Sec71 functions as a GEF for the small GTPase Arf1 to govern dendrite pruning of *Drosophila* sensory neurons

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Abstract

Pruning, whereby neurons eliminate their exuberant neurites, is central for the maturation of the nervous system. In *Drosophila*, sensory neurons, ddaCs, selectively prune their larval dendrites without affecting their axons during metamorphosis. However, it is unknown whether the secretory pathway plays a role in dendrite pruning. Here, we show that the small GTPase Arf1, an important regulator of secretory pathway, is specifically required for dendrite pruning of ddaC/D/E sensory neurons but dispensable for apoptosis of ddaF neurons. Analyses of the GTP and GDP-locked forms of Arf1 indicate that the cycling of Arf1 between GDP-bound and GTP-bound forms is essential for dendrite pruning. We further identified Sec71 as a guanine nucleotide exchange factor for Arf1 that preferentially interacts with its GDP-bound form. Like Arf1, Sec71 is also important for dendrite pruning, but not apoptosis, of sensory neurons. Arf1 and Sec71 are interdependent for their localizations on Golgi. Finally, we show that Sec71/Arf1-mediated trafficking process is a prerequisite for Rab5-dependent endocytosis to facilitate endocytosis and degradation of the cell adhesion molecule Neuroglian (Nrg).
Introduction

In the developing nervous systems, neurons often extend excessive neurites and form superfluous connections at early stages. Subsequent removal of those exuberant or inappropriate neurites without causing the death of parental neurons, a process known as pruning, is crucial for the refinement of neural circuits at late developmental stages (Luo and O’Leary, 2005; Riccomagno and Kolodkin, 2015; Schuldiner and Yaron, 2015). Neuronal pruning is a conserved process widely occurring in both vertebrates and invertebrates. In vertebrates, many neurons in the neocortex, neuromuscular system and hippocampal dentate gyrus prune their unwanted neurites to control the proper wiring of the nervous systems (Bagri et al., 2003; O’Leary and Koester, 1993; Tapia et al., 2012). In invertebrates, such as *Drosophila*, the nervous systems undergo drastic remodelling during metamorphosis, a transition stage from a larva to an adult fly (Kanamori et al., 2015a; Truman, 1990; Yu and Schuldiner, 2014). In the central nervous system (CNS), mushroom body (MB) γ neurons prune their dorsal and medial axon branches as well as entire dendrites (Lee et al., 1999). In the peripheral nervous system (PNS), some dorsal dendritic arborization (da) neurons, ddaC, ddaD and ddaE, selectively eliminate their larval dendrites without affecting their axons (Kuo et al., 2005; Williams and Truman, 2005), whereas ddaF neurons are apoptotic during early metamorphosis (Williams and Truman, 2005). The pruning event involves both local degeneration and retraction (Luo and O’Leary, 2005), resembling neurodegeneration associated with brain injury and neurodegenerative diseases. Thus, a complete understanding of cellular and molecular mechanisms of developmental pruning would shed some light on pathological neurodegeneration following neurological diseases and injury.

In *Drosophila*, ddaC sensory neurons have emerged as an attractive model system to elucidate the molecular and cellular mechanisms of dendrite-specific pruning during early metamorphosis. In response to the steroid-molting hormone 20-hydroxyecdysone (ecdysone)
at the late larval stage, ddaC neurons sever the proximal region of their dendrites and subsequently undergo rapid fragmentation of the severed dendrites as well as dendritic clearance via phagocytosis (Figure 1A) (Kuo et al., 2005; Williams and Truman, 2005). It has been well documented that the Ecdysone Receptor and its co-receptor Ultraspiracle are required to activate the expression of several downstream targets to initiate dendrite pruning (Yu and Schuldiner, 2014). We and others have recently reported the identification of endocytic pathways that are critical for dendrite pruning (Kanamori et al., 2015b; Zhang et al., 2014). Rab5/Avalanche and ESCRT complexes, the components of the endocytic pathways, are required for downregulation of the L1-type cell adhesion molecule (L1-CAM) Neurogian (Nrg) (Zhang et al., 2014). Nrg is drastically redistributed to endosomes and its protein levels are strongly downregulated prior to pruning, suggesting that massive Nrg endocytosis promotes dendrite pruning (Zhang et al., 2014). It is conceivable that Nrg endocytosis might be triggered by secreted ligands/signals through the secretory pathway. However, it is completely unknown whether the secretory pathway, an opposite route of the endocytic pathway, also plays a role in dendrite pruning of ddaC neurons.

The primary sites of the secretory pathway consist of the endoplasmic reticulum (ER), the Golgi apparatus and the trans-Golgi network in eukaryotic cells (Lippincott-Schwartz et al., 2000). Newly synthesized membrane proteins and lipids exit from the ER, pass through the Golgi complexes and are delivered to the plasma membrane via the post-Golgi exocytosis (Pfenninger, 2009). In developing neurons, the continuous addition of membrane proteins and lipids via the secretory pathway plays a key role in the outgrowth and elongation of dendrites and axons (Horton and Ehlers, 2004). Disruption of the ER-to-Golgi transport leads to the inhibition of dendritic or axonal growth in Drosophila sensory neurons and rodent hippocampal neurons (Aridor and Fish, 2009; Ye et al., 2007). In an attempt to isolate novel players of dendrite pruning, we carried out a large-scale RNA interference (RNAi) screen and
identified ADP-ribosylation factor 1 (Arf1), also known as ADP-ribosylation factor at 79F, as an important player for dendrite pruning in ddaC sensory neurons. Arf1 is a small GTPase and belongs to the Class I Arf family (D'Souza-Schorey and Chavrier, 2006; Gillingham and Munro, 2007). It has been reported that Arf1 can recruit COPI coat proteins on cis-Golgi and clathrin adaptor proteins, such as AP-1, AP-3, and GGAs, on trans-Golgi in a GTP-dependent manner, and thereby facilitate vesicle formation and trafficking (Cherfils, 2014). Studies from yeast and mammals indicate that Arf1 is activated by two conserved families of guanine nucleotide exchange factors (GEFs), including the Gea1/GBF1 family on cis-Golgi and Sec7/BIG family on trans-Golgi (Gillingham and Munro, 2007). Arf1 cycles between GDP- and GTP-bound forms, and both the GTP- and GDP-locked forms can interfere its functions and disrupt secretory trafficking (Dascher and Balch, 1994). In mammalian hippocampal neurons, overexpression of the GTP-locked form of Arf1 (Arf1\textsuperscript{Q71L}), which abolishes Arf1 activity, inhibits dendrite growth (Horton et al., 2005). The mammalian Arf1GEF, BIG2, is required for vesicle trafficking and mutations in human BIG2 gene lead to autosomal recessive periventricular heterotopia with microcephaly (ARPHM), a brain disorder characterized by defective neural proliferation and migration (Ferland et al., 2009; Sheen et al., 2004). Thus, various studies have documented that the secretory pathway plays a critical role in neurite growth and extension in developing neurons. However, very little is known about its role in regulating neurite pruning, a developmental degenerative process.

Here, we report the identification of Arf1 as an important player of dendrite pruning in ddaC sensory neurons. The cycling of Arf1 between GDP-bound and GTP-bound forms is essential for dendrite pruning. We further identified Sec71 as a GEF for Arf1 in Drosophila. We show that Sec71, like Arf1, is cell-autonomously required for dendrite pruning of ddaC/D/E sensory neurons but not for apoptosis of ddaF neurons during early metamorphosis. Arf1 and Sec71 co-localize on Golgi apparatus and regulate secretory vesicle
biogenesis in ddaC neurons. Furthermore, we show that Sec71/Arf1-dependent secretory pathway acts upstream of Rab5-dependent endocytosis and facilitates the internalization and downregulation of the cell adhesion molecule Nrg prior to dendrite pruning. Thus, our study demonstrates a novel and important role of Arf1/Sec71-mediated secretory pathway in promoting developmental pruning via the regulation of Nrg endocytosis.

Results

Arf1 GTPase is critical for dendrite pruning but not for neuronal apoptosis

To identify novel players of dendrite pruning in ddaC sensory neurons, we conducted a genome-wide RNAi screen by crossing the class IV da neuron driver ppk-Gal4 (Grueber et al., 2003) and UAS-Dicer2 with a large collection of RNAi lines from Vienna and Bloomington Drosophila stock centers (Kirilly et al., 2009). From this screen, we isolated two distinct RNAi lines, v23082 (#1) and v103572 (#2), corresponding to Arf1. In Drosophila, Arf1 was reported to regulate planar cell polarity, blood cell homeostasis and lamellipodium formation (Carvajal-Gonzalez et al., 2015; Humphreys et al., 2012; Khadilkar et al., 2014). The expression of these Arf1 RNAi transgenes, via ppk-Gal4, led to consistent dendrite pruning defects in the vast majority of ddaC neurons at 16 h after puparium formation (APF) (90% and 100%, respectively; Figure 1C, S1A). On average, 3.2 primary and secondary dendrites remained attached to Arf1 RNAi ddaC neurons at 16 h APF (Figure 1J). Those larval dendrites were eventually pruned away at 32 h APF (Figure S1D). In contrast, all larval dendrites were completely eliminated in the control neurons expressing an irrelevant RNAi line at 16 h APF (Figure 1B). Development of larval dendrite arbors was also impaired in Arf1 RNAi ddaC neurons, as the numbers of primary and secondary dendrites at the white prepupal (WP) stage were reduced, and moreover elaboration of high-order dendrites was also severely affected (Figures 1C, J). To validate the RNAi knockdown
phenotypes, we generated a synthetic RNAi-resistant transgene which bears minimal homology to the Arf1 dsRNA sequence (#1) but encodes the wild-type Arf1 protein. Both dendrite pruning and morphology defects observed in Arf1 RNAi (#1) ddaC neurons were fully rescued by co-expression of this RNAi-resistant Arf1 transgene (Figures 1D, J), confirming the specificity of Arf1 RNAi effect.

