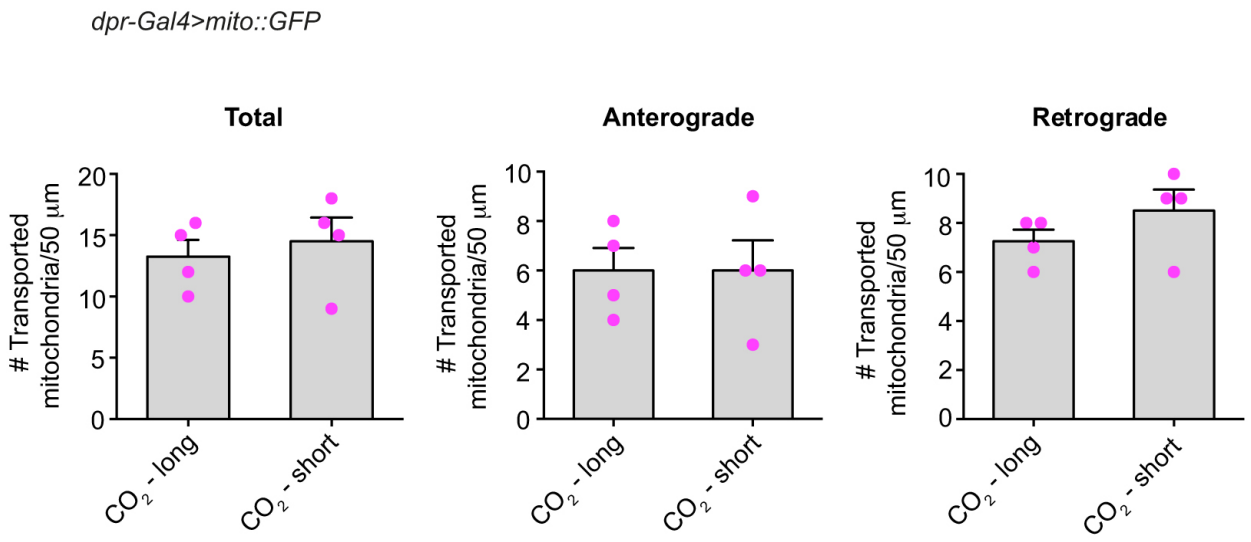
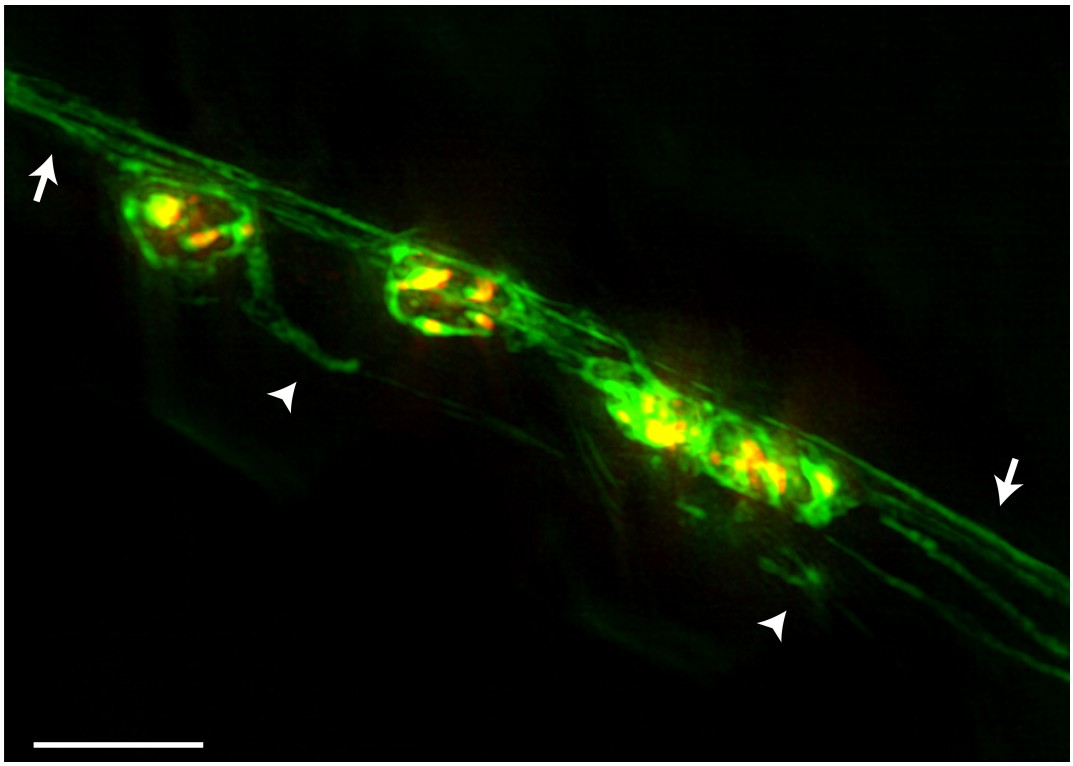
SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Assessment of the effects of CO_2 exposure on cargo transport. Quantification of the number of total mitochondria that are motile in *dpr*$^{+}$ wing neurons in a 3 min time window when flies are exposed to CO_2 for 5 min followed by mounting and immediate imaging (CO_2 – long) or exposed to CO_2 for 20–30 s followed by mounting and a ~ 5 min recovery period before imaging (CO_2 – short). Values are mean ± standard error of the mean, with number of flies per condition shown above bars. For the same parameter, there were no statistically significant differences between the two conditions.



