

SUPPLEMENTARY ONLINE DATA

Characterization of *Staphylococcus aureus* EssB, an integral membrane component of the Type VII secretion system: atomic resolution crystal structure of the cytoplasmic segment

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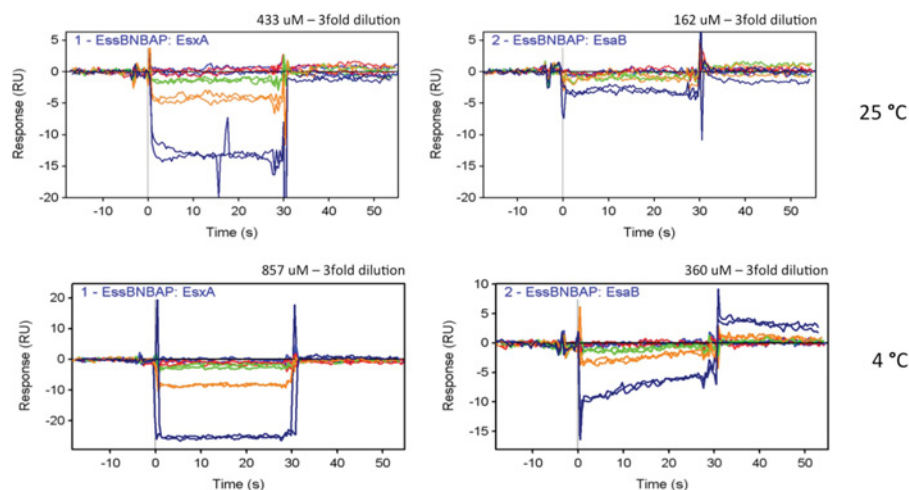


Figure S1 SPR analysis of potential EssB-N interactions

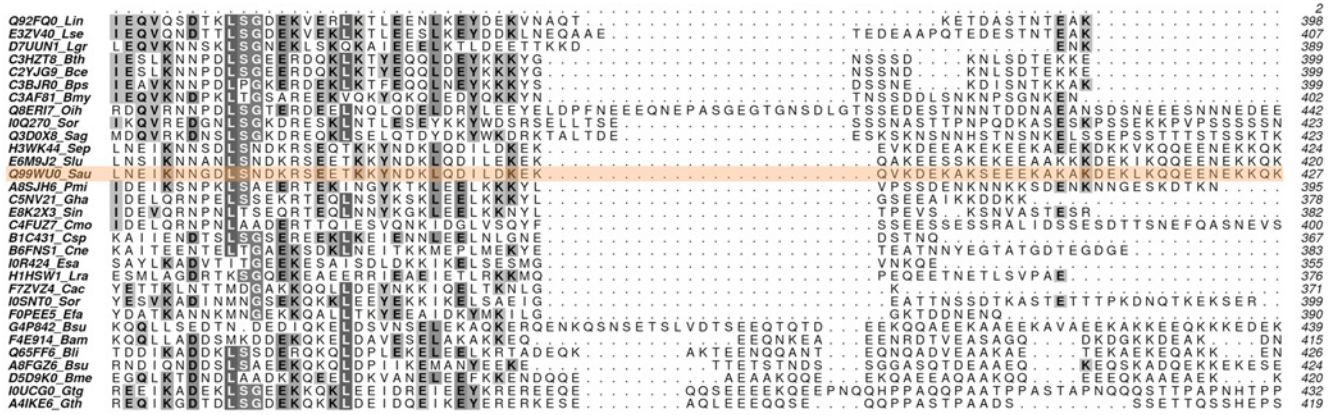
BAP-tagged EssB-N was captured on a streptavidin chip (see the Experimental section of the main text). Binding of EsxA (left-hand panels) and EsaB (right-hand panels) was measured at 25 °C (upper panels) and 4 °C (lower panels). The curves correspond to a dilution series starting from the concentration indicated (dark blue curve). Shown are the binding curves after subtracting binding to the empty reference chip. The negative response signals obtained suggest non-specific binding (see the Results and discussion section of the main text).

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The atomic co-ordinates and structure factors have been deposited in the PDB under code 4ANN.



