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Supplemental information

Comparative transcriptomic analysis of *in situ* and onboard fixed deep-sea limpets reveals sample preparation-related differences

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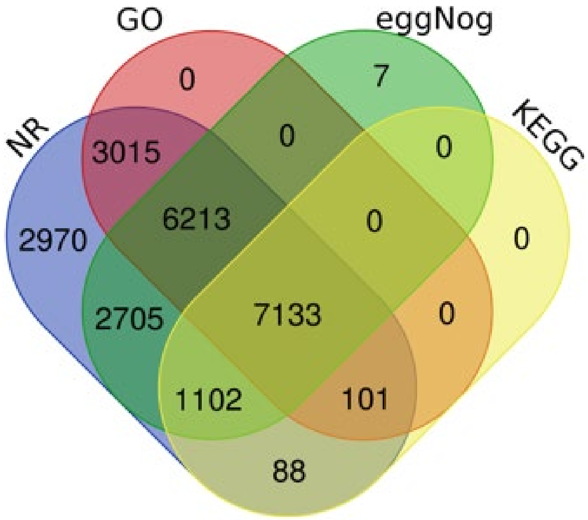

Figure S1. A Veen diagram showing the gene annotation results against four public databases. Related to Table 1,Table 2, Figure 3 and Figure 5.

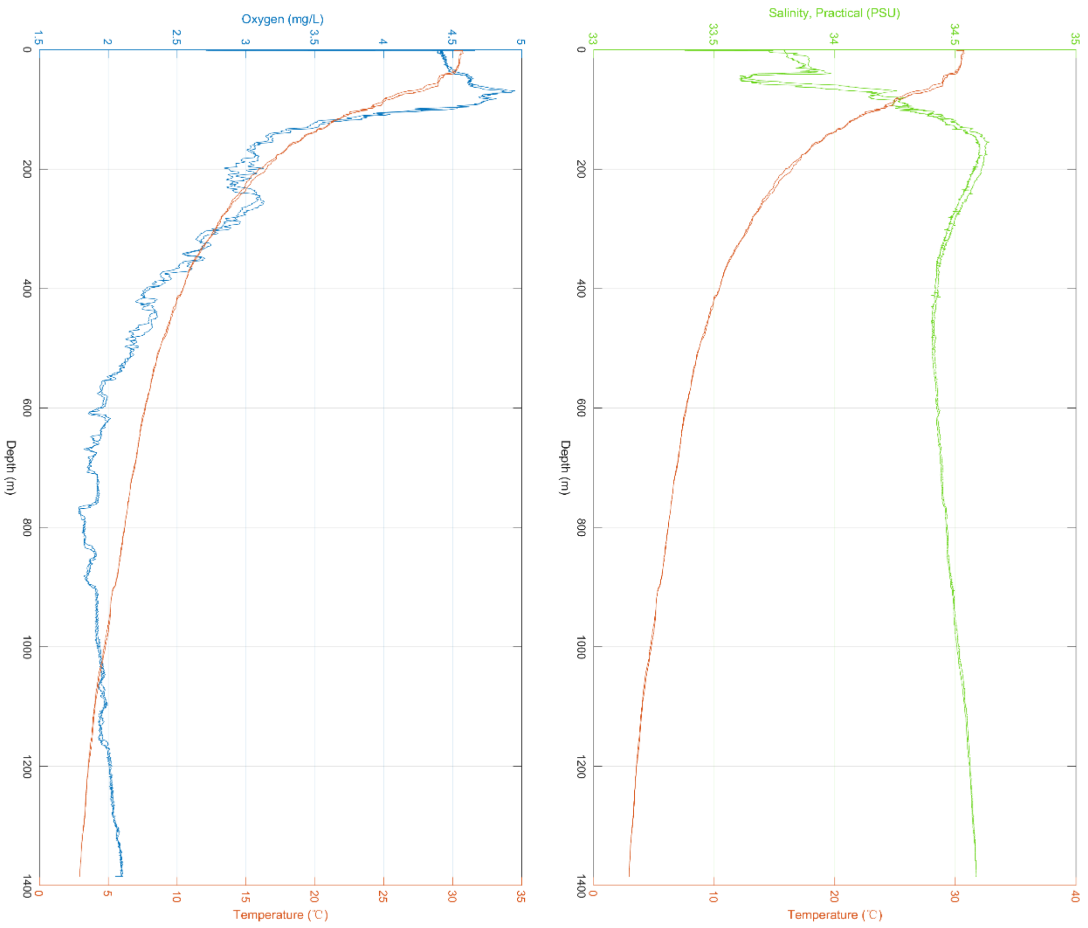

Figure S2. The CTD data showing the variation curve of salinity, temperature, and dissolved oxygen with the depth at the sampling site. Related to Figure 1 and STAR Methods.

