

Table S1. Sequence analysis of *sid-1* alleles

Allele	Mutation location (domain)	DNA change	Amino acid change	RNAi phenotype†	Reference
<i>qt6</i>	N terminus	AT <u>G</u> -ATA	M1I	Weak	This study and (6)
<i>qt84</i>	N terminus	CC <u>G</u> -TCG	P108S	Strong	This study
<i>qt95, qt117, [pk3321]*</i>	N terminus	GA <u>T</u> -AA <u>T</u>	D130N	Weak	This study and (15)
<i>qt9, qt81</i>	N terminus	CA <u>G</u> -I <u>A</u> G	Q154stop	Strong	This study and (6)
<i>qt109</i>	N terminus	CG <u>G</u> -I <u>G</u> G	R172W	Strong	This study
<i>qt11*, qt98, qt120</i>	N terminus	GC <u>C</u> -A <u>C</u> C	A173T	Weak	This study and (6)
<i>qt100, qt110</i>	N terminus	CA <u>A</u> -I <u>A</u> A	Q174stop	Strong	This study
<i>qt10</i>	N terminus	CC <u>G</u> -C <u>I</u> G	P199L	Weak	This study and (6)
<i>qt97, qt104</i>	N terminus	GG <u>A</u> -G <u>A</u> A	G201E	Weak	This study
<i>qt113</i>	N terminus	GG <u>A</u> -A <u>G</u> A	G201R	Strong	This study
<i>qt86</i>	N terminus	CA <u>A</u> -T <u>A</u> A	Q213stop	Strong	This study
<i>qt5*, qt107</i>	N terminus	CG <u>A</u> -I <u>G</u> A	R275stop	Strong	This study and (6)
<i>qt91</i>	N terminus	TAC <u>-</u> TAA	Y314stop	Strong	This study
<i>qt7*, qt8*, qt82</i>	TM1	CC <u>G</u> -C <u>I</u> G	P328L	Strong	This study and (6)
<i>qt127</i>	IC loop between TM1 and TM2	CA <u>G</u> -I <u>A</u> G	Q373stop	Strong	This study
<i>qt92</i>	IC loop between TM1 and TM2	CA <u>A</u> -I <u>A</u> A	Q402stop	Strong	This study
<i>qt96</i>	EC loop between TM2 and TM3	TGG <u>-</u> TG <u>A</u>	W452stop	Strong	This study
<i>qt99, qt103</i>	EC loop between TM2 and TM3	TG <u>C</u> -T <u>A</u> C	C464Y	Strong	This study
<i>qt102</i>	EC loop between TM2 and TM3	CG <u>A</u> -I <u>G</u> A	R478stop	Strong	This study
<i>qt111</i>	TM3	GG <u>A</u> -G <u>A</u> A	G488E	Strong	This study
<i>qt80</i>	TM3	GG <u>A</u> -A <u>G</u> A	G493R	Strong	This study
<i>qt114</i>	TM4	CA <u>A</u> -I <u>A</u> A	Q532stop	Strong	This study
<i>qt85</i>	TM4	A <u>I</u> T-A <u>A</u> T	I534N	Weak	This study
<i>qt2*</i>	TM4	A <u>G</u> T-A <u>I</u> T	S536I		(6)
<i>qt118</i>	MR5	CA <u>A</u> -I <u>A</u> A	Q556stop	Strong	This study
<i>qt112</i>	MR5	TG <u>T</u> -T <u>A</u> T	C559Y	Strong	This study
<i>qt4, qt93, qt115</i>	EC loop between MR5 and TM6	CG <u>T</u> -I <u>G</u> T	R565C	Strong	This study and (6)
<i>qt125</i>	EC loop between MR5 and TM6	CG <u>T</u> -I <u>G</u> T	R570C	Strong	This study
<i>qt87</i>	IC loop between MR7 and MR8	CG <u>A</u> -I <u>G</u> A	R633stop	Strong	This study
<i>qt90</i>	EC loop between MR10 and MR11	CA <u>C</u> -I <u>A</u> C	H740Y	Strong	This study
<i>qt101</i>	EC loop between MR10 and MR11	TGG <u>-</u> TG <u>A</u>	W743stop	Strong	This study
<i>qt130</i>	MR11	GG <u>A</u> -A <u>G</u> A	G747R	Strong	This study
<i>qt12*</i>	Splice donor site	G482A	Non-coding		(6)
<i>qt129</i>	Splice acceptor site	G524A	Non-coding	Strong	This study
<i>qt89, qt108</i>	Splice donor site	G5855A	Non-coding	Strong	This study
<i>qt106</i>	G to A, 24bp downstream of TAG	3'UTR	Non-coding	Strong	This study
<i>qt78</i>	385bp deletion from 3430-3815	Deletion		Strong	This study
<i>qt83</i>	338bp deletion from 5323-5661	Deletion		Strong	This study
<i>qt88</i>	465bp deletion from 2003-2468	Deletion		Strong	This study
<i>qt116</i>	158bp deletion from 5477-5635	Deletion		Strong	This study
<i>qt119</i>	378bp deletion from 6101-6479	Deletion		Strong	This study
<i>qt126</i>	Deletion from ~3600-4000	Deletion		Strong	This study
<i>qt128</i>	372bp deletion from 2005-2377	Deletion		Strong	This study

