Supplementary Information

Title: Proteomic profiles of white sucker (*Catostomus commersonii*) sampled from within the Thunder Bay Area of Concern reveal up-regulation of proteins associated with tumor formation and exposure to environmental estrogens.

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Summary:

Table S1	Page S1
Table S2	Page S2
Table S3	Page S2
Figure S1	Page S3
Figure S2	Page S4
Figure S3	Page S5

Table S1. Method variables used for separation and detection by LC-MS/MS.

Injector (h-ALS-SL, Model G1367C)	
Injection volume	2 µl
Injection type	Needle wash
Wash time	10 sec
Flush Out factor	5
Wash location	FlushPort
Wash solution	60% ACN 0.1 % Formic Acid
Pump (BinPump-SL, Model G1312B)	
Stop time	50 min
Post time	OFF
Flow	0.100 ml/min
Solvent A	5% ACN 0.1% Formic Acid
Solvent B	95% ACN 0.1% Formic Acid
Source (Dual ESI)	
Polarity	Positive
Gas Temperature	325 °C
Gas Flow	8 l/min
Nebulizer	35 psi
VCap	4500
Fragmentor	180
Skimmer	65
Octopole RF Peak	750
Detector (Q-TOF Mass Spectrometer)	
Mode	Targeted MS2
MS scan range	200-3000
MS scan rate	1
MS/MS scan range	200-3000
MS/MS scan rate	1
Isolation width	Narrow
Ramped Collision Energy	Slope: 3 Offset: 2

Targeted MS/MS

Precursor m/z	Z	Retention Time Window (min)	Acquisition time (ms/spectra)
449.254	3	10-14	974.5
451.26	3	10-14	974.5

Table S2. Spectrum Mill MS/MS Search Statistics

Location	*n*	*filtered spectra*	*validated spectra*	*% yield*	*total unique proteins*	*x̄ proteins per replicate*	*total IPA identified*
Gravel male	8	13695	564	4.1	151	48	112
TBH male	8	12556	704	5.6	209	67	127
Kam male	8	13802	673	4.9	185	60	117
Gravel female	7	11059	470	4.2	132	45	91
TBH female	9	15924	692	4.3	190	59	118
Kam female	9	11697	708	6.1	219	64	151

Table S3. P-values obtained for functional analyses using Fisher's one-tailed exact test; $p < 0.05$ is considered a significant association.

	Males			**females**		
	Gravel	*TBH*	*Kam*	*Gravel*	*TBH*	*Kam*
Genetic Disorder	6.19E-05	1.06E-05	4.11E-06	1.36E-04	2.38E-04	7.82E-07
Cancer, tumorigenesis	5.98E-06	1.04E-05	1.55E-06	6.76E-04	8.93E-07	1.41E-08
Tissue Morphology	3.74E-05	1.21E-03	4.92E-04	2.28E-04	8.64E-05	8.70E-07
Small Molecule Biochemistry	3.08E-04	2.29E-06	1.15E-04	2.66E-04	3.95E-04	1.83E-04
DNA Replication, Recombination and Repair	8.53E-04	2.73E-05	6.24E-03	9.59E-03	2.98E-03	3.95E-04
Gastrointestinal Disease	1.00E-02	3.44E-04	8.11E-04	1.27E-03	5.05E-04	2.40E-03
Molecular transport	9.39E-06	1.90E-05	1.29E-05	5.90E-06	1.37E-04	2.92E-05
Reproductive System Disease	3.45E-04	1.96E-03	2.13E-04	3.70E-03	3.97E-05	2.61E-04
Neurological Disease	1.66E-03	1.49E-04	1.28E-03	2.00E-03	1.27E-04	2.01E-03
Immunological Disease	1.50E-04	1.17E-04	2.18E-04	3.32E-04	2.19E-05	5.59E-05
Cell Cycle	8.53E-04	4.21E-06	2.17E-03	2.28E-05	1.14E-03	1.26E-04

