Supplementary Information for
Phagocytosis and self-destruction break down dendrites of Drosophila sensory neurons at distinct steps of Wallerian degeneration.

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- Legends for Videos S1 to S12
- SI References

Other supplementary materials for this manuscript include the following:

- Videos S1 to S12
SI Methods

Molecular cloning and transgenic flies

ey-Cas9: Three tandem copies of a 211bp ey enhancer (corresponding to nucleotides 2577-2787 in GenBank accession number AJ131630) were inserted into XhoI/Sall sites of pENTR11 (Thermo Fisher Scientific). The resulting entry vector was combined with a Cas9 destination vector which is similar to pDEST-APIC-Cas9 (Addgene 121657) but does not contain Inr, MTE, and DPE in the Hsp70 core promoter to generate the pAPIC-ey-Cas9 expression vector through a Gateway LR reaction.

shot-Cas9: A 3851 bp epidermis-specific enhancer (R38F11) of shot was amplified from R38F11-Gal4 genomic DNA using primers ggggACAAGTTTGTACAAMAAAGCAGGCTACGGAGATGCACTC and ggggACCACGTGTACAAGAAAGCTGGGTAATGCTGCTTGGGAAAG. The PCR product was combined with pDONR221 (Thermo Fisher Scientific) to make pENTR-shot through a Gateway BP reaction. pENTR-shot was then combined with a Cas9 destination vector to generate the expression vector pAPIC-shot-Cas9. The Cas9 destination vector is similar to pDEST-APIC-Cas9 (Addgene 121657) but does not contain Inr, MTE, and DPE in the Hsp70 core promoter.

LexAop-myr-tdTom-GFP(1-10): pAPLO-CD4-tdTom (1) was digested by BglII/Ascl, blunted, and religated to remove an XbaI site before 13xLexAop2. The resulting construct is called pAPLOm-CD4-tdTom. An myr-GFP fragment was isolated from pJFRC176-10XUAS-rox-dSTOP-rox-myr-GFP (Addgene 32147) by XhoI/XbaI digestion and ligated to XhoI/XbaI sites of pAPLOm-CD4-tdTom to make pAPLO-myr-GFP. pAPLO-myr-GFP was then digested by BamHI/XbaI and subsequently assembled with a tdTom PCR fragment and a GFP(1-10) gBlock fragment (synthesized by IDT) through NEBuilder DNA Assembly to generate pAPLO-myr-tdTom-GFP1-10.

UAS-sGFP(11)x7: A DNA fragment containing GFP(11)x7 was PCR amplified from a gBlock DNA fragment and cloned into NheI/XbaI sites of pIHEU-sfGFP-LactC1C2 using NEBuilder DNA Assembly. The resulting construct pIHEU-sGFP11x7 inherits the signal peptide sequence from pIHEU-sfGFP-LactC1C2 but removes the sfGFP-LactC1C2 coding sequence.

LexAop-WldS: The WldS coding sequence was PCR amplified from UAS-WldS genomic DNA and cloned into XhoI/XbaI sites of pAPLOm-CD4-tdTom via restriction cloning to make pAPLO-WldS.

UAS-ATP8A: A fragment encoding a FLAG tag and the ER exit signal of Kir2.1 (2) was cloned into the EcoRI and Xbal sites of pACU (2) by ligating with annealed oligos. The ATP8A coding region that is shared by all isoforms was amplified by PCR from cDNA clone GH28327 (AA26 to AA1091, BDGP) and inserted into an NheI site before the FLAG tag.

gRNA expression vectors: For Nmnat, Sarm, and axed, gRNAs were cloned into pAC-U63-tgRNA-Rev as described (3). The first trNA spacer in each final construct is trNA Gly but the remaining trNA spacers are trNA Gin (4). gRNA-peb and gRNA-drpr was constructed in pAC-U63-QtgRNA2.1-BR (4). The gRNA target sequences are listed in Table S2.

The above constructs were injected by Rainbow Transgenic Flies to transform flies through φC31 integrase-mediated integration into attP docker sites.

