Supporting Information

Systematic study of tissue section thickness for MALDI MS profiling and imaging

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Supporting information

Extraction all proteins from tissues and trypsin digestion.

The tissue was frozen in liquid nitrogen and ground into powder. 2 mL of lysate solution (1% sodium dodecyl sulfate, 100 mM ethylenediaminetetraacetic acid, and 0.1 mM phenylmethyl sulfonyl fluoride) was added into the tissue sample. The mixture was vibrated by pulsed ultrasonication for 5 min (ultrasonic 2 s/stop 3 s), then was kept at 4 °C for 1 h, followed by centrifugation at 13,000 r/min for 20 min. The supernatant was collected and ready for the trypsin digestion.

300 μL 0.15 M dithiotreitol solution was added to 150 μL protein extract (about 50-100 μg protein) and the mixture was incubated at 56 °C for 1 h. The mixture was then added to a filter centrifuge tube with 30 KDa molecular weight cutoff (Microcon YM-30, Merck Millipore, Ltd. Tullagreen, Ireland) and centrifuged at 14,000 g for 15 min. After centrifugation, the liquid in the tube was discarded.

For the following washing steps, centrifugation was all conducted at 14,000 g for 10-15 min and the liquid in the tube was discarded after each centrifugation if without mentioned. (1) 200 μL washing buffer UA (8 M urea, 0.1M Tris-HCl, pH 8.5) was added to the filter tube and centrifuged. (2) 100μL of 10 mg/mL iodoacetamide solution was added into the filter tube. It was stored in dark for 1h and then centrifuged. (3) The filter was washed twice by adding 100 μL UA and being centrifuged. (4) The filter was further washed twice by adding 100 μL NH_4HCO_3 and being centrifuged.

The outer filter tube was replaced by a new tube and 40 μL 0.05 mg/mL trypsin solution in 50 mM NH_4HCO_3 was added and the mixture was vortexed, followed by an incubation at 37 °C for 18 h and centrifugation. Additional 40 μL of NH_4HCO_3 solution was added to the filter tube and centrifuged to collect the residue peptides left on the filter. The eluted sample was desalted and lyophilized, then dissolved in 0.1% formic acid, followed by filtration.

LC-MS/MS detection of all peptide in tissues and establishment of whole peptide information database library

The whole process was largely followed the literature [1] . The peptides were resuspended in 0.1% formic acid (FA) and analyzed by a Orbitrap Exploris 480 mass spectrometer (ThermoFisher Scientific) coupled online to an Easy-nLC 1200 in the data-dependent mode. Briefly, 2 μL of peptide sample (1 μg/μL) was injected into a 25 cm long, 150μm inner diameter capillary analytic column packed with C18 particles of 1.9 μm diameter. The mobile phases for the LC included buffer A (2% ACN and 0.1% FA) and buffer B (98% ACN and 0.1% FA). The peptides were separated using a 90 min nonlinear gradient consisting of 3–8% B for 10 min, 8–20% B for 60 min, 20–30% B for 8 min, 30–100% B for 2 min, and 100% B for 10 min with a flow rate of 600 nL/min. The source voltage and current were set at 2.5 kV and 100 μA, respectively. All MS measurements were performed in the positive ion mode and acquired across the mass range of m/z 300–1800 Da. The fifteen most intense ions from each MS scan were isolated and fragmented by HCD. The mass spectrometry proteomics data have been deposited to the ProteomeX change Consortium (http://proteomecentral. proteomexchange.org) via the PRIDE partner repositorywith the dataset identifier PXD001246 [2]. The raw MS files were analyzed by the software MaxQuant version 1.4.1.2.

