

Supplementary information

Changes in the lipid profile of hamster liver after *Schistosoma mansoni* infection, characterized by mass spectrometry imaging and LC-MS/MS analysis

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Chemicals

Table S1: Used chemicals and their specifications.

Chemical name	Quality grade	manufacturer
1,5-diaminonaphthalene (DAN)	97%	Acros Organics, Geel, Belgium
2,5-dihydroxy benzoic acid (DHB)	for synthesis	Merck, Darmstadt, Germany
2-propanol	for HPLC	Chemsolute, Renningen, Germany
acetone	HiPerSolv	VWR International, Fontenay-sous-Bois, France
ammonium formate	99.995%	Sigma-Aldrich, Steinheim, Germany
acetonitrile	HiPerSolv	VWR International, Fontenay-sous-Bois, France
Eosin Y solution		Sigma-Aldrich, Steinheim, Germany
ethanol	Uvasol	Merck, Darmstadt, Germany
Eukitt quick hardening medium		Sigma-Aldrich, Steinheim, Germany
formic acid	for mass spectrometry	Honeywell, Morris Plains, NJ, USA
Mayer's hematoxylin solution		Sigma-Aldrich, Steinheim, Germany
methanol	LiChroSolv	Merck, Darmstadt, Germany
Methyl-tert-butylether (MTBE)	for HPLC	Sigma-Aldrich, Steinheim, Germany
Oil Red		Sigma-Aldrich, Steinheim, Germany
paraformaldehyde		Roth, Karlsruhe, Germany
phosphate buffered saline (PBS)		Gibco, Carlsbad, CA, USA
trifluoro acetic acid	Uvasol	Merck, Darmstadt, Germany
water	HiPerSolv	VWR International, Fontenay-sous-Bois, France
xylene	for analysis	Merck, Darmstadt, Germany

MALDI MSI measurements

Table S2: Settings used for data acquisition with MALDI MSI.

Parameter	Setting
m/z	250-1000
Ion mode	Positive and negative
Resolution	240,000 at m/z 200
Ion injection time	500 ms
Scan rate	1 / s
Spray voltage	3 kV
Capillary temperature	250°C
Lock mass	m/z 716.12461 [5 DHB – 4 H ₂ O + NH ₄] ⁺ , none for DAN
Pixel size	10 μ m

Mode	2D pixel mode
20% filter	On
Attenuator	15-20°

LC-MS/MS measurements

Table S3: LC gradient.

Time / min	Mobile phase A / %	Mobile phase B / %
0	70	30
2	57	43
2.1	45	55
12	35	65
18	15	85
20	0	100
25	0	100
25.1	70	30
28	70	30

Table S4: Source parameters for LC-MS measurements.

Parameter	Positive-ion mode	Negative-ion mode
Sheath gas / a.u.	40	45
Auxiliary gas / a.u.	15	12
Sweep gas / a.u.	2	1
Spray voltage / kV	3.5	3.5
Capillary temperature / °C	300	320
S-lens RF	50	55
Auxiliary heater temperature / °C	300	320

Table S5: MS parameters for LC-MS measurements, in () for MS/MS.

Parameters	Positive-ion mode	Negative-ion mode
Run time / min	28	28
Internal lock mass	391.28421	-
Charge state	1	1
Exclusion list [47]	On	-
Resolution	70k (35k)	70k (35k)
AGC target	10 ⁶ (10 ⁵)	10 ⁶ (10 ⁵)
Maximum injection time / ms	250 (75)	250 (75)
Scan range	200-1800	200-1800
Top N	15	15
Isolation window / m/z	1	1
Stepped NCE	25, 30	20, 30, 40
Underfill ration / %	0.6	0.6
Intensity threshold	8·10 ³	8·10 ³
Exclude isotopes	On	On
Dynamic exclusion / s	8	8

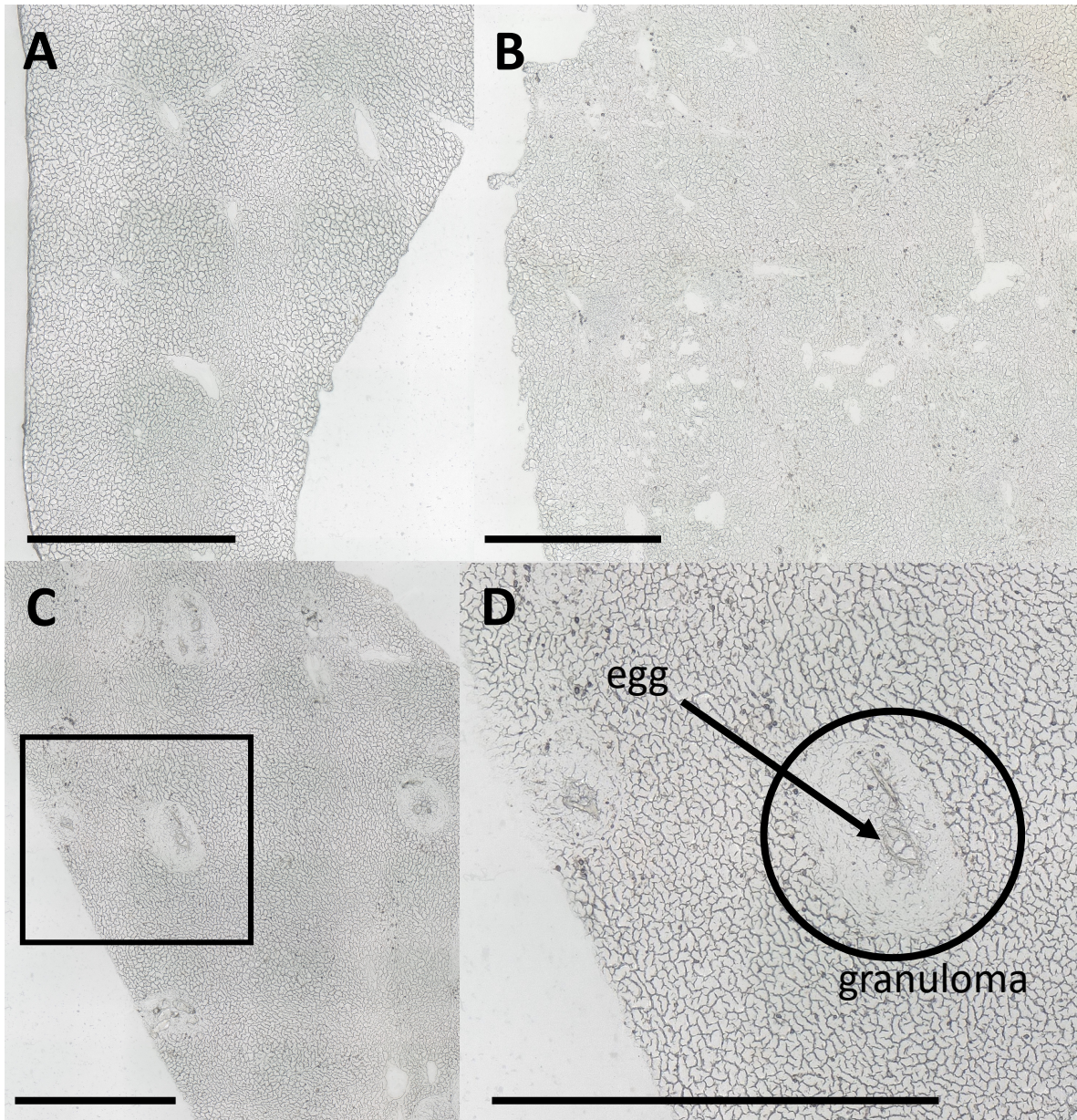


Figure S1: Light microscopic images of hamster liver cryosections of 20 μm thickness. (A) Non-infected, (B) monosex-infected, (C) bisex-infected, (D) zoomed area of (C), showing a nested egg and inflammation area around egg; scale bars are 1 mm.

