

Supplementary Table 1. *S. equi* survival after exposure to saline solutions for 24 and 48 hrs. At each time point, the CFU observed for the wild type after incubation in the absence of NaCl was set to 100%. Data from triplicate biological replicates.

	% NaCl (w/v)	Wild type	$\Delta prtM_{138-213}$
% Survival after 24 hr exposure	0	100	60
	0.9	147	99
	14.7	5	0
	29.4	1	0
% Survival after 48 hr exposure	0	100	25
	0.9	192	75
	14.7	8	0
	29.4	0	0

Supplementary Figure 1. Alignment showing conserved residues in representative *Firmicutes* PrsA family lipoproteins. Alignment produced with Clustal Omega, with minor manual adjustments. Proteins are aligned from the lipidated cysteine at the N-terminus of the mature protein. The central parvulin domain cloned as rSEQ0694parv is highlighted in bold. Reference sequences are those selected by Jakob *et al.*²⁶ Abbreviations and UniProt accession codes for the sequences are: Ba_PrsA, *Bacillus anthracis* PrsA1 (Q81U45); Bsu_PRSA (Q81U45); *Bacillus subtilis* PrsA (P24327); Lla_PrsA, *Lactococcus lactis* PrsA (P0C2B5); Lmo_PrsA1, *Listeria monocytogenes* PrsA1 (Q71ZM6); LMO_PrsA2, *L. monocytogenes* PrsA2 (Q71XE6); Sau_PrsA, *Staphylococcus aureus* PrsA (A6QI23); SEQ0694, *Streptococcus equi* PrsA (C0M9L5); Sgo_PrsA, *Streptococcus gordonii* PrsA (A8AYJ0); and Spn_PrsA, *Streptococcus pneumoniae* PrsA/PpmA (B2IPD4).

