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Working title: a *Leptospira* LRR protein binds human cadherins

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SUMMARY

Pathogenic *Leptospira* bacteria are the causative agents of leptospirosis, a zoonotic disease affecting animals and humans worldwide. These pathogenic species have the ability to rapidly cross host tissue barriers by a yet unknown mechanism. A comparative analysis of pathogens and saprophytes revealed a higher abundance of genes encoding proteins with Leucine Rich Repeat (LRR) domains in the genomes of pathogens. In other bacterial pathogens, proteins with LRR domains have been shown to be involved in mediating host cell attachment and invasion. One protein from the pathogenic species *L. interrogans*, LIC10831, has been previously analyzed via X-ray crystallography, with findings suggesting it may be an important bacterial adhesin. Herein we show that LIC10831 elicits an antibody response in infected animals, is actively secreted by the bacterium, and binds human E-and VE-cadherins. These results provide biochemical and cellular evidence of LRR protein-mediated host-pathogen interactions and identify a new multi-receptor binding protein from this infectious *Leptospira* species.

INTRODUCTION

Leptospirosis is a bacterial zoonotic disease caused by infection with pathogenic members of the genus *Leptospira*. Climate change and continuous growth of urban populations living in slums have likely influenced the emergence of leptospirosis worldwide (Mwachui, Crump, Hartskeerl, Zinsstag, & Hattendorf, 2015), including in Europe (Pijnacker et al., 2016). Recent estimates place the worldwide burden of leptospirosis at one million severe cases per year, including cases that result in an estimated 60,000 annual deaths (Costa et al., 2015). Additional effects on local agriculture and other industries are secondary, but also severe, as pathogenic *Leptospira* are known cattle abortifacients (Ellis, 1994). The majority of human cases are in the developing world (Lau, Smythe, Craig, & Weinstein, 2010), but, even so, the disease is often under-reported (Allan et al., 2015). Despite its worldwide distribution and high case burden, *Leptospira* remains a highly understudied bacterial genus, partly due to the lack of efficient genetic tools for and fastidious *in vitro* culture requirements of pathogenic *Leptospira* species (Picardeau, 2017).

In addition to pathogenic species, other members of the *Leptospira* genus referred to as intermediates or saprophytes cause mild to no disease in humans, respectively (Chiriboga et al., 2015; Ko, Goarant, & Picardeau, 2009). Genomic comparisons between these species groups have revealed a number of important differences. One striking contrast between the pathogenic and other bacterial types is a large disparity in the number of genes coding for leucine-rich-repeat domain proteins (Fouts et al., 2016). For instance, the pathogen *Leptospira interrogans* encodes at least 20 LRR-containing proteins while the non-pathogenic *L. biflexa* genome contains only one annotated LRR-protein-encoding gene (Picardeau, 2017). The association between the number of LRR proteins and pathogenicity (**Fig. 1**) suggests that these LRR proteins may be potential virulence factors of the bacterium. Furthermore, the number of LRR-protein-encoding genes in pathogenic *Leptospira* spp. greatly exceeds the number of such genes in almost all other pathogenic bacteria (Bierne, Sabet, Personnic, & Cossart, 2007), suggesting that these proteins may be important for leptospiral pathogenesis.

LRR proteins are characterized by the presence of multiple regions of approximately 20-29 residues, each containing several leucine residues (Kobe & Deisenhofer, 1994). These motifs repeat anywhere from 3-30 times within the protein (Helft et al., 2011), and form proteins with a classical solenoid structure, similar to a horseshoe, in which the outer surface is formed by is formed by α-helices and the curved interior is composed of β-strands (Kobe & Kajava, 2001). This shape allows for a particularly large surface area to allow for substrate recognition and binding. The LRR domain is widely distributed; in fact LRR proteins can be found in all of the kingdoms of life (Helft et al., 2011; Soanes & Talbot, 2010). Despite their common structure and wide distribution, LRR proteins can also have diverse biochemical and biological functions. One of the most well studied functions of LRR proteins is as immune receptors (e.g. Toll-like and NOD-like receptors) in mammals (Ng et al., 2011) and plants (Zipfel & Felix, 2005).

Many bacteria are known to produce LRR proteins including other infectious spirochetes from the genus *Treponema* (Ikegami, Honma, Sharma, & Kuramitsu, 2004; Shevchenko et al., 1997). Notably, all members of the internalin family of proteins from *Listeria monocytogenes* contain LRR domains (Marino, Braun, Cossart, & Ghosh, 2000). Internalin A (InlA) allows bacterial uptake into intestinal epithelium cells through interaction with E-cadherin (Lecuit, Ohayon, Braun, Mengaud, & Cossart, 1997), a host protein facilitating cell-cell junctions in epithelia (van Roy & Berx, 2008). Other important pathogens also use LRR proteins to establish themselves in hosts. These bacterial LRR proteins can be effectors secreted directly into the host cell (Leung, Reisner, & Straley, 1990; Miao Edward A. et al., 2002), or act as bacterial adhesins (Bierne et al., 2007; Bober, Mörgelin, Olin, von Pawel-Rammingen, & Collin, 2011).

In *Leptospira* spp. the majority of LRR proteins lack any other identifiable functional domain (Miras et al., 2015). Furthermore, bioinformatic predictions suggest that the majority of leptospiral LRRs are extracellular proteins (Viratyosin, Ingsriswang, Pacharawongsakda, & Palittapongarnpim, 2008). In our recent work, we solved the crystal structures of four *L. interrogans* LRR proteins forming a novel subfamily of LRR proteins with the characteristic

curved solenoid structure formed by 23-residue repeating leucine rich motifs (Miras et al., 2015). This structural analysis suggested that the specific LRR protein LIC10831 has a binding pocket that may accommodate binding to human E-cadherin (hE-Cad).

In the present study, we confirmed that LIC10831 is produced along with several other LRR proteins during a mammalian infection, and determined LIC10831's cellular localization and capacity to bind E-and VE-cadherins using *in vitro* and *in situ* assays. Our results indicate that LIC10831 may be a pathogenicity-associated bacterial extracellular protein meriting further study.