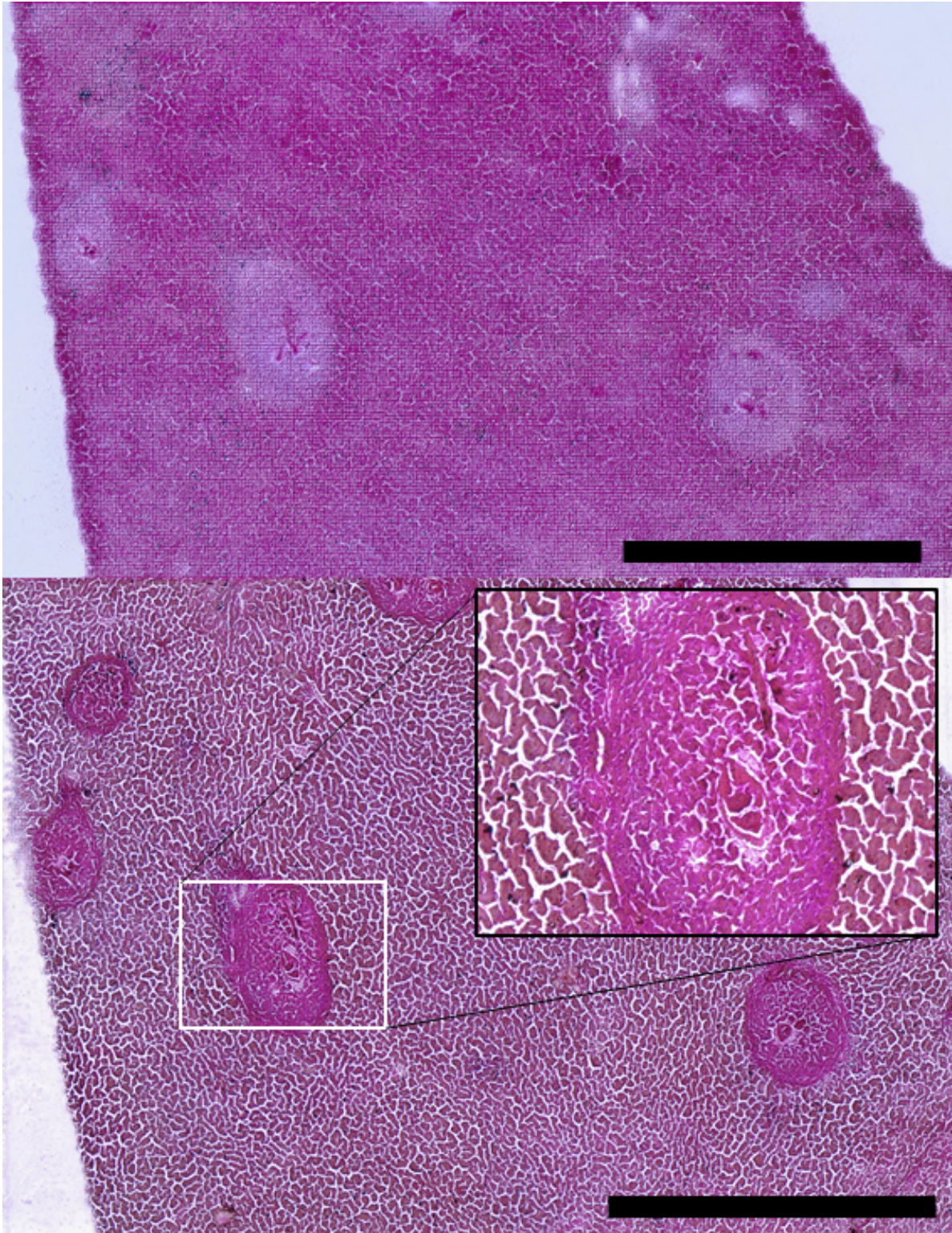


Figure S2: H&E stained sections of bisex-infected sample 2. Staining was performed after MSI measurements, therefore laser ablation spots are clearly visible. However, granuloma can still be identified around the eggs.

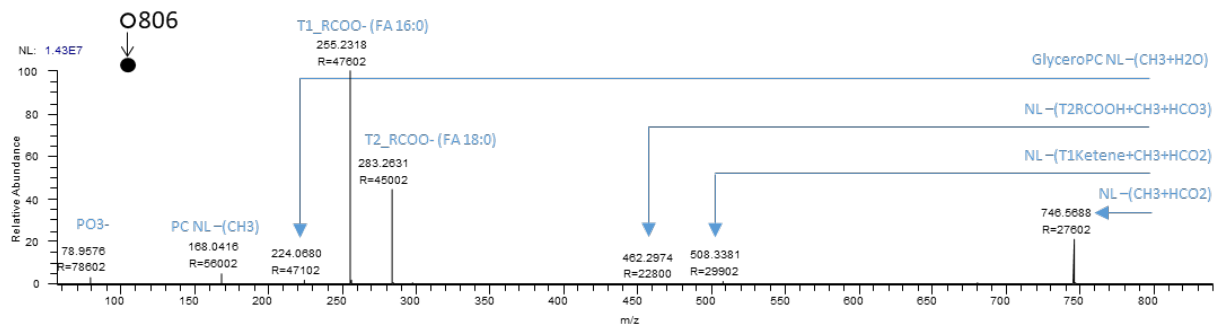
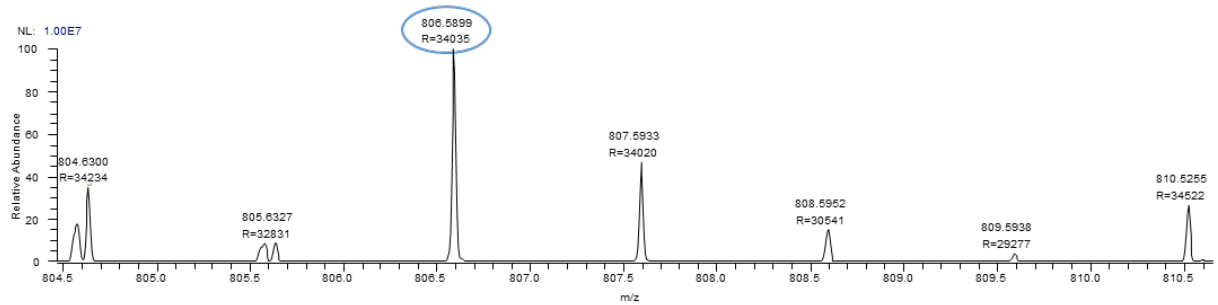
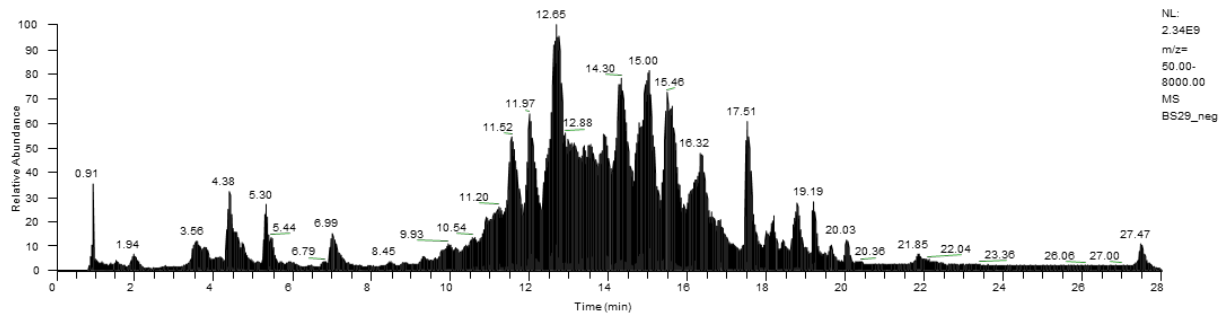