Arf1 is a small GTPase required for secretory membrane biogenesis and transport (D'Souza-Schorey and Chavrier, 2006). It can cycle between an inactive GDP-bound form and an active GTP-bound form (Gillingham and Munro, 2007). We expressed Arf1T31N, a GDP-locked form of Arf1, in ddaC neurons. Consistently, the expression of Arf1T31N, via two copies of ppk-Gal4 driver, also resulted in a dendrite pruning defect at 16 h APF (Figures 1E, J). The mild pruning defect is probably due to an initial dendrite development defect, as few WP dendrites were present in the vicinity of Arf1T31N ddaC neurons (Figures 1E, J). The cytological location of the Arf1 gene near the centromere precludes our MARCM clonal analysis. To circumvent the requirement of Arf1 for initial dendrite development, we induced Arf1T31N expression at the middle third instar larval stage (90-96 h after egg laying, AEL) using the Gene-Switch system. Full penetrance of dendrite pruning defect was observed in the Arf1T31N-expressing ddaC neurons derived from animals fed with RU486 for 6 h (100%; Figure 1G), in contrast to no pruning defect observed in non-fed animals (Figure 1F). Approximately 9.4 primary and secondary dendrites persisted with the attachment to the soma of Arf1T31N-induced ddaC neurons at 16 h APF (Figure 1J), indicating a severe dendrite pruning defect. Moreover, pulse expression of Arf1T31N did not impair initial ddaC dendritic development and morphology as shown at WP stage (Figures 1G, J). These data highlight that the Arf1T31N-associated dendrite pruning defect is not a secondary consequence of its initial dendrite arborization defect. Thus, Arf1 is required to promote dendrite pruning in ddaC sensory neurons.
Both the GTP- and GDP-locked forms of Arf1 can interfere its functions in the secretory pathway (Dascher and Balch, 1994). Indeed, like that of Arf1\textsuperscript{T31N}, pulse expression of Arf1\textsuperscript{Q71L}, a GTP-locked form of Arf1, also led to strong dendrite severing defects with the persistence of 10.6 primary and secondary dendrites at 16 h APF in RU486-fed animals (100%, Figure 1H). However, no obvious pruning defect phenotype was observed in ddaC neurons expressing wild-type Arf1 protein (Arf1\textsuperscript{WT}) at 16 h APF with two copies of the continuously expressing driver ppk-Gal4 (Figure S1A) or the Gene-Switch system (Figure 1I, J). These results strongly support the notion that the cycling of Arf1 between GDP-bound and GTP-bound forms is essential for normal progression of dendrite pruning. In addition to ddaC neurons, ddaD/E sensory neurons also completely eliminated their dendrites by 19 h APF (Figure S1B). Arf1\textsuperscript{T31N}-expressing mutant ddaD/E neurons retained some of their larval dendrites attached to their soma (83%, Figure S1B). Wild-type ddaF neurons are apoptotic during early metamorphosis (Williams and Truman, 2005). Interestingly, ddaF neurons expressing Arf1\textsuperscript{T31N} or Arf1 RNAi constructs were eliminated (Figure S1B), similar to wild-type ddaF neurons, suggesting that Arf1 is dispensable for ddaF apoptosis.

Collectively, Arf1 plays an important role in regulating dendrite pruning, rather than apoptosis, of sensory neurons; the cycling of GDP-Arf1 and GTP-Arf1 is essential for normal dendrite pruning.

**Arf1 is localized on the Golgi compartments in ddaC sensory neurons**

To assess subcellular localization of Arf1 in *Drosophila*, we generated an antibody against Arf1 and performed the immune-staining with the anti-Arf1 antibody in ddaC sensory neurons. The anti-Arf1 antibody is specific, as its signals were completely eliminated by both *Arf1* RNAi knockdowns (Figure S2A). We observed many Arf1-positive punctate structures in wild-type ddaC soma and these discrete puncta were co-localized with...
galactosyltransferase-GFP (GalT-GFP), a trans-Golgi marker (Figure 2A). Arf1 punctate structures were partially co-localized and juxtaposed with the cis-Golgi marker GM130 (Figure 2B). Likewise, Arf1 was also co-localized with another Golgi protein Lava Lamp (Lva) (Figure S2B). Arf1-positive puncta were also localized next to the ER exit site marker Sec31-mCherry (Figure 2C). However, Arf1 did not overlap with the recycling endosomal marker Rab4-mRFP, the early endosomal marker GFP-Rab5 and the mitochondrial marker Mito-GFP (Figures S2C-E).

We then determined whether Arf1 regulates the integrity of the Golgi apparatus in ddaC neurons. We observed that GalT-GFP punctate structures were completely disrupted, as GalT-GFP signals became diffused in the soma of either Arf1T31N Gene-Switch (Figure 2D) or Arf1 RNAi (Figure S2F) mutant ddaC neurons, compared to those bright puncta in wild type (Figure 2A’). Similarly, GalT-GFP-positive (Figure S2H) or ManII-VENUS (Figure 2H’) signals were also diffused in the dendrites of Arf1 RNAi ddaC neurons, compared to the controls (Figures S2G, 2G’, respectively). The GM130 marker was also strongly reduced in size and number in Arf1T31N neurons (Figure 2E). These data indicate that the integrity of Golgi apparatus is disrupted in Arf1 mutant neurons. In contrast, Sec31-mCherry marker was still distributed as many discrete punctate structures, although the numbers of their respective puncta were slightly reduced (Figure 2F). These data indicate that Arf1 regulates the integrity of the Golgi apparatus.

Thus, Arf1 is predominantly localized on the Golgi compartments and is important for the integrity of the Golgi structures in ddaC neurons.
Sec71, a Sec7 domain-containing protein, is required for the regulation of dendrite pruning, rather than apoptosis, in sensory neurons

We then took advantage of RNAi and biochemical approaches to identify an important Arf1GEF that is also involved in dendrite pruning. Arf1 can be activated from GDP-bound state to GTP-bound state by Sec7 domain-containing GEFs (Gillingham and Munro, 2007). We first examined the potential requirements of six Sec7 domain containing GEFs from the *Drosophila* genome via RNAi. Among them, we isolated two independent RNAi transgenes, BL32366 (#1) and v100300 (#2), which both target Sec71. Sec71 is a GEF with a catalytic Sec7 domain, which is more closely related to the BIG1/BIG2 family (Christis and Munro, 2012). Mutations in BIG2 gene, a human counterpart of Sec71, lead to defective vesicle trafficking, impaired cell adhesion and ARPHM disease (Sheen et al., 2004). Notably, RNAi knockdown of Sec71 led to complete penetrance of dendrite pruning defects in all ddaC neurons with 5.7 and 4.7 primary and secondary dendrites attached to their soma at 16 h APF (Figures 3B, 3D, 3G). Those larval dendrites were eventually pruned away at 32 h APF (Figure S1D). In contrast, all larval dendrites were completely pruned in the control neurons expressing an irrelevant RNAi line at 16 h APF (Figures 3A, G). Similar to Arf1 mutants, Sec71 RNAi ddaC neurons exhibited simplified dendrite arbors at larval and WP stages (WP: Figures 3B, D, G; wL3: Figure S3E). Co-expression of an RNAi-resistant Sec71 transgene fully rescued both dendrite pruning and morphology defects associated with Sec71 RNAi (#1) ddaC neurons (Figure 3C, G), confirming that the RNAi transgene specifically targets Sec71.

Sec7 domain-containing ArfGEFs activate Arf proteins from the GDP-bound state to the GTP-bound state. The Glutamic acid-to-Lysine substitution in the conserved Sec7 domain behaves as a catalytically inactive form, as shown for the corresponding mutation in the
human ArfGEF GBF1 (E794K) (Garcia-Mata et al., 2003). As described below, the E-K change indeed abolished the GEF activity of the Sec71 protein in a GEF assay (Figures 4E, F), thus behaving as a dominant-negative form (hereafter referred to as Sec71\textsuperscript{DN}). To determine a specific requirement of Sec71 function for dendrite pruning, we used the Gene-Switch system to pulse induce the expression of Sec71\textsuperscript{DN} at the middle third instar larval (90-96 h AEL) stage, which did not affect initial dendrite development and morphology in ddaC neurons at WP stage (Figure 3F). Strong dendrite severing defects were observed in all Sec71\textsuperscript{DN}-expressing ddaC neurons derived from RU486-induced animals and 9.7 primary and secondary dendrites remained unpruned at 16 h APF (Figures 3F, G), in contrast to no larval dendrites present in non-induced animals (Figures 3E, G). Some larval dendrites in Sec71\textsuperscript{DN}-expressing ddaC neurons remained attached by 24 h APF (data not shown). Continuous expression of Sec71\textsuperscript{DN} via ppk-Gal4 also caused simplified dendrite arbors in ddaC neurons (data not shown). Thus, the Gene-Switch experiments strongly suggest that the Sec71\textsuperscript{DN} dendrite pruning defect is not a secondary effect of its initial dendrite arborization defect. Thus, Sec71 regulates dendrite pruning of ddaC neurons, which is separable from its role in initial dendrite growth.

To further verify the functions of Sec71 in dendrite pruning, we generated a strong mutant allele, Sec71\textsuperscript{EX11}, from the mobilization of the P-element insertion Sec71\textsuperscript{GS16990} (Figure S3A). MARCM clones of Sec71\textsuperscript{EX11} consistently exhibited simple dendrite arbors and dendrite pruning defects (Figure S3B). Reintroduction of the Sec71 protein into Sec71\textsuperscript{EX11} mutant ddaC neurons fully restored the complex dendrite arbors and rescued the pruning defects (Figure S3B). In addition to ddaC neurons, ddaD/E neurons expressing Sec71\textsuperscript{DN} also exhibited dendrite pruning defects, as their larval dendrites were attached to their soma by 19 h APF (50%, Figure S3C). Sec71\textsuperscript{DN}-expressing ddaF neurons were eliminated by 16 h APF (Figure S3C), similar to wild-type neurons. Other known ArfGEFs, namely Steppke and
Arf6GEF (encoded by the loner gene), are dispensable for ddaC dendrite pruning (Figure S3D).

Sec71 is an Arf1GEF that preferentially interacts with GDP-bound Arf1

To examine the possibility that Sec71 functions as an Arf1GEF, we first determined the physical interaction between Arf1 and Sec71. We overexpressed the full-length Sec71 protein in S2 cells and performed GST pull-down experiments using various GST fusion proteins containing either wild-type Arf1 (Arf1WT), GTP-bound form (Arf1Q71L) or GDP-bound form (Arf1T31N). GEF proteins preferentially bind to the nucleotide-free form or the GDP-bound form but not the GTP-bound form. Importantly, Sec71 possessed a strong binding affinity for Arf1WT and GDP-Arf1 but not for GTP-Arf1 (Figure 4A). To confirm these interactions, we conducted co-immunoprecipitation experiments for Sec71 and Arf1. Similarly, we observed that Sec71 interacted strongly with Arf1WT and GDP-Arf1 but weakly with GTP-Arf1 (Figure 4B). Moreover, the interaction between Sec71 and wild-type Arf1 was attenuated in the presence of excess γ-GTP, a non-hydrolyzable GTP analog (Figure S4A). Thus, Sec71 preferentially interacts with the nucleotide-free form or the GDP-bound form of Arf1.

To further substantiate that Sec71 is an Arf1GEF, we investigated whether Sec71 is able to specifically catalyze the guanine nucleotide exchange of Arf1. We monitored the quantum yield of a non-hydrolyzable and fluorescent analogue of GTP, which increases upon binding to GTPase. His-Arf1 was incubated with different GST proteins, and the guanine nucleotide exchange of Arf1 was measured via the increase of fluorescence intensity. Sec71 tagged with GST resulted in rapid kinetics of fluorescence increase (Figure 4D), compared to the GST protein control (Figure 4C). The inverse of time constant, 1/τ value, reflects the intrinsic exchange activity of an GEF. The exchange activity of GST-Sec71 on Arf1 was
significantly higher than that of the GST control (Figure 4F). This exchange activity was abolished by the catalytically incompetent form of Sec71 (GST-Sec71\textsuperscript{EK}) (Figure 4E, F). As a control, we did not observe any exchange activity of GST-Sec71 on another small GTPase Arl1 (Figure S4B). Thus, Sec71 possesses specific GEF activity toward Arf1.

