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(71) Applicant(s)
Valneva Austria GmbH

(72) Inventor(s)
Comstedt, Par;Lundberg, Urban;Meinke, Andreas;Hanner, Markus;Schuler, Wolfgang;Wizel, Benjamin;Reinisch, Christoph;Grohmann, Brigitte;Schlegl, Robert

(74) Agent / Attorney
Watermark Intellectual Property Pty Ltd, L 1 109 Burwood Rd, Hawthorn, VIC, 3122, AU

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(71) Applicant: **INTERCELL AUSTRIA AG** [AT/AT]; Campus Vienna Biocenter 3, A-1030 Vienna (AT).

(72) Inventors: **COMSTEDT, Pär**; Viktorgasse 18/26, A-1040 Wien (AT). **LUNDBERG, Urban**; Badgasse 6a/1/02, A-3013 Pressbaum (AT). **MEINKE, Andreas**; Piettegasse 26/1, A-3013 Pressbaum (AT). **HANNER, Markus**; Terrassengasse 12, A-3013 Pressbaum (AT). **SCHÜLER, Wolfgang**; Landstraßer Hauptstr. 112/2/26, A-1030 Wien (AT). **WIZEL, Benjamin**; 12952 Carmel Creek Road, San Diego, 92130 (US).

(74) Agent: **BÖSL, Raphael Konrad**; Isenbruck Bösl Hörschler LLP, Patentanwälte, Prinzregentenstr. 68, 81675 München (DE).

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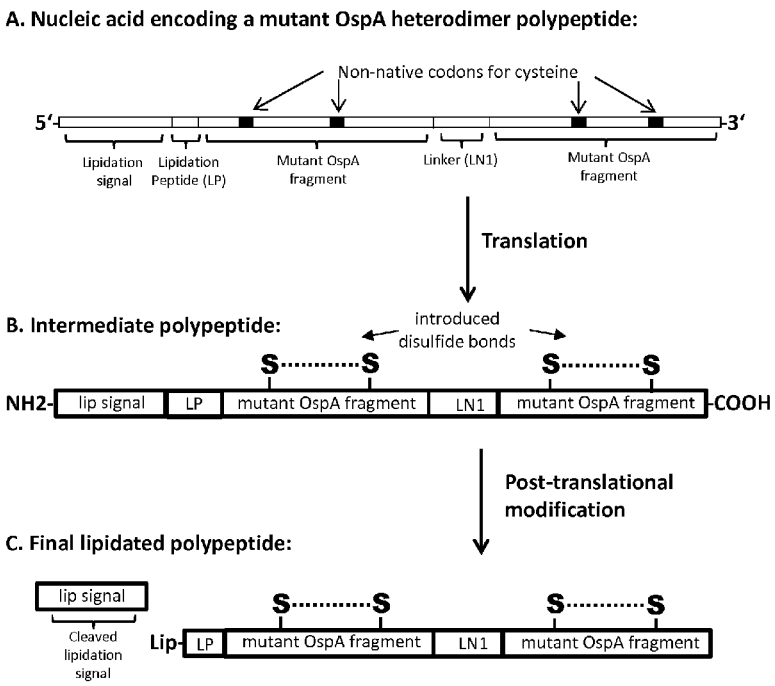
[Continued on next page]

(54) Title: MUTANT FRAGMENTS OF OspA AND METHODS AND USES RELATING THERETO

Figure 2



(57) Abstract: The present invention relates to a polypeptide comprising a mutant fragment of an outer surface protein A (OspA), a nucleic acid coding the same, a pharmaceutical composition (particularly for use as a medicament of in a method of treating or preventing a *Borrelia* infection) comprising the polypeptide and/or the nucleic acid, a method of treating or preventing a *Borrelia* infection and a method of immunizing a subject.

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MUTANT FRAGMENTS OF OspA AND METHODS AND USES RELATING THERETO

FIELD OF THE INVENTION

The present invention relates to compositions and methods for the prevention and treatment of *Borrelia* infection. Particularly, the present invention relates to a polypeptide comprising a mutant fragment of an outer surface protein A (OspA), a nucleic acid coding the same, a pharmaceutical composition (particularly for use as a medicament of in a method of treating or preventing a *Borrelia* infection) comprising the polypeptide and/or the nucleic acid, a method of treating or preventing a *Borrelia* infection and a method of immunizing a subject.

BACKGROUND OF THE INVENTION

Lyme borreliosis, or Lyme disease, is the most commonly reported tick-borne disease in Europe and North America. The disease is caused by the arthropod-borne gram-negative-like spirochete, *Borrelia burgdorferi* sensu lato (*B. burgdorferi* s.l.), and is an infection that can involve multiple organs or tissues, resulting in skin, cardiac, musculoskeletal and neurological disorders. In most countries, Lyme borreliosis is not a notifiable disease and no exact data regarding annual incident rates are available. In the United States, the causative agent is *B. burgdorferi* sensu stricto (*B. burgdorferi* s.s.) and Lyme borreliosis is localized to north-eastern, mid-Atlantic and upper north-central states. In 2010, a total of about 30,000 cases of Lyme borreliosis were reported for the US to the Centers for Disease Control and Prevention (CDC). In Europe, *B. afzelii* and *B. garinii* are the main causative agents of Lyme borreliosis, as well as *B. burgdorferi* s.s. and *B. bavariensis*, which contribute to a lesser extent depending on the geographic location. The prevalence of Lyme borreliosis varies considerably in different European countries with an overall increased prevalence from west to east. In much of Europe, the number of reported cases of Lyme borreliosis has increased since the early 1990s (*e.g.,* the Czech Republic, Estonia, Lithuania; see Lyme borreliosis in Europe, WHO report of 2006), and the geographic distribution of cases has also expanded.

Borrelia belongs to the family *Spirochaetaceae,* which is subdivided into the medically important genera *Treponema, Leptospira* and *Borrelia*. *B. burgdorferi* s.l. is a spiral-shaped, vigorously motile gram-negative bacterium, about 10-20 µm long and 0.2-0.5 µm wide, that grows under microaerophilic conditions. The spirochetal cell wall consists of a cytoplasmic membrane surrounded by peptidoglycan and several flagella and then by a loosely-associated outer membrane.

Lyme borreliosis generally occurs in stages characterized by different clinical manifestations, with remissions and exacerbations. Stage 1, early infection, consists of a localized infection of the skin,

followed within days or weeks by stage 2, disseminated infection, and months to years later by stage 3, persistent infection. However, the infection is variable; some patients have only localized infections of the skin, while others display only later manifestations of the illness, such as arthritis. Different clinical syndromes of Lyme borreliosis are also caused by infection with diverse *B. burgdorferi* s.l. species. *B. burgdorferi* s.s. more often causes joint manifestations (arthritis) and heart problems, *B. afzelii* causes mainly dermal symptoms (erythema migrans; EM and acrodermatitis chronica atrophicans; ACA), whereas *B. garinii* is implicated in most cases of neuroborreliosis.

Localized infection - The most common symptom of stage 1 of an infection is erythema migrans, which occurs in 70-80% of infected people. This skin lesion is often followed by flu-like symptoms, such as myalgia, arthralgia, headache and fever. These non-specific symptoms occur in 50% of patients with erythema migrans.

Disseminated infection - During stage 2, the bacteria move into the blood stream from the site of infection to distal tissues and organs. Neurological, cardiovascular and arthritic symptoms that occur in this stage include meningitis, cranial neuropathy and intermittent inflammatory arthritis.

Persistent infection - Stage 3 of the infection is chronic and occurs from months to years after the tick bite. The most common symptom in North America is rheumatoid arthritis, caused by an infection with *B. burgdorferi* s.s. Persistent infection of the central nervous system with *B. garinii* causes more severe neurological symptoms during stage 3, and a persistent infection of the skin with *B. afzelii* results in acrodermatitis chronica atrophicans.

In some risk groups, such as farmers, forestry workers, hikers, runners or vacationers, seroprevalence and disease incidence rates have increased, as in children under 15 years of age and adults between 39 and 59, without gender preference. This increased incidence of Lyme borreliosis is linked to changes in forest habitats as well as social factors. Environmental changes, such as forest fragmentation, have led to a sharp reduction of rodent predators such as foxes and birds of prey, which in turn has led to an increase in the mouse population, with a subsequent increase in the tick population. More recently, patchy reforestation has increased the number of deer and thus the number of ticks. Suburban sprawl and the increasing use of woodland areas for recreation such as camping and hiking has brought humans into greater contact with the larger number of tick *Borrelia* vectors. All of these factors together have contributed to a wider distribution of *Borrelia* and a higher incidence of Lyme borreliosis.

Antimicrobial agents are the principle method of treatment of *Borrelia* infection. The antibiotic used depends on the stage of the disease, symptoms, and the patient's allergies to medication. The length of the antibiotic course also depends on the stage of the disease and the severity of symptoms. Early Lyme borreliosis is typically treated with oral tetracyclines, such as doxycycline, and semi-synthetic penicillins, such as amoxicillin or penicillin V. Arthritic and neurological disorders are treated with high-dose intravenous penicillin G or ceftriaxone. Up to 30% of Lyme borreliosis patients do not display the early characteristic symptoms of infection with *Borrelia*, making diagnosis and treatment problematic. The antibiotic course can be long (up to several months) and sometimes ineffective and is thus debated in the *Borrelia* field, especially during later-stage disease. Even in the case of effective treatment of *Borrelia*, patients can be left with debilitating fatigue, pain, or neurological symptoms for years afterwards referred to as post-treatment Lyme disease syndrome. In general, the use of antibiotics can have undesirable consequences, such as the development of resistance by the target micro-organisms. Finally, antibiotic therapy may effectively cure Lyme borreliosis, but provides no protection against subsequent infections.

A monovalent serotype 1-OspA-based vaccine (LYMErix™) was approved and marketed in the USA for the prevention of Lyme disease caused by *Borrelia burgdorferi* s.s. However, heterogeneity in OspA sequences across different serotypes in Europe and elsewhere precludes efficient protection with a vaccine based on OspA from only a single serotype.

Chimeric OspA molecules comprising the proximal portion from one OspA serotype, together with the distal portion form another OspA serotype, while retaining antigenic properties of both of the parent polypeptides, may be used in the prevention and treatment of Lyme disease or borreliosis (WO2011/143617, WO2011/143623).

Currently, there is no preventative medicament for Lyme borreliosis on the market and thus there is a need in the art for the development of such a medicament that can provide effective protection against *Borrelia* that are present in the USA, Europe and elsewhere, especially for the development of a medicament that can provide effective protection against several *Borrelia* serotypes simultaneously..

SUMMARY OF THE INVENTION

The present invention relates to a polypeptide comprising a mutant fragment of *Borrelia* outer surface protein A (OspA), a nucleic acid encoding the same, a vector which comprises such nucleic acid molecule, and a host cell comprising such vector. Furthermore, the invention provides a process for producing such polypeptide and a process for producing a cell which expresses such polypeptide. Moreover, the present invention provides antibodies specifically binding to such polypeptide, a

2013285364 12 May 2017

hybridoma cell producing such antibodies, methods for producing such antibodies, a pharmaceutical composition comprising such polypeptide, nucleic acid molecule, vector or antibody, the use of such polypeptide, nucleic acid molecule, vector or antibody for the preparation of a medicament or a pharmaceutical composition (particularly for use as a vaccine or in a method of treating or preventing a *Borrelia* infection), methods for diagnosing an infection and methods for treating or preventing a *Borrelia* infection and methods of immunizing a subject.

Efforts to develop a subunit vaccine for prevention of Lyme borreliosis have been focused in large part on the use of borrelial outer surface protein A (OspA) as an antigen. The OspA protein is expressed by *Borrelia* only when it is in the gut of the tick vector. Thus, OspA antibodies produced by vaccination do not fight infection in the body, but rather enter the gut of the tick when it takes a blood meal. There, the antibodies neutralise the spirochetes and block the migration of bacteria from the midgut to the salivary glands of the tick, the route through which *Borrelia* enters the vertebrate host. Thus, OspA-specific antibodies prevent the transmission of *Borrelia* from the tick vector to the human host.

The lipidated form of OspA from *B. burgdorferi* s.s., strain ZS7, together with aluminium hydroxide was commercially developed as a vaccine against *Borrelia* (LYMErix™) by SmithKline Beecham, now GlaxoSmithKline (GSK) for the US market. Three doses of LYMErix™ over a period of one year were needed for optimal protection. After the first two doses, vaccine efficacy against Lyme borreliosis was 49%, and after the third dose 76%. However, shortly after LYMErix™ was commercially available, it was withdrawn from the market in 2002. Reasons cited were matters of practical application of the vaccine, for example the need for booster injections every year or every other year, as well as the relatively high cost of this preventive approach compared with antibiotic treatment of early infection. In addition, there was a concern that LYMErix™ could trigger autoimmune reactions in a subgroup of the population due to sequence homology with a human protein, though this was never proven. In addition, cross-protection against other clinically important *Borrelia* species was not provided by this vaccine.

Accordingly, in one embodiment, the present invention provides an improved vaccine for the prevention of Lyme borreliosis. Preferably, the vaccine is easily produced while being protective, safe and more effective than existing therapies and/or provides protection against more than one *Borrelia* species.

The problem underlying the present invention is solved by a polypeptide comprising a mutant fragment of an outer surface protein A (OspA), wherein the mutant fragment consists of a C-

2013285364 12 May 2017

terminal domain of an OspA protein of *Borrelia* and differs from the corresponding wild-type fragment at least by the introduction of at least one disulfide bond.

Surprisingly, it was found that the introduction of at least one disulfide bond in a mutant fragment increases the protective capacity of the polypeptide comprising the mutant OspA fragment relative to a polypeptide comprising the wild-type OspA fragment, as shown in an *in vivo* model of infection. As shown in the Examples, the introduction of at least one disulfide bond into the *B. afzelii* OspA C-terminal fragment increased its protective capacity relative to the wild-type OspA fragment without a disulfide bond. Tables 2 and 3 provide data demonstrating the protective capacity of mutant fragments with an introduced disulfide bond ("S2D1-5") as compared to the wild-type OspA fragment ("S2D0"), as fewer animals were infected after immunization with mutant OspA fragments in comparison to wild-type OspA fragments. Some of the mutant OspA fragments tested provided protection comparable to that conveyed by the positive control antigen, the non-lipidated full-length OspA protein.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a polypeptide comprising a mutant fragment of an outer surface protein A (OspA), wherein the mutant fragment consists of a C-terminal domain of an OspA of *Borrelia* and differs from the corresponding wild-type fragment at least by the introduction of at least one disulfide bond.

Accordingly, in a first aspect, the present invention relates to a polypeptide comprising the polypeptide consisting of SEQ ID NO: 186; or any functional variant of said amino acid sequence - with a sequence identity of at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95% to the sequence of SEQ ID NO: 186, and - with a difference in protective capacity (Δpc) between the functional variant and the placebo (negative) control of at least 50%, especially at least 60%, preferably at least 70%, more preferably at least 80%, even more preferably 90%, even more preferably 95%, most preferably at least 95%.

The term *B. burgdorferi* s.l. encompasses at least 13 *Borrelia* species (Table A-1). These species occur in different geographic regions, and live in nature in enzootic cycles involving ticks of the *Ixodes ricinus* complex (also called *Ixodes persulcatus* complex) and a wide range of animal hosts. Four *Borrelia* species are responsible for the majority of infections in humans: *B. burgdorferi* s.s., *B. afzelii, B. bavariensis* and *B. garinii*. Three other species, *B. lusitaniae, B. bissettii* and *B. spielmanii*, have occasionally been detected in humans, but their role in Lyme borreliosis is uncertain at present. New species of *Borrelia* are still being reported.

2013285364 12 May 2017

Table A-1.

Pathogenic species (4)	Principal tick vector	Location
Borrelia burgdorferi (*Borrelia burgdorferi* s.s.)	*Ixodes scapularis*	Northeastern/north-central US
	Ixodes pacificus	Western US
	Ixodes ricinus	Europe
	Ixodes persulcatus	Asia
Borrelia garinii	*Ixodes ricinus*	Europe
	Ixodes persulcatus	Asia
Borrelia afzelii	*Ixodes ricinus*	Europe
	Ixodes persulcatus	Asia
Borrelia bavariensis	*Ixodes ricinus*	Europe

	Ixodes persulcatus	Asia
Minimally pathogenic or non-pathogenic species (9)	**Principal tick vector**	**Location**
Borrelia andersonii	*Ixodes dentatus*	Eastern US
Borrelia bissettii	*Ixodes spinipalpis* *Ixodes pacificus* *Ixodes ricinus*	Western US Europe
Borrelia valaisiana	*Ixodes ricinus* *Ixodes columnae*	Europe and Asia
Borrelia lusitaniae	*Ixodes ricinus*	Europe
Borrelia spielmanii	*Ixodes ricinus*	Europe
Borrelia japonica	*Ixodes ovatus*	Japan
Borrelia tanukii	*Ixodes tanuki*	Japan
Borrelia turdi	*Ixodes turdus*	Japan
Borrelia sinica	*Ixodes persulcatus*	China

As detailed above, *Borrelia* outer surface protein A (OspA) is an abundant immunogenic lipoprotein of *Borrelia* of particular interest because of its potential as a vaccine candidate. OspA of *B. burgdorferi* s.l. is a basic lipoprotein that has a molecular mass of approximately 30 kDa and is encoded on a linear plasmid. An important aspect of the OspA protein is its N-terminal lipidation; that is, the N-terminal cysteine residue is substituted with fatty acids with a chain length of between C14 and C19 with or without double-bonds, a feature that enhances the immunogenicity of the OspA protein. It has been shown that poorly-immunogenic synthetic peptides induce stronger antibody responses when lipidated; for example, when covalently coupled to Pam_3Cys (Bessler and Jung, Research Immunology (1992) 143:548-552), a fatty acid substitution found at the amino terminus of many bacterial lipoproteins that are synthesized with a signal sequence specifying lipid attachment. Additionally, the Pam_3Cys moiety was shown to enhance immune responses to OspA in mice, partially through its interaction with TLR-2 (Yoder, *et al.* (2003) Infection and Immunity 71:3894–3900). Therefore, lipidation of a C-terminal fragment of OspA would be expected to enhance the immunogenicity and protective capacity of the fragment.

Analysis of isolates of *B. burgdorferi* s.l. obtained in North America and Europe has revealed that OspA has antigenic variability and that several distinct groups can be defined based on serology. Anti-OspA mAbs which bind to specific N- and C-terminal antigenic determinants have been reported. X-ray crystallography and NMR analysis have been used to identify immunologically important hypervariable domains in OspA and have mapped the LA-2 epitope to C-terminal amino acids 203-257 (Ding et al., Mol. Biol. 302: 1153-64, 2000). Previous studies have shown that the production of antibodies against the C-terminal epitope LA-2 correlates with protective immunity after vaccination with OspA (Van Hoecke *et al.* Vaccine (1996) 14(17-18):1620-6 and Steere *et al.*, N Engl J Med (1998) 339:209-215). Antibodies to LA-2 were shown to block the transmission of *Borrelia* from tick to host (Golde *et al.*, Infect Immun (1997) 65(3):882-889). These studies

suggested that the C-terminal portion of the OspA protein may be sufficient for inducing protective immunity. It should be noted that the sequence of the C-terminal portion of OspA is less highly-conserved between *Borrelia* serotypes than is the N-terminal portion (see Fig. 1).

Based on information from the studies outlined above, along with others, truncated forms of OspA comprising the C-terminal portion (also referred to herein as "OspA fragment" or "monomer") were used in the current invention. These truncated forms of OspA proved to be less protective than the full-length OspA protein. Surprisingly, however, it was found in the course of the current invention that the introduction of a disulfide bond in the truncated form (also referred to herein as "mutant OspA fragment" or "mutant fragment") overcomes this disadvantage. While not being limited to a specific mechanism, it is thought that improved protection is due to increased stability of the OspA fragment, as shown in assays measuring thermal stability.

In accordance with the present invention, the mutant OspA fragment may be derived from any *Borrelia* species; however, due to their relevance in the medical field, particularly for humans, *B. burgdorferi* s.s., *B. afzelii, B. bavariensis* and *B. garinii* are preferred. In this regard, these four *Borrelia* species can be further classified according to their OspA serotypes, which have been determined by analysis with monoclonal antibodies specific to the respective OspA protein. Serotypes 1-7, which account for the majority of human *Borrelia* infections, along with their rates of prevalence, are shown in Table A-2 below.

Table A-2. Serotype designation and prevalence of *B. burdorferi* s.s., *B. afzelii, B. bavariensis* and *B. garinii. Borrelia* isolated from human cerebrospinal fluid or skin or from tick vectors were serotyped by probing whole-cell lysates with mouse monoclonal antibodies, each specific to a particular epitope of OspA (as described by Wilske *et al.*, J. of Clin Microbiol (1993) 31(2):340-350 and presented by Baxter Bioscience at "Climate change effect on ticks and tick-borne diseases", Brussels, 06 Feb 2009).

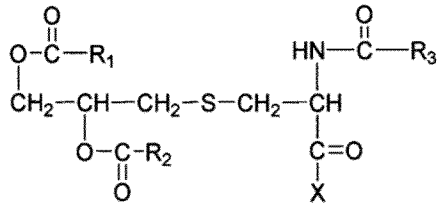
Borrelia sp.	**OspA serotype defined by mAb testing**	**Prevalence in human disease**	**Strain source for sequence**	**Seq ID No:**
B. burgdorferi s.s.	**1**	**11%**	B31	20
B. afzelii	**2**	**63%**	K78	19
B. garinii	**3**	**1.5%**	PBr	21
B. bavariensis	**4**	**4%**	PBi	22
B. garinii	**5**	**6%**	PHEi	23
B. garinii	**6**	**13%**	DK29	24
B. garinii	**7**	**0.5%**	T25	25

The structure of the OspA protein from *B. burgdorferi* s.s. strain B31 was determined by Li *et al.* (Proc Natl Acad Sci (1997) 94:3584-3589). It is composed of N-terminal (β-strands 1 to 4) and central β-sheets (β-strands 5 to 14n [N-terminal part]), barrel sheet 1 (β-strands 14c [C-terminal part] to 16), barrel sheet 2 (β-strands 17 to 21) and a C-terminal α-helix. The term "OspA C-terminal domain" or "C-terminal domain" or "wild-type fragment" or "C-terminal portion" with respect to OspA as used throughout the present specification shall mean the C-terminal amino acid sequence of OspA, *i.e.,* OspA lacking at least the N-terminal β-sheet (including β-strands 1 to 4). In OspA from *B. burgdorferi* s.s. strain B31, the N-terminal sheet consists of amino acids 17 to 70 (following post-translational cleavage of the 16 aa long lipidation signal peptide).

The C-terminal OspA fragment of the current invention may also include a lipidation signal sequence at the N-terminus, *e.g.,* the lipidation signal sequence of amino acids 1 to 16 of OspA (SEQ ID NO: 14) or OspB (SEQ ID NO: 15) from *B. burgdorferi* s.s. strain B31, a lipidation signal sequence from *E. coli*, referred to herein as the "lpp lipidation signal" (SEQ ID NO: 16), or any other signal sequence, *e.g.,* as defined below.

Lipidation of a protein with an N-terminal lipidation signal sequence, such as those present on a nascent OspA polypeptide, occurs in the *E. coli* expression vector by the step-wise action of the enzymes diacylglyceryl transferase, signal peptidase II and transacylase, respectively. The first step is the transfer of a diacylglyceride to the cysteine sulphydryl group of the unmodified prolipoprotein, followed by the cleavage of the signal peptide by signal peptidase II and, finally, the acylation of the α-amino group of the N-terminal cysteine of the apolipoprotein. The result is the placement of one lipid and a glycerol group substituted with two further lipids on the N-terminal cysteine residue of the polypeptide. The lipidation signal sequence, which is cleaved off during lipidation, is not present in the final polypeptide sequence.

According to the current invention, the mutant OspA fragment may be a lipidated protein, also lipoprotein, wherein the lipid moieties, along with the glycerol group, is also referred to as "Lip". According to the invention, Lip comprises one to three lipids such as C_{14-20} alkyl and/or C_{14-20} alkenyl attached to a glycerol and the N-terminal cysteine of the polypeptide of the invention, or preferably wherein Lip is a moiety of formula (I) below,



Formula (I),

in which one of R_1, R_2 or R_3 is C_{14}-C_{20} alkyl or alkenyl, and each of the others, independently is C_{14}-C_{20} alkyl or C_{14}-C_{20} alkenyl, and X is an amino acid sequence attached to the cysteine residue shown in Formula (I). More preferably, Lip plus the N-terminal cysteine of the polypeptide is N-palmitoyl-S-(2RS)-2,3-bis-(palmitoyloxy) propyl cysteine (referred to herein as "Pam_3Cys") and is connected via the carbonyl C of the cysteine to said amino acid sequence of the invention. In Formula (I) above R_1, R_2 and R_3 would be palmitoyl moieties and X is an amino acid sequence attached to the cysteine residue.

In accordance with the current invention, the C-terminal domain of an OspA from a strain other than *B. burgdorferi* s.s. B31 is defined by (i) lacking at least amino acids 17 to 70 and/or (ii) by lacking at least the N-terminal domain homologous to amino acids 17 to 70 of OspA from *B. burgdorferi* s.s. B31. Additionally, the OspA C-terminal domain according to the present invention may also lack further portions of the central sheet as defined by Li and co-workers (Li *et al.*, supra), particularly further strands such as the amino acid portions from amino acid 17 to 82, 93, 105, 118 or 119, preferably 17 to 129, more preferably 1 to 125, 1 to 129 or 1 to 130 of any *Borrelia*, particularly *B. burgdorferi* s.s. B31, or homologous portions of an OspA protein from a *Borrelia sp.* other than *B. burgdorferi* s.s. B31.

In the context of the present invention, the OspA C-terminal domain is also referred to as "OspA fragment" or "fragment of OspA".

The "mutant fragment" in the context of the polypeptide of the present invention and as used throughout the present specification shall mean the OspA C-terminal fragment, as defined above, which differs from the wild-type fragment at least by at least two introduced cysteines that can form a disulfide bond. Without being bound to that theory, it is assumed that the disulfide bond stabilizes the fragment in a conformation conducive to the induction of antibody binding. The fold of the wild-type C-terminal fragment of OspA shows reduced temperature stability in comparison to the full-length protein (Koide *et al.*, Structure-based Design of a Second-generation Lyme Disease Vaccine Based on a C-terminal Fragment of *Borrelia burgdorferi* OspA, J. Mol. Biol. (2005) 350:290-299). For the

present invention, the sequence of the C-terminal domain of the *B. burgdorferi* s.s. B31 OspA has been *in silico* analyzed to determine positions for introduced disulfide bridges that may enhance the stability of the fold of this C-terminal domain. The results of the analysis have been transferred to homologous OspA fragments of other *Borrelia* species with the assumption that the fold is conserved across species.

Typically, the disulfide bond may be introduced by the introduction of one or more cysteine residues, wherein a disulfide bond (S-S bridge) is formed between the thiol groups of two cysteine residues. Only one cysteine residue need be introduced if a disulfide bond is formed with a cysteine residue present in the wild-type fragment. The one, or preferably two, cysteine(s) may be introduced by amino acid addition or, preferably, substitution.

The OspA mutant fragment may also comprise further mutations relative to the wild-type. As detailed above, the structure and surface domain of OspA are known in the art. Accordingly, the mutant fragment may comprise further mutations, particularly at sites not on the surface of the protein and/or not involved in the immune response and, therefore not impacting antigenic capacity. These can include one or more amino acid deletion(s), particularly small (*e.g.*, up to 10 amino acids) deletions, one or more amino acid addition(s) (particularly C- or N-terminally), one or more amino acid substitution(s), particularly one or more conservative amino acid substitutions. Examples of conservative amino acid substitutions include, but are not limited to, those listed below:

Ala	Ser	Leu	Ile; Val
Arg	Lys	Lys	Arg; Gln; Asn
Asn	Gln; His	Met	Leu; Ile
Asp	Glu	Phe	Met; Leu; Tyr
Cys	Ser	Ser	Thr
Gln	Asn	Thr	Ser
Glu	Asp	Trp	Tyr
His	Asn; Gln	Tyr	Trp; Phe
Ile	Leu, Val	Val	Ile; Leu

Preferred mutations include changes in selected portions of the fragment, for example, wherein the sequence with sequence similarity to human leukocyte function-associated antigen (hLFA-1), which exists in *B. burgdorferi* s.s., is modified, for example, replaced by a homologous sequence from an OspA protein from another *Borrelia sp.* The rationale for this modification is to reduce the risk for inducing immunological cross-reaction with human proteins. Also possible is the addition of a signal sequence for lipidation in the final, or an intermediate, fragment, or the addition of a marker protein (*e.g.*, for identification or purification).

In some embodiments, the mutant OspA fragment has an amino acid sequence that has 60%, preferably at least 70%, more preferably at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to the wild-type fragment. In another embodiment, the sequence differs by at most 10%, at most 9%, at most 8%, at most 7%, at most 6%, 5%, 4%, 3%, 2%, most preferably at most 1%, due to a sequence addition, deletion or substitution.

Identity, as known in the art and as used herein, is the relationship between two or more polypeptide sequences, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. Identity can be readily calculated. While a number of methods exist to measure identity between two polynucleotides or two polypeptide sequences, the term is well known to skilled artisans (e.g. *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in computer programs. Preferred computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux, J. *et al.,* 1984), BLASTP, BLASTN, and FASTA (Altschul, S. *et al.,* 1990).

In contrast to the mutant OspA fragment, the "wild-type fragment" in the context of the present invention relates to a fragment of a naturally-occurring OspA of *Borrelia*. The wild-type fragment is obtained by N-terminal deletions, but it does not comprise internal deletions (except from signal sequences as detailed herein) or mutations. In relation to the mutant OspA fragment, the wild-type fragment consists of an identical part of the OspA (identical length and same strain of OspA, etc.) and differs only in the mutation(s) detailed above, particularly in the introduction of at least one disulfide bond or the replacement of a sequence with human homology, for example hLFA-1 (see above).

According to a preferred embodiment of the present invention, the polypeptide of the present invention does not comprise or consist of the full-length OspA polypeptide having at least one disulfide bond introduced.

In one embodiment of the present invention, the mutant OspA fragment may differ from the respective wild-type fragment only by the introduction of at least one, preferably exactly one, disulfide bond.

A polypeptide is a single linear polymer of amino acids linked by peptide bonds, in some cases also by disulfide bonds. In accordance with the present invention, the polypeptide may also compromise

one or more posttranslational modifications; *i.e.,* an attached biochemical functional group, such as an attached acetate, phosphate, lipid or carbohydrate, preferably a lipid or lipids attached to the N-terminal cysteine along with a glycerol, more preferably 1 to 3 C_{14}-C_{20} alkyl or alkenyl moieties, even more preferably 1 to 3 palmitoyl groups, most preferably three palmitoyl groups (Pam_3).

In accordance with the present invention, the polypeptide of the present invention comprises the above-described mutant OspA fragment. According to the present invention, it does not comprise (i) the N-terminal sheet as defined above and (ii) optionally one or more further strands of the central sheet as defined above. However, the polypeptide may comprise one or more functional sequences such as a signal sequence, *e.g.,* a lipidation signal sequence or a posttranslational modification, such as lipidation.

In a further embodiment of the present invention, the polypeptide of the present invention consists of (i) one or more mutant OspA fragments, optionally joined by linkers, *e.g.,* as defined below and (ii) optionally one or more amino acids heterologous to OspA, particularly a signal sequence and (iii) optionally a posttranslational modification, such as lipidation.

The polypeptide of the present invention has protective capacity. As detailed above, the introduction of a disulfide bond into the mutant OspA fragment increases the protective capacity of the polypeptide relative to a polypeptide comprising the respective fragment without the disulfide bond(s). In some embodiments, the protective capacity is increased by at least 10%, more preferably by at least 20%, more preferably by at least 30%, more preferably by at least 40%, more preferably by at least 50%, more preferably by at least 60%, more preferably by at least 70%, more preferably by at least 80%, even more preferably by at least 90% relative to a polypeptide comprising the respective fragment without the disulfide bond(s).

The term protective capacity describes the ability to protect a subject against a *Borrelia* infection. With respect to the polypeptide of the invention, protective capacity relates to the ability of the polypeptide to induce an immune response that protects a subject against a *Borrelia* infection. Protective capacity can be tested by administering to a subject the polypeptide in a manner to induce an immune reaction against the polypeptide. Thereafter, the subject may be challenged with *Borrelia*. The subject's reaction to the infection is monitored. Particularly, the presence of *Borrelia* in the subject may be determined. For example, the polypeptide is protective if *Borrelia* cannot be detected in the subject. The presence of *Borrelia* can be determined by detecting *Borrelia*-specific nucleic acids (*e.g.,* by PCR) or *Borrelia*-specific antibodies (*e.g.,* by ELISA or Western blot) or by detecting *Borrelia* itself (*e.g.,* culturing organs or tissues in growth medium and verifying the presence of

Borrelia by microscopy). In particular, the protective capacity ("pc"), reported as a percentage, for a particular dose is defined as follows:

pc (%) = [(number of total tested subjects – number of *Borrelia*-infected subjects) / number of total tested subjects] x 100

Differences in protective capacity (Δpc) may be determined by, e.g. comparing the protective capacity (pc) of a mutant OspA fragment with a disulfide bond(s) (pc [with bond]) to the protective capacity of an OspA fragment without a disulfide bond(s) (pc [w/o bond]). In accordance with the present invention, the polypeptides to be compared differ only in the introduction of at least one disulfide bond. The change in protective capacity (Δpc) by the introduction of the disulfide bond(s) is determined as follows:

Δpc = (pc [sample] – pc [control])

e.g. Δpc = (pc [with bond] – pc [w/o bond])

If Δpc is greater than zero (> 0), assuming all other parameters (e.g., dose and assay) are the same, then the protective capacity of the sample (e.g. the mutant OspA fragment with a disulfide bond(s)) is better than the protective capacity of the control (e.g. the OspA fragment without a disulfide bond(s)). Conversely, if Δpc is less than zero (< 0) and assuming all other parameters (e.g., dose and assay) are the same, then the protective capacity of the sample (e.g. the mutant OspA fragment with a disulfide bond(s)) is less than the protective capacity of the comparison (e.g., the OspA fragment without a disulfide bond(s)).

Preferably, the polypeptide of the present invention is assessed for its protective capacity by an *in vivo* challenge assay wherein mice immunized with the polypeptide of the invention or with a placebo control are challenged with *Borrelia* introduced into the immunized subjects with a hypodermic needle (Needle Challenge Method) or by introduction by a tick vector (Tick Challenge Method).

The Needle Challenge Method is carried out for the desired *Borrelia* strain (e.g., *B. burgdorferi*, strain N40) by subcutaneously introducing *Borrelia* at a dose between 20 and 50 times the Infectious Dose $(ID)_{50}$ to mice that are immunized with said first polypeptide of the first aspect or with an appropriate placebo (negative) control, such as buffer or adjuvant alone and comparing the rates of infection in the challenged mice. The ID_{50} is defined as the dose at which 50% of the challenged mice are infected. The dose of *Borrelia* is measured in numbers of bacteria. The challenge dose can vary widely and is strain-dependent; therefore, the virulence of the strain must first be assessed by challenge experiments

for determination of ID_{50}. Four weeks after needle challenge, blood and tissues are collected for readout methods to determine the infection status. These readout methods can be e.g. VlsE ELISA on sera or qPCR on collected tissues for identification of *Borrelia*, as described herein, or other methods.

The Tick Challenge Method is carried out by applying at least one tick nymph (e.g., *I. ricinus*) infected with *Borrelia* (*e.g., B. afzelii*, strain IS1), to a mouse that is immunized with said first polypeptide of the first aspect; and b) applying at least one infected tick nymph to a second mouse that is immunized with said second polypeptide of the first aspect; and c) comparing the rates of infection in the two mice, generally six weeks after challenge. Preferably, the assay or test is done with a group of mice per polypeptide to be tested. A suitable test is also described and illustrated in the Examples. Assessment of infection status can be done using VlsE ELISA on sera or qPCR on collected tissues, or using other suitable methods.

In a preferred embodiment of the present invention, the products of the invention such as, e.g. the polypeptides of the invention comprising the mutant OspA fragment with a disulfide bond(s) administered 3 times to a subject at a dose of 30 µg, preferably 10 µg, preferably 5.0 µg, preferably 1.0 µg, preferably 0.3 µg or lower have a protective capacity of 50% or more, preferably 60% or more, more preferably 70% or more, more preferably 80% or more, more preferably 90% or more, even more preferably 95% or more, most preferred 99% or more. In one embodiment, the protective capacity is assessed in an in vivo challenge method, preferably a Tick Challenge Method, more preferably a Needle Challenge Method, e.g. as described in the Examples. It has been surprisingly observed that immunization with an OspA mutant fragment of one serotype can provide cross-protection against other another serotype (Example 4, Table 4). Based on this finding, it might be anticipated that the dose of polypeptide of the present invention could be even further reduced.

In a preferred embodiment, the difference in protective capacity (Δpc) between the polypeptides of the invention comprising the mutant OspA fragment with a disulfide bond(s) and the placebo (negative) control is at least 50%, especially at least 60%, preferably at least 70%, more preferably at least 80%, even more preferably 90%, even more preferably 95%, most preferably at least 95%, when administered 3 times to a subject at a dose of 30 µg, preferably 10 µg, preferably 5.0 µg, preferably 1.0 µg, preferably 0.3 µg or lower.

In a preferred embodiment of the present invention, the C-terminal domain is defined as a fragment consisting of at least the C-terminal 150 amino acids of the OspA protein. In one embodiment, the C-terminal domain is between 140 and 152 amino acids in length. In a further embodiment, the C-terminal domain consists of no more than the last 152 amino acids of the OspA protein, preferably the

last 151 amino acids, more preferably the last 150 amino acids. In an alternative embodiment, the C-terminal domain consists of no less than the last 140 amino acids of the OspA protein, preferably the last 141 amino acids, preferably the last 142 amino acids, most preferably the last 143 amino acids. The last amino acids of the OspA protein are defined herein as the most C-terminal contiguous amino acid sequence of the OspA protein.

In another embodiment, the C-terminal domain of an OspA protein of *Borrelia* comprises, essentially consists of or consists of (i) the amino acids from position 126, 131 or 130 to position 273 of the OspA of *B. afzelii*, strain K78 or (ii) the homologous domain to amino acids of OspA from a *Borrelia* strain other than *B. afzelii*, strain K78.

The polypeptide of the present invention may comprise or essentially consists of or consist of (i) one or more of these mutant fragments, optionally joined by linkers, *e.g.,* as defined below and (ii) optionally one or more amino acids heterologous to OspA, particularly a signal sequence or site for a post-translational modification such as lipidation and (iii) optionally a posttranslational modification, such as lipidation.

