A multimodal desorption electrospray ionisation workflow enabling visualisation of lipids and biologically relevant elements in a single tissue section

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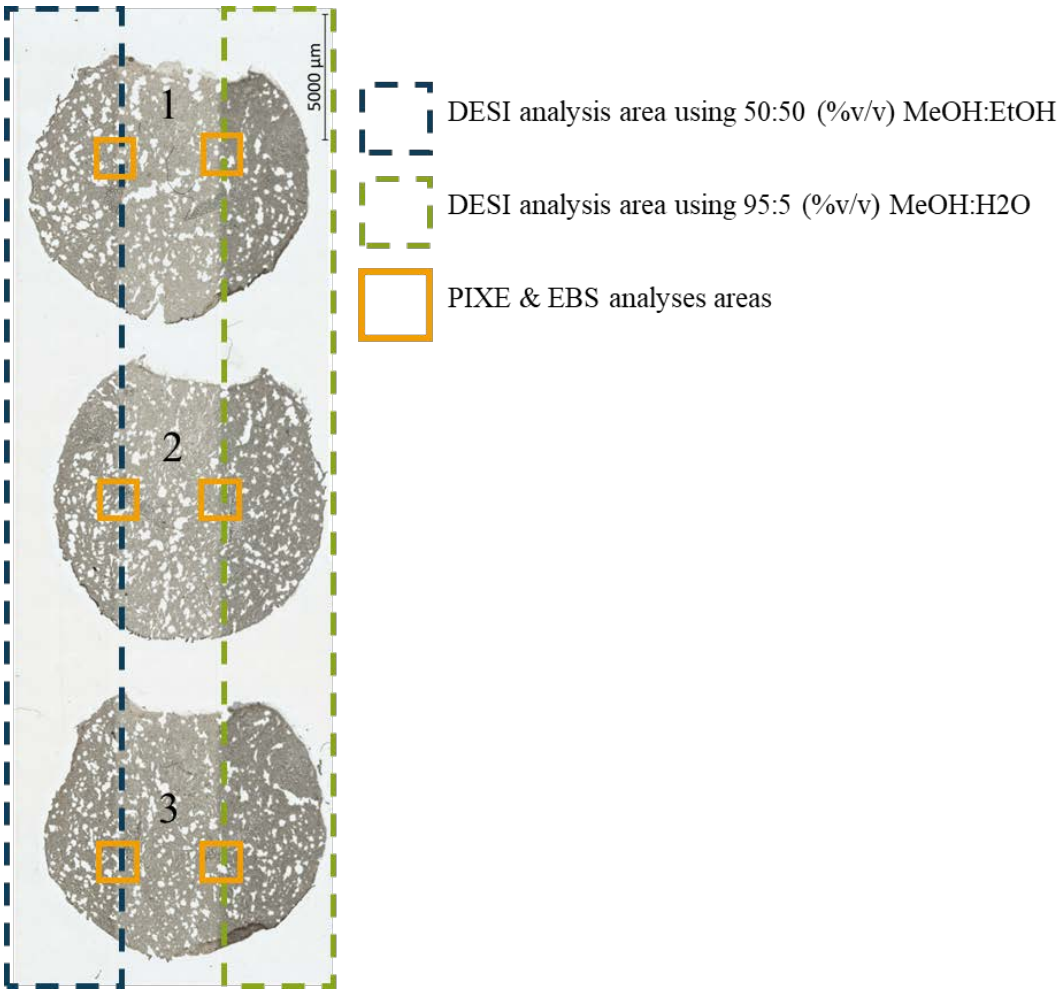

Figure S1. Areas analysed by DESI, PIXE & EBS on liver tissue homogenate sections.

