en face* by spinning disk confocal microscopy at approximately 37°C with low laser power until no more than 4 hours after euthanasia. Files were acquired and scored using the SlideBook software by Intelligent Imaging Innovations (3i). For scoring, centriole groups below the apical surface were identified in a 4D volume view and measured in a maximum intensity projection over the y axis with respect to a group of nearby mature OSN centrioles as a fiducial mark. Locations for each group were scored in the first and last frame during which they were visible in the maximum projection, and average rates were calculated from the difference between those measurements.

Kymograph analysis was performed using Dynamic Kymograph in FIJI (Zhou et al., 2020).

Culture and analysis of septum explants grown on air-liquid interface

Details about growing olfactory epithelium explants can be found at github.com/katieching/Protocols and is partly based on the procedure described by Oberland and Neuhaus (2014). In brief, septum epithelia from 1- to 4-month-old mice were dissected mechanically in cold Tyrode's buffer and placed apical side up on transwell polycarbonate filters with 0.4 μ m pore size with the bottom compartment containing additional Tyrode's buffer. All experiments were performed in explants taken from both male and female mice. Once all

samples were placed, buffer was aspirated from the bottom compartment and replaced with warm explant medium (15% fetal bovine serum and 0.02% L-ascorbic acid in Waymouth's media (Thermo Fisher Scientific #11220035)) (based on Farbman, 1977). No medium was added to the top compartment, preserving the air-liquid interface. Relevant treatments (10 μ M EdU (Thermo Fisher Scientific #A10044), 15 μ M paclitaxel (EMD Millipore #580555), or an equivalent volume of DMSO) were mixed into media by pipetting and vortexing before addition. Samples were incubated in a 33°C, 5% CO₂, humidified incubator for 1 hour or 6 hours before forceps were used to transfer them from the filters to 4% PFA in PBS for overnight fixation. For Fig 3B, 3C, samples within each replicate were obtained from the same animal. For Fig 3D-F, samples were matched such that explants taken from the two conditions came from the same animal at the same time point. Where explants were taken from the same animal, conditions were randomized between the right and left halves of the septum. Samples were then washed and processed by the same method of immunofluorescent staining described above. To score explants treated with paclitaxel (Fig 3F), sample size was selected by (1) measuring the average length between areas of high amplification (approximately 250 μ m), (2) scoring a length greater than that for the condition with the most limited sample, and (3) scoring progressively (i.e. not selecting regions) along the epithelium of all other conditions until the length was matched. This was then repeated in samples from the opposite sex.

Acknowledgements

This project was supported by the National Science Foundation Graduate Research Fellowship under Grant No. D-G16E5 6518 (to K.C.), and by the NIGMS of the National Institutes of Health under award numbers 1R35GM130286 (to T.S.), K99 GM131024 (to J.T.W.), and T32GM007276 (CMB Training Grant). We thank members of the Stearns lab for helpful feedback and suggestions.

636

637

638 **Competing interests**

639 The authors declare that no competing interests exist.

640

641

642 **Author contributions**

643 Conceptualization and writing: K.C. (ORCID: 0000-0002-0517-2421), J.T.W. (ORCID: 0000-

644 0002-8506-5182), and T.S. (ORCID: 0000-0002-0671-6582); Investigation: K.C. and J.T.W.;

645 Supervision, funding acquisition, and project management: T.S.

646

647

648

Figure Legends

Figure 1. Centrioles in olfactory sensory neurons (OSNs) migrate tens of micrometers to the apical surface.

(A) Overview of the olfactory epithelium. Single plane fluorescence image of a side view of the olfactory epithelium (left), corresponding to a schematic of cell types in the olfactory epithelium (right). Yellow: eGFP-centrin2. Cyan: staining for acetylated tubulin, strongly marking olfactory cilia and, faintly, neuronal microtubules. Magenta: staining for β -tubulin III, marking neuronal microtubules. White: DAPI, marking DNA. The apical surface is oriented at the top of this image and all subsequent side view images. Dashed line: apical surface.

Double solid line: basal lamina. Boxes show the relative positions of critical stages of OSN differentiation: (i) The subapical compartment of the olfactory epithelium, defined as the space between the bottom of the sustentacular cell nuclei and the apical surface with olfactory cilia. (ii) A group of centrioles migrating through the middle of the olfactory epithelium, below the sustentacular cell nuclei. (iii) A progenitor cell near the basal lamina. Scale bar = 10 μ m.

(B) Mature olfactory sensory neurons. Single plane, *en face* fluorescence image of the apical surface of the olfactory epithelium. Green: eGFP-centrin2. Magenta: dye-conjugated phalloidin, marking an enrichment of F-actin at the apical borders of sustentacular cells. Scale bar = 10 μ m.

(C) Schematic of Fig 1B depicting a sustentacular cell wrapping around the dendrites of nearby OSNs. Green: OSN dendrite. Gray: sustentacular cell cytoplasm. Magenta: F-actin. Wavy black lines: multiple OSN cilia.

