

Type of file: figure

Label: Fig 1

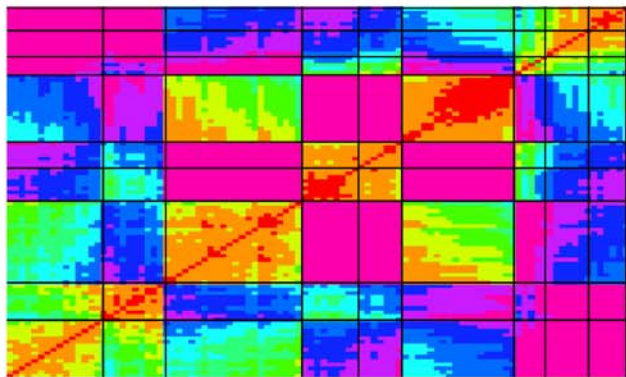
Filename: 2008 McColl JBC supplemental figures.pdf

SUPPLEMENTAL INFORMATION

Supplemental Experimental Procedures. *mRNA quantitation by real time polymerase chain reaction* - Sample preparation and total RNA extraction was performed as described for microarray analysis. 450 ng of this total RNA was then reverse transcribed using a MessageSensor RT kit (Ambion Inc) using random decamers, and diluted to a 3ng/ul equivalent template for real-time PCR. TaqMan Gene Expression Assay 20X primers for each gene were purchased from Applied Biosystems and used with their “Two-Step RT-PCR” protocol. The volume per reaction was reduced to 10ul, and all samples were run in triplicate. Real-time PCR was carried out in a 384-well format on a 7900HT PCR machine (Applied Biosystems) using conditions recommended for the primers used.

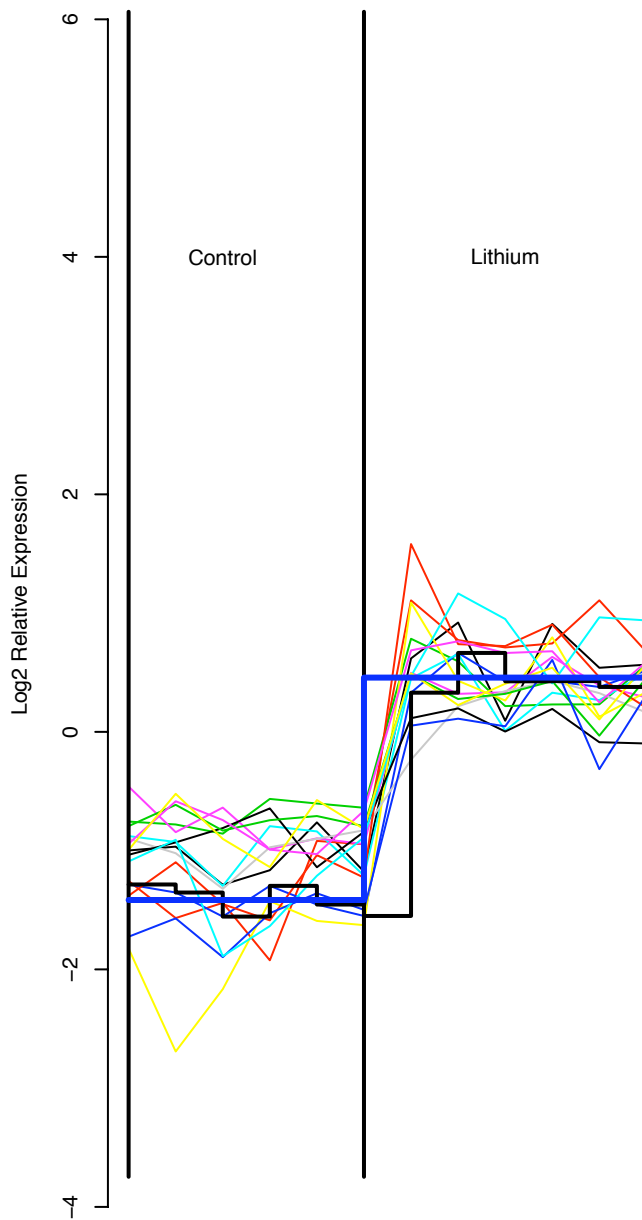
Supplemental FIGURE 1. **Ordered distance matrix and clusters (black outlines) based on HOPACH of differentially expressed genes in response to 10mM LiCl.** A total of 99 genes at a FWER cut-off of < 0.05 form 9 associated 1st level clusters as shown with colors red to purple representing distances close to far.

Supplemental FIGURE 2. **Graph of randomly chosen genes within each cluster showing \log_2 relative expression across all replicates.** The thick blue line is the medoid of the respective cluster and is the representative profile. Replicates are ordered by treatment group.

