Comprehensive LESA mass spectrometry imaging of intact proteins by integration of cylindrical FAIMS

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Supporting information

File S1. Protein masses detected in testes imaging dataset.

File S2. Protein masses detected in kidney imaging dataset.

File S3. Protein masses detected in brain imaging dataset.

Figure S1. Schematic showing the workflow for tissue analysis via liquid extraction surface analysis coupled to cylindrical FAIMS mass spectrometry imaging.

Figure S2. UpSet plot showing set intersections of the filtered Xtract results for intact proteins across the testes image and all compensation field values.

Figure S3. UpSet plot showing set intersections of the filtered Xtract results for intact proteins across the kidney image and all compensation field values.

Figure S4. UpSet plot showing set intersections of the filtered Xtract results for intact proteins across the brain image and all compensation field values.

Figure S5. LESA FAIMS EThCD MS/MS of ions with m/z 661 (14+, 9242.00 Da) detected in kidney (CV = -50 V).

Figure S6. LESA FAIMS EThCD MS/MS of ions detected in testes: A) m/z 706 (7+, 4933.53 Da) (CV = -70 V); B) m/z 558 (5+, 2788.48 Da) (CV = -70 V); C) m/z 1036 (20+, 20698.49 Da) (CV = -30 V).

Table S1. Fragment ion assignments for high mobility group nucleasomal binding domain 2 (precursor m/z 661; 14+)

Table S2. Fragment ion assignments for β-thymosin 10 (precursor m/z 706; 7+)

Table S3. Fragment ion assignments for N-terminal region peptide of serum albumin (precursor m/z 558; 5+)

Table S4. Fragment ion assignments for phosphatidyl-ethanolamine binding protein (precursor m/z 1036; 20+)

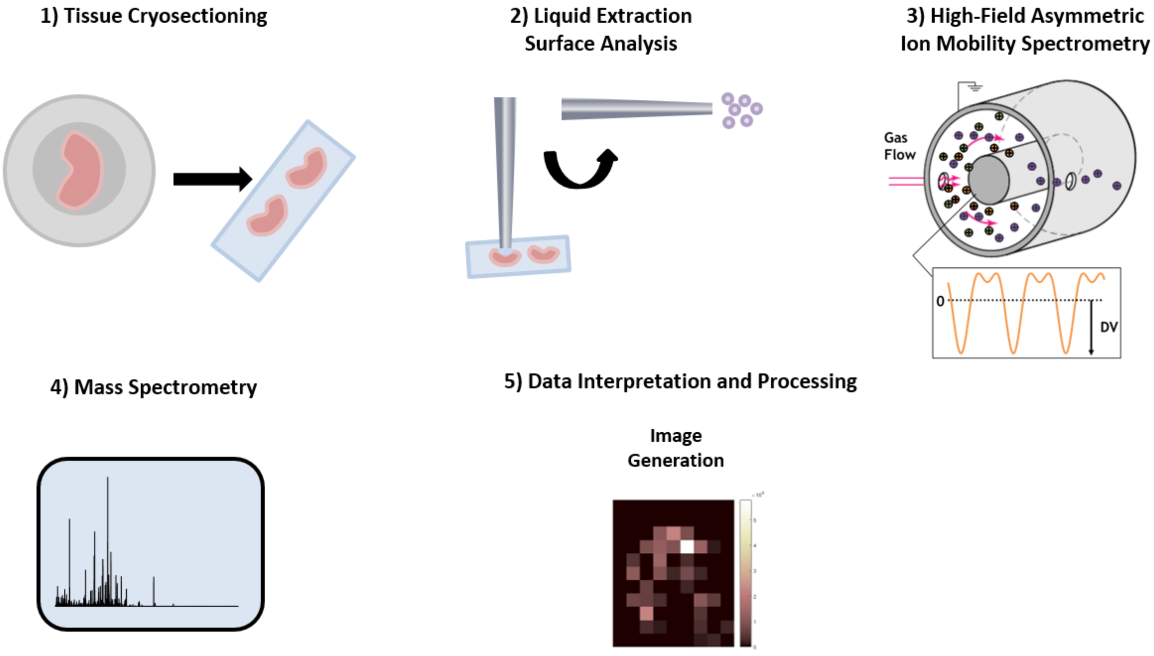

Figure S1. Schematic showing the workflow for tissue analysis via liquid extraction surface analysis coupled to cylindrical FAIMS mass spectrometry imaging.