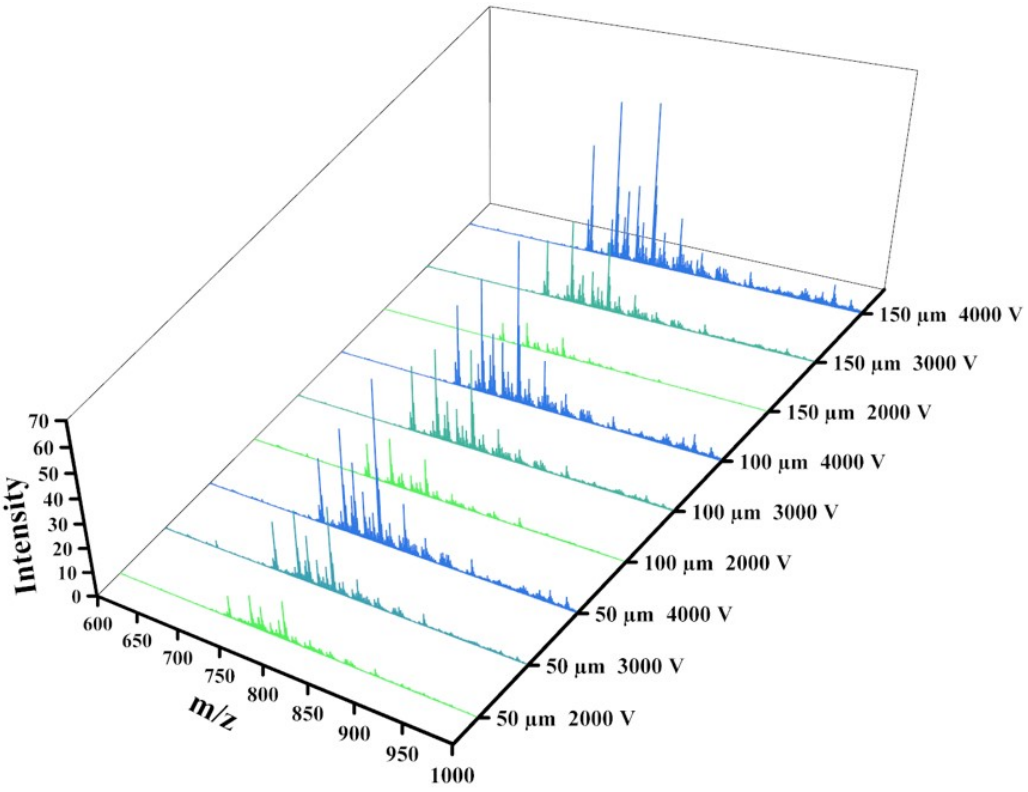

Figure S1. AP MALDI mean mass spectra of there different thickness consecutive mouse brain tissue section (50, 100, 150 µm) at different voltages (2000, 3000, 4000 V)

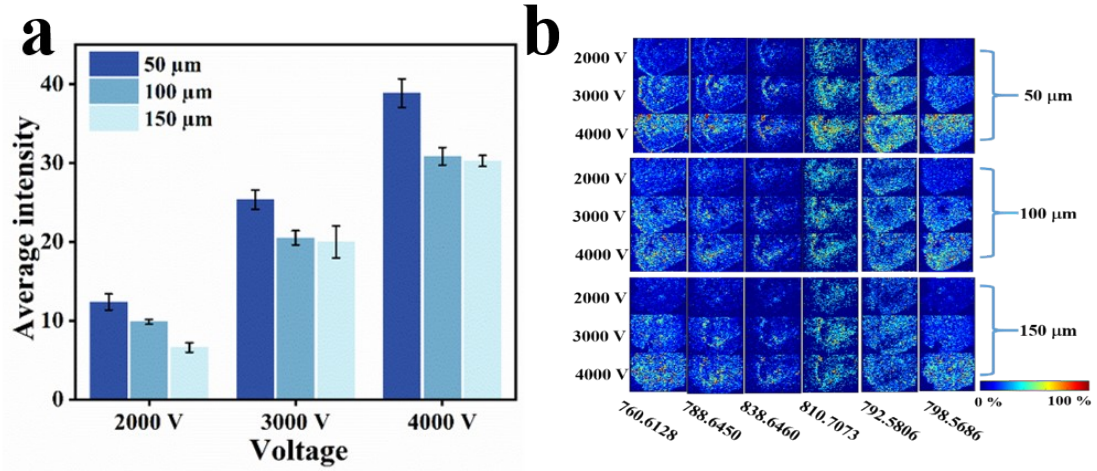

Figure S2. a. AP MALDI mean ions signal intensity **b.** AP MALDI MSI of there different thickness consecutive mouse half brain tissue section (50, 100, 150 µm) at different voltages(2000, 3000, 4000 V)

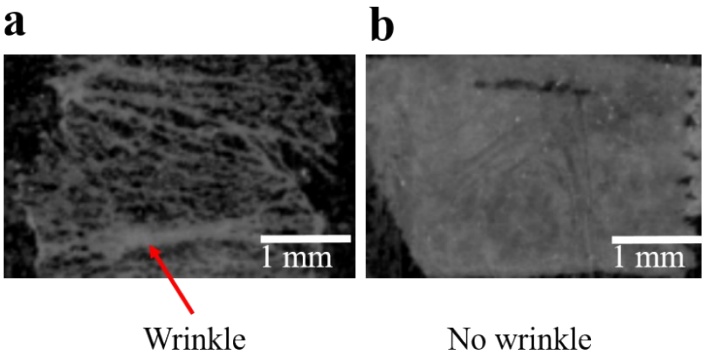

Figure S3. a. Optical images of the winkle tissues (0.4 μm) **b.** optical images of the no winkle tissues (10 μm)

