

Supplemental Information

**Reduced peroxisomal import triggers
peroxisomal retrograde signaling**

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Figure S1

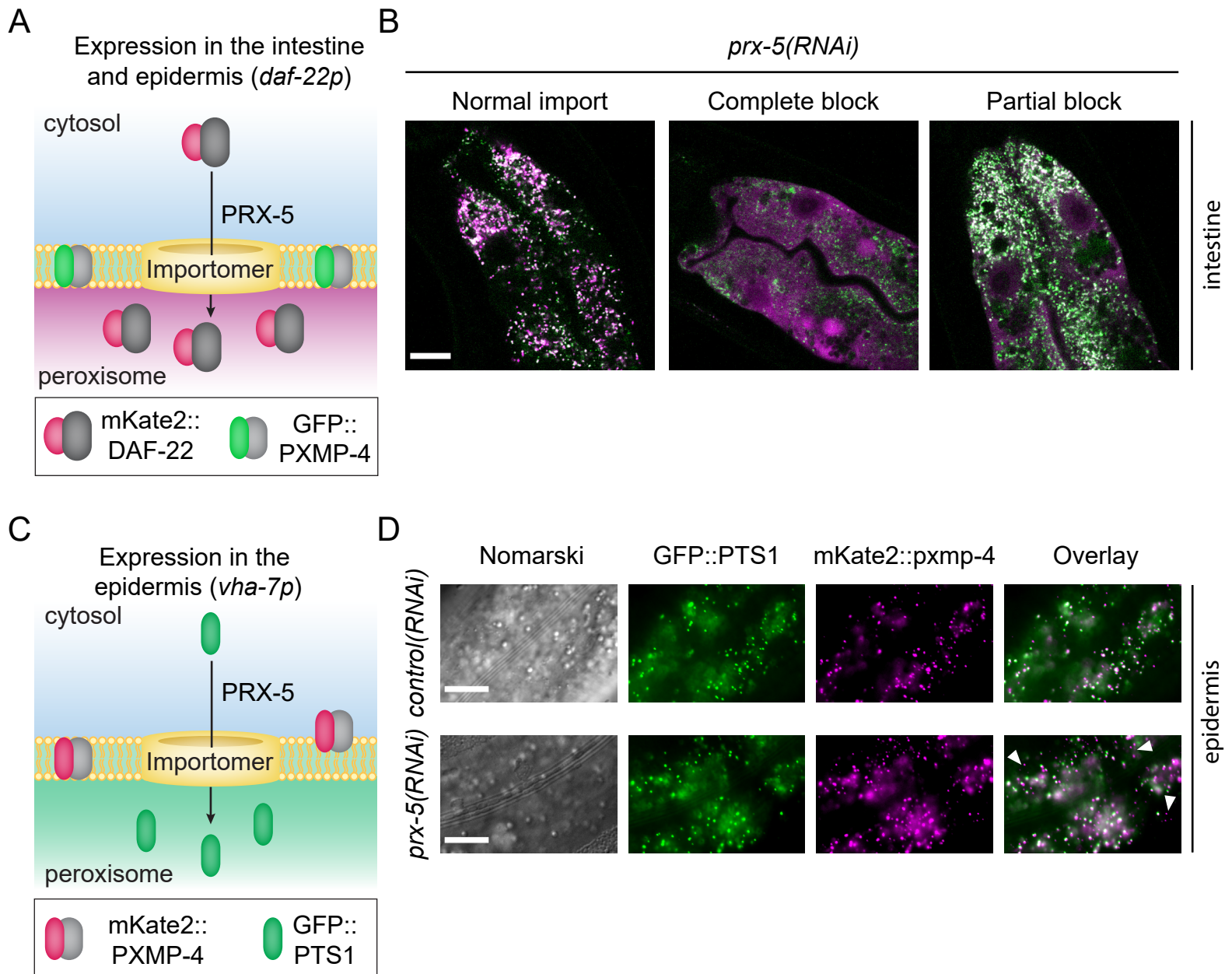


Figure S1: Effect of *prx-5(RNAi)* on peroxisomes in intestinal cells (A, B) and epidermal cells (C, D). (Related to Figure 1.) **(A)** Schematic representation of the *daf-22p::mKate2::daf-22* and *daf-22p::gfp::pxmp-4* reporters used in panel B. **(B)** Representative images of the different phenotypes observed in intestinal cells upon *prx-5(RNAi)*: normal import, complete block of import and partial block of import of mKate2::DAF-22. The scale bar represents 10 μ m. **(C)** Schematic representation of the *vha-7p::gfp::PTS1* and *vha-7p::mKate2::pxmp-4* reporters used in panel D. **(D)** L4 larvae carrying the *vha-7p::gfp::PTS1* and *vha-7p::mKate2::pxmp-4* reporters were subjected to *control(RNAi)* or *prx-5(RNAi)*. Adults of the next generation were analyzed by Nomarski and fluorescent microscopy. The scale bar represents 10 μ m. Arrowheads indicate structures labeled with mKate2::PXMP-4 but not GFP::PTS1.

Figure S2

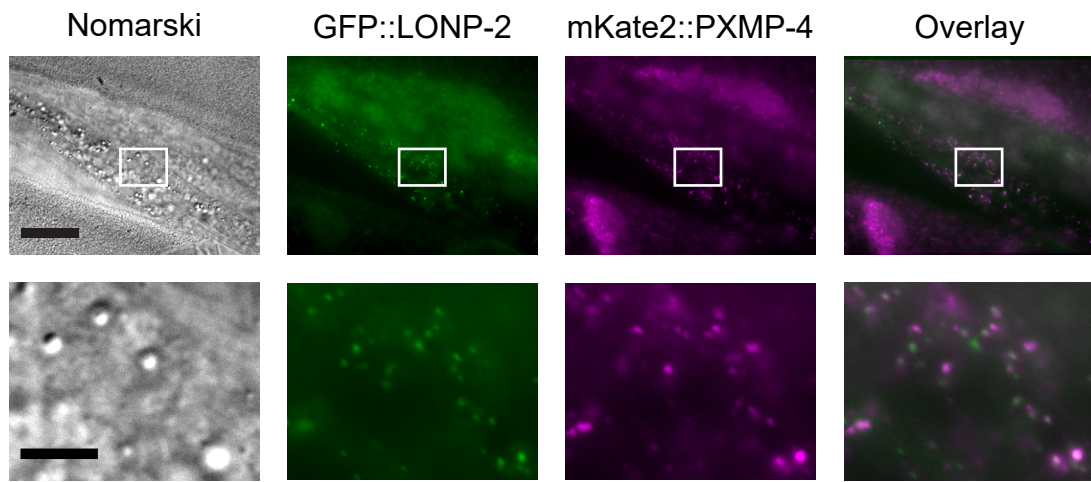


Figure S2: The *C. elegans* peroxisomal Lon protease localizes to peroxisomes. (Related to Figure 2.) Representative images of the reporter strain expressing *vha-7p::GFP::lonp-2* and *vha-7p::mKate2::pxmp-4* in epidermal cells. The scale bar represents 20 μm (top panels) or 5 μm in the enlarged images (bottom panels).

