Supporting Information for Holmes-Hampton *et al*. 2012

Table of Contents:

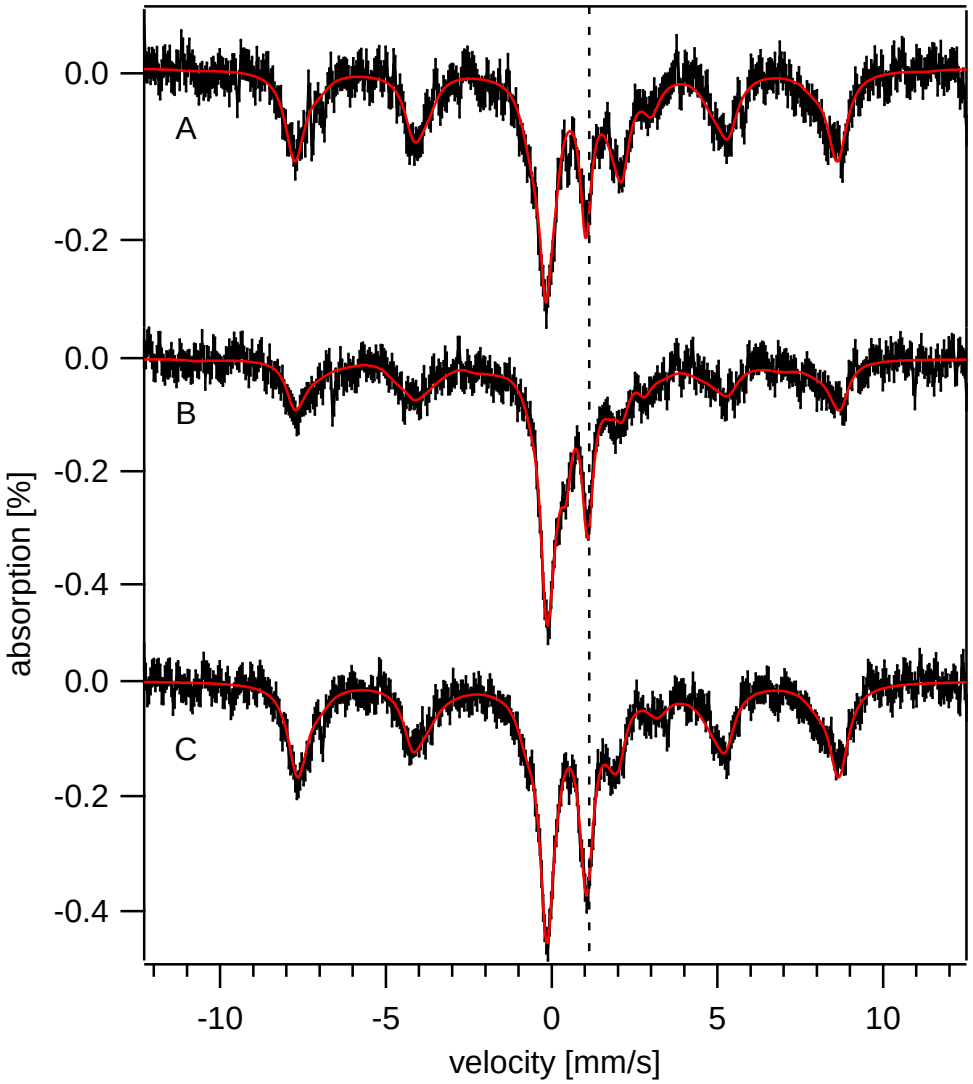

Figure S1: Corrected Mössbauer spectra of mouse brains at A, 1 wk, B, 2 wks, and C, 24 wks. The dashed line indicates the position of the high energy line of the central doublet. Simulations in red used parameters indicated in the text and percentages from Table 1. Spectra were collected at 6 K with a 0.05 T field applied parallel to the radiation.

