Supplementary Material

Characterization of spatial heterogeneity in metastasized colorectal cancer by MALDI imaging

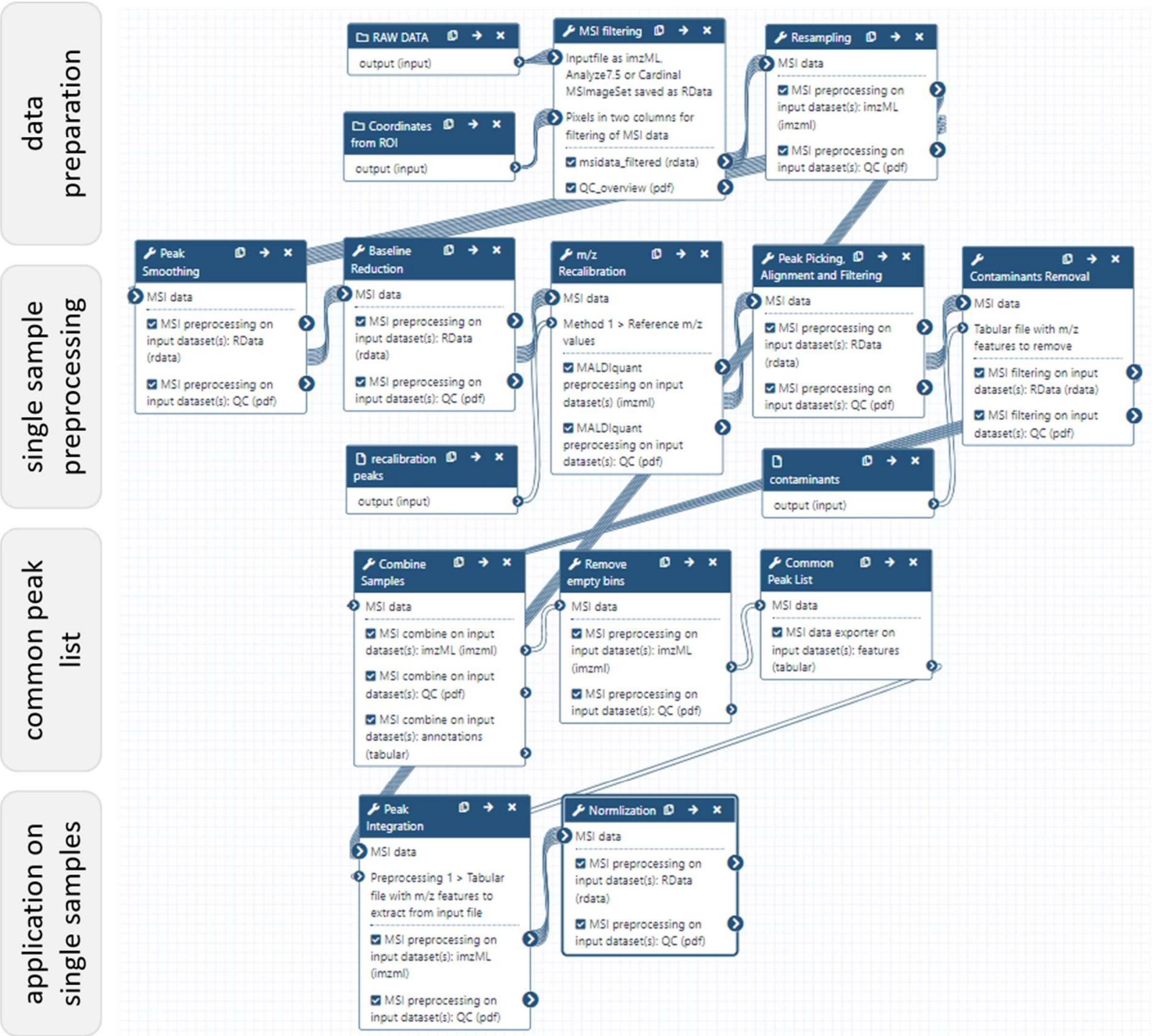

Figure S1: Final pre-processing workflow in Galaxy after evaluation of multiple possible approaches. At first, the data is imported into Galaxy and background spectra are removed to keep only tissue spectra. Each sample is then pre-processed with the same parameters, to finally combine all samples. The combined file is used to extract a common peak list, which is needed to integrate the intensities of these peaks all spectra of all raw files. Finally, the intensities are normalized. This procedure enables comparison of all files with each other.

