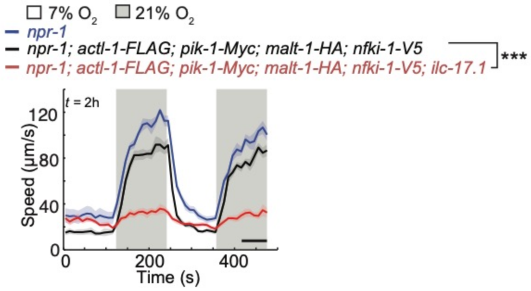
MALT1 MEDIATES IL-17 NEURAL SIGNALING TO REGULATE *C. ELEGANS* BEHAVIOR, IMMUNITY AND LONGEVITY

Flynn et al.

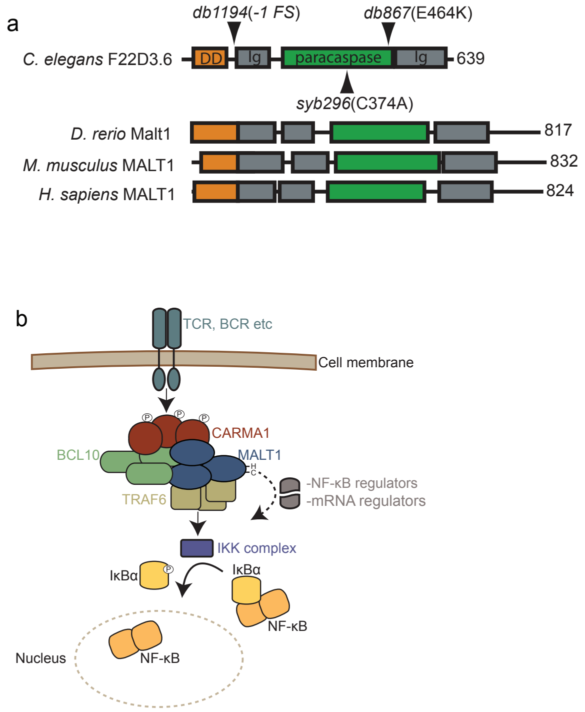
Supplementary Information

Supplementary Figures



Supplementary Figure 1. Related to Figure 1. Knocking in epitope tags into endogenous *actl-1*, *pik-1*, *malt-1*, and *nfki-1* does not disrupt their function**.**

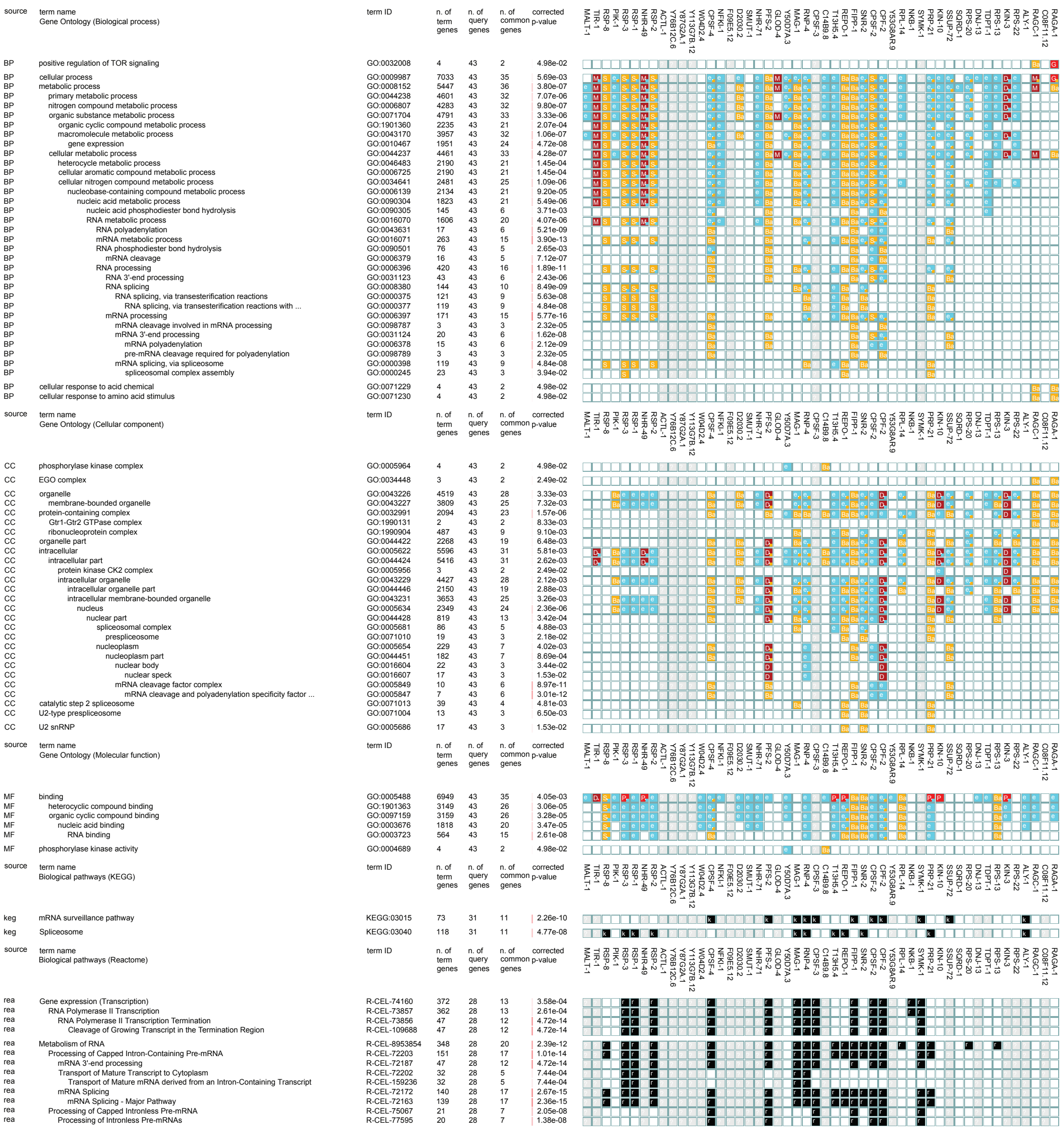
A strain co-expressing *actl1-1-HA*, *pik-1-Myc*, *malt-1-HA* and *nfki-1-V5* in an *npr-1* background responds to 21% O_2 like *npr-1* controls. Arousal is abolished when these knock-in alleles are crossed into an *ilc-17.1(tm5218)* background. n = 69 animals (*npr-1*), n = 81 animals (*npr-1; actl-1-FLAG; pik-1-Myc; malt-1-HA; nfki-1-V5*), n = 113 animals (*npr-1; actl-1-FLAG; pik-1-Myc; malt-1-HA; nfki-1-V5; ilc-17.1*) ***, P = 1.18e-27, Mann-Whitney *U* test.



