This journal is © The Royal Society of Chemistry 2012

Supplementary Information

- **Protein ID/QT Methods**
- **Table S1.** Proteins found de-regulated in 6 dpf zebrafish larvae exposed to 5 µg/L of MeHg.
- **Table S2.** Proteins found de-regulated in 6dpf zebrafish larvae exposed to 25 µg/L of MeHg.
- **Table S3.** References supporting the organ-specific location of proteins found deregulated upon 25 µg/L MeHg exposure based on in-situ hybridation studies.
- **Figure S1.** Number of identified proteins in the different SCX and IEF fractions in both biological replicates (ZFA and ZFB).
- **Figure S2.** Top network generated by IPA. The associated network functions include cellular assembly and organization; cellular function and maintenance; and cellular movement.
- **Figure S3**. Differences in the size of the swim bladder of control (A) and ZF exposed to 5 (B) and 25 µg/L (C) of MeHg.
- **Figure S4.** Morphological and histological analyses of the eye of control (A, D) and ZF exposed to 5 (B, E) and 25 µg/L (C, F) of MeHg .

This journal is © The Royal Society of Chemistry 2012

Protein ID/QT Methods

Protein identification and relative quantification were performed with the ProteinPilot™ software (version 3.0; ABSCIEX) using the Paragon™ algorithm as the search engine. Each MS/MS spectrum was searched against the NCBI *Danio rerio* database (released on 08/31/2010) containing 27587 entries, downloaded from ftp://ftp.ncbi.nih.gov/genomes/D_rerio/protein/. The target plus reversed protein database contained 55174 proteins. The search parameters allowed for cysteine modification by methyl methane-thiosulfonate and the 220 biological modifications programmed in the algorithm (i.e., phosphorylations, amidations, semitryptic fragments, etc.). The detected protein threshold (Unused ProtScore (conf)) in the software was set to 1.3 to achieve 95% confidence and identified proteins were grouped by the ProGroup algorithm (Applied Biosystems) to minimize redundancy. The bias correction option was executed so the ratios of each analysis were automatically normalized.

To compare the performance of each separation technique at the protein identification level, the complete set of raw data files of each approach was analysed independently using ProteinPilot, which includes Paragon and ProGroup algorithms). A comparison between the gi Accession numbers of identified proteins from SCX and IEF approaches was carried out using Venn diagrams (*Oliveros, J.C. (2007) VENNY.* An interactive tool for comparing lists with Venn Diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html).

The peptide and protein selection criteria for relative quantitation were performed as follows. Proteins were identified on the basis of having at least two peptides with an ion score above 95% confidence. Only peptides unique for a given protein were considered for relative quantitation, excluding those

This journal is © The Royal Society of Chemistry 2012

common to other isoforms or proteins of the same family. Among the identified peptides, some of them were excluded from the quantitative analysis for one of the following reasons: i) the peaks corresponding to the iTRAQ labels were not detected, ii) they were identified with low ID confidence (< 1.0%), iii) because of shared MS/MS spectra, when either the same peptide sequence is claimed by more than one protein or when more than one peptide is fragmented at the same time, iv) the peptide ratios where the sum of the signal-to-noise ratio for all of the peak pairs was <9, v) peptides with a combined feature probability less than 30%, including semi-tryptic peptides, peptides missing an iTRAQ reagent label, peptides with low probability modifications and peptides with large delta masses. The protein sequence coverage (95%) was estimated for specific proteins by the percentage of matching amino acids from the identified peptides having confidence greater than or equal to 95% divided by the total number of amino acids in the sequence.

Two quantitative estimates provided for each protein by the ProteinPilot^{TM} were utilized: the fold change ratios of differential expression between labelled protein extracts and the *P* value, representing the probability that the observed ratio is different to 1 by chance. To estimate and minimize the false discovery rate (FDR), defined as the percentage of decoy proteins identified against the total protein identification, a target-decoy search strategy was followed using a protein sequence database that combines forward and reverse amino acid orientation of every NCBI zebrafish protein sequence. The estimated FDR was less than 0.3% in both biological replicates, indicating high reliability in the proteins identified.

This journal is © The Royal Society of Chemistry 2012

The results were then exported into Excel for manual data interpretation. Although relative quantification and statistical analysis were provided by the ProteinPilot 3.0 software, an additional 1.5-fold change cutoff for all iTRAQ ratios (ratio <0.67 or >1.5) was selected to classify proteins as up- or down-regulated. Proteins with iTRAQ ratios below the low range (0.67) were considered to be under-expressed, while those above the high range (1.5) were considered overexpressed. The average ratio provided by ProteinPilot includes correction from experimental bias and is inversely weighted by the % Error on each distinct peptide. For further information about how the average ratio is calculated, please refer to the manual.

