

(51) International Patent Classification:
A61K 39/00 (2006.01)(21) International Application Number:
PCT/US2013/044488(22) International Filing Date:
6 June 2013 (06.06.2013)

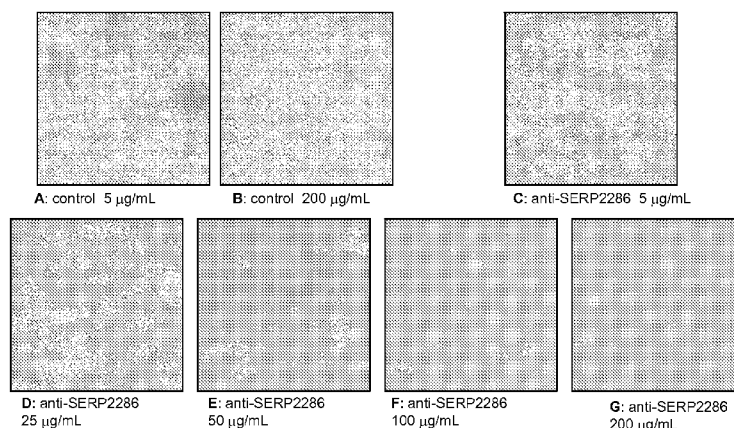
(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/656,427 6 June 2012 (06.06.2012) US(71) Applicant: **SANOFI PASTEUR BIOLOGICS, LLC**
[US/US]; 38 Sydney Street, Cambridge, MA 02139 (US).(72) Inventors: **LAM, Hubert**; Sanofi Pasteur Biologics, LLC,
38 Sydney Street, Cambridge, MA 02139 (US).
YETHON, Jeremy; Sanofi Pasteur Biologics, LLC, 38
Sydney Street, Cambridge, MA 02139 (US).(74) Agent: **HALLORAN, Patrick, J.**; 3141 Muirfield Road,
Center Valley, PA 18034 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).**Published:**— without international search report and to be republished
upon receipt of that report (Rule 48.2(g))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: IMMUNOGENIC COMPOSITIONS AND RELATED METHODS

Figure 1

(57) **Abstract:** This disclosure relates to polypeptides that may be used as immunogens in immunogenic compositions (e.g., vaccines) for eliciting an immune response in a subject against staphylococci (such as coagulase-negative staphylococci (CoNS) and/or *S. aureus* species). The immune response elicited (e.g., antibodies) inhibits biofilm development and may be sufficient for treating and/or preventing disease resulting from an infection by a *Staphylococcus* species. Binding agents that bind to these polypeptides are also provided. Exemplary polypeptides include but are not limited to SEQ ID NOs: 1-38 and fragments and derivatives thereof. Immunogenic compositions including one or more polypeptides are provided, as are methods for their use (e.g., to induce an immune response to staphylococci in a subject by administering to the subject a composition as described herein). Administration of the compositions of the present disclosure to a subject may elicit an immune response against a number of *Staphylococcus* species and/or strains.

IMMUNOGENIC COMPOSITIONS, BINDING MOLECULES AND RELATED METHODS**RELATED APPLICATIONS**

This application claims priority to U.S. Ser. No. 61/656,427 filed June 6, 2012, which is hereby incorporated into this disclosure in its entirety.

FIELD OF DISCLOSURE

The present disclosure relates to staphylococcal proteins, compositions and methods for eliciting an immune response in a subject against staphylococci (such as e.g., *Staphylococcus epidermidis*), to compositions comprising one or more such proteins and to antibodies against these proteins.

BACKGROUND

Staphylococci are gram-positive bacteria which typically reside on healthy human skin and mucous membranes. The genus *Staphylococcus* includes both coagulase-positive species (that produce free coagulase) and coagulase-negative species (that are unable to produce free coagulase).

Coagulase-negative staphylococci (CoNS) comprise a group of more than 40 recognized *Staphylococcus* species. The group of coagulase-positive staphylococci includes *Staphylococcus aureus* (e.g., methicillin-resistant *S. aureus* (MRSA)). Both groups have been increasingly recognized as causing clinically significant infections; the CoNS group for example, is the most common cause of nosocomial bloodstream infection, typically as a result of infections of intravascular catheters. The primary virulence mechanism of the CoNS and an important virulence mechanism for many coagulase-positive staphylococci is their ability to form biofilms on indwelling medical devices, especially catheters.

Biofilms are communities of individual cells, held together by a secreted matrix of polymers. Patients receiving or who have received medical devices are particularly susceptible to such biofilm formation as implanted devices may breach skin and/or provide a surface for attachment.

Device-related infections are initially localized and resulting clinical syndromes are dependant on device type and site of insertion; examples include, prosthetic valve endocarditis following prosthetic valve implantation, keratitis due to contact lens use, bacteriuria following urinary catheter use, intravascular catheter associated infection, prosthesis-related infection including septic loosening of joint prostheses following joint arthroplasty, and post-operative endophthalmitis

associated with intraocular lens implantation. The bacteria causing these infections can be introduced during the original surgery to place the device, or can be seeded via the blood from a distal infection. If left untreated, bacteria may disperse from the biofilm to seed secondary infections on other indwelling devices or may result in bacteremia and clinical sepsis. Confirmed staphylococcal colonization of a catheter, for example, presents a serious threat of spreading and establishing a biofilm on other indwelling devices or even on native heart valves, with potentially catastrophic consequences. In neonates – a high-risk population for CoNS-related infections – an infection may cause wound abscesses, pneumonia, urinary tract infections, meningitis, enterocolitis, and omphalitis.

The majority of CoNS-related clinical infections are caused by *Staphylococcus epidermidis* (>70%). Besides *S. epidermidis*, several other species of CoNS have documented pathogenic potential including *S. lugdunensis*, *S. haemolyticus*, *S. capitis*, *S. hominis*, and *S. saprophyticus*.

Staphylococcal infections, particularly those involving biofilm formation, tend to be difficult to eradicate. Infections are often unresponsive to antimicrobials due, in part, to the high degree of antibiotic resistance among species and the reduced killing of bacteria within a biofilm by antibiotics. The individual bacteria within a biofilm are protected from host defences and antibiotics. In light of these difficulties, prevention of infections is considered by many as important.

A number of staphylococcal proteins have been identified and suggested as possible vaccine candidates against staphylococci (including e.g., against *S. epidermidis*) but to date, no such vaccine is currently available on the market. There remains a need for prophylactic and/or therapeutic treatments for disease resulting from staphylococcal infections and in particular, from CoNS infections (including, *S. epidermidis* infections).

SUMMARY

This disclosure relates to polypeptides, compositions and methods for eliciting an immune response in a subject against staphylococci (such as e.g., *S. epidermidis*). More particularly, the present disclosure relates to compositions comprising an effective amount of at least one isolated *Staphylococcus* polypeptide (or fragment or derivative thereof). Exemplary polypeptides include, but are not limited to those set out in **Table 1**. In one example, compositions comprise an effective amount of at least one isolated *Staphylococcus* protein selected from the group consisting of: PhnD, LipL, LytD, SdrG and EmbP or a fragment or a derivative thereof. As described further herein, these proteins (and fragments and derivatives thereof) are highly immunogenic *in vivo* and elicit antibodies that inhibit formation of *Staphylococcus* (e.g., *S. epidermidis*) biofilms. The immune response

elicited may be sufficient for treating and/or preventing a disease resulting from an infection by a *Staphylococcus* species, such as for example, a CoNS species (e.g., *S. epidermidis*).

In another aspect, the present disclosure provides methods for inhibiting development of a *Staphylococcus* biofilm (e.g., on an indwelling medical device) in a subject. These methods may involve administering to a subject in need thereof an effective amount of a *Staphylococcus* polypeptide (or fragment or derivative thereof) to induce an immune response against *Staphylococcus*.

The present disclosure further relates to binding molecules (binding agents) that specifically bind to a *Staphylococcus* polypeptide (or fragments or derivatives thereof). These binding molecules may include for example, aptamers, antibodies and antibody mimetics. Methods of inhibiting development of a *Staphylococcus* biofilm in a subject by administering one or more of such binding molecules are provided. Binding molecules may for example be used prophylactically to inhibit biofilm formation and/or therapeutically to treat *Staphylococcus* biofilms to inhibit further growth or expansion after initial seeding and/or inhibit, reduce or eliminate metastatic spread.

At the time of administration, the subject may already have or may be at risk of developing a symptomatic staphylococcal infection. For example, in some embodiments, at the time of administration the subject may have received an indwelling medical device; in others, the subject may be scheduled to receive such a device. Administration may be performed sometime prior to the implantation of the device, in conjunction with implantation or sometime following implantation. In some embodiments, administration is performed 7 days or more before implantation or up to 24 hours after implantation.

In some embodiments the subject may have a symptomatic staphylococcal infection. For example, the subject may have been diagnosed with a primary staphylococcal infection (manifesting as a confirmed staphylococcal biofilm on an indwelling device or wounded skin and soft tissue) and may, therefore, be at risk of metastasis or further spread of the bacteria within the biofilm. Such subjects may be at risk of endocarditis resulting from the formation of a *Staphylococcus* biofilm on a native or prosthetic heart valve and/or at risk of premature prosthetic joint loosening or failure resulting from the formation of a *Staphylococcus* biofilm on the prosthetic joint.

BRIEF DESCRIPTION OF FIGURES

The present disclosure will be further understood from the following description with reference to the drawings, in which:

Figure 1. Panels A to G depict the inhibition of biofilm formation of *S. epidermidis* by anti-SERP2286 antibodies in a dose response manner under conditions of flow. *S. epidermidis* 1457 was grown in Bioflux flow cell channels with the indicated concentrations of control or SERP2286 antibodies under 0.4 dyne/cm² of flow pressure and imaged at 20 min intervals for 18 hr. Representative images are shown at the 10 hr timepoint.

Figure 2. Panels A to D depict the inhibition of biofilm formation of *S. epidermidis* by anti-SERP2286 antibodies under static growth conditions. *S. epidermidis* 1457 (A and C) and RP62A (B and D) were normalized to OD_{600nm} of 0.05 and incubated in triplicate with no antibody or 100 µg/mL of control or anti-SERP2286 antibody. After 8 hr of static growth, biofilms were stained with crystal violet. Microtiter plates were scanned (A and B) as well as measured at OD_{490nm} (C and D).

Figure 3. Panels A to C depict the anti-biofilm effect of anti- SERP2286 antibodies in the presence and absence of exogenously-added purified SERP2286 protein. *S. epidermidis* 1457 was grown in Bioflux flow cell channels with 100 µg/mL of control (A) or SERP2286 antibodies (B, C), and 75 µg/mL of purified SERP2286 protein (A, C) as indicated, under 0.4 dyne/cm² of flow pressure and imaged at 20 min intervals for 18 hr. Representative images shown at the 14 hr timepoint are provided.

Figure 4. Depicts the anti-biofilm effect of anti- SERP2286 antibodies against a non-PNAG producing *S. epidermidis* clinical isolate. *S. epidermidis* 7291 was grown in Bioflux flow cell channels with 100 µg/mL of control (A) or anti-SERP2286 antibody (B) under 0.3 dyne/cm² of flow pressure and imaged at 20 min intervals for 36 hr. Representative images shown at 30 hr timepoint are provided.

Figure 5. Depicts upregulation of SERP2286 during stationary and biofilm phase of growth. *S. epidermidis* 1457 was grown to the indicated optical density in broth or grown statically to form biofilms. After normalization by cell density, bacteria were lysed, crude protein was subjected to SDS-PAGE, and SERP2286 was detected by western blot using a 1/20000 dilution of rabbit affinity-purified anti-SERP2286 antibody. A representative western blot is shown.

Figure 6. Panels A-D depict the surface accessibility of SERP2286 in the biofilm mode of growth. Representative images were obtained using the Bioflux system after labeling with control (C, D) or anti-SERP2286 antibody (A, B) followed by fluorescent detection with anti-rabbit antibody conjugated to FITC (B, D).

Figure 7. Depicts quantitatively the anti-biofilm effects of protein-specific antibodies against *S. epidermidis*. A fluorescent reporter strain of *S. epidermidis* was grown in Bioflux flow cell

channels with 100 µg/mL of control or protein-specific antibody under 0.3 dyne/cm² of flow pressure for about 18 hours. Average pixel intensity (A.U.) was measured at specific time intervals over the 18 hr period.

Figure 8. Depicts the anti-biofilm effects of SERP2286- and SERP0237-specific antibodies against an encapsulated strain of *S. aureus* (strain Lowenstein), suggesting that *S. epidermidis* protein-specific antibodies were able to cross-react with *S. aureus* antigens and prevent biofilm formation. *S. aureus* strain Lowenstein was grown in Bioflux flow cell channels in half-strength TSB with 100 µg/mL of control or anti-protein antibodies as indicated, under 0.3 dyne/cm² of flow pressure and imaged at 20 min intervals for 6 hr. Representative images shown at the 6 hr timepoint are provided.

Figure 9. Depicts the cross-reactivity of *S. epidermidis* SERP2286-specific antibodies with whole cell lysates of other staphylococcal species of clinical relevance (*S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*). After normalization by cell density, bacteria were lysed, crude protein was subjected to SDS-PAGE, and SERP2286-reactive protein was detected by western immunoblotting. A representative western blot is shown.

Figure 10. Assessing biofilm inhibition by antibodies added post-attachment. Panel (A) depicts the biofilm development cycle. Possible antibody intervention points include the following: prophylactic – prior to bacterial attachment, early therapeutic –before intercellular aggregation, or late therapeutic – after biofilm establishment and maturation. Panel (B) is a schematic representation of the assay for biofilm inhibition, in which antibodies are introduced post-attachment (model for early therapeutic intervention). Biofilm formation is monitored for the duration of the experiment. Wavy arrows indicate the direction of flow.

Figure 11. Depicts the therapeutic effect of protein-specific antibodies against *S. epidermidis* biofilm when introduced to the system post-initial attachment of cells. A fluorescent reporter *S. epidermidis* strain was grown in Bioflux flow cell channels with 100 µg/mL of control or protein-specific antibody as indicated, under 0.3 dyne/cm² of flow pressure for about 18 hours. Average pixel intensity (A.U.) was measured at specific time intervals over the 18 hr period.

Figure 12. Monoclonal antibodies to PhnD inhibit *S. epidermidis* biofilm formation. *S. epidermidis* (1457-FL) grown for 21 hr at 37°C with monoclonal antibodies. Polyclonal control antibodies (PhnD, positive; rabbit anti-rat IgG, negative) were included as comparators. All antibodies were administered in prophylactic mode at 100 µg/mL. Longitudinal quantification of the experiment using average pixel intensity of images captured at 20 min intervals for 21 hr.

DETAILED DESCRIPTION

This disclosure provides staphylococcal polypeptides (including fragments and derivatives thereof) and binding molecules that specifically bind to these polypeptides which are useful prophylactically and/or therapeutically against symptomatic staphylococcal infections that are caused by, originate from, or otherwise involve a staphylococcal biofilm. Exemplary polypeptides include, but are not limited to, those set out in **Table 1**. Preferred polypeptides include but are not limited to the *Staphylococcus* proteins SERP2286 (PhnD), SERP0237, SERP1891, SERP0207, SERP1011 (EmbP), and SERP2288 and fragments and derivatives thereof. Exemplary binding molecules include, but are not limited to binding molecules that specifically bind to any of these proteins.

Compositions comprising one or more of these polypeptides and binding molecules that specifically bind to one or more of these are also provided. As described further below, compositions comprising an effective amount of at least one isolated staphylococcal polypeptide or a fragment or a derivative thereof may be administered to a subject to elicit an immune response (i.e., antibodies) that inhibits staphylococcal biofilm development (e.g., inhibits biofilm formation and/or treats an existing *Staphylococcus* biofilm to inhibit further growth or expansion after initial seeding and/or inhibits, reduces or eliminates metastatic spread).

This disclosure is based in part on the surprising finding that certain staphylococcal proteins are highly immunogenic and elicit an immune response (e.g., antibodies) *in vivo* that inhibits the formation of *Staphylococcus* biofilms and/or treat existing *Staphylococcus* biofilms to inhibit further growth or expansion after initial seeding and/or inhibit, reduce or eliminate metastatic spread. As disclosed further herein, binding molecules (e.g., antibodies) to these proteins surprisingly inhibited formation of *Staphylococcus* biofilms and/or treated existing *Staphylococcus* biofilms to inhibit further growth or expansion after initial seeding and/or inhibited, reduced or eliminated metastatic spread.

Examples of the polypeptides, compositions, binding molecules and methods are described further herein.

Polypeptides

Exemplary polypeptides include, but are not limited to, any of SEQ ID NOs. 1 to 38, as set out in **Table 1**.

The term “polypeptide” may also refer to fragments and/or derivatives (variants) of a polypeptide. Exemplary polypeptides include, for example, the full length protein (i.e., comprising

the full-length amino acid sequence, including the signal sequence), the mature full length protein (i.e., comprising the full-length amino acid sequence but lacking the signal sequence), derivatives thereof (naturally occurring or otherwise, e.g, synthetically derived) and fragments thereof. Set out in **Table 1** is the full length amino acid sequence (including signal sequence) of the respective exemplary proteins (SEQ ID NOs: 1, 3, 5, 7, 9-13, 15, 17, 18, 20, 22, 24, 27, 28, 30, 32, 34, 36, 37) in the *S. epidermidis* strain RP62A genome (GenBank Accession No. CP000029, RefSeq Accession Number NC_002976.3) although those derived from, or corresponding to, any *S. epidermidis* strain may also be suitable for use. In **Table 1**, amino acids underlined with a single line depict a predicted signal sequence that would be cleaved upon processing in *S. epidermidis* and amino acids underlined with a double line depict the sequence of the mature full length sequence or of exemplary fragments.

Preferred polypeptides comprise an amino acid sequence having 50% or more identity (e.g, 60, 65, 70, 75, 80, 85, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5% or more) to the amino acid sequence as set out in any of SEQ ID NOs: 1 to 38. Most preferred polypeptides comprise an amino acid sequence having at least 80%, 85%, 90%, 95% or more sequence identity when aligned globally to the amino acid sequence as set out in any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

A preferred protein is SERP2286 (PhnD). Exemplary SERP2286 (PhnD) polypeptides comprise the full-length PhnD amino acid sequence (in the presence or absence of the signal sequence), fragments thereof, and derivatives thereof. PhnD polypeptides suitable for use in the compositions described herein include, for example, those of GenBank Accession No. CP000029, RefSeq Accession Number NC_002976.3, and those described herein and in the Examples below, among others. The amino acid sequence of full length (including signal sequence) PhnD in the *S. epidermidis* RP62A genome is SEQ ID NO.1.

Table 1

Gene ID*	Gene Name (annotation)	SEQ ID NO	Amino Acid Sequence [∞]
SERP2286	<i>phnD</i> (phosphonate ABC transporter substrate-binding protein)	1	<u>MKNLKFLVVLVLSVVI</u> <u>FAAACGNSSSLDNNKSSDSGSDSKSG</u> <u>SYTPKELTVQFVPSQ</u> <u>NADTLEAKAKPLEKLLSDKLGIPVKVS</u> <u>VSTNYNTIVEAMKSKKVDVGFLPPTAYTLAHDQKAADLLLKA</u> <u>ERYDVNEDGSPSKKLVD</u> <u>DKSEILVKKDSGINSKDLKGKKI</u> <u>ALQDVTSTAGYTFPLVTLKKEAGIDATKDMKIVNVKGHDQAV</u> <u>ISLLNGDVDAAAVFNDARTVVKKDQPNVFKD</u> <u>TKILKLT</u> <u>KPI</u> <u>NDTISVRPDMDKKFQDKLKKAFKEISKTKKGHKII</u> <u>SEVYSHE</u> <u>GYVDTKDSDFDIVRQY</u> <u>EKAVHDMK</u>
		2	(20-318 aa)

SERP1891	(N-acetylmuramoyl-L-alanine amidase)	3	<u>MLKNTRLRMTTLFIISILVILAILFLIFDTNLFKNDVKHTFK</u> <u>EAVSLQTSSEGNHTKEVNGKFIYASKODIEKAMQIKHSDNDL</u> <u>KYMDISEKVPMSSEKEVNHILKGKGIENKSTFIKAQDKYEVN</u> <u>IILYLISHALVETGNGQSDLSKGIKEGNHHYYNFFGIGAFDED</u> <u>AVKTGKSFAKQKKWTTPEKAIMGGAWFVRYHYFKNNQLSLYQ</u> <u>MRWNPNPQGOHQASDIQWANNIADLMEKYYDKYGIKKDHIRK</u> <u>KYYK</u>
		4	(34-258aa)
SERP1011	Extracellular matrix-binding protein Ebh (EmbP) (cell wall associated fibronectin-binding protein) <i>Alternate start amino acid: M at residue 765 (bold, underlined)</i>	5	MKSKPKLNGRNICSFLLSKCMSYSLSKLSTLKTYNFQITSNN KEKTSRIGVAIALNNRDKLQKFSIRKYAIGTFSTVIATLVFM GINTNHASADELNQONQKLIKQLNQTDDDDSNTHSQEIENNKO NSSGKTESLRSSSTSONQANARLSDQFKDTNETSQQLPTNVSD DSINQSHSEANMNNEPLKVDNSTMQAHSKIVSDSDGNASENK HHKLTENVLAESRASKNDKEKENLQEKDKSQQVHPPLDKNAL QAFFDASYHNYRMIDRDRADATEYQKVKSTFDYVNDLLGNNQ NIPSEQLVSAYQQLEKALELARTLPQQSTTEKRGRRSTRSVV ENRSSRSYDLARTEYYVSKDDDDSGFPPTFFHASNRRWPY NLPRSRNILRASDVQGNAYITTKRLKDGQWDILFNSNHKGH EYMYWFGLPDQTPPTGPTFTIINRDGSSSTGGVVGFGSGA PLPQFWSAGAINSSVANDFKHGSATNYAFYDGVNNSDFAR GGELYFDREGATQTNKYYGDENFALLNSEKPDQIRGLDTIYS FKGSGDVSYRISFKTQGAFTARLYYAAGARSGEYKQATNYNQ LYVEPYKNYRNVRVQSNVQVKNRTLHLKRTIRQFDPTLQRTTD VPILDSGSGSIDSVYDPLSYVKNVTGTVLGIYPSYLPYNQE RWQGANAMNAYQIEELFSQENLQNAARSGRPIQFLVGFVED SHHNPEITLLPVNLYVKPELKHTIELYHDNEKQNRKEFSVSKR AGHGVFQIMSGTLHNTVGGSGILPYQQEIRIKLTSNEPIKDSE WSITGYPNTLTQLNAVGRTNNAATEKNLALVGHIDPGNYFITV KFGDKVEQFEIRSKPTPPRIITTANELRGNSNHKPEIRVTDI PNDTTAKIKLVMGGTDGDHDPEINPYTVPENYTVVAEAYHDN DPSKNGVLTFRSSDYLDKDLPLSGELKAIVYYNQYVQSNFSNS VPFSSDTPPTINEPAGLVHKYRGRDHVEITLPTVNTGSSG LRDNNVNLPPQGWTKTFTINPNNNTEGTLKLIGNIPSNEAYNT TYHFNITATDNSGNTTNPAKTFILNVGKLADDLNPVGLSRDQ LQLVTDPPSSLSNSEREVVKRKISEANANIRSYLLQNNPILAG VNGDVTFFYRDGSVDVIDAENVITYEPERKSIFSENGNTNKK EAVITITARGQNYTIGPNLRKYFSLSNGLPNRDFTSISAIG SLPSSEISRLNVGNYNRVNAKNAYHKTQQELNLKLKIVEV NAPTGNRRVYRVSTYNLTNDEINKIKQAFKAANSGLNLNDND ITVSNNFDHRNVSSVTVTIRKGDLIKEFSSNLNMMNFRWVN IRDDYTISWTSSKIQRNTDGGLEWSPDHKSLIYKYDATLGR QINTNDVLTLLQATAKNSNLRNSNINSNEKQLAERGSNGYSKS IIRDDGEKSYLLNSNPIQVLDLVEPDNGYGGRRQVSHSNVIYN EKNSSIVNGQVPEANGASAFNIDKVVKANAAANNIMGVYKA QLYLAPYSPKGYIEKLGQNLNNTNNVINVFVPSDKVNPSIT VGNYDHHTVYSGETFKNNTINVNDNYGLNTVASTSDSAITMTR NNNELVGQAPNVTNSTNKIVVKATDKSGNESIVSFTVNIKP LNEKYRITTSNNQTPVRIISNIQNNANLSIEDQNRVKSSLSM TKILGTRNYVNESNNDVRSQVVSQVNRSGNNATVNVTTTFS GTTNTITVPVKHVLLEVVPTRTTVRGQQFPTGKGTSPNDEF SLRTGGPVDARIVWVNQGPDINSNQIGRDLTLHAEIFFDGE TTPIRKDDTTYKLSQSIPKQIYETTINGRFNSSGDAYPGNFVQ AVNQYWPEHMDFRWAQGSPTSSRNAGSFTKTVTVVYQNGQT ENVNVLFKVKPNKPVIDSNSVISKGQLNGQQILVRNVQNAQ VTLYQSNGTVIPNTNTTIDSNGIATVTIQGTLPNTGNTAKTS MTNNVITYTKQNSSGIASNTTEDISVFSSENSDQVNVTAGMQAK

