

# TEMI: tissue-expansion mass-spectrometry imaging

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## ***Supplementary Information***

### **TEMI: Tissue Expansion Mass Spectrometry Imaging**

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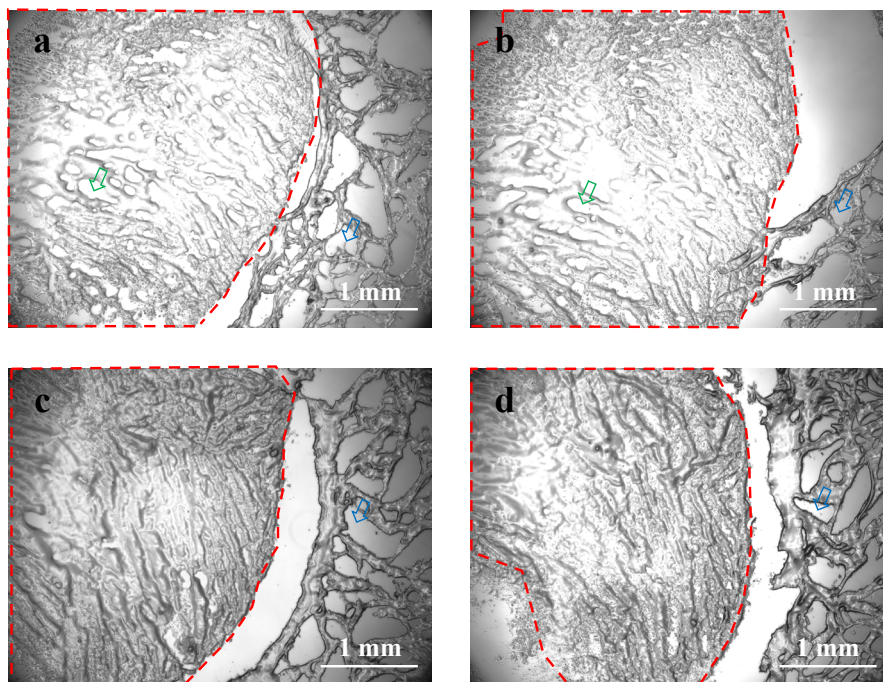
**Supplementary Table 10. The LC-MS gradient used for the proteomics analysis.**

**Supplementary Note 1. Measurement of expansion non-uniformity.**

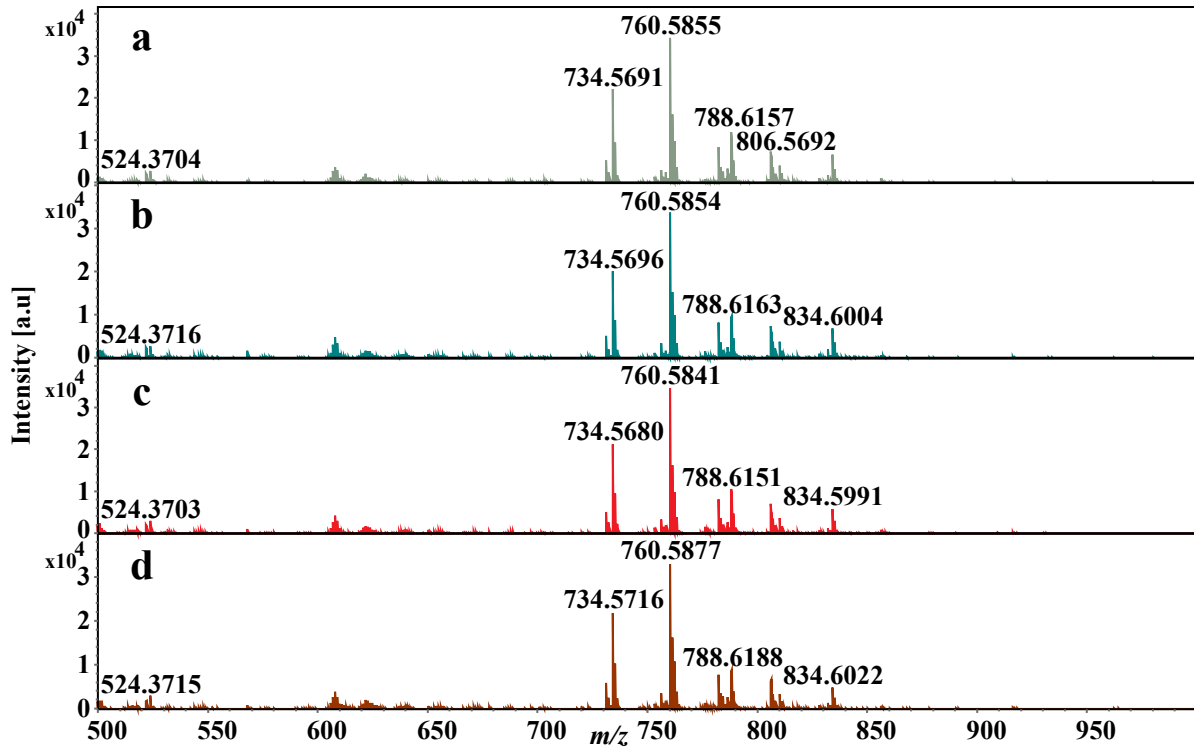
**Supplementary Note 2. Comparison among TEMI, GAMSI, Ex-MSI.**

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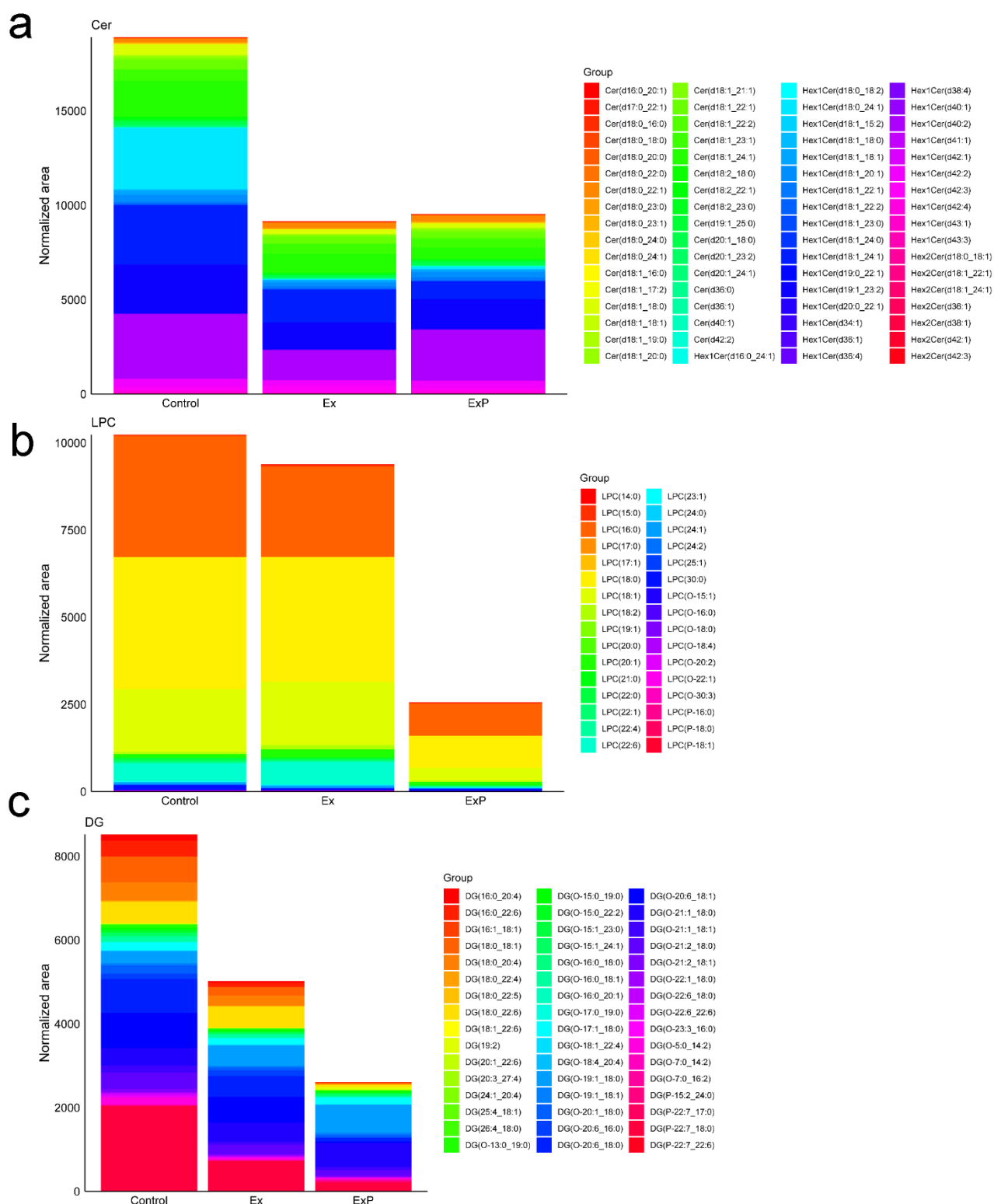




**Supplementary Figure 1. Cryosectioning of mouse cerebellum after tissue expansion treatment:** bright-field microscope images of expanded tissue sections cryosectioned with different thickness a. 10  $\mu\text{m}$ . b. 12  $\mu\text{m}$ . c. 20  $\mu\text{m}$ . d. 30  $\mu\text{m}$ . The dashed red circle represents the expanded tissue regions; the green hollow arrows on the tissue indicate where tears form due to shrinkage of the tissue upon drying; the blue arrows denote similar tears in the hydrogel adjacent to the tissue.



**Supplementary Figure 2. MALDI-MS spotting mass spectra of expanded mouse brain tissue sections with varied sectioning thickness.** a. 10  $\mu\text{m}$ . b. 12  $\mu\text{m}$ . c. 20  $\mu\text{m}$ . d. 30  $\mu\text{m}$ . MALDI-MS spotting of these tissue sections indicated that the signal intensities exhibit no significant decline as the sectioning thickness increases from 10 to 30  $\mu\text{m}$ .

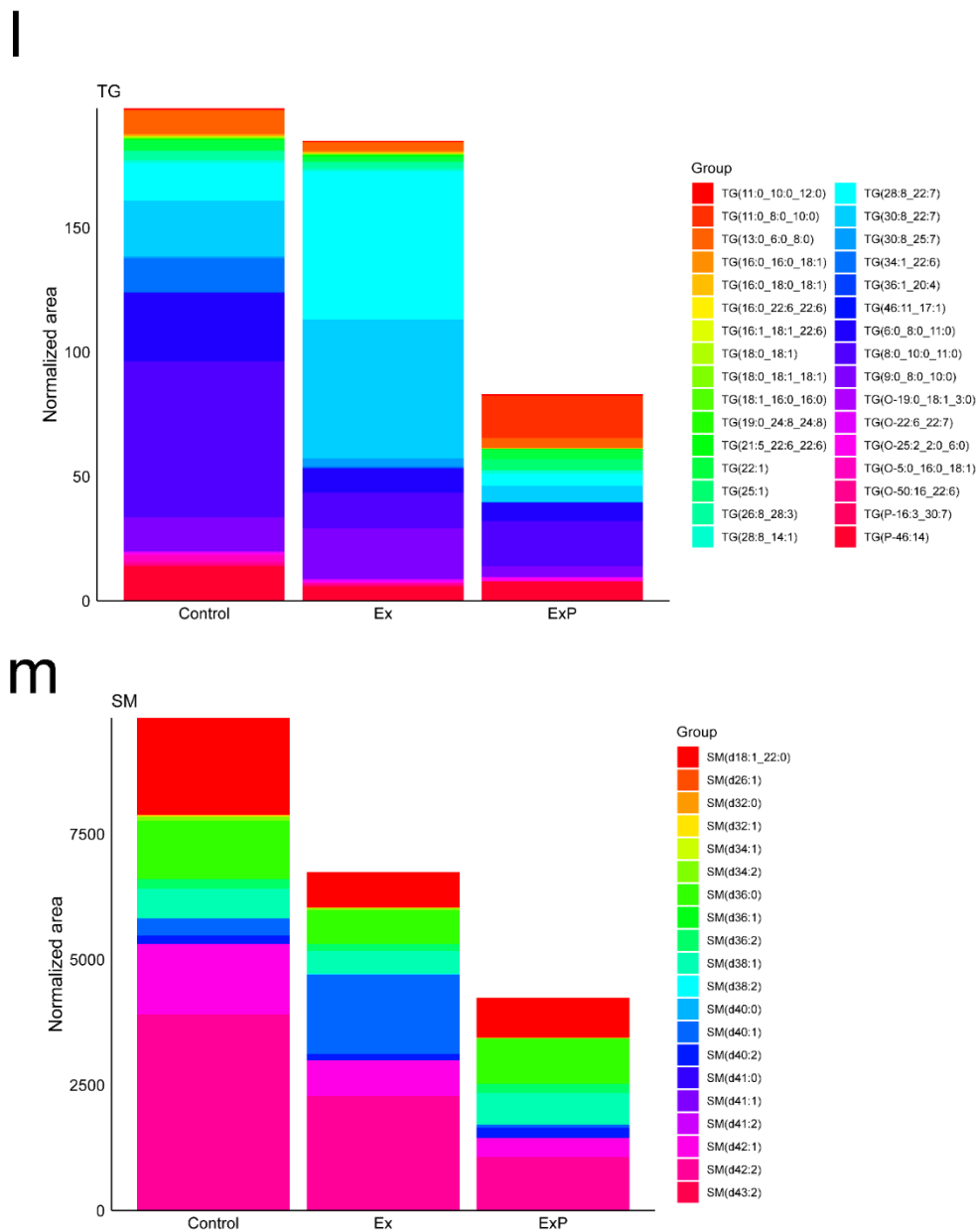


**Supplementary Figure 3. Lipidomics results.** Three groups: control (without expansion), Ex (expanded without applying ProK), ExP (expanded with applying ProK). a. Ceramide (Cer)-type lipids. b. Lysophosphatidylcholine (LPC)-type lipids. c. Diacylglycerol (DG)-type lipids.

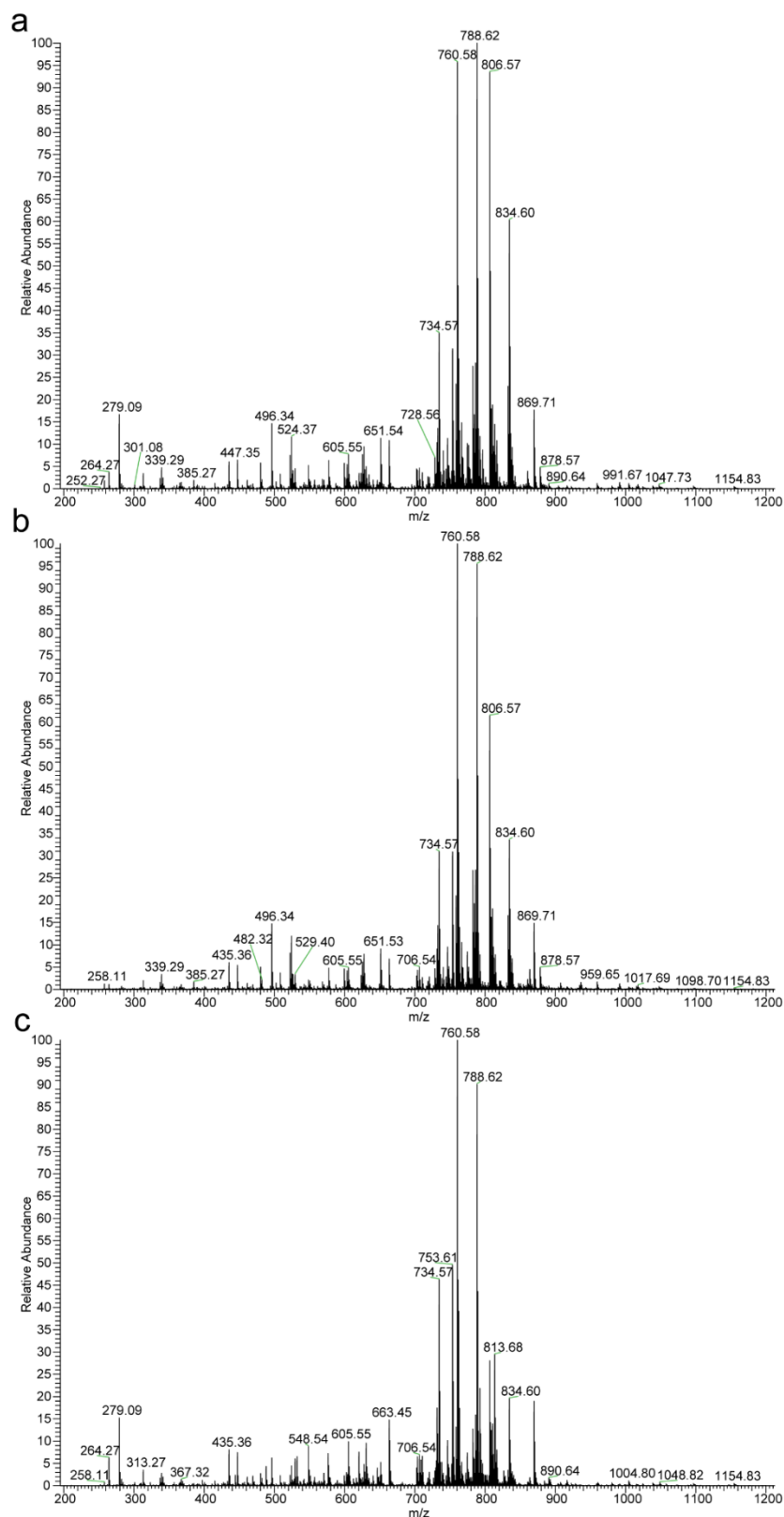


S-7

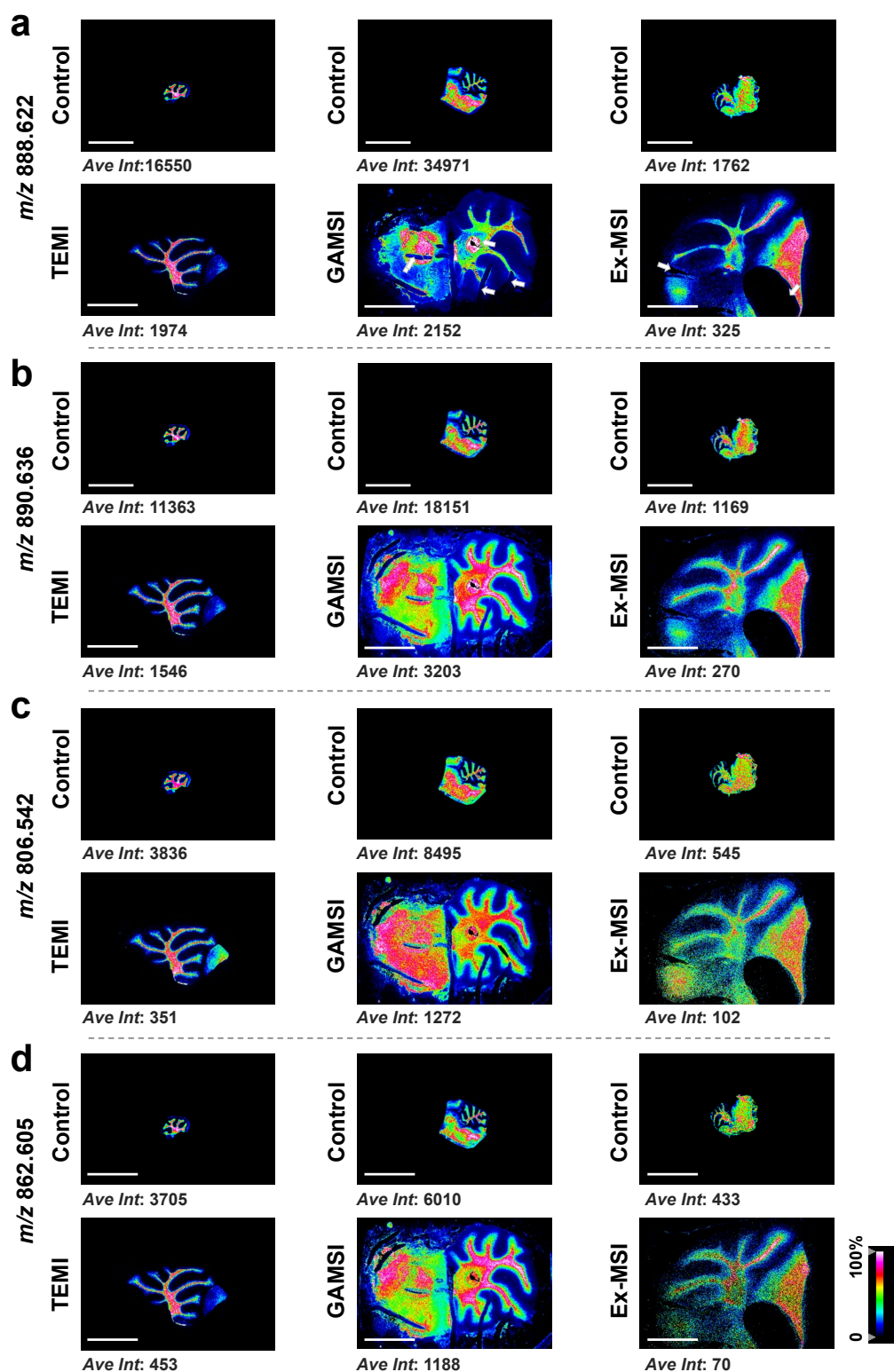




**Supplementary Figure 3 (continued). Lipidomics results\*** l. Triacylglycerol TG-type lipids. m. Sphingomyelin (SM)-type lipids. \*: The detailed information of detected lipids and significantly changed lipids among Control, Ex, and ExP groups is shown in **Table S4**.

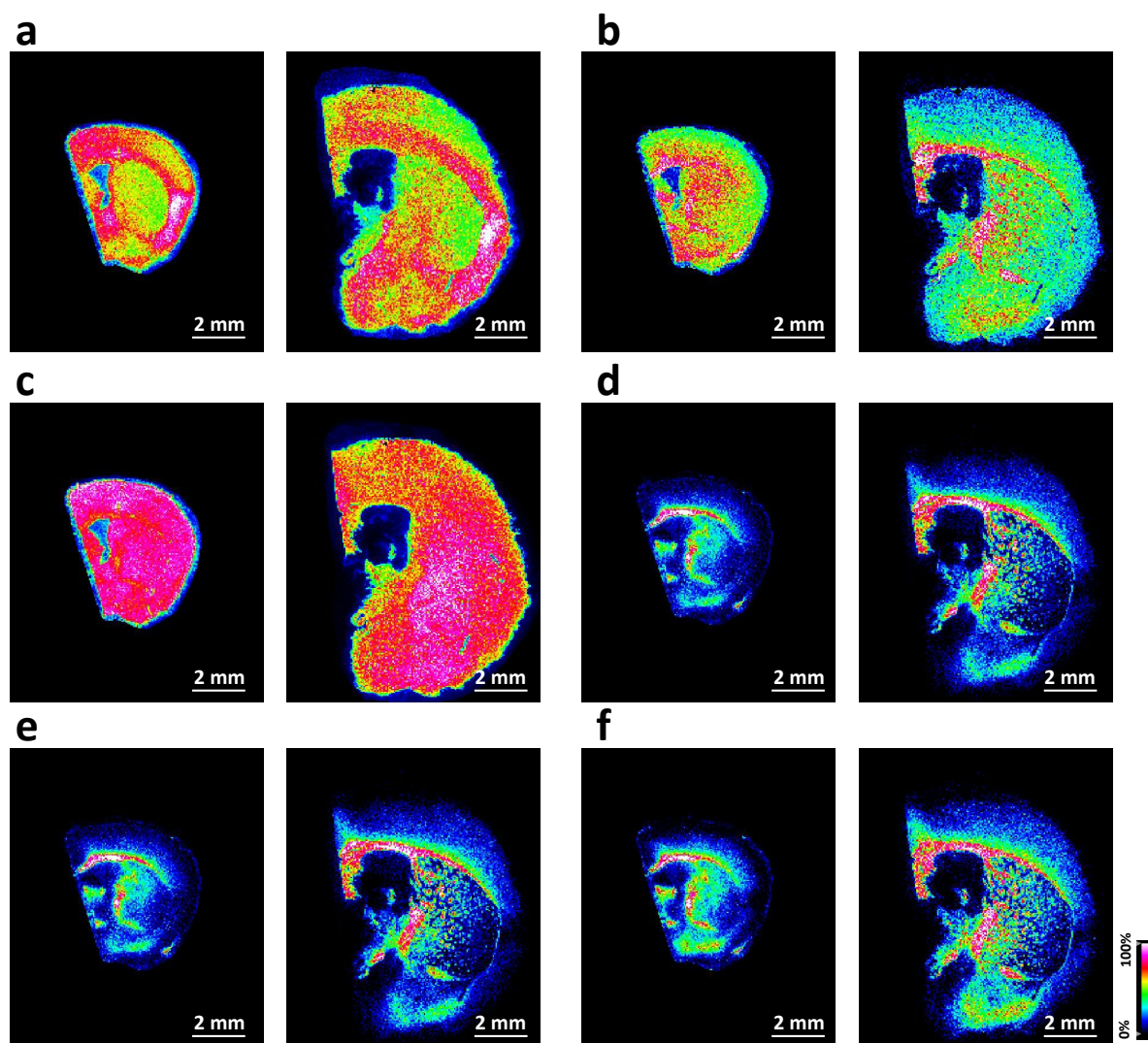


**Supplementary Figure 4. Representative MS spectra of LC-MS lipidomics among Control, Ex, and ExP groups.** a. The average MS spectrum of the sample control-1. b. The average MS spectrum of the sample Ex-1. c. The average MS spectrum of the sample ExP-1. The average MS spectra was generated by selecting the entire time region of the TIC using Qual Browser (Thermo Scientific).

