Supplementary Data A

Proteomic data and MS/MS fragmentation spectra from 2-DE and GeLC-MS/MS analysis of the bovine teat canal lining and the outer teat skin epithelium

Table A1: 2-DE proteins spots identified by nanoLC-ESI-Q\TOF MS\MS.

Spot #	Identified protein (species)	NCBI accession code	Predicted MW (kDa)	Predicted pI	No. peptides matched	Sequence coverage (%)	Mowse score
1	heat shock cognate 71 kDa protein	gi|148887198	71.2	5.2	31	51.8	2138.0
2	serum albumin precursor	gi|30794280	69.3	5.8	51	70.5	3372.3
3	Chain A, Crystal Structure Of Bovine Serum Albumin	gi|367460260	66.4	5.5	47	75.1	3023.4
4	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 4-like	gi|296473654	64.1	9.3	16	25.1	1124.3
5	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	41	53.3	2870.5
6	keratin, type I cytoskeletal 10	gi|27805977	54.8	4.9	40	63.7	2859.4
7	keratin, type I cytoskeletal 10	gi|296476308	54.5	4.8	40	59.5	3249.4
8	keratin, type I cytoskeletal 10	gi|27805977	54.8	4.9	43	64.1	2819.8
9	keratin, type I cytoskeletal 10	gi|27805977	54.8	4.9	32	54.8	2742.7
10	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	26	34.7	1472.5
	PREDICTED: keratin, type II cytoskeletal 3	gi|358421417	64.7	9	21	23.3	1177.6
11	PREDICTED: keratin, type II cytoskeletal 3	gi|358421417	64.7	9	26	31.8	1373.9
	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	23	40.6	1218.3
12	PREDICTED: keratin, type II cytoskeletal 3	gi|358421417	64.7	9	27	36.7	1554.4
	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	26	44.9	1505.5
13	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	52	58.4	3737.4
14	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	38	53.1	2663.4
15	PREDICTED: keratin, type II cytoskeletal 3	gi|358421417	64.7	9	49	50.6	2867.9
	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	28	46.9	1682.7
16	PREDICTED: keratin, type II cytoskeletal 1	gi|297474460	63.1	9	55	64.5	4058.3
17	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	41	59.9	2516.1
	PREDICTED: keratin, type II cytoskeletal 3	gi|358421417	64.7	9	33	40.6	1767.0

18	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	62	64.5	4539.8
19	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	49	64.4	3279.9
20	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	44	60.7	3178.0
21	PREDICTED: SERPIN B3-like	gi|359079289	41.7	5.8	27	62.3	1945.8
22	annexin A2	gi|73586982	38.5	8.5	25	64	1393.0
23	keratin, type I cytoskeletal 17	gi|160395544	48.7	4.9	43	67.3	3166.4
24	keratin, type II cytoskeletal 4	gi|148356276	58	8.6	49	57.7	3306.3
25	Chain A, The Structure Of Crystalline Profilin-Beta-Actin	gi|313507212	41.7	5.1	23	60.5	1503.9
26	keratin, type II cytoskeletal 4	gi|148356276	58	8.6	45	56.3	2778.6
	keratin, type I cytoskeletal 14	gi|262118301	51.8	4.9	24	50.3	1519.4
27	keratin, type II cytoskeletal 4	gi|148356276	58	8.6	35	58.8	1952.5
	keratin, type II cytoskeletal 5	gi|296487899	62.4	7.6	27	38.9	1414.4
28	keratin, type II cytoskeletal 4	gi|148356276	58	8.6	39	60.5	2217.8
	keratin 6A	gi|296487872	60.8	9	34	46.6	2014.5
	keratin, type II cytoskeletal 5	gi|296487899	62.4	7.6	19	25.5	1049.9
	keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	13	16.3	719.8
	SERPIN B4 protein	gi|151557009	38.6	6.2	11	32.4	612.9
29	keratin, type II cytoskeletal 4	gi|148356276	58	8.6	35	60.3	2132.8
	keratin, type II cytoskeletal 5	gi|296487899	62.4	7.6	24	39	1361.0
	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	16	27.6	948.1
	keratin, type II cytoskeletal 6A	gi|134085706	60.8	9	16	24.5	914.9
	SERPIN B4 protein	gi|151557009	38.6	6.2	13	42.2	737.2
30	keratin, type II cytoskeletal 5	gi|296487899	62.4	7.6	54	55.1	3314.8
	keratin, type II cytoskeletal 4	gi|148356276	58	8.6	33	57.7	1884.9
	keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	28	46.2	1615.2
	keratin 6A	gi|296487872	60.8	9	22	30.5	1241.5
	PREDICTED: keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	20	33.3	1194.0

31	PREDICTED: SERPIN B3-like	gi|359079289	41.7	5.8	28	60.7	1983.0
32	PREDICTED: keratin, type II cytoskeletal 6A isoform 3	gi|76617862	60.8	9.4	33	47.3	2108.2
	keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	33	42.1	1938.2
	keratin, type II cytoskeletal 6A	gi|134085706	60.8	9	31	43.4	1959.8
33	keratin 6A	gi|296487872	60.8	9	38	53.4	2356.4
	keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	34	43.9	2014.5
34	keratin, type II cytoskeletal 4	gi|148356276	58	8.6	46	65.9	3006.0
	keratin, type II cytoskeletal 6A	gi|134085706	60.8	9	43	55.5	2776.1
	PREDICTED: keratin, type II cytoskeletal 6A isoform 3	gi|76617862	60.8	9.3	38	49.7	2351.1
	keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	35	53.8	2149.8
35	keratin 6A	gi|296487872	60.8	9	52	57.1	3157.8
	PREDICTED: keratin, type II cytoskeletal 6A isoform 3	gi|76617862	60.8	9.4	47	55.5	2735.3
	keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	40	54.2	2402.0
	keratin, type II cytoskeletal 4	gi|148356276	58	8.6	31	55.9	1557.5
36	keratin, type II cytoskeletal 6A	gi|134085706	60.8	9	46	60.1	3067.9
	PREDICTED: keratin, type II cytoskeletal 6A isoform 3	gi|76617862	60.8	9.4	43	57.1	2787.0
	keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	40	60.7	2541.2
37	keratin 6A	gi|296487872	60.8	9	36	56.9	2587.8
	PREDICTED: keratin, type II cytoskeletal 6A isoform 3	gi|76617862	60.8	9.4	36	56.6	2501.9
	keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	35	57.2	2437.7
38	keratin, type II cytoskeletal 6A	gi|134085706	60.8	9	44	58.7	2899.4
	PREDICTED: keratin, type II cytoskeletal 6A isoform 3	gi|76617862	60.8	9.4	40	54.8	2594.0
	keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	39	59.6	2423.5
39	EEF1A1 protein	gi|86827651	50.1	9.7	13	34.6	804.0
40	PREDICTED: SERPIN B3-like	gi|359079289	41.7	5.8	25	56.4	1639.4
	PREDICTED: SERPIN B4	gi|358420938	44.5	6.1	23	45.9	1516.0
41	PREDICTED: SERPIN B4-like	gi|119923094	44.3	6.5	31	66.2	2167.7

42	PREDICTED: SERPIN B4-like	gi|119923094	44.3	6.5	44	72.1	3037.9
43	alpha S1 casein, partial	gi|159793209	22.4	4.6	13	46.7	760.3
44	caspase-14	gi|329663440	27.5	4.8	17	62.4	1267.2
45	14-3-3 protein sigma (ovis aries)	gi|57163961	27.8	4.5	27	68.1	1787.3
	PREDICTED: keratin, type II cytoskeletal 1	gi|297474460	63.1	9	10	10.1	474.0
46	*spot not identified*						
47	PREDICTED: SERPIN B3-like	gi|359079289	41.7	5.8	11	27.4	747.0
48	PREDICTED: SERPIN B3-like	gi|119916469	44.2	6.5	12	31.5	787.7
49	annexin I	gi|74	38.9	6.5	20	51.7	1396.7
50	annexin A2	gi|73586982	38.5	8.5	36	74.6	2154.9
51	glyceraldehyde-3-phosphate dehydrogenase	gi|77404273	35.8	9.3	22	51.4	1190.5
52	glyceraldehyde-3-phosphate dehydrogenase	gi|77404273	35.8	9.3	37	64.3	2358.7
53	glyceraldehyde-3-phosphate dehydrogenase	gi|77404273	35.8	9.3	22	51.1	1312.1
54	gasdermin-A	gi|118151260	49.9	5.3	23	38.3	1426.5
55	heat shock protein beta-1	gi|71037405	22.7	5.7	15	76.5	1015.2
56	phosphoglycerate mutase 1	gi|77404217	28.8	6.8	20	58.3	1299.2
57	triosephosphate isomerase	gi|61888856	26.7	6.5	20	84.7	1383.8
58	protein S100A9	gi|114052490	16.4	6.3	19	66.7	1314.3
59	protein S100A9	gi|114052490	16.4	6.3	26	63.9	2124.6
60	PREDICTED: non-specific cytotoxic cell receptor protein 1 homolog	gi|76641449	29.5	6.4	15	56.5	1104.6
61	Chain X, The Cys121ser Mutant Of Beta-Lactoglobulin	gi|49259423	18.3	4.6	18	77.2	1191.4
62	Chain X, The Cys121ser Mutant Of Beta-Lactoglobulin	gi|49259423	18.3	4.6	8	45.1	400.2
63	calmodulin	gi|640294	16.3	3.9	9	34	455.6
64	caspase-14	gi|329663440	27.5	4.8	14	39.7	917.1
65	PREDICTED: LOW QUALITY PROTEIN: aspartic peptidase, retroviral-like 1	gi|358414487	36.5	5.9	6	24.6	506.7
66	calmodulin-like 5	gi|148234364	16.7	4	6	31.1	482.4
67	fatty acid-binding protein, adipocyte	gi|27805811	14.7	5.4	17	72	1417.0

68	PREDICTED: galectin-7	gi|119910404	15.4	6.1	7	61.2	541.1
69	fatty acid-binding protein, epidermal	gi|27805805	15.1	9	17	67.4	913.6
70	hemoglobin subunit beta	gi|27819608	15.9	7.9	16	82.8	1140.6
71	Chain A, A Novel Allosteric Mechanism In Haemoglobin.	gi|576142	15	9.2	10	64.5	680.9
72	protein S100A7	gi|27807077	11.5	5.1	8	58.4	474.2
	PREDICTED: protein S100A7-like	gi|76612440	11.6	5.4	4	35.6	289.9
73	protein S100A7	gi|27807077	11.5	5.1	9	75.2	565.0
74	PREDICTED: protein S100A7-like	gi|76612440	11.6	5.4	5	67.3	323.1
75	protein S100A7	gi|27807077	11.5	5.1	12	80.2	719.0
76	protein S100A7	gi|27807077	11.5	5.1	4	58.4	236.8
77	PREDICTED: protein S100A7-like	gi|76612440	11.6	5.4	9	57.4	650.7
78	PREDICTED: protein S100A7-like	gi|76612440	11.6	5.4	8	81.2	415.6
79	protein S100A7	gi|27807077	11.6	5.6	10	72.3	503.8
80	PREDICTED: protein S100A7-like	gi|76612440	11.6	5.4	7	41.6	283.6
81	PREDICTED: protein S100A7-like	gi|76612440	11.6	5.4	6	60.4	378.0
	protein S100A7	gi|27807077	11.5	5.1	6	54.5	368.3
82	S100 calcium binding protein A11 (calgizzarin)	gi|296489536	11.2	6.1	8	75.5	612.1
83	protein S100A8	gi|165973998	10.5	5	7	84.3	390.4
84	protein S100A8	gi|165973998	10.5	5	10	85.4	551.5
85	protein S100A8	gi|165973998	10.5	5	15	91	928.0
86	protein S100A12	gi|27807183	10.7	5.9	10	71.7	621.8
87	protein S100A12	gi|27807183	10.7	5.9	18	71.7	1131.1
88	Chain F, Crystal Structure Of Human Stam1 Vhs Domain In Complex With Ubiquitin	gi|290560476	8.3	5.7	4	58.9	289.7
89	PREDICTED: galectin-7	gi|119910404	15.4	6.1	8	72.7	644.9

Table A2: Quantitative changes in 43 differentially expressed teat canal lining and teat skin proteins by 2-DE ($p<0.05$).

		Group A - TCL		Group B - Teat skin			
SSP	**Identified protein**	**Average Spot intensity**	**CV (%)**	**Average Spot intensity**	**CV (%)**	**Fold change**	***p*-value (n=3)**
105	calmodulin-like 5	4.0	21.2	30.3	34.2	0.13	0.012
106	14-3-3 protein sigma	11.7	52.4	109.4	22.7	0.11	0.003
112	S100A7	17.6	70.8	83.0	29.3	0.21	0.014
704	keratin, type I cytoskeletal 10	6.7	48.6	157.4	53.6	0.04	0.036
1009	S100A8	68.2	57.9	3.8	117.9	17.95	0.049
1010	S100A8	43.4	34.7	16.7	5.7	2.60	0.038
1104	caspase-14	34.8	49.7	6.0	41.7	5.80	0.046
1120	S100A7-like	379.5	33.8	97.0	88.5	3.91	0.034
1415	keratin, type I cytoskeletal 10	7.7	142.0	44.4	20.3	0.17	0.011
2002	S100A8	157.9	49.6	24.8	28.2	6.37	0.043
2009	S100A7-like	220.1	29.6	5.7	44	38.61	0.005
3106	S100A7-like	151.3	21.4	4.5	67.5	33.62	0.001
4011	S100A12	317.1	11.9	85.4	14.6	3.71	0.001
4507	keratin, type II cytoskeletal 4	45.5	39.7	4.7	51.3	9.68	0.018
4508	keratin, type II cytoskeletal 5	60.7	46.1	9.0	71.3	6.74	0.035
4711	keratin, type II cytoskeletal 1	4.2	79.4	78.0	14.2	0.05	3.9E-04
4712	keratin, type II cytoskeletal 3	3.2	29.5	53.2	40.8	0.06	0.016
4713	keratin, type II cytoskeletal 1	6.7	86.7	64.5	47.1	0.10	0.032
5102	galectin-7	20.6	54.7	125.9	15.5	0.16	0.001
5509	keratin, type II cytoskeletal 5	64.2	31.0	5.0	154	12.84	0.009
5518	keratin, type II cytoskeletal 4	65.9	61.0	0.6	102.6	109.83	0.048

5613	keratin, type II cytoskeletal 5	67.5	24.4	27.4	33.8	2.46	0.021
5708	keratin, type II cytoskeletal 1	38.2	85.6	106.4	14.8	0.36	0.031
6007	S100A12	768.5	32.7	338.9	11.1	2.27	0.043
6104	S100A9	188.7	29.1	21.4	61	8.82	0.007
6107	S100A9	57.9	26.5	19.4	24.4	2.98	0.014
6316	SERPIN B4-like	35.0	46.0	2.0	52.3	17.50	0.024
6510	keratin, type II cytoskeletal 6A	59.4	46.5	1.0	25.4	59.40	0.022
6611	keratin, type II cytoskeletal 6A	71.3	54.1	1.1	89.3	64.82	0.035
6612	keratin, type II cytoskeletal 6A	38.4	39.1	5.8	94.7	6.62	0.024
7305	SERPIN B4-like	63.6	26.3	2.7	22.7	23.56	0.003
7507	keratin, type II cytoskeletal 6A	157.7	42.8	1.0	75.6	157.70	0.016
7509	keratin, type II cytoskeletal 6A	46.2	34.2	1.2	57.4	38.50	0.008
7609	keratin, type II cytoskeletal 1	437.6	23.8	127.1	53.3	3.44	0.012
7611	keratin, type II cytoskeletal 1	470.7	50.0	60.9	35.3	7.73	0.040
7722	keratin, type II cytoskeletal 3	179.3	12.4	65.1	12.7	2.75	0.001
8102	hemoglobin subunit beta	66.5	26.6	11.5	67.3	5.78	0.008
8110	hemoglobin subunit alpha	79.0	18.3	39.5	8.2	2.00	0.028
8512	keratin, type II cytoskeletal 6A	91.0	20.3	4.1	44.3	22.20	0.001
8630	keratin, type II cytoskeletal 6A	43.0	48.0	2.0	62.6	21.50	0.026
8726	keratin, type II cytoskeletal 1	130.7	15.2	49.4	56	2.65	0.014
9704	keratin, type II cytoskeletal 1	120.5	5.0	60.0	27.9	2.01	0.048
9707	keratin, type II cytoskeletal 1	22.0	43.8	83.2	27.3	0.26	0.013

Spot intensity is an average optical density (OD) of the same spot from three replicate samples belonging to the same experimental group. Coefficient of variation (CV) shows spot variation across these three samples. Fold change is calculated from spot intensity with teat skin as the denominator.

Figure A1: PDQuest histograms of 2-DE spot intensities for differentially expressed teat canal lining and teat skin protein spots.
The standard spot (SSP) number is displayed beneath each histogram. The number at the upper right hand corner of the histogram is the normalised quantity of the maximum bar in the graph. The other bars are drawn proportionally to the highest bar. Intensity bars from individual teat canal lining and teat skin spots are labelled in green and red, respectively.

