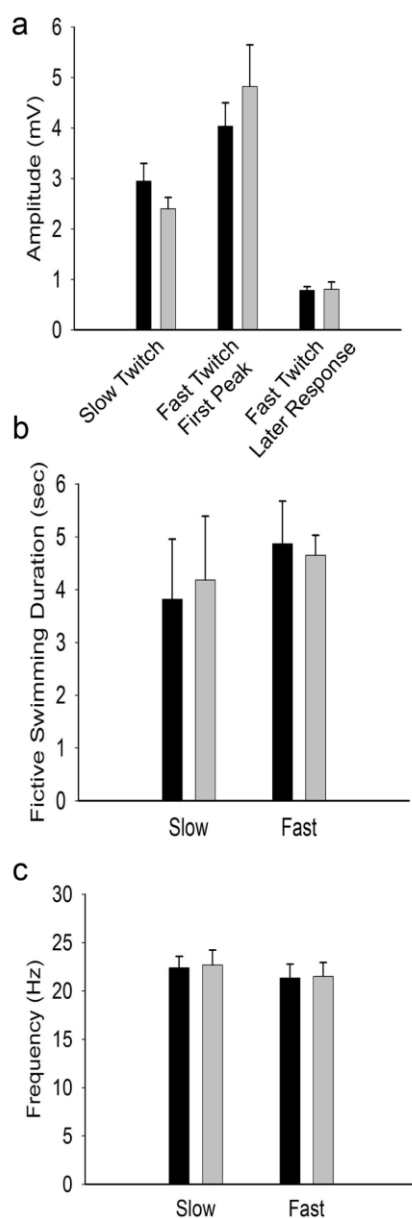
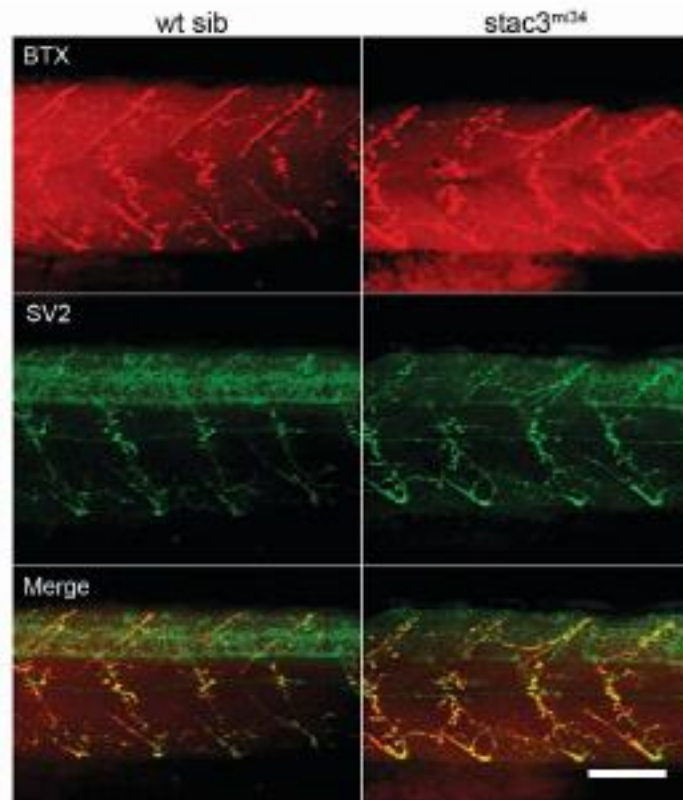


