

Proteomics of hyposaline stress in blue mussel congeners (genus *Mytilus*): implications for biogeographic range limits in response to climate change

Lars Tomanek*, Marcus J. Zuzow, Lauren Hitt, Loredana Serafini and
Jacob J. Valenzuela

SUPPLEMENTAL MATERIAL

California Polytechnic State University
Department of Biological Sciences
Center for Coastal Marine Science
Environmental Proteomics Laboratory
1 Grand Ave.
San Luis Obispo, CA 93407-0401
U.S.A.

* Author for correspondence:

E-mail: ltomanek@calpoly.edu

Phone: 1-805-756-2437

Fax: 1-805-756-1419

Figure legends:

Figure S1: Principal component analysis of hyposaline treatments for (A) *M. trossulus* and (B) *M. galloprovincialis*, using proteins that were significant for the interaction effect between salinity and time of recovery (two-way ANOVA). Each symbol represents a mussel treated to a different salinity (for 4 h) without (0 h) and with a 24 h recovery at 1000 mOsm/kg. In each panel the horizontal axis represents PC1, and the vertical axis represents PC2. Percentages represent the proportion of total variation in the dataset described by each component. For matching positive and negative protein loadings (above or below ± 1.0 only) of the two main effects (salinity and time of recovery; see also PCAs of the main effects in Figs 2 and 3 in the paper) and the interaction effect of proteins contributing to PC1 and PC2 see Figs S2-S4. For a more detailed description of the role of positive and negative loadings see the publication by Fields et al. (2012).

Figure S2: Hierarchical clustering using Pearson's correlation of protein spots from (A) *Mytilus trossulus* and (B) *M. galloprovincialis* that changed significantly with hyposaline stress (salinity main effect only) and were identified with tandem mass spectrometry. Blue coloring represents a lower than average protein abundance (standardized volume), whereas orange represents greater than average protein abundance. The columns show individual mussels, which cluster according to treatment ($N= 4-6$ for each treatment for *M. trossulus* and $N=6$ for *M. galloprovincialis*). The rows represent the standardized protein abundances, which are identified to the right. Clusters talked about in the text are labeled for species (T versus G), main effect (S=salinity), and cluster (starting with A). Clusters do not adhere to specific criteria other than that they show similar changes in

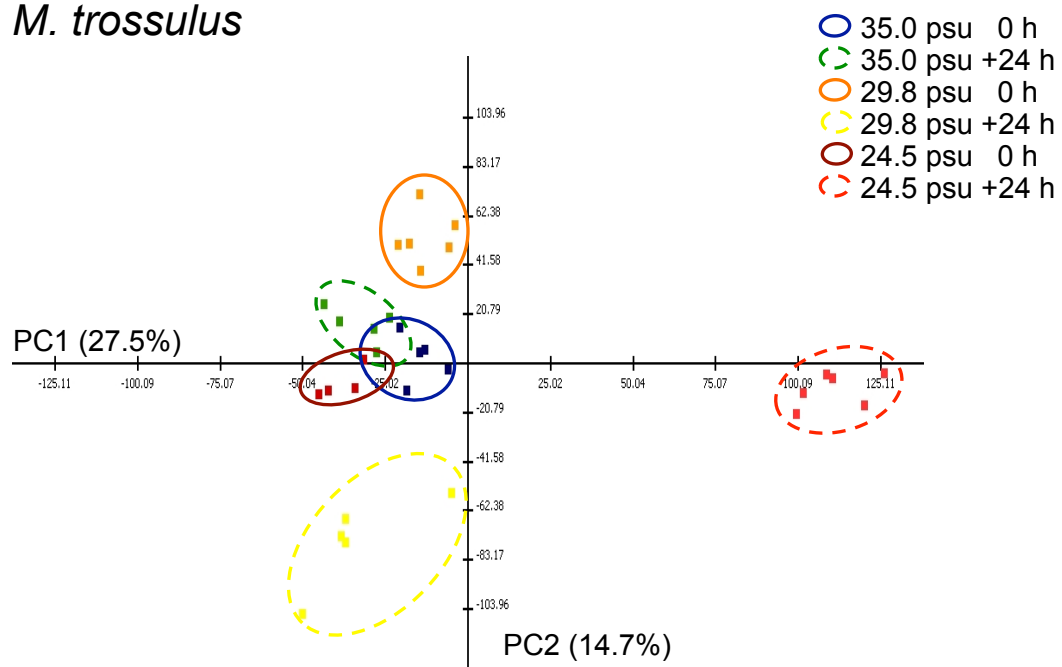
protein abundance. Positive and negative loadings (above or below 1) for PC1 and PC2 as well as putative protein functions are given in columns to the right of the protein identifications.

Figure S3: Hierarchical clustering of protein spots from (A) *Mytilus trossulus* and (B) *M. galloprovincialis* that changed significantly with hyposaline stress (time during recovery main effect only). For further details see Fig S2.

Figure S4: Hierarchical clustering of protein spots from (A) *Mytilus trossulus* and (B) *M. galloprovincialis* that changed significantly during recovery from hyposaline stress (salinity and time of recovery interaction effect). For further details see Fig. S2.