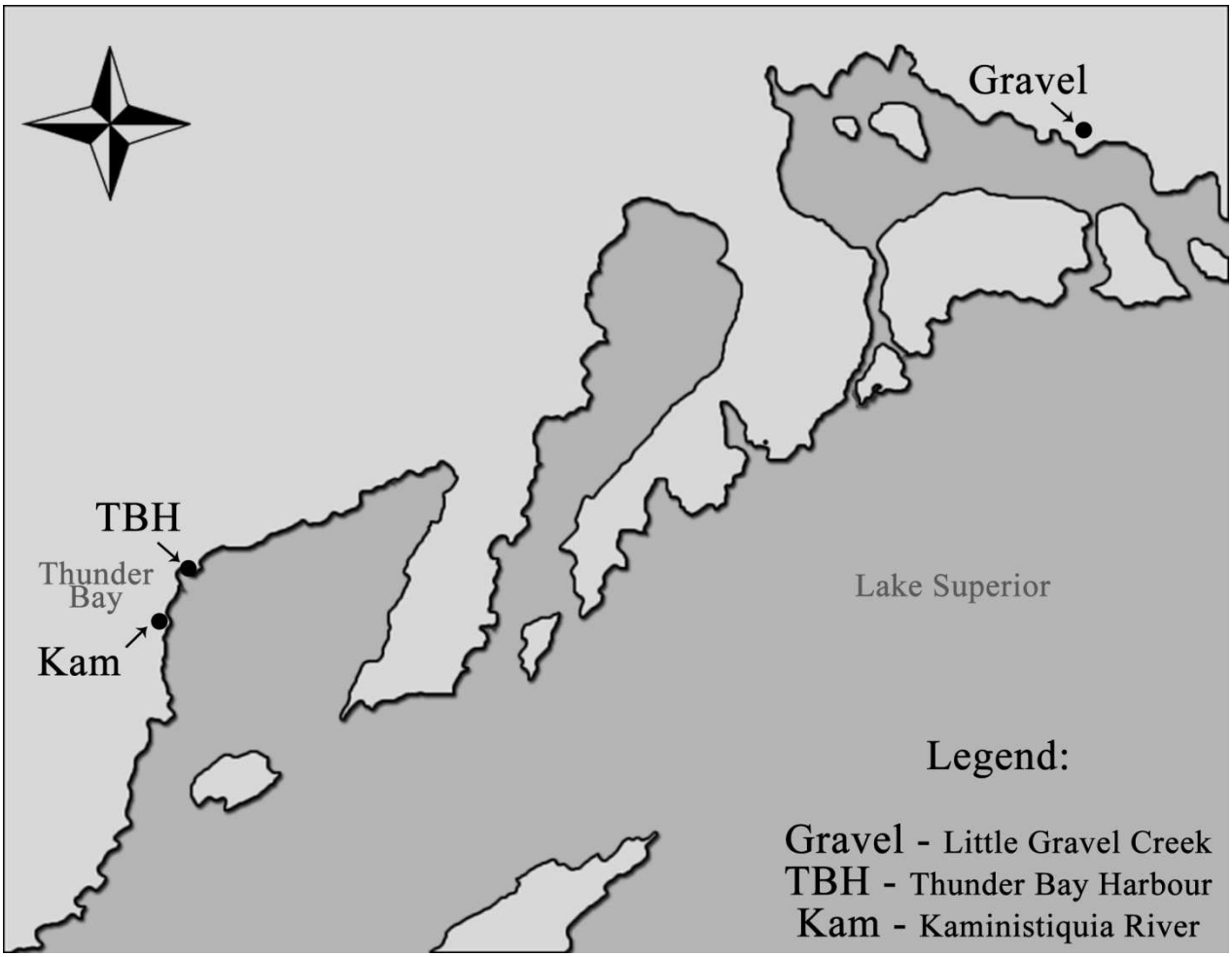

Figure S1. Map of white sucker sampling (Kam, TBH) and reference (Gravel) locations in the Thunder Bay Area of Concern.

