








The mouse metallomic landscape of aging and metabolism

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Organic elements make up 99% of an organism but without the remaining inorganic bioessential elements, termed the metallome, no life could be possible. The metallome is involved in all aspects of life, including charge balance and electrolytic activity, structure and conformation, signaling, acid-base buffering, electron and chemical group transfer, redox catalysis energy storage and biomineralization. Here, we report the evolution with age of the metallome and copper and zinc isotope compositions in five mouse organs. The aging metallome shows a conserved and reproducible fingerprint. By analyzing the metallome in tandem with the phenome, metabolome and proteome, we show networks of interactions that are organ-specific, age-dependent, isotopically-typified and that are associated with a wealth of clinical and molecular traits. We report that the copper isotope composition in liver is age-dependent, extending the existence of aging isotopic clocks beyond bulk organic elements. Furthermore, iron concentration and copper isotope composition relate to predictors of metabolic health, such as body fat percentage and maximum running capacity at the physiological level, and adipogenesis and OXPHOS at the biochemical level. Our results shed light on the metallome as an overlooked omic layer and open perspectives for potentially modulating cellular processes using careful and selective metallome manipulation.

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Knowledge of the metallome – briefly, the set of inorganic elements in an organism, (for a complete definition see Lobinski et al.¹) and its interactions with other omic layers is elemental for understanding how physiological perturbations cause or result from alterations of metal concentrations and stable isotope compositions. Isotopic fractionation, the partitioning of stable isotopes due to slight differences in mass-dependent quantum zero-point energies^{2,3}, is an emerging means for characterizing the involvement of the metallome in biological processes^{4–6}. So far, interactions of the metallome with other omic layers have been mostly studied in plants to optimize crop production^{7–9}. Scarcer studies in yeast¹⁰ and cultured human cells¹¹ reveal a wealth of metallome-dependent biological traits. The present study is the first comprehensive analysis of the interactions between the metallome and other omic layers (phenome, metabolome, and proteome) in organs of mice as a function of age.

Results and discussion

The metallome and isotope compositions fingerprints are organ-specific. We measured the metallome (K, Mg, Na, P, S, Ca, Fe, Cu, Rb, Zn, Se, Co, Mo, and Cd) and the Cu and Zn stable isotope compositions (denoted hereafter $\delta^{65}\text{Cu}$ and $\delta^{66}\text{Zn}$, respectively) in five organs (brain, heart, kidney, liver, and muscle, Supplementary data 1 and table S1) of 49 C57BL/6 male mice aged 6-, 16-, and 24-months old (mo) (Fig. 1a). Principal Component Analysis (PCA) shows organ-specific metallomic and isotopic signatures (Fig. 1b) as described before^{12–14}. The first principal component is driven oppositely by protein-bound transition metals (Fe, Zn, Se, Mn, Co, and Cu) and free alkali and alkaline earth metals (Ca, K, and Mg) (Fig. 1b). The $\delta^{65}\text{Cu}$ and $\delta^{66}\text{Zn}$ values are also organ-specific, suggesting that isotope fractionation depends on fine, organ-specific processes (Fig. 1c, d). The $\delta^{66}\text{Zn}$ value in heart, brain, muscle and liver follows a linear relationship with Zn concentration, a relationship that is known to indicate identical Zn routing in these organs¹⁵, obviously distinct in kidneys where glomerular filtration probably drives isotopic fractionation through a distillation process¹⁶ (Fig. 1c). Copper isotope fractionation is more intense than that of Zn. Cu exhibits a unique concentration vs isotopic composition signature for each organ, depending on the occurrence of its oxidation state, Cu(II) compounds being isotopically heavier than Cu(I) compounds¹⁷ (Fig. 1d).

We further explored the metallome evolution during aging. Major elements, i.e. electrolytes (K, Mg, and Na) or molecular constituents (S and P), show a remarkable homogeneity among organs and stability over time (Fig. S1A). Minor elements (Ca, Cu, Fe, Rb, Zn), with countless biological roles¹⁸, and especially ultra-trace elements – Se for selenoproteins, Co for vitamin B12, Mo for molybdoenzymes, and Cd with unknown biological role¹⁸ – exhibit a more heterogeneous distribution in the body than major elements and vary with age (Fig. S1B–S1C). Such ultra-trace elements are mainly present in the liver and to a lesser extent in the kidney and brain. The gastrocnemius striated muscle and the myocardial muscle are depleted in ultra-trace elements, probably reflecting their limited involvement in specific biochemical synthesis (Fig. S1C).

The metallome and isotope compositions fingerprints are age-dependent. Of all the organs, metal concentrations in the brain seem to vary the most with age (Fig. 1e, Rb, Fe, Ca, Cu, and Co at p -value < 0.05). We find an age-dependent accumulation of Fe and Cu in a healthy brain (Fig. 1f), which complements previous observations^{19–22} in the mouse. As high levels of transition metals such as Fe, Cu, and Zn are known to be associated with amyloid- β

plaques and α -synuclein accumulation in neurodegenerative diseases^{23,24}, all these results may suggest that metal accumulation precedes the formation of the protein aggregates. The accumulation of Cd in kidney (Fig. 1f) may be illustrative of chronic toxicity²⁵. Rubidium, which is the K minor element analog²⁶, decreases between 6 m.o. and 24 m.o. in brain, muscle, and kidney (Figs. 1f and S2), suggesting unprecedented evidence of disrupted K metabolism in old mice. These results confirm the value of the metallome as a biomarker for aging¹⁴, but our data extend this observation to isotope compositions, notably the decrease of the liver $\delta^{65}\text{Cu}$ value during aging (Fig. 1f). Isotopic aging “clocks” have been described in yeast (D/H, $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$,²⁷), worm ($^{65}\text{Cu}/^{63}\text{Cu}$,²⁸), and in human blood ($^{65}\text{Cu}/^{63}\text{Cu}$, $^{66}\text{Zn}/^{64}\text{Zn}$ ²⁹). Furthermore, metallomic or isotopic aging clocks are much easier to handle than DNA methylation clocks (performed for a subset of livers of the same animals³⁰) which require a predetermined profile of methylation sites in the organism and organ of interest. To find metal correlations not solely driven by age, we hereafter used linear regression to correct metallomic concentrations for age effects. This correction is applied hereafter except if specified otherwise and ultra-trace elements are excluded to compare organs with the same set of metals. The concentration of major elements Mg, K, P, and S, but not Na, are positively and strongly correlated in all organs and conserved after age correction, highlighting the fundamental interplay throughout life between cationic electrolytes (Mg^{2+} and K^{+}), ATP (PO_4) and glutathione (S)³¹ (Figs. 1g and S3). Minor elements Ca and, to a lesser extent Fe, are negatively correlated to other elements in many organs (Fig. 1g).

The metallome fingerprint is highly conserved across different studies. We compared our results with a recent mouse study¹⁴, which measured a smaller subset of the metallome in mouse organs at different ages. With the same set of metals and organs, the respective PCAs agree almost perfectly, demonstrating a highly conserved metallome distribution in mouse organs (Fig. 2a, b). For a given organ, age effects converged in both studies despite very different timepoints (16 timepoints at 2 months intervals¹⁴ versus 3 timepoints in this study) and different diets (NIH-31¹⁴ vs Teklad 18% protein) (Fig. 2c). After age correction, the correlation networks generated with both datasets still converge in most organs (Figs. S4 and 2d). This suggests a tight control of the metallome, reflective of the duality of metals, being bioessential at normal concentrations and toxic outside. From an evolutionary perspective, the metallome homeostasis likely evolved billions of years ago when early life had to adapt to an inorganic environment driven by ocean chemistry³². Our work reveals that not only basal metal concentrations in organs are conserved across unrelated studies, but those subtle variations are also conserved, as evidenced by the similar interaction network and reproducible response to aging. Given this, we asked whether these metal variations would relate to perturbations in other biological layers, notably the phenome, metabolome, and proteome.

The metallome and isotope compositions are associated with metabolic health and mitochondrial content. We recorded cardiometabolic phenotypic traits, including body weight, fat content, O_2 consumption, glucose level, and blood pressure, and compared these with the metallome and $\delta^{65}\text{Cu}$ and $\delta^{66}\text{Zn}$ profiles through pairwise correlations (Figs. 3a, b and S5). Amongst the metallome-phenotype associations, the most significant is a negative correlation between the liver $\delta^{65}\text{Cu}$ value and body weight (Fig. 3b), and to a lesser extent fat% and a slower return to glucose homeostasis after intraperitoneal injection (IPGTT AUC,

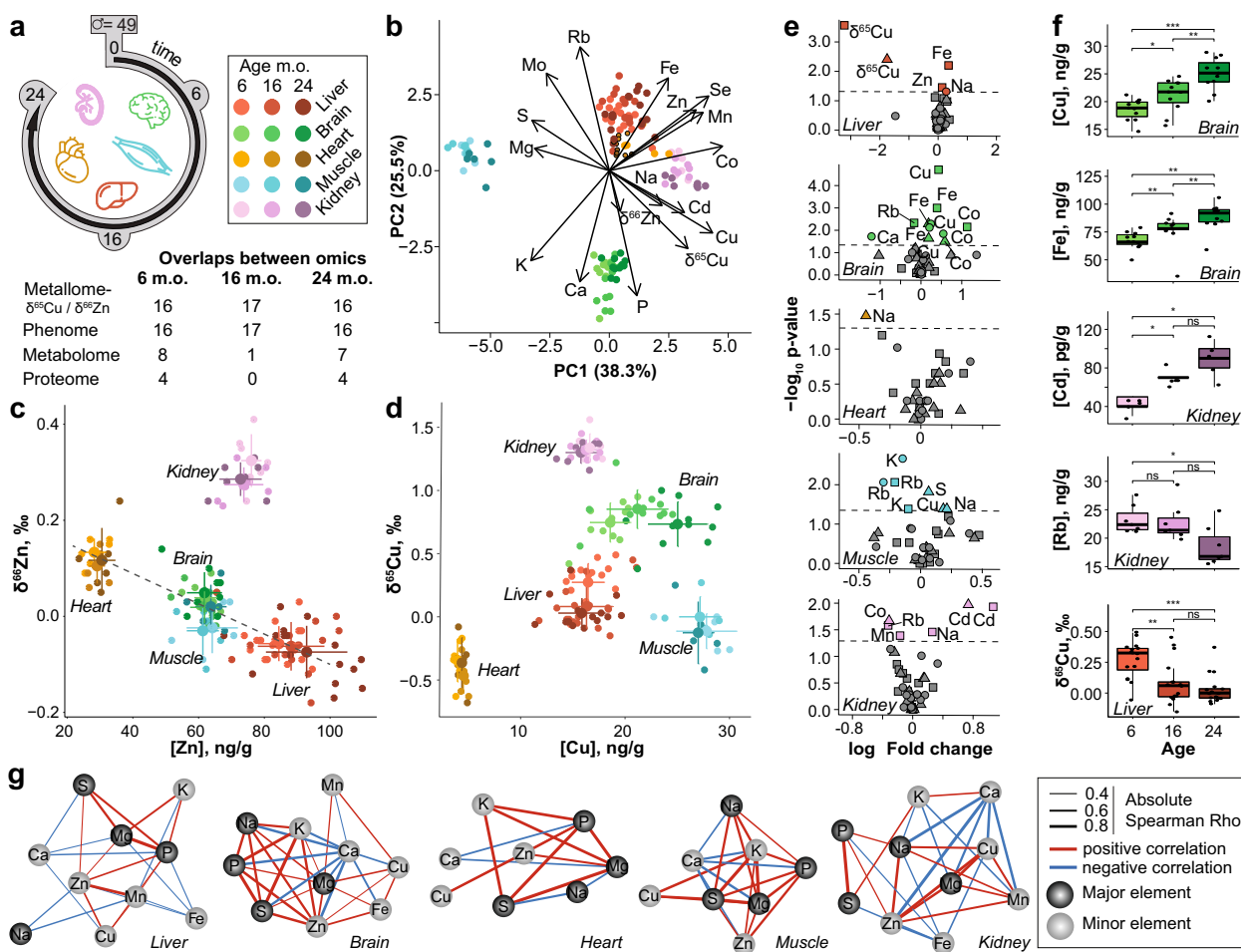


Fig. 1 Organs have distinct metallomic fingerprints, that evolve with aging. **a** Study design and overlapping samples between omic layers. Icons are designed by www.freepik.com, and assembled by VB on Adobe Illustrator. **b** Principal component analysis of the metal concentration and isotope composition of each sample. Clear organ signatures are visible, with small shifts with aging. **c, d** Relation between isotope composition and concentrations of Cu (**c**) and Zn (**d**). Error bars indicate standard deviation. The dashed line indicates a linear relationship between $\delta^{66}\text{Zn}$ and Zn between the liver, brain, muscle, and heart, indicative of identical Zn routing in these organs. **e** Volcano plots of the effect of aging on metal concentrations. Triangles: 16 vs 6 m.o., circles 16 vs 24 m.o., squares: 24 vs 6 m.o. The dotted line indicates the threshold of $\text{Adj.}P < 0.05$, FDR-corrected (limma) moderated t -test, two-sided. **f** Boxplot of the most age-dependent metal concentrations and isotope compositions. The lower and upper hinges correspond to the first and third quartiles, and center line is the median. The whiskers extend from the hinge to the largest value no further than $1.5 \times$ inter-quartile range. $***\text{Adj.}P < 0.001$, $**\text{Adj.}P < 0.01$, $*\text{Adj.}P < 0.05$, FDR-corrected (limma) moderated t -test, two-sided. **g** Network of Spearman Rho correlations between metals. Only correlated metals with $**\text{Adj.}P < 0.001$, $**\text{Adj.}P < 0.01$, $*\text{Adj.}P < 0.05$, (limma) moderated t -test, with age as a covariate, are pictured. The correlation coefficient and directionality of the correlation are pictured through line thickness and color, respectively. Source data are provided as a Source Data file.

