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Proteomic analysis of embryonic Fasciola hepatica: Characterization and antigenic potential of a developmentally regulated heat shock protein

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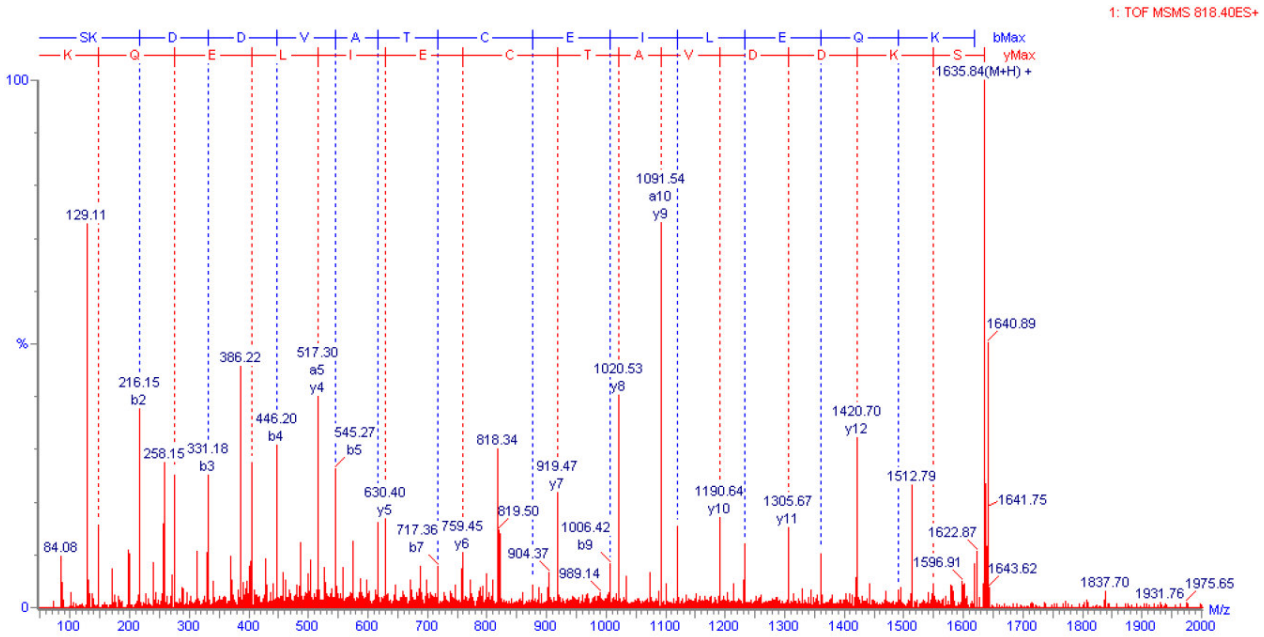

Figure S1. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 869.7 taken from spot 1. Interpretation of the b and y ion series revealed the peptide sequence SKDDVATCEILQK, matching a peptide from a ferritin-like protein expressed by *Fasciola hepatica*

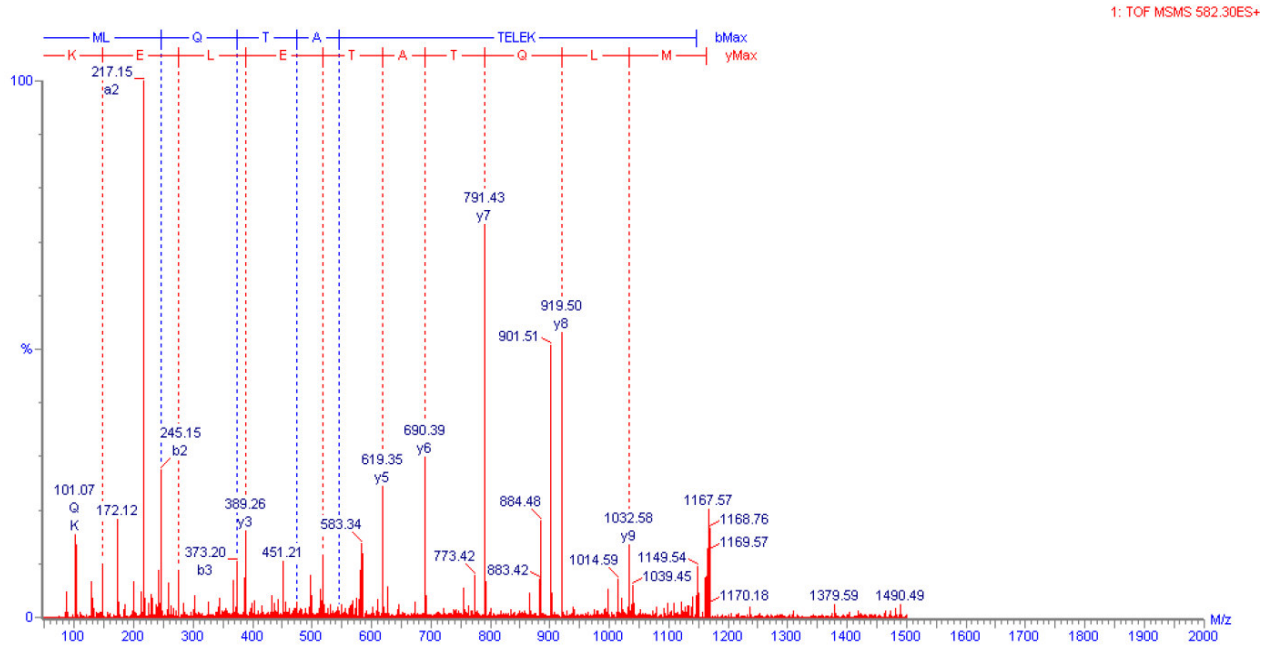

Figure S2. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 583.2 taken from spot 1. Interpretation of the b and y ion series revealed the peptide sequence MLQTATELEK, matching a peptide from a ferritin-like protein expressed by *Fasciola hepatica*

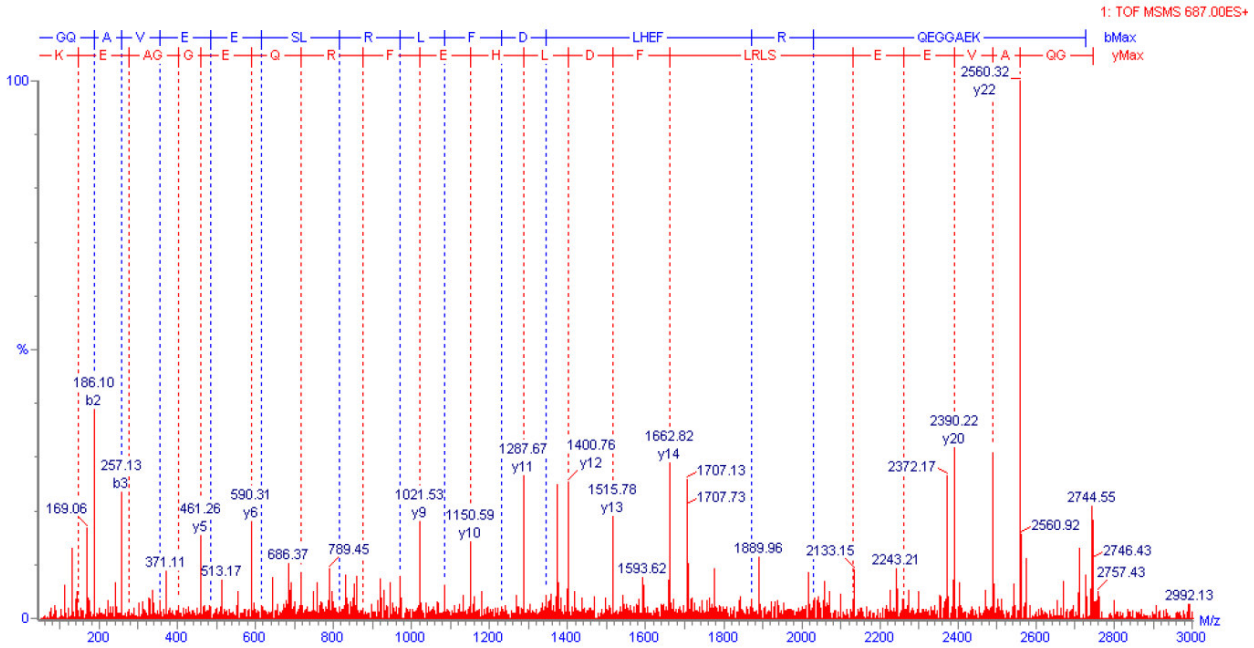

Figure S3. MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 687.07 with a 4+ charge taken from spot 4. Interpretation of the b and y ion series revealed the peptide sequence GQAVEESLRLFDLHEFRQEGGAEK, underlined text denotes deviation from expected protein cleavage specificity. A mass error of 0.3 Da was observed. BLAST analysis against an in-house translated database of *F. hepatica* transcript revealed matches (e 0.42) to clones showing significant homology to a thioredoxin peroxidase expressed by *F. gigantica* (GenBank accession number ABY85785, BLAST score 1e-112).

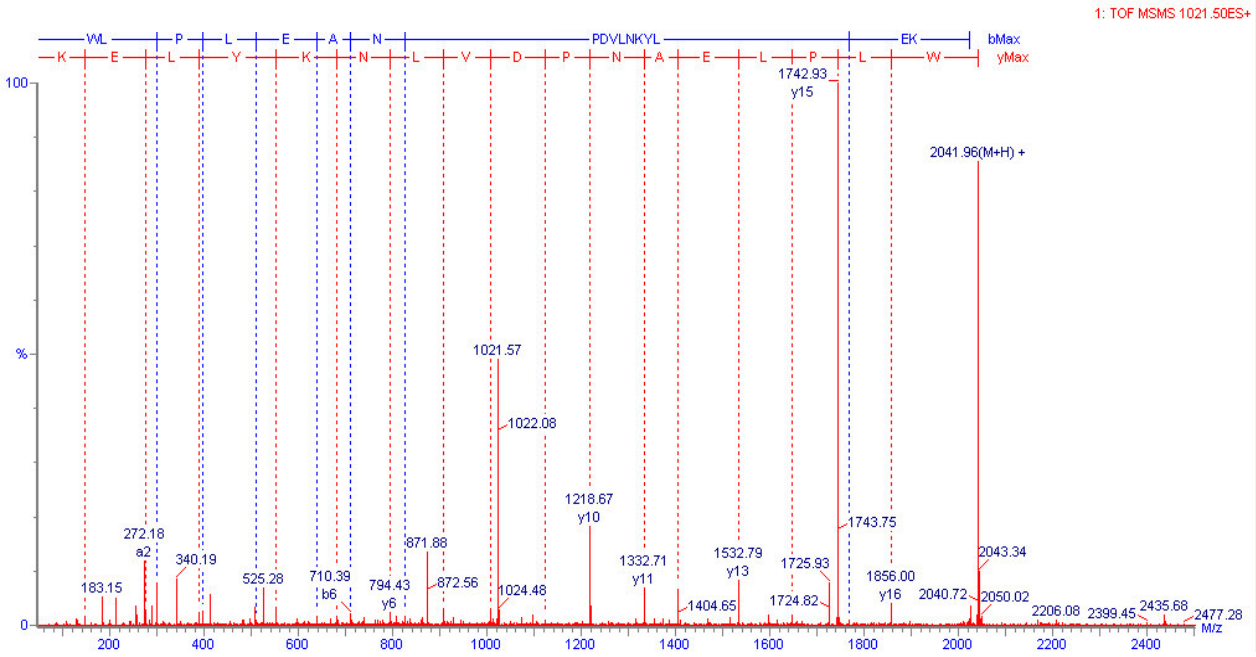

Figure S4. MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 1021.5 with a 2+ charge taken from spot 4. Interpretation of the b and y ion series revealed the peptide sequence WLPEANPDVLNKYLEK, underlined text denotes deviation from expected protein cleavage specificity. A mass error of 0.02 Da was observed. BLASTp analysis revealed significant match to a peptide from a ubiquitin carboxyl-terminal hydrolase expressed by *Culex quinquefasciatus* (GenBank accession number XP_001866570 , BLAST score 3e-08).

