

Supporting Information:

A New Enzymatic Approach to Distinguish Fucosylation Isomers of N-linked Glycans in Tissues Using MALDI Imaging Mass Spectrometry

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Supplementary Figure 1: Hematoxylin and Eosin Stain of Prostate Cancer Tissue

Supplementary Figure 2: Multiple Tissue Types Treated with Endo F3

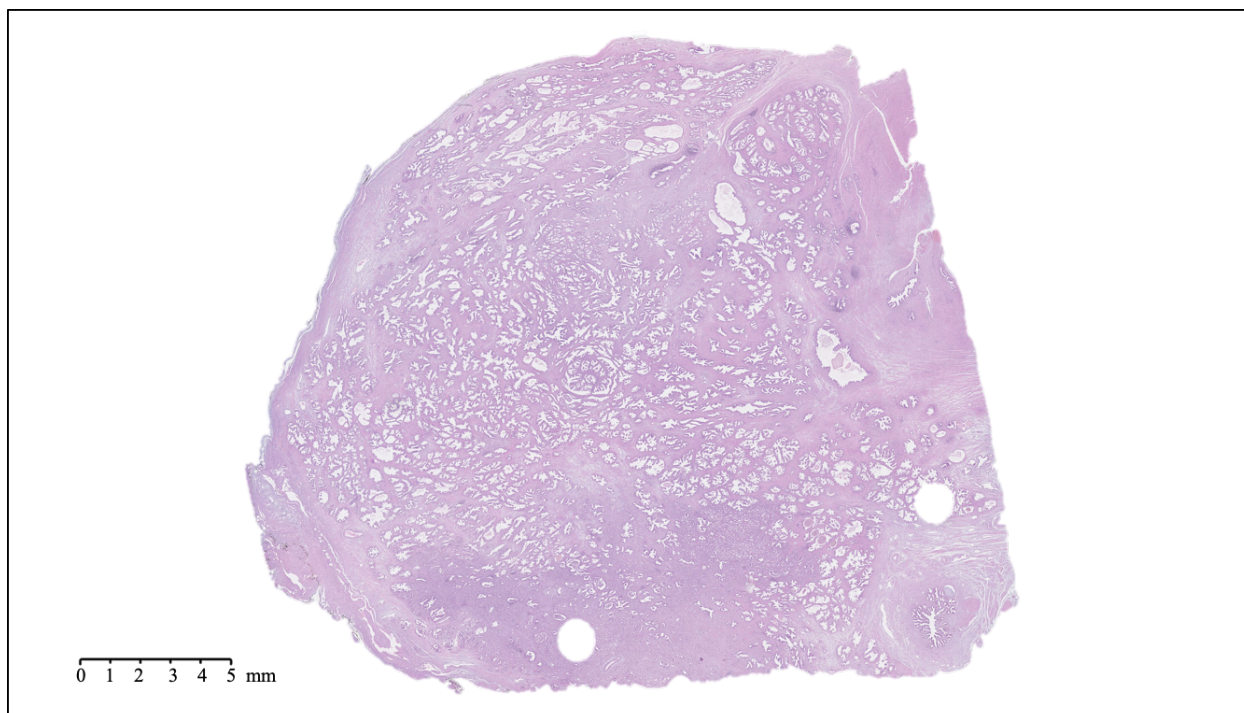
Supplementary Figure 3: Mixture of PNGase F and Endo F3

Supplementary Figure 4: Survival Probability for Non-Core Fucosylated N-Linked Glycan

