



**Figure S7** Mitochondrial Calcium Transients within DLM Neuromuscular Presynaptic Terminals. Imaging of mitochondrial calcium transients was performed at WT and *dp32<sup>EC1</sup>* (*dp32EC1/Df*) synapses expressing the GCaMP3 targeted to mitochondria (mito-GCaMP3). (A) Example images of mito-GCaMP3 at WT synapses before, during and after DLM motor axon stimulation (40 Hz for 5 sec) at 20 °C. Neuronal expression of the membrane-associated mCD8-mRFP protein provided a red fluorescent marker for nerve terminals. Times indicated are relative to the beginning of the time-lapse imaging and axon stimulation was initiated at 5 seconds. Note that mito-GCaMP3 exhibits a punctate distribution consistent with its mitochondrial localization (compare with the distribution of cytosolic GCaMP3 in Figure 9B). (B) Mito-GCaMP3 fluorescence changes ( $\Delta F/F$ ) elicited at WT and *dp32<sup>EC1</sup>* (*dp32EC1/Df*) synapses by DLM motor axon stimulation (40 Hz for 5 seconds; bar over X axis) at 20 °C. Calcium transients observed in *dp32<sup>EC1</sup>* closely resembled those of WT. Note that fluorescence intensity measurements were made in regions of interest corresponding to individual mito-GCaMP3 puncta (see panel A) and thus both the initial peak in  $\Delta F/F$  as well as the sustained fluorescence increase following axon stimulation appear to reflect mitochondrial rather than cytosolic calcium changes.