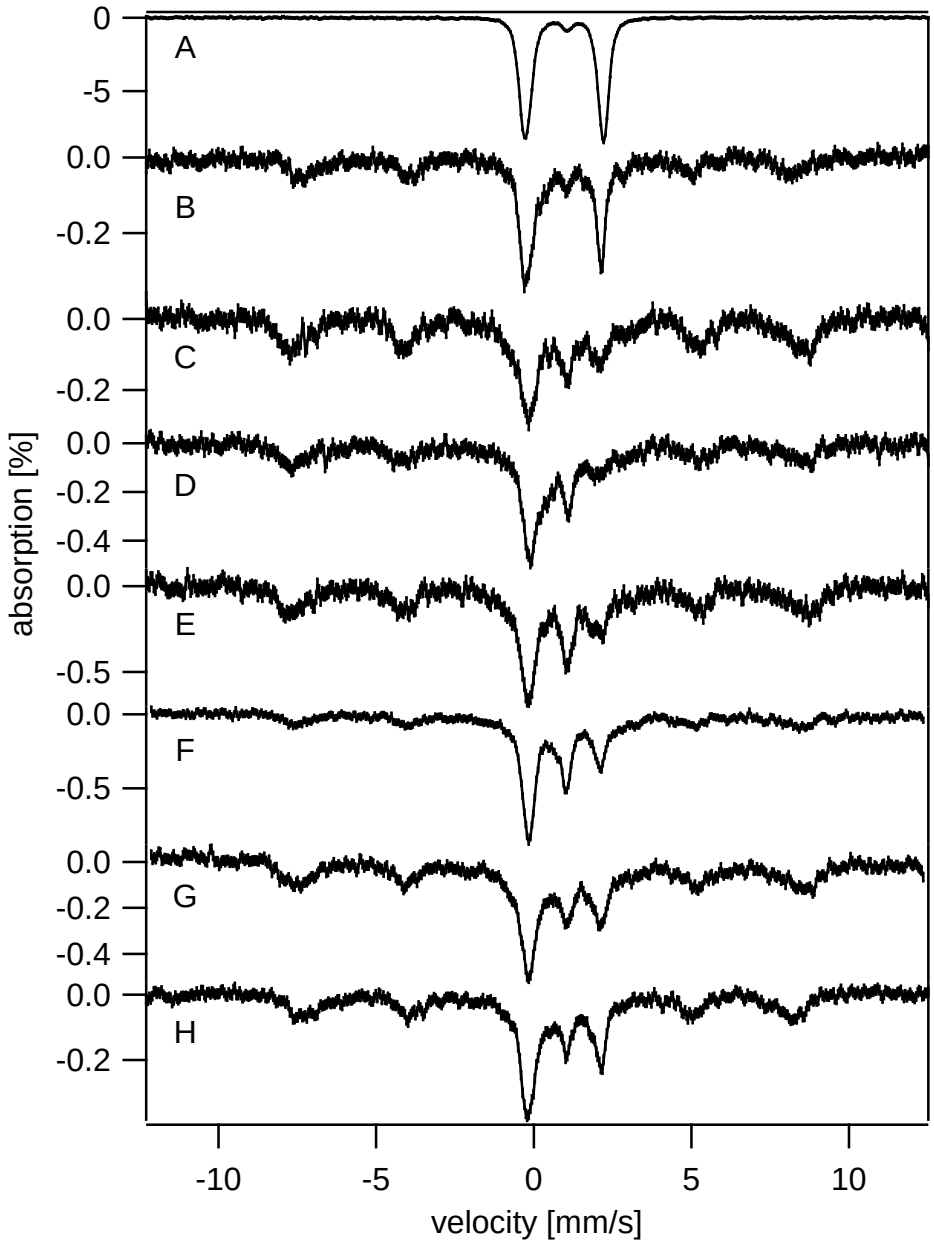

Figure S2. Uncorrected Mössbauer spectra of mouse brains at different ages. A, blood from a 4 wk mouse; **B,** unperfused prenatal brain (corresponds to corrected spectrum, Fig. **2E**); **C**, uncorrected spectrum of perfused 1 wk brain (corrected spectrum is Fig. **S1A**); **D,** uncorrected spectrum of perfused 2 wk brain (corrected spectrum is Fig. **S1B**); **E,** uncorrected spectrum of perfused 3 wk brain (corrected spectrum is Fig. **2A**); **F,** uncorrected spectrum of perfused 4 wk brain (corrected spectrum is Fig. **2F**); **G,** uncorrected spectrum of perfused 24 wk

brain (corrected spectrum, Fig. **S1C**); **H,** uncorrected spectrum of perfused 58 wk brain (corrected spectrum, Fig. **2G**); Spectra were collected at 5-6 K and 0.05 T magnetic field applied parallel to the direction of the radiation. Total Mössbauer data collection times (in hrs), including 70 K data, were **A**, 40; **B**, 330; **C**, 435; **D**, 390; **E**, 240; **F**, 390; **G**, 570; and **H**, 400.