(D) Centriole migration in the olfactory epithelium. Single plane fluorescence image of a side view of the subapical compartment of the olfactory epithelium. Green:eGFP-centrin2.

Magenta: dye-conjugated phalloidin. Arrows: groups of migrating centrioles. Scale bar = 10 μm .

(E) Mature olfactory sensory neurons, as imaged by expansion microscopy. Single plane fluorescence image of the *en face* apical surface. White: staining for acetylated tubulin, marking centrioles, cilia, and, faintly, neuronal microtubules. Multiple cilia can be seen protruding from mature OSNs. A green arrow marks a dendrite with a group of migrating centrioles arriving at the apical tip. Scale bar = 2 μm .

(F) Centriole migration, as imaged by expansion microscopy. Single plane fluorescence image of a side view of the subapical compartment. White: staining for acetylated tubulin, marking centrioles, cilia, and, faintly, neuronal microtubules. Cilia can be seen at the apical surface, and green arrows mark groups of migrating centrioles. Scale bar = 2 μm .

Figure 1-figure supplement 1. Centriole and cilium numbers in OSN progenitors and mature OSNs.

(A) Example of Imaris segmentation of centrioles in progenitor cells. (i) 3D image of centrioles in a progenitor cell. Green: staining for acetylated tubulin. (ii) The same image after segmentation in Imaris. Segmented objects are color-coded with a LUT to encode z-depth. After automated counting, each image was visually inspected, and counts were corrected where objects and centrioles or cilia did not correspond. Scale bar = 2 μm .

(B) Example of Imaris segmentation of centrioles and cilia in mature OSNs. (i) 3D image of a mature OSN dendritic knob. Green: staining for acetylated tubulin. (ii) The same image after segmentation in Imaris. Segmented objects are color-coded with a LUT to encode z-depth. After automated counting, each image was visually inspected, and counts were corrected where objects and centrioles or cilia did not correspond. Scale bar = 2 μm .

(C) Plot of the number of centrioles per cell in progenitor cells and mature OSNs. Red dots: sample 1, three-month-old female. Black dots: sample 2, one-month-old male. Mean and

standard error of the mean (SEM) are graphed. In progenitors: mean = 26.40 centrioles, SEM = 2.234 and standard deviation = 9.992. In mature OSNs: mean = 36.46 centrioles, SEM = 1.665, and standard deviation = 8.156

(D) Plot of centriole and cilium numbers in mature OSNs. Black dots: percentage of centrioles that have cilia. Purple squares: number of cilia in each cell. Each dot and square in the same column belong to the same dendritic knob. Measurements were arranged along the x-axis in order of increasing percentage of centrioles with cilia. Mean: 85% of centrioles at a dendritic knob nucleated cilia (SEM=2.47; standard deviation = 12.10). Mean cilia number per dendritic knob: 30.54 cilia (SEM=1.455; standard deviation = 7.126).

Figure 2. Centrioles migrate in multiple groups during dendrite elongation.

- (A) Within a single dendrite, centrioles migrate in multiple groups. Expansion microscopy - side view of expanded olfactory epithelium. In all side-view images, apical is oriented toward the top of the image. White: staining for acetylated tubulin. Maximum z-projection of confocal stack. Mature OSNs have multiple cilia, which are visible at the apical surface. Green arrows: groups of centrioles migrating separately within the dendrite of a single OSN. Scale bar = 2 μ m.
- (B) Centriole migration occurs concomitantly with dendrite elongation. Fluorescence image of a side view of the olfactory epithelium. The leftmost image is a maximum intensity projection, and the right panels show a single plane. Cyan: staining for GAP43. Magenta: staining for β -tubulin III. Yellow: eGFP-centrin2. The OSN dendrite is outlined and bears a growth cone at apical tip.
- (C) A centriole group migrates toward the apical surface. i) Live time lapse imaging of olfactory epithelium. Maximum x-projection image, showing a side view. White: eGFP-centrin2. Blue: Hoechst, marking DNA in the most apical layer of nuclei, which are mostly sustentacular cells. Green arrows: a group of centrioles moving toward the apical surface at 0.18

µm/minute (fastest rate of all observed groups). See Figure 2 – Source Data 1 for migration rates. ii) Kymograph illustrating migration of the centriole group. Apical and basal direction labels indicate orientation of the sample in the kymograph.

(D) A centriole group with no net movement. i) Live time lapse imaging of olfactory epithelium, highlighting a different centriole group from the same acquisition as Fig 2C. Maximum x-projection image, showing a side view. White: eGFP-centrin2. Blue: Hoechst. Green arrows: a group of centrioles that have no net movement. See Figure 2 – Source Data 1 for migration rates. ii) Kymograph illustrating a lack of total migration of the centriole group. Apical and basal direction labels indicate orientation of the sample in the kymograph.

