

# Two-Dimensional Differential Gel Electrophoresis of a Cell Line derived from a Breast Cancer Micrometastasis Revealed a Stem/Progenitor Cell Protein Profile

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## Supporting Information

Supplementary experimental procedures

**1-D SDS-PAGE and Western Blot Analysis.** 1-D protein separation was done with the Novex XCell Sure-Lock mini system (Invitrogen, Groningen, Netherlands) using 10% polyacrylamide separation gels<sup>1</sup>. Samples were diluted in SDS-sample buffer, heat denatured at 95°C for 5 min and loaded onto the gel under the following running conditions: 84 V for 1 h and 130 V until the dye front reached the edge of the gel. The molecular size standard was the peqGOLD protein-marker V (Peqlab, Erlangen, Germany). Gel staining was performed according to Neuhoff<sup>2</sup>.

For Western Blot analysis samples (25 µg of protein; except for CK5, where 5 µg were applied) were separated by SDS-PAGE as described and transferred to a PDVF membrane under semi-dry conditions. Cytokeratins were detected using the following primary antibodies: anti-cytokeratin 5 [XM26] antibody (abcam, Cambridge, United Kingdom), 1: 5000 dilution; anti-cytokeratin 7 clone OV-TL 12/30 (DAKO, Glostrup; Denmark), 1:10000 dilution; anti-Keratin K8 clone K8.2 (Progen, Heidelberg, Germany), 1:10000 dilution; anti-Keratin K17 clone Ks17.E3 (Progen, Heidelberg, Germany), 1:500 dilution; anti-Keratin K18 clone K18.04 (Progen, Heidelberg, Germany), 1:10000 dilution, and anti-Cytokeratin 19, clone BA 17 (Millipore GmbH, Schwalbach), 1:10000 dilution. Other antibodies: anti-vimentin

antibody (clone V9) from Sigma, 1:5000 dilution; anti-HSP 90 antibody (Cell Signaling Technology, Beverly, MA, USA), 1:100000 dilution; anti-c-ErbB2/c-Neu (Ab-3) clone 3B5 antibody (Calbiochem, Darmstadt, Germany), 1:1000 dilution; anti-gelsolin antibody (DAKO, Glostrup; Denmark), 1:30000 dilution, and anti-Hyou1 (M01) clone 6F7 (Abnova, Heidelberg, Germany), 1:9000 dilution. Detection of alpha-tubulin, which served as a loading control, was done using the anti-alpha-tubulin (11H10) antibody (Cell Signaling Technology) at 1:45000 dilution. The appropriate secondary antibodies conjugated with horseradish peroxidase (all DAKO) were applied at 1:1000 dilution for CK7, CK17, CK19, Her-2 and vimentin; for CK5, CK8, CK18, gelsolin, HSP 90, Hyou1 and alpha-tubulin a 1:1500 dilution was used. Bands were visualized using the enhanced chemiluminescence detection system and X-ray films (both GE Healthcare, Uppsala, Sweden) in accordance to the manufacturer's instructions.

**Mass Spectrometry.** Proteins of interest were manually excised from a colloidal Coomassie stained preparative gel. Protein spots were destained with 50% methanol for 1 h and dried with acetonitrile for 30 min. In-gel reduction, alkylation with iodoacetamide, tryptic digest and extraction of the peptides was performed as described.<sup>3</sup> For MALDI-TOF analysis of tryptic peptides, 1 µl of the peptide mixture was deposited on a stainless steel sample stage (Bruker Daltonics GmbH, Bremen, Germany) and allowed to evaporate at ambient temperature. Thereafter, 1 µl of matrix solution (20 mg/ml 2,5-dihydroxybenzoic acid in 30% [v/v] aqueous acetonitrile) was added on the dried peptides and allowed to dry out. Tryptic peptide mass fingerprints were obtained on a Reflex IV MALDI-TOF mass spectrometer (Bruker) in the positive ion reflector mode. The ion acceleration voltage was 20 kV. Raw data were generated using the software FlexControl 2.4 and further processed by FlexAnalysis 2.4 (both software Bruker). The mass spectra were internally calibrated using the trypsin autoproteolysis peaks preferentially using the signals at 805.41 Da and 2163.05 Da and all known contaminant signals were excluded. For analysis of the MALDI spectra the following parameters were used: the signal-to-noise threshold was set greater 10 and the resolution to greater 5000 with a mass accuracy of 30 ppm. Proteins were searched using the non-redundant UniProtKB/Swiss-Prot database and Mascot Peptide Mass Fingerprint (www.matrixscience.com). The following parameters for protein identification were used: The species

was limited to *homo sapiens*, one missed cleavage site for trypsin was allowed, carbamidomethyl cysteine was set as fixed modification, and oxidation of methionine was set as variable modification. The deviation of experimental peptide mass values was limited to 80 ppm. The probability scores of the search results were higher than the score fixed as significant with a significance threshold ( $p < 0.05$ ).

For protein identification using nanoelectrospray mass spectrometry, experiments were carried out using a quadrupole time-of-flight mass spectrometer (Q-ToF-2 electrospray mass spectrometer, Waters, Saint-Quentin, France) in the positive ion mode (ESI(+)). In-gel reduction, alkylation with iodoacetamide, tryptic digest and extraction of the peptides was performed as described for MALDI-TOF MS. Tryptic digest was carried out using modified porcine trypsin at 10 ng/ $\mu$ l (Promega, Mannheim, Germany). Prior to nanoelectrospray mass spectrometry experiments, peptides were purified with ZipTipC18, Size P10 (Millipore; Woburn, USA) according to the manufacturer's instructions. Raw data were acquired and analyzed using the software MassLynx 3.5 (Micromass, Manchester, UK). The capillary tip was set to a potential of 0.78 kV and the cone voltage was 40 V. For collision-induced dissociation (CID) experiments, ions were selected within a precursor mass window of  $\pm 1$  Da in the quadrupole analyzer and fragmented in the collision cell using a collision gas (Ar) and collision energies of 27 to 35 eV. The resolution of the peaks was more than 4000 with a signal-to-noise threshold of more than 10. For database research the NCBI nr database and Mascot MS/MS Ions Search ([www.matrixscience.com](http://www.matrixscience.com)) was used. The search parameters (if applicable) were identical as for MALDI-TOF except the following: The precursor mass tolerance was limited to 1.2 Da, and the MS/MS fragment tolerance was 0.6 Da and no missed cleavage was allowed. The probability scores of the search results for each protein were at least for two peptides higher than the score fixed as significant with a significance threshold ( $p < 0.05$ ) indicating identity or extensive homology.

**Molecular Size Standard used in 2-DE.** For 2-DE experiments, the molecular size standard peqGOLD protein-marker V was applied for an initial estimation of the protein masses. The molecular masses specified here for a more direct estimation are the theoretical masses of the unmodified proteins identified by peptide mass fingerprints (PMF) from the gels. For the pH gradient 4-7, the following

proteins were selected as size standard: vinculin (123 kDa), gelsolin (82 kDa), HSP60 (58 kDa), actin (41 kDa), inorganic pyrophosphatase (32 kDa) and peroxiredoxin2 (21 kDa). For the pH gradient 6-11 the following proteins were selected as size standard: elongation factor 2 (95 kDa), heterogeneous nuclear ribonucleoprotein M (77 kDa), cytokeratin 5 (62 kDa), annexin A2 (38 kDa) and GTP-binding nuclear protein Ran (24 kDa).