Table A3: Summary of total MS/MS spectra from each tryptic digest fraction for the teat canal lining group from 6 cows

	Cow #							
Fraction #	**005**	**390**	**452**	**872**	**910**	**1048**	**Average**	**SD**
1	991	1112	870	1105	1111	1003	1032	97
2	866	1028	1053	915	1030	1051	991	80
3	992	907	876	1040	990	1042	975	69
4	1009	873	850	998	1152	1109	999	121
5	1162	1016	996	1124	1163	1125	1098	73
6	1175	1059	992	970	1090	1125	1069	78
7	1173	1097	1064	1116	1141	1094	1114	38
8	1177	972	935	1126	937	935	1014	109
Total	**8545**	**8064**	**7636**	**8394**	**8614**	**8484**	**8289**	**373**
% CV								**8.1%**

SD, standard deviation; CV, coefficient of variation

Table A4: Summary of total MS/MS spectra from each tryptic digest fraction for the teat skin group from 6 cows

	Cow #							
Fraction #	**005**	**390**	**452**	**872**	**910**	**1048**	**Average**	**SD**
1	1347	1261	1199	1177	1155	1152	1215	76
2	1072	1105	1102	1162	1130	1114	1114	30
3	1154	889	790	1141	1092	1013	1013	147
4	785	858	880	1209	1182	1183	1016	195
5	1191	1229	1064	1167	1092	1071	1136	69
6	1071	1111	1156	1157	1075	1067	1106	42
7	1131	1093	925	1178	1192	1158	1113	99
8	1091	1051	986	1188	1078	1064	1076	66
Total	**8842**	**8597**	**8102**	**9379**	**8996**	**8822**	**8789**	**425**
% CV								**8.4%**

SD, standard deviation; CV, coefficient of variation

Table A5: Proteins with 2 or more peptides identified by GeLC-MS/MS from TCL and teat skin replicate samples at 5 % FDR

				Total unique peptide count											
				Teat canal lining						Teat skin					
Identified proteins (species)	**Accession**	**MW [kDa]**	**pI**	**Cow 005**	**Cow 390**	**Cow 452**	**Cow 872**	**Cow 910**	**Cow 1048**	**Cow 005**	**Cow 390**	**Cow 452**	**Cow 872**	**Cow 910**	**Cow 1048**
keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	40	44	39	48	42	44	56	60	55	66	69	62
keratin, type I cytoskeletal 10	gi|27805977	54.8	4.9	48	39	37	47	51	42	52	54	56	64	57	58
keratin, type II cytoskeletal 6A	gi|134085706	60.8	9	36	40	34	43	36	42	19	15	16	19	20	21
keratin, type II cytoskeletal 6A isoform 3	gi|76617862	60.8	9.4	31	35	30	38	33	37						
keratin, type II cytoskeletal 4	gi|148356276	58	8.6	39	28	29	36	34	33	13	10	11	13	14	13
keratin, type II cytoskeletal 5	gi|296487899	62.4	7.6	30	37	33	33	29	33	47	39	41	40	40	39
keratin, type I cytoskeletal 14	gi|262118301	51.8	4.9	36	34	33	39	31	32	44	38	40	46	44	47
keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	30	28	30	29	32	30						
keratin, type I cytoskeletal 17	gi|157427776	48.7	4.9	25	26	24	24	28	29						
keratin, type II cytoskeletal 3	gi|358421417	64.7	9	36	25	24	30	34	26	44	35	41	43	44	41
profilaggrin-like	gi|296489626	55.8	11.4	22	16	17	19	19	25	12	13	16	11	8	12
SERPIN B3-like	gi|359079289	41.7	5.8	17	16	16	16	20	23				2	3	
SERPIN B3-like	gi|119923094	44.3	6.5	18	13	13	19	17	23						
SERPIN B4-like protein	gi|611962707	38.6	6.2	17	15	14	18	19	22			4			
SERPIN B3-like	gi|119916469	44.2	6.5	18	15	14	16	17	22						4
filaggrin	gi|359063845	176.6	10.3	21	12	14	21	18	20	11	11	12	7	6	10
SERPIN B3-like	gi|119924103	44.5	7				16		20		6				
bovine serum albumin	gi|367460260	66.4	5.5	14	18	16	11	18	16	3	3	1	3	1	1
SERPIN B3	gi|358420938	44.5	6.1				13	15	16						
14-3-3 protein sigma (ovis aries)	gi|57163961	27.8	4.5		10	12	14		14	24	23	24	16	19	19
caspase-14	gi|329663440	27.5	4.8	12	9	7	13	9	12	2	2	2	2	1	2
junction plakoglobin	gi|109658166	81.8	5.7	2	9	11	13	9	11	29	19	21	25	26	26
S100A12	gi|27807183	10.7	5.9	10	10	8	9	11	10	11	10	8	11	11	12

S100A9	gi|114052490	16.4	6.3	15	11	9	13	11	10	3	6	2	6	8	7
keratin, type II cytoskeletal 2 oral	gi|358421415	62.7	5.2	12	10	10	12	10	10	11	9	10	7	7	7
annexin A1	gi|73853762	38.9	6.4	16	6	6	9	16	8	2	1	2	5	2	4
beta-lactoglobulin	gi|2194088	18.3	4.7	12	9	9	9	9	8	7	9	7	4	5	5
14-3-3 protein zeta	gi|253706	27.7	4.6	1				1	8	14	8	14	9	10	11
hemoglobin subunit beta	gi|27819608	15.9	7.9	14	11	11	10	12	7	15	14	14	15	15	14
hornerin	gi|528942932	174.7	8.1	7	6	6	4	7	7	7	4	7	4	3	4
alpha-S1-casein precursor	gi|30794348	24.5	4.8	9	5	7	8	9	6	10	10	11	2	4	3
desmoplakin	gi|300797856	332.2	6.5	3	3	4	16	5	6	68	56	69	54	64	57
keratin, type II cytoskeletal 2 epidermal	gi|119892108	64.3	9.2	5	7	7	8	5	6	52	49	52	45	47	46
ubiquitin	gi|51701999	8.6	7.6	5	3	4	5	4	6	5	6	5	4	4	3
fatty acid-binding protein, epidermal	gi|27805805	15.1	9	6	6	6	6	7	5	7	8	7	9	10	9
pancreatic adenocarcinoma upregulated factor-like	gi|296473587	17	9.3	4	3	1	5	7	5						
glyceraldehyde-3-phosphate dehydrogenase	gi|77404273	35.8	9.3	6	3	6	8	5	4						
nucleoside diphosphate kinase B	gi|296488274	17	9.4	4	5	5	5	5	5	2		2	3		
S100A8	gi|165973998	10.5	5	5	2	3	5	4	5	2	2	1	6	5	5
alpha-S2-casein precursor	gi|27806963	26	9.2	7	1		11	7	4	7	11	8		2	
S100A7	gi|27807077	11.5	5.1	6	7	4	8	5	4	5	5	5	8	10	8
beta-casein precursor	gi|162797	25.1	5.3	4	4	4	4	4	4	5	9	5	2	3	2
S100A7-like	gi|528901741	11.6	5.6	4	5	4	5	4	4	4	5	4	3	4	5
malate dehydrogenase, cytoplasmic	gi|77736203	36.4	6.2	3	2	1	4	3	4	2	2	2	3	3	2
triosephosphate isomerase	gi|61888856	26.7	6.5				4		4	5	2	5	3	1	1
actin, beta	gi|148744172	41.7	5.2	1	10	10	11	9	3	9	9	6	5	7	4
hemoglobin subunit alpha	gi|116812902	15.2	9.1	9	8	6	7	8	3	7	8	7	7	7	6
gasdermin 1	gi|296476376	49.9	5.2	6	2	2	3	6	3	1	2	1	1	1	1
annexin A2	gi|73586982	38.5	8.5	4	2	2	4	5	3	2	1	2	2	3	2
heat shock cognate 71 kDa protein	gi|76253709	60.9	6	1	2	3	3	4	3	1	5	3			
IGL@ protein	gi|74353860	22.5	5.9	3	3	4	2	3	3	2	4	2	1		
L-lactate dehydrogenase A chain	gi|27806559	36.6	9.1	3	3	3	3	3	3	6	3	6	4	1	2

tropomyosin 4-like	gi|296486791	29	4.9	3	2	2	3	3	3						
casein para kappaA	gi|229416	12.3	9.5	2	1	1	3	2	3	2	3	3	1	1	
repetin	gi|528942936	78.3	5.8	4	2	2	2	2	3						
S100A2	gi|77735683	10.9	4.5	1	1	2	4	2	3	3	2		3		1
transitional endoplasmic reticulum ATPase isoform 3 (canis lupus familiaris)	gi|73971210	89.2	5	2	2	3	1	2	3	2	1	5	2	4	3
SERPIN B12	gi|297464091	45.9	5.1			1	2		3				1	1	1
heat shock 27kDa protein 1	gi|61553385	17.5	6.6	2	2	4	5	3	2	6	7	9	7	6	7
insulin-degrading enzyme isoform X1 (IDE)	gi|529008257	113.3	5.9	3	3	2	1	3	2	2		2			
proteasome subunit beta type-2 isoform 2	gi|426215156	20.3	7	2	2	2	2	2	2	2	1	2	1	1	
ganglioside GM2 activator precursor	gi|329663176	20.9	5.2		1		1	1	2						
retroviral-like aspartic protease 1	gi|358414487	34.1	5.3	1			2	1	2	1	1	1	2	2	1
annexin A8	gi|27806317	36.8	5.2			1	2		2	4		4	2	3	4
purine nucleoside phosphorylase, chain A	gi|157834116	31.3	6		1	1	2		2				2	2	3
chloride intracellular channel protein 1	gi|62751970	27	5				2		2						
collagen, type VI, alpha 3-like isoform 4	gi|296488814	276	9.2		3	3		3	1	6		6			
F-box only protein 50	gi|76641449	29.5	6.4	4	3	1	4	3	1		1	1			
tubulin alpha-1B chain (mus musculus)	gi|34740335	50.1	4.8	1	3	2	3	3	1	1	1	1	2	2	2
alpha-actinin 1	gi|145286437	102.8	5.1		1		1	2	1	2	3	2	3	3	2
calmodulin-like 5	gi|148234364	16.7	4					1	1	4	4	4	1	2	2
IgM heavy chain constant region	gi|2232299	47.9	5.6	3	1	1	1	1	1						
thioredoxin	gi|296484386	10.9	5.2	1	1	1	2	1	1	2	2	1	2	2	2
elongation factor 2	gi|115497900	95.3	6.4			1	1		1	2			3	3	4
fatty acid-binding protein, adipocyte	gi|296480594	14.6	5.4		4	2	8	8		7	2		6	6	6
galectin-7	gi|297485581	15.4	6.1		5	4	7	6	2	8	9	8	6	8	7
peptidylprolyl isomerase A	gi|28189771	16.9	9.5		2	3	5	4	1	6	6	6	4	3	4
cystatin-A	gi|268607697	11.1	4.9			1	2	3		2	3	2			
histone H2A type 2-C	gi|296489557	12.9	11.5		2	2	2	3					3	4	4
histone H2B	gi|229329	13.8	10.9		4	4	3	3		5	6	5	3	2	4
vimentin	gi|289450	53.6	5.1		2	2	2	3	1						

peroxiredoxin-1	gi|27806081	22.2	9.6		3	2	5	2	2	8	6	6	6	6	5
apolipoprotein A-I precursor	gi|162678	30.3	5.8	2	2	2		1	1		1				
elongation factor 1 alpha	gi|28189681	19.3	6.2		2	1	1	1		2	2	1	4	3	3
profilin	gi|253723066	15	9.5		1	1	1	1		2	1	1	3	3	1
protein DJ-1	gi|62751849	20	7.7	1			2	1		1	1		3	1	
S100A11 (calgizzarin)	gi|296489536	11.2	6.1		2	2	2	1		2	2	1	3		2
S100A14	gi|118601864	11.4	4.5				1	1		2	2			2	1
14-3-3 protein beta/alpha (homo sapiens)	gi|4507949	28.1	4.6											6	
14-3-3 protein epsilon (homo sapiens)	gi|5803225	29.2	4.5								4		5	5	6
40S ribosomal protein S7 (homo sapiens)	gi|4506741	22.1	10.6		1	1	1			2	2	1			
60S ribosomal protein L11 isoform 2 (homo sapiens)	gi|315221152	20.1	10.3				2			1	2	1		1	
60S ribosomal protein L12 isoform X1	gi|528969841	20.5	9.4				3								
ADP-ribosylation factor 3	gi|296487799	15.9	5.3							1		1	2	2	2
AHNAK nucleoprotein 2	gi|296475271	149.2	4.9							6	7	6	4	2	3
aldose reductase	gi|113594	35.9	5.7							8	6	8			
enolase (alpha)	gi|4927286	47.2	6.5				2		2						
angiogenin	gi|108522	14.6	10.1							2					
annexin A4	gi|48374083	34.9	5.1							1	2	1	3		
annexin V=CaBP33 isoform [cattle, brain, peptide, 320 aa]	gi|260137	35.9	4.8							3		3	4	2	3
band 6 polypeptide B6P, partial	gi|913808	33.1	7.7				2								
basigin precursor	gi|115496127	29.8	5.6												2
beta-enolase	gi|77736349	47.1	8.6		1	1									
cofilin-1 (sus scrofa)	gi|51592135	18.5	9.1		1	1	2		1	7	5	2	3	2	2
collagen alpha-2(VI) chain isoform X1	gi|528938209	109.3	6.3			2									
cornulin-like	gi|296489625	164	9.6	3									2	3	3
cullin 1-like	gi|296488584	89.1	8.9											3	
cystatin-B	gi|82697343	11.2	6.4				1			3	3		3		1
desmocollin	gi|162971	93.4	5							8	4	8	9	5	8

desmoglein	gi|436062	107	4.7				1			4	3		6	6	7
desmosomal cadherin	gi|545008	112.1	4.8									4			
epiplakin	gi|528914337	375.6	5.6							16	11	16	15	10	13
F-actin-binding protein	gi|545605	8.5	5		2										
fatty acid synthase isoform X1	gi|528996770	274.2	5.9							2	1	2			
gamma-glutamylcyclotransferase	gi|83035087	21.2	5.4										2		2
gelsolin isoform b	gi|77736201	80.7	5.5							4		1		1	
glutathione S-transferase Mu 1 isoform X1	gi|297465561	21	9.9							1	2				
glutathione S-transferase P	gi|29135329	23.6	7.7				2			2	4	2	3		
heat shock protein HSP 90-alpha	gi|60592792	84.7	4.8				3								
heat shock protein HSP 90-beta	gi|296474454	78.6	5							2			1		
histone H2A type 1	gi|529004004	27.5	11.4							6	4	4			
histone H3 family 3A	gi|28189807	10.2	12.3		2	2	4	1		3		3			2
histone H4	gi|359071049	10	11.2										2	3	3
keratin 15	gi|195539527	49	4.7							20	22	19	24	25	24
keratin 77-like	gi|296487879	62.9	6.3							7	8	6	9	8	8
keratin, type I cytoskeletal 16	gi|528921408	46.1	4.7		7										
keratin, type I cytoskeletal 19	gi|62751472	43.9	4.8							11		10			
keratin, type I cytoskeletal 27	gi|115494902	49.9	4.9							4	4	4	6	7	8
keratin, type II cytoskeletal 73	gi|162287381	58.8	7.6												10
lactoferrin	gi|504	75.1	9.6	4						2	4				
L-lactate dehydrogenase B chain	gi|27806561	36.6	6		2	2	1		1					2	2
mediator of RNA polymerase II transcription subunit 1	gi|329663655	167.7	9.5											2	3
mitochondrial malate dehydrogenase 2, NAD	gi|89574145	29.9	9							2		2			
myosin regulatory light chain 12B-like isoform 2	gi|358422992	13.7	5.1				2			1					
macrophage migration-inhibitory factor [cattle, Peptide, 114 aa]	gi|693791	12.2	9.5								2				
peroxiredoxin-2	gi|27807469	21.9	5.3			1				4	2	3	3	5	4
phosphatidylethanolamine binding protein	gi|157829678	20.7	6.6			1	4			5	6	4	1		2

plakophilin-1	gi|27806479	80.1	10.1							6	3	10	5	7	6
plasmalemmal porin	gi|437027	30.7	9.4							2		2			
putative RNA-binding protein 3	gi|77735675	9.7	4.9		1		2			1		1			
ras-related protein Rab-10 (mus musculus)	gi|7710086	22.5	9.4							1	3	1	2	2	2
ras-related protein Rab-11A isoform X1	gi|528962105	17.8	8.6							1			2		
ras-related protein Rab-1A	gi|75812956	19	8.9							1		1	2		
ras-related protein Rab-1B	gi|296471468	21.9	5.4								3				
ribosomal protein L12	gi|28189683	15.7	10.6							2		2	1	1	1
secernin-3	gi|296490700	48.2	5.4								2		1	2	1
SERPIN B13 isoform 1	gi|297464093	42.9	5.3										2	1	
suprabasin isoform X1	gi|528991569	47.6	6.7							2	1				
titin	gi|297471578	3713.5	6										2		
transgelin-2	gi|61888874	22.4	9.3		3	3	4	1	1	3	2		1		1
tyrosine 3/tryptophan 5 -monooxygenase activation protein	gi|296475944	27.7	4.6			5	8		6						
ZBTB42 protein-like	gi|296475269	69.8	11.6		1	1							1	1	2
zinc finger protein 638	gi|296482727	215	5.8	2	2										
zymogen granule protein 16 homolog B	gi|529006436	24.2	5.8				3								

Cells highlighted in grey correspond to biological replicates (n = 6) where no peptides were identified in either the TCL or teat skin group.