Figure S2 Multiple sequence alignment of ESB-N orthologues



<i>Q92FQ0_Lin</i>	398
<i>E32V40_Lse</i>	407
<i>D7UUNT_Lgr</i>	389
<i>C3HZT8_Bth</i>	399
<i>C2YJG9_Bce</i>	399
<i>C3BJR0_Bps</i>	399
<i>C3JAF1_Bmy</i>	402
<i>Q8ER17_Oih</i>	442
<i>I0Q270_Sor</i>	423
<i>Q3D0X8_Sag</i>	425
<i>H3WK44_Sep</i>	441
<i>E6M9J2_Slu</i>	437
<i>Q89WU0_Sau</i>	444
<i>A8SJH6_Pmi</i>	395
<i>C5NV21_Gha</i>	378
<i>E8K2X3_Sin</i>	382
<i>C4FUZ7_Cmo</i>	414
<i>B1C431_Csp</i>	367
<i>B6FNS1_Cne</i>	383
<i>I0R424_Esa</i>	355
<i>H1HSW1_Lra</i>	376
<i>F7ZV24_Cac</i>	371
<i>ISONT0_Sor</i>	399
<i>F0PEE5_Eta</i>	390
<i>G4P842_Bsu</i>	452
<i>F4E914_Bam</i>	429
<i>G65FF6_Bli</i>	438
<i>A8FGZ6_Bsu</i>	438
<i>D5D9K0_Bme</i>	434
<i>IUUCG0_Gtg</i>	446
<i>A4IKE6_Gth</i>	428

Figure S2 Multiple sequence alignment of EssB-N orthologues (cont.)

EssB-N secondary structure elements and the predicted transmembrane span (TM) are indicated. Selected EssB-N sequences are titled with a UniProt identifier and species abbreviation. Lin, *Listeria innocua*; Lse, *Listeria seeligeri*; Lgr, *Listeria grayi*; Bth, *Bacillus thuringiensis*; Bce, *Bacillus cereus*; Bps, *Bacillus pseudomycoloides*; Bmy, *Bacillus mycoloides*; Oih, *Oceanobacillus ihayensis*; Sor, *Streptococcus oralis*; Sag, *Streptococcus agalactiae*; Sep, *Staphylococcus epidermis*; Slu, *Staphylococcus lugdunensis*; Sau, *Staphylococcus aureus*; Pmi, *Parvimonas micra*; Gha, *Gemella haemolysans*; Sin, *Streptococcus infantis*; Cmo, *Catonella morbi*; Csp, *Clostridium spiroforme*; Cne, *Clostridium nexile*; Esa, *Eubacterium saburreum*; Lra, *Lachnospiraceae bacterium*; Cac, *Clostridium acetobutylicum*; Eta, *Enterococcus faecalis*; Bsu, *Bacillus subtilis*; Bam, *Bacillus amyloliquefaciens*; Bli, *Bacillus licheniformis*; Bme, *Bacillus megaterium*; Gtg, *Geobacillus thermoglucosidans*; and Gth, *Geobacillus thermodenitrificans*.

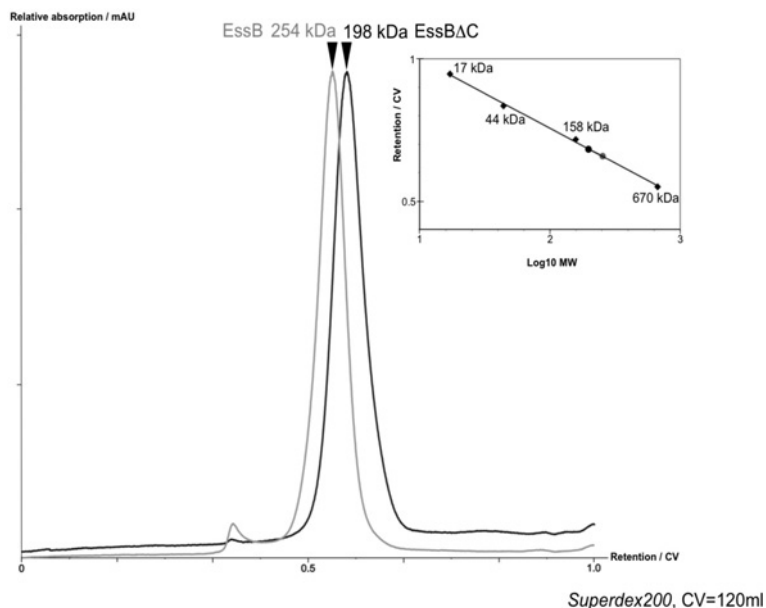


Figure S3 Analytical gel filtration of detergent-solubilized EssB

Overlay of size-exclusion chromatography chromatograms for EssB and truncated EssB Δ C. Protein (5 mg) was loaded on to a CV of 120 ml Superdex 200 (prep grade; GE Healthcare) column equilibrated with 10 mM sodium phosphate, 50 mM NaCl, 1 mM DTT, 1 μ g/ml DOPC and 0.02% DDM. Peaks are labelled with apparent molecular masses calculated from a previously determined calibration curve (inset; see the Experimental section of the main text).

