Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

In situ metabolite and lipid analysis of GluN2D$^{-/-}$ and wild-type mice after ischemic stroke using MALDI MSI

William T. Andrews, Deborah Donahue, Adam Holmes, Rashna Balsara,
Francis J. Castellino, Amanda B. Hummon

Table S1 Analyte that was successfully detected and annotated in knockout control, knockout stroke, wild type control, and wild type stroke mouse brains. Species were detected by MALDI FT-ICR imaging mass spectrometry and were annotated in Metaspace. Asterisk indicates multiple possible annotations within a subclass of molecule

All Four Conditions				
m/z	Ion Mode	Brain Region	Annotation	Database
762.6007	Positive	B	PC/PE*	HMDB-v4
718.5392	Negative	B	PC/PE*	HMDB-v4
782.567	Positive	B	PC/PE*	HMDB-v4
784.5827	Positive	B	PC/PE*	HMDB-v4
890.6396	Negative	B	Ceramide	HMDB-v4
885.5498	Negative	B	PI*	HMDB-v4
546.353	Positive	B	LysoPC*	HMDB-v4
673.4814	Negative	B	PA*	HMDB-v4
459.2482	Positive	B	LysoPA*	HMDB-v4
474.1305	Positive	B	Dihydronaphthalene*	PAMDB-v1.0
697.4779	Positive	B	PA*	HMDB-v4
495.1864	Positive	C	Doxepin N-oxide glucuronide	HMDB-v4
798.5408	Positive	B	Docosahexaenoate*	BraChemDB
788.5447	Negative	B	PS*	HMDB-v4
496.3398	Positive	B	LysoPC(16:0)	HMDB-v4
772.5252	Positive	B	PE*	HMDB-v4
713.4776	Positive	C	19'-Hexanoyloxymytiloxanthin	HMDB-v4
497.1936	Positive	B	Hydroxystrobilurin D*	HMDB-v4
760.5851	Positive	B/C	Ethyl phosphate*	BraChemDB
558.2576	Positive	C	Vignatic acid B	HMDB-v4
551.5034	Positive	B	Artemoin*	HMDB-v4
744.5549	Negative	B	PC/PE*	HMDB-v4
553.1704	Positive	C	Cucumerin	BraChemDB
734.5694	Positive	B/C	PC/PE*	HMDB-v4
693.1097	Negative	C	Guavin B	HMDB-v4
495.1861	Positive	C	Oxane-2-carboxylic acid*	HMDB-v4
734.5975	Positive	C	Aluminum;tetradecanoate	BraChemDB
741.5581	Positive	C	Menaquinol 8	PAMDB-v1.0
534.2956	Positive	B	LysoPC(16:0)	HMDB-v4
404.9958	Positive	C	Phosphatidylglycerophosphate	PAMDB-v1.0
699.497	Negative	B	PA*	HMDB-v4
790.5392	Negative	B	PC/PE*	HMDB-v4
546.3166	Positive	C	PE(10:0/10:0)	PAMDB-v1.0
701.5127	Negative	B	PA*	HMDB-v4
518.3217	Positive	B	LysoPC(16:0)	HMDB-v4
492.9921	Positive	C	2'-Deoxyinosine triphosphate	PAMDB-v1.0
810.5983	Positive	B	PC/PE*	HMDB-v4
647.4657	Negative	B	PA*	HMDB-v4
772.5253	Positive	B	PC/PE*	HMDB-v4
497.2017	Positive	C	Oxyneolignan 4-xyloside	HMDB-v4
693.0711	Negative	B	Oxane-2-carboxylic acid*	HMDB-v4
742.5392	Negative	B	Phosphinic acid*	HMDB-v4

Table S2 Annotated species that were detected in knockout control mice after MALDI FT-ICR MSI analysis followed by a metabolite search using Metaspace. Species were only annotated in knockout control mice. Asterisk indicates multiple possible annotations within a subclass of molecule