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SEQ0694      CQST-N--DNTSVITMKGDTISVSDFYNETKNTEISQRAMLNLLVSRVFEDQY GKKVSKK
Spn_PrsA     CSKG-S--EGADLISMKGDVITEHQFYEQVKSNPAAQVLLNMTIQKFVEKQY GSELDDK
Sgo_PrsA     CGKTS--SDKDIITMKGDTITVSEFYDKVKSNPAAQVLLNLTIKEVFEKQY GKKVTDK
Sau_PrsA     CGASATDSKENTLISSKAGDVTADTMKKIGKDQIANASF-TEMLNKILADKYKNKVNDK
Lla_PrsA     CQSNQT---DQTVATYSGGKVTESSLYKELKQSPTTKTMLANMLIYRALNHAYGKSVSTK
Ban_PrsA1    CGTSSS---DK-IVTSKAGDITKDEFYEQMKTQAG-KQVLNNMVMMEKVLKNY--KVEDK
Bsu_PrsA     CSSGDK---EV-IAKTDAGDVTIKGELYTNMKTQAG-ASVLTQLVQEKVLDKKY--KVSDK
Lmo_PrsA1    CGS-----SA-VVKTDAGSVTQDELYEAMKTTYG-NEVVQQQLTFKKILEDKY--TVTEK
Lmo_PrsA2    CGGG-----GD-VVKTDSDGVTQDELYDAMKDKYG-SEFVQQQLTFEKILGDKY--KVSDE
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SEQ0694      RTEEAYNKSAEQY--ASFSAALAQSGLTDDTYKRQIRSAMLVEYAVKEAAKKELTDADY
Spn_PrsA     EVDDTIAEEKKQY--ENYQRVLSQAGMTLETRKAQIRTSKLVELAVKKVAEAEELTDEAY
Sgo_PrsA     EVEEAYEKSSKAY--DNFARVLAQAGLTEDAYREQIRTNKLVEYAVKKAEEKELTDENY
Sau_PrsA     KIDEQIEKMQKQYGGKDKFEKALQQQGLTADKYKENLRTAAYHKELLSDK--IKISDSEI
Lla_PrsA     TVNDAYDSYKQY--ENFDAFLSQNGFSRSSFKESLRTNFLSEVALKKL--KKVSESQ
Ban_PrsA1    EVDKKYDEMKKQY--DQFDTLKQQGIKEETLKTGVRAQLAQEKAIEKT--IT--D---
Bsu_PrsA     EIDNKLKEYKTQLG--DQYTALEKQY--GKDYLKEQVKYELLTQKAAKDN--IKVTDADI
Lmo_PrsA1    EVNAEYKKYEEQY--DSFESTLSSNNLTKTSFKENLEYNLLVQKATEAN--MNVSESKL
Lmo_PrsA2    DVDKKFNEYKSQY--DQFSAVLAQSGLTEKSFKSQKYNLLVQKATEAN--TDTSDKTL
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SEQ0694      KKAYESYTPMTTQVTTLDNEE-----TAKAVLGEVKAEGADFAAIAKEK
Spn_PrsA     KKAFFDEYTPDVTAQIIRLNNED-----KAKEVLEKAKAEGADFAQLAKDN
Sgo_PrsA     KAAYEAYTPEVTAQIIKVDSED-----KAKEVLAAAKAEGADFAQLAKDN
Sau_PrsA     KEDS-----KKASHILIKVSKSKSDKEGLDDKEAKQKAEIQQEVSKDPSKGEIAKKE
Lla_PrsA     KAAWKTYQPKVTVQHILTSDED-----TAKQVISD-LAAGKDFAMLAKT
Ban_PrsA1    KELKDNYPKPEIKASHILVKDEA-----TAKKVKEE-LGQKSFEEELAKQY
Bsu_PrsA     KEYWEGLKGKIRASHILVADKK-----TAEVEKK-LKKGEKFTDLAKEY
Lmo_PrsA1    KTYKTYWEPDITVRHILVDDEA-----TAKEIQTK-LKNGEKFTDLAKEY
Lmo_PrsA2    KKYETWQPDITVSHILVADEN-----KAKEVEQK-LKDGAKFADLAKEY
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SEQ0694      TTAAD---KKVDYKFDSGDTKLPAVVIKASGLK-EGDISEVVSVLDPATYQNKFYIVKV
Spn_PrsA     STDEKTKENGGEITFDSASTEVPEQVKKAFAFAD-VDGVSDVITATGTQAYSSQYIVKL
Sgo_PrsA     STDGDTKDKGGEIKFDSAATNVPDAVKKAFAFGL-ANAVSDLVTVRSNQG-QASQYIVKL
Sau_PrsA     SMDTGSAAKKGELGYV-LKGQTDKDFEKAFLFKL-KDEVSEVVK-----SFGYHIIKA
Lla_PrsA     SIDTATKDNKGKISFELNNKTLDFATFKDAAYKLNKGDYDTQTPVKV-----TDGYEVIKM
Ban_PrsA1    SEDTGSKEKGGDLGFF-GAGKMVKEFEDAAFKL-KDEVSEPVKS-----QFGYHIIKV
Bsu_PrsA     STDS-SASKGDLGWFAKEGQMDFTFSKAFAFKL-KDEVSDPVKT-----QYGYHIIKK
Lmo_PrsA1    STDATSTNGGLLDPF-GPGEMDETFEKAAYALENKDDVSGIVKS-----TYGYHIIQL
Lmo_PrsA2    STDATKDNQGQLAPF-GPGKMDPAFEKAAYALKNKGDISAPVKT-----QYGYHIIQM
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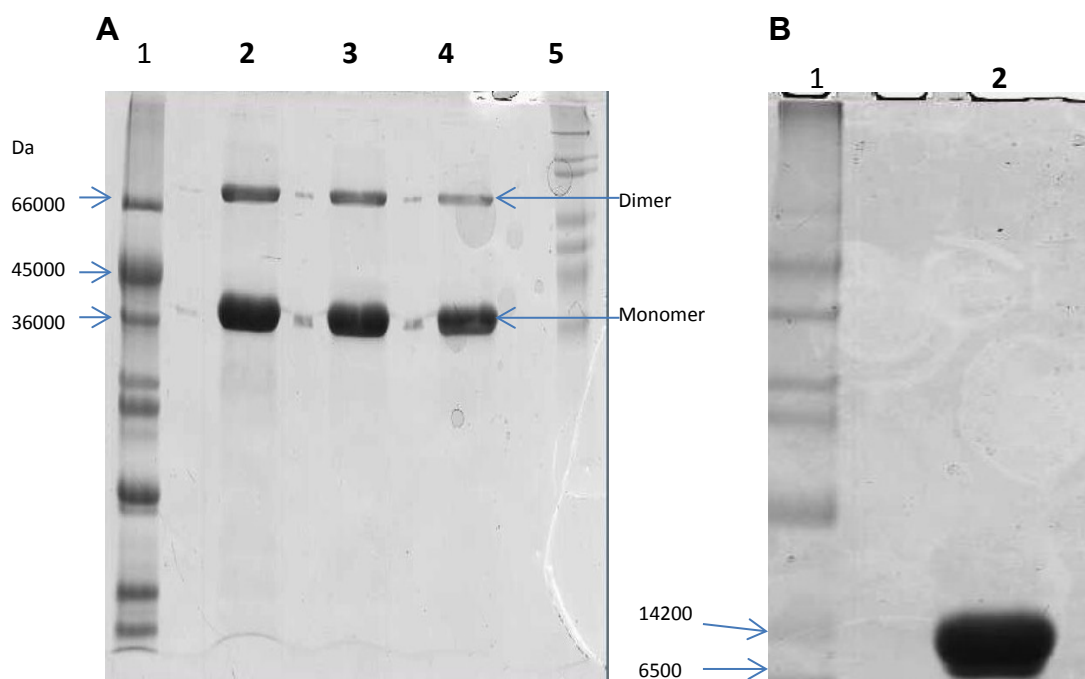
SEQ0694	TKK AEKASDWKKY KK R L KEIVLA E KTQNI-DFQNKVIAKALDKANVKIK D QAFANILAQY
Spn_PrSA	TKKTEKSSNIDDY K EK L KT V ILTQKQ N DS-TFVQSIIGKELQAANIKVK D QAFQNI F TQY
Sgo_PrSA	VSKTEKSSKWEDY K DK L KQVILTAKQ N NT-SFIQSVVAKELKDANIKVK D AA F QNL F SQY
Sau_PrSA	DKPT----DFNSEKQSLKEKLVDQKVQKNPKLLTDAYKD L LKEYDVDFK D RD I KSVVEDK
Lla_PrSA	INHP-AKGTFTSSK K ALTASVYAKWSRDS-SIMQ R VISQVLKNQHVTIK D KDLADALDSY
Ban_PrSA1	TDIKEPEKSFEQSKADIKKELVAKKSQDG-EFMNDLMMKEIKKADV K VD D KDLKDLFEEK
Bsu_PrSA	T--E-ERGKYDDMK K ELKSEVLEQKLNDN-AAVQEAVQKVMKKADIEVK D KDLKDTFNTS
Lmo_PrSA1	VKKT-EKGT Y AKEKANVKAAYIESQLTTE--NMTAALKKELKAANIDIK D SDLKDAFADY
Lmo_PrSA2	DKPA-TKTTFEK D KKAVKASYLESQ L TTE--NMQKTLKKEYKDANVKVE D KDLKDAFKDF

