

# Golgi Outposts Locally Regulate Microtubule Orientation in Neurons but Are Not Required for the Overall Polarity of the Dendritic Cytoskeleton

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**ABSTRACT** Microtubule-organizing centers often play a central role in organizing the cellular microtubule networks that underlie cell function. In neurons, microtubules in axons and dendrites have distinct polarities. Dendrite-specific Golgi “outposts,” in particular multicompartment outposts, have emerged as regulators of acentrosomal microtubule growth, raising the question of whether outposts contribute to establishing or maintaining the overall polarity of the dendritic microtubule cytoskeleton. Using a combination of genetic approaches and live imaging in a *Drosophila* model, we found that dendritic microtubule polarity is unaffected by eliminating known regulators of Golgi-dependent microtubule organization including the *cis*-Golgi matrix protein GM130, the fly AKAP450 ortholog pericentrin-like protein, and centrosomin. This indicates that Golgi outposts are not essential for the formation or maintenance of a dendrite-specific cytoskeleton. However, the overexpression of GM130, which promotes the formation of ectopic multicompartment units, is sufficient to alter dendritic microtubule polarity. Axonal microtubule polarity is similarly disrupted by the presence of ectopic multicompartment Golgi outposts. Notably, multicompartment outposts alter microtubule polarity independently of microtubule nucleation mediated by the  $\gamma$ -tubulin ring complex. Thus, although Golgi outposts are not essential to dendritic microtubule polarity, altering their organization correlates with changes to microtubule polarity. Based on these data, we propose that the organization of Golgi outposts is carefully regulated to ensure proper dendritic microtubule polarity.

**KEYWORDS** *Drosophila*; Golgi; microtubules; neuron

**P**ROPER neuronal structure and function depends on the underlying microtubule cytoskeleton, which is uniquely organized in axons and dendrites. The compartment-specific orientation of microtubules is thought to contribute to the specific morphologies and functions of these compartments. In axons, microtubules are uniformly oriented with their plus-ends positioned distal to the cell body. In contrast, microtubule polarity in dendrites is mixed to varying degrees, and the percentage of microtubules of a particular orientation differs locally within the dendritic arbor. How the distinctly organized cytoskeletons in axons and dendrites are created and maintained remains an open question. Microtubules are often

generated at and organized by cellular structures called microtubule-organizing centers (MTOCs), which anchor and stabilize microtubules and support microtubule nucleation (Sanchez and Feldman 2017; Wu and Akhmanova 2017). The centrosome is one example of a well-studied MTOC. Although neurons have a centrosome, recent work indicates that the neuronal centrosome does not have a major role in either generating or anchoring dendritic or axonal microtubules (Stiess *et al.* 2010; Nguyen *et al.* 2011; Sánchez-Huertas *et al.* 2016). However, the centrosome is not the only organelle that functions as an MTOC (Sanchez and Feldman 2017; Wu and Akhmanova 2017). The Golgi apparatus and nonconventional Golgi structures such as Golgi elements and Golgi outposts have emerged as potential MTOCs in several cell types, including epithelia, muscles, and neurons (Rios 2014; Sanders and Kaverina 2015; Martin and Akhmanova 2018). Unlike centrosomes, which support the nucleation of microtubules radially, Golgi-based microtubule nucleation can create asymmetric microtubule arrays (Efimov *et al.*

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2007; Zhu and Kaverina 2013). Such asymmetric Golgi-derived microtubule arrays have also been shown to contribute to cell polarity (Zhu and Kaverina 2013; Rios 2014). Thus, Golgi in neurons have the potential to shape the polarity of the microtubule cytoskeleton, and influence neuronal polarity, by selectively seeding or stabilizing microtubules in a particular orientation.

In developing flies and mammals, many neurons have Golgi in the form of mini stacks called outposts that localize specifically to dendrites and have a different structure than the somatic Golgi (Gardiol *et al.* 1999; Pierce *et al.* 2001; Horton and Ehlers 2003; Ye *et al.* 2007; Liu *et al.* 2017; Rao *et al.* 2018; Tann and Moore 2019). The term Golgi outposts is used to refer generally to a heterogeneous population of dendritic Golgi composed of one or more compartments. In studies using fluorescently tagged End-binding 1 (EB1::GFP), which marks growing microtubule ends, Golgi outposts have been shown to correlate with microtubule growth initiation sites in developing dendrites (Ori-McKenney *et al.* 2012; Zhou *et al.* 2014; Yalgin *et al.* 2015). Decreasing the levels of proteins involved in microtubule nucleation, such as  $\gamma$ -tubulin and its interactors centrosomin (*cnn*, the fly ortholog of CDK5RAP2) and pericentrin-like protein (*plp*, the *Drosophila* ortholog of AKAP450), disrupts the correlation between microtubule growth initiation sites and outposts, and perturbs dendrite branch growth (Ori-McKenney *et al.* 2012; Yalgin *et al.* 2015). This correlation has led to the model that Golgi outposts function as MTOCs that support acentrosomal nucleation and the directional growth of microtubules during dendrite branch formation (Delandre *et al.* 2016). These studies have focused on the connection between Golgi outposts, microtubule growth, and dendrite growth, leaving unanswered the broader question of whether outposts have a role in creating and/or maintaining the distinct polarity of the dendritic microtubule cytoskeleton. Notably, the link between microtubule growth and Golgi outposts relies on the compartmental organization of outposts: multicompartment dendritic Golgi outposts are more likely than single-compartment outposts to correlate with microtubule growth start sites (Zhou *et al.* 2014), and dragging individual Golgi compartments into the axon is not sufficient to alter axonal microtubule organization (Nguyen *et al.* 2014). The dendrite-specific localization of Golgi outposts and their MTOC potential raises the possibility that Golgi outposts, and in particular multicompartment outposts, may play a role in establishing and/or maintaining the unique polarity of the dendritic microtubule cytoskeleton. However, this idea has not been tested.

Here, we investigated whether Golgi outposts are necessary for the compartment-specific orientation of dendritic microtubules. In particular, we asked whether outposts are necessary for the formation of the minus-end-distal microtubules that are specific to the dendritic cytoskeleton. To test this notion, we leveraged a combination of genetics and live imaging, and used the class IV dendritic arborization (*da*) neurons in *Drosophila* as a model. In the class IV *da* neurons,

dendritic microtubules are predominately oriented with their minus-ends distal to the cell body, providing an advantageous paradigm to detect changes in microtubule orientation. If Golgi outposts regulate the overall polarity of the dendritic microtubule cytoskeleton, then blocking the MTOC activity of Golgi should disrupt the stereotyped minus-end-distal orientation of dendritic microtubules. We targeted the *cis*-Golgi matrix protein GM130, which has two key roles. First, work done in mammalian cells has shown that GM130 recruits AKAP450, which in turn recruits protein complexes that nucleate, tether, and stabilize microtubules (Rivero *et al.* 2009; Hurtado *et al.* 2011; Roubin *et al.* 2013; Wu and Akhmanova 2017). Second, GM130 is needed for proper Golgi structure and connects Golgi compartments to form multicompartment units, including outposts (Nakamura *et al.* 1995; Barr *et al.* 1997; Kondylis *et al.* 2005; Zhou *et al.* 2014; Liu *et al.* 2017; Lowe 2019). We found that the global orientation of dendritic microtubules is unaffected by the loss of GM130 or the fly AKAP450 ortholog *plp*. *Cnn*, which is proposed to orient Golgi outpost-associated microtubule growth (Yalgin *et al.* 2015), is also dispensable. This suggests that Golgi outposts do not have an essential role in establishing the overall polarity of the dendritic microtubule cytoskeleton. However, our studies of GM130 overexpression reveal that inducing the formation of ectopic multicompartment units correlates with a disruption in microtubule polarity. This suggests that compartment connectedness is critical to the microtubule organization capacity of outposts. Interestingly, the ability of multicompartment outposts to organize microtubules is independent of  $\gamma$ -tubulin ring complex ( $\gamma$ -TuRC)-mediated microtubule nucleation, which suggests that outposts can regulate microtubule polarity via microtubule anchoring or stabilizing.