Figure 2-figure supplement 1. A centriole group migrates toward the apical surface.

Movie of the same centriole group as depicted in Fig 2C. Maximum x-projection image, showing a side view. White: eGFP-centrin2. Blue: Hoechst, marking DNA in the most apical layer of nuclei, which are mostly sustentacular cells. Time between frames = 2 minutes.

Figure 2-figure supplement 2. A centriole group with no net movement.

Movie of the same centriole group as depicted in Fig 2D. Maximum x-projection image, showing a side view. White: eGFP-centrin2. Blue: Hoechst. Time between frames = 2 minutes.

Figure 3. Composition of centriole groups during OSN differentiation.

(A) The cohesion fiber and striated rootlet protein rootletin is absent during centriole migration but is gained at the mature dendritic knob. Expansion microscopy, maximum z-projection of confocal stack. In all side-view images, apical is oriented toward the top of the image. Cyan: staining for Rootletin (CROCC). Magenta: staining for acetylated tubulin. Yellow: staining for centrin. (i) Side view of migrating centrioles. Centrioles below the apical surface, in the

dendrite of an immature OSN. (ii) Centrioles at the apical surface in the same sample as those shown in Fig 3Ai. Scale bars = 2 μ m.

(B-F) Expansion microscopy - Single plane fluorescence images of centrioles in progenitor cells (left column), immature OSNs with migrating centrioles (middle column, white lines outline an OSN dendrite), and mature OSNs imaged *en face* (right column). Scale bars = 2 μ m.

(B) The immature centriole protein STIL is present in progenitors and immature OSNs. Green: staining for STIL. Magenta: staining for acetylated tubulin.

(C) The immature centriole protein SASS6 is present in progenitors and immature OSNs. Green: staining for SASS6. Magenta: staining for centrin.

(D) The pericentriolar material protein gamma-tubulin is present throughout OSN differentiation. Green: staining for gamma-tubulin. Magenta: staining for acetylated tubulin.

(E) The pericentriolar material protein CDK5RAP2 is present throughout OSN differentiation. Green: staining for CDK5RAP2. Magenta: staining for acetylated tubulin.

(F) The pericentriolar material protein pericentrin (PCNT) is present in progenitors and immature OSNs. Green: staining for pericentrin (PCNT). Magenta: staining for acetylated tubulin.

Figure 4. A single cilium forms prior to formation of multiple cilia.

(A) An OSN bearing a single cilium in the subapical compartment of the olfactory epithelium.

Expansion microscopy - single plane fluorescence image of a side view of the subapical compartment. In all side-view images, apical is oriented toward the top of the image. Cyan: staining for CEP164, marking the location of distal appendages. Magenta: staining for acetylated tubulin, marking centrioles, cilia, and, faintly, neuron microtubules. Yellow: staining for centrin, marking centrioles. White: DAPI, marking DNA. A box marks the location of the inset, A'. Scale bar = 10 μ m. (A') Immature OSN with a single cilium, inset from panel A. Magenta: staining for acetylated tubulin. Yellow: staining for centrin. Cyan: staining for CEP164, marking distal appendages at the base of the cilium. Arrow: Cep164 is only found

at the base of the cilium, and not present on other centrioles within the dendrite. Scale bar = 2 μ m.

(B) During centriole migration in immature OSNs, a single centriole bears Cep164. Expansion microscopy - single plane fluorescence image of migrating centrioles in a side view of the olfactory epithelium. Green: staining for CEP164. Magenta: staining for acetylated tubulin. Scale bar = 2 μ m.

(C) In mature OSNs, multiple cilia bear Cep164. Expansion microscopy - single plane fluorescence image of a mature OSN with multiple cilia. Green: staining for CEP164, marking distal appendages at the base of cilia. Magenta: staining for acetylated tubulin. Scale bar = 2 μ m.

(D) In OSNs bearing a single cilium, the centriole at the base of the cilium is surrounded by the subdistal appendage/basal foot marker centriolin. Expansion microscopy - maximum z-projection of a confocal stack. Magenta: staining for acetylated tubulin. Green: staining for centriolin, marking subdistal appendages on the mother centriole at the base of the cilium. Scale bar = 2 μ m.

(E) In mature OSNs, a single cilium is surrounded by centriolin. Expansion microscopy - mature OSN imaged *en face*, maximum z-projection of a confocal stack. Magenta: staining for acetylated tubulin. Green: staining for centriolin. Arrow: centriolin surrounds the base of one cilium. Other cilia of the same dendrite are only associated with one centriolin punctum. Scale bar = 2 μ m.

Figure 4-figure supplement 1. Centriole maturation in progenitors and migrating centriole groups.

(A) During centriole migration in immature OSNs, a single centriole bears distal and subdistal appendages. Expansion microscopy - single plane fluorescence image of

centrioles below the apical surface in a side view of the expanded olfactory epithelium.

