Supplemental Figures

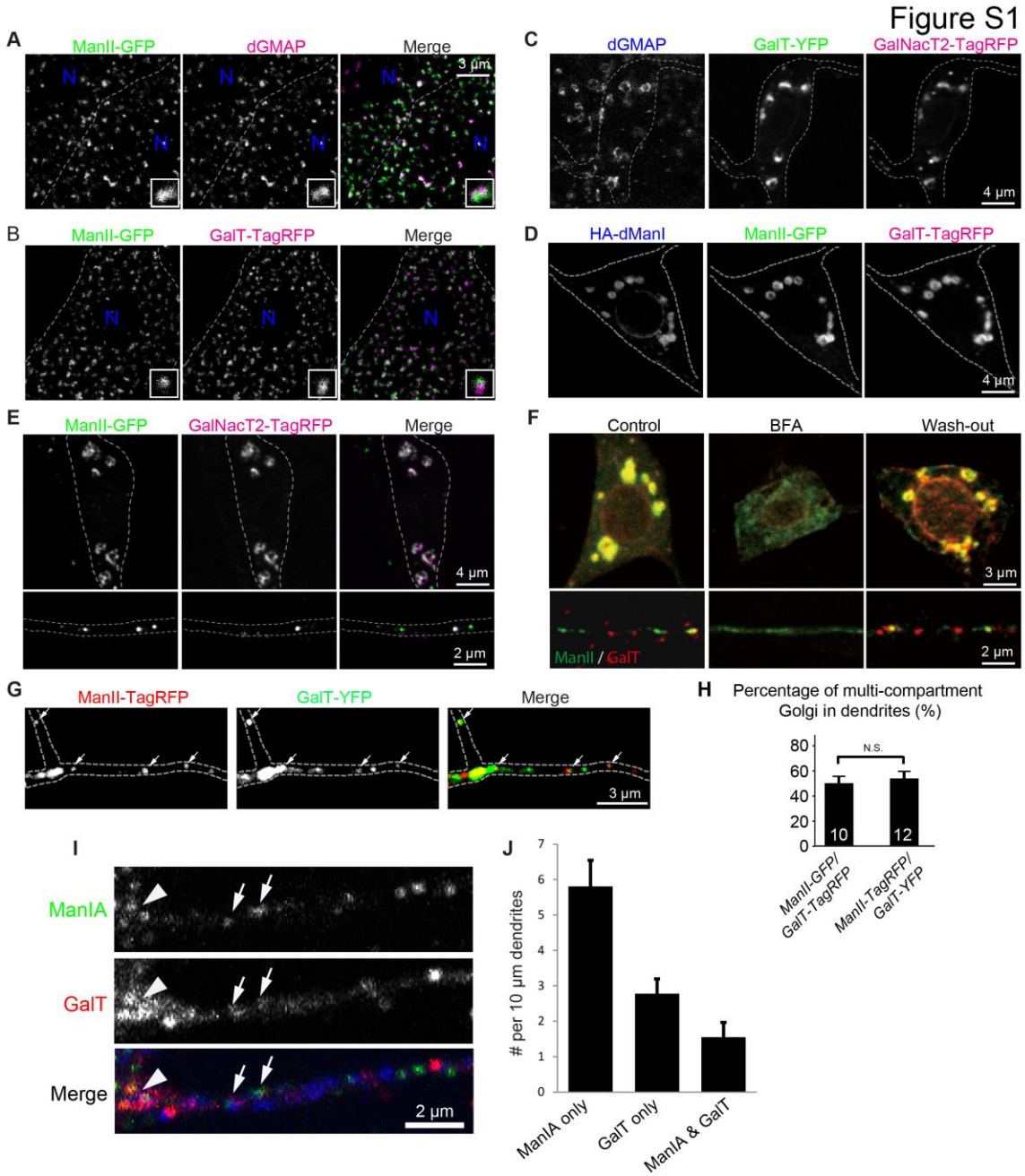

Figure S1. Golgi compartments in *Drosophila* larval epithelial cells, *Drosophila* da neurons, and cultured cortical neurons from mouse embryos. (Related to Figure 1)

(A and B) Golgi mini-stacks in larval epithelial cells. (A) The Golgi were labeled by the transgenic marker ManII-GFP *(medial)* and immunostained for endogenous dGMAP *(cis)*. (B) The Golgi were labeled by ManII-GFP (*medial*) and GalT-TagRFP (*trans*). The distinct compartments are juxtaposed to each other in the Golgi mini-stacks, which is visible in the single mini-stacks shown in the insets.