Supplementary Figure 2. Related to Figure 1. MALT1.

a MALT1 paracaspase domain organization. Black arrows indicate the impact of *malt-1* mutations. DD, death domain; Ig, Immunoglobulin-like fold.

b Schematic of MALT1 signaling in mammalian B and T cells. BCR/TCR stimulation induces formation of the CARMA1-BCL10-MALT1 (CBM) signalosome via protein kinase C β (PKCβ)/PKCθ-mediated phosphorylation of CARMA1. MALT1 recruits TRAF6, whose ubiquitin ligase activity leads to the recruitment and activation of the IKK complex. This culminates in IKK-mediated phosphorylation of IκBα, triggering its degradation and releasing NF-κB for nuclear translocation. MALT1 also promotes lymphocyte activation by cleaving negative regulators of NF-κB, and factors that regulate mRNA stability. Blast searches do not find nematode orthologs of BCL10 or CARMA.



source	term name Gene Ontology (Biological process)	term ID	n. of term genes	n. of query genes	n. of common genes	corrected p-value
BP	positive regulation of TOR signaling	GO:0032008	4	43	2	4.98e-02
BP	cellular process	GO:0009987	7033	43	35	5.69e-03
BP	metabolic process	GO:0008152	5447	43	36	3.80e-07
BP	primary metabolic process	GO:0044238	4601	43	32	7.07e-06
BP	nitrogen compound metabolic process	GO:0006807	4283	43	32	9.80e-07
BP	organic substance metabolic process	GO:0071704	4791	43	33	3.33e-06
BP	organic cyclic compound metabolic process	GO:1901360	2235	43	21	2.07e-04
BP	macromolecule metabolic process	GO:0043170	3957	43	32	1.06e-07
BP	gene expression	GO:0010467	1951	43	24	4.72e-08
BP	cellular metabolic process	GO:0044237	4461	43	33	4.28e-07
BP	heterocycle metabolic process	GO:0046483	2190	43	21	1.45e-04
BP	cellular aromatic compound metabolic process	GO:0006725	2190	43	21	1.45e-04
BP	cellular nitrogen compound metabolic process	GO:0034641	2481	43	25	1.09e-06
BP	nucleobase-containing compound metabolic process	GO:0006139	2134	43	21	9.20e-05
BP	nucleic acid metabolic process	GO:0090304	1823	43	21	5.49e-06
BP	nucleic acid phosphodiester bond hydrolysis	GO:0090305	145	43	6	3.71e-03
BP	RNA metabolic process	GO:0016070	1606	43	20	4.07e-06
BP	RNA polyadenylation	GO:0043631	17	43	6	5.21e-09
BP	mRNA metabolic process	GO:0016071	263	43	15	3.90e-13
BP	RNA phosphodiester bond hydrolysis	GO:0090501	76	43	5	2.65e-03
BP	mRNA cleavage	GO:0006379	16	43	5	7.12e-07
BP	RNA processing	GO:0006396	420	43	16	1.89e-11
BP	RNA 3'-end processing	GO:0031123	43	43	6	2.43e-06
BP	RNA splicing	GO:0008380	144	43	10	8.49e-09
BP	RNA splicing, via transesterification reactions	GO:0000375	121	43	9	5.63e-08
BP	RNA splicing, via transesterification reactions with ...	GO:0000377	119	43	9	4.84e-08
BP	mRNA processing	GO:0006397	171	43	15	5.77e-16
BP	mRNA cleavage involved in mRNA processing	GO:0098787	3	43	3	2.32e-05
BP	mRNA 3'-end processing	GO:0031124	20	43	6	1.62e-08
BP	mRNA polyadenylation	GO:0006378	15	43	6	2.12e-09
BP	pre-mRNA cleavage required for polyadenylation	GO:0098789	3	43	3	2.32e-05
BP	mRNA splicing, via spliceosome	GO:0000398	119	43	9	4.84e-08
BP	spliceosomal complex assembly	GO:0000245	23	43	3	3.94e-02
BP	cellular response to acid chemical	GO:0071229	4	43	2	4.98e-02
BP	cellular response to amino acid stimulus	GO:0071230	4	43	2	4.98e-02

source	term name Gene Ontology (Cellular component)	term ID	n. of term genes	n. of query genes	n. of common genes	corrected p-value
CC	phosphorylase kinase complex	GO:0005964	4	43	2	4.98e-02
CC	EGO complex	GO:0034448	3	43	2	2.49e-02
CC	organelle	GO:0043226	4519	43	28	3.33e-03
CC	membrane-bounded organelle	GO:0043227	3809	43	25	7.32e-03
CC	protein-containing complex	GO:0032991	2094	43	23	1.57e-06
CC	Gtr1-Gtr2 GTPase complex	GO:1990131	2	43	2	8.33e-03
CC	ribonucleoprotein complex	GO:1990904	487	43	9	9.10e-03
CC	organelle part	GO:0044422	2268	43	19	6.48e-03
CC	intracellular	GO:0005622	5596	43	31	5.81e-03
CC	intracellular part	GO:0044424	5416	43	31	2.62e-03
CC	protein kinase CK2 complex	GO:0005956	3	43	2	2.49e-02
CC	intracellular organelle	GO:0043229	4427	43	28	2.12e-03
CC	intracellular organelle part	GO:0044446	2150	43	19	2.88e-03
CC	intracellular membrane-bounded organelle	GO:0043231	3653	43	25	3.26e-03
CC	nucleus	GO:0005634	2349	43	24	2.36e-06
CC	nuclear part	GO:0044428	819	43	13	3.42e-04
CC	spliceosomal complex	GO:0005681	86	43	5	4.88e-03
CC	prespliceosome	GO:0071010	19	43	3	2.18e-02
CC	nucleoplasm	GO:0005654	229	43	7	4.02e-03
CC	nucleoplasm part	GO:0044451	182	43	7	8.69e-04
CC	nuclear body	GO:0016604	22	43	3	3.44e-02
CC	nuclear speck	GO:0016607	17	43	3	1.53e-02
CC	mRNA cleavage factor complex	GO:0005849	10	43	6	8.97e-11
CC	mRNA cleavage and polyadenylation specificity factor ...	GO:0005847	7	43	6	3.01e-12
CC	catalytic step 2 spliceosome	GO:0071013	39	43	4	4.81e-03
CC	U2-type prespliceosome	GO:0071004	13	43	3	6.50e-03
CC	U2 snRNP	GO:0005686	17	43	3	1.53e-02

source	term name Gene Ontology (Molecular function)	term ID	n. of term genes	n. of query genes	n. of common genes	corrected p-value
MF	binding	GO:0005488	6949	43	35	4.05e-03
MF	heterocyclic compound binding	GO:1901363	3149	43	26	3.06e-05
MF	organic cyclic compound binding	GO:0097159	3159	43	26	3.28e-05
MF	nucleic acid binding	GO:0003676	1818	43	20	3.47e-05
MF	RNA binding	GO:0003723	564	43	15	2.61e-08
MF	phosphorylase kinase activity	GO:0004689	4	43	2	4.98e-02

source	term name Biological pathways (KEGG)	term ID	n. of term genes	n. of query genes	n. of common genes	corrected p-value
keg	mRNA surveillance pathway	KEGG:03015	73	31	11	2.26e-10
keg	Spliceosome	KEGG:03040	118	31	11	4.77e-08

