Supplemental Material Information

Information on contents of each supplemental file submitted to *Genome Research* from "Dynamic metabolic and molecular changes during seasonal shrinking in *Sorex araneus*".

Supplemental Tables

Supplemental Table 1 – Mean body and organ mass by season. Significant change (t-test, $p<0.05$) in mass from prior season indicated by an asterisk.

	Summer Juvenile (g)	Autumn Juvenile (g)	Winter Juvenile (g)	Spring Adult (g)	Summer Adult (g)
Body	7.88	7.61	6.88	10.60	12.11
Liver	0.70	0.61	0.68	1.21	1.03
Brain	0.28	0.24	0.23	0.30	0.25
Spleen	0.10	0.06	0.05	0.11	0.14
Heart	0.16	0.13	0.17	0.17	0.22

Supplemental Table 2 – Significant change (t-test, $p<0.05$) in body mass from between seasons of Dehnel's phenomenon.

Body Mass (p<0.05*)	Summer Juvenile	Autumn Juvenile	Winter Juvenile	Spring Adult	Summer Adult
Summer Juvenile	-	-	-	-	-
Autumn Juvenile	1.0000	-	-	-	-
Winter Juvenile	0.0279*	0.2284	-	-	-
Spring Adult	0.0003*	0.0003*	<0.0001*	-	-
Summer Adult	<0.0001*	<0.0001*	0.0002*	0.0126*	-

Supplemental Table 3 – Significant change (t-test, $p<0.05$) in liver mass from between seasons of Dehnel's phenomenon.

Liver Mass (p<0.05*)	Summer Juvenile	Autumn Juvenile	Winter Juvenile	Spring Adult	Summer Adult
Summer Juvenile	-	-	-	-	-
Autumn Juvenile	0.3796	-	-	-	-
Winter Juvenile	1.0000	1.0000	-	-	-
Spring Adult	0.0018*	0.0010*	0.0010*	-	-
Summer Adult	0.0272*	0.0097*	0.01888	0.3737	-

Supplemental Table 4 – List of significant metabolites that are differentially concentrated between seasons of Dehnel's phenomenon (one-way ANOVA, p<0.05).

Metabolite	F.stat	p.value	-LOG10(p)	FDR
Butorphanol	349.3	0.0000	16.77	0.0000
8-Hydroxyquinoline	23.4	0.0000	6.40	0.0001
Thymidine	19.5	0.0000	5.79	0.0001
Cystine	12.9	0.0000	4.52	0.0019
3-(2,7-dimethylpyrazolo[1,5-a]pyrimidin-6-yl)-5-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-5-ol	12.4	0.0000	4.39	0.0020
Kynurenic acid	9.4	0.0002	3.65	0.0093
Glutathione disulfide	9.2	0.0003	3.58	0.0094
N-Acetyl-ornithine	8.8	0.0003	3.48	0.0104
Acetyl-methylcholine.1	8.3	0.0005	3.33	0.0129
Adipic acid	7.6	0.0008	3.10	0.0186
Carnitine	7.5	0.0008	3.09	0.0186
Methyprylon	7.2	0.0011	2.97	0.0221
2-Ethylhexyl sulfate	7.0	0.0012	2.90	0.0225
Rhamnose	7.0	0.0013	2.90	0.0225
Ethyl palmitoleate	6.8	0.0014	2.84	0.0240
L-Tyrosine methyl ester	6.6	0.0017	2.78	0.0262
Asparagine	6.2	0.0023	2.64	0.0335
Galactonic acid	6.1	0.0026	2.59	0.0347
Gluconolactone	6.0	0.0026	2.58	0.0347
Docosahexanoic acid	5.9	0.0030	2.52	0.0370
Arachidonic acid	5.8	0.0032	2.49	0.0370
N-Acetyl-glutamine	5.8	0.0033	2.49	0.0370
Serine	5.6	0.0037	2.43	0.0401
Quinoline.1	5.5	0.0041	2.39	0.0421
N-Acetylneuraminic acid	5.5	0.0042	2.38	0.0421
Homoarginine	5.4	0.0045	2.35	0.0432
Threonic acid	5.2	0.0054	2.26	0.0487
D-Alanine methyl ester	5.2	0.0055	2.26	0.0487

Supplemental Table 5 – Significant KEGG pathway enrichments ($p_{adj} < 0.01$) of gene expression clusters.

