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Chapter 9

*Ixodes scapularis* dystroglycan-like protein promotes *Borrelia burgdorferi* migration from the gut

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Abstract

The causative agent of Lyme borreliosis, *Borrelia burgdorferi*, is transmitted by *Ixodes* ticks. During tick feeding, *B. burgdorferi* migrates from the tick’s gut to the salivary glands from where transmission to the host occurs. *B. burgdorferi*-interacting tick proteins might serve as vaccine targets to thwart *B. burgdorferi* transmission. A previous screening for *B. burgdorferi*-interacting *Ixodes scapularis* gut proteins identified an *I. scapularis* putative dystroglycan protein (ISCW015049). Here we describe ISCW015049’s protein structure and its cellular location in the tick gut in relation to *B. burgdorferi* migration. Secondly, in vivo *B. burgdorferi*-tick attachment murine models were performed to study the role of ISCW015049 during *B. burgdorferi* migration and transmission. In silico analysis confirmed ISCW015049 is similar to dystroglycan and was named “*Ixodes scapularis* dystroglycan-like protein” (ISDLP). Confocal microscopy of gut tissue showed that ISDLP is expressed on the surface of gut cells, is upregulated during tick feeding, and is expressed significantly higher in infected ticks compared to uninfected ticks. Inhibition of ISDLP by RNA interference resulted in lower *B. burgdorferi* transmission to mice. In conclusion, we have identified a dystroglycan-like protein in *Ixodes scapularis* gut that can bind to *B. burgdorferi* and promotes *B. burgdorferi* migration from the tick gut.
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**Introduction**

In the United States, *Ixodes scapularis* is the vector of *Borrelia burgdorferi*, the causative agent of Lyme borreliosis [59, 259]. *B. burgdorferi* colonization of the tick gut can occur when uninfected *Ixodes* larvae acquire *B. burgdorferi* when feeding on a *B. burgdorferi*-infected animal [71]. *B. burgdorferi* anchors itself to the tick gut wall by expressing outer surface protein A (OspA), which binds to the tick receptor OspA (TROSPA) [194]. When a *B. burgdorferi*-infected *I. scapularis* nymph feeds on a vertebrate host, *B. burgdorferi* becomes metabolically active, changes its outer surface proteins and migrates from the tick gut to the salivary glands [207]. Motility of *B. burgdorferi* appears not to be essential for exiting the gut, as described in a newly proposed model called “adherence-mediated migration” by Dunham-Ems et al [69]. Dunham-Ems and colleagues observed that during tick feeding *B. burgdorferi* spirochetes initially replicate in the lumen of the gut and remain non-motile. After approximately 24 hours, *B. burgdorferi* spirochetes transition into aggregates or a “network of non-motile organisms” at the basal lamina of the gut. From here, a small percentage of *B. burgdorferi* penetrate the gut, followed by migration via the hemolymph to the salivary glands into the skin of the host [69].

The close interaction of *B. burgdorferi* with tick gut epithelial cells suggests potential interactions between the tick gut proteins and *B. burgdorferi* proteins that might be critical for *B. burgdorferi* growth in the tick gut, and its egress from the gut – a critical step for successful transmission to the vertebrate host. *B. burgdorferi*-interacting tick gut proteins might thus be vaccine targeted to prevent spirochete migration from the gut and preempt transmission. The advantages of using tick gut proteins as anti-tick vaccines is that migration of *B. burgdorferi* can be targeted early in tick feeding – even before spirochetes have been transmitted to the host. Of note, a vaccine against Lyme disease is currently not available for humans [204, 233]. Since the last decade, tick salivary gland proteins and tick gut proteins have become a target of vaccine development as the tick plays a central role in *B. burgdorferi* transmission [114]. Immunization against salivary gland proteins introduced into the skin that facilitate tick feeding provided (partial) protection against *B. burgdorferi* transmission [52, 53, 231], as well as immunization against tick gut proteins [175, 194]. One limitation of vaccine targeting tick gut proteins is that gut proteins might not provide an anamnestic response, since they are not presented to the host during a tick bite. Nonetheless, future development of cocktail vaccines combining tick gut proteins that facilitate spirochete migration
from the gut and salivary antigens that facilitate survival at the bite site might provide a robust impairment of \textit{B. burgdorferi} transmission by simultaneously targeting spirochete egress from the gut and survival at the bite site. Recently, we used a yeast surface display approach to screen for \textit{B. burgdorferi}-interacting tick gut proteins and identified three putative \textit{B. burgdorferi}-interacting gut proteins – ISCW008121, ISCW015049, and ISCW015135 [175]. One of them, ISCW008121, was shown to be a transmembrane fibronectin domain containing \textit{I. scapularis} protein that enabled \textit{B. burgdorferi} adherence to the basal lamina of the gut to facilitate transmission [175]. In this study we report the characterization of ISCW015049 and examine its vivo role in the context of \textit{B. burgdorferi} transmission and assess its ability to serve as a transmission-thwarting vaccine.