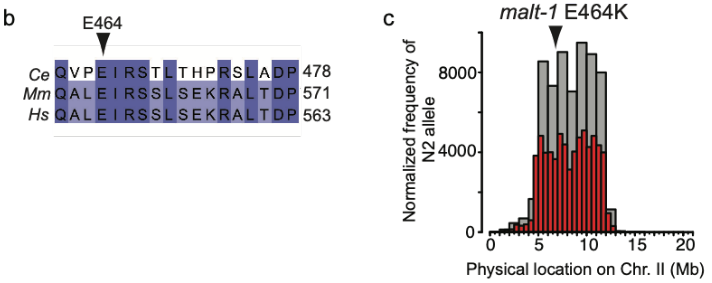
source	term name Biological pathways (Reactome)	term ID	n. of term genes	n. of query genes	n. of common genes	corrected p-value
rea	Gene expression (Transcription)	R-CEL-74160	372	28	13	3.58e-04
rea	RNA Polymerase II Transcription	R-CEL-73857	362	28	13	2.61e-04
rea	RNA Polymerase II Transcription Termination	R-CEL-73856	47	28	12	4.72e-14
rea	Cleavage of Growing Transcript in the Termination Region	R-CEL-109688	47	28	12	4.72e-14
rea	Metabolism of RNA	R-CEL-8953854	348	28	20	2.39e-12
rea	Processing of Capped Intron-Containing Pre-mRNA	R-CEL-72203	151	28	17	1.01e-14
rea	mRNA 3'-end processing	R-CEL-72187	47	28	12	4.72e-14
rea	Transport of Mature Transcript to Cytoplasm	R-CEL-72202	32	28	5	7.44e-04
rea	Transport of Mature mRNA derived from an Intron-Containing Transcript	R-CEL-159236	32	28	5	7.44e-04
rea	mRNA Splicing	R-CEL-72172	140	28	17	2.67e-15
rea	mRNA Splicing - Major Pathway	R-CEL-72163	139	28	17	2.36e-15
rea	Processing of Capped Intronless Pre-mRNA	R-CEL-75067	21	28	7	2.05e-08
rea	Processing of Intronless Pre-mRNAs	R-CEL-77595	20	28	7	1.38e-08

Supplementary Figure 3. Related to Figure 1. The MALT-1-interacting proteome is enriched in factors involved in RNA metabolism.

Gene ontology and biological pathway enrichment analysis of the 50 factors that specifically co-immunoprecipitate with MALT-1 (Supplementary Data 1), performed with g:Profiler[79].

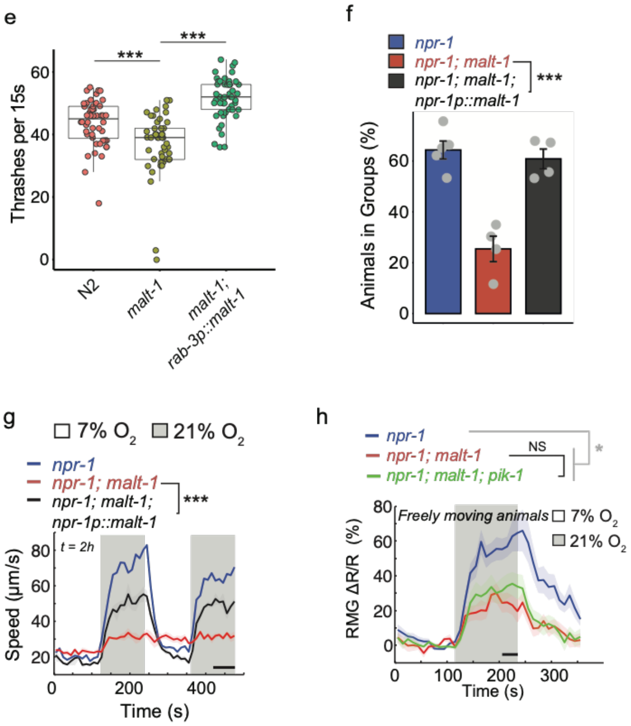
a

Strain	Position	Reference	Alteration	Effect	aa change
AX3324	6948747	G	A	STOP_GAINED	W495*
AX3621	6948842	G	A	NON_SYNONYMOUS_CODING	E464K
AX3713	6948859	G	A	NON_SYNONYMOUS_CODING	C458Y
AX3868	6951060	G	A	NON_SYNONYMOUS_CODING	G63R



d

Strain	Allele	Position	Reference	Alteration	Effect	aa change
AX5877	*db1194*	6950732	CCACCG-	--ACCTA	FRAME_SHIFT	-1



Supplementary Figure 4. Related to Figure 1. MALT-1 promotes escape from 21 O_2.

a *malt-1* mutations isolated in a forward genetic screen for loss of aggregation behavior.

b The MALT-1 E464 residue mutated in AX3621 is conserved from *Ce* to human, and corresponds to E549 in human, which is known to be required for proteolytic activity. *Mm = Mus musculus, Hs = Homo sapiens*.

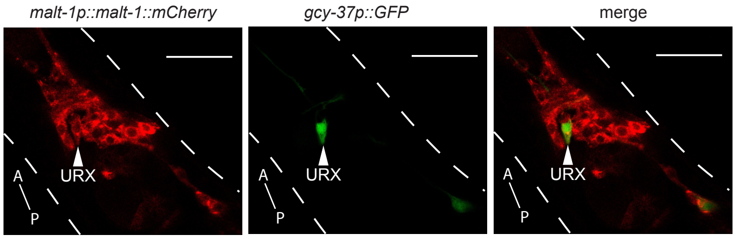
c The aggregation defect of AX3621 maps close to *malt-1*. Mapping used CloudMap (see Methods), which measures the relative levels of SNPs derived from two genetic backgrounds in aggregation-defective recombinants. N2 single nucleotide polymorphisms (SNPs) are derived from AX3621; Hawaiian SNPs are derived from the AX288 (*lon-2 npr-1*) Hawaiian background strain. Aggregation-defective recombinants show an enrichment of N2 Bristol SNPs on chromosome II, flanking the physical location of the *malt-1* mutation at 6.9 Mb.

d The *malt-1(db1194)* allele, generated using CRISPR/Cas9 genome editing.

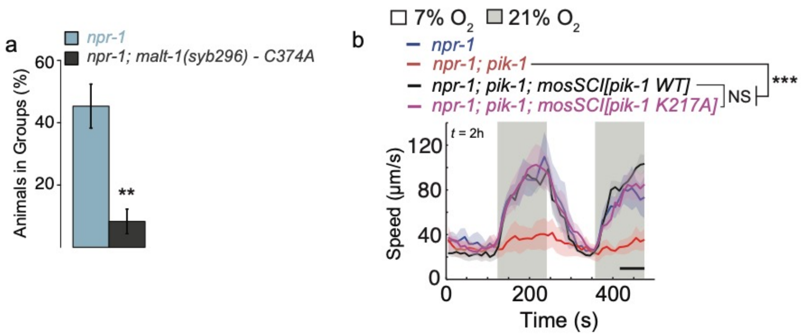
e *malt-1* mutants exhibit minor defects in thrashing rate. Data are presented as median (centre) and interquartile range (box; the lower and upper bounds of the box represent the 25th and 75th percentiles respectively). Whiskers represent ±1.5x interquartile range. n = 48 animals. ***, $P < 0.001$, one-way ANOVA with Tukey's post hoc HSD.

f and **g** The aggregation phenotype (**f**) and arousal defect in 21% O_2 (**g**) of *malt-1(db1194)* mutants is rescued by expressing *malt-1* cDNA from the *npr-1* promoter, which drives expression in a broad subset of neurons including URX and RMG[29,47]. (**f**) N = 5 assays (*npr-1)*, N = 4 assays (*npr-1; malt-1*) and (*npr-1; malt-1; npr-1p::malt-1*), ***, $P < 0.001$, ANOVA with Tukey's post hoc HSD (**g**) n = 44 animals (*npr-1*), n = 84 animals (*npr-1; malt-1*), n = 77 animals (*npr-1; malt-1; npr-1p::malt-1*). ***, P = 5.33e-08, Mann-Whitney *U* test.

h *malt-1* RMG Ca^{2+} transients (reported by YC2.60) in freely moving animals are not further reduced by loss-of-function mutations in *pik-1*. n = 20 animals (*npr-1*), n = 17 animals (*npr-1; malt-1*), n = 17 animals (*npr-1; malt-1; pik-1*). NS, P = 0.3, *, $P < 0.05$, two-sided Mann-Whitney *U* test.