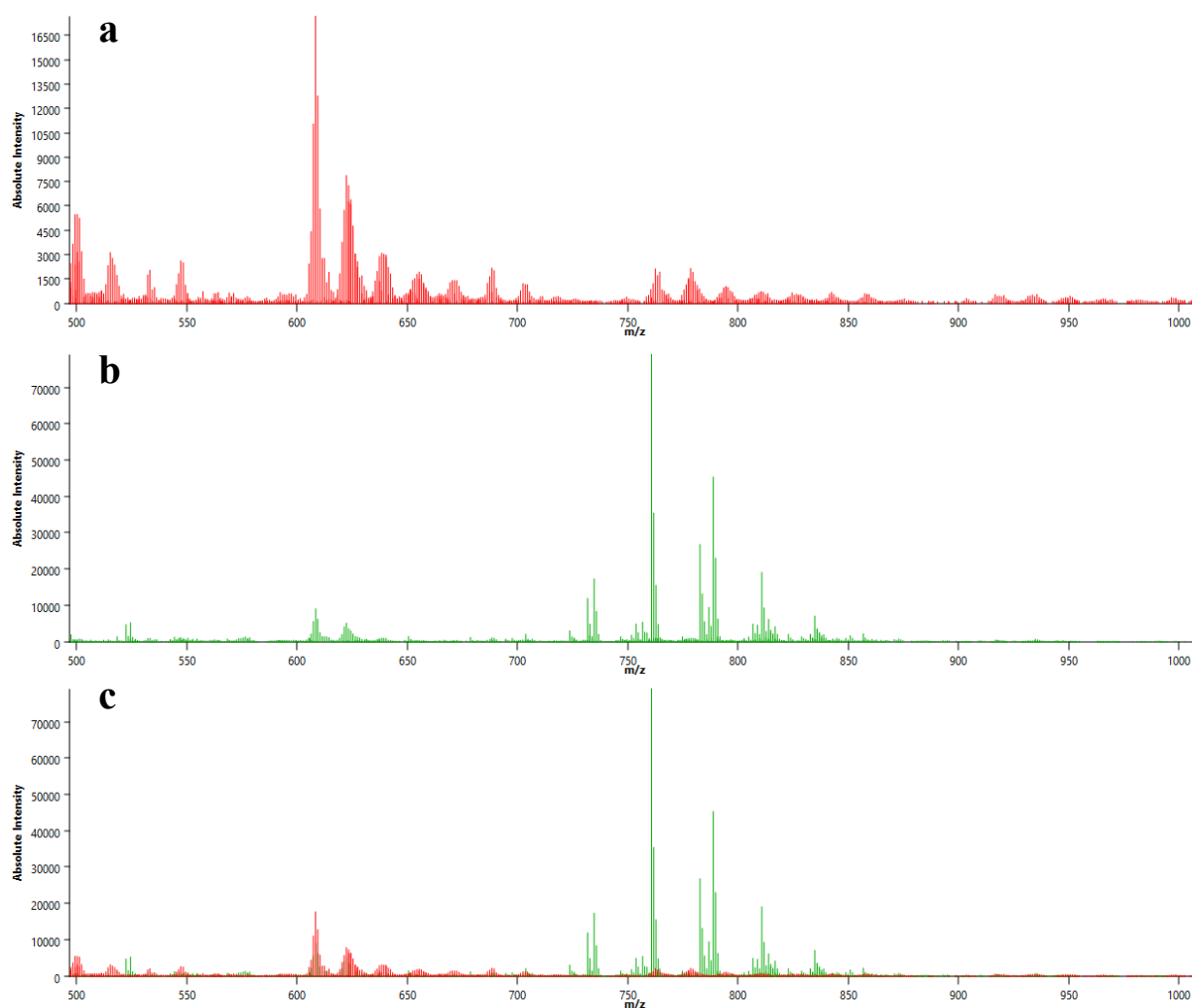


**Supplementary Figure 5. Ion images of dominant lipid species obtained from the mouse cerebellum using different methodologies including TEMI (left), GAMSI (middle), and Ex-MSI (right). The ion images of a. SHexCer (42:2;O<sub>2</sub>) ([M-H]<sup>-</sup>,  $m/z$  888.622). b. PE (46:6;O) ([M-H]<sup>-</sup>,  $m/z$  890.636). c. PE (40:6;O) ([M-H]<sup>-</sup>,  $m/z$  806.542). d. SHexCer (40:1;O<sub>2</sub>) ([M-H]<sup>-</sup>,  $m/z$  862.605). The scale bar is 6 mm, and all the experiments were carried out under 50  $\mu$ m laser beam raster scanning. The “Ave Int” at the bottom of each ion image denotes the average signal intensity of the corresponding ion in the tissue region.**

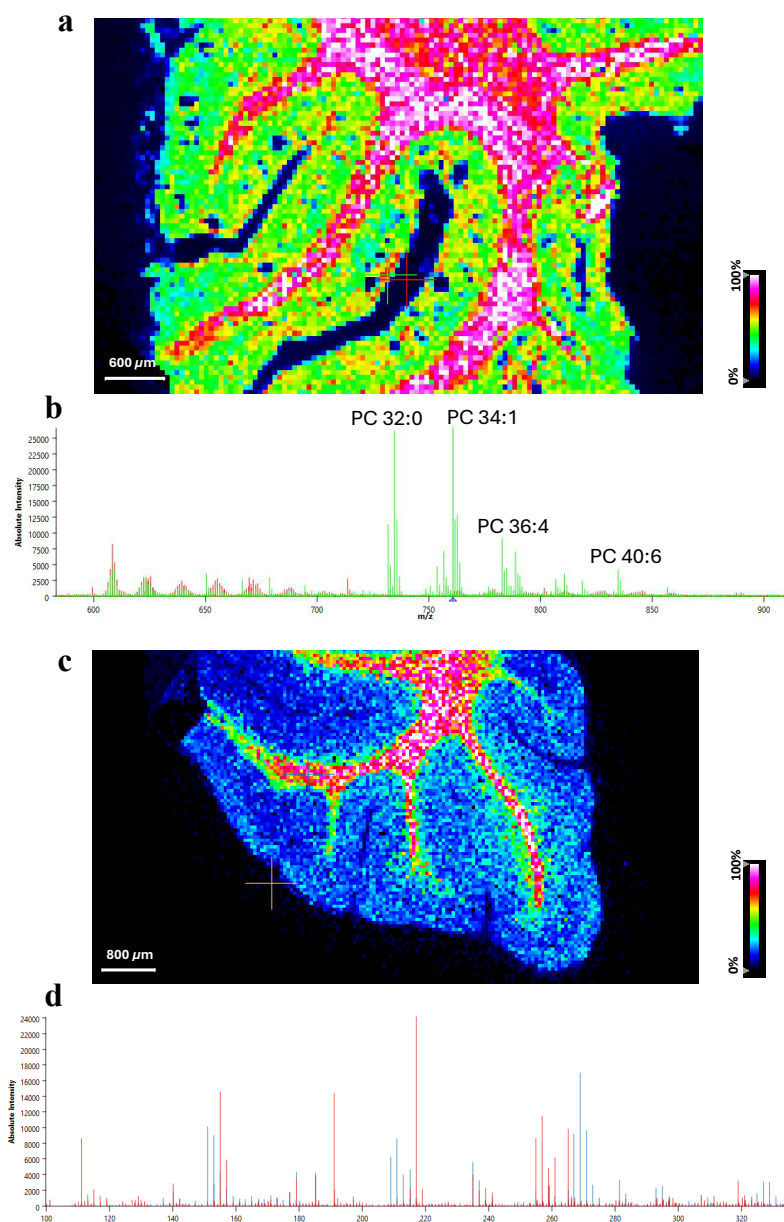




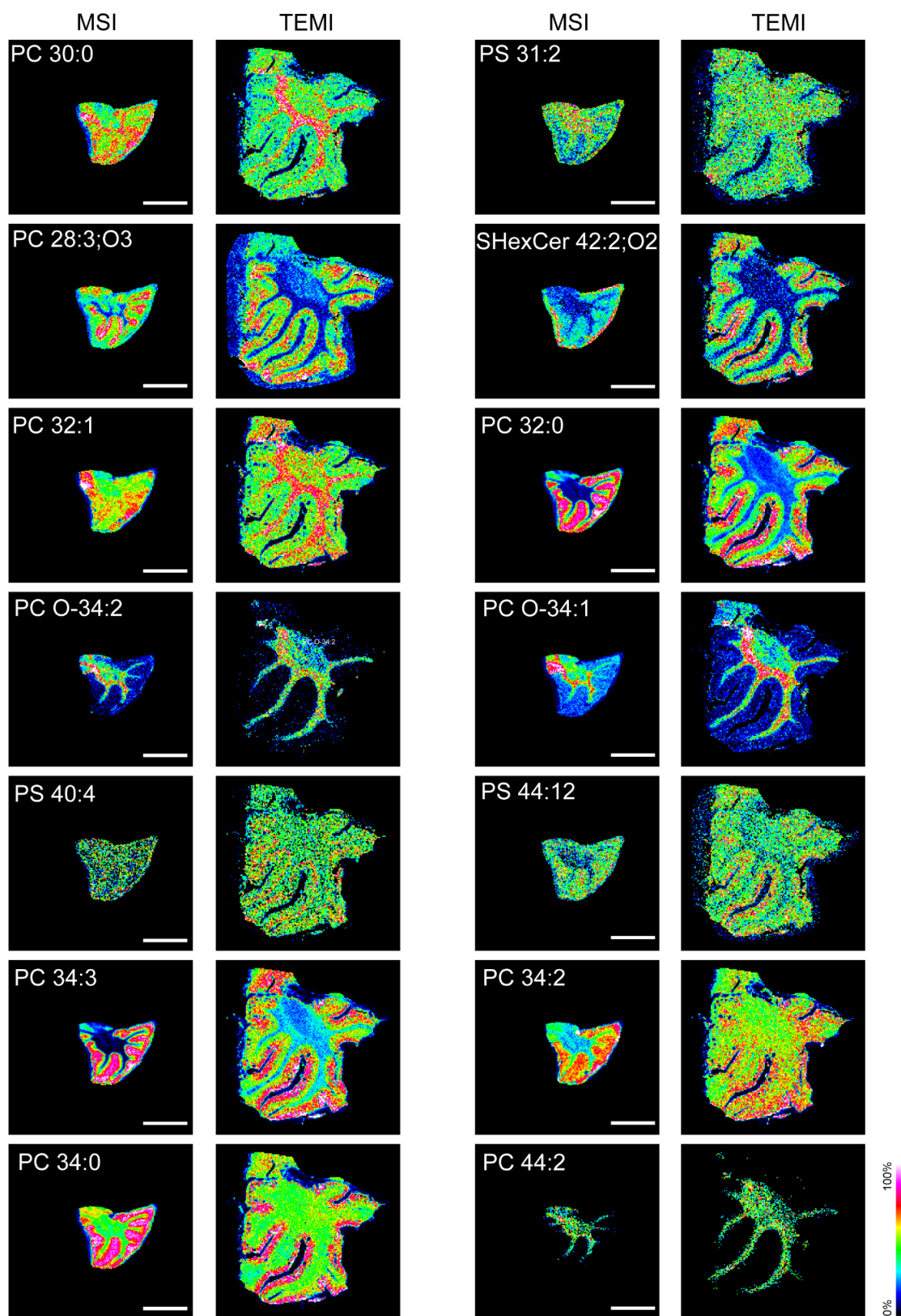
**Supplementary Figure 6. MS imaging of a half mouse brain coronal tissue expanded with a single round of gel embedding.** MS images of representative lipid species detected from control (left panel) and tissue expansion treated coronal tissue section (right panel). a. MS image of PC (32:1) ( $[M + H]^+$ ,  $m/z$  732.552). b. MS image of PC (34:5) ( $[M + H]^+$ ,  $m/z$  752.532). c. MS image of PC (34:1) ( $[M + H]^+$ ,  $m/z$  760.593). d. MS image of PC(O-40:5) ( $[M + H]^+$ ,  $m/z$  822.645). e. MS image of PE (42:0) ( $[M + H]^+$ ,  $m/z$  832.672). f. MS image of PC (O-42:6) ( $[M + H]^+$ ,  $m/z$  848.660).



**Supplementary Figure 7. Representative mass spectra from surrounding gelatin region and  $\sim 3.5$ -fold expanded tissue region under positive mode with DAN matrix.** a. The gelatin region, in which the dominant peaks observed are from the DAN matrix. b.  $\sim 3.5$ -fold expanded tissue region, in which the dominant peaks observed are lipids species from the tissue; c. overlapped spectra from the gelatin region (red) and tissue region (green).

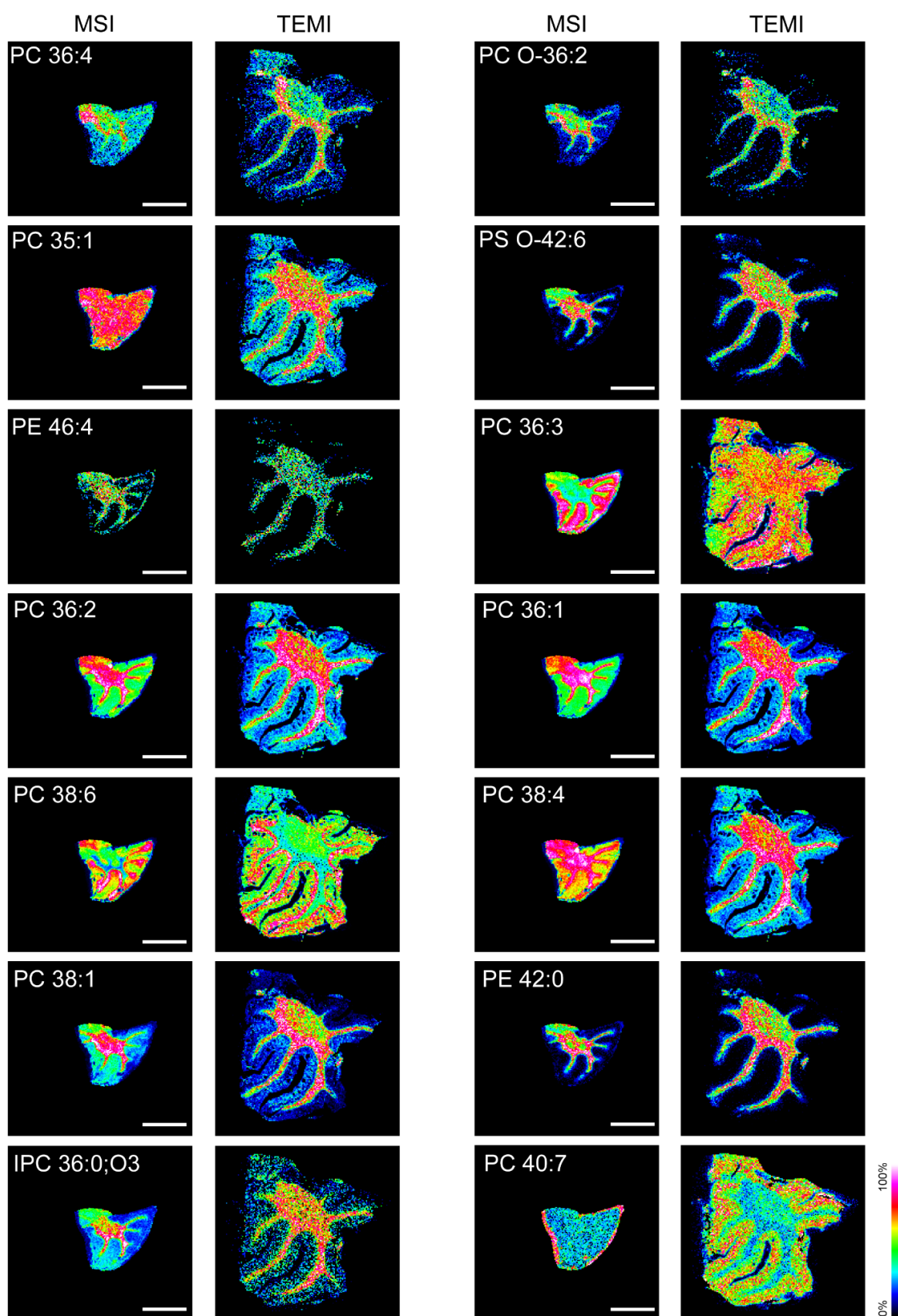


**Supplementary Figure 8. TEMI of mouse cerebellum tissue.** a. The MSI image of PC (34:1) ( $[M + H]^+$ ,  $m/z$  760.5901) from a ~2.5-fold linear expanded mouse cerebellum using DAN matrix under positive ionization mode, the scale bar is 600  $\mu\text{m}$ . b. The representative single mass spectrum from the tissue regions (in green color) and its adjacent blank hydrogel area (in red color), corresponding single spectrum from the pixels highlighted on the above MS image. c. The MSI image of FA (18:1) ( $[M - H]^-$ ,  $m/z$  281.2498) from a ~2.5-fold linear expanded mouse cerebellum using NEDC matrix under negative ionization mode, the scale bar is 800  $\mu\text{m}$ . d. The representative single mass spectrum from the tissue regions (in blue color) and its adjacent blank hydrogel area (in red color), corresponding single spectrum from the pixels highlighted on the above MS image. It is noted that high background signals from the MALDI matrix were observed during MS imaging of the tissue in the low mass range; however, most target small biomolecules were still resolvable due to the high mass resolution. Target lipid and small metabolite signals were solely observed from the tissue area whereas no signal of these lipids or metabolites was observed in the adjacent blank hydrogel area, indicating the delocalization of these biomolecules was minimal. MALDI-MSI performed with a 50  $\mu\text{m}$  laser beam raster scanning.

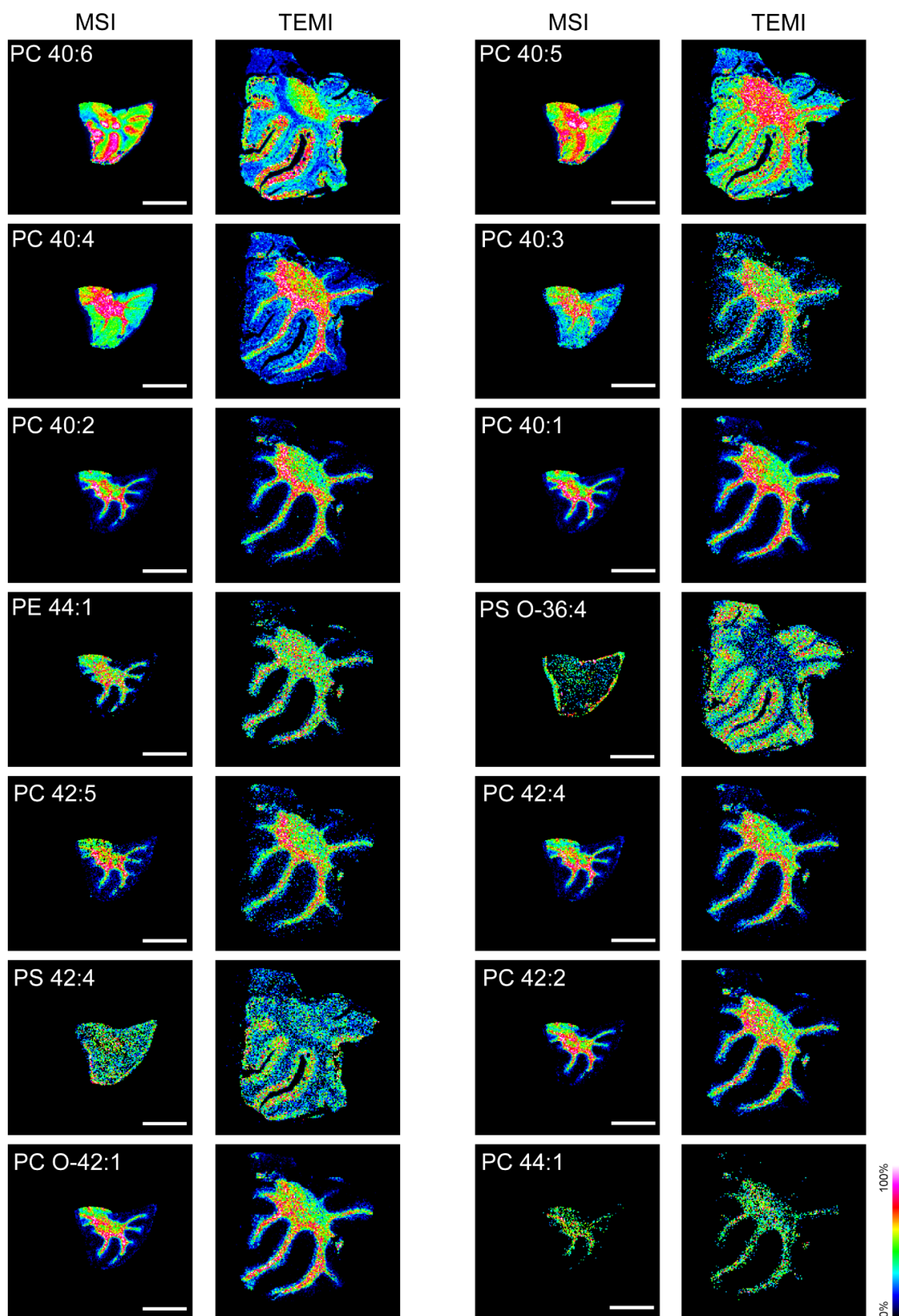


**Supplementary Figure 9\***. MS imaging of ~2.5-fold linear expanded mouse cerebellum tissue under **positive mode**. MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. \*: Detailed information in **Supplementary Table 1**. The scale bar is 2 mm.

