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Supplementary Information for

Cell-type-specific, multi-color labeling of endogenous proteins with split fluorescent protein tags in *Drosophila*

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This PDF file includes:

Other supplementary materials for this manuscript include the following:

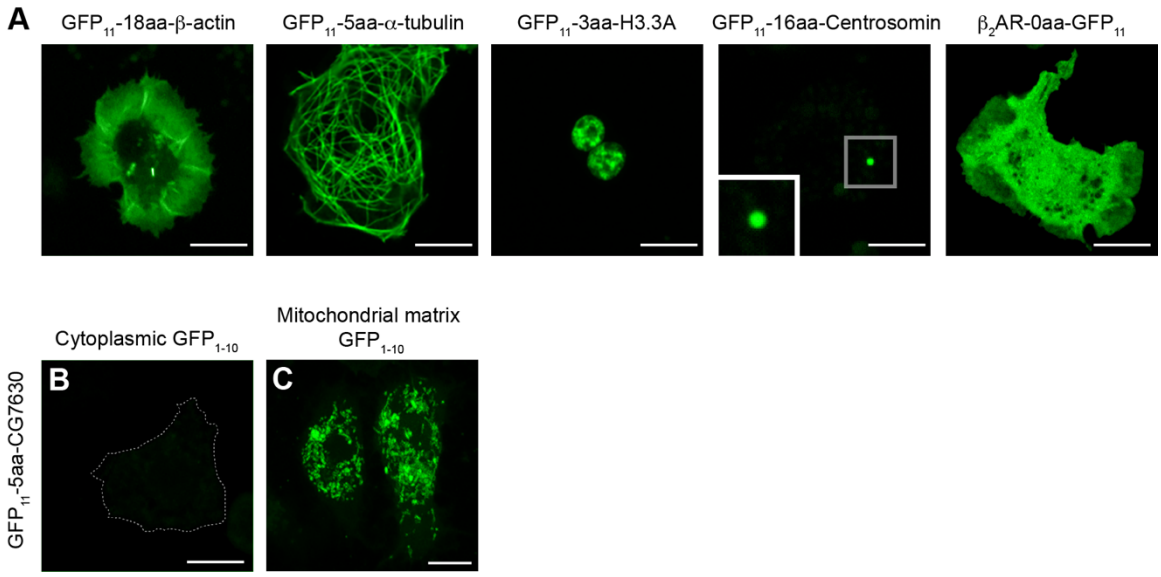

Fig. S1 | Labeling of cellular proteins with GFP_{11}-tag in S2 cells

(**A**) Representative images of S2 cells co-expressing GFP_{1-10} with GFP_{11} fused to various cellular proteins. For each fusion, the linker length is indicated in the figure. (**B**) The expression of cytoplasmic GFP_{1-10} shows that there is no reconstituted signal from GFP_{11}-tagged CG7630. CG7630 is known to localize to the mitochondrial matrix (1). (**C**) CG7630-GFP_{11} in S2 cells co-expressing $GFP_{1-10}^{\text{mitochondrial matrix}}$ leads to localization pattern similar to that obtained by the CG7630-HA fusion (1). Scale bars, 10 μm.

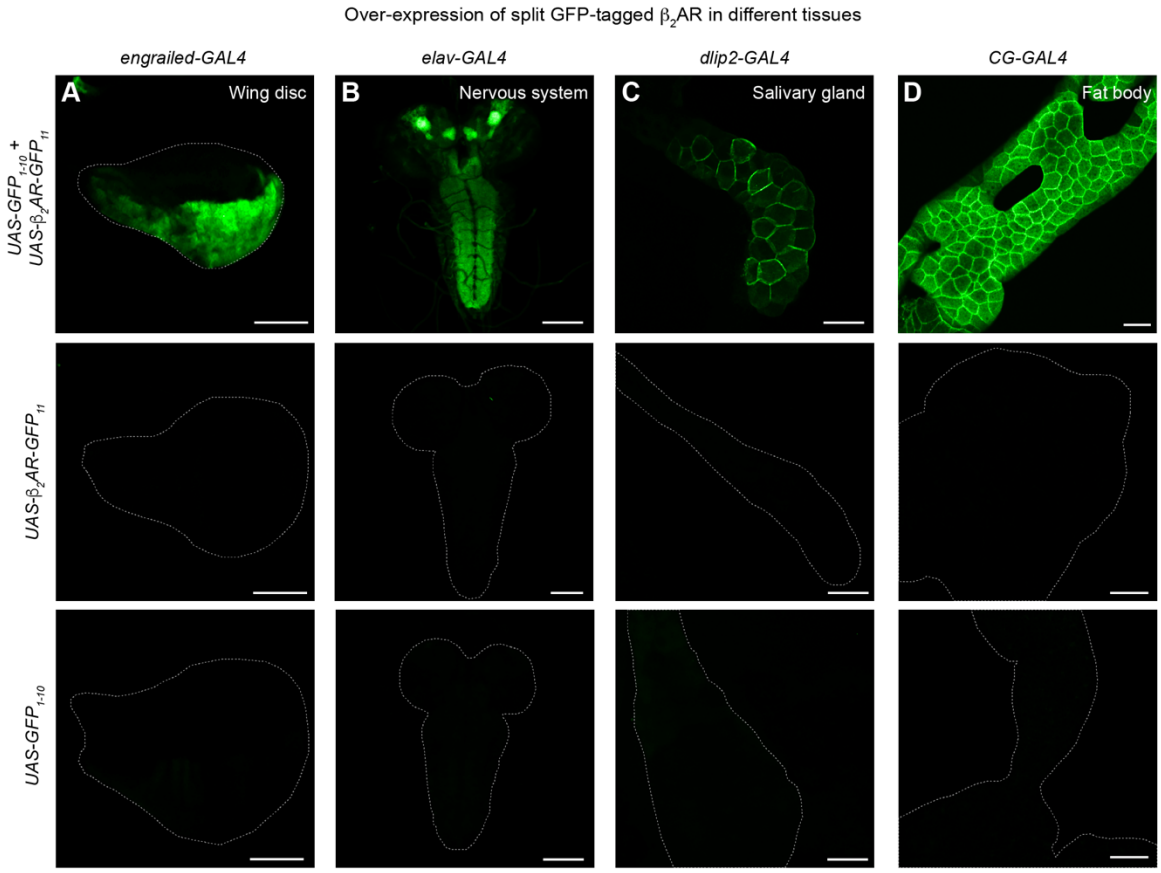

Fig. S2 | Validation of the split GFP$_{1\text{-}10/11}$ system in flies

Top panels (**A-D**): Confocal projections showing *GAL4*-driven split GFP expression in larval tissues. We simultaneously expressed *UAS-GFP$_{1\text{-}10}$* and *UAS-β_2AR-GFP$_{11}$* under the control of various *GAL4* drivers. Middle and bottom panels (**A-D**): Representative images of larval tissues over-expressing either *UAS-β_2AR-GFP$_{11}$* (middle panels) or *UAS-GFP$_{1\text{-}10}$* (bottom panels) under the control of these *GAL4* drivers. We show that neither GFP$_{1\text{-}10}$ nor β_2AR-GFP$_{11}$ produces fluorescence in these tissues. The images were taken with the same acquisition settings. The tissues (outlined by white dash lines) are oriented to the anterior to the top. Scale bars, 100 μm.

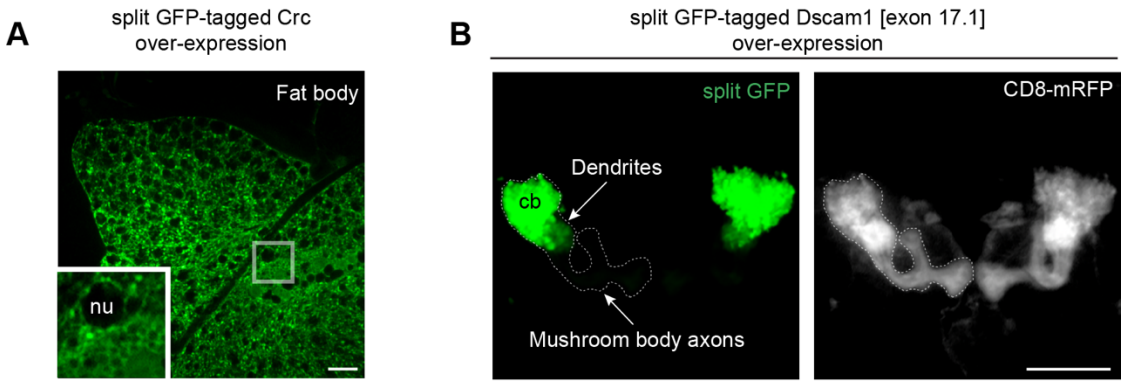

Fig. S3 | Tagging an ER luminal protein and the extracellular domain of a transmembrane protein with GFP_{11}-tag

(**A**-**B**) An ER-targeted GFP_{1-10} (GFP_{1-10}^{sec}) labels an ER luminal protein (GFP_{11}-Calreticulin) in the larval fat body or the extracellular domain of a plasma membrane protein (GFP_{11}-Dscam1 [exon 17.1]) in the larval brain. (**A**, inset) Calreticulin is localized to the ER and the nuclear membrane (nu). (**B**) *CD8* (a cell membrane maker) and *dscam1 [exon 17.1]* are co-expressed under the control of *201Y-GAL4*. Dscam1 [exon 17.1] is preferentially localized in dendrites and cell bodies (cb), while CD8 is distributed throughout cell bodies, dendrites, and axons. Images for different fluorophores were collected sequentially to avoid crosstalk between color channels. Scale bars, 100 μm.