Fig. 3b), an indicator of diabetic-like symptoms. Iron and $\delta^{65}\text{Cu}$ are further associated with an increase in the activity of mitochondrial complex I and a reduction of the mtDNA/nuclear DNA ratio in the liver, suggesting that liver mitochondrial activity is associated to these changes in metal levels. A high liver $\delta^{65}\text{Cu}$ is therefore associated with both younger (Fig. 1f) and metabolically healthier (Fig. 3b) animals. While increased Cu concentration³³ and isotope composition^{4,34,35} have been previously associated with metabolic activity and growth of tumors, our study shows that $\delta^{65}\text{Cu}$ may be a more general indicator of metabolic fitness. Importantly, these liver metal concentrations may not be causal in metabolic fitness, but rather represent a biomarker of liver health in metabolically healthy animals, as opposed to mildly obese or diabetic animals which develop liver dysfunction. Increased liver Fe further correlated with fat percentage and muscle ROS production (Fig. 3c), which are produced primarily by mitochondria during muscle exercise³⁶. In addition, liver calcium was strongly correlated with the activity of mitochondrial

complex IV. Calcium has been shown to bind and inhibit mitochondrial complex IV in vitro³⁷, but these results suggest that interactions between Ca and complex IV may also be relevant in vivo. Kidney Fe also correlated with respiratory capacity (VO_2 increase, Fig. 3c) reflecting the importance of Fe signaling in kidney erythropoietin production, a critical regulator of hemoglobin and erythrocyte production³⁸.

The metallome and isotope compositions fingerprints integrate with overlapping biological pathways in the proteome and metabolome. To examine the interplay between metallomics and other omics layers, we used previously published metabolomics³⁹ and new proteomics analyses performed on liver samples from the same animals. A Metabolite Set Enrichment Analysis (MSEA) and Gene Set Enrichment Analysis (GSEA) based on correlations between the metallome, the metabolome (Fig. 4a), and the proteome (Fig. 4b) define four groups of correlated metals. Group I

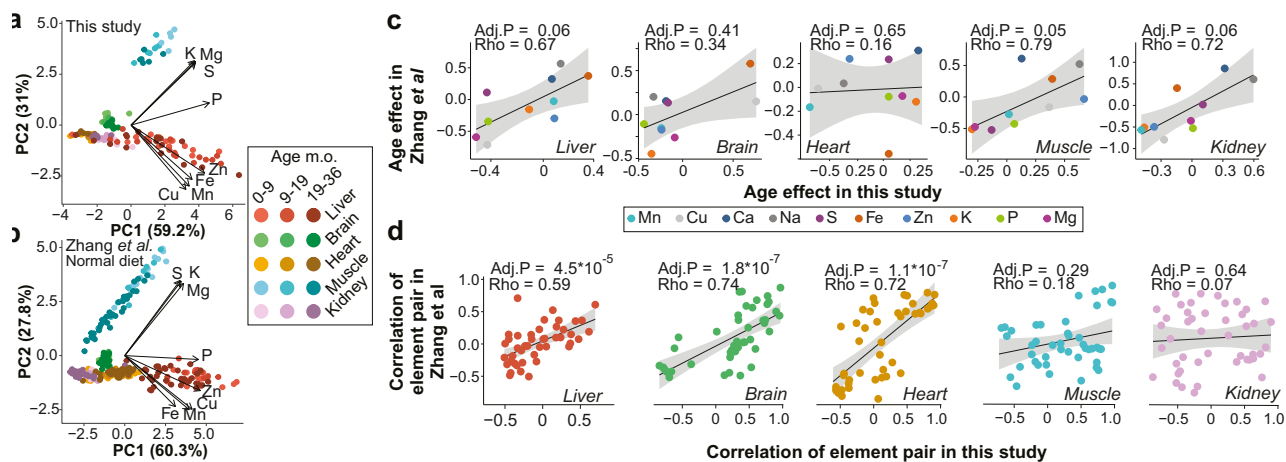


Fig. 2 The metallo-organ signature and its response to stimuli are conserved across datasets. **a**, Robustness of PCA analysis of the mouse metallome across different data sets. **a** PCA for the present study. **b** PCA analysis for the study of Zhang et al.¹⁴ **c** Scatterplots of Spearman Rho correlation coefficients, and Spearman test FDR-corrected *p*-value calculated between metals and age in the data from Zhang et al. vs. those of the present study. The Zhang dataset measured metals at 16 timepoints at 2 months interval, as opposed to our studies' 3 timepoints, yet trends converge towards the same age effects in the liver, muscle, and kidney. The effects of age in the heart do not match, but are very low in both studies. **d** Scatterplots of Spearman Rho correlation coefficients and Spearman test FDR-corrected *p*-value calculated between pairs of metals from Zhang et al. vs. those of the present study. To enable comparisons between the two studies, data were normalized to Na in **a, b** and total metal concentrations in **c, d**. In panels **c** and **d**, the black line and gray error band represent a linear regression and its 95% confidence interval, respectively. Source data are provided as a Source Data file.

(S, Mg, P, Rb, K, Cu, Zn, and Mn) displays strong positive normalized enrichment scores (NES) for amino, organic, and carboxylic acids and derivatives and negative NES for fatty acids, lipids, and derivatives, with opposite enrichments in Group II (Na and Ca) (Fig. 4a). This pattern is independently confirmed by the GSEA on hallmark gene sets in the proteome, where groups I and II oppositely correlate with adipogenesis and fatty acid metabolism (Fig. 4b). The MSEA for cellular locations indicates that group I associates with cytoplasmic components but negatively correlates with membrane components (Fig. 4a). Group I also negatively correlates with the mitochondria (Fig. S8), in accordance with the Hallmark GSEA concerning the oxidative phosphorylation pathway (OXPHOS, Fig. 4b). Group III consists of $\delta^{65}\text{Cu}$ only and is positively associated for amino, organic and carboxylic acids and derivatives in the chemical classes MSEA (Fig. 4a). The previously observed negative phenotypic correlation between the $\delta^{65}\text{Cu}$ value and fat % (Fig. 3b) is strengthened by the Hallmark GSEA (Fig. 4b) implicating adipogenesis. Group IV (Fe and $\delta^{66}\text{Zn}$) had comparably fewer enrichments with the MSEA (Fig. 4a) but reveals many significant enrichments with the GSEA (Figs. 4b and S8). This group associates with mitochondrial content, in accordance with the role of Fe in heme synthesis, electron transport and oxidative phosphorylation, and the mitochondrial biosynthesis of the critical Fe-S cluster proteins^{40,41}. The discovery that the $\delta^{66}\text{Zn}$ value varies in opposition to Zn concentration, notably with mitochondrial proteins (GO CC, Figs. 4b and S8), emphasizes the added value of determining stable isotope composition in addition to concentration to highlight metal-based molecular pathways. The KEGG GSEA (Fig. S8) links $\delta^{66}\text{Zn}$ and neurodegenerative diseases, predicting a connection that was experimentally confirmed in the APP^{swe}/PSEN1^{dE9} mouse model of Alzheimer disease^{42,43}.

We summarize the results of the study in a network analysis gathering correlations between metals, phenotypes, as well as the MSEA and GSEA results in the liver (Fig. 4c). The resulting network recapitulates all the above observations (e.g. $\delta^{65}\text{Cu}$ and body weight, Fe and OXPHOS) and shows that the metallo-organ significantly interacts with all the studied omic layers. The network highlights the similar roles of K and Rb which strengthen the postulated status of Rb as a proxy for K metabolism.

Perspectives. Overall, our findings establish that the metallo-organ and the isotopic compositions are biologically relevant and highly integrated omic layers with an unprecedented level of conservation in both basal signature and evolution with age. Subtle variations in metal concentrations are conserved across studies and associated with markers of metabolic health at both the physiological (body weight, fat percentage, and insulin resistance in the IPGTT) and biochemical levels (OXPHOS, lipid synthesis among others). A limitation of this study is that it is mostly based on correlations, and we do not imply that alterations in metals are causal in the phenotypes observed. However, the high reproducibility of metallomics suggests that changes in metal concentration may represent a source of reliable and affordable biomarkers of organismal, tissular, cellular, and subcellular aging and metabolism.

Methods

Mouse sample collection. All animal experiments were performed according to Swiss ethical guidelines and approved by the local animal experimentation committee of the Canton de Vaud under license 2172. Male C57BL/6J mice (Janvier, St Berthevin, France) were fed with a standard chow diet (containing 18% protein, 50% carbohydrate, and 6.0% fat; Harlan Laboratories, Madison, WI, USA) at the Laboratory of Integrative Systems Physiology (LIPS) at EPFL (Ecole Polytechnique Fédérale de Lausanne). The mice were kept with a 12-h light cycle and temperature was regulated at 23 °C and 40–60% humidity. After in vivo phenotyping, organs (i.e. liver, brain, heart, muscle (i.e. quadriceps), and kidney) were collected at three different time-points in their life i.e. 6 months (mature adult), 16 months (middle age) and 24 months (old). The organs were flash frozen in liquid nitrogen (N_2), and stored at $-80\text{ }^\circ\text{C}$, to promote their conservation before chemical and metabolomics analyses.