**Immunocytochemical double staining of bone marrow samples.** Bone marrow from breast cancer patients was aspirated at the University Medical Center Hamburg-Eppendorf at the time of primary surgery. Written informed consent was obtained beforehand from all patients. For immuno-double staining, slides with  $7 \times 10^5$  mono-nuclear cells isolated from bone marrow<sup>4</sup> of three different breast cancer patients were fixed for 10 min with Fixation Solution B (Epimet, AS Diagnostik, Germany), washed with PBS three times, and blocked for unspecific binding for 20 min using Proteinblock (Dako). This was followed by a 45 min incubation with CK5 antibody diluted 1:100 in Dako Antibody Diluent. Subsequently slides were washed again three times with PBS, and Alexa 488 rabbit anti- mouse secondary antibody was applied for 45 min diluted 1:200 in Dako Antibody Diluent (with Background reducing components, #I2270). After another washing step, a protein block followed as described above to prevent any interference between primary and secondary antibody, and A45-Cy3 at a dilution of 1:300 was added for 45 min. After another washing step, slides were covered with Vectashield Mounting Medium containing Dapi (Vector Laboratories, Burlingame, U.S.A.). For CK5 detection, the anti-cytokeratin 5 [XM26] antibody (abcam) was used. The A45-B/B3 (AS Diagnostics, Hückeswagen, Germany) is a mouse IgG1 antibody reactive with a common epitope on several cytokeratins including CK8, CK18 and CK19<sup>5</sup>. Slides were evaluated manually.

## Supplementary tables

**Supplementary Table 1.** Detailed information of proteins identified by MALDI-TOF MS tryptic peptide mass fingerprint (A) and Q-TOF-MS/MS (B). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.

Supplementary Table 1A.

Spot no.	Swiss Prot Acc no.	Protein name	Observed Mass [kDa]	Observed pI	Theor. Mass [kDa]	Theor. pI	MASCOT score	Expect MASCOT	Number of mass values searched	sequence coverage (%) / matched peptides
1	P08238	Heat shock protein HSP 90-beta	85	4.6	83.0	4.97	139	2.6e-10	36	27/19
2	P68363	Tubulin alpha-ubiquitous chain	55	5.0	50.2	4.94	141	1.6e-10	21	38/12
3	P06396	Gelsolin	85	5.9	82.9	5.72	116	5.1e-08	21	13/12
4	P18206	Vinculin	120	6.2	123.6	5.51	283	1e-24	55	31/37
5	P15311	Ezrin (Cyto villin)	65	6.4	69.2	5.95	200	2e-16	34	29/21
6	P08729	Keratin, type II cytoskeletal 7	50	5.5	51.2	5.50	128	3.2e-09	25	30/12
7	P05787	Keratin, type II cytoskeletal 8	50	5.6	53.5	5.52	159	2.6e-12	23	30/16
8	Q04695	Keratin, type I cytoskeletal 17	45	5.0	47.9	4.97	118	3.2e-08	23	25/10
9	P05783	Keratin, type I cytoskeletal 18	45	5.4	47.9	5.34	151	1.6e-11	44	38/17
10	P08727	Keratin, type I cytoskeletal 19	40	5.0	44.1	5.05	251	1.6e-21	23	38/18
11	P31947	14-3-3 protein sigma (Stratiffin)	25	4.8	27.8	4.68	134	8.1e-10	24	35/13
12	P13639	Elongation factor 2	90	7.1	95.1	6.42	103	1e-06	13	18/9
13	P13647	Keratin, type II cytoskeletal 5	55	7.9	62.4	8.14	138	3.2e-10	34	24/15
14	P25705	ATP synthase subunit alpha	50	8.3	55.2	8.28	139	2.6e-10	15	24/10
15	P07355	Annexin A2	35	7.8	38.5	7.56	193	1e-15	27	45/18
16	P68104	Elongation factor 1-alpha 1 (EF-1-alpha-1)	45	9.4	50.1	9.1	89	2.5e-05	19	26/10
17	P52272	Heterogeneous nuclear ribonucleoprotein M	70	9.1	77.3	8.85	63	0.0095	18	15/12

Supplementary Table 1B.

Spot no.	Swiss Prot Acc no.	Protein name		Theor. Mass [kDa]/ Observed Mass [kDa]		Theor. pI / Observed pI	
18	Q9Y4L1	Hypoxia up-regulated protein 1		107.6 / 140		5.07 / 5.6	
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	762.0039 [2+]	1521.9933	1520.8239	1.1694	28	0.04	R.DAVVYPILVE FTR.E
	Individual ions scores > 19 indicate peptides with significant homology. Individual ions scores > 27 indicate identity or extensive homology (p<0.05).						
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	829.1039 [2+]	1656.1933	1655.7501	0.4432	49	0.0003	R.VEFEELCADL FER.V
	Individual ions scores > 20 indicate peptides with significant homology. Individual ions scores > 27 indicate identity or extensive homology (p<0.05).						
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	869.0039 [2+]	1735.9933	1734.8708	1.114	29	0.046	K.LGNTISSLFGG GTTPDAK.E
	Individual ions scores > 22 indicate peptides with significant homology. Individual ions scores > 28 indicate identity or extensive homology (p<0.05)						
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	1047.994 [2+]	2093.9733	2094.1109	-0.137	85	$3.9 \times 10^{-08}$	K.VLQLINDNTA TALSYGVR.R
	Individual ions scores > 24 indicate identity or extensive homology (p<0.05).						

	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	1171.994 [2+]	2341.9733	2341.1060	0.8672	27	0.022	R.VESVFETLVE DSAEESTLTK.L
	Individual ions scores > 23 indicate identity or extensive homology (p<0.05).						
Spot no.	Swiss Prot Acc no.	Protein name		Theor. Mass [kDa]/ Observed Mass [kDa]		Theor. pI / Observed pI	
19 a)	P08670	Vimentin		53.5 / 45		5.06 / 4.9	
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	648.8039 [2+]	1295.5933	1294.6591	0.9342	27	0.093	K.MALDIEIATY R.K
	Individual ions scores > 27 indicate peptides with significant homology. Individual ions scores > 30 indicate identity or extensive homology (p<0.05).						
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	785.9039 [2+]	1569.7933	1569.8878	-0.094	64	$1.8 \times 10^{-05}$	R.ISLPLPNFSSL NLR.E
	Individual ions scores > 20 indicate peptides with significant homology. Individual ions scores > 29 indicate identity or extensive homology (p<0.05).						
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	1063.503 [2+]	2124.9933	2125.0579	-0.064	121	$2.3 \times 10^{-11}$	R.LLQDSVDFSL ADAINTEFK.N
	Individual ions scores > 25 indicate peptides with significant homology. Individual ions scores > 27 indicate identity or extensive homology (p<0.05).						
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide

	1093.904 [2+]	2185.7933	2185.9586	-0.165	121	$1.2 \times 10^{-11}$	R.EMEENFAVEA ANYQDTIGR.L
	Individual ions scores > 22 indicate peptides with significant homology. Individual ions scores > 24 indicate identity or extensive homology (p<0.05).						
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	1101.904 [2+]	2201.7933	2201.9535	-0.160	153	$8.8 \times 10^{-15}$	R.EMEENFAVEA ANYQDTIGR.L + Oxidation (M)
	Individual ions scores > 23 indicate peptides with significant homology. Individual ions scores > 25 indicate identity or extensive homology (p<0.05).						
Spot no.	Swiss Prot Acc no.	Protein name		Theor. Mass [kDa]/ Observed Mass [kDa]		Theor. pI / Observed pI	
19 b)	P68363	Tubulin alpha-ubiquitous chain		50.2 / 55		4.94 / 5.0	
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	912.9039 [2+]	1823.7933	1823.9782	-0.185	36	0.01	K.VGINYQPPTV VPGDLAK.V
	Individual ions scores > 22 indicate peptides with significant homology. Individual ions scores > 28 indicate identity or extensive homology (p<0.05).						
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	940.9039 [2+]	1879.7933	1879.8920	-0.099	43	0.0019	R.AVCMLSNTTA IAEAWAR.L + Oxidation (M)
	Individual ions scores > 19 indicate peptides with significant homology. Individual ions scores > 28 indicate identity or extensive homology (p<0.05).						
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide



