PNAS
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Supplementary Information for

P47 Licenses Activation of the Immune Deficiency Pathway in the Tick *Ixodes scapularis*

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www.pnas.org/cgi/doi/10.1073/pnas.1808905115

Supplementary Materials and Methods

Bacteria

Escherichia coli BL21 (DE3) cultures were grown overnight at 37°C in lysogeny broth supplemented with 100 µg/ml ampicillin. *Anaplasma phagocytophilum* HZ was cultured in the human HL-60 cell line (ATCC, CCL-240) (1). Host-free *A. phagocytophilum* was obtained by syringe lysis. Briefly, infected HL-60 cells were collected at 2300 x g for 10 minutes at 4°C. The pellet was resuspended in PBS and lysed four times by passing through a 25-gauge needle. Cellular debris was collected by centrifuging at 750 x g for 5 minutes at 4°C. The supernatant was kept and subjected to syringe lysis as above. Host-free *A. phagocytophilum* were collected by centrifuging at 2300 x g for 10 minutes at 4°C. Host-free bacteria were enumerated by this formula: number of infected HL-60s * 5 morulae/cell * 19 bacteria/morula * 0.5 (50% recovery) (2).

Low passage *Borrelia burgdorferi* B31 clone MSK5 was grown in Barbour-Stoenner-Kelly (BSK)-II medium supplemented with 6% normal rabbit serum at 37°C, as described elsewhere (3, 4). Profiling to ensure the presence of necessary virulence plasmids was performed, as described elsewhere (3).

Data Availability

All proteins and genes referred to the supplementary information (SI) Appendix, Tables S1-3 are freely available from Universal Protein Resource (Uniprot) or the National Center for Biotechnology Information (NCBI) databases.

Enzyme-linked Immunosorbent Assay (ELISA)

A high-binding 96-well ELISA plate (Costar) was coated with either no protein, 11.4 pmol bovine serum albumin (BSA), 11.4 pmol p47-His, 50 ng recombinant WT, Δ ubiquitin-binding domain (UBA), or Δ ubiquitin-like domain (UBX) p47-His (for recombinant protein binding experiments), or 200 ng pooled flat tick lysate (for *in vivo* binding experiments) in 500 mM carbonate-bicarbonate buffer (pH 9.5) at 4°C overnight. Plates were washed with phosphate buffered saline pH 7.4 with 0.05% Tween-20 (PBS-T) three times and blocked with 10% heat-inactivated fetal bovine serum (FBS) for 1 hour at room temperature, after which they were incubated with the indicated concentrations of glutathione S-transferase (GST) (Thermo Scientific, 20237), X-linked inhibitor of apoptosis (XIAP)-GST, or Kenny-GST for two hours at room temperature. Plates were washed five times with PBS-T and subsequently incubated with α-GST (SI Appendix, Table S3) followed by an additional 5 washes and incubation with α-mouse IgG-HRP for one hour at room temperature (Abcam, 1:10,000). Plates were washed seven times in PBS-T and developed with 3,3',5,5'-tetramethylbenzidine (TMB) (BD Biosciences) for 10-30 minutes. The reaction was stopped with 1 M H_2SO_4 and the absorbance was read at 450 nm with a 595 nm correction (Bio-Rad iMark). Background signal (BSA or no protein) was subtracted from the p47-His-coated well signal prior to graphing and statistical analysis.

Eukaryotic Cell Culture

All cell cultures were verified by PCR to be *Mycoplasma*-free (Southern Biotech, 13100-01). The human cell line HL-60 (ATCC, CCL-240) was cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% heat-inactivated FBS (Gemini Bio-Products) and 1X GlutaMAX (Gibco). HL-60s were maintained in suspension cultures at densities ranging from 1 x 10^5 to 1 x 10^6 cells/ml. Human embryonic kidney (HEK) 293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with GlutaMAX, 10% heat-inactivated FBS, and 1% penicillin and streptomycin antibiotics (Corning). Mammalian cell cultures were maintained at 37°C and 5% CO_2. The *I. scapularis* ISE6 and IDE12 cell lines were

cultured in L15C300 medium supplemented with 10% heat-inactivated FBS (Sigma), 10% tryptose phosphate broth (BD, 260300), and 0.1% bovine lipoprotein concentrate (MP Biomedicals,191476) at 34°C. ISE6 and IDE12 cells were grown to confluence and either seeded at 3 x 10^6 cells/well in 6-well plates (IDE12) (Sigma) or 1 x 10^6 cells/well in 24-well plates (ISE6) (Celltreat), or subcultured at 1:1-1:20 in capped T25 flasks (Cellstar) (5). The S2* cell line was cultured in Schneider's *Drosophila* medium supplemented with 10% heat-inactivated FBS and 1% penicillin and streptomycin antibiotics (6). The loosely adherent culture was maintained at a density between 1 x 10^6 and 1 x 10^7 cells/ml at 28°C.

