Lipidomic Signatures of CNS Ischemic Injury and Their Modulation by Immunomodulatory Hydrogels

Mary F. Wang[1], Yunxin Ouyang[2], Tatiana Segura[2], David C. Muddiman[1]

[1] Biological Imaging Laboratory for Disease and Exposure Research (BILDER), Department of Chemistry, North Carolina State University, Raleigh, North Carolina, 27695, United States,
[2]Department of Biomedical Engineering, Duke University, Durham, North Carolina, 27705

Supporting Information

Submitted to: *RCS Analyst*

Submitted: January 2nd, 2026

Revised: April 20th, 2026

Manuscript: 33 Pages/7 Figures/3 Tables/10 Supplemental Pages/5 Supplemental Figures/2 Supplemental Tables

Keywords: IR-MALDESI MSI, Ischemic Stroke, Hydrogel, Lipids, Metabolites

*Authors for Correspondence

David C. Muddiman, Ph.D. / Tatiana Segura, Ph.D.

Biological Imaging Laboratory for Disease and Exposure Research (BILDER) /

Department of Chemistry / Department of Biomedical Engineering

Phone: 919-513-0084 / +1-919-660-2901

Email: dcmuddim@ncsu.edu / tatiana.segura@duke.edu

Table of Contents

Table S1. Hydrogel-treated tissues organized according to replicate and hydrogel type. Additional details are included below regarding cage number, sex, and age at the time of the stroke.

Cage ID	Cage Code	Sex	Type	Date of Birth	Date of Stroke	Age at Time of Stroke	Hydrogel Type
C87	1734579	M	C57BL/6J	1/24/2025	3/22/2025	8 weeks	PSA
C87	1734579	M	C57BL/6J	1/24/2025	3/22/2025	8 weeks	PSA
C93	1751449	M	C57BL/6J	3/26/2025	5/21/2025	8 weeks	PSA
C87	1734579	M	C57BL/6J	1/24/2025	3/22/2025	8 weeks	HA
C85	1734572	M	C57BL/6J	1/5/2025	3/22/2025	11 weeks	HA
C88	1734581	M	C57BL/6J	1/28/2025	4/17/2025	11 weeks	HA

Table S2. The up- and down-regulated metabolites and lipids found in this study in addition to all putative MS1 annotations sorted by METASPACE. If lipid and metabolite annotations overlap with components found in the gel, the last column in the spreadsheet will list that annotation as “yes” for fully in the gel or “edge” for something with detection on the edge of the gel spot. Additionally, “Replicate Detection” columns denote whether ion abundance at a given *m/z* value is localized to the tissue or the infarct/peri-infarct site. Ion abundances listed for tissue replicates with a “Replicate Detection” column value of “0” may occur from background noise. Control stroke replicates highlighted in red were randomly excluded technical replicates as highlighted in **Figure S3**. This table is attached as a separate excel file due to the size of the table.