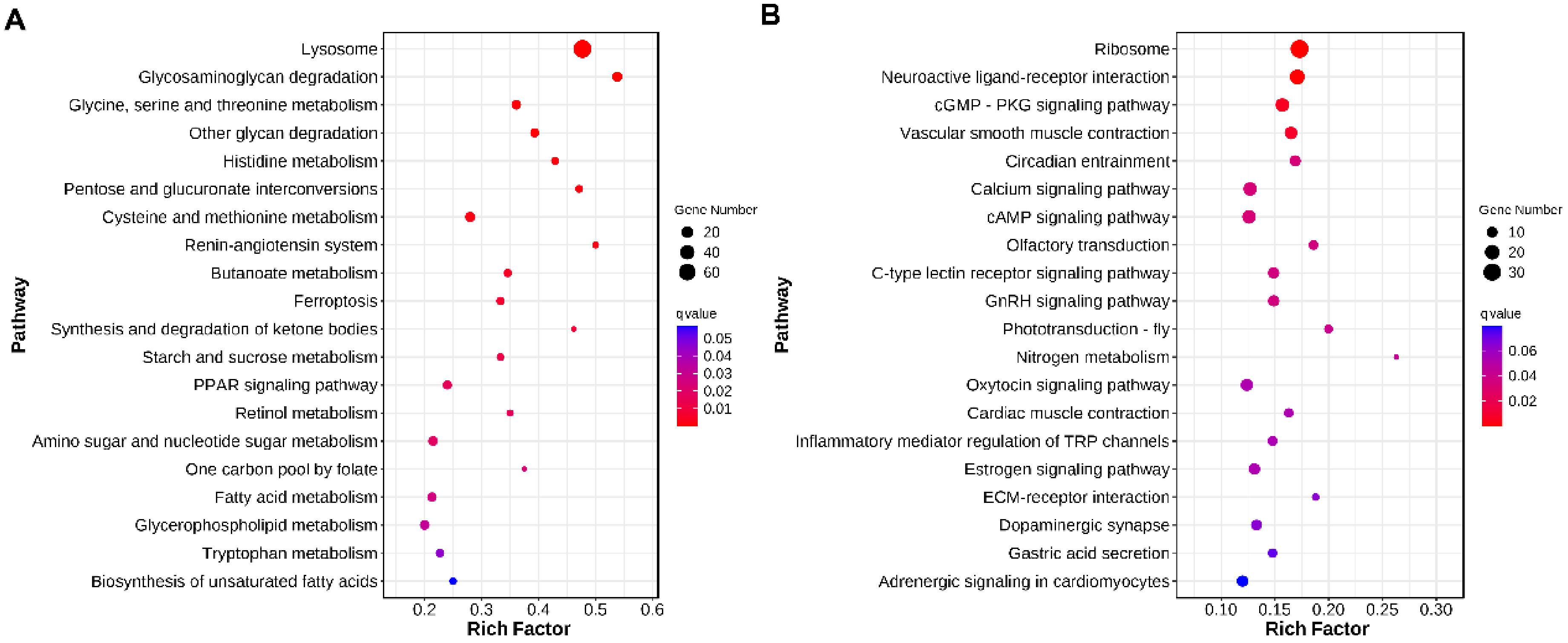

Figure S3. Separate KEGG enrichment analysis of down-regulated transcripts (A) and up-regulated transcripts (B) between the *in situ* and onboard fixed groups. Related to Figure 3.

Table S1. Summary of the transcriptome sequencing, assembly and annotation results. Related to Figure 2.

Sequencing	***In situ* fixation**				**Onboard fixation**		
Individual	ins1	ins2	ins3	ins4	non1	non2	non3
Clean reads number	85,780,516	100,772,664	99,217,744	94,157,418	75,073,948	100,332,550	93,123,332
Assembly Statistics							
Total reads				648,458,172			
Transcript number				27,674			
Transcript mean length (nt)				2,393			
Transcript N50 (nt)				3,610			
Functional Annotation							
NR				23,327			
KEGG				8,424			
Gene Ontology				16,462			
EggNog				17,160			

Table S2. List of differentially expressed collagen-encoding transcripts. Related to Table 1.