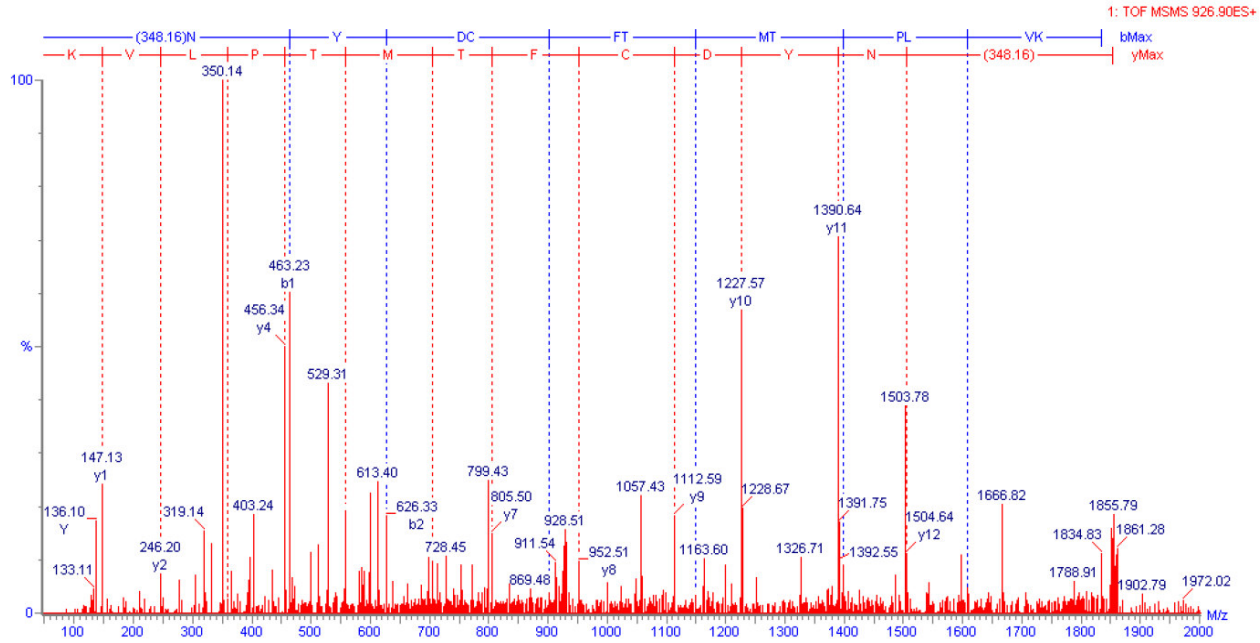

Figure S5. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 926.9 taken from spot 6. Interpretation of the b and y ion series revealed the sequence tag NYDCMTMTPLVK, matching a peptide from a ferritin-like protein expressed by *Fasciola hepatica*

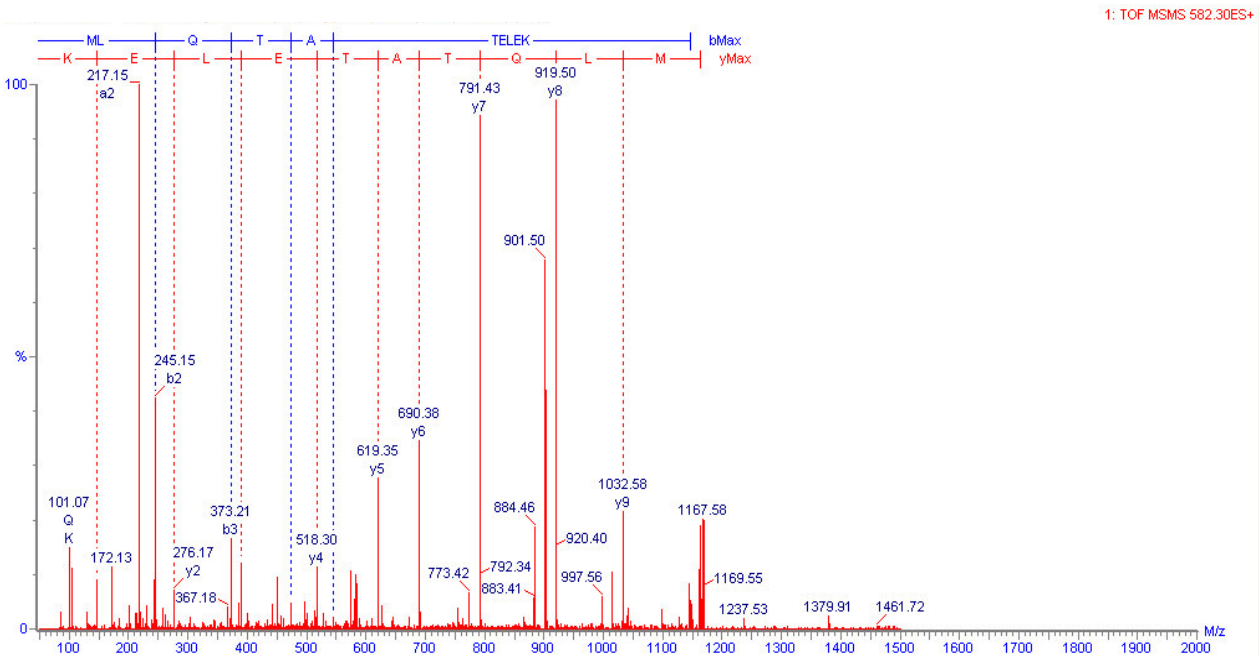

Figure S6. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 528.3 taken from spot 6. Interpretation of the b and y ion series revealed the peptide sequence MLQTATELEK, matching a peptide from a ferritin-like protein expressed by *Fasciola hepatica*

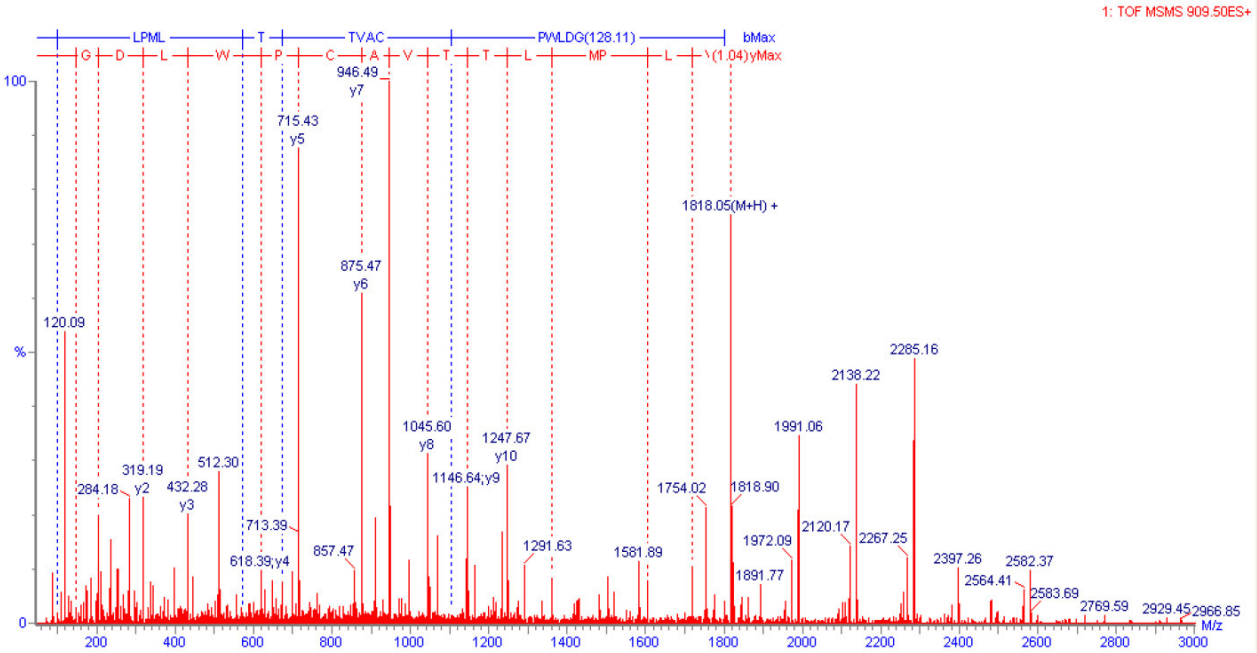

Figure S7. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 909.5 taken from spot 10. Interpretation of the b and y ion series revealed the sequence tag VLPMLTTVACPWLDG, matching a peptide from a protein similar to Mal s 6 allergen expressed by *Ciona intestinalis*

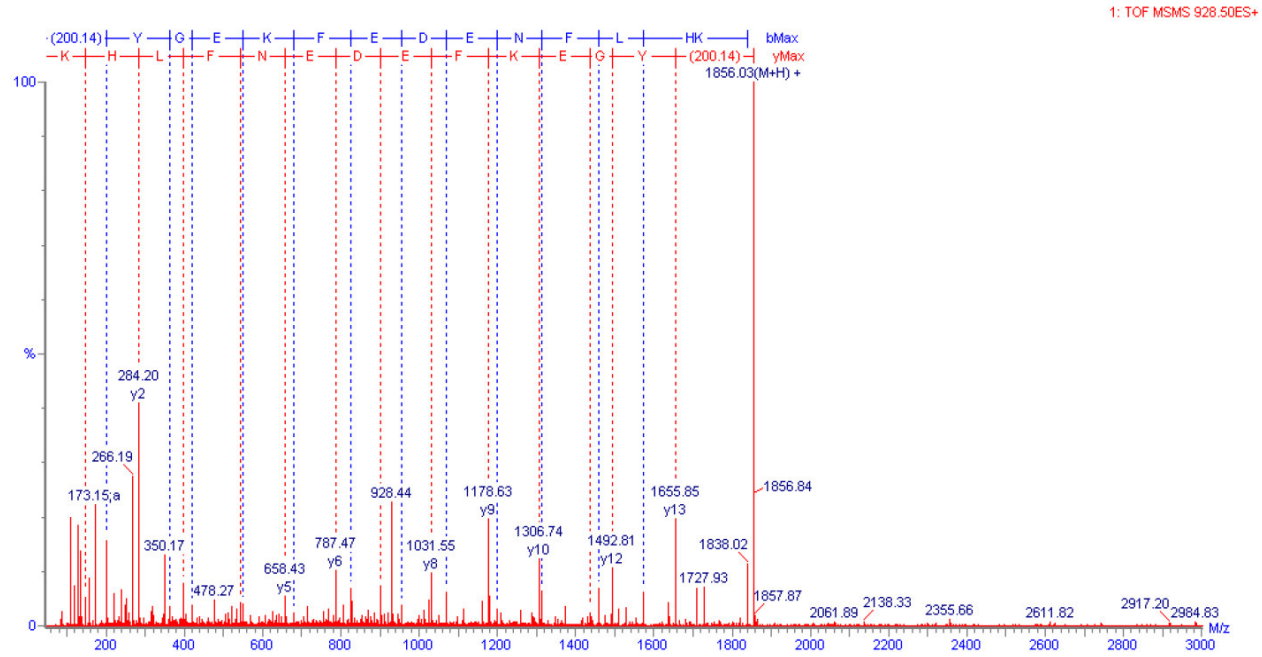

Figure S8. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 928.5 taken from spot 10. Interpretation of the b and y ion series revealed the sequence tag YGEKFEDENFLHK, matching a peptide from a protein similar to Mal s 6 allergen expressed by *Ciona intestinalis* Underlined text denotes deviation from expected protein cleavage specificity.

