Supporting Information

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SI Materials and Methods

Strains. *Caenorhabditis elegans* were maintained on nematode growth media (NGM) plates seeded with *Escherichia coli* OP50-1, as described (1). We used N2 wild-type animals for all RNAi spore shedding experiments. The following transgenic strains were used in this study:

ERT106	*jyIs17[vha-6p::mCherry::ACT-5, ttx-3p::RFP] IV; dkIs166[opt-2p::PGP-1::GFP]*
HC722	*gk505[dyf-2(-) & sid-2(-)]; qtIs5(SID-2::GFP)]* (2)
MZE1	*unc-119(ed3); cbgIs91[pept-1p::PEPT-1::DsRed, unc-119(+)]; cbgIs98[pept-1p::GFP::RAB-11.1, unc-119(+)]* (3)
ERT213	*jyIs17[vha-6p::mCherry::ACT-5, ttx-3p::RFP] IV; cbgIs98[pept-1p::GFP::RAB-11.1, unc-119(+)]*
RT1102	*unc-119(ed3); pwIs428[vha-6p::RFP::RAB-11.1, unc-119(+)]* (4)
ERT260	*unc-119(ed3); cbgIs98[pept-1p::GFP::RAB-11.1, unc-119(+)]* 2x backcrossed
RT1239	*unc-119(ed3); pwIs480[vha-6p::RFP::RAB-5, unc-119(+)]* (4)
RT525	*unc-119(ed3); pwIs206[vha-6p::GFP::RAB-10, unc-119(+)]* (4)
GK288	*unc-119(ed3); dkIs166[opt-2p::PGP-1::GFP, unc-119(+)]* (5)
ERT197	*unc-119(ed3); pwIs428[vha-6p::RFP::RAB-11.1, unc-119(+); dkIs166[opt-2p::PGP-1::GFP]*
MZE4	*unc-119(ed3); cbgIs91[pept-1p::PEPT-1::DsRed, unc-119(+)];cbgIs103[pept-1p::SP12::GFP, unc-119(+)]* (3)
DA2123	*N2; adIs2122[lgg-1p::GFP::LGG-1; rol-6(su1006)]*) (6, 7)

Strains ERT106 and ERT213 contain an intestinally expressed actin *vha-6p::mCherry::ACT-5* (pET187) transgene that was made by PCR amplifying the *act-5* cDNA with its endogenous 3′ UTR and cloning it into the Gateway 3′ element vector pDONR P2R-P3. This vector was then recombined in a three-fragment Gateway LR recombination reaction with the *vha-6* promoter in the Gateway 5′ element vector pDONR P4-P1R, and mCherry in the Gateway middle element vector pDONR221, into the destination vector pDEST R4-R3. This *vha-6p::mCherry::ACT-5* transgene was injected into N2 worms at 10 ng/μL along with *ttx-3p::RFP* as a co-injection marker to generate an extrachromosomal array strain that was integrated with UV/psoralen treatment to create integrant *jyIs17[vha-6p::mCherry::ACT-5]IV*.

***Nematocida parisii* Spore Preps.** Spore preps were prepared as described (8). Briefly, *N. parisii* was cultured inside of *C. elegans*, and when animals were heavily infected, they were mechanically disrupted with silicon beads to isolate spores, which were then filtered through a 5-μm filter to remove intact *C. elegans* larvae and eggs. Spores were quantified by staining with Calcofluor white (Sigma-Aldrich) and counting with a hemocytometer (Cell-Vu). Aliquots of spores were stored at −80 °C before use.

Luminal Access Assays. To stain spores in contact with the lumen, Calcofluor white was fed to infected animals by applying 300 μL of Calcofluor white to 6-cm NGM/OP50 plates. Calcofluor white was spread evenly over the entire surface of the plate, the plate was dried in a sterile hood, and worms were allowed to feed on the dye for 2 h before imaging.

