

S.aureus strain SH682	(27-247)	IVTKD---YSGKSQVNAGSKNGTLIDSRYLNSALYLEDYIIYAIGLTKNYEYGDNIYKE
S.aureus strain Newman	(27-263)	IVTKD---YSGKSQVNAGSKNGTLIDSRYLNSALYLEDYIIYAIGLTKNYEYGDNIYKE
S.aureus strain 6850	(27-239)	VVSGEKNPYKSESLKLTGKRSNS-VTPVMYKENL----EKLIASLSFADYEKYEPEYKE
S.pseudintermedius 081661	(27-247)	IVSGEKNPYTSKALSIMEKSSTSSVTTEQYKKSLSL----EKLMARLTIGGYEKYDEPEYAD
S.pseudintermedius 063228	(27-250)	IVSGEENPYKSKALSITEKSSTSSYTSEQYKKSLSL----EDLIYKLTISGYEKYDEPEYAA
		:* : *...: . . : . :. * : : : : . :* : *
S.aureus strain SH682		AKDRLLEKVLREDQYLLERKKSQYEDYKQWYANYKKENPRTDLKMANFHKYNLEELSMKE
S.aureus strain Newman		AKDRLLEKVLREDQYLLERKKSQYEDYKQWYANYKKENPRTDLKMANFHKYNLEELSMKE
S.aureus strain 6850		AVKTYQQKFMAEDAAL----KKFFREEKHIVEN-----TNTN-----EMLGLTEER
S.pseudintermedius 081661		VVKYKQRFMAEMNAM----NQFLQEEKAKEKK---NGAIST-----DAIGLTHQR
S.pseudintermedius 063228		AVKTYQQRFMAEMEAM----NQFLQGEKVKERKRKNSQPIPD-----DIIGLTYQR
		. . :...: * : :. * : . . : * : :
S.aureus strain SH682		YNELQDALKRALDDFHREVVDIKDKNSDLKTFNAAEEDKATKEVYDLVSEIDTLVVSY-Y
S.aureus strain Newman		YNELQDALKRALDDFHREVVDIKDKNSDLKTFNAAEEDKATKEVYDLVSEIDTLVVSY-Y
S.aureus strain 6850		YQHIYDKLKSNEWFMKEIKNIQERYQDLKDFDEKQQHDADV KINELNKV--LMLGYTF
S.pseudintermedius 081661		YDTVYKSLEENKKDFEREIKKLNEQHSDLKTFNRQEONDADQKLNLENQA--LMLGQTF
S.pseudintermedius 063228		YKAVYDALEENKKDFDREIAELNDKHPKELKTFDSKHQSEADQKLNLENQV--LMLGKTF
		*. : . * : . * .*: :...: **: * : : . * : : : * . : * : . :
S.aureus strain SH682		GDKDYGEHAKELRAKLDLILKDTDNPH---KITNERIKKEMIDDLNSIIK-----
S.aureus strain Newman		GDKDYGEHAKELRAKLDLILGDTDNPH---KITNERIKKEMIDDLNSIIDDFMETKQNR
S.aureus strain 6850		GNV--GGARTNLYSKLDLILGYEDYERKYKQPTNSRMLNEKMEDLETIIDEFFEIEIGKQR
S.pseudintermedius 081661		IYQ--GDAIFNLYNKLDMSIGNLDYEREKKAINKRMLDKKVEDLETIIDEFFEIEIGLAR
S.pseudintermedius 063228		IRK--DEARASLYQKLDLSIGYTDNERKEKKAINKRMLDRKIEDLETIIDEFFEIEADLAR
		. * ***: : * . : *.*: . :***:***.
S.aureus strain SH682		-----YNPTTHNYKTNSDNK--PNFDKLVVEE
S.aureus strain Newman		PKSITKYNPTTHNYKTNSDNK--PNFDKLVVEE
S.aureus strain 6850		PINIPTLA-SEKEKETNAKN-----ANKLRAD
S.pseudintermedius 081661		PKEIPVLT-RENEKDSAVKAKLRQDAQDAKND
S.pseudintermedius 063228		PKEIPVLT-AENEYDNAVKNKLRDANKAKAD
		: : . . . : . :

Figure S1. Clustal W alignment of amino acid sequences from *S. pseudintermedius* coagulase (residues 27 to 247) and prothrombin binding domains from *S. aureus*. Asterisks denote conserved residues, and two dots represent similar residues.

A.