In accordance with the present invention, the polypeptide of the present invention may comprise or essentially consists of or consist of of the elements as defined herein, particularly the one or more mutant OspA fragments and optionally one or more further elements such as homologous domain, a linker peptide, a signal sequence or a site for lipidation. “Essentially consists” in this context means that the element(s) may have some minor amino acid changes with respect to the above sequences, such as amino acid additions, substitutions or deletions, preferably relating to at most 10%, 5%, 4%, 3%, 2% or 1% of the amino acids of the elementes as defined herein.

In accordance with the present invention, at least one disulfide bond is introduced into an OspA fragment. This may preferably be achieved by introducing into the fragment at least 1 or 2 cysteine(s), particularly 2 cysteines, in order to allow for the formation of the at least one disulfide bond. Only one cysteine may be introduced, if another cysteine in the fragment is available for a disulfide bond. However, preferably two cysteines are introduced. The cysteine(s) is/are introduced by amino acid addition or substitution, preferably substitution. In case of addition, the cysteine is inserted into the amino acid sequence between two amino acids, whereas in case of substitution one amino acid is replaced with the cysteine.

In accordance with the present invention, the OspA may be from any *Borrelia* strain, particularly from those specified herein such as *B. burgdorferi* s.s., *B. garinii*, *B. afzelii*, *B. andersoni*, *B. bissettii*, *B.*

valaisiana, *B. lusitaniae*, *B. spielmanii*, *B. japonica*, *B. tanukii*, *B. turdi* or *B. sinica*, *B. bavariensis*, preferably from *B. burgdorferi* s.s., *B. afzelii*, *B. bavariensis* or *B. garinii*. Preferably, the OspA is from *B. afzelii*, particularly strain K78, OspA serotype 2 (SEQ ID NO: 19); *B. burgdorferi* s.s., particularly strain B31, OspA serotype 1 (SEQ ID NO: 20); *B. garinii*, particularly strain PBr, OspA serotype 3 (SEQ ID NO: 21); *B. bavariensis*, particularly strain PBi, OspA serotype 4 (SEQ ID NO: 22); *B. garinii*, particularly strain PHei, OspA serotype 5 (SEQ ID NO: 23); *B. garinii*, particularly strain DK29, OspA serotype 6 (SEQ ID NO: 24) or *B. garinii*, particularly strain T25, OspA serotype 7 (SEQ ID NO: 25). The amino acid sequences of these OspA proteins (full-length) are given below.

Table A-3. Accession numbers of OspA sequences from selected strains of *Borrelia* species. Abbreviations: **Baf**=*Borrelia afzelii*, **Bbu**=*Borrelia burgdorferi* s.s., **Bga**=*Borrelia garinii*, **Bsp**=*Borrelia spielmanii*, **Bbi**=*Borrelia bissettii*, **Bva**=*Borrelia valaisiana*, **Btu**=*Borrelia turicatae*, **Bdu**=*Borrelia duttonii*, **Blu**=*Borrelia lusitaniae*, **Bja**=*Borrelia japonica*, **gb**=GenBank, **emb**=EMBL, **tr**=UniProt/tremble, **sp**=UniProt/Swissprot, **prf**=Protein Research Foundation, **dbj**=DNA Databank of Japan (DDBJ), **pdb**=Protein Data Bank, **db**=database

Organism_Strain	db|accession.version	Organism_Strain	db|accession.version	Organism_Strain	db|accession.version
Bbu_156a (serotype 1)	gb|ACL33776.1	Bbu_K48	emb|CAA44492.1	Bga_Mng4702	gb|ABF29559.1
Baf_K78 (serotype 2)	emb|CAA49828.1	Bbu_N40	gb|ACS94765.1	Bga_N34	emb|CAB64763.1
Bga_PBr (serotype 3)	emb|CAA56549.1	Bbu_P0A3N6.1	sp|P0A3N6.1	Bga_Nov1006	gb|ACD02016.1
Bga_PBi (serotype 4)	emb|CAA56550.1	Bbu_PBo	emb|CAA56468.1	Bga_Nov105	gb|ABF29551.1
Bbu_PHei (serotype 5)	tr|Q06228	Bbu_PBre	emb|CAA59742.1	Bga_Nov14506	gb|ACD02013.1
Bbu_DK29 (serotype 6)	emb|CAA45010.1	Bbu_PHei	emb|CAA56544.1	Bga_Nov14606	gb|ACD02017.1
Bga_T25 (serotype 7)	emb|CAA56547.1	Bbu_PKa	emb|CAA56467.1	Bga_Nov2005	gb|ABF29553.1
Baf_ACA-1	gb|ACJ73559.1	Bbu_PKo	emb|CAA46550.1	Bga_Nov2006	gb|ACD02018.1
Baf_K78	(sequenced)	Bbu_Poti_B1	emb|CAB64754.1	Bga_Nov3305	gb|ABF29554.1
Baf_Khab_625	gb|AAR96311.1	Bbu_Poti_B2	emb|CAB64755.1	Bga_Nov405	gb|ABF29552.1
Baf_Khab2-Sakh	gb|AAP94134.1	Bbu_Poti_B3	emb|CAB64756.1	Bga_Nov7006	gb|ACD02014.1
Baf_Khab470	gb|AAO91923.1	Bbu_PTro	emb|CAA56471.1	Bga_Nov9906	gb|ACD02015.1
Baf_Khab505	gb|AAO91925.1	Bbu_PWudI	emb|CAA56469.1	Bga_PBi	gb|AAT93773.1
Baf_LU192	(sequenced, partial)	Bbu_PWudI/6	emb|CAA56470.1	Bga_PBr	emb|CAA56549.1
Baf_Mng3602	gb|ABF29573.1	Bbu_PWudII	emb|CAA56546.1	Bga_Q1HLH6	gb|ABF29564.1
Baf_Mng4302	gb|ABF29574.1	Bbu_Q04851.1	sp|Q04851.1	Bga_T25	emb|CAA56547.1
Baf_Mng6702	gb|ABF29578.1	Bbu_Q04968.1	sp|Q04968.1	Bga_TIsI	emb|CAA59727.1
Baf_Mng702	gb|ABF29572.1	Bbu_Q09086.1	sp|Q09086.1	Bga_TN	emb|CAA56545.1
Baf_Nov1105	gb|ABF29569.1	Bbu_Q09087.1	sp|Q09087.1	Bga_Tom1003	gb|ABF29564.1
Baf_Nov11506	gb|ACD02019.1	Bbu_Q44738	tr|Q44738	Bga_Tom1805	gb|ABF29567.1
Baf_Nov3005	gb|ABF29570.1	Bbu_Q44956	emb|CAA56937.1	Bga_Tom203	gb|ABF29562.1
Baf_P0A3N7.1	sp|P0A3N7.1	Bbu_Q44962	dbj|BAA06133.1	Bga_Tom2903	gb|ABF29565.1
Baf_PHo	emb|CAA59724.1	Bbu_Q45039	emb|CAR95556.1	Bga_Tom3005	gb|ABF29568.1
Baf_PKo	gb|ABH02138.1	Bbu_Q45040	tr|Q45040	Bga_Tom303	gb|ABF29563.1
Baf_PLe	emb|CAA59970.1	Bbu_S-1-10	gb|AAB96354.1	Bga_Tom3101	gb|ABF29557.1
Baf_PLj7	emb|CAA59725.1	Bbu_T.R.O.	emb|CAA46549.1	Bga_Tom3803	gb|ABF29566.1
Baf_PLud	emb|CAA59726.1	Bbu_T255	emb|CAA59730.1	Bga_Tom5102	gb|ABF29560.1
Baf_Tom1103	gb|ABF29581.1	Bbu_UK	emb|CAB64758.1	Bga_Tom5202	gb|ABF29561.1
Baf_Tom1303	gb|ABF29582.1	Bbu_VS116	emb|CAB64757.1	Bga_Tom7105	gb|ABF29556.1
Baf_Tom1503	gb|ABF29583.1	Bbu_VS461	emb|CAA82329.1	Bga_VS100	emb|CAB64765.1
Baf_Tom2303	gb|ABF29584.1	Bbu_WI91-23	ref|ZP_03091138.1	Bga_VS307	emb|CAB64764.1
Baf_Tom2403	gb|ABF29585.1	Bbu_ZQ1	emb|CAA01704.1	Bga_WABSou	emb|CAA59728.1
Baf_Tom2504	gb|ABF29577.1	Bbu_ZS7	gb|ACK74228.1	Bja_Cow611	emb|CAB64759.1
Baf_Tom2803	gb|ABF29586.1	Bga_BgVir-1	gb|ABF29555.1	Bja_F63	emb|CAB64760.1
Baf_Tom3401	gb|ABF29571.1	Bga_Far04	ref|ZP_03328706.1	Bja_HO14	emb|CAB64762.1
Baf_Tom3703	gb|ABF29587.1	Bga_FujiP2	gb|AAA92301.1	Bja_IKA2	emb|CAB64761.1
Baf_Tom4703	gb|ABF29588.1	Bga_IP90	emb|CAJ75754.1	Blu_A8D057	gb|ABR22627.1

Baf_Tom5403	gb|ABF29575.1	Bga_Ip90	emb|CAJ75754.1	Blu_A8D060	gb|ABR22625.1
Baf_Tom603	gb|ABF29579.1	Bga_JEM1	gb|AAB81567.1	Blu_A8D075	gb|ABR22628.1
Baf_Tom6303	gb|ABF29576.1	Bga_JEM2	gb|AAB81569.1	Blu_A8D079	gb|ABR22629.1
Baf_Tom703	gb|ABF29580.1	Bga_JEM3	gb|AAB81571.1	Blu_ABR22624.1	gb|ABR22624.1
Baf_XJ23	gb|AAB95225.1	Bga_JEM4	dbj|BAA19222.1	Blu_ABR22626.1	gb|ABR22626.1
Bbu_118a	ref|ZP_02720644.1	Bga_JEM5	gb|AAB81573.1	Bsp_A14S	gb|AAD16455.1
Bbu_156a	gb|ACL33776.1	Bga_JEM6	gb|AAB81575.1	Btu_Ya501	dbj|BAA32513.1
Bbu_19857	emb|CAA48196.1	Bga_JEM7	gb|AAB81577.1	Bva_AR-2	gb|AAF00571.1
Bbu_2005348A	prf|2005348A	Bga_JEM8	gb|AAB81579.1	Bva_M19	gb|AAF00573.1
Bbu_2005348B	prf|2005348B	Bga_Khab3155	gb|AAR96310.1	Bva_M49	gb|AAF00574.1
Bbu_297	emb|CAA59729.1	Bga_Khab550	gb|AAR96306.1	Bva_M52	gb|AAF00575.1
Bbu_29805	ref|ZP_03092996.1	Bga_Khab616	gb|AAR96307.1	Bva_M53	gb|AAF00576.1
Bbu_64b	ref|ZP_03097520.1	Bga_Khab648	gb|AAR96308.1	Bva_M7	gb|AAF00572.1
Bbu_72a	ref|ZP_02724465.1	Bga_Khab722	gb|AAR96309.1	Bva_Q9RM88	emb|CAB56150.1
Bbu_80a	ref|ZP_03088001.1	Bga_Khab23	gb|AAP94125.1	Bva_QLZSP1	gb|ACA13516.1
Bbu_94a	ref|ZP_02725946.1	Bga_Khab24	gb|AAP94126.1	Bva_QSDS4	gb|ACA13517.1
Bbu_AAB23809.1	gb|AAB23809.1	Bga_Khab31	gb|AAP94127.1	Bva_QSYSP3	gb|ACA13518.1
Bbu_AAB23810.1	gb|AAB23810.1	Bga_Khab31a	gb|AAP94128.1	Bva_QSYSP4	gb|ACA13519.1
Bbu_B29	gb|AAA18508.1	Bga_Khab-466	gb|AAP94129.1	Bva_QTMP2	gb|ACA13520.1
Bbu_B31	gb|AAC66260.1	Bga_Khab489	gb|AAP94130.1	Bva_QX-S13	gb|ACA13521.1
Bbu_Bol26	ref|ZP_02531917.1	Bga_Khab5-Sakh	gb|AAO91932.1	Bva_UK	gb|AAF00570.1
Bbu_C-1-11	gb|AAB96351.1	Bga_Khab506	gb|AAP94132.1	Bva_VS116	gb|AAF00569.1
Bbu_CA-11.2a_1	ref|ZP_03094587.1	Bga_Khab516	gb|AAP94133.1	Bsp_10MT	dbj|BAA32516.1
Bbu_CA-11.2a_2	ref|ZP_03094587.1	Bga_Khab721	gb|AAP94131.1	Bsp_5MT	dbj|BAA32515.1
Bbu_CA-11.2a_CA-112a	ref|ZP_03094587.1	Bga_Khab2119	gb|AAO91928.1	Bsp_Am501	dbj|BAA32514.1
Bbu_CAA00316.1	emb|CAA00316.1	Bga_Khab2559	gb|AAO91929.1	Bsp_LV5	gb|AAB96353.1
Bbu_CAA42842.1	emb|CAA42842.1	Bga_Khab2560	gb|AAO91930.1	Bsp_PAnz	emb|CAJ43585.1
Bbu_CAA44258.1	emb|CAA44258.1	Bga_Khab2594	gb|AAO91931.1	Bsp_PHaP_PHap	emb|CAJ43582.1
Bbu_CAR95597.1	emb|CAR95597.1	Bga_Khab430	gb|AAO91919.1	Bsp_PJes	emb|CAJ43586.1
Bbu_DK1	gb|AAA22955.1	Bga_Khab448	gb|AAO91920.1	Bsp_PMai	emb|CAJ43584.1
Bbu_DK29	emb|CAA45010.1	Bga_Khab457	gb|AAO91921.1	Bsp_PMew	emb|CAJ43583.1
Bbu_DK6_Danish_isolate	emb|CAA58601.1	Bga_Khab468	gb|AAO91922.1	Bsp_PSigII	emb|CAJ43581.1
Bbu_G2	gb|AAA88846.1	Bga_Khab492	gb|AAO91924.1	Bsp_SV1	ref|ZP_03095680.1
Bbu_G25	emb|CAA82328.1	Bga_Khab511	gb|AAO91926.1	Bbi_25015	gb|AAB21761.1
Bbu_H.E.	emb|CAA46551.1	Bga_Khab560	gb|AAO91927.1	Bbi_DN127	emb|CAB64766.1
Bbu_HB19	gb|AAC18776.1	Bga_LV4	gb|AAB96352.1	Bbi_Q09087.1	gb|AAB21761.1

In accordance with the present invention, the disulfide bond may be formed between cysteines that have been introduced at any position of the OspA fragment allowing or supporting appropriate folding of the fragment. The positions may be selected, as detailed above, based on the known structure of the OspA. In a preferred embodiment, the polypeptide of the current invention contains at least one disulfide bond between any of positions 182 +/- 3 and any of positions 269 +/- 3 (disulfide bond type 1); any of positions 182 +/- 3 and any of positions 272 +/- 3 (disulfide bond type 2); any of positions 244 +/- 3 and any of positions 259 +/- 3 (disulfide bond type 3); any of positions 141 +/- 3 and any of positions 241 +/- 3 (disulfide bond type 4); any of positions 165 +/- 3 and any of positions 265 +/- 3 (disulfide bond type 5); any of positions 185 +/- 3 and any of positions 272 +/- 3 (disulfide bond type 6); any of positions 199 +/- 3 and any of positions 223 +/- 3 (disulfide bond type 7); any of positions 243 +/-3 and any of positions 262 +/- 3 (disulfide bond type 8); any of positions 184 +/- 3 and any of positions 204 +/- 3 (disulfide bond type 9); any of positions 201 +/- 3 and any of positions 214 +/- 3 (disulfide bond type 10); any of positions 246 +/-3 and any of positions 259 +/- 3 (disulfide bond type

11); and/or any of positions 167 +/- 3 and any of positions 178 +/-3 (disulfide bond type 12) of a *B. afzelii*, particularly *B. afzelii* K78 serotype 2 OspA, or the homologous amino acids of an OspA from a *Borrelia sp.* other than *B. afzelii*, such as *B. burgdorferi* s.s., particularly strain B31, serotype 1; *B. garinii*, particularly strain PBr, serotype 3; *B. bavariensis,* particularly strain PBi, serotype 4; *B. garinii*, particularly strain PHei, serotype 5; *B. garinii*, particularly strain DK29, serotype 6 or *B. garinii*, particularly strain T25, serotype 7.

More particularly, the polypeptide of the current invention contains the at least one disulfide bond between any of positions 182 and 269 (disulfide bond type 1); positions 182 and 272 (disulfide bond type 2); positions 244 and 259 (disulfide bond type 3); positions 141 and 241 (disulfide bond type 4); positions 165 and 265 (disulfide bond type 5); positions 185 and 272 (disulfide bond type 6); positions 199 and 223 (disulfide bond type 7); positions 243 and 262 (disulfide bond type 8); positions 184 and 204 (disulfide bond type 9); positions 201 and 214 (disulfide bond type 10); positions 246 and 259 (disulfide bond type 11); and/or positions 167 and 178 (disulfide bond type 12) of a *B. afzelii*, particularly *B. afzelii* K78 serotype 2 OspA, or the homologous amino acids of an OspA from a *Borrelia* other than *B. afzelii*, such as *B. burgdorferi* s.s., particularly strain B31, serotype 1; *B. garinii*, particularly strain PBr, serotype 3; *B. bavariensis,* particularly strain PBi, serotype 4; *B. garinii*, particularly strain PHei, serotype 5; *B. garinii*, particularly strain DK29, serotype 6 or *B. garinii*, particularly strain T25, serotype 7.

Table A-4. Disulfide bond types with nomenclature and the position of the cysteine substitutions in the serotype 2 OspA protein.

Disulfide bond type	**Nomenclature**	**Position of cysteines in *B. afzelii* K78 serotype 2 OspA**
wild-type sequence	D0	No cysteine substitutions
1	D1	182 and 269
2	D2	182 and 272
3	D3	244 and 259
4	D4	141 and 241
5	D5	165 and 265
6	D6	185 and 272
7	D7	199 and 223
8	D8	243 and 262
9	D9	184 and 204
10	D10	201 and 214
11	D11	246 and 259
12	D12	167 and 178

Even more preferred are disulfide bond types 1 to 5, especially disulfide bond types 1 to 4.

It is noted that:

Position 182 +/- 3 is an abbreviation for position 179, 180, 181, 182, 183, 184 or 185, preferably 182.

Position 269 +/- 3 is an abbreviation for position 266, 267, 268, 269, 270, 271 or 272, preferably 269.

Position 272 +/- 3 is an abbreviation for position 269, 270, 271, 272, 273, 274 or 275, preferably 272.

Position 244 +/- 3 is an abbreviation for position 241, 242, 243, 244, 245, 246 or 247, preferably 244.

Position 259 +/- 3 is an abbreviation for position 256, 257, 258, 259, 260, 261 or 262, preferably 259.

Position 141 +/- 3 is an abbreviation for position 138, 139, 140, 141, 142, 143 or 144, preferably 141.

Position 241 +/- 3 is an abbreviation for position 238, 239, 240, 241, 242, 243 or 244, preferably 241.

Position 165 +/- 3 is an abbreviation for position 162, 163, 164, 165, 166, 167 or 168, preferably 165.

Position 265 +/- 3 is an abbreviation for position 262, 263, 264, 265, 266, 267 or 268, preferably 265.

Position 185 +/- 3 is an abbreviation for position 182, 183, 184, 185, 186, 187 or 188, preferably 185.

Position 199 +/- 3 is an abbreviation for position 196, 197, 198, 199, 200, 201 or 202, preferably 199.

Position 223 +/- 3 is an abbreviation for position 220, 221, 222, 223, 224, 225 or 226, preferably 223.

Position 243 +/- 3 is an abbreviation for position 240, 241, 242, 243, 244, 245 or 246, preferably 143.

Position 262 +/- 3 is an abbreviation for position 259, 260, 261, 262, 263, 264 or 265, preferably 262.

Position 184 +/- 3 is an abbreviation for position 181, 182, 183, 184, 185, 186 or 187, preferably 184.

Position 204 +/- 3 is an abbreviation for position 201, 202, 203, 204, 205, 206 or 207, preferably 204.

Position 201 +/- 3 is an abbreviation for position 198, 199, 200, 201, 202, 203 or 204, preferably 201.

Position 214 +/- 3 is an abbreviation for position 211, 212, 213, 214, 215, 216 or 217, preferably 214.

Position 246 +/- 3 is an abbreviation for position 243, 244, 245, 246, 247, 248 or 249, preferably 246.

Position 167 +/- 3 is an abbreviation for position 164, 165, 166, 167, 168, 169 or 170, preferably 167.

Position 178 +/- 3 is an abbreviation for position 175, 176, 177, 178, 179, 180 or 181, preferably 178.

In a preferred embodiment, the mutant fragment is derived from the amino acids from position 126, 130 or 131 to position 273 of the wild-type sequence of the OspA of *B. afzelii* strain K78, serotype 2 (SEQ ID NO: 18) and differs only by the introduction of at least one disulfide bond, particularly wherein the at least one disulfide bond is between positions 182 and 269 (disulfide bond type 1); positions 182 and 272 (disulfide bond type 2); positions 244 and 259 (disulfide bond type 3); positions 141 and 241 (disulfide bond type 4); positions 165 and 265 (disulfide bond type 5); positions 185 and 272 (disulfide bond type 6); positions 199 and 223 (disulfide bond type 7); positions 243 and 262 (disulfide bond type 8); positions 184 and 204 (disulfide bond type 9); positions 201 and 214 (disulfide bond type 10); positions 246 and 259 (disulfide bond type 11); and/or

positions 167 and 178 (disulfide bond type 12), or the homologous fragments and positions of an OspA from a *Borrelia sp.* other than *B. afzelii*, such as *B. burgdorferi* s.s., particularly strain B31, serotype 1; *B. garinii*, particularly strain PBr, serotype 3; *B. bavariensis*, particularly strain PBi, serotype 4; *B. garinii*, particularly strain PHei, serotype 5; *B. garinii*, particularly strain DK29, serotype 6 or *B. garinii*, particularly strain T25, serotype 7.

In a still more preferred embodiment, the mutant fragment has an amino acid sequence selected from the group consisting of SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178 and an amino acid sequence that has 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to at least one of sequences with SEQ ID NOs: 2 to 13, wherein the cysteines are not replaced. Further details on mutations and sequence identity are given above.

As detailed above, the polypeptide of the present invention may comprise signal sequences. It has been shown that lipidation confers adjuvant properties on OspA. Accordingly, lipidated forms of the polypeptide of the invention or polypeptides comprising a lipidation signal are preferred. In a preferred embodiment, the polypeptide of the current invention comprises a lipidation signal, preferably a lipidation signal of a *Borrelia* outer surface protein, OspA or OspB (SEQ ID NOs: 14 and 15, respectively) or more preferably an *E. coli* lpp lipidation signal sequence (SEQ ID NO: 16). The OspA fragment of the invention comprising a lipidation signal is lipidated during processing and the lipidation signal peptide is cleaved off. Therefore the signal peptide is no longer present in the mature lipidated protein.

Lipidated proteins according to the current invention are labeled with "Lip" at the N-terminus to indicate the addition of 3 fatty acid groups and a glycerol to the polypeptide (see Fig. 4). Suitable lipidation signals as described above include MKKYLLGIGLILALIA (SEQ ID NO: 14), MRLLIGFALALALIG (SEQ ID NO: 15) and MKATKLVLGAVILGSTLLAG (SEQ ID NO: 16). Because lipid moieties and a glycerol are attached to the N-terminal cysteine residue which is present in the full-length wild-type OspA protein, OspA C-terminal fragments for lipidation may additionally comprise a peptide comprising a cysteine residue followed by additional amino acids, herein referred to as "Lipidation Peptide" or "LP" (see Figs. 1 and 2). For example, sequences such as CSS or CKQN (SEQ ID NO: 211) immediately C-terminal to the lipidation signal sequence provide an N-terminal cysteine residue for lipidation upon cleavage of the lipidation signal peptide. The lipidated cysteine-containing peptides are present in the final lipidated polypeptide of the invention.

It has been found that the OspA protein of *B. burgdorferi* s.s. comprises a sequence with the capacity to bind to a T-cell receptor that also has the capacity to bind to human leukocyte function-associated antigen (hLFA-1) (herein referred to also as "hLFA-1-like sequence"). The similarity of this OspA region to hLFA-1 may result in an immune response with cross-reactivity upon administration of *B. burgdorferi* s.s. OspA to a human subject and may induce autoimmune diseases, particularly autoimmune arthritis, in susceptible individuals. Accordingly, in a preferred embodiment, the polypeptide of the current invention does not comprise a sequence with binding capacity to the T-cell receptor that has a binding capacity to the human leukocyte function-associated antigen (hLFA-1), and particularly does not comprise the amino acid sequence GYVLEGTLTAE (SEQ ID NO: 17). To this end, the hLFA-1-like sequence, particularly the amino acid sequence GYVLEGTLTAE (SEQ ID NO: 17), may be replaced with a homologous sequence from an OspA protein of another *Borrelia sp.*, particularly with NFTLEGKVAND (SEQ ID NO: 18).

In a preferred embodiment, the polypeptide of the current invention comprising at least one disulfide bond essentially establishes the same protective capacity with said polypeptide against a *Borrelia* infection relative to at least one of the wild-type full-length OspA proteins derived from at least one *Borrelia* strain, particularly *B. afzelii* K78, OspA serotype 2 (SEQ ID NO: 19); *B. burgdorferi* s.s., particularly strain B31, serotype 1 (SEQ ID NO: 20); *B. garinii*, particularly strain PBr, serotype 3 (SEQ ID NO: 21); *B. bavariensis*, particularly strain PBi, serotype 4 (SEQ ID NO: 22); *B. garinii*, particularly strain PHei, serotype 5 (SEQ ID NO: 23); *B. garinii,* particularly strain DK29, serotype 6 (SEQ ID NO: 24) or *B. garinii*, particularly strain T25, serotype 7 (SEQ ID NO: 25).

In order to provide cross-protection against different *Borrelia* species or OspA serotypes, the development of a multivalent vaccine is desirable. Accordingly, in another preferred embodiment, the polypeptide of the first aspect comprises at least two mutant fragments from two different *Borrelia* serotypes as defined above. In a preferred embodiment, the polypeptide of the first aspect comprises at least two mutant OspA fragments which are selected from the group consisting of

- fragment with disulfide bond type 1 and fragment with disulfide bond type 2;
- fragment with disulfide bond type 1 and fragment with disulfide bond type 3;
- fragment with disulfide bond type 1 and fragment with disulfide bond type 4;
- fragment with disulfide bond type 1 and fragment with disulfide bond type 5;
- fragment with disulfide bond type 1 and fragment with disulfide bond type 6;
- fragment with disulfide bond type 1 and fragment with disulfide bond type 7;
- fragment with disulfide bond type 1 and fragment with disulfide bond type 8;
- fragment with disulfide bond type 1 and fragment with disulfide bond type 9;
- fragment with disulfide bond type 1 and fragment with disulfide bond type 10;

- fragment with disulfide bond type 1 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 1 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 3;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 4;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 5;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 6;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 7;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 8;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 9;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 10;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 2 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 3 and fragment with disulfide bond type 4;
- fragment with disulfide bond type 3 and fragment with disulfide bond type 5;
- fragment with disulfide bond type 3 and fragment with disulfide bond type 6;
- fragment with disulfide bond type 3 and fragment with disulfide bond type 7;
- fragment with disulfide bond type 3 and fragment with disulfide bond type 8;
- fragment with disulfide bond type 3 and fragment with disulfide bond type 9;
- fragment with disulfide bond type 3 and fragment with disulfide bond type 10;
- fragment with disulfide bond type 3 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 3 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 4 and fragment with disulfide bond type 5;
- fragment with disulfide bond type 4 and fragment with disulfide bond type 6;
- fragment with disulfide bond type 4 and fragment with disulfide bond type 7;
- fragment with disulfide bond type 4 and fragment with disulfide bond type 8;
- fragment with disulfide bond type 4 and fragment with disulfide bond type 9;
- fragment with disulfide bond type 4 and fragment with disulfide bond type 10;
- fragment with disulfide bond type 4 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 4 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 5 and fragment with disulfide bond type 6;
- fragment with disulfide bond type 5 and fragment with disulfide bond type 7;
- fragment with disulfide bond type 5 and fragment with disulfide bond type 8;
- fragment with disulfide bond type 5 and fragment with disulfide bond type 9;
- fragment with disulfide bond type 5 and fragment with disulfide bond type 10;

- fragment with disulfide bond type 5 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 5 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 6 and fragment with disulfide bond type 7;
- fragment with disulfide bond type 6 and fragment with disulfide bond type 8;
- fragment with disulfide bond type 6 and fragment with disulfide bond type 9;
- fragment with disulfide bond type 6 and fragment with disulfide bond type 10;
- fragment with disulfide bond type 6 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 6 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 7 and fragment with disulfide bond type 8;
- fragment with disulfide bond type 7 and fragment with disulfide bond type 9;
- fragment with disulfide bond type 7 and fragment with disulfide bond type 10;
- fragment with disulfide bond type 7 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 7 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 8 and fragment with disulfide bond type 9;
- fragment with disulfide bond type 8 and fragment with disulfide bond type 10;
- fragment with disulfide bond type 8 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 8 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 9 and fragment with disulfide bond type 10;
- fragment with disulfide bond type 9 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 9 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 10 and fragment with disulfide bond type 11;
- fragment with disulfide bond type 10 and fragment with disulfide bond type 12;
- fragment with disulfide bond type 11 and fragment with disulfide bond type 12;
- and

particularly wherein

- the fragment with disulfide bond type 1 has the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 2, wherein the cysteines are not replaced;
- the fragment with disulfide bond type 2 has the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 3, wherein the cysteines are not replaced;
- the fragment with disulfide bond type 3 has the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably

90%, even more preferably 95% sequence identity to SEQ ID NO: 4, wherein the cysteines are not replaced;

- the fragment with disulfide bond type 4 has the amino acid sequence of SEQ ID NO: 5 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 5, wherein the cysteines are not replaced;
- the fragment with disulfide bond type 5 has the amino acid sequence of SEQ ID NO: 6 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 6, wherein the cysteines are not replaced;
- the fragment with disulfide bond type 6 has the amino acid sequence of SEQ ID NO: 7 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 7, wherein the cysteines are not replaced;
- the fragment with disulfide bond type 7 has the amino acid sequence of SEQ ID NO: 8 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 8, wherein the cysteines are not replaced;
- the fragment with disulfide bond type 8 has the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 9, wherein the cysteines are not replaced;
- the fragment with disulfide bond type 9 has the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 10, wherein the cysteines are not replaced;
- the fragment with disulfide bond type 10 has the amino acid sequence of SEQ ID NO: 11 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 11, wherein the cysteines are not replaced;
- the fragment with disulfide bond type 11 has the amino acid sequence of SEQ ID NO: 12 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably 90%, even more preferably 95% sequence identity to SEQ ID NO: 12, wherein the cysteines are not replaced; and/or
- the fragment with disulfide bond type 12 has the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence that has at least 80%, more preferably 85%, more preferably

90%, even more preferably 95% sequence identity to SEQ ID NO: 13, wherein the cysteines are not replaced.

Please note that further details on mutations and sequence identity are given above.

Table A-5. Nomenclature and SEQ ID NOs. of mutant OspA fragment heterodimers, non-lipidated and lipidated, described in the current invention.

Mutant OspA fragment heterodimer*	SEQ ID NO:	Lipidated mutant OspA fragment heterodimer*	SEQ ID NO:
S1D4-S2D4	43	Lip-S1D4-S2D4	185
S1D1-S2D1	47	Lip-S1D1-S2D1	186
S3D4-S4D4	51	Lip-S3D4-S4D4	187
S3D1-S4D1	55	Lip-S3D1-S4D1	188
S5D4-S6D4	59	Lip-S5D4-S6D4	189
S5D1-S6D1	63	Lip-S5D1-S6D1	190
S2D4-S1D4	67	Lip-S2D4-S1D4	191
S2D1-S1D1	71	Lip-S2D1-S1D1	192
S4D4-S3D4	75	Lip-S4D4-S3D4	193
S4D1-S3D1	79	Lip-S4D1-S3D1	194
S6D4-S5D4	83	Lip-S6D4-S5D4	195
S6D1-S5D1	87	Lip-S6D1-S5D1	196
S1D4-S2D1	91	Lip-S1D4-S2D1	197
S1D1-S2D4	95	Lip-S1D1-S2D4	198
S3D4-S4D1	99	Lip-S3D4-S4D1	199
S3D1-S4D4	103	Lip-S3D1-S4D4	200
S5D4-S6D1	107	Lip-S5D4-S6D1	201
S5D1-S6D4	111	Lip-S5D1-S6D4	202
S2D4-S1D1	115	Lip-S2D4-S1D1	203
S2D1-S1D4	119	Lip-S2D1-S1D4	204
S4D4-S3D1	123	Lip-S4D4-S3D1	205
S4D1-S3D4	127	Lip-S4D1-S3D4	206
S6D4-S5D1	131	Lip-S6D4-S5D1	207
S6D1-S5D4	135	Lip-S6D1-S5D4	208

*S=Serotype (1-6) (see Table A-2); D=Disulfide Bond Type (see Table A-4); Lip=lipidation: the N-terminal addition of glycerol and fatty acid residues.

In another preferred embodiment, the polypeptide according to the first aspect comprises at least two or three mutant fragments which are connected via one or more linkers. A linker is a rather short amino acid sequence employed to connect two fragments. It should be designed in order to avoid any

negative impact on the fragments, their interaction in subjects to be treated or vaccinated or upon their protective capacity. Preferred are short linkers of at most 21 amino acids, particularly at most 15 amino acids, especially at most 12 or 8 amino acids. More preferably, the one or more linkers is/are composed of small amino acids in order to reduce or minimize interactions with the fragments, such as glycine, serine and alanine. Examples or preferred linkers include linkers comprising or consisting of polyG, such as $(G)_8$ (SEQ ID NO: 36) $(G)_{12}$ (SEQ ID NO: 37), GAGA (SEQ ID NO: 38), $(GAGA)_2$ (SEQ ID NO: 39), $(GAGA)_3$ (SEQ ID NO: 40), $(GGGS)_2$ (SEQ ID NO: 41), or $(GGGS)_3$ (SEQ ID NO: 42). A more preferred linker is the "LN1" peptide linker, a fusion of two separate loop regions of the N-terminal half of OspA from *B. burgdorferi* s.s., strain B31 (aa 65-74 and aa 42-53, with an amino acid exchange at position 53 of D53S) which has the following sequence: GTSDKNNGSGSKEKNKDGKYS (SEQ ID NO: 184).

In another preferred embodiment, the polypeptide according to the first aspect comprises a polypeptide with a total size of at most 500 amino acids, comprising two or three different mutant fragments as defined in preferred embodiments of the first aspect; or a polypeptide which consists of essentially two or three different mutant fragments, one or two linkers and, optionally, an N-terminal cysteine; and/or a polypeptide which consists of essentially two or three different mutant fragments, an N-terminal extension of the fragment consisting of at most 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12 or 11 amino acids, preferably at most 10, 9, 8, 7 or 6 amino acids, still more preferably at most 5, 4, 3, 2 or 1 amino acid(s), wherein the N-terminal extension is located directly N-terminally from the fragment in the respective *Borrelia* OspA and, optionally, an N-terminal cysteine. The N-terminal cysteine may optionally be followed by a short peptide linker from 1-10 amino acids long, and preferably takes the form of an N-terminal CSS peptide or CKQN peptide (SEQ ID NO: 211).

In a second aspect, the present invention relates to a nucleic acid encoding for the polypeptide according to the first aspect.

The invention further provides a nucleic acid encoding a polypeptide of the invention. For the purposes of the invention the term "nucleic acid(s)" generally refers to any polyribonucleotide or polydeoxynbonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double-stranded regions/forms.

The term "nucleic acid encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a peptide or polypeptide of the invention. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the peptide or polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion

sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal similarity to the nucleotide sequence of any native (i.e., naturally occuring) gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention, for example polynucleotides that are optimized for human and/or primate and/or E. coli codon selection.

Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. ef al., Nucl. Acids Res. Symp. Ser. pp. 215-223 (1980), Horn et al., Nucl. Acids Res. Symp. Ser. pp. 225-232 (1980)). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge et al., Science 269:202-204 (1995)) and automated synthesis may be achieved, for example, using the ASI 431 A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In a further aspect of the invention the present invention relates vector comprising a nucleic acid of the invention linked to an inducible promoter such that when the promoter is induced a polypeptide encoded by the nucleic acid is expressed. In a preferred embodiment, the vector is pET28b(+).

A further aspect of the invention comprises said vector wherein the inducible promoter is activated by addition of a sufficient quantity of IPTG (Isopropyl β-D-l-thiogalactopyranoside) preferably to the growth medium. Optionally this is at a concentration of between 0.1 and 10 mM, 0.1 and 5 mM, 0.1 and 2.5 mM, 0.2 and 10 mM, 0.2 and 5 mM, 0.2 and 2.5 mM, 0.4 and 10 mM, 1 and 10 mM, 1 and 5

mM, 2.5 and 10 mM, 2.5 and 5 mM, 5 and 10 mM. Alternatively the promoter may be induced by a change in temperature or pH.

Nucleic acid molecule as used herein generally refers to any ribonucleic acid molecule or deoxyribonucleic acid molecule, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, nucleic acid molecule as used herein refers to at least single- and double-stranded DNA, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or a mixture of single- and double-stranded regions. As used herein, the term nucleic acid molecule includes DNA or RNA molecules as described above that contain one or more modified bases. Thus, DNA or RNA molecules with backbones modified for stability or for other reasons are "nucleic acid molecule" as that term is intended herein. Moreover, DNA or RNA species comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are also nucleic acid molecules as defined herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA molecules that serve many useful purposes known to those of skill in the art. The term nucleic acid molecule as used herein embraces such chemically, enzymatically or metabolically modified forms of nucleic acid molecules, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, *inter alia*. The term nucleic acid molecule also encompasses short nucleic acid molecules often referred to as oligonucleotide(s). The terms "polynucleotide" and "nucleic acid" or "nucleic acid molecule" are used interchangeably herein.

The nucleic acids according to the present invention may be chemically synthesized. Alternatively, the nucleic acids can be isolated from *Borrelia* and modified by methods known to one skilled in the art. The same applies to the polypeptides according to the present invention.