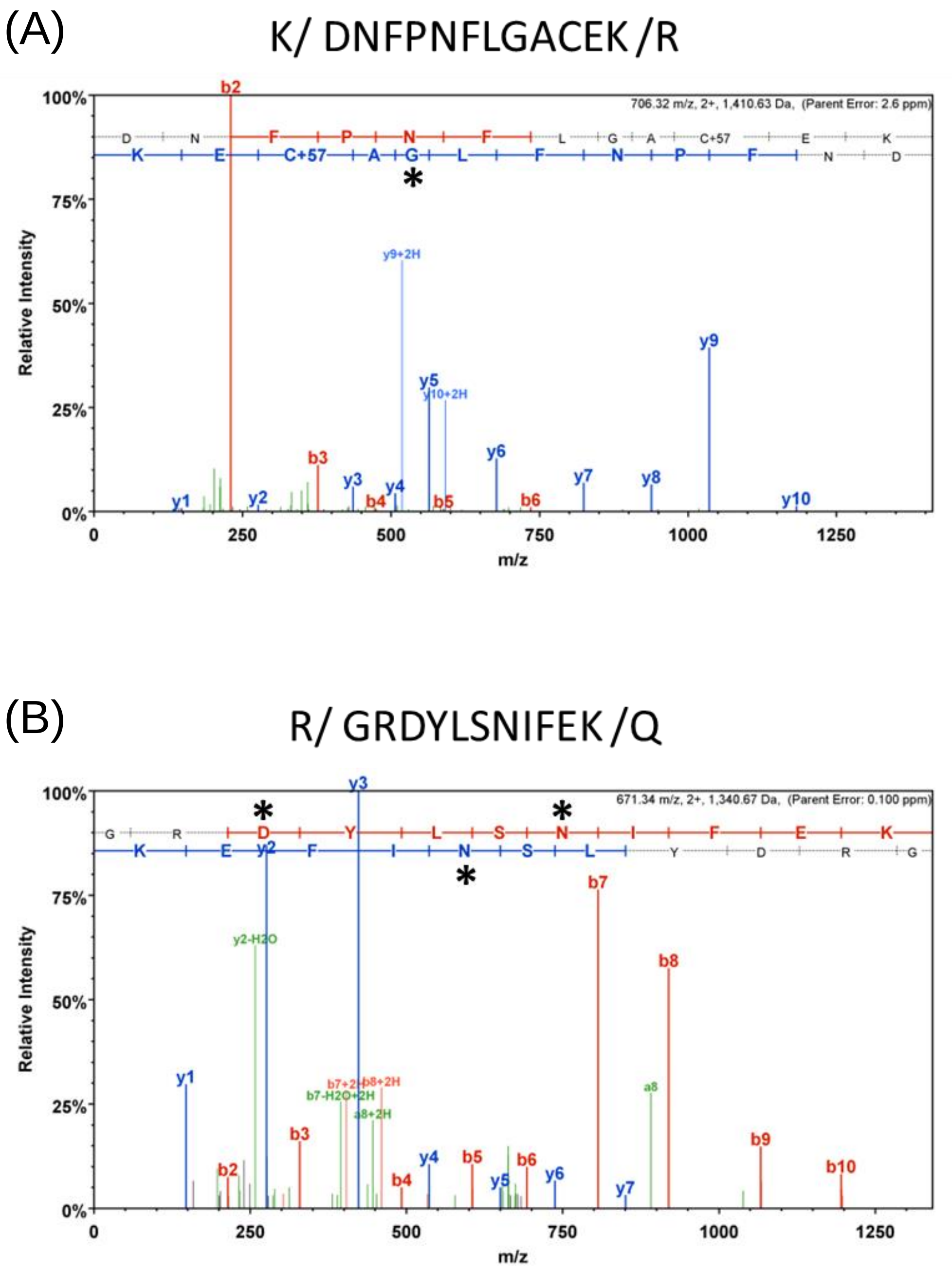
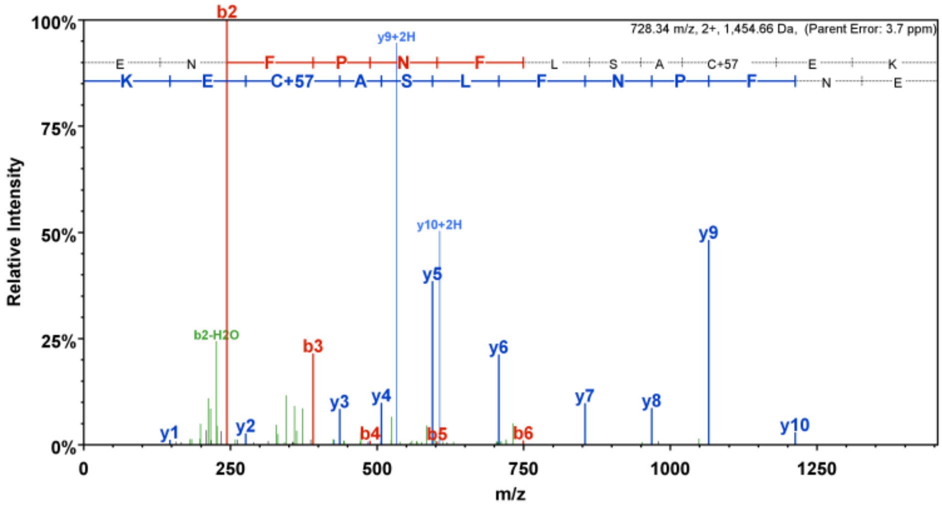


Figure A2: Representative MS/MS spectra identifying the two diagnostic peptides for S100A7.

(A) Fragmentation pattern for precursor ion at *m/z* 706.32; sequence: DNFPNFLGACEK. (B) Fragmentation pattern for precursor ion at *m/z* 671.34; sequence: GRDYLSNIFEK. Peaks corresponding to *b* (red) and *y* (blue) ions are marked. Diagnostic amino acids detected are labelled with an asterisk.

(A) K/ ENFPNFLSACEK /R



(B) R/ QYLSDIFEK /K

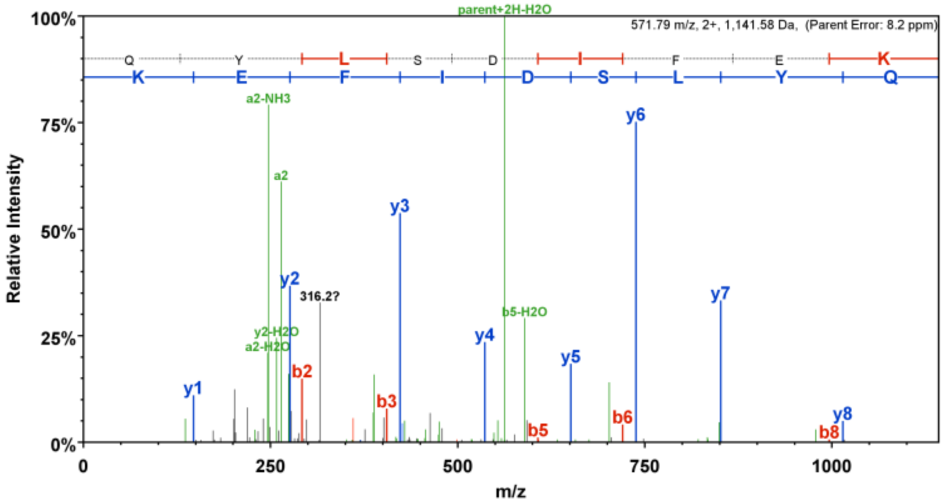


Figure A3: Representative MS/MS spectra identifying the two diagnostic peptides for S100A7-like protein.

(A) Fragmentation pattern for precursor ion at *m/z* 728.34; sequence: ENFPNFLSACEK. (B) Fragmentation pattern for precursor ion at *m/z* 571.79; sequence: QYLSDIFEK. Peaks corresponding to *b* (red) and *y* (blue) ions are marked. Diagnostic amino acids detected are labelled with an asterisk

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Table A6: Differential expression of keratin proteins in the teat canal lining and teat skin proteomes.

	Average peptide count (n=6)			
Identified keratin protein	**Teat canal lining**	**Teat skin**	**Fold change**	***p*-value**
keratin, type II cytoskeletal 1	42.8	61.3	1.4	3.3×10^{-5}
keratin, type II cytoskeletal 2 epidermal	6.3	48.5	7.7	2.2×10^{-11}
keratin, type II cytoskeletal 2 oral	10.7	8.5	0.79	2.6×10^{-2}
keratin, type II cytoskeletal 3	29.2	41.3	1.4	5.9×10^{-4}
keratin, type II cytoskeletal 4	33.2	12.3	0.37	4.3×10^{-7}
keratin, type II cytoskeletal 5	32.5	41.0	1.26	5.1×10^{-4}
keratin, type II cytoskeletal 6A	38.5	18.3	0.47	5.0×10^{-4}
keratin, type II cytoskeletal 6A isoform 3	34.0	0.5*	0.01	
keratin, type I cytoskeletal 10	44.0	56.8	1.3	1.0×10^{-3}
keratin, type I cytoskeletal 14	34.2	43.2	0.79	6.8×10^{-4}
keratin 15	0.5*	22.3	44.6	
keratin, type I cytoskeletal 16	1.2	0.5*	0.41	
keratin, type I cytoskeletal 17	26.0	0.5*	0.02	
keratin, type I cytoskeletal 19	0.5*	10.5	21	
keratin, type I cytoskeletal 27	0.5*	5.5	11	
keratin, type II cytoskeletal 73	0.5*	10.0	20	
keratin 77-like	0.5*	7.7	15.4	
keratin, type II cytoskeletal 79	29.8	0.5*	0.02	

* Proteins absent in either of the two samples were given a value of 0.5 so that a fold change could be calculated. Fold change was calculated from the average peptide count with TCL as the denominator. *p*-values were derived from the six teat canal lining and six teat skin samples and were calculated as a two-tailed test with even distribution.

Table A7: Proteins identified in all 12 GeLC samples from teat canal lining and teat skin

Identified proteins	Accession number	Average peptide count Teat canal lining	Teat skin	Fold change
Keratins				
keratin, type II cytoskeletal 1	gi|358421409	43	61	0.7
keratin, type I cytoskeletal 10	gi|27805977	44	57	0.8
keratin, type II cytoskeletal 2 epidermal	gi|119892108	6	49	0.1
keratin, type II cytoskeletal 2 oral	gi|358421415	11	9	1.3
keratin, type II cytoskeletal 3	gi|358421417	29	41	0.7
keratin, type II cytoskeletal 4	gi|148356276	33	12	2.7
keratin, type II cytoskeletal 5	gi|296487899	33	41	0.8
keratin, type II cytoskeletal 6A	gi|134085706	39	18	2.1
keratin, type I cytoskeletal 14	gi|262118301	34	43	0.8
Cytoskeleton				
desmoplakin	gi|300797856	6	61	0.1
hornerin	gi|528942932	6	5	1.3
junction plakoglobin	gi|109658166	9	24	0.4
filaggrin	gi|359063845	18	10	1.9
profilaggrin-like	gi|296489626	20	12	1.6
tubulin alpha-1B chain (mus musculus)	gi|34740335	2	2	1.4
Major milk proteins				
alpha-S1-casein precursor	gi|30794348	7	7	1.1
beta-casein precursor	gi|162797	4	4	0.9
beta-lactoglobulin	gi|2194088	9	6	1.5
S100 calcium-binding proteins				
S100A7	gi|27807077	6	7	0.8
S100A7-like	gi|528901741	4	4	1.0
S100A8	gi|165973998	4	4	1.1
S100A9	gi|114052490	10	5	1.9
S100A12	gi|27807183	10	11	0.9
Cellular proteins				
actin, beta	gi|148744172	7	7	1.1
annexin A1	gi|73853762	10	3	3.8
annexin A2	gi|73586982	3	2	1.7
ubiquitin	gi|51701999	5	5	1.0
fatty acid-binding protein, epidermal	gi|27805805	6	8	0.7
heat shock 27kDa protein 1	gi|61553385	3	7	0.4
L-lactate dehydrogenase A chain	gi|27806559	3	4	0.8
malate dehydrogenase, cytoplasmic	gi|77736203	3	2	1.2
thioredoxin	gi|296484386	1	2	0.6
transitional endoplasmic reticulum ATPase isoform 3 (canis lupus familiaris)	gi|73971210	2	3	0.8
Serum proteins				
bovine serum albumin	gi|367460260	15	2	7.3
hemoglobin subunit alpha	gi|116812902	7	7	1.0
hemoglobin subunit beta	gi|27819608	11	15	0.7
Apoptosis				
galectin-7	gi|297485581	4	8	0.5
caspase-14	gi|329663440	10	2	5.6
gasdermin 1	gi|296476376	4	1	3.1

Fold change is calculated from average peptide counts with teat skin counts used as the denominator.

Supplementary Data B

Experiments to determine monoclonal and polyclonal antibody specificity

B1: Immunohistochemical staining of super mammary lymph node.

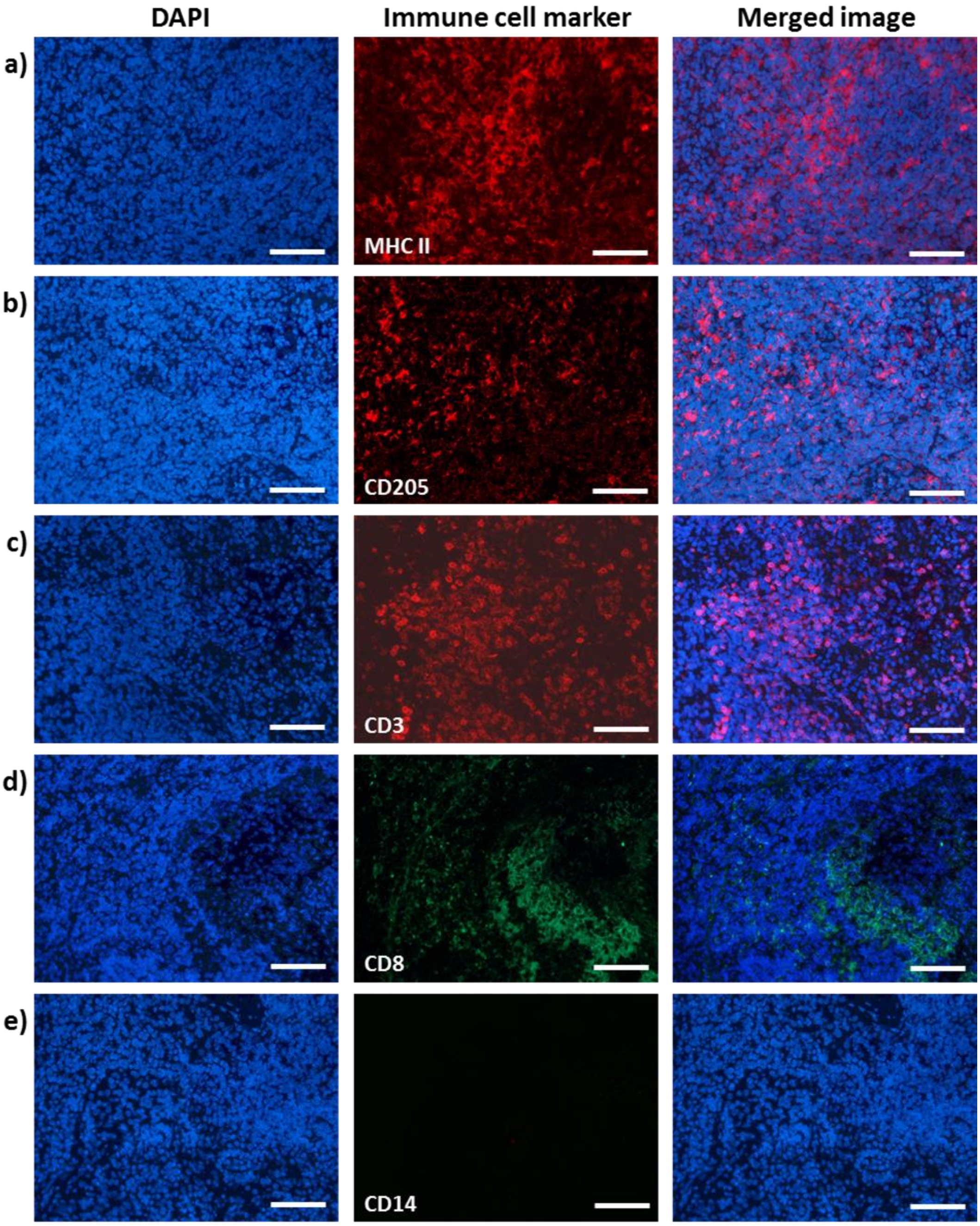

Figure B2: Immune cell staining of super mammary lymph node tissue.
Cryosections of super mammary lymph node tissues were probed with anti-MHC class II monoclonal antibody (a), anti-CD205 monoclonal antibody (b), and anti-CD3 monoclonal antibody (c). The bound anti-MHC class II antibody was detected using Alexa Fluor-594-IgG2a while the anti-CD205 and anti-CD3 antibodies were detected with Alexa Fluor-594-IgG (red signal). Cryosections probed with anti-CD8 monoclonal antibody (d), and anti-CD14 monoclonal antibody (e) were detected with Alexa Fluor-488-IgM labelled secondary antibody (green signal). Nuclei were counterstained with DAPI (blue signal). Section thickness: 5 µm. Scale bar = 100 µm.

