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Identification of the major proteins of an immune modulating fraction from adult Fasciola hepatica released by Nonidet P40

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Supplementary Table 1. Identification of tegumental associated proteins from FhTeg preparations by MSMS – Full Data. Peptide sequences were used to search against Genbank or a translated EST library for the identification of each protein. Sequences derived from MSMS analysis were interpreted either, automated or manually (where manually interpreted using Masslynx version 3.5 sequences are denoted by a *). Sequenced amino acids that match exactly with those found in the Genbank database or translated EST database are underlined>. A) Sequence coverage was calculated on the EST if available otherwise the NCBI sequence was used. B) Results from sequence analysis looking for signal peptides (SP), exportation to mitochondria (EM) and trans-membrane domains (TM). If TM was denoted the number of domains is reported in parentheses. C) The accession number reported here relates to the top hit, where many sequences matched 100 %. Data in red type did not score significantly and consequently were not used in identification but were used to give confidence in the overall 'hit'.

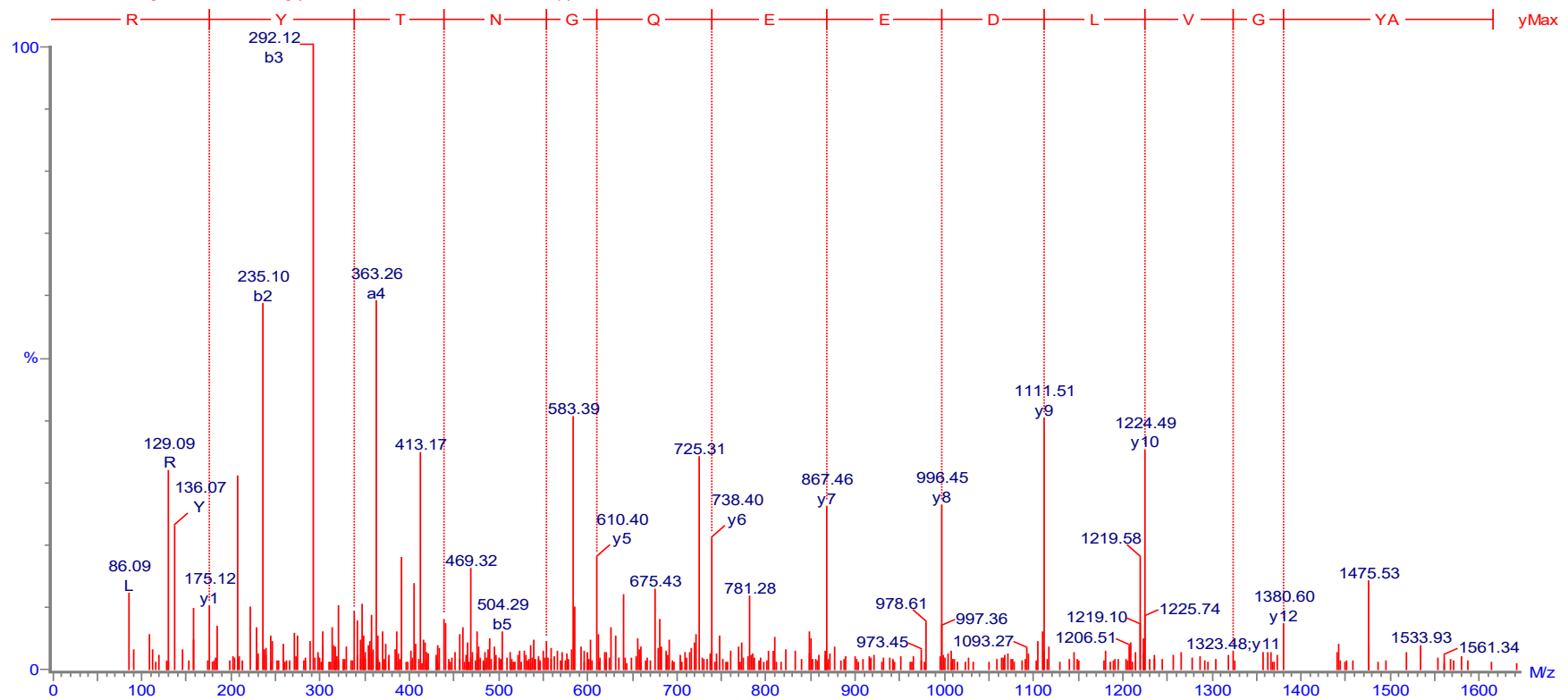
Precursor

Spot Number	Sequence	Mass	Charge	BLAST Score	EST qtk	EST BLAST Score	Accession Number	Identification	Species	Unique Peptides	Sequence Coverage [%JA]	MOTIF ^(B)	% Contribution
1	-	-	-	-	-	-	-	-	Unidentified	-	-	-	-
2	1- <u>TFWEEFEETDP*</u>	670.81	4	13	Fhep46d02.qtk	-	Q7M4G0	FABP Type I	<i>Fasciola hepatica</i>	1	4.6	-	3.580
	1- <u>NEVGEVYSDP*</u>	960.41	2	0.001	Fhep15h04.qtk	1.00E-32	XP_001149111	Heat Responsive Protein 12	<i>Pan troglodytes</i>	2	14.8	EM, TM ⁽¹⁾	-