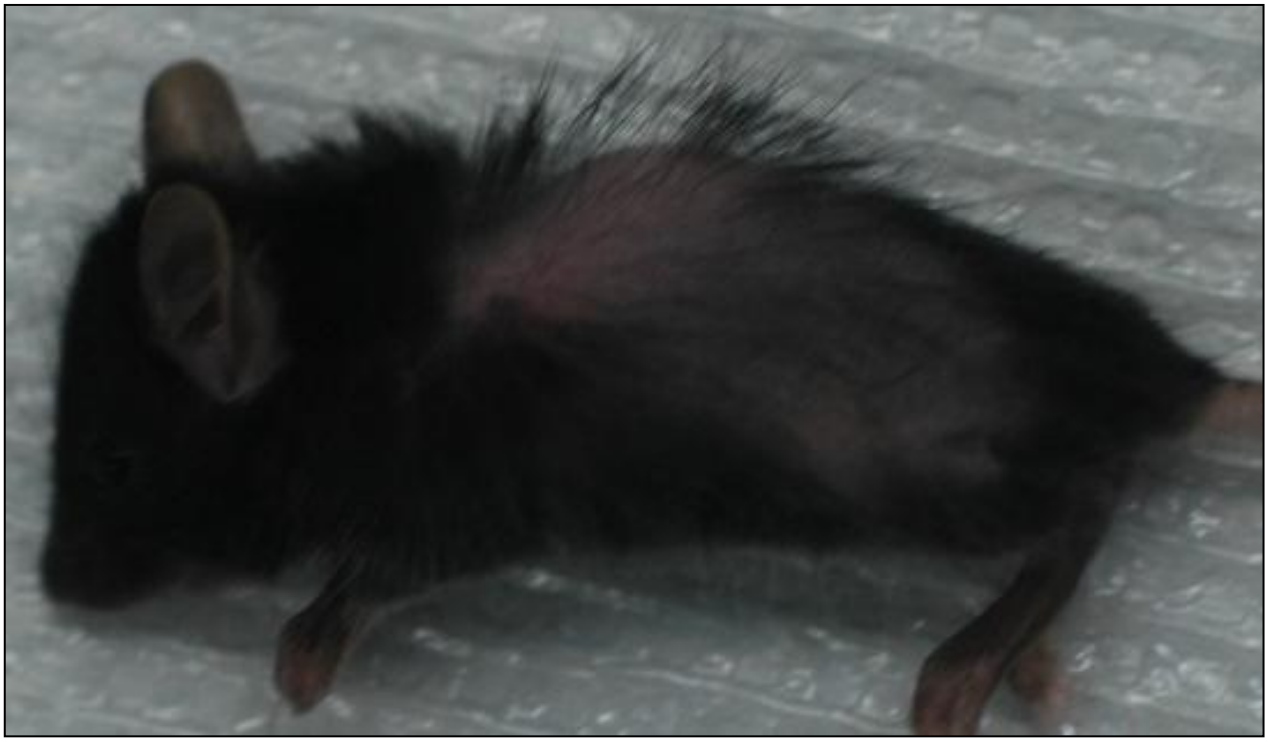

Figure S3. Photograph of an iron-deficient mouse used in this study. This 3 wk old mouse was nursed by a mother whose chow had been switched from ^{57}Fe-supplemented to Fe-deficient *ca*. 3 days prior to giving birth. The pup displayed significant alopecia (thinning of hair) indicating Fe deficiency.

