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Supplemental Information

Selenium Deficiency Is Associated with Pro-longevity Mechanisms

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Figure S6. Dietary Se regulates miRNA expression in liver. Related to Figure 6, Table S3, and File S3.

SUPPLEMENTAL TABLES

Table S1. Commonly enriched metabolic pathways in brain and liver in response to selenium diets ($p<0.05$) (A) and ($p<0.01$) (B). Related to Figures 2 and 3, Figures S1 and S3, and File S1.

Table S2. Metabolites associated with selenium deficiency in liver. Related to Figure 5, Figure S1 and S2, and File S1.

Table S3. microRNAs differentially expressed in response to selenium diets. Related to Figure 6, Figure S6, and File S3.

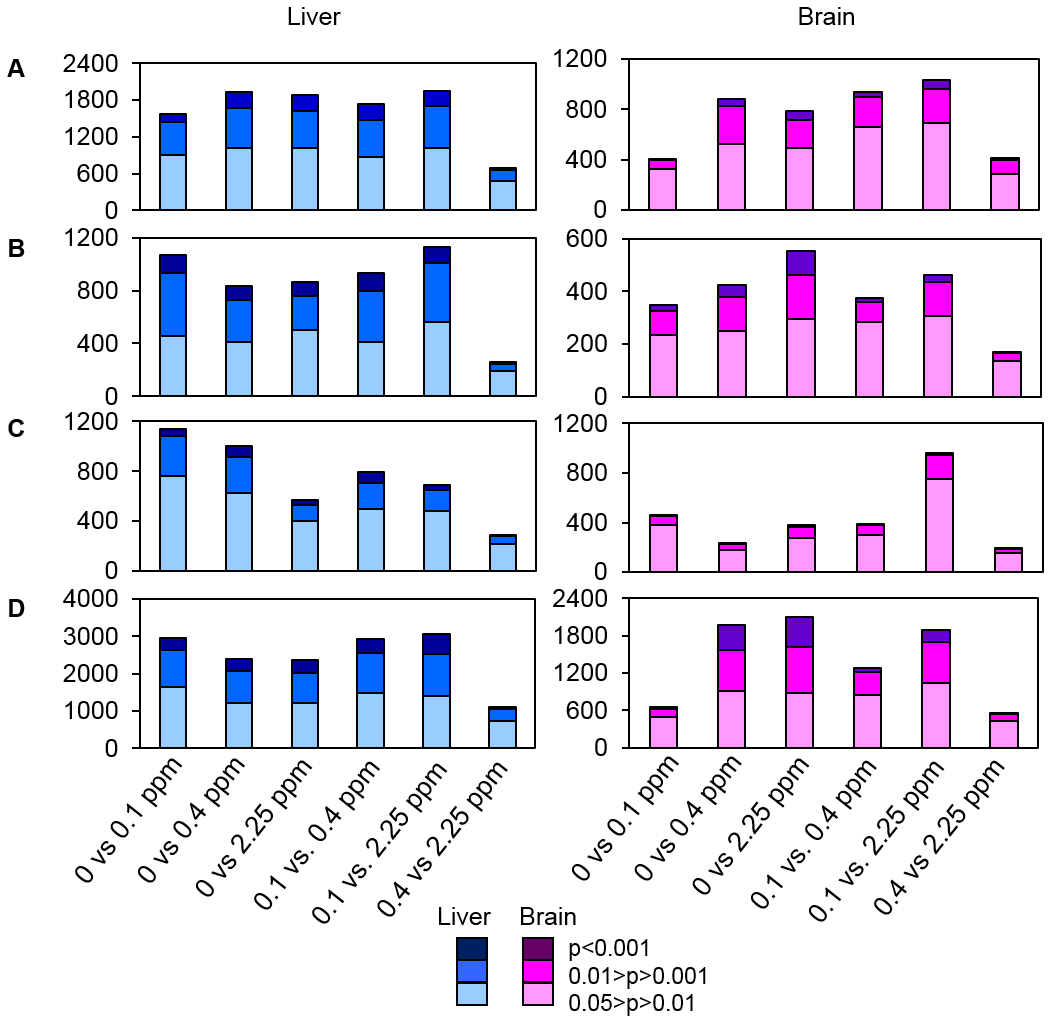
SUPPLEMENTAL FILES (SEPARATE EXCEL FILES)

File S1. Metabolite profiling data. Related to Figures 2-5, Figures S1and S2, and Tables S1 and S2.

File S2. Transcriptome data. Related to Figure 6 and Figures S3-S5.

File S3. miRNA data. Related to Figure 6, Figure S6, and Table S3.

