

Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figures

Supplemental Figure 1: A screen of 102 genes that encode actin binding and regulatory proteins reveals the importance of the actin cytoskeleton in *C. elegans* reproduction and somatic gonad function. Spermathecae in live animals were visualized on a dissection scope using a line that expresses GFP::ACT-1 under a spermatheca specific promoter. This line allows for easy visualization of the spermatheca to distinguish spermathecae undergoing ovulation that are occupied by an oocyte and appear distended, “occupied,” from spermathecae between ovulations that contain only sperm and appear compact, “empty.” Animals with occupied spermathecae but no embryos in the uterus were scored as, “occupied on first,” and animals with empty spermathecae and no embryos in the uterus were scored as, “no entry.” At least 100 animals were scored for each condition. For the number of animals screened for each gene and for a description of the known gene functions and orthologs see Supplemental Table 1. Genes that result in a 2 to 4-fold increase in the percent of animals with occupied spermathecae are classified as resulting in a mild contractility defect. Genes that result in a greater than 4-fold increase are classified as resulting in a severe contractility defect. Genes that result in a “no entry,” phenotype were classified as producing in a mild entry defect if fewer than 50% of animals had the “no entry” phenotype and a severe entry defect if 50% or more did.

Supplemental Figure 2: The screening method used to identify contractility defects is highly reproducible. Animals treated with control RNAi were scored on 11 different days over 2 years. Spermathecae in live animals were visualized on a dissection scope using a line that expresses GFP::ACT-1 under a spermatheca specific promoter. This line allows for easy visualization of the spermatheca to distinguish spermathecae undergoing ovulation that are occupied by an oocyte and appear distended, “occupied,” from spermathecae between ovulations that contain only sperm and appear compact, “empty.” The number of animals scored on each day is indicated (n). The average percent of animals with each phenotype and the SD across experiments is indicated.

Supplemental Figure 3: The *unc-70* gene has 4 predicted splice variants and only isoform c is detected in the spermathecae. (A) Schematic of the different splice variants for the *unc-70* locus, K11C4.3. The sequence used to generate the RNAi construct is indicated and is common to all for transcripts. (B) Schematic of the expression constructs used to determine *unc-70* expression pattern. The top constructs reports the expression of all isoforms except c and is not detected in the spermatheca (Krieg *et al.*, 2014). The bottom construct is expressed in the spermatheca and SP-UT valve (this work). (C) Protein alignment for the predicted amino acid sequence of each isoform. Alternative splicing results in a unique N-terminal extension in isoform c. Unique residues are highlighted in red. Yellow indicates two unstructured regions predicted using the online Simple Modular Architecture Research Tool (SMART) and blue shows the start of the calponin homology (CH) domain conserved across all isoforms.

Supplemental Figure 4: Labeled SPC-1/ α is functional in the somatic gonad. (A) Scoring of ovulation defects in WT unlabeled animals and animals expressing SPC-1::mKate2 using a widefield microscope. Expression of labeled SPC-1/ α does not significantly alter the percentages of gonad arms with empty spermathecae, occupied spermathecae, and spermathecae occupied by a pinched oocyte. (B) Scoring of contractility defects in animals expressing GFP labeled actin in the spermatheca with unlabeled SPC-1/ α and SPC-1::mKate2 using a dissection microscope. Expression of labeled mKate2 in the background of this

potentially sensitized line does not result in significant changes in the percent of animals with empty and occupied spermathecae. In (A) n = the number of gonad arms scored and in (B) n = the number of animals scored. Chi-square test, P value: ns $P > 0.05$.

Supplemental Figure 5: SPC-1/α is faintly expressed in the sheath cells but is not required for F-actin organization in the sheath. (A) A confocal maximum intensity projection of an excised gonad of an animal expressing SPC-1::mKate2 labeled at the endogenous locus. The image is colored to highlight differences in fluorescence intensity. SPC-1::mKate2 is prominently expressed in the spermatheca and faint expression is also present in the sheath (white arrowheads). (B) Confocal maximum intensity projections of excised and fixed gonads from WT, N2, animals stained with phalloidin to label F-actin. Animals were treated with control RNAi or RNAi against *spc-1* and *unc-70* to disrupt the SBMS. Knock down of SPC-1/α or UNC-70/β does not result in notable alterations to sheath F-actin morphology.

Supplemental Figure 6: Spectrin is not required for myosin association with actomyosin bundles.(A) Confocal maximum intensity projections of spermathecal cells in intact animals expressing *moeABD::mCherry*, to label F-actin, and *GFP::NMY-1*, to label myosin. (B) Central sagittal cross sections of the spermathecae shown in (A). Note, actin and myosin are both enriched at the basal cell surface. In cross section images, basal is at the bottom and apical is at the top. (C) Fluorescence intensity across the dashed line on the WT cell shown in (A). (D) Fluorescence intensity across the dashed line on the *unc-70(RNAi)* cell shown in (A). Line scan analysis shows that peaks of actin fluorescence coincide with peaks of myosin fluorescence in both WT and *unc-70(RNAi)* cells. Scale bar 5 μm.