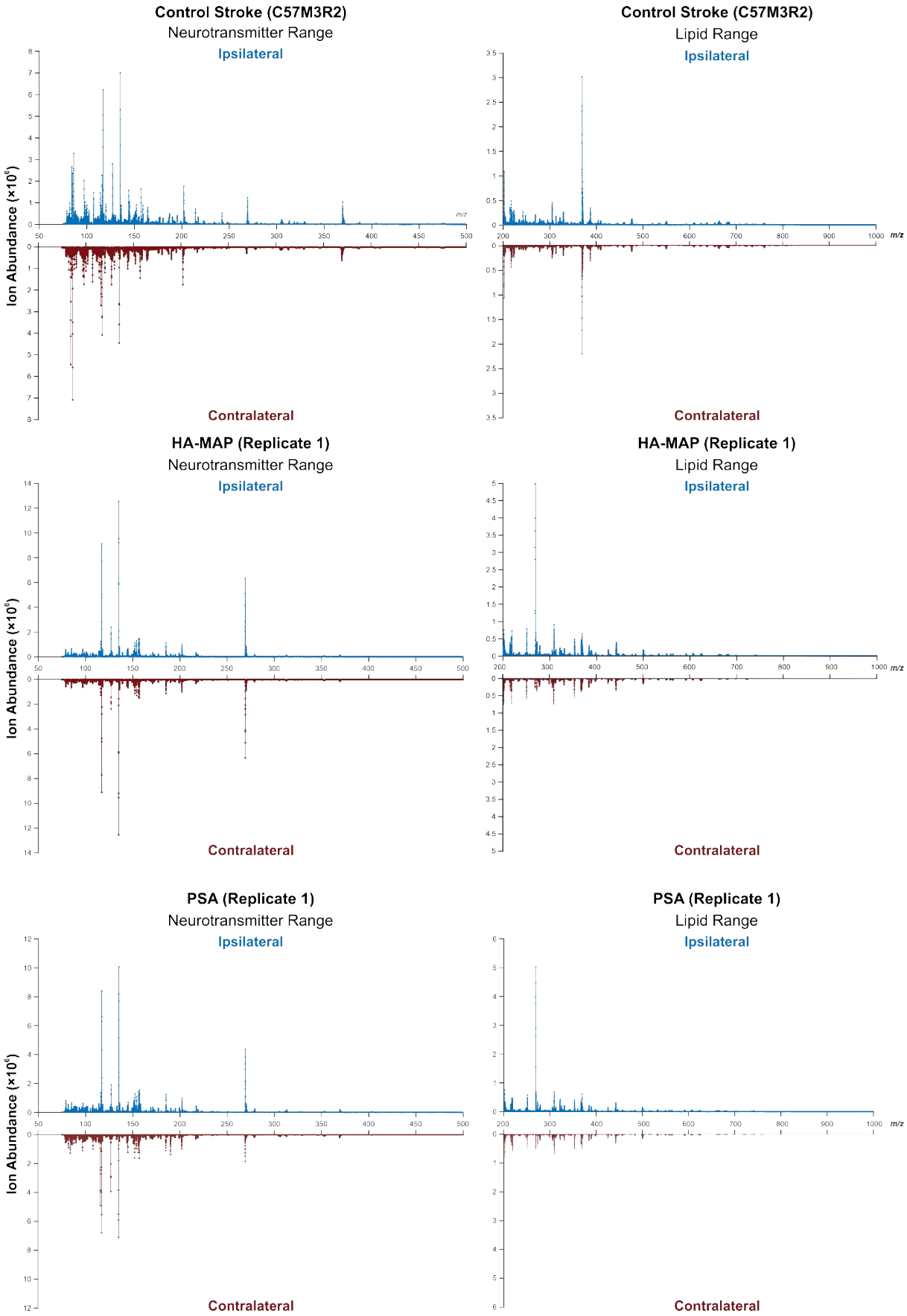


Figure S1. Reflection plots prepared using MSiReader "Generate Mass Spectrum" tool for an ROI within the ipsilateral (lesion) region and in the contralateral (healthy) region. The spectra are averaged across all voxels in the ROI selected. ROIs are similar size for both ipsilateral and contralateral within

each tissue. Ipsilateral refers to the lesion including the infarct and peri-infarct regions and contralateral is the same size ROI on the opposite hemisphere with healthy tissue.

