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Q8N4F7|RN175_HUMAN      MAAGTAARKAAPVLEAPPQQEQLSHTKLSAEDTWNLQQERMYKMHRGHDS 50
Q9H920|RN121_HUMAN      MAAVVEVEVGGGAAGER-ELDEVMSDLSPEEQWRVEHARMHAKHRGHEA 49
sp|Q09251|RN121_CAEEEL  -----MGQHGAIRLQNEVQEGMPPPELHTEEEQWAEEHKRMHEKHKHGHEA 45
                          . . . : . * : * * : : * : * : * :

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Q8N4F7|RN175_HUMAN      MHVEMILIFLCVLVIAQIVLVQWRQRHGRSYNLVTLTQMWWVPLYFTIKL 100
Q9H920|RN121_HUMAN      MHAEMVLIIATLVVAQLLLVQWKQRHPRSYNMVTLFQMWWVPLYFTVKL 99
sp|Q09251|RN121_CAEEEL  MHMEMMVI FMSIVVGGQIFLV TWKRKHFKSYQMCTLIGMLTIPVYVCFNR 95
                          ** * : : * : : : : : : : : : * : : : : * : * : * : : :

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Q8N4F7|RN175_HUMAN      YWWRFSLMWGMFVSVITSYILFRATRKLPLSGRTPRLVYKWFLLIYKLSYAF 150
Q9H920|RN121_HUMAN      HWWRFVLVIWILFSAVTAFTVFRATRKLPLVQTTPRLVYKWFLLIYKISYAT 149
sp|Q09251|RN121_CAEEEL  SWYRFLATWLVFCIFSAF IWLKASQAHTISGGT PRFVYKWFVFLHKLSYVL 145
                          * : * * * * * * : * . : : : : : : : : : * : * : * : * : * : * :

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Q8N4F7|RN175_HUMAN      GVVGYLAIMFTMCGENLFFKIKARDSMDFGIVSLFYGLYYGVMGRDFAEI 200
Q9H920|RN121_HUMAN      GIVGYMAVMFTLFLGNLLFKIKPEDAMDFGISLLFYGLYYGVLERDFAEM 199
sp|Q09251|RN121_CAEEEL  GVVGYLIMMGALLGFHVLFVGSQPTLMDAGILFMFYGVYYGVLGRDFAHI 195
                          * : * * * : * : : : * : : : * : . * * * * : * * : * * : * : * : :

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Q8N4F7|RN175_HUMAN      CSDYMASTIGFYSVSRPLPTRSLSDNICAVCG-----OKIIVEL 238
Q9H920|RN121_HUMAN      CADYMASTIGFYSVSGMPTKHLSDSVCVAVCG-----QQIFVDV 237
sp|Q09251|RN121_CAEEEL  CTARMASRIGYTYPEGLPKKHLEDGVCVAVCGGRLLDDSEHVDADAVVTTK 245
                          * : * * * * * : * : . * : * : * : * : * : * : * : * : * : * :

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Q8N4F7|RN175_HUMAN      DEEGLIENTYQLSCNHVFHEFCIRGWCIVGKKQTCPYCKEKVDLKRMI SN 288
Q9H920|RN121_HUMAN      SEEGI IENTYRLSCNHVFHEFCIRGWCIVGKKQTCPYCKEKVDLKRMF SN 287
sp|Q09251|RN121_CAEEEL  MVEDEDEKLYKLSGSHVFHEFCIRGWVVVVKLQTCPYCKERVDLQRMFKN 295
                          * . * : * : * * * . * * * * * * * * * * : * * * * * * * : * * : * : *

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Q8N4F7|RN175_HUMAN      PWERTHFLYQILDWLRYLVAWQPVVIGIVQGINYSLGLE 328
Q9H920|RN121_HUMAN      PWERPHVMYQLLDWRYLVAWQPVIIGVVQGINYILGLE 327
sp|Q09251|RN121_CAEEEL  PWEKPHLFYGKLLDWIRYLVCWQPLIVTAVQGLTTWMLGLE 335
                          * * : . * : * * : * * : * * : * * : * * : * * : * * : * * : * *