Supplemental Table 1: This table contains a list of the genes screened in this paper, the known orthologs, and gene functions in *C. elegans*. Rows one and two contain the *C. elegans* gene name and the number of animals screened for spermathecal contractility defects with RNAi of the indicated gene. Row three lists known orthologs or related protein families based on conserved protein domains. Row four includes a list of the biological function gene ontology terms associated with that gene in *C. elegans*. Information for the 3rd and 4th rows is from WormBase. For details on the spermathecal contractility phenotypes associated with each gene see Supplemental Figure 1. Genes listed in this table are in the same order as the genes in Supplemental Figure 1.

| C. elegans Gene | Animals Scored | Orthologs and Related Protein Families | C. elegans Biological Function Gene Ontology (GO) |
|------------------------|-----------------------|---|---|
| Empty vector | 628 | | |
| <i>vps-16</i> | 200 | <i>S. cerevisiae</i> Vps16p (vacuolar sorting protein) | intracellular protein transport, vacuole organization, embryo development ending in birth or egg hatching, protein transport, endosomal transport, regulation of vacuole fusion, (non-autophagic), regulation of SNARE complex assembly, and digestive tract development |
| <i>tth-1</i> | 170 | Thymosin-beta | actin filament organization, and nervous system development |
| <i>Y65B4A.4</i> | 141 | Human CAP2 (CAP, adenylate cyclase-associated protein, 2 (yeast)) | cell morphogenesis, cytoskeleton organization, establishment or maintenance of cell polarity, signal transduction, actin polymerization or depolymerization, and regulation of adenylate cyclase activity |
| <i>ttn-1</i> | 203 | Titin | protein phosphorylation, striated muscle contraction, actin filament organization, phosphorylation, sarcomere organization, and striated muscle myosin thick filament assembly |
| <i>ced-12</i> | 126 | ELMO (Engulfment and Cell Motility) | establishment of mitotic spindle orientation, phagocytosis, engulfment, apoptotic process, actin filament organization, cell migration, regulation of cell migration, actin cytoskeleton reorganization, gonad morphogenesis, engulfment of apoptotic cell, cytoskeletal rearrangement involved in phagocytosis and engulfment, left/right axis specification, positive regulation of engulfment of apoptotic cell, apoptotic process involved in development, and positive regulation of distal tip cell migration |
| <i>arx-7</i> | 185 | p16Arc (subunit of the actin related protein of the conserved Arp2/3 complex) | epithelial cell migration, morphogenesis of embryonic epithelium, regulation of actin filament polymerization, and Arp2/3 complex-mediated actin nucleation |
| <i>tmd-2</i> | 169 | Human LMOD1-3 (leiomodin 1-3) and members of the TMOD (Tropomodulins) family | actin filament organization, myofibril assembly, and pointed-end actin filament capping |

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|-----------------|-----|---|--|
| <i>dys-1</i> | 395 | Human DMD (Duchenne muscular dystrophy) | drug transmembrane transport, synaptic transmission (cholinergic), locomotory behavior, acetylcholine transport, positive regulation of synaptic transmission (cholinergic), positive regulation of locomotion, forward locomotion, sarcomere organization, muscle cell cellular homeostasis, and ammonium transmembrane transport |
| <i>daam-1</i> | 236 | Drosophila DAAM and human DAAM1 (Disheveled-Associated Activator of Morphogenesis) | cellular component organization and actin cytoskeleton organization |
| <i>atn-1</i> | 174 | Alpha-actinin | striated muscle thin filament and striated muscle dense body |
| <i>arx-5</i> | 216 | p21Arc (subunit of the actin related protein of the conserved Arp2/3 complex) | epithelial cell migration, morphogenesis of embryonic epithelium, regulation of actin filament polymerization, and Arp2/3 complex-mediated actin nucleation |
| <i>pfn-3</i> | 191 | Profilin | sequestering of actin monomers and muscle thin filament assembly |
| <i>myo-6</i> | 132 | Human myosin-13, striated muscle conventional myosin heavy chain | microtubule-based movement |
| <i>mig-2</i> | 196 | RhoG family GTP-binding proteins; homologous to Human RAC1 | neuron migration, regulation of protein phosphorylation, actin filament organization |
| <i>C46H11.3</i> | 163 | Human Arpc5 (actin related protein 2/3 complex subunit 5) and ARPC5L (actin related protein 2/3 complex subunit 5 like) | cell migration, regulation of actin filament polymerization, and Arp2/3 complex-mediated actin nucleation |
| <i>pxl-1</i> | 284 | Paxillin | nematode larval development, pharyngeal pumping, and muscle structure development |
| <i>frm-1</i> | 302 | Human EPB41L1 and 3 (erythrocyte membrane protein band 4.1 like 1 and 3) | actomyosin structure organization, |