	1004.904 [2+]	2007.7933	2006.8858	0.9075	32	0.01	K.TIGGGDDSFN TFFSETGAGK.H
Individual ions scores > 25 indicate identity or extensive homology (p<0.05).							
Spot no.	Swiss Prot Acc no.	Name		Theor. Mass [kDa]/ Observed Mass [kDa]		Theor. pI / Observed pI	
20	P23246	Splicing factor, proline- and glutamine-rich		76.1 / 85		9.45 / 9.7	
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	572.8039 [2+]	1143.5933	1142.6196	0.9736	28	0.071	R.FATHAAALSV R.N
Individual ions scores > 18 indicate peptides with significant homology. Individual ions scores > 29 indicate identity or extensive homology (p<0.05).							
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	905.0039 [2+]	1807.9933	1806.9040	1.0893	38	0.0067	R.LFVGNLPADIT EDEFK.R
Individual ions scores > 21 indicate peptides with significant homology. Individual ions scores > 28 indicate identity or extensive homology (p<0.05).							
	precursor ion mass [m/z] and charge	Mr(expt)	Mr(calc)	Delta	individual ion score	Expect	Peptide
	1320.094 [2+]	2638.1733	2638.2915	-0.118	36	0.0021	R.NLSPYVSNEL LEEAFSQFGPIE R.A
Individual ions scores > 22 indicate identity or extensive homology (p<0.05).							

**Supplementary Table 2.** Peak lists of proteins identified by MALDI-TOF MS tryptic peptide mass fingerprint. Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.

Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)
Heat shock protein HSP 90-beta (P08238)	1194.6932	Tubulin alpha-ubiquitous (P68363)	2007.893	Gelsolin (P06396)	1254.6994
	1242.7661		2191.0528		998.5336
	951.4725		1701.923		1722.9643
	1311.6084		1718.9054		850.4919
	1151.6362		1487.8824		1275.7247
	901.5239		2409.2106		1320.671
	1847.6507		1756.9753		847.425
	1527.6874		1584.7497		882.5092
	1348.6909		1824.9951		1074.5246
	829.5015		1864.9223		887.4741
	1236.6504		1880.9109		839.415
	1513.7824		2346.0234		1078.5444
	891.4403				
	886.5234				
	1160.6302				
	1249.6462				
	1009.5399				
	1376.6355				
	1782.8095				

Supplementary Table 2 (continued).

Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)
Vinculin (P18206)	1560.7021	Ezrin (P15311)	823.5308	Keratin, type II cytoskeletal 7 (P08729)	2246.1447
	1090.6128		976.598		1104.6185
	934.4857		848.4439		2449.233
	938.4739		894.5828		1082.613

1305.6404	1236.6289	906.5043
1001.5491	898.4474	1045.5856
1191.6048	1175.6462	1773.0599
1748.7407	1104.6228	1220.6921
1470.6499	965.5754	1348.8011
1493.6306	959.5575	1441.8478
1509.6315	1087.7241	1385.7926
1457.7798	1310.7215	1277.7836
1037.5124	1182.6325	
1345.5996	924.4559	
916.4515	1002.5694	
1105.6291	987.5663	
1314.7074	1416.6969	
1230.5968	874.4896	
838.4261	1651.8455	
1183.5801	1070.6574	
1484.7416	1445.8354	
930.4418		
1173.6397		
1074.5258		
1170.6531		
1752.6582		
1145.5918		
1269.679		
944.4625		
1550.6963		
1566.6231		
868.4867		
1200.5697		
1132.5866		
1303.6916		
880.4694		
822.3902		

Supplementary Table 2 (continued).

Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)
Keratin, type II cytoskeletal (P05787)	1352.7264	Keratin, type I cytoskeletal (Q04695)	1886.915	Keratin, type I cytoskeletal (P05783)	975.4931
	1496.7385		1034.5118		1092.6794
	1797.8276		1064.56		848.4298
	1813.8129		1036.5345		1004.5352
	1419.7393		994.445		924.5747
	2109.0066		3023.6008		982.5018
	2125.0012		2068.1679		2059.1334
	1320.6294		807.4039		1041.6561
	1079.5239		1222.6574		807.4222
	1428.6592		1342.7605		1255.7256
	1137.5475				1267.7196
	1341.7315				1884.0915
	1344.6677				1680.9658
	1129.6201				965.5131
	1277.7003				1878.0518
	1405.7959				1473.8775
					1292.7736

Supplementary Table 2 (continued).

Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)
Keratin, type I cytoskeletal (P08727)	1554.7681	14-3-3 protein sigma (Stratifin) (P31947)	816.4554	Elongation factor 2 (P13639)	2220.1428
	850.4667		1918.8474		1849.0258
	1120.5567		1934.8114		2759.3167
	1064.6224		1137.4582		2143.0688
	1008.563		1592.7323		1742.8929
	1073.6155		1546.6947		1534.8225
	1041.6359		928.4817		3005.3802

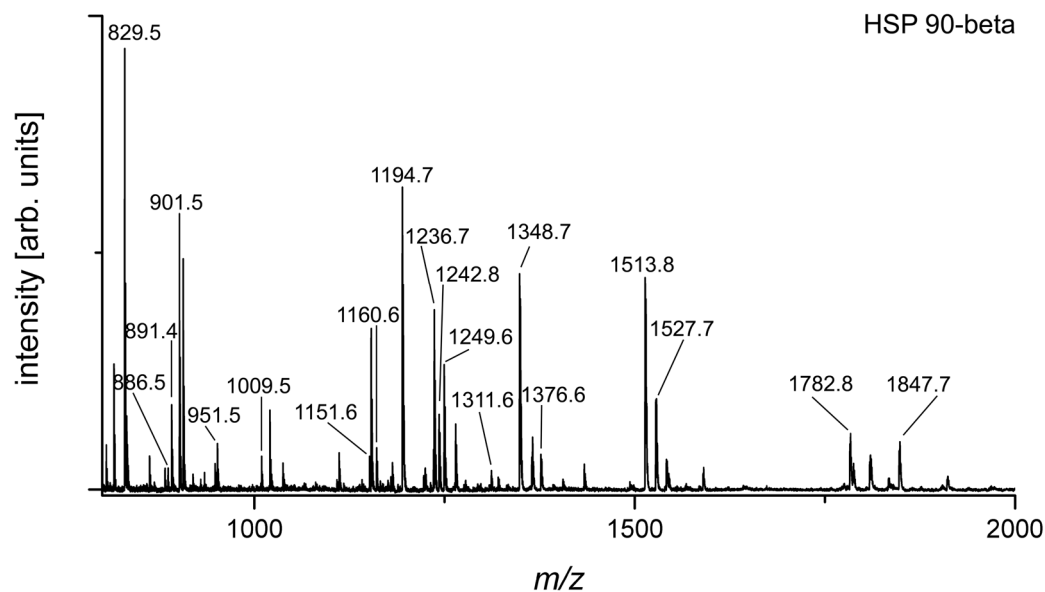
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1222.6684	1386.6137	1444.7617
993.5255	1198.6392	
1029.6114	1054.556	
1227.6184	1070.5423	
1370.6235	1205.6592	
1498.7019		
1210.6345		
1082.5303		
1501.7178		
1389.7133		

Supplementary Table 2 (continued).

