Requirement for endocytosis and intracellular trafficking in *C. elegans* necrotic neurodegeneration

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Supplementary Figures and Legends

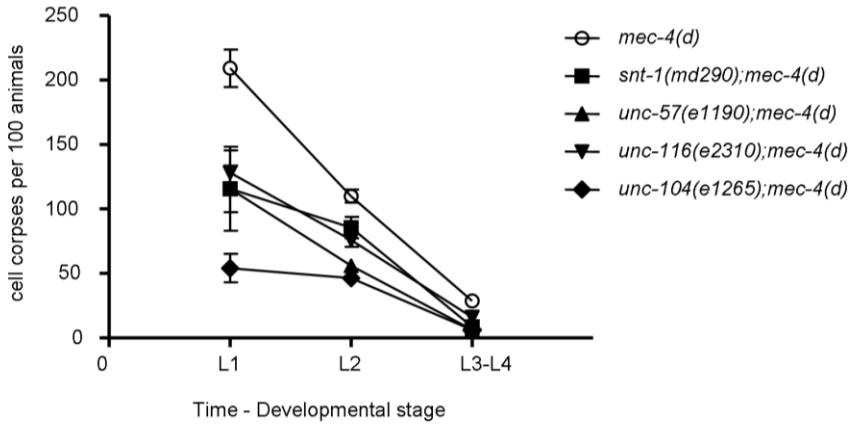

Figure S1 Time-course analysis of *mec-4(d)*-induced degeneration in *mec-4(d)* animals and in double mutants combining mutations in *mec-4* and the endocytic genes *snt-1* and *unc-57* or the kinesin genes *unc-116* and *unc-104*. The number of touch receptor neuron corpses at the indicated developmental stage, per 100 *mec-4(d)* animals is shown. Error bars denote S.E.M. values ($n>250$; $P<0.001$, compared to control animals, unpaired t-test).

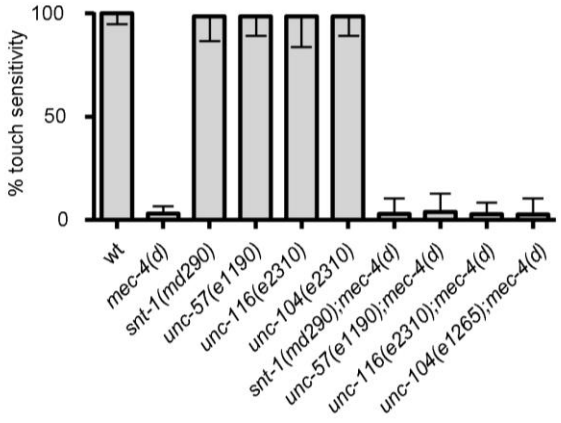

Figure S2 Responsiveness of wild type animals and the indicated single and double mutants to light mechanical stimuli. Animals, which respond to three repeated gentle mechanical stimuli in the middle area of the body and the tail, are scored as sensitive to touch. Error bars denote S.E.M. values (n>150 for all populations examined; P<0.001, compared to control animals, unpaired t-test).

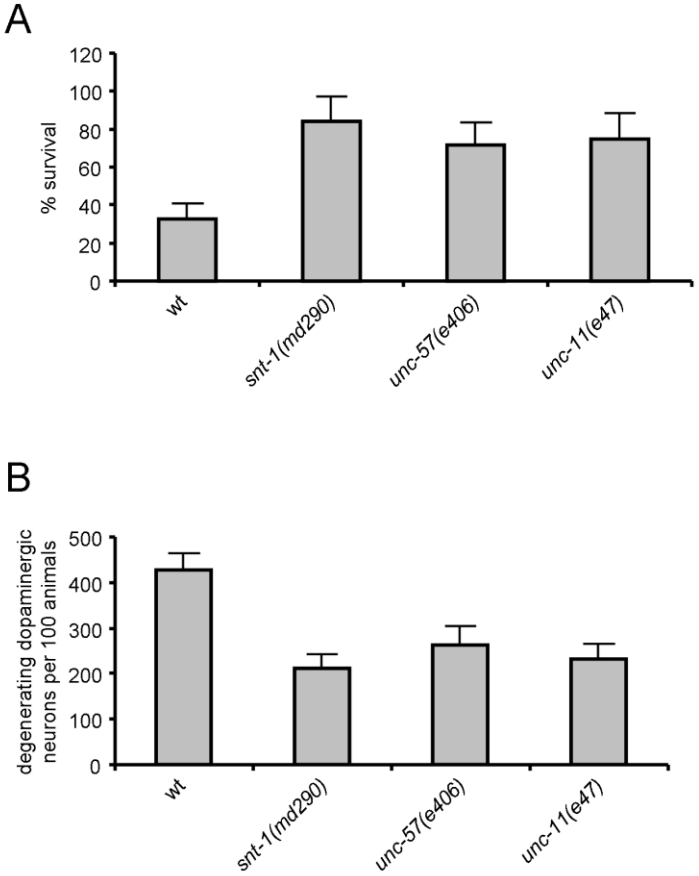

Figure S3 (A) The endocytic pathway mutants *snt-1(md290)*, *unc-57(e406)* and *unc-11(e47)* are more resistant to hypoxic conditions, compared to wild type animals. The percentage of adult animals 2 days post-L4 that survive exposure to <0.5 % oxygen at 25^{o}C for 24 hrs. **(B)** Downregulation of the endocytic system protects dopaminergic neurons from neurotoxin-induced death. The number of degenerating dopaminergic neurons per 100 animals carrying mutations in the endocytic genes *snt-1*, *unc-57* and *unc-11* after treatment with 6-OHDA (6-hydroxydopamine) is shown. Error bars denote S.E.M. values (n>250 for all populations examined; P<0.001, compared to control animals, unpaired t-test).