Supplementary Data C

Proteomic data and MS/MS fragmentation spectra from 2-DE and GeLC-MS/MS analysis of late-lactating teat canal lining and 14 d involution teat canal lining

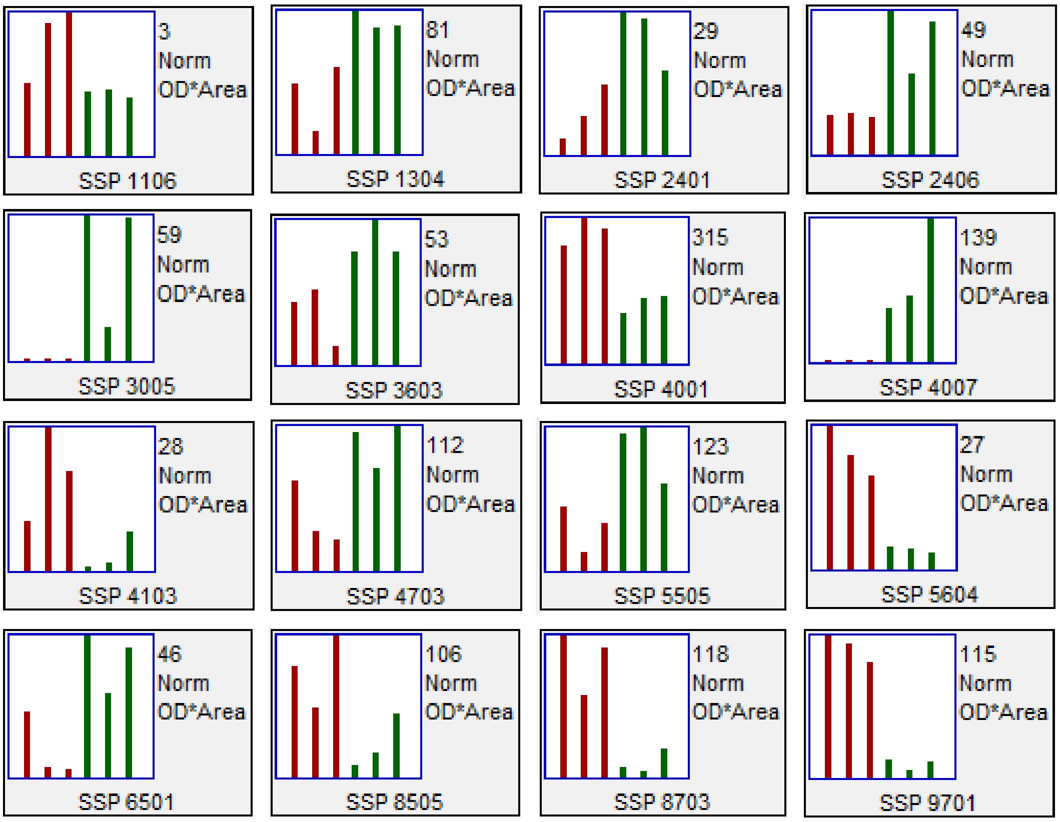

Figure C1: PDQuest histograms of 2-DE spot intensities for differentially expressed late-lactating and 14 d involution teat canal lining protein spots.

The standard spot (SSP) number is displayed beneath each histogram. The number at the upper right hand corner of the histogram is the normalised quantity of the maximum bar in the graph. The other bars are drawn proportionally to the highest bar. Intensity bars from individual late-lactating and 14 d involution teat canal lining spots are labelled in red and green, respectively.

Table C1: Summary of LC-MS/MS results for the four spiked proteins in *E. coli* lysate[a].

Gel slice	SpC				Peptide count				Sequence coverage			
Conalbumin	**fmol**	**Ave**	**SD**	**SEM**	**fmol**	**Ave**	**SD**	**SEM**	**fmol**	**Ave**	**SD**	**SEM**
	200	69.5	7.0	3.5	200	34.3	4.1	2.1	200	54.6	10.0	5.0
	100	39.8	4.9	2.4	100	22.8	1.3	0.6	100	36.6	2.2	1.1
	50	25.8	4.3	2.1	50	16.3	2.1	1.0	50	27.1	2.7	1.3
	25	16.5	6.2	3.1	25	12.0	3.4	1.7	25	18.4	4.7	2.4
	12.5	4.8	2.8	1.4	12.5	3.8	1.5	0.8	12.5	4.9	2.0	1.0
	SpC				**Peptide count**				**Sequence coverage**			
Ovalbumin	**fmol**	**Ave**	**SD**	**SEM**	**fmol**	**Ave**	**SD**	**SEM**	**fmol**	**Ave**	**SD**	**SEM**
	200	32.5	3.7	1.8	200	15.3	1.5	0.8	200	36.4	2.8	1.4
	100	19.5	0.6	0.3	100	10.5	0.6	0.3	100	33.2	1.9	0.9
	50	12.3	2.5	1.3	50	8.5	1.7	0.9	50	28.6	5.8	2.9
	25	7.0	0.8	0.4	25	5.3	0.5	0.3	25	17.2	2.6	1.3
	12.5	4.0	1.8	0.9	12.5	3.5	1.3	0.6	12.5	11.4	4.9	2.4
	SpC				**Peptide count**				**Sequence coverage**			
Carbonic Anhydrase	**fmol**	**Ave**	**SD**	**SEM**	**fmol**	**Ave**	**SD**	**SEM**	**fmol**	**Ave**	**SD**	**SEM**
	200	42.0	8.0	4.0	200	13.0	1.8	0.9	200	57.2	4.0	2.0
	100	26.0	2.4	1.2	100	10.3	0.5	0.3	100	45.0	1.2	0.6
	50	21.0	0.8	0.4	50	10.0	0.0	0.0	50	45.5	1.1	0.6
	25	14.8	1.0	0.5	25	8.3	0.5	0.3	25	42.7	0.8	0.4
	12.5	10.0	2.9	1.5	12.5	6.8	1.7	0.9	12.5	31.4	6.9	3.5
	SpC				**Peptide count**				**Sequence coverage**			
Lysozyme	**fmol**	**Ave**	**SD**	**SEM**	**fmol**	**Ave**	**SD**	**SEM**	**fmol**	**Ave**	**SD**	**SEM**
	200	15.5	1.7	0.9	200	7.3	1.0	0.5	200	45.0	3.1	1.6
	100	10.0	2.2	1.1	100	5.8	1.3	0.6	100	42.6	6.4	3.2
	50	6.3	0.5	0.3	50	4.3	0.5	0.3	50	35.1	6.1	3.1
	25	1.8	0.5	0.3	25	1.0	0.0	0.0	25	9.3	0.0	0.0
	12.5	0.0	0.0	0.0	12.5	0.0	0.0	0.0	12.5	0.0	0.0	0.0

SpC, spectral count; Ave, average; SD, standard deviation; SEM, standard error of the mean

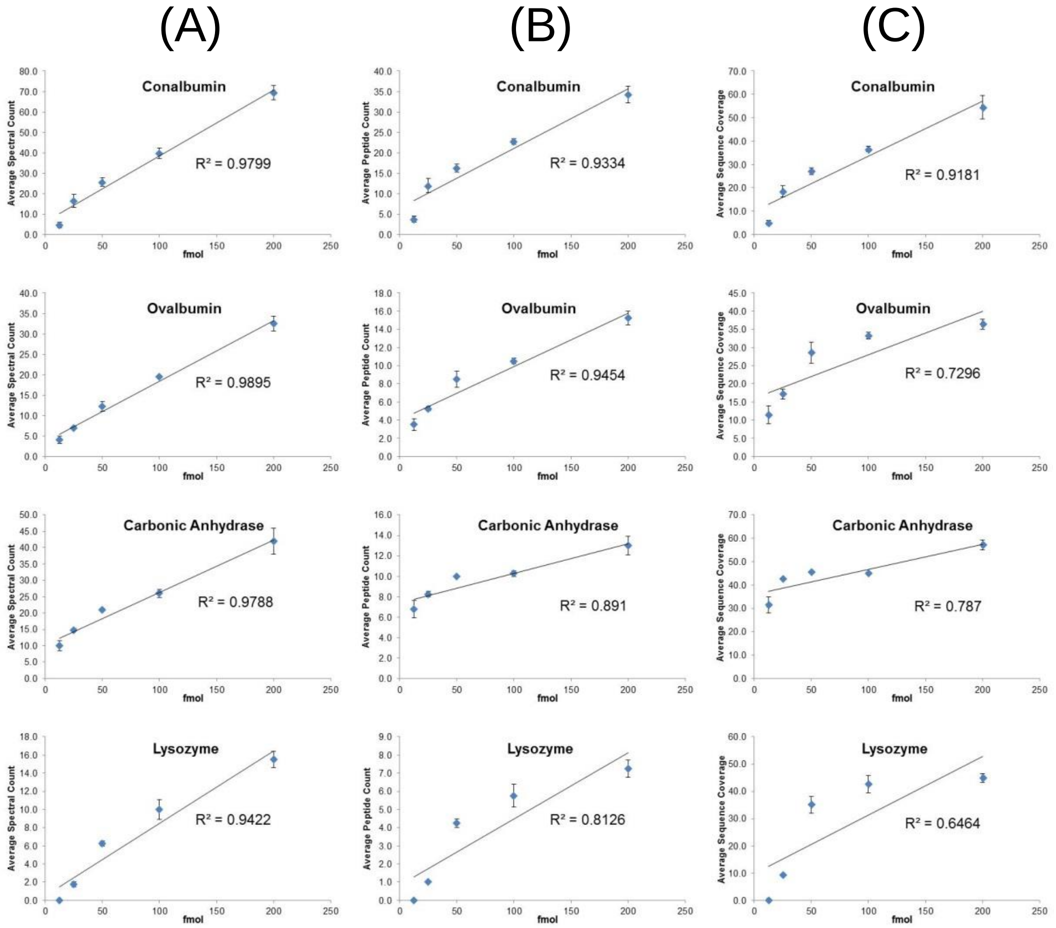

Figure C2: Correlation between SpC, peptide count and sequence coverage with protein abundance level.

In each experiment four non- *E. coli* proteins were added to *E. coli* lysate at different concentrations representing different protein abundance levels. For all added proteins, a linear correlation was observed between SpC and relative protein abundance (A). Regression coefficients decreased between peptide count and relative protein abundance (B) and sequence coverage and relative protein abundance (C). The results are an average of four technical replicates. Error bars represent the standard error of the mean.

Table C2: Summary of LC-MS/MS results for the *E. coli* proteins in each of the five dilution series lysates.

The results are from the four technical replicates. Abbreviations used for *E. coli* proteins are as follows: PFL, pyruvate formate-lyase [2PFL_A]; DnaK, chaperone protein DnaK [ZP_07123178]; TEF Tu, translation elongation factor Tu [EKW71632]; PPH, phosphopyruvate hydratase [ZP_11503972]; Oxidoreductase, [ZP_07115779]; OMP A, outer membrane protein A [ZP_03085789]; Global, global DNA-binding transcriptional dual regulator H-NS [WP_001287378.1]; Stress F, universal stress protein F [WP_001082294.1]. Overall coefficient of variation (CV) was 14.9 %.

Gel slice	**SpC (PFL)**					**SpC (DnaK)**				
Conalbumin	**fmol**	**Ave.**	**SD**	**SEM**	**%CV**	**fmol**	**Ave.**	**SD**	**SEM**	**%CV**
	200	50.0	4.5	2.3	9 %	200	43.5	10.3	5.2	24 %
	100	50.3	5.6	2.8	11 %	100	38.3	3.4	1.7	9 %
	50	53.5	2.1	1.0	4 %	50	41.8	4.9	2.5	12 %
	25	58.5	9.3	4.6	16 %	25	45.8	12.3	6.1	27 %
	12.5	51.0	6.5	3.2	13 %	12.5	39.3	3.6	1.8	9 %
	SpC (TEF Tu)					**SpC (PPH)**				
Ovalbumin	**fmol**	**Ave.**	**SD**	**SEM**	**%CV**	**fmol**	**Ave.**	**SD**	**SEM**	**%CV**
	200	53.3	2.5	1.3	5 %	200	24.8	0.5	0.3	2 %
	100	57.0	6.7	3.3	12 %	100	27.5	1.3	0.6	5 %
	50	50.3	9.9	5.0	20 %	50	26.3	0.5	0.3	2 %
	25	52.5	3.7	1.8	7 %	25	25.5	1.9	1.0	8 %
	12.5	53.3	4.2	2.1	8 %	12.5	27.0	0.8	0.4	3 %
	SpC (Oxireductase)					**SpC (OMP A)**				
Carbonic Anhydrase	**fmol**	**Ave.**	**SD**	**SEM**	**%CV**	**fmol**	**Ave.**	**SD**	**SEM**	**%CV**
	200	13.3	4.7	2.4	36 %	200	13.5	2.5	1.3	19 %
	100	7.3	0.5	0.3	7 %	100	8.5	1.0	0.5	12 %
	50	8.8	2.1	1.0	24 %	50	6.8	2.1	1.2	31 %
	25	11.5	7.9	3.9	68 %	25	10.8	1.0	0.6	9 %
	12.5	16.5	4.8	2.4	29 %	12.5	10.8	1.7	1.0	16 %
	SpC (Global)					**SpC (Stress F)**				
Lysozyme	**fmol**	**Ave.**	**SD**	**SEM**	**%CV**	**fmol**	**Ave.**	**SD**	**SEM**	**%CV**
	200	12.5	0.6	0.3	5 %	200	7.5	1.3	0.6	17 %
	100	12.0	1.8	0.9	15 %	100	6.8	1.3	0.6	19 %
	50	15.5	2.1	1.0	13 %	50	9.8	2.2	1.1	23 %
	25	11.5	2.1	1.0	18 %	25	7.3	0.5	0.3	7 %
	12.5	13.0	0.8	0.4	6 %	12.5	8.3	1.7	0.9	21 %

SpC, spectral count; Ave, average; SD, standard deviation; SEM, standard error of the mean; CV, coefficient of variation

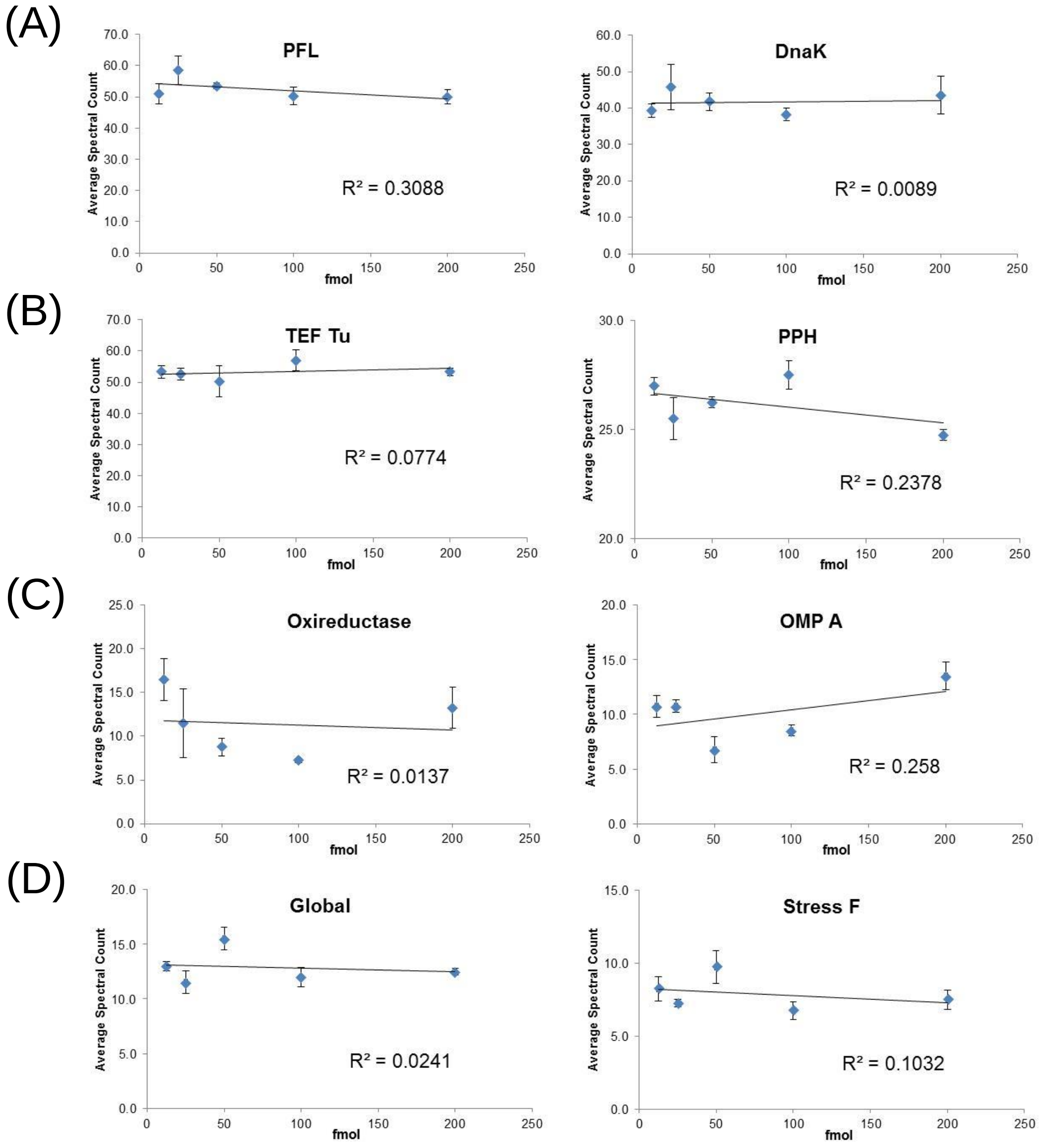

Figure C3: Correlation between *E. coli* protein SpC with added protein abundance levels. In each experiment two of the most abundant *E. coli* proteins were identified and their SpC plotted relative to the concentration of the added non-*E. coli* proteins. For all *E. coli* proteins, a nonlinear correlation was observed between SpC and relative added protein abundance for conalbumin (A), ovalbumin (B), carbonic anhydrase (C), and lysozyme (D). The results are an average of four technical replicates. Error bars represent the standard error of the mean. Abbreviations used for *E. coli* proteins are as follows: PFL, pyruvate formate-lyase [2PFL_A]; DnaK, chaperone protein DnaK [ZP_07123178]; TEF Tu, translation elongation factor Tu [EKW71632]; PPH, phosphopyruvate hydratase [ZP_11503972]; Oxidoreductase, [ZP_07115779]; OMP A, outer membrane protein A [ZP_03085789]; Global, global DNA-binding transcriptional dual regulator H-NS [WP_001287378.1]; Stress F, universal stress protein F [WP_001082294.1].

