SUPPLEMENTARY INFORMATION

**Supplementary Figure 1.** Pulse labeling of dextran in RGC axons. A. Axons were pulse-labeled with dextran for 2 min without chase or with 15 min chase. Without chasing, the dex+ vesicles predominantly localized to the growth cone (arrowhead, insets), while a 15-minute chase resulted in a shift of the localization of some dex+ vesicles into the shaft (arrows, insets). The insets show 1.6X magnification of the images. Scale bar: 5 μm. B, C. By 2-minute pulse labeling, high concentration of Shh was found to significantly increase the percentage of dex+ axons as well as the number of dex+ vesicles/axon. * p< 0.05 compared with vehicle control, Student’s t-test. Data are represented as mean ± SEM. Numbers in parentheses indicate the total number of axons scored.

**Supplementary Figure 2.** The dex+ vesicles do not appear to associate closely with the Shh or Smo proteins. RGC axons were treated with either vehicle control or high Shh for 5 minutes with FITC-dextran. The samples were then fixed and stained by antibodies specific to Shh (A) or Smo proteins (B). Scale bar: 5 μm.

**Supplementary Figure 3.** Control- or high Shh-treated RGC axons were stained with phalloidin to visualize F-actin. Note that F-actin is rapidly reorganized by the treatment of high Shh, with increased association to the reverse shadowcast vesicles (arrows) away from filopodia.
Supplementary Figure 4. A. RGC axons were pre-treated for 5 minutes with jasplakinolide (jasp). Note that the growth cone failed to collapse indicated by remaining filopodia (arrows) despite retrograde movement of the axon core domains. Scale bar = 10 µm. B, C. Time-laspe microscopy was carried out to analyze growth cone collapse and axon retraction in the presence of high Shh or high Shh with dynasore (B), or in the presence of calyculin A (C). Note that calyculin A induced rapid growth cone collapse and axon retraction.

Supplementary Figure 5. FM1-43 can be used to label macropinosomes. RGC axons were incubated with FITC-dextran and FM1-43 for 5 minutes, washed and live imaging was performed. Note that FM1-43 labeled all the large dex+ vesicles (arrows), and some large dex- vesicles (arrowhead). At the level of magnification and set exposure, small vesicles were hardly visible.

Supplementary Figure 6. Rab34 did not label newly formed dex+ vesicles. RGC axons were treated with either vehicle or high Shh with dextran for 5 min, fixed, and stained by an anti-Rab34 antibody. Rab34 staining did not associate with the dex+ vesicles in both the vehicle- and high Shh-treated samples (arrows). Scale bars: 5 µm.

Supplementary video 1. Time-lapse movie of axons treated with a high concentration of Shh for 15 minutes.
Supplementary video 2. Time-lapse movie of axons pre-treated with dynasore for 3 min and then a high concentration of Shh was added for 15 minutes.