ID	ins_average	non_average	logFC	FDR	NR
TRINITY_DN2739_c0_g2_i7	374.505585	3291.004708	-2.438092	0.005967436	short-chain collagen C4-like
TRINITY_DN66877_c0_g2_i1	188.8543038	2795.193084	-3.031169	0.000688849	short-chain collagen C4-like
TRINITY_DN1166_c2_g1_i3	119.376141	572.1078237	-1.733253	0.010574941	putative procollagen type VI alpha 4
TRINITY_DN2445_c0_g1_i2	101.471243	4047.630077	-4.092714	1.52155E-05	short-chain collagen C4-like
TRINITY_DN31486_c0_g1_i2	89.41657	0.723511667	7.169146	4.93475E-67	collagen alpha-1(XIV) chain
TRINITY_DN3306_c0_g2_i5	52.20564825	1762.778486	-4.059277	1.18784E-06	collagen alpha-5(VI) chain-like isoform X2
TRINITY_DN11657_c0_g1_i6	40.029205	10.341137	2.0655507	0.0396453	short-chain collagen C4
TRINITY_DN9869_c0_g1_i9	26.5620095	1.254437	4.7293103	2.76305E-21	short-chain collagen C4-like
TRINITY_DN3471_c0_g1_i6	17.360009	244.1116303	-3.241606	1.20004E-11	collagen alpha-1(I) chain-like isoform X1
TRINITY_DN3306_c0_g1_i1	15.27345425	204.829712	-2.97146	2.25933E-05	collagen alpha-5(VI) chain-like isoform X1
TRINITY_DN1992_c0_g1_i4	14.51716875	105.9711457	-2.265902	2.41773E-05	collagen alpha-6(VI) chain-like
TRINITY_DN1159_c0_g1_i1	14.49481175	160.2004253	-2.80572	2.73032E-05	collagen alpha-2(IV) chain-like
TRINITY_DN142003_c0_g1_i1	11.807539	135.3141027	-2.799168	9.12594E-05	collagen alpha-5(IV) chain-like
TRINITY_DN49793_c0_g2_i3	11.5652965	301.6931797	-3.800327	3.65765E-07	collagen alpha-1(I) chain-like
TRINITY_DN397_c0_g1_i1	9.80269975	2.815728	2.2775464	7.30999E-07	collagen alpha-4(VI) chain-like isoform X1
TRINITY_DN5487_c0_g1_i1	8.3963765	48.45862233	-1.826645	0.020902696	collagen alpha-3(VI) chain-like isoform X11
TRINITY_DN2445_c0_g1_i11	3.68032625	74.90905333	-3.755026	3.90263E-13	short-chain collagen C4
TRINITY_DN2739_c0_g3_i1	3.3063385	258.5454493	-5.733037	9.21306E-55	short-chain collagen C4-like
TRINITY_DN2445_c0_g1_i3	2.84928825	130.43682	-4.958833	6.48615E-23	short-chain collagen C4
TRINITY_DN16191_c0_g3_i4	1.01123675	3.585083333	-1.520571	0.02751357	collagen alpha-4(VI) chain
TRINITY_DN80209_c0_g1_i1	0.326606	5.353581667	-3.44875	1.52155E-05	short-chain collagen C4-like
TRINITY_DN23676_c0_g1_i5	0.10889075	0.889313333	-2.354926	0.030902781	putative collagen alpha-1(XXVII) chain A-like
TRINITY_DN280056_c0_g1_i1	0	1.040623667	-3.646896	0.018412965	collagen alpha-1(I) chain-like

Table S3. List of fatty acid desaturase- and fatty acid elongation protein-encoding transcripts. Related to Figure 4.

ID	ins_average	non_average	logFC	FDR	NR
TRINITY_DN8681_c2_g1_i2	79.1281105	44.16025067	1.363289442	0.022497794	acyl-CoA desaturase-like
TRINITY_DN1131_c0_g1_i1	13.332562	2.634848333	2.568548318	3.68785E-07	fatty acid desaturase 6-like
TRINITY_DN27677_c0_g4_i1	9.88089425	4.811776	1.519700259	0.023147565	omega-3 desaturase-like A
TRINITY_DN34195_c0_g1_i2	6.38398875	2.152945	2.001986541	0.000873805	omega-3 desaturase-like B
TRINITY_DN4934_c0_g4_i1	30.8760765	18.28340633	1.184519027	1.34709E-06	putative fatty acid elongation protein 3
TRINITY_DN4934_c0_g3_i1	23.073005	7.576264	1.957399036	3.52743E-11	putative fatty acid elongation protein 3
TRINITY_DN9376_c0_g2_i1	10.322831	4.145861667	1.630677057	0.012182098	putative fatty acid elongation protein 3

Table S4. List of toxin- and cysteine-rich venom protein-encoding transcripts. Related to Figure 4.