Cluster	KEGG Term	Count	FDR
4	Protein processing in endoplasmic reticulum	37	<0.0001
4	Proteasome	11	0.0095
1	Cytoskeleton in muscle cells	36	0.0000
1	Cardiac muscle contraction	15	0.0001
1	Dilated cardiomyopathy	16	0.0002
1	Adrenergic signaling in cardiomyocytes	19	0.0002
1	Hypertrophic cardiomyopathy	15	0.0002
1	Calcium signaling pathway	22	0.0049
1	Metabolic pathways	78	0.0055

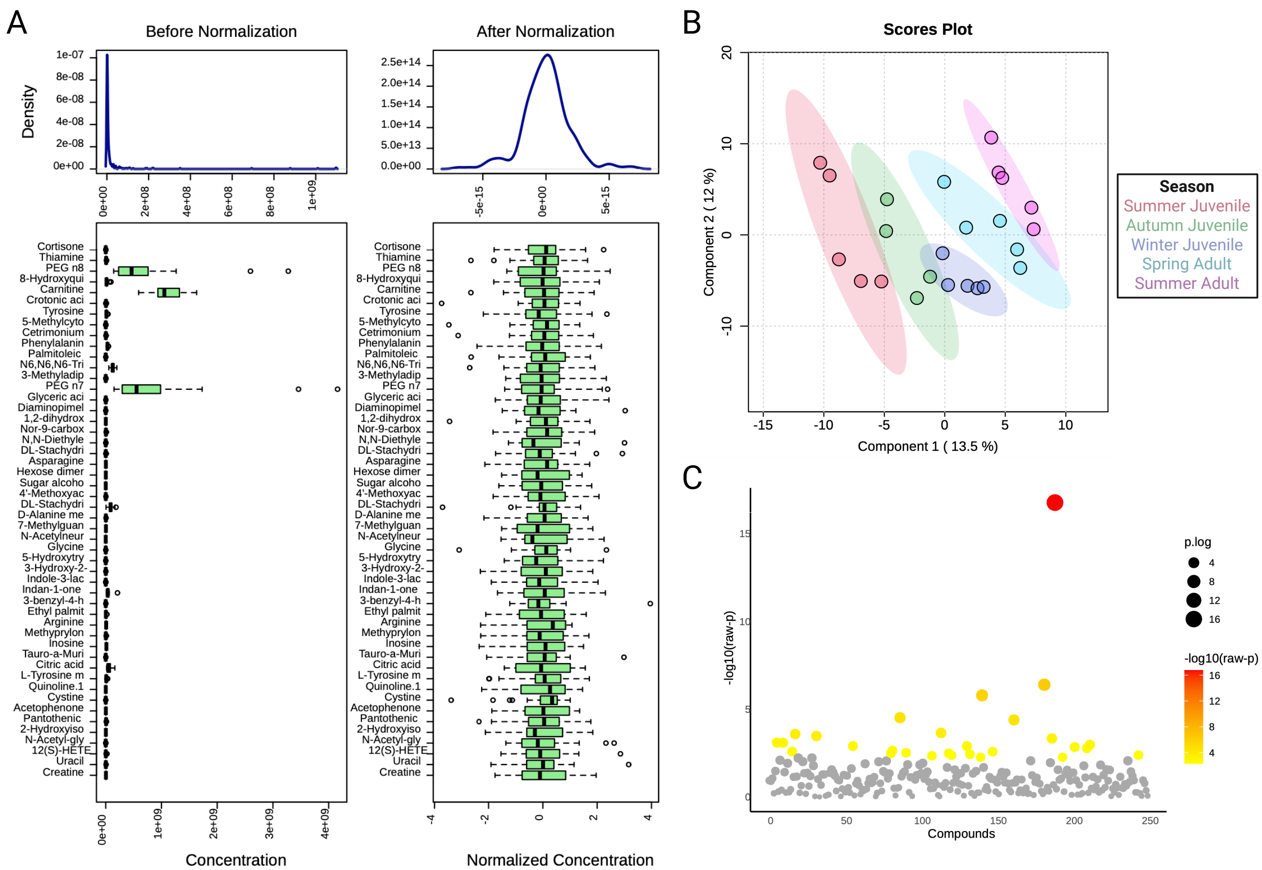
Supplemental Table 6 – Overlap of significant genes (FDR/$p_{adj} < 0.05$) between Dehnel's phenomenon and hibernation (GSE199814) results.

