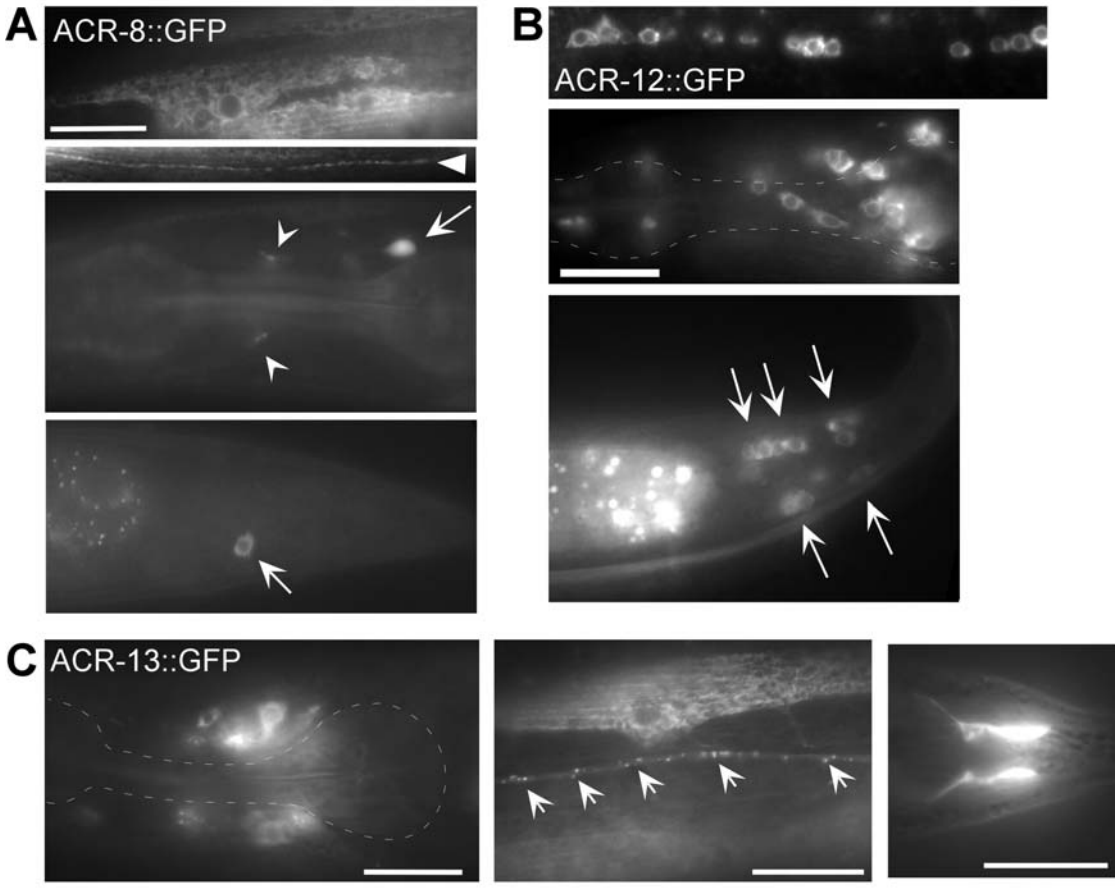


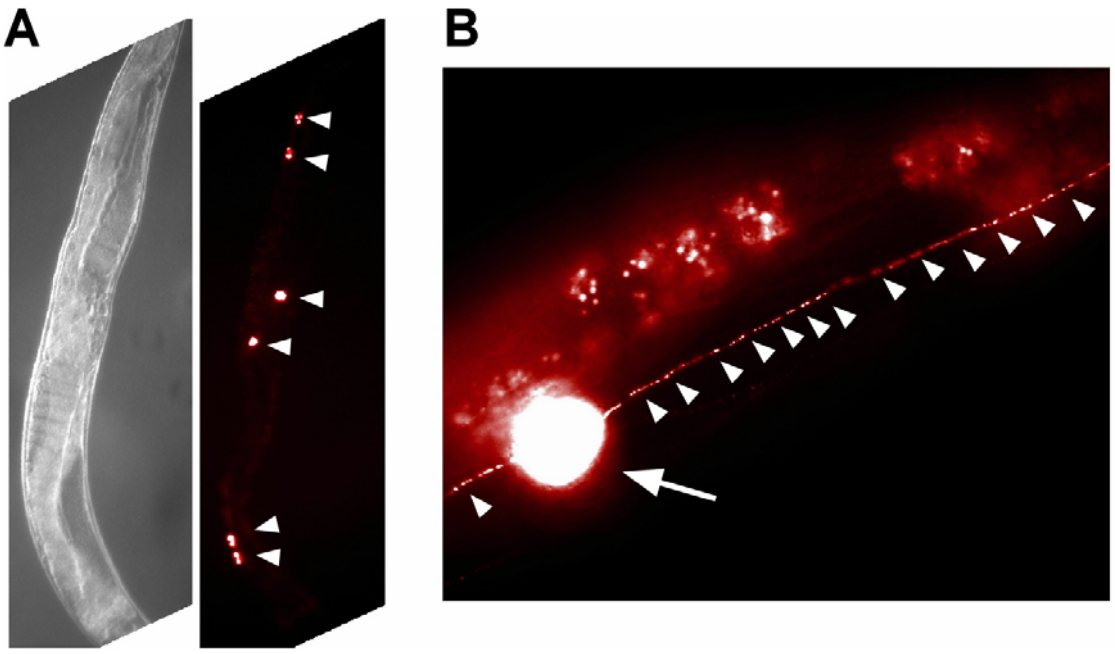
Supplementary Figure-1 Gottschalk et al.

Supplementary Fig. 1: Expression patterns of translational fusions of ACR-8, ACR-12 and ACR-13 to GFP

A: ACR-8::GFP (strain AQ1010) is expressed in body muscle (first panel), punctate sites along nervecords (arrowhead; second panel), head neurons (arrow) and nerve ring processes (open arrowheads; third panel), and in tail neurons (arrow, fourth panel).

B: ACR-12::GFP (strain AQ1012) is expressed in ventral cord motorneurons (upper panel), multiple neurons in the head (middle panel; the pharynx, the two-lobed feeding organ of the animal, is outlined with a dashed line), and tail neurons (lower panel, open arrows).

C: A truncated ACR-13::GFP (missing part of the cytoplasmic loop and TM4; strain AQ1014), is expressed in head neurons (left panel; the pharynx is outlined with a dashed line), body muscles and punctate sites along the nervecords (arrowheads, middle panel), and in the anal depressor muscle (right panel). Anterior is left in all images, young adult animals were observed. Scale bars = 20 μm.