## Materials and Methods

### Fly stocks

The following alleles and transgenic fly strains from the Bloomington *Drosophila* Stock Center (BDSC) and individual laboratories were used as follows: *cnn*<sup>HK21</sup> (Megraw *et al.* 1999; Vaizel-Ohayon and Schejter 1999) (BDSC 5039), which produces a drastically truncated  $\sim$ 106 amino acid-long protein that is not detectable by western blot, and *Df(2R)BSC306* (BDSC 23689) were used to eliminate *cnn*; *GM130* <sup>$\Delta$ 23</sup>, a protein null allele (Zhou *et al.* 2014) (BDSC 65255), and *Df(2R)Exel7170* (BDSC 7901) were used to eliminate GM130; *UAS-GM130::eBFP* (Zhou *et al.* 2014) (BDSC 65254) and *ppk-Gal4* (BDSC 32079) were used to overexpress GM130; *dGrip75*<sup>175</sup> (Schnorrer *et al.* 2002) (Conduit laboratory, University of Cambridge and Raff laboratory, University of Oxford) and *Df(2L)Exel7048* (BDSC 7999) were used to eliminate *dGrip75*; *Khc*<sup>E177K</sup> (Kelliher *et al.* 2018) was used *in trans* to the null allele *Khc*<sup>27</sup> (Brendza *et al.* 1999) (Saxton laboratory, University of California, Santa Cruz); *nudE*<sup>39A</sup> (Wainman *et al.* 2009) (Goldberg laboratory, Cornell

University), a protein null allele, and *Df(3L)BSC673* (BDSC 26525) were used to eliminate *nudE*; *plp<sup>5</sup>* (BDSC 9567) (Martinez-Campos *et al.* 2004), an EMS-induced loss-of-function allele that strongly reduces *plp* levels, and *Df(3L)Brd15*, *pp* (BDSC 5354) were used to eliminate *plp* activity; *ppk-ManII::GFP* (Jenkins *et al.* 2017) and *UAS-GalNacT2::TagRFP* (Zhou *et al.* 2014) (Ye laboratory, University of Michigan) were used to label *medial* and *trans* Golgi compartments, respectively; *ppk-CD4::GFP* (BDSC 35842 and BDSC 35843) and *ppk-CD4::tdTom* (Han *et al.* 2011) (BDSC 35844 and BDSC 35845) were used to visualize neuron morphology; and *ppk-EB1::GFP* (Arthur *et al.* 2015) was used to analyze microtubule polarity and dynamics.

### Live imaging

Fly crosses for live imaging were set up using five to eight virgin females and four to six young males; larvae were collected in 12-hr intervals and aged to the desired developmental stage (larvae produced in the first 24–48 hr after mating were not used). Larvae of the desired genotype were washed with 1× phosphate-buffered saline (PBS), mounted in a 50:50 1× PBS:glycerol solution on a slide between two strips of vacuum grease, and immobilized by pressing on a coverslip mounted on top of the larva and vacuum-grease spacers. The dorsal class IV ddaC neurons within abdominal segments 2–4 were imaged with a 40 × 1.3 numerical aperture oil immersion objective. All imaging was performed on a Leica SP5 (Leica Microsystems) using hybrid detector (HyD) photodetectors.

### Microtubule polarity and growth analysis

Microtubule polarity and growth were analyzed by scoring EB1::GFP comets within 150 μm of the cell body in dendrites or axons. EB1::GFP comet trajectories were captured at a resolution of 1024 × 512 pixels and a rate of 0.86 sec per frame for 5–7 min. One or two ddaC neurons per larva were imaged at 96–120 hr after egg laying (AEL). Videos were stabilized with the stabilizer plugin in FIJI ImageJ (ImageJ; National Institutes of Health); kymographs were generated in Metamorph (Molecular Devices). Movies that did not contain at least two comets were excluded. Comet trajectories were manually traced, and the position and time coordinates were recorded to calculate comet direction (microtubule orientation) and frequency. A comet trajectory was included only if it could be clearly traced in at least 12 continuous frames (~11 sec). Anterograde comets traveled away from the cell body whereas retrograde comets traveled toward the cell body. The frequency of EB1::GFP comets was calculated as the number of comets present in a 100-μm segment (axons) per minute. To calculate microtubule polarity in dendrites, EB1::GFP comets were scored in a segment ≥ 30 μm between two branch points. To identify the origin of anterograde EB1::GFP comets in dendrites, dendrite segments within 100 μm of the cell body that contained at least one comet were selected for analysis. Comet trajectories were identified in kymographs, and any anterograde comets were then traced back to their origin in the corresponding movie.

### Axon branching analysis

Axon morphology was visualized at late third instar (120–144 hr AEL) using CD4::GFP and CD4::Tomato. Images were captured at 1024 × 1024 resolution and 1-μm z-steps (5–15 steps in total). Analysis was performed on axons within 150 μm of the cell body. Z-stacks 5–15-μm thick were maximally projected for analysis. Axon branching was assessed manually by determining whether an axon had split into one or more branches.

### Golgi compartment analysis

Images of ManII::GFP and GalNacT2::TagRFP puncta in the dendrites and axons of neurons in 96–120 hr AEL larvae were captured at a resolution of 1024 × 1024 pixels and 0.75-μm z-steps over 5–15 μm. Analysis of Golgi outposts in axons included outposts within 100 μm of the cell body. For dendrite analysis, Golgi outposts throughout the entire arbors were included and split into two groups: (1) those within a radius of 95 μm of the cell body and (2) those outside this radius. One to two ddaC neurons were imaged per larva. Analysis was performed on the maximally projected images. Signals outside the regions of interest (ROI) in axons and dendrites were masked. Masked images were subjected to a threshold gray value of 100 for segmentation. The segmented signals were quantified with the ImageJ particle analysis function with the size cutoff of 0.10–15 μm<sup>2</sup>. Puncta outlines were saved as ROIs. The resulting particle numbers and sizes were exported to Excel for analysis. For multicompartment analysis, overlapping compartments were manually scored by overlaying the puncta outlines from each channel, namely ManII::GFP and GalNacT2::TagRFP. Golgi units were scored as multicompartment when the ManII::GFP and GalNacT2::TagRFP signals overlapped.

### Statistical analysis

Statistical analyses were performed using GraphPad Prism8. The Anderson–Darling and Shapiro–Wilk tests were used to determine whether data were normally distributed. For normally distributed data, Student's unpaired *t*-tests were used to compare two groups; one-way ANOVA with a *post hoc* Tukey test was used for multiple comparisons. For non-normally distributed data, a Mann–Whitney *U*-test was used to compare two groups and a Kruskal–Wallis test followed by a *post hoc* Dunn test was used for multiple comparisons. Fisher's exact test was used for comparing proportions. *P* = 0.05 was used as a cutoff for significance. Significance levels are represented as: \**P* = 0.05–0.01, \*\**P* = 0.01–0.001, \*\*\**P* = 0.001–0.0001, and \*\*\*\**P* < 0.0001. n.s. indicates not significant.

### Data availability

All strains are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are represented fully within the article and its figures.

