Supplemental Materials

Molecular Biology of the Cell

Qadota et al.

Supplemental Table 1: List of oligonucleotide primers and their purposes

Name	Property	Sequence
GK1	UNC-89 from ATG, +BamHI, Forward	GGCGGATCCATGGCTAGTCGACGCCAAAAG
GK2	deltaSH3, Forward	TCCAGTGTACATTGCGCCGACCGAGTTTTACA
GK3	deltaDH, Forward	ACAGGCGGCTCTTGTCCTAGAATTCACCAATA
GK4	deltaPH, Forward	GCAACATCCAGGAGATGCAGAGGAGCAGGAGA
GK5	until 1500 bp, +STOP, +XhoI, Reverse	CGGCTCGAGTTAACCAGGGAACAAGCTGCTCTT
GK6	deltaSH3, Reverse	TGTAAAACTCGGTCGGCGCAATGTACACTGGA
GK7	deltaDH, Reverse	TATTGGTGAATTCTAGGACAAGAGCCGCCTGT
GK8	deltaPH, Reverse	TCTCCTGCTCCTCTGCATCTCCTGGATGTTGC
GK9	until SH3+27aa +XhoI, Reverse	CGCCTCGAGTTAGACAAGAGCCGCCTGTTCATC
GK10	UNC-15 from aa221, +BamHI, Forward	GCGGGATCCCAGAAGGTTCAACTCGATAAC
GK11	until end of UNC-15, +SalI, Reverse	CGCGTCGACTTAATAATCGTCTTCCGTGAC
GK12	sequencing	ACAGAAGGAGGCTCTTGC
GK13	sequencing	GCTCTTGCTCGCGAGAAC
GK14	sequencing	AGCGGCGACCTTGCGTTG
GK15	-unc-15(-446)-SalI	CGCGTCGACTTAGGCGTCGGCAAGAGCCTCCTT
GK16	BamHI-unc-15(294-)	GCGGGATCCGAGATCACCCAGTGGAAGAGC
GK17	-unc-15(-373)-SalI	CGCGTCGACTTATCTGGCACGCTCCAAGAGAGC
GK18	-unc-15(-376)-SalI	CGCGTCGACTTAGAGTTGTTCTCTGGCACGCTC
GK19	BamHI-unc-15(365-)	GCGGGATCC ACCATTGCTCTCTTGGAGCGT
GK20	BamHI-58-	GCGGGATCCGGATCGGAATCCCGATCTTAT
GK21	-131-STOP-XhoI	CGCCTCGAGTTATTGCTTGTAAAACTCGGTCGG
GK22	PK2-SalI	CGCGTCGACTTATTTTCCATATTTCGACTC
GK23	SmaI-IK(1/3)	GCGCCCGGGTCTCCACGCCGTTCCACTCCA
GK24	MscI-BamHI-SmaI-IK(1/3), with IK-PK2-4	GCGTGGCCAGGATCCGCGGCCGCCCCGGGTCTCCACGCCGTTCCACTCCA
GK25	SH3+27-SmaI, with BamHI-unc-89(N)	CGCCCCGGGGACAAGAGCCGCCTGTTCATC
GK26	EcoRV-ATG	GCGGATATCATGGCTAGTCGACGCCAAAAG

Supplemental Table 2: Unc-89 SH3/DH/PH Region Yeast Two Hybrid

Table 2A: Number of Colonies

# Colonies Screened	442,600
# His+	1315
# His+ and Ade+	85
# His+ Only (retransformation)	4
# Ade+ Only (retransformation)	3
# His+ and Ade+ (retransformation)	15

