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Supplemental information

A tick C1q protein alters infectivity of the Lyme disease agent by modulating interferon γ

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Supplementary materials

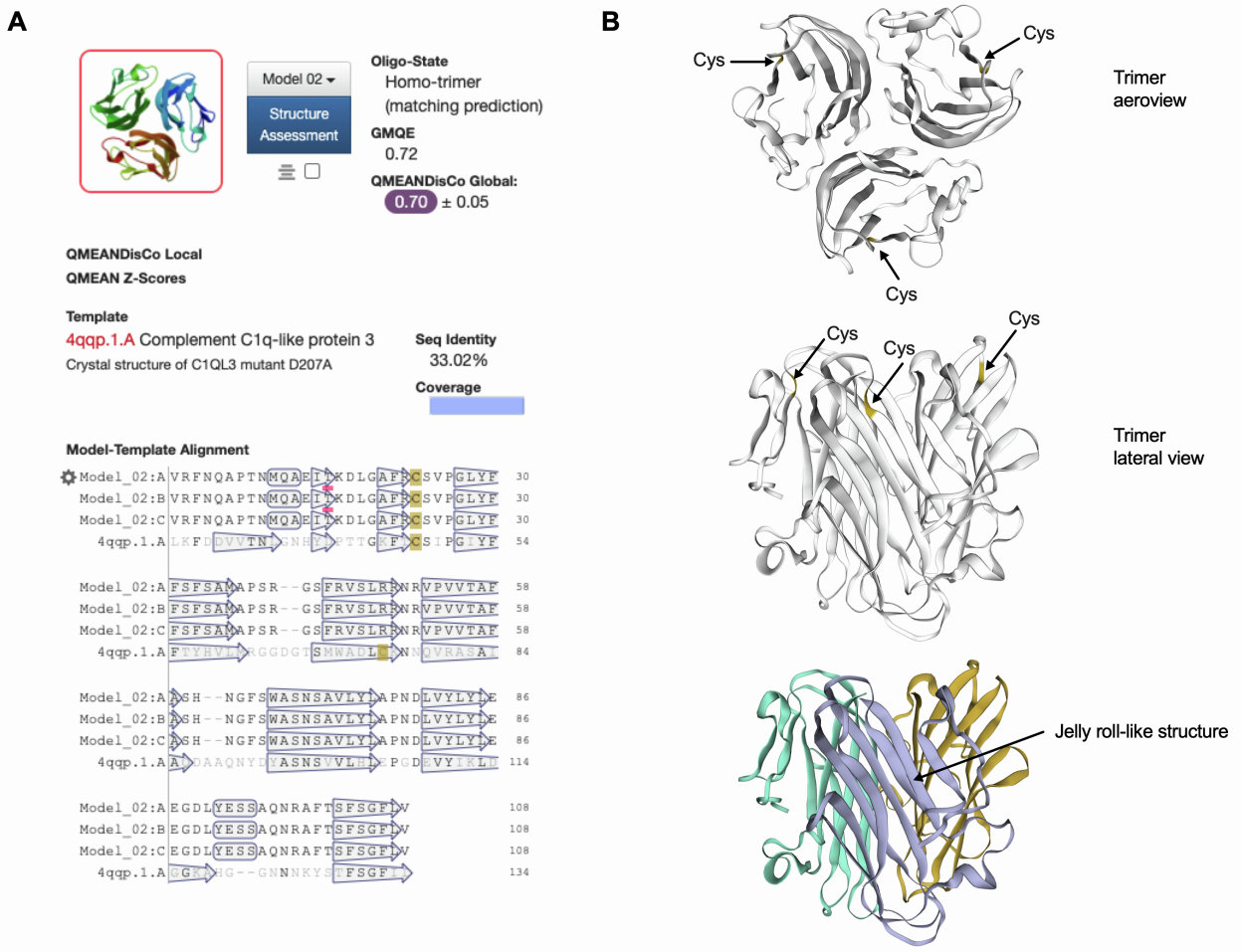
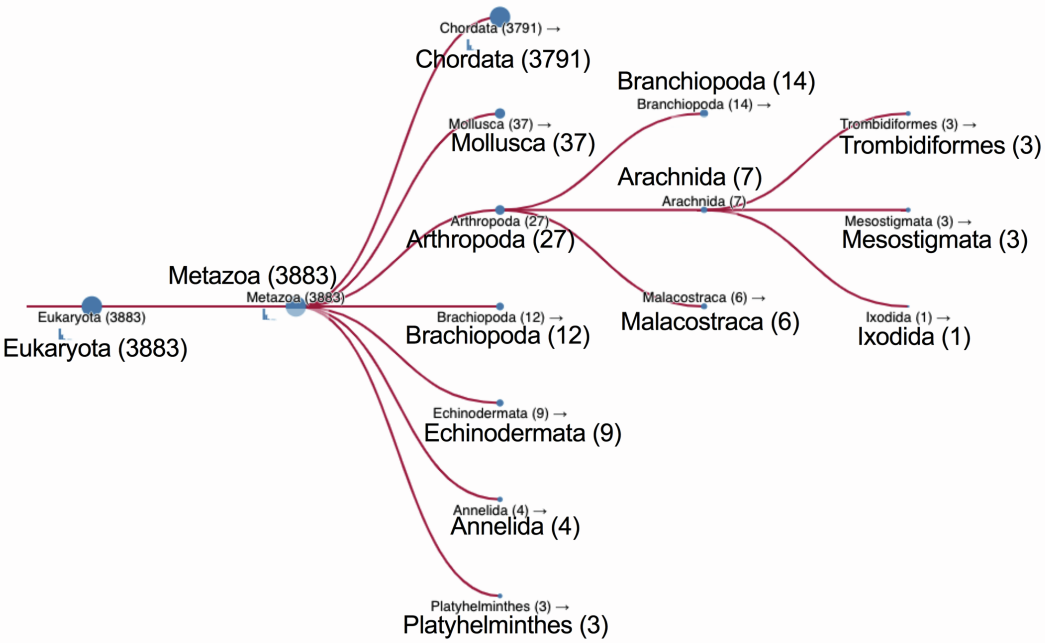