\section*{Material and methods}

\textit{Animal experiments}\n
The rabbit immunized against rISDLP and mice used in the RNA-interference experiments were housed and handled under the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal experimental protocol was approved by the Yale University’s Institutional Animal Care & Use Committee (protocol number 2008-07941, approval date: 3/31/2014). All animal infection experiments were performed in a Bio-safety Level 2 animal facility, according to the regulations of Yale University. In addition, the mice that were used for immunization experiments were housed and handled under the approval of the Animal Care and Use Committee of the University of Amsterdam (DIX103179).

\textit{Ticks}\n
\textit{I. scapularis} nymphs and larvae were obtained from a tick colony at the Connecticut Agricultural Experiment Station in New Haven CT, USA and ticks maintained as described earlier [179]. Ticks were allowed to feed to repletion and RNA isolated from guts and salivary glands using Trizol (Invitrogen, CA) as described earlier [22]. cDNA was synthesized using the iScript RT-PCR kit (Bio-Rad, CA) and analyzed by quantitative PCR for the expression of tick actin and \textit{B. burgdorferi} flab using gene-specific primers and the iQ SYBR Green Supermix (Bio-Rad, Hercules, CA).
Identification of the full-length transcript of ISCW015049 and purification of rISDLP
First strand cDNA was synthesized from total I. scapularis gut RNA using a 3’-RACE Adapter. The RLM-RACE kit was used to identify the sequence at the 3’-end and 5’end according to the manufacturer’s instructions (Invitrogen, CA). The identification of the sequence at the 5’end was performed using ISDLP-specific primers. The full-length sequence was assembled using the web-based software SMART [236] (http://smart.embl-heidelberg.de). Purification of rISDLP was performed as described previously [175].

ELISA assessment of rISDLP binding to Borrelia burgdorferi membrane extract
B. burgdorferi membrane extract purified as described previously [175] was coated (1 µg/ml) on high binding microtiter plates (Microlon, Greiner, Germany) overnight at RT. Wells were blocked with PBS/1 % BSA at RT for 1 h and incubated with rISDLP or rTSLPI (3-100 pmol/ml) diluted in PBS/0.05 % Tween20/1 % BSA for 1 h. Wells were washed and incubated with 1:5000 diluted mouse anti-V5 HRP IgG. Bound antibody was detecting using TMB as substrate (Thermoscientific, IL).

Confocal microscopy
Confocal microscopy to detect native ISDLP was performed as previously described [175]. Briefly, guts from nymphal ticks (B. burgdorferi-infected or uninfected) were dissected and fixed in 4 % PFA. Washed guts were incubated with rabbit anti-rISDLP antibody and bound antibodies detected using FITC-labeled affinity purified goat anti rabbit IgG antibody (Sigma, MO) and nuclei stained with propidium idodide or with TOPRO-3 iodide (Invitrogen, CA). Control guts were incubated with IgG purified from rabbit anti-ovalbumin sera. Stained guts were visualized under a Zeiss LSM510 Confocal microscope.