HEK 293T Transfection and Co-immunoprecipitation

HEK 293T cells were transfected, as described elsewhere (7). Briefly, 4 µg pCMV-*xiap*-HA, pCMV-*kenny*-HA, pCMV-*p47*-FLAG, or pCMV-**p47*-FLAG plasmids were transfected into 1 x 10^6 HEK 293T cells with Lipofectamine 2000 (Invitrogen) using Opti-MEM I Reduced Serum Medium (Invitrogen). After five hours, the transfection reagent was removed, and cells were left in complete medium for 48 hours. Cells were collected, washed once with PBS, and lysed in immunoprecipitation lysis buffer (Thermo Scientific). The cell lysate (250 µg to 1 mg) was incubated with 30-60 µl α-FLAG-M2 (Sigma, A2220) or α-HA (Pierce, 26181) cross-linked agarose beads overnight at 4°C. Beads were washed three times (XIAP/p47 and Kenny/p47 co-immunoprecipitations) or six times (Kenny/p47 and Kenny/*p47 co-immunoprecipitations) with 50 mM Tris, 150 mM NaCl, pH 7.5 and boiled for 5 minutes in 2X Laemmli buffer to elute proteins. Boiled beads and input samples were analyzed by western blot for XIAP-HA, Kenny-HA, or p47/*p47-FLAG expression.

Mouse Infections

All mouse experiments were approved by the Institutional Biosafety (IBC, 00002247) and Animal Care and Use (IACUC, 0216015) committees at the University of Maryland School of Medicine and complied with National Institutes of Health (NIH) guidelines (Office of Laboratory Animal Welfare [OLAW] assurance numbers A3200-01, A323-01, A3270-1). Female eight- to 10-week old mice were used for tick experiments. C57BL/6 mice were infected intraperitoneally with 1 x 10^7 host-free *A. phagocytophilum* HZ. C3H/HeJ mice (Jackson Laboratories, 000659) were infected intradermally with 1 x 10^5 low-passage *B. burgdorferi* B31 MSK5.

P47 Identification by Mass Spectrometry

The XIAP-GST co-immunoprecipitation was performed by crosslinking recombinant XIAP-GST to glutathione agarose beads with bis(sulfosuccinimidyl)suberate (BS3, ThermoFisher, 21580). ISE6 cells infected with *A. phagocytophilum* multiplicity of infection (MOI) 50 for 30 minutes were sonicated in 20 mM Tris pH 8.9, 150 mM NaCl, 0.01% Triton X-100 with protease inhibitors. The lysate was incubated with crosslinked XIAP-GST for 1 hour at 4°C. Beads were washed four times and eluted in 20 mM Tris pH 8.9, 150 mM NaCl, 10 mM DTT, 5 mM EDTA, 0.01% Triton X-100 with PreScission Protease (GE Healthcare, 27-0843-01). Eluted proteins were precipitated, trypsinized overnight, desalted, and analyzed by the University of Maryland School of Pharmacy Mass Spectrometry Facility. Identified proteins were filtered to remove contaminants (*e.g.* keratin), XIAP, GST, and other associated proteins, as well as proteins involved in metabolism, DNA replication, RNA transcription, and protein synthesis (SI Appendix, Table S1).

Plasmids

The plasmids pCMV-HA (Christopher A. Walsh, plasmid #32530), pCMV-FLAG (Joan Massague, plasmid #15738), pMT/V5/His (Cyril Gueydan, plasmid #63752), and pCoPURO (Francis Castellino, plasmid #17533) were obtained from Addgene and were received as gifts

from the depositing scientists. All constructs were amplified from tick or IDE12 cDNA using high-fidelity polymerase. *Bendless* and *xiap* were cloned into pGEX-6P-2 as previously reported (7, 8). *Xiap* was cloned into pCMV-HA as previously reported for expression in HEK 293T (7). *Kenny* cDNA cloned into pCMV-HA using the *EcoRI* and *NotI* restriction enzyme sites for expression in HEK 293T cells. GenScript performed codon optimization of tick *kenny* for expression in *E. coli*. The codon-optimized insert was digested with *BamHI* and *EcoRI* and subcloned into the pGEX-6P-2 vector.