This journal is © The Royal Society of Chemistry 2012

Table S1. Proteins found de-regulated in 6 dpf zebrafish larvae exposed to 5 µg/L of MeHg.

Accession number (gi|)	Protein name	Common name	Peptides (95%)	iTRAQ ratio 5:Contr	PVMeHg 5:Contr
47550733	SERPINE1 mRNA binding protein 1	serbp1	6	2.905	0.008
18858587	elongation factor 1-alpha	eef1a	25	1.764	0.011
18858197	glutathione S-transferase pi	gstp1	11	2.129	0.0001
18859297	parvalbumin-2	pvalb2	18	1.677	0.0003
157787181	muscle creatine kinase b	ckmb	42	1.659	0.0053
33636707	parvalbumin 9	pvalb9	5	1.623	0.0062
55742597	eukaryotic translation initiation factor 5A-2	eif5a2	3	1.589	0.0355
31795559	troponin T3b, skeletal, fast isoform 2	tnnt3b	13	1.538	0.0102
18858947	keratin 4	krt4	58	1.537	0.0010
113678458	vitellogenin 2 isoform 1	vtg2	650	0.647	0.000
156713467	vitellogenin 7	vtg7	795	0.641	0.001
68448530	vitellogenin 5	vtg5	878	0.639	0.000
189523697	PREDICTED: titin b	ttnb	18	0.636	0.017
303227889	vitellogenin 6	vtg6	957	0.633	0.004
68444085	PREDICTED: microfibrillar-associated protein 4-like	mfap4	2	0.557	0.027
66472252	slow myosin heavy chain 1	smyhc1	21	0.539	0.005
189523699	PREDICTED: titin a	ttna	10	0.415	0.001
238776848	myosin, heavy polypeptide 1.2, skeletal muscle	myhz1.2	69	0.261	0.000

This journal is © The Royal Society of Chemistry 2012

Table S2. Proteins found de-regulated in 6dpf zebrafish larvae exposed to 25 µg/L of MeHg.

Accession number (gi|)	Protein name	Common name	Peptides (95%)	iTRAQ ratio 25:Contr	PVMeHg 25:Contr
189521249	PREDICTED: hypothetical protein		7	15.100	0.038
24308537	zona pellucida glycoprotein 2.4	zp2.4	20	7.442	0.000
47086603	ZPA domain containing protein isoform 2	si:dkeyp-50f7.2	9	7.234	0.002
41055329	acidic chitinase	chia	5	6.041	0.006
94536701	ZPA domain containing protein isoform 1	si:dkeyp-50f7.2	9	6.038	0.003
156739289	hypothetical protein LOC569326	im:6910535	4	5.993	0.001
125816799	PREDICTED: si:dkey-46g23.2	si:dkey-46g23.2	26	5.828	0.001
61651682	fibronectin 1b	fn1b	6	3.654	0.006
226442998	hatching enzyme 1b	he1b	8	3.115	0.002
288856329	myosin, heavy polypeptide 1.1, skeletal muscle	myhz1.1	47	3.049	0.000
238776848	myosin, heavy polypeptide 1.2, skeletal muscle	myhz1.2	69	2.304	0.000
50355968	calreticulin like	calrl	10	2.154	0.047
41054259	protein disulfide-isomerase A4	pdia4	12	2.136	0.017
82658290	high-mobility group box 2	hmgb2	10	2.105	0.017
292610077	PREDICTED: complement component 3	c3	5	2.082	0.027
53749651	peptidyl-prolyl cis-trans isomerase B	ppib	12	2.061	0.016
18858197	glutathione S-transferase pi	gstp1	11	2.046	0.001
18859107	nucleoside diphosphate kinase A	nme2b.1	8	2.007	0.003
125827065	PREDICTED: si:dkey-88l16.3	si:dkey-88l16.3	2	1.982	0.036
33636707	parvalbumin 9	pvalb9	5	1.948	0.041
292618718	PREDICTED: vitellogenin 3, phosvitinless	vtg3	61	1.862	0.000
41152406	FK506 binding protein 1A, 12kDa	fkbp1aa	4	1.861	0.029