Figure S2 – UpSet plot for testes

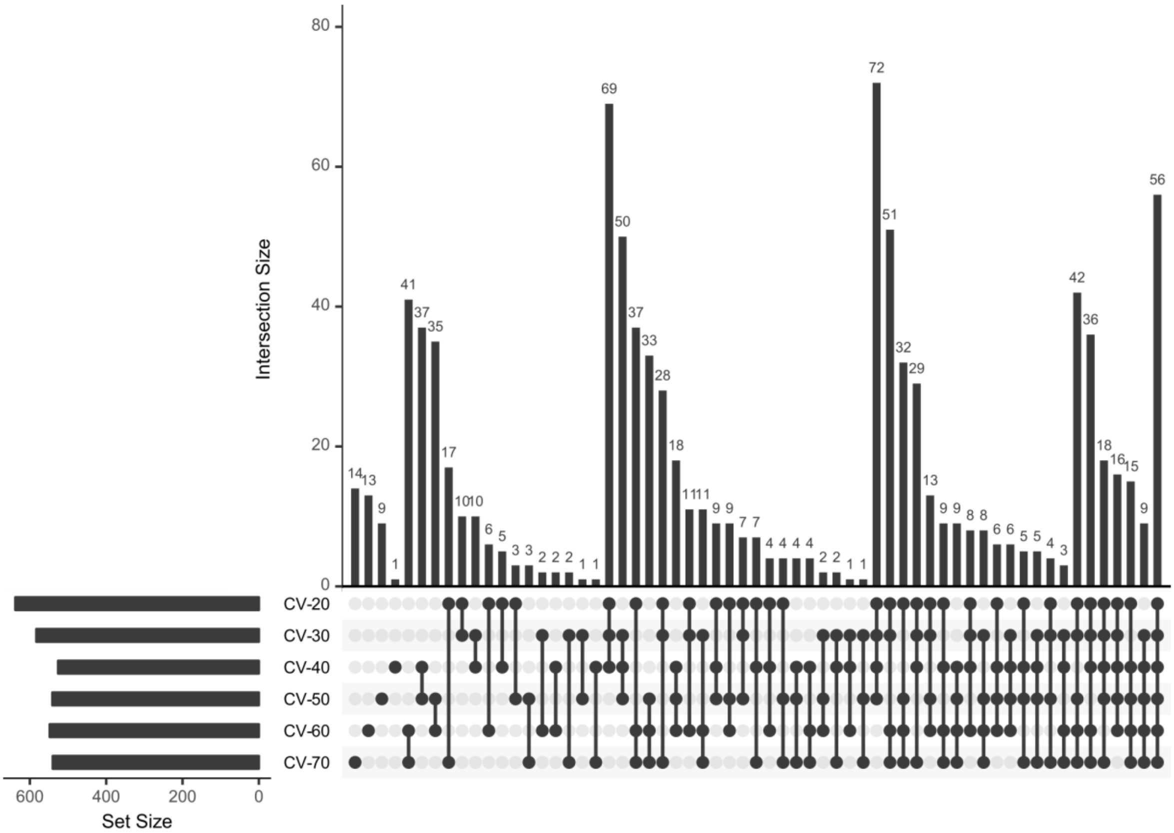

Figure S2 – UpSet plot showing set intersections of the filtered Xtract results for intact proteins across the testes image and all compensation field values. The histogram shows the number of intact proteins belonging to the set. The sets are shown below the histogram and are ordered according to the degree of the set overlap.