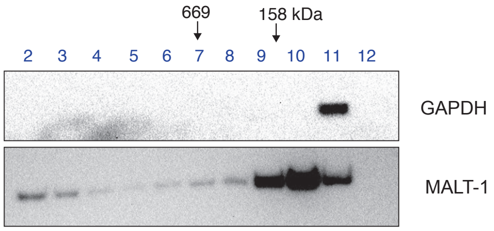
Supplementary Figure 5. Related to Figure 2. MALT-1 is expressed in O_2-sensing neurons. MALT-1::mCherry translational fusion, expressed from its endogenous promoter (4kb), is expressed in URX neurons in the head which are labelled with a *gcy-37p::gfp* reporter. Similar results were obtained in two experiments. Scale bars: 20µm.



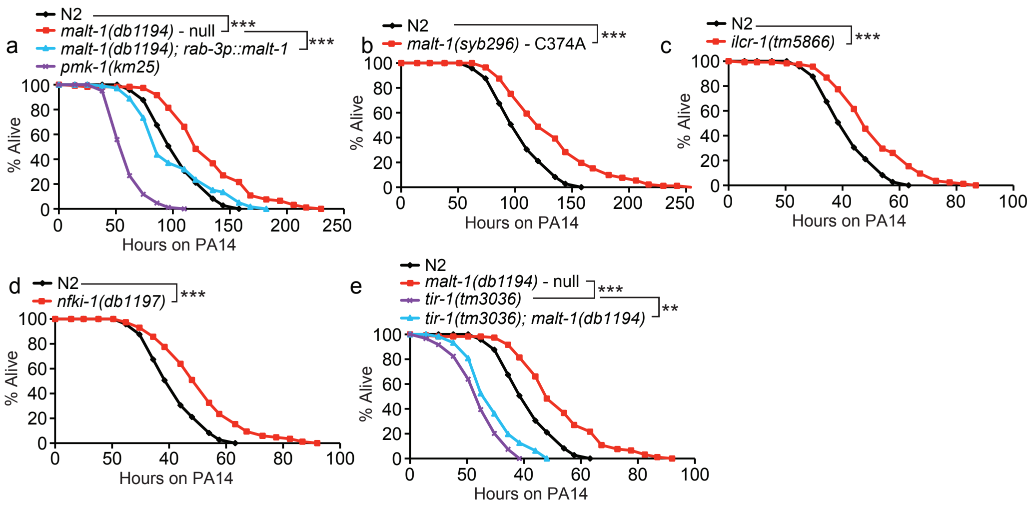
Supplementary Figure 6. Related to Figure 5. Targeted disruption of the protease activity of MALT-1 and kinase activity of PIK-1/IRAK.

a *malt-1(syb296)* mutants expressing catalytically inactive MALT-1 C374A exhibit strong aggregation defects compared to *npr-1* animals. N = 5 assays, **, P = 0.0018, one-way ANOVA with Tukey's post hoc HSD.

b PIK-1 ATP-binding may not be required for avoidance of 21% O_2. Single copy transgenes (MosSCI) expressing *pik-1* WT and *pik-1 K217A* cDNA rescue the O_2-response defect of *pik-1(tm2167)* mutants equally well. K217 corresponds to the lysine residue coordinating ATP binding in kinase active sites. n = 40 animals (*npr-1*), n = 69 animals (*npr-1; pik-1*), n = 70 animals (*npr-1; pik-1; mosSCI[pik-1 K217A]*), n = 56 animals (*npr-1; pik-1; mosSCI[pik-1 WT]*). Plots show average speed (line) and SEM (shaded regions). NS, P = 0.09, ***, P < 0.001, two-sided Mann-Whitney U test.



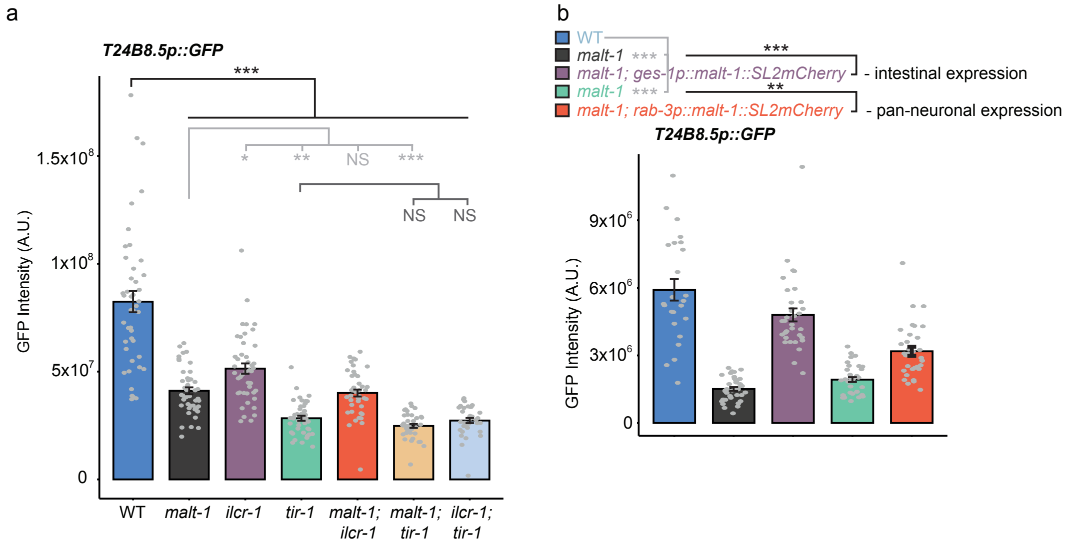
Supplementary Figure 7. Related to Figure 6**.** The elution profile MALT-1 and GAPDH proteins in *C. elegans* extract run on a Superose 6 Gel Filtration column and visualized by immunoblot. Unlike GAPDH control, MALT-1 is found in high-molecular weight fractions. This experiment was performed once.



Supplementary Figure 8. Related to Figure 8. *P. aeruginosa* PA14 small lawn assays.

a-d Mutants defective in *malt-1* and other IL-17 signaling components are resistant to *P. aeruginosa* PA14. The enhanced survival in PA14 small lawn assays of *malt-1* mutants compared to N2 controls is rescued by pan-neuronal expression of *malt-1* gDNA. n ≥ 74 animals. *, P, < 0.05, **, P, < 0.01, ***, P, < 0.001, logrank test. Precise n numbers and P values are provided in Supplementary Table 2.

e The enhanced resistance of *malt-1* mutants to PA14 requires TIR-1. Like *tir-1* mutants, *malt-1; tir-1* double mutants are hypersensitive to PA14 infection. n ≥ 95 animals. ***, P < 0.001, logrank test. Precise n numbers and P values are provided in Supplementary Table 2.