SUPPLEMENTAL FIGURES



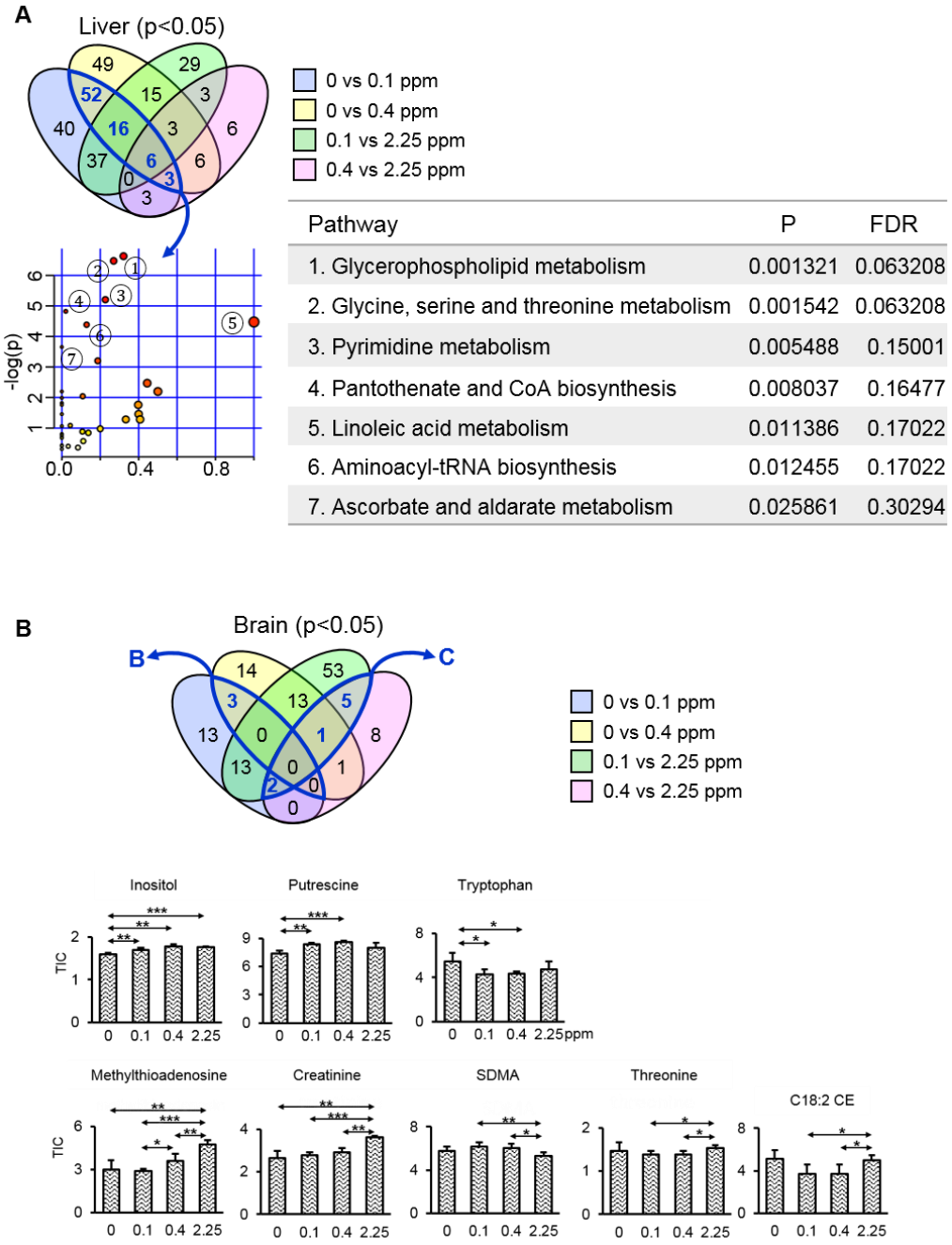
E

Targeted metabolite	0 vs. (0.1 or 0.4) ppm		2.25 vs. (0.1 or 0.4) ppm	
TTEST	Liver	Brain	Liver	Brain
p<0.001	25	1	1	2
0.001<p<0.01	74	5	20	3
0.01<p<0.05	87	8	41	20

Non-targeted metabolite	0 vs. (0.1 or 0.4) ppm		2.25 vs. (0.1 or 0.4) ppm	
TTEST	Liver	Brain	Liver	Brain
p<0.001	1035	181	319	59
0.001<p<0.01	2129	814	1061	441
0.01<p<0.05	2980	1616	2525	1434

Figure S1. Effect of Se diets on mouse liver and brain metabolomes. Related to Figure 2, Figure S2, Tables S1 and S2, and File S1.

Fractions of significant metabolites that represents the numbers of metabolites with altered levels detected in each comparison. Note that the four metabolite profiling platforms (**A, B, C,** and **D**) detect different numbers of features. The Y-axis corresponds to the number of features detected. (**E**) Number of metabolites that are altered in the comparisons of dietary groups with indicated statistical significance are shown. Targeted metabolites (upper table) and non-targeted metabolite (lower table) from all four metabolite profiling platforms in both liver and brain are summarized.



Pathway	P	FDR
1. Glycerophospholipid metabolism	0.001321	0.063208
2. Glycine, serine and threonine metabolism	0.001542	0.063208
3. Pyrimidine metabolism	0.005488	0.15001
4. Pantothenate and CoA biosynthesis	0.008037	0.16477
5. Linoleic acid metabolism	0.011386	0.17022
6. Aminoacyl-tRNA biosynthesis	0.012455	0.17022
7. Ascorbate and aldarate metabolism	0.025861	0.30294

Figure S2. Metabolites and metabolic pathways altered in response to Se deficiency and toxicity in liver (A) and brain (B). Related to Figures 2 - 5, Figures S1 and S3, Tables S1 and S2, and File S1.