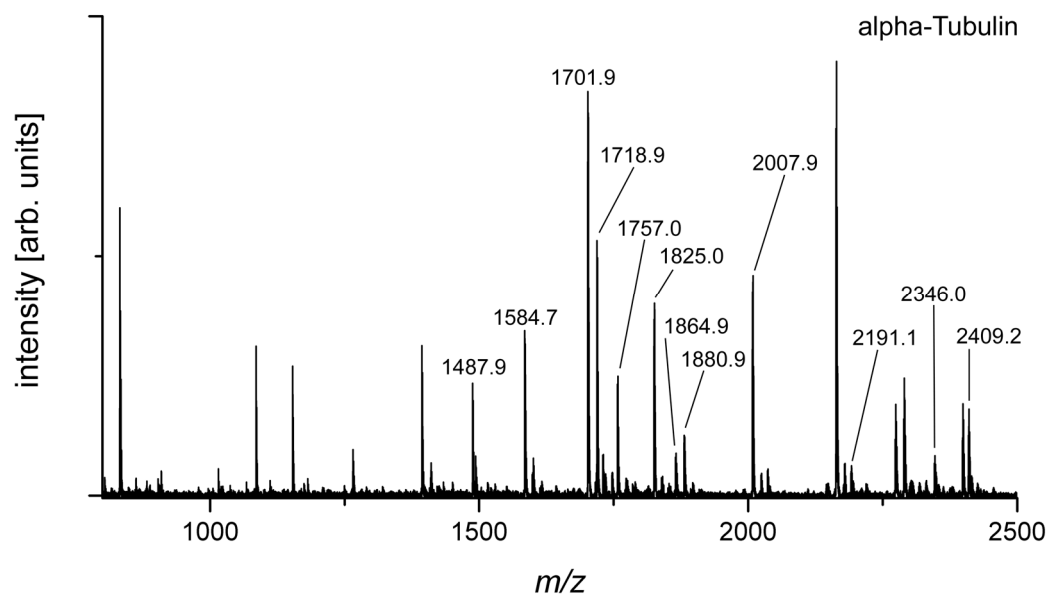
Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)
Keratin, type II cytoskeletal 5 (P13647)	865.3866	ATP synthase subunit alpha (P25705)	1575.757	Annexin A2 (P07355)	1844.8678
	2257.002		1667.7902		1086.4324
	1410.6786		1624.8875		2154.9842
	938.4911		1710.8886		1542.8111
	1082.5499		1120.6792		1777.8276
	1462.7663		1229.6443		1244.5766
	1759.9373		1287.653		1811.8389
	1203.5985		1553.7305		2064.9207
	1426.6799		1438.8145		1908.8436
	2418.0586		1310.6121		1632.7535
	1537.7839				1035.4858
	1553.761				1051.4721
	1133.4976				1460.6214
	1439.6797				1476.6166
	2392.0928				1588.7215
					1604.7285
					1421.6491

Supplementary Table 2 (continued).

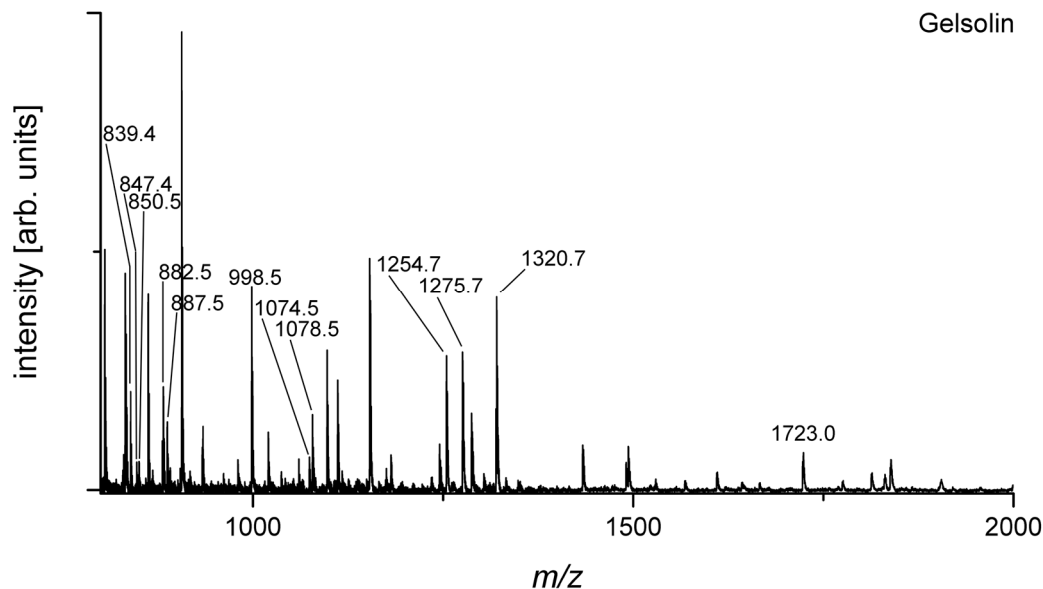
Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)	Protein name and Swiss Prot Acc no.	observed peptide masses (m/z)(Da)
Elongation factor 1-alpha 1 (P68104)	1588.8016	Heterogeneous nuclear ribonucleoprotein M (P52272)	1629.7181
	1120.5638		1125.4458
	1404.6662		1141.4965
	1314.6794		1157.4901
	1600.8126		1556.7795
	2515.3498		1572.7658
	2531.3185		1427.6594
	3011.3004		1443.6528
	3027.2832		1399.562
	910.4295		2034.9762
			1021.561
			1663.799



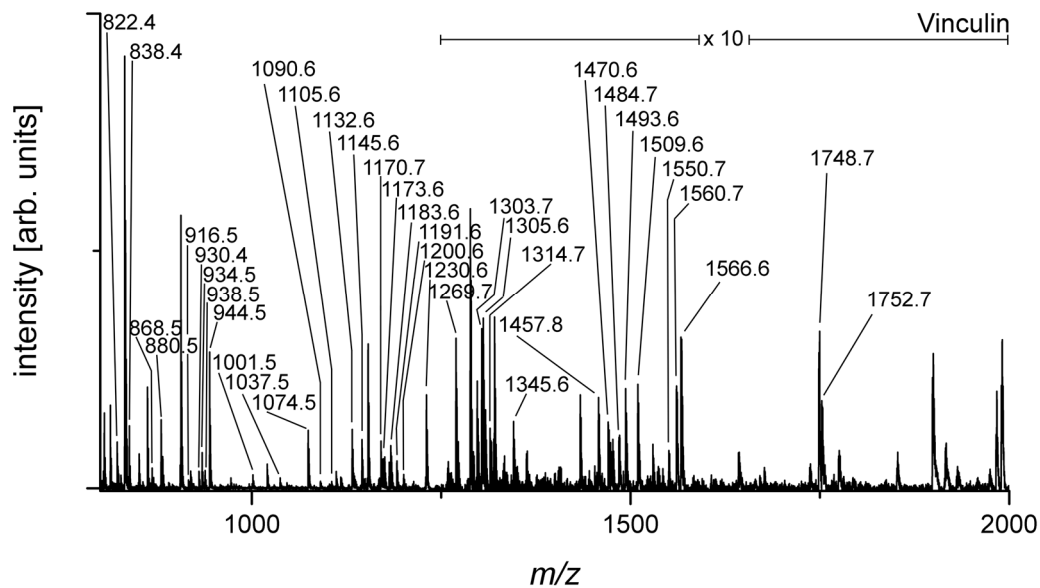
**Supplementary Figure 1.** MALDI-TOF MS tryptic peptide mass fingerprint of Heat shock protein HSP 90-beta (Swiss Prot Acc no. P08238). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



**Supplementary Figure 2.** MALDI-TOF MS tryptic peptide mass fingerprint of Tubulin alpha-chain (Swiss Prot Acc no. P68363). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.