Count	GeneName	Dehnel_padj	Dehnel_LFC	Hibernation_FDR	Hibernation_LFC
1	CA2	5.40E-09	1.550670361	0.008859465	0.926596986
2	ETNPPL	4.35E-07	1.352929964	2.25E-07	1.698525095
3	GPD2	0.000266591	1.236207064	0.017794767	0.737199555
4	ALDH1B1	0.003192451	0.810292065	0.033411395	0.616653772
5	MPV17L	0.005252221	0.931150187	0.000323814	1.120171681
6	FZD4	0.01566118	0.694984138	0.004779974	0.723562336
7	GPI	0.020139568	0.596342371	0.001522743	0.778615748
8	PAH	0.029770601	0.652278158	0.042597722	0.565841831
9	ACY1	0.044420039	0.745746993	0.038772282	0.521107839
10	JCHAIN	1.51E-12	4.741964724	9.97E-05	-1.452798982
11	LIN7A	2.73E-11	3.282128813	0.002479783	-0.813033662
12	MZB1	6.08E-05	2.7508683	6.23E-05	-1.413407045
13	ALDH8A1	0.003545633	0.634083856	0.013645796	-0.620074539
14	RASGRP3	0.003639426	1.531903188	0.000523394	-0.827194113
15	ABCB11	0.015984461	0.71355314	0.043671419	-0.594849207
16	FABP3	0.026392137	1.233509894	0.03121992	-1.11963061
17	RAP1GAP2	0.049217545	0.656388965	0.002206204	-1.024665142
18	GAA	2.48E-06	-0.687116903	0.009965112	0.585984644
19	FEZ1	0.000727521	-1.513182793	0.000617611	1.487436622
20	MMP15	0.000727521	-0.949182075	0.040448376	0.52563468
21	CD14	0.002971773	-1.354351904	0.004536963	0.945691507
22	SORBS3	0.010634057	-0.694323318	0.007736876	0.71001204
23	BTG2	0.018313358	-1.320268397	0.036937948	1.234723635
24	REEP4	0.022348276	-0.792949687	1.70E-05	1.123813636
25	ATG16L2	0.035851785	-1.296126053	0.046548824	0.573913056
26	APOA1	0.037705326	-0.444132736	0.039378898	0.609308145
27	HNRNPL	0.041027922	-0.501927172	0.00376513	0.673822724
28	SLC1A4	0.047727581	-0.854488846	0.000632152	1.441759786
29	NUDT19	1.04E-05	-0.989104734	0.03476183	-0.534009649
30	SIX5	3.40E-05	-1.513474523	0.023058756	-0.676690493
31	TMEM64	5.18E-05	-1.872298839	0.016967619	-0.722603179
32	INHBE	0.000866251	-3.119300531	3.18E-06	-1.843128085
33	MBL2	0.001504569	-0.866627278	0.034548373	-0.78284658
34	SREBF2	0.00494666	-0.969424032	0.023026063	-0.523193873
35	ZNF467	0.005464956	-1.304370467	2.89E-05	-0.870990481
36	ADAMTS4	0.005772044	-1.467728614	9.73E-05	-1.341904756
37	CYTH4	0.006821679	-1.090116424	0.000192208	-0.992710462
38	INSIG1	0.009275886	-1.133959775	0.001470542	-1.143292774
39	INHBA	0.010784514	-1.84557613	6.85E-07	-1.411794493
40	NSDHL	0.019666224	-1.233472156	0.001919304	-0.766836682
41	MVD	0.021792931	-1.756739578	0.033411395	-0.855165656
42	BAAT	0.022905188	-0.865923215	0.043795182	-0.577866331
43	UBE2L6	0.025693818	-1.569067984	0.02146075	-1.514261943
44	TBC1D8	0.03209886	-0.717462721	0.014151066	-0.75615761
45	IDI1	0.040622447	-1.992957336	2.57E-11	-2.027388842
46	THRB	0.041288823	-1.506647995	0.015748163	-0.582943257
47	CSAD	0.041582536	-0.898491433	0.015875729	-0.655585899
48	SULT1B1	0.046561224	-1.133748288	0.008892679	-0.627597777
49	FDPS	0.047368969	-1.149589701	0.005938536	-0.798149827
50	PPP1R10	0.049527672	-0.579255261	1.87E-11	-2.212734511

