**SUPPLEMENTAL INFORMATION (Ko et al.)**

**Age-dependent autophagy induction after injury promotes axon regeneration by limiting NOTCH**

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**Supplemental Figure Legends**

**Figure S1.** PLM morphology at day 1 in the mutants of autophagy-related genes.(**A**) A simplified representation of the autophagy pathway. *C. elegans* orthologous genes are listed in rounded brackets for selected molecules in the pathway. (**B**) Autophagy gene mutants were crossed to *zdIs5* (P*mec-4::GFP*) or *muIs32* (P*mec-7::GFP*) reporter. Images were taken at day 1 adulthood using 60x objectives, multiple images of the same animals were then stitched to generate one image showing the morphology of entire PLM neurons.Except for *unc-51* mutants, mutants of all other autophagy genes tested displayed normal PLM morphology. Illustrative diagrams for PLM neurons in wildtype and *unc-51* mutant animals were provided to show the abnormal morphology in *unc-51* mutants. Scale bar: 50 µm.

**Figure S2.** Axon injury activates autophagy in DD motor neurons.(**A**) Representative images of DD2 neurons at day 1 in transgenic animals expressing P*unc-25*-mCherry::GFP::LGG-1. Laser axotomy was performed to cut the axons at the midline position of the commissures. Axotomy was performed on day 1 of adulthood. Images were taken 24 h post-axotomy (injured). For the uninjured controls, animals underwent sham injury at day 1 neurons were imaged 24 h later. Scale bar: 5 µm. (**B**) Quantification of APs and ALs in injured and uninjured DD2 neurons. Statistics: One-way ANOVA; mean ± SEM; *\*\*p*<0.01;

**Figure S3.** Sequence alignment of the Bec sequence of Beclin 1 proteins from various species.Tat-ceBec and Tat-scr control peptides were designed based on the sequence alignment.

**Figure S4.** Relative DLK-1 expression level in DLK-1 overexpression line.Representative images and relative expression level of DLK-1 overexpression line (*Pmec-4-GFP::dlk-1*) and single-copy transgenic line (*Pdlk-1-GFP::dlk-1*). P*mec-4-mKate2* was injected into the single-copy MosSCI line to label touch neurons. Scale bar: 10 µm.

**Figure S5.** Blocking intracellular Ca2+ release impairs injury-induced autophagy activation.(**A**) Representative images of APs and ALs in PLM cell bodies in day 1 animals expressing P*mec-4*-mCherry::GFP::LGG-1 reporter and high-affinity (“super sponge” or the low-affinity “control sponge”) ITR-1 IP3-binding domain mutant forms in touch neurons. Blocking intracellular Ca2+ release by “super sponge” was sufficient to abolish autophagy induction in response to axon injury. The defect in autophagy induction in the presence of “super sponge” could be rescued by rapamycin treatment.(**B**) Quantification of APs and ALs in PLM cell bodies in day 1 animals expressing autophagy reporter and “super sponge” (or “control sponge”) with indicated treatments. Scale bar: 5 µm.Statistics: One-way ANOVA; mean ± SEM; *\*\*\*p*<0.001. ns, Not significant.

**Figure S6.** Co-localization between LIN-12 and autophagic vesicles is specific.(**A**) Representative images of intact and injured PLM neurons from day 1 animals co-expressing mCherry::LGG-1 and GFP-tagged inhibitors of axon regrowth (EFA-6, ARF-6 or HDA-3) in touch neurons. None of the three proteins showed co-localization with mCherry::LGG-1 puncta. Axotomy was performed on day 1 of adulthood. Images were taken 24 h post axotomy (injured). For the uninjured controls, animals underwent sham injury at Day 1 and PLM neurons were imaged 24 h later.(**B**) Representative images of Day 1 PLM neurons co-expressing mCherry::LGG-1G116A and GFP::LIN-12 with indicated treatments. For rapamycin treatment, L4 and day 9 animals were treated for 24 h prior laser axotomy. After axotomy, animals were cultured for 24 h in the presence of rapamycin before imaging. For BA1 treatment, BA1 or 0.2% DMSO was injected to day 1 and day 10 animals 1 h prior laser axotomy, and animals were recovered for 24 h before imaging. For the uninjured controls, animals underwent sham injury and imaged 24 h later.(**C**) Quantification of mCherry::LGG-1G116A puncta and GFP::LGG-1 puncta. Mutant LGG-1 formed puncta, but the number of puncta was not affected by axotomy or autophagy drugs. Scale bar: 5 µm. Statistics: One-way ANOVA; mean ± SEM; *\*\*\*p*<0.001. ns, Not significant.

**Figure S7.** Relative LIN-12 expression level in LIN-12 overexpression line. Representative images and relative expression level of LIN-12 overexpression line (*Pmec-4-GFP::LIN-12*) and single-copy transgenic line (*Plin-12-GFP::LIN-12*). *Pmec-4-mKate2* was injected into the single-copy MosSCI line to label touch neurons. Scale bar: 10 µm.