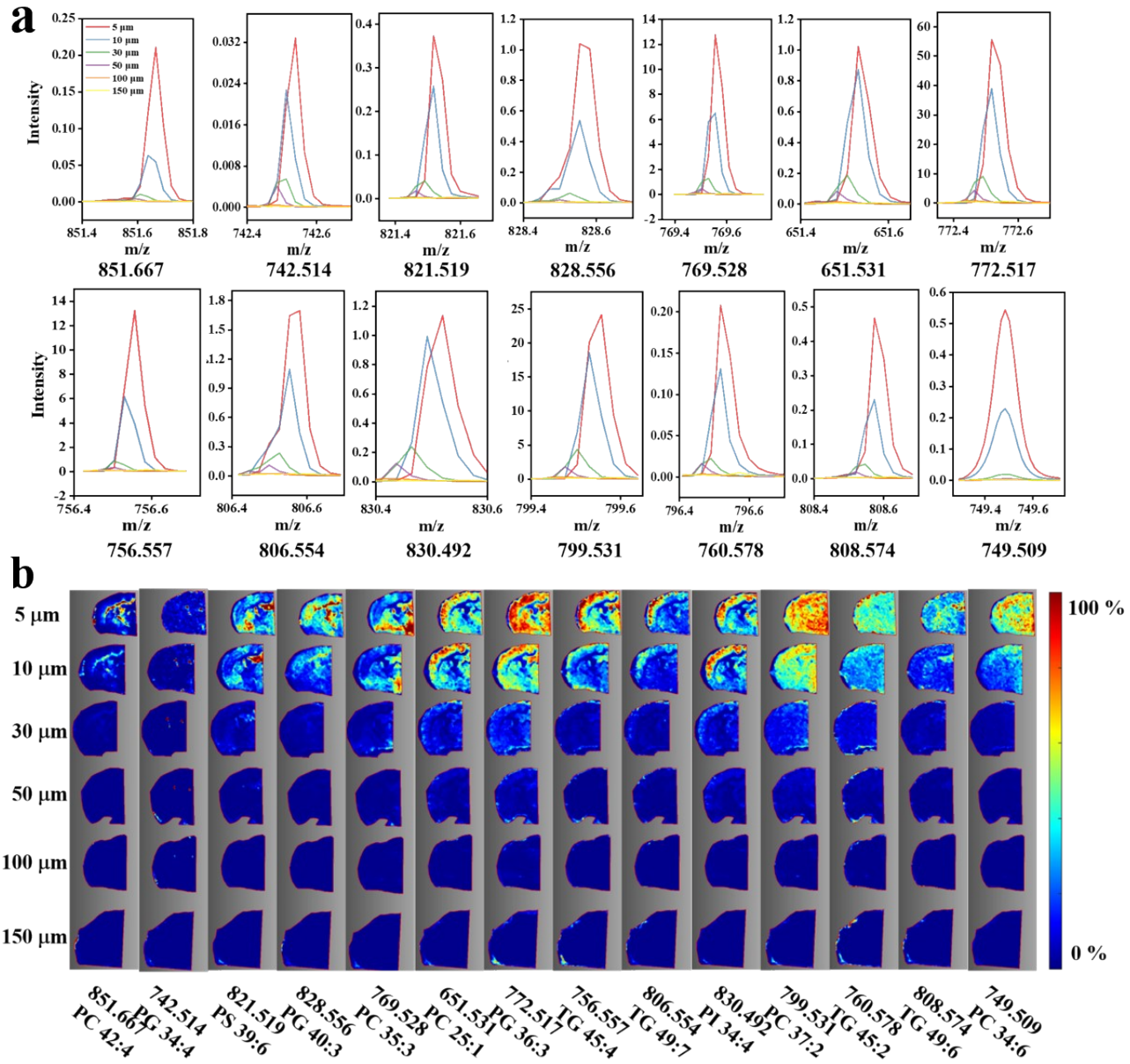

Figure S4. a. 14 representative lipids ions vacuum MALDI MS peaks acquired from continuous mouse half brain tissue section with different thicknesses: 5, 10, 30, 50, 100, 150 μm **b.** Vacuum MALDI MS images of the above 14 representative lipids.