## Results

### ***Golgi outposts are not essential to the overall polarity of the dendritic microtubule cytoskeleton***

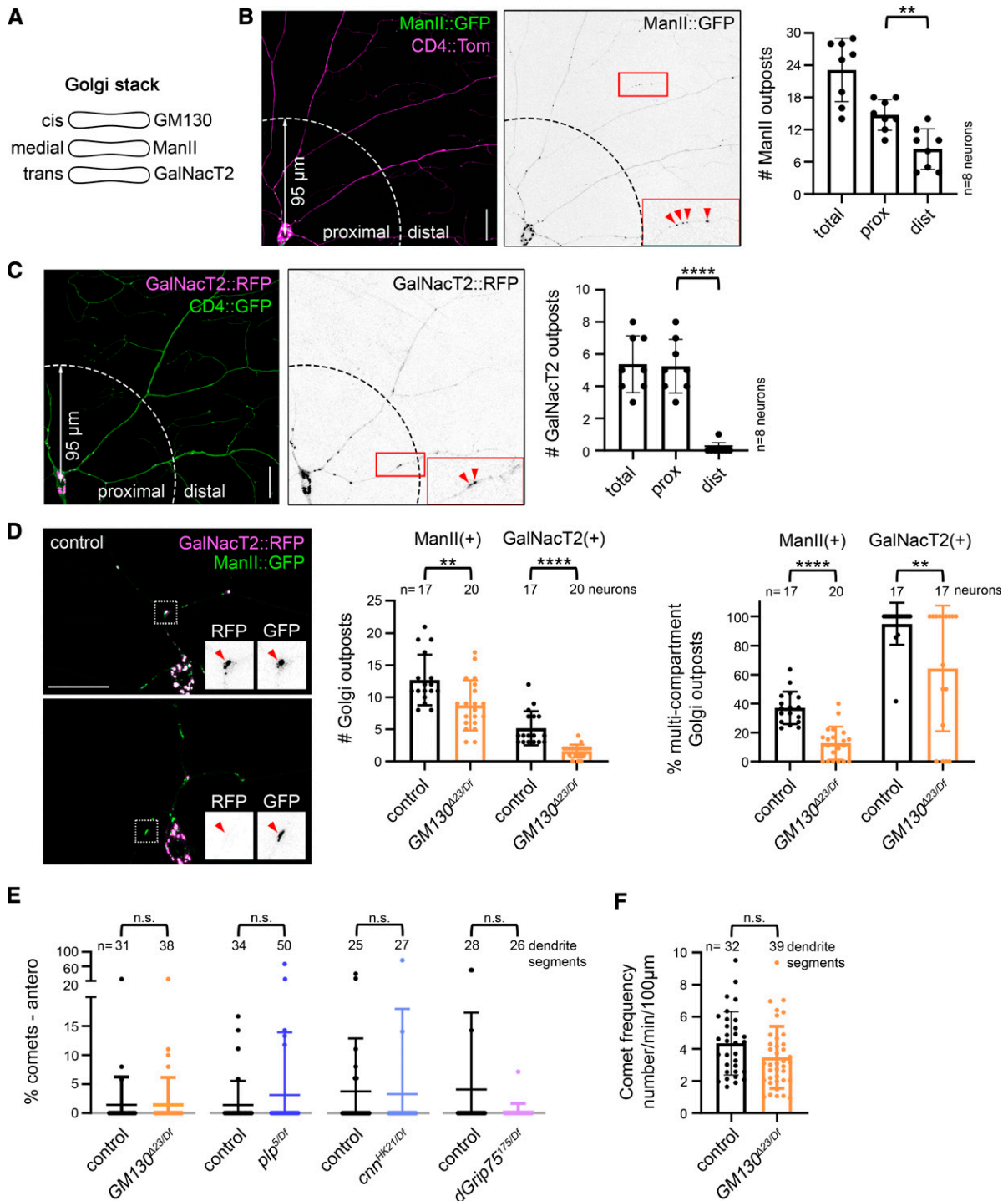
To determine whether Golgi outposts play a critical role in determining the unique polarity of the dendritic microtubule cytoskeleton, we turned to the class IV da neurons in *Drosophila* as a model. The da neurons are an ideal model as they are easily accessible for live imaging, the orientation of microtubules in the dendritic microtubule cytoskeleton is well defined, and there is a wealth of tools for labeling and manipulating Golgi, microtubules, and microtubule regulators. In the class IV da neurons, Golgi compartments are present in the cell body and throughout the dendritic arbor, but most are found close to the cell body (Figure 1, A–C). Indeed, GalNacT2-positive *trans* Golgi compartments are seldom present beyond 95  $\mu\text{m}$  of the cell body. Next, we quantified the number of multicompartment outposts in the arbor. Consistent with previous work, we defined multicompartment Golgi outposts as units with at least two compartments (Zhou *et al.* 2014). Here, we used markers of the *medial* and *trans* Golgi, namely ManII::GFP and GalNacT2::TagRFP, respectively. Fewer than one-half of the ManII-positive Golgi outposts colocalized with GalNacT2, indicating ManII::GFP predominantly labels single-compartment Golgi outposts (Figure 1D). In contrast, the majority of GalNacT2-positive outposts colocalized with ManII::GFP (Figure 1D), which suggests that most multicompartment Golgi outposts cluster relatively close to the cell body. Given that Golgi-associated microtubule growth correlates predominantly with multicompartment outposts (Zhou *et al.* 2014), this suggests that outposts in the proximal arbor may be most likely to regulate microtubule organization.

To determine whether Golgi outposts function to create or maintain the dendrite-specific orientation of microtubules, we analyzed microtubule polarity in neurons in which we eliminated the *cis*-Golgi matrix protein GM130. GM130 both recruits the protein machinery for MTOC activities (microtubule nucleation, anchoring, and stabilization) and contributes to forming multicompartment Golgi units (Nakamura *et al.* 1995; Barr *et al.* 1997; Kondylis *et al.* 2005; Zhou *et al.* 2014; Sanders and Kaverina 2015; Liu *et al.* 2017; Martin and Akhmanova 2018; Lowe 2019). We found that the percentage of multicompartment outposts decreases when GM130 is absent, which supports the model that GM130 participates in connecting Golgi compartments in neurons (Figure 1D) (Zhou *et al.* 2014). The loss of GM130 also reduces the number of ManII- and GalNacT2-positive Golgi outposts, consistent with previous reports (Liu *et al.* 2017). To readout microtubule orientation, we used EB1::GFP, whose binding to growing microtubule ends produces a comet-like trajectory. The majority of microtubule growth occurs at plus-ends, which also grow faster than minus-ends, enabling a clear distinction of plus- and minus-end growth and thus microtubule polarity (Feng *et al.* 2019). As previously reported, in control neurons dendritic microtubules are oriented

predominantly minus-ends-distal (Figure 1E). Strikingly, eliminating GM130 had no effect on the overall polarity of microtubules within the proximal dendritic arbor where multicompartment Golgi outposts clustered (Figure 1E). The overall frequency of microtubule growth was also unaffected by the loss of GM130 (Figure 1F). In mammalian cells, GM130 affects microtubule organization through the recruitment of AKAP450, whose fly ortholog is plp (Martinez-Campos *et al.* 2004; Rios 2014; Sanders and Kaverina 2015; Martin and Akhmanova 2018). Plp in fly neurons has likewise been implicated in the MTOC activity of Golgi outposts (Ori-McKenney *et al.* 2012). Similar to the loss of GM130, eliminating plp had no effect on dendritic microtubule polarity (Figure 1E). Plp is proposed to regulate microtubule growth at Golgi outposts in conjunction with *cnn* and  $\gamma$ -tubulin, the latter of whose activity is controlled by the  $\gamma$ -TuRC (Ori-McKenney *et al.* 2012; Yalgin *et al.* 2015). Eliminating either *cnn* or the  $\gamma$ -TuRC component dGrip75 does not alter dendritic microtubule polarity (Figure 1E). Thus, the results of our experiments provide compelling evidence that Golgi outposts do not play an essential role in creating or maintaining the overall polarity of the dendritic microtubule cytoskeleton.