CRISPR-TRiM

The efficiency of transgenic gRNA lines was validated by the Cas9-LEThAL assay (3). Homozygous males of each gRNA line were crossed to Act-Cas9 w lig4 homozygous females. gRNA-Nmnat crosses caused lethality of all progeny at the 2nd instar larval stage; gRNA-Sarm crosses yielded viable female
progeny and male lethality between 3rd instar larvae to prepupae; *gRNA-axed* crosses caused lethality at the embryonic stage for all progeny; *gRNA-peb* crosses caused lethality from embryonic stage to the 2nd instar for all progeny; *gRNA-drpr* crosses resulted in lethality in late pupae. These results suggest that all gRNAs are efficient.

C4da-specific gene knockout was carried out using *ppk-Cas9* (3). Tissue-specific knockout in da neuron precursor cells were carried out with *SOP-Cas9* (3). Epidermis-specific knockout of *drpr* was induced by *shot-Cas9* (this study). Gene knockout in the precursor cells of Or22a ORNs was carried out using *ey-Cas9* (this study).

**Live imaging**

Animals were reared at 25°C in density-controlled vials (60-100 embryos/vial) on standard yeast-glucose medium (doi:10.1101/pdb.rec10907). Larvae at 125 hours AEL (wandering stage) or stages specified were mounted in 100% glycerol under coverslips with vacuum grease spacer and imaged using a Leica SP8 microscope equipped with a 40X NA1.30 oil objective. Larvae were lightly anesthetized with isoflurane before mounting. For consistency, we imaged dorsal ddaC neurons from A1-A3 segments (2-3 neurons per animal) on one side of the larvae. Unless stated otherwise, confocal images shown in all figures are maximum intensity projections of z stacks encompassing the epidermal layer and the sensory neurons beneath, which are typically 8–10 μm for 3rd instar larvae.

**Injury assay**

Injury assay at the larval stage was done as described previously (5). Briefly, larvae at 90 hr AEL were lightly anesthetized with isoflurane, mounted in a small amount of halocarbon oil under coverslips with grease spacers. The laser ablation was performed on a Zeiss LSM880 Confocal/Multiphoton Upright Microscope, using a 790 nm two-photon laser at primary dendrites of ddaC neurons in A1 and A3 segments. Animals were recovered on grape juice agar plates following lesion for appropriate times before imaging.

ORN axon injury assay was performed on 7-day-old male flies by removing the outer segments of both antennae as described in (6). The injured males were recovered for 7 days by transferring to fresh yeast-glucose medium every day. To examine Or22a axon degeneration, brains were dissected in PBST (0.2% Triton-X in PBS), fixed in 4% formaldehyde in PBS for 20 min, and then rinsed with PBST three times, 20 minutes each. Then the brains were mounted in SlowFade® Diamond Antifade Mountant (Thermo Fisher Scientific) and imaged using a Leica SP8 microscope with a 40x NA1.3 oil objective.

**Long-term time-lapse imaging**

Long-term time-lapse imaging at the larval stage was done as described previously (5, 7). Briefly, a layer of double-sided tape was placed on the coverslip to define the position of PDMS blocks. A small amount of UV glue was added to the groove of PDMS and to the coverslip. Anesthetized larvae were placed on top of the UV glue on the coverslip and then covered by PDMS blocks with the groove side contacting the larva. Glue was then cured by 365nm UV light. The coverslip with attached PDMS and larvae was mounted on an aluminum slide chamber that contained a piece of moisturized Kimwipes (Kimtech Science) paper. Time-lapse imaging was performed on a Leica SP8 confocal equipped with a 40x NA1.3 oil objective and a resonant scanner at digital zoom 0.75 and a 3-min or 2-sec interval. For imaging after ablation, larvae were pre-mounted in the imaging chamber and subjected to laser injury. The larvae were then imaged 0.5-1 hours after ablation. For calcium imaging before and immediately
after ablation, images were captured on a Zeiss LSM880 Confocal/Multiphoton Upright Microscope on which the ablation was performed.