Fig. S1

(A) *M. trossulus*



(B) *M. galloprovincialis*

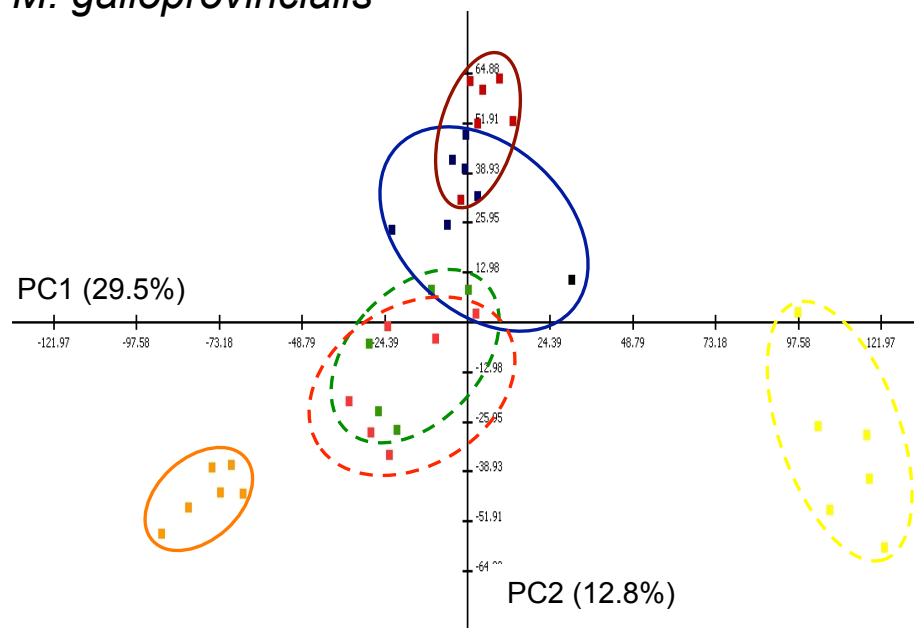


Fig. S2A

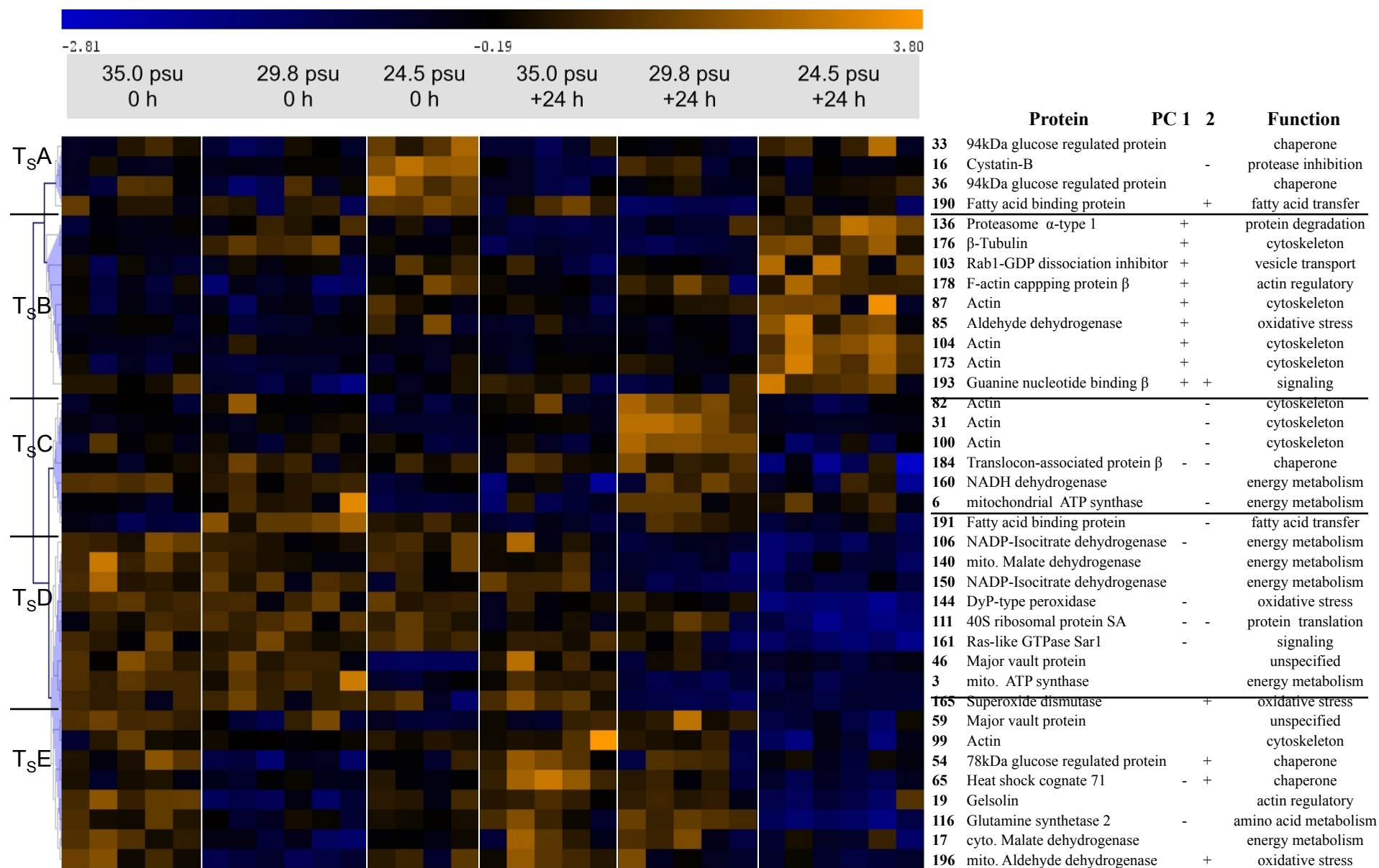