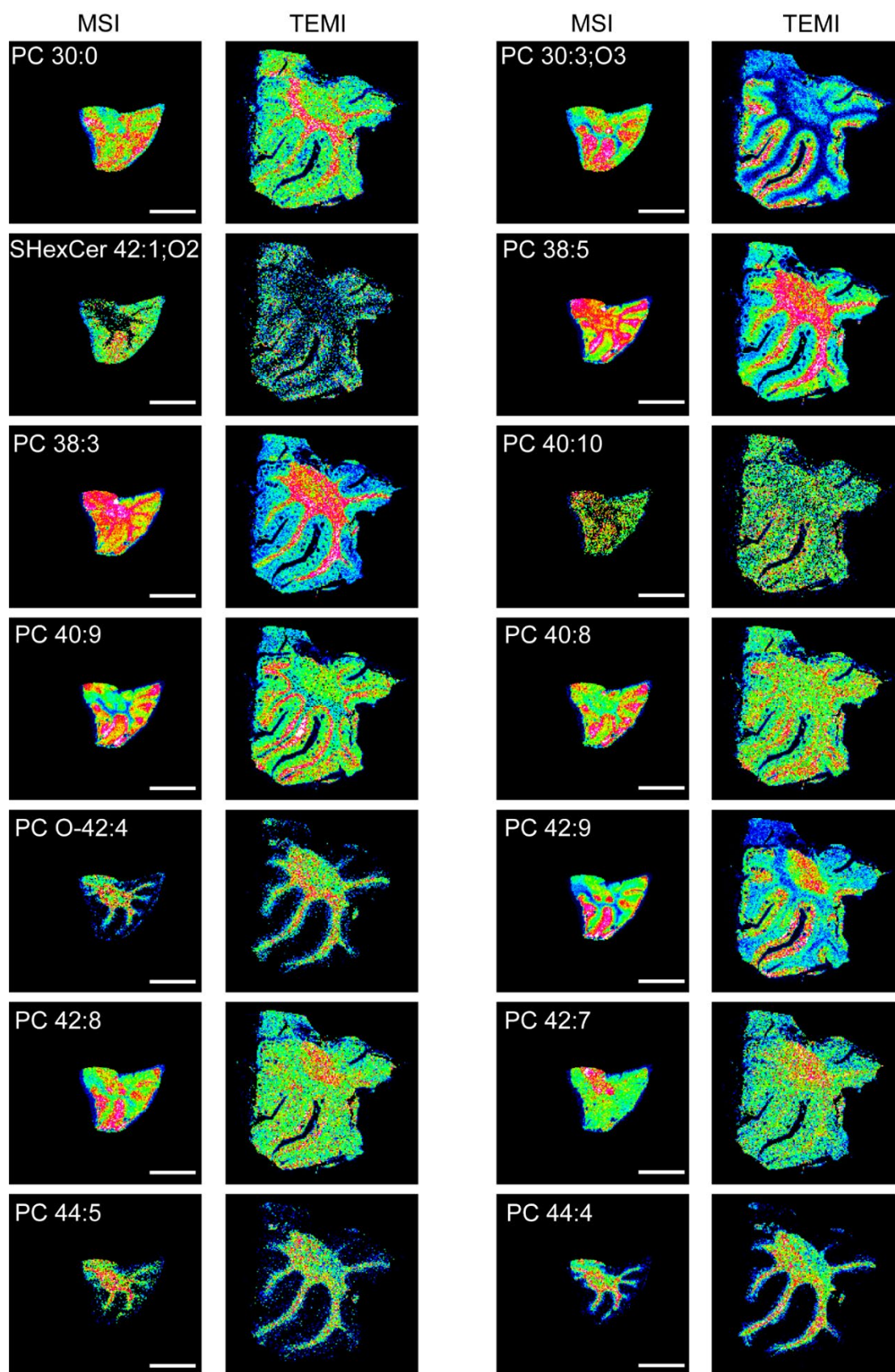




**Supplementary Figure 9\***. (continued) MS imaging of ~2.5-fold linear expanded mouse cerebellum tissue under positive mode. MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. \*: Detailed information in **Supplementary Table 1**. The scale bar is 2 mm.

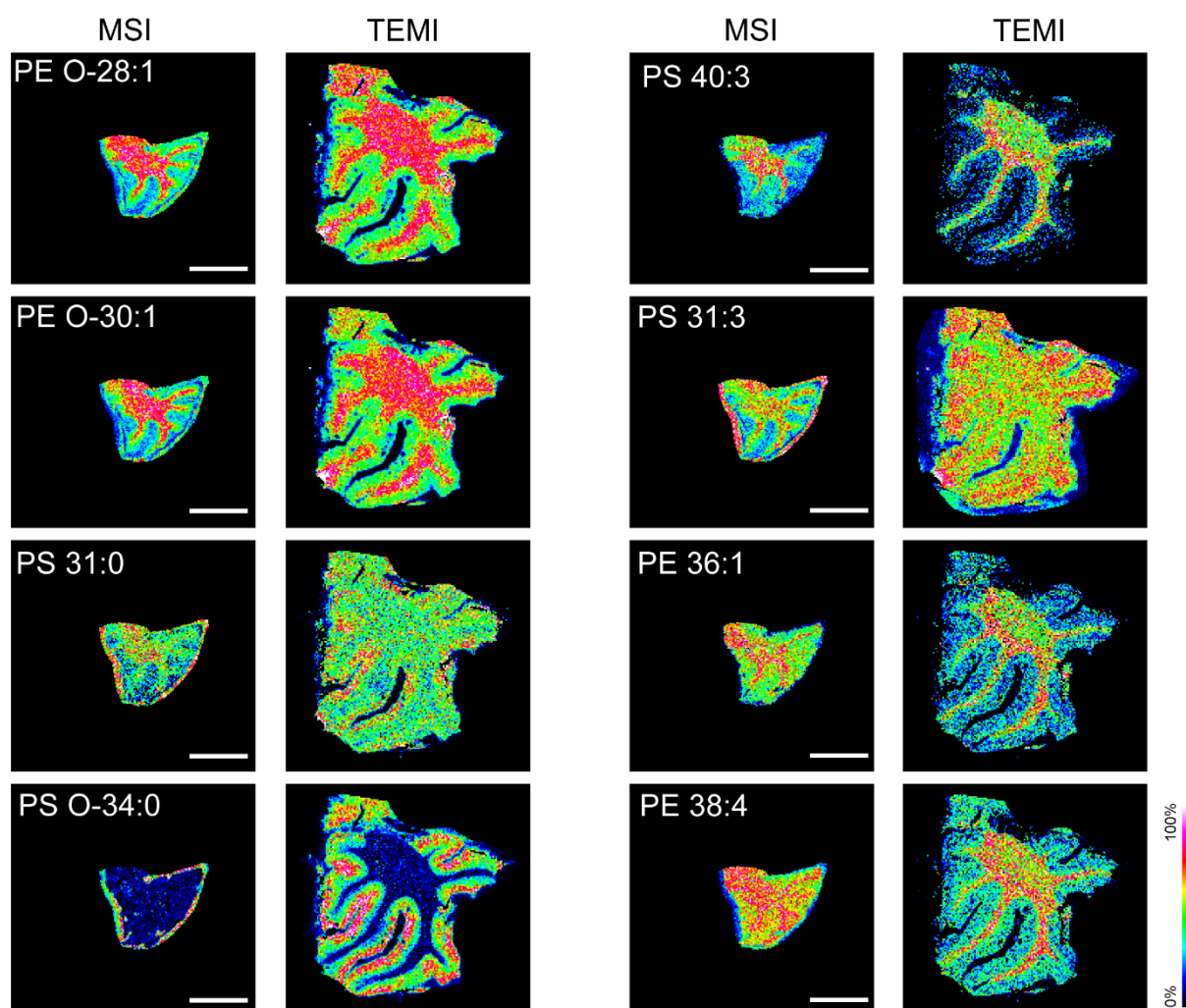


**Supplementary Figure 9\*.** (continued) MS imaging of ~2.5-fold linear expanded mouse cerebellum tissue under positive mode. MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. \*: Detailed information in **Supplementary Table 1**. The scale bar is 2 mm.



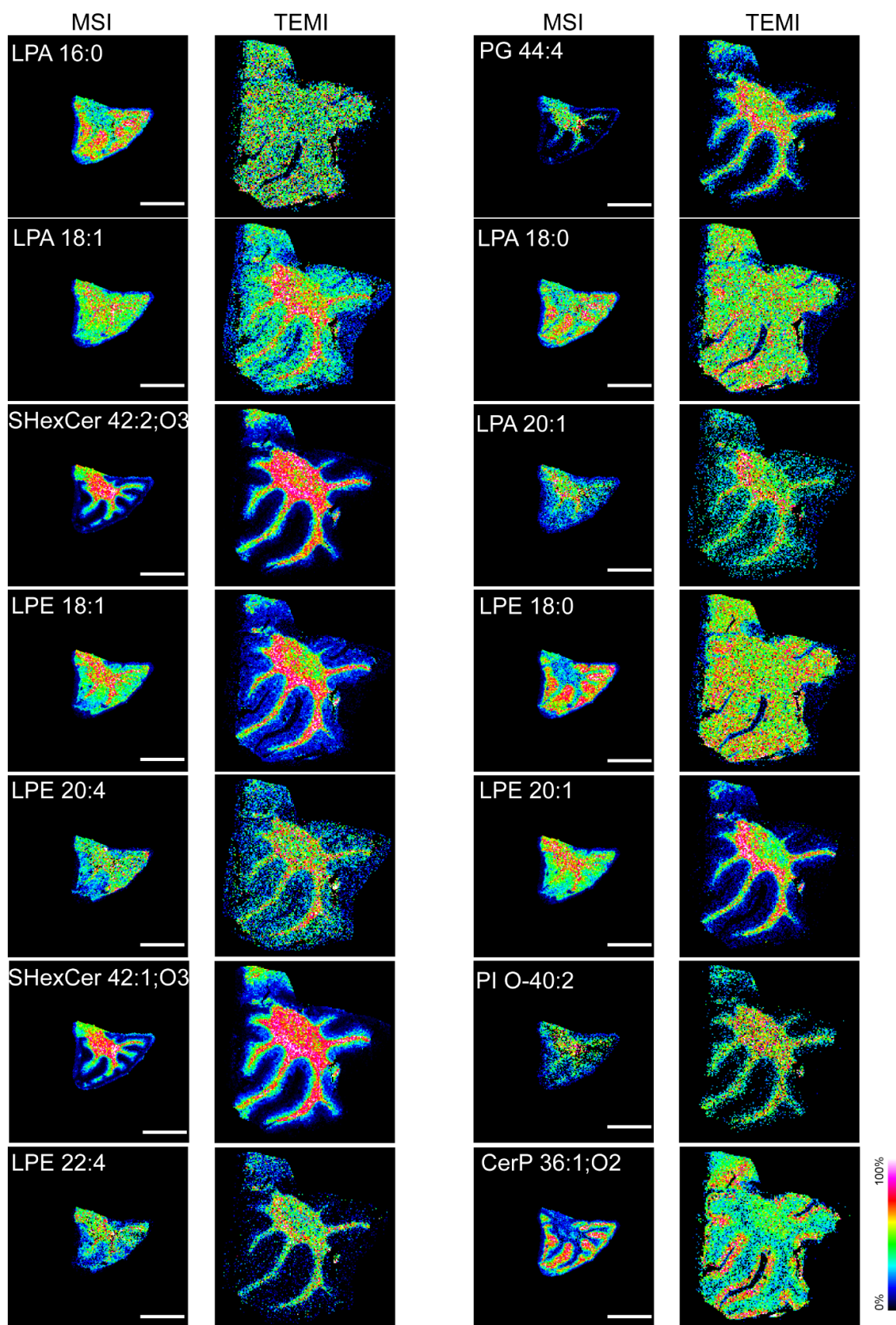
**Supplementary Figure 9\*.** (continued) MS imaging of ~2.5-fold linear expanded mouse cerebellum tissue under positive mode. MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. \*: Detailed information in **Supplementary Table 1**. The scale bar is 2 mm.



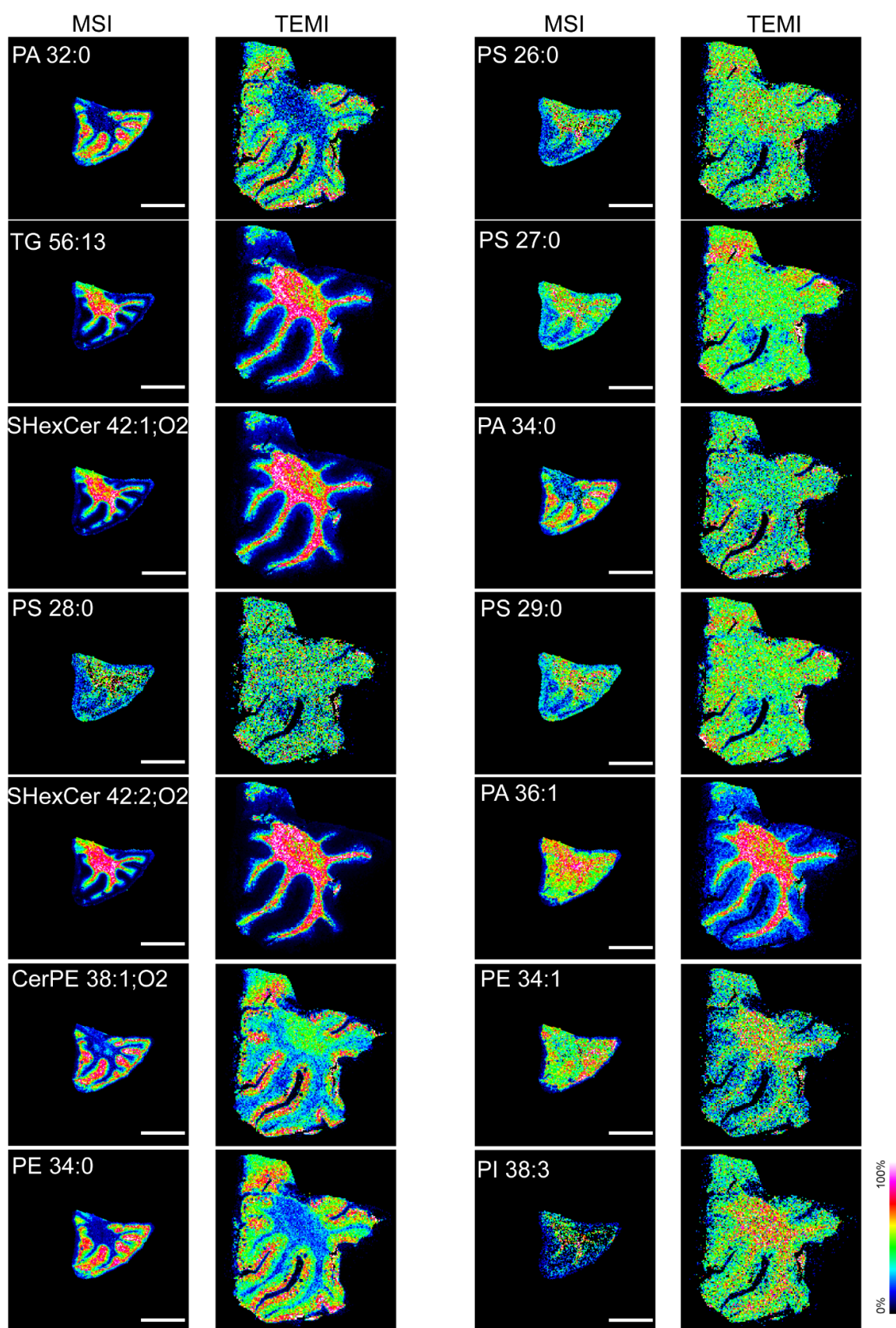


**Supplementary Figure 9\*.** (continued) MS imaging of ~2.5-fold linear expanded mouse cerebellum tissue under positive mode. MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. \*: Detailed information in **Supplementary Table 1**. The scale bar is 2 mm.

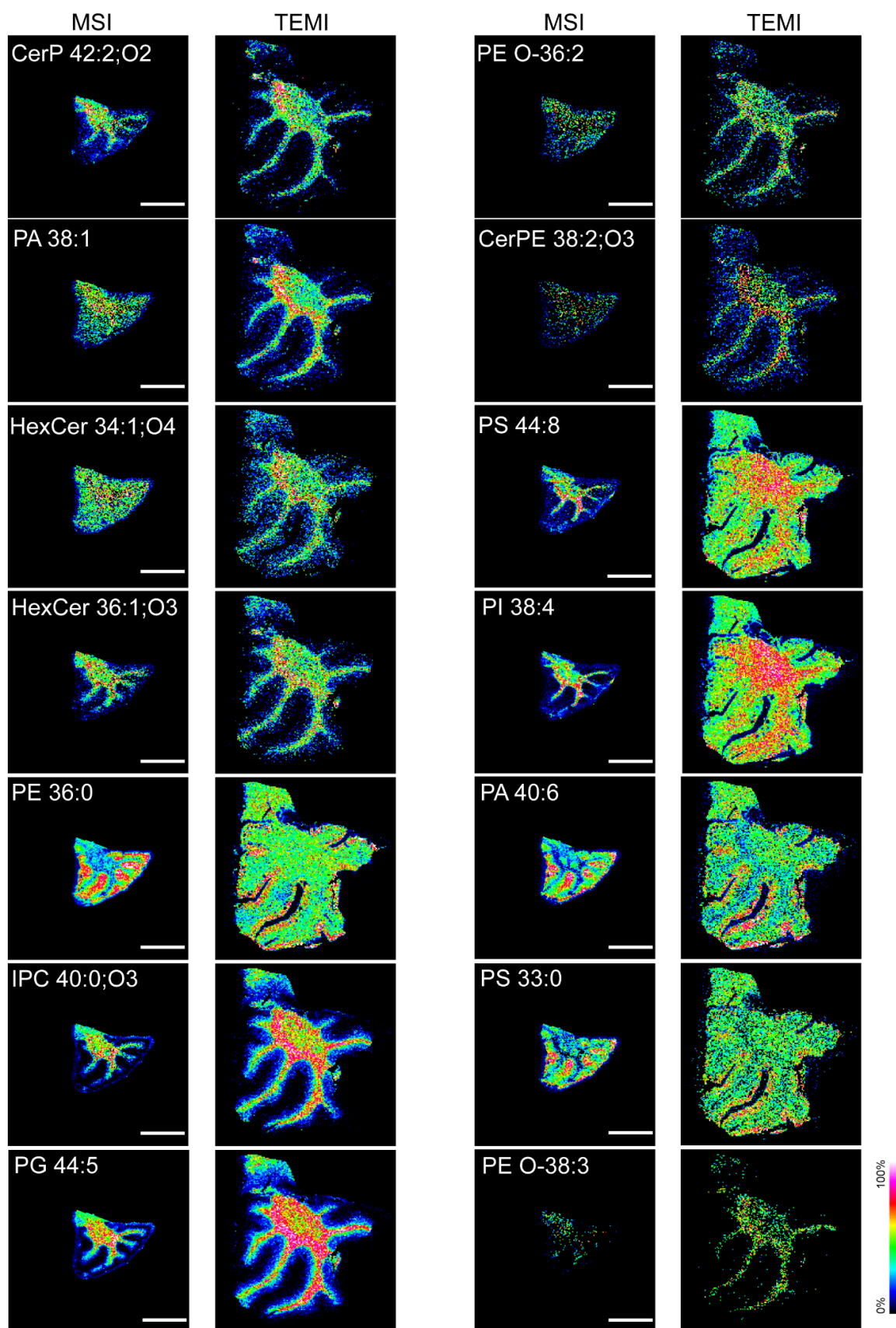




**Supplementary Figure 10\***. MS imaging of ~2.5-fold linear expanded mouse cerebellum tissue under **negative mode**. MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. \*: Detailed information in **Supplementary Table 2**. The scale bar is 2 mm.

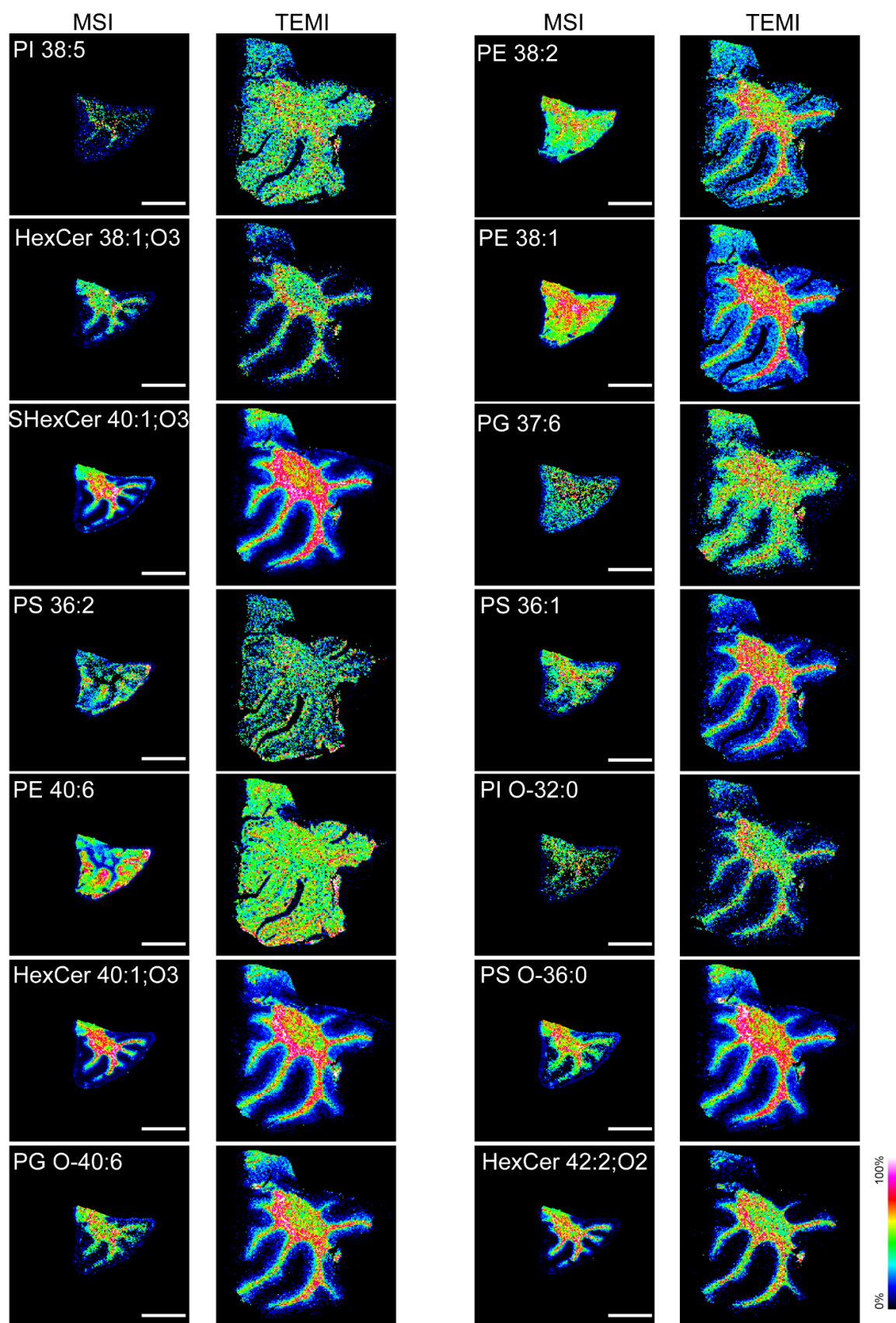


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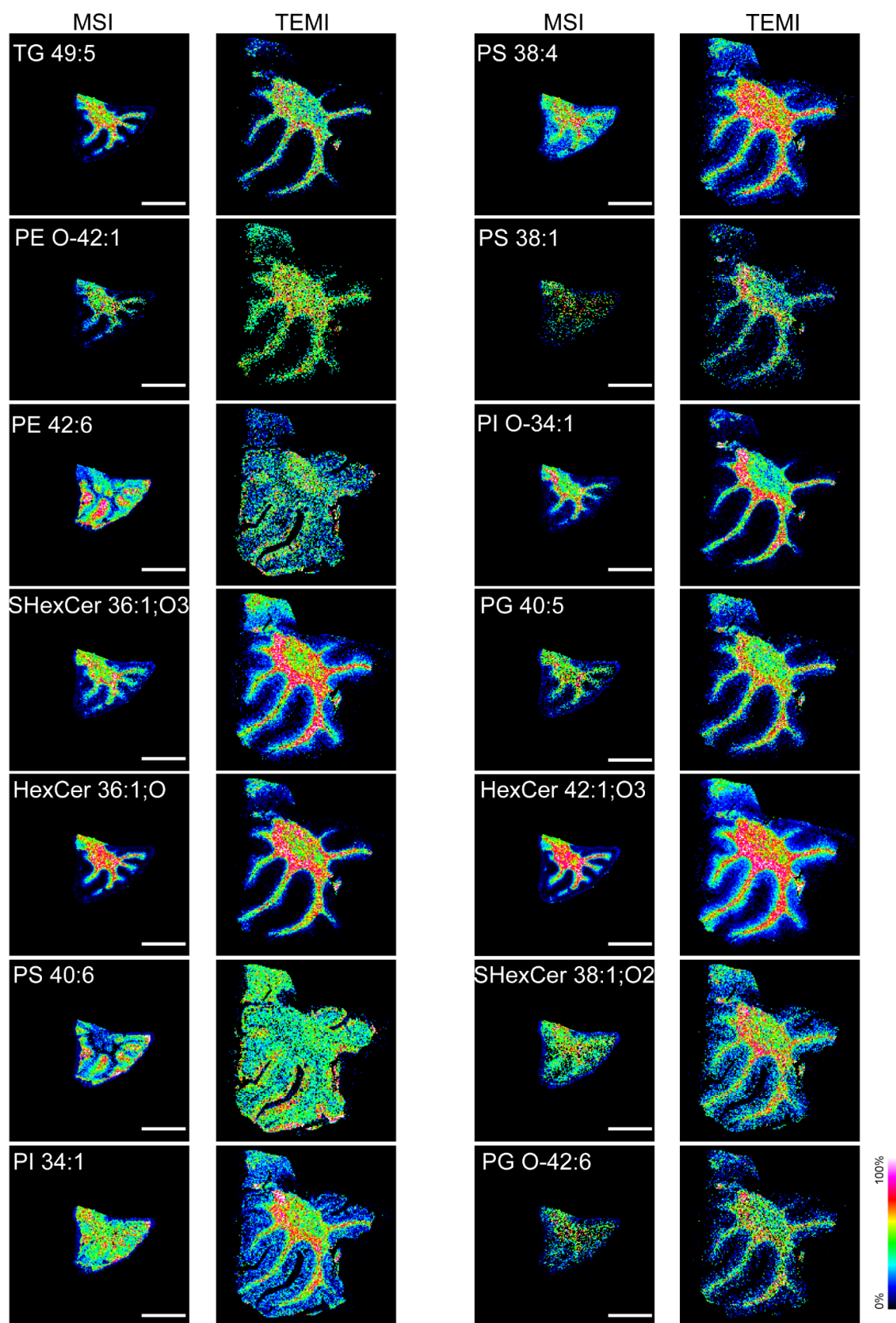


**Supplementary Figure 10<sup>\*</sup>. MS imaging of ~2.5-fold linear expanded mouse cerebellum tissue under negative mode.** MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. \*: Detailed information in **Supplementary Table 2**. The scale bar is 2 mm.

