Eisner et al., http://www.jcb.org/cgi/content/full/jcb.201312066/DC1

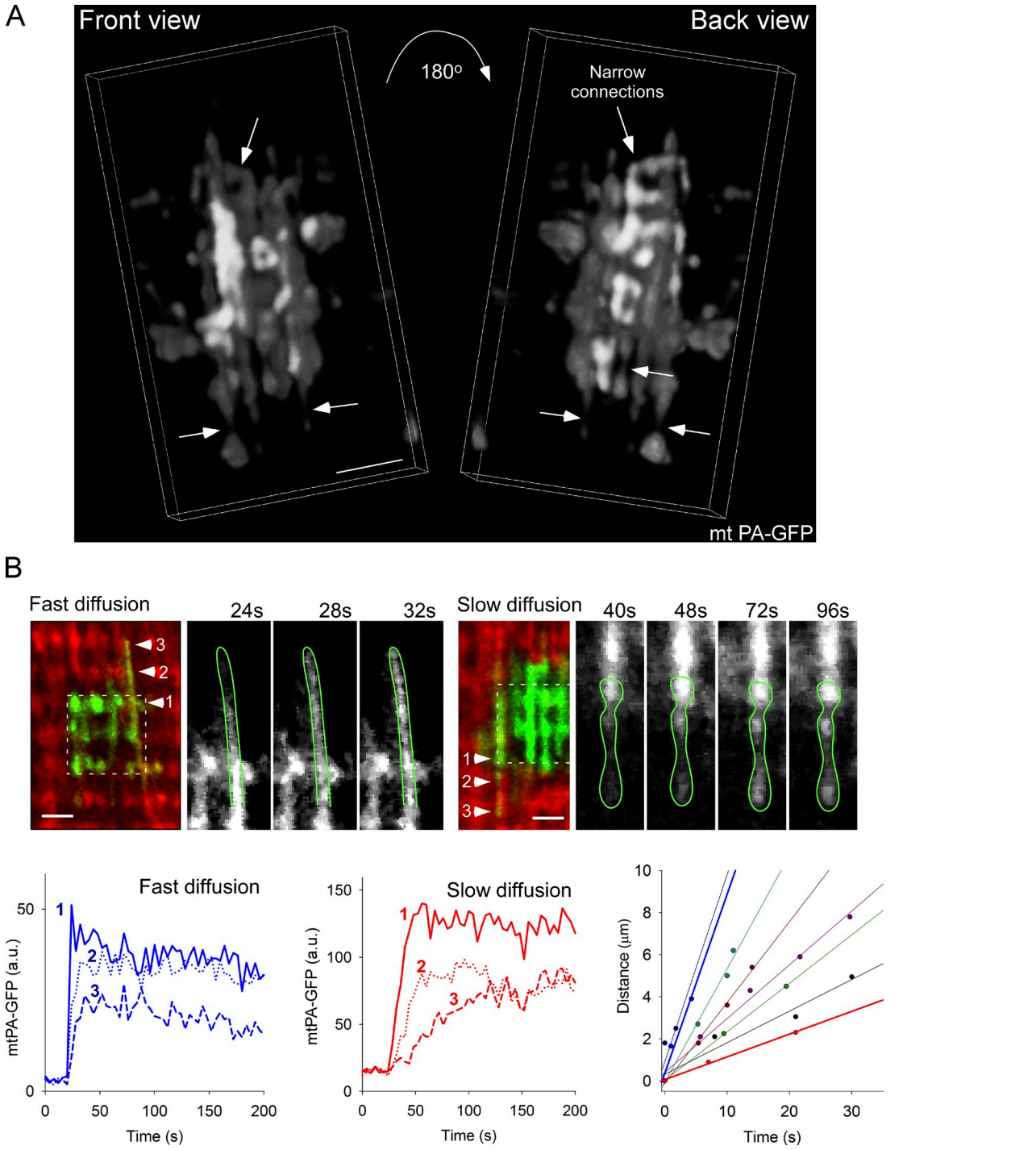


Figure S1. **Mitochondrial continuity in adult skeletal muscle.** (A) Front and back views of 3D reconstruction of the distribution of photoactivated mtPA-GFP in an FDB fiber show complex interconnected mitochondria with examples of transversal and longitudinal continuity. Both longitudinal and transversal matrix connections are illustrated; several connectors appear as narrowing intermitochondrial domains (arrows). Bar, 2 µm. (B) Evaluation of mtPA-GFP diffusion kinetics in individual mitochondria of different elongated morphology, outside of the photoactivated area (examples from a single representative experiment out of three repeats). Left panel and plot below (blue traces): example of an elongated mitochondrion with apparent constant diameter and fast mtPA-GFP diffusion kinetics reflected in the essentially synchronous increase in PA-GFP fluorescence at points of different distance (1, 2, and 3) from the photoactivation area. Top right panel and bottom center plot (red traces): an elongated mitochondrion showing a narrowing domain displays slow mtPA-GFP diffusion kinetics. Bottom right: the plot shows the diverse diffusion kinetics of eight longitudinal oriented organelles (half-time of fluorescence increase vs. distance from the edge of the photoactivation area and linear fits to the data points).