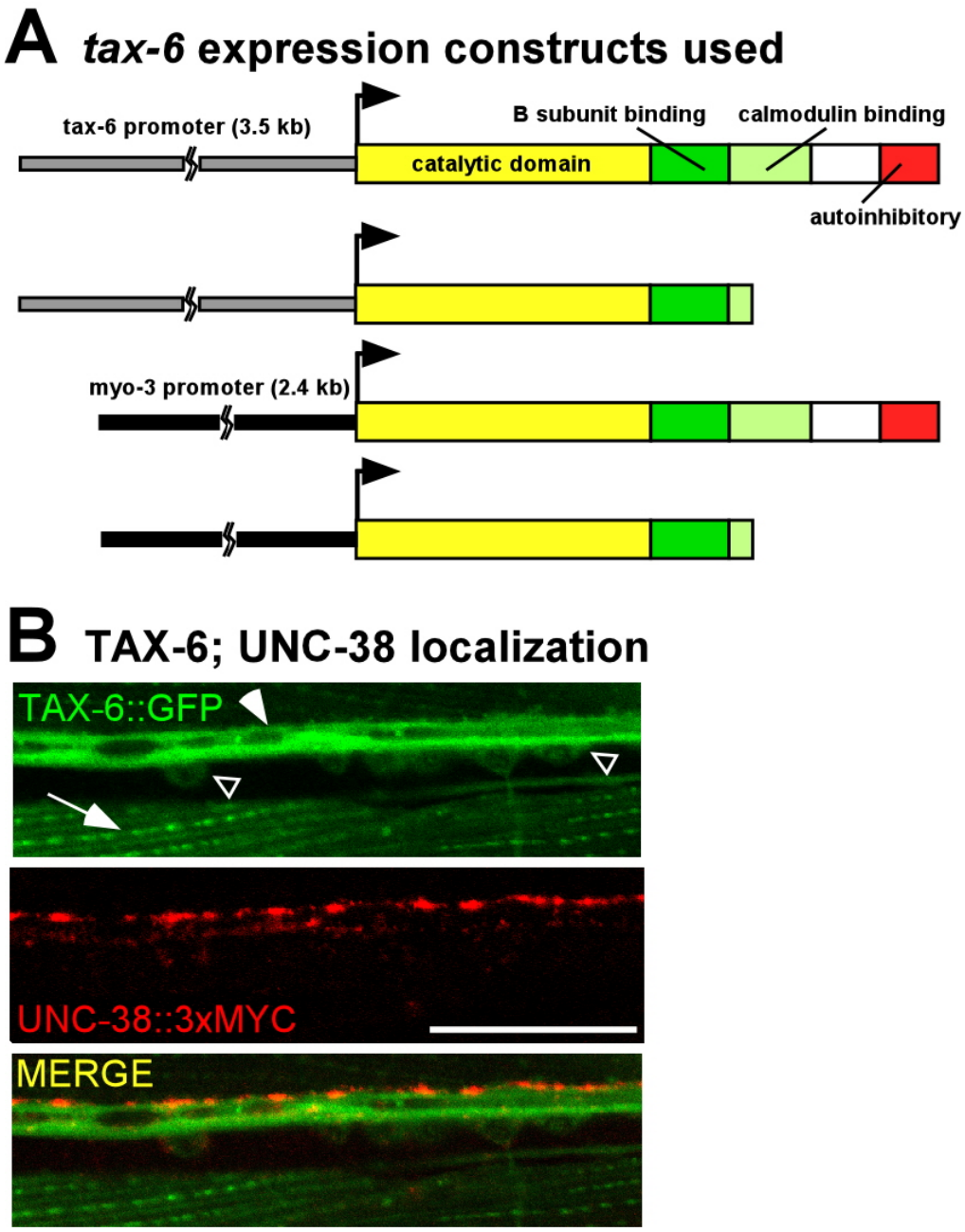


Supplementary Figure-2 Gottschalk et al.

Supplementary Figure 2: Antibodies injected into the pseudocoelom are removed from the pseudocoelomic fluid by coelomocytes

A: Nomarski and fluorescence image of a young adult animal injected 6 hours earlier with fluorescent anti-HA antibodies, showing bright fluorescence of the 6 coelomocytes.

B: Fluorescent antibodies stain punctate sites along the dorsal nervecord in the midbody region of a young adult animal expressing HA-tagged LEV-1 (arrowheads; in strain AQ881). Also shown is a brightly fluorescent coelomocyte (arrow) that took up excess (unbound) fluorescent antibody from the pseudocoelomic fluid. The auto fluorescence of gut granules can be seen as well.

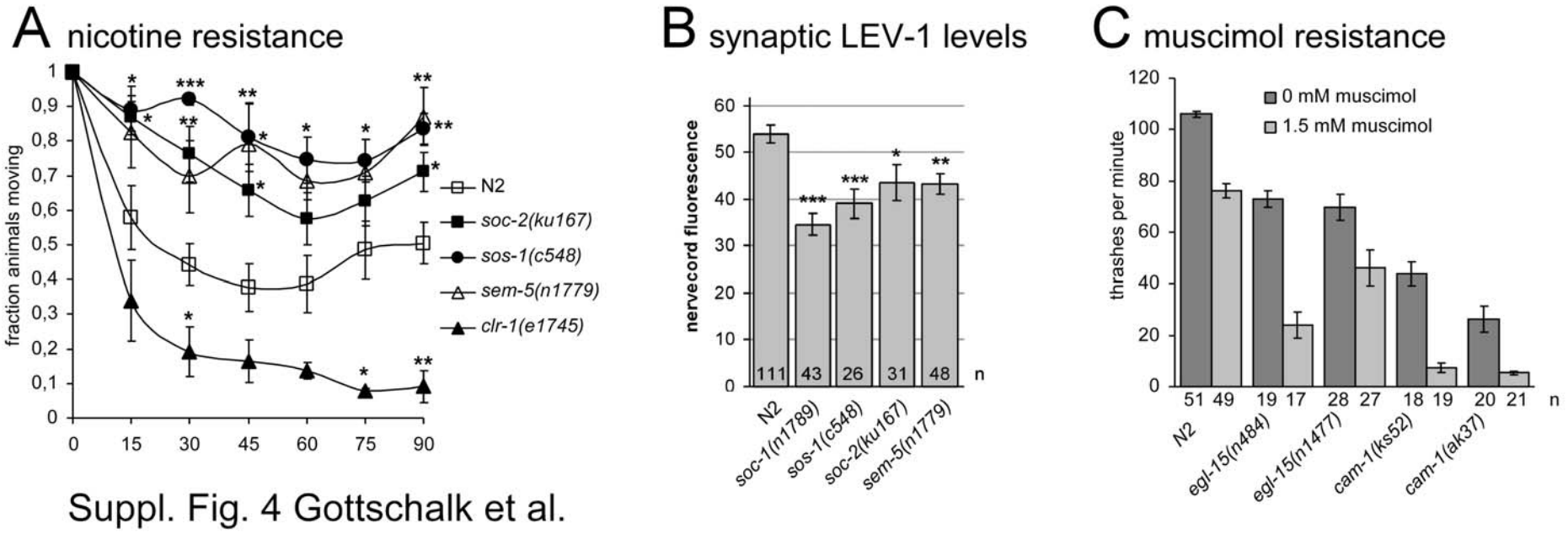


Supplementary Figure-3 Gottschalk et al.

Supplementary Fig. 3: *tax-6* constructs used for rescue experiments. Co-expression of TAX-6::GFP with the levamisole receptor.

A: Constructs used for the *tax-6* rescue experiments. cDNA constructs encoding either wild-type TAX-6 or a C-terminally truncated gain-of-function protein were placed under the control of either the *tax-6* or the muscle-specific *myo-3* promoters.

B: Co-localization of TAX-6::GFP and UNC-38::3xMYC, the latter stained by α-MYC-Cy3 antibodies injected into the pseudocoelom of a young adult animal of strain AQ1020. TAX-6 is found in muscles (arrow), ventral cord neurons (open arrows) and the ventral cord processes (closed arrowhead). Note that TAX-6 is cytosolic, thus it does not appear in clusters (like UNC-38), but uniformly along the nervecord. Confocal images obtained near the vulva, single focal plane; scale bar = 20 μm.