**Image analysis and quantification**

Image processing and analyses were done in Fiji/ImageJ. Methods for tracing and measuring C4da neuron dendrite length have been previously described (1). Briefly, the images were segmented by Auto Local Threshold and reduced to single pixel skeletons before measurement of skeleton length by pixel distance. The dendrite debris measurement has been described previously (5). Briefly, a dendrite mask was first generated from projected images by Auto Local Threshold in order to create a region of interest (ROI) by dilation to map areas within one-epidermal-cell diameter (40 μm) from dendrites. Dendrite debris within the ROI was converted to binary masks based on fixed thresholds. Different thresholds were used for ppk-C4-tdTom and ppk-Gal4 UAS-CD-tdTom as they have different brightness. The dendrite pixel area (ADen), debris pixel area (ADeb), and ROI area (AROI) were measured and dendrite coverage ratio was calculated based on the following formula: 100·Adeb·AROI/(AROI·ADen)·ADen. For measuring Lact-GFP, two regions at empty epidermal regions were measured as background levels. TdTom signals on dendrites were used to generate dendrite masks for measurement of GFP within the masks. For kymographs, we used a custom macro based on the Straighten function to extract a strip of pixels centered at the selected dendrite branch. The maximum intensity pixel in the strip at each distance was used to generate a single-pixel line for each time frame. The final kymographs were displayed using the Fire lookup table (LUT).

**Statistical Analysis**

R was used to conduct statistical analyses and generate graphs. (*p < 0.05, **p < 0.01, and ***p < 0.001). Statistical significance was set at p < 0.05. Data acquisition and quantification were performed non-blinded. Acquisition was performed in ImageJ (batch processing for debris coverage ratio and fragmentation ratio, manually by hand for GFP-Lact binding) and Microsoft Excel. Statistical analyses were performed using R. We used the following R packages: car, stats, multcomp for statistical analysis and ggplot2 for generating graphs. Some graphs were made in Excel using its native plotting functions. For the statistical analysis we ran the following tests, ANOVA (followed by Tukey’s HSD) when dependent variable was normally distributed and there was approximately equal variance across groups. When dependent variable was not normally distributed and variance was not equal across groups, we used Kruskal-Wallis (followed by Dunn’s test, p-values adjusted with Benjamini-Hochberg method) to test the null hypothesis that assumes that the samples (groups) are from identical populations. To check whether the data fit a normal distribution, we generated qqPlots to analyze whether the residuals of the linear regression model is normally distributed. We used the Levene’s test to check for equal variance within groups. The state of neuronal degeneration or fragmentation was compared using the Freeman-Halton extension of Fisher’s exact test.

**Replication**

For all larval and adult imaging experiments, at least 3 biological replications were performed for each genotype and/or condition.
SI Figures

Figure S1: Injured dendrites expose PS
(A) GFP-Lact labeling on injured dendrites (yellow arrowheads) of a wildtype control C4da neuron at 5 hours AI.
Scale bar represents 20 μm. Blue asterisks indicate the injury site.
Figure S2: TMEM16F OE and CDC50 KO causes ectopic PS exposure on injured dendrites of Wld^s OE neurons

(A) GFP-Lact labeling on injured dendrites (yellow arrowheads) of a Wld^s OE + TMEM16F OE neuron at 7 hours AI.

(B) GFP-Lact labeling on injured dendrites (yellow arrowheads) of a Wld^s OE + CDC50 KO neuron at 6 hours AI.

Scale bars represent 10 μm (A and B). Blue asterisks indicate injury sites (A and B).
Figure S3: *drpr* KO in epidermal cells blocks engulfment of multiple classes of da neurons after injury

(A) Injured dendrites of a C1da (ddaE) neuron (cyan arrows) and a C4da (ddaC) neuron (yellow arrowhead) were not engulfed at 24 hours AI in epidermal KO of *drpr*. Dendrites of all da neurons were labeled by *RabX4-Gal4 UAS-MApHS*. Epidermis-specific *drpr* KO was induced by *shot-Cas9 gRNA-drpr*. The MApHS marker (8) consists of an extracellular pHluorin, a CD4 transmembrane domain, and an intracellular tdTom. Quenching of the pHluorin signal indicates engulfment, while the presence of both pHluorin and tdTom signals indicates the lack of engulfment. All injured dendrites fragmented but maintained pHluorin signals, indicating that injured dendrites were not engulfed.