We further provided genetic evidence to strengthen that Arf1 functions downstream of Sec71 to regulate dendrite pruning. We had observed that the expression of Arf1\textsuperscript{Q71L}, like Arf1\textsuperscript{T31N}, caused a block of dendrite pruning, whereas the expression of wild-type Arf1 (Arf1\textsuperscript{WT}) did not (Figures 1G, H, I). The GTP-locked form Arf1\textsuperscript{Q71L} was unable to recue sec71 RNAi knockdown in terms of its dendrite pruning defects (data not shown) because excess GTP-Arf1, induced by Arf1\textsuperscript{QL}, is not functional. We therefore overexpressed Arf1\textsuperscript{WT} in Sec71 RNAi ddaC neurons to investigate its potential rescue effect on dendrite pruning. Importantly, the expression of wild-type Arf1 in Sec71 RNAi mutant ddaC (Figures 4H, I) fully rescued the dendrite pruning defects, compared to the control UAS transgene (Figures 4G, I). Moreover, overexpression of Arf1 also rescued the initial dendrite morphology defects at WP stage (Figures 4H-I). These data suggest that elevated level of Arf1 may enhance basal levels of activation/inactivation cycling, which is sufficient to override the requirement of the Arf1GEF Sec71 for dendrite pruning. As a control, the expression of another small GTPase Arf6\textsuperscript{WT} was unable to suppress pruning defects in Sec71 RNAi ddaC neurons (Figure S4C).

Collectively, Sec71 is an important GEF for Arf1 and facilitates the Arf1 cycling between GDP-bound and GTP-bound forms during dendrite pruning.

**Sec71 is co-localized with Arf1 on Golgi and their localizations are interdependent**

We next assessed the subcellular localization of Sec71 and its co-localization with Arf1. An antibody against the N-terminal epitope of Sec71 (Figure 6A) was generated and the specificity was confirmed in Sec71 RNAi and Sec71\textsuperscript{Es11} mutant neurons (Figure S5A).
Using the anti-Sec71 antibody, we observed that endogenous Sec71 was distributed on punctate structures and co-localized with the *trans*-Golgi marker GalT-GFP (Figure 5A-A”). The Sec71 signals were also co-localized with Arf1 (Figure 5B-B”), partially with the *cis*-Golgi marker GM130 (Figure 5C-C”) and adjacent to the ER exit marker Sec31-mCherry (Figure 5D-D”). Likewise, Sec71 is also important for the integrity of the Golgi apparatus in ddaC neurons. In Sec71\textsuperscript{DN} or Sec71 RNAi-expressing ddaC neurons, the *trans*-Golgi marker GalT-GFP was completely diffused in their somas (Figures 5E, S2F), compared to those in the wild type (Figure 5A’). The *cis*-Golgi compartments, indicated by GM130 signals, were also strongly reduced in size and number in Sec71\textsuperscript{DN} expressing ddaC neurons (Figure 5F). The ER exit marker Sec31-mCherry remained largely similar in Sec71\textsuperscript{DN}-expressing ddaC neurons (Figure 5G). These data suggest that the Golgi apparatus, particularly *trans*-Golgi, is severely disrupted in Sec71 mutant neurons, similar to that in Arf1 mutant neurons. Thus, Sec71, like Arf1, is localized on Golgi and also required for the integrity of the Golgi compartment in ddaC neurons.

To determine the dependency of Arf1 and Sec71 localizations, we checked their endogenous proteins in either Sec71 or Arf1 mutants. In Sec71 RNAi (Figure 5H’) and Sec71\textsuperscript{DN} Gene-Switch (Figure 5H’’) mutant ddaCs, Golgi localization of Arf1 was almost completely disrupted, compared to wild-type neurons (Figure 5H). Likewise, Sec71-positive puncta were largely absent in Arf1 RNAi (Figure 5I’) or Arf1\textsuperscript{T31N} Gene-Switch (Figure 5I’’) ddaC neurons, compared to wild-type ddaC neurons (Figure 5I). Occasionally, a couple of Sec71-labelled puncta were observed in Arf1 RNAi ddaC (Figure 5I’). Hence, Arf1 and Sec71 are mutually dependent on one another for their localizations on Golgi compartments. Formally, we cannot exclude the possibility that their localization interdependence is due to the disruption of Golgi integrity caused by loss of either gene.
To investigate whether Arf1 and Sec71 regulate the formation of secretory vesicles in ddaC neurons, we made use of a GFP-tagged version of Sec15 (Sec15-GFP), a component of the exocyst complex, to monitor the distribution of secretory vesicles in sensory neurons. Sec15-GFP has been previously used to visualize a subset of secretory vesicles in neurons (Jafar-Nejad et al., 2005). In wild-type ddaC neurons, Sec15-GFP was distributed as discrete punctate structures in the somas (Figure S5B), and was also enriched at dendritic branch points along major dendrites at WP stage (data not shown). Consistent with our observations, a previous study also reported Sec15 distribution in larval sensory neurons (Peng et al., 2015). Some Sec15-positive puncta were localized adjacent to or partially co-localized with the Arf1-positive Golgi apparatus (Figure S5B). Notably, Sec15-GFP-positive puncta were completely absent in all Arf1 RNAi (Figure S5C) or Sec71 RNAi (Figure S5C) ddaC neurons.

As a control, the ER exit marker Sec31-mCherry remained present in either Arf1 T31N (Figures 2F) or Sec71 DN (Figure 5G) mutant neurons. Thus, Arf1 and Sec71 appear to regulate biogenesis of secretory vesicles in ddaC sensory neurons.

To examine whether Arf1 and Sec71 regulate protein transport to the cell surface, we conducted a trafficking assay by detecting the levels of extracellular mCD8 epitope in a detergent-free condition. Via the Gene-Switch system, we pulse induced the expression of mCD8-GFP (murine CD8 fused to GFP) at the middle L3 stage and measured the levels of newly synthesized mCD8-GFP protein at WP stage in ddaC neurons. In wild-type WP ddaC neurons, the extracellular mCD8 epitope was robustly detectable on the dendrites by the anti-mCD8 antibody in the absence of the detergent (Figure S5D). We observed no or strongly reduced mCD8 signal on the surface of the dendrites in either Arf1 T31N (Figure S5D) or Sec71 DN (Figure S5D) ddaC neurons in the detergent-free condition. We found that overall GFP fluorescence representing both surface and internal pools of the protein in the soma of
Sec71<sup>DN</sup> and Arf1<sup>T21N</sup> ddaC neurons was similar to that in wild-type neurons. In the trafficking assays, mCD8 signals were absent on the soma, axons and proximal regions of dendrites of ddaC neurons presumably because these structures are tightly wrapped by glia and the antibody was not able to penetrate into the wrapped parts with the absence of the detergent.

Taken together, Arf1 and Sec71 co-localize on Golgi and regulate protein secretion in sensory neurons.

The DCB domain of Sec71 is essential for its Golgi localization

Sec71 contains DCB (dimerization and cyclophilin binding) and HUS (homology upstream of Sec7) domains in its N-terminal portion, a Sec7 domain in the middle portion, and HDS1-4 (homology downstream of Sec7) domains in its C-terminal portion (Figure 6A). We found that the Sec7 domain is essential for the Sec71 function in regulating dendrite pruning (Figure 3F). To elucidate whether other domains of Sec71 are also required for its function, we generated a series of RNAi-resistant transgenes expressing various truncated proteins and examined their abilities to rescue sec71 RNAi phenotypes (Figure S6A). While the expression of full-length Sec71 fully rescued the Sec71 RNAi phenotypes in terms of dendrite arborization and pruning (Figures 6C and 6G), the expression of DCB-deleted Sec71 protein was unable to significantly rescue the pruning defect in sec71 RNAi ddaC neurons at 16 h APF (Figures 6D, G) but partially restored the dendrite morphology (Figures 6D, G). Sec71<sup>ΔDCB</sup> did not appear to localize on Golgi (Figure S6B), suggesting that the DCB domain of Sec71 is essential for its Golgi localization as well as for its function in regulating dendrite pruning. We observed that HDS2-4 domains are not important for Sec71 function, as the Sec71 variants deleting either HDS2-4 (Figures 6E, G) or HDS3-4 domains (Figures 6F, G), like the full-length protein, completely rescued both dendrite pruning and arborization.
defects. Except Sec71<sup>ADC</sup>B, all the other Sec71 variants were able to localize on Golgi (Figure S6B). Thus, the DCB domain of Sec71 is important for proper function and Golgi localization of Sec71 during dendrite pruning.

**Arf1/Sec71-mediated trafficking is a prerequisite for downregulation of the cell adhesion molecule Nrg prior to dendrite pruning**

We previously reported that Rab5/ESCRT-dependent endocytic pathways downregulate the L1-CAM Nrg to promote dendrite pruning during early metamorphosis. In wild-type ddaC neurons, Nrg is drastically redistributed to early endosomes at 6 h APF prior to dendrite pruning and concomitantly its protein levels are also strongly reduced in the somas, dendrites and axons (Zhang et al., 2014). In *Rab5<sup>DN</sup>* ddaC neurons, Nrg protein levels were significantly increased in the soma, dendrites and axons (Figures 7B, E) (Zhang et al., 2014), compared to wild-type neurons (Figures 7A, E). We therefore examined whether Arf1/Sec71-dependent secretory pathway regulates the protein levels of Nrg at 6 h APF. Surprisingly, Nrg protein levels were significantly elevated in the dendrites, axons or soma of Arf1<sup>T31N</sup> or Sec71<sup>DN</sup> Gene-Switch ddaC neurons (Figures 7C-E), similar to those in *Rab5<sup>DN</sup>* neurons. Nrg protein levels in Arf1<sup>T31N</sup> or Sec71<sup>DN</sup> Gene-Switch ddaC neurons were comparable to those in Rab5<sup>DN</sup>-expressing neurons (Figures 7B-E). Moreover, Arf1 and Sec71 also regulate endosomal localization of Nrg prior to dendrite pruning. In wild-type neurons, Nrg protein mainly co-localized with the early endosomal marker GFP-2xFYVE at 6 h APF (Figure S7A). In contrast, in Arf1<sup>T31N</sup> or Sec71<sup>DN</sup> Gene-Switch ddaC neurons, Nrg did not co-localize with those GFP-2xFYVE puncta (Figure S7A), suggesting a block of Nrg endocytosis. Thus, Arf1 and Sec71, like Rab5, are required to facilitate Nrg endocytosis and downregulation prior to dendrite pruning.
To examine whether Arf1 and Sec71 regulate dendrite pruning upstream of Nrg function, we knocked down Nrg, via two independent nrg RNAi transgenes, in Arf1 RNAi or Sec71 RNAi-expressing ddaC neurons. Importantly, the expression of two independent nrg RNAi transgenes, both of which efficiently knocked down Nrg protein (Zhang et al., 2014), dramatically suppressed the dendrite severing defects in Arf1 RNAi (Figures 7G-I) or Sec71 RNAi (Figures 7K-M) ddaC neurons, further supporting the notion that similar to Rab5, Arf1 and Sec71 facilitate Nrg endocytosis and degradation to promote dendrite pruning. There are at least two possibilities about how Arf1 and Sec71 regulate Nrg endocytosis: they may act in a pathway either upstream of or parallel to Rab5 to facilitate endocytosis. To distinguish these two possibilities, we conducted double mutants for Rab5 and Arf1/Sec71 and examine their epistasis. Pulse expression of Rab5\textsuperscript{DN} at the late larval stage via the Gene-Switch system led to severe accumulation of ubiquitinated protein deposits on 2-3 enlarged endosomes which were positively labelled by Ubiquitin (Figure S7B), the early endosomal marker Avl (Figure S7C) and Nrg (Figure S7C). These data suggest that Rab5 does not regulate initial formation of early endosomes but is important for subsequent endosomal maturation as well as Nrg downregulation (Zhang et al., 2014). In contrast, Ubiquitin and Avl were not accumulated in Arf1\textsuperscript{T31N} or Sec71\textsuperscript{DN}-expressing ddaC neurons (Figures S7B-C). Importantly, double mutants of Arf1\textsuperscript{T31N} and Rab5\textsuperscript{DN} did not exhibit any ubiquitinated aggregates (Figure S7B) and Avl-positive enlarged endosomes (Figure S7C) in ddaC neurons, resembling Arf1\textsuperscript{T31N} single mutant neurons. Likewise, co-expression of Sec71\textsuperscript{DN} and Rab5\textsuperscript{DN} did not result in formation of ubiquitinated protein deposits (Figure S7B) and Avl-positive endosomes (Figure S7C), resembling Sec71\textsuperscript{DN} mutant neurons. Thus, these epistatic data support the first possibility that Arf1 and Sec71 act upstream of Rab5 to facilitate initial formation of endosomes and robust endocytosis prior to dendrite pruning.
Discussion