smGTPase RNAi Screen and Spore Shedding Assays. Feeding RNAi experiments were performed as described (8, 9). A library of 41 smGTPase RNAi clones was generated based on a list from ref. 10, and was divided roughly into quarters and prepped as 4 separate batches, each with their own L4440 empty vector controls, which were used to normalize the relative number of spores shed across the four batches. All RNAi clones were sequence verified. All clones were used undiluted, except for *rab-5* and *rab-11.1* RNAi clones, which were diluted 1:10 with L4440 (vector alone) control RNAi bacteria to allow for normal development. All experiments with these clones used these dilutions. Synchronized L2 animals were infected with 5.8×10^6 spores on 10-cm RNAi plates and incubated at 25 °C for 40 h to allow the infection to progress until the spore shedding stage of infection. Animals were washed three times in M9 at 24 h after infection (hpi) and 39 hpi and replated on fresh RNAi plates (at 24 hpi) or OP50 plates (at 39 hpi) to remove spores from cuticles. At 40 hpi, 50 animals were picked off of *E. coli* plates into microfuge tubes with 500 μL of 1:1 *E. coli* OP50 and M9. Spores excreted from 40 to 48 hpi were collected and quantified by staining with Calcofluor white and counting at 400×. All genes were tested in three biological replicates and screen hits were verified in triplicate in an independent biological replicate experiment.

To determine whether RNAi clones affected cell polarity, localization of the apically localized plasma membrane marker *PGP-1::GFP* in strain ERT106 was assessed at 630× in >30 RNAi-treated animals per RNAi clone.

RAB-11 Antibody Staining. Antibody staining with anti–RAB-11 antibodies was performed as described (11). Briefly, *C. elegans* intestines were dissected out, fixed in paraformaldehyde, washed, then incubated with 1:500 dilution of primary anti–RAB-11 peptide antibody (12) overnight at 4 °C, washed, then stained with 1:500 dilution of secondary antibody Cy3-labeled goat anti-rabbit IgG (Jackson Immunoresearch) for 2 h at room temperature, washed, then mounted in Vectashield with DAPI for viewing by fluorescence microscopy.

Spore Production Assays. The pathogen load in individual RNAi-treated animals was semiquantitatively measured by counting spores in ∼40 animals per treatment group fixed with acetone at 45 hpi, using a Zeiss AxioImager with Nomarski optics. The pathogen load in a population of animals was quantitatively determined by hydrolyzing infected animals and counting the spores released after host tissue was dissolved. RNAi-treated infected animals were fixed at 44 hpi with acetone and 50 animals were hydrolyzed for 30 min with 200 mM NaOH, 0.1% SDS, and 1:100 Calcofluor white. To ensure complete homogenization, infected *C. elegans* were repeatedly pipetted with

a 200-μL pipette tip. The suspension was neutralized by adding 100 μM of 1 M Tris·HCl at pH 7.4. *N. parisii* spores were then counted by using a Cell-Vu counting chamber. Three biological replicates were counted for each RNAi treatment.

Defecation Assays. The defecation contents of infected animals were analyzed for the ratio of *N. parisii* spores to control fluorescent beads. Briefly, spore-stage infected animals were fed a blend of 0.5-μm fluorescent beads (Polysciences; catalog no. 19507) and Calcofluor white (to label extracellular spores) for 2 h on OP50. Animals were then removed from the bead-feeding plate, briefly transferred to a clean OP50 plate to remove beads from the cuticle, and then transferred to a fresh OP50 plate and allowed to defecate undisturbed for 35 min, at which point they were removed from the plates and the presence of intracellular spores was verified with Nomarski optics at 630×. Each RNAi clone was tested on three independent plates, each containing eight animals during each of three independent biological experiments. The defecated materials were imaged with fixed exposure at 100×, and fluorescence intensity above background was quantified with ImageJ64 (version 1.46r) for the blue (Calcofluor white-stained spores) and red (beads) channels. For each defecation spot, the blue fluorescence value was divided by the total fluorescence (red plus blue). These values represent the fraction of each defecation spot that is comprised of spores and were normalized to the values obtained from analyzing defecated material from L4440 control RNAi animals.

Autophagy Assays. Autophagy pathway perturbation was assessed by quantifying the number of *GFP::LGG-1* puncta greater than 1.2 μm in size after feeding RNAi treatment. Animals were fixed in 4% (vol/vol) PFA at 24 hpi and imaged by using confocal microscopy then analyzed in ImageJ.