(C and D) Somal Golgi in da neurons. (C) The localization of the *cis* Golgi marker dGMAP, *trans* Golgi markers GalT-YFP and GalNacT2-TagRFP in the soma of class III da neurons. (D) The localization of the *medial* Golgi markers HA-ManI and ManII-GFP and the *trans* Golgi marker GalT-TagRFP in the soma of class III da neurons.

(E) Golgi compartments are disconnected in dendritic shafts. Whereas the *medial* Golgi marker ManII-GFP (green) and the *trans* Golgi marker GalNacT2-TagRFP (magenta) colocalize in the soma (top) but are often disconnected in dendritic shafts (bottom).

(F) BFA disperses the Golgi labeled by ManII-GFP and GalT-TagRFP transgenic markers in both soma (top) and dendrites (bottom). Both somal Golgi and dendritic Golgi outposts re-appear after 30 min of washout.

(G-H) Switching the fluorescent protein tags of the Golgi markers does not change the localizations of the markers. (G) ManII was tagged with TagRFP-T and GalT with YFP. Arrows points to multi-compartment Golgi outposts in dendrites. (H) Quantification of percentage of multi-compartment outposts in dendritic shafts between ManII-TagRFP/GalT-YFP and ManII-GFP/GalT-TagRFP pairs.

(I) Both single and multi-compartment Golgi exist in the dendrites of mouse cortical neurons in culture. Cultured neurons were stained with antibodies against endogenous Golgi proteins: anti-ManIA for *medial* Golgi (green) and anti-GalT for *trans* Golgi (red). An anti-MAP2 antibody (blue) was used to identify dendrites.

(J) Quantitation of single and multi-compartment of dendritic Golgi outposts in mouse cultured cortical neurons.