Pixel intensity quantification
Pixel intensities in the TRITC channel (as a measure of anti-rISDLP serum binding to tick gut ISDLP) or in the FITC channel of confocal images were quantified using ImageJ 1.47t software. Confocal images of four individual guts were examined in each control and experimental group and mean pixel intensities representing the average intensity of pixels in the region of interest were obtained in five different regions of each tick gut.
**Immunisation of rabbits and mice against ISDLP**

New Zealand white rabbits 4-6 weeks old were immunized subcutaneously with 30 μg of rISDLP or ovalbumin in complete Freund’s adjuvant (CFA) and boosted twice with 30 μg of rISDLP or ovalbumin at week 3 and 6 in incomplete Freund’s adjuvant (IFA). Test bleeds were obtained from ear veins 2 weeks after the final boost and reactivity to recombinant rISDLP and ovalbumin was assessed by Western Blot. Rabbits were euthanized and serum was obtained by cardiac puncture. Polyclonal IgG was purified from the sera using the Melon Gel IgG purification kit (Thermoscientific, IL).

For immunization of mice, animals were immunized with 10 μg of rISDLP or ovalbumin in CFA and boosted twice with 10 μg of rISDLP or ovalbumin at week 2 and 4 in IFA. To address the role of rISDLP in *B. burgdorferi* transmission, eight *B. burgdorferi* N40 infected nymphs were placed on each immunized mouse. Nymphs were allowed to feed to repletion. Salivary glands and guts were dissected and combined in pools of 2-3 ticks for quantitative RT-PCR as described earlier [231]. DNA was isolated from skin punch-biopsies at 7, 14 and 21 days and from heart and joints 21 days post tick-detachment and *Borrelia* burden was assessed by quantitative PCR as described [231].

**RNAi silencing of isdlp in Borrelia burgdorferi-infected Ixodes scapularis nymphs**

RNAi silencing of *isdlp* in ticks was performed as described before [231] using primers specific for *isdlp* with an T7 promoter sequence. ds *isdlp* dsRNA was synthesized using the MEGAscript RNAi kit (Ambion/Invitrogen, CA). ds *isdlp* RNA or ds *gfp* RNA (5 nl, 3x10^{12} molecules/ml) was injected into the anal pore of *Borrelia*-infected nymphs as described earlier [231]. dsRNA-injected ticks were allowed to feed until repletion and weighed to assess feeding efficiency, and guts and salivary glands dissected for mRNA isolation and quantitative RT-PCR as described above. *B. burgdorferi* burden in mice was assessed by quantitative PCR as described earlier [231].

**Statistical analysis**

The significance of the difference between the mean values of the groups was analyzed using a non-parametric two-tailed Mann-Whitney test or a two-tailed student *t* test with Prism 5.0 software (GraphPad Software, San Diego, CA), and *p* ≤ 0.05 was considered significant.
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The significance of the difference between the mean values of the groups was analyzed using a non-parametric two-tailed Mann-Whitney test or a two-tailed student t test with Prism 5.0 software (GraphPad Software, San Diego, CA), and p ≤ 0.05 was considered significant.