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Transmembrane domains

RING finger domain

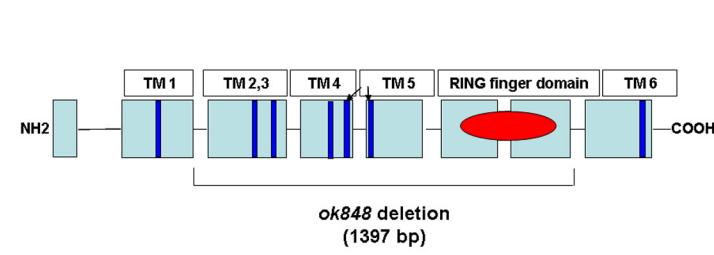
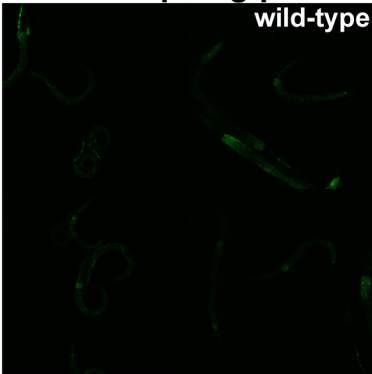


Figure S1. Protein sequence and gene structure.
(A) Clustal W multiple alignment showing homology of the predicted transmembrane domains and the RING finger in the worm and human orthologs. Human RNF121 has 51% identity and 68% similarity and human RNF175 has 47% identity and 68% similarity to the *C. elegans* RNF-121. The cysteine and histidine residues of the RING-H2 domain are labeled. (B) The genomic structure of the *rnf-121* gene. Exons are shown in light blue boxes. The blue lines are the predicted transmembrane domains. The deleted region in the *rnf-121(ok848)* mutant strain is marked.

hsp-4::gfp

wild-type



rnf-121(ok848)

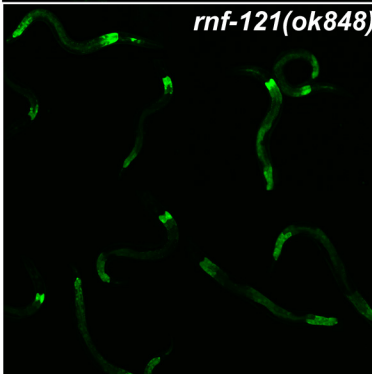


Figure S2. Induction of hsp-4::gfp expression in *rnf-121(ok848)* worms.

Elevated basal expression of the hsp-4::gfp transcriptional reporter in *rnf-121(ok848)* mutant.

Worms were grown under normal conditions at 20°C.

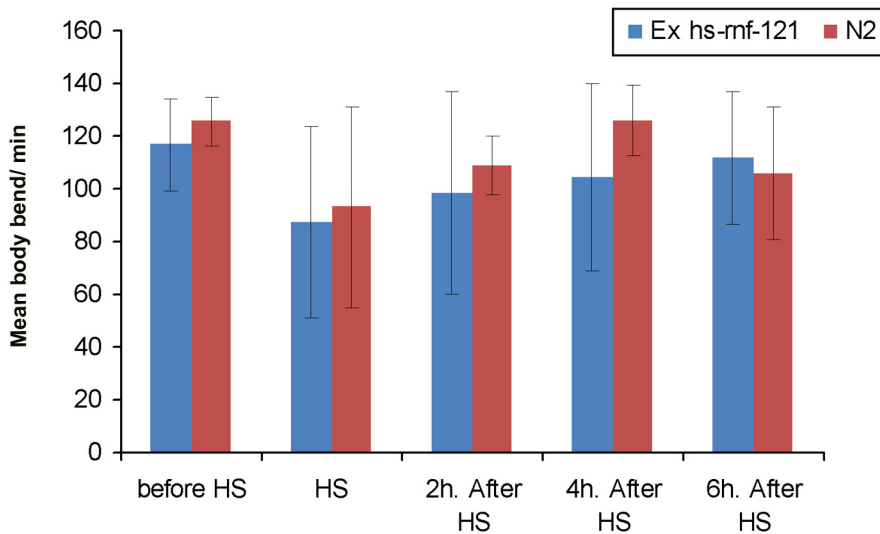


Figure S3. Ectopic expression of RNF-121 has moderate effect on motility.