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|------------------|-----|--|--|
| <i>tnt-4</i> | 120 | Troponin | nematode larval development, muscle contraction, regulation of muscle contraction, pharynx development, and pharyngeal gland morphogenesis |
| <i>eps-8</i> | 237 | Cell signaling adaptor protein containing PTB, SH3, and actin-binding domains; homologous to <i>Drosophila</i> CG8907 and human BAG65598 | nematode larval development, intermediate filament organization, maintenance of protein location, and digestive tract morphogenesis |
| <i>Y50D7A.10</i> | 254 | Human GMFG and B (glia maturation factor gamma and beta) | negative regulation of Arp2/3 complex-mediated actin nucleation |
| <i>tnt-4</i> | 112 | Troponin | muscle contraction, pharyngeal pumping, and embryo development ending in birth or egg hatching |
| <i>samp-1</i> | 138 | Mammalian NET5/Samp1 (spindle associated membrane protein) | embryo development ending in birth or egg hatching and nuclear migration along microtubule |
| <i>myo-2</i> | 165 | Human myosin-3, conventional myosin heavy chain | microtubule-based movement |
| <i>unc-53</i> | 330 | Human NAV1-3 (Neuron Navigator 1-3) | signal transduction, multicellular organism development, axon guidance, mating behavior, motor neuron axon guidance, mesodermal cell migration, determination of muscle attachment site, oviposition, actin cytoskeleton organization, positive regulation of cell migration, positive regulation of multicellular organism growth, backward locomotion, positive regulation of axon extension, positive regulation of axon extension involved in axon guidance, and cell projection morphogenesis |
| <i>pfn-1</i> | 207 | Profilin | sequestering of actin monomers |
| <i>cap-1</i> | 250 | Human F-actin-capping protein subunit alpha-1 | barbed-end actin filament capping |

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|---------------|-----|--|---|
| <i>feh-1</i> | 325 | Fe65 protein family homologous to human Isoform 2 of Amyloid beta A4 precursor protein-binding family B member 2 | nematode larval development, transcription (DNA-templated), regulation of transcription (DNA-templated), feeding behavior, and embryo development ending in birth or egg hatching |
| <i>aip1-1</i> | 196 | AIP1 (Actin Interacting Protein 1) cooperates with ADF/cofilin | actin filament organization, actin filament depolymerization, positive regulation of actin filament depolymerization, regulation of protein localization, locomotion, and sarcomere organization |
| <i>zoo-1</i> | 195 | Zonula occludens (ZO) subfamily of membrane-associated guanylate kinases (MAGUKs) | actin filament organization, embryo development ending in birth or egg hatching, and embryonic morphogenesis |
| <i>gsnl-1</i> | 145 | Gelsolin | actin filament depolymerization, actin filament severing, barbed-end actin filament capping, actin filament capping, and positive regulation of synapse disassembly |
| <i>cor-1</i> | 333 | Coronin | actin cytoskeleton organization |
| <i>frl-1</i> | 280 | Human formin FMNL1 | cellular component organization and actin cytoskeleton organization |
| <i>spe-15</i> | 210 | Human unconventional myosin VI heavy chain | microtubule-based movement, spermatogenesis, spermatid development, and organelle localization |
| <i>hipr-1</i> | 535 | Mammalian Huntingtin-interacting protein 1-related (Hip1r) | endocytosis, neurotransmitter secretion, ultradian rhythm, protein localization, defecation, and developmental process |
| <i>cas-2</i> | 342 | Human CAP1-2 (adenylate cyclase associated protein 1-2) | cell morphogenesis, cytoskeleton organization, establishment or maintenance of cell polarity, signal transduction, actin polymerization or depolymerization, and regulation of adenylate cyclase activity |
| <i>plst-1</i> | 182 | Human PLS1 and 3 (plastin 1 and 3) and LCP1 (lymphocyte cytosolic protein 1 (L-plastin)) | actin filament bundle assembly, actin filament network formation, and actin crosslink formation, |