**Sec71 is an important GEF for Arf1 in *Drosophila***

The small G protein Arf1 regulates vesicular trafficking in eukaryotes and is activated on *cis*-Golgi by the Gea1/GBF1 family of Arf1GEF or on *trans*-Golgi by the Sec7/BIG1 family (Cherfils, 2014). It has been reported that *Drosophila* Arf1 regulates hematopoietic niche maintenance, blood cell precursor differentiation *in vivo*, planar cell polarity, and lamellipodium formation in S2 cells (Carvajal-Gonzalez et al., 2015; Humphreys et al., 2012; Khadilkar et al., 2014). Arf1 and other Golgi proteins were reported to exhibit upregulation of their transcripts in axon pruning of MB γ neurons during the larval-pupal transition (Hoopfer et al., 2008). In this study, we report an important role of Arf1 in regulating dendrite pruning of ddaC sensory neurons. We found that Arf1 puncta overlap with the *trans*-Golgi marker GalT and partially with the *cis*-Golgi marker GM130, suggesting that Arf1 is primarily localized on *trans*-Golgi in ddaC sensory neurons. A specific GEF for Arf1 has not been identified in *Drosophila*. Our *in vivo* and *in vitro* data provide compelling evidence that Sec71 is a GEF for Arf1 in *Drosophila*. First, Sec71 was co-localized with Arf1 on Golgi and their localizations were inter-dependent in ddaC neurons. Second, Sec71 preferentially bound to the GDP-bound form of Arf1 instead of GTP-bound form. Third, in the GEF assays Sec71 accelerated the release of GDP from Arf1. Fourth, both Arf1 and Sec71 are required for dendrite pruning, as loss of *Sec71* or *Arf1* led to comparable pruning defects in ddaC sensory neurons. Finally, the expression of Arf1 fully restored WP dendrite morphology and rescued the pruning defects in *Sec71* RNAi ddaC neurons. Thus, Sec71 is specifically required for the GDP-to-GTP exchange of Arf1.

Our structure-function analyses indicate that DCB domain of Sec71 is important for its targeting on Golgi (Figure S6). In contrast, another small GTPase Arl1 was reported to bind to the N-terminal region of Sec71 (DCB and HUS1 domains) and recruit Sec71 on
trans-Golgi apparatus in *Drosophila* S2 cells, and mammalian Arl1 is required for the recruitment of BIG1/2 (mammalian homologues of Sec71) on trans-Golgi (Christis and Munro, 2012). Unexpectedly, we found that mutant ddaC neurons derived from arl11, a null arl1 mutant (Torres et al., 2014), did not exhibit delocalization of Sec71 nor dendrite pruning defects in ddaC neurons (Figure S1C), suggesting an Arl1-independent mechanism regulating Sec71 localization and function in ddaC sensory neurons.

**Secretory pathway plays a novel and important role in governing neurite pruning**

Extensive studies have attempted to understand roles of post-Golgi trafficking in outgrowth and elaboration of dendrites in growing neurons. Post-Golgi trafficking is polarized toward apical dendrites of rodent hippocampal neurons and selectively regulates the growth of dendrites (Horton and Ehlers, 2003; Horton et al., 2005). The dynamics of the Golgi outposts, mediated by the Golgin Lava Lamp, dynein-dynactin complex and Leucine-rich repeat kinase (Lrrk), is important for dendrite growth in *Drosophila* class IV da neurons (Lin et al., 2015; Ye et al., 2007). A small GTPase Rab10, which mediates post-Golgi vesicle trafficking, regulates dendrite growth and branching of multi-dendritic sensory neurons in both *C. elegans* and *Drosophila* (Taylor et al., 2015; Zou et al., 2015).

In this study, we provide compelling evidence to demonstrate that post-Golgi trafficking plays a crucial role in proper dendrite pruning in sensory neurons. First, we identified the key small GTPase Arf1 that is important for post-Golgi trafficking regulates secretory vesicle biogenesis and dendrite pruning in sensory neurons during metamorphosis. Second, a Sec7-domain-containing protein Sec71 acts as a specific GEF for Arf1 and co-localizes with Arf1. Like Arf1, Sec71 is also an essential factor for regulating dendrite pruning. Third, given that both Arf1 and Sec71 also regulate dendrite growth and arborization in ddaC neurons, we further confirmed a critical role of Arf1 and Sec71 in dendrite pruning
using the Gene-Switch system. Pulse induction of Arf1\textsuperscript{T31N} or Sec71\textsuperscript{DN} at the middle third instar larval stage when the complete larval dendrite arbors form in ddaC neurons consistently caused much more severe dendrite pruning defects. These results highlight that the secretory pathway play separable roles in two distinct processes, namely dendrite growth and dendrite pruning. Arf1 was reported to regulate post-Golgi secretion by recruiting its downstream effectors, including the clathrin adaptors AP-1 and AP-3, and GGA (Cherfils, 2014). Post-Golgi trafficking pathways include the transport from Golgi to plasma membrane (exocyst complex-mediated), from Golgi to early/sorting endosomes (AP-1-mediated), from Golgi to late endosomes (Golgi-localized Gamma-ear containing Arf-binding protein or GGA-mediated) as well as from Golgi to lysosomes (AP-3-mediated). It is conceivable that at least one of these post-Golgi trafficking routes is involved in dendrite pruning of sensory neurons.

**Arf1/Sec71-dependent post-Golgi trafficking is a prerequisite for endocytosis and downregulation of Nrg prior to dendrite pruning**

We previously reported that Rab5/ESCRT-dependent endocytic pathways facilitate dendrite pruning by downregulating the L1-CAM Nrg in ddaC neurons during metamorphosis (Zhang et al., 2014). In MB γ neurons, the JNK pathway promotes axon pruning by downregulating another adhesion molecule Fasciclin II (Bornstein et al., 2015). These studies suggest a general mechanism whereby cell adhesion molecules are internalized and downregulated to destabilize dendrites and/or axons during neurite pruning. However, the mechanism that triggers Nrg endocytosis is poorly understood. In this study, we demonstrate that Arf1/Sec71-mediated secretory pathway promotes endocytosis and downregulates Nrg prior to dendrite pruning. First, while Nrg levels were strongly reduced prior to dendrite pruning, loss of *Arf1* or *Sec71* led to elevated levels of Nrg protein in
dendrites, axons and soma, comparable to Rab5 mutant neurons. Second, Nrg was no longer redistributed on FYVE-positive endosomes in Arf1 or Sec71 mutant ddaC neurons, suggesting a blockage of Nrg endocytosis. Third, while Rab5 mutant neurons exhibited robust ubiquinated protein aggregates and enlarged endosomes, further removal of either Arf1 or Sec71 suppressed these rab5 mutant phenotypes, suggesting that the secretory pathway acts upstream of Rab5 to positively regulates endocytosis. Finally, knockdown of Nrg significantly suppressed the dendrite pruning defect in Arf1 or Sec71 mutant neurons, supporting the notion that the secretory pathway promotes Nrg endocytosis and downregulation. Thus, the secretory pathway not only secretes the cell adhesion molecules to the dendrite surface and stabilize dendrites (Taylor et al., 2015; Zou et al., 2015), but also unexpectedly promotes the internalization and turnover of the adhesion molecules (this study). It is conceivable that in response to ecdysone pulse, the secretory pathway might be required to specifically secrete an as-yet-unidentified ligand to trigger massive endocytosis of the L1-CAM Nrg and thereby leads to degeneration of larval dendrites. Further studies may continue to elucidate what ligand or secreted protein promotes Nrg endocytosis.

**Materials and Methods**

**Fly Strains**

The fly stocks used in this study were maintained on standard food at 25 °C. See supplementary Materials and Methods for further details of fly stocks used in this study.

**MARCM analysis of ddaC neurons**

We carried out MARCM clonal analysis, dendrite imaging, and branch quantification as previously described (Kirillo et al., 2009). See supplementary Materials and Methods for further details.
RU486/mifepristone treatment for the Gene-Switch system

Wild-type and mutant embryos were collected at 3 h intervals and were reared on standard food to the middle 3rd instar larval stage before being transferred to the standard culture medium containing 240 µg/ml mifepristone (Sigma Aldrich M8046).

Generation of Sec71 mutants

We crossed Sec71\textsuperscript{GS16990} flies with a fly strain carrying the Δ2–3 transposase to induce imprecise excision as previously described (Kirilly et al., 2009). See supplementary Materials and Methods for further details.

Generation of Arf1, Sec71 and other transgenes

The Arf1 and Sec71 full-length cDNA were PCR from EST LD24904 and LD29171 (DGRC) into Topo Entry and pDonor, respectively (Life Tech). The variants of Sec71 and Arf1 were generated by site mutagenesis (Agilent Tech). The cDNA fragment encoding the aa 1-100 portion of rat ManII protein were used to generate ManII-Venus transgene (Bestgene Inc). See supplementary Materials and Methods for further details.

Arf1 and Sec71 antibody production

The full-length Arf1 and the aa 60-345 fragment of Sec71 were used for their respective antibody generation. See supplementary Materials and Methods for further details.

Co-Immunoprecipitation (Co-IP) and GST pull-down assay

Transfected S2 cells were homogenized with the lysis buffer. The supernatants were used for immunoprecipitation, followed by incubation with protein A/G beads (Pierce Chemical Co.). Protein A/G beads were washed four times. Bound proteins were separated by SDS-PAGE and analysed by Western blotting with anti-Myc, anti-Flag HRP-conjugated antibody. See supplementary Materials and Methods for further details.
**Trafficking Assay**

The fillets were incubated with rat monoclonal anti-CD8α (1:100, CALTAG Laboratories) in PBS and washed for three times with PBS in the detergent-free condition. See supplementary Materials and Methods for further details.