Statistical Analyses. For all datasets, *P* values reported were calculated in Prism 6 software and ns = not significant; $^{*}P < 0.05$; $^{**}P << 0.001$. *P* values for spore shedding assays (Fig. 3*A* and Figs. S2*B* and S3), spore production in population assay (Fig. 3*C*), defecation contents assay (Fig. 3*D*), coat quantification assays (Fig. 5*A* and Fig. S6*D*), and LGG-1 puncta quantification assay (Fig. S2*D*) were calculated by using ANOVA with Dunnett's correction for multiple comparisons. *P* values reported in spore production in individual animals assay (Fig. 3*B*) were from Friedman's Test, ANOVA with Dunn's correction for multiple comparisons. Contagiousness assay (Fig. 5*B*) *P* values were calculated by a two-sided Fisher's exact test.

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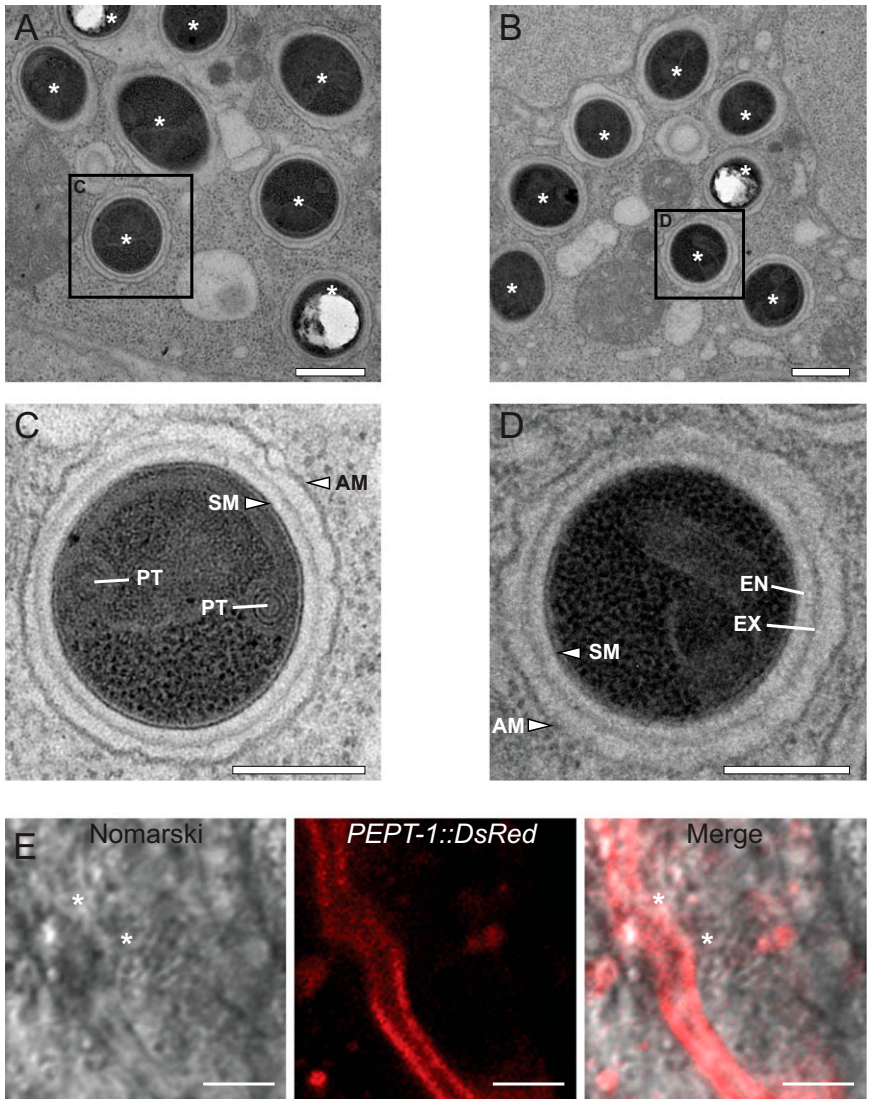


Fig. S1. *N. parisii* spores are surrounded by an additional membrane, and PEPT-1 does not localize to *N. parisii* spores. (*A* and *B*) Representative fields of view showing cross-sections of intracellular spores (asterisks) that are contained in an additional membrane. Boxed regions are expanded in *C* and *D*, respectively. (*C* and *D*) Magnified view of spores showing the spore membrane (SM) and an additional membrane (AM) surrounding spores. EN, endospore; EX, exospore; PT, polar tube. (*E*) *PEPT-1::DsRed* does not localize to *N. parisii* spores, which are marked with asterisks in Nomarski optics. (Scale bars: *A* and *B*, 500 nm; *C* and *D*, 250 nm; *E*, 5 μm.)