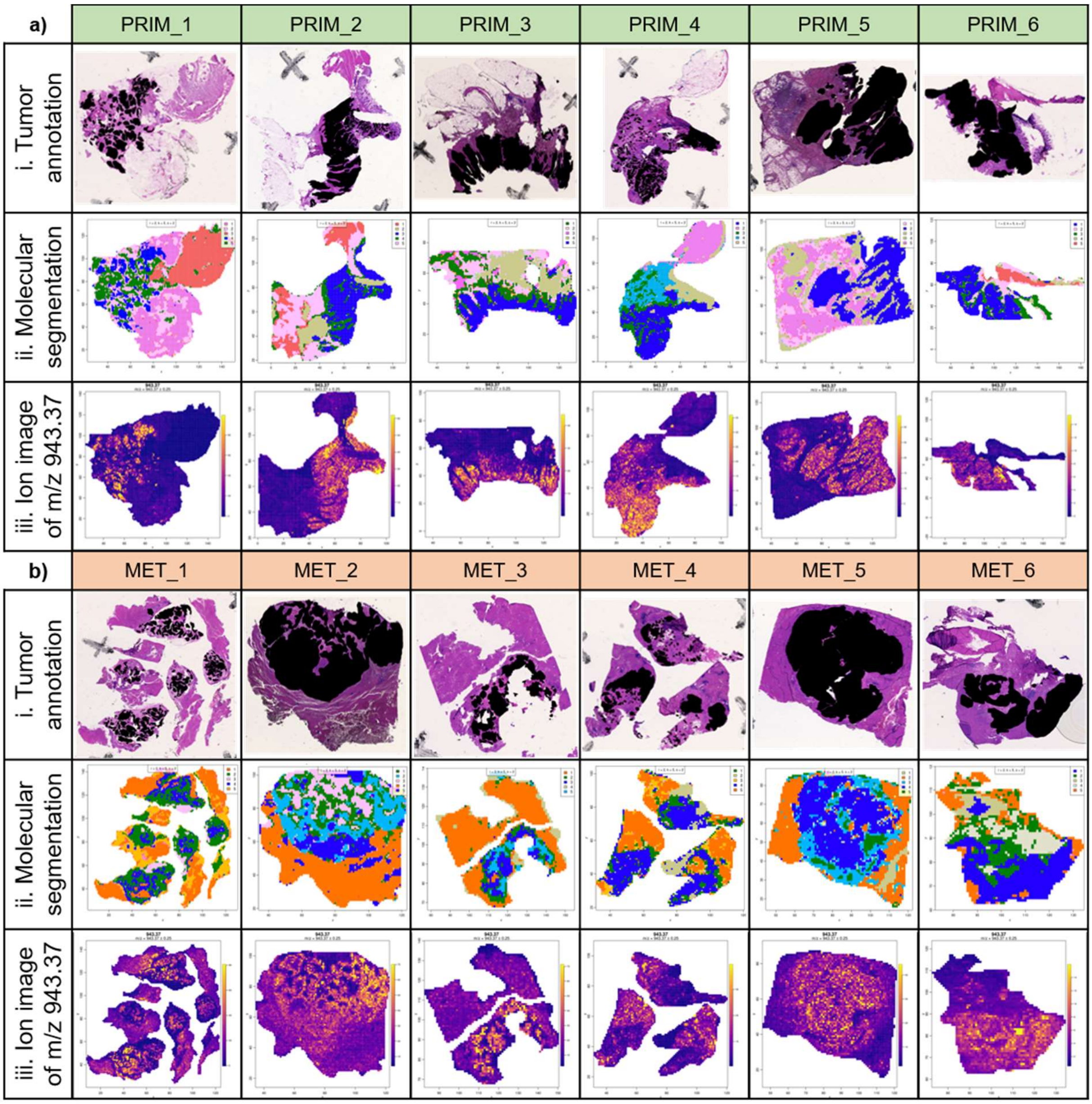

Figure S2. Detailed tumor annotations and spatial proteome segments. i. Detailed annotation of the vital tumor tissue in the H&E image in black. ii. MALDI imaging segments with different proteomic composition. The dark blue cluster correlates with the vital tumor tissue areas. For primary tumors the clusters resembled the annotations very well, with the exception of two samples (PRIM_2, PRIM_6) in which the healthy colon epithelium could not be separated reliably from tumor tissue. In metastasis samples the small areas outside of the tumor region were more frequently included in the tumor cluster. One metastasis was missing a significant part of the tumor tissue in the tumor cluster (MET_4). iii. Ion image shows the distribution of