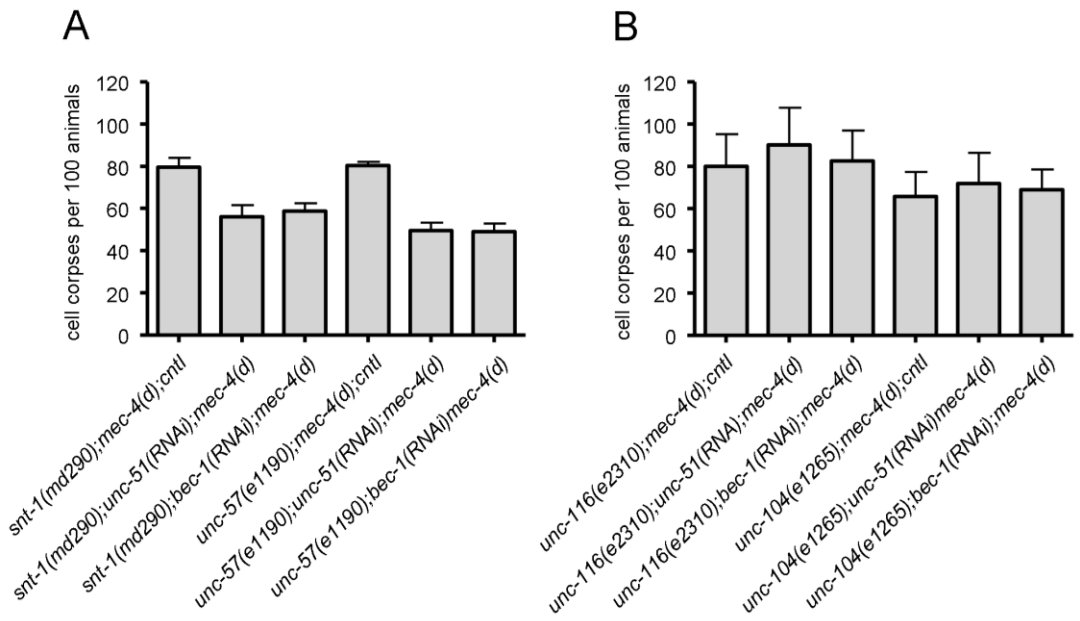

Figure S4 (A) Synaptotagmin or endophilin depletion further ameliorates *mec-4(d)*-induced necrosis in autophagy-deficient *unc-51(RNAi)* and *bec-1(RNAi)* animals. **(B)** Mutations in the kinesin genes *unc-116* or *unc-104* do not significantly enhance protection of touch receptor neurons in animals with impaired autophagy. The number of touch receptor neuron corpses at the L1 stage of development, per 100 *mec-4(d)* animals is shown. Error bars denote S.E.M. values (n>250 for all populations examined; P>0.5, compared to double mutants subjected to control RNAi bacteria, unpaired t-test).

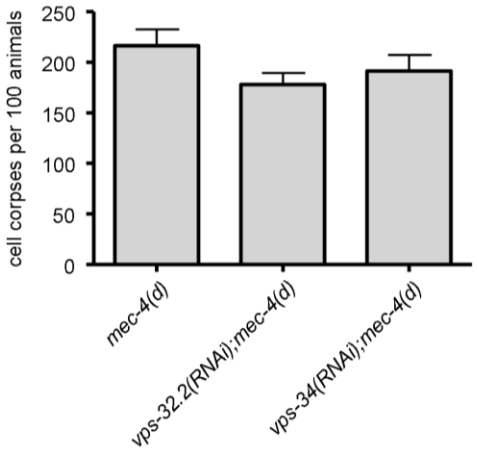

Figure S5 Downregulation of *vps-32.2*, a component of the ESCRT-III, or *vps-34*, which encodes for a class III PI3K, does not suppress *mec-4(d)*-induced neurodegeneration, indicating that the ESCRT complex (endosomal sorting complex required for transport) is not required for necrotic cell death in *C. elegans*. The number of touch receptor neuron corpses at the L1 stage of development, per 100 *mec-4(d)* animals is shown. Error bars denote S.E.M. values (n>250 for all populations examined; P<0.001, compared to control animals, unpaired t-test).