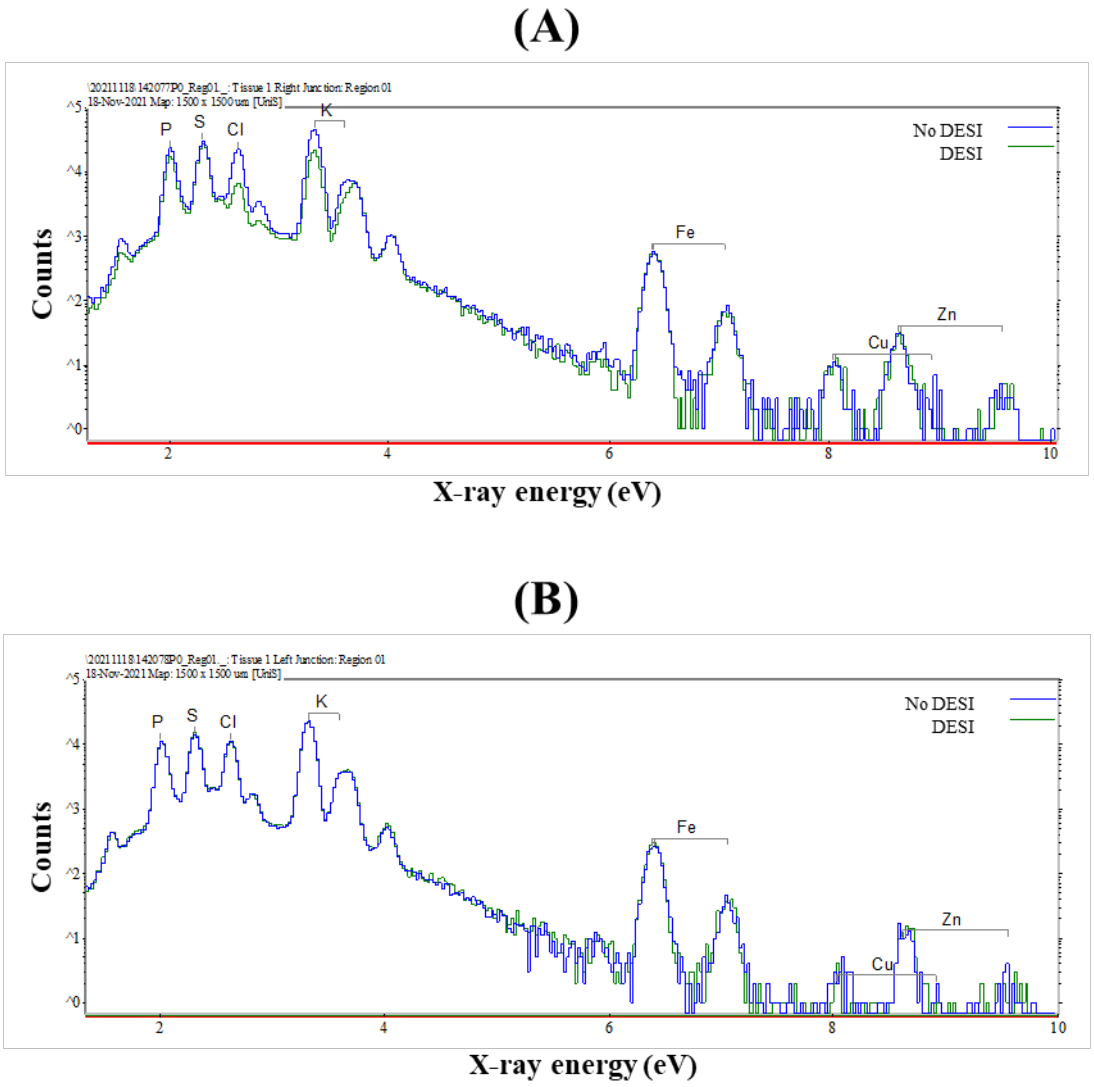

Figure S2. Overlay of X-ray spectra taken from regions of interest on areas not analysed (Reg01; blue) and analysed by DESI (Reg02; green) using (A) 95:5 (%v/v) MeOH:H_2O and (B) 50:50 (%v/v) MeOH:EtOH.

Table S1. T-test results between the average (n=3) of the ratios of the elemental peak area measured by PIXE after DESI analysis with two different solvents – MeOH/H_2O and MeOH/EtOH.

Table Analyzed	P
Column B	MeOH/EtOH
vs.	vs.
Column A	MeOH/H2O
Unpaired t test	
P value	0.0004
P value summary	***
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=11.28, df=4
How big is the difference?	
Mean of column A	0.6233
Mean of column B	1.007
Difference between means (B - A) ± SEM	0.3833 ± 0.03399
95% confidence interval	0.2890 to 0.4777
R squared (eta squared)	0.9695
F test to compare variances	
F, DFn, Dfd	13.86, 2, 2
P value	0.1346
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	3
Sample size, column B	3

Table Analyzed	S
Column B	MeOH/EtOH
vs.	vs.
Column A	MeOH/H2O
Unpaired t test	
P value	0.0016
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=7.566, df=4
How big is the difference?	
Mean of column A	0.7667
Mean of column B	1.087
Difference between means (B - A) ± SEM	0.3200 ± 0.04230
95% confidence interval	0.2026 to 0.4374
R squared (eta squared)	0.9347
F test to compare variances	
F, DFn, Dfd	39.25, 2, 2
P value	0.0497
P value summary	*
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	3
Sample size, column B	3

Table Analyzed	Cl
Column B	MeOH/EtOH
vs.	vs.
Column A	MeOH/H2O
Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=25.22, df=4
How big is the difference?	
Mean of column A	0.2567
Mean of column B	0.96
Difference between means (B - A) ± SEM	0.7033 ± 0.02789
95% confidence interval	0.6259 to 0.7808
R squared (eta squared)	0.9938
F test to compare variances	
F, DFn, Dfd	9.000, 2, 2
P value	0.2
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	3
Sample size, column B	3

Table Analyzed	K
Column B	MeOH/EtOH
vs.	vs.
Column A	MeOH/H2O
Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=21.57, df=4
How big is the difference?	
Mean of column A	0.4
Mean of column B	1.027
Difference between means (B - A) ± SEM	0.6267 ± 0.02906
95% confidence interval	0.5460 to 0.7073
R squared (eta squared)	0.9915
F test to compare variances	
F, DFn, Dfd	7.444, 2, 2
P value	0.2368
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	3
Sample size, column B	3

Table Analyzed	Fe
Column B	MeOH/EtOH
vs.	vs.
Column A	MeOH/H2O
Unpaired t test	
P value	0.0067
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=5.163, df=4
How big is the difference?	
Mean of column A	0.8067
Mean of column B	1.09
Difference between means (B - A) ± SEM	0.2833 ± 0.05487
95% confidence interval	0.1310 to 0.4357
R squared (eta squared)	0.8695
F test to compare variances	
F, DFn, Dfd	2.430, 2, 2
P value	0.583
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	3
Sample size, column B	3

Table Analyzed	Zn
Column B	MeOH/EtOH
vs.	vs.
Column A	MeOH/H2O
Unpaired t test	
P value	0.0232
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.577, df=4
How big is the difference?	
Mean of column A	0.87
Mean of column B	1.107
Difference between means (B - A) ± SEM	0.2367 ± 0.06616
95% confidence interval	0.05296 to 0.4204
R squared (eta squared)	0.7618
F test to compare variances	
F, DFn, Dfd	5.254, 2, 2
P value	0.3198
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	3
Sample size, column B	3