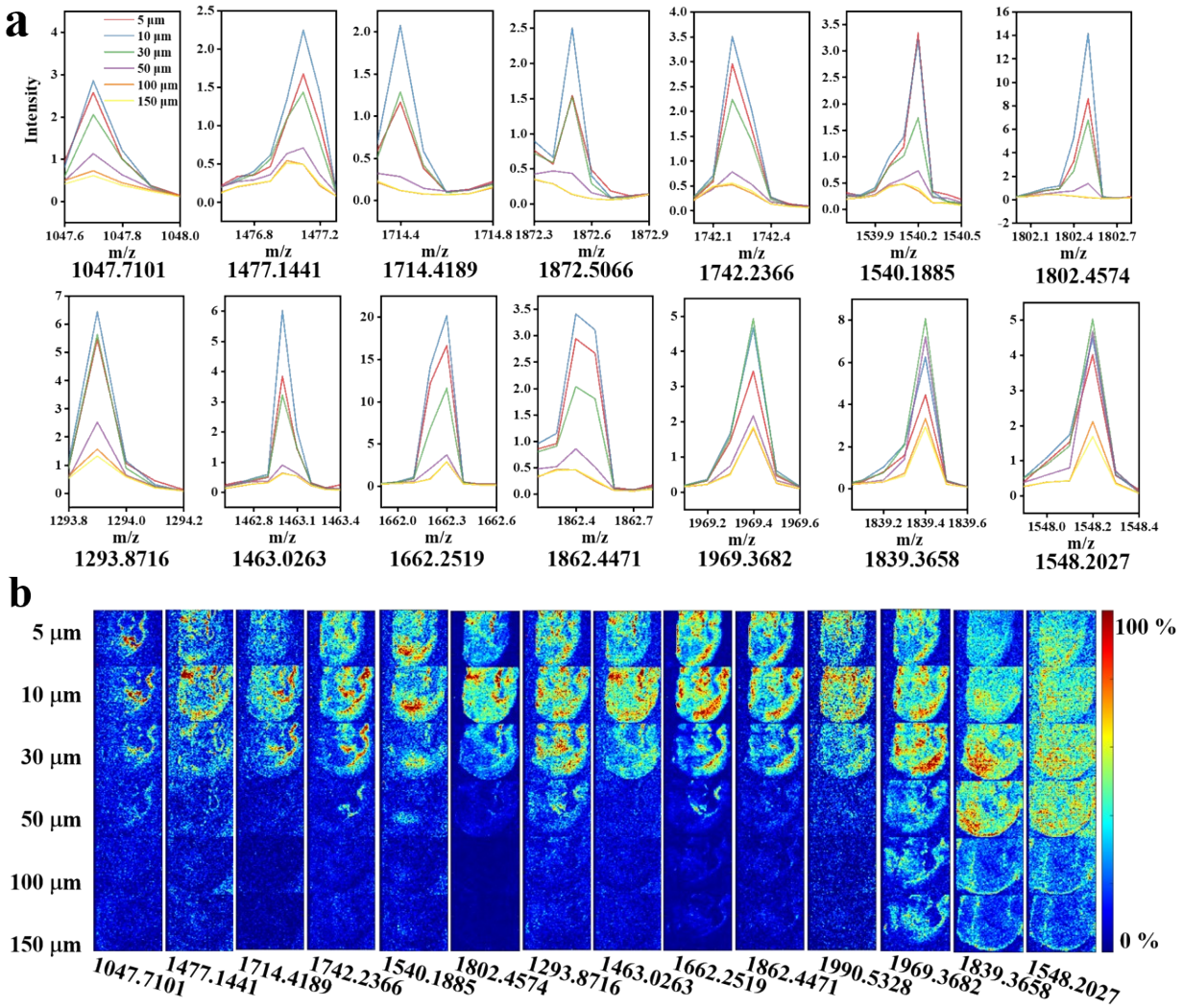

Figure S5. a. 14 representative peptide ions AP MALDI MS peaks acquired from continuous mouse half brain tissue section with different thicknesses: 5, 10, 30, 50, 100, 150 µm **b.** AP MALDI MS images of the above 14 representative peptides

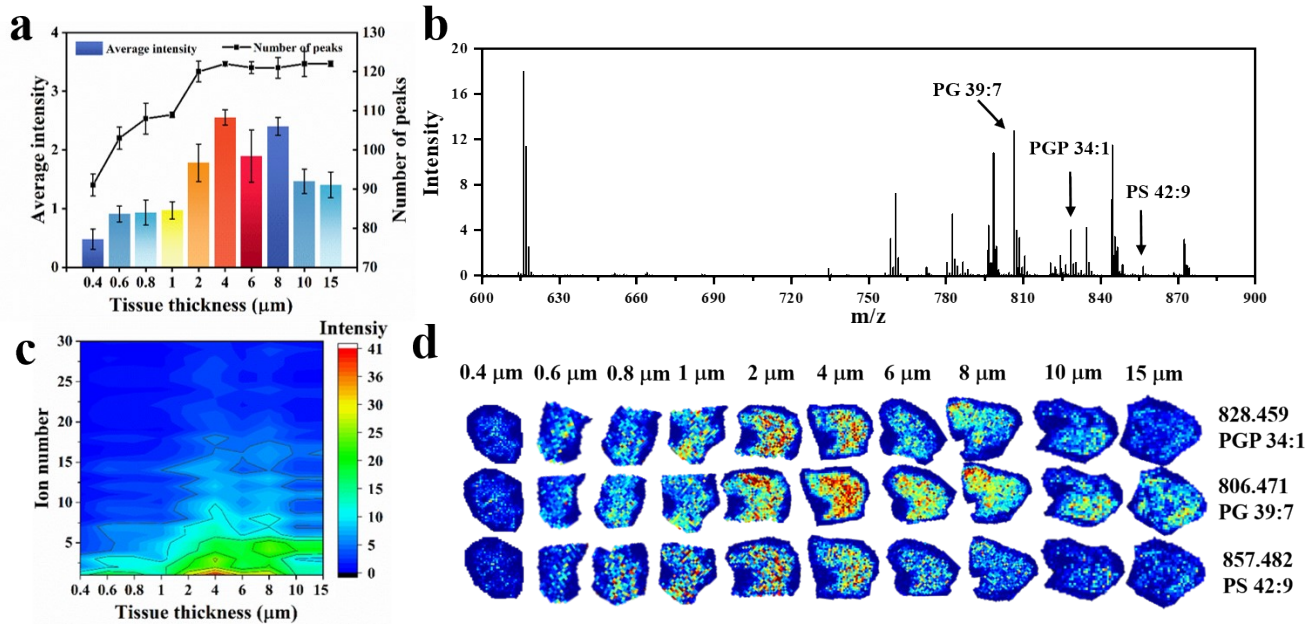

Figure S6. Vacuum MALDI MS data obtained from serial mouse heart tissue slices with different thicknesses. The area of section is about 4 square millimeters. **a.** Average signal intensity and number of lipid ions. **b.** Average mass spectra of all sections. **c.** Heatmap composed of 30 ions with the strongest signal intensity **d.** MALDI MS images of 6 representative lipids