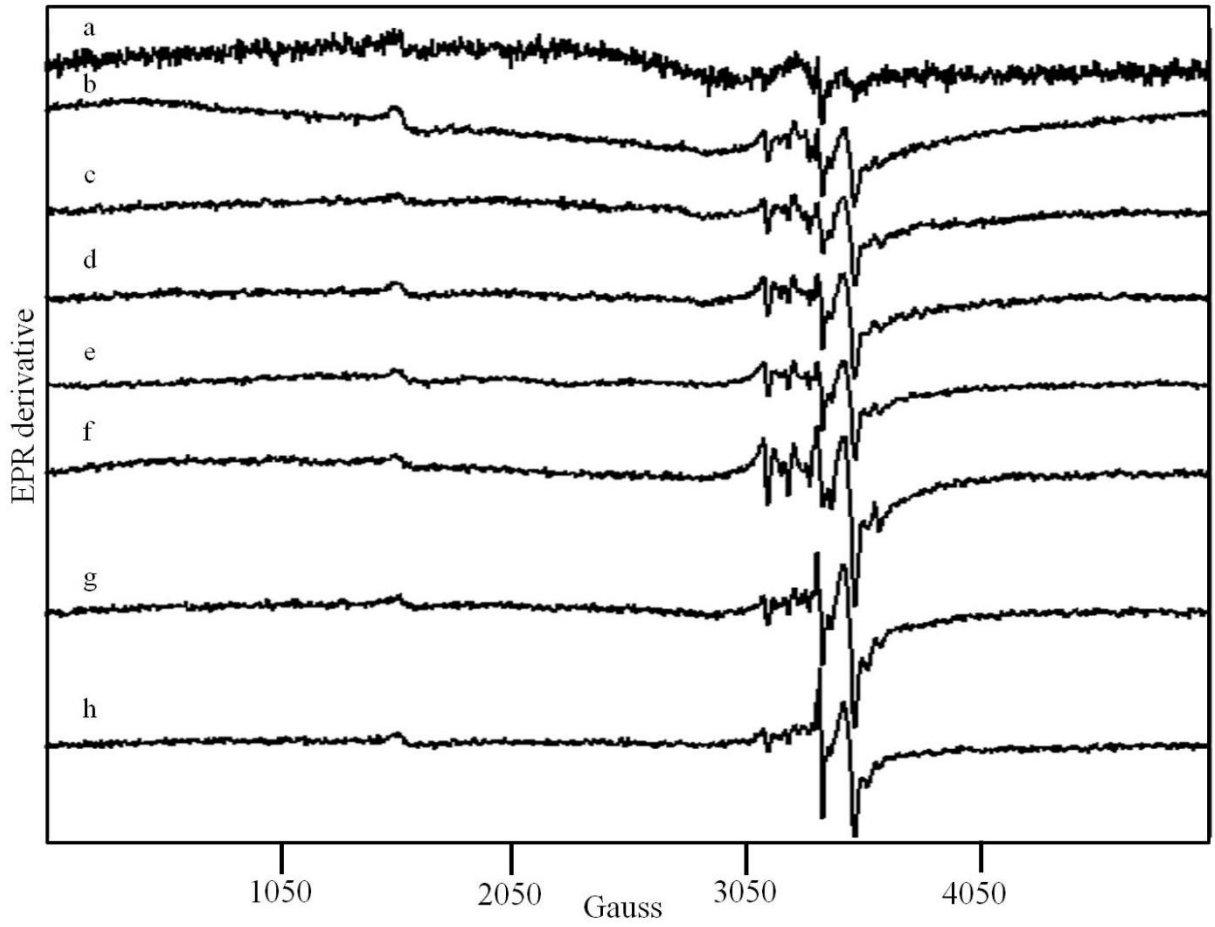

Figure S4. EPR spectra of packed mouse brain homogenates from mice euthanized at different ages. a, -1 wk; **b**, 1 wk; **c**, 2 wks; **d**, 3 wks; **e**, 3 wks, Fe-deficient; **f**, 4 wks; **g**, 24 wks; and **h**, 58 wks. EPR conditions are the same as in the Fig. 3 legend.