Furthermore, the nucleic acid of the present invention can be functionally linked, using standard techniques such as cloning, to any desired sequence(s), whether a *Borrelia* regulatory sequence or a heterologous regulatory sequence, heterologous leader sequence, heterologous marker sequence or a heterologous coding sequence to create a fusion gene.

Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA or cRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthesis techniques or by a combination thereof. The DNA may be triple-stranded, double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The nucleic acid of the present invention may be comprised in a vector or in a cell. The vector may comprise the above-mentioned nucleic acid in such a manner that the vector is replicable and can express the protein encoded by the nucleotide sequence in a host cell.

For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or nucleic acid of the invention. Introduction of a nucleic acid into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, et al., BASIC METHODS IN MOLECULAR BIOLOGY, (1986) and Sambrook, et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, conjugation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include gram negative bacterial cells, such as cells of E. coli, Acinetobacter, Actinobacillus, Bordetella, Brucella, Campylobacter, Cyanobacteria, Enterobacter, Erwinia, Franciscella, Helicobacter, hemophilus, Klebsiella, Legionella, Moraxella, Neisseria, Pasteurella, Proteus, Pseudomonas, Salmonella, Serratia, Shigella, Treponema, Vibrio, Yersinia. In one embodiment the host cell is an Escherichia coli cell. In a preferred embodiment, the host cell is an E. coli BL21 (DE3) cell or an E. coli BL21 Star™ (DE3) cell.

Alternatively gram positive bacterial cells may also be used. A great variety of expression systems can be used to produce the polypeptides of the invention. In one embodiment the vector is derived from bacterial plasmids. Generally any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL, (supra).

In one embodiment of the current invention, the cells are grown under selective pressure, such as in the presence of antibiotics, preferably kanamycin. In another embodiment, cells are grown in the absence of antibiotics.

A great variety of expression vectors can be used to express the polypeptides according to the present invention. Generally, any vector suitable to maintain, propagate or express nucleic acids to express a polypeptide in a host may be used for expression in this regard. In accordance with this aspect of the

invention the vector may be, for example, a plasmid vector, a single- or double-stranded phage vector or a single- or double-stranded RNA or DNA viral vector. Starting plasmids disclosed herein are either commercially available, publicly available, or can be constructed from available plasmids by routine application of well-known, published procedures. Preferred among vectors, in certain respects, are those for expression of nucleic acid molecules and the polypeptides according to the present invention. Nucleic acid constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides according to the present invention can be synthetically produced by conventional peptide synthesizers.

In addition, the present invention relates to a host cell comprising this vector. Representative examples of appropriate host cells include bacteria, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis*; fungi, such as yeast and *Aspergillus*; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; mammalian cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 or Bowes melanoma cells; and plant cells. Cell-free translation systems can also be employed to produce such proteins using RNA derived from the DNA construct of the present invention.

In order to express the desired amino acid sequence practically by introducing the vector according to the present invention into a host cell, the vector may contain, in addition to the nucleic acid sequence according to the present invention, other sequences for controlling the expression (*e.g.*, promoter sequences, terminator sequences and enhancer sequences) and gene markers for selecting microorganisms, insect cells, animal culture cells, or the like (*e.g.*, neomycin resistance genes and kanamycin resistance genes). Furthermore, the vector may contain the nucleic acid sequence according to the present invention in a repeated form (*e.g.*, in tandem). The vector may be constructed based on procedures and manners which are conventionally used in the field of genetic engineering.

The host cells may be cultured in an appropriate medium, and the protein according to the present invention may be obtained from the culture product. The protein according to the present invention may be recovered from the culture medium and purified in the conventional manner.

The problem underlying the present invention is furthermore solved by a method for producing a polypeptide as defined above, characterized by the following steps:

a) introducing a vector encoding the polypeptide into a host cell,

b) growing the host cell under conditions allowing for expression of said polypeptide,

c) homogenizing said host cell, and

d) subjecting the host cell homogenate to purification steps.

The invention further relates to a method for producing a polypeptide as defined above, characterized by the following steps:

a) introducing a nucleic acid encoding a polypeptide into a vector,
b) introducing said vector into a host cell,
c) growing said host cell under conditions allowing for expression of polypeptide,
d) homogenizing said host cell,
e) enriching polypeptide in the lipid phase by phase separation, and
f) further purifying over a gel filtration column.

The invention further relates to a method for producing a polypeptide as defined above, characterized by the following steps:

a) introducing a nucleic acid encoding a polypeptide into a vector,
b) introducing said vector into a host cell,
c) growing said host cell under conditions allowing for expression of polypeptide,
d) homogenizing said host cell,
e) enriching polypeptide in the lipid phase by phase separation,
g) purifying over a gel filtration column, and
h) optionally, further processing over a buffer exchange column.

The problem underlying the present invention is solved in a further aspect by an antibody, or at least an effective part thereof, which specifically binds to at least a selective part of a polypeptide, as defined above.

In a preferred embodiment the antibody is a monoclonal antibody.

In another preferred embodiment said effective part comprises an Fab fragment, an F(ab) fragment, an F(ab)N fragment, an $F(ab)_2$ fragment or an F_v fragment.

In still another embodiment of the invention the antibody is a chimeric antibody.

In yet another embodiment the antibody is a humanized antibody.

In a preferred aspect, antibodies of the invention bind specifically to mutant OspA fragment polypeptides of the invention, but not to corresponding wild-type OspA fragment polypeptides. In a more preferred aspect, the antibody binds specifically to the disulfide bond of the mutant OspA fragment of the invention.

The term "specificity" refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule or antigen-binding protein (such as a Nanobody or a polypeptide of the invention) molecule can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity. The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding protein (K_D), is a measure for the binding strength between an antigenic determinant and an antigen-binding site on the antigen-binding protein: the lesser the value of the K_D, the stronger the binding strength between an antigenic determinant and the antigen-binding molecule (alternatively, the affinity can also be expressed as the affinity constant (K_A), which is $1/K_D$).

As will be clear to the skilled person (for example on the basis of the further disclosure herein), affinity can be determined in a manner known per se, depending on the specific antigen of interest. Avidity is the measure of the strength of binding between an antigen-binding molecule (such as an antibody or an effective part thereof of the invention) and the pertinent antigen. Avidity is related to both the affinity between an antigenic determinant and its antigen binding site on the antigen-binding molecule and the number of pertinent binding sites present on the antigen-binding molecule. Typically, antigen-binding proteins (such as an antibody or an effective part thereof of the invention) will bind to their antigen with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles). Any K_D value greater than 10^4 mol/liter (or any K_A value lower than 10^4 M^{-1}) liters/mol is generally considered to indicate non-specific binding. Preferably, a monovalent immunoglobulin sequence of the invention will bind to the desired antigen with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner known per se, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known per se in the art, as well as the other techniques mentioned herein.

The dissociation constant may be the actual or apparent dissociation constant, as will be clear to the skilled person. Methods for determining the dissociation constant will be clear to the skilled person, and for example include the techniques mentioned herein. In this respect, it will also be clear that it may not be possible to measure dissociation constants of more than 10^{-4} moles/liter or 10^{-3} moles/liter (e.g., of 10^{-2} moles/liter). Optionally, as will also be clear to the skilled person, the (actual or apparent) dissociation constant may be calculated on the basis of the (actual or apparent) association constant (K_A), by means of the relationship [$K_D = 1/K_A$].

The affinity denotes the strength or stability of a molecular interaction. The affinity is commonly given as by the K_D, or dissociation constant, which has units of mol/liter (or M). The affinity can also be expressed as an association constant, K_A, which equals $1/K_D$ and has units of $(mol/liter)^{-1}$ (or M^{-1}). In the present specification, the stability of the interaction between two molecules (such as an amino acid sequence, Nanobody or polypeptide of the invention and its intended target) will mainly be expressed in terms of the K_D value of their interaction; it being clear to the skilled person that in view of the relation $K_A = 1/K_D$, specifying the strength of molecular interaction by its K_D value can also be used to calculate the corresponding K_A value. The K_D value characterizes the strength of a molecular interaction also in a thermodynamic sense as it is related to the free energy (DG) of binding by the well known relation $DG = RT.\ln(K_D)$ (equivalently $DG = -RT.\ln(K_A)$), where R equals the gas constant, T equals the absolute temperature and ln denotes the natural logarithm.

The K_D for biological interactions which are considered meaningful (e.g. specific) are typically in the range of 10^{-10} M (0.1 nM) to 10^{-5} M (10000 nM). The stronger an interaction, the lower its K_D.

In a preferred embodiment, the K_D of the antibody of the invention is between 10^{-12} M and 10^{-5} M, preferably less than 10^{-6}, preferably less than 10^{-7}, preferably less than 10^{-8} M, preferably less than 10^{-9} M, more preferably less than 10^{-10} M, even more preferably less than 10^{-11} M, most preferably less than 10^{-12} M.

The K_D can also be expressed as the ratio of the dissociation rate constant of a complex, denoted as k_{off}, to the rate of its association, denoted k_{on} (so that $K_D = k_{off}/k_{on}$ and $K_A = k_{on}/k_{off}$). The off-rate k_{off} has units s^{-1} (where s is the SI unit notation for second). The on-rate k_{on} has units $M^{-1}\,s^{-1}$. The on-rate may vary between $10^2\,M^{-1}\,s^{-1}$ to about $10^7\,M^{-1}s^{-1}$, approaching the diffusion-limited association rate constant for bimolecular interactions. The off-rate is related to the half-life of a given molecular interaction by the relation $t_{1/2} = \ln(2)/k_{off}$. The off-rate may vary between $10^{-6}\,s^{-1}$ (near irreversible complex with a $t_{1/2}$ of multiple days) to $1\,s^{-1}$ ($t_{1/2} = 0.69$ s).

The affinity of a molecular interaction between two molecules can be measured via different techniques known per se, such as the well known surface plasmon resonance (SPR) biosensor technique (see for example Ober et al., Intern. Immunology, 13, 1551-1559, 2001) where one molecule is immobilized on the biosensor chip and the other molecule is passed over the immobilized molecule under flow conditions yielding k_{on}, k_{off} measurements and hence K_D (or K_A) values. This can for example be performed using the well-known BIACORE instruments.

It will also be clear to the skilled person that the measured K_D may correspond to the apparent K_D if the measuring process somehow influences the intrinsic binding affinity of the implied molecules for example by artefacts related to the coating on the biosensor of one molecule. Also, an apparent K_D may be measured if one molecule contains more than one recognition sites for the other molecule. In such situation the measured affinity may be affected by the avidity of the interaction by the two molecules.

Another approach that may be used to assess affinity is the 2-step ELISA (Enzyme-Linked Immunosorbent Assay) procedure of Friguet et al. (J. Immunol. Methods, 77, 305-19, 1985). This method establishes a solution phase binding equilibrium measurement and avoids possible artefacts relating to adsorption of one of the molecules on a support such as plastic.
However, the accurate measurement of K_D may be quite labor-intensive; therefore, apparent K_D values are often determined in order to assess the binding strength of two molecules. It should be noted that as long all measurements are made in a consistent way (e.g. keeping the assay conditions unchanged), apparent K_D measurements can be used as an approximation of the true K_D and hence in the present document K_D and apparent K_D should be treated with equal importance or relevance.

Finally, it should be noted that in many situations the experienced scientist may judge it to be convenient to determine the binding affinity relative to some reference molecule. For example, to assess the binding strength between molecules A and B, one may e.g. use a reference molecule C that is known to bind to B and that is suitably labelled with a fluorophore or chromophore group or other chemical moiety, such as biotin for easy detection in an ELISA or flow cytometry or other format (the fluorophore for fluorescence detection, the chromophore for light absorption detection, the biotin for streptavidin-mediated ELISA detection). Typically, the reference molecule C is kept at a fixed concentration and the concentration of A is varied for a given concentration or amount of B. As a result an Inhibitory Concentration $(IC)_{50}$ value is obtained corresponding to the concentration of A at which the signal measured for C in absence of A is halved. Provided $K_{D\ ref}$, the K_D of the reference molecule, is known, as well as the total concentration c_{ref} of the reference molecule, the apparent K_D for the interaction A-B can be obtained from following formula: $K_D = IC_{50}/(1 + c_{ref}/K_{Dref})$. Note that if

$c_{ref} << K_{Dref}$, $K_D \approx IC_{50}$. Provided the measurement of the IC_{50} is performed in a consistent way (e.g. keeping c_{ref} fixed) for the binders that are compared, the strength or stability of a molecular interaction can be assessed by the IC_{50} and this measurement is judged as equivalent to K_D or to apparent K_D throughout this text.

Another aspect of the invention relates to a hybridoma cell line, which produces an antibody as defined above.

The problem underlying the present invention is furthermore solved by a method for producing an antibody as defined above, characterized by the following steps:

a) initiating an immune response in a non-human animal by administering a polypeptide as defined above to said animal,
b) removing an antibody containing body fluid from said animal, and
c) producing the antibody by subjecting said antibody containing body fluid to further purification steps.

The invention further relates to a method for producing an antibody as defined above, characterized by the following steps:

a) initiating an immune response in a non-human animal by administering a polypeptide as defined above to said animal,
b) removing the spleen or spleen cells from said animal,
c) producing hybridoma cells of said spleen or spleen cells,
d) selecting and cloning hybridoma cells specific for said polypeptide,
e) producing the antibody by cultivation of said cloned hybridoma cells, and
f) optionally conducting further purification steps.

Another aspect of the present invention is related to a pharmaceutical composition comprising an antibody as specified above.

Still another aspect relates to an antibody as defined above or a pharmaceutical composition comprising an antibody as defined above for the treatment or prevention of an infection with *Borrelia* species, more preferably pathogenic *Borrelia* species as disclosed herein more preferably comprising *B. burgdorferi* s.s., *B. afzelii, B. bavariensis* and *B. garinii.*

The problem underlying the present invention is solved in another aspect by the use of an antibody as defined above for the preparation of a pharmaceutical composition for treating or preventing infections with *Borrelia* species, more preferably pathogenic *Borrelia* species as disclosed herein more preferably comprising *B. burgdorferi* s.s., *B. afzelii, B. bavariensis* and *B. garinii.*

In a third aspect the present invention relates to a pharmaceutical composition comprising the polypeptide according to the first aspect and/or the nucleic acid according to the second aspect. The pharmaceutical composition may optionally contain any pharmaceutically acceptable carrier or excipient, such as buffer substances, stabilisers or further active ingredients, especially ingredients known in connection with pharmaceutical compositions and/or vaccine production. Preferably, the pharmaceutical composition is used as a medicament, particularly as a vaccine or for preventing or treating an infection caused by *Borrelia* species, more preferably pathogenic *Borrelia* species as disclosed herein more preferably comprising *B. burgdorferi* s.s., *B. afzelii, B. bavariensis* and *B. garinii,* and/or other pathogens against which the antigens have been included in the vaccine.

In one embodiment the pharmaceutical composition further comprises an adjuvant. The choice of a suitable adjuvant to be mixed with bacterial toxins or conjugates made using the processes of the invention is within the knowledge of the person skilled in the art. Suitable adjuvants include an aluminium salt such as aluminium hydroxide or aluminum phosphate, but may also be other metal salts such as those of calcium, magnesium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatized saccharides, or polyphosphazenes. In a preferred embodiment, the pharmaceutical composition is adjuvanted with aluminium hydroxide.

In a further embodiment, the pharmaceutical composition further comprises an immunostimulatory substance, preferably selected from the group consisting of polycationic polymers, especially polycationic peptides, immunostimulatory oligodeoxynucleotides (ODNs), especially oligo(dIdC)$_{13}$ (SEQ ID NO: 32), peptides containing at least two LysLeuLys motifs, especially peptide KLKLLLLLKLK (SEQ ID NO: 33), neuroactive compounds, especially human growth hormone, aluminium hydroxide, aluminium phosphate, Freund's complete or incomplete adjuvants, or combinations thereof. Preferably, the immunostimulatory substance is a combination of either a polycationic polymer and immunostimulatory deoxynucleotides or of a peptide containing at least two LysLeuLys motifs and immunostimulatory deoxynucleotides, preferably a combination of KLKLLLLLKLK (SEQ ID NO: 33) and oligo(dIdC)$_{13}$ (SEQ ID NO: 32). More preferably, said polycationic peptide is polyarginine.

In a further embodiment, the pharmaceutical composition comprises sodium phosphate, sodium chloride, L-methionine, sucrose and Tween-20 at a pH of 6.7 +/- 0.2. Preferably, the pharmaceutical composition also comprises aluminium hydroxide, preferably at a concentration of 0.15%.

In one embodiment, the formulation comprises between 5 mM and 50 mM sodium phosphate, between 100 and 200 mM sodium chloride, between 5 mM and 25 mM L-Methionine, between 2.5% and 10% Sucrose, between 0.01% and 0.1% Tween 20 and between 0.1% and 0.2% (w/v) aluminium hydroxide. More preferably, the formulation comprises 10 mM sodium phosphate, 150 mM sodium chloride, 10 mM L-Methionine, 5% Sucrose, 0.05% Tween 20 and 0.15% (w/v) aluminium hydroxide at pH 6.7 ± 0.2. Even more preferably, the formulation comprises at least one, at least two, at least three mutant OspA heterodimers according to the invention.

In one embodiment, the pharmaceutical composition comprises 3 heterodimers, preferably Lip-S1D1-S2D1 (SEQ ID NO: 186), Lip-S4D1-S3D1 (SEQ ID NO: 194) and Lip-S5D1-S6D1 (SEQ ID NO: 190). Preferably, the three heterodimers are mixed at a molar ratio of 1:2:1, 1:3:1, 1:1:2, 1:1:3, 1:2:2, 1:2:3, 1:3:2, 1:3:3, 2:1:1, 2:1:2, 2:1:3, 2:2:3, 2:2:1, 2:3:1, 2:3:2, 2:3:3, 3:1:1, 3:1:2, 3:1:3, 3:2:1, 3:2:2, 3:2:3, 3:3:1, 3:3:2, most preferably 1:1:1.

In a further embodiment, the pharmaceutical composition comprises two heterodimers, preferably Lip-S1D1-S2D1 (SEQ ID NO: 186) and Lip-S5D1-S6D1 (SEQ ID NO: 190), Lip-S1D1-S2D1 (SEQ ID NO: 186) and Lip-S4D1-S3D1 (SEQ ID NO: 194) or Lip-S4D1-S3D1 (SEQ ID NO: 194) and Lip-S5D1-S6D1 (SEQ ID NO: 190) in a molar ratio of 1:2, 1:3, 2:1, 3:1, 2:3, 3:2, preferably 1:1.

In one embodiment the pharmaceutical composition or vaccine of the invention further comprises at least one additional antigen (herein referred to generically as “combination vaccine”). In a preferred embodiment, the at least one additional antigen is derived from a *Borrelia* species causing Lyme borreliosis. In various aspects, the at least one additional antigen is derived from another pathogen, preferably a tick-borne pathogen. In a further aspect, the pathogen causes Rocky Mountain spotted fever, Human granulocytic ehrlichiosis (HGE), Sennetsu Fever, Human Monocytic Ehrlichiosis (HME), Anaplasmosis, Boutonneuse fever, Rickettsia parkeri Rickettsiosis, Southern Tick-Associated Rash Illness (STARI), Helvetica Spotted fever, 364D Rickettsiosis, African spotted fever, Relapsing fever, Tularemia, Colorado tick fever, Tick-borne encephalitis (TBE, also known as FSME), Crimean-Congo hemorrhagic fever, Q fever, Omsk hemorrhagic fever, Kyasanur forest disease, Powassan encephalitis, Heartland virus disease or Babesiosis. In a further aspect, the disease is Japanese encephalitis.

In a further embodiment, the at least one additional antigen is derived from a vector-borne, preferably a tick-borne, pathogen selected from the group comprising *Borrelia hermsii*, *Borrelia parkeri*, *Borrelia duttoni*, *Borrelia miyamotoi*, *Borrelia turicatae*, *Rickettsia rickettsii*, *Rickettsia australis*, *Rickettsia conori*, *Rickettsia helvetica*, *Francisella tularensis*, *Anaplasma phagocytophilum, Ehrlichia sennetsu*, *Ehrlichia chaffeensis*, *Coxiella burnetii* and *Borrelia lonestari*, Tick-borne encephalitis virus (TBEV aka FSME virus), Colorado tick fever virus (CTFV), Crimean-Congo hemorrhagic fever virus (CCHFV), Omsk Hemorrhagic Fever virus (OHFV), Japanese encepalitis virus (JEV) and *Babesia* spp.

In another aspect, a combination vaccine of the invention comprises any vaccine composition discussed herein in combination with at least a second vaccine composition. In some aspects, the second vaccine composition protects against a vector-borne disease, preferably a tick-borne disease. In various aspects, the second vaccine composition has a seasonal immunization schedule compatible with immunization against *Borrelia* infection or Lyme borreliosis. In other aspects, combination vaccines are useful in the prevention of multiple diseases for use in geographical locations where these diseases are prevalent.

In one aspect, the second vaccine composition is a vaccine selected from the group consisting of a tick-borne encephalitis vaccine, a Japanese encephalitis vaccine, and a Rocky Mountain Spotted Fever vaccine. In a preferred aspect, the vaccine composition is FSME-IMMUN® (Baxter), Encepur® (Novartis Vaccines), EnceVir® (Microgen NPO) or TBE Moscow Vaccine® (Chumakov Institute of Poliomyelitis and Viral Encephalitides of Russian Academy of Medical Sciences). In another preferred aspect, the vaccine composition is IXIARO®/JESPECT® (Valneva SE), JEEV® (Biological E, Ltd.) or IMOJEV® (Sanofi Pasteur).

There is further provided a vaccine comprising the pharmaceutical composition, this vaccine may further comprise a pharmaceutically acceptable excipient. In a preferred embodiment, the excipient is L-methionine.

The invention also includes immunogenic compositions. In some aspects, an immunogenic composition of the invention comprises any of the compositions discussed herein and a pharmaceutically acceptable carrier. In various aspects, the immunogenic composition has the property of inducing production of an antibody that specifically binds an outer surface protein A (OspA) protein. In certain aspects, the immunogenic composition has the property of inducing production of an antibody that specifically binds *Borrelia*. In particular aspects, the immunogenic composition has the property of inducing production of an antibody that neutralizes *Borrelia*. In some

aspects, the antibody is produced by an animal. In further aspects, the animal is a mammal. In even further aspects, the mammal is human.

The vaccine preparations containing pharmaceutical compositions of the present invention may be used to protect a mammal susceptible to *Borrelia* infection or treat a mammal with a *Borrelia* infection, by means of administering said vaccine via a systemic or mucosal route. These administrations may include injection via the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. Although the vaccine of the invention may be administered as a single dose, components thereof may also be co-administered together at the same time or at different times.

In one aspect of the invention is provided a vaccine kit, comprising a vial containing a pharmaceutical composition of the invention, optionally in lyophilised form, and further comprising a vial containing an adjuvant as described herein. It is envisioned that in this aspect of the invention, the adjuvant will be used to reconstitute the lyophilised immunogenic composition. In a further aspect, the pharmaceutical composition of the invention may be pre-mixed in a vial, preferably in a syringe.

A further aspect of the invention is a method of preventing or treating *Borrelia* infection comprising administering to the host an immunoprotective dose of the pharmaceutical composition or vaccine or kit of the invention. In one embodiment there is provided a method of preventing or treating primary and/or recurrence episodes of *Borrelia* infection comprising administering to the host an immunoprotective dose of the pharmaceutical composition or vaccine or kit of the invention.

A further aspect of the invention is a pharmaceutical composition of the invention for use in the treatment or prevention of Borrelial disease. In one embodiment there is provided a pharmaceutical composition for use in the treatment or prevention of *Borrelia* infection.

A further aspect of the invention is the use of the pharmaceutical composition or vaccine or kit of the invention in the manufacture of a medicament for the treatment or prevention of *Borrelia* infection. In one embodiment there is provided a pharmaceutical composition of the invention for use in the manufacture of a medicament for the treatment or prevention of *Borrelia* infection.

The invention also includes methods for inducing an immunological response in a subject. In various aspects, such methods comprise the step of administering any of the immunogenic compositions or vaccine compositions discussed herein to the subject in an amount effective to induce an

immunological response. In certain aspects, the immunological response comprises production of an anti-OspA antibody.

The invention includes methods for preventing or treating a *Borrelia* infection or Lyme boreliosis in a subject. In various aspects, such methods comprise the step of administering any of the vaccine compositions discussed herein or any of the combination vaccines discussed herein to the subject in an amount effective to prevent or treat the *Borrelia* infection or Lyme borreliosis.

The invention includes uses of polypeptides, nucleic acids, antibodies, pharmaceutical compositions or vaccines of the invention for the preparation of medicaments. Other related aspects are also provided in the instant invention.

The terms "comprising", "comprise" and "comprises" herein are intended by the inventors to be optionally substitutable with the terms "consisting of", "consist of" and "consists of", respectively, in every instance. The term "comprises" means "includes". Thus, unless the context requires otherwise, the word "comprises", and variations such as "comprise" and "comprising" will be understood to imply the inclusion of a stated compound or composition (e.g., nucleic acid, polypeptide, antibody) or step, or group of compounds or steps, but not to the exclusion of any other compounds, composition, steps, or groups thereof. The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example".

Embodiments herein relating to "vaccine compositions" of the invention are also applicable to embodiments relating to "pharmaceutical compositions" of the invention, and vice versa.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Definitions of common terms in molecular biology can be found in Benjamin Lewin, Genes V, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew ef al. (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1 -56081 -569-8).

The singular terms "a", "an", and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. The term "plurality" refers to two or more. It is further to be understood that all base sizes

or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Additionally, numerical limitations given with respect to concentrations or levels of a substance, such as an antigen, may be approximate.

A preferable carrier or excipient for the polypeptides according to the present invention in their diverse embodiments, or a nucleic acid molecule according to the present invention is an immunostimulatory compound such as an adjuvant for further stimulating the immune response to the polypeptide according to the present invention or a coding nucleic acid molecule thereof.

Adjuvants which may be used in compositions of the invention include, but are not limited to:

A. Mineral-containing compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.,* oxyhydroxides), phosphates (*e.g.,* hydroxyphosphates, orthophosphates), sulphates, etc., or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.,* gel, crystalline, amorphous, etc.), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt.

A useful aluminium phosphate adjuvant is amorphous aluminium hydroxyphosphate with PO_4/ Al molar ratio between 0.84 and 0.92. Another useful aluminium-based adjuvant is AS04, a combination of aluminium hydroxide + monophosphoryl lipid A (MPL).

B. Oil Emulsions

Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-in-water emulsions, such as MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer), AS03 (squalene, DL-α-tocopherol and Tween 80) and AF03 (squalene, Montane® 80 and Eumulgon® B1 PH). Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

Useful oil-in-water emulsions typically include at least one oil and at least one surfactant, with the oil(s) and surfactant(s) being biodegradable (metabolizable) and biocompatible. The oil droplets in the emulsion are generally less than 1 μm in diameter, with these small sizes being achieved with a microfluidizer to provide stable emulsions. Droplets with a size less than 220 nm are preferred as they can be subjected to filter sterilization.

The emulsion can comprise oils such as those from an animal (such as fish) or vegetable source. Sources for vegetable oils include nuts, seeds and grains. Peanut oil, soybean oil, coconut oil, and olive oil, the most commonly available, exemplify the nut oils. Jojoba oil can be used *e.g.,* obtained from the jojoba bean. Seed oils include safflower oil, cottonseed oil, sunflower seed oil, sesame seed oil and the like. In the grain group, corn oil is the most readily available, but the oil of other cereal grains such as wheat, oats, rye, rice, teff, triticale and the like may also be used. 6-10 carbon fatty acid esters of glycerol and 1,2-propanediol, while not occurring naturally in seed oils, may be prepared by hydrolysis, separation and esterification of the appropriate materials starting from the nut and seed oils. Fats and oils from mammalian milk are metabolizable and may therefore be used in the practice of this invention. The procedures for separation, purification, saponification and other means necessary for obtaining pure oils from animal sources are well known in the art. Most fish contain metabolizable oils which may be readily recovered. For example, cod liver oil, shark liver oils, and whale oil such as spermaceti exemplify several of the fish oils which may be used herein. A number of branched chain oils are synthesized biochemically in 5-carbon isoprene units and are generally referred to as terpenoids. Shark liver oil contains a branched, unsaturated terpenoid known as squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, which is particularly preferred herein. Squalane, the saturated analog to squalene, is also a preferred oil. Fish oils, including squalene and squalane, are readily available from commercial sources or may be obtained by methods known in the art. Other preferred oils are the tocopherols (see below). Mixtures of oils can be used.

Surfactants can be classified by their 'HLB' (hydrophile/lipophile balance). Preferred surfactants of the invention have a HLB of at least 10, preferably at least 15, and more preferably at least 16. The invention can be used with surfactants including, but not limited to: the polyoxyethylene sorbitan esters surfactants (commonly referred to as the Tweens), especially polysorbate 20 and polysorbate 80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWF AX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-l,2-ethanediyl) groups, with octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol) being of particular interest; (octylphenoxy) polyethoxyethanol (IGEPAL CA-630/NP-40); phospholipids such as phosphatidylcholine (lecithin); nonylphenol ethoxylates, such as the Tergitol™ NP series; polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); and sorbitan esters (commonly known as the SPANs), such as sorbitan trioleate (Span 85) and sorbitan monolaurate. Non-ionic surfactants are preferred. Preferred surfactants for including in the emulsion are Tween 80 (polyoxyethylene sorbitan monooleate), Span 85 (sorbitan trioleate), lecithin and Triton X-100.

Mixtures of surfactants can be used *e.g.,* Tween 80/Span 85 mixtures. A combination of a polyoxyethylene sorbitan ester such as polyoxyethylene sorbitan monooleate (Tween 80) and an octoxynol such as t-octylphenoxypolyethoxyethanol (Triton X-100) is also suitable. Another useful combination comprises laureth 9 plus a polyoxyethylene sorbitan ester and/or an octoxynol.

Preferred amounts of surfactants (% by weight) are: polyoxyethylene sorbitan esters (such as Tween 80) 0.01 to 1%, in particular about 0.1%; octyl- or nonylphenoxy polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001 to 0.1%, in particular 0.005 to 0.02%; polyoxyethylene ethers (such as laureth 9) 0.1 to 20%, preferably 0.1 to 10% and in particular 0.1 to 1% or about 0.5%.

Preferably, substantially all (e.g. at least 90% by number) of the oil droplets have a diameter of less than l μm, e.g. <750 nm, <500 nm, <400 nm, <300 nm, <250 nm, <220 nm, <200 nm, or smaller. One specific useful submicron emulsion consists of squalene, Tween 80, and Span 85. The composition of the emulsion by volume can be about 5% squalene, about 0.5% polysorbate 80 and about 0.5% Span 85. In weight terms, these ratios become 4.3% squalene, 0.5% polysorbate 80 and 0.48% Span 85. The MF59 emulsion advantageously includes citrate ions e.g. 10 mM sodium citrate buffer.

C. Saponin formulations

Saponin formulations may also be used as adjuvants in the invention. Saponins are a heterogeneous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the Quillaia saponaria Molina tree has been widely studied as adjuvant. Saponin can also be commercially obtained from Smilax ornata (sarsaprilla), Gypsophilla paniculata (brideal veil), and Saponaria officianalis (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. QS21 is marketed as Stimulon™.

Saponin compositions have been purified using HPLC and RP-HPLC. Specific purified fractions using these techniques have been identified, including QS7, QS 17, QS l8, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. Saponin formulations may also comprise a sterol, such as cholesterol.

Combinations of saponins and cholesterols can be used to form unique particles called immunostimulating complexes (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs.

Preferably, the ISCOM includes one or more of QS7, QS 17, QS l8, QS21, QH-A, QH-B and QH-C. Optionally, the ISCOMS may be devoid of additional detergent.

D. Virosomes and virus-like particles

Virosomes and virus-like particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retroviruses, Norwalk virus, Human Papilloma virus, HIV, RNA-phages, Qβ-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein pi).

E. Bacterial or microbial derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof.

Non-toxic derivatives of LPS include monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 de-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 μm membrane. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives e.g. RC-529 and the synthetic phospholipid dimer, E6020.

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a dinucleotide sequence containing an unmethylated cytosine linked by a phosphate bond to a guanosine). Double-stranded RNAs and oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. The CpG sequence may be specific for inducing a Th1 immune

response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". A particularly useful adjuvant based around immunostimulatory oligonucleotides is known as IC31®. Thus an adjuvant used with the invention may comprise a mixture of (i) an oligonucleotide (e.g. between 15-40 nucleotides) including at least one (and preferably multiple) CpI motifs (i.e. a cytosine linked to an inosine to form a dinucleotide), and (ii) a polycationic polymer, such as an oligopeptide (e.g. between 5-20 amino acids) including at least one (and preferably multiple) Lys-Arg-Lys tripeptide sequence(s). The oligonucleotide may be a deoxynucleotide comprising the 26-mer sequence 5'-$(dIdC)_{13}$-3' (SEQ ID NO: 32). The polycationic polymer may be a peptide comprising the 11-mer amino acid sequence KLKLLLLLKLK (SEQ ID NO: 33).

Polycationic compounds derived from natural sources include HIV-REV or HIV-TAT (derived cationic peptides, antennapedia peptides, chitosan or other derivatives of chitin) or other peptides derived from these peptides or proteins by biochemical or recombinant production. Other preferred polycationic compounds are cathelin or related or derived substances from cathelin. For example, mouse cathelin is a peptide, which has the amino acid sequence NH_2-RLAGLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPE-COOH (SEQ ID NO: 31). Related or derived cathelin substances contain the whole or parts of the cathelin sequence with at least 15-20 amino acid residues. Derivations may include the substitution or modification of the natural amino acids by amino acids which are not among the 20 standard amino acids. Moreover, further cationic residues may be introduced into such cathelin molecules. These cathelin molecules are preferred to be combined with the antigen. These cathelin molecules surprisingly have turned out to be also effective as an adjuvant for an antigen without the addition of further adjuvants. It is therefore possible to use such cathelin molecules as efficient adjuvants in vaccine formulations with or without further immune activating substances.

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (*E. coli* heat labile enterotoxin "LT"), *Vibrio cholerae* (Cholera toxin "CT"), or *Bordetella pertussis* (Pertussis toxin "PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants and as parenteral adjuvants is known. The toxin or toxoid is preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, LT-G192 or dmLT. A useful CT mutant is CT-E29H.

F. Human immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g. interferon-γ), macrophage colony stimulating factor and tumor necrosis factor. A preferred immunomodulator is IL-12.

G. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres or mucoadhesives such as cross-linked derivatives of polyacrylic acid, polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention.

H. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (i.e. a particle of ~100 nm to ~150 μm in diameter, more preferably ~200 nm to ~30 μm in diameter, and most preferably ~500 nm to ~10 μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.,* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, a poly(lactide-co-glycolide) etc.), wherein poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (e.g. with SDS) or a positively-charged surface (*e.g.,* with a cationic detergent, such as CTAB).

I. Liposomes

Examples of liposome formulations suitable for use as adjuvants are known.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

K. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(l'-2'-dipalmitoyl-5n-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use as adjuvants in the invention include Imiquimod and its homologues (*e.g.*, "Resiquimod 3M").

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above.

Preferably, the immunostimulatory compound in the pharmaceutical preparation according to the present invention is selected from the group of polycationic substances, especially polycationic peptides, immunostimulatory nucleic acids molecules, preferably immunostimulatory deoxynucleotides, oil-in-water or water-in-oil emulsions, MF59, aluminium salts, Freund's complete adjuvant, Freund's incomplete adjuvant, neuroactive compounds, especially human growth hormone, or combinations thereof.

The use of an aluminium hydroxide and/or aluminium phosphate adjuvant is particularly preferred, and antigens are generally adsorbed to these salts.

Also, the pharmaceutical composition in accordance with the present invention is a pharmaceutical composition which comprises at least any of the following compounds or combinations thereof: the nucleic acid molecules according to the present invention, the polypeptides according to the present invention in their diverse embodiments, the vector according to the present invention, the cells according to the present invention and the antibody according to the present invention. In connection therewith, any of these compounds may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

In one embodiment, the pharmaceutical composition comprises a stabilizer. The term "stabilizer" refers to a substance or vaccine excipient which protects the immunogenic composition of the vaccine

from adverse conditions, such as those which occur during heating or freezing, and/or prolongs the stability or shelf-life of the immunogenic composition in a stable and immunogenic condition or state. Examples of stabilizers include, but are not limited to, sugars, such as sucrose, lactose and mannose; sugar alcohols, such as manitol; amino acids, such as glycine or glutamic acid; and proteins, such as human serum albumin or gelatin.

The pharmaceutical compositions of the present invention may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, intratracheal or intradermal routes, among others. In a preferred embodiment, the pharmaceutical compositions are administered subcutaneously or intramuscularly, most preferably intramuscularly.

In therapy or as a prophylactic, the active agent of the pharmaceutical composition of the present invention may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition, preferably the pharmaceutical composition may be formulated for topical application, for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

In a preferred embodiment the pharmaceutical composition is a vaccine composition. Preferably, such vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination with a protein antigen is for adults between 0.02 µg and 3 µg antigen per kg body weight and for children between 0.2 µg and 10 µg antigen per kg body weight, and such dose is preferably administered 1 to 3 times at intervals of 2 to 24 weeks.

At the indicated dose range, no adverse toxicological effects are expected with the compounds of the invention, which would preclude their administration to suitable individuals.

As an additional aspect, the invention includes kits which comprise one or more pharmaceutical formulations for administration to a subject packaged in a manner which facilitates their use for administration to subjects. In a preferred embodiment, the kits comprise the formulation in a final volume of 2 mL, more preferably in a final volume of 1 mL.

In a specific embodiment, the invention includes kits for producing a single dose administration unit. The kits, in various aspects, each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

In another embodiment, such a kit includes pharmaceutical formulation described herein (e.g., a composition comprising a therapeutic protein or peptide), packaged in a container such as a sealed bottle or vessel, with a label affixed to the container or included in the package that describes use of the compound or composition in practicing the method. In one embodiment, the pharmaceutical formulation is packaged in the container such that the amount of headspace in the container (e.g., the amount of air between the liquid formulation and the top of the container) is very small. Preferably, the amount of headspace is negligible (i.e., almost none).

In one aspect, the kit contains a first container having a therapeutic protein or peptide composition and a second container having a physiologically acceptable reconstitution solution for the composition. In one aspect, the pharmaceutical formulation is packaged in a unit dosage form. The kit optionally further includes a device suitable for administering the pharmaceutical formulation according to a specific route of administration. In some aspects, the kit contains a label that describes use of the pharmaceutical formulations.