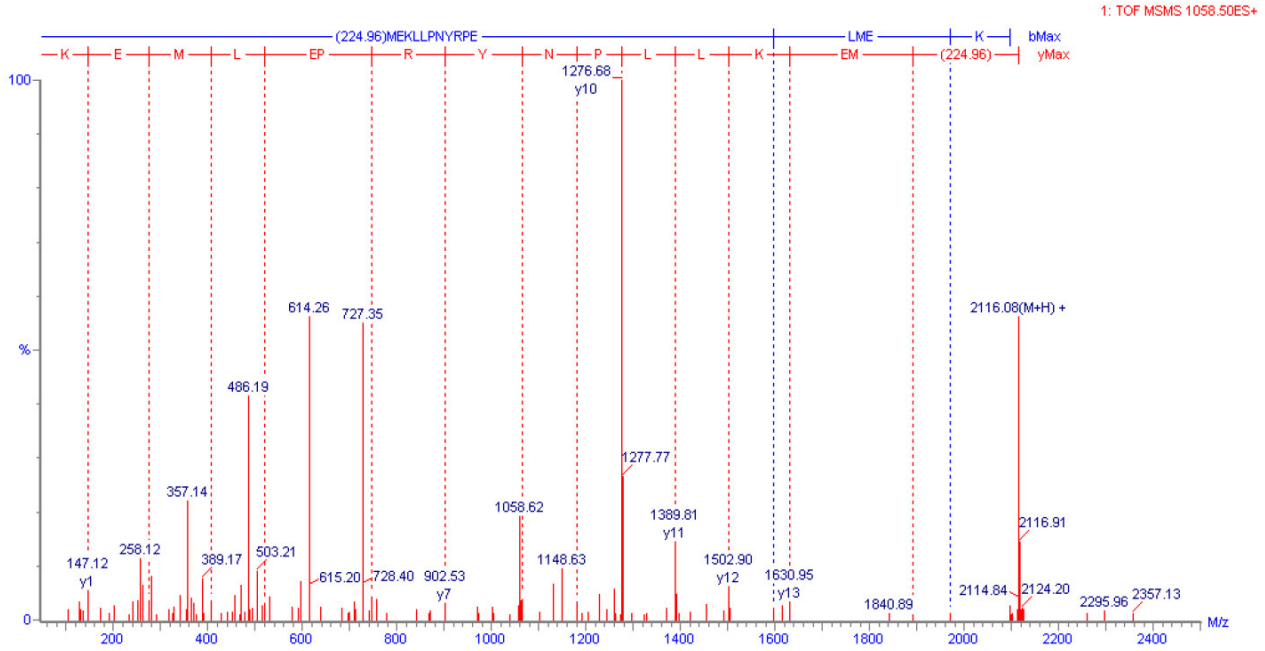

Figure S9. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 1058.5 taken from spots 12/13. Interpretation of the b and y ion series revealed the sequence tag ME*K*LLPNYRPELMEK. Underlined text denotes deviation from expected protein cleavage specificity. Residue in italics was annotated by the software as a lysine, whereas EST clones express glutamine at this position. Mass difference between K and Q is ~0.04 Da, thus Q was accepted as the true moiety. Significant matches to an unannotated protein of *Paragonimus westermani* were noted

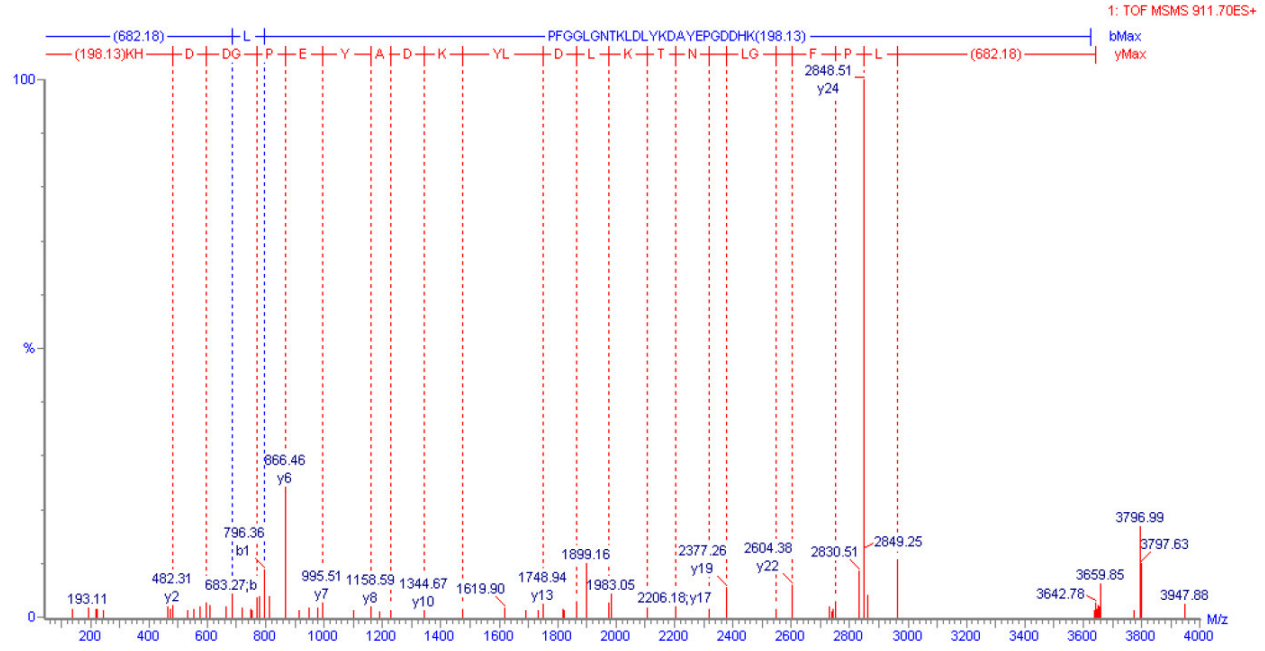

Figure S10. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 911.7 taken from spots 12/13. Interpretation of the b and y ion series revealed the sequence tag IPFGGLGNT*K*LDYLKDAYEPGDDHK Underlined text denotes deviation from expected protein cleavage specificity. Residue in italics was annotated by the software as a lysine, whereas EST clones express glutamine at this position. Mass difference between K and Q is ~0.04 Da, thus Q was accepted as the true moiety. Significant matches to an unannotated protein of *Paragonimus westermani* were noted

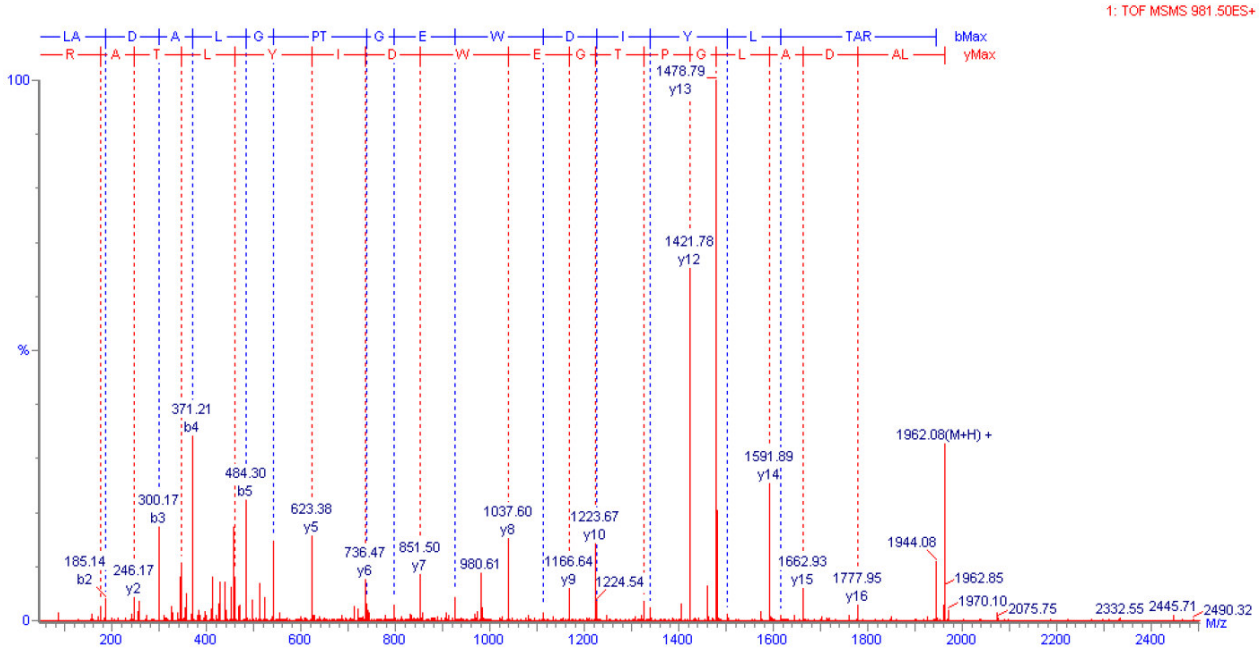

Figure S11. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 981.5 taken from spot 24. Interpretation of the b and y ion series revealed the peptide sequence LADALGPTGEWDIYLTAR, matching a peptide from a carbonyl reductase expressed by *Schistosoma mansoni.*