Figure S1. IsC1ql3 protein structure homology-modelling based on the platform of Swiss-Model. (A) The predicted protein structure of IsC1ql3 has a high degree of similarity to mammal complement C1q-like protein 3 with the global model quality estimation (GMQE) value of 0.72. (B) IsC1ql3 is predicted to form a trimer and have a jelly roll-like structure. The cysteine (cys) residue is highlighted with yellow color.

A



B

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Ixodes_scapularis          ------------------------------------------------------------  0
Ixodes_persulcatus         ------------------------------------------------------------  0
Dermacentor_silvarum       ------------------------------------------------------------  0
Rhipicephalus_sanguineus   ------------------------------------------------------------  0
Rhipicephalus_microplus    MKAVLVLAVFFVIAYVAPQGATEEHQHESHEGHHGDHIDEHGRHRPHHGSVCNLSEQSLA  60

Ixodes_scapularis          ----------------------------------------------MQTWVVL----  7
Ixodes_persulcatus         ------------------------------------------------------------  0
Dermacentor_silvarum       ---------------------------------------MCSIRDATAAAGWRLLP-VL  19
Rhipicephalus_sanguineus   ---------------------------------------MGATSNAITFASRLLLPVLV  20
Rhipicephalus_microplus    AVVECVVRNVTEEIRGKLESVKESLRCDTILYRRTSGMDPTDATRRAITFPGRLLLAVLL  120

Ixodes_scapularis          AVHFCCALCAEARIGFTMTQGSVDGTYVRFNQAPTNMQAEITKDLGAFRCSVPGLYFFSF  67
Ixodes_persulcatus         ----------------MTQGSVDGTYVRFNQAPTNMQAEITKDLGAFRCSVPGLYFFTF  43
Dermacentor_silvarum       LLGFFAVQEASAVVGFTMTQGTQDGTYIRFKRVPTNMLAEVTKELGAFRCSQPGLYYFSF  79
Rhipicephalus_sanguineus   VVGFFATQEASAMVGFTMTQATQDGTYIRFNRVLTNMIADITKELGAFRCSQPGLYYFSF  80
Rhipicephalus_microplus    VFGLFATQEASAMVGFTMTQGTQDGTYIRFNRVPTNMIADVTKELGAFRCSQPGLYYFSF  180
                                     ***.: ****:**::. *** *::**:******* ****:*:*

Ixodes_scapularis          SAMAPSRGSFRVSLRRNRVPVVTAFASHNGFSWASNSAVLYLAPNDLVYLYLEEGDLYES  127
Ixodes_persulcatus         SAMAPSRGSFRVSLRRNRVPVVTAFASHNGFSWASNSVVLYLAPNDLVYLYLEEGDIYES  103
Dermacentor_silvarum       TAMAPSPGSCRISLRRNRVPVVTAFASNSGFSWISSGALLYLAQNDLVYLYLEDGTIHES  139
Rhipicephalus_sanguineus   TAMAPSQGSCRISLRRNRIPMVTAFASNNGFSWISSGAVLYLSQGDVVYLYLEDGAIHES  140
Rhipicephalus_microplus    TAMAPSQGSCRISLRKNRVPIVTAFASNSGFSWISSGAVLYLSQGDVVYPYVEDGAIHES  240
                           :***** ** *:***:**:*:******:.**** *...:***: .*:** *:*:* ::**

Ixodes_scapularis          SAQNRAFTSFSGFLVGDESHHLLGRN-P----VEEEMGAPDPADEIFQKLALIRRKGTV-  181
Ixodes_persulcatus         SAQNRAFTSFSGFLVGDESHHLLGRN-P----VEEEMGAPDPTDDIFQKLAIIHRKGTV-  157
Dermacentor_silvarum       SATNRAFTSFTGFAVGTDML--MGRT-PDTDAIEGPLPSPDPFDDIAERMYRVKLSKNAT  196
Rhipicephalus_sanguineus   SVPNRAFTSFSGFTVSTDSL--MARNGPVVDVTEGPLPSPDPYDDIEERMYRVKLAKNAT  198
Rhipicephalus_microplus    SAPNRAFTSFSGFTVSTDSL--MARNGPVVDATEGPLTSPDPYDDIEERMYRVELAKNAT  298
                           *. *******:** *. :    :.*. *     *  : :*** *:* :::  :.   ..

Ixodes_scapularis          -------   181
Ixodes_persulcatus         -------   157
Dermacentor_silvarum       AGTSASL   203
Rhipicephalus_sanguineus   TAASHS-   204
Rhipicephalus_microplus    KSA----   301
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Figure S2. Characterization of *Ixodes scapularis* IsC1ql3. (A) InterProScan search of IsC1ql3 homologs. (B) Protein sequence comparison of IsC1ql3 and homologs in ticks. gC1q domain regions are marked by upper lines. (*) indicates positions which have a single, fully conserved residue (dark grey). (:) indicates conservation between groups of strongly similar properties (light grey). (.) indicates conservation between groups of weakly similar properties (white grey).