The pharmaceutical composition can contain a range of different antigens. Examples of antigens are whole-killed or attenuated organisms, subfractions of these organisms, proteins, or, in their most simple form, peptides. Antigens can also be recognized by the immune system in the form of glycosylated proteins or peptides and may also be or contain polysaccharides or lipids. Short peptides can be used, since cytotoxic T-cells (CTL) recognize antigens in the form of short, usually 8-11 amino acids long, peptides in conjunction with major histocompatibility complex (MHC). B cells can

recognize linear epitopes as short as 4 to 5 amino acids, as well as three-dimensional structures (conformational epitopes).

In a preferred embodiment, the pharmaceutical composition of the third aspect additionally comprises a hyperimmune serum-reactive antigen against a *Borrelia* protein or an active fragment or variant thereof, such as, *e.g.,* the antigens, fragments and variants as described in WO 2008/031133.

According to the invention, the pharmaceutical composition according to the third aspect may be used as a medicament, particularly as a vaccine, particularly in connection with particularly a disease or diseased condition which is caused by, linked or associated with *Borrelia.*

The pharmaceutical composition of the present invention may be used as a medicament, particularly as a vaccine, particularly in connection with a disease or disease condition which is caused by, linked with or associated with *Borrelia,* more preferably any pathogenic *Borrelia* species and more preferably in a method for treating or preventing a *Borrelia* infection, particularly a *B. burgdorferi* s.s., *B. garinii*, *B. afzelii*, *B. andersoni*, *B. bavariensis*, *B. bissettii*, *B. valaisiana*, *B. lusitaniae*, *B. spielmanii*, *B. japonica*, *B. tanukii*, *B. turdi* or *B. sinica* infection, preferably a *B. burgdorferi* s.s., *B. afzelii* or *B. garinii* infection.

In connection therewith, it should be noted that the various *Borrelia* species, including *B. burgdorferi* s.l., comprise several species and strains including those disclosed herein. A disease related, caused or associated with the bacterial infection to be prevented and/or treated according to the present invention includes Lyme borreliosis (Lyme disease). Further aspects, symptoms, stages and subgroups of Lyme borreliosis as well as specific groups of patients suffering from such disease as also disclosed herein, including in the introductory part, are incorporated herein by reference. More specifically, Lyme borreliosis generally occurs in stages, with remission and exacerbations with different clinical manifestation at each stage. Early infection stage 1 consists of localized infection of the skin, followed within days or weeks by stage 2, disseminated infection, and months to years later by stage 3, persistent infection. However, the infection is variable; some patients have only localized infections of the skin, while others display only later manifestations of the illness, such as arthritis.

In a fourth aspect, the present invention relates to a method of treating or preventing a *Borrelia* infection in a subject in need thereof, comprising the step of administering to the subject a therapeutically effective amount of a pharmaceutical composition according to the third aspect.

The term "subject" is used throughout the specification to describe an animal, preferably a mammal, more preferably a human, to whom a treatment or a method according to the present invention is provided. For treatment of those infections, conditions or disease states which are specific for a specific animal such as a human patient, the term patient refers to that specific animal. Preferably, the subject is a human; however, the medical use of the composition may also include animals such as poultry including chicken, turkey, duck or goose, livestock such as horse, cow or sheep, or companion animals such as dogs or cats.

The term "effective amount" is used throughout the specification to describe an amount of the present pharmaceutical composition which may be used to induce an intended result when used in the method of the present invention. In numerous aspects of the present invention, the term effective amount is used in conjunction with the treatment or prevention. In other aspects, the term effective amount simply refers to an amount of an agent which produces a result which is seen as being beneficial or useful, including in methods according to the present invention where the treatment or prevention of a *Borrelia* infection is sought.

The term effective amount with respect to the presently described compounds and compositions is used throughout the specification to describe that amount of the compound according to the present invention which is administered to a mammalian patient, especially including a human patient, suffering from a *Borrelia*-associated disease, to reduce or inhibit a *Borrelia* infection.

In a preferred embodiment, the method of immunizing a subject according to the fourth aspect comprises the step of administering to the subject a therapeutically effective amount of a pharmaceutical composition of the third aspect of the current invention.

The method comprises inducing an immunological response in an individual through gene therapy or otherwise, by administering a polypeptide or nucleic acid according to the present invention *in vivo* in order to stimulate an immunological response to produce antibodies or a cell-mediated T cell response, either cytokine-producing T cells or cytotoxic T cells, to protect said individual from disease, whether or not that disease is already established within the individual.

The products of the present invention, particularly the polypeptides and nucleic acids, are preferably provided in isolated form, and may be purified to homogeneity. The term “isolated” as used herein means separated “by the hand of man” from its natural state; i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally-occurring nucleic acid molecule or a polypeptide naturally present in a living organism in its natural state is not

"isolated", but the same nucleic acid molecule or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. As part of or following isolation, such nucleic acid molecules can be joined to other nucleic acid molecules, such as DNA molecules, for mutagenesis, to form fusion genes, and for propagation or expression in a host, for instance. The isolated nucleic acid molecules, alone or joined to other nucleic acid molecules such as vectors, can be introduced into host cells, in culture or in whole organisms. Introduced into host cells in culture or in whole organisms, such DNA molecules still would be isolated, as the term is used herein, because they would not be in their naturally-occurring form or environment. Similarly, the nucleic acid molecules and polypeptides may occur in a composition, such as medium formulations, solutions for introduction of nucleic acid molecules or polypeptides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein remain isolated nucleic acid molecules or polypeptides within the meaning of that term as it is employed herein.

The invention is not limited to the particular methodology, protocols and reagents described herein because they may vary. Furthermore, the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention. As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Similarly, the words "comprise", "contain" and "encompass" are to be interpreted inclusively rather than exclusively.

Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of the invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods, and materials are described herein.

The present invention is further illustrated by the following Figures, Tables, Examples and the Sequence listing, from which further features, embodiments and advantages may be taken. As such, the specific modifications discussed are not to be construed as limitations on the scope of the invention. It will be apparent to the person skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is thus to be understood that such equivalent embodiments are to be included herein.

In connection with the present invention

Fig. 1 shows the amino acid alignment of OspA serotypes 1-6 from *Borrelia*.

Fig. 2 schematically shows the production of mutant OspA fragment heterodimers according to the current invention.

Fig. 3 schematically represents the polypeptide components of one possible pharmaceutical composition of the current invention comprising three different mutant OspA heterodimers, a "combination vaccine".

Fig. 4 shows the chemical structure of Pam_3Cys, an example of a fatty acid substituted cysteine, such as would be found at the N-terminus of lipidated polypeptides of the current invention.

Fig. 5 shows the binding of antibodies from mice immunized with mutant OspA fragment heterodimer polypeptides of the invention to the cell surface of *Borrelia* of OspA serotypes 1-6.

Table 1 shows the thermal stability of the folding of mutant serotype 2 OspA fragments with disulfide bond types from D1 to D5 (for nomenclature, see Table A-4) compared to the wild-type serotype 2 OspA fragment without disulfide bonds (D0).

Table 2 shows the protection of mice from *B. afzelii* (strain IS1) infection by the Tick Challenge Method following immunization with mutant serotype 2 OspA fragments with disulfide bond types D1 to D5 (for nomenclature, see Table A-4), including control groups of mice immunized with PBS, full-length OspA or the wild-type serotype 2 OspA fragment (S2D0-His).

Table 3 shows the protection of mice from *B. afzelii* (strain IS1) infection by the Tick Challenge Method following immunization with lipidated mutant serotype 2 OspA fragments with disulfide bond types D1, D3 and D4 (Lip-S2D1-His, Lip-S2D3-His and Lip-S2D4-His), including control groups of mice immunized with PBS or full-length OspA protein.

Table 4 shows the protective capacity of mutant OspA heterodimers of the invention in *in vivo* Borrelia challenge models. Mice were immunized with Lip-S1D1-S2D1-His, Lip-S4D1-S3D1-His, Lip-S4D1-S3D1 or Lip-S5D1-S6D1-His and challenged with the indicated *Borrelia* OspA serotype via Tick or Needle Challenge Method, as indicated. The control group in each experiment was immunized with $Al(OH)_3$ adjuvant alone.

Table 5 shows the protective capacity of the combination vaccine of the invention against challenge *in vivo* with OspA serotype 1 *Borrelia* (strain N40 in needle challenge method) and OspA serotype 2 *Borrelia* (strain IS1 in the tick challenge method). Mice were immunized with the three antigens Lip-S1D1-S2D1, Lip-S4D1-S3D1 and Lip-S5D1-S6D1 together in a 1:1:1 ratio (combination vaccine) or with the indicated control antigens and challenged with *Borrelia* via Tick or Needle Challenge Method, as indicated. The control group in each experiment was immunized with $Al(OH)_3$ adjuvant alone.

The figures and tables which may be referred to in the specification are described below in more detail.

Fig. 1 Amino acid sequence alignment of OspA serotypes one through six. The alignment illustrates that the membrane-associated N-terminal portion of the protein has a more highly-conserved amino acid sequence than the more exposed C-terminal portion.

Fig. 2 Production of a mutant OspA heterodimer of the invention comprising mutant OspA C-terminal fragments from two different OspA serotypes of *Borrelia sp.* **(A)** Schematic representation of a nucleic acid encoding a lipidated mutant OspA heterodimer. The components, from 5' to 3', comprise the coding sequences for a lipidation signal sequence (Lip signal), a small cysteine-containing peptide for N-terminal lipidation (Lipidation peptide = LP), a mutant C-terminal fragment of OspA with two non-native cysteines, a short linker peptide (LN1), followed by a second mutant OspA C-terminal fragment with two non-native cysteines. **(B)** The intermediate mutant OspA heterodimer polypeptide comprises the nascent product directly following translation of the nucleic acid construct. From the N- to the C-terminus, this polypeptide consists of a lipidation signal sequence (Lip signal), a cysteine-containing peptide for lipidation (LP), a mutant OspA fragment with a non-native disulfide bond, a short linker peptide (LN1), followed by a second mutant OspA fragment with a non-native disulfide bond. **(C)** The final lipidated mutant OspA heterodimer polypeptide after post-translational modification. The heterodimer, from the N- to the C-terminus, consists of a short cysteine-containing peptide with the N-terminal cysteine lipidated (indicated by "Lip"), a mutant OspA fragment stabilized by a disulfide bond, a linker peptide (LN1), and a second mutant OspA fragment stabilized by a disulfide bond. The lipidation signal sequence is cleaved off during post-translational modification of the polypeptide as shown.

Fig. 3 An example of a preferred pharmaceutical composition according to the current invention. Three mutant OspA heterodimers, each comprising mutated OspA fragments from two different *Borrelia* OspA serotypes are present in the composition, together providing OspA antigens from six

different *Borrelia* OspA serotypes. Such a pharmaceutical composition enables simultaneous immunization against six *Borrelia* serotypes.

Fig. 4 Illustration of the chemical structure of Pam_3Cys, an example of a fatty acid substitution of the N-terminal cysteine of full-length wild-type OspA protein as well as of lipidated mutant OspA fragment monomers and heterodimers of the invention. During post-translational modification of a full-length OspA protein or polypeptides of the invention, the N-terminal lipidation signal sequence is cleaved off and fatty acids, most commonly three palmitoyl moieties ("Pam_3"), are enzymatically covalently attached to the N-terminal cysteine residue (the sulfur atom, "S", is indicated by an arrow). The remaining residues of the polypeptide chain, which are located C-terminally from the Pam_3Cys residue, are represented by "Xn". (Modified from Bouchon, *et al.* (1997) Analytical Biochemistry 246: 52–61.)

Fig. 5 Binding of antibodies from immunized mice to the cell surface of *Borrelia* spirochetes. Mice were immunized three times with 1 μg each of the indicated antigens: Lipidated and His-tagged full-length OspA proteins of OspA serotypes 1-6; Lip-S1D1-S2D1, Lip-S4D1-S3D1 or Lip-S5D1-S6D1 alone, or Lip-S1D1-S2D1, Lip-S4D1-S3D1 and Lip-S5D1-S6D1 together in a 1:1:1 ratio ("combination vaccine") at two week intervals and sera were collected at one week after the last dose. Several dilutions of the sera were tested for binding to the cell surface of *Borrelia* via cell staining and flow cytometry. Fluorescent intensity values observed when staining with sera collected from control mice immunized with $Al(OH)_3$ adjuvant alone were subtracted to account for non-specific binding. (*Borrelia* used were: *B. burgdorferi*, OspA serotype 1, strain N40; *B. afzelii*, OspA serotype 2, strain "C"; *B. garinii*, OspA serotype 3, strain "D"; *B. bavariensis*, OspA serotype 4, strain Fin; *B. garinii*, OspA serotype 5, strain "E"; *B. garinii*, OspA serotype 6, strain "B".)

Table 1. Thermal stability of non-lipidated, His-tagged *B. afzelii* K78 mutant serotype 2 OspA fragments with different placement of disulfide bonds. Mutant serotype 2 OspA fragments with different cysteine bond types (see Table A-4) were solubilized in 50 mM Tris-HCl, 150 mM NaCl (pH 8.0) and tested for thermal stability compared with the wild-type serotype 2 OspA fragment (S2D0). The presence of a disulfide bond resulted in an increased melting temperature compared to the wild-type serotype 2 OspA fragment.

Serotype 2 OspA mutant fragment	SEQ ID NO:	Melting temperature (°C)
S2D0-His*	1	47.6
S2D1-His	2	70.4
S2D2-His	3	54.6

S2D3-His	4	58.6
S2D4-His	5	58.4
S2D5-His	6	53.8

*see Tables A-4 and A-5 for nomenclature.

Table 2. Protective capacity of decreasing doses of non-lipidated His-tagged mutant serotype 2 OspA fragments against *B. afzelii* (serotype 2) infection by the Tick Challenge Method. Five non-lipidated His-tagged mutant serotype 2 OspA fragments were tested for protective capacity at two different doses (30 µg and 5 µg) and compared with the wild-type serotype 2 OspA fragment. Groups of mice immunized with $Al(OH)_3$ adjuvant alone or with non-lipidated full-length serotype 2 OspA served as negative and positive controls, respectively. All antigens were His-tagged and non-lipidated. The data presented combine the results of several experiments performed under identical conditions.

Immunogen	Tick challenge (OspA serotype 2: *B.afzelii*, strain IS1)	3 × 30 µg (11 experiments)	3 × 5 µg (4 experiments)
		Infected/total	Infected/total
$Al(OH)_3$ adjuvant alone	Tick (OspA-ST2)	58/62	20/23
Full-length OspA K78-His (SEQ ID NO: 209)	Tick (OspA-ST2)	1/72	1/25
S2D0-His (SEQ ID NO: 1)	Tick (OspA-ST2)	15/20	8/16
S2D1-His (SEQ ID NO: 2)	Tick (OspA-ST2)	1/26	1/25
S2D2-His (SEQ ID NO: 3)	Tick (OspA-ST2)	0/26	4/26
S2D3-His (SEQ ID NO: 4)	Tick (OspA-ST2)	0/34	1/21
S2D4-His (SEQ ID NO: 5)	Tick (OspA-ST2)	2/30	4/27
S2D5-His (SEQ ID NO: 6)	Tick (OspA-ST2)	5/35	2/11

Table 3. Protective capacity of decreasing doses of lipidated His-tagged mutant serotype 2 OspA fragments against *B. afzelii* infection by the Tick Challenge Method. Three lipidated His-tagged mutant serotype 2 OspA fragments with different disulfide bond types were tested for protective capacity at three different doses (3.0 µg, 1.0 µg and 0.3 µg). Groups of mice immunized with $Al(OH)_3$ adjuvant alone or with non-lipidated full-length serotype 2 OspA served as negative and positive controls, respectively. The data presented combine the results of several experiments performed under identical conditions.

Immunogen	Tick challenge (OspA serotype 2: *B.afzelii*, strain IS1)	3 × 3.0 µg (5 experiments)	3 × 1.0 µg (5 experiments)	3 × 0.3 µg (4 experiments)
		Infected/total	Infected/total	Infected/total
$Al(OH)_3$ adjuvant alone (control for all doses)	Tick (OspA-ST2)	58/59	-	-
Full-length OspA K78-His (SEQ ID NO: 209)	Tick (OspA-ST2)	0/14	0/21	1/20
Lip-S2D1-His (SEQ ID NO: 141)	Tick (OspA-ST2)	0/17	5/31	1/29
Lip-S2D3-His (SEQ ID NO: 143)	Tick (OspA-ST2)	1/15	1/12	5/19
Lip-S2D4-His (SEQ ID NO: 144)	Tick (OspA-ST2)	0/8	0/25	0/34

Table 4. Protective capacity of mutant OspA heterodimers of the invention against *in vivo Borrelia* challenge via Needle or Tick Challenge Methods. Groups of mice were immunized three times at two week intervals with the indicated doses of OspA heterodimer or $Al(OH)_3$ adjuvant alone. Immunogens used were Lip-S1D1-S2D1-His (challenged with *Borrelia* OspA-ST1, Experiments 1-3), Lip-S1D1-S2D1-His, Lip-S4D1-S3D1-His and Lip-S5D1-S6D1-His, separately (challenged with *Borrelia* OspA-ST2, Experiments 4-6), Lip-S4D1-S3D1 (challenged with *Borrelia* OspA-ST4, Experiments 7 and 8) and Lip-S5D1-S6D1-His (challenged with *Borrelia* OspA-ST5, Experiments 9 and 10; challenged with *Borrelia* OspA-ST6, Experiments 11 and 12). Immunized mice were challenged two weeks after the last immunization via Tick or Needle Challenge Models as indicated.

Immunogen	Dose	Needle challenge (OspA-serotype 1: *B. burgdorferi* s.s., strain N40)	Infected/Total		
			Exp. 1	Exp. 2	Exp. 3
Lip-S1D1-S2D1-His (SEQ ID NO: 49)	3 × 5.0 µg	Needle (OspA-ST1)	0/10***	0/9***	4/10**
$Al(OH)_3$ adjuvant alone	-	Needle (OspA-ST1)	10/10	8/10	10/10
Immunogen	**Dose**	**Tick challenge (OspA-serotype 2: *B.afzelii*, strain IS1)**	**Exp. 4**	**Exp. 5**	**Exp. 6**
Lip-S1D1-S2D1-His (SEQ ID NO: 49)	3 × 2.0 µg	Tick (OspA-ST2)	0/10***	0/9***	0/6***
Lip-S4D1-S3D1-His (SEQ ID NO: 81)	3 × 2.0 µg	Tick (OspA-ST2)	0/9***	2/7*	0/6***
Lip-S5D1-S6D1-His (SEQ ID NO: 65)	3 × 2.0 µg	Tick (OspA-ST2)	0/7***	0/9***	0/6***
$Al(OH)_3$ adjuvant alone	-	Tick (OspA-ST2)	9/9	8/8	7/7
Immunogen	**Dose**	**Needle challenge (OspA-serotype 4: *B. bavariensis*, strain Scf)**	**Exp. 7**	**Exp. 8**	

Lip-S4D1-S3D1 (Seq ID No: 194)	3 × 5.0 µg	Needle (OspA-ST4)	2/10**	1/10***	-
$Al(OH)_3$ adjuvant alone	-	Needle (OspA-ST4)	9/10	9/10	-
Immunogen	**Dose**	**Needle challenge (OspA-serotype 5: *B. garinii*)**	**Exp. 9**	**Exp. 10**	
Lip-S5D1-S6D1-His (SEQ ID NO: 65)	3 × 5.0 µg	Needle (OspA-ST5)	1/10	2/10	-
$Al(OH)_3$ adjuvant alone	-	Needle (ST5)	6/10	6/10	-
Immunogen	**Dose**	**Needle challenge (OspA-serotype 6: *B. garinii*)**	**Exp. 11**	**Exp. 12**	
Lip-S5D1-S6D1-His (SEQ ID NO: 65)	3 × 5.0 µg	Needle (OspA-ST6)	2/10**	2/10***	-
$Al(OH)_3$ adjuvant alone	-	Needle (OspA-ST6)	9/10	10/10	-

P-value; Fisher's exact test, two tailed. *significant (<0.05), **highly significant (<0.01), ***extremely significant (<0.001)

Table 5. Protective capacity of the mutant OspA heterodimer combination vaccine of the invention against OspA serotype 1 and serotype 2 *Borrelia* challenge. Groups of mice were immunized three times with the indicated doses of immunogen or $Al(OH)_3$ adjuvant alone at two-week intervals. Immunogens used were a 1:1:1 combination of the mutant OspA heterodimers Lip-S1D1-S2D1, Lip-S4D1-S3D1 and Lip-S5D1-S6D1 (combination vaccine), Lip-S1D1-S2D1, Lip-OspA1-His and Chimeric OspA ST1/ST2. Immunized mice were challenged two weeks after the last immunization via the Tick Challenge Method (ST2, Experiments 13 and 14) or the Needle Challenge Method (ST1, Experiments 15 and 16).

Immunogen	**Dose**	**Tick challenge (OspA-serotype 2: *B afzelii*, strain IS1)**	**Infected/Total**	
			Exp. 13	**Exp. 14**
Lip-S1D1-S2D1 (SEQ ID NO: 186)	3 × 5.0 µg	Tick (OspA-ST2)	0/6***	0/7**
Combination vaccine: Lip-S1D1-S2D1 (Seq ID No: 186) Lip-S4D1-S3D1 (Seq ID No: 194) Lip-S5D1-S6D1 (Seq ID No: 190)	 3 × 5.0 µg 3 × 5.0 µg 3 × 5.0 µg	Tick (OspA-ST2)	0/9***	0/6**
$Al(OH)_3$ adjuvant alone	-	Tick (OspA-ST2)	7/7	6/7
Immunogen	**Dose**	**Needle challenge (OspA-serotype 1: *B. burgdorferi* s.s., strain ZS7)**	**Exp. 15**	**Exp. 16**

Lip-S1D1-S2D1 (Seq ID No: 186)	3 × 1.0 µg	Needle (OspA-ST1)	0/10***	0/10***
Lip-OspA1-His (Seq ID No: 210)	3 × 1.0 µg	Needle (OspA-ST1)	0/10***	0/10***
Chimeric OspA ST1/ST2 (Seq ID No: 212)	3 × 1.0 µg	Needle (OspA-ST1)	0/10***	0/10***
Combination vaccine: Lip-S1D1-S2D1 (Seq ID No: 186) Lip-S4D1-S3D1 (Seq ID No: 194) Lip-S5D1-S6D1 (Seq ID No: 190)	3 × 1.0 µg 3 × 1.0 µg 3 × 1.0 µg	Needle (OspA-ST1)	0/10***	0/10***
$Al(OH)_3$ adjuvant alone	-	Needle (OspA-ST1)	10/10	10/10

P-value; Fisher's exact test, two tailed. *significant (<0.05), **highly significant (<0.01), ***extremely significant (<0.001)

EXAMPLES

Example 1. Assessment of thermal stability of mutant serotype 2 OspA fragments

Experimental procedures

Thermal stability

The melting temperatures (T_m) of non-lipidated mutant serotype 2 OspA fragment monomers were determined by the fluorescence-based thermal shift assay described by Pantoliano, *et al.* (J. Biomol Screen 6:429–440 (2001)). The fluorescent dye SYPRO® Orange protein gel stain (supplied as a 5000x concentrate in DMSO by Sigma, U.S.A.) was used to monitor protein unfolding. In each well, 7.5 µL of SYPRO® Orange (diluted 1:1000 from the stock solution) and 17.5 µL of a solution of protein (1 µg or 2 µg) in buffer were combined. The protein samples were heated from 25°C to 95°C at a rate of 0.2°C/10 sec in the CFX96 Real-time Detection System (Bio-Rad, USA) and fluorescent changes were monitored. Fluorescence intensity was measured with excitation and emission wavelengths of 490 and 575 nm, respectively. The T_m was determined using the Bio-Rad CFX Manager 2.0 program. The T_m values of non-lipidated His-tagged serotype 2 OspA mutant fragments were measured in four different buffer systems: 50 mM Tris-HCl, 150 mM NaCl (pH 9.0); 50 mM Tris-HCl, 150 mM NaCl (pH 8.0); PBS (pH 7.4); and 25 mM HEPES, 150 mM NaCl (pH 6.5), using the non-lipidated serotype 2 OspA wild-type fragment (S2D0) as a control.

Results

In all cases, mutant serotype 2 OspA fragments with an introduced cysteine bond had higher T_m than the wild-type serotype 2 OspA fragment (S2D0) (see Table 1). The T_m was tested in four different

buffer systems with similar results (data for proteins dissolved in 50 mM Tris-HCl, 150 mM NaCl (pH 8.0) is shown in Table 1), indicating that the stability of the proteins is similar over a wide pH range. This result lends credence to the hypothesis that the introduced disulfide bond stabilizes the OspA fragment.

Example 2. Assessment of the protective capacity of non-lipidated His-tagged mutant serotype 2 OspA fragment monomers in the Tick Challenge Method (ST2, *B. afzelii*)

Experimental procedures

Cloning and expression of recombinant proteins

The the wild-type serotype 2 OspA fragment as well as the mutant serotype 2 OspA fragments with cysteine bond types 1-5 (SEQ ID NOs: 1, 2, 3, 4, 5 and 6, respectively), were codon-optimized for *E. coli* expression by GenScript, USA. The non-lipidated mutant serotype 2 OspA fragments were C-terminally His-tagged for purification purposes. Gene fragments were cloned into the pET28b(+) vector (Novagen, USA), a vector containing a Kanamycin resistance cassette as well as a T7 promoter. The monomers were expressed in BL21 Star™ (DE3) cells (Invitrogen, USA) at 37°C by the addition of IPTG. Cells were collected after 4 h by centrifugation and the pellet was stored at -70°C for up to 12 months prior to further processing.

Purification of non-lipidated His-tagged wild-type and mutant OspA fragment monomer proteins

Cells were disrupted mechanically by high-pressure homogenization and the soluble fraction containing the His-tagged OspA fragments was applied to a Ni-sepharose column (Ni Sepharose™ 6 Fast Flow; GE Healthcare, United Kingdom) and the His-tagged OspA fragments were eluted on an Imidazole gradient (0-250 mM). Pooled fractions were further purified over a gel filtration column (Superdex 200, GE Healthcare) followed by a buffer exchange column (Sephadex G-25, GE Healthcare). His-tagged OspA fragment peaks were pooled on the basis of the analytical size exclusion column and reversed phase chromatography. After sterile filtration, the purified proteins were stored at -20°C until formulation.

Immunization of mice

Female C3H/HeN (*H-2*^{k}) mice were used for all studies (Harlan, Italy). Prior to each challenge, groups of five 8-week-old mice were bled via the tail vein and pre-immune sera were prepared and pooled. Five non-lipidated mutant serotype 2 OspA fragment proteins (S2D1-5, SEQ ID NOs: 2, 3, 4, 5 and 6, respectively), were tested in fifteen separate experiments. Three subcutaneous (s.c.) immunizations of 100 µL, were administered at two week intervals. Doses used were 30 and 5 µg of the respective protein, tested in 11 and 4 experiments respectively. All formulations included

aluminium hydroxide ($Al(OH)_3$) at a final concentration of 0.15%. One week after the third immunization, blood was collected and hyper-immune sera were prepared. In each experiment, one group injected with PBS formulated with $Al(OH)_3$ was included as a negative control and one group of mice was immunized with S2D0, the wild-type C-terminal OspA fragment from *B. afzelii* strain K78 (SEQ ID NO: 1). Another group immunized with a non-lipidated full-length wild-type OspA protein from *B. afzelii*, strain K78 (SEQ ID NO: 209), also formulated with 0.15% $Al(OH)_3$, was included as positive control in each animal study. All animal experiments were conducted in accordance with Austrian law (BGB1 Nr. 501/1989) and approved by "Magistratsabteilung 58".

Tick challenge of immunized mice and collection of sera and tissues (herein referred to also as "Tick Challenge Method")

Tick challenge of immunized mice was done two weeks after the last immunization. In order to challenge the immunized mice with *B. afzelii*, the hair on the back of each mouse was removed with Veet® Cream (Reckitt Benckiser, United Kingdom) and a small ventilated container was glued to the skin with super glue (Pattex, Germany). Thereafter, one or two *I. ricinus* nymphs infected with *B. afzelii*, strain IS1, were applied per mouse, allowed to attach and feed to depletion. The feeding status was monitored daily for each individual tick and only mice where at least one fully-fed tick was collected were included in the final readout. No distinction was made between mice where one or two fully-fed ticks were collected.

Six weeks after the tick application, blood was collected by orbital bleeding and final sera were prepared and used for VlsE ELISA analysis to determine infection status. The mice were then sacrificed by cervical dislocation and one ear from each mouse was collected, DNA extracted and subjected to nested PCR analysis to identify *Borrelia* in tissue.

Infection readout

Only mice where the applied tick(s) fed to completion and could be collected were included in the final readout of the experiment. The mice were sacrificed 6 weeks after tick application and organs as well as final sera were collected. The final infection readout was based on two different analyses (nested PCR targeting the 16S-23S intergenic spacer and VlsE (IR6) ELISA as described in detail below).

Nested PCR targeting the 16S-23S intergenic spacer

One ear from each mouse was subjected to DNA extraction and purification using the DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions, with the following modification. Each ear was digested over night at 60°C in recombinant Proteinase K, PCR grade

(Roche, 14-22 mg/mL). The DNA was eluted in 50 µL deionized sterile water and stored at −20°C until further analysis. As a negative control, one empty purification column was included in each DNA extraction and purification and the eluate subjected to nested PCR. All DNA extracts were screened for the presence of *Borrelia* DNA by a nested PCR procedure, comprising 40 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 60 s using the primers; Forward 5′-GTATGTTTAGTGAGGGGGGTG-3′ (SEQ ID NO: 26) and Reverse 5′-GGATCATAGCTCAGGTGGTTAG-3′ (SEQ ID NO: 27). From the reaction volume of 10 µL, 1 µL was used as template for the nested PCR reaction. The nested PCR step comprised 25 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 60 s using the primers; Forward nested 5′-AGGGGGGTGAAGTCGTAACAAG-3′ (SEQ ID NO: 28) and Reversed nested 5′-GTCTGATAAACCTGAGGTCGGA-3′ (SEQ ID NO: 29). Of the final reaction volume, 5 µL was separated on a 1% agarose gel containing ethidium bromide and bands were visualized in UV-light.

In each PCR analysis, DNA purified from an *in vitro* grown culture of *B. afzelii* strain K78 was used as a positive control template. In addition, PBS was used instead of extracted DNA as negative control. Five microliters of the final product was separated on a 1% agarose gel containing ethidium bromide and bands were visualized in UV-light.

ELISA with the Invariable Region 6 (IR6) of the Variable major protein-like Sequence E protein (VlsE)

A biotinylated 25-mer peptide (MKKDDQIAAAMVLRGMAKDGQFALK) (SEQ ID NO: 30) derived from the sequence of *B. garinii* strain IP90 was used for analysis (Liang FT, *et al.* (1999) J Immunol. 163:5566-73). Streptavidin pre-coated 96-well ELISA plates (Nunc, Denmark) were coated with 100 µL/well (1 µg/mL) biotinylated peptide in PBS supplemented with 0.1% Tween 20 (PBS/0.1T). The plates were incubated overnight at 4°C. After coating with the peptide, the plates were washed once with PBS/0.1T. The plates were then blocked for one hour at room temperature (RT) with 100 µL/well of PBS + 2% BSA, before being washed again with PBS/0.1T. Reactivity of post-challenge sera to the peptide was tested at 1:200, 1:400 and 1:800 dilutions in PBS + 1% BSA. Plates were incubated for 90 min at RT before being washed three times with PBS/0.1T. Each well then received 50 µL of 1.3 µg/mL polyclonal rabbit anti-mouse IgG conjugated to HRP (Dako, Denmark) in PBS + 1% BSA. The plates were then incubated for 1 h at RT. After three washes with PBS/0.1T, ABTS (50 µL/well) was added as substrate (Sigma-Aldrich, USA) and color was allowed to develop for 30 min. Absorbance was measured at 405 nm. All sera were tested in duplicate; negative controls included PBS instead of sera, as well as plates not coated with the peptide. Sera from mice shown to be culture positive for *B. afzelii* infection were used as positive controls.

Results

Levels of protection in the tick challenge method

High levels of protection were observed for all five stabilized OspA *B. afzelii* fragments at both of the doses tested (30 µg and 5 µg, see Table 2). The high infection rates in the PBS control group indicate that the ticks were infected with high frequency. Additionally, the positive control, non-lipidated full-length OspA from *B. afzelii* strain K78, was very protective. Together these control groups indicate the high reliability of the experimental readout.

Protection results from experiments testing 30 µg doses (11 total experiments) and 5 µg doses (4 total experiments) are summarized in Table 2. The two methods employed to verify infection, namely VlsE ELISA and nested PCR, gave virtually identical results (data not shown), demonstrating the robustness of these readout methods for assessing infection in the Tick Challenge Method.

Example 3. Assessment of the protective capacity of lipidated His-tagged mutant serotype 2 OspA fragment monomers against *in vivo Borrelia* challenge via the Tick Challenge Method (ST2, *B. afzelii*)

Experimental procedures

Cloning and expression of lipidated His-tagged mutant OspA fragment proteins

The serotype 2 mutant OspA fragments with cysteine bond types 1, 3 and 4 (SEQ ID NOs: 141, 143 and 144, respectively) were modified by the addition of a lipidation signal sequence derived from OspA (SEQ ID NO: 14) and followed directly C-terminally by a CKQN peptide (SEQ ID NO: 211) to provide an N-terminal cysteine for lipidation. All mutant OspA fragments were C-terminally histidine-tagged for purification purposes. Gene fragments were cloned into the pET28b(+) vector (Novagen), a vector containing a Kanamycin resistance cassette as well as a T7 promoter. The lipidated monomers were expressed in BL21 Star™(DE3) cells (Invitrogen) and after induction by IPTG, the growth temperature of the cells was lowered from 37°C to 25°C to promote efficient post-translational processing of the proteins. Cells were collected after 4 h by centrifugation and the pellet was stored at -70°C for up to 12 months prior to further processing.

Purification of lipidated His-tagged wild-type and mutant OspA fragment monomer proteins

Cells were disrupted mechanically by high-pressure homogenization and the lipidated His-tagged OspA fragment monomer polypeptides were enriched in the lipid phase by phase separation, using Triton X-114 as detergent. Subsequently, the diluted detergent phase (20 to 30 fold) was applied to a Ni-sepharose column (Ni Sepharose™ 6 Fast Flow; GE Healthcare) and the lipidated His-tagged OspA fragments were eluted by Imidazole gradient (0-250 mM) elution. Pooled fractions were further

purified over a gel filtration column (Superdex 200, GE Healthcare) followed by a buffer exchange column (Sephadex G-25, GE Healthcare). Lipidated His-tagged OspA fragment peaks were pooled on the basis of the analytical size exclusion column and reversed phase chromatography. After sterile filtration, the purified proteins were stored at -20°C until formulation.

Immunization of mice

Three lipidated mutant OspA proteins (Lip-S2D1-His, Lip-S2D3-His and Lip-S2D4-His) were expressed and purified as described above. *In vivo* protection studies were performed as described in Example 2 using $Al(OH)_3$–adjuvant alone and non-lipidated full-length serotype 2 OspA as negative and positive controls, respectively. All immunogens were formulated with 0.15% $Al(OH)_3$. Mice were injected subcutaneously three times at two week intervals with formulations containing 3.0 µg, 1.0 µg or 0.3 µg antigen and challenged with *B. afzelii*-infected ticks (strain IS1) two weeks after the last immunization. Mice were sacrificed six weeks following tick challenge and infection was assessed.

Results

Levels of protection in the tick challenge method

All three lipidated mutant OspA fragments conferred very high levels of protection from *B. afzelii* challenge even at the lowest tested dose (Table 3). Infection rates in the $Al(OH)_3$–adjuvant alone immunized mice were high, indicating that the ticks were infected to a high frequency. The positive control antigen, full-length non-lipidated OspA from *B. afzelii* strain K78, was also very protective. Together, these control groups indicate the high reliability of the method of infection and thus give high credibility to the results observed following immunization with the lipidated mutant OspA fragments.

Example 4. Assessment of the protective capacity of mutant OspA heterodimers of the invention against *in vivo Borrelia* challenge via the Needle or Tick Challenge Methods

Experimental procedures

Cloning and expression of lipidated His-tagged mutant OspA fragment heterodimers

The mutant OspA fragment monomers from *B. burgdorferi* s.s. strain B31, *B. afzelii* strain K78, *B. garinii* strain PBr, *B. bavariensis* strain PBi, *B. garinii* strain PHEi and *B. garinii* strain DK29 were codon-optimized for *E. coli* expression by GenScript, USA. The hLFA-1-like epitope (aa 164-174, SEQ ID NO: 17) of the OspA from *B. burgdorferi* s.s. strain B31 was replaced by a non-hLFA-1-like sequence NFTLEGKVAND from *B. afzelii* strain K78 (SEQ ID NO: 18). The lipidation signal sequence added to the mutant OspA fragment heterodimers was derived from the *E. coli* major outer membrane lipoprotein, Lpp, and was followed directly C-terminally by a CSS peptide to provide an

N-terminal cysteine for lipidation. The mutant OspA fragment heterodimers were generated by fusing different mutant OspA fragment monomers as described above via a 21 amino acid linker sequence, originating from two separate loop regions of the N-terminal half of OspA from *B. burgdorferi* s.s. strain B31 ("LN1"; aa 65-74 and aa 42-53 with an amino acid exchange of D53S, SEQ ID NO: 184). The heterodimers were constructed with a His-tag for purification purposes. Gene fragments were cloned into the pET28b(+) vector (Novagen), a vector containing a Kanamycin resistance cassette as well as a T7 promoter. The lipoproteins of the stabilized heterodimers were expressed in BL21 Star™ (DE3) cells (Invitrogen) and after induction by IPTG, the growth temperature of the cells was lowered from 37°C to 25°C to promote efficient post-translational processing of the proteins. Cells were collected after 4 h by centrifugation and the pellet was stored at -70°C for up to 12 months prior to further processing.

Purification of lipidated His-tagged mutant OspA fragment heterodimers

Cells were disrupted mechanically by high-pressure homogenization and the lipidated His-tagged mutant OspA fragment heterodimers were enriched in the lipid phase by phase separation, using Triton X-114 as detergent. Subsequently, the diluted detergent phase (20 to 30 fold) was applied to a Ni-sepharose column (Ni Sepharose™ 6 Fast Flow; GE Healthcare) and the lipidated His-tagged OspA heterodimers were eluted by Imidazole gradient (0-250 mM) elution. Pooled fractions were further purified over a gel filtration column (Superdex 200, GE Healthcare) followed by a buffer exchange column (Sephadex G-25, GE Healthcare). The lipidated His-tagged mutant OspA heterodimer peaks were pooled on the basis of the analytical size exclusion column and reversed phase chromatography. After sterile filtration, the purified heterodimers were stored at -20°C until formulation.