**Supplemental Experimental Procedures**

***Transgene construction.***

To construct the P*mec-4::mCherry::gfp::lgg-1* vector, *mCherry::gfp::lgg-1* sequence was amplified by PCR from the lysate of MAH215 (Chang et al., *eLife,* 2017) and PCR product was inserted into TOPO TA vector (ThermoFisher, K250020). Next, *mCherry::gfp::lgg-1* entry vector was recombined with the P*mec-4*-SI-GW using Gateway LR Clonase II Enzyme mix (ThermoFisher, 11791020). The P*mec-4::mCherry::gfp::lgg-1G116A* mutation was generated by mutagenesis. We used the mutagenesis method. Point mutant was generated by PCR using *mCherry::gfp::lgg-1* entry vector and recombined with the P*mec-4* destination vector. An expression plasmid for touch neuron-specific P*mec-4-gfp-lin-12* was generated by gateway cloning technology using P*mec-4-gfp* destination vector and *lin-12* entry vector. The WT lin-12 cDNA was amplified by PCR from mRNA of N2, and the fragment was cloned with TOPO TA vector. We made single copy insertions using MosSCI (<http://www.wormbuilder.org/>), on chromosomes V (EG8083).

**Table S1.** Plasmids used in the study.

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| **pCHN#** | **Name** | **Description** |
| pCHN14 | P*mec-4*-SI-GW | P*mec-4* destination vector |
| pCHN55 | *lgg-1*-pCR8 | PCR from cDNA and cloned into PCR8 |
| pCHN74 | P*mec-4*-*gfp*-gGW | P*mec-4* destination vector |
| pCHN102 | *mCherry-gfp-lgg-1*-PCR8 | PCR form genomic DNA (MAH215) and cloned into pCR8 |
| pCHN103 | P*mec-4-mCherry-gfp-lgg-1* | LR using pCHN14 and pCHN102 |
| pCHN104 | P*unc-25*-GW | P*unc-25* destination vector |
| pCHN105 | P*unc-25-mCherry-gfp-lgg-1* | LR using pCHN102 and pCHN104 |
| pCHN109 | *mCherry-gfp-lgg-1G116A-*pCR8 | mutagenesis using pCHN102 |
| pCHN110 | P*mec-4-mCherry-gfp-lgg-1G116A* | LR using pCHN14 and pCHN109 |
| pCHN113 | *lin-12*-pCR8 | PCR from mRNA and cloned into pCR8 |
| pCHN114 | P*mec-4-mCherry-lgg-1* | LR using pCHN55 and P*mec-4*-*mCherry*-GW |
| pCHN115 | P*mec-4-gfp-lin-12* | LR using pCHN113 and pCHN74 |
| pLC594 | *dlk-1*-pCR8 | PCR from mRNA and cloned into pCR8 |
| pCHN164 | P*mec-4-dlk-1* | LR using pLC594 and pCHN14 |
| pCHN189 | *lgg-1G116A*-pCR8 | PCR from pCHN110 and cloned into pCR8 |
| pCHN190 | P*mec-4-mCherry-lgg-1G116A* | LR using pCHN189 and P*mec-4-mCherry*-GW |
| pCHN204 | P*lin-12*-pCR8 | PCR from genomic DNA (N2) and cloned into pCR8 |
| pCHN207 | Pdlk-1-pCR8 | PCR from genomic DNA (N2) and cloned into pCR8 |
| pCHN208 | P*dlk-1-dlk-1-gfp-unc54*UTR-pCR8 | Gibson assembly (3 fragments: *dlk-1* cDNA from pCHN164, *gfp*-UTR from P*col-19-ebp-2-gfp-unc-*54UTR Mossci, P*dlk-1*-pCR8 from pCHN207) |
| pCHN209 | P*lin-12-lin-12-gfp-unc-54*UTR-pCR8 | Gibson assembly (3 fragments: *lin-12* from P*mec-4-lin-12*, *gfp-*UTR from pCHN58, Plin-12-pCR8 from pCHN204) |
| pCHN210 | P*dlk-1-dlk-1-gfp-unc54*UTR-Mossci | LR using pCHN208 and pCZGY1030 |
| pCHN211 | P*lin-12-lin-12-gfp-unc54*UTR-Mossci | LR using pCHN209 and pCZGY1030 |
| pCHN213 | *mKate2*-pCR8 | PCR from P*mec-4-mKate2-lgg-1* and cloned into pCR8 |
| pCHN214 | P*mec-4-mKate2* | LR using pCHN14 and pCHN213 |