RESULTS

LIC10831 is expressed during a mammalian infection

To characterize the abundance of various LRR proteins, the pathogenic *L. interrogans* serovars Manilae, Lai, and Copenhageni were subjected to protein immunoblot analyses using rabbit antisera raised against the recombinant leptospiral LRR protein rLIC10831 after growth at both 30°C–the normal incubation temperature for optimized leptospiral growth in laboratory conditions–and 37°C–a temperature used to mimic host temperatures and known to affect the expression of leptospiral genes (Lo, Cordwell, Bulach, & Adler, 2009; Xue et al., 2010). These experiments revealed several protein bands with apparent molecular masses ranging from 12 to 100kDa in the *L. interrogans* strains (**Fig. 2**). This result is unsurprising, as many of the proteins possess high sequence identity with one another. However, some inter-strain differences can be observed in the banding pattern of LRR proteins. This could be due to the different repertoire of LRR-encoding genes encoded by each genome (Xu et al., 2016), but may also be a result of varying in-culture expression profiles of LRR genes between the serovars. Differences could also be seen between the two temperatures used. Notably, one protein migrating at approximately 80kDa increased in abundance at 37°C relative to 30°C in all three species tested, while the amount of another protein of approximately 50kDa was reduced. These disparate regulatory outcomes suggest that the LRR proteins of *Leptospira* may also be diverse in function. Unfortunately the

complexity of the genome and abundance of LRR protein-encoding genes precludes the identification of individual bands as specific LRR proteins. Additionally, the secretome of *L. interrogans* remains incompletely characterized, and many of the LRR proteins may be actively secreted—a phenomenon excluding such proteins from detection via this assay.

To verify cross reactivity of the rabbit-derived LRR antibodies, recombinant LRR proteins rLIC12234, rLIC12512, rLIC10831, rLIC11098, and rLIC12759 were analyzed via protein immunoblot analyses (**Fig. S1**). Strong cross-reactivity was observed with all tested leptospiral recombinant LRR proteins, but not with the recombinant *Listeria monocytogenes* LRR-containing protein InlA or with the recombinant *Leptospira* outer membrane protein Loa22, which lacks LRR motifs.

Subsequently, we determined whether sera from guinea pigs infected with *L. interrogans* recognized our recombinant LRR proteins to indirectly assess protein production during an animal infection. Several LRR proteins showed very low recognition by antibodies in infected guinea pig serum, but recombinant LIC10831 was strongly recognized by both IgG and IgM (**Fig. 3**). This suggests that the LIC10831 protein is not only immunoreactive, but also present during a host infection. rLIC12234 was also recognized by IgG and IgM guinea pig serum—although not as strongly as rLIC10831—as well as rLIC12759, which additionally showed mild cross-reactivity with uninfected guinea pig serum. Overall, these results suggested a potential role for LIC10831 during a host infection.

Extracellular localization of LIC10831

Bioinformatic analyses of LRR protein sequences predicted extracellular localization for the majority of these proteins (Miras et al., 2015) and some of these predictions have been confirmed experimentally in a previous study quantifying *Leptospira* exoproteins (Eshghi et al., 2015). In that study LIC10831 was characterized as enriched in the culture medium, suggesting active export. Solely examining whole cell lysates (**Fig. 2**) did not allow us to conclusively state whether LIC10831 was present or absent from this population due to the

promiscuity of anti-LIC10831 antibodies. To conclusively determine whether an N-terminal signal peptide targets LIC10831 to the extracellular space, we genetically engineered *L. interrogans* serovar Manilae to constitutively produce LIC10831 with a C-terminal FLAG™ peptide (Einhauer & Jungbauer, 2001) for specific detection. We also created another LIC10831 construct with the same affinity tag, but lacking the predicted N-terminal signal peptide (-SP) (**Fig. 4A**). Constitutive synthesis of LIC10831 with the native signal peptide resulted in a band that migrated at approximately 40kDa (the expected molecular weight of rLIC10831 monomer) in the culture supernatant but not in the cell pellet samples, while generation of LIC10831 lacking a signal peptide resulted in a band migrating at approximately 40kDa in cell pellet samples, but not in the culture supernatant (**Fig. 4B**). Antiserum against a previously identified extracellular protein LβP52 (Eshghi et al., 2015) was used as a positive control. As expected, this control protein was secreted into the extracellular environment (**Fig. 4B**). In contrast, we were unable to detect the abundant periplasmic flagella protein FlaA1 in the culture supernatant, further increasing our confidence that viable *Leptospira* actively target LIC10831 to the extracellular space via an N-terminal signal peptide and this localization was not simply a byproduct of bacterial lysis or membrane perturbation.

Leptospira LRR protein LIC10831 binds human E-cadherin

Our previous study on the structure of several leptospiral LRR proteins suggested a similarity in structure and binding pocket of LIC10831 to InlA from *Listeria monocytogenes* (Miras et al., 2015), a well-studied human epithelial cadherin (hE-Cad)-binding protein (Bierne et al., 2007; Wollert, Heinz, & Schubert, 2007). Due to this similarity, we decided to investigate whether LIC10831 is also capable of interacting with this host protein. Surface plasmon resonance (SPR) was used to quantify the binding between rLIC10831 and rhE-Cad (**Fig. 5A**). Our results indicated that rLIC10831 did, indeed, bind rhE-Cad in a dose-

dependent manner. The K_D that we determined for the interaction was 2.3 ± 0.3μM, which is lower than that of wild-type InlA—which has a K_D of approximately 8μM for the same receptor (Wollert, Pasche, et al., 2007).

Display of hE-Cad by CHO cells enhances rLic10831 binding

Chinese hamster ovary (CHO) cells do not produce endogenous cadherins, and are, therefore, useful in the study of specific cadherin functions (Guo, Johnson, Randolph, & Pierce, 2009; Kovacs, Goodwin, Ali, Paterson, & Yap, 2002). To verify the capacity of hE-Cad binding by LIC10831 *in situ*, we transfected CHO-K1 cells with pCDNA3 vector encoding hE-Cad. Cells transfected with hE-Cad-pcDNA3 produce hE-Cad, which was localized to the cell surface—as determined by a protease protection assay, but the protein was absent from the empty vector-transfected controls (**Fig. S2**). Using immunofluorescence microscopy, we were able to observe that CHO cells producing hE-Cad displayed increased His-rLIC10831 binding when compared to cells transfected with an empty pCDNA3.1 vector as a control (**Fig. 5B**). When taken in concert with the SPR data, these results suggested that soluble His-rLIC10831 does, in fact, recognize hE-Cad.

LIC10831 also binds human VE-cadherin

Interestingly, cell based assays using HMEC-1 cells, a human dermal microvascular endothelial cell line (Ades et al., 1992) also showed a specific binding by His-tagged recombinant LIC10831 at cell-cell junctions (**Fig. 6**). Endothelial cells do not produce E-Cad, but instead produce neural and vascular endothelial cadherins (N- & VE-Cad) (Navarro, Ruco, & Dejana, 1998). VE-Cad is an adherens junction protein extracellularly localized between cells while N-Cad is normally distributed evenly across the cell surface (Navarro et al., 1998), suggesting a preference for hVE-Cad by the leptospiral protein. Although LIC10831 immunostaining overlapped with hVE-cadherin staining at cell junctions, the

leptospiral protein did not exclusively co-localize with hVE-cadherin. This could be due to obstruction of some hVE-Cad antibody sites by His-rLIC10831, or it could be a result of His-rLIC10831 interacting with other binding partners (**Fig. S3**). A control protein, His-rLIC12234, which did not interact with cadherins, but did bind other host factors *in vitro* (**Fig. S3**), did not localize to cell junctions (**Fig. 6**).