71143098	tenascin C	tnc	3	1.845	0.036
40254675	lamin-B1	lmnb1	6	1.844	0.006
189523699	PREDICTED: titin a	ttna	10	1.832	0.007
82524272	complement component c3b	c3b	4	1.805	0.018
189535920	PREDICTED: filamin A, alpha	flna	30	1.802	0.000
41393111	ubiquitin carboxyl-terminal hydrolase isozyme L1	uchl1	12	1.793	0.016
292619135	PREDICTED: filamin B, like, partial	flnb	5	1.776	0.007
30410758	keratin, type I cytoskeletal 18	krt18	6	1.726	0.004
67972636	heterogeneous nuclear ribonucleoprotein A/B	hnrnpab	8	1.713	0.049
18859297	parvalbumin-2	pvalb2	18	1.619	0.006
56118638	profilin-2	pfn2l	4	1.618	0.004
41151982	major vault protein	mvp	8	1.595	0.017
55925229	ribose-5-phosphate isomerase	rpia	5	1.575	0.010
65301457	thioredoxin-dependent peroxide reductase, mitochondrial	prdx3	3	1.569	0.034
48597012	60S ribosomal protein L23a	rpl23a	4	1.566	0.004
41152461	60S ribosomal protein L7a	rpl7a	2	1.560	0.008
41386743	eukaryotic translation elongation factor 2b	eef2b	33	1.558	0.038
292611632	PREDICTED: hypothetical protein LOC325896	BX901973.4	5	1.557	0.045
68442739	PREDICTED: histone H4 replacement-like	his4r	13	1.549	0.024
41053774	endoplasmic reticulum resident protein 44	erp44	3	1.542	0.043
41152439	60S ribosomal protein L10a	rpl10a	5	0.666	0.006
71834286	apolipoprotein B, like	apobl	67	0.653	0.000
292619319	PREDICTED: retinol-binding protein 4, plasma-like	rbp4	2	0.653	0.015
45387573	parvalbumin isoform 1d	pvalb1	10	0.649	0.007
51571925	adenylate kinase isoenzyme 1	AK1	5	0.639	0.048
292627608	PREDICTED: ATPase, H+ transporting, lysosomal V1 subunit A	atp6v1a	2	0.639	0.008
41054770	WD repeat-containing protein 1	wdr1	2	0.634	0.007
48762657	alpha-enolase	eno1	9	0.625	0.020
54261787	interleukin enhancer-binding factor	ilf2	5	0.621	0.043

	2 homolog				
292617604	PREDICTED: crystallin, gamma C-like	crygc	7	0.609	0.018
18858425	keratin 5	krt5	16	0.595	0.008
41054746	glycine amidinotransferase, mitochondrial	gatm	2	0.590	0.043
47550793	NAD(P) transhydrogenase, mitochondrial	nnt	4	0.476	0.046
33504543	V-type proton ATPase subunit B, kidney isoform	atp6v1ba	3	0.467	0.022
62632719	hemoglobin beta embryonic-3	hbbe3	5	0.451	0.042
27545277	elongation factor 1-gamma	eef1g	8	0.407	0.035

This journal is © The Royal Society of Chemistry 2012

Table S3. References supporting organ-specific localization of proteins found de-regulated upon MeHg exposure based on in-situ hybridation studies.

Accession Number (gi)	Gene name	Protein Name	iTRAQ ratio	Location	Reference
189521249		hypothetical protein	15.10		
24308537	zp2.4	zona pellucida glycoprotein 2.4	7.44		
47086603	si:dkeyp-50f7.2	ZPA domain containing protein isoform 2	7.23		
41055329	chia	acidic chitinase	6.04	gut	Rauch, G.J., Lyons, D.A., Middendorf, I., Friedlander, B., Arana, N., Reyes, T., and Talbot, W.S. (2003) Submission and Curation of Gene Expression Data. ZFIN Direct Data Submission (http://zfin.org).
94536701	si:dkeyp-50f7.2	ZPA domain containing protein isoform 1	6.04		
156739289	im:6910535	hypothetical protein LOC569326	5.99		
125816799	si:dkey-46g23.2	si:dkey-46g23.2	5.83		
61651682	fn1b	fibronectin 1b	3.65	notocord, liver, epidermis	Thisse, B, Pfumio, S., Fürthauer, M., Loppin B., Heyer, V., Degrave, A., Woehl, R., Lux, A., Steffan, T., Charbonnier, X.Q. and Thisse, C. Expression of the zebrafish genome during embryogenesis. ZFIN on-line publication, 2001
226442998	he1b	hatching enzyme 1b	3.12		
288856329	myhz1.1	myosin, heavy polypeptide 1.1, skeletal muscle	3.05	fast muscle	Rauch, G.J., Lyons, D.A., Middendorf, I., Friedlander, B., Arana, N., Reyes, T., and Talbot, W.S. (2003) Submission and Curation of Gene Expression Data. ZFIN Direct Data Submission (http://zfin.org).