Table 2B: Positive Preys

Clone	His+ and/or Ade+	Gene	Region (nucleotide #)	Region (amino acid #)
1-1A	His+	unc-15	681-2619	227-873
5-2A	Ade+	unc-15	880-2619	294-873
6-1A	Ade+	unc-15	880-2619	294-873
6-2A	His+ and Ade+	unc-15	693-2619	231-873
7-2A	His+ and Ade+	unc-15	746-2619	249-873
10-1A	His+ and Ade+	unc-15	831-? *	277-?
12-2A	His+ and Ade+	unc-15	746-2619	249-873
14-2A	Ade+	unc-15	746-2619	249-873
4-6A	His+ and Ade+	unc-15	750-2619	250-873
6-5A	His+ and Ade+	unc-15	887-? **	291-?
7-4A	His+ and Ade+	unc-15	720-2619	240-873
8-4A	His+ and Ade+	spd-2	242-1685	161-562
8-10A	His+ and Ade+	lev-11	Isoform C: 407-771; Isoform G: 564-855	Isoform C: 136-257; Isoform G: 188-348
9-3A	His+ and Ade+	unc-15	663-2619	221-873
9-8A	His+ and Ade+	unc-15	741-2619	247-873
10-5A	His+ and Ade+	unc-15	831-? *	277-?
10-6A	His+ and Ade+	unc-15	831-? *	277-?
10-8A	His+	unc-15	?-2619	?-873
10-8B	His+	unc-15	880-2619	294-873
12-3A	His+	unc-15	845-?***	282-?
14-3A	His+ and Ade+	unc-15	750-2619	250-873
14-4A	His+ and Ade+	unc-15	746-2619	249-873

* DNA sequencing of 10-1A 3` yielded Y41D4A.5 mRNA

** No results from DNA sequencing found for 6-5A 3`

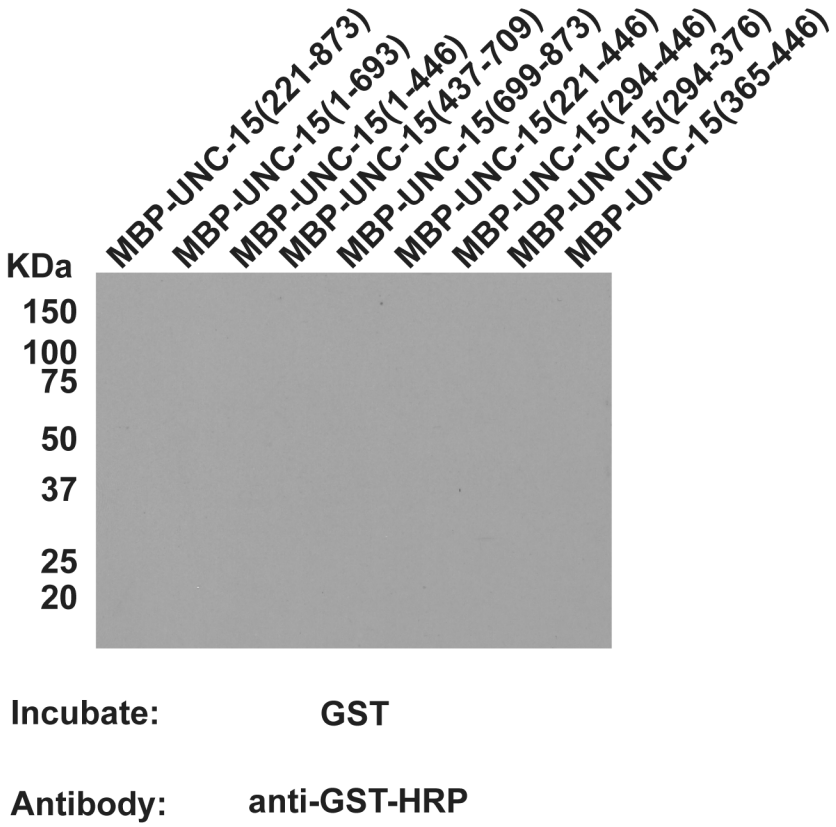
*** 3' sequence is not aligned

ONLINE SUPPLEMENTAL MATERIAL

Table 1. List of oligonucleotide primers and their purposes.

