Supplemental Figure Legends

Figure S1. Analysis of three biological replicate preparations of released Caco-2 cell membrane glycans after infection with *S.* Typhimurium for 60 minutes. Overlaid total compound chromatograms (TCCs) of each analysis are shown to demonstrate the inter-sample reproducibility.

Figure S2. Global compositional profiling by relative abundances of glycan signals according to glycan type in uninfected (blue) vs. infected (red) cells. Complex/hybrid (C/H) glycans were categorized by their decoration. Bar graphs show the relative abundances and line graphs show the number of compositions summed together for each type.

Figure S3. Isomer separation and differentiation by PGC-LC/MS. Extracted ion chromatogram shows two distinct isomers of an N-glycan structure *m/z* 883.68 eluting at 23.3 min (*a*) and 25.6 min (*b*), respectively.

Figure S4. Tandem mass spectra of identified N-glycans. Precursor ions are indicated by a blue diamond. Putative structures are drawn above the glycan composition name.

Figure S5. Effects on kifunensine concentration on cell surface glycosylation. Distribution of high mannose structures on Caco-2 with increasing additions of kifunensine.

Figure S1.

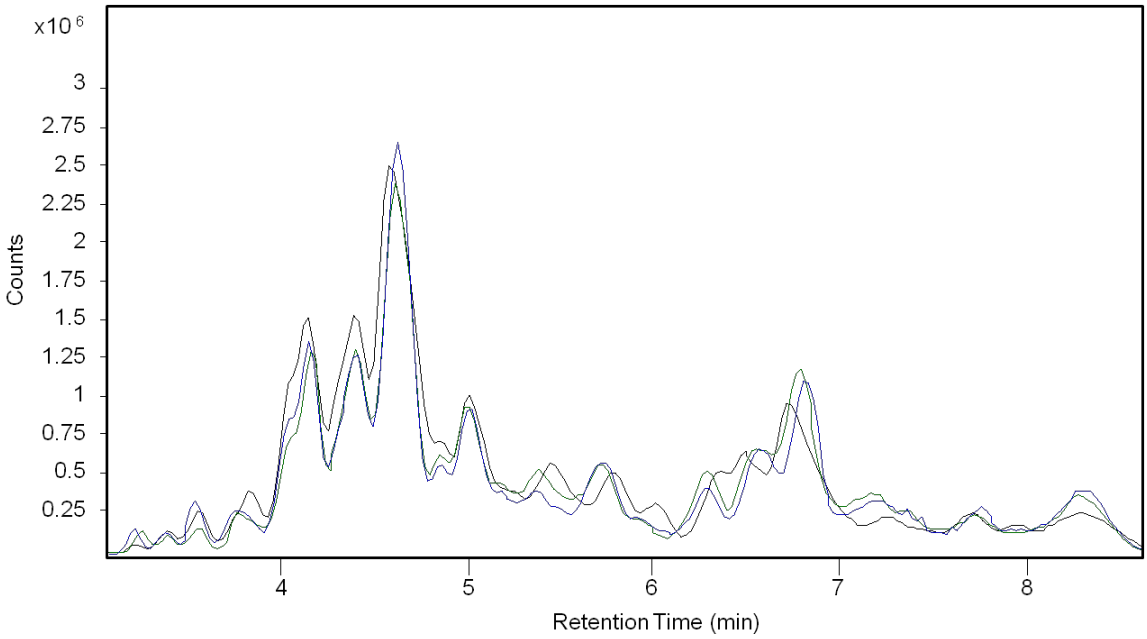

x10 6
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
Counts
4
5
6
7
8
Retention Time (min)

Figure S2.

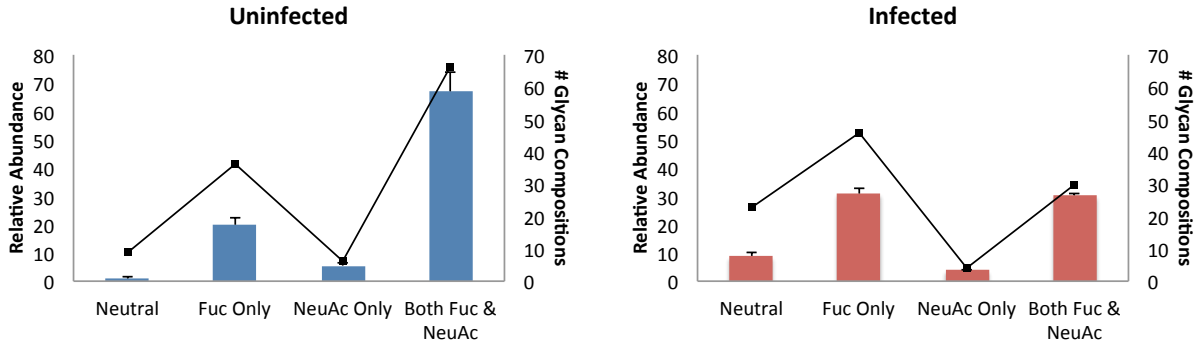

Uninfected
Relative Abundance
Glycan Compositions
0
10
20
30
40
50
60
70
80
Neutral
Fuc Only
NeuAc Only
Both Fuc & NeuAc
Infected
Relative Abundance
Glycan Compositions
0
10
20
30
40
50
60
70
80
Neutral
Fuc Only
NeuAc Only
Both Fuc & NeuAc

Figure S3.

