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Supplemental information

On-tissue spatially resolved glycoproteomics guided by N-glycan imaging reveal global dysregulation of canine glioma glycoproteomic landscape

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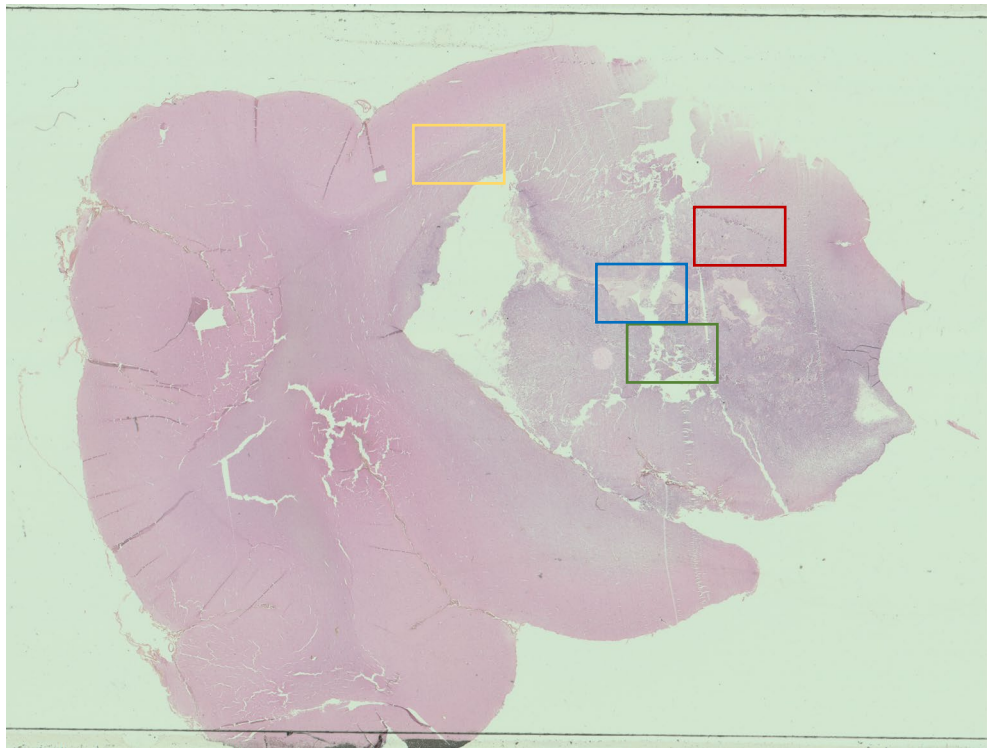
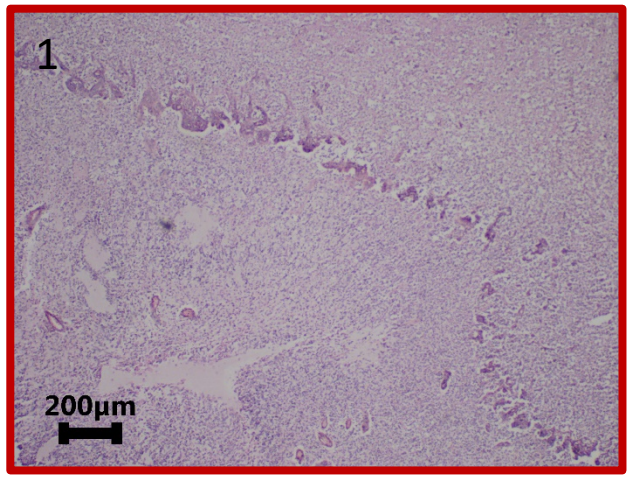
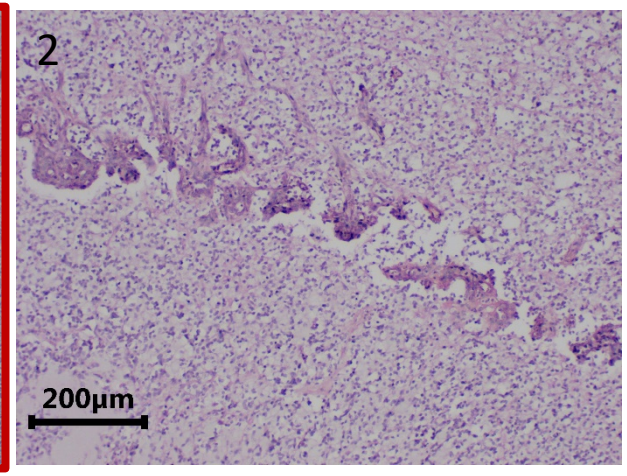
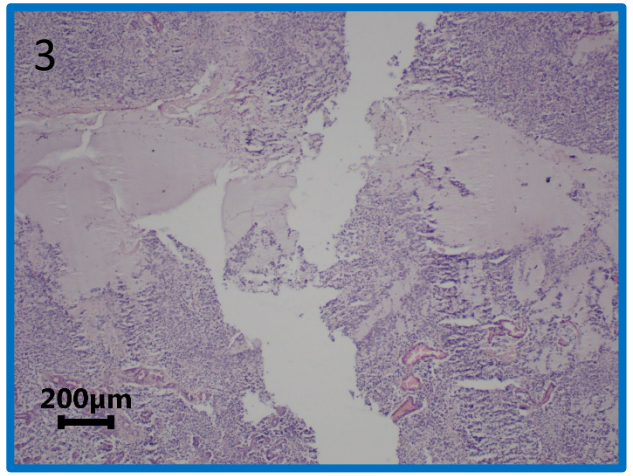
Supplementary Data 1. Magnified H&E stained images of anaplastic oligodendroglioma and glioblastoma specimens. A: Magnified images of the pseudo-glomerular vessels (panels 1-2), necrotic region (3-4), margin (5-6) and tumor+necrotic region (7-8) taken at 40X, then at 100X, 200X or 400X magnification, in an anaplastic oligodendroglioma biopsy. Colored boxes indicate magnified regions in relation to the whole section. B: Magnified images of the pseudopalisading necrosis (panels 1-2), necrotic region (3-4), margin (5-6) and tumor+necrotic region (7-8), taken at 40X, then at 100X or 200X magnification. Colored boxes indicate magnified regions in relation to the whole section. Related to Figures 1 and 2.