Supplementary Figure S1



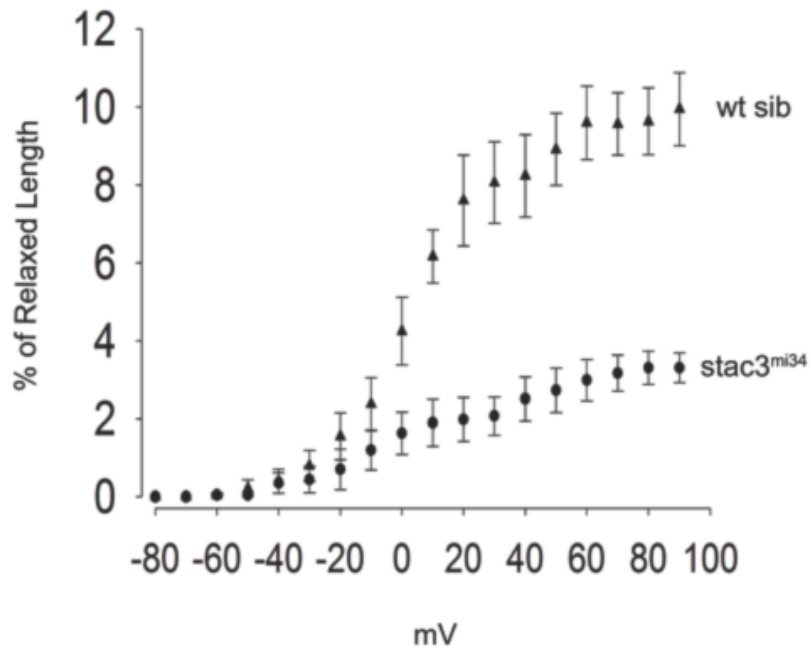
Supplementary Fig. S1 The synaptic responses of skeletal muscles initiated by tactile stimulation in 48 hpf embryos are comparable between *wtsib* and *stac3^{mi34}*. All recordings were made in 6 mM of curare to minimize muscle contractions (n=5 for slow twitch; n=6 for fast twitch) and *stac3^{mi34}* (n=6 for slow; n=4 for fast). Muscles responded to tactile stimulation with a burst of excitatory endplate potentials (epp; see Fig. 1b). (A) Histograms showing that the amplitude of epps in slow twitch muscles during a burst (left), peak amplitude of the 1st epp in a burst in fast twitch muscles (middle) and the peak amplitude of epps recorded for a duration of 1 sec starting 1 sec after the 1st epp (right) were comparable between *wtsib* and mutants. (B) Histograms showing that the duration of the synaptic bursts in slow and fast twitch muscles were comparable between *wtsib* and mutants. (C) Histograms showing that the frequencies of epps within bursts in slow and fast twitch muscles were comparable. Error bars represent standard error of the means.

Supplementary Figure S2



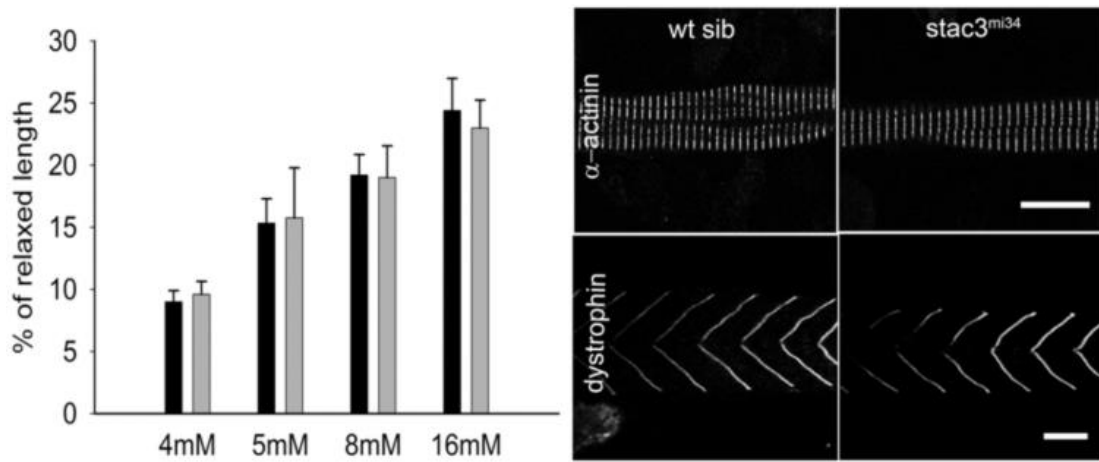
Supplementary Fig. S2 Distribution of the neuromuscular junction (NMJ) is unperturbed in *stac3^{mi34}* embryos. Sideview of the trunk of a 48 hpf wt sib and *stac3^{mi34}* embryo showing bungarotoxin-Alexa594 (BTX) labeled distribution of AChRs (red) is normal (top) as is the anti-SV2 (green) labeled presynaptic terminals (middle). The BTX and anti-SV2 labeled panels are merged to show co-localization (bottom). Scale: 60 μ m.