In vivo phenotyping. All phenotype data used in this paper was previously published in Houtkooper et al.³⁹, but a brief description of the procedures is provided here. Body composition was determined by Echo-MRI (Echo Medical Systems, Houston, TX, USA) and oxygen consumption (VO_2), respiratory exchange ratios (RER), activity levels and activity were monitored by indirect calorimetry using the comprehensive laboratory animal monitoring system (CLAMS) (Columbus Instruments, Columbus, OH, USA). Glucose tolerance was analyzed by measuring blood glucose and insulin following intraperitoneal injection of 2 g/kg glucose after an overnight fast. Maximum respiration potential ($\text{VO}_{2\text{max}}$) was determined in a metabolic treadmill (Columbus instruments, Columbus, OH, USA) with an incremental speed protocol. During the run, VO_2 and VCO_2 are measured. The experiment is stopped when mice are exhausted, RER is above 1 for more than one

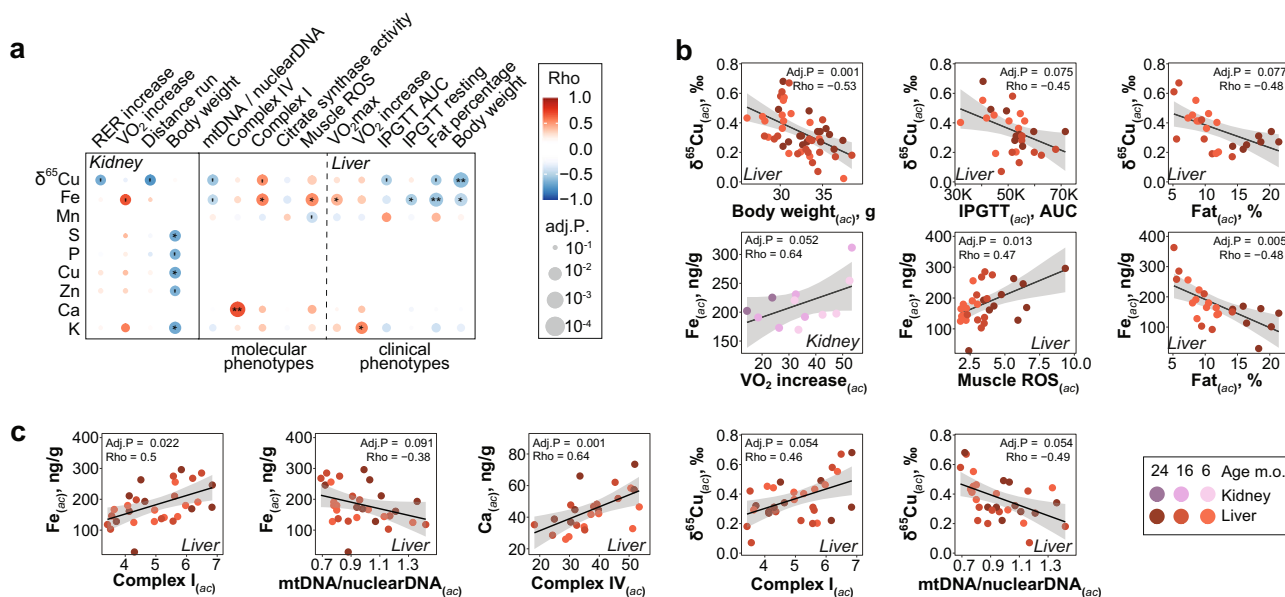


Fig. 3 Metals and isotope compositions, notably Fe and $\delta^{65}\text{Cu}$ in liver and kidney associate with metabolic traits. **a** Significant correlations between metabolic phenotypes and metals in the liver and kidney. There were no significant associations in other organs except for a single one in the heart. This is consistent with the central role of liver and kidney in the control of metabolic traits. ***Adj.P < 0.001, **Adj.P < 0.01, *Adj.P < 0.05, 'Adj.P < 0.1. **b, c** Dot plots of the individual correlations of $\delta^{65}\text{Cu}$ (**b**) and Fe (**c**) with indicators of obesity (body weight, fat %), diabetes (Intra-peritoneal glucose tolerance test, area under the curve, IPGTT AUC), and muscle activity (Muscle ROS) and running performance (VO_2 increase), as well as the activity of mitochondrial complex I. The subscripts (ac) stand for “age corrected”. In all panels, Rho indicates the Spearman Rho coefficient, and Adj.P is computed by a moderated t-test (limma), two-sided and FDR-corrected with age as a covariate. In panels **b** and **c**, the black line and gray error band represent a simple linear regression and its 95% confidence interval, respectively. Source data are provided as a Source Data file.

interval, or the VO_2 reaches a clear plateau. Liver mitochondrial complex I and IV activity (Mitosciences, Eugene, OR, USA), citrate synthase (Sigma) were determined according to the manufacturer’s protocols. Liver and muscle ROS were determined by 4-HNE detection in PBS tissue lysates using an ELISA-based method (Cell Biolabs, San Diego, CA, USA).

Analyses of major and trace element concentrations. All the chemical analyses were carried out in a clean laboratory below laminar flow clean hoods using ultra-pure water at 18.2 M Ω .cm and acids that were doubly distilled to ensure low trace element contents and avoid any exogenous contaminations. Blanks were also run to quantify and integrate potential contamination from the material and/or acids used to perform experiments. The samples were crushed under liquid N_2 , freeze-dried, weighted, and then dissolved in a concentrated $\text{HNO}_3\text{-H}_2\text{O}_2$ 30% mixture in Savillex beakers for about 72 h at 120 °C. Once dissolved, major (Ca, K, Na, P, S, and Mg) and trace elements (Mn, Co, Cu, Zn, Fe, Se, Rb, Mo, and Cd) were measured using the ICP-AES, iCAP 6000 Radial and the quadrupole ICP-MS Thermo iCap-Q respectively at the Ecole Normale Supérieure (ENS) of Lyon. The concentrations were calculated using calibration curves determined based on multi-elemental solutions (SP-33MS for trace element and in-house solution made from mono-elemental solutions for major element) following the procedure detailed in Garçon et al.⁴⁴. Both accuracy and reproducibility were monitored by complete duplicate analyses, replication of an in-house (sheep plasma; OEP) and certified standards (bovine liver, SRM-1577c) as well as re-run analyses over the course of an analytical session. Given our long-term reproducibility on the in-house/certified standards and duplicate analyses, the 2 standard deviations (2 SD) analytical uncertainty adopted in this study for both trace and major concentrations are on average better than 10% (Tables S1 and S2).

Analyses of copper and zinc isotopic compositions. Copper and zinc isotopic compositions were measured at the Ecole Normale Supérieure (ENS) of Lyon following the procedure described by Maréchal et al.⁴⁵. Briefly, before each isotopic measurement, the sample solutions were purified by ion-exchange chromatography using quartz columns filled with 1.8 mL of Bio-Rad AGMP-1 (100–200 mesh) anion-exchange resin. Both copper and zinc were successively eluted with 20 mL of $\text{HCl } 7\text{ N} + 0.001\% \text{ H}_2\text{O}_2$ and 10 mL of HNO_3 0.5 M, respectively. On the day of the analyses, Zn and Cu purified solutions, previously evaporated to dryness, were dissolved in a Cu (Cu SRM 976, National Institute of Standards and Technology, Gaithersburg, MD, USA) or Zn-doped solution (Zn JMC 3-0749 L, Johnson Matthey Royston, UK) respectively to reach a Zn or Cu sample concentration of

about 300 ppb, which is similar to the concentration of the standard solution that was run between each sample.

Copper and Zn isotopic compositions are expressed as:

$$\delta^{65}\text{Cu}_{\text{sample}}(\text{‰}) = \left[\frac{(\frac{65\text{Cu}}{63\text{Cu}})_{\text{sample}}}{(\frac{65\text{Cu}}{63\text{Cu}})_{\text{standard}}} - 1 \right] * 1000 \quad (1)$$

and

$$\delta^{66}\text{Zn}_{\text{sample}}(\text{‰}) = \left[\frac{(\frac{66\text{Zn}}{64\text{Zn}})_{\text{sample}}}{(\frac{66\text{Zn}}{64\text{Zn}})_{\text{standard}}} - 1 \right] * 1000 \quad (2)$$

Copper and Zn isotopic compositions were measured on a Nu Plasma (Nu 500) MC-ICP-MS in wet plasma conditions. Zn JMC 3-0749L (also called JMC-Lyon; Johnson Matthey, Royston, UK) and Cu SRM 976 were used as reference standards for $\delta^{66}\text{Zn}$ and $\delta^{65}\text{Cu}$, respectively. Instrumental mass fractionation was corrected with an exponential law using an elemental-doping method and instrumental drift over time was controlled with standard sample bracketing⁴⁵.

The accuracy of isotopic compositions was assessed by the analysis of in-house and commercial standard solutions (i.e. sheep plasma; OEP and bovine liver SRM-1577c certified reference material⁴⁶) during each analytical sequence. For Zn, the average $\delta^{66}\text{Zn}$ value measured is $+0.76 \pm 0.08\text{‰}$ ($n = 3$, 2 SD) for OEP and $-0.18 \pm 0.05\text{‰}$ ($n = 4$, 2 SD) for SRM-1577c which is in good agreement with the average values $0.73 \pm 0.09\text{‰}$ ($n = 17$, 2 SD) for OEP and $-0.19 \pm 0.06\text{‰}$ ($n = 20$, 2 SD) for SRM-1577c (4). For Cu, we measured the OEP $\delta^{65}\text{Cu}$ value at $-1.10 \pm 0.15\text{‰}$ ($n = 3$, 2 SD) and the SRM-1577c $\delta^{65}\text{Cu}$ value at $+0.43 \pm 0.04\text{‰}$ ($n = 4$, 2 SD) which is also in good agreement with previously estimated values ($\delta^{65}\text{Cu}_{\text{OEP}} = -1.14 \pm 0.13 \text{‰}$ (2 s, $n = 35$) and $\delta^{65}\text{Cu}_{\text{SRM-1577c}} = +0.37 \pm 0.14 \text{‰}$ ($n = 19$, 2 SD, (4), Table S1). Based on re-run samples, complete duplicate analyses and standard measurements, we estimate the precision of our measurements at $\pm 0.12\text{‰}$ (2 SD). The long-term precision based on the repeated measurements of standard Zn JMC 3-0749L and Cu SRM 976 alone is however better than $\pm 0.06\text{‰}$ ($n = 140$, 2 SD).

Metabolomics. The liver metabolomics was performed by Metabolon (Durham, NC, USA) and has already been published⁴⁷. Briefly, sample preparation was conducted using a proprietary series of organic and aqueous extractions to remove the protein fraction while allowing maximum recovery of small molecules. The extracted samples were split into equal parts for analysis on the GC/MS and LC-MS/MS platforms. For LC-MS/MS, samples were split into two aliquots that were

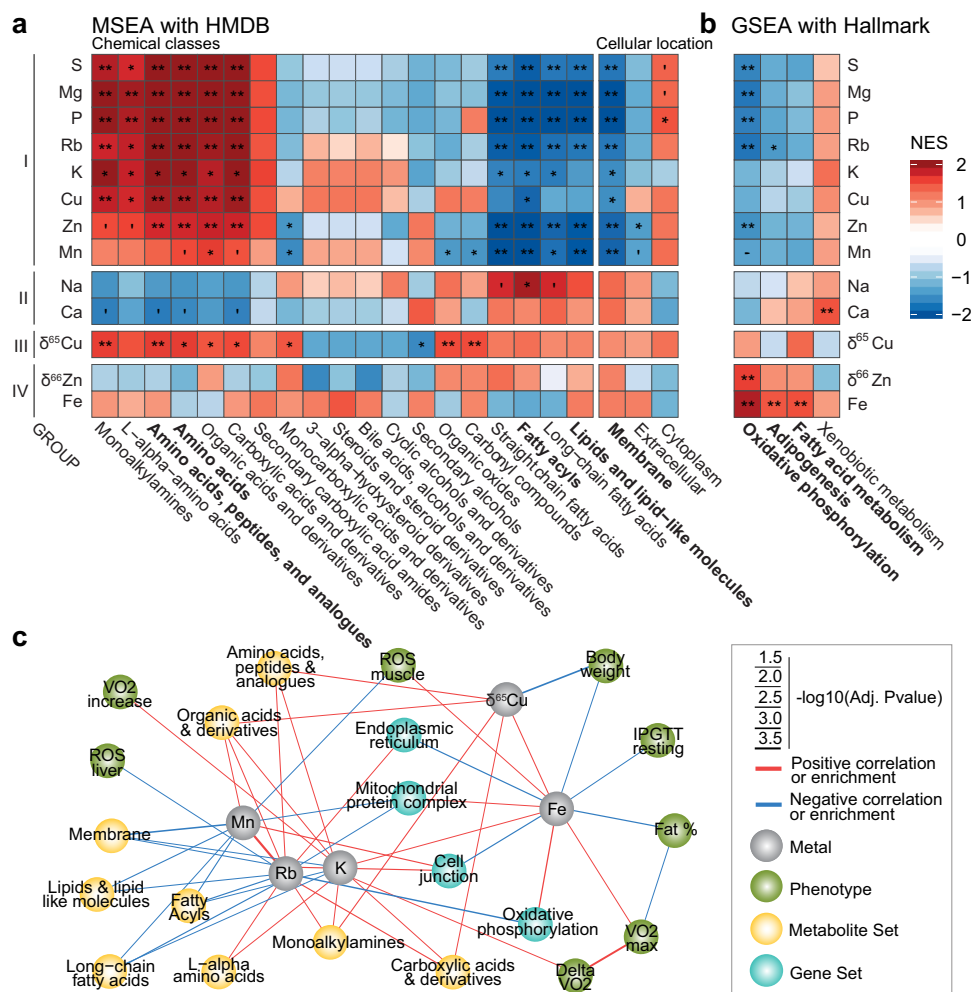


Fig. 4 Enrichment analyses in the liver metabolome and proteome converge towards common associations between ions and mitochondrial and fatty acid metabolism. **a**, **b** Metabolites and proteins were sorted on their correlation with individual metals in the liver, then metabolite or gene -set enrichment analyses (MSEA, (**a**), GSEA, (**b**)) were performed. For each metal, the top three gene or metabolite sets are pictured. FDR-corrected p -values. **Adj. $P < 0.01$, *Adj. $P < 0.05$, 'Adj. $P < 0.1$ (**c**) Network representation, combining the most significant correlations between metals, between metals and phenotypes (Spearman Rho and limma test, both), as well as the metabolite and gene set enrichment analyses. All tests were two-sided when applicable and p -values are FDR corrected. To limit the number of nodes, only nodes with very significant associations are pictured (Adj. $P < 0.01$), but all edges with Adj. $P < 0.05$ are drawn. Source data are provided as a Source Data file.

either analyzed in positive (acidic solvent) or negative (basic solvent) ionization mode. GC-MS was performed on bistrimethyl-silyl-trifluoroacetamide derivatized samples in a 5% phenyl GC column.