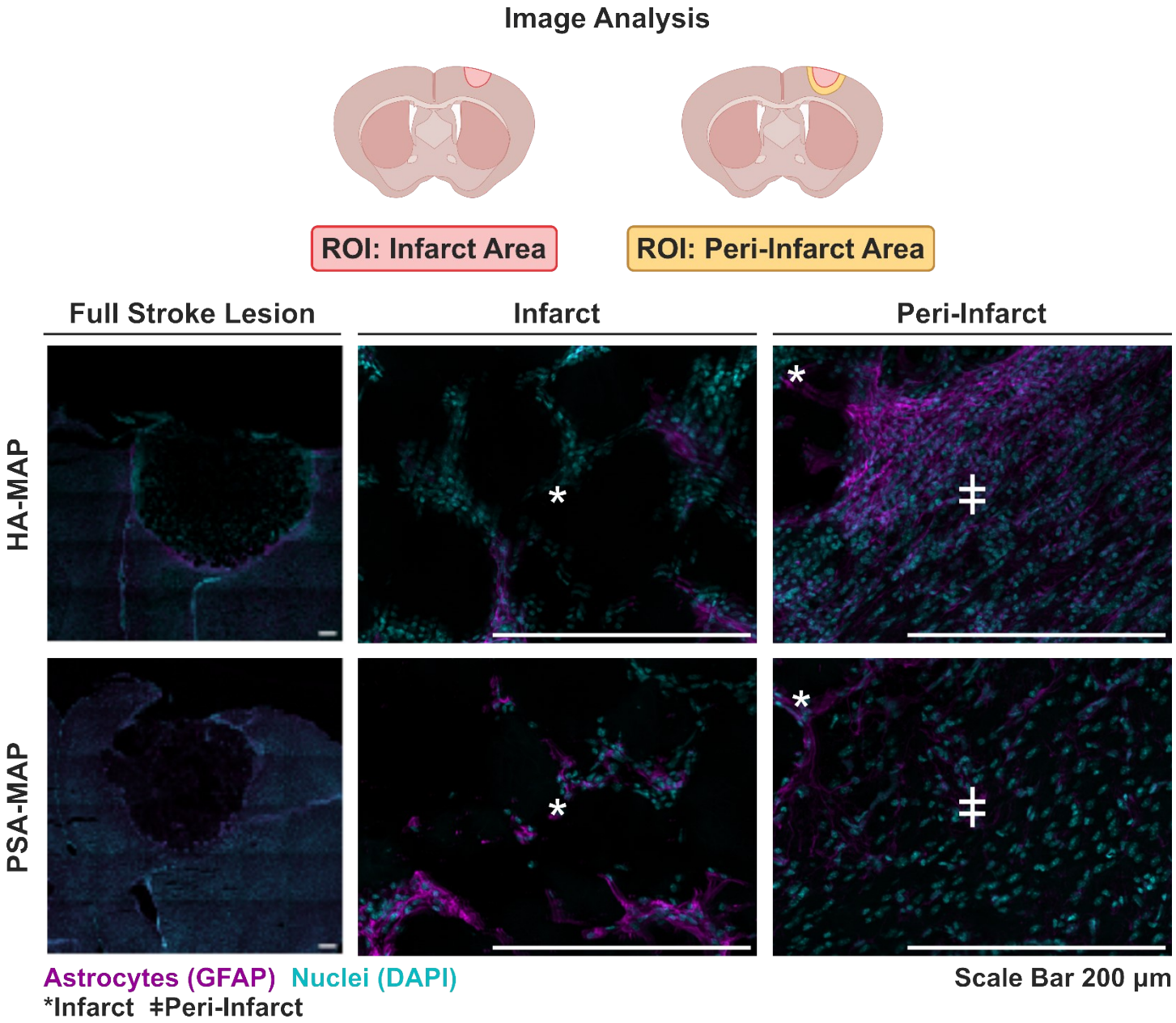


Figure S2. Immunofluorescence data for the three hydrogel-treated PT stroke tissues. One representative tissue was used for each treatment to show the full stroke lesion, the infarct region, and the peri-infarct region. GFAP and DAPI stains were selected to highlight the astrocyte and nuclei responses to injury respectively.