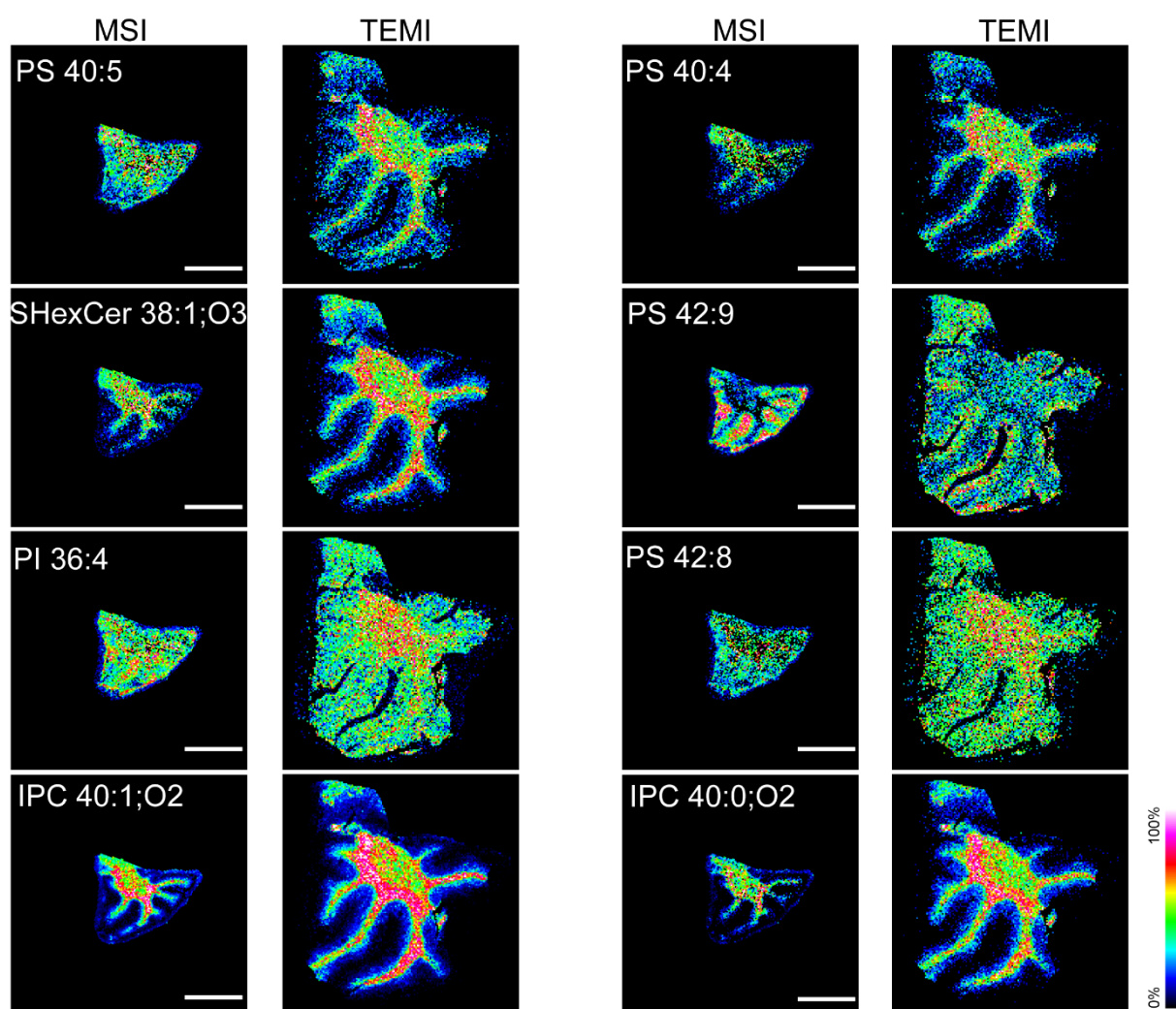




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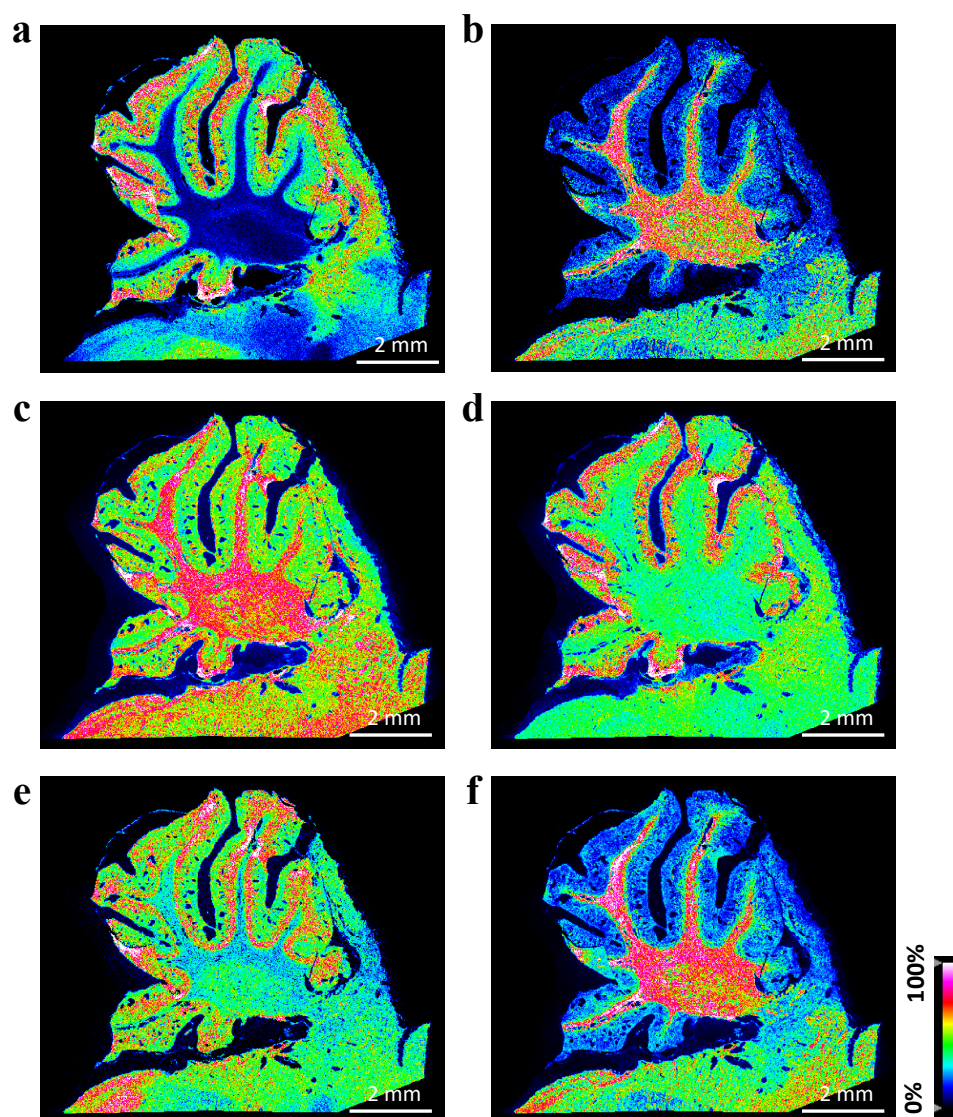


**Supplementary Figure 10\***. MS imaging of ~2.5-fold linear expanded mouse cerebellum tissue under **negative mode**. MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. \*: Detailed information in **Supplementary Table 2**. The scale bar is 2 mm.

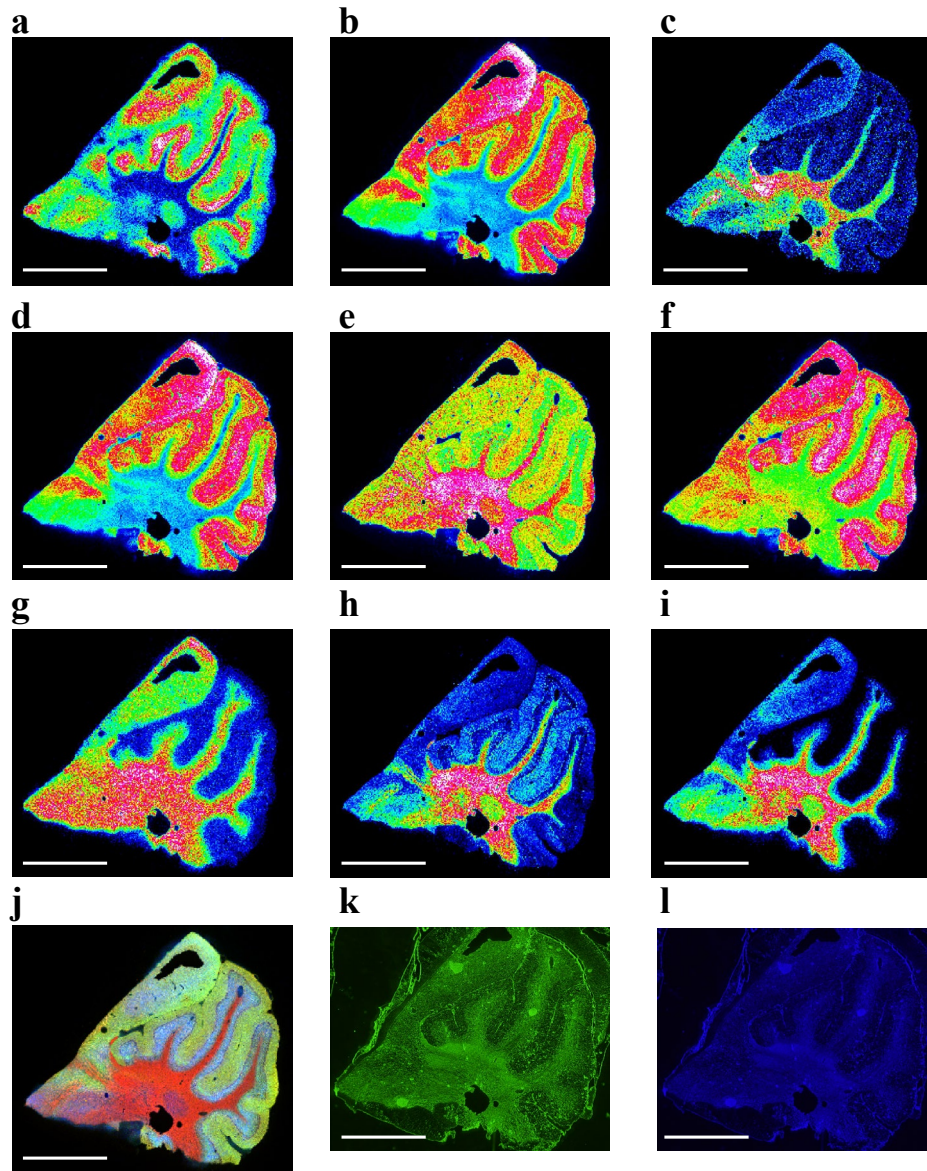
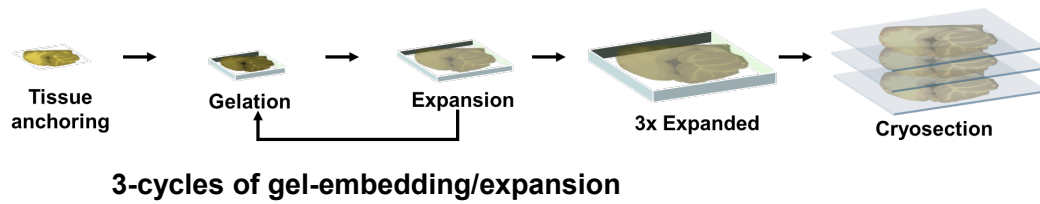


**Supplementary Figure 10\***. MS imaging of ~2.5-fold linear expanded mouse cerebellum tissue under **negative mode**. MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. \*: Detailed information in **Supplementary Table 2**. The scale bar is 2 mm.



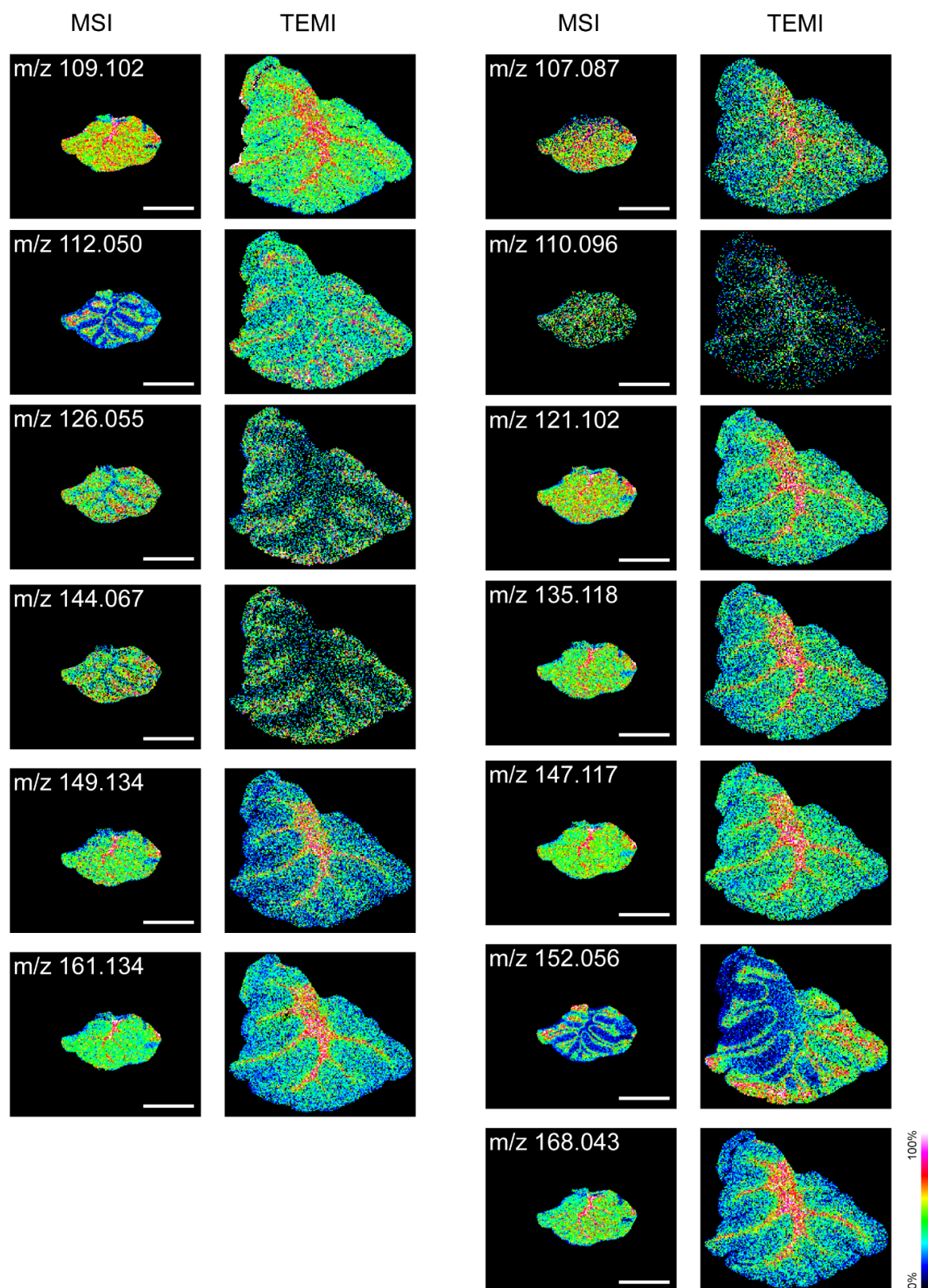


**Supplementary Figure 11. Representative TEMI images of a ~2.5 linearly expanded mouse cerebellum tissue through a 20  $\mu$ m laser beam raster scanning under positive mode. a. PC (32:0) ( $[M + H]^+$ ,  $m/z$  734.5694). b. PC (34:5) ( $[M + H]^+$ ,  $m/z$  752.529). c. PC (34:1) ( $[M + H]^+$ ,  $m/z$  760.586). d. PC (34:0) ( $[M + H]^+$ ,  $m/z$  762.597). e. PC (38:6) ( $[M + H]^+$ ,  $m/z$  806.565). f. PC (38:4) ( $[M + H]^+$ ,  $m/z$  810.596). The MSI images obtained with mass error tolerance of 10 ppm.**

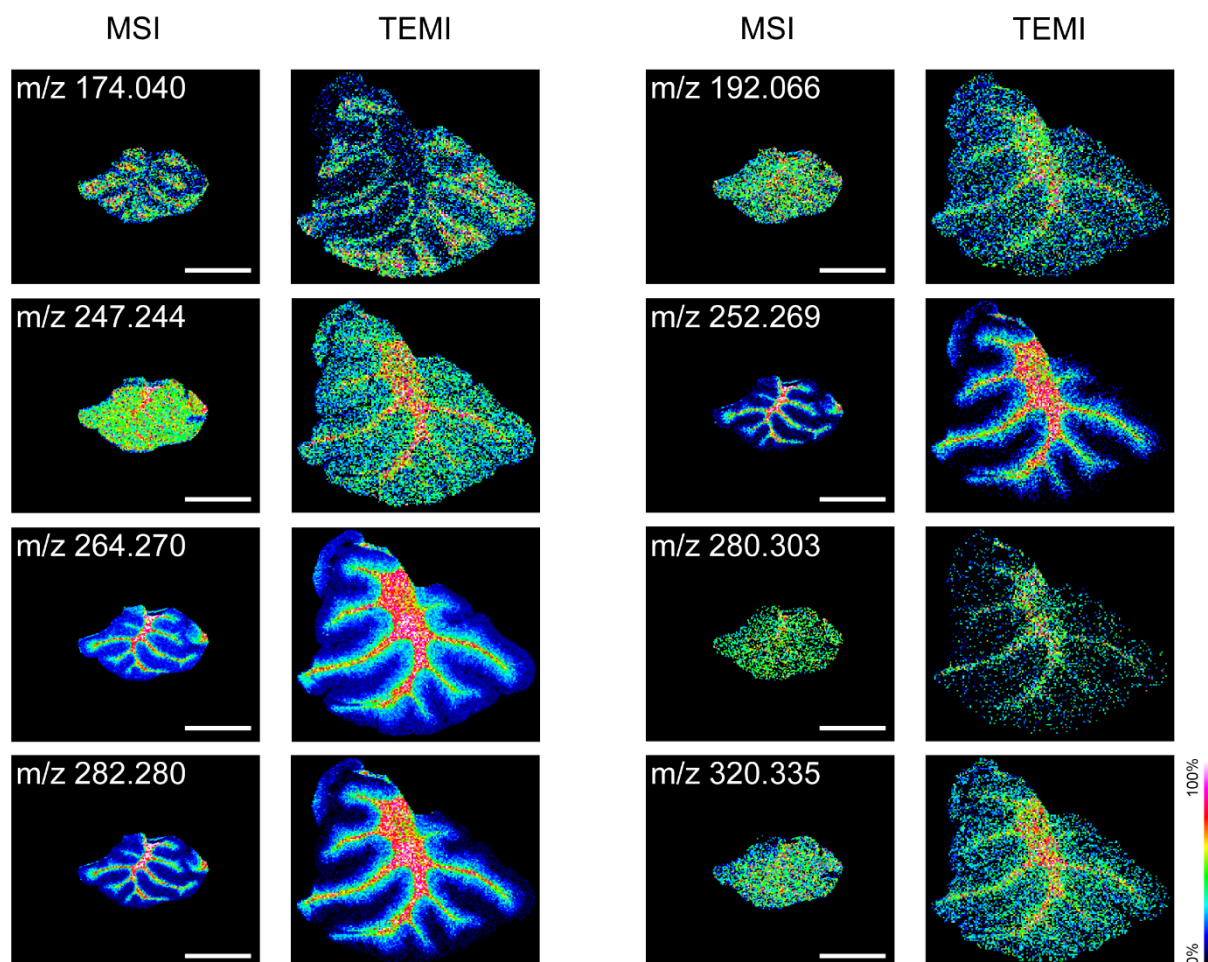


**Supplementary Figure 12. TEMI results from mouse cerebellum tissue with 3 times gel-embedding and expansion treatment.** On the top is the workflow of the 3 times gel-embedding-expansion with the cerebellum tissue; on the bottom are the TEMI and fluorescent images obtained from the ~3.5-fold-expanded cerebellum tissue section, including a. PC 28:3;O3 ( $m/z$  720.432). b. PC 32:0 ( $m/z$  734.567). c. PC O-34:1 ( $m/z$  746.602). d. PC 34:3 ( $m/z$  756.548). e. PC 34:1 ( $m/z$  760.584). f. PC 34:0 ( $m/z$  762.595). g. PE 38:7;O ( $m/z$  778.509). h. PC 38:1 ( $m/z$  816.646). i. PC O-40:5 ( $m/z$  822.638). j. Overlay of TEMI images of PC 28:3;O3 ( $m/z$  720.432) (blue), PC 32:0 ( $m/z$  734.567) (green), and PC 34:1 ( $m/z$  760.584) (red). k. Alexa Fluor 488 staining fluorescent image of the expanded cerebellum tissue. l. DAPI staining fluorescent image of the expanded cerebellum tissue. The MSI experiments were carried out using a 50  $\mu$ m laser raster scanning and the MSI images obtained with mass error tolerance of 10 ppm. The scale bar is 4 mm.

