

Figure S1

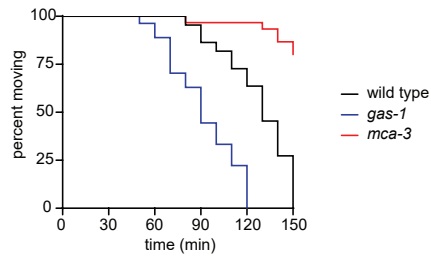


Figure S1: Use of either the swim-based or plate-based levamisole assay results in the same phenotype

Sensitivity to 0.5 mM levamisole in a plate-based assay performed as described (Krajacic *et al.* 2013). The *gas-1(fc21)* mutant exhibited hypersensitivity while the *mca-3(ar492)* mutant showed resistance compared to the wild type. This is consistent with data in Figures 2A and 4F, which were collected with the swim-based assay. Thus, these two different levamisole assays produced the same results for mutants that were already known to have a levamisole phenotype (Figure 1A; Lewis *et al.* 1980; Krajacic *et al.* 2013) and loss of genes that were identified in our screen.

Figure S2

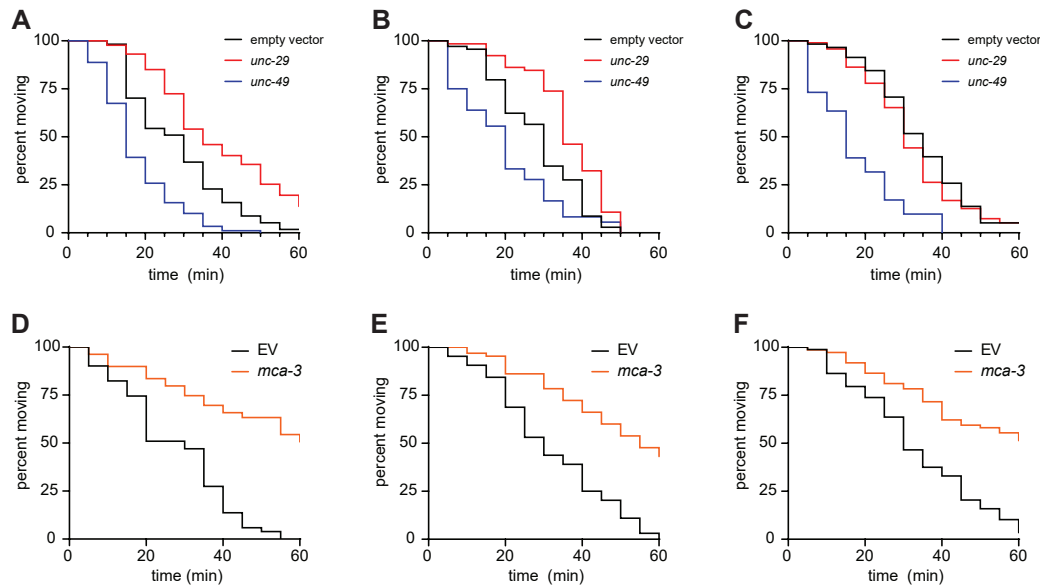


Figure S2: The *unc-29* RNAi clone caused inefficient knockdown, while the *unc-49* and *mca-3* clones produced consistent results

unc-29 mutations cause nearly complete resistance in both plate-based levamisole assays (Lewis *et al.* 1980; Krajacic *et al.* 2013) and our swim-based levamisole assay (this work, Figure 1A). Yet in three separate time course assays, knockdown of *unc-29* (red) caused (A) significant resistance ($p < 0.001$), but far weaker than the mutant (Figure 1A), (B) weak resistance ($p < 0.01$) that would not have been identified in the RNAi screen due to similarity to empty vector (black) at 40 minutes, and (C) no significant difference compared to the empty vector ($p = 0.34$). (D-F) Knockdown of *mca-3* (orange) resulted in delayed paralysis in all assays, indicating that levamisole resistance can be scored reproducibly with this swim-based assay. Thus, it is likely that inefficient knockdown prevented us from identifying *unc-29* in our screen. We note that knockdown of *unc-49* (blue) in (A-C) caused significant hypersensitivity in all three assays ($p < 0.001$), consistent with the *unc-49* mutant phenotype (Krajacic *et al.* 2013 and this work, Figure 1A). However, we did not identify *unc-49* in our screen, suggesting that the two point screening method likely missed some knockdowns that cause robust levamisole hypersensitivity.