Table C3: Summary of total MS/MS spectra from each tryptic digest fraction for the 14 d involution teat canal lining group from 7 cows

Fraction #	115	160	331	358	499	837	911	Average	SD
1	1267	1195	1248	1258	1246	1191	1266	1032	97
2	1278	868	1217	1184	1306	1292	1204	991	80
3	1136	1042	1187	1076	888	1040	1190	975	69
4	952	891	1185	1179	955	1184	890	999	121
5	1151	908	1153	1273	1267	1286	1231	1098	73
6	1169	1185	1022	896	1170	1180	1020	1069	78
7	871	918	1106	1241	1245	1217	1242	1114	38
8	871	1456	1205	1243	1043	1197	1079	1014	109
Total	**8695**	**8463**	**9323**	**9350**	**9120**	**9587**	**9122**	**9094**	**392**
% CV									**8.1%**

SD, standard deviation; CV, coefficient of variation

Table C4: 86 proteins with 2 or more peptides identified by GeLC-MS/MS from 14 d TCL replicate samples at 5 % FDR

				Total unique peptide count					
				14 d Involution TCL					
Identified proteins	**Accession**	**MW [kDa]**	**pI**	**Cow 005**	**Cow 390**	**Cow 452**	**Cow 872**	**Cow 910**	**Cow 1048**
keratin, type II cytoskeletal 1	gi|358421409	63.1	9.2	58	51	47	54	56	53
keratin, type I cytoskeletal 10	gi|27805977	54.8	4.9	44	43	48	46	46	49
keratin, type II cytoskeletal 6A	gi|134085706	60.8	9	37	39	38	38	41	35
keratin, type II cytoskeletal 4	gi|148356276	58	8.6	35	28	33	36	38	31
keratin, type II cytoskeletal 79	gi|115496454	57.7	7.8	33	31	33	33	36	28
keratin, type II cytoskeletal 3	gi|358421417	64.7	9	32	18	26	26	27	31
keratin, type II cytoskeletal 6C	gi|296487883	58.9	9.2	31	32	33	35	38	30
keratin, type I cytoskeletal 14	gi|262118301	51.8	4.9	30	26	34	28	28	34
keratin, type II cytoskeletal 5	gi|56710317	62.9	8.6	26	22	31	31	34	29
profilaggrin-like	gi|296489626	55.8	11.4	26	22	21	18	21	22
keratin, type I cytoskeletal 17	gi|160395544	48.7	4.9	24	19	25	21	23	25
filaggrin-like	gi|296489624	180.2	10.3	20	24	18	14	21	24
filaggrin-like, partial	gi|528942934	39	10	19	15	12	13	15	15
serpin B3-like	gi|359079289	41.7	5.8	18		16	17	15	17
serpin B3-like	gi|119923094	44.3	6.5	17	19		16	16	15
serpin B4-like protein	gi|611962707	38.6	6.2	17	16	15	20	16	17
serpin B3-like	gi|119916469	44.2	6.5	17	14	16	18	15	18
lactoferrin	gi|504	75.1	9.6	17	13	19	10	9	16
keratin, type II cytoskeletal 2, oral	gi|358421415	62.7	5.2	13	9	11	10	12	9
serum albumin precursor	gi|30794280	69.3	5.8	12	4	19	11	7	16

keratin, type II cytoskeletal 2, epidermal	gi|119892108	64.3	9.2	11	8	9	8	8	11
annexin A1	gi|73853762	38.9	6.4	11	8	6	8	7	8
protein S100A9	gi|296489689	16.4	6.3	10	8	13	10	11	8
protein S100A12	gi|27807183	10.7	5.9	9	10	9	9	10	9
annexin A2	gi|73586982	38.5	8.5	9	6	3	4	4	2
glyceraldehyde-3-phosphate dehydrogenase	gi|77404273	35.8	9.3	9	3	1	2	2	2
caspase-14	gi|329663440	27.5	4.8	8	13	5	11	9	11
beta-lactoglobulin	gi|6729725	18.3	4.7	7	7	6	4	7	7
hemoglobin subunit beta	gi|27819608	15.9	7.9	6	12	7	8	5	12
hornerin	gi|528942932	174.7	8.1	6	8	8	8	9	9
cytokeratin 8 (370 AA)	gi|481	42.4	5	6					
alpha S1 casein, partial	gi|159793187	13.8	5.3	5	5	8	4	6	3
protein S100A7	gi|27807077	11.5	5.1	5	5	4	4	3	5
hemoglobin alpha chain	gi|6006425	15.2	9	4	8	4	3	2	6
protein S100A8	gi|165973998	10.5	5	4	5	8	6	5	5
protein S100A7-like	gi|528901741	11.6	5.6	4	4	3	4	3	4
ubiquitin	gi|290560476	8.3	5.7	4	4	2		4	4
imunoglobulin light chain, lambda	gi|513137422	22.5	5.9	4	3	5	3	4	5
fatty acid-binding protein, epidermal	gi|27805805	15.1	9	4	3	2	4	4	5
desmoplakin	gi|300797856	332.2	6.5	4			2	3	3
hornerin-like	gi|528901784	164	9.6	3	5	1	5	6	5
beta-casein	gi|119388700	18.5	5.9	3	3	7	1	4	3
gasdermin 1	gi|296476376	49.9	5.2	3	2	2	4	4	2
malate dehydrogenase, cytoplasmic	gi|77736203	36.4	6.2	3	1	3	1	5	5
IgM heavy chain constant region	gi|2232299	47.9	5.6	3		3	1	1	2
F-box only protein 50	gi|76641449	29.5	6.4	2	4		1	2	4
protein S100A2	gi|77735683	10.9	4.5	2	2	1	1	1	1

cystatin-A	gi|268607697	11.1	4.9	2	2		1	2	
cathelicidin-1 precursor	gi|27807341	17.6	9.3	2	2				
nucleoside diphosphate kinase B	gi|296488274	17	9.4	2	2				2
transitional endoplasmic reticulum ATPase 3	gi|73971210	89.2	5	2		3	2	1	3
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 12-like	gi|296473768	45.9	5.1	2		1	1	1	1
lactate dehydrogenase-A	gi|217590	35.6	9.1	2		1		1	2
S100 calcium binding protein A11	gi|296489536	11.2	6.1	2			1		
beta actin	gi|6224858	9.1	6.2	2				1	
L-lactate dehydrogenase B chain	gi|27806561	36.6	6	2					3
serotransferrin precursor	gi|296490958	77.6	7.8	2					2
pancreatic adenocarcinoma upregulated factor-like	gi|296473587	17	9.3	1	3	1	2		6
proteasome subunit beta type-2 isoform 2	gi|426215156	20.3	7	1	2	2	1	1	2
mammalian 20s proteasome	gi|21465643	25.8	7.7	1	2	1		1	1
protein DJ-1	gi|62751849	20	7.7	1	2			1	2
lactoperoxidase precursor	gi|27806851	80.6	9.7	1	2				
alpha-S2-casein precursor	gi|27806963	26	9.2	1	1	4		1	1
casein para kappaA	gi|229416	12.3	9.5	1	1	2	1	2	2
IgG1 heavy chain constant region, partial	gi|7547266	35.8	6.1	1	1	2			1
thioredoxin	gi|296484386	10.9	5.2	1	1		1	2	1
junction plakoglobin isoform X1	gi|528996002	81.6	6.7	1		3	2		
apolipoprotein A-I precursor	gi|162678	30.3	5.8	1			2		
histone H2B	gi|157785601	7.4	10.3	1			1		3
serpin B3-like	gi|119924103	44.5	7		15	16			18

serpin B3	gi|358420938	44.5	6.1		12				
hypothetical protein BOS_17317	gi|296478164	48.7	11.6		1	2	2	1	2
keratin, type I cytoskeletal 16	gi|528921408	46.1	4.7			9	7		
keratin, type II cytoskeletal 78	gi|156120801	57.4	9.4			4	5	4	4
keratin, type I cytoskeletal 24	gi|329664418	55.1	4.8			3	3	3	1
proteasome subunit alpha type-7 isoform X3	gi|528973440	23.7	7.8			1	2	1	1
protein S100A14	gi|118601864	11.4	4.5			1		1	
polyubiquitin-C	gi|329665078	77.5	7.8				5		
cathepsin O preproprotein-like	gi|296478683	41.6	9.9				2		1
heat shock cognate 71 kDa protein	gi|76253709	60.9	6					1	2
14-3-3 protein sigma	gi|57163961	27.8	4.5						10
zymogen granule protein 16 homolog B	gi|528925568	24.2	5.6						4
galectin-7	gi|297485581	15.4	6.1						3
tropomyosin alpha-3 chain isoform 8	gi|499137520	26.4	4.6						3
heat shock 27kDa protein 1	gi|61553385	17.5	6.6						2
cyclophilin I	gi|7767529	10.3	6.3						2

Table C5: Normalised total SpCs of 25 proteins showing ≥ two-fold change in protein abundance between late-lactating and 14 d involution TCL ($p < 0.05$)

	Late-lactating TCL						**14 d involution TCL**										
Identified proteins	**Cow 005**	**Cow 390**	**Cow 452**	**Cow 872**	**Cow 910**	**Cow 1048**	**Cow 115**	**Cow 160**	**Cow 331**	**Cow 358**	**Cow 499**	**Cow 837**	**Cow 911**	**LL Ave**	**d14 Ave**	***p*-value**	**Fc**
lactoferrin	3	0	0	0	0	0	25	23	37	13	10	21	45	0.5	24.9	0.0006	49.71
vimentin	0	0	2	2	2	2	0	0	0	0	0	0	0	1.3	0.5	0.0055	0.38
cofilin-1	0	2	1	3	0	2	0	0	0	0	0	0	0	1.3	0.5	0.0136	0.38
glyceraldehyde-3-phosphate dehydrogenase	6	10	11	7	8	9	12	4	0	1	2	2	1	8.5	3.1	0.0136	0.37
fatty acid binding protein, epithelial	8	10	10	13	14	9	3	4	2	7	3	4	3	10.7	3.7	0.0001	0.35
repetin	5	2	8	3	2	5	4	0	1	0	2	2	1	4.2	1.4	0.0234	0.34
transgelin-2	0	2	1	5	1	2	0	0	0	0	0	0	0	1.8	0.5	0.0161	0.27
purine nucleoside phosphorylase	0	1	2	4	0	4	0	0	0	0	0	0	0	1.8	0.5	0.0220	0.27
histone H2A type 1-B/E	0	2	4	3	4	0	0	0	0	0	0	0	0	2.2	0.5	0.0093	0.23
tubulin alpha-1B chain	0	0	2	5	4	5	0	0	0	0	0	0	0	2.7	0.5	0.0112	0.19
histone H2B type 1-D	0	4	3	5	4	0	1	0	0	2	0	0	0	2.7	0.4	0.0263	0.16
collagen, type VI, alpha 3-like isoform 2	0	6	6	0	6	1	0	0	0	0	0	0	0	3.2	0.5	0.0206	0.16
nucleoside diphosphate kinase B	7	8	5	7	10	7	3	2	0	0	0	2	1	7.3	1.1	0.0000	0.16
L-lactate dehydrogenase A chain	2	3	4	3	2	3	1	0	1	0	1	0	0	2.8	0.4	0.0000	0.15
peroxiredoxin-1	0	3	2	8	3	4	0	0	0	0	0	0	0	3.3	0.5	0.0066	0.15

Table B5 continued:

14-3-3 protein sigma	2	8	9	23	2	23	2	0	0	2	0	6	0	11.2	1.4	0.0240	0.13
Junction plakoglobin	2	11	18	19	11	9	2	0	3	1	0	0	3	11.7	1.3	0.0012	0.11
insulin-degrading enzyme precursor	4	3	4	0	3	2	0	0	1	0	1	0	0	2.7	0.3	0.0022	0.11
14-3-3 protein zeta	0	0	6	12	0	12	0	0	0	0	0	0	0	5.0	0.5	0.0451	0.10
peptidylprolyl isomerase A	0	4	3	9	2	2	0	0	0	0	0	2	0	3.3	0.3	0.0270	0.09
aspartic peptidase, retroviral-like 1	2	0	0	5	1	3	0	0	0	1	0	0	0	1.8	0.1	0.0442	0.08
galectin-7	0	7	5	12	8	1	0	0	0	0	0	2	0	5.5	0.3	0.0114	0.05
heat shock protein beta-1	0	6	8	10	3	7	0	0	0	0	0	2	0	5.7	0.3	0.0026	0.05
heat shock cognate 71 kDa protein	1	2	6	4	3	3	0	0	0	0	0	1	0	3.2	0.1	0.0008	0.05
beta-actin	0	2	8	11	6	11	1	0	0	0	1	0	0	6.3	0.3	0.0051	0.05

Fc, fold count

Supplementary Data D

Microbiological methods and proteomic GeLC-MS/MS analysis of the bovine teat canal lining 24 h post bacterial challenge

D1 Preparation of cultures for teat canal inoculation

The extent of turbidity, or optical density (OD), was used to measure the cell biomass indirectly as the bacteria grew in liquid culture. Two cultures were made for each of the three microbes. One culture for measuring OD and the other culture for glycerol stocks. All three bacterial cultures exhibited standard, *in vitro*, temporal bacterial growth curves with characteristic lag, exponential and stationary phases (Figure D1). Both the *E.coli* and *S. aureus* cultures were stopped and harvested for glycerol-stock storage at late-log phase when their absorbance reading reached an OD of 0.8. *S. uberis* not only grew slower than the other two cultures, but its stationary phase was also lower (OD of 0.4). Based on this growth curve, the *S. uberis* culture was stopped and 0.5 mL aliquots harvested for glycerol-stock storage at late-log phase when its absorbance reading reached an OD of 0.25 - 0.3. Glycerol stocks of bacteria were stored at -80 °C until required.

In preparation for teat infusion, bacteria were recovered from frozen glycerol stock solutions, washed in 1 mL of one-quarter strength ringer’s solution containing 0.1 % (*w/v*) proteose-peptone for 1 h at 37 °C. One hundred microlitres of this suspension was diluted into 9.9 mL of sterile skim milk. After 16 h growth at 37 °C, ten-fold serial dilutions were performed to estimate the bacterial concentration in the skim milk suspension (See Methods Figure 2.2). The suspensions were then adjusted by dilution to give final inoculum concentrations of 2.5 x 10^8 cfu/mL (~500,000 cfu in 2 µL of skim milk), 1.25 x 10^6 cfu/mL (~2500 cfu in 2 µL of skim milk), and 1.25 x 10^6 cfu/mL (~2500 cfu in 2 µL of skim milk), for *E. coli*, *S. aureus*, and *S, uberis*, respectively.