**In vitro guanine nucleotide exchange assay**

We carried out the in vitro guanine nucleotide exchange assays as previously described (Mahajan et al., 2013). GST fusion proteins for the Sec7 domain of Sec71 (aa554-790), its dominant negative form (E-K) and Sec7 domain of hBIG1 were expressed using the GST expression vector pGEB. 6xHis-tagged Arf1WT and 6xHis-tagged Arl1WT were expressed via the vector pET37b. See supplementary Materials and Methods for further details.

**Quantification of immunolabeling**

We quantified the immunolabeling intensities of Nrg at 6 h APF, as described previously (Zhang et al., 2014). One-way ANOVA and Bonferroni tests were utilized for comparison between different conditions (*p<0.05, **p<0.01, ***p<0.001, n.s., not significant). See supplementary Materials and Methods for further details.

**Quantification of ddaC dendrites**

Live confocal images of ddaC neurons were shown at WP and 16h APF. Statistical significance was determined using either two-tailed Student’s T test (two samples) or one-way ANOVA and Bonferroni test (multiple samples) (*p<0.05, **p<0.01, ***p<0.001, n.s., not significant). See supplementary Materials and Methods for further details.
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Competing interests: The authors declare that no competing interests exist.

Author Contributions

F.Y. and Y.W. conceived and designed the study. H.Z. generated anti-Arf1 antibody and performed Nrg staining. M.S. and L. L. conducted the GEF assays. Y.W., H.Z., M.S., Y.L., L.L. and F.Y. analyzed the data. F.Y. and Y.W. wrote the paper.

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References


Figures

A

White pupa 5-8 h APF 10-12 h APF 16-18 h APF >24 h APF
Severing Fragmentation Clearance Regrowth

B

Control Arf1 RNAi #1 Arf1 RNAi #1 Rescue Arf1T11V 2 X pnp-Gal4

WP

16 h APF

C

D

E

Arf1T11V GeneSwitch

F

Non-induced Induced

WP

16 h APF

G

H

Induced

I

Induced

J

Number of primary and secondary branches

N.S.  N.S.  N.S.  N.S.  ***  ***  ***  ***

WP  16 h APF
Figure 1. Arf1 GTPase is critical for dendrite pruning in ddaC sensory neurons.

(A) A schematic representation of dendrite pruning in ddaC neurons during early metamorphosis. (B–I) Dendrites of control RNAi (B), Arf1 RNAi #1 (C), Arf1 RNAi #1 rescue (D), Arf1T31N (E), GeneSwitch-Gal4-2295-driven Arf1T31N (F, non-induced; G, induced), Arf1Q71L (H) and Arf1WT (I) ddaC neurons at WP and 16 h APF stages. Red arrowheads point to the ddaC somas. (J) Quantification of the average number of primary and secondary ddaC dendrites. Scale bar in (B) represents 50 μm. Error bars represent S.E.M.. N.S. , not significant; ***p < 0.001 as assessed by one-way ANOVA and Bonferroni test.
Figure 2. Arf1 is localized on the Golgi compartments in ddaC sensory neurons.

(A-A”) Distribution of Arf1 and trans-Golgi marker GalT-GFP in wild-type (WT) ddaC neurons expressing a UAS-GalT-GFP transgene. (B-B”) Distribution of Arf1 and cis-Golgi marker GM130 in wild-type ddaC neurons. (C-C”) Distribution of Arf1 and ER exit point marker Sec31-mcherry in WT ddaC neurons expressing a UAS-Sec31-mCherry transgene. (D-F) Distribution of GalT-GFP (D), GM130 (E) and Sec31-mcherry (F) in Arf1T31N expressing ddaC neurons. (G-H”) ManII-Venus-positive dendritic Golgi outpost in wild-type (G-G”), Arf1 RNAi (H-H”). GS denotes Gene-Switch. Scale bar in (A) and (G) represents 10 μm. ddaC somas are marked by dashed lines.
Figure 3. Sec71 is crucial for the regulation of dendrite pruning in sensory neurons.

(A–E) Dendrites of wild-type (A), Sec71 RNAi #1 (B), Sec71 RNAi #1 and Sec71 RNAi resistant full-length construct-coexpressing (C), Sec71 RNAi #2 (D), and GeneSwitch-Gal4-
2295-driven Sec71\textsuperscript{DN} (E, non-induced; F, induced) ddaC neurons at WP and 16 h APF stages. Red arrowheads point to the ddaC somas. (G) Quantification of the average number of primary and secondary ddaC dendrites. Scale bar in (A) represents 50 μm. Error bars represent S.E.M. N.S., not significant; ***p < 0.001 as assessed by one-way ANOVA and Bonferroni test.
Figure 4. Sec71 is an Arf1GEF that preferentially interacts with GDP-bound Arf1. 

(A) Sec71 associated strongly with Arf1WT and Arf1T31N, weakly with Arf1Q71L, but not with GST alone in GST pull down assays (n=3). Myc-Sec71 was transfected and expressed in S2 cells. (B) Sec71 associated strongly with Arf1WT and Arf1T31N, and weakly with Arf1Q71L (n=3). S2 cells were co-transfected with Myc-Sec71 and different forms of Flag-Arf1. (C-E) Kinetics of fluorescence increases of GTP analog. Incubation of GST-tagged Sec7 domain of Sec71 (Sec71) (D) led to more rapid kinetics towards Arf1, compared to the GST protein control (C) and GST-tagged mutated Sec7 domain (Sec71EK) (E). (F) Quantification of the exchange activity reflected by inverse of time constant, 1/τ value. (G-H) Dendrites of Sec71 RNAi ddaC neurons coexpressing the UAS control (G) or Arf1WT (H) at WP and 16 h APF. Red arrowheads point to the ddaC somas. (I) Quantification of the average number of primary and secondary ddaC dendrites. Scale bars in (G) represent 50 μm. Error bars represent S.E.M.. N.S., not significant; **p < 0.001 as assessed by one-way ANOVA and Bonferroni test.
Figure 5. Sec71 is co-localized with Arf1 on Golgi and their Golgi localizations are interdependent. (A-A”) Distribution of Sec71 and GaIT-GFP in wild-type (WT) ddaC neurons expressing a UAS-GaIT-GFP transgene. (B-B”) Distribution of Sec71 and Arf1 in wild-type ddaC neurons. (C-C”) Distribution of Sec71 and GM130 in wild-type ddaC neurons. (D-D”) Distribution of Sec71 and Sec31-mcherry in WT ddaC neurons expressing a UAS-Sec31-mCherry transgene. (E-G) Distribution of GaIT-GFP (E), GM130 (F) and Sec31-mcherry (G) in Sec71DN expressing ddaC neurons. (H-H”) Distribution of Arf1 in wild-type (H), Sec71 RNAi (H’) and Sec71DN (H”) expressing ddaC neurons. (I-I”) Distribution of Sec71 in wild-type (I), Arf1 RNAi (I’) and Arf1T31N (I”) expressing ddaC neurons. GS denotes Gene-Switch. Scale bar in (A) represents 10 μm. ddaC somas are marked by dashed lines.
Figure 6. Structure-function analysis of the Sec71 protein.

(A) A schematic representation of the full-length Sec71 protein. (B–F) Dendrites of ddaC neurons co-expressing Sec71 RNAi #1 and UAS control (B), Sec71 RNAi #1 and Sec71 full length (C), Sec71 RNAi #1 and Sec71<sup>ΔDCB</sup> (D), Sec71 RNAi #1 and Sec71<sup>ΔHDS2-4</sup> (E), or Sec71 RNAi #1 and Sec71<sup>ΔHDS3-4</sup> (F) at WP and 16 h APF. Red arrowheads point to the ddaC somas. (G) Quantification of the average number of primary and secondary ddaC dendrites. Scale bar in (B) represents 50 μm. Error bars represent S.E.M.. N.S., not significant; ***p < 0.001 as assessed by one-way ANOVA and Bonferroni test.
Figure 7. Arf1/Sec71-mediated secretory pathway is required for downregulation of the cell adhesion molecule Nrg prior to dendrite pruning.

(A–D) Distribution of Nrg in wild-type (A), Rab5DN (B), Arf1T31N (C), and Sec71DN (D) ddaC neurons at 6 h APF. ddaC somas are marked by dashed lines, axons by arrows, and proximal dendrites by curly brackets. ddaE somas are marked by asterisks. White arrowheads point to apoptotic ddaF neurons. (E) Quantification of Nrg immunostaining intensity. (F–H) Dendrites of Arf1 RNAi ddaC neurons co-expressing the control RNAi line (F) or nrg RNAi #1 (G) or #2 (H) at 16 h APF. Red arrowheads point to the ddaC somas. (I) Quantification of the average number of primary and secondary ddaC dendrites. (J–L) Dendrites of Sec71 RNAi ddaC neurons co-expressing the control RNAi line (J) or nrg RNAi #1 (K) or #2 (L) at 16 h APF. (M) Quantification of the average number of primary and secondary ddaC dendrites. Scale bars represent 20 μm in (A) and 50 μm in (F). Error bars represent S.E.M.. N.S., not significant, *p<0.05, **p<0.01, ***p < 0.001 as assessed by one-way ANOVA and Bonferroni test.
Supplemental Information

Sec71 functions as a GEF for the small GTPase Arf1 to govern dendrite pruning of Drosophila sensory neurons

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Figure S1
Figure S1. Arf1 is required for dendrite pruning in da sensory neurons.

(A-D) Live confocal images of ddaC or ddaD/E neurons expressing mCD8-GFP at WP stage, 16 h or 19 h APF. (A) While the control neurons that expressed an irrelevant RNAi line pruned all the dendrites, ddaC neurons overexpressing Arf1 RNAi #2 by ppk-Gal4 exhibited simple dendrite arbors at WP stage and dendrite pruning defects at 16 h APF. Arf1DR-expressing ddaC neurons by two copies of ppk-Gal4 driver at WP stage exhibited normal dendrite morphology and pruned their larval dendrites at 16 h APF. Red arrowheads point to the ddaC somas. (B) While wild-type class I ddaD/ddaE neurons pruned their larval dendrites at 19 h APF, Arf1T31N mutant ddaD and ddaE clones retained some of their larval dendrites at the same time point. Red arrows point to the ddaD somas and open arrowheads to the ddaE somas. Wild-type ddaF underwent apoptosis and disappeared at 16 h APF. Similarly, Arf1T31N ddaF labelled by GSG2295-driven mCD8-GFP and Arf1 RNAi #1 mutant ddaF neurons were also removed at 16 h APF. Red arrowheads point to the ddaC somas, and blue arrowheads point to the ddaF somas. (C) Dendrites of Arf6DN-expressing ddaC neurons and Arl1MARCM ddaC clones (n=5) were pruned at 16 h APF, similar to the wild-type controls. (D) Similar to the control RNAi-expressing ddaC neurons, ddaC neurons overexpressing Arf1 RNAi #1 (n=14) and Sec7 RNAi #1 (n=12) by ppk-Gal4 pruned all the dendrites at 32 h APF. Red arrowheads point to the ddaC somas. Scale bar in (A) represents 50 μm. See genotypes in Supplemental list of fly strains.
Figure S2
Figure S2. Arf1 does not localize on endosomes and mitochondria in ddaC sensory neurons.