Supplementary Figure 9*.* Related to Figure 8. MALT-1 regulates innate immune response gene expression.

a Expression from *T24B8.5p::GFP*, a reporter of the innate immune response, is inhibited in *malt-1(db1194)*, *ilcr-1(tm5866)*, and *tir-1(tm3036)* mutants compared to N2. n = 44 animals (WT, *malt-1*, and *ilcr-1*), n = 42 animals (*tir-1*), n = 43 animals (*malt-1*; *ilcr-1*), n = 39 animals (*malt-1*; *tir-1*), n = 35 animals (*ilcr-1*; *tir-1*). *, P < 0.05, **, P < 0.01, ***, P < 0.001, one-way ANOVA with Tukey's post hoc HSD.

b *T24B8.5p::GFP* expression in *malt-1* mutants is restored by tissue-specific expression of WT *malt-1* cDNA in the intestine (*ges-1* promoter) or the nervous system (*rab-3* promoter). n = 42 animals (blue bars), n = 37 animals (black bars), n = 33 animals (purple bars), n = 24 animals (green and red bars). **, P < 0.01, ***, P < 0.001, one-way ANOVA with Tukey's post hoc HSD.

Fig. 6a

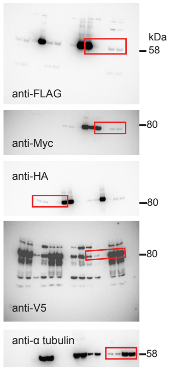


Fig. 6d

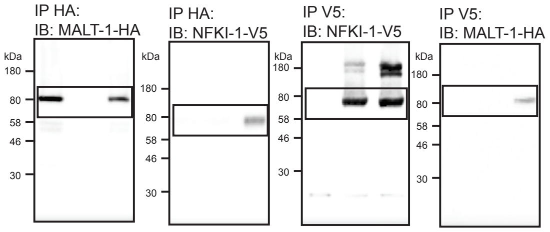


Fig. 6e

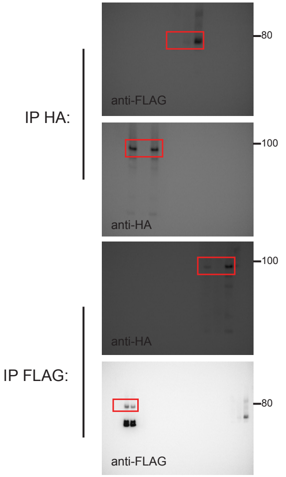


Fig. 6g

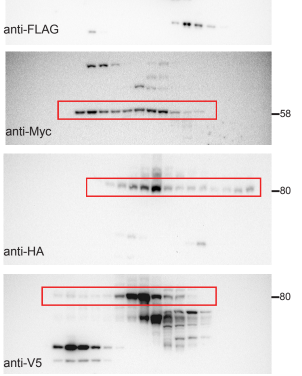
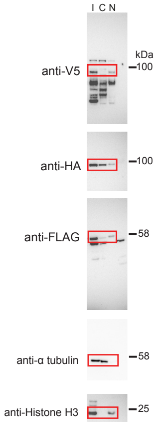
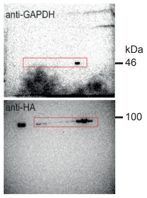


Fig. 7a



Supplementary Fig. 7



Supplementary Figure 10. Uncropped Western blots (original scans).

Supplementary Tables

Supplementary Table 1 Related to Fig. 8**.** Summary of PA14 big lawn Survival data analysis of IL-17 pathway mutants.

Strain	**Mean lifespan ±s.e.m. (hours)**	**Age in hours at 50% mortality**	**n (died/total)**	**Bonferroni *p* value vs. N2**
N2	48.47 ± 0.8	48	83/120	
malt-1(db1194)	56.95 ± 1.35	54	91/120	6.9e-7
malt-1 (db1194); rab-3p:: malt-1	45.25 ± 0.86	48	86/120	0.069 (0*)
pmk-1 (km25)	36.91 ± 0.62	38	104/120	0
malt-1(syb296)	62.16 ± 1.36	62	81/120	0
ilcr-1 (tm5866)	57.82 ± 1.25	54	87/120	0
nfki-1 (db1197)	57.72 ± 1.31	54	85/120	7.7e-8
tir-1(tm3036)	41.1 ± 0.81	38	98/120	1.6e-7 (0*)

malt-1(db1194); tir-1(tm3036	45.2 ± 1.48	38	98/120	0.0319 (0.0000014*) (0.2221**)

**p* value against *malt-1(db1194)*. ** *p* value against *tir-1(tm3036)*.

Supplementary Table 2 Related to Fig. 8**.** Summary of PA14 small-lawn survival data analysis of IL-17 pathway mutants.

Strain	**Mean lifespan ±s.e.m. (hours)**	**Age in hours at 50% mortality**	**n (died/total)**	**Bonferroni *p* value vs. N2**
N2	105.18 ± 2.31	110	95/120	
malt-1(db1194)	132.14 ± 3.67	120	96/120	0
malt-1 (db1194); rab-3p:: malt-1	101.31 ± 3.74	86	74/120	1 (1e-7*)
pmk-1(km25)	61.73 ± 1.34	62	112/120	0
malt-1(syb296)	132.92 ± 3.92	120	98/120	2.9e-8
ilcr-1(tm5866)	127.20 ± 3.46	120	91/120	6.8e-7
nfki-1(db1197)	129.17 ± 3.54	135	94/120	2.1e-7
tir-1(tm3036)	73.82 ± 2.1	74	100/120	0 (0*)
malt-1(db1194); tir-	62.19 ± 1.88	62	99/120	0

1(tm3036				(0*) (0.0039**)

*p value against *malt-1(db1194)*.   ** p value against *tir-1(tm3036)*.

Supplementary Table 3 Related to Fig. 8. Summary of lifespan of IL-17 pathway mutants on OP50.

Strain	**Mean lifespan ±s.e.m. (days)**	**Age in hours at 50% mortality**	**n (died/ total)**	**Bonferroni *p* value vs. N2**	**Independent replicate 1 (n; mean lifespan ±s.e.m. (days); Bonferroni *p value*)**	**Independent replicate 2 (n; mean lifespan ±s.e.m. (days); Bonferroni *p value*)**	**Independent replicate 3 (n; mean lifespan ±s.e.m. (days); Bonferroni *p value*)**
N2	18.68 ± 0.31 (Figure 6i-l)	19	157/ 180		134/180; 17.32 ± 0.37	142/180; 18.7 ± 0.31; (Figure 6m)	143/180; 20.25 ± 0.54
malt-1 (db1194)	22.76 ± 0.41 (Figure 6j)	23	132/ 180	0	112/180; 20.84 ± 0.58; 2.9e-7	146/180; 21.29 ± 0.3; 1.3e-7 (Figure 6m)	155/180; 25.62 ± 0.63; 0
malt-1 (db1194); rab-3p:: malt-1	18.56 ± 0.38 (Figure 6j)	19	109/ 180	1 (0*)	146/180; 18.78 ± 0.46; 0.0105 (0.0115*)		
ilc-17.1 (tm5218)	20.72 ± 0.35 (Figure 6k)	21	140/ 180	0.0001	117/180; 19.00 ± 0.45; 0.0097		112/180; 24.06 ± 0.83;; 0.0001
illc-17.1 (tm5218); ilc-17.1p::	13.78 ± 0.25 (Figure 6k)	13	116/ 180	0 (0*)	104/180; 13.84 ± 0.31; 0 (0**)	96/180; 16.11 ± 0.32; 0.000006	105/180; 16.56 ± 0.5; 0.000004

ilc-17.1						(Figure 6m)	(0**)
ilc-17.1 (tm5218); malt-1 (db1194)	21.62 ± 0.41 (Figure 6l)	23	111/150	1.2e-7 (0.2044*) (0.3839**)			131/180; 23.95 ± 0.64; 0.0001 (0.1232*) (1**)
malt-1(db1194); ilc-17.1p:: ilc-17.1						92/180; 21.48 ± 0.34; 4.5e-8 (1*) (0***) (Figure 6m)	116/180; 23.95 ± 0.71; 0.0002 (0.1847*) (0***)

**p* value against *malt-1(db1194)*. ***p* value against *ilc-17.1(tm5218)*.

****p* value against *ilc-17.1(tm5218)*; *ilc-17.1p:: ilc-17.1*

Supplementary Table 4 Knockin alleles used in this study.