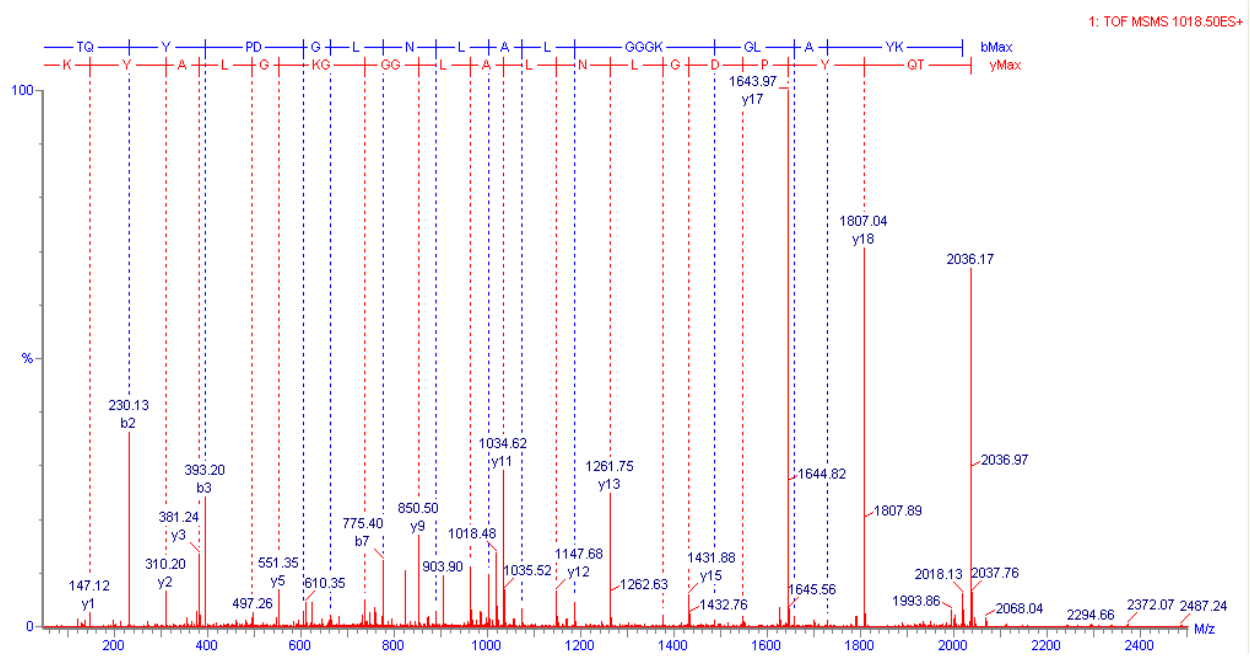

Figure S12. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 1018.5 taken from spot 24. Interpretation of the b and y ion series revealed the peptide sequence TQYPDGINIAIGGGKGIAYK matching a peptide from a carbonyl reductase expressed by *Schistosoma mansoni.* Underlined text denotes deviation from anticipated peptide cleavage specificity.

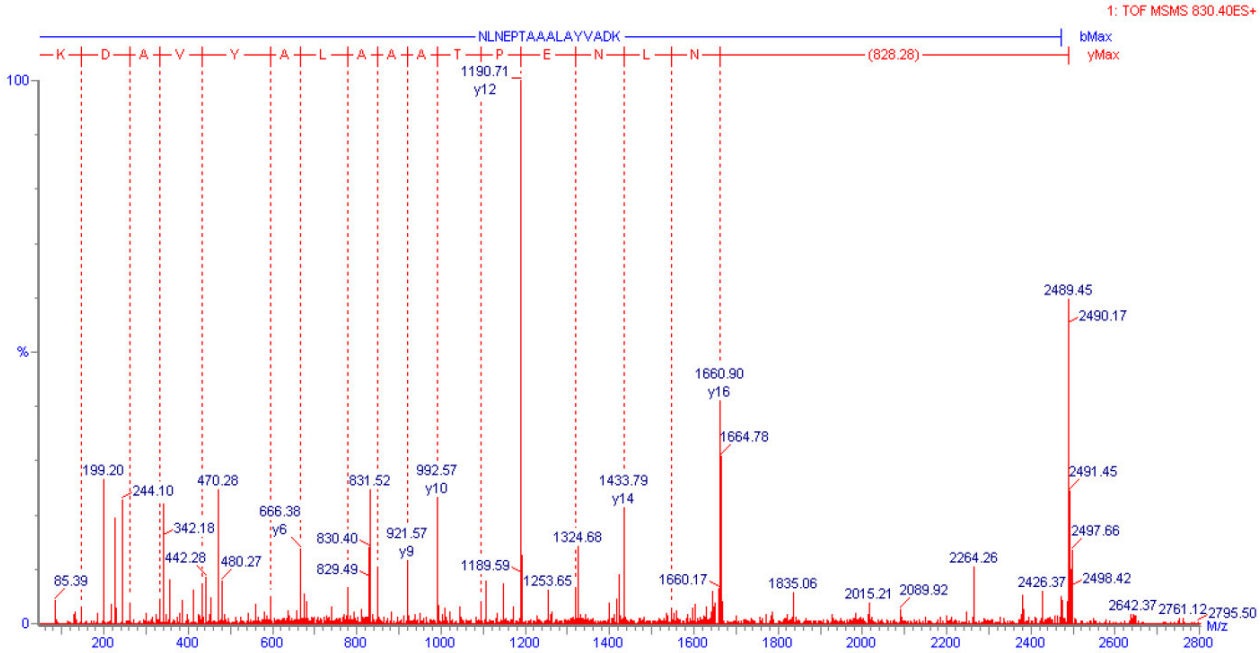

Figure S13. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 830.4 taken from spot 27. Interpretation of the b and y ion series revealed the peptide sequence NINEPTAAAIAYVADK, matching a peptide from a HSP 70 expressed by *Fasciola gigantica*

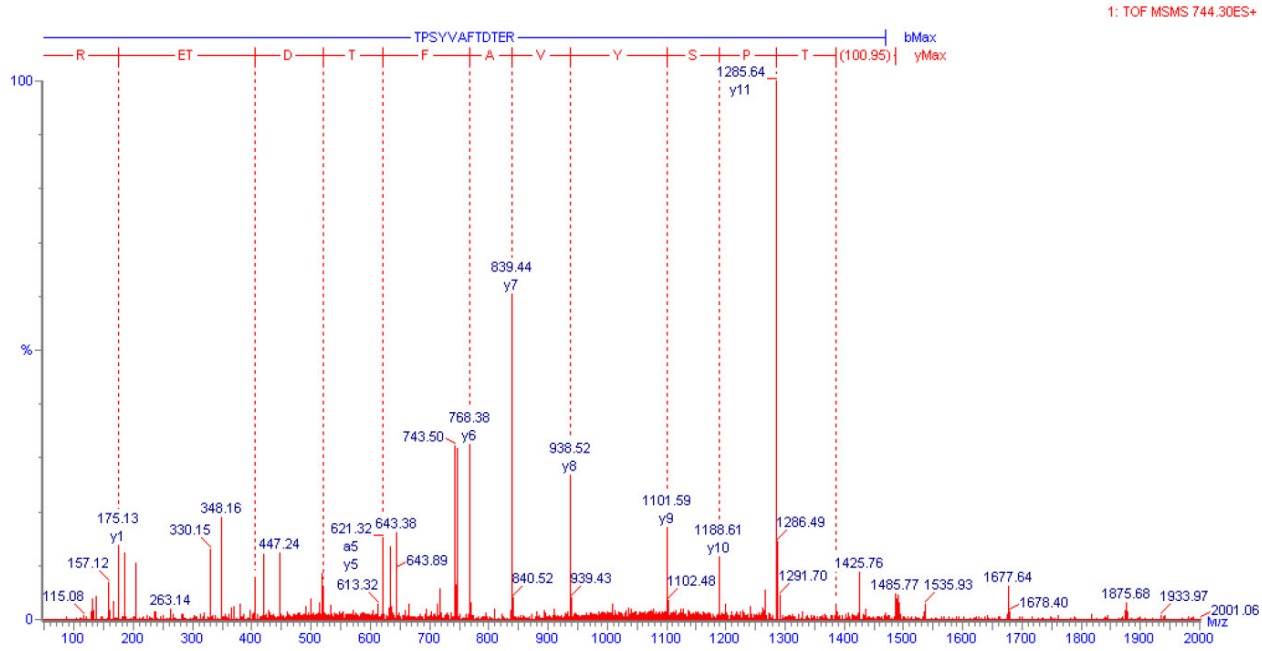

Figure S14. Representative MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 744.3 taken from spot 27. Interpretation of the b and y ion series revealed the peptide sequence TPSYVAFTDTER matching a peptide from a HSP 70 a HSP 70 expressed by *Fasciola gigantica*

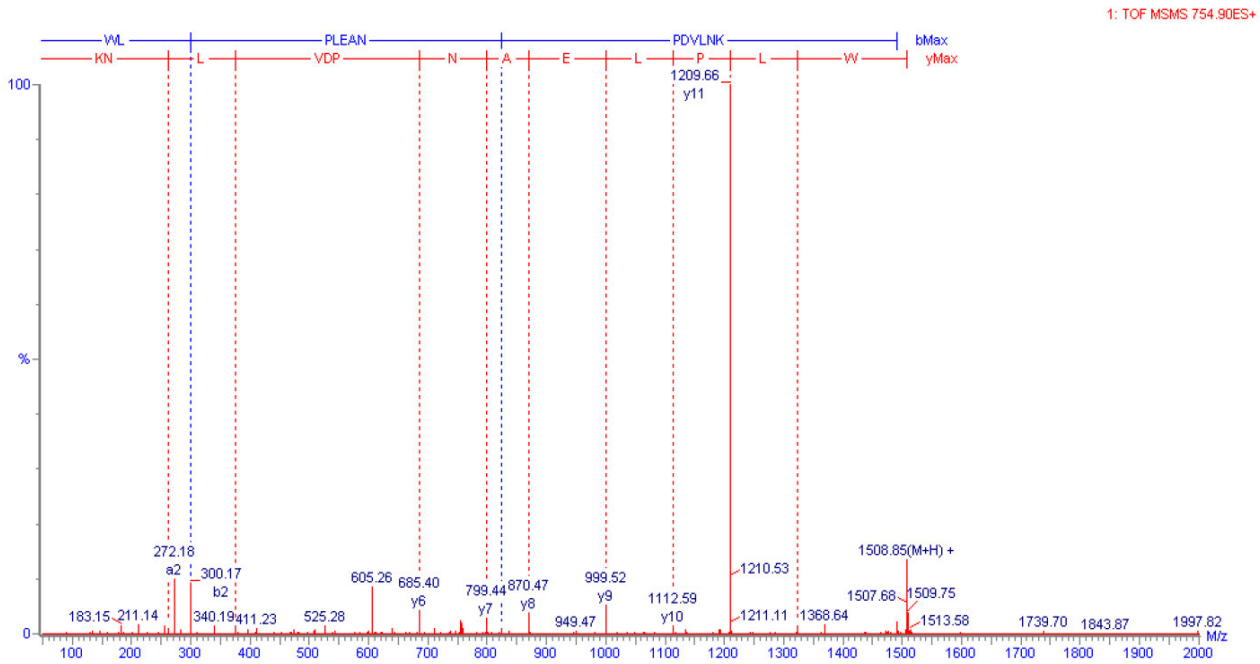

Figure S15. MSMS sequence analysis using peptide sequencing software (MassLynx v. 5.0, Micromass, UK) of a precursor ion *m/z* 754.9 with a 2+ charge taken from spot 31. Interpretation of the b and y ion series revealed the peptide sequence WLPLEANNPDVLNK. A mass error of 0.02 Da was observed. BLAST analysis revealed significant homology to ubiquitin carboxyl-terminal hydrolase expressed by *Culex quinquefasciatus* (GenBank accession number XP_001866570 , BLAST score 4e-04).