GenScript performed codon optimization of *p47* cDNA for expression in *Drosophila* S2* cells. Codon-optimized *p47* was cloned into the pCMV-FLAG vector using the EcoRI and SalI. For expression in S2* cells, wild-type (WT) and mutant *p47* constructs were cloned into the pMT/V5/His vector using the *EcoRI* and *AgeI* sites. Domain deletion of the *p47* variant versions was generated from the full-length template using the primers indicated in SI Appendix, Table S2, and cloned into the pCMV-FLAG and pMT/V5/His vectors. PCMV-**p47*-FLAG was generated by mutating codons encoding lysine to arginine residues at positions 109, 139, 141, 154, and 300 (New England Biolabs, E0554). All plasmids were verified by sequencing.

Protein Extraction and Subcellular Fractionation

Cell pellets or pooled flat ticks were lysed with radioimmunoprecipitation (RIPA) buffer (Thermo Scientific, 89900) buffer with protease inhibitors (Roche, 11836170001), and incubated at 4°C for 20 minutes prior to centrifugation at 12,000xg for 15 minutes at 4°C. The supernatant was kept at -80°C until western blotting. Cytoplasmic and nuclear protein extracts were prepared from 3 x 10^6 IDE12 cells using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific, 78833) and stored at -80°C. Concentrated protein extracts were obtained by following the kit protocol for 10 µl packed cell volume.

Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR)

The PureLink RNA Mini kit (Ambion, 12183025) was used to extract RNA from cells or engorged ticks preserved in Trizol. 500 ng RNA was used to synthesize cDNA with the Verso cDNA Synthesis Kit (ThermoFisher, AB-1453). *P47* silencing and bacterial burdens were quantified by qRT-PCR using the primers listed in SI Appendix, Table S2.

Recombinant Proteins

Recombinant XIAP and Bendless were produced as described previously (7). *E. coli* BL21 (DE3) was transformed with the pGEX-6P-2-*xiap*, pGEX-6P-2-*bendless*, or pGEX-6P-2-*kenny* plasmids and induced with 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 18 hours at 18-22°C. Cells were collected by centrifugation, resuspended in 20 mM Tris pH 8.9 (XIAP) or pH 8.1 (Bendless and Kenny), 300 mM NaCl, 5% glycerol, and lysed three times with the Microfluidics LV2 homogenizer. Lysates were clarified by centrifugation, and the resulting supernatant was incubated with glutathione agarose beads (Thermo Scientific, 16100) for 1 hour at room temperature. Beads were washed three times (50 mM Tris pH 8.0 and 150 mM NaCl) prior to elution with 50 mM Tris pH 8.0, 150 mM NaCl, and 10 mM reduced L-glutathione. Eluates were concentrated on 3 kDa (Bendless-GST), 30 kDa (XIAP-GST), or 50 kDa molecular weight cut off (MWCO) (Kenny-GST) spin filter concentrators (Amicon EMD Millipore). Concentrated eluates were dialyzed against 1X PBS on 7 kDa MWCO desalting columns (Thermo Scientific, 89889).

Recombinant full-length and deletion mutant p47-His proteins were produced by inducing stably transfected pMT-*p47*-His S2* cells with 500 µM $CuSO_4$ for 48 hours. Cells were collected by centrifugation, resuspended in RIPA buffer with protease inhibitors and incubated in ice for 30 minutes with periodic brief agitation. The lysate was clarified by centrifugation at 3200 x g, 4°C, 30 minutes, and the supernatant was incubated with prepared Hi60 Ni Superflow resin (Clontech, 635659) for 1 hour at room temperature or overnight at 4°C. The resin was washed

once with binding buffer (50 mM Tris pH 8.0, 100 mM NaCl, 5 mM imidazole, 0.1 mM EDTA) followed by three buffer washes (50 mM Tris pH 8.0, 300 mM NaCl, 10 mM imidazole, 0.1 mM EDTA). Recombinant p47-His was eluted by incubating the resin for 5 minutes in 50 mM Tris pH 8.0, 50 mM NaCl, 300 mM imidazole, 0.1 mM EDTA with protease inhibitors. All eluates were concentrated and dialyzed against 1X PBS.