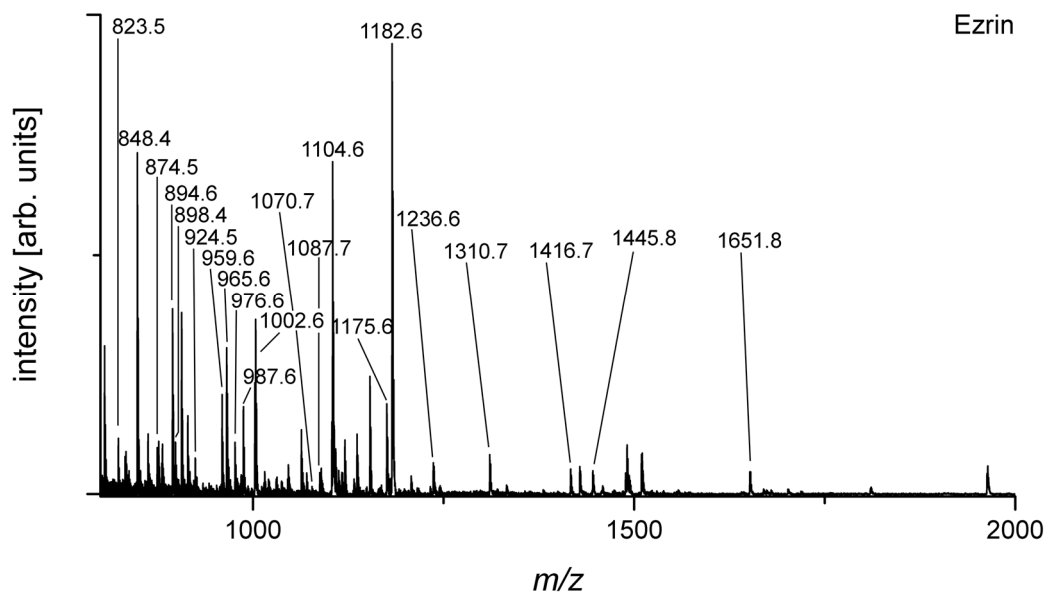


**Supplementary Figure 3.** MALDI-TOF MS tryptic peptide mass fingerprint of Gelsolin (Swiss Prot Acc no. P06396). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.

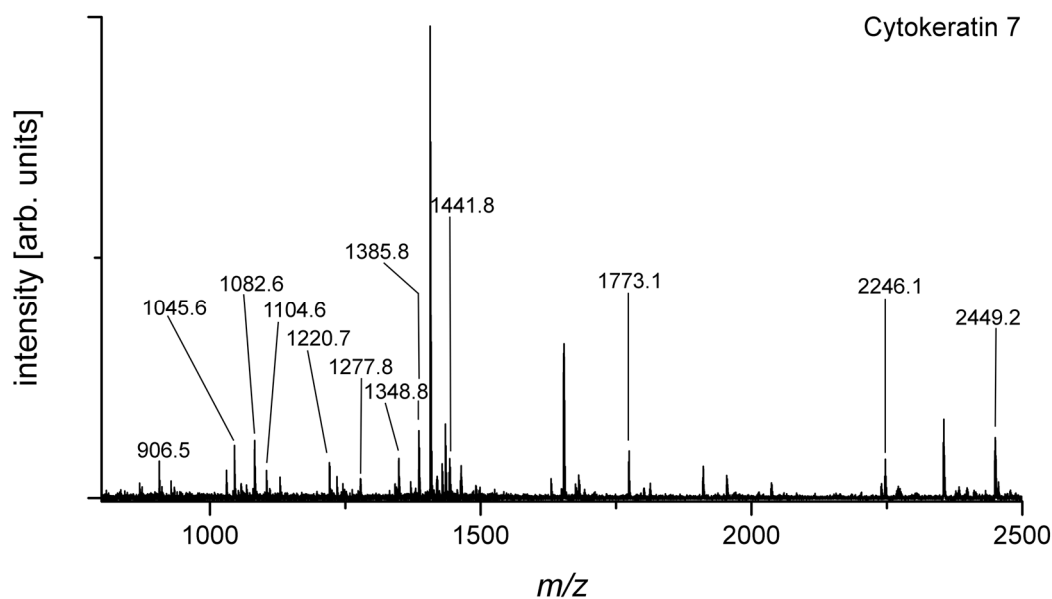


**Supplementary Figure 4.** MALDI-TOF MS tryptic peptide mass fingerprint of Vinculin (Swiss Prot Acc no. P18206). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.