Minimal cell death (< 3 %) was observed when the stock skim milk suspensions were stored overnight at 4 °C (*data not shown*). Stock skim milk suspensions were therefore made two days preceding the on-farm inoculations, allowing for a 16 h growth phase and then a 24 h storage phase. This storage phase enabled an accurate bacterial count for each inoculum to be determined prior to administration (Table D1). Dilutions of the inoculums were also replated after the on-farm inoculations. The results from this analysis showed negligible change (< 5.0 %) from the preceding dilution series (*data not shown*).

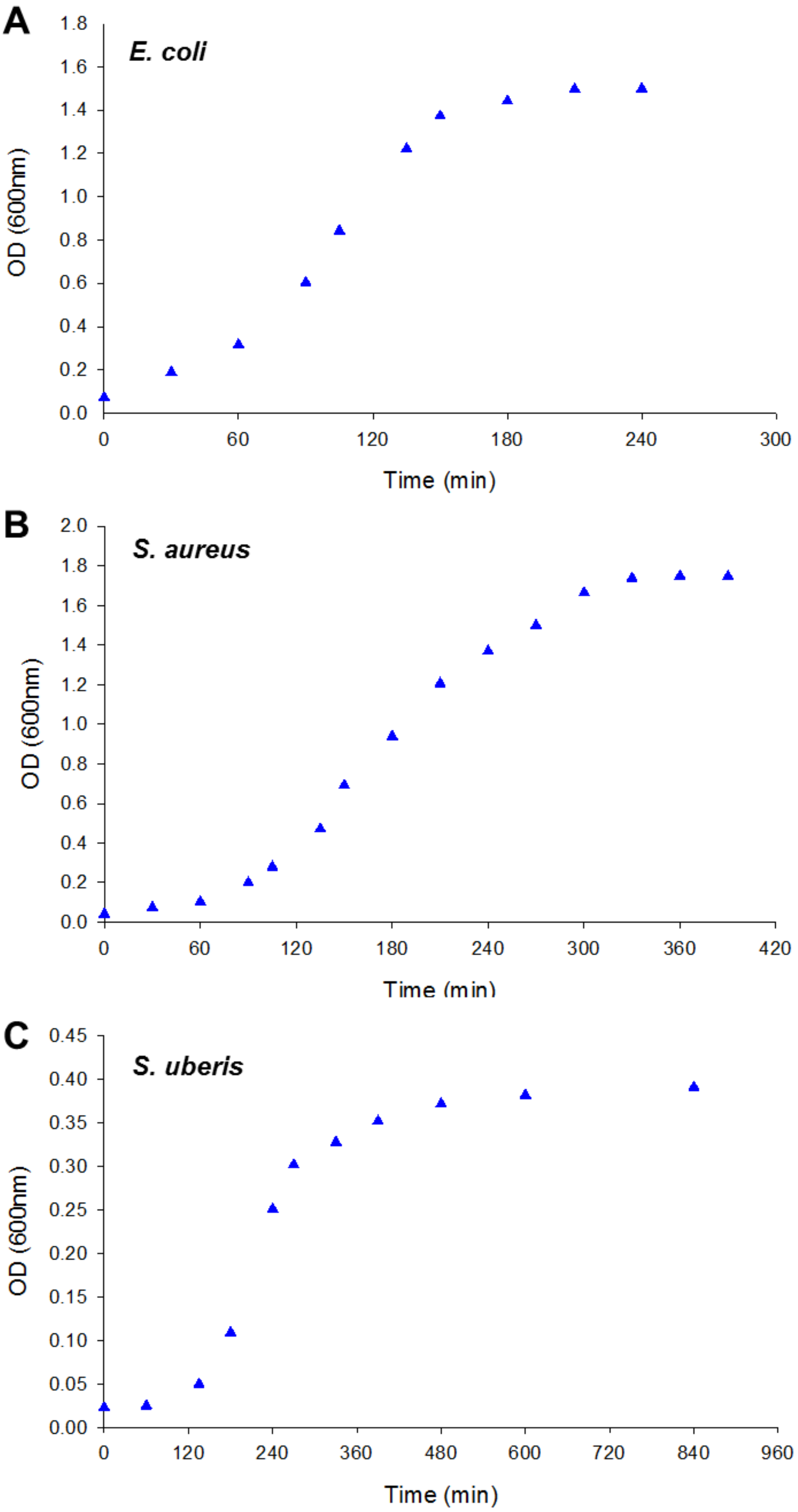

Figure D1: Growth curves for *E.coli, S. aureus* and *S. uberis* in brain heart infusion broth.

Table D1: Bacterial concentrations for teat canal inoculations.

Day 1	**Colony counts prior to teat canal inoculations**						
Bacteria	**Number of colonies**			**Average**	**Dilution**	**cfu[a]/mL**	**SD[b]**
E. coli	*TMTC[c]*	*TMTC*	*TMTC*		1 x 10^{-3}	2.1 x 10^{8}	1.0 x 10^{7}
	TMTC	*TMTC*	*TMTC*		1 x 10^{-4}		
	20	21	22	22.60	1 x 10^{-5}		
	1	0	0	0.33	1 x 10^{-6}		
S. uberis	*TMTC*	*TMTC*	*TMTC*		1 x 10^{-2}	1.3 x 10^{6}	1.7 x 10^{5}
	12	12	15	13.00	1 x 10^{-3}		
	2	1	2	1.67	1 x 10^{-4}		
	0	0	0	0.00	1 x 10^{-5}		
S. aureus	*TMTC*	*TMTC*	*TMTC*		1 x 10^{-2}	1.6 x 10^{6}	2.1 x 10^{5}
	14	17	18	16.33	1 x 10^{-3}		
	2	3	3	2.67	1 x 10^{-4}		
	0	0	0	0.00	1 x 10^{-5}		

Day 2	**Colony counts prior to teat canal inoculations**						
Bacteria	**Number of colonies**			**Average**	**Dilution**	**cfu/mL**	**SD**
E. coli	*TMTC*	*TMTC*	*TMTC*		1 x 10^{-4}	2.7 x 10^{8}	2.0 x 10^{7}
	29	25	27	27.00	1 x 10^{-5}		
	3	2	4	3.00	1 x 10^{-6}		
	1	0	0	0.33	1 x 10^{-7}		
S. uberis	*TMTC*	*TMTC*	*TMTC*		1 x 10^{-2}	1.6 x 10^{6}	3.2 x 10^{5}
	14	20	15	16.33	1 x 10^{-3}		
	1	2	1	1.33	1 x 10^{-4}		
	0	0	0	0.00	1 x 10^{-5}		
S. aureus	*TMTC*	*TMTC*	*TMTC*		1 x 10^{-2}	1.6 x 10^{6}	4.0 x 10^{7}
	20	16	12	16.00	1 x 10^{-3}		
	2	1	1	1.33	1 x 10^{-4}		
	0	0	0	0.00	1 x 10^{-5}		

a) cfu, colony forming units; b) SD, standard deviation; c) TMTC, Too many to count

Table D2: Significantly altered 2-DE proteins spots from Control verses *E. coli* infection groups

SSP	Con-1	Con-2	Con-3	*E. coli*-1	*E. coli*-2	*E. coli*-3	(Ave) Con	SD	(Ave) *E. coli*	SD	Fc	*p*-value (n = 3)	Identified protein
102	16.2	17.8	14.9	22	24.6	30.7	16.3	1.5	25.8	4.5	1.58	0.025	caspase-14
104	5.9	3.9	3.7	9.1	9.5	8.9	4.5	1.2	9.2	0.3	2.04	0.003	β-lactoglobulin
1507	33.6	51.1	28.6	7.9	12.8	11	37.8	11.8	10.6	2.5	0.28	0.017	keratin 14
2401	15.2	19.3	20.4	10.9	11.7	2.1	18.3	2.7	8.2	5.3	0.45	0.043	β-actin
4201	6	5.2	5.6	4.4	4.6	3.6	5.6	0.4	4.2	0.5	0.75	0.021	heat shock protein beta-1
4301	11.4	14.2	16.6	26.9	22.1	28	14.1	2.6	25.7	3.1	1.82	0.008	*spot not identified*
4302	4.5	3.6	4.9	2.7	3.4	2.8	4.3	0.7	3.0	0.4	0.68	0.036	serpin B3-like
4402	5.8	5.9	6.9	5	5.2	5.1	6.2	0.6	5.1	0.1	0.82	0.036	serpin B3-like
4901	18.5	18.3	25.7	8.5	8.2	5.7	20.8	4.2	7.5	1.5	0.36	0.007	serum albumin
5003	1102	926	1156	1288.9	1267.6	1299.6	1061.3	120.3	1285.4	16.3	1.21	0.033	S100A12
5501	5	4.4	4.9	6.8	6	5.5	4.8	0.3	6.1	0.7	1.28	0.034	serpin B3-like
5702	14.3	21.1	12.8	30.1	32.2	29.5	16.1	4.4	30.6	1.4	1.90	0.006	keratin 4/ keratin 5
6503	2.7	2.4	2.4	4.2	3.6	3.6	2.5	0.2	3.8	0.3	1.52	0.004	keratin 6A
7001	23.3	22.9	18.6	12.6	13.2	10.8	21.6	2.6	12.2	1.2	0.56	0.005	ubiquitin
8101	8.7	12.3	14.1	16.1	20.4	18.3	11.7	2.7	18.3	2.2	1.56	0.031	hemoglobin subunit beta
9201	6	1.6	3.5	9.4	10.8	12.9	3.7	2.2	11.0	1.8	2.98	0.011	*spot not identified*

Table D3: Significantly altered 2-DE proteins spots from Control verses *S. aureus* infection groups

SSP	Con-1	Con-2	Con-3	*S.aureus*-1	*S.aureus*-2	*S.aureus*-3	(Ave) Con	SD	(Ave) *S. aureus*	SD	Fc	*p*-value	Identified protein
1103	14.5	16.3	13.1	17.3	18.6	20.4	14.6	1.6	18.8	1.6	1.28	0.033	caspase-14
3101	3.7	4.3	4	6.1	7.2	6.8	4.0	0.3	6.7	0.6	1.68	0.002	*spot not identified*
3801	3.9	2.7	3.7	5.6	4.6	5.2	3.4	0.6	5.1	0.5	1.50	0.023	heat shock cognate 71 kDa protein
4702	8	8.2	8.8	12.5	12.2	14.6	8.3	0.4	13.1	1.3	1.57	0.004	keratin 4/ keratin 5
4802	16.5	16.7	20.5	30.5	29.1	29.9	17.9	2.3	29.8	0.7	1.67	0.001	serum albumin
6502	4.2	5	4.7	2.3	2.6	2	4.6	0.4	2.3	0.3	0.50	0.001	*spot not identified*
6504	7.3	15.5	10.6	1.6	1.1	2	11.1	4.1	1.6	0.5	0.14	0.016	serpin B3-like
6801	28.6	20.7	30.9	20.3	9.6	7.8	26.7	5.4	12.6	6.8	0.47	0.047	keratin 1
7001	20.1	20.2	15.8	6.7	5.1	5.2	18.7	2.5	5.7	0.9	0.30	0.001	ubiquitin
7103	10.9	9.2	10.6	5.9	6.6	6.1	10.2	0.9	6.2	0.4	0.61	0.002	beta hemoglobin
8103	2.8	3	3.3	2.3	1.9	2.2	3.0	0.3	2.1	0.2	0.70	0.009	*spot not identified*
8201	6.1	5.3	6.3	5.1	4.7	4.1	5.9	0.5	4.6	0.5	0.79	0.040	*spot not identified*
8708	24.7	26.5	32.3	97.6	71.9	99.6	27.8	4.0	89.7	15.4	3.22	0.003	keratin 6A
9206	8.2	8.2	4.2	12	10.4	10.6	6.9	2.3	11.0	0.9	1.60	0.044	non-specific cytotoxic cell receptor protein 1 homolog

Table D4: Significantly altered 2-DE proteins spots from Control verses *S. uberis* infection groups

SSP	Con-1	Con-2	Con-3	*S.uberis*-1	*S.uberis*-2	*S.uberis*-3	(Ave) Con	SD	(Ave) *S. uberis*	SD	Fc	*p*-value	Identified protein
2	12.8	19.4	15.7	8.6	10.7	9.2	16.0	3.3	9.5	1.1	0.59	0.032	*spot not identified*
4	1.8	46.4	24.9	57.2	65.2	75.6	24.4	22.3	66.0	9.2	2.71	0.040	*spot not identified*
901	1.4	0.4	0.2	2.1	2.3	2.9	0.7	0.6	2.4	0.4	3.65	0.016	*spot not identified*
1101	5.1	3.2	3.2	7.1	6.8	6.9	3.8	1.1	6.9	0.2	1.81	0.008	*spot not identified*
1102	13.4	14.4	12.3	19	18.4	18.9	13.4	1.1	18.8	0.3	1.40	0.001	caspase-14
1106	6.2	1.1	1.8	9.7	7	7.9	3.0	2.8	8.2	1.4	2.70	0.044	*spot not identified*
1110	2.9	0.5	3.4	8.6	12.9	11.2	2.3	1.6	10.9	2.2	4.81	0.005	β-lactoglobulin
2501	27.8	31.1	20.6	13.1	14.1	10.1	26.5	5.4	12.4	2.1	0.47	0.013	keratin 4
4002	74.3	46.6	113.3	209.6	136	151.6	78.1	33.5	165.7	38.8	2.12	0.041	S100A12
4901	15.3	14.8	22.1	7.2	8.2	11.3	17.4	4.1	8.9	2.1	0.51	0.033	serum albumin
5505	1.5	1.1	1.4	2.2	2.5	1.9	1.3	0.2	2.2	0.3	1.65	0.015	*spot not identified*
5604	11.9	14.5	2.2	48	19.5	37.8	9.5	6.5	35.1	14.4	3.68	0.049	keratin 4/ keratin 5
5701	29.4	19	35.4	11.2	7.6	17.1	27.9	8.3	12.0	4.8	0.43	0.045	keratin 1
6505	4	4.7	4.6	2.9	2.8	2.9	4.4	0.4	2.9	0.1	0.65	0.002	*spot not identified*
7001	19.7	18.5	15.5	4.9	7.4	10.7	17.9	2.2	7.7	2.9	0.43	0.008	ubiquitin
7104	3.5	2.7	8	9.2	10.6	10.2	4.7	2.9	10.0	0.7	2.11	0.036	S100A9
7205	3.4	2.5	2.6	0.4	0.5	2	2.8	0.5	1.0	0.9	0.34	0.034	*spot not identified*
8108	4.1	2.9	3.1	1.1	2.2	0.4	3.4	0.6	1.2	0.9	0.37	0.029	*spot not identified*
8204	2.1	0.4	1.6	5.2	2.7	3.9	1.4	0.9	3.9	1.3	2.88	0.043	annexin A2
9101	5.4	2.8	3.6	6.1	6.7	7.6	3.9	1.3	6.8	0.8	1.73	0.032	non-specific cytotoxic cell receptor protein 1 homolog
9704	7.4	8.3	3	11.8	10.5	16	6.2	2.8	12.8	2.9	2.05	0.049	keratin 1

Table D5: Summary of total MS/MS spectra from each teat canal lining tryptic digest fraction from the bacterial challenge study group of 4 cows

Cow #	**225**				**708**			
Treatment	**Con**	**EC**	**SA**	**SU**	**Con**	**EC**	**SA**	**SU**
Quarter	**FL**	**BL**	**FR**	**BR**	**FL**	**BR**	**FR**	**BL**
Fraction 1	1119	1324	1227	1184	965	1194	1204	1020
Fraction 2	1223	1294	1148	1123	919	1148	1201	870
Fraction 3	1009	1180	1061	1040	887	1056	1003	1048
Fraction 4	924	992	997	999	915	911	960	933
Fraction 5	1243	1242	1217	1234	1179	1070	1173	1013
Fraction 6	1097	1186	1101	1149	1138	850	1134	983
Fraction 7	1114	1183	1212	1189	1119	819	989	936
Fraction 8	1061	1141	1050	1056	579	603	487	1052
Total	**8790**	**9542**	**9013**	**8974**	**7701**	**7651**	**8151**	**7855**

Cow #	**1193**				**1348**			
Treatment	**Con**	**EC**	**SA**	**SU**	**Con**	**EC**	**SA**	**SU**
Quarter	**FR**	**BR**	**FL**	**BL**	**FR**	**BR**	**BL**	**FL**
Fraction 1	1267	1305	1275	1061	1141	1325	726	1063
Fraction 2	1126	1254	1012	967	1011	1143	1193	872
Fraction 3	1034	982	1004	1061	1079	1079	1049	1039
Fraction 4	947	957	952	957	891	995	916	997
Fraction 5	877	1311	1061	949	1146	1069	1145	1231
Fraction 6	948	1080	1064	1179	1094	1176	637	984
Fraction 7	1073	687	1224	1145	920	823	1158	1191
Fraction 8	721	927	1049	1050	578	1121	660	1057
Total	**7993**	**8503**	**8641**	**8369**	**7860**	**8731**	**7484**	**8434**