RNAi Silencing and Tick Experiments

P47 and *relish* siRNA and the scrambled controls (scRNA) were synthesized with the Silencer siRNA construction kit (Ambion, AM1620) using the primers indicated in SI Appendix, Table S2. To silence *p47* or *relish,* 3 µg or 10 µg siRNA or scRNA was transfected into 1 x 10^6 ISE6 or 3 x 10^6 IDE12 cells using Lipofectamine 2000, respectively. After 24 hours, the transfection reagents were removed, and cells were infected with *A. phagocytophilum* MOI 50 (ISE6) or MOI 150 (IDE12) for 18 hours (ISE6) or times indicated (IDE12). IDE12 cells were harvested, washed twice with PBS, and stored at -80°C. ISE6 cells were harvested in Trizol (Ambion, 15596018) and stored at -80°C. *I. scapularis* ticks were microinjected with 10 ng siRNA or scRNA, as described elsewhere (7). After resting overnight, ticks were placed on infected, anesthetized mice seven (*A. phagocytophilum)* or 10 (*B. burgdorferi*) days post-infection and allowed to attach for 30 minutes. The anesthesia was subsequently removed and ticks were allowed to feed until repletion. Fully engorged ticks were collected, weighed, and frozen in single tubes at -80°C until analysis. Ticks were snap frozen in liquid nitrogen and homogenized prior to RNA or protein extraction.

S2* Transfection

3 x 10^6 S2* cells were seeded in 6-well plates and grown overnight at 28°C. $CaCl_2$ (240 mM final concentration) was mixed with 19 µg plasmid DNA. Sterile H_2O was added to bring the solution volume to 300 µl. The DNA/$CaCl_2$ solution was added dropwise to HEPES-buffered saline (50 mM HEPES, 1.5 mM Na_2HPO_4, 280 mM NaCl, pH 7.1). The resulting solution was incubated at room temperature for 30 minutes, vortexed briefly, and added dropwise to the S2* cells. After incubating for 24h, cells were washed twice followed by the addition of fresh complete medium. Stably transfected S2* cell lines were generated by co-transfecting the pMT-His plasmid of interest and 1 µg pCoPURO. After 24 hours, cells were washed twice and grown in fresh complete medium for 48 hours. Successfully transfected cells were selected with 5 µg/ml puromycin.

Surface Plasmon Resonance (SPR)

The Biacore 2000 instrument (GE Healthcare) was used to perform SPR. XIAP-GST or GST alone was captured to a Biacore CM5 chip (GE Healthcare) coated in α-GST antibodies. To determine the association and dissociation constants ($K_D = K_d/K_a$) of the protein-protein interaction, increasing concentrations of p47-His diluted in HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20) were flowed over the chip. Non-specific binding was corrected for by subtracting the GST:p47-His signal from the XIAP-GST:p47-His signal. Data were fitted with a 1:1 binding mode with mass transfer using Biacore T200 Evaluation software to determine the kinetic rate constants and the K_D for the XIAP:p47 interactions.

Ticks

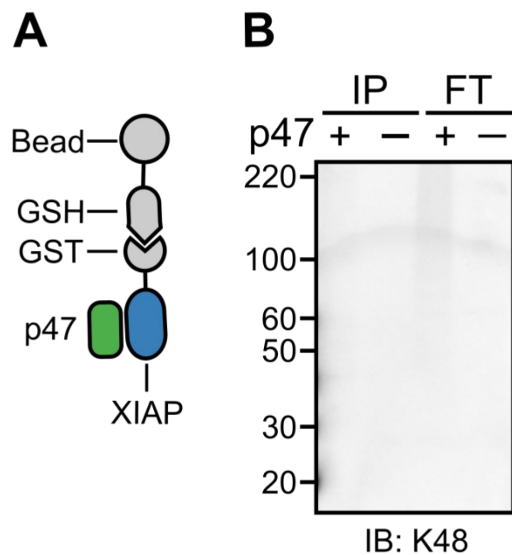
I. scapularis nymphs were supplied by the NIH Biodefense and Emerging Infectious Diseases (BEI) Research Resources Repository. Ticks were housed upon arrival at 23°C with 85% relative humidity and a 12/10-hour light/dark photoperiod regimen.