### ***Loss of GM130 significantly reduces misoriented microtubules in nudE<sup>-</sup> axons***

The results of our GM130 loss-of-function experiments in da neurons suggest that the overall polarity of the dendritic microtubule cytoskeleton does not depend on Golgi outposts. However, microtubule polarity varies within the dendritic arbors of da neurons (Stone *et al.* 2008), and it is possible that Golgi outposts are nevertheless sufficient to locally influence microtubule polarity as previously reported (Ori-McKenney *et al.* 2012; Yalgin *et al.* 2015; Delandre *et al.* 2016). To test the idea that outposts have the capacity to affect microtubule polarity, we used a paradigm in which Golgi outposts are ectopically localized to axons, a compartment from which outposts are normally excluded and in which microtubules are uniformly oriented with their plus-ends distal. If Golgi outposts are capable of regulating microtubule organization, we reasoned that their ectopic presence in axons should disrupt the uniform plus-ends-distal array of axonal microtubules. To ectopically localize Golgi outposts to axons, we relied on mutations that disrupt the activity of the molecular motors dynein and kinesin-1, which transport outposts (Ye *et al.* 2007; Zheng *et al.* 2008; Arthur *et al.* 2015; Lin *et al.* 2015; Kelliher *et al.* 2019). While disrupting the activity of either motor results in Golgi outposts invading axons, we and others have previously shown that these ectopic outposts do not always correlate with a change in axonal microtubule polarity (Ye *et al.* 2007; Nguyen *et al.* 2014; Kelliher *et al.* 2019). Analyzing these different motor mutants and the outposts in their axons enables us to determine whether Golgi outposts are sufficient to affect microtubule polarity, and to then identify the factors that are essential for this activity. The loss of dynein activity alters both Golgi outpost localization and axonal microtubule polarity (Zheng *et al.* 2008; Arthur



**Figure 1** Global dendritic microtubule polarity does not depend on Golgi outposts. (A) Cartoon showing the compartmental distribution of GM130, ManII, and GalNacT2 in a Golgi stack. (B–D) ManII::GFP-positive outposts are present throughout the class IV da neuron dendritic arbor, but fewer than one-half of these outposts are multicompartment units (B and D). Multicompartment outposts are defined as those that have overlapping ManII::GFP and GalNacT2::TagRFP signals. In contrast to ManII::GFP-positive outposts, GalNacT2::TagRFP-positive outposts cluster in the proximal arbor (prox) and nearly all GalNacT2::TagRFP-positive outposts are multicompartment (C and D). Eliminating GM130 reduces the overall number of Golgi outposts and the percentage of outposts that are multicompartment in the prox. The prox encompasses a radius of 95  $\mu\text{m}$  from the cell body; distal (dist) is beyond this radius. Red arrowheads indicate Golgi outposts. The left side of each graph represents the fraction of ManII::GFP-positive outposts that are multicompartment and the right side of each graph represents GalNacT2::TagRFP-positive outposts that are multicompartment (D).  $^{**}P = 0.01\text{--}0.001$ , and  $^{****}P < 0.0001$ ; Student's unpaired *t*-tests. (E) Dendritic microtubule polarity is not affected by the loss of GM130, plp, cnn, or dGrip75. Mann–Whitney *U*-test. (F) EB1::GFP comet frequency is normal in the dendrites of neurons lacking GM130. Student's unpaired *t*-test. Microtubule polarity and EB1::GFP comet frequency were quantified in the proximal dendritic arbor, which contained the majority of multicompartment outposts. Scale bars, 25  $\mu\text{m}$ . All data are mean  $\pm$  SD. Antero, anterograde; cnn, centrosomin; n.s., not significant; plp, pericentrin-like protein; RFP, red fluorescent protein.

*et al.* 2015; del Castillo *et al.* 2015; Klinman *et al.* 2017; Rao *et al.* 2017). We first asked whether the misoriented microtubules in the axons of dynein loss-of-function neurons depend on the ectopic Golgi outposts. The multisubunit dynein motor complex has several cofactors that are important for its activity (Reck-Peterson *et al.* 2018); in *Drosophila* neurons, this includes the conserved cofactor nudE (Arthur *et al.* 2015). In the absence of nudE, axons are infiltrated by multicompartment Golgi outposts (Figure 2, A and B) (Arthur *et al.* 2015). In the axons of these *nudE<sup>39A/Df</sup>* mutant neurons, EB1::GFP comets travel both anterograde and retrograde, which indicates a disruption in axonal microtubule polarity (Figure 2, C and D). Although EB1::GFP can also bind to slowly growing microtubule minus-ends (Feng *et al.* 2019), the speed of the retrograde comets in the *nudE<sup>39A/Df</sup>* mutant axons is indicative of microtubule plus-end growth (Figure 2E). Thus, the ectopic axonal Golgi outposts in the *nudE<sup>39A/Df</sup>* mutant neurons correlate with a change in axonal microtubule polarity.

To determine whether ectopic Golgi outposts might contribute to the alteration in axonal microtubule polarity in *nudE<sup>39A/Df</sup>* mutant axons, we eliminated GM130. By itself, the loss of GM130 did not affect either the orientation of axonal microtubules or the localization of Golgi outposts (Figure 2, A–D). However, eliminating GM130 significantly reduced the number of misoriented minus-end-distal microtubules in *nudE<sup>39A/Df</sup>* mutant axons (Figure 2, C and D). ManII-positive Golgi were still present in the *nudE<sup>39A/Df</sup>* GM130<sup>Δ23/Df</sup> double-mutant axons, but, strikingly, the loss of GM130 suppressed the axonal mislocalization of GalNacT2-positive outposts in *nudE<sup>39A/Df</sup>* mutant neurons (Figure 2, A and B). A little over one-half of the ManII-positive outposts in the *nudE<sup>39A/Df</sup>* mutant axons were multicompartment, but there were virtually no multicompartment outposts in the axons of *nudE<sup>39A/Df</sup>* GM130<sup>Δ23/Df</sup> double-mutant neurons, likely because the loss of GM130 suppressed the axonal mislocalization of the GalNacT2-positive compartments. These data suggest that microtubule polarity may be affected by multicompartment, but not single-compartment, Golgi outposts.

Notably, the loss of GM130 did not completely suppress the appearance of misoriented microtubules. This may be due to the “microtubule gatekeeper” role that dynein is proposed to play in maintaining axonal microtubule polarity. In addition to carrying cargo, dynein also transports, or slides, microtubules (Rao and Baas 2018). Dynein anchored in the proximal axon translocates microtubules into or out of the axon, and prevents the entry of minus-end-distal microtubules (del Castillo *et al.* 2015; Rao *et al.* 2017; Rao and Baas 2018). Our data suggest that dynein also maintains axonal microtubule polarity by excluding Golgi outposts that have the capacity to induce changes in microtubule organization.

The presence of ectopic Golgi outposts in the *nudE<sup>39A/Df</sup>* mutant axons also correlates with the formation of ectopic axonal branches (Arthur *et al.* 2015). The axons of neurons lacking nudE develop multiple fine branches that run parallel to the main axon but terminate before reaching the ventral

nerve cord; occasionally ectopic branches even extend back toward the cell body and dendrites (Figure 2, F and G). Since dendritic Golgi outposts have been correlated with dendrite branch formation, and stability and loss of GM130 decreases branch number (Ye *et al.* 2007; Ori-McKenney *et al.* 2012; Zhou *et al.* 2014; Yalgin *et al.* 2015; Liu *et al.* 2017), we tested whether Golgi outposts might be implicated in the formation of ectopic branches that sprout from the *nudE<sup>39A/Df</sup>* mutant axons. We found that loss of GM130 suppressed the axonal morphology defects of the *nudE<sup>39A/Df</sup>* mutant axons, implicating the mislocalized Golgi outposts in the growth of ectopic axonal branches (Figure 2, F and G). Together, these data suggest that the ectopic Golgi outposts may be a key contributing factor to both the cytoskeletal and morphological defects of the *nudE<sup>39A/Df</sup>* mutant axons. Thus, Golgi outposts are likely capable of inducing changes both in the microtubule cytoskeleton and neurite branching.