(A-E) Confocal images of wild-type and mutant ddaC neurons at the late wL3 stage immunostained with anti-Arf1 or anti-Lva. ddaC somas are marked by dashed lines. (A) While Arf1 exhibited punctate structures in wild-type ddaCs, Arf1 staining in Arf1 #1 RNAi or Arf1 #2 RNAi ddaC neurons was abolished, verifying the specificity of the anti-Arf1 antibody. (B) Arf1-positive puncta (in red) largely co-localized with the Golgi marker Lva (in green) in wild-type ddaC somas. (C) Arf1-positive puncta (in red) did not overlap with the recycling endosomal marker Rab4-mRFP (in green) in wild-type ddaC neurons. (D) Arf1-positive puncta (in red) did not overlap with the early endosomal marker GFP-Rab5 (in green) in wild-type ddaC neurons. (E) Arf1-positive puncta (in red) did not overlap with the mitochondrial marker Mito-GFP (in green) in wild-type ddaC neurons. (F-H) Live confocal images of ddaC neurons expressing GalT-GFP and mCD8-RFP at WP stage. (F) GalT-GFP signals in soma were largely disrupted in Arf1 RNAi and Sec71 RNAi-expressing ddaC neurons. (G-H) GalT-GFP-positive dendritic Golgi outposts were disrupted in Arf1 RNAi ddaC neurons (H), compared to those in wild-type ddaC neurons (G). Scale bars in (A) and (G) represent 10 μm. See genotypes in Supplemental list of fly strains.
Figure S3. Sec71 is crucial for the regulation of dendrite pruning in sensory neurons.

(A) A schematic diagram of the Sec71 gene and the deleted region of the Sec71<sup>Ex11</sup> mutant. The P-element insertion P{GSV6}GS16990, which is inserted in the first exon of the gene, was used to generate imprecise excision mutants. The start site of the Sec71 open reading frame is shown in an arrow. (B-E) Live confocal images of ddaC or ddaD/E neurons expressing mCD8-GFP at wL3, WP, 16 h or 19 h APF stages. (B) Sec71<sup>Ex11</sup> MARCM ddaC clones exhibited simple arbors at WP stage and pruning defects at 16 h APF. Both dendrite morphological defects and pruning defects could be rescued by expressing full-length Sec71 protein in Sec71<sup>Ex11</sup> MARCM ddaC neurons. Red arrowheads point to the ddaC somas. Quantification of the average number of primary and secondary ddaC dendrites. Error bars represent S.E.M.. ***p < 0.001 as assessed by two-tailed Student’s T test. (C) While wild-type class I ddaD/ddaE neurons pruned their larval dendrites at 19 h APF, Sec71<sup>DN</sup> ddaD or ddaE MARCM mutant clones failed to prune their larval dendrites at 19 h APF. Open arrowheads point to the ddaE somas. Wild-type ddaFs underwent apoptosis and were gone by 16 h APF. Similarly, Sec71<sup>DN</sup> ddaFs labelled by GSG2295-driven mCD8-GFP also underwent apoptosis by 16 h APF. Red arrowheads point to the ddaC somas, and blue arrowheads point to the ddaF somas. (D) Dendrites of loner<sup>T1032</sup> MARCM and Step<sup>DN</sup>-expressing ddaC neurons were removed by 16 h APF. (E) While control RNAi-expressing ddaC neurons exhibited complex arbors at 96 h AEL, Sec71 RNAi-expressing ddaC neurons exhibited simplified arbors. Red arrowheads point to the ddaC somas. Scale bar in (B) and (E) represents 50 μm. See genotypes in Supplemental list of fly strains.
Figure S4

A

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B

C

16 h APF

Sec71 RNAi #1 + UAS control  
Sec71 RNAi #1 + Arf\textsuperscript{WT}  

Number of primary and secondary dendrites attached to soma

- 16 h APF

19 23  

N.S.
Figure S4. Sec71 is an Arf1GEF that preferentially interacts with GDP-bound Arf1.

(A) GST-Arf1 WT was associated with Myc-Sec71 but not with the Myc-tagged control protein, and this association was reduced in the presence of γ-GTP (n=3). Myc-tagged Sec71 and control protein were transfected and expressed in S2 cells. Error bars represent S.E.M.. ***p < 0.001 as assessed by one-way ANOVA and Bonferroni test. (B) Kinetics of fluorescence increases of GTP analog. Incubation of GST-tagged Sec7 domain of Sec71 (Sec71) led to slow kinetics towards Arl1, similar to the control GST-tagged Sec7 domain of hBIG1. Quantification of the exchange activity reflected by inverse of time constant, 1/τ value. N.S. not significant, as assessed by a Student’s T-test. (C) The expression of Arf6 WT was unable to suppress the pruning defects in Sec71 RNAi ddaC neurons at 16 h APF. Red arrowheads point to the ddaC somas. Quantification of the average number of primary and secondary ddaC dendrites. Scale bar in (C) represents 50 μm. Error bars represent S.E.M.. N.S. not significant, as assessed by two-tailed Student’s T test. See genotypes in Supplemental list of fly strains.
Figure S5
Figure S5. Arf1 and Sec71 regulate protein transport to the dendrite surface.

(A) Confocal images of wild-type and mutant ddaC neurons at the late wL3 stage immunostained with anti-Sec71. ddaC somas are marked by dashed lines. While Sec71 was distributed as punctate structures in wild-type ddaCs, Sec71 signals were completely abolished in Sec71 RNAi #1 ddaC neurons and Sec71Ex11 MARCM clones, verifying the specificity of the Sec71 antibody. (B) Sec15-positive puncta were localized adjacent to or partially colocalized with the Arf1-positive Golgi apparatus in wild-type ddaC neurons. (C) Sec15-GFP puncta were disrupted in Arf1 RNAi and Sec71 RNAi knockdown ddaC neurons, compared to the wild type. ddaC somas are marked by dashed lines. (D) mCD8 signals on the surface of the dendrites in wild-type ddaC neurons were detected by the antibody against its extracellular epitope in the detergent-free condition. However, the mCD8 signals were greatly reduced in Sec71DN, and Arf1T31N expressing ddaC neurons. The graph represents quantification of normalized dendritic mCD8/GFP intensity. The scale bars represent 10 μm in (A) and 20 μm in (D). Error bars represent S.E.M.. ***p < 0.001 as assessed by one-way ANOVA and Bonferroni test. See genotypes in Supplemental list of fly strains.
Figure S6

A

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B

![GalT-GFP](image1)

![Sec71](image2)

![Merge](image3)

![GalT-GFP](image4)

![Sec71](image5)

![Merge](image6)
Figure S6. Structure-function analysis of the Sec71 protein.

(A) A summary of structure-function analysis of Sec71. Sec71 contains multiple domains: Dimerization/Cyclophilin Binding domain (DCB), Homology Upstream of Sec7 domain (HUS), Sec7 domain and Homology Downstream of Sec7 domain (HDS). (B) Confocal images of ddaC neurons at the late wL3 stage immunostained with anti-Sec71. Similar to full-length RNAi-resistant Sec71, Sec71^{ΔHUS}, Sec71^{ΔHDS1}, Sec71^{ΔHDS2-4} and Sec71^{ΔHDS3-4}, but not Sec71^{ΔDCB}, were localized on Golgi compartments labelled by GalT-GFP in ddaC neurons. Scale bar in (B) represents 10 μm. See genotypes in Supplemental list of fly strains.
Figure S7

A

Wild type GeneSwitch  Arf1^{13N} GeneSwitch  Sec71^{10N} GeneSwitch

6 h APF 6 h APF 6 h APF

B

Lbs

Rab5^{DN} GeneSwitch  Arf1^{13N} GeneSwitch  Rab5^{DN} GeneSwitch  Rab5^{DN}; Arf1^{13N} GeneSwitch  Sec71^{10N} GeneSwitch  Rab5^{DN}; Sec71^{10N} GeneSwitch

6 h APF 6 h APF 6 h APF 6 h APF

C

Adh

Nrg

Rab5^{DN} GeneSwitch  Arf1^{13N} GeneSwitch  Rab5^{DN}; Arf1^{13N} GeneSwitch  Sec71^{10N} GeneSwitch  Rab5^{DN}; Sec71^{10N} GeneSwitch

6 h APF 6 h APF 6 h APF 6 h APF 6 h APF
Figure S7. Arf1/Sec71-mediated secretory pathway is required for downregulation of the cell adhesion molecule Nrg prior to dendrite pruning

(A-C) Confocal images of ddaC neurons at 6 h APF stage immunostained for anti-Nrg (in red), anti-Ubiquitin (in red) or anti-Avl (in blue). (A) While Nrg proteins in wild-type ddaC neurons were redistributed to the early endosome labelled by GFP-2xFYVE at 6 h APF, Nrg in Arf1T31N or Sec71DN-expressing ddaC neurons did not co-localize with those GFP-2xFYVE puncta and remained on the plasma membrane. (B) 2-3 ubiquitinated protein deposits were accumulated in Rab5DN expressing ddaC neurons but not in Arf1T31N, Rab5DN and Arf1T31N co-expressing, Sec71DN, or Rab5DN and Sec71DN co-expressing ddaC neurons at 6 h APF. (C) Avl/Nrg-positive aberrant endosomes were present in Rab5DN expressing ddaC neurons but absent in Arf1T31N-expressing, Rab5DN and Arf1T31N-co-expressing, Sec71DN-expressing, or Rab5DN and Sec71DN-co-expressing ddaC neurons at 6 h APF. ddaC somas are marked by dashed lines. The scale bars in (A) and (B) represent 10 μm. See genotypes in Supplemental list of fly strains.
Supplemental Material and Methods

Fly Strains

The following fly stocks were used in this study: *UAS-Rab5<sup>DN</sup>* (M. Gonzalez-Gaitan)(Wucherpfennig et al., 2003), *UAS-GFP-Rab5* (M. Gonzalez-Gaitan)(Wucherpfennig et al., 2003), *UAS-MicalN<sup>ter</sup>* (A. Kolodkin)(Terman et al., 2002), *UAS-GFP-2xFYVE* (M. Gonzalez-Gaitan)(Wucherpfennig et al., 2003), *ppk-Gal4* on II and III (Y. Jan) (Grueber et al., 2003), *SOP-flp* (#42) (a kind gift from T. Uemura), *UAS-Sec31-mCherry* (S. Luschnig)(Forster et al., 2010), *UAS-Sec15-GFP* (H. Bellen)(Jafar-Nejad et al., 2005), *UAS-Arf6<sup>DN</sup>* (E. Chen), *UAS-Arf6* (E. Chen), *loner<sup>T1032</sup>*(E. Olson)(Chen et al., 2003), *UAS-Step<sup>DN</sup>* (T. Harris)(Lee and Harris, 2013), *Arl1<sup>1</sup>* (J. Kennison)(Tamkun et al., 1991).

The following stocks were obtained from Bloomington Stock Center (BSC): *Gal4<sup>109(2)80</sup>, elav-Gal4*, Control RNAi (BL#50613), *nrg* RNAi #1 (BL#38215), *nrg* RNAi #2 (BL#37496), *ppk-CD4-tdGFP* (BL#35843), *GS2295-Gal4* (BL#40266), *Sec71* RNAi #1 (BL#32366), *UAS-GalT-GFP* (BL#30902), *UAS-Rab4-mRFP* (BL#8505), *UAS-mito-HA-GFP* (BL#8442).