Supplemental Table 1: Genes identified in the RNAi screen for altered sensitivity to levamisole.Knockdown of the genes shown in **red** caused levamisole resistance; knockdown of genes in **black** resulted in hypersensitivity.

		<u># times / 6</u>	<u>muscle expression</u>	<u>lethal</u>	<u>known lev. phenotype</u>	<u>Description</u>	<u>Ref for muscle expressed</u>	<u>Ref for lev phenotype</u>
Acetylcholine receptor and clustering								
Y110A7A.3	<i>unc-63</i>	6	yes		resistant	Acetylcholine receptor, nicotinic	Culetto et al, 2004	Culetto et al, 2004
Y105E8A.7	<i>lev-10</i>	4	yes		resistant	Required for AChR clustering	Gally et al, 2004	Lewis et al, 1980
Receptor trafficking, endocytosis and recycling								
T04C10.2	<i>epn-1</i>	5	yes	lethal		Epsin	Shen et al, 2013	
Y67D8C.10	<i>mca-3</i>	5	yes			Plasma membrane Ca2+ ATPase	Bednarek, 2007	
ATP synthesis								
T06D8.6	<i>cchl-1</i>	5				Cytochrome c-type heme lyase		
K08F11.4	<i>yars-2</i>	4		lethal		Mitochondrial tyrosyl-tRNA synthetase		
F26E4.6	<i>cox-7C</i>	5		lethal		Cytochrome c oxidase subunit 7C		
W09C5.8	<i>cox-4</i>	5		lethal		Cytochrome c oxidase subunit 4 isoform 1		
Y57G11C.12	<i>nuo-3</i>	5	yes	lethal		NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6	Hunt-Newbury et al, 2007	
F32D1.2	<i>hpo-18</i>	6		lethal		ATP synthase subunit epsilon		
F53F4.10		6		lethal		NADH dehydrogenase [ubiquinone] flavoprotein 2		
E04A4.7	<i>cyc-2.1</i>	6	yes	lethal		Cytochrome c	Giacomotto et al, 2013	
C33A12.1		6	yes	lethal		NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5	Dupuy et al, 2007	
K09A9.5	<i>gas-1</i>	6	yes			NADH dehydrogenase [ubiquinone] iron-sulfur protein 2	Kayser et al, 2001	
F42G8.10		6		lethal		NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11	Fox et al, 2007	
Y53G8AL.2	<i>nduf-9</i>	5	yes	lethal		NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9	Hunt-Newbury et al, 2007	
D2030.4		6		lethal		NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7	Fox et al, 2007	
K07A12.3	<i>asg-1</i>	6		lethal		ATP synthase subunit G	Fox et al, 2007	
Muscle structural								
F09F7.2	<i>mlc-3</i>	4	yes			Myosin light chain	Meissner et al, 2011	
C37C3.6	<i>mig-6</i>	5	yes			Proteoglycan Like Sulfated Glycoprotein PAPLN	Kawano et al, 2009	
F42E11.4	<i>tni-1</i>	6	yes			Troponin I	Ruksana et al, 2005	
Y71G12B.11	<i>tln-1</i>	5	yes			Talin 1	Spencer et al, 2011	
F20H11.3	<i>mdh-2</i>	5	yes	lethal		Mitochondrial malate dehydrogenase	Meissner et al, 2011	
F14D12.2	<i>unc-97</i>	5	yes			LIM zinc finger domain containing 1	Hobert et al, 1999	
C29F9.7	<i>pat-4</i>	6	yes	lethal		Integrin linked kinase ILK	Mackinnon et al, 2002	
C36E6.3	<i>mlc-1</i>	5	yes			Myosin light chain	Rushforth et al, 1998	
C38C3.5	<i>unc-60</i>	6	yes			Cofilin	Ono et al, 1999	
F55C7.7	<i>unc-73</i>	4	yes		hypersensitive	Kalirin RhoGEF Kinase	Steven et al, 2005	Hu et al, 2011
Y53F4B.22	<i>arp-1</i>	6		lethal		Alpha-centractin ACTR1A		
C31H2.1	<i>tbc-7</i>	6				TBC1 domain family member 24		
ZK617.1	<i>unc-22</i>	6	yes		resistant	Titin	Moerman et al, 1988	Lewis et al, 1980
F15G9.4	<i>him-4</i>	4	yes			Hemicentin 1	Spencer et al, 2011	
DNA Structure and Replication								
T23G5.1	<i>rnr-1</i>	5	yes	lethal		Ribonucleoside-diphosphate reductase	Hunt-Newbury et al, 2007	
W02D9.1	<i>pri-2</i>	6		lethal		DNA primase large subunit		
F32D1.10	<i>mcm-7</i>	4	yes	lethal		Component of the Mcm2-7 helicase complex	Hunt-Newbury et al, 2007	
Y111B2A.11	<i>epc-1</i>	4	yes	lethal		Component of the NuA4 histone acetyltransferase (HAT) complex	Dupuy et al, 2007	
C47D12.1	<i>trr-1</i>	4		lethal		Component of the NuA4 histone acetyltransferase complex		