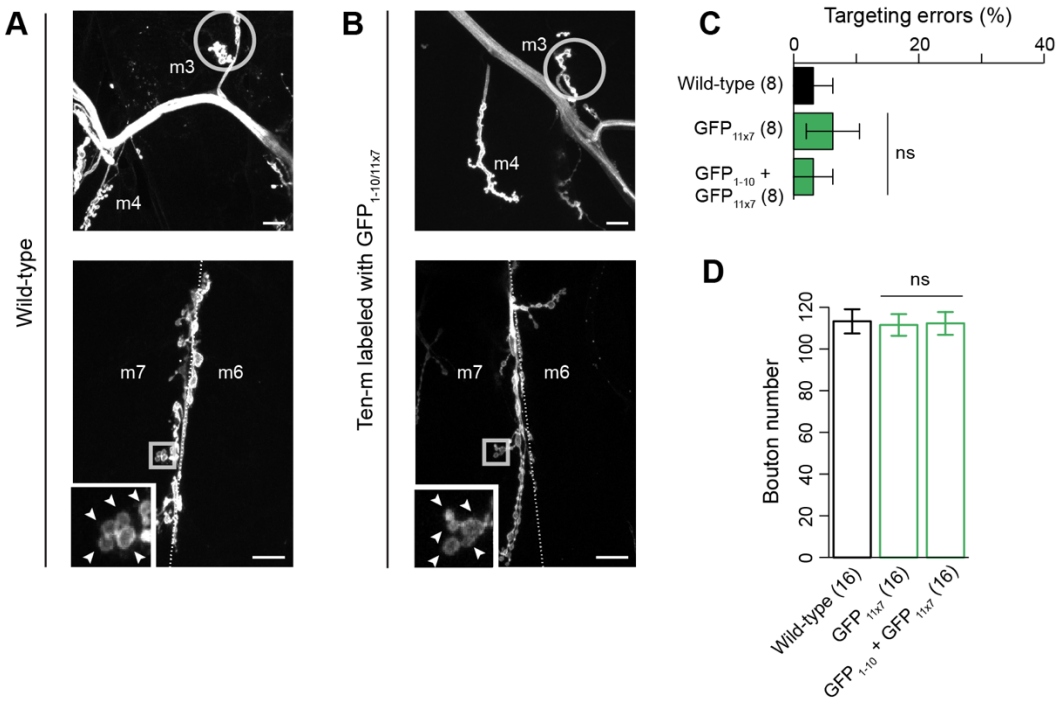

Fig. S4 | Evaluation of the function of Ten-m in homozygous protein trap lines (**A**-**B**) Representative NMJs in segment A2 stained with antibodies to the enzyme horseradish peroxidase (HRP). Larvae with either GFP_{11x7} or $GFP_{1\text{-}10/11x7}$-labeled Ten-m do not show any phenotype at the NMJs, suggesting that the tandem tag does not disturb the function of Ten-m. Circles indicate the innervation on muscle 3. Arrowheads in the zoomed insets indicate individual boutons. (**C**) Quantification of the hemisegment percentage with failed 'muscle 3' innervation. (**D**) Quantification of bouton numbers on muscles 6 and 7. For each quantification, *n* = 8 larvae; 16 NMJs. ns, not significant as compared to wild-type. Scale bars, 20 μm.

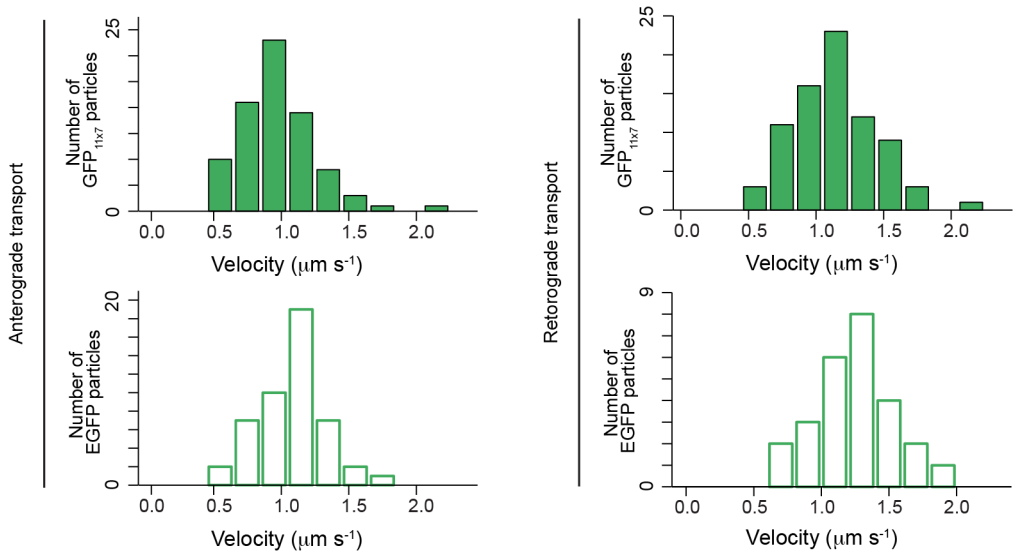

Fig. S5 | Quantification of Ten-m transport along larval motor axons

Velocity frequency distribution of Ten-m tagged with either GFP_{11x7} or full-length EGFP. There is no statistical difference between the two different labeling approaches for either anterograde or retrograde velocity. A summary graph is shown in **Fig. 1J**.

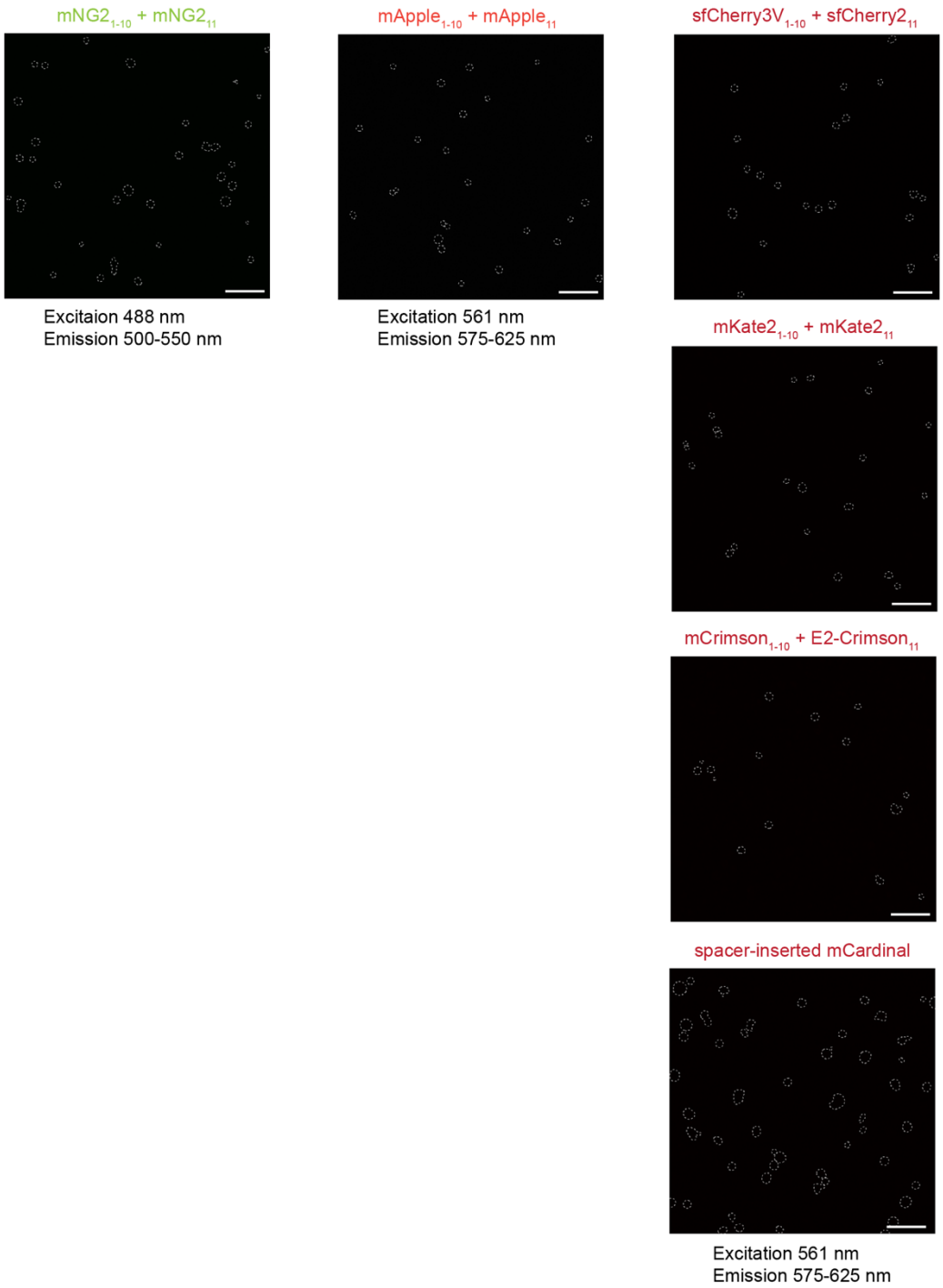

Fig. S6 | Engineering new split FPs for protein labeling in *Drosophila* cells

Fluorescence images of S2 cells co-expressing CD8-mIFP (an infrared FP-tagged membrane marker) with mNeonGreen2$_{1-10/11}$, mApple$_{1-10/11}$, sfCherry3V$_{1-10}$/sfCherry2$_{11}$, mKate2$_{1-10/11}$, E2-Crimson$_{1-10/11}$, or spacer inserted mCardinal. Dashed lines encircle IFP-positive cells. Scale bars, 100 μm.