Proteomics. Proteomics was performed as described in Yu et al.⁴⁸. Briefly, total proteome was isolated using a RIPA-M buffer (pH 7.5; nonidet NP-40, 0.1% sodium doxycholate, 150 mM NaCl, 1 mM EDTA, 50 mM Tris, protease inhibitor cocktail, 10 mM NaF, 5 mM 2-glycerophosphate) and lysed with a 8 M urea buffer (pH 8.1, 75 mM NaCl, 10 mM NaF, 5 mM 2-glycerophosphate; 8 M urea; protease inhibitor cocktail). Tissues were ground using a pestle grinder in the Ripa-M buffer before the samples were lysed in the urea buffer. After proteins were isolated, samples were trypsin digested overnight in the dark (~22 h @ 37 °C in a mild shaker) in a urea buffer with dithiothreitol and indole-3-acetic acid. After trypsinization, samples were passed through a C18 column and eluted in a 2% ACN solution with 0.1% FA. Samples were then run on a TripleTOF 5600 in SWATH mode. The search library used was generated from that of an earlier study⁴⁹. For the data processing, we removed decoy peptides and peptides mapping only to reverse proteins, then summed raw intensities over different charge states and chemical modifications. The resulting peptide-level intensities were \log_2 transformed and normalized by robust linear regression normalization, separately for each organ. We then removed peptides that are identified in only one sample, as well as proteins identified by a single unique peptide sequence. Finally, peptide-level intensities were summarized to the protein level with the MsqRobSum R package⁵⁰, (proteins were grouped by gene annotations).

Data analysis. Outlier measurements were removed using the 'normal' method (differences between each value and the mean divided by standard deviation) through the R package 'outliers'. Less than 5% of measurements were removed this way. All correlation analyses were performed by parallel linear regression using the 'limma' R package using age as a covariate⁵¹. P -values were corrected for multiple testing through the Benjamini-Hochberg false discovery rate method. Pearson correlation coefficients were computed separately through the 'cor.test' function. Age-corrected values were computed by estimating the effect of age through a linear model value ~age and extracting the intercept + residuals of the model. Those values were used for visualization only, but statistical analyses were performed using age as a covariate instead.

MSEA. In order to use the most complete and up-to-date repository of metabolite information, we based our analysis on the Human Metabolome Database (HMDB). We downloaded the database and parsed information about biological processes and cellular components from the relevant entries in the database, then generated a search file for the ClusterProfiler R package⁵². For MSEA, metabolites were sorted based on their degree of correlation with each metal (calculated with limma package, see 'analysis' section) using the following formula: $-\log_{10}(P\text{-value})^*$ Pearson correlation coefficient. Metabolite Set enrichment analysis (MSEA) was performed using the ClusterProfiler package in R software.

GSEA. Gene set databases were downloaded from the Molecular Signatures Database (MSigDB) v7.2 through the MSigDRr R package. Similar to GSEA, all

proteins were sorted by $-\log_{10}(P\text{-value})$ * Pearson correlation coefficient and GSEA was performed with the ClusterProfiler R package.

Networks. All network representations show the correlation analyzes and MSEA/GSEA results outlined above. Only correlations with Adj. P -values < 0.005 were drawn. Network visualizations were generated using the igraph and ggraph R packages.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Raw metallomic data are included in this study as supplementary data 1. Proteomics has been submitted to the PRIDE repository under the identifier [PXD011142](https://www.ebi.ac.uk/pride/archive/study/MSK011142)⁵³. Metabolomics data have been previously published and are available in Houtkooper et al.³⁹. In addition, source data files are provided for every figure panel.

Code availability

Data analysis was performed in R software version 3.5.2. The following open-source packages were used in analysis and figure generation: MsqRobSum: proteomics; limma: differential analyzes and parallel linear regressions; ClusterProfiler: Gene and Metabolite set enrichment analyzes; ggplot2: figure generation; igraph, ggraph: network visualizations. All code used in the paper is available on request.

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Author contributions

V.B. and J.A. conceived the project. R.H. performed mouse clinical phenotyping and tissue collection. L.S. performed the metallomic measurements. R.A. and E.W. generated the proteomics dataset. J.D.M., V.B., and L.J.E.G. analyzed the data with the help of L.S. and P.J.; V.B. and J.D.M. wrote the manuscript with comments from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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The mouse metallomic landscape of aging and metabolism

Supplementary information

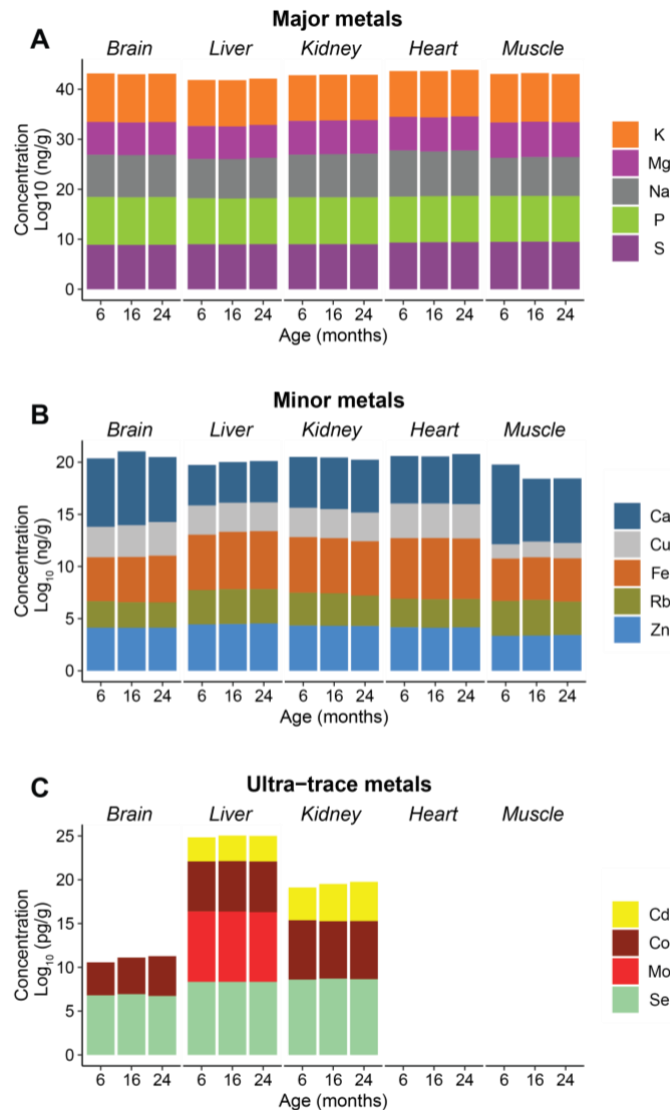


Fig. S1.

Barplots showing the average concentration distribution of (A) Major, (B) Minor and (C) ultra-trace metals in each measured organ and age. Due to the enormous differences in scale between different element, concentrations were converted to log₁₀ to enable comparisons on the same plot. Variations between ages and organs were greater for less abundant metals, and several ultra-trace metals were below the detection limit in heart and muscle which are typically <1 ng/g for the ICP-AES (major metals, A) and < 0.1 pg/g for the ICP-MS (minor and ultra-trace metals, B and C).

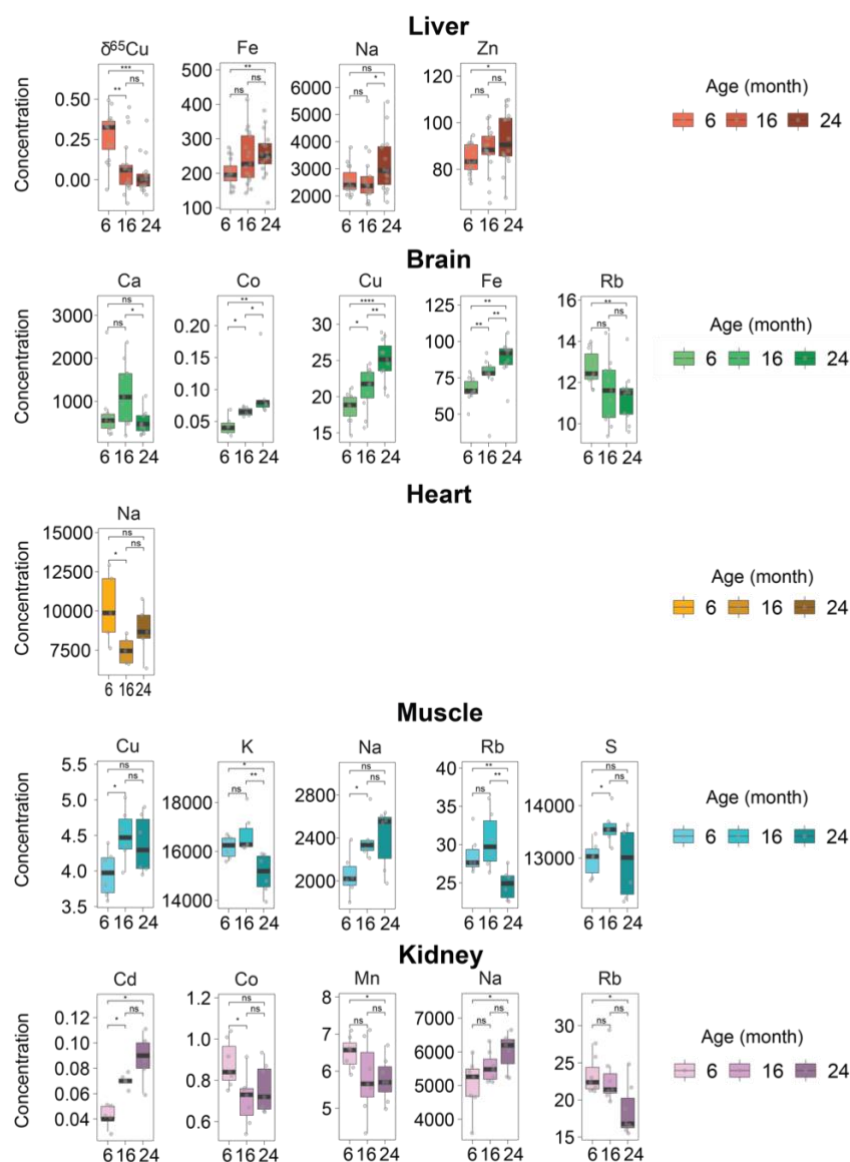


Fig. S2.