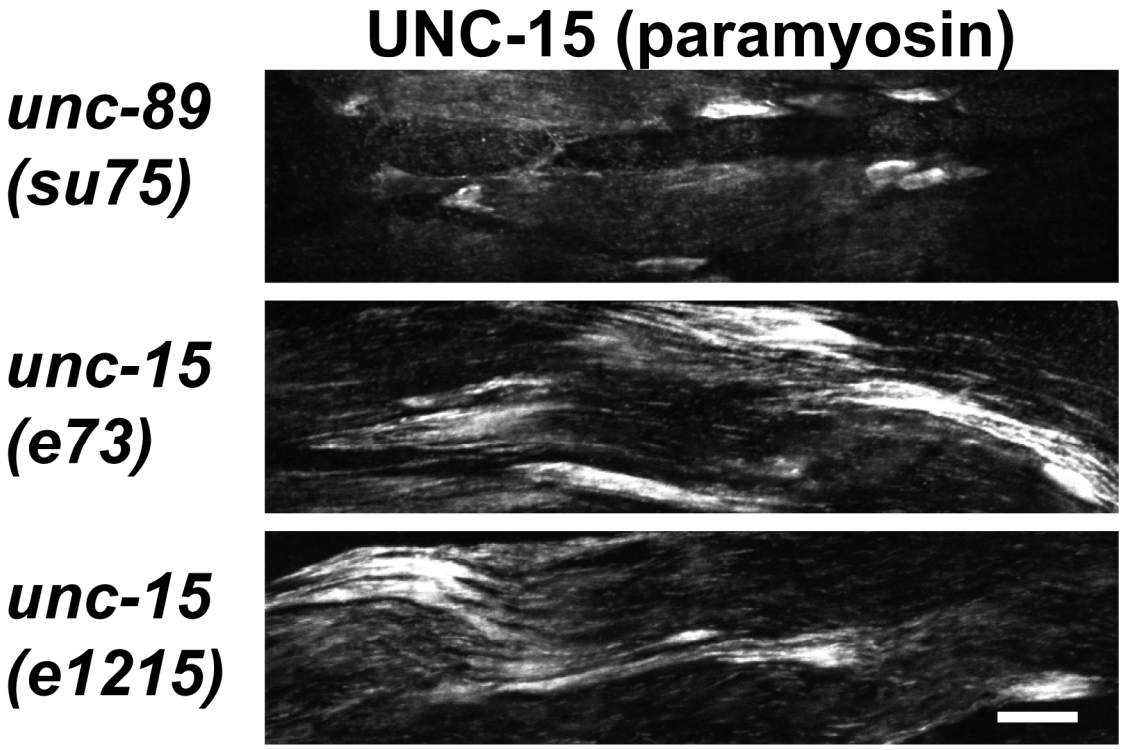
Table 2. Results of yeast 2-hybrid library screen using UNC-89 SH3-DH-PH

Supplemental figure 1



Supplemental Figure 1. Far western shows that GST does not interact with any portion of paramyosin. The indicated portions of UNC-15 (paramyosin) as MBP fusion proteins were separated by SDS-PAGE, transferred to a membrane, incubated with GST in solution, washed, incubated with anti-GST-horseradish peroxidase, washed and detected by ECL. Positions of molecular weight standards are indicated on the left.