ID	ins_average	non_average	logFC	FDR	NR
TRINITY_DN1545_c0_g1_i11	263.584355	3667.379167	-3.09515788	5.71536E-06	toxin CrTX-A-like
TRINITY_DN1545_c0_g1_i8	205.894272	1376.098092	-2.100527052	0.007813826	toxin A precursor
TRINITY_DN1098_c0_g1_i7	11.367106	74.49964433	-2.253755492	5.34871E-05	toxin CaTX-A
TRINITY_DN3423_c0_g1_i3	10.69046775	221.7810273	-3.047137947	0.008880859	toxin CrTX-A
TRINITY_DN1098_c0_g1_i1	10.25435825	114.5756337	-2.769168519	3.21232E-06	toxin CrTX-A
TRINITY_DN1545_c0_g1_i10	6.42776075	55.566296	-2.504581043	2.22238E-05	toxin A precursor
TRINITY_DN24304_c0_g1_i2	6.417995	75.048983	-2.799422799	1.61019E-05	toxin CrTX-A
TRINITY_DN3423_c0_g1_i4	5.37948375	320.2994623	-4.864915323	4.8517E-13	toxin A precursor
TRINITY_DN2402_c0_g1_i1	5.30510775	23.350461	-1.589377417	0.011974893	echotoxin A
TRINITY_DN3423_c0_g1_i8	4.28620875	212.1040213	-4.651938285	3.06984E-11	toxin CrTX-A
TRINITY_DN14485_c0_g1_i1	3.615971	41.25895767	-2.670287138	0.006703202	toxin CrTX-A-like
TRINITY_DN7820_c0_g1_i1	1.5903425	43.214034	-3.818111936	1.83842E-05	toxin CrTX-A-like
TRINITY_DN2402_c0_g1_i2	1.5702685	11.124286	-2.416689185	0.00295809	echotoxin A
TRINITY_DN1545_c0_g1_i1	0.84208225	24.77773967	-3.953319611	4.74591E-07	toxin A precursor
TRINITY_DN6429_c0_g1_i2	64.68668925	2581.833468	-4.772973124	1.62926E-37	cysteine-rich venom protein-like
TRINITY_DN5002_c2_g1_i1	27.3255255	2111.976827	-5.61847009	1.19305E-38	cysteine-rich venom protein-like
TRINITY_DN641_c0_g1_i5	21.07691975	1735.902122	-5.78386213	2.71205E-43	cysteine-rich venom protein-like
TRINITY_DN641_c0_g1_i3	7.75205875	641.3594397	-5.583565902	4.07389E-29	cysteine-rich venom protein-like
TRINITY_DN6429_c0_g1_i8	5.0319705	303.0628087	-4.819345902	6.80517E-11	cysteine-rich venom protein-like
TRINITY_DN2277_c0_g1_i2	2.80523275	982.6851947	-7.546232119	5.38278E-51	cysteine-rich venom protein Mr30-like
TRINITY_DN5650_c1_g2_i1	2.167595	91.22677967	-4.706513391	1.8616E-15	cysteine-rich venom protein
TRINITY_DN25568_c0_g3_i3	0.554169	4.775297333	-2.683742657	0.000171501	cysteine-rich venom protein VAR8-like

Table S5. List of top 10% most abundant transcripts that mapped to lysosome (ko04142). Related to Figure 5.