Motility assays for wild-type (N2) worms and transgenic *hsp-16p::RNF-121* worms were performed before heat shock, immediately after heat shock (HS) and at the indicated time points following heat shock. $n=40$ for each group.

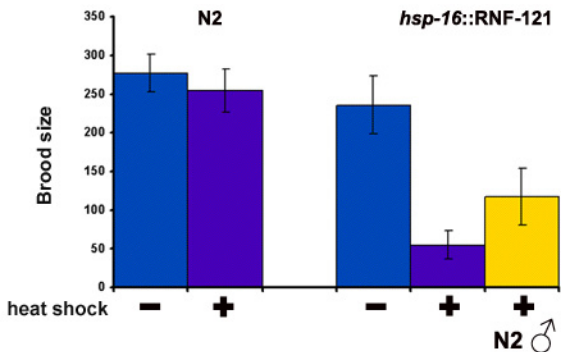


Figure S4. Effect of RNF-121 ectopic expression on fertility.

Heat shock of N2 (wild type) and *hsp-16p::RNF-121* worms at the mid-L2 stage (+) and control (-). The total number of progeny of each worm was counted. $n > 60$ worms were analyzed for each strain and condition in 3 independent experiments. Mean and standard error values are shown.

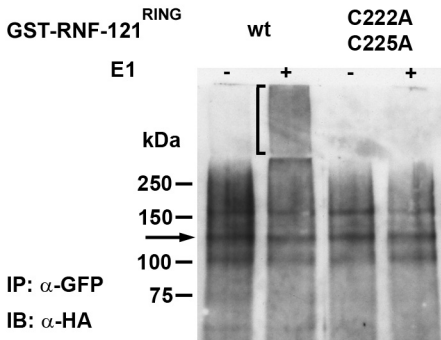


Figure S5. In vitro ubiquitination of immunopurified PAT-3::GFP
 Equal aliquots of immunopurified PAT-3::HA::GFP from PAT-3::HA::GFP worms (wild-type background) were used in each reaction in the presence of bacterially expressed and purified GST-RNF-121RING or GST-RNF-121C222AC225A and E2, HA-ubiquitin and ATP, with or without E1 as indicated. Reactions were incubated at 37°C for 20 min and terminated with 8M urea buffer. Black arrow indicate PAT-3::HA::GFP. The ubiquitinated forms of PAT-3::HA::GFP are indicated by parentheses.

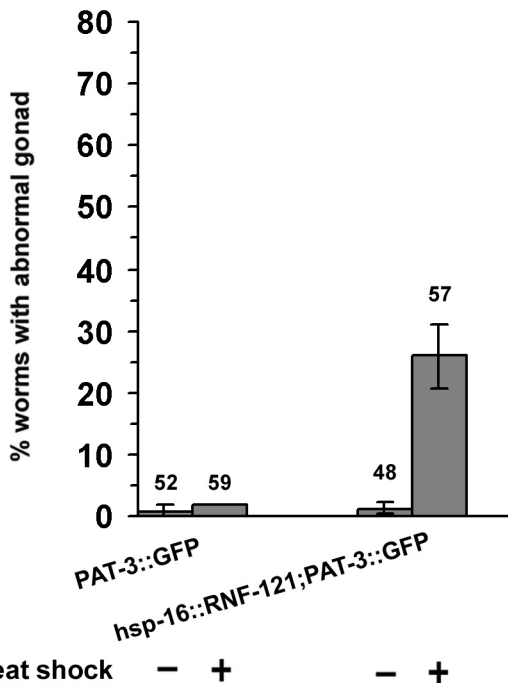


Figure S6. High levels of PAT-3 suppress the gonad defects induced by RNF-121.

