SUPPLEMENTARY MATERIALS

Interaction between mitsugumin 29 and TRPC3 participates in regulating Ca^{2+} transients in skeletal muscle

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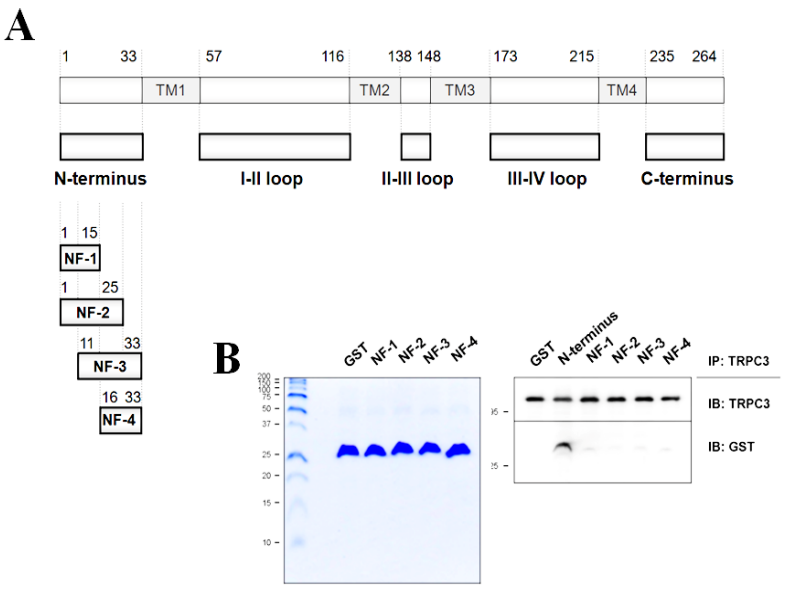
Supplementary Material 1. PCR primers for the cloning of GST-fused MG29 portions or GFP-fused MG29 deletion mutants.

	PCR primers	
GST-N-terminus	forward	5'– GCGAATTCATGTCCTCGACGGAGAGCCCC –3'
	backward	5'– GCGTCGACTTACTCCTCCAGGC –3'
GST-I-II loop	forward	5'– GCGAATTCATGAGCGGGGAGAC –3'
	backward	5'– GCGTCGACTTACTCGGCGGGG –3'
GST-II-III loop	forward	5'– CGAATTCATGCGCTTCCACAAACTC –3'
	backward	5'– GCGTCGACTTAGCGTTTGTTCTCTGT –3'
GST-III-IV loop	forward	5'– GCGAATTCATGAAGGGCTTGACTGACGTC –3'
	backward	5'– GCGTCGACTTAAGAGAGGTTAGCCAGCCCC –3'
GST-C-terminus	forward	5'– GCGAATTCATGAAAGAGACCCCG –3'
	backward	5'– GCGTCGACTTACTGCTTCTCCACTGCCCCC –3'
GST-NF-1	forward	5'– GCGAATTCATGTCCTCGACGGAGAGCCCC –3'
	backward	5'– GCGTCGACTTACGGAGACTTGTCC –3'
GST-NF-2	forward	5'– GCGAATTCATGTCCTCGACGGAGAGCCCC –3'
	backward	5'– GCGTCGACTTACCCCAGGAGCAGGC –3'
GST-NF-3	forward	5'– GCGAATTCATGTCGGACAAGTCTCC–3'
	backward	5'– GCGTCGACTTACTCCTCCAGGC –3'
GST-NF-4	forward	5'– GGGAATTCATGCGCCAGCAGGTGGACCG –3'
	backward	5'– GCGTCGACTTACTCCTCCAGGC –3'
GFP-Δ33-MG29	forward	5'– GGGGATCCGGATGCCGCTGGGCTTCATC –3'
	backward	5'– GCGATATCTTACTGCTTCTCCACTGCCC –3'
GFP-Δ116-MG29	forward	5'– GCGGATCCGGATGTTCTTTGTGACCCTT –3'
	backward	5'– GCGATATCTTACTGCTTCTCCACTGCCC –3'

Supplementary Material 2. Co-immunoprecipitation of TRPC3 with each smaller portion of the N-terminus in MG29. (A) Schematic diagrams of the smaller portions of the N-terminus in MG29. Numbers indicate the sequence of amino acids. TM indicates transmembrane domain. **(B)** Immobilized GST-fused smaller portions of the N-terminus were separated on SDS-PAGE gel (12%), and the gel was stained with Coomassie Blue. Each portion was successfully expressed in E. coli (left). Co-immunoprecipitation of TRPC3 with each smaller portion of the N-terminus was conducted using a solubilized triad sample containing TRPC3, the lysate sample of E. coli expressing each GST-fused smaller portion, and anti-TRPC3 antibody. The protein complex was separated on SDS-PAGE gel (12%), and the gel was subjected to immunoblot assay with anti-TRPC3 or anti-GST antibody (right). GST was used as a negative control. IP or IB translates to immunoprecipitation or immunoblot. Three independent experiments were conducted and a representative result is presented. None of the smaller portions of the N-terminus were bound to TRPC3, suggesting that the intact N-terminus of MG29 is required for the binding of MG29 to TRPC3.