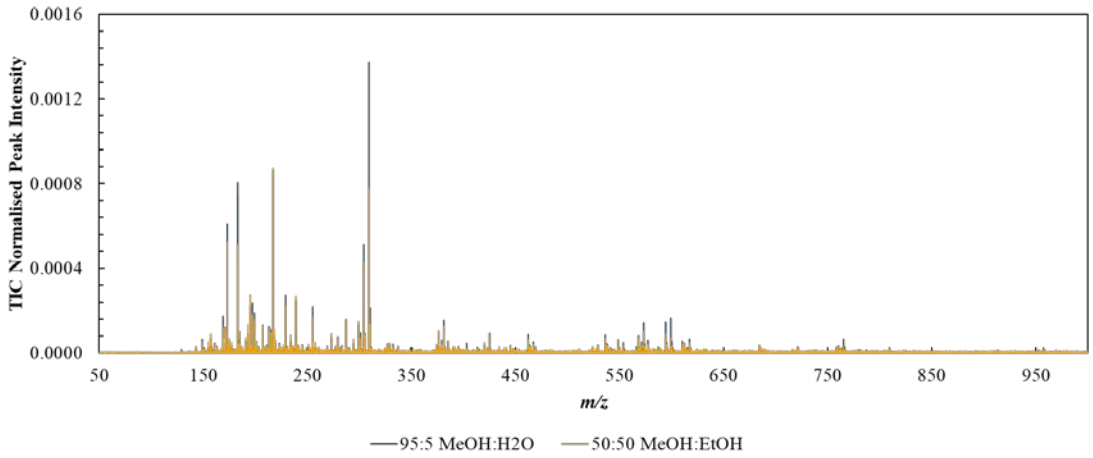

Figure S3. Spectra acquired with DESI using the two solvent systems 95:5 (%v/v) methanol/water (top) or 50:50 (%v/v) methanol/ethanol from the background (PET-only).

Table S2. Top 50 lipid features, adducts formed and measured *m/z* detected using each spray solvent system.

95:5 (%v/v) MeOH:H_2O		
Assignment	**Adduct**	**Measured m/z**
PC 36:4	$[M+K]^+$	820.538
PC 38:4	$[M+K]^+$	848.568
PC 34:2	$[M+K]^+$	796.534
PC 38:7	$[M+H]^+$	804.562
PC 40:7	$[M+H]^+$	832.592
PC 36:5	$[M+H]^+$	780.560
PC 34:1	$[M+K]^+$	798.550
PC 36:4	$[M+H]^+$	782.574
PC 36:2	$[M+K]^+$	824.566
PA 42:3	$[M+K]^+$	821.542
PG 42:7	$[M+H]^+$	849.570
PC 38:5	$[M+H]^+$	808.592
PC 36:3	$[M+K]^+$	822.546
PA 40:1	$[M+K]^+$	797.540
PC 38:5	$[M+K]^+$	846.548
FA 18:2	$[M+K]^+$	319.226
PC 38:6	$[M+H]^+$	806.572
PC 38:6	$[M+K]^+$	844.536
PC 38:4	$[M+H]^+$	810.606
PA 42:1	$[M+K]^+$	825.572
PA 40:0	$[M+Na]^+$	783.582
PC 38:3	$[M+K]^+$	850.574
PG 38:4	$[M+H]^+$	799.554
PC 38:5	$[M+Na]^+$	830.578
PC 38:6	$[M+Na]^+$	828.552
LPC 16:0	$[M+K]^+$	534.302
FA 20:5	$[M+H_3O]^+$	321.244
PC 36:1	$[M+K]^+$	826.572
PC 34:2	$[M+H]^+$	758.578
PG 40:5	$[M+Na]^+$	847.556
PC 40:6	$[M+H]^+$	834.606
PG 40:6	$[M+H]^+$	823.550
LPC 18:3	[M+H]+	518.328
PC 40:6	$[M+K]^+$	872.564
PC 32:1	$[M+K]^+$	770.516
LPC 20:4	$[M+K]^+$	582.302
PG 40:6	$[M+Na]^+$	845.536
PS 36:1	$[M+Na]^+$	812.542

50:50 (%v/v) MeOH:EtOH		
Assignment	**Adduct**	**Measured m/z**
PC 36:4	$[M+K]^+$	820.538
PC 38:4	$[M^+K]^+$	848.568
PC 38:7	$[M^+H]^+$	804.562
PC 40:7	$[M^+H]^+$	832.596
PC 34:2	$[M+K]^+$	796.54
PC 36:4	$[M^+H]^+$	782.58
PA 42:3	$[M^+K]^+$	821.542
PC 34:1	$[M^+K]^+$	798.552
PI 32:0	$[M^+K]^+$	849.576
PC 36:2	$[M+K]^+$	824.572
PC 38:5	$[M^+H]^+$	808.596
PC 36:3	$[M^+K]^+$	822.55
PA 40:1	$[M^+K]^+$	797.544
PC 38:5	$[M+K]^+$	846.554
PC 38:6	$[M^+H]^+$	806.578
PC 38:4	$[M^+H]^+$	810.612
PC 38:5	$[M^+Na]^+$	830.578
PC 38:6	$[M^+K]^+$	844.536
PC 38:3	$[M^+K]^+$	850.578
PA 40:0	$[M^+Na]^+$	783.582
PC 40:6	$[M^+H]^+$	834.606
PA 42:1	$[M^+K]^+$	825.572
PG 38:4	$[M+H]^+$	799.558
PG 40:5	$[M+Na]^+$	847.56
PC 36:1	$[M^+K]^+$	826.578
FA 18:2	$[M+K]^+$	319.23
PG 40:6	$[M^+H]^+$	823.55
PG 40:6	$[M^+Na]^+$	845.542
PA 42:0	$[M^+Na]^+$	811.616
LPC 18:3	$[M^+H]^+$	518.33
PC 36:3	$[M^+H]^+$	784.592
PC32:1	$[M+K]^+$	770.522
PC 36:2	$[M^+H]^+$	786.612
PE O-40:6	$[M^+Na]^+$	800.556
PC 34:1	$[M^+H]^+$	760.592
FA 20:5	$[M^+H]^+$	303.234
LPC 20:4	$[M^+Na]^+$	566.33
LPC 20:3	$[M^+H]^+$	546.362