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|-----------------|-----|--|--|
| <i>hum-5</i> | 182 | Human unconventional myosin-Ib heavy chain | microtubule-based movement |
| <i>viln-1</i> | 177 | Human SVIL (supervillin) | cytoskeleton organization |
| <i>pfn-2</i> | 175 | Profilin | sequestering of actin monomers and muscle thin filament assembly |
| <i>spe-26</i> | 239 | Kelch motif-containing protein related to Drosophila proteins kelch and diablo | protein ubiquitination |
| <i>cas-1</i> | 239 | Human CAP1 (adenylyl cyclase-associated protein 1) | cell morphogenesis, cytoskeleton organization, establishment or maintenance of cell polarity, signal transduction, actin polymerization or depolymerization, and regulation of adenylyl cyclase activity |
| <i>M04F3.5</i> | 230 | I-BAR domain-containing protein related to human MTSS1L (MTSS1-like protein) | plasma membrane organization, cell projection assembly, and membrane organization |
| <i>Y73B3B.1</i> | 183 | Human PLS1 and 3 (plastin 1 and 3) and LCP1 (lymphocyte cytosolic protein 1 (L-plastin)) | actin filament bundle assembly, actin filament network formation, and actin crosslink formation, |
| <i>ketn-1</i> | 282 | Kettin, invertebrate paralog of titin | No biological process GO available; Cellular component GO term: I band |
| <i>anc-1</i> | 242 | Mammalian SYNE1-2 (Spectrin repeat-containing nuclear envelope protein 1-2) | nucleus organization, cytoskeleton organization, and pronuclear migration |
| <i>add-1</i> | 160 | Human ADD1 (alpha-adducin) | memory, short-term memory, long-term memory, barbed-end actin filament capping, receptor localization to synapse, and regulation of actin cytoskeleton reorganization |
| <i>hum-8</i> | 154 | Human unconventional myosin VI | microtubule-based movement |

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|------------------------------------|-----|--|---|
| <i>add-2</i> | 246 | Contains an alpha-adducin-like domain related to human ADD1 (alpha-adducin) | No biological process GO available; cellular compartment GO term predicts ADD-2 is associated with the cytoskeleton and cellular membrane |
| <i>myo-5</i> | 151 | Human myosin-3, conventional myosin heavy chain | microtubule-based movement and Golgi vesicle transport |
| <i>hum-2</i> | 150 | Human Myosin-5B, unconventional myosin heavy chain type V | microtubule-based movement |
| <i>syg-1</i> | 163 | Immunoglobulin superfamily with homology to human KIRREL2 (Kin of IRRE-like protein 2) | cell adhesion, cell-cell signaling, nervous system development, synapse assembly, synaptic target recognition, collateral sprouting, branching morphogenesis of a nerve, synapse organization, and actin filament bundle assembly |
| <i>tni-3</i> | 348 | Troponin | muscle contraction, oviposition, and post-embryonic body morphogenesis |
| Mild exit defect (13 genes) | | | |
| <i>ehbp-1</i> | 176 | Human EH domain-binding protein 1 (EHBP1) | receptor recycling, protein secretion, and endocytic recycling |
| <i>exc-6</i> | 201 | Human FHDC1 (FH2 domain containing 1) | epithelial cell development, regulation of microtubule polymerization, regulation of tube size, regulation of tube size, epithelial tube morphogenesis, and positive regulation of actin filament binding |
| <i>nab-1</i> | 256 | Mammalian Neurabin/Spinophilin proteins | synapse assembly |
| <i>frg-1</i> | 236 | Vertebrate facioscapulohumeral muscular dystrophy (FSHD) region gene 1 (FRG1) | rRNA processing, muscle organ development, ribosome biogenesis, and actin filament bundle assembly, |

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|-------------------------------------|-----|--|--|
| <i>cap-2</i> | 236 | Human F-actin-capping protein subunit beta | embryonic axis specification, cell morphogenesis, regulation of lamellipodium assembly, actin cytoskeleton organization, barbed-end actin filament capping, negative regulation of filopodium assembly, and actin filament capping |
| <i>ttn-1</i> | 104 | Troponin | muscle contraction, backward locomotion |
| <i>rac-2</i> | 179 | RhoG family GTP-binding proteins; homologous to Human RAC1 | neuron migration, actin filament organization, motor neuron axon guidance, dorsal/ventral axon guidance, neuron projection morphogenesis, and axon extension involved in axon guidance |
| <i>twf-1</i> | 250 | Human TWF1 (twinfilin actin binding protein 1) | negative regulation of actin filament polymerization |
| <i>abi-1</i> | 123 | Human Abl interactor 1 (ABI1) | neuron migration, actin polymerization or depolymerization, cell migration, cell projection morphogenesis, positive regulation of engulfment of apoptotic cell, positive regulation of clathrin-dependent endocytosis |
| <i>unc-27</i> | 185 | Troponin | muscle contraction, locomotion, and sarcomere organization |
| <i>wsp-1</i> | 235 | Human WASP (Wiskott-Aldrich syndrome protein) | epithelial cell migration, morphogenesis of embryonic epithelium, regulation of protein stability, microvillus organization, regulation of Arp2/3 complex-mediated actin nucleation, embryonic ectodermal digestive tract morphogenesis, positive regulation of oviposition, and positive regulation of clathrin-dependent endocytosis |
| <i>dbn-1</i> | 188 | Human DBNL (drebrin like) and DBN1 (drebrin 1) | No biological process GO available; cellular component GO term indicates actin binding |
| <i>hum-6</i> | 118 | Human Unconventional myosin-VIIa heavy chain | microtubule-based movement and signal transduction |
| Strong exit defect (6 genes) | | | |
| <i>unc-87</i> | 254 | Similar structure to calponin but with no obvious orthologs outside of nematodes | actomyosin structure organization, myosin filament assembly, actin filament bundle assembly, negative regulation of plus-end directed microfilament motor activity, and negative regulation of muscle filament sliding |
| <i>unc-115</i> | 175 | Human ABLIM1 (Actin-binding LIM protein 1) | cytoskeleton organization, regulation of lamellipodium assembly, neuron projection morphogenesis, and regulation of filopodium assembly |