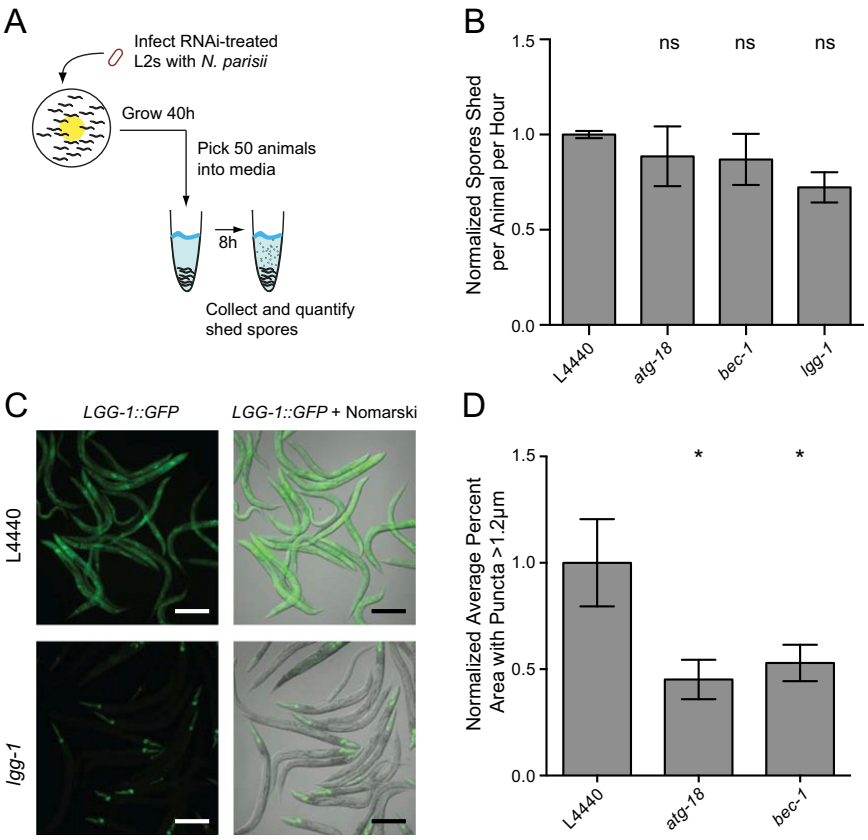


Fig. S2. RNAi against autophagy components *bec-1*, *atg-18*, and *lgg-1* does not significantly reduce spore shedding. (*A*) Spore shedding assay. (*B*) Spores shed by animals treated with control L4440 or RNAi against *bec-1*, *atg-18*, or *lgg-1*. The mean and SEM combined from three independent experiments are shown, normalized to the value for the L4440 RNAi control. $n = 150$ animals per treatment. (*C*) Intestinal expression of *GFP::LGG-1* is greatly reduced by *lgg-1* RNAi treatment. Remaining GFP expression is in the pharynx. (Scale bars: 200 µm.) (*D*) The number of *GFP::LGG-1* puncta greater than 1.2 µm in area is significantly reduced in *atg-18* and *bec-1* RNAi-treated animals. All results are normalized to the average L4440 control RNAi sample values. Mean and SEM from three combined independent experiments are shown. $n = 57$–64 animals per treatment group. ns, not significant; $^*P < 0.05$.