LPC 18:0	$[M+K]^+$	562.334
PC 42:11	$[M+H]^+$	852.538
PA 42:0	$[M+Na]^+$	811.610
PC 40:6	$[M+Na]^+$	856.584
PS 38:3	$[M+Na]^+$	836.536
LPC 20:4	$[M+Na]^+$	566.330
PC 36:3	$[M+H]^+$	784.590
PC 42:8	$[M+K]^+$	896.558
PE O-40:6	$[M+Na]^+$	800.554
PC 34:1	$[M+H]^+$	760.592
LPC 20:3	$[M+H]^+$	546.360
PC 36:2	$[M+H]^+$	786.610

PC 34:5	$[M+K]^+$	790.548
PG 42:6	$[M^+H]^+$	851.582
LPC 20:4	$[M^+K]^+$	582.304
FA 18:1	$[M+K]^+$	321.244
LPC 18:0	$[M^+K]^+$	562.334
PI 34:2	$[M+K]^+$	873.574
PE 38:2	$[M^+H]^+$	772.538
PC 40:5	$[M^+K]^+$	874.58
FA 22:6	$[M^+K]^+$	367.204
FA 20:4	$[M^+Na]^+$	327.234
FA 18:2	$[M^+K]^+$	319.208
PE 38:1	$[M^+K]^+$	812.548

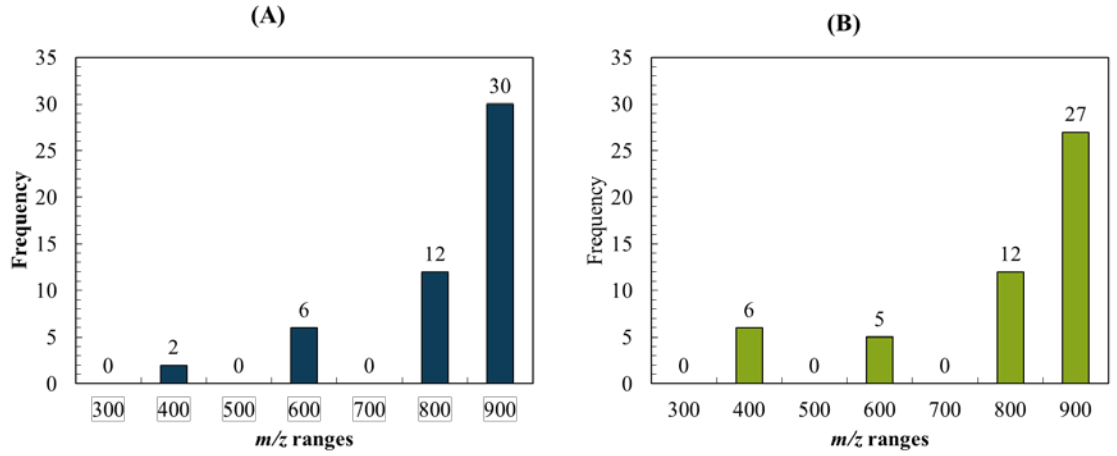

Figure S4. Histogram of the number of lipid features in the top 50 detected per m/z range (bin size 100) for each spray solvent – (A) MeOH/H_2O and (B) MeOH/EtOH.

Table S3. T-test results comparing the measured normalised (to TIC) peak intensities for the top 10 lipids measured using the two solvent systems.

	Discovery?	P value	Mean of 95:5 (%v/v) MeOH:H2O	Mean of 50:50 (%v/v/) MeOH:EtOH	Difference	SE of difference	t ratio	df	q value
PC 36:4 $[M+K]^+$	Yes	<0.000001	0.01117	0.01398	-0.002816	0.0003404	8.27	16	<0.000001
PC 38:4 $[M+K]^+$	Yes	<0.000001	0.01014	0.01349	-0.003351	0.0003131	10.7	16	<0.000001
PC 34:2 $[M+K]^+$	Yes	0.000786	0.009648	0.01054	-0.0008878	0.000215	4.13	16	0.000893
PC 38:7 $[M+H]^+$	Yes	<0.000001	0.007512	0.01219	-0.004677	0.0002612	17.91	16	<0.000001
PC 40:7 $[M+H]^+$	Yes	<0.000001	0.006606	0.0115	-0.004899	0.0002348	20.86	16	<0.000001
PC 34:1 $[M+K]^+$	Yes	0.006748	0.007	0.007458	-0.0004578	0.0001472	3.109	16	0.006816
PC 36:4 $[M+H]^+$	Yes	<0.000001	0.006516	0.008541	-0.002026	0.0001099	18.44	16	<0.000001
PC 36:2 $[M+K]^+$	Yes	0.000004	0.006268	0.007224	-0.0009567	0.000139	6.884	16	0.000005
PA 42:3 $[M+K]^+$	Yes	0.000001	0.00551	0.006727	-0.001217	0.0001635	7.439	16	0.000002