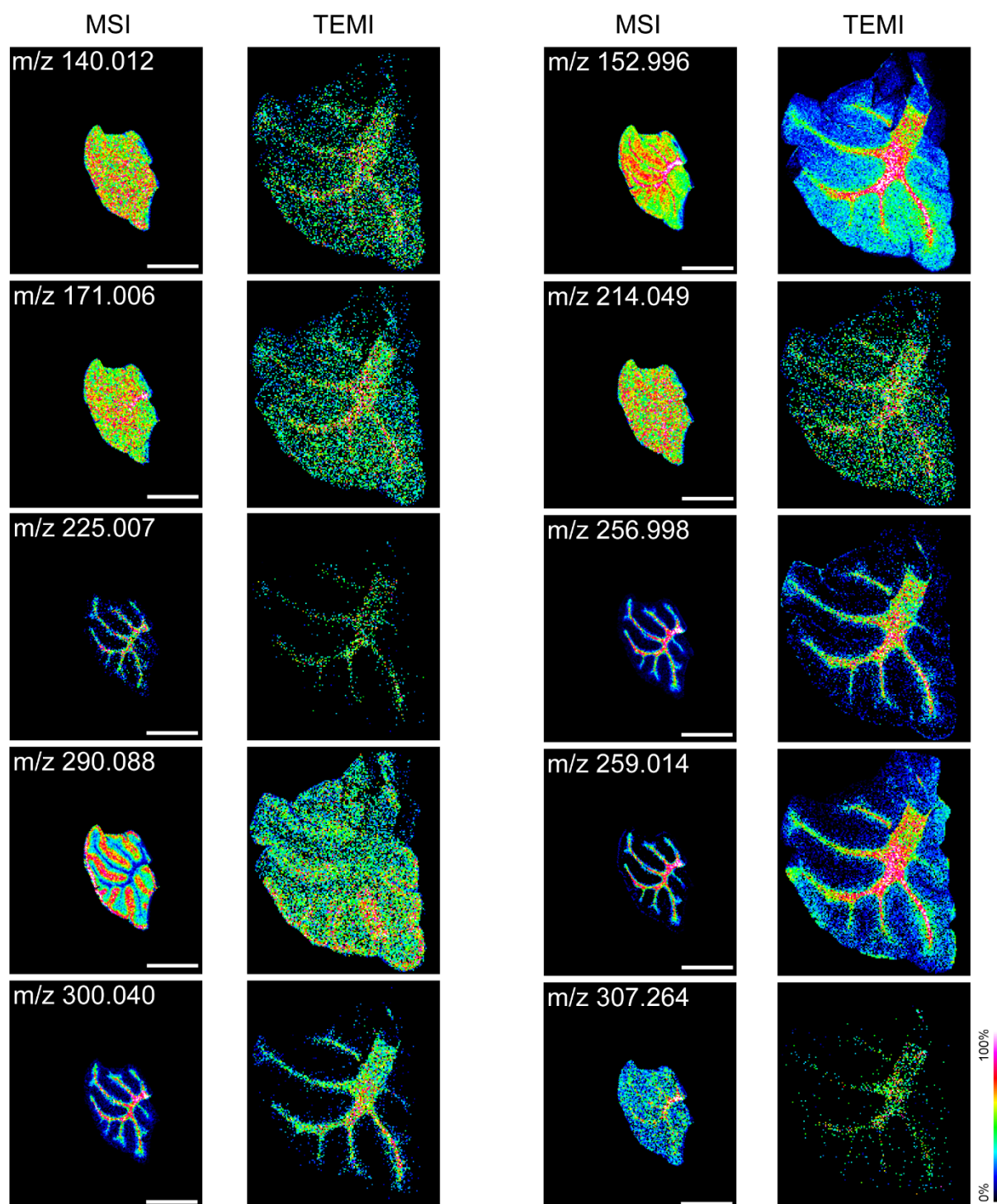




**Supplementary Figure 13. Representative mass spectrometry images of the other unknown small molecules observed from the ~2.5-fold linearly expanded mouse cerebellum under positive mode, the control (left panel, MSI) and tissue expansion treated (right panel, TEMI),  $m/z$   $[M+H]^+$ . The MSI experiments were carried out using a 50  $\mu$ m laser raster scanning and the MSI images obtained with mass error tolerance of 10 ppm. The scale bar is 2 mm.**

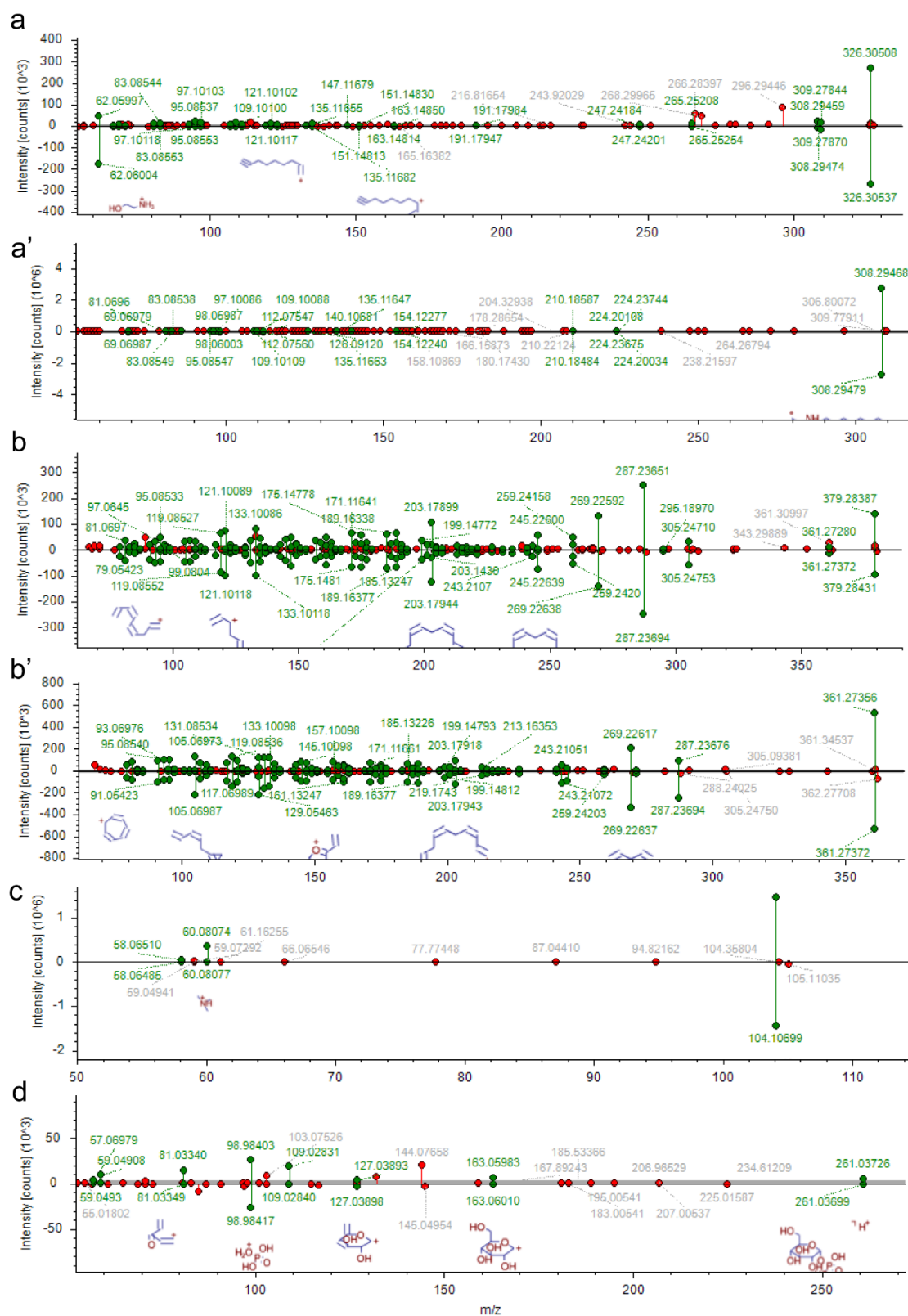


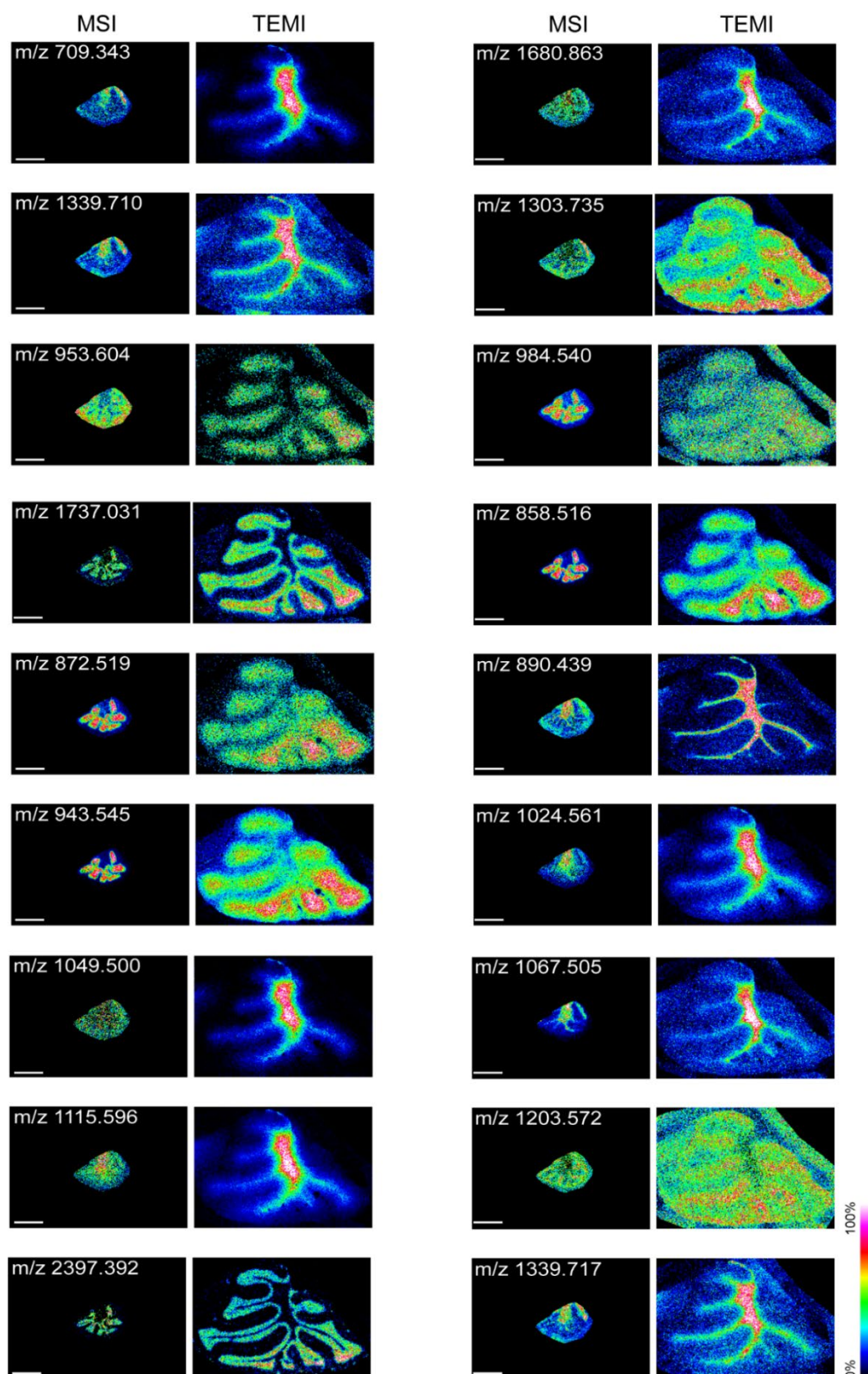
**Supplementary Figure 13. (Continued) Representative of other unknown small molecules observed from the ~2.5-fold linearly expanded mouse cerebellum under positive mode, the control (left panel, MSI) and tissue expansion treated (right panel, TEMI). The MSI experiments were carried out using a 50  $\mu$ m laser raster scanning and the MSI images obtained with mass error tolerance of 10 ppm. The scale bar is 2 mm.**



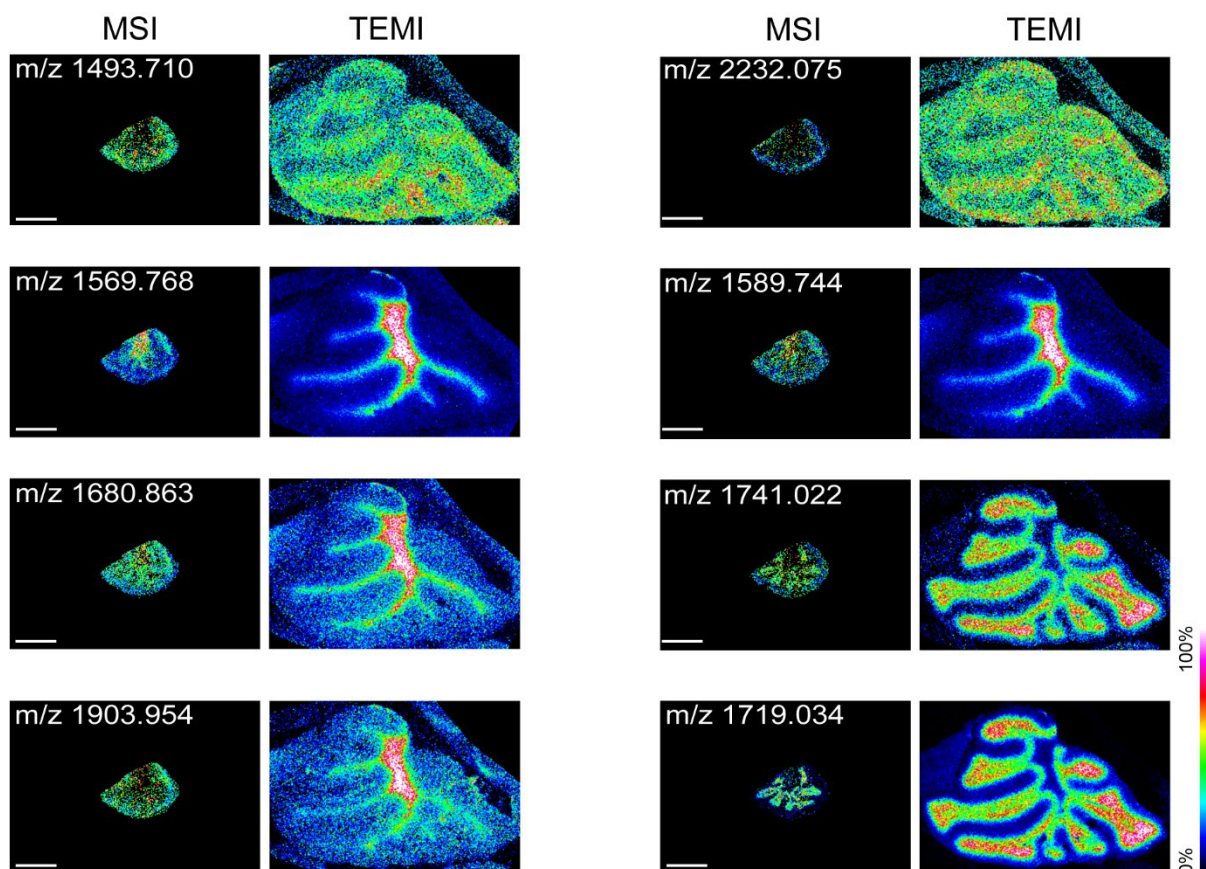
**Supplementary Figure 14. Representative mass spectrometry images of other unknown small molecules observed from the ~2.5-fold linearly expanded mouse cerebellum under negative mode, the control (left panel, MSI) and tissue expansion treated (right panel, TEMI). The MSI experiments were carried out using a 50  $\mu$ m laser raster scanning and the MSI images obtained with mass error tolerance of 10 ppm. The scale bar is 2 mm.**



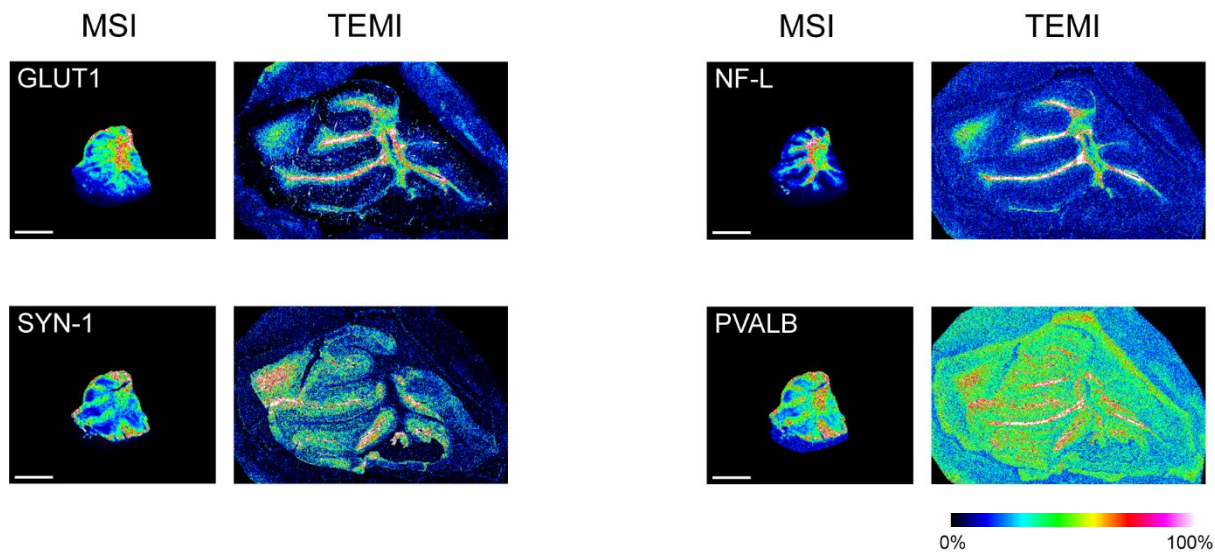




**Supplementary Figure 16. Representative MS images of on-tissue digested peptides of ~3.5-fold linearly expanded mouse cerebellum tissue under positive mode.** MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. The  $m/z$  corresponding peptides information is in **Supplementary Table 4**. The scale bar is 2 mm.

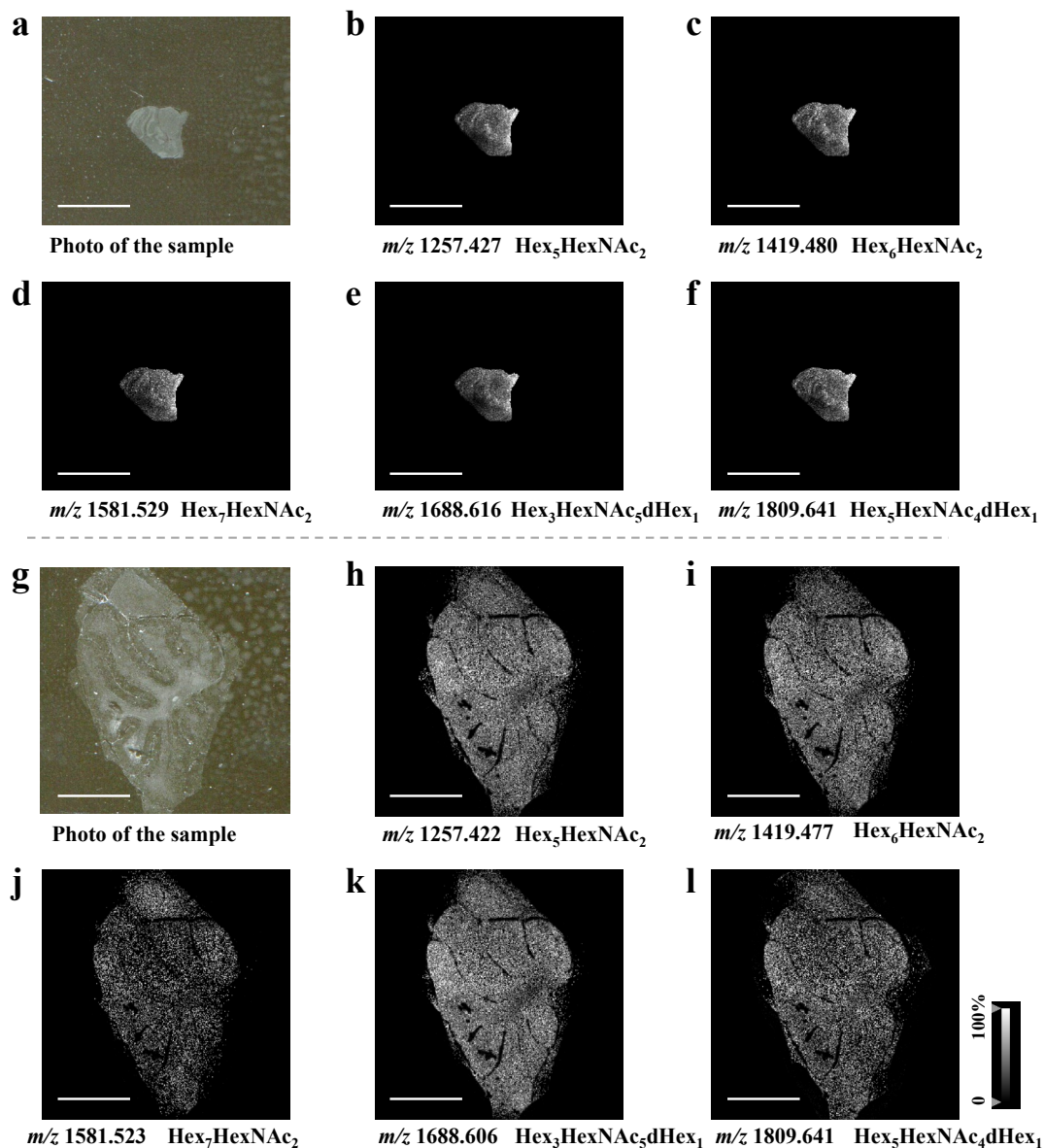


**Supplementary Figure 16. (Continued) Representative MS images of on-tissue digested peptides of ~3.5-fold linearly expanded mouse cerebellum tissue under positive mode.** MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. The *m/z* corresponding peptides information is in **Supplementary Table 4**. The scale bar is 2 mm.



**Supplementary Figure 17. Representative MS images of unexpanded and TEMI samples by IHC-MALDI-MSI.** Photocleavable antibody mass tags of solute carrier family 2, facilitated MS identification and imaging of glucose transporter member 1 (GLUT1):  $[M + H]^+$ ,  $m/z$  856.823; Neurofilament light polypeptide (NF-L):  $[M + H]^+$ ,  $m/z$  1346.031; Synapsin-1 (SYN-1):  $[M + H]^+$ ,  $m/z$  1483.26; Parvalbumin alpha (PVALB):  $[M + H]^+$ ,  $m/z$  1540.283. MSI: MS imaging of unexpanded tissue, TEMI: MS imaging of expanded tissue. The scale bar is 2 mm.





**Supplementary Figure 18. Comparative spatial mapping of N-glycans from the mouse cerebellum between unexpanded control and TEMI.** a. Bright field photo image of an unexpanded cerebellum tissue section before MALDI matrix deposition, b-f. N-glycans from the unexpanded cerebellum tissue section, g. Bright field photo image of an ~3.5-fold linearly expanded cerebellum tissue section before MALDI matrix deposition, h-l. TEMI of N-glycans from the cerebellum. The scale bar is 4 mm. The experiments were carried out using a 50  $\mu$ m laser raster scanning and the MSI images obtained with mass error tolerance of 10 ppm. Hex, Hexose (Galactose, Mannose); HexNAc, N-Acetylhexosamine; NeuAc, N-Acetyl neuraminic acid; dHex, Fucose.



