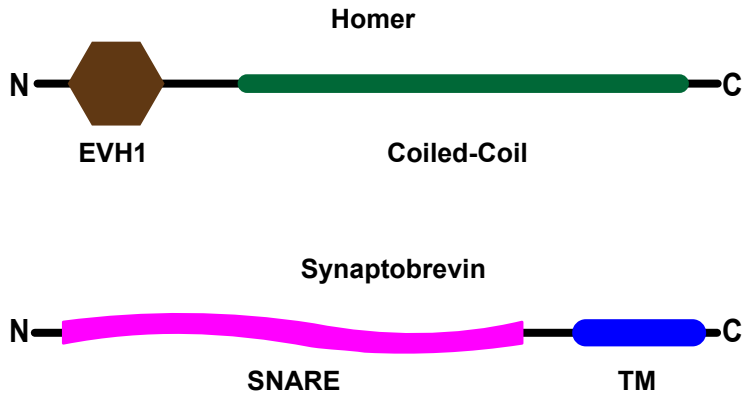
Figure S1

A



B

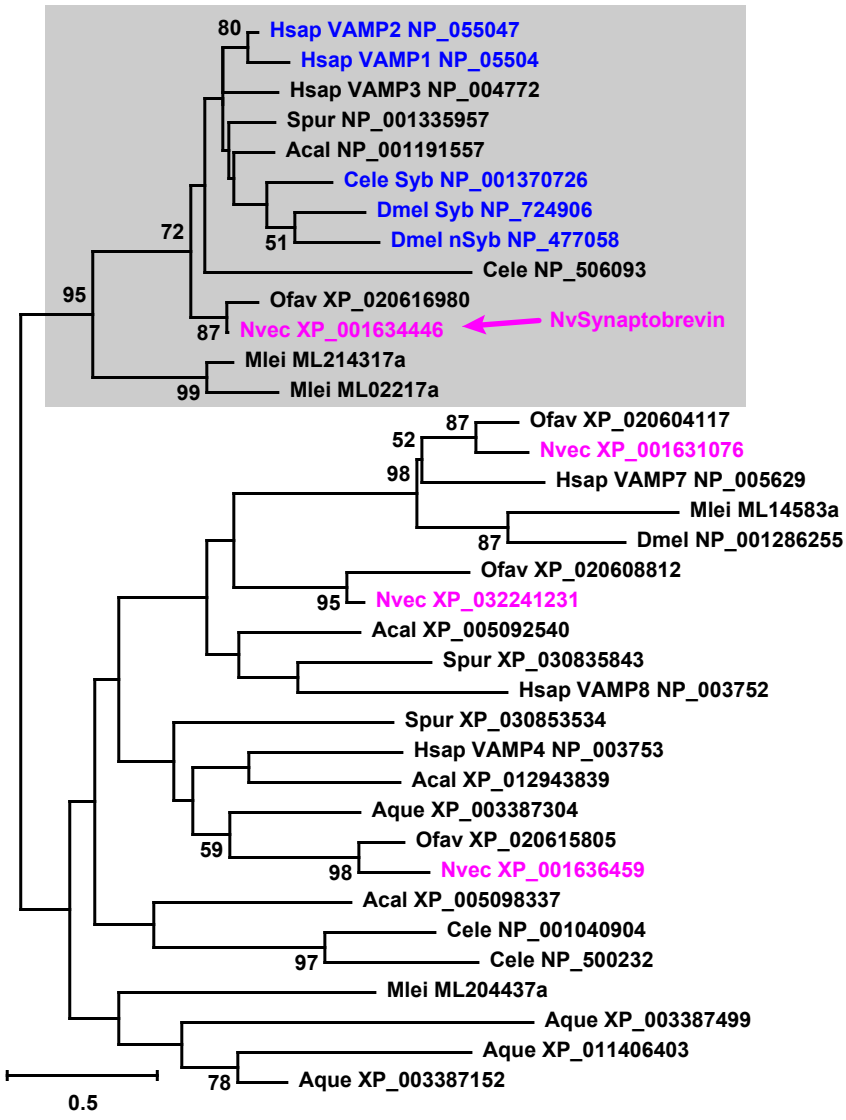


Figure S1. Domain structure and conservation of synaptic markers in *Nematostella*. (A) Domain structures are shown for Homer proteins (top) and V-SNAREs (Bottom). Homer proteins contain an N-terminal EVH1 domain for binding target proteins and a C-terminal Coiled-coil domain that mediates tetramerization. V-SNAREs, the vesicular component of the SNARE complex that is required for exocytosis, have an N-terminal SNARE domain followed by a C-terminal transmembrane domain to anchor them in the vesicle. V-SNAREs do not share sufficient homology with other members of the SNARE complex for detection in BLAST searches. (B) Maximum Likelihood phylogeny of metazoan V-SNARE proteins based on the conserved protein core extending from the N-terminus of the SNARE domain to the C-terminus of the transmembrane domain (85 amino acids). The phylogeny was inferred in MEGA X (Kumar et al., 2018) using the Le Gascuel substitution model (Le and Gascuel, 2008), and the tree with the highest log likelihood (-4289.26) is displayed. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories, parameter = 1.3175). Numbers at nodes indicate percent support in 500 Bootstrap replications; only nodes with > 50% support are labeled. The highly supported Synaptobrevin clade is outlined with a gray box and NvSynaptobrevin is marked with an arrow. Branch lengths reflect substitutions/site and a scale bar is given. Branch labels give a species prefix, protein name if available, and Genbank accession number; *Nematostella* V-SNARES are highlighted with magenta. Species prefixes are as follows: Acal, *Aplysia californica* (lophotrochozoan); Aque, *Amphimedon queenslandica* (sponge); Cele, *Caenorhabditis elegans* (protosome); Dmel, *Drosophila melanogaster* (protostome); Hsap, *Homo sapiens* (deuterostome); Mlei, *Mnemiopsis leidyi* (ctenophore); Nvec, *Nematostella vectensis* (cnidarian); Ofav, *Orbicella faveolata* (cnidarian); and Spur, *Stronglyocentrototus purpuratus* (deuterostome).

