Supporting Information

Brennan et al. 10.1073/pnas.1711530114

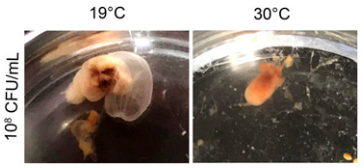

Fig. S1. *V. coralliilyticus* disease progression in *N. vectensis* is temperature-dependent. Anemones infected with 10^8 cfu/mL *V. coralliilyticus* were kept at the normal husbandry temperature (19 °C) or at 30 °C for 9 d. On day 9, infected anemones housed at 19 °C were alive (*Left*), but anemones at 30 °C were dead and exhibited severe to complete ectodermal tissue degradation (*Right*).

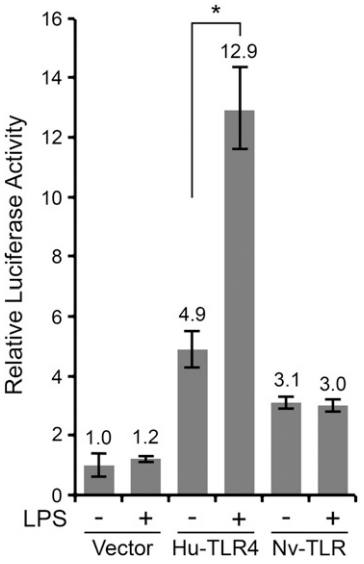

Fig. S2. LPS does not stimulate an Nv-TLR–to–NF-κB pathway in human cells. An NF-κB–responsive reporter assay was performed in HEK 293 cells transfected with the human coreceptors MD-2 and CD-14. Cells were also transfected with Nv-TLR, Hu-TLR4, or the control vector and then were treated with LPS for 6 h. Luciferase activity values were normalized to the vector control (1.0) and are the averages of duplicates. Data are expressed as means ± SEM. Statistical significance was determined using Student's *t* test; $*P < 0.05$.

