

Supplementary Information

for

N-glycomic profiling of microsatellite unstable colorectal cancer

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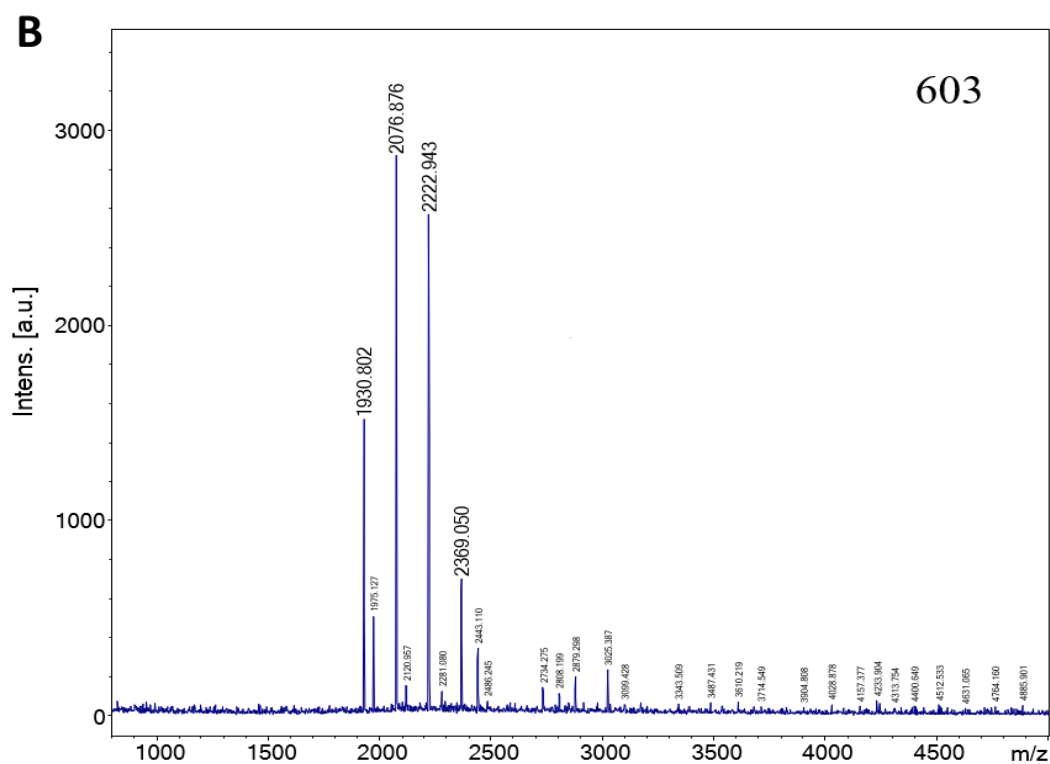
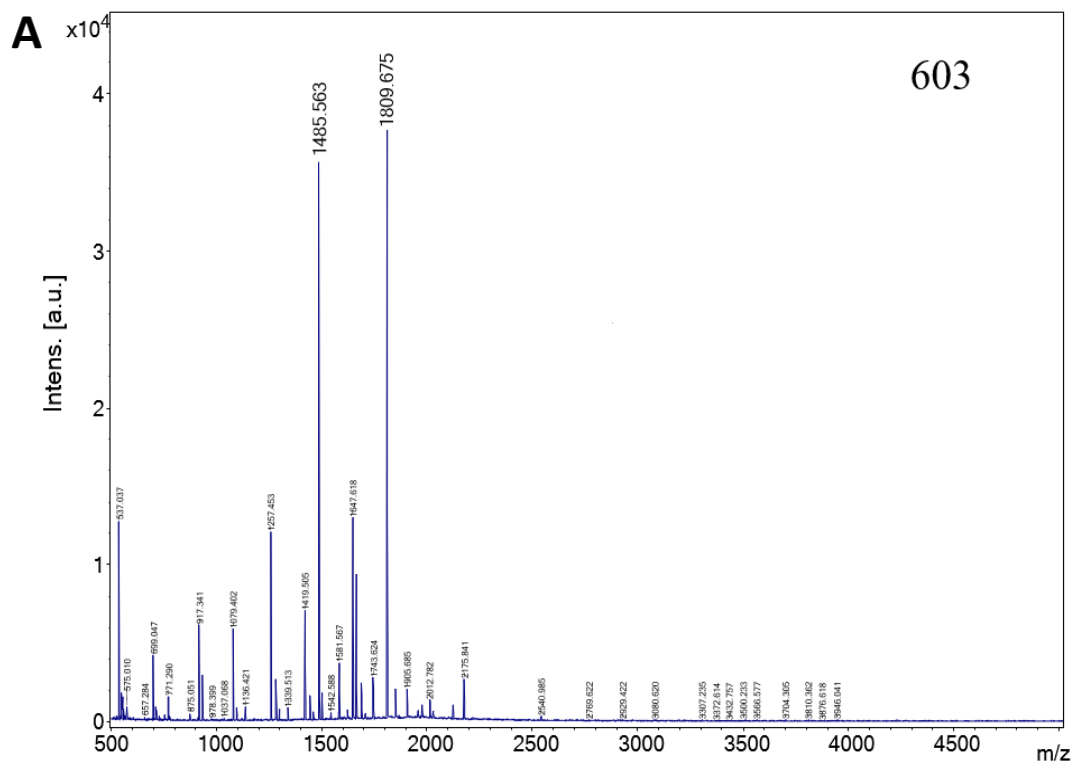
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Supplementary Figure S1. Representative unprocessed MALDI-TOF mass spectra of (A) neutral and (B) acidic N-linked glycans isolated from a tumor tissue sample from a patient with stage II MSI *BRAF*^{V600E} wild-type colon cancer. Note the different scales on y-axes.