Figure S3: Exemplary LC-MS/MS spectra. In the upper part, the chromatogram of the LC separation is shown. In the middle, a zoom of the full MS spectra can be found. The peak of interest is marked with a blue circle. The lower part shows the MS/MS spectra of the peak of interest. Fragment ions and losses are stated in blue.

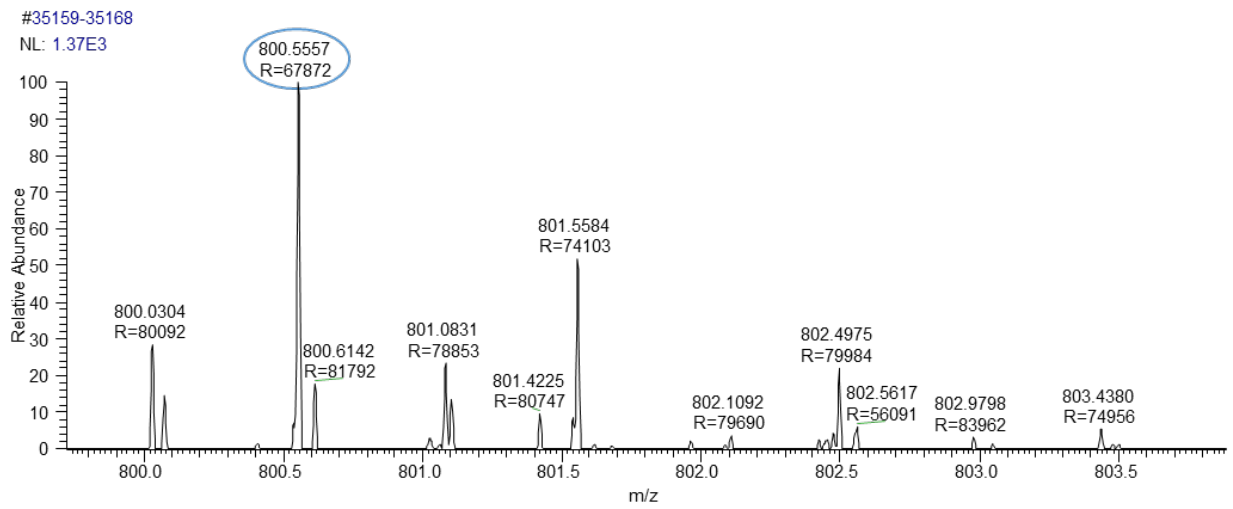
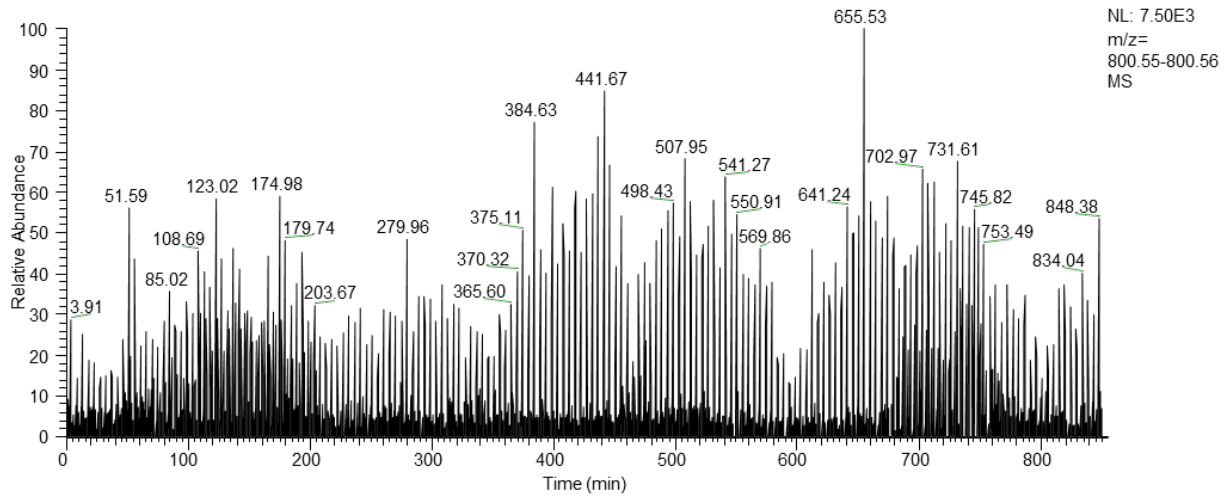


Figure S4: Exemplary MALDI spectrum. In the upper part, the extracted ion chromatogram of m/z 800.5557 is shown. The lower part shows the summed spectrum. The ion of interest is marked with a blue circle. According to the LC-MS/MS data, this peak belongs to PC(16:0_18:0) $[M+K]^+$.