Figure S3

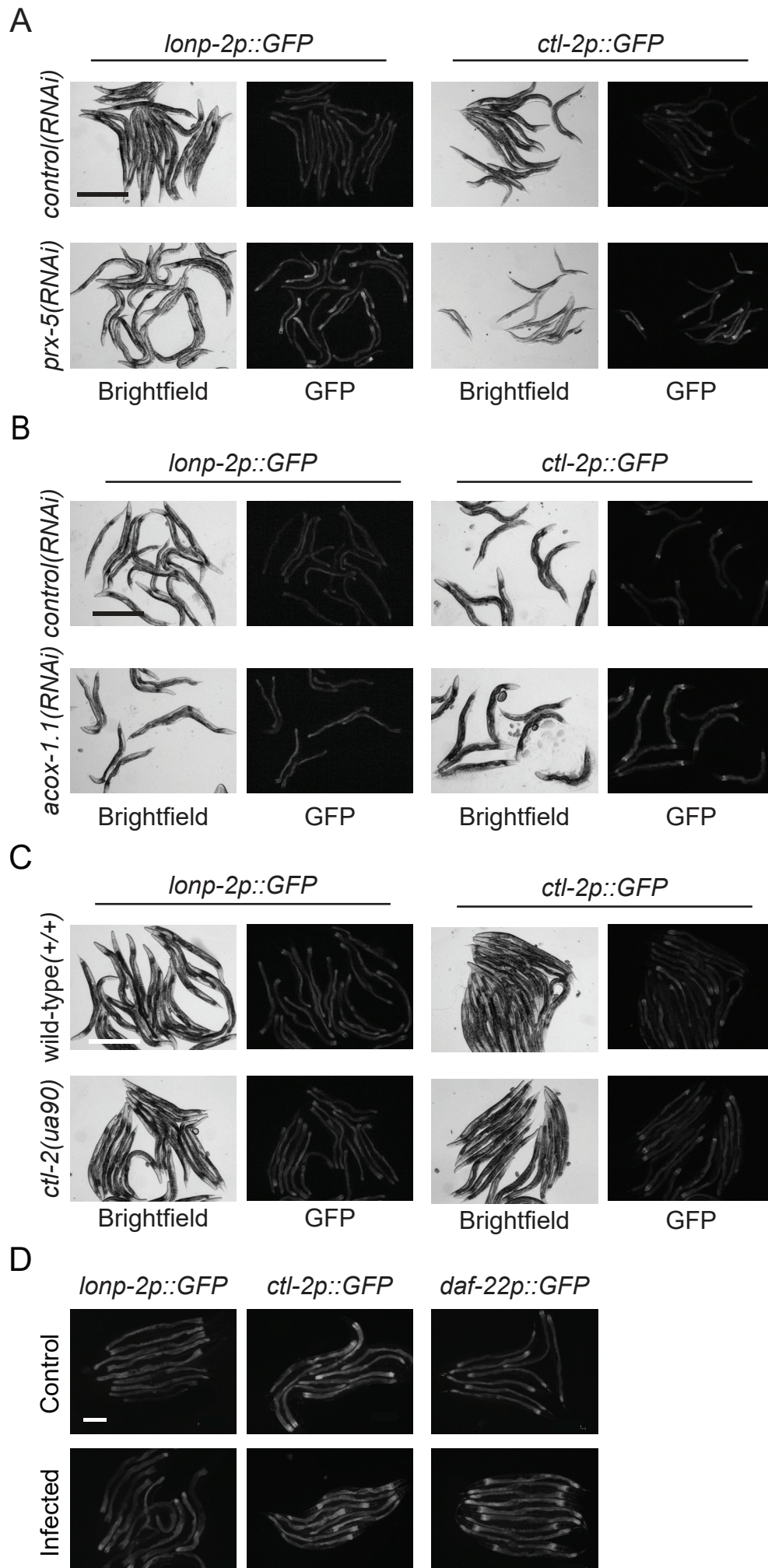


Figure S3: Normalized expression of the transcriptional reporters. (Related to Figure 2,4,5 and 7.) Images of Figure 2A (**A**), Figure 4A (**B**), Figure 5B (**C**) and Figure 7B (**D**) were rescaled with a minimum displayed value of 0 and a maximum displayed value that equals two times the maximum pixel value of the control of this experiment. By this scaling, none of the pixels in the representative images are saturated (scale bar: 0.5 mm).