	2- <u>FQVACLPL*</u>	552.79	2	0.79	Fhep15h04.qtk	1.00E-32	XP_001149111						
3	1- <u>TLTETGEVYFEETDPLK</u>	1038.95	2	0.025	Fhep46d02.qtk	2.00E-47	Q9UAS2	FABP Type I	<i>F. hepatica</i>	2	13.8	-	2.570
	2- <u>CVGIDWK*</u>	932.48	2	29	Fhep46d02.qtk	2.00E-47	Q9UAS2						
	3- <u>TTTTTGEFFFEETDPL*</u>	894.08	3	4.00E-07	-	-	Q7M4G0						
4	1- <u>TTTTTGEFFFEETDPL*</u>	1093.01	2	2.00E-09	-	-	Q7M4G1	FABP Type II	<i>F. hepatica</i>	4	49.2	-	7.012
	2- <u>KMMITIVGIDWK*</u>	911.01	2	0.006	-	-	-						
	3- <u>WIKLHSENMDGQVWK</u>	798.37	3	2.00E-06	-	-	-						
	4- <u>FEVGSWIKLHSENMDGQVWK*</u>	793.05	3	4.00E-11	-	-	-						
	5- <u>PKDFTFELGKHMILK*</u>	724.39	3	2.00E-07	-	-	-						
5	1- <u>PEVEFAKVDVQNEFAAAK*</u>	777.72	3	2.00E-09	-	-	AAF14217	Thioredoxin	<i>F. hepatica</i>	3	41.3	-	1.407
	2- <u>YSVTAMPTEVPIK</u>	760.41	2	2.00E-04	-	-	-						
	3- <u>MPTVWIDGK</u>	607.32	3	2.00E-02	Fhep41b07.qtk	1.80E-42	-						
6	1- <u>FVQSESTSDVQK</u>	746.35	2	8.00E-04	-	-	AAD80361	Cu/Zn Superoxide Dismutase	<i>F. hepatica</i>	3	26.0	-	0.657
	2- <u>AMVWHENRDLGR</u>	750.8	2	2.00E-04	-	-	-						
	3- <u>SMGSSGYDGLVQ*</u>	753.35	2	4.80E-02	-	-	-						
7	1- <u>NKLSBLCGEGSPWIK</u>	755.43	2	5.00E-03	Fhep33a10.qtk	7.00E-40	NP_082902	Methylmalonyl-CoA Epimerase, Mitochondrial Precursor	<i>Mus. Musculus</i>	3	19.5	SP, EM	0.599
	2- <u>DSGMLTELFAGASAPQ</u>	808.39	2	0.75	Fhep33a10.qtk	7.00E-40	-						
	3- <u>SGVTELEFTHSK</u>	715.35	2	2.2	Fhep33a10.qtk	7.00E-40	-						
	4- <u>FHGVVAVVHVEGNTK</u>	821.91	2	6.00E-05	Fhep33a10.qtk	7.00E-40	-						
	5- <u>DSGMLTELET*</u>	539.26	3	0.15	Fhep33a10.qtk	7.00E-40	-						
8	1- <u>DDFTGAADPK</u>	654.36	2	1.00E-02	Fhep22d10.qtk	5.00E-36	AA11353	Myoglobin 2	<i>Paragonimus westermani</i>	3	19.1	-	4.186