the tumor enriched m/z 943.37, which correlates with the tumor annotation in i. Primary tumors showed almost exclusively high intensities for m/z 943.37 in the tumor regions, whereas in liver metastases intensities were high in tumor regions but also moderate in liver parenchyma.

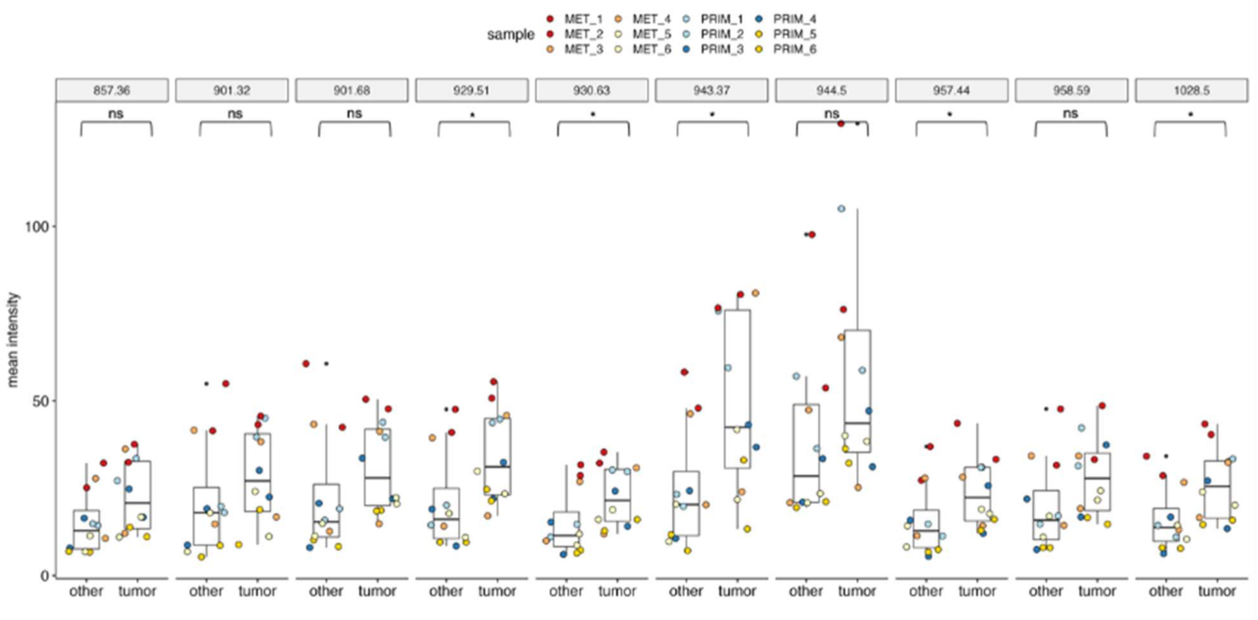

Figure S3: Mean intensities of the top tumor m/z features per sample. The tumor m/z features show consistently higher intensities in tumor areas in comparison to the rest of the imaged samples when compared patient-matched (same colors represent matched pairs of primary tumors and metastasis of the same patient). Significance tested using Wilcoxon rank sum test, corrected for multiple testing using "Benjamin Hochberg“ method. * adjusted p-value < 0.05.