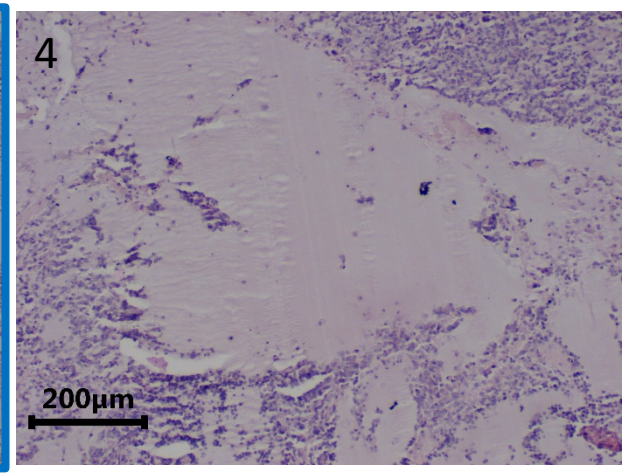
SD.1A

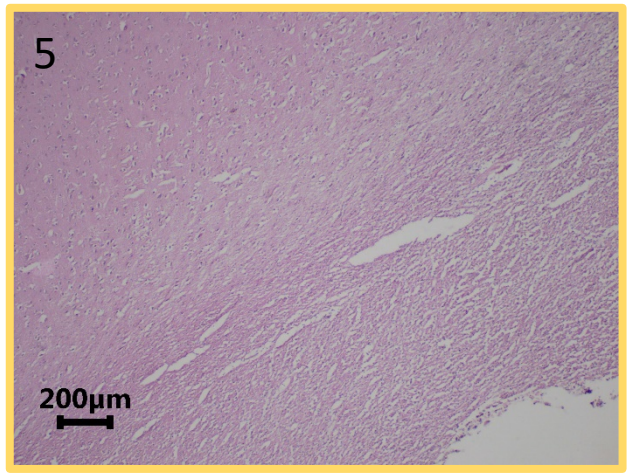


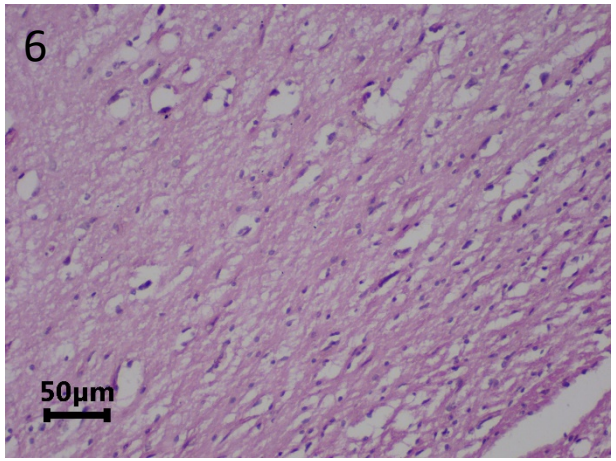


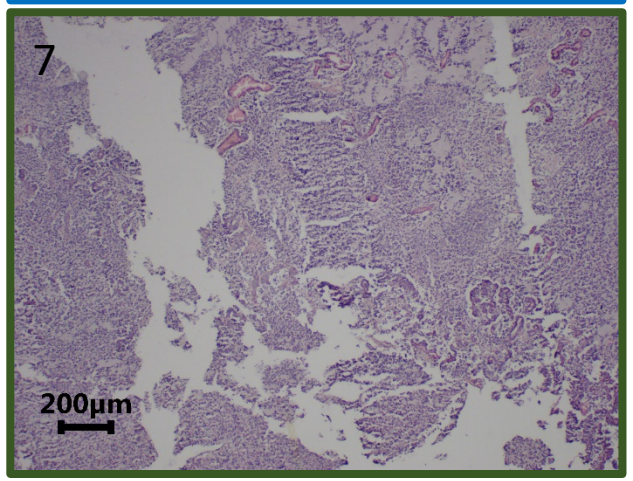


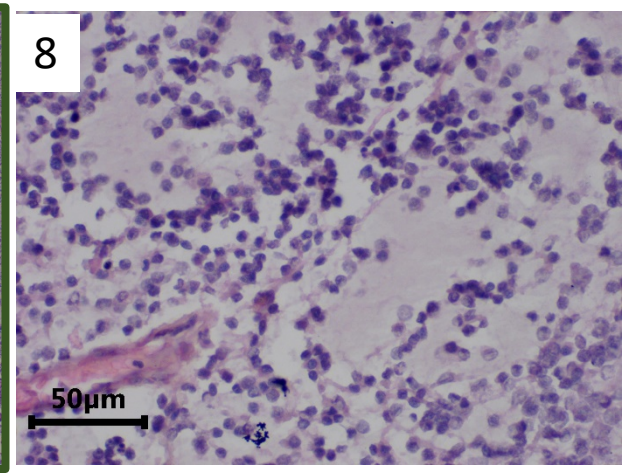




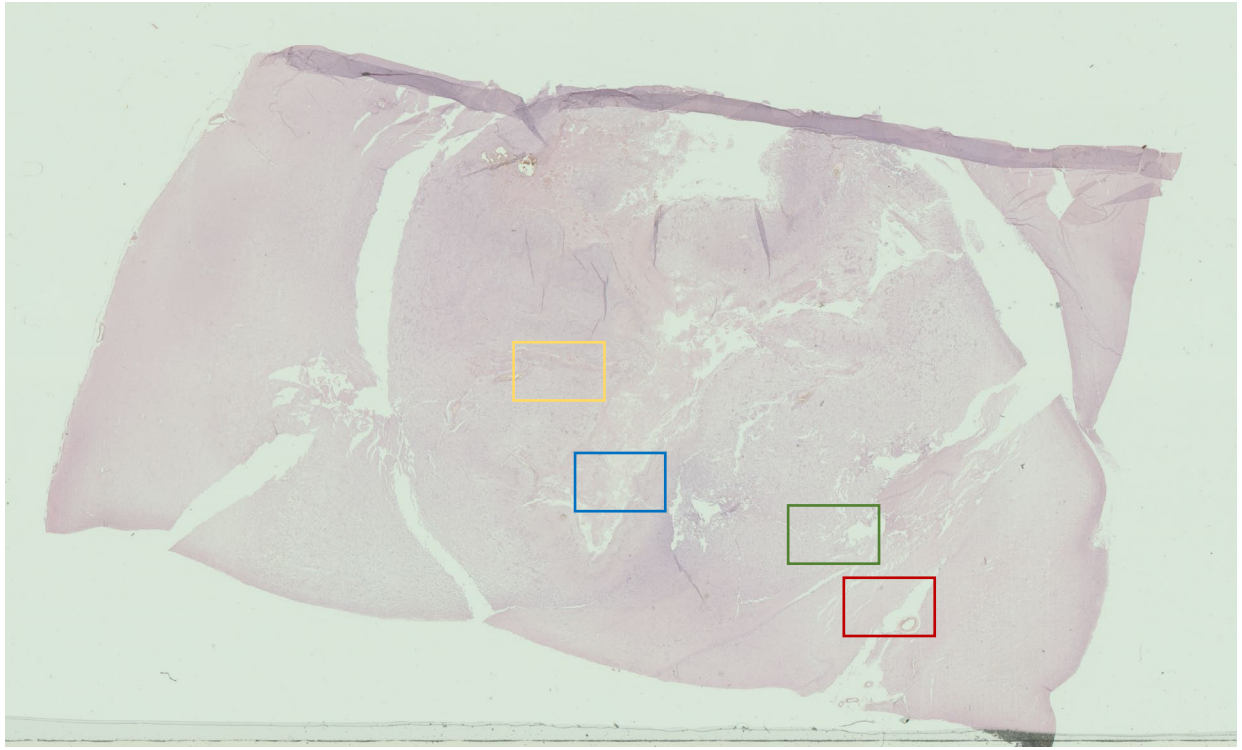


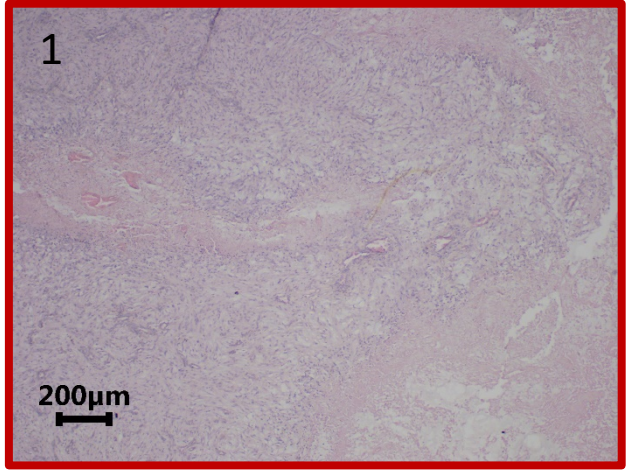


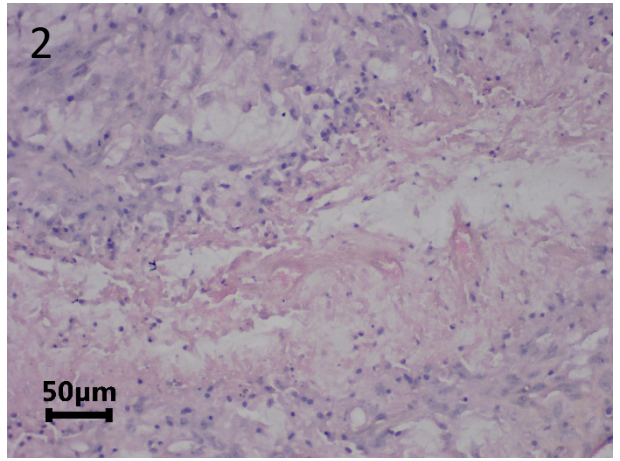


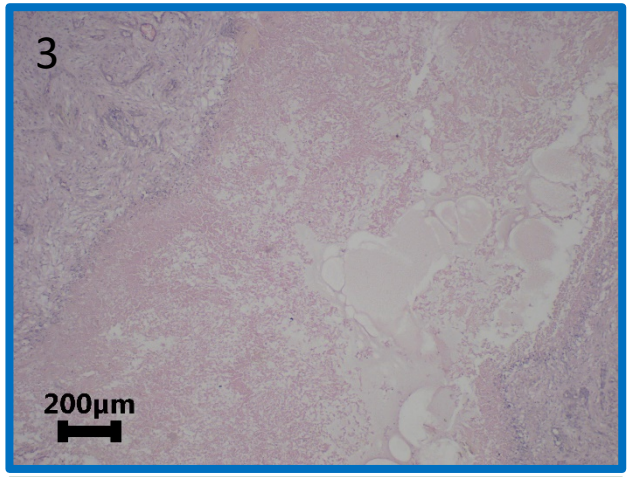


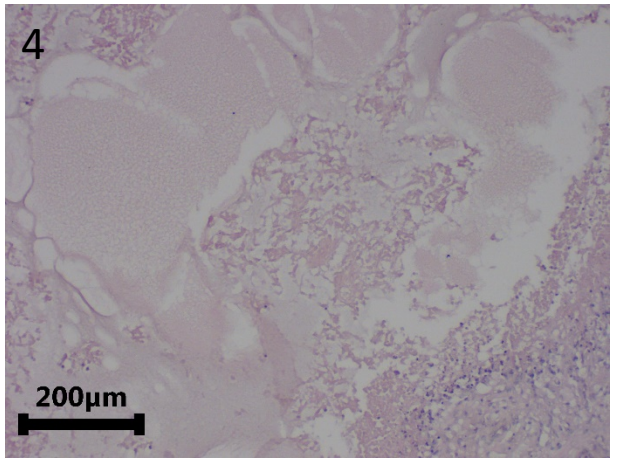
SD.1B

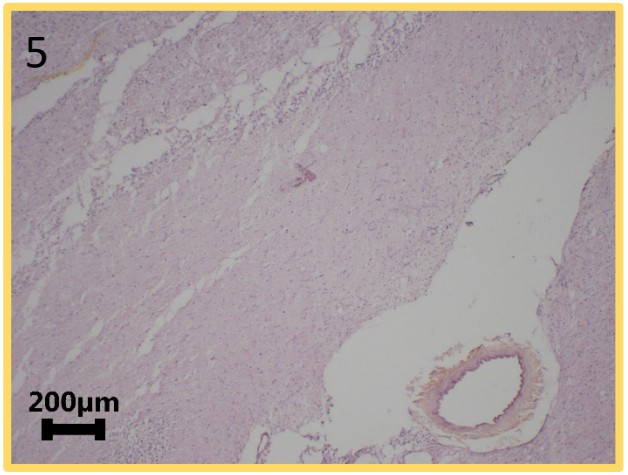


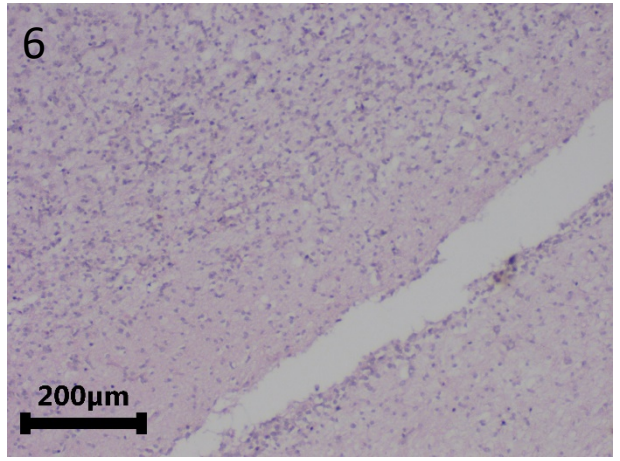


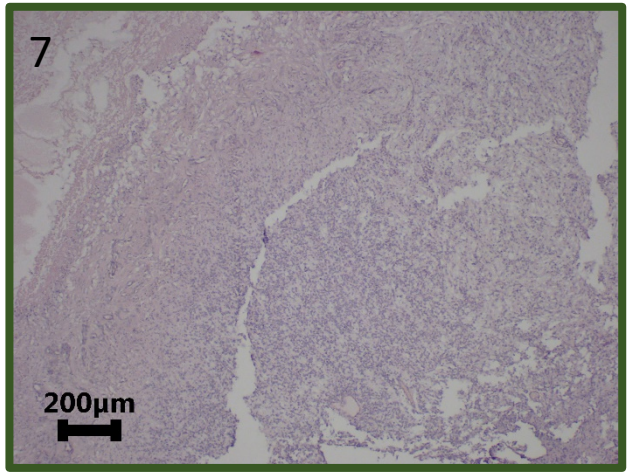


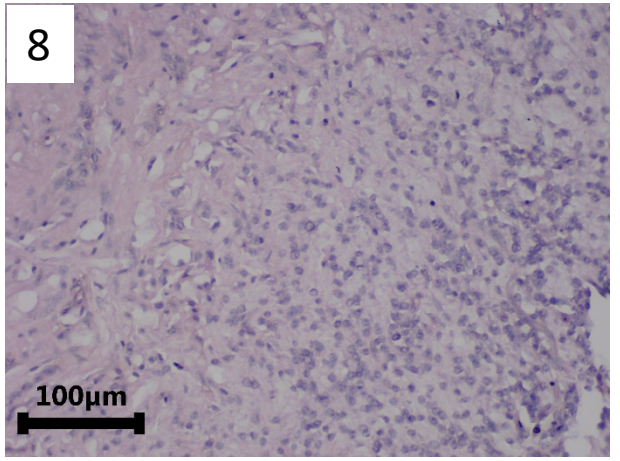






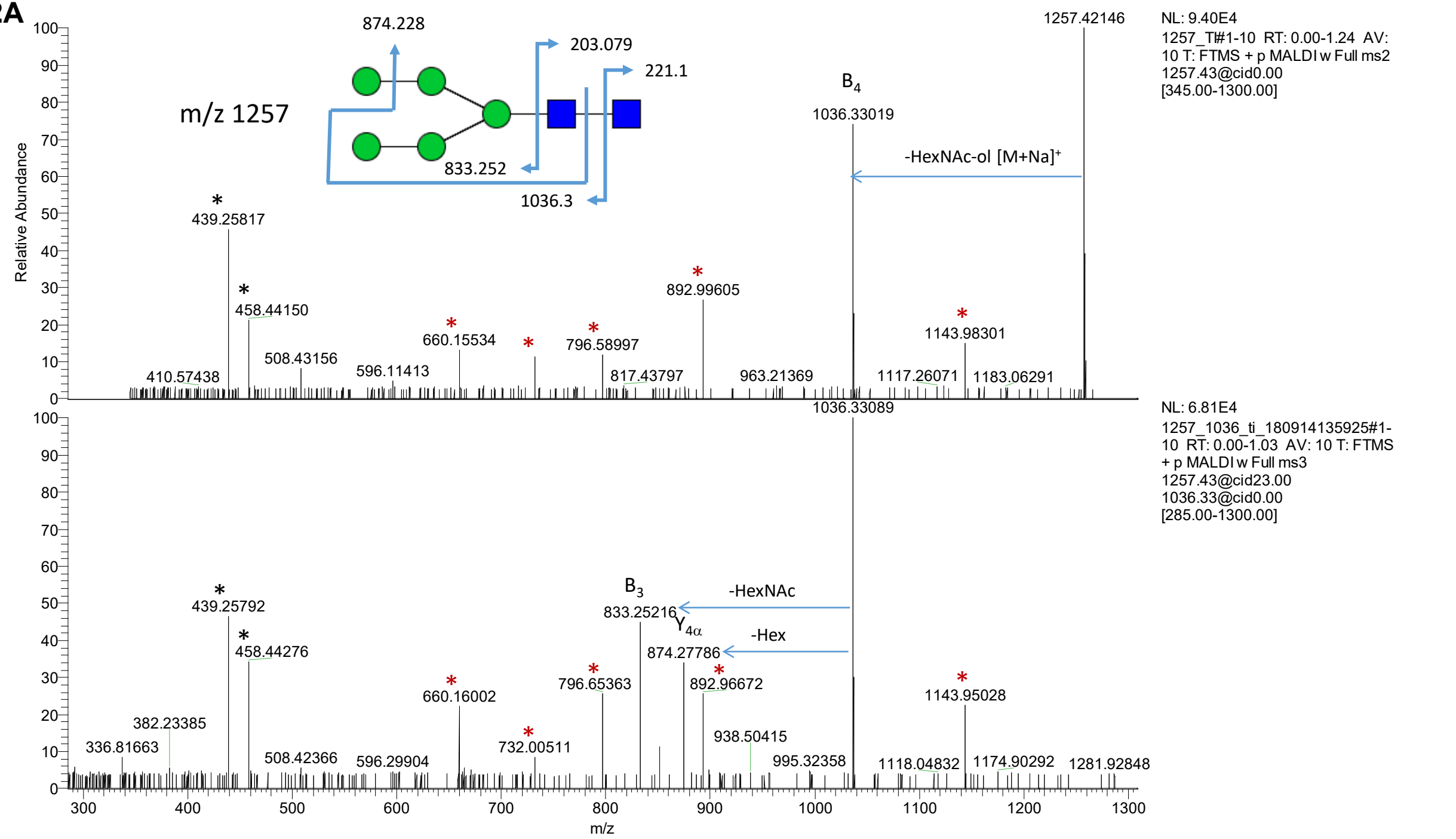




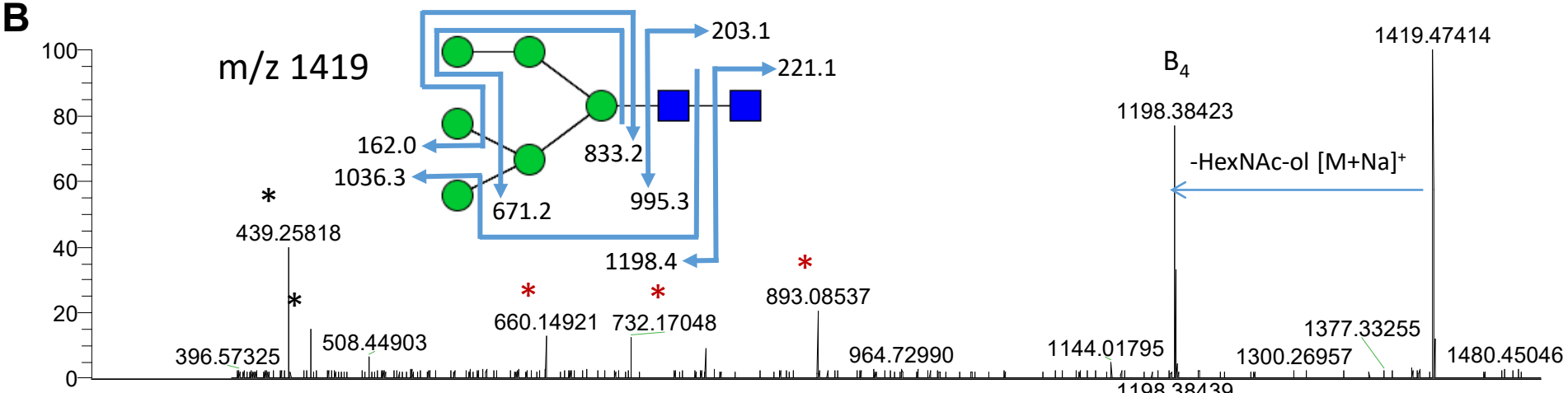


Supplementary Data 2. MSn assignments. MSn spectra were acquired directly on tissue using a MALDI LTQ Orbitrap instrument after images had been obtained. The MALDI LTQ Orbitrap XL is equipped with a commercial N2 laser (LTB Lasertechnik, Berlin, Germany) operating at λ = 337 nm with a maximum repetition rate of 60 Hz. The hybrid configuration replaces the heated capillary of the electrospray source with a q00 that sends packets of ions into a linear trap for collision-induced fragmentation (CID), with the fragment ions then being concentrated in a C-trap and transferred to the orbitrap for high-resolution mass analysis. The maximum energy per pulse was set to 12 μJ. Precursor ion isolation was performed using an isolation window between ±1 and ±3 Da and the fragments scanned with a maximum accumulation time of 120 ms. Succeeding MSn of the daughter ions were performed with a maximum accumulation time of 180 ms. External calibration was performed using the ProteoMass MALDI Calibration Kit (Sigma-Aldrich, St. Quentin-Fallavier, France). Related to Figure 1.

SD 2A


m/z 1257
874.228
203.079
221.1
833.252
1036.3
NL: 9.40E4
1257_T#1-10 RT: 0.00-1.24 AV: 10 T: FTMS + p MALDI w Full ms2 1257.43@cid0.00 [345.00-1300.00]
1257.42146
B4
1036.33019
-HexNAc-ol [M+Na]+
439.25817
458.44150
660.15534
796.58997
892.99605
1143.98301
410.57438
508.43156
596.11413
817.43797
963.21369
1117.26071
1183.06291
NL: 6.81E4
1257_1036_ti_180914135925#1-10 RT: 0.00-1.03 AV: 10 T: FTMS + p MALDI w Full ms3 1257.43@cid23.00 1036.33@cid0.00 [285.00-1300.00]
1036.33089
B3
-HexNAc
833.25216
Y4α
-Hex
874.27786
439.25792
458.44276
660.16002
796.65363
892.96672
1143.95028
382.23385
336.81663
508.42366
596.29904
732.00511
938.50415
995.32358
1118.04832
1174.90292
1281.92848
Relative Abundance
m/z

SD 2B



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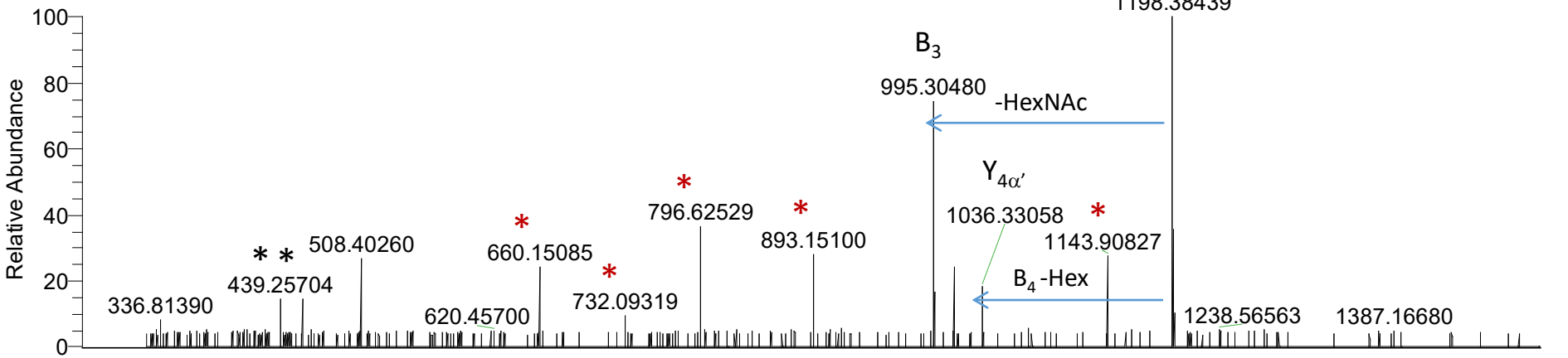
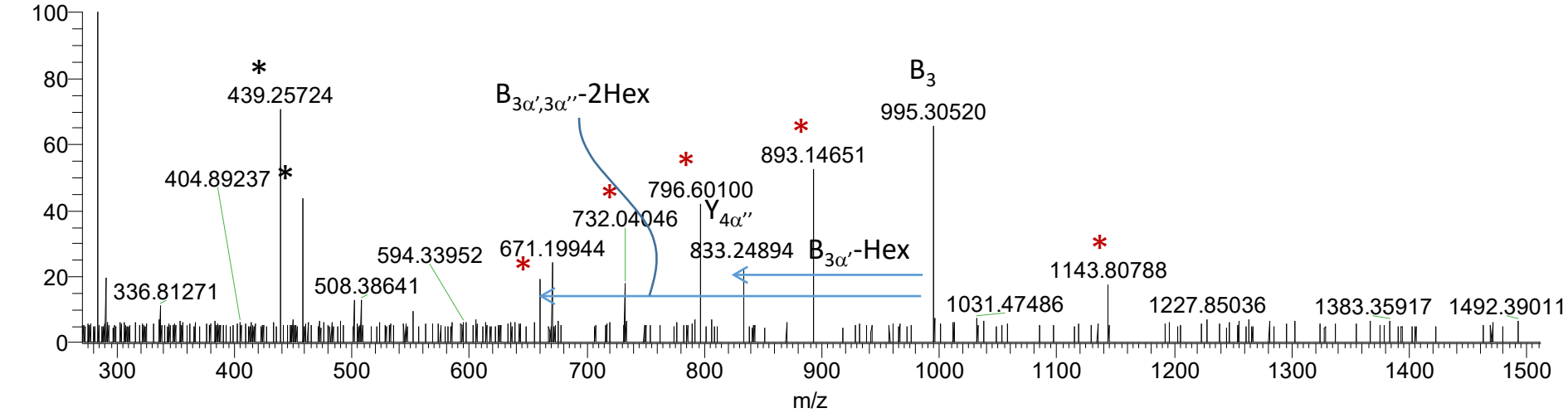
1419_T#1-10 RT: 0.00-1.36 AV: 10 T: FTMS + p MALDI w Full ms2 1419.48@cid0.00 [390.00-1500.00]