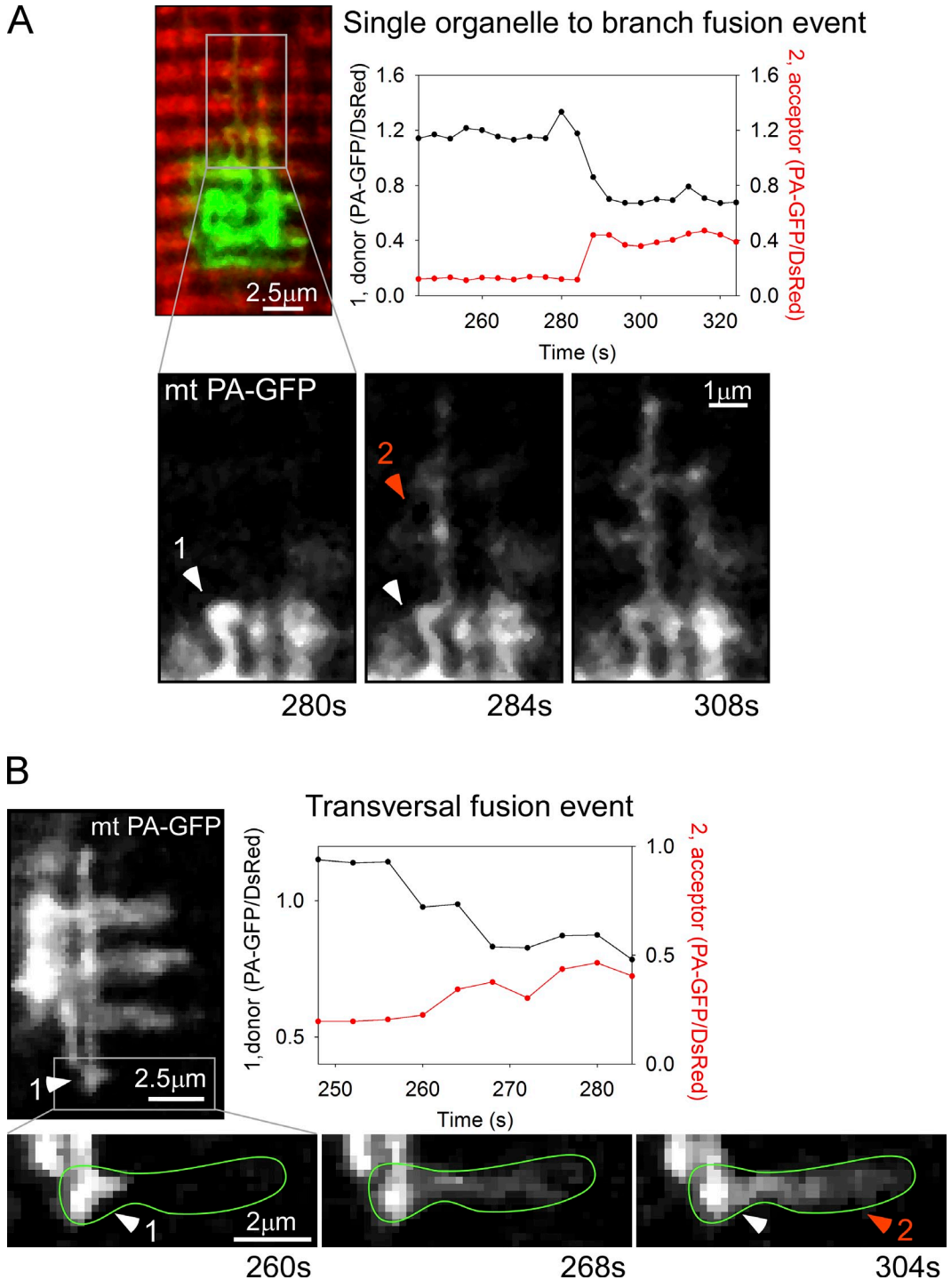


Figure S2. **Diversity of mitochondrial fusion events in adult FDB fibers.** (A) Example of a single mitochondrion to network fusion event. Mitochondrion 1, located in the immediate vicinity of the photoactivation area (left; bar, 2.5 µm) fuses with mitochondrion 2, a large complex mitochondrion that displays extensive branching in both longitudinal and transversal directions. The graph shows the ratio of mtPA-GFP/mtDsRed at different time points for mitochondrion 1 (PA-GFP donor) and 2 (acceptor) to illustrate the complementary redistribution of the two fluorescent proteins. (B) Example of a transversal fusion event. Mitochondrion 1 adjacent to the photoactivation area (top; bar, 2.5 µm) fuses with mitochondrion 2; mtPA-GFP diffuses in transversal direction, unveiling a new interconnected organelle (highlighted by green outline). Graph shows mtPA-GFP/mtDsRed ratio for each organelle.