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S.pseudintermedius 081661(403-430)      -----ETEIIITENH-VVDIDEST-TFQKSGYLYGV-
S.pseudintermedius 063228(406-433)      -----ETETITENH-VVDIDEST-TFQKSGYLYGV-
S.aureus Efb-A(fg1)of Efb protein (30-67)  SEGYPREKKPVSINHNIVEYNDGTFKYQSRPKFNSTP
                                           *.: :. ** :*: :.:* .:* . : ..
    
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B.

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S.pseudintermedius 081661(431-467)      SESDTSGYTEREKRAIRRNH-VREAEALVNQYVETHRY----
S.pseudintermedius 063228(434-470)      SESDTSGYTEREKRAIRRNH-VREAEALVNQYVETHRY----
S.aureus Efb-A(fg1)of Efb protein (30-67)  SE----GYGPREKKPVSINHNIVEYNDGTFKYQSRPKFNSTP
**      **   ***.:. ** : * : . :* . .:
    
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Figure S2. *S. pseudintermedius* coagulase Efb-like fibrinogen binding motifs. (A and B) Comparison of amino acid sequences of Efb-A with Coa (residues 403 to 430) (A) and with Coa (residues 431 to 467) (B) Asterisks denote conserved residues, and two dots represent similar residues.

A.

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S.aureus Newman Sbi-I(IgG binding domain) (47-95)  YVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERA----QEVFSESLKDSK
S.pseudintermedius 081661(280-331)                RVLKQQST---DAPSQPSDQEVAPSVNSIEIKAPQAATPQQPVHTETIVHTP
S.pseudintermedius 063228(283-332)                RALKQQSI---DAPSQPSDQEVAPTSVNSIEIKAPQAATPQQPVHTETIVHTP
. .**.          :  :*  :  ::::  :. .*  * *.*:*:  :
```

B.

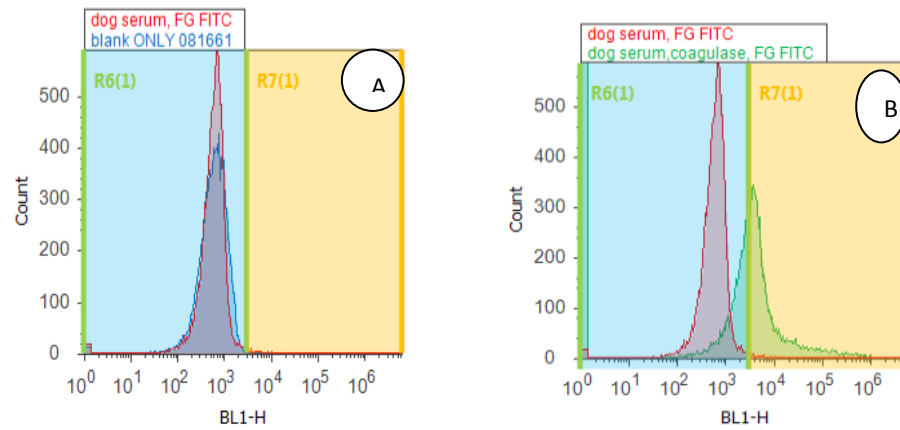
```
S.aureus Newman Sbi-II(IgG binding domain) (99-148) RRVAQQNAFYNVLKNLNLTEQEKNNYIAQIKENPDRSQQ-VWVESVQSSKA
S.pseudintermedius 081661(280-331)                RVLKQQSTDAPSQPSDQEVAPSVNSIEIKAPQAATPQQPVHTETIVHTP-
S.pseudintermedius 063228(283-332)                RALKQQSIDAPSQPSDQEVAPTSVNSIEIKAPQAATPQQPVHTETIVHTP-
* : **.          .*: . * . * * . : . ** *.*:*:  :
```

Figure S3. Comparison of amino acid sequences of the IgG binding site of *S. aureus* Sbi protein and *S. pseudintermedius* coagulase (residues 280 to 331). (A and B) Comparison of amino acid sequences to Sbi-I (47-95) (A). Comparison of amino acid sequences to Sbi-II (99-148) (B). Asterisks denote conserved residues, and two dots represent similar residues

S.aureus Newman Sbi-IV(C3 binding domain) (228-251)
 S.pseudintermedius 081661(468-491)
 S.pseudintermedius 063228(471-494)

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ENRRLAQREVNKAPMDVKEHLQKQ
QDRMAAQQKVNTLSKAHQKRFNKM
QDRMAAQQKVNTLSKAHQKRFNKM
::*  **.:** . .  ::::*
```