K07A1.12	<i>lin-53</i>	4	yes		Histone binding protein	Muthel et al, 2019		
F54F2.2	<i>zfp-1</i>	4			Histone lysine methyltransferase DOT1L cofactor MLLT10			
W05B10.1	<i>his-74</i>	4			H3 histone 3A			
ZK632.1	<i>mcm-6</i>	5		lethal	DNA replication licensing factor MCM6			
Transcription								
M04G12.4	<i>somi-1</i>	4	yes		Suppressor of overexpressed micro-RNA	Hayes et al, 2011		
F23B12.7		4	yes	lethal	CCAAT enhancer binding protein zeta	Spencer et al, 2011		
F21H11.3	<i>tbx-2</i>	4	yes	lethal	T-box family TBX2	Chowdhuri et al 2006		
W04A8.7	<i>taf-1</i>	4			TATA-box binding protein associated factor 1			
C48E7.2	<i>let-611</i>	4	yes	lethal	RNA polymerase III subunit C	Spencer et al, 2011		
D1043.1	<i>ints-7</i>	5			Integrator complex subunit 7			
Y48G10A.4	<i>ints-8</i>	6		lethal	Integrator complex subunit 8			
W10C8.2	<i>pop-1</i>	5		lethal	Transcription factor 7 TCF7			
T14F9.4	<i>peb-1</i>	6	yes	lethal	Transcription factor; pharyngeal enhancer binding	Beaster-Jones et al, 2004		
C49H3.5	<i>ntl-4</i>	6			CCR4-NOT transcription complex subunit 4			
F20H11.6	<i>let-765</i>	5		lethal	Strawberry Notch Homolog 1			
F35F11.1	<i>cdc-73</i>	4	yes		Parafibromin	Hunt-Newbury et al, 2007		
H43I07.2	<i>rpac-40</i>	4	yes	lethal	DNA-directed RNA polymerases I and III subunit RPAC1	Spencer et al, 2011		
B0261.1		6	yes		Subunit of RNA polymerase III transcription initiation factor IIIB	Spencer et al, 2011		
K02B12.3	<i>sec-12</i>	5		lethal	Prolactin regulatory element-binding protein			
mRNA processing								
Y32F6A.3	<i>pap-1</i>	4	yes		Poly(A) polymerase alpha	Hunt-Newbury et al, 2007		
D1054.14	<i>prp-38</i>	4	yes	lethal	Pre-mRNA-splicing factor 38A	Ma et al,2016		
F53B7.3	<i>isy-1</i>	4		lethal	Pre-mRNA-splicing factor ISY1			
Y71F9B.4	<i>snr-7</i>	5	yes	lethal	Small nuclear ribonucleoprotein G	Spencer et al, 2011		
F58D5.1	<i>hrpr-1</i>	6		lethal	Heterogeneous nuclear ribonucleoprotein Q SYNCRIP			
T01D1.2	<i>etr-1</i>	4	yes	lethal	CUGBP Elav-like family member 1	Milne et al, 1999		
Nuclear / cytoplasmic transport								
R06A4.4	<i>imb-2</i>	6		lethal	Transportin 2 TNPO2			
Y71F9AM.5	<i>nxt-1</i>	6	yes		Nuclear transport factor 2	Spencer et al, 2011		
Y41D4B.19	<i>npp-8</i>	4		lethal	Nucleoporin 160			
Translation								
W04D2.5	<i>mrps-11</i>	4	yes		Mitochondrial ribosomal protein	Spencer et al, 2011		
B0511.8	<i>mrps-30</i>	5		lethal	Mitochondrial ribosomal protein			
K02B2.5	<i>rps-25</i>	5			Ribosomal protein			
Y41E3.4	<i>qars-1</i>	5	yes		GlutaminyI-tRNA synthetase	Spencer et al, 2011		
Y65B4BR.5	<i>icd-2</i>	6	yes	lethal	Nascent polypeptide-associated complex subunit alpha	Guo et al, 2014		
F09G8.3	<i>mrps-9</i>	6		lethal	Mitochondrial ribosomal protein			
G protein signaling								
R06A10.2	<i>gsa-1</i>	4	yes	lethal	hypersensitive	Heterotrimeric G protein alpha subunit G(s)	Korswagen et al, 1997	Hu et al, 2011
M01D7.7	<i>egl-30</i>	4				Heterotrimeric G protein alpha subunit G(q)		
F13D12.7	<i>gpb-1</i>	5	yes	lethal		Heterotrimeric G protein beta subunit	Zwaal et al, 1996	
Cholinergic synaptic transmission								
M01A10.2	<i>tom-1</i>	4			hypersensitive	Tomosyn		Vashlishan et al, 2008
ZK637.8	<i>unc-32</i>	4				ATPase H+ transporting V0 subunit		