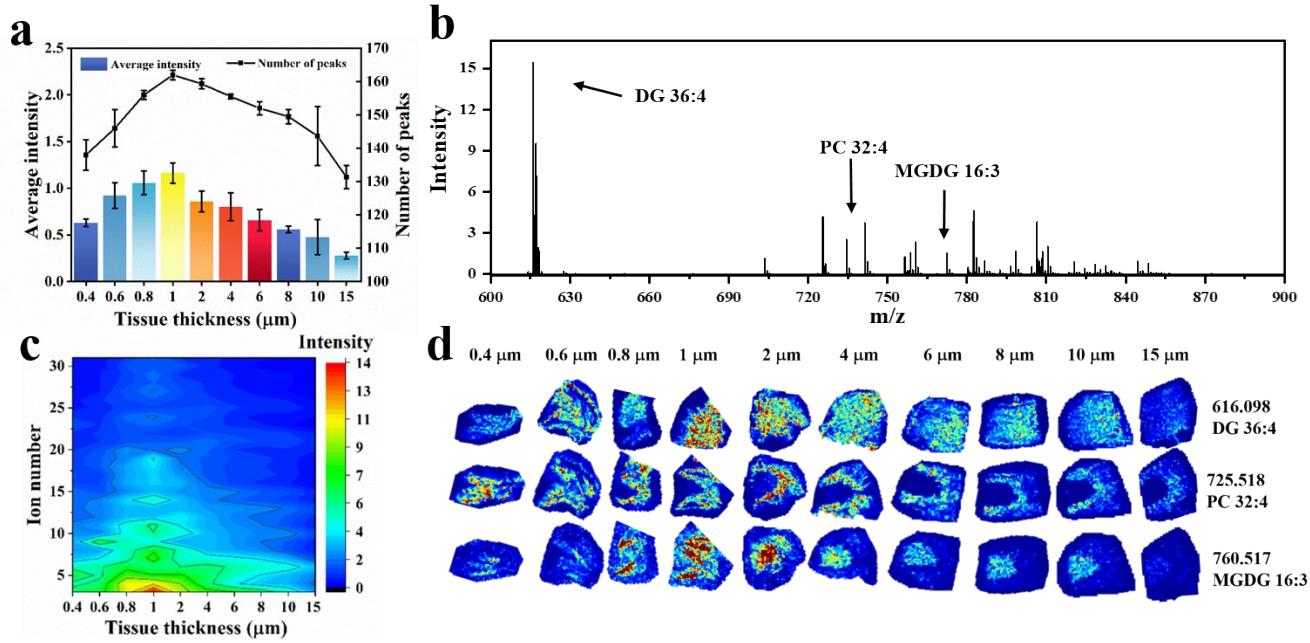

Figure S7. Vacuum MALDI MS data obtained from serial mouse liver tissue slices with different thicknesses. The area of section is about 4 square millimeters. **a.** Average signal intensity and number of lipid ions. **b.** Average mass spectra of all sections. **c.** Heatmap composed of 30 ions with the strongest signal intensity **d.** MALDI MS images of 6 representative lipids.

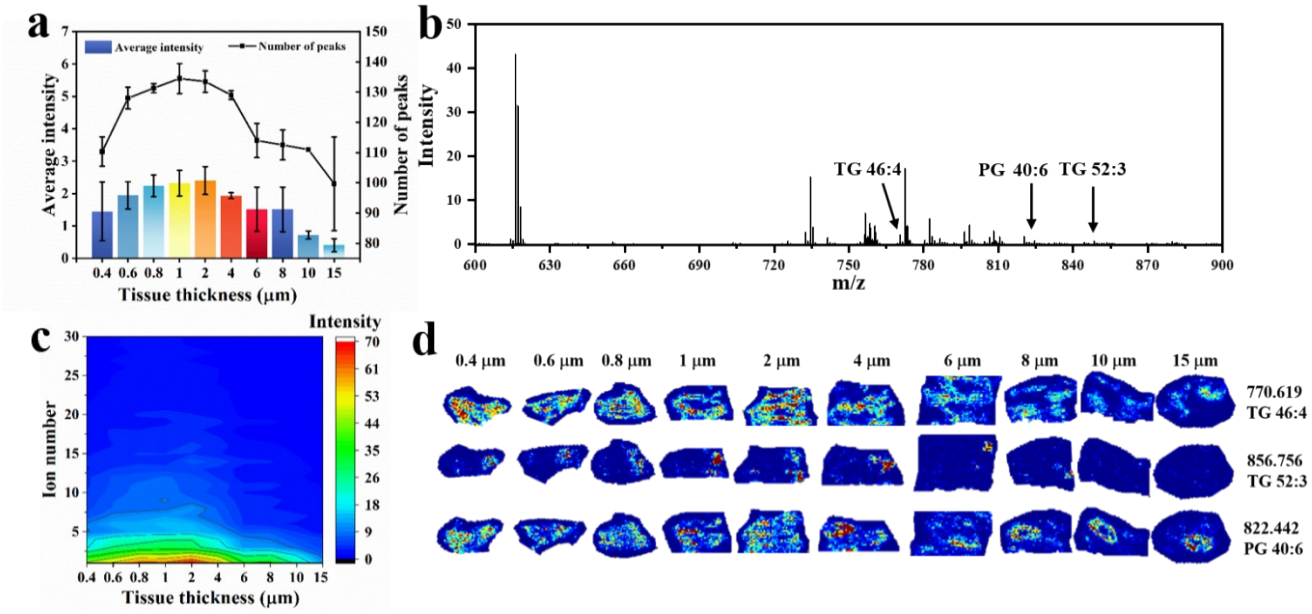

Figure S8. Vacuum MALDI MS data obtained from serial mouse lung tissue slices with different thicknesses. The area of section is about 4 square millimeters. **a.** Average signal intensity and number of lipid ions. **b.** Average mass spectra of all sections. **c.** Heatmap composed of 30 ions with the strongest signal intensity **d.** MALDI MS images of 6 representative lipids.