Western Blotting

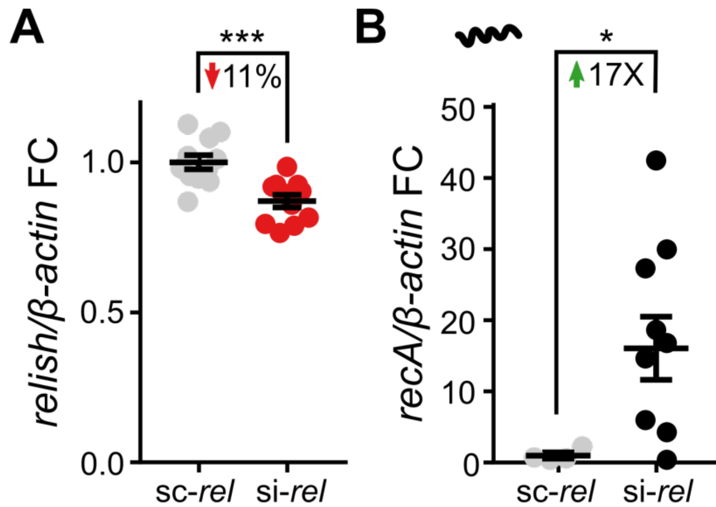
The full western blotting procedure was described elsewhere (7). Briefly, protein lysates were quantified by bicinchoninic acid (BCA) assay (Thermo Scientific, 23227). Equal amounts of protein were boiled in 6X Laemmli sample buffer (VWR) containing 5% β-mercaptoethanol. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Biorad mini-PROTEAN gels) and transferred onto PVDF membranes (Biorad). Membranes were blocked for 1 hour with 5% skim milk (Biorad) in PBS-T, followed by overnight primary antibody incubation at 4°C in 0.25% skim milk PBS-T at the dilutions indicated in SI Appendix, Table S3. Blots were washed three times in PBS-T and incubated with secondary antibodies for 1 hour at room temperature. Blots were next washed three times in PBS-T before 1-2 minutes incubation in fresh enhanced chemiluminescence (ECL) substrate solution prior to visualization (Thermo Scientific, 32106). If needed, blots were incubated in stripping buffer (Thermo Scientific, 21059) for 15 minutes at room temperature, and then washed three times in PBS-T prior to blocking and re-probing with the primary antibody.

Ubiquitylation assays

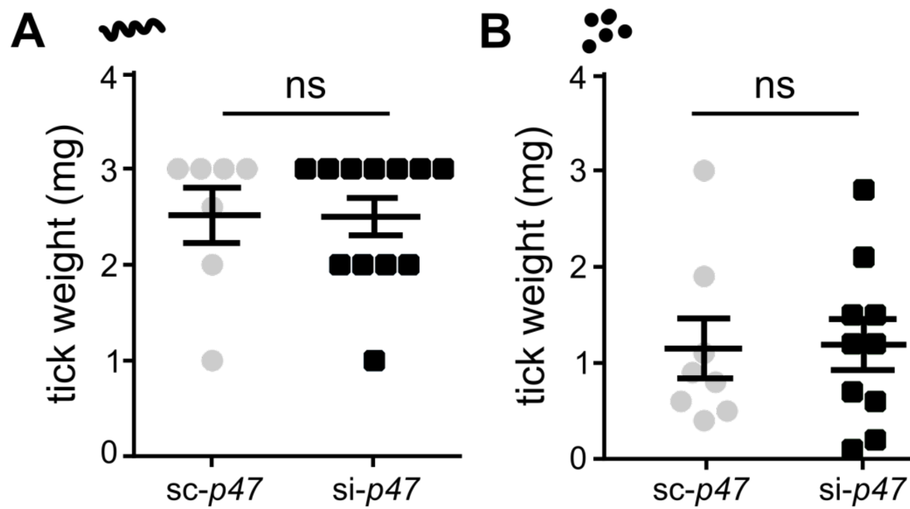
The *in vitro* ubiquitylation reactions contained reaction buffer (500 mM Tris pH 7.4, 10 mM DTT), 1.5 mM Mg-ATP (Boston Biochemical, B-20), 155 nM Ube1 (Boston Biochemical, E-305), 250 nM Uev1a (Boston Biochemical, E2-662), 375 nM Bendless-GST, 2.4 mM XIAP-GST, 10 mM p47-His, and 50 mM ubiquitin (Boston Biochemical, U-100H) and were incubated at 37°C for 1 hour prior to stop buffer addition (Boston Biochemical, SK-10). Reactions were dialyzed against PBS. P47-His was isolated from the reactions using the protocol for p47-His purification from S2* cells. The eluate and flow-through were dialyzed against PBS to remove imidazole and subjected to western blotting to assess K63- and K48-specific ubiquitylation.