Table S1. Galaxy tools and parameters applied for spectra pre-processing.

Pre-processing step	**Galaxy tool**	**Method**	**Setting**
Import data	MSI filtering	Pixel filtering	Coordinates from tabular file
Resampling	MSI preprocessing	Data reduction, resample	Step size: 0.05 m/z
Tissue area extraction + stripe removal	MSI filtering	Pixel filtering	Coordinates from tabular file: tabular file with coordinates of tissue area from co-registration workflow
Smoothing	MSI preprocessing	Peak Smoothing, Guassian	Standard deviation: 3 Window Size: 12
Baseline reduction	MSI preprocessing	Baseline Reduction	Blocks: 850 Spar value: 1
m/z recalibration	MALDIquant preprocessing	Align spectra, Lowess	Tolerance: 0.0002 Half window size: 9 Noise estimation: MAD S/N: 3

Peak picking	MSI preprocessing	Peak Picking	S/N: 6 Number of blocks: 100 Window size: 6 Method: adaptive Spar value: 1
Peak alignment	MSI preprocessing	Peak alignment, diff	Diff.max: 200 Units: ppm
Peak filtering	MSI preprocessing	Peak filtering	Freq. min: 1% (unique value each sample)
Contaminants removal	MSI filtering	Removal of m/z features	Window: 200 Units: ppm Tabular: contaminant tabular from Keller + added int. calibrants
Combine samples	MSI combine		Different m/z axis: yes
Remove empty bins	MSI preprocessing	Peak filtering	Processed: 200 ppm Freq. min: 1
Common peak list	MSI data exporter	m/z feature output	
Peak integration	MSI preprocessing	Data reduction, peaks: Common peak list tabular	Intensity value: height
Normalization	MSI preprocessing	TIC	

Table S2. List of vital tumor area enriched peptide m/z features

Primary tumor, tumor enriched m/z	**Liver metastasis, tumor enriched m/z**
801.32	801.32
802.28	802.28
803.57	803.57
804.53	804.53
810.35	810.35
811.32	811.32
814.57	814.57
815.55	815.55
816.53	816.53
817.51	817.51
818.49	818.49
822.43	822.43
823.42	823.42
827.38	827.38

828.37	828.37
831.36	831.36
833.36	833.36
834.36	834.36
835.36	835.36
838.37	836.36
839.38	837.37
842.41	838.37
843.42	839.38
845.44	840.39
847.47	841.39
850.53	842.41
851.55	843.42
852.57	845.44
853.26	847.47
853.6	850.53
854.28	851.55
854.62	852.57
855.31	853.26
857.36	853.6
858.39	854.28
859.42	857.36
867.37	858.39
870.49	859.42
871.54	867.37
872.59	868.41
873.63	869.45
874.33	870.49
874.68	871.54
875.38	872.59
876.43	873.63
882.41	874.33
888.43	874.68
890.21	875.38
890.57	876.43
900.6	886.3
901.32	887.37
901.68	890.21
902.4	890.57
911.47	898.44
912.57	899.52
914.39	900.6
915.49	901.32
916.59	901.68
917.32	902.4
917.69	911.47

919.53	912.57
920.63	914.39
926.54	915.49
928.4	916.59
929.51	917.32
930.63	917.69
931.37	919.53
931.75	920.63
932.49	926.54
933.61	928.4
940.36	929.51
941.49	930.63
942.62	931.37
943.37	931.75
944.5	932.49
945.64	933.61
946.4	940.36
947.53	941.49
948.29	942.62
950.57	943.37
951.33	944.5
951.71	945.64
957.44	946.4
958.59	947.53
959.35	948.29
960.51	950.57
961.66	951.33
962.43	951.71
964.36	957.44
965.51	958.59
966.67	959.35
967.45	960.51
971.71	961.66
972.49	962.43
974.44	964.36
975.61	965.51
976.39	966.67
977.56	967.45
978.34	971.71
985.41	972.49
986.6	974.44
987.39	975.61
992.53	976.39
993.73	977.56
994.52	978.34
1002.51	985.41