(A) *M. trossulus*- Salinity

Fig. S2B

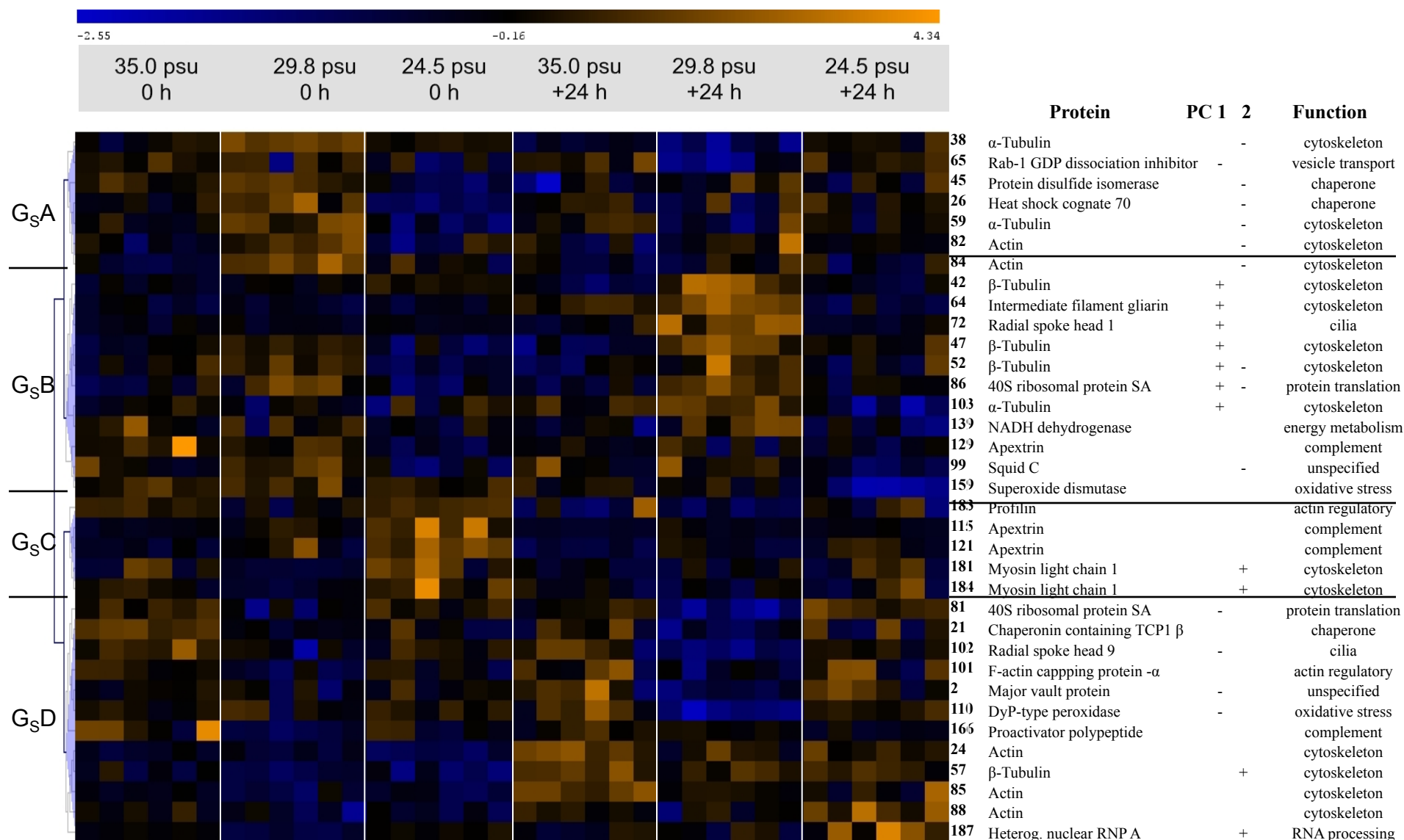
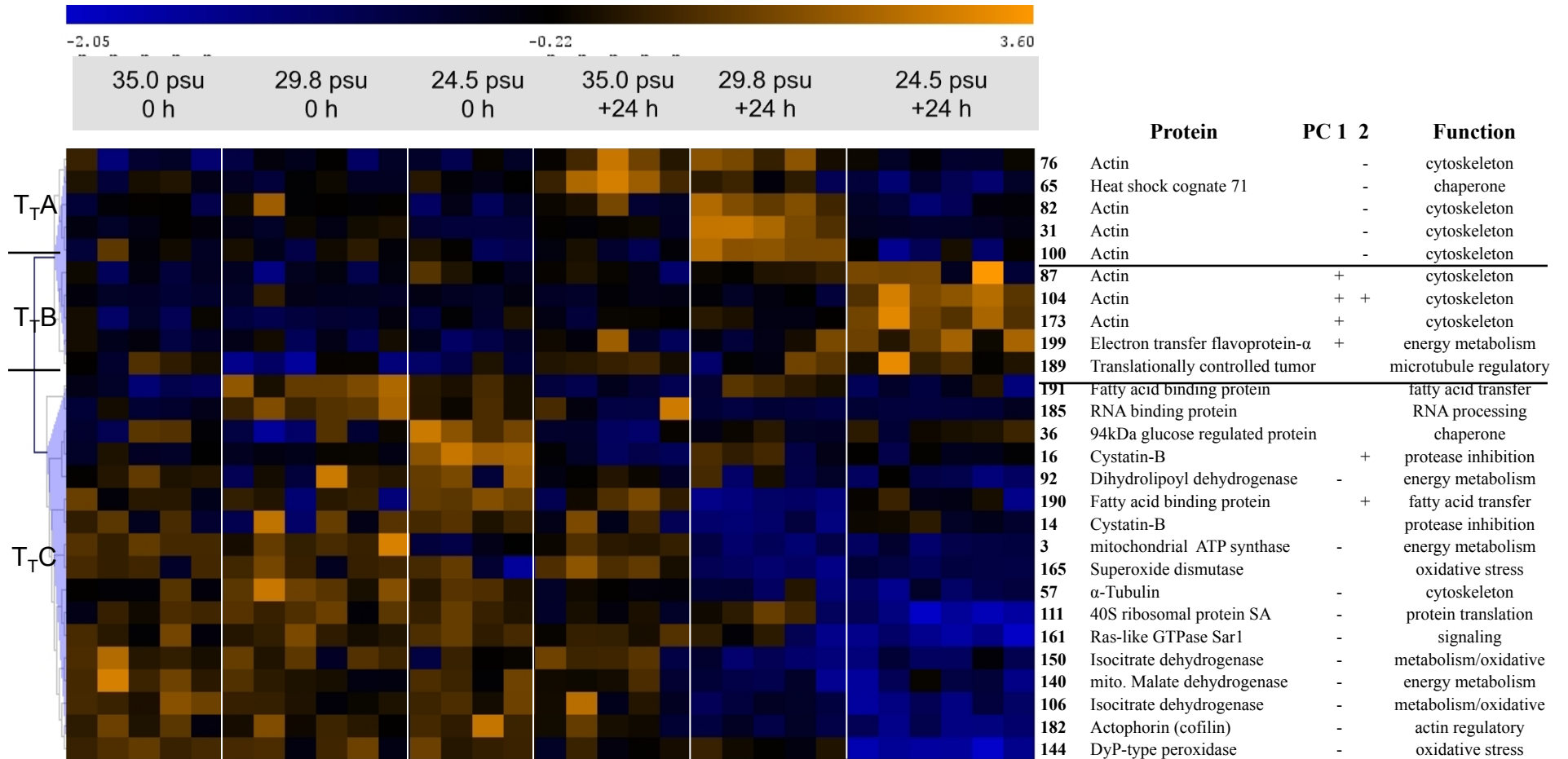
(B) *M. galloprovincialis* - Salinity