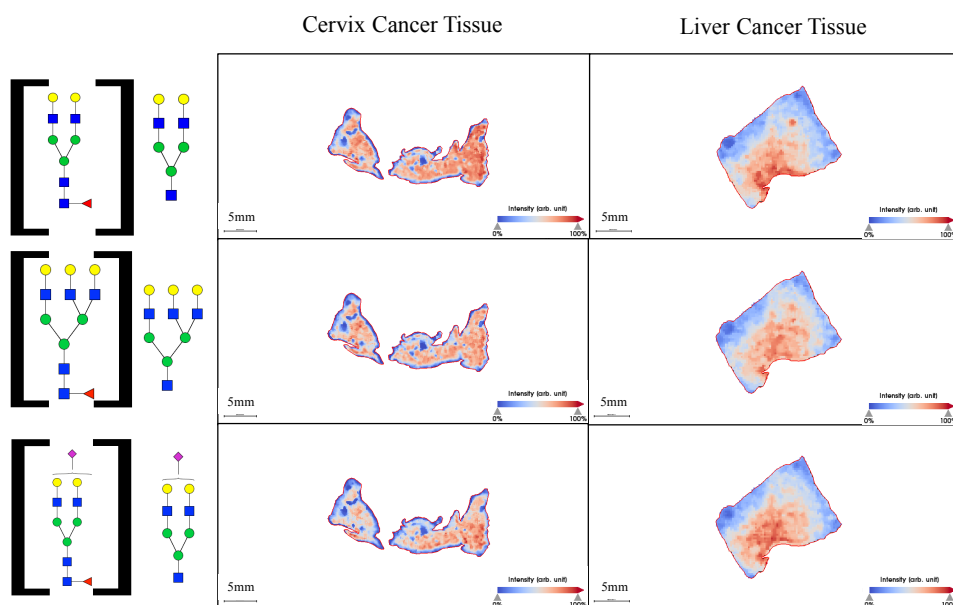
Supplementary Figure 5: Full SDS-PAGE Gel Image from Figure 1

Supplementary Table 1: Master List of N-Linked Glycans with PNGase F

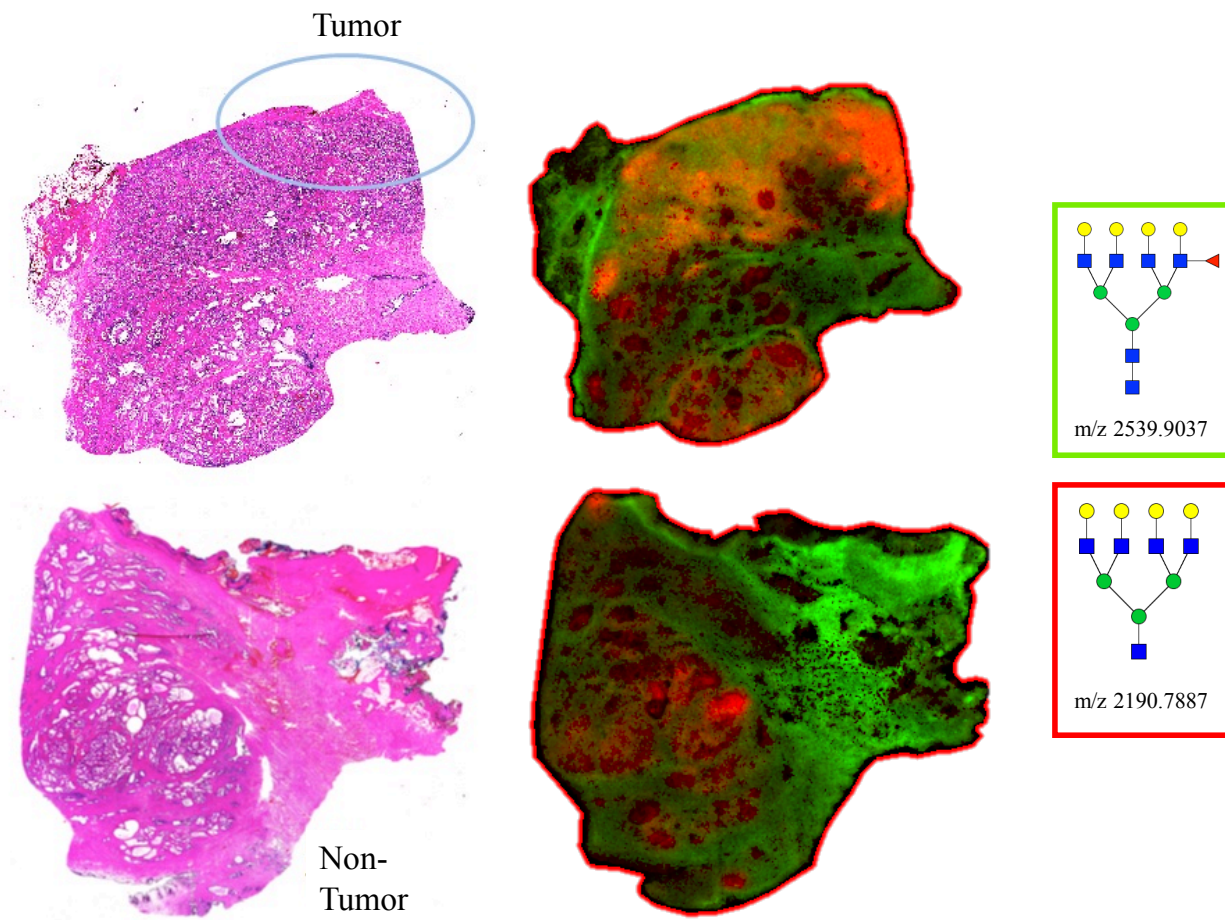
Supplementary Table 2: Master List of N-Linked Glycans with Endo F3