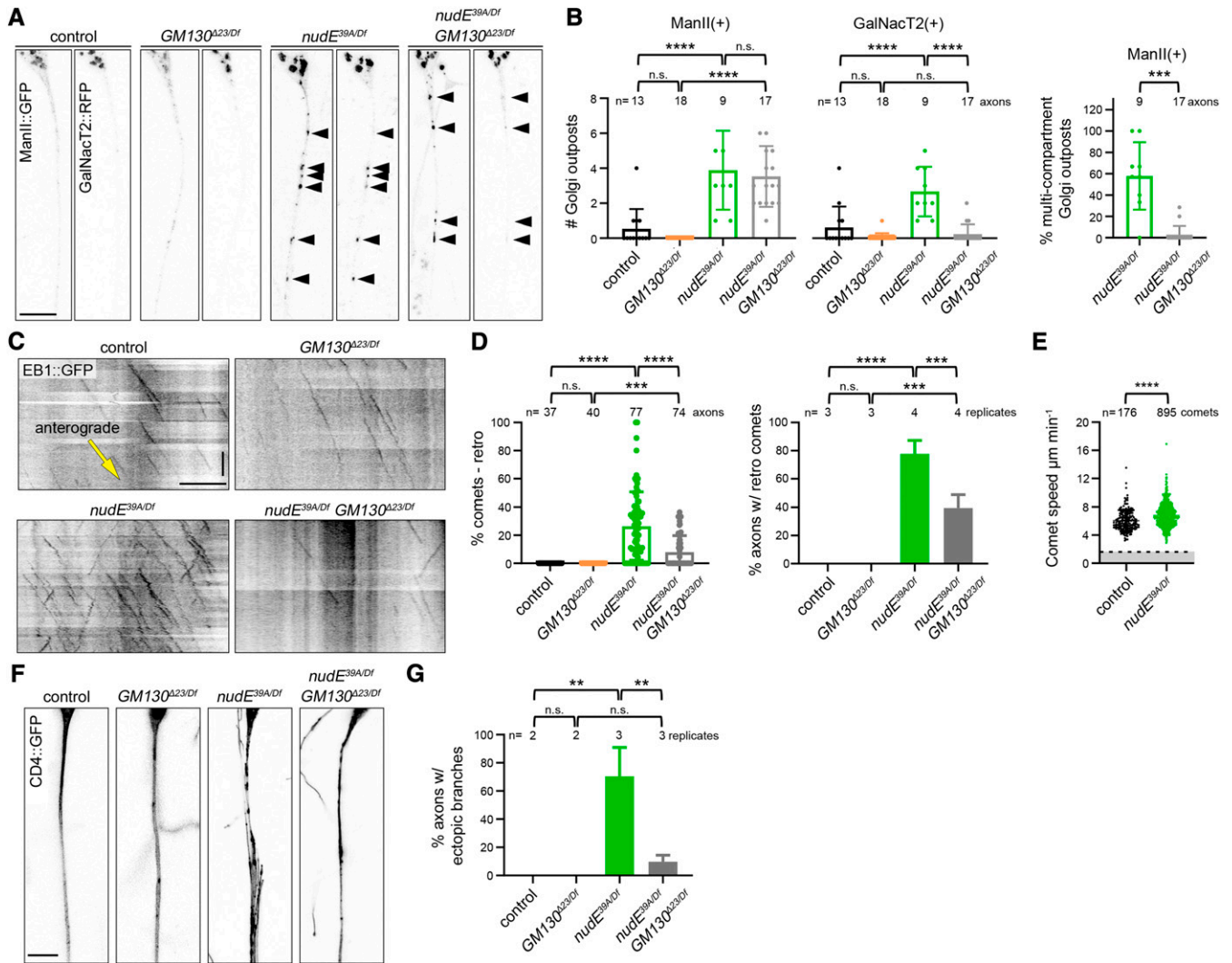
### **Golgi outposts affect microtubule polarity independently of $\gamma$ -TuRC-mediated microtubule nucleation**

Dendritic Golgi outposts have been reported to serve as platforms for oriented microtubule growth during dendrite branch extension (Ori-McKenney *et al.* 2012; Yalgin *et al.* 2015). This suggests that Golgi outposts might influence microtubule polarity by controlling microtubule nucleation. Therefore, we tested whether the misoriented microtubules in the *nudE<sup>39A/Df</sup>* mutant axons resulted from ectopic nucleation at Golgi outposts. Microtubule nucleation at Golgi membranes is templated by  $\gamma$ -tubulin whose nucleation activity is regulated by additional proteins, including *cnn* and  $\gamma$ -TuRC components (Sanders and Kaverina 2015; Martin and Akhmanova 2018; Tann and Moore 2019). In dendrites, *cnn* has been implicated in regulating the directional growth of Golgi-derived microtubules during branching (Yalgin *et al.* 2015). Thus, we focused on whether  $\gamma$ -TuRC-mediated microtubule nucleation might mediate the Golgi-induced change in microtubule polarity in *nudE<sup>39A/Df</sup>* mutant axons.

We have previously shown that reducing  $\gamma$ -tubulin does not suppress the appearance of minus-end-distal microtubules in *nudE<sup>39A/Df</sup>* mutant axons (Arthur *et al.* 2015). Nonetheless, we followed up our earlier findings by testing the  $\gamma$ -tubulin regulator *cnn* and the  $\gamma$ -TuRC component GCP4, known as dGrip75 in *Drosophila* (Vérollet *et al.* 2006). Consistent with our prior report, we found that eliminating either *cnn* (*cnn<sup>HK21/Df</sup>*) or dGrip75 (*dGrip75<sup>175/Df</sup>*) does not suppress the formation of misoriented microtubules in *nudE<sup>39A/Df</sup>* mutant axons (Figure 3, A and B). Altogether, our results suggest that the ectopic Golgi outposts have the capacity to affect microtubule polarity but do so through a pathway that is independent of  $\gamma$ -TuRC-mediated microtubule nucleation.

### **Elevating GM130 levels increases the number of multicompartment Golgi and alters microtubule polarity**

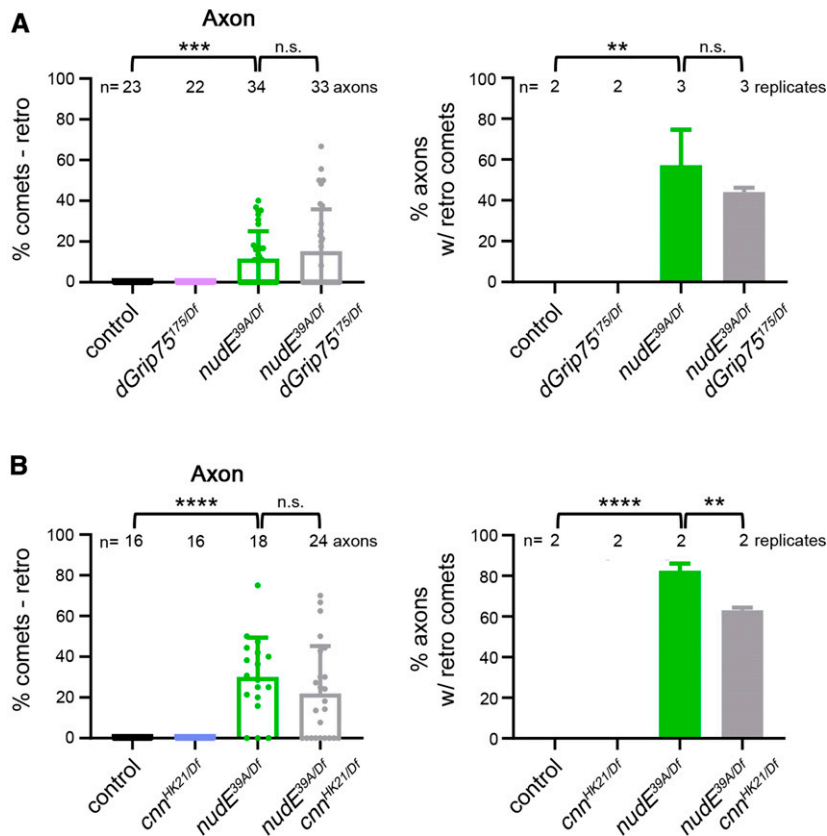
Our manipulations of GM130 in *nudE<sup>39A/Df</sup>* mutant neurons indicate that ectopic multicompartment, but not single-compartment,



