Supplementary Methods

Limits of detection were calculated from the standard deviation of the gelatine standard blank (σ_B) and the slope of the calibration curve (S) for elements with a mean blank count greater than five.

$$LOD = \frac{3\sigma_B}{S}$$

For elements with a mean blank count (N_B) below ten and above zero,[1] Poisson statistics were used.[2]

$$LOD = \frac{2.33}{S}\sqrt{N_B}$$

Supplementary Table 1. Linearity of the matrix-matched gelatine standards when measured using the addition of 0 and 8 ml min^{-1} of nitrogen.

Element	r^2 (0 ml min^{-1})	r^2 (8 ml min^{-1})	Element	r^2 (0 ml min^{-1})	r^2 (8 ml min^{-1})
Al	0.9954	0.9979	Ba	0.9996	0.9998
Sc	0.9997	0.9997	La	0.9994	0.9990
Ti	0.9957	0.9948	Ce	0.9995	0.9996
Cr	0.9988	0.9986	Pr	0.9986	0.9989
Mn	0.9994	0.9846	Nd	0.9998	0.9998
Fe	0.9859	0.9907	Sm	0.9998	0.9996
Co	0.9998	0.9996	Eu	0.9999	0.9995
Ni	0.9994	0.9992	Gd	0.9988	0.9997
Cu	0.9984	0.9984	Tb	0.9988	0.9992
Zn	0.9711	0.9736	Dy	0.9997	0.9995
Ga	0.9997	0.9997	Ho	0.9987	0.9979
Ge	0.9862	0.9841	Er	0.9994	0.9994
As	0.9998	0.9998	Tm	0.9989	0.9984
Se	0.9970	0.9971	Yb	0.9996	0.9990
Sr	0.9997	0.9997	Lu	0.9990	0.9989
Y	0.9998	0.9997	Hf	0.9947	0.9963
Zr	0.9935	0.9959	Ta	0.9536	0.9602
Nb	0.9961	0.9959	W	0.9986	0.9985
Mo	0.9988	0.9997	Re	0.9997	0.9992
Ru	0.9917	0.9898	Os	0.9940	0.9824
Rh	0.9951	0.9924	Ir	0.9892	0.9855
Ag	0.9969	0.9972	Pt	0.9906	0.9844
Cd	0.9973	0.9950	Tl	0.9998	0.9996
In	0.9998	0.9996	Pb	0.9995	0.9992
Sn	0.9841	0.9856	Bi	0.9996	0.9993
Sb	0.9994	0.9995	Th	0.9998	0.9999
Te	0.9930	0.9955	U	0.9995	0.9993

Supplementary Table 2. List of antibodies, their clone and manufacturers, and isotopic label.

antibody	Clone	manufacturer	Isotopic label
DNA intercalator		Fluidigm (Cat. No.: 201103A)	Rh103
Alpha1-syntrophin	EPR14828	Abcam	Nd144
Alpha-dystroglycan	IIH6	Santa Cruz Biotechnology	Sm152
Gamma-sarcoglycan	n/a (Rabbit polyclonal)	GeneTex	Eu153
Anti-dystrophin	Mandys8	Santa Cruz Biotechnology	Gd158
Alpha dystroglycan	VIA41	Santa Cruz Biotechnology	Tb159
nNOS	EP1855Y	Abcam	Ho165
Myosin, fast	MY-32	Abcam	Tm169
Sarcospan	E2	Santa Cruz Biotechnology	Yb173
Alpha2-laminin	4H8-2	Abcam	Yb176

Supplementary Table 3: Matrix-matched gelatine standard concentrations.