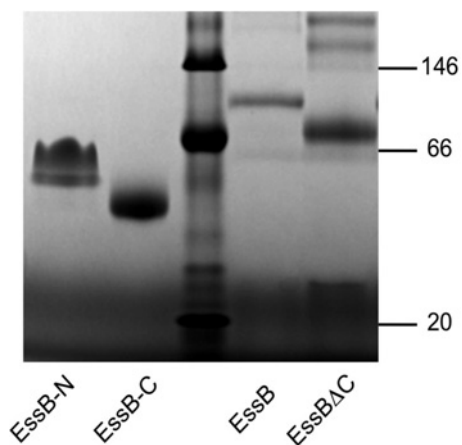


Figure S4 BN-PAGE analysis of the EssB constructs

BN-PAGE of purified EssB-N (far-left lane), EssB-C (near-left lane), EssB (near-right lane) and EssB Δ C (far-right lane). All constructs are migrating as dimers with apparent molecular masses of 58 kDa, 42 kDa, 98 kDa and 72 kDa, derived by comparison with the molecular mass standards (centre lane, masses given in kDa on the right-hand side; see the Experimental section of the main text). For EssB-N an additional band at 51 kDa is visible that corresponds to the degradation product (see the Results and discussion section of the main text).

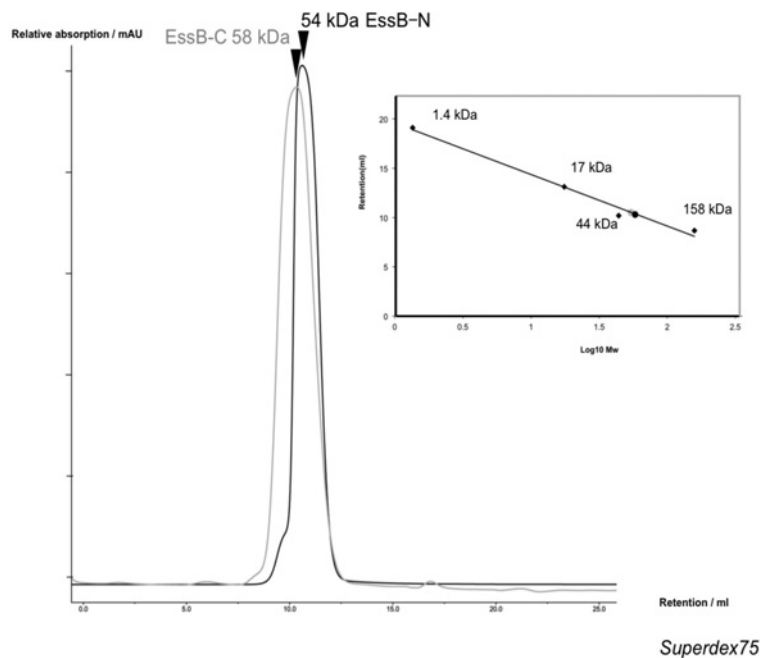


Figure S5 Analytical gel filtration of EssB isolated soluble domains

Overlay of size-exclusion chromatography chromatograms for EssB-N and EssB-C. Protein (8 mg) was loaded on a CV of 24 ml Superdex 75 (prep grade, GE Healthcare) column equilibrated with 10 mM sodium phosphate, 50 mM NaCl and 1 mM DTT. Peaks are labelled with apparent molecular masses calculated from the calibration curve (inset; see the Experimental section of the main text).

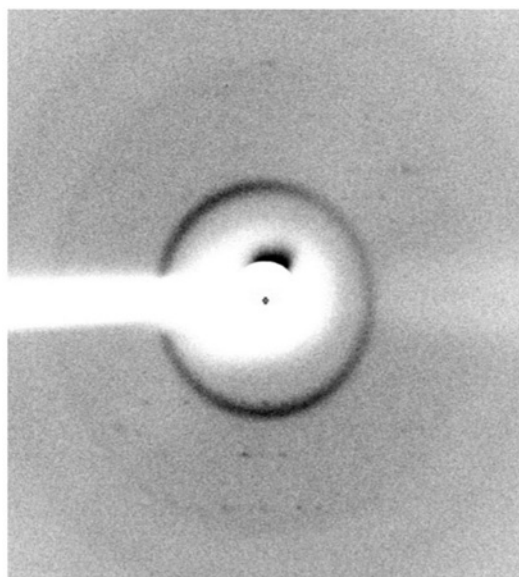


Figure S6 Diffraction pattern from EssB-C crystals

An example diffraction image of EssB-C crystals recorded using in-house facilities (see the Experimental section of the main text).