Figure S3 – UpSet plot for kidney

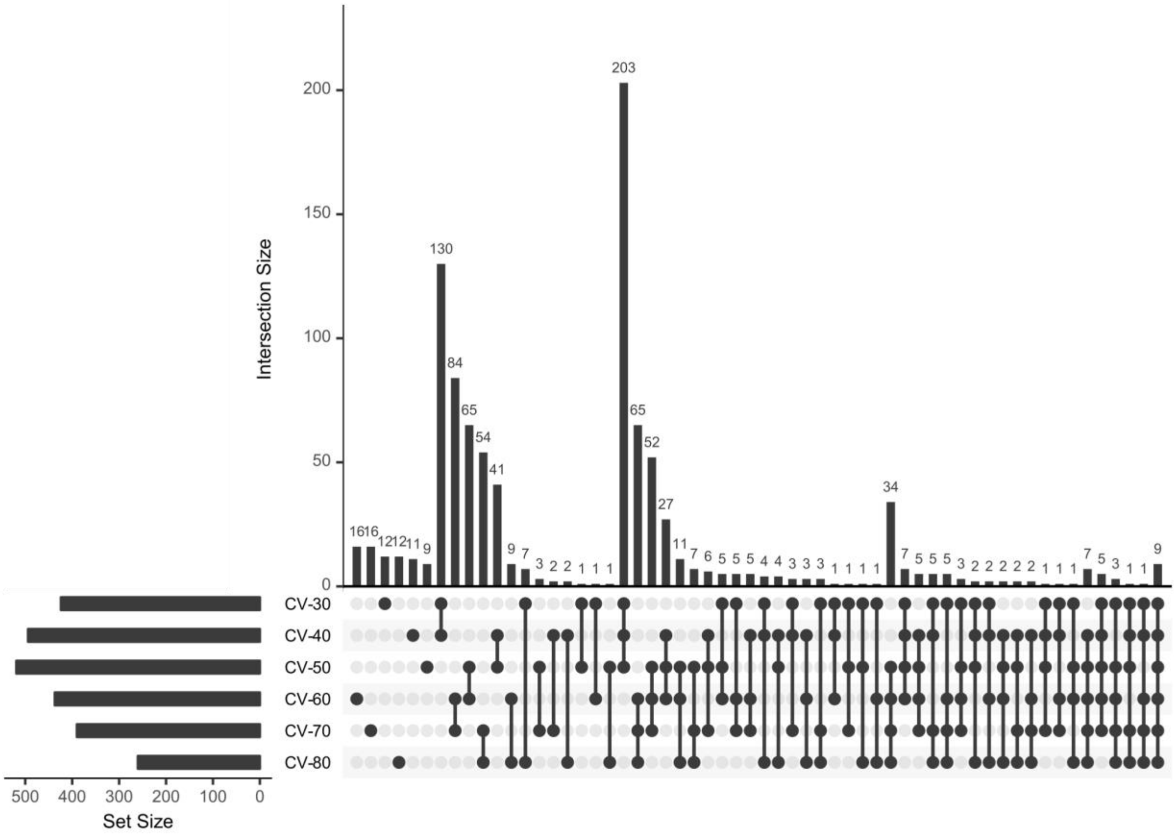

Figure S3 – UpSet plot showing set intersections of the filtered Xtract results for intact proteins across the kidney image and all compensation field values. The histogram shows the number of intact proteins belonging to the set. The sets are shown below the histogram and are ordered according to the degree of the set overlap.