The scale bar represents 25 μm. Blue asterisks indicate the injury sites. Yellow dash lines encircle regions covered by injured dendrites.
Figure S4: Axed is not involved in PS-mediated phagocytosis

(A-F) Axons of control NI (A), control 7 days AI (B), axed KO NI (C), axed KO 7 days AI (D), peb KO NI (E), and peb KO 7 days AI (F) Or22a neurons in 14-day-old adult brains. Or22a neurons were labeled by Or22a>CD8-GFP and KO was induced by ey-Cas9. Scale bars, 25 μm.

(G) Quantification of GFP brightness of Or22a glomeruli. n = number of brains: control NI 14 days (n = 19); control 7 days AI 14 days (n = 15); axed KO NI 14 days (n = 16); axed KO 7 days AI 14 days (n = 14); peb KO NI 14 days (n = 18); peb KO 7 days AI 14 days (n = 20). Kruskal-Wallis (One-way ANOVA on ranks) and Dunn’s test, p-values adjusted with the Benjamini-Hochberg method. *p ≤ 0.05, ***p < 0.001, n.s., not significant. The significance level above each genotype is for comparison with the control. Statistical comparisons for peb KO glomeruli were not performed due to the variation within the group of peb KO NI 14 days. Black bar, mean; red bar, SD.

(H-J) Injured axons of wildtype 24 hrs AI (H), axed KO AI 24 hrs AI (I), and axed KD AI 24 hrs AI (J). Neurons were labeled by ppk-MApHS. Neuronal-specific KO was induced by SOP-Cas9, and neuronal-specific KD was driven by 21-7 Gal4. Scale bars, 25 μm.

(K) Quantification of axon fragmentation showing percentages of neurons undergoing no fragmentation, and complete fragmentation of injured axons at 24 hrs AI. n = number of neurons: wildtype 24 hrs AI (n = 20, 12 animals); axed KO 24 hrs AI (n = 17, 9 animals); axed KD 24 hrs AI (n = 20, 8 animals). Freeman-Halton extension of Fisher’s exact test. For all quantifications, ***p ≤ 0.001; n.s., not significant.

(L-O) Partial dendritic fields of Nmnat KO + axed KO (L and N) and Nmnat KO + Sarm KO (M and O) ddaC C4da neurons. Neurons were labeled by ppk>CD4-tdTom (L-O). The C4da-specific KO was induced by ppk-Cas9 (L-M) or by SOP-Cas9 (N-O). Scale bars, 25 μm.

(P) Quantification of dendrite length. n = number of neurons: Nmnat KO (ppk-Cas9) (n = 19, 10 animals); Nmnat KO + axed KO (ppk-Cas9) (n = 17, 9 animals); Nmnat KO + Sarm KO (ppk-Cas9) (n = 16, 8 animals); Nmnat KO + axed KO (SOP-Cas9) (n = 14, 7 animals); Nmnat KO + Sarm KO (SOP-Cas9) (n = 15, 8 animals). One-way ANOVA and Tukey’s test.

(Q) Quantification of debris coverage ratio, which is the percentage of debris area normalized by dendrite area ratio. Number of neurons: same as in (P). Kruskal-Wallis (One-way ANOVA on ranks) and Dunn’s test, p-values adjusted with the Benjamini-Hochberg method.

For all quantifications, **p ≤ 0.01; ***p ≤ 0.001; n.s., not significant. The significance level above each genotype is for comparison with the control. Black bar, mean; red bar, SD.

(R-S) Partial dendritic fields of peb KO NI (R) and peb KO 24 hrs AI (S) ddaC neurons. Yellow dots outline regions covered by injured dendrites in (S). Neurons were labeled by ppk>CD4-tdTom and neuronal-specific KO was induced by SOP-Cas9. Scale bars, 25 μm.