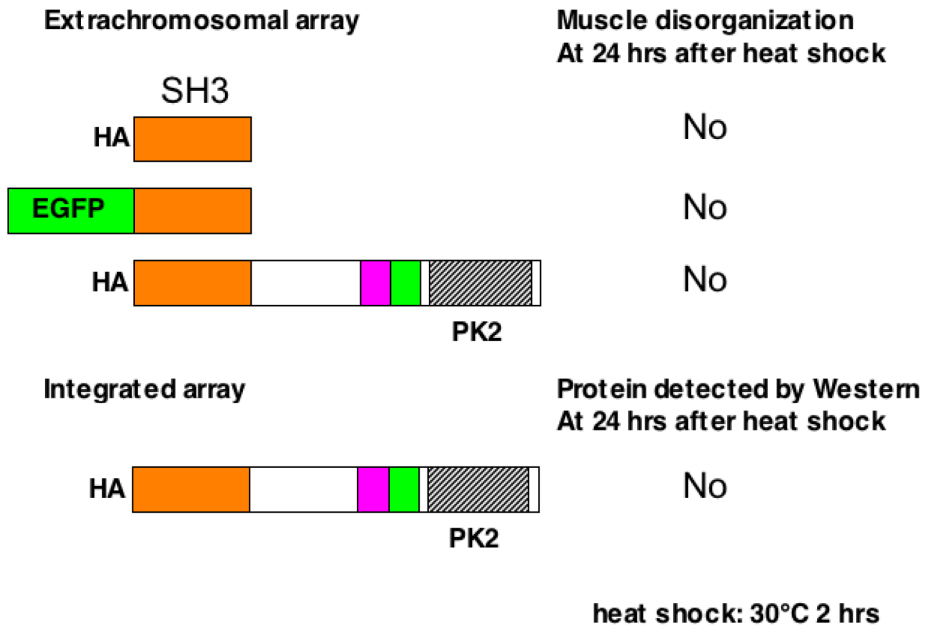
Supplemental figure 2



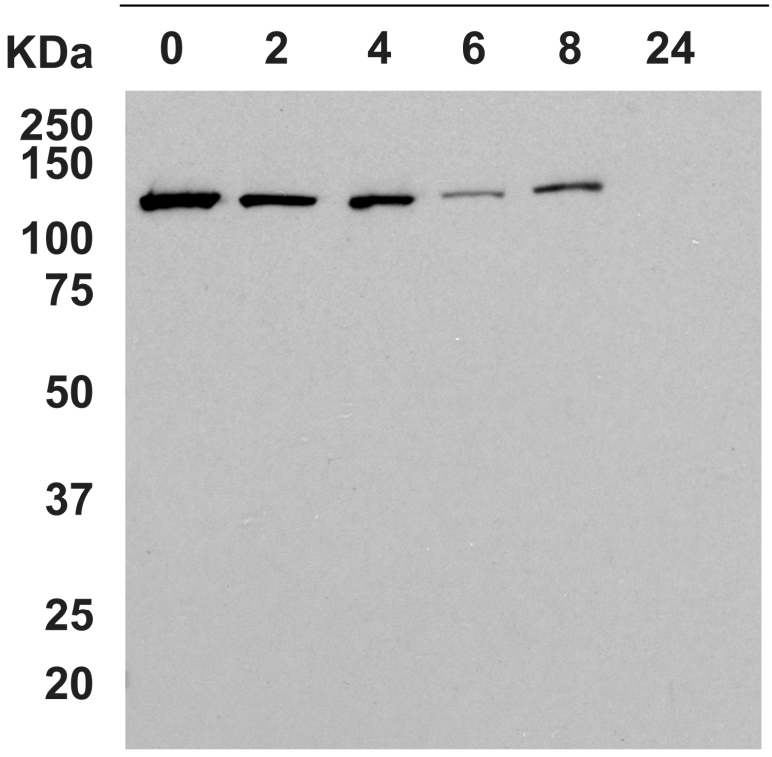
Supplemental Figure 2. Less severe accumulations of UNC-15 (paramyosin) in *unc-89(su75)* than there is in two *unc-15* missense alleles. Immunostaining of the indicated strains with anti-paramyosin. Several body wall muscle cells are shown. Scale bar, 10 μm.