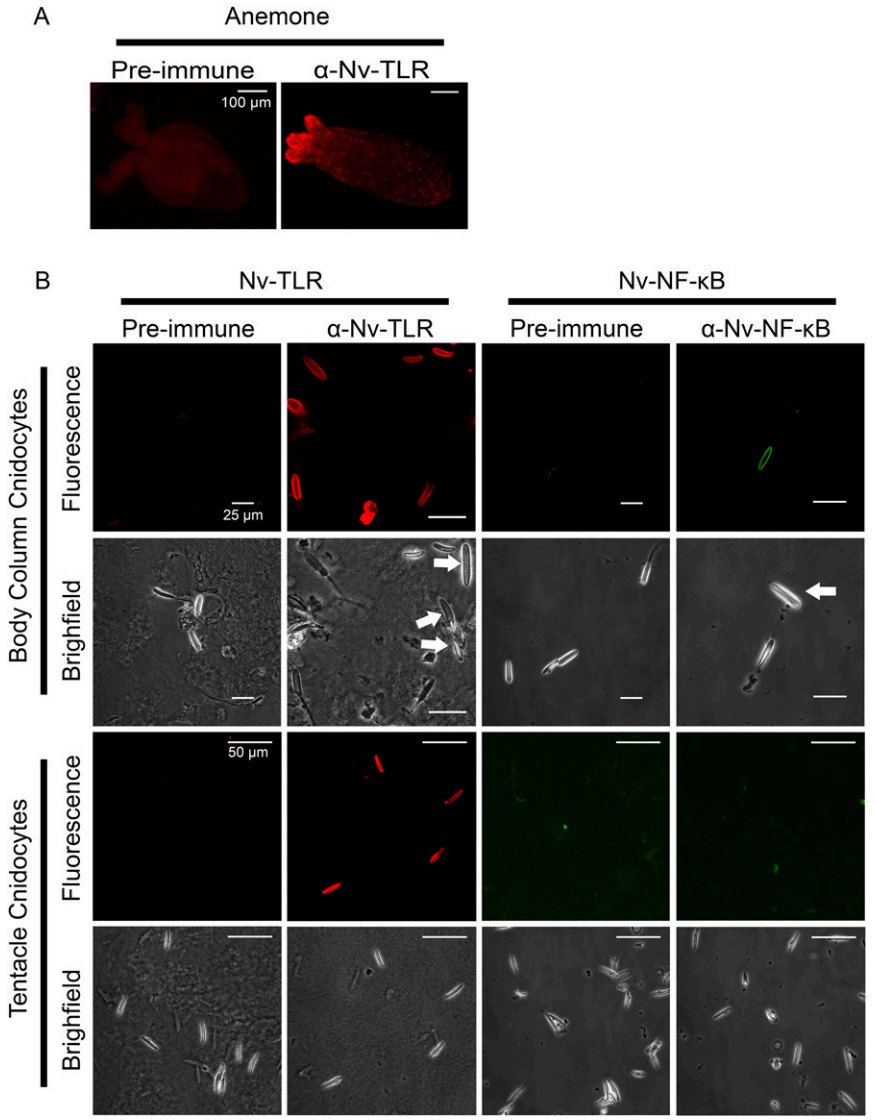

Fig. S3. Nv-TLR and Nv-NF-κB antisera specifically stain anemone cnidocytes in vitro and in vivo. (*A*) Whole-mount immunofluorescence of a four-tentacle–stage anemone was performed with preimmune guinea pig serum or Nv-TLR antiserum and then was visualized with Texas Red-conjugated secondary antiserum. (*B*) Immunofluorescence of isolated body column and tentacle cnidocytes was performed with preimmune guinea pig serum, Nv-TLR antiserum, preimmune rabbit serum, or Nv-NF-κB antiserum. Brightfield images show the cnidocytes that were subjected to staining. Nv-TLR is not detected with preimmune guinea pig serum in body column and tentacle cnidocytes (false-colored red, *Left Column*), but is detected with Nv-TLR antiserum in body column and tentacle cnidocytes (false-colored red, *Second Column from Left*); arrows indicate cnidocytes that do not stain positive for Nv-TLR. Nv-NF-κB is not detected with preimmune rabbit serum in body column and tentacle cnidocytes (false-colored green, *Third Column from Left*) but is detected with Nv-NF-κB antiserum in body column but not tentacle cnidocytes (false-colored green, *Right Column*); the arrow identifies a cnidocyte that does not stain positive for Nv-NF-κB.

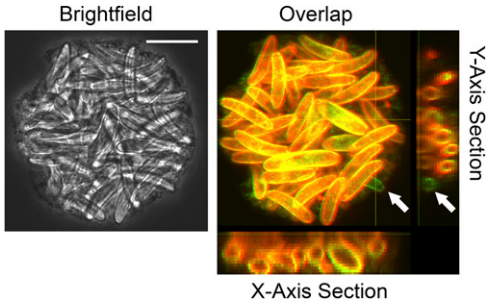

Fig. S4. Orthogonal rendering of nematosome. (*Left*) Brightfield image of an isolated nematosome. (*Right*) Indirect immunofluorescence staining for Nv-TLR and Nv-NF-κB was performed. The image represents a merge of Nv-TLR (false-colored red) and Nv-NF-κB (false-colored green) staining. Yellow lines in the panel on the *Right* mark *x* and *y* axes that were used to make the optical slices that are shown to the right and below the merged image. Arrows identify a cnidocyte that stains only for Nv-NF-κB. (Scale bar: 15 μm.)