(T) Quantification of dendrite fragmentation showing percentages of neurons undergoing complete fragmentation of injured dendrites at 24 hrs AI. n = number of neurons: wildtype 24 hrs AI (n = 21, 12 animals); peb KO 24 hrs AI (n = 12, 7 animals). Freeman-Halton extension of Fisher’s exact test. For all quantifications, n.s., not significant.
Figure S5: Membrane rupture assay on uninjured dendrites.

(A) Uninjured dendrites of a wildtype neuron that was laser-severed at another dendrite branch (injury site is outside the frame). The dendrites lacked reconstituted GFP signal. Scale bar, 10 μm.
Figure S6: WldS overexpression and loss of axed changed calcium flashes in injured dendrites. (A-C) Representative plots of GCaMP6s brightness in injured dendrites of wildtype (A), WldS-overexpressing (B), and axed KO (C) neurons over time with 3-min interval. (D-F) Representative plots of GCaMP6s brightness in uninjured dendrites of wildtype (A), WldS-overexpressing (B), and axed KO (C) neurons over time with 3-min interval.
Figure S7. Potential roles of NAD$^+$ reduction in eliciting distinct responses in dendrites. See Discussion for details.
**SI Tables**

**Table S1. Key Resource Table.**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER | ADDITIONAL INFORMATION |
|---------------------|--------|------------|------------------------|
| **Experimental Models: Organisms/Strains** | | | |
| ppk-Gal4 | (9) | ppk-Gal4VK00037 | |
| UAS-CD4-tdTom | (2) | RRID:BDSC_35841 | UAS-CD4-tdTom^{SM1} |
| ppk-CD4-tdTom | (2) | ppk-GFP(11)-CD4-tdTom | |
| ppk-Cas9 | (3) | ppk-Cas9^{7D} | |
| gRNA-Nmnat | this study | gRNA-NmnatVK00027 | |
| ppk-LexA | (1) | ppk-LexA.GAD | |
| LexAop-Wld^{S} | this study | LexAop-WldS{VK00027} | |
| UAS-Wld^{S-dead} | (10) | | |
| Sarm^{*} | (11) | Sarm^{705} | |
| Sarm^{*} | (11) | Sarm^{621} | |
| UAS-wnd | Bloomington Drosophila Stock Center | RRID:BDSC_51642 | UAS-wnd.C^{2} |
| UAS-Cas9 | Bloomington Drosophila Stock Center | RRID:BDSC_58986 | UAS-Cas9.P2^{attP2} |
| drpr^{*} | (5) | drpr^{indel3} | |
| UAS-JNK-RNAi | Bloomington Drosophila Stock Center | RRID:BDSC_57035 | TRiP.HMS04479^{attP40} |
| Dcg-Gal4 | (5) | | |
| UAS-GFP-Lact | (5) | UAS-GFP-LactC1C2VK00018 | |
| ppk-MApHS | (8) | ppk-MApHS^{1} | |
| SOP-Cas9 | (3) | SOP-Cas9^{3A} | |
| gRNA-Sarm | this study | gRNA-SarmVK00027 | |
| R16A03-LexA | (5) | R16A03-LexAp65VK00027 | |
| LexAop-GFP-LactC1C2 | (5) | LexAop2-GFP-LactC1C2^{VK00033} | |
| Or22a-Gal4 | (6) | | |
| UAS-mCD8-GFP | (6) | | |
| ey-Cas9 | this study | ey-Cas9^{VK00005} | |
| gRNA-axed | this study | gRNA-axed^{VK00027} | |
| gRNA-peb | this study | gRNA-peb(BR)^{VK00027} | |
| gRNA-drpr | this study | gRNA-drpr(BR)^{VK00027} | |
| Gene/Vector | Institution | RRID | Notes |
|------------|-------------|------|-------|
| R38F11-Gal4 | Bloomington Drosophila Stock Center | RRID:BDSC_50014 | R38F11-Gal4\textsuperscript{attP2} |
| shot-Cas9 | this study | | |
| RabX4-Gal4 | Bloomington Drosophila Stock Center | RRID:BDSC_51602 | \textit{RabX4-Gal4.\textsuperscript{ORF(loxP)VK00033, 3xP3-RFP was removed}} |
| UAS-MApHS | (8) | | \textit{UAS-MApHSVK00019} |
| UAS-TMEM16F | (5) | | \textit{UAS-TMEM16F(D430G)VK00016} |
| UAS-Wld\textsuperscript{S} | (6) | | |
| gRNA-CDC50 | (5) | | gRNA-CDC50\textsuperscript{mP2} |
| Df(drpr) | Bloomington Drosophila Stock Center | RRID:BDSC_9693 | \textit{Df(3L)BSC181} |
| UAS-Sarm\textsuperscript{GOF} | (12) | | \textit{UAS-Sarm\textsuperscript{ΔArm}} |
| UAS-JNK\textsuperscript{DN} | Bloomington Drosophila Stock Center | RRID:BDSC_6409 | \textit{UAS-bsk.DN}\textsuperscript{2} |
| \textit{LexAop-myrmtdTom-GFP(1-10)} | this study | | \textit{LexAop2-myrttdTom-GFP(1-10)VK00005} |
| UAS-sGFP(11)\textit{x7} | this study | | \textit{UAS-sGFP(11)\textit{x7VK00027}} |
| \textit{LexAop-myrgCaMP6s} | (13) | | 13x\textit{LexAop2-myrmGCaMP6s} |
| \textit{Gal4}\textsuperscript{21-7} | (14) | | |
| \textit{UAS-AnnexinV-mCard} | (5) | | \textit{UAS-AnnexinV-mCardVK00037} |

