

**Figure S2** Localization of dP32 in Mitochondria at DLM Neuromuscular Synapses. Confocal immunofluorescence and native DsRed fluorescence images of adult DLM neuromuscular synapses exhibiting neuronal expression of mito-GCaMP3 and mito-DsRed. Mito-GCaMP3 consists of the first 71 amino acids of dP32 fused to the N-terminus of the calcium indicator, GCaMP3. Mito-GCaMP3 was recognized by an anti-GFP antibody. Mito-DsRed, which includes the previously characterized mitochondrial targeting domain of human COX8 (see Methods), serves as a mitochondrial marker. Anti-HRP labels the neuronal plasma membrane. Colocalization of mito-GCaMP3 and mito-DsRed demonstrates that mito-GCaMP3 was efficiently targeted to mitochondria.

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