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SEQ0694	ANTDKKASKANTSKSDQKSSSDSSKDSQSSKSKSEK
Spn_PrSA	IGGGDSSSSSSTSNE-----
Sgo_PrSA	TQTNTSSSSSK-----
Sau_PrSA	ILNPEKLQGGGAQGGQSGMSQ-----
Lla_PrSA	KKLATTN-----
Ban_PrSA1	KADAKKEEK-----
Bsu_PrSA	STSNSTSSSSSNSK-----
Lmo_PrSA1	TSTSSTSTTTSN-----
Lmo_PrSA2	DGSSSDSDSSK-----

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Supplementary Figure 2. SDS-PAGE of concentrated IMAC-purified recombinant proteins: A. Production of rSEQ0694. 1, low molecular weight protein standards; 2, 3 and 4, rSEQ0694 preparations; 5, high molecular weight protein standards. B. Production of rSEQ0694parv. 1, low molecular weight protein standards; 2, rSEQ0694parv fraction. Protein bands were excised, trypsinised and analysed by mass spectroscopy. Peptide mass fingerprints of the dimer (upper band in A 2-4) gave a Mascot score of 1304, with 26 peptide matches and 56% protein sequence coverage to SEQ0694. The monomer (lower band in A 2-4) gave a Mascot score of 1475, with 36 peptide matches and 59% protein sequence coverage to SEQ0694. The rSEQ0694parv protein (B) gave a Mascot score of 312, with 6 peptide matches and 51% protein sequence coverage of the central domain of SEQ0694.



Supplementary Figure 3. Recombinant protein stability to chymotrypsin treatment. A, SDS-PAGE analysis of the stability of rSEQ0694. B, SDS-PAGE analysis of the stability of rSEQ0694parv. Lanes in each panel are as follows: (1) High molecular weight protein size standards. (2) PMSF-inactivated chymotrypsin was incubated with the recombinant protein in assay buffer. Recombinant protein incubated with chymotrypsin in assay buffer for 20 s (3), 2 min (4) and 5 min (5) prior to addition of PMSF. (6) Chymotrypsin and PMSF in reaction buffer, no recombinant protein. (7) Recombinant protein and PMSF only. (8) Recombinant protein only. (9) Chymotrypsin only. (10) Low molecular weight protein size standards.