Figure S4

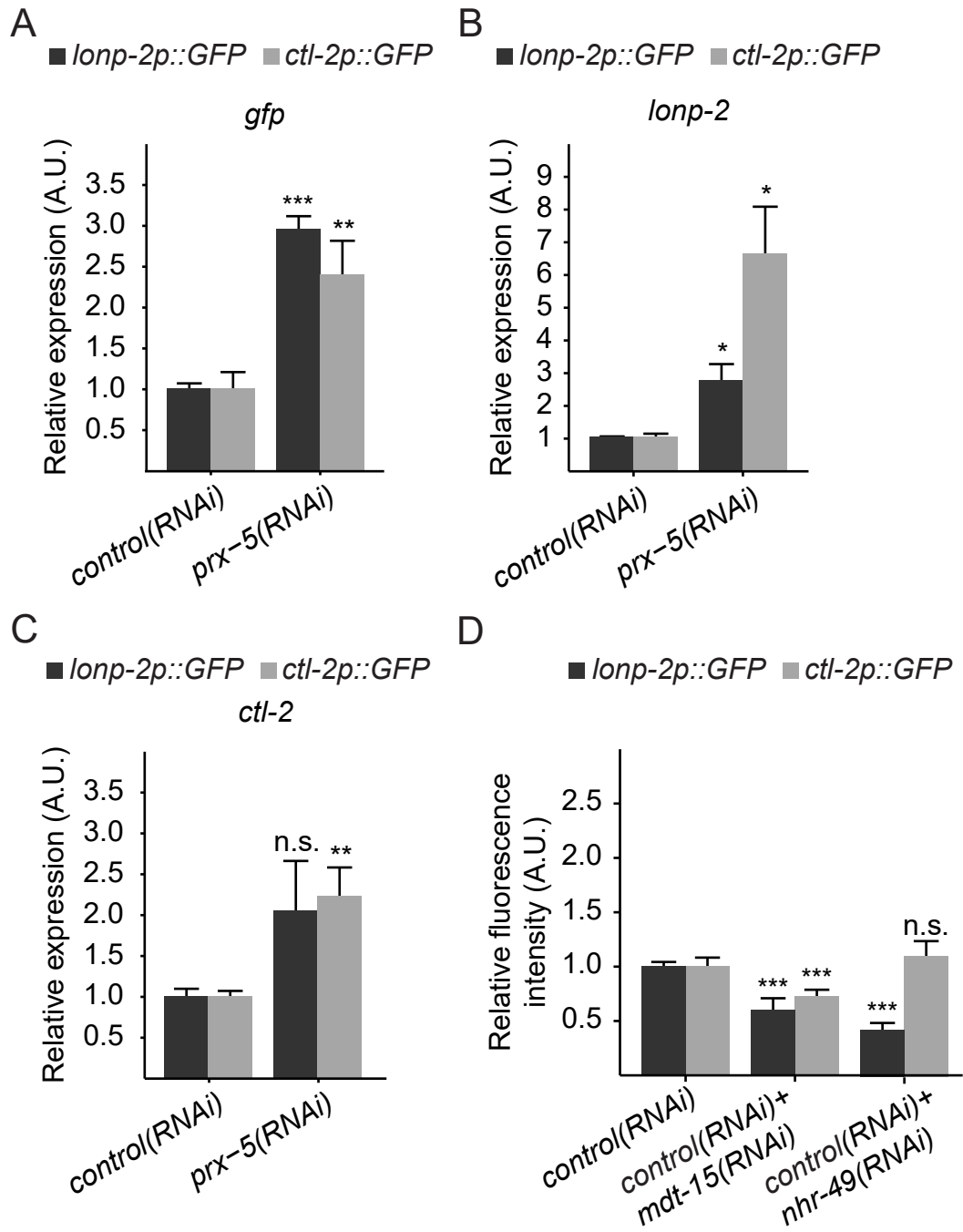


Figure S4: The transcriptional regulation of the peroxisomal Lon protease or the peroxisomal catalase. (Related to Figure 2.) **(A-C)** Analysis of relative gene expression in the *lonp-2p::GFP* and the *ctl-2p::GFP* reporter strains by RT-qPCR. L4 larvae carrying the reporters were subjected to *prx-5(RNAi)* and the relative gene expression of *gfp* **(A)**, *lonp-2* **(B)** and *ctl-2* **(C)** was analyzed in adults of the next generation and quantified relative to *control(RNAi)*. (n=3 (n=number of biological replicates); mean and SD are shown; n.s.: not significant, * p<0.05, ** p<0.01, *** p<0.001 by a (Welch's) two sample t-test.) **(D)** Analysis of the role of NHR-49 and MDT-15 in the basal expression of the *lonp-2p::GFP* and the *ctl-2p::GFP* reporters. L4 larvae carrying the reporters were subjected to different RNAi (dilution 1:2) and the expression of the reporters in adults of the next generation was monitored by fluorescence microscopy and quantified relative to *control(RNAi)*. (n=6-12 (n=number of biological replicates); mean and SD are shown; n.s.=not significant and *** p<0.001 by one-way ANOVA with Dunnett's multiple comparison to *control(RNAi)*.)