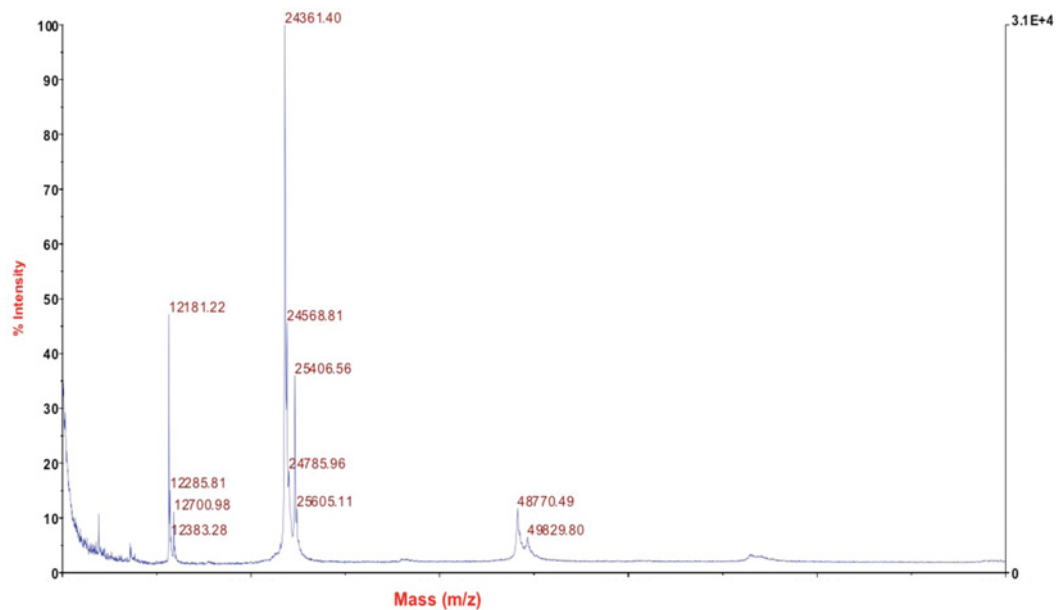


Figure S7 ESI-Q-TOF-MS analysis of purified EssB-N

ESI-Q-TOF-MS analysis of the EssB-N sample prior to crystallization (see the Results and discussion section of the main text).

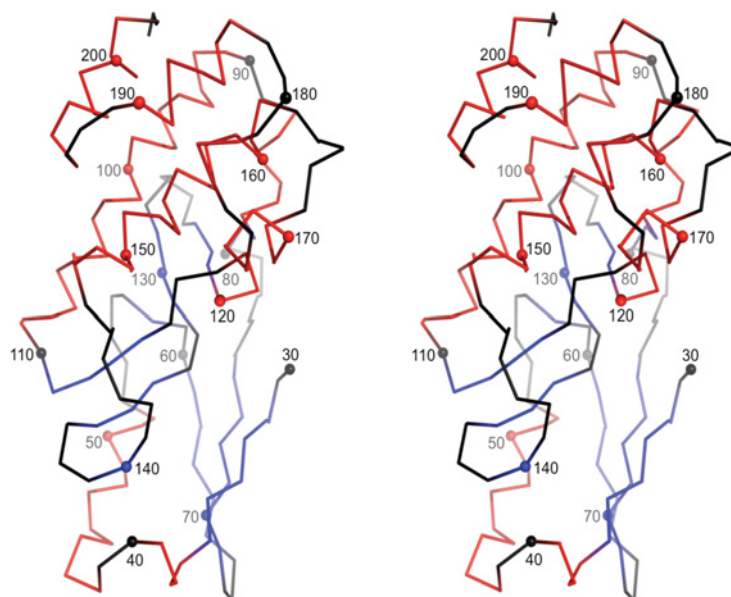


Figure S8 EssB-N structure, stereo view of the C α trace

The chain is coloured by secondary structure elements (black, loop; red, helix; and blue, strand) and residue numbers are indicated at every tenth C α (shown as spheres).