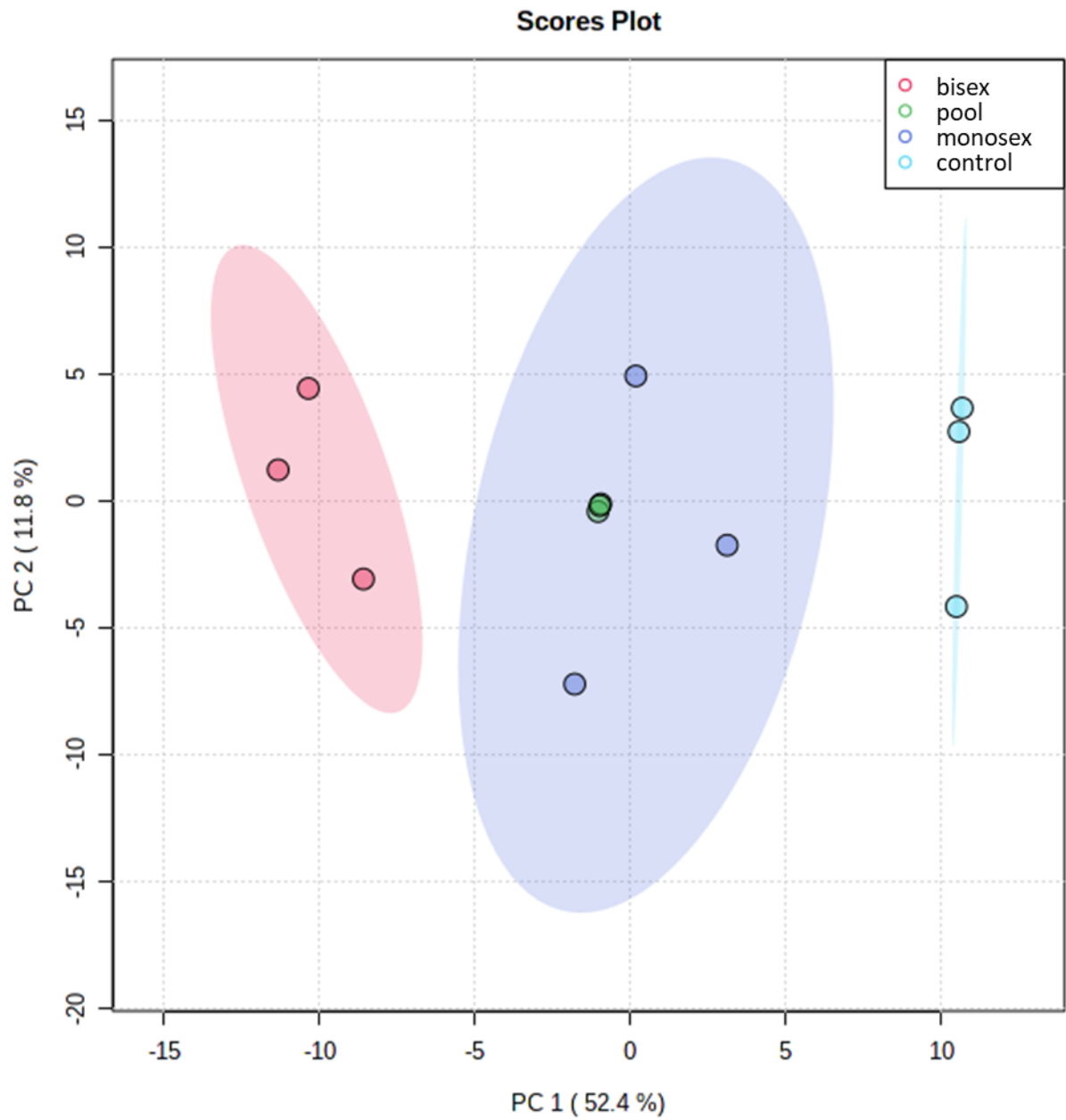


Figure S5: PCA scores plot from MetaboAnalyst[36], showing that the three sample groups are clearly distinguishable. The pool samples group nicely on nearly the same point, what is reasonable since they are only technical replicates.

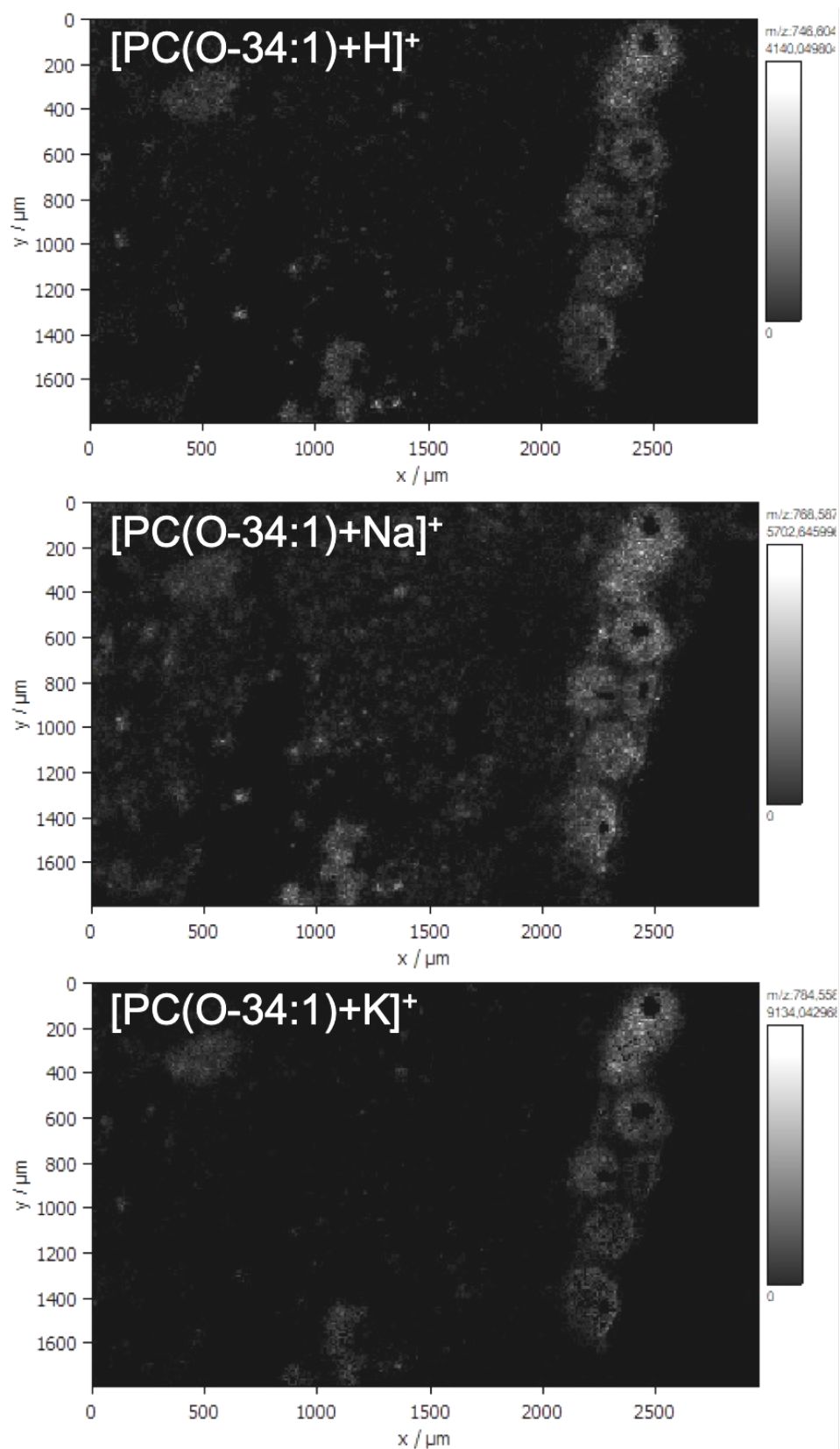


Figure S6: Comparison of different adducts for one lipid. Nearly the same distribution patterns were found. However, for most of the lipids, not all adducts were observed.