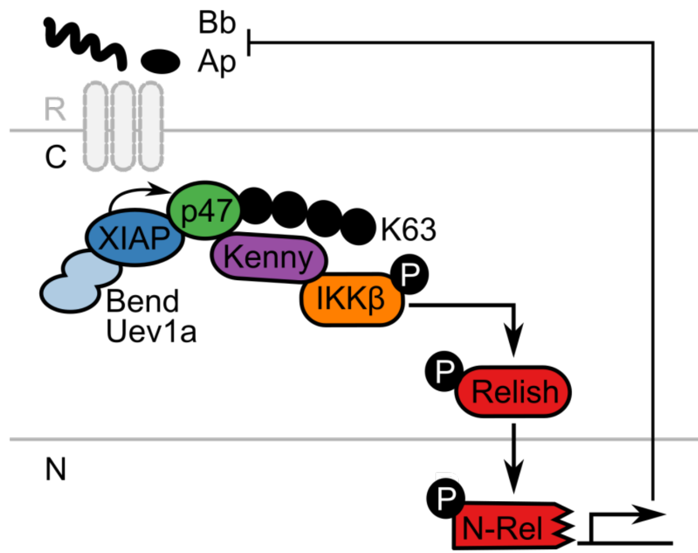
SI Appendix, Fig S1. XIAP does not ubiquitylate the substrate p47 in a lysine (K)48-dependent manner. **(A)** Co-immunoprecipitation of *Ixodes scapularis* XIAP-glutathione S-transferase (GST) incubated with *Anaplasma phagocytophilum*-infected tick cell lysates led to the identification of *I. scapularis* p47 through mass spectrometry. **(B)** Ubiquitylation reactions were performed in the presence of an E1 activating enzyme (Ube1), the heterodimeric E2 conjugating enzyme complex (Uev1a and Bendless), the E3 ubiquitin ligase (XIAP) and its substrate (p47). P47 is not ubiquitylated in a K48-dependent manner by XIAP, as demonstrated by the absence of the K48-specific signal. Western blot is representative of at least two experiments. GSH, reduced glutathione; IP, immunoprecipitation; IB, immunoblot; FT, flow-through.



SI Appendix, Fig S2. *Relish* silencing increases *Borrelia burgdorferi* acquisition in *Ixodes scapularis*. **(A)** ISE6 cells were transfected with 3 µg sc-*relish* (n=11) or si-*relish* (n=11) for 24h followed by an 18h *A. phagocytophilum* multiplicity of infection (MOI) 50 infection. *Relish* expression was quantified by quantitative real-time polymerase chain reaction (qRT-PCR). **(B)** *I. scapularis* nymphs were silenced by microinjecting 10 ng small interfering RNA (siRNA) (20 ticks for either si-*relish* or sc-*relish* per experiment) and allowed to feed to repletion (sc-*relish* n=4, si-*relish* n=9) on *B. burgdorferi*-infected mice. RNA was extracted from cells or single ticks and subjected to qRT-PCR to determine *relish* expression and *B. burgdorferi* burden. To calculate fold change, all values were divided by the mean sc-*rel* value. Means ± standard error of the mean (SEM) are graphed. Two-tailed Student's t tests were performed to determine statistical significance. *$P < 0.05$; *** $P < 0.001$; *recA,* recombinase A; FC, fold change.



SI Appendix, Fig. S3. *P47* silencing does not impact tick feeding success. *Ixodes scapularis* nymphs were microinjected with 10 ng sc-*p47* control or si-*p47* constructs to knock down *p47* expression. Recovered ticks (n=7-12) were weighed after feeding to repletion on **(A)** *B. burgdorferi*- or **(B)** *A. phagocytophilum*-infected mice. Two-tailed Student's t tests were performed to determine statistical significance. These data are representative of three independent experiments per infection. ns, not significant.