This journal is © The Royal Society of Chemistry 2012

238776848	myhz1.2	myosin, heavy polypeptide 1.2, skeletal muscle	2.30	fast muscle	Kotani, T., and Kawakami, K. (2008) misty somites, a maternal effect gene identified by transposon-mediated insertional mutagenesis in zebrafish that is essential for the somite boundary maintenance. Dev. Biol. 316(2): 383-396.
50355968	calrl	calreticulin like	2.15		
41054259	pdia4	protein disulfide-isomerase A4	2.14		
82658290	hmgb2	high-mobility group box 2	2.11	expressed ubiquitously	Thisse, B., Thisse, C. (2004) Fast Release Clones: A High Throughput Expression Analysis. ZFIN Direct Data Submission (http://zfin.org)
292610077	c3	complement component 3	2.08	liver	Thisse, B., Thisse, C. (2004) Fast Release Clones: A High Throughput Expression Analysis. ZFIN Direct Data Submission (http://zfin.org).
53749651	ppib	peptidyl-prolyl cis-trans isomerase B	2.06		
18858197	gstp1	glutathione S-transferase pi	2.05	liver	Cheng, W., Guo, L., Zhang, Z., Soo, H.M., Wen, C., Wu, W., and Peng, J. (2006) HNF factors form a network to regulate liver-enriched genes in zebrafish. Dev. Biol. 294(2): 482-496.
18859107	nme2b.1	nucleoside diphosphate kinase A	2.01	whole body, heart	Hippe, H.J., Wolf, N.M., Abu-Taha, I., Mehringer, R., Just, S., Lutz, S., Niroomand, F., Postel, E.H., Katus, H.A., Rottbauer, W., and Wieland, T. (2009) The interaction of nucleoside diphosphate kinase B with Gbetagamma dimers controls heterotrimeric G protein function. Proc. Natl. Acad. Sci. USA 106(38): 16269-16274.
125827065	si:dkey-88l16.3	si:dkey-88l16.3	1.98		
33636707	pvalb9	parvalbumin 9	1.95		

This journal is © The Royal Society of Chemistry 2012

292618718	vtg3	vitellogenin 3, phosvitinless	1.86		
41152406	fkbp1aa	FK506 binding protein 1A, 12kDa	1.86		
71143098	tnc	tenascin C	1.85		
40254675	lmnb1	lamin-B1	1.84		
189523699	ttna	titin a	1.83	musculature system, myotome, pectoral fin musculature	Thisse, B., Thisse, C. (2004) Fast Release Clones: A High Throughput Expression Analysis. ZFIN Direct Data Submission (http://zfin.org)
82524272	c3b	complement component c3b	1.81	liver	Wang, Z., Zhang, S., and Wang, G. (2008) Response of complement expression to challenge with lipopolysaccharide in embryos/larvae of zebrafish Danio rerio: Acquisition of immunocompetent complement. Fish Shellfish Immunol. 25(3): 264-270
189535920	flna	filamin A, alpha	1.80		
41393111	uchl1	ubiquitin carboxyl-terminal hydrolase isozyme L1	1.79		
292619135	flnb	filamin B, like, partial	1.78		
30410758	krt18	keratin, type I cytoskeletal 18	1.73		
67972636	hnrnpab	heterogeneous nuclear ribonucleoprotein A/B	1.71	brain, lateral line system	Rauch, G.J., Lyons, D.A., Middendorf, I., Friedlander, B., Arana, N., Reyes, T., and Talbot, W.S. (2003) Submission and Curation of Gene Expression Data. ZFIN Direct Data Submission (http://zfin.org)
18859297	pvalb2	parvalbumin-2	1.62		
56118638	pfn2l	profilin-2	1.62		