Serine - threonine phosphatase

C02F4.2	<i>tax-6</i>	6	yes		Serine/threonine-protein phosphatase 2B catalytic subunit	Kuhara et al, 2002
F56C9.1	<i>gsp-2</i>	5	yes	lethal	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	Hunt-Newbury et al, 2007

Kinase

C14B9.4	<i>plk-1</i>	4		lethal	Serine/threonine-protein kinase PLK1	
ZC504.4	<i>mig-15</i>	5	yes		Misshapen Like Kinase 1	Poinat et al, 2002

Lipid synthesis, metabolism and storage

K09H9.6	<i>lpd-6</i>	5	yes	lethal	Suppressor of SWI4 1 PPAN	Spencer et al, 2011
T05H4.5	<i>hpo-19</i>	5			NADH-cytochrome b5 reductase 3	
F41H10.7	<i>elo-5</i>	5		lethal	Elongation of very long chain fatty acids protein 3	
Y80D3A.5	<i>cyp-42a1</i>	6		lethal	Omega-hydroxylase Cytochrome P450 4V2	
Y47G6A.23	<i>lpd-3</i>	5			Transmembrane protein KIAA1109	
B0395.2	<i>mboa-1</i>	5	yes	lethal	Acyl Co-A cholesterol acyltransferase	Dupuy et al, 2007

Collagen - structural stability of basement membranes

F46C8.6	<i>dpy-7</i>	5			Collagen XXIV alpha 1 chain	
C31H2.2	<i>dpy-8</i>	6			Collagen VI alpha 5 chain	
T21D12.2	<i>dpy-9</i>	6			Collagen VI alpha 5 chain	
Y41E3.2	<i>dpy-4</i>	6			Macrophage receptor with collagenous structure	
F27C1.8	<i>dpy-5</i>	5			Collagen VI alpha 5 chain	
C36B1.1	<i>cle-1</i>	6	yes		Collagen XV alpha 1 chain	Ackley, 2001