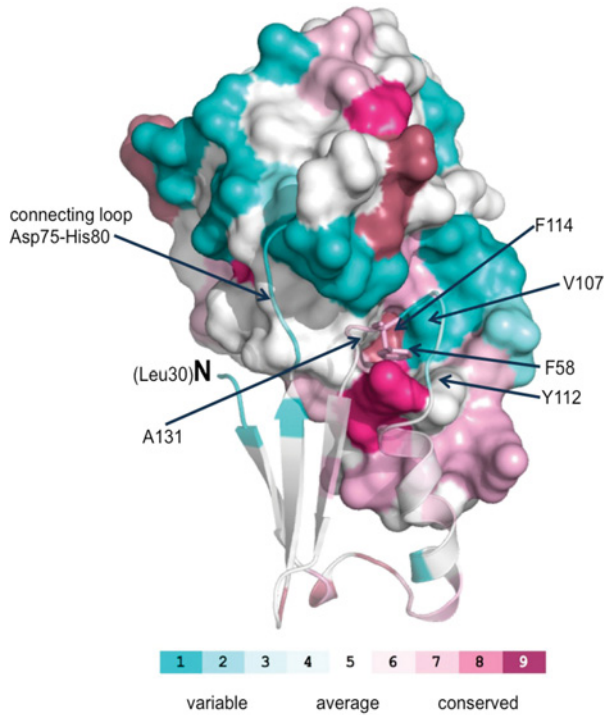


Figure S9 Conservation mapping on to the EssB-N C-terminal domain surface

The continuous conservation scores are normalized and partitioned into nine discrete conservation bins (darkest pink indicates the most conserved), reflecting the relative degree of conservation of each amino acid position (see the Experimental section of the main text).

Table S1 Primers used to generate samples for the bacterial two-hybrid experiments

Restriction sites are in bold.

Construct	Primers (forward/reverse)
pT18EssB-N	5'-GCGC CTCGAG GAAAAACCATAACCCGAAAAACGAAATG-3' 5'-GCGC AAAGCTT GCCACGGTATGGCCACTTTGCGCAC-3'
pT25EsxA	5'-GCGC GGATCC CGCGATGATTAATAAGAGCCGGAAG-3' 5'-GCGC GGTACC TACTGCAGGCCAAAGTTGTTGCTCAGC-3'
pT25EsxB	5'-GCGC GGATCC CGGCGGCTATAAAGGCATTAAGC-3' 5'-GCGC GGTACC TACGGGTTACGCGATCCAGGCCCTG-3'
pT25EsxB	5'-GCGC GGATCC CAATCAGCACGTAAGTAAC-3' 5'-GCGC GGTACC TACAGCAGTTTCAGAATATCGCCATC-3'
pT25EsaB	5'-GCGC GGATCC CAACTTTAACGATATTGAAACGATG-3' 5'-GCGC GGTACC TAGTTCATCGCTTTGTTAAATATTCGCTCG-3'

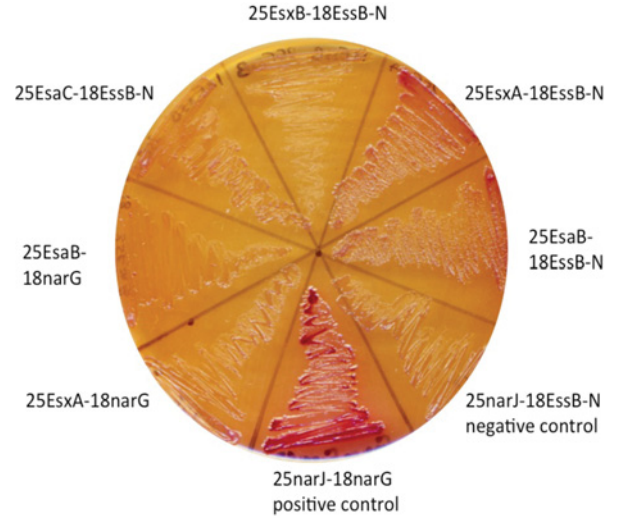


Figure S10 Bacterial two-hybrid assay

E. coli BTH101 cells were co-transformed with pT25 constructs encoding EsxA, EsxB, EsaB and EsaC fusions in combination with pT18EssB or either pT25narJ or pT18narG as a negative control. The combination pT25narJ/pT18narG, producing a stable dimer, served as a positive control. Initial colonies were re-streaked on to fresh MacConkey/maltose plates and incubated for 36 h at 25 °C.

Table S2 Primers used to generate samples for the SPR experiments

Restriction sites are in bold.

