Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Figures to

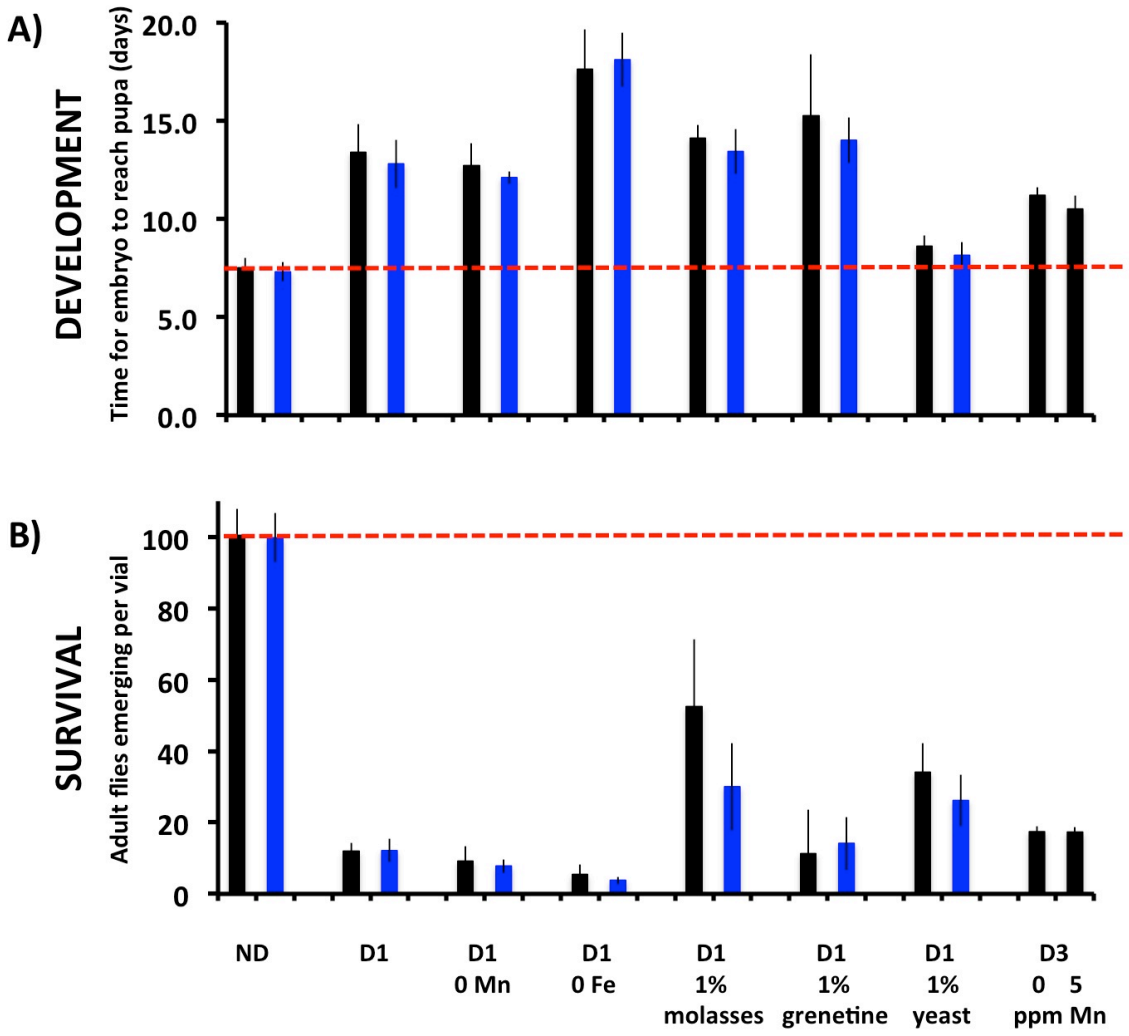
Vásquez-Procopio *et al*.