Table S2. Strong *sid-1* alleles on tissue-specific environmental RNAi

Mutation	Strain or Allele	<i>act-5 RNAi</i> gut		<i>bli-1 RNAi</i> hypodermis		<i>unc-22 RNAi</i> muscle		<i>unc-45 RNAi</i> muscle, germline			<i>fkh-6 RNAi</i> somatic gonad	
		% Arr	(n)	% Bli	(n)	% Unc	(n)	% Unc	Fertility	(n)	Fertility	(n)
CONTROLS												
	N2	100	(111)	100	(115)	100	(173)	100	Sterile	(237)	Sterile	(173)
	HC57	100	(64)	100	(34)	100	(43)	100	Sterile	(48)	Sterile	(12)
NONSENSE AND NON-CODING, STRONG												
Q154stop	<i>qt9</i>	0	(98)	0	(37)	0	(60)	0	Fertile	(24)	Fertile	(35)
Q154stop	<i>qt81</i>	0	(57)	0	(33)	0	(58)	0	Fertile	(68)	Fertile	(24)
Q174stop	<i>qt100</i>	0	(18)	6	(32)	0	(35)	0	Fertile	(24)	Fertile	(29)
Q174stop	<i>qt110</i>	0	(24)	0	(19)	0	(81)	0	Fertile	(74)	Fertile	(50)
Q213stop	<i>qt86</i>	0	(50)	ND		0	(31)	0	Fertile	(62)	Fertile	(57)
R275stop	<i>qt107</i>	0	(18)	0	(20)	0	(48)	0	Fertile	(42)	Fertile	(47)
Y314stop	<i>qt91</i>	0	(25)	ND		0	(51)	0	Fertile	(33)	Fertile	(38)
Q373stop	<i>qt127</i>	0	(23)	0	(17)	0	(19)	0	Fertile	(14)	Fertile	(17)
Q402stop	<i>qt92</i>	0	(16)	6	(33)	0	(17)	0	Fertile	(31)	Fertile	(33)
R478stop	<i>qt102</i>	0	(20)	0	(19)	0	(18)	0	Fertile	(22)	Fertile	(18)
W452stop	<i>qt96</i>	0	(35)	0	(34)	6	(35)	0	Fertile	(30)	Fertile	(35)
Q532stop	<i>qt114</i>	0	(27)	0	(22)	0	(22)	0	Fertile	(24)	Fertile	(18)
Q556stop	<i>qt118</i>	0	(23)	0	(9)	0	(15)	0	Fertile	(14)	Fertile	(25)
R633stop	<i>qt87</i>	0	(43)	ND		0	(44)	0	Fertile	(41)	Fertile	(35)
W743stop	<i>qt101</i>	0	(20)	0	(33)	0	(30)	0	Fertile	(27)	Fertile	(41)
Δ2003-2468	<i>qt88</i>	0	(65)	ND		0	(58)	0	Fertile	(50)	Fertile	(41)
Δ2005-2377	<i>qt128</i>	0	(14)	0	(18)	0	(23)	0	Fertile	(20)	Fertile	(16)
Δ3430-3815	<i>qt78</i>	0	(30)	0	(10)	0	(16)	4	Fertile	(23)	Fertile	(11)
~Δ3600-4000	<i>qt126</i>	0	(21)	0	(17)	0	(21)	0	Fertile	(14)	Fertile	(11)
Δ5323-5661	<i>qt83</i>	0	(57)	0	(53)	0	(67)	0	Fertile	(63)	Fertile	(32)