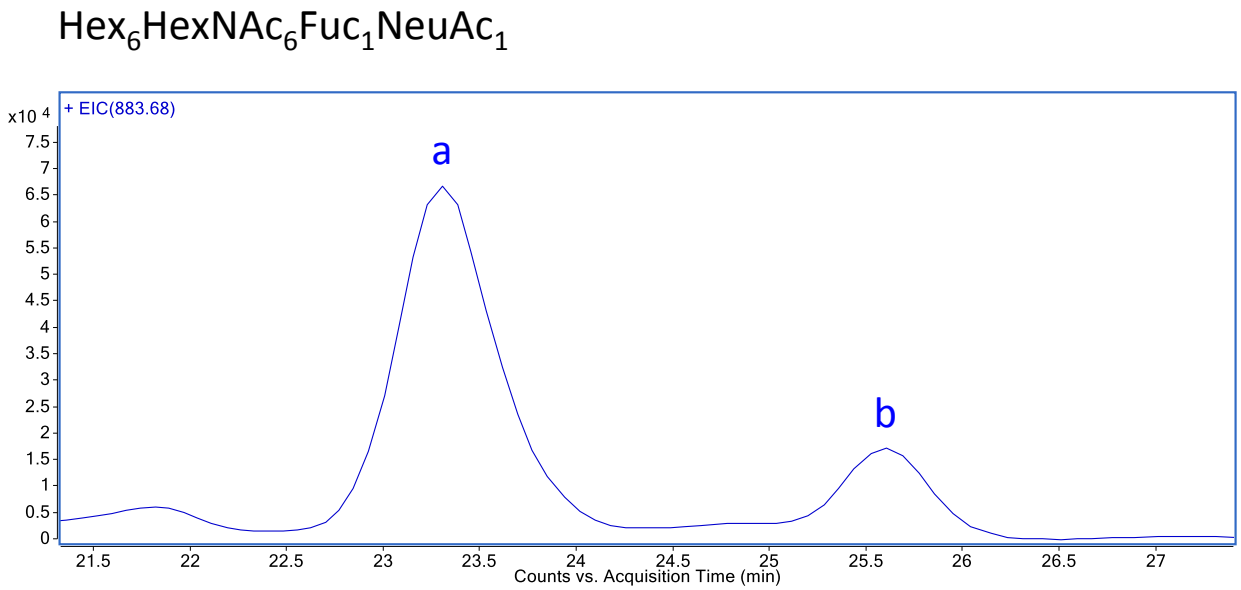
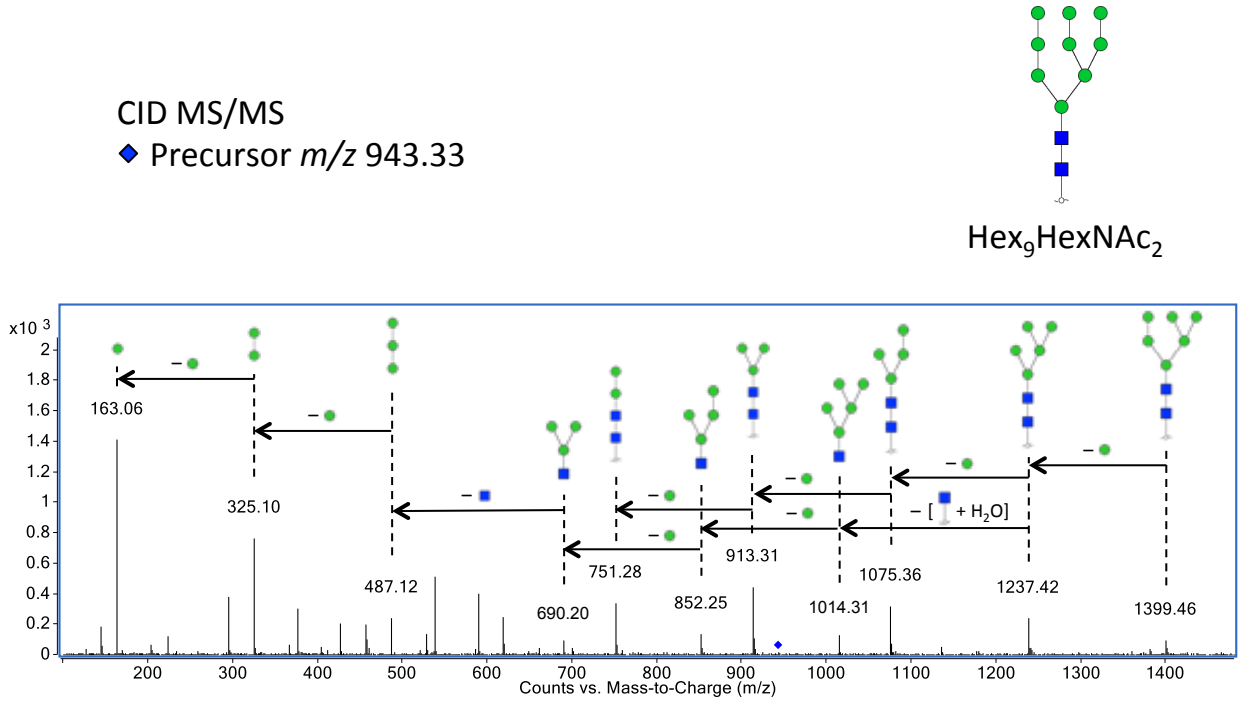