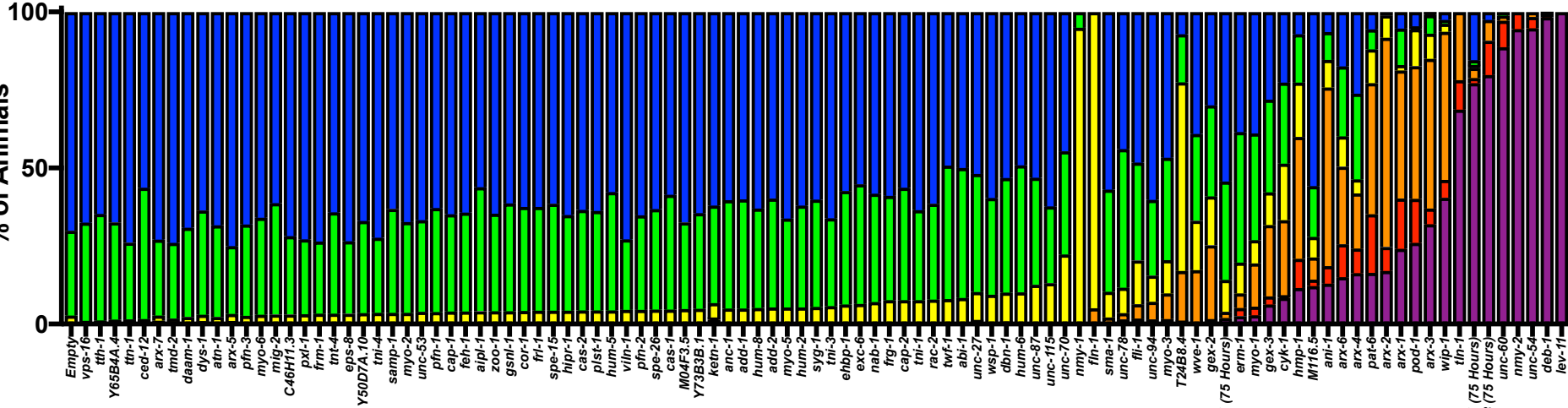
| | | | |
|-------------------------------------|-----|--|--|
| <i>unc-70</i> | 103 | beta-spectrin | nematode larval development, cytoskeleton organization, embryonic body morphogenesis, dendrite development, regulation of locomotion, muscle cell cellular homeostasis, neuron development, and actin filament capping |
| <i>nmy-1</i> | 178 | Drosophila zipper (type II non-muscle myosin heavy chain) | nematode larval development, microtubule-based movement, protein localization, regulation of embryonic cell shape, and locomotion |
| <i>fln-1</i> | 198 | Human FLNA (filamin A) | actin filament organization, axon guidance, axonal fasciculation, uterus morphogenesis, and semaphorin-plexin signaling pathway |
| <i>sma-1</i> | 232 | beta-H spectrin | epithelial cell development, nematode larval development, body morphogenesis, embryonic body morphogenesis, regulation of tube size, regulation of locomotion, positive regulation of multicellular organism growth, positive regulation of cell size, positive regulation of organ growth, pharynx development, and pharyngeal gland morphogenesis |
| Mild entry defect (23 genes) | | | |
| <i>unc-78</i> | 198 | AIP1 (Actin Interacting Protein 1) cooperates with ADF/cofilin | skeletal muscle thin filament assembly, positive regulation of actin filament depolymerization, and negative regulation of actin filament polymerization |
| <i>fli-1</i> | 172 | Drosophila and human Flightless I | multicellular organism development, actin cytoskeleton organization, and actin filament severing |
| <i>unc-94</i> | 334 | Tropomodulin | nematode larval development, embryo development ending in birth or egg hatching, and negative regulation of actin filament depolymerization |
| <i>myo-3</i> | 122 | Human myosin-13, striated muscle conventional myosin heavy chain | microtubule-based movement, locomotory behavior, and positive regulation of ovulation |
| <i>T24B8.4</i> | 324 | Contains two tandem WH2 domains with no obvious orthologs outside of nematodes | No biological process GO; molecular GO predicts actin binding |
| <i>wve-1</i> | 133 | SCAR/WAVE | neuron migration, actin filament organization, motor neuron axon guidance, actin cytoskeleton organization, embryonic morphogenesis, embryonic ectodermal digestive tract morphogenesis, collateral sprouting, positive regulation of oviposition, positive regulation of protein localization to synapse, and positive regulation of clathrin-dependent endocytosis |