Knockout Control Annotations				
m/z	Ion Mode	Brain Region	Annotation	Database
909.5498	Negative	B	PI*	HMDB-v4
794.5705	Negative	B	PC/PE*	HMDB-v4
788.5236	Negative	B	Phosphinic acid*	BraChemDB
490.9776	Negative	C	2'-Deoxyinosine triphosphate	PAMDB-v1.0
856.5134	Negative	B	Propanoic acid*	BraChemDB
822.1341	Negative	B	Propionyl-CoA	PAMDB-v1.0
810.529	Negative	B	PS*	HMDB-v4
786.529	Negative	B	Propanoic acid*	BraChemDB
835.5342	Negative	B	PI*	BraChemDB
700.565	Negative	B	Dihydrogen phosphate	BraChemDB
834.5079	Negative	B	PE	HMDB-v4
417.0639	Positive	C	Sulfonic acid*	HMDB-v4
772.5862	Negative	B	Phosphinic acid*	BraChemDB

Table S3 Annotated species that were detected in knockout stroke mice after MALDI FT-ICR MSI analysis followed by a metabolite search using Metaspace. Species were only annotated in knockout stroke mice. Asterisk indicates multiple possible annotations within a subclass of molecule

Knockout Stroke Annotations				
m/z	Ion Mode	Brain Region	Annotation	Database
454.0072	Negative	C	4-Methylthio-3-butenylglucosinolate	BraChemDB
693.4701	Positive	C	PG*	PAMDB-v1.0
707.4858	Positive	C	PG*	PAMDB-v1.0
806.5705	Negative	C	Ethyl phosphate*	BraChemDB
741.4677	Positive	C	PG*	PAMDB-v1.0
550.0542	Positive	C	Oxane-2-carboxylate	HMDB-v4
764.5437	Positive	C	Octadecenamide*	BraChemDB
762.5279	Positive	C	PS*	PAMDB-v1.0
863.2981	Positive	C	Precorrin 2*	PAMDB-v1.0
747.5171	Positive	C	PG*	PAMDB-v1.0
733.5014	Positive	C	PG*	PAMDB-v1.0
715.452	Positive	C	PG*	PAMDB-v1.0

Table S4 Annotated species that were detected in wild type control mice after MALDI FT-ICR MSI analysis followed by a metabolite search using Metaspace. Species were only annotated in wild type control mice. Asterisk indicates multiple possible annotations within a subclass of molecule

Wild Type Control Annotations				
m/z	Ion Mode	Brain Region	Annotation	Database
741.5307	Positive	B	Palmitoyl sphingomyelin*	HMDB-v4
296.0643	Positive	B	5-Methylcytidine	BraChemDB
459.2272	Positive	B	Methyl stearate	BraChemDB
732.4212	Positive	B	PS*	PAMDB-v1.0
546.2956	Positive	B	Phosphinic acid*	BraChemDB
562.3269	Positive	B	Trimethylazanium*	BraChemDB
688.395	Positive	B	PE*	PAMDB-v1.0
826.5723	Positive	B	Ethyl Phosphate*	BraChemDB
800.5566	Positive	B	Ethyl Phosphate*	BraChemDB
506.3252	Negative	B	Phosphinic acid*	BraChemDB
848.6376	Positive	B	Glucosylceramide*	HMDB-v4
564.2487	Positive	B	LysoPE*	HMDB-v4
296.0642	Positive	B	5-Aminoimidazole ribonucleotide	PAMDB-v1.0
568.28	Positive	B	Phosphinic acid*	BraChemDB
723.5113	Positive	B	2-Octaprenyl-6-methoxy-1,4-benzoquinol	PAMDB-v1.0
820.5253	Positive	B	Ethyl Phosphate*	BraChemDB
525.9702	Negative	B	dATP	PAMDB-v1.0
560.3113	Positive	B	LysoPC*	HMDB-v4

Table S5 Annotated species that were detected in wild type stroke mice after MALDI FT-ICR MSI analysis followed by a metabolite search using Metaspace. Species were only annotated in wild type stroke mice. Asterisk indicates multiple possible annotations within a subclass of molecule