>malt-1(syb296)

C374A

Synonymous mutation

ttaccccactagcactactcctccttcaataattttttatttcagAAAATGAACTACAAAACAGTAACTCTCGCTGATCTGACACTTGATGAAATGAGATATTTTATTAGAGTTTATCAGAAATTGATTGGAAATGGAGTATACGgtaggtatatttgatttttaaaaatataaaaaagaacacttaatttagCTGTATTCTATTTTGTTGGTCATGGATTTGAAGTGAATGGTCAATGTTATTTACTTGGTGTTGATGCTCCAGCAGATGCTCATCAACCACAACATTCAATGAGTATGGATTGGgtaaatacctataaaaactatatttagagaatctaaatccaattccagCTTTTGTCAATTTTCCGTCATAAAACTCCTGATTTAAACTTGCTTCTTCTTGATGTC**GCG**CGCAAATTTGTTCCgtaagtattaaaactgaattcgcgtaactctgtcaaattcgttaaatttaaaataattcgaaaagatattatgatcagaacatttttatttgaaaaccggcaacaaatttctcaaattccacatttgccaaaaatttggcagtatgccaaaactttagtatacggtatttagaaaaatcgccagcttgaacagttgaatatttccagATATGATGCGATCAGTGCATTTGTGGAATATTCAGAACAATTCAAAAAATTCCATCGAGCCCATAGAAATATGGTTTATGGATACTCTACAAGCGGTGGAGTCGGAGCATATGAGgtttctttttcatttgaaaaaagtttataaaaattgattattttcagGTTAAAGGAGAAGTGAATGGTGTCTTTATGAAATATTTGAAGAATCATGTTCAATTGGAAATATCTGTTATTGATATGTTGAATAAAGTACTATTAGgtaagtatatgaactttttctctgagaactatacactatccaaagtttctactctgatttatatttttgcagATATTGGGGATGATCAAAAAGTTTGTGATCTTCAAGTTCCCGAG

>malt-1(syb573)

3XHA + Twin-Strep

Synonymous mutation

CTCAACAATTAGTCGCTACGATTTGGTTTCAATTTTGTGGAAATTTCACAAACAAGgtttctagaaaatgtcctcaattctttaaagtataaaatattcagGTTTACGTATTCGCATCAATATCAGACTTCCGTCCTTGCCAGGAAGACACCGATATGGGAGAAAATGAAGAATTAAGTGAAAATGCATTGAATCATCGAGCATTTGTTGAATTTCCTGAAGAGCTTCATTGTTCTGATGTTCGAGAATATAATGATGATGAAGAAGGTGTCTCAATGTTATGGATTCTATCAGGATTACAAAAAATCAAGAAAGAAGCCGGATTGACATGTGAAGTTCATTTAAGACACGTTGATGATCCA**GA**AAAGACGATTGAGATGAAGAATGTT**GA**CATTGGTCATATTTTGATTACAAGAATCAAATGTCTACAGTATCCATACGACGTCCCAGATTATGCTTACCCGTACGACGTTCCAGACTACGCATATCCATATGATGTCCCAGACTACGCTTGGTCGCACCCGCAATTTGAGAAAGGATCCGCCGGATCCGCCGCCGGATCCGGAGCCGGATGGTCCCATCCACAGTTCGAAAAGTAAtgcattgccatgtgaacacctacgggtccgaaaatccattattaattatttttgccaattgaaatgtagtactaatgaaacgaaatctcatttattttacaatgaataaaatgcgtaaagtaatggtgtatgaattaatataaaacgaataaaccgatggaagg agaaaaaaatgaacgaatacgccatcgtgaatgaatgaaacagacaaagcaatgggaaagaaacgacaaaaaggatgagaaagagattttcgc

gtcaatatatagagaaaatgattggacggtgtggaga

>pik-1(syb378)

linker-2XMyc

Synonymous mutation

AAAAGCATCCATTAATTGCTTCACATATCAAGGGAACACTTGCTTATCTTGCACCAGAATTCATTACATC

AAAGATTCTTACCACAAAACTTGATGTCTATAGTTTTGGAATAGTACTTTTGGAAATTGCATCTGGTCAAC

GGGCATATTCGGATTCTCGTGAAACTCGGGGGCTCGTTGAATATTGTCAGGTTAATAAGGAATTGGCAG

CACATCGGAAGATTCCAGTCAGAGAGATTTTTATTGATCGACGAGCGCCGCCACTTGTTGGTGATGAG

GAAAAATCATTTTTGGATGCTCTGATTGAAGTTGGATTAGCTGGAGCGAATAACGATCGGAAAGTTCGA

CCGACTATGTCACAAATTGTTGAATATCTTTGTAAAAATTCAATTCCGCCAGTTGTCGGAGGCGGT

GAACAAAAGTTGATATCCGAAGAGGACCTTGAGCAGAAGCTGATAAGTGAA

GAAGACCTGTAAttaatactgtgtaccttgccatattcctcgaactcgaaattttgccatttttgagtcaaaactacggtagcgggtctcgacac

gaccgcctatagagattactgtagcaggggctttttggcaaatttttatatccggcaatttgcctatttgccggaaatttcaattctggcaatttgccaatttgcct

gaaagtttcatttccggcaattttccgatttgccgaaagttttcatatccggcaatatgctgatttgccggaaatttttattttctgaaa

>actl-1(syb412)

3XFLAG

Synonymous mutation

tagagttttctcattacgaacgtcggaattttgtgatcatttttggtctgataaaataaagactcaaatatagctgctccaccttctacaaaattttttgattaatttgt

cttaacatcacatttaaaaacttcgtttttaattaaagattgcagtatctttctgaaaatacatggctggacatctaactctctggcttccaaaaatgtttacactg

tgtgaaatgtgtattatagGAAGTGGAAAAAGTAACGGACACGTACTGGGCAGCTGTAACGAATCAATATCCATTC

CCCGGAAGTTTTGAACCATTTGTGAAGCGTCTTCTGAGAGATGGAAAAGTGAAGAAACAATCACACAAT

AATGCAAATGATCATCACGAGGATTCCATGAACTACAGTATTACACAAGGTGGTGGTGATTACA

AGGATCACGACGGAGACTATAAAGATCATGATATCGACTACAAGGATGATG

ATGATAAGTAGacaccatgtggtattttgagccaataatctatacattgtagcatctgatgaacttttttttcattgtacactttttgatgacagtcac

gtgtttttttagatttctcagacagtctttactatccacatttttcatattacaaaaacagtatttttctttcaattttctcagttaatctattaagatgttgtttccgactttc

aatttccagattttattatacactaatgaaataaatcttacagaaggttc

>nkfi-1(syb617)