Supplemental figure 3

A

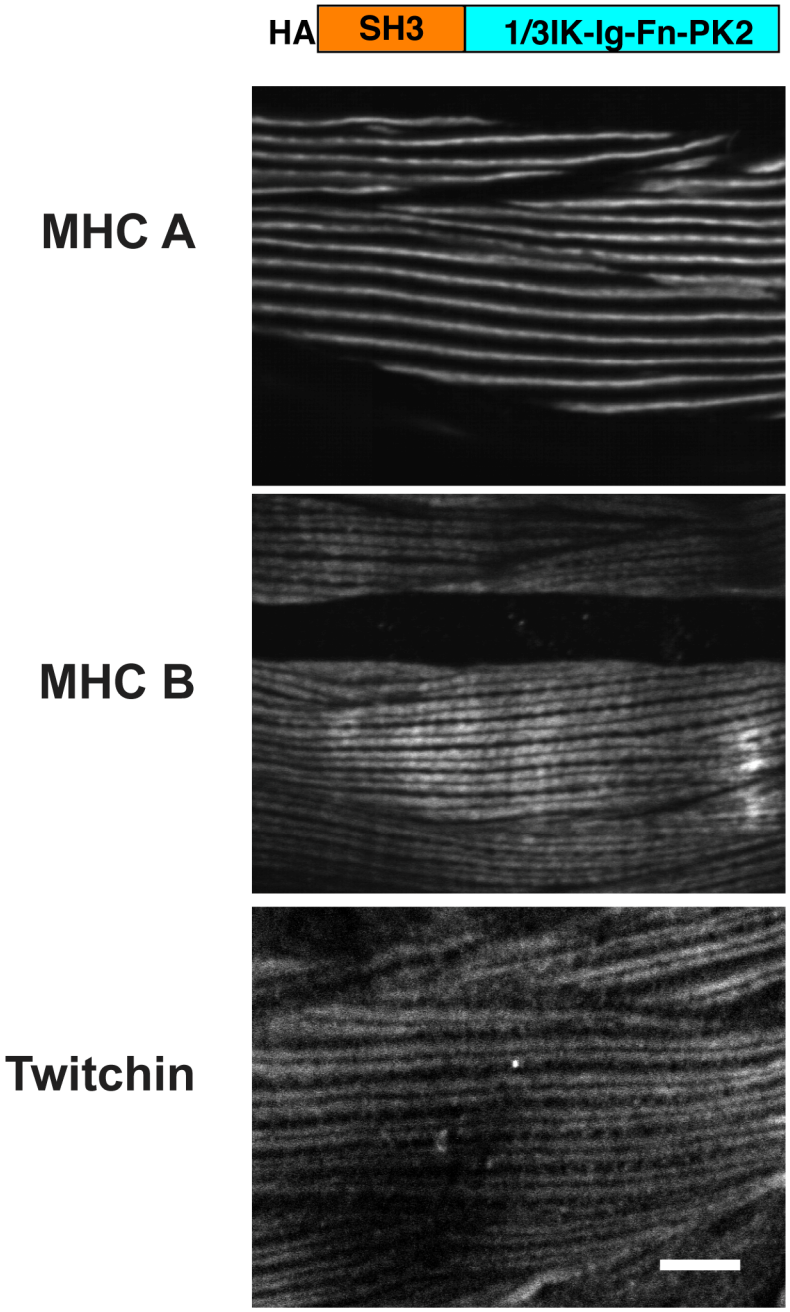


B



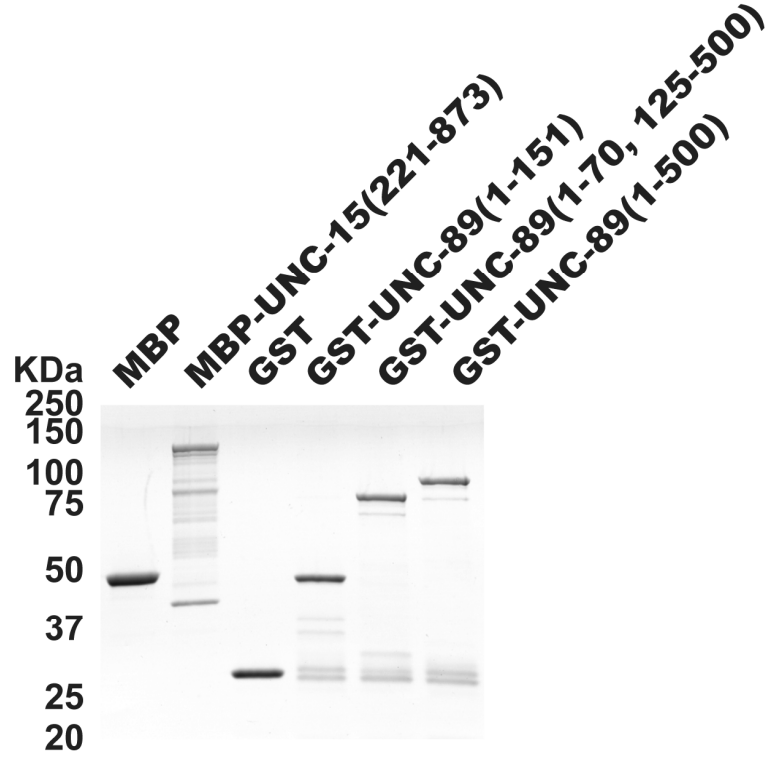
Supplemental Figure 3. Portions of UNC-89 that were expressed as HA or EGFP tagged fusion proteins in transgenic nematodes. (A) The SH3 domain by itself, or when artificially fused to 1/3IK-Ig-Fn-PK2 were expressed either from extrachromosomal arrays or as integrated arrays, as indicated. Expression was induced by heat shock (30^0 for 2 hours) and then allowed to recover at 20^0 for 24 hours before extracts were made. Note that either muscle disorganization did not occur, or that the fusion protein was not detected by western blot. (B) Western blot detecting heat-shock-induced expression (30^0 for 2 hours) of HA-SH3-1/3IK-Ig-FN-PK2, after recovery at 20^0 at the indicated times after heat shock.