Wild Type Stroke Annotations				
m/z	Ion Mode	Brain Region	Annotation	Database
520.3398	Positive	B	LysoPC*	BraChemDB
711.1203	Negative	B	2",6"-Digalloyliriflophenone 3-C-glucoside	HMDB-v4
702.4316	Positive	B	PS*	PAMDB-v1.0
689.3436	Negative	C	PGP(12:0/12:0)	PAMDB-v1.0
872.6386	Negative	B	PS*	HMDB-v4
510.3554	Positive	B	Phosphinic acid*	BraChemDB
457.2326	Positive	B	Phosphonic acid	BraChemDB
591.4959	Positive	B	DG*	PAMDB-v1.0
615.4959	Positive	B	DG*	PAMDB-v1.0
754.5357	Positive	B	PE*	PAMDB-v1.0
741.5007	Positive	B	2-Demethylmenaquinone 8	PAMDB-v1.0
590.1147	Positive	C	Phosphinic acid*	BraChemDB
415.222	Positive	B	Methyl palmitate	BraChemDB
613.4802	Positive	B	DG*	HMDB-v4
716.4473	Positive	B	PS*	PAMDB-v1.0
837.682	Positive	B	Trimethylazanium	BraChemDB
809.6507	Positive	B	Trimethylazanium*	BraChemDB
607.0847	Positive	B	Cinnamoyl-galloyl-quercetin*	BraChemDB
409.3441	Positive	B	5alpha-Cholestanone*	BraChemDB
812.5201	Positive	B	PE(22:5(7Z,10Z,13Z,16Z,19Z)/18:2(9Z,12Z))	PAMDB-v1.0
575.5034	Positive	B	Montecristin	HMDB-v4
423.3233	Positive	B	25-Hydroxytachysterol3*	BraChemDB

Table S6 All possible annotations for phosphatidylinositol species obtained by searching MALDI-MSI data for knockout control, knockout stroke, wild type control, and wild type stroke mouse brains against the Human Metabolome Database using Metaspace

Phosphatidylinositols		
m/z	Polarity	Annotation
885.5498	Negative	PI(16:0/22:4(10Z,13Z,16Z,19Z))
		PI(16:0/22:4(7Z,10Z,13Z,16Z))
		PI(18:0/20:4(5Z,8Z,11Z,14Z))
		PI(18:0/20:4(8Z,11Z,14Z,17Z))
		PI(18:1(11Z)/20:3(5Z,8Z,11Z))
		PI(18:1(11Z)/20:3(8Z,11Z,14Z))
		PI(18:1(9Z)/20:3(5Z,8Z,11Z))
		PI(18:1(9Z)/20:3(8Z,11Z,14Z))
		PI(18:2(9Z,12Z)/20:2(11Z,14Z))
		PI(20:2(11Z,14Z)/18:2(9Z,12Z))
		PI(20:3(5Z,8Z,11Z)/18:1(11Z))
		PI(20:3(5Z,8Z,11Z)/18:1(9Z))
		PI(20:3(8Z,11Z,14Z)/18:1(11Z))
		PI(20:3(8Z,11Z,14Z)/18:1(9Z))
		PI(20:4(5Z,8Z,11Z,14Z)/18:0)
		PI(20:4(8Z,11Z,14Z,17Z)/18:0)
		PI(22:4(10Z,13Z,16Z,19Z)/16:0)
		PI(22:4(7Z,10Z,13Z,16Z)/16:0)
857.5185	Negative	PI(16:0/20:4(5Z,8Z,11Z,14Z))
		PI(16:0/20:4(8Z,11Z,14Z,17Z))
		PI(18:1(11Z)/18:3(6Z,9Z,12Z))
		PI(18:1(11Z)/18:3(9Z,12Z,15Z))
		PI(18:1(9Z)/18:3(6Z,9Z,12Z))
		PI(18:1(9Z)/18:3(9Z,12Z,15Z))
		PI(18:2(9Z,12Z)/18:2(9Z,12Z))
		PI(18:3(6Z,9Z,12Z)/18:1(11Z))
		PI(18:3(6Z,9Z,12Z)/18:1(9Z))
		PI(18:3(9Z,12Z,15Z)/18:1(11Z))
		PI(18:3(9Z,12Z,15Z)/18:1(9Z))
		PI(20:4(5Z,8Z,11Z,14Z)/16:0)
		PI(20:4(8Z,11Z,14Z,17Z)/16:0)
883.5342	Negative	PI(16:0/22:5(4Z,7Z,10Z,13Z,16Z))
		PI(16:0/22:5(7Z,10Z,13Z,16Z,19Z))
		PI(16:2(9Z,12Z)/22:3(10Z,13Z,16Z))
		PI(18:1(11Z)/20:4(5Z,8Z,11Z,14Z))
		PI(18:1(11Z)/20:4(8Z,11Z,14Z,17Z))
		PI(18:1(9Z)/20:4(5Z,8Z,11Z,14Z))