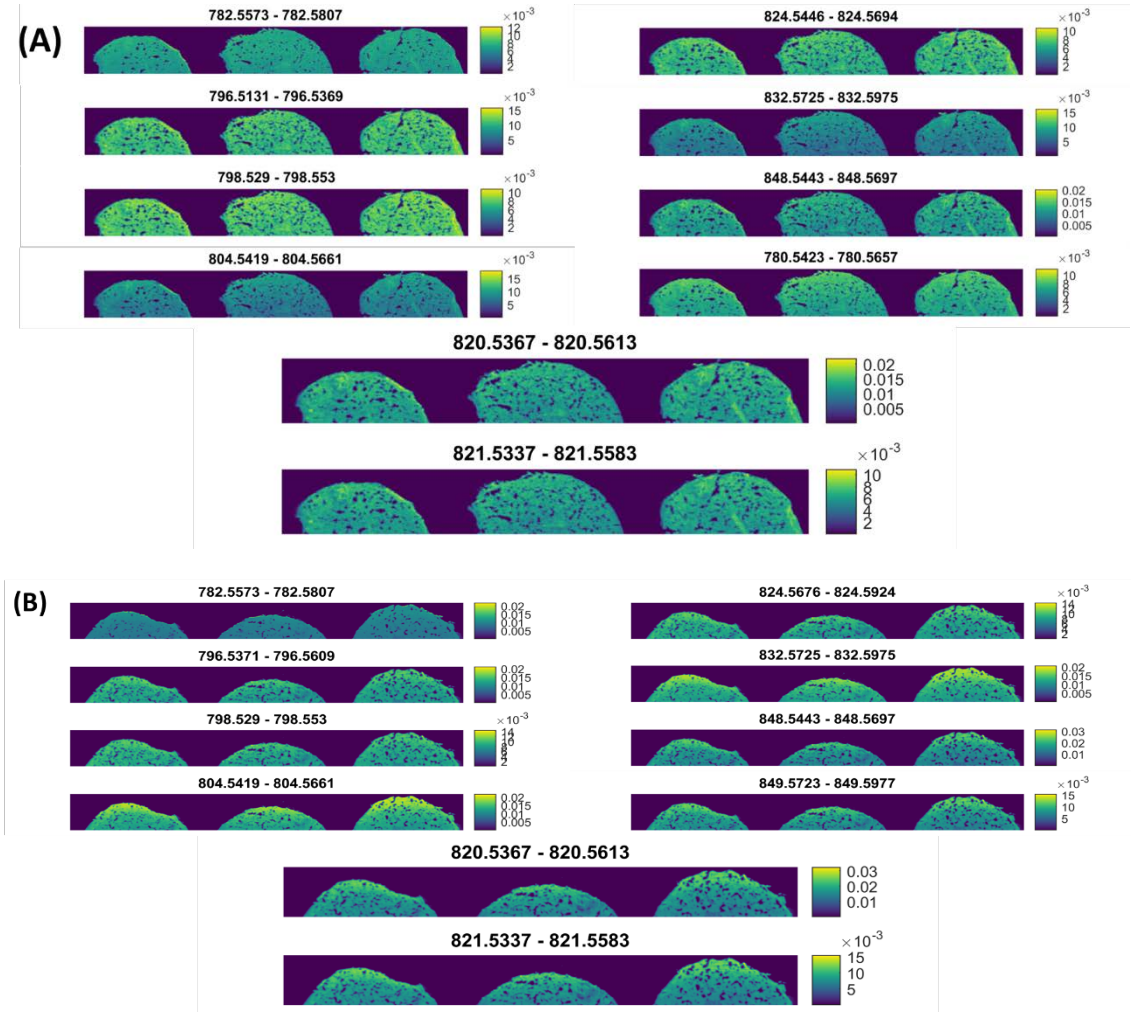

Figure S5. Resulting DESI ion maps for the top 10 most abundant lipids imaged using (A) 95:5 (%v/v) MeOH:H_2O or (B) 50:50 (%v/v) MeOH:EtOH spray solvents on liver tissue homogenates.