SI Appendix, Fig. S4. The immune deficiency pathway of *Ixodes scapularis.* Immune deficiency signaling in *I. scapularis* ticks is triggered by *A. phagocytophilum* and *B. burgdorferi* and leads to the cleavage and nuclear translocation of Relish (N-Rel) to promote antibacterial responses. The E3 ubiquitin ligase XIAP, together with the heterodimer Bendless:Uev1a, binds to p47 and regulates signal transduction through K63-dependent polyubiquitylation. Ubiquitylated p47 is required for recruiting Kenny in the tick IMD network. IKKβ, Inhibitor of nuclear factor-κB kinase β; Bend, Bendless; R, unidentified receptor; C, cytoplasm; N, nucleus; P, phosphorylated.

SI Appendix, Table S1. Putative interacting partners of *I. scapularis* XIAP*

Protein IDs	FASTA name	Ubiquitylation Prediction
B7PEL3	Protein tyrosine phosphatase (p47)	Yes
B7QF96	Putative uncharacterized protein	No
B7Q4P0	Putative uncharacterized protein	Yes
B7Q0Q1	Putative uncharacterized protein	Yes
B7PIP9	Ankyrin 2,3/unc44, putative	Yes
B7PJK3	Putative uncharacterized protein	No
B7PSX9	Tropomyosin invertebrate, putative	No
B7PU84	Putative uncharacterized protein	Yes
B7Q121	Putative uncharacterized protein	No
B7Q2T7	Putative uncharacterized protein	No
B7Q740	Putative uncharacterized protein	No
B7Q815	Secreted protein	Yes
B7QD48	Putative uncharacterized protein	No
B7QGQ3	Putative uncharacterized protein	No

*After removal of contaminant proteins, XIAP, and glutathione S-transferase (GST). See SI Appendix Materials & Methods for details.

SI Appendix, Table S2. Primers used in this study.

Name	Target Accession		Sequences (5'→3')
A. phagocytophilum 16S rRNA (qPCR)	NC_007797	F	CAGCCACACTGGAACTGAGA
		R	5CCCTAAGGCCTTCCTCACTC
I. scapularis actin (qPCR)	AF426178	F	GGTATCGTGCTCGACTC
		R	ATCAGGTAGTCGGTCAGG
Borrelia burgdorferi recA (qPCR)	NC_001318, BB_0131	F	GTGGATCTATTGTATTAGATGAGGCT
		R	GCCAAAGTTCTGCAACATTAACACCT
I.scapularis p47 (qPCR)	XM_002433590*	F	GCCAGGGCCAAGCTTTACC
		R	CTTGGACGCTCCAGCGAC
*I. scapularis p47*_scrambled	XM_002433590	F	AAGCTACGCATCGCCACAATACCTGTCTC
		R	AATATTGTGGCGATGCGTAGCCCTGTCTC
*I. scapularis p47*_silenced	N/A	F	AAGCCTCGCAGACTTAACCAACCTGTCTC
		R	AATTGGTTAAGTCTGCGAGGCCCTGTCTC
*I. scapularis relish*_scrambled	GBBN01020499.1**	F	AACCTCACACCATTTGCCTTTCCTGTCTC
		R	AAAAAGGCAAATGGTGTGAGGCCTGTCTC
*I. scapularis relish*_scrambled	N/A	F	AAGACGCCTATCGCCTACATTCCTGTCTC
		R	AAAATGTAGGCGATAGGCGTCCCTGTCTC
I. scapularis relish (qPCR)	GBBN01020499.1**	F	AGAATGTCCGCCACCGTTTTTTTCTGC
		R	CACGTGCACCGCCTCACCATGAAGG
pGEX-6P-*bendless*	XM_002400659	F	GGATCCATGGCCGCTTTAGGAAG
		R	CTCGAGTCAGGCGTTCTGGG
pGEX-6P-*xiap*	XM_002433822	F	GGAATTCCCGTTGTCATCAGCATGGCG
		R	AAGGAAAAAAGCGGCCGCTCATGAAAGAAAAGCCTTAAT
pCMV-*xiap*-HA	XM_002433822	F	GATTGCAGGAATTCATGGTTGTCATCAGTATGGCG
		R	GTATAGTTGCGGCCGCTCATGAAAGAAAAGCCTTAATGTTC
pCMV-*p47*-FLAG	XM_002433590*	F	GGCCGAATTCATGGCAGATTGTGCGGGGCG
		R	GGCCGTCGACTCACTTGATACGCTGGACGATAA
pCMV-UBA-*p47*-FLAG	XM_002433590*	F	GGCCGAATTCATGACCGATGAAAGCCTGGAACG
		R	GGCCGTCGACTCACTTGATACGCTGGACGATAA