Cyan: staining for ODF2, marking subdistal appendages. Yellow: staining for Cep164, marking distal appendages. Magenta: staining for acetylated tubulin. Within the group, a single centriole bears both ODF2 and Cep164. Scale bar = 2 μ m.

(B) During centriole migration in immature OSNs, a single centriole bears centriolin.

Expansion microscopy - Single plane fluorescence image of centrioles below the apical surface in expanded olfactory epithelium. Green: staining for centriolin. Magenta: staining for acetylated tubulin. Scale bar = 2 μ m.

(C) In progenitor cells, a single centriole bears distal and subdistal appendages. Expansion microscopy - Single plane fluorescence image of centriole rosettes in expanded olfactory epithelium. Cyan: staining for ODF2. Yellow: staining for CEP164. Magenta: staining for acetylated tubulin. Arrow: subdistal appendages and distal appendages are found on a single centriole. Scale bar = 2 μ m.

(D) In cells with multiple motile cilia (MMCCs), centriolin is found in a single puncta corresponding to the basal foot. Expansion microscopy - Single plane fluorescence image of an MMCC imaged *en face* in expanded nasal epithelium. Green: staining for centriolin, marking a basal foot on each centriole. Magenta: staining for acetylated tubulin. Scale bars = 2 μ m.

Figure 5. Centriole progression toward the apical surface can be altered by stabilizing microtubules.

(A) Schematic: workflow from sample collection through imaging of olfactory epithelium explants. Septal olfactory epithelium was taken from adult mice expressing eGFP-centrin2 and Arl13b-mCherry and plated on transwell filters with an air-liquid interface. After 1 or 6 hours, samples were fixed and processed for sectioning. Stained sections were analyzed by confocal microscopy.

- (B) Progenitor cells synthesize DNA by 1 hour *ex vivo*. Fluorescence image of an olfactory epithelium explant grown in EdU for 1 hour, maximum intensity projection. Gray: DAPI, marking all nuclei. Cyan: EdU, conjugated to dye after fixation, marks cells that synthesized DNA *ex vivo*. Dashed line: the apical surface. Double solid line: basal lamina. Box: location of the inset, B'. Scale bar = 10 μm . (B') Inset from panel B showing a progenitor cell positive for EdU. Magenta: staining for β -tubulin III, showing that the cell is neuronally-fated. Yellow: eGFP-centrin 2, showing signal consistent with centriole amplification. Cyan: EdU. Scale bar = 2 μm .
- (C) More cells synthesize DNA by 6 hours *ex vivo*. Fluorescence image of an olfactory epithelium explant grown in EdU for 6 hours, maximum intensity projection. Tissue was taken from the same animal as that shown in panel B. Gray: DAPI, marking all nuclei. Cyan: EdU, conjugated to dye after fixation, shows an increased number of cells that have synthesized DNA *ex vivo*, compared to one hour treatment. Box: location of the inset, C'. Scale bar = 10 μm . (C') Inset from panel C showing a progenitor cell positive for EdU. Magenta: staining for β -tubulin III, showing that the cell is neuronally-fated. Yellow: eGFP-centrin 2 shows signal consistent with centriole amplification. Cyan: EdU. Scale bar = 2 μm .
- (D) Control image of centriole group position in explants treated with DMSO for 6 hours. Single plane fluorescence image. Green: eGFP-centrin 2. Magenta: dye-conjugated phalloidin. Dashed line: apical surface. Arrows: migrating centriole groups. Scale bar = 10 μm .
- (E) Centriole group position in explants treated with paclitaxel for 6 hours. Single plane fluorescence image. Tissue was taken from the same animal as that shown in Fig 5D. Green: eGFP-centrin 2. Magenta: dye-conjugated phalloidin. Arrows: migrating centriole groups. Compared to Fig 5D, many centrioles are found below the apical surface. Dashed line: apical surface. Scale bar = 10 μm .
- (F) Bar plot summarizing centriole group position in explants grown for 1 or 6 hours in the presence of DMSO or paclitaxel. Green bars: migrating centriole groups in the subapical

compartment of the epithelium (defined in Fig 1A). Black bars: centriole groups in the middle and basal regions of the epithelium (defined in Fig 1A). Magenta circles: normalized number of groups for the female sample. Magenta squares: normalized number of groups for the male sample. Counts were normalized to the lateral length of the basal lamina. Length of epithelium scored for each timepoint: 1 hour DMSO = 988.17 μm , 1 hour paclitaxel = 905.37 μm , 6 hours DMSO = 1126.44 μm , 6 hours paclitaxel = 1019.76 μm . N=2. See Figure 5 – Source Data 1 for values.

Figure 6. Summary of centriole migration and maturation during OSN differentiation.