"Intestinal response to dietary manganese depletion in Drosophila"

Metallomics

Gene RNAi	Full Genotype	BDSC
Vha13	*y^1 sc* v^1; P{TRiP.HMS01677}attP40*	38233
Vha26	*y^1 sc* v^1; P{TRiP.HMS01912}attP2*	38996
Vha44	*y^1 sc* v^1; P{TRiP.HMS00821}attP2*	33884
Vha55	*y^1 v^1; P{TRiP.HMS02132}attP40/Cyo*	40884
VhaSFD	*y^1 sc* v^1; P{TRiP.HMS02144}attP40/Cyo*	40896
Vha68-1	*y^1 sc* v^1; P{TRiP.HMS02581}attP40/Cyo*	42888
Vha68-2	*y^1 sc* v^1; P{TRiP.HMS01056}attP2*	34582
Vha68-3	*y^1 sc* v^1; P{TRiP.HMS02647}attP40*	42954
Vha14-2	*y^1 sc* v^1; P{TRiP.HMC06115}attP40/Cyo*	65363
Vha16-1	*y^1 sc* v^1; P{TRiP.HMS02171}attP40*	40923
Vha16-2	*y^1 sc* v^1; P{TRiP.HMC06043}attP40/Cyo*	65167
Vha16-3	*y^1 sc* v^1; P{TRiP.HMC04786}attP40*	57474
Vha16-5	*y^1 v^1; P{TRiP.JF01821}attP2*	25803
Vha36-3	*y1 sc* v1; P{TRiP.HMC06023}attP40*	65075
VhaPPA1-2	*y^1 sc* v^1; P{TRiP.HMC06080}attP40/Cyo*	65217
VhaM9.7-a	*y^1 v^1; P{TRiP.JF02029}attP2*	26004
VhaM9.7-b	*y^1 w^{67c23}; P{SUPor-P}VhaM9.7-$b^{KG03854}$ ry^{506}*	13351
VhaM9.7-d	*y^1 sc* v^1; P{TRiP.HMS05645}attP40/Cyo*	67822
Vha100-1	*y^1 v^1; P{TRiP.JF02059}attP2*	26290
Vha100-2	*y^1 sc* v^1; P{TRiP.HMC05732}attP40*	64859
Vha100-5	*y^1 v^1; P{TRiP.HMJ30067}attP40/Cyo*	62990
VhaAC39-1	*y^1 sc* v^1; P{TRiP.HMS01442}attP2*	35029
VhaAC39-2	*y^1 sc* v^1; P{TRiP.HMS05622}attP40*	67809
VhaAC45	*y^1 sc* v^1; P{TRiP.HMS01717}attP40*	38522
VhaAC45-2	*y^1 sc* v^1; P{TRiP.HMC04789}attP40*	57476
Sod1	*w*; P{UAS-Sod1.IR}4*	24491
Sod2	*w^1; P{UAS-Sod2.dsRNA.K}15/SM5*	24489

Supplementary Figure 1. List of RNAi lines used in this study. Underlined subunits correspond to the V_1 complex. Subunits in bold are only encoded by a single gene and should therefore affect all V-ATPase complexes. More information over these lines is available at www.flybase.org using the identifier at Bloomington Drosophla Stock Center (BDSC).



Supplementary Figure 2. Days to appearance of first pupa and survival to adulthood of larvae raised on different chemical diets. Ten mated females were allowed to lay their eggs for 3 days on the media indicated (Supplementary Figure 3). Black bars refer to isogenic w^+ and blue bars to isogenic w^* genotypes. Measurements are from 8-10 vials per condition; the standard deviation from the mean is shown.

A) Developmental timing from embryo to pupae measured as time of appearance of the first pupae in 10 different vials. Note that diet 1 almost doubles developmental time from embryo to pupa; removal of Fe further extends this phenotype; addition of yeast fully reverts it. Diet 3 extends this period of development from 7.4 to 10.9 days irrespective of manganese concentration.

B) Total progeny from the vials. The numbers of eggs laid were not determined, but the data show fewer flies being recovered per vial on the chemically defined media (roughly 20% from Normal Diet in the case of diet 3).