Figure S5

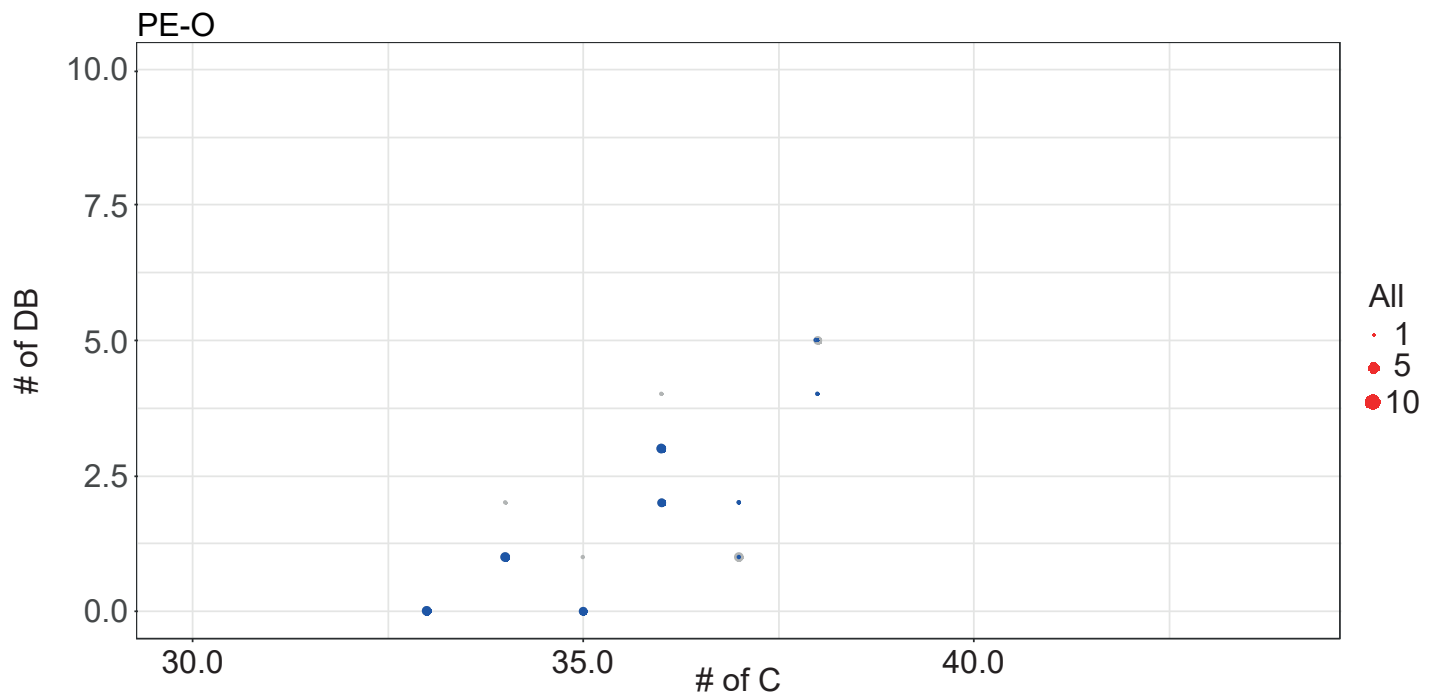