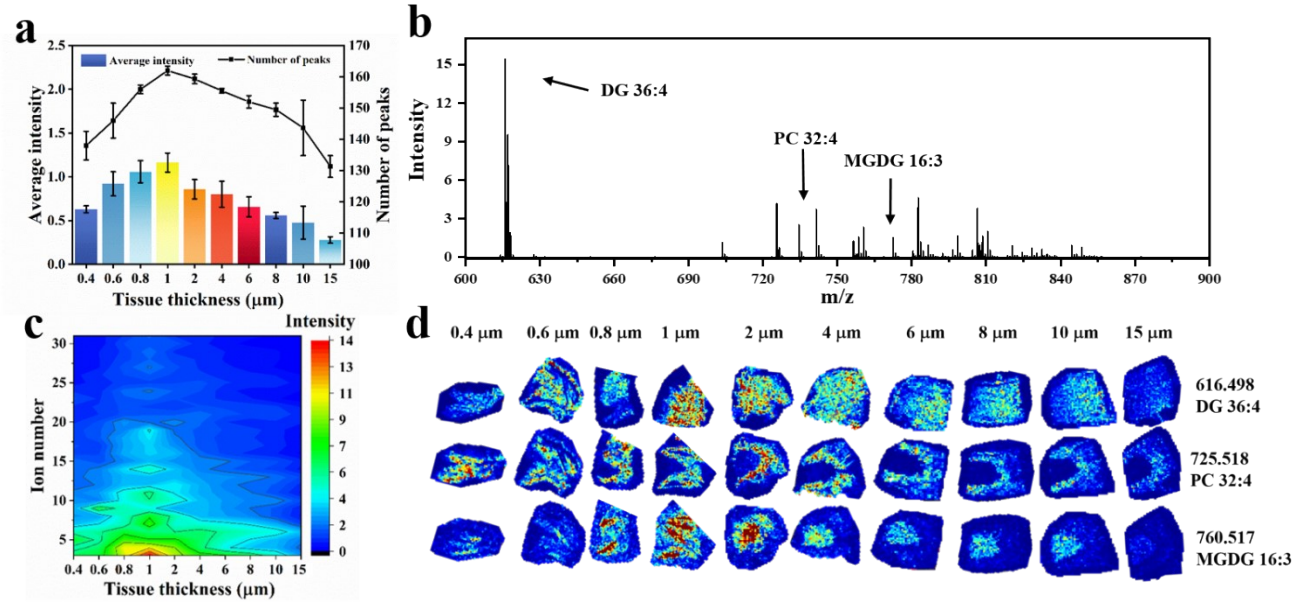

Figure S9. Vacuum MALDI MS data obtained from serial mouse kindy tissue slices with different thicknesses.The area of section is about 4 square millimeters. **a.** Average signal intensity and number of lipid ions. **b.** Average mass spectra of all sections. **c.** Heatmap composed of 30 ions with the strongest signal intensity **d.** MALDI MS images of 6 representative lipids.