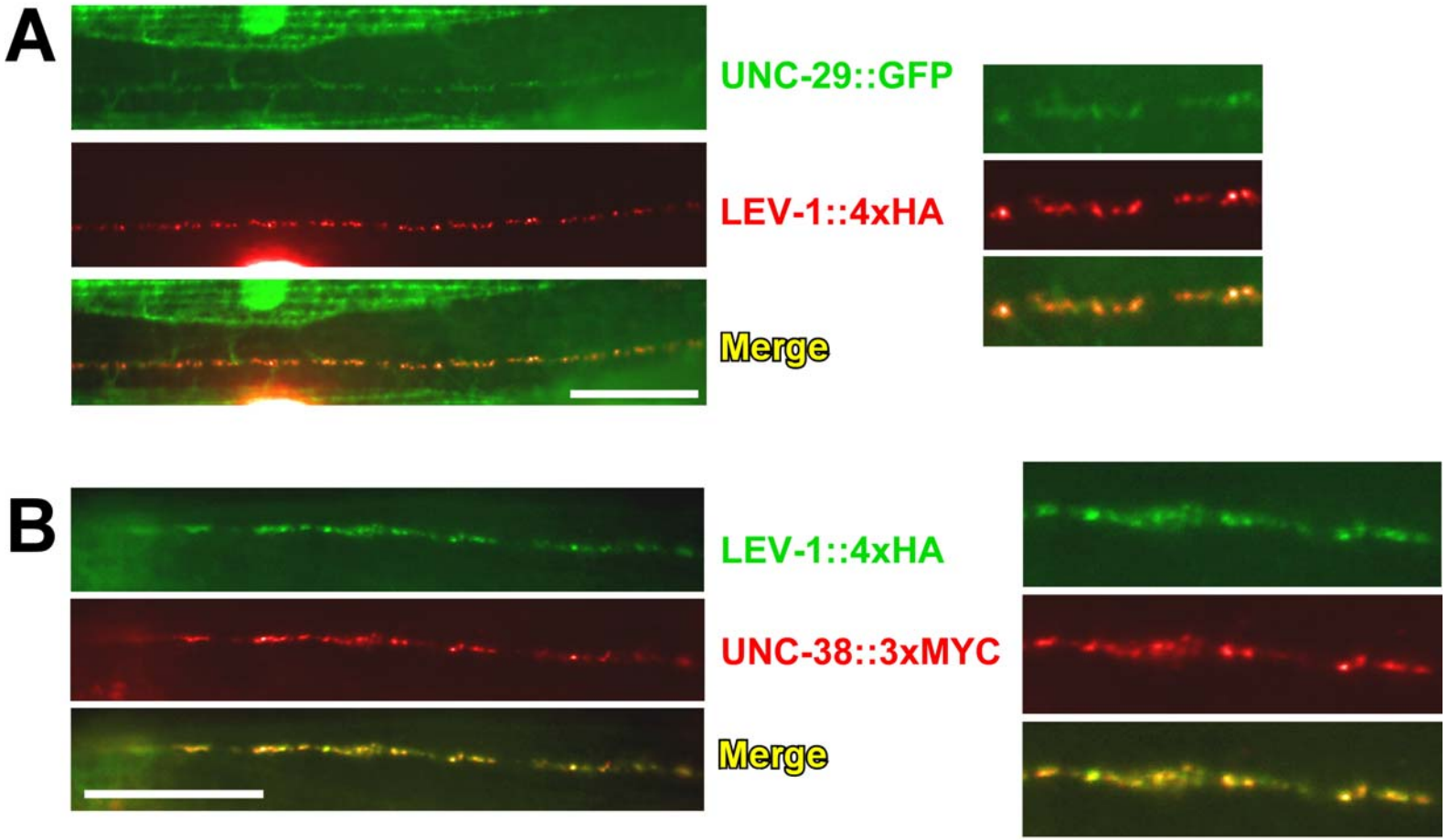
Suppl. Fig. 4 Gottschalk et al.

Supplementary Fig. 4: Mutations in the signalling pathway downstream of the FGF receptor EGL-15 affect sensitivity to nicotine but not muscimol and reduce synaptic levamisole receptor expression.

A: Paralysis after exposure to 31 mM nicotine was assayed in *sem-5(n1779)*, *sos-1(c548)*, *soc-2(ku167)* and *clr-1(e1745)* mutants and compared to the wild-type (N2). Significant resistance was observed for mutants of the positive regulators of FGF signalling, *sem-5*, *sos-1* and *soc-2*, while *clr-1* mutants, defective in a protein tyrosine phosphatase acting antagonistically to EGL-15, were nicotine resistant.

B: Synaptic expression levels of the levamisole receptor subunit LEV-1, as a 4xHA-tagged version, were quantified along the ventral nervecord of adult animals near the vulva. *sem-5(n1779)*, *sos-1(c548)*, *soc-2(ku167)* (and *soc-1(n1789)*) mutants all showed reduced synaptic expression levels when compared to the wild-type (N2).

C: Mutants in *egl-15(n484* and *n1477)* and *cam-1(ks52* and *ak37)* were studied in thrashing assays in the absence and presence of 1.5 mM muscimol, a GABA receptor agonist, and compared to the wild-type (N2). All mutants displayed significant uncoordinated *(unc)* phenotypes in this assay ($p<0.001$ in all cases). When subjected to muscimol, all mutants showed a stronger effect than wildtype animals, demonstrating that they were not muscimol resistant. ***$p<0.001$, **$p<0.01$; *$p<0.05$, error bars=s.e.m. in all panels.

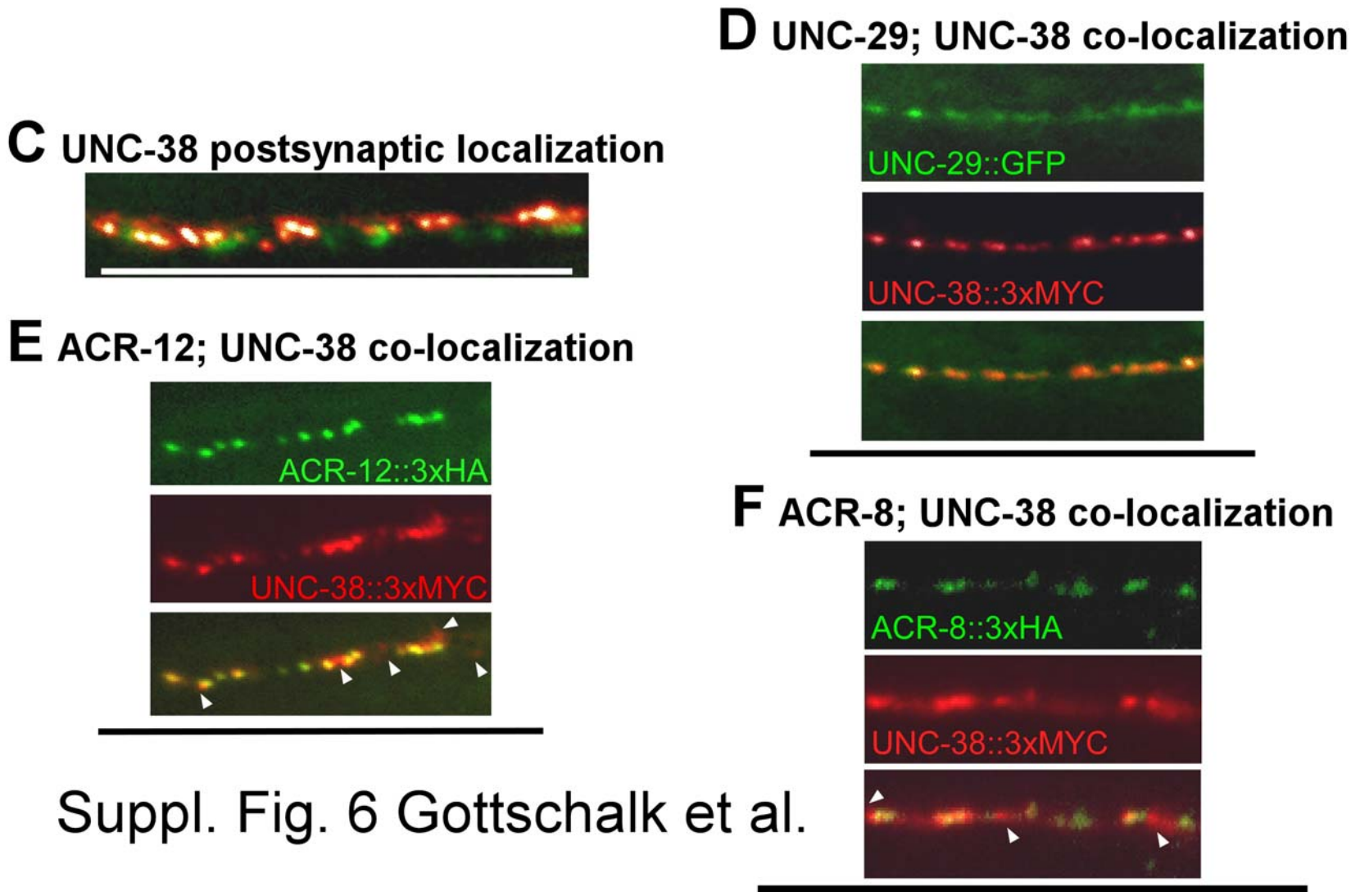


Suppl. Fig. 5 Gottschalk et al.