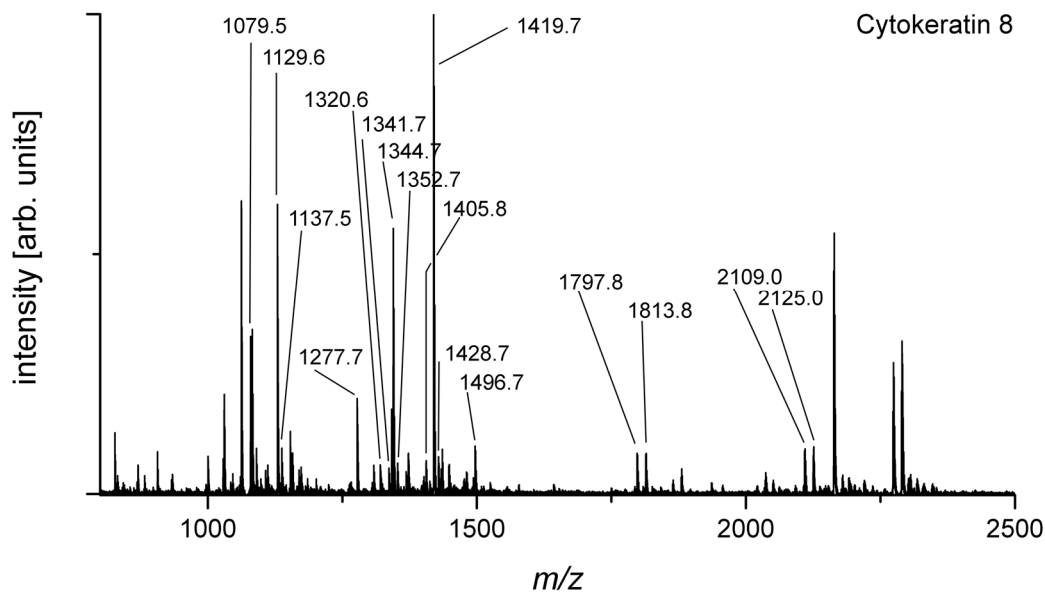




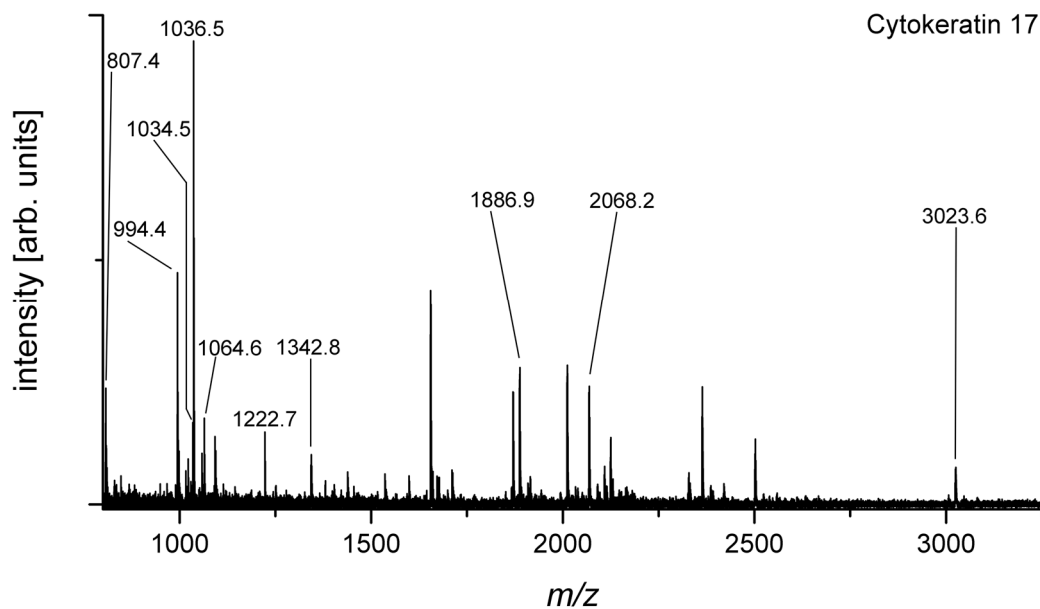
**Supplementary Figure 5.** MALDI-TOF MS tryptic peptide mass fingerprint of Ezrin (Cytovillin) (Swiss Prot Acc no. P15311). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



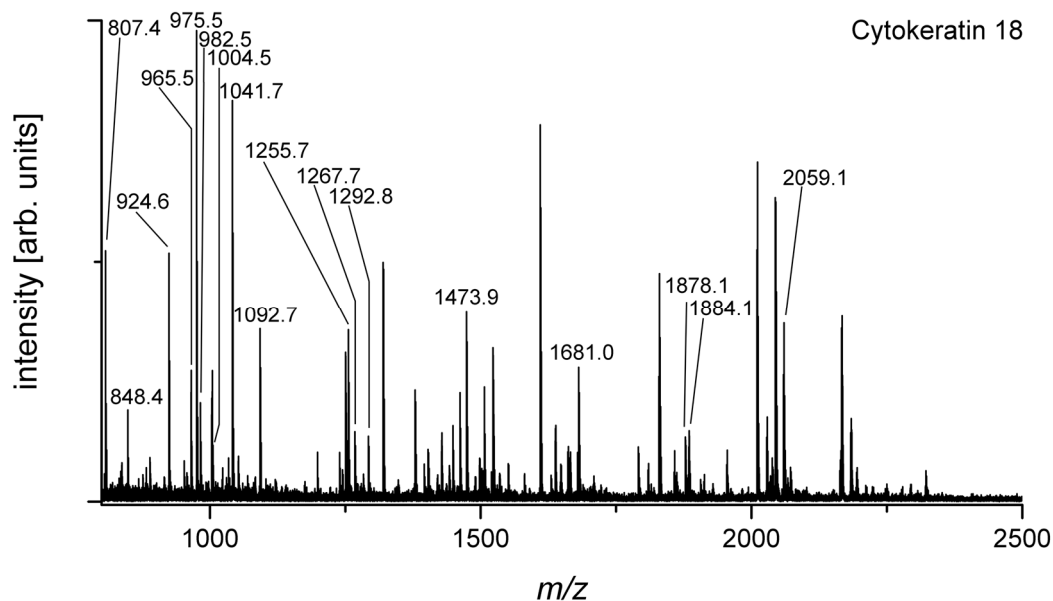
**Supplementary Figure 6.** MALDI-TOF MS tryptic peptide mass fingerprint of Keratin, type II cytoskeletal 7 (Swiss Prot Acc no. P08729). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



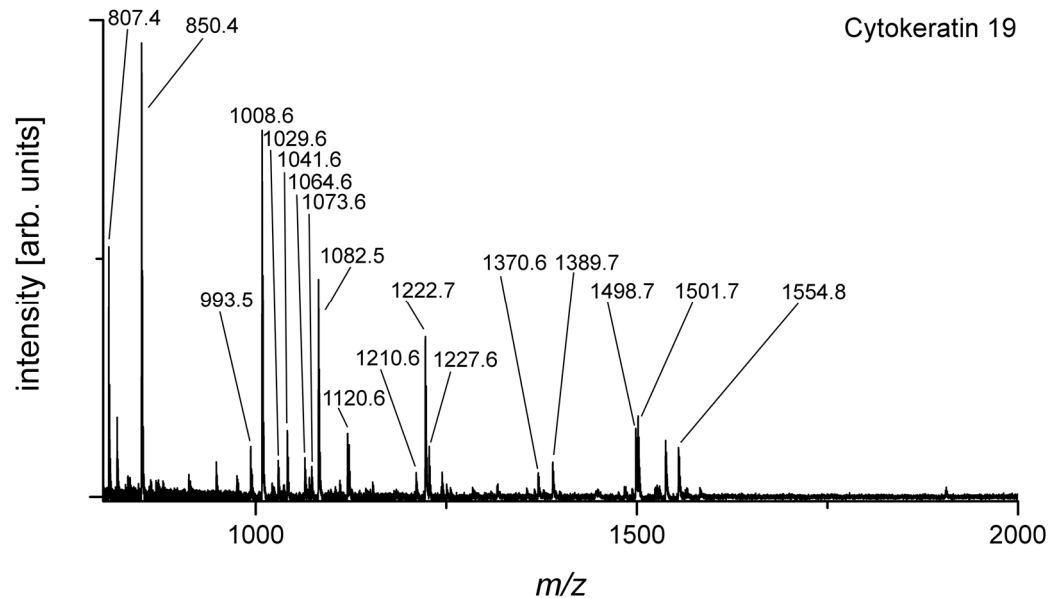
**Supplementary Figure 7.** MALDI-TOF MS tryptic peptide mass fingerprint of Keratin, type II cytoskeletal 8 (Swiss Prot Acc no. P05787). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



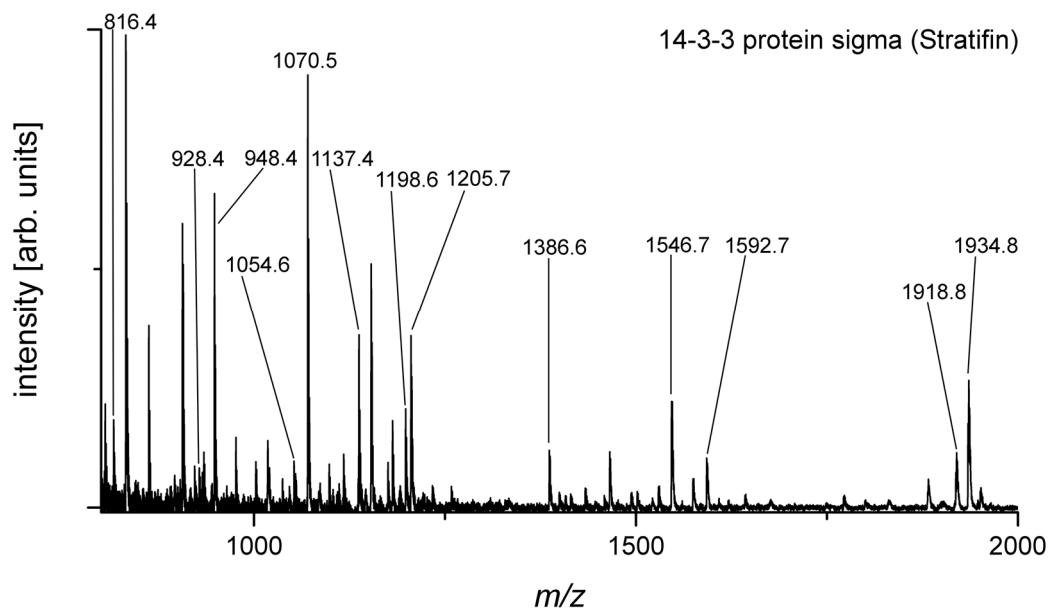
**Supplementary Figure 8.** MALDI-TOF MS tryptic peptide mass fingerprint of Keratin, type I cytoskeletal 17 (Swiss Prot Acc no. Q04695). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