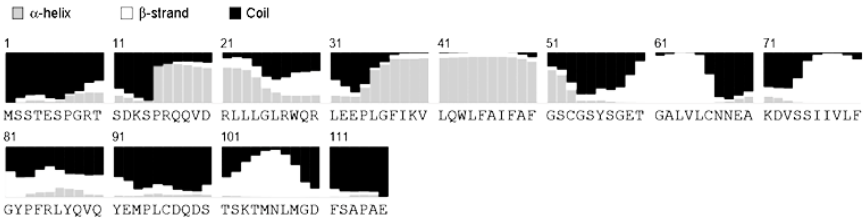


Supplementary Material 3. Prediction of the secondary and three-dimensional (3D) structure or the ordered status of the TRPC3-binding region in MG29 (1-116 amino acids), and the prediction of phosphorylation site in the un-structured random coil in the N-terminus of MG29. Based on mouse MG29 cDNA (GenBank accession No. AB010140.1), the secondary **(A)** and 3D structures of the TRPC3-binding region in MG29 (1 to 116 amino acids) **(B)** were predicted using RaptorX[†] which is a prediction program/server for the 3D structure of proteins by homology searches in amino acid sequences with other proteins with 3D structures that have been deposited in the Protein Data Bank (PDB). **(A)** The α-helix, β-strand, and coil (β-turn or un-structured random coil) are colored in gray, white, and black, respectively. Numbers indicate the sequence of amino acids. **(B)** The 3D structure of the TRPC3-binding region in MG29 was predicted on the basis of the structure of a malate dehydrogenase (PDB accession No. 3NEP), and was presented as a ribbon diagram using the RasMol program (http://www.rasmol.org/). There are an un-structured random coil and a short α-helix in the N-terminus, and 3 tandem β-strands in the I-II loop. The α-helix, β-strand, β-turn, or un-structured random coil are presented as red helix, yellow strand, blue loop, or white loop, respectively. N or C translates as the N- or C-terminal end. TM1 is the first transmembrane. The right-hand image is the 90° counterclockwise rotation of the left-hand image along the vertical axis of the view. **(C)** The ordered status of each amino acid in the TRPC3-binding region in MG29 was predicted using the RaptorX program/server. The un-structured random coil in the N-terminus (20 amino acids from the first of MG29, colored in reddish brown) was predicted to exist as an intrinsically disordered state with high confidence scores. **(D)** The phosphorylation site for the un-structured random coil in the N-terminus was predicted using the NetPhos program/server[‡] that is a sequence- and structure-based prediction program of eukaryotic protein phosphorylation sites. Position numbers indicate the sequence of amino acids. Four amino acids in the un-structured random coil (20% of the total amino acids) were predicted as possible phosphorylation sites with high confidence scores: three serines (6th, 11th, or 14th amino acid colored in purple) and one threonine (10th amino acid colored in green).

[†] Kallberg, M., Wang, H., Wang, S., Peng, J., Wang, Z., Lu, H. & Xu, J. 2012. Template-based protein structure modeling using the RaptorX web server. *Nat Protoc*, **7,** 1511-22.

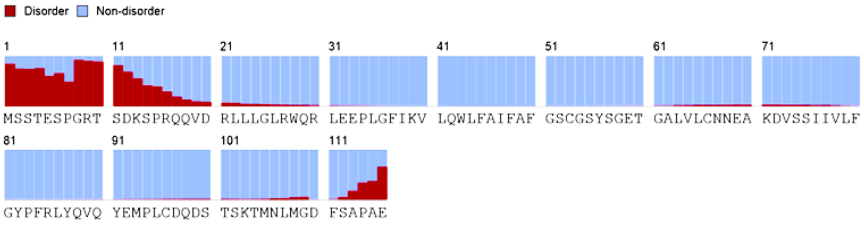
[‡] Blom, N., Gammeltoft, S. & Brunak, S. 1999. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. *J Mol Biol*, **294,** 1351-62.

A



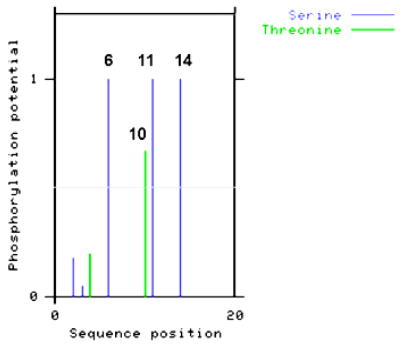
B

C



D

	Position	context	Confidence score	Predicted residue
		v		
Sequence	6	SSTESPGRT	0.997	*S*
Sequence	10	SPGRTSDKS	0.666	*T*
Sequence	11	PGRTSDKSP	0.998	*S*
Sequence	14	TSDKSPRQQ	0.998	*S*
		^		



Supplementary Material 4. Normal formations of junctional membrane complex (JMC) in mouse primary skeletal myotubes expressing Δ116-Mg29. (A) JMC in the myotubes expressing wild-type MG29 or Δ116-MG29 was observed using a transmission electron microscopy using methods as previously described[§]. The SR indicates sarcoplasmic reticulum, and the white arrowhead indicates transverse (t)-tubule. Normal existence of the juxtaposed t-tubule and SR membranes was found in the myotubes expressing Δ116-MG29. **(B)** In the myotubes expressing Δ116-MG29, evenly spaced feet[#] between the t-tubule and the SR membranes (indicated by black arrows) were also easily found. Therefore, there was no significant change in the overall JMC formations by Δ116-MG29. *Bars* represent 0.2 μm.

[§] J.S. Woo, C.H. Cho, K.J. Lee, H. Kim do, J. Ma, E.H. Lee, Hypertrophy in skeletal myotubes induced by junctophilin-2 mutant, Y141H, involves an increase in store-operated Ca^{2+} entry via Orai1, J Biol Chem 287 (2012) 14336-14348.

[#] C. Franzini-Armstrong, STUDIES OF THE TRIAD : I. Structure of the Junction in Frog Twitch Fibers, J Cell Biol 47 (1970) 488-499.