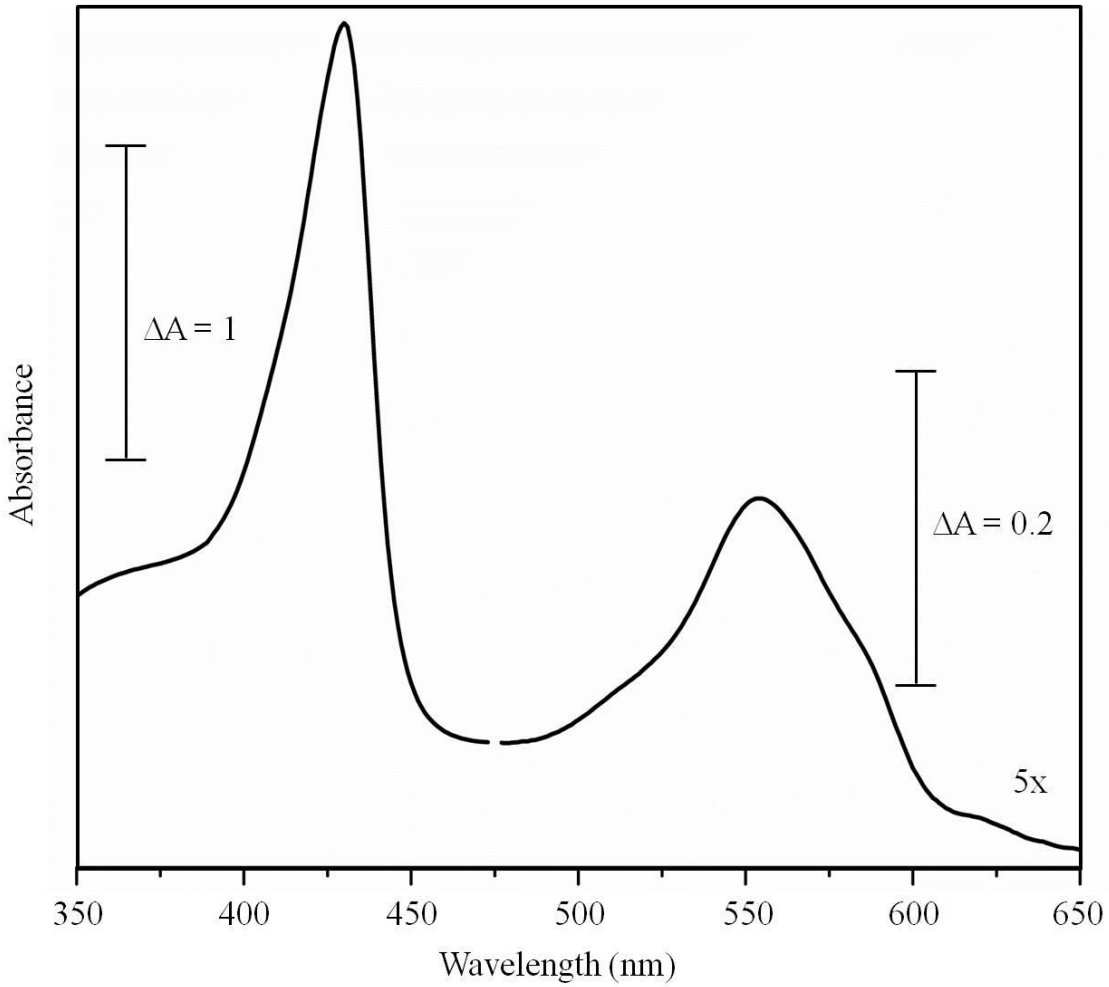

Figure S5. UV-vis spectrum of Blood. The sample (Sample 5, Table S9) was diluted 100:1 with Ringer's buffer plus heparin and was prepared under anaerobic conditions.

Table S1. **Masses of organs used for elemental analysis.** *These numbers also apply to Tables S2 - S8.

Age at Euthanization (wks)	Brain	Liver	Heart	Kidney	Spleen	Duodenum	Number of animals*
4	370 ± 2	890 ± 220	100 ± 10	160 ± 15	64 ± 26	82 ± 35	2
5	380 ± 27	740 ± 300	110 ± 12	270 ± 49	72 ± 21	110 ± 49	5
6	390 ± 17	1100 ± 77	120 ± 14	260 ± 21	71 ± 27	94 ± 44	5
8	400 ± 12	1100 ± 240	120 ± 17	300 ± 40	64 ± 11	120 ± 33	5
12	420 ± 1	900 ± 22	100 ± 7	280 ± 33	63 ± 5	110 ± 8	2
16	420 ± 26	1200 ± 320	140 ± 52	344 ± 210	410 ± 320	140 ± 37	2
28	400 ± 17	1700 ± 170	150 ± 27	340 ± 31	80 ± 10	140 ± 37	3

Table S2. Iron concentrations of mouse organs (μM) used in the ^{57}Fe enrichment study, at different ages, along with standard deviations.