41151982	mvp	major vault protein	1.60	blastema mesenchymal cell	Yoshinari, N., Ishida, T., Kudo, A., and Kawakami, A. (2009) Gene expression and functional analysis of zebrafish larval fin fold regeneration. Dev. Biol. 325(1): 71-81
55925229	rpia	ribose-5-phosphate isomerase	1.58		
65301457	prdx3	thioredoxin-dependent peroxide reductase, mitochondrial	1.57		
48597012	rpl23a	60S ribosomal protein L23a	1.57		
41152461	rpl7a	60S ribosomal protein L7a	1.56		
41386743	eef2b	eukaryotic translation elongation factor 2b	1.56	epidermis	Rauch, G.J., Lyons, D.A., Middendorf, I., Friedlander, B., Arana, N., Reyes, T., and Talbot, W.S. (2003) Submission and Curation of Gene Expression Data. ZFIN Direct Data Submission (http://zfin.org).
292611632	BX901973.4	hypothetical protein LOC325896	1.56		
68442739	his4r	histone H4 replacement-like	1.55		
41053774	erp44	endoplasmic reticulum resident protein 44	1.54		
41152439	rpl10a	60S ribosomal protein L10a	-1.49		
71834286	apobl	apolipoprotein B, like	-1.54		
292619319	rbp4	retinol-binding protein 4, plasma-like	-1.54	liver	Cheng, W., Guo, L., Zhang, Z., Soo, H.M., Wen, C., Wu, W., and Peng, J. (2006) HNF factors form a network to regulate liver-enriched genes in zebrafish. Dev. Biol. 294(2): 482-496
45387573	pvalb1	parvalbumin isoform 1d	-1.54	musculature system, myotome	Thisse, C., and Thisse, B. (2005) High Throughput Expression Analysis of ZF-Models Consortium Clones. ZFIN Direct Data Submission (http://zfin.org).
51571925	AK1	adenylate kinase isoenzyme 1	-1.56		

This journal is © The Royal Society of Chemistry 2012

292627608	atp6v1a	ATPase, H+ transporting, lysosomal V1	-1.56	central nervous system	Thisse, B., Thisse, C. (2004) Fast Release Clones: A High Throughput Expression Analysis. ZFIN Direct Data Submission (http://zfin.org).
41054770	wdr1	WD repeat-containing protein 1	-1.59		
48762657	eno1	alpha-enolase	-1.59		
54261787	ilf2	interleukin enhancer-binding factor 2	-1.61	blastema m esenchymal cell	Yoshinari, N., Ishida, T., Kudo, A., and Kawakami, A. (2009) Gene expression and functional analysis of zebrafish larval fin fold regeneration. Dev. Biol. 325(1): 71-81.
292617604	crygc	crystallin, gamma C-like	-1.64		
18858425	krt5	keratin 5	-1.67	epidermal cell	Reischauer, S., Levesque, M.P., Nüsslein-Volhard, C., and Sonawane, M. (2009) Lgl2 executes its function as a tumor suppressor by regulating ErbB signaling in the zebrafish epidermis. PLoS Genet. 5(11): e1000720
41054746	gatm	glycine amidinotransferase, mitochondrial	-1.69	Intestine, liver, pancreas	Cheng, W., Guo, L., Zhang, Z., Soo, H.M., Wen, C., Wu, W., and Peng, J. (2006) HNF factors form a network to regulate liver-enriched genes in zebrafish. Dev. Biol. 294(2): 482-496.
47550793	nnt	NAD(P) transhydrogenase, mitochondrial	-2.08		
33504543	atp6v1ba	V-type proton ATPase subunit B	-2.13		
62632719	hbbe3	hemoglobin beta embryonic-3	-2.22		
27545277	eef1g	elongation factor 1-gamma	-2.44	whole organism	Thisse, B., Thisse, C. (2004) Fast Release Clones: A High Throughput Expression Analysis. ZFIN Direct Data Submission (http://zfin.org).