Proteins for most species were collected from REFseq (Pruitt et al., 2012) using BLASTP (Altschul et al., 1997) searches with human VAMP1, VAMP4 and VAMP7 as queries. *Mnemiopsis leidyi* was the lone exception where the Mnemiopsis genome portal (Ryan et al., 2013) was used for BLAST searches of predicted proteins. Note we also searched the *Nematostella* genome draft but found no additional V-SNARE sequences. In all cases, default BLAST search parameters were sufficient to comprehensively identify V-SNARE sequences. Sequences were aligned using the MUSCLE algorithm (Edgar, 2004) as implemented in MEGA X (Kumar et al., 2018). File S2 contains the alignment used for phylogenetic analysis in FASTA format.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389-402.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792-7.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol* **35**, 1547-1549.

Le, S. Q. and Gascuel, O. (2008). An improved general amino acid replacement matrix. *Mol Biol Evol* **25**, 1307-20.

Pruitt, K. D., Tatusova, T., Brown, G. R. and Maglott, D. R. (2012). NCBI Reference Sequences (RefSeq): current status, new features and genome annotation policy. *Nucleic Acids Res* **40**, D130-5.

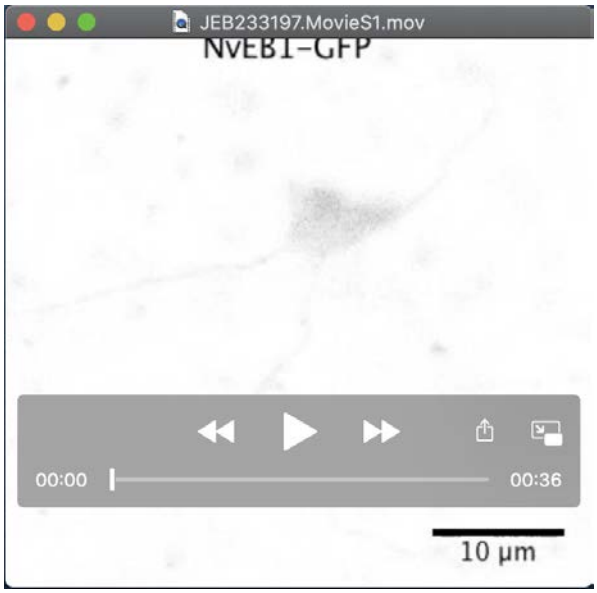
Ryan, J. F., Pang, K., Schnitzler, C. E., Nguyen, A. D., Moreland, R. T., Simmons, D. K., Koch, B. J., Francis, W. R., Havlak, P., Program, N. C. S. et al. (2013). The genome of the ctenophore Mnemiopsis leidyi and its implications for cell type evolution. *Science* **342**, 1242592.

