

Supplemental material

Lee et al., <https://doi.org/10.1083/jcb.201801044>

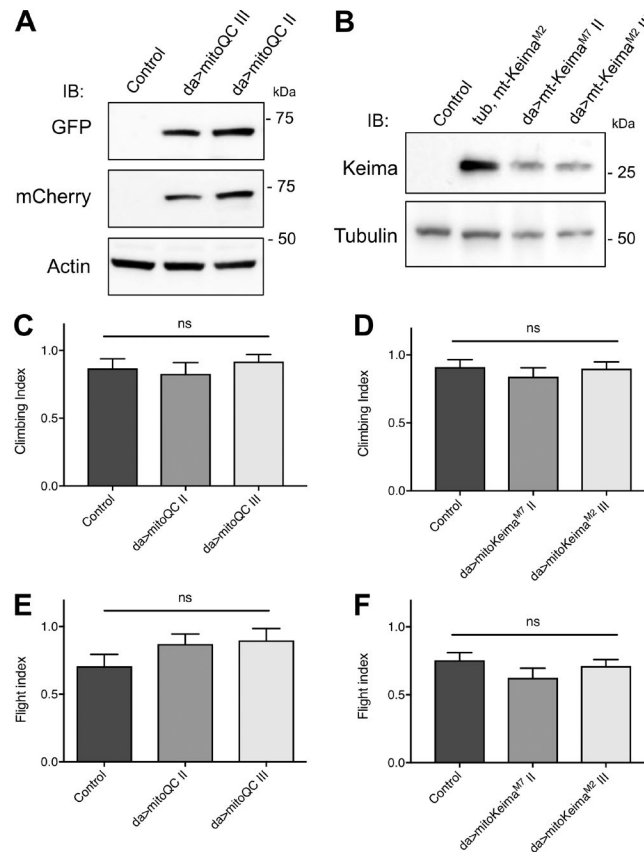


Figure S1. **Expression of mito-QC and mt-Keima is benign in *Drosophila*.** (A and B) Expression analysis of independent transgenic lines expressing (A) mito-QC and (B) mt-Keima induced by tubulin (tub)- or daughterless (da)-GAL4 lines. Immunoblots were probed with the indicated antibodies. Control genotype is nontransgenic *w¹¹¹⁸*. (C–F) Locomotor assays for climbing (C and D) and flight ability (E and F) of the same genotypes analyzed above. Charts show mean \pm 95% confidence interval. $n > 50$ animals (2 d old). Statistical analysis was determined by Kruskal–Wallis nonparametric test with Dunn’s post-hoc correction for multiple comparisons; ns, nonsignificant. Control genotype for behavior is *da-GAL4/+*.