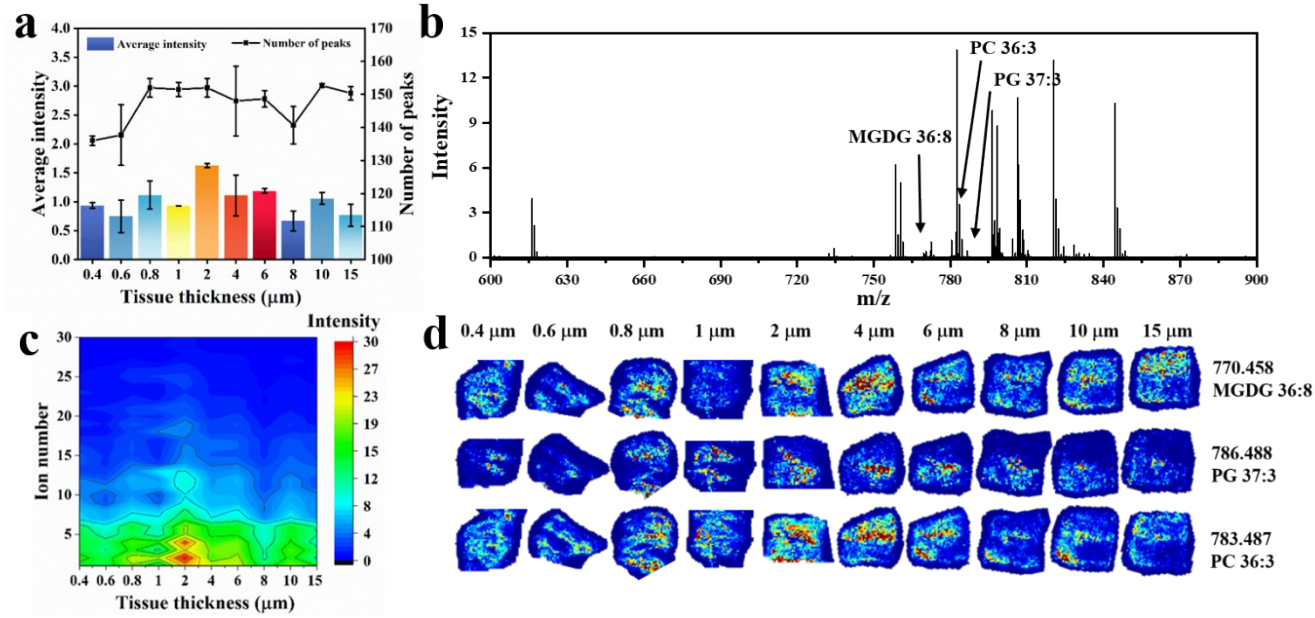

Figure S10. Vacuum MALDI MS data obtained from serial mouse muscel tissue slices with different thicknesses.The area of section is about 4 square millimeters. **a.** Average signal intensity and number of lipid ions. **b.** Average mass spectra of all sections. **c.** Heatmap composed of 30 ions with the strongest signal intensity **d.** MALDI MS images of 6 representative lipids.

Table S1. Percentage reduction in average peak intensity and peak number relative to 5 μm (%)

	Tissue thickness(μm)	10	30	50	100	150	Average reduction
Intensity	Vacuum	35.5	81.3	93.5	98.4	98.3	81.4
	AP	25.6	42.7	63.9	86.9	99.6	61.7
Number	Vacuum	5.5	21.3	75.6	91.5	97.0	58.1
	AP	3.0	9.9	30.0	82.2	95.1	41.0

Table S2. Representative lipids detected in mouse brain tissue section by AP MALDI MS in positive ion mode

m/z detected	Compound name	Molecular Formula	compound_id	exact Mass	delta
730.6560	DG 44:3	$C_{47}H_{86}O_5$	LMGL02010272	730.6475	-0.0084
735.7129	Cer 47:1	$C_{47}H_{93}NO_4$	LMSP02010106	735.7104	-0.0024
746.6524	TG 44:2	$C_{47}H_{86}O_6$	LMGL03012647	746.6424	-0.0099
748.6115	TG 44:1	$C_{47}H_{88}O_6$	LMGL03012646	748.6581	0.0465
765.6120	PC 36:5	$C_{44}H_{80}NO_7P$	LMGP01020058	765.5672	-0.0447

768.6391	TG 46:5	$C_{49}H_{84}O_6$	LMGL03012657	768.6268	-0.0123
769.6450	Cer 39:1	$C_{45}H_{87}NO_8$	LMSP0501AA31	769.6432	-0.0018
788.7041	TG 47:2	$C_{50}H_{92}O_6$	LMGL03012729	788.6894	-0.0147
788.7680	FA 53:1	$C_{53}H_{104}O_3$	LMFA01160026	788.7985	0.0305
792.6427	TG 48:7	$C_{51}H_{84}O_6$	LMGL03012833	792.6268	-0.0159
793.6627	PC 38:5	$C_{46}H_{84}NO_7P$	LMGP01020066	793.5985	-0.0641
798.7682	TG 48:4	$C_{51}H_{90}O_6$	LMGL03012671	798.6737	-0.0944
810.7718	TG 49:5	$C_{52}H_{90}O_6$	LMGL03012958	810.6737	-0.0980
822.8016	TG 50:6	$C_{53}H_{90}O_6$	LMGL03012782	822.6737	-0.1279
827.6511	PC 39:2	$C_{47}H_{90}NO_8P$	LMGP01011746	827.6404	-0.0107
838.7812	TG 51:5	$C_{54}H_{94}O_6$	LMGL03010081	838.705	-0.0762
847.7593	PS 41:0	$C_{47}H_{94}NO_9P$	LMGP03020065	847.6666	-0.0926
849.7370	PC 42:5	$C_{50}H_{92}NO_7P$	LMGP01030103	849.6611	-0.0758
865.7287	PC 42:4	$C_{50}H_{92}NO_8P$	LMGP01011804	865.6561	-0.0726
896.7771	TG 55:4	$C_{58}H_{104}O_6$	LMGL03010380	896.7832	0.0061

Table S3. Representative lipids detected in mouse brain tissue section by vacuum MALDI MS in positive ion mode

m/z detected	Compound name	Molecular Formula	LM ID	m/z exact	delta
616.498	DG 36:4	$C_{39}H_{68}O_5$	LMGL02010063	616.507	0.009
651.531	PC 25:1	$C_{33}H_{66}NO_9P$	LMGP01020279	651.448	-0.083
725.518	PC 32:4	$C_{40}H_{72}NO_8P$	LMGP01010499	725.500	-0.018
742.514	PG 34:4	$C_{40}H_{71}O_{10}P$	LMGP04010067	742.478	-0.036
749.509	PC 34:6	$C_{42}H_{72}NO_8P$	LMGP01010447	749.500	0.009
756.557	TG 45:4	$C_{48}H_{84}O_6$	LMGL03013198	756.627	0.070
760.517	MGDG 16:3	$C_{43}H_{68}O_{11}$	LMGL05010027	760.476	-0.041
760.578	TG 45:2	$C_{48}H_{88}O_6$	LMGL03013196	760.658	0.080
769.528	PC 35:3	$C_{43}H_{80}NO_8P$	LMGP01011423	769.562	0.034
770.619	TG 46:4	$C_{49}H_{86}O_6$	LMGL03012656	770.642	0.023
770.458	MGDG 36:8	$C_{45}H_{70}O_{10}$	LMGL05010017	770.497	0.039
772.433	PG 36:3	$C_{42}H_{77}O_{10}P$	LMGP04010136	772.525	0.092
772.517	PG 36:3	$C_{42}H_{77}O_{10}P$	LMGP04010136	772.525	0.008
783.487	PC 36:3	$C_{44}H_{82}NO_8P$	LMGP01010622	783.578	0.091
783.487	PC 36:3	$C_{44}H_{82}NO_8P$	LMGP01010622	783.578	0.091