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|---------------|-----|--|---|
| <i>gex-2</i> | 281 | Mammalian p140/Sra-1 (specifically Rac1-associated protein) | multicellular organism development, embryonic body morphogenesis, cell migration, positive regulation of oviposition, and positive regulation of clathrin-dependent endocytosis, |
| <i>nfm-1</i> | 254 | Human merlin/schwannomin (NF2) | negative regulation of cell proliferation |
| <i>erm-1</i> | 234 | ERM (Ezrin, Radixin, Moesin) family of cytoskeletal linkers | morphogenesis of an epithelium, nematode larval development, actin filament organization, tube formation, and protein localization to basolateral plasma membrane |
| <i>myo-1</i> | 108 | Human myosin-3, conventional myosin heavy chain | muscle contraction, microtubule-based movement, pharyngeal pumping, and inositol lipid-mediated signaling |
| <i>gex-3</i> | 192 | Mammalian NAP1/NCKAP1 (NCK Associated Protein 1) | actin filament organization, multicellular organism development, embryonic body morphogenesis, cell migration, oviposition, embryonic ectodermal digestive tract morphogenesis, collateral sprouting, positive regulation of oviposition, positive regulation of protein localization to synapse, and positive regulation of clathrin-dependent endocytosis |
| <i>cyk-1</i> | 177 | Formin Homology protein homologous to Drosophila diaphanous and human DIAPH1 | actin filament organization, cell cycle, cellular component organization, actin cytoskeleton organization, and pronuclear migration |
| <i>hmp-1</i> | 225 | alpha-catenin | cytoskeletal anchoring at plasma membrane, cell adhesion, multicellular organism development, embryonic body morphogenesis, cell migration, regulation of protein localization, regulation of actin cytoskeleton organization, and cell migration involved in gastrulation |
| <i>M116.5</i> | 197 | Human plectin | No biological process GO available; molecular function GO terms indicate actin and microtubule binding activity |
| <i>ani-1</i> | 124 | Anillin | cell cycle, multicellular organism development, protein localization, embryo development ending in birth or egg hatching, first cell cycle pseudocleavage, cortical cytoskeleton organization, septin ring organization, collagen and cuticulin-based cuticle development, locomotion, hermaphrodite genitalia development, polar body extrusion after meiotic divisions, nematode male tail tip morphogenesis, actin filament bundle assembly, cell division, and meiotic cell cycle |

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|--------------------------------------|-----|--|--|
| <i>arx-6</i> | 246 | p20/Arc subunit of the actin related protein of the conserved Arp2/3 complex | epithelial cell migration, morphogenesis of embryonic epithelium, regulation of actin filament polymerization, and Arp2/3 complex-mediated actin nucleation |
| <i>arx-4</i> | 153 | p34/Arc subunit of the actin related protein of the conserved Arp2/3 complex | epithelial cell migration, morphogenesis of embryonic epithelium, regulation of actin filament polymerization, and Arp2/3 complex-mediated actin nucleation |
| <i>pat-6</i> | 250 | alpha-parvin | cell adhesion, actin cytoskeleton reorganization, striated muscle cell development, and positive regulation of sarcomere organization |
| <i>arx-2</i> | 194 | Arp-2 subunit of the actin related protein of the conserved Arp2/3 complex | actin filament organization, gastrulation, epithelial cell migration, morphogenesis of embryonic epithelium, and Arp2/3 complex-mediated actin nucleation |
| <i>arx-1</i> | 112 | Arp-3 subunit of the actin related protein of the conserved Arp2/3 complex | actin filament organization, gastrulation, epithelial cell migration, morphogenesis of embryonic epithelium, and Arp2/3 complex-mediated actin nucleation |
| <i>pod-1</i> | 127 | Coronin-like protein similar to human Coronin-7 | No biological process GO available; molecular function GO terms indicate association with the Golgi, cell cortex, and cleavage furrow |
| <i>arx-3</i> | 100 | p41/Arc subunit of the actin related protein of the conserved Arp2/3 complex | regulation of actin filament polymerization and Arp2/3 complex-mediated actin nucleation |
| <i>wip-1</i> | 158 | WIP (Wiskott-Aldrich syndrome protein (WASP)-Interacting Protein) | embryonic body morphogenesis, regulation of cell migration, and regulation of protein stability |
| Strong entry defect (8 genes) | | | |
| <i>tlh-1</i> | 288 | Human TLN1-2 (talin 1-2) | cytoskeletal anchoring at plasma membrane, cell adhesion, integrin-mediated signaling pathway, regulation of vulval development, negative regulation of epidermal growth factor receptor signaling pathway, and vulval cell fate specification |
| <i>ctn-1</i> | 123 | alpha-catenin family of catenin-like proteins | cell adhesion, Rho protein signal transduction, and regulation of locomotion |