Supplementary Figure 2. Structured illumination microscopy (SIM) of L1 vein neurons. Representative SIM image of the anterior wing margin showing cell bodies and processes of neurons in the L1 vein. Wings were mounted using the procedures described in this article. The genotype of the fly is *Appl-Gal4>mCD8::GFP, RedStinger::NLS*, which leads to cell membranes and nuclei marked in green and red, respectively. Arrowheads, dendrites; arrows, bundled axons. Note the improved detail in the SIM image compared to the image taken by spinning disk microscopy of the same genotype (Fig. 1b). The image is a projection of a z-stack taken on a Zeiss Elyra imaging system using a 63x/1.4 NA PlanApo oil-immersion objective. Scale bar: 5 µm.

SUPPLEMENTARY VIDEO LEGENDS

Supplementary Video 1. Movie capturing the procedure used to mount a fly in the imaging chamber (Steps 8–19), with the chamber part-assembled in advance). Note that the movie does not show the process of inspecting wings for damage or the whole anaesthetisation procedure.

Supplementary VIdeo 2. Representative time-lapse movie of mitochondrial dynamics in axons of *dpr*$^{+}$ neurons in the wing arch (L1 vein) at 1 day after eclosion. The cell body is to the left and thorax to the right in this and other movies. Note that, as in many other neuronal cell types, a significant fraction of mitochondria is stationary at any one time-point. Genotype: *dpr-Gal4 UAS-mito::GFP*. Width: 50 µm. Movie represents 3 min of real-time.

Supplementary Video 3. Representative time-lapse movie of mitochondrial dynamics in axons of *nSyb*$^{+}$ neurons in the L3 vein at 2 days after eclosion. Genotype: *nSyb-lexA lexAop-mCherry::mito*. Width: 50 µm. Movie represents 3 min of real-time.

Supplementary Video 4. Time-lapse movie showing an example of mitochondrial fission in axons of *dpr*$^{+}$ neurons in the wing arch. A stationary mitochondrion (red arrow) undergoes fission to produce a new mitochondrion (yellow arrow) that is motile. Genotype: *dpr-Gal4 UAS-mito::GFP*. Width: 17 µm. Movie represents 68 s of real-time.

Supplementary Video 5. Time-lapse movie showing an example of mitochondrial fusion in axons of *dpr*$^{+}$ neurons in the wing arch. A motile mitochondrion (red arrow) undergoes fusion with a stationary mitochondrion (yellow arrow). The orange arrow marks the movement of the new elongated mitochondrion produced by the fusion event. Genotype: *dpr-Gal4 UAS-mito::GFP*. Width: 50 µm. Movie represents 266 s of real-time.

Supplementary Video 6. Representative time-lapse movie of ANF::EMD dynamics in axons of *dpr*$^{+}$ neurons in the wing arch at 2 days after eclosion. The ANF::EMD signal on dense-core vesicles is masked by cytoplasmic GFP encoded by a *UAS-GFP* transgene on the *dpr-Gal4* chromosome. Genotype: *dpr-Gal4 UAS-ANF::EMD*. Width: 50 µm. Movie represents 1 min of real-time.

Supplementary Video 7. Representative time-lapse movie of ANF::EMD dynamics in axons of *Appl*$^{+}$ neurons in the wing arch at 2 days after eclosion. This genotype allows dense-core vesicles to be visualised clearly. Genotype: *Appl-Gal4 UAS-ANF::GFP*. Width: 50 µm. Movie represents 2 min of real-time.

Supplementary Video 8. Representative time-lapse movie of Rab4::RFP dynamics in axons of *dpr*$^{+}$ neurons in the wing arch at 2 days after eclosion. Genotype: *dpr-Gal4 UAS-Rab4::RFP*. Width: 50 µm. Movie represents 1 min of real-time