Supplementary Figure S3



Supplementary Fig. S3 Depolarization initiated muscle contraction is decreased in *stac3^{mi34}* embryos at 48 hpf. Muscle fibers were voltage clamped *in vivo* to different membrane potentials for 200 ms and imaged at 60 Hz to measure the amount of contraction as a % of the relaxed fiber length. Wt sib fibers (n=5) and *stac3^{mi34}* fibers (n=4) are denoted by triangles and circles, respectively. The difference in contraction was first significant ($p < 0.01$) starting at 10 mV through to 90 mV. Error bars represent standard error of the means.

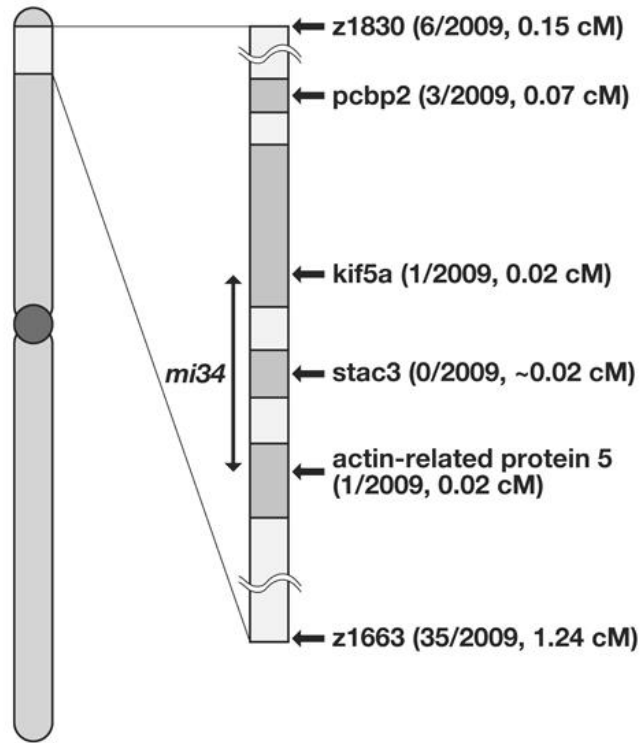
Supplementary Figure S4



Supplementary Fig. S4 Caffeine induces skeletal muscle contractions that were similar between wt sib and *stac3^{mi34}* embryos at 48 hpf. Left, histogram of contraction induced by caffeine in the presence of 50 mM curare (n=5 each for wtsib and mut for 4mM, 8mM and 16 mM caffeine; n=4 each for wtsib and mut for 5mM caffeine). Right, the distribution of α -actinin and dystrophin are comparable in *stac3^{mi34}* and wt sib embryos. Anti- α -actinin labeling is shown on dissociated muscle fibers (scale: 10 μ m) while anti-dystrophin is on wholemounted embryos (scale: 60 μ m). Error bars represent standard error of the means.