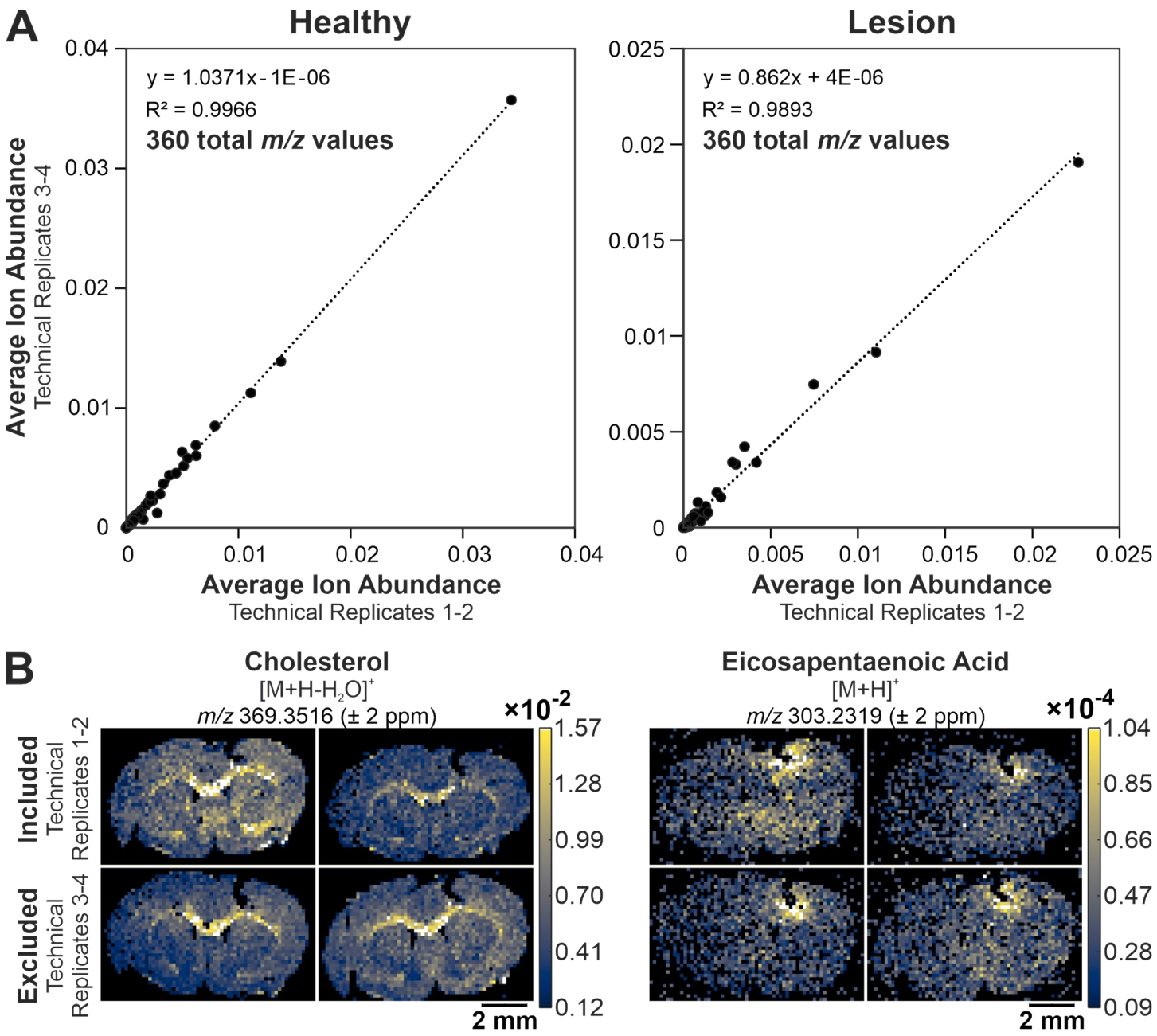
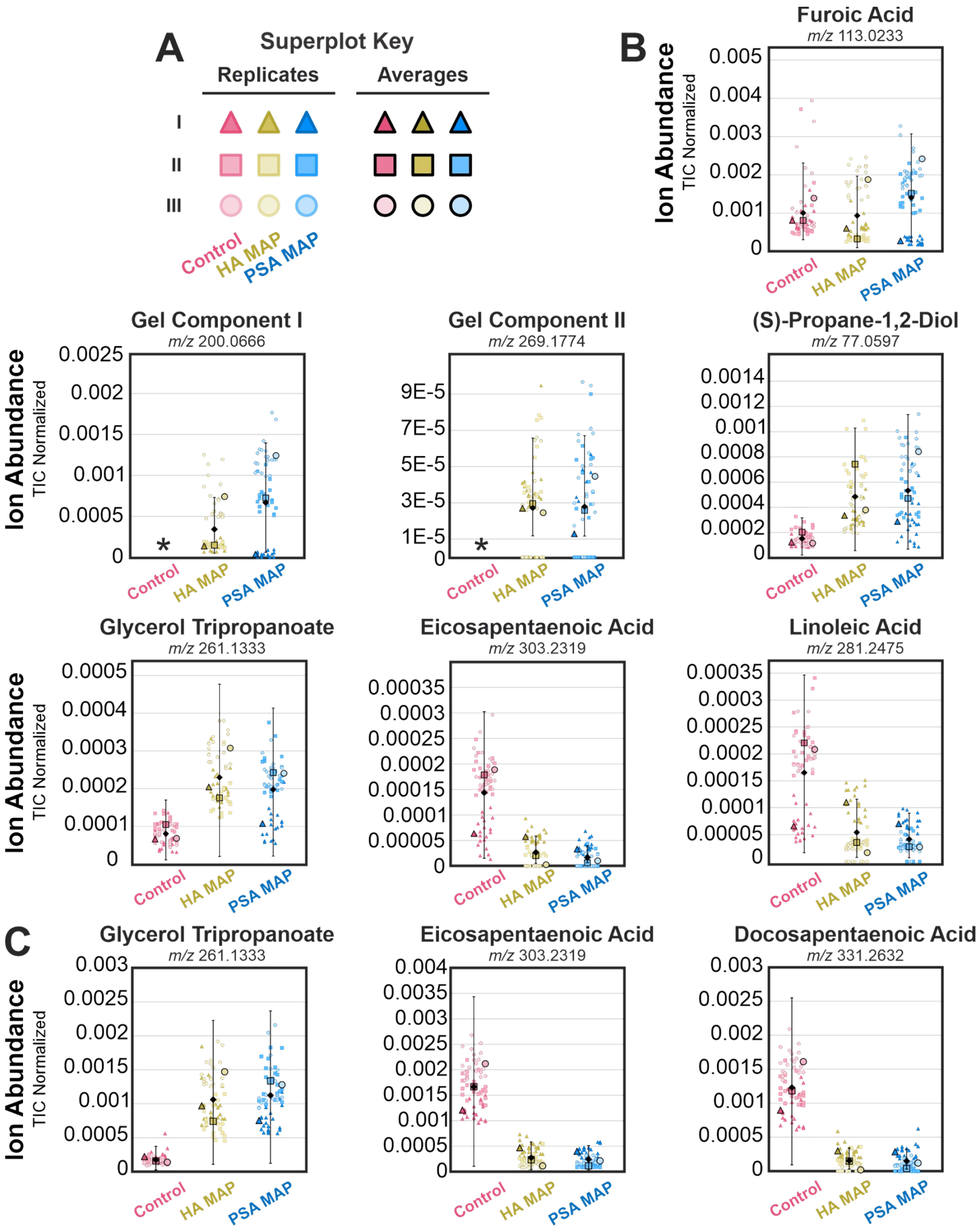