**Figure 2** Misoriented microtubules in *nudE* mutant axons are significantly reduced when GM130 is eliminated. (A and B) Golgi outposts (marked by ManII::GFP and GalNacT2::RFP; arrowheads) mislocalize to axons in *nudE*<sup>39A/Df</sup> mutant neurons. In *nudE*<sup>39A/Df</sup> mutant neurons, the loss of GM130 does not affect the mislocalization of ManII-positive Golgi outposts, but suppresses the mislocalization of GalNacT2-positive outposts; as a result, the percentage of multicompartment ManII-positive outposts is dramatically reduced.  $***P = 0.001-0.0001$  and  $****P < 0.0001$ ; one-way ANOVA with Tukey's *post hoc* analysis. Scale bar, 10  $\mu\text{m}$ . (C and D) In the absence of *nudE*, axonal microtubule polarity is perturbed. Eliminating GM130 reduces the number of misoriented microtubules in *nudE*<sup>39A/Df</sup> mutant axons. Cell body is to the left; yellow arrow indicates the direction of anterograde comet movement.  $***P = 0.001-0.0001$ , and  $****P < 0.0001$ ; Kruskal-Wallis test with *post hoc* Dunn's multiple comparison analysis (% retro comets) and one-way ANOVA with Tukey's *post hoc* analysis (% axons). Scale bars, 10  $\mu\text{m}$  (x-axis) and 30 sec (y-axis). (E) The speed of EB1::GFP comets in control and *nudE*<sup>39A/Df</sup> mutant axons is consistent with microtubule plus-end growth, indicating *nudE*<sup>39A/Df</sup> mutant axons indeed contain microtubules with mixed polarity (comets moving at speeds below the dotted line would be consistent with microtubule minus-end growth).  $****P < 0.0001$ , Mann-Whitney *U*-test. (F and G) The ectopic branches that sprout from *nudE*<sup>39A/Df</sup> mutant axons are suppressed by removing GM130.  $n = 16$  (control), 27 (*GM130* <sup>$\Delta$ 23/Df</sup>), 59 (*nudE*<sup>39A/Df</sup>), and 41 (*GM130* <sup>$\Delta$ 23/Df</sup>; *nudE*<sup>39A/Df</sup>) axons in replicates as indicated (G).  $**P = 0.01-0.001$ ; one-way ANOVA with Tukey's *post hoc* analysis. Scale bar, 10  $\mu\text{m}$ . All data are mean  $\pm$  SD. Antero, anterograde; n.s., not significant; retro, retrograde; RFP, red fluorescent protein.

Golgi outposts disrupt axonal microtubule polarity (Figure 2, A-D). As previously mentioned, GM130 is implicated in both the MTOC activity of Golgi and in promoting the formation of multicompartment Golgi units (Zhou *et al.* 2014; Martin and Akhmanova 2018). Connectedness between Golgi compartments may be important to the coordinated regulation of microtubules by protein complexes that are present on the *cis*- and *trans*-Golgi compartments (Rios 2014; Sanders and

Kaverina 2015). Thus, our results and the work of others suggest that the ability of Golgi to regulate microtubule polarity may depend on the formation of multicompartment units. We asked whether increasing GM130 levels would be sufficient to increase the number of multicompartment Golgi outposts and to alter microtubule polarity in dendrites. We found that GM130 overexpression increased the number of multicompartment outposts in dendrites as previously



**Figure 3** Appearance of misoriented microtubules in *nudE* mutant axons does not depend on microtubule nucleation machinery. (A and B) Eliminating either dGrip75 (A) or *cnn* (B) does not affect the microtubule polarity phenotype of *nudE<sup>39A/Df</sup>* mutant axons.  $n = 23$  (control), 22 (*dGrip75<sup>175/Df</sup>*), 34 (*nudE<sup>39A/Df</sup>*), and 33 (*dGrip75<sup>175/Df</sup>; nudE<sup>39A/Df</sup>*) axons (A) and  $n = 16$  (control), 16 (*cnn<sup>HK21/Df</sup>*), 18 (*nudE<sup>39A/Df</sup>*), and 24 (*cnn<sup>HK21/Df</sup>; nudE<sup>39A/Df</sup>*) axons (B) in replicates as indicated.  $**P = 0.01-0.001$   $***P = 0.001-0.0001$ , and  $****P < 0.0001$ ; Kruskal-Wallis test with *post hoc* Dunn's multiple comparison analysis. ANOVA with Tukey's *post-hoc* analysis. All data are mean  $\pm$  SD. *cnn*, centrosomin; n.s., not significant.

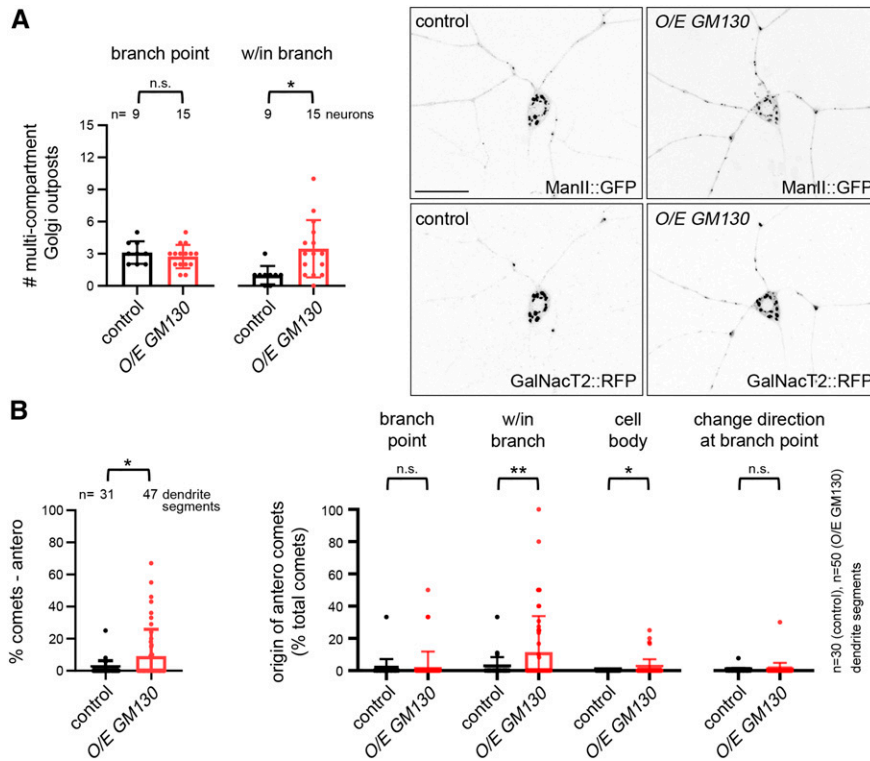
reported (Zhou *et al.* 2014) and that these additional outposts were more prevalent in dendrite branches than branch points (Figure 4A). Thus, our results provide additional support to the idea that GM130 is integral to the connectedness of Golgi compartments in neurons (Zhou *et al.* 2014; Liu *et al.* 2017); this is significant given that the role of GM130 in Golgi stack formation in other cell types has been debated (Kondylis and Rabouille 2003; Puthenveedu *et al.* 2006; Marra *et al.* 2007; Baschieri *et al.* 2014; Tormanen *et al.* 2019). Notably, this increase in multicompartment units within branches correlated with an increase in anterograde EB1::GFP comets that originated within branches (there was also a mild but significant increase in the anterograde comets that originated from the cell body; Figure 4B). There was no significant increase in anterograde comets that originated at branch points and the frequency of comets that changed direction at branch points was also unaffected (Figure 4B). Others have suggested that multicompartment Golgi outposts are prime sites of EB1::GFP comet initiation (Zhou *et al.* 2014), leading us to propose that the additional anterograde EB1::GFP comets we observe in the neurons overexpressing GM130 originate from ectopic multicompartment outposts. Altogether, these data are consistent with the model that the formation of ectopic multicompartment outposts is sufficient to alter microtubule polarity in dendrites.