Diagram summarizing the migration and maturation of centrioles over the course of OSN differentiation. A double solid line marks the basal lamina, and a dashed line is drawn above the apical surface. Newly-formed centrioles lacking appendages are represented as green dots, centrioles with appendages as magenta dots, and the parental centriole as a magenta dot with a yellow outline. After centriole amplification and mitosis in the progenitor, the immature OSN extends a dendrite toward the apical surface. Centrioles migrate primarily in groups during dendrite elongation. At the apical surface, a single cilium forms from the appendage-bearing, parental centriole, before multiple cilia form in the mature OSN.

References

- Al Jord, A., Shihavuddin A., d'Aout R.S., Faucourt M., Genovesio A., Karaïskou A., Sobczak-Thépot J., Spassky N., Meunier A. (2017). Calibrated mitotic oscillator drives motile ciliogenesis. *Science* 358(6364):803-806.
- Anderson R.G.W., Brenner R.M. (1971). The formation of basal bodies (centrioles) in the rhesus monkey oviduct. *J. Cell Biol.* 50:10-34.
- Arquint C., Nigg E.A. (2014). STIL microcephaly mutations interfere with APC/C-mediated degradation and cause centriole amplification. *Curr Biol.* 24(4):351-60.
- Bangs, F.K., Schrode, N., Hadjantonakis, A.K., and Anderson, K. V. (2015). Lineage specificity of primary cilia in the mouse embryo. *Nat. Cell Biol.* 17, 113–122.
- Baas, P.W., and Buster, D.W. (2004). Slow Axonal Transport and the Genesis of Neuronal Morphology. *J. Neurobiol.* 58, 3–17.
- Baas, P.W., Rao, A.N., Matamoros, A.J., and Leo, L. (2016). Stability properties of neuronal microtubules. *Cytoskeleton (Hoboken).* 73, 442–460
- Boixvieux-Ulrich E., Laine M-C, Sandoz D. (1989). In vitro effects of taxol on ciliogenesis in quail oviduct. *J. Cell Sci.* 92 (Pt 1):9-20.
- Bolivar J., Huynh J.R., Lopez-Schier H., Gonzalez C., St. Johnston D., Gonzalez-Reyes A. (2001). Centrosome migration into the *Drosophila* oocyte is independent of BicD and egl, and of the organisation of the microtubule cytoskeleton. *Development* 128(10):1889-97.
- Breslow D.K., Holland A.J. (2019). Mechanism and Regulation of Centriole and Cilium Biogenesis. *Annu Rev Biochem.* 88:691-724.
- Burakov, A. V., and Nadezhdina, E.S. (2020). Centering and Shifting of Centrosomes in Cells. *Cells* 9(6): 1351.
- Burton, P.R. (1985). Ultrastructure of the olfactory neuron of the bullfrog: the dendrite and its

900 microtubules. *J. Comp. Neurol.* 242, 147–160.

901 Cajanek L., and Nigg E.A. (2014). Cep164 triggers ciliogenesis by recruiting Tau tubulin kinase
902 2 to the mother centriole. *Proc. Nat. Acad. Sci.* 111 (28) E2841-E2850.

903 Challis R.C., Tian H., Wang J., He J., Jiang J., Chen X., Yin W., Connelly T., Ma L., Yu C.R.,
904 Pluznick J.L., Storm D.R., Huang L., Zhao K., Ma M. (2015). An Olfactory Cilia Pattern in
905 the Mammalian Nose Ensures High Sensitivity to Odors. *Curr Biol.* 25(19):2503-12.

906 Ching, K., and Stearns, T. (2020). Centrioles are amplified in cycling progenitors of olfactory
907 sensory neurons. *PLoS Biol.* 18, 1–17.

908 Choi Y-W., Liu P., Sze S.K., Dai C., Qi R.Z. (2010). CDK5RAP2 stimulates microtubule
909 nucleation by the γ -tubulin ring complex. *J. Cell Biol.* 191 (6): 1089–1095.

910 Cuschieri A., Bannister L.H. (1975). The development of the olfactory mucosa in the mouse:
911 Electron microscopy. *J. Anat.* 119:471-498.

912 Dawe, H. R., Farr, H., & Gull, K. (2007). Centriole/basal body morphogenesis and migration
913 during ciliogenesis in animal cells. *Journal of Cell Science*, 120(1), 7–15.

914 Delaval B., Doxsey S.J. (2010). Pericentrin in cellular function and disease. *J. Cell Biol.* 188(2):
915 181–190.

916 Farbman, A. I. (1977). Differentiation of olfactory receptor cells in organ culture. *The Anatomical*
917 *Record*, 189(2), 187–199.

918 Fong K-W., Choi Y-W., Rattner J.B., Qi R.Z. (2008). CDK5RAP2 is a pericentriolar protein that
919 functions in centrosomal attachment of the gamma-tubulin ring complex. *Mol. Biol. Cell.*
920 19(1):115-25.