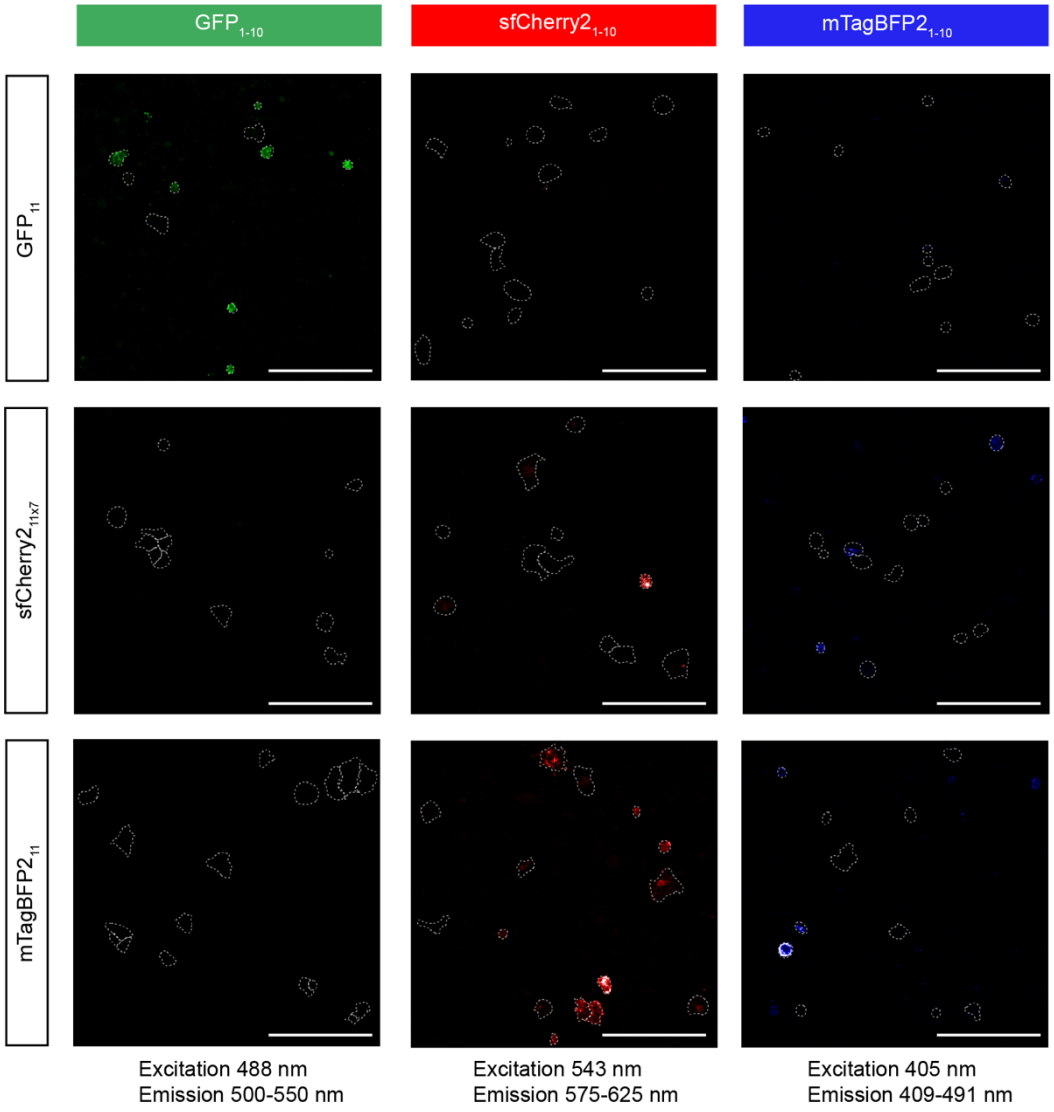

Fig. S7 | Characterizing the binding properties of the three $FP_{1-10/11}$ pairs (i.e., $GFP_{1-10/11}$, $sfCherry2_{1-10/11}$, and $mTagBFP2_{1-10/11}$)

We tested each of the FP_{11} fragments to complement all of the FP_{1-10} fragments. We calculated the percentage of split FP-positive cells in the IFP-positive population. A summary chart is shown in **Fig. 3A**. Dashed lines encircle IFP-positive cells. Scale bars, 100 μm.

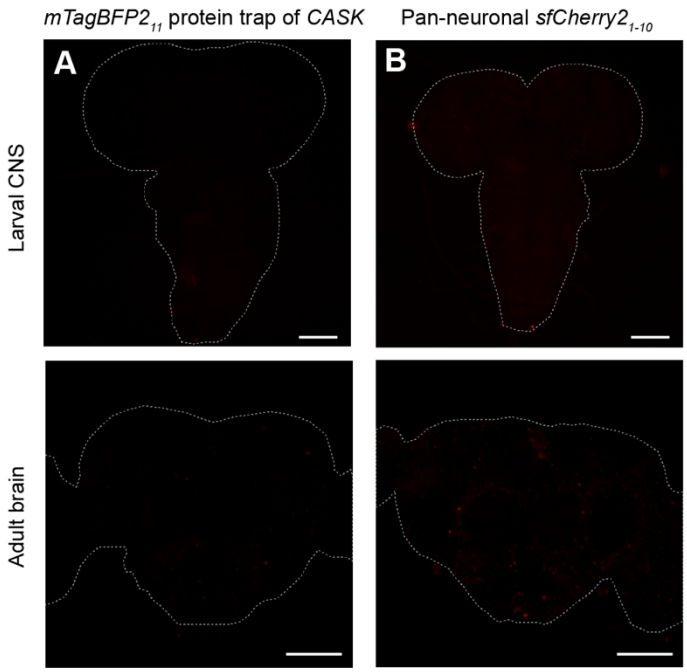

Fig. S8 | Background fluorescence in larval and adult brains from sfCherry2$_{1-10}$ or mTagBFP2$_{11}$

(**A**-**B**) Fluorescence images of larval (top panels) and adult fly brains (bottom panels). We show that neither mTagBFP2$_{11}$ nor sfCherry2$_{1-10}$ by themselves produce visible fluorescence as negative controls. Dashed lines encircle the brains. Images in **A**-**B** and **Fig. 4** were taken with the same acquisition settings. Scale bars, 100 μm.

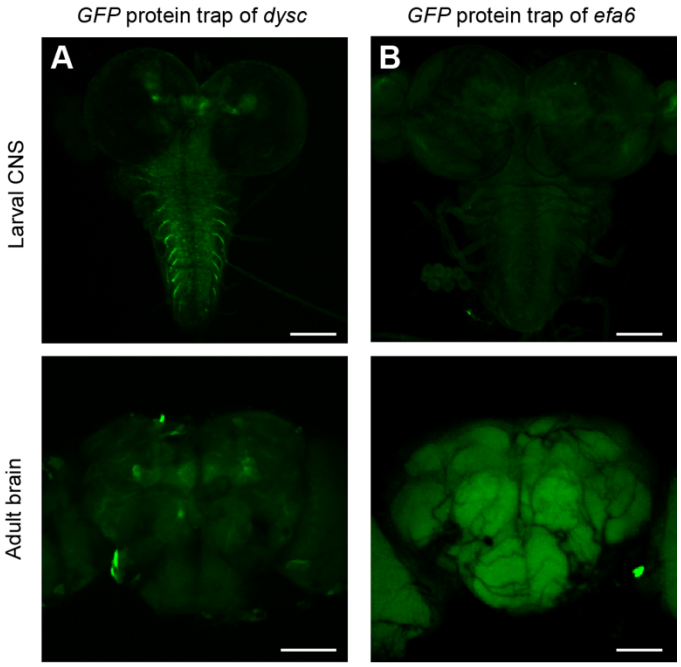

Fig. S9 | *GFP* protein trap lines of *dysc* and *efa6*

(**A**-**B**) Fluorescence images of two different GFP trap lines. Expression and localization of GFP-tagged Dysc and Efa6 proteins are detected in neuronal tracts throughout the CNS during the larval (top panels) and adult (bottom panels) stages. Scale bars, 100 μm.

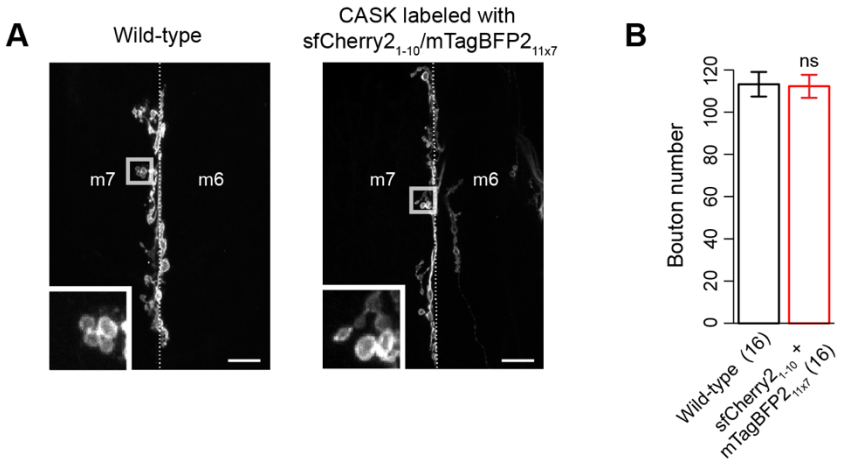

Fig. S10 | Function of sfCherry2$_{1-10}$/mTagBFP2$_{11x7}$-tagged CASK at NMJs

(**A**) Representative NMJs stained for the presynaptic membrane maker HRP. (**B**) Quantification of bouton numbers on muscles 6 and 7 in segment A2. In a previous study, Sun *et al*. have demonstrated that *CASK* mutations cause an increase in bouton number (2). In contrast, the homozygous *TagBFP$_{11x7}$* line of *CASK* with a pan-neuronal expression of *sfCherry2$_{1-10}$* has shown no significant difference in bouton number from wild-type. Scale bars, 20 μm.