(A) Metabolites associated with Se deficiency (0 vs 0.1 ppm, and 0 vs 0.4 ppm) and Se toxicity (0.1 vs. 2.25 ppm, and 0.4 vs. 2.25 ppm) with p<0.05. Metabolites associated with Se deficiency were quantified and enriched pathways were assessed using MetaboAnalyst 4.0. **(B)** Three metabolites (inositol, putrescine, and tryptophan) were altered in response to Se deficiency. Eight metabolites were commonly altered in response to Se toxicity (0.1 vs 2.25 ppm and 0.4 vs 2.25 ppm). In addition to the metabolites shown in Figure S2B, methionine sulfoxide, 5-adenosylhomocysteine, and methionine were also altered by Se toxicity (Figure 3A). TIC represents total ion count.

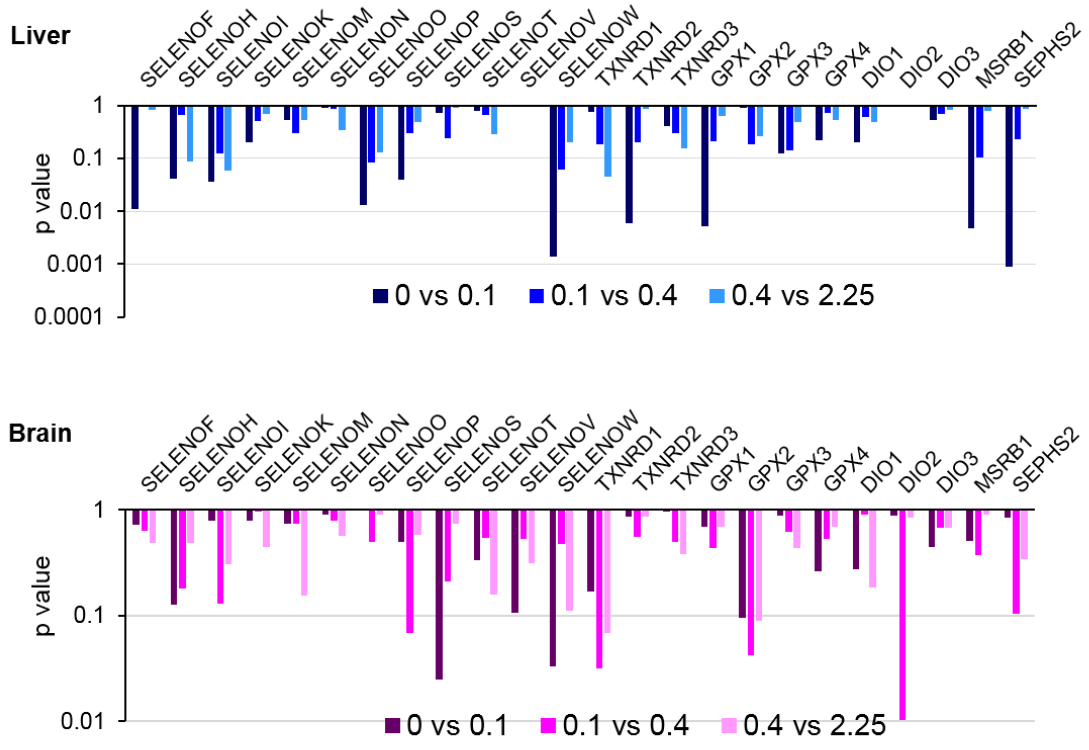


Figure S3. Selenoprotein gene expression in response to Se diets. Related to Figure 6, Figure S4, and File S2.

Selenoprotein transcript expression was assessed by the comparison between 0 vs 0.1, 0.1 vs 0.4, and 0.4 vs 2.25 ppm diets. Upper panel shows significance for the liver, and lower panel for the brain. The Y-axis shows p values of Student t-test. Selenoprotein transcripts in liver were more significantly modified by dietary Se than in brain.