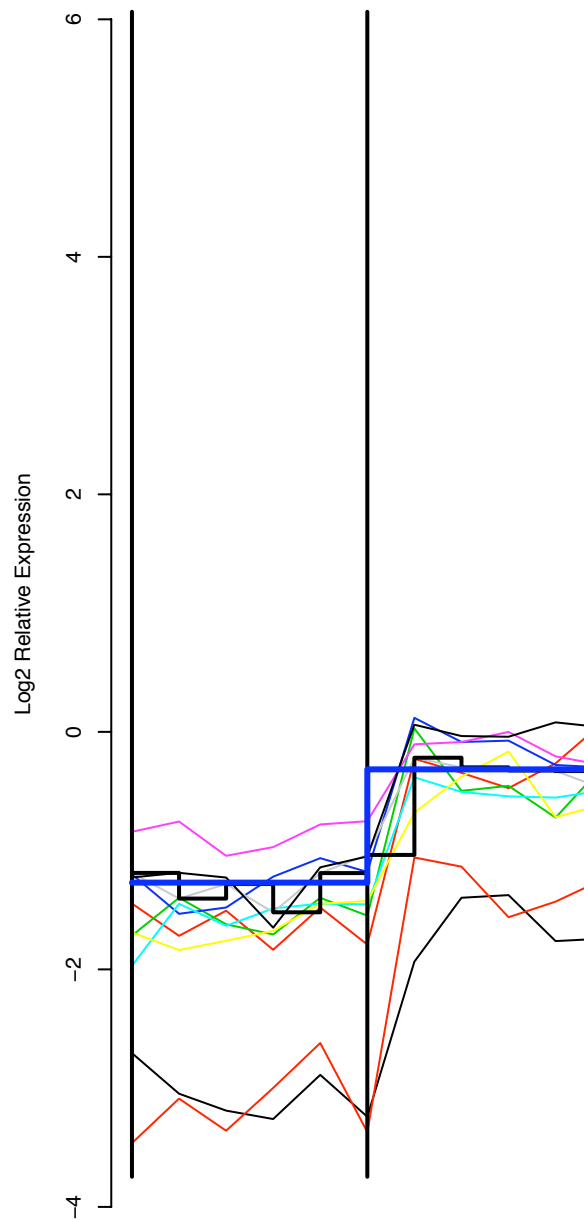


Supplemental Fig. 1

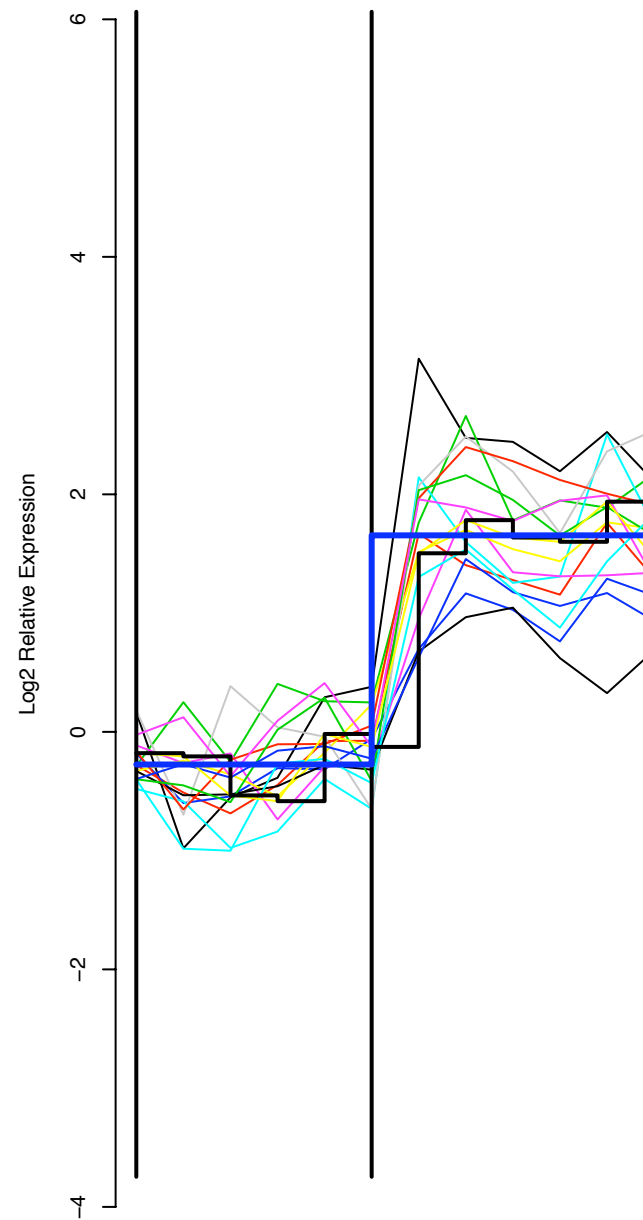
Cluster 1



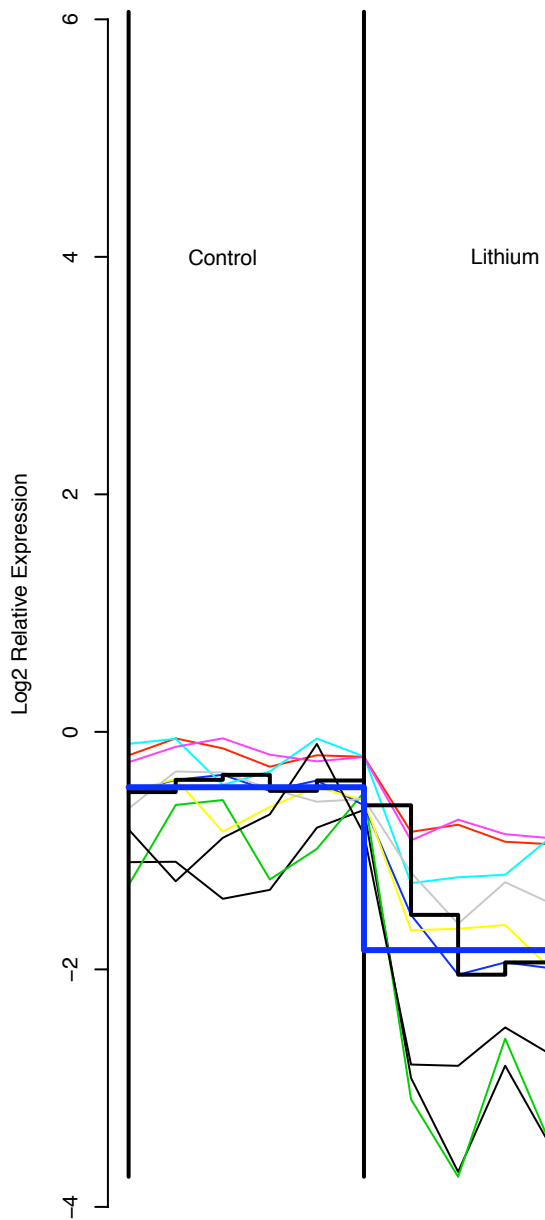
Cluster 2



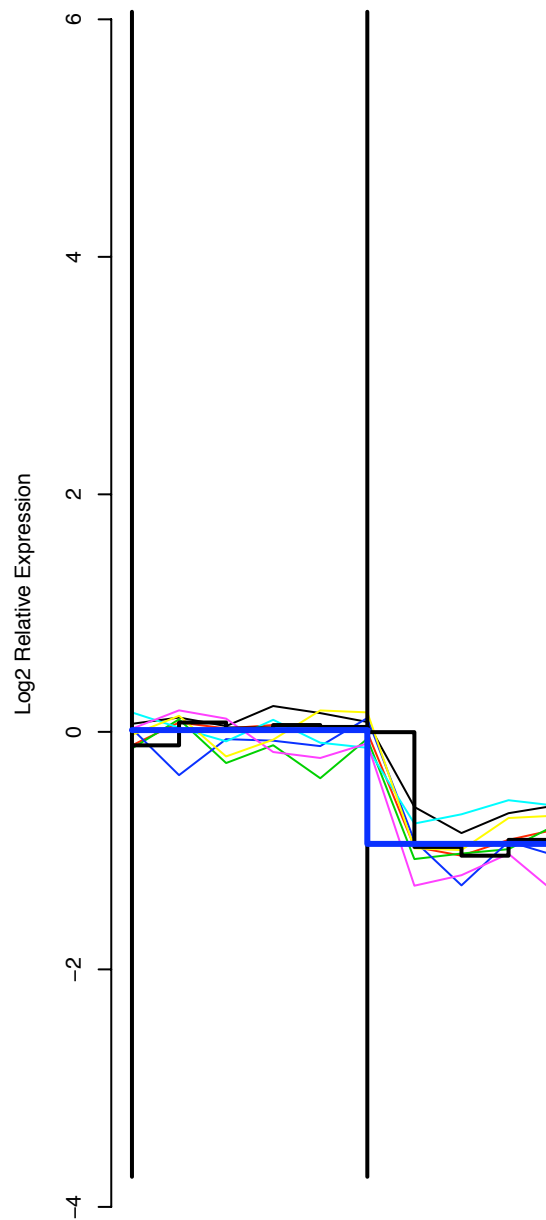
Cluster 3



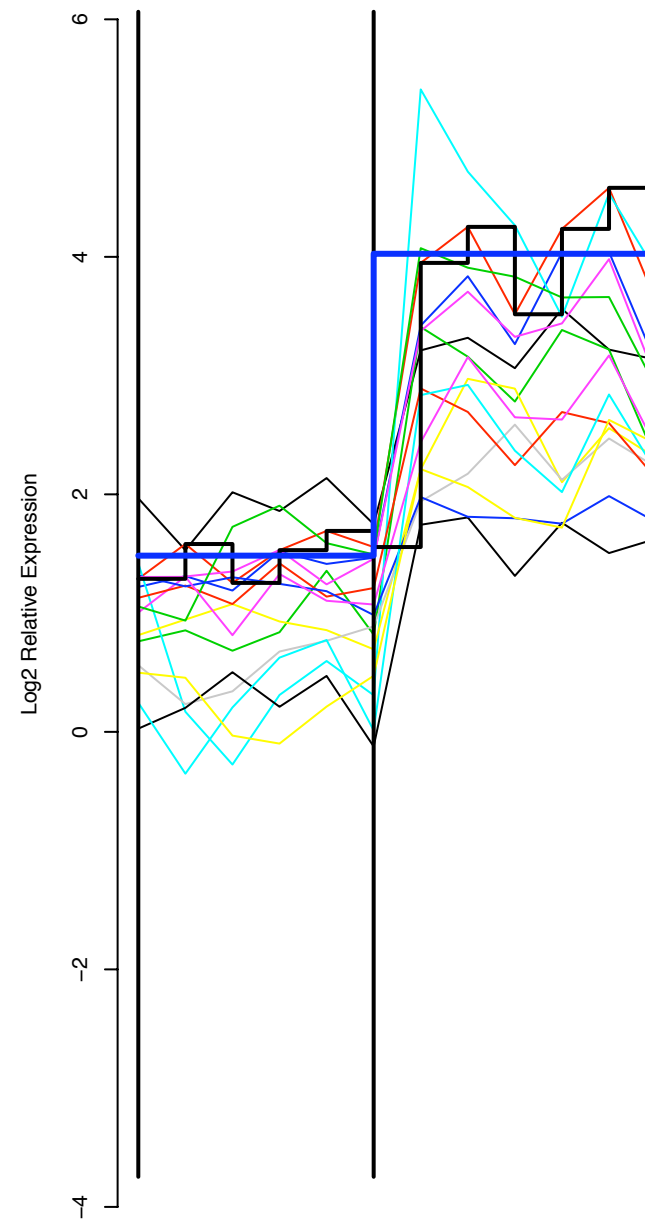
Cluster 4



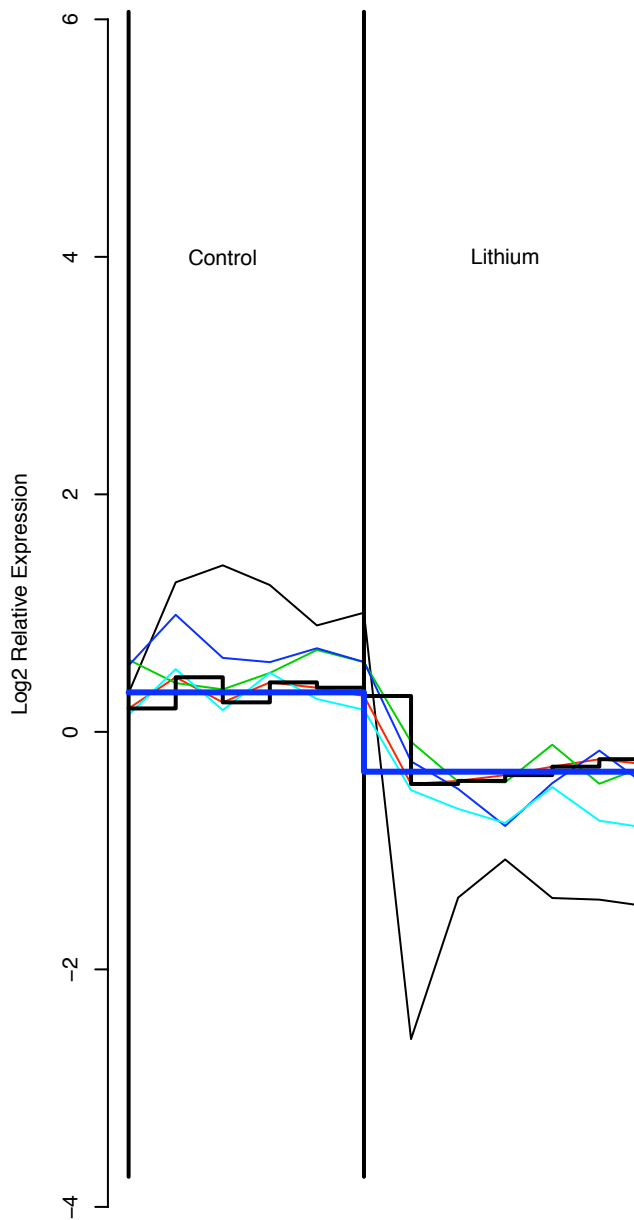