Δ5477-5635	<i>qt116</i>	0	(25)	0	(26)	0	(18)	0	Fertile	(14)	Fertile	(21)
Δ6101-6479	<i>qt119</i>	0	(22)	0	(25)	0	(44)	0	Fertile	(32)	Fertile	(29)
SAS G524A	<i>qt129</i>	0	(28)	0	(33)	0	(27)	0	Fertile	(30)	Fertile	(21)
SDS G5855A	<i>qt89</i>	0	(33)	ND		0	(43)	0	Fertile	(34)	Fertile	(35)
SDS G5855A	<i>qt108</i>	0	(18)	0	(12)	0	(56)	0	Fertile	(45)	Fertile	(28)
3'UTR G6649A	<i>qt106</i>	0	(12)	0	(14)	0	(39)	0	Fertile	(40)	Fertile	(13)
MISSENSE, STRONG												
P108S	<i>qt84</i>	0	(75)	0	(31)	0	(66)	0	Fertile	(60)	Fertile	(32)
R172W	<i>qt109</i>	0	(24)	0	(18)	0	(34)	0	Fertile	(56)	Fertile	(35)
G201R	<i>qt113</i>	0	(30)	0	(20)	0	(33)	0	Fertile	(14)	Fertile	(18)
P328L	<i>qt82</i>	0	(21)	0	(27)	0	(33)	0	Fertile	(28)	Fertile	(9)
C464Y	<i>qt99</i>	0	(20)	0	(36)	0	(36)	0	Fertile	(42)	Fertile	(33)
C464Y	<i>qt103</i>	0	(20)	0	(41)	3	(35)	0	Fertile	(38)	Fertile	(24)
G488E	<i>qt111</i>	0	(9)	0	(20)	0	(44)	0	Fertile	(51)	Fertile	(47)
G493R	<i>qt80</i>	0	(48)	0	(51)	0	(86)	0	Fertile	(52)	Fertile	(45)
C559Y	<i>qt112</i>	0	(28)	0	(23)	0	(50)	0	Fertile	(53)	Fertile	(48)
R565C	<i>qt93</i>	0	(28)	0	(20)	0	(24)	0	Fertile	(23)	Fertile	(16)
R565C	<i>qt115</i>	0	(36)	0	(37)	0	(24)	0	Fertile	(27)	Fertile	(31)
R570C	<i>qt125</i>	0	(38)	0	(15)	0	(28)	0	Fertile	(19)	Fertile	(16)
H740Y	<i>qt90</i>	0	(46)	ND		0	(45)	0	Fertile	(41)	Fertile	(46)
G747R	<i>qt130</i>	0	(18)	0	(29)	0	(33)	0	Fertile	(38)	Fertile	(24)

L1-L2 staged larvae were placed on bacteria expressing dsRNA at 20°C and were scored as young adults for the RNAi phenotypes. N2, wild type strain. HC57, background strain for all *sid-1* mutants. Arr, arrested; Bli, blistered cuticle; Unc, uncoordinated (*unc-45* RNAi animals are paralyzed, *unc-22* RNAi animals are twitching). Fertility was scored qualitatively: sterile (no progeny), fertile (many progeny), decreased (few progeny). (n), number of animals scored. ND, not determined.