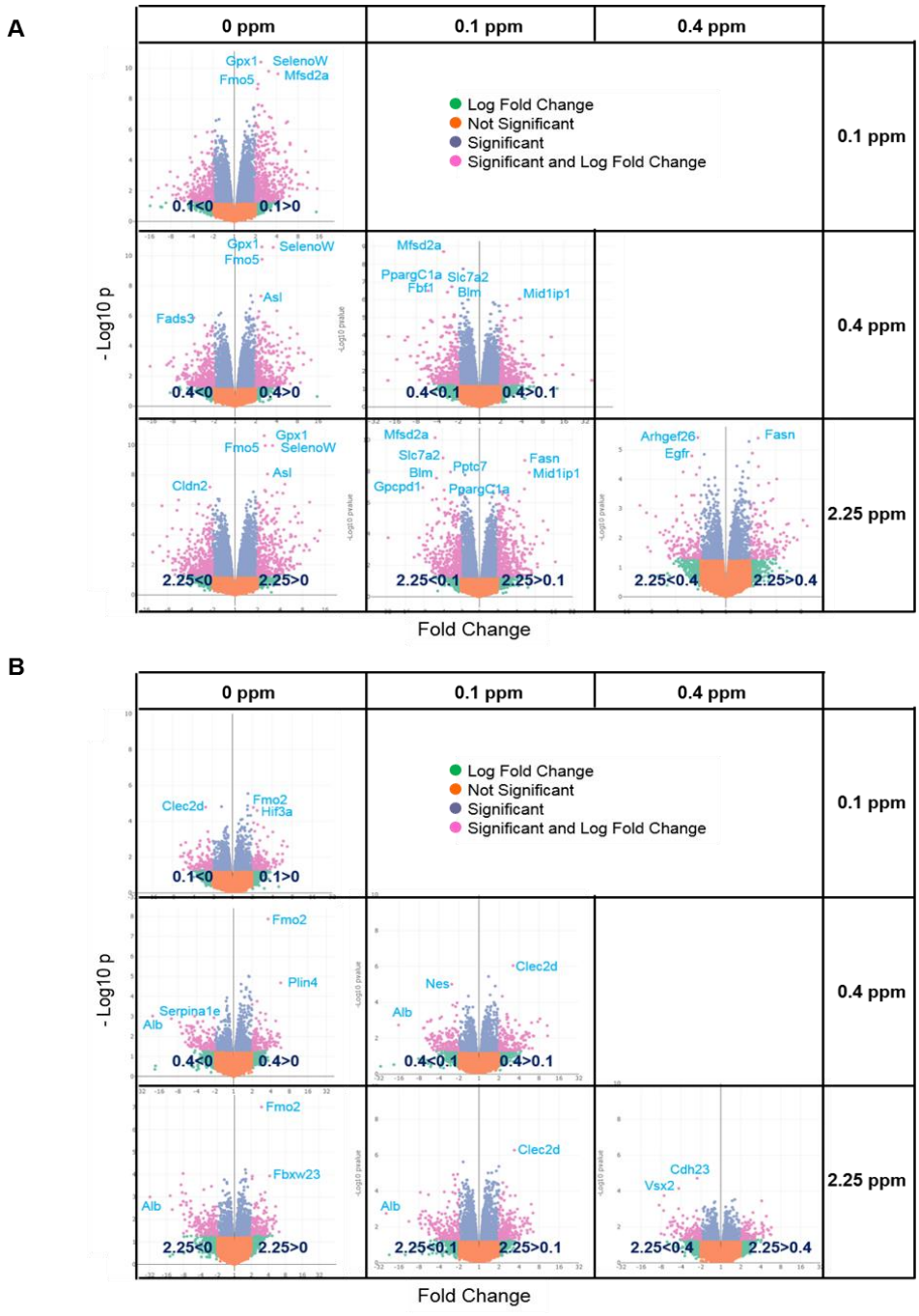
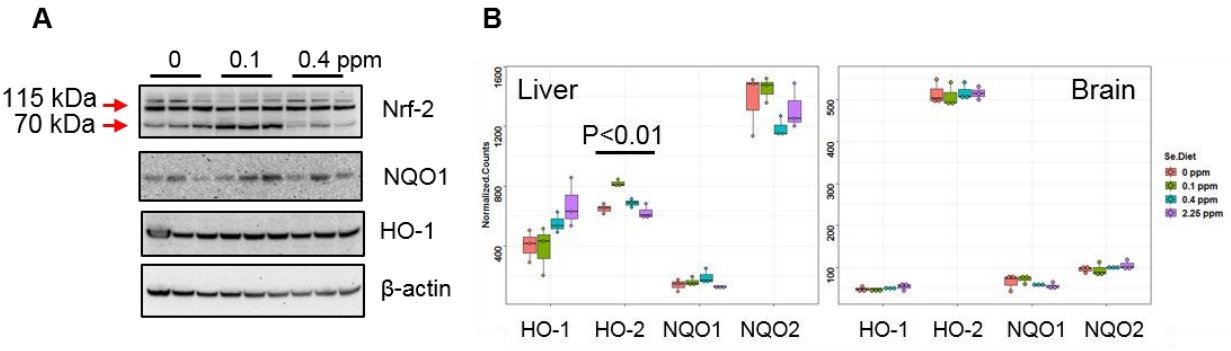
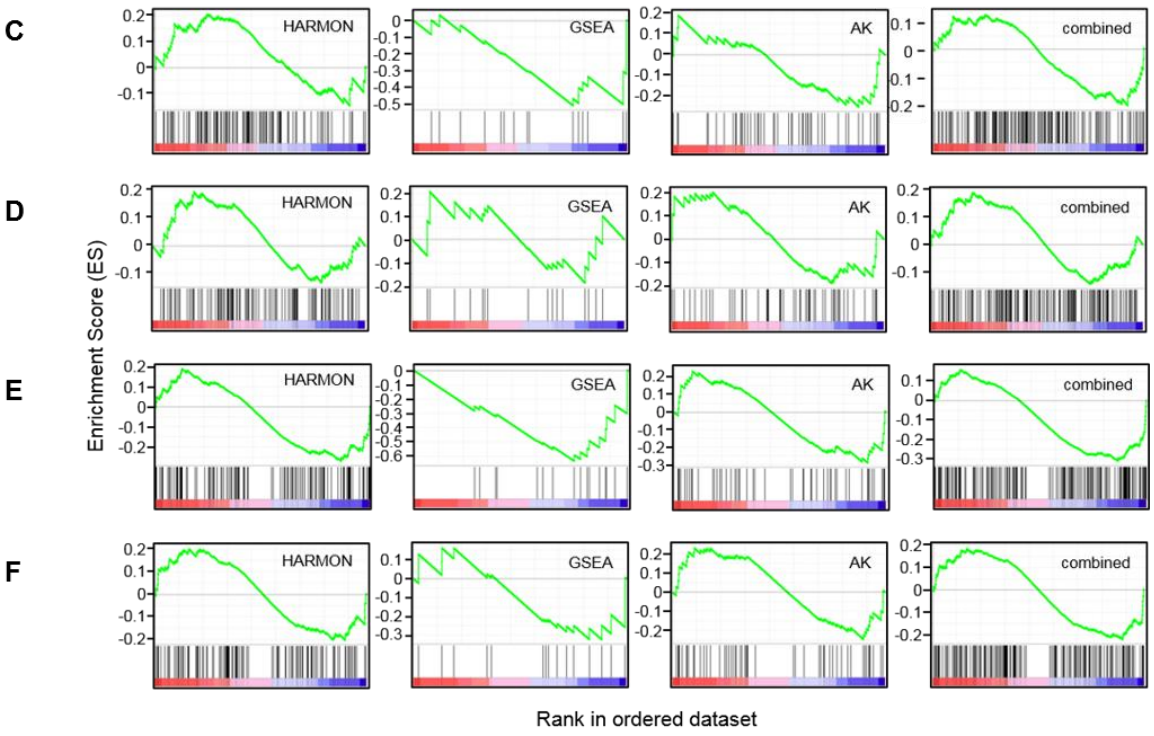