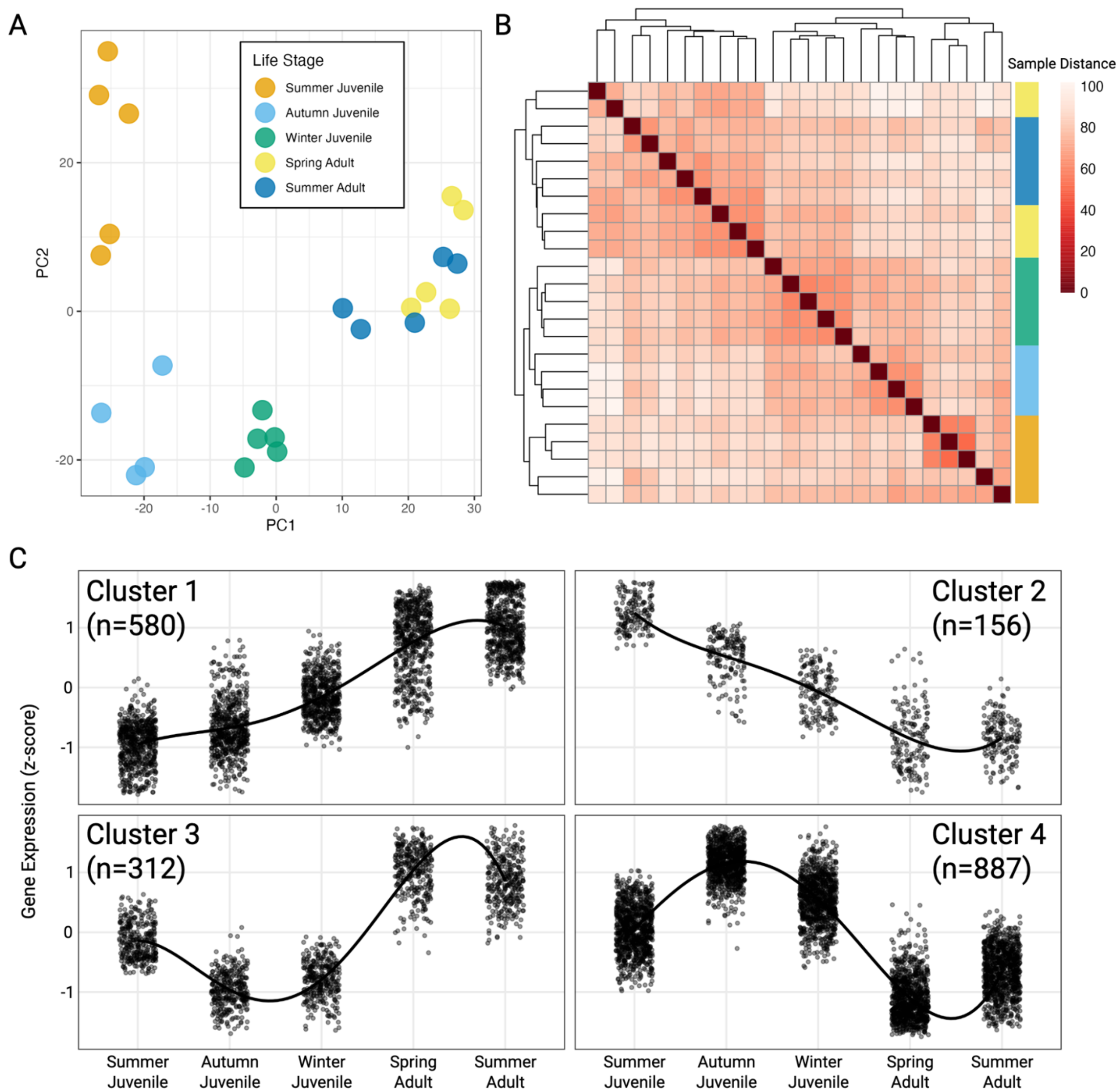
Supplemental Table 7 – Top 10 significantly enriched KEGG pathways from the red and blue WGCNA modules.

Module	KEGG Term	Count	FDR
Red	Polycomb repressive complex	29	5.81E-05
Red	Insulin resistance	29	0.00540236
Red	Prostate cancer	27	0.00540236
Red	FoxO signaling pathway	33	0.00540236
Red	Pathways in cancer	94	0.00741984
Red	Neurotrophin signaling pathway	30	0.00814246
Red	Thyroid hormone signaling pathway	30	0.00946895
Red	RNA degradation	22	0.00979166
Red	Longevity regulating pathway	24	0.00995183
Blue	Shigellosis	50	0.00995183
Blue	Nucleocytoplasmic transport	33	4.96E-04
Blue	Autophagy - animal	43	0.00152884
Blue	Protein processing in endoplasmic reticulum	42	0.00275968
Blue	Endocytosis	56	0.00275968
Blue	Renal cell carcinoma	22	0.00668506
Blue	Spinocerebellar ataxia	35	0.01224237
Blue	Adherens junction	25	0.02154757
Blue	Metabolic pathways	240	0.04720624
Blue	Mitophagy - animal	26	0.04729203