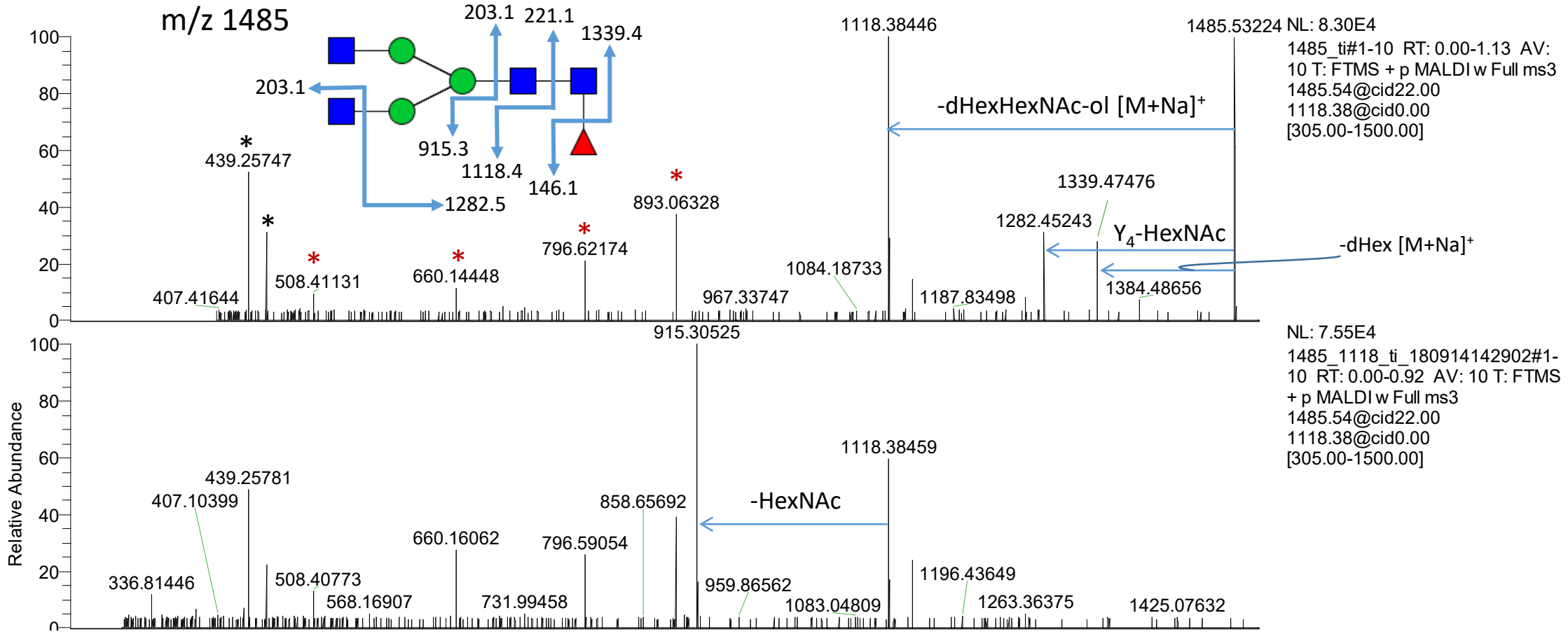


NL: 6.14E4
1419_1198_ti#1-10 RT: 0.00-1.06 AV: 10 T: FTMS + p MALDI w Full ms3 1419.48@cid28.00 1198.38@cid0.00 [325.00-1500.00]

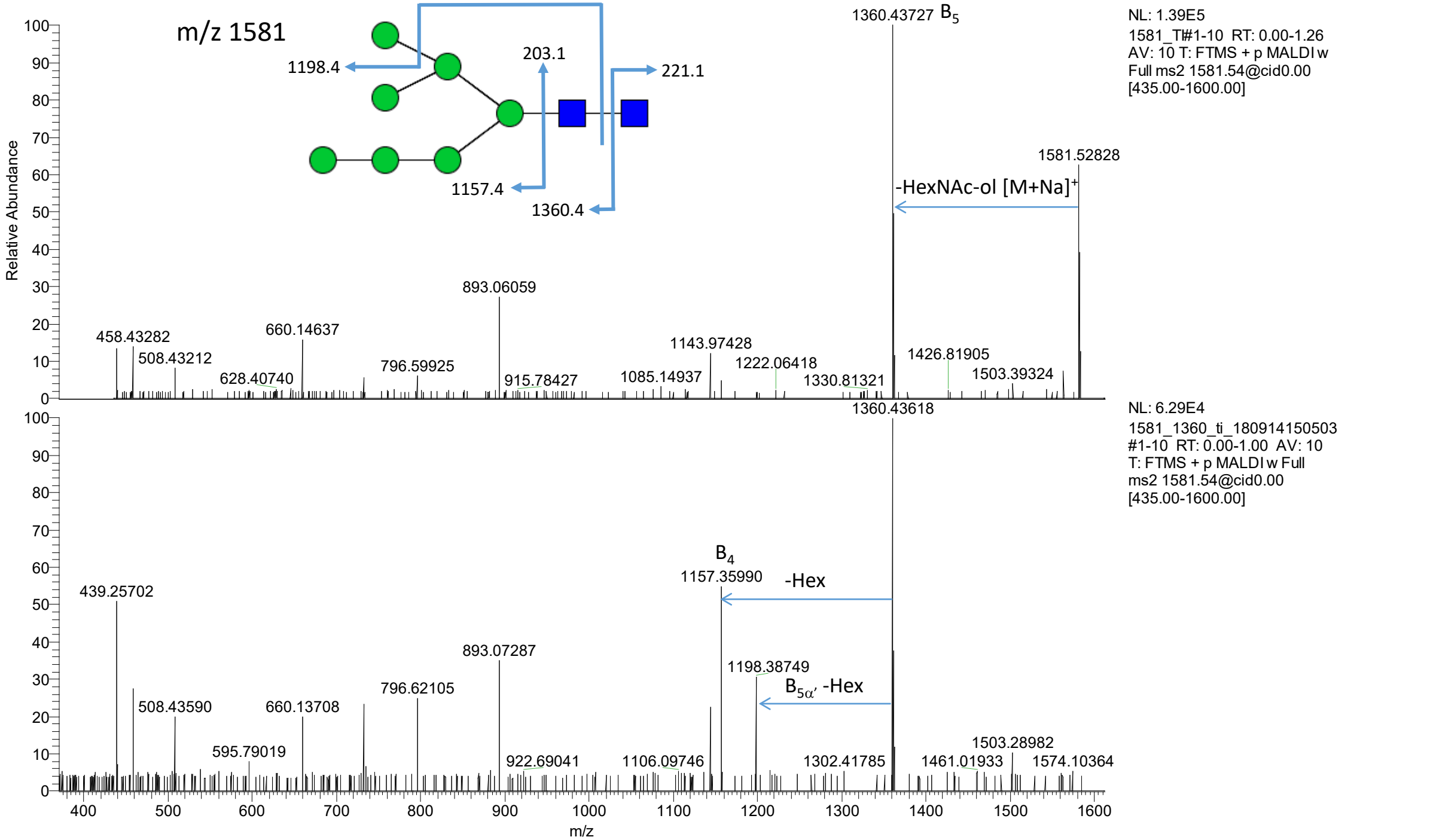


NL: 4.95E4
1419_1198_995_ti#1-10 RT: 0.00-1.03 AV: 10 T: FTMS + p MALDI w Full ms4 1419.48@cid28.00 1198.38@cid28.00 995.30@cid22.00 [270.00-1500.00]

SD 2C

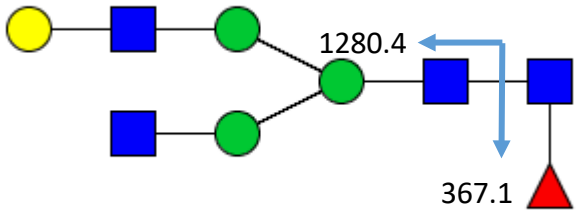

m/z 1485
203.1
221.1
1339.4
203.1
915.3
1118.4
146.1
1282.5
439.25747
893.06328
796.62174
508.41131
660.14448
407.41644
967.33747
1084.18733
1187.83498
1118.38446
1485.53224
-dHexHexNAc-ol [M+Na]+
1339.47476
1282.45243
Y4-HexNAc
-dHex [M+Na]+
1384.48656
NL: 8.30E4
1485_ti#1-10 RT: 0.00-1.13 AV: 10 T: FTMS + p MALDI w Full ms3
1485.54@cid22.00
1118.38@cid0.00
[305.00-1500.00]
915.30525
439.25781
407.10399
858.65692
660.16062
796.59054
336.81446
508.40773
568.16907
731.99458
959.86562
1083.04809
1118.38459
-HexNAc
1196.43649
1263.36375
1425.07632
NL: 7.55E4
1485_1118_ti_180914142902#1-10 RT: 0.00-0.92 AV: 10 T: FTMS + p MALDI w Full ms3
1485.54@cid22.00
1118.38@cid0.00
[305.00-1500.00]
Relative Abundance

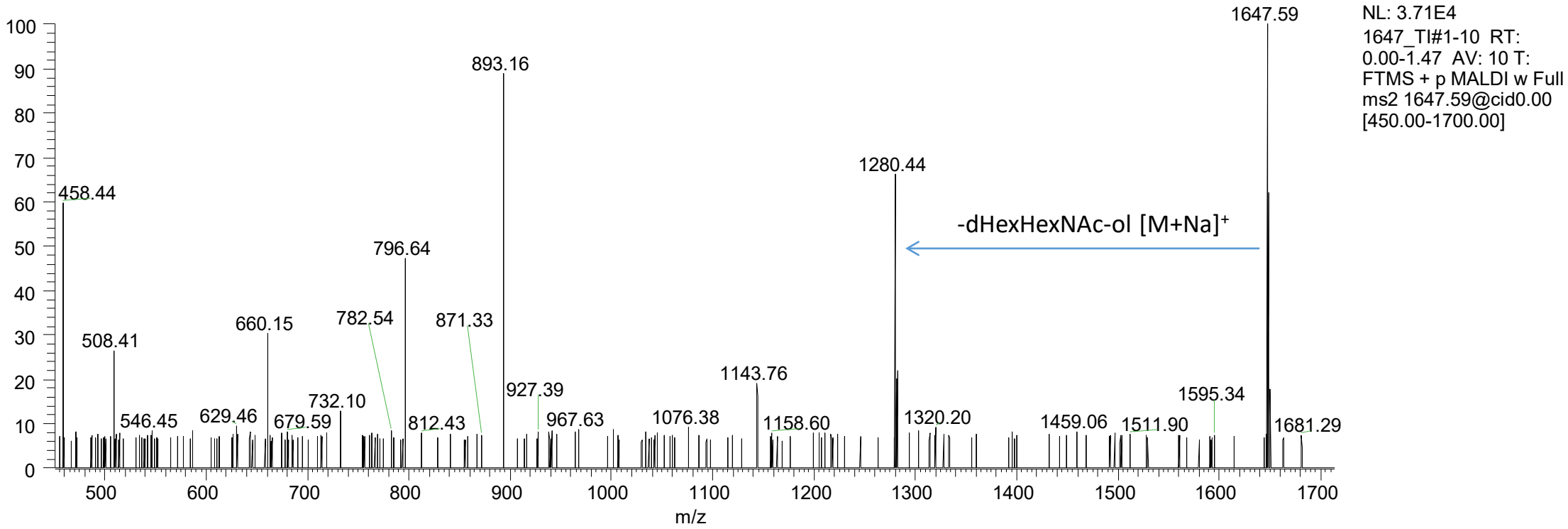
SD 2D


m/z 1581
1198.4
203.1
221.1
1157.4
1360.4
1360.43727 B5
NL: 1.39E5
1581_TI#1-10 RT: 0.00-1.26
AV: 10 T: FTMS + p MALDI w
Full ms2 1581.54@cid0.00
[435.00-1600.00]
1581.52828
-HexNAc-ol [M+Na]+
893.06059
660.14637
458.43282
508.43212
628.40740
796.59925
915.78427
1085.14937
1143.97428
1222.06418
1330.81321
1426.81905
1503.39324
Relative Abundance
1360.43618
NL: 6.29E4
1581_1360_ti_180914150503
#1-10 RT: 0.00-1.00 AV: 10
T: FTMS + p MALDI w Full
ms2 1581.54@cid0.00
[435.00-1600.00]
B4
1157.35990
-Hex
439.25702
893.07287
1198.38749
B5α' -Hex
796.62105
508.43590
660.13708
595.79019
922.69041
1106.09746
1302.41785
1503.28982
1461.01933
1574.10364
m/z

SD 2E

m/z 1647


1280.4
367.1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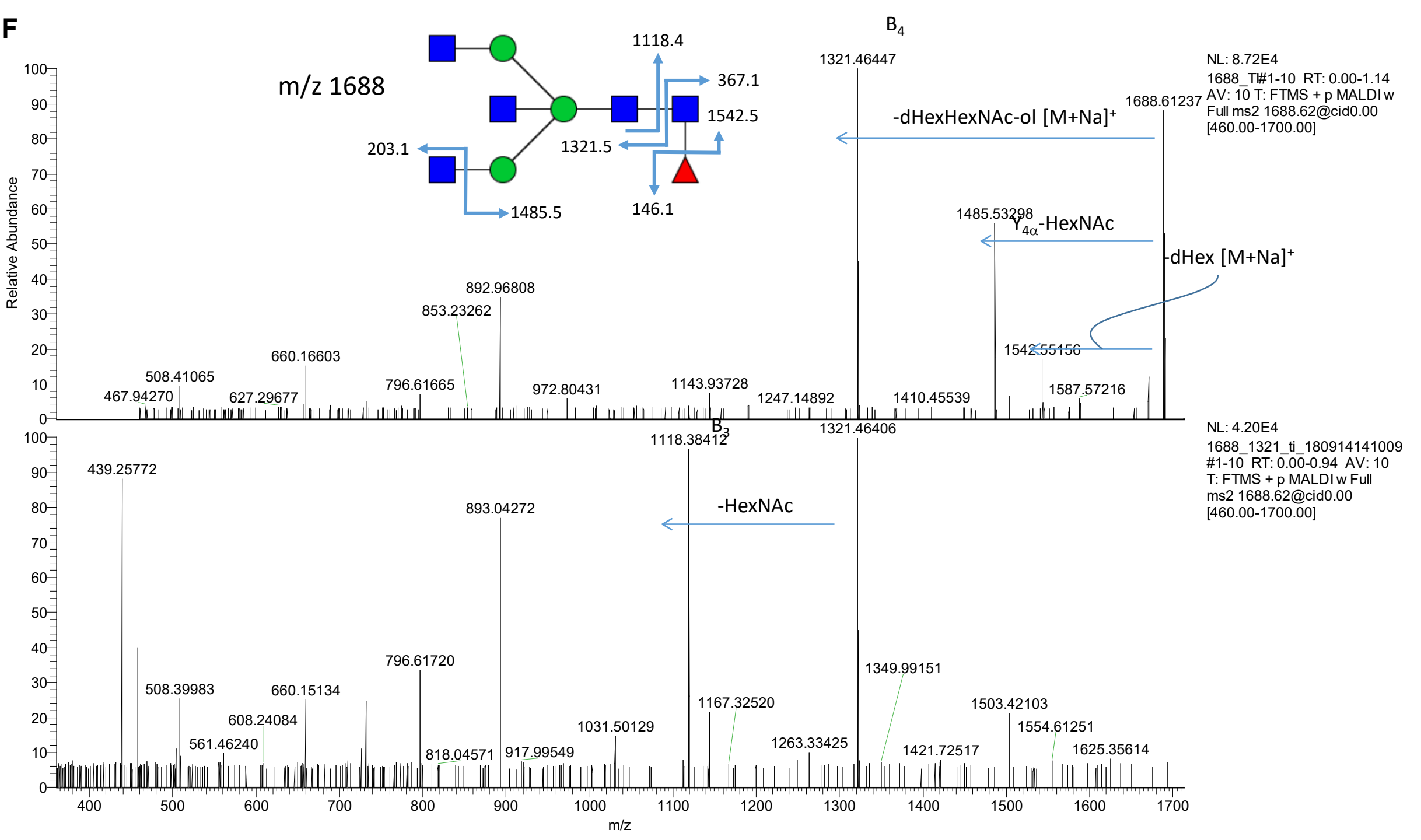
-dHexHexNAc-ol [M+Na]+
1647.59
893.16
1280.44
458.44
796.64
660.15
782.54
871.33
508.41
927.39
1143.76
732.10
1595.34
546.45
629.46
679.59
812.43
967.63
1076.38
1158.60
1320.20
1459.06
1511.90
1681.29
m/z