Cluster 5



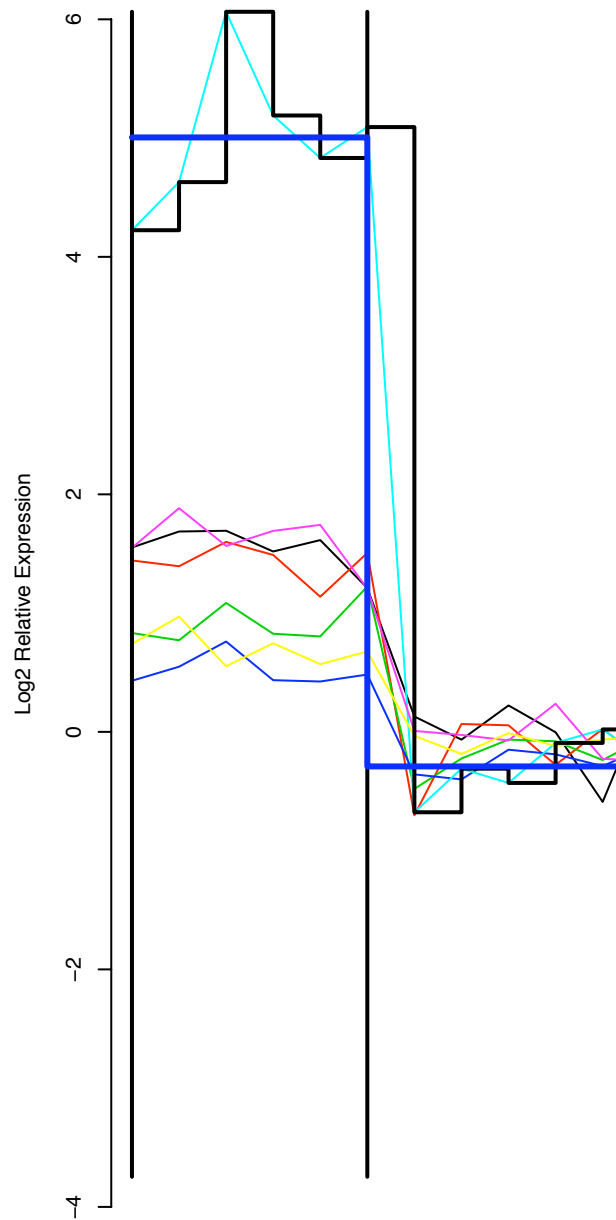
Cluster 6



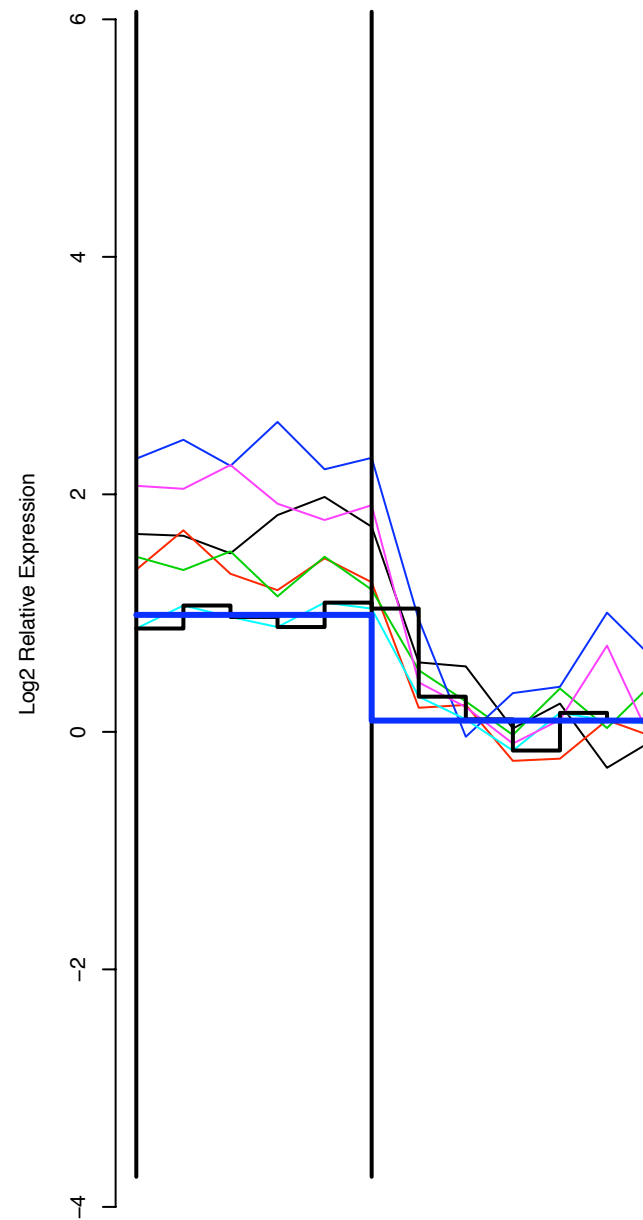
Cluster 7



Cluster 8



Cluster 9



Type of file: table

Label: Supp Table 1

Filename: 2008 McColl JBC supplemental tabel 1.pdf

SUPPLEMENTAL TABLE I

Mean elemental content of Nematode Growth Media (NGM) with and without 10mM LiCl.

Element	Control NGM		10 mM LiCl NGM		
	mM	SEM	mM	SEM	
B	1.58E-01	4.00E-06	1.59E-01	1.02E-06	
Ca	1.34E+00	2.83E-05	1.37E+00	9.68E-06	
Fe	8.72E-03	4.15E-07	8.84E-03	4.48E-08	
Li	-	-	1.07E+01	1.25E-04	***
Mg	2.24E+00	5.94E-05	2.29E+00	1.58E-05	
Na	6.74E+01	2.06E-03	7.00E+01	2.74E-04	
Rb	2.21E-03	8.91E-08	2.35E-03	1.89E-08	
S	4.78E+00	1.22E-04	4.96E+00	3.52E-05	
Si	1.66E-01	5.27E-06	1.60E-01	1.03E-06	
Sr	1.61E-03	4.88E-08	1.63E-03	9.86E-09	
Zn	8.32E-04	6.49E-08	1.19E-03	3.38E-07	

-, below detection limit of 4.60E-02 mM; ***, p<0.0001.