Dataset 1. Sequences of transgenic marker plasmids used in this study.

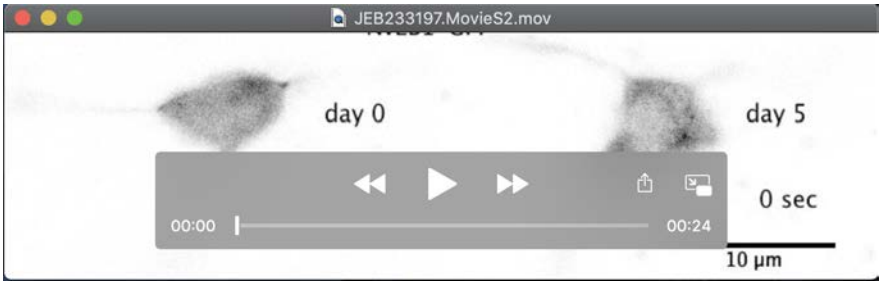
Click here to Download Dataset 1

Dataset 2. V-SNARE alignment used for the phylogeny in Figure S2.

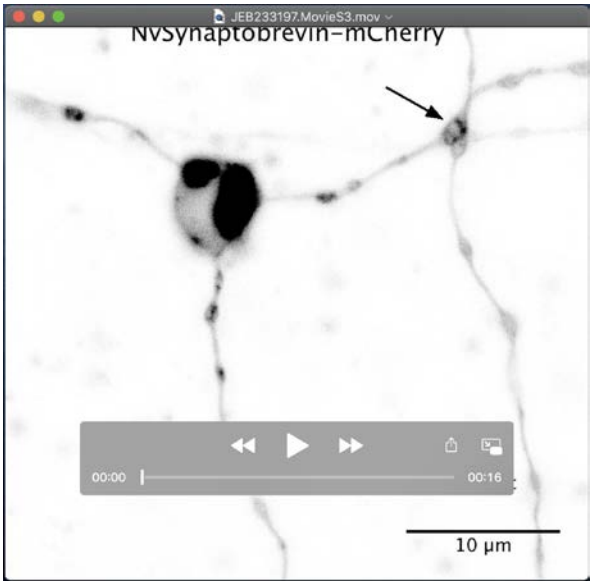
Click here to Download Dataset 2



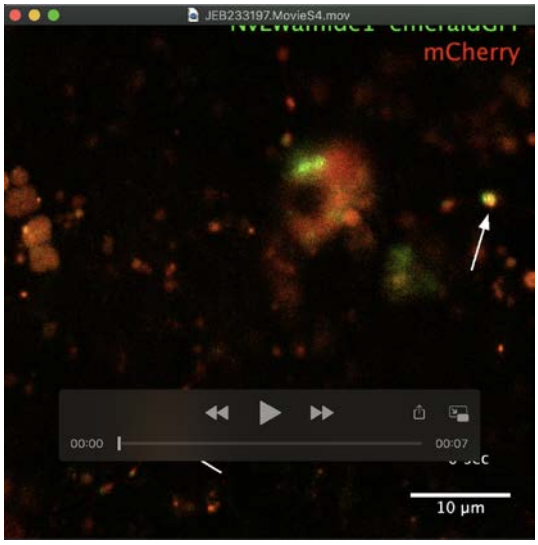
Movie 1. LWamide1+ tripolar ganglion neuron expressing NvEB1-GFP. In all three neurites the majority of NvEB1-GFP comets can be seen moving away from the cell body, indicating a plus-end out microtubule orientation.



Movie 2. Microtubule organizing center in LWamide1+ tripolar ganglion neuron expressing NvEB1-GFP. Comets can be seen radiating from a single point in the cell body at day 0. This MTOC is still present at day 5.



Movie 3. LWamide1+ tripolar ganglion neuron expressing NvSynaptobrevin-mCherry. The black arrow points to the site of two neurites crossing and NvSynaptobrevin-mCherry can be seen localized to the varicosities where they cross. Vesicles can be seen moving at these varicosities.



Movie 4. LWamide1+ tripolar ganglion neuron expressing LWamide1-Emerald and cytoplasmic mCherry. LWamide1-Emerald can be seen localizing to varicosities along the neurites as indicated by the white arrows. Vesicles can be seen moving at these varicosities.