	2- <u>LQGLTFPWGQSEGLR</u>	572.31	3	6.40E-02	Fhep22d10.qtk	5.00E-36	-						
	3- <u>LLKRTNDSVLEER</u>	509.62	3	2.8	Fhep22d10.qtk	5.00E-36	-						
9	1- <u>JIEELVITLPIK</u>	669.37	2	0.49	Fhep25c09.qtk	3.00E-66	AAT73777	TRIM5/ Cyclophilin A Fusion Protein	<i>Aotus trivirgatus</i>	5	37.2	-	6.171
	2- <u>HVVFGVITPQVYDVK</u>	823.42	2	5.00E-03	Fhep25c09.qtk	3.00E-66	-						
	3- <u>MEAGSASGQITRK</u>	641.8	2	1.40E-02	Fhep25c09.qtk	5.10E-68	XP_721313	Cyclophilin Type Peptidyl-Prolyl Gs-Trans Isomerase	<i>Candida albicans</i>				
	4- <u>HVVFGVTEGSDVQK</u>	831.42	2	1.00E-04	-	-	ATT91286	Putative Cyclophilin	<i>Paxillus involutus</i>				
	5- <u>IRDQVESGIGDIEGATKTRGK</u>	1063	2	4.60E-02	-	-	POC12	Cyclophilin E	<i>Rhizopus oryzae</i>				
	6- <u>FERTADGSLG*</u>	734.33	2	1.30E-02	Fhep25c09.qtk	5.10E-68	XP_721313	Cyclophilin Type Peptidyl-Prolyl Gs-Trans Isomerase	<i>Candida albicans</i>				
10	1- <u>IAIVYDQKAW*</u>	692.34	3	4.70E-02	-	-	Q9U1G6	FABP Type III	<i>F. hepatica</i>	1	9.1	-	4.863
11	1- <u>GRGTAMPFAK</u>	553.29	2	2.00E-02	Fhep05f07.qtk	5.00E-50	AAW26651	SiCHG C05973 Protein (GAtase1_D1)	<i>Schistosoma japonicum</i>	5	34.3	TM ⁽¹⁾	0.305
	2- <u>ITSYGEEFK</u>	578.77	2	5.20E-02	Fhep05f07.qtk	5.00E-50	-						
	3- <u>KTSYVGFEEK</u>	642.83	2	1.50E-02	Fhep05f07.qtk	5.00E-50	-						
	4- <u>WTVYCSFGVWVGNLVTSR</u>	1058.99	2	1.20E-02	Fhep05f07.qtk	5.00E-50	-						
	5- <u>IAVACADIGLQPLAAALGR</u>	682.72	3	2.00E-03	Fhep05f07.qtk	5.00E-50	-						
12	1- <u>JEIGLEGLVQK*</u>	622.87	2	1.60E-02	Fhep29e10.qtk	9.00E-63	XP_001174161	Peptidylprolyl Isomerase B	<i>P. Troglodytes</i>	2	13.9	-	0.324
	2- <u>FEMLDGGLDFTDGDITL*</u>	917.4	2	3.00E-04	-	-	O93826	Peptidyl-Prolyl Cis-Trans Isomerase B	<i>Arthroderma benhamiae</i>				