Fig. S3A

(A) *M. trossulus*- Recovery



(B) *M. galloprovincialis* - Recovery

Fig. S3B

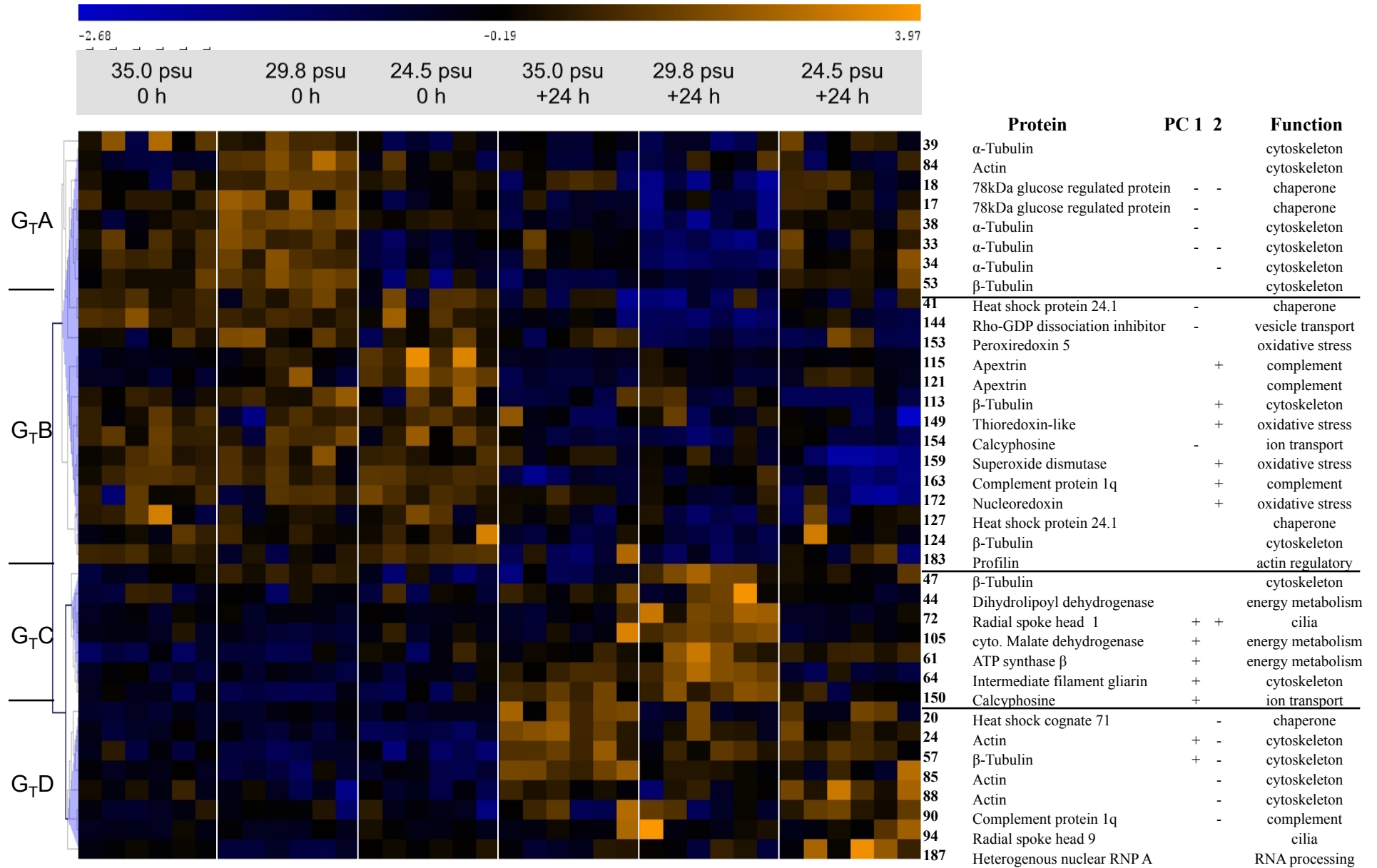


Fig. S4A

(A) *M. trossulus*- Interaction between salinity and recovery

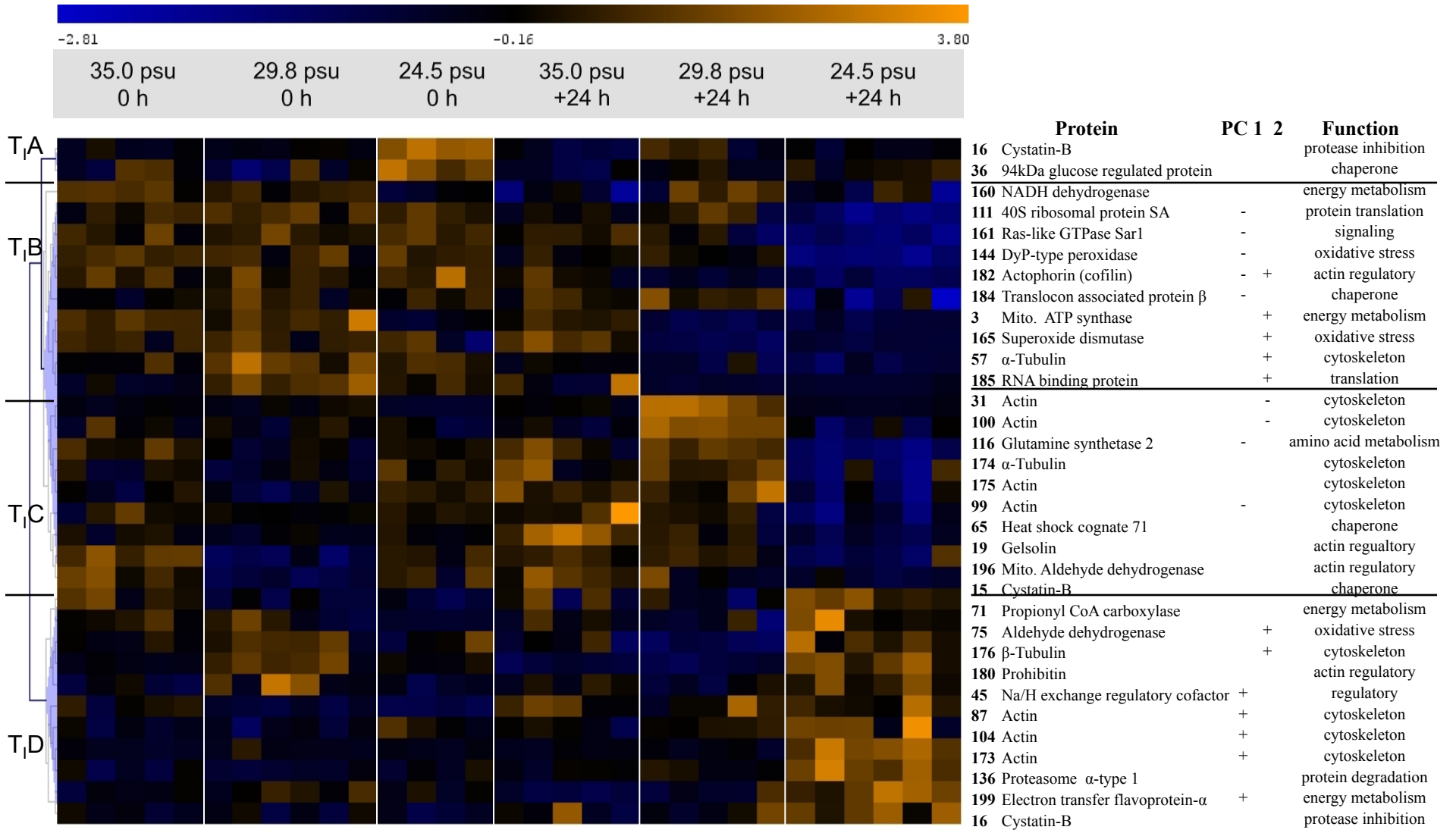


Fig. S4B

(A) *M. galloprovincialis* – Interaction between salinity and recovery