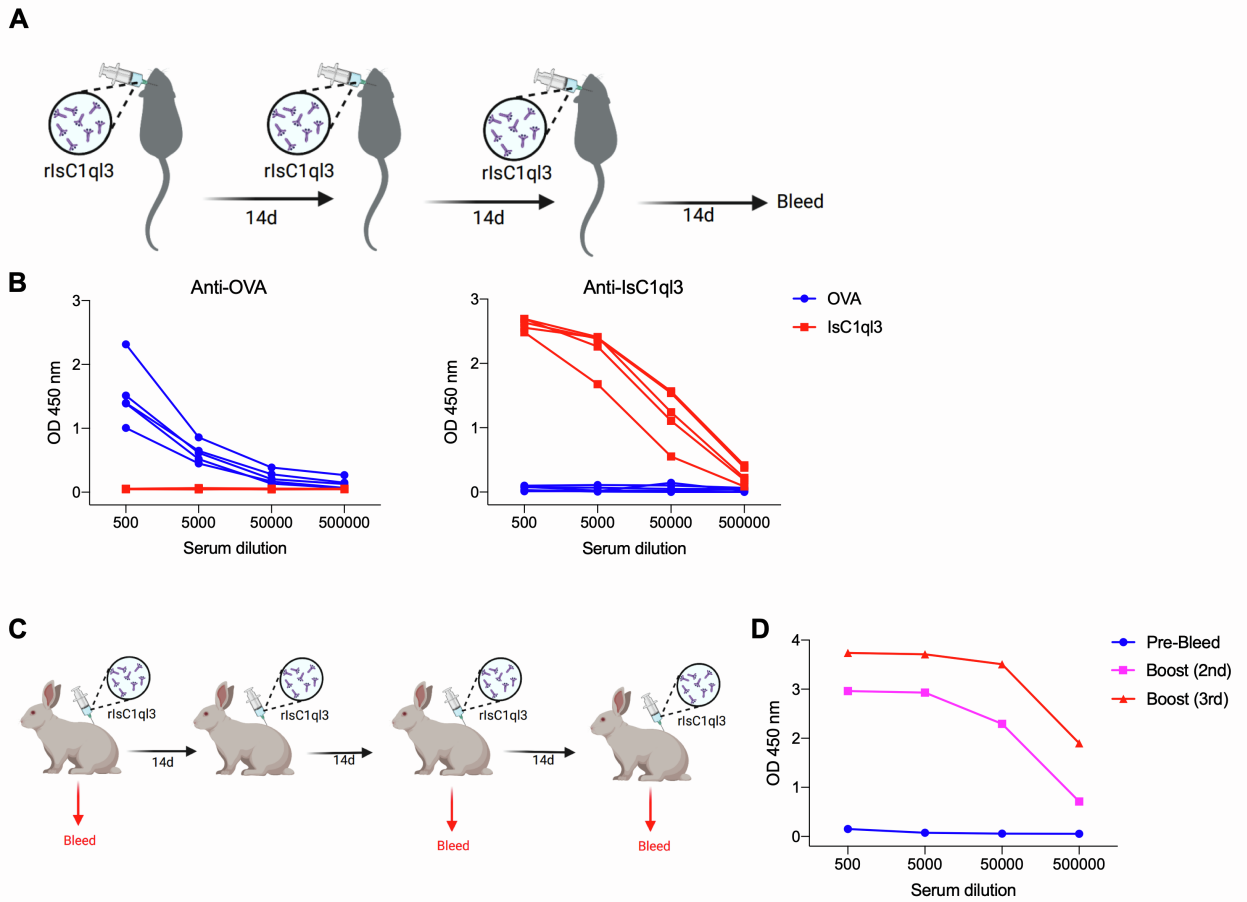
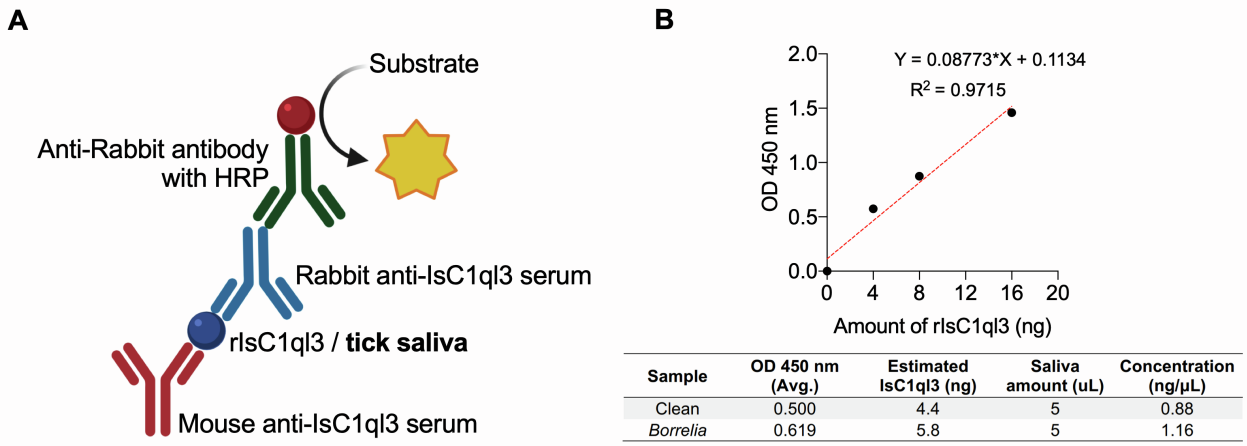