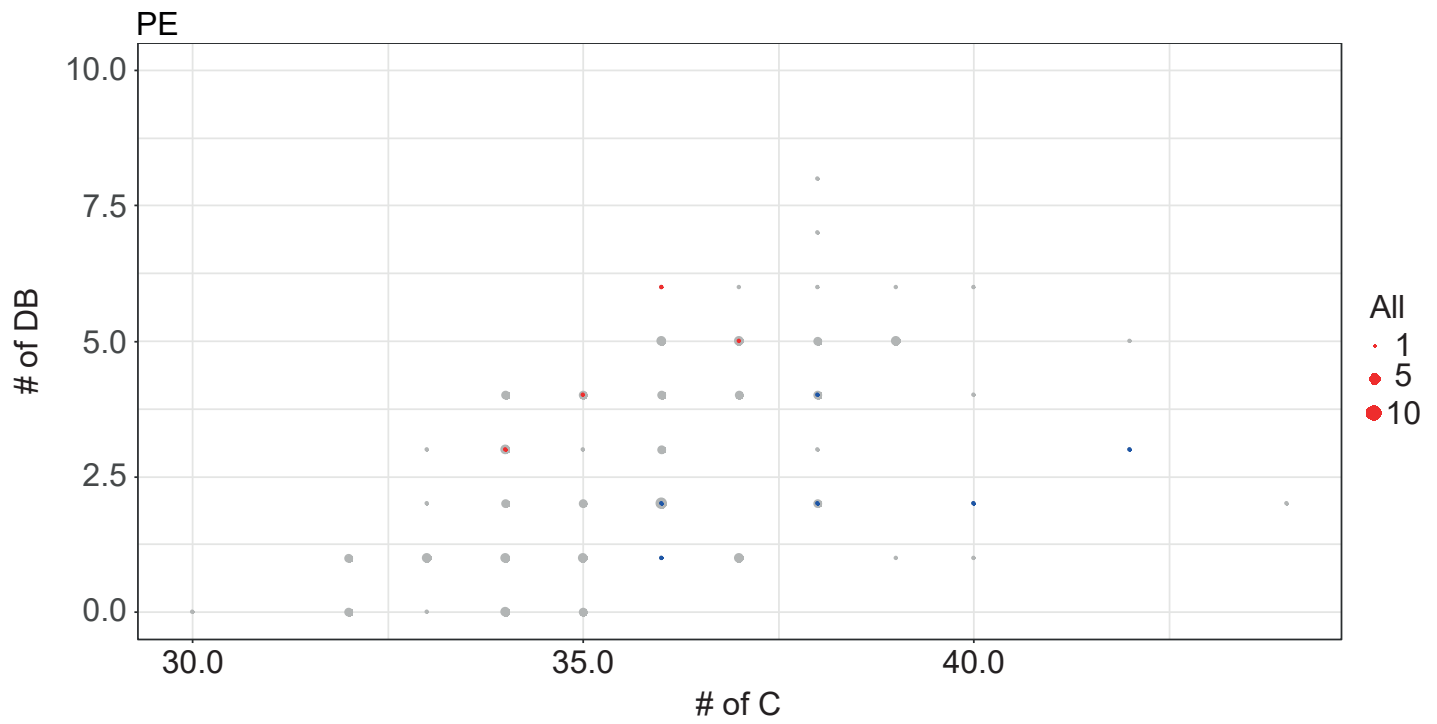