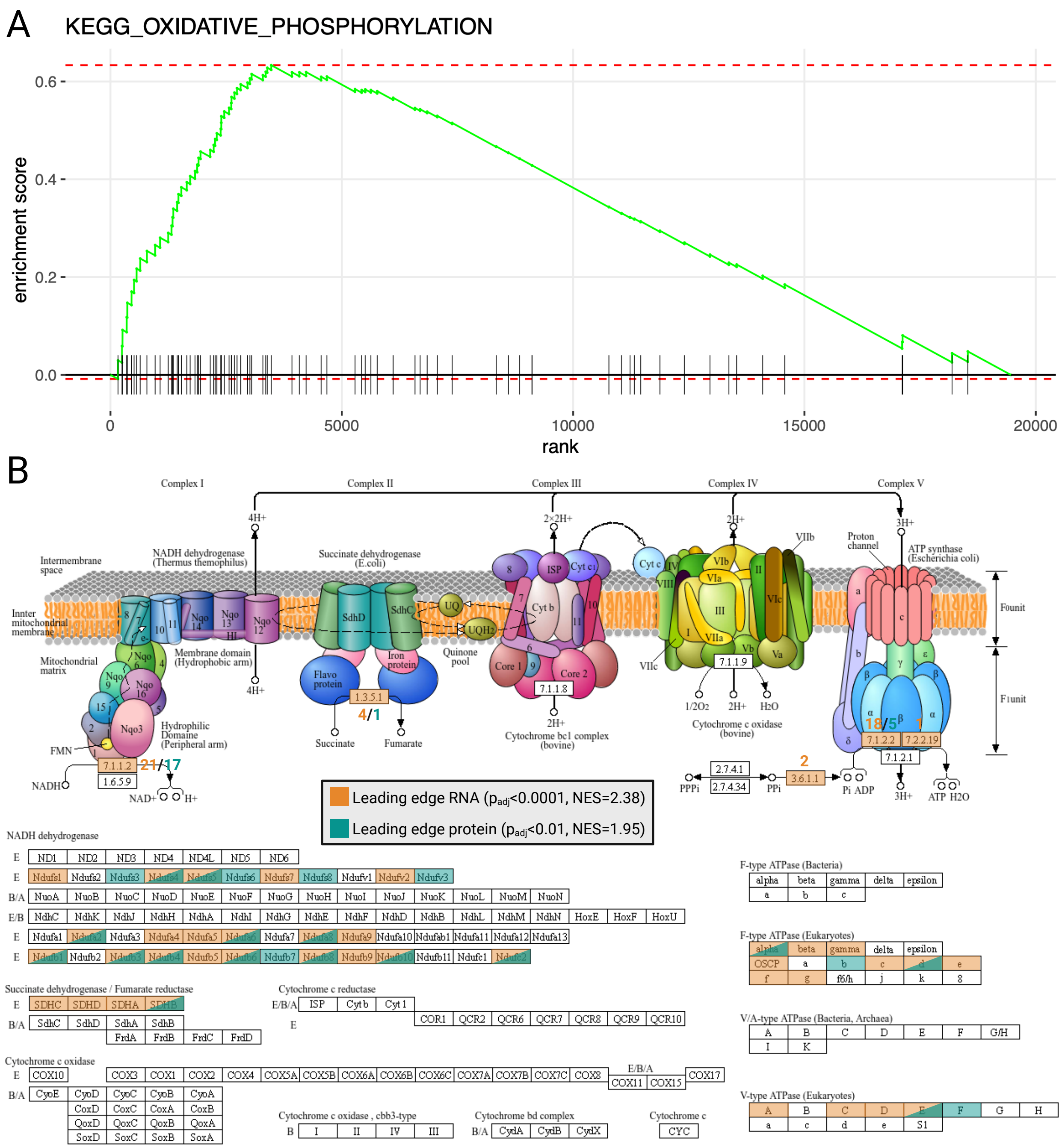
Supplemental Figures



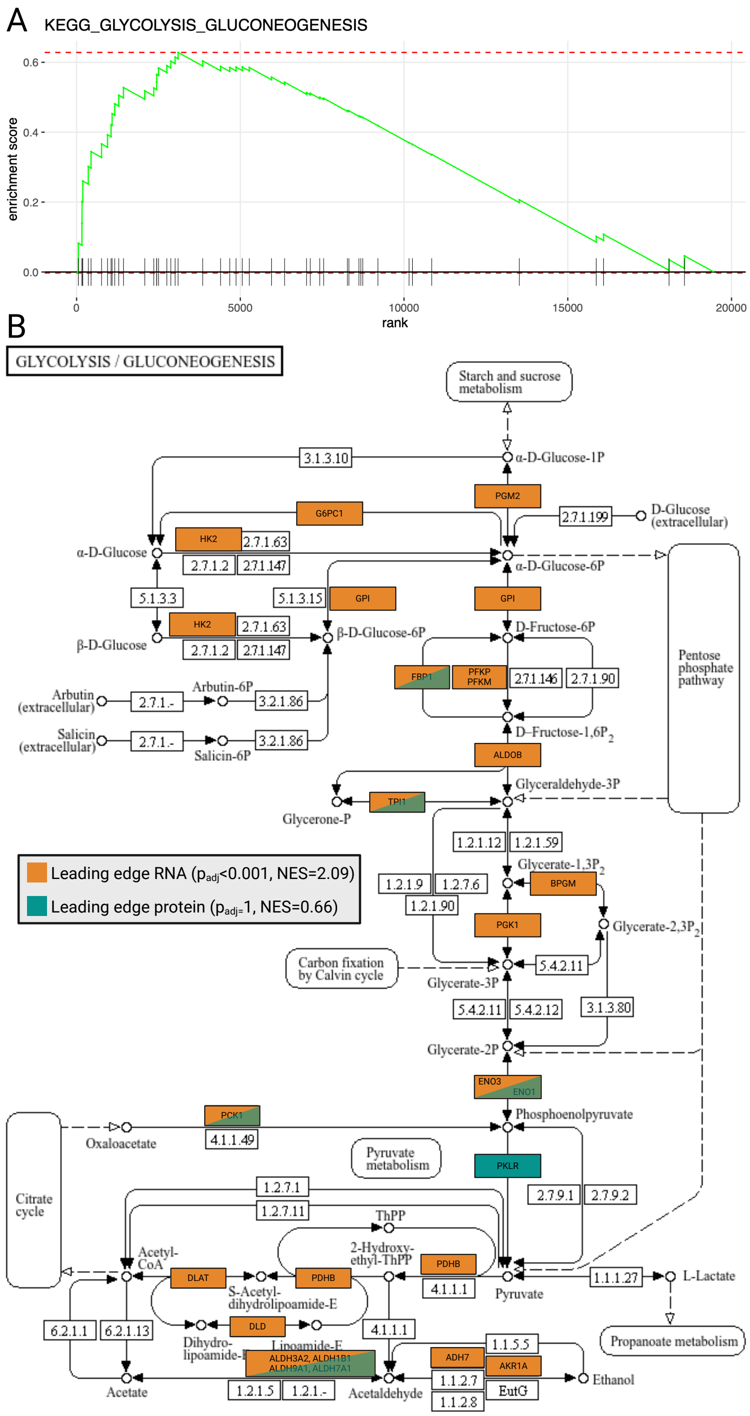
Supplemental Figure 1 – (**A**) Normalization of 250 metabolites concentrations by summer adults, log transformed and auto scaled (50 shown for space). **(B)** Partial least squares discriminant analysis identifies clustering of samples by season (**C**) One-way ANOVA of metabolites testing for differential concentration between seasons. Significant metabolites are colored ($p<0.05$).