Supplementary Fig. 5: Co-localization of levamisole receptor subunits in vivo.

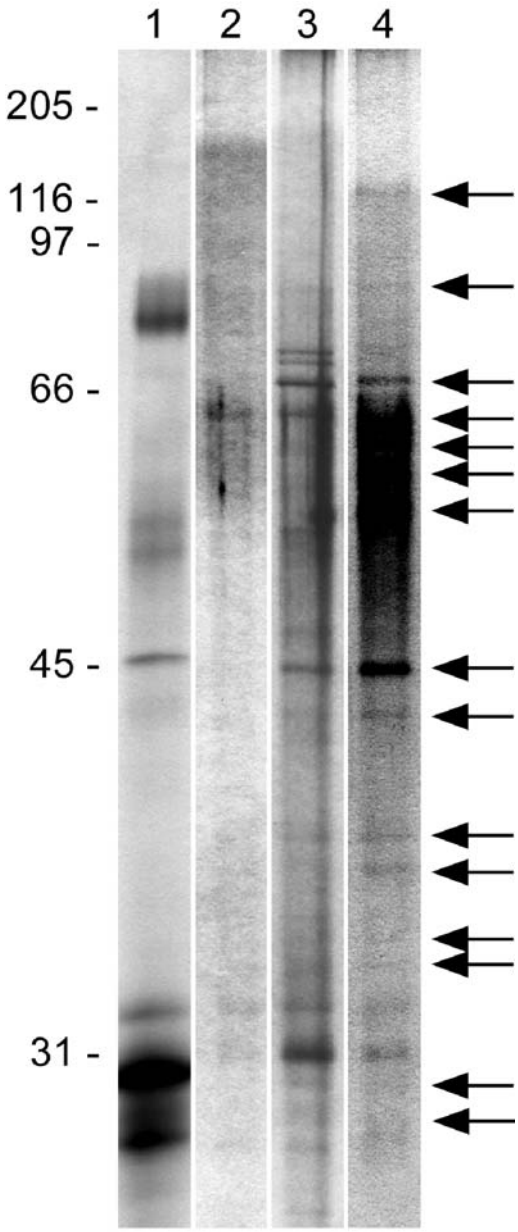
A: UNC-29::GFP (green) and LEV-1::4xHA were co-expressed (strain AQ658) and LEV-1 was labeled with injected anti-HA antibodies (red). Both proteins co-localize completely in clusters along the nervecords (merge), while UNC-29::GFP can also be seen in the ER of a muscle cell, as well as along muscle arms. Right panels show a 2.5x magnification of a small portion of the pictures in the right panel.

B: LEV-1::4xHA (green) and UNC-38::3xMYC (red) were co-expressed (strain AQ839) and labeled with injected green anti-HA and red anti-MYC antibodies in vivo. Both subunits of the levamisole receptor show complete co-localization, and no intracellular staining is visible. Right panels show a 2.5x magnification of a small portion of the pictures in the right panel. Scale bars = 20 µm in A and B.



Suppl. Fig. 6 Gottschalk et al.

Supplementary Fig. 6: Magnification of panels C-F in Fig. 2 of the main manuscript. These panels are provided to allow better visibility of the co-localization of the proteins described in this figure. Arrows point to clusters containing only UNC-38::3xMYC, but not the other nAChR subunit. Scale bars = 20 µm in all panels.



Supplementary Fig. 7: Part of Fig. 1 D of the main manuscript (purification of the levamisole receptor) with stronger contrast. This figure is provided to allow better visualization of relevant portions of the gels that contain silver stained protein bands. Lanes 2 (mock purification) and 4 (purification of the levamisole receptor using the split TAP-tag) were enhanced for contrast.

Supplementary Table I: Proteins causing nicotine hypersensitivity when depleted, that may have been unspecifically co-purified due to high abundance in *C. elegans*

The extent of the nicotine sensitivity phenotype is indicated by: + + + (strongly resistant) to - - - (strongly hypersensitive). Homologies to the best human candidate were taken from wormbase (http://www.wormbase.org).

† RNAi could target also C41C4.8

‡ RNAi could target also K01H12.2 and T01B11.4

§ RNAi could target also hsp-4

visible phenotypes abbreviated: unc – uncoordinated

Gene	No. of peptides identified	Identity/Features	RNAi phenotype in *rrf-3(pk1426)*	resistant (+) or sensitive (-) to nicotine after RNAi	No of peptides whole proteome mass spec. (Mawuenyega et al., 2003)	conservation to human homologue e-Value	over % length
RNAi confers sensitivity to nicotine, proteins are abundant in whole proteome, thus may be contaminants							
T04F3.1	1	1-aminocyclopropane-1-carboxylate synthase	few adults, else only L1, slightly unc	-	1	1.1e-42	14.1
F53A9.10	3	TNT-2, Troponin T	strongly unc, sick	- - -	2	4.1e-22	65.0
C06A1.1 †	2	transitional ER ATPase, p97 homologue	few, small, sick, unc	- - -	3	0	98.8
Y67D8C.10a	3	MCA-3, Plasma membrane Ca^{2+} ATPase	normal	- / - - (feed / soak)	3	0	93.7
R05C11.3	2	Cation transporting, E1-E2 ATPase	small adults, few, unc	- - -	3	0	94.1
Y71H2AM.23	3	Elongation factor-Tu family	few worms, L1-L3 arrest	- - -	3	2.4e-122	83.9
T22D1.4	1	Glycosyltransferase	small, unc	- - -	3	4.5e-105	92.0
F08B6.4a	1	UNC-87, Calponin	slightly unc	- -	5	5.4e-17	25.8
T22E5.5	6	MUP-2, Troponin T like	strongly unc	- - -	5	1.5e-20	66.4
C09D1.1	1	UNC-89, Ig-domains	unc	-	6	0	93.7
F01G12.5a	1	let-2, alpha-2 type IV collagen	normal, small adults, low broods.	- -	8	0	92.7
F38B2.1a	1	IFA-1; intermediate filament protein	normal	-	10	5.8e-66	91.3
K01H12.2	2	ADP/ATP carrier protein	normal	-	10	3.4e-107	92.0
T27E9.1 ‡	6	ADP/ATP carrier protein	slow growth, or arrest at L1/L2	- - -	21	2.4e-106	95.7
C15H9.6 §	4	HSP-3, BiP; associates with nAChRs in ER	arrest at L2/L3, few, unc	- - -	25	2.9e-275	98.9
K07H8.6	4	VIT-6; Vitellogenin	normal	- -	101	3.1e-6	10.0

Supplementary Table II: Proteins co-purified with the levamisole receptor that did not cause altered sensitivity to nicotine, when depleted by RNAi.

Proteins are ordered by functional categories. Proteins that were found in a whole proteome mass spectrometric analysis of *C. elegans* (Mawuenyega et al., 2003) are listed at the end of the table by increasing abundance (i.e. number of individual peptides identified).