Cloning and expression of lipidated non-His-tagged mutant OspA fragment heterodimers

The constructs made as described as above were used for the generation of constructs without a His-tag by the introduction of a stop codon by PCR amplification. Gene fragments were cloned into the pET28b(+) vector (Merck Millipore), a vector containing a kanamycin resistance cassette as well as a T7 promoter. The lipoproteins of the stabilized heterodimers were expressed in BL21 Star™ (DE3) cells (Invitrogen) and after induction by IPTG, the growth temperature of the cells was lowered from 37°C to 25°C to promote efficient post-translational processing of the proteins. Cells were collected after 4 h by centrifugation and the pellet was stored at -70°C for up to 12 months prior to further processing.

Purification of lipidated non-His-tagged mutant OspA fragment heterodimers

Cells were disrupted mechanically by high-pressure homogenization and the lipidated mutant OspA fragment heterodimers were enriched in the lipid phase by phase separation, using Triton X-114 as detergent. Subsequently, the diluted detergent phase was subjected to anion exchange chromatography operated in non-binding mode. The resulting flow-through was loaded on a hydroxyapatite column (Bio-Rad) and the lipidated proteins were eluted from the column by a linear salt gradient. The eluate was subjected to further purification over a DEAE-Sepharose column (GE Healthcare) in non-binding mode followed by gel filtration column (Superdex 200, GE Healthcare) for buffer exchange. The lipidated mutant OspA heterodimer peaks were pooled on the basis of the analytical size exclusion column and SDS-PAGE. After sterile filtration, the purified heterodimers were stored at -20°C until formulation.

Immunization of mice

Female C3H/HeN mice (Janvier, France) were used for all studies. Prior to each challenge, groups of ten 8-week-old mice were bled via the facial vein and pre-immune sera were prepared and pooled. Three subcutaneous (s.c.) immunizations of 100 µL each were administered at two week intervals. Each dose contained the amount of immunogen indicated in Table 4 (dose), formulated with aluminium hydroxide ($Al(OH)_3$) at a final concentration of 0.15%. One week after the third immunization, blood was collected from the facial vein and hyper-immune sera were prepared. In each experiment, one group immunized with $Al(OH)_3$ alone was included as a negative control. All animal experiments were conducted in accordance with Austrian law (BGB1 Nr. 501/1989) and approved by "Magistratsabteilung 58".

Tick challenge of immunized mice and collection of sera and tissues (herein referred to also as "Tick Challenge Method")

In order to challenge the immunized mice with *B. afzelii*, the hair of the back of each mouse was removed with Veet® Cream (Reckitt Benckiser) and a small ventilated container was glued to the skin with super glue (Pattex). Thereafter, one or two *I. ricinus* nymphs infected with *B. afzelii*, strain IS1, were applied per mouse, allowed to attach and feed until they were fully engorged and dropped off. The feeding status was monitored daily for each individual tick and only mice from which at least one fully-fed tick was collected were included in the final readout.

Needle challenge of immunized mice with in vitro *grown Borrelia*

Two weeks after the last immunization, the mice were challenged s.c. with *Borrelia* diluted in 100 µL *Borrelia* growth medium (BSK II). The challenge doses were strain-dependent, the virulence of the

individual strains being assessed by challenge experiments for determination of ID_{50}. Doses employed for needle challenge experiments ranged from 20 to 50 times the ID_{50}.

Sacrifice of mice and collection of material

Four weeks after needle challenge with the indicated *Borrelia spp.* or six weeks after tick challenge with *B. afzelii*, mice were sacrificed by cervical dislocation. The blood was collected by orbital bleeding and final sera were prepared and used for VlsE ELISA to determine infection status. In addition, one ear from each mouse was collected, and DNA was extracted and subjected to quantitative PCR (qPCR) for identification of *Borrelia*. The final infection readout was based on two different analyses (VlsE ELISA and qPCR targeting *recA*).

ELISA with the Invariable Region 6 (IR6) of VlsE

A biotinylated 25-mer peptide (MKKDDQIAAAMVLRGMAKDGQFALK) derived from the sequence of *B. garinii* strain IP90 was used for the analysis (Liang FT, Alvarez AL, Gu Y, Nowling JM, Ramamoorthy R, Philipp MT. An immunodominant conserved region within the variable domain of VlsE, the variable surface antigen of *Borrelia burgdorferi*. J Immunol. 1999;163:5566-73). Streptavidin pre-coated 96-well ELISA plates (Nunc), were coated with 100 µL/well (1 µg/mL) peptide in PBS supplemented with 0.1% Tween (PBS/0.1T). The plates were incubated overnight at 4°C. After coating with the peptide, the plates were washed once with PBS/0.1T. The plates were then blocked for one hour at room temperature (RT) with 100 µL/well of PBS + 2% BSA, before being washed again with PBS/0.1T. Reactivity of post-challenge sera to the peptide was tested at 1:200, 1:400 and 1:800 dilutions in PBS + 1% BSA. Plates were incubated for 90 min at RT before being washed three times with PBS/0.1T. Each well then received 50 µL of 1.3 µg/mL polyclonal rabbit anti-mouse IgG conjugated to HRP (Dako) in PBS + 1% BSA. The plates were then incubated for 1 h at RT. After three washes with PBS/0.1T, ABTS (50 µL/well) was added as substrate (Sigma-Aldrich) and color was allowed to develop for 30 min. Absorbance was measured at 405 nm. All sera were tested in duplicate. Negative controls included PBS instead of sera as well as plates not coated with the peptide. Sera from mice shown to be culture positive for *B. afzelii* infection were used as positive controls.

qPCR targeting recA

Oligonucleotide primers were designed for the *recA* gene in a manner that they could be used in qPCR for identification of all relevant *Borrelia* species causing Lyme borreliosis (forward: CATGCTCTTGATCCTGTTTA, reverse: CCCATTTCTCCATCTATCTC). The *recA* fragment was cloned from the *B. burgdorferi* s.s. strain N40 into pET28b(+), to be used as standard in each reaction. The chromosomal DNA extracted from mouse ears was diluted 1:8 in water in order to reduce matrix

effects observed with undiluted DNA. A master mix consisting of 10 µL SSoAdvanced™ SYBR® Green Supermix, 0.3 µL of each primer (10 µM), and 7.4 µL water was prepared for each experiment. Eighteen µL of master mix was mixed with 2 µL of the diluted DNA extracted from either bladder or ear in micro-titer plates and the DNA was amplified using a CFX96 real-time PCR detection system (Bio-Rad, USA). The DNA was denatured for 3 minutes at 95°C, followed by 50 cycles of 15 seconds at 95°C and 30 seconds at 55°C. After amplification, the DNA was prepared for the melting curve analysis by denaturation for 30 seconds at 95°C followed by 2 minutes at 55°C. The melting curve analysis was performed by 5 seconds incubation at 55°C, with a 0.5°C increase per cycle, and 5 seconds at 95°C. On each plate, four no-template controls (NTC) were included as well as a standard curve in duplicate with template copy numbers ranging from 10 to 10,000.

Results

Lipidated mutant OspA fragment heterodimers were tested for protective capacity in twelve separate experiments. Mice were challenged with either *B. burgdorferi* s.s., strain N40, OspA serotype 1 (ST1, needle challenge) or *B. afzelii* strain IS1, OspA serotype 2 (ST2, tick challenge) in three experiments each or *B. bavariensis*, strain Scf, OspA serotype 4 (ST4, needle challenge), *B. garinii*, strain "A", OspA serotype 5 (ST5, needle challenge) or *B. garinii*, strain "B", OspA serotype 6 (ST6, needle challenge) in two experiments each. In all experiments, a group of mice immunized with $Al(OH)_3$ adjuvant alone served as a negative control group. For challenge with ticks, 1-2 ticks were applied per mouse and only mice from which at least one tick fed until fully engorged were included in the final readout. However, no distinction was made between mice from which one or two fully-fed ticks were collected. The protection data from the twelve experiments are summarized in Table 4.

The lipidated His-tagged OspA heterodimer (Lip-S1D1-S2D1-His) showed highly statistically-significant protection (Fisher's exact test, two-tailed) in all six experiments against both OspA serotype 1 and OspA serotype 2 challenge as compared to the negative control group. Surprisingly, immunization with Lip-S4D1-S3D1-His and Lip-S5D1-S6D1-His also conferred a high protective capacity against OspA serotype 2 challenge (Experiments 4-6), indicating that there can be a cross-protective effect of immunization with other serotypes of the mutant OspA fragments. Furthermore, immunization with Lip-S4D1-S3D1 conferred statistically-significant protection against needle challenge with OspA serotype 4 *Borrelia* (Experiments 7 and 8). Finally, immunization with Lip-S5D1-S6D1-His conferred protection against needle challenge with both OspA serotype 5 (Experiments 9 and 10) and OspA serotype 6 (Experiments 11 and 12). The infectious status of each mouse was determined using VlsE ELISA in combination with *recA* qPCR. A mouse was regarded as infected when at least one method gave a positive result.

In conclusion, immunization with mutant OspA fragment heterodimer polypeptides of the invention confers protection against all *Borrelia* serotypes tested and also may provide cross-protection in some cases.

The lipidated His-tagged OspA heterodimer (Lip-S1D1-S2D1-His) showed highly statistically-significant protection (Fisher's exact test, two-tailed) in all six experiments against both OspA serotype 1 and OspA serotype 2 challenge as compared to the negative control group. Surprisingly, immunization with Lip-S4D1-S3D1-His and Lip-S5D1-S6D1-His also conferred a high protective capacity against OspA serotype 2 challenge (Experiments 4-6), indicating that there can be a cross-protective effect of immunization with other serotypes of the mutant OspA fragments. Furthermore, immunization with Lip-S4D1-S3D1 conferred statistically-significant protection against needle challenge with OspA serotype 4 *Borrelia* (Experiments 7 and 8). Finally, immunization with Lip-S5D1-S6D1-His conferred protection against needle challenge with both OspA serotype 5 (Experiments 9 and 10) and OspA serotype 6 (Experiments 11 and 12). The infectious status of each mouse was determined using VlsE ELISA in combination with *recA* qPCR. A mouse was regarded as infected when at least one method gave a positive result.

In conclusion, immunization with mutant OspA fragment heterodimer polypeptides of the invention confers protection against all *Borrelia* serotypes tested and also may provide cross-protection in some cases.

Example 5. Assessment of the protective capacity of a 1:1:1 combination vaccine of the mutant OspA heterodimers of the invention against *in vivo* OspA serotype 1 and serotype 2 *Borrelia* challenge via the Needle Challenge or Tick Challenge Methods

Experimental procedures

Immunization of mice

Female C3H/HeN mice (Janvier, France) were used for all studies. Prior to each challenge, groups of ten 8-week-old mice were bled via the facial vein and pre-immune sera were prepared and pooled. Three s.c. immunizations of 100 µL each were administered at two week intervals. Groups of mice were immunized with the combination vaccine consisting of 1 µg each of Lip-S1D1-S2D1, Lip-S4D1-S3D1 and Lip-S5D1-S6D1. Three other OspA-based antigens were included in the challenge experiments: Lip-OspA1-His (full-length serotype 1 OspA, lipidated and his-tagged), lipidated chimeric OspA ST1/ST2* and Lip-S1D1-S2D1 alone. The negative (placebo) control was $Al(OH)_3$-advuvant alone. All antigens were formulated in PBS with aluminium hydroxide ($Al(OH)_3$) at a final concentration of 0.15%.

*(Chimeric OspA ST1/ST2 (SEQ ID NO: 212) is an OspA chimera consisting of the first 10 amino acids of the N-terminal portion of OspB (strain B31), amino acids 11-200 of serotype 1 OspA, fused with the last 201-255 amino acids from the C-terminal portion of serotype 2 OspA and wherein the hLFA-1-like sequence of the serotype 1 OspA (146-170) is replaced with the homologous sequence from a serotype 2 OspA The serotype 2 OspA sequence is followed by two amino acids which are added because of the cloning site (XhoI) ahead of the stop codon in the vector.)

One week after the third immunization, blood was collected from the facial vein and hyper-immune sera were prepared. All animal experiments were conducted in accordance with Austrian law (BGB1 Nr. 501/1989) and approved by "Magistratsabteilung 58".

Needle challenge of immunized mice with in vitro *grown* Borrelia

Two weeks after the last immunization, the mice were challenged s.c. with *Borrelia* spirochetes diluted in 100 µL growth medium (BSKII). The challenge doses were strain-dependent, the virulence of the individual strains were assessed by challenge experiments for determination of ID_{50}. Doses employed for needle challenge experiments ranged from 20 to 50 times the ID_{50}. Four weeks after needle challenge, mice were sacrificed and blood and tissues were collected for readout methods to determine the infection status.

Tick challenge of immunized mice and collection of sera and tissues (herein referred to also as "Tick Challenge Method")

In order to challenge the immunized mice with *B. afzelii*, the hair of the back of each mouse was removed with Veet® Cream (Reckitt Benckiser, United Kingdom) and a small ventilated container was glued to the skin with super glue (Pattex, Germany). Thereafter, one or two *I. ricinus* nymphs infected with *B. afzelii*, strain IS1, were applied per mouse, allowed to attach and feed until they are fully engorged and drop off. The feeding status was monitored for each individual tick and only mice where at least one fully-fed tick was collected were included in the final readout.

Results

Lipidated mutant OspA fragment heterodimers that were not His-tagged were combined at a 1:1:1 ratio and tested for protective capacity against *Borrelia* challenge. Immunized mice were challenged with *B. afzelii* (ST2, strain IS1, tick challenge) or with *B. burgdorferi* s.s. (ST1, strain ZS7, needle challenge) in two experiments each. Other OspA-based antigens included Lip-S1D1-S2D2 in all four experments and Lip-OspA1-His and lipidated chimeric OspA ST1/ST2 in Experiments 15 and 16. A group of mice immunized with $Al(OH)_3$ adjuvant alone served as a negative control group in each experiment. For challenge with ticks, 1-2 ticks were applied per mouse and only mice from which at

least one tick fed until fully engorged were included in the final readout. However, no distinction was made between mice from which one or two fully fed ticks were collected. The protection data from the four experiments are summarized in Table 5.

The combination vaccine containing three lipidated mutant OspA fragment heterodimers at a 1:1:1 ratio conferred statistically-significant protection (Fisher's exact test, two-tailed) in all four challenge experiments as compared to the negative control group. The infectious status of each mouse was determined using VlsE ELISA in combination with *recA* qPCR. A mouse was regarded as infected when at least one method gave a positive result.

Example 6 Binding of antibodies from the sera of mice immunized with mutant OspA fragment heterodimers to the cell surface of *Borrelia*

Experimental procedures

Immunization of mice

Female C3H/HeN mice were used for all studies. Prior to each challenge, groups of twenty 8-week-old mice were bled via the facial vein and pre-immune sera were prepared and pooled. Three s.c. immunizations of 100 µL each were administered at two week intervals. Each dose contained 1 µg of each of the respective proteins: Lip-S1D1-S2D1, Lip-S4D1-S3D1 and Lip-S5D1-S6D1 (combination vaccine), or 1 µg lipidated full-length OspA protein (ST1-ST6 as indicated) or 1 µg OspA heterodimer alone (Lip-S1D1-S2D1, Lip-S4D1-S3D1 or Lip-S5D1-S6D1, as indicated) adjuvanted with aluminium hydroxide at a final concentration of 0.15%. The negative (placebo) control was $Al(OH)_3$ adjuvant alone. One week after the third immunization, blood was collected from the facial vein and hyper-immune sera were prepared. All animal experiments were conducted in accordance with Austrian law (BGB1 Nr. 501/1989) and approved by "Magistratsabteilung 58".

Flow cytometry to assess binding to Borrelia

Spirochetes ($1x10^6$) were mixed with an equal volume of 4% paraformaldehyde and incubated for 2 hours at room temperature in a 96-well plate (Nunclon 96U, Nunc). The plate was centrifuged for 5 minutes at 2,000 *g* and the supernatant was discarded. Cells were washed with 150 µL HBSS with 2% BSA (HBSS-B), centrifuged as above and the supernatant was discarded. Mouse sera were heat inactivated by incubating them at 56°C for 35 minutes. Heat-inactivated sera were diluted in HBSS-B and sterile filtered by centrifuging 4,000 *g* for 3 minutes using Costar spin-X centrifuge tube filters (0.22 µm, Corning, USA). Spirochetes were dissolved in 100 µL serum and incubated for 45 minutes at room temperature. The plate was centrifuged for 15 minutes at 2,000 *g* and the supernatant was discarded. The cells were washed once with 150 µL HBSS-B and then dissolved in 100 µL HBSS-B.

One microliter secondary antibody (PE conjugated goat anti-mouse IgG, Beckman Coulter, USA) was added to the cells and incubated at room temperature for 45 minutes in the dark. Spirochetes were washed once with 150 µL HBSS-B and then dissolved in 200 µL HBSS containing 2.5 µM SYTO-17 DNA dye and incubated for 10 minutes at room temperature in the dark. The stained spirochetes were pelleted by centrifuging for 5 minutes at 2000 g and subsequently dissolved in 200 µL HBSS. Labelled spirochetes were measured with a FC500 (Beckman Coulter) flow cytometer, gated for SYTO-17 positive events. Values obtained with sera from the placebo-immunized group were subtracted from the values observed with sera from the heterodimer-immunized groups to control for non-specific binding.

Results

Binding of antibodies from hyperimmune mouse sera was observed in the case of different *Borreliae* expressing all six OspA serotypes, indicating that the antibodies generated in response to all of the antigens are functionally active and can bind native OspA *in situ*. The fluorescence intensity was linear over a large range of serum dilutions. For most OspA serotypes, the observed fluorescence intensity with heterodimer-generated sera was comparable to the fluorescence intensity seen with sera generated with lipidated full-length OspA.

Example 7 Formulation studies

Studies regarding the formulation of the combination vaccine of the invention were carried out in order to optimize stability. Different types of buffers and stabilizers were tested at various concentrations in combination with alumninium hydroxide and antigen. An optimal formulation of 40 µg/mL each of three heterodimers (120 µg total protein), 10 mM sodium phosphate, 150 mM sodium chloride, 10 mM L-Methionine, 5% Sucrose, 0.05% Tween 20 (polysorbate 20) and 0.15% (w/v) aluminium hydroxide at pH 6.7 ± 0.2 was determined.#

SEQUENCES

SEQ ID NO: 1
S2D0-His: amino acids of positions 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2, wild-type sequence, C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNALKGLEHHHHHH

SEQ ID NO: 2
S2D1-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 1 (aa 182 and 269), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVK**C**GTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
L**C**NALKGLEHHHHHH

SEQ ID NO: 3
S2D2-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 2 (aa 182 and 272), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVK**C**GTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNA**C**KGLEHHHHHH

SEQ ID NO: 4
S2D3-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 3 (aa 244 and 259), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTI**C**VQKYDSAGTNLEGT**C**VEIKTLDE
LKNALKGLEHHHHHH

SEQ ID NO: 5
S2D4-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 4 (aa 141 and 241), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRE**C**GTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQ**C**TITVQKYDSAGTNLEGTAVEIKTLDE
LKNALKGLEHHHHHH

SEQ ID NO: 6
S2D5-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 5 (aa 165 and 265), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKN**C**TLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKT**C**DE
LKNALKGLEHHHHHH

SEQ ID NO: 7
S2D6-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA sero type 2 with disulfide bond type 6 (aa 185 and 272), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGT**C**TLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNA**C**KGLEHHHHHH

SEQ ID NO: 8
S2D7-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 7 (aa 199 and 223), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
CALNDTNTTQATKKTGAWDSKTST**C**TISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNALKGLEHHHHHH

SEQ ID NO: 9
S2D8-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 8 (aa 243 and 262), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDT**C**TVQKYDSAGTNLEGTAVE**C**KTLD
ELKNALKGLEHHHHHH

SEQ ID NO: 10
S2D9-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 9 (aa 184 and 204), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEG**C**VTLSKEIAKSGEVT
VALND**C**NTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNALKGLEHHHHHH

SEQ ID NO: 11
S2D10-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 10 (aa 201 and 214), C-terminal His tag (GLEHHHHHH)

ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VA**C**NDTNTTQATKKT**C**AWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNALKGLEHHHHHH

SEQ ID NO: 12
S2D11-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 11 (aa 246 and 259), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITV**C**KYDSAGTNLEGT**C**VEIKTLDE
LKNALKGLEHHHHHH

SEQ ID NO: 13
S2D12-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 12 (aa 167 and 178), C-terminal His tag (GLEHHHHHH)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFT**C**EGKVANDKVT**C**EVKEGTVTLSKEIAKSGEV
TVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLD
ELKNALKGLEHHHHHH

SEQ ID NO: 14
Borrelia OspA lipidation signal
MKKYLLGIGLILALIA

SEQ ID NO: 15
Borrelia OspB lipidation signal
MRLLIGFALALALIG

SEQ ID NO: 16
E. coli lpp lipidation signal
MKATKLVLGAVILGSTLLAG

SEQ ID NO: 17
hLFA-1-like sequence from *B. burgdorferi* s.s. strain B31
GYVLEGTLTAE

SEQ ID NO: 18
Non-hLFA-1-like sequence from *B. afzelii* strain K78
NFTLEGKVAND

SEQ ID NO: 19
B. afzelii (strain K78; OspA serotype 2)
MKKYLLGIGLILALIACKQNVSSLDEKNSASVDLPGEMKVLVSKEKDKDGKYSLKATVDKIELKGTSDK
DNGSGVLEGTKDDKSKAKLTIADDLSKTTFELFKEDGKTLVSRKVSSKDKTSTDEMFNEKGELSAKT
MTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVTVALNDT
NTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELKNALK

SEQ ID NO: 20
B. burgdorferi s.s. (strain B31, OspA serotype 1)
MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGEMKVLVSKEKNKDGKYDLIATVDKLELKGTSDK
NNGSGVLEGVKADKSKVKLTISDDLGQTTLEVFKEDGKTLVSKKVTSKDKSSTEEKFNEKGEVSEKIIT
RADGTRLEYTGIKSDGSGKAKEVLKGYVLEGTLTAEKTTLVVKEGTVTLSKNISKSGEVSVELNDTDS
SAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEIKNALK

SEQ ID NO: 21
B. garinii (strain PBr, OspA serotype 3)
MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGGMKVLVSKEKDKDGKYSLMATVEKLELKGTSD
KSNGSGVLEGEKADKSKAKLTISQDLNQTTFEIFKEDGKTLVSRKVNSKDKSSTEEKFNDKGKLSEKV
VTRANGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTEGTVTLSKNISKSGEITVALNDT
ETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKENTITVQNYNRAGNALEGSPAEIKDLAELKAALK

SEQ ID NO: 22
B. bavariensis (strain PBi, OspA serotype 4)
MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGEMKVLVSKEKDKDGKYSLMATVDKLELKGTSD
KSNGSGTLEGEKSDKSKAKLTISEDLSKTTFEIFKEDGKTLVSKKVNSKDKSSIEEKFNAKGELSEKTIL
RANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNSGEITVELNDSNST
QATKKTGKWDSNTSTLTISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIKTLDELKNALK

SEQ ID NO: 23
B. garinii (strain PHei, OspA serotype 5)
MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGGMKVLVSKEKDKDGKYSLMATVEKLELKGTSD
KNNGSGTLEGEKTDKSKVKLTIAEDLSKTTFEIFKEDGKTLVSKKVTLKDKSSTEEKFNEKGEISEKTIV
RANGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKSGEITVALDDTDS
SGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 24
B. garinii (strain DK29, OspA serotype 6)
MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGGMTVLVSKEKDKDGKYSLEATVDKLELKGTSDK
NNGSGTLEGEKTDKSKVKSTIADDLSQTKFEIFKEDGKTLVSKKVTLKDKSSTEEKFNGKGETSEKTIV
RANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVVLSKNILKSGEITAALDDSDT
TRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKEDTITVQRYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 25
B. garinii (strain T25, OspA serotype 7)
MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGEMKVLVSKEKDKDGKYSLEATVDKLELKGTSDK
NNGSGVLEGVKAAKSKAKLTIADDLSQTKFEIFKEDGKTLVSKKVTLKDKSSTEEKFNDKGKLSEKVV
TRANGTRLEYTEIQNDGSGKAKEVLKSLTLEGTLTADGETKLTVEAGTVTLSKNISESGEITVELKDTE
TTPADKKSGTWDSKTSTLTISKNSQKTKQLVFTKENTITVQKYNTAGTKLEGSPAEIKDLEALKAALK

SEQ ID NO: 26
Forward Primer
GTATGTTTAGTGAGGGGGGTG

SEQ ID NO: 27
Reverse Primer
GGATCATAGCTCAGGTGGTTAG

SEQ ID NO: 28
Forward Nested Primer
AGGGGGGTGAAGTCGTAACAAG

SEQ ID NO: 29
Reversed Nested Primer
GTCTGATAAACCTGAGGTCGGA

SEQ ID NO: 30
25-mer peptide
MKKDDQIAAAMVLRGMAKDGQFALK

SEQ ID NO: 31
Mouse cathelin
RLAGLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPE

SEQ ID NO: 32
5'-$(dIdC)_{13}$-3'
dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC

SEQ ID NO: 33
KLK peptide
KLKLLLLLKLK

SEQ ID NO: 34
B. afzelii (strain K78, serotype 2), OspA aa 126-273
FNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAK
SGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEI
KTLDELKNALK

SEQ ID NO: 35
B. afzelii (strain K78, serotype 2), OspA aa 131-273
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNALK

SEQ ID NO: 36
peptide linker
GGGGGGGG

SEQ ID NO: 37
peptide linker
GGGGGGGGGGGG

SEQ ID NO: 38
peptide linker
GAGA

SEQ ID NO: 39
peptide linker
GAGAGAGA

SEQ ID NO: 40
peptide linker
GAGAGAGAGAGA

SEQ ID NO: 41
peptide linker
GGGSGGGS

SEQ ID NO: 42
peptide linker
GGGSGGGSGGGS

SEQ ID NO: 43
S1D4-S2D4_aa: Heterodimer fusion protein of OspA serotypes 1 and 2 both with disulfide bond type 4, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
FNEKGEVSEKIITRACGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSKNISKS
GEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKECTITVQQYDSNGTKLEGSAVEIT
KLDEIKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRECGTKLEYTEMKSDGTGKAKE
VLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNS
KKTTQLVFTKQCTITVQKYDSAGTNLEGTAVEIKTLDELKNALK

SEQ ID NO: 44
Lip-S1D4-S2D4_nt: Coding sequence for fusion proteins of OspA serotypes 1 and 2 both with disulfide bond type 4, *E. coli* lpp lipidation signal, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAGTCTCGGAAAAAATCATTACCCGTGCTTGCGGCACCCGTCT
GGAATACACCGGCATTAAATCGGATGGCAGCGGCAAAGCGAAGGAAGTTCTGAAAAACTTTACC
CTGGAAGGCAAAGTCGCAAATGATAAGACCACCCTGGTGGTGAAAGAAGGCACCGTTACGCTGA

GCAAAAACATTAGTAAGTCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGC
CACCAAAAAGACGGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATTCCAAA
AAGACCAAAGATCTGGTCTTCACGAAAGAATGCACCATCACGGTGCAGCAATATGACAGCAACG
GTACCAAACTGGAAGGCTCTGCGGTGGAAATCACGAAACTGGATGAAATCAAAAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAATGCGGCACCAAACTGGAATAT
ACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAAGAAGGCACCGTTACGCTGTCAAAAGA
AATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCGACCAAG
AAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAACAGCAAGAAAACCA
CGCAGCTGGTCTTCACCAAACAATGTACGATCACCGTGCAGAAATACGATAGTGCGGGTACCAA
CCTGGAAGGCACCGCTGTTGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAA

SEQ ID NO: 45

Lip-S1D4-S2D4_His_aa: Heterodimer fusion protein of OspA serotypes 1 and 2 both with disulfide bond type 4, N-terminal CSS for addition of lipids, N-terminal lipidation, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)

LipCSSFNEKGEVSEKIITRACGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSK
NISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKECTITVQQYDSNGTKLEG
SAVEITKLDEIKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRECGTKLEYTEMKSDGT
GKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTL
TISVNSKKTTQLVFTKQCTITVQKYDSAGTNLEGTAVEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 46

Lip-S1D4-S2D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotypes 1 and 2 both with disulfide bond type 4, *E. coli* lpp lipidation signal, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAGTCTCGGAAAAAATCATTACCCGTGCTTGCGGCACCCGTCT
GGAATACACCGGCATTAAATCGGATGGCAGCGGCAAAGCGAAGGAAGTTCTGAAAAACTTTACC
CTGGAAGGCAAAGTCGCAAATGATAAGACCACCCTGGTGGTGAAAGAAGGCACCGTTACGCTGA
GCAAAAACATTAGTAAGTCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGC
CACCAAAAAGACGGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATTCCAAA
AAGACCAAAGATCTGGTCTTCACGAAAGAATGCACCATCACGGTGCAGCAATATGACAGCAACG
GTACCAAACTGGAAGGCTCTGCGGTGGAAATCACGAAACTGGATGAAATCAAAAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAATGCGGCACCAAACTGGAATAT
ACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAAGAAGGCACCGTTACGCTGTCAAAAGA
AATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCGACCAAG
AAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAACAGCAAGAAAACCA
CGCAGCTGGTCTTCACCAAACAATGTACGATCACCGTGCAGAAATACGATAGTGCGGGTACCAA
CCTGGAAGGCACCGCTGTTGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAAGGCCTC
GAGCACCACCACCACCACCAC

SEQ ID NO: 47

S1D1-S2D1_aa: Heterodimer fusion protein of OspA serotype 1 and OspA serotype 2 with disulfide bond type 1, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND

FNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSKNISKS
GEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEIT
KLDEICNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKE
VLKNFTLEGKVANDKVTLEVKCGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNS
KKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELCNALK

SEQ ID NO: 48

Lip-S1D1-S2D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 1 and OspA serotype 2 with disulfide bond type 1, *E. coli* lpp lipidation signal, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAGTCAGCGAAAAAATCATTACCCGCGCAGACGGCACCCGCCT
GGAATACACCGGCATCAAATCGGACGGCAGCGGCAAAGCGAAAGAAGTTCTGAAAAACTTTACC
CTGGAAGGCAAAGTCGCAAATGATAAAACCACCCTGGTGGTGAAATGCGGCACCGTTACGCTGA
GCAAAAACATTAGTAAATCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGC
CACCAAGAAAACCGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATAGCAAG
AAAACCAAAGATCTGGTCTTCACGAAAGAAAACACCATCACGGTGCAGCAATATGACAGCAATGG
TACCAAACTGGAAGGCTCCGCTGTGGAAATCACGAAACTGGATGAAATCTGTAATGCTCTGAAAG
GTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATTC
AACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAAAACGGCACCAAACTGGAATATA
CGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAGG
CAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAATGCGGCACCGTTACGCTGTCAAAAGAA
ATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCGACCAAGA
AAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAATAGCAAGAAAACCAC
GCAGCTGGTCTTCACCAAACAAGATACGATCACCGTGCAGAAATACGACAGTGCGGGTACCAAC
CTGGAAGGCACGGCTGTTGAAATCAAAACCCTGGACGAACTGTGTAACGCCCTGAAA

SEQ ID NO: 49

Lip-S1D1-S2D1_His_aa: Heterodimer fusion protein of OspA serotype 1 and OspA serotype 2 with disulfide bond type 1, N-terminal CSS for addition of lipids, N-terminal lipidation, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)

LipCSSFNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSK
NISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEG
SAVEITKLDEICNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRENGTKLEYTEMKSDGT
GKAKEVLKNFTLEGKVANDKVTLEVKCGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTL
TISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELCNALKGLEHHHHHH

SEQ ID NO: 50

Lip-S1D1-S2D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 1 and OspA serotype 2 with disulfide bond type 1, *E. coli* lpp lipidation signal, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAGTCAGCGAAAAAATCATTACCCGCGCAGACGGCACCCGCCT
GGAATACACCGGCATCAAATCGGACGGCAGCGGCAAAGCGAAAGAAGTTCTGAAAAACTTTACC
CTGGAAGGCAAAGTCGCAAATGATAAAACCACCCTGGTGGTGAAATGCGGCACCGTTACGCTGA
GCAAAAACATTAGTAAATCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGC
CACCAAGAAAACCGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATAGCAAG
AAAACCAAAGATCTGGTCTTCACGAAAGAAAACACCATCACGGTGCAGCAATATGACAGCAATGG
TACCAAACTGGAAGGCTCCGCTGTGGAAATCACGAAACTGGATGAAATCTGTAATGCTCTGAAAG
GTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATTC
AACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAAAACGGCACCAAACTGGAATATA
CGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAGG
CAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAATGCGGCACCGTTACGCTGTCAAAAGAA
ATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCGACCAAGA
AAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAATAGCAAGAAAACCAC
GCAGCTGGTCTTCACCAAACAAGATACGATCACCGTGCAGAAATACGACAGTGCGGGTACCAAC
CTGGAAGGCACGGCTGTTGAAATCAAAACCCTGGACGAACTGTGTAACGCCCTGAAAGGCCTCG
AGCACCACCACCACCACCAC

SEQ ID NO: 51

S3D4-S4D4_aa: Heterodimer fusion protein of OspA serotype 3 and OspA serotype 4 with disulfide bond type 4, LN1 linker sequence

FNEKGKLSEKVVTRACGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTEGTVTLSKNIS
KSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKECTITVQNYNRAGNALEGSPA

EIKDLAELKAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRACGTRLEYTEIKSDGTGKAK
EVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVN
SKKTKNIVFTKECTITVQKYDSAGTNLEGNAVEIKTLDELKNALK

SEQ ID NO: 52
Lip-S3D4-S4D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 3 and OspA serotype 4 with disulfide bond type 4, *E. coli* lpp lipidation signal, LN1 linker sequence
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCAAACTGTCAGAAAAAGTGGTCACCCGCGCTTGTGGCACCCGCCT
GGAATACACCGAAATCAAAAACGACGGCTCGGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCC
CTGGAAGGTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGGAAGGCACCGTTACG
CTGTCTAAAAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCC
GGCTGACAAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCG
CAGAAACCGAAGCAACTGGTCTTCACCAAAGAATGCACGATCACCGTGCAGAACTATAATCGTG
CCGGTAATGCTCTGGAAGGCTCCCCGGCTGAAATCAAGGACCTGGCGGAACTGAAGGCGGCAC
TGAAAGGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTA
CTCATTCAACGCTAAAGGTGAACTGTCGGAAAAAACCATCCTGCGCGCCTGTGGCACCCGCCTG
GAATACACGGAAATCAAGTCGGACGGCACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCTC
TGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTGA
GCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGCG
ACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAAA
GACCAAAAATATTGTGTTCACGAAGGAATGCACGATCACCGTTCAAAAATATGATTCCGCAGGTA
CCAACCTGGAAGGCAACGCTGTGGAAATCAAAACCCTGGACGAACTGAAAAATGCTCTGAAG

SEQ ID NO: 53
Lip-S3D4-S4D4_His_aa: Heterodimer fusion protein of OspA serotype 3 and OspA serotype 4 with disulfide bond type 4, N-terminal CSS for addition of lipids, N-terminal lipidation, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGKLSEKVVTRACGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTEGTVTL
SKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKECTITVQNYNRAGNALE
GSPAEIKDLAELKAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRACGTRLEYTEIKSDGT
GKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTL
TISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGNAVEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 54
Lip-S3D4-S4D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 3 and OspA serotype 4 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCAAACTGTCAGAAAAAGTGGTCACCCGCGCTTGTGGCACCCGCCT
GGAATACACCGAAATCAAAAACGACGGCTCGGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCC
CTGGAAGGTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGGAAGGCACCGTTACG
CTGTCTAAAAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCC
GGCTGACAAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCG
CAGAAACCGAAGCAACTGGTCTTCACCAAAGAATGCACGATCACCGTGCAGAACTATAATCGTG
CCGGTAATGCTCTGGAAGGCTCCCCGGCTGAAATCAAGGACCTGGCGGAACTGAAGGCGGCAC
TGAAAGGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTA
CTCATTCAACGCTAAAGGTGAACTGTCGGAAAAAACCATCCTGCGCGCCTGTGGCACCCGCCTG
GAATACACGGAAATCAAGTCGGACGGCACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCTC
TGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTGA
GCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGCG
ACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAAA
GACCAAAAATATTGTGTTCACGAAGGAATGCACGATCACCGTTCAAAAATATGATTCCGCAGGTA
CCAACCTGGAAGGCAACGCTGTGGAAATCAAAACCCTGGACGAACTGAAAAATGCTCTGAAGGG
TCTCGAGCACCACCACCACCACCAC

SEQ ID NO: 55

S3D1-S4D1_aa: Heterodimer fusion protein of OspA serotypes 3 and 4 both with disulfide bond type 1, LN1 linker sequence

FNEKGKLSEKVVTRANGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTCGTVTLSKNIS
KSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKENTITVQNYNRAGNALEGSPA
EIKDLAELCAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRANGTRLEYTEIKSDGTGKAK
EVLKDFALEGTLAADKTTLKVTCGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVN
SKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIKTLDELCNALK

SEQ ID NO: 56

Lip-S3D1-S4D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotypes 3 and 4 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCAAACTGTCGGAAAAAGTGGTCACCCGCGCAAATGGCACCCGCCT
GGAATACACGGAAATCAAAAACGATGGTAGCGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCC
CTGGAAGGTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGTGCGGCACCGTTACG
CTGTCTAAAAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCC
GGCTGACAAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCG
CAGAAACCGAAGCAACTGGTCTTCACCAAAGAAAACACGATCACCGTGCAGAACTATAATCGTGC
CGGTAATGCTCTGGAAGGCTCACCGGCTGAAATCAAGGACCTGGCTGAACTGTGTGCGGCACT
GAAAGGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTAC
TCATTCAACGCTAAAGGTGAACTGAGCGAAAAAACGATCCTGCGTGCGAATGGCACCCGTCTGG
AATACACCGAAATCAAATCCGATGGTACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCTCTG
GAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTGAGC
AAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGCAAC
CAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAAAGA
CCAAAAATATTGTGTTCACGAAGGAAGATACGATCACCGTTCAAAAATATGACTCCGCGGGCACC
AACCTGGAAGGCAATGCCGTCGAAATCAAAACCCTGGATGAACTGTGTAATGCTCTGAAG

SEQ ID NO: 57

Lip-S3D1-S4D1_His_aa: Heterodimer fusion protein of OspA serotypes 3 and 4 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, N-terminal lipidation, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)

LipCSSFNEKGKLSEKVVTRANGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTCGTVTL
SKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKENTITVQNYNRAGNALE
GSPAEIKDLAELCAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRANGTRLEYTEIKSDGT
GKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTL
TISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIKTLDELCNALKGLEHHHHHH

SEQ ID NO: 58

Lip-S3D1-S4D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotypes 3 and 4 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCAAACTGTCGGAAAAAGTGGTCACCCGCGCAAATGGCACCCGCCT
GGAATACACGGAAATCAAAAACGATGGTAGCGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCC
CTGGAAGGTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGTGCGGCACCGTTACG
CTGTCTAAAAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCC
GGCTGACAAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCG
CAGAAACCGAAGCAACTGGTCTTCACCAAAGAAAACACGATCACCGTGCAGAACTATAATCGTGC
CGGTAATGCTCTGGAAGGCTCACCGGCTGAAATCAAGGACCTGGCTGAACTGTGTGCGGCACT
GAAAGGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTAC
TCATTCAACGCTAAAGGTGAACTGAGCGAAAAAACGATCCTGCGTGCGAATGGCACCCGTCTGG
AATACACCGAAATCAAATCCGATGGTACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCTCTG
GAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTGAGC
AAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGCAAC
CAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAAAGA
CCAAAAATATTGTGTTCACGAAGGAAGATACGATCACCGTTCAAAAATATGACTCCGCGGGCACC
AACCTGGAAGGCAATGCCGTCGAAATCAAAACCCTGGATGAACTGTGTAATGCTCTGAAGGGTC

TCGAGCACCACCACCACCACCAC

SEQ ID NO: 59
S5D4-S6D4_aa: Heterodimer fusion protein OspA serotypes 5 and 6 both with disulfide bond type 4, LN1 linker sequence
FNEKGEISEKTIVRACGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKS
GEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKECTITVQNYDSAGTNLEGKAVEITT
LKELKNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGKAKEVL
KDFTLEGTLAADGKTTLKVTEGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQ
KTKNLVFTKECTITVQRYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 60
Lip-S5D4-S6D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins OspA serotypes 5 and 6 both with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAATCAGTGAAAAAACCATTGTGCGTGCGTGTGGCACCCGTCT
GGAATATACCGACATCAAGAGCGATAAAACGGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGC
TGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCGAAGGTACCGTTACGC
TGTCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGG
CAACAAAAAGAGCGGTACCTGGGACTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACG
AAAACCAAGCAGCTGGTCTTCACGAAAGAATGCACGATCACCGTGCAAAACTATGATAGCGCAG
GTACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGGCAAAGGTGAAACGAGTGAAAAAACGATTGTTCGCGCCTGTGGCACCCGCCTGGAATAC
ACGGATATCAAGTCGGATGGTTCGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACGCTGGAAG
GTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTGTCAA
AAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGCGTGCTAC
GAAAAAGACCGGTAAATGGGACAGCAAGACCTCTACGCTGACCATTAGTGTCAACTCCCAGAAA
ACGAAGAATCTGGTGTTCACCAAAGAATGCACGATCACCGTTCAACGCTATGATAGTGCGGGCA
CCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAA

SEQ ID NO: 61
Lip-S5D4-S6D4_His_aa: Heterodimer fusion protein OspA serotypes 5 and 6 both with disulfide bond type 4, N-terminal CSS for addition of lipids, N-terminal lipidation, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGEISEKTIVRACGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLS
KNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKECTITVQNYDSAGTNLEGK
AVEITTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGK
AKEVLKDFTLEGTLAADGKTTLKVTEGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTIS
VNSQKTKNLVFTKECTITVQRYDSAGTNLEGKAVEITTLKELKNALKGLEHHHHHH

SEQ ID NO: 62
Lip-S5D4-S6D4_His_nt: Coding sequence for heterodimer fusion protein OspA serotypes 5 and 6 both with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAATCAGTGAAAAAACCATTGTGCGTGCGTGTGGCACCCGTCT
GGAATATACCGACATCAAGAGCGATAAAACGGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGC
TGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCGAAGGTACCGTTACGC
TGTCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGG
CAACAAAAAGAGCGGTACCTGGGACTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACG
AAAACCAAGCAGCTGGTCTTCACGAAAGAATGCACGATCACCGTGCAAAACTATGATAGCGCAG
GTACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGGCAAAGGTGAAACGAGTGAAAAAACGATTGTTCGCGCCTGTGGCACCCGCCTGGAATAC
ACGGATATCAAGTCGGATGGTTCGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACGCTGGAAG
GTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTGTCAA

AAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGCGTGCTAC
GAAAAAGACCGGTAAATGGGACAGCAAGACCTCTACGCTGACCATTAGTGTCAACTCCCAGAAA
ACGAAGAATCTGGTGTTCACCAAAGAATGCACGATCACCGTTCAACGCTATGATAGTGCGGGCA
CCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAAGG
TCTCGAGCACCACCACCACCACCAC

SEQ ID NO: 63
S5D1-S6D1_aa: Heterodimer fusion protein of OspA serotypes 6 both with disulfide bond type 1, LN1 linker sequence
FNEKGEISEKTIVRANGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLSKNISKS
GEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITT
LKELCNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVL
KDFTLEGTLAADGKTTLKVTCGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQ
KTKNLVFTKEDTITVQRYDSAGTNLEGKAVEITTLKELCNALK

SEQ ID NO: 64
Lip-S5D1-S6D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotypes 6 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAATCTCAGAAAAAACCATCGTCCGCGCTAACGGCACCCGCCT
GGAATACACCGACATCAAATCAGACAAGACCGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGC
TGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCTGCGGTACCGTTACGC
TGTCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGG
CAACAAAAAGAGCGGTACCTGGGATTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACG
AAAACCAAGCAGCTGGTCTTCACGAAAGAAGATACGATCACCGTGCAAAACTATGACAGCGCAG
GTACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGGCAAAGGTGAAACGAGCGAAAAGACCATCGTGCGTGCGAACGGTACCCGCCTGGAATA
TACGGACATTAAATCGGACGGCAGCGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACGCTGGAA
GGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTGTCA
AAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGCGTGCTA
CGAAAAAGACCGGTAAATGGGATAGCAAGACCTCTACGCTGACCATTAGTGTCAACTCCCAGAA
AACGAAGAATCTGGTGTTCACCAAAGAAGATACGATCACCGTTCAACGCTATGACAGTGCGGGC
ACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAA

SEQ ID NO: 65
Lip-S5D1-S6D1_His_aa: Heterodimer fusion protein of OspA serotypes 6 both with disulfide bond type 1, N-terminal CSS for addition of lipids, N-terminal lipidation, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGEISEKTIVRANGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLS
KNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGK
AVEITTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGK
AKEVLKDFTLEGTLAADGKTTLKVTCGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTIS
VNSQKTKNLVFTKEDTITVQRYDSAGTNLEGKAVEITTLKELCNALKGLEHHHHHH

SEQ ID NO: 66
Lip-S5D1-S6D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotypes 6 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAATCTCAGAAAAAACCATCGTCCGCGCTAACGGCACCCGCCT
GGAATACACCGACATCAAATCAGACAAGACCGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGC
TGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCTGCGGTACCGTTACGC
TGTCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGG
CAACAAAAAGAGCGGTACCTGGGATTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACG
AAAACCAAGCAGCTGGTCTTCACGAAAGAAGATACGATCACCGTGCAAAACTATGACAGCGCAG
GTACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT

CAACGGCAAAGGTGAAACGAGCGAAAAGACCATCGTGCGTGCGAACGGTACCCGCCTGGAATA
TACGGACATTAAATCGGACGGCAGCGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACGCTGGAA
GGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTGTCA
AAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGCGTGCTA
CGAAAAAGACCGGTAAATGGGATAGCAAGACCTCTACGCTGACCATTAGTGTCAACTCCCAGAA
AACGAAGAATCTGGTGTTCACCAAAGAAGATACGATCACCGTTCAACGCTATGACAGTGCGGGC
ACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAAG
GTCTCGAGCACCACCACCACCACCAC

SEQ ID NO: 67
S2D4-S1D4_aa: Heterodimer fusion protein of OspA serotypes 2 and 1 both with disulfide bond type 4, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
FNEKGELSAKTMTRECGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAK
SGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQCTITVQKYDSAGTNLEGTAVEI
KTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRACGTRLEYTGIKSDGSGKAKEV
LKNFTLEGKVANDKTTLVVKEGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSK
KTKDLVFTKECTITVQQYDSNGTKLEGSAVEITKLDEIKNALK

SEQ ID NO: 68
Lip-S2D4-S1D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotypes 2 and 1 both with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAATGCGGCACCAAACT
GGAATATACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCC
TGGAAGGCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAAGAAGGCACCGTTACGCTGTC
AAAAGAAATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCG
ACCAAGAAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAACAGCAAGA
AAACCACGCAGCTGGTCTTCACCAAACAATGTACGATCACCGTGCAGAAATACGATAGTGCGGG
TACCAACCTGGAAGGCACCGCTGTTGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAA
GGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAAGTCTCGGAAAAAATCATTACCCGTGCTTGCGGCACCCGTCTGGAATAC
ACCGGCATTAAATCGGATGGCAGCGGCAAAGCGAAGGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCAAATGATAAGACCACCCTGGTGGTGAAAGAAGGCACCGTTACGCTGAGCAAAAA
CATTAGTAAGTCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGCCACCAAA
AAGACGGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATTCCAAAAAGACCA
AAGATCTGGTCTTCACGAAAGAATGCACCATCACGGTGCAGCAATATGACAGCAACGGTACCAA
ACTGGAAGGCTCTGCGGTGGAAATCACGAAACTGGATGAAATCAAAAATGCACTGAAA

SEQ ID NO: 69
Lip-S2D4-S1D4_His_aa: Heterodimer fusion protein of OspA serotypes 2 and 1 both with disulfide bond type 4, N-terminal CSS for addition of lipids, N-terminal lipidation, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGELSAKTMTRECGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTL
SKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQCTITVQKYDSAGTNLE
GTAVEIKTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRACGTRLEYTGIKSDGS
GKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTL
TITVNSKKTKDLVFTKECTITVQQYDSNGTKLEGSAVEITKLDEIKNALKGLEHHHHHH

SEQ ID NO: 70
Lip-S2D4-S1D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotypes 2 and 1 both with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAATGCGGCACCAAACT

GGAATATACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCC
TGGAAGGCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAAGAAGGCACCGTTACGCTGTC
AAAAGAAATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCG
ACCAAGAAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAACAGCAAGA
AAACCACGCAGCTGGTCTTCACCAAACAATGTACGATCACCGTGCAGAAATACGATAGTGCGGG
TACCAACCTGGAAGGCACCGCTGTTGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAA
GGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAAGTCTCGGAAAAAATCATTACCCGTGCTTGCGGCACCCGTCTGGAATAC
ACCGGCATTAAATCGGATGGCAGCGGCAAAGCGAAGGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCAAATGATAAGACCACCCTGGTGGTGAAAGAAGGCACCGTTACGCTGAGCAAAAA
CATTAGTAAGTCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGCCACCAAA
AAGACGGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATTCCAAAAAGACCA
AAGATCTGGTCTTCACGAAAGAATGCACCATCACGGTGCAGCAATATGACAGCAACGGTACCAA
ACTGGAAGGCTCTGCGGTGGAAATCACGAAACTGGATGAAATCAAAAATGCACTGAAAGGTCTC
GAGCACCACCACCACCACCAC

SEQ ID NO: 71
S2D1-S1D1_aa: Heterodimer fusion protein of OspA serotypes 2 and 1 both with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
FNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKCGTVTLSKEIAK
SGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEI
KTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEV
LKNFTLEGKVANDKTTLVVKCGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSK
KTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEICNALK

SEQ ID NO: 72
Lip-S2D1-S1D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotypes 2 and 1 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAAAACGGCACCAAACT
GGAATATACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCC
TGGAAGGCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAATGCGGCACCGTTACGCTGTC
AAAAGAAATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCG
ACCAAGAAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAATAGCAAGA
AAACCACGCAGCTGGTCTTCACCAAACAAGATACGATCACCGTGCAGAAATACGACAGTGCGGG
TACCAACCTGGAAGGCACGGCTGTTGAAATCAAAACCCTGGACGAACTGTGTAACGCCCTGAAA
GGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAAGTCAGCGAAAAAATCATTACCCGCGCAGACGGCACCCGCCTGGAATAC
ACCGGCATCAAATCGGACGGCAGCGGCAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCAAATGATAAAACCACCCTGGTGGTGAAATGCGGCACCGTTACGCTGAGCAAAAA
CATTAGTAAATCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGCCACCAAG
AAAACCGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATAGCAAGAAAACCA
AAGATCTGGTCTTCACGAAAGAAAACACCATCACGGTGCAGCAATATGACAGCAATGGTACCAAA
CTGGAAGGCTCCGCTGTGGAAATCACGAAACTGGATGAAATCTGTAATGCACTGAAA

SEQ ID NO: 73
Lip-S2D1-S1D1_His_aa: Heterodimer fusion protein of OspA serotypes 2 and 1 both with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKCGTVTL
SKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLE
GTAVEIKTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRADGTRLEYTGIKSDGS
GKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTL
TITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEICNALKGLEHHHHHH

SEQ ID NO: 74
Lip-S2D1-S1D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotypes 2 and 1 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAAAACGGCACCAAACT
GGAATATACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCC
TGGAAGGCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAATGCGGCACCGTTACGCTGTC
AAAAGAAATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCG
ACCAAGAAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAATAGCAAGA
AAACCACGCAGCTGGTCTTCACCAAACAAGATACGATCACCGTGCAGAAATACGACAGTGCGGG
TACCAACCTGGAAGGCACGGCTGTTGAAATCAAAACCCTGGACGAACTGTGTAACGCCCTGAAA
GGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAAGTCAGCGAAAAAATCATTACCCGCGCAGACGGCACCCGCCTGGAATAC
ACCGGCATCAAATCGGACGGCAGCGGCAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCAAATGATAAAACCACCCTGGTGGTGAAATGCGGCACCGTTACGCTGAGCAAAAA
CATTAGTAAATCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGCCACCAAG
AAAACCGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATAGCAAGAAAACCA
AAGATCTGGTCTTCACGAAAGAAAACACCATCACGGTGCAGCAATATGACAGCAATGGTACCAAA
CTGGAAGGCTCCGCTGTGGAAATCACGAAACTGGATGAAATCTGTAATGCACTGAAAGGTCTCG
AGCACCACCACCACCACCAC

SEQ ID NO: 75
S4D4-S3D4_aa: Heterodimer fusion protein of OspA serotypes 4 and 3 both with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence

FNAKGELSEKTILRACGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNS
GEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGNAVEIK
TLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRACGTRLEYTEIKNDGSGKAKEV
LKGFALEGTLTDGGETKLTVTEGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNS
QKPKQLVFTKECTITVQNYNRAGNALEGSPAEIKDLAELKAALK

SEQ ID NO: 76
Lip-S4D4-S3D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotypes 4 and 3 both with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGCTAAAGGTGAACTGTCGGAAAAAACCATCCTGCGCGCCTGTGGCACCCGCCT
GGAATACACGGAAATCAAGTCGGACGGCACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCT
CTGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTG
AGCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGC
GACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAA
AAGACCAAAAATATTGTGTTCACGAAGGAATGCACGATCACCGTTCAAAAATATGATTCCGCAGG
TACCAACCTGGAAGGCAACGCTGTGGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAG
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
TAACGATAAGGGCAAACTGTCAGAAAAAGTGGTCACCCGCGCTTGTGGCACCCGCCTGGAATAC
ACCGAAATCAAAAACGACGGCTCGGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCCCTGGAAG
GTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGGAAGGCACCGTTACGCTGTCTAA
AAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCCGGCTGAC
AAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCGCAGAAAC
CGAAGCAACTGGTCTTCACCAAAGAATGCACGATCACCGTGCAGAACTATAATCGTGCCGGTAAT
GCTCTGGAAGGCTCCCCGGCTGAAATCAAGGACCTGGCGGAACTGAAGGCGGCACTGAAA

SEQ ID NO: 77
Lip-S4D4-S3D4_His_aa: Heterodimer fusion protein of OspA serotypes 4 and 3 both with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)

LipCSSFNAKGELSEKTILRACGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSK
HIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGN
AVEIKTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRACGTRLEYTEIKNDGSG
KAKEVLKGFALEGTLTDGGETKLTVTEGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLT
ISKNSQKPKQLVFTKECTITVQNYNRAGNALEGSPAEIKDLAELKAALKGLEHHHHHH

SEQ ID NO: 78
Lip-S4D4-S3D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotypes 4 and 3 both with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGCTAAAGGTGAACTGTCGGAAAAAACCATCCTGCGCGCCTGTGGCACCCGCCT
GGAATACACGGAAATCAAGTCGGACGGCACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCT
CTGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTG
AGCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGC
GACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAA
AAGACCAAAAATATTGTGTTCACGAAGGAATGCACGATCACCGTTCAAAAATATGATTCCGCAGG
TACCAACCTGGAAGGCAACGCTGTGGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAG
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
TAACGATAAGGGCAAACTGTCAGAAAAAGTGGTCACCCGCGCTTGTGGCACCCGCCTGGAATAC
ACCGAAATCAAAAACGACGGCTCGGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCCCTGGAAG
GTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGGAAGGCACCGTTACGCTGTCTAA
AAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCCGGCTGAC
AAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCGCAGAAAC
CGAAGCAACTGGTCTTCACCAAAGAATGCACGATCACCGTGCAGAACTATAATCGTGCCGGTAAT
GCTCTGGAAGGCTCCCCGGCTGAAATCAAGGACCTGGCGGAACTGAAGGCGGCACTGAAAGGT
CTCGAGCACCACCACCACCACCAC

SEQ ID NO: 79
S4D1-S3D1_aa: Heterodimer fusion protein of OspA serotypes 4 and 3 both with disulfide bond type 1, LN1 linker sequence
FNAKGELSEKTILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSKHIPNS
GEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIK
TLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRANGTRLEYTEIKNDGSGKAKEV
LKGFALEGTLTDGGETKLTVTCGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNS
QKPKQLVFTKENTITVQNYNRAGNALEGSPAEIKDLAELCAALK

SEQ ID NO: 80
Lip-S4D1-S3D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotypes 4 and 3 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAATGCTAAGGGCGAACTGAGCGAAAAAACGATCCTGCGTGCGAATGGCACCCGTCT
GGAATACACCGAAATCAAATCCGATGGTACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCT
CTGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTG
AGCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGC
AACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAA
AGACCAAAAATATTGTGTTCACGAAGGAAGATACGATCACCGTTCAAAAATATGACTCCGCGGGC
ACCAACCTGGAAGGCAATGCCGTCGAAATCAAAACCCTGGATGAACTGTGTAACGCCCTGAAGG
GTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATTT
AACGATAAGGGCAAACTGTCGGAAAAAGTGGTCACCCGCGCAAATGGCACCCGCCTGGAATACA
CGGAAATCAAAAACGATGGTAGCGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCCCTGGAAGG
TACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGTGCGGCACCGTTACGCTGTCTAAA
AACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCCGGCTGACA
AAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCGCAGAAACC
GAAGCAACTGGTCTTCACCAAAGAAAACACGATCACCGTGCAGAACTATAATCGTGCCGGTAATG
CTCTGGAAGGCTCACCGGCTGAAATCAAGGACCTGGCTGAACTGTGTGCGGCACTGAAA

SEQ ID NO: 81

Lip-S4D1-S3D1_His_aa: Heterodimer fusion protein of OspA serotypes 4 and 3 both with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)

LipCSSFNAKGELSEKTILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSK
HIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGN
AVEIKTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRANGTRLEYTEIKNDGSG
KAKEVLKGFALEGTLTDGGETKLTVTCGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLT
ISKNSQKPKQLVFTKENTITVQNYNRAGNALEGSPAEIKDLAELCAALKGLEHHHHHH

SEQ ID NO: 82

Lip-S4D1-S3D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotypes 4 and 3 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAATGCTAAGGGCGAACTGAGCGAAAAAACGATCCTGCGTGCGAATGGCACCCGTCT
GGAATACACCGAAATCAAATCCGATGGTACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCT
CTGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTG
AGCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGC
AACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAA
AGACCAAAAATATTGTGTTCACGAAGGAAGATACGATCACCGTTCAAAAATATGACTCCGCGGGC
ACCAACCTGGAAGGCAATGCCGTCGAAATCAAAACCCTGGATGAACTGTGTAACGCCCTGAAGG
GTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATTT
AACGATAAGGGCAAACTGTCGGAAAAAGTGGTCACCCGCGCAAATGGCACCCGCCTGGAATACA
CGGAAATCAAAAACGATGGTAGCGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCCCTGGAAGG
TACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGTGCGGCACCGTTACGCTGTCTAAA
AACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCCGGCTGACA
AAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCGCAGAAACC
GAAGCAACTGGTCTTCACCAAAGAAAACACGATCACCGTGCAGAACTATAATCGTGCCGGTAATG
CTCTGGAAGGCTCACCGGCTGAAATCAAGGACCTGGCTGAACTGTGTGCGGCACTGAAAGGTCT
CGAGCACCACCACCACCACCAC

SEQ ID NO: 83

S6D4-S5D4_aa: Heterodimer fusion protein of OspA serotypes 6 and 5 both with disulfide bond type 4, LN1 linker sequence

FNGKGETSEKTIVRACGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVVLSKNILK
SGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKECTITVQRYDSAGTNLEGKAVEI
TTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRACGTRLEYTDIKSDKTGKAKEV
LKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRT
KTKQLVFTKECTITVQNYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 84

Lip-S6D4-S5D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotypes 6 and 5 both with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGGCAAAGGTGAAACGAGTGAAAAAACGATTGTTCGCGCCTGTGGCACCCGCCT
GGAATACACGGATATCAAGTCGGATGGTTCGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACG
CTGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTT
CTGTCAAAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGC
GTGCTACGAAAAAGACCGGTAAATGGGACAGCAAGACCTCTACGCTGACCATTAGTGTCAACTC
CCAGAAAACGAAGAATCTGGTGTTCACCAAAGAATGCACGATCACCGTTCAACGCTATGATAGTG
CGGGCACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGAAGAATGCTCT
GAAAGGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACT
CATTCAACGAAAAAGGCGAAATCAGTGAAAAAACCATTGTGCGTGCGTGTGGCACCCGTCTGGA
ATATACCGACATCAAGAGCGATAAAACGGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGCTGG
AAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCGAAGGTACCGTTACGCTGT
CCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGGCAA
CAAAAAGAGCGGTACCTGGGACTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACGAAA

ACCAAGCAGCTGGTCTTCACGAAAGAATGCACGATCACCGTGCAAAACTATGATAGCGCAGGTA
CCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAA

SEQ ID NO: 85
Lip-S6D4-S5D4_His_aa: Heterodimer fusion protein of OspA serotypes 6 and 5 both with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVVL
SKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKECTITVQRYDSAGTNLE
GKAVEITTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRACGTRLEYTDIKSDKTG
KAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTI
SKNRTKTKQLVFTKECTITVQNYDSAGTNLEGKAVEITTLKELKNALKGLEHHHHHH

SEQ ID NO: 86
Lip-S6D4-S5D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotypes 6 and 5 both with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGGCAAAGGTGAAACGAGTGAAAAAACGATTGTTCGCGCCTGTGGCACCCGCCT
GGAATACACGGATATCAAGTCGGATGGTTCGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACG
CTGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTT
CTGTCAAAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGC
GTGCTACGAAAAAGACCGGTAAATGGGACAGCAAGACCTCTACGCTGACCATTAGTGTCAACTC
CCAGAAAACGAAGAATCTGGTGTTCACCAAAGAATGCACGATCACCGTTCAACGCTATGATAGTG
CGGGCACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGAAGAATGCTCT
GAAAGGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACT
CATTCAACGAAAAAGGCGAAATCAGTGAAAAAACCATTGTGCGTGCGTGTGGCACCCGTCTGGA
ATATACCGACATCAAGAGCGATAAAACGGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGCTGG
AAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCGAAGGTACCGTTACGCTGT
CCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGGCAA
CAAAAAGAGCGGTACCTGGGACTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACGAAA
ACCAAGCAGCTGGTCTTCACGAAAGAATGCACGATCACCGTGCAAAACTATGATAGCGCAGGTA
CCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAAGG
TCTCGAGCACCACCACCACCACCAC

SEQ ID NO: 87
S6D1-S5D1_aa: Heterodimer fusion protein of OspA serotypes 6 and 5 both with disulfide bond type 1, LN1 linker sequence
FNGKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVVLSKNILK
SGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKEDTITVQRYDSAGTNLEGKAVEI
TTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRANGTRLEYTDIKSDKTGKAKEV
LKDFTLEGTLAADGKTTLKVTCGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRT
KTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITTLKELCNALK

SEQ ID NO: 88
Lip-S6D1-S5D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotypes 6 and 5 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGGCAAAGGTGAAACGAGCGAAAAGACCATCGTGCGTGCGAACGGTACCCGCC
TGGAATATACGGACATTAAATCGGACGGCAGCGGCAAAGCAAAGGAAGTCCTGAAAGATTTTAC
GCTGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGT
TCTGTCAAAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACG
CGTGCTACGAAAAAGACCGGTAAATGGGATAGCAAGACCTCTACGCTGACCATTAGTGTCAACT
CCCAGAAAACGAAGAATCTGGTGTTCACCAAAGAAGATACGATCACCGTTCAACGCTATGACAGT
GCGGGCACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGTGTAATGCTC
TGAAAGGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTAC
TCATTCAACGAAAAAGGCGAAATCTCAGAAAAAACCATCGTCCGCGCTAACGGCACCCGCCTGG
AATACACCGACATCAAATCAGACAAGACCGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGCTG

GAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCTGCGGTACCGTTACGCTG
TCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGGCA
ACAAAAAGAGCGGTACCTGGGATTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACGAAA
ACCAAGCAGCTGGTCTTCACGAAAGAAGATACGATCACCGTGCAAAACTATGACAGCGCAGGTA
CCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAA

SEQ ID NO: 89
Lip-S6D1-S5D1_His_aa: Heterodimer fusion protein of OspA serotypes 6 and 5 both with disulfide bond type 1, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVVL
SKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKEDTITVQRYDSAGTNLE
GKAVEITTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRANGTRLEYTDIKSDKT
GKAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTL
TISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITTLKELCNALKGLEHHHHHH

SEQ ID NO: 90
Lip-S6D1-S5D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotypes 6 and 5 both with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGGCAAAGGTGAAACGAGCGAAAAGACCATCGTGCGTGCGAACGGTACCCGCC
TGGAATATACGGACATTAAATCGGACGGCAGCGGCAAAGCAAAGGAAGTCCTGAAAGATTTTAC
GCTGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGT
TCTGTCAAAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACG
CGTGCTACGAAAAAGACCGGTAAATGGGATAGCAAGACCTCTACGCTGACCATTAGTGTCAACT
CCCAGAAAACGAAGAATCTGGTGTTCACCAAAGAAGATACGATCACCGTTCAACGCTATGACAGT
GCGGGCACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGTGTAATGCTC
TGAAAGGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTAC
TCATTCAACGAAAAAGGCGAAATCTCAGAAAAAACCATCGTCCGCGCTAACGGCACCCGCCTGG
AATACACCGACATCAAATCAGACAAGACCGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGCTG
GAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCTGCGGTACCGTTACGCTG
TCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGGCA
ACAAAAAGAGCGGTACCTGGGATTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACGAAA
ACCAAGCAGCTGGTCTTCACGAAAGAAGATACGATCACCGTGCAAAACTATGACAGCGCAGGTA
CCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAAGGT
CTCGAGCACCACCACCACCACCAC

SEQ ID NO: 91
S1D4-S2D1_aa: Heterodimer fusion protein of OspA serotype 1 with disulfide bond type 4 and OspA serotype 2 with disulfide bond type 1, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
FNEKGEVSEKIITRACGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSKNISKS
GEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKECTITVQQYDSNGTKLEGSAVEIT
KLDEIKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKE
VLKNFTLEGKVANDKVTLEVKCGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNS
KKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELCNALK

SEQ ID NO: 92
Lip-S1D4-S2D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 1 with disulfide bond type 4 and OspA serotype 2 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAGTCTCGGAAAAAATCATTACCCGTGCTTGCGGCACCCGTCT
GGAATACACCGGCATTAAATCGGATGGCAGCGGCAAAGCGAAGGAAGTTCTGAAAAACTTTACC
CTGGAAGGCAAAGTCGCAAATGATAAGACCACCCTGGTGGTGAAAGAAGGCACCGTTACGCTGA
GCAAAAACATTAGTAAGTCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGC
CACCAAAAAGACGGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATTCCAAA
AAGACCAAAGATCTGGTCTTCACGAAAGAATGCACCATCACGGTGCAGCAATATGACAGCAACG

GTACCAAACTGGAAGGCTCTGCGGTGGAAATCACGAAACTGGATGAAATCAAAAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAAAACGGCACCAAACTGGAATAT
ACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAATGCGGCACCGTTACGCTGTCAAAAGA
AATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCGACCAAG
AAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAATAGCAAGAAAACCA
CGCAGCTGGTCTTCACCAAACAAGATACGATCACCGTGCAGAAATACGACAGTGCGGGTACCAA
CCTGGAAGGCACGGCTGTTGAAATCAAAACCCTGGACGAACTGTGTAACGCCCTGAAA

SEQ ID NO: 93
Lip-S1D4-S2D1_His_aa: Heterodimer fusion protein of OspA serotype 1 with disulfide bond type 4 and OspA serotype 2 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGEVSEKIITRACGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSK
NISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKECTITVQQYDSNGTKLEG
SAVEITKLDEIKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRENGTKLEYTEMKSDGT
GKAKEVLKNFTLEGKVANDKVTLEVKCGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTL
TISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELCNALKGLEHHHHHH

SEQ ID NO: 94
Lip-S1D4-S2D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 1 with disulfide bond type 4 and OspA serotype 2 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAGTCTCGGAAAAAATCATTACCCGTGCTTGCGGCACCCGTCT
GGAATACACCGGCATTAAATCGGATGGCAGCGGCAAAGCGAAGGAAGTTCTGAAAAACTTTACC
CTGGAAGGCAAAGTCGCAAATGATAAGACCACCCTGGTGGTGAAAGAAGGCACCGTTACGCTGA
GCAAAAACATTAGTAAGTCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGC
CACCAAAAAGACGGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATTCCAAA
AAGACCAAAGATCTGGTCTTCACGAAAGAATGCACCATCACGGTGCAGCAATATGACAGCAACG
GTACCAAACTGGAAGGCTCTGCGGTGGAAATCACGAAACTGGATGAAATCAAAAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAAAACGGCACCAAACTGGAATAT
ACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAATGCGGCACCGTTACGCTGTCAAAAGA
AATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCGACCAAG
AAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAATAGCAAGAAAACCA
CGCAGCTGGTCTTCACCAAACAAGATACGATCACCGTGCAGAAATACGACAGTGCGGGTACCAA
CCTGGAAGGCACGGCTGTTGAAATCAAAACCCTGGACGAACTGTGTAACGCCCTGAAAGGCCTC
GAGCACCACCACCACCACCAC

SEQ ID NO: 95
S1D1-S2D4_aa: Heterodimer fusion protein of OspA serotype 1 with disulfide bond type 1 and OspA serotype 2 with disulfide bond type 4, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
FNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSKNISKS
GEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEIT
KLDEICNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRECGTKLEYTEMKSDGTGKAKE
VLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNS
KKTTQLVFTKQCTITVQKYDSAGTNLEGTAVEIKTLDELKNALK

SEQ ID NO: 96
Lip-S1D1-S2D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 1 with disulfide bond type 1 and OspA serotype 2 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAGTCAGCGAAAAAATCATTACCCGCGCAGACGGCACCCGCCT
GGAATACACCGGCATCAAATCGGACGGCAGCGGCAAAGCGAAAGAAGTTCTGAAAAACTTTACC
CTGGAAGGCAAAGTCGCAAATGATAAAACCACCCTGGTGGTGAAATGCGGCACCGTTACGCTGA
GCAAAAACATTAGTAAATCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGC
CACCAAGAAAACCGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATAGCAAG
AAAACCAAAGATCTGGTCTTCACGAAAGAAAACACCATCACGGTGCAGCAATATGACAGCAATGG
TACCAAACTGGAAGGCTCCGCTGTGGAAATCACGAAACTGGATGAAATCTGTAATGCTCTGAAAG
GTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATTC
AACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAATGCGGCACCAAACTGGAATATA
CGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAGG
CAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAAGAAGGCACCGTTACGCTGTCAAAAGAA
ATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCGACCAAGA
AAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAACAGCAAGAAAACCAC
GCAGCTGGTCTTCACCAAACAATGTACGATCACCGTGCAGAAATACGATAGTGCGGGTACCAAC
CTGGAAGGCACCGCTGTTGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAA

SEQ ID NO: 97

Lip-S1D1-S2D4_His_aa: Heterodimer fusion protein of OspA serotype 1 with disulfide bond type 1 and OspA serotype 2 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)

LipCSSFNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSK
NISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEG
SAVEITKLDEICNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRECGTKLEYTEMKSDGT
GKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTL
TISVNSKKTTQLVFTKQCTITVQKYDSAGTNLEGTAVEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 98

Lip-S1D1-S2D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 1 with disulfide bond type 1 and OspA serotype 2 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAGTCAGCGAAAAAATCATTACCCGCGCAGACGGCACCCGCCT
GGAATACACCGGCATCAAATCGGACGGCAGCGGCAAAGCGAAAGAAGTTCTGAAAAACTTTACC
CTGGAAGGCAAAGTCGCAAATGATAAAACCACCCTGGTGGTGAAATGCGGCACCGTTACGCTGA
GCAAAAACATTAGTAAATCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGC
CACCAAGAAAACCGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATAGCAAG
AAAACCAAAGATCTGGTCTTCACGAAAGAAAACACCATCACGGTGCAGCAATATGACAGCAATGG
TACCAAACTGGAAGGCTCCGCTGTGGAAATCACGAAACTGGATGAAATCTGTAATGCTCTGAAAG
GTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATTC
AACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAATGCGGCACCAAACTGGAATATA
CGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAGG
CAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAAGAAGGCACCGTTACGCTGTCAAAAGAA
ATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCGACCAAGA
AAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAACAGCAAGAAAACCAC
GCAGCTGGTCTTCACCAAACAATGTACGATCACCGTGCAGAAATACGATAGTGCGGGTACCAAC
CTGGAAGGCACCGCTGTTGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAAGGCCTCG
AGCACCACCACCACCACCAC

SEQ ID NO: 99

S3D4-S4D1_aa: Heterodimer fusion protein of OspA serotype 3 with disulfide bond type 4 and OspA serotype 4 with disulfide bond type 1, LN1 linker sequence

FNEKGKLSEKVVTRACGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTEGTVTLSKNIS
KSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKECTITVQNYNRAGNALEGSPA
EIKDLAELKAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRANGTRLEYTEIKSDGTGKAK
EVLKDFALEGTLAADKTTLKVTCGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVN
SKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIKTLDELCNALK

SEQ ID NO: 100
Lip-S3D4-S4D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 3 with disulfide bond type 4 and OspA serotype 4 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCAAACTGTCAGAAAAAGTGGTCACCCGCGCTTGTGGCACCCGCCT
GGAATACACCGAAATCAAAAACGACGGCTCGGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCC
CTGGAAGGTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGGAAGGCACCGTTACG
CTGTCTAAAAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCC
GGCTGACAAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCG
CAGAAACCGAAGCAACTGGTCTTCACCAAAGAATGCACGATCACCGTGCAGAACTATAATCGTG
CCGGTAATGCTCTGGAAGGCTCCCCGGCTGAAATCAAGGACCTGGCGGAACTGAAGGCGGCAC
TGAAAGGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTA
CTCATTCAACGCTAAAGGTGAACTGAGCGAAAAAACGATCCTGCGTGCGAATGGCACCCGTCTG
GAATACACCGAAATCAAATCCGATGGTACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCTCT
GGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTGAG
CAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGCAA
CCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAAA
GACCAAAAATATTGTGTTCACGAAGGAAGATACGATCACCGTTCAAAAATATGACTCCGCGGGCA
CCAACCTGGAAGGCAATGCCGTCGAAATCAAAACCCTGGATGAACTGTGTAATGCTCTGAAG

SEQ ID NO: 101
Lip-S3D4-S4D1_His_aa: Heterodimer fusion protein of OspA serotype 3 with disulfide bond type 4 and OspA serotype 4 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGKLSEKVVTRACGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTEGTVTL
SKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKECTITVQNYNRAGNALE
GSPAEIKDLAELKAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRANGTRLEYTEIKSDGT
GKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTL
TISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIKTLDELCNALKGLEHHHHHH

SEQ ID NO: 102
Lip-S3D4-S4D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 3 with disulfide bond type 4 and OspA serotype 4 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCAAACTGTCAGAAAAAGTGGTCACCCGCGCTTGTGGCACCCGCCT
GGAATACACCGAAATCAAAAACGACGGCTCGGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCC
CTGGAAGGTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGGAAGGCACCGTTACG
CTGTCTAAAAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCC
GGCTGACAAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCG
CAGAAACCGAAGCAACTGGTCTTCACCAAAGAATGCACGATCACCGTGCAGAACTATAATCGTG
CCGGTAATGCTCTGGAAGGCTCCCCGGCTGAAATCAAGGACCTGGCGGAACTGAAGGCGGCAC
TGAAAGGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTA
CTCATTCAACGCTAAAGGTGAACTGAGCGAAAAAACGATCCTGCGTGCGAATGGCACCCGTCTG
GAATACACCGAAATCAAATCCGATGGTACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCTCT
GGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTGAG
CAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGCAA
CCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAAA
GACCAAAAATATTGTGTTCACGAAGGAAGATACGATCACCGTTCAAAAATATGACTCCGCGGGCA
CCAACCTGGAAGGCAATGCCGTCGAAATCAAAACCCTGGATGAACTGTGTAATGCTCTGAAGGG
TCTCGAGCACCACCACCACCACCAC

SEQ ID NO: 103
S3D1-S4D4_aa: Heterodimer fusion protein of OspA serotype 3 with disulfide bond type 1 and OspA serotype 4 with disulfide bond type 1, LN1 linker sequence
FNEKGKLSEKVVTRANGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTCGTVTLSKNIS
KSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKENTITVQNYNRAGNALEGSPA

EIKDLAELCAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRACGTRLEYTEIKSDGTGKAK
EVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVN
SKKTKNIVFTKECTITVQKYDSAGTNLEGNAVEIKTLDELKNALK

SEQ ID NO: 104
Lip-S3D1-S4D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 3 with disulfide bond type 1 and OspA serotype 4 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCAAACTGTCGGAAAAAGTGGTCACCCGCGCAAATGGCACCCGCCT
GGAATACACGGAAATCAAAAACGATGGTAGCGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCC
CTGGAAGGTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGTGCGGCACCGTTACG
CTGTCTAAAAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCC
GGCTGACAAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCG
CAGAAACCGAAGCAACTGGTCTTCACCAAAGAAAACACGATCACCGTGCAGAACTATAATCGTGC
CGGTAATGCTCTGGAAGGCTCACCGGCTGAAATCAAGGACCTGGCTGAACTGTGTGCGGCACT
GAAAGGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTAC
TCATTCAACGCTAAAGGTGAACTGTCGGAAAAAACCATCCTGCGCGCCTGTGGCACCCGCCTGG
AATACACGGAAATCAAGTCGGACGGCACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCTCT
GGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTGAG
CAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGCGA
CCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAAA
GACCAAAAATATTGTGTTCACGAAGGAATGCACGATCACCGTTCAAAAATATGATTCCGCAGGTA
CCAACCTGGAAGGCAACGCTGTGGAAATCAAAACCCTGGACGAACTGAAAAATGCTCTGAAG

SEQ ID NO: 105
Lip-S3D1-S4D4_His_aa: Heterodimer fusion protein of OspA serotype 3 with disulfide bond type 1 and OspA serotype 4 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGKLSEKVVTRANGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTCGTVTL
SKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKENTITVQNYNRAGNALE
GSPAEIKDLAELCAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRACGTRLEYTEIKSDGT
GKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTL
TISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGNAVEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 106
Lip-S3D1-S4D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 3 with disulfide bond type 1 and OspA serotype 4 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCAAACTGTCGGAAAAAGTGGTCACCCGCGCAAATGGCACCCGCCT
GGAATACACGGAAATCAAAAACGATGGTAGCGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCC
CTGGAAGGTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGTGCGGCACCGTTACG
CTGTCTAAAAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCC
GGCTGACAAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCG
CAGAAACCGAAGCAACTGGTCTTCACCAAAGAAAACACGATCACCGTGCAGAACTATAATCGTGC
CGGTAATGCTCTGGAAGGCTCACCGGCTGAAATCAAGGACCTGGCTGAACTGTGTGCGGCACT
GAAAGGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTAC
TCATTCAACGCTAAAGGTGAACTGTCGGAAAAAACCATCCTGCGCGCCTGTGGCACCCGCCTGG
AATACACGGAAATCAAGTCGGACGGCACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCTCT
GGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTGAG
CAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGCGA
CCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAAA
GACCAAAAATATTGTGTTCACGAAGGAATGCACGATCACCGTTCAAAAATATGATTCCGCAGGTA
CCAACCTGGAAGGCAACGCTGTGGAAATCAAAACCCTGGACGAACTGAAAAATGCTCTGAAGGG
TCTCGAGCACCACCACCACCACCAC

SEQ ID NO: 107

S5D4-S6D1_aa: Heterodimer fusion protein of OspA serotype 5 with disulfide bond type 4 and OspA serotype 6 with disulfide bond type 1, LN1 linker sequence
FNEKGEISEKTIVRACGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKS
GEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKECTITVQNYDSAGTNLEGKAVEITT
LKELKNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVL
KDFTLEGTLAADGKTTLKVTCGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQ
KTKNLVFTKEDTITVQRYDSAGTNLEGKAVEITTLKELCNALK

SEQ ID NO: 108
Lip-S5D4-S6D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 5 with disulfide bond type 4 and OspA serotype 6 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAATCAGTGAAAAAACCATTGTGCGTGCGTGTGGCACCCGTCT
GGAATATACCGACATCAAGAGCGATAAAACGGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGC
TGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCGAAGGTACCGTTACGC
TGTCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGG
CAACAAAAAGAGCGGTACCTGGGACTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACG
AAAACCAAGCAGCTGGTCTTCACGAAAGAATGCACGATCACCGTGCAAAACTATGATAGCGCAG
GTACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGGCAAAGGTGAAACGAGCGAAAAGACCATCGTGCGTGCGAACGGTACCCGCCTGGAATA
TACGGACATTAAATCGGACGGCAGCGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACGCTGGAA
GGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTGTCA
AAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGCGTGCTA
CGAAAAAGACCGGTAAATGGGATAGCAAGACCTCTACGCTGACCATTAGTGTCAACTCCCAGAA
AACGAAGAATCTGGTGTTCACCAAAGAAGATACGATCACCGTTCAACGCTATGACAGTGCGGGC
ACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAA

SEQ ID NO: 109
Lip-S5D4-S6D1_His_aa: Heterodimer fusion protein of OspA serotype 5 with disulfide bond type 4 and OspA serotype 6 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGEISEKTIVRACGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLS
KNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKECTITVQNYDSAGTNLEGK
AVEITTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGK
AKEVLKDFTLEGTLAADGKTTLKVTCGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTIS
VNSQKTKNLVFTKEDTITVQRYDSAGTNLEGKAVEITTLKELCNALKGLEHHHHHH

SEQ ID NO: 110
Lip-S5D4-S6D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 5 with disulfide bond type 4 and OspA serotype 6 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAATCAGTGAAAAAACCATTGTGCGTGCGTGTGGCACCCGTCT
GGAATATACCGACATCAAGAGCGATAAAACGGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGC
TGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCGAAGGTACCGTTACGC
TGTCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGG
CAACAAAAAGAGCGGTACCTGGGACTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACG
AAAACCAAGCAGCTGGTCTTCACGAAAGAATGCACGATCACCGTGCAAAACTATGATAGCGCAG
GTACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGGCAAAGGTGAAACGAGCGAAAAGACCATCGTGCGTGCGAACGGTACCCGCCTGGAATA
TACGGACATTAAATCGGACGGCAGCGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACGCTGGAA
GGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTGTCA
AAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGCGTGCTA
CGAAAAAGACCGGTAAATGGGATAGCAAGACCTCTACGCTGACCATTAGTGTCAACTCCCAGAA
AACGAAGAATCTGGTGTTCACCAAAGAAGATACGATCACCGTTCAACGCTATGACAGTGCGGGC

ACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAAG
GTCTCGAGCACCACCACCACCACCAC

SEQ ID NO: 111
S5D1-S6D4_aa: Heterodimer fusion protein of OspA serotype 5 with disulfide bond type 1 and OspA serotype 6 with disulfide bond type 4, LN1 linker sequence
FNEKGEISEKTIVRANGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLSKNISKS
GEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITT
LKELCNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGKAKEVL
KDFTLEGTLAADGKTTLKVTEGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQ
KTKNLVFTKECTITVQRYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 112
Lip-S5D1-S6D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 5 with disulfide bond type 1 and OspA serotype 6 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAATCTCAGAAAAAACCATCGTCCGCGCTAACGGCACCCGCCT
GGAATACACCGACATCAAATCAGACAAGACCGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGC
TGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCTGCGGTACCGTTACGC
TGTCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGG
CAACAAAAAGAGCGGTACCTGGGATTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACG
AAAACCAAGCAGCTGGTCTTCACGAAAGAAGATACGATCACCGTGCAAAACTATGACAGCGCAG
GTACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGGCAAAGGTGAAACGAGTGAAAAAACGATTGTTCGCGCCTGTGGCACCCGCCTGGAATAC
ACGGATATCAAGTCGGATGGTTCGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACGCTGGAAG
GTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTGTCAA
AAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGCGTGCTAC
GAAAAAGACCGGTAAATGGGACAGCAAGACCTCTACGCTGACCATTAGTGTCAACTCCCAGAAA
ACGAAGAATCTGGTGTTCACCAAAGAATGCACGATCACCGTTCAACGCTATGATAGTGCGGGCA
CCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAA

SEQ ID NO: 113
Lip-S5D1-S6D4_His_aa: Heterodimer fusion protein of OspA serotype 5 with disulfide bond type 1 and OspA serotype 6 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGEISEKTIVRANGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLS
KNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGK
AVEITTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGK
AKEVLKDFTLEGTLAADGKTTLKVTEGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTIS
VNSQKTKNLVFTKECTITVQRYDSAGTNLEGKAVEITTLKELKNALKGLEHHHHHH

SEQ ID NO: 114
Lip-S5D1-S6D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 5 with disulfide bond type 1 and OspA serotype 6 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAGGGCGAAATCTCAGAAAAAACCATCGTCCGCGCTAACGGCACCCGCCT
GGAATACACCGACATCAAATCAGACAAGACCGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGC
TGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCTGCGGTACCGTTACGC
TGTCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGG
CAACAAAAAGAGCGGTACCTGGGATTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACG
AAAACCAAGCAGCTGGTCTTCACGAAAGAAGATACGATCACCGTGCAAAACTATGACAGCGCAG
GTACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAA
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGGCAAAGGTGAAACGAGTGAAAAAACGATTGTTCGCGCCTGTGGCACCCGCCTGGAATAC
ACGGATATCAAGTCGGATGGTTCGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACGCTGGAAG
GTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTGTCAA

AAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGCGTGCTAC
GAAAAAGACCGGTAAATGGGACAGCAAGACCTCTACGCTGACCATTAGTGTCAACTCCCAGAAA
ACGAAGAATCTGGTGTTCACCAAAGAATGCACGATCACCGTTCAACGCTATGATAGTGCGGGCA
CCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAAGG
TCTCGAGCACCACCACCACCACCAC

SEQ ID NO: 115
S2D4-S1D1_aa: Heterodimer fusion protein of OspA serotype 2 with disulfide bond type 4 and OspA serotype 1 with disulfide bond type 1, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
FNEKGELSAKTMTRECGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAK
SGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQCTITVQKYDSAGTNLEGTAVEI
KTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEV
LKNFTLEGKVANDKTTLVVKCGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSK
KTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEICNALK

SEQ ID NO: 116
Lip-S2D4-S1D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 2 with disulfide bond type 4 and OspA serotype 1 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAATGCGGCACCAAACT
GGAATATACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCC
TGGAAGGCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAAGAAGGCACCGTTACGCTGTC
AAAAGAAATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCG
ACCAAGAAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAACAGCAAGA
AAACCACGCAGCTGGTCTTCACCAAACAATGTACGATCACCGTGCAGAAATACGATAGTGCGGG
TACCAACCTGGAAGGCACCGCTGTTGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAA
GGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAAGTCAGCGAAAAAATCATTACCCGCGCAGACGGCACCCGCCTGGAATAC
ACCGGCATCAAATCGGACGGCAGCGGCAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCAAATGATAAAACCACCCTGGTGGTGAAATGCGGCACCGTTACGCTGAGCAAAAA
CATTAGTAAATCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGCCACCAAG
AAAACCGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATAGCAAGAAAACCA
AAGATCTGGTCTTCACGAAAGAAAACACCATCACGGTGCAGCAATATGACAGCAATGGTACCAAA
CTGGAAGGCTCCGCTGTGGAAATCACGAAACTGGATGAAATCTGTAATGCACTGAAA

SEQ ID NO: 117
Lip-S2D4-S1D1_His_aa: Heterodimer fusion protein of OspA serotype 2 with disulfide bond type 4 and OspA serotype 1 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNEKGELSAKTMTRECGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTL
SKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQCTITVQKYDSAGTNLE
GTAVEIKTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRADGTRLEYTGIKSDGS
GKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTL
TITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEICNALKGLEHHHHHH

SEQ ID NO: 118
Lip-S2D4-S1D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 2 with disulfide bond type 4 and OspA serotype 1 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAATGCGGCACCAAACT
GGAATATACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCC
TGGAAGGCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAAGAAGGCACCGTTACGCTGTC
AAAAGAAATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCG

ACCAAGAAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAACAGCAAGA
AAACCACGCAGCTGGTCTTCACCAAACAATGTACGATCACCGTGCAGAAATACGATAGTGCGGG
TACCAACCTGGAAGGCACCGCTGTTGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAA
GGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAAGTCAGCGAAAAAATCATTACCCGCGCAGACGGCACCCGCCTGGAATAC
ACCGGCATCAAATCGGACGGCAGCGGCAAAGCGAAAGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCAAATGATAAAACCACCCTGGTGGTGAAATGCGGCACCGTTACGCTGAGCAAAAA
CATTAGTAAATCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGCCACCAAG
AAAACCGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATAGCAAGAAAACCA
AAGATCTGGTCTTCACGAAAGAAAACACCATCACGGTGCAGCAATATGACAGCAATGGTACCAAA
CTGGAAGGCTCCGCTGTGGAAATCACGAAACTGGATGAAATCTGTAATGCACTGAAAGGTCTCG
AGCACCACCACCACCACCAC

SEQ ID NO: 119

S2D1-S1D4_aa: Heterodimer fusion protein of OspA serotype 2 with disulfide bond type 1 and OspA serotype 1 with disulfide bond type 4, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND

FNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKCGTVTLSKEIAK
SGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEI
KTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRACGTRLEYTGIKSDGSGKAKEV
LKNFTLEGKVANDKTTLVVKEGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSK
KTKDLVFTKECTITVQQYDSNGTKLEGSAVEITKLDEIKNALK

SEQ ID NO: 120

Lip-S2D1-S1D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 2 with disulfide bond type 1 and OspA serotype 1 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAAAACGGCACCAAACT
GGAATATACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCC
TGGAAGGCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAATGCGGCACCGTTACGCTGTC
AAAAGAAATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCG
ACCAAGAAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAATAGCAAGA
AAACCACGCAGCTGGTCTTCACCAAACAAGATACGATCACCGTGCAGAAATACGACAGTGCGGG
TACCAACCTGGAAGGCACGGCTGTTGAAATCAAAACCCTGGACGAACTGTGTAACGCCCTGAAA
GGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAAGTCTCGGAAAAAATCATTACCCGTGCTTGCGGCACCCGTCTGGAATAC
ACCGGCATTAAATCGGATGGCAGCGGCAAAGCGAAGGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCAAATGATAAGACCACCCTGGTGGTGAAAGAAGGCACCGTTACGCTGAGCAAAAA
CATTAGTAAGTCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGCCACCAAA
AAGACGGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATTCCAAAAAGACCA
AAGATCTGGTCTTCACGAAAGAATGCACCATCACGGTGCAGCAATATGACAGCAACGGTACCAA
ACTGGAAGGCTCTGCGGTGGAAATCACGAAACTGGATGAAATCAAAAATGCACTGAAA

SEQ ID NO: 121

Lip-S2D1-S1D4_His_aa: Heterodimer fusion protein of OspA serotype 2 with disulfide bond type 1 and OspA serotype 1 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)

LipCSSFNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKCGTVTL
SKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLE
GTAVEIKTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRACGTRLEYTGIKSDGS
GKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTL
TITVNSKKTKDLVFTKECTITVQQYDSNGTKLEGSAVEITKLDEIKNALKGLEHHHHHH

SEQ ID NO: 122

Lip-S2D1-S1D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 2 with disulfide bond type 1 and OspA serotype 1 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-

terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGAAAAAGGCGAACTGTCGGCGAAAACGATGACGCGTGAAAACGGCACCAAACT
GGAATATACGGAAATGAAAAGCGATGGCACCGGTAAAGCGAAAGAAGTTCTGAAAAACTTTACCC
TGGAAGGCAAAGTCGCCAATGACAAAGTCACCCTGGAAGTGAAATGCGGCACCGTTACGCTGTC
AAAAGAAATTGCAAAATCGGGTGAAGTGACCGTTGCTCTGAACGATACGAATACCACGCAAGCG
ACCAAGAAAACCGGCGCCTGGGACAGCAAAACCTCTACGCTGACCATTAGTGTTAATAGCAAGA
AAACCACGCAGCTGGTCTTCACCAAACAAGATACGATCACCGTGCAGAAATACGACAGTGCGGG
TACCAACCTGGAAGGCACGGCTGTTGAAATCAAAACCCTGGACGAACTGTGTAACGCCCTGAAA
GGCACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
CAACGAAAAAGGCGAAGTCTCGGAAAAAATCATTACCCGTGCTTGCGGCACCCGTCTGGAATAC
ACCGGCATTAAATCGGATGGCAGCGGCAAAGCGAAGGAAGTTCTGAAAAACTTTACCCTGGAAG
GCAAAGTCGCAAATGATAAGACCACCCTGGTGGTGAAAGAAGGCACCGTTACGCTGAGCAAAAA
CATTAGTAAGTCCGGTGAAGTCTCTGTGGAACTGAATGATACCGACAGCTCTGCGGCCACCAAA
AAGACGGCAGCTTGGAACTCAGGCACCTCGACGCTGACCATTACGGTTAATTCCAAAAAGACCA
AAGATCTGGTCTTCACGAAAGAATGCACCATCACGGTGCAGCAATATGACAGCAACGGTACCAA
ACTGGAAGGCTCTGCGGTGGAAATCACGAAACTGGATGAAATCAAAAATGCACTGAAAGGTCTC
GAGCACCACCACCACCACCAC

SEQ ID NO: 123

S4D4-S3D1_aa: Heterodimer fusion protein of OspA serotype 4 with disulfide bond type 4 and OspA serotype 3 with disulfide bond type 1, LN1 linker sequence

FNAKGELSEKTILRACGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNS
GEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGNAVEIK
TLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRANGTRLEYTEIKNDGSGKAKEV
LKGFALEGTLTDGGETKLTVTCGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNS
QKPKQLVFTKENTITVQNYNRAGNALEGSPAEIKDLAELCAALK

SEQ ID NO: 124

Lip-S4D4-S3D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 4 with disulfide bond type 4 and OspA serotype 3 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGCTAAAGGTGAACTGTCGGAAAAAACCATCCTGCGCGCCTGTGGCACCCGCCT
GGAATACACGGAAATCAAGTCGGACGGCACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCT
CTGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTG
AGCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGC
GACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAA
AAGACCAAAAATATTGTGTTCACGAAGGAATGCACGATCACCGTTCAAAAATATGATTCCGCAGG
TACCAACCTGGAAGGCAACGCTGTGGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAG
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
TAACGATAAGGGCAAACTGTCGGAAAAAGTGGTCACCCGCGCAAATGGCACCCGCCTGGAATAC
ACGGAAATCAAAAACGATGGTAGCGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCCCTGGAAG
GTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGTGCGGCACCGTTACGCTGTCTAA
AAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCCGGCTGAC
AAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCGCAGAAAC
CGAAGCAACTGGTCTTCACCAAAGAAAACACGATCACCGTGCAGAACTATAATCGTGCCGGTAAT
GCTCTGGAAGGCTCACCGGCTGAAATCAAGGACCTGGCTGAACTGTGTGCGGCACTGAAA

SEQ ID NO: 125

Lip-S4D4-S3D1_His_aa: Heterodimer fusion protein of OspA serotype 4 with disulfide bond type 4 and OspA serotype 3 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal CSS for addition of lipids, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)

LipCSSFNAKGELSEKTILRACGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSK
HIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGN
AVEIKTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRANGTRLEYTEIKNDGSG

KAKEVLKGFALEGTLTDGGETKLTVTCGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLT
ISKNSQKPKQLVFTKENTITVQNYNRAGNALEGSPAEIKDLAELCAALKGLEHHHHHH

SEQ ID NO: 126

Lip-S4D4-S3D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 4 with disulfide bond type 4 and OspA serotype 3 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGCTAAAGGTGAACTGTCGGAAAAAACCATCCTGCGCGCCTGTGGCACCCGCCT
GGAATACACGGAAATCAAGTCGGACGGCACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCT
CTGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTTCTG
AGCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGC
GACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAA
AAGACCAAAAATATTGTGTTCACGAAGGAATGCACGATCACCGTTCAAAAATATGATTCCGCAGG
TACCAACCTGGAAGGCAACGCTGTGGAAATCAAAACCCTGGACGAACTGAAAAACGCCCTGAAG
GGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATT
TAACGATAAGGGCAAACTGTCGGAAAAAGTGGTCACCCGCGCAAATGGCACCCGCCTGGAATAC
ACGGAAATCAAAAACGATGGTAGCGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCCCTGGAAG
GTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGTGCGGCACCGTTACGCTGTCTAA
AAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCCGGCTGAC
AAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCGCAGAAAC
CGAAGCAACTGGTCTTCACCAAAGAAAACACGATCACCGTGCAGAACTATAATCGTGCCGGTAAT
GCTCTGGAAGGCTCACCGGCTGAAATCAAGGACCTGGCTGAACTGTGTGCGGCACTGAAAGGT
CTCGAGCACCACCACCACCACCAC

SEQ ID NO: 127

S4D1-S3D4_aa: Heterodimer fusion protein of OspA serotype 4 with disulfide bond type 1 and OspA serotype 3 with disulfide bond type 4, LN1 linker sequence

FNAKGELSEKTILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSKHIPNS
GEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIK
TLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRACGTRLEYTEIKNDGSGKAKEV
LKGFALEGTLTDGGETKLTVTEGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNS
QKPKQLVFTKECTITVQNYNRAGNALEGSPAEIKDLAELKAALK

SEQ ID NO: 128

Lip-S4D1-S3D4_nt: Heterodimer fusion protein of OspA serotype 4 with disulfide bond type 1 and OspA serotype 3 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAATGCTAAGGGCGAACTGAGCGAAAAAACGATCCTGCGTGCGAATGGCACCCGTCT
GGAATACACCGAAATCAAATCCGATGGTACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCT
CTGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTG
AGCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGC
AACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAA
AGACCAAAAATATTGTGTTCACGAAGGAAGATACGATCACCGTTCAAAAATATGACTCCGCGGGC
ACCAACCTGGAAGGCAATGCCGTCGAAATCAAAACCCTGGATGAACTGTGTAACGCCCTGAAGG
GTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATTT
AACGATAAGGGCAAACTGTCAGAAAAAGTGGTCACCCGCGCTTGTGGCACCCGCCTGGAATACA
CCGAAATCAAAAACGACGGCTCGGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCCCTGGAAG
GTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGGAAGGCACCGTTACGCTGTCTAA
AAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCCGGCTGAC
AAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCGCAGAAAC
CGAAGCAACTGGTCTTCACCAAAGAATGCACGATCACCGTGCAGAACTATAATCGTGCCGGTAAT
GCTCTGGAAGGCTCCCCGGCTGAAATCAAGGACCTGGCGGAACTGAAGGCGGCACTGAAA

SEQ ID NO: 129

Lip-S4D1-S3D4_His_aa: Heterodimer fusion protein of OspA serotype 4 with disulfide bond type 1 and OspA serotype 3 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)

LipCSSFNAKGELSEKTILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSK
HIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGN
AVEIKTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRACGTRLEYTEIKNDGSG
KAKEVLKGFALEGTLTDGGETKLTVTEGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLT
ISKNSQKPKQLVFTKECTITVQNYNRAGNALEGSPAEIKDLAELKAALKGLEHHHHHH

SEQ ID NO: 130

Lip-S4D1-S3D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 4 with disulfide bond type 1 and OspA serotype 3 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAATGCTAAGGGCGAACTGAGCGAAAAAACGATCCTGCGTGCGAATGGCACCCGTCT
GGAATACACCGAAATCAAATCCGATGGTACGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTGCT
CTGGAAGGTACCCTGGCGGCCGACAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGTTCTG
AGCAAACATATTCCGAACTCTGGTGAAATCACCGTTGAACTGAACGATAGCAATTCTACGCAGGC
AACCAAAAAGACGGGCAAATGGGACAGTAATACCTCCACGCTGACCATTTCAGTCAACTCGAAAA
AGACCAAAAATATTGTGTTCACGAAGGAAGATACGATCACCGTTCAAAAATATGACTCCGCGGGC
ACCAACCTGGAAGGCAATGCCGTCGAAATCAAAACCCTGGATGAACTGTGTAACGCCCTGAAGG
GTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACTCATTT
AACGATAAGGGCAAACTGTCAGAAAAAGTGGTCACCCGCGCTTGTGGCACCCGCCTGGAATACA
CCGAAATCAAAAACGACGGCTCGGGCAAAGCGAAGGAAGTTCTGAAAGGCTTTGCCCTGGAAG
GTACCCTGACGGATGGCGGTGAAACCAAACTGACCGTGACGGAAGGCACCGTTACGCTGTCTAA
AAACATTAGCAAGTCTGGTGAAATCACGGTCGCACTGAATGATACCGAAACCACGCCGGCTGAC
AAAAAGACCGGCGAATGGAAAAGTGACACCTCCACGCTGACCATTTCAAAGAACTCGCAGAAAC
CGAAGCAACTGGTCTTCACCAAAGAATGCACGATCACCGTGCAGAACTATAATCGTGCCGGTAAT
GCTCTGGAAGGCTCCCCGGCTGAAATCAAGGACCTGGCGGAACTGAAGGCGGCACTGAAAGGT
CTCGAGCACCACCACCACCACCAC

SEQ ID NO: 131

S6D4-S5D1_aa: Heterodimer fusion protein of OspA serotype 6 with disulfide bond type 4 and OspA serotype 5 with disulfide bond type 1, LN1 linker sequence

FNGKGETSEKTIVRACGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVVLSKNILK
SGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKECTITVQRYDSAGTNLEGKAVEI
TTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRANGTRLEYTDIKSDKTGKAKEV
LKDFTLEGTLAADGKTTLKVTCGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRT
KTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITTLKELCNALK

SEQ ID NO: 132

Lip-S6D4-S5D1_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 6 with disulfide bond type 4 and OspA serotype 5 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGGCAAAGGTGAAACGAGTGAAAAAACGATTGTTCGCGCCTGTGGCACCCGCCT
GGAATACACGGATATCAAGTCGGATGGTTCGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACG
CTGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTT
CTGTCAAAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGC
GTGCTACGAAAAAGACCGGTAAATGGGACAGCAAGACCTCTACGCTGACCATTAGTGTCAACTC
CCAGAAAACGAAGAATCTGGTGTTCACCAAAGAATGCACGATCACCGTTCAACGCTATGATAGTG
CGGGCACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGAAGAATGCTCT
GAAAGGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACT
CATTCAACGAAAAAGGCGAAATCTCAGAAAAAACCATCGTCCGCGCTAACGGCACCCGCCTGGA
ATACACCGACATCAAATCAGACAAGACCGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGCTGG
AAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCTGCGGTACCGTTACGCTGT
CCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGGCAA
CAAAAAGAGCGGTACCTGGGATTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACGAAAA
CCAAGCAGCTGGTCTTCACGAAAGAAGATACGATCACCGTGCAAAACTATGACAGCGCAGGTAC
CAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAA

SEQ ID NO: 133

Lip-S6D4-S5D1_His_aa: Heterodimer fusion protein of OspA serotype 6 with disulfide bond type 4 and OspA serotype 5 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)

LipCSSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVVL
SKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKECTITVQRYDSAGTNLE
GKAVEITTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRANGTRLEYTDIKSDKTG
KAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTI
SKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITTLKELCNALKGLEHHHHHH

SEQ ID NO: 134

Lip-S6D4-S5D1_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 6 with disulfide bond type 4 and OspA serotype 5 with disulfide bond type 1, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGGCAAAGGTGAAACGAGTGAAAAAACGATTGTTCGCGCCTGTGGCACCCGCCT
GGAATACACGGATATCAAGTCGGATGGTTCGGGCAAAGCAAAGGAAGTCCTGAAAGATTTTACG
CTGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGGAAGGCACCGTGGTT
CTGTCAAAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACGC
GTGCTACGAAAAAGACCGGTAAATGGGACAGCAAGACCTCTACGCTGACCATTAGTGTCAACTC
CCAGAAAACGAAGAATCTGGTGTTCACCAAAGAATGCACGATCACCGTTCAACGCTATGATAGTG
CGGGCACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGAAGAATGCTCT
GAAAGGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTACT
CATTCAACGAAAAAGGCGAAATCTCAGAAAAAACCATCGTCCGCGCTAACGGCACCCGCCTGGA
ATACACCGACATCAAATCAGACAAGACCGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGCTGG
AAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCTGCGGTACCGTTACGCTGT
CCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGGCAA
CAAAAAGAGCGGTACCTGGGATTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACGAAAA
CCAAGCAGCTGGTCTTCACGAAAGAAGATACGATCACCGTGCAAAACTATGACAGCGCAGGTAC
CAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGTGTAATGCTCTGAAAGGTC
TCGAGCACCACCACCACCACCAC

SEQ ID NO: 135

S6D1-S5D4_aa: Heterodimer fusion protein of OspA serotype 6 with disulfide bond type 1 and OspA serotype 5 with disulfide bond type 4, LN1 linker sequence

FNGKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVVLSKNILK
SGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKEDTITVQRYDSAGTNLEGKAVEI
TTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRACGTRLEYTDIKSDKTGKAKEV
LKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRT
KTKQLVFTKECTITVQNYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 136

Lip-S6D1-S5D4_nt: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 6 with disulfide bond type 1 and OspA serotype 5 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence

ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGGCAAAGGTGAAACGAGCGAAAAGACCATCGTGCGTGCGAACGGTACCCGCC
TGGAATATACGGACATTAAATCGGACGGCAGCGGCAAAGCAAAGGAAGTCCTGAAAGATTTTAC
GCTGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGT
TCTGTCAAAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACG
CGTGCTACGAAAAAGACCGGTAAATGGGATAGCAAGACCTCTACGCTGACCATTAGTGTCAACT
CCCAGAAAACGAAGAATCTGGTGTTCACCAAAGAAGATACGATCACCGTTCAACGCTATGACAGT
GCGGGCACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGTGTAATGCTC
TGAAAGGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTAC
TCATTCAACGAAAAAGGCGAAATCAGTGAAAAAACCATTGTGCGTGCGTGTGGCACCCGTCTGG
AATATACCGACATCAAGAGCGATAAAACGGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGCTG
GAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCGAAGGTACCGTTACGCTG
TCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGGCA
ACAAAAAGAGCGGTACCTGGGACTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACGAA
AACCAAGCAGCTGGTCTTCACGAAAGAATGCACGATCACCGTGCAAAACTATGATAGCGCAGGT

ACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAA

SEQ ID NO: 137
Lip-S6D1-S5D4_His_aa: Heterodimer fusion protein of OspA serotype 6 with disulfide bond type 1 and OspA serotype 5 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation, C-terminal His tag (GLEHHHHHH)
LipCSSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVVL
SKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKEDTITVQRYDSAGTNLE
GKAVEITTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRACGTRLEYTDIKSDKT
GKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTL
TISKNRTKTKQLVFTKECTITVQNYDSAGTNLEGKAVEITTLKELKNALKGLEHHHHHH

SEQ ID NO: 138
Lip-S6D1-S5D4_His_nt: Coding sequence for heterodimer fusion protein of OspA serotype 6 with disulfide bond type 1 and OspA serotype 5 with disulfide bond type 4, *E. coli* lpp lipidation signal, N-terminal CSS for addition of lipids, LN1 linker sequence, C-terminal His tag (GLEHHHHHH)
ATGAAAGCTACTAAACTGGTACTGGGCGCGGTAATCCTGGGTTCTACTCTGCTGGCAGGTTGCT
CAAGCTTCAACGGCAAAGGTGAAACGAGCGAAAAGACCATCGTGCGTGCGAACGGTACCCGCC
TGGAATATACGGACATTAAATCGGACGGCAGCGGCAAAGCAAAGGAAGTCCTGAAAGATTTTAC
GCTGGAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACGTGCGGCACCGTGGT
TCTGTCAAAAAACATTCTGAAGTCGGGTGAAATCACCGCAGCTCTGGATGACAGCGATACCACG
CGTGCTACGAAAAAGACCGGTAAATGGGATAGCAAGACCTCTACGCTGACCATTAGTGTCAACT
CCCAGAAAACGAAGAATCTGGTGTTCACCAAAGAAGATACGATCACCGTTCAACGCTATGACAGT
GCGGGCACCAACCTGGAAGGCAAAGCCGTTGAAATTACCACGCTGAAAGAACTGTGTAATGCTC
TGAAAGGTACTAGTGACAAAAACAATGGCTCTGGTAGCAAAGAGAAAAACAAAGATGGCAAGTAC
TCATTCAACGAAAAAGGCGAAATCAGTGAAAAAACCATTGTGCGTGCGTGTGGCACCCGTCTGG
AATATACCGACATCAAGAGCGATAAAACGGGTAAAGCGAAGGAAGTTCTGAAAGATTTTACGCTG
GAAGGTACCCTGGCAGCAGACGGTAAAACCACGCTGAAGGTGACCGAAGGTACCGTTACGCTG
TCCAAAAACATTAGTAAGTCCGGCGAAATCACGGTCGCCCTGGATGACACCGATAGCTCTGGCA
ACAAAAAGAGCGGTACCTGGGACTCAGGCACCTCGACGCTGACCATTTCTAAAAATCGTACGAA
AACCAAGCAGCTGGTCTTCACGAAAGAATGCACGATCACCGTGCAAAACTATGATAGCGCAGGT
ACCAATCTGGAAGGCAAAGCTGTGGAAATTACCACGCTGAAAGAACTGAAGAATGCTCTGAAAG
GTCTCGAGCACCACCACCACCACCAC

SEQ ID NO: 140
Lip-S2D0-His: amino acids of positions 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2, wild-type sequence, N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 141
Lip-S2D1-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 1 (aa 182 and 269), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVK**C**GTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDEL**C**NALKGLEHHHHHH

SEQ ID NO: 142
Lip-S2D2-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 2 (aa 182 and 272), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVK**C**GTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNA**C**KGLEHHHHHH

SEQ ID NO: 143
Lip-S2D3-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 3 (aa 244 and 259), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)

LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTI**C**VQKYDSAGTNLEGT**C**
VEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 144
Lip-S2D4-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 4 (aa 141 and 241), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRE**C**GTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQ**C**TITVQKYDSAGTNLEGTA
VEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 145
Lip-S2D5-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 5 (aa 165 and 265), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKN**C**TLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKT**C**DELKNALKGLEHHHHHH

SEQ ID NO: 146
Lip-S2D6-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA sero type 2 with disulfide bond type 6 (aa 185 and 272), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGT**C**TLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNA**C**KGLEHHHHHH

SEQ ID NO: 147
Lip-S2D7-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 7 (aa 199 and 223), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVT**C**ALNDTNTTQATKKTGAWDSKTST**C**TISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 148
Lip-S2D8-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 8 (aa 243 and 262), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDT**C**TVQKYDSAGTNLEGT
AVE**C**KTLDELKNALKGLEHHHHHH

SEQ ID NO: 149
Lip-S2D9-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 9 (aa 184 and 204), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEG**C**VTLSKEI
AKSGEVTVALND**C**NTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 150
Lip-S2D10-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 10 (aa 201 and 214), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVA**C**NDTNTTQATKKT**C**AWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 151
Lip-S2D11-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 11 (aa 246 and 259), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITV**C**KYDSAGTNLEGT**C**
VEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 152
Lip-S2D12-His: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 12 (aa 167 and 178), N-terminal CKQN for addition of lipids, C-terminal His tag (GLEHHHHHH)
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFT**C**EGKVANDKVT**C**EVKEGTVTLSKEI AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA VEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 153
Lip-S2D0: amino acids of positions 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2, wild-type sequence, N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA VEIKTLDELKNALK

SEQ ID NO: 154
Lip-S2D1: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 1 (aa 182 and 269), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVK**C**GTVTLSKEI AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA VEIKTLDEL**C**NALK

SEQ ID NO: 155
Lip-S2D2: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 2 (aa 182 and 272), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVK**C**GTVTLSKEI AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA VEIKTLDELKNA**C**K

SEQ ID NO: 156
Lip-S2D3: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 3 (aa 244 and 259), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTI**C**VQKYDSAGTNLEGT**C** VEIKTLDELKNALK

SEQ ID NO: 157
Lip-S2D4: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 4 (aa 141 and 241), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRE**C**GTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQ**C**TITVQKYDSAGTNLEGTA VEIKTLDELKNALK

SEQ ID NO: 158
Lip-S2D5: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 5 (aa 165 and 265), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKN**C**TLEGKVANDKVTLEVKEGTVTLSKEI AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA VEIKT**C**DELKNALK

SEQ ID NO: 159
Lip-S2D6: aa 131-273 of *Borrelia afzelii* strain K78, OspA sero type 2 with disulfide bond type 6 (aa 185 and 272), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGT**C**TLSKEI AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA VEIKTLDELKNA**C**K

SEQ ID NO: 160

Lip-S2D7: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 7 (aa 199 and 223), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVT**C**ALNDTNTTQATKKTGAWDSKTST**C**TISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNALK

SEQ ID NO: 161
Lip-S2D8: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 8 (aa 243 and 262), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDT**C**TVQKYDSAGTNLEGT
AVE**C**KTLDELKNALK

SEQ ID NO: 162
Lip-S2D9: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 9 (aa 184 and 204), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEG**C**VTLSKEI
AKSGEVTVALND**C**NTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNALK

SEQ ID NO: 163
Lip-S2D10: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 10 (aa 201 and 214), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVA**C**NDTNTTQATKKT**C**AWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNALK

SEQ ID NO: 164
Lip-S2D11: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 11 (aa 246 and 259), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITV**C**KYDSAGTNLEGT**C**
VEIKTLDELKNALK

SEQ ID NO: 165
Lip-S2D12: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 12 (aa 167 and 178), N-terminal CKQN for addition of lipids
LipCKQNELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFT**C**EGKVANDKVT**C**EVKEGTVTLSKEI
AKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTA
VEIKTLDELKNALK

SEQ ID NO: 166
S2D0: amino acids of positions 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2, wild-type sequence
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNALK

SEQ ID NO: 167
S2D1: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 1 (aa 182 and 269)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVK**C**GTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
L**C**NALK

SEQ ID NO: 168
S2D2: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 2 (aa 182 and 272)

ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVK**C**GTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNA**C**K

SEQ ID NO: 169
S2D3: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 3 (aa 244 and 259)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTI**C**VQKYDSAGTNLEGT**C**VEIKTLDE
LKNALK

SEQ ID NO: 170
S2D4: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 4 (aa 141 and 241)
ELSAKTMTRE**C**GTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQ**C**TITVQKYDSAGTNLEGTAVEIKTLDE
LKNALK

SEQ ID NO: 171
S2D5: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 5 (aa 165 and 265)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKN**C**TLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKT**C**DE
LKNALK

SEQ ID NO: 172
S2D6: aa 131-273 of *Borrelia afzelii* strain K78, OspA sero type 2 with disulfide bond type 6 (aa 185 and 272)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGT**C**TLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNA**C**K