Supplementary Table S3. Significantly different neutral and acidic monosaccharide compositions between controls (n=4 pools) and MSI CRC samples (n=40)

	Ctrls		MSI CRC		Fold change	p-adjusted ^a
	Average (%)	SEM	Average (%)	SEM		
NEUTRAL						
H6N2	11.3	0.7	8.9	0.4	0.8	0.032
H9N2	6.7	0.5	4.4	0.3	0.7	0.032
H10N2	0.2	0.0	0.1	0.0	0.5	0.008
H1N2	0.1	0.0	0.3	0.0	3.0	0.028
H3N2	3.6	0.3	2.6	0.2	0.7	0.043
H1N2F1	0.1	0.0	0.9	0.1	9.0	0.005
H2N2F1	3.4	0.4	11.7	0.9	3.4	0.006
H2N3F1	0.1	0.0	0.2	0.0	2.0	0.013
H3N3F1	0.9	0.1	1.8	0.1	2.0	0.013
H4N3F1	0.6	0.0	1.1	0.1	1.8	0.025
H4N3F2	0.1	0.0	0.3	0.0	3.0	0.029
H3N5	1.2	0.3	0.5	0.1	0.4	0.012
H3N5F1	4.3	0.2	1.6	0.2	0.4	0.004
H4N5F1	2.6	0.2	1.3	0.1	0.5	0.009
H5N5F1	2.2	0.4	0.9	0.1	0.4	0.004
H4N5F2	0.9	0.1	0.4	0.1	0.4	0.045
H5N5F2	1.2	0.3	0.2	0.0	0.2	0.008
H4N5F3	0.6	0.1	0.2	0.0	0.3	0.011
ACIDIC						
H5N4F3P1	1.0	0.4	0.3	0.1	0.3	0.025
H4N5F3P1	1.6	0.7	0.1	0.0	0.1	<0.001
H5N5F2P1	1.4	0.7	0.0	0.0	None in CRC	<0.001
H5N5F3P1	1.3	0.6	0.1	0.0	0.1	0.002
H5N6F4P1	0.6	0.3	0.1	0.1	0.2	0.001
H6N6F3P1	1.1	0.5	0.1	0.0	0.1	0.004

^aBenjamini-Hochberg

CRC, colorectal cancer; F, deoxyhexose; H, hexose; MSI, microsatellite instability; N, N-acetylhexosamine; P, acid ester; SEM, standard error of mean

Supplementary Table S4. Significantly different neutral monosaccharide compositions between MSS BRAFwt stage II and MSI BRAFwt stage II samples