Figure S2

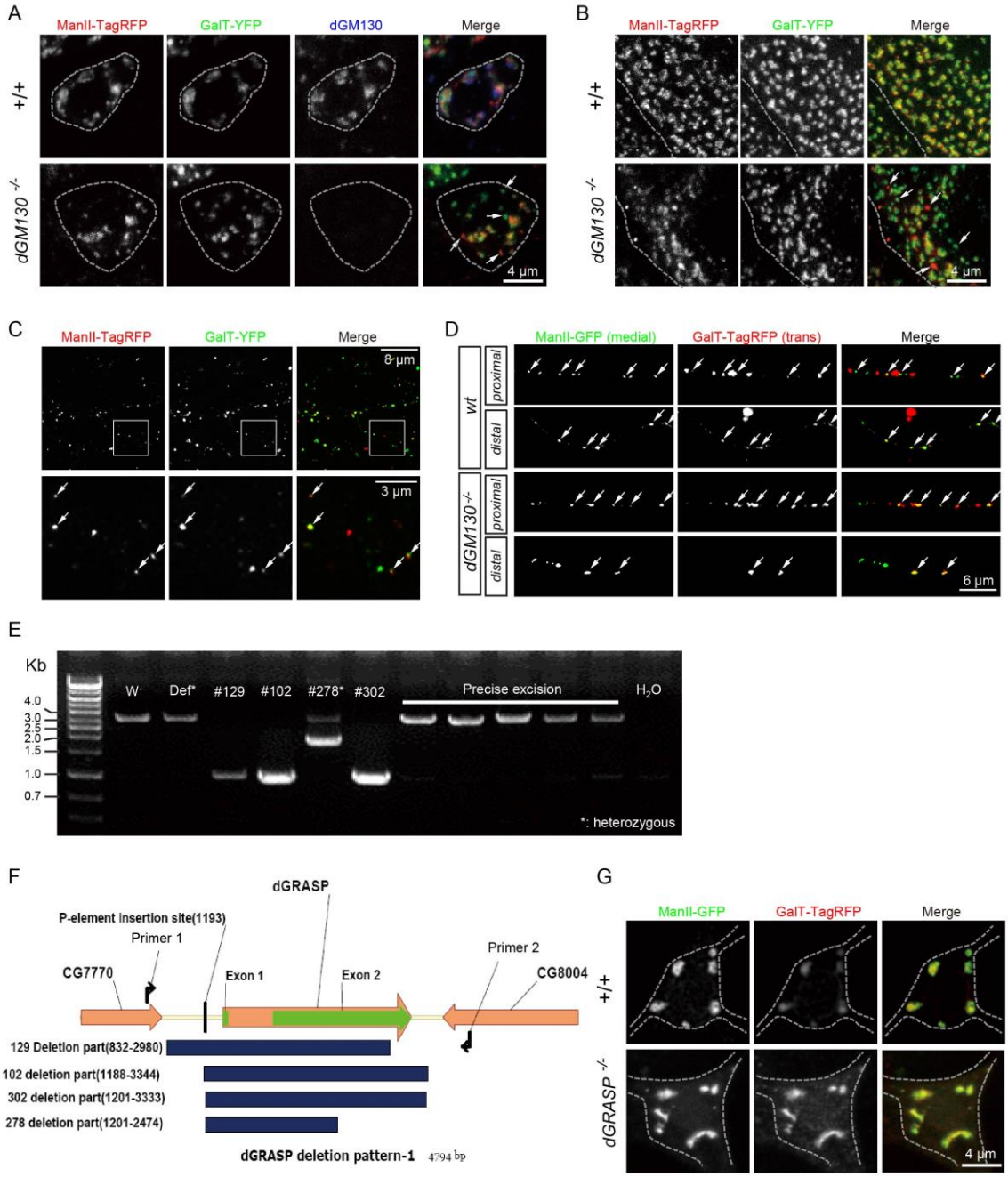
A
ManII-TagRFP
GalT-YFP
dGM130
Merge
+/+
dGM130 -/-
4 μm
B
ManII-TagRFP
GalT-YFP
Merge
+/+
dGM130 -/-
4 μm
C
ManII-TagRFP
GalT-YFP
Merge
8 μm
3 μm
D
ManII-GFP (medial)
GalT-TagRFP (trans)
Merge
wt
proximal
distal
dGM130 -/-
proximal
distal
6 μm
E
Kb
4.0
3.0
2.5
2.0
1.5
1.0
0.7
W-
Def*
#129
#102
#278*
#302
Precise excision
H2O
*: heterozygous
F
dGRASP
P-element insertion site(1193)
Primer 1
Primer 2
CG7770
Exon 1
Exon 2
CG8004
129 Deletion part(832-2980)
102 deletion part(1188-3344)
302 deletion part(1201-3333)
278 deletion part(1201-2474)
dGRASP deletion pattern-1 4794 bp
G
ManII-GFP
GalT-TagRFP
Merge
+/+
dGRASP -/-
4 μm

Figure S2. The roles of dGM130 and dGRASP in organizing Golgi architecture.

(Related to Figure 2)

(A and B) dGM130 is required for connecting the *cis*, *medial*, and *trans* compartments in the lobula plate tangential cells (A) and epithelial cells (B). Loss of *dGM130* function led to a separation of Golgi compartments. Arrows indicate single Golgi compartments in *dGM130* mutant cells.

(C) Single and multi-compartment Golgi outposts are present in the dendrites of lobula plate tangential cells in adult brains. Lower panels show the magnified images of the boxed region in the upper panels. Arrows point to multi-compartment outposts.

(D) Loss of *dGM130* decreased the percentage of multi-compartment Golgi in the distal, but not, proximal dendrites. Arrows point to multi-compartment outposts.