Figure S4. Comparison of the amino acid sequences of the complement C3 binding site of *S. aureus* Sbi protein and *S. pseudintermedius* coagulase (residues 468 to 491). Asterisks denote conserved residues, and two dots represent similar residues



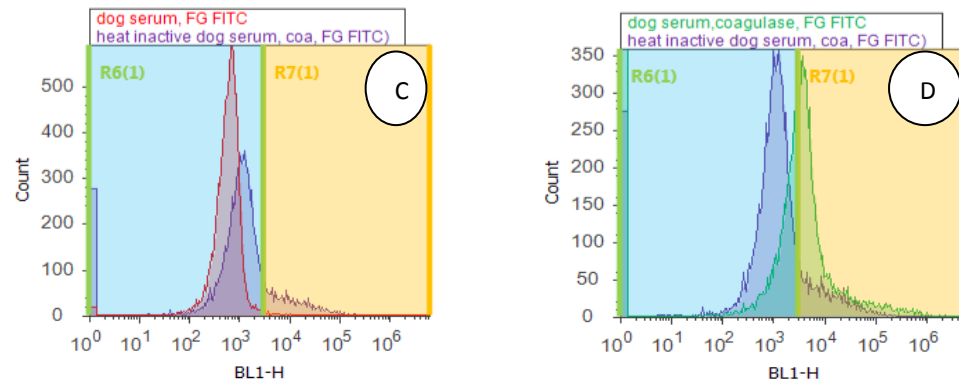


Figure S 5. Coagulase mediated binding of fibrinogen to the bacterial surface opsonized with dog serum.

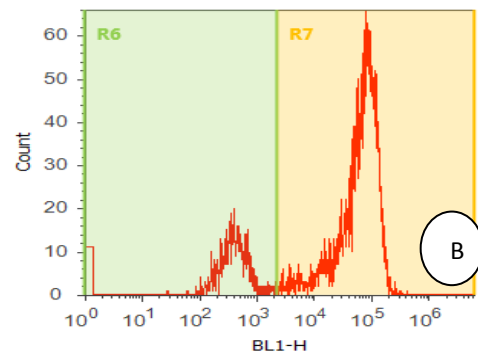
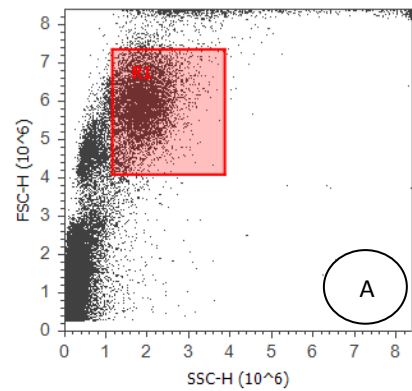
Fluorescence intensity is shown on the X axis and the number of events is shown on the Y axis.

A) Representative overlay histogram of fibrinogen FITC conjugate deposited on the bacterial surface when preincubated with dog serum compared to bacteria without the fibrinogen FITC conjugate (blank).

B) Representative overlay histogram of fibrinogen FITC conjugate deposited on the bacterial surface when preincubated with dog serum and recombinant *S. pseudintermedius* coagulase compared with dog serum only.

C) Representative overlay histogram of fibrinogen FITC conjugate deposited on the bacterial surface when preincubated with heat inactivated dog serum and recombinant *S. pseudintermedius* coagulase compared with dog serum only.

D) Representative overlay histogram of fibrinogen FITC conjugate deposited on the bacterial surface when preincubated with heat inactivated dog serum and recombinant *S. pseudintermedius* coagulase compared with dog serum and recombinant *S. pseudintermedius* coagulase.