SD 2F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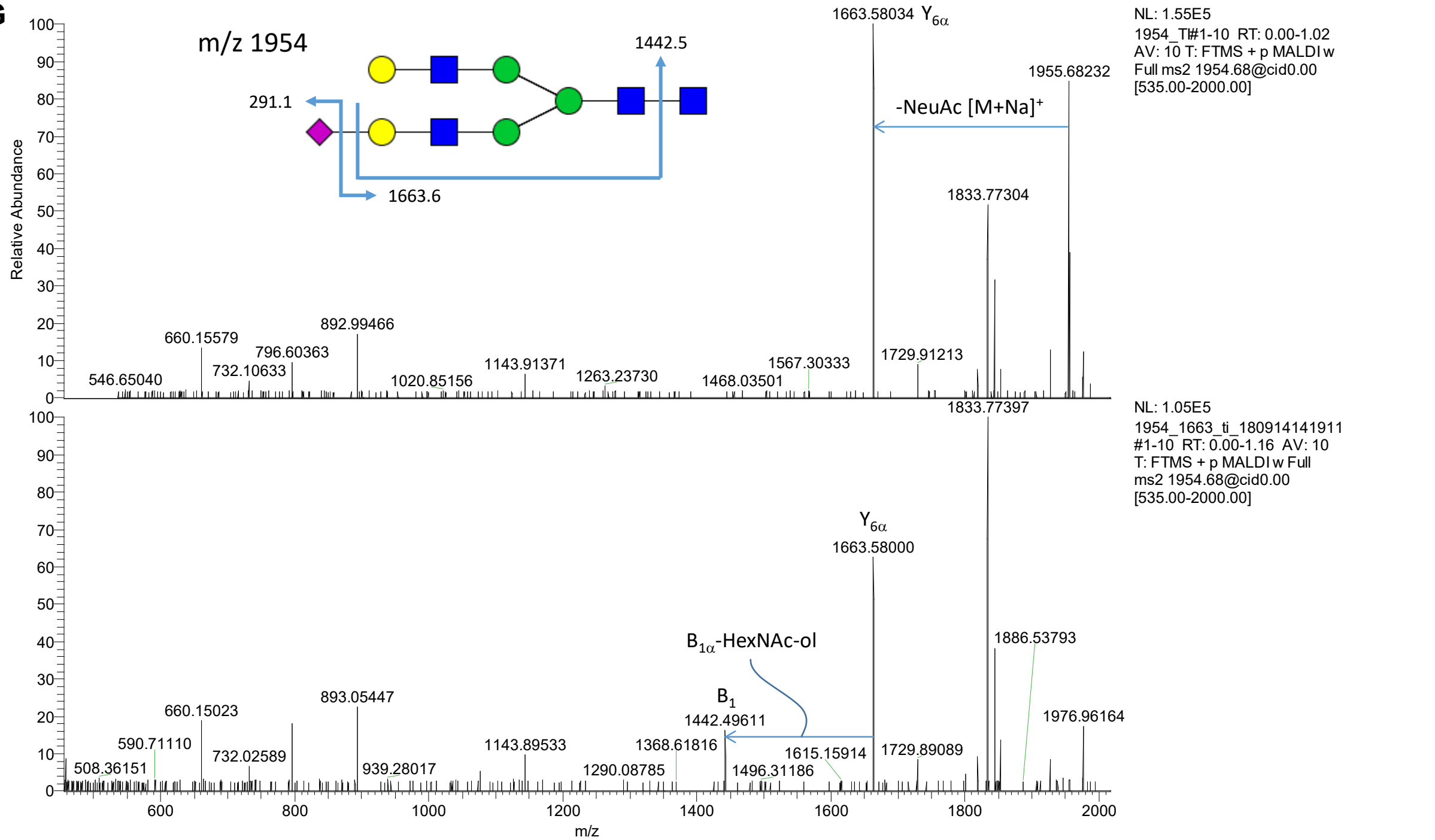
1542.5
1321.5
203.1
1485.5
146.1
B4
1321.46447
1688.61237
-dHexHexNAc-ol [M+Na]+
1485.53298
Y4α-HexNAc
-dHex [M+Na]+
1542.55156
892.96808
853.23262
660.16603
508.41065
467.94270
627.29677
796.61665
972.80431
1143.93728
1247.14892
1410.45539
1587.57216
Relative Abundance
NL: 8.72E4
1688_TI#1-10 RT: 0.00-1.14
AV: 10 T: FTMS + p MALDI w
Full ms2 1688.62@cid0.00
[460.00-1700.00]
B3
1118.38412
1321.46406
439.25772
893.04272
-HexNAc
796.61720
508.39983
660.15134
608.24084
561.46240
818.04571
917.99549
1031.50129
1167.32520
1263.33425
1349.99151
1421.72517
1503.42103
1554.61251
1625.35614
NL: 4.20E4
1688_1321_ti_180914141009
#1-10 RT: 0.00-0.94 AV: 10
T: FTMS + p MALDI w Full
ms2 1688.62@cid0.00
[460.00-1700.00]
m/z

SD 2G


m/z 1954
1442.5
291.1
1663.6
1663.58034 Y6α
1955.68232
-NeuAc [M+Na]+
1833.77304
892.99466
660.15579
796.60363
732.10633
546.65040
1020.85156
1143.91371
1263.23730
1468.03501
1567.30333
1729.91213
Relative Abundance
NL: 1.55E5
1954_TI#1-10 RT: 0.00-1.02
AV: 10 T: FTMS + p MALDI w
Full ms2 1954.68@cid0.00
[535.00-2000.00]
1833.77397
NL: 1.05E5
1954_1663_ti_180914141911
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T: FTMS + p MALDI w Full
ms2 1954.68@cid0.00
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Y6α
1663.58000
B1α-HexNAc-ol
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1442.49611
1886.53793
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893.05447
660.15023
590.71110
732.02589
508.36151
939.28017
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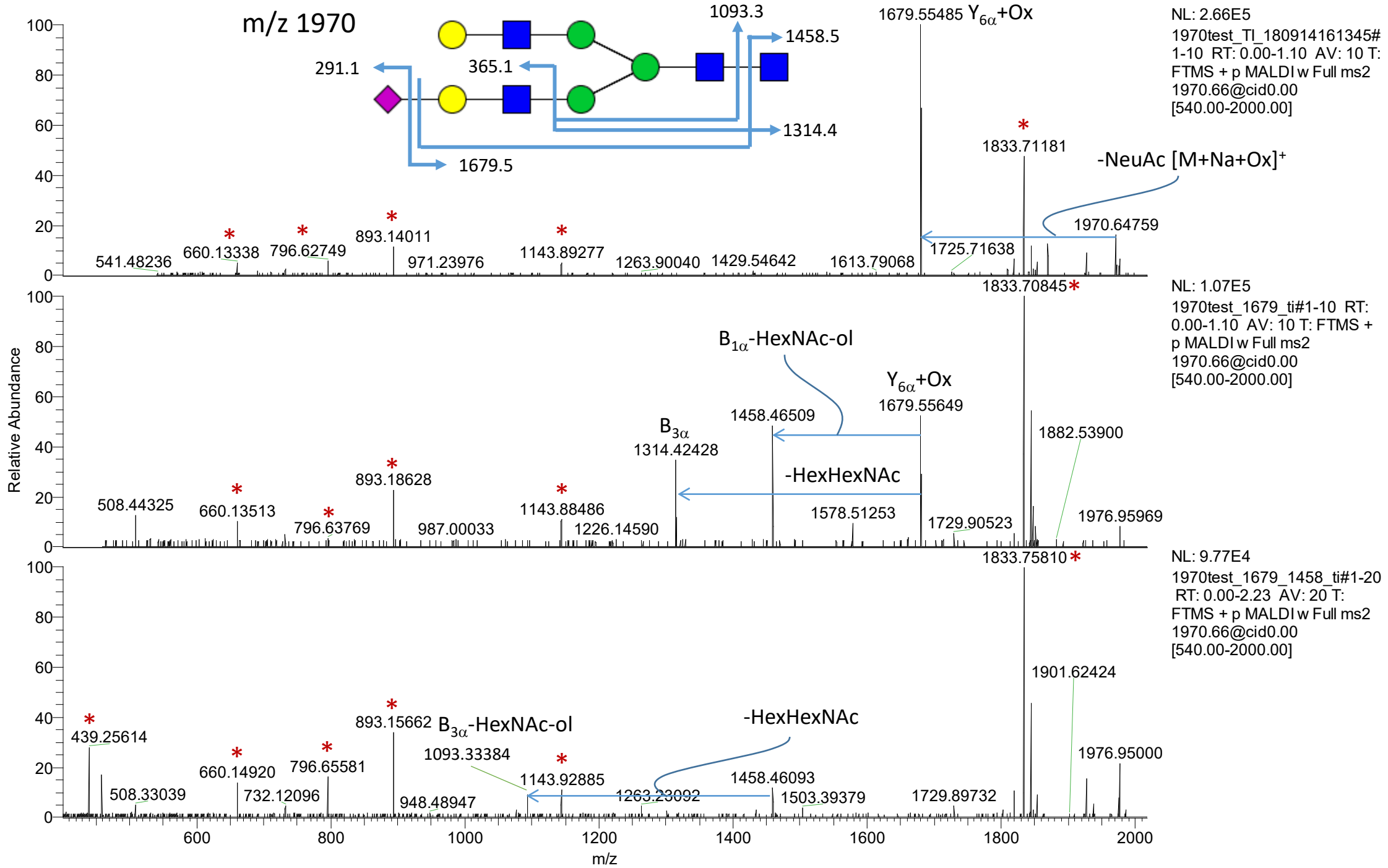
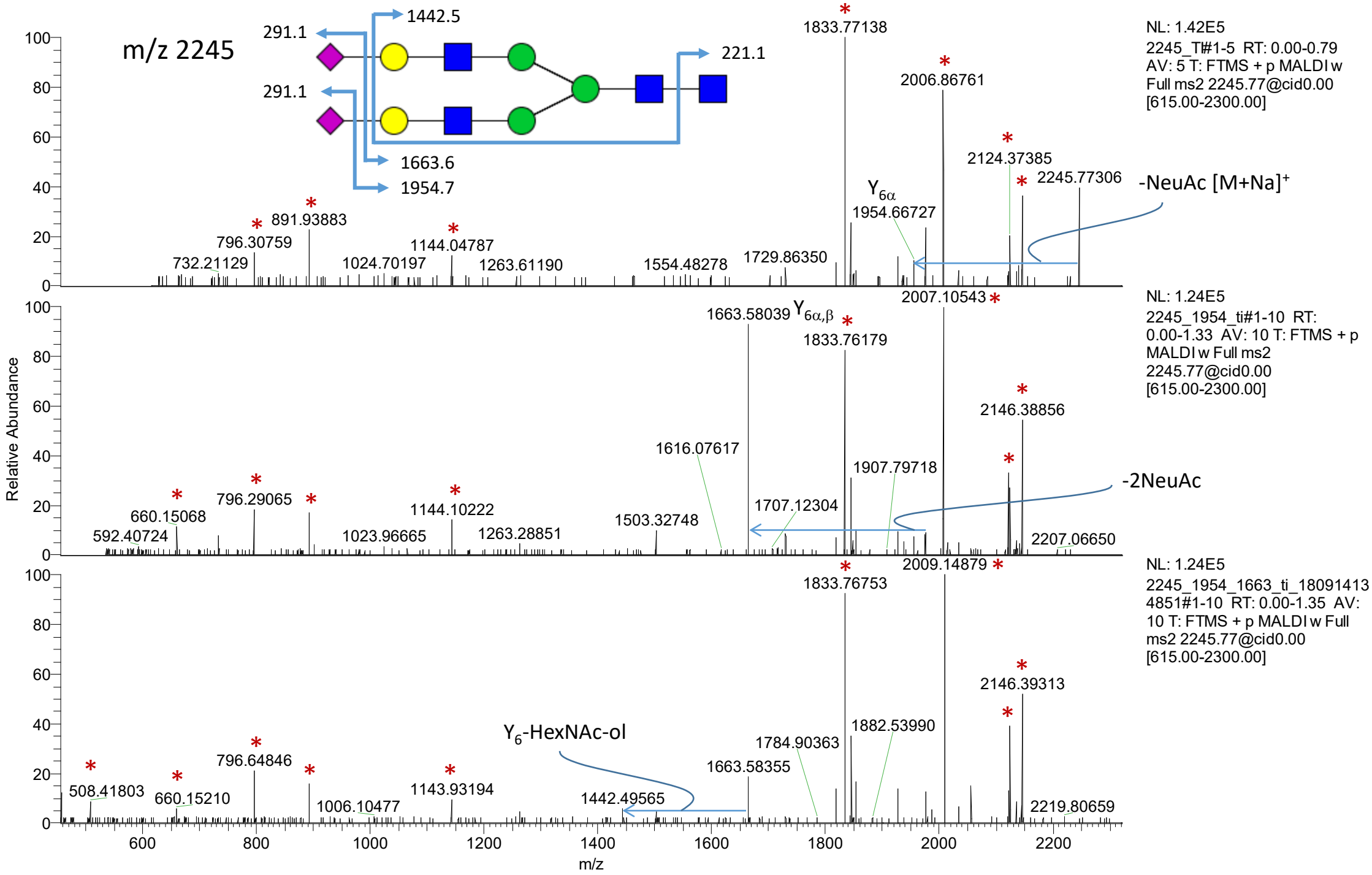
1615.15914
1496.31186
1729.89089
m/z

SD 2H


m/z 1970
1093.3
1458.5
291.1
365.1
1314.4
1679.5
1679.55485 Y6α+Ox
NL: 2.66E5
1970test_TI_180914161345#
1-10 RT: 0.00-1.10 AV: 10 T:
FTMS + p MALDI w Full ms2
1970.66@cid0.00
[540.00-2000.00]
1833.71181
-NeuAc [M+Na+Ox]+
1970.64759
1725.71638
541.48236
660.13338
796.62749
893.14011
971.23976
1143.89277
1263.90040
1429.54642
1613.79068
1833.70845
NL: 1.07E5
1970test_1679_ti#1-10 RT:
0.00-1.10 AV: 10 T: FTMS +
p MALDI w Full ms2
1970.66@cid0.00
[540.00-2000.00]
B1α-HexNAc-ol
Y6α+Ox
1679.55649
1458.46509
B3α
1314.42428
1882.53900
-HexHexNAc
508.44325
660.13513
796.63769
893.18628
987.00033
1143.88486
1226.14590
1578.51253
1729.90523
1976.95969
1833.75810
NL: 9.77E4
1970test_1679_1458_ti#1-20
RT: 0.00-2.23 AV: 20 T:
FTMS + p MALDI w Full ms2
1970.66@cid0.00
[540.00-2000.00]
1901.62424
439.25614
893.15662
B3α-HexNAc-ol
-HexHexNAc
1976.95000
660.14920
796.65581
1093.33384
1143.92885
1458.46093
508.33039
732.12096
948.48947
1263.23092
1503.39379
1729.89732
Relative Abundance
m/z
600
800
1000
1200
1400
1600
1800
2000

SD 2l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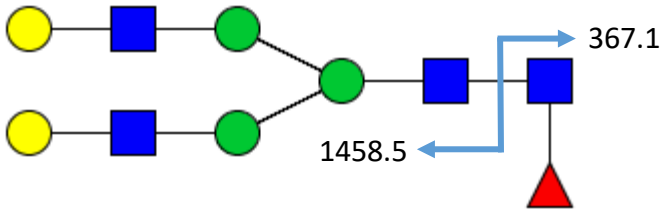
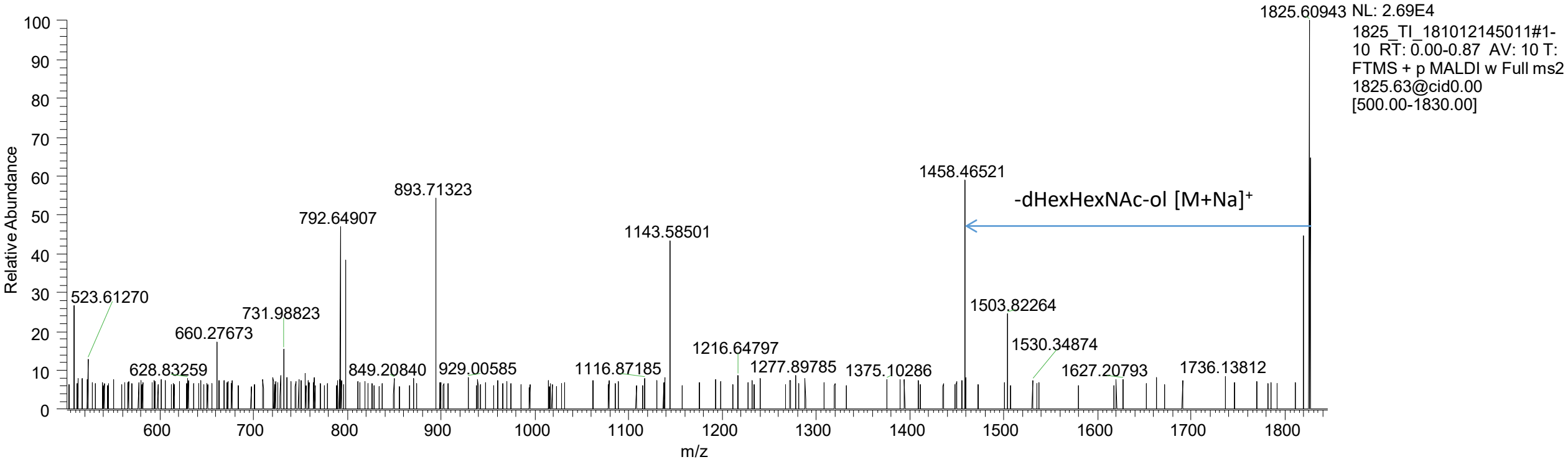
1442.5
291.1
291.1
221.1
1663.6
1954.7
1833.77138
2006.86761
2124.37385
2245.77306
-NeuAc [M+Na]+
Y6α
1954.66727
891.93883
796.30759
732.21129
1144.04787
1024.70197
1263.61190
1554.48278
1729.86350
NL: 1.42E5
2245_T#1-5 RT: 0.00-0.79 AV: 5 T: FTMS + p MALDI w Full ms2 2245.77@cid0.00 [615.00-2300.00]
2007.10543
1663.58039
Y6α,β
1833.76179
2146.38856
1616.07617
1907.79718
-2NeuAc
796.29065
660.15068
592.40724
1144.10222
1023.96665
1263.28851
1503.32748
1707.12304
2207.06650
NL: 1.24E5
2245_1954_ti#1-10 RT: 0.00-1.33 AV: 10 T: FTMS + p MALDI w Full ms2 2245.77@cid0.00 [615.00-2300.00]
2009.14879
1833.76753
2146.39313
Y6-HexNAc-ol
1882.53990
1784.90363
1663.58355
796.64846
508.41803
660.15210
1143.93194
1006.10477
1442.49565
2219.80659
NL: 1.24E5
2245_1954_1663_ti_18091413 4851#1-10 RT: 0.00-1.35 AV: 10 T: FTMS + p MALDI w Full ms2 2245.77@cid0.00 [615.00-2300.00]
Relative Abundance
m/z