Other

K04F10.2		4	yes		Ciliary protein KIAA0556	Dupuy et al, 2007
W02F12.5	<i>dlst-1</i>	6		lethal	Component of 2-oxoglutarate dehydrogenase complex	
Y71H10B.1		5	yes		Cytosolic purine 5'-nucleotidase	Hunt-Newbury et al, 2007
C42C1.5	<i>tag-335</i>	6		lethal	Mannose-1-phosphate guanylttransferase beta	
LLC1.3	<i>dld-1</i>	6		lethal	Dihydrolipoyl dehydrogenase	
T09B4.9	<i>tin-44</i>	6	yes	lethal	Mitochondrial import inner membrane translocase subunit TIM44	Dupuy et al, 2007
B0273.4	<i>unc-5</i>	4	no		unc-5 netrin receptor C	
C17E4.9	<i>nkb-1</i>	4	yes	lethal	Sodium/potassium-transporting ATPase	Frederic et al, 2013
F09B9.3	<i>erd-2.1</i>	5	yes		ER lumen protein retaining receptor 2	Hunt-Newbury et al, 2007
Y113G7B.17	<i>prmt-1</i>	5	yes		Protein arginine methyltransferase 1	Li et al, 2013
K08E3.6	<i>cyk-4</i>	5		lethal	Rac GTPase-activating protein 1	
F54D7.2	<i>ept-1</i>	5	yes	lethal	Ethanolaminephosphotransferase 1	Hunt-Newbury et al, 2007
F47B7.2		6	yes		Sulfhydryl oxidase 1	Hunt-Newbury et al, 2007
C07A12.4	<i>pdi-2</i>	6	yes	lethal	Protein disulfide-isomerase	Dupuy et al, 2007
F33C8.1	<i>atrnr-1</i>	6			Attractin like 1	Vashlishan et al, 2008
F25B4.9	<i>clcc-1</i>	6			C-type lectin	
F18E2.3	<i>scc-3</i>	6		lethal	Cohesin subunit SA-2	
Y11D7A.9		6			Post-GPI Attachment To Proteins 2	
R03E1.1	<i>sym-4</i>	6	yes		WD repeat-containing protein 44	Hunt-Newbury et al, 2007
M7.1	<i>let-70</i>	5	yes	lethal	Ubiquitin-conjugating enzyme E2 D2	Zhen et al, 1996
H04M03.4	<i>glf-1</i>	6	yes		UDP-galactopyranose mutase	Thoenke et al, 2005
Y51H7C.6	<i>cogc-4</i>	4	yes	lethal	Conserved oligomeric Golgi complex subunit 4	Novelli et al, 2009
BE10.2	<i>agmo-1</i>	6			Alkylglycerol monooxygenase	
F57H12.1	<i>arf-3</i>	5	yes	lethal	ADP-ribosylation factor 5	Li et al, 2004
B0001.4		5	yes		Uridine-cytidine kinase 2	Spencer et al, 2011

H15N14.2		4		lethal	N-ethylmaleimide sensitive factor		
K04D7.1	<i>rack-1</i>	6			Receptor For Activated C Kinase 1		
D1081.4		4			Dexamethasone-induced Ras-related protein 1		
C54D1.6	<i>bar-1</i>	5		(hypersensitive)	Junction plakoglobin		Vashlishan et al, 2008
R07B7.5	<i>kmo-1</i>	6			Kynurenine 3-monooxygenase		
ZK792.7		4	yes	lethal	Metallophosphoesterase 1	Fox et al, 2007	
ZK484.1	<i>oaz-1</i>	5		lethal	Ornithine decarboxylase antizyme 2		
Unknown							
Y71F9B.10	<i>sop-3</i>	4					
F16F9.2	<i>dpy-6</i>	6					
Y43C5A.3		6		lethal			
F40F11.2	<i>mig-38</i>	6	yes	lethal		Hunt-Newbury et al, 2007	
C03D6.1		4		lethal			
F21C3.6		5					
Y37H9A.2		6					
ZK686.1		4					
Y37D8A.16		6					
K07A12.5		4				Fox et al, 2007	
F58E1.5	<i>fbxb-17</i>	6					

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