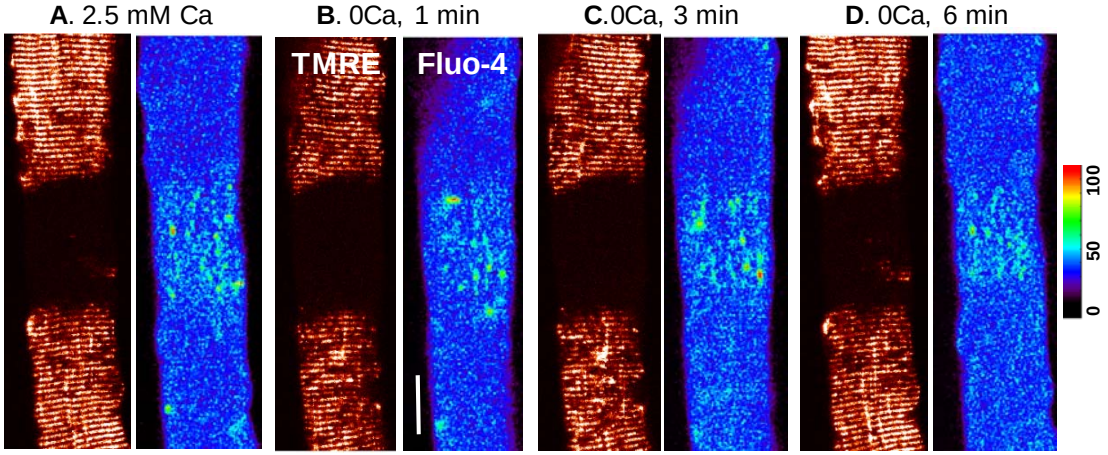
**Supplemental Data**

**Data 1**, **Eliminating external Ca does not stop osmotic stress-induced hyperactive Ca release in fiber segments with depolarized mitochondria in G93A muscle**.




*Supplemental figure 1* A G93A muscle fiber loaded with TMRE and fluo-4 AM was first exposed to 170-mosM hypotonic solution for 2 min, and then returned to Krebs. **A**, the induced release activity is widespread, but clearly greater in the area of lesion. Then Krebs was replaced with 0Ca solution*. **B**, **C** and **D** are images taken at 1, 3 and 6 min after the change. Note, the increased Ca activity in the fiber segment with depolarized mitochondria maintained in 0Ca solution. Bar, 20 μm.

*0Ca solution: a modified Krebs solution without addition of 2.5 mM $CaCl_2$, and 3.75 mM NaCl added to maintain the osmolarity.

*Note on data 1* Although our data show that the enhanced Ca release activity is likely due to the reduced mitochondrial Ca uptake, the present study does not exclude other potential contributions to the abnormal Ca transient in G93A muscle. One distinct possibility is that the increased Ca level is a consequence of increased membrane permeability to Ca in the area of mitochondrial lesion. We evaluated this possibility by testing whether eliminating external Ca could stop the osmotically-induced hyperactivity in the fiber segment with depolarized mitochondria. Ca release was first induced by osmotic shock. Then we quantified the effect of perfusion with a 0Ca solution on this activity by measuring *D/N*, the ration of mean fluorescence in the area of lesion over that in the normal area. On average in 4 experiments, *D/N* was 1.273 ± 0.048 in Krebs and 1.283 ± 0.083, 1.195 ± 0.023 and 1.152 ± 0.022 respectively after 1, 3 and 6 minutes in 0Ca. Thus, eliminating external Ca does not stop the hyperactive Ca release, suggesting that Ca entry is not a major contributor to the increased activity in the area of lesion.

**Data 2**, **Human SOD1 proteins enter mitochondria in muscle of adult mice**

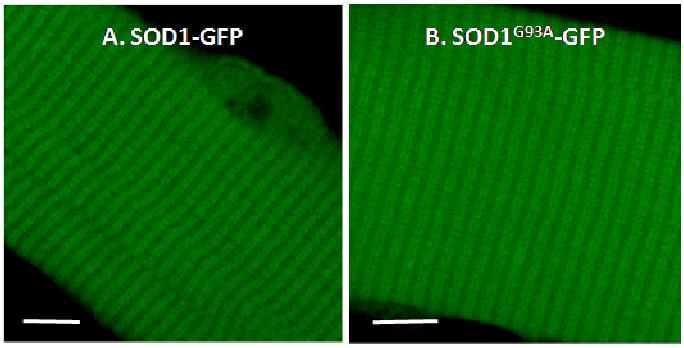
*Construction of the plasmid cDNA*

The pcDNA3.1/NT-SOD1-GFP and pcDNA3.1/NT-$SOD1^{G93A}$-GFP were generated using PCR. SOD1 and $SOD1^{G93A}$ cDNAs were amplified from pBK/SOD1 and pBK/$SOD1^{G93A}$ and ligated respectively with pcDNA3.1/NTGFP vector (Invitrogen). The resulting constructs were in frame fusion genes with GFP at the 3' terminal. Both constructs were confirmed by sequencing.

*Acute expression of foreign genes in skeletal muscle of adult mice by electroporation*

Using our established method (1), we acutely expressed SOD1-GFP and $SOD1^{G93A}$-GFP in FDB muscles of adult mice. Under anesthesia, 10 μl hyaluronidase (2 mg/ml) in saline are injected subcutaneously at the center of each paw. 1 hr later, 10 μl of saline containing 5 μg plasmid DNA are injected at the same site. 15 min later, 20 pulses of 100V/cm with duration of 20 ms are applied at 1 Hz.

*Supplemental figure 2*




Four days after the electroporation, transfected FDB muscles were dissected and digested. The enzyme-dissociated single muscle fibers were investigated using confocal microscopic imaging (**A, B**). Although the cytoplasm shows a basal green fluorescence, both SOD1-GFP and $SOD1^{G93A}$-GFP appear in regular arrays, as expected due to the localization of mitochondria in FDB muscle fibers, indicating more accumulation of these fusion proteins inside mitochondria. Bar: 10 μm.

*Note on data 2* SOD1 is a soluble protein residing largely in the cytoplasm with only a small portion transported into mitochondria (2). Normally, SOD1 is not essential for mitochondrial integrity, as mice with SOD1 deficiency do not show ALS-like symptoms or mitochondrial defects (3). The pathogenesis of ALS is probably due to a toxic effect of the mutant, rather than loss of SOD1 function (4). Studies in spinal cord neurons of transgenic mice (5, 6), show that WT SOD1 and $SOD1^{G93A}$ are co-localized in mitochondria at similar or higher concentrations as in the cytoplasm, which indicate that the mutant SOD1 was toxic inside mitochondria. The G93A mice express human SOD1 at high levels in muscle (7). A recent study showed that transgenic mice with muscle-restricted expression of $SOD1^{G93A}$ developed mitochondrial dysfunction in muscle (8). The mechanism by which mutant SOD1 leads to defective mitochondria in muscle is not yet known. The first question is whether mutant SOD1 reaches inside mitochondria in muscle. To test this possibility, we constructed human SOD1-GFP fusion genes and examined their expression in live muscle of mice. Four days after the transfection, both SOD1-GFP and $SOD1^{G93A}$-GFP were observed in muscle fibers with higher concentration inside mitochondria similar to one observed in the spinal cord. Our data provide a new line of evidence that SOD1 enters muscle mitochondria, where mutant SOD1 may exert a role in disrupting function.

*Supplemental references*

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