(E-G) *dGRASP* mutant da neurons do not show significant change in Golgi compartmental organization. (E) The P-element excision sites in the mutant lines were identified by DNA sequencing of PCR-amplified genomic DNA. (F) A schematic showing the deleted regions in the imprecise excision lines based on results from sequencing of the genomic DNA. In the $dGRASP^{129}$ line, the promoter and coding region except for the last 34 amino acids were deleted. In the $dGRASP^{102}$ and $dGRASP^{302}$ lines, the entire coding region was deleted. (G) The compartmental organization of somal Golgi in $dGRASP^{302}$ is similar to that in wild-type neurons. As we discussed in the main text, this is possibly due to maternal contribution of wild-type dGRASP.

Figure S3

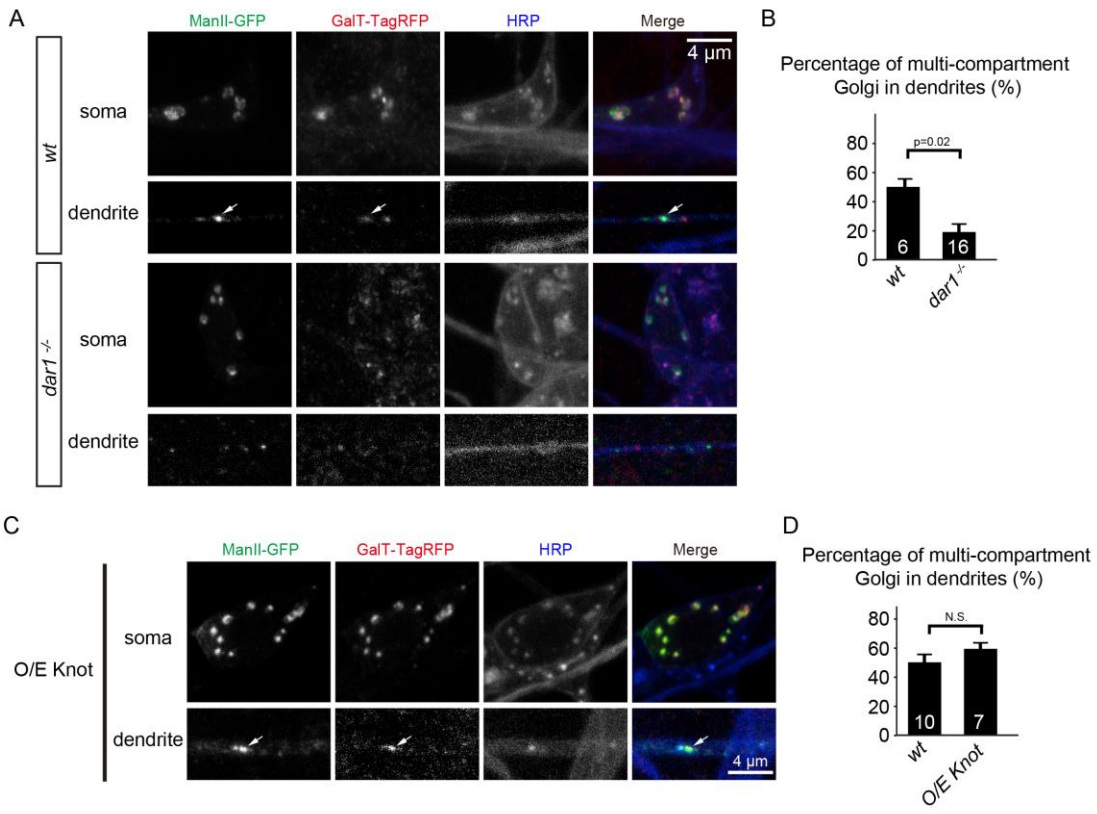

Figure S3. **Compartmental organization of somal and dendritic Golgi in mutant neurons with decreased or increased dendritic branching.** (Related to Figure 3)

(A) *dar1* null mutant class III da neurons, which dramatically reduces dendritic branching, displayed a significantly reduced percentage of multi-compartment of Golgi outposts in dendrites, although somal Golgi appeared to be normal. MARCM analysis was used. Arrows point to multi-compartment outposts.