#	Gene	No. of peptides	No. of. peptides in whole proteome mass spec. (Mawuen-yega et al., 2003)	MW[Da]	Identity / features	visual RNAi phenotype in *rrf-3*	**addtl. comments:** -paralys. experiment performed ? -strain in RNAi libr. ? -bacteria growing ?
					proteins enriched as compared to whole proteome mass spectrometry experiment		
					Ca2+ -binding domains		
1	T03F1.11	1	0	8165	calcium binding EF-hand	normal	yes
					kinases / phosphatases		
2	F22D6.9	1	0	42652	serine/threonine protein phosphatase	normal	yes
3	C46H11.4a	1	0	56840	IFE-2; Inositol polyphosphate kinase	normal	yes
4	T06D10.2	1	0	55812	unknown; similar to Protein-tyrosine phosphatase		NOT IN LIBRARY
5	F22F1.2	1	0	33656	serine/threonine protein kinase	normal	yes
6	F46C5.6	1	0	96888	possibly protein phosphatase PP2A subunit A	normal	yes
7	T04C10.1	1	0	108571	MBK-1 serine/threonine kinase	grow slowly, unc	yes
8	C07A9.3a	1	0	139865	serine/threonine kinase	very few progeny, small, sick, unc	ND
9	H05L14.1	1	0	89743	casein kinase	normal	yes
10	Y75B8A.24	1	0	238185	Phosphatidylinositol 3- and 4-kinases		NOT IN LIBRARY
11	T10A3.1a	1	0	175478	UNC-10, *C. elegans* RIM protein	normal	yes
12	C16D9.2	1	0	270446	tyrosine-protein kinase, Fibronectin type III domain	normal	yes
					Ion pumps / transporter proteins / channels		
13	F01G4.6	3	0	36674	mitochondrial phosphate carrier protein	almost no progeny, slow growth	yes
14	C01G6.1a	1	0	31426	glycerol uptake facilitator		no bacteria
15	C13C4.5	1	0	58114	putative sugar transporter	normal	yes
16	ZC168.1	1	0	89515	NCX-3, sodium/calcium exchanger like		no bacteria
17	R10D12.1	1	0	52674	sodium/phosphate transporter	normal	yes
18	F52D10.1	1	0	80278	HCO3- transporter	normal	yes
19	Y73F8A.1	1	0	92011	PKD-2, polycystic kidney disease related protein, Ca2+/Na+ channel, pore region	normal	yes
					Myosin / Troponin complex		
20	F29G9.7	1	0	34009	unknown, maybe unconventional myosin	normal	yes
21	T20B3.2	1	0	29839	TNI-3, Troponin I	normal, but less progeny than WT	yes
					G-protein coupled 7TM chemoreceptors		
22	F55C5.9	1	0	30052	seven-TM chemoreceptor	normal, low broodsize	yes
23	Y94A7B.3	1	0	34278	seven-TM chemoreceptor	normal, slightly unc	yes
24	Y60A3A.5	1	0	34629	seven-TM chemoreceptor		NOT IN LIBRARY
25	T05B11.6	1	0	43929	SRR-10, 7 TM chemoreceptor		NOT IN LIBRARY
26	C13D9.1	1	0	45779	SRR-6, 7 TM chemoreceptor	normal	yes
					G-protein and -like or involved in G-protein signaling		
27	Y39A1A.1	1	0	49628	G-protein beta WD-40 repeat	normal	yes
28	Y71G12B.1a	1	0	55212	G-protein beta WD-40 repeat	no progeny, dead eggs or L1 arrest	yes
					proteases / ubiquitination factors		
29	Y39B6A.20	2	0	42693	ASP-1, Aspartic protease A1	normal	soaking
30	C42D8.5	2	0	100725	peptidase	L1 arrest, few progeny	yes
31	F57G8.7	1	0	14782	unknown, maybe metalloprotease	normal	yes
32	F13B12.1	1	0	57254	unknown; weak similarity to calpain-like protease	very few progeny, unc	yes
33	C56A3.1	1	0	37490	unknown, weak similarity to calcium-dependent proetase	normal	yes
34	K09F5.3a	1	0	113346	UVT-1: Eukaryotic thiol (cysteine) protease	normal	yes
35	M01D1.8	1	0	33677	F-box domain	few progeny, unc (slow worms)	yes
36	W08F4.2	1	0	65782	F-box domain	normal	yes
37	Y56A3A.15	1	0	33917	F-box domain	normal	yes
					DNA-binding / transcription / RNA processing		
38	B0432.9	1	0	21607	Zinc finger	small adults	yes
39	Y48G8AL.9	1	0	33264	C2H2-type zinc finger		no bacteria
40	F49E8.2	1	0	47795	Glutaredoxin, Zinc finger	normal	yes
41	T09A5.12	1	0	79001	Zinc finger, C2H2 type (2 domains)	normal	yes
42	B0336.9a	1	0	86310	SWP-1; Surp module	normal	yes
43	Y50D4C.3	1	0	66406	Tudor domain	normal	yes
44 *	W02H5.3	1	0	58264	Transposase	normal	yes
45 *	W02H5.4	1	0	58264	CENPB, Putative DNA-binding domain in centromere protein B	some abnormal morph., rol	yes
46	F21H12.5	1	0	71805	FBF-2, Fem-3 mRNA Binding Factor	normal	yes
47	Y59A8A.2	1	0	69574	Zn-finger-like, PHD finger		NOT IN LIBRARY
48	T10D4.6	1	0	89745	AT hook motif	normal	yes
49	C36B1.9	1	0	77729	C3HC4 type Zinc (RING) finger, H+-transporting ATPase		no bacteria
50	T12F5.4	1	0	147583	LIN-59; transcription factor	normal	yes
51	Y73F8A.33	1	0	99187	Zn-finger, C2H2 type; EGF-like domain		NOT IN LIBRARY
52	M7.3	1	0	20240	KH domain family of RNA binding proteins	normal	yes
53	ZC376.7a	1	0	56019	DNA-binding, leucine zipper		no bacteria
54	ZK1055.1	1	0	166762	HCP-1	normal	yes
					Ribosomal proteins		
55	Y105E8A.16	2	0	13234	Ribosomal protein S10p/S20e		no bacteria
56	K07A12.7	1	0	38940	Ribosomal protein S15		NOT IN LIBRARY