This journal is © The Royal Society of Chemistry 2012

Fig. S1

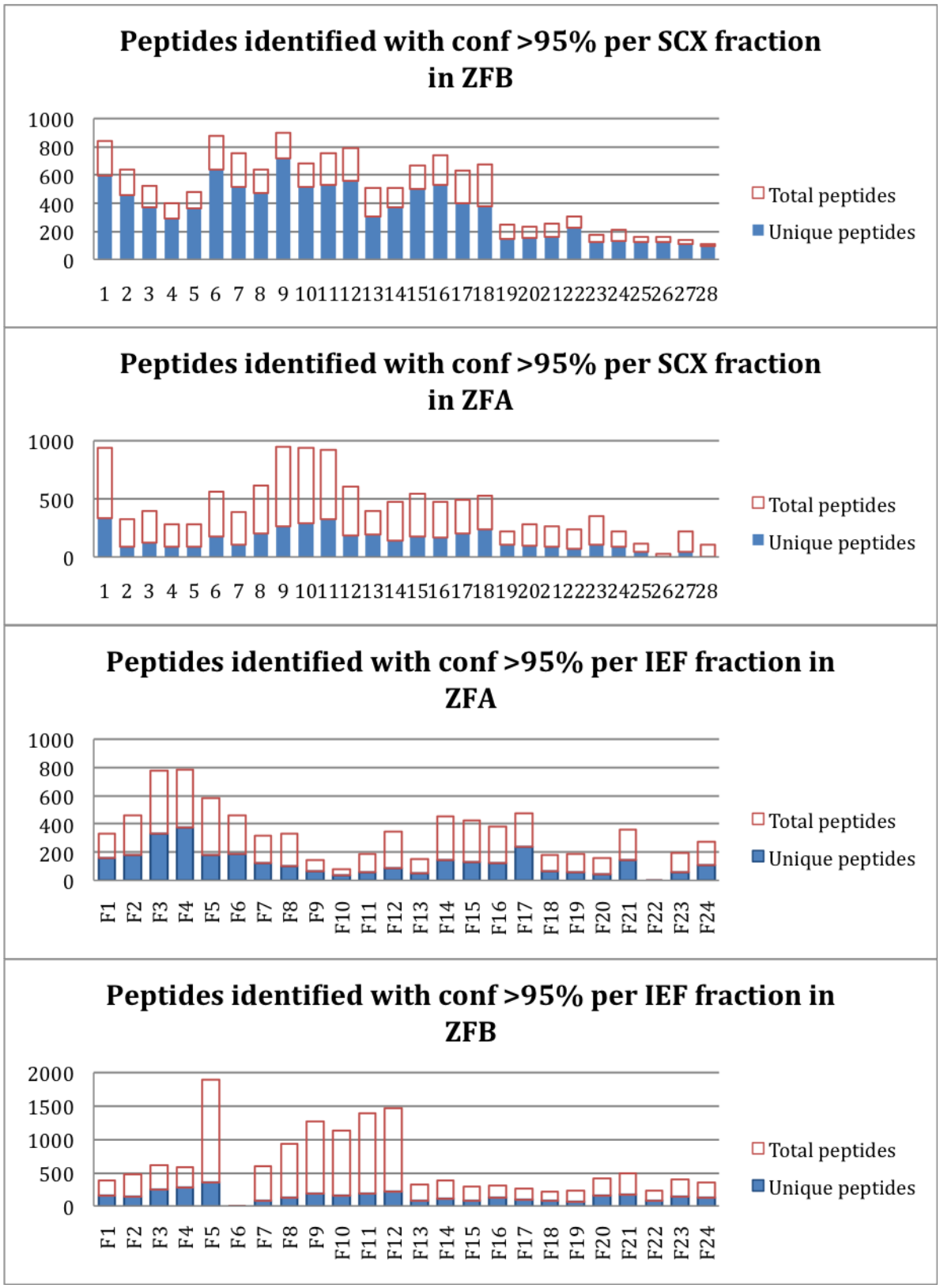
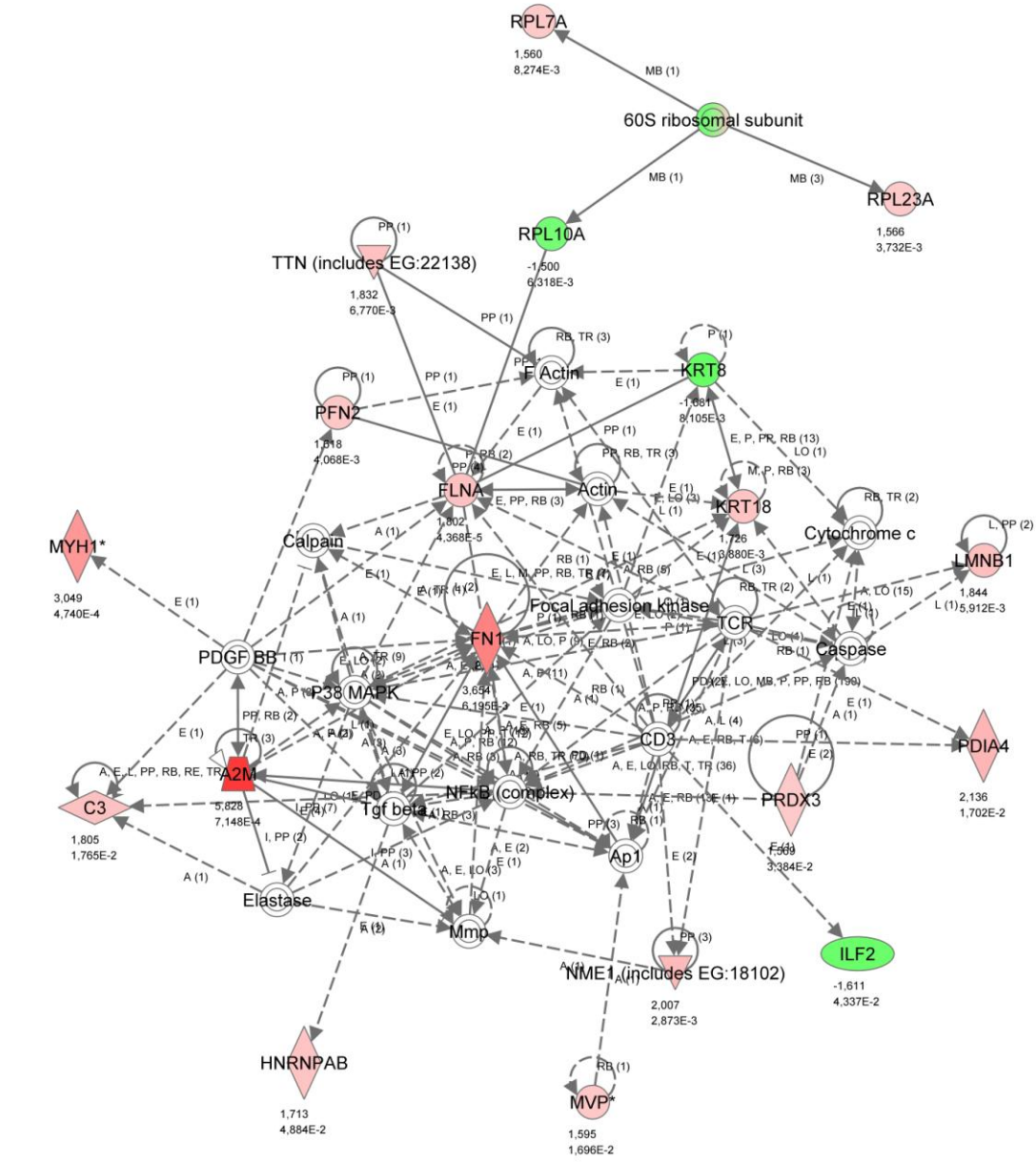