(B) Quantification of percentage of multi-compartment Golgi outposts in the dendrites of wild type and *dar1* mutant neurons.

(C) Overexpressing Knot in class III da neurons, which increases dendritic branching, does not change the compartmental organization of somal and dendritic Golgi.

(D) Quantification of percentage of multi-compartment Golgi outposts in the dendrites of wild type and Knot-overexpressing neurons.

Figure S4

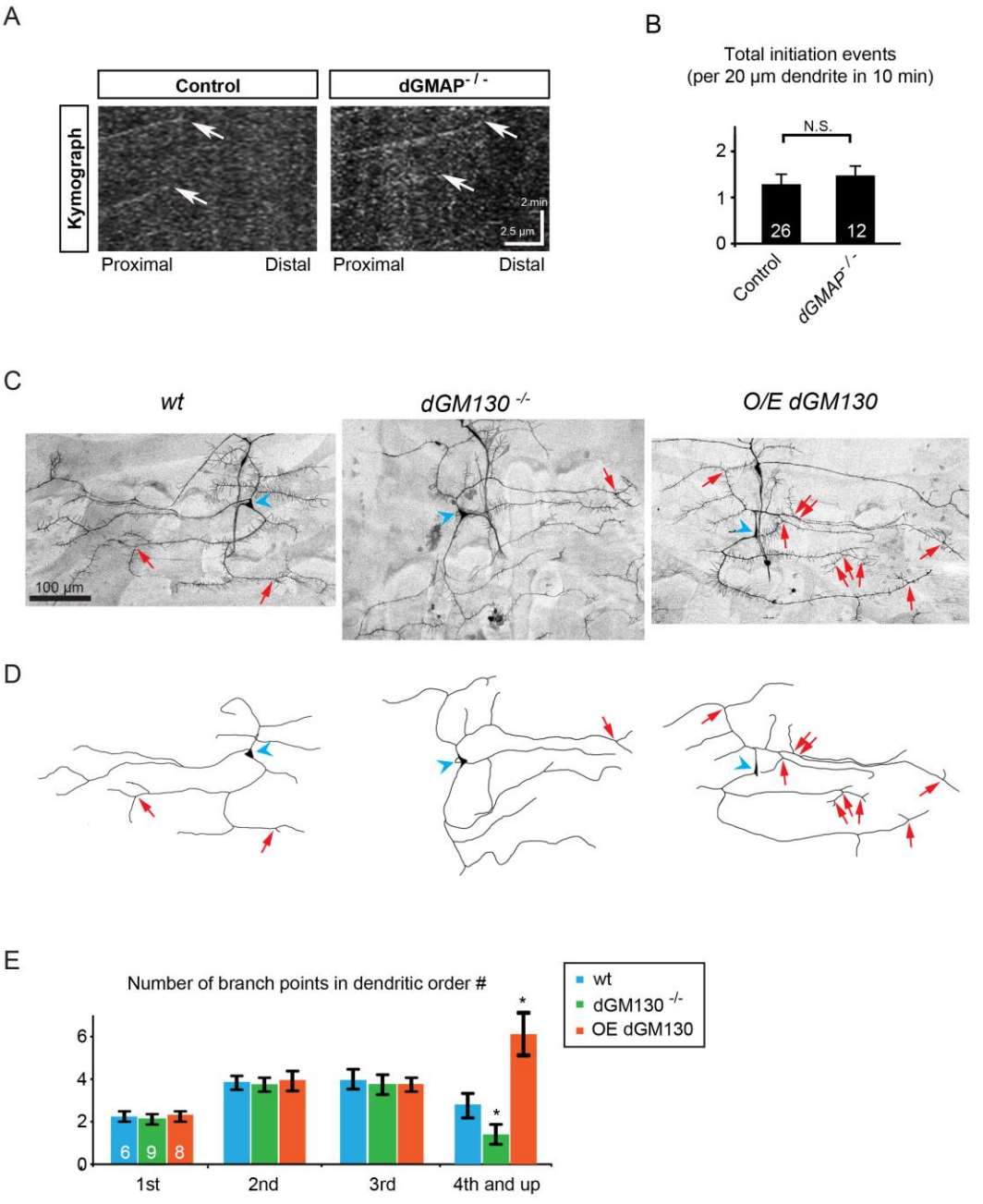

Figure S4. dGM130 regulates dendritic branching. (Related to Figure 4)