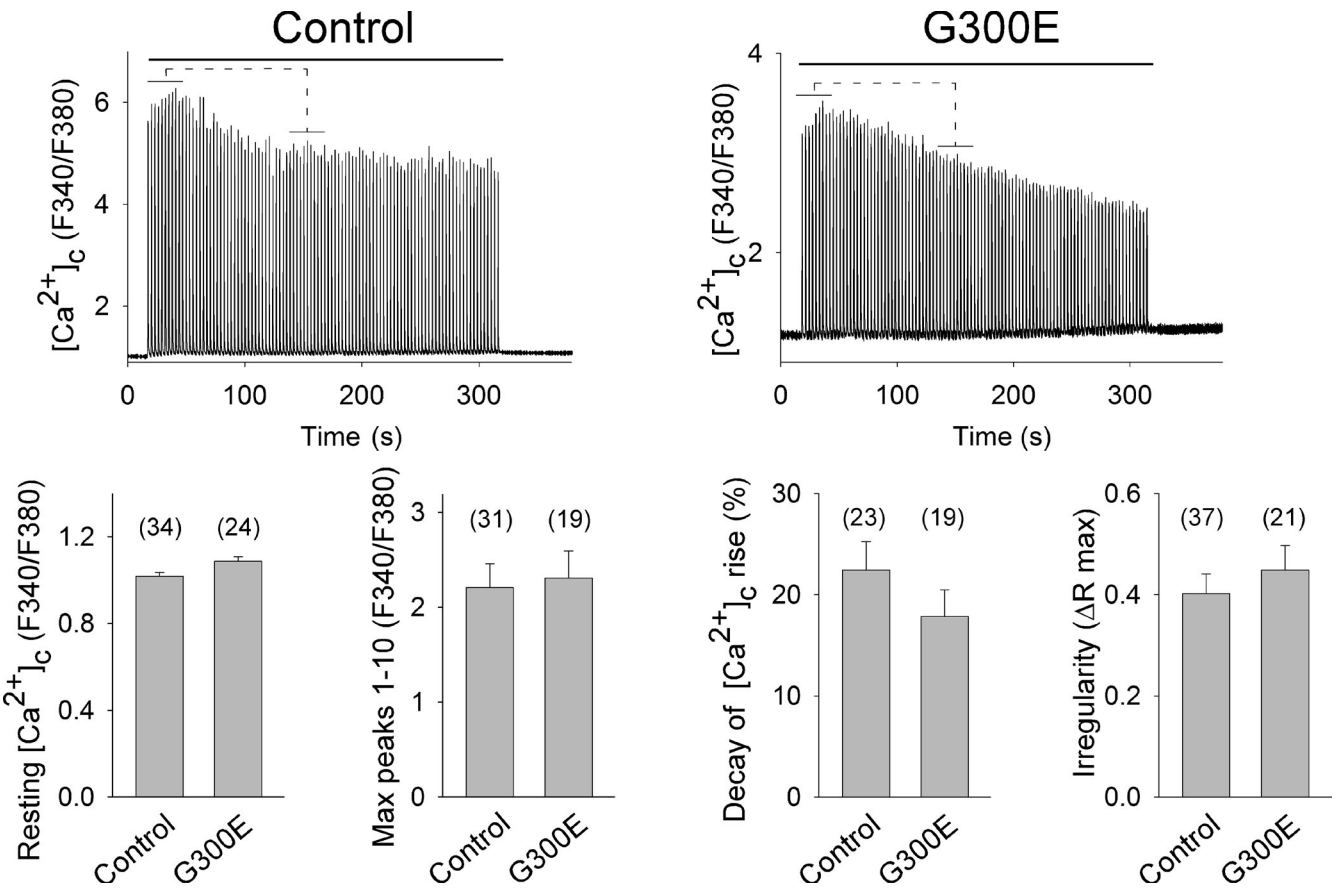


Figure S3. **Short-term expression of Opa1 G300E does not alter trains of $[Ca^{2+}]_c$ transients in skeletal muscle fibers.** FDB muscles from control animals were cotransfected with mtDsRed and mock or G300E Opa1 cDNA. After 7–10 d, fibers were isolated and loaded with Fura2. mtDsRed-positive fibers that responded to single electric pulses were picked for each experiment. Fibers were challenged by tetanic electrical stimulation. Top panel displays representative $[Ca^{2+}]_c$ transients. Bottom bar charts show from left to right: $[Ca^{2+}]_c$ levels preES, max amplitude (peaks 1–10), evaluation of the $[Ca^{2+}]_c$ transients amplitude decay, for the time