Figure S4. Regulation of gene expression by dietary Se in liver (A) and brain (B). Related to Figure 6, Figure S3, and File S2.

(A) Volcano plots of RNAseq data (in triplicate) showing the overall view on how gene expression is affected by Se diets in liver. The X-axis shows log fold changes and the Y-axis -log p values. Pink dots represent the transcripts that are significant in both p-value and fold change. Some significant transcripts are indicated on the plot in blue. **(B)** Volcano plots of RNAseq data (in triplicate) showing the overall view on how gene expression is affected by Se diets in brain. The X-axis shows log fold changes and the Y-axis -log p-values. Pink dots represent the transcripts that are significant in both p-value and fold change. Some significant transcripts are indicated on the plot in blue.





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	A. Brain (0 vs 0.1 ppm)				B. Brain (0 vs 0.4 ppm)			
	HARMON	GSEA	AK	combined	HARMON	GSEA	AK	combined
ES	0.2	-0.51	-0.25	-0.21	0.19	0.21	0.2	0.19
NES	0.91	-1.21	-0.91	-1	0.81	0.46	0.79	0.83
Nominal p-value	0.611	0.101	0.516	0.393	0.591	0.897	0.707	0.732
FDR q-value	0.706	0.204	0.608	**0.494**	0.697	1	0.816	**0.822**

	C. Liver (0 vs 0.1 ppm)				D. Liver (0 vs 0.4 ppm)			
	HARMON	GSEA	AK	combined	HARMON	GSEA	AK	combined
ES	-0.27	-0.64	-0.29	-0.31	-0.2	-0.32	-0.25	-0.22
NES	-1.1	-1.5	-0.73	-1.11	-0.83	-1.02	-0.61	-0.83
Nominal p-value	0.306	0	0.686	0.184	0.605	0.384	0.906	0.719
FDR q-value	0.4	0.097	0.797	**0.269**	0.702	0.471	1	**0.819**

Enrichment Score (ES), Normalized Enrichment Score (NES)

Figure S5. Nrf2 target analysis. NQO1 and HO-1 protein levels and mRNA expression in response to Se diets (A) and Nrf2 target gene set enrichment analysis (GSEA) in liver and brain of mice subjected to Se diets (B). Related to Figure 6 and File S2.