Age at Euthanization (wks)	Brain	Liver	Heart	Kidney	Spleen	Duodenum
4	410 ± 12	1700 ± 370	970 ± 180	1600 ± 260	3700 ± 2400	1100 ± 220
5	350 ± 60	1600 ± 250	1400 ± 290	1900 ± 460	3200 ± 1300	890 ± 210
6	380 ± 76	2400 ± 480	1400 ± 210	1800 ± 570	3100 ± 800	2000 ± 890
8	300 ± 48	1800 ± 340	1600 ± 230	2200 ± 340	4300 ± 1000	990 ± 240
12	290 ± 48	2200 ± 64	1300 ± 180	1500 ± 680	4300 ± 1000	870 ± 84
16	330 ± 44	2300 ± 18	1400 ± 18	1400 ± 440	5100 ± 1500	910 ± 33
28	410 ± 30	2200 ± 310	2000 ± 320	2700 ± 450	8700 ± 2700	1400 ± 370
Average	350 ± 60	2000 ± 400	1500 ± 300	2000 ± 500	4000 ± 1400	1200 ± 600

Table S3. Percentage of the total Fe that was ^{57}Fe in mouse organs used in the ^{57}Fe enrichment study. Numbers are reported as %, with standard deviations.

Age at Euthanization (wks)	Brain	Liver	Heart	Kidney	Spleen	Duodenum
4	6.5 ± 5	3 ± 0	3.5 ± 0.7	3 ± 0	3 ± 0	3 ± 0
5	9.4 ± 2	30 ± 5	17 ± 3	23 ± 4	25 ± 5	62 ± 7
6	16 ± 1	43 ± 3	30 ± 3	36 ± 4	31 ± 4	74 ± 5
8	21 ± 3	48 ± 9	38 ± 5	35 ± 11	31 ± 4	75 ± 5
12	23 ± 2	48 ± 1	39 ± 0	48 ± 2	39 ± 2	79 ± 2
16	30 ± 7	66 ± 2	47 ± 6	60 ± 11	54 ± 16	82 ± 1
28	53 ± 10	53 ± 3	66 ± 6	78 ± 7	76 ± 10	88 ± 5

Table S4. Copper concentrations of mouse organs used in the enrichment study, at different ages. Concentrations and standard deviations are reported in µM.

Age at Euthanization (wks)	Brain	Liver	Heart	Kidney	Spleen	Duodenum
4	4 ± 1	6 ± 2	90 ± 48	64 ± 8	52 ± 42	36 ± 10
5	5 ± 2	8 ± 2	130 ± 83	84 ± 15	18 ± 7	36 ± 13
6	6 ± 3	12 ± 3	90 ± 31	86 ± 26	16 ± 5	39 ± 8
8	4 ± 1	8 ± 2	100 ± 19	97 ± 13	20 ± 5	48 ± 6
12	4 ± 1	9 ± 0	110 ± 7	86 ± 7	12 ± 6	41 ± 7
16	4 ± 1	8 ± 2	100 ± 6	83 ± 11	15 ± 8	32 ± 3
28	6 ± 1	7 ± 1	110 ± 4	87 ± 2	17 ± 2	34 ± 3
Averages	5 ± 2	8 ± 3	110 ± 40	90 ± 20	20 ± 10	40 ± 10

Table S5. Manganese concentrations of mouse organs used in the enrichment study, at different ages. Concentrations and standard deviations are reported in µM.

Age at Euthanization (wks)	Brain	Liver	Heart	Kidney	Spleen	Duodenum
4	21 ± 8	32 ± 4	8 ± 2	27 ± 1	6 ± 1	40 ± 22
5	18 ± 4	48 ± 17	18 ± 7	53 ± 19	5 ± 2	42 ± 17
6	17 ± 2	44 ± 12	18 ± 8	50 ± 18	5 ± 2	110 ± 30
8	13 ± 5	27 ± 10	20 ± 4	54 ± 16	6 ± 2	66 ± 29
12	11 ± 1	33 ± 1	26 ± 6	36 ± 8	4 ± 2	38 ± 5
16	10 ± 1	24 ± 4	27 ± 13	37 ± 1	4 ± 1	70 ± 30
28	10 ± 1	19 ± 2	14 ± 1	34 ± 4	6 ± 1	56 ± 12
Averages	14 ± 5	30 ± 10	18 ± 7	40 ± 20	5 ± 2	60 ± 30

Table S6. Zinc concentrations of mouse organs used in the enrichment study, at different ages. Concentrations and standard deviations are reported in μM.