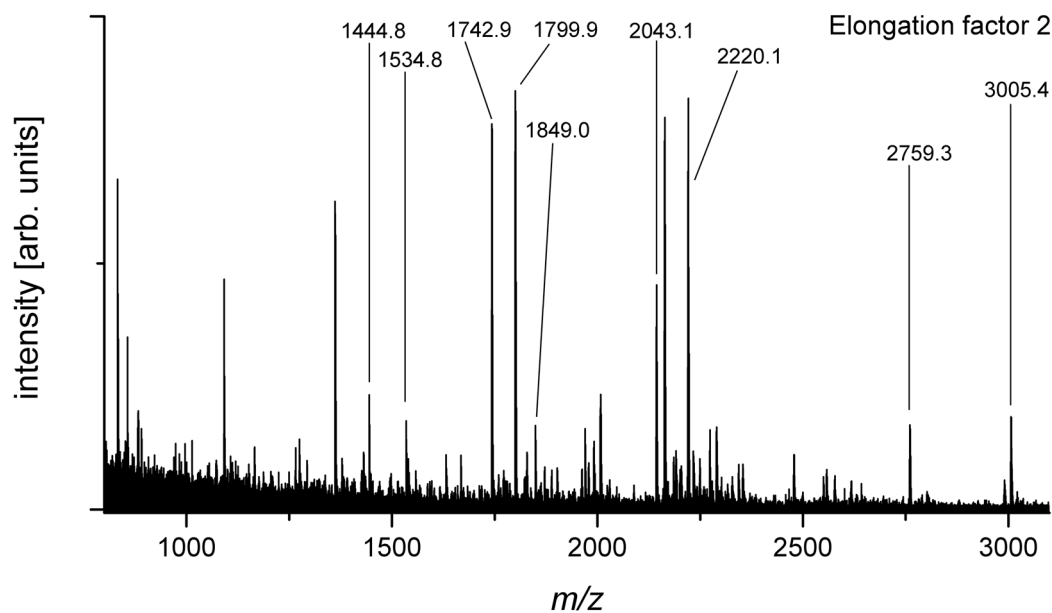
**Supplementary Figure 9.** MALDI-TOF MS tryptic peptide mass fingerprint of Keratin, type I cytoskeletal 18 (Swiss Prot Acc no. P05783). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



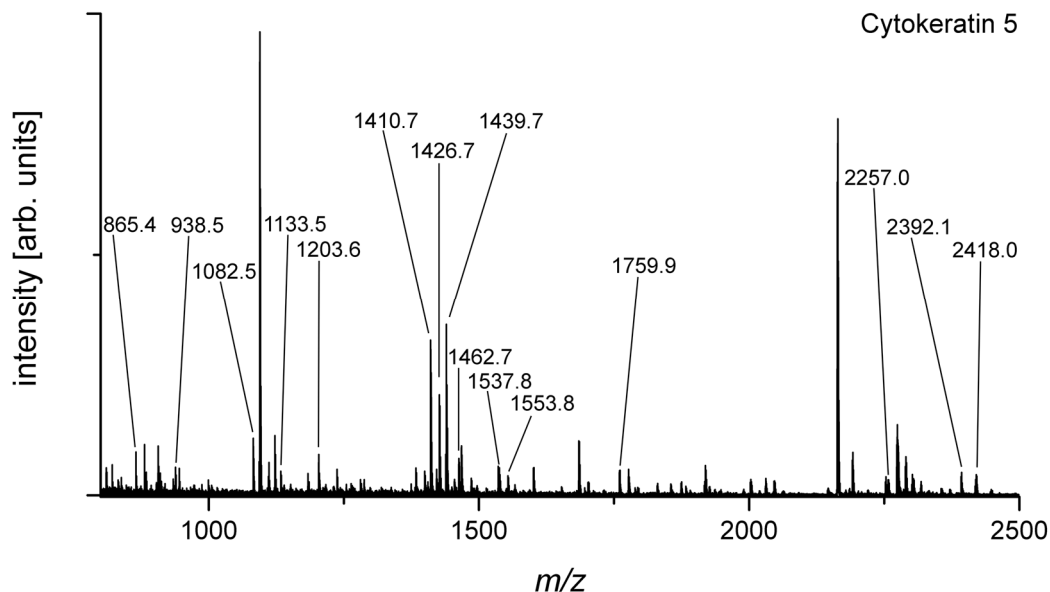
**Supplementary Figure 10.** MALDI-TOF MS tryptic peptide mass fingerprint of Keratin, type I cytoskeletal 19 (Swiss Prot Acc no. P08727). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



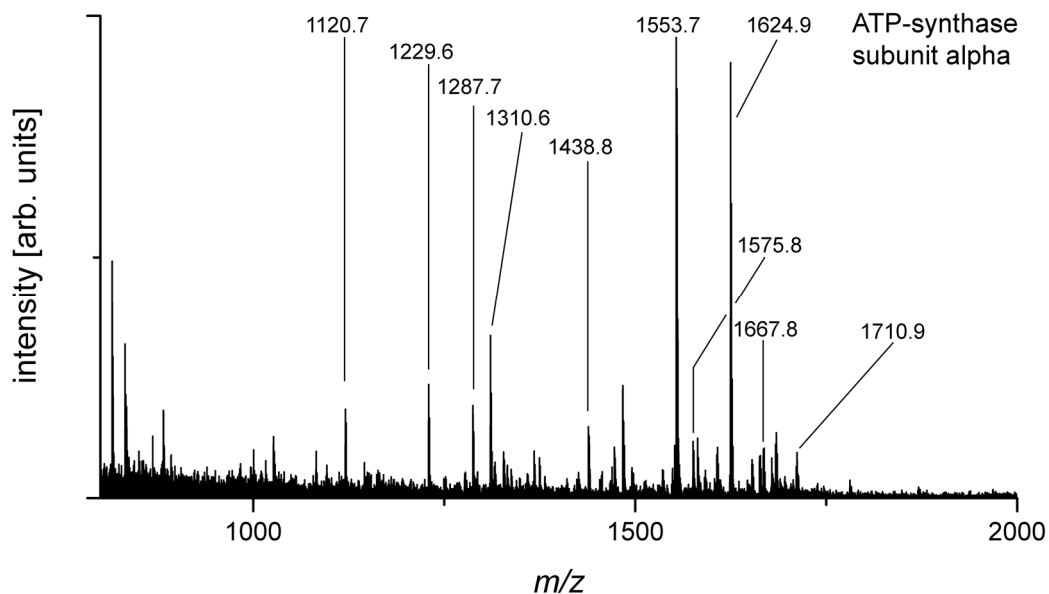
**Supplementary Figure 11.** MALDI-TOF MS tryptic peptide mass fingerprint of 14-3-3 protein sigma (Stratifin) (Swiss Prot Acc no. P31947). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