SEQ ID NO: 173
S2D7: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 7 (aa 199 and 223)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
CALNDTNTTQATKKTGAWDSKTST**C**TISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNALK

SEQ ID NO: 174
S2D8: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 8 (aa 243 and 262)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDT**C**TVQKYDSAGTNLEGTAVE**C**KTLD
ELKNALK

SEQ ID NO: 175
S2D9: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 9 (aa 184 and 204)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEG**C**VTLSKEIAKSGEVT
VALND**C**NTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNALK

SEQ ID NO: 176
S2D10: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 10 (aa 201 and 214)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT
VA**C**NDTNTTQATKKT**C**AWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDE
LKNALK

SEQ ID NO: 177
S2D11: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 11 (aa 246 and 259)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVT VALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITV**C**KYDSAGTNLEGT**C**VEIKTLDE LKNALK

SEQ ID NO: 178
S2D12: aa 131-273 of *Borrelia afzelii* strain K78, OspA serotype 2 with disulfide bond type 12 (aa 167 and 178)
ELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFT**C**EGKVANDKVT**C**EVKEGTVTLSKEIAKSGEV TVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLD ELKNALK

SEQ ID NO: 179
B. burgdorferi s.s. (strain B31, serotype 1), OspA_aa 126-273 with replaced hLFA-like sequence from serotype 1 OspA
FNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSKNISKS GEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEIT KLDEIKNALK

SEQ ID NO: 180
B. garinii (strain PBr, serotype 3), OspA_aa 126-274
FNDKGKLSEKVVTRANGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTEGTVTLSKNIS KSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKENTITVQNYNRAGNALEGSPA EIKDLAELKAALK

SEQ ID NO: 181
B.bavariensis (strain PBi, serotype 4), OspA_aa 126-273
FNAKGELSEKTILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNS GEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIK TLDELKNALK

SEQ ID NO: 182
B. garinii (strain PHei, serotype 5), OspA_aa 126-273
FNEKGEISEKTIVRANGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKS GEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITT LKELKNALK

SEQ ID NO: 183
B. garinii (strain DK29, serotype 6), OspA_aa 126-274
FNGKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVVLSKNILK SGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKEDTITVQRYDSAGTNLEGKAVEI TTLKELKNALK

SEQ ID NO: 184
LN1 peptide linker constructed from two separate loop regions of the N-terminal half of OspA from *B. burgdorferi* s.s. *strain* B31 (aa 65-74 and aa 42-53, amino acid exchange at position 53: D53S)
GTSDKNNGSGSKEKNKDGKYS

SEQ ID NO: 185
Lip-S1D4-S2D4_aa: Heterodimer fusion protein of OspA serotypes 1 and 2 both with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND
LipCSSFNEKGEVSEKIITRACGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSK NISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKECTITVQQYDSNGTKLEG SAVEITKLDEIKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRECGTKLEYTEMKSDGT

GKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTL
TISVNSKKTTQLVFTKQCTITVQKYDSAGTNLEGTAVEIKTLDELKNALK

SEQ ID NO: 186
Lip-S1D1-S2D1_aa: Heterodimer fusion protein of OspA serotype 1 and OspA serotype 2 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation
LipCSSFNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSK
NISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEG
SAVEITKLDEICNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRENGTKLEYTEMKSDGT
GKAKEVLKNFTLEGKVANDKVTLEVKCGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTL
TISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELCNALK

SEQ ID NO: 187
Lip-S3D4-S4D4_aa: Heterodimer fusion protein of OspA serotype 3 and OspA serotype 4 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNEKGKLSEKVVTRACGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTEGTVTL
SKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKECTITVQNYNRAGNALE
GSPAEIKDLAELKAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRACGTRLEYTEIKSDGT
GKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTL
TISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGNAVEIKTLDELKNALK

SEQ ID NO: 188
Lip-S3D1-S4D1_aa: Heterodimer fusion protein of OspA serotypes 3 and 4 both with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNEKGKLSEKVVTRANGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTCGTVTL
SKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKENTITVQNYNRAGNALE
GSPAEIKDLAELCAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRANGTRLEYTEIKSDGT
GKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTL
TISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIKTLDELCNALK

SEQ ID NO: 189
Lip-S5D4-S6D4_aa: Heterodimer fusion protein OspA serotypes 5 and 6 both with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNEKGEISEKTIVRACGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLS
KNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKECTITVQNYDSAGTNLEGK
AVEITTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGK
AKEVLKDFTLEGTLAADGKTTLKVTEGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTIS
VNSQKTKNLVFTKECTITVQRYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 190
Lip-S5D1-S6D1_aa: Heterodimer fusion protein of OspA serotypes 6 both with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNEKGEISEKTIVRANGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLS
KNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGK
AVEITTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGK
AKEVLKDFTLEGTLAADGKTTLKVTCGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTIS
VNSQKTKNLVFTKEDTITVQRYDSAGTNLEGKAVEITTLKELCNALK

SEQ ID NO: 191
Lip-S2D4-S1D4_aa: Heterodimer fusion protein of OspA serotypes 2 and 1 both with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation
LipCSSFNEKGELSAKTMTRECGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTL
SKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQCTITVQKYDSAGTNLE
GTAVEIKTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRACGTRLEYTGIKSDGS
GKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTL
TITVNSKKTKDLVFTKECTITVQQYDSNGTKLEGSAVEITKLDEIKNALK

SEQ ID NO: 192
Lip-S2D1-S1D1_aa: Heterodimer fusion protein of OspA serotypes 2 and 1 both with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation
LipCSSFNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKCGTVTL
SKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLE
GTAVEIKTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRADGTRLEYTGIKSDGS
GKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTL
TITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEICNALK

SEQ ID NO: 193
Lip-S4D4-S3D4_aa: Heterodimer fusion protein of OspA serotypes 4 and 3 both with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNAKGELSEKTILRACGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSK
HIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGN
AVEIKTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRACGTRLEYTEIKNDGSG
KAKEVLKGFALEGTLTDGGETKLTVTEGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLT
ISKNSQKPKQLVFTKECTITVQNYNRAGNALEGSPAEIKDLAELKAALK

SEQ ID NO: 194
Lip-S4D1-S3D1_aa: Heterodimer fusion protein of OspA serotypes 4 and 3 both with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNAKGELSEKTILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSK
HIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGN
AVEIKTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRANGTRLEYTEIKNDGSG
KAKEVLKGFALEGTLTDGGETKLTVTCGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLT
ISKNSQKPKQLVFTKENTITVQNYNRAGNALEGSPAEIKDLAELCAALK

SEQ ID NO: 195
Lip-S6D4-S5D4_aa: Heterodimer fusion protein of OspA serotypes 6 and 5 both with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVVL
SKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKECTITVQRYDSAGTNLE
GKAVEITTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRACGTRLEYTDIKSDKTG
KAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTI
SKNRTKTKQLVFTKECTITVQNYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 196
Lip-S6D1-S5D1_aa: Heterodimer fusion protein of OspA serotypes 6 and 5 both with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVVL
SKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKEDTITVQRYDSAGTNLE
GKAVEITTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRANGTRLEYTDIKSDKT
GKAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTL
TISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITTLKELCNALK

SEQ ID NO: 197
Lip-S1D4-S2D1_aa: Heterodimer fusion protein of OspA serotype 1 with disulfide bond type 4 and OspA serotype 2 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation
LipCSSFNEKGEVSEKIITRACGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSK
NISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKECTITVQQYDSNGTKLEG
SAVEITKLDEIKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRENGTKLEYTEMKSDGT
GKAKEVLKNFTLEGKVANDKVTLEVKCGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTL
TISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELCNALK

SEQ ID NO: 198

Lip-S1D1-S2D4_aa: Heterodimer fusion protein of OspA serotype 1 with disulfide bond type 1 and OspA serotype 2 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation

LipCSSFNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSK
NISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEG
SAVEITKLDEICNALKGTSDKNNGSGSKEKNKDGKYSFNEKGELSAKTMTRECGTKLEYTEMKSDGT
GKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTL
TISVNSKKTTQLVFTKQCTITVQKYDSAGTNLEGTAVEIKTLDELKNALK

SEQ ID NO: 199

Lip-S3D4-S4D1_aa: Heterodimer fusion protein of OspA serotype 3 with disulfide bond type 4 and OspA serotype 4 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation

LipCSSFNEKGKLSEKVVTRACGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTEGTVTL
SKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKECTITVQNYNRAGNALE
GSPAEIKDLAELKAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRANGTRLEYTEIKSDGT
GKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTL
TISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIKTLDELCNALK

SEQ ID NO: 200

Lip-S3D1-S4D4_aa: Heterodimer fusion protein of OspA serotype 3 with disulfide bond type 1 and OspA serotype 4 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation

LipCSSFNEKGKLSEKVVTRANGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTCGTVTL
SKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLTISKNSQKPKQLVFTKENTITVQNYNRAGNALE
GSPAEIKDLAELCAALKGTSDKNNGSGSKEKNKDGKYSFNAKGELSEKTILRACGTRLEYTEIKSDGT
GKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTL
TISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGNAVEIKTLDELKNALK

SEQ ID NO: 201

Lip-S5D4-S6D1_aa: Heterodimer fusion protein of OspA serotype 5 with disulfide bond type 4 and OspA serotype 6 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation

LipCSSFNEKGEISEKTIVRACGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLS
KNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKECTITVQNYDSAGTNLEGK
AVEITTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGK
AKEVLKDFTLEGTLAADGKTTLKVTCGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTIS
VNSQKTKNLVFTKEDTITVQRYDSAGTNLEGKAVEITTLKELCNALK

SEQ ID NO: 202

Lip-S5D1-S6D4_aa: Heterodimer fusion protein of OspA serotype 5 with disulfide bond type 1 and OspA serotype 6 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation

LipCSSFNEKGEISEKTIVRANGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLS
KNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTISKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGK
AVEITTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGK
AKEVLKDFTLEGTLAADGKTTLKVTEGTVVLSKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTIS
VNSQKTKNLVFTKECTITVQRYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 203

Lip-S2D4-S1D1_aa: Heterodimer fusion protein of OspA serotype 2 with disulfide bond type 4 and OspA serotype 1 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation

LipCSSFNEKGELSAKTMTRECGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTL
SKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQCTITVQKYDSAGTNLE
GTAVEIKTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRADGTRLEYTGIKSDGS

GKAKEVLKNFTLEGKVANDKTTLVVKCGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTL
TITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEICNALK

SEQ ID NO: 204
Lip-S2D1-S1D4_aa: Heterodimer fusion protein of OspA serotype 2 with disulfide bond type 1 and OspA serotype 1 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, aa 164-174 of OspA serotype 1 replaced by non-hLFA-1-like sequence NFTLEGKVAND, N-terminal lipidation
LipCSSFNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKCGTVTL
SKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLE
GTAVEIKTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEVSEKIITRACGTRLEYTGIKSDGS
GKAKEVLKNFTLEGKVANDKTTLVVKEGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTL
TITVNSKKTKDLVFTKECTITVQQYDSNGTKLEGSAVEITKLDEIKNALK

SEQ ID NO: 205
Lip-S4D4-S3D1_aa: Heterodimer fusion protein of OspA serotype 4 with disulfide bond type 4 and OspA serotype 3 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNAKGELSEKTILRACGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTEGTVVLSK
HIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKECTITVQKYDSAGTNLEGN
AVEIKTLDELKNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRANGTRLEYTEIKNDGSG
KAKEVLKGFALEGTLTDGGETKLTVTCGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLT
ISKNSQKPKQLVFTKENTITVQNYNRAGNALEGSPAEIKDLAELCAALK

SEQ ID NO: 206
Lip-S4D1-S3D4_aa: Coding sequence for intermediate and final heterodimer fusion proteins of OspA serotype 4 with disulfide bond type 1 and OspA serotype 3 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNAKGELSEKTILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTCGTVVLSK
HIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKEDTITVQKYDSAGTNLEGN
AVEIKTLDELCNALKGTSDKNNGSGSKEKNKDGKYSFNDKGKLSEKVVTRACGTRLEYTEIKNDGSG
KAKEVLKGFALEGTLTDGGETKLTVTEGTVTLSKNISKSGEITVALNDTETTPADKKTGEWKSDTSTLT
ISKNSQKPKQLVFTKECTITVQNYNRAGNALEGSPAEIKDLAELKAALK

SEQ ID NO: 207
Lip-S6D4-S5D1_aa: Heterodimer fusion protein of OspA serotype 6 with disulfide bond type 4 and OspA serotype 5 with disulfide bond type 1, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNGKGETSEKTIVRACGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVVL
SKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKECTITVQRYDSAGTNLE
GKAVEITTLKELKNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRANGTRLEYTDIKSDKTG
KAKEVLKDFTLEGTLAADGKTTLKVTCGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTLTI
SKNRTKTKQLVFTKEDTITVQNYDSAGTNLEGKAVEITTLKELCNALK

SEQ ID NO: 208
Lip-S6D1-S5D4_aa: Heterodimer fusion protein of OspA serotype 6 with disulfide bond type 1 and OspA serotype 5 with disulfide bond type 4, N-terminal CSS for addition of lipids, LN1 linker sequence, N-terminal lipidation
LipCSSFNGKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTCGTVVL
SKNILKSGEITAALDDSDTTRATKKTGKWDSKTSTLTISVNSQKTKNLVFTKEDTITVQRYDSAGTNLE
GKAVEITTLKELCNALKGTSDKNNGSGSKEKNKDGKYSFNEKGEISEKTIVRACGTRLEYTDIKSDKT
GKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKSGEITVALDDTDSSGNKKSGTWDSGTSTL
TISKNRTKTKQLVFTKECTITVQNYDSAGTNLEGKAVEITTLKELKNALK

SEQ ID NO: 209
B. afzelii (strain K78; OspA serotype 2) aa 17-273, lipidation signal sequence removed (aa 1-16: MKKYLLGIGLILALIA), C-terminal His tag (GLEHHHHHH)
CKQNVSSLDEKNSASVDLPGEMKVLVSKEKDKDGKYSLKATVDKIELKGTSDKDNGSGVLEGTKDDK
SKAKLTIADDLSKTTFELFKEDGKTLVSRKVSSKDKTSTDEMFNEKGELSAKTMTRENGTKLEYTEMK

SDGTGKAKEVLKNFTLEGKVANDKVTLEVKEGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDS
KTSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELKNALKGLEHHHHHH

SEQ ID NO: 210
B. burgdorferi (OspA serotype 1, strain ZS7) aa 17-273, lipidation signal sequence removed (aa 1-16: MKKYLLGIGLILALIA), C-terminal His tag (LEHHHHHH)
CSSFKQNVSSLDEKNSVSVDLPGEMKVLVSKEKNKDGKYDLIATVDKLELKGTSDKNNGSGVLEGVK
ADKSKVKLTISDDLGQTTLEVFKEDGKTLVSKKVTSKDKSSTEEKFNEKGEVSEKIITRADGTRLEYTGI
KSDGSGKAKEVLKGYVLEGTLTAEKTTLVVKEGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNS
GTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEIKNALKLEHHHHHH

SEQ ID NO: 211
Cysteine-containing peptide from OspA
CKQN

SEQ ID NO: 212
Chimeric OspA Serotype1/Serotype2, N-terminal lipidation
LipCAQKGAESIGSVSVDLPGEMKVLVSKEKDKNGKYDLIATVDKLELKGTSDKNNGSGVLEGVKTNK
SKVKLTISDDLGQTTLEVFKEDGKTLVSKKVTSKDKSSTEEKFNEKGEVSEKIITMADGTRLEYTGIKS
DGTGKAKYVLKNFTLEGKVANDKTTLEVKEGTVTLSMNISKSGEVSVELNDTDSSAATKKTAAWNSK
TSTLTISVNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELKNALKLE

SEQ ID NO: 213
amino acids of positions 126-274 of *B. garinii* strain T25, OspA serotype 7
FNDKGKLSEKVVTRANGTRLEYTEIQNDGSGKAKEVLKSLTLEGTLTADGETKLTVEAGTVTLSKNISE
SGEITVELKDTETTPADKKSGTWDSKTSTLTISKNSQKTKQLVFTKENTITVQKYNTAGTKLEGSPAEI
KDLEALKAALK

SEQ ID NO: 214
Forward oligonucleotide primer for the RecA gene of *Borrelia*
CATGCTCTTGATCCTGTTTA

SEQ ID NO: 215
Histidine tag
GLEHHHHHH

SEQ ID NO: 216
Reverse oligonucleotide primer for the RecA gene of *Borrelia*
CCCATTTCTCCATCTATCTC

The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference.

2013285364 12 May 2017

CLAIMS:

1. A polypeptide comprising the polypeptide consisting of SEQ ID NO: 186; or any functional variant of said amino acid sequence - with a sequence identity of at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95% to the sequence of SEQ ID NO: 186, and - with a difference in protective capacity (Δpc) between the functional variant and the placebo (negative) control of at least 50%, especially at least 60%, preferably at least 70%, more preferably at least 80%, even more preferably 90%, even more preferably 95%, most preferably at least 95%.

2. The polypeptide according to claim 1 consisting of SEQ ID NO: 186.

3. A nucleic acid encoding the polypeptide according to claim 1 or 2.

4. A vector comprising the nucleic acid molecule according to claim 3.

5. A host cell comprising the nucleic acid of claim 3 or the vector as defined in claim 4, wherein said host cell is preferably *E. coli*.

6. A method for producing a polypeptide according to claim 1 or 2, characterized by the following steps:
   a) introducing a vector encoding the polypeptide into a host cell,
   b) growing the host cell under conditions allowing for expression of said polypeptide,
   c) homogenizing said host cell, and
   d) subjecting the host cell homogenate to purification steps.

7. A pharmaceutical composition comprising a polypeptide according to claim 1 or 2 and/or the nucleic acid according to claim 3 and optionally a pharmaceutically acceptable excipient.

8. A pharmaceutical composition comprising SEQ ID NO: 186 and optionally a pharmaceutically acceptable excipient.

9. The pharmaceutical composition according to claim 7 or 8, further comprising SEQ ID NO: 190 and optionally a pharmaceutically acceptable excipient.

2013285364 12 May 2017

10. The pharmaceutical composition according to any one of claims 7 to 9, wherein the pharmaceutically acceptable excipient comprises L-methionine.

11. The pharmaceutical composition according to any one of claims 7 to 10 further comprising at least one additional antigen from *Borrelia* or a pathogen other than *Borrelia*.

12. The pharmaceutical composition of claim 11, wherein the additional antigen is from a tick-borne pathogen.

13. The pharmaceutical composition of claim 12, wherein said tick-borne pathogen is selected from the group comprising *Borrelia hermsii*, *Borrelia parkeri*, *Borrelia duttoni*, *Borrelia miyamotoi*, *Borrelia turicatae*, *Rickettsia rickettsii*, *Rickettsia australis*, *Rickettsia conorii*, *Rickettsia helvetica*, *Rickettsia parkeri*, *Francisella tularensis*, *Anaplasma phagocytophilum*, *Ehrlichia sennetsu*, *Ehrlichia chaffeensis*, *Coxiella burnetii* and *Borrelia lonestari*, Tick-borne encephalitis virus (TBEV), Colorado tick fever virus (CTFV), Crimean-Congo hemorrhagic fever virus (CCHFV), Kyasanur forest disease virus (KFDV), Powassan virus, Heartland virus, Omsk Hemorrhagic Fever virus (OHFV) and *Babesia* spp.

14. The pharmaceutical composition of any one of claims 11 to 13, wherein the at least one additional antigen is comprised in a second vaccine composition.

15. The pharmaceutical composition of claim 14, wherein said second vaccine composition is a tick-borne encephalitis vaccine, a Japanese encephalitis vaccine or a Rocky Mountain spotted fever vaccine.

16. A pharmaceutical composition according to any one of claims 7 to 15, characterized in that it further comprises an immunostimulatory substance selected from the group consisting of polycationic polymers polycationic peptides, immunostimulatory oligodeoxynucleotides (ODNs), oligo(dIdC)$_{13}$ (SEQ ID NO: 32), peptides containing at least two LysLeuLys motifs, peptide KLKLLLLLKLK (SEQ ID NO: 33), neuroactive compounds, human growth hormone, aluminium hydroxide or aluminium phosphate, Freund's complete or incomplete adjuvants or combinations thereof.

17. The pharmaceutical composition of any one of claims 7 to 16, wherein said pharmaceutical composition is a vaccine.

2013285364 12 May 2017

18. The polypeptide according to claim 1 or 2, the nucleic acid according to claim 3 or the pharmaceutical composition according to any one of claims 7 to 17 for use as a medicament.

19. The polypeptide according to claim 1 or 2, the nucleic acid according to claim 3 or the pharmaceutical composition according to any one of claims 7 to 17 for use in a method of treating or preventing a *Borrelia* infection, wherein said infection is a *B. burgdorferi* s.s., *B. garinii*, *B. afzelii*, *B. andersoni*, *B. bavariensis*, *B. bissettii*, *B. valaisiana*, *B. lusitaniae*, *B. spielmanii*, *B. japonica*, *B. tanukii*, *B. turdi* or *B. sinica* infection.

20. Use of a polypeptide according to claim 1 or 2, a nucleic acid according to claim 3 or a pharmaceutical composition according to any one of claims 7 to 17 in the manufacture of a medicament for use in a method of treating or preventing a *Borrelia* infection, wherein said infection is a *B. burgdorferi* s.s., *B. garinii*, *B. afzelii*, *B. andersoni*, *B. bavariensis*, *B. bissettii*, *B. valaisiana*, *B. lusitaniae*, *B. spielmanii*, *B. japonica*, *B. tanukii*, *B. turdi* or *B. sinica* infection.

VALNEVA AUSTRIA GmbH

WATERMARK INTELLECTUAL PROPERTY PTY LTD

P39873AU00

Figure 1

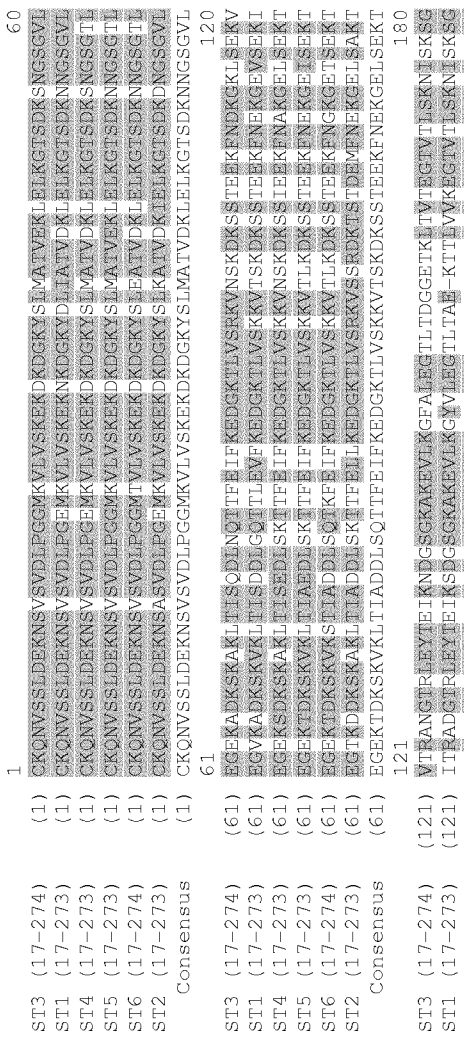

1 60
ST3 (17-274) (1) CKQNVSSLDEKNSVSVDLPGGMKVLVSKEKDKDGKYSLVATVEKLELKGTSDKSNGSGVL
ST1 (17-273) (1) CKQNVSSLDEKNSVSVDLPGEMKVLVSKEKNKDGKYDLIATVDKLELKGTSDKNNGSGVL
ST4 (17-273) (1) CKQNVSSLDEKNSVSVDLPGEMKVLVSKEKDKDGKYSLVATVDKLELKGTSDKSNGSGTL
ST5 (17-273) (1) CKQNVSSLDEKNSVSVDLPGGMKVLVSKEKDKDGKYSLVATVEKLELKGTSDKNNGSGTL
ST6 (17-274) (1) CKQNVSSLDEKNSVSVDLPGGMTVLVSKEKDKDGKYSLEATVDKLELKGTSDKNNGSGTL
ST2 (17-273) (1) CKQNVSSLDEKNSASVDLPGEMKVLVSKEKDKDGKYSLKATVDKIELKGTSDKDNGSGVL
Consensus (1) CKQNVSSLDEKNSVSVDLPGGMKVLVSKEKDKDGKYSLMATVDKLELKGTSDKNNGSGVL
61 120
ST3 (17-274) (61) EGEKADKSKAKLTISQDNQTTFEIFKEDGKTLVSRKVNSKDKSSTEEKFNDKGKLSEKV
ST1 (17-273) (61) EGVKADKSKVKLTISDDGQTTLEVFKEDGKTLVSKKVTSKDKSSTEEKFNEKGEVSEKI
ST4 (17-273) (61) EGEKSDKSKAKLTISEDSKTTFEIFKEDGKTLVSKKVNSKDKSSIEEKFNAKGELSEKT
ST5 (17-273) (61) EGEKTDKSKVKLTIADDLSKTTFEIFKEDGKTLVSKKVTLKDKSSTEEKFNGKGETSEKT
ST6 (17-274) (61) EGEKTDKSKAKLTIADDLSQTKFEIFKEDGKTLVSKKVTLKDKSSTEEKFNGKGETSEKT
ST2 (17-273) (61) EGTKDDKSKAKLTIADDLSKTTFEILKEDGKTLVSRKVSSKDKTSTDEMFNEKGELSAKT
Consensus (61) EGEKTDKSKVKLTIADDLSQTTFEIFKEDGKTLVSKKVTSKDKSSTEEKFNEKGELSEKT
121 180
ST3 (17-274) (121) VTRANGTRLEYTEIKNDGSGKAKEVLKGFALEGTLTDGGETKLTVTEGTVTLSKNLSKSG
ST1 (17-273) (121) ITRADGTRLEYTEIKSDGSGKAKEVLKGMVLEGTLTAE-KTTLVVKEGTVTLSKNISKSG
ST4 (17-273) (121) ILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAAD-KTTLKVTEGTVVLSKHIPNSG
ST5 (17-273) (121) IVRANGTRLEYTDIKSDKTGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNLSKSG
ST6 (17-274) (121) IVRANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVVLSKNILKSG
ST2 (17-273) (121) MIRENGTRLEYTEMKSDGTGKAKEVLKKFTLEGKVAND-KVTLEVKEGTVTLSKEIAKSG
Consensus (121) ITRANGTRLEYTEIKSDGSGKAKEVLKDFTLEGTLAADGKTTLKVTEGTVTLSKNISKSG
181 240
ST3 (17-274) (181) EITVALNDTETTPADKKTGEWKSDTSTITISKNSQKPKQLVFTKENTTVQNTNRAGNAI
ST1 (17-273) (180) EVSVELNDTDSSAATKKTAAWNSGTSTITIVNSKKTKDLVFTKENTITVQQTDSNGTKL
ST4 (17-273) (180) EITVELNDSNSTQATKKTGKWDSNTSTITISVNSKKTKNAVFTKEDTITVQKTDSAGTNL
ST5 (17-273) (181) EITVALDDTDSS-GNKKSGTWDSGTSTITISKNRTKTKQLVFTKEDTITVQNTDSAGTNL
ST6 (17-274) (181) EITAALDDSDTTRATKKTGKWDSKTSTITISVNSQKTKNLVFTKEDTITVQRTDSAGTNL
ST2 (17-273) (180) EVTVALNDTNTTQATKKTGAWDSKTSTITISVNSKKTTQLVFTKQYTITVKQTDSAGTNL
Consensus (181) EITVALNDTDST ATKKTG WDS TSTLTISVNSKKTKQLVFTKEDTITVQNTDSAGTNL
241 258
ST3 (17-274) (241) EGSPAEIKDLAELKAALK
ST1 (17-273) (240) EGSAVEITKLDEIKNALK
ST4 (17-273) (240) EGNAVEIKTLDELKNALK
ST5 (17-273) (240) EGKAVEITTLKELKNALK
ST6 (17-274) (241) EGKAVEITTLKELKNALK
ST2 (17-273) (240) EGTAVEIKTLDELKNALK
Consensus (241) EGSAVEITTLDELKNALK

Figure 2

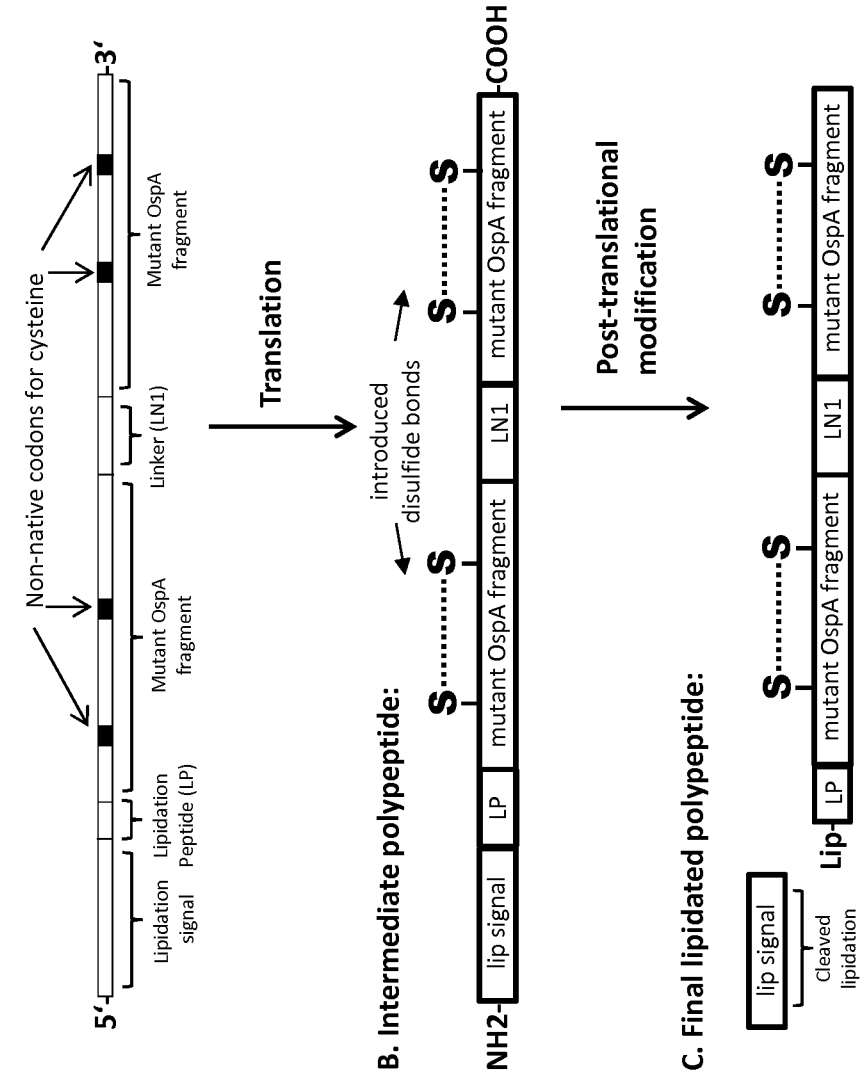
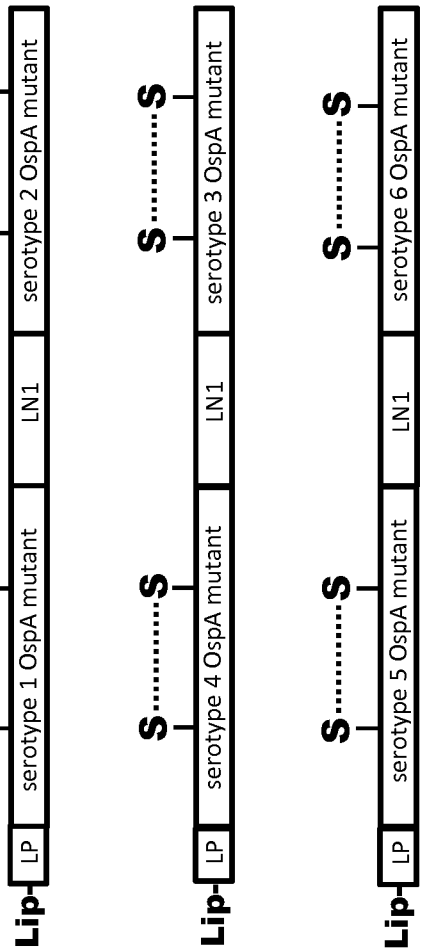

A. Nucleic acid encoding a mutant OspA heterodimer polypeptide:
5'
3'
Non-native codons for cysteine
Lipidation signal
Lipidation Peptide (LP)
Mutant OspA fragment
Linker (LN1)
Mutant OspA fragment
Translation
B. Intermediate polypeptide:
introduced disulfide bonds
NH2-
lip signal
LP
mutant OspA fragment
LN1
mutant OspA fragment
-COOH
Post-translational modification
C. Final lipidated polypeptide:
lip signal
Cleaved lipidation signal
Lip-
LP
mutant OspA fragment
LN1
mutant OspA fragment

Figure 3


Lip
LP
serotype 1 OspA mutant
LN1
serotype 2 OspA mutant
S
S
S
S
Lip
LP
serotype 4 OspA mutant
LN1
serotype 3 OspA mutant
S
S
S
S
Lip
LP
serotype 5 OspA mutant
LN1
serotype 6 OspA mutant
S
S
S
S

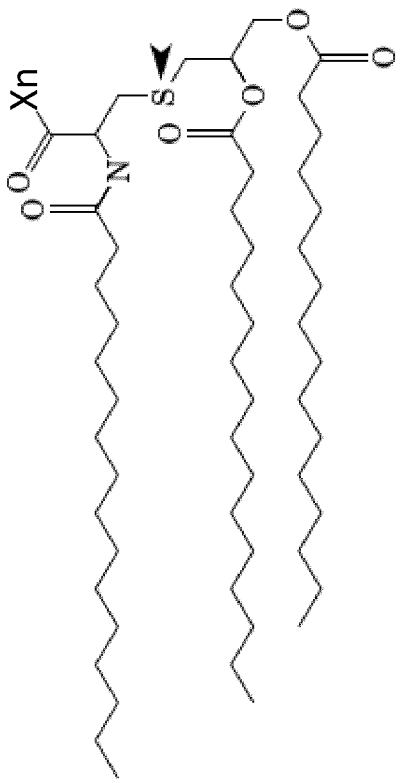
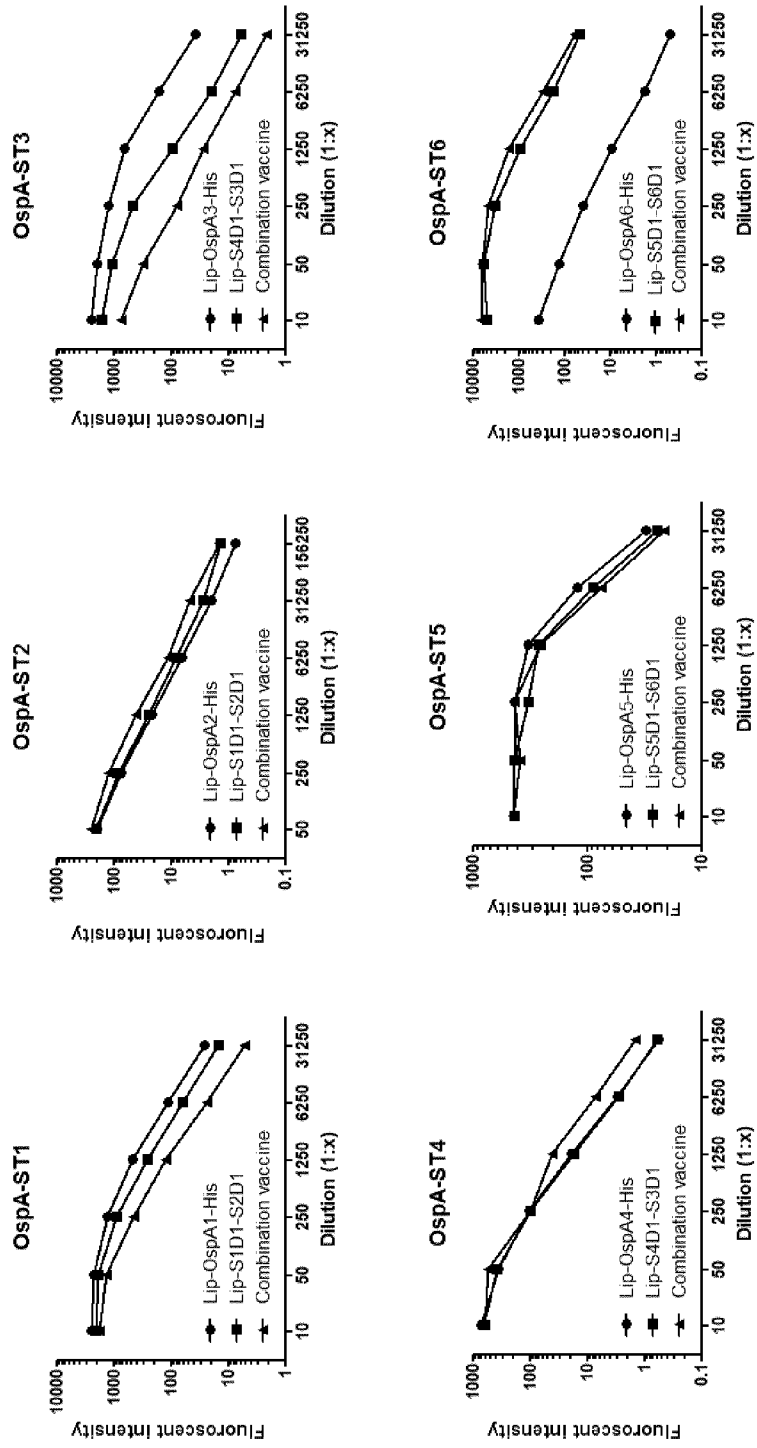


Figure 4

Figure 5


OspA-ST1
Fluorescent Intensity
Dilution (1:x)
Lip-OspA1-His
Lip-S1D1-S2D1
Combination vaccine
OspA-ST2
Lip-OspA2-His
Lip-S1D1-S2D1
Combination vaccine
OspA-ST3
Lip-OspA3-His
Lip-S4D1-S3D1
Combination vaccine
OspA-ST4
Lip-OspA4-His
Lip-S4D1-S3D1
Combination vaccine
OspA-ST5
Lip-OspA5-His
Lip-S5D1-S6D1
Combination vaccine
OspA-ST6
Lip-OspA6-His
Lip-S5D1-S6D1
Combination vaccine