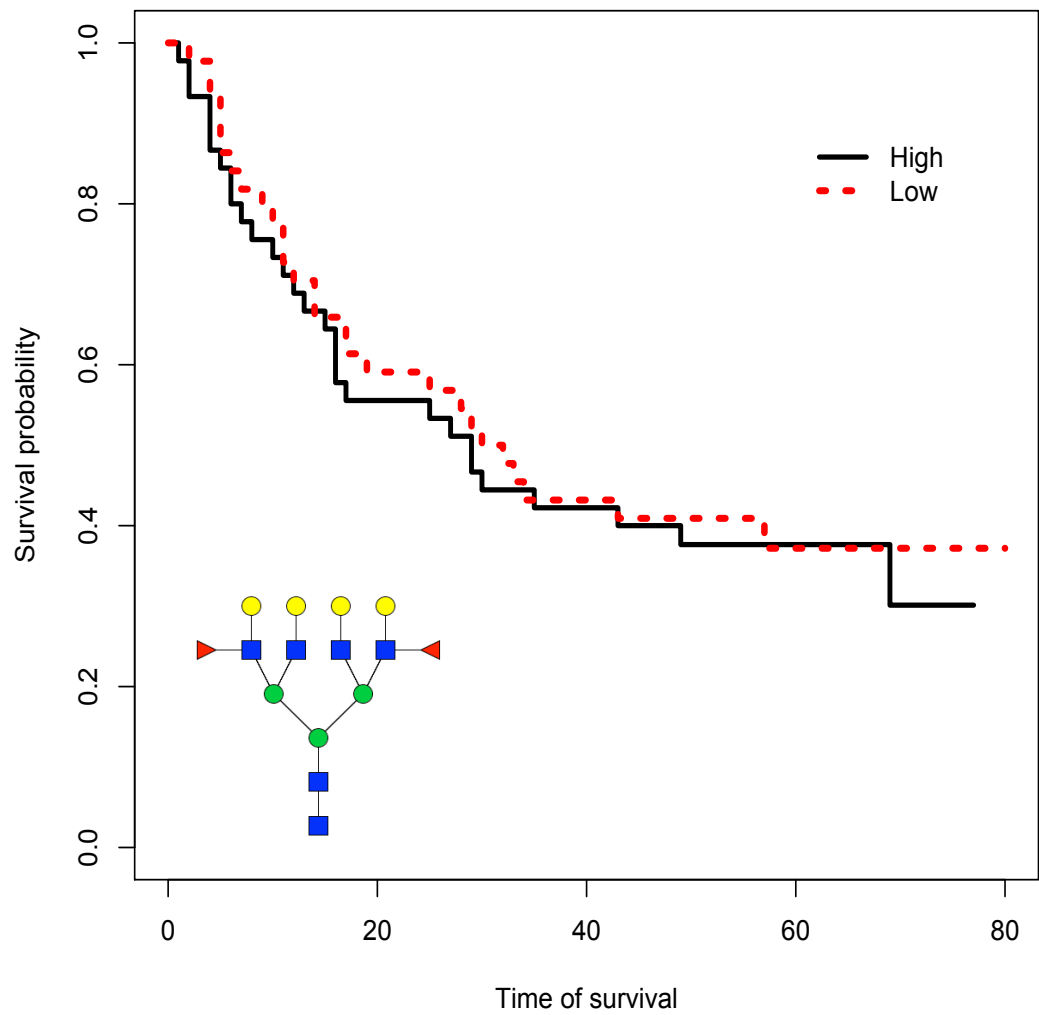
Supplementary Figure 1: Hematoxylin and Eosin Stain of Prostate Cancer Tissue