Results

**Full length ISCW015049 encodes a potential transmembrane dystroglycan-like protein**

The protein sequence present in the YSD screening yeast colony “Clone 3” matched amino acids 1 to 201 of the protein annotated on Vectorbase as “putative dystroglycan, ISCW015049”. The complete sequence of ISCW015049 was confirmed from *I. scapularis* gut extract using 3’end and 5’end RLM RACE. Using mRNA from guts of fed *I. scapularis* nymphs, the start and stop codons of ISCW015049 were identified and the complete transcript of 2904 bp was sequenced (Figure 1A). Identification of the full sequence revealed that the full length of ISCW015049 was 968 amino acids long, since one fragment of ISCW015049 that annotated as an intron (www.vectorbase.org) was actually found to be part of the ISCW015049 transcript. In silico analysis showed that full length ISCW015049 has a potential transmembrane domain and is similar to dystroglycan, a widely distributed protein involved in the linkage between the extracellular matrix and the cytoskeleton [117]. The protein sequence of full length ISCW015049 was 27.0 %, 28.7 % and 26.7 % identical to *Drosophila melanogaster* dystroglycan (NP_725523.3), *Homo sapiens* dystroglycan (AA81779.1) and *Mus musculus* dystroglycan (NP_001263423.1) respectively. Online programs for protein modeling (SMART) predicted that ISCW015049 has three Dystroglycan-type cadherin-like domains (CADG) and two Dystrophin-associated glycoprotein 1 domains (DAG1) (Figure 1B). The 201 aa region identified in the YSD screen was predicted to be located on the extracellular region, which consists of one CADG domain and a C-terminal domain of α-dystroglycan (Figure 1B-C). Based on the similarity with dystroglycan, full length ISCW015049 is henceforth referred to as *Ixodes Scapularis Dystroglycan Like Protein* (ISDLP) and has been submitted to Genbank (Accession number KR782315).

**Production of recombinant ISDLP and confirmation of binding to Borrelia burgdorferi**

We expressed the full-length protein transcript of ISDLP in a *Drosophila melanogaster* expression system. Binding of purified recombinant ISDLP (rISDLP)
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Figure 1. Full-length sequence of ISDLP (Genbank accession number KR782315). A. Guts from three fed I. scapularis were dissected and RNA was extracted. Reverse transcriptase–polymerase chain reaction (RT-PCR) was performed using primers based on the 3’end and 5’end of ISCW015049 which were identified using 3’end and 5’END RLM RACE. The complete sequence was identified by sequencing of three contigs (bp 1-604, bp 472-1575 and bp 1272-2904), which were cloned into a PGEMT-easy vector. B. Protein domains were predicted by SMART domain prediction (http://smart.embl-heidelberg.de) [236], TMHMM v2.0 software and the conserved domain database of NCBI. If available, E-values are provided for each domain in brackets. A potential transmembrane domain was found at aa 828-853, three CADG-1 domains at aa 4-102 (7.63-3), 346-450 (3.47-10) and 576-680 (2.01-6), two DAG-1 domains at aa 454-588 (2.20-17) and 683-968 (7.30-70) and a C-terminal domain of alpha dystroglycan was identified at aa 111-236.

C. A 3d-model of ISDLP was creating using using Phyre2 software (http://www.sbg.bio.ic.ac.uk/phyre2) [128]. 676 residues (70 %) were modelled at >90 % accuracy. D. Purified Drosophila-expressed recombinant full length ISDLP electrophoresed on SDS 10 % polyacrylamide gel and stained with Coomassie blue. E. ELISA assessment of dose-dependent binding of ISDLP to B. burgdorferi membrane protein extract-coated plates compared to rTSLPI, a tick protein that is known not to bind to B. burgdorferi.

(Figure 1D) to B. burgdorferi was confirmed by an ELISA-based binding assay. We observed a dose-dependent increase of rISDLP binding to B. burgdorferi membrane extract compared to the control tick protein rTSLPI (Figure 1E).