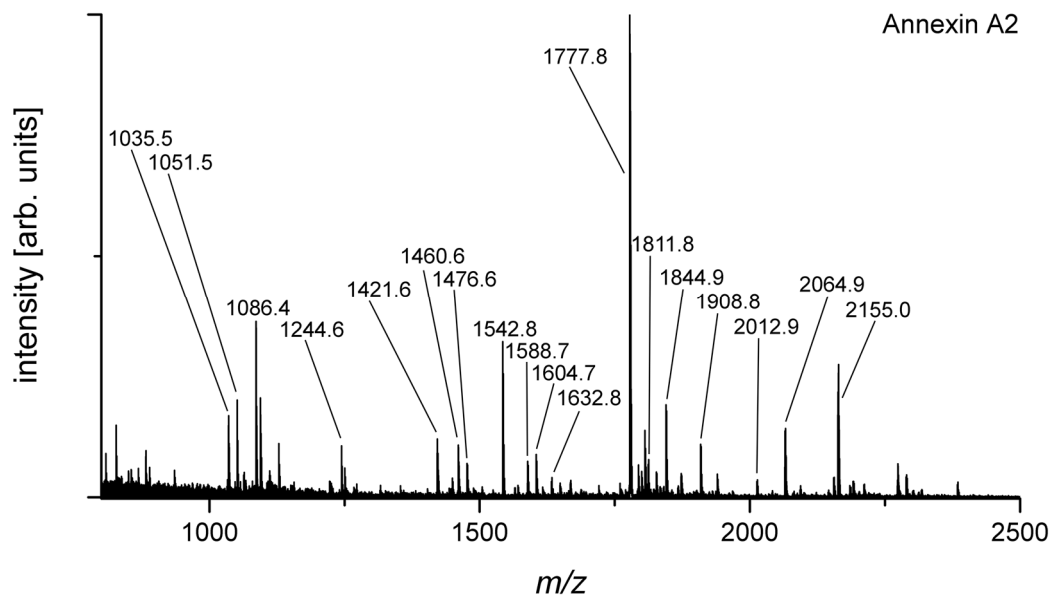
**Supplementary Figure 12.** MALDI-TOF MS tryptic peptide mass fingerprint of Elongation factor 2 (Swiss Prot Acc no. P13639). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



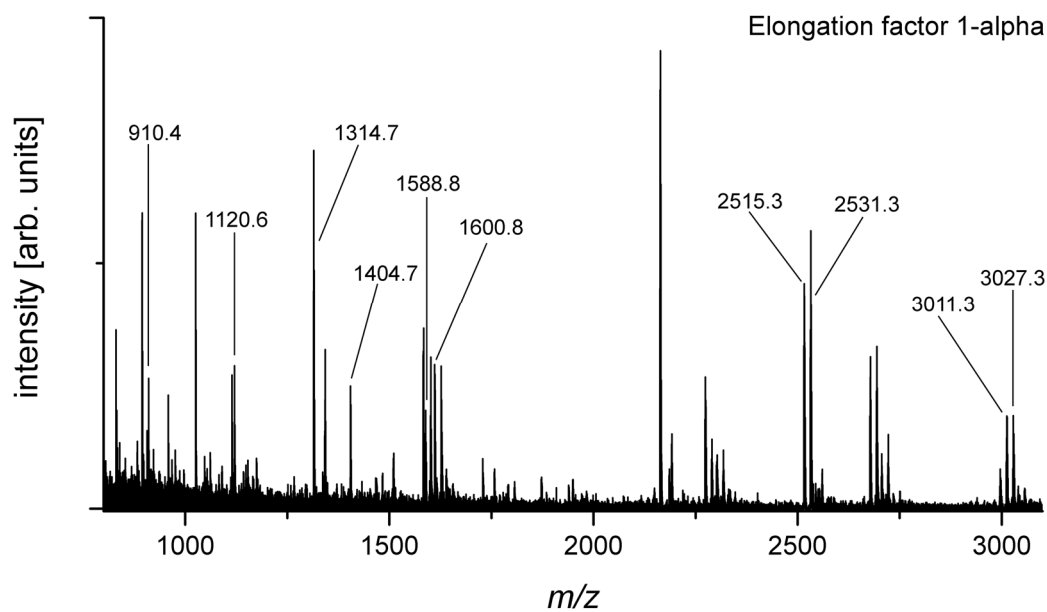
**Supplementary Figure 13.** MALDI-TOF MS tryptic peptide mass fingerprint of Keratin, type II cytoskeletal 5 (Swiss Prot Acc no. P13647). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



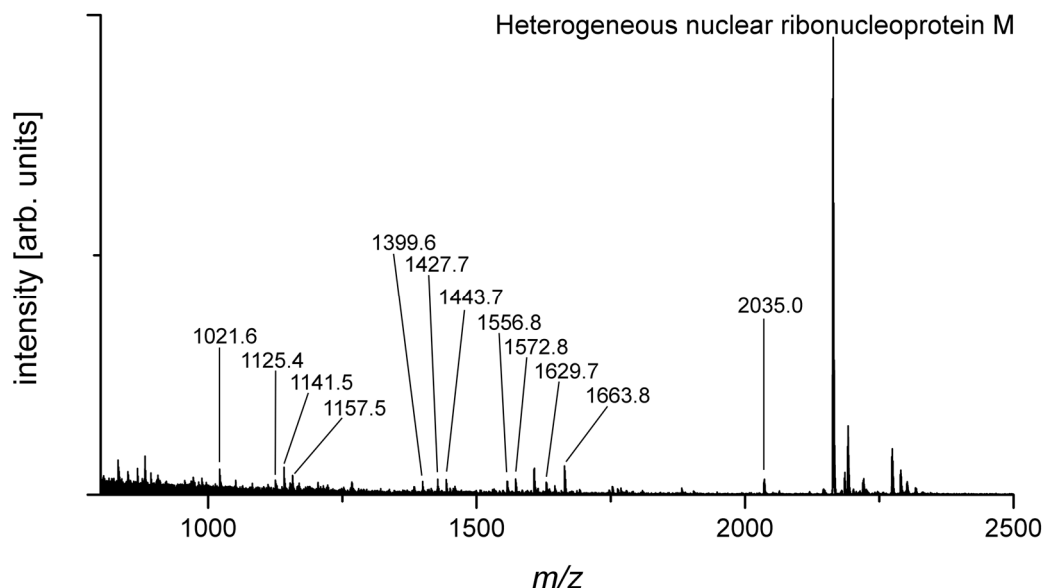
**Supplementary Figure 14.** MALDI-TOF MS tryptic peptide mass fingerprint of ATP synthase subunit alpha (Swiss Prot Acc no. P25705). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



**Supplementary Figure 15.** MALDI-TOF MS tryptic peptide mass fingerprint of Annexin A2 (Swiss Prot Acc no. P07355). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



**Supplementary Figure 16.** MALDI-TOF MS tryptic peptide mass fingerprint of Elongation factor 1-alpha 1 (Swiss Prot Acc no. P68104). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.



**Supplementary Figure 17.** MALDI-TOF MS tryptic peptide mass fingerprint of Heterogeneous nuclear ribonucleoprotein M (Swiss Prot Acc no. P52272). Spot numbers refer to designated protein spots in Fig. 3 and 4 of the main text.

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