Boxplots of the mouse metallome as a function of age and organ. Only metals with significant (Adj. P -value < 0.05) variations are shown. Cu and Fe significantly increase with age in brain, a factor that may relate to cognitive decline, and Cd significantly increase with age in brain. Of note, is the decrease between 6 and 24 mo for Rb and K in several organs, suggesting evidence of increasing K dysregulation during aging. ns non-significant, * Adj. $P < 0.05$, ** Adj. $P < 0.01$, *** Adj. $P < 0.001$, **** Adj. $P < 10^{-4}$.

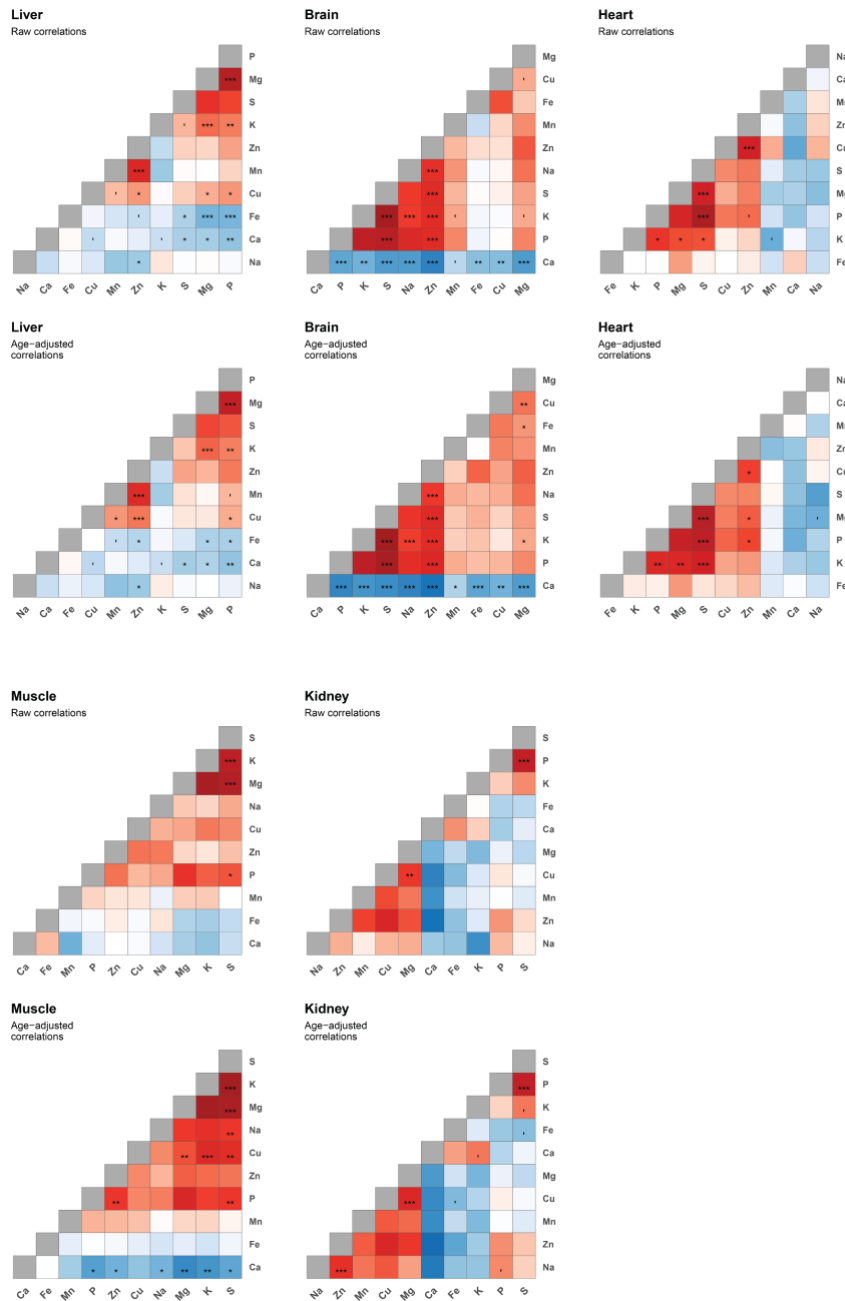


Fig. S3.

Heatmaps of Spearman Rho coefficients of the mouse metallome for each organ. For a given organ, heatmaps are provided for raw concentrations in the upper panel and for age-corrected concentrations in the lower panel. Metals are ordered by hierarchical clustering, based on the age-adjusted data in each organ. Generally, age correction does not affect correlations between elements, notably the positive S vs P and negative Ca vs Mg correlations. * Adj.*P* < 0.05, ** Adj.*P* < 0.01, *** Adj.*P* < 0.001.

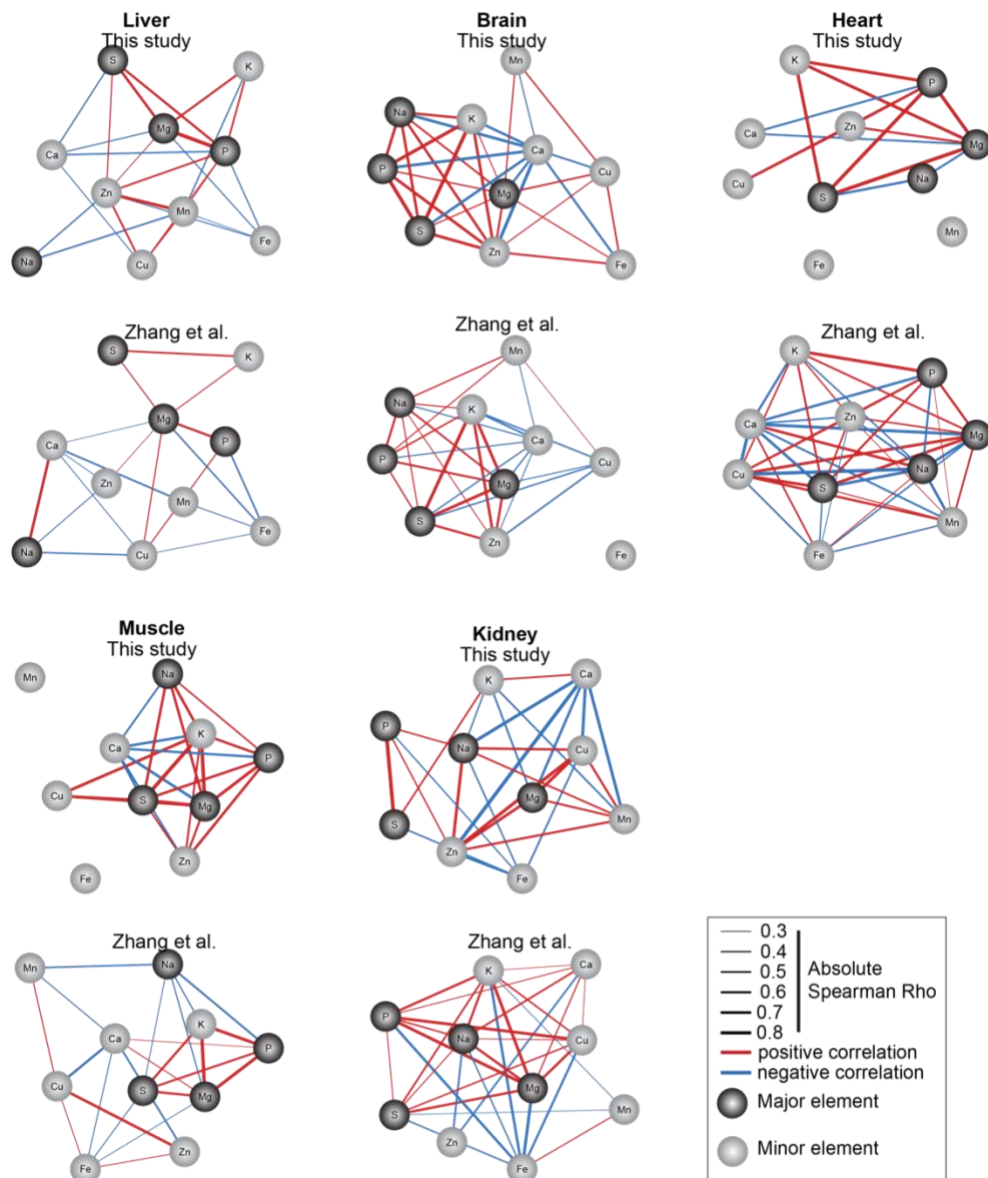


Fig. S4.

Networks of Spearman Rho correlations for the mouse metallome in different organs, showing conservation between data from this study (upper network) and from Zhang *et al.* (10) (lower network). Only significant ($Adj.P < 0.05$) correlations are considered, and concentrations are age-corrected. A more systematic comparison between the correlation coefficients of both networks can be found on Fig 2B. The networks in liver, brain and heart are very similar, while muscle shows intermediate, and kidney poor, conservation.

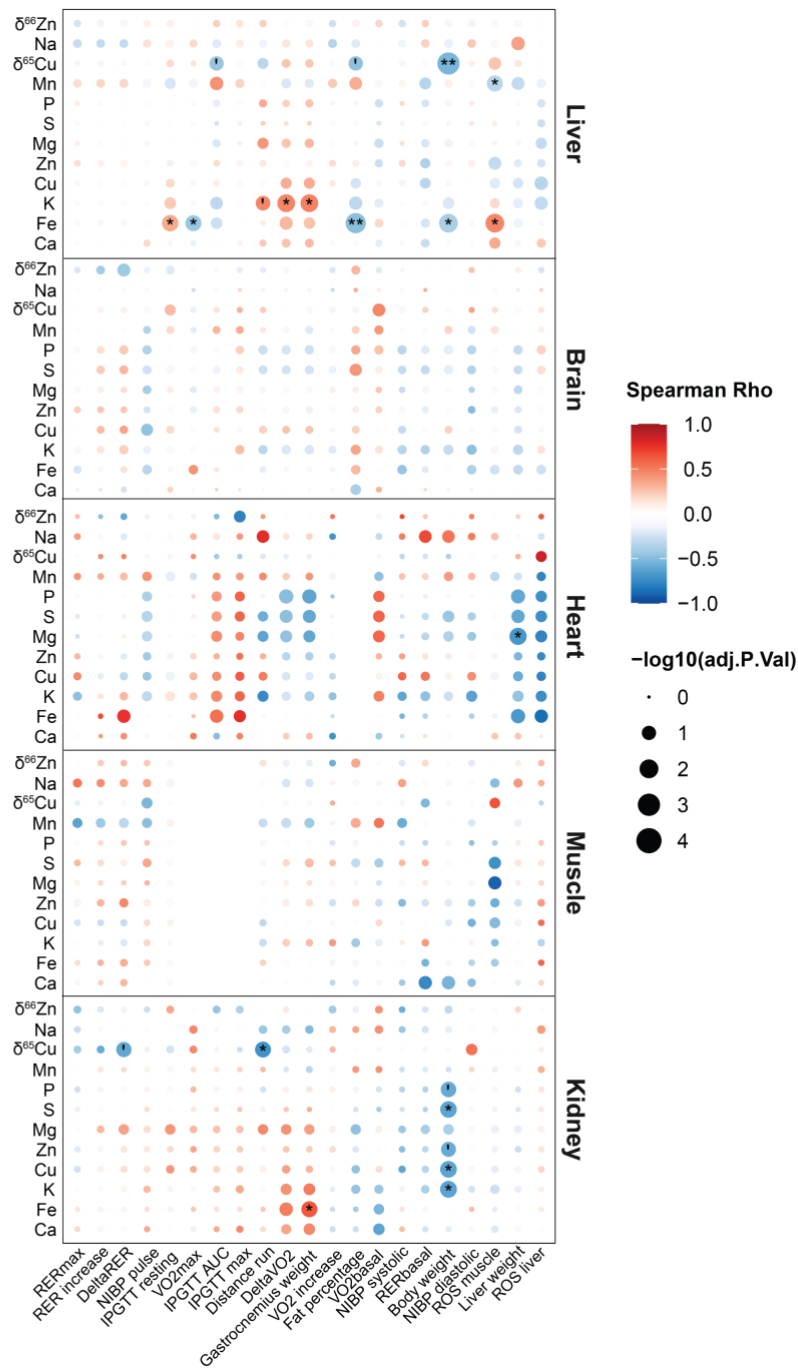


Fig. S5. Heatmaps of Spearman Rho coefficients between the mouse metallome of each organ, and the phenotypes. The kidney and liver are the only organs with significant correlations (and a single one for the heart), which is consistent with the fact that all the measured phenotypic traits are metabolic in essence and are therefore more likely to involve the liver or kidney. ***Adj.P < 0.001, **Adj.P < 0.01, *Adj.P < 0.05, Adj.P < 0.1 (limma) moderated t-test with age as a covariate, corrected for multiple testing.