	Provider (Diets 1-3)	quantity given in g / L							
Chemical component		**Diet 1**	**Diet 2**	**Diet 3**	**B&H 2005**	**Troen 2007**	**L&M 2013**	**Reis 2016**	**Piper 2017**
Protein and sugar									
Casein	BD Biosciences 223050	53.2	21.6	21.6	53.2	amino acids	amino acids	73.3	amino acids
Sucrose	Sigma-Aldrich S0389	9.7	19.3	19.3	9.7	-	63.7	13.3	17.2
Inorganic salts									
Sodium phosphate dibasic	Sigma-Aldrich S7907	1.82	-	-	25.2	-	-		-
Potassium phosphate monobasic	Merck 4873	0.69	0.10	0.10	9.47	0.45	0.61	7.10	-
Potassium phosphate dibasic	Sigma-Aldrich P2222	3.61	0.54	0.54	49.73	-	0.61	37.3	3.0
Sodium bicarbonate	Sigma-Aldrich S6297	0.96	0.06	0.06	13.33	0.005	-	10.0	1.0
Magnesium sulfate heptahydrate	Sigma-Aldrich M5921	0.60	0.50	0.50	8.27	0.02	0.25	6.20	0.25
Calcium chloride dehydrate	Sigma-Aldrich C5670	0.19	0.15	0.15	0.27	0.005	0.013	-	0.25
Zinc acetate dihydrate	Sigma-Aldrich 379786	0.19	0.006	0.006	0.27	0.010	0.018	-	0.025
Ferric ammonium citrate	Sigma-Aldrich F5879	0.010	0.008	0.008	0.13	0.003	0.013	-	0.025
Manganese sulfate monohydrate	Sigma-Aldrich M7634	0.013	0.002	0.002	0.172	0.003	0.010	-	0.001
Copper sulfate	Sigma-Aldrich C1297	0.0050	0.0005	0.0005	0.0667	0.0047	0.0085	-	0.0025
Ammonium molybdate	Merck 321182	0.0050	0.0010	0.0010	-	-	-	-	-
Vitamins									
B1. Thiamine	Sigma-Aldrich T1270	0.0120	0.0060	0.0060	0.0116	0.0015	0.0015	0.0200	0.0014
B2. Riboflavin	Sigma-Aldrich R9504	0.0100	0.0050	0.0050	0.0097	0.0024	0.0024	0.1000	0.0007
B3. Niacin	Sigma-Aldrich N4126	0.0460	0.0230	0.0230	0.0464	0.0100	0.0100	0.1200	0.0084
B5. Calcium pantothenate	Sigma-Aldrich C8731	0.0190	0.0190	0.0190	0.0193	0.0600	0.0060	0.1600	0.0108
B6. Pyridoxine hydrochloride	Sigma-Aldrich P6280	0.0060	0.0030	0.0030	0.0058	0.0300	0.0030	0.0250	0.0017
B8. Biotin	Sigma-Aldrich B4639	0.0006	0.0003	0.0003	0.0006	0.00002	0.0000	0.0020	0.0001
B9. Folic acid	Sigma-Aldrich F8758	0.0030	0.0030	0.0030	0.0029	0.0060	0.0059	0.5000	0.0005
C. Ascorbic acid	Sigma-Aldrich A5960	0.54	0.54	0.54	0.54	-	-	-	-
Other									
Yeast RNA	Sigma-Aldrich R6625	3.9	2.5	-	3.9	1.0	1.0	-	-
Cholesterol	Sigma-Aldrich C8667	0.29	0.29	0.29	0.29	0.10	0.08	0.40	0.10
Tryptophan	Sigma-Aldrich T0254	2.4	0.24	0.24	2.42	0.09	0.07	-	0.03
L-carnitine hydrochloride	Sigma-Aldrich C0283	0.010	0.010	0.010	0.010	-	-	-	-
Choline	Sigma-Aldrich C1879	0.058	0.058	0.058	0.058	0.016	0.036	0.32	0.050
Lecithin	Sigma-Aldrich P3644	-	0.40	0.40	-	0.0001	0.79	-	-
Putrescine	Sigma-Aldrich P5780	-	0.16	0.16	-	-	-	-	-
Lipoic acid	Sigma-Aldrich T1395	-	0.052	0.052	-	-	-	-	-
Myo-inositol	Sigma-Aldrich I7508	-	-	0.005	-	0.042	0.042		0.005
Uridine	Sigma-Aldrich U3750	-	-	0.060	-	-	-	0.76	0.060
Inosine	Sigma-Aldrich I4125	-	-	0.065	-	-	-	0.85	0.065
Yeast	Grupo La Florida	10	-	-	-	-	35	-	-
Agar	Oxoid LP0011	29	16	16	29	20	9	5	20
Propionic acid	Sigma-Aldrich P5561	10 mL/L	10 mL/L	10 mL/L	-	-	-	-	6 mL/L