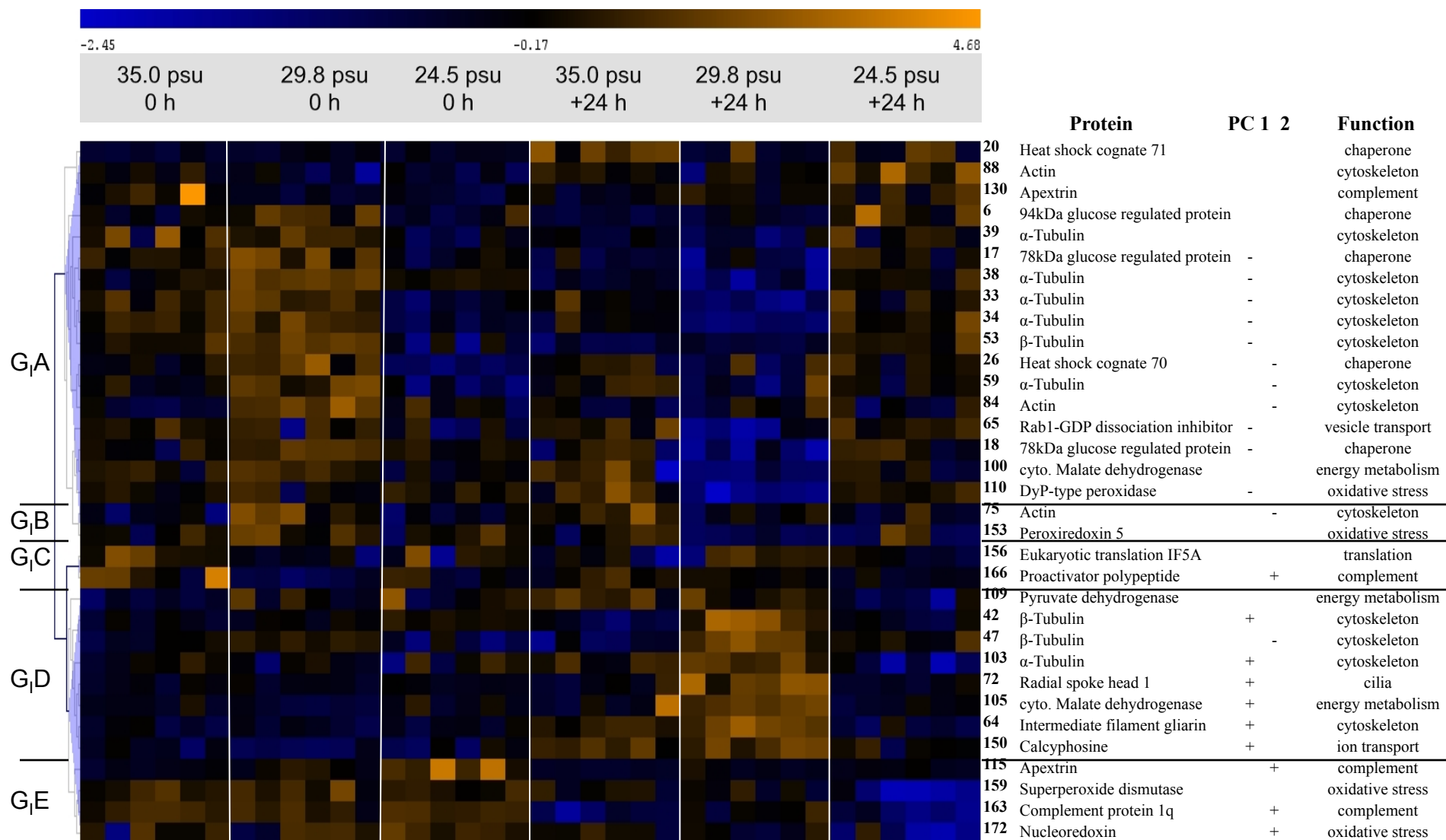


Table S1: Protein identifications for *M. trassulus* with estimated and predicted molecular weight (kDa) and isoelectric point, score, peptide matches, sequence coverage, average levels (NR=no recovery), and putative function.