Table S1. Identified leucine-rich repeat motifs, NF motif, CF motifs, and putative transmembrane domain in Nv-TLR

Motif	Residues	Sequence
Leucine-rich repeat motif	216–247	LQALDLNNNSLT [1] PPD [8] SLPRNL [1] R
Leucine-rich repeat motif	248–288	L [19] RFLVLDYNQI [2] LSQYPFTGH
Leucine-rich repeat motif	289–312	LQKLSIKGNGLRVIGG [7] G
Leucine-rich repeat motif	312–336	VNIIDLSNNEIR [4] QLFRG [2] S
Leucine-rich repeat motif	337–360	MLELRFNHNFL [2] LPNRVFTDM [1] R
Leucine-rich repeat motif	361–384	LKRLYLNNNRLQ [5] LLYGN [1] E
Leucine-rich repeat motif	385–408	IETLTLNDNDLT [2] ENNALPEN [1] N
Leucine-rich repeat motif	410–432	LKTLTLQRNRLT [1] VPRAVFLL [1] N
Leucine-rich repeat motif	475–500	LK [4] ELNLANNGI [3] DIGSL
CF motif	520–575	PLICNCKLTALFRLLKRLTADYPDVTHAQFDSWICSQPTRLRNVALLRVPENQFQC
NF motif	582–615	CPRECTCAVRE [6] VDCSERGLH RLPF [1] MPA
Leucine-rich repeat motif	639–662	ITVLELSNNEIK [5] FVDSL [1] R
Leucine-rich repeat motif	663–685	VVNLAINDNKLK [2] PRGVTNL [1] A
CF motif	697–748	FFVCDCYASWMRDWL [1] NN [1] DK [1] EDT [1] SILCASG [6] IISVP [3] FNCS
Leucine-rich repeat region	216–697	—
Predicted transmembrane domain	765–787	LLAIVLAVLLVLSVVAFVMTYCF

Leucine-rich repeat structural motifs, an N-terminal LRR cysteine cluster (NF motif), and two C-terminal LRR cysteine clusters (CF motifs) were predicted using NCBI BLAST. Cysteines in each cluster are in red font. Bracketed numbers represent gaps in alignment with canonical domains and motifs. Transmembrane domain prediction was performed with the Center for Biological Sequence Analysis's TMHMM Server version 2.0 (www.cbs.dtu.dk/services/TMHMM/).

Table S2. Quantification of Nv-TLR$^+$ cells following morpholino injection

	Morpholino	
Data classification	Control	Nv-TLR
No. of animals	55	55
Range	2–282	0–139
Median	81	10
Mean	92.3	14.5
SD	65.6	19.3
SE	8.8	2.6

Anemones were injected with either control or Nv-TLR morpholinos. Representative images of these injected anemones are shown in Fig. 7*B*. Optical cross-sections were taken through the ectoderm with a FluoView FV10i confocal microscope. The number of Nv-TLR–positive cells was counted from 55 anemones (5 d postinjection) for both the control and Nv-TLR morpholino-injected groups. The range, median, and mean numbers of Nv-TLR–positive cells, along with SDs and SEs for these counts, are reported. Nv-TLR$^+$ cells were identified and counted using FIJI image processing software (61).

Table S3. Mammalian and bacterial expression vectors used in this study

Plasmid name	Description and/or source
pUC57-Nv-TLR	pUC57 with a full-length codon-optimized Nv-TLR cDNA with a 5′ EcoRI restriction site and a 3′ NotI restriction site. Synthesized by GenScript.
pcDNA-FLAG	Mammalian expression vector with 5′ FLAG (34)
FLAG-Nv-TLR	EcoRI-NotI fragment containing codons 2–969 of Nv-TLR from pUC57-Nv-TLR was subcloned into EcoRI-NotI–digested pcDNA-FLAG.
FLAG-Nv-TLRΔTIR	PCR-amplified from FLAG-Nv-TLR using Gibson primers to insert codons 2–814 into pcDNA-FLAG. Primers: NvTLR-FWD-FLAG and NvTLRΔTIR-REV
FLAG-Nv-NF-κB	(34)
FLAG-MAL	From Jan Tavernier, Ghent University, Ghent, Belgium
FLAG-MyD88	From Claudio Sette, University of Rome, Rome
pMSCV-puro	Retroviral mammalian expression vector with puromycin resistance
pMSCV-Nv-TLR	PCR amplified from FLAG-Nv-TLR using Gibson primers to insert codons 1–969 into pMSCV-puro. Primers: NvTLRpMSCV-FWD and NvTLRpMSCV-REV.
pcDNA3.1+	(Invitrogen)
pcDNA-Nv-TLR	PCR amplified from FLAG-Nv-TLR using Gibson primers to insert codons 1–969 into pcDNA3.1+. Primers: NvTLR-FWD-pcDNA3.1+ and NvTLR-REV-pcDNA3.1+.
3x-κB site LUC	(34)
RSV-βGAL	(55)
HA-TLR4	From Adeline Hajjar, University of Washington, Seattle
CD14	From Adeline Hajjar, University of Washington, Seattle
MD-2	From Adeline Hajjar, University of Washington, Seattle
pGEX-KG	Bacterial expression vector with 5′ GST (32)
GST-Nv-TIR	EcoRI-XhoI fragment containing Nv-TLR codons 748–969 was subcloned in EcoRI-XhoI –digested pGEX-KG. Primers: GST-NvTIR-FWD and GST-NvTIR-REV. PCR amplified from FLAG-Nv-TLR.