Figure S3. Comparison of the four technical replicates for one of the control stroke biological replicates. **(A)** shows the similarities between technical replicates 1-2 (included in analyses) and 3-4 (excluded from analyses) across 360 total lipids and metabolites. These comparisons were completed in the neurotransmitter mass range comparing C59M1R1, C59M1R2, C59M1R3, and C59M1R4 (R1-4 correspond to replicate numbers). The TIC-normalized ion abundances were extracted from the healthy region of the tissue and averaged for each tissue replicate. Replicates 1-2 and 3-4 were averaged for each *m/z* value and plotted against each other in increasing ion abundance order. **(B)** shows the TIC-normalized ion images for two representatives down- and up-regulated molecules of interest for all four technical replicates.


A
Superplot Key
Replicates
Averages
I
II
III
Control
HA MAP
PSA MAP
B
Furoic Acid
m/z 113.0233
Ion Abundance
TIC Normalized
0.005
0.004
0.003
0.002
0.001
0
Gel Component I
m/z 200.0666
0.0025
0.002
0.0015
0.001
0.0005
Gel Component II
m/z 269.1774
9E-5
7E-5
5E-5
3E-5
1E-5
(S)-Propane-1,2-Diol
m/z 77.0597
0.0014
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
Glycerol Tripropanoate
m/z 261.1333
Eicosapentaenoic Acid
m/z 303.2319
Linoleic Acid
m/z 281.2475
0.00035
0.0003
0.00025
0.0002
0.00015
0.0001
0.00005
C
Glycerol Tripropanoate
m/z 261.1333
Eicosapentaenoic Acid
m/z 303.2319
Docosapentaenoic Acid
m/z 331.2632
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005