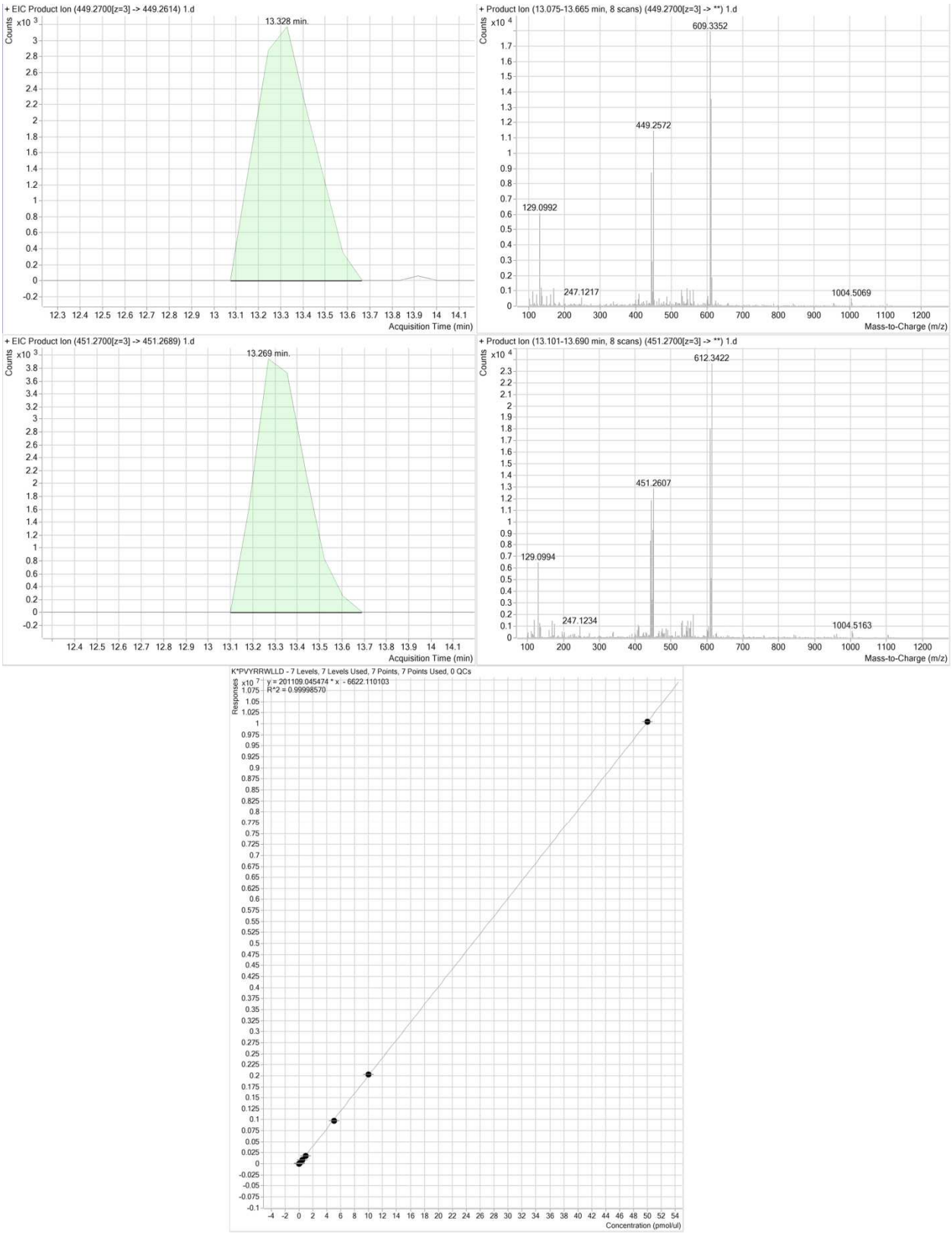

Figure S2. Example chromatograms, spectra, and linear range.