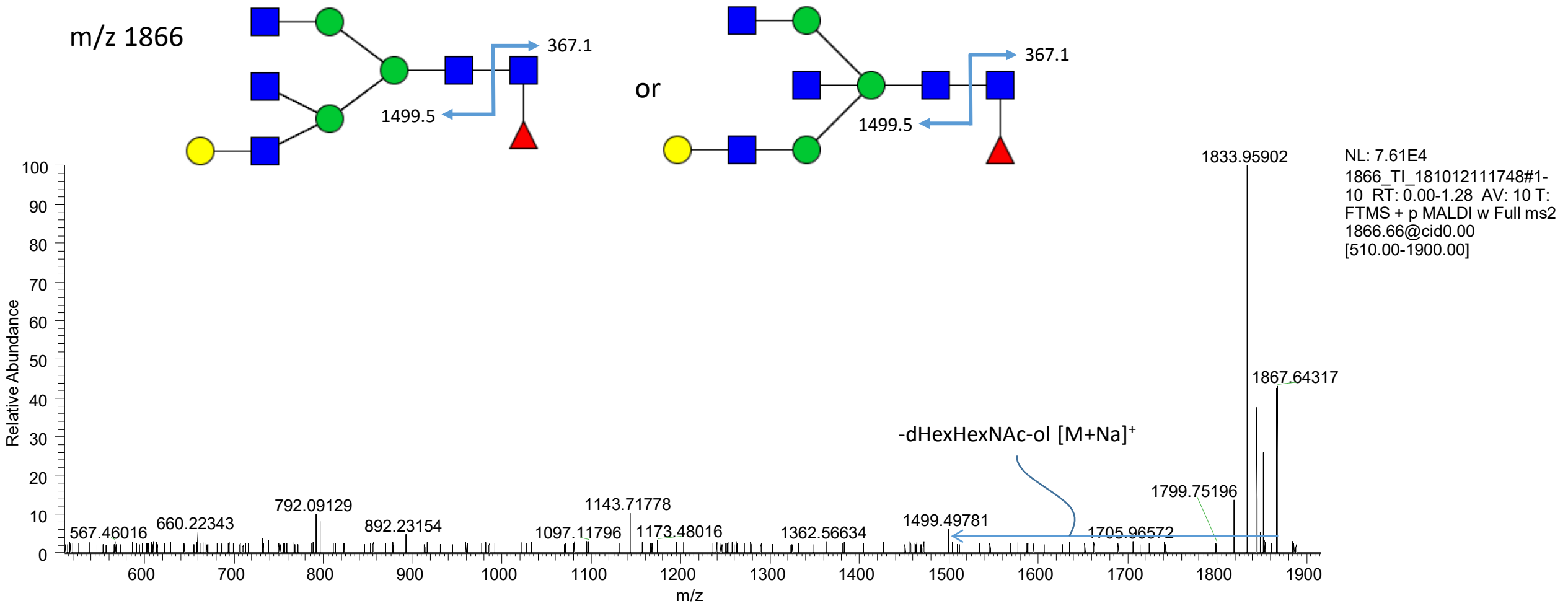
SD 2J

m/z 1825

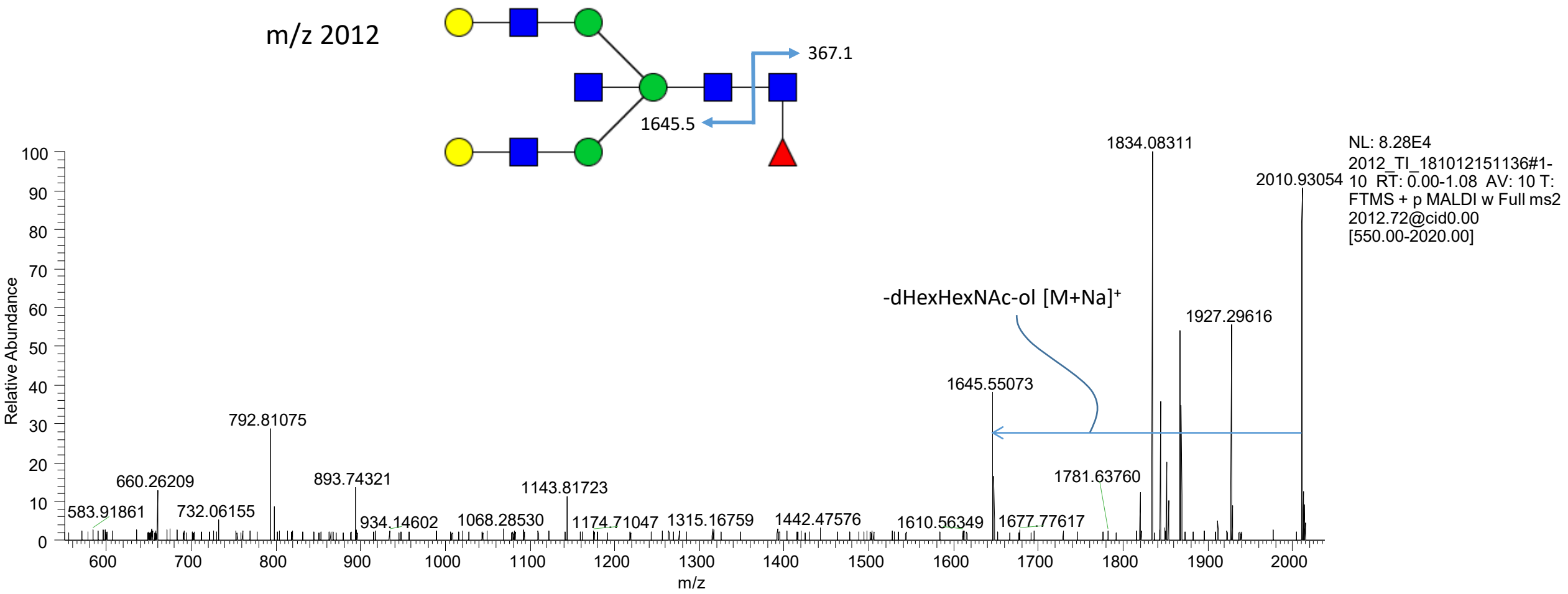

367.1
1458.5


1825.60943 NL: 2.69E4
1825_TI_181012145011#1-10 RT: 0.00-0.87 AV: 10 T: FTMS + p MALDI w Full ms2 1825.63@cid0.00 [500.00-1830.00]
1458.46521
-dHexHexNAc-ol [M+Na]+
893.71323
792.64907
1143.58501
523.61270
731.98823
660.27673
628.83259
849.20840
929.00585
1116.87185
1216.64797
1277.89785
1375.10286
1503.82264
1530.34874
1627.20793
1736.13812
Relative Abundance
m/z

SD 2K


m/z 1866
367.1
1499.5
or
367.1
1499.5
NL: 7.61E4
1866_TI_181012111748#1-10 RT: 0.00-1.28 AV: 10 T: FTMS + p MALDI w Full ms2 1866.66@cid0.00 [510.00-1900.00]
1833.95902
1867.64317
-dHexHexNAc-ol [M+Na]+
1799.75196
1499.49781
1705.96572
1143.71778
792.09129
660.22343
892.23154
567.46016
1097.11796
1173.48016
1362.56634
Relative Abundance
m/z

SD 2L

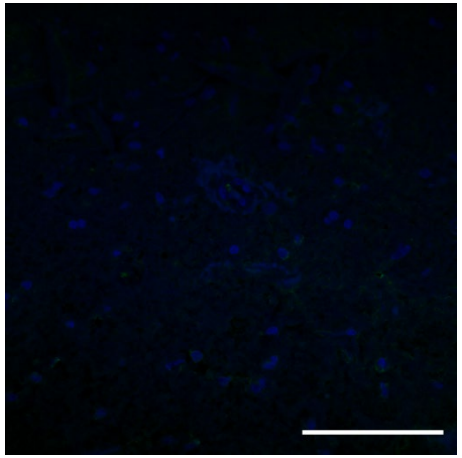

m/z 2012
367.1
1645.5
NL: 8.28E4
2012_TI_181012151136#1-10 RT: 0.00-1.08 AV: 10 T: FTMS + p MALDI w Full ms2 2012.72@cid0.00 [550.00-2020.00]
-dHexHexNAc-ol [M+Na]+
1834.08311
2010.93054
1927.29616
1645.55073
1781.63760
792.81075
660.26209
893.74321
583.91861
732.06155
934.14602
1068.28530
1143.81723
1174.71047
1315.16759
1442.47576
1610.56349
1677.77617
Relative Abundance
m/z

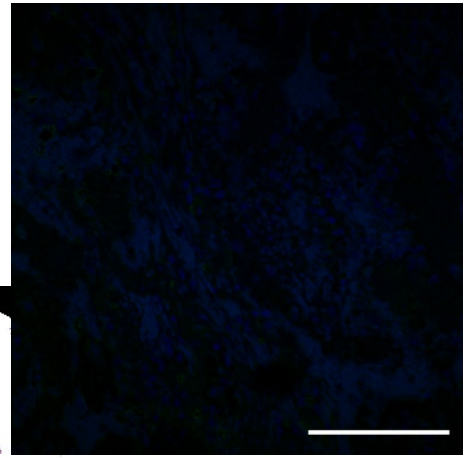
Supplementary Data 4. Lectin staining. Using a Dako delimiting pen (Agilent Technologies, Santa Clara, CA), dams were created around tissue sections. The sections were then incubated in 1% BSA (w/v) in approximately 300 µL of PBS for 30 min at room temperature, then incubated in 10 µg/mL of SNA lectin for 2 h. They were then rinsed for 10 min three times with 1% BSA in PBS. The sections were then incubated in approximately 300 µL of DAPI for 20 min and rinsed with PBS for 5 min. Finally, two drops of Vectashield fluorescence mounting medium (Dako, Agilent Technologies) was added and the sections were cover-slipped and sealed with nail polish. Confocal images were obtained using a fluorescence microscope (Leica Biosystems). Adjacent tissue sections incubated in 1% BSA in PBS served as controls. Zeiss LSM700 confocal microscope connected to a Zeiss Axiovert 200 M with an EC Plan-Neofluar 40x/1.30 numerical aperture oil immersion objective (Carl Zeiss AG, Oberkochen, Germany). Processing of the images was performed using Zen software and applied on the entire images as well as on controls. Scale bar = 100 μm. Related to Figure 2.

SD 4A

VH14-0622

Glioblastoma (Grade IV WHO)





Necrosis+Tumor with Microhemmorhages

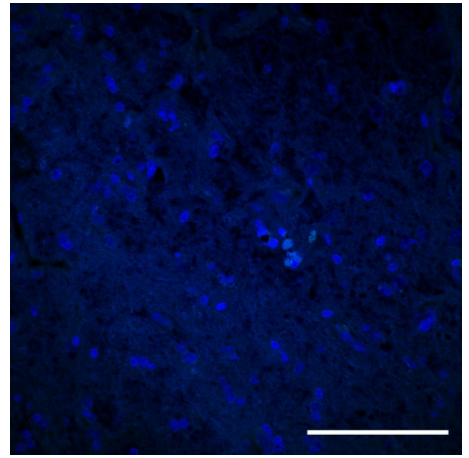
Tumor

Margin

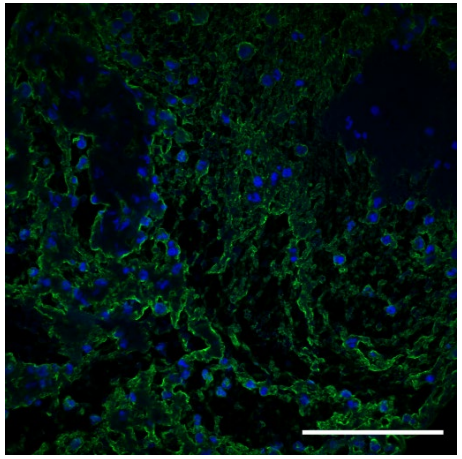
Benign

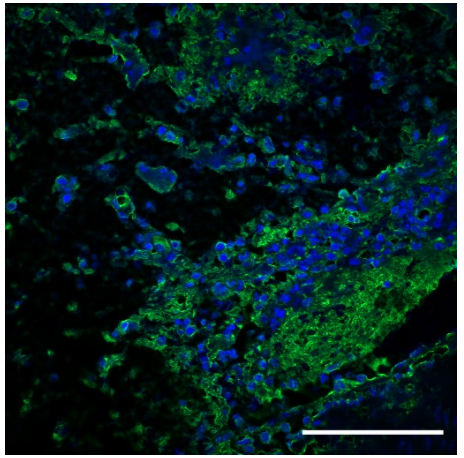
Tumor

Necrosis



Control: separate section



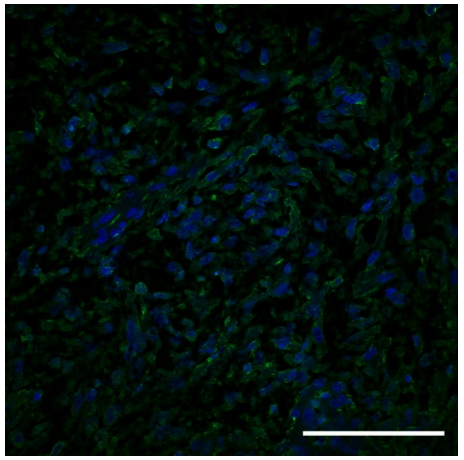


Green=positive for SNA lectin staining
Blue=DAPI

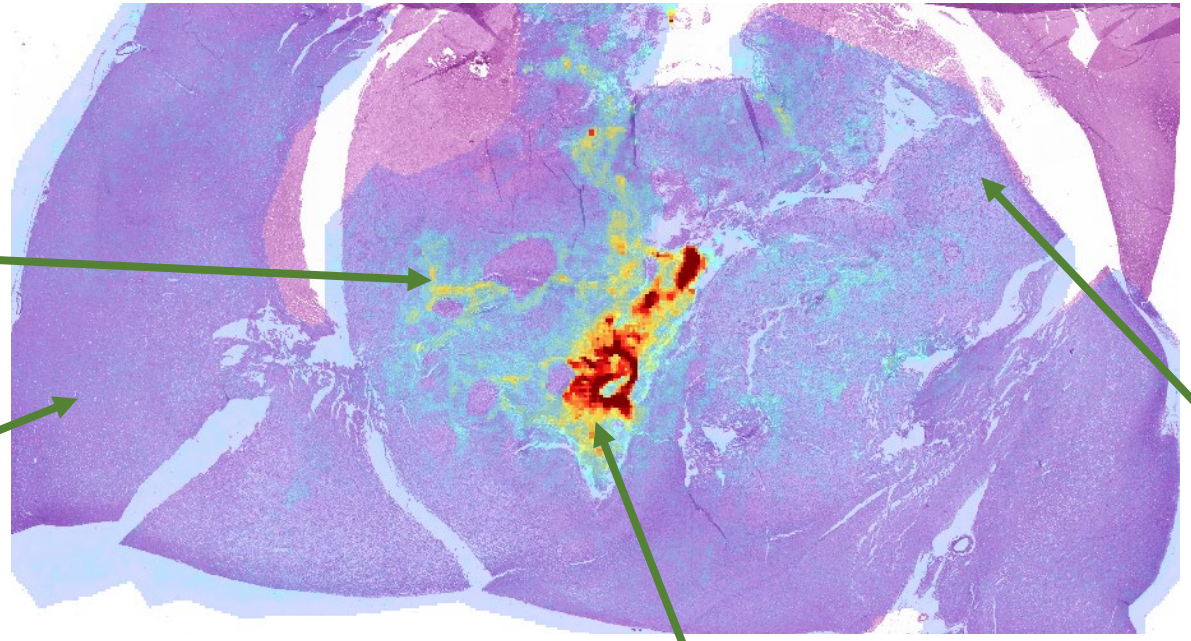
SD 4B

VH15-1139A

Glioblastoma (Grade IV WHO)



