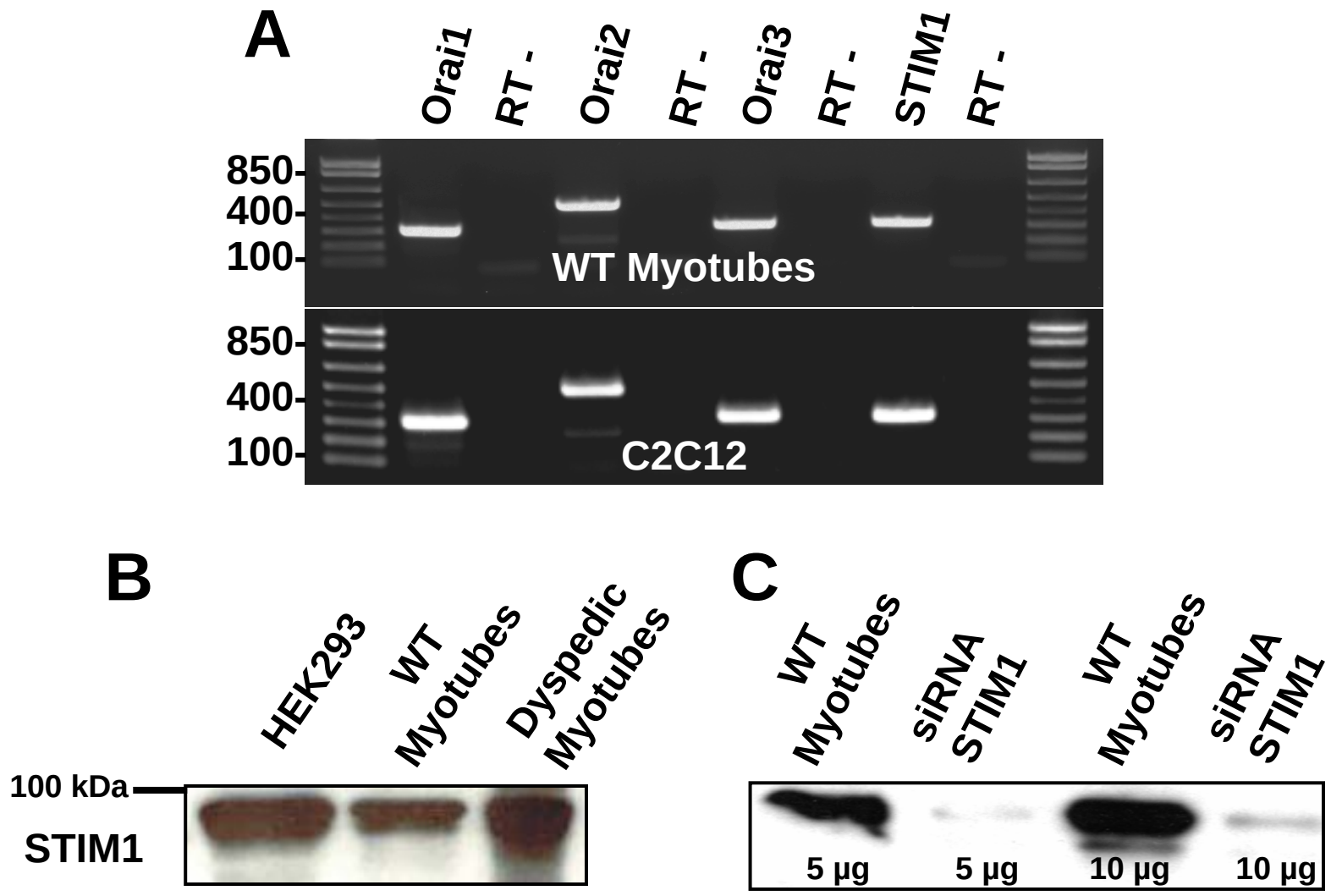
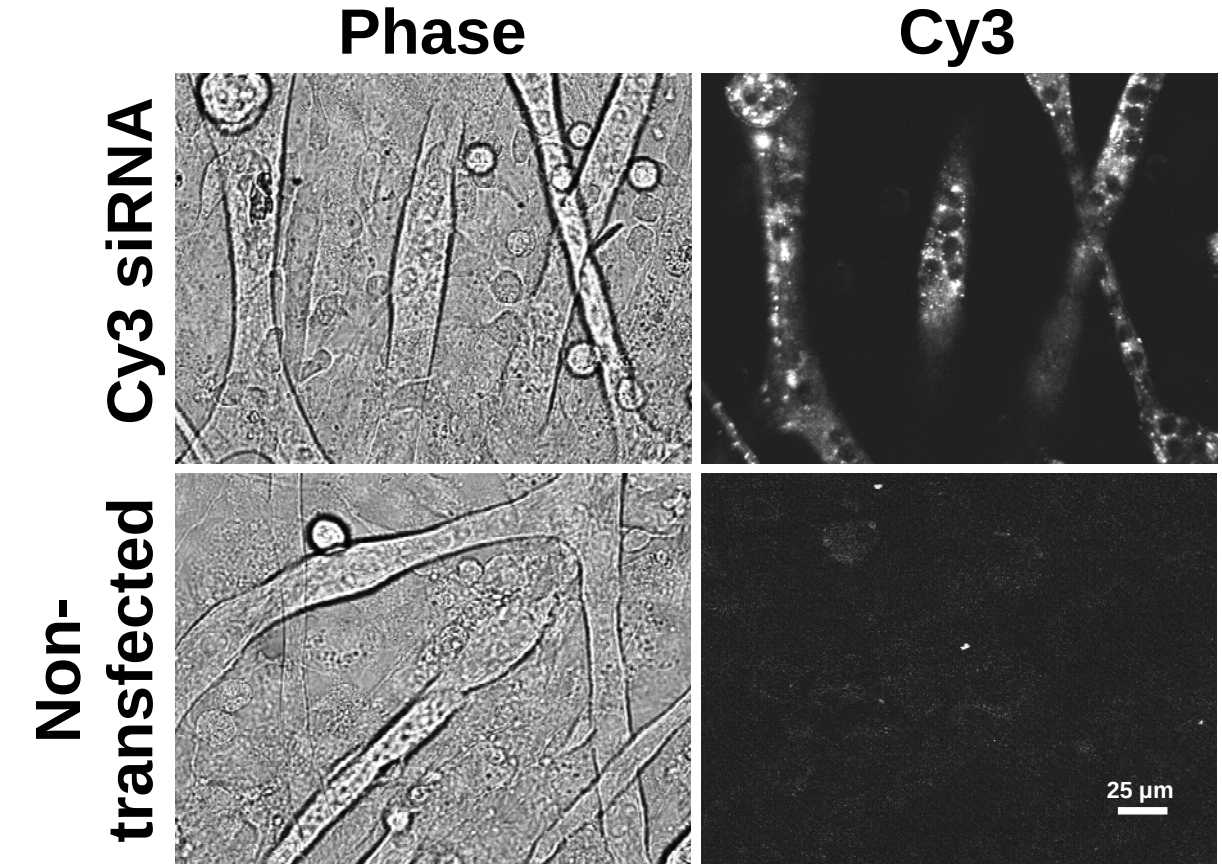