			NDGIKIIKGTNYNFNDFNSFISNIPAHSTLTWNEEPNSWKNN IGTTTKTVTVTLPNHQGTRTVDIPITITYPTVTAKNPVRDQKG RNLTNGTDVYNYIIFENNNRLGGTASWKDNRPDKNIAGVQN LIALVNYPGISTPLEVPVKVWVYNFDFTOPIYKIQVGDTFPK GTWAGYYKHLENGEGLPIDGWKFYWNQQSTGTTSDQWQSLAY TRTPFVKTGTYDVVNPSNWGVWQTSQSAKFIVTNAKPNQPTI TQSKTGDTVTPGAVRNILISGTNDYIQASADKIVINKNGNK LTTTFVKNNDRWTVETGSPDINGIGPTNNGTAISLSRLAVRP GDSIEAIAATEGSGETISTSATSEIYIVKAPQPEQVATHTYDN GTFDILPDNSRNSLNPTERVEINYTEKLNNGNETQKSFTITKN NNGKWTINNKNPYVEFNQDNGKVVFSAANTIKPNSQITITPKA GQGTENTNPTVIQAPAQHTLTINEIVKEQGQNVNTDDINNA VQVPKNRVAIKQGNALPTNLGGSTSHIPVVIYSDGSSEE ATETVRTKVNKTELINARRRLDEEISKENKTPSSIRNFDQAM NRAQSQINTAKSDADQVIGTEFATPQQVNSALSKVQAAQNKI NEAKALLQNKADNSQLVRAKEQLQSSIQPAASTDGMTQDSTR NYKNKRQAAEQAIQHANSVINNGDATSQQINDAKNTVEQQR DYVEAKSNLRADKSQLQSAYDTLNRDVLTDNKKPASVRRYNE AISNIRKELDTAKADASSTLRNTNPSVEQVRDALNKINTVQP KVNQAIALLQPKENNELVQAKKRLQDAVNDIPQTQGMTQQT INNYNDKQREAEALTSARVIDNGDATTQEIITSEKSKVEQA MQALTNAKSNLRADKNELQTAYNKLIENVSTNGKKPASIRQY ETAKARIQNQINDAKNEAERILGNDNPQVSQVTQALNKKIKAI QPKLTEAINMLQNKENNTLVNAKNRLENVNDTDPHGMTQ ETINNYNAKKREAQNEIQKANMIINNGDATAQDISSEKSKVE QVLQALQNAKNDLRADKRELQTAYNKLIQNVNTNGKKPSSIQ NYKSARRNIENQYNTAKNEAHNVLENTNPTVNAVEDALRKIN AIQPEVTKAINILQDKEDNSELVRAKEKLDQAINSQPSLNGM TQESINNYTTKRREAQNIASSADTIINNGDASIEQITENKIR VEEATNALNEAKQHLTADTTSLKTEVRKLSRRGDTNNKKPSS VSAYNNTIHSLSQSEITQTENRANTIINKPIRSVEEVNNALHE VNQLNQRLTDTINLLQPLANKESLKEARNRLESKINETVQTD GMTQQSVENYKQAKIKAQNESSIAQTLINNGDASDQEVSTEI EKLNQKLSELTNSINHLTVNKEPLETAKNQLQANIDQKPSTD GMTQQSVQSYERKLQEAQDKINSINNVLANNPVDNAIRTNKV ETEQINNELTQAKQGLTVDKQPLINAKTALQQSLDNQPSTTG MTEATIQNYNAKRQKAEQVIQONANKI IENAQPSVQQVSDEKS KVEQALSELNNAKSALRADKQELQQAYNQLIQPTDLNNKKPA SITAYNQRYQQFSNELNSTKTNTDRILKEQNPSVADVNNALN KVREVQQKLNEARALLQNKEDNSALVRAKEQLQQAQVDPST EGMTQQTKDDYNSKQQAQQEISKAAQVIDNGDATTQQISNA KTNVERALEALNNAKTGLRADKEELQONAYNQLTQNIIDTSGKT PASIRKYNEAKSRIQTQIDSANEANSILTDNPNQVSQVTAA LNKIKAVQPELDAIAMLKNKENNNALVQAKQQLQQIVNEVD PTQGMTTDTANNYKSKKREAEDIEQKAQQIINNGDATEQQIT NETNRVNQAINAINAKNDLRADKSQLENAYNQLIQNVDTNG KKPASIQOYQAAEQAIETQYNNAKSEAHQILENSNPSVNEVA QALQKVEAVQLKVNDIAIHILQNKENNSALVTAKNQLQQSVND QPLTTGMTQDSINNYEAKRNEAQSAIRNAEAVINNGDATAKQ ISDEKSKVEQALAHLDNAKQQLTADTTELQTAVQQLNRRGDT NNKKPRSINAYNKAIQSLETQITSAKDNANAVIQKPIRTVQE VNNALQQVNQLNQQLTEAINQLQPLSNNDALKAARLNLENKI NQTVQTDGMTQQSIEAYQNAKRVAQNESNTALALINNGDADE QQITTETDRVNQQTTLNLQAINGLTVNKEPLETAKTALQNNI DQVPSTDGMTQQSVANYNQKLQIAKNEINTINNVLANNPVDN AIKTNKAEAEERISNDLTQAKNNLQVDTQPLEKIKRQLQDEID QGTNTDGMTQDSVDNYNDSLAAIEKGKVNKLLKRNPTVEQ
--	--	--	---

			<p> VKESVANAAQQVIQDLQNARTSLVPDKTQLQEAKNRLENSINQ QTDTDGMTQDSLNNYNDKLAKARQNLKISKVLGGQPTVAEI RQNTDEANAHKQALDTARSQTLNREPYINHINNESHNNAAQ KDNFKAQVNSAPNHNTLETIKNKADTLNQSMTALSSESADIYE NQKQQENYLDASNNKRQDYDNAVNAAGILNQTSPTMSADV IDQKAEDVKRTKTALDGNQRLEVAQQAALNHLNLTNDLNDQAQ RQTLTDTINHSPNINSVNAKEKANTVNTAMTQLKQTIANYD DELHDGNYINADKDKKDAYNNNAVNNAKQLINQSDANQAQLDP AEINKVTQRVNTTKNDLNGNDKLAEAKRDANTTIDGLTYLNE AQRNKAENVGKASTKTNITSQLQDYNQNLIAMQALRNSVND VNNVKANSNYINEDNGPKAEYNQAVTHAQTLLINAQSNPEMSR DVVNQKTQAVNTAHQNLHGQKLEQAQSSANTEIGNLPNLTN TQKAKEKELVNSKQTRTEVQEQLNQAKSLDSSMGTLLKSLVAK QPTVQKTSVYINEDQPEQSAYNDSITMGQTIINKTADPVLDK TLVDNAISNISSTKENALHGEQKLTTAKTEAINALNTLADLNT PQKEAIKTAINTAHTRTDVTAEQSKANQINSAMHTLRQNI SD NESVTNESNYINAEPEKQHAFTREALNNAKEIVNEQQATLDAN SINQKAQAILTTKNALDGEEQLRRAKENADQEIINTLNQLTDA QRNSEKGLVNSSQTRTEVASQLAKAKELNKVMEQLNHLINGK NQMINSSKFINEDANQQQAYSNAIASAEALKNKSONPELDKV TIEQAINNINSAINNLNGEAKLTKAKEDAVASINNLSGLTNE QKPKENQAVNGAQTRDQVANKLRDAEALDQSMQTLRDLVNNQ NAIHSTSNYFNEDSTQKNYTDNAIDNGSTYITGQHNPELNKS TIDQTI SRINTAKNDLHGVEKLQRDKGTANQEIGQLGYLNDP QKSGEESLVNGSNTRSEVEEHLNEAKSLNNAMQQLRDKVAEK TNVKQSSDYINDSTEHQRGYDQALQEAENIINEIGNPTLNKS EIEQKLQQLTDAQNALQGSLLLEEAKNNAITGINKLTALNDA QRQKAIENVQAQQTIPAVNQQLTLTREINTAMQALRDKVGGQ NNVHQSSNYFNEDEQPKHNYDNSVQAGQTIIDKLQDPIMNKN EIEQAINQINTTQTALSGENKLHTDQESTNRQIEGLSSLNTA QINAEKDLVNQAKTRTDVAQKLAAAKEINSAMSNLRDGIQNK EDIKR SAYINADPTKVTAYDQALQNAENIINATPNVELNKA TIEQALSRVQQAQDLDGVQQLANAKQQAQTQTVNGLNSLNDG QKRELNLLINSANTRTKVQEELNKATELNHAMEALRNSVQNV DQVKQSSNYVNEDQPEQHNYDNAVNEAQATINNNAQPVLDKL AIERLTQTVNTTKDALHGAQKLTQDQQAETGIRGLTSLNEP QKNAEVAKVTAATTRDEVNRIRQEATTLDTAMLGLRKS IKDK NDTKNSSKYINEDHDQQQAYDNAVNNAQQVIDETQATLSSDT INQLANAVTQAKSNLHGDTKLQHDKDSAKQTI AQLQNLNSAQ KHMEDSLIDNESTRTQVQHDLTEAQAALDGLMGALKESIKDYT NIVSNGNYINAEPSKKQAYDAVQNAQNIINGTNQPTINKGN VTTATQTVKNTKDALDGDHRLLEEAKNNANQTI RNLSNLNNAQ KDAEKNLVNSASTLEQVQQNLQTAQQQLDNAMGELRQSI AKKD QVKADSKYLNEDPQIKQNYDDAVQRVETIINETQNPPELLKAN IDQATQSVQNAEQALHGAEKLNDQDKQTSSTELDGLTDLTDAQ REKLREQINTSNSRDDIKQKIEQAKALNDAMKKLKEQVAQKD GVHANSDYTNEDSAQKDAYNNALKQAEDIINNSSNPNLNAQD ITNALNNIKQAQDNLHGAQKLQDDKNTTNQAIGNLNHLNQPD KDALIQAINGATS RDQVAEKLKEAEALDEAMKQLEDQVNDQDD QISNSSPFINEDSDKQKTYNDKIQAAKEIINQTSNPTLDKQK IADTLQNIKDAVNNLHGDQKLAQSKQDANNQLNHLDDLTEEQ KNHFKPLINNADTRDEVNKQLEIAKQLNGDMSTLHKVINDKD QIQHLSNYINADNDKKQNYDNAIKEAEDLIHNHPDTLDHKAL <u>QDLLNKIDQAHNELNGESRFGQALDNALNDIDSLNSLNVQOR</u> <u>QTVKDNINHVTTLES LAQELQAKELNDAMKAMRDSIMNQE</u> <u>IRKNSNYTNEDLAQQONAYNHAVDKINNIIGEDNATMDPQIIK</u> <u>QATQDINTAINGLDQKLQDAKTDKQOITNFTGLTEPQKQ</u> </p>
--	--	--	---

			<p> <u>ALENI INQOTSRANVAKOLSHAKFLNGKMEELKVAVAKASLV</u> <u>RONSNYINEDVSEKEAYEQAIKGOEINSENNP TISSTDIN</u> <u>RTIOEINDAEQNLHGDNKLROAQEIAKNEIQNL DGLNSAQIT</u> <u>KLIQDIGRTTTKPAVTOKLEEKAINQAMQOLKOSIADKDAT</u> <u>LNSSNYLNEDSEKKLAYDNAVSQAEQLINQNLDP TMDISNIQ</u> <u>AITOKVIOAKDSLHGANKLAQNOQADSNLIINQSTNLNDKQKQ</u> <u>ALNDLINHAQTKQQVAEIIAQANKLNNEMGTLKTLVVEEQSNV</u> <u>HQOSKYINEDPQVQNIYND SIQKGREILNGTTDDVLNNNKIA</u> <u>DAIQNIHLTKNDLHGDQKLQKAQODATNELNYLTNLNNSQRO</u> <u>SEHDEINSAPSRTEVSNDLNHAKALNEAMROLENEVALENSV</u> <u>KKLSDFINEDEAAQNEYSNALOKAKDIINGVPSSTL DKATIE</u> <u>DALLELONARES LHGEOKLOEAKNOQVAEIDNLQALNPGQVL</u> <u>AEKTLVNOASTKPEVQOEALOKAKELNEAMKALKTEINKKEQI</u> <u>KADSRVYNADSGLQANYNSALNYGSQIIATTOPPELNKDVIN</u> <u>RATQTIKTAENNLNGQSKLAEAKSDGNQSI EHLQGLTQSQKD</u> <u>KQHDLINQAQTKQQVDDIVNNSKQLDN SMNQLQQIVNNDNTV</u> <u>KQNSDFINEDSSQQDAYNHAIQAAKDLITAHPTIMDKNQIDQ</u> <u>AIENIKQALNDLHG SNKLSSEDKKEASEQLQNLNSLTNGQKDT</u> <u>ILNHIFSAPTRSQVGEKIASAKQLNNTMKALRDSIADNNEIL</u> <u>QSSKYFNEDSEQQAYNQAVNKA KNIINDQPTVMA NDEIQS</u> <u>VLNEVKQTKDNLHGDQKLANDKTD AQATLNALNYLNQAQRGN</u> <u>LETKVQNSNSRPEVQKVQ LANQLNDAMKKLDDAL TGNDAIK</u> <u>QTSNYINEDTSQQVNFDEYTD RGKNIVAEQTNPNMSP TNINT</u> <u>IADKITEAKNDLHGVQKLKQAQQQSINTINQMTGLNQAQKEQ</u> <u>LNQEIQQTQTRSEVHVINKAQALND SMNLTLRQSI TDEHEVK</u> <u>QTSNYINETVGNQTAYNNAVDRVKQIINQTSNPTMNP LEVER</u> <u>ATSNVKISKDALHGERELNDNKN SKTFAVNHLDNLNQAQKEA</u> <u>LTHEIEQATIVSQVNNIYNKAKALNNDMKKLKDIVAQQDNVR</u> <u>QSNNYINEDSTPQNM YNDTINHAQSIIDQVANPTM SHDEIEN</u> <u>AINNIKHAINALDGEHKLQQA KENANLLINSLNDLNAPQRDA</u> <u>INRLVNEAQ TREKVAEQLOSAQALNDAMKHLRNSIQNQSSVR</u> <u>QESKYINASDAKKEQYNHAVREVENI INEQHPTLDKEI IKQL</u> <u>TDGVNQANNDLNGVELLDADKQNAHQSIPTLMHLNQAQQNAL</u> <u>NEKINNAVTRTEVA AIIQOAKLLDHAMENLEESIKDK EQVKQ</u> <u>SSNYINEDSDVQETYDNAVDHVTEILNQTVNPTLSIEDIEHA</u> <u>INEVNQAKKQLRGKQKLYQTIDLADKELSKLDDLT SQSSSI</u> <u>SNQIYTAKTRTEVAQAIEKAKSLNHAMKALNKVYKNADK VLD</u> <u>SSRFINEDQPEKKAYQQA INHVDSIIHRQTNPEMDPTVIN SI</u> <u>THELETAQNNLHGDQKL AHAQQDAANVINGLIHLNVAQREVM</u> <u>INTNTNATTREKVAKNL DNAQALDKAMETLQQVVAHKNNILN</u> <u>DSKYL NEDSKYQQQYDRVIADAEQLLNQTTNPTLEPYKV DI V</u> <u>KDNVLANEKILFGAEKLSYDKSNANDEIKHMNYLNNAQKQSI</u> <u>KDMI SHAALRTEVKQLLQQA KILDEAMKSLEDKTQVVITD TT</u> <u>LPNYTEASEDKKEKVDQTVSHAQAIIDKINGSNVSLDQVRQA</u> <u>LEQLTQASENL DGDQRVEEAKVHANQTIDQLTHLNSLQQQTA</u> <u>KESVK NATKLEEIATVSNN AQALNKVMGKLEQFINHADSVEN</u> <u>SDNYRQADDDKIIAYDEALEHGQDIQKT NATQNETKQALQQL</u> <u>IYAETSLNGFERLNHARPRALEYIKSLEKINNAQKSALEDKV</u> <u>TQSHDLLELHIVNEG TNLNDIMGELANAIVNNYAPT KASIN</u> <u>YINADNLRKDNFTQAINNARDALNKTOGQNLDFNAIDTFKDD</u> <u>IFKTKDALNGIERLTA AKSKAEKLIDSLKFINKAQFTHANDE</u> <u>IINTNSIAQLSRIVNQAFDLNDAMKSLRDELNNQAFPVQASS</u> <u>NYINSD EDLKQQFDHALSNARKVLAKENGKNLDEKQIQGLKQ</u> <u>VIEDTKDALNGIQR LSKAKAKAIQYVQSLSYINDAQRHIAEN</u> <u>NIHNSDDLSSLANTLSKASDLNAMKDLRDTIESNSTSV P NS</u> <u>VNYINADKNLQIEFDEALQQASATSSKTSENPATIEEVLGLS</u> <u>QAIYDTKNALNGEQR LATEKSKDLKLIKGLKDLNKAQLEDVT</u> </p>
--	--	--	--

		6	<p> NKVNSANTLTLSQLTQSTLELNDKMKLLRDKLKTIVNPVKA SLNYRNADYNLKRQFNKALKEAGVNLKNSGTNVNINDIQHL LTQIDNAKDQLNGERRLKEHQKSEVFIIKELDILNNAQKAA IINQIRASKDIKIINQIVDNAIELNDAMQGLKEHVAQLTATT KDNIEYLNADHDHKLQYDYAINLANNVLDKENGNTKNDANII GMIQNMDDARALLNGIERLKDAQTKAHNDIKDTLKRQLDEIE HANATSNSKAQAKQMVNEEARKALSNINDATSNDLVNQAKDE GQSAIEHIHADELPAKAKLDANQMIDQKVEDINHLISQNPPLS NEEKNKLISQINKLVNGIKNEIQQAINKQQIENATTKLDEVI ETTKKLI IAKAEAKQMIKELSQKKRDAINNNTDLTPSQKAHA LADIDKTEKDALQHIENSNSIDINNNKEHAFNTLAHII IWD TDQQPLVFELPELSLQNALVTSEVVVHRDETISLESIIIGAMT LTDELKVNIVSLPNTDKVADHLTAKVKVILADGSYVTNVVPV KVVEKELQIAKKDAIKTIDVLVKQKIKDIDSNNELTSTQRED AKAEIERLKKQAIDKVNHSKSIKDIE TVKRTDFEEDQFDPK RFTLNKAKKDIITDVNTQIQNGFKEIETIKGLTSNEKTQFDK QLTALQKEFLEKVEHAHNLVELNQLQQEFNNRYKHILNQAHL LGEKHIAEHKLGYYVVVNKTQQIILNNQSASYFIKQWALDRIKQ IQLETMNSIRGAHTVQDVHKALLQGIEQILKVNVSIIINQSFN DSLHNFNYLHSKFDARLREKDVANHIVQTETTFKEVLKGTGVE PGKINKETQQPKLHKNDNDSL FKHLDNFVKTVGVITLTGLL SSFWLVLAARRRKEEEEKQSIKNHHKDIRLSDTDKIDPIVIT KRKIDKEEQIQNDKHSIPVAKHKKSKKEQLSEEDIHSIPVV KRKQNSDNKDTKQKKVTSKKKKTPQSTKKVVKTKKRSKK </p> <p>(6600-7340aa)</p>
SERP0207	(SdrG protein)	7	<p> MFGLGHNEAKAEENTVQDVKDSNMDELSDSNDQSSNEEKND VINNSQSINTDDDNQIKKEETNSNDAIENRSKDI TQSTTNVD ENEATFLQKTPQDNTQLKEEVVKEPSSVESSNSSMDTAQQPS HTTINSEASIQTSNEENS RVSDFANSKIIESNTESNKEENT IEQPNKVREDSITSQPSSYKNIDEKISNQDELLNLPINEYEN KVRPLSTTSAQPSSKRVTVNQLAAEQGSNVNHLIKVTDQSIT <u>EGYDDSDGIIKAHDAENLIYDVTFEVDDKVKSGDTMTVNI</u> <u>DK</u> <u>NTVPSDLTDSFAIPKIKDNSGEIIATGTYDNTNKQITYTFTD</u> <u>YVDKYENIKAHLKLTSYIDKSKVPNNNTKLDVEYKTALSSVN</u> <u>KTITVEYQKPENRTANLQSMFTNIDTKNHTVEQTIYINPLR</u> <u>YSAKETNVNISGNGDEGSTIIDDSTIIKVYKVGDNQNLPSN</u> <u>RIYDYSEYEDVTNDDYAQLGNNNDVNINFGNIDSPYIIKVIS</u> <u>KYDPNKDDYTTIQOTVTMOTTINEYTGEFRTASYDNTIAFST</u> <u>SSGOGQGDLPPEKTYKIGDYVWEDVDKDGIQNTNDNEKPLSN</u> VLVTLTYPDGTSKSVRTDEEGKYQFDGLKNGLTYKITFETPE GYTPTLKHSGTNPALDSEGNSVWVTINGQDDMTIDSGFYQTP KYSLGNYVWYDTNKDGIQGDDEKGISGVKVTCLKDENGNIIST TTTDENGKYQFDNLNSGNYIVHFDKPSGMTQT TTDSDGDDDEQ DADGEEVHVTITDHDDFSIDNGYYDDSDSDSDSDSDSDSD SDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSG LDNSSDKNTKDKLPDTGANEDHDSKGTLLGALFAGLGALLG KRRKNRKNKN </p> <p>(233-559aa)</p>