		PI(18:1(9Z)/20:4(8Z,11Z,14Z,17Z))
		PI(18:2(9Z,12Z)/20:3(5Z,8Z,11Z))
		PI(18:2(9Z,12Z)/20:3(8Z,11Z,14Z))
		PI(20:3(5Z,8Z,11Z)/18:2(9Z,12Z))
		PI(20:3(8Z,11Z,14Z)/18:2(9Z,12Z))
		PI(20:4(5Z,8Z,11Z,14Z)/18:1(11Z))
		PI(20:4(5Z,8Z,11Z,14Z)/18:1(9Z))
		PI(20:4(8Z,11Z,14Z,17Z)/18:1(11Z))
		PI(20:4(8Z,11Z,14Z,17Z)/18:1(9Z))
		PI(22:3(10Z,13Z,16Z)/16:2(9Z,12Z))
		PI(22:5(4Z,7Z,10Z,13Z,16Z)/16:0)
		PI(22:5(7Z,10Z,13Z,16Z,19Z)/16:0)
835.5342	Negative	PI(16:0/18:1(11Z))
		PI(16:0/18:1(9Z))
		PI(16:1(9Z)/18:0)
		PI(18:0/16:1(9Z))
		PI(18:1(11Z)/16:0)
		PI(18:1(9Z)/16:0)
909.5498	Negative	PI(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))
		PI(18:3(6Z,9Z,12Z)/22:3(10Z,13Z,16Z))
		PI(18:3(9Z,12Z,15Z)/22:3(10Z,13Z,16Z))
		PI(20:3(5Z,8Z,11Z)/20:3(5Z,8Z,11Z))
		PI(20:3(5Z,8Z,11Z)/20:3(8Z,11Z,14Z))
		PI(20:3(8Z,11Z,14Z)/20:3(5Z,8Z,11Z))
		PI(20:3(8Z,11Z,14Z)/20:3(8Z,11Z,14Z))
		PI(22:3(10Z,13Z,16Z)/18:3(6Z,9Z,12Z))
		PI(22:3(10Z,13Z,16Z)/18:3(9Z,12Z,15Z))
		PI(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/18:0)