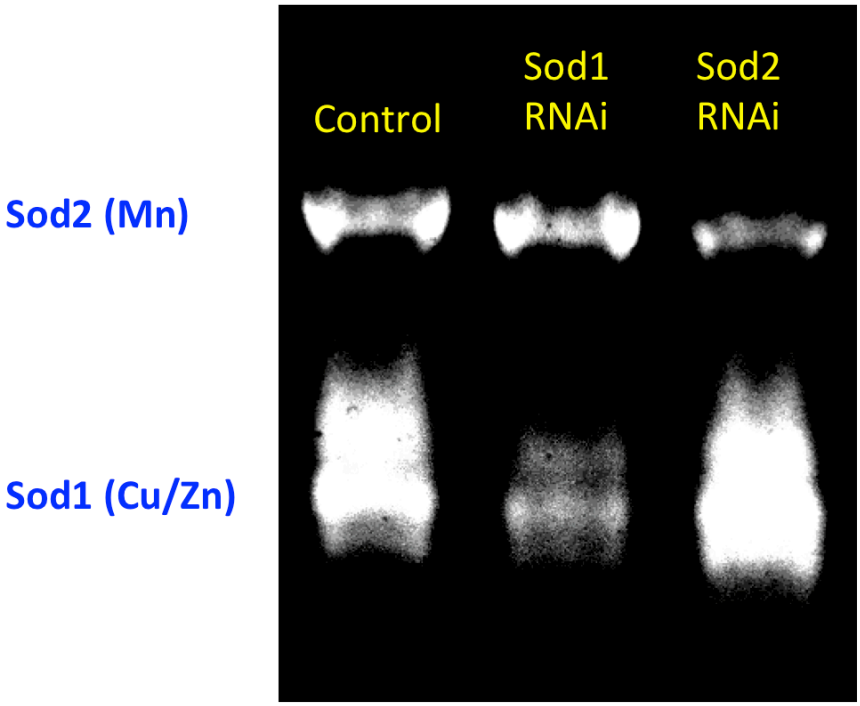
Supplementary Figure 3. Concentration of chemicals in the defined diets used here and comparison to other published protocols. References 90-96 in main text.

mg metal / g dry weight	Mn	Cu	Fe	Zn	Na	K	Ca	Mg	P
Standard diet	0.012	0.004	0.025	0.052	0.6	3.7	0.8	0.6	1.9
Diet 1	**0.020**	0.031	0.034	0.105	-	-	-	-	-
Diet 1 - 0 ppm Mn	**0.006**	0.017	0.025	0.112	-	-	-	-	-
Diet 1 - 2 ppm Fe	**0.017**	0.007	0.027	0.103	-	-	-	-	-
Diet 2 - 0 ppm Mn	**0.001**	0.006	0.041	0.025	**17.0**	4.7	0.8	0.8	6.3
Diet 2 - 5 ppm Mn	**0.019**	0.007	0.042	0.026	**16.5**	4.7	0.8	0.8	6.5
Diet 3 - 0 ppm Mn	**0.000**	0.007	0.024	0.021	11.4	5.1	0.7	0.6	4.8
Diet 3 - 5 ppm Mn	**0.023**	0.006	0.027	0.022	11.9	5.5	0.8	0.7	5.3