Figure S4 UpSet plot for brain

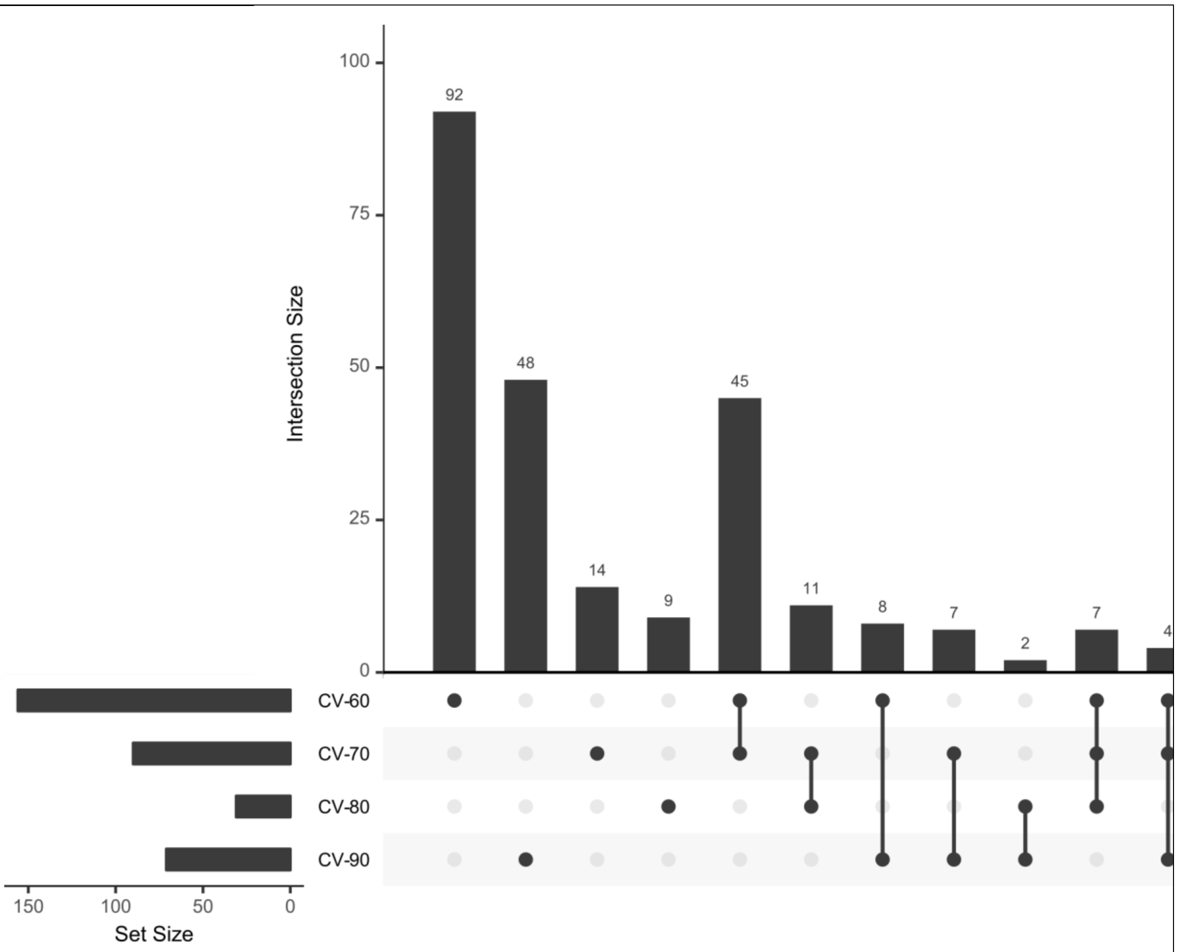

Figure S4 – UpSet plot showing set intersections of the filtered Xtract results for intact proteins across the brain image and all compensation field values. The histogram shows the number of intact proteins belonging to the set. The sets are shown below the histogram and are ordered according to the degree of the set overlap.

Figure S5 - LESA FAIMS EThcD MS/MS of ions with m/z 661 (14+, 9242.00 Da) detected in kidney (CV = -50 V), assigned as high mobility group nucleosomal binding domain 2 (Q4KLJ0).

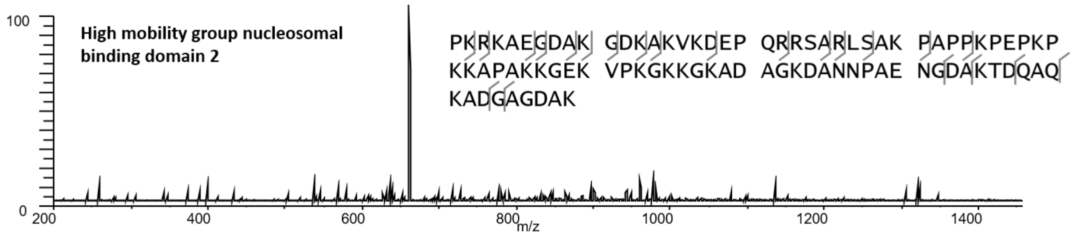

Table S1 - Fragment ion assignments for high mobility group nucleasomal binding domain 2 (precursor m/z 661; 14+)