Level	Concentration (mg/kg)																	
	Al	Sc	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Sr	Y	Zr	Nb
Blank	4.1	0.09	2.69	0.92	0.05	1.76	0.01	0.13	0.18	1.19	0	0.22	0	0.22	0.14	0	0.11	0.1
1	5.65	0.97	3.77	1.74	1.01	3.73	0.97	1.17	1.33	4.3	1.01	1.06	0.93	1.69	1.11	0.97	1.01	0.89
2	7.83	3.84	6.57	4	3.75	5.96	3.77	3.98	4.48	5.2	3.88	3.88	3.88	4.3	3.84	3.74	3.89	3.59
3	13	8.6	10.9	8.45	8.5	11.8	8.56	8.94	9.13	9.99	8.87	8.09	9.08	9.17	8.61	8.49	8.29	7.68
4	17.1	12.8	14.3	13	13	15.9	13.1	13.8	13.9	14.7	13.7	11.4	14.2	14.3	13.1	13.2	11.7	11
5	25.3	19.9	23.6	21.1	21.3	25	21.4	22.3	22.8	24	22.5	19.8	23.2	23	21.4	21.4	20.3	19.3
	Mo	Ru	Rh	Ag	Cd	In	Sn	Sb	Te	Ba	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb
Blank	0.02	0.02	0.05	0	0	0	0	0.05	0.02	0.07	0	0	0	0	0	0	0	0
1	0.96	5.22	5.03	0.87	0.99	0.99	0.96	1.13	0.95	1.08	0.95	0.96	0.96	0.99	0.99	0.93	0.97	0.94
2	3.96	13.2	12.6	3.74	3.82	3.82	3.7	4.13	3.97	3.88	3.67	3.68	3.68	3.77	3.77	3.63	3.77	3.62
3	8.47	33.7	32.1	7.99	8.61	8.61	8.3	8.61	8.48	8.78	8.29	8.28	8.3	8.55	8.57	8.17	8.46	8.14
4	11.9	42	41.4	11.3	13.3	13.3	12.8	12.2	12.1	13.4	13.2	13.4	13.4	13.2	13.1	12.5	13.1	13.3
5	20.8	57.6	60.7	19.7	21.6	21.6	22.1	21.2	20.7	21.9	22	22	22	21.5	21.5	20.4	21.4	21.7
	Dy	Ho	Er	Tm	Yb	Lu	Hf	Ta	W	Re	Os	Ir	Pt	Tl	Pb	Bi	Th	U
Blank	0	0	0	0	0	0	0.04	0.01	0.04	0	0.46	0.1	0.02	3.75	0.04	0.02	0	0
1	0.93	0.94	0.94	0.94	0.99	0.93	0.91	0.76	0.95	0.93	8.81	5.51	5.92	2.62	0.77	0.96	0.9	0.63
2	3.58	3.59	3.68	3.64	3.79	3.61	3.81	3.06	3.93	3.53	22.2	13.7	15	2.37	3.92	3.59	3.51	3.01
3	8.06	8.09	8.26	8.17	8.53	8.14	8.13	5.9	8.46	7.94	56.4	33.9	37.2	4.57	9.25	7.98	7.96	7.27
4	12.4	13.2	12.6	13.4	13.1	13.3	11.5	6.9	12	12.2	69	41.3	45.6	5.9	14.4	12.2	12.2	11.4
5	20.2	21.4	20.6	21.9	21.4	21.8	20	12.2	20.8	20	98.4	64.4	65.4	9.2	23.6	20.1	21.3	20.2

Supplementary Table 4: Mean signal intensity and standard deviations for all endogenous elements investigated across the murine brain section.

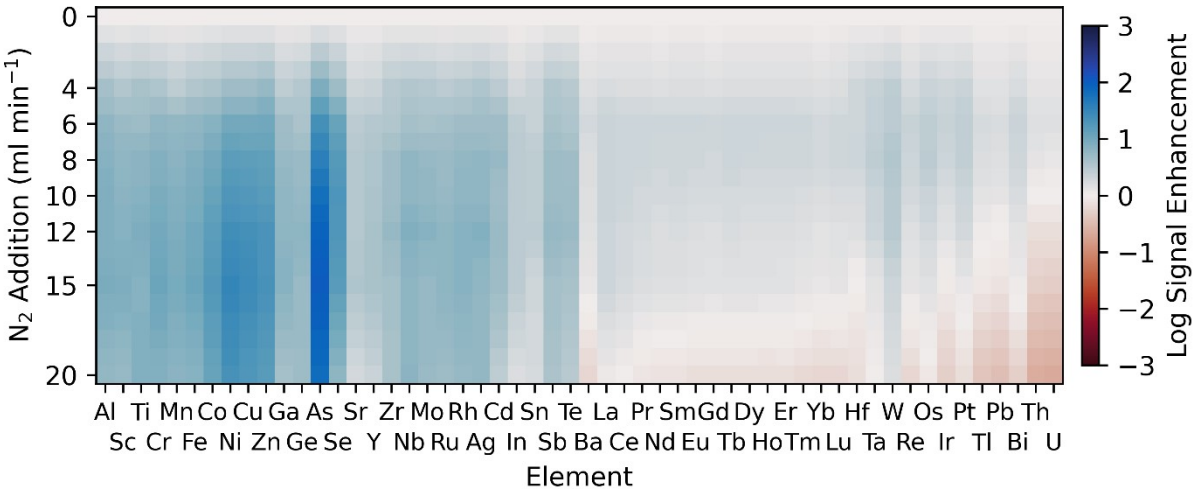
Element	N2 Flow (ml/min)	Signal Mean	%RSD
Na23	0	632582.0	10
	8	1206924.3	15
Mg24	0	5613.7	28
	8	10830.8	45
P31	0	4672.8	8
	8	24534.7	11
Mn55	0	185.1	27
	8	11965.6	9
Fe56	0	5193.5	43
	8	15321.1	43
Ni60	0	2.7	1727
	8	7.1	2023
Cu63	0	294.9	95
	8	1026.7	147
Zn66	0	599.4	40
	8	1948.6	39
Mo95	0	5.4	65
	8	11.3	59

Supplementary Table 5: Mean signal intensity and standard deviations for all elements investigated across the human quadriceps section.