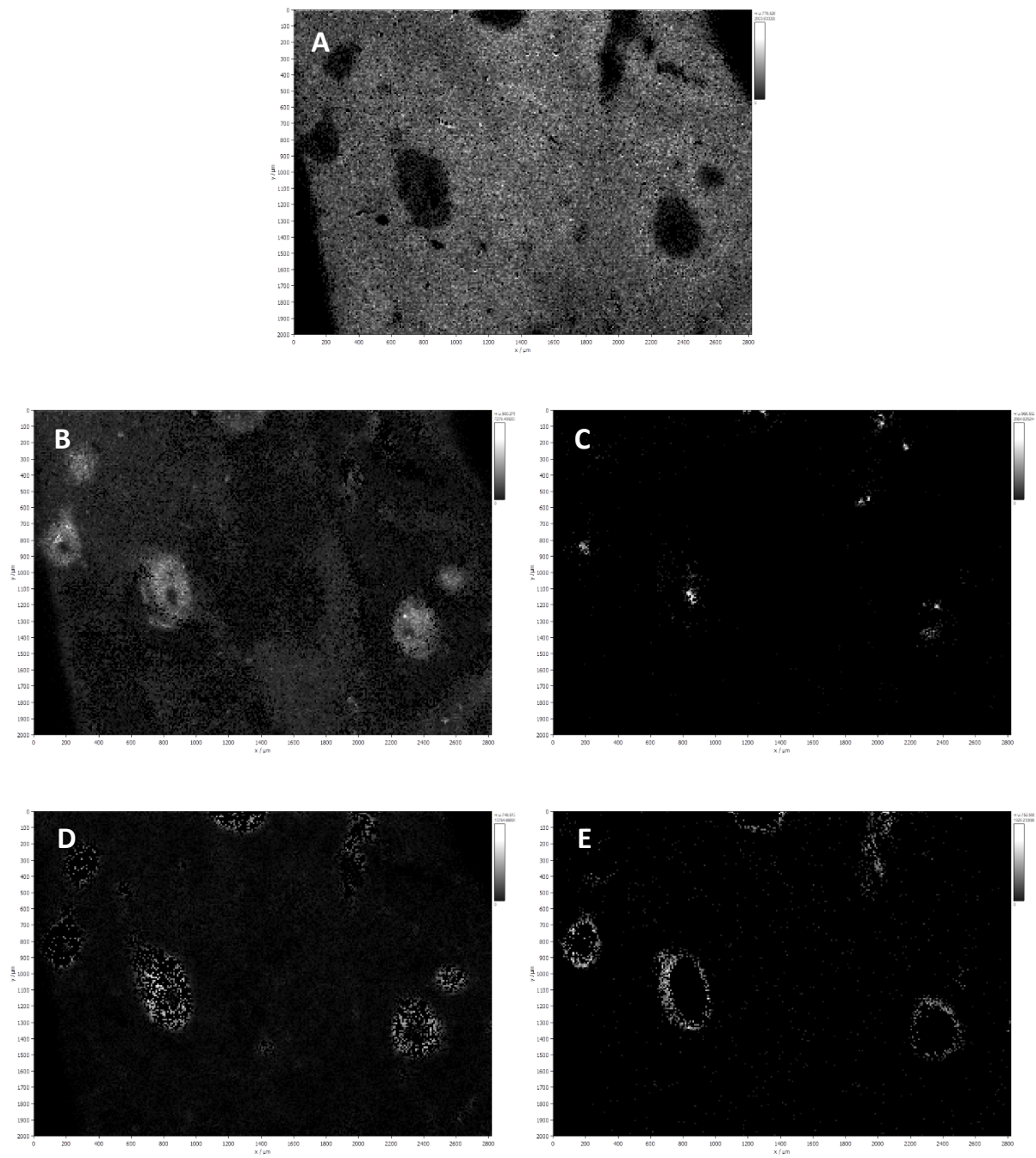


Figure S7: Single-ion images that were used for overlays of a bisex-infected sample as shown in Figure 5. A: m/z 776.526946, MMPE(16:0_22:6), $[M-H]^-$, B: m/z 500.275684, LPE(20:4), $[M-H]^-$, C: m/z 866.592639, PS(42:4), $[M-H]^-$, D: m/z 746.511353, plasmeyl-PE(P-16:0/22:6), $[M-H]^-$, E: m/z 752.555581, plasmanylyl-PE(O-18:0/20:4), $[M-H]^-$.

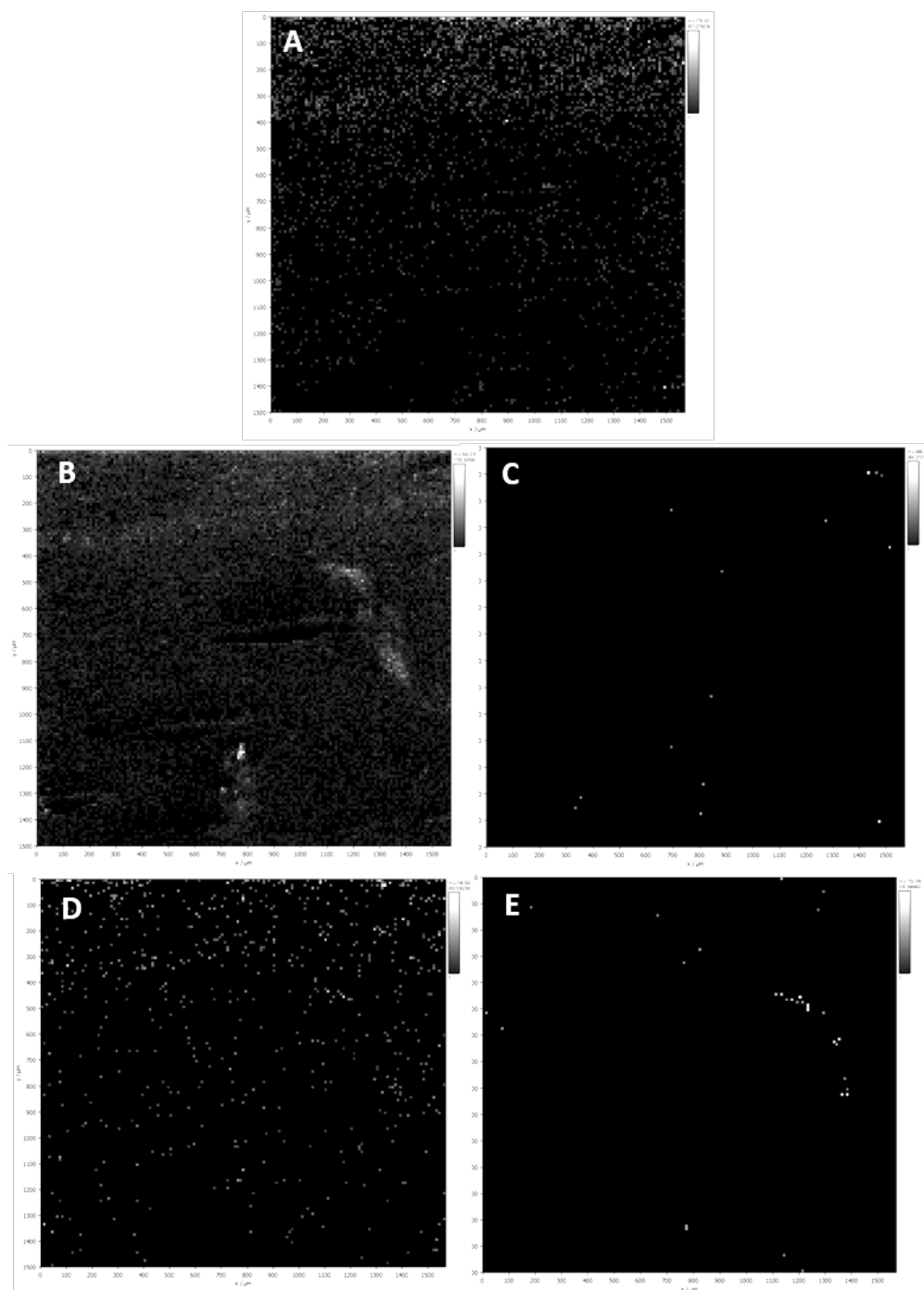


Figure S8: Single-ion images that were used for overlays of a control sample as shown in Figure 5. A: m/z 776.526946, MMPE(16:0_22:6), $[M-H]^-$, B: m/z 500.275684, LPE(20:4), $[M-H]^-$, C: m/z 866.592639, PS(42:4), $[M-H]^-$, D: m/z 746.511353, plasmeyl-PE(P-16:0/22:6), $[M-H]^-$, E: m/z 752.555581, plasmany-PE(O-18:0/20:4), $[M-H]^-$.