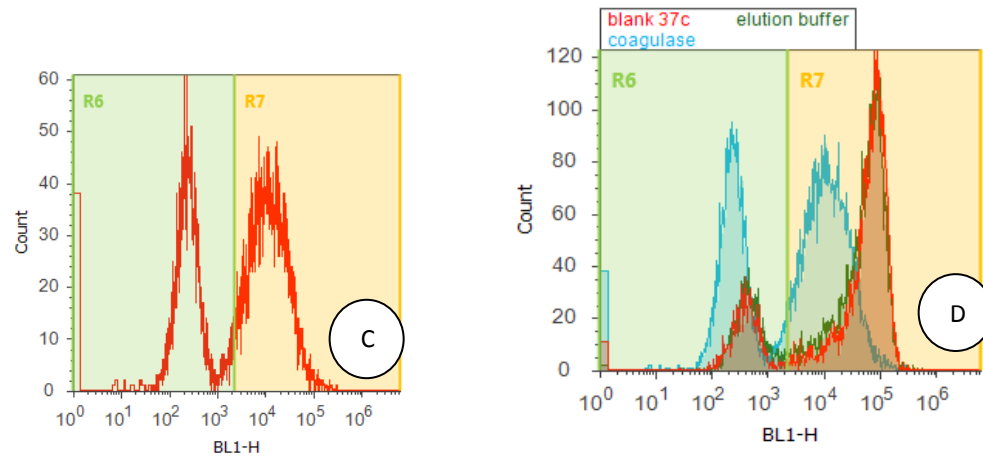


Figure S 6. Recombinant *S. pseudintermedius* coagulase blockage of phagocytosis by canine neutrophils.

A) Dot plot of neutrophils gated based on forward and side scatter properties.

B) Representative histogram of ex vivo phagocytosis of PHrodo labeled *S. pseudintermedius* incubated with normal dog whole blood (normalized phagocytosis).

C) Representative histogram of ex vivo phagocytosis of PHrodo labeled *S. pseudintermedius* incubated with dog whole blood, rabbit plasma, and recombinant *S. pseudintermedius* coagulase.

D) Overlay histogram of phagocytosis of PHrodo labeled *S. pseudintermedius* incubated with normal dog whole blood (blank 37c), normal dog whole blood with buffer control (elution buffer) or dog whole blood, rabbit plasma, and recombinant *S. pseudintermedius*.

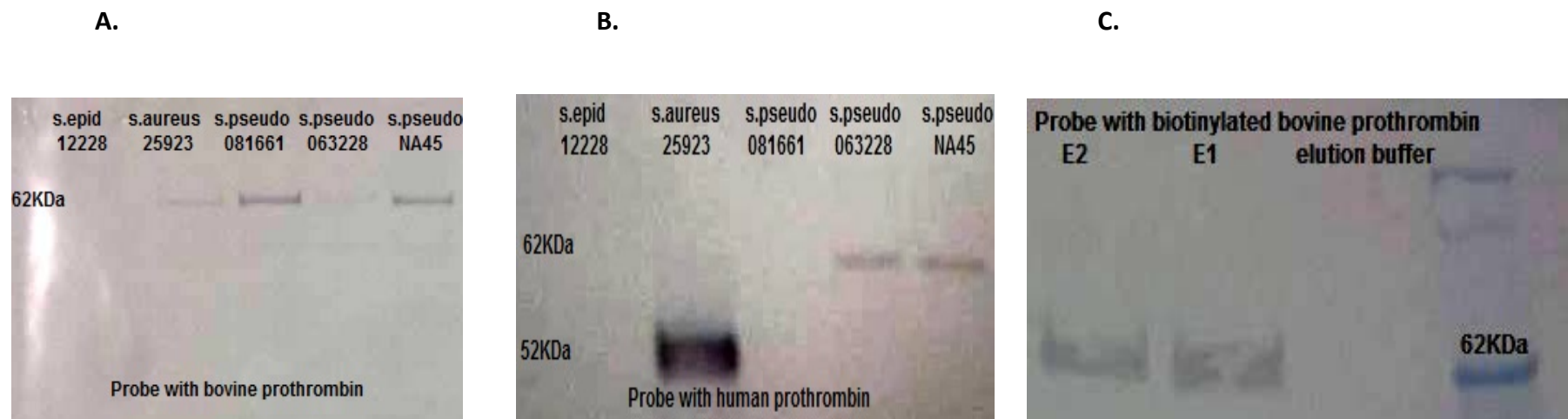


Figure S7. (A) Bovine and human prothrombin binding to overnight concentrated culture supernatant of *Staphylococcus pseudintermedius* strains 081661, 063228 and NA45 along with *S.epidermidis* strain 12228 and *S.aureus* strain 25923 as coagulase positive and negative controls, respectively. Overnight supernatants were concentrated, separated by SDS-PAGE and the immunoreactive bands were detected by western blot analysis using (HRP)-streptavidin conjugate for the biotinylated bovine prothrombin and (B) goat anti-human prothrombin and

HRP rabbit anti-goat IgG (H+L) to detect human prothrombin. (C) Recombinant coagulase from *S. pseudintermedius* strain 081661 detected in a western blot probed with bovine prothrombin (E1 and E2) and stained in a SDS-PAGE gel.