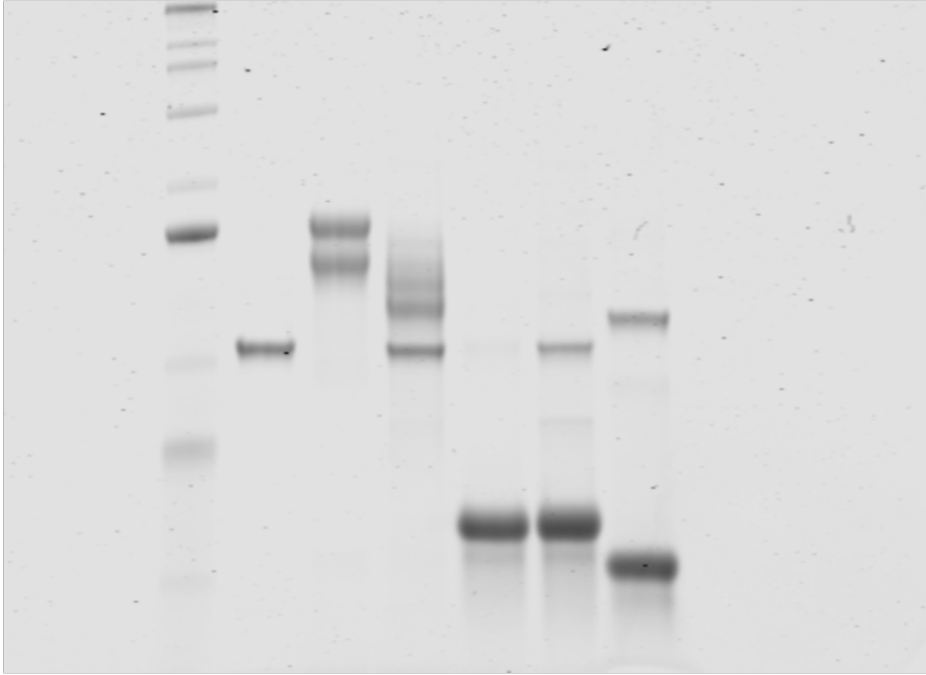
Supplementary Figure 2: Multiple Tissue Types Treated with Endo F3. Cervical cancer tissue as well as hepatocellular carcinoma tissue were treated with Endo F3 and analyzed for core fucosylated glycans shown to the left with the parent glycan structures shown in the brackets.



Supplementary Figure 3: Mixture of PNGase F and Endo F3: Representative images of Endo F3 and PNGase F mixed at an approximate ratio of 1:20 respectively (middle). Hemotoxylin and eosin stain included (left), as well as structure and m/z (right) for distribution of the green N-glycan (m/z 2539.9037) and red N-glycan (m/z 2190.7887)



Supplementary Figure 4: Survival Probability for Non-Core Fucosylated N-Linked Glycan



Supplementary Figure 5: Full SDS-PAGE Gel Image from Figure 1

Supplementary Table 1: Master List of N-Linked Glycans with PNGase F. Master list of all N-linked glycans found using PNGase F treatment, along with the corresponding mass error and compositional structure.