Type of file: table

Label: Supp Table 2

Filename: 2008 McColl JBC supplemental tabel 2.pdf

SUPPLEMENTAL TABLE IIMean elemental content of *C. elegans* treated with 10 mM LiCl.

Element	control		10mM LiCl		
	nmols/worm	SEM	nmols/worm	SEM	
Ca	7.41E-02	1.80E-03	7.49E-02	4.37E-04	
Cu	2.02E-05	7.90E-07	1.95E-05	4.55E-07	
Fe	1.42E-03	3.23E-05	1.39E-03	6.77E-06	
K	2.87E-01	5.74E-03	2.68E-01	1.91E-03	*
Li	-	-	6.03E-03	4.59E-05	***
Mg	7.84E-02	1.14E-03	7.58E-02	5.37E-04	
Mn	1.32E-04	1.23E-06	1.27E-04	6.00E-07	
Na	7.29E-02	1.85E-04	6.35E-02	6.69E-04	**
P	5.89E-01	5.72E-03	5.87E-01	5.30E-03	
S	1.64E-01	2.39E-03	1.57E-01	2.50E-04	*
Sr	4.45E-05	1.13E-06	4.46E-05	2.87E-07	
Zn	9.30E-04	8.12E-06	8.95E-04	5.18E-06	*

-, below detection limits of 4.50E-04 nmols/worm; *, p<0.05; **, p<0.01; ***, p<0.0001.

Type of file: table

Label: Supp Table 3

Filename: 2008 McColl JBC supplemental tabel 3.pdf

SUPPLEMENTAL TABLE III part i.

Gene transcript changes (down-regulated) in response to 10mM LiCl.

Gene	Description	Geo. Mean ratio*	Raw p-value**	FWER (Bonf)***	FDR (BH)****
F21F3.3	Predicted protein S isoprenylsysteine O- methyltransferase	0.03	2.97E-09	6.65E-05	2.22E-05
<i>lea-1</i>	Predicted protein to be hydrophilic and heat-resistant, may participate in anhydrobiosis	0.17	2.11E-06	0.04723	0.000477
ZK813.1	Predicted chorion protein	0.19	1.35E-06	0.030089	0.000397
Y62H9A.4	Unknown function	0.22	9.23E-07	0.020646	0.000358
C04H5.7	Unknown function	0.24	7.62E-07	0.017049	0.000334
T08B6.5	RNA binding protein	0.28	1.07E-06	0.023937	0.000364
K09E3.7	Putative coiled coil-4 domain protein	0.29	2.71E-07	0.006062	0.000225
F14H3.5	Putative coiled coil-4 domain protein	0.32	6.89E-08	0.001542	0.00014
T08D10.2	Ortholog of the human histone (H3K4) demethylase LSD1	0.33	3.81E-07	0.008523	0.000251
F38C2.5	CCCH-type Zn-finger protein	0.34	1.91E-06	0.042671	0.000462
C35E7.1	Uncharacterized coiled-coil containing protein	0.34	1.60E-06	0.035897	0.000436
T08A9.6	Predicted F-box containing protein	0.38	2.18E-07	0.004869	0.000203
<i>cdh-4</i>	Cadherin superfamily member	0.39	3.19E-08	0.000714	0.000102
<i>sax-3</i>	Protein involved in sensory axon guidance.	0.46	1.06E-06	0.023815	0.000364
K09E9.3	Putative coiled coil-4 domain protein	0.46	7.37E-07	0.016484	0.000334
<i>dhc-1</i>	Cytoplasmic dynein heavy chain homolog	0.47	7.62E-07	0.017041	0.000334
<i>tag-52</i>	Frabin actin binding protein	0.47	1.72E-06	0.03848	0.000447
F45C12.15	Homeobox domain containing protein	0.47	1.62E-06	0.036204	0.000436
ZK856.11	Predicted translation initiation factor related to eIF-1A	0.51	1.13E-06	0.025344	0.000364
<i>ten-1</i>	Tenurin	0.51	1.13E-06	0.025366	0.000364
C54H2.1	Novel protein, synthetic lethal with <i>mec-8</i>	0.51	4.85E-10	1.09E-05	5.43E-06
F22D6.2	Predicted Zn-finger protein	0.52	1.06E-06	0.023657	0.000364
<i>ugt-45</i>	UDP-glucuronosyl transferase	0.52	9.28E-07	0.020768	0.000358
F52D2.7	Unknown function	0.54	2.03E-07	0.004543	0.000203
C17G1.4	Unknown function	0.55	1.51E-06	0.033773	0.000423

R05F9.6	Predicted mitochondrial phosphoglucomutase	0.56	1.21E-06	0.027106	0.000371
<i>pfn-1</i>	Profilin.	0.57	2.04E-06	0.045736	0.000468
<i>rde-3</i>	Nucleotidyltransferase	0.58	4.03E-07	0.009005	0.000256
<i>ced-7</i>	Predicted ATP-binding cassette (ABC) transporter protein	0.59	3.82E-08	0.000855	0.000107
<i>cit-1.1</i>	Cyclin T	0.59	8.38E-07	0.018735	0.000353
Y65B4BR.5	Predicted transcription factor	0.62	8.94E-08	0.002	0.00014
T08G11.1b	chorein A	0.63	8.72E-07	0.019506	0.000355
<i>npp-10</i>	Nuclear pore complex protein	0.63	1.94E-07	0.004329	0.000203
<i>cua-1</i>	Putative copper ATPase	0.64	1.15E-06	0.025723	0.000364

* Represents the geometric mean ratio of expression in the two treatment groups,

** Based on two-sample t-test

*** Adjusted p-value based on familywise error rate control, using the Bonferroni method

**** Adjusted q-value based on the false discovery rate using the Benjamin-Hochberg Method

SUPPLEMENTAL TABLE III part ii.