SERP0237	(lipoate-protein ligase A)	9	<u>MDLATKYFNQINWRYVDHSSGLEPMQSFADDDTFSESVGKDL</u> <u>SCNVVRTWIHQHTVILGIHDSRLPFLSDGIRFLTDEQGYNAI</u> <u>VRNSGGLGVVLDQGIILNISLIFKGQETTTIDEAFTVMYLLIS</u> <u>KMFEDEDVSI DTKEIEQSYCPGKFDLSINDKKFAGISORRVR</u> <u>GGIAVQIYLCIEGSGSERALMMQOFYQORALKGQTTKFHYPI</u> <u>DPSCMASLETLLNREIKVQDVMFLLLYALKDLGANLNMDFIT</u> <u>EDEWTRYEGYYDKMLERNAKINEKLDF</u> (1-279aa)
SERP2288	(5'-nucleotidase)	10	<u>MSQIAFYILSDVHGFIPTDFSSREKDLPMGLLKANHLIEQD</u> <u>CVHYDGSVKIDNGDFLQGSPLCNYLVSKLKSSSLPTSINRL</u> <u>GFDFGIVGNHEFNIDLPYLKQAINQLHPVLCANI ENTQPF</u> <u>TGOGIHYFKVNDLTIGTIGLTTOYIPHWEQPDYIKTLTFNSA</u> <u>VHTLKSELPTLREKSDIVVVSYHGGFERDLDSGLPTEALTGE</u> <u>NEGYDILSQFSDSIDVLITGHQHRDIATIKNQTAI IQPGSKG</u> <u>TKVGKIVIEYTHDKKVLIKECNLMNVNNNTFFKPNDEIALR</u> <u>NQLEDWLDTOIAELPYAMRINNSFEARKSPHAFVNLNLYILL</u> <u>EKSGADIACTALFDSANGFDEKVTMRDI INNYPFNTFKVIE</u> <u>LSGKDIKLAIERSASYFDIVNHKITVNKEFLEPKPOHFNYDI</u> <u>FAGIQYTIHVSHPHYGERVSDLLINDAPIQSDQIYITICVNNYR</u> <u>AVGGGNYDMYVNKPVIKDIQIEGAQLLIDYLSHNDLSQIPQV</u> <u>IDFNVVK</u> (1-511aa)
SERP0442	<i>gapA-1</i> (glyceraldehyde 3- phosphate dehydrogenase)	11	<u>MAIKVAINGFGRIGRLAFRRIQDVEGLEVVAVNDLTDDMLA</u> <u>HLLKYDTMQGRFTGEVEVIEGGFRVNGKEIKSFDEPDAGKLP</u> <u>WGDLDIDVVELECTGFYTDKEKAQAHIDAGAKKVLISAPAKGD</u> <u>VKTIVFENTNHDTLDGSETVVSGASCTTNSLAPVAKVLSDEFG</u> <u>LVEGFMTTIIHAYTGDQNTQDAPHRKGDKRRARAAAENI IPNS</u> <u>TGAAKAIGKVIPEIDGKLDGGAQRVPVATGSLTELTVVLDKQ</u> <u>DVTVDQVNSAMKQASDESFGYTEDEIVSSDIVGMTYGSLEDA</u> <u>TQTRVMTVGDRQLVKVAAWYDNEMSYTAQLVRTLAHLAELSK</u> (1-336aa)
SERP0306	(iron ABC transporter ATP- binding protein)	12	<u>MSRLSGEQVKIGYGDSTIINNLDVAIPDGKVTSIIGPNGCGK</u> <u>STLLKALSRLLSIKEGKINLDGKSIHATSTKEIAKKIAILPQ</u> <u>SPEVPDGLTVGELVSYGRFPHQKGFGRLTAEDKIDWALSV</u> <u>TGTSEFRHRTINDLSGGQRQRVWIAMALAQRDTDII FLDEPTT</u> <u>YLDICHQLEIILNLVKKLNEEEGCTIVMVLHDINQAIRFSDHL</u> <u>ITMKAGDIVATGQTDVLTDKDILEKVFENIDGVLDIDPRTGKP</u> <u>ILVTYDLFCQ TYS</u> (1-265aa)
SERP0574	<i>oppA</i> (oligopeptide ABC transporter oligopeptide- binding protein)	13	<u>MKGFKVLI ILLSVCII LSACSNKQSLYSDQGQVFRKVITQDM</u> <u>TTLDTALITDAVSGDIAAQAFEGLYTLNKEDKAEP AI AKSFP</u> <u>KKSNGGKTLTINLRKNAKWSNGDSVTAYDFVYAWRKVVNPKT</u> <u>ASEFAYIMSDIKNADEVNAGKKSVDLGIKAIGKYKLQVDLE</u> <u>RPVPYINELLALNTFNPNQNEKVAKKFGEQYGTAEKAVYNGP</u> <u>FEVTNWKVEDKIQLVKNEQYWDKKNVKLDKVNYKVLKDQQAG</u> <u>ASLYDTGSVDDTIITSEQVDKYRGESALNYRLTAATFFIKMN</u> <u>OKTVPEFKNKHRLRAISQAINKKGYVNSVLNDGSLPSNFTG</u> <u>VGTADTPDGKDFARTIKSPLKFNPD LAKKNWREAQKELGKNK</u> <u>FTFTMNTQDTPASKIAAEYIKSQVEKNLPGVTLKIKQMPFKQ</u> <u>KTTLELANNYEASYSWSPDYPDPTAFLOTMTKD NAQNNTDW</u> <u>SNKEYDQLLKEANSKLLRKPGERN TSLQKA EYILLHEAPVAP</u> <u>VYQKGAEHLTNPOVKGLQYHKVGPETTLKHVYIDKSINRETG</u> <u>K</u>
		14	(20-547aa)

SERP2252	<i>sepA</i> (extracellular elastase precursor)	15	<u>MKNFSKFALTSIAALTVASPLVNTEVDAKDKVSATONIDAKV</u> <u>TOESQATNALKELPKSENIKKHYKDYKVTDTTEKDNKGFTHT</u> <u>LQPKVGNTYAPDKEVKVHTNKEGKVVLVNGDTPDAKKVQPTNK</u> <u>VAISKESATDKAFEAIKIDROKAKNLKSDVIKTNKVEIDGK</u> <u>NKYVYNIEIITTSFKISHWNVKIDAETGQVVDKLNMIKEAAT</u> <u>TGTGKGVLDGTDKQININSVSGGYTLQDLTQOQTL SAYNYDAN</u> <u>TGQAYLMQDKDKNFVDDEQRAGVDANY YAKETYDYKNTFGR</u> <u>ESYDNQGSPIISIAHVNNFQGDNRNNAAWIGDKMIYGDGDG</u> <u>RTFTALSGANDVVAHEITHGVTQOTANLVYRSQS GALNESFS</u> <u>DVFGYFIDDEDFLMGEDVYTPGVGGDALRSMNPERFGQPSH</u> <u>MNDFVYTNSDNGGVHTNSGIPNKAAAYNTIRSIGKORSEQIYY</u> <u>RALTVYLTSNSDFQDAKASLOQAAFDLYGDGIAQQVGQAWDS</u> <u>VGV</u>
		16	(28-507aa)
SERP2391	<i>sspC</i> (SspC protein)	17	<u>MHNYNINIVSDDSKYQEI CWFHTLEGIWHPVEVETSPLNIT</u> <u>FNKEITPNYVCTLINE DSRKII LSNVDNPNII IEMILINSKK</u> <u>LVFNAISKEGLGTSPKITFTK</u> (1-105aa)
SERP0290	<i>sitC</i> (ABC transporter substrate-binding protein)	18	<u>MKKILALAI AFLII LAACGNHSNHEHHSHEGKLVVTTNSIL</u> <u>YDMVKRVGGNKVDVHSIVPVGQDPHEYEVKPKDIKALTDADV</u> <u>VFYNGLNLETGNWFEKALDQAGKSTKDKNVIAASN NVKPIY</u> <u>INGEEGNKNKQDPHAWLSLENGIKYVKTIQKSLEHHDKKDKS</u> <u>TYEKQGNAYISKLEELNKDSKNKFDDIPKNQRAMMTSEGAFK</u> <u>YFAQQFDVKPGYIWEINTEKOGTPGOMKQAIKFVKDNHLKHL</u> <u>LVETSVDDKAMQSLSEETKKDIYGEVFTDSIGKEGTKGDSYY</u> <u>KMMKSNIDTIH GSMK</u>
		19	(18-309aa)
SERP1316	(cell wall surface anchor family protein)	20	<u>MNLFRKQKFSIRKFNIGIFSALIATVAFLAHPGQATASELEP</u> <u>SQNNDTTAQSDGGLENTSQSNPISEETTNTLSGQTVPSSTEN</u> <u>KOTQNVPNHNAOPIAINTEEAESAQTASYTNINENNDTSDDG</u> <u>LHVNPAPKHHIEAQSEDTVNTHTNSNHSNSSIPENKATTESSS</u> <u>KPKKRGKRS LDTNNGNDTTSTTONTDPNLSNTGPNGINTVIT</u> <u>FDDLGIKTSTNRSRPEVKVVDLSLNGFTMVNGGKVLG LNSVLE</u> <u>RTSVFDSADPKNYQAIDNVVALGRIGKNDPNDH DGFNGIEKE</u> <u>FVSNPNSEIIFSFNTMTAKNRKGGTQLVLRNAENNQEI ASTD</u> <u>IQGGGVYRLFKLPD NVHRLKVQFLPMNEIHSDFKRIQQLHDG</u> <u>YRYYSFIDTIGVNSGSHLYVKSRQVNKNVKNNGKEFEVNTRIE</u> <u>NNGNFAAAIGQNELTYKVTLPENFEYVDNSTEVSVNGNVPN</u> <u>STVNPFSVNFDRQNHTLTFSSNGLNLGRSAQDVARELPNKIL</u> <u>NIRYKL RPNVISTPREVTFNEAIKYKTFSEYYINTNDNTVTG</u> <u>QOTPF SINVMNKDDLSEQVNKDIIPSNYTLASYNKYNKLKE</u> <u>RAQTVLDEETNNTPFNQRYSTQIDDLLHELQTTLINRV SAS</u> <u>REINDKAQEMTDAVYDSTELTTEEKDTLVDQIENHKNEISNN</u> <u>IDDELTDG DVERVKEAGLHTLES DTPHPVTKPNARQV VNNRA</u> <u>DQOKTLIRNNHEATTEEQNEAIRQVEAHSSDAIAKIGEAETD</u> <u>TTVNEARDNGTKLIATDVPNP TKKAEARAAVTNSANSKIKDI</u> <u>NNNTQATLDERNDALVNRSKDEAIQNI NTAQGNDDVTEAQ</u> <u>NNGTNTIQQVPLTPVKRQNAIATINAKADEQKRLIQANN NAT</u> <u>TEEKADAERKVN EAVITANQNITNATTNRD VDDQAQTTGSGII</u> <u>SAISPATKIKEDARA AVEAKAIAQNQQINSNNMATTEEKEDA</u> <u>LNQVEAHKQAAIATINQAQSTQQVSEAKNNGINTINQDQ PNA</u> <u>VKKNNTKII LEQKGNEKKS AIAQTPDATTEEKQEAVSAVSQA</u> <u>VTNGITHINQANSNDDVDQELSNAEQIITQTNVNVQKKPQAR</u> <u>QALIAKTNERQSTINTDNEGTIEEKQKAIQSLNDAKNLADEQ</u>

			<p> ITQAASNQNVNDNALNIGISNISKIQTNFTKKQQARDQVNQKF QEKEAELNSTPHATQDEKQDALTRLTQAKETALNDINQAQTN QNVDTALTSGIQNIQNTQVNVVRKKQEAKTTINDIVQQHKQTI QNNDDATTEEKEVANNLVNASQQNVISKIDNATNNQIDGIV SDGRQSINAITPDTSIKRNAKNDIDIKAADKKIKIQRINDAT DEEIQEANRKIEEAKIEAKDNIQRNSTRDQVNEAKTNGINKI ENITPATTVKSEARQAVQNKANEQINHIQNTPDATNEEKQEA INRVSAELARVQAQINAEHTTQGVKTIKDDAITSLSRINAQV VEKESARNAIEQKATQQTQFINNNDNATDEEKEVANNLVIA KQKSLDNINSLSSNNDVENAKVAGINEIANVLPATAVSKAK KDIDQKLAQQINQIQTHQTATTEEKEAAIQLANQKSNEARTA IQNEHSNNGVAQAKSNGIHEIELVMPDAHKKSDAKQSIDNKY NEQSNITINTTPDATDEEKQKALDKLKIADAGYNKVDQAQTN QQVSDAKTEAIDTITNIQANVAKKPSARVELDSKFEDLKRQI NATPNATEEEKQDAIQRLNGKRDEVKNLINQDRRDNEVEQHK NIGLQELETIHANPTRKSDALQELQTKFISQTELINNNKD NEEKDEAKRLLEISKNTITNINQAQTNNQVDNAKDNMGNEI ATIIIPATTIKTDAKTAIDKKAEEQVTTINGNNDATDEEKAEA RKLVEKAKIEAKSNITNSDTEREVNGAKTNGLEKINNIQPS QTKTNAKQEIINDKAQEQLIQINNTPDATNEEKQEAATNRVNAG LAQAIQININNAHSTQEVNESKTSIATIKSVQPNVKKPTAI NSLTQEAANNQKTLIGNDGNATDDEKEAAKQLVTQKLNEQIQK IHSTQDNQVDNVKAQAITAIKLINANAHKRQDAINILTNLA ESKKS DIRANQDATTEEKNTAIQSIDDTLAQARNNINGANTN ALVDENLEDGKQKLQRIVLSTQTKTQAKADIAQAIGQQRSTI DQONQATTEEKQEALERLNQETNGVNDRIQAALANQNVTDEK NNILETIRNVEPIVIVKPKANEIRKKAAEQTTLINQNQDAT LEEKQIALGKLEEVKNEALNQVSQAHSNNDVKIVENNGIAKI SEVHPETIIKRNAKQEI EQDAQSQIDTINANNKSTNEEKSA IDRVNVAKIDAINNITNATTTQLVNDAKNSGNTSISQILPST AVKTNALAALASEAKNKNAIIDQTPNATAEEKEEANNKVDRL QEEADANILKAHTTDEVNNIKNQAVQINAVQVEVIKKQNAK NQLNQFIDNQKKIIENTPDATLEEKAEANRLQNVLTSDE IANVDHNNEVDQALDKARPKIEAIVPQVSKKRDALNAIQEAF NSQTQEIQENQEATNEEKTEALNKINQLLNQAKVNIQQAQSN KDVDSAKTRS IQDIEQIQPHPQTKATGRHRLNEKANQQQSTI ATHPNSTIEERQEASAKLQEVLLKAIKIDKGQTNDDVEKTV VNGIAEIEENILPATTVKDKAKADVNAEKEEKNLQINSNDEAT TEEKLVASDNLNHVETTNAIEDAPDTNQVNVEKNKGIGTI RDIQPLVVKKPTAKSKIESAVEKKKTEINQTNATHDEVREG LNQLNQIHEKAKNDVNQSQTNQQVENAEQNSLDQINNFRPDF SKKRNAVAEIVKAQQNKIDEIEQEFSATQEEKDNALQHLDEQ VKEIINSINQANTDNEVDNAKTSGLNNITEYRPEYNKKKNAI LKLYDVSDTQEAIINGYPDATEDELQEANSKLNKILLDAKKQ IGLAHTNNEVDDIYNEVSQMKMTILPRVDTKAVARSVLNALA KQLIKTFENTADVTHEERNDAINHVKEQLSLVFNAIEKDRKD IQVAQDELFLNELNSIFINITQKPTARKAISGMASQLNNSI NNTPYATEEERQIALNKVKAIVDDANEKIREANTDSEVLGTK SNAITLLQAI SADVQVKPQAFEEINAQAEIQREERINGNSDAT REEKEEALKQVDTLVNHSFITTNNVKNQEVYDTKDKTIEAI HKIKPISTIKPQALNEITIQLDQTDRLIKNNKESTVEEKASA IDKLIKTAARIAESIDKAQTNEEVKNIKKQSIDEISKILPVI EIKSAARNEIHQKAEVIRGLINDNEEATKEEKDIALNQLDTT LTQANVSIDQALTNEAVNRAKEIANSEINKISVIAIKKPEAI AEIQELADKKLNKFKQSQEATIEEKQSAINLEQALKSAINH IHQSQNNESVSAALKESISLIDSIEIQAHKKLEAKAYIDGYS DDKINDISSRATNEEKQIFVSKLKALINRTHKQIDEAETFVS </p>
--	--	--	---

		21	VETIVRNFKVEADKLNSIVRKKAKASKEIELEADHVKQMINA NLSASTRVKQNARTLINEIVSNALSQLNKVTNKEVDEIVNE TIEKLKSIQIREDKILSSQRSSTSMTEKSNQCYSENNTIKS LPEAGNADKSLPLAGVTLISGLAIMSSRKKKKDKKVND (40-570aa)
SERP2398	<i>aap</i> (accumulation associated protein)	22	MGKRRQGPINKKVDFLPNKLNKYSIRKFTVGTASILLGSTLI FGSSSHEAKAAEEKQVDPITOANQNDSSERSLENTNQPTVNN <u>EAPQMSSTLQAEEGSNAEAPQSEPTKAEEGGNAEAAQSEPTK</u> <u>AEEGGNAEAPQSEPTKAEEGGNAEAAQSEPTKTEEGSNVCAA</u> <u>QSEPTKAEEGSNAEAPQSEPTKTEEGSNAKAAQSEPTKAEEG</u> <u>GNAEAAQSEPTKTEEGSNAEAPQSEPTKAEEGGNAEAPQSEP</u> <u>TKTEEGGNAEAPNVPTIKANSNDNTOTQFSEAPTRNDLARKE</u> <u>DIPAVSKNEELOSSQPNNTDSKIEPTTSEPVNLNYSFPMSLL</u> <u>SMPADSSSNNTKNTIDIPPTTVKGRDNYDFYGRVDIESNPTD</u> <u>LNATNLTRYNYGQPPGTTTAGAVQFKNOVSFDDKDFENIRVA</u> <u>NNRQSNTTGADGWGFMFSKKDGDDFLKNGGILREKGTPSAAG</u> <u>FRIDTGYNNNDPLDKIQKQAGQGYRGYGTFFVKNDSSQNTSKV</u> <u>GSGTTPSTDFLNYADNTTNDLDGKFHGOGLNNVNLKYNASNOT</u> <u>FTATYAGKTWTATLSELGLSPTDSYNFLVTSSQYGNNGSGTY</u> <u>ASGVMRADLDGATLTYTPKAVDGDPIISTKEIPFNKKREFDP</u> NLAPGTEKVVQKGEPIETTTTPTYVNPNTGEKVGEGETE ITKQPVDEIVHYGGEEIKPGHKDEFDPNAPKGSQTTQPGKPG VKNPDTGEVVTTPVDDVTKYGPVDGDPITSTEEIPFDKKREF NPDLKPGEERVVKQKGEPTGTITTTPTTKNPLTGEKVGEGET EKITKQPVDEITEYGGEEIKPGHKDEFDPNAPKGSQEDVPGK PGVKNPDTGEVVTTPVDDVTKYGPVDGDPITSTEEIPFDKKR EFNPDLKPGEERVVKQKGEPTGTITTTPTTKNPLTGEKVGE PTEKVTKQPVDEITEYGGEEIKPGHKDEFDPNAPKGSQEDVP GKPGVKNPDTGEVVTTPVDDVTKYGPVDGDPITSTEEIPFDK KREFDPNLAPGTEKVVQKGEPTGTITTTPTTKNPLTGEKVGE GETEKTQPVDEIVHYGGEEIKPGHKDEFDPNAPKGSQED VPGKPGVKNPDTGEVVTTPVDDVTKYGPVDGDPITSTEEIPF DKKREFDPNLAPGTEKVVQKGEPTGTITTTPTTKNPLTGEKV GEGETEKTQPVDEIVHYGGEEIKPGHKDEFDPNAPKGSQ EDVPGKPGVKNPDTGEVVTTPVDDVTKYGPVDGDPITSTEEI PFDDKKREFNPDLKPGEERVVKQKGEPTGTITTTPTTKNPLT GEKVGEGETEKTQPVDEITEYGGEEIKPGHKDEFDPNAPKG SQTTPGKPGVKNPDTGEVVTTPVDDVTKYGPVDGDPITSTE EIPFDKKREFNPDLKPGEERVVKQKGEPTGTITTTPTTKNPLT GEKVGEGETEKTQPVDEITEYGGEEIKPGHKDEFDPNAP KGSQEDVPGKPGVKNPDTGEVVTTPVDDVTKYGPVDGDPITS TEEIPFDKKREFNPDLKPGEERVVKQKGEPTGTITTTPTTKNP LTGEKVGEGETEKTQPVDEITEYGGEEIKPGHKDEFDPN APKGSQEDVPGKPGVKNPDTGEVVTTPVDDVTKYGPVDGDP ITSTEEIPFDKKREFDPNLAPGTEKVVQKGEPTGTITTTPTTK NPLTGEKVGEGETEKTQPVDEITEYGGEEIKPGHKDEFDP NAPKGSQEDVPGKPGVKNPDTGEVVTTPVDDVTKYGPVDG DPITSTEEIPFDKKREFDPNLAPGTEKVVQKGEPTGTITTTPT TKNPLTGEKVGEGETEKTQPVDEIVHYGGEEIKTGHKDE FDPNAPKGSQTTQPGKPGVKNPDTGEVVTTPVDDVTKYGPV DGPITSTEEIPFDKKREFDPNLAPGTEKVVQKGEPTGTITTT PTTKNPLTGEKVGEGETEKTQPVDEIVHYGGEEIKPGHK DEFDPNAPKGSQEDVPGKPGVKNPDTGEVVTTPVDDVTKYGP VDGDSITSTEEIPFDKKREFDPNLAPGTEKVVQKGEPTGTIT TTPTTKNPLTGEKVGEGETEKTQPVDEIVHYGGEQIPQG HKDEFDPNAPVDSKTEVPGKPGVKNPDTGEVVTTPVDDVTKY