13	1- <u>AVPKDIDR</u>	550.27	2	1.00E-01	Fhep36f01.qtk	0	AAR99518 ^(C)	Cathepsin L1 Protease	<i>F. hepatica</i>	2	7.4	SP	0.985
	2- <u>MCGIASIASLPMVAF*</u>	890.42	2	6.00E-06	-	-	-						
14	1- <u>ISMIFGAAMDRL*</u>	669.83	2	5.70E-03	-	-	ADP09370 ^(C)	Mu Class GST (GST 7, 26, 27, 28 or 47)	<i>F. hepatica</i>	1	5.5	-	3.681
15	1- <u>ISMIFGAAMDRL</u>	669.81	2	5.70E-03	-	-	ADP09370	Mu Class GST (GST 51)	<i>F. hepatica</i>	3	16.0	-	1.884
	2- <u>HGMLGSTPEER</u>	615.28	2	3.60E-02	-	-	-						
	3- <u>WAPDCLPEEIK</u>	740.85	2	1.00E-03	-	-	-						
16	1- <u>AVGLDFEAGNLTYP*</u>	807.88	3	4.00E-05	-	-	CAA06158	Thiol-Specific Antioxidant Protein (TPX)	<i>F. hepatica</i>	1	7.2	TM ⁽¹⁾	1.604

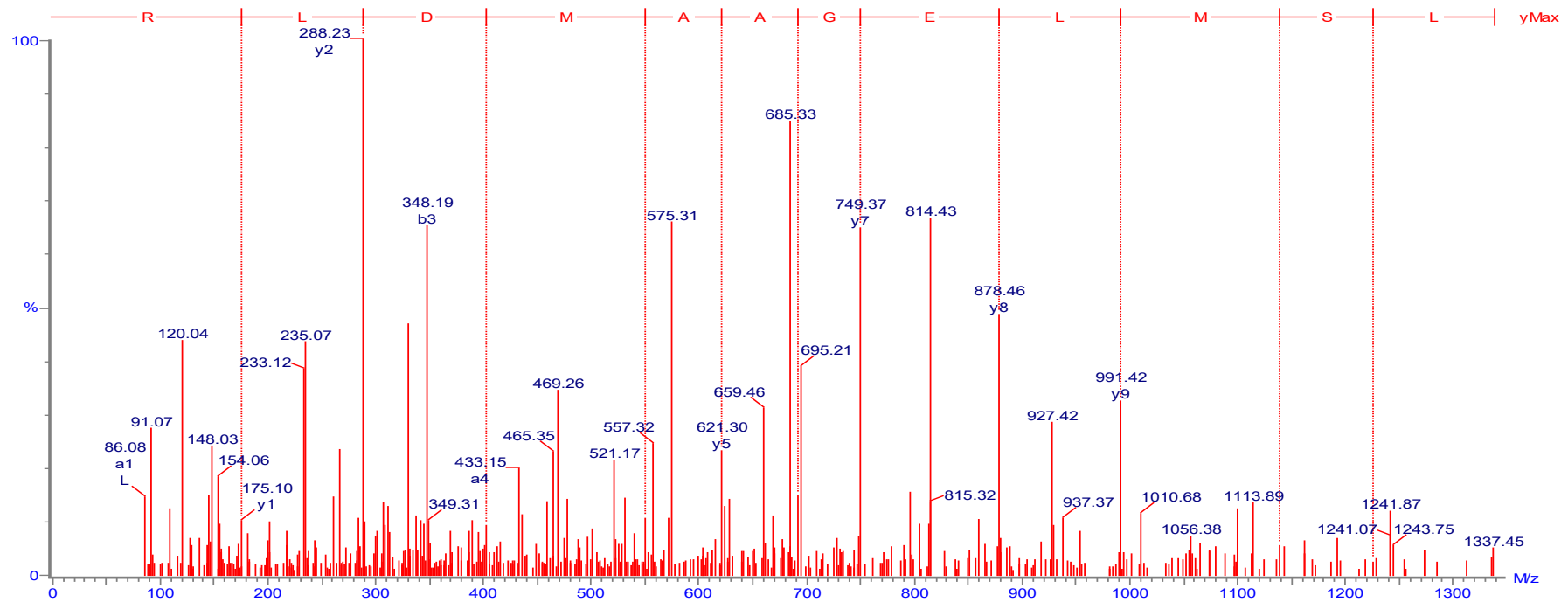
Supplementary Table 1 Cont. Identification of tegumental associated proteins from FhTeg preparations by MSMS – Full Data. Peptide sequences were used to search against Genbank or a translated EST library for the identification of each protein. Sequences derived from MSMS analysis were interpreted either, automated or manually (where manually interpreted using Masslynx version 3.5 sequences are denoted by a *). Sequenced amino acids that match exactly with those found in the Genbank database or translated EST database are underlined. A) Sequence coverage was calculated on the EST if available otherwise the NCBI sequence was used. B) Results from sequence analysis looking for signal peptides (SP), exportation to mitochondria (EM) and trans-membrane domains (TM). If TM was denoted the number of domains is reported in parentheses. C) The accession number reported here relates to the top hit, where many sequences matched 100 %. Data in red type did not score significantly and consequently were not used in identification but were used to give confidence in the overall 'hit'.