period indicated by brackets above the representative traces (([mean peaks 1–10] − [mean peaks 30–40])/ [mean peaks 1–10] × 100), and irregularity in amplitude of the $[Ca^{2+}]_c$ transients ([max − min]/[max]), among peaks 1–100 (four or more independent experiments, the number of fibers is shown in the bar charts).

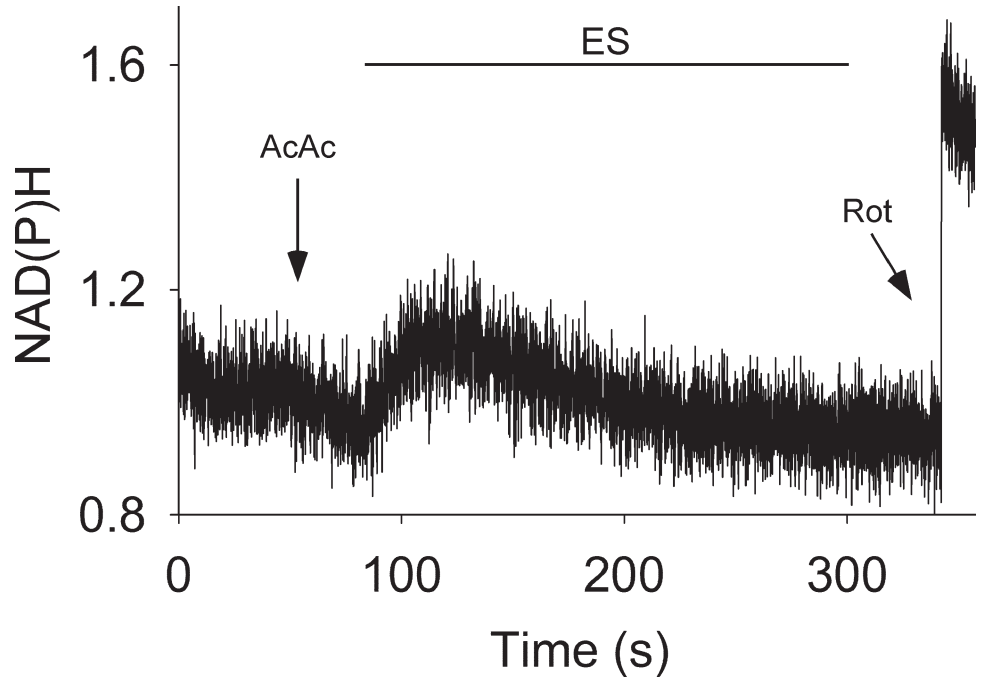


Figure S4. **Representative NADH transient induced by repetitive tetanic stimulation of a FDB fiber that does not express cytoRCaMP.** This experiment was completed with *n* = 3 fibers. The fiber responded to each tetanus with slight contraction that did not affect the NADH autofluorescence quantification. AcAc, acetoacetate; Rot, rotenone.