57	Y71F9AL.6	1	0	31749	unknown; weakly similar to ribosomal protein L10a		NOT IN LIBRARY
					metabolic enzymes		
58	Y60A3A.10	1	0	30742	DHS-24, dehydrogenase		NOT IN LIBRARY
59	C05C8.1	1	0	22772	similar to mitochondrial ATP synthase regulatory component factor B from mouse		NOT IN LIBRARY
60	F27C8.2	1	0	37852	weakly similar to acetyltransferase	normal	yes
61	F27E5.1	1	0	45235	Choloylglycine hydrolase		no bacteria
62	Y48G1C.4	1	0	51701	Phospholipase D/Transphosphatidylase		NOT IN LIBRARY
63	Y48A6B.9	1	0	37944	similar to zinc-binding dehydrogenase	normal	yes
64	ZK1320.6	1	0	61202	ARC-1, ADP-ribosylation factor, C3HC4 Zinc (RING) finger		no bacteria
65	T07C4.1	1	0	54805	UMP synthase	normal, but few progeny	yes
66	E04F6.5	1	0	66172	Isovaleryl-CoA dehydrogenase		no bacteria
67	C24G6.6	1	0	59805	Flavin containing amine oxidoreductase	normal	yes
68	C49F5.1	1	0	43582	s-adenosylmethionine synthetase	L1/L2 arrest	ND
69	ZC410.3	1	0	60837	Man(9)-alpha-mannosidase ; Calcium ion binding		no bacteria
70	F10F2.2	1	0	148629	Phosphoribosylformylglycinamidine synthase	slow growth or even L1 arrest	yes
71	E02H1.4	1	0	61268	ADP-Ribosyltransferase	normal	yes
72	C05D2.4	1	0	58450	Pyridoxal-dependent decarboxylase	normal	yes
73	C33H5.18b	1	0	53460	Phosphatidate cytidylyltransferase	few progeny, L1/L2 arrest	ND
					other proteins		
74	F54D8.2	2	0	14743	Cytochrome C oxidase	slow growth or even L1 arrest, few progeny	yes
75	Y39G10AR.17	1	0	62541	unknown		NOT IN LIBRARY
76	F47C10.3	1	0	42584	nuclear hormone receptor	normal	yes
77	F52B5.5	1	0	74569	CEP-1, C.Elegans P-53-like protein	normal	yes
78	ZK1193.2	1	0	137831	Lectin C-type, von Willebrand factor type A, EGF-like domain	normal	yes
79	F49E2.5a	1	0	133819	similar to unc-89	normal	yes
80	ZK783.1	1	0	271206	EGF-like domain, Kunitz domains	no progeny, dead eggs, few L1s	ND
81	F32A7.3a	1	0	52623	Galactose binding lectin domain	normal	yes
82	F35B12.7	1	0	14299	NLP-24, neuropeptide like protein	normal	yes
83	F22F4.2	1	0	50265	UNC-7/INX-3, innexin	few worms	yes
84	R144.1	1	0	103589	KLP-6: kinesin-like protein	normal	yes
85	F35G2.1a	1	0	69535	thioredoxin	normal	yes
86	Y56A3A.21	1	0	17416	translocon-associated protein delta subunit	normal	yes
87	C50C3.2	1	0	246123	Spectrin alpha chain	normal	yes
88	Y71G12A.3	1	0	36286	Tubby-like protein		NOT IN LIBRARY
89	Y46E12BL.2	1	0	169305	SPRY domain		NOT IN LIBRARY
90 ◊	F52B11.4	1	0	30024	cuticle collagen	normal	yes
					unknown proteins		
91	W05H9.1	2	0	47474	unknown; 1 TM domain	normal	yes
92	C52D10.1	1	0	14233	unknown	normal	yes
93	T07E3.2	1	0	9729	unknown	normal, slight unc (slow)	yes
94	F21C10.5	1	0	10965	unknown	normal	yes
95	F31F7.1	1	0	36660	unknown	normal	yes
96	F32B6.4	1	0	24582	unkown		NOT IN LIBRARY
97	Y82E9BL.12	1	0	38926	Domain of unknown function DUF13		no bacteria
98	T06D4.1	1	0	50457	unknown, carboxyl transferase domain	normal	yes
99	ZK678.6	1	0	26078	unknown, 6 TM domains	normal	yes
100	D1014.6	1	0	56138	Domain of unknown function DUF138	normal	yes
101	F26D11.2	1	0	44462	unknown		no bacteria
102	D1054.13	1	0	53408	unknown	normal	yes
103	C35A5.6	1	0	31143	unknown, 2 TM domains		no bacteria
104	C08F1.5	1	0	104733	unknown, MATH domain	normal	yes
105	B0024.11	1	0	64631	Uncharacterized protein family UPF0024	normal	yes
106	C32H11.4	1	0	37959	Domain of unknown function DUF141	normal	yes
107	Y42H9AR.4	1	0	67255	unknown; maybe transporter protein		NOT IN LIBRARY
108	C29G2.4	1	0	48453	Domain of unknown function DUF32, 8 TM domains	normal, slight unc (slow)	yes
109	C40H5.4	1	0	44615	unknown	normal	yes
110	T23G4.3	1	0	55447	unknown	normal, but low broodsize	yes
111	B0412.3	1	0	122903	unknown; but conserved protein	normal	yes
112	R13F6.10	1	0	109226	unknown; but conserved	normal, but low broodsize	yes
113	M01F1.4	1	0	89899	Domain of unknown function DUF20, transmembrane domain	normal	yes
114	B0379.2	1	0	25657	TM domain, unknown	normal	yes
115	H10D18.5	1	0	38644	no homologies		no bacteria
					proteins with increasing abundance in whole protein mass spectrometry experiment; more likely to be contaminants		
116	F54C1.7	1	1	18517	PAT-10/TNC-1, Troponin C	strongly paralyzed, slow growth	ND, paralyzed
117	Y47G6A.12	1	1	144121	SEP-1, Separase	L1 arrest, few progeny	ND
118	Y51H4A.3	1	1	21635	RHO-1, p21 ras-related rho (RhoA)		NOT IN LIBRARY
119	B0250.5	1	1	31217	3-hydroxyisobutyrate dehydrogenase precurcor	normal	yes
120	C48B4.4a	1	1	189584	CED-7 ATP-binding (ABC) transporter	normal	yes
121	C01G8.9	1	2	184939	ARID/BRIGHT DNA binding domain	sterile, no eggs	ND
122 ‡	F25B5.4	1	2	94004	rpl-40.2 ubq-1, ubiquitin fused to ribosomal protein L40	no progeny, dead eggs	ND
123	C53A5.1	2	2	16495	unknown, one TM domain	no progeny, dead eggs	ND
124	ZK1058.4	1	2	50791	unknown, conserved family in all organisms		no bacteria
125	ZK721.2	1	3	35183	UNC-27, Troponin I	normal	yes
126	T13F2.8	1	3	26291	CAV-1, caveolin, component of caveolae & lipid rafts, binds G protein $\tilde{\alpha}$-subunits	some worms sick/abnormal, slightly unc	yes
127	C24A3.6	1	3	17734	TWK-2, potassium channel	normal	yes
128	F56H1.4	2	3	48157	RPT-5, AAA-ATPase	no progeny, dead eggs	ND
129	F42C5.8	1	3	23750	RPS-8, 40S ribosomal protein S8	very few, L1 arrest	ND
130	F29G9.5	2	4	49791	RPT-2, AAA-ATPase		no bacteria
131	C37H5.8	1	4	70863	HSP-6, heat shock protein 70	no progeny	ND
132	C09D4.5	1	4	23652	RPL-19, 60S ribosomal protein L19	L1 arrest, dead eggs	ND
133	T25B9.9	1	5	53196	6-phosphogluconate dehydrogenase	normal	yes
134 †	K09C4.3	7	6	33862	HSP-2, heat shock protein	no progeny, dead eggs, few L1s	ND
135	C12C8.1	7	6	70472	hsp-70 heat shock protein		no bacteria
136	F44E5.4	6	6	70687	hsp-70 heat shock protein		NOT IN LIBRARY
137	C54G4.8	1	6	30967	cytochrome C1	no progeny, no eggs	ND
138	C27A2.2a	1	6	14946	RPL-22, ribosomal protein L22	L1 arrest, dead eggs	ND
139	F56F3.5	1	6	28961	RPS-1, Ribosomal protein S3a	no progeny	ND
140	F47B10.1	7	7	47437	succinate-CoA ligase	normal	yes
141	T28D6.2	1	7	49560	TBA-7, Tubulin	normal	yes
142	Y37E3.17	1	7	91822	Sugar transporter superfamily		NOT IN LIBRARY
143	C44B12.5	1	9	42627	unknown; 1 TM domain	normal	yes
144	F09F7.2a	3	9	17144	MLC-3, myosin light chain	slightly unc, otherwise normal	soaking
145 §	F44F4.11	1	9	49960	TBA-4, Tubulin	few prog., slow growth, unc/slow movement	yes
146	Y69A2AR.18a	3	9	32382	H+-transporting two-sector ATPase, gamma subunit		NOT IN LIBRARY
147	B0393.1	4	10	30703	RPS-0 40S ribosomal protein	L1 arrest, few progeny	ND