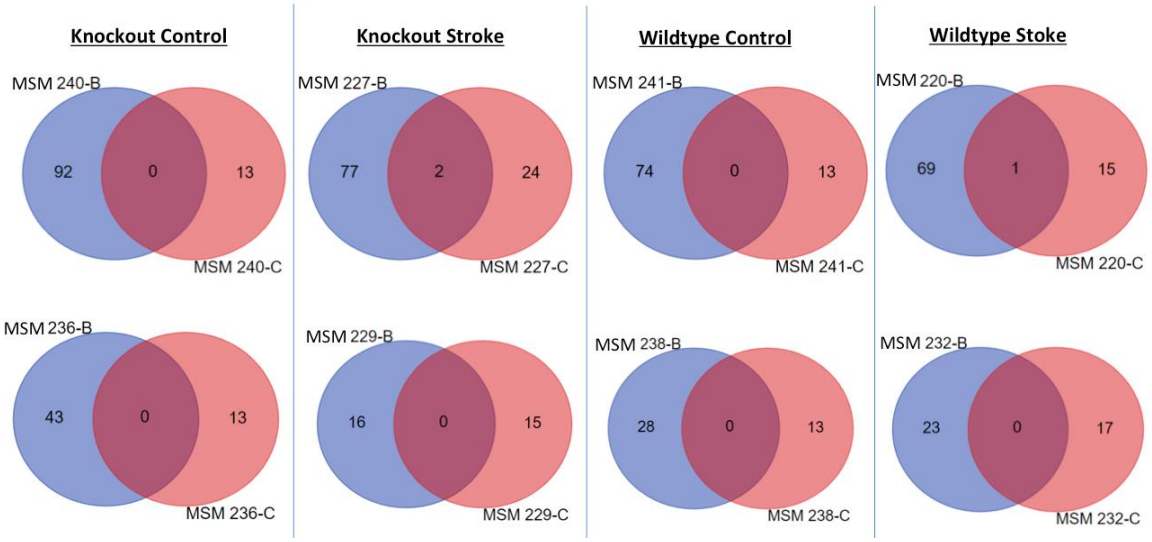

Fig. S1 Comparison of annotations obtained from B and C regions of mouse brain sections using Metaspace. Metaspace annotations obtained from data in both positive and negative MALDI MSI data were combined and compared with annotations from a different region of the same mouse brain. Knockout stroke mice shared *m/z* 734.5694 (PE) and 760.5851 (PE), while wild type stroke mice shared *m/z* 756.5514 (PE). MALDI MSI analysis was conducted using a 15T FT-ICR mass spectrometer with a mass window of ±10 mDa