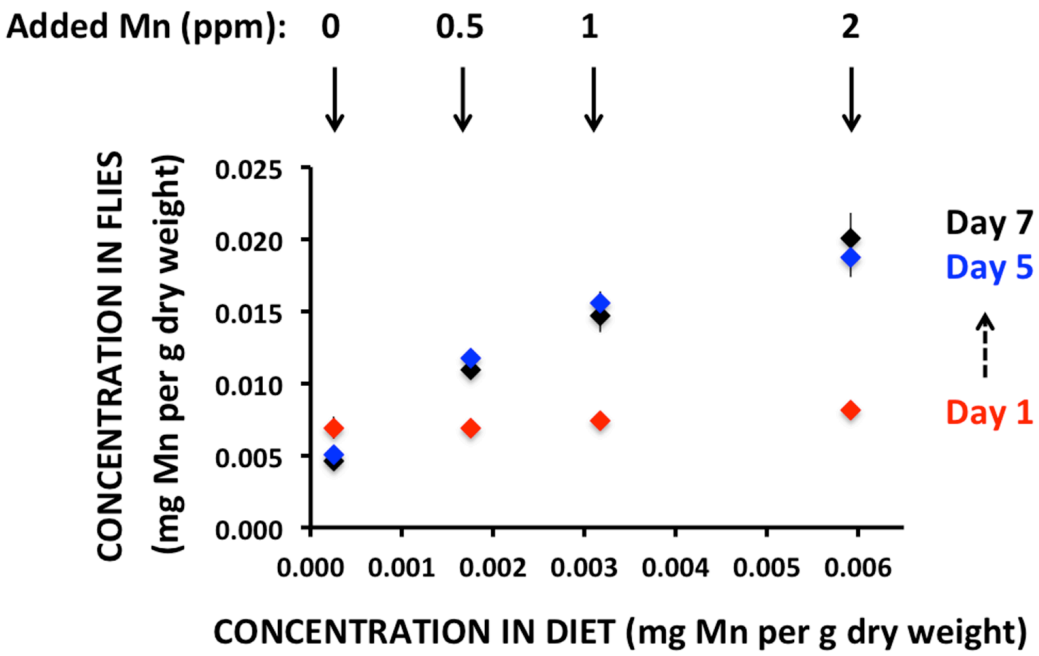
Supplementary Figure 4. Metal analysis by ICP-OES of the chemical diets used in this study. Mean values in mg [element] per g [dry weight diet] are shown for metal determinations in the various diets used in this study. Red font depicts manganese concentration in the depleted diets (0.000 in Diet 3 is equal to 0.00025). The pink shadow depicts the condition in which our label-free proteomic experiment was performed. The yellow shadow depicts the high sodium concentration, attributed to the casein protein source we used.

Gene	Primers
sug	CCAGCGATTTCGTATGCAACT GCGGCAATAGTAGAGTCCGTC
CG1946	TCCTGCTGTACTACGGCGAA GCTGCGGTAGAATAACCAACCA
Lip3	AAAACGGGTGAATCTTCCAACC CCAGCATATAGGCCAGGGA
Amyrel	TTGCCCAGGAGTGTGAGAGT TCCTCATTACCAGACCGAGTG
prt	CCTGATTGCCGTGATTGTTTACC CGGACGATGTATCCGCCTC
ZnT63C	TTGCGGCCCTGGTCATATC GGCCCAGCCAAAAGTGTTCT