148 ¥	B0272.1	6	11	49921	Tubulin beta chain	normal	yes
149	T21H3.3	5	12	16825	CMD-1, calmodulin	no progeny	ND
150 ¥	C54C6.2	8	13	50514	BEN-1, Tubulin beta chain	normal	yes
151	F41C3.5	2	13	53646	Serine carboxypeptidase	normal	soaking
152 †	F43E2.8	3	14	72289	HSP-4, heat shock protein		no bacteria
153	F26E4.8	2	16	50009	TBA-1, Tubulin	no progeny, dead eggs	ND
154 §	C47B2.3a	1	18	49913	TBA-2, Tubulin	no progeny	ND
155	Y105E8B.1d	1	21	32937	LEV-11, Tropomyosin		NOT IN LIBRARY
156	C36E6.3	1	21	29684	MLC-1, Myosin light chain	move slowly, unc	yes
157	C36E6.5	1	23	18603	MLC-2, Myosin light chain	normal, but less progeny than WT	yes
158	K11D9.2a/b	1	24	115511	SCA-1, SERCA (Sarco-Endoplasmic Reticulum Calcium ATPase)	no progeny	ND
159 ¥	K01G5.7	19	27	50372	Beta tubulin	normal	yes
160 ¥	C36E8.5	19	31	50504	Tubulin beta chain	no progeny, dead eggs	ND
161	F31E3.5	3	39	50668	EFT-3 Elongation factor 1-alpha	normal	yes
162	T18D3.4	6	43	223046	MYO-2, Myosin heavy chain C		no bacteria
163 $	T25C8.2	4	47	41873	ACT-5, actin	very few, sick, dead eggs, larval arrest	ND
164 †	F26D10.3	7	49	69723	HSP-1, heat shock protein	L1 arrest, low broodsize, sick, unc, dpy	ND
165	R06C7.10	4	49	223321	MYO-1 myosin heavy chain	low broodsize, else normal	yes
166 $	T04C12.4	7	68	41796	ACT-3, actin	no progeny, dead eggs, larval arrest	ND
167 $	T04C12.6	7	68	41796	ACT-1, actin	no progeny, dead eggs, few l1/L2	ND
168 $	M03F4.2a	7	69	41778	ACT-4, actin	no progeny	ND
169	F11C3.3	30	188	224753	UNC-54, myosin heavy chain	slightly unc, otherwise normal	yes

* same protein encoded by adjacent genes
$ RNAi could mutually affect act-1, -3, -4, -5
¥ RNAi could mutually affect B0272.1, C36E8.5, C54C6.2, K01G5.7 and ZK154.3
§ RNAi could mutually affect C44B11.3, F26E4.8, F44F4.11 and C47B2.3
† RNAi could mutually affect C15H9.6, F26D10.3, F43E2.8 and K09C4.3
‡ RNAi could affect also ZK1010.1
◊ RNAi could also affect F23H12.4, W05B2.1, W05B2.5, W05B2.6 and ZC513.8

SUPPLEMENTARY MATERIALS AND METHODS

Genetics

For normal maintenance, animals were cultivated on NGM, as described (Brenner, 1974). Strains used were: **N2**: wild-type, **AQ516**: *unc-29(e1072)I; unc-38(sy576)I; lev-1(e211)IV*, **AQ559**: *unc-29(x29)I*, **ZZ427**: *lev-1(x427)IV*, **AQ353**: *unc-29(x29)I*; *lev-1(e211)IV*, **AQ801**: *acr-8(cxP821)X*, **RB1195**: *acr-8(ok1240)X*, **VC188**: *acr-12(ok367)X*, **ZZ15**: *acr-13/lev-8(x15)*, **PR675**: *tax-6(p675)IV*, **AQ1008**: *tax-6(p675)IV; lin-15(n765ts)X*, **KJ300**: *cnb-1(jh103)V*, **NL2099**: *rrf-3(pk1426)II*, **RM509**: *ric-3(md158)IV*, **FK163**: *cam-1(ks52)II*, **MT5267**: *soc-1(n1789)V*, **MT1079**: *egl-15(n484)X*, **VM1095**: *cam-1(ak37)II*, **MT3456**: *egl-15(n1477ts-)X*, **MT1019**: *soc-2(ku167)IV*, **MT185**: *sem-5(n1779)X*, **UP604**: *sos-1(c548)V*, **CB3241**: *clr-1(e1745)II*, **RB1068**: *T28F3.1/nra-1(ok1025)IV*, **TM1395**: *plk-2(tm1395)I*, **TM1453**: *T05F1.1/nra-2(tm1453)I*, **TM1649**: *C17G1.4/nra-3(tm1649)X*

We created these transgenic strains (some extrachromosomal arrays were integrated by γ-irradiation): **AQ748**: AQ559; *ljIs3 [punc-29::unc-29::TAP; rol-6d]*, **AQ803**: AQ516; *ljIs5 [punc-29::unc-29::TAP; punc-38::unc-38::MYC::6xHIS-2xMYC; plev-1::lev-1::HA-6xHIS-3xHA; punc-63::unc-63::VSV-6xHIS-3xVSV; rol-6d]*, **AQ658**: AQ516; *ljEx47[punc-29::unc-29::GFP; plev-1::lev-1-HA-6xHIS-3xHA; punc-38::unc-38-MYC-6xHIS-2xMYC; punc-63::unc-63-VSV-6xHIS-3xVSV; rol-6d]*, **AQ839**: AQ353*; ljIs6 [punc-29::unc-29::TEV-ProtA; plev-1::lev-1::CBP; rol-6d]*, **AQ881**: N2; *ljEx41[plev-1::lev-1-HA-6xHIS-3xHA; rol-6d]*, **AQ884**: FK163; *ljEx41*, **AQ887**: RM509; *ljEx41*, **AQ898**: N2; *ysIs42X [punc-4::snb-1::GFP; lin-15+]; ljEx46[plev-1::lev-1-HA-6xHIS-3xHA; punc-38::unc-38-MYC-6xHIS-2xMYC; rol-6d]*; **AQ1010**: N2; *ljEx84 [pacr-8::acr-8::GFP; rol-6d]*, **AQ1011**: N2; *ljEx85 [pacr-8::acr-8::6xHIS-3xHA; punc-38::unc-38::MYC-6xHIS-2xMYC; rol-6d]*, **AQ1012**:

N2; *ljEx86 [pacr-12::acr-12::GFP; rol-6d]*, **AQ1013**: N2; *ljEx87 [pacr-12::acr-12::6xHIS-3xHA; punc-38::unc-38::MYC-6xHIS-2xMYC; rol-6d]*, **AQ1014**: N2; *ljEx88 [pacr-13::acr-13Δ412–531::GFP; rol-6d]*, **AQ1015**: AQ1008*; ljEx89 [ptax-6::tax-6 cDNA WT; lin-15+]*, **AQ1016**: AQ1008*; ljEx90 [ptax-6::tax-6 cDNA g.o.f.; lin-15+]*, **AQ1017**: PR675; *ljEx91 [pmyo-3::tax-6 cDNA WT; rol-6d]*, **AQ1018**: AQ1008; *ljEx92 [pmyo-3::tax-6 cDNA g.o.f.; lin-15+]*, **AQ1019**: NL2099; *ljEx93 [plev-1::lev-1::GFP; punc-38::unc-38::MYC-6xHIS-2xMYC; rol-6d]*, **AQ1020**: N2; *ljEx94 [ptax-6::tax-6::GFP; punc-38::unc-38::MYC-6xHIS-2xMYC; rol-6d]*, **AQ1056**: MT5267; *ljEx41*, **AQ1057**: MT1079; *ljEx41*, **ZX97**: VM1095; *ljEx41*, **ZX188**: RB1068; *ljEx41*, **ZX189**: UP604; ljEx41, **ZX190**: MT1019; ljEx41, **ZX187**: CB3241; ljEx41, **ZX198**: MT185; ljEx41, **ZX174**: MT3456; ljEx41, **ZX214**: N2; *zxEx11[pnra-1::nra-1::GFP; rol-6d]*, **ZX206**: N2; *zxEx4[pnra-1::nra-1::GFP; plev-1::lev-1-HA::6xHIS-3xHA; rol-6d]*, **FY386**: *unc-49(e407); lin-15(n765ts)X; grEx[punc-49::3xMYC::unc-49; lin-15(+)]*, **ZX276**: *nra-1(ok1025); unc-49(e407); lin-15(n765ts)X; grEx[punc-49::3xMYC::unc-49; lin-15(+)]*, **ZX277**: *soc-1(n1789); unc-49(e407); lin-15(n765ts)X; grEx[punc-49::3xMYC::unc-49; lin-15(+)]*