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|---------------|-----|--|--|
| <i>nuo-3</i> | 119 | encodes two non-overlapping proteins; one is homologous to human C3orf10/BRICK1 (WAVE1 complex component) the other to human NDUFA6 (NADH-ubiquinone oxidoreductase alpha subunit) | mitochondrial electron transport, NADH to ubiquinone and response to oxidative stress |
| <i>unc-60</i> | 177 | Actin depolymerizing factor(ADF)/cofilin | embryo development ending in birth or egg hatching, actin filament depolymerization, skeletal muscle thin filament assembly, positive regulation of actin filament polymerization, locomotion, actin filament severing, and muscle thin filament assembly |
| <i>nmy-2</i> | 145 | Drosophila Mhc (type II non-muscle myosin heavy chain) | mitotic cytokinesis, nematode larval development, microtubule-based movement, embryo development ending in birth or egg hatching, polarity specification of anterior/posterior axis, regulation of embryonic cell shape, asymmetric protein localization involved in cell fate determination, and asymmetric neuroblast division |
| <i>unc-54</i> | 192 | Human myosin-13, striated muscle conventional myosin heavy chain | muscle contraction, microtubule-based movement, oviposition, skeletal muscle myosin thick filament assembly, locomotion, pharyngeal pumping, and inositol lipid-mediated signaling |
| <i>deb-1</i> | 242 | Vinculin | cytoskeletal anchoring at plasma membrane, cell adhesion, and. positive regulation of ovulation |
| <i>lev-11</i> | 239 | Tropomyosin | muscle contraction, actin filament organization, embryo development ending in birth or egg hatching, regulation of actin filament polymerization, negative regulation of actin filament depolymerization, spicule insertion, locomotion, and regulation of protein binding |