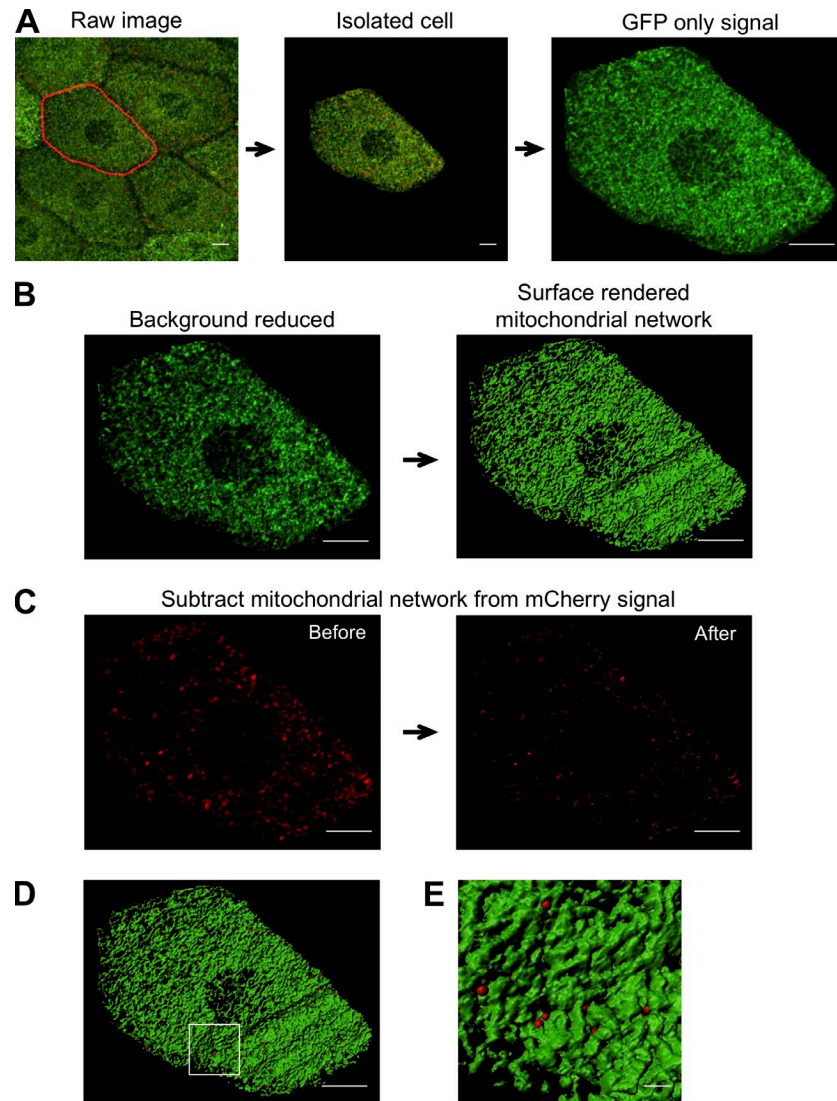


Figure S2. **Mitolysosome quantification workflow for larval epidermis (mito-QC only)**. Z-stack images were acquired by spinning disk microscopy for quantification. **(A)** Where possible, individual cells were isolated for workflow analysis. **(B)** The contrast of the mitochondrial network (GFP only) was adjusted to reduce the background, and a rendered 3D surface corresponding to the mitochondrial network was generated. **(C)** This surface was then subtracted from the mCherry channel to discard the mCherry signal overlapping the GFP-labeled mitochondrial network, leaving the red-only puncta. **(D and E)** Imapris-defined mitolysosomes (red) outside of the mitochondrial network (green). Genotype shown is *da-GAL4/UAS-mito-QC*. Bars: (A–D) 10 μ m; (E) 2 μ m.

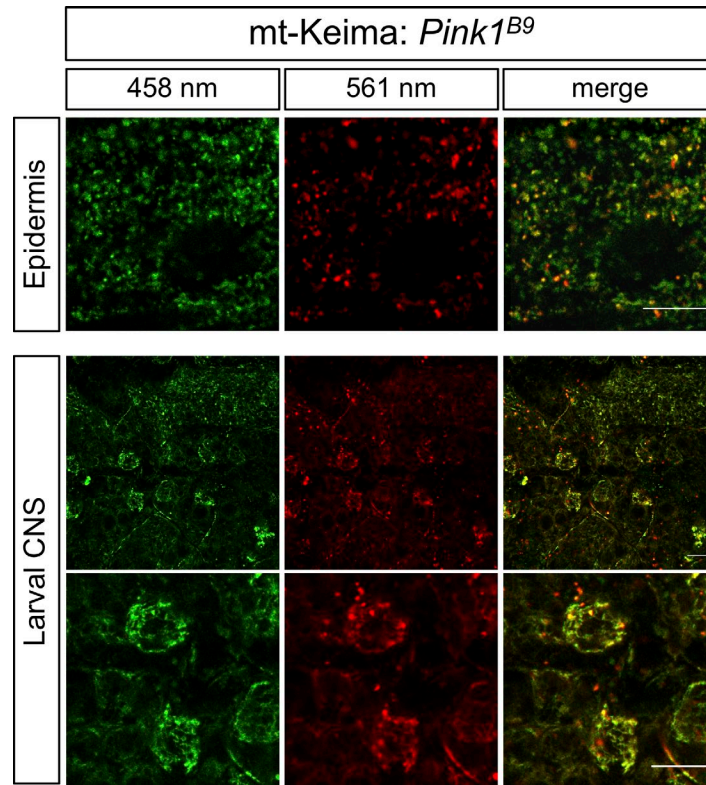


Figure S3. **Mitolysosome analysis with mt-Keima in *Pink1* mutant larval tissues.** Live confocal microscopy of larval epidermal cells and ventral ganglion of the CNS to visualize mt-Keima in *Pink1*^{B9} mutant animals (compare with Fig. 2). Genotype analyzed is *Pink1*^{B9}; *tub-GAL4*, *UAS-mt-Keima*. Bars, 10 μ m.