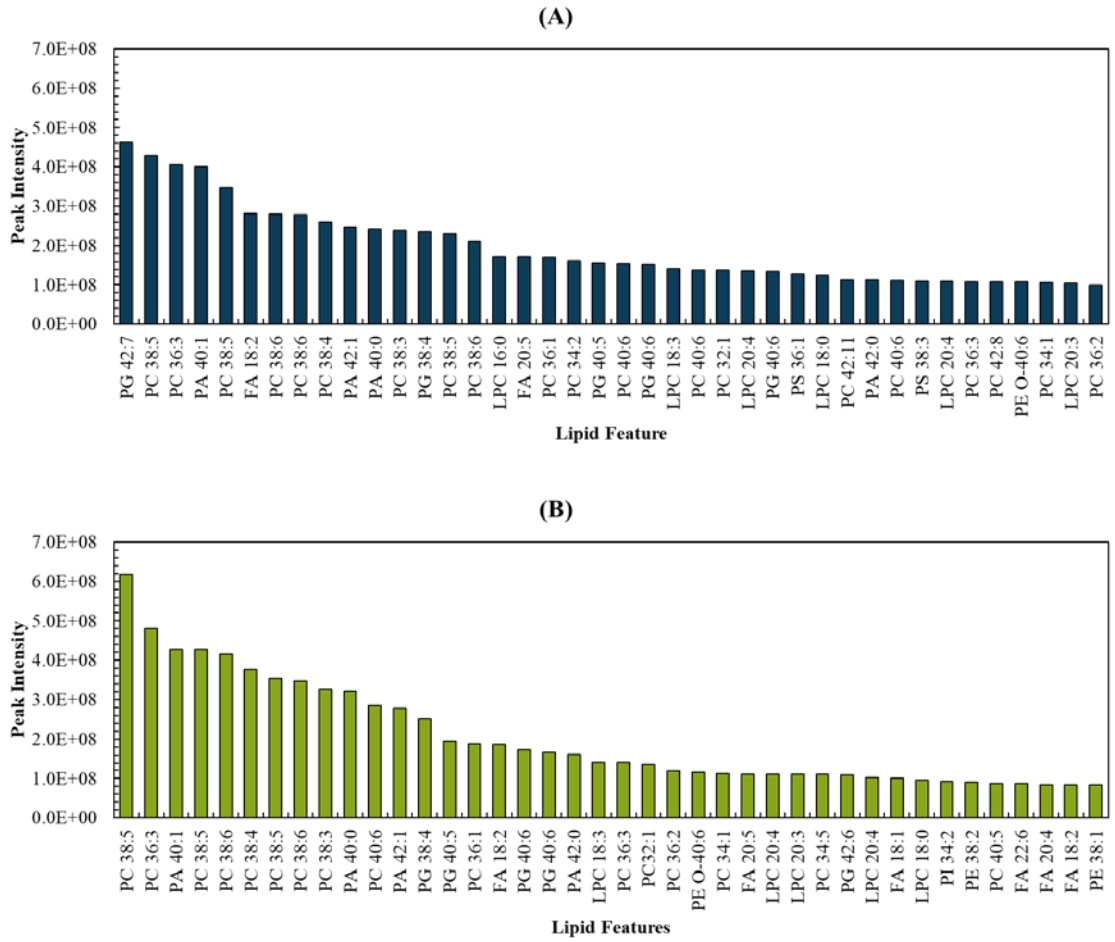

Figure S6. Remaining 40 features in the top 50 most intense lipid features and their respective intensities for (A) MeOH:H2O and (B) MeOH:EtOH.

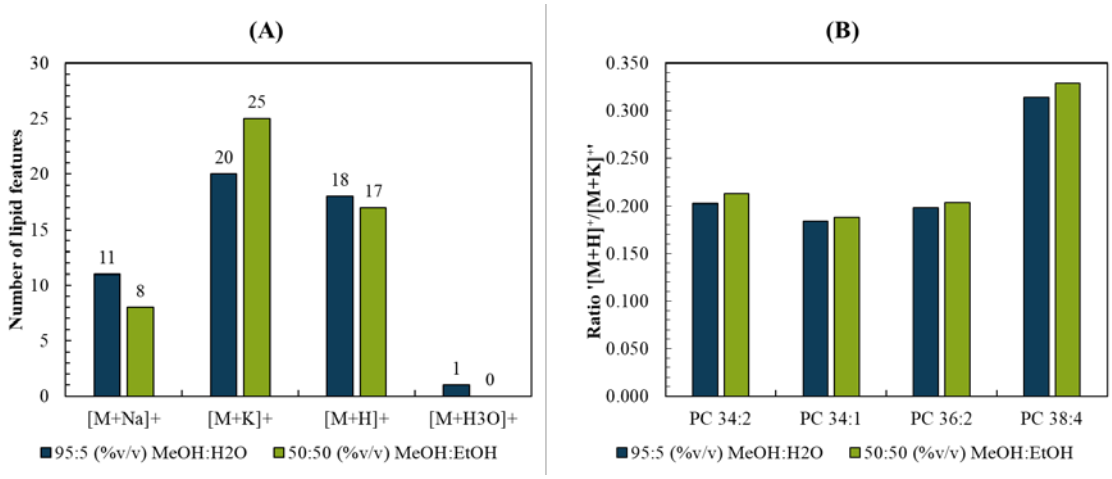

Figure S7. (A) Number of lipid features detected in the top 50 lipids with a specific adduct for each of the two solvent mixtures – 95:5 (%v/v) MeOH:H_2O (blue) and 50:50 (%v/v) MeOH:EtOH (green). (B) Ratio of average (n=9) peak intensities measured for $[M+H]^+$ and $[M+K]^+$ ions formed for a selection of PC lipids using the two solvent mixtures – 95:5 (%v/v) MeOH:H_2O (blue) and 50:50 (%v/v) MeOH:EtOH (green).

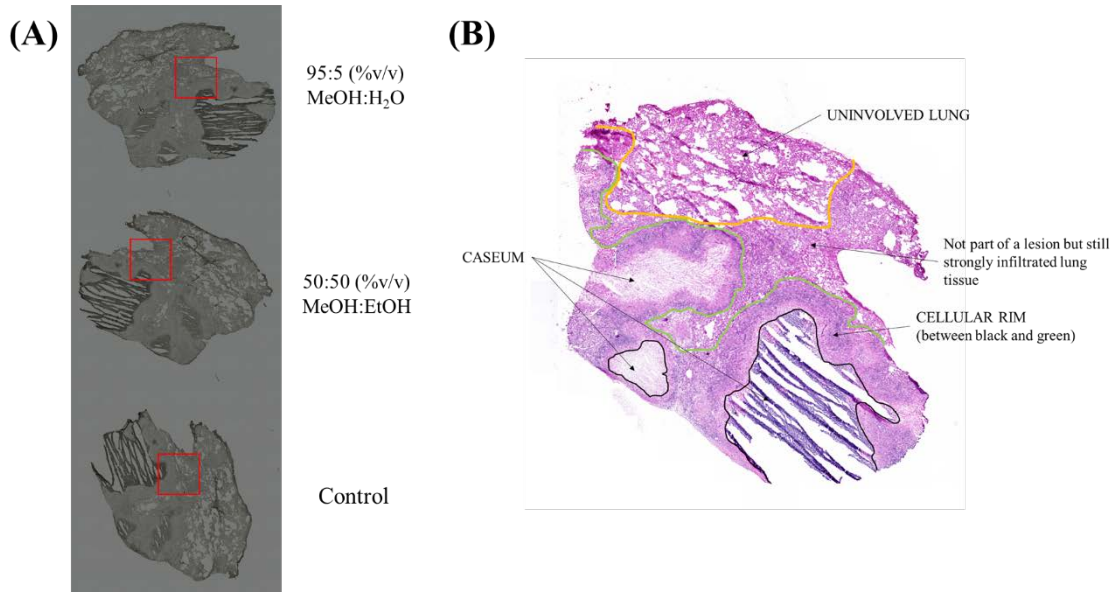

Figure S8. (A) Optical images of sequential fresh frozen rabbit lung tissues containing a caseous granuloma (a lesion caused by tuberculosis) showing the regions analysed using ion beam analysis; (B) Hematoxylin and eosin (H&E) stained section and the different regions of the granuloma.