Percentage of animals with abnormal gonads. Analysis was performed to PAT-3::GFP and PAT-3::GFP; hsp-16p::RNF-121 transgenic lines. Worms were treated with heat shock at mid-L2 stage to induce transgene expression (+) or non-treated controls (-). n=total number of worms analyzed in 3 independent experiments (indicated above each bar). Compare values to Figure 4l.

Table S1. Mean and STD values of developmental stage distributions of N2 and *rnf-121(ok848)* worms following treatment with tunicamycin

Tunicamycin ($\mu\text{g/ml}$)	Developmental stage (72hr at 20°C)	N2		<i>rnf-121(ok848)</i>	
		% ^(*)	n	% ^(*)	n
0	L4 and adult	100	1331	99.5 \pm 1.2	1273
	L3 and younger	0		0.2 \pm 0.1	
	Larval lethality	0		0.3 \pm 0.1	
2	L4 and adult	68.9 \pm 22.5	1404	23.7 \pm 12.1	1186
	L3 and younger	22.4 \pm 16.5		67.6 \pm 15.2	
	Larval lethality	8.7 \pm 7.4		8.7 \pm 5.8	
5	L4 and adult	0	732	0	1171
	L3 and younger	62.3 \pm 4.1		51.8 \pm 8.5	
	Larval lethality	37.7 \pm 3.9		48.2 \pm 8.7	
7.5	L4 and adult	0	562	0	1056
	L3 and younger	41.2 \pm 9.7		35.9 \pm 11.0	
	Larval lethality	58.8 \pm 9.8		64.1 \pm 12.1	

^(*) Mean values of 3-6 independent experiments; n= total number of worms. For experimental conditions see Material and Methods.

Table S2. Mean and STD values of developmental stage distributions of N2 worms fed with control vector (L4440) or *rnf-121(RNAi)* following treatment with tunicamycin

Tunicamycin ($\mu\text{g/ml}$)	Developmental stage (72hr at 20°C)	L4440		<i>rnf-121(RNAi)</i>	
		% ^(*)	n	% ^(*)	n
0	L4 and adult	100	1330	99.9 \pm 0.3	1765
	L3 and younger	0		0.1 \pm 0.3	
	Larval lethality	0		0	
1	L4 and adult	99.1 \pm 1.3	1525	93.8 \pm 8.3	1601
	L3 and younger	0.5 \pm 1.1		3.4 \pm 4.8	
	Larval lethality	0.4 \pm 0.7		2.8 \pm 3.5	
2	L4 and adult	80.5 \pm 12.1	1398	67.9 \pm 8.7	1351
	L3 and younger	14.2 \pm 10.0		24.5 \pm 6.8	
	Larval lethality	5.3 \pm 4.1		7.6 \pm 2.1	

3	L4 and adult	55.2±8.3	1306	34.6±17.1	1372
	L3 and younger	26.1±6.5		46.8±12.4	
	Larval lethality	18.7±4.2		18.6±5.2	
5	L4 and adult	10.1±3.6	1089	7.8±5.3	1040
	L3 and younger	42.4±5.2		44.1±7.4	
	Larval lethality	47.5±5.6		48.1±9.1	

(*) Mean values of 3-6 independent experiments; n= total number of worms. For experimental conditions see Material and Methods.