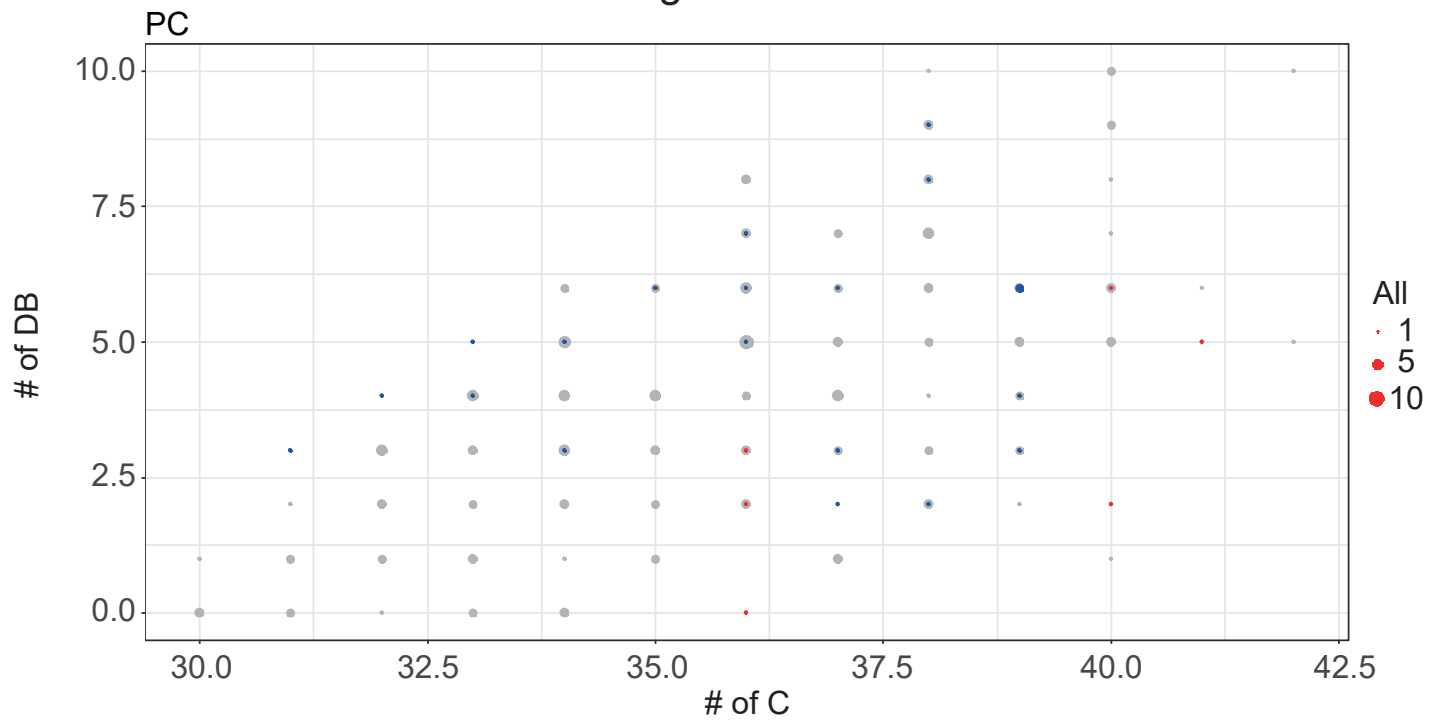