To support the hypothesis that hVE-Cad is a substrate of LIC10831, we sought to verify and quantify the binding of rLIC10831 to hVE-Cad *in vitro*, as with hE-Cad. Using SPR as previously, we were able to confirm the binding of rLIC10831 to rhVE-Cad (**Fig. 5A**). Furthermore, the K_D of rLIC10831/rhVE-Cad was determined as 5.8 ± 0.6μM (approximately twofold higher than that of rLIC10831/rhE-Cad). To investigate the veracity of this binding, a modified ELISA was performed to determine if E-and VE-cadherin would compete for binding to LIC10831 (**Fig. S4**). Addition of rhVE-Cad to the solution of rLIC10831 significantly reduced binding to immobilized rhE-Cad (**Fig. 7**), further demonstrating a specificity of binding for both host receptors.

LIC10831 uses Zn to dimerize and enhance binding to hE-Cad

Structural characterization of rLIC10831 identified an interaction of the zinc cation with rLIC10831 (Miras et al., 2015), prompting us to hypothesize that zinc might alter the binding capacity of LIC10831 for hE-Cad. In SPR experiments, inclusion of zinc increased the R_{max} in a concentration-dependent manner (**Fig. 8A**) and reduced the K_D of rLIC10831 binding to rhE-Cad approximately 3 fold to 0.8μM at the highest Zn concentration tested (500μM). This is a 10 fold lower K_D for human E-cadherin when compared to that reported for *Listeria monocytogenes* InlA (8μM) (Wollert, Heinz, et al., 2007).

The mechanism by which this increased affinity is achieved was not readily apparent. However, the fact that the R_{max} signal at high Zn^{2+} concentrations was two times higher than in the absence of Zn^{2+} prompted us to hypothesize that LIC10831 might undergo a monomer to dimer transition in the presence of Zn ions. We investigated this hypothesis by analytical ultracentrifugation, a technique for determining a molecule's size and shape in solution (Cole,

Lary, Moody, & Laue, 2008). Comparing rLIC10831 in buffer with or without supplemented Zn^{2+} revealed a clear shift of the protein to a dimeric form in the presence of this cation (**Fig. 8B**). LIC10831 indeed undergoes a conformational change from a monomeric form, with a sedimentation coefficient of 2.8S and a frictional ratio (f/f0) of 1.3, to a dimeric form with a sedimentation coefficient of 3.9S and an f/f0 of 1.4.

DISCUSSION

While previous works have identified E-cadherin as a potential ligand of whole *Leptospira* (K. Evangelista, Franco, Schwab, & Coburn, 2014; K. V. Evangelista et al., 2014), this study is the first that identifies a specific leptospiral protein capable of binding this specific human host receptor. Furthermore, we showed that LIC10831-E-Cad interactions, while not particularly strong, occur with a K_D in the same order of magnitude as the well-known adhesin InlA, from *L. monocytogenes* (Lecuit et al., 1999; Wollert, Pasche, et al., 2007). The strength of this adhesion might also be highly dependent on the extracellular milieu during a leptospiral infection, and various compounds might impact the affinity of LIC10831 for its cadherin receptor. Through the use of analytical ultracentrifugation, we were able to definitively identify zinc as a facilitator of LIC10831 homo-dimerization. This dimerization strengthens the affinity of LIC10831 for hE-Cad, most probably by stabilizing the LIC10831-cadherin interaction. Dimerization is not a phenomenon unique to this LRR protein, notably, the well-studied Toll-like receptor family of LRR proteins are known to typically form dimers upon ligand interaction (Botos, Segal, & Davies, 2011).

Epithelial- and vascular endothelial cadherins are specific to separate types of mammalian cells (Brasch, Harrison, Honig, & Shapiro, 2012; Navarro et al., 1998; Nollet, Kools, & van Roy, 2000; Prozialeck, Lamar, & Appelt, 2004). Regardless, VE-Cad is another target of the same protein, promoting the potential for LIC10831 to be useful during multiple loci of the leptospiral mammalian infection. While the relative affinities of LIC10831 for these two ligands are slightly different, they are both within the same order of magnitude. To further support the *in situ* function of LIC10831 CHO-K1 cells expressing hE-Cad were bound by

rLIC10831 more strongly than empty vector-transfected control CHO-K1 cells. Additionally, His-rLIC10831, but not His-rLIC12234, localized to HMEC-1 cell junctions, even though both leptospiral proteins share some substrates outside of cadherins. Surprisingly, E-and VE-Cad are remarkably different in their primary structures (Nollet et al., 2000). Even so, incubation of rLIC10831 with soluble rhVE-Cad prevented the binding to rhE-Cadherin coated wells. Whether LIC10831 uses the same binding site to interact with both cadherins or whether there exist several potential binding sites within LIC10831 for host receptors remains an open question meriting future investigation. However, the inhibition of E-Cad binding by VE-Cad blocking suggests that the host proteins are at least sterically competitive.

While LRR-protein-encoding genes are overrepresented in the sequenced pathogenic strains of *Leptospira* when compared to the intermediates and saprophytes (Picardeau, 2017), this is the first study to identify an *in vitro* function for a member of this protein family in *Leptospira*. Numerous studies to date reveal that multiple members of the spirochete family produce apparently redundant adhesins (Brissette & Gaultney, 2014; Visser & Ellen, 2011). This is true as well for *Leptospira spp*. (Fernandes et al., 2016; Murray, 2015; Picardeau, 2017). However, even with their basic similarities in structure—each of the crystalized leptospiral LRR proteins forms the classic solenoid structure (Miras et al., 2015)—there may also be clearly defined roles for the different members of the *Leptospira* LRR protein family. Notably, only one of our tested LRR proteins was able to interact with hE-Cad, suggesting specificity by sequence variations in the binding pocket. Again, this phenomenon is typical of many known LRR proteins (Botos et al., 2011; Ng et al., 2011).

The cross-reactivity of IgG purified anti-LIC10831 to the other leptospiral LRR proteins and the lack of InlA recognition suggested a homology in antigenic regions between the specific leptospiral LRR proteins tested. While the rabbit-derived IgG (purified) anti-LRR antibodies appeared to cross-react with multiple LRR proteins when blotted against the available recombinant proteins, the antibodies of Guinea pig origin appeared to show more specificity for the targeted recombinant proteins, especially when comparing IgM reactivity, which displayed the highest reactivity with rLIC10831. The guinea pig combined IgG/IgM

response was stronger against LIC10831 than against any of the other recombinant proteins assessed, suggesting some sequence specificity for rLIC10831, indicative of *in vivo* expression of the protein during active infection. However, a LIC10831 deletion mutant should confirm these results (i.e. sera from guinea pigs infected with LIC10831-deficient spirochetes should not contain antibodies reactive against rLIC10831). Additionally, whether these antibodies produced during an infection are able to inhibit the activity of the LRR proteins may also be an interesting avenue for exploration in future studies.