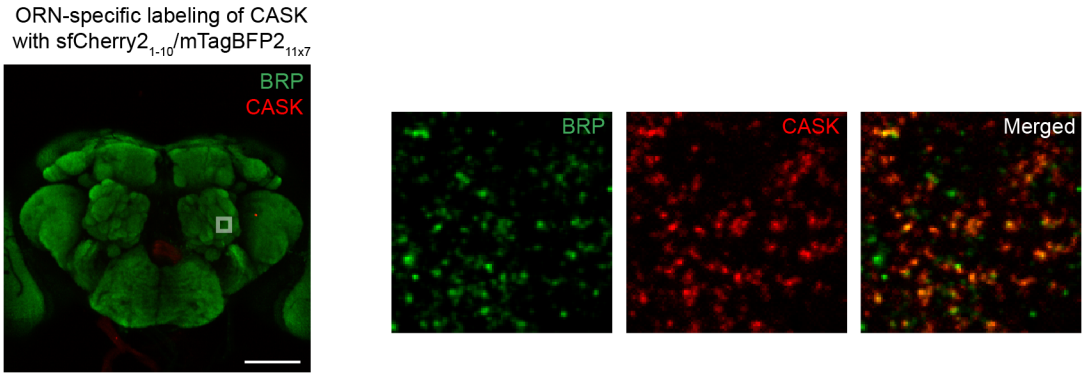

Fig. S11 | Co-labeling of presynaptic sites in Or47b-expressing ORNs using anti-BRP and cell-type-specific CASK labeling

Representative images of ORNs stained with anti-BRP and expressing sfCherry2$_{1-10}$ via *Or47b-GAL4* in an *mTagBFP2$_{11x7}$* line of *CASK*. A boxed region is shown in higher magnification in the right columns. Scale bar, 100 μm.

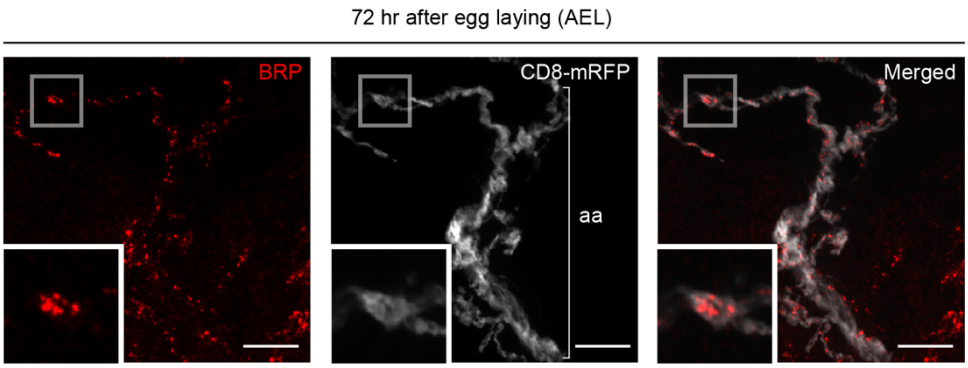

Fig. S12 | BRP puncta accumulation at IPC axonal terminals

Partial view of the axonal terminals of IPCs. IPCs expressing *mCD8-mRFP* were co-labeled with anti-BRP. The inset shows a higher magnification confocal image of BRP puncta in the axonal terminals. aa, anterior aorta. Scale bars, 10 μm.

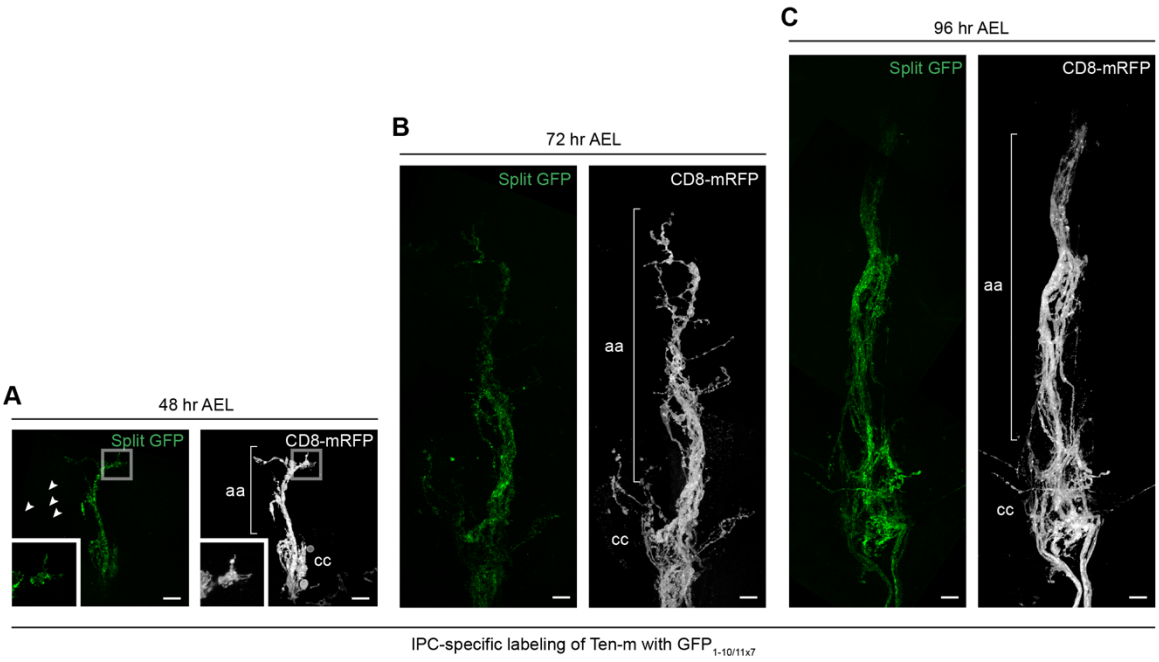

Fig. S13 | Localization of Ten-m in IPC axons at different stages of larval development

(**A**-**C**) A *GFP*$_{11x7}$ line of *Ten-m* was crossed with an IPC-specific line of *GFP*$_{1-10}$. Ten-m is localized to axons of IPC during larval stages. Arrowheads (**A**, inset) mark Ten-m puncta in the terminals. aa, anterior aorta; cc, corpora cardiaca. Scale bars, 10 μm.

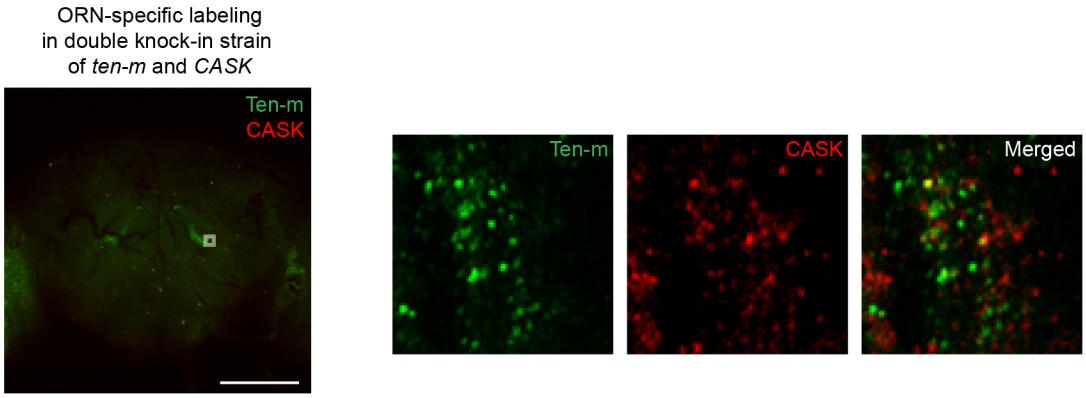

Fig.S14 | Dual-color labeling of Ten-m and CASK in Or47b-expressing ORNs
Representative images of Or47b-expressing ORNs. Ten-m and CASK's localization patterns labeled with two orthogonal split FP systems are shown with higher magnification in the right panels (the inset at the left panel). Ten-m puncta only slightly overlap with CASK puncta in the axonal terminals. Scale bars, 100 μm.

Table S1

Properties of red-colored split FPs used in this study

	sfCherry2$_{1-10}$ + sfCherry2$_{11}$	**sfCherry2$_{1-10}$ + TagBFP2$_{11}$**
EC at peak ($M^{-1}cm^{-1}$)	82,500	76,200
QY	0.22	0.19
Brightness [a]	18,150	14,478

EC, extinction coefficient; QY, quantum yield. [a] Calculated as the product of EC and QY.