m/z expt	m/z calc	ppm error	z	assignment
226.1548	226.1550	-0.9	1	b2
243.1814	243.1816	-0.8	1	c2
399.2824	399.2827	-0.8	1	c3
445.2164	445.2167	-0.7	1	z5
485.7748	485.7751	-0.6	2	c9
502.2377	502.2382	1.0	1	z6
549.8221	549.8225	-0.7	2	c10
572.279	572.2794	-0.7	2	z12
578.3329	578.3333	-0.7	2	c11
684.1907	684.192	-1.9	5	c31
699.8937	699.8942	-0.7	2	c13
727.4569	727.4573	-0.5	1	c6
730.9122	730.9130	-1.1	4	c26
735.4119	735.4128	-1.2	1	c14
737.2203	737.2205	-0.3	5	c34
744.3639	744.3642	-0.4	2	z15
784.4782	784.4788	-0.8	1	c7
780.941	780.942	-1.3	4	c28
816.3971	816.3972	-0.1	1	z9
817.4572	817.4577	-0.6	3	c22
837.3957	837.3962	-0.6	2	z17
845.405	845.4056	-0.7	2	y17
922.1802	922.1811	-1.0	3	c25
970.5543	970.5554	-1.1	2	c18

Figure S6 – LESA FAIMS EThCD MS/MS of ions detected in testes: A) m/z 706 (7+, 4933.53 Da) (CV = -70 V); B) m/z 558 (5+, 2788.48 Da) (CV = -70 V); C) m/z 1036 (20+, 20698.49 Da) (CV = -30 V).