Supplemental figure 4

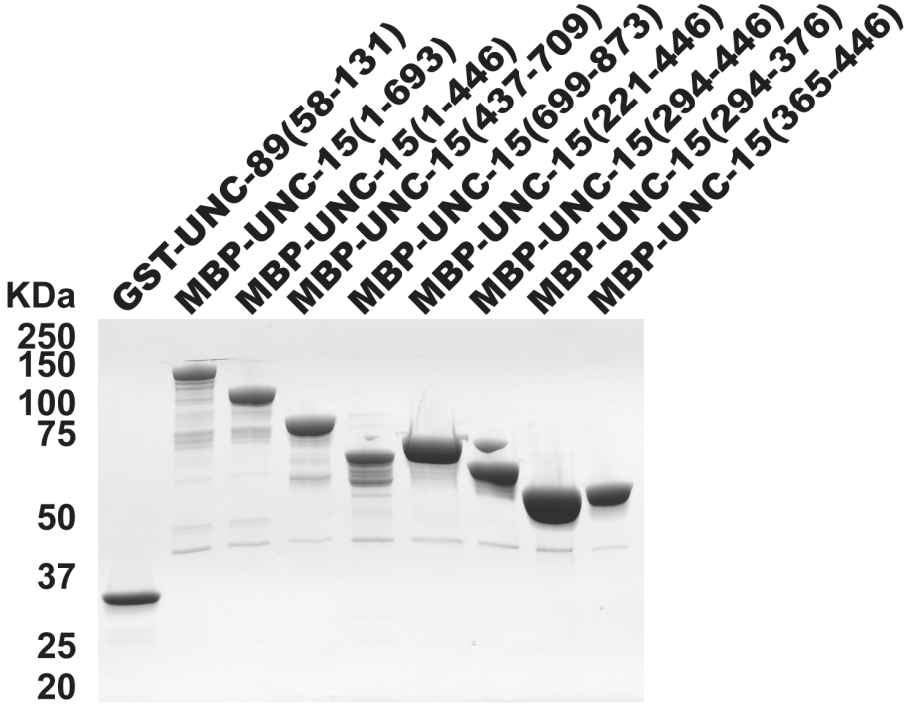


Supplemental Figure 4. Heat shock induced by 24-hours at 30^0 of HA-SH3-1/3IK-Ig-Fn-PK2 has no effect on muscle structure. Each panel shows staining with antibodies to MHC A, MHC B and twitchin. Localization patterns are normal. Scale bar, 10 μm.

Supplemental figure 5



CBB staining



CBB staining

Supplemental Figure 5. SDS PAGE stained with Coomassie Blue of bacterially expressed recombinant fusion proteins used in *in vitro* binding experiments. Approx. 2 μg of each protein was separated on a 10% polyacrylamide gel. Positions of molecular weight standards are indicated.