Table S4. T-test results of the elemental peak area measured by PIXE from a lung tissue section after DESI analysis with two different solvents – MeOH/H_2O and MeOH/EtOH.

Table Analyzed	Phosphorus
Column B	MeOH:H2O
vs.	vs.
Column A	Control
Unpaired t test	
P value	0.0042
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=4.484, df=6
How big is the difference?	
Mean of column A	169825
Mean of column B	103629
Difference between means (B - A) ± SEM	-66197 ± 14763
95% confidence interval	-102321 to -30073
R squared (eta squared)	0.7702
F test to compare variances	
F, DFn, Dfd	1.472, 3, 3
P value	0.7585
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	4

Table Analyzed	Sulphur
Column B	MeOH:H2O
vs.	vs.
Column A	Control
Unpaired t test	
P value	0.0641
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=2.265, df=6
How big is the difference?	
Mean of column A	166966
Mean of column B	131636
Difference between means (B - A) ± SEM	-35330 ± 15598
95% confidence interval	-73496 to 2836
R squared (eta squared)	0.4609
F test to compare variances	
F, DFn, Dfd	2.191, 3, 3
P value	0.536
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	4

Table Analyzed	Chlorine
Column B	MeOH:H2O
vs.	vs.
Column A	Control
Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=9.781, df=6
How big is the difference?	
Mean of column A	239292
Mean of column B	20466
Difference between means (B - A) ± SEM	-218826 ± 22373
95% confidence interval	-273572 to -164081
R squared (eta squared)	0.941
F test to compare variances	
F, DFn, Dfd	737.9, 3, 3
P value	0.0002
P value summary	***
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	4
Sample size, column B	4

Table Analyzed	Potassium
Column B	MeOH:H2O
vs.	vs.
Column A	Control
Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=11.11, df=6
How big is the difference?	
Mean of column A	327173
Mean of column B	90625
Difference between means (B - A) ± SEM	-236548 ± 21288
95% confidence interval	-288637 to -184459
R squared (eta squared)	0.9537
F test to compare variances	
F, DFn, Dfd	7.637, 3, 3
P value	0.129
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	4

Table Analyzed	Iron
Column B	MeOH:H2O
vs.	vs.
Column A	Control
Unpaired t test	
P value	0.354
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.004, df=6
How big is the difference?	
Mean of column A	2756
Mean of column B	2233
Difference between means (B - A) ± SEM	-523.1 ± 520.8
95% confidence interval	-1797 to 751.2
R squared (eta squared)	0.1439
F test to compare variances	
F, DFn, Dfd	13.91, 3, 3
P value	0.0578
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	4

Table Analyzed	Phosphorus
Column C	MeOH:EtOH
vs.	vs.
Column A	Control
Unpaired t test	
P value	0.0556
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=2.369, df=6
How big is the difference?	
Mean of column A	169825
Mean of column C	121313
Difference between means (C - A) ± SEM	-48512 ± 20475
95% confidence interval	-98613 to 1588
R squared (eta squared)	0.4834
F test to compare variances	
F, DFn, Dfd	2.231, 3, 3
P value	0.527
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column C	4

Table Analyzed	Sulphur
Column C	MeOH:EtOH
vs.	vs.
Column A	Control
Unpaired t test	
P value	0.3721
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.9643, df=6
How big is the difference?	
Mean of column A	166966
Mean of column C	146604
Difference between means (C - A) ± SEM	-20362 ± 21115
95% confidence interval	-72029 to 31306
R squared (eta squared)	0.1342
F test to compare variances	
F, DFn, Dfd	4.848, 3, 3
P value	0.2273
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column C	4

Table Analyzed	Chlorine
Column C	MeOH:EtOH
vs.	vs.
Column A	Control
Unpaired t test	
P value	0.2547
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.259, df=6
How big is the difference?	
Mean of column A	239292
Mean of column C	195866
Difference between means (C - A) ± SEM	-43427 ± 34487
95% confidence interval	-127814 to 40961
R squared (eta squared)	0.209
F test to compare variances	
F, DFn, Dfd	1.379, 3, 3
P value	0.7979
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column C	4

Table Analyzed	Potassium
Column C	MeOH:EtOH
vs.	vs.
Column A	Control
Unpaired t test	
P value	0.1484
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.658, df=6
How big is the difference?	
Mean of column A	327173
Mean of column C	259388
Difference between means (C - A) ± SEM	-67785 ± 40881
95% confidence interval	-167818 to 32248
R squared (eta squared)	0.3142
F test to compare variances	
F, DFn, Dfd	3.171, 3, 3
P value	0.3686
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column C	4

Table Analyzed	Iron
Column C	MeOH:EtOH
vs.	vs.
Column A	Control
Unpaired t test	
P value	0.3264
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.068, df=6
How big is the difference?	
Mean of column A	2756
Mean of column C	2127
Difference between means (C - A) ± SEM	-629.8 ± 589.4
95% confidence interval	-2072 to 812.5
R squared (eta squared)	0.1599
F test to compare variances	
F, DFn, Dfd	2.680, 3, 3
P value	0.4396
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column C	4