	MSS St II BRAFwt		MSI St II BRAFwt		Fold change	p-adjusted ^a
	Average (%)	SEM	Average (%)	SEM		
H5N2	15.5	1.3	8.5	0.8	0.5	0.001
H6N2	12.5	0.7	8.1	0.8	0.6	0.003
H7N2	8.1	0.7	4.9	0.5	0.6	0.004
H8N2	8.8	0.6	5.5	0.8	0.6	0.006
H10N2	0.4	0.1	0.1	0.1	0.3	0.011
H2N2	4.3	0.3	2.4	0.3	0.6	0.005
H3N2	5.0	0.5	2.6	0.5	0.5	0.008
H4N2	2.7	0.2	0.9	0.1	0.3	<0.001
H3N3	0.8	0.1	0.5	0.1	0.6	0.025
H5N3	0.7	0.1	0.3	0.0	0.4	0.002
H6N3	0.7	0.1	0.4	0.0	0.6	0.020
H2N2F1	5.4	0.8	11.4	2.4	2.1	0.037
H4N2F1	0.3	0.0	0.2	0.0	0.7	0.024
H2N3F1	0.0	0.0	0.1	0.0	None in MSS	0.003
H5N3F1	0.4	0.1	0.1	0.0	0.3	0.003
H6N3F1	0.4	0.1	0.1	0.0	0.3	0.014
H3N4	0.7	0.1	0.5	0.1	0.7	0.020
H5N4	1.6	0.3	4.0	0.8	2.5	0.016
H3N4F1	1.6	0.2	6.5	1.9	4.1	0.002
H4N4F1	1.2	0.1	4.1	0.8	3.4	<0.001
H5N4F1	1.7	0.2	11.5	2.0	6.8	<0.001
H5N5F1	0.3	0.1	0.8	0.1	2.7	0.005
H6N5F1	0.3	0.0	1.4	0.3	4.7	<0.001
H7N6F1	0.0	0.0	0.3	0.1	None in MSS	<0.001

^aBenjamini-Hochberg

F, deoxyhexose; H, hexose; MSI, microsatellite instability; MSS, microsatellite stable; N, N-acetylhexosamine; St, stage; mut, mutated; wt, wild-type

Supplementary Table S5. Significantly different acidic monosaccharide compositions between MSS BRAFwt stage II and MSI BRAFwt stage II samples

	MSS St II BRAFwt		MSI St II BRAFwt		Fold change	p-adjusted ^a
	Average (%)	SEM	Average (%)	SEM		
S1H4N4F1	1.6	0.2	0.4	0.1	0.3	0.001
S1H5N4F1	14.8	2.1	25.6	2.1	1.7	0.006
S1H4N5F1	1.5	0.2	0.3	0.1	0.2	<0.001
S1H5N4F2	2.2	0.3	4.8	0.6	2.2	0.005
S1H4N5F2	0.3	0.1	0.0	0.0	None in MSI	0.010
S1H5N5F3	0.6	0.4	0.0	0.0	None in MSI	0.010
S2H5N4	4.7	0.7	18.0	1.7	3.8	<0.001
S2H5N4F1	3.1	0.7	7.4	1.1	2.4	0.005
S2H6N5F3	0.1	0.1	0.6	0.2	6.0	0.027
S3H6N5	0.1	0.1	1.2	0.2	12.0	0.002
S3H6N5F1	0.1	0.1	1.1	0.2	11.0	0.001
H4N5F3P1	0.4	0.1	0.1	0.1	0.3	0.018
H5N5F2P1	0.7	0.3	0.0	0.0	None in MSI	0.026
H5N6F2P1	0.9	0.3	0.0	0.0	None in MSI	0.002
H5N6F3P1	1.2	0.3	0.1	0.1	0.1	0.002
H6N6F3P1	0.7	0.3	0.1	0.1	0.1	0.041

^aBenjamini-Hochberg

F, deoxyhexose; H, hexose; MSI, microsatellite instability; MSS, microsatellite stable; N, N-acetylhexosamine; St, stage; mut, mutated; wt, wild-type

Supplementary Table S6. Significantly different neutral monosaccharide compositions between MSI subgroups