Gene transcript changes (up-regulated) in response to 10mM LiCl.

Gene	Description.	Geo. Mean ratio	Raw p-value	FWER (Bonf)	FDR (BH)
W09D6.4	Putative membrane protein	15.58	6.24E-07	0.01396	0.000308
<i>pgp-6</i>	P-glycoprotein subclass of the transporters	9.89	5.30E-07	0.011854	0.000304
F54D7.3	Putative ortholog of human gonadotropin releasing hormone receptor	6.42	1.98E-06	0.044323	0.000462
<i>pgp-5</i>	P-glycoprotein subclass of the transporters	6.33	1.63E-06	0.036451	0.000436
<i>his-64</i>	H4 histone	5.82	6.64E-08	0.001486	0.00014
F19G12.3	Protein related to argonaute and Dicer protein. PAZ family member	5.32	6.32E-07	0.014149	0.000308
<i>dhs-4</i>	Predicted mitochondrial short- chain dehydrogenase	5.26	1.75E-06	0.039247	0.000451
<i>acr-18</i>	Acetylcholine Receptor	5.10	1.01E-06	0.022558	0.000364
T24F1.4	Putative nuclear protein	4.97	4.85E-09	0.000108	2.71E-05
F56A4.11	Putative protein with at least 17 transmembrane domains.	4.91	7.85E-07	0.017563	0.000338
K07D4.4	Unknown function	4.85	1.72E-06	0.038371	0.000447
<i>his-68</i>	H2A histone	4.80	1.44E-07	0.003218	0.000179
W02B12.4	Putative protein containing carboxylesterase domain	4.66	2.05E-06	0.045815	0.000468
<i>his-8</i>	H2B histone	4.58	2.17E-07	0.004857	0.000203
<i>ser-4</i>	G-protein-coupled 7- transmembrane domain serotonin receptor	4.45	8.51E-07	0.019039	0.000353
<i>his-45</i>	H3 histone	4.44	1.02E-07	0.002271	0.00014
Y71D11A.3a	Predicted 2-amino-3- carboxylmuconate-6- semialdehyde decarboxylase	4.44	1.07E-07	0.002385	0.00014
<i>sri-40</i>	Serpentine receptor, class I.	4.25	1.51E-06	0.033853	0.000423
W02D9.10	Unknown function	4.24	1.77E-06	0.039649	0.000451
<i>ugt-39</i>	UDP-glucuronosyl transferase	4.18	1.86E-06	0.04155	0.000462
W01A11.7	Unknown function	4.06	1.28E-06	0.028609	0.000387
F40E3.5	Putative protein containing serine/threonine specific protein phosphatase domain	3.81	1.00E-06	0.022383	0.000364
<i>his-7</i>	H2A histone	3.81	1.62E-08	0.000363	7.26E-05