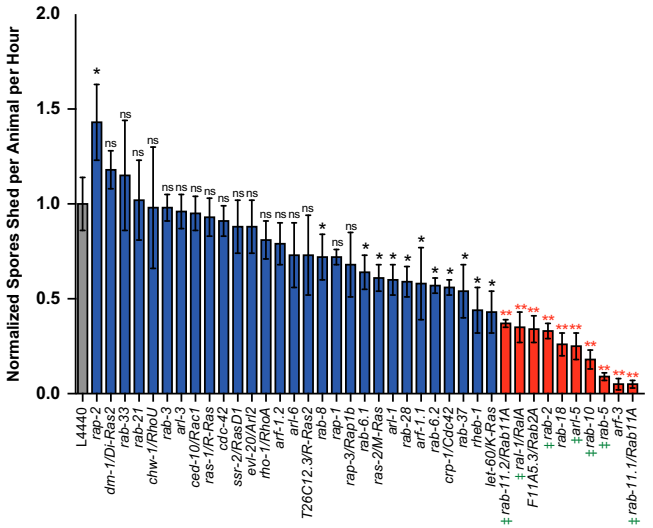


Fig. S3. Spore shedding screen using RNAi against 41 *C. elegans* predicted smGTPases. The mean and SD of biological triplicate samples is shown. $n = 50$ animals per treatment. L4440 is the empty vector control for RNAi to which all values are normalized. Red denotes samples scored as screen hits; blue denotes nonhits. Note that a lack of effect in this screen could be due to ineffective RNAi knockdown against the gene of interest. Green crosses denote characterized members of the recycling endosome pathway. When *C. elegans* gene names differ from those of human homologs, human gene names are listed after the *C. elegans* gene name. ns, not significant; $^*P < 0.05$; $^{**}P << 0.001$.

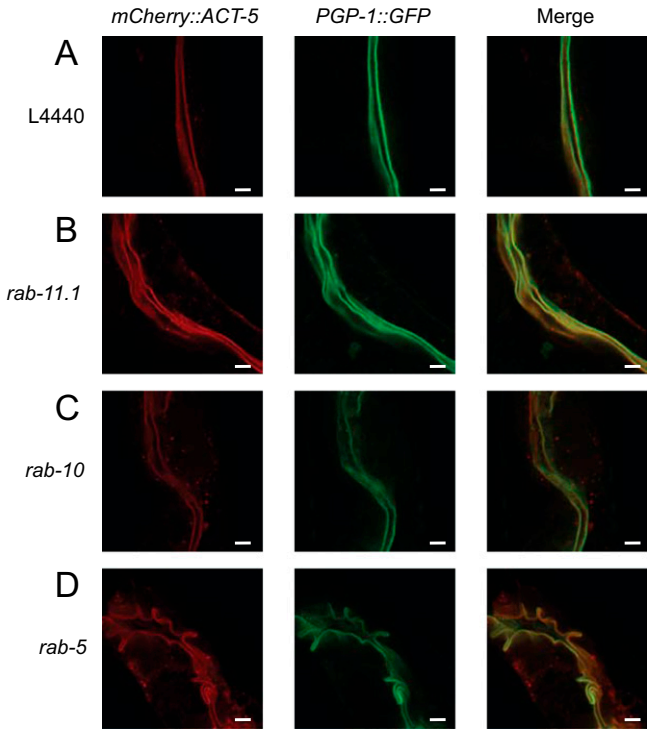


Fig. S4. Markers for intestinal cell polarity are unaffected by smGTPase RNAi treatment. The apical localizations of *mCherry::ACT-5* and *PGP-1::GFP* were unperturbed by treatment for each smGTPase spore shedding screen hit: (*A*) L4440 control, (*B*) *rab-11.1*, RNAi, (*C*) *rab-10* RNAi, (*D*) *rab-5* RNAi. Images are shown of *N. parisii*-infected animals, which is the reason for some actin localization to the basolateral side of intestinal cells (8). All genes included in screen were tested for polarity disruption, and representative images are shown. (Scale bars: 5 μm.)

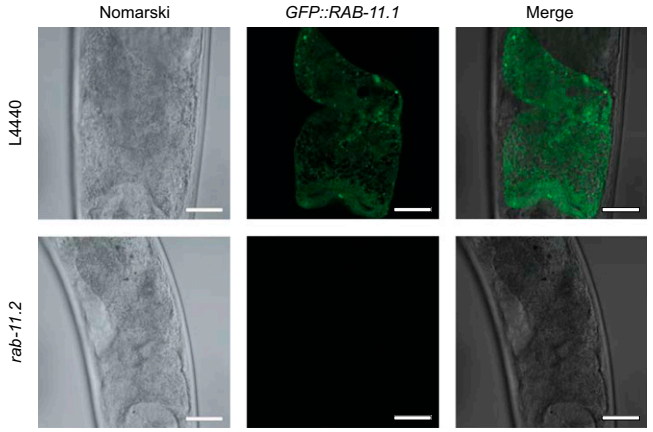


Fig. S5. *GFP::RAB-11.1* is knocked down by *rab-11.2* RNAi. *GFP::RAB-11.1* fluorescence is greatly reduced by *rab-11.2* RNAi. (Scale bars: 20 μm.)