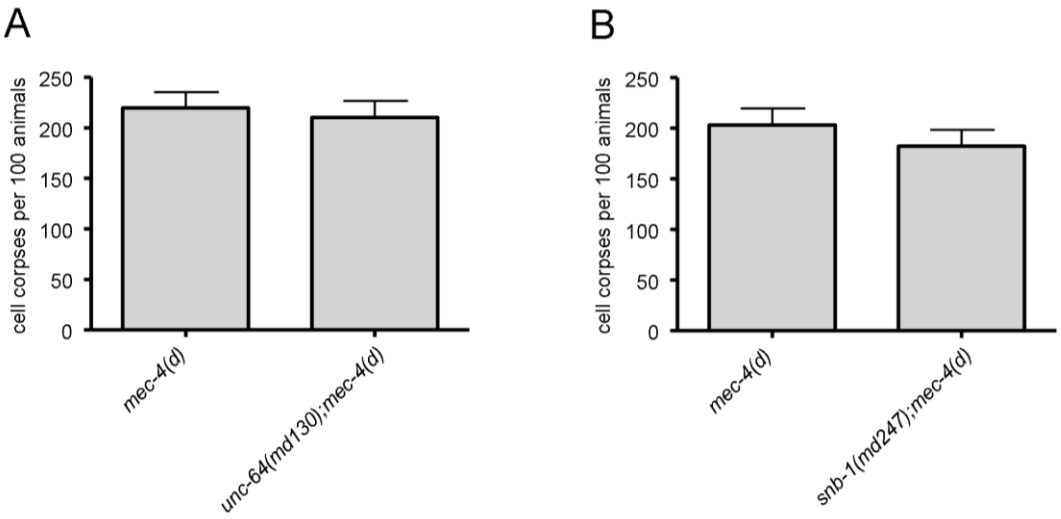

Figure S6 Impairment of synaptic neurotransmitter exocytosis does not significantly reduce *mec-4(d)*-induced neurodegeneration. **(A)** Knock down of *unc-64*, which encodes for syntaxin, a SNARE protein of the plasma membrane necessary for neurotransmission, does not suppress touch receptor neuron necrosis. **(B)** Similarly, depletion of SNB-1, the *C. elegans* v-SNARE synaptobrevin, does not ameliorate neurodegeneration. The number of touch receptor neuron corpses at the L1 stage of development, per 100 *mec-4(d)* animals is shown. Error bars denote S.E.M. values (n>250 for all populations examined; P<0.001, compared to control animals, unpaired t-test).

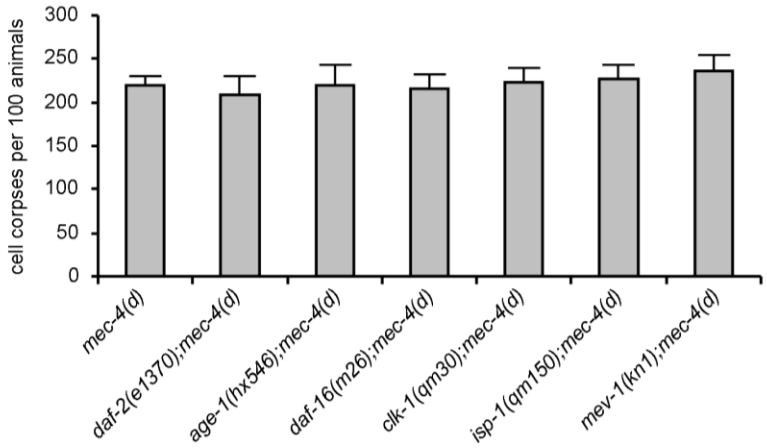

Figure S7 Knock down of genes encoding components of the insulin/IGF-1 signalling pathway (*daf-2*, *age-1*, *daf-16*) or genes encoding proteins involved in mitochondrial energy metabolism (*clk-1*, *isp-1*, *mev-1*) does not suppress necrotic cell death induced by the hyperactive ion channel MEC-4(d). The number of touch receptor neuron corpses at the L1 stage of development, per 100 *mec-4(d)* animals is shown. Error bars denote S.E.M. values (n>250; P<0.001, compared to control animals, unpaired t-test).

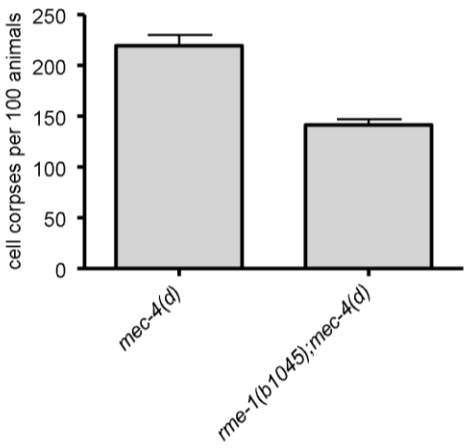

Figure S8 Mutation in the *rme-1* gene, which encodes for a protein involved in vesicle trafficking between the endocytic recycling compartment (ERC) and the plasma membrane, ameliorates *mec-4(d)*-induced neurodegeneration. The number of touch receptor neuron corpses at the L1 stage of development, per 100 *mec-4(d)* animals is graphed. Error bars denote S.E.M. values ($n>250$; $P<0.001$, compared to control animals, unpaired t-test).