Figure S3. Active immunization and antibody preparation of IsC1ql3. (A) Mice were Immunized by injecting 10 μg of rIsC1ql3 protein or ovalbumin (control) three times at 2-week intervals. Two weeks after the final immunization with IsC1ql3 or OVA, the sera from the immunized mice were examined for specific antibodies by ELISA. (B) Sera from IsC1ql3-immunized mice recognized IsC1ql3 (right panel), but not ovalbumin, OVA (left panel). (C) Rabbit anti-IsC1ql3 antibody preparation. A rabbit was immunized by injecting 150 μg of rIsC1ql3 protein three times at 2-week intervals. Two weeks after the final immunization with IsC1ql3, the serum from the immunized rabbit was examined for specific antibodies with ELISA. (D) Sera from IsC1ql3-immunized mice recognized IsC1ql3.



Sample	OD 450 nm (Avg.)	Estimated IsC1ql3 (ng)	Saliva amount (uL)	Concentration (ng/μL)
Clean	0.500	4.4	5	0.88
Borrelia	0.619	5.8	5	1.16

Figure S4. Quantification of the IsC1ql3 concentration in tick saliva by capture ELISA. (A) Schematic diagram of the capture ELISA to quantify the IsC1ql3 concentration in tick saliva**.** (B) Standard curve comparing the OD value of diluted samples for known amounts of recombinant IsC1ql3 and the OD value corresponding to IsC1ql3 in saliva from *B. burgdorferi*-uninfected and infected ticks.

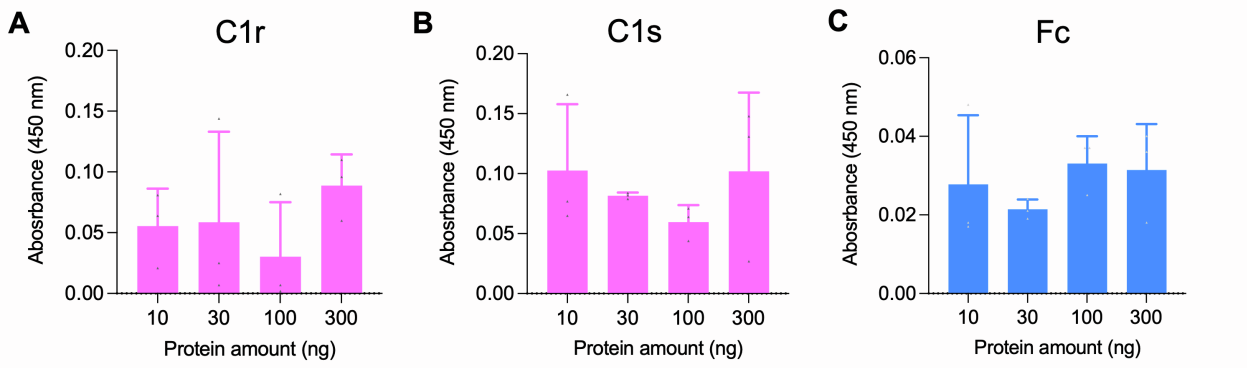


Figure S5. IsC1ql3 is not involved in classical complement pathway cascade. (A) ELISA result shows no interactions between IsC1ql3 and human C1r. Data are represented as mean ± SD. (B) ELISA result shows no interactions between IsC1ql3 and human C1s. Data are represented as mean ± SD. (C) ELISA result shows no interactions between IsC1ql3 and antibody Fc region. Data are represented as mean ± SD.

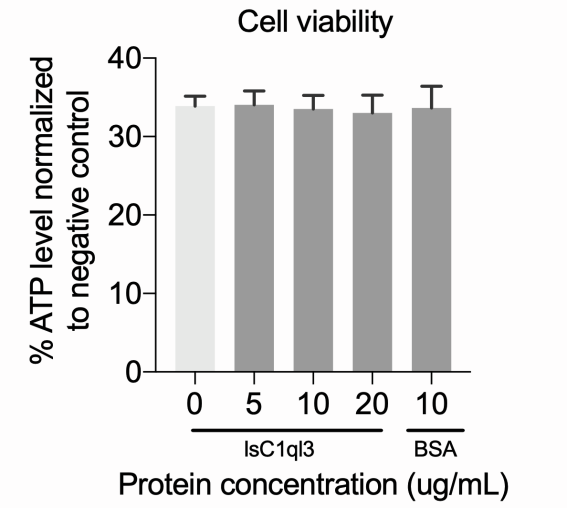


Figure S6. No effect of rIsC1ql3 on splenocytes viability as determined by CellTiter-Glo luminescent cell viability assay. Data are represented as mean ± SD.