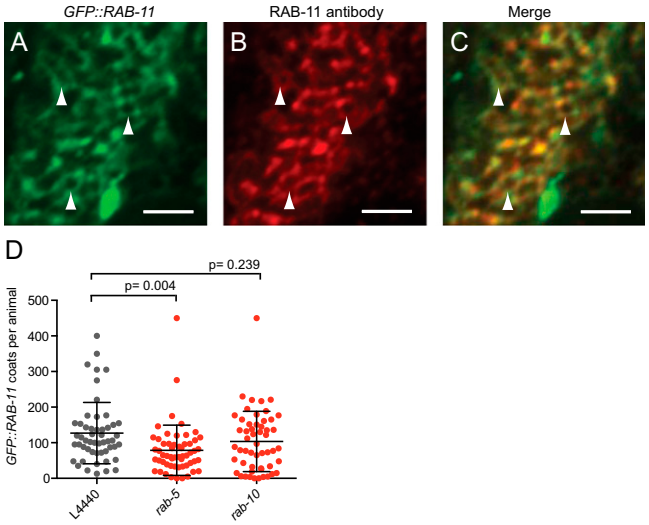


Fig. S6. Antibody against endogenous RAB-11 colocalizes with *GFP::RAB-11*–coated spores, and *rab-5* or *rab-10* RNAi knockdown does not substantially alter the number of *GFP::RAB-11*–coated spores. (*A*) The *GFP::RAB-11* transgene localizes to rod-shaped *N. parisii* spores (examples of spores shown in cross-section are highlighted by arrowheads), as shown in Fig. 4*E*. (*B*) RAB-11 antibody colocalizes with *GFP::RAB-11* transgene, as seen in the merged image in *C*. (Scale bars: 2.5 μm.) (*D*) Neither *rab*-5 nor *rab*-10 RNAi block the formation of *GFP::RAB-11* coats on SCCs. $n = 60$ animals per treatment group combined from two independent experiments. Each point represents the number of *GFP::RAB-11* coats in a single animal. Average is shown with error bars as SD.

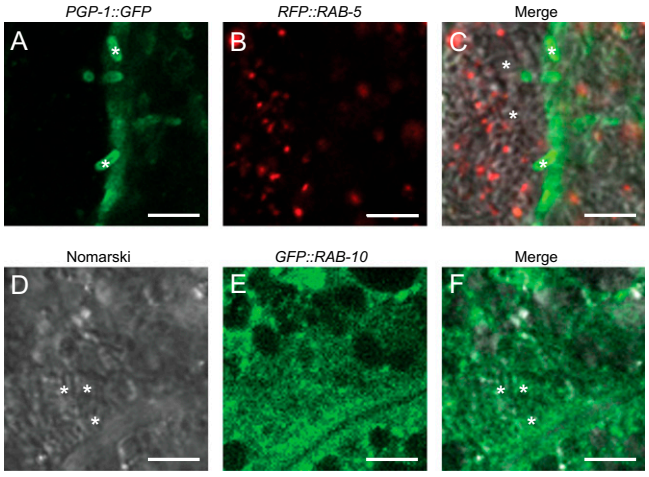


Fig. S7. RAB-5 and RAB-10 do not localize to spores. (*A*–*C*) *RFP::RAB-5* does not localize to *N. parisii* spores. Examples of spores are marked with asterisks in *A* and *C*. (*D*–*F*) *GFP::RAB-10* does not localize to *N. parisii* spores. Examples of spores are marked with asterisks in *D* and *F*. (Scale bars: 5 μm.)