Precursor

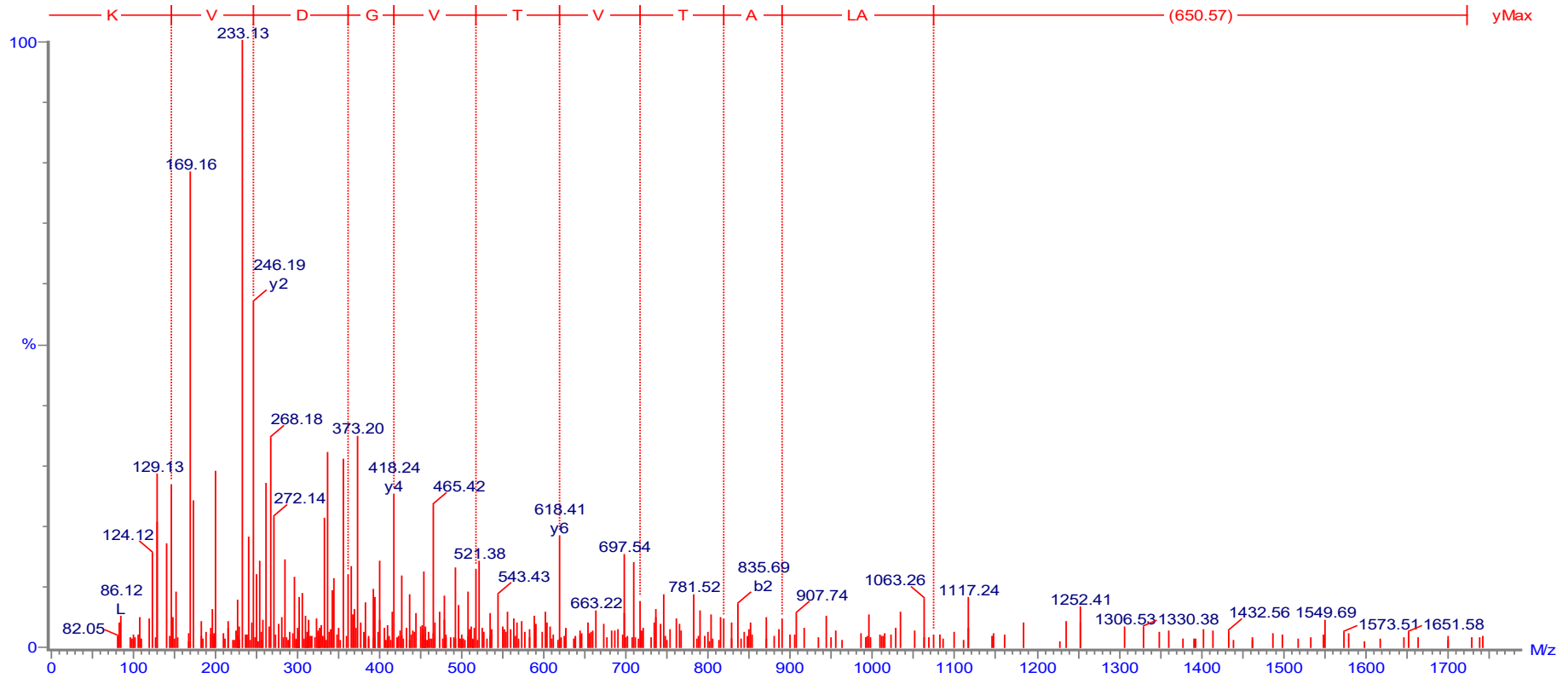
Spot Number	Sequence	Mass	Charge	BLAST Score	EST qtk	EST BLAST Score	Accession Number	Identification	Species	Unique Peptides	Sequence Coverage (%) ^(A)	MOTIF ^(B)	% Contribution
17	1- <u>VAWEPDWAVGTRK</u> *	801.91	2	5.00E-06	Fhep26g11.q1k	1.00E-87	AA257433	Triose-Phosphate Isomerase	<i>Orientobilhazia turkesstanicum</i>	4	19.9	-	1.617
	2- <u>VAGAFITGFKDAMR</u>	811.88	2	7.00E-04	Fhep26g11.q1k	1.00E-87							
	3- <u>IFGESPELIGERK</u> *	737.33	2	1.60E-02	Fhep26g11.q1k	1.00E-87							
4- <u>IIVGGSVTAANK</u>	677.32	2	1.00E-03	-	-	BAF62292	Triose Phosphate Isomerase	<i>Schistosoma haematobium</i>					
18	1- <u>VMLGASGGLQEGVLLK</u>	890.99	2	6.00E-03	-	-	ABD77293	Mitochondrial Malate Dehydrogenase 2	<i>Canis familiaris</i>	2	13.1	EM, TM ⁽¹⁾	1.038
	2- <u>ADGSATLSMAVSGMR</u>	757.83	2	2.00E-02	-	-	AA135230	Mitochondrial Malate Dehydrogenase	<i>Clonorchis sinensis</i>				
19	1- <u>ATEQWAFETVK</u> *	635.79	2	6.50E-02	-	-	AAO85503	Aldolase	<i>Pecten maximus</i>	3	18.2	-	0.441
	2- <u>PTEAQYLQDFQFAK</u>	825.34	2	5.00E-04	Fhep10d01.q1k	3.00E-93	ABF55380	Fructose-1, 6-Bisphosphate Aldolase	<i>S. japonicum</i>				
3- <u>LVNHLDQVIRGK</u>	815.92	2	8.00E-04	Fhep10d01.q1k	3.00E-93								
20	1- <u>VETADQVVDLTK</u>	766.35	2	1.00E-04	-	-	AAG23287	Glyceraldehyde Phosphate Dehydrogenase	<i>F. hepatica</i>	2	10.0	EM, TM ⁽¹⁾	0.583