921 Gambarotto, D., Zwettler, F.U., Le Guennec, M., Schmidt-Cernohorska, M., Fortun, D., Borgers,
922 S., Heine, J., Schloetel, J.G., Reuss, M., Unser, M., et al. (2019). Imaging cellular
923 ultrastructures using expansion microscopy (U-ExM). *Nat. Methods* 16, 71–74.

924 Hatch, E.M., Kulukian, A., Holland, A.J., Cleveland, D.W., and Stearns, T. (2010). Cep152
925 interacts with Plk4 and is required for centriole duplication. *J. Cell Biol.* 191, 721–729.

926 Heist, H.E., and Mulvaney, B.D. (1968). Centriole migration. *J Ultrastruct Res* 24, 86–101.

927 Izquierdo D., Wang W-J, Uryu K., Tsou M.F.B. (2014). Stabilization of cartwheel-less centrioles
928 for duplication requires CEP295-mediated centriole-to-centrosome conversion. *Cell Rep.*
929 8(4):957-65

930 Jain R., Pan J., Driscoll J.A., Wisner J.W., Huang T., Gunsten S.P., You Y., Brody S.L. (2010).
931 Temporal Relationship between Primary and Motile Ciliogenesis in Airway Epithelial
932 Cells. *Am J Respir Cell Mol Biol.* 43(6): 731–739.

933 Jenkins P.M., McEwen, D.P., Martens, J.R. (2009). Olfactory Cilia: Linking Sensory Cilia
934 Function and Human Disease. *Chem Senses*, 34(5):451-464.

935 Jurczyk A., Gromley A., Redick S., San Agustin J., Witman G., Pazour G.J., Peters D.J.M.,
936 Doxsey S. (2004). Pericentrin forms a complex with intraflagellar transport proteins and
937 polycystin-2 and is required for primary cilia assembly. *J. Cell Biol.* 166(5): 637–643.

938 Klimenkov, I. V., Sudakov, N.P., Pastukhov, M. V., Svinov, M.M., and Kositsyn, N.S. (2018).
939 Rearrangement of Actin Microfilaments in the Development of Olfactory Receptor Cells
940 in Fish. *Sci. Rep.* 8, 1–12.

941 King S.J., Schroer T.A. (1999). Dynactin increases the processivity of the cytoplasmic dynein
942 motor. *Nat Cell Biol* 2, 20–24.

943 Kong D., Farmer V., Shukla A., James J., Gruskin R., Kiriya S., Loncarek J. (2014). Centriole
944 maturation requires regulated Plk1 activity during two consecutive cell cycles. *J. Cell*
945 *Biol.* 206(7):855-65.

946 Kulaga, H.M., Leitch, C.C., Eichers, E.R., Badano, J.L., Lesemann, A., Hoskins, B.E., Lupski,
947 J.R., Beales, P.L., Reed, R.R., and Katsanis, N. (2004). Loss of BBS proteins causes
948 anosmia in humans and defects in olfactory cilia structure and function in the mouse.
949 *Nat. Genet.* 36, 994–998.

950 Leask, A., Obrietan, K., and Stearns, T. (1997). Synaptically coupled central nervous system
951 neurons lack centrosomal γ - tubulin. *Neurosci. Lett.* 229, 17–20.

952 Lemullois M., Boisvieux-Ulrich E., Laine M-C, Chailley B., Sandoz D. (1988). Development and
953 functions of the cytoskeleton during ciliogenesis in metazoa. *Biology of the Cell*. 63,195-
954 208.

955 Lee Y.L., Santé J., Commerci C.J., Cyge B., Menezes L.F., Li F.Q., Germino G.G., Moerner W.E.,
956 Takemaru K., Stearns T. (2014) Cby1 promotes Ahi1 recruitment to a ring-shaped
957 domain at the centriole-cilium interface and facilitates proper cilium formation and
958 function. *Molecular Biology of the Cell* 25:2919–2933.

959 Li, W., Yi, P., Zhu, Z., Zhang, X., Li, W., and Ou, G. (2017). Centriole translocation and
960 degeneration during ciliogenesis in *Caenorhabditis elegans* neurons. *EMBO J*.
961 e201796883.

962 Liang, F. (2018). Olfactory receptor neuronal dendrites become mostly intra-sustentacularly
963 enwrapped upon maturity. *J. Anat.* 232, 674–685.

964 Liang, X., Kokes, M., Fetter, R.D., Sallee, M.D., Moore, A.W., Feldman, J.L., and Shen, K.
965 (2020). Growth cone-localized microtubule organizing center establishes microtubule
966 orientation in dendrites. *Elife* 9, 1–28.

967 Liu, Z., Nguyen, Q. P. H., Nanjundappa, R., Delgehyr, N., Megherbi, A., Doherty, R., Thompson,
968 J., Jackson, C., Albulescu, A., Heng, Y. M., Lucas, J. S., Dell, S. D., Meunier, A.,
969 Czymmek, K., Mahjoub, M. R., & Mennella, V. (2020). Super-Resolution Microscopy and
970 FIB-SEM Imaging Reveal Parental Centriole-Derived, Hybrid Cilium in Mammalian
971 Multiciliated Cells. *Developmental Cell*, 55(2), 224-236.