What remains unexplored is the *in-vivo* function of LIC10831 as well as other LRR proteins in *Leptospira*. While it is apparent that the protein is produced by the spirochete during a mammalian infection, as evidenced by the aforementioned production of anti-LIC10831 antibodies during a guinea pig model of leptospirosis, the exact timing of the LRR protein production remains unclear. While the spirochete does exist in two distinct ecosystems (animal hosts vs. an external aqueous environment) (Ko et al., 2009), it would be unwise to assume that the control of transcription is an on-or-off phenomenon in the bacterium. *Leptospira* might likely tailor the expression of some genes for use in highly specific host microenvironments, as is well characterized in many other pathogens (Hautefort & Hinton, 2000). This process would most likely be controlled by the spirochete's large complement of two-component regulatory systems, which, again, remain largely uncharacterized (Fouts et al., 2016).

One mildly surprising finding is the active secretion of LIC10831 by *L. interrogans*. Other bacteria with E-Cad-binding proteins will often use the host receptor as a means to facilitate bacterial cell binding or invasion, necessitating the placement of the bacterial protein in the membrane (Anderton et al., 2007; Bierne et al., 2007; Rubinstein et al., 2013). In *Leptospira*, the LIC10831 protein is found soluble in the extracellular milieu, complicating the prediction of its function. It may be that the protein is secreted, but subsequently re-bound by another bacterial protein or proteins upon recognition of its ligand target. The functionality of a two-step binding scheme like this is unknown, but it may allow for adhesins that are immunoreactive, such as LIC10831, to remain separate from the bacterial cell,

preventing opsonisation. To test whether LIC10831 can enhance whole leptospiral binding, we conducted a preliminary experiment, but *L. interrogans* L495 did not more efficiently bind rLIC10831-treated CHO-hE-Cad than cells exposed to the control rLIC12234 (data not shown). This lack of effect could be due to experimental conditions, and we still cannot rule out the hypothesis. More likely, however, is that LIC10831 serves to inhibit or otherwise alter the function of E- or VE-Cad as a means of advancing bacterial goals independent of whole-cell adhesion. Indeed, antibodies against VE-Cadherin have been known to induce vascular permeability in mice, potentially to the point of hemorrhage (Corada et al., 1999), a phenomenon also observed during leptospirosis (Plank & Dean, 2000). Furthermore, pathogenic, but not saprophytic, *Leptospira* have been shown to interrupt cell-to-cell junctions *in vitro* (Sato & Coburn, 2017). Enhanced vascular permeability facilitated by LIC10831 may allow the bacterium to escape host blood vessels to take up residence in preferred host tissues (Barnett et al., 1999; Ozuru et al., 2017).

Ultimately, a LIC10831 knockout strain of *L. interrogans* would be ideal for determining the role of the protein in pathogenesis, however, direct study of virulence factors in *L. interrogans* can be challenging due to limited genetic manipulation methodologies. Our group has previously developed a transposase system to allow for untargeted mutagenesis (Murray et al., 2009) which has been used to generate a library of genetically modified *Leptospira* strains, including clones with insertions in several LRR protein-encoding genes. However, a *lic10831*-disrupted clone has not been produced to date (**Table S1**), limiting further characterization of this gene product as a leptospiral virulence factor and verification of the antibody response from guinea pigs as LRR antigen-specific.

A potential avenue for elucidation of LIC10831 function is the use of transcriptional fusions to the LIC10831 promoter (Buckley et al., 2015). By infecting animals with *L. interrogans* $P_{lic10831}$-luciferase (Ratet et al., 2014), we can track the over-time expression of the gene in an animal model and determine at which time points or tissues the promoter is most active. Ours and previous labs' results indicate that the expression of several LRR protein genes is at least partially controlled by temperature (Lo et al., 2009); however, the

problem remains that the bacterial culture medium remains a poor substitute for the bacterium's *in-vivo* growth conditions, even when the temperature of incubation is altered. Therefore, more tailored approaches such as the aforementioned transcriptional fusion can help clarify the complex regulon of *Leptospira*.

In summary, we have identified a new protein of interest from a ubiquitous protein family in pathogenic *Leptospira*. This is also the first known hE-cadherin-binding protein of the bacterium, a phenomenon that is enhanced by zinc cation *in vitro* and can occur in intact mammalian cells in culture. The expression and the secretion of LIC10831 during the host infection, suggest that this LRR protein may be effectively used as an antigen for the development of potential vaccine candidates and diagnostic tools that remain underdeveloped for this neglected tropical disease. Lastly, future studies detailing the function of LIC10831 and other members of the LRR protein family in *Leptospira* may reveal insights into the pathogenic mechanisms and disease processes of this enigmatic spirochete.

MATERIALS AND METHODS

Strains, cultures, and proteins

The saprophyte *L. biflexa* serovar Patoc strain Patoc 1 and the pathogens *L. interrogans* serovar Copenhageni strain Fiocruz L1-130, *L. interrogans* serovar Lai strain 56601, and *L. interrogans* serovar Manilae strain L495 were used in this study. *Leptospira* strains were grown at 30°C in EMJH (Ellinghausen & Mccullough, 1965; Johnson & Harris, 1967) medium. Spectinomycin and kanamycin, when required, were added to the cultures at a final concentration of 50µg/ml and 30µg/ml, respectively.

The FLAG^{TM}-tagged LIC10831 coding sequence with or without the signal peptide sequence under the control of a strong promoter were synthesized de novo (Geneart, ThermoFisher). Genes were then digested with SacI and XbaI, purified, and inserted between the corresponding restriction sites into pMaOri (Pappas, Benaroudj, & Picardeau, 2015). Plasmid constructs were propagated in *Escherichia coli* π1, and introduced into *L. interrogans* serovar Manilae strain L495 by conjugation with *E. coli* β2163 as described

previously (Picardeau, 2008). The transformants were plated onto EMJH plates containing 40µg/ml of spectinomycin and incubated at 30°C for 30 days.

Recombinant *Leptospira* LRR proteins were expressed and purified as previously described (Miras et al., 2015). Proteins used for *in vitro* binding assays and antibody production were further treated by TEV protease to remove the histidine tag as described earlier (Miras et al., 2015), but proteins used for immunofluorescence were used with the His tag still attached to allow for ease of detection. When referred to in the text, proteins preceded by a His-prefix were used without removal of the tag. Polyclonal rabbit antiserum was prepared against *L. interrogans* rLIC12234 (amino acids 2-167) and rLIC10831 (amino acids 30-377) proteins by Proteogenix (Oberhausbergen, France).