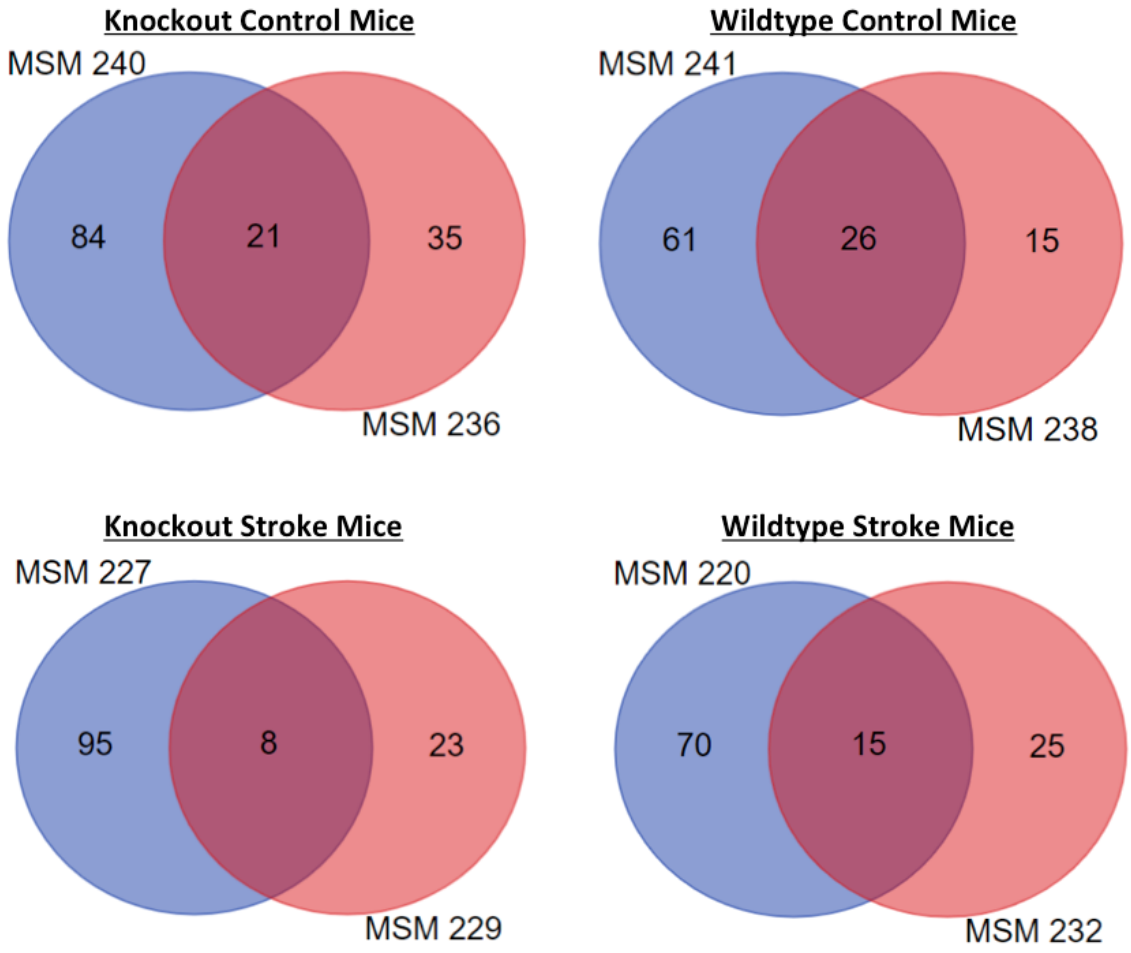

Fig. S2 Comparison of annotations obtained from knockout control, knockout stroke, wild type control, and wild type stroke mouse brain sections using Metaspace. Metaspace annotations obtained from data in both positive and negative MALDI MSI data from B and C regions of the brain were combined and compared with annotations from a different mouse of the same cohort. MALDI MSI analysis was conducted using a 15T FT-ICR mass spectrometer with a mass window of ±10 mDa

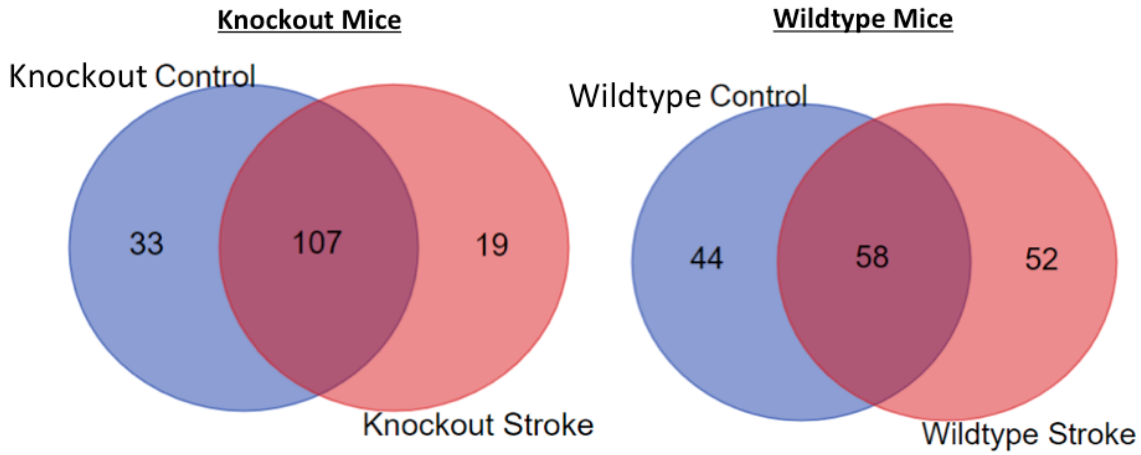

Fig. S3 Comparison of annotations obtained from knockout wild type mouse brain sections using Metaspace. Metaspace annotations obtained from data in both positive and negative MALDI MSI data from B and C regions of the brain from mice of each cohort were combined and compared with annotations from mice of a different cohort with the same genotype. MALDI MSI analysis was conducted using a 15T FT-ICR mass spectrometer with a mass window of ±10 mDa