Table S3. Sequences of primers used in this study.

A. For amplification and sequencing of the *sid-1* gene, C04F5.1

Primer	C04F5.1 starting nucleotide	Sequence
LGV W F	5' UTR	ggctatgagagggtcgagag
C04F5.1 F1	5' UTR	ttttcccttcattttccatttt
C04F5.1 F2	283	gcgaaatcggtgattcttc
C04F5.1 F3	796	gcgaatgtacggtatttgct
C04F5.1 F4	1352	cggaagaaaaattacctgattgg
C04F5.1 F5	1889	tcgatcagtgattccgataaa
C04F5.1 F6	2263	gatttgagccatgatgagca
C04F5.1 F7	2719	cgaattgtcggagtggaaat
C04F5.1 F8	4268	cgagcccagcgatacagtag
C04F5.1 F9	4750	tgggttgacgtaaatgcaa
C04F5.1 F10	5297	cccataaacatgaactgtcc
C04F5.1 F11	5781	ttgtggagctctgtcactgc
C04F5.1 R1	3' UTR	gcaaacgagcaattgtgaag
C04F5.1 R2	6526	gtgccataaatcgtgggaac
C04F5.1 R3	6000	cgggaaattaccgaaattga
C04F5.1 R4	5539	gatcgatccaacgacaatga
C04F5.1 R5	5021	aatcgcaataagacagcctga
C04F5.1 R6	4483	ggtgaaaattggcgaagaa
C04F5.1 R7	3062	tgaaacggccgacttttact
C04F5.1 R8	2471	tcaagccaaatatccgaag
C04F5.1 R9	2012	cgggagctatgaagacgaag
C04F5.1 R10	1550	tttgaaaaatcaagcaaaaa
C04F5.1 R11	1004	tgatcgattcaaacaaaattcg
C04F5.1 R12	500	tttaggccaaaagcctacc
C04F5.1 new genomic F	3769	cacaatcatgctgtgtctc
C04F5.1 new genomic R	3877	tcatgacggcctcttctaca
<i>sid-1</i> Exon 5 F	3473	cgcgccatcatagatttta
<i>sid-1</i> Exon 5 R	4002	catcactggggcaaatatga
<i>sid-1</i> Exon 3 R	827	ttgaaatttaagcaaaatccgta

B. For amplification of the *cbn-sid-1* promoter, gene (CBN00847) and 3' UTR

Primer	Sequence
AW179_cbn-sid-1 genomic_F1	tggcatgagttctgaaatgaa
AW180_cbn-sid-1 genomic_R1	tgtcaagttcaagtggaag

C. For cloning *sid-1* homolog cDNAs onto pPacPL and site-directed mutagenesis of the *sid-1* cDNA sequence on pH235.

Primer	Sequence
AW168_cbn-sid-1 cDNA+overlap_F	tagactcgagccgcaatgaaaggcagcggctcc
AW169_cbn-sid-1 cDNA+overlap_R	ctgtcgtcgtcgtcctttagtagcatacagttaattgcggattttg
AW170_pPacPL backbone plus flag_F	gactacaaggacgacgacg

AW171_pPacPL backbone plus flag_F	tgcggtcgagtctagag
AW205_tag-130 cDNA+overlap_F	tagactcgagccgcaatgaggacctcacaggcg
AW206_tag-130 cDNA+overlap_R	cttgctgctgctccttgtagtcgaaaactgaattgtattcctcatc
3428 qt95 Forward (D130N substitution)	caatccgctcgttgagcaaaattcggggcgacagaaag
3429 qt95 Reverse (D130N substitution)	cttctgtgcgccccgaaatttgctcaacgagcggattg

D. For gut and muscle rescue of *sid-1*

Primer	Sequence
P5	cattggcgaggagccatg
P23	gtacgacagaaagcacttcg
P37	gcggcatggtttggaac
P38	cagttcaagacagttgggtg