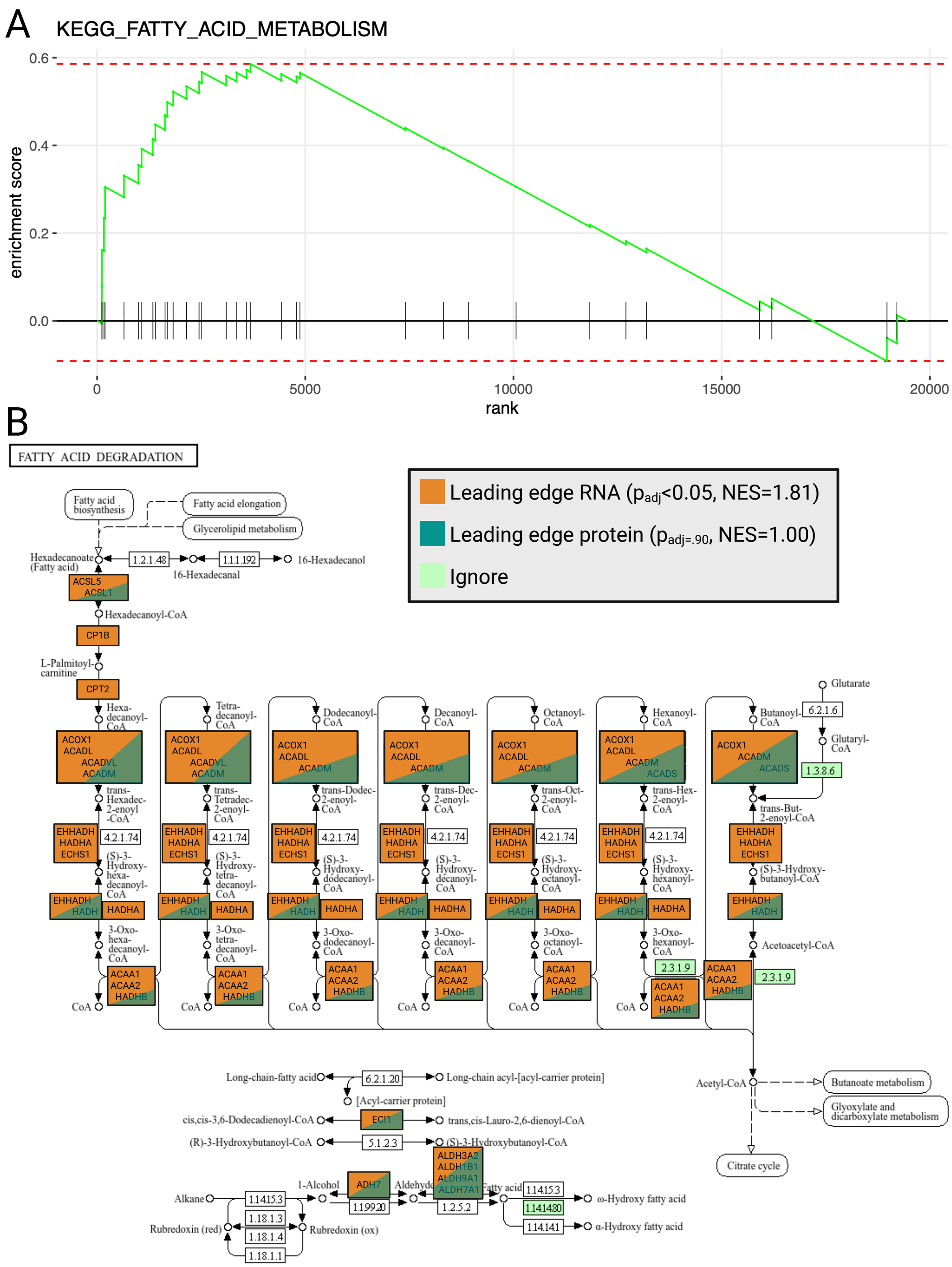
Supplemental Figure 2 – (**A**) Principal component analysis (PCA) based off 3336 significant genes from seasonal likelihood ratio test (LRT). PC1 explains 22.3% of the variation, while PC2 explains 13.4%. **(B**) Hierarchical clustering reveals grouping of samples into three major clusters: summer juveniles, shrinking autumn and winter juveniles, spring and summer adults. (**C**) Based on significant LRT genes, temporal clustering identified four groups (>150 genes) with similar expression patterns across seasons.