972 Mahowald, A. P., & Strassheim, J. M. (1970). Intercellular migration of centrioles in the
973 germarium of *Drosophila Melanogaster*: An electron microscopic study. *Journal of Cell*
974 *Biology*, 45(2), 306–320.

975 McClintock, T. S., Glasser, C. E., Bose, S. C., & Bergman, D. A. (2008). Tissue expression
976 patterns identify mouse cilia genes. *Physiological Genomics*, 32(2), 198–206.

977 Menco, B. P. M., Leunissen, J. L. M., Bannister, L. H., & Dodd, G. H. (1978). Bovine olfactory

978 and nasal respiratory epithelium surfaces - High-voltage and scanning electron
979 microscopy, and cryo-ultramicrotomy. *Cell and Tissue Research*, 193(3), 503–524.

980 Menco BP (1997) Ultrastructural aspects of olfactory signaling. *Chem Senses* 22: 295–311

981 McEwen DP, Koenekoop RK, Khanna H, Jenkins PM, Lopez I, Swaroop A, Martens JR. (2007).
982 Hypomorphic CEP290/NPHP6 mutations result in anosmia caused by the selective loss
983 of G proteins in cilia of olfactory sensory neurons. *Proc Natl Acad Sci USA*.104:15917–
984 15922.

985 McEwen D.P., Jenkins P.M., Martens J.R. (2008). Olfactory Cilia: Our Direct Neuronal
986 Connection to the External World. *Curr. Top. Dev. Biol.* 85:333-370.

987 McIntyre, J.C., Titlow, W.B., and McClintock, T.S. (2010). Axon Growth and Guidance Genes
988 Identify Nascent, Immature, and Mature Olfactory Sensory Neurons. *J Neurosci Res* 88,
989 3243–3256.

990 Moulton, D. G., & Beidler, L. M. (1967). “Physiological Reviews.” *The American Physiological*
991 *Society*, 47(4), 1–52.

992 Mulvaney, B.D., and Heist, H.E. (1971). Centriole migration during regeneration and normal
993 development of olfactory epithelium. *J. Ultrastructure Res.* 35, 274–281.

994 Nigg E.A., Stearns T. (2011). The centrosome cycle: Centriole biogenesis, duplication and
995 inherent asymmetries. *Nat. Cell Biol.* 13(10):1154-60.

996 Oberland, S., and Neuhaus, E.M. (2014). Whole Mount Labeling of Cilia in the Main Olfactory
997 System of Mice. *J. Vis. Exp.* 6–11.

998 Presley J.F., Cole N.B., Schroer T.A., Hirschberg K., Zall K.J.M., Lippincott-Schwartz J. (1997).
999 ER-to-Golgi transport visualized in living cells. *Nature* 389, 81–85.

1000 Rodriguez-Gil, D.J., Bartel, D.L., Jaspers, A.W., Mobley, A.S., Imamura, F., and Greer, C.A.
1001 (2015). Odorant receptors regulate the final glomerular coalescence of olfactory sensory
1002 neuron axons. *Proc. Natl. Acad. Sci.* 112, 5821–5826.

1003 Roy S. (2020). Finding order in slow axonal transport. *Current Opinion in Neurobiology* 63, 87-

1004 94.

1005 Sahabandu N., Kong D., Magidson V., Nanjundappa R., Sullenberger C., Mahjoub M.R.,

1006 Loncarek J. (2019). Expansion microscopy for the analysis of centrioles and cilia. *J*

1007 *Microsc.* 276(3):145-149.

1008 Sanchez-Huertas C., Freixo F., Viais R., Lacasa C., Soriano E., Luders J. (2016). Non-

1009 centrosomal nucleation mediated by augmin organizes microtubules in post-mitotic

1010 neurons and controls axonal microtubule polarity. *Nat Comm* 7:12187.

1011 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch,

1012 S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: An open-source platform for

1013 biological-image analysis. *Nat. Methods* 9, 676–682.

1014 Shukla A., Kong D., Sharma M., Magidson V., Loncarek J. (2015). Plk1 relieves centriole block

1015 to reduplication by promoting daughter centriole maturation. *Nat. Comm.* 6:8077.

1016 Sorokin S.P. (1968). Reconstructions of centriole formation and ciliogenesis in mammalian

1017 lungs. *J. Cell Sci.* 3,207-230.