V5

Synonymous mutation

TGATGGATGAGCAGACACAGCACAAAATTGACAACAAGTCCATCATCGAAATGCTCATCAAGGCTGGAG
CAGATCCAACGATTGCTgtaagtggattatgtgcttttgatgattaccaatacagctcttttcagGAAACTTCAACTGGAAAGACAA
TTGTACATCATGCTGTCGACAAGATGGACGTGGAACTTTTAGATgtaagttccgaatttcctcgagtttccttaataaataaat
atttaaaattttcagTTCTTAAAAACCGTTGTCAACGAAGACACGTTCACCGAGTTGGCAAATCTCTCCGACTTC
CATGGAGACACCGCAGTCGACCTTTTGTGTAGTTCCACCCAGAACGAGGACACGAATAATGTCCGCGA
AAATCTATACATCCGACTCCTGACC**AGT**GGTGCAGTCCCGAACAAGTCGAGAGCTGGAAAGCCAA
TCCCAAACCCACTTCTTGGACTCGACTCCACCTGAgctctattcagcttcattcacattttttttgacgttgttg
cataatgccatcaaaaccacctatatttgactcttcgttatagtttattttttattttgttgatctcaaaaccacccctctaatctaaaaaattaaactttgaaaataa
tcaaaaaattattttttaaaagtacttcattaaaattaatgtgttcatttttccttacaacgaccgttaagctattccactcaacgattgttaagtctacagttcaac
ctctcaattgaatattgaaaataattatttaaaacgtttacacctccaacttcaacg

Supplementary Table 5 Strain list.

Strain	**Genotype**
N2	Wild-type Bristol strain
AX204	*npr-1(ad609)* X
PHX412	*actl-1(syb-412) npr-1(ad609)* X
AX7213	*pik-1(syb-378)* II; *npr-1(ad609)* X
AX6130	*npr-1(ad609) nfki-1(db1198)* X*; dbIs38[nfki-1p::nfki-1::GFP, ccRFP]*
AX6145	*npr-1(ad609)* X*; dbIs38[nfki-1p::GFP, ccRFP]*
AX6969	*malt-1(db1194)* II; *npr-1(ad609)* X; *dbIs16[rab-3p::malt-1(gDNA)::GFP, ccRFP]*
AX288	[*lon-2*(*e678*) *npr-1*(*ad609*)]
AX5877	*malt-1(db1194)* II; *npr-1(ad609)* X
AX6742	*malt-1(db1194)* II; *npr-1(ad609)* X; *dbEx983[rab-3p::malt-1(cDNA)::SL2mCherry, ccGFP]*
AX6392	*malt-1(db1194)* II; *npr-1(ad609)* X; *dbEx925[rab-3p::malt-1(gDNA)::GFP, ccRFP]*
AX7502	*dbEx1097[malt-1p(4kb)::malt-1(gDNA)::GFP, ccRFP]*
AX7591	*ynIs49[flp-5::GFP]; dbEx1119[malt-1p::(4kb)::malt-1(gDNA)::GFP, rol-6(su1006)]*
AX6740	*malt-1(db1194)* II; *npr-1(ad609)* X*; dbEx982[gcy-32p::malt-1(cDNA)::SL2mKate, ccGFP]*
AX7109	*malt-1(db1194)* II; *npr-1(ad609)* X*; dbEx1054[flp-5p::malt-1(cDNA):SL2mKate, ccRFP]*

AX7132	*malt-1(db1194)* II; *npr-1(ad609)* X*; dbEx982[gcy-32p::malt-1(cDNA)::SL2mKate, ccGFP]; dbEx1054[flp-5p::malt-1(cDNA):SL2mKate, ccRFP]*
AX5995	*malt-1(db1194)* II; *npr-1(ad609)* X*; dbEx637*[*RMGp::YC2.60, ccRFP*]
AX6836	*malt-1(db1194)* II; *npr-1(ad609)* X; *dbEx637*[*RMGp::YC2.60, ccRFP*]; *dbEx996[npr-1p::malt-1::SL2mCherry, ccRFP]*
AX5989	*malt-1(db1194)* II; *npr-1(ad609)* X*; dbEx614*[*gcy-37p::YC2.60, ccRFP]*
AX5797	*npr-1(ad609) ilc-17.1(tm5218)* X*; dbEx614*[*gcy-37p::YC2.60, ccRFP]*
AX5811	*npr-1(ad609) ilc-17.1(tm5218)* X*; dbEx614*[*RMGp::YC2.60, ccRFP]*
AX6984	*malt-1(db1194)* II*; npr-1(ad609) ilc-17.1(tm5218)* X; *dbEx614*[*RMGp::YC2.60, ccRFP]*
AX5689	*npr-1(ad609) ilc-17.1(tm5218)* X
AX6727	*malt-1(db1194)* II; *npr-1(ad609)* X; *dbEx978[hsp-16.41p::malt-1::SL2mCherry, ccRFP]*
AX7149	*malt-1(syb296)* II; *npr-1(ad609)* X
AX7221	*malt-1(syb296)* II; *npr-1(ad609)* X; *dbEx1070[rab-3p::malt-1(cDNA)::SL2mKate, ccRFP]*
AX6415	*malt-1(db1194)* II; *npr-1(ad609)* X; *dbEx930[rab-3p::malt-1(C374A)::SL2mCherry, ccRFP]*

AX6991	*malt-1(db1194)* II; *npr-1(ad609)* X; *dbEx1026[rab-3p::malt-1(C374A gDNA)::GFP, ccRFP]*
AX7006	*malt-1(db1194)* II; *npr-1(ad609)* X; *dbIs18[rab-3p::malt-1(C374A gDNA)::GFP, ccRFP]*
AX7462	*malt-1(syb573)* II; *pik-1(syb378)* IV; *npr-1(ad609) actl-1(syb412) nfki-1(syb617)* X
AX7464	*malt-1(syb573)* II; *pik-1(syb378)* IV; *npr-1(ad609) actl-1(syb412) ilc-17.1(tm5218) nfki-1(syb617)* X
AX7229	*pik-1(syb378)* IV; *npr-1(ad609) actl-1(syb412)*
PHX617	*nfki-1(syb617)* X
AX6250	*malt-1(db1194)* II; *npr-1(ad609) nfki-1(db1198)* X; *dbIs38[nfki-1p::nfki-1::GFP, ccRFP]*
AX6142	*pik-1(tm2167)* IV; *npr-1(ad609) nfki-1(db1198)* X; *dbIs38[nfki-1p::nfki-1::GFP, ccRFP]*
AX7849	*npr-1(ad609) ilc-17.1(tm5218)* X; *dbIs16[rab-3p::malt-1(gDNA)::GFP, ccRFP]*
AX5663	*ilcr-1(tm5866)* IV*; npr-1(ad609)* X
AX7850	*ilcr-1(tm5866)* IV*; npr-1(ad609)* X; *dbIs16[rab-3p::malt-1(gDNA)::GFP, ccRFP]*
AX6008	*npr-1(ad609) actl-1(db1203)* X
AX7040	*npr-1(ad609) actl-1(db1203)* X; *dbIs16[rab-3p::malt-1(gDNA)::GFP, ccRFP]*
AX5909	*pik-1(tm2167)* IV*; npr-1(ad609)* X