	Fraction 1	**Fraction 2**	**Fraction 3**	**Fraction 4**	**Fraction 5**	**Fraction 6**	**Fraction 7**	**Fraction 8**
Average	1150	1094	1038	953	1135	1050	1049	887
SD	157.8	135.0	60.2	35.6	119.3	144.5	166.1	234.6
% CV	13.7	12.3	5.8	3.7	10.5	13.8	15.8	26.4

SD, standard deviation; CV, coefficient of variation

Table D6: Normalised raw SpC data for experimentally induced bacterial challenge study

				Normalised SpCs																		
				Control				*E. coli*				*S. aureus*				*S. uberis*				Ratio		
#	Protein Acc. No.	Protein Name	Mwt	225	708	1193	1348	225	708	1193	1348	225	708	1193	1348	225	708	1193	1348	*E. coli* /Con	*S. aureus* /Con	*S. uberis* /Con
1	gi|358421409	keratin, type II cytoskeletal 1	63 kDa	554	457	453	489	619	497	453	510	548	486	395	466	547	478	406	466	1.06	0.97	0.97
2	gi|151553925	keratin, type I cytoskeletal 10	55 kDa	510	544	438	414	538	575	450	534	493	416	388	428	436	467	462	410	1.10	0.91	0.93
3	gi|134085706	keratin, type II cytoskeletal 6A	61 kDa	313	255	294	344	337	239	303	254	297	253	271	301	238	282	272	259	0.94	0.93	0.87
4	gi|296487883	cytokeratin type II, component IV isoform 2	59 kDa	269	231	287	301	295	219	252	231	253	217	248	263	204	252	249	230	0.92	0.90	0.86
5	gi|358421417	keratin, type II cytoskeletal 3	65 kDa	267	155	210	221	287	189	204	206	257	204	174	206	283	162	187	211	1.04	0.99	0.99
6	gi|148356276	keratin, type II cytoskeletal 4	58 kDa	254	126	135	154	284	108	156	136	233	153	123	115	217	133	100	116	1.02	0.93	0.85
7	gi|146186887	keratin, type II cytoskeletal 5	63 kDa	236	177	191	242	258	196	180	190	219	170	202	208	180	189	169	165	0.97	0.94	0.83
8	gi|115496454	keratin, type II cytoskeletal 79	58 kDa	212	189	241	258	251	173	260	196	195	143	211	235	154	218	218	184	0.98	0.87	0.86
9	gi|296489626	profilaggrin-like	56 kDa	203	189	278	159	288	168	283	238	215	157	190	190	198	154	250	215	1.18	0.91	0.99
10	gi|119892108	keratin, type II cytoskeletal 2 (epidermal)	64 kDa	114	42	44	49	61	136	44	97	58	72	36	50	95	52	33	68	1.36	0.87	1.00
11	gi|262118301	keratin, type I cytoskeletal 14	52 kDa	106	87	98	117	95	84	119	88	108	110	92	105	89	102	86	107	0.95	1.02	0.94
12	gi|296489624	filaggrin-like	180 kDa	83	84	133	75	105	76	130	94	101	71	80	81	84	79	109	103	1.08	0.89	1.00
13	gi|114052490	S100-A9	16 kDa	63	71	65	87	53	41	77	87	52	45	69	73	42	35	67	77	0.90	0.84	0.77
14	gi|27807183	S100-A12	11 kDa	60	49	70	26	71	61	44	72	45	19	59	53	47	30	16	59	1.21	0.86	0.74
15	gi|119923094	serpin B3-like	44 kDa	47	34	59	31	58	30	67	47	39	29	62	56	42	35	54	54	1.18	1.09	1.08
16	gi|157427776	keratin, type I cytoskeletal 17	49 kDa	41	50	80	57	52	22	97	41	50	41	79	88	38	51	53	64	0.93	1.13	0.90
17	gi|159793191	alpha S1 casein, partial	24 kDa	40	31	31	27	34	112	31	39	60	25	36	47	43	38	48	47	1.67	1.30	1.36
18	gi|119916469	serpin B3-like	44 kDa	39	48	54	37	40	29	59	44	30	32	55	54	28	37	59	57	0.97	0.96	1.02
19	gi|359079289	serpin B3-like	42 kDa	37	40	53	38	38	30	60	49	25	31	53	56	27	40	53	47	1.05	0.98	0.99
20	gi|332205893	serpin B4	44 kDa	36	46	56	42	38	30	61	46	29	35	56	51	28	38	58	55	0.97	0.95	0.99
21	gi|528942932	hornerin	175 kDa	32	29	41	16	19	21	38	21	25	26	41	42	24	28	39	44	0.84	1.14	1.14
22	gi|165973998	S100-A8	10 kDa	29	15	21	7	27	29	16	27	31	3	31	25	25	6	5	26	1.38	1.25	0.86
23	gi|122539	hemoglobin beta-A chain	16 kDa	28	3	14	30	49	19	9	43	24	5	29	32	28	6	28	18	1.60	1.20	1.07
24	gi|329663440	caspase-14	28 kDa	23	20	16	7	24	13	19	20	16	11	28	16	16	20	20	22	1.15	1.08	1.18
25	gi|109658166	junction plakoglobin	82 kDa	19	13	27	18	11	6	20	19	2	0	35	22	6	5	25	28	0.73	0.77	0.83
26	gi|2194088	beta-lactoglobulin	18 kDa	18	19	18	24	22	19	19	23	26	23	15	14	24	23	21	13	1.05	0.99	1.03

27	gi|300797856	desmoplakin	332 kDa	16	25	60	24	9	6	14	11	5	7	67	69	1	21	23	42	0.32	1.18	0.70
28	gi|30794280	serum albumin precursor	69 kDa	15	6	18	5	21	26	20	34	16	47	40	57	25	36	25	19	2.30	3.64	2.39
29	gi|27807077	S100-A7	12 kDa	14	18	8	7	16	14	18	16	12	7	12	16	16	11	15	19	1.36	1.00	1.30
30	gi|297472640	S100-A7 isoform X2	12 kDa	14	14	12	7	16	14	14	16	8	6	8	14	14	11	10	17	1.28	0.77	1.11
31	gi|115496892	nucleoside diphosphate kinase B	17 kDa	13	5	7	9	8	0	12	5	4	4	11	11	5	3	8	12	0.74	0.88	0.82
32	gi|116812902	hemoglobin subunit alpha	15 kDa	12	0	9	12	18	1	5	15	9	9	13	7	13	2	15	5	1.18	1.15	1.06
33	gi|528901784	hornerin-like	164 kDa	11	14	18	7	6	7	23	9	9	12	25	26	8	7	19	21	0.90	1.44	1.10
34	gi|77404273	glyceraldehyde-3-phosphate dehydrogenase	36 kDa	11	9	16	11	4	4	12	6	5	6	7	5	4	7	9	11	0.55	0.49	0.66
35	gi|1293786	fatty acid binding protein, epidermal	15 kDa	11	6	6	8	6	7	12	7	11	6	20	8	6	3	9	12	1.03	1.45	0.97
36	gi|57163961	14-3-3 protein sigma	28 kDa	11	5	5	21	0	3	1	1	0	16	26	18	0	10	4	5	0.12	1.43	0.45
37	gi|125489161	kappa casein	18 kDa	10	5	4	5	11	22	6	9	8	6	8	6	8	6	11	6	2.00	1.17	1.29
38	gi|118151260	gasdermin-A	50 kDa	10	5	15	6	8	13	9	9	7	7	12	10	5	6	10	5	1.08	1.00	0.72
39	gi|83406093	beta casein	25 kDa	9	8	11	11	9	28	11	13	8	7	7	9	4	17	14	5	1.56	0.79	1.03
40	gi|296477713	galectin-7-like	15 kDa	9	8	2	9	0	4	6	0	0	5	14	2	0	8	6	6	0.36	0.75	0.71
41	gi|27806963	alpha-S2-casein precursor	26 kDa	8	8	5	6	11	23	5	13	25	4	12	14	17	4	6	13	1.93	2.04	1.48
42	gi|34538498	immunoglobulin mu heavy chain constant region	50 kDa	8	7	2	0	12	9	1	0	4	3	4	5	8	6	3	3	1.29	0.94	1.18
43	gi|74353860	IGL@ protein	25 kDa	8	4	7	13	6	8	4	7	5	8	12	17	6	10	9	10	0.78	1.31	1.09
44	gi|296480594	fatty acid-binding protein, adipocyte	15 kDa	8	8	1	4	0	8	8	0	0	2	5	1	0	6	8	0	0.76	0.38	0.67
45	gi|27806505	polyubiquitin-B	34 kDa	7	7	6	6	8	7	6	8	6	4	6	6	6	8	4	7	1.12	0.85	0.96
46	gi|528924507	histone H2A type 1-like	34 kDa	6	10	2	8	0	4	2	2	4	10	15	14	1	1	5	5	0.31	1.65	0.46
47	gi|4507953	14-3-3 protein zeta/delta	28 kDa	6	6	0	10	0	0	0	0	0	8	18	10	0	4	3	6	0.00	1.64	0.59
48	gi|151556908	retroviral-like aspartic protease 1	34 kDa	6	6	0	4	4	3	5	2	2	0	4	3	0	4	6	3	0.88	0.56	0.81
49	gi|268607697	cystatin-A	11 kDa	6	5	6	5	6	6	6	6	6	0	6	12	5	8	4	6	1.09	1.09	1.05
50	gi|118601864	S100-A14	11 kDa	6	5	3	2	3	6	5	3	2	0	2	3	3	1	3	3	1.06	0.44	0.63
51	gi|10800138	histone H2B type 1-D	14 kDa	5	1	1	4	0	4	2	1	1	4	12	7	0	0	6	4	0.64	2.18	0.91
52	gi|529007450	heat shock protein beta-1	22 kDa	5	5	2	0	0	0	1	8	22	18	15	17	7	8	3	9	0.75	6.00	2.25
53	gi|77735683	S100-A2	11 kDa	4	4	6	2	4	4	4	4	4	2	4	4	4	4	3	4	1.00	0.88	0.94
54	gi|74	annexin I	39 kDa	5	9	15	21	3	6	12	12	1	11	18	28	5	9	3	10	0.66	1.16	0.54
55	gi|313507212	beta-actin	42 kDa	4	4	8	20	0	2	7	7	0	12	17	8	0	6	4	7	0.44	1.03	0.47
56	gi|77736203	malate dehydrogenase, cytoplasmic	36 kDa	4	4	12	7	2	0	8	8	5	0	10	8	3	3	1	4	0.67	0.85	0.41
57	gi|62751339	proteasome subunit beta type-2	23 kDa	3	3	4	6	2	1	4	6	2	3	4	4	4	4	2	4	0.81	0.81	0.88

58	gi|76641449	F-box only protein 50	30 kDa	3	2	10	6	2	1	6	6	1	3	6	6	5	3	4	6	0.71	0.76	0.86
59	gi|329663176	ganglioside GM2 activator precursor	21 kDa	3	0	6	3	3	0	4	3	3	1	3	3	2	1	3	3	0.83	0.83	0.75
60	gi|27806559	L-lactate dehydrogenase A chain	37 kDa	3	2	2	5	0	0	2	2	0	1	4	5	0	2	4	2	0.33	0.83	0.67
61	gi|62751849	protein DJ-1	20 kDa	3	2	3	2	1	0	2	2	1	1	4	4	0	0	0	4	0.50	1.00	0.40
62	gi|27807289	annexin A2	39 kDa	3	2	6	8	0	2	5	4	1	0	6	7	0	0	4	3	0.58	0.74	0.37
63	gi|156120801	keratin, type II cytoskeletal 78	57 kDa	3	0	4	2	3	0	5	0	0	1	2	3	4	0	1	0	0.89	0.67	0.56
64	gi|296473587	pancreatic adenocarcinoma upregulated factor-like	17 kDa	2	1	2	0	4	4	3	6	8	4	3	4	7	2	3	4	3.40	3.80	3.20
65	gi|148238040	alpha-actinin-4	105 kDa	2	1	2	0	2	0	0	2	0	2	2	5	0	1	2	4	0.80	1.80	1.40
66	gi|595763493	peroxiredoxin 1	22 kDa	2	1	1	13	0	1	3	13	0	4	7	7	8	4	4	7	1.00	1.06	1.35
67	gi|28189771	peptidylprolyl isomerase A	17 kDa	2	1	0	7	1	0	3	0	0	4	9	3	2	4	4	3	0.40	1.60	1.30
68	gi|27806783	thioredoxin	12 kDa	2	3	4	4	2	2	3	2	1	3	1	3	3	2	1	3	0.69	0.62	0.69
69	gi|528942936	repetin	78 kDa	2	0	3	4	1	2	9	5	4	2	9	18	2	1	1	0	1.89	3.67	0.44
70	gi|296473768	serpin B12	46 kDa	2	0	4	0	0	0	4	0	0	0	2	1	1	1	0	0	0.67	0.50	0.33
71	gi|115495235	insulin-degrading enzyme precursor	118 kDa	1	0	0	1	2	0	5	6	2	0	5	8	0	0	5	6	6.50	7.50	5.50
72	gi|27881410	desmoglein-1 precursor	112 kDa	1	0	1	0	1	0	0	0	1	0	6	5	0	0	2	5	0.50	6.00	3.50
73	gi|77735541	transitional endoplasmic reticulum ATPase	89 kDa	1	4	8	3	6	1	2	7	4	5	8	7	4	4	7	7	1.00	1.50	1.38
74	gi|61888856	triosephosphate isomerase	27 kDa	1	2	0	3	0	0	0	0	0	3	9	4	0	2	0	2	0.00	2.67	0.67
75	gi|548494982	heat shock cognate 71 kDa protein	71 kDa	1	6	3	5	0	0	0	5	1	1	18	1	0	0	4	5	0.33	1.40	0.60
76	gi|154757495	PSMA7 protein	27 kDa	1	1	4	2	0	0	0	0	1	0	1	3	0	1	2	1	0.00	0.63	0.50
77	gi|114155144	tropomyosin alpha-3 chain isoform 4	29 kDa	1	2	1	3	2	1	1	1	2	1	1	3	0	1	0	2	0.71	1.00	0.43
78	gi|118572666	L-lactate dehydrogenase B chain	37 kDa	1	0	1	4	0	0	0	0	0	2	2	2	0	1	1	0	0.00	1.00	0.33
79	gi|253723066	profilin	15 kDa	1	4	0	3	0	1	2	0	0	0	4	4	0	1	0	0	0.38	1.00	0.13
80	gi|157830389	beta2-microglobulin	12 kDa	0	0	0	0	0	0	0	0	3	0	3	4	4	0	0	4	0.00	5.00	4.00
81	gi|296475271	AHNAK nucleoprotein 2	149 kDa	0	0	0	0	0	0	0	0	0	0	7	7	0	0	0	1	0.00	7.00	0.50
82	gi|114326282	serotransferrin precursor	78 kDa	0	0	0	0	1	1	2	1	3	7	3	4	2	2	1	0	2.50	8.50	2.50
83	gi|27806351	ezrin	69 kDa	0	0	0	0	0	0	0	1	0	0	3	1	0	0	2	2	0.50	2.00	2.00
84	gi|17986258	myosin light polypeptide 6 isoform 1	17 kDa	0	0	0	0	0	0	0	0	0	4	4	1	0	0	0	0	0.00	4.50	0.00
85	gi|296489622	cornulin-like	87 kDa	0	0	0	0	3	0	0	3	0	1	0	3	0	0	0	0	3.00	2.00	0.00
86	gi|528981276	fibrinogen gamma-B chain isoform X1	49 kDa	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0.00	4.00	0.00