**Table S2.** Strains used in the study.

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| **Strain #** | **Genotype** |
| CHN121 | *Pmec-4-mCherry-gfp-lgg-1(lizEx84)* |
| CHN122 | *Punc-25-mCherry-gfp-lgg-1(lizEx85)* |
| CHN123 | *Pmec-7-GFP(muIs32)II; atg-9(bp564)V* |
| CHN124 | *Pmec-7-GFP(muIs32)II; bec-1(ok691)IV/nT1(qIs51)* |
| CHN126 | *Pmec-7-GFP(muIs32)II; lgg-2(tm6544)IV/nT1* |
| CHN127 | *Pmec-4-GFP(zdIs5)I; lgg-1(tm3489)II/mIn1* |
| CHN128 | *Pmec-7-GFP(muIs32)II; lgg-2(tm5755)IV* |
| CHN129 | *Pmec-4-GFP(zdIs5)I; klf-3(ok1975)II* |
| CHN130 | *unc-119(ed3)III; Pmec-4-mCherry-gfp-lgg-1(lizSi5)I* |
| CHN133 | *Pmec-4-mCherry-gfp-lgg-1(G116A)(lizEx87)* |
| CHN134 | *Pmec-7-GFP(muIs32)II; bec-1(ok700)IV/nT1* |
| CHN135 | *Pmec-4-GFP(zdIs5)I; klf-3(gk612)II/mIn1* |
| CHN136 | *dlk-1(tm4024)I; Pmec-4-mCherry-gfp-lgg-1(lizEx85)* |
| CZ15956 | *Pmec-7-GFP(muIs32)II; dlk-1(tm4024)I* |
| CHN137 | *Pmec-4-mCherry-gfp-lgg-1(lizIs1)* |
| CHN156 | *Pmec-7-GFP(muIs32)II; Pmec-4-gfp-lin-12 (lizEx92)* |
| CHN157 | *Pmec-4-GFP(zdIs5)I; lgg-1(tm3489)II/mIn1; Pmec-4-gfp-lgg-1 (lizEx93)* |
| CHN158 | *Pmec-4-Control Sponge(low-affinity IP3); Pmec-4-mCherry-gfp-lgg-1 (lizEx94)* |
| CHN159 | *Pmec-4-Super Sponge(high-affinity IP3); Pmec-4-mCherry-gfp-lgg-1 (lizEx95)* |
| CHN160 | *Pmec-4-gfp-lin-12 (lizEx96)* |
| CHN161 | *Pmec-4-gfp-lin-12; Pmec-4-mcherry-lgg-1 (lizEx97)* |
| CHN289 | *mkk-4(ju91)X; Pmec-4-mcherry-gfp-lgg-1(lizEx195)* |
| CHN291 | *pmk-3(ok169)IV; Pmec-4-mcherry-gfp-lgg-1(lizEx195)* |
| CHN292 | *mak-2(gk1110)IV; Pmec-4-mcherry-gfp-lgg-1(lizEx195)* |
| CHN293 | *cebp-1(tm2807)X; Pmec-4-mcherry-gfp-lgg-1(lizEx195)* |
| CHN294 | *adEx1750; Pmec-4-mcherry-gfp-lgg-1(lizEx195)* |
| CHN295 | *unc-51(e369)V; Pmec-4-mcherry-gfp-lgg-1(lizEx195)* |
| CHN296 | *atg-9(bp564)V; Pmec-4-mcherry-gfp-lgg-1(lizEx195)* |
| CHN297 | *mkk-4(ju91)X; Pmec-4-mcherry-gfp-lgg-1 + Pmec-4-dlk-1 (lizEx196)* |
| CHN299 | *pmk-3(ok169)IV; Pmec-4-mcherry-gfp-lgg-1 + Pmec-4-dlk-1 (lizEx196)* |
| CHN300 | *mak-2(gk1110)IV; Pmec-4-mcherry-gfp-lgg-1 + Pmec-4-dlk-1 (lizEx196)* |
| CHN301 | *cebp-1(tm2807)X; Pmec-4-mcherry-gfp-lgg-1 + Pmec-4-dlk-1 (lizEx196)* |
| CHN302 | *unc-51(e369)V; Pmec-4-mcherry-gfp-lgg-1 + Pmec-4-dlk-1 (lizEx196)* |
| CHN303 | *atg-9(bp564)V; Pmec-4-mcherry-gfp-lgg-1 + Pmec-4-dlk-1 (lizEx196)* |
| CHN304 | *Pdlk-1-dlk-1-gfp (lizSi14)* |
| CHN306 | *Plin-12-lin-12-gfp (lizSi16)* |
| CHN308 | *Pmec-4-gfp-dlk-1 (lizEx197)* |
| CHN309 | *Pdlk-1-dlk-1-gfp (lizSi14); Pmec-4-mKate2 (lizEx198)* |
| CHN310 | *Plin-12-lin-12-gfp (lizSi16); Pmec-4-mKate2 (lizEx198)* |