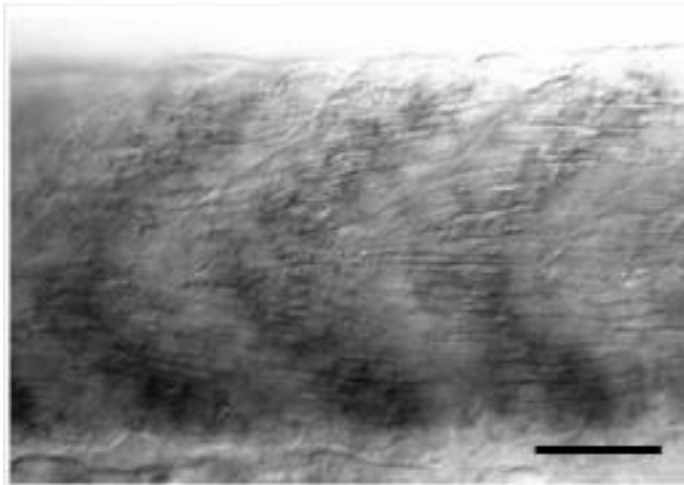
Supplementary Figure S5

Chromosome 9



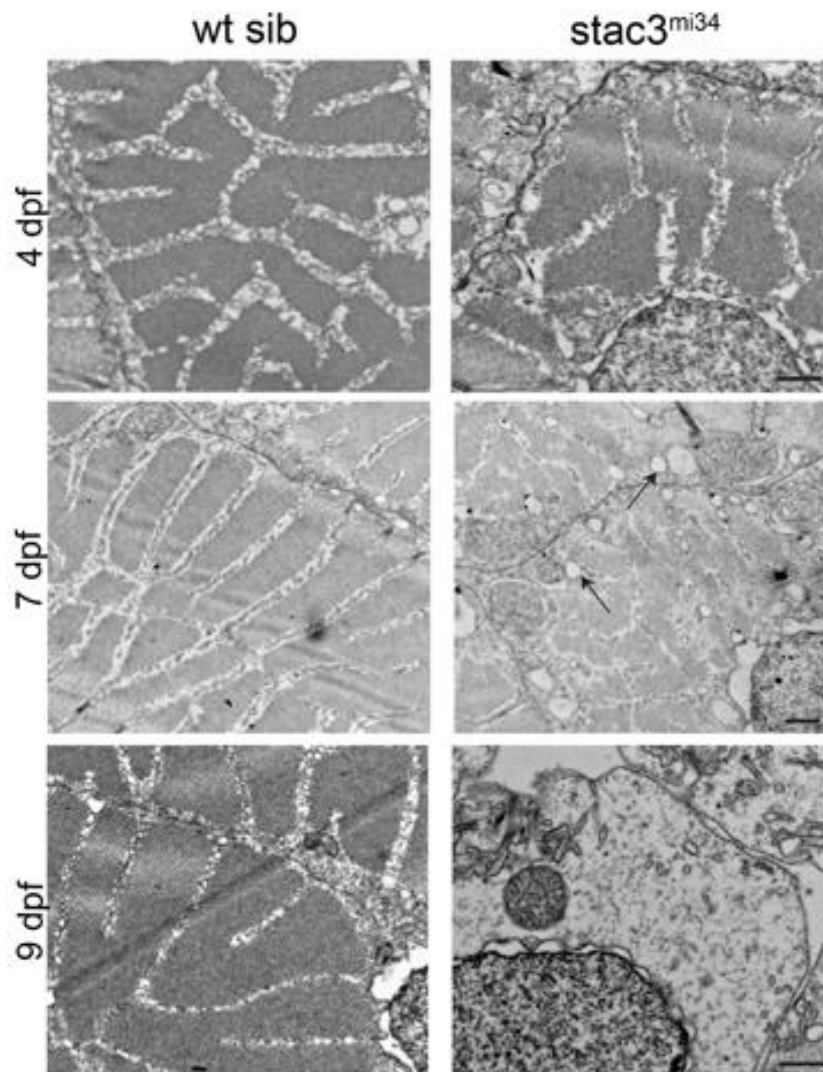
Supplementary Fig. S5 Meiotic mapping of the *stac3*^{mi34} locus to the *stac3* gene. Initial meiotic mapping of *stac3*^{mi34} located it to chromosome 9 between markers z1830 and z1663. Numbers in parentheses denote x recombinants in y mutants. A sequenced 156 kb BAC (CR848672) was identified that contained z1830 and high resolution mapping identified new markers (zh1, zh34 and zh16) that flanked the mutation within the BAC. A 4th marker, zh31, located in the *stac3* gene showed no recombination with the mutation out of 2009 mutant embryos suggesting that the mutant locus was *stac3*.