87	gi|201067368	peptidoglycan recognition protein 1	21 kDa	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	3	0.00	3.00	1.50
88	gi|7710086	ras-related protein Rab-10	23 kDa	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	3	0.00	3.00	1.50
89	gi|300794325	ATP-dependent RNA helicase DDX3X	73 kDa	0	0	0	0	0	0	0	0	0	0	4	0	0	0	1	1	0.00	2.00	1.00
90	gi|4838363	creatine kinase M chain	43 kDa	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0.00	0.00	2.50
91	gi|528942126	prelamin-A/C isoform X1	74 kDa	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0.00	2.00	0.00
92	gi|4506697	40S ribosomal protein S20 isoform 2	13 kDa	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0.00	2.00	0.00
93	gi|77735675	putative RNA-binding protein 3	10 kDa	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0.00	1.50	0.50
94	gi|529014820	protein POF1B	57 kDa	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0.00	2.00	0.00
95	gi|27807341	cathelicidin-1 precursor	18 kDa	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0.00	1.00	0.00
96	gi|27806939	myoglobin	17 kDa	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0.00	0.00	1.50
97	gi|163285	lactoferrin	78 kDa	0	0	5	0	6	4	8	3	29	21	10	9	13	33	6	6	4.20	13.80	11.60
98	gi|115497900	elongation factor 2	95 kDa	0	1	0	1	0	0	0	1	0	0	13	1	0	0	7	7	0.50	7.00	7.00
99	gi|297298444	calmodulin-like isoform 2	17 kDa	0	1	0	0	1	0	2	2	1	2	3	0	2	0	2	2	5.00	6.00	6.00
100	gi|1620375	xanthine dehydrogenase	147 kDa	0	0	1	0	0	0	0	1	0	0	0	2	0	1	2	2	1.00	2.00	5.00
101	gi|119894421	histone H3.1	15 kDa	0	1	0	0	0	0	0	0	0	2	6	1	0	1	3	0	0.00	9.00	4.00
102	gi|29135329	glutathione S-transferase P	24 kDa	0	0	0	1	0	0	0	1	0	0	3	3	1	0	0	3	1.00	6.00	4.00
103	gi|162678	apolipoprotein A-I precursor	30 kDa	0	0	0	4	0	1	0	4	0	3	11	11	0	4	0	11	1.25	6.25	3.75
104	gi|13385408	60S ribosomal protein L11	20 kDa	0	0	0	2	0	0	0	2	0	0	4	4	3	0	0	4	1.00	4.00	3.50
105	gi|329664418	keratin, type I cytoskeletal 24	55 kDa	0	6	8	0	8	5	5	7	0	0	6	2	0	11	13	8	1.79	0.57	2.29
106	gi|528999775	heat shock protein HSP 90-alpha isoform X1	89 kDa	0	0	1	1	0	0	0	3	0	0	6	2	0	0	3	3	1.50	4.00	3.00
107	gi|149642995	tripeptidyl-peptidase 2	138 kDa	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	3	0.00	0.00	3.00
108	gi|28189683	ribosomal protein L12	16 kDa	0	1	0	2	0	0	0	2	0	0	4	4	2	0	0	4	0.67	2.67	2.00
109	gi|329664500	pyruvate kinase isozymes M1/M2	58 kDa	0	1	0	6	1	0	5	1	0	0	6	8	1	0	7	5	1.00	2.00	1.86
110	gi|4506741	40S ribosomal protein S7	22 kDa	0	2	0	3	0	0	0	3	0	0	4	4	1	1	3	4	0.60	1.60	1.80
111	gi|51592135	cofilin-1	19 kDa	0	4	1	6	0	0	0	6	0	0	9	9	5	4	1	8	0.55	1.64	1.64
112	gi|296471700	AHNAK nucleoprotein	587 kDa	0	3	4	0	0	0	0	0	0	0	11	11	0	2	1	7	0.00	3.14	1.43
113	gi|114052248	plastin-3	72 kDa	0	2	1	0	0	0	0	1	0	2	4	2	0	0	2	2	0.33	2.67	1.33
114	gi|296474125	eukaryotic translation elongation factor 1 alpha 1-like	50 kDa	0	1	0	4	0	0	2	0	1	0	7	5	0	1	2	3	0.40	2.60	1.20
115	gi|27806789	transthyretin precursor	16 kDa	0	0	0	2	0	0	2	0	0	0	4	4	0	0	0	2	1.00	4.00	1.00
116	gi|61888874	transgelin-2	22 kDa	0	2	1	9	0	0	0	9	0	2	2	2	6	2	2	2	0.75	0.50	1.00
117	gi|34740335	tubulin alpha-1B chain	50 kDa	0	3	1	5	0	0	3	1	0	2	10	8	0	0	3	5	0.44	2.22	0.89

118	gi|528996064	ATP-citrate synthase isoform X1	123 kDa	0	0	2	1	0	0	0	1	0	0	3	3	0	0	1	1	0.33	2.00	0.67
119	gi|77736139	proteasome subunit beta type-3	23 kDa	0	0	2	1	0	0	2	1	0	0	1	1	0	0	1	1	1.00	0.67	0.67
120	gi|75812940	phosphatidylethanolamine-binding protein 1	21 kDa	0	3	1	5	0	2	0	5	0	1	0	0	5	0	0	0	0.78	0.11	0.56
121	gi|139949054	short-chain dehydrogenase/ reductase family 9C member 7	35 kDa	0	0	4	2	0	0	1	1	1	0	0	2	0	2	1	0	0.33	0.50	0.50
122	gi|528914337	epiplakin	376 kDa	0	1	5	0	0	0	0	0	0	0	5	5	0	0	0	2	0.00	1.67	0.33
123	gi|109932864	annexin A5	36 kDa	0	0	2	4	0	0	0	0	0	0	3	3	0	0	2	0	0.00	1.00	0.33
124	gi|300795444	myosin-9	227 kDa	0	0	9	0	0	0	0	0	0	0	5	4	0	1	0	1	0.00	1.00	0.22
125	gi|296471095	filamin A, alpha	276 kDa	0	2	3	0	0	1	0	0	0	2	2	3	0	0	0	1	0.20	1.40	0.20
126	gi|134024776	actin, alpha 1, skeletal muscle	42 kDa	0	0	0	17	0	0	0	0	0	13	0	4	0	0	0	0	0.00	1.00	0.00
127	gi|27806689	clathrin heavy chain 1	192 kDa	0	0	1	0	0	0	0	0	0	0	3	3	0	0	0	0	0.00	6.00	0.00
128	gi|4927286	enolase, alpha	47 kDa	0	0	0	1	0	0	2	0	0	0	3	1	0	0	0	0	2.00	4.00	0.00
129	gi|110347570	vimentin	54 kDa	0	0	0	2	0	0	0	0	0	2	3	3	0	0	0	0	0.00	4.00	0.00
130	gi|61888854	malate dehydrogenase, mitochondrial	30 kDa	0	0	0	1	0	0	0	0	0	0	2	1	0	0	0	0	0.00	3.00	0.00
131	gi|1042206	purine nucleoside phosphorylase	32 kDa	0	0	0	4	0	0	0	0	0	0	3	4	0	0	0	0	0.00	1.75	0.00
132	gi|296488812	collagen, type VI, alpha 3-like isoform 2	319 kDa	0	0	9	0	0	0	0	0	0	5	5	5	0	0	0	0	0.00	1.67	0.00
133	gi|27806317	annexin A8	37 kDa	0	0	0	3	0	0	0	0	0	0	1	4	0	0	0	0	0.00	1.67	0.00
134	gi|528938209	collagen alpha-2(VI) chain isoform X1	109 kDa	0	0	3	0	0	0	0	0	0	2	1	1	0	0	0	0	0.00	1.33	0.00
135	gi|115497120	kallikrein-10 precursor	31 kDa	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0.00	1.00	0.00
136	gi|156120901	myosin-11	228 kDa	0	3	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0.67	0.67	0.00
137	gi|1063258	annexin IV	36 kDa	0	0	3	1	0	0	0	0	0	0	0	2	0	0	0	0	0.00	0.50	0.00
138	gi|77735487	proteasome subunit beta type-4 precursor	29 kDa	0	0	0	2	0	1	0	2	0	0	0	0	0	0	0	0	1.50	0.00	0.00
139	gi|27807469	peroxiredoxin-2	22 kDa	0	2	0	4	0	0	0	4	0	0	0	0	0	0	0	0	0.67	0.00	0.00
140	gi|528966315	proteasome subunit alpha type-3 isoform X1	28 kDa	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0.50	0.00	0.00

Table D7: Normalised total, EmPAI and NSAF ratios for significantly regulated teat canal lining proteins

		Average normalised SpC				Normalised total ratio			*p*-value verses control		
#	**Protein name**	**Control**	***E.coli***	***S. aureus***	***S. uberis***	***E. coli* /Control**	***S. aureus* /Control**	***S. uberis* /Control**	***E.coli***	***S. aureus***	***S. uberis***
28	serum albumin precursor	8.8	25.3	40.0	26.3	2.89	4.57	3.00	0.047	0.065	0.028
34	glyceraldehyde-3-phosphate dehydrogenase	11.8	6.5	5.8	7.5	0.55	0.49	0.64	0.004	0.016	0.088
52	heat shock protein beta-1	3.0	2.3	18.0	6.8	0.75	6.00	2.25	0.822	0.001	0.128
64	pancreatic adenocarcinoma upregulated factor-like	1.3	4.3	4.8	4.3	3.40	3.80	3.40	0.069	0.044	0.046
82	serotransferrin precursor	0.0	1.3	4.3	1.3	2.50	8.50	2.50	0.015	0.021	0.080
97	lactoferrin	1.3	5.8	17.8	18.5	4.60	14.2	14.8	0.006	0.050	0.049
103	apolipoprotein A-I precursor	1.0	1.3	7.3	4.0	1.25	7.25	4.00	0.391	0.040	0.154

		Average EmPAI value				EmPAI ratio			*p*-value verses control		
#	**Protein name**	**Control**	***E.coli***	***S. aureus***	***S. uberis***	***E. coli* /Con**	***S. aureus* /Con**	***S. uberis* /Con**	***E.coli***	***S. aureus***	***S. uberis***
28	serum albumin precursor	0.55	1.28	2.46	1.30	2.33	4.48	2.36	0.049	0.055	0.041
34	glyceraldehyde-3-phosphate dehydrogenase	0.70	0.36	0.33	0.56	0.52	0.47	0.80	0.022	0.095	0.529
52	heat shock protein beta-1	0.54	0.19	2.23	1.24	0.34	4.13	2.30	0.500	0.001	0.054
64	pancreatic adenocarcinoma upregulated factor-like	0.19	0.95	0.90	0.65	5.07	4.80	3.44	0.061	0.122	0.149
82	serotransferrin precursor	0.00	0.12	0.41	0.17	11.8*	41.3*	17.0*	0.017	0.021	0.083
97	lactoferrin	0.08	0.36	1.05	1.12	4.83	14.0	15.0	0.016	0.094	0.164
103	apolipoprotein A-I precursor	0.06	0.09	0.82	0.52	1.64	14.9	9.50	0.391	0.092	0.179

		Average NSAF value				NSAF ratio			*p*-value verses control		
#	**Protein name**	**Control**	***E.coli***	***S. aureus***	***S. uberis***	***E. coli* /Con**	***S. aureus* /Con**	***S. uberis* /Con**	***E.coli***	***S. aureus***	***S. uberis***
28	serum albumin precursor	0.022	0.056	0.096	0.060	2.52	4.36	2.72	0.070	0.064	0.071
34	glyceraldehyde-3-phosphate dehydrogenase	0.036	0.018	0.017	0.024	0.48	0.47	0.66	0.002	0.025	0.128
52	heat shock protein beta-1	0.021	0.008	0.076	0.040	0.37	3.71	1.95	0.523	0.001	0.114
64	pancreatic adenocarcinoma upregulated factor-like	0.009	0.027	0.028	0.026	2.93	3.05	2.88	0.118	0.057	0.099
82	serotransferrin precursor	0.000	0.002	0.007	0.002	2.35*	6.63*	2.40*	0.024	0.010	0.076
97	lactoferrin	0.002	0.013	0.044	0.045	5.71	20.0	20.1	0.006	0.062	0.102
103	apolipoprotein A-I precursor	0.003	0.004	0.023	0.013	1.20	7.61	4.38	0.682	0.029	0.133

* For serotransferrin fold calculations, the control value was set to 0.01 and 0.001 to calculate EmPAI and NSAF ratios, respectively.

Table D8: **Quickscore summary of MHC class II signal in teat-end tissue regions from 24 h bacteria challenge cows.**

Cow #	Tissue region	Treatment			
		Control	*E. coli*	*S. aureus*	*S. uberis*
428	Teat canal	++	++	++	+
	Fürstenberg's rosette	++	++	++	++
	Teat sinus	++	++	++	++
862	Teat canal	++	++	++	++
	Fürstenberg's rosette	+++	+++	+++	+++
	Teat sinus	++	++	++	++
1238	Teat canal	+	+	++	+
	Fürstenberg's rosette	+++	+++	+++	+++
	Teat sinus	++	++	++	++
1312	Teat canal	+	+	+	+
	Fürstenberg's rosette	++	+++	++	++
	Teat sinus	++	++	++	++

Immunofluorescent signal intensities in the cryosections were subjectively graded as weakly positive (+), mildly positive (++), moderately positive (+++) and strongly positive (++++).

Table D9: **Quickscore summary of CH138A signal in teat-end tissue regions from 24 h bacteria challenge cows.**

Cow #	Tissue region	Treatment			
		Control	*E. coli*	*S. aureus*	*S. uberis*
428	Teat canal	++	+	++	++
	Fürstenberg's rosette	+++	+++	+++	+++
	Teat sinus	++	+++	+++	+++
862	Teat canal	++	++	++	++
	Fürstenberg's rosette	+++	+++	+++	+++
	Teat sinus	+++	+++	+++	+++
1238	Teat canal	++	++	++	++
	Fürstenberg's rosette	+++	+++	+++	+++
	Teat sinus	+++	+++	+++	+++
1312	Teat canal	++	++	++	+
	Fürstenberg's rosette	+++	+++	+++	+++
	Teat sinus	++	++	+++	+++

Immunofluorescent signal intensities in the cryosections were subjectively graded as weakly positive (+), mildly positive (++), moderately positive (+++) and strongly positive (++++).

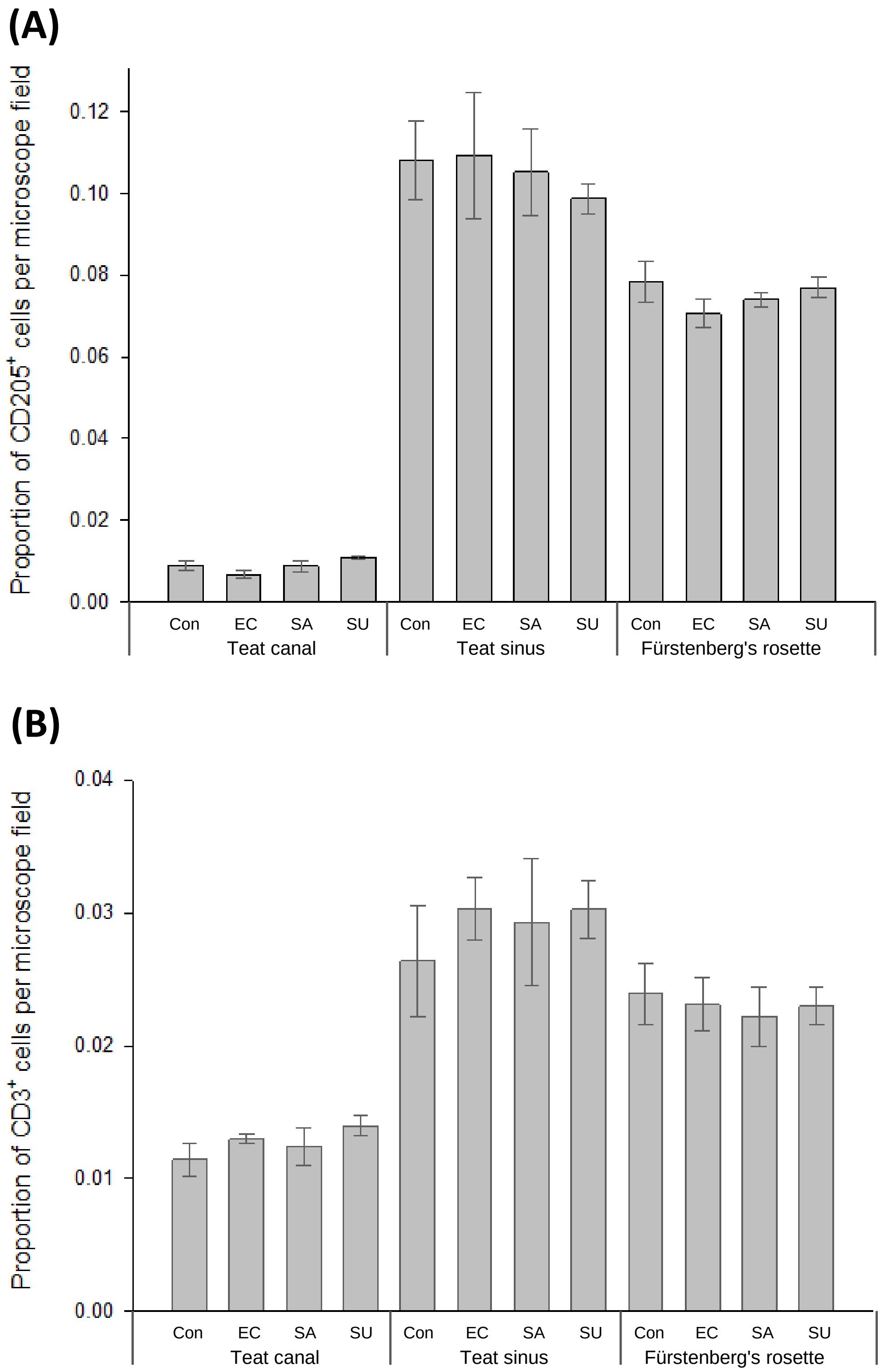

Figure D2: Semi-quantitative comparison of the proportion CD205$^+$ and CD3$^+$ cells in 24 h challenged teat-end tissues.

Positively stained CD205 expressing cells associated with the epithelial bilayer (A), and CD3 expressing cells located within the epithelial layer (B) were counted from seven nonadjacent fields per slide at 200 x magnification and then averaged. The results are cumulative data from four late-lactating cows and are expressed as a proportion of the total number of nuclei in each field. Con; Control inoculation, EC; *E. coli*, SA; *S. aureus*, SU; *S. uberis*. Error bar: ± SEM.