The following Stocks were obtained from Vienna *Drosophila* RNAi Center (VDRC): *Sec71* RNAi #2 (v100300), *Arfl* RNAi #1 (v23082), *Arfl* RNAi #2 (v103572).

The following stocks were obtained from Kyoto Stock Center: *Sec71<sup>GS16990</sup>* (#206863).

Generation of *Sec71* mutants

We crossed *Sec71<sup>GS16990</sup>* flies with a fly strain carrying the Δ2–3 transposase to induce imprecise excision. About 150 lines were established on the basis of the absence of the *w+* marker. 56 lethal lines were isolated and subjected to genomic PCR and DNA sequencing analysis. The line with a 1461-bp deletion was named *Sec71<sup>Ext11</sup>* (Figure S3A). The
Sec71<sup>GSI6990</sup> insertion line probably hopped from the original insertion site to the second intron and created this imprecise excision mutant.

**Immunohistochemistry and antibodies**

The following primary antibodies were used for immunohistochemistry at the indicated dilution: guinea pig anti-Avl (1:500; Yu Lab) (Zhang et al., 2014), rabbit anti-GM130 (1:200, Abcam ab52649), mouse anti-Ubiquitin (1:500; FK2, Enzo Life Sciences BML-PW0150-0100), mouse anti-Nrg (1:20, BP104, DSHB), rabbit anti-Lva antibody (1:1000, a gift from W. Sullivan) (Sisson et al., 2000), guinea pig anti-Arf1 (1:200, Yu Lab), mouse and guinea pig anti-Sec71 (1:200, Yu Lab), rabbit anti-GFP (1:1000, Invitrogen A11122), Cy3-, Cy5- or fluorescein isothiocyanate (FITC) conjugated secondary antibodies (Jackson Laboratories, Cat#: 111-165-003, 112-095-003, 111-175-003) were used at 1:400 dilution. For immunostaining, pupae or larvae were dissected in PBS and fixed with 4% formaldehyde for 20 min. Mounting was performed in VectaShield mounting medium, and the samples were directly visualized by confocal microscopy.

**Generation of Arf1, Sec71 and other transgenes**

The Arf1 and Sec71 full-length cDNA were PCR from EST LD24904 and LD29171 (DGRC) into Topo Entry and pDonor, respectively (Life Tech). The dominant-negative variant of Sec71 was generated by E677K site mutagenesis (Agilent Tech). The GDP-locked and GTP-locked constructs of Arf1 were generated by T31N and Q71L site mutagenesis, respectively. Sec71 RNAi-resistant construct against Sec71 RNAi #1 was generated by site mutagenesis using following two primers CGTCATTTCGAATGGGTTTAAGTTCAACGAGTCC and GGACTCGTTGAACTTAAACCATTCGAAATGACG.

The GATEWAY pTW vector containing the respective fragment of the cDNA were constructed by LR reaction (Life Tech) and several transgenic lines were established by the Bestgene Inc. An Arf1 RNAi-resistant cDNA was synthesized with a silent mutation on the
third base of each codon (First Base Tech), and was subjected to subcloning into pUAST and generation of transgenic lines. The Sec71 variants were generated by site mutagenesis (Agilent Tech) and subcloned into pTWF-attB and targeted to the attP2 site.

The cDNA fragment encoding the aa 1-100 portion of rat ManII protein were amplified by PCR, subcloned into Topo entry and pTWV to generate ManII-Venus transgene (Bestgene Inc).

**Arf1 and Sec71 antibody production**

The cDNA fragment corresponding to the aa 60-345 fragment of Sec71 was amplified by PCR and verified by DNA sequencing. The product was expressed using the MBP expression vector (pMAL, NEB) and the purified protein was used to immunize guinea pigs and mice to generate antibodies against Sec71. The Arf1 full-length cDNA was amplified by PCR and verified by DNA sequencing. The product was expressed using the GST expression vector (pGEX 4T-1, Pharmacia) and the purified protein was used to immunize guinea pigs to generate antibodies against Arf1. The specificity of these antibodies was verified in respective RNAi and/or mutant ddaC neurons.

**MARCM analysis of ddaC neurons**

We carried out MARCM clonal analysis, dendrite imaging, and branch quantification as previously described (Kirilly et al., 2009). Briefly, ddaC or da clones were picked up and imaged at the WP stage according to their location and the dendritic arbor morphology. The same da neurons were examined for dendrite pruning defects at 16 h APF.

**Co-Immunoprecipitation (Co-IP) and GST pull-down assay**

We carried out S2 cell culture and Western blotting as described below. Myc-Sec71, Flag-Arf1 WT, Flag-Arf1 T31N and Flag-Arf1 Q71L expression vectors were generated by Gateway cloning and were transfected into S2 cells using Effectene Transfection Reagent (Qiagen). Transfected S2 cells were homogenized with the lysis buffer (25 mM Tris pH8/27.5 mM
NaCl/20 mM KCl/25 mM sucrose/1 mM DTT/10% (v/v) glycerol/0.5% Nonidet P40) with protease inhibitors (Complete, Boehringer; PMSF 10 µg/ml, Sodium orthovanadate 10 µg/ml). The supernatants were used for immunoprecipitation with anti-Myc (1:2,000, ab32, Abcam) overnight at 4°C, followed by incubation with protein A/G beads (Pierce Chemical Co.) for 2 h. Protein A/G beads were washed four times with cold PBS. Bound proteins were separated by SDS-PAGE and analysed by Western blotting with anti-Myc, anti-Flag HRP-conjugated antibody.

Arf1WT, Arf1T31N and Arf1Q71L were inserted into (pGEX 4T-1, Pharmacia). The GST pull-down assays were carried out as previously described. Briefly, glutathione-sepharose 4B beads (GE Healthcare) bounded GST-Arf1 fusion protein were incubated for 3 h with Myc-Sec71 protein extracts derived from S2 cell cultures, respectively. Bound beads were washed one time with the lysis buffer, two times with PBS and subjected to immunoblotting. γ-GTP (G0635, Sigma-Aldrich, 0.2mM) was pre-incubated with GST-Arf1 at room temperature for 30 min and also added into the lysis buffer. The blot bands were quantified in ImageJ software.

** Trafficking Assay  

eL3 staged larvae were fed with RU486 for 12 h. For immunostaining, larvae were dissected in PBS and fixed with 4% formaldehyde for 20 min. The fillets were incubated with rat monoclonal anti-CD8α (1:100, CALTAG Laboratories) in PBS and washed for three times with PBS in the detergent-free condition, which was followed by Cy3- secondary antibody incubation and wished for three times in the detergent-free condition. The intensity of immunofluorescence was measured in the same confocal setting for both mutant and control.

**In vitro guanine nucleotide exchange assay**
We carried out the in vitro guanine nucleotide exchange assays as previously described (Mahajan et al., 2013). GST fusion proteins for the Sec7 domain of Sec71 (aa554-790), its dominant negative form (E-K) and Sec7 domain of hBIG1 were expressed using the GST expression vector pGEB. 6xHis-tagged Arf1WT and 6xHis-tagged Arl1WT were expressed via the vector pET37b. The 6xHis-Arf1 fusion proteins were subjected to Tobacco Etch Virus protease cleavage to remove the N-terminal 6xHis-tag followed by gel filtration. All fusion proteins were dialyzed against HK buffer (50 mM HEPES pH 7.5, 120 mM KCl). The exchange assays were conducted in a black 96-well plate (Greiner Bio-One). Each reaction had 150 µl HKM buffer (HK buffer supplemented with 1 mM MgCl2 and 1 mM DTT) containing 1 µM Mant-GMPPNP, 1 µM 6xHis-Arf1WT/6xHis-Arl1WT, and 1 µM EDTA and 0.7 mM testing GST proteins (GST, GST-Sec71, GST-Sec71E-K, or GST-Sec7-hBIG1). The fluorescence was monitored by Tecan Infinite M200 pro at 25 °C with the excitation at 360 nm and emission at 440 nm. The fluorescence data were collected every 30 or 50 seconds depending on the total number of samples per plate. The exchange kinetic traces with significant spikes were rejected. Single exponential curve fitting were analyzed in OriginPro 8.5 (Origin Lab).

Quantification of immunolabeling

We quantified the immunolabeling intensities of Nrg at 6 h APF, as described previously (Zhang et al., 2014). Basically, soma/dendrite/axon contours were outlined on the individual fluorescent channel according to the GFP channel in ImageJ software. After subtracting the background (Rolling Ball Radius=50) on the entire image of that channel, we measured the mean grey value in the marked areas in ddaC or ddaE on the same images and calculated their ratios. The ratios were normalized to corresponding average control values and subjected to one-way ANOVA and Bonferroni tests for comparison between different conditions (*p<0.05, **p<0.01, ***p<0.001, n.s., not significant). Graphs display the average
values of ddaC/ddaE ratios and the standard error of means (S.E.M). The number of samples (n) in each group is shown on the bars.

**Quantification of ddaC dendrites**

Live confocal images of ddaC neurons expressing UAS-mCD8-GFP driven by ppk-GAL4 were shown at WP and 16h APF. The average number of primary and secondary dendrites attached to soma was counted from wild type or mutant ddaC neurons. Primary dendrites are attached to the soma, while secondary dendrites are attached to the soma via their respective primary dendrites. The number of samples (n) in each group is shown on the bars. Statistical significance was determined using either two-tailed Student’s T test (two samples) or one-way ANOVA and Bonferroni test (multiple samples) (*p<0.05, **p<0.01, ***p<0.001, n.s., not significant). Error represent S.E.M. Dorsal is up in all images.

**Genotypes of fly strains**

**Figure 1:** (B) w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Control RNAi. (C) w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Arf1 RNAi #1. (D) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Arf1 RNAi #1 / UAS-Arf1 (RNAi Resistant). (E) w*; ppk-Gal4 / UAS-Arf1T31N; ppk-Gal4, UAS-mCD8GFP / +. (F, G) w*; UAS-Arf1T31N / GSG2295-Gal4, ppk-tdGFP. (H) w*; UAS-Arf1Q71L / GSG2295-Gal4, ppk-tdGFP. (I) w*; UAS-Arf1WT / GSG2295-Gal4, ppk-tdGFP.

**Figure 2:** (A-A”) w*; ppk-Gal4 / +; UAS-GalT-GFP / +. (B-B”) w*; ppk-Gal4 / ppk-Gal4; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. (C-C”) w*; ppk-Gal4 / +; UAS-Sec31-mCherry / +. (D) w*; GSG2295-Gal4 / UAS-Arf1T31N; UAS-GalT-GFP / +. (E) w*; UAS-Arf1T31N / GSG2295-Gal4, ppk-tdGFP. (F) w*; GSG2295-Gal4 / UAS-Arf1T31N, UAS-Sec31-mCherry / +. (G-G”) w*; ppk-Gal4, UAS-mCD8RFP, UAS-Dcr2 / +; UAS-ManII-Venus / UAS-Arf1 RNAi #1.