(A) Protein expression levels of Nrf2 and its downstream targets, NQO1 and HO-1, in livers of animals fed with diets containing 0, 0.1 and 0.4 ppm Se. Three animals per diet group were evaluated by Western blot analyses. β-actin served as a loading control. **(B)** Expression of Nrf2 target transcripts, HO-1 and NQO1, in the liver and brain of animals fed with diets containing 0, 0.1 0.4, and 2.25 ppm Se. Data were obtained from RNAseq analysis, and three animals per diet group were evaluated. **(C-F)** The mRNA expression data generated by RNAseq was analyzed using GSEA (http://software.broadinstitute.org/gsea/index.jsp) to test for Nrf2 target enrichment. Three Nrf2 target gene lists, HARMON (https://amp.pharm.mssm.edu/Harmonizome/gene_set/nrf2/GeneRIF+Biological+Term+Annotations), GSEA (http://software.broadinstitute.org/gsea/msigdb/geneset_page.jsp?geneSetName=BIOCARTA_ARENRF2_PATHWAY) and AK (Genes Nutr. 2010 5(4):297-307, PMID: 21189866) as well as the combined dataset were tested. In each panel, the green curve represents the evolution of density of genes identified in the RNAseq data. X-axis indicates rank in ordered dataset (among ~22,000 genes) and Y-axis shows enrichment score (ES) for genes in each gene set. Each barcode plot indicates the position of genes in each gene set; red and blue colors represent positive and negative Pearson correlation with the Nrf2 target gene expression, respectively. The FDR (false discovery rate) was calculated by comparing the Se diet RNAseq data with 2,500 Monte-Carlo permutations. The NES (Normalized enrichment score) computed the density of modified genes in the Nrf2 target gene lists with random expectancies. It was further normalized by the number of genes found in a given gene list (i.e. Harmon, GSEA, AK, or combined) to take into account the cluster size. **(C)** Comparison of 0 vs 0.1 ppm in the brain. Three Nrf2 target gene lists and the combined set are shown. The FDR of all Nrf2 combined gene is 0.494. **(D)** Comparison of 0 vs 0.4 ppm in the brain. Three Nrf2 target gene lists and the combined list are shown. The FDR of all Nrf2 combined gene is 0.822. **(E)** Comparison of 0 vs 0.1 ppm in the liver. Three Nrf2 target gene lists and the combined list are shown. The FDR of all Nrf2 combined gene is 0.269. **(F)** Comparison of 0 vs 0.4 ppm in the liver. Three Nrf2 target gene lists and the combined list are shown. The FDR of all Nrf2 combined gene is 0.819. **(G)** Statistics of the Ntf2 response to response to Se deficiency in liver and brain.