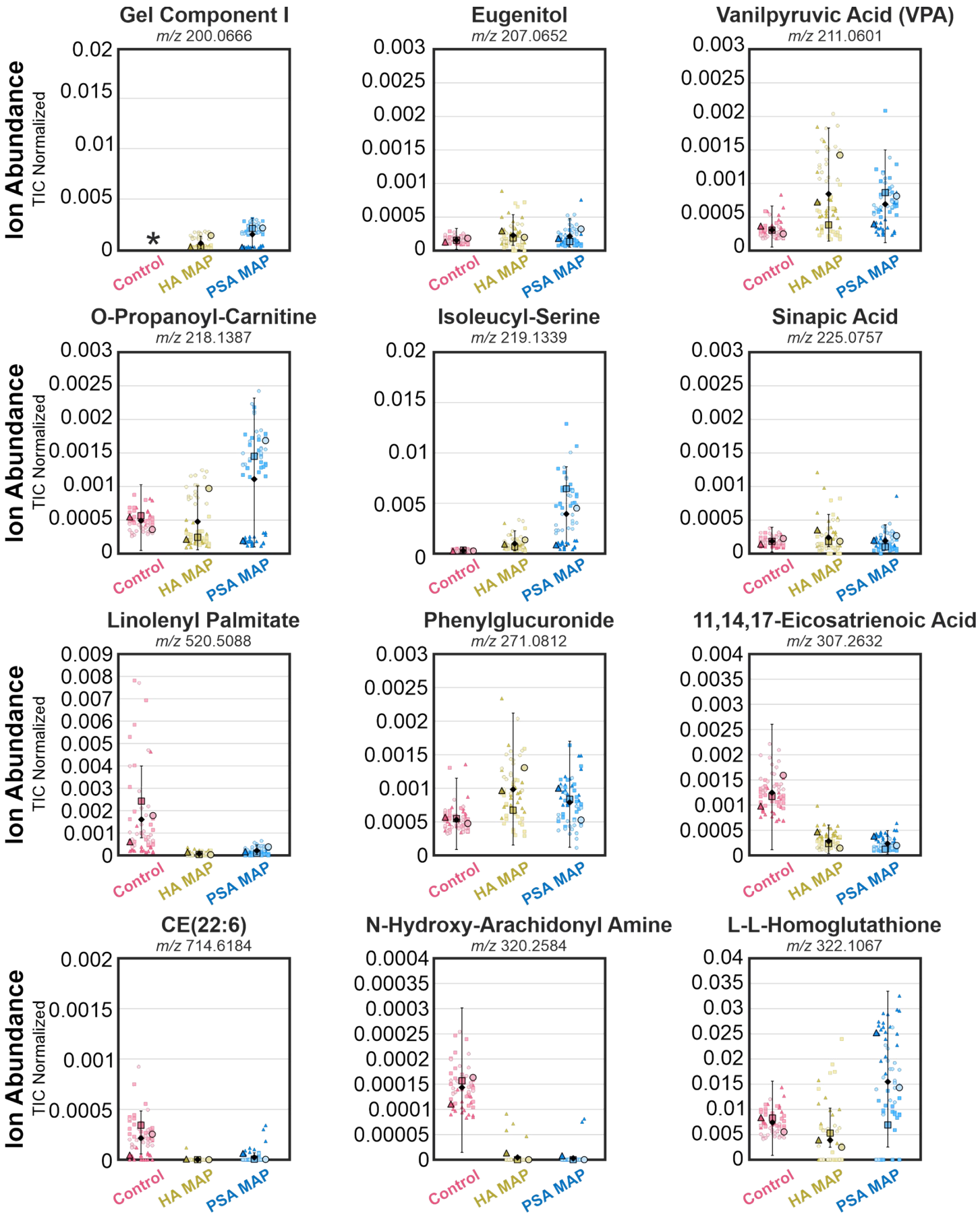


Figure S4. Super plots constructed for lipid and metabolites species with increased ion abundance detected by all three treatments/conditions. The “Superplot Key” is shown in part **(A)**, part **(B)** shows

the overlapping up-regulated species in the neurotransmitter range, and **(C)** shows the overlapping species in the lipid mass range. All error bars correspond to the 95% confidence interval with respect to the three averages for the biological replicates. The 95% confidence intervals are centered at the average of the three biological replicate averages. Each plot accounts for the 20 voxels/scans with the highest TIC-normalized ion abundance within the ROI extracted from the ipsilateral (lesion) region. The asterix (*) indicates a value of zero or no detection. The control conditions consist of the three biological replicates with two technical replicates averaged for each of the top 20 scans where the additional two technical replicates removed according to **Figure S3**.