Age at Euthanization (wks)	Brain	Liver	Heart	Kidney	Spleen	Duodenum
4	360 ± 35	380 ± 28	210 ± 35	320 ± 29	380 ± 55	310 ± 97
5	330 ± 94	460 ± 100	330 ± 87	380 ± 55	370 ± 140	380 ± 130
6	370 ± 29	640 ± 160	360 ± 130	470 ± 110	420 ± 150	710 ± 110
8	300 ± 86	430 ± 30	330 ± 100	410 ± 89	460 ± 83	450 ± 150
12	260 ± 11	470 ± 7	310 ± 2	340 ± 8	310 ± 59	250 ± 78
16	260 ± 2	400 ± 47	290 ± 3	340 ± 78	280 ± 100	280 ± 120
28	360 ± 57	460 ± 24	300 ± 17	380 ± 16	380 ± 10	440 ± 45
Averages	320 ± 70	470 ± 110	320 ± 80	390 ± 80	380 ± 110	430 ± 170

Table S7. Molybdenum concentrations of mouse organs used in the enrichment study, at different ages. Concentrations and standard deviations are reported in µM.

Age at Euthanization (wks)	Brain	Liver	Heart	Kidney	Spleen	Duodenum
4	0.1 ± 0	5 ± 0	0.6 ± 0.1	3 ± 0	0.7 ± 0.1	2 ± 0
5	0.5 ± 0.1	10 ± 3	0.7 ± 0.1	5 ± 1	0.5 ± 0.1	3 ± 1
6	0.5 ± 0.1	13 ± 3	0.7 ± 0.3	5 ± 2	0.6 ± 0.2	6 ± 2
8	0.3 ± 0.1	9 ± 1	0.6 ± 0.1	6 ± 0	0.6 ± 0.1	3 ± 1
12	0.3 ± 0.1	11 ± 1	0.6 ± 0.1	4 ± 1	0.5 ± 0.1	2.5 ± 1
16	0.3 ± 0.1	9 ± 1	0.5 ± 0.1	4 ± 0	0.5 ± 0.1	2.5 ± 1
28	0.4 ± 0.1	8 ± 1	0.5 ± 0.1	4 ± 1	0.6 ± 0.1	3 ± 0
Averages	0.4 ± 0.1	9 ± 2	0.6 ± 0.2	5 ± 1	0.6 ± 0.1	3 ± 2

Table S8. Phosphorus concentrations of mouse organs used in the enrichment study, at different ages. Concentrations and standard deviations are reported in mM.

Age at Euthanization (wks)	Brain	Liver	Heart	Kidney	Spleen	Duodenum
4	170 ± 36	110 ± 18	61 ± 8	110 ± 4	140 ± 7	84 ± 27
5	170 ± 38	140 ± 32	110 ± 43	140 ± 37	150 ± 66	120 ± 61
6	210 ± 12	180 ± 37	130 ± 26	180 ± 28	200 ± 30	240 ± 86
8	160 ± 44	130 ± 26	110 ± 33	160 ± 34	190 ± 37	130 ± 33
12	140 ± 16	150 ± 7	110 ± 18	150 ± 30	110 ± 42	83 ± 10
16	140 ± 6	120 ± 11	140 ± 54	150 ± 33	140 ± 50	94 ± 28
28	170 ± 33	130 ± 9	110 ± 7	160 ± 5	190 ± 11	160 ± 27
Averages	160 ± 40	130 ± 30	110 ± 30	150 ± 30	170 ± 50	140 ± 70

Table S9. Elemental analysis of blood at indicated ages.

BLOOD (Age in wks)	[Fe] (μM)	[Cu] (μM)	[Mn] (μM)	[Zn] (μM)	[Mo] (μM)	[P] (mM)
Sample 1 (4)	7500	6.8	0.15	71	1.9	320
Sample 2 (5)	7600	7.1	0.18	70	2.2	320
Sample 3 (5)	7600	6.5	0.18	69	1.5	320
Sample 4 (18)	8400	5.1	0.22	65	2.0	330
Sample 5 (18)	8300	5.3	0.20	54	2.9	370
Sample 6 (18)	7500	5.4	0.19	61	2.4	370
Averages	7800 ± 400	6 ± 1	0.19 ± 0.02	65 ± 7	2.1 ± 0.5	340 ± 20