		23	GPVDGDSITSTEEIPFDKKREFDPNLAPGTEKVVQKGEPTK TITPTTKNPLTGEKVGEGKSTEKVTQPVDEIVEYGPTKAE PGKPAEPGKPAEPGKPAEPGTAEFGKPAEPGTAEFGKPAE PGKPAEPGKPAEPGKPAEPGTAEFGTAEFGKPAEPGTAE PGKPAEPGTAEFGKPAESGKPVETPGTAPQSGAPEQPNRSMH STDNKNQLPDTGENRQANEGTLVGSLLAIVGSLFIFGRRKKG NEK (50-600aa)
SERP0636	<i>atlE</i> (bifunctional autolysin, amidase domain, glucosaminidase domain)	24	MAKKFNYKLPSMVALTLFGTAFTAHQANAAEQPNQSNHKNV LDDQTALKQAEKAKSEVTQSTTNVSGTQTYQDPTQVQPKQDT QSTTYDASLDEMSTYNEISSNQKQQLSTDDANQNQTNVTK NQOETNDLTQEDKTSTDNTNQLQETQSVAKENEKDLGANANN EQQDKKMTASQPSENQAIETQTASNDNESQQKSQQVTSEQNE TATPKVSNTNASGYNFDYDDEDDSSSDHLEPISLNNVNATS KQTTSYKYKEPAQRVTTNTVKKETASNQATIDTKQFTPFSA AQPRTVYSVSSQKTSSLPKYTPKVNSSINNYIRKKNMKAPRI <u>EEDYTSYFPKYGYRNGVGRPEGIVVHDTANDNSTIDGEIAFM</u> <u>KRNYTNAFVHAFFVDGNRIIETAPTDLVSWGAGPYGNQRFINV</u> <u>EIVHTHDYDSFARSMNNYADYAATQLOYYNLKPDSEAENDGRG</u> <u>TVWTHAAISNFLGGTDHADPHOYLRSHNYSYAELYDLIYEKY</u> <u>LIKTKQVAPWGTSTTKPSQPSKPSGGTNKLTVSANRGVAQI</u> <u>KPTNNGLYTTVYDSKGHKTDQVQKTLSTVTKTATLGNNKFYLV</u> <u>EDYNSGKKYGVVKGQDVVYNTAKAPVKVNQTYNVKAGSTLYT</u> <u>VPWGTGPKQVASKVSGTGNQTFKATKQQQIDKATYLYGTVNGK</u> <u>SGWISKYYLTTASKPSNPTKPSTNNQLTVTNNSGVAQINAKN</u> <u>SGLYTTVYDTKGKTTNQIQRTLSTVTKAATLGDKKFYLVGDYN</u> <u>TGTNYGVWKQDEVIYNTAKSPVKINQTYNVKPGVKLHTVPWG</u> <u>TYNQVAGTVSGKGQDTFKATKQQQIDKATYLYGTVNGKSGWI</u> <u>SKYYLTAPSKVQALSTQSTPAPKQVKPSTQTVNQIAQVKANN</u> <u>SGIRASVYDKTAKSGTKYANRTFLINKQRTQGNNTYVLLQDG</u> <u>TSNTPLGWVNINDVTTQNIQKQTSIGKYSVKPTNNGLYSIA</u> <u>WGTKNQQLAPNTLANQAFNASKAVYVVGKDLVLYGTVNNRTG</u> <u>WIAAKDLIQNSTDAQSTPYNYTFVINNSKSYFYMDPTKANRY</u> <u>SLKPYEQTFTVIKQKNINGVKWYYGQLLDGKYVWIKSTDIV</u> <u>KEKIKYAYTGMTLNNAINIQSRLKYKPOVONEPLKWSNANYS</u> <u>QIKNAMDTKRLANDSSLKYQFLRLDQPOYLSAQALNKLKKGK</u> <u>GVLENQGAASFQAARKYGLNEIYLISHALVETGNGTSQOLAKG</u> <u>GDVSKGKFTTKTGHKYHNVFGIGAFDNNALVDGIKYAKNAGW</u> <u>TSVSKAIIIGGAKFIGNSYVKAGQNTLYKMRWNPANPGTHQYA</u> <u>TDINWANVNAQVLKQFYDKIGEVGKYFEIPTYK</u> (302-845aa)
		26	(846-1335aa)
		27	<u>MTKSQQKVSSIEKLSNQEGIIISALAFDQORGALKRMAEHQSE</u> <u>TPTVEQIEQLKVLVSEELTOYASSILLDPEYGLPASDARNND</u> <u>CGLLLAYEKTGYDVNAKGRLPDCLVEWSAKRLKEQGANAVKE</u> <u>LLYYDVDDTEEINIQQKAYIERIGSECVAEIDIPFFLEVLTID</u> <u>DNIIPDNKSAEFAKVKPRKVNEAMKLFSEDRFNVVDLVKEVPV</u> <u>NMNFVEGFSEGEVYVYTKEEAAQHFRDQDAATHLPYIYLSAGV</u> <u>SAELFQDTLKFADHSGAQFNGVLGGRATWSGAVKVYIEEGEQ</u> <u>AAREWLRTVGFKNIDDLNTVLKTTATSWKNK</u> (1-325aa)
SERP1792	<i>lacD</i> (tagatose 1,6- diphosphate aldolase)		

SERP0641	(Fmt protein)	28	<u>MKLNKFKIVFFIIIVLLTLVVSIGILGVEWTRHLELKKOTLSQ</u> <u>ESGNTNYIEKRDKTVEKPKKIKTKYDKKDPTSKSINKYLEKT</u> <u>QFNGTVAVFDNGKVKMNKGYGYODIEKGKKNNTANTMYLIGSA</u> <u>OKFTTGLMLKQLEVENKVNLODSVTKYIPWFKTNKEITIKDL</u> <u>MLHKSGLYKYEASTNIKNLEQAVRAIQARGIDDTVYHKHQYN</u> <u>DANYLVLAQVIENVVTGKPYVKNYERLGNKYNLKHAFYDEK</u> <u>PLQSEMAKGYKFKNNTFSFLKPNILDQYYGAGNLYMTPHDMG</u> <u>KLIYTLQONKIFNARQTRPILHEFGTOEYPEEYRYGFYITPY</u> <u>LNRVNGVFFGQIFTVYFNDRYIVILGTNVSNTPGLVSNEDKM</u> <u>RHIFYNILDQKKPYNTAGVKVE</u>
		29	(30-400aa)
SERP0689	<i>potD</i> (spermidine/ putrescine-binding protein)	30	<u>MKRFFQLIIISAIVIGILCLMISHWFKSKDNTHSNEKLYVYNW</u> <u>GEYIDPSLIKKFKKETGIQVVYETFDSDNEAMEAKIRNGGTHY</u> <u>DVAFPSSEYTVQKLKKAKLLETLNHDKIPNIRNLNDYMNLSY</u> <u>DPNNRYSIPYFFGTGILYDKEKYPNETFDSWDDLYHSQFKN</u> <u>DILLVDGAREIIGMGLNKLGYSLNDKNPTHIHQAEDLHNLA</u> <u>PQVRGIVGDEITMMLQONEGHVAVVWSGVAAPLVQENTRYNY</u> <u>VIPKEGSNLWFDNMVIPKTAQONKEGAYKFMNFFLLDAQNSAQN</u> <u>TEWVGATPNKAARSKLPKKVRNDYRFYPSNOEQORLEVYKD</u> <u>LGOTSLSEYNESFLNFKMSLK</u>
		31	(27-357aa)
SERP2081	(adhesion lipoprotein)	32	<u>MKKLIIVSIIILMLSGCSSFDHRKRESINDKNMKVYTTVYA</u> <u>FQSLTQQIGGKYVDVQSIYPDGADLHSYEPTQKMDIDIAKSD</u> <u>LFVYSSHQLDPVAAKITNSMTNNSMKLALAEGLKQSDFIDSK</u> <u>GHDEDHEHHSHHEESNQDPHVWLDPVLNOKFAFMIKEKLEK</u> <u>DPKHQAYYNKNYKMNKDIVHIDQQLQSITKHSKRDKVVIS</u> <u>DSLGYLAHRYGFGKQGVKGMNDEEPSQKEILNIVKDIQHSHA</u> <u>PYVLYEQNITSKITDVIKKETDTKPLSFHNLAFLTKEEQND</u> <u>SISYQSLMKKNIIYVLNRALNN</u>
		33	(17-315aa)
SERP0679	(conserved hypothetical lipoprotein)	34	<u>MNFKKTVAIVLTSAVLLAGCTIDKKEIKKYDDQVQKAMDQEK</u> <u>TVNQVSKKINELEKKQKLFKKVNDKDQSTRKKAEDIVENV</u> <u>KORQKEFEKEEKALDNSEKAFKQAKOYLEHVENKAKKKEVEQ</u> <u>LDSAIKEKYKSHDAYAKAYKKALNKEKELFSYLNEDNATQSE</u> <u>VDGKSKDLSKAYKEMNNKFNAYSKAIEKVKREKQDQDQK</u>
		35	(20-208aa)
SERP0846	(M16 family peptidase)	36	<u>MKTHQYELIDEKVFEHEFDNGLKLFIIIPKPGFQKTYVTTYTQ</u> <u>FGSLDNHFKPIGSQQFVKVPDGVAFHLEHKLFEKEDEDLFTA</u> <u>FAEENAQANAFTSFDRTSYLSATSNIENIKRLNMYVETPY</u> <u>FTEETVNKEKGIIAEEIKMYQEOPGYKLMFNTLRAMYSKHPI</u> <u>RVDIAGSVESIYEITKDDLYLCYETFYHPSNMVLFVVGDVSP</u> <u>QSI IKLVEKHENQRNKTYQPRIERAQIDEPREINQRFVSEKM</u> <u>KLQSPRLMLGFKNEPLDESATKVFVORDLEMFFYELVFGEET</u> <u>EFYQQLLNKDLIDETFGYQFVLEPSYSFSIITSATQOPDLFK</u> <u>QLIMDELRYKGNLKDQEAFDLLKKQFIGEFISSLNSPEYIA</u> <u>NOYAKLYFEGVSVFDMLDIVENITLESVNETSEFLNFDQLV</u> <u>DSRLEMENR</u> (1-429aa)

SERP2103	<i>dep</i> (gamma-glutamyl- transpeptidase)	37	<u>MNIYNKVTL</u> <u>SVILVITIIISFLFYFYTTEKKVDKDKLYENKI</u> <u>TNHKQAGKPKNYGVASNNKIATKVGNKIIEDGGNAVDAAIGV</u> <u>SYALAVTEPHSSGLGGGGATLTYNKENETPKAYEYKTMSSY</u> <u>EYKEGDKIGVPGFVRGLHDMHAKGKMDDEKKILDYVIPLAKD</u> <u>GFEVDSELERSLKLYGRDIDHNSPFYKGNKSVREGDIVKQDK</u> <u>LANTLTKIKDKGPDYFYEDIGKSVSKQLDNKLTERDFKEFKT</u> <u>EEKEAVSTDYKNNQVYSAPNPLGGTLMLOGLKIDENVDNM</u> <u>DRNNFITAMIKSRDVMYKNRDIVNGNEPSNEOHLTDDYLLGE</u> <u>LNKVNIGENTDNGSDFDQIRTDNTSTTHFVVIDKNGKLASTT</u> <u>NTLSSYFGTGDYMKEGFYMNNSLGDFS</u> <u>SKDKSSPNHGE</u> <u>PHKAP</u> <u>RSFISPSVIVGPNFYMGIGTPGGNKIPTILNEVIVDYLN</u> <u>SDG</u> <u>SLQESINKPRFYNDGGTIFYENAMTDEDINI</u> <u>FKSLGYGVEEK</u> <u>HNDPNFGSVQGA</u> <u>VYDKDKNTVDVGH</u> <u>DVGNR</u>
		38	(27-534aa)

* The digit gene identifier (“GeneID”) provided in **Table 1** refers to the *S. epidermidis* strain RP62A genomic identifier (GenBank Accession Number CP000029.1, RefSeq Accession Number NC_002976.3)

5 ∞ Amino acids underlined with a single line depict a predicted signal sequence that would be
 cleaved upon processing in *S. epidermidis*. Amino acids underlined with a double line depict the
 polypeptides expressed as C-terminal fusions to the following vector-encoded amino acid sequence:
 MH[∞]HHHHHSSGLVPRGSGMKETAAKFERQHMDSPDLGTDDDDKM. The corresponding
 amino acid sequence of the full length mature polypeptide is also identified in brackets (e.g., SEQ ID
 NO.: 2 is amino acids 20 – 318 of SEQ ID NO:1). A person skilled in the art would understand that
 10 these polypeptides could be constructed without the vector-derived sequence and would be able to do
 so without undue experimentation. In such cases, the polypeptide may also include a methionine at
 its N-terminus.

As used herein, a “fragment” of a polypeptide preferably has at least about 40 amino acid residues, or 60 residues, and preferably at least about 100 residues in length. Fragments may represent, for example, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the original sequence (such as e.g., SEQ ID NOs:1 to 38). Exemplary fragments may include peptides of, for example, about 3, 4, 5, or 50 or more consecutive amino acids of the amino acid sequence set out in any of SEQ ID NOs. 1 to 38. Preferred fragments comprise an epitope from any of SEQ ID NOs.1 to 38. More preferred fragments are capable of eliciting an immune response specific for the corresponding full-length mature amino acid sequence. Fragments of staphylococcal polypeptides can be generated by methods known to those skilled in the art.

The polypeptides and fragments thereof, described herein, include (derivatives) variants. Such variants are selected for their immunogenic capacity using methods well known in the art and

may comprise one or more conservative amino acid modifications. Variants of the polypeptides include an amino acid sequence having about 60 to about 99% sequence identity (or any identity in between 60 and 99% identity) to any of the disclosed sequences (i.e., SEQ ID NOs:1 to 38).

Amino acid sequence modifications include substitutional, insertional or deletional changes.

5 Substitutions, deletions, insertions or any combination thereof may be combined in a single derivative so long as the derivative is an immunogenic polypeptide. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Deletions are characterized by the removal of one or
10 more amino acid residues from the protein sequence. Typically no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These derivatives ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in a recombinant cell culture. Alternately, the DNA coding sequence containing the desired mutations may be prepared by
15 chemical synthesis. Techniques for making substitution mutations are predetermined sites in DNA having a known sequence are well known and include, but are not limited to, M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues but can occur at a number of different locations at once. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions may be
20 in accordance with **Table 2**. Others are well known to those of skill in the art.

An amino acid may be substituted with a conservative or non-conservative alternative. The specific amino acid substitution selected may depend on the location of the site selected.

Conservative amino acid substitutions may involve a substitution of a native amino acid residue with a non-native residue such that there is little or no effect on the size, polarity, charge,
25 hydrophobicity, or hydrophilicity of the amino acid residue at that position and, in particular, does not result in decreased immunogenicity. Suitable conservative amino acid substitutions are shown in **Table 2**.

TABLE 2

Original Residues	Exemplary Conservative Substitutions	Preferred Conservative Substitution
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

A non-conservative substitution may include any one or more of the following: from one type of charge to another (e.g., arginine to glutamate); from charge to noncharged (e.g., glutamate to proline); the number of cysteine residues and formation of disulfide bonds (e.g., glutamate to cysteine and threonine to cysteine); from hydrophobic to hydrophilic residues (e.g., alanine to serine); from hydrophilic residues to hydrophobic residues (e.g., aspartate to leucine); size of the amino acid (e.g., glycine to valine); to a conformationally restrictive amino acid or analog (e.g., glutamate to proline); and / or to a non-naturally occurring amino acid or analog.

Structural similarity of two polypeptides can be determined by aligning the residues of the two polypeptides (for example, a candidate polypeptide and the polypeptide of, for example, SEQ ID NO:1) to optimize the number of identical amino acids along the length of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. A candidate polypeptide is the polypeptide being compared to the reference polypeptide. A candidate polypeptide can be isolated, for example, from a microbe, or can be produced using a recombinant techniques, or chemically or enzymatically synthesized.

A pair-wise comparison analysis of amino acids sequences can be carried out using a global algorithm, for example, Needleman-Wunsch. Alternatively, polypeptides may be compared using a

local alignment algorithm such as the Blastp program of the BLAST 2 search algorithm, as described by Tatiana et al., (FEMS Microbiol. Lett, 174, 247-250 (1999), and available on the National Centre for Biotechnology Information (NCBI) website. The Smith and Waterman algorithm is another local alignment tool that can be used (1988).

5 In the comparison of two amino acid sequences, structural similarity may be referred to by percent "identity" or may be referred to by percent "similarity." The term "identity" refers to the presence of identical amino acids whereas the term "similarity" refers to the presences of not only identical amino acid but also the presence of conservative substitutions.

10 Modified polypeptides may also exhibit at least one change in a biological function (e.g., toxicity) compared to the original sequence (e.g., any of SEQ ID NOs. 1-38; **Table 1**). Polypeptides and / or fragments may be produced by direct modification (e.g., using protein synthesis or modification techniques) and / or by modifying a nucleic acid sequence encoding the same and then expressing that modified nucleic acid sequence. For example, modified nucleic acid sequences may be generated using a variety of methods for manipulating nucleic acid sequences including, but not
15 limited to, site-directed mutagenesis, random mutagenesis, conventional mutagenesis, in vitro mutagenesis, spontaneous mutagenesis and chemical synthesis. Methods of mutagenesis can be found in Sambrook et al., A Guide to Molecular Cloning, Cold Spring Harbour, N.Y. (1989) and Sambrook and Russel. Molecular Cloning: A Laboratory Manual (2001), for instance.

20 A skilled artisan will be able to determine suitable derivatives of the polypeptides and /or fragments described and provided herein using well-known techniques. For identifying suitable areas of the molecule that may be changed without destroying biological activity (i.e., MHC (including human HLA) binding, immunogenicity), one skilled in the art may target areas not important for that activity. For example, when polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a polypeptide to
25 such similar polypeptides. By performing such analyses, one can identify residues and portions of the molecules that are conserved. It will be appreciated that changes in areas of the molecule that are not conserved relative to such similar polypeptides may be less likely to adversely affect the biological activity and/or structure of a polypeptide. Similarly, the residues required for binding to MHC may be known (e.g., amino acid residues two and / or nine of a nine amino acid peptide), and may be
30 changed (e.g., substituted) to improve binding to MHC molecules. However, modifications resulting in decreased binding to MHC may not be appropriate in most situations. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining the desired characteristics of the

polypeptide. Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the structure of the polypeptide.

In other embodiments, the polypeptides described herein may include fusion polypeptide segments that assist in purification or detection of the polypeptides. Fusions can be made either at the amino terminus or at the carboxy terminus of the subject polypeptide variant thereof. Fusions may be direct with no linker or adapter molecule or may be through a linker or adapter molecule. A linker or adapter molecule may be one or more amino acid residues, typically from about two to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein. Suitable fusion segments include, among others, metal binding domains (e.g., a poly histidine segment), immunoglobulin binding domains (i.e., Protein A, Protein G, T cell, B cell, Fc receptor, or complement protein antibody binding domains), sugar binding domains (e.g., a maltose binding domain), and/or a "tag" domain (i.e., at least a portion of β -galactosidase, a strepavidin-derived tag peptide, a T7 tag peptide, a FLAG peptide, or other peptides that can be purified using compounds that bind to the domain, such as monoclonal antibodies). This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of the sequence of interest polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified sequence of interest polypeptide by various means such as using certain peptidases for cleavage.

In certain embodiments, the polypeptide may be directly or indirectly labeled (e.g., using an antibody) or tagged in a manner which enables it to be detected. Exemplary labels may include fluorochromes such as fluorescein, rhodamine, phycoerythrin, Europium and Texas Red, chromogenic dyes such as diaminobenzidine, radioisotopes, macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, binding agents such as biotin and digoxigenin, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded, for example in a FACS, ELISA, Western blot, TRFIA, immunohistochemistry, evanescence, Luminex bead array, dipstick and / or other lateral flow assay format. Suitable antibody-binding molecules for use in such methods may include immunoglobulin-binding antibodies, may include, for example, anti-human antibodies, anti-goat antibodies, anti-chicken

antibodies, and / or antibodies specific for protein A or G. Exemplary fluorescent tag proteins may include fluorescent proteins such as green fluorescent protein (GFP), the red-shifted variant EGFP, the cyan shifted variant ECFP, and / or the yellow shifted variant EYFP. EGFP may be particularly suitable as it typically exhibits bright fluorescence combined with minimal effect on the antigenic properties of the target antigen. Alternative fluorescent marker proteins are commercially available. Biologically or chemically active agents include enzymes, which catalyse reactions that develop or change colours or cause changes in electrical properties, for example, and may also be utilized. These may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions and may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed. Further examples include horseradish peroxidase and chemiluminescence.

In some embodiments, a tagged polypeptide may be detected by, for example, immunoprecipitation. The tag may then be detected to determine that protein has been precipitated (qualitative determination) or to determine the amount of protein precipitated (quantitative determination). For example, to identify antibodies against a polypeptide in a bodily fluid for example, a fluorescence-tagged polypeptide may be incubated with such fluid (e.g., patient sera) for an appropriate period of time such as overnight at 4°C (typically 10 - 15µl of serum to 300 - 500µl of extract or less) to allow antibodies to bind thereto. Protein A or Protein G Sepharose beads, pre-incubated with low IgG fetal calf serum (Sigma) to block non-specific binding, are then added to the extract/serum mix containing the tagged protein/antibody complexes, and mixed with gentle rotation for 1 to 2 hours at room temperature. The antibodies within the serum, including those that specifically bind the tagged protein, are bound by the protein A/G beads. The protein A/G Sepharose beads are then washed in a suitable buffer (typically 10 mM Tris-HCl pH 7.4, 100 mM NaCl/ ImM EDTA/ 1% Triton X-100) to remove any unbound tagged protein. This may be achieved by, for example, repeated rounds of centrifugation, removal of the supernatant, and resuspension in buffer. The beads, some with tagged polypeptide attached, are then collected and placed in a fluorescence reader, for example a Spectra Max Gemini XS plate reader from Molecular Devices Inc. The presence of antibodies reactive against the polypeptide in the sample may be quantitated. In the case of GFP, excitation at wavelength 472nm and emission at 512nm may be used. The fluorescence excitation will depend upon the fluorophore/tag that is used but it would be possible to combine several different tagged proteins in the same time. For example, different polypeptides may be separately tagged and separately or simultaneously assayed. The sensitivity of the method is dependent on the detection device and can be considerably enhanced by using more sensitive detection devices. Various modifications of these methods could also be utilized.

The polypeptides of the present disclosure can be produced using standard molecular biology techniques and expression systems (see for example, *Molecular Cloning: A Laboratory Manual*, Third Edition by Sambrook et. al., Cold Spring Harbor Press, 2001). For example, a fragment of a gene that encodes an immunogenic polypeptide may be isolated and the polynucleotide encoding the immunogenic polypeptide may be cloned into any commercially available expression vector (such as, e.g., pBR322, and pUC vectors (New England Biolabs, Inc., Ipswich, MA)) or expression/purification vectors (such as e.g., GST fusion vectors (Pfizer, Inc., Piscataway, N.J.)) and then expressed in a suitable prokaryotic, viral or eukaryotic host. Purification may then be achieved by conventional means, or in the case of a commercial expression/purification system, in accordance with manufacturer's instructions.

Alternatively, the polypeptides, including fragments or derivatives thereof, may be isolated for example, but without limitation, from wild-type or mutant *S. epidermidis* cells, and through chemical synthesization using commercially automated procedures, such as for example, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or solution synthesis.