Supplementary Figure S6



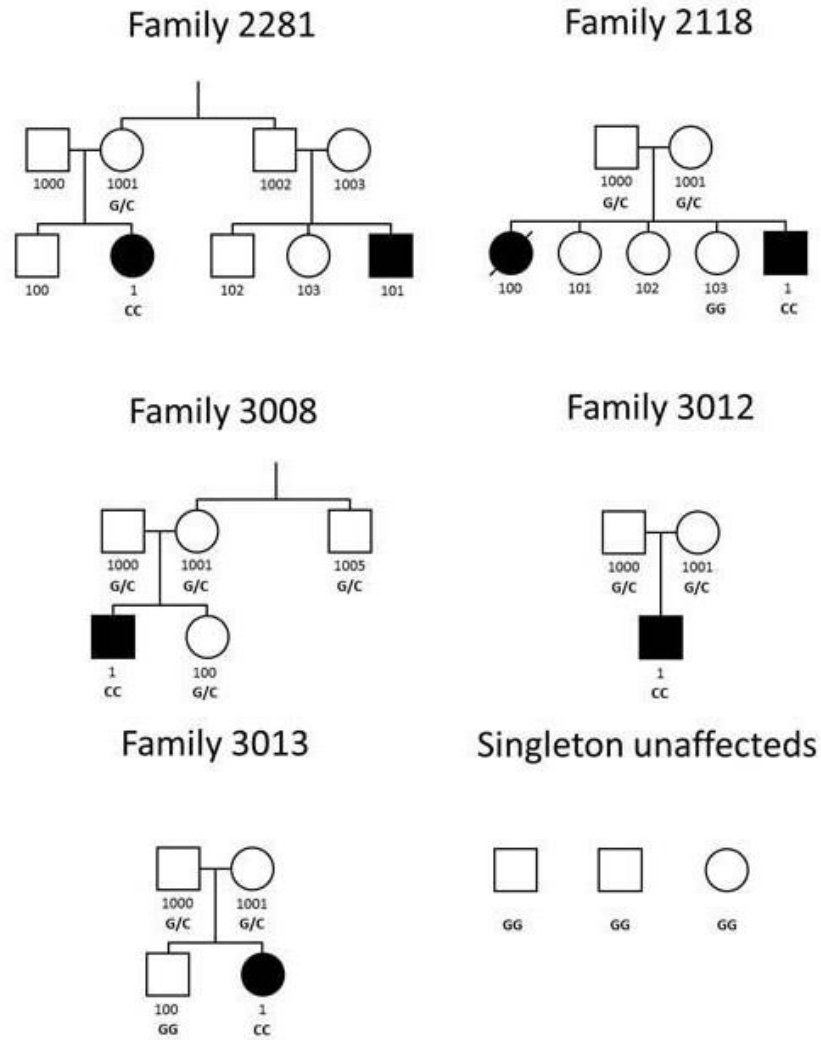
Supplementary Fig. S6 *stac3* is expressed specifically by skeletal muscles in zebrafish embryos. Sideview of the trunk of a wildtype embryo (27 hpf) labeled with an antisense riboprobe against *stac3* mRNA showing several segments of *stac3*-positive skeletal muscles. *stac3* was not expressed in any other cells at this stage. The sense riboprobe did not label any cells (not shown). Scale: 60 μ m.