Plasmid construction

Sequences of primers used are available from the authors, on request. For TAP- or TEV-ProtA-tagged versions of *unc-29*, the respective PCR products, amplified from the TAP-plasmid pBS1479 (Rigaut et al., 1999) were inserted 3' of the *unc-29* coding region of plasmid LJH5 (Fleming et al., 1997), replacing the GFP coding sequence that is present in this plasmid, to yield pAG17 (punc-29::unc-29::TAP) and pAG18 (punc-29::unc-29::TEV-ProtA). For the CBP-tagged version of *lev-1*, 2 kb preceding the start codon of *lev-1* and subsequently the whole *lev-1* coding region, created by

PCR from genomic DNA, were cloned into plasmid pPD95.79 (1995 Fire lab vector kit), to yield pAG5. Then the GFP coding region of the resulting plasmid, downstream of the *lev-1* coding region, was replaced by a PCR product encoding the CBP tag amplified from plasmid pBS1479, to yield pAG19.

GFP-tagged versions of levamisole receptor subunits were created similarly: Promoter (usually 2 kb or more of the genomic region upstream of the respective genes' start codon) and coding regions were amplified from genomic DNA. DNA encoding for single copies of the epitope tags (HA or cMYC) was included at the 3'-end as part of the downstream primer used to amplify the coding regions. These products were subsequently cloned into pPD95.79, to yield GFP-tagged versions, namely pAG6 (punc-38::unc-38-MYC::GFP), pAG7 (punc-63::unc-63-VSV::GFP) and pAG5 (plev-1:lev-1-HA::GFP). To create epitope-tagged versions, the GFP coding sequence of the respective constructs was replaced by PCR-products encoding a hexa-histidine tag and 2 or 3 copies of the respective epitope tags (amplified from plasmids pU6H2MYC, pU6H3HA, or pU6H3VSV, a gift from A. DeAntoni; De Antoni and Gallwitz, 2000), to yield pAG8 (plev-1::lev-1-HA-6xHIS-3xHA), pAG9 (punc-38::unc-38-MYC-6xHIS-2xMYC) and pAG10 (punc-63::unc-63-VSV-6xHIS-3xVSV). ACR-8, ACR-12 and ACR-13 GFP fusions were prepared using pAG5, by amplifying the respective promoter (3.07 kb preceding the start codon for *acr-8*, 1.52 kb for *acr-12* and 3.39 kb for *acr-13*) and full coding regions and inserting them to replace the LEV-1 coding region and promoter. For ACR-13, the coding region was C-terminally truncated, removing the last 119 amino acids. These plasmids were pAG20 (pacr-8::acr-8::GFP), pAG21 (pacr-12::acr-12::GFP) and pAG22 (pacr-13::acr-13Δ412–531::GFP). To create 3xHA-tagged ACR-8 and ACR-12, their promoter and coding regions were subcloned into pAG8, replacing all *lev-1* sequence

and one of the HA-tag copies, to yield pAG23 (pacr-8::acr-8::6xHis-3xHA) and pAG24 (pacr-12::acr-12:: 6xHis-3xHA).

For the *tax-6* constructs, we modified plasmids that were a generous gift from I. Mori (Kuhara et al., 2002). A wild-type genomic clone of tax-6 (pAK043), fused to GFP, was used to amplify the 3.5 kb *tax-6* promoter. This promoter, or the *myo-3* promoter amplified from plasmid pPD96.52 (A. Fire), were used to replace the *gcy-8*-promoter in two plasmids that contained either a wild-type *tax-6* cDNA (pAK049) or a *tax-6* cDNA that was truncated at the 3'-end, so that the auto inhibitory peptide and part of the calmodulin binding region were removed (pAK50). The plasmids created were pAG25 (ptax-6::tax-6cDNA WT), pAG26 (ptax-6::tax-6cDNA g.o.f.), pAG27 (pmyo-3::tax-6cDNA WT) and pAG28 (pmyo-3::tax-6cDNA g.o.f.).

GFP-tagged copine NRA-1 was made by amplifying the promoter (1.7 kB) and coding region, omitting the stop codon, of T28F3.1, and fusing it C-terminally to GFP, in vector pPD95.79, between PstI and XmaI sites, to yield plasmid pAG30.

An N-terminally 3xcMYC-tagged version of the GABA receptor subunit UNC-49 was kindly provided by A. Benham and B. Bamber, University of Utah, Salt Lake City, USA. This construct was made by an in-frame insertion of three copies of the c-myc epitope (EQKLISEEDL) between the fourth and fifth amino acids following the predicted signal peptide cleavage site. Strain FY386 was constructed by injecting overlapping fragments of *unc-49* genomic DNA as described in (Bamber et al., 1999) into strain EG1892 (*unc-49(e407);lin-15(n765ts)*), along with the *lin-15* rescue plasmid pEK1 at 40 ng/µl. Non-shrinker non-muvs were isolated and a line was established.

Large-scale culture of *C. elegans*

Nematodes were cultivated on egg-plates, as described (Mains and McGhee, 1999). Typically, ca. 50 15cm plates were seeded with the respective strains. Animals were harvested 7-9 days later, depending on health of the culture and appearance of dauer larvae. Animals were washed off the plate with water, and pelleted by centrifugation. The tight-packed worm pellet was cleared off the soft egg-pellet by gentle shaking with fresh water. Residual animals present in the egg-pellet were cleaned by sucrose floatation. Yields varied between 40-120 g of animals.

Peptide generation, multi-dimensional liquid chromatography, mass spectrometry and database searching

The TCA-precipitated sample was reduced and alkylated using TRIS 2-carboxyethyl phosphine HCl and Iodoacetamide. The sample was then sequentially digested with endoproteinase Lys-C (Roche Diagnostics, Indianapolis, IN) and sequencing grade soluble Trypsin (McCormack et al., 1997) (Promega, Madison, WI). The resulting peptide mixture was then analysed by multidimensional protein identification technology (MudPIT) as described (Link et al., 1999; Washburn et al., 2001) with modifications as described (McDonald et al., 2002). Tandem mass spectra were searched against the Wormpep database (available at www.wormbase.org) of predicted *C. elegans* open reading frames to which common contaminants such as keratin and trypsin were added. Search results were filtered and grouped using the DTASelect program (Tabb et al., 2002) and identifications confirmed through manual evaluation of spectra.

Fluorescence microscopy

Fluorescence microscopy was performed on a Zeiss Axioskop 2 FS, using excitation and emission filters for GFP and rhodamine, and Hamamatsu C4742-95 or Zeiss Axiocam MRm digital cameras. Images were obtained using MetaVue (Universal Imaging Corporation) or Zeiss Axiovision software. Worms were mounted on wet agarose pads containing 30 mM NaN_3 as anaesthetic. Images of TAX-6::GFP co-localised with antibody-stained UNC-38::3xMYC were taken on a Leica SP2-AOBS confocal microscope. For double labelling (red and green labelled antibodies or GFP), identical images were taken with the respective filters and subsequently coloured and overlayed using Adobe Photoshop and Canvas (DENEBA).

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