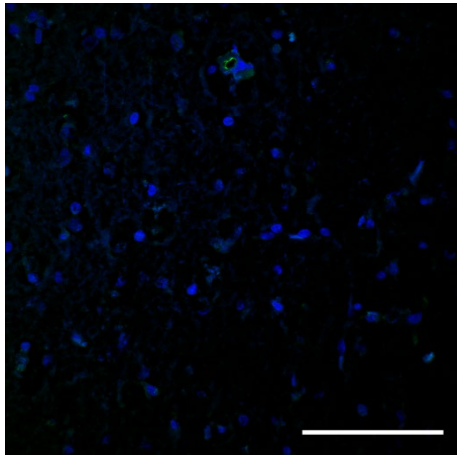
Pseudopalisading

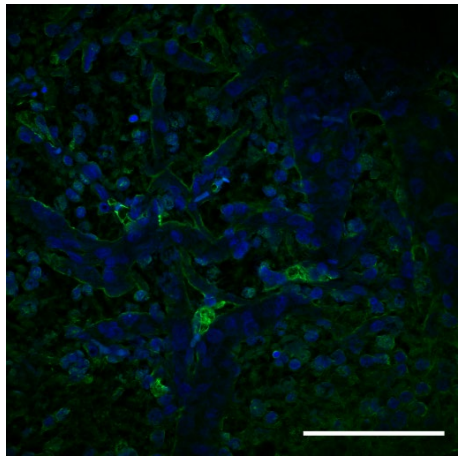


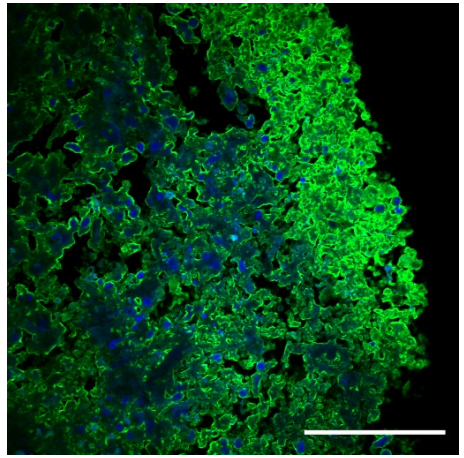
Benign cortex

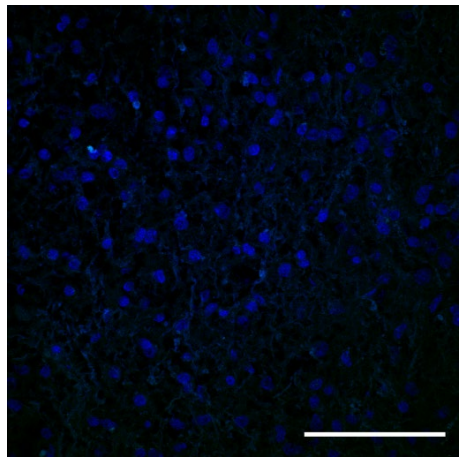
Tumor

Necrosis







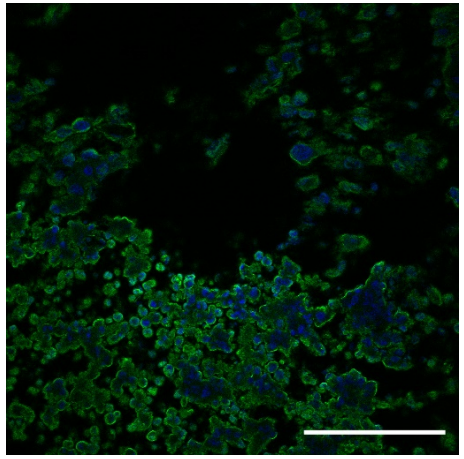


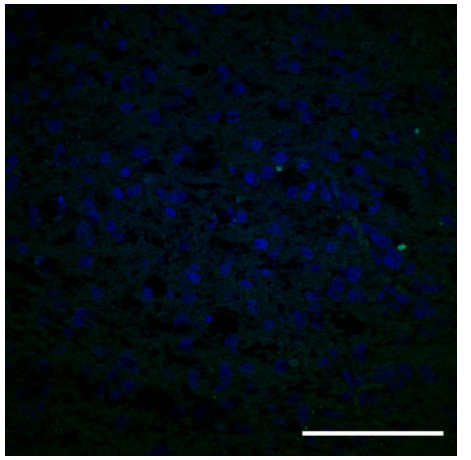
Control: separate section

SD 4C

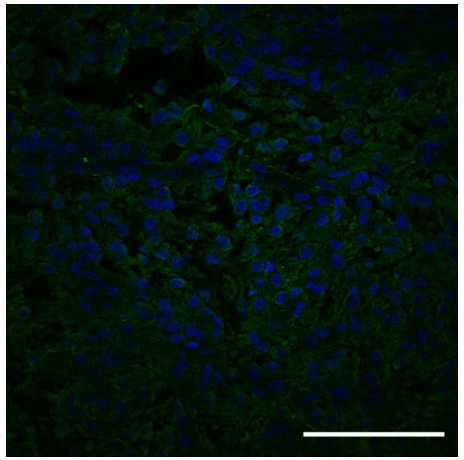
VH15-1139D

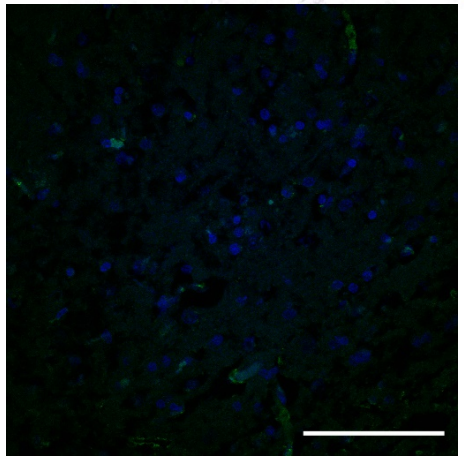
Glioblastoma (Grade IV WHO)

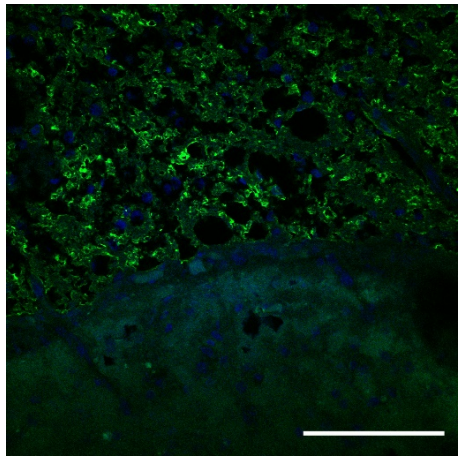




Control: separate section



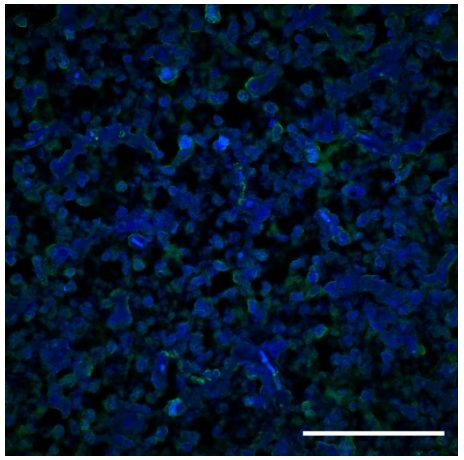




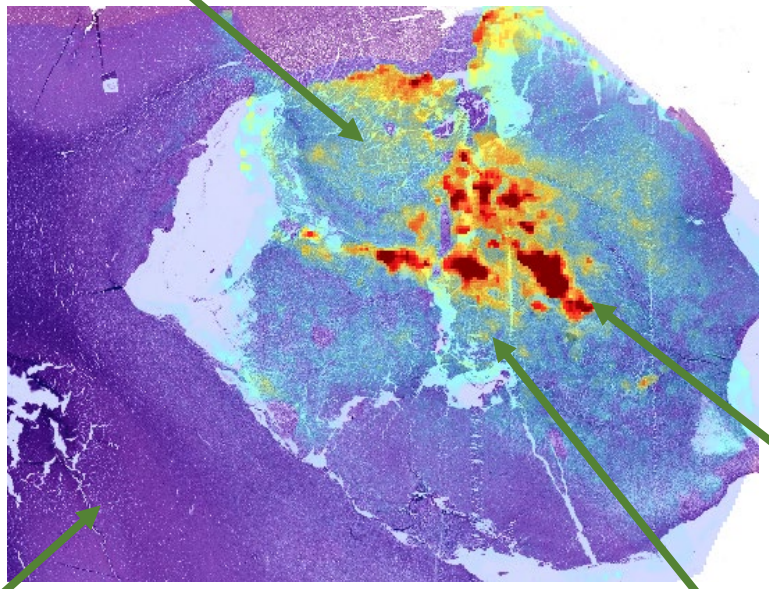
SD 4D

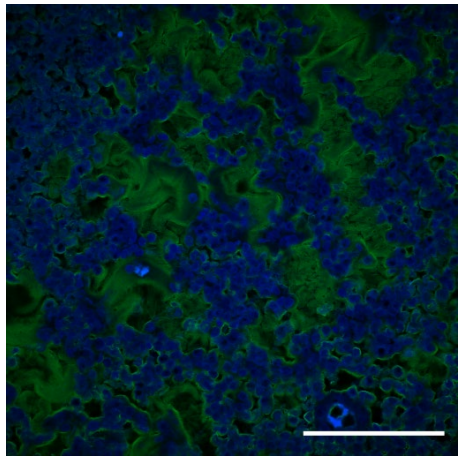
VH15-3520A

Anaplastic Oligodendroglioma (Grade III WHO)

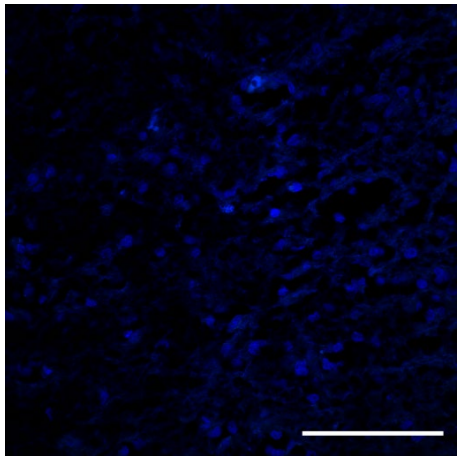


Tumor

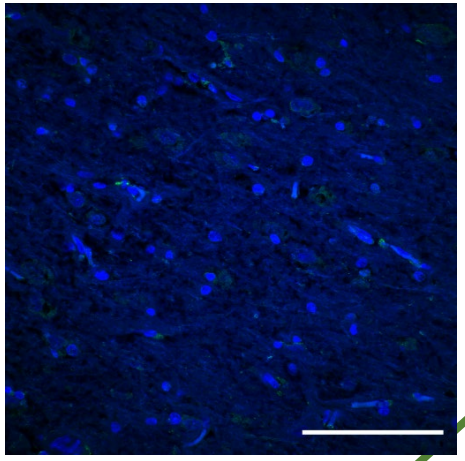




Signal fills empty spaces on necrotic zones

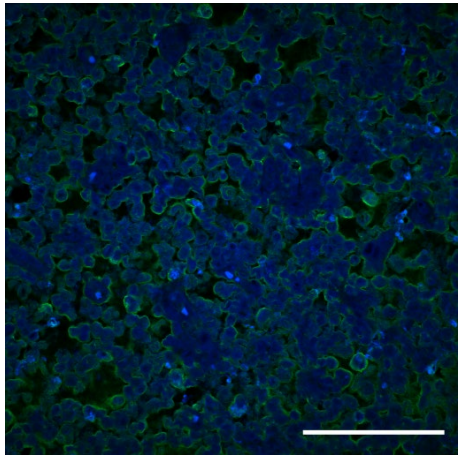


Control: separate section



Benign cortex

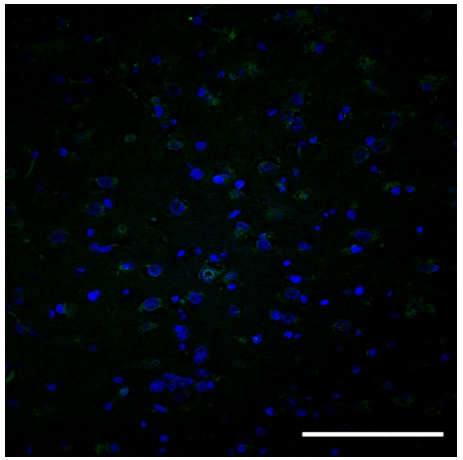
Necrosis+Tumor



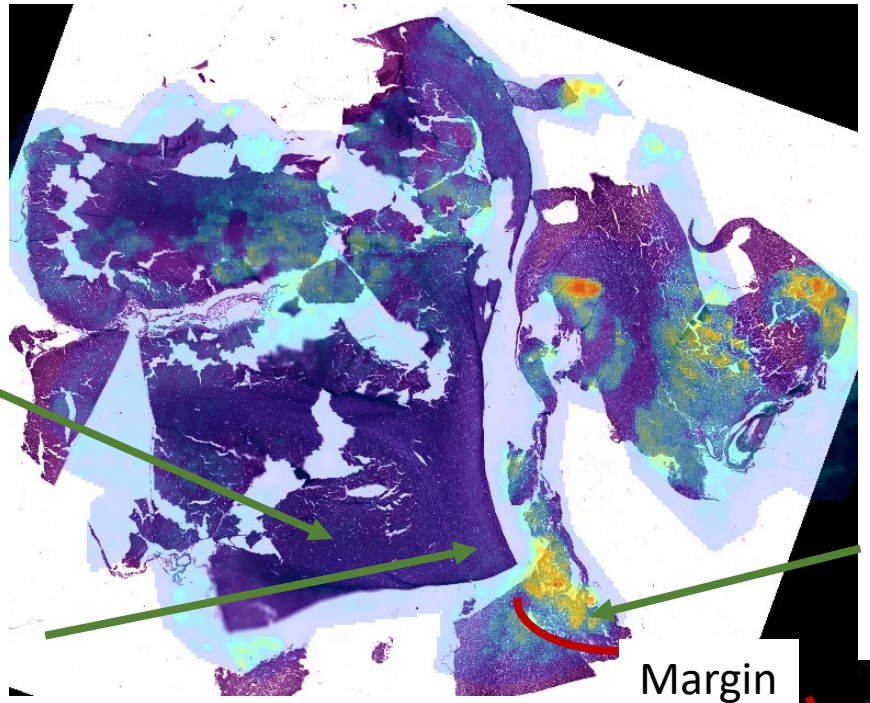
SD 4E

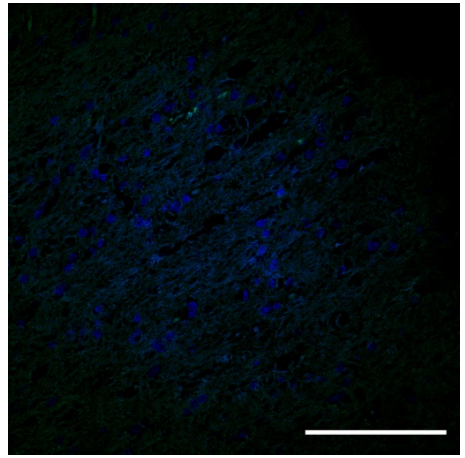
VH16-0703A

Anaplastic Oligodendroglioma (Grade III WHO)