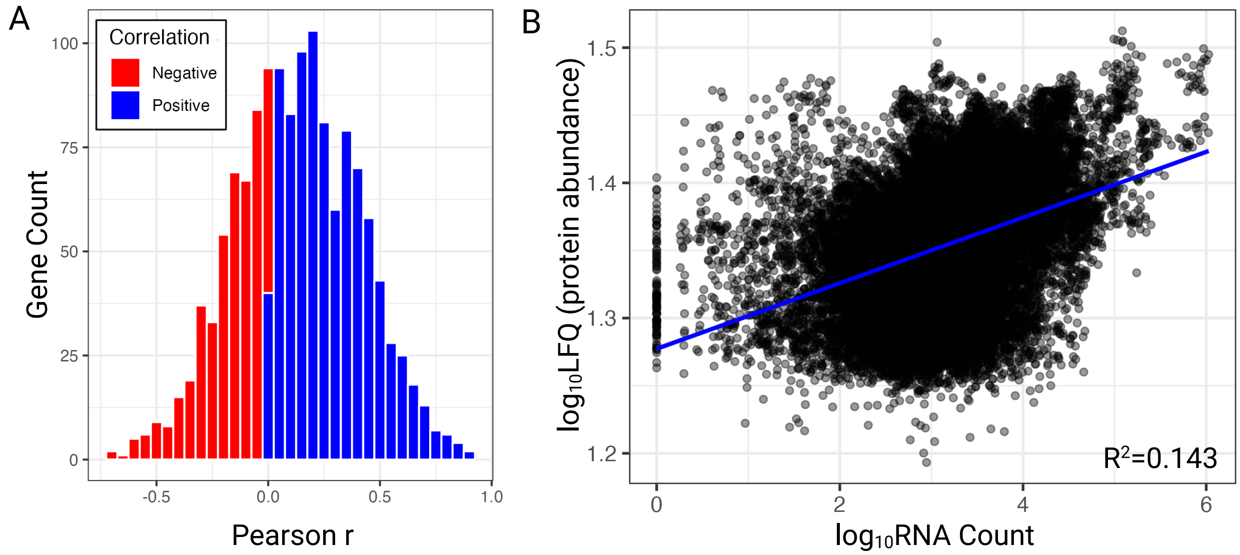
Supplemental Figure 3 – (**A**) Cumulative enrichment score increase for genes (ranked) in the KEGG oxidative phosphorylation pathway **(B)** Upregulated genes and proteins appear to occur Complex I and Complex 5 of mitochondrial oxidative phosphorylation.