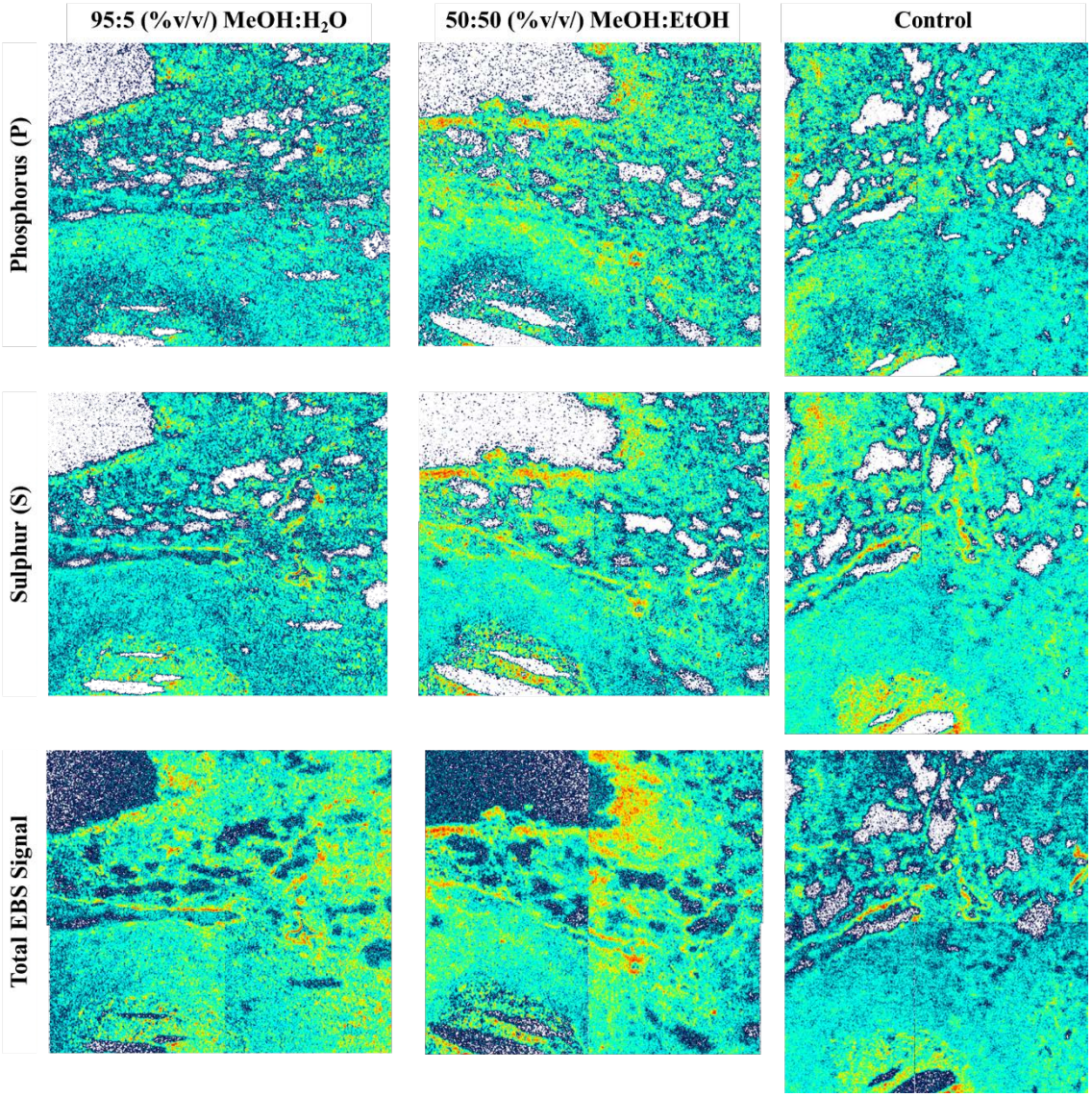

Figure S9. Phosphorus (P), sulphur (S) PIXE maps and total EBS maps taken from fresh frozen lung tissue sections after DESI analysis using 95:5 (%v/v) MeOH:H2O or 50:50 (%v/v) MeOH:EtOH. A third section (Control) was also analysed – no DESI measurements were taken on this sample.

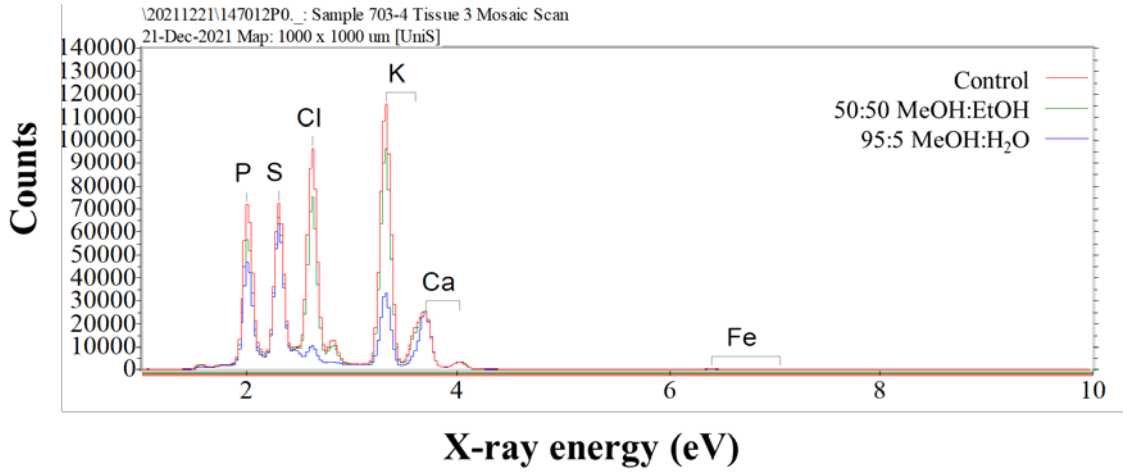

Figure S10. Overlay of the X-ray spectra taken from the squares containing the caseum in each of the lung tissue section samples.