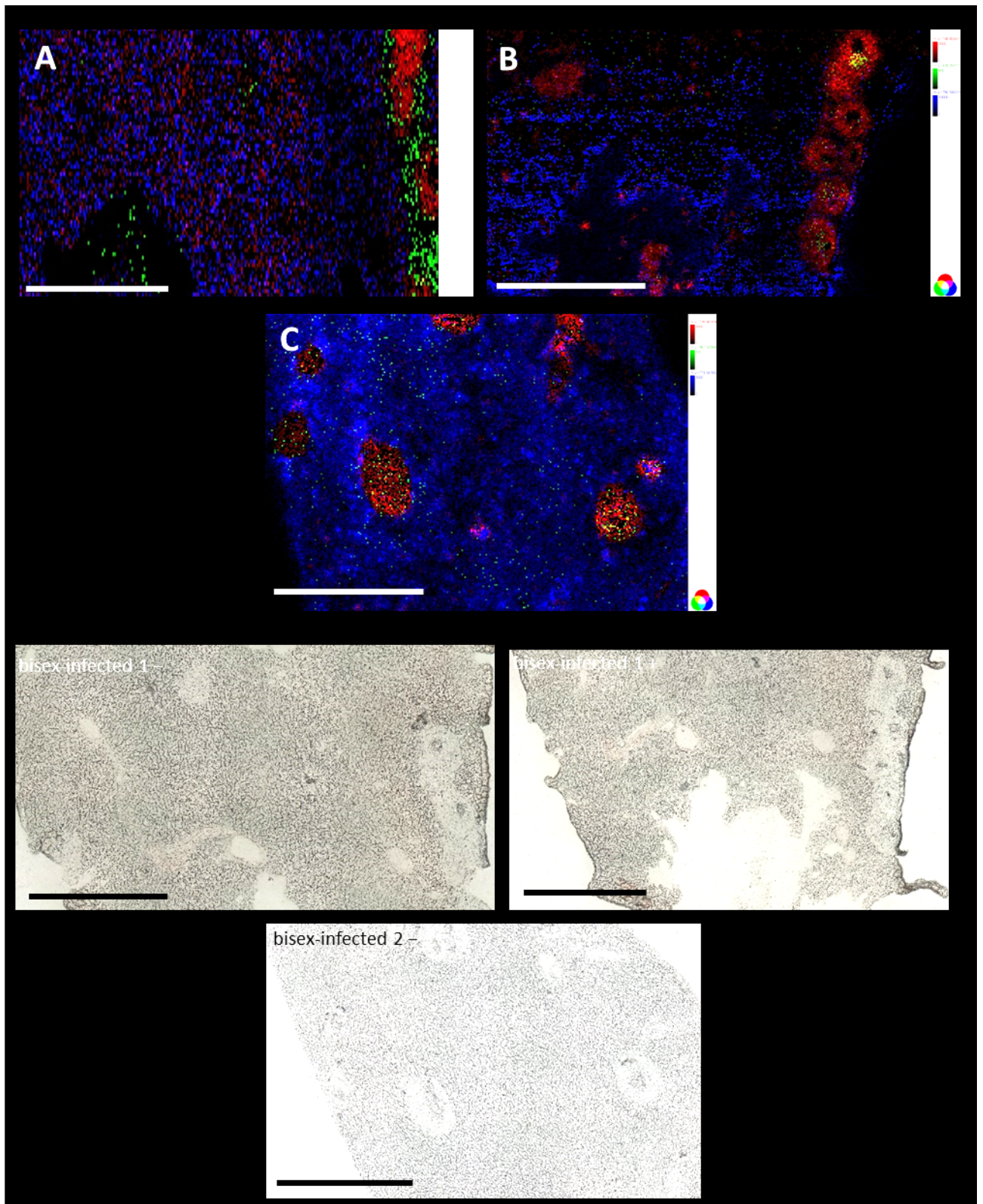


Figure S9: Upper part: Overlay MS images based on markers found by LC-MS/MS analysis. For A, m/z 836.545472, tentatively assigned to PS(40:5), $[M-H]^-$ was found to be a marker for granuloma, m/z 788.529391, DMPE(18:3_20:4), $[M-H]^-$ a marker for surrounding tissue and m/z 752.557091, plasmanyl-PE (O-18:0_20:4), $[M-H]^-$ a marker for the outer part of the granuloma. For B, m/z 746.604414, plasmanyl-PC(O-16:1_18:0), $[M+H]^+$ was found to be a marker for granuloma, m/z 780.550319, PE(17:0_22:5), $[M+H]^+$ a marker for surrounding tissue and m/z 438.297017, plasmeryl-LPE (P-16:0), $[M+H]^+$ as a marker for an enrichment inside the granuloma. For C, m/z 728.561836, plasmeryl-PE(P-18:0_18:1) or plasmanyl-PE(O-18:0_18:2), $[M-H]^-$ as a marker for granuloma and m/z 867.520849, PG(22:5_22:6), $[M-H]^-$ as a marker for an enrichment inside the granuloma. m/z 774.541653, plasmeryl-PE(P-18:0_22:6), $[M-H]^-$ was taken as a marker for surrounding tissue. Lower part: Corresponding microscopically images. Scale bars are 1 mm.