A
Orai1
RT -
Orai2
RT -
Orai3
RT -
STIM1
RT -
850
400
100
WT Myotubes
850
400
100
C2C12
B
HEK293
WT Myotubes
Dyspedic Myotubes
100 kDa
STIM1
C
WT Myotubes
siRNA STIM1
WT Myotubes
siRNA STIM1
5 µg
5 µg
10 µg
10 µg

Supplementary Figure 1. STIM1 and Orai1-3 expression in skeletal muscle cells. *A)* Representative RT-PCR results from total RNA isolated from primary skeletal myotubes (*upper*) and C2C12 cells (*lower*) using primers specific for Orai1 (lanes 2 and 3), Orai2 (lanes 4 and 5), Orai3 (lanes 6 and 7), and STIM1 (lanes 8 and 9). Lanes 1 and 10 are standards. RT-PCR reactions were run both in the presence (lanes 2, 4, 6, and 8) and absence of reverse transcriptase (RT-; lanes 3, 5, 7, and 9) to control for potential amplification of genomic DNA. *B)* Representative STIM1 Western blot in HEK293 cells (lane 1), naïve myotubes (lane 2), and dyspedic myotubes (lane 3). *C)* Representative Western blot for STIM1 expression in parallel cultures of naïve myotubes (lanes 1 and 3) and STIM1 siRNA-transfected myotubes (lanes 2 and 4). 5 μg of total protein was added to lanes 1 and 2 and 10 μg of total protein was added to lanes 3 and 4. STIM1 siRNAs transfection resulted in >90% reduction in STIM1 protein.


Phase
Cy3
Cy3 siRNA
Non-transfected
25 µm

Supplementary Figure 2. Efficiency of siRNA transfection of primary myotubes. Representative phase (*left*) and fluorescence (*right*) micrographs of Cy3-labeled siRNA transfected (*upper*) and mock transfected (*lower*) myotubes. Approximately 100% of Cy3-transfected myotubes are fluorescent during 543 nM excitation while non-transfected myotubes are not significantly fluorescent under identical conditions.

Supplementary Table 1

Table 1. siRNA Sequences

Target	siRNA sequences
STIM1	Sense: UACAGUGGCUCAUUACGUAUU Antisense: 5'-P.UACGUAAUGAGCCACUGUAUU Sense: GAUCGGAGCCACAGGCAGAUU Antisense: 5'-P.UCUGCCUGUGGCUCCGAUCUU Sense: AAACAUAGCACCUUCCAUGUU Antisense: 5'-P. CAUGGAAGGUGCUAUGUUUUU Sense: GAAGUAGGCAGACUAGGGUUU Antisense: 5'-P. ACCCUAGUCUGCCUACUUCUU
Negative Control	Sense: 5'-UAACGACGCGACGACGUAA-3' Antisense: 5'-UUACGUCGUCGCGUCGUUA-3'

Supplementary Table 2

Table 2. Isoform-Specific STIM1 and Orai1 Primers

Protein	Primers
STIM1	Forward: TGGAGCTGCCACAGTATGAG Reverse: CCCTTCCAGATCCTTCATCA
Orai1	Forward: GCCAGAGTTACTCCGAGGTG Reverse: ACCGAGTTGAGGTTGTGGAC
Orai2	Forward: CACTGTCCTGGAGGAAGCTC Reverse: CGAAGATGAGACCCACAGGT
Orai3	Forward: GCTAAGCTCAAAGCCTCCAG Reverse: TTCAGCCAGGAAGAGAAAGG