Table S2

Nucleotide sequences of *mTagBFP2*$_{1\text{-}10/11}$, *GFP*$_{1\text{-}10/11}$, *mNeonGreen2*$_{1\text{-}10/11}$, *mApple*$_{1\text{-}10/11}$, *sfCherry2*$_{1\text{-}10/11}$, *mKate2*$_{1\text{-}10/11}$, *E2-Crimson*$_{1\text{-}10/11}$, and *spacer-inserted mCardinal*

FP$_{1\text{-}10/11}$	**Sequence (5'-3')**
mTagBFP2$_{1\text{-}10}$	ATGAGCGAGCTGATTAAGGAGAACATGCACATGAAGCT GTACATGGAGGGCACCGTGGACAACCATCACTTCAAGT GCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCAC CCAGACCATGAGAATCAAGGTGGTCGAGGGCGGCCCTC TCCCCTTCGCCTTCGACATCCTGGCTACTAGCTTCCTCT ACGGCAGCAAGACCTTCATCAACCACACCCAGGGCATC CCCGACTTCTTCAAGCAGTCCTTCCCTGAGGGCTTCACA TGGGAGAGAGTCACCACATACGAAGACGGGGGCGTGC TGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGC CTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCACA TCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTG GGAGGCCTTCACCGAGACGCTGTACCCCGCTGACGGC GGCCTGGAAGGCAGAAACGACATGGCCCTGAAGCTCGT GGGCGGGAGCCATCTGATCGCAAACGCCAAGACCACAT ATAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCTG GCGTCTACTATGTGGACTACAGACTGGAAAGAATCAAG GAGGCCAACAACTAA
mTagBFP2$_{11}$	TCTGAGACCTACGTCGAGCAGCACGAGGTGGCAGTGGC CAGATACTGCGACCTCCCTAGCAAACTGGGGCACAAGC TTAAT
GFP$_{1\text{-}10}$	ATGTCCAAAGGAGAAGAACTGTTTACCGGTGTTGTGCCA ATTTTGGTTGAACTCGATGGTGATGTCAACGGACATAAG TTCTCAGTGAGAGGCGAAGGAGAAGGTGACGCCACCAT TGGAAAATTGACTCTTAAATTCATCTGTACTACTGGTAAA CTTCCTGTACCATGGCCGACTCTCGTAACAACGCTTACG TACGGAGTTCAGTGCTTTTCGAGATACCCAGACCATATG AAAAGACATGACTTTTTTAAGTCGGCTATGCCTGAAGGT TACGTGCAAGAAAGAACAATTTCGTTCAAAGATGATGGA AAATATAAAACTAGAGCAGTTGTTAAATTTGAAGGAGATA CTTTGGTTAACCGCATTGAACTGAAAGGAACAGATTTTA AAGAAGATGGTAATATTCTTGGACACAAACTCGAATACA ATTTTAATAGTCATAACGTATACATCACTGCTGATAAGCA AAAGAACGGAATTAAAGCGAATTTCACAGTACGCCATAA TGTAGAAGATGGCAGTGTTCAACTTGCCGACCATTACCA ACAAAACACCCCTATTGGAGACGGTCCGGTACTTCTTCC TGATAATCACTACCTCTCAACACAAACAGTCCTGAGCAA AGATCCAAATGAAAAATAA
GFP$_{11}$	CGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGCT GGGATTACA

(Table continues)

mNeonGreen2$_{1\text{-}10}$	ATGGTGAGCAAGGGTGAGGAGGATAACATGGCCTCTCT CCCAGCGACTCATGAGTTACACATCTTTGGCTCCATCAA CGGTGTGGACTTTGACATGGTGGGTCAGGGTACCGGCA ATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCA CCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTC CCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTAC CCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAGA TGGCTCCGGATACCAAGTCCATCGCACAATGCAGTTTGA AGATGGTGCCTCCCTTACTGTTAACTACCGCTACACCTA CGAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGATG GGGACTGGTTTCCCTGCTGACGGTCCTGTGATGACCAA CACGCTGACCGCTGCGGACTGGTGCATGTCGAAGAAGA CTTACCCCAACGACAAAACCATCATCAGTACCTTTAAGT GGAGTTACACCACTGTAAATGGCAAACGCTACCGGAGC ACTGCGCGGACCACCTACACCTTTGCCAAGCCAATGGC GGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCC GTAAGACGGAGCTCAAGCACTCCATGTAA
mNeonGreen2$_{11}$	ACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTAC CGATATGATG
mApple$_{1\text{-}10}$	ATGGTGAGCAAGGGCGAGGAGAATAACATGGCCATCAT CAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCT CCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGG CGAGGGCCGCCCCTACGAGGCCTTTCAGACCGCTAAGC TGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGG GACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGTC TACATTAAGCACCCAGCCGACATCCCCGACTACTTCAAG CTGTCCTTCCCCGAGGGCTTCAGGTGGGAGCGCGTGAT GAACTTCGAGGACGGCGGCATTATTCACGTTAACCAGG ACTCCTCCCTGCAGGACGGCGTGTTCATCTACAAGGTG AAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGT AATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCGAG GAGCGGATGTACCCCGAGGACGGCGCCCTGAAGAGCG AGATCAAGAAGAGGCTGAAGCTGAAGGACGGCGGCCA CTACGCCGCCGAGGTCAAGACCACCTACAAGGCCAAGA AGCCCGTGCAGCTGCCCGGCGCCTACATCGTCGACATC AAGTTGGACATCGTGTCCCACAACGAGGACTAA
mApple$_{11}$	TACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCG CCACTCCACCGGCGGCATGGACGAGCTGTACAAG

(Table continues)

sfCherry2$_{1\text{-}10}$	ATGGAGGAGGACAACATGGCCATCATCAAGGAGTTCAT GAGATTCAAGGTGCACATGGAGGGCAGCGTGAACGGC CACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCACC CCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGAC CAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGA GCCCCCAGTTCATGTACGGCAGCAAGGCCTACGTGAAG CACCCCGCCGACATCCCCGACTACCTGAAGCTGAGCTT CCCCGAGGGCTTCACCTGGGAGAGAGTGATGAACTTCG AGGACGGCGGCGTGGTGACCGTGACCCAGGACAGCAG CCTGCAGGACGGCCAGTTCATCTACAAGGTGAAGCTGC TGGGCATCAACTTCCCCAGCGACGGCCCCGTGATGCAG AAGAAGACCATGGGCTGGGAGGCCAGCACCGAGAGAA TGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAA CCAGAGACTGAAGCTGAAGGACGGCGGCCACTACGAC GCCGAGGTGAAGACCACCTACAAGGCCAAGAAGCCCGT GCAGCTGCCCGGCGCCTACAACGTGGACATCAAGCTGG ACATCACCAGCCACAACGAGGACTAA
sfCherry2$_{11}$	TACACCATCGTGGAGCAGTATGAACGTGCAGAGGCTCG CCATTCCACA
mKate2$_{1\text{-}10}$	ATGGTGAGCGAGCTGATTAAGGAGAACATGCACATGAA GCTGTACATGGAGGGCACCGTGAACAACCACCACTTCA AGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGG CACCCAGACCATGAGAATCAAGGCGGTCGAGGGCGGC CCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTC ATGTACGGCAGCAAAACCTTCATCAACCACACCCAGGG CATCCCCGACTTCTTTAAGCAGTCCTTCCCCGAGGGCTT CACATGGGAGAGAGTCACCACATACGAAGACGGGGGC GTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACG GCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAAC TTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTC GGCTGGGAGGCCTCCACCGAGACCCTGTACCCCGCTG ACGGCGGCCTGGAAGGCAGAGCCGACATGGCCCTGAA GCTCGTGGGCGGGGGCCACCTGATCTGCAACTTGAAGA CCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGA TGCCCGGCGTCTACTATGTGGACAGAAGACTGGAAAGA ATCAAGGAGGCCGACAAATAA
mKate2$_{11}$	GAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCA GATACTGCGACCTCCCTAGCAAACTGGGGCACAGA

(Table continues)

E2-Crimson$_{1\text{-}10}$	ATGGATAGCACTGAGAACGTCATCAAGCCCTTCATGCG CTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACG AGTTCGAGATCGAGGGCGTGGGCGAGGGCAAGCCCTA CGAGGGCACCCAGACCGCCAAGCTGCAAGTGACCAAG GGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCC CCAGTTCTTCTACGGCTCCAAGGCGTACATCAAGCACC CCGCCGACATCCCCGACTACCTCAAGCAGTCCTTCCCC GAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGA CGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTG CAGGACGGCACCCTCATCTACCACGTGAAGTTCATCGG CGTGAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGA AGACTCTGGGCTGGGAGCCCTCCACTGAGCGCAACTAC CCCCGCGACGGCGTGCTGAAGGGCGAGAACCACATGG CGCTGAAGCTGAAGGGCGGCGGCCACTACCTGTGTGA GTTCAAGTCCATCTACATGGCCAAGAAGCCCGTGAAGC TGCCCGGCTACCACTACGTGGACTACAAGCTCGACATC ACCTCCCACAACGAGGACTAA
E2-Crimson$_{11}$	TACACCGTGGTGGAGCAGTACGAGCGCGCCGAGGCCC GCCACCACCTGTTCCAG
Spacer-inserted mCardinal	ATGGTGAGCAAGGGCGAGGAGCTGATCAAGGAGAACAT GCACATGAAGCTGTACATGGAAGGCACCGTGAACAACC ACCACTTCAAGTGCACCACCGAAGGGGAGGGCAAGCCC TACGAGGGCACCCAGACCCAGAGGATTAAGGTGGTGGA GGGAGGCCCCCTGCCGTTCGCATTCGACATCCTGGCCA CCTGCTTTATGTACGGGAGCAAGACCTTCATCAACCACA CCCAGGGCATCCCCGATTTCTTTAAGCAGTCCTTCCCTG AGGGCTTCACATGGGAGAGAGTCACCACATACGAAGAC GGGGGCGTGCTTACCGTTACCCAGGACACCAGCCTCCA GGACGGCTGCTTGATCTACAACGTCAAGCTCAGAGGGG TGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAA CACTCGGCTGGGAGGCCACCACCGAGACCCTGTACCC CGCTGACGGCGGCCTGGAAGGCAGATGCGACATGGCC CTGAAGCTCGTGGGCGGGGGCCACCTGCACTGCAACC TGAAGACCACATACAGATCCAAGAAACCCGCTAAGAACC TCAAGATGCCCGGCGTCTACTTTGTGGACCGCAGACTG GAAAGAATCAAGGAGGCCGACAAT**GGTGGTGGCGGATC** **AGAAGGAGGCGGTAGCGGGGGCCCTGGTTCGGGAGG** **GGAAGGTTCTGCTGGGGGAGGGAGCGCTGGCGGGGG** **GTCT**GAGACCTACGTCGAGCAGCACGAGGTGGCTGTGG CCAGATACTGCGACCTCCCTAGCAAACTGGGGCACAAA CTTAATGGCATGGACGAGCTGTACAAGTAA