AX7637	*pik-1(tm2167)* IV; *npr-1(ad609)* X; *dbIs16[rab-3p::malt-1(gDNA)::GFP, ccRFP]*
AX7235	*npr-1(ad609) nfki-1(db1197)* X; *dbIs16[rab-3p::malt-1(gDNA)::GFP, ccRFP]*
AX6010	*npr-1(ad609) nfki-1(db1197)* X
AX7586	*ilc-17.1(tm5218)* X
AX7450	*ilc-17.1(tm5218)* X; *dbIs39[ilc-17.1p::ilc-17.1::mCherry]*
AX7585	*malt-1(db1194)* II
AX7449	*malt-1(db1194)* II; *dbIs16[rab-3p::malt-1(gDNA)::GFP, ccRFP]*
KU25	*pmk-1(km25)* IV
PHX296	*malt-1(syb296)* II
AX5949	*ilcr-1(tm5866)* IV
AX7587	*nfki-1(db1197)* X
TM3036	*tir-1(tm3036)* III
AX7649	*malt-1(db1194)* II*; tir-1(tm3036)* III
AX7847	*malt-1(db1194)* II*; dbIs39[ilc-17.1p::ilc-17.1::mCherry]*
AX7848	*malt-1(db1194)* II*; ilc-17.1(tm5218)*
AX6859	*malt-1(db1194)* II; *npr-1(ad609)* X*; dbEx996[npr-1p::malt-1(cDNA)::SL2mCherry, ccRFP]*
AX6134	*malt-1(db1194)* II; *pik-1(tm2167)* IV; *npr-1(ad609)* X*; dbEx637*[*RMGp::YC2.60, ccRFP*]
AX7185	*ttTi5605 II; unc-119(ed3) III; pik-1(tm2167)* IV; *npr-1(ad609)* X; *MosSCI[unc-119(+); pik-1p::pik-1(K214A)::unc-54 3’UTR]*

AX6666	*ttTi5605 II; unc-119(ed3) III; npr-1(ad609); pik-1(tm2167); MosSCI[unc-119(+); pik-1p::pik-1::unc-54 3’UTR]*
AX6829	malt-1(db1195); npr-1(ad609); *iaIs25[gcy-37p::GFP(+) unc-119(+)] dbEx979[malt-1p(4kb)::malt-1(gDNA)::mCherry, ccGFP]*
AX7479	*agIs219[T24B8.5p::GFP::unc-54-3’ UTR + ttx-3p::GFP::unc-54-3’ UTR]* III
AX7480	*malt-1(db1194)* II; *agIs219[T24B8.5p::GFP::unc-54-3’ UTR + ttx-3p::GFP::unc-54-3’ UTR]* III
AX7852	*ilcr-1(tm5866)* IV; *agIs219[T24B8.5p::GFP::unc-54-3’ UTR + ttx-3p::GFP::unc-54-3’ UTR]* III
AX7595	*tir-1(tm3036)* III; *agIs219[T24B8.5p::GFP::unc-54-3’ UTR + ttx-3p::GFP::unc-54-3’ UTR]* III
AX7871	*malt-1(db1194)* II; *ilcr-1(tm5866)* IV; *agIs219[T24B8.5p::GFP::unc-54-3’ UTR + ttx-3p::GFP::unc-54-3’ UTR]* III
AX7872	*malt-1(db1194)* II; *tir-1(tm3036)* III; *agIs219[T24B8.5p::GFP::unc-54-3’ UTR + ttx-3p::GFP::unc-54-3’ UTR]* III
AX7854	*tir-1(tm3036)* III; *ilcr-1(tm5866)* IV; *agIs219[T24B8.5p::GFP::unc-54-3’ UTR + ttx-3p::GFP::unc-54-3’ UTR]* III
AX7572	*malt-1(db1194)* II; *agIs219[T24B8.5p::GFP::unc-54-3’ UTR + ttx-3p::GFP::unc-54-3’ UTR]* III; *dbEx110[rab-3p::malt-1::SL2mCherry, ccRFP]*

AX7573	*malt-1(db1194)* II*; agIs219[T24B8.5p::GFP::unc-54-3’ UTR* + *ttx-3p::GFP::unc-54-3’ UTR]* III; *dbEx111[ges-1p::malt-1::SL2mCherry, ccRFP]*

Supplementary Table 6 Primers used in this study.

Primers for cloning *malt-1* promoter, cDNA and gDNA:

malt-1 promoter F	ggggACAACTTTGTATAGAAAAGTTGctgccggtggattccaacatattg
malt-1 promoter R	ggggACTGCTTTTTTGTACAAACTTGtctgaaattggggttcaagaaatttatttttgatttttaaaata
malt-1 ORF F	ggggACAAGTTTGTACAAAAAAGCAGGCTtttcagaaaaatgaacacaaacttggcggagtt
malt-1 ORF R	ggggACCACTTTGTACAAGAAAGCTGGGTATTACTGTAGACAT
malt-1(C374A) F	TCTTGATGTCgcCAGAAAATTTGTTCCATATG
malt-1(C374A) R	gcgcgtcaagttgtGCCTGACGACGAGTTGTGCTGTTTTAGAGCTAGAA

Oligos for generating sgRNA expression plasmid used to make *db1194*:

malt-1 sgRNA EcoRI 1	gcgcgtcaagttgtGgatcaggtatccaccgtagGTTTTAGAGCTAGAA
malt-1 sgRNA EcoRI 2	TTCTAGCTCTAAAACctacggtggatacctgatcCacaacttgacgcgc

Primers for cloning with *E. coli* expression plasmids:

MALT-1-HA F	ATATATGCTCTTCTAGTAACACAAACTTGGCGGAGTTACCTG
MALT-1-HA R	TATATAGCTCTTCATGCAGCATAATCTGGTACGTCGTATGGGTATCCTCCTCCCTGTAGACATTTGATTCTTGTAATCAAAATATGACC
ACTL-1-FLAG F	ATATATGCTCTTCTAGTACTAAGATGAAAATGGACGTAACAATTGAGTTGG
ACTL-1-FLAG R	TATATAGCTCTTCATGCCTTGTCATCGTCGTCCTTGTAATCTCCTCCTCCTTGTGTAATACTGTAGTTCATGGAATCCTCG
NFKI-1-V5 F	ATATATGCTCTTCTAGTGCAACCGTTGCCCCCAAGGGAAACTGC
NFKI-1-V5 R	TATATAGCTCTTCATGCCGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCTCCTCCTCCAGCTCTCGACTTGTTCGGGACTGC

Primers for cloning with *S. cerevisiae* Y2H plasmids:

NFKI-1(full) EcoRI/BamHI 1	GCGAATTCATGGCAACCGTTGCCCC
NFKI-1(full) EcoRI/BamHI 2	GCGGATCCTCAAGCTCTCGACTTGTTCGG
NFKI-1(1-374) EcoRI/BamHI 1	GCGAATTCATGGCAACCGTTGCCCC
NFKI-1(1-374) EcoRI/BamHI 2	GCGGATCCTCATGCATCGCGGTTAGTAAG
MALT-1(full) XmaI/XhoI 1	GTTCCCGGGGATGAACACAAACTTGGCGG
MALT-1(full) XmaI/XhoI 2	CGGCTCGAGTTACTGTAGACATTTGATTCTTGT
MALT-1(1-81) XmaI/XhoI 1	GTTCCCGGGgATGAACACAAACTTGGCGG
MALT-1(1-81) XmaI/XhoI 2	CGGCTCGAGTTATCTTGAAAGAAACTGCAATCT
MALT-1(248-639) XmaI/XhoI 1	GTTCCCGGGgCGAGCAGCAGATAAAGTTG
MALT-1(248-639) XmaI/XhoI 1	CGGCTCGAGTTACTGTAGACATTTGATTCTTGT