Supplementary table 1: Ion data for automatically generated peptides used for MASCOT analysis of cytosolic *F. hepatica* egg proteins

Spot	MS/MS Peptides	Precursor ion mass (Da)	Precursor ion charge	Mr (Expt) Da	Mr (Calc) Da	Difference (Delta)	Missed cleavages
7	**R.VPTADVSVVDLTCR.L**	766.3039	+2	1530.7712	1530.5933	-0.1780	0
	K.LISWYDNEFGYSCR.V	743.4039	+2	1484.7933	1484.8385	-0.0452	0
9	**R.SGETEDNFIADLVVGLR.T**	918.0039	+2	1833.9933	1833.9109	0.0824	0
	K.IQIVGDDLTVTNPLR.V	827.4039	+2	1652.7933	1652.9097	-0.1165	0
	K.LGANAILGVSLAVCK.A	743.4039	+2	1484.7933	1484.8385	-0.0452	0
11	K.SYELPDGQVITIGNER.F	895.9039	+2	1789.7933	1789.8846	-0.0914	0
14	**R.AYGVLDEEQGNTYR.G**	807.9039	+2	1613.7933	1613.7321	0.0611	0
	K.TIVPTPDGSKAYFSSAN	877.9039	+2	1753.7933	1753.8523	-0.0590	1
17	**K.SGISSLVQQAVDVER.K**	794.4039	+2	1586.7933	1586.8264	-0.0331	0
	K.QSQSPIMYDQESILR.L	897.9039	+2	1793.7933	1793.8618	-0.0685	0
22	**K.SLMSLCGEAEFER.A**	764.8039	+2	1527.5933	1527.6697	-0.0765	0
23	**R.ISMIEGAAMDLR.V**	653.8039	+2	1298.5933	1305.6421	-0.0488	
	K.FEEVKEEYVK.E	650.3039	+2	1298.5933	1298.6394	-0.0461	1
28	R.GPGTAMPFALK.L	545.3039	+2	1088.5933	1088.5688	0.0244	0
	K.LLEILVDKR.T	549.8039	+2	1097.5933	1097.6808	-0.0875	1
29	K.LAPALAMGNTVVMK.V	724.4039	+2	1446.7933	1446.7574	0.0358	0
30	R.LEKLDGGYESGFNK.V	778.9039	+2	1555.7933	1555.7518	0.0415	1
32	K.QSQSPIMYDQESILR.L	897.9039	+2	1793.7933	1793.8618	-0.0685	0
	R.SQGQCYFMGENDEMIDPK.H	1074.9039	+2	2147.7933	2147.8598	-0.0665	0
33	K.SIYGEKFEDENFIHK.H	928.4039	+2	1854.7933	1854.8788	-0.0855	1
34	R.ILEFFGMTSSDVPGYR.M	909.9039	+2	1817.7933	1817.8658	-0.0726	0
	K.QLAPIWDELGEAYK.T	816.9039		1631.7933	1631.8195	-0.0262	0
36	K.WEDPEGVPISAILFGGR.R	921.9039	+2	1841.7933	1841.9312	-0.1379	0
38	R.AYGVLDEEQGNTYR.G	807.9039	+2	1613.7933	1613.7321	0.0611	0
46	R.VPTADVSVVDLTCR.L	766.9039	+2	1531.7933	1530.7712	1.0220	0
47	R.FATSLMEAMTGR.A	665.8039	+2	1329.5933	1329.6057	-0.0124	0
	K.NGVYDPHR.L	479.7039	+2	957.3933	956.4464	0.9468	0
48	K.GRPMYAIPFSMGPVGSPLGK.V	1031.6039	+2	2061.1933	2061.0540	0.1393	0

Table S2. Statistical analysis of protein spots following matching of average gels from days 0 and 1 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Normalised vols day 0	Normalised vols day 1	F	P
32	119.4912826	26.46758102	1.68	0.264
	56.20691093	29.71793231		
	224.1970044	119.2304229		
41	69.39988978	164.6301856	43.31	0.003
	15.75986182	160.2747754		
	64.35535662	163.1757772		
78	19.56395666	587.9832652	9.79	0.035
	56.69634763	322.0935871		
	11.45602884	218.6057168		
106	543.9163141	210.8980815	1.52	0.285
	150.9422791	114.6399262		
	220.223255	127.2521939		
127	164.877959	298.6349072	1.8	0.251
	62.1193063	159.9507184		
	42.50942682	95.8128304		
154	358.2822531	60.37172805	0.7	0.449
	31.04986441	49.25666267		
	20.85733847	20.12916458		
155	144.7179297	23.11563131	5.51	0.079
	52.52634692	45.27266789		
	95.25368145	31.09059083		
156	54.83868591	74.75031012	5.32	0.082
	40.01634481	179.1272674		
	13.41382734	92.59913952		
167	46.40851525	158.4757861	0	>1
	100.2953691	144.2816098		
	30.89832483	69.85418004		
173	14.34832077	55.66434513	15.25	0.017
	31.53930112	51.83005642		
	29.27005677	43.5218447		
192	372.3325375	54.63861188	3.18	0.149
	254.7224375	92.07030986		
	75.09417215	88.51351701		
199	897.0893728	259.3639773	4.15	0.111
	349.6144254	96.41648598		
	374.6179584	133.8788743		
215	1833.029911	438.3361116	0.68	0.456
	333.1693522	168.4905733		
	138.7323155	359.9582908		

Table S3. Statistical analysis of protein spots Spot following matching of average gels from days 1 and 2 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Normalised vols day 1	Normalised vols day 2	F	P
9	34.85661361	88.5265982	3.72	0.126
	100.6292269	106.1758702		
	41.15517312	170.8679067		
68	643.830777	902.3045741	5.15	0.086
	908.4842438	1995.642327		
	653.9736378	1546.373126		
73	164.6301856	25.38243357	37.14	0.004
	160.2747754	38.68202041		
	163.1757772	85.95197184		
105	20.07506491	81.68386841	4.3	0.107
	107.7394185	166.0246009		
	36.34709297	147.5473012		
156	416.2095802	277.998082	1.51	0.286
	1136.772863	331.2124226		
	317.8813614	306.8428706		
264	504.0929392	12.16485296	0.98	0.378
	14.81131072	28.65968769		
	27.90181229	27.1910832		
298	230.2038465	58.28992042	4.84	0.093
	182.9968892	85.12326616		
	93.29668483	90.13521541		
306	12.2172156	73.92487566	2.76	0.172
	50.47664193	29.55352002		
	24.46391041	87.06620027		
308	5.82469949	20.15778519	10.44	0.032
	17.17502054	23.58195935		
	7.598261381	22.38230788		
352	2236.867771	443.8221834	4.65	0.097
	2253.111074	338.1348687		
	726.9667717	943.9860523		

Table S4. Statistical analysis of protein spots following matching of average gels from days 2 and 3 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Normalised vols day 2	Normalised vols day 3	F	P
1	88.5265982	33.75150063	5.24	0.084
	106.1758702	21.29905487		
	170.8679067	88.20462776		
15	36.86496305	14.58680761	9.26	0.038
	29.40137835	16.65164626		
	52.91607636	21.7170485		
23	53.65012073	24.47087838	0	1
	65.99145075	26.90604988		
	168.1116575	43.64291478		
27	97.41629844	13.65042195	38.33	0.03
	152.027567	12.154761		
	132.3977042	33.36908029		
34	19.6119264	6.658742417	3.76	0.124
	48.47614063	11.08228208		
	41.91062716	28.42009905		
102	155.3163197	119.1498721	2.9	0.164
	127.114368	34.90260317		
	313.7823634	114.3903766		
103	30.54859709	7.719979489	4.45	0.102
	32.17796388	20.75340771		
	58.07671329	26.12310353		
188	25.38243357	85.41918007	1.35	0.309
	38.68202041	81.71536688		
	85.95197184	682.6670669		
192	23.10152364	46.15340838	1.38	0.305
	53.66797522	67.34038638		
	62.84639287	191.089145		
232	3055.074153	1620.36335	30.54	0.005
	3165.744918	484.7980988		
	3054.569268	1390.601085		
234	378.4750886	196.349667	18.24	0.013
	391.7077554	51.25320271		
	383.6268927	222.3491658		
278	853.6646573	950.0985	4.95	0.09
	2048.435488	189.2831201		
	1637.739857	635.0566145		
283	4653.30969	856.2726577	10.24	0.003
	3145.148739	985.4199653		
	2346.486876	1521.65512		

Spot	Normalised vols day 2	Normalised vols day 3	F	P
284	3205.2633	812.7411291	19.91	0.011
	3964.754951	1046.60771		
	2229.629727	584.4809496		
287	788.2590777	258.0262687	5.98	0.071
	802.7945564	177.9562376		
	856.040198	732.5953967		
293	51.01830158	9.634367934	5.06	0.088
	28.75477624	23.59453605		
	51.23495979	31.51060211		
296	32.08869867	23.57610987	3.52	0.134
	154.5379046	29.333239		
	106.2817537	38.06748021		
298	171.7505682	110.0149099	11.56	0.027
	305.7667277	53.7556535		
	214.8897035	91.81717525		
300	220.1565456	39.39062311	4.89	0.092
	240.9353572	188.6810267		
	360.8340796	168.6412343		
306	38.50253941	55.18432778	3.66	0.128
	51.15763762	240.1600145		
	39.81900538	141.7872686		
307	970.0495485	320.2855102	2.05	0.226
	208.8524819	137.653609		
	837.4501764	469.2761836		
309	50.16052349	9.155770823	15.72	0.017
	31.68350345	19.11646621		
	41.67605275	19.75416143		
311	132.1563112	20.53805864	12.45	0.024
	117.8337259	72.571073		
	158.767777	80.14426168		
319	25.96728227	60.88587597	1.53	0.284
	66.86626538	61.92154557		
	45.40969538	236.4861291		
341	52.34395864	17.04221887	35.47	0.004
	60.95175783	17.29137052		
	67.20556725	31.44795677		
345	351.6695232	235.5946301	2.16	0.216
	350.8957515	1193.53732		
	389.5303486	920.9072654		

Table S5. Statistical analysis of protein spots following matching of average gels from days 3 and 4 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Normalised vols day 3	Normalised vols day 4	F	P
5	21.84899856	16.35599415	0.51	0.513
	42.50403265	25.53478566		
	65.44349039	264.5825899		
12	20.53805864	19.77685567	4.41	0.104
	13.79170249	61.89562085		
	17.89568324	71.77093714		
43	27.63378103	216.9040008	1.31	0.317
	32.28725086	58.85243407		
	24.36903423	17.20478399		
48	20.20512152	32.8367072	1.33	0.313
	36.7653297	105.8994022		
	94.44827921	172.2502491		
51	7.719979489	12.52534192	2.79	0.17
	20.75340771	73.61363892		
	26.12310353	92.9733033		
67	288.0738438	36.86334628	5.57	0.078
	342.5911582	83.93249066		
	112.9912975	109.6720642		
99	57.70216476	84.20308098	1.52	0.285
	113.9273651	385.5402843		
	138.0285487	180.4140878		
132	52.8745765	31.44698221	2.74	0.173
	19.71855063	172.3423136		
	53.4155866	268.7657139		
151	44.90489417	104.5500802	4.46	0.102
	27.33880453	103.4858402		
	31.92823765	38.47461989		
160	105.7075359	163.4886735	7.78	0.049
	57.87623037	457.9121631		
	93.88447122	392.0497984		
161	6.658742417	26.44040884	3.49	0.135
	11.08228208	32.63555482		
	28.42009905	67.09865757		
170	51.14746519	7.34059868	1.53	0.283
	77.36900497	262.7984173		
	108.9611145	386.7534237		
178	9.634367934	22.02179604	3.73	0.126
	23.59453605	110.2892865		
	31.51060211	93.41185662		