	2- <u>EPDTHIDNFK</u>	677.31	2	1.20E-02	Fhep15c10.q1k	4.00E-147	AAA16243	Glyceraldehyde-3-Phosphate Dehydrogenase	<i>S. japonicum</i>				
21	1- <u>AVANVNSQIARNLK</u>	776.44	2	3.00E-05	-	-	Q27655/A53665	Enolase (2-Phosphoglycerate Dehydrogenase)/Phosphopyruvate Hydratase	<i>F. hepatica</i>	1	3.0	-	1.345
22	1- <u>QNVESGGRVHHR</u> *	743.84	2	3.00E-04	-	-	AAW25537	SICH G CD6318 Protein Actin	<i>S. japonicum</i>	2	7.7	TM ⁽²⁾	4.769
	2- <u>SYELPDGQVITGRNER</u>	895.94	2	7.00E-07	-	-	ABQ65136	Actin	<i>Trichoderma atroviride</i>			TM ⁽¹⁾	
23	1- <u>GTAITLGGTCLNVGQPSK</u> *	903.42	2	1.50E-06	-	-	YP_001202603	Dihydroliipoamide Dehydrogenase	<i>Badythis obium</i> sp.	1	3.4	TM ⁽¹⁾	0.814
24	1- <u>LVSPTGALNTQANR</u>	910.94	2	2.00E-07	-	-	AAV59016	Leucyl Aminopeptidase	<i>F. hepatica</i>	2	6.5	-	0.448
	2- <u>NSVGNDSYAKELVAR</u>	889.91	2	7.00E-06	-	-							
25	1- <u>NOAVNDSVQVDFK</u>	839.88	2	1.30E-03	-	-	ABS52704	Heat Shock Protein 70	<i>F. hepatica</i>	3	14.3	-	2.386
	2- <u>TAEEDHLLGSLTFRNR</u> *	846.35	2	9.00E-03	Fhep13a09.q1k	0							
3- <u>TPSYVETDFER</u> *	744.34	2	7.00E-03	Fhep13a09.q1k	0								



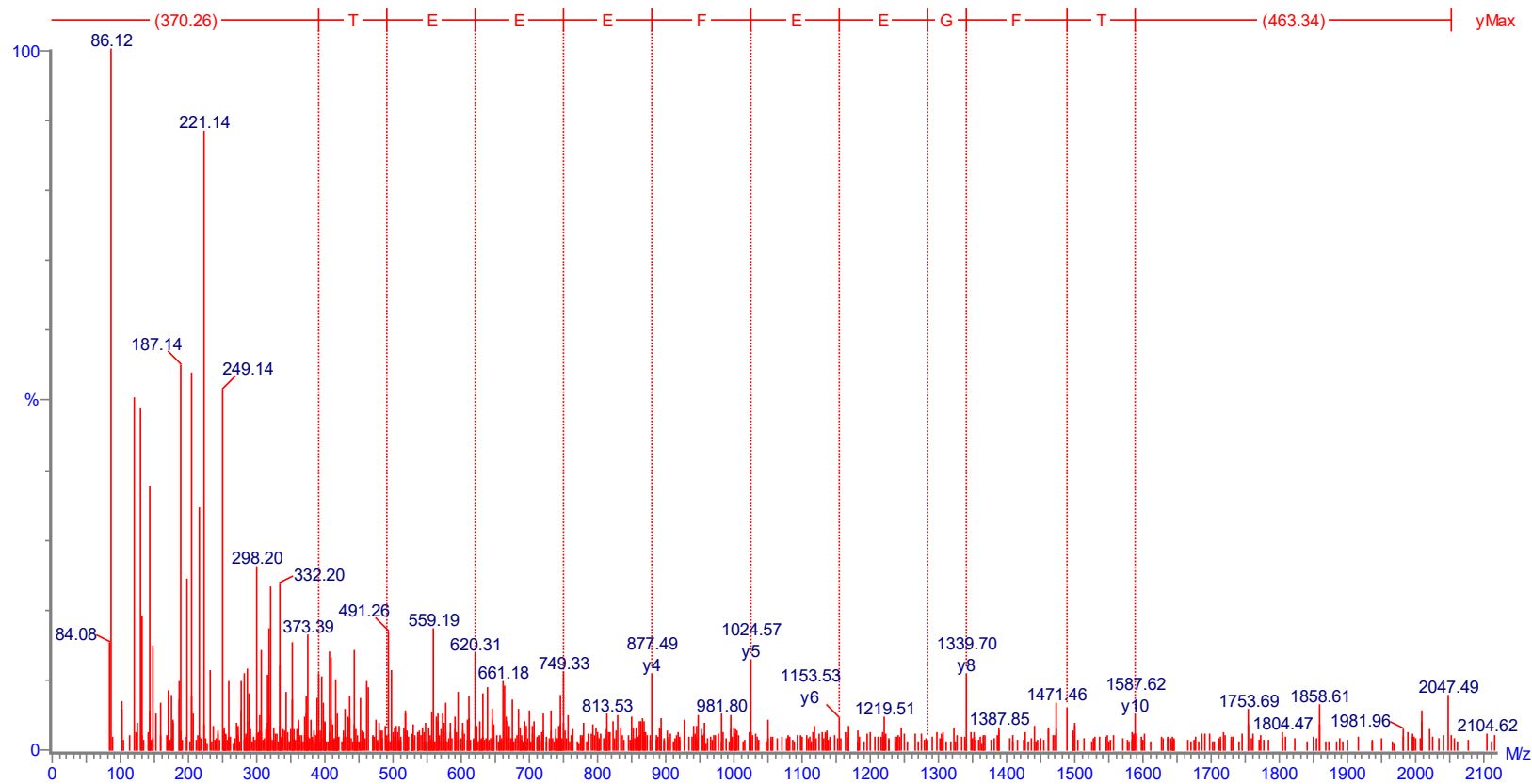
Supplementary Figure 1. MS/MS sequence analysis using peptide sequencer (MassLynx v. 5.0, Micromass, UK) from the fragmentation of a precursor ion m/z 807.89 (2^+) representative of the antioxidant Thioredoxin peroxidase (TPx) from *Fasciola hepatica*. Interpretation of the γ and b ion series provided the peptide sequence AYGVLD EEQNTYR.