30-aa spacer
Start or stop codon

Table S3

Amino acid sequences of mTagBFP2$_{1\text{-}10/11}$, GFP$_{1\text{-}10/11}$, and sfCherry2$_{1\text{-}10/11}$

FP$_{1\text{-}10/11}$	**aa. sequence**
mTagBFP2$_{1\text{-}10}$	MSELIKENMHMKLYMEGTVDNHHFKCTSEGEGKPYEGTQTMRI KVVEGGPLPFAFDILATSFLYGSKTFINHTQGIPDFFKQSFPEGFT WERVTTYEDGGVLTATQDTSLQDGCLIYNVKIRGVNFTSNGPVM QKKTLGWEAFTETLYPADGGLEGRNDMALKLVGGSHLIANAKTT YRSKKPAKNLKMPGVYYVDYRLERIKEANN
mTagBFP2$_{11}$	SETYVEQHEVAVARYCDLPSKLGHKLN
mTagBFP2$_{11x7}$	MSETYVEQHEVAVARYCDLPSKLGHKLN**GGSGG**SETYVEQHEV AVARYCDLPSKLGHKLN**GGSGG**SETYVEQHEVAVARYCDLPSK LGHKLN**GGSGG**SETYVEQHEVAVARYCDLPSKLGHKLN**GGSG** **G**SETYVEQHEVAVARYCDLPSKLGHKLN**GGSGG**SETYVEQHEV AVARYCDLPSKLGHKLN**GGSGG**SETYVEQHEVAVARYCDLPSK LGHKLN
GFP$_{1\text{-}10}$	MSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTL KFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAM PEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKE DGNILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSV QLADHYQQNTPIGDGPVLLPDNHYLSTQTVLSKDPNEK
GFP$_{11}$	RDHMVLHEYVNAAGIT
GFP$_{11x7}$	MRDHMVLHEYVNAAGIT**GGSGG**RDHMVLHEYVNAAGIT**GGSG** **G**RDHMVLHEYVNAAGIT**GGSGG**RDHMVLHEYVNAAGIT**GGSG** **G**RDHMVLHEYVNAAGIT**GGSGG**RDHMVLHEYVNAAGIT**GGSG** **G**RDHMVLHEYVNAAGIT
sfCherry2$_{1\text{-}10}$	MEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGHPYEGTQ TAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSF PEGFTWERVMNFEDGGVVTVTQDSSLQDGQFIYKVKLLGINFPS DGPVMQKKTMGWEASTERMYPEDGALKGEINQRLKLKDGGHY DAEVKTTYKAKKPVQLPGAYNVDIKLDITSHNED
sfCherry2$_{11}$	YTIVEQYERAEARHST
sfCherry2$_{11x7}$	MYTIVEQYERAEARHST**GGSGG**YTIVEQYERAEARHST**GGSGG** YTIVEQYERAEARHST**GGSGG**YTIVEQYERAEARHST**GGSGG**Y TIVEQYERAEARHST**GGSGG**YTIVEQYERAEARHST**GGSGG**YTI VEQYERAEARHST

5-aa spacer

Table S4
List of the oligonucleotides and gene fragments used in this study

Oligonucleotide or gene fragment	Sequence (5'-3')
GFP_{11}-Histone H3.3A gene fragment	GGGAATTGGGAATTCACCATGGCACGTAC CAAGCAAACAGCCCGTAAATCGACCGGAG GCAAGGCGCCCCGCAAGCAGCTGGCCACC AAGGCGGCCCGTAAATCGGCGCCATCCAC CGGCGGAGTGAAGAAGCCACATCGCTACC GTCCTGGAACGGTGGCCCTGCGTGAGATT CGTCGCTACCAGAAGTCCACGGAGCTGCT CATCCGCAAGCTGCCGTTCCAGCGTCTGG TGCGCGAGATAGCCCAGGACTTCAAGACC GATCTGCGCTTCCAGTCGGCGGCCATTGG AGCCCTACAGGAGGCCAGCGAGGCGTACC TGGTCGGTCTGTTCGAGGACACCAATCTGT GCGCCATTCACGCCAAGCGCGTCACCATT ATGCCCAAGGACATCCAGCTGGCCAGACG CATCCGTGGCGAGCGGGCCGGTGGATCG **CGTGACCACATGGTCCTTCATGAGTATGT AAATGCTGCTGGGATTACA**TAGATCTGCG GCCGCGG
pACUH-Centrosomin [1-1440] (F)	GGGAATTGGGAATTCACCATGAATAGTAAT CGAACGTC
pACUH-Centrosomin [1-1440] (R)	AATAGAGTACAGAATGCCAGC
Centrosomin [1420-1440]-GFP_{11} gene fragment	CTGGCATTCTGTACTCTATTAGCAGCGGTG GAGCAAGCGCAGCCAGTGGTAGCGCGGAT CCACCGGTCGCCACC**CGTGACCACATGGT CCTTCATGAGTATGTAAATGCTGCTGGGAT TACA**TAAGATCTGCGGCCGCGG
pACUH-β_2AR-GFP_{11} (F)	TTAACAGATCTTGCGGCCGCATGCGGCTCT GCATCCCGCAGGTGCTGTTG
pACUH-β_2AR-GFP_{11} (R)	CACAAAGATCCTCTAGATTA**TGTAATCCCA GCAGCATTTACATACTCATGAAGGACCAT GTGGTCACG**CAGCAGTGAGTCATTTGTACT ACAATTCCT
[1] pACUH-GFP_{1-10}^{sec} (F)	ACTTCCGCCGCCACCTGTTC
[1] pACUH-GFP_{1-10}^{sec} (F)	GGTGGCGGCGGAAGTTAGTCTAGAGGATC TTTGTG

(Table continues)

pACUH-GFP$_{1-10}^{\text{mitochondrial matrix}}$ (F)	ATGTCCAAAGGAGAAGAACT
pACUH-GFP$_{1-10}^{\text{mitochondrial matrix}}$ (R)	TCGAGCCGCGGCCGCTTACAGATCCTCTT CTGAGATGAGTTTTTGTTCACTTCCTCCGC CACCTTTTTCATTTGGATCTTTGC
COXVIII gene fragment	ATGTCCGTCCTGACGCCGCTGCTGCTGCG GGGCTTGACAGGCTCGGCCCGGCGGCTC CCAGTGCCGCGCGCCAAGATCCATTCGTT G
[2] pACUH-mCherry-GFP$_{11}$(F)	GTGAGCAAGGGCGAGGAGGAT
[2] pACUH-mCherry-GFP$_{11}$(R)	TCGAGCCGCGGCCGCTA**TGTAATCCCAG CAGCATTTACA**TACTCATGAAGGACCATG TGGTCACGACTTCCTCCGCCACCCTTGTA CAGCTCGTCCATGC
[2] CG7630 gene fragment	ATGTTGGTTAAGCACATTGTCAAGCAGGG GCTTCTGCTCAAGAACGCTGGCGCTTTGT CGCGTGCCGCTTACCACGGTGGACATGGT CCCCACTCCACCATGAACGATCTGCCCGT GCCCGCTGGAGACTGGAAGGAGCAGCAC AGCCAGAAGAACGCCAAGTACAATGCCGC GCTCATCACTGGCATTCTCGTCCTGGCCG GCACTATTGGATTCGTGAAATCTTCTGGTA TCATCCACTTCAACTACTACGCGCCCAAG AGCCTGGAC
[3] pBS-Dscam1 [1-1684] (F)	GGGAATTGGGAATTCACCATGAATATGCC CAACGAACGC
[3] pBS-Dscam1 [1-1684] (R)	AGCTAGGGTCTGACTACCAC
[3] pBS-GFP$_{11}$-Dscam1 [1-1684] (F)	GGTGCGGGCGCAGGAGCC**CGTGACCACA TGGTCCTTCA**
[3] pBS-GFP$_{11}$-Dscam1 [1-1684] (R)	TGCGCCGGCTCCAGCCCC**TGTAATCCCA GCAGCATTTACA**
[3] pACUH-GFP$_{11}$-Dscam1 [1-1684] (F)	GCCAATCCCCCAGATGCC
[3] pACUH-GFP$_{11}$-Dscam1 [1-1684] (R)	GCTCCCAGACAATCGAATCG
[3] pACUH-GFP$_{11}$-Dscam1 [1685-6463] (F)	CGATTCGATTGTCTGGGAGC
[3] pACUH-GFP$_{11}$-Dscam1 [1685-6463] (R)	GCGGCCGCAGATCTGTTAACTTACACTGC CATAGTATCGTAGGC
[4] pACUH-sfCherry2$_{11}$-β-actin (F)	TCCGGACTCAGATCTGGC
[4] pACUH-sfCherry2$_{11}$-β-actin (R)	TGTGGAATGGCGAGCCTC
[4] pACUH-sfCherry2$_{11x7}$-β-actin (F)	TCCGGACTCAGATCTGGC
[4] pACUH-sfCherry2$_{11x7}$-β-actin (R)	CGTGGAGTGTCGTGCCTC