% of Animals



Both Empty
One Occupied
Both Occupied
Both On First
One No Entry
Both No Entry

No defect
51.0%

Mild exit defect
12.7%

Severe exit defect
5.9%

Mild entry defect
22.5%

Severe entry defect
7.8%

Both Empty

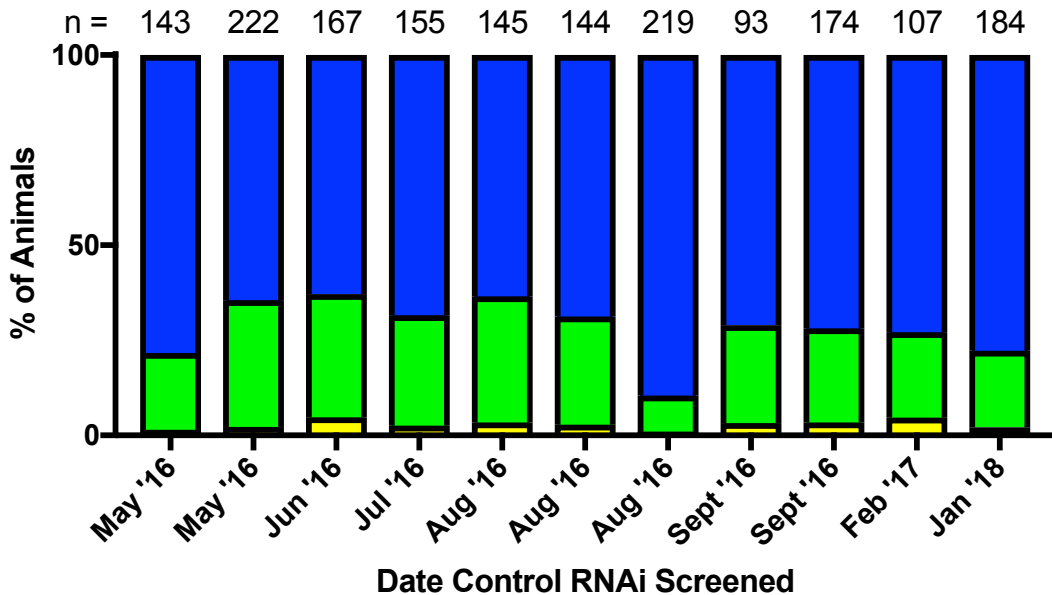
One Occupied

Both Occupied

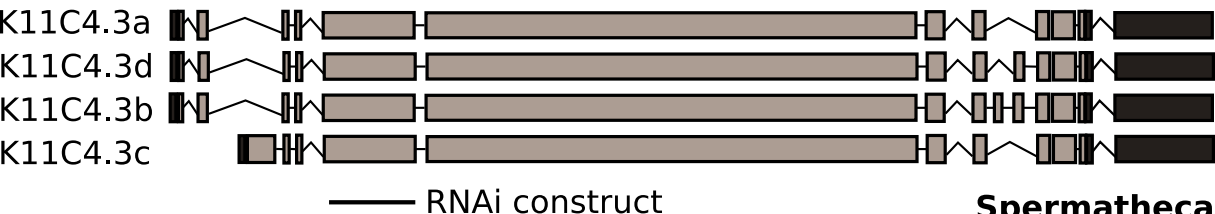
Average (SD) 71.7 (7.9)

25.4 (7.0)

2.8 (1.3)

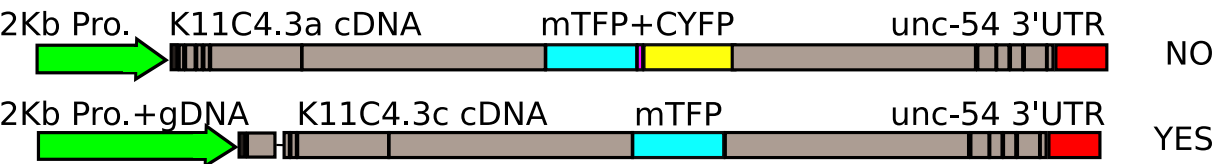


A *unc-70* mRNA Isoforms



**Spermatheca
Expression**

B *UNC-70* Expression Constructs



C

K11C4.3a MATVD-..... GNSGAYTQDEED--Y 17

K11C4.3d MATVD-..... GNSGAYTQDEED--Y 17

K11C4.3b MATVD-..... GNSGAYTQDEED--Y 17

K11C4.3c **MSSVDD****DEHHP****SI****EVD****I****PDV****Y****ESA****SD****CE****KT****ED****CT****Y****L****G****S****I****L****K****A****K****S****L****R****K****T****K****V****A****S****P****G****I****Y****P****G****S****A****G****A****L****S****S****L****S****N****G****L****A** 72

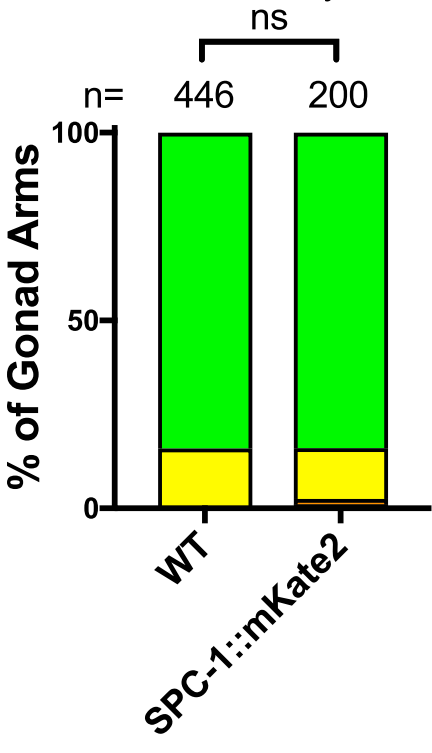
K11C4.3a DDNSSARLFERS-.....RIKALADERELVQKKTFTKWVNSHLVRV SCKVQDLYMDMRDGKMLL 75

K11C4.3d DDNSSARLFERS-.....RIKALADERELVQKKTFTKWVNSHLVRV SCKVQDLYMDMRDGKMLL 75

K11C4.3b DDNSSARLFERS-.....RIKALADERELVQKKTFTKWVNSHLVRV SCKVQDLYMDMRDGKMLL 75

K11C4.3c **DENR****SPT****SA****EV****SD****S****V****CG****Q****K****S****I****NS****V****D****L****R****FR****G****L****R****D****E****R****E****L****V****Q****K****K****T****F****T****K****W****N****S****H****L****V****R****V****S****C****K****V****Q****D****L****Y****M****D****M****R****D****G****K****M****L****L** 14

- A**
- Empty
 - Occupied
 - Pinched Oocyte



- B**
- Both Empty
 - One Occupied
 - Both Occupied