Hex6HexNAc6Fuc1NeuAc1
+ EIC(883.68)
x10 4
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
a
b
21.5
22
22.5
23
23.5
24
24.5
25
25.5
26
26.5
27
Counts vs. Acquisition Time (min)

Figure S4.

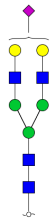
A


CID MS/MS
Precursor m/z 943.33
Hex9HexNAc2
x10 3
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
163.06
325.10
487.12
690.20
751.28
852.25
913.31
1014.31
1075.36
1237.42
1399.46
+ H2O]
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
Counts vs. Mass-to-Charge (m/z)

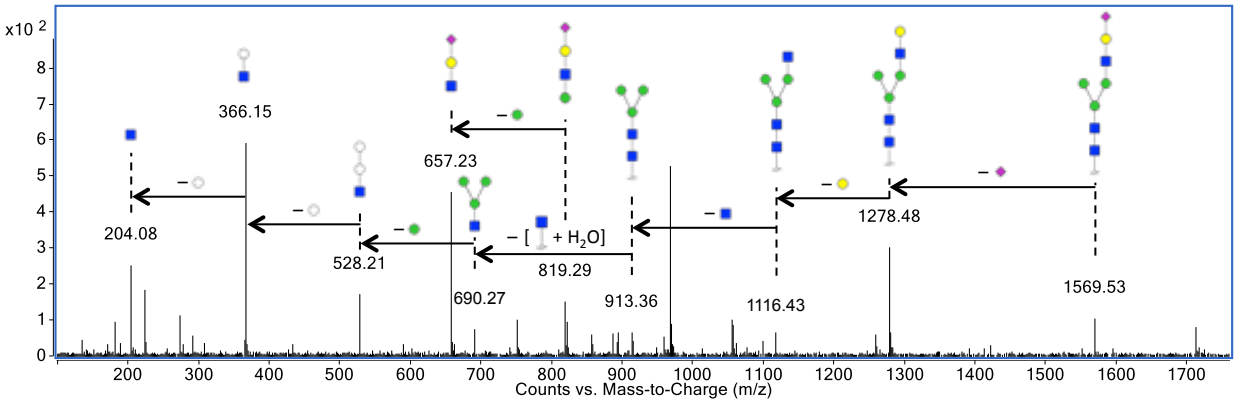
B

CID MS/MS

◆ Precursor *m/z* 967.87



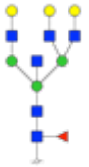
$Hex_5HexNAc_4NeuAc_1$



C

CID MS/MS

◆ Precursor *m/z* 786.64



$Hex_6HexNAc_6Fuc_1$

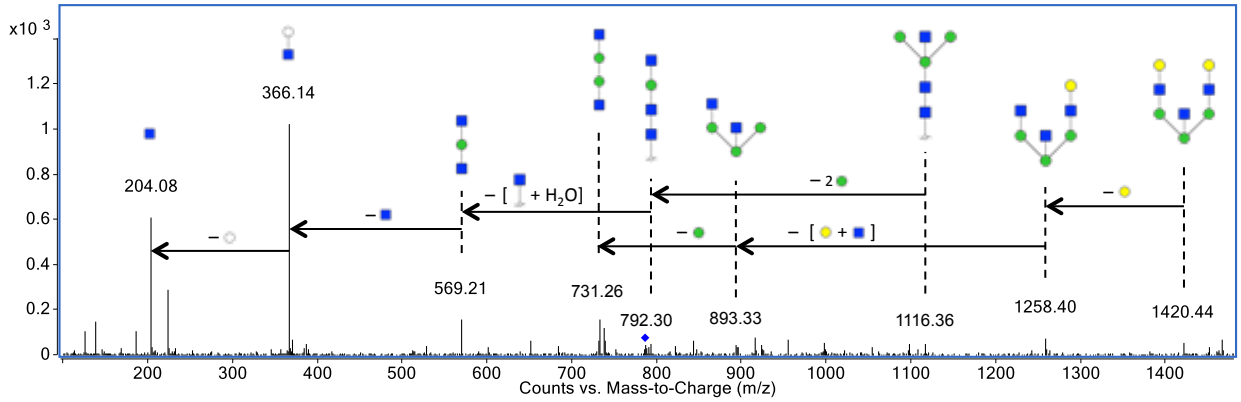