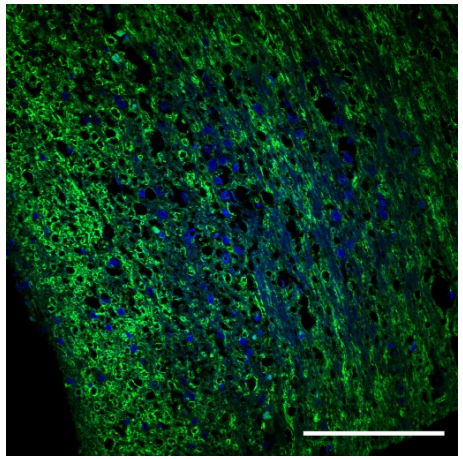


Benign – No signal on Cortex





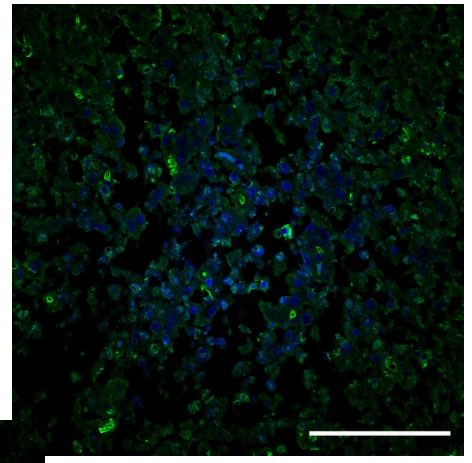
Control: separate section

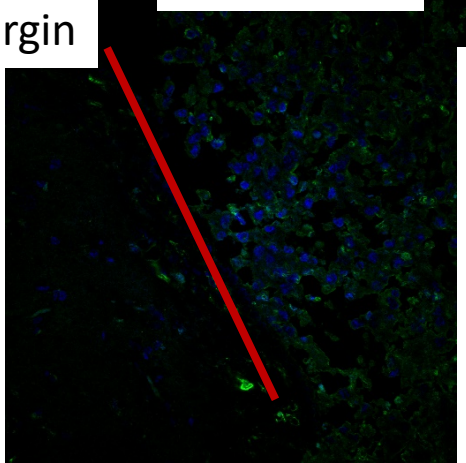


Benign – Intense signal on corpus callosum

Necrosis

Margin

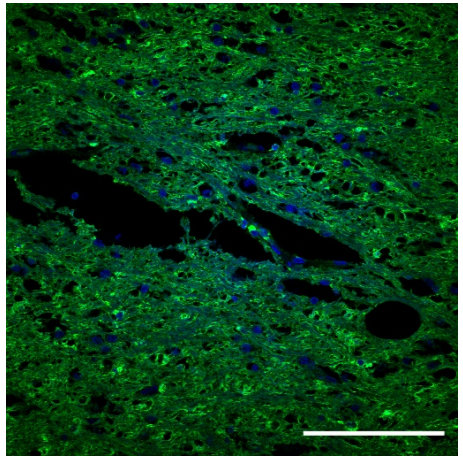




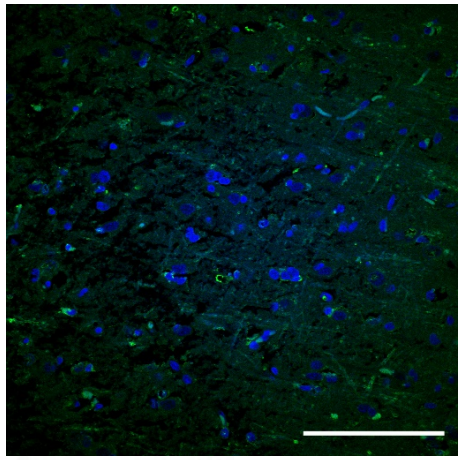
SD 4F

VH16-0703B

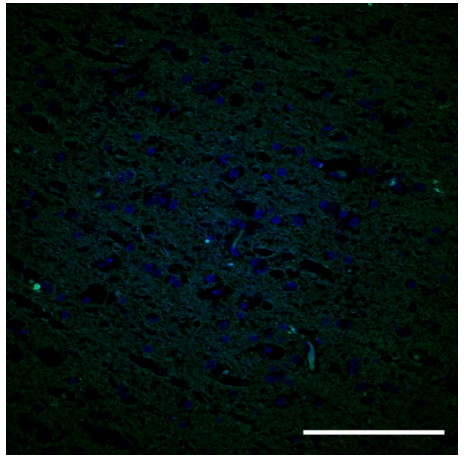
Anaplastic Oligodendroglioma (Grade III WHO)



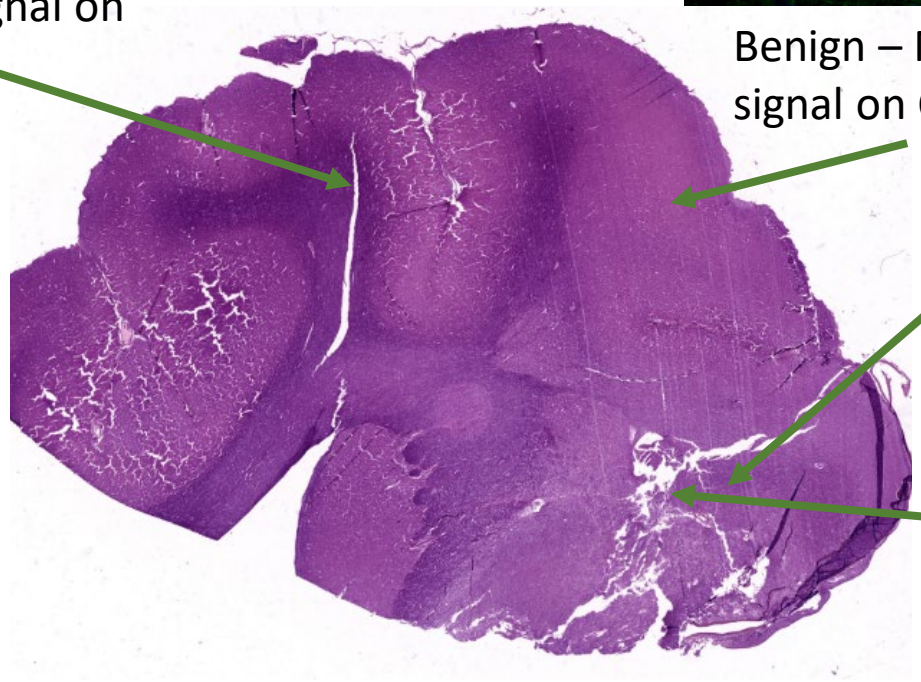
Benign – Intense signal on corpus callosum



Benign – No signal on Cortex

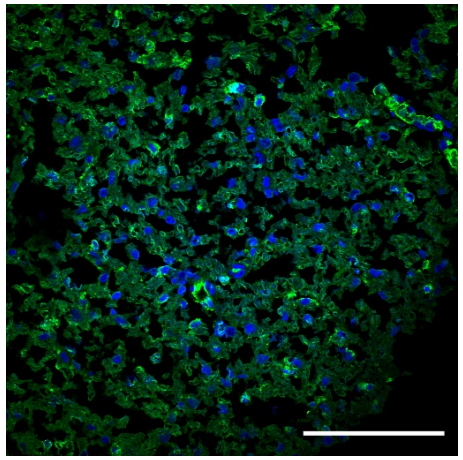


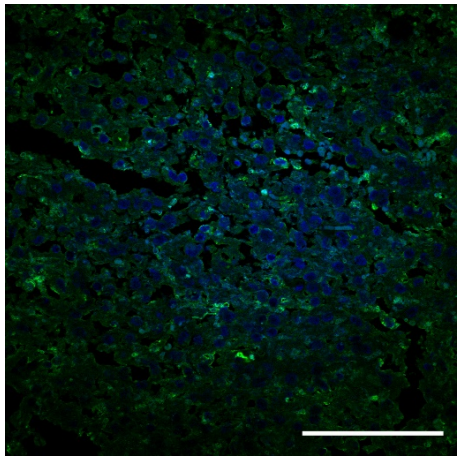
Control: separate section



Tumor

Necrosis

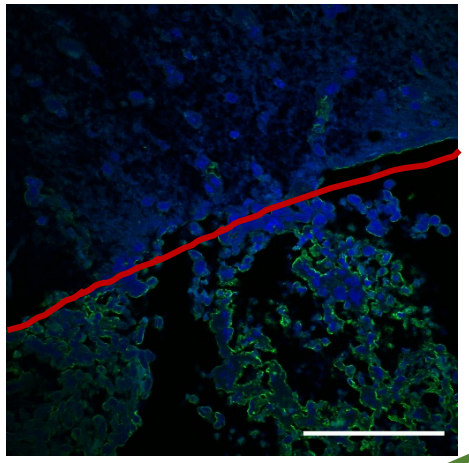


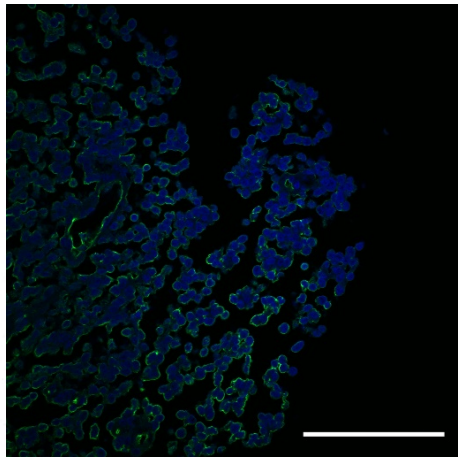


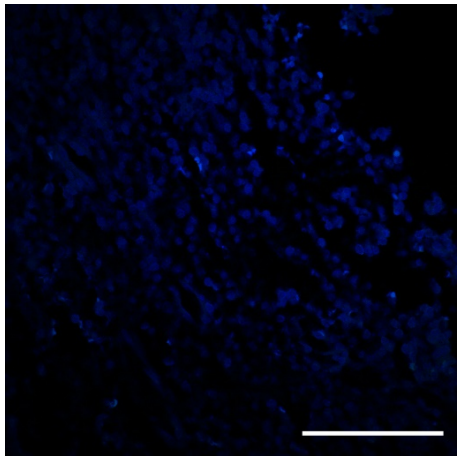
SD 4G

VH13-0935

Anaplastic Oligodendroglioma (Grade III WHO)





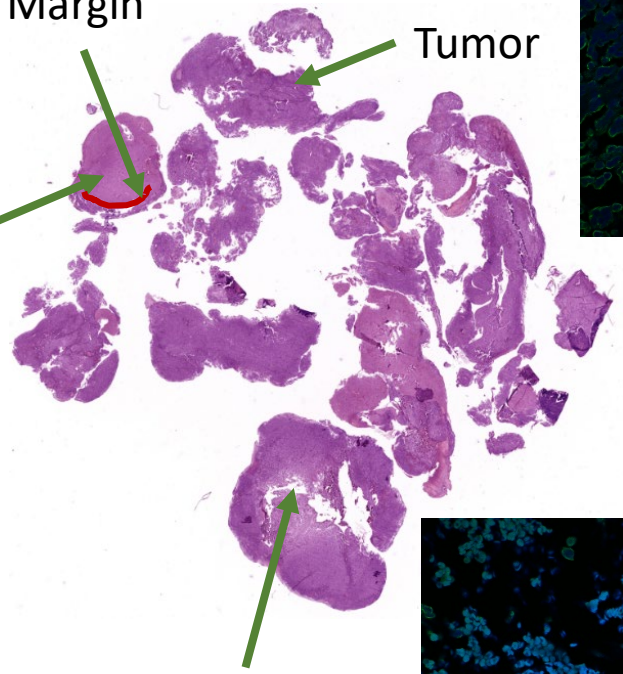


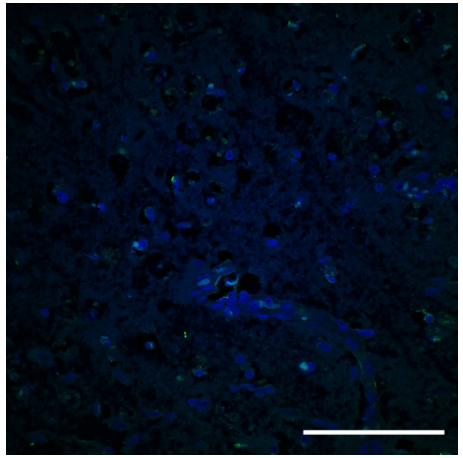
Control: separate section

Margin

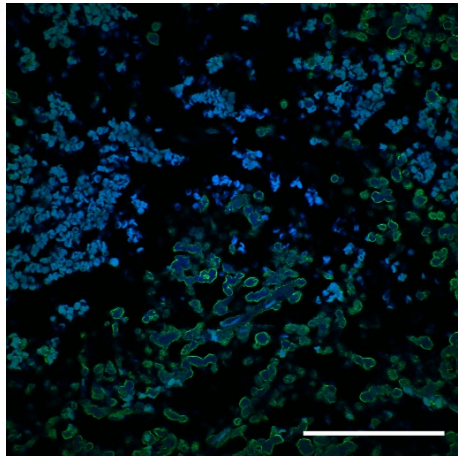
Tumor

Benign





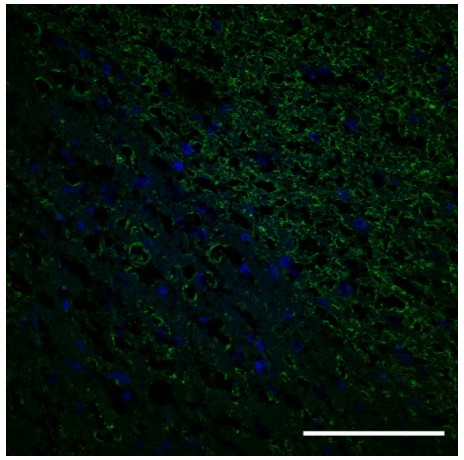
Necrosis

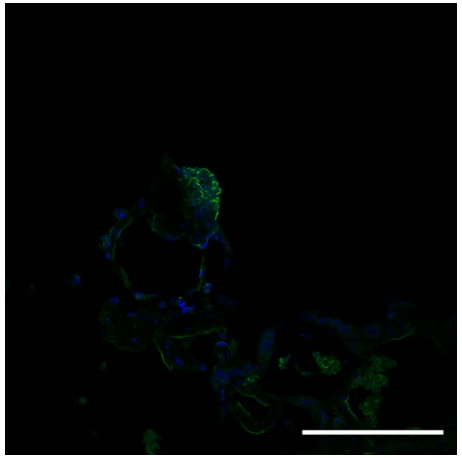


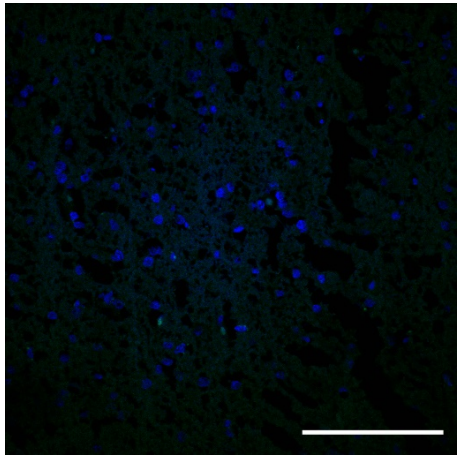
SD 4H

VH16-0440C

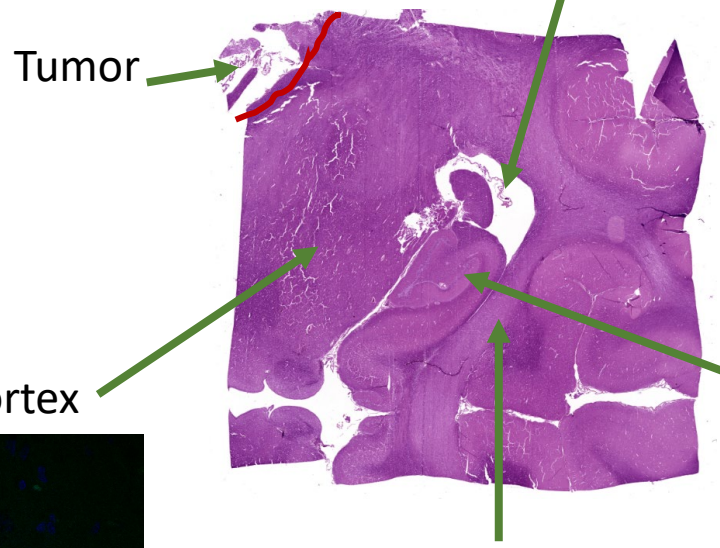
Anaplastic Oligodendroglioma (Grade III WHO)