Supplementary Figure 2. MSMS sequence analysis using peptide sequencer (MassLynx v. 5.0, Micromass, UK) from the fragmentation of a precursor ion m/z 669.83 (2^+) representative of the phase II detoxification enzyme Mu Class glutathione transferase (GST) from *Fasciola hepatica*. Interpretation of the y and b ion series provided the peptide sequence ISMIEGAAMDRLR.



Supplementary Figure 3. MS/MS sequence analysis using peptide sequencer (MassLynx v. 5.0, Micromass, UK) from the fragmentation of a precursor ion m/z 575.35 (3^+) representative of the type III Fatty Acid Binding Protein (FABP 3) from *Fasciola hepatica*. Interpretation of the γ and b ion series provided the peptide sequence IATVTVGDVK.



Supplementary Figure 4. MSMS sequence analysis using peptide sequencer (MassLynx v. 5.0, Micromass, UK) from the fragmentation of a precursor ion m/z 683.90 (3^+) representative of the type I Fatty Acid Binding Protein (FABP 1) from *Fasciola hepatica*. Interpretation of the y and b ion series provided the peptide sequence TFGEEFEET.

Supplementary Table 2. Location and function of the identified FhTeg proteins. The location and function as reported in the literature of the identified proteins found in the FhTeg samples. SE – Surface expressed; S – Soluble Preparations; SB – Sub-tegumental; ES - Excretory/secretory products; Ma – Mitochondrial Matrix; Me – Mitochondrial membrane.

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