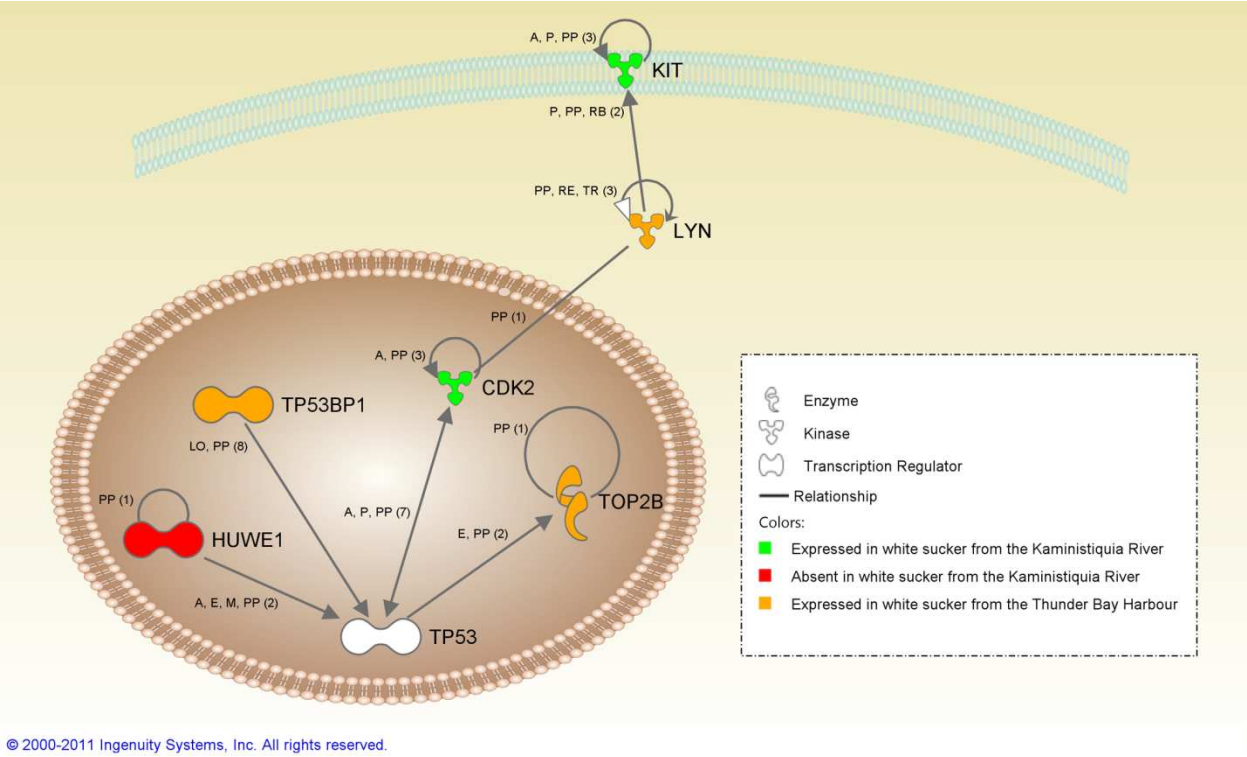

Figure S3. Relationship(s) between DNA topoisomerase 2-beta (TOP2B), tumour suppressor p53-binding protein 1 (TP53BP1), mast/stem cell growth factor receptor (KIT), cyclin-dependent kinase 2 (CDK2), E3 ubiquitin-protein ligase (HUWE1), and tyrosine-protein kinase (LYN) to the cellular tumour antigen p53 (TP53). PP = protein-protein interaction, E = expression, A = activation, M = modification, P = phosporylation, RB = regulation of binding, LO = localization, RE = reaction, TR = translocation.