(Table continues)

[4] pACUH-sfCherry2$_{11x7}$-sfGFP-β-act (F)	GGGAATTGGGAATTCATGTACACCATCGTG
[4] pACUH-sfCherry2$_{11x7}$-sfGFP-β-act (R)	GGTGGCGACCTCGAGCGGTAGCGCCGTGGAGTGTCGTGCCTCTGC
pACUH-β_2AR-sfCherry$_{11}$ (F)	TTAACAGATCTTGCGGCCGCATGCGGCTCTGCATCCCGCAGGTGCTGTTG
pACUH-β_2AR-sfCherry$_{11}$ (R)	CTTCACAAAGATCCTCTAGATTAGCCGCC**GGTGCTGTGTCTGCCCTCGGCTCTCTCGTACTGCTCCACGATGGTGTA**CAGCAGTGAGTCATTTGTACTAC
pACUH-CD8-sfCherry$_{11}$ (F)	CGTTAACAGATCTTGCGGCCGCATGGCCTCACC
pACUH-CD8-sfCherry$_{11}$ (R)	CTTCACAAAGATCCTCTAGACTAGCCGCC**GGTGCTGTGTCTGCCCTCGGCTCTCTCGTACTGCTCCACGATGGTGTA**GCGGCTGTGGTAGCAGATGAGAG
[5] pACUH-CD4-IFP-T2A-HO-mNG2$_{11}$ (F)	GGGAATTCGTTAACAGATCTATGAATCCCAAGAGCGAAGTCCTCATTGCA
[5] pACUH-CD4-IFP-T2A-HO-mNG2$_{11}$ (R)	CCTTCACAAAGATCCTCTAGATTA**CATCATATCGGTAAAGGCCTTTTGCCACTCCTTGAAGTTGAGCTCGGT**CATAGCATAGAGCCCCACTGCA
[5] pACUH-CD4-IFP-T2A-HO-mKT2$_{11}$ (F)	GGGAATTCGTTAACAGATCTATGAATCCCAAGAGCGAAGTCCTCATTGCA
[5] pACUH-CD4-IFP-T2A-HO-mKT2$_{11}$ (R)	CCTTCACAAAGATCCTCTAGATTAT**CTGTGCCCCAGTTTGCTAGGGAGGTCGCAGTATCTGGCCACAGCCACCTCGTGCTGCTCGACGTAGGTCT**CCATAGCATAGAGCCCCACTGCA
[5] pACUH-CD4-IFP-T2A-HO-mCrim$_{11}$ (F)	GGGAATTCGTTAACAGATCTATGAATCCCAAGAGCGAAGTCCTCATTGCA
[5] pACUH-CD4-IFP-T2A-HO-mCrim$_{11}$ (R)	CCTTCACAAAGATCCTCTAGATTA**CTGGAACAGGTGGTGGCGGGCCTCGGCGCGCTCGTACTGCTCCACCACGGTGTA**CATAGCATAGAGCCCCACTGCA
[6] pACUH-mApple$_{11}$-sfGFP (F)	ATAGGGAATTGGGAATTC**TACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAG**ATGGTGAGCAAGGGCGAGGAGCTG
[6] pACUH-mApple$_{11}$-sfGFP (R)	TCACGGCGGCCGCAAGATCTCTTGTACAGCTCGTCCATGCCGTGAGT

(Table continues)

[7] pACUH-TagBFP2$_{11}$-GFP/Emerald (F)	GGGAATTCGTTAACAGATCTATG**TCTGAGA CCTACGTCGAGCAGCACGAGGTGGCAGT GGCCAGATACTGCGACCTCCCTAGCAAAC TGGGGCACAAGCTTAAT**GGTGGCTCTATG GTGAGCAAG
[7] pACUH-mTagBFP2$_{11}$-Emerald-Clc (R)	CCTTCACAAAGATCCTCTAGACTAGCGGGA CAGTG
[7] pACUH-mTagBFP2$_{11}$-sfGFP-β-act (R)	CCTTCACAAAGATCCTCTAGACTAGAAGCA TTTGC
[7] pACUH-mTagBFP2$_{11}$-β-actin (F)	TCCGGACTCAGATCTGGC
[7] pACUH-mTagBFP2$_{11}$-β-actin (R)	ATTAAGCTTGTGCCCCAG
[8] pACUH-mEmerald-Tub-TagBFP2$_{11}$ (F)	GGGAATTCGTTAACAGATCTATGGTGAGCA AGGGC
[8] pACUH-mEmerald-Tub-TagBFP2$_{11}$ (R)	CCTTCACAAAGATCCTCTAGATTA**ATTAAG CTTGTGCCCCAGTTTGCTAGGGAGGTCGC AGTATCTGGCCACTGCCACCTCGTGCTGC TCGACGTAGGTCTCAGA**GTATTCCTCTCCT TCTTCCTCA
mTagBFP2$_{11}$-Histone H3.3A gene fragment	GGGAATTGGGAATTCACCATGGCACGTAC CAAGCAAACAGCCCGTAAATCGACCGGAG GCAAGGCGCCCCGCAAGCAGCTGGCCACC AAGGCGGCCCGTAAATCGGCGCCATCCAC CGGCGGAGTGAAGAAGCCACATCGCTACC GTCCTGGAACGGTGGCCCTGCGTGAGATT CGTCGCTACCAGAAGTCCACGGAGCTGCT CATCCGCAAGCTGCCGTTCCAGCGTCTGG TGCGCGAGATAGCCCAGGACTTCAAGACC GATCTGCGCTTCCAGTCGGCGGCCATTGG AGCCCTACAGGAGGCCAGCGAGGCGTACC TGGTCGGTCTGTTCGAGGACACCAATCTGT GCGCCATTCACGCCAAGCGCGTCACCATT ATGCCCAAGGACATCCAGCTGGCCAGACG CATCCGTGGCGAGCGGGCCGGTGGATCG **GAGACCTACGTCGAGCAGCACGAGGTGG CAGTGGCCAGATACTGCGACCTCCCTAGC AAACTGGGGCACAAGCTTAAT**TAGATCTG CGGCCGCGG

Split FP$_{11}$-tag
Start or stop codon
(F)orward primer
(R)everse primer

[1] *pACUH-GFP$_{1-10}^{sec}$* was generated by deleting the SEHDEL sequence of *pACUH-SP-GFP$_{1-10}$-SEHDEL* used in previous work (3).

[2] *mCherry* was amplified by PCR from *pcDNA3.1-mCherry-CAAX* with a 3' primer encoding *GFP$_{11}$* and cloned together with the *CG7630* gene fragment into the EcoRI/NotI sites of *pACUH*.

[3] *pACUH-GFP$_{11}$-Dscam1* was generated in two steps. First, DNA from the extracellular domain was subcloned into *pBluescript* (*pBS-Dscam1 [1-1684 nucleotide]*), and PCR-based mutagenesis of the plasmid using Q5 polymerase was used to create *pBS-GFP$_{11}$-Dscam1 [1-1684]*. Second, *GFP$_{11}$-Dscam1 [1-1684]* and the remainder of *Dscam1* (*Dscam1 [1685-6463]*) were individually amplified by PCR. The two PCR products were ligated into the EcoRI/BglII sites of *pACUH*

[4] An *sfCherry2$_{11x7}$-β-actin* construct was generated from *pACUH-sfGFP-β-actin* (3). The *sfCherry2$_{11x7}$* fragment was introduced into this plasmid, replacing *sfGFP*. An *sfCherry2$_{11}$-β-actin* construct was made by replacing *sfCherry2$_{11x7}$* with *sfCherry2$_{11}$*.

[5] Using *pACU2_CD4-mIFP T2A HO1* (plasmid 72441, Addgene) as a template, the *CD4-mIFP T2A HO1* region was amplified by PCR with a 3' primer encoding *FP$_{11}$*, and inserted between the BglII/XbaI sites of *pACUH*.

[6] *sfGFP* was amplified by PCR from *pACUH-β_2AR-sfGFP* with a 5' primer encoding *mApple$_{11}$*, and cloned into the EcoRI/BglII sites of *pACUH*.

[7] *mTagBFP2$_{11}$-mEmerald-Clc* and *mTagBFP2$_{11}$-sfGFP-β-actin* were PCR-amplified from *mEmerald-Clathrin-15* (plasmid 54040, Addgene) and *pACUH-sfGFP-β-actin* (3), respectively, and inserted into the BglII/XbaI sites of *pACUH*. *pACUH-mTagBFP2$_{11}$-β-actin* was constructed by deleting the *sfGFP* sequence in *pACHU-mTagBFP2$_{11}$-sfGFP-β-actin*.

[8] *mEmerald-α-tubulin* was PCR-amplified from *mEmerald-Tubulin-C-18* (plasmid 54292, Addgene) with a 3' primer encoding *mTagBFP2$_{11}$*. We cloned the resulting fragment into the BglII/XbaI sites of *pACUH*.