Polypeptides of the present disclosure preferably have immunogenic activity. The term "immunogenic activity" refers to the ability of a polypeptide to elicit an immunological response in a subject. An immunological response to a polypeptide is the development in a subject of a cellular and / or antibody-mediated immune response to the polypeptide. Usually, an immunological response includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, suppressor T cells and / or cytotoxic T cells, directed to an epitope or epitopes of the polypeptide. The term "epitope" refers to the site on an antigen to which specific B cells and / or T cells respond so that antibody is produced. The immunogenic activity may be protective. The term "protective immunogenic activity" refers to the ability of a polypeptide, or a fragment or a derivative thereof to elicit an immunological response in a subject that prevents or inhibits a symptomatic staphylococcal infection (i.e., a staphylococcal infection resulting in disease).

Nucleic acids encoding the polypeptides may be isolated for example, but without limitation from wild type or mutant staphylococcal (e.g., *S. epidermidis*) cells or alternatively, may be obtained directly from the DNA of an staphylococcal strain carrying the applicable DNA gene (e.g., *PhnD*), by using the polymerase chain reaction (PCR) or by using alternative standard techniques that are recognized by one skilled in the art. Possible strains of use include for example, *S. epidermidis* and *S. aureus* strains. In preferred embodiments the polypeptides are recombinantly derived from a *S. epidermidis* strain (e.g., clinical isolates RP62A, 1457 or 7291).

Binding Molecules (e.g., Aptamers, Antibodies, Antibody Mimics)

This disclosure further relates to binding molecules (or agents) capable of binding to the polypeptides described herein (e.g., SEQ ID NOs. 1-38; **Table 1**) or fragments or derivatives thereof. Preferred binding molecules include those that specifically bind to a SERP2286 (PhnD), SERP0237, SERP1891, SERP0207, SERP1011, or SERP2288 polypeptide and fragments and derivatives thereof. In some examples, such binding molecules bind staphylococcal microorganisms (e.g., *S. epidermidis*) or a polypeptide and/or factor expressed thereby to inhibit and/or otherwise affect staphylococcal biofilm development by, for example, inhibiting biofilm formation and/or inhibiting further growth or expansion of a biofilm after initial seeding and/or inhibiting, reducing or eliminating metastatic spread of a biofilm.

Binding molecules may include, but are not limited to: oligonucleotide aptamers (such as e.g., DNA or RNA aptamer); peptide aptamers; antibodies and antibody mimetics, that specifically bind to polypeptides described herein (e.g., SEQ ID NOs. 1-38; **Table 1**) or fragments or derivatives thereof. Antibodies and antibody mimetics may include, but are not limited to polyclonal antibodies, monoclonal antibodies, humanized antibodies, hybrid antibodies, chimeric antibodies, antibody fragments, single chain antibodies, nanobodies, unibodies, affibodies, DARPs, anticalins, adnectins and avimers.

In some examples, such binding molecules may be and/or comprise antibodies, against staphylococcal species (e.g., *S. epidermidis* and/or *S. aureus*) or a polypeptide expressed thereby. The term "antibody" or "antibodies" includes whole or fragmented antibodies, or derivatives thereof, in unpurified or partially purified form (e.g., hybridoma supernatant, ascites, polyclonal antisera) or in purified form. A "purified" antibody is one that is separated from at least about 50% of the proteins with which it is initially found (i.e., as part of a hybridoma supernatant or ascites preparation). Preferably, a purified antibody is separated from at least about 60%, 75%, 90%, or 95% of the proteins with which it is initially found. Suitable derivatives may include, for example, Fab, Fab2, Fab' single chain antibody, Fv, single domain antibody, mono-specific antibody, bi-specific antibody, tri-specific antibody, multivalent antibody, chimeric antibody, and / or humanized antibodies, as are known in the art. The antibodies may be of any suitable origin or form including, for example, murine (i.e., produced by murine hybridoma cells), or expressed as humanized antibodies, chimeric antibodies, human antibodies, and the like. In some embodiments, the antibodies may be neutralizing and/or protective against one or more *Staphylococcus species* (e.g., *S. epidermidis* and/or, *S. aureus*). In some embodiments, such antibodies may be generated using the polypeptides described herein such as for example, any one of SEQ ID NOs. 1-38, (or, as described

above, fragments and/or derivatives thereof). The antibodies may elicit either or both active and passive immunity. The polypeptides (or fragments, or derivatives thereof) may also be used to identify and isolate antibodies, which may be protective and/or neutralizing, that are cross-reactive with those elicited by the polypeptides (or fragments, or derivatives thereof).

Also provided are methods for eliciting the production of antibodies, which may be protective and / or neutralizing, and / or may be reactive to the polypeptides (e.g., SEQ ID NOs. 1-38; **Table 1**). Methods of preparing and utilizing various types of antibodies are well-known to those of skill in the art and would be suitable for use (see, for example, Harlow, et al. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988; Harlow, et al. *Using Antibodies: A Laboratory Manual*, Portable Protocol No. 1, 1998; Kohler and Milstein, *Nature*, 256:495 (1975)); Jones et al. *Nature*, 321:522-525 (1986); Riechmann et al. *Nature*, 332:323-329 (1988); Presta (*Curr. Op. Struct. Biol.*, 2:593-596 (1992); Verhoeyen et al. (*Science*, 239:1534-1536 (1988); Hoogenboom et al., *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991); Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J. Immunol.*, 147(1):86-95 (1991); Marks et al., *Bio/Technology* 10, 779-783 (1992); Lonberg et al., *Nature* 368 856-859 (1994); Morrison, *Nature* 368 812-13 (1994); Fishwild et al., *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14, 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13 65-93 (1995); as well as U.S. Pat. Nos. 4,816,567; 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and, 5,661,016). In certain applications, the antibodies may be contained within hybridoma supernatant or ascites and utilized either directly as such or following concentration using standard techniques. In other applications, the antibodies may be further purified using, for example, salt fractionation and ion exchange chromatography, or affinity chromatography using Protein A, Protein G, Protein A/G, and / or Protein L ligands covalently coupled to a solid support such as agarose beads, or combinations of these techniques. The antibodies may be stored in any suitable format, including as a frozen preparation (i.e., about -20°C or -70°C), in lyophilized form, or under normal refrigeration conditions (i.e., about 4°C). When stored in liquid form, it is preferred that a suitable buffer such as Tris-buffered saline (TBS) or phosphate buffered saline (PBS) is utilized. Antibodies and their derivatives may be incorporated into compositions and/or formulated as pharmaceutical formulations as described herein for use *in vitro* or *in vivo*. Other methods for making and using antibodies available to one of skill in the art may also be suitable for use.

Binding molecules (e.g., antibodies and their derivatives) may be incorporated into compositions and/or formulated as pharmaceutical formulations as described herein for use *in vitro* or

in vivo. In certain examples, binding molecules (e.g., antibodies) may be administered by intravenous infusion as a solution in sterile physiological saline.

Binding molecules (e.g., antibodies) may be administered to target a single staphylococcal polypeptide including any of SEQ ID NOs: 1 to 38 or fragments or derivatives thereof. Binding molecules may also be administered to target two, three or more staphylococcal polypeptides. The one or more binding molecules are administered in appropriate doses to achieve the desired concentration of molecules in circulation, which depends on the body weight of the recipient. The amounts of each binding molecule (e.g. monoclonal antibody) can be administered in a range of doses of about 5 mg/kg of body weight to 150 mg/kg of body weight. Preferably the dose range for each specific binding molecule is about 5 or 6 mg/kg of body weight to 50 mg/kg of body weight.

In certain examples, binding molecules (e.g., antibodies) may be used to provide passive protection. For instance, the binding molecules (e.g., antibodies) may be administered (e.g., with a pharmaceutically acceptable carrier such as saline) to inhibit and/or otherwise affect staphylococcal biofilm development by, for example, inhibiting biofilm formation and/or inhibiting further growth or expansion of a biofilm after initial seeding and/or inhibiting, reducing or eliminating metastatic spread of a biofilm. In some cases, the antibodies administered may reduce or inhibit the lethality of the applicable staphylococcal species.

Compositions

The disclosed polypeptides may be used to produce immunogenic compositions such as, for example, vaccine compositions. An immunogenic composition is one that, upon administration to a subject (e.g., a mammal), induces or enhances an immune response directed against the antigen and/or immunogen contained within the composition. This response may include the generation of antibodies (e.g, through the stimulation of B cells) or a T cell-based response (e.g., a cytolytic response). These responses may or may not be protective or neutralizing. A protective or neutralizing immune response is one that is detrimental to the corresponding infectious organism (e.g, from which the antigen or immunogen was derived) and beneficial to the subject (e.g., by reducing or preventing symptomatic infection). As used herein, protective or neutralizing antibodies may be reactive to the corresponding wild-type staphylococcal polypeptide (or fragment thereof) and may reduce or inhibit the lethality of the corresponding wild-type staphylococcal *species* when tested in animals. An immunogenic composition that, upon administration to a host, results in a protective or neutralizing immune response may be considered a vaccine.

The immunogenic compositions may include one or more staphylococcal polypeptides including any of SEQ NOs: 1 to 38 or fragments or derivatives thereof. The immunogenic compositions may comprise an effective amount of at least one or more isolated polypeptides including any of SEQ ID NOs: 1 to 38 or fragments or derivatives thereof. Exemplary compositions are described in the examples. The one or more polypeptides or binding molecules are included in effective amounts (i.e., sufficient to elicit an immune response) when administered to a subject.

In compositions that are comprised of two, three or more polypeptides, the polypeptide components are preferably compatible and are combined in appropriate ratios to avoid antigenic interference and to optimize any possible synergies. For example, the amounts of each component can be in the range of about 5 µg to about 500 µg per dose, 5 µg to about 100 µg per dose; or 25 µg to about 50 µg per dose. Preferably the range can be 5 or 6 µg to 50 µg per antigenic component per dose.

In certain embodiments, the compositions include one, two or more polypeptides selected from the group consisting of SEQ ID NOs: 1 – 38, or a fragment or a derivative thereof. These polypeptides may optionally be used in combination with *Staphylococcus* saccharides or other staphylococcal polypeptides.

Compositions may be administered by an appropriate route such as for example, percutaneous (e.g., intramuscular, intravenous, intraperitoneal or subcutaneous), transdermal, mucosal (e.g., intranasal) or topical, in amounts and in regimes determined to be appropriate by those skilled in the art. For example, 1– 250 µg or 10-100 µg of the composition can be administered. For the purposes of prophylaxis or therapy, the composition can be administered 1, 2, 3, 4 or more times. In one example, the one or more administrations may occur as part of a “prime-boost” protocol. When multiple doses are administered, the doses can be separated from one another by, for example, one week, one month or several months.

Compositions (e.g., vaccine compositions) may be administered in the presence or absence of an adjuvant. Adjuvants generally are substances that can enhance the immunogenicity of antigens. Adjuvants may play a role in both acquired and innate immunity (e.g., toll-like receptors) and may function in a variety of ways, not all of which are understood.

Many substances, both natural and synthetic, have been shown to function as adjuvants. For example, adjuvants may include, but are not limited to, mineral salts, squalene mixtures, muramyl peptide, saponin derivatives, mycobacterium cell wall preparations, certain emulsions, monophosphoryl lipid A, mycolic acid derivatives, nonionic block copolymer surfactants, Quil A,

cholera toxin B subunit, polyphosphazene and derivatives, immunostimulating complexes (ISCOMs), cytokine adjuvants, MF59 adjuvant, lipid adjuvants, mucosal adjuvants, certain bacterial exotoxins and other components, certain oligonucleotides, PLG, and others. These adjuvants may be used in the compositions and methods described herein.

5 In certain embodiments, the composition is administered in the presence of an adjuvant that comprises an oil-in-water emulsion comprising at least squalene, an aqueous solvent, a polyoxyethylene alkyl ether hydrophilic nonionic surfactant, a hydrophobic nonionic surfactant, wherein said oil-in-water emulsion is obtainable by a phase inversion temperature process and wherein 90% of the population by volume of the oil drops has a size less than 200 nm, and optionally
10 less than 150 nm. Such an adjuvant is described in WO2007006939 (Vaccine Composition Comprising a Thermoinversable Emulsion) which is incorporated herein in its entirety. The composition may also include the product E6020 (having CAS Number 287180-63-6), in addition to, or instead of the described squalene oil-in-water emulsion. Product E6020 is described in US2007/0082875 (which is incorporated herein by reference in its entirety).

15 In certain embodiments, the composition includes a TLR agonist (*e.g.*, TLR4 agonist) alone or together in combination with an adjuvant. For example, the adjuvant may comprise a TLR4 agonist (*e.g.*, TLA4), squalene, an aqueous solvent, a nonionic hydrophilic surfactant belonging to the polyoxyethylene alkyl ether chemical group, a nonionic hydrophobic surfactant and which is thermoreversible. Examples of such adjuvants are described in WO2007080308 (Thermoreversible
20 Oil-in-Water Emulsion) which is incorporated herein in its entirety. In one embodiment, the composition is adjuvanted with a combination of CpG and an aluminum salt adjuvant (*e.g.*, Alum).

Aluminum salt adjuvants (or compounds) are among the adjuvants of use in the practice of the disclosure. Examples of aluminum salt adjuvants of use include aluminum hydroxide (*e.g.*, crystalline aluminum oxyhydroxide $\text{AlO}(\text{OH})$, and aluminum hydroxide $\text{Al}(\text{OH})_3$). Aluminum
25 hydroxide is an aluminum compound comprising Al^{3+} ions and hydroxyl groups ($-\text{OH}$). Mixtures of aluminum hydroxide with other aluminum compounds (*e.g.*, hydroxyphosphate or hydroxysulfate) may also be of use where the resulting mixture is an aluminum compound comprising hydroxyl groups. In particular embodiments, the aluminum adjuvant is aluminum oxyhydroxide (*e.g.*, Alhydrogel[®]). The polypeptides (individually or in combination) may be adsorbed to the aluminum
30 compound. It is well known in the art that compositions with aluminum salt adjuvants should not be exposed to extreme temperatures, *i.e.* below freezing (0°C) or extreme heat (*e.g.*, $\geq 70^\circ\text{C}$) as such exposure may adversely affect the stability and the immunogenicity of both the antigen and adjuvant.

In one embodiment, polypeptides and/or derivatives and/or fragments thereof may be covalently coupled to bacterial polysaccharides to form polysaccharide conjugates. Such conjugates may be useful as immunogens for eliciting a T cell dependent immunogenic response directed against the bacterial polysaccharide conjugated to the polypeptides and/or derivatives and/or fragments thereof.

The compositions may be in liquid form, or lyophilized (as per standard methods) or foam dried (as described e.g., in WO2009012601, Antigen-Adjuvant Compositions and Methods). Liquid formulations may be in any form suitable for administration including for example, a solution, or suspension and may include a liquid medium (e.g., saline or water), which may be buffered. In certain embodiments, an immunization dose may be formulated in a volume of between 0.5 and 1.0 ml.

The pH of the formulation is preferably between about 6.4 and about 8.4. More preferably, the pH is about 7.4. An exemplary pH range of the compositions is 5-10, e.g., 5-9, 5-8, 5.5-9, 6-7.5, or 6.5-7. The pH may be maintained by the use of a buffer.

Pharmaceutical formulations may also optionally include one or more excipients (e.g., pharmaceutically acceptable excipient) (e.g., diluents, thickeners, buffers, preservatives, surface active agents, adjuvants, detergents and/or immunostimulants) which are well known in the art. Suitable excipients will be compatible with the antigen and with the adjuvant (in adjuvanted compositions) as is known in the art. Examples of diluents include binder, disintegrants, or dispersants such as starch, cellulose derivatives, phenol, polyethylene glycol, propylene glycol or glycerin. Pharmaceutical formulations may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents and anesthetics. Examples of detergents include Tween (polysorbate) such as Tween 80. Suitable excipients for inclusion in compositions are known in the art.

Compositions (e.g., immunogenic compositions, binding molecule formulations) may be presented in a kit form. Such kits may include, in addition to the composition, instructions for use and/or the device for administration of the composition (e.g. hypodermic syringe, microneedle array). The kit may comprise an immunogenic composition and an adjuvant or a reconstitution solution comprising one or more pharmaceutically acceptable diluents to facilitate reconstitution of the composition for administration to a subject (e.g., a mammal) using conventional or other devices. Such a kit would optionally include a device for administration of a liquid form of the composition (e.g., hypodermic syringe, microneedle array) and/or instructions for use.

The compositions and vaccines disclosed herein may also be incorporated into various delivery systems. In one example, the compositions may be applied to a "microneedle array" or "microneedle patch" delivery system for administration. These microneedle arrays or patches generally comprise a plurality of needle-like projections attached to a backing material and coated with a dried form of a vaccine. When applied to the skin of a mammal, the needle-like projections pierce the skin and achieve delivery of the vaccine, effecting immunization of the subject mammal.

Methods

The polypeptides, binding molecules and compositions provided herein find use in methods of eliciting an immune response in a subject by administering the polypeptides, binding molecules, compositions, or formulations thereof, to subjects. In preferred embodiments the immune response elicited in a subject inhibits development of a *Staphylococcus* biofilm (e.g., on an indwelling medical device) and in some cases may treat an existing *Staphylococcus* biofilm. This may be achieved by the administration of a pharmaceutically acceptable formulation of the polypeptides, binding molecules or compositions to the subject to effect exposure of the polypeptide, binding molecule and/or composition to the immune system of the subject. The administrations may occur once or may occur multiple times.

The pharmaceutically acceptable formulation administered includes the applicable polypeptides, binding molecules, or compositions in therapeutically effective amounts. A therapeutically effective amount refers to an amount that provides a therapeutic effect for a given condition and administration regimen. A therapeutically effective amount can be determined by the ordinary skilled medical worker based on patient characteristics (age, weight, gender, condition, complications other diseases etc.). The therapeutically effective amount will be further influenced by the route of administration.

Methods provided include methods of treating and/or inhibiting development of a *Staphylococcus* biofilm (e.g., on an indwelling medical device) in a subject. In one aspect, these methods involve administering to a subject in need thereof an effective amount of a Staphylococcal polypeptide selected from the group consisting of SEQ ID NOs: 1-38, or a fragment or a derivative thereof, or a binding molecule that specifically binds to any of these, to induce an immune response against *Staphylococcus*.

At the time of administration, the subject may already have or may be at risk of developing a symptomatic staphylococcal infection. For example, in some embodiments, at the time of administration the subject may have received an indwelling medical device; in others, the subject

may be scheduled to receive such a device. Administration may be performed sometime prior to the implantation of the device, in conjunction with implantation or sometime following implantation. In some embodiments, administration is performed 1, 2, or 3 hours before or after implantation.

The polypeptides, binding molecules, and compositions also find use in methods of preventing or treating or reducing the risk of a disease or symptoms associated with, or resulting from, a staphylococcal infection. The terms disease, disorder and condition are used interchangeably herein. The prophylactic and therapeutic methods involve administration of one or more of the disclosed polypeptides in a therapeutically effective amount in, for example, carrying out the treatment itself, or in preventing subsequent symptomatic infection.

As used herein, preventing a disease is intended to mean protecting the subject from the development of the particular disease associated with, or resulting from, a staphylococcal infection. Similarly, treating a disease is intended to mean administration to a subject that is afflicted with a disease caused by *Staphylococcus* or that has been exposed to *Staphylococcus* where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the condition or the symptoms of the disease. Diseases associated with, or resulting from, infection by *Staphylococcus* include, for example, but are not limited to, prosthetic valve endocarditis, keratitis, bacteriuria, intravascular catheter associated infection, prosthesis-related infection including septic loosening of joint prostheses following joint arthroplasty, and post-operative endophthalmitis (associated with intraocular lens implantation), bacteremia and sepsis. The risk of any one or more of these diseases may be reduced by the methods described herein. One example provides a method of reducing the risk of bacteremia and/or sepsis in a subject comprising administering to the subject an immunogenic composition comprising an effective amount of a staphylococcal polypeptide selected from the group consisting of SEQ ID NOs: 1-38, or a fragment or a derivative thereof, or a binding molecule that specifically binds to any of these. In other preferred methods, the composition comprises an effective amount of a PhnD polypeptide (e.g., SEQ ID NOs:1 or 2), or a fragment or a derivative thereof, or a binding molecule that specifically binds to any of these.

In some embodiments the subject may have a symptomatic staphylococcal infection. For example, the subject may have been diagnosed with a primary staphylococcal infection (manifesting as a *Staphylococcus* biofilm) and may, therefore, be at risk of metastasis or further spread of the bacteria within the biofilm (i.e., a secondary staphylococcal infection). In these subjects, there may be a risk of a biofilm forming on an indwelling device or an organ. Metastasis or spread of bacteria from a biofilm to the heart may result in endocarditis should a *Staphylococcus* biofilm form on a native or prosthetic heart valve. Similarly, metastasis or spread of bacteria from a biofilm to a

prosthetic joint may result in the premature prosthetic joint loosening or failure should a *Staphylococcus* biofilm form on the prosthetic joint.

Assays, Methods for the Evaluation of Materials (e.g., Polypeptides, Binding Molecules, Compositions)

5 The properties (e.g., immunogenicity) of any of the materials (e.g., polypeptides, binding molecules, compositions) including the suitability of the material for an intended purpose (such as e.g., in inducing a protective immune response) may be evaluated using the assays and/or animal models described herein (such as e.g., in the Examples) and/or using any other suitable assay or animal model known to those of skill in the art. It is to be understood that the methods (assays,
10 animal models) described herein are exemplary and non-limiting; other methods may also be suitable.

 In certain embodiments, a polypeptide or binding molecule preferably exhibits certain immunogenic properties (e.g., induces neutralizing and/or protective immune responses following administration to a subject), either alone or as part of an immunogenic composition (e.g., vaccine). The ability of a polypeptide or binding molecule (alone or as part of an immunogenic composition) to
15 elicit a neutralizing and/or protective immune response may be demonstrated by showing that an infection by *Staphylococcus* is affected (e.g., reduced) in subjects to whom it is administered in comparison to subjects to whom it is not. Suitable animal models that may be used to make such a determination may include, for example, the sub-lethal mouse model of infection described in the Examples. As described in the Examples, one or more test animals (e.g., mouse) may be
20 administered (e.g., subcutaneously, intravenously, intramuscularly, intradermally, intranodally, intranasally) a polypeptide or binding molecule described herein, and following a suitable amount of time (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks) challenged by a pathogenic *Staphylococcus* species (e.g., *S. epidermidis*). Following challenge, infection (measured as CFU per spleen) in all mouse groups is assessed and protection is determined by comparing the fold reduction in CFU per
25 spleen in immunized group(s) to that of the placebo control. The data may be analysed statistically (e.g., using Fisher's exact test, Wilcoxon test, Mann-Whitney Test) to determine efficacy.

 The ability of a polypeptide or binding molecule (alone or as part of an immunogenic composition) to elicit antibodies that inhibit *Staphylococcus* biofilm development may be evaluated using one or more *in vitro* assays such as those described in the Examples. For example, polyclonal
30 antibodies specific against a polypeptide may be raised in an appropriate animal species (e.g., mouse, rabbit, human) and then evaluated for antagonistic effects against biofilm formation using cell-based assays which examine *in vitro* biofilm formation under, e.g., static growth conditions and/or

conditions of shear flow mimicing the flow conditions experienced by staphylococcal (*e.g.*, *S. epidermidis*, *S. aureus*) cells on catheters or other implanted devices, such as described in the Examples.