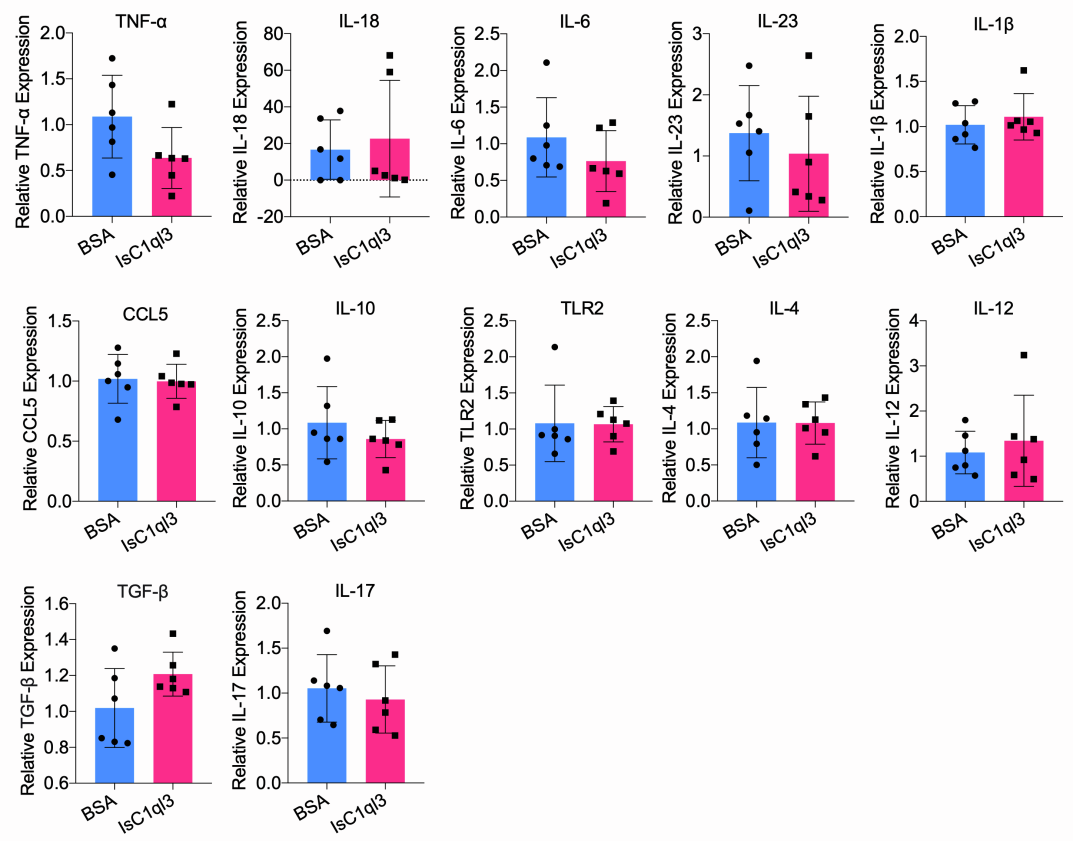


Figure S7. The effects of lsC1ql3 on gene expression of cytokines and chemokines in splenocytes upon LPS stimulation. Data are represented as mean ± SD.

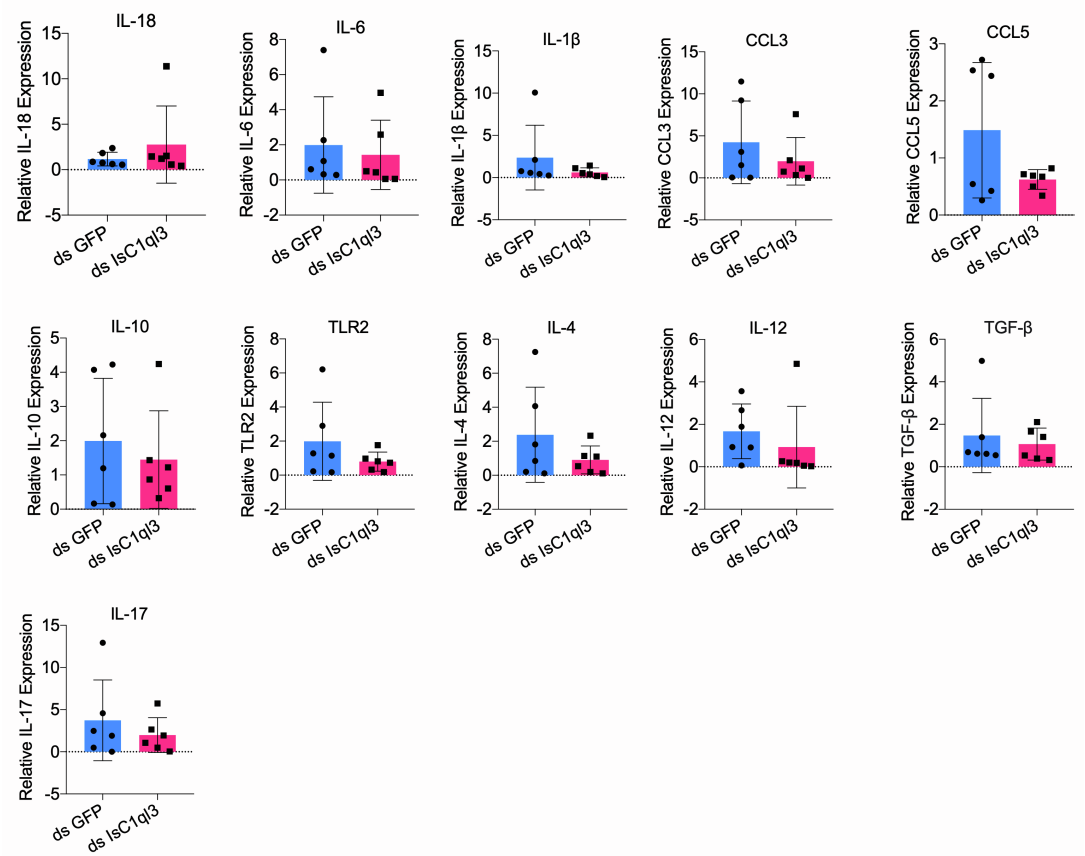


Figure S8. Gene expression of cytokines and chemokines at the tick bite of mice after challenged by control (GFP) or *IsC1ql3*-silenced *B. burgdorferi*-infected ticks. Data are represented as mean ± SD.