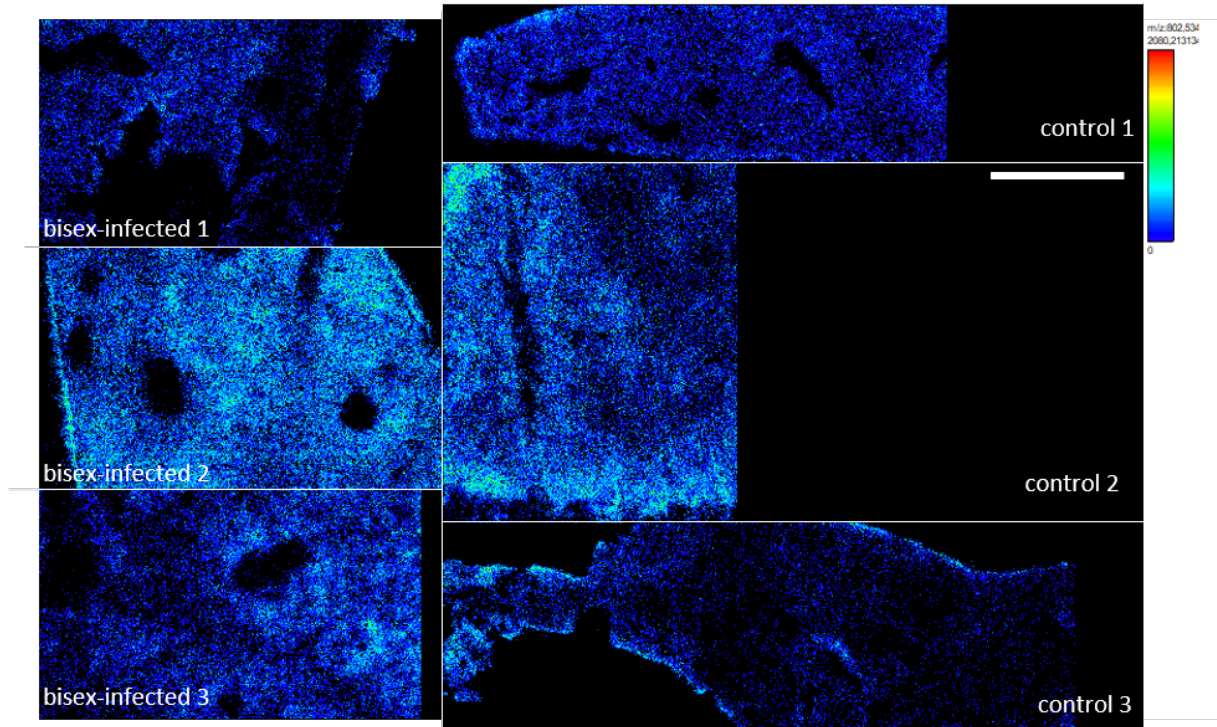


Figure S10: Lateral distribution of PC(16:0_20:5) at m/z 802.535727 as $[M+Na]^+$. While the lipid is depleted in the area of granulomas of liver samples of bisex-infected hamsters (left), it is evenly distributed in the control samples (right). Scale bars are 1 mm.

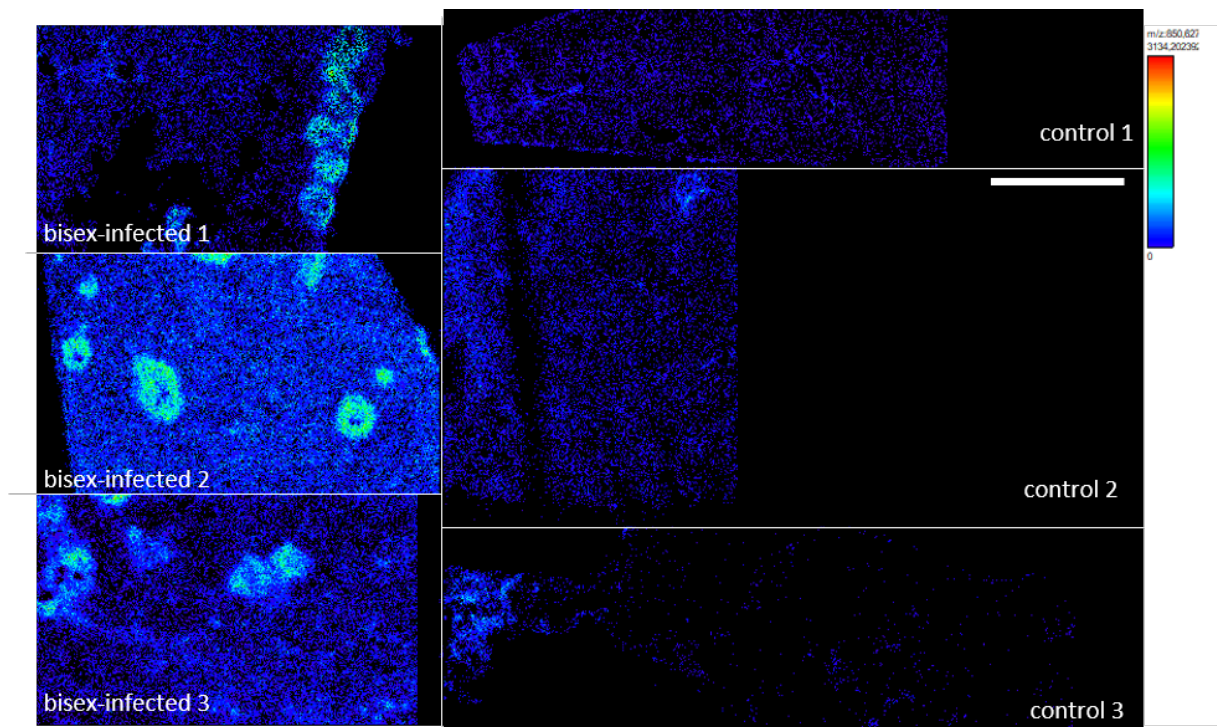


Figure S11: Lateral distribution of PC(19:0_20:2) at m/z 850.629627 as $[M+Na]^+$. While the lipid is evenly distributed in the control samples (right), enrichment in the granulomas is recognizable in liver samples of bisex-infected hamsters (left). However, the lipid is still detectable in the non-affected parts of the tissue. Scale bars are 1 mm.

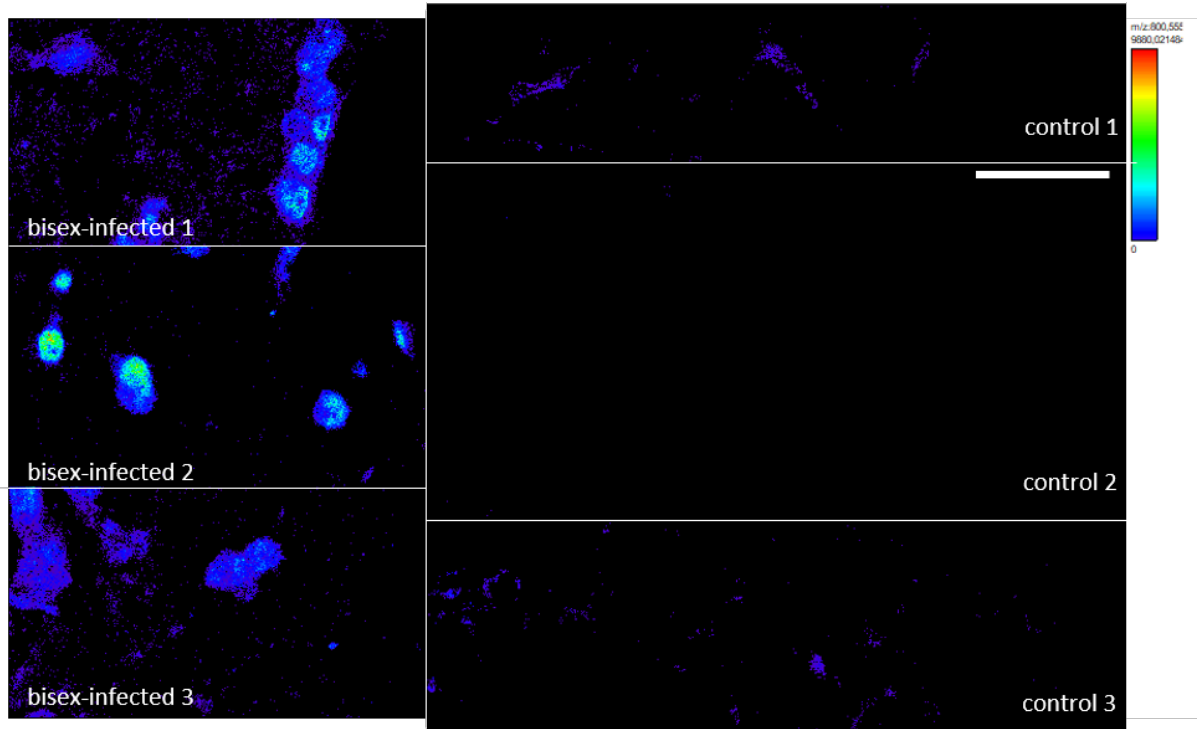


Figure S12: Lateral distribution of PC(16:0_18:0) at m/z 800.55617 as $[M+K]^+$. While the lipid was not found in the control samples (right), strong enrichment in the granulomas is recognizable in liver samples of bisex-infected hamsters (left). The lipid species was found accumulated especially in direct contact around the eggs. Scale bars are 1 mm.