ID	ins_average	non_average	logFC	FDR	NR	Category
TRINITY_DN70299_c1_g4_i1	1670.513326	9.472547	7.424905189	3.57955E-36	cathepsin L	Protease
TRINITY_DN36136_c0_g2_i1	1385.177848	15.79139233	6.599741148	5.65518E-46	cathepsin L	
TRINITY_DN11409_c0_g1_i2	753.2047893	12.80984267	6.037736543	7.81905E-24	cathepsin L	
TRINITY_DN6362_c0_g1_i3	509.9510235	103.9105057	2.69042151	9.14243E-10	cathepsin B-like	
TRINITY_DN13270_c0_g2_i2	414.3957145	8.981670333	5.817147509	6.88928E-48	cathepsin Z	
TRINITY_DN30657_c0_g2_i1	304.40586	63.47053133	2.721992263	1.96532E-09	cathepsin D	
TRINITY_DN435_c1_g2_i2	165.1709825	40.309032	2.285938496	2.18142E-06	cathepsin Z-like	
TRINITY_DN11409_c0_g2_i5	151.7490458	190.845807	0.073936081	0.915173758	cathepsin L1-like isoform X1	
TRINITY_DN117902_c0_g1_i2	107.2758508	6.761294	4.387237542	8.23174E-42	cathepsin L1-like	
TRINITY_DN4494_c0_g1_i8	379.9328985	123.9757037	1.987358657	1.03933E-06	putative cysteine proteinase CG12163 isoform X1	
TRINITY_DN3833_c0_g1_i2	208.3072073	21.206951	3.7334029	1.09343E-26	legumain-like isoform X2	
TRINITY_DN2700_c0_g1_i11	116.6007678	54.25417467	1.498360597	0.000176089	lysosomal protective protein-like	
TRINITY_DN282_c1_g1_i4	90.65786	26.454285	2.144080111	1.37615E-13	dipeptidyl peptidase 1	
TRINITY_DN4748_c0_g5_i1	39.90610775	21.38673767	1.30982242	0.010974398	dipeptidyl peptidase 1-like isoform X2	
TRINITY_DN8386_c0_g2_i2	261.6817585	39.486903	3.102213759	6.89618E-22	alpha-N-acetylgalactosaminidase-like isoform X2	Glycosidase
TRINITY_DN27046_c0_g1_i1	112.6329405	40.48725767	1.861755338	6.00779E-10	alpha-L-fucosidase isoform X2	
TRINITY_DN228524_c0_g1_i1	106.7871465	0.695212667	7.546174993	1.1211E-68	alpha-galactosidase A-like isoform X1	
TRINITY_DN94_c0_g2_i1	101.1914368	19.19534733	2.85662343	1.9947E-10	lysosomal alpha-mannosidase-like	
TRINITY_DN1901_c1_g1_i2	82.122367	4.837692	3.883897071	2.11748E-07	beta-hexosaminidase subunit beta-like isoform X3	
TRINITY_DN9823_c0_g1_i5	71.597267	15.84770167	2.570241385	1.02367E-14	alpha-L-fucosidase	
TRINITY_DN27046_c0_g2_i1	58.44602675	50.332806	0.609479881	0.031995071	alpha-L-fucosidase-like isoform X1	
TRINITY_DN31161_c0_g1_i3	56.414978	18.08420067	2.07421756	2.39463E-13	alpha-galactosidase A-like isoform X1	
TRINITY_DN144_c0_g1_i5	49.882435	0.504982667	6.744576206	1.01978E-44	lysosomal alpha-glucosidase-like	
TRINITY_DN7236_c0_g1_i6	45.38700975	2.417165333	4.576383406	1.52143E-45	beta-glucuronidase-like isoform X1	