pCMV-UBX-*p47*-FLAG	XM_002433590*	F	GGCCGAATTCATGGCAGATTGTGCGGGGCG
		R	GGCCGTCGACTCATGGGATGGCCTGCTGGGCGG
pCMV-SEP-UBX-*p47*-FLAG	XM_002433590*	F	GGCCGAATTCATGGCAGATTGTGCGGGGCG
		R	GGCCGTCGACTCACACGCGCACCACAGGGGCTG
pCMV-*kenny*-HA	XM_002411821*	F	TCCGGGCCGAATTCATGTCCACGGAAGCGGCGAC
		R	TGCTTAGCGGCCGCTTAGCGGAAGCCGGCGTCGCTGT
pMT-*p47*-His	XM_002433590*	F	GAGCGAATTCCACCATGGCAGATTGTGCGG
		R	GAGCACCGGTGCCGCCGCCCTTGATACGCTGGACGAT
pMT-UBA-*p47*-His	XM_002433590*	F	GGCCGAATTC ATG ACCGATGAAAGCCTGGAACG
		R	GGCCACCGGTCTTGATACGCTGGACGATAA
pMT-UBX-*p47*-His	XM_002433590*	F	GGCCGAATTCATGGCAGATTGTGCGGGGCG
		R	GGCCACCGGTTGGGATGGCCTGCTGGGCGG
pCMV-*p47*-KDBL139-141-FLAG***	XM_002433590*	F	ACCTGGCAAACGTCAGCGTGATAATTTTGTGGTGGAAGCCTTC
		R	CCCAGCACCTGCTGTCCG
pCMV-*p47*-K300-FLAG***	XM_002433590*	F	TCGCCGTGGCCGTATGGCCTTCG
		R	GGGGCCACAAACTGTTCAT
pCMV-*p47*-K154-FLAG***	XM_002433590*	F	GGCCGCCAAACGTCATGGTGCACAG
		R	TTGAAGGCTTCCACCACA
pCMV-*p47*-K109-FLAG***	XM_002433590*	F	CGATCTGACCCGTGACGAGAGCGGCAAC
		R	GCCAGGCCTGCAATACGG

*These sequences were codon-optimized for expression in *Escherichia coli,* human embryonic kidney (HEK) 293T, and *Drosophila melanogaster* hosts. *Kenny*-HA was not codon-optimized; however, the *kenny* sequence subcloned into the pGEX-6p-2 vector was codon-optimized for *E. coli* expression.

** Primers were designed based on the relish sequence derived from an *I. scapularis* transcriptome (NCBI project number: PRJNA230499).

***These primers were used to generate the **pCMV-p47-FLAG* construct, where codons encoding lysines 109, 139, 141, 154, and 300 were mutated to arginine.

SI Appendix, Table S3: Antibodies used in this study.

Reagent	Catalog	Usage	Origin	Origin accession	Tick accession	Identity	Predicted Size
α-Actin	Sigma A2103	1:4000	human	NP_001135417	XP_002408110	95%	42 kDa
α-FLAG	Sigma F3165	1:1000	synthetic	N.A.	N.A.	N.A.	varies
α-GST	Calbiochem OB03*	1:400	unknown	unknown	N.A.	N.A.	26 kDa
α-HA	Sino Biological 100028-MM10	1:1000	synthetic	N.A.	N.A.	N.A.	varies
α-His	Novus Biologicals NB100-64768	1:1000	synthetic	N.A.	N.A.	N.A.	varies
α-K48Ub	Millipore 05-1307	1:1000	human	P0CG47	XP_002415681	54%	varies
α-K63Ub	Millipore 05-1308	1:1000	human	P0CG47	XP_002415681	54%	varies
α-Kenny	Gift from Neal Silverman	1:500	fly	NP_523856	XP_002411866	19%	61 kDa
α-Lamin A + C	Abcam ab108922	1:1000	human	NP_733821	XP_002402839	36%	62-66 kDa
α-p-IKKβ (Y188)	Abcam ab194519	1:500	human	NP_001177649	XP_002410053	53%	46 kDa
α-Relish	Thermo Scientific custom	1:500	tick	REDGRATFPS MSIVFQQKK	AIT40202**	100%	40-50 kDa

*No longer commercially available, **From *Rhipicephalus microplus*

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