<i>his-61</i>	H2A histone	3.80	3.36E-07	0.007527	0.000239
W10G11.19	Unknown function	3.78	1.83E-06	0.04083	0.000459
<i>gst-24</i>	Glutathione S-transferase.	3.71	1.04E-06	0.023163	0.000364
<i>feh-1</i>	Ortholog of mammalian FE65.	3.66	1.16E-10	2.59E-06	2.59E-06
<i>his-62</i>	H2B histone	3.60	2.56E-08	0.000574	9.56E-05
<i>acdH-2</i>	Acyl CoA dehydrogenase	3.55	3.31E-07	0.007411	0.000239
<i>his-67</i>	H4 histone	3.54	1.35E-06	0.030139	0.000397
<i>mif-4</i>	Macrophage migration inhibitory factor related protein	3.46	3.52E-07	0.007885	0.000239
<i>his-4</i>	H2B histone	3.42	1.75E-07	0.003909	0.000195
W04B5.3a	Unknown function	3.35	5.64E-07	0.012606	0.000306
Y71H2AM.10	GBP protein like	3.35	1.16E-06	0.025877	0.000364
Y46G5A.19	Putative spermidine synthase	3.32	3.49E-07	0.007809	0.000239
<i>mrp-8</i>	Multidrug Resistance Protein family member.	3.19	3.21E-07	0.007186	0.000239
T20G5.4	Putative membrane protein, family 8 (53.1 kD)	3.18	4.56E-07	0.010191	0.000275
F43D9.1	Putative patched family member Delta/Serrate/Lag-2 domain protein.	3.17	6.02E-07	0.013458	0.000306
<i>dsl-3</i>	SKp1 related protein	3.07	1.21E-06	0.027111	0.000371
<i>skr-11</i>	Predicted immunoglobulin-like and EGF-like domain containing protein	3.00	1.94E-06	0.043481	0.000462
F28E10.2	Cuticular collagen family member	2.70	1.03E-07	0.002307	0.00014
<i>dpy-9</i>	Unknown function	2.69	5.84E-07	0.013055	0.000306
F23C8.13	Insulin related protein	2.66	5.46E-07	0.012222	0.000306
<i>ins-17</i>	Putative nuclear protein	2.61	9.48E-08	0.00212	0.00014
H14A12.5	Predicted spinster-like family member	2.59	2.63E-07	0.005874	0.000225
C39E9.10	Unknown function	2.57	7.26E-07	0.016238	0.000334
C09F5.2	Putative nuclear protein	2.55	1.00E-06	0.022469	0.000364
C56G7.3	Putative protein with at least 7 transmembrane domains	2.53	1.12E-06	0.024947	0.000364
C14A6.2	MAP kinase activated protein kinase	2.52	7.27E-07	0.016273	0.000334
<i>mak-2</i>	Predicted diacylglycerol O-acyltransferase 2 family member	2.40	5.12E-08	0.001146	0.000127
F59A1.10	I Kappa B homolog. encodes an ortholog of human BCL3 that physically interacts with the checkpoint protein MRT-2	2.40	9.88E-08	0.00221	0.00014
<i>ikb-1</i>	Metridin-like ShK toxin	2.32	1.95E-06	0.043652	0.000462
F23B12.4	Putative endoplasmic reticulum protein, with at least 4 transmembrane domains	2.31	2.58E-07	0.005768	0.000225
F39B2.8	Putative cytoplasmic protein	2.29	1.64E-06	0.036658	0.000436
F32B5.6a	H2A histone	2.27	1.48E-06	0.033133	0.000423
<i>his-35</i>	Unknown function	2.27	4.13E-07	0.009231	0.000256
Y57A10A.26	Unknown function	2.24	1.37E-06	0.030733	0.000399
C34B2.11	Unknown function	2.20	1.13E-06	0.025212	0.000364

Y66D12A.24	Putative protein, with at least 2 transmembrane domains	2.18	1.93E-06	0.043134	0.000462
<i>atg-1</i>	Amino acid transporter glycoprotein subunit.	2.17	4.72E-07	0.010567	0.000278
Y18H1A.9	Predicted carboxypeptidase a2 precursor family member	2.10	1.68E-07	0.003763	0.000195
C15F1.2	Putative secreted or extracellular protein precursor, with 2 coiled coil-4 domains	1.94	9.23E-07	0.020645	0.000358
C13B9.1	Putative mitochondrial protein	1.94	2.89E-07	0.006473	0.000231
Y47G6A.22a	Predicted short-chain dehydrogenase/reductase	1.67	6.00E-07	0.013423	0.000306
ZK1307.9	Putative nuclear protein	1.56	1.96E-06	0.04393	0.000462

Type of file: table

Label: Supp Table 4

Filename: 2008 McColl JBC supplemental tabel 4.pdf

SUPPLEMENTAL TABLE IV

Gene ontologies (GO) for which average expression (across the probes of constituent genes) is associated with treatment with 10mM LiCl.

GO ID	GO description	Geo. mean ratio*	Raw p-value**	FWER***	FDR****
GO:0000786	nucleosome	1.35	6.04E-05	0.009789	0.005259
GO:0006334	nucleosome assembly	1.34	6.49E-05	0.010519	0.005259
GO:0008415	acyltransferase activity	0.88	0.000117	0.018875	0.006292
GO:0005783	endoplasmic reticulum	0.85	0.000158	0.025509	0.006377
GO:0007001	chromosome organization and biogenesis	1.32	0.000254	0.041187	0.008237

* Represents the geometric mean ratio of expression representing the average expression of probes of genes within over outside the GO.

** Based on two-sample t-test

*** Adjusted p-value based on family wise error rate control, using the Bonferroni method

**** Adjusted p-value based on the false discovery rate using the Benjamin-Hochberg Method

Type of file: table

Label: Supp Table 5

Filename: 2008 McColl JBC supplemental tabel 5.pdf

SUPPLEMENTAL TABLE V

Real time-PCR validation of transcripts identified from microarray analysis as responsive to 10mM LiCl.

Gene	Geo mean ratio	Fold	<i>p</i> value
<i>lea-1</i>	0.17	0.27	0.003
ZK813.1	0.19	0.75	0.075
WO9D6.4	15.58	42.64	7.40E-07
<i>pgp-6</i>	9.89	25.10	1.34E-05

Geometric mean ratio values are taken from microarray data (Supplemental Table III). Fold ($2^{\Delta\Delta C_T}$, where ΔC_T denotes the difference between the cycle-threshold (C_T) for 10mM LiCl treated versus Control samples) indicates relative fold change as assessed by real time-PCR. Statistical significance was defined using Student's *t*-test, where genes with $p < 0.05$ indicate confirmation by RT-PCR.