Table S5

Amino acid sequences of spacer-inserted red-colored split FPs

$FP_{1\text{-}10/11}$	**aa. sequence**
Spacer-inserted sfCherry$2_{1\text{-}10/11}$	MEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGHPYEGTQ TAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSF PEGFTWERVMNFEDGGVVTVTQDSSLQDGQFIYKVKLLGINFPS DGPVMQKKTMGWEASTERMYPEDGALKGEINQRLKLKDGGHY DAEVKTTYKAKKPVQLPGAYNVDIKLDITSHNED**GGGGSEGGGS** **GGPGSGGEGSAGGGSAGGGS**YTIVEQYERAEARHST*HHHHHH*
Spacer-inserted sfCherry$2_{1\text{-}10}$/ mTagBFP2_{11}	MEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGHPYEGTQ TAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSF PEGFTWERVMNFEDGGVVTVTQDSSLQDGQFIYKVKLLGINFPS DGPVMQKKTMGWEASTERMYPEDGALKGEINQRLKLKDGGHY DAEVKTTYKAKKPVQLPGAYNVDIKLDITSHNED**GGGGSEGGGS** **GGPGSGGEGSAGGGSAGGGS**SETYVEQHEVAVARYCDLPSKL GHKLN*HHHHHH*

30-aa spacer
His_{x6}-tag

Table S6
Specific genotypes in each experiment used in this study

Figure	Genotype
Fig. 1C	*w ; elav-GAL4 / UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11}$.1}Ten-m[MI07828-GFP $_{11}$.1]*
Fig. 1D	*w ; elav-GAL4 / UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1]*
Fig. 1F	*w ; Or47b-Gal4, UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], UAS-mCD8-mRFP*
Fig. 1G	*w ; dlip2-GAL4, UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], UAS-mCD8-mRFP*
Fig. 1H	*w ; ato-GAL4, UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], UAS-mCD8-mRFP*
	w ; elav-GAL4 / UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], UAS-mCD8-mRFP **(top)**
Fig. 1I	*yw ; ; Mi{PT-GFSTF.1}Ten-m[MI02844-GFSTF.1]* **(bottom)**
Fig. 2B	*w ; UAS-sfCherry2 $_{1\text{-}10}$ / + ; UAS-mCD8-sfCherry $_{11}$ / elav-GAL4*
Fig. 4A	*w ; elav-GAL4, UAS-sfCherry2 $_{1\text{-}10}$ / UAS-sfCherry2 $_{1\text{-}10}$; elav-GAL4 / Mi{PT-mTagBFP2 $_{11}$.0}CASK[MI01748-mTagBFP2 $_{11}$.0]*
Fig. 4B	*yw ; Mi{PT-GFSTF.0}CASK[MI00969-GFSTF.0] / TM6*
Fig. 4C	*w ; elav-GAL4, UAS-sfCherry2 $_{1\text{-}10}$ / UAS-sfCherry2 $_{1\text{-}10}$; + / Mi{PT-mTagBFP2 $_{11\text{x}7}$.0}CASK[MI01748-mTagBFP2 $_{11\text{x}7}$.0]*
Fig. 4D	*w ; elav-GAL4, UAS-sfCherry2 $_{1\text{-}10}$ / UAS-sfCherry2 $_{1\text{-}10}$; elav-GAL4 / Mi{PT-mTagBFP2 $_{11\text{x}7}$.0}dysc[MI01684-mTagBFP2 $_{11\text{x}7}$.0]*
Fig. 4E	*w ; elav-GAL4, UAS-sfCherry2 $_{1\text{-}10}$ / UAS-sfCherry2 $_{1\text{-}10}$; elav-GAL4 / Mi{PT-mTagBFP2 $_{11\text{x}7}$.0}efa6[MI00261-mTagBFP2 $_{11\text{x}7}$.0]*
Fig. 4G	*w ; Or47b-GAL4, UAS-sfCherry2 $_{1\text{-}10}$; UAS-CD4-tdGFP / Mi{PT-mTagBFP2 $_{11\text{x}7}$.0}CASK[MI01748-mTagBFP2 $_{11\text{x}7}$.0]*
Fig. 5A	*w ; elav-GAL4, UAS-sfCherry2 $_{1\text{-}10}$ / UAS-sfCherry2 $_{1\text{-}10}$; elav-GAL4, UAS-GFP $_{1\text{-}10}$ / Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], Mi{PT-mTagBFP2 $_{11\text{x}7}$.0}CASK[MI01748-mTagBFP2 $_{11\text{x}7}$.0]*
Fig. 5C	*w ; dlip2-GAL4, UAS-sfCherry2 $_{1\text{-}10}$ / dlip2-GAL4, UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], Mi{PT-mTagBFP2 $_{11\text{x}7}$.0}CASK[MI01748-mTagBFP2 $_{11\text{x}7}$.0]*
	w ; engrailed-GAL4, UAS-GFP $_{1\text{-}10}$ / CyO ; UAS- β_2AR-GFP $_{11}$ / TM6B **(top)**
	w ; engrailed-GAL4 / CyO ; UAS- β_2AR-GFP $_{11}$ / TM6B **(middle)**
Fig. S2A	*w ; engrailed-GAL4, UAS-GFP $_{1\text{-}10}$ / CyO* **(bottom)**
	w ; elav-GAL4, UAS-GFP $_{1\text{-}10}$ / CyO ; UAS- β_2AR-GFP $_{11}$ / TM6B **(top)**
	w ; elav-GAL4 / CyO ; UAS- β_2AR-GFP $_{11}$ / TM6B **(middle)**
Fig. S2B	*w ; elav-GAL4, UAS-GFP $_{1\text{-}10}$ / CyO* **(bottom)**
	w ; dlip2-GAL4, UAS-GFP $_{1\text{-}10}$ / CyO ; UAS- β_2AR-GFP $_{11}$ / TM6B **(top)**
	w ; dlip2-GAL4 / CyO ; UAS- β_2AR-GFP $_{11}$ / TM6B **(middle)**
Fig. S2C	*w ; dlip2-GAL4, UAS-GFP $_{1\text{-}10}$ / CyO* **(bottom)**
	w ; CG-GAL4, UAS-GFP $_{1\text{-}10}$ / CyO ; UAS- β_2AR-GFP $_{11}$ / TM6B **(top)**
	w ; CG-GAL4 / CyO ; UAS- β_2AR-GFP $_{11}$ / TM6B **(middle)**
Fig. S2D	*w ; CG-GAL4, UAS-GFP $_{1\text{-}10}$ / CyO* **(bottom)**
Fig. S3A	*w ; CG-GAL4 / UAS-GFP $^{1\text{-}10\ \text{sec}}$; UAS-Calreticulin-GFP $_{11}$ / TM6B*
Fig. S3B	*w ; 201Y-GAL4 / UAS-GFP $^{1\text{-}10\ \text{sec}}$; UAS-GFP $_{11}$-Dscam1 [exon 17.1] / TM6B*
Fig. S4A	*yw ;*
Fig. S4B	*w ; elav-GAL4 / UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1]*
Fig. S8A	*w ; If / CyO ; Mi{PT-mTagBFP2 $_{11}$.0}CASK[MI01748-mTagBFP2 $_{11}$.0] / TM3*
Fig. S8B	*w ; elav-GAL4 / + ; UAS-sfCherry2 $_{1\text{-}10}$ / +*
Fig. S9A	*yw ; Mi{PT-GFSTF.0}dysc[MI02729-GFSTF.0]*
Fig. S9B	*yw ; Mi{PT-GFSTF.0}Efa6[MI00261-GFSTF.0] / TM6*
	yw ; **(left)**
Fig. S10A	*w ; elav-GAL4, UAS-sfCherry2 $_{1\text{-}10}$ / CyO ; Mi{PT-GFP11x7.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], Mi{PT-mTagBFP2 $_{11\text{x}7}$.0}CASK[MI01748-mTagBFP2 $_{11\text{x}7}$.0]* **(right)**
Fig. S11	*w ; Or47b-GAL4, UAS-sfCherry2 $_{1\text{-}10}$; Mi{PT-mTagBFP2 $_{11\text{x}7}$.0}CASK[MI01748-mTagBFP2 $_{11\text{x}7}$.0] / TM3*
Fig. S12-13	*w ; dlip2-GAL4, UAS-GFP $_{1\text{-}10}$ / dlip2-GAL4, UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], UAS-mCD8-mRFP*
Fig. S14	*w ; Or47b-GAL4, UAS-sfCherry2 $_{1\text{-}10}$; UAS-GFP $_{1\text{-}10}$ / Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], Mi{PT-mTagBFP2 $_{11\text{x}7}$.0}CASK[MI01748-mTagBFP2 $_{11\text{x}7}$.0]*
Video S1	*w ; elav-GAL4 / UAS-GFP $_{1\text{-}10}$; UAS-GFP $_{11}$-actin*
	w ; elav-GAL4 / UAS-GFP $_{1\text{-}10}$; Mi{PT-GFP $_{11\text{x}7}$.1}Ten-m[MI07828-GFP $_{11\text{x}7}$.1], UAS-mCD8-mRFP
Video S2	*yw ; ; Mi{PT-GFSTF.1}Ten-m[MI02844-GFSTF.1]*

Legends for Movies S1 to S2

Movie S1. Movie of split GFP-tagged actin in axonal growth cones

Time-lapse movie shows dynamic filopodia activity in SNb (segmental nerve b) motor growth cones in an hour 13 AEL embryo co-expressing *GFP_{1-10}* and *GFP_{11}-β-actin* under the control of *elav-GAL4*. Images were acquired every 10 sec in a single plane. Arrowheads indicate individual filopodia. Scale bar, 10 μm.

Movie S2. Movie of Ten-m axonal transport in motor axons of 3rd instar larva

Time-lapse movie shows retrograde and anterograde transport of Ten-m tagged with $GFP_{1-10/11x7}$ (top) and full-length GFP (bottom) in larval motor axons. Axon bundles are aligned with cell bodies on the left and axon terminals on the right. Images were acquired every 0.5 sec. Arrowheads indicate Ten-m puncta which are transported along axons. Scale bar, 5 μm.

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