Spot ID	Protein ID	MW (kDa)		pI		GenBank ID	Mascot Score	Peptide Matches	Sequence Coverage(%)	Avg. Levels (relative to 100NR)					Functional Category
		Estimated	Predicted	Estimated	Predicted					29.8-0	24.5-0	35.0-24	29.8-24	24.5-24	
3	mitochondrial ATP synthase	18.00	4.45	17.71	5.02	gii58305969	146	2	11	1.08	0.41	0.82	0.23	0.28	energy metabolism
6	mitochondrial ATP synthase	16.00	4.44	17.71	5.02	gii58305969	96	2	11	1.90	0.57	0.73	1.88	1.56	energy metabolism
14	Cystatin-B	16.00	5.11	11.20	5.92	gii164594924	108	3	31	1.07	1.18	0.92	0.36	0.78	protease inhibition
15	Cystatin-B	15.00	5.12	11.20	5.92	gii164594924	151	2	20	0.45	0.40	0.79	0.57	1.20	protease inhibition
16	Cystatin-B	15.00	5.13	11.20	5.92	gii164594924	122	3	38	1.04	4.73	0.42	1.76	0.92	protease inhibition
17	cytosolic Malate dehydrogenase	49.00	5.89	35.50	6.01	gii145901989	58	2	6	0.30	0.56	0.85	0.80	0.46	energy metabolism
19	Gelsolin	60.00	5.79	41.20	5.13	gii212814711	248	2	16	0.20	0.68	0.73	0.69	0.30	actin regulatory
31	Actin	103.00	5.25	41.60	5.3	gii145892505	108	2	9	1.44	0.08	1.35	7.26	0.59	cytoskeletal
33	94kDa glucose regulated protein	96.00	4.90	92.00	4.70	gii238629046	73	4	15	0.56	1.80	0.60	0.70	1.38	molecular chaperone
36	94kDa glucose regulated protein	96.00	4.87	92.00	4.70	gii238639046	101	3	15	0.65	2.00	0.56	0.83	1.04	molecular chaperone
45	Na(+)/H(+) exchange regulatory cofactor (NHE-RF1)	95.00	5.52	38.60	5.63	gii238645041	55	2	11	0.30	0.07	1.49	1.38	1.56	cytoskeletal
46	Major vault protein	96.00	5.63	95.20	5.33	gii223026493	78	2	10	0.86	0.01	0.92	0.45	0.33	signaling
54	78kDa glucose regulated protein	87.00	4.88	73.00	5.02	gii238639954	122	3	19	0.32	0.67	1.16	0.78	0.59	molecular chaperone
57	α -Tubulin	81.00	5.49	30.70	5.23	gii154349830	53	2	11	1.85	1.38	0.83	0.58	0.40	cytoskeletal
59	Major vault protein	83.00	4.79	95.20	5.33	gii223026493	40	2	11	0.57	0.32	0.63	0.95	0.31	signaling
62	Na(+)/H(+) exchange regulatory cofactor (NHE-RF1)	82.00	4.82	38.60	5.63	gii238645041	146	5	28	0.75	0.94	0.63	1.20	0.52	cytoskeletal
65	Heat shock cognate 71	80.00	5.30	71.20	5.28	gii212812325	45	2	9	0.77	0.98	2.30	1.11	0.46	molecular chaperone
71	Propionyl CoA carboxylase	78.00	5.37	77.40	6.48	gii223020299	78	2	21	0.90	0.54	0.84	0.60	1.35	energy metabolism
75	Aldehyde dehydrogenase	74.00	5.55	55.60	6.69	gii58307245	180	3	17	1.45	1.14	0.88	0.43	1.58	oxidative stress
76	Actin	71.00	5.29	31.40	4.94	gii212827318	45	2	7	1.03	1.05	2.30	2.43	1.20	cytoskeletal
82	Actin	68.00	5.25	41.60	5.3	gii145892505	188	4	15	1.38	0.82	1.31	2.17	0.86	cytoskeletal
85	Aldehyde dehydrogenase	69.00	5.38	55.60	6.69	gii58307245	43	2	9	0.85	1.75	0.93	0.91	2.88	oxidative stress
87	Actin	68.00	5.44	17.20	5.20	gii58307494	103	2	8	0.94	1.31	1.10	1.32	1.76	cytoskeletal
92	Dihydrolypoyl dehydrogenase	68.00	6.44	54.40	6.68	gii145896736	105	3	10	0.97	1.38	0.70	0.60	0.28	energy metabolism
99	Actin	55.00	5.30	41.60	5.3	gii145892505	246	5	16	0.89	1.01	1.28	0.95	0.57	cytoskeletal
100	Actin	61.00	5.25	41.60	5.3	gii145892505	158	3	10	0.94	0.79	0.82	2.05	0.69	cytoskeletal
103	Rab1 - GDP dissociation inhibitor	59.00	6.22	50.20	5.31	gii58306730	121	4	28	1.09	1.54	1.04	1.11	2.02	vesicle transport
104	Actin	57.00	5.40	41.60	5.3	gii145892505	359	6	21	1.36	1.15	1.18	1.19	5.15	cytoskeletal
106	Isocitrate dehydrogenase (NADP)	56.00	6.54	50.85	8.52	gii223026036	115	2	7	0.71	0.93	0.78	0.37	0.20	oxidative stress
111	40S ribosomal protein SA	56.00	4.63	24.18	7.66	gii58307365	206	5	28	1.16	1.12	0.79	0.98	0.32	translation
116	Glutamine synthetase 2	56.00	6.11	41.50	6.03	gii145886483	158	3	18	0.70	0.85	0.99	1.17	0.37	nitrogen metabolism
136	Proteasome α -type 1	43.00	5.35	30.70	5.23	gii145888141	79	3	14	1.33	1.11	0.73	1.04	2.05	protein degradation
140	mitochondrial Malate dehydrogenase	44.00	6.53	36.30	8.70	gii223024468	85	2	10	0.69	0.84	0.66	0.37	0.37	energy metabolism
144	DyP-type peroxidase	41.00	6.37	45.00	5.49	gii238638759	246	6	35	0.89	0.96	0.70	0.75	0.17	oxidative stress
150	Isocitrate dehydrogenase (NADP)	39.00	6.57	50.85	8.52	gii223026036	126	4	20	0.91	0.61	0.90	0.24	0.30	energy metabolism
160	NADH dehydrogenase	33.00	4.75	23.80	5.74	gii223028674	79	2	14	0.92	0.75	0.65	0.97	0.74	oxidative stress
161	Ras-like GTPase Sar1	27.00	6.43	21.79	6.52	gii145895499	108	4	12	1.04	1.17	0.99	0.70	0.25	vesicle transport
165	Superoxide dismutase	18.00	6.26	15.80	5.85	gii154349389	98	3	12	1.07	0.77	1.26	0.27	0.42	oxidative stress
173	Actin	57.00	5.50	41.60	5.3	gii145892505	220	6	21	0.80	1.22	1.29	1.56	3.82	cytoskeletal
174	α -Tubulin	61.00	5.65	49.98	4.97	gii238782438	210	4	22	0.86	1.25	1.32	1.47	0.72	cytoskeletal
175	Actin	62.00	5.54	41.60	5.3	gii145892505	243	5	18	0.87	1.39	1.55	1.68	0.68	cytoskeletal
176	β -Tubulin	45.00	5.62	27.80	4.81	gii145889387	69	2	10	2.00	1.25	0.57	0.54	2.39	cytoskeletal
178	F-actin capping protein- β	38.00	5.06	30.79	5.45	gii321468824	100	3	11	0.52	1.90	1.13	1.69	2.21	actin regulatory
180	Prohibitin	39.00	5.38	29.90	5.36	gii238643256	221	6	25	2.42	0.94	1.58	1.12	2.44	molecular chaperone
182	Actophorin (cofilin)	18.00	6.46	16.15	5.94	gii212817000	85	2	13	0.95	1.25	0.82	0.44	0.23	actin regulatory
184	Translocon associated protein β	31.00	5.74	20.00	8.64	gii223024827	168	2	11	1.22	1.20	1.21	1.38	0.62	molecular chaperone
185	RNA binding protein	14.00	5.91	110.16	6.93	gii164573751	105	4	15	5.72	3.22	3.26	0.24	0.39	RNA binding
189	Translationally controlled tumor protein	29.00	4.72	22.39	5.48	gii223020329	108	5	21	0.53	0.68	1.11	1.01	1.29	molecular chaperone
190	Fatty acid binding protein	21.00	4.67	13.80	6.58	gii223024166	99	4	21	0.80	1.55	1.07	0.25	0.73	fatty acid transfer
191	Fatty acid binding protein	20.00	4.67	13.80	6.58	gii223024166	49	4	21	2.87	2.00	1.06	1.98	1.15	fatty acid transfer
193	Guanine nucleotide binding protein- β	45.00	5.94	37.30	5.76	gii223020331	176	6	15	0.59	0.85	0.93	0.79	1.25	signaling
196	mitochondrial Aldehyde dehydrogenase	64.00	6.00	57.10	7.53	gii145898855	86	3	10	0.25	0.82	1.02	0.63	0.37	oxidative stress
199	Electron transfer flavoprotein- α	41.00	5.92	35.00	7.74	gii58307490	188	4	32	0.97	0.61	1.50	1.51	2.86	energy metabolism