Spot	Normalised vols day 3	Normalised vols day 4	F	P
182	10.57075359	7.251513745	2.3	0.204
	26.86841904	79.89239786		
	18.68919078	116.8575917		
186	62.52975301	298.8086904	2.3	0.204
	44.21623582	121.9198567		
	120.9890183	106.5178538		
193	124.9970803	43.17055971	7.54	0.052
	175.5102331	62.49026654		
	162.7108095	123.0985427		
207	41.42986297	287.9937793	6	0.07
	80.26657958	140.9660085		
	156.1121679	268.8163162		
208	134.4441711	983.7293081	0.56	0.497
	112.2339773	108.7676932		
	342.7326126	153.9996842		
232	88.47803986	294.6573325	5.03	0.088
	212.5389786	687.8126819		
	164.6110513	1136.072369		
241	113.1153868	338.006062	61.02	0.001
	159.6864653	419.4875575		
	137.9241398	343.9607404		
243	172.7111314	250.5781064	8.61	0.043
	89.56139681	515.2604933		
	197.6251414	488.7676723		
244	33.33532922	168.7803187	5.46	0.08
	152.9693605	470.3297643		
	135.0215727	349.8474753		
267	172.7527486	31.98149182	0.75	0.435
	21.07326984	34.80426264		
	30.06975946	29.16379561		
279	15.21106471	13.13111948	2.05	0.226
	25.476078	92.78221775		
	38.27629798	86.52994302		

Table S6. Statistical analysis of protein spots following matching of average gels from days 4 and 5 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Normalised vols day 4	Normalised vols day 5	F	P
14	287.5305376	2855.42892	0.77	0.43
	783.9179197	451.8588692		
	759.9622968	610.2145202		
16	137.921297	643.6198541	1.76	0.256
	145.390872	279.9425129		
	207.1658402	155.4921837		
21	71.60647111	839.993556	1.25	0.326
	172.0100116	305.1019525		
	133.3202085	33.15291269		
26	31.98149182	25.80371173	2.78	0.171
	34.80426264	264.5824222		
	29.16379561	521.9072164		
27	36.86334628	292.2420375	6.22	0.067
	83.93249066	496.5392342		
	109.6720642	983.4810622		
30	36.97024821	365.725335	11.13	0.029
	47.04696809	228.4181582		
	28.86018178	150.5192468		
33	10.03096373	726.468135	1.64	0.27
	27.266254	101.3765983		
	31.47463424	64.99980154		
34	7.073343874	300.9160125	2.7	0.176
	22.03686981	96.06861759		
	38.30504554	51.46236219		
40	118.2869773	966.9300891	1.29	0.32
	241.3561932	305.6463608		
	162.0623182	127.6387138		
43	12.52534192	104.7059705	0.97	0.38
	73.61363892	29.04807016		
	92.9733033	249.149162		
45	18.72565343	51.18918148	1.14	0.345
	13.18714273	52.1465356		
	60.28421371	931.9987		
49	557.422258	1359.322804	3.02	0.157
	188.3102994	503.4998828		
	411.7340953	737.9034657		
53	32.8367072	572.3005043	0.66	0.464
	105.8994022	102.6792895		
	172.2502491	50.65865522		
54	50.79623019	252.4726805	2.35	0.2
	28.77035781	60.0404556		
	103.7009921	146.5760594		
56	46.66268918	476.3776153	0.68	0.455
	57.92548637	67.95381876		
	114.1081997	31.49526705		

Spot	Normalised vols day 4	Normalised vols day 5	F	P
59	42.44006324	774.4386717	4.78	0.094
	100.4776326	188.8902287		
	57.8384356	375.9841689		
77	408.5613309	5253.792094	2.81	0.169
	427.987493	1277.356804		
	1142.650669	1822.581375		
92	794.6732581	4754.883965	**6.16**	**0.068**
	270.791155	2312.529698		
	828.8151677	1779.306778		
95	2079.456197	647.6204295	1.53	0.283
	462.1446412	333.1584219		
	566.9819697	98.68014693		
96	75.16986852	416.350799	2.24	0.209
	150.3229334	83.52778409		
	181.5610734	453.4414285		
106	1551.18253	4811.164788	5.35	0.082
	2150.53615	2513.844102		
	1416.358541	2936.293199		
108	17.08649062	264.5107758	2.22	0.211
	41.83507349	71.58968832		
	74.13237808	84.71573825		
111	183.9425747	1421.986363	3.42	0.138
	425.9237227	341.96617		
	312.5535759	933.1289129		
120	64.58657819	907.4214369	5.33	0.082
	66.46040102	431.1907979		
	110.6503755	255.6541653		
121	212.0221463	3656.27139	1.54	0.282
	700.317731	484.6011384		
	474.1438059	962.539565		
124	32.08839375	668.0961021	20.29	0.011
	283.2087563	560.2933319		
	211.8549872	502.4926698		
129	176.2812702	496.3077549	3.12	0.152
	155.989557	145.2014645		
	185.2550418	522.6606917		
131	34.06607931	112.4889082	1.81	0.25
	544.992778	25.17888277		
	209.442944	36.29239305		
138	7.34059968	1569.716705	1	0.373
	262.7984173	174.4245231		
	386.7534237	294.9855754		
140	10.99308103	407.8768528	0.86	0.406
	38.49456386	54.16862348		
	195.8140562	124.3736543		

Table S6 continued. Statistical analysis of protein spots following matching of average gels from days 4 and 5 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Normalised vols day 4	Normalised vols day 5	F	P
151	30.03944023	218.5050801	10.91	0.03
	31.76107586	107.0928852		
	24.81199731	116.0351944		
153	19.17107811	206.5751693	1.26	0.324
	63.64457877	34.80324337		
	48.71315311	71.58015239		
157	25.44265756	132.9100275	2.47	0.191
	44.30110416	45.10811431		
	54.16133471	73.99127331		
162	534.0998219	6071.727932	2.09	0.222
	1042.064109	1749.825415		
	913.2366813	1297.735605		
163	3535.086225	6138.246591	2.74	0.173
	1150.167198	2067.565417		
	851.1307846	4571.485269		
165	106.9197395	260.7647824	8.11	0.047
	118.8067116	150.1983548		
	109.1829086	291.1428514		
166	415.9197466	4968.714724	1.16	0.343
	821.3631088	534.7255861		
	1028.20512	1237.156192		
169	775.3596441	804.5702787	2.58	0.183
	243.6823072	502.1388622		
	89.80222547	1246.449054		
173	2092.373513	7983.275625	2.61	0.182
	1715.552825	2508.205587		
	1717.459128	3317.752621		
184	536.4338472	4077.841122	0.64	0.467
	929.37875	780.0592871		
	696.4226681	217.8799375		
187	4749.723686	11517.58402	2.63	0.18
	1967.927454	3285.445617		
	1599.032865	6200.523959		
188	6900.073674	13546.98503	3.2	0.148
	1231.266377	4295.342413		
	2712.975159	10114.77785		
193	3917.795108	18731.45806	6	0.071
	1635.345615	7117.554916		
	945.3354106	8424.682546		
195	112.9240642	8026.263627	1.17	0.341
	743.7618458	1145.687774		
	708.3816797	329.1431218		
202	310.8173397	4765.376384	1.39	0.303
	641.8675573	591.6551373		
	613.299947	950.3332653		

Spot	Normalised vols day 4	Normalised vols day 5	F	P
219	338.006062	54.88062156	68.53	0.001
	419.4875575	84.03330606		
	343.9607404	130.2507615		
220	27.63414697	62.60900598	1.19	0.337
	65.06123469	109.8149266		
	107.0407443	678.4542654		
229	7.251513745	1310.243017	2.47	0.191
	79.89239786	289.1002378		
	116.8575917	248.1194124		
254	457.1126207	5902.030794	0.45	0.538
	910.1577025	1099.976922		
	2109.104108	186.5102497		
255	46.28853245	724.0859742	0.81	0.42
	92.78221775	97.07966153		
	130.7394909	41.59183592		
262	66.93842049	269.5660484	1.54	0.282
	4.354905225	32.45062189		
	11.50359087	66.28070953		
272	136.1039644	2823.187919	1.09	0.354
	271.8755089	447.9313524		
	769.1044468	458.3641337		
274	125.0217984	489.3067479	3.3	0.143
	167.4977002	364.8702039		
	79.88417352	1521.467534		
291	1042.008673	19870.20368	3.28	0.144
	877.8194705	6213.564974		
	2566.869434	4746.291536		
299	20.86369188	818.7723216	0.82	0.416
	108.2255162	228.5737034		
	417.0979398	156.7479758		
328	28.57844729	90.84943185	1.1	0.354
	106.2142146	8.691089266		
	211.2983618	63.59331433		
337	9.033212453	726.0862619	2.4	0.196
	454.921445	374.8834276		
	126.7756436	335.9495152		
347	2137.129784	25503.85041	1.31	0.317
	4401.759808	5271.835872		
	5604.087302	4934.333852		
350	92.18509119	1709.609555	3.44	0.137
	909.8953588	711.1138676		
	311.8282761	1023.822222		
356	232.6185834	1465.31987	2.13	0.218
	134.7572079	335.1416234		
	141.5346495	352.0738864		

Table S6 continued. Statistical analysis of protein spots following matching of average gels from days 4 and 5 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Normalised vols day 4	Normalised vols day 5	F	P
357	41.86991966	128.9821898	3.24	0.146
	69.39865033	58.36834447		
	64.7709515	167.8240626		
387	294.6573325	402.4760759	3.57	0.132
	687.8126819	62.60695176		
	1136.072369	161.3441751		
388	630.9885977	9293.245876	0.9	0.396
	1328.00124	476.1044807		
	2002.333243	2024.814142		
392	1343.347375	13092.33781	1.13	0.348
	1038.811047	1604.079543		
	3321.38355	2841.079038		
395	40.62273056	179.6440227	9.06	0.04
	33.70241915	75.63386408		
	34.91559104	197.3854097		

Table S7. Statistical analysis of protein spots following matching of average gels from days 5 and 6 using Progenesis PG v. 2006 imageanalysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant (p <0.05) differences in normalised spot volumes are shown in red.