**Figure 3:** (A) w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Control RNAi. (B) w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Sec71 RNAi #1. (C) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Sec71 (RNAi-Resistant) / UAS-Sec71 RNAi #1. (D) w*; ppk-Gal4 / UAS-Sec71 RNAi #2; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2. (E, F) w*; UAS-Sec71DN / GSG2295-Gal4, ppk-tdGFP.
Figure 4: (G) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Mical<sup>Nter</sup> / UAS-Sec71 RNAi #1. (H) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Arf1<sup>WT</sup>; UAS-Sec71 RNAi #1 / +.

Figure 5: (A-A") w*; ppk-Gal4 / +; UAS-Arf1<sup>T31N</sup> / +. (B-B") w*; ppk-Gal4 / ppk-Gal4; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. (C-C") w*; ppk-Gal4 / ppk-Gal4; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. (D-D") w*; ppk-Gal4 / +; UAS-Sec31-mcherry / +. (E) w*; GSG2295-Gal4 / UAS-Sec71<sup>DN</sup>; UAS-GalT-GFP / +. (F) w*; UAS-Sec71<sup>DN</sup> / GSG2295-Gal4, ppk-tdGFP. (G) w*; GSG2295-Gal4 / UAS-Sec71<sup>DN</sup>; UAS-Sec31-mcherry / +. (H) w*; ppk-Gal4 / ppk-Gal4; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. (H') w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Sec71 RNAi #1. (H") w*; UAS-Sec71<sup>DN</sup> / GSG2295-Gal4, ppk-tdGFP. (I) w*; ppk-Gal4 / ppk-Gal4; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. (I') w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Arf1 RNAi #1. (I") w*; UAS-Arf1<sup>T31N</sup>/ GSG2295-Gal4, ppk-tdGFP.

Figure 6: (B) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2; UAS-Mical<sup>Nter</sup> / UAS-Sec71 RNAi #1. (C) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Sec71 (RNAi-Resistant) / UAS-Sec71 RNAi #1. (D) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Sec71<sup>ADC8</sup> / UAS-Sec71 RNAi #1. (E) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Sec71<sup>HDS2-4</sup> / UAS-Sec71 RNAi #1. (F) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Sec71<sup>HDS3-4</sup> / UAS-Sec71 RNAi #1.

Figure 7: (A) w*; ppk-Gal4 / ppk-Gal4; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. (B) w*; ppk-Gal4, UAS-mCD8GFP / +; ppk-Gal4, UAS-mCD8GFP / +. (C) w*; UAS-Arf1<sup>T31N</sup> / GSG2295-Gal4, ppk-tdGFP. (D) w*; UAS-Sec71<sup>DN</sup> / GSG2295-Gal4, ppk-tdGFP. (F) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Control RNAi / UAS-Arf1 RNAi #1. (G) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-nrg RNAi #1; UAS-Arf1 RNAi #1 / +. (H) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-nrg RNAi #2; UAS-Arf1 RNAi #1 / +. (J) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Control RNAi / UAS-Sec71 RNAi #1. (K) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-nrg RNAi #1; UAS-Sec71 RNAi #1 / +. (L) w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-nrg RNAi #2; UAS-Sec71 RNAi #1 / +.

Figure S1: (A) Control: w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Control RNAi. Arf1 RNAi #2: w*; ppk-Gal4, UAS-mCD8GFP / UAS-Arf1 RNAi #2; UAS-Dcr2 / +. Arf1<sup>WT</sup>: w*; ppk-Gal4 / UAS-Arf1<sup>WT</sup>; ppk-Gal4, UAS-mCD8GFP / +. (B) Wild type: w*; Gal4<sup>1892</sup>; UAS-mCD8GFP, SOP-flp / +; FRT82B / FRT82B, tubP-Gal80. Arf1<sup>T31N</sup>: w*; Gal4<sup>1892</sup>; UAS-mCD8GFP, SOP-flp / UAS-Arf1<sup>WT</sup>; FRT82B / FRT82B, tubP-Gal80. Wild type: w*; GSG2295-Gal4 / +; UAS-mCD8GFP / UAS-Mical<sup>Nter</sup>. Arf1<sup>T31N</sup>: w*; GSG2295-Gal4 / UAS-Arf1<sup>T31N</sup>; UAS-mCD8GFP / +. Arf1 RNAi #1: w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Arf1 RNAi #1. (C) Wild type: w*; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. Arf6<sup>DN</sup>: w*; ppk-Gal4 / UAS-Arf6<sup>DN</sup>; ppk-Gal4, UAS-mCD8GFP / +. Arf1<sup>Δ</sup>: w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp #42/+; Arf1<sup>Δ</sup>, FRT2A; tubP-Gal80,FRT2A. (D) Control RNAi: w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Control RNAi.
Arf6 RNAi #1: w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Arf6 RNAi #1. Sec71 RNAi #1: w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Sec71 RNAi #1.

**Figure S2:** (A) **Wild type:** w*; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP; UAS-Dcr2 / UAS-Dcr2. **Arf6 RNAi #1:** w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Arf6 RNAi #1. **Arf6 RNAi #2:** w*; ppk-Gal4, UAS-mCD8GFP / UAS-Arf6 RNAi #2; UAS-Dcr2 / +. (B) **Wild type:** w*; ppk-Gal4 / ppk-Gal4; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. (C) w*; ppk-Gal4 / UAS-Rab4-mRFP. (D) w*; ppk-Gal4 / UAS-GFP-Rab5. (E) w*; ppk-Gal4 / UAS-mito-HA-GFP. (F) **Wild type:** w*; ppk-Gal4, UAS-mCD8RFP, UAS-Dcr2 / +; UAS-GalT-GFP / +. **Arf6 RNAi:** ppk-Gal4, UAS-mCD8RFP, UAS-Dcr2 / +; UAS-GalT-GFP / UAS-Arf6 RNAi #1. **Sec71 RNAi:** ppk-Gal4, UAS-mCD8RFP, UAS-Dcr2 / +; UAS-GalT-GFP / UAS-Sec71 RNAi #1. (G) w*; ppk-Gal4, UAS-mCD8RFP, UAS-Dcr2 / +; UAS-GalT-GFP / +. (H) w*; ppk-Gal4, UAS-mCD8RFP, UAS-Dcr2 / +; UAS-GalT-GFP / UAS-Arf6 RNAi #1.

**Figure S3:** (B) **Sec71**\textsuperscript{Ex11}: w*; Sec71\textsuperscript{Ex11}, FRT40A / tubP-Gal80, FRT40A; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +. **Sec71**\textsuperscript{Ex11} **Rescue:** elav-Gal4, UAS-mCD8GFP, hs-FLP, w* / Gal4\textsuperscript{5-40}, UAS-Venus:pm, SOP-flp #42; Sec71\textsuperscript{Ex11}, FRT40A / tubP-Gal80, FRT40A; UAS-Sec71 FL / +. (C) **Wild type:** w*; Gal4\textsuperscript{109(2)80}, UAS-mCD8GFP, SOP-flp / +; FRT82B / FRT82B, tubP-Gal80. **Sec71**\textsuperscript{DN}: w*; Gal4\textsuperscript{109(2)80}, UAS-mCD8GFP, SOP-flp / UAS-Sec71\textsuperscript{DN}; FRT82B / FRT82B, tubP-Gal80. (D) **Wild type:** w*; GSG2295-Gal4 / +; UAS-mCD8GFP / UAS-Mical\textsuperscript{N-Ter}. **Sec71**\textsuperscript{DN}: w*; GSG2295-Gal4 / UAS-Sec71\textsuperscript{DN}; UAS-mCD8GFP / +. **loner**\textsuperscript{T1032}: elav-Gal4, UAS-mCD8GFP, hs-FLP, w* / Gal4\textsuperscript{5-40}, UAS-Venus:pm, SOP-flp #42; FRT82B, loner\textsuperscript{T1032} / FRT82B, tubP-Gal80. **Step**\textsuperscript{DN}: w*; ppk-Gal4 / +; ppk-Gal4, UAS-mCD8GFP / UAS-step\textsuperscript{DN}. (E) **Control RNAi:** w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Control RNAi. **Sec71 RNAi #1:** w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Sec71 RNAi #1.

**Figure S4:** (C) **Sec71 RNAi #1+UAS control:** w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Mical\textsuperscript{N-Ter} / UAS-Sec71 RNAi #1. **Sec71 RNAi #1 + Arf6 WT:** w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Arf6 WT; UAS-Sec71 RNAi #1 / +.

**Figure S5:** (A) **Wild type:** w*; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP; UAS-Dcr2 / UAS-Dcr2. **Sec71 RNAi #1:** w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-Sec71 RNAi #1. **Sec71**\textsuperscript{Ex11}: w*; Sec71\textsuperscript{Ex11}, FRT40A / tubP-Gal80, FRT40A; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +. (B) **Wild type:** w*; ppk-Gal4 / UAS-Sec15-GFP. (C) **Wild type:** w*; ppk-Gal4, UAS-Dcr2 / UAS-Sec15-GFP. **Arf6 RNAi:** w*; ppk-Gal4, UAS-Dcr2 / UAS-Sec15-GFP; UAS-Arf6 RNAi #1 / +. **Sec71 RNAi:** w*; ppk-Gal4 / UAS-Sec71 FL / +; UAS-Sec71 RNAi #1 / +. (D) **Wild type:** w*; GSG2295-Gal4 / +; UAS-mCD8GFP / UAS-Mical\textsuperscript{N-Ter}. **Arf6**\textsuperscript{T31N}: w*; GSG2295-Gal4 / UAS-Arf6\textsuperscript{T31N}; UAS-mCD8GFP / +. **Sec71**\textsuperscript{DN}: w*; GSG2295-Gal4 / UAS-Sec71\textsuperscript{DN}; UAS-mCD8GFP / +.

**Figure S6:** (B) **Sec71**\textsuperscript{FL}: w*; ppk-Gal4 / +; UAS-GalT-GFP / UAS-Sec71 (RNAi-Resistant). **Sec71**\textsuperscript{4DCB}: w*; ppk-Gal4 / +; UAS-GalT-GFP / UAS-Sec71\textsuperscript{4DCB}. **Sec71**\textsuperscript{4HUS}: w*; ppk-Gal4 /

Figure S7: (A) Wild type: w*; GSG2295-Gal4 / +; UAS-GFP-2×FYVE / UAS-MicalN-Ter. Arf1T31N: w*; GSG2295-Gal4 / UAS-Arf1T31N; UAS-GFP-2×FYVE / +. Sec71DN: w*; GSG2295-Gal4 / UAS-Sec71DN; UAS-GFP-2×FYVE / +. (B-C) Rab5DN: w*; UAS-Rab5DN / GSG2295-Gal4; ppk-tdGFP / UAS-MicalN-Ter. Arf1T31N: w*; UAS-Arf1T31N / GSG2295-Gal4, UAS-Rab5DN; ppk-tdGFP / +. Sec71DN: w*; UAS-Sec71DN / GSG2295-Gal4; ppk-tdGFP / UAS-MicalN-Ter. Rab5DN; Sec71DN: w*; UAS-Sec71DN / GSG2295-Gal4, UAS-Rab5DN; ppk-tdGFP / +.

Supplemental References