TRINITY_DN160_c0_g1_i10	40.72058825	52.65429967	0.10587938	0.934108347	hexosaminidase	
TRINITY_DN8387_c0_g1_i12	39.1863405	11.75121567	2.057737281	3.0245E-07	galactocerebrosidase-like isoform X1	
TRINITY_DN574_c0_g1_i6	37.6958385	11.05328967	2.140710504	7.37496E-11	beta-mannosidase-like isoform X2	
TRINITY_DN160_c0_g1_i7	34.85720925	1.706204333	2.741905922	0.086230458	hexosaminidase	
TRINITY_DN107_c0_g2_i1	31.02668275	52.59167533	-0.327019443	0.373656869	lysosomal alpha-mannosidase	
TRINITY_DN574_c0_g1_i2	30.105067	2.238477667	4.164104634	2.39082E-32	beta-mannosidase-like isoform X2	
TRINITY_DN9166_c0_g1_i4	29.96046825	5.442944	2.803412521	7.89279E-16	alpha-N-acetylglucosaminidase-like isoform X3	
TRINITY_DN978_c0_g1_i1	28.833394	7.128599	2.457501734	6.18759E-21	alpha-L-iduronidase isoform X4	
TRINITY_DN2928_c1_g1_i10	28.68580125	38.92979567	-0.049719845	0.936728816	alpha-L-fucosidase isoform X1	
TRINITY_DN2291_c0_g1_i4	111.3868428	14.703239	3.261491968	1.1945E-13	arylsulfatase B-like isoform X4	Sulfatase
TRINITY_DN1371_c0_g1_i12	46.27857875	15.254502	2.009669746	2.37726E-08	arylsulfatase B	
TRINITY_DN1762_c0_g3_i2	115.4104535	4.797551	4.925275747	1.11023E-54	tartrate-resistant acid phosphatase type 5-like	Phosphatase
TRINITY_DN1225_c0_g1_i3	55.9688475	6.961143667	3.446334451	2.78034E-16	prostatic acid phosphatase-like	
TRINITY_DN2509_c3_g1_i1	54.238148	2.737971	4.538710304	2.504E-20	sphingomyelin phosphodiesterase-like	Sphingomyelinase
TRINITY_DN16778_c0_g1_i1	32.3193325	10.16454867	2.033173183	2.66846E-10	N(4)-(Beta-N-acetylglucosaminyl)-L-asparaginase-like	Aspartylglucosaminidase
TRINITY_DN10570_c1_g1_i7	288.7476185	266.316892	0.538854276	0.052824114	prosaposin-like isoform X2	Other lysosomal enzymes and activators
TRINITY_DN15312_c1_g1_i3	254.2697955	4.850341667	6.017696244	8.21977E-63	prosaposin-like isoform X2	
TRINITY_DN279836_c0_g1_i1	248.8749608	0.632121667	8.605573507	3.14223E-81	ganglioside GM2 activator-like	
TRINITY_DN582_c3_g1_i1	172.7626653	18.70695367	3.459047492	6.95492E-11	ganglioside GM2 activator-like	
TRINITY_DN45191_c0_g1_i1	120.942684	148.4633013	0.113495308	0.827306803	ganglioside GM2 activator-like	
TRINITY_DN465_c0_g1_i1	37.1543635	28.44172267	0.85148292	0.164878695	palmitoyl-protein thioesterase 1-like	
TRINITY_DN39_c2_g1_i5	29.967425	8.385254667	2.287394826	0.000702277	lysosomal thioesterase PPT2-A-like isoform X1	
TRINITY_DN7053_c0_g1_i1	254.335812	234.5838973	0.488950989	0.192533513	CD63 antigen-like	Major lysosomal membrane protein
TRINITY_DN6449_c1_g1_i1	76.564016	43.99766767	1.283637143	0.000410471	lysosome-associated membrane glycoprotein 1	
TRINITY_DN343_c2_g2_i3	300.8739698	420.2746927	-0.147829248	0.834696542	cell death-inducing p53-target protein 1 homolog	Minor lysosomal membrane protein
TRINITY_DN24856_c0_g1_i3	79.701395	1.848526	5.793625488	1.34121E-81	integumentary mucin C.1-like	

TRINITY_DN3287_c0_g1_i5	49.72707075	71.453596	-0.193079506	0.759814672	epididymal secretory protein E1	
TRINITY_DN343_c2_g2_i2	37.58832975	21.65853933	1.217860455	0.000137675	cell death-inducing p53-target protein 1 homolog	
TRINITY_DN454_c0_g1_i1	36.387883	4.869285333	3.109651442	6.51404E-05	heparan-alpha-glucosaminide N-acetyltransferase-like	
TRINITY_DN14264_c0_g1_i3	35.5827055	41.93823733	0.153093308	0.696232975	cell death-inducing p53-target protein 1 homolog	
TRINITY_DN83056_c0_g1_i6	26.6495155	15.85372633	1.262426868	0.030306124	sulfatase-modifying factor 1-like	FGE
TRINITY_DN7178_c0_g2_i3	33.28410675	1.659780333	4.569486115	1.06161E-14	cation-dependent mannose-6-phosphate receptor	MPR
TRINITY_DN370_c0_g1_i2	82.72759325	135.2689543	-0.337682307	0.351736622	clathrin light chain A isoform X2	Clathrin
TRINITY_DN370_c0_g1_i3	79.322643	55.615864	0.985530139	0.020040995	clathrin light chain B isoform X2	
TRINITY_DN17786_c0_g1_i2	74.6604545	70.387984	0.516458425	0.01271069	clathrin heavy chain 1	
TRINITY_DN6110_c1_g1_i3	31.412089	46.82801167	-0.251231966	0.622640557	AP-3 complex subunit sigma-1-like isoform X2	AP-3
TRINITY_DN6213_c0_g1_i3	81.20926925	39.23457333	1.525184986	1.20402E-05	V-type proton ATPase subunit d 1-like	ATPeV
TRINITY_DN247_c0_g1_i14	47.32874125	22.75766	1.487000979	1.08384E-18	V-type proton ATPase subunit H	
TRINITY_DN6657_c0_g2_i4	44.5397185	35.822305	0.691588886	0.017280352	V-type proton ATPase 21 kDa proteolipid subunit-like	
TRINITY_DN37_c4_g1_i1	37.13446175	24.34637133	1.040634409	7.21674E-05	V-type proton ATPase subunit S1-like	