**Localization of native ISDLP in the tick gut using confocal microscopy**

To study the expression and protein localization of native ISDLP, we generated antibodies against rISDLP by immunizing a rabbit with rISDLP. Unfed and partially fed (24h and 48h) B. burgdorferi and uninfected I. scapularis guts were collected, fixed with PFA and probed with anti-ISDLP rabbit serum or anti-ova rabbit serum as a control. Binding of antibodies in the gut was visualized with immunofluorescence confocal microscopy. Comparing the binding of anti-rISDLP antibodies at the different time points by mean pixel intensity using ImageJ
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Figure 2. ISDLP is a membrane bound protein increasingly expressed in the gut during tick feeding. Gut nuclei and ISDLP were stained with TO-PRO-3 (blue) and anti-rISDLP rabbit serum (TRITC-red), respectively. A-C. Confocal microscopy of guts of unfed, 24 h and 48 h fed uninfected and B. burgdorferi-infected I. scapularis nymphs. Magnification x20. Guts stained with anti-ovalbumin IgG (TRITC-red) served as antibody control. D. A Z-stack (magnification x63) of a B. burgdorferi infected gut after 48 h of feeding gut. E. Mean pixel intensities of regions of interest in the TRITC channel (representing anti-rISDLP rabbit serum binding to ISDLP) of the confocal images obtained in A-C, as measured by ImageJ software. Each data point represents one region of interest. Error bars represent mean ± SEM and mean values significantly different in a two-tailed non-parametric Mann-Whitney test are indicated by an asterisk (p<0.05) or by three asterisks (p<0.0001).
Figure 3. Immunization against ISDLP does not have an effect on *B. burgdorferi* transmission to murine skin. Mice were actively immunized with rIxofin3D-PF or Ovalbumin. Eight *B. burgdorferi* N40 ticks / mouse were placed and fed until repletion. Mice were sacrificed after 14 days of *B. burgdorferi*-infection. A. Mean IgG titer in serum from animals vaccinated against rISDLP, diluted 1:10² to 1:10⁷ on ELISA coated plates with rISDLP. Error bars represent mean ± SEM. Cut-off for titer was calculated as OD value of ova-immune serum + 3 SD. B. Engorgement weights of ticks post-feeding. Each data point represents one tick. A tick was considered female when > 3.5 mg. C. qRT-PCR assessment of *B. burgdorferi* burden in tick guts and salivary glands. D-E. qPCR assessment of *B. burgdorferi* burden in murine skin at 7 days and at skin, bladder and heart at 14 days post-tick feeding. The experiment has been performed once with eight mice per group.

software showed ISDLP expression increases significantly during tick feeding (Figure 2A-C, E). Furthermore, increased binding was observed in *B. burgdorferi*-infected ticks compared to uninfected ticks. In line with our computer-based modelling of ISDLP (Figure 1), Z-stack imaging suggested that ISDLP is represented both on the cell surface and in the cytosol (Figure 2D).

**Immunization against rISDLP does not prevent *Borrelia burgdorferi* transmission**

To test whether immunization against rISDLP would prevent *B. burgdorferi* transmission we immunized eight mice against rISDLP and eight mice against ovalbumin. We achieved good IgG titer levels against rISDLP in the sera of mice that were immunized against rISDLP after immunization with Complete Freund...
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Figure 4. Silencing of ISDLP expression by RNA interference results in decreased *B. burgdorferi* burden in the salivary glands and in murine skin. Double-stranded isldp (ds isldp) or ds gfp as a control was injected through the anal pore 3 h prior to *B. burgdorferi*-infected *I. scapularis* challenge (5 ticks/mouse, 5 mice per experiment). Mice were sacrificed after 14 days. A. qRT-PCR assessment of isldp expression in the gut. B. Engorgement weights of ticks post-feeding. Each data point represents one tick. A tick was considered female when > 3.5 mg. C. *B. burgdorferi* burden in tick guts and salivary glands post-feeding. D-E. qPCR assessment of *B. burgdorferi* burden in murine skin at 7 days and at 14 days in skin, bladder and heart post-tick feeding. Error bars represent mean ± SEM and mean values that were significantly different in a two-tailed non-parametric Mann-Whitney test are indicated by an asterisk (p≤0.05). The pooled results of two independent mouse experiments are shown.

Adjuvants and two boosters with incomplete Freund adjuvant (Figure 3A). Two weeks after the second IFA boost, we placed eights ticks per mouse which were allowed to feed until repletion – ranging from three to five days. No difference was found in post-feeding tick weight compared to the ovalbumin group (Figure 3B), nor did we observe a difference in *B. burgdorferi* migration to the salivary glands or in *B. burgdorferi* transmission to the host by RT-qPCR (Figure 3C). Based on qPCR analysis, *B. burgdorferi* loads in skin tissue from the tick bite site (ears) as well as deeper tissue were similar between the rISDLP immunized animals and the ovalbumin immunized animals (Figure 3D-E).
**isdlp RNA-interference reduces *Borrelia burgdorferi* migration to the salivary glands and transmission to the murine host**