Construct	Primers (forward/reverse)
pT18EssB-N	5'-GCGC CTCGAG GAAAAACCATAACCCGAAAAACGAAATG-3' 5'-GCGC AAAGCTT GCCACGGTATGGCCACTTTGCGCAC-3'
pT25EsxA	5'-GCGC GGATCC CGCGATGATTAATAAGAGCCGGAAG-3' 5'-GCGC GGTACC TACTGCAGGCCAAAGTTGTTGCTCAGC-3'
pT25EsxB	5'-GCGC GGATCC CGGCGGCTATAAAGGCATTAAGC-3' 5'-GCGC GGTACC TACGGGTTACGCGATCCAGGCCCTG-3'
pT25EsxB	5'-GCGC GGATCC CAATCAGCACGTAAGTAAC-3' 5'-GCGC GGTACC TACAGCAGTTTCAGAATATCGCCATC-3'
pT25EsaB	5'-GCGC GGATCC CAACTTTAACGATATTGAAACGATG-3' 5'-GCGC GGTACC TAGTTCATCGCTTTGTTAAATATTCGCTCG-3'

Table S3 Tryptic fragments identified from analysis of EssB-N crystals

MALDI-TOF-MS analysis of EssB-N after tryptic digest (for details see the Experimental section of the main text). The N- and C-terminal peptides are highlighted in blue and red respectively. $M_r(\text{calc})$, calculated M_r ; $M_r(\text{expt})$, expected M_r .