Definitions

5 The term “antigen” as used herein refers to a substance that is capable of initiating and mediating the formation of a corresponding immune body (antibody) when introduced into a subject or can be bound by a major histocompatibility complex (MHC) and presented to a T-cell. An antigen may possess multiple antigenic determinants such that the exposure of the subject to an antigen may produce a plurality of corresponding antibodies with differing specificities. Antigens may include,
10 but are not limited to proteins, peptides, polypeptides, nucleic acids and fragments, derivatives (variants) and combinations thereof.

The term “immunogen” is a substance that is able to induce an immune response.

The terms peptides, proteins and polypeptides are used interchangeably herein.

An “isolated” polypeptide is one that has been removed from its natural environment. For
15 instance, an isolated polypeptide is a polypeptide that has been removed from the cytoplasm or from the membrane of a cell, and many of the polypeptides, nucleic acids, and other cellular material of its natural environment are no longer present. An “isolatable” polypeptide is a polypeptide that could be isolated from a particular source. A “purified” polypeptide is one that is at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which
20 they are naturally associated. Polypeptides that are produced outside the organism in which they naturally occur, *e.g.* through chemical or recombinant means, are considered to be isolated and purified by definition, since they were never present in a natural environment.

As used in the specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to
25 a fragment may include mixtures of fragments and reference to a pharmaceutical carrier or adjuvant may include mixtures of two or more such carriers or adjuvants.

As used herein, a subject is meant to be an individual mammal (*e.g.*, human).

Optional or optionally means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and
30 instances where it does not. For example, the phrase, “optionally the composition can comprise a combination” means that the composition may comprise a combination of different molecules or may

not include a combination such that the description includes both the combination and the absence of the combination (i.e., individual members of the combination).

Ranges may be expressed herein as from about one particular value, and/or to about another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent about or approximately, it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

When the terms prevent, preventing, and prevention are used herein in connection with a given treatment for a given condition (e.g., preventing *S. epidermidis* infection), it is meant to convey that the treated subject either does not develop a clinically observable level of the condition at all, or develops it more slowly and/or to a lesser degree than he/she would have absent the treatment. These terms are not limited solely to a situation in which the subject experiences no aspect of the condition whatsoever. For example, a treatment will be said to have prevented the condition if it is given during exposure of a patient to a stimulus that would have been expected to produce a given manifestation of the condition, and results in the subject's experiencing fewer and/or milder symptoms of the condition than otherwise expected. A treatment can "prevent" infection by resulting in the subject's displaying only mild overt symptoms of the infection; it does not imply that there must have been no penetration of any cell by the infecting microorganism.

Similarly, reduce, reducing, and reduction as used herein in connection with the risk of infection with a given treatment (e.g., reducing the risk of a *S. epidermidis* infection) refers to a subject developing an infection more slowly or to a lesser degree as compared to a control or basal level of developing an infection in the absence of a treatment (e.g., administration of an immunogenic polypeptide). A reduction in the risk of infection may result in the subject displaying only mild overt symptoms of the infection or delayed symptoms of infection; it does not imply that there must have been no penetration of any cell by the infecting microorganism.

All references cited within this disclosure are hereby incorporated by reference in their entirety.

EXAMPLES

The following examples are provided solely for purposes of illustration and are not intended to limit the scope of the disclosure. Changes in form and substitution of equivalents are contemplated

as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry, and immunology used, but not explicitly described in this disclosure and these Examples, are amply reported in the scientific literatures and are well within the ability of those skilled in the art

Example 1

The published genome sequence of *S. epidermidis* strain RP62A was screened to identify antigens of interest (GenBank Accession Number CP000029.1, RefSeq Accession Number NC_002976.3). In brief, predicted protein sequences were triaged using a suite of bioinformatics tools to predict subcellular localization, physical properties (e.g. molecular weight, isoelectric point, domain architecture), prevalence and sequence conservation, human sequence similarity, and other relevant data. An initial short list of 150 antigens was generated. The genes encoding these antigens (or fragments thereof) were PCR-amplified from the *S. epidermidis* strain RP62A genome using a high-fidelity polymerase and primers designed for ligation independent cloning into pET-30 Ek/LIC (a T7 promoter-based expression plasmid designed for high-level production of 6xHis-tagged fusion proteins; Novagen). Sequences encoding predicted signal peptides were not cloned since these are processed and removed from the mature antigens. For some very large (i.e. MW>100 kDa) or multidomain proteins, gene fragments were cloned in place of the complete open reading frames. Cloned inserts were verified by DNA sequencing, and then tested for expression in *E. coli* strain BL21(DE3) (Novagen) using Overnight Express™ Autoinduction System 1 medium (Novagen). Purifications for each of the expressed polypeptides were then attempted by Ni-NTA affinity chromatography following conventional protocols (Qiagen); 84 proteins were successfully isolated in this fashion. A number of these proteins (and antisera to each) were evaluated in the studies as described further in this example and in examples 3 to 5). The amino acid sequence of the expressed constructs identified in these examples are set out in **Table 1** (e.g., SEQ ID NOs: 2, 4, 6, 8, 9, 10, 11, 12, 14, 16, 19, 21, 23, 25, 26, 29, 31, 33, 35, 38).

Antisera to individual purified proteins were generated in mice using TiterMax Gold adjuvant. In brief, protein at a concentration of 1 mg/mL in buffer (50 mM phosphate pH 8.0, 300 mM NaCl, 250 mM imidazole) was emulsified with an equal volume of TiterMax Gold, then 100 microliters of

emulsion was injected intramuscularly on day 0 followed by a 50 microliter boost of the same (freshly-prepared) on day 28. Terminal bleeds were performed under anesthesia on day 49. All protocols were approved by the institutional Animal Care Committee. Confirmation of a specific antibody response in the sera was determined by western immunoblotting against the corresponding purified immunogen. The resulting antisera were used to assess whether antigens were accessible to antibody binding on the surface of intact cells using a flow cytometry-based assay.

Flow Cytometry-Based Surface Accessibility Assay

Surface accessibility assays were performed to determine the ability of antibodies in hyperimmune mouse sera to bind live cells of *S. epidermidis*. In brief, cultures of *S. epidermidis* (clinical isolate RP62A) were grown from frozen stocks to an OD600 of 0.3–0.4 in tryptic soy broth medium. Bacteria were harvested by centrifugation, washed twice in PBS, and then resuspended in PBS to 5 percent of the original culture volume. Aliquots of the washed and concentrated cells were mixed in a 2:1 ratio with undiluted antisera and incubated for 30 minutes to allow antibody binding. Hyperimmune antiserum raised against an irrelevant (i.e. non-*S. epidermidis*) antigen was used as a negative control. Antibody bound to bacteria was detected using a secondary antibody (FITC-conjugated goat anti-mouse IgG) and evaluated using flow cytometry. Bacteria were scored positive when a fluorescent signal was detected with the cells, and data are reported as the percentage of cells positive in the population as a whole.

Thirteen proteins were identified as positive in this assay (i.e., antigen-specific antibodies bound to the surface of live *S. epidermidis*). Results are set out in **Table 3**. In relation to each of the thirteen proteins, these data indicate that the corresponding native protein is surface accessible and that the antibodies raised against the recombinant protein recognize native protein all of which confirms the candidacy of each as a vaccine component.

TABLE 3

Genes encoding proteins that can be detected on the surface of intact cells of S. epidermidis using antigen-specific antiserum.

GENE ID*	% Cells Positive for Surface Accessibility	Gene Name (Annotation)
SERP1316	20%	(cell wall surface anchor family protein)
SERP2398	89%	<i>aap</i> (accumulation associated protein)
SERP0636	32%	<i>atlE</i> (bifunctional autolysin, glucosaminidase domain)
SERP0636	25%	<i>atlE</i> (bifunctional autolysin, amidase domain)

SERP0442	35%	<i>gapA-1</i> (glyceraldehyde 3-phosphate dehydrogenase)
SERP1792	19%	<i>lacD</i> (tagatose 1,6-diphosphate aldolase)
SERP0641	15%	(fmt protein)
SERP2252	15%	<i>sepA</i> (extracellular elastase precursor)
SERP0689	15%	<i>potD</i> (spermidine/putrescine-binding protein)
SERP2081	15%	(adhesion lipoprotein)
SERP0679	15%	(conserved hypothetical lipoprotein)
SERP0237	17%	(lipoate-protein ligase A)
SERP0846	16%	(M16 family peptidase)
SERP2103	16%	<i>dep</i> (gamma-glutamyltranspeptidase)

*The 4 digit gene identifier provided in **Table 3** refers to the *S. epidermidis* strain RP62A genomic identifier (GenBank Accession Number CP000029.1, RefSeq Accession Number NC_002976.3)

EXAMPLE 2

Enhanced Bacterial Clearance Following Challenge with *S. epidermidis*

5 This example describes the protective capacity of a number of proteins (and corresponding formulations/compositions) against challenge with *S. epidermidis* in a mouse sub-lethal infection model.

Purified proteins were evaluated for *in vivo* efficacy using a mouse model of infection. The mouse model differs from those previously described, both in the route of challenge (IV) and in the preparation of the challenge inoculum (cells from a freshly-dispersed biofilm). These changes were implemented to better reproduce the scenario of bloodstream infections that occur in hospital patients with indwelling catheters contaminated with *S. epidermidis* biofilms, where it is predicted that bacteria enter the bloodstream directly as they disseminate from the biofilm nidus. In this model, groups of female BALB/c mice (N = 7 per group) were immunized subcutaneously with a composition containing 10 µg/dose of purified recombinant protein formulated in Alhydrogel (final aluminum content = 2 mg/mL). The injection volume was 100 µL per dose. Adjuvant mixed with PBS was administered as a negative control. Animals were immunized on days 0, 21, and 35. On day 42, the animals were challenged intravenously via the tail vein with 200 µL of PBS containing approximately 1×10^8 CFU of *S. epidermidis* strain RP62A prepared from a freshly-dispersed biofilm. Sample bleeds were taken from all animals on day 0 (prior to the first immunization), on day 28 (1 week post second immunization), and then again on day 41 (one day before challenge). Sera were analyzed for total protein-specific IgG response by means of an antibody ELISA assay. Exactly 24 hours following challenge, mice were euthanized and their spleens were harvested into ice-cold PBS. Serial dilutions of the spleen homogenates were plated on tryptic soy agar plates containing 50 µg/mL kanamycin, and incubated overnight at 37°C. Bacterial burden in the spleens

was determined from CFU counts, and protection was determined by comparing fold reduction in CFU per spleen in the immunized groups relative to the placebo control (p values <0.05 using a one-sided Mann-Whitney test were considered significant). A total of 36 purified proteins were screened in this model. **Table 4** shows the proteins that provided statistically significant protection in the initial screen. A repeat study was performed for a subset (11 of 13) of the proteins positive in the initial screen and the results for this repeat study are also set out in **Table 4**.

As shown in **Table 4**, a number of proteins provided statistically significant reduction in the level of infection in the first study which was later confirmed for some in the second study. It was observed during the development of the model that healthy mice were able to rapidly clear the bacterial challenge by innate immune mechanisms. Consequently, the measured impact of vaccination occurs against a backdrop of rapid clearance by non-antibody-mediated means (representing a source of inherent variability in the model). The fact that a number of antigens did not give statistically significant protection in the repeat study is a reflection of the inherent variability due to competing mechanisms of clearance in the animals. Thus, further confirmation of the candidacy of these proteins as vaccine antigens was sought through *in vitro* testing.

The group of proteins assessed in the sub-lethal mouse model included the thirteen proteins that had tested positive for surface accessibility (as described in Example 2) and a number of proteins that had tested negative. As the assay utilized in Example 2 detects surface-exposed antigens that are expressed under the standard *in vitro* growth conditions (i.e., growth at 37°C in tryptic soy broth with aeration by shaking at 250 rpm), antigens negative for surface accessibility by that assay may nonetheless be protective *in vivo*. Indeed, among the proteins for which statistically significant protection was observed (see **Table 2**), a number had tested negative for surface accessibility (e.g., SERP2288, SERP2286/PhnD).

TABLE 4

Genes encoding antigens that showed a statistically significant reduction in the level of infection in an in vivo mouse model.

GENE ID*	Gene Name (Annotation)	Fold Reduction in CFU per Spleen**	
		Expt. 1	Expt. 2
SERP2288	(5'-nucleotidase)	13.9	P >0.05
SERP0207	(SdrG protein)	1.8	1.9
SERP0237	(lipoate-protein ligase A)	2.0	4.0
SERP0442	<i>gapA-1</i> (glyceraldehyde 3-phosphate dehydrogenase)	1.5	1.8
SERP0306	(iron ABC transporter ATP-binding protein)	1.9	3.1
SERP0574	<i>oppA</i> (oligopeptide ABC transporter oligopeptide-binding protein)	2.2	P >0.05
SERP2252	<i>sepA</i> (extracellular elastase precursor)	1.5	not done
SERP2391	<i>sspC</i> (SspC protein)	1.6	P >0.05
SERP2286	<i>phnD</i> (phosphonate ABC transporter substrate-binding protein)	1.5	P >0.05
SERP1891	(N-acetylmuramoyl-L-alanine amidase)	1.7	P >0.05
SERP1011	<i>ebh</i> (cell wall associated fibronectin-binding protein)	2.2	P >0.05
SERP0290	<i>sitC</i> (ABC transporter substrate-binding protein)	1.9	P >0.05
SERP1316	(cell wall surface anchor family protein)	2.0	not done

*The 4 digit gene identifier provided in **Table 4** refers to the *S. epidermidis* strain RP62A genomic identifier (GenBank Accession Number CP000029.1, RefSeq Accession Number NC_002976.3)

**All reported values for fold-reduction are statistically significant ($p < 0.05$ by one-sided Mann Whitney test). The indication ' $p > 0.05$ ' is used when fold reduction versus the control was not statistically significant in the repeat study.

EXAMPLE 3

This example describes the inhibition of *S. epidermidis* biofilm formation by SERP2286 (PhnD) specific antibodies under both static growth conditions and those of shear flow.

A. Antibodies against SERP2286 exhibit a dose response inhibition of S. epidermidis biofilm formation

Rabbit affinity purified antibodies were generated by Genscript USA Inc (Piscatawa, NJ, SC1247 - Basic Affinity-Purified Polyclonal Antibody Package) using immunizations with recombinant purified SERP2286 (20-318aa, SEQ ID NO:2) RP62A protein.

The Bioflux 1000 (Fluxion, San Francisco, CA) is an automated system for imaging live cell-based experiments under conditions of shear flow which mimic the flow conditions that *S. epidermidis* cells experience on catheters or implanted devices. *S. epidermidis* strains RP62A and 1457 were grown for 3.5 hr in tryptic soy broth (TSB) at 250 rpm and 37°C. Cultures were normalized to an OD_{600nm} of 1.0 in TSB and sonicated using a Branson Sonifier 250 (Emerson, Danbury, CT) with two 5 sec pulses at 5% output. Sonicated cultures were diluted to an OD_{600nm} of 0.15 in TSB and pre-incubated on a nutator at 25°C with control or test antibody at varying concentration between 5 and 200 µg/mL. After 20 min, 165 µl of culture was placed in the outlet well of a pre-primed 48-well 0-20 dyne Bioflux plate (Fluxion, 910-0047). Priming media consisted of 200 µl TSB with indicated concentration of control or test (rabbit anti-SERP2286) antibody. Using the Bioflux 1000 system, bacteria were driven into the viewing window of the channel from the outlet well with 2 dyne/cm² of pressure for 3 sec. Bacteria were incubated for 1.5 hr in the temperature controlled Bioflux chamber at 37°C for 1.5 hr to allow for initial attachment. Remaining culture was then aspirated from the outlet well and 800 µl of TSB containing the indicated concentration of control or test antibody was placed in the inlet well. A flow pressure of 0.4 dyne/cm² and a temperature of 37°C was used to flow media over the attached bacteria to generate shear conditions. Images were acquired at 20 min intervals for 18 hr through automated acquisition using Bioflux Montage software (**Fig. 1**).

An alternative assay to examine *in vitro* biofilm formation under static growth conditions by crystal violet staining was also used. In this assay, *S. epidermidis* strain RP62A and 1457 were grown for 3.5 hr in TSB broth at 250 rpm and 37°C. Cultures were normalized to OD_{600nm} of 0.05 in TSB and pre-incubated on a nutator at 25°C with no antibody or 100 µg/mL of control or anti-SERP2286 antibody. After 20 min, 200 µl of each sample was spotted in triplicate on a 96-well Nunc Surface microtiter plate (Nalge Nunc, Cat# 167008, Rochester, NY). The microtiter plate was incubated under static growth for 8 hr at 37°C. Non-bound cells and media were decanted and the attached biofilms were washed four times with 150 µL of PBS. After 20 min of drying, the plate was stained with 150 µL of 0.4% crystal violet for 5 min. Crystal violet was decanted and stained biofilms were washed three times with 150 µL PBS. After 20 min of drying, the biofilms were scanned and measured at OD_{490 nm} using an Infinite M200 Pro plate reader (Tecan, San Jose, CA). Visual and quantitative results are shown in **Figure 2 (A-D)**.

These results of these experiments show that rabbit affinity-purified polyclonal antibodies against SERP2286 demonstrated an antagonistic effect against *S. epidermidis* 1457 (clinical isolate) *in vitro* biofilms formed under conditions of shear flow which mimic the conditions that *S.*

epidermidis cells might experience on catheters or implanted devices. Dose-dependent anti-biofilm activity was observed at concentrations between 5-200 µg/mL of anti-SERP2286, with higher concentrations of antibody nearly abolishing biofilm formation (**Fig. 1**). Anti-SERP2286 antibodies were also effective at inhibiting *S. epidermidis* clinical isolate RP62A (ATCC35984) under flow conditions (data not shown). Control antibodies against a non-specific viral protein (HPV L1 major capsid protein) had no effect (Fig. 1). Results from the crystal violet assay were supportive (Fig. 2). Treatment of *S. epidermidis* 1457 and RP62A with 100 µg/mL of anti-SERP2286 elicited a 65.3% and 85.2% reduction in biofilm formation, respectively, under these conditions (**Fig. 2**).

In order to better mimic the environmental conditions experienced by bacteria during an *in vivo* catheter or indwelling device infection, the Bioflux assay was optimized to evaluate *S. epidermidis* biofilm formation when bacteria are grown in 100% human plasma (as opposed to TSB) in the presence of PhnD affinity-purified polyclonal antibodies and PhnD monoclonal antibodies. Both the PhnD affinity-purified polyclonal antibodies and the PhnD monoclonal antibodies were able to inhibit biofilm formation under these growth conditions (data not shown). This confirms that PhnD binding molecules (e.g., the antibodies tested here) inhibit biofilm formation under conditions that mimic both the *in vivo* environment and flow.

B. Biofilm inhibition by anti-SERP2286 antibody is target specific

To confirm that the anti-biofilm effect of the polyclonal anti-SERP2286 antibody was due to the interaction with the SERP2286 target, *S. epidermidis* was co-incubated with anti-SERP2286 antibody and excess purified SERP2286 protein. The protocol for *S. epidermidis* biofilm formation under flow conditions was equivalent to that described above in this example, aside from the addition of SERP2286 purified protein in the indicated samples. The excess SERP2286 protein bound and titrated free anti-SERP2286 antibody and abrogated the anti-biofilm activity of the antibody under flow conditions (**Fig. 3**). This confirmed that the interaction between anti-SERP2286 antibody and SERP2286 target on *S. epidermidis* mediated biofilm inhibition.

C. Anti-SERP2286 antibodies inhibit biofilm formation against a range of S. epidermidis clinical isolates

Screening of clinical isolates showed that 62 of 100 *S. epidermidis* isolates tested were negative for the *ica* locus, which produces the biofilm matrix polysaccharide, poly-N-acetyl glucosamine (PNAG). An *ica* negative clinical isolate 7291 (clinical isolate) was tested, and anti-SERP2286 antibodies also inhibited biofilm formation of this isolate under conditions of flow (**Fig.**

4). The protocol for *S. epidermidis* biofilm formation under flow conditions was equivalent to that described above in this example, aside from the duration of the experiment and a lower flow pressure. The biofilms formed by the *ica* negative clinical isolate 7291 have weaker surface attachment and develop slower than either 1457 or RP62A and therefore required reduction of flow pressure to 0.3 dyne/cm² and an increased incubation time of 36 hr. These results, in addition to those shown for 1457 and RP62A (**Figs. 1 and 2**), demonstrate that anti-SERP2286 antibodies are efficacious against the two *ica* positive *S. epidermidis* strains evaluated (clinical isolates 1457, RP62A) and the *ica* negative *S. epidermidis* strain evaluated (clinical isolate 7291); consequently, anti-SERP2286 is efficacious against a range of *S. epidermidis* clinical isolates. This suggests that the anti-biofilm activity of PhnD antibodies is independent of the matrix, which implies coverage against a broad range of clinically relevant species.

D. SERP2286 is expressed and surface accessible in S. epidermidis biofilms

Expression of SERP2286 was assessed during planktonic and biofilm phase of growth by western analysis. *S. epidermidis* 1457 was grown in TSB at 250 rpm and 37°C to the indicated optical density or alternatively, grown statically in Petri dishes to form biofilms for either 20 or 44 hr. For planktonic grown bacteria, cells were normalized at each time point to OD_{600nm} of 2.0 and pelleted at 16,000 x g. For biofilm grown bacteria, cells were scraped from Petri plates, resuspended in 10 mL of PBS, and sonicated using a Branson Sonifier 250 with two 10 sec pulses at 5% output. Sonicated cultures were normalized to OD_{600nm} of 2.0 and pelleted at 16,000 x g. *S. epidermidis* pellets were resuspended in 300 µl of 100 µg/mL of lysostaphin (Sigma, L9043) in PBS and lysed overnight at 37°C. Crude extracts were run under standard SDS-PAGE conditions. Proteins were transferred onto nitrocellulose membrane employing the iBlot system (Invitrogen, IB301001) and using recommended manufacturer conditions. Nitrocellulose membranes were blocked overnight with 5% milk solution and incubated with rabbit affinity-purified anti-SERP2286 antibody at a 1/20000 concentration for 1 hr, followed by incubation with anti-rabbit-HRP antibody (Rockland, KCB003, Gilbertsville, PA) at a 1/10000 concentration for 1hr. SERP2286 labeled protein was detected using SuperSignal West Dura Substrate (ThermoScientific, 34075) and scanned on a Carestream Imaging Scanner 4000 (Carestream, Rochester, NY). Although SERP2286 appears to be ubiquitously expressed during all growth conditions tested, the protein is upregulated in stationary phase and biofilm phase of growth (**Fig. 5**).