Table S2: Protein identifications for *M. galloprovincialis* with estimated and predicted molecular weight (kDa) and isoelectric point, score, peptide matches, sequence coverage, average levels (NR=no recovery), and putative function.

Spot ID	Protein ID	MW (kDa)		pI		GenBank ID	Mascot Score	Peptide Matches	Sequence Coverage(%)	Avg. Levels (relative to 100 NR)					Functional Category
		Estimated	Predicted	Estimated	Predicted					29.8-0	24.5-0	35.0-24	29.8-2	24.5-24	
2	Major vault protein	96.00	5.85	95.20	5.33	gi 223026493	88	3	13	0.83	1.03	1.52	0.49	1.47	signaling
6	94kDa glucose regulated protein	96.00	5.07	92.00	4.70	gi 238629046	92	4	15	2.55	0.86	0.35	0.55	2.63	molecular chaperone
17	78kDa glucose regulated protein	84.00	5.04	73.00	5.02	gi 238639954	189	4	24	1.39	0.69	0.61	0.44	0.92	molecular chaperone
18	78kDa glucose regulated protein	83.00	5.07	73.00	5.02	gi 238639954	136	4	24	1.57	1.10	1.02	0.38	1.17	molecular chaperone
20	Heat shock cognate 71	81.00	5.54	71.20	5.28	gi 149382715	127	3	18	1.40	1.33	5.51	1.91	3.37	molecular chaperone
21	Chaperonin containing TCP1 subunit 2 (β) variant 1	80.00	6.04	29.30	8.86	gi 149381836	52	2	8	0.56	0.61	0.73	0.42	0.69	molecular chaperone
24	Actin	76.00	5.51	41.70	5.30	gi 145892505	238	6	21	0.96	0.61	2.69	1.65	1.89	cytoskeletal
26	Heat shock cognate 70	76.00	5.58	35.00	5.35	gi 58307085	67	2	13	1.76	0.43	1.13	0.97	1.16	molecular chaperone
33	α-Tubulin	68.00	5.30	50.00	4.97	gi 238782438	451	6	25	1.14	0.59	0.83	0.42	0.89	cytoskeletal
34	α-Tubulin	69.00	5.26	30.70	5.23	gi 145887917	204	2	4	1.21	0.46	0.70	0.24	0.98	cytoskeletal
38	α-Tubulin	69.00	5.12	50.00	4.97	gi 238782438	492	6	25	1.72	1.12	0.77	0.48	1.12	cytoskeletal
39	α-Tubulin	69.00	5.33	50.00	4.97	gi 238782438	590	7	27	0.96	0.47	0.52	0.39	0.76	cytoskeletal
41	Heat shock protein 24.1	70.00	6.00	28.50	5.61	gi 2387642855	128	3	21	1.00	0.98	0.64	0.48	0.70	molecular chaperone
42	β-Tubulin	62.00	5.06	43.20	5.76	gi 164592164	300	7	25	1.05	1.16	0.92	1.50	1.00	cytoskeletal
44	Dihydropyridyl dehydrogenase	67.00	6.47	53.90	6.68	gi 196005079	120	2	4	0.96	0.99	1.44	1.69	1.00	energy metabolism
45	Protein disulfide isomerase	66.00	4.73	56.70	4.70	gi 212819041	329	5	24	1.12	0.76	0.76	0.98	0.90	molecular chaperone
47	β-Tubulin	64.00	4.98	43.20	5.76	gi 164592164	197	5	20	1.30	0.83	0.81	1.64	1.20	cytoskeletal
52	β-Tubulin	65.00	4.90	27.80	4.81	gi 145889387	129	3	13	1.24	0.72	0.82	1.30	0.94	cytoskeletal
53	β-Tubulin	66.00	4.94	51.40	4.77	gi 145890511	270	5	20	1.43	0.60	0.51	0.57	1.06	cytoskeletal
57	β-Tubulin	61.00	5.12	50.00	4.79	gi 238638721	127	6	18	0.78	0.71	1.69	1.39	1.45	cytoskeletal
59	α-Tubulin	65.00	5.30	50.00	4.97	gi 238782438	510	6	25	1.53	0.67	1.18	0.88	1.02	cytoskeletal
61	ATP synthase β	60.00	5.01	56.60	5.38	gi 212812392	587	7	38	1.06	1.29	1.35	1.82	1.53	energy metabolism
64	Intermediate filament gliarin	62.00	5.28	71.30	5.39	gi 212812257	155	4	18	0.99	1.06	1.62	2.33	0.93	cytoskeletal
65	Rab1 - GDP dissociation inhibitor	60.00	6.52	50.20	5.31	gi 58306730	146	4	28	0.98	0.78	1.07	0.55	1.05	vesicle transport
72	Radial spoke head 1	59.00	4.70	35.00	4.51	gi 145223031	134	2	7	0.99	1.04	1.15	3.70	0.94	cytoskeletal
75	Actin	56.00	5.56	41.70	5.30	gi 145892505	190	2	9	1.99	1.05	1.83	1.24	1.20	cytoskeletal
81	40S ribosomal protein SA	54.00	4.83	33.50	5.24	gi 58307365	168	5	28	0.57	0.95	0.85	0.23	1.14	translation
82	Actin	53.00	5.60	41.70	5.30	gi 145892505	214	5	18	1.42	0.99	1.02	1.26	1.06	cytoskeletal
84	Actin	52.00	5.41	41.70	5.30	gi 145892505	224	5	18	2.33	1.33	1.09	1.34	1.21	cytoskeletal
85	Actin	52.00	5.37	31.40	4.94	gi 212827318	149	3	11	0.83	0.85	1.89	1.23	1.35	cytoskeletal