Query	Observed	$M_r(\text{expt})$	$M_r(\text{calc})$	p.p.m.	Miss	Score	Expect	Rank	Peptide
367	582.810440	1163.606328	1163.600861	4.70	0	75	3.4e-08	1	K.ALVICAFNEK.Q
368	582.810440	1163.606328	1163.600861	4.70	0	(56)	2.5e-06	1	K.ALVICAFNEK.Q
369	582.810440	1163.606328	1163.600861	4.70	0	(37)	0.00018	1	K.ALVICAFNEK.Q
370	582.810440	1163.606328	1163.600861	4.70	0	(44)	4.5e-05	1	K.ALVICAFNEK.Q
373	583.309937	1164.605322	1163.600861	863	0	(9)	0.12	1	K.ALVICAFNEK.Q
415	614.743591	1227.472630	1227.468002	3.77	0	48	1.5e-05	1	K.QEQDYSQNYA.- + Gln->pyro-Glu (N-term)
416	614.743591	1227.472630	1227.468002	3.77	0	(28)	0.0016	1	K.QEQDYSQNYA.- + Gln->pyro-Glu (N-term)
420	623.257599	1244.500646	1244.494537	4.91	0	(26)	0.0027	1	K.QEQDYSQNYA.-
421	623.257599	1244.500646	1244.494537	4.91	0	(33)	0.00046	1	K.QEQDYSQNYA.-
422	623.257599	1244.500646	1244.494537	4.91	0	(21)	0.0086	1	K.QEQDYSQNYA.-
423	623.257599	1244.500646	1244.494537	4.91	0	(20)	0.0095	1	K.QEQDYSQNYA.-
425	623.752838	1245.491124	1244.494537	801	0	(10)	0.096	1	K.QEQDYSQNYA.-
426	623.752838	1245.491124	1244.494537	801	0	(12)	0.058	1	K.QEQDYSQNYA.-
607	841.926209	1681.837866	1681.831146	4.00	0	(51)	7.6e-06	1	K.QSFDALVEGNLELHK.G + glycine-> pyroglutamate (N-terminus)
608	841.926209	1681.837866	1681.831146	4.00	0	(70)	1e-07	1	K.QSFDALVEGNLELHK.G + glycine-> pyroglutamate (N-terminus)
609	841.926209	1681.837866	1681.831146	4.00	0	(28)	0.0016	1	K.QSFDALVEGNLELHK.G + glycine-> pyroglutamate (N-terminus)
615	850.441223	1698.867894	1698.857681	6.01	0	75	2.9e-08	1	K.QSFDALVEGNLELHK.G
616	850.441223	1698.867894	1698.857681	6.01	0	(22)	0.0057	1	K.QSFDALVEGNLELHK.G
617	850.441223	1698.867894	1698.857681	6.01	0	(47)	1.9e-05	1	K.QSFDALVEGNLELHK.G
618	850.441223	1698.867894	1698.857681	6.01	0	(17)	0.022	1	K.QSFDALVEGNLELHK.G
333	567.298391	1698.873345	1698.857681	9.22	0	(12)	0.064	1	K.QSFDALVEGNLELHK.G
334	567.298391	1698.873345	1698.857681	9.22	0	(9)	0.13	1	K.QSFDALVEGNLELHK.G
335	567.298391	1698.873345	1698.857681	9.22	0	(11)	0.079	1	K.QSFDALVEGNLELHK.G
336	567.298391	1698.873345	1698.857681	9.22	0	(26)	0.0023	1	K.QSFDALVEGNLELHK.G
619	567.298391	1698.873345	1698.857681	9.22	0	(54)	4.1e-06	1	K.QSFDALVEGNLELHK.G
620	567.298391	1698.873345	1698.857681	9.22	0	(33)	0.00053	1	K.QSFDALVEGNLELHK.G
621	567.298391	1698.873345	1698.857681	9.22	0	(39)	0.00014	1	K.QSFDALVEGNLELHK.G
622	567.298391	1698.873345	1698.857681	9.22	0	(22)	0.0063	1	K.QSFDALVEGNLELHK.G
623	567.298391	1698.873345	1698.857681	9.22	0	(26)	0.0025	1	K.QSFDALVEGNLELHK.G
624	567.298391	1698.873345	1698.857681	9.22	0	(21)	0.0072	1	K.QSFDALVEGNLELHK.G
626	567.298391	1698.873345	1698.857681	9.22	0	(12)	0.061	1	K.QSFDALVEGNLELHK.G
337	567.629924	1699.867944	1698.857681	595	0	(19)	0.014	1	K.QSFDALVEGNLELHK.G
338	567.629924	1699.867944	1698.857681	595	0	(23)	0.0056	1	K.QSFDALVEGNLELHK.G
339	567.629924	1699.867944	1698.857681	595	0	(49)	1.3e-05	1	K.QSFDALVEGNLELHK.G
345	573.634064	1717.880364	1717.871567	5.12	0	(52)	6.1e-06	1	R.YTFVLAPDELFFTR.D
633	573.634064	1717.880364	1717.871567	5.12	0	(51)	7.1e-06	1	R.YTFVLAPDELFFTR.D
634	573.634064	1717.880364	1717.871567	5.12	0	(55)	3.1e-06	1	R.YTFVLAPDELFFTR.D
635	573.634064	1717.880364	1717.871567	5.12	0	(20)	0.0092	1	R.YTFVLAPDELFFTR.D
636	859.948171	1717.881790	1717.871567	5.95	0	61	8e-07	1	R.YTFVLAPDELFFTR.D
637	859.948171	1717.881790	1717.871567	5.95	0	(51)	8.1e-06	1	R.YTFVLAPDELFFTR.D
638	859.948171	1717.881790	1717.871567	5.95	0	(45)	3.3e-05	1	R.YTFVLAPDELFFTR.D
639	859.948171	1717.881790	1717.871567	5.95	0	(17)	0.022	1	R.YTFVLAPDELFFTR.D
640	859.948171	1717.881790	1717.871567	5.95	0	(36)	0.00023	1	R.YTFVLAPDELFFTR.D
641	859.948171	1717.881790	1717.871567	5.95	0	(12)	0.061	1	R.YTFVLAPDELFFTR.D
643	860.451172	1718.887792	1717.871567	592	0	(18)	0.015	1	R.YTFVLAPDELFFTR.D
684	621.960266	1862.858970	1862.856750	1.19	0	(21)	0.0089	1	-GASTQDMLTPLDAEEAAK.T + oxidation (M)
685	932.437927	1862.861302	1862.856750	2.44	0	87	2.1e-09	1	-GASTQDMLTPLDAEEAAK.T + oxidation (M)
686	932.437927	1862.861302	1862.856750	2.44	0	(51)	7.2e-06	1	-GASTQDMLTPLDAEEAAK.T + oxidation (M)
741	659.353699	1975.039269	1975.020355	9.58	1	57	1.9e-06	1	R.TRYTFVLAPDELFFTR.D
742	659.353699	1975.039269	1975.020355	9.58	1	(21)	0.0073	1	R.TRYTFVLAPDELFFTR.D
459	659.687622	1976.041038	1975.020355	517	1	(23)	0.005	1	R.TRYTFVLAPDELFFTR.D
504	695.376800	2083.108572	2083.094971	6.53	0	(37)	0.00021	1	R.GLQNVVDPLPVSEAEFLTR.Y
749	695.376800	2083.108572	2083.094971	6.53	0	(62)	5.8e-07	1	R.GLQNVVDPLPVSEAEFLTR.Y
750	695.376800	2083.108572	2083.094971	6.53	0	(87)	2.2e-09	1	R.GLQNVVDPLPVSEAEFLTR.Y
751	695.376800	2083.108572	2083.094971	6.53	0	104	4.4e-11	1	R.GLQNVVDPLPVSEAEFLTR.Y
752	695.376800	2083.108572	2083.094971	6.53	0	(60)	9.8e-07	1	R.GLQNVVDPLPVSEAEFLTR.Y
753	695.376800	2083.108572	2083.094971	6.53	0	(40)	0.0001	1	R.GLQNVVDPLPVSEAEFLTR.Y
754	1042.563375	2083.112198	2083.094971	8.27	0	(94)	3.9e-10	1	R.GLQNVVDPLPVSEAEFLTR.Y
755	1042.563375	2083.112198	2083.094971	8.27	0	(68)	1.7e-07	1	R.GLQNVVDPLPVSEAEFLTR.Y
756	1042.563375	2083.112198	2083.094971	8.27	0	(70)	9.7e-08	1	R.GLQNVVDPLPVSEAEFLTR.Y
757	1042.563375	2083.112198	2083.094971	8.27	0	(90)	1e-09	1	R.GLQNVVDPLPVSEAEFLTR.Y
758	1042.563375	2083.112198	2083.094971	8.27	0	(76)	2.6e-08	1	R.GLQNVVDPLPVSEAEFLTR.Y
759	1042.563375	2083.112198	2083.094971	8.27	0	(27)	0.0019	1	R.GLQNVVDPLPVSEAEFLTR.Y
505	695.709351	2084.106225	2083.094971	485	0	(39)	0.00011	1	R.GLQNVVDPLPVSEAEFLTR.Y
760	695.709351	2084.106225	2083.094971	485	0	(31)	0.00074	1	R.GLQNVVDPLPVSEAEFLTR.Y
761	1043.061279	2084.108006	2083.094971	486	0	(44)	3.8e-05	1	R.GLQNVVDPLPVSEAEFLTR.Y