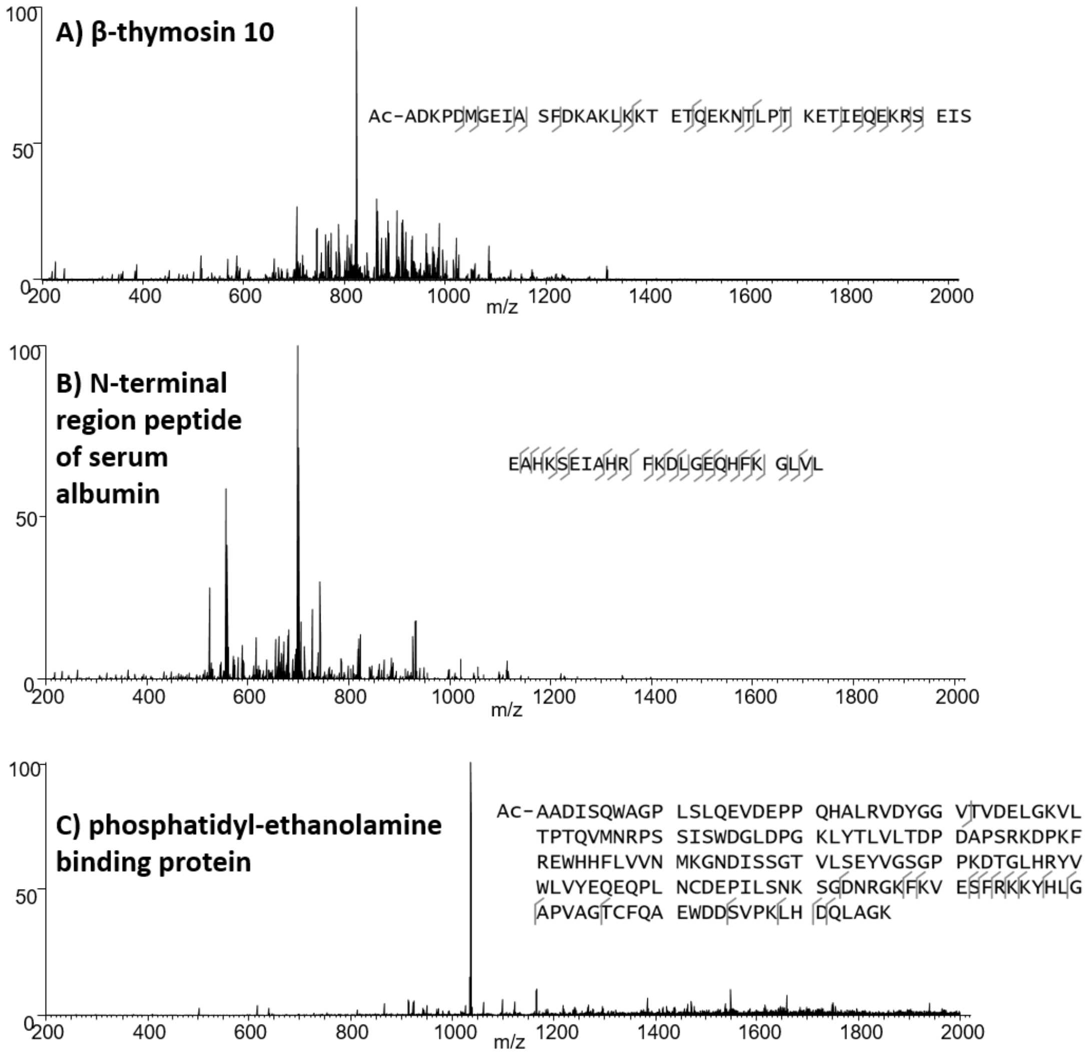

Table S2 - Fragment ion assignments for β-thymosin 10 (precursor m/z 706; 7+)

m/z expt	m/z calc	ppm error	z	assignment
500.2259	500.2262	-0.6	2	b9
535.7450	535.7448	0.4	2	b10
569.2566	569.2566	0.0	1	b5
586.2834	586.2831	0.5	1	c5
592.311	592.3109	0.2	3	y15
645.0156	645.0153	0.5	6	y33
656.8552	656.8548	0.6	6	y34
660.3438	660.3437	0.2	2	y11
663.3435	663.3434	0.2	3	b18
669.0187	669.0189	-0.3	3	c18
675.7019	675.7021	-0.3	6	y35
717.3235	717.3236	-0.1	1	c6
724.8651	724.865	0.1	2	y12
741.6345	741.634	0.7	4	c26
744.4007	744.4008	-0.1	3	y19
756.4114	756.4105	1.2	5	y32
766.8958	766.8959	-0.1	4	c27
773.8164	773.8169	-0.6	5	y33
788.0242	788.0243	-0.1	5	y34
790.9107	790.9103	0.5	4	b28
801.088	801.0882	-0.2	6	a42
810.6414	810.6411	0.4	5	y35
819.42997	819.4301	-0.2	4	c29
822.0965	822.0966	-0.1	3	c22
833.0451	833.0443	1.0	5	y36
839.4363	839.4363	0.0	2	y14
844.6922	844.6921	0.1	4	c30
864.783	864.7828	0.2	3	c23
886.3612	886.3611	0.1	1	b8
887.9626	887.9627	-0.1	2	y15
904.2769	904.2769	0.0	5	c39
921.6834	921.6833	0.1	5	c40
934.2391	934.2384	0.7	4	c33
936.4952	936.4954	-0.2	2	z16
938.9775	938.9773	0.2	2	c17
977.8398	977.8392	0.6	3	z25
994.7706	994.77	0.6	4	c35
1003.025	1003.0248	0.2	2	c18
999.4452	999.4452	0.0	1	b9
1016.4715	1016.4717	-0.2	1	c9
1022.1918	1022.1922	-0.4	3	c27
1026.7838	1026.7847	-0.9	4	c36
1052.0499	1052.05	-0.1	2	y18

1054.211	1054.2113	-0.3	3	b28
1059.0462	1059.0453	0.8	4	c37
1087.5086	1087.5088	-0.2	1	c10
1091.0682	1091.0691	-0.8	4	c39
1125.9193	1125.9203	-0.9	3	c30
1223.8851	1223.8854	-0.29	4	y43
1232.6406	1232.6412	-0.5	2	c22
1236.6397	1236.6387	0.8	2	z21
1321.6091	1321.6093	-0.2	1	c12