Supplementary Figure S7



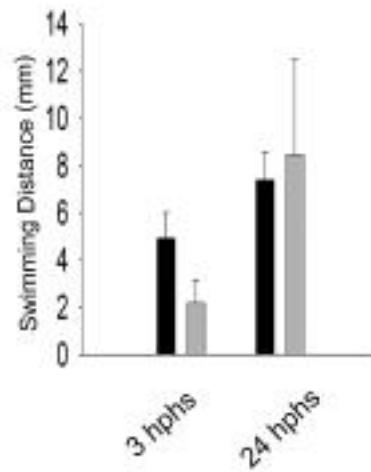
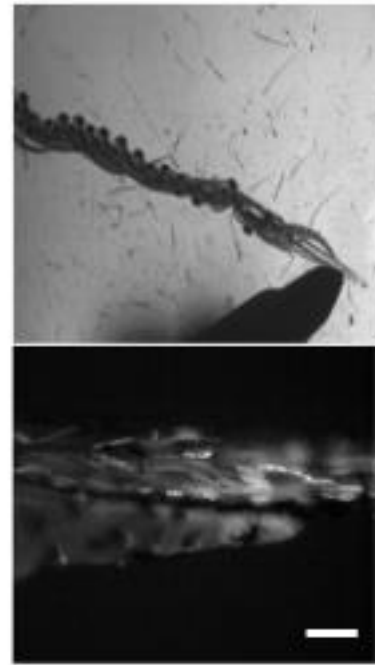
Supplementary Fig. S7 Skeletal axial muscles from *stac3^{mi34}* mutants exhibit progressively defective SR. Electron micrographs of SR (arrayed circular profiles) from wtsib and mutant skeletal muscles showing that mutant muscles have relatively normal SR at 4 dpf and swollen ones (arrows) by 7 dpf. Mutant myofibers are breaking down at 9 dpf. Scale: 500 nm.

Supplementary Figure S8



Supplementary Fig. S8 Pedigrees of 5 families with individuals exhibiting Native American myopathy (black) and 3 unaffected singletons. Sequenced individuals and their genotype are denoted as G/C, GG & CC. Five of the 18 family members were affected and 13 not.

Supplementary Figure S9



Supplementary Fig. S9 Expression of human wt *stac3* by skeletal muscles of *stac3^{mi34}* embryos rescues the mutant behavior. Top, superimposed frames (30 Hz) showing swimming by a *stac3^{mi34}* mutant embryos injected with heat inducible constructs for human *stac3* fused to EGFP following heat induction. Middle, sideview of the trunk of a *pHSP70:hstac3^{wt}-egfp* injected *stac3^{mi34}* embryo showing expression of human Stac3-EGFP in myotomes in the embryo shown above (scale: 60 μ m). Bottom, histogram showing that mutant embryos expressing human Stac3-EGFP (gray, n=4) swim as effectively as mutant embryos expressing zebrafish Stac3-EGFP (black, n=32). Error bars represent standard error of the means.

Supplementary Table S1

Selected Identified Proteins by MS/MS

Selected Identified Proteins	Accession Number	Molecular Weight	# Unique Peptides Matched
Stac3	IPI00493641	39 kDa	220
Green Fluorescent Protein	CON_GFP	27 kDa	206
RyR1b	IPI00771673	575 kDa	9
DHPR α 2 δ 1	IPI00498816	121 kDa	5
DHPR α 1 β	IPI00493269	209 kDa	4
RyR3	IPI00897644	551 kDa	3
DHPR β 1	IPI00627451	57 kDa	2

Supplementary Table S1. Selected zebrafish proteins identified from MS/MS analysis after co-immunoprecipitation with anti-GFP of skeletal muscle lysates from transgenic *α -actin:stac3-gfp* zebrafish. Peptides were identified at greater than 95% probability and proteins were identified if they contained at least two identified peptides with each peptide identified in the protein at greater than 95% probability. Probabilities of protein assignments were determined by the Protein Prophet algorithm (see Methods).