

Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: **Dynamics of mitochondrial entry into the axon upon *Mff* knockdown.** In 10DIV control neurons, mitochondria enter the axon already small and at a rate of approximately eight mitochondria per hour. In *Mff* knockdown neurons of the same age mitochondria enter with a much larger size and at a reduced rate of approximately three mitochondria per hour. Related to **Figures 2 and 3.**

File Name: Supplementary Movie 2

Description: **Mitochondrial fission and fusion dynamics along the axon upon *Mff* knockdown.** In 10DIV control axons, mitochondria are maintained at a small size by regulating the fission and fusion rate to around one. Upon loss of *Mff*, the fission fusion rate plummets causing increased mitochondrial size along the axon. Related to **Figures 2 & 3.**

File Name: Supplementary Movie 3

Description: **Axonal mitochondrial motility upon *Mff* knockdown.** Loss of *Mff* does not prevent mitochondrial movement along the axon, even though mitochondrial length is strongly increased. Related to **Figures 2,3 & Supplemental Figure 4.**

File Name: Supplementary Movie 4

Description: **Presynaptic mitochondrial Ca^{2+} uptake upon evoked activity in *Mff* knockdown axons.** Elongation of axonal mitochondria upon loss of *Mff* increases their capacity for calcium buffering at the presynaptic site during neuronal activity. Calcium uptake is visualized via mitochondrial matrix targeted GCaMP. Related to **Figure 6.**