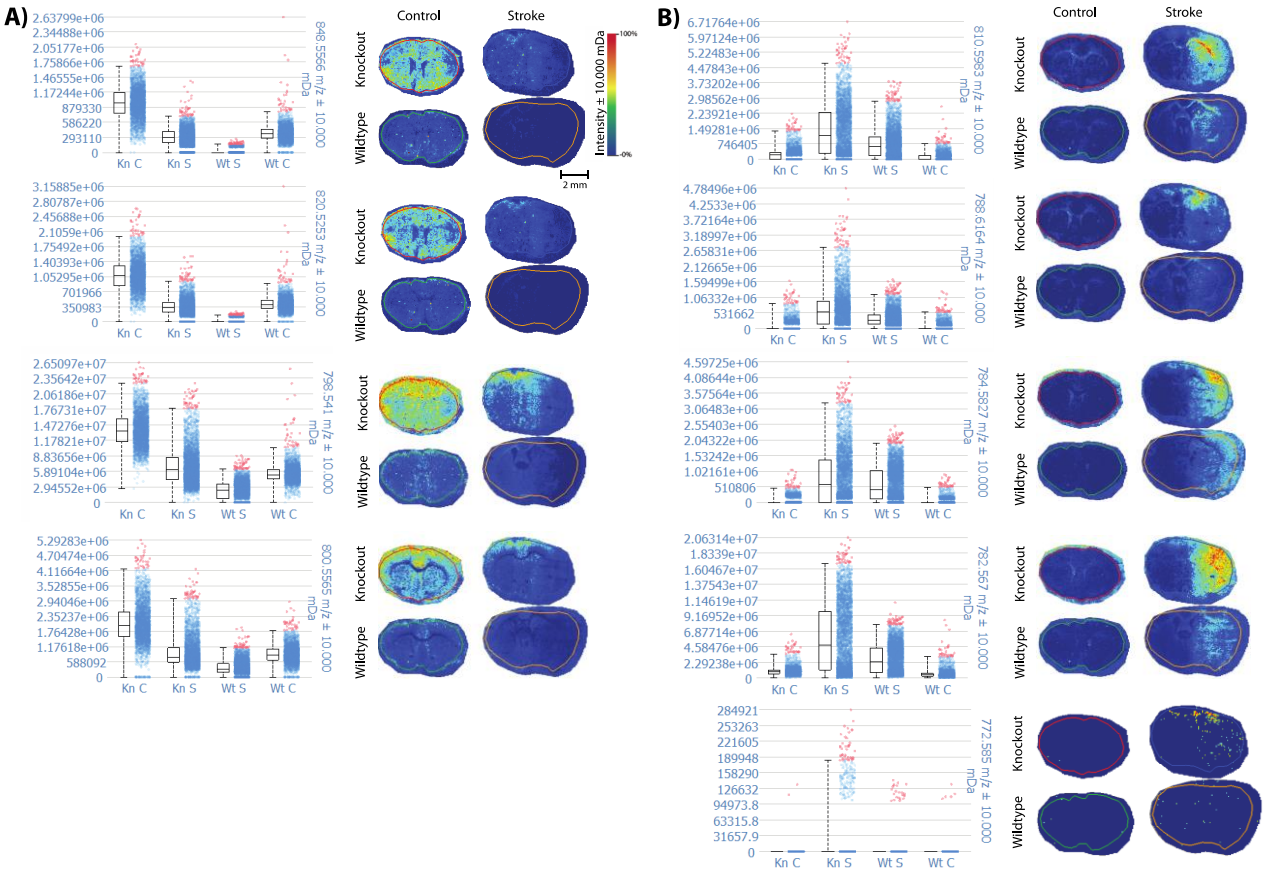

Fig. S4 MALDI MSI ion heat maps and intensity plots for PC species in wild type and knockout mice that had and had not undergone ischemic stroke. All PC species displayed above appeared more intense in knockout mice when compared to wildtype. a) Four PC species appeared more intense in knockout control mice, while b) five PC species appeared more intense in knockout stroke mice. MALDI MSI analysis was conducted using a 15T FT-ICR mass spectrometer with a mass window of ±10 mDa