Spot	normalised vols day 5	normalised vols day 6	F	P
3	62.60900598	32.45096609	1.24	0.328
	109.8149266	95.44866176		
	678.4542654	59.35821628		
13	476.3776153	58.91824218	0.72	0.444
	67.95381876	113.1730358		
	31.49526705	36.52650991		
18	2855.42892	446.9146057	0.92	0.391
	451.8588692	408.191575		
	610.2145202	795.6068919		
20	2113.994996	229.0933926	1.01	0.371
	291.7639497	46.27047709		
	148.886717	350.1354009		
23	5253.792004	769.7855797	2.36	0.199
	1277.356804	823.968474		
	1822.581375	1015.145776		
25	184.2992378	26.86453352	2.03	0.228
	54.82969067	42.14911766		
	99.68478065	89.02677373		
34	2076.298665	303.3805315	1.77	0.254
	818.9650357	332.8693065		
	317.3135598	476.8281597		
36	363.6886784	59.3403282	2.21	0.212
	98.26569385	73.7546348		
	147.3797664	107.3427822		
37	292.2420375	412.0056093	2.17	0.215
	496.5392342	68.31848585		
	983.4610622	282.885264		
38	1095.812172	138.3200705	2.68	0.177
	281.2257609	215.5243425		
	532.2549437	338.8883589		
44	4754.883965	1158.72543	4.07	0.114
	2312.529698	977.3437276		
	1779.306778	1151.819494		
45	647.6204295	553.6030535	3.62	0.13
	333.1584219	1009.960621		
	98.68014603	704.0690524		
46	3656.27139	517.6015991	1.22	0.332
	484.6011384	603.0838358		
	962.539565	708.8801699		
47	907.4214369	282.3010592	1.84	0.247
	431.1907979	100.5308289		
	255.6541653	360.4328803		
49	1421.986363	314.0568248	2.46	0.192
	341.96617	246.725309		
	933.1289129	587.9058883		

Spot	normalised vols day 5	normalised vols day 6	F	P
51	966.9390891	304.7212753	0.79	0.423
	305.6463608	115.7014771		
	127.6387138	279.3402301		
52	2823.187919	421.4404731	1.26	0.324
	447.9313524	327.1297446		
	458.3641337	313.4400799		
54	489.3067479	268.1239348	2.76	0.172
	364.8702039	57.47147237		
	1521.467534	196.4750629		
56	3433.803026	388.9895071	<0.001	1
	500.0195585	454.33563		
	45.93687674	1127.173068		
61	104.7059705	42.33274459	2.17	0.215
	29.04807016	20.15167773		
	249.149162	33.8466331		
62	324.8103586	99.98472872	2.93	0.162
	87.66917562	78.12883837		
	173.5002431	41.4431343		
68	1146.364899	165.9294617	1.45	0.295
	174.6383977	225.6633924		
	375.7330104	232.7061533		
70	25503.85041	4383.835024	1.32	0.315
	5271.835872	3232.005467		
	4934.333852	4649.417455		
75	1310.243017	71.77945139	2.31	0.203
	289.1002378	37.67377643		
	248.1194124	145.747316		
78	1569.716705	203.9420317	1.39	0.304
	174.4245231	99.72172768		
	294.9855754	154.7998132		
79	407.8768528	62.12113019	1.02	0.369
	54.16862348	79.8481785		
	124.3736543	113.0190567		
81	1667.548959	96.70735495	1.39	0.303
	281.6729535	10.34132521		
	99.40850637	179.91047		
84	286.3684653	37.91325571	0.44	0.545
	22.76793184	92.49038536		
	31.84688885	35.53474448		
85	1532.274955	50.25306455	1.08	0.358
	92.2383165	89.73438426		
	57.28923776	28.48687948		
86	157.2953534	18.19935589	0.67	0.46
	11.87976631	37.95190498		
	23.60889237	21.48121727		

Table S7 continued. Statistical analysis of protein spots following matching of average gels from days 5 and 6 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	normalised vols day 5	normalised vols day 6	F	P
89	18731.45806	4601.159666	3.46	0.136
	7117.554916	3043.130897		
	8424.682546	5682.710429		
90	6138.246591	1797.440887	4.28	0.107
	2067.565417	1928.239958		
	4571.485269	1679.269893		
95	534.6041727	37.73945559	1.08	0.357
	53.215909	187.0288082		
	233.2759493	136.926934		
97	1465.31987	108.6250778	<0.001	1
	335.1416234	297.7492559		
	352.0738864	273.57955		
98	1834.245665	180.0817575	2.31	0.203
	637.6576366	474.992996		
	451.2312343	250.178106		
107	253.7455909	39.75057132	1.46	0.293
	36.61145657	48.11623929		
	116.9895964	84.38446744		
108	206.5751693	41.73685845	0.97	0.381
	34.80324337	48.01510164		
	71.58015239	67.35564393		
110	11517.58402	2338.455846	3.24	0.146
	3285.445617	3075.74779		
	6200.523959	2528.643133		
111	4968.714724	1148.942965	0.7	0.45
	534.7255861	1107.179192		
	1237.156192	1029.494723		
112	13546.98503	2259.848532	4.2	0.11
	4295.342413	4829.398872		
	10114.77785	3650.435345		
130	1610.686234	241.2345727	0.46	0.533
	50.7466286	155.9289794		
	177.4685462	408.7972713		
137	2390.525683	35.23176808	0.89	0.399
	49.52170998	240.7581874		
	118.9486322	99.70407819		
144	19870.20368	1361.202575	2.91	0.163
	6213.564974	2777.720406		
	4746.291536	1945.759376		
145	202.6291471	26.98867646	1.64	0.27
	42.61939076	39.14027242		
	47.14243721	23.48584953		
155	252.4726805	26.41761891	3.37	0.14
	60.0404556	70.49294543		
	146.5760594	47.87905892		

Spot	normalised vols day 5	normalised vols day 6	F	P
158	9982.326815	1042.378661	1.18	0.338
	1198.417604	1228.89836		
	1482.010545	993.601255		
159	9293.245876	1792.922084	0.69	0.452
	476.1044807	1941.792404		
	2024.814142	1254.752085		
160	8483.820353	778.6742146	0.71	0.446
	683.6018065	2021.994564		
	1184.513385	1088.663027		
161	8026.263627	734.7276117	0.81	0.419
	1145.687774	999.6951493		
	329.1431218	1172.773176		
167	5902.030794	1130.222209	0.65	0.464
	1099.976922	931.5030855		
	186.5102497	817.7422523		
168	1838.08258	635.289112	3.71	0.126
	576.8588981	251.0236593		
	1015.031677	295.4616937		
170	389.4014679	82.1081445	1.74	0.258
	146.9902346	15.1200794		
	46.48942529	74.69892842		
174	839.993556	87.47111977	1.11	0.351
	305.1019525	101.4410678		
	33.15291269	226.2702287		
183	140.4201987	64.87710359	3.02	0.157
	141.3906066	22.37670613		
	33.73057707	29.96397694		
190	369.9804925	297.545813	1.53	0.283
	250.1750461	98.68506671		
	1524.807941	223.6958588		
200	365.725335	168.7599208	4.33	0.106
	228.4181582	21.59288931		
	150.5192468	79.34123471		
204	179.6440227	33.54342401	3.31	0.143
	75.63386408	124.6521596		
	197.3854097	21.16469639		
207	167.7332185	52.61178052	4.09	0.113
	42.24997086	13.17317954		
	190.5287846	45.53680439		
226	1042.131723	172.6580093	0.83	0.413
	160.5809983	223.0590978		
	204.8448151	225.6582883		
228	614.7066042	16.13858298	3.12	0.152
	59.86546723	55.42343485		
	283.4574035	30.70252566		

Table S7 continued. Statistical analysis of protein spots following matching of average gels from days 5 and 6 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	normalised vols day 5	normalised vols day 6	F	P
239	689.7355785	172.7821523	1.69	0.263
	164.489072	85.96700862		
	197.184483	122.1559595		
242	1221.448426	278.0553705	2.2	0.212
	408.8506154	442.0221205		
	517.1352062	265.0967903		
244	593.7581363	32.50062327	1.19	0.337
	32.31451982	120.4296625		
	201.8560297	122.4302776		
246	740.1064604	56.5843548	1.62	0.272
	61.40147629	212.869479		
	659.3913407	322.2393804		
249	264.5107758	54.44909612	2.28	0.206
	71.58968832	34.79135327		
	84.71573825	48.68091183		
250	95.95925778	23.41335962	3.33	0.142
	22.72904553	21.99743993		
	62.13659544	19.41328083		
255	239.6162857	41.19062949	2.31	0.203
	68.18713659	40.0252269		
	73.08710297	42.75142062		
256	154.4040284	57.32921247	0.97	0.379
	58.17391294	16.86470395		
	18.10852276	31.71539249		
261	4765.376384	295.3857258	1.4	0.302
	591.6551373	651.250644		
	950.3332653	605.5677536		
273	283.4771403	41.88582999	1.02	0.371
	31.26458957	61.16299677		
	79.0144419	56.23521024		
277	82.8846498	9.6583212	1.5	0.288
	17.187747	23.18580737		
	30.28970659	22.02985347		
291	1549.277401	163.5459171	2.15	0.217
	529.2815034	212.8189102		
	247.1901263	209.2414051		
298	70.33739039	546.8496772	0.73	0.44
	20.3958672	44.32357724		
	87.3780176	27.72722936		
309	627.6539211	181.9935589	8.94	0.04
	368.311642	236.8896721		
	372.4679509	139.8600276		
324	4077.841122	520.4817155	0.97	0.38
	780.0592871	185.7645875		
	217.8799375	775.3284539		

Spot	normalised vols day 5	normalised vols day 6	F	P
328	726.0862619	144.6761893	4.13	0.112
	374.8834276	196.2576192		
	335.9495152	293.7735823		
332	785.8403118	2001.060147	1.46	0.293
	577.2477611	793.8041685		
	295.5632398	562.7530288		
338	818.7723216	190.9070224	1.45	0.296
	228.5737034	103.590243		
	156.7479758	147.6253399		

Table S8. Statistical analysis of protein spots following matching of average gels from days 6 and 7 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	normalised vols day 6	normalised vols day 7	F	P
38	353.9315391	752.3383145	**6.47**	**0.064**
	264.3485454	379.5518596		
	216.4580813	580.4864313		
49	23.41335962	604.9809426	1.19	0.336
	21.99743993	34.18899904		
	19.41328083	44.09551691		
84	31.58196547	36.73664469	2.38	0.198
	34.74078444	206.1664691		
	13.44158684	578.3851514		
96	99.98472872	450.9335108	2.12	0.219
	78.12883837	138.75055		
	41.4431343	111.4932878		
111	141.4484727	36.03242785	**12.48**	**0.024**
	104.6269039	60.23552542		
	133.2552918	87.09335025		
114	66.36681894	38.02770888	**7.84**	**0.049**
	44.60170579	27.19632799		
	86.6845192	24.77628617		
139	165.9294617	433.885595	5.64	0.076
	225.6633924	554.8379425		
	232.7061533	261.217326		
143	154.0365674	493.3332325	3.47	0.136
	161.466266	628.988414		
	241.3577241	185.1948985		
144	144.6761893	472.6175207	**8.22**	**0.046**
	196.2576192	848.154681		
	293.7735823	464.8846708		
146	71.77945139	208.7122633	<0.001	1
	37.67377643	467.5234158		
	145.747316	250.0836784		
148	62.12113019	134.1826487	3.51	0.134
	79.8481785	262.8587284		
	113.0190567	123.2855455		
172	53.20766666	67.54613104	0.89	0.4
	74.63958928	473.1785625		
	46.69738096	33.5263923		
173	52.51246617	21.80137939	0.85	0.409
	20.70793483	172.1417275		
	54.63150443	61.78390349		
174	67.63307699	251.1413274	3.63	0.129
	256.3586706	216.538149		
	56.3407172	331.0300007		
176	138.3945562	44.74711114	0.69	0.453
	27.53472653	579.3826872		
	87.12764843	70.09493619		