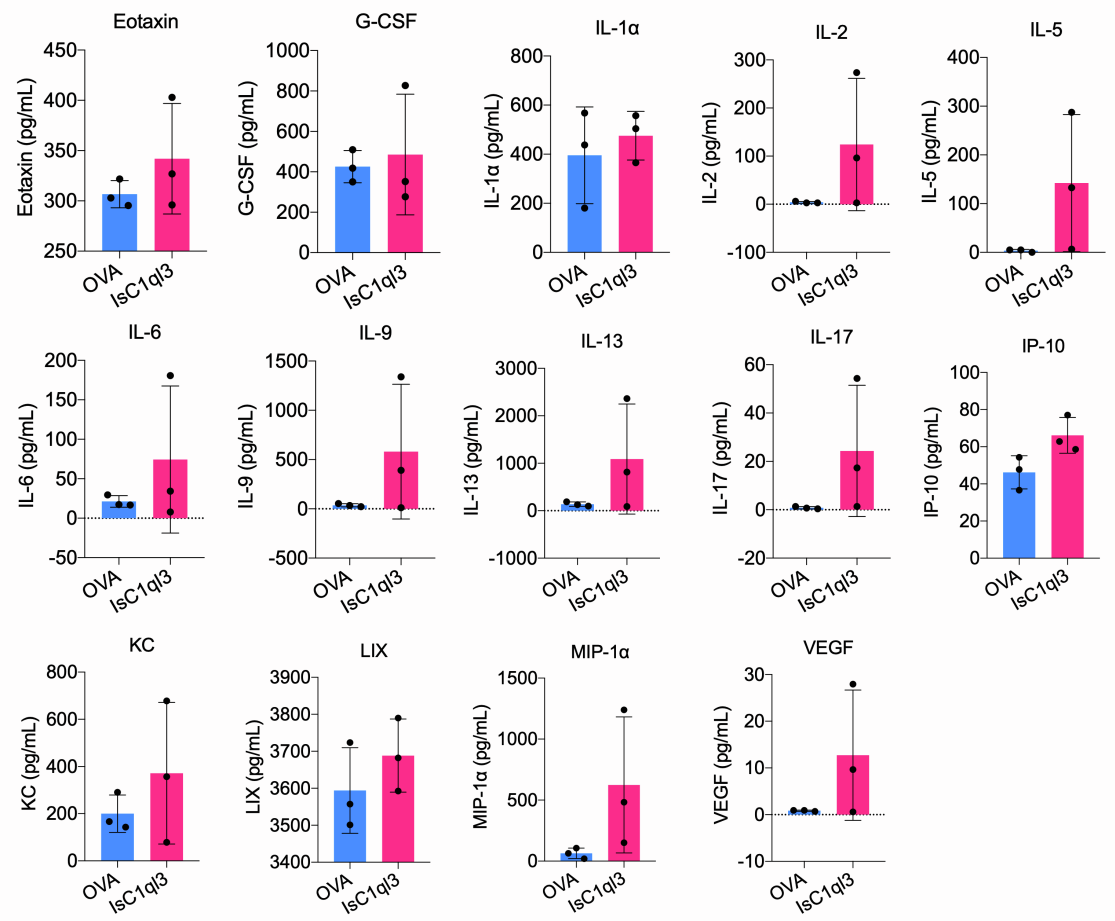


Figure S9. Serum cytokines and chemokines production in control or IsC1ql3-immunized mice after challenged by *B. burgdorferi*-infected ticks. Data are represented as mean ± SD.