Table S3 Continued

Query	Observed	M_r (expt)	M_r (calc)	p.p.m.	Miss	Score	Expect	Rank	Peptide
762	1043.061279	2084.108006	2083.094971	486	0	(24)	0.0036	1	R.GLQNVDPPLPVSEAEFLTR.Y
801	1141.108002	2280.201452	2280.188904	5.50	0	127	1.8e-13	1	K.VIEAATLDLLTAFLDEQYQK.Q
802	1141.108002	2280.201452	2280.188904	5.50	0	(104)	3.9e-11	1	K.VIEAATLDLLTAFLDEQYQK.Q
803	1141.108002	2280.201452	2280.188904	5.50	0	(105)	2.9e-11	1	K.VIEAATLDLLTAFLDEQYQK.Q
804	1141.108002	2280.201452	2280.188904	5.50	0	(85)	3.4e-09	1	K.VIEAATLDLLTAFLDEQYQK.Q
805	761.075882	2280.205818	2280.188904	7.42	0	(88)	1.6e-09	1	K.VIEAATLDLLTAFLDEQYQK.Q
806	761.075882	2280.205818	2280.188904	7.42	0	(89)	1.2e-09	1	K.VIEAATLDLLTAFLDEQYQK.Q
807	761.075882	2280.205818	2280.188904	7.42	0	(76)	2.5e-08	1	K.VIEAATLDLLTAFLDEQYQK.Q
808	761.075882	2280.205818	2280.188904	7.42	0	(77)	1.8e-08	1	K.VIEAATLDLLTAFLDEQYQK.Q
558	761.411194	2281.211754	2280.188904	449	0	(49)	1.2e-05	1	K.VIEAATLDLLTAFLDEQYQK.Q
559	761.411194	2281.211754	2280.188904	449	0	(42)	6.6e-05	1	K.VIEAATLDLLTAFLDEQYQK.Q
828	805.104858	2412.292746	2412.272934	8.21	1	23	0.0047	1	R.YTFVLAPDELFFTRDGLPIAK.T
829	805.104858	2412.292746	2412.272934	8.21	1	(23)	0.005	1	R.YTFVLAPDELFFTRDGLPIAK.T
830	609.037720	2432.121776	2432.103302	7.60	0	(14)	0.043	1	R.DSFQIHYDINDNHTPFQDNK.S
831	811.714905	2432.122887	2432.103302	8.05	0	37	0.0002	1	R.DSFQIHYDINDNHTPFQDNK.S
832	1217.069397	2432.124242	2432.103302	8.61	0	(9)	0.13	1	R.DSFQIHYDINDNHTPFQDNK.S
906	911.717468	3642.840768	3642.818039	6.24	0	32	0.0006	1	K.SSIKPEHFHLMYLLLEQHSPLYFIDAE TELR.D
907	729.577667	3642.851955	3642.818039	9.31	0	(15)	0.033	1	K.SSIKPEHFHLMYLLLEQHSPLYFIDAE TELR.D
908	729.577667	3642.851955	3642.818039	9.31	0	(15)	0.031	1	K.SSIKPEHFHLMYLLLEQHSPLYFIDAE TELR.D
910	738.368835	3686.807795	3684.828598	537	0	(2)	0.7	1	K.SSIKPEHFHLMYLLLEQHSPLYFIDAE TELR.D acetyl

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