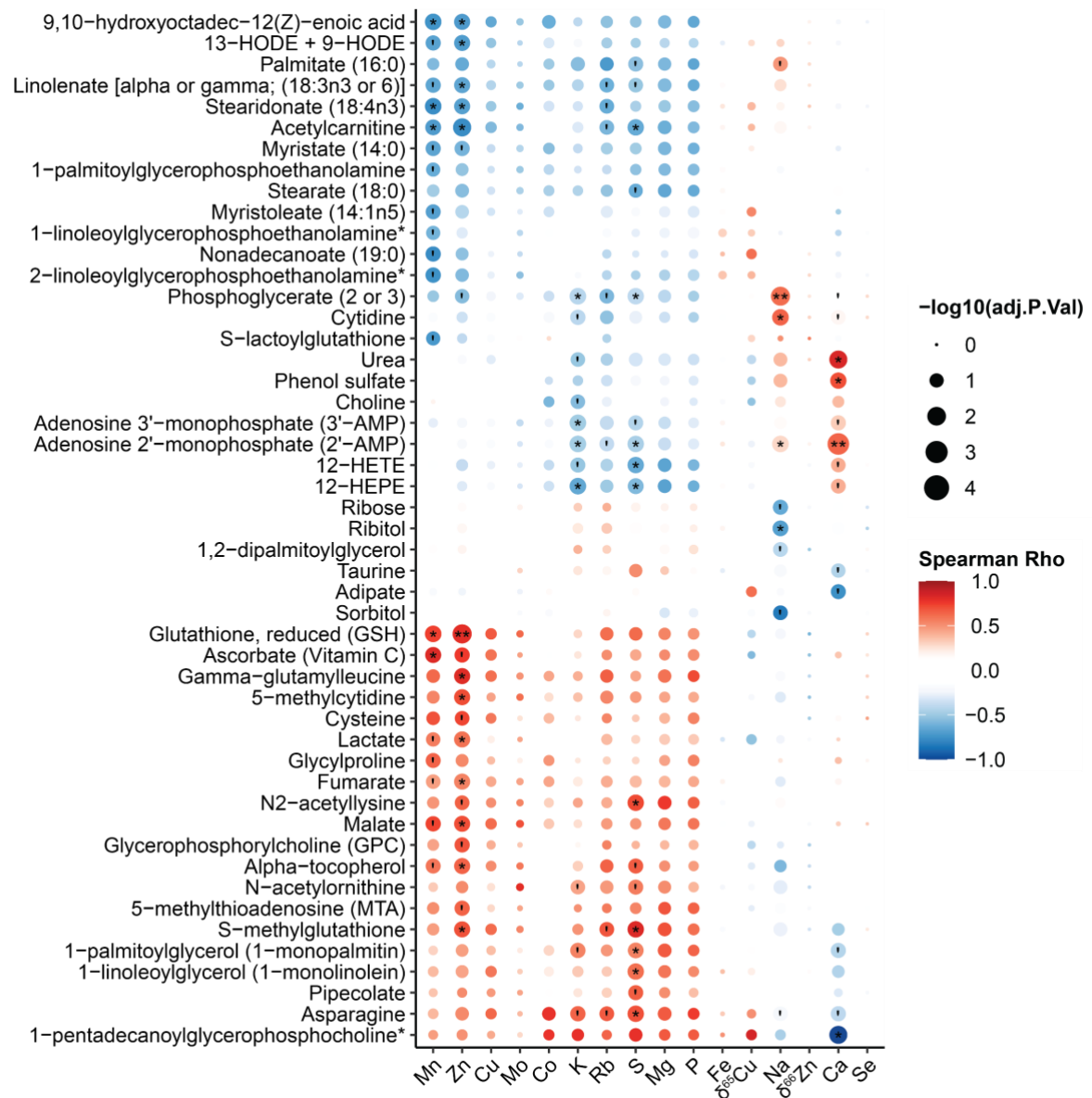


Fig. S6. Heatmaps of Spearman Rho coefficients between the liver metallome and the metabolome. Only metabolites with at least one correlation at Adj.P < 0.1 are displayed. Except for ultra-trace metals and $\delta^{66}\text{Zn}$, most of the metals exhibit numerous associations, of which the strongest mostly involve Zn and S. ***Adj.P < 0.001, **Adj.P < 0.01, *Adj.P < 0.05, Adj.P < 0.1 (limma) moderated t-test with age as a covariate, corrected for multiple testing.

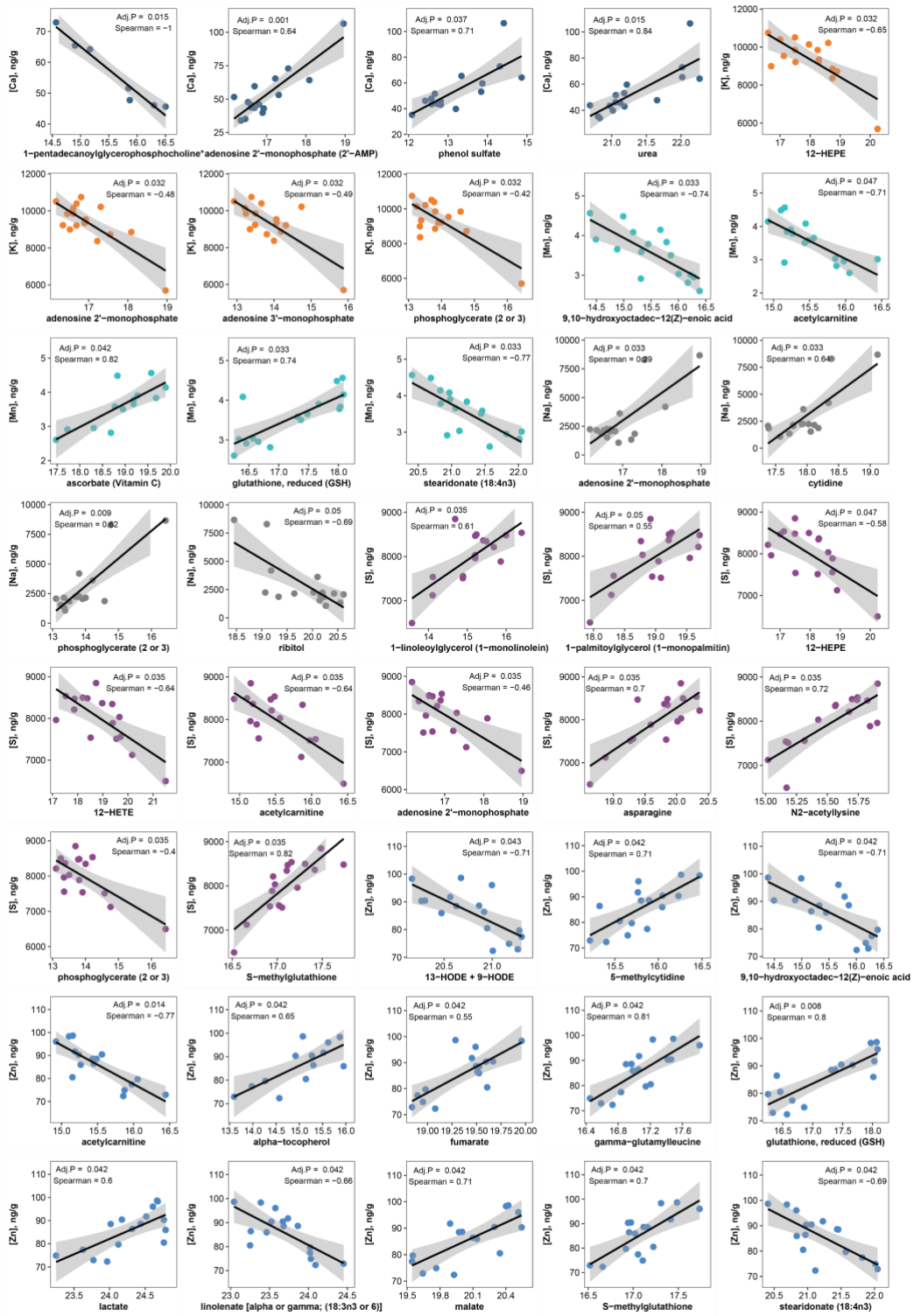


Fig. S7.

Scatterplots between the mouse metallome and metabolome. Only significant correlations

(adj. $P < 0.05$) are shown.

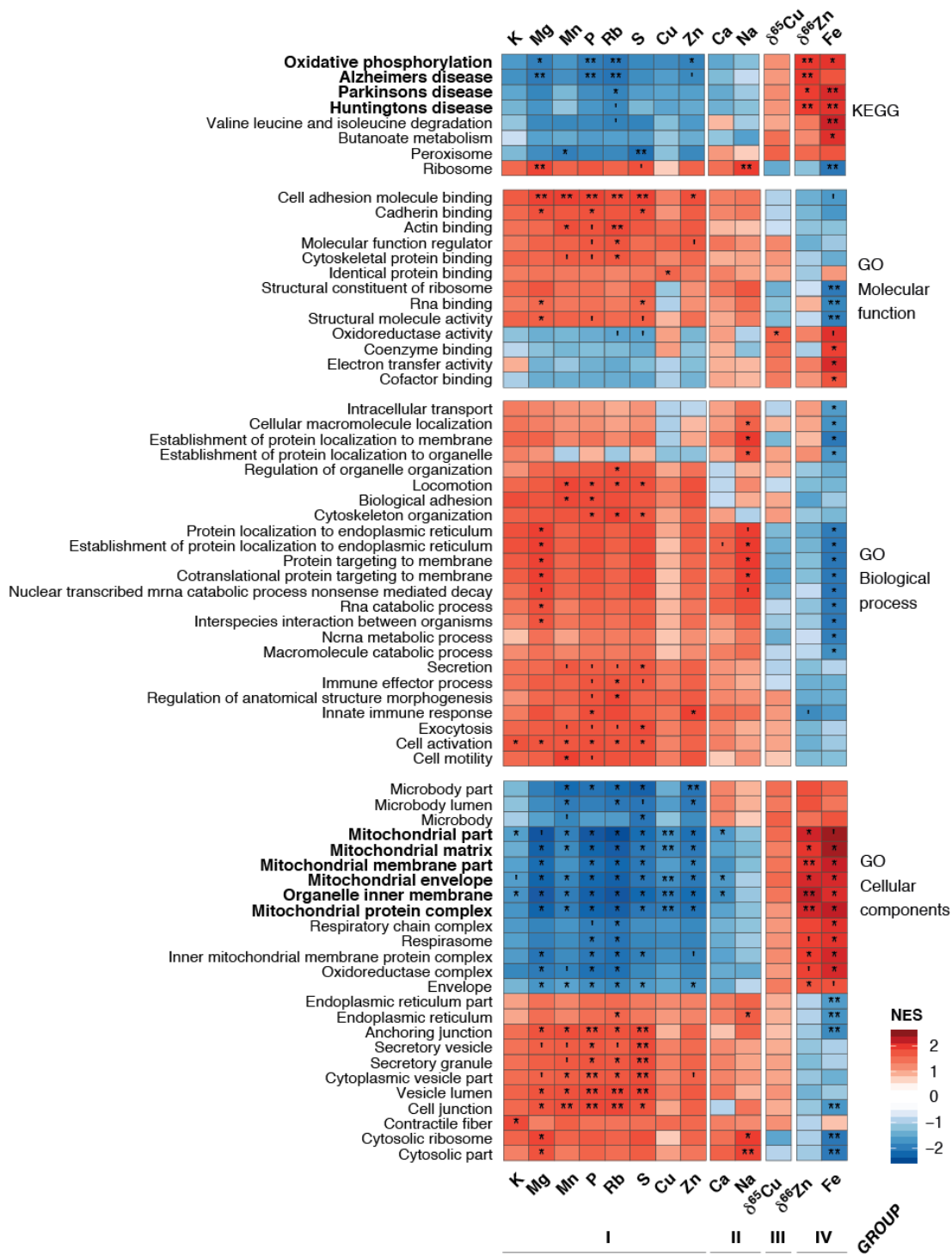


Fig. S8. Proteins were sorted on their correlation with individual metals in the liver, then gene-set enrichment analysis (GSEA) was performed. For each metal, the top five gene sets are selected in each category and included in the heatmap. Gene sets discussed in the main text are highlighted in bold. ' Adj.*P* < 0.1, * Adj.*P* < 0.05, ** Adj.*P* < 0.01, *** Adj.*P* < 0.001, *** Adj. < 10⁻⁴.