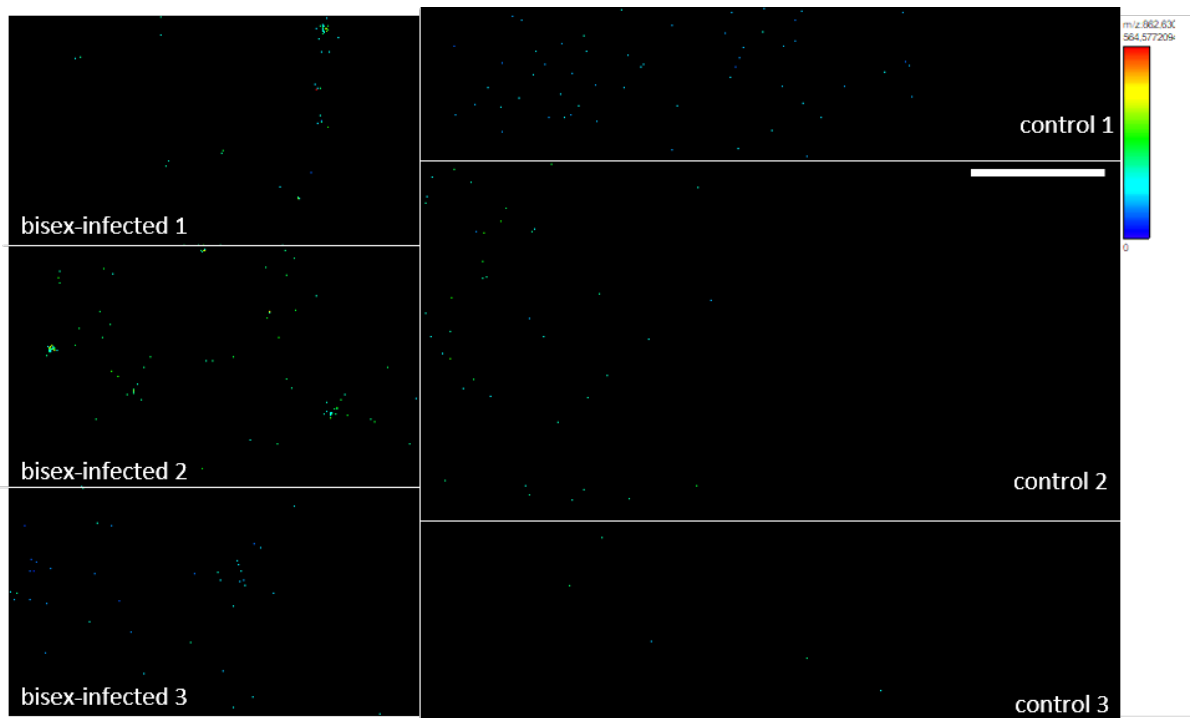


Figure S13: Lateral distribution of PC(20:0_20:3) at m/z 862.630305 as $[M+Na]^+$. The lipid was neither found in the control samples, nor in the non-affected tissue of liver samples of bisex-infected hamsters but was only found in and directly around the eggs. Scale bars are 1 mm.

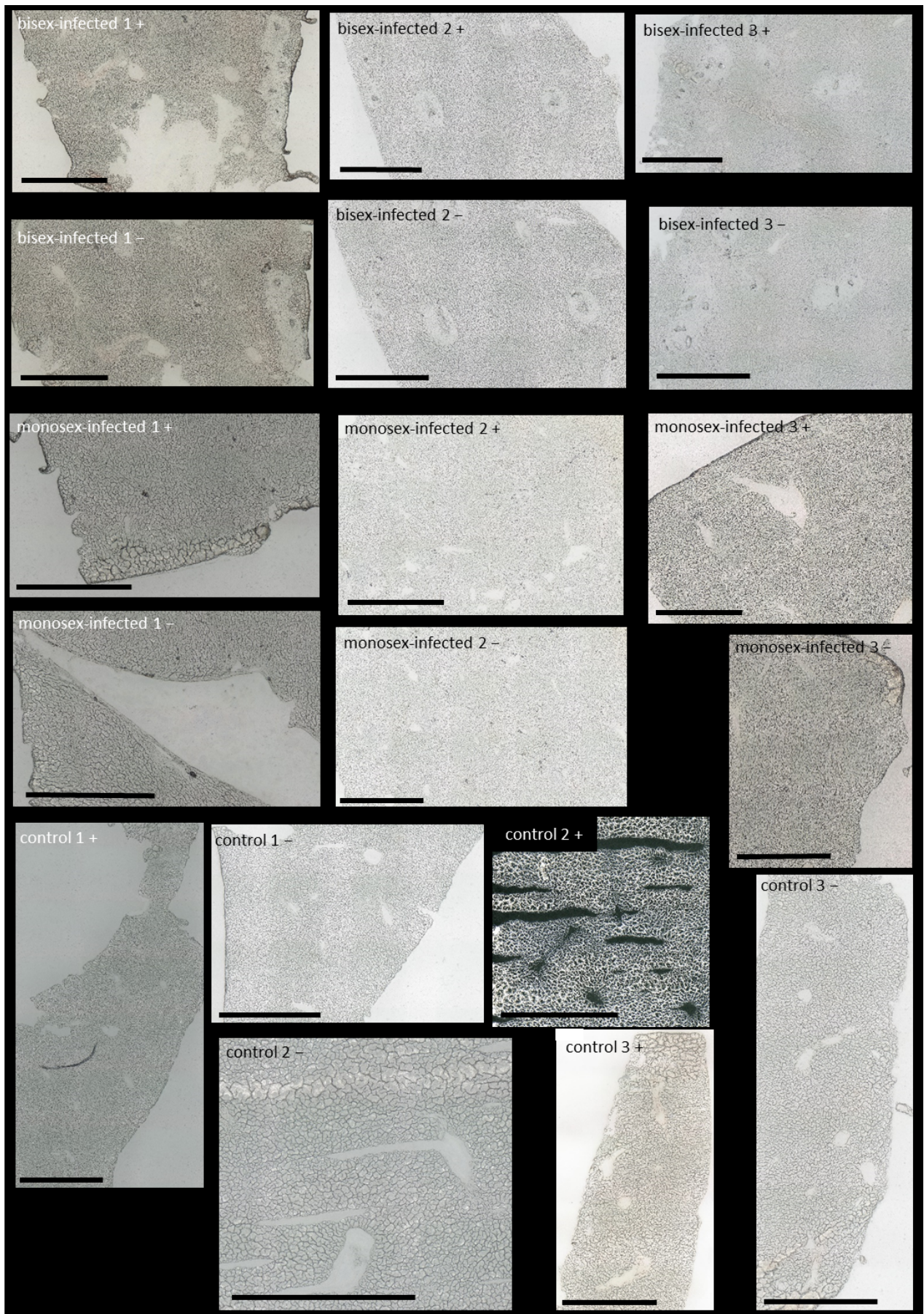


Figure S14: Microscopical images of all samples used for MALDI MSI experiments. Pictures were taken before matrix application and measurement. Scale bars are 1 mm.