Figure S5: Effect of *prx-5(RNAi)* on different classes of lipids. (Related to Figure 3.)

Scatterplot indicating the distribution and changes in the levels of different class of lipid (Phosphatidylcholines (PCs) (top panel), Phosphatidylethanolamines (PEs) (middle panel), Ether-PE (PE-O) (bottom panel)) in *prx-5(RNAi)* in comparison to *control(RNAi)*. The x-axis labels the number of carbons (# of C) and the y-axis the number of double bonds (# of DB) in the acyl side chains. Red and blue dots indicate up-regulated and down-regulated lipid respectively.

Figure S6

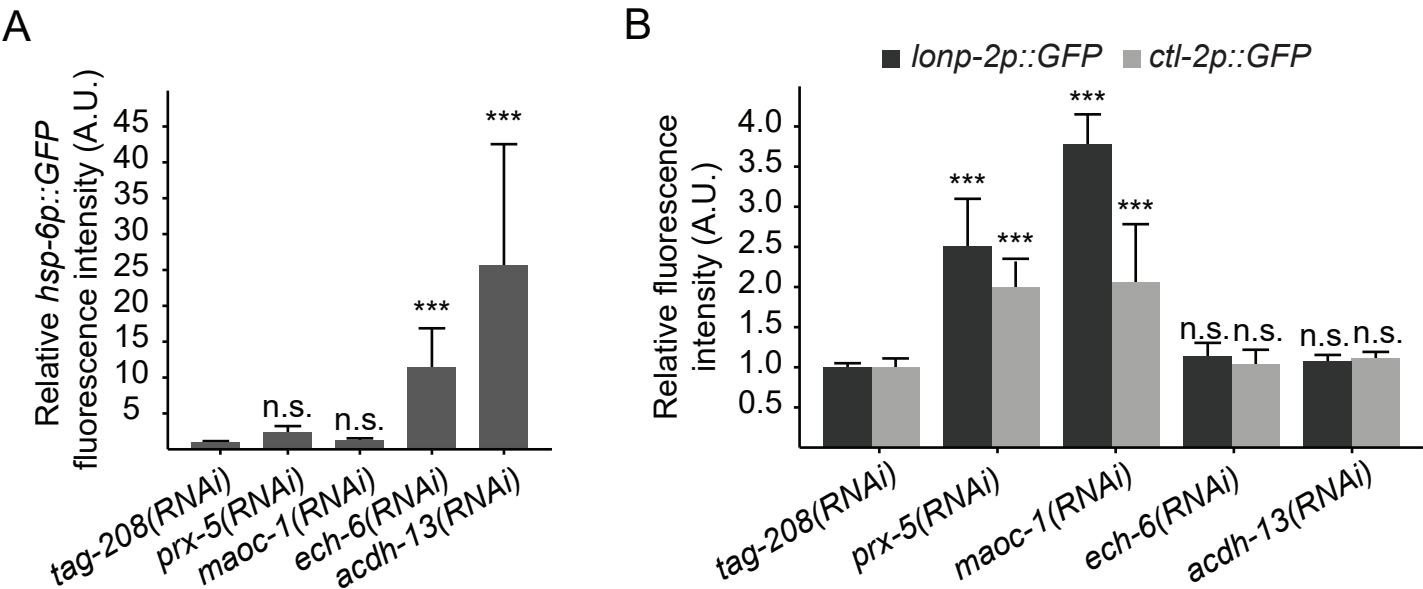


Figure S6: Peroxisomal retrograde signaling is specifically activated by a perturbation of peroxisomal beta-oxidation. (Related to Figure 4.) L4 larvae carrying the *hsp-6p::GFP* reporter (**A**) or *lonp-2p::GFP* and *ctl-2p::GFP* reporters (**B**) were subjected to different RNAi conditions and the expression of the reporters in adults of the next generation was monitored by fluorescence microscopy and quantified relative to the *control(RNAi)* control (n=6-8 (n=number of biological replicates); mean and SD are shown; n.s.=not significant and *** p<0.001 by Kruskal-Wallis test with Dunn's post hoc test to *control(RNAi)*.)

Figure S7

■ *lonp-2p::GFP* ■ *ctl-2p::GFP*

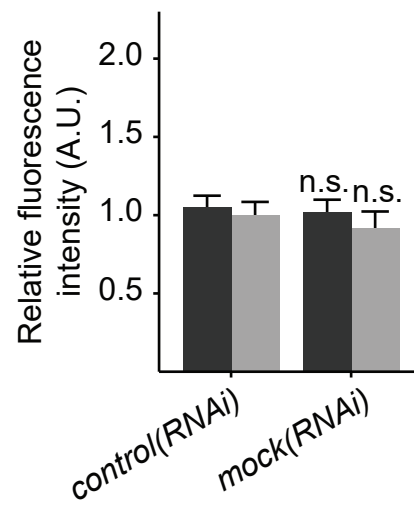


Figure S7: *tag-208(RNAi)* does not induce the *lonp-2p::GFP* and *ctl-2p::GFP* reporters expression. (Related to Star Methods.) L4 larvae carrying the *lonp-2p::GFP* or the *ctl-2p::GFP* reporters were subjected to *mock(RNAi)* (RNAi bacteria containing the empty L4440 vector) and the expression of the reporters in adults of the next generation was monitored by fluorescence microscopy and quantified relative to the *tag-208(RNAi)* control. (n=6 (n=number of biological replicates); mean and SD are shown; n.s.: the differences are not significant by a two sample t-test.)