Animal experiments and collection of animal sera

Leptospira positive guinea pig sera were obtained as previously described (Eshghi et al., 2015). Serum was collected pre and 14 days post-infection (two animals) for use in immunoblot experiments. Protocols for animal experiments conformed to the guidelines of the Animal Care and Use Committees of the Institut Pasteur (Comité d'éthique d'expérimentation animale CETEA # 2016-0019), agreed by the French Ministry of Agriculture.

Preparation of culture supernatants

Culture supernatants were prepared as previously described (Eshghi et al., 2015). Briefly, strains were cultured to mid exponential phase and pelleted by centrifugation at 3,200 x *g* for 15 min and washed with 20 ml albumin free-0.01%-Tween 80 EMJH. Centrifugation and washing were repeated twice. The pellets were resuspended to OD_{420nm} of 0.150 in albumin free-0.01%-Tween 80 EMJH in a total volume of 90 ml for each strain and incubated overnight at 30°C. After overnight incubation, OD_{420nm} was measured and bacterial motility was ensured by dark field microscopy. *Leptospira* were then pelleted via centrifugation at 3,200*g* for 15 min. The pellets and culture supernatants were separated and the pellets were

resuspended in 250µl lysing buffer (2% SDS-1x protease inhibitor cocktail), sonicated, and the OD_{280nm} was measured to estimate total protein concentration. Lysates were then mixed 1:1 with Laemmli sample buffer (Bio-Rad) containing 1% 2-Mercaptoethanol and stored at room temperature until use. The 90 ml supernatants were applied to separate 15 ml Amicon Ultra Centrifugal Filters Ultracel-100K (Millipore, Molsheim Cedex, France) and centrifuged at 5,000 x *g* until all 90 ml from each culture passed through the filter. Each flow through was then applied to 15 ml Amicon Ultra Centrifugal Filters Ultracel-3k (Millipore) and concentrated down to a volume of 250µl. The concentrated samples were further concentrated to a volume of 20µl using 0.5 ml Amicon Ultra Centrifugal Filters Ultracel-3k (Millipore) and protein concentrations were estimated at OD_{280nm}. Samples consisting of 15µg of protein from the cell pellet, and one quarter of the final volume of the respective supernatant samples were mixed 1:1 with Laemmli sample buffer (Bio-Rad) containing 1% 2-Mercaptoethanol and were separated via 12% SDS-PAGE electrophoresis, and transferred to a PVDF membrane for blotting. Immunoblot experiments were performed using anti-FLAG™ monoclonal antibody from mouse, LβP52 and FlaA2 rabbit antiserum with dilutions of 1:1,000, 1:500 and 1:2,000, respectively. Detection of mouse or rabbit IgG was then performed in each case using the appropriate secondary anti-IgG HRP conjugate antibody (Sigma). Blotting was performed using SuperSignal West Pico substrate (Pierce), and developed on a C-DiGit scanner with accompanying Image Studio Version 5 software (LI-COR Biosciences).

Antibody response to LRRs as determined by immunoblot

For immunoblot experiments, either 50-100ng of each recombinant protein or 20µg of total *Leptospira* protein were separated by electrophoresis in 12% SDS-polyacrylamide gels. The amount of recombinant protein loaded on SDS-PAGE was normalized by densitometry of protein bands in a Coomassie stained SDS-polyacrylamide gel. Total and relative protein concentrations in crude *Leptospira* lysates were estimated using absorbance at 280nm. Three different combinations of immunoblots were conducted: 1) leptospiral recombinant protein specific rabbit antiserum with whole cell extracts, 2) recombinant proteins, or 3) whole

leptospiral-specific guinea pig antisera with recombinant proteins. Immunoblots were performed as previously described (Eshghi et al., 2012). Rabbit antisera against recombinant LIC10831, LIC12234, and FlaA2 were used at a dilution of 1:2,000. Pooled guinea pig sera were used at a dilution factor of 50. Goat polyclonal secondary antibody to rabbit IgG-whole molecule (HRP) (Sigma) was used at a dilution of 1:100,000. Goat polyclonal secondary antibody to guinea pig IgG-Fc (HRP) (Abcam) and IgM-Fc (HRP) (Acris) were used at a dilution of 1:20,000. Detection was performed as detailed above.

Temperature shift experiments

For temperature shift experiments, *Leptospira* strains were grown to OD_{420nm} of ~0.2 in EMJH at 30°C. Cultures were diluted to OD_{420nm} of ~0.04, corresponding to approximately 8 x 10^7 *Leptospira* per ml in 30 ml total in duplicate for each strain. One set of cultures was incubated at 30°C and the second set at 37°C overnight. The cells were pelleted via centrifugation at 3,200 x *g* and washed with 20 ml 1x PBS-5mM $MgCl_2$. Washing and centrifugation was repeated a total of three times. Washed *Leptospira* pellets were then resuspended in 250µl lysis buffer (2% SDS-1x protease inhibitor cocktail (Roche)) and lysed by sonication. Protein concentration was estimated at OD_{280nm} and samples were mixed with 250µl of Laemmeli sample buffer (Bio-Rad) containing 1% 2-Mercaptoethanol. For SDS-PAGE separation and immunoblot analyses 10µg and 15µg of total protein was loaded per lane for temperature shift samples and osmotic shift samples, respectively. Immunoblots were performed as previously described (Eshghi et al., 2012; King et al., 2013), with the following antiserum dilutions: LIC12234, stress response protein GroEL and flagella filament protein FlaA2 rabbit antisera at 1:2,000, 1:8,000 and 1:2,000 dilutions, respectively. Detection was performed as detailed above.

Construction and microscopy of CHO-K1 cell strains

CHO-K1 cells (Sigma) were transfected with either FLAG-HA-pcDNA3.1 or hE-Cad-pcDNA3 vectors using the DNA-in CHO transfection reagent according to manufacturer's instructions (MTI-GlobalStem, USA). FLAG-HA-pcDNA3.1- was a gift from Adam Antebi

(Addgene plasmid # 52535) (Horn et al., 2014), and hE-cadherin-pcDNA3 was a gift from Barry Gumbiner (Addgene plasmid # 45769) (Gottardi, Wong, & Gumbiner, 2001). Briefly, plasmid DNA for each vector was linearized by treatment with PvuI (Thermo Scientific), then DNA was purified using the PureLink™ PCR purification kit (Thermo Scientific). DNA concentration was estimated using absorbance at 260nm, and 0, 0.125 0.25, 0.5 0.75, and 1µg DNA from hE-Cad-pcDNA3 or the empty vector was added to CHO cells in separate wells of a 24-well in OPTI-MEM medium with 4% FBS. As a control, untreated cells were left in other wells. After 48 hours, cells were supplemented with 500µg/mL G418 solution to select for successful integrative transformants. After one week, cells were split into individual wells to screen for hE-Cad-producing transformants.