Composition	Theoretical m/z	Observed m/z	Error in PPM
Hex5HexNAc2 + 1Na	1257.4226	1257.4337	8.8443
Hex6HexNAc2 + 1Na	1419.4754	1419.4974	15.5276
Hex4dHex1HexNAc3 + 1Na	1444.5071	1444.5368	20.5544
Hex3dHex1HexNAc4 + 1Na	1485.5337	1485.5605	18.0144
Hex4HexNAc4 + 1Na	1501.5286	1501.5592	20.4065
Hex7HexNAc2 + 1Na	1581.5282	1581.5788	31.9634
Hex4dHex1HexNAc4 + 1Na	1647.5865	1647.6356	29.7714
Hex5HexNAc4 + 1Na	1663.5814	1663.6206	23.5883
Hex3dHex1HexNAc5 + 1Na	1688.6131	1688.6569	25.9154
Hex4HexNAc5 + 1Na	1704.6080	1704.6473	23.0792
Hex8HexNAc2 + 1Na	1743.5810	1743.6242	24.7944
Hex5dHex1HexNAc4 + 1Na	1809.6393	1809.7042	35.8806
Hex4dHex1HexNAc5 + 1Na	1850.6659	1850.7334	36.4955
Hex9HexNAc2 + 1Na	1905.6338	1905.7078	38.8432
Hex5HexNAc4NeuAc1 + 2Na	1976.6666	1976.7341	34.1848
Hex5dHex1HexNAc5 + 1Na	2012.7187	2012.7656	23.3222
Hex6HexNAc5 + 1Na	2028.7136	2028.7927	38.9661
Hex5dHex1HexNAc4NeuAc1 + 2Na	2122.7245	2122.7869	29.3924
Hex5dHex2HexNAc5 + 1Na	2158.7766	2158.8272	23.4304
Hex6dHex1HexNAc5 + 1Na	2174.7715	2174.8578	39.6828
Hex4dHex2HexNAc5NeuAc1 + 1Na	2287.8192	2287.8214	0.9577
Hex6HexNAc5NeuAc1 + 1Na	2319.8090	2319.8982	38.4562
Hex6dHex2HexNAc5 + 1Na	2320.8294	2320.9157	37.2026
Hex6HexNAc5NeuAc1 + 2Na	2341.7988	2341.8272	12.1240
Hex6dHex1HexNAc6 + 1Na	2377.8509	2377.8963	19.0975
Hex7HexNAc6 + 1Na	2393.8458	2393.9259	33.4779
Hex9HexNAc3NeuAc1 + 2Na	2421.7984	2421.9534	64.0235
Hex6dHex3HexNAc5 + 1Na	2466.8873	2466.9264	15.8422
Hex6dHex1HexNAc5NeuAc1 + 2Na	2487.8567	2487.9189	25.0022
Hex7dHex1HexNAc6 + 1Na	2539.9037	2539.9544	19.9736
Hex7dHex2HexNAc6 + 1Na	2685.9616	2685.9904	10.7265
Hex8HexNAc7 + 1Na	2758.9780	2759.0200	15.2230
Hex6dHex3HexNAc5NeuAc1 + 2Na	2779.9725	2779.9808	3.0043
Hex7dHex3HexNAc6 + 1Na	2832.0195	2832.0103	3.2376
Hex8dHex1HexNAc7 + 1Na	2905.0359	2905.0798	15.1017

Supplementary Table 2: Master List of N-Linked Glycans with Endo F3. Master list of all N-linked glycans found using Endo F3 treatment, along with the corresponding mass error and compositional structure.