To further test the idea that Golgi compartmentalization correlates with an effect on microtubule polarity, we turned to

a mutation in *Kinesin heavy chain* (*Khc<sup>E177K</sup>*) that enhances kinesin-1 activity by disrupting motor autoinhibition (Kelliher *et al.* 2018). Golgi outposts mislocalize to *Khc<sup>E177K</sup>* mutant axons; however, unlike in *nudE<sup>39A/Df</sup>* mutant axons, the polarity of axonal microtubules is not significantly affected (Kelliher *et al.* 2018). Our results indicate that the compartmental organization of Golgi outposts is key to their ability to affect microtubule polarity in dendrites. This led us to characterize the compartmental organization of the ectopic Golgi outposts in the *Khc<sup>E177K</sup>* and *nudE<sup>39A/Df</sup>* mutant axons to determine whether the differences in axonal microtubule polarity in the two mutants might correlate with differences in Golgi compartmentalization. More specifically, we reasoned that there may be a higher number of multicompartment Golgi outposts in *nudE<sup>39A/Df</sup>* mutant axons, which have altered axonal microtubule polarity, than in the *Khc<sup>E177K</sup>* mutant axons, which do not.

We analyzed the distribution of ManII::GFP and GalNacT2::TagRFP in *Khc<sup>E177K</sup>* and *nudE<sup>39A/Df</sup>* mutant axons. Consistent with the idea that compartment connectedness enables Golgi to influence microtubule polarity, we found that there was a higher percentage of multicompartment Golgi outposts in the *nudE<sup>39A/Df</sup>* mutant axons than the *Khc<sup>E177K/27</sup>* mutant axons (Figure 5, A and B). Notably, *Khc<sup>E177K/27</sup>* mutant axons contained equal numbers of GalNacT2-positive outposts and more ManII-positive outposts than *nudE<sup>39A/Df</sup>* mutant axons (Figure 5B). This indicates that the *Khc<sup>E177K/27</sup>* mutant axons





**Figure 4** Multicompartment Golgi are sufficient to alter microtubule polarity. (A) The overexpression of GM130 increases the number of multi-compartment Golgi outposts within dendrite branches but not branch points. Scale bar, 25  $\mu$ m. \* $P$  = 0.05–0.01; Student’s unpaired  $t$ -tests. (B) The percentage of anterograde comets increases in the dendrites of neurons overexpressing GM130. The percentage of anterograde comets that originate from within branches increases, paralleling the increase in multicompartment outposts within branches. The graph on the right represents the number of anterograde comets that fall into a designated category divided by the total number of comets in the dendrite segment (% total comets). \* $P$  = 0.05–0.01 and \*\* $P$  = 0.01–0.001; Mann–Whitney  $U$ -test. All data are mean  $\pm$  SD. n.s., not significant; O/E, overexpressed; RFP, red fluorescent protein.

have just as many Golgi compartments as the *nudE<sup>39A/Df</sup>* mutant axons, but that the compartments are not as connected. Correspondingly, microtubule polarity is largely normal in *Khc<sup>E177K/27</sup>* mutant axons, which is in contrast to the *nudE<sup>39A/Df</sup>* mutant axons (Figure 5C). Combined, these results suggest that *nudE* (and dynein activity) are needed for the proper localization, but not the formation, of multicompartment Golgi outposts. In contrast, enhancing kinesin-1 activity both perturbs Golgi localization and antagonizes the connectedness of Golgi compartments.

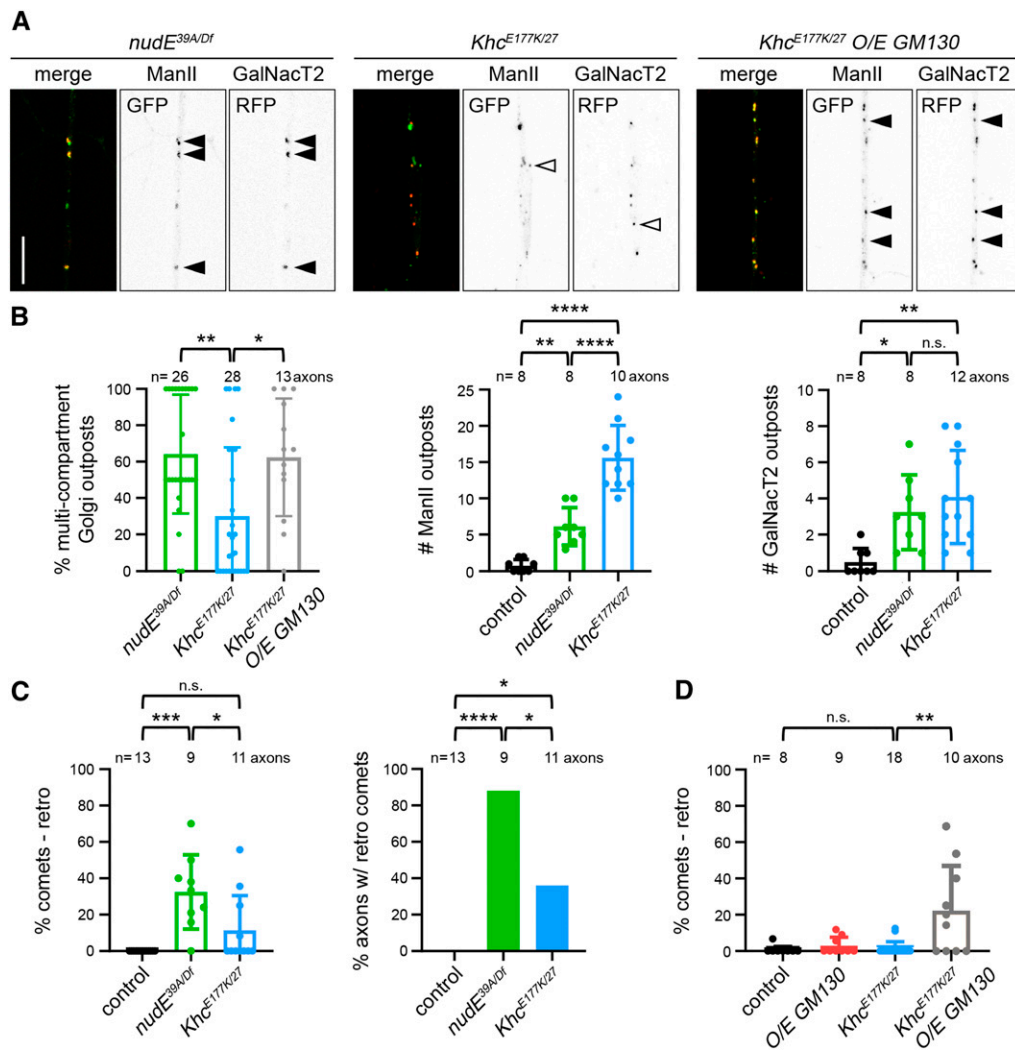
We then asked whether increasing GM130 might alter the polarity of axonal microtubules in the *Khc<sup>E177K/27</sup>* mutant axons, which contain predominantly single-compartment Golgi outposts. In control neurons, the overexpression of GM130 alone did not affect axonal microtubule polarity (Figure 5D). The overexpression of GM130 in *Khc<sup>E177K/27</sup>* mutant neurons both increased the percentage of multicompartment outposts and resulted in the appearance of ectopic minus-end-distal microtubules (Figure 5, A and D). Altogether, our data support the idea that multicompartment Golgi outposts have the capacity to remodel microtubule polarity locally even if they are not essential to the overall polarity of the dendritic cytoskeleton.