Table S6. List of transcripts involved in xenobiotic detoxification. Related to Figure 6.

ID	ins_average	non_average	logFC	FDR	NR
TRINITY_DN84_c1_g3_i10	89.18405475	90.811631	0.372316132	0.151883109	cytochrome P450 3A12-like
TRINITY_DN484_c0_g1_i7	58.80033775	87.41447967	-0.009175092	0.995753828	cytochrome P450 3A5-like isoform X9
TRINITY_DN1580_c0_g1_i10	48.2983525	5.715049667	3.520694926	4.02501E-31	cytochrome P450 1A1-like
TRINITY_DN159_c0_g1_i1	42.1674405	34.06700367	0.731779621	0.066838348	cytochrome P450 2U1-like
TRINITY_DN2389_c0_g2_i6	41.307914	43.65940633	0.31668239	0.447356161	cytochrome P450 2J6-like isoform X1
TRINITY_DN484_c0_g1_i5	41.26078575	38.81213	0.548231117	0.214676449	cytochrome P450 3A24-like
TRINITY_DN159_c0_g1_i13	30.71038225	22.29538267	0.819248294	0.023985903	cytochrome P450 2U1-like
TRINITY_DN2491_c1_g1_i1	494.7612928	177.1774047	1.91101098	5.97063E-09	glutathione S-transferase A
TRINITY_DN10742_c0_g1_i2	159.5684028	125.2398117	0.699629751	0.203656048	microsomal glutathione S-transferase 1
TRINITY_DN228044_c0_g1_i1	155.7405245	261.0610823	-0.382418862	0.434982549	microsomal glutathione S-transferase 1
TRINITY_DN235_c2_g2_i3	152.9840718	126.4794873	0.699821921	0.061364456	glutathione S-transferase A
TRINITY_DN6438_c0_g2_i1	125.2524858	60.731002	1.392314501	0.001154828	glutathione S-transferase P 1-like
TRINITY_DN235_c2_g2_i1	92.25908075	111.248237	0.056255642	0.976190609	glutathione S-transferase A-like
TRINITY_DN4982_c0_g1_i1	82.50143175	148.665964	-0.489262587	0.162461033	microsomal glutathione S-transferase 3-like
TRINITY_DN55415_c0_g1_i2	62.72769825	70.159324	0.354268814	0.763086764	glutathione S-transferase A
TRINITY_DN3888_c0_g1_i4	57.16741325	46.671111	0.605817593	0.074245718	glutathione S-transferase alpha
TRINITY_DN6904_c0_g1_i6	51.9286665	46.31327433	0.548929142	0.162307591	probable glutathione S-transferase 7
TRINITY_DN13424_c1_g1_i3	41.305482	55.14324833	-0.113774659	0.872010802	glutathione S-transferase Mu 3-like
TRINITY_DN15099_c0_g1_i4	33.870615	28.15952133	0.648484837	0.041961407	microsomal glutathione S-transferase 2-like
TRINITY_DN1080_c0_g1_i9	312.6857608	218.053308	1.045445061	0.216350981	superoxide dismutase [Cu-Zn]
TRINITY_DN1965_c1_g1_i1	206.999128	253.5149307	0.079114139	0.853770607	superoxide dismutase [Cu-Zn]
TRINITY_DN8529_c0_g3_i1	75.015949	31.61692933	1.743576079	0.010832894	superoxide dismutase [Cu-Zn]-like
TRINITY_DN129587_c0_g2_i3	382.5238453	17.48332867	4.683637878	4.81543E-29	glutathione peroxidase 6-like