Table S3 - Fragment ion assignments for N-terminal region peptide of serum albumin (precursor m/z 558; 5+)

m/z expt	m/z calc	ppm error	z	assignment
231.1702	231.1703	-0.4	1	y2
466.2408	466.2409	-0.2	1	b4
471.282	471.2820	0.0	2	y8
472.9211	472.9213	-0.4	3	b12 -H2O
483.2673	483.2674	-0.2	1	c4
490.5008	490.5012	-0.8	4	b17 -H2O
518.6866	518.6868	-0.4	5	y22
524.7658	524.7660	-0.4	4	b18 - H2O
526.885	526.8848	0.4	5	a23
528.8815	528.8817	-0.4	5	b23 - H2O
548.9582	548.9584	-0.4	3	b14 - H2O
555.1006	555.1006	0.0	5	M- H2O
570.2994	570.2994	0.0	1	c5
579.6444	579.6445	-0.2	3	c15
588.8152	588.8153	-0.2	2	c10
602.3161	602.3161	0.0	4	c20
610.9796	610.9797	-0.2	3	b16 - H2O
613.8422	613.8419	0.5	4	y21
620.8558	620.8561	-0.5	2	y11
622.6588	622.6587	0.2	3	c16
633.587	633.5871	-0.2	4	a22
636.0833	636.0832	0.2	4	b22 - H2O
640.5859	640.5858	0.2	4	b22
642.0231	642.0232	-0.2	3	y15
648.1067	648.1067	0.0	4	y22
660.8501	660.8503	-0.3	4	b23 - H2O
662.3496	662.3495	0.2	2	c11
665.3534	665.3529	0.8	4	b22
669.6094	669.6096	-0.3	4	c23
676.4392	676.4392	0.0	1	y6
678.3689	678.3695	-0.9	2	y12
711.0312	711.0312	0.0	3	c18
726.397	726.3969	0.1	2	c12
748.3752	748.375	0.3	3	b19 - H2O
760.0539	760.0540	-0.1	3	c19
770.0818	770.0823	-0.6	3	z20
775.3973	775.3971	0.3	2	b13
783.9101	783.9104	-0.4	2	c13
791.0732	791.0733	-0.1	3	b20 - H2O
802.7519	802.7524	-0.6	3	c20
807.9415	807.9419	-0.5	2	z14
810.0807	810.0805	0.2	3	b21

815.9515	815.9512	0.4	2	y14
818.1202	818.1202	0.0	3	y21
821.7596	821.7595	0.1	3	c21
840.4522	840.4524	-0.2	2	c14
847.7754	847.7752	0.2	3	b22 - H2O
853.7779	853.7787	-0.9	3	b22
858.4662	858.4669	-0.8	3	z22
863.8064	863.8065	-0.1	3	y22
868.9634	868.9632	0.2	2	c15
882.1461	882.1459	0.2	3	z23
883.4630	883.4632	-0.2	2	c8
885.9921	885.9924	-0.3	2	z16
892.4778	892.477	0.9	3	c23
933.4845	933.4845	0.0	2	c16
975.5015	975.5007	0.8	1	a9
997.5134	997.5138	-0.4	2	c17
1020.5216	1020.5221	-0.5	1	c9
1054.5802	1054.5806	-0.4	1	z9
1066.0434	1066.0432	0.2	2	c18
1111.1039	1111.1037	0.2	2	z19
1218.6666	1218.6672	-0.5	2	z21

Table S4 - Fragment ion assignments for phosphatidyl-ethanolamine binding protein (precursor m/z 1036; 20+)

m/z expt	m/z calc	ppm error	z	assignment
500.2951	500.2953	-0.4	1	z5
615.3219	615.3222	-0.5	1	z6
638.8600	638.8603	-0.5	1	z12
865.4649	865.4652	-0.3	1	z8
913.2161	913.2169	-0.9	4	z33
923.1126	923.1139	-1.4	3	z26
942.1199	942.1210	-1.2	3	z27
949.9829	949.9840	-1.2	4	z34
971.7410	971.7420	-1.0	4	z35
992.3059	992.3074	-1.5	5	z44
1025.5014	1025.5020	-0.6	3	z29
1060.7931	1060.7935	-0.4	4	z38
1097.5606	1097.5606	0.0	4	z39
1120.5565	1120.5600	-3.1	3	c31
1122.5541	1122.5548	-0.6	3	z31
1165.2520	1165.2531	-0.9	3	z32
1186.5577	1186.5587	-0.8	2	z21
1384.1646	1384.1671	-1.8	2	z26
1747.3749	1747.3760	-0.6	2	z32