**Recombinant DNA**

| Vector | Institution | RRID | Notes |
|--------|-------------|------|-------|
| pJFRC176-10XUAS-rox-dSTOP-rox-myrgFP | Addgene | RRID:Addgene_32147 | |
| pAC-U63-tgRNA-Rev | (3) | RRID:Addgene_112811 | |
| pAC-U63-QtgRNA2.1-BR | (4) | RRID:Addgene_170513 | |
| pHEU-sfGFP-LactC1C2 | (5) | | |
| pAPLO-CD4-tdTom | (1) | | |
| pDEST-APIC-Cas9 | (3) | RRID:Addgene_121657 | |
| pENTR11 | Thermo Fisher Scientific | | #A10467 |

**Software and Algorithms**

| Software | Website | RRID | Notes |
|----------|---------|------|-------|
| Fiji | https://fiji.sc/ | SCR 002285 | |
| Software                  | Manufacturer                | RRID: SCR_001905 |
|--------------------------|-----------------------------|------------------|
| Adobe Photoshop          | Adobe                       | RRID: SCR_014199 |
| Adobe Illustrator        | Adobe                       | RRID: SCR_010279 |

**Other**

| Other Products                  | Manufacturer                        | Code |
|---------------------------------|--------------------------------------|------|
| Gateway™ LR Clonase™ II Enzyme mix | Thermo Fisher Scientific,           | #11791020 |
| NEBuilder® HiFi DNA Assembly Master Mix | New England Biolabs Inc. | #E2621 |
| Gene | Target sequence 1 | Target sequence 2 |
|------|------------------|------------------|
| Sarm | GCATCTGTCAACACTCCG | CGATTCAATATCAGCCCAG |
| Nmnat | GGAACCCACAGAGTGATGGAGG | TAAGAGCCGCCGAATCAACG |
| axed | GTCGGTACTCGAGCGAGCG | TGGACCCGATGGCCATCACG |
| peb | ATTTCTTGACTGCTCGGTG | ACGCATGTGACGCAACCAGGG |
| drpr | CCATGCCGTAGAATCCAGGT | ACGGACAAAGGATGCGCCAG |

Table S2. gRNA target sequences.
SI Video Legends

Video S1 (separate file). GFP-Lact labeling of degenerating Nmnat KO dendrites, related to Figure 2.