Organ	Mouse_ID	Age	Mn	Fe	Co	Cu	Zn	Se	Rb	Mb	Cd	Ca	K	Na	P	S	Mg	$\delta^{65}\text{Cu}$	$\delta^{66}\text{Zn}$
LIVER	RHO-2860	6	3.66	163	0.28	16.25	91.4	3.87	28.85	3.31		59.66	10571	2401	10099	8472	753	0.12	-0.09
	RHO-2861	6	2.76	160	0.31	14.9	82.47	3.5	27.84	2.84		43.48	9388	2231	9355	7541	665	0.32	-0.06
	RHO-2862	6	3.07	147	0.31	16.51	83.3	4.62	25.88	2.99		47.74	9168	2324	9359	7965	688	0.33	-0.01
	RHO-2863	6	4.24	275	0.39	16.82	89.2	3.32	30.47	2.95		46.1	10922	2245	10436	8216	727	0.35	-0.07
	RHO-2864	6	3.17	184	0.26	14.37	75.7	5.21	15.52	2.61		106.48	5870	8843	8866	6500	597	0.28	-0.07
	RHO-2865	6	3.91	242	0.33	17.77	94.6	4.28	31.25	3.14		84.47	11066	1943	10799	9445	788	0.33	-0.11
	RHO-2866	6	3.11	199	0.3	15.77	80.2	5.17	26.37	2.7		35.21	10021	2040	9298	7514	662	0.37	-0.09
	RHO-2867	6	3.8	183	0.3	16.11	87	4.16	27.76	3.04		36.64	9670	2039	9869	8056	703	0.11	-0.10
	RHO-2868	6	3.74	199	0.35	18.4	91.3	5.28	29.45	3.08		43.01	9712	3790	10234	8540	728	0.38	-0.03
	RHO-2869	6	2.97	210	0.29	17.36	77.7	3.85	24.97	3.18	0.01	34.01	9403	2324	9027	8347	667	0.47	-0.05
	RHO-2870	6	3.27	215	0.31	16.34	90.6	3.8	29.53	3.26	0.02	73.46	12250	3100	11229	9177	815	0.09	0.03
	RHO-2871	6	3.57	256	0.32	19.08	90.5	4.73	29.52	3.64	0.02	65.23	12354	3257	10800	8877	815	0.22	-0.03
	RHO-2872	6	2.98	259	0.25	19.11	78.9	4.29	31.97	3.26	0.02	34.58	11217	2624	9944	7801	703	0.49	-0.05
	RHO-2873	6	2.79	192	0.27	14.77	83.2	3.33	27.25	3.33	0.01	74.02	9908	2559	9646	8153	694	-0.06	-0.05
	RHO-2874	6	3.22	186	0.24	14.83	80.9	3.61	25.46	3.16	0.02	28.9	10105	3106	9458	7926	678	0.21	-0.09
	RHO-2876	6	3.01	144	0.29	14.91	73.9	4.12	24.23	3.11	0.01	27.3	9757	2585	8463	7411	626	0.36	-0.04
	RHO-2880	16	2.62	309	0.24	13.47	75.7	4.17	22.63	2.91	0.02	32.58	9232	3791	8653	6415	635	0.07	-0.06
	RHO-2883	16	3.02	201	0.26	15.43	87.8	4.44	27.96	3.39	0.01	39.36	11258	2399	9912	8084	715	-0.05	-0.08
	RHO-2884	16	2.63	143	0.27	11.76	65.5	3.52	20.75	2.61	0.02	26.24	10245	3105	8606	6662	609	-0.03	0.05
	RHO-2886	16	2.61	414	0.27	21.09	94.2	5.67	39.11	3.48	0.02	65.01	13282	2716	10612	8597	743	0.39	-0.18
	RHO-2887	16	3.54	261	0.38	18.36	97.7	4.73	29.53	3.71	0.02	55.17	12839	3648	10653	8328	755	0.07	-0.05
	RHO-2888	16	2.47	154	0.24	14.34	70.1	3.49	22.48	2.74	0.02	31.94	9865	2211	8572	6515	610	0.20	-0.09
	RHO-2889	16	3.33	336	0.35	20.74	90.2	4.85	29.95	3.6	0.02	51.56	12127	5499	10891	8975	761	0.45	-0.06
	RHO-2890	16	3.48	319	0.31	16.3	88.3	4.8	27.87	3.01		38.25	9284	2104	9317	8060	650	0.36	-0.09
	RHO-2891	16	4.23	326	0.32	16.59	90.6	3.7	27.47	2.42		50.09	10341	2468	10050	8359	693	0.09	-0.06
	RHO-2892	16	4.15	180	0.33	14.64	86.3	3.92	27.93	2.79		35.29	9806	1699	9328	7412	659	-0.02	-0.08
	RHO-2893	16	4.5	249	0.48	18.41	101.9	4.77	32.56	3.25		74.32	11355	2081	10885	8608	767	0.06	0.00
	RHO-2894	16	4.91	197	0.32	18.56	102.7	3.38	34.72	3.46		51.06	10359	2602	10470	8127	732	-0.15	-0.03
	RHO-2895	16	4.14	227	0.36	17.43	95.6	4.5	31.09	3.13		45.05	10912	2106	10255	7838	712	-0.09	-0.01
	RHO-2896	16	4.01	220	0.33	14.42	83.2	3.9	24.51	2.78		77.29	8688	2238	9125	7885	636	-0.02	-0.08
	RHO-2897	16	3.28	276	0.24	15.16	87.7	3.62	25.25	2.69		63.95	9172	1692	9083	7426	655	0.09	-0.08
	RHO-2898	16	4.06	188	0.39	15.53	87.6	3.59	27.07	2.84		38.57	9704	2371	9627	7114	673	0.00	-0.04
	RHO-2899	16	4.2	169	0.27	15.05	93.4	4.31	28	3.13		65.42	8832	1806	9077	7565	629	-0.04	-0.12
	RHO-2900	24	2.62	199	0.23	11.37	67.7	2.92	19.37	2.4	0.01	25.49	9208	2117	8045	6765	568	-0.06	-0.04
	RHO-2902	24	3.26	245	0.32	14.99	84.9	3.22	29.31	3.12	0.03	72.87	11481	2722	9799	7485	722	-0.07	0.02
	RHO-2903	24	3.64	187	0.26	15.85	87.9	4.24	23.46	3.34	0.01	40.69	12014	2769	10159	8407	733	0.03	-0.08
	RHO-2904	24	5.19	115	0.39	18.35	109.8	5.39	37.86	3.14		51.59	11208	2937	11661	8859	796	0.00	-0.15
	RHO-2905	24	3.22	332	0.29	14.79	101.2	2.75	28.26	2.88	0.02	58.3	14534	3858	12164	10198	852	0.37	-0.17
	RHO-2906	24	2.55	382	0.23	12.78	76.4	2.71	21.91	2.45	0.02	80.35	12149	2291	9776	8382	734	0.18	-0.12
	RHO-2907	24	3.22	239	0.32	15.49	93.1	3.68	26.36	3.2	0.02	39.46	11039	3900	10532	9207	777	0.05	0.00
	RHO-2908	24	3.18	283	0.29	14.61	86	3.37	26.03	3.02	0.02	66.73	11490	3775	9815	8663	734	-0.04	-0.04
	RHO-2910	24	3.72	232	0.45	14.33	85.9	4.85	23.55	3		37.7	7804	3373	8312	7371	575	-0.04	-0.03
	RHO-2911	24	3.66	255	0.32	16.08	83.5	4.88	24.41	2.69		72.92	9420	8993	8858	7135	622	-0.09	-0.04
	RHO-2913	24	3.36	247	0.3	14.58	86.9	4.47	20.53	2.38		51.61	7387	5474	8442	6745	587	-0.04	-0.04
	RHO-2915	24	5.11	279	0.39	17.71	109.5	4.98	33.49	3.07		45.66	10338	2242	10226	8509	721	0.03	-0.09
	RHO-2916	24	4.53	260	0.36	16.89	101.5	3.98	24.71	2.79		64.26	9555	4892	9726	7898	694	0.00	-0.06
	RHO-2917	24	4.77	214	0.31	19.65	107.2	6.27	31.55	3.25		43.81	10549	2961	10778	8492	750	0.01	-0.11
	RHO-2918	24	4.28	349	0.41	19.38	101.6	5.86	31.09	3		53.19	10915	2543	10399	8043	718	0.17	-0.14
	RHO-2919	24	4.46	297	0.31	17.33	102.9	3.17	29.77	2.29		39.82	10040	1770	9938	8376	699	-0.03	-0.1
	RHO-2860		1.74	65		18.85	63.8		11.65				381.05	16768	4667	14206	7206	674	0.64
RHO-2861		1.31	62		14.63	59.2		12.12				366.67	16583	4623	14065	7331	681	0.42	
RHO-2862		1.64	71		16.72	65.6		12.31				239.29	19137	4989	15643	8173	752	0.69	0.05
RHO-2863		1.73	66		19.5	64.5		12.43				504.4	18403	4975	15283	8000	742	0.61	0.03
RHO-2864		1.78	66		17.89	63.1		11.65				250.98	17696	5000	14843	7685	712	0.68	0.03
RHO-2870		1.67	63	0.03	19.81	58.9	1.24	12.2				679.06	14248	4808	13290	6506	655	0.91	0.06
RHO-2871		1.72	74	0.03	21.21	61.7	0.71	12.85				821.39	15806	3771	14361	7006	714	0.95	0.02
RHO-2872		2	75	0.07	18.73	66.7	0.55	13.98				2604.29	15791	4472	14573	6749	742	0.78	0.01
RHO-2873		1.69	79	0.04	20.02	60.3	1.44	13.1				722.96	15653	4615	14044	6994	703	0.83	0.02
RHO-2874		1.95	70	0.04	20.3	62.7	0.76	13.68				552.71	16047	4626	13870	6932	734	0.86	0.03
RHO-2876		1.9	50	0.05	16.7	54.9	0.68	13.84				676.92	15395	4264	13231	6608	600	0.84	0.05
RHO-2880		1.74	35	0.06	16.58	45.3	0.92	10.38				928.45	12998	3623	11765	5917	542	0.85	0.05
RHO-2883		1.73	78	0.07	21.69	64.2	0.64	12.57				1646.01	15482	5134	14463	7018	762	0.82	0.00
RHO-2884		1.68	86	0.07	23.36	63.2	0.99	12.1				1645.63	15249	4785	14180	6845	741	0.80	0.02
RHO-2886		1.28	78	0.06	23.72	65.3	0.99	14.4				1101.18	16077	4851	14055	6909	774	0.82	-0.02
RHO-2887		1.77	92	0.06	23.38	64.1	1.13	12.94				483.18	15994	5017	14343	7239	772	0.89	0.01
RHO-2888		1.77	83	0.07	24.55	63	1.5	12.63				203.17	15653	4945	14147	7113	762	0.81	-0.02
RHO-2889		1.13	81		15.69	51.6		9.39				2375.44	12860	3688	12202	5834	580	0.56	0.04
RHO-2890		1.29	74		20.48	55.7		9.86				2002.9	14715	4173	13638	6758	638	0.81	0.08

Table S1.