1018 Stiess, M., Maghelli, N., Kapitein, L.C., Gomis-Ruth, S., Wilsch-Bräuninger, M., Hoogenraad,

1019 C.C., Tolic-Norrelykke, I.M., and Bradke, F. (2010). Axon Extension Occurs

1020 Independently of Centrosomal Microtubule Nucleation. *Science.* 327, 704–707.

1021 Strnad P., Leidel S., Vinogradova T., Euteneuer U., Khodjakov A., Gonczy P. (2007). Regulated

1022 HsSAS-6 Levels Ensure Formation of a Single Procentriole per Centriole during the

1023 Centrosome Duplication Cycle. *Dev. Cell* 13(2)203-213.

1024 Schwarzenbacher, K., Fleischer, J., & Breer, H. (2005). Formation and maturation of olfactory

1025 cilia monitored by odorant receptor-specific antibodies. *Histochemistry and Cell Biology*,

1026 123(4–5), 419–428.

1027 Tamm S., Tamm S.L. (1988). Development of macrociliary cells in Beroe. I. Actin bundles and

1028 centriole migration. *J Cell Sci* 89 (1): 67–80.

1029 Tanos B.E., Yang H-J., Soni R., Wang W-J., Macaluso F.P., Asara J.M., Tsou M-F B. (2013).

1030 Centriole distal appendages promote membrane docking, leading to cilia initiation.
1031 Genes and Dev. 2013. 27: 163-168.

1032 Uytingco C.R., Williams C.L., Xie C., Shively D.T., Green W.W., Ukhanov K., Zhang L.,
1033 Nishimura D.Y., Sheffield V.C., Martens J.R. (2019). BBS4 is required for intraflagellar
1034 transport coordination and basal body number in mammalian olfactory cilia. J. Cell Sci.
1035 132(5):jcs222331.

1036 Ukhanov K., Uytingco C., Green W., Zhang L., Schurmans S., Martens J. (2022). INPP5E
1037 controls ciliary localization of phospholipids and the odor response in olfactory sensory
1038 neurons. J. Cell Sci. 135(5):jcs258364.

1039 Vladar E.K., Bayly R.D., Sangoram A., Scott M.P., Axelrod J.D. (2012). Microtubules Enable the
1040 Planar Cell Polarity of Airway Cilia. Curr Biol. 22(23):2203-12.

1041 Vlijm, R., Li, X., Panic, M., Rthnick, D., Hata, S., Hermannsdrfer, F., Kner, T., Heilemann,
1042 M., Engelhardt, J., Hell, S.W., et al. (2018). STED nanoscopy of the centrosome linker
1043 reveals a CEP68-organized, periodic rootletin network anchored to a C-Nap1 ring at
1044 centrioles. Proc. Natl. Acad. Sci. U. S. A. 201716840.

1045 Williams, C. L., McIntyre, J. C., Norris, S. R., Jenkins, P. M., Zhang, L., Pei, Q., ... Martens, J.
1046 R. (2014). Direct evidence for BBSome-associated intraflagellar transport reveals distinct
1047 properties of native mammalian cilia. *Nature Communications*, 5, 5813.

1048 Wilkes, O.R., and Moore, A.W. (2020). Distinct microtubule organizing center mechanisms
1049 combine to generate neuron polarity and arbor complexity. Front. Cell. Neurosci.
1050 19;14:594199.

1051 Wang W-J, Soni R.K., Uryu K., Tsou M.F.B. (2011). The conversion of centrioles to
1052 centrosomes: essential coupling of duplication with segregation. J. Cell Biol. 193(4):
1053 727–739.

1054 Yang, J., Liu, X., Yue, G., Adamian, M., Bulgakov, O., and Li, T. (2002). Rootletin, a novel
1055 coiled-coil protein, is a structural component of the ciliary rootlet. J. Cell Biol. 159, 431–

1056 440.

1057 Ying G., Avasthi P., Irwin M., Gerstner C.D., Frederick J.M., Lucero M.T., Baehr W. (2014).

1058 Centrin 2 Is Required for Mouse Olfactory Ciliary Trafficking and Development of

1059 Ependymal Cilia Planar Polarity. *J. Neurosci.* 34(18): 6377–6388.

1060 Yonezawa S., Shigematsu M., Hirata K., Hayashi K. (2015). Loss of γ -tubulin, GCP-WD/NEDD1

1061 and CDK5RAP2 from the Centrosome of Neurons in Developing Mouse Cerebral and

1062 Cerebellar Cortex. *Acta Histochem Cytochem.*48(5): 145–152.

1063 Zhao H., Chen Q., Li F., Cui L., Xie L., Huang Q., Liang X., Zhou J., Yan X., Zhu X. (2021).

1064 Fibrogranular materials function as organizers to ensure the fidelity of multiciliary

1065 assembly. *Nat Comm.* 12(1):1273.

1066 Zhou R., Liu H., Ju T., Dixit R. (2020). Quantifying the polymerization dynamics of plant cortical

1067 microtubules using kymograph analysis. *Methods Cell Biol.*160:281-293.

1068

Figure 1

Figure 1 - Supplement 1

Figure 2

A

B

C

D

Figure 3

Figure 4

Figure 4 - Supplement 1

Figure 5**A** **B** **D** **E** **F**

Figure 6