Table S4. Primers used in this study

Primers	Sequence
NvTLR-FWD-pcDNA3.1+	5′-TAGTCCAGTGTGGTGGATGAAAGGGAGTATTCTGC GCC-3′
NvTLR-REV-pcDNA3.1+	5′-TGCTGGATATCTGCAGTTAGACTTTGGACATTTCGA C-3′
NvTLR-FWD-FLAG	5′-ACGTGCTGCAGATGCGAATTCAAAAGGGAGTATTC TGC-3′
NvTLR-REV-FLAG	5′-GCTCGAGGGATCCCTGTTAGACTTTGGACATTTCG AC-3′
GST-NvTIR-FWD	5′-GCGCGCGAATTCTAGCCTACCGGCCACCCGTGCCA-3′
GST-NvTIR-REV	5′-GCGCGCTCGAGTTAGACTTTGGACATTTCGAC-3′
NvTLRΔTIR-REV	5′-GCTCGAGGGATCCCTGTTAGATCTTGCTCACGTC GGTATC−3′
NvTLRpMSCV-FWD	5′-GATCTCTCGAAGTTAACGATGAAAGGGAGTATTC TGCGCC-3′
NvTLRpMSCV-REV	5′ GCCTCCCCTACCCGGTAGTTAGACTTTGGACATT TCGAC-3′
GAPDH-FWD	5′-TGGTATCGTGGAAGGACTCATGAC-3′
GAPDH-REV	5′-ATGCCAGTGAGCTTCCCGTTCAGC-3′
NvTLRqPCR-FWD	5′-ACCTGGCCATCAACGACAAT-3′
NvTLRqPCR-REV	5′-CCAGCTGGCATAACAATCGC-3′
EMSA NF-κB-consensus	5′-TCGAGAGGTCGGGGAATTCCCCCCCCG-3′
	5′-TCGACGGGGGGGGAATTCCCCGACCTC-3′

Dataset S1. Native, codon-optimized, and translated Nv-TLR sequences

Dataset S1

A black sequence indicates the nucleotide sequence of the codon-optimized Nv-TLR used in this paper. A red sequence indicates nucleotides in the native Nv-TLR sequence. A dash indicates that the original nucleotide at that position was not changed in the codon-optimized sequence. The translated amino acid sequence is in blue.

Dataset S2. *N. vectensis* TLR–to–NF-κB and cGAS–STING homolog sequences

Dataset S2

cDNA sequences were used as a reference to map raw nematosome RNA-seq reads (41) and to determine the relative expression (in RPKM) of TLR–to–NF-κB and cGAS–STING pathway homologs. Partial sequences are indicated.

Dataset S3. TIR domain sequences for phylogenetic analysis

Dataset S3

Full-length TLR sequences from *Homo sapiens*, *N. vectensis*, *D. melanogaster*, *C. elegans*, and *A. queenslandica* are included with their respective TIR domains in bold font. These truncated and culled TIR domain sequences were later aligned by Clustal Omega (53), and neighbor-joining analysis was performed by PAUP* (Fig. 1*B*) (54).