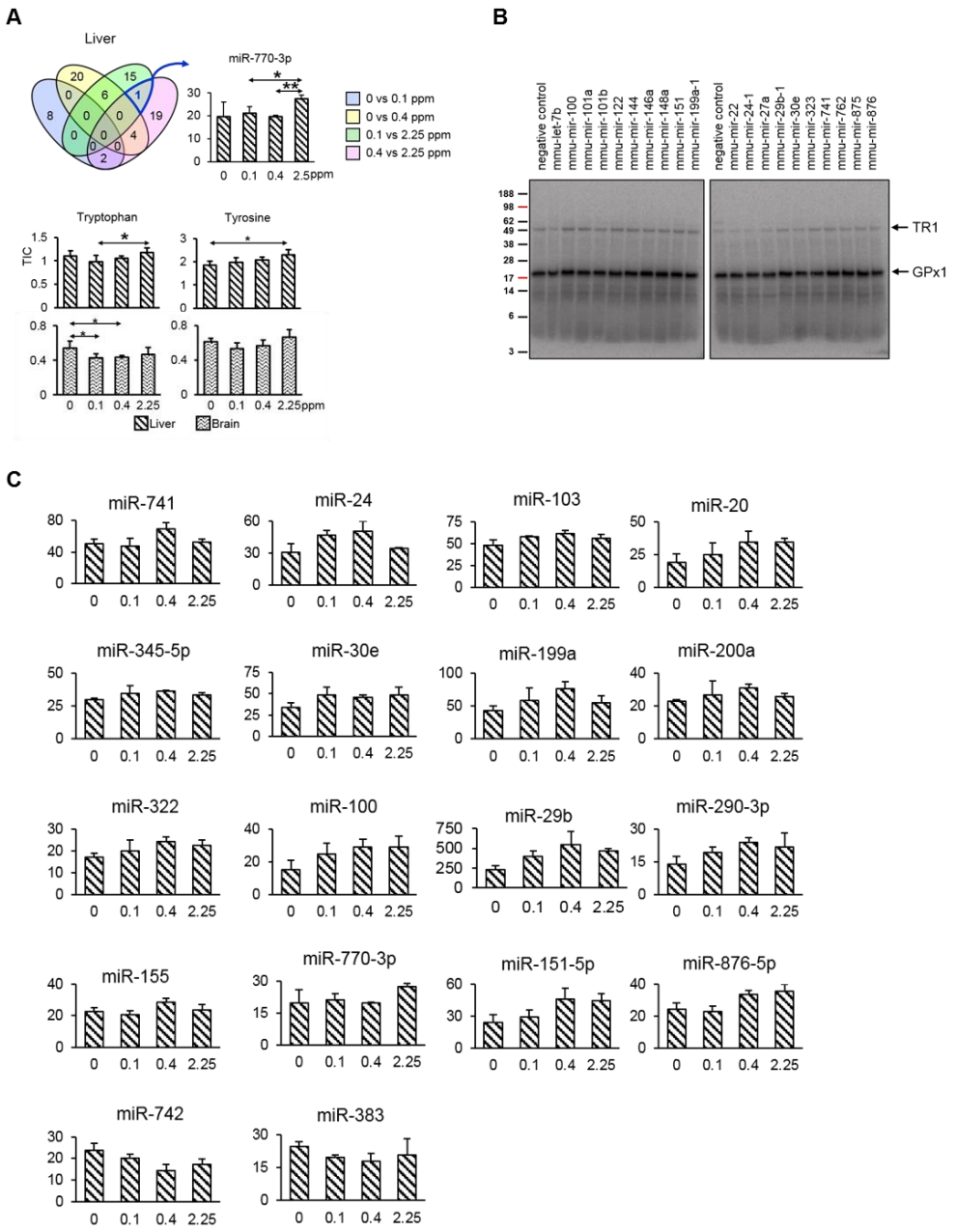

Figure S6. Dietary Se regulates miRNA expression in liver. Related to Figure 6, Table S3, and File S3.

(A) Ten miRNAs were found to be changed in the 0 vs 0.1 ppm comparison and 30 in the 0 vs 0.4 ppm comparison. Se toxicity altered 22 and 26 miRNAs relative to 0.4 ppm and 0.1 ppm samples, respectively. Expression of miR-770-3p was increased by Se toxicity which potentially target Szrd1 (ENSMUSG00000040842), Pttg1ip (ENSMUSG00000009291), Rbm14 (ENSMUSG00000006456), and (MaoB, ENSMUSG00000040147) that target tyrosine (p=2.2132E-10) and tryptophan (p=2.8886E-04). TIC represents total ion count. **(B)** ^{75}Se-labeling patterns (representing selenoprotein expression) of mouse primary hepatocytes are shown which were provided with miRNA Hairpin inhibitors. Cells were labeled with ^{75}Se for 24 h, proteins were separated by SDS-PAGE, and incorporation of ^{75}Se was visualized with a PhosphorImager. Migration of two major liver selenoproteins, Txnrd1 (TR1) and GPx1, is indicated. **(C)** Differentially expressed miRNAs. miRNAs with altered expression in response to dietary Se, as assessed by NanoString nCounter miRNA expression assays, are shown. Data are shown in mean count ± SD for three independent experiments. miRNAs that differed in at least one comparison are shown.

SUPPLEMENTAL TABLES

Table S1. Commonly enriched metabolic pathways in brain and liver in response to selenium diets (p<0.05) (A) and (p<0.01) (B). Related to Figures 2 and 3, Figures S1 and S3, and File S1.

(A) Targeted metabolite profiling method detected 335 metabolites in liver and 156 in brain that were altered in any concentration of dietary selenium. Among these, 114 metabolites were commonly altered in both liver and brain and further analyzed for metabolic pathway enrichment using MetaboAnalyst 4.0. Significantly enriched pathways (1st to 7th) are marked in the Figure 2E and listed. **(B)** Targeted metabolite profiling method detected 169 metabolites in liver and 46 in brain that were altered in any concentration of dietary selenium. Among these, 22 metabolites were commonly altered in both liver and brain and further analyzed for metabolic pathway enrichment using MetaboAnalyst 4.0. Significantly enriched pathways (1st to 5th) are listed.

A: PATHWAY (P<0.05)	TOTAL	HITS	P	-LOG(P)	FDR
aminoacyl-trna biosynthesis ①	69	13	2.78e-05	10.491	0.002279
valine, leucine and isoleucine biosynthesis ②	11	4	0.001712	6.37	0.055661
purine metabolism ③	68	10	0.002036	6.1966	0.055661
alanine, aspartate and glutamate metabolism ④	24	5	0.006607	5.0196	0.12875
nitrogen metabolism ⑤	9	3	0.009151	4.6939	0.12875
glutathione metabolism ⑥	26	5	0.009421	4.6648	0.12875
cysteine and methionine metabolism ⑦	27	5	0.011099	4.5009	0.13001
lysine biosynthesis	4	2	0.015078	4.1945	0.15455
pyrimidine metabolism	41	6	0.017278	4.0583	0.15742
glycine, serine and threonine metabolism	31	5	0.019841	3.92	0.1627
arginine and proline metabolism	44	6	0.023953	3.7317	0.17856
beta-alanine metabolism	17	3	0.054848	2.9032	0.3748
biosynthesis of unsaturated fatty acids	42	5	0.063642	2.7545	0.40143

B: PATHWAY (P<0.01)	TOTAL	HITS	P	-LOG(P)	FDR
cysteine and methionine metabolism ①	27	3	0.004164	5.4813	0.19251
ascorbate and aldarate metabolism ②	9	2	0.005207	5.2577	0.19251
valine, leucine and isoleucine biosynthesis ③	11	2	0.007837	4.849	0.19251
aminoacyl-trna biosynthesis ④	69	4	0.009391	4.668	0.19251
starch and sucrose metabolism ⑤	19	2	0.022941	3.7748	0.37623
alanine, aspartate and glutamate metabolism	24	2	0.035665	3.3336	0.42172
galactose metabolism	26	2	0.041372	3.1851	0.42172
inositol phosphate metabolism	28	2	0.047404	3.049	0.42172
phenylalanine, tyrosine and tryptophan biosynthesis	4	1	0.049903	2.9977	0.42172
purine metabolism	68	3	0.051429	2.9675	0.42172

Table S2. Metabolites associated with selenium deficiency in liver. Related to Figure 5, Figure S1 and S2, and File S1.