(A-B) Loss of *dGMAP* does not change the number of microtubule initiation sites in dendrites. (A) Kymograph. (B) Quantification of total initiation events of EB1 comets between control and *dGMAP* null mutant.

(C) The morphologies of the class III da neuron ddaAs that are wild-type (wt), *dGM130* mutant (*dGM130*$^{-/-}$), and dGM130-overexpression (O/E dGM130). Cyan arrowheads point to the soma and red arrows point to the branch points that are higher than the 4th order.

(D) Tracings of the microtubule-based dendritic branches. The numerous F-actin-based "dendritic spikes" of class III da neurons were not included in the tracings.

(E) Quantification of the number of branch points in different orders. The F-actin-based dendritic spikes were excluded from the quantification.

Supplemental Experimental Procedures

Imprecise excision to generate *dGM130* and *dGRASP* mutants

Imprecise excision of P{RS3}GM130$^{CB-6408-3}$ was performed to generate the *dGM130* null mutants. The initial screen for deletion mutants was based on loss of the *white* transgene. A secondary screen on the candidate lines was performed by using PCR. The exact region deleted in each of the mutants was identified by DNA sequencing. Although null alleles of *dGM130* survived to adulthood and were fertile, 18.9% homozygous mutants died at the pupal stage (n=479), compared to 0% in wild-type (n = 836). These results suggest that *dGM130* mutant flies are not completely normal.

The *dGRASP* null mutants were generated similarly by imprecise excision of p{EPgy2}[EY00794].

Mouse neuronal cultures and immunostaining

Cortical neurons were prepared from mouse embryos (embryonic day 17), and plated at 30,000 cells per well on poly-D-lysine coated coverslips placed in the wells of 12-well plates. At 5 days *in vitro* (D.I.V.), the neurons were fixed in 100% cold methanol (-20 °C) at 4 °C for 10 min, followed by permeablization and washing in wash buffer (1X PBS containing 0.1% Triton X-100). The neurons were then blocked in wash buffer containing 5% bovine serum albumin, and incubated with the goat anti-β-1,4-GalT1 (1:100; Santa Cruz Biotechnology, Dallas, TX), rabbit anti-α-1,2-mannosidase IA (1:100; Abcam, Cambridge, MA), and chicken anti-MAP2 (1:200; Aves Labs, Inc., Tigard, OR). The goat anti- β-1,4-GalT1 antibody was pre-absorbed with poly-D-lysine-coated coverslips as this antibody tended to give a background staining on these coverslips. The

incubations with primary and secondary antibodies were done at 4 °C overnight in the blocking buffer.

Image processing and quantification

Image processing of somal Golgi and dendritic Golgi outposts was performed with ImageJ. To remove the background, the intensity threshold was set as the mean intensity of background plus three times the standard deviation of background. Threshold levels were determined independently for each data set.

Statistical analysis

Two-tailed Student's t-test was used throughout the manuscript. P-values less than 0.05 were considered statistically significant. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Data are presented as mean ± s.e.m.

Brefeldin A (BFA)-treatment of *Drosophila* da neurons in situ

Third instar larvae were dissected in sylgard-coated dishes and treated with insect saline containing either 10 μg/ml BFA (Cell Signaling Technology, MA) dissolved in DMSO or the same volume of DMSO only as negative control. The BFA-treatment lasted for 30 minutes. In the wash-out experiments, the larval samples were treated with 10 μg/ml BFA for 30 minutes, washed 3 times with insect saline, and then incubated in insect saline for 30 minutes. After the treatments, the larval samples were fixed and immunostained as described above.