**Supplementary Table 1. List of the identified lipid species from mouse brain samples using TEMI**

**under positive mode**

| Name       | Type               | Molecular Formula | Experimental $m/z$ | Theoretical adduct $m/z^a$ | Error <sup>b</sup> $\Delta m/z$ [ppm] | Enriched in cerebellum layer <sup>c</sup> |
|------------|--------------------|-------------------|--------------------|----------------------------|---------------------------------------|-------------------------------------------|
| PE 28:1;O  | [M+H] <sup>+</sup> | C33H64NO9P        | 650.444            | 650.439                    | 6.872                                 | WML                                       |
| PE 30:1;O  | [M+H] <sup>+</sup> | C35H68NO9P        | 678.476            | 678.470                    | 8.696                                 | WML                                       |
| PC 30:0    | [M+H] <sup>+</sup> | C38H76NO8P        | 706.543            | 706.538                    | 7.416                                 | WML                                       |
| PS 31:3    | [M+H] <sup>+</sup> | C37H66NO10P       | 716.454            | 716.450                    | 6.504                                 | WML, GrCL                                 |
| PS 31:2    | [M+H] <sup>+</sup> | C40H80NO7P        | 718.467            | 718.465                    | 1.990                                 | WML, GrCL                                 |
| PC 28:3;O3 | [M+H] <sup>+</sup> | C40H82NO7P        | 720.442            | 720.445                    | -3.984                                | GrCL, ML                                  |
| PS 31:0    | [M+H] <sup>+</sup> | C37H72NO10P       | 722.502            | 722.497                    | 6.699                                 | GrCL                                      |
| PC 32:3    | [M+H] <sup>+</sup> | C40H74NO8P        | 728.519            | 728.519                    | 0.000                                 | WML                                       |
| PC 32:1    | [M+H] <sup>+</sup> | C40H78NO8P        | 732.559            | 732.554                    | 7.754                                 | WML                                       |
| PC 32:0    | [M+H] <sup>+</sup> | C40H80NO8P        | 734.568            | 734.569                    | -1.361                                | ML                                        |
| PC O-34:2  | [M+H] <sup>+</sup> | C42H82NO7P        | 744.594            | 744.590                    | 5.547                                 | WML                                       |
| PE 36:1    | [M+H] <sup>+</sup> | C41H80NO8P        | 746.574            | 746.569                    | 5.961                                 | WML                                       |
| PC O-34:1  | [M+H] <sup>+</sup> | C42H84NO7P        | 746.611            | 746.606                    | 7.206                                 | WML                                       |
| PC 30:3;O3 | [M+H] <sup>+</sup> | C38H70NO11P       | 748.473            | 748.476                    | -4.462                                | GrCL                                      |
| PS O-34:0  | [M+H] <sup>+</sup> | C40H80NO9P        | 750.571            | 750.564                    | 9.220                                 | ML                                        |
| PC 34:4    | [M+H] <sup>+</sup> | C42H76NO8P        | 754.541            | 754.538                    | 3.896                                 | WML                                       |
| PC 34:3    | [M+H] <sup>+</sup> | C42H78NO8P        | 756.558            | 756.554                    | 5.631                                 | ML                                        |
| PC 34:2    | [M+H] <sup>+</sup> | C42H80NO8P        | 758.573            | 758.569                    | 5.787                                 | ML, GrCL                                  |
| PC 34:1    | [M+H] <sup>+</sup> | C42H82NO8P        | 760.587            | 760.585                    | 2.261                                 | WML                                       |
| PC 34:0    | [M+H] <sup>+</sup> | C42H84NO8P        | 762.605            | 762.601                    | 4.590                                 | ML                                        |
| PE 38:4    | [M+H] <sup>+</sup> | C43H78NO8P        | 768.557            | 768.554                    | 3.982                                 | WML                                       |
| PC O-36:4  | [M+H] <sup>+</sup> | C44H82NO7P        | 768.595            | 768.590                    | 6.883                                 | WML                                       |
| PS O-36:4  | [M+H] <sup>+</sup> | C42H76NO9P        | 770.535            | 770.533                    | 2.064                                 | ML                                        |
| PC O-36:2  | [M+H] <sup>+</sup> | C44H86NO7P        | 772.626            | 772.621                    | 5.915                                 | ML                                        |
| PC 35:1    | [M+H] <sup>+</sup> | C43H84NO8P        | 774.606            | 774.601                    | 6.494                                 | ML                                        |
| PC 36:5    | [M+H] <sup>+</sup> | C44H78NO8P        | 780.558            | 780.554                    | 5.624                                 | WML                                       |
| PC 36:4    | [M+H] <sup>+</sup> | C44H80NO8P        | 782.575            | 782.569                    | 7.450                                 | WML                                       |
| PC 36:3    | [M+H] <sup>+</sup> | C44H82NO8P        | 784.585            | 784.585                    | -0.370                                | ML, GrCL                                  |
| PC 36:2    | [M+H] <sup>+</sup> | C44H84NO8P        | 786.608            | 786.601                    | 8.416                                 | WML                                       |
| PC 36:1    | [M+H] <sup>+</sup> | C44H86NO8P        | 788.620            | 788.616                    | 5.034                                 | WML                                       |
| PC 38:6    | [M+H] <sup>+</sup> | C46H80NO8P        | 806.573            | 806.569                    | 5.096                                 | GrCL                                      |
| PC 38:5    | [M+H] <sup>+</sup> | C46H82NO8P        | 808.588            | 808.585                    | 3.611                                 | WML                                       |
| PC 38:4    | [M+H] <sup>+</sup> | C46H84NO8P        | 810.604            | 810.601                    | 3.602                                 | WML                                       |
| PC 38:3    | [M+H] <sup>+</sup> | C46H86NO8P        | 812.613            | 812.616                    | -4.615                                | WML                                       |
| PC 38:1    | [M+H] <sup>+</sup> | C46H90NO8P        | 816.653            | 816.650                    | 3.943                                 | WML                                       |

|                 |                    |             |         |         |        |               |
|-----------------|--------------------|-------------|---------|---------|--------|---------------|
| PC 40:10        | [M+H] <sup>+</sup> | C48H76NO8P  | 826.541 | 826.538 | 3.533  | ML            |
| IPC 36:0;O3     | [M+H] <sup>+</sup> | C42H84NO12P | 826.578 | 826.580 | -2.649 | WML           |
| PC 40:9         | [M+H] <sup>+</sup> | C48H78NO8P  | 828.556 | 828.554 | 2.776  | GrCL          |
| PC 40:8         | [M+H] <sup>+</sup> | C48H80NO8P  | 830.568 | 830.569 | -1.950 | GrCL          |
| PC 40:7         | [M+H] <sup>+</sup> | C48H82NO8P  | 832.589 | 832.585 | 4.228  | ML, GrCL      |
| PE 42:0         | [M+H] <sup>+</sup> | C47H94NO8P  | 832.672 | 832.679 | -8.671 | GrCL          |
| PC 40:6         | [M+H] <sup>+</sup> | C48H84NO8P  | 834.606 | 834.601 | 5.775  | ML            |
| PC 40:5         | [M+H] <sup>+</sup> | C48H86NO8P  | 836.620 | 836.616 | 4.363  | ML, WML       |
| PC 40:4         | [M+H] <sup>+</sup> | C48H88NO8P  | 838.634 | 838.632 | 2.552  | WML           |
| PS 40:4         | [M+H] <sup>+</sup> | C46H82NO10P | 840.578 | 840.575 | 3.486  | GrCL          |
| PC 40:3         | [M+H] <sup>+</sup> | C48H90NO8P  | 840.645 | 840.648 | -3.485 | WML           |
| PS 40:3         | [M+H] <sup>+</sup> | C46H84NO10P | 842.593 | 842.591 | 3.359  | ML, GrCL, WML |
| PC 40:2         | [M+H] <sup>+</sup> | C48H92NO8P  | 842.669 | 842.663 | 7.073  | WML           |
| PC 40:1         | [M+H] <sup>+</sup> | C48H94NO8P  | 844.686 | 844.679 | 7.873  | WML           |
| PC 40:0         | [M+H] <sup>+</sup> | C48H96NO8P  | 846.690 | 846.695 | -5.516 | WML           |
| PS O-42:6       | [M+H] <sup>+</sup> | C48H84NO9P  | 850.602 | 850.596 | 7.736  | ML            |
| PC O-42:4       | [M+H] <sup>+</sup> | C50H94NO7P  | 852.689 | 852.684 | 5.406  | WML           |
| PC 42:9         | [M+H] <sup>+</sup> | C50H82NO8P  | 856.586 | 856.585 | 0.876  | ML, GrCL, WML |
| PC 42:8         | [M+H] <sup>+</sup> | C50H84NO8P  | 858.593 | 858.601 | -9.422 | ML, GrCL, WML |
| PE 44:1         | [M+H] <sup>+</sup> | C49H96NO8P  | 858.700 | 858.695 | 6.219  | WML           |
| PC 42:7         | [M+H] <sup>+</sup> | C50H86NO8P  | 860.617 | 860.616 | 0.662  | WML           |
| PC 42:5         | [M+H] <sup>+</sup> | C50H90NO8P  | 864.650 | 864.648 | 2.059  | WML           |
| PC 42:4         | [M+H] <sup>+</sup> | C50H92NO8P  | 866.667 | 866.663 | 4.338  | WML           |
| PS 42:4         | [M+H] <sup>+</sup> | C48H86NO10P | 868.610 | 868.606 | 4.686  | ML            |
| PC 42:2         | [M+H] <sup>+</sup> | C50H96NO8P  | 870.702 | 870.695 | 8.258  | WML           |
| PC 42:1         | [M+H] <sup>+</sup> | C50H98NO8P  | 872.715 | 872.710 | 5.890  | WML           |
| PS 44:12        | [M+H] <sup>+</sup> | C50H74NO10P | 880.517 | 880.512 | 5.644  | ML            |
| PE 46:4         | [M+H] <sup>+</sup> | C51H94NO8P  | 880.685 | 880.679 | 6.336  | WML           |
| SHexCer 42:2;O2 | [M+H] <sup>+</sup> | C48H91NO11S | 890.644 | 890.639 | 5.816  | ML            |
| SHexCer 42:1;O2 | [M+H] <sup>+</sup> | C48H93NO11S | 892.650 | 892.654 | -4.727 | ML            |
| PC 44:5         | [M+H] <sup>+</sup> | C52H94NO8P  | 892.681 | 892.679 | 2.498  | WML           |
| PC 44:4         | [M+H] <sup>+</sup> | C52H96NO8P  | 894.696 | 894.695 | 2.112  | WML           |
| PC 44:2         | [M+H] <sup>+</sup> | C52H100NO8P | 898.732 | 898.726 | 6.442  | WML           |
| PC 44:1         | [M+H] <sup>+</sup> | C52H102NO8P | 900.747 | 900.742 | 6.128  | WML           |

<sup>a</sup>Theoretical adduct *m/z* refers to databases of Human Metabolome Database ([http:// www.hmdb.ca](http://www.hmdb.ca)) and LIPID MAPS ([http:// www.lipidmaps.org](http://www.lipidmaps.org))

<sup>b</sup>Error = ((experimental adduct mass - theoretical adduct mass)/ theoretical adduct mass)\*1000000

<sup>c</sup>Enriched in cerebellum layers (Molecular layer (ML), granular cell layer (GrCL), and white matter layer (WML)): estimated dominant distribution of the corresponding lipid.

**Supplementary Table 2. List of the identified lipid species from mouse brain samples using TEMI**

**under negative mode**

| Name           | Type               | Molecular Formula | Experimental $m/z$ | Theoretical adduct $m/z^a$ | Error <sup>b</sup> $\Delta m/z$ [ppm] | Enriched in cerebellum layers <sup>c</sup> |
|----------------|--------------------|-------------------|--------------------|----------------------------|---------------------------------------|--------------------------------------------|
| LPA 16:0       | [M-H] <sup>-</sup> | C19H39O7P         | 409.236            | 409.236                    | -0.635                                | ML                                         |
| LPA 18:1       | [M-H] <sup>-</sup> | C21H41O7P         | 435.254            | 435.252                    | 4.733                                 | WML                                        |
| LPA 18:0       | [M-H] <sup>-</sup> | C21H43O7P         | 437.269            | 437.267                    | 3.408                                 | ML                                         |
| LPE 16:0       | [M-H] <sup>-</sup> | C21H44NO7P        | 452.281            | 452.278                    | 6.832                                 | WML                                        |
| LPA 20:1       | [M-H] <sup>-</sup> | C23H45O7P         | 463.284            | 463.283                    | 2.698                                 | WML                                        |
| LPE 18:1       | [M-H] <sup>-</sup> | C23H46NO7P        | 478.296            | 478.294                    | 4.621                                 | WML                                        |
| LPE 18:0       | [M-H] <sup>-</sup> | C23H48NO7P        | 480.311            | 480.310                    | 3.456                                 | ML, GrCL                                   |
| LPE 20:4       | [M-H] <sup>-</sup> | C25H44NO7P        | 500.280            | 500.278                    | 3.178                                 | WML                                        |
| LPE 20:1       | [M-H] <sup>-</sup> | C25H50NO7P        | 506.327            | 506.325                    | 4.483                                 | WML                                        |
| LPE 20:0       | [M-H] <sup>-</sup> | C25H52NO7P        | 508.342            | 508.341                    | 1.436                                 | WML                                        |
| LPE 22:6       | [M-H] <sup>-</sup> | C27H44NO7P        | 524.281            | 524.278                    | 4.711                                 | WML                                        |
| LPE 22:4       | [M-H] <sup>-</sup> | C27H48NO7P        | 528.312            | 528.310                    | 3.710                                 | WML                                        |
| CerP 36:1;O2   | [M-H] <sup>-</sup> | C36H72NO6P        | 644.505            | 644.503                    | 3.507                                 | ML                                         |
| PA 32:0        | [M-H] <sup>-</sup> | C35H69O8P         | 647.468            | 647.466                    | 3.923                                 | ML                                         |
| PS 26:0        | [M-H] <sup>-</sup> | C32H62NO10P       | 650.406            | 650.404                    | 3.183                                 | WML                                        |
| PS O-28:1      | [M-H] <sup>-</sup> | C34H66NO9P        | 662.443            | 662.440                    | 3.729                                 | WML                                        |
| PS 27:0        | [M-H] <sup>-</sup> | C33H64NO10P       | 664.423            | 664.420                    | 4.560                                 | WML, GrCL, ML                              |
| PA 34:1        | [M-H] <sup>-</sup> | C37H71O8P         | 673.485            | 673.481                    | 4.677                                 | WML                                        |
| PA 34:0        | [M-H] <sup>-</sup> | C37H73O8P         | 675.499            | 675.497                    | 3.035                                 | ML                                         |
| PS 28:0        | [M-H] <sup>-</sup> | C34H66NO10P       | 678.437            | 678.435                    | 2.506                                 | WML                                        |
| PS 29:0        | [M-H] <sup>-</sup> | C35H68NO10P       | 692.454            | 692.451                    | 4.159                                 | WML                                        |
| PA 36:2        | [M-H] <sup>-</sup> | C39H73O8P         | 699.500            | 699.497                    | 4.232                                 | WML                                        |
| PA 36:1        | [M-H] <sup>-</sup> | C39H75O8P         | 701.516            | 701.513                    | 4.333                                 | WML                                        |
| CerPE 38:1;O2  | [M-H] <sup>-</sup> | C40H81N2O6P       | 715.578            | 715.576                    | 3.396                                 | ML                                         |
| PE 34:1        | [M-H] <sup>-</sup> | C39H76NO8P        | 716.527            | 716.524                    | 4.047                                 | WML                                        |
| PE 34:0        | [M-H] <sup>-</sup> | C39H78NO8P        | 718.542            | 718.539                    | 3.451                                 | ML                                         |
| PA 38:4        | [M-H] <sup>-</sup> | C41H73O8P         | 723.499            | 723.497                    | 2.184                                 | WML                                        |
| CerP 42:2;O2   | [M-H] <sup>-</sup> | C42H82NO6P        | 726.585            | 726.581                    | 5.753                                 | WML                                        |
| PE O-36:2      | [M-H] <sup>-</sup> | C41H80NO7P        | 728.563            | 728.560                    | 3.706                                 | WML                                        |
| PA 38:1        | [M-H] <sup>-</sup> | C41H79O8P         | 729.549            | 729.544                    | 6.675                                 | WML                                        |
| CerPE 38:2;O3  | [M-H] <sup>-</sup> | C40H79N2O7P       | 729.561            | 729.555                    | 8.334                                 | WML                                        |
| HexCer 34:1;O4 | [M-H] <sup>-</sup> | C40H77NO10        | 730.547            | 730.548                    | -0.684                                | WML                                        |
| PE 36:2        | [M-H] <sup>-</sup> | C41H78NO8P        | 742.542            | 742.539                    | 4.417                                 | WML                                        |

|                    |                    |             |         |         |        |           |
|--------------------|--------------------|-------------|---------|---------|--------|-----------|
| HexCer<br>36:1;O3  | [M-H] <sup>-</sup> | C42H81NO9   | 742.586 | 742.584 | 2.680  | WML       |
| PE 36:1            | [M-H] <sup>-</sup> | C41H80NO8P  | 744.558 | 744.555 | 3.855  | WML       |
| PE 36:0            | [M-H] <sup>-</sup> | C41H82NO8P  | 746.571 | 746.571 | 1.179  | ML        |
| PA 40:6            | [M-H] <sup>-</sup> | C43H73O8P   | 747.500 | 747.497 | 4.134  | ML        |
| PS 33:0            | [M-H] <sup>-</sup> | C39H76NO10P | 748.507 | 748.513 | -8.764 | ML        |
| PE O-38:3          | [M-H] <sup>-</sup> | C43H82NO7P  | 754.578 | 754.576 | 3.048  | WML       |
| PE 38:4            | [M-H] <sup>-</sup> | C43H78NO8P  | 766.542 | 766.539 | 3.261  | WML       |
| PE 38:2            | [M-H] <sup>-</sup> | C43H82NO8P  | 770.574 | 770.571 | 4.075  | WML       |
| HexCer<br>38:1;O3  | [M-H] <sup>-</sup> | C44H85NO9   | 770.617 | 770.615 | 1.856  | WML       |
| PE 38:1            | [M-H] <sup>-</sup> | C43H84NO8P  | 772.589 | 772.586 | 3.663  | WML       |
| PG 37:6            | [M-H] <sup>-</sup> | C43H73O10P  | 779.488 | 779.487 | 2.040  | WML, GrCL |
| PS 36:2            | [M-H] <sup>-</sup> | C42H78NO10P | 786.532 | 786.529 | 3.725  | ML        |
| PS 36:1            | [M-H] <sup>-</sup> | C42H80NO10P | 788.548 | 788.545 | 3.602  | WML       |
| PE 40:6            | [M-H] <sup>-</sup> | C45H78NO8P  | 790.542 | 790.539 | 4.124  | ML        |
| PI O-32:0          | [M-H] <sup>-</sup> | C41H81O12P  | 795.532 | 795.539 | -9.629 | WML       |
| HexCer<br>40:1;O3  | [M-H] <sup>-</sup> | C46H89NO9   | 798.650 | 798.647 | 4.558  | WML       |
| PS 36:0;O          | [M-H] <sup>-</sup> | C42H82NO11P | 806.548 | 806.555 | -8.716 | WML       |
| PG O-40:6          | [M-H] <sup>-</sup> | C46H81O9P   | 807.554 | 807.555 | -0.421 | WML       |
| HexCer<br>42:2;O2  | [M-H] <sup>-</sup> | C48H91NO8   | 808.670 | 808.667 | 3.574  | WML       |
| TG 49:5            | [M-H] <sup>-</sup> | C52H90O6    | 809.674 | 809.667 | 9.522  | WML       |
| PS 38:4            | [M-H] <sup>-</sup> | C44H78NO10P | 810.529 | 810.529 | -0.407 | WML       |
| PE O-42:1          | [M-H] <sup>-</sup> | C47H94NO7P  | 814.674 | 814.670 | 5.880  | WML       |
| PS 38:1            | [M-H] <sup>-</sup> | C44H84NO10P | 816.578 | 816.576 | 2.608  | WML       |
| PE 42:6            | [M-H] <sup>-</sup> | C47H82NO8P  | 818.571 | 818.571 | 0.098  | ML        |
| PI O-34:1          | [M-H] <sup>-</sup> | C43H83O12P  | 821.548 | 821.555 | -8.666 | WML       |
| SHexCer<br>36:1;O3 | [M-H] <sup>-</sup> | C42H81NO12S | 822.545 | 822.541 | 5.605  | WML       |
| PG 40:5            | [M-H] <sup>-</sup> | C46H81O10P  | 823.556 | 823.550 | 8.293  | WML       |
| HexCer<br>36:1;O   | [M-H] <sup>-</sup> | C48H91NO9   | 824.665 | 824.662 | 3.092  | WML       |
| HexCer<br>42:1;O3  | [M-H] <sup>-</sup> | C48H93NO9   | 826.680 | 826.678 | 2.710  | WML       |
| PS 40:6            | [M-H] <sup>-</sup> | C46H78NO10P | 834.531 | 834.529 | 2.073  | ML        |
| SHexCer<br>38:1;O2 | [M-H] <sup>-</sup> | C44H85NO11S | 834.579 | 834.577 | 2.432  | WML       |
| PI 34:1            | [M-H] <sup>-</sup> | C43H81O13P  | 835.533 | 835.534 | -1.508 | WML       |
| PG O-42:6          | [M-H] <sup>-</sup> | C48H85O9P   | 835.584 | 835.586 | -2.729 | WML       |
| PS 40:5            | [M-H] <sup>-</sup> | C46H80NO10P | 836.539 | 836.545 | -6.587 | WML       |
| PS 40:4            | [M-H] <sup>-</sup> | C46H82NO10P | 838.558 | 838.560 | -3.268 | WML       |
| SHexCer<br>38:1;O3 | [M-H] <sup>-</sup> | C44H85NO12S | 850.576 | 850.572 | 4.573  | WML       |
| PS 42:9            | [M-H] <sup>-</sup> | C48H76NO10P | 856.515 | 856.513 | 1.354  | ML        |
| PI 36:4            | [M-H] <sup>-</sup> | C45H79O13P  | 857.519 | 857.519 | 0.431  | WML       |



|                 |                    |             |         |         |        |     |
|-----------------|--------------------|-------------|---------|---------|--------|-----|
| PS 42:8         | [M-H] <sup>-</sup> | C48H78NO10P | 858.523 | 858.529 | -7.268 | WML |
| IPC 40:1;O2     | [M-H] <sup>-</sup> | C46H90NO11P | 862.611 | 862.618 | -8.497 | WML |
| IPC 40:0;O2     | [M-H] <sup>-</sup> | C46H92NO11P | 864.626 | 864.634 | -9.010 | WML |
| SHexCer 40:1;O3 | [M-H] <sup>-</sup> | C46H89NO12S | 878.606 | 878.603 | 3.119  | WML |
| PG 44:5         | [M-H] <sup>-</sup> | C50H89O10P  | 879.611 | 879.612 | -1.648 | WML |
| IPC 40:0;O3     | [M-H] <sup>-</sup> | C46H92NO12P | 880.621 | 880.628 | -8.925 | WML |
| PG 44:4         | [M-H] <sup>-</sup> | C50H91O10P  | 881.622 | 881.628 | -6.590 | WML |
| PI 38:5         | [M-H] <sup>-</sup> | C47H81O13P  | 883.535 | 883.534 | 0.555  | WML |
| PI 38:4         | [M-H] <sup>-</sup> | C47H83O13P  | 885.552 | 885.550 | 2.417  | WML |
| PS 44:8         | [M-H] <sup>-</sup> | C50H82NO10P | 886.557 | 886.560 | -3.373 | WML |
| PI 38:3         | [M-H] <sup>-</sup> | C47H85O13P  | 887.562 | 887.566 | -4.067 | WML |
| SHexCer 42:2;O2 | [M-H] <sup>-</sup> | C48H91NO11S | 888.626 | 888.624 | 2.723  | WML |
| SHexCer 42:1;O2 | [M-H] <sup>-</sup> | C48H93NO11S | 890.640 | 890.640 | 0.404  | WML |
| TG 56:13        | [M-H] <sup>-</sup> | C59H88O6    | 891.645 | 891.651 | -6.325 | WML |
| PI O-40:2       | [M-H] <sup>-</sup> | C49H93O12P  | 903.642 | 903.633 | 9.705  | WML |
| SHexCer 42:2;O3 | [M-H] <sup>-</sup> | C48H91NO12S | 904.621 | 904.619 | 2.454  | WML |
| SHexCer 42:1;O3 | [M-H] <sup>-</sup> | C48H93NO12S | 906.636 | 906.635 | 1.831  | WML |

<sup>a</sup>Theoretical adduct m/z refers to databases of Human Metabolome Database ([http://: www.hmdb.ca](http://www.hmdb.ca)) and LIPID MAPS ([http://: www.lipidmaps.org](http://www.lipidmaps.org)).

<sup>b</sup>Error = ((experimental adduct mass - theoretical adduct mass)/ theoretical adduct mass)\*1000000.

<sup>c</sup>Enriched in cerebellum layers (Molecular layer (ML), granular cell layer (GrCL), and white matter layer (WML)): estimated dominant distribution of the corresponding lipid.

**Supplementary Table 3. Details of detected lipid species in Purkinje neuron cells using TEMI under positive mode**

| Name    | Type               | Molecular Formula | Experimental $m/z$ | Theoretical adduct $m/z^a$ | Error <sup>b</sup> $\Delta m/z$ [ppm] | Enriched in Purkinje neuron cells |
|---------|--------------------|-------------------|--------------------|----------------------------|---------------------------------------|-----------------------------------|
| PC 32:0 | [M+H] <sup>+</sup> | C40H80NO8P        | 734.575            | 734.569                    | 7.217                                 | No                                |
| PC 34:1 | [M+H] <sup>+</sup> | C42H82NO8P        | 760.585            | 760.588                    | -3.57                                 | No                                |
| PC 36:1 | [M+H] <sup>+</sup> | C44H87NO8P        | 788.614            | 788.616                    | 2.54                                  | Yes                               |
| PC 36:0 | [M+H] <sup>+</sup> | C44H89NO8P        | 790.631            | 790.632                    | 1.26                                  | Yes                               |
| PC 38:4 | [M+H] <sup>+</sup> | C46H85NO8P        | 810.601            | 810.601                    | 0.74                                  | Yes                               |
| PC 38:3 | [M+H] <sup>+</sup> | C46H87NO8P        | 812.614            | 812.616                    | 2.83                                  | Yes                               |
| PC 38:2 | [M+H] <sup>+</sup> | C46H89NO8P        | 814.636            | 814.632                    | 4.79                                  | Yes                               |
| PC 38:1 | [M+H] <sup>+</sup> | C46H91NO8P        | 816.647            | 816.648                    | 1.47                                  | Yes                               |
| PC 40:6 | [M+H] <sup>+</sup> | C48H85NO8P        | 834.603            | 834.601                    | 2.76                                  | Yes                               |
| PC 40:5 | [M+H] <sup>+</sup> | C48H87NO8P        | 836.617            | 836.616                    | 0.48                                  | Yes                               |
| PC 40:4 | [M+H] <sup>+</sup> | C48H89NO8P        | 838.636            | 838.632                    | 4.17                                  | Yes                               |
| PC 42:9 | [M+H] <sup>+</sup> | C50H83NO8P        | 856.583            | 856.585                    | 2.57                                  | Yes                               |

<sup>a</sup>Theoretical adduct  $m/z$  refers to databases of Human Metabolome Database ([http://: www.hmdb.ca](http://www.hmdb.ca)) and LIPID MAPS ([http://: www.lipidmaps.org](http://www.lipidmaps.org)).

<sup>b</sup>Error = ((experimental adduct mass - theoretical adduct mass)/ theoretical adduct mass)\*1000000.