Spot	normalised vols day 6	normalised vols day 7	F	P
184	116.9426552	67.07665316	0.4	0.563
	87.50935585	489.7685573		
	139.2480872	57.54998117		
187	295.3857258	788.8402202	2.19	0.213
	651.250644	1998.261345		
	605.5677536	693.265577		
189	41.19062949	73.26789279	1.52	0.285
	40.0252269	172.0478661		
	42.75142062	32.20917202		
191	54.44909612	76.08476012	2.23	0.21
	34.79135327	193.6359781		
	48.68091183	59.33763726		
199	122.9015166	190.8134188	3.03	0.157
	19.59542063	456.6589638		
	152.2254434	171.3327232		
201	304.7212753	172.7091778	1.64	0.269
	115.7014771	498.2160793		
	279.3402301	1116.030561		
212	1792.922084	1034.377155	<0.001	1
	1941.792404	9595.868832		
	1254.752085	1491.156079		
217	403.861832	1437.746688	1.06	0.362
	50.46768977	574.1264512		
	301.0113599	39.98704414		
218	229.0933926	212.5267711	1.97	0.233
	46.27047709	1181.198239		
	350.1354009	498.9128613		
234	26.41761891	190.6373646	6.92	0.058
	70.49294543	608.9490144		
	47.87905892	323.8480139		
251	168.7599208	130.1627443	1.46	0.293
	21.59288931	179.7679627		
	79.34123471	126.9235825		
307	203.9420317	23.73797568	0.7	0.45
	99.72172768	793.4100449		
	154.7998132	224.8996812		
311	58.32235604	71.83011676	1.99	0.231
	55.19587513	207.3632014		
	53.68194178	76.36741371		
323	308.1972778	35.94440075	0.9	0.397
	25.89123964	43.71916826		
	114.3062416	119.72016		
353	297.545813	559.001452	<0.001	1
	98.68506671	878.2841764		
	223.6958588	79.03321665		

Table S9. Statistical analysis of protein spots following matching of average gels from days 7 and 8 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Day 7	Day 8	F	P
13	646.1189425	137.6329289	7.85	0.049
	1181.258239	238.0872121		
	498.9128613	191.2420569		
22	20.48097284	10.97527194	2.3	0.204
	83.93978152	28.86106346		
	102.3981954	45.38261842		
23	753.89346	304.5260621	0.01	0.938
	964.6856245	1082.234675		
	614.4205347	1013.384213		
49	381.9789461	97.20339079	10.85	0.03
	475.3145862	258.7779668		
	462.5638541	295.7854864		
104	124.9984875	1290.446169	0.94	0.386
	370.090661	245.0871793		
	166.8165394	191.7619887		
109	30.54540505	214.0070216	1.12	0.349
	31.91448221	76.40342736		
	84.61572163	36.56234684		
113	604.9809426	3.579361773	1.19	0.336
	34.19073572	39.83577542		
	44.09551691	21.00153086		
114	124.7050638	9.681526722	<0.001	>1
	80.65446718	35.92727639		
	87.37561174	29.41328461		
116	13.76157057	139.2069856	1.22	0.332
	32.38381283	27.16075597		
	18.72334537	24.73389842		
123	11.2674693	90.62685259	10.81	0.03
	35.90379249	117.2108071		
	60.12169695	78.50970159		
124	71.83011676	31.02832284	3.7	0.127
	207.3737348	36.58973385		
	76.36741371	31.34445986		
130	21.68400992	109.7958443	2.34	0.201
	57.00020389	56.4192939		
	15.30484513	40.68466324		
149	276.7572146	51.03824889	3.56	0.132
	675.1086314	305.5695456		
	466.2332534	269.4175166		
171	180.4849053	83.14469274	7.09	0.056
	192.3316884	123.9016274		
	329.1482574	102.4265643		
186	190.8134188	67.10225202	2.07	0.223
	456.6821605	213.3996306		
	171.3327232	96.44734864		

Spot	Day 7	Day 8	F	P
201	10.12311695	237.2297483	1.12	0.349
	59.67538843	43.05973507		
	32.2718968	36.26524295		
202	172.7091778	66.84350298	9.89	0.035
	392.2430663	73.66526985		
	434.2122558	90.8580818		
247	11.20878456	211.6998427	60.17	0.001
	37.66378232	308.086884		
	23.14544202	240.4684568		
261	24.61824672	69.90536667	1.45	0.295
	284.5082223	78.0374891		
	183.5013296	71.10067346		
301	182.7149253	59.72790428	2.93	0.162
	39.56457133	51.42878102		
	169.9527782	45.25263547		
311	27.87524956	380.9648421	1.27	0.323
	26.42331395	74.28356348		
	73.95250986	41.66881986		
323	391.3098191	167.2596944	48.34	0.002
	392.008401	247.8695006		
	380.3943987	161.3831165		
327	493.3332325	45.23795782	5.91	0.072
	629.0203645	196.1536547		
	185.1948985	36.43236389		
330	139.1121665	1311.081405	<0.001	>1
	141.3154499	70.77253893		
	53.31605885	27.14786748		
336	53.10968601	253.8759368	1.06	0.362
	17.43563256	82.1888892		
	68.5581792	22.2085154		
334	78.93096981	630.4204828	8.08	0.047
	577.4174627	835.7784168		
	216.1182127	1065.06172		
340	1034.377155	821.1400905	0.55	0.5
	9596.35627	2737.737956		
	1491.156079	2253.830071		
351	202.9611592	52.91417946	9.24	0.038
	464.8015803	41.02819885		
	218.7840156	11.53134453		
362	174.6457741	77.10721505	3.82	0.122
	181.1146866	39.83577542		
	57.01682058	50.65621238		

Table S10. Statistical analysis of protein spots following matching of average gels from days 8 and 9 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Day 8	Day 9	F	P
10	14.94275728	57.98914981	2.35	0.2
	105.9048664	227.9924045		
	36.98943367	130.8903727		
12	45.71233107	83.74781872	8.04	0.047
	66.576975	163.6121024		
	37.06370964	196.6132615		
13	137.6329289	1069.306207	6.04	0.07
	238.0872121	415.1038694		
	191.2420569	560.4562313		
14	20.98023497	49.93152958	7.54	0.052
	26.78536341	44.81222405		
	11.77274144	28.66946132		
21	10.04808787	21.7298588	7.45	0.052
	8.413209776	34.82941218		
	6.22061259	59.66633278		
29	68.2450603	500.5796565	4.01	0.116
	103.3212823	344.4070093		
	68.83525633	99.6554707		
34	33.29237697	72.4542925	1.41	0.301
	97.71247574	163.6341882		
	115.5919802	615.599983		
49	97.20339079	417.4104435	13.25	0.022
	258.7779668	517.5602062		
	295.7854864	455.0880267		
53	15.56806747	415.8889247	2.03	0.227
	127.4347339	146.6943283		
	151.7458092	152.3924687		
64	30.59707443	166.0169784	7.36	0.053
	102.084695	351.1211128		
	154.5311581	320.2569254		
69	36.20330371	327.1693829	8.03	0.049
	214.0400062	406.5124671		
	162.515825	257.840017		
82	4333.981796	3966.813582	3.63	0.129
	3223.805078	616.1073445		
	5281.46721	1653.360429		
119	99.98494301	1007.759704	11.8	0.251
	187.5417077	222.4046802		
	175.662672	264.3197384		
142	90.13091692	929.7765125	16.59	0.015
	339.5756954	784.180792		
	100.0683022	536.5209338		
147	56.38572913	475.6567514	0.72	0.444
	20.11662495	18.3754413		
	39.06916087	12.61562089		

Spot	Day 8	Day 9	F	P
166	66.13194311	213.6126553	41.17	0.003
	81.94598813	191.8157545		
	42.22588964	159.6391776		
178	105.0521118	88.24808533	1.84	0.247
	76.86714758	337.4057894		
	40.35042137	122.612198		
181	589.7537581	2141.719742	5.76	0.074
	961.4907613	3435.390347		
	1235.246539	1507.844399		
182	922.1384673	2218.138555	8.85	0.041
	1706.269605	3659.91527		
	1326.383156	2501.727874		
186	67.10225202	502.0368857	1.67	0.266
	213.3996306	103.6489736		
	96.44734864	253.2116445		
202	66.84350298	345.491897	18.77	0.012
	73.66526985	589.8693346		
	90.8580818	717.4241768		
207	20.11773816	177.803391	3.91	0.119
	78.43496358	117.3863829		
	34.12980878	61.88795155		
212	12.57089104	70.63275601	4.18	0.11
	59.28994291	134.1053841		
	28.74480086	60.35397668		
228	1121.828042	366.7717291	173.11	<0.001
	1095.837135	460.2032097		
	1040.884892	291.931286		
232	1054.294541	4401.967934	10.18	0.033
	1394.693778	4488.091109		
	2284.07896	2627.196439		
233	406.9044338	3933.040153	12.02	0.026
	1765.625793	3967.01925		
	2187.130249	2980.11645		
240	25.93959164	41.76676015	2.29	0.205
	27.86737726	123.8575419		
	24.0097077	38.74608933		
248	40.66672472	106.8491873	7.01	0.057
	91.30872027	221.0574423		
	83.09624281	180.2949426		
254	77.49533862	198.0760206	10.05	0.034
	85.52325843	259.4868508		
	103.8935148	141.5753011		
256	44.54796037	57.17481585	5.43	0.08
	30.47304328	89.46984702		
	16.02504079	156.1745102		

Table S10 continued. Statistical analysis of protein spots following matching of average gels from days 8 and 9 using Progenesis PG v. 2006 image analysis software (Non-Linear Dynamics, UK). Spots showing a 2 fold change in protein expression were Normalised spot volumes from all replicate gels are shown, spot numbers are arbitrary and do not refer to those mentioned in the main text. Normalised volumes were analysed by one-way ANOVA, resultant F and p statistics are shown. Significant ($p < 0.05$) differences in normalised spot volumes are shown in red.

Spot	Day 8	Day 9	F	P
257	34.65080945	156.1378217	5.91	0.072
	38.93041689	57.79871381		
	51.58466202	127.3992575		
265	454.0830094	691.1766625	7.65	0.051
	257.4530519	812.2519289		
	251.3684556	466.1961205		
267	97.31120289	381.7726178	6.25	0.067
	164.8414986	523.4129609		
	89.81821821	191.0592145		
278	6.943099342	39.47376716	5.62	0.077
	35.26481893	125.8010982		
	34.11123979	122.6386459		
280	1.401557321	43.67401601	3.56	0.132
	34.84526254	187.7077832		
	57.61958468	115.4448327		
281	3.40686241	126.9932379	5.08	0.087
	103.2992003	224.591181		
	97.20867734	148.7691143		
336	253.8759368	888.6097879	0.69	0.453
	82.1888892	152.9446286		
	22.2085154	14.25538713		
337	208.9829777	251.9720708	2.29	0.205
	130.5703659	362.2523897		
	191.3534709	1039.42666		