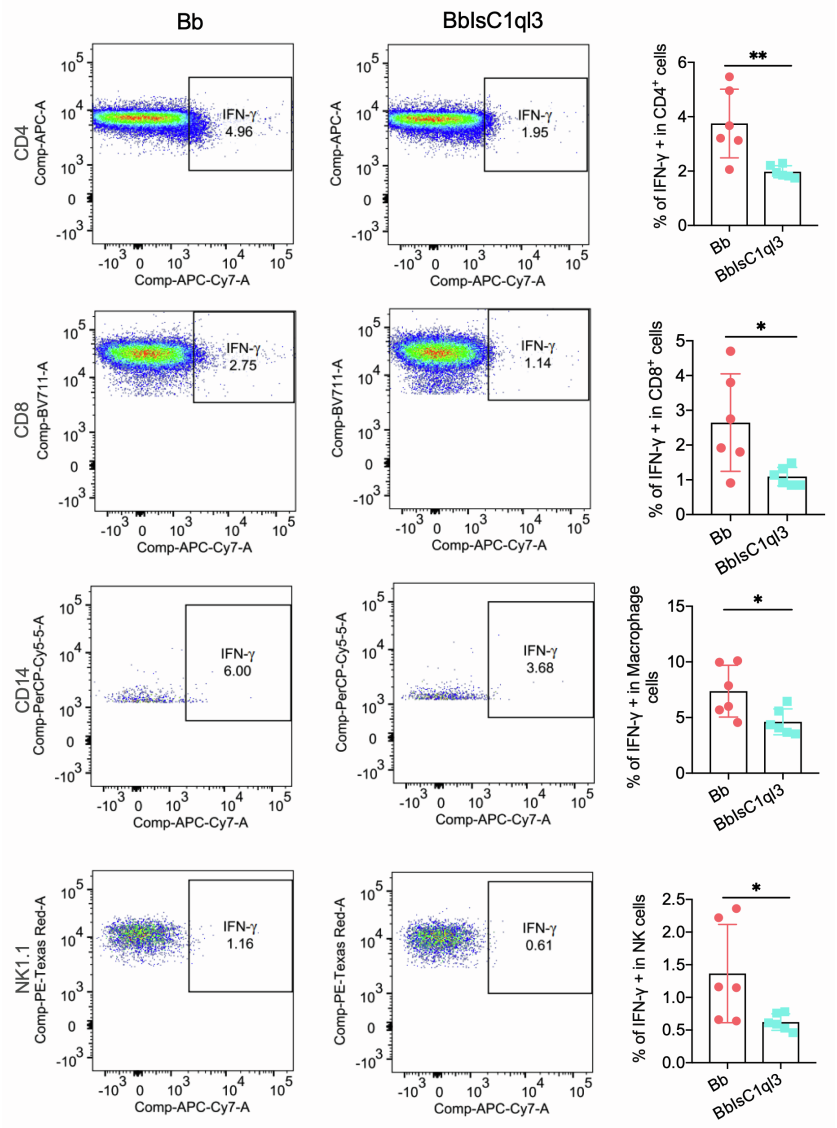


Figure S10. Tick salivary protein IsC1ql3 attenuates the host immune response. Percentage of $CD4^+$, $CD8^+$ T cells, macrophages and NK cells expressing IFN-γ. IsC1ql3 significantly inhibits IFN-γ production in the immune cells upon *B. burgdorferi* infection and IL-12 stimulation. Data are represented as mean ± SD. Statistical significance was assessed using a non-parametric Mann-Whitney test (*, $p < 0.05$; **, $p < 0.01$).

Table S1. Summary of differently expressed genes of transcriptome data from splenocytes that were stimulated by *B. burgdorferi*, with or without IsC1ql3.

Gene ID	Gene name	*p*-value	Fold change
Serpina3f	serine (or cysteine) peptidase inhibitor, clade A, member 3F	0.0000	-4.6483
Iigp1	Interferon-γ-inducible 47-kDa GTPase	0.0000	-3.3747
Tifab	TRAF-interacting protein with forkhead-associated domain, family member B	0.0002	-3.3408
Gm10291	predicted pseudogene 10291	0.0013	-3.1083
Cxcl9	chemokine (C-X-C motif) ligand 9	0.0000	-3.0660
Gbp2	guanylate binding protein 2	0.0000	-3.0367
Gbp11	guanylate binding protein 11	0.0000	-2.8262
Gbp10	guanylate-binding protein 10	0.0000	-2.8140
Xcl1	chemokine (C motif) ligand 1	0.0000	-2.6498
Itgad	integrin, alpha D	0.0018	-2.6153
Srgap3	SLIT-ROBO Rho GTPase activating protein 3	0.0023	-2.5747
Serpina3g	serine (or cysteine) peptidase inhibitor, clade A, member 3G	0.0000	-2.5561
Gm12185	predicted gene 12185	0.0000	-2.5301
Ffar2	free fatty acid receptor 2	0.0052	-2.5140
Gm43302	predicted gene 43302	0.0000	-2.4890
Ankrd61	ankyrin repeat domain 61	0.0025	-2.3738
Tgtp1	T cell specific GTPase 1	0.0000	-2.3571
Gm49730	predicted gene 49730	0.0080	-2.3461
Rps2-ps5	ribosomal protein S2, pseudogene 5	0.0000	-2.3354
Gm18852	predicted gene 18852	0.0002	-2.3254
Gm18180	predicted gene, 18180	0.0012	-2.3104
Dnaaf3	dynein, axonemal assembly factor 3	0.0111	-2.2805
Gbp8	guanylate-binding protein 8	0.0000	-2.2804
Igf2bp2	insulin-like growth factor 2 mRNA binding protein 2	0.0082	-2.2498
Rnd3	Rho family GTPase 3	0.0031	-2.2069
Eaf2	ELL associated factor 2	0.0109	-2.1957
Gm2654	predicted gene 2654	0.0162	-2.1707
Rpl23a-ps14	Ribosomal Protein L23a	0.0086	-2.1634
Gbp4	guanylate binding protein 4	0.0000	-2.1628
Gm15801	predicted gene 15801	0.0314	-2.1592
Gm28177	predicted gene 28177	0.0002	-2.1587