For the confirmation of surface localization of hE-Cad in transfected cells, cells were gently mechanically removed from the growing surface of a T-75 flask (Corning) and placed in conical tubes. Cells were pelleted at 125 x g for 5 min, then the medium was removed and cells resuspended in either serum-free medium or 0.5% Trypsin-EDTA for 5 min with gentle agitation. Cells were again pelleted and resuspended in 1X Laemmli buffer, boiled for 5 min, and run on a 12% SDS page gel. The proteins were then blotted onto a PVDF membrane and blotted with anti-β-actin (Life, #AM4302) or anti-pan-cadherin (Thermo, #PA5-16766) antibodies. Detection was performed as above.

For microscopy, both CHO-K1 strains were seeded onto Poly-L-lysine-coated coverslips held in 12-well plates overnight to allow binding. The next day, the cells were washed once with PBS, and the medium was replaced with serum-free F-12 medium supplemented with 500µM $ZnCl_2$. The cells were then treated for 2 hours with 4µM His-rLIC10831 or His-rLIC12234 at 37°C with 5% CO_2. After the incubation, cells were rinsed 3 times for 5 minutes each with the Serum-free medium, then once with Dulbecco's PBS. Cells were then fixed for 15 min with a 10% formalin solution, and subsequently rinsed 3 times with PBS to remove excess formalin. Cells were blocked with 2.5% BSA in PBS, and probed with anti-LIC10831 rabbit serum (1:500), then anti-mouse IgG Alexa Fluor 488 antibody (Thermo, #A-11029) at 1:1000. Both antibody incubations were carried out at room temperature for

one hour in PBS with 0.1% BSA and washed 3 times for 5 minutes each with the same buffer. Cells were lastly rinsed one time with PBS, and coverslips were mounted using ProLong Gold antifade reagent with DAPI (Thermo, #P36931).

Microscopy was performed with a Olympus BX53 microscope equipped with a C11440 digital camera (Hamamatsu), X-cite series 120Q excitation light source (Excelitas Technologies), and DAPI-49000/FITC-390002 filter sets (Chroma Technology Corporation). Image acquisition, analysis, and artificial color addition was performed using CellSens Dimension software (Olympus). To compare binding between cells, all exposure and image processing settings were held constant between the different conditions.

Analytical Ultracentrifugation

The sedimentation velocity experiment was performed on a ProteomeLab XL-A/XL-I (Beckman-Coulter, USA) ultracentrifuge. The rLIC10831 protein at 400µg/mL in the absence or presence of 500µM $ZnCl_2$ was centrifuged at 20°C in an AN60-Ti rotor at 42,000 rpm, and the ProteomeLab software (Beckman-Coulter, USA) was used to monitor the experiment. 400 scans were collected at 280nm with 3 min of interval time with a radial step size of 0.002cm. Profiles were analyzed using the normalized continuous (s) distribution model of the software Sedfit (Schuck, 2000). The partial specific volume of the rLIC10831 protein, the viscosity and the density of the buffer were theoretically calculated with the software Sednterp (Spin Analytical, USA).

HMEC-1 immunofluorescence microscopy

The dermal human microvascular endothelial cell line (HMEC-1) was acquired and grown as described previously (Sato & Coburn, 2017). To use HMEC-1 for protein binding tests, cells were seeded at 4.8 x 10^5/well on sterile coverslips placed in wells of 12-well plates for 2-day growth.

To examine the binding of His-tagged recombinant LIC10831 and LIC12234 proteins to HMEC-1 cells, cells were washed with PBS twice, placed in 500µl cell culture medium

supplemented with 50μM zinc sulfate. Each His-tagged recombinant protein (2μM) was added to endothelial cells for 1 h incubation at 37°C. Unbound protein was washed away once with PBS (-Ca^{2+}, -Mg^{2+}) prior to fixation with 2% *para*-formaldehyde for 15 min and then rinsed with PBS three times prior to immunofluorescence procedures. The samples were blocked with 3% BSA/PBS for 1 h and then incubated in a rabbit anti-His (H.15; Santa Cruz Biotechnology, CA) antibody (1:400) diluted in 3% BSA/PBS for 1 h. For VE-cadherin labeling, a mouse anti-cadherin 5 antibody (1:300, BD Biosciences) was used. Unbound primary antibodies were rinsed off with 3% BSA/PBS three times prior to incubation with an anti-mouse-IgG antibody conjugated with Alexa Fluor 488 and an anti-rabbit-IgG antibody conjugated with Alexa Fluor 568 (both from Molecular Probes) for detection. After 1 h incubation, the unbound secondary antibodies were rinsed with 3% BSA/PBS twice and then with PBS twice. Coverslips were mounted on glass slides using ProLong Diamond containing DAPI (Molecular Probes). The mountant was cured in the dark for 12 h or longer before sealing of the coverslips with nail polish.

Fluorescence microscopy images were acquired by a Nikon Eclipse Ti-U inverted microscope equipped with a CoolSNAP ES2 CCD camera (Photometrics) and a multifluorescent Sedat Quad ET filter set (multichromic splitter, Chroma) using the 20× Plan Apo (N.A. 0.75) or 60× Plan Apo VC (N.A. 1.40, oil immersion) objective lens (Nikon). NIS-Elements software (Nikon) was used for image acquisition, processing, and analysis.

ELISA and SPR

Host factors tested included recombinant human E-Cad (Gln23 - Pro621 fused to human IgG Fc; accession # AAI41839; ACROBiosystems, London, UK), recombinant human VE-cad (Asp48-Gln593 fused to human IgG Fc; accession # P33151; R&D Systems) laminin from human placenta (Sigma), fibronectin from human plasma (Sigma), superfibronectin (Sigma), BSA (Sigma) and dextran sulfate from *Leuconostoc mesenteroids* (Sigma).