**Supplementary Table 4. List of the identified on-tissue tryptic peptides matched with LC-MS**

**proteomics results from mouse cerebella using TEMI under positive mode.**

| Experimental adduct mass, $m/z$ $[M + H]^+$ | Theoretical adduct mass, $m/z$ $[M + H]^+$ | Mass Error (ppm) * | Putative Peptides | Modification                | Putative Gene Name |
|---------------------------------------------|--------------------------------------------|--------------------|-------------------|-----------------------------|--------------------|
| 709.3432                                    | 709.3416                                   | -2.26              | FSWGGR            |                             | Mbp                |
| 858.5155                                    | 858.5155                                   | 0.00               | QLATKAAR          |                             | H3c1               |
| 872.5193                                    | 872.52                                     | 0.80               | TGIVLANGK         |                             | Map1a              |
| 890.4391                                    | 890.4407                                   | 1.80               | FGYQFTK           |                             | Tmod2              |
| 917.5064                                    | 917.505                                    | -1.53              | GTVGSIILDR        |                             | Abce1              |
| 935.4981                                    | 935.4945                                   | -3.85              | DGYAQILR          |                             | Aco2               |
| 938.4828                                    | 938.4829                                   | 0.11               | EAIDSYIK          |                             | Cltc               |
| 943.5454                                    | 943.5458                                   | 0.42               | LTVPSADLK         |                             | Slc6a11            |
| 953.6042                                    | 953.6029                                   | -1.36              | LLLPGELAK         |                             | H2bc26             |
| 984.5395                                    | 984.536                                    | -3.55              | AVTKYTSSK         |                             | H2bc26             |
| 1006.52                                     | 1006.5203                                  | 0.40               | ANEFLEVVGK        |                             | Eif3a              |
| 1024.561                                    | 1024.5574                                  | -3.32              | FIRNFAEK          |                             | Atp5mg             |
| 1039.52                                     | 1039.5241                                  | 4.04               | FGNMGSLSVK        |                             | Lama1              |
| 1049.5                                      | 1049.501                                   | 0.86               | SHFESSTVR         |                             | Epb4113            |
| 1067.505                                    | 1067.5004                                  | -4.03              | EYQLNDSAK         |                             | Gnao1              |
| 1087.59                                     | 1087.5928                                  | 3.03               | SPQMVSIVR         |                             | Ctnnb1             |
| 1115.596                                    | 1115.5942                                  | -1.61              | AVDEAADALLK       |                             | Immt               |
| 1179.633                                    | 1179.6368                                  | 3.14               | VPQIEVETHK        |                             | Dync1h1            |
| 1203.572                                    | 1203.5739                                  | 1.91               | VAVEEVDEEGK       |                             | Lmna               |
| 1215.674                                    | 1215.6731                                  | -0.58              | FKVPGVEASGPK      |                             | Ahnak              |
| 1225.616                                    | 1225.617                                   | 0.90               | ASHLELNNGTK       | 1A(30.0105),<br>2S(12.0000) | Akr1b1             |
| 1229.679                                    | 1229.6848                                  | 4.39               | ELIIGDRQTGK       |                             | Atp5fla            |
| 1263.718                                    | 1263.7168                                  | -1.19              | LNIPVNQVNPR       |                             | Atp1a1             |
| 1288.694                                    | 1288.6929                                  | -0.85              | VVDITDQLVMR       |                             | Sugp2              |
| 1303.735                                    | 1303.7368                                  | 1.30               | DLTPEHLPLLR       |                             | Dcps               |
| 1311.575                                    | 1311.5699                                  | -3.51              | EDQTEYLEER        |                             | Hsp90a1            |
| 1339.71                                     | 1339.7076                                  | -1.94              | HRDTGILDSIGR      |                             | Mbp                |
| 1347.739                                    | 1347.7379                                  | -0.67              | ALIAAQYSGAQVR     |                             | Eef1g              |
| 1358.7                                      | 1358.6964                                  | -2.58              | FAGHNFRNPSVL      |                             | Eno2               |
| 1363.764                                    | 1363.758                                   | -4.03              | ITVTSEVPFSKR      |                             | Rpl22              |
| 1367.772                                    | 1367.7753                                  | 2.27               | AVDSLVPPIRGQR     |                             | Atp5fla            |
| 1411.783                                    | 1411.7791                                  | -2.55              | LEEGPPVTTVLTR     |                             | Pdha1              |
| 1415.692                                    | 1415.6913                                  | -0.14              | TWGDAGAAAGGGIPSK  |                             | Prkar2b            |

|          |           |       |                        |                 |              |
|----------|-----------|-------|------------------------|-----------------|--------------|
| 1440.742 | 1440.7369 | -3.33 | VEEFLYEKLDLR           |                 | Sh3glb2      |
| 1493.71  | 1493.7118 | 0.94  | TGENVEDAFLEAAK         |                 | Rab14        |
| 1498.857 | 1498.8587 | 1.00  | SGKAPILIATDVASR        |                 | Ddx17        |
| 1503.865 | 1503.8641 | -0.33 | NLLSVAYKNVVGAR         |                 | Ywhaz        |
| 1515.761 | 1515.7649 | 2.31  | EQGVDENETLLLR          |                 | Tln2         |
| 1569.768 | 1569.7655 | -1.53 | KAEAGAGSATEFQFR        |                 | Rps10        |
| 1575.863 | 1575.8588 | -2.41 | SSLLDVTVTIPSSR         |                 | Map1a        |
| 1589.744 | 1589.7441 | 0.31  | HQEGEIFDTEKEK          |                 | Rpl6         |
| 1649.905 | 1649.9009 | -2.42 | VEFLRPSFIDGTIR         |                 | Nectin1      |
| 1680.863 | 1680.8564 | -4.16 | GLSLSRFSWGGRDSR        |                 | Mbp          |
| 1696.857 | 1696.8514 | -3.18 | TTHYGSLPQKSQHGR        |                 | Mbp          |
| 1707.871 | 1707.866  | -2.93 | SVHHALSDVQAEVSTK       |                 | Mmrn2        |
| 1714.913 | 1714.9122 | -0.64 | SSGPTSLFAVTVAPPGAR     |                 | Hnrnpu       |
| 1719.034 | 1719.0288 | -3.08 | AGLQFPVGRVHRLLR        |                 | H2ac15       |
| 1737.031 | 1737.0268 | -2.59 | EVQTAVRLLLPGELAK       |                 | H2bc26       |
| 1741.858 | 1741.8602 | 1.09  | ELDALDANDELTPLGR       |                 | Dhx9         |
| 1751.045 | 1751.0435 | -1.09 | EVQTAVRLLLPGELAK       | 1E(14.01<br>67) | H2bc26       |
| 1764.941 | 1764.9391 | -1.30 | AAALEFLNRFEEAKR        |                 | Stip1        |
| 1816.938 | 1816.934  | -1.93 | IQPNDGPVFFKVDGQR       |                 | Cnrip1       |
| 1903.954 | 1903.9508 | -1.79 | SAYSSYSAPVSSLSVRR      |                 | Nefl         |
| 2063.064 | 2063.0596 | -1.89 | TWNDPSVQQDIKFLPFK      |                 | Hspa5        |
| 2232.075 | 2232.0785 | 1.39  | QQAAMGSQGNLSAEVEQATTR  |                 | Atp6v1<br>g2 |
| 2397.392 | 2397.385  | -3.04 | ETVSIEVKEVVKPLLSTLGEK  |                 | Mecp2        |
| 2402.242 | 2402.2323 | -4.16 | HRDTGILDSIGRFFSGDRGAPK |                 | Mbp          |

\*Error = ((experimental adduct mass - theoretical adduct mass)/ theoretical adduct mass)\*1000000.

**Supplementary Table 5. N-glycans detected from mouse cerebellum using TEMI**

| Experimental mass<br>[M + Na] <sup>+</sup> <i>m/z</i> | Identified N-glycans <sup>a</sup> | Theoretical adduct mass <sup>b</sup><br>[M + Na] <sup>+</sup> <i>m/z</i> | Mass Error<br>(ppm) <sup>c</sup> |
|-------------------------------------------------------|-----------------------------------|--------------------------------------------------------------------------|----------------------------------|
| 1257.4287                                             | Hex5HexNAc2                       | 1257.4230                                                                | 4.53                             |
| 1282.4520                                             | Hex3HexNAc3dHex1                  | 1282.4550                                                                | -2.34                            |
| 1419.4773                                             | Hex6HexNAc2                       | 1419.4760                                                                | 0.92                             |
| 1444.5151                                             | Hex4HexNAc3dHex1                  | 1444.5080                                                                | 4.92                             |
| 1485.5330                                             | Hex3HexNAc4dHex1                  | 1485.5342                                                                | -0.83                            |
| 1542.5526                                             | Hex3HexNAc5                       | 1542.5557                                                                | -2.03                            |
| 1581.5233                                             | Hex7HexNAc2                       | 1581.5290                                                                | -3.60                            |
| 1606.5572                                             | Hex5HexNAc3dHex1                  | 1606.5604                                                                | -2.01                            |
| 1647.5897                                             | Hex4HexNAc4dHex1                  | 1647.5870                                                                | 1.64                             |
| 1663.5742                                             | Hex5HexNAc4                       | 1663.5820                                                                | -4.69                            |
| 1688.6061                                             | Hex3HexNAc5dHex1                  | 1688.6136                                                                | -4.46                            |
| 1743.5763                                             | Hex8HexNAc2                       | 1743.5820                                                                | -3.27                            |
| 1768.6177                                             | Hex6HexNAc3dHex1                  | 1768.6132                                                                | 2.52                             |
| 1809.6406                                             | Hex5HexNAc4dHex1                  | 1809.6398                                                                | 0.42                             |
| 1825.6448                                             | Hex6HexNAc4                       | 1825.6350                                                                | 5.37                             |
| 1850.6709                                             | Hex4HexNAc5dHex1                  | 1850.6664                                                                | 2.41                             |
| 1905.6309                                             | Hex9HexNAc2                       | 1905.6340                                                                | -1.63                            |
| 1955.6901                                             | Hex5HexNAc4dHex2                  | 1955.6977                                                                | -3.90                            |
| 1971.7039                                             | Hex6HexNAc4dHex1                  | 1971.6926                                                                | 5.71                             |
| 1996.7244                                             | Hex4HexNAc5dHex2                  | 1996.7243                                                                | 0.03                             |
| 2012.7120                                             | Hex5HexNAc5dHex1                  | 2012.7190                                                                | -3.48                            |
| 2053.7513                                             | Hex9HexNAc2                       | 2053.7458                                                                | 2.66                             |
| 2158.7793                                             | Hex5HexNAc5dHex2                  | 2158.7771                                                                | 1.00                             |
| 2174.7700                                             | Hex6HexNAc5dHex1                  | 2174.7720                                                                | -0.92                            |
| 2304.8551                                             | Hex5HexNAc5dHex3                  | 2304.8350                                                                | 8.71                             |
| 2320.8251                                             | Hex6HexNAc5dHex2                  | 2320.8299                                                                | -2.08                            |
| 2523.9184                                             | Hex6HexNAc6dHex2                  | 2523.9093                                                                | 3.59                             |
| 2539.9074                                             | Hex7HexNAc6dHex1                  | 2539.9042                                                                | 1.25                             |
| 2669.9707                                             | Hex6HexNAc6dHex3                  | 2669.9672                                                                | 1.30                             |

<sup>a</sup> Hex, hexose (galactose, mannose); HexNAc, N-acetylhexosamine; NeuAc, N-acetyl neuraminic acid; dHex, fucose

<sup>b</sup> Theoretical adduct *m/z* refers to databases of GlycoWorkbench

(<https://code.google.com/archive/p/glycoworkbench/>)

<sup>c</sup> Error = ((experimental mass – theoretical mass)/ theoretical mass)\*1000000



**Supplementary Table 6. The expansion factor and the maximum measurement error of different tissue types under 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> embeddings.**

|            |                           | pre-expansion | 1st embedding | 2nd embedding | 3rd embedding |
|------------|---------------------------|---------------|---------------|---------------|---------------|
| Cerebellum | expansion factor          | 1             | 1.7           | 2.4           | 3.5           |
|            | maximum deformation error | 0             | 9.7%          | 5.6%          | 5.4%          |
| Kidney     | expansion factor          | 1             | 1.3           | 2.1           | 3             |
|            | maximum deformation error | 0             | 9.5%          | 11.6%         | 11.9%         |
| Pancreas   | expansion factor          | 1             | 1.4           | 2.5           | 3.5           |
|            | maximum deformation error | 0             | 9.7%          | 6.9%          | 8.3%          |

**Supplementary Table 7. The LC-MS gradient used for the lipidomic analysis\***

| Time | Flow rate (mL/min) | %B  |
|------|--------------------|-----|
| 0    | 0.15               | 30  |
| 3.5  | 0.15               | 43  |
| 3.64 | 0.15               | 55  |
| 20.8 | 0.15               | 65  |
| 31.2 | 0.15               | 85  |
| 35   | 0.15               | 100 |
| 43   | 0.15               | 100 |
| 43.5 | 0.15               | 30  |
| 52   | 0.15               | 30  |

\*: The mobile phase A: 60:40 (v:v) acetonitrile/water with 10 mM ammonium formate and 0.1% formic acid, the mobile phase B: 90:10 (v:v) IPA/acetonitrile with 10 mM ammonium formate and 0.1% formic acid. Column: Accucore™ Vanquish™ C18+ UHPLC Column (2.1 x 150 mm, 1.5 μm). Temperature: 55 °C. Flow rate: 150 μL/min.

**Supplementary Table 8. The significantly changed lipids from the lipidomic analysis\***

| LipidMolec      | Adjusted p-value | Significant pairs |
|-----------------|------------------|-------------------|
| PC(18:1_22:6)   | 0.0135           | Ex_ExP;ctrl_ExP   |
| PC(18:1_20:4)   | 0.0422           | Ex_ExP;ctrl_ExP   |
| PE(16:0_20:4)   | 0.0349           | Ex_ExP;ctrl_ExP   |
| PE(P-18:0_22:6) | 0.0314           | ctrl_ExP          |
| DG(O-20:6_18:0) | 0.0234           | Ex_ExP;ctrl_ExP   |
| PE(18:0_20:4)   | 0.0349           | ctrl_ExP          |
| PC(22:6_22:6)   | 0.0322           | Ex_ExP;ctrl_ExP   |

|                 |        |                 |
|-----------------|--------|-----------------|
| PC(44:12)       | 0.0104 | Ex_Exp;ctrl_Exp |
| LPE(20:1)       | 0.0373 | Ex_Exp;ctrl_Exp |
| LPE(22:6)       | 0.0350 | Ex_Exp;ctrl_Exp |
| PE(22:6_18:0)   | 0.0349 | Ex_Exp;ctrl_Exp |
| PC(40:8)        | 0.0110 | Ex_Exp;ctrl_Exp |
| LPC(22:6)       | 0.0349 | ctrl_Exp;Ex_Exp |
| PA(24:5_18:0)   | 0.0367 | ctrl_Exp        |
| PS(18:0_22:6)   | 0.0079 | Ex_Exp;ctrl_Exp |
| PC(42:10)       | 0.0079 | Ex_Exp;ctrl_Exp |
| PC(42:8)        | 0.0260 | Ex_Exp;ctrl_Exp |
| PC(16:0_20:5)   | 0.0322 | Ex_Exp;ctrl_Exp |
| PE(19:0_15:0)   | 0.0441 | ctrl_Exp        |
| DG(18:0_20:4)   | 0.0190 | Ex_Exp;ctrl_Exp |
| LPE(20:4)       | 0.0256 | ctrl_Exp;Ex_Exp |
| PC(16:1_22:6)   | 0.0073 | Ex_Exp;ctrl_Exp |
| PC(20:4_22:6)   | 0.0104 | Ex_Exp;ctrl_Exp |
| PE(22:6_22:6)   | 0.0078 | Ex_Exp;ctrl_Exp |
| PC(22:4_22:6)   | 0.0260 | Ex_Exp;ctrl_Exp |
| DG(O-20:6_18:1) | 0.0484 | ctrl_Exp;Ex_Exp |
| DG(16:0_22:6)   | 0.0292 | Ex_Exp;ctrl_Exp |
| DG(O-20:6_16:0) | 0.0233 | ctrl_Exp;Ex_Exp |
| PC(O-18:0_22:6) | 0.0474 | ctrl_Exp        |
| PC(23:6_18:1)   | 0.0322 | Ex_Exp;ctrl_Exp |
| PC(18:0_24:6)   | 0.0107 | Ex_Exp;ctrl_Exp |
| PC(16:1_20:4)   | 0.0320 | Ex_Exp;ctrl_Exp |
| PI(20:4_18:1)   | 0.0349 | Ex_Exp;ctrl_Exp |
| PC(34:3)        | 0.0349 | Ex_Exp;ctrl_Exp |
| PS(22:4_22:6)   | 0.0079 | Ex_Exp;ctrl_Exp |
| PA(19:1_22:6)   | 0.0115 | Ex_Exp;ctrl_Exp |
| DG(O-22:6_22:6) | 0.0373 | ctrl_Exp        |
| PC(44:5)        | 0.0285 | Ex_Exp;ctrl_Exp |
| PE(44:11)       | 0.0237 | Ex_Exp;ctrl_Exp |
| PC(14:0_20:4)   | 0.0178 | Ex_Exp;ctrl_Exp |
| PC(20:3_22:6)   | 0.0322 | ctrl_Exp;Ex_Exp |
| PA(P-19:4_21:1) | 0.0424 | ctrl_Exp        |
| PE(20:4_22:6)   | 0.0174 | Ex_Exp;ctrl_Exp |
| PG(O-17:2_23:6) | 0.0104 | Ex_Exp;ctrl_Exp |
| PC(35:4)        | 0.0349 | Ex_Exp;ctrl_Exp |
| AcCa(16:1)      | 0.0046 | Ex_Exp;ctrl_Exp |
| PE(P-17:0_20:4) | 0.0387 | ctrl_Exp        |
| LPE(16:1)       | 0.0256 | Ex_Exp;ctrl_Exp |
| LPE(24:4)       | 0.0413 | Ex_Exp;ctrl_Exp |
| DG(18:1_22:6)   | 0.0256 | Ex_Exp;ctrl_Exp |
| PC(44:7)        | 0.0349 | Ex_Exp;ctrl_Exp |
| PC(O-18:4_22:1) | 0.0473 | ctrl_Exp;Ex_Exp |
| AcCa(18:2)      | 0.0182 | Ex_Exp;ctrl_Exp |

|                    |        |                   |
|--------------------|--------|-------------------|
| AcCa(20:4)         | 0.0260 | Ex_Exp;ctrl_Exp   |
| PA(21:2_22:6)      | 0.0320 | ctrl_Exp;Ex_Exp   |
| PA(25:7_18:0)      | 0.0292 | ctrl_Exp;ctrl_Exp |
| LPE(24:2)          | 0.0256 | Ex_Exp;ctrl_Exp   |
| PS(24:0_18:1)      | 0.0484 | ctrl_Exp          |
| PI(20:4_16:0)      | 0.0486 | ctrl_Exp;Ex_Exp   |
| PC(O-17:2_22:6)    | 0.0276 | Ex_Exp;ctrl_Exp   |
| LPE(24:3)          | 0.0398 | Ex_Exp;ctrl_Exp   |
| PE(O-18:1_22:6)    | 0.0078 | Ex_Exp;ctrl_Exp   |
| PS(20:1_22:6)      | 0.0473 | Ex_Exp;ctrl_Exp   |
| PI(22:6_16:0)      | 0.0474 | Ex_Exp;ctrl_Exp   |
| PS(19:3_18:1)      | 0.0191 | Ex_Exp;ctrl_Exp   |
| PE(24:6_22:6)      | 0.0322 | Ex_Exp;ctrl_Exp   |
| PE(O-18:2_20:4)    | 0.0181 | Ex_Exp;ctrl_Exp   |
| PA(18:0_22:6)      | 0.0187 | ctrl_Exp;Ex_Exp   |
| LPE(24:6)          | 0.0329 | Ex_Exp;ctrl_Exp   |
| PE(O-18:1_20:4)    | 0.0237 | Ex_Exp;ctrl_Exp   |
| PC(O-16:1_20:4)    | 0.0256 | Ex_Exp;ctrl_Exp   |
| PE(18:1_18:1)      | 0.0203 | Ex_Exp;ctrl_Exp   |
| LPC(24:2)          | 0.0182 | Ex_Exp;ctrl_Exp   |
| PS(21:5_18:0)      | 0.0255 | Ex_Exp;ctrl_Exp   |
| PI(18:1_20:4)      | 0.0165 | ctrl_Exp;Ex_Exp   |
| PC(40:9)           | 0.0191 | ctrl_Exp;Ex_Exp   |
| PE(16:1_18:2)      | 0.0205 | Ex_Exp;ctrl_Exp   |
| PE(37:6)           | 0.0104 | Ex_Exp;ctrl_Exp   |
| PE(O-18:2_22:6)    | 0.0237 | Ex_Exp;ctrl_Exp   |
| PA(22:4_25:5)      | 0.0243 | Ex_Exp;ctrl_Exp   |
| PS(19:4_18:1)      | 0.0320 | ctrl_Exp;Ex_Exp   |
| PC(16:0_20:3)      | 0.0441 | ctrl_Exp;Ex_Exp   |
| PE(24:6_18:0)      | 0.0084 | Ex_Exp;ctrl_Exp   |
| PA(P-19:4_21:2)    | 0.0398 | Ex_Exp;ctrl_Exp   |
| PS(18:0_22:4)      | 0.0095 | Ex_Exp;ctrl_Exp   |
| PA(23:4_18:0)      | 0.0037 | Ex_Exp;ctrl_Exp   |
| PE(22:6_14:0)      | 0.0079 | Ex_Exp;ctrl_Exp   |
| TG(16:0_18:0_18:1) | 0.0285 | ctrl_Exp;Ex_Exp   |
| PC(45:6)           | 0.0276 | Ex_Exp;ctrl_Exp   |
| PE(O-16:0_18:1)    | 0.0349 | ctrl_Exp          |
| PA(18:0_20:4)      | 0.0203 | Ex_Exp;ctrl_Exp   |
| PA(18:1_18:1)      | 0.0152 | ctrl_Exp;Ex_Exp   |
| PI(40:8)           | 0.0037 | Ex_Exp;ctrl_Exp   |
| DG(P-22:7_22:6)    | 0.0365 | ctrl_Exp;Ex_Exp   |
| PS(16:0_18:1)      | 0.0214 | ctrl_Exp;Ex_Exp   |
| PA(22:6_28:5)      | 0.0349 | Ex_Exp;ctrl_Exp   |
| PE(O-18:0_22:6)    | 0.0115 | Ex_Exp;ctrl_Exp   |
| PE(O-16:1_16:1)    | 0.0396 | ctrl_Exp          |
| PG(22:6_22:6)      | 0.0039 | Ex_Exp;ctrl_Exp   |

|                    |        |                         |
|--------------------|--------|-------------------------|
| PI(18:1_22:6)      | 0.0473 | ctrl_ExP;Ex_ExP         |
| TG(16:0_22:6_22:6) | 0.0237 | Ex_ExP;ctrl_ExP         |
| PC(47:6)           | 0.0258 | Ex_ExP;ctrl_ExP         |
| PC(18:1_20:3)      | 0.0078 | Ex_ExP;ctrl_ExP         |
| PE(18:0_20:3)      | 0.0443 | ctrl_ExP;Ex_ExP         |
| PE(O-17:1_22:6)    | 0.0182 | Ex_ExP;ctrl_ExP         |
| PC(O-14:0_22:7)    | 0.0118 | ctrl_ExP;Ex_ExP         |
| PA(18:0_22:4)      | 0.0392 | ctrl_ExP;Ex_ExP         |
| PS(O-16:1_22:6)    | 0.0314 | ctrl_ExP;Ex_ExP         |
| PC(20:2_22:6)      | 0.0078 | Ex_ExP;ctrl_ExP         |
| PE(P-18:1_20:5)    | 0.0410 | ctrl_ExP;Ex_ExP         |
| PI(16:0_20:5)      | 0.0349 | ctrl_ExP;Ex_ExP         |
| PS(22:1_22:6)      | 0.0320 | Ex_ExP;ctrl_ExP         |
| PS(42:4)           | 0.0448 | Ex_ExP;ctrl_ExP         |
| PI(17:0_22:6)      | 0.0258 | ctrl_ExP;Ex_ExP         |
| PS(22:6_22:6)      | 0.0188 | Ex_ExP;ctrl_ExP         |
| PC(O-14:0_20:5)    | 0.0205 | ctrl_ExP;Ex_ExP         |
| PC(O-42:5)         | 0.0174 | Ex_ExP;ctrl_ExP         |
| PG(25:8_18:1)      | 0.0392 | Ex_ExP;Ex_ctrl          |
| PS(16:0_22:5)      | 0.0152 | ctrl_ExP;Ex_ExP         |
| PG(23:6_18:0)      | 0.0118 | ExP_ctrl;Ex_ctrl        |
| PG(18:0_25:6)      | 0.0256 | Ex_ctrl;Ex_ExP          |
| PC(18:1_18:2)      | 0.0104 | Ex_ctrl;Ex_ExP          |
| PC(O-46:8)         | 0.0020 | ExP_ctrl;Ex_ctrl;Ex_ExP |
| Cer(d18:0_16:0)    | 0.0256 | ExP_Ex;ExP_ctrl         |
| PA(O-28:6_20:4)    | 0.0102 | ExP_ctrl;Ex_ctrl;Ex_ExP |

\*: The ANOVA test was performed for significance analysis, followed by post hoc Tukey's HSD test. The Benjamini-Hochberg adjusted p value that less than or equal to 0.05 was considered as significance. Ex: expanded without applying proteinase K, ExP: expanded with applying proteinase K, ctrl: Control (without expansion).