Continued on next page.

Organ	Mouse_ID	Age	Mn	Fe	Co	Cu	Zn	Se	Rb	Mb	Cd	Ca	K	Na	P	S	Mg	$\delta^{65}\text{Cu}$	$\delta^{66}\text{Zn}$
	RHO-2891		1.66	82		19.14	63.3		10.21			1552.02	17106	4769	14803	7434	704	1.22	0.05
	RHO-2892		1.46	76		22.56	62.3		11.13			479.44	16500	4594	13806	7383	663	0.93	0.02
	RHO-2893		1.55	78		21.75	63.7		11.61			577.4	16825	4684	14842	7814	702	0.86	-0.02
	RHO-2900		1.5	85		26.07	56.5		9.61			471.62	15568	4485	13351	7176	645	0.77	0.00
	RHO-2902		1.56	96		21.19	66.4		14.1			715.92	17169	4606	14055	7736	700	0.38	0.04
	RHO-2903		1.64	106		26.08	62.9		10.41			240.2	16431	4698	14299	7578	675	0.73	0.04
	RHO-2904		1.3	94	0.19	23.63	63.2		11.7			377.14	14986	4410	13324	6962	631	0.58	0.10
	RHO-2905		1.52	82		23.48	59.6		11.46			253.33	15906	4821	14528	7533	673	1.00	0.03
	RHO-2911		1.93	59	1.06	20.09	48.8	0.45	9.87	2.73		679.33	14026	3887	12106	6224	538	0.76	0.14
	RHO-2913		1.86	92	0.08	25.14	65.6	0.79	11.5			656.21	15897	5304	14732	7394	797	0.96	0.07
	RHO-2915		1.74	83	0.07	24.56	60.7	1.11	11.72			1127.07	14573	4567	13803	6752	761	0.72	0.03
	RHO-2916		1.78	87	0.08	27.92	63.6	0.93	10.5			213.36	15668	4645	13981	7072	770	0.59	0.02
	RHO-2917		1.77	96	0.08	28.37	65.8	0.78	11.54			474.03	15801	4578	14651	7439	823	0.71	0.00
	RHO-2919		1.9	94	0.07	28.87	66.6	0.92	12.07			399.83	15192	4874	14599	7384	826	0.87	0.07
	RHO-2864		2.89	227	0.12	22.74	51.8	1.36	14.22			67.48	8160	7621	8267	10000	704	0.00	-0.05
	RHO-2870		3.7	325		30.01	67.5		11.83			89.66	7666	12920	9866	10748	782	-0.26	0.03
	RHO-2871		5.39	461	0.42	28.78	70.7		18.19			108.93	10827	8643	10387	12565	936	0.00	-0.04
	RHO-2872		2.74	300		28.85	69.5		18.63			81.2	10675	9872	10316	12162	910	-0.27	0.03
	RHO-2873		3.58	348		28.58	61.3		14.52			131.94	9879	12065	9957	10971	786	-0.04	-0.09
	RHO-2889		3.26	361		27.71	62.6		16.85			119.05	10638	6683	9981	12267	924	-0.09	-0.01
	RHO-2890		2.43	323		27.3	61.6		15.03			93.33	10505	8106	9933	11914	894	0.27	0.01
HEART	RHO-2891		3.06	383		29.01	66.7		14.79			84.4	10830	7454	10702	13433	1000	-0.03	-0.05
	RHO-2892		3.14	452		28.07	62.3		14.89			102.9	11235	8571	10303	12371	932	-0.14	-0.11
	RHO-2895		2.92	267	0.15	23.81	53.3	0.9	15.45			61.62	9495	6621	8944	10793	816	-0.01	0.01
	RHO-2900		2.59	371		27.6	66.2		14.38			106.86	10941	8260	10490	12510	968	-0.42	0.02
	RHO-2905		2.82	273	0.2	24.8	56.4	0.92	17.8			147.75	10107	6343	9478	11556	808	0.25	0.03
	RHO-2906		2.26	350		27.25	67.9		15.06			155.13	11829	9733	10751	12897	969	-0.06	-0.03
	RHO-2907		2.8	355		28.81	66.8		15.43			110.1	10646	10768	10677	12374	936	-0.21	0.09
	RHO-2911		2.51	304		26.55	61.4		13.49			89.25	10774	8669	9955	11914	891	-0.19	-0.01
	RHO-2870		0.76	48		3.66	27		27.62			347.07	16517	1942	8934	13041	1159	-0.31	0.16
	RHO-2871		1.98	118	0.16	4.2	32.9		27.65				15982	2170	10608	13021	1198	-0.19	0.16
	RHO-2872		0.95	36	0.02	4.39	25.4	0.87	33.37			276.54	16553	2029	8458	12615	1086	-0.17	0.09
	RHO-2873		0.6	57		3.58	24.4		26.92			505.04	15739	1801	8798	12571	1101	-0.34	0.13
	RHO-2874		1.16	41		4.15	27		29.93			287.07	16694	2009	9378	13463	1387	-0.43	0.11
	RHO-2876		0.7	45		3.8	32.8		26.48				15592	2384	11786	13217	1269	-0.63	0.15
	RHO-2880		0.55	84		3.98	26.3		26.36			348.87	16176	2763	9132	13545	1145	-0.38	0.09
	RHO-2883		0.69	57		4.78	30.1		30.6			394.4	16157	2368	9288	13413	1133	-0.51	0.13
MUSCLE	RHO-2884		0.74	56		4.58	30		27.49			390.36	16264	2278	9438	13543	1157	-0.43	0.15
	RHO-2886		0.44	68		5.03	32.8		33.95			590.85	17160	2298	9850	14141	1199	-0.63	0.07
	RHO-2887		0.79	54		4.29	28.9		28.81			355.32	16286	2381	9158	13387	1142	-0.59	0.13
	RHO-2889			39	0.03	4.36	26.7	0.3	36.05			414.97	18159	2212	9309	13697	1380	-0.26	0.06
	RHO-2903		0.64	54		3.95	31.9		22.56			428.03	15596	2545	8952	13487	1108	-0.33	0.09
	RHO-2906			48	0.03	4.05	23.6	0.68	22.72			435.45	13944	2097	8101	12172	947	-0.08	0.12
	RHO-2907		0.69	59		4.54	29.2		27.61			480	15873	2561	9267	13489	1136	-0.33	0.07
	RHO-2908		0.9	78		4.79	30.3		25.99			401.87	14475	1977	8277	12235	1038	-0.3	0.05
	RHO-2910		1.03	71		4.03	36		24.13				14791	2635	13026	12532	1222	-0.46	0.24
	RHO-2913		0.96	67		4.9	32.5		25.71			713.43	15898	2604	9424	13637	1136	-0.68	0.13
	RHO-2860		6.1	389	0.75	15.61	74.6	5.12	21.19	1.1		161.47	10000	4658	12048	8459	765	1.39	0.24
	RHO-2861		6.28	212	0.82	16.62	78.9	5.46	22.99	1.31		123.08	9509	5568	13368	8799	812	1.56	0.3
	RHO-2870		6.57	246	0.78	16.09	73	5.23	22.38		0.04	155.64	9037	3587	10900	7597	863	1.25	0.35
	RHO-2871		6.91	387	0.92	17.83	81	6.44	27.62		0.04	102.76	9868	5413	12402	8473	951	1.25	0.32
	RHO-2872		6.61	161	1.01	17.66	76.8	4.62	25.8		0.05	119.52	9344	5262	12027	7961	862	1.35	0.41
	RHO-2873		5.91	305	1.04	16.16	72.5	5	21.71		0.05	168.08	9395	4705	11162	7827	858	1.29	0.36
	RHO-2874		7.1	151	0.84	16.37	75.7	5.78	21.28		0.03	91.34	8352	5981	11095	7469	847	1.28	0.29
	RHO-2883		5.55	168	0.76	14.8	65.8	4.98	19.81		0.07	134.9	8188	5255	10227	7068	778	1.26	0.24
	RHO-2884		6.06	165	0.67	16.4	74.3	7.14	21.4		0.08	105.36	8514	5584	11267	7706	861	1.24	0.27
	RHO-2886		4.33	289	0.91	17.59	75.5	6.59	29.41		0.07	208.85	10895	5106	12087	8709	963	1.3	0.28
KIDNEY	RHO-2887		6.95	208	0.76	17.25	80.5	6.18	24.56		0.07	135.48	9454	5988	12348	8413	945	1.26	0.31
	RHO-2888		5.66	150	0.54	14.8	67.4	5.74	20.64		0.06	112.04	7811	5480	10664	7131	791	1.35	0.23
	RHO-2889		5.06	169	0.59	15.83	71.7	5.1	22.52	1.17		152.6	10203	5116	12057	8688	770	1.45	0.33
	RHO-2890		7.11	228	0.73	17.28	81.2	6.27	21.22	1.45		144.1	10482	6318	13703	9549	852	1.39	0.26
	RHO-2900		5.18	159	0.65	14.52	68.6	5.51	15.92	1.11		146.77	8607	6035	11577	8269	722	1.23	0.28
	RHO-2902		5.97	196	0.65	16.59	77	5.58	24.82	1.29		247.26	11030	5262	13097	9473	862	1.16	0.33
	RHO-2913		5.7	173	0.84	15.31	68	5.77	16.79		0.09	117.62	7566	6195	10881	7337	799	1.34	0.31
	RHO-2915		6.27	210	0.93	17.51	80.7	6.4	21.68		0.11	193.66	9676	6649	12769	8859	951	1.37	0.24
	RHO-2916		6.7	195	0.67	17.07	78.1	5.97	18.8		0.1	167.27	8884	6401	12146	8401	936	1.37	0.31
	RHO-2917		5.7	179	0.87	15.96	74.1	6.19	16.59		0.08	125.63	7301	6331	11825	7777	863	1.38	0.29
	RHO-2919		4.98	157	0.72	13.44	62.9	4.3	15.51		0.06	113.15	6822	5229	10102	6795	750	1.25	0.24

Table S1.

Element concentrations (ppm) and Cu-Zn isotopic compositions of mice organs.

	This study		Reference values	
	OEP	SRM-1577 c	OEP	SRM-1577 c
Mn	n/a	9.9±0.2 (4)	n/a	10.5±0.5**
Fe	20.1±4.6	191±5 (4)	19.5±3.6 (15)*	198±1**
Co	0.17±0.03 (3)	0.29±0.01 (4)	0.17±0.04 (15)*	0.30±0.02**
Cu	10.7±1.2 (3)	270±5 (4)	10.5±1.4 (15)*	273±5**
Zn	7.3±1.0 (3)	177±4 (4)	7.2±1.2 (15)*	181±1**
Se	1.33±0.22 (3)	1.67±0.19 (4)	1.48±0.77(15)*	2.03±0.04**
Rb	1.10±0.29 (3)	31.8±0.6 (4)	1.03±0.18(15)*	35.3±1.1**
Mo	2.70±0.66 (3)	3.7±0.2 (4)	2.61±0.39(15)*	3.3±0.1**
Cd	n/a	0.08±0.02 (4)	n/a	0.10±0.01 **
Ca	900±171 (3)	134±6 (4)	870±144(15)*	131±10**
K	2079±395 (3)	10355±261 (4)	1989±405(15)*	10230±640**
Na	36667±7751 (3)	1985±58 (4)	34494±8248(15)*	2033±64**
P	1085±101 (3)	11070±249 (4)	1068±152(15)*	11750±270**
S	10156±1734 (3)	7162±178 (4)	9792±1477 (15)*	7490±340**
Mg	257±38 (3)	587±18 (4)	272±66(15)*	620±42**
$\delta^{65}\text{Cu}$	-1.10±0.15 (3)	0.43±0.04 (4)	-1.14±0.13 (35)*	0.37±0.14 (45)*
$\delta^{66}\text{Zn}$	0.76±0.08 (3)	-0.18±0.05 (4)	0.73±0.09 (35)*	-0.19±0.06 (45)*

Table S2.

Element concentrations (ppm) and Cu-Zn isotopic compositions (‰) of in-house and certified reference standards. * long-term in-lab average. ** reference value from National Institute of Standards and Technology.