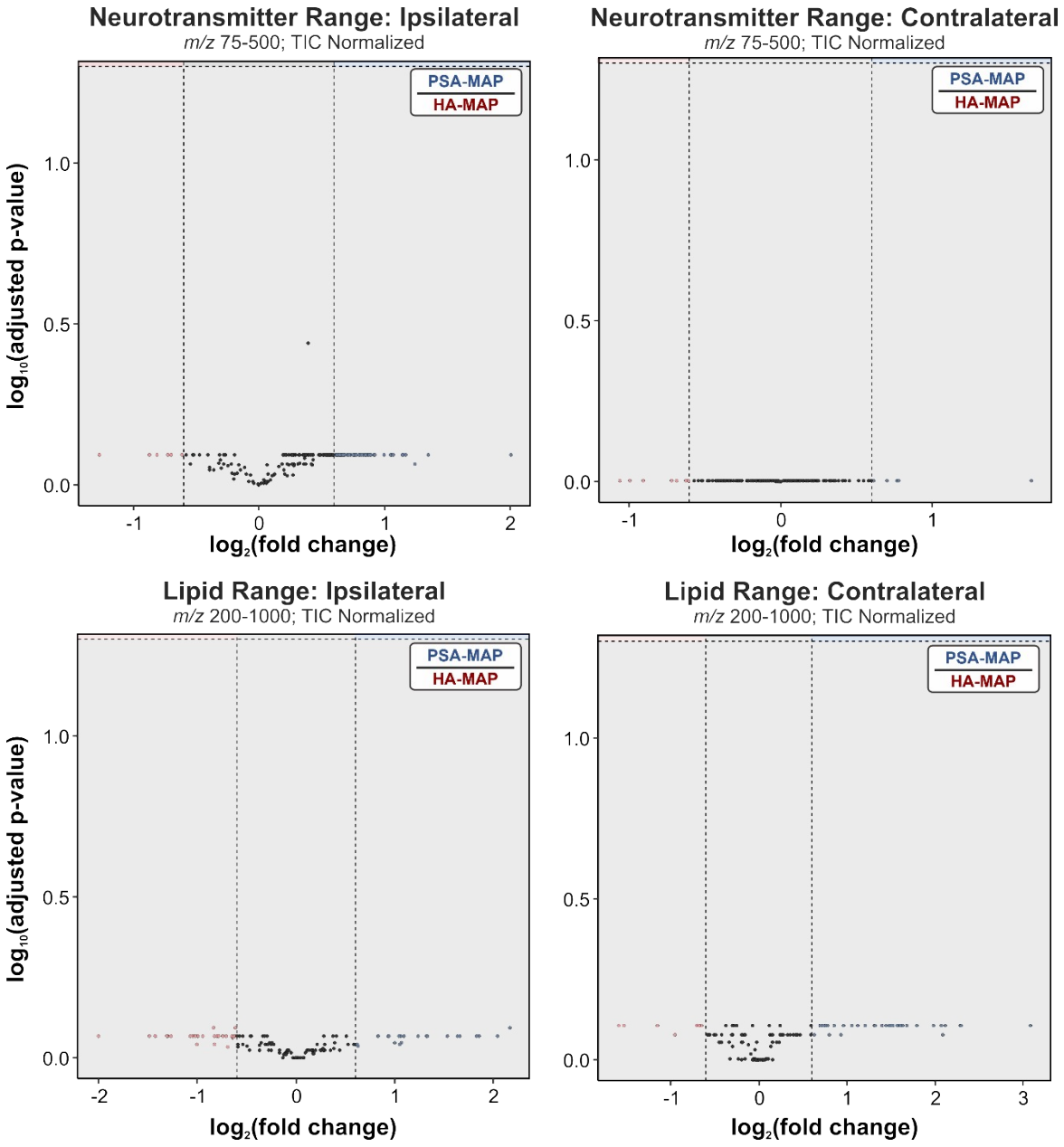


Figure S5. Volcano plots for two mass ranges *m/z* 75-500 and *m/z* 200-1000 in **(A)** control stroke (no hydrogel treatment) samples and **(B)** HA- and PSA-MAP-treated stroke models. The plots compare the log2(fold change) between the ipsilateral (lesion) region and contralateral (healthy) region of the brain. These regions consist of the infarct and per-infarct region of the brain (lesion) and a similar sized region of interest (ROI) from the contralateral side of the brain. The size of ROIs corresponds to the sample/replicate and varies accordingly to account for only the affected area and the opposite hemisphere of the brain (see **Figure 5**). All p-values were adjusted according to a Benjamini-Hochberg correction with a cutoff of log10(p-value) = 1.3 (p-value of 0.05). Key metabolites and lipids of interest are labeled accordingly according to tentative annotations in **Table S2**. All $-\log_{10}$(Benjimi-Hochberg corrected p-values) and $\log_2$(fold changes) are included in **Table S2**.