To determine if SERP2286 was surface accessible in *S. epidermidis* biofilms, bacterial biofilms grown under flow conditions were incubated with either control or anti-SERP2286 antibody followed

by secondary labeling with anti-Rabbit antibodies conjugated to fluorescein isothiocyanate (FITC). The protocol to form *S. epidermidis* biofilms under flow conditions was equivalent to that described earlier in this example, aside from that no antibody was added initially and biofilms were formed at 30°C. After 15 hr of biofilm growth, media and culture from inlet and outlet wells was aspirated and

5 100 µg/mL of control or anti-SERP2286 antibody in 100 µl of TSB was placed in the inlet well and flowed over biofilm bacteria for 40 min at 0.4 dyne/cm². Media from the inlet well was replaced with 20 µg/mL of anti-rabbit-FITC in 100 µl of PBS and flowed over biofilm bacteria for 20 min. After washing with PBS for 20 min at 0.4 dyne/cm², images of labeled biofilms were acquired using the Bioflux system (**Fig. 6**). Whereas anti-SERP2286 antibodies mediated FITC labeling of *S.*

10 *epidermidis* biofilms, control antibody resulted in negligible labeling. These results indicate that the anti-biofilm activity of anti-SERP2286 antibodies is likely mediated by the interaction between antibody and target antigen on the surface of *S. epidermidis*. Although SERP2286 appears to be surface accessible in the biofilm phase of growth, an alternative surface accessibility assay carried out on planktonic *S. epidermidis* did not detect surface protein (see Example 1; anti-SERP2286 was not

15 detected and therefore is absent from **Table 3**). This may indicate that SERP2286 protein, while present on the bacterial surface, is present at this location at low levels and/or only accessible during biofilm phase of growth.

EXAMPLE 4

To provide a quantitative readout of biofilm inhibition an *S. epidermidis* fluorescent reporter strain was generated. A plasmid was constructed expressing the red fluorescent protein from the promoter of the *S. aureus* *ftsZ* gene. The plasmid was transformed into *S. epidermidis* 1457 and was stably maintained through a staphylococcal pE194 origin of replication. Performance of the reporter strain was evaluated: (i) biofilm development was shown to occur normally; (ii) the reporter plasmid was stable even without selection and (iii) fluorescent signal correlated with cell density

20 (e.g., using Bioflux system, cell density was quantifiable via pixel intensity).

To quantify anti-biofilm activity, rabbit affinity-purified polyclonal antibodies against a number of proteins were tested under flow pressure. Rabbit affinity purified antibodies were generated by Genscript USA Inc (Piscataway, NJ, SC1247 - Basic Affinity-Purified Polyclonal Antibody Package (Rabbit)) using immunization with recombinant purified RP62A proteins

30 (SERP2286 – 20-318aa (SEQ ID NO.:2), SERP0207 – 223-559aa (SEQ ID NO.: 8), SERP1011 (EmbP) – 6599-7340aa (SEQ ID NO.: 6), SERP2288 – 1-511aa (SEQ ID NO.: 10), SERP0237 – 1-279aa (SEQ ID NO.: 9), SERP0574 – 20-547aa (SEQ ID NO.: 14), SERP0442 – 1-336aa (SEQ ID

NO.: 11), SERP0306 – 1-265aa (SEQ ID NO.: 12), SERP2264 – 34-649aa (SEQ ID NO:40) SERP1891 – 34-258aa (SEQ ID NO.: 4)) or immunization with KLH-conjugated peptides (SERP2398 – 2118-2137 (PGKPGVKNPDTGEVVTTPVD (SEQ ID NO.: 49)). The *S. epidermidis* fluorescent reporter strain was grown for 3.5 hr in TSB broth at 250 rpm and 37°C.

5 Cultures were normalized to an OD_{600nm} of 1.0 in TSB and sonicated using a Branson Sonifier 250 (Emerson, Danbury, CT) with two 5 sec pulses at 5% output. Sonicated cultures were diluted to an OD_{600nm} of 0.15 in TSB and pre-incubated on a nutator at 25°C with control or test antibody at 100 µg/mL. After 20 min, 165 µl of culture was placed in the outlet well of a pre-primed 48-well 0-20 dyne Bioflux plate (Fluxion, 910-0047). Priming media consisted of 200 µl TSB with indicated

10 concentration of control or test antibody. Using the Bioflux 1000 system, bacteria were driven into the viewing window of the channel from the outlet well with 2 dyne/cm² of pressure for 3 sec. Bacteria were incubated for 1.5 hr in the temperature controlled Bioflux chamber at 30°C for 1.5 hr to allow for initial attachment. Remaining culture was then aspirated from the outlet well and 800 µl of TSB containing the indicated concentration of control or test antibody was placed in the inlet well.

15 A flow pressure of 0.4 dyne/cm² and a temperature of 30°C was used to flow media over the attached bacteria to generate shear conditions. Brightfield and RFP fluorescent images (200ms exposure) were acquired at 20 min intervals for 18 hr through automated acquisition using Bioflux Montage software and average pixel intensity (A.U.) was determined. Results are summarized in Figure 7.

20 As shown in **Figure 7**, a number of protein-specific antibodies strongly inhibited *S. epidermidis* biofilm formation (>90% reduction versus control) including for example, anti-SERP2286 (PhnD), anti-SERP0237, anti-SERP1891, anti-SERP0207, and anti-SERP1011. In regards to SesC (SERP2264: SEQ ID NO:39 (full-length amino acid sequence), SEQ ID NO:40 (cloned amino acid sequence), longitudinal quantification showed that antibodies to SesC caused an initial

25 delay in biofilm establishment, but this effect was eventually overwhelmed. Aap antibodies seemed to increase biofilm formation; given the repetitive structure of the Aap protein, a plausible explanation for this biofilm enhancement is that antibodies cross-linked neighboring cells and increased bacterial aggregation. Thus, antibody binding to the cell surface may not be, by itself, sufficient to prevent biofilm formation in all cases, and inhibition of target function may also play a

30 role.

The inhibition of *S. aureus* biofilm formation by *S. epidermidis* SERP2286 and SERP0237 specific antibodies under flow conditions was also evaluated. While Bioflux biofilm assay conditions were substantially the same as for *S. epidermidis*, slight modifications were made for *S. aureus*

(Lowenstein strain) biofilms. Growth media consisted of 50% TSB diluted in sterile MilliQ H₂O and image acquisition was performed at 20 min intervals for only 6 hr, due to faster biofilm development time. *S. epidermidis* SERP2286 (PhnD) and SERP0237 protein-specific antibodies both inhibited biofilm formation of *S. aureus*, suggesting that *S. epidermidis* protein-specific antibodies were able to cross-react with *S. aureus* antigens (**Fig. 8**).

Protein-specific antibodies to the applicable proteins (e.g., anti-SERP2286 (PhnD), anti-SERP0237, anti-SERP1891, anti-SERP0207, anti-SERP1011 (EmbP) and anti-SERP2288) could be similarly evaluated quantitatively for inhibition of *S. aureus* biofilm formation using for example, a *S. aureus* strain with a fluorescent reporter plasmid. Indeed, each of SERP2286 (PhnD), SERP0237, SERP1891, SERP0207, SERP1011 (EmbP) and SERP2288 has a homolog in *S. aureus* as set out in **Table 5**.

Table 5

Target	AA used for immunization	biofilm inhibition vs. SE 1457 (%)	Distribution among 21 <i>S. aureus</i> strains *	% identity (SE vs SA)	% similarity (SE vs SA)
SERP2286	20-318	93	21	86	92
SERP0237	1-279	93	21	82	92
SERP1891	34-258	94	21	66	85
SERP0207	233-559	91	21	54	68
SERP1011	6600 -7340	91	17	36	56
SERP2288	1-511	64	21	62	78

*strains: Mu50; MW2; N315; RF122; COL; ED98; ED133; JH1; JH9; JKD6008; JKD6159; MRSA252; MSSA476; Mu3; NCTC 8325; Newman; TCH60; TW20; USA300; USA300_TCH1516; 04-02981.

Consistent with the ability of anti-SERP2286 (PhnD) antibodies to cross-react with the *S. aureus* PhnD homolog, Western immunoblot analysis with these same antibodies showed specific detection of a single band at the predicted molecular weight for PhnD in whole cell lysates of multiple staphylococcal species (*S. aureus* strain MRSA252, *S. epidermidis* strain 1457, *S. haemolyticus* strain ATCC29940, and *S. hominis* strain R22; see **Fig. 9**). Whole cell lysates for the different staphylococcal species were generated from cells growing in the logarithmic phase of growth in tryptic soy broth at 37°C with aeration by shaking at 250 rpm. The cultures were normalized by optical density, and equivalent numbers of cells were harvested by centrifugation. Lysates were generated by enzymatic digestion of the cell wall with lysostaphin in the presence of protease inhibitors. Samples were prepared for SDS-PAGE by heating at 70°C for 10 minutes in

reducing NuPAGE LDS sample buffer, then separated on a NuPAGE Bis-Tris (4-12%) gel in MES buffer run for 35 minutes at 200V. Western transfer and immunodetection were performed using the iBlot system as per the manufacturer's recommendations (Life Technologies). Anti-SERP2286 antibodies were used at a concentration of 4 µg/mL for primary detection.

EXAMPLE 5

To evaluate the use of protein-specific antibodies in early therapeutic intervention, the protocol for antibody treatment was modified, such that antibodies were introduced at different stages of biofilm development (prophylactic, early therapeutic, or late therapeutic stages as illustrated in **Figures 10A and B**). The *S. epidermidis* fluorescent reporter strain was grown for 3.5 hr in TSB broth at 250 rpm and 37°C. The culture was grown to an OD_{600nm} of 1.0 in TSB, sonicated, and normalized to OD_{600nm} of 0.15 in TSB as described above. However, pre-incubation with antibody was omitted and 165 µl of culture was placed in the outlet well in the absence of antibody. Priming media consisted of 200 µl TSB, also in the absence of antibody. Bacteria were driven into the viewing window of the channel from the outlet well with 2 dyne/cm² of pressure for 3 sec. Bacteria were incubated for 1.5 hr in the temperature controlled Bioflux chamber at 30°C for 1.5 hr to allow for initial attachment in the absence of antibody. Remaining culture was then aspirated from the outlet well and 800 µl of TSB containing 100 µg/mL of control or test antibody was placed in the inlet well. A flow pressure of 0.4 dyne/cm² and a temperature of 30°C was used to flow media over the attached bacteria to generate shear conditions. Brightfield and RFP fluorescent images (200ms exposure) were acquired at 20 min intervals for 18 hr through automated acquisition using Bioflux Montage software and average pixel intensity (A.U.) was determined. Since *S. epidermidis* initial attachment stages proceeded in the absence of antibody, this assay assesses the ability of antibody to prevent further accumulation/development of a pre-existing biofilm. In this way, the assay measures the ability of antibodies to inhibit at the aggregation stage. Results are summarized in **Figure 11**.

As shown therein, a number of protein-specific antibodies were able to inhibit *S. epidermidis* biofilm formation when added at the early therapeutic stage (post seeding), including for example, anti-SERP2286 (PhnD), anti-SERP0237, and anti-SERP1011 (EmbP). Under the conditions tested, anti-SERP2286 antibody treatment still lead to a significant decrease in biofilm development (85% endpoint reduction versus control). Anti-SERP1011 (EmbP) and anti-SERP0237 antibodies also reduced biofilm formation (65% and 69% versus control, respectively), whereas certain other antibodies either had less or no effect (anti-GapA(SERP0442)) or, in some cases, led to increased biofilm (anti-SesC and anti-Aap). Intervention in the late therapeutic window was also tested by

adding antibodies to a mature biofilm but no effect was observed for any candidate (data not shown). These data show that anti-SERP2286 (PhnD) and anti-SERP1011 (EmbP) antibodies inhibit biofilm formation at both the initial attachment and early aggregation stages of development but may not affect maturation or dispersal. Taken together, these quantitative data support the use of one or more of these proteins as targets for antibody-mediated biofilm intervention strategies.

EXAMPLE 6

To investigate the anti-biofilm mechanism of SERP2286 (PhnD) antibodies, a *phnD* knockout strain was generated in the *S. epidermidis* 1457 background ($\Delta phnD$ strain). Biofilm formation by the mutant strain was defective, but not completely abolished. The $\Delta phnD$ strain formed biofilms that were less dense and only loosely attached to the channel surface. It is not intuitive that PhnD antibodies should be more inhibitory to biofilm formation than deletion of their target. One possibility is that anti-biofilm activity is a cumulative effect of both functional target inhibition and steric interference of surface attachment by antibody binding. A second possibility is that deletion of *phnD* leads to compensating mutations or changes in gene expression that alleviate the genetic defect. These results show that deletion of *phnD* results in defective biofilm formation.

EXAMPLE 7

In a human infection, antibodies work in concert with other components of the immune system, often as opsonins to promote pathogen uptake by professional phagocytes. However, multiple studies have shown that staphylococcal biofilms impede the normal phagocytic activity of human neutrophils (reviewed in Otto M. (2012), *Semin Immunopathol* 34:201-214). It is possible that the PhnD antibodies circumvent the matrix defense and cooperate with neutrophils to promote biofilm clearance. To test this hypothesis, biofilms were formed and then freshly-isolated human neutrophils were introduced in the presence of control or PhnD antibodies (all under conditions of flow). Addition of anti-SERP2286 (PhnD) antibodies led to an immediate and marked increase in neutrophil binding and active engulfment of *S. epidermidis* biofilm versus control (data not shown). In four independent experiments, treatment with anti-SERP2286 (PhnD) antibodies increased neutrophil binding more than eight-fold versus control. Tracking of individual neutrophils showed that a single cell was capable of moving toward and engulfing large numbers of biofilm bacteria. These results show that anti-SERP2286 (PhnD) antibodies promote phagocytosis of *S. epidermidis* biofilms. Anti-SERP2286 (PhnD) antibodies may enhance neutrophil phagocytosis of staphylococcal biofilms *in vivo*, which would act as a complement to their direct anti-biofilm effects on the bacteria themselves.

EXAMPLE 8

A number of monoclonal antibodies targeting PhnD (SERP2286) were generated and tested for biofilm inhibition. Mouse monoclonal antibodies were derived using typical methods (GenScript USA Inc - Piscatawa, NJ, SC1040 - Basic Monoclonal Antibody Package (Mouse)): spleen cells from a mouse immunized with PhnD were fused to myeloma cells, then cloned by limiting dilution. Linear epitopes recognized by PhnD mAbs were mapped using a 15-mer peptide library with 10 amino acid overlap. ELISAs were performed to identify peptides bound by each mAb. Briefly, the biotinylated peptides were captured in 96-well microtiter plates pre-coated with streptavidin. The peptide plates were blocked, washed, and then incubated with each mAb at 1 µg/ml at 37°C for 2 h. A plate without test antibody (PBS) was run as a control for non-specific binding by the detection antibody. Positive and negative controls were also included on each plate; (i) as positive controls, GST (detected with GST mAb), and purified full-length PhnD protein; (ii) as a negative control, streptavidin. Following incubation, the plates were washed and then incubated with secondary antibody (HRP-conjugated) at 37°C for 1 h. After a final wash with PBS-T, the plate was incubated with TMB substrate for colorimetric detection. The reaction was stopped with HCl, and absorbance was measured at 450 nm. Under these conditions, peptides with a signal to noise ratio >3 were considered positive for binding. Linear epitopes could be identified for all PhnD mAbs except 4E4E4. **Table 6** lists the linear epitopes identified. The epitope of 2C1D3 is within PSKKLVDDYK; the epitope of 9B8G7 is within SDFDIVRQYEKAVHD; the epitope of 5B2F5, 6B9D6, 8C3D8, 8H1E4, 9F1A5, and 11C9E7 is within FDIVRQYEKAVHD; and the epitope of 11E3D8 is within FDIVRQYE. In regards to mAb 4E4E4, it is possible that the epitope is conformational. All mAbs reacted with SERP2286 and its *S. aureus* homolog SAR0145 by ELISA (data not shown).

All of the mAbs generated were able to inhibit *S. epidermidis* biofilm development to varying degree (**Figure 12**). Importantly, mAbs 8H1E4 and 4E4E4 had an effect comparable to the polyclonal antisera, confirming the validity of targeting a single PhnD epitope. These results further support the PhnD as a target for antibody-mediated biofilm intervention either by vaccination or through passive administration of monoclonal antibodies. In addition, humanized versions of 8H1E4 or 4E4E4 could be used for passive immunoprophylaxis of staphylococcal biofilms.

Table 6
Sequence Overlap of Binding Peptides

mAb	Binding Peptide #	Epitope Sequence
2C1D3	23	NEDGSPSKKLVDYK (SEQ ID NO.: 41)
	24	PSKKLVDDYKSEILV (SEQ ID NO.: 42)
9B8G7	57	SDFDIVRQYEKAVHD (SEQ ID NO.: 43)
5B2F5/ 6B9D6/ 8C3D8/ 8H1E4/ 9F1A5/ 11C9E7	57	SDFDIVRQYEKAVHD (SEQ ID NO.: 44)
	58	FDIVRQYEKAVHDMK (SEQ ID NO.: 45)
11E3D8	56	VDTKDSDFDIVRQYE (SEQ ID NO.: 46)
	57	SDFDIVRQYEKAVHD (SEQ ID NO.: 47)
	58	FDIVRQYEKAVHDMK (SEQ ID NO.: 48)

While certain embodiments have been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the following claims.

CLAIMS

What is claimed is:

1. An immunogenic composition comprising an effective amount of at least one isolated protein selected from the group consisting of:
 - 5 (i) SERP2286 or a fragment or a derivative thereof;
 - (ii) SERP0237 or or a fragment or a derivative thereof;
 - (iii) SERP1891 or a fragment or a derivative thereof;
 - (iv) SERP0207 or a fragment or a derivative thereof;
 - (v) SERP1011 or a fragment or a derivative thereof; and
 - 10 (vi) SERP2288 or a fragment or a derivative thereof.
2. The immunogenic composition of claim 1 wherein the at least one isolated protein, has an amino acid sequence having at least 80% sequence identity when aligned globally to SEQ ID No. 1, 3, 5, 7, 9 or 10.
3. The immunogenic composition of claim 1 wherein the at least one isolated protein, has an amino acid sequence having at least 80% sequence identity when aligned globally to SEQ ID No. 2, 4, 6, or 8.
4. The immunogenic composition of claim 2 wherein the at least one isolated protein has an amino acid sequence having at least 85%, 90%, 95% or more sequence identity when aligned globally to SEQ ID No. 1, 3, 5, 7, 9 or 10.
- 20 5. The immunogenic composition of claim 3 wherein the at least one isolated protein has an amino acid sequence having at least 85%, 90%, 95% or more sequence identity when aligned globally to SEQ ID No. 2, 4, 6, 8.
6. The immunogenic composition of claim 1 comprising at least two or more isolated proteins selected from the group.
- 25 7. The immunogenic composition of claim 1 comprising an isolated SERP2286 protein or a fragment or a derivative thereof.
8. The immunogenic composition of claim 3 wherein the at least one isolated protein has an amino acid sequence having at least 80% sequence identity to when aligned globally to SEQ ID No. 2.
9. The immunogenic composition of claim 8 wherein the at least one isolated protein has an amino acid sequence as set out in SEQ ID NO: 2 with one or more conservative amino acid substitutions.
- 30

10. The immunogenic composition of claim 9 wherein the at least one isolated protein has an amino acid sequence as set out in SEQ ID NO: 2.
11. The immunogenic composition according to any one of claims 1 to 10 wherein the composition comprises a pharmaceutically acceptable excipient.
- 5 12. The immunogenic composition of claim 11 wherein the composition is in liquid form.
13. The immunogenic composition of according to claim 11, wherein the composition is in dry powder form, freeze dried, spray dried or foam dried.
14. The immunogenic composition according to any one of claims 1 to 13 wherein the composition comprises an adjuvant.
- 10 15. The immunogenic composition of claim 14 wherein the adjuvant is an aluminum compound.
16. A kit comprising an immunogenic composition according to any one of claims 1 to 15 and instructions for use.
17. A method of eliciting an immune response in a subject, comprising administering to the subject an effective amount of at least one isolated protein selected from the group consisting of:
 - 15 (i) SERP2286 or a fragment or a derivative thereof;
 - (ii) SERP0237 or or a fragment or a derivative thereof;
 - (iii) SERP1891 or a fragment or a derivative thereof;
 - (iv) SERP0207 or a fragment or a derivative thereof;
 - (v) SERP1011 or a fragment or a derivative thereof; and
 - 20 (vi) SERP2288 or a fragment or a derivative thereof.
18. The method of claim 17 wherein the immune response elicited inhibits the development of a *Staphylococcus* biofilm in the subject.
19. The method of claim 17 wherein the immune response elicited inhibits the development of a *Staphylococcus* biofilm on an indwelling medical device in the subject.
- 25 20. The method of claim 19, wherein administration occurs prior to the implantation of the device into the subject.

21. The method of claim 19, wherein administration occurs sometime following the implantation of the device into the subject.
22. The method of claim 20 wherein the device is a catheter, cannula, prosthesis, contact lens, or intraocular lens.
- 5 23. The method of claim 21, wherein the device is a central venous catheter, a peritoneal dialysis catheter, orthopedic prosthesis, orthopedic mesh, an artificial valve, a pacemaker, a stent, a cholelear implant, breast implant, endotracheal tube, or voice prostheses.
24. The method of claim 17 wherein the immune response elicited is for treating a *Staphylococcus* biofilm in a subject.
- 10 25. The method of any of claims 17 to 24 wherein the subject is a human.
26. The method of claim 25 wherein the subject has or is at risk of developing a symptomatic staphylococcal infection.
27. The method of claim 26 wherein the subject has a symptomatic staphylococcal infection.
28. The method of claim 26 wherein the subject has a symptomatic nosocomial CoNS infection.
- 15 29. The method of claim 26 wherein the subject has a *Staphylococcus* biofilm.
30. The method of claim 29 wherein the *Staphylococcus* biofilm is a *S. epidermidis* biofilm.
31. The method of claim 29 wherein the *Staphylococcus* biofilm is a *S. aureus* biofilm.
32. A high affinity binding molecule for use in inhibiting development of a staphylococcal bacterial biofilm in a subject that specifically binds to a protein or peptide selected from the group
- 20 consisting of:
- (i) SERP2286 or a fragment or a derivative thereof;
 - (ii) SERP0237 or or a fragment or a derivative thereof;
 - (iii) SERP1891 or a fragment or a derivative thereof;
 - (iv) SERP0207 or a fragment or a derivative thereof;
 - 25 (v) SERP1011 or a fragment or a derivative thereof;
 - (vi) SERP2288 or a fragment or a derivative thereof;
 - (vii) SEQ ID NO.: 41;
 - (viii) SEQ ID NO.: 42;
 - (ix) SEQ ID NO.: 43;

- (x) SEQ ID NO.: 44;
- (xi) SEQ ID NO.: 45;
- (xii) SEQ ID NO.: 46;
- (xiii) SEQ ID NO.: 47; and,
- 5 (xiv) SEQ ID NO.: 48.

33. The high affinity binding molecule of claim 32 wherein the molecule is an aptamer, antibody or antibody mimetic.

34. The high affinity binding molecule of claim 33 wherein the antibody or antibody mimetic is selected from the group consisting of polyclonal antibody, monoclonal antibody, humanized
10 antibody, hybrid antibody, chimeric antibody, antibody fragment, single chain antibody, nanobody, unibody, affibody, DARPin, anticalin, adnectin or avimer.