Sixty-three metabolites are altered in the comparison of 0 vs 0.1 ppm and 76 metabolites are altered in 0 vs 0.4 ppm. Among these, 25 metabolites are commonly altered in both comparisons with p value less than 0.01 (Figure 5A).

HMDB	COMPOUND NAME
HMDB00696	L-Methionine
HMDB00187	L-Serine
HMDB00168	L-Asparagine
HMDB00056	Beta-Alanine
HMDB01185	s-adenosylmethionine
HMDB29416	L-Targinine
HMDB03337	Oxidized glutathione
HMDB02005	Methionine sulfoxide
HMDB01520	Flavin Mononucleotide
HMDB00691	Malonic acid
HMDB00300	Uracil
HMDB00157	Hypoxanthine
HMDB01539	ADMA
HMDB01202	dCMP
HMDB10393	LysoPC(20:3(5Z,8Z,11Z))
HMDB10382	LysoPC(16:0)
HMDB11503	LysoPE(16:0/0:0)
HMDB06726	CE(20:4(5Z,8Z,11Z,14Z)
HMDB11504	LysoPE(16:1(9Z)/0:0)
HMDB42105	C53:3 TAG
HMDB05363	TG(16:0/16:0/20:4(5Z,8Z,11Z,14Z))[iso3]
HMDB11130	LysoPE(18:0/0:0)
HMDB08006	PC(16:1(9Z)/18:2(9Z,12Z))
HMDB05462	C56:7 TAG
HMDB11507	C18:2 LPE

Table S3. microRNAs differentially expressed in response to selenium diets. Related to Figure 6, Figure S6, and File S3.

Comparisons are made with the Student t-test. All listed microRNAs are with $p<0.05$, microRNAs with $p<0.01$ are marked ¶, microRNAs marked § are shown in the Figure S6.

0 vs 0.1 ppm	0 vs 0.4ppm	0 vs 2.5 ppm	0.1 vs 0.4 ppm	0.1 vs 2.5 ppm	0.4 vs 2.5 ppm
miR-1894-3p	miR-452¶	miR-1274a¶	miR-667¶	miR-466f-5p¶	miR-770-3p§¶
miR-222	miR-450a-3p¶	miR-29b§¶	miR-669e¶	miR-466j¶	miR-713¶
miR-767	miR-345-5p§¶	miR-1191¶	miR-546¶	miR-m108-2-5p.2	miR-200c
miR-29b§	miR-M87-1	miR-466j	miR-296-3p¶	miR-876-5p§	miR-1963
miR-383§	miR-322§	miR-M23-2	miR-342-5p	miR-1968	miR-340-3p
miR-666-3p	miR-376b	miR-669m	miR-155§	let-7b	miR-10a
miR-188-5p	miR-433	miR-466f-5p	miR-582-5p	miR-1966	miR-450a-5p
miR-327	miR-713	miR-151-5p§	miR-452	miR-323-3p	miR-298
miR-466f-5p	miR-298	miR-3072	miR-199a-5p§	miR-1224	miR-210
miR-615-3p	miR-27a	miR-1944	miR-291a-5p	miR-1192	miR-615-5p
	miR-199a-3p§	miR-297b-3p	miR-876-5p§	miR-743a	miR-471
	miR-742§	miR-15b	miR-450a-3p	miR-2133	miR-188-3p
	miR-290-3p§	miR-1929	miR-297b-3p	miR-1894-3p	miR-664
	miR-200a§	miR-876-5p§	miR-1953	miR-297b-3p	miR-2139
	miR-362-3p	miR-9	miR-139-5p	miR-194	miR-9
	miR-155§	miR-1903	miR-191	miR-122	miR-1953
	miR-741§	miR-615-5p	miR-210	miR-133b	miR-376a
	miR-100§	miR-20a§	miR-713	miR-1187	miR-1943
	miR-471	(miR-20b)	miR-2183	miR-151-5p§	miR-741§
	miR-210	miR-101a	miR-376b	miR-24§	miR-670
	miR-291a-3p	miR-487b	miR-330	miR-540-3p	miR-669e
	miR-450a-5p	miR-135b	miR-671-3p	miR-296-3p	miR-32
	miR-103§	miR-322§	miR-188-5p	miR-546	
	miR-487b	miR-122	miR-450a-5p	miR-487b	
	miR-667	miR-323-3p	miR-802	miR-1903	
	miR-876-5p§		miR-1192	miR-770-3p§	
	miR-30e§		miR-487b		
	miR-297b-3p		miR-741§		
	miR-151-5p§		miR-M1-1		
	miR-142-5p		miR-686		
			miR-369-5p		
			miR-2133		
			miR-488		