86	40S ribosomal protein SA	52.00	4.83	33.50	5.24	gi 58307365	201	5	28	1.96	0.82	0.93	2.17	1.20	translation
88	Actin	51.00	5.42	41.70	5.30	gi 145892505	197	3	10	0.79	0.82	1.07	0.94	1.43	cytoskeletal
90	Complement component 1q	47.00	5.10	26.90	5.09	gi 58308171	259	4	25	1.02	0.93	1.43	1.20	1.72	complement
94	Radial spoke head 9	50.00	6.10	31.20	5.41	gi 223025866	190	5	18	0.97	1.68	3.88	5.41	4.98	cytoskeletal
99	Squid, isoform C	47.00	5.59	33.00	6.47	gi 58307067	53	2	6	1.37	0.60	1.06	1.20	0.56	translation
100	cytosolic Malate dehydrogenase	47.00	6.39	36.40	6.02	gi 223021440	221	5	17	1.15	0.84	1.04	0.57	0.90	energy metabolism
101	F-actin capping protein -α	46.00	6.53	38.00	5.76	gi 223022543	113	2	14	0.70	0.88	1.08	0.73	1.12	actin regulatory
102	Radial spoke head 9	45.00	6.14	31.20	5.41	gi 223025866	93	2	12	0.71	0.86	0.92	0.52	0.83	cytoskeletal
103	α-Tubulin	46.00	6.00	30.70	5.23	gi 154349830	169	4	19	0.91	1.01	1.16	1.40	0.67	cytoskeletal
105	cytosolic Malate dehydrogenase	45.00	6.10	36.40	6.02	gi 145901989	60	2	6	0.54	1.20	1.71	3.38	0.91	energy metabolism
109	Pyruvate dehydrogenase	43.00	5.59	39.40	5.52	gi 212834721	109	3	10	1.27	1.32	1.50	1.40	0.97	energy metabolism
110	TyrA (Dyp-type peroxidase)	42.00	6.56	41.62	7.00	gi 145886780	150	3	12	1.00	1.07	1.20	0.48	1.10	oxidative stress
113	β-Tubulin	40.00	5.23	54.40	4.92	gi 145889387	87	2	9	1.29	1.11	0.74	0.96	0.50	cytoskeletal
115	Apexrin	40.00	6.22	24.30	8.42	gi 238641395	245	6	21	2.41	7.82	0.50	1.38	1.19	immune response
121	Apexrin	38.00	6.21	24.30	8.42	gi 238641395	260	5	18	1.68	3.02	0.71	1.20	1.59	immune response
124	β-Tubulin	37.00	5.25	43.20	5.76	gi 164592164	250	7	25	1.04	1.34	0.54	0.41	1.11	cytoskeletal
127	Heat shock protein 24.1	36.00	5.95	28.50	5.61	gi 212814271	125	3	13	0.67	0.89	0.53	0.39	0.62	molecular chaperones
129	Apexrin	35.00	5.91	24.30	8.42	gi 238641395	51	4	15	0.77	0.29	0.49	0.55	0.40	immune response
130	Apexrin	35.00	5.87	24.30	8.42	gi 238641395	59	2	6	0.32	0.32	0.44	0.61	0.78	immune response
139	NADH dehydrogenase	32.00	5.14	23.80	5.74	gi 223028674	125	3	12	0.97	0.77	0.71	1.18	0.63	energy metabolism
144	Rho - GDP dissociation inhibitor	30.00	5.15	23.20	5.01	gi 149382257	68	2	12	0.89	0.91	0.48	0.22	0.53	signaling
149	Thioredoxin-like superfamily	25.00	4.71	26.00	4.15	gi 238645469	203	3	14	0.90	1.02	0.85	0.88	0.71	oxidative stress
150	Calcyphosine	25.00	5.71	21.40	4.99	gi 238642964	136	3	13	0.51	0.91	2.61	3.19	1.55	signaling
153	Peroxisome oxidoreductin 5	22.00	6.64	19.87	8.88	gi 212815268	145	2	8	1.61	1.10	1.09	0.31	0.81	oxidative stress
154	Calcyphosine	22.00	5.70	21.40	4.99	gi 238642964	209	8	33	0.98	0.99	0.56	0.51	0.57	signaling
156	Eukaryotic translation initiation factor 5A	21.00	5.49	17.00	5.30	gi 37650329	144	4	17	0.80	0.90	0.79	0.94	0.82	translation
159	Superoxide dismutase	19.00	6.10	15.80	5.84	gi 154349389	425	5	23	1.05	0.96	0.83	0.78	0.31	oxidative stress
163	Complement component 1q	18.00	6.43	19.50	5.64	gi 238646846	112	4	29	0.95	1.05	0.48	0.77	0.44	complement
166	Proactivator polypeptide	17.00	4.53	61.30	4.90	gi 223023145	80	2	11	0.19	0.53	0.64	0.54	0.39	complement
172	Nucleoredoxin	16.00	5.34	46.70	4.86	gi 145896033	51	2	9	1.05	1.22	0.95	0.85	0.50	oxidative stress
181	Myosin light chain 1	13.00	4.38	20.63	4.69	gi 164595445	227	3	24	0.25	1.82	0.42	0.63	0.95	cytoskeletal
183	Profilin	12.00	6.87	15.27	6.10	gi 37650202	209	4	17	0.62	1.16	0.59	0.27	0.68	actin regulatory
184	Myosin light chain 1	13.00	4.47	20.63	4.69	gi 164595445	156	3	24	0.42	1.95	0.64	0.53	0.92	cytoskeletal
187	Heterogenous nuclear ribonucleoprotein A	11.00	4.98	31.45	6.14	gi 37649755	116	4	22	0.31	1.19	0.95	0.99	2.74	translation