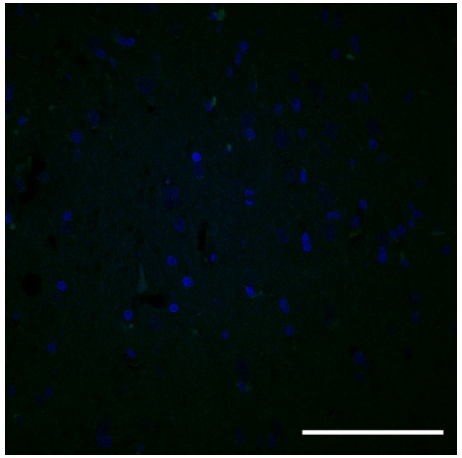


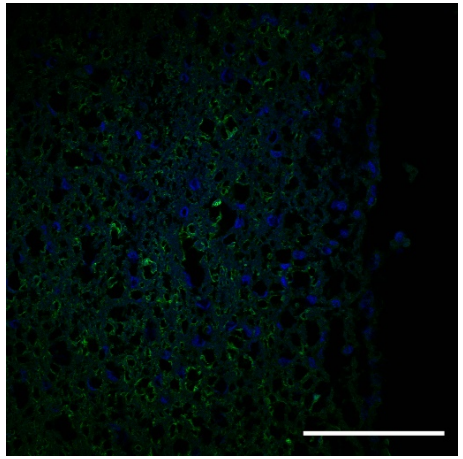


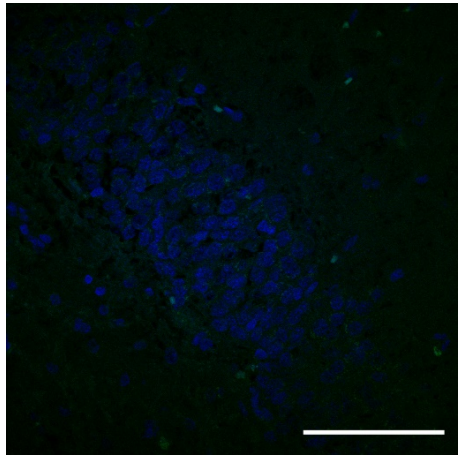


Control: separate section







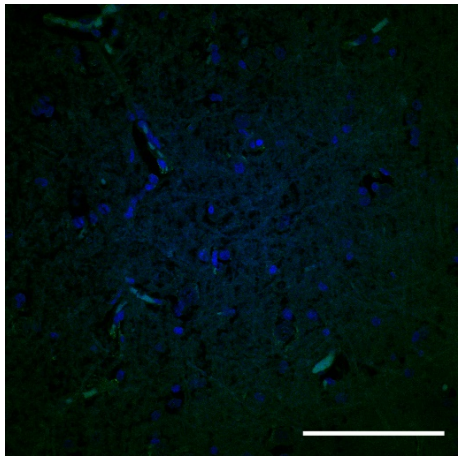


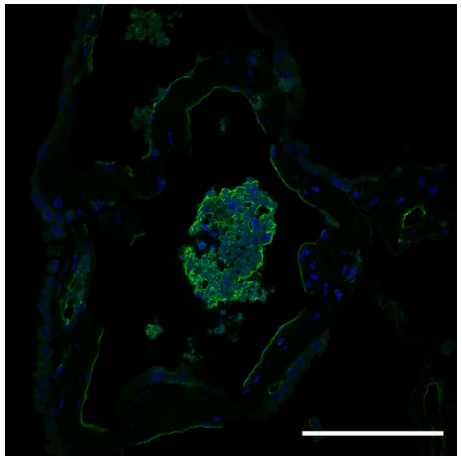
SD 4l

VH16-0440D

Anaplastic Oligodendroglioma (Grade III WHO)

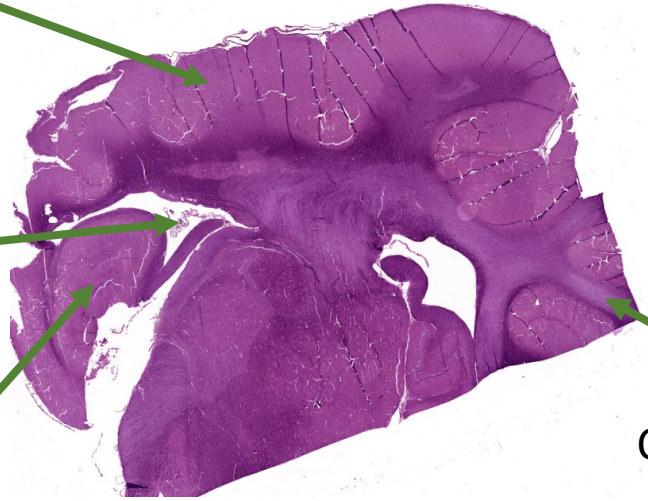
No visible tumor/necrotic zone



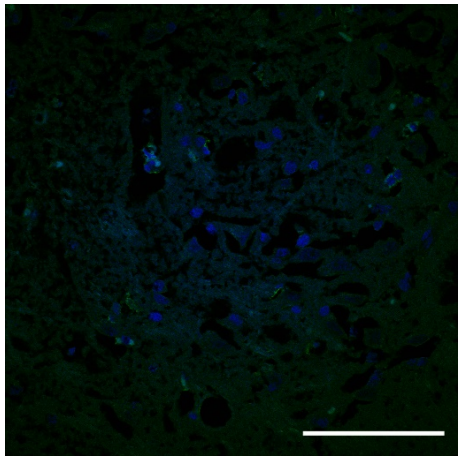


Cortex

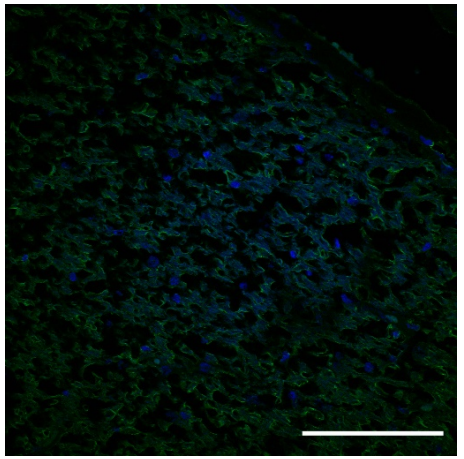
Choroid plexus

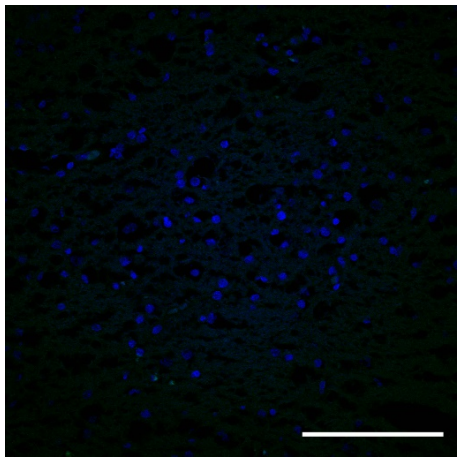


Hippocampus

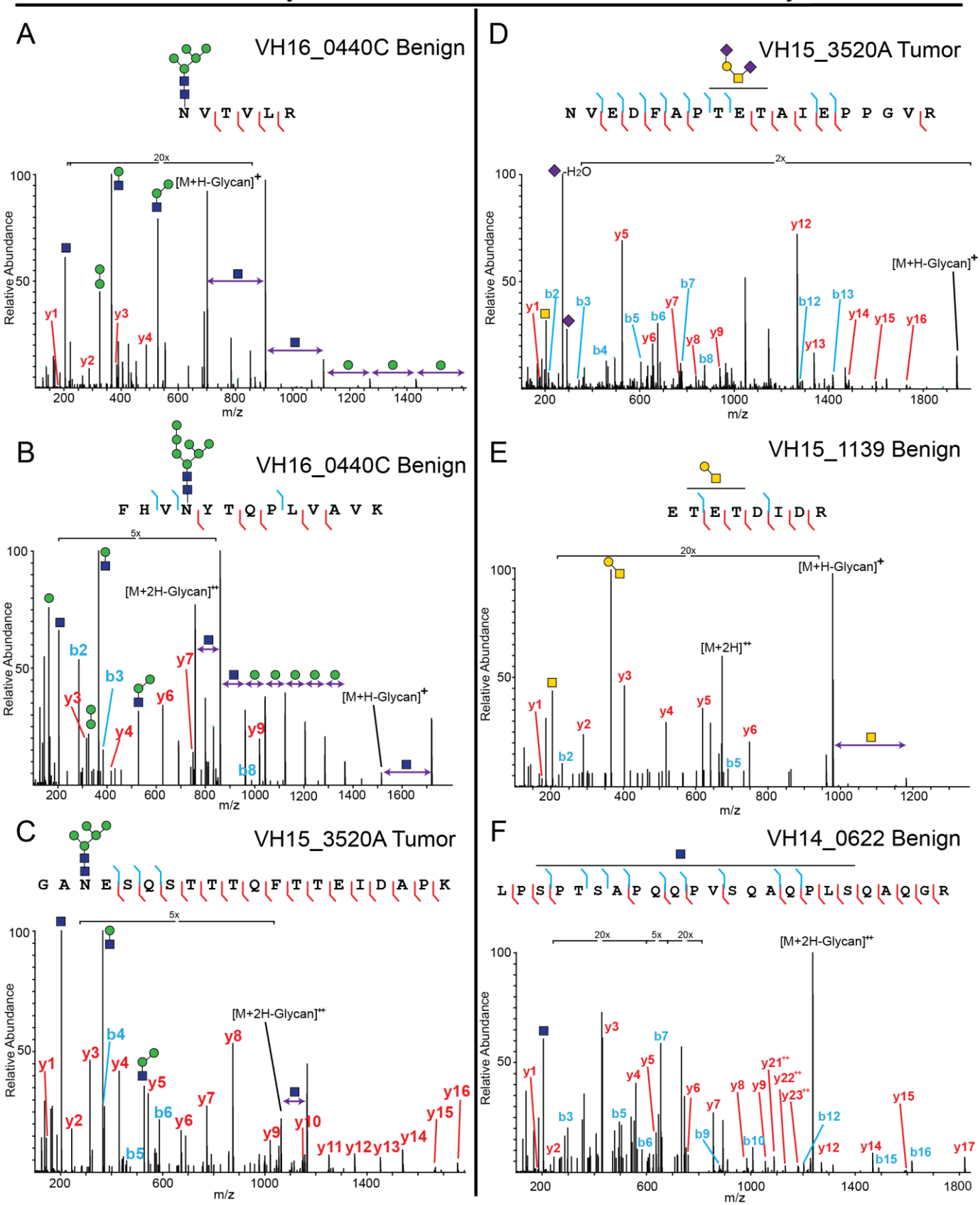


Corpus callosum





Control: separate section



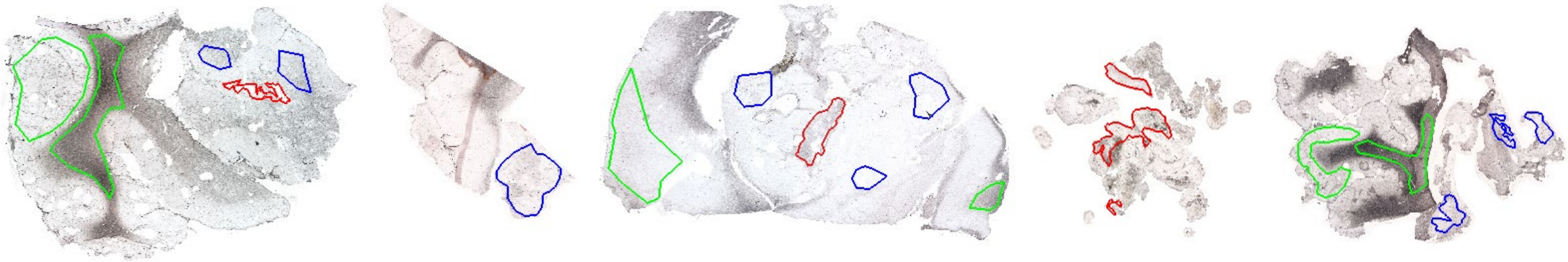
Supplementary Data 5. Additional annotated glycopeptide MS2 spectra. All microdigested samples were subjected to LC-MS/MS analysis on a Thermo Orbitrap Fusion Tribrid, and peptides were subjected to HCD fragmentation. Spectra were annotated manually to confirm glycan composition, peptide sequence, and (if possible) site localize the glycan. (Left) N-glycopeptide spectra, (right) O-glycopeptide spectra. Related to Figure 4.

Supplemental Data 7: Clinical data of Glioma Samples, Related to Figures 1-5

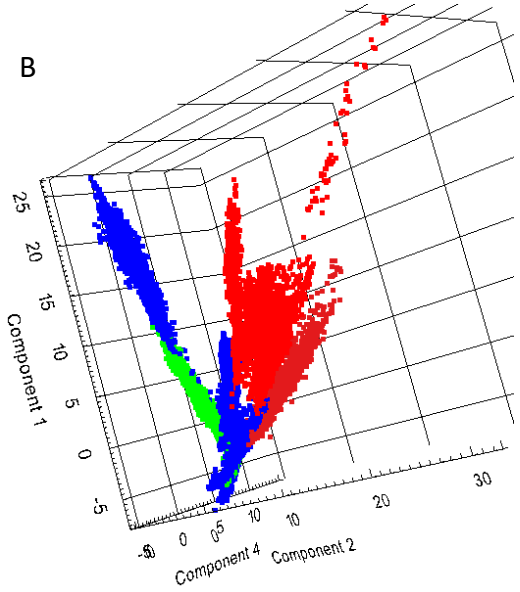
Code	Breed	Age (years)	Sex	N/US	Type of Cancer	Area of brain
VH 15 1139A	Boxer	9	F	Neutered	Glioblastoma (Grade IV WHO)	Cortex left hemisphere
VH 15 1139D						
VH 16 0440C	French bulldog	7	M	Unspayed	Anaplastic Oligodendroglioma (Grade III WHO)	Cortex left hemisphere
VH 16 0440D						
VH 16 0703A	French bulldog	8	M	Unspayed	Anaplastic Oligodendroglioma (Grade III WHO)	Cortex Right hemisphere
VH 16 0703B						
VH 13 0935	French bulldog	8	F	Neutered	Anaplastic Oligodendroglioma (Grade III WHO)	Left parietal lobe (cranial aspect)
VH 14 0622	Border Collie	13	F	Unspayed	Glioblastoma (Grade IV WHO)	junction between parietal and frontal lobe left hemisphere
VH 15 3520A	English bulldog	5	F	Unspayed	Anaplastic Oligodendroglioma (Grade III WHO)	Left rhinencephalus

Supplementary Data 8. Principal component analysis results. H&E images were uploaded on SCiLS software and co-registered with the ion images to help define the regions of interest (ROIs). The ROIs are shown in **A**, marked with green, red and blue outlines corresponding to benign, necrosis and tumor regions, respectively. Spectra from these ROIs were extracted and subjected to PCA. Panels in **B** show different views of the 3D scores plot using PC1, PC2 and PC4. The first five principal components explain about 90% of the variance observed, more than 50% of which are explained by the first component (**C**). Related to Figure 1.

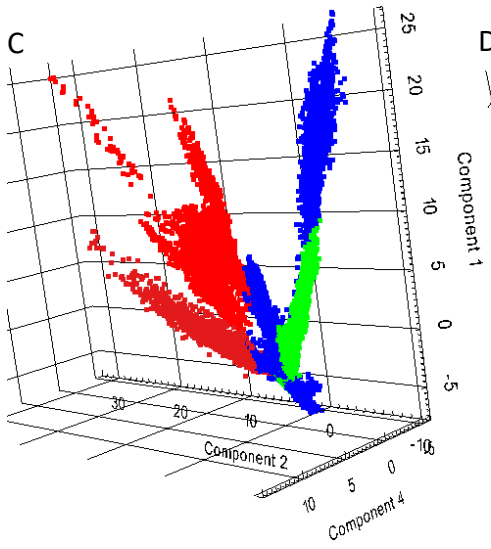
A



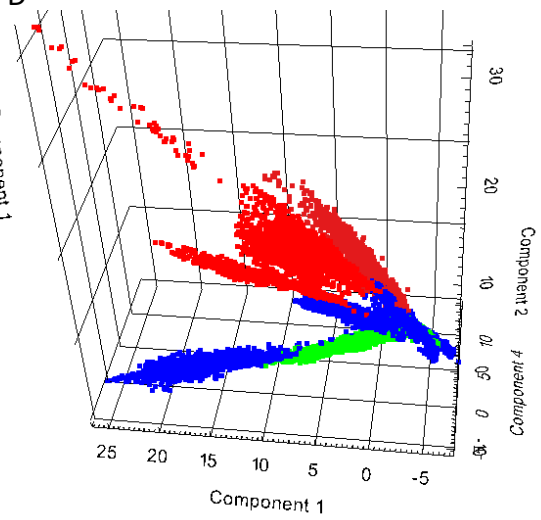
B


Component 1
Component 4
Component 2

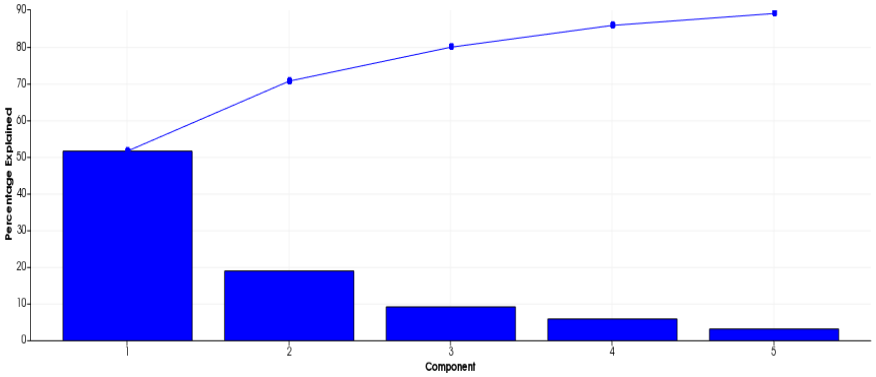
C


Component 1
Component 2
Component 4

D


Component 2
Component 1

E


Percentage Explained
Component