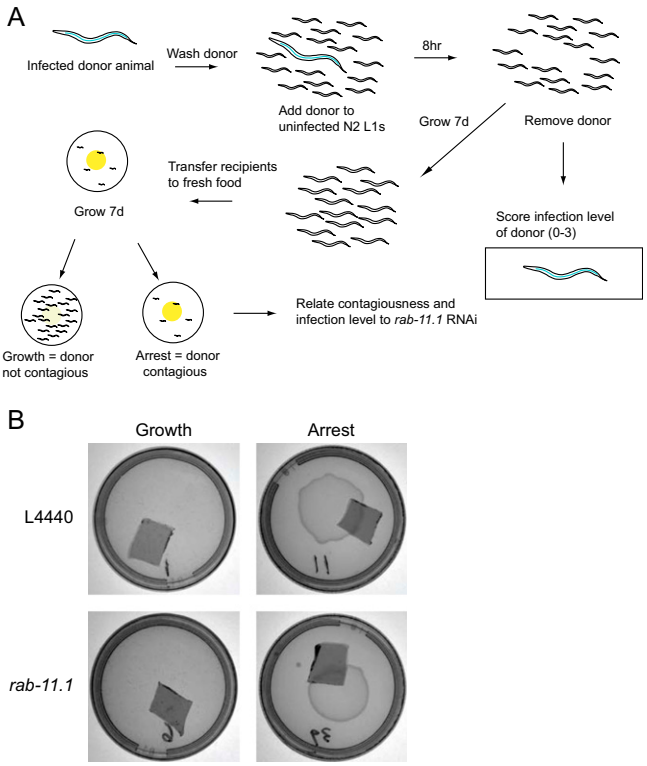
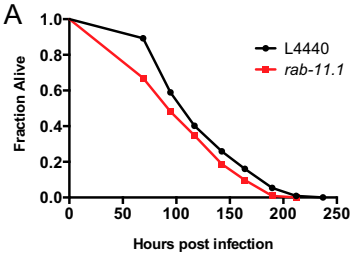


Fig. S8. Contagiousness assay design. (*A*) Experimental design for contagiousness assays. Donor animals were treated with either L4440 empty vector control RNAi bacteria, or *rab-11.1* RNAi bacteria. (*B*) Representative images of plates scored as growth-positive or arrested. The dark squares on the plates are chunks of agar used to transfer recipients to fresh food plates. The circle on arrested plates is an uneaten bacterial lawn, whereas on the growth-positive plates all bacteria have been consumed by the animals on the plate.



B

EXPERIMENTAL REPLICATE 1	Fraction Alive	
	L4440	*rab-11.1*
hpi	(n =112)	(n =112)
0.00	1.00	1.00
69.00	0.89	0.67
94.50	0.59	0.48
117.00	0.40	0.35
142.50	0.26	0.19
164.00	0.16	0.10
189.50	0.05	0.01
212.00	0.01	0.00
237.00	0.00	
median	117 hpi	94.5 hpi

C

EXPERIMENTAL REPLICATE 2	Fraction Alive	
	L4440	*rab-11.1*
hpi	(n =119)	(n=120)
0.00	1.00	1.00
65.50	0.90	0.74
87.25	0.66	0.55
109.75	0.45	0.45
134.25	0.37	0.29
158.00	0.24	0.16
182.25	0.10	0.08
206.25	0.06	0.03
230.25	0.01	0.01
255.00	0.00	0.00
median	109.75 hpi	109.75 hpi

D

EXPERIMENTAL REPLICATE 3	Fraction Alive	
	L4440	*rab-11.1*
hpi	(n = 94)	(n = 108)
0.00	1.00	1.00
60.50	0.89	0.83
85.25	0.67	0.59
92.75	0.63	0.56
130.50	0.46	0.28
158.50	0.33	0.13
179.10	0.16	0.06
182.00	0.05	0.03
205.50	0.01	0.00
230.50	0.00	
median	130.5 hpi	130.5 hpi

E

Uninfected	L4440	*rab-11.1*
Experiment 1	260.25	211.58
Experiment 2	263.75	190.92
Experiment 3	278.58	209.25

Fig. S9. *rab-11.1* RNAi effects on host survival in absence and presence of *N. parisii* infection. (*A*) Representative survival curve of independent biological replicates of infected *rab-11.1* RNAi and control animals. $n = 120$ animals per treatment group per experiment (three plates of 40 animals each). (*B–D*) Summary survival curve data for replicate experiments showing the fraction of animals alive at each time point animals were scored. The median survival time point for treatment groups is shown at the bottom of each table. *A* and *B* are data from the same experiment. (*E*) Summary of three experimental replicates comparing median survival time of uninfected *rab-11.1* RNAi and uninfected control animals. $n = 120$ animals per treatment group per experiment (three plates of 40 animals each).