To determine whether LRR proteins bind host proteins, we used 1μM of each recombinant *Leptospira* LRR protein and performed ELISA experiments as previously

described (K. Evangelista et al., 2014), with the following modifications. Host factors were added to 96 well microplates in duplicate at a concentration range of 0.2μM to 2pM using 10-fold serial dilutions. *Leptospira* LRR proteins and host factors were all reconstituted in HBSC buffer (25mM HEPES pH 7.6, 150mM NaCl, 1mM $MgCl_2$, 1mM $ZnCl_2$, and 0.25mM $CaCl_2$) and blocking was performed with 2% (w/v) BSA in PBS. Upon addition of host factors to the 96 well plates, the plates were incubated overnight at 4˚C and all other subsequent incubations were performed at 37˚C and all washes were performed four times using 300μl PBS per well. *Leptospira* LRR proteins were incubated with host factors for 3 h followed by washing. Rabbit antiserum against LRR proteins was used at 1:2,000 in PBS/0.05% tween 20 for a 2-hour incubation period, followed by washing. Anti-rabbit HRP conjugate antibodies (Sigma) were used at 1:5,000 in PBS for a 45 min incubation period, followed by washing. The ABTS substrate (Roche) was reconstituted according to the manufacturer's instructions and 100μl was added per well. Optical densities were measured at 415nm, every 15 min for 1 h using a Bio-Rad model 680 spectrophotometer. For the inhibition assay, steps were performed as above, except wells were coated with 1μg rhE-Cad, and rhVE-Cad or human IgG Fc were added simultaneously with rLIC10831 to asses binding inhibition. All other steps are as above. See **Figure S4** for a graphical representation of the experimental layout for the inhibition assay.

Surface plasmon resonance (SPR) experiments were performed as previously described (Merkulova-Rainon, England, Ding, Demerens, & Tobelem, 2003) with the following modifications. The binding studies were performed on a Biacore T200 instrument (GE Healthcare, Velizy-Villacoublay, France) equilibrated at 25°C in running buffer (25mM HEPES, 150mM NaCl, pH 7.4, 1 mg/ml BSA). The flowcells of a CM5 sensor chip (GE healthcare, #100399) were functionalized with anti-IgG Fc capture antibody according to kit directions (GE healthcare, #BR100839), after which IgG Fc, rhE-Cad, or rhVE-Cad (20μg/mL each) were added to individual flowcells. rLIC10831 in twofold dilutions of concentrations ranging from 5μM to 0 was then applied at 30μl/min over the channels in a random order. Between binding cycles, the coated surfaces were regenerated by two injections of 3 M

$MgCl_2$. Binding curves were then obtained using the Biacore T200 analysis software, by subtracting the blank values obtained by running the rLIC10831 over the IgG Fc tag control alone. The concentration dependences of steady-state responses were then analyzed with the non-linear least squares algorithm, and K_D are reported as the calculated values ± standard error (SEM). The curves of best fit were calculated assuming a 1:1 ratio of rLIC10831 binding both recombinant cadherins, an assumption that yielded the highest R^2 value, indicating truest fit.

For the Zn-mediated affinity change experiments, the procedure was as follows. The first flowcell of a HLC 200 m sensor chip (XanTec bioanalytics, Düsseldorf, Germany) was left empty while E-cadherin was immobilized on a second one. rLIC10831 protein in metal-free Biacore running buffer or buffer containing 250μM or 500μM zinc was injected over the immobilized rhE-cadherin at a twofold decreasing concentrations as above. Analysis steps were performed as detailed above.

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Table S1. List of LRR-encoding genes in *L. interrogans* serovar Manilae and the available transposon mutants

CDS [a]	PSORTB [b]	other domain [b]	MW (kDa) [b]	mutants [c]	infection experiments [d]
LIMLP_02930	extracellular	WGR domain	185	m1722	virulent
LIMLP_02940	extracellular		30	m2175	virulent
LIMLP_03670	extracellular		49		
LIMLP_04100	unknown		26		
LIMLP_04110	unknown	WGR domain	76		
LIMLP_05075	extracellular	WGR domain	78	m1866	virulent
LIMLP_05305	extracellular		47		
LIMLP_05310	extracellular		49		
LIMLP_05715	extracellular		33		
LIMLP_07325	extracellular		54		
LIMLP_07330	extracellular		71	m2164	unknown
LIMLP_07335	extracellular		39	m449	virulent
LIMLP_07350	extracellular		31		
LIMLP_09465	cytoplasmic		17		
LIMLP_09470	unknown		12		
LIMLP_09475	unknown		9		
LIMLP_11080	extracellular		14	m1267	virulent
LIMLP_13525	extracellular		56		
LIMLP_13530‡	extracellular		44		
LIMLP_13535	extracellular		44		
LIMLP_13540	extracellular		58		
LIMLP_13545	extracellular		44		
LIMLP_13550	extracellular		39		
LIMLP_13555	extracellular		31	m476	virulent
LIMLP_18630	extracellular		25		
LIMLP_11955	extracellular		25		

[a] complete genome of *L. interrogans* serovar Manilae (Satou et al., 2015) available at http://www.genoscope.cns.fr/agc/microscope/home/index.php
[b] as defined by genoscope (http://www.genoscope.cns.fr/agc/microscope/home/index.php)
[c] library of transposon mutants in *L. interrogans* serovar Manilae (Murray et al., 2009)
[d] infection of 10^6 bacteria in groups of 4 gerbils
‡The gene corresponding to *lic10831* in *L. interrogans* serovar Manilae

FIGURE LEGENDS

Figure 1) Genes encoding LRR proteins are more highly represented in pathogenic *Leptospira*

A) Phylogenetic tree based on concatenated single copy genes present in all leptospiral genomes and in *Leptonema illini* (outgroup), rightmost horizontal bars represent the number of LRR domains identified per genome. The genus *Leptospira* currently consists of 22 species that are divided into three distinct clades: the pathogens (shaded in red) that can infect and cause disease in humans and animals, the intermediates (shaded in yellow) that have been isolated from humans and animals that may cause various mild clinical manifestations of leptospirosis, and the saprophytes (shaded in green) that do not cause disease. The most virulent species, *L. interrogans*, *L. kirschneri*, and *L. noguchi*, each encode over 15 LRR proteins, whereas only 1-2 LRR protein-encoding genes are present in each saprophytic and intermediate species. **B**) Boxplot summarizing the occurrence distribution of LRR domains in the three distinct leptospiral clades.

Figure 2) Differential LRR protein production at physiological (37˚C) and culturing (30˚C) temperatures as well as between infectious strains

Cells grown overnight at either 30°C or 37°C were harvested and used for protein immunoblot analyses. Serovars and incubation temperatures are indicated at the top and protein molecular weight markers to the left, within the figure. SDS-PAGE and immunoblot of FlaA2 were used as loading controls, and anti-GroEL as a temperature response positive control (Lo et al., 2009). Anti-LIC10831 antibodies detected different levels of LRR proteins between strains, and a strain-dependent temperature response in several of the LRR proteins.