Composition	Parent m/z	Endo F3 Theoretical m/z	Observed m/z	Error in PPM
Hex4HexNAc3 + 1Na	1647.5865	1298.4495	1298.4477	1.4086
Hex3HexNAc4 + 1Na	1688.6131	1339.4761	1339.4328	32.2955
Hex4dHex1HexNAc3 + 1Na	1793.6444	1444.5074	1444.5008	4.5891
Hex5HexNAc3 + 1Na	1809.6393	1460.5023	1460.5117	6.4505
Hex3dHex1HexNAc4 + 1Na	1834.6710	1485.5340	1485.4574	51.5565
Hex4HexNAc4 + 1Na	1850.6659	1501.5289	1501.5338	3.2906
Hex3HexNAc5 + 1Na	1891.6925	1542.5555	1542.5491	4.1678
Hex4dHex1HexNAc4 + 1Na	1996.7238	1647.5868	1647.5796	4.3694
Hex5HexNAc4 + 1Na	2012.7187	1663.5817	1663.6029	12.7262
Hex4HexNAc5 + 1Na	2053.7453	1704.6083	1704.5639	26.0758
Hex5HexNAc3NeuAc1 + 1Na	2100.7347	1751.5977	1751.5814	9.2995
Hex5dHex1HexNAc4 + 1Na	2158.7766	1809.6396	1809.6271	6.9180
Hex6HexNAc4 + 1Na	2174.7715	1825.6345	1825.6245	5.4660
Hex5dHex2HexNAc4 + 1Na	2304.8345	1955.6975	1955.6420	28.3935
Hex6dHex1HexNAc4 + 1Na	2320.8294	1971.6924	1971.6772	7.6934
Hex5HexNAc4NeuAc1 + 2Na	2325.8039	1976.6669	1976.6640	1.4408
Hex5dHex1HexNAc5 + 1Na	2361.8560	2012.7190	2012.7185	0.2579
Hex6HexNAc5 + 1Na	2377.8509	2028.7139	2028.7039	4.9386
Hex5HexNAc3NeuAc2 + 1Na	2391.8301	2042.6931	2042.6896	1.7178
Hex5HexNAc3NeuAc2 + 2Na	2413.8199	2064.6829	2064.6713	5.5883
Hex6HexNAc4NeuAc1 + 1Na	2465.8669	2116.7299	2116.7179	5.6875
Hex6HexNAc4NeuAc1 + 2Na	2487.8567	2138.7197	2138.7127	3.2580
Hex5HexNAc5NeuAc1 + 1Na	2506.8935	2157.7565	2157.7176	18.0368
Hex6dHex1HexNAc5 + 1Na	2523.9088	2174.7718	2174.7546	7.8900
Hex7HexNAc5 + 1Na	2539.9037	2190.7667	2190.8064	18.1128
Hex6dHex2HexNAc5 + 1Na	2669.9667	2320.8297	2320.8643	14.9046
Hex7dHex1HexNAc5 + 1Na	2685.9616	2336.8246	2336.7482	32.6850
Hex6HexNAc5NeuAc1 + 2Na	2690.9361	2341.7991	2341.8213	9.4936
Hex7HexNAc5 + 1Na	2742.9831	2393.8461	2393.8870	17.0942
Hex6dHex1HexNAc5NeuAc1 + 1Na	2815.0042	2465.8672	2465.8742	2.8392
Hex7dHex2HexNAc5 + 1Na	2832.0195	2482.8825	2482.8719	4.2729
Hex7dHex1HexNAc6 + 1Na	2889.0410	2539.9040	2539.9865	32.4937
Hex8HexNAc6 + 1Na	2905.0359	2555.8989	2555.9095	4.1516
Hex7dHex2HexNAc6 + 1Na	3035.0989	2685.9619	2685.9424	7.2745
Hex7dHex1HexNAc6NeuAc1 + 1Na	3180.1364	2830.9994	2831.1097	38.9442
Hex7dHex1HexNAc6NeuAc1 + 2Na	3202.1262	2852.9892	2853.0869	34.2560
Hex9HexNAc7 + 1Na	3270.1681	2921.0311	2921.1289	33.4885