35. The high affinity binding molecule method of claim 34, wherein the antibody is a monoclonal antibody.

36. The high affinity binding molecule of claim 35 wherein the protein is PhnD or a fragment or a
15 derivative thereof.

37. A method of treating and/or inhibiting development of a *Staphylococcus* bacterial biofilm in a subject, the method comprising administering to the subject an effective amount of the binding molecule as claimed in any one of claims 32 to 36.

38. The method of claim 37 wherein the subject is a human.

20 39. The method of claim 37 wherein the subject has or is at risk of developing symptomatic staphylococcal infection.

40. The method of claim 39 wherein the subject has a symptomatic staphylococcal infection.

41. The method of claim 40 wherein the subject has a symptomatic nosocomial CoNS infection.

42. A method of inhibiting development of a *Staphylococcus* bacterial biofilm on an indwelling
25 medical device in a subject, the method comprising administering to the subject prior to the implantation of the implant into the subject an effective amount of the binding molecule as claimed in any one of claims 32 to 36.

43. The method of claim 42 wherein the device is a catheter, cannula, prosthesis, contact lens, or intraocular lens.

44. The method of claim 42 wherein the subject is a human.
45. The method of claim 43 wherein the subject has or is at risk of developing symptomatic staphylococcal infection.
46. The method of claim 45 wherein the subject has a symptomatic staphylococcal infection.
- 5 47. The method of claim 46 wherein the subject has a symptomatic nosocomial CoNS infection.
48. The method of claim 47 wherein the subject has a *Staphylococcus* bacterial biofilm.
49. The composition of claim 47 wherein the symptomatic nosocomial CoNS infection is a central line associated blood stream infection.
50. An isolated polypeptide selected from the group consisting of SEQ ID NOs. 1-38.
- 10 51. The isolated polypeptide of claim 50 selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9, 10, 11, 12, 14, 16, 19, 21, 23, 25, 26, 27, 29, 31, 33, 35 and 38.
52. A composition comprising the isolated polypeptide of claim 50 or 51, or a fragment or derivative thereof, and a pharmaceutically acceptable excipient.
53. A peptide having the amino acid sequence selected from the group consisting of SEQ ID NO.: 41, SEQ ID NO.: 42, SEQ ID NO.: 43, SEQ ID NO.: 44, SEQ ID NO.: 45, SEQ ID NO.: 46, 15 SEQ ID NO.: 47, and SEQ ID NO.: 48.
54. A composition comprising a peptide of claim 56.

Figure 1

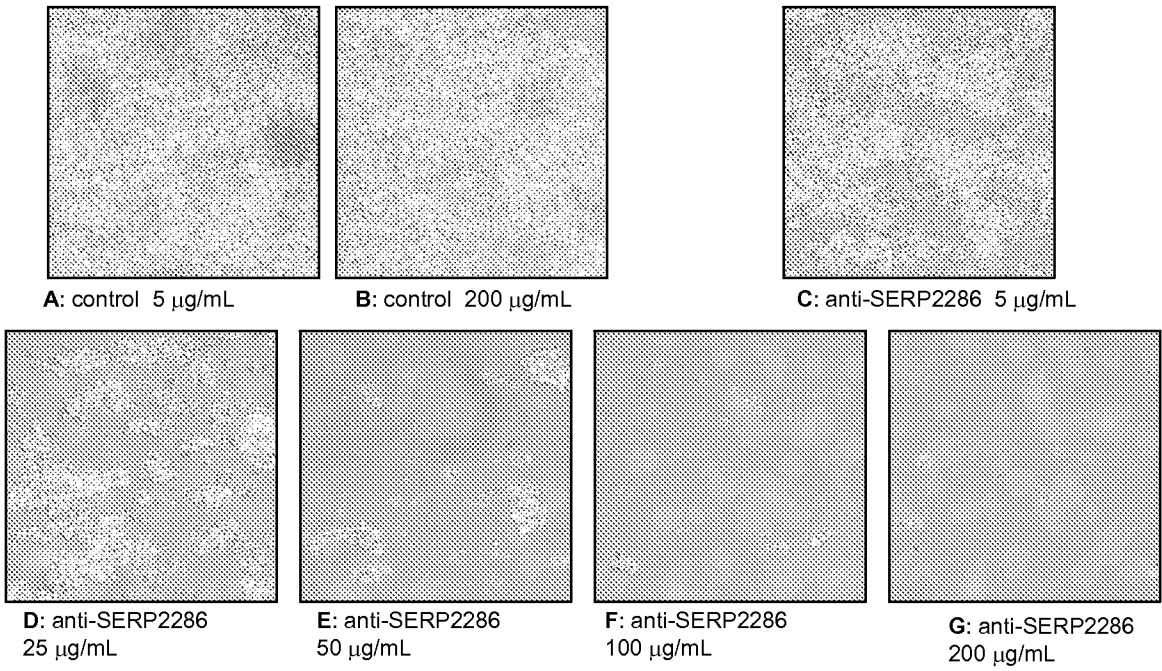
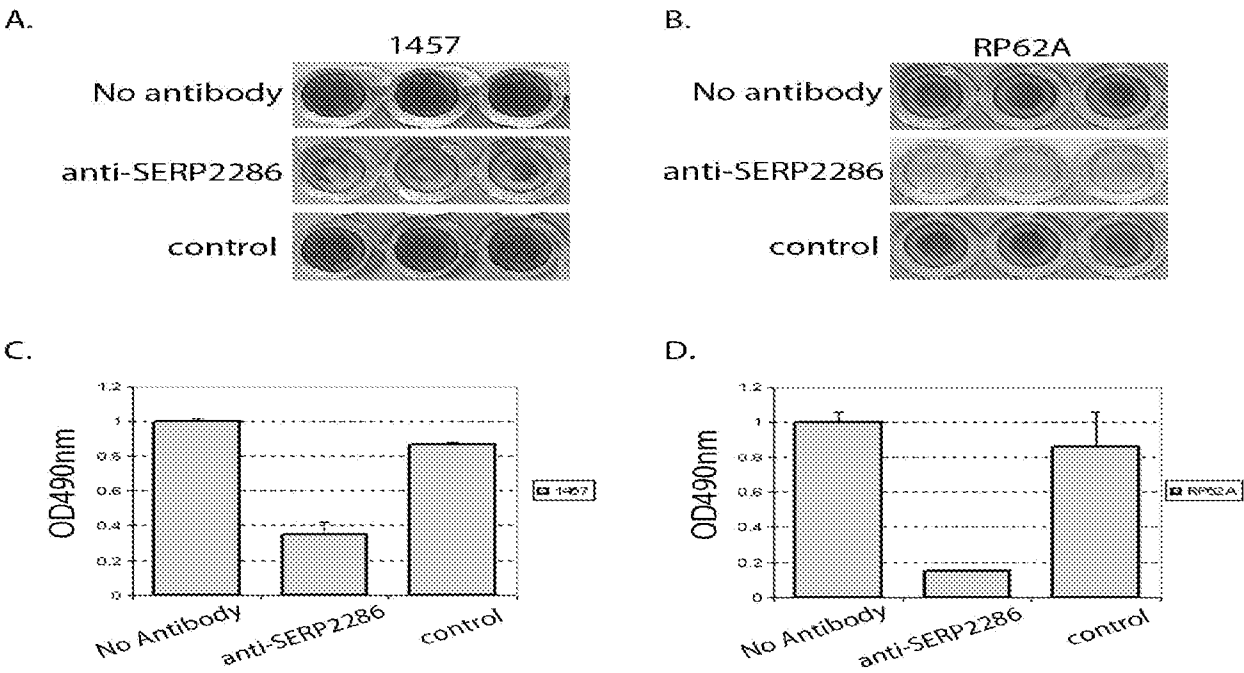


Figure 2



2/8

Figure 3

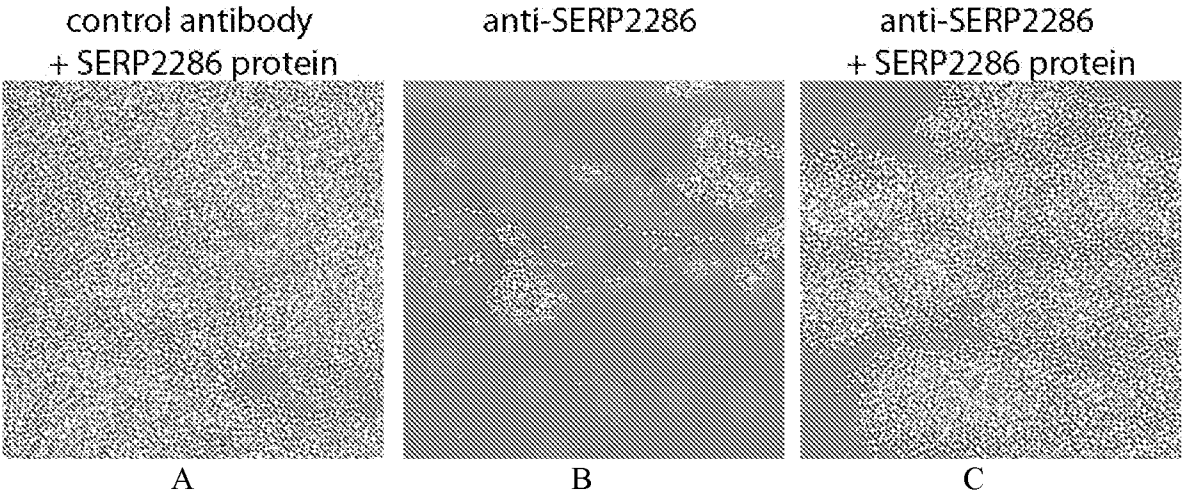


Figure 4

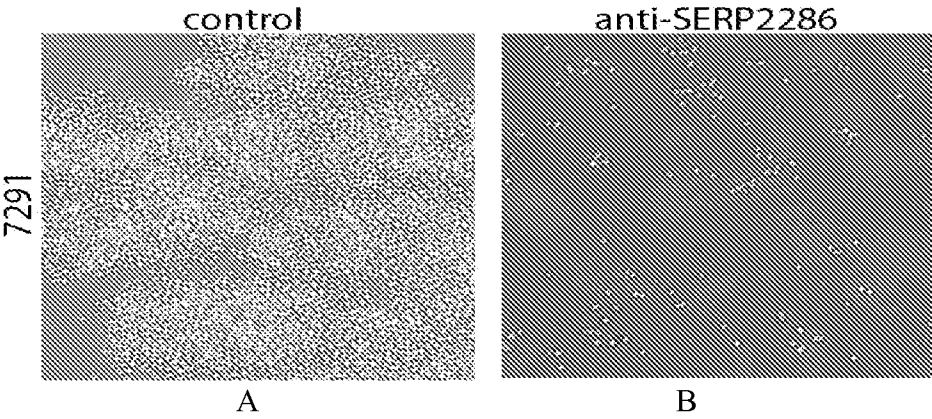
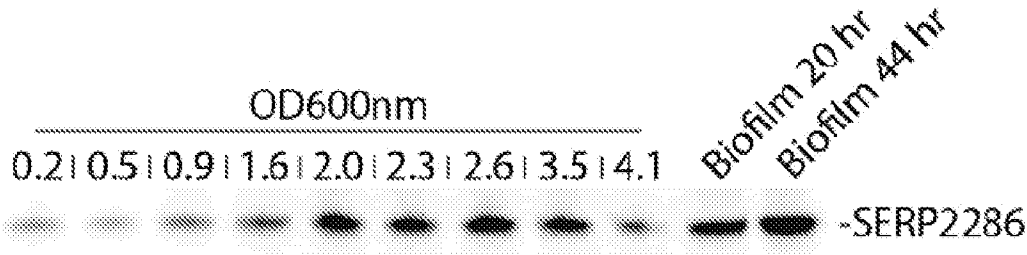
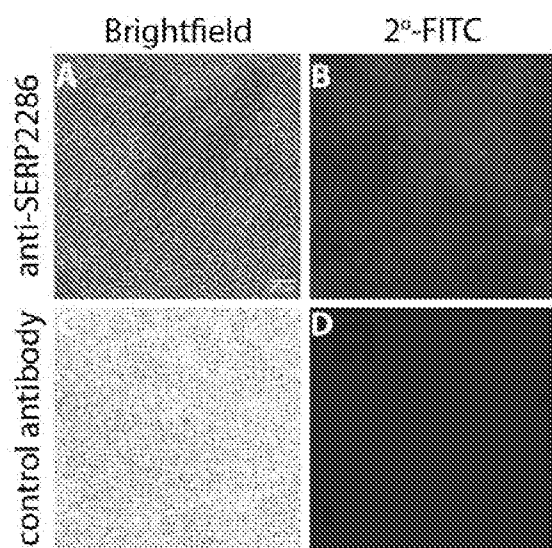


Figure 5



3/8

Figure 6

4/8

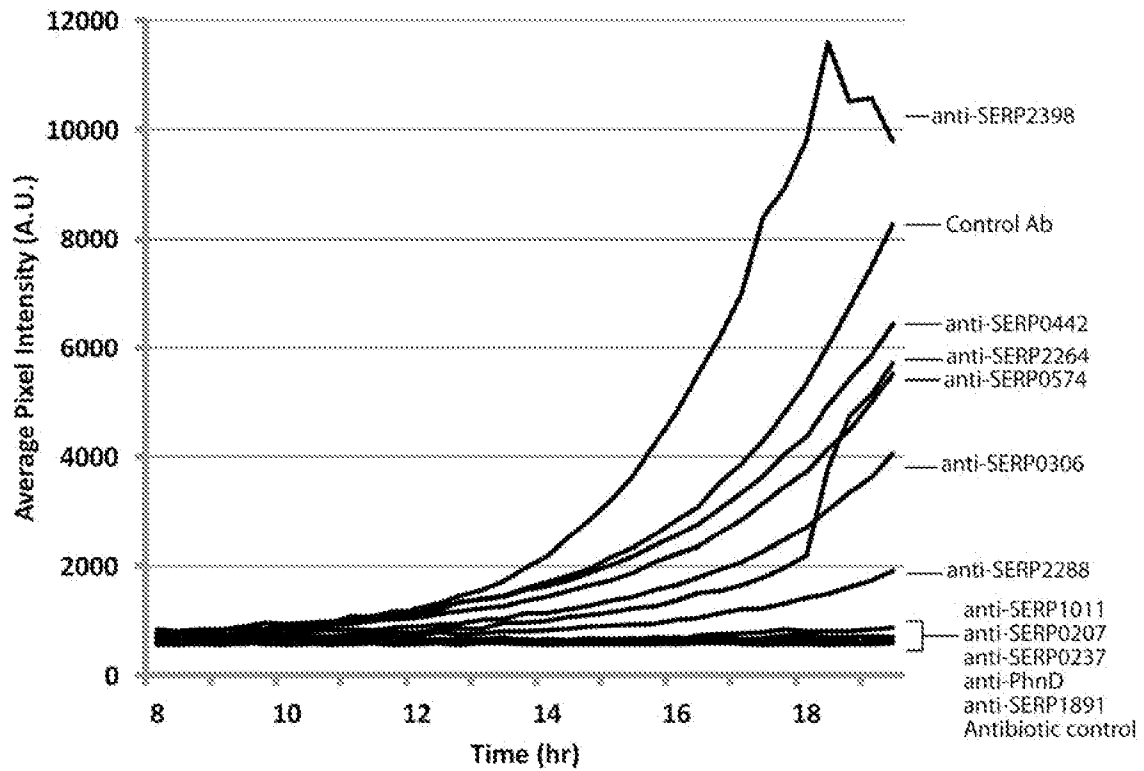
Figure 7

Figure 8

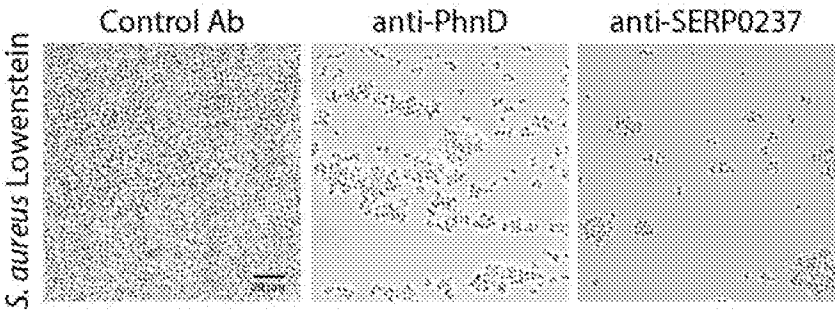
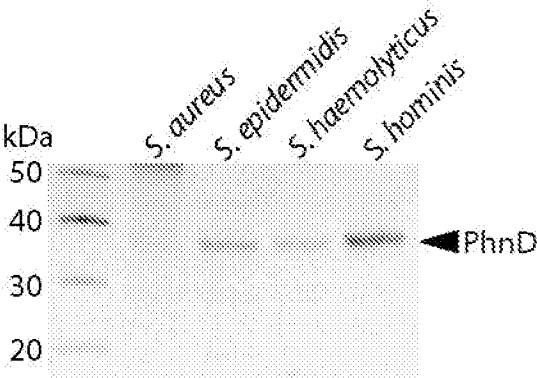
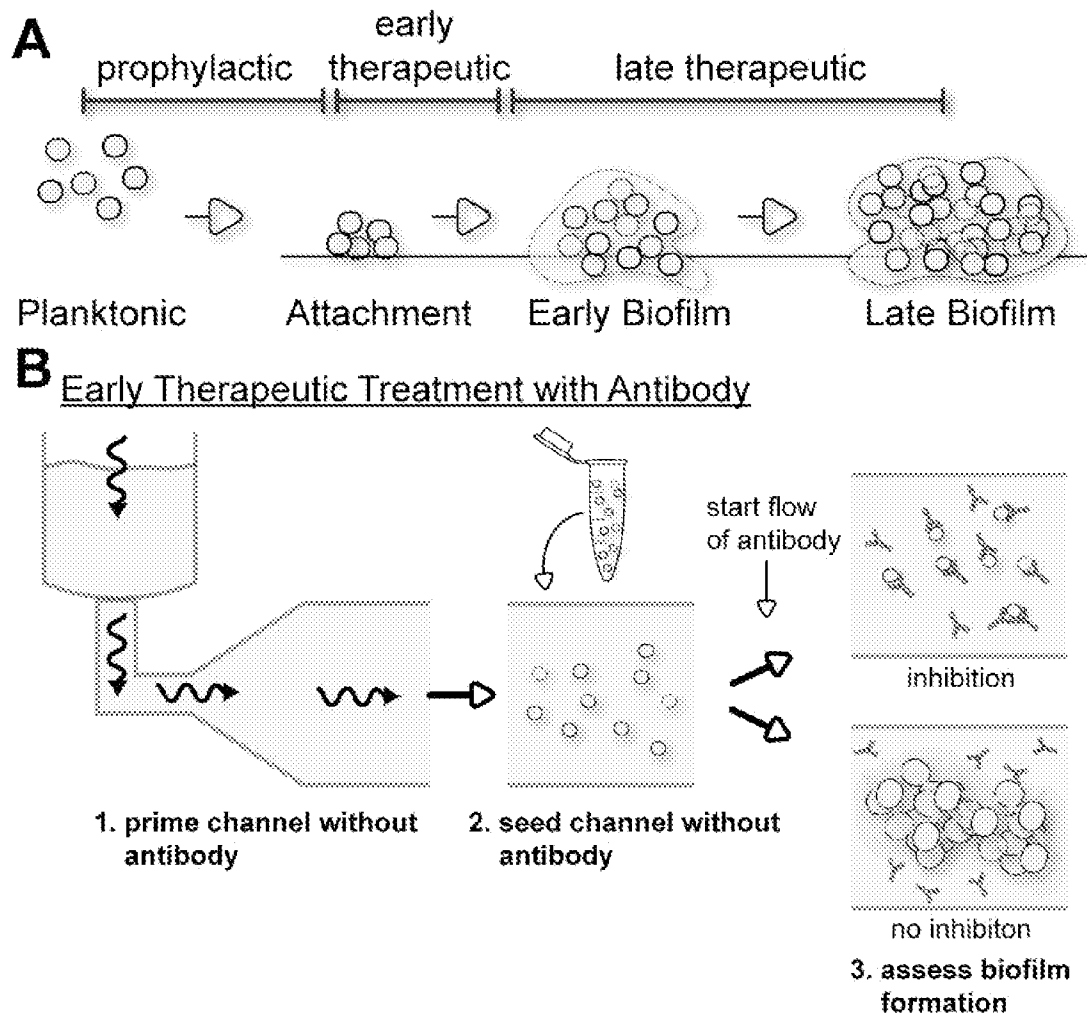


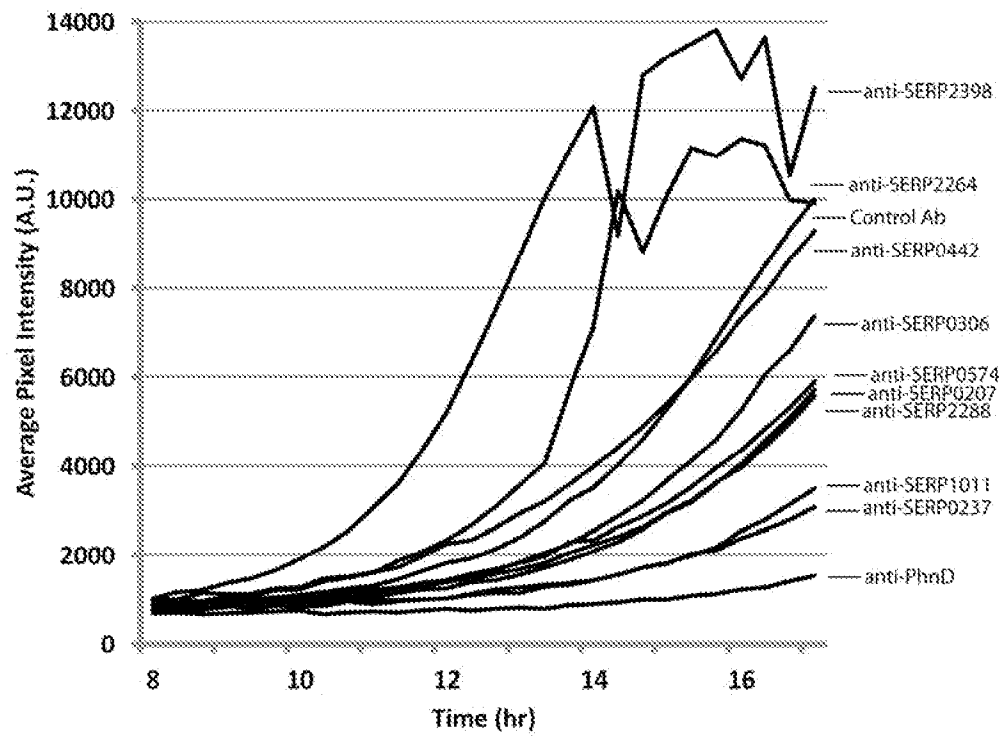
Figure 9



6/8

Figure 10

7/8

Figure 11

8/8

Figure 12