Time-lapse movie of GFP-Lact labeling on distal dendrites of a Nmnat KO C4da neuron before and during degeneration. Imaging started around 96 hours AEL. Dcg-Gal4 drives GFP-Lact expression in both fat bodies and hemocytes (mobile cells in the GFP-Lact channel). Timestamp is relative to the first frame, with a 3-min interval between each frame.

Video S2 (separate file). Dendrite dynamics of Nmnat KO neurons in drpr mutant larvae, related to Figure 2.

Time-lapse movie of dendrites of a Nmnat KO neuron exhibiting dynamic extension and retraction behaviors in a drpr mutant larva. Imaging started around 120 hours AEL. Timestamp is relative to the first frame, with a 3-min interval between each frame.

Video S3 (separate file). Cytoplasmic calcium dynamics in uninjured wildtype dendrites, related to Figure 8.

Time-lapse movie of an uninjured wildtype C4da neuron showing low baseline GCaMP6s signals and occasional local rises in dendrites. Timestamp is relative to the first frame, with a 3-min interval between each frame.

Video S4 (separate file). Cytoplasmic calcium dynamics in dendrites of Nmnat KO neurons, related to Figure 8.

Time-lapse movie of a Nmnat KO C4da neuron showing calcium dynamics in uninjured dendrites. Timestamp is relative to the first frame, with a 3-min interval between each frame.

Video S5 (separate file). Calcium dynamics in injured wildtype dendrites, related to Figure 8.

Time-lapse movie of laser-injured C4da dendrites from 1 to 11 hrs AI showing calcium dynamics in both severed dendrites and those attached to the soma. Timestamp is relative to the first frame, with a 3-min interval between each frame.

Video S6 (separate file). AV-mCard labeling and calcium dynamics of injured dendrites, related to Figure 8.

Time-lapse movie of laser-injured C4da dendrites from 1 to 6 hrs AI showing labeling of injured dendrites by the PS sensor AV-mCard and GCaMP6s signals. Timestamp is relative to the first frame, with a 3-min interval between each frame.

Video S7 (separate file). Calcium dynamics in injured WldS OE dendrites, related to Figure 8.

Time-lapse movie of laser-injured WldS OE C4da dendrites from 1 to 13 hours AI showing GCaMP6s signals in severed dendrites. Timestamp is relative to the first frame, with a 3-min interval between each frame.

Video S8 (separate file). Calcium dynamics in injured wildtype dendrites with a higher temporal resolution, related to Figure 8.

High temporal-resolution time-lapse movie of laser-injured wildtype C4da dendrites around 1 hr AI showing calcium dynamics. Timestamp is relative to the first frame, with a 2-sec interval between each frame.
Video S9 (separate file). Calcium dynamics in injured Wld\textsuperscript{S} OE dendrites at a higher temporal resolution, related to Figure 8.

High temporal-resolution time-lapse movie of laser-injured Wld\textsuperscript{S} OE C4da dendrites around 2 hrs AI showing calcium dynamics. Timestamp is relative to the first frame, with a 2-sec interval between each frame.

Video S10 (separate file). Irregular and infrequent cytoplasmic calcium flashes in injured Wld\textsuperscript{S} OE dendrites at a higher temporal resolution, related to Figure 8.

High temporal-resolution time-lapse movie of laser-injured Wld\textsuperscript{S} OE C4da dendrites around 2 hrs AI showing irregular and infrequent GCaMP6s signals in severed dendrites. Timestamp is relative to the first frame, with a 2-sec interval between each frame.

Video S11 (separate file). Calcium dynamics in injured \textit{axed} KO dendrites, related to Figure 8.

Time-lapse movie of laser-injured \textit{axed} KO C4da dendrites from 0.5 to 5.5 hours AI showing GCaMP6s signals in severed dendrites. Timestamp is relative to the first frame, with a 3-min interval between each frame.

Video S12 (separate file). Calcium dynamics in injured \textit{axed} KO dendrites at a higher temporal resolution, related to Figure 8.

High temporal-resolution time-lapse movie of laser-injured \textit{axed} KO C4da dendrites around 10 min AI showing calcium dynamics. Timestamp is relative to the first frame, with a 2-sec interval between each frame.

SI References

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