It is likely that in the active immunization experiment the function of ISDLP in relation to *B. burgdorferi* migration and transmission remained unaffected by murine anti-ISDLP antibodies present in the gut during tick feeding. To circumvent the use of antibodies to provide insight in the role of ISDLP during *B. burgdorferi* transmission, we performed another experiment in which *isdlp* expression in ticks was silenced by RNAi. We injected double stranded (ds) *isdlp* RNA or ds *gfp* RNA as a control in *B. burgdorferi*-infected *I. scapularis* nymphs before placing 4-5 ticks on C3H/H3N mice. The decrease of *isdlp* expression in the tick gut was confirmed by RT-qPCR (Figure 4A). No difference in tick engorgement weights was observed between *isdlp* and the control ticks, indicating that reduced *isdlp* expression does not influence successful tick feeding (Figure 4B). In contrast with the active immunization, significantly lower *B. burgdorferi* loads were detected in the salivary glands (Figure 4C). Furthermore, qPCR on DNA from skin tissue showed significantly lower *B. burgdorferi* numbers in mice on which *isdlp* silenced ticks fed compared to the control group (Figure 4D). However, *B. burgdorferi* loads were not significantly different at a later time point (t=14 days) or in deeper tissues such as joint and heart indicating *B. burgdorferi* growth in the murine host is not affected by the reduced expression of *isdlp* in the tick (Figure 4E).

**Discussion**

During tick feeding, while the tick gut adapts to cope with the uptake of blood, *B. burgdorferi* becomes metabolically active, replicates and binds to hypertrophic and differentiating gut cells in order to cross the gut barrier [69, 227]. The molecular mechanisms that direct the growth and migration from the tick gut and entry into salivary glands are only beginning to unfold [207]. *B. burgdorferi* has been shown to bind to host and tick proteins to facilitate its survival and dissemination [43]. To better understand vector-*B. burgdorferi* interactions we performed a yeast surface display screening to identify *I. scapularis* gut proteins that interact with *B. burgdorferi*. We identified four *B. burgdorferi*-interacting tick proteins of which one, Ixofin3D, has been previously described [175]. Here we characterize one of the other three proteins, referred to as ISDLP, and assess its role in *B. burgdorferi* transmission.
Computer-based protein structure and function predictions showed that ISDLP is similar to the conserved transmembrane protein dystroglycan. Recombinant ISDLP binds to *B. burgdorferi* and is abundantly expressed on the surface of gut epithelial cells during tick feeding, which was in accordance with previous assessment of ISDLP expression by RT-qPCR [175]. The function of ISDLP for *I. scapularis* has not yet been described. In other organisms, dystroglycan is part of the dystrophin-associated protein complex and is cleaved posttranslationally into two subunits, α and β dystroglycan, that together form the dystroglycan complex [117]. The dystroglycan complex can bind to the extracellular matrix by binding to laminin. Furthermore, studies have shown that the dystroglycan complex is involved in cell adhesion-mediated signaling, tissue remodeling and cell polarity and that β-dystroglycan is involved in MAPK signaling [45, 250]. The functional role of ISDLP on cell metabolism, cell signaling or tissue remodeling during tick feeding remains to be defined.

RNAi-mediated decrease in expression of ISDLP reduced *B. burgdorferi* transmission to the murine host. While *B. burgdorferi* load in the gut was not altered, *B. burgdorferi* load in the salivary glands was significantly reduced suggesting that ISDLP might have a role in *B. burgdorferi* migration from the gut. There have been no previous reports on *B. burgdorferi* interactions with human dystroglycan, which is identified as a receptor for a number of viruses as well as for *Mycobacterium leprae* [34, 210]. *B. burgdorferi* is known to bind to extracellular matrix proteins such as decorin and fibronectin among others [95, 227], and we speculate that human dystroglycan could be a ligand for *B. burgdorferi* in humans or other vertebrates. The specific binding partner of *B. burgdorferi* that binds ISDLP and the mechanism by which ISDLP promotes *B. burgdorferi* migration and transmission remains to be understood. Although silencing of ISDLP by RNA-interference did not impair tick feeding, it cannot be excluded that the effect on *B. burgdorferi* transmission is the result of ISDLP mediated processes in the feeding gut, e.g. altered gut tissue remodeling or a reduced barrier.