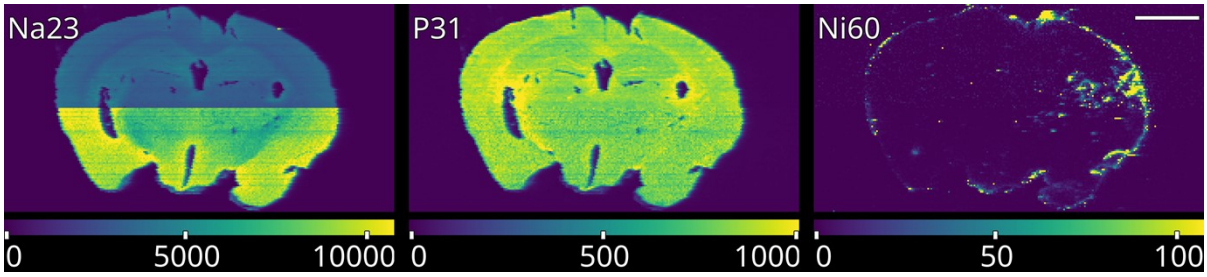
Element	N2 Flow (ml/min)	Signal Mean	%RSD
Rh103	0	291.9	42
	8	506.1	38
Nd144	0	98.2	44
	8	117.7	47
Sm152	0	13.8	77
	8	15.8	57
Eu153	0	23.0	56
	8	29.3	52
Gd158	0	38.9	56
	8	52.0	55
Tb159	0	9.6	62
	8	11.6	65
Ho165	0	11.2	50
	8	13.9	50
Tm169	0	225.1	151
	8	316.7	142
Yb173	0	75.1	61
	8	77.9	61
Yb176	0	90.6	62
	8	112.4	58

Supplementary Table 6: Limits of detection determined for elements with sufficient background counts at zero and optimal N_2 flow. Limits are calculated using the signal of the un-spiked gelatine standard and Gaussian or Poisson statistics where appropriate. * = alternate flow as optimal flow of 0 ml/min.

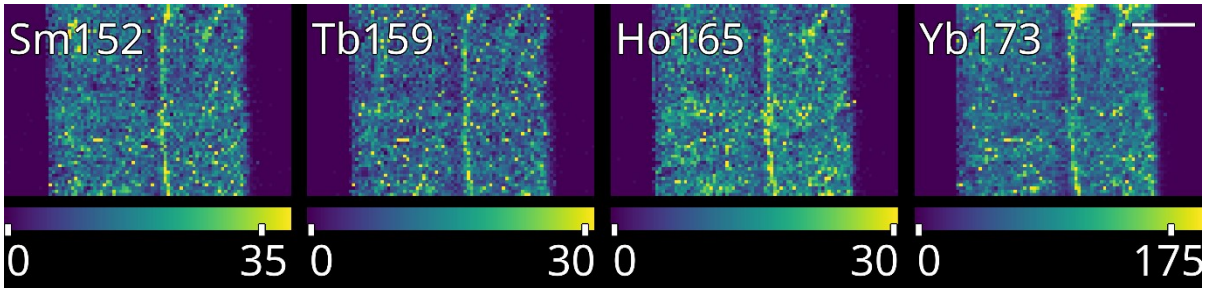
Element	N_2 Flow (ml/min)	LOD (mg/kg)
Al	0	1.3
	6	0.86
Sc	0	0.19
	*4	0.34
Cr	0	0.20
	*4	0.32
Mn	0	0.32
	*4	8.3
Fe	0	0.55
	*4	0.52
Ni	0	0.27
	20	0.14
Cu	0	0.81
	20	0.27
Zn	0	0.51
	12	0.26
Ga	0	0.21
	8	0.05
As	0	0.36
	10	0.12
Se	0	0.85
	*4	0.79
Sr	0	0.14
	12	0.12
Zr	0	0.066
	12	0.048
Sn	0	0.19
	12	0.14
Sb	0	0.10
	12	0.069
Ba	0	0.29
	6	0.26



Supplementary Figure 1. Signal enhancement for measured elements at different nitrogen addition flow rates. Values between those measured (0, 4, 6, 8, 10, 12, 15 and 20 ml min^{-1}) are interpolated at a 1 ml min^{-1} step.



Supplementary Figure 2. Comparison of the nitrogen addition and standard method for analysing endogenous metals in mouse brain. The top half of each image was collected using standard conditions and the bottom half with 8 ml min-1 nitrogen. Images were formed by dividing the signal collected with the mean background intensity, to show improvements in SNRs. Scale bar is 2 mm and image units signal/background.



Supplementary Figure 3. Raw signal for multiplexed imaging of lanthanide tagged antibodies in murine quadriceps muscle tissue sections. The top half of each image was collected using standard conditions and the bottom half with 8 ml min-1 nitrogen. Scale bar is 200 µm and the image units are in counts.

Supplementary References

1 T. E. Lockwood, R. G. de Vega and D. Clases, An interactive Python-based data processing platform for single particle and single cell ICP-MS, *Journal of Analytical Atomic Spectrometry*, 2021, **36**, 2536–2544.
2 L. A. Currie, Limits for qualitative detection and quantitative determination. Application to radiochemistry, *Anal. Chem.*, 1968, **40**, 586–593.