1006.53	986.6
1010.56	987.39
1011.37	992.53
1019.49	993.73
1028.5	994.52
1029.33	1002.51
1029.74	1006.53
1032.63	1010.56
1033.45	1011.37
1034.69	1019.49
1039.67	1028.5
1040.5	1029.33
1043.42	1029.74
1053.49	1032.63
1055.6	1033.45
1056.44	1034.69
1059.4	1039.67
1060.68	1040.5
1061.52	1043.42
1062.37	1053.49
1063.65	1055.6
1064.5	1056.44
1066.63	1059.4
1071.76	1060.68
1074.34	1061.52
1081.67	1062.37
1082.54	1063.65
1084.7	1064.5
1087.74	1066.63
1088.61	1071.76
1089.49	1074.34
1093.41	1079.51
1094.29	1081.67
1094.73	1082.54
1116.4	1084.7
1117.74	1087.74
1118.63	1088.61
1129.42	1089.49
1132.59	1093.41
1134.86	1094.29
1141.69	1094.73
1160.56	1095.6
1161.49	1096.48
1163.35	1097.36
1165.68	1097.8
1166.61	1098.68

1173.63	1105.29
1181.64	1105.73
1185.43	1106.61
1187.8	1107.5
1188.75	1110.61
1189.71	1116.4
1190.66	1120.42
1197.82	1121.32
1198.78	1127.62
1199.74	1132.59
1209.38	1133.49
1209.86	1134.4
1220.56	1138.49
1221.53	1141.69
1222.51	1149.48
1242.72	1160.56
1254.71	1161.49
1255.72	1165.68
1264.79	1166.61
1265.3	1173.63
1270.88	1181.64
1273.93	1184.48
1280.57	1185.43
1285.7	1187.8
1286.73	1188.75
1293.44	1189.71
1301.74	1190.66
1303.83	1197.82
1314.83	1198.78
1315.88	1199.74
1316.93	1209.86
1325.92	1220.56
1326.98	1221.53
1328.04	1222.51
1328.57	1223.49
1330.7	1224.47
1334.43	1240.74
1336.57	1242.72
1337.64	1255.72
1338.71	1267.83
1341.93	1268.84
1342.46	1270.88
1343.54	1273.93
1354.87	1274.44
1356.5	1275.46
1363.57	1285.7

1364.66	1286.73
1365.75	1301.74
1366.85	1302.79
1367.39	1303.83
1367.94	1304.87
1368.49	1306.44
1369.58	1307.49
1370.68	1314.83
1371.78	1315.88
1372.87	1316.93
1377.82	1320.63
1385.56	1321.68
1386.67	1322.74
1387.78	1325.92
1410.73	1326.98
1430.62	1328.04
1445.57	1328.57
1446.73	1330.7
1481.87	1334.43
1483.06	1336.57
1483.65	1337.64
1489.6	1338.71
1503.97	1341.93
1505.77	1354.87
1506.98	1363.57
1508.79	1364.66
1509.99	1365.75
1510.6	1366.85
1511.81	1367.94
1543.58	1368.49
1551.01	1369.58
1554.74	1370.68
1555.98	1371.78
1570.99	1372.87
1571.62	1377.82
1572.88	1385.56
1584.88	1386.67
1589.95	1387.78
1593.78	1408.47
1598.88	1409.6
1613.02	1410.73
1613.66	1429.47
1629.88	1432.91
1641.0	1445.57
1718.94	1446.73
1720.31	1459.52

1721.0	1460.69
1847.26	1461.85
1848.0	1477.73
1850.22	1478.91
	1479.5
	1481.87
	1482.46
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