Table S4. Strains used in this study

Strain	Genotype
N2 Bristol	wild type
HC195	<i>nrls20 [sur-5::NLS-gfp] IV</i>
HC196	<i>sid-1(qt9) V</i>
HC393	<i>sid-1(qt9) V; qtEx89 [sur-5::NLS-gfp + sid-1(+)]</i>
HC566	<i>nrls20 [sur-5::NLS-gfp] IV; sid-1(qt9) V</i>
HC757	<i>nrls20 [sur-5::NLS-gfp] IV; sid-1(qt95) V</i>
HC57	<i>ccls4251[myo-3::NLS-gfp, myo-3::MITO-gfp] I; qtIs3 [myo-2::GFP dsRNA] III; mIs11 [myo-2::GFP] IV</i>
HC303	<i>sid-1(qt6) V</i>
HC94	<i>sid-1(qt10) V</i>
HC653	<i>sid-1(qt78) V</i>
HC655	<i>sid-1(qt80) V</i>
HC656	<i>sid-1(qt81) V</i>
HC657	<i>sid-1(qt82) V</i>
HC658	<i>sid-1(qt83) V</i>
HC659	<i>sid-1(qt84) V</i>
HC660	<i>sid-1(qt85) V</i>
HC661	<i>sid-1(qt86) V</i>
HC662	<i>sid-1(qt87) V</i>
HC663	<i>sid-1(qt88) V</i>
HC664	<i>sid-1(qt89) V</i>
HC665	<i>sid-1(qt90) V</i>
HC666	<i>sid-1(qt91) V</i>
HC667	<i>sid-1(qt92) V</i>
HC668	<i>sid-1(qt93) V</i>
HC670	<i>sid-1(qt95) V</i>
HC671	<i>sid-1(qt96) V</i>
HC672	<i>sid-1(qt97) V</i>
HC673	<i>sid-1(qt98) V</i>
HC674	<i>sid-1(qt99) V</i>
HC675	<i>sid-1(qt100) V</i>
HC676	<i>sid-1(qt101) V</i>
HC677	<i>sid-1(qt102) V</i>
HC678	<i>sid-1(qt103) V</i>
HC679	<i>sid-1(qt104) V</i>
HC681	<i>sid-1(qt106) V</i>
HC682	<i>sid-1(qt107) V</i>
HC683	<i>sid-1(qt108) V</i>
HC684	<i>sid-1(qt109) V</i>
HC685	<i>sid-1(qt110) V</i>
HC686	<i>sid-1(qt111) V</i>
HC687	<i>sid-1(qt112) V</i>
HC688	<i>sid-1(qt113) V</i>
HC689	<i>sid-1(qt114) V</i>
HC690	<i>sid-1(qt115) V</i>

HC691	<i>sid-1(qt116) V</i>
HC692	<i>sid-1(qt117) V</i>
HC693	<i>sid-1(qt118) V</i>
HC694	<i>sid-1(qt119) V</i>
HC709	<i>sid-1(qt120) V</i>
HC714	<i>sid-1(qt125) V</i>
HC715	<i>sid-1(qt126) V</i>
HC716	<i>sid-1(qt127) V</i>
HC717	<i>sid-1(qt128) V</i>
HC718	<i>sid-1(qt129) V</i>
HC719	<i>sid-1(qt130) V</i>
HC935	<i>sid-1(qt95) V; qtEx157[sid-2::sid-1 cDNA, myo-3::dsRed]</i>
HC936	<i>sid-1(qt95) V; qtEx157[sid-2::sid-1 cDNA, myo-3::dsRed]</i>
HC937	<i>sid-1(qt95) V; qtEx158[myo-3::sid-1 cDNA, myo-3::dsRed]</i>
HC938	<i>sid-1(qt95) V; qtEx158[myo-3::sid-1 cDNA, myo-3::dsRed]</i>
HC1034	<i>sid-1(qt9) V; qtEx195[cbn-sid-1 promoter::cbn-sid-1::3'UTR; pHC183 myo-3::dsRed]</i>
PB2801	<i>Caenorhabditis brenneri</i> , outcrossed x20

Strains HC94 and HC653 through HC719 are in the HC57 strain background.