Table S3. Mean and STD values of developmental stage distributions of *ire-1(v33)* worms fed with control vector (L4440) or *rnf-121(RNAi)* following treatment with tunicamycin

Tunicamycin (µg/ml)	Developmental stage (72hr at 20°C)	L4440		<i>rnf-121(RNAi)</i>	
		%(*)	n	%(*)	n
0	L4 and adult	94.3±7.8	1360	85.5±19.5	1126
	L3 and younger	5.7±7.9		13.6±19.6	
	Larval lethality	0.05±0.2		0.9±3.3	
1	L4 and adult	92.2±6.8	1076	86.2±14.1	864
	L3 and younger	7.2±7.0		13.4±13.1	
	Larval lethality	0.6±1.1		0.4±0.3	
2	L4 and adult	73.3±11.3	930	40.1±19.7	882
	L3 and younger	24.6±11.44		48.1±12.6	
	Larval lethality	2.1±2.4		11.8±13.0	
3	L4 and adult	39.6±12.5	568	9.1±9.8	454
	L3 and younger	55.3±14.7		84.6±11.2	
	Larval lethality	5.1±3.3		6.3±4.6	
5	L4 and adult	1.7±3.1	935	1.2±1.7	972
	L3 and younger	53.6±7.5		47.2±7.8	
	Larval lethality	44.7±6.8		51.6±7.6	

(*) Mean values of 3-6 independent experiments; n= total number of worms. For experimental conditions see Material and Methods.

Table S4. Mean and STD values of developmental stage distributions of *atf-6(ok551)* worms fed with control vector (L4440) or *rnf-121(RNAi)* following treatment with tunicamycin

Tunicamycin ($\mu\text{g/ml}$)	Developmental stage (72hr at 20°C)	L4440		<i>rnf-121(RNAi)</i>	
		% ^(*)	n	% ^(*)	n
0	L4 and adult	100	1537	100	1806
	L3 and younger	0		0	
	Larval lethality	0		0	
1	L4 and adult	98.6 \pm 4.4	1563	98.8 \pm 1.3	1625
	L3 and younger	1.4 \pm 4.4		1.2 \pm 1.3	
	Larval lethality	0		0	
2	L4 and adult	95.9 \pm 5.5	1531	73.1 \pm 8.8	1561
	L3 and younger	3.8 \pm 5.0		16.3 \pm 3.9	
	Larval lethality	0.3 \pm 0.8		10.6 \pm 5.2	
3	L4 and adult	87.4 \pm 3.4	2002	54.8 \pm 9.4	1464
	L3 and younger	8.5 \pm 2.4		29.8 \pm 6.3	
	Larval lethality	4.1 \pm 3.8		15.4 \pm 5.3	
5	L4 and adult	13.6 \pm 4.6	1255	12.3 \pm 5.4	1140
	L3 and younger	40.1 \pm 8.5		35.1 \pm 9.8	
	Larval lethality	46.3 \pm 8.8		52.6 \pm 10.6	

^(*) Mean values of 3-6 independent experiments; n= total number of worms. For experimental conditions see Material and Methods.

Table S5. Mean and STD values of developmental stage distributions of *pek-1(ok275)* worms fed with control vector (L4440) or *rnf-121(RNAi)* following treatment with tunicamycin

Tunicamycin ($\mu\text{g/ml}$)	Developmental stage (72hr at 20°C)	L4440		<i>rnf-121(RNAi)</i>	
		% ^(*)	n	% ^(*)	n
0	L4 and adult	99.7±0.9	1789	99.6±0.8	1650
	L3 and younger	0.3±0.9		0.4±0.8	
	Larval lethality	0		0	
1	L4 and adult	94.9±5.4	1413	89.6±5.7	1512
	L3 and younger	3.9±4.5		6.2±3.3	
	Larval lethality	1.2±1.6		4.1±3.1	
2	L4 and adult	29.5±10.7	1097	33.4±8.2	1175
	L3 and younger	50.9±11.0		44.6±4.1	
	Larval lethality	19.6±3.4		22.0±6.9	
3	L4 and adult	4.6±2.3	998	8.9±2.8	1257
	L3 and younger	75.2±6.3		60.4±2.6	
	Larval lethality	20.2±5.7		30.7±2.5	
5	L4 and adult	3.2±4.9	1127	3.0±3.3	1146
	L3 and younger	49.4±7.7		47.4±8.8	
	Larval lethality	47.4±7.7		49.6±8.2	

^(*) Mean values of 3-6 independent experiments; n= total number of worms. For experimental conditions see Material and Methods.