**Supplementary Table 9. The LC-MS gradient used for the metabolomics analysis\***

| Time | Flow rate (mL/min) | %B  |
|------|--------------------|-----|
| 0    | 0.2                | 100 |
| 8    | 0.2                | 100 |
| 30.8 | 0.2                | 70  |
| 38   | 0.2                | 40  |
| 41   | 0.2                | 30  |
| 51   | 0.2                | 100 |
| 68   | 0.2                | 100 |

\*: The mobile phase A: water with 10mM ammonium formate and 0.125% formic acid. The mobile phase B: acetonitrile/water (95/5, v/v) with 10mM ammonium formate and 0.125% formic acid. Column: Waters Acquity UPLC BEH Amide column (150 mm× 2.1 mm, 1.7 μm). Temperature: 45 °C. Flow rate: 200 μL/min.

**Supplementary Table 10. The LC-MS gradient used for the proteomics analysis\***

| Time   | Flow rate (nL/min) | %B |
|--------|--------------------|----|
| 0      | 200                | 2  |
| 7.4    | 200                | 8  |
| 88.8   | 200                | 27 |
| 118.4  | 200                | 42 |
| 119.4  | 200                | 99 |
| 124.95 | 200                | 99 |
| 126.3  | 200                | 99 |
| 130    | 200                | 50 |

\*: The mobile phase A: water with 0.1% formic acid. The mobile phase B: acetonitrile/water (80/20, v/v) with 0.1% formic acid. Column: Aurora Ultimate XT 25×75 C18 UHPLC column (25 cm× 75 μm, 1.7 μm). Temperature: 35 °C. Flow rate: 200 nL/min.



## Supplementary Note 1

### Comparison among TEMI, GAMSI, and Ex-MSI

Sample preparation of GAMSI (middle) and Ex-MSI (right) were strictly following the literature of *Nat Commun* 15, 5036 (2024) and *CCS Chemistry* 6, 2662–2670 (2024), respectively. Overall, TEMI outperforms the other two methods—severe cracks (as indicated by the white arrows in a) were observed in the expanded tissue prepared by GAMSI and Ex-MSI methods. We found the tissue samples cracking in GAMSI and Ex-MSI was associated with the drying of the thick tissue-gel material on the ITO slide (a required step for MS imaging), as no cryosection was performed following tissue expansion in these two methods. In addition, chemical delocalization was found in both GAMSI and Ex-MSI, with more severe chemical diffusion observed in Ex-MSI. The chemical diffusion may be associated with proteolysis digestion of the tissue during expansion treatment, where GAMSI used a 2-day trypsin digestion in PBS buffer treatment, and Ex-MSI used 3-hour Proteinase K digestion at 60 °C for more extensive tissue digestion during sample preparation. It is worth mentioning that, since the GAMSI and Ex-MSI experiments strictly adhered to the published studies (refs. GAMSI, *Nat Commun* 15, 5036 (2024) and Ex-MSI, *CCS Chemistry* 6, 2662–2670 (2024)), the sample preparation strategies were different among the different methods. Certain important experimental conditions, such as thickness of the tissue slice (used for tissue expansion), MALDI matrix and laser energy, were different among different methods. Specifically, for MALDI matrix used in **Figure S5**, 9-AA matrix was used in GAMSI, NEDC matrix was used in Ex-MSI, and DAN matrix was used in TEMI for the control and corresponding expanded tissue samples; the laser energy was adjusted based on their MALDI matrix applied. For the thickness of the tissue slices, GAMSI used ~25  $\mu\text{m}$ -thick mouse brain slices, while Ex-MSI used 50  $\mu\text{m}$ -thick mouse brain slices for tissue expansion. Protease digestion was included in GAMSI and Ex-MSI, where GAMSI used a 2-day trypsin digestion in PBS buffer, and Ex-MSI used 3-hour Proteinase K digestion at 60 °C during sample preparation. Neither GAMSI nor Ex-MSI performed cryosection after tissue expansion; instead, the expanded tissue-hydrogel was directly dried on the ITO slide. In contrast, TEMI used a different strategy—a 300  $\mu\text{m}$ -thick tissue slice was subjected to tissue expansion without any harsh denaturation (e.g., detergent treatment, protease digestion, or heating), and the expanded tissue-hydrogel was subjected to cryosection to obtain robust tissue sections for MS imaging. Thus, all these different conditions could contribute to the signal variations observed among the three methods.

## Supplementary Note 2

### Measurement of expansion non-uniformity.

Expansion non-uniformity is quantified as the deviation of point-to-point distance measurements from the expected values under the assumption of uniform expansion. For any pair of points in the expanded image, the measurement error is defined as the difference between the observed distance between the points and the distance that would have been expected if the expansion were uniform. These values are calculated using the code deposited at the end of this supplementary information. The expanded tissue image was registered to the pre-expansion tissue image using landmarks manually picked with BigWarp. The landmarks are exported as a csv file ("landmarks.csv"). The landmarks file is used by the Fiji script "createSimOrigAndThinPlateCoords.groovy" to generate three arrays of coordinates: one tiling the pre-expansion specimen space, one tiling the post-expansion specimen space assuming uniform expansion, and a third corresponding to the true nonrigid deformation of the expansion process, using a thin plate spline model. The MATLAB script "AllParts\_measurement\_error\_analysis\_createMask.m" compares the true coordinate array with the hypothetical uniform coordinate array to calculate the measurement errors for all pairs of points. These errors are then binned based on measurement length, and the mean and standard deviation of the measurements are presented as a function of measurement length shown in Figure S7. The Fiji script "bigwarpSimilarityPart.groovy" uses the "landmarks.csv" file to extract the average expansion factor for each specimen, which the script reports as the average scale of the non-rigid deformation.

## Supplementary Note 3

### Codes for Figure S7 and expansion factor

#### Fiji script: createSimOrigAndThinPlateCoords.groovy

```
import java.io.*
import java.nio.file.*
import java.util.*
import bigwarp.landmarks.*
import bigwarp.transforms.*
import net.imglib2.realtransform.*
import net.imglib2.realtransform.inverse.*

//USER: modify path to correspond to folder containing landmarks.csv for one pair of images
def homePath = "C:/Users..."
def exptFolderName = "/test"
def folderPath = homePath + exptFolderName

// Create coords_similarity, using the max and min x and y values of the landmarks as a bounding rectangle
// This confines the analyzed area to the minimum rectangular bounding box that contains all of the
// manually picked landmarks
def landmarksFile = new File(folderPath, 'landmarks.csv')
def data = landmarksFile.readLines().collect { line ->
    def values = line.split(',')
    return values[2..-1].collect { it.toDouble() } // Skip the first two columns
}

def pointsFixed = data.collect { [it[2], it[3]] }
def pointsMoving = data.collect { [it[0], it[1]] }

def XMax = Math.ceil(pointsFixed.collect { it[0] }.max()).intValue()
def XMin = Math.floor(pointsFixed.collect { it[0] }.min()).intValue()
def YMax = Math.ceil(pointsFixed.collect { it[1] }.max()).intValue()
def YMin = Math.floor(pointsFixed.collect { it[1] }.min()).intValue()
def XRange = XMax - XMin
def YRange = YMax - YMin

def dXYstep = (int) Math.round(Math.sqrt(XRange * YRange) / 30)

def Xpoints = (XMin..(XMax + dXYstep)).step(dXYstep)
def Ypoints = (YMin..(YMax + dXYstep)).step(dXYstep)
def xyzSim = []
Xpoints.each { x ->
```

```

Ypoints.each { y ->
  xyzSim << [x, y]
}
}

// Write to CSV
def outputFile = new File(folderPath, 'coords_similarity.csv')
def writer = new FileWriter(outputFile)
xyzSim.each { coords ->
  writer.write(coords.join(',') + '\n')
}
writer.close()

def buildTransform(File landmarksPath, String transformType, int nd, boolean needInverse, double
invTolerance, int maxIters) {
  LandmarkTableModel ltm = new LandmarkTableModel(nd)
  try {
    ltm.load(landmarksPath)
  } catch (IOException e) {
    e.printStackTrace()
    return null
  }

  BigWarpTransform bwTransform = new BigWarpTransform(ltm, transformType)
  RealTransform xfm = bwTransform.getTransformation()

  if (xfm instanceof Wrapped2DTransformAs3D)
    xfm = ((Wrapped2DTransformAs3D) xfm).getTransform()

  if (needInverse) {
    if (transformType.equals("Thin Plate Spline")) {
      xfm.getOptimizer().setMaxIters(maxIters)
      xfm.getOptimizer().setTolerance(invTolerance)
    }
    xfm = xfm.inverse()
  }

  return xfm
}

def transformPoints(File landmarksPath, File inCsv, File outCsv, String inverseOrForward,

```

```

        String transformType, double invTolerance, int invMaxIters,
        boolean csvHasHeader, double sx, double sy, double sz) {

    boolean needInverseTransform = inverseOrForward.equals("Moving to target")

    // Read the input points
    List<String> lines
    try {
        lines = Files.readAllLines(Paths.get(inCsv.getAbsolutePath()))
    } catch (IOException e) {
        e.printStackTrace()
        return
    }

    // Get the transformation to apply
    int nd = lines.get(0).split(",").length
    RealTransform transform = buildTransform(landmarksPath, transformType, nd, needInverseTransform,
    invTolerance, invMaxIters)

    // Transform all points
    List<String> outputLines = []
    double[] result = new double[nd]
    boolean firstLine = true
    for (String l : lines) {
        // Add the first line to the output if it's a header
        if (firstLine && csvHasHeader) {
            outputLines.add(l)
            firstLine = false
            continue
        }

        // Parse line
        double[] pt = Arrays.stream(l.split(",")).mapToDouble(Double::parseDouble).toArray()
        double[] scale = [sx, sy, sz] as double[]

        // Elementwise multiplication of pt and scale
        double[] scaledpt = new double[pt.length]
        for (int i = 0; i < pt.length; i++) {
            scaledpt[i] = pt[i] * scale[i]
        }

        // Transform point
        try {

```

```

        transform.apply(scaledpt, result)
    } catch (Exception e) {
        System.err.println("Warning: failed to transform " + Arrays.toString(scaledpt))
        Arrays.fill(result, Double.NaN)
    }

    outputLines.add(Arrays.toString(result).replaceAll("[\\[\\]]", "").replaceAll(" ", ""))
}

// Write output
try {
    Files.write(Paths.get(outCsv.getAbsolutePath()), outputLines)
} catch (IOException e) {
    e.printStackTrace()
}
}

```

#### **MATLAB script: AllParts\_measurement\_error\_analysis\_createMask.m**

% this code was adapted from the code displaying deformation fields  
 % used in Jurriens, D., van Batenburg, V., Katrukha, E. A., & Kapitein, L. C. (2020). Mapping the neuronal  
 cytoskeleton using expansion microscopy. In Methods in Cell Biology.  
<https://doi.org/10.1016/bs.mcb.2020.04.018>

```

homeFolder = "C:\Users\...";
experimentFolderName = "\test";
experimentFolder = homeFolder + experimentFolderName;

runPart = 2;

% Instructions:
% 1) generate registration landmarks manually using BigWarp and save them
% as landmarks.csv
% 2) run createSimOrigAndThinPlateCoords.groovy, with the "folderPath"
% updated to correspond to the folder containing landmkars.csv
% 3) update "experimentFolder" above to correspond to the same folder and
% run this script to use "coords_thin_plate.csv" to calcualte the
% deformation field and measurement errors matrix.

% change runPart to 1 to manually choose analysis steps
% to run below if needed

previousDir = pwd;

```



```

cd(experimentFolder);

%% stages of analysis (1 = run, 0 = skip):

if runPart == 1 %manually set individual parts
    calculateDeformationField = 0;
    collectMeasurements = 0;
    measureErrors = 0;
elseif runPart == 2
    calculateDeformationField = 1;
    collectMeasurements = 1;
    measureErrors = 1;
end

%% settings:

% number of dimensions in the nonrigid transformation (2D vs 3D data).
% only 2D supported by this script
nD = 2;

%% size of expanded (target) image in scaled units (e.g. um)
detectSize = 1;

if detectSize
    landmarks=importdata('landmarks.csv');
    if size(landmarks.data,2) == 4
        nD = 2;
    elseif size(landmarks.data,2) == 6
        disp('error: detectSize not set up for 3D');
    else
        disp('error: landmarks.data should have 4 or 6 columns');
    end

    pointsFixed = landmarks.data(:,3:4);
    pointsMoving = landmarks.data(:,1:2);
    XMax = ceil(max(pointsFixed(:,1)));
    XMin = floor(min(pointsFixed(:,1)));
    YMax = ceil(max(pointsFixed(:,2)));
    YMin = floor(min(pointsFixed(:,2)));
    ZMax = 1;
    XRange = XMax - XMin;
    YRange = YMax - YMin;

```

```

else % manually set the size of the expanded image
    XMax=8178;
    YMax=7028;
    XMin=0;
    YMin=0;
    XRange = XMax - XMin;
    YRange = YMax - YMin;
    ZMax=1;
    if nD == 2; ZMax=1; end
end

%%
%image sampling: increase the step size to sample the image more sparsely
dXYstep = round(sqrt(XRange*YRange)/30);
dZstep = 1;

% create a mask using the convex hull containing all landmarks
bUseMask = 1;

%step size along meas length axis in final plot. This controls how much
%binning is done before displaying measurement error plot
%in scaled units
dMeasStep = 10;

%% calculateDeformationField
% generate a vector field that indicates the nonrigid component of
% transformation from post-expansion image to ideal uniformly expanded
% coordinates
% optional: export slices of the deformation field

if calculateDeformationField

    %number of tiff images in the mask sequence (# of Z planes)
    nFR=floor(ZMax / dZstep);
    % whether to export deformation field in XY as z-stack
    bExportXYdefField=true;

    % read coordinates from similarity array
    xySim=importdata('coords_similarity.csv');
    sz=size(xySim);

    boolmarks=zeros(sz(1),1);

```

```

if (bUseMask)
    %generate mask using convex hull of landmarks
    hullIndices = convhull(pointsFixed(:,1), pointsFixed(:,2));
    in_hull = inpolygon(xySim(:,1), xySim(:,2), pointsFixed(hullIndices,1), pointsFixed(hullIndices,2));
    boolmarks = double(in_hull); % Convert logical to numeric
else
    boolmarks=boolmarks+1;
end

% import transformed coordinates in thin plate
xySpline=importdata('coords_thin_plate.csv');

%vector difference between coordinates in 'similarity' and 'thin plate'
diffvect=xySim-xySpline;

% calculate absolute length of deformation vectors
totNFilt=sum(boolmarks);
displall=zeros(totNFilt,1);
displx=zeros(totNFilt,1);
displz=zeros(totNFilt,1);
count=0;
for i=1:sz(1)
    if(boolmarks(i))
        count=count+1;
        displall(count)=norm(diffvect(i,:));
        displx(count)=norm(diffvect(i,1:2));
        if nD == 3
            displz(count)=abs(diffvect(i,3));
        end
    end
end

%export deformation field in 2D (XY)
if (bExportXYdefField)
    h1=figure;
    if nD==3
        filt=(abs(xySim(:,3)-k)<0.00001);
        filt=filt&boolmarks;
    else
        filt = 1&boolmarks;
    end
end

```

```

xy=xySpline(filt,1:2);
xyvect=diffvect(filt,1:2);

quiver(xy(:,1),xy(:,2),xyvect(:,1),xyvect(:,2),'AutoScale','off');

axis equal
set(gca,'Ydir','reverse');
xlim([0 XMax]);
ylim([0 YMax]);
set(gcf,'Units','pixels');
set(gcf,'Position',[20 20 600 500]);
set(gca,'Units','pixels');
set(gca,'Position',[20 20 550 550]);
saveas(gcf, strcat('XY_sim_thinpl.png'));
end

end

%% collectMeasurements
%for each pair of points in the image, find the correct distance between
%them assuming uniform expansion (similarity transformation). Find the
%'measured' distance between them (after nonrigid transformation). The
%difference between these lengths is the measurement error for that pair of
%points

if collectMeasurements
    boolmarksDec = boolmarks;

    nPointsDec = sum(boolmarksDec);

    xySimDec = NaN(nPointsDec,nD);
    diffvectDec = NaN(nPointsDec,nD);
    j = 1;

    for i = 1:length(boolmarks)
        if boolmarksDec(i)
            xySimDec(j,:) = xySim(i,:);
            diffvectDec(j,:) = diffvect(i,:);
            j = j + 1;
        end
    end
end
if j ~= nPointsDec + 1

```

```

    disp('error: indices do not match!!');
end

nMeasDec = nPointsDec * (nPointsDec - 1);
allMeasurements = NaN(nMeasDec,3);
%col 1 is meas length
%col 2 is meas length bin (i.e. which index in measLengths array)
%col 3 is meas error
%one row for every measurment (i.e. for every pair of points in the
%masked region)

measNum = 1;

for i = 1:nPointsDec
    for j = 1:nPointsDec
        if i ~= j
            measLength = norm(xySimDec(j,:)-xySimDec(i,:));
            measError = norm(diffvectDec(j,:)-diffvectDec(i,:));
            allMeasurements(measNum,:) = [measLength NaN measError];
            measNum = measNum + 1;
        end
    end
end
end

%% measureErrors
%bucket measurement errors by measurement length, and find the average and
%S.D. for each measurement length bucket

if measureErrors

    %compute bucket number for each length measurement
    allMeasurements(:,2) = round(allMeasurements(:,1)/dMeasStep + 0.5);

    maxMeas = max(allMeasurements(:,1));
    measLengths = dMeasStep/2:dMeasStep:ceil(maxMeas+dMeasStep);
    %measLength 0 only exists if dMeasStep>min pix spacing after decimation
    %in any direction

    measErrMtx = zeros(length(measLengths),4);
    measErrMtx(:,1) = measLengths;
    %each row is a measurement length bucket
    %for row n:

```

```

%col 1: middle of bucket n (bucket 1 starts at measLength 0)
%col 2: number of measurements in this bucket
%col 3: average of measurement errors for this bucket
%col 4: std dev of measurement errors for this bucket

%add up number of measurements (col 2) and average error (col 3) for each bucket
nMeasDec = size(allMeasurements,1);

for i = 1:nMeasDec
    bucket = allMeasurements(i,2);
    measErrMtx(bucket,2) = measErrMtx(bucket,2) + 1;
    measErrMtx(bucket,3) = measErrMtx(bucket,3) + allMeasurements(i,3);
end
measErrMtx(:,3) = measErrMtx(:,3)./measErrMtx(:,2); %error is inf when N = 0

%add up SD (col 4) for each bucket
for i = 1:nMeasDec
    bucket = allMeasurements(i,2);
    %sum up squared difference between measurement error and average error
    difference = allMeasurements(i,3)-measErrMtx(bucket,3);
    measErrMtx(bucket,4) = measErrMtx(bucket,4) + difference^2;
end
measErrMtx(:,4) = sqrt(measErrMtx(:,4)./measErrMtx(:,2)); %div by N and take sqrt

if nMeasDec ~= sum(measErrMtx(:,2))
    disp('Error: nMeasDec does not equal sum(measErrMtx(:,2))')
end

figure;
hold on;

% Filter out NaNs
iKeep = ~isnan(measErrMtx(:,3));

% Define the upper and lower bounds for the shaded area (mean  $\pm$  S.D.)
upperBound = measErrMtx(iKeep,3) + measErrMtx(iKeep,4);
lowerBound = measErrMtx(iKeep,3) - measErrMtx(iKeep,4);
% Plot the mean line
plot(measErrMtx(iKeep,1), measErrMtx(iKeep,3), 'b', 'LineWidth', 1.5);
% Create the shaded area
xValues = measErrMtx(iKeep,1); % x-axis values
fill([xValues; flipud(xValues)], [upperBound; flipud(lowerBound)], 'b', 'FaceAlpha', 0.2, 'EdgeColor', 'none');

% Add labels and legend if needed

```



```

xlabel('Measurement Distance (μm)', 'FontName', 'Arial', 'FontSize', 12);
ylabel('Measurement Error (μm)', 'FontName', 'Arial', 'FontSize', 12);

legend('Mean', 'Standard Deviation');
set(gca, 'FontName', 'Arial', 'FontSize', 12);
hold off;

% Extract x-axis and mean y values
xValues = measErrMtx(iKeep, 1);    % x-axis values
meanValues = measErrMtx(iKeep, 3); % mean values for y-axis

% Combine x and y mean values into a single matrix
meanData = [xValues, meanValues];

% Save to a CSV file
csvwrite('mean_points.csv', meanData); % Saves to a file named 'mean_points.csv'

end

cd(previousDir)

```

### **Fiji script: bigwarpSimilarityPart.groovy**

```

#@ File (label="Landmark file") landmarksPath
#@ Integer (label="Number of dimensions", value=2) nd
#@ String (label="Direction", choices={"Forward", "Inverse" }, value="Forward") direction

```

```

import mpicbg.models.*;
import bigwarp.landmarks.LandmarkTableModel;
import net.imglib2.util.*;

```

```

// load landmarks
tableModel = new LandmarkTableModel( nd );
try
{
    tableModel.load( landmarksPath );
} catch ( IOException e )
{
    e.printStackTrace();
    return;
}

```

```

def getModel( final int numDims )
{

```

```

    if( numDims == 2 )
        return new SimilarityModel2D();
    else if( numDims == 3 )
        return new SimilarityModel3D();
    else
        return null;
}

/**
 * Determinant of a 2d transform stored as a row-major 3d homogeneous matrix array
 */
def det2d( final double[][] mtx )
{
    return (mtx[0][0] * mtx[1][1]) - (mtx[0][1] * mtx[1][0]);
}

/**
 * Determinant of a 3d transform stored as a row major 4d homogeneous matrix array
 */
def det3d( final double[][] mtx )
{
    return mtx[0][0] * mtx[1][1] * mtx[2][2] +
           mtx[1][0] * mtx[2][1] * mtx[0][2] +
           mtx[2][0] * mtx[0][1] * mtx[1][2] -
           mtx[0][2] * mtx[1][1] * mtx[2][0] -
           mtx[1][2] * mtx[2][1] * mtx[0][0] -
           mtx[2][2] * mtx[0][1] * mtx[1][0];
}

/**
 * Need this until Fiji's mpicbg versions are updated
 */
def double[][] toMatrix2d( final SimilarityModel2D model )
{
    a = new double[ 6 ];
    model.toArray( a );
    return [[ a[0], -a[1], a[4] ],
            [ a[1], a[0], a[5] ]] as double[][];
}

/**
 * Need this until Fiji's mpicbg versions are updated

```

```

*/
def double[][] toMatrix3d( final SimilarityModel3D model )
{
    mtx = new double[ 3 ][ 4 ];
    model.toMatrix( mtx );
    return mtx;
}

model = getModel( nd );
if( model == null )
{
    println( "Only works for 2 or 3 dimensions");
    return;
}

// fit the model
int numActive = tableModel.numActive();
int ndims = tableModel.getNumdims();

mvgPts = new double[ ndims ][ numActive ];
tgtPts = new double[ ndims ][ numActive ];

tableModel.copyLandmarks( mvgPts, tgtPts );

double[] w = new double[ numActive ];
Arrays.fill( w, 1.0 );

try {
    model.fit( mvgPts, tgtPts, w );
} catch (NotEnoughDataPointsException e) {
    e.printStackTrace();
} catch (IllDefinedDataPointsException e) {
    e.printStackTrace();
}

themodel = model;
if( direction.equals( "Inverse" ) )
{
    themodel = model.createInverse();
    println( themodel )
}

if( nd == 2 )

```

```

{
    det = det2d( toMatrix2d( themodel ));
    avgscale = Math.sqrt( det );
}
else if( nd == 3 )
{
    det = det3d( toMatrix3d( themodel ));
    avgscale = Math.cbrt( det );
}

println( 'determinant : ' + det );
println( 'avgerage scale : ' + avgscale );

```