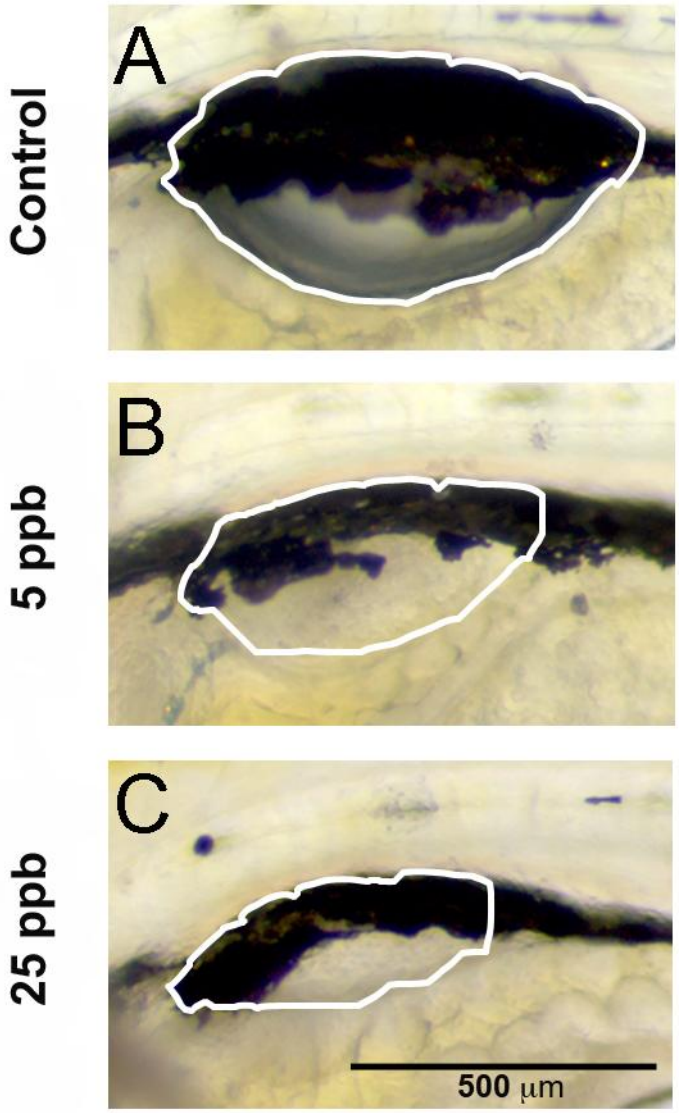
Fig. S2



© 2000-2012 Ingenuity Systems, Inc. All rights reserved.

This journal is © The Royal Society of Chemistry 2012

Fig. S3



This journal is © The Royal Society of Chemistry 2012

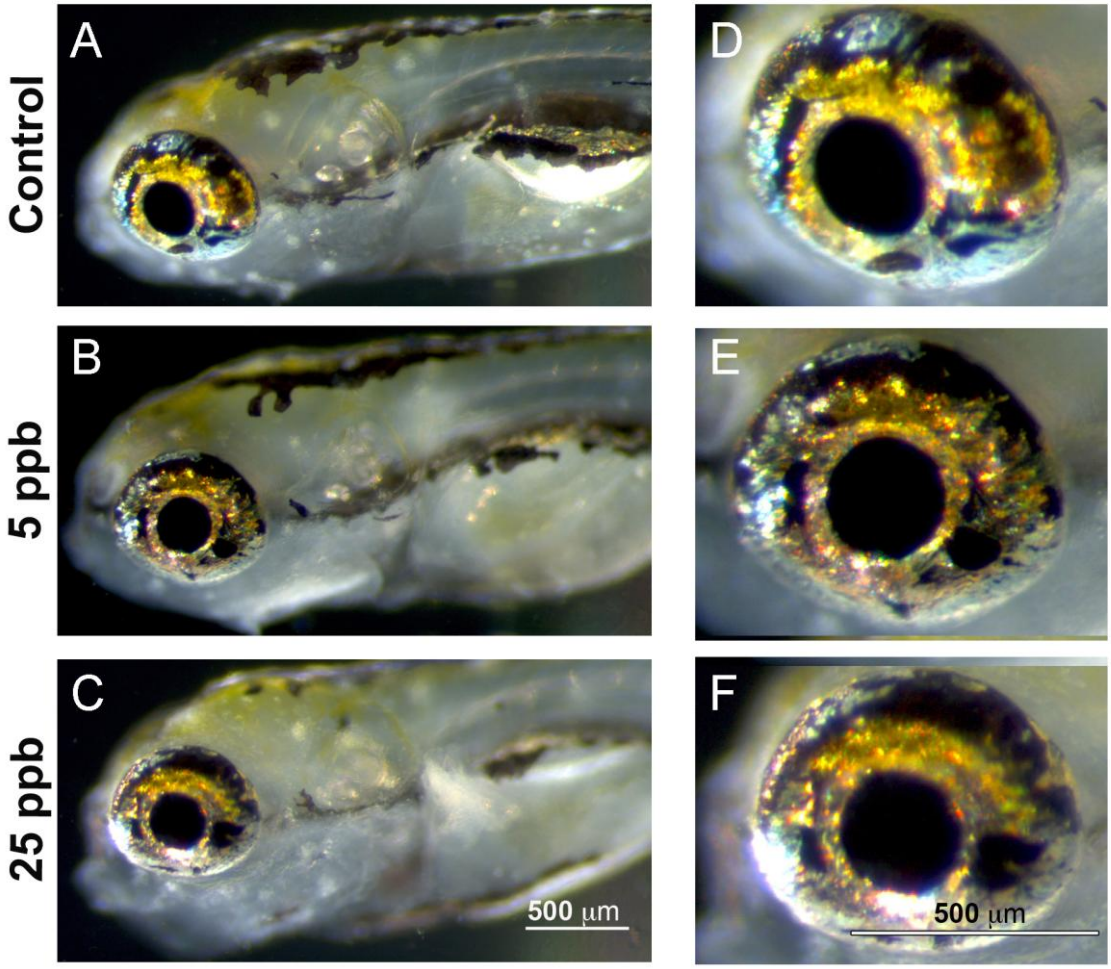



Fig. S4