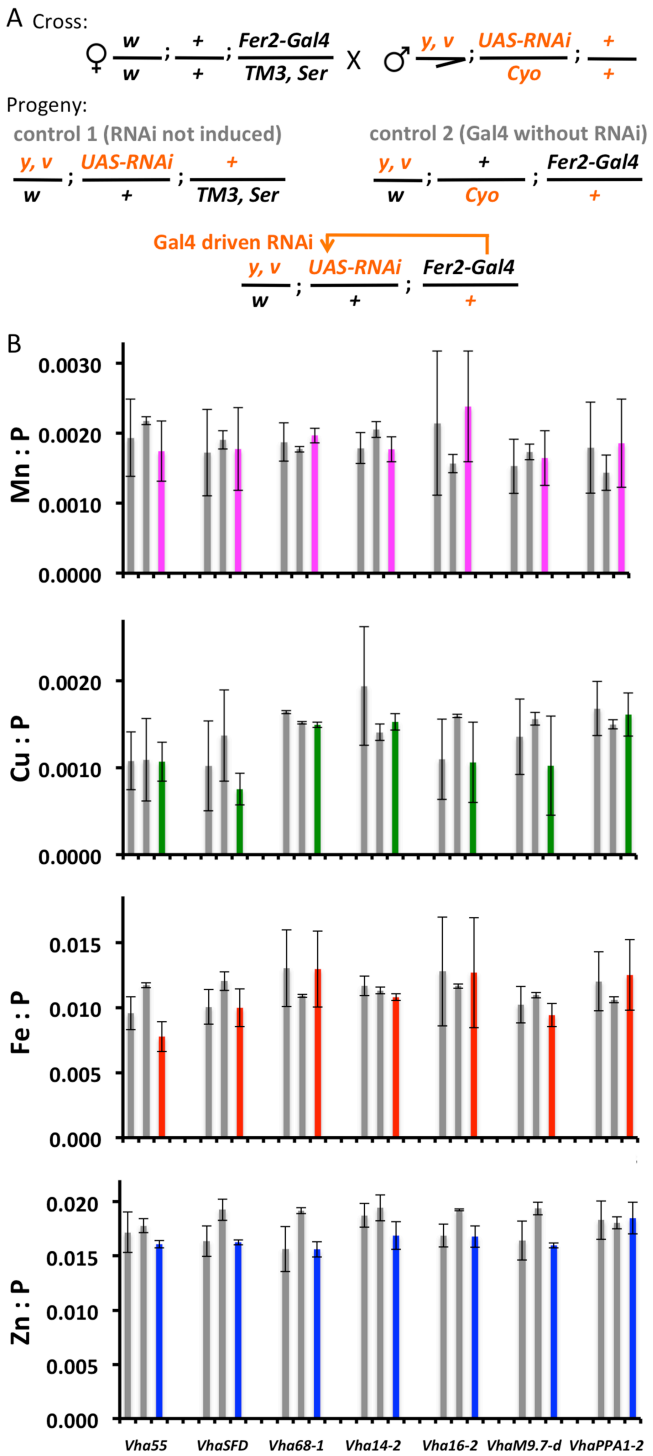
Supplementary Figure 5. List of primers used for the qPCR experiments. Obtained from the Fly Primer Bank for PCR Primers in *Drosophila melanogaster* (Hu *et al*. 2013).



Supplementary Figure 6. Validation of in-gel Sod assay through RNA interference against Sod1 and Sod2. Lanes 2 and 3 show the progeny of RNAi lines crossed to the Da-Gal4 driver (shown as control on lane 1).



Supplementary Figure 7. Diet 3 supplemented with 0.5 ppm Mn leads to Mn accumulation in five-day-old flies. Three diets were prepared and Mn content was determined (plotted on the X-axis). Metal content was measured in flies grown on a normal diet, but transferred immediately on eclosion to the above three diets, where they were kept for 1 day (red rhomboids), 5 days (blue rhomboids) or 7 days (black rhomboids). Manganese accumulation was observed with as little as 1 ppm added to the diet. Values appear slightly higher on the X-axis because media are dried prior to measurement. At 0.5 ppm Mn there was detectable increase in concentration of Mn. Standard deviations indicate triplicate measurements on different pools of flies from a single experiment.



Supplementary Figure 8. Metallomes of flies in which RNAi against specific v-ATPase subunits was driven by Fer2-Gal4 compared to two genetically similar sibling controls.

A) A generic scheme for the parental crosses and selected genotypes used.

B) Results from the three indicated genotypes were plotted; the first grey bar indicates control 1 (RNAi line not induced), followed by control 2 also in grey (Gal4 line alone), followed by the RNAi flies in colour. The affected V-ATPase subunit is shown at the bottom of the figure.