Figure 3) Leptospira-positive guinea pig serum reacts with several LRR proteins

Guinea pig serum was collected pre- and 14-days post-infection by *L. interrogans* serovar Manilae L495. Sera from two guinea pigs were pooled and used to probe the recombinant LRR proteins listed in the top panel. As controls, we employed *L. monocytogenes* InlA (*L.m.* LRR) and the abundant *Leptospiral* virulence factor Loa22 (Ristow et al., 2007). Anti-guinea pig IgM and IgG conjugated to HRP were used to detect bound immunoglobulins. Multiple LRR proteins showed reactivity with the sera, suggesting production of these proteins during a leptospiral infection. rLIC10831, when cumulating the IgM and IgG data, appears to display the strongest reactivity.

Figure 4) An N-terminal signal peptide mediates export of LIC10831

L. interrogans serovar Manilae strains expressing LIC10831 with a signal peptide (+SP) or lacking a signal peptide (-SP) **(A)** were grown in albumin free media overnight and both cell pellets and supernatants used to determine localization of constitutively expressed constructs. **B)** Protein immunoblots comparing anti-FLAG™ tag reactivity in cell pellet samples (Cell pellet) and culture supernatants from the two strains. Antisera against the secreted protein LβP52 and the flagellar filament protein (FlaA1) were used to control for protein localization in the culture supernatant and cell pellet, respectively. This experiment was performed in duplicate, and both replicates are presented in the figure.

Figure 5) LIC10831 binds hE- and hVE-cadherins

(**A**) The binding of rLIC10831 to rhE-Cad and rhVE-Cad was assessed by surface plasmon resonance. Data displayed are normalized SPR response values over time and inserts are best curve fits of steady-state values vs. concentration as calculated by Biacore 200 analysis software. rLIC10831 quantifiably bound both host receptors, but with different K_D values, as reported in the text (±standard error). **B**) CHO-K1 cells transfected with the gene encoding hE-Cad or an empty vector (pcDNA3.1) control were assessed for their capacity to bind His-rLIC10831 via immunofluorescence microscopy. Cells incubated with 2μM rLIC10831 were fixed and stained with anti-LIC10834 antibody/Alexa Fluor 488 anti-rabbit IgG (green). DAPI

was used to localize cell nuclei. CHO-K1 cells transfected with hE-Cad displayed a higher relative fluorescent intensity than the empty-vector controls, indicating stronger rLIC10831 binding in the presence of this receptor. Neither subtype of CHO-K1 cell untreated with rLIC10831 displayed fluorescence other than that conferred by DAPI under the conditions used in this experiment.

Figure 6) LIC10831 localizes to the HMEC-1 cell surface

HMEC-1 cells were treated with either His-rLIC12234 (control, see Figure S3) or His-rLIC10831, and bound LRR proteins were detected via anti-His (Red) immunofluorescent staining and microscopy. Simultaneously, cells were strained with anti-VE-Cad antibodies (Green), and nuclei were stained with DAPI (Blue). A clear difference in the staining pattern can be observed between the two LRR proteins used, with His-rLIC10831 showing a clear preference for cell-cell junctions, and partial co-localization with anti-VE-Cad staining.

Figure 7) hVE-Cad competes with hE-Cad for LIC10831 binding

A competition assay was performed as detailed in Figure S4. Soluble rhVE-Cadherin was able to reduce the binding of rLIC10831 to immobilized rhE-Cad statistically more strongly than human IgG-Fc (used as a negative control because it is present as an affinity tag on both recombinant cadherins). The data presented are blanked against rLIC10831-treated empty wells with equal amounts of rhVE-Cad to account for non-specific binding and non-specific inhibition. Data were normalized to wells with 0 inhibitor. *: $p<0.05$ as determined by point-by-point Student's t-tests with a Holm-Sidak correction as calculated by Graphpad Prism software (Graphpad Software; La Jolla, CA). Results presented are representative of 3 independent ELISAs, all with similar results.

Figure 8) LIC10831 dimerizes and binds hE-Cad more strongly in the presence of zinc

A) The affinity between rLIC10831 and rhE-Cad was compared using SPR in the presence of increasing concentrations of $ZnCl_2$ (up to 500μM). A strengthening of the affinity of the

complex was observed, as well as an increase in the R_{max} (maximal response). To determine the effect of zinc treatment on rLIC10831, we performed analytical ultracentrifugation in the presence or absence of 500μM $ZnCl_2$ (**B**). The results indicate a strong shift towards rLIC10831 dimerization in the presence of Zn, suggesting this as the most likely cause of the observed increase in affinity and R_{max}.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1) Antisera against *L. interrogans* LRR proteins display cross reactivity with various LRR motif-containing leptospiral proteins

Recombinant *L. interrogans* LRR proteins, Loa22, and *L. monocytogenes* internalin InlA were probed with rLIC10831-specific antiserum. Similar results were obtained with the rLIC12234 specific antiserum (data not shown). Cross reactivity was also observed with the other leptospiral LRR motif containing proteins tested, but not with rLoa22 or rInlA.

Figure S2) hE-Cad is successfully produced and surface-localized in hE-Cad-pcDNA3-transfected CHO cells

A protease protection assay was performed comparing CHO-K1 cells transfected with either empty vector pcDNA3.1 (left panel) or hE-Cad-pcDNA3 (right). No band was seen at the expected size in the empty vector clone, but a lower MW-band, suggesting some cross-reactivity with our antibody, was observed. hE-Cad transfects showed a band at the expected size, which was approximately 85% degraded upon addition of 0.25% Trypsin/EDTA for 5 minutes. β-actin, conversely, showed no degradation, suggesting membrane integrity was not compromised, and that the hE-Cad was digested as a result of being surface exposed.

Figure S3) *Leptospira* LRR proteins can bind host proteins

Host factors were bound to 96 well protein-binding plates in duplicate at the indicated concentrations and subsequently used to test LRR binding via ELISA. Substrates tested were human E-cadherin (CDH1), laminin, fibronectin, superfibronectin and dextran. Bovine

serum albumin (BSA) was used as a negative control. Background optical density was measured without the addition of LRR proteins. The values displayed are background subtracted. Results of the binding assays are indicated for **A)** rLIC12234, **B)** rLIC12512, **C)** rLIC10831, **D)** rLIC11098 and **E)** rLIC12759. The rLIC12234 displayed binding to laminin and fibronectin and rLIC10831 to laminin, fibronectin and hE-Cad. No significant binding was observed for rLIC12512, rLIC11098, and rLIC12759 against any of the host proteins tested.

Figure S4) A schematic of the rhE-Cad-rhVE-Cad competition assay

The graphic depicts the scheme used to detect competition for rLIC10831 binding by rhE-Cad and rhVE-Cad. Note the presence of the human IgG Fc as an affinity tag on both of the recombinant cadherins. The resulting data are presented in the text and in Figure 7. Clip art for this figure was obtained from the somersault18:24 library of science and medical illustrations (http://www.somersault1824.com/science-illustrations/).