786.488	PG 37:3	$C_{43}H_{79}O_{10}P$	LMGP04010189	786.541	0.053
796.475	FA 47:11	$C_{47}H_{72}O_{10}$	LMFA01031214	796.513	0.038
799.531	PC 37:2	$C_{45}H_{86}NO_8P$	LMGP01011428	799.609	0.078
806.471	PG 39:7	$C_{45}H_{75}O_{10}P$	LMGP04010277	806.510	0.039
806.554	TG 49:7	$C_{52}H_{86}O_6$	LMGL03013321	806.642	0.088
808.574	TG 49:6	$C_{52}H_{88}O_6$	LMGL03013294	808.658	0.084
820.473	PG 40:7	$C_{46}H_{77}O_{10}P$	LMGP04010043	820.525	0.052
821.519	PS 39:6	$C_{45}H_{76}NO_{10}P$	LMGP03010892	821.521	0.002
822.442	PG 40:6	$C_{46}H_{79}O_{10}P$	LMGP04010040	822.541	0.099
826.483	PG 40:4	$C_{46}H_{83}O_{10}P$	LMGP04010364	826.572	0.089
828.459	PGP 34:1	$C_{40}H_{78}O_{13}P_2$	LMGP05010001	828.492	0.033
828.556	PG 40:3	$C_{46}H_{85}O_{10}P$	LMGP04010338	828.588	0.032
830.492	PI 34:4	$C_{43}H_{75}O_{13}P$	LMGP06010035	830.495	0.003
850.491	SQDG 36:0	$C_{45}H_{86}O_{12}S$	LMGL05010062	850.584	0.093
851.667	PC 42:4	$C_{50}H_{94}NO_7P$	LMGP01020243	851.677	0.010
856.756	TG 52:3	$C_{55}H_{100}O_6$	LMGL03010099	856.752	-0.004
857.482	PS 42:9	$C_{48}H_{76}NO_{10}P$	LMGP03010619	857.521	0.039

Table S4. Peptides percentage reduction relative to 5 μm (%)

Tissue thickness(μm)	10	30	50	100	150	Average reduction
Average intensity	-2.7	21.1	49.1	67.3	74.2	41.8
Peak number	-1.6	21.8	37.5	68.7	76.6	40.6

Table S5. 14 representative peptides detected in mouse brain tissue section by AP MALDI MS in positive ion mode

m/z detected	m/z library	ΔM	Peptide Sequence	Protein
1047.7101	1047.6309	0.0791	LLNIQPPPR	Q91WD5
1293.8716	1293.7525	0.1190	LAILGIHNEVSK	A1BN54
1463.0263	1463.6543	-0.6280	NLLSCENSDQGAR	Q548L6
1477.1441	1477.6554	-0.5112	SASESYTQSFQSR	Q2TBE6
1477.1441	1477.6554	-0.5112	SASESYTQSFQSR	Q2TBE6
1540.1885	1540.7312	-0.5426	NVEGQDMLYQSLK	Q5SWR1; Q5SVG5
1548.2027	1548.69	-0.4872	YAFSFAGAQEACAR	Q61361
1662.2519	1662.7468	-0.4949	GFGFVCFSSPEEATK	P29341; Q99LF8
1714.4189	1714.6802	-0.2613	GESEDDFWWCIDR	Q80SW1; F8WGT1
1802.4574	1802.82	-0.3626	GADIMYTGTLDCWRK	Q8BVI9; P48962

1839.3658	1839.0011	0.3647	HKQEFLDKPEDVLLK	E9PV14
1862.4471	1862.0858	0.3612	TVLQRPLSLIQGPPGTGK	Q9EPU0
1969.3682	1969.0502	0.3180	HIAIISGAGVSAESGVPTFR	A0A1Y7VM56
1990.5328	1990.1345	0.3983	APIRPDIVNFVHTNLRK	Q9D8E6

Table S6. Theoretical optimal MALDI MSI slice thickness range of various tissues in mice

Tissue	brain	heart	liver	lung	kidney	muscle
thickness(μm)	2-8	4-8	0.8-2	1-4	0.8-2	2-6

References

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2. J. A. Vizcaino, R. G. Cote, A. Csordas, J. A. Dianes, A. Fabregat, J. M. Foster, J. Griss, E. Alpi, M. Birim, J. Contell, G. O'Kelly, A. Schoenegger, D. Ovelleiro, Y. Perez-Riverol, F. Reisinger, D. Rios, R. Wang and H. Hermjakob, *Nucleic Acids Res*, 2013, **41**, D1063-1069.