Based on our findings that ISDLP interacts with *B. burgdorferi*, and that ISDLP is expressed during tick feeding, we speculated that ISDLP could be a target to impair transmission. Tick gut antigens could be useful to target *B. burgdorferi* migration from the gut and derail transmission early in the process, i.e. “nipping it in the bud”. While RNAi mediated interference of ISDLP expression decreased *B. burgdorferi* transmission, active immunization against ISDLP did not impair *B.
*burgdorferi* migration to the salivary glands and did not reduce transmission to the murine host. There are several explanations for the discrepancy between our RNAi experiment and immunization experiment. While RNAi-mediated silencing is initiated prior to and during tick feeding, sufficient antibody uptake by the tick from the host might take more than 24-36 h coincident with the arrival of blood-meal into the tick gut [7]. Thus delayed entry of antibodies might allow *B. burgdorferi* to exploit the gut ISDLP and continue its migration from the gut. In order to prevent *B. burgdorferi* egress from the gut, anti-ISDLP antibodies have to significantly neutralize ISDLP’s interaction with *B. burgdorferi*. Immunofluorescence microscopy suggests that ISDLP is ubiquitously represented on the tick gut. Affinity of the antibody binding as well as amounts of antibody that enter the gut would determine the successful abrogation of *B. burgdorferi*-ISDLP interaction. In addition, other possibilities that impaired a protective effect of anti-ISDLP antibodies could be the inaccessibility of the protective epitope within the tick or the inability of the generated antibodies to block the interaction between ISDLP and *B. burgdorferi*. The latter possibility is supported by *in vitro* observations that binding of ISDLP to *B. burgdorferi* could not be blocked by anti-ISDLP antibodies (Supplemental Figure 1). More research to identify specific regions of ISDLP that interact with *B. burgdorferi* as well as the *B. burgdorferi* ligand that interacts with ISDLP would be informative for effectively blocking ISDLP-*B. burgdorferi* interaction through antibodies.

While several tick proteins with pharmacological functions critical for tick feeding and *B. burgdorferi* transmission have been identified [110], vaccine targeting has been confounded by the functional and structural paralogy of the tick transcriptome [214]. Recent efforts have increased our understanding of tick-*B. burgdorferi* interactions that facilitate *B. burgdorferi* migration within the tick. Vaccines targeting these *B. burgdorferi*-interacting tick proteins provide another avenue to interrupt *B. burgdorferi* transmission. It is becoming evident that *B. burgdorferi* exploits multiple tick proteins to temporally and spatially orchestrate its migration from the gut, entry into salivary glands and transmission to the host. Elucidation of *B. burgdorferi*-interacting tick proteins that facilitate the various aspects of transmission would help design an optimal combination of vaccine targets that would provide a synergistic impairment of transmission to the vertebrate host.
ISDLP facilitates *B. burgdorferi* migration from the *Ixodes* gut

**Supplemental Figure 1.** Anti-ISDLP antibodies do not block binding of rISDLP to *B. burgdorferi*. *B. burgdorferi* membrane extract purified as described in the manuscript was coated (1 µg/ml) on high binding microtiter plates (Microlon, Greiner, Germany) overnight at RT. Wells were blocked with PBS/1 % BSA at RT for 1 h and incubated with rTSLPI or rISDLP (100 pmol/ml) diluted in PBS/0.05 % Tween20/1 % BSA for 1 h. In addition, rISDLP was preincubated with 1:25 anti-ISDLP or anti-ovalbumin rabbit serum for 30 minutes before adding it to the *B. burgdorferi* extracted coated wells. Wells were washed and incubated with 1:5000 diluted mouse anti-V5 HRP IgG. Bound antibody was detecting using TMB as substrate (Thermoscientific, IL)