Gm28539	predicted gene 28539	0.0068	-2.1554
Gm5630	predicted pseudogene 5630	0.0056	-2.1479
Gm11739	predicted gene 11739	0.0135	-2.1358
Gm6257	predicted gene 6257	0.0128	-2.1231
Gbp6	guanylate binding protein 6	0.0000	-2.0585
Gbp7	guanylate binding protein 7	0.0000	-2.0339
AA413626	expressed sequence AA413626	0.0100	-2.0292
Tstd1	thiosulfate sulfurtransferase (rhodanese)-like domain containing 1	0.0129	-2.0211
Gm16069	predicted gene 16069	0.0259	-2.0193
Gm20302	predicted gene, 20302	0.0199	-2.0065
Gm5561	predicted gene 5561	0.0031	-2.0051
Gm8979	predicted gene 8979	0.0000	-2.0029
Cd163l1	CD163 Molecule Like 1	0.0160	2.0161
Amot	angiomotin	0.0157	2.0296
4930594C11Rik	RIKEN cDNA 4930594C11 gene	0.0124	2.0301
Tmem220	transmembrane protein 220	0.0117	2.0317
Slpi	secretory leukocyte peptidase inhibitor	0.0000	2.0435
Tarm1	T cell-interacting, activating receptor on myeloid cells 1	0.0008	2.0664
Ptgr1	prostaglandin reductase 1	0.0244	2.0736
Tnfsf15	tumor necrosis factor (ligand) superfamily, member 15	0.0050	2.0855
Gm17173	predicted gene 17173	0.0092	2.1432
Trav14n-1	T cell receptor alpha variable 14N-1	0.0411	2.1527
Lrrc6	leucine rich repeat containing 6	0.0122	2.1808
Gm10074	predicted gene 10074	0.0038	2.1857
Gm10416	predicted pseudogene 10416	0.0200	2.2002
Gm6361	predicted gene 6361	0.0088	2.4020
Rufy4	RUN and FYVE domain containing 4	0.0271	2.4905
Mmp13	matrix metallopeptidase 13	0.0052	2.5401
Zfp708	zinc finger protein 708	0.0073	2.5899
Ptges	prostaglandin E synthase	0.0000	2.6199
Slc7a2	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	0.0008	2.6946
Pcdhgb7	protocadherin gamma subfamily B, 7	0.0010	2.8856

"-" indicates downregulation of genes in the spirochetes with IsC1ql3 after *B. burgdorferi* stimulation.

Table S2. The primers used in this study.

Gene name	Primer sequence
Tick *actin*	F: GGCGACGTAGCAG R: GGTATCGTGCTCGACTC
Mouse *β-actin*	F: AGCGGGAAATCGTGCGTG R: CAGGGTACATGGTGGTGCC
Borrelia flaB	F: TTCAATCAGGTAACGGCACA R: GACGCRRGAGACCCTGAAAG
ds *GFP*	F: *TAATACGACTCACTATAGGGAGA*GCGACGTAAACGGCCACAAG TT R: *TAATACGACTCACTATAGGGAGA*CGGGTCTTGTAGTTGCCGTC
ds *IsC1ql3*	F: *TAATACGACTCACTATAGGGAGA*GAACATGCAGGCAGAAATCA R: *TAATACGACTCACTATAGGGAGA*ACGAGAAAGCCCGAGAAAG
IsC1ql3 qPCR	F: ACGAGAGCCATCACCTCCT R: TCCCCTTTCTGCGAATAAGA
IsC1ql3_pMT	F: CTCGCTCGGGAGATCTATGCAGACCTGGGTTGTTCTTG R: GCCCTCTAGACTCGAGTACCGTCCCCTTTCTGCGAAT
IsC1ql3_pET-28a	F: CTGGTGCCGCGCGGCAGCCATATGGTGCGCTTCAACCAGGCG CCGA R: GTGCTCGAGTGCGGCCGCAAGCTTCACGAGAAAGCCCGAGAA AGA
TNF-α qPCR	F: AGGCACTCCCCCAAAAGATG R: TGGTGGTTTGTGAGTGTGAGG
IL-18 qPCR	F: GACTCTTGCGTCAACTTCAAGG R: CAGGCTGTCTTTTGTCAACGA
IL-6 qPCR	F: ATACCACTCCCAACAGACCT R: CCAGTTTGGTAGCATCCATC
IL-1β qPCR	F: GCAGTGGTTCGAGGCCTAAT R: GCTGCTTCAGACACTTGCAC
CCL3 qPCR	F: GCCAGGTGTCATTTTCCTGAC R: CTCAAGCCCCTGCTCTACAC
IFN-γ qPCR	F: GAGGAACTGGCAAAAGGATGG R: ACCTGTGGGTTGTTGACCTC
CCL5 qPCR	

	F: GACAGCACATGCATCTCCCA R: GTGTCCGAGCCATATGGTGA
IL-10 qPCR	F: GTACAGCCGGGAAGACAATAAC R: GCATTAAGGAGTCGGTTAGCAG
TLR2 qPCR	F: AAGAGGAAGCCCAAGAAAGC R: AATGGGAATCCTGCTCACTG
IL-4 qPCR	F: CGGATGCGACAAAAATCAC R: CGTTTGGCACATCCATCTC
IL-12 qPCR	F: ATCGTTTTGCTGGTGTCTCC R: CTTCTTCAGGCGTGTCACAG
TGF-β qPCR	F: TGGAGCAACATGTGGAACTC R: TGCCGTACAACTCCAGTGAC
IL-17 qPCR	F: TCATCTGTGTCTCTGATGCTGTTG R: TCGCTGCCTTCACTGT
Dae2 qPCR	F: CATCGAGGGCTCAAGTTTTC R: CGTGGTGTAGTCCCTCAGGT
gC1qR qPCR	F: CATTTGATGGTGAGGAGGAG R: AAAGTTGGGAGTTGATGTCC

The underlines indicate restriction enzymes sites. The italicized letters indicate T7 promoter sequence.