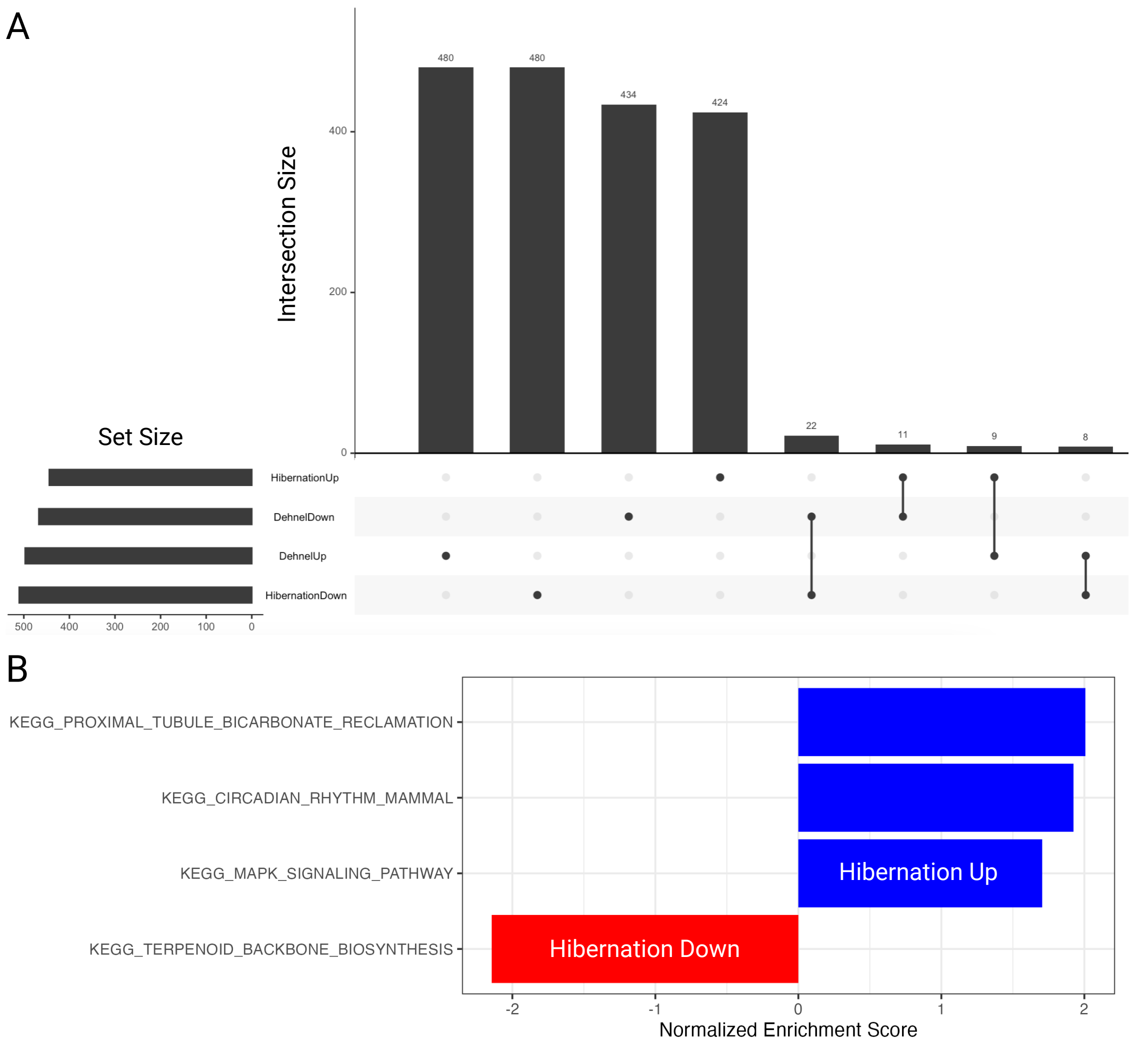
Supplemental Figure 4 – (**A**) Cumulative enrichment score increase for genes (ranked) in the KEGG glycolysis / gluconeogenesis pathway **(B)** Upregulated genes and proteins appear to occur in the linear path related to the production and breakdown of glucose.



Supplemental Figure 5 – (**A**) Cumulative enrichment score increase for genes (ranked) in the KEGG fatty acid metabolism pathway (**B**) Upregulated genes and proteins in fatty acid pathway are associated with activated fatty acid oxidation.



Supplemental Figure 6 – (**A**) Histogram showing Pearson correlation coefficients between normalized RNA counts and protein abundances across all samples for genes present in both datasets. A total of 912 genes showed positive correlation, while 463 were negatively correlated. **(B**) Scatterplot of log-transformed RNA expression versus protein abundance, with a fitted linear regression line (blue) illustrating a modest correlation between transcriptomic and proteomic data (R^2=0.143).



Supplemental Figure 7 – (**A**) Upset plot showing overlap in significant (p_{adj}<0.05) differentially expressed genes between Dehnel's phenomenon and hibernation (GSE199814). **(B**) Pathway enrichment analysis for differentially expressed genes during hibernation.

Code Steps

1) Step 1: Get raw sequencing RNA data.
    a. get_rawseq.sh
2) Step 2: Quality control, filtering and trimming of raw data.
    a. fastp.sh
3) Step 3: Mapping and quantification.
    a. kallisto.sh
4) Step 4: Protein to gene mapping
    a. protein_db2.sh
5) Step 5: Exploratory analyses, differential expression, pathway enrichments, hibernation and protein comparisons, gene clustering, gene networks, and figure creation.
    a. Dehnel_Liver_v2.R
6) Step6: Plotting metabolomics data
    a. metabolomics_figures.R

Code can be found in Supplemental Code. README file and code can be found on GitHub: https://github.com/wrthomas315/Dehnels_Seasonal_RNAseq2