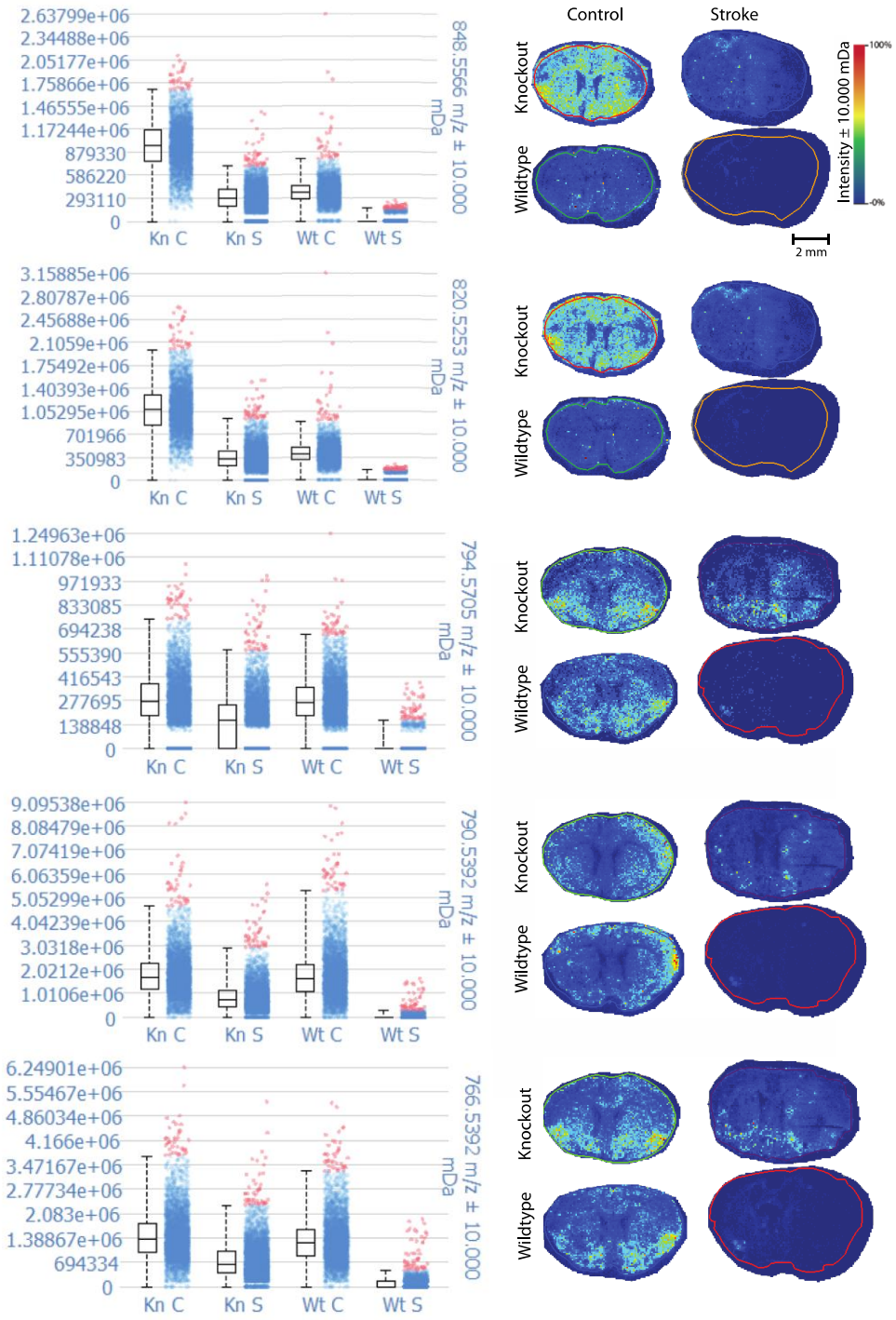

Fig. S5 MALDI MSI ion heat maps for PC species that are less abundant in wild type stroke mouse brains when compared to the other three conditions. MALDI MSI analysis was conducted using a 15T FT-ICR mass spectrometer with a mass window of ±10 mDa