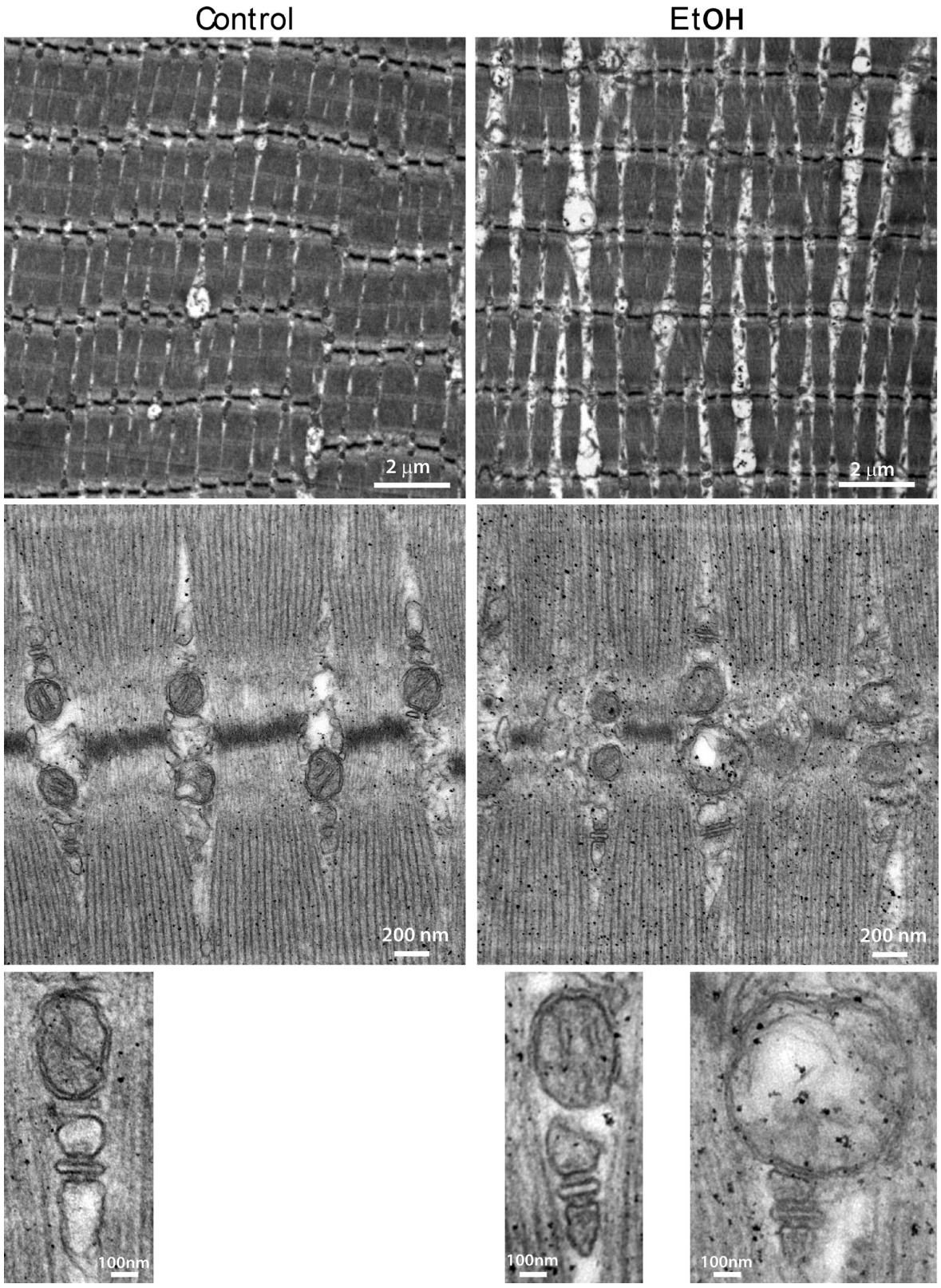
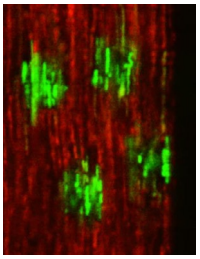
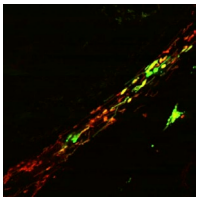


Figure S5. **TEM representative images of longitudinal sections of FDB muscles from control and EtOH-fed paired rats.** Top: 1,650×; middle: 21,000×; bottom: digital zoom on triads and associated mitochondria from 21,000× images.



Video 1. **Mitochondrial matrix dynamics in an adult FDB fiber.** Single fiber was isolated from rat FDB skeletal muscle transfected in vivo with mtDsRed and mtPA-GFP. Four regions of 5 × 5 μm^2 were exposed to 2P photoactivation and time-lapse imaged for a total time of 8 min using a laser-scanning confocal microscope (LSM780MP; Carl Zeiss). Frames were taken every 4 s.



Video 2. **Mitochondrial matrix dynamics in a rat myotube.** FDB fibers plated onto polylysine-coated (Sigma-Aldrich) and laminin-coated (Invitrogen) coverslips (100 µg/ml each; ∼20 coverslips/rat). Satellite cells were infected with the adenoviruses AdmtDsRed and AdmtPA-GFP and were allowed to differentiate to myotubes. Two regions of 5 × 5 μm^2 were exposed to 2P photoactivation and recorded for 8 min using a laser-scanning confocal microscope (LSM780MP; Carl Zeiss). Frames were taken every 4 s.