## Discussion

Microtubules in axons and dendrites have distinct polarities. In a variety of cell types microtubule orientation is regulated by MTOCs, raising the question of whether neurons have

MTOCs that carry out a similar function. In dendrites, Golgi outposts are likely candidates (Ori-McKenney *et al.* 2012; Zhou *et al.* 2014; Yalgin *et al.* 2015). The compartmental organization of Golgi outposts and their correlation with microtubule growth initiation sites were recently shown to depend on the *cis*-Golgi matrix protein GM130, the fly AKAP450 ortholog *plp*, and *cnn* (Ori-McKenney *et al.* 2012; Zhou *et al.* 2014; Yalgin *et al.* 2015). We found that the elimination of these factors does not affect the predominantly minus-end-distal orientation of microtubules in class IV da neuron dendrites, suggesting that Golgi outposts are not necessary for the unique polarity of the dendritic microtubule network. This raises the question of whether Golgi outposts might have any capacity to affect microtubule orientation. Our analysis of outposts in dendrites and outposts mislocalized to axons suggests that ectopic multicompartment Golgi outposts are sufficient to alter microtubule polarity, and likely do so independently of microtubule nucleation. We propose that the unique polarity of the dendritic microtubule cytoskeleton is established and maintained independently of Golgi outposts, but that multicompartment Golgi outposts may have the capacity to locally influence microtubule polarity during events such as dendrite branch extension.

Studies carried out in mammalian cells have shown that Golgi serve as platforms for microtubule nucleation, anchoring, and stabilization (Sanders and Kaverina 2015; Martin and Akhmanova 2018; Fu *et al.* 2019). These activities arise from distinct protein complexes, whose recruitment to the *cis*-Golgi depends on GM130. Golgi are generally thought



**Figure 5** Inducing the formation of multicompartment Golgi alters microtubule polarity in the axons of *Khc* mutant neurons. (A and B) Axons of *Khc<sup>E177K/27</sup>* mutant neurons contain fewer multicompartment Golgi outposts than *nudE<sup>39A/Df</sup>* mutants, despite have similar or more numbers of ManII- and GalNacT2-positive compartments. Over-expressing GM130 increases the percentage of multicompartment outposts in *Khc<sup>E177K/27</sup>* mutant axons. \* $P = 0.05-0.01$ , \*\* $P = 0.01-0.001$  and \*\*\*\* $P < 0.0001$ ; Kruskal-Wallis test with *post hoc* Dunn's multiple comparison analysis (% multicompartment Golgi outposts) and one-way ANOVA with Tukey's *post hoc* analysis (# outposts). Scale bar, 10  $\mu\text{m}$ . Closed arrowheads indicate multicompartment outposts and open arrowheads indicate single compartments. (C) In contrast to *nudE<sup>39A/Df</sup>* mutants, *Khc<sup>E177K/27</sup>* mutant axons have normal microtubule polarity. \* $P = 0.05-0.01$ , \*\*\* $P = 0.001-0.0001$ , and \*\*\*\* $P < 0.0001$ ; Kruskal-Wallis test with *post hoc* Dunn's multiple comparison analysis (% comets); Fisher's Exact test (% axons). (D) The increase in multicompartment Golgi outposts in *Khc<sup>E177K/27</sup>* mutant axons that results from the overexpression of GM130 is accompanied by an increase in misoriented axonal microtubules. \*\* $P = 0.01-0.001$ ; Kruskal-Wallis test with *post hoc* Dunn's multiple comparison analysis. All data are mean  $\pm$  SD. n.s., not significant; RFP, red fluorescent protein.

to anchor and stabilize microtubules that have been generated at Golgi membranes, although the molecular mechanism by which Golgi would selectively capture these microtubules is unclear (Zhu and Kaverina 2013; Martin and Akhmanova 2018). Our studies indicate that  $\gamma$ -TuRC-mediated microtubule nucleation is dispensable for Golgi outposts to affect microtubule polarity. Consistently, a new study indicates that  $\gamma$ -tubulin only rarely associates with outposts (Mukherjee *et al.*, 2019 *preprint*). One possibility is that microtubules are nucleated at Golgi independently of  $\gamma$ -tubulin. For example, it was recently reported that the mammalian tubulin polymerization promoting protein (TPPP) nucleates microtubules at Golgi outposts independently of  $\gamma$ -tubulin; however, TPPP is enriched in glia cells, not neurons, in the mammalian nervous system (Fu *et al.* 2019). Another possibility is that Golgi outposts may be able to capture and stabilize microtubules that are growing in the vicinity of outposts in the relatively confined spaces of

dendrites (and axons). For example, in the *nudE* mutant axons, ectopic multicompartment Golgi outposts may alter microtubule polarity by stabilizing nearby misoriented microtubules that would otherwise be eliminated. Thus, outposts may influence microtubule polarity by tethering and stabilizing microtubules that are not generated at Golgi.

Our results raise the question of what molecular players may organize microtubules at Golgi outposts in neurons. A key component of the complexes that tether and stabilize microtubules on Golgi in vertebrate cells is myomegalin (Roubin *et al.* 2013; Wang *et al.* 2014; Wu *et al.* 2016). However, there is no clear *Drosophila* ortholog of myomegalin, making it difficult to directly test this model. The most closely related family member in flies is *cnn*, which is implicated in activating  $\gamma$ -tubulin-templated microtubule assembly (Choi *et al.* 2010; Roubin *et al.* 2013) and which we have shown is not necessary for Golgi outposts to alter axonal microtubule polarity. Moreover, new findings call into question whether *cnn*

strongly associates with Golgi in fly neurons (Mukherjee *et al.*, 2019 *preprint*). Another component of the Golgi-associated complex that anchors and stabilizes microtubules is the microtubule minus-end-binding protein CAMSAP2, whose fly ortholog is Patronin. Recent studies using *da* neurons have shown that Patronin is needed for minus-end-distal microtubules in dendrites, and likely acts by antagonizing the kinesin-13 microtubule depolymerase Klp10A (Feng *et al.* 2019; Wang *et al.* 2019). However, our preliminary analysis of Patronin localization makes it unclear whether Patronin localizes to or functions at Golgi outposts. In work using mitotic mammalian cells, GM130 has also been implicated in the stabilization of microtubules on Golgi through a mechanism that depends on the microtubule-associated protein TPX2; however, TPX2 structure and function are likely not conserved between mammals and flies (Goshima 2011; Hayward *et al.* 2014; Wei *et al.* 2015). Thus, additional studies are needed to identify the molecular players that tether and stabilize microtubules on Golgi in *Drosophila* neurons.

The organization of the Golgi apparatus into a multicompartment stack gives it morphological and functional polarity. Correspondingly, microtubules associated with Golgi are proposed to be oriented in a particular direction relative to the Golgi compartments (Efimov *et al.* 2007; Zhu and Kaverina 2013; Martin and Akhmanova 2018). In *da* neurons, the correlation between microtubule polarity and Golgi compartment organization is supported by findings that microtubule growth typically initiates in a single direction from an outpost (Ori-McKenney *et al.* 2012; Yalgin *et al.* 2015). This suggests a relationship between the polarity of the Golgi stack and the associated microtubules. Thus, the relative orientation of the Golgi outpost stack likely influences the polarity of Golgi-associated microtubules (Delandre *et al.* 2016). Our finding that elevated GM130 levels altered microtubule polarity in dendrites may suggest that GM130 instigated the formation of misoriented Golgi stacks that in turn stabilized misoriented microtubules. Given the potential of multicompartment outposts to influence microtubule polarity, it will be interesting to determine how Golgi outpost compartmentalization in dendrites is controlled to ensure proper dendritic microtubule polarity.

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