	MSI St II BRAFwt		MSI St II BRAFmut		Fold change	p-adjusted ^a
	Average (%)	SEM	Average (%)	SEM		
H2N3F1	0.1	0.0	0.3	0.1	3.0	0.011
H3N3F1	1.3	0.2	2.0	0.1	1.5	0.017
	MSI St II		MSI St IV		Fold change	p-adjusted ^a
	Average (%)	SEM	Average (%)	SEM		
H3N4	0.4	0.0	0.7	0.1	1.8	0.033
H7N6F1	0.2	0.0	0.1	0.0	0.5	0.018
	MSI St II BRAFwt		MSI St IV BRAFwt		Fold change	p-adjusted ^a
	Average (%)	SEM	Average (%)	SEM		
H2N3F1	0.1	0.0	0.3	0.0	3.0	0.040
H3N3F1	1.3	0.2	2.2	0.2	1.7	0.016
H4N6F1	0.0	0.0	0.2	0.0	None in St II	0.018
H7N6F1	0.3	0.1	0.1	0.0	0.3	0.006
	MSI St II BRAFmut		MSI St IV BRAFmut		Fold change	p-adjusted ^a
	Average (%)	SEM	Average (%)	SEM		
H2N3F1	0.3	0.1	0.2	0.0	0.7	0.026
	MSI St IV BRAFwt		MSI St IV BRAFmut		Fold change	p-adjusted ^a
	Average (%)	SEM	Average (%)	SEM		
H2N3F1	0.3	0.0	0.2	0.0	0.7	0.043

^aBenjamini-Hochberg

F, deoxyhexose; H, hexose; MSI, microsatellite instability; N, N-acetylhexosamine; St, stage; mut, mutated; wt, wild-type

Supplementary Table S7. Significantly different acidic monosaccharide compositions between MSI subgroups

MSI St II BRAFwt			MSI St II BRAFmut		Fold change	p-adjusted ^a
Average (%)	SEM		Average (%)	SEM		
S1H4N5F1	0.3	0.1	0.0	0.0	None in BRAFmut	0.032
S2H5N4	18.0	1.7	33.2	5.4	1.8	0.043
S1H5N5F1	1.0	0.2	0.2	0.2	0.2	0.040
S1H6N5	0.7	0.1	0.0	0.0	None in BRAFmut	0.002
S2H6N5	0.4	0.2	0.0	0.0	None in BRAFmut	0.045
S3H6N5	1.2	0.2	0.2	0.1	0.2	0.009
S2H6N5F3	0.6	0.2	0.0	0.0	None in BRAFmut	0.022
MSI St II			MSI St IV		Fold change	p-adjusted ^a
Average (%)	SEM		Average (%)	SEM		
S1H4N4F1	0.3	0.1	0.8	0.1	2.7	0.010
S2H6N5	0.2	0.1	0.6	0.1	3.0	0.010
S2H7N6F1	0.1	0.1	0.5	0.1	5.0	0.008
MSI St II BRAFwt			MSI St IV BRAFwt		Fold change	p-adjusted ^a
Average (%)	SEM		Average (%)	SEM		
S1H4N4F1	0.4	0.1	1.0	0.2	2.5	0.040
S1H6N5	0.7	0.1	0.2	0.1	0.3	0.009
MSI St II BRAFmut			MSI St IV BRAFmut		Fold change	p-adjusted ^a
Average (%)	SEM		Average (%)	SEM		
S1H4N5F1	0.0	0.0	0.5	0.2	None in St II	0.008
S2H5N4	33.2	5.4	17.5	2.3	0.5	0.045
S1H5N5F1	0.2	0.2	1.3	0.1	6.5	0.016
S1H6N5	0.0	0.0	0.7	0.2	None in St II	0.003
S2H6N5	0.0	0.0	0.7	0.1	None in St II	0.001
S1H6N5F2	0.0	0.0	0.3	0.1	None in St II	0.030
S3H6N5	0.2	0.1	1.2	0.3	6.0	0.020
S2H7N6F1	0.0	0.0	0.5	0.2	None in St II	0.017
MSI St IV BRAFwt			MSI St IV BRAFmut		Fold change	p-adjusted ^a
Average (%)	SEM		Average (%)	SEM		
S1H4N4F1	1.0	0.2	0.5	0.1	0.5	0.043
S1H5N5F1	0.8	0.1	1.3	0.1	1.6	0.042
S1H6N5	0.2	0.1	0.7	0.2	3.5	0.018

^aBenjamini-Hochberg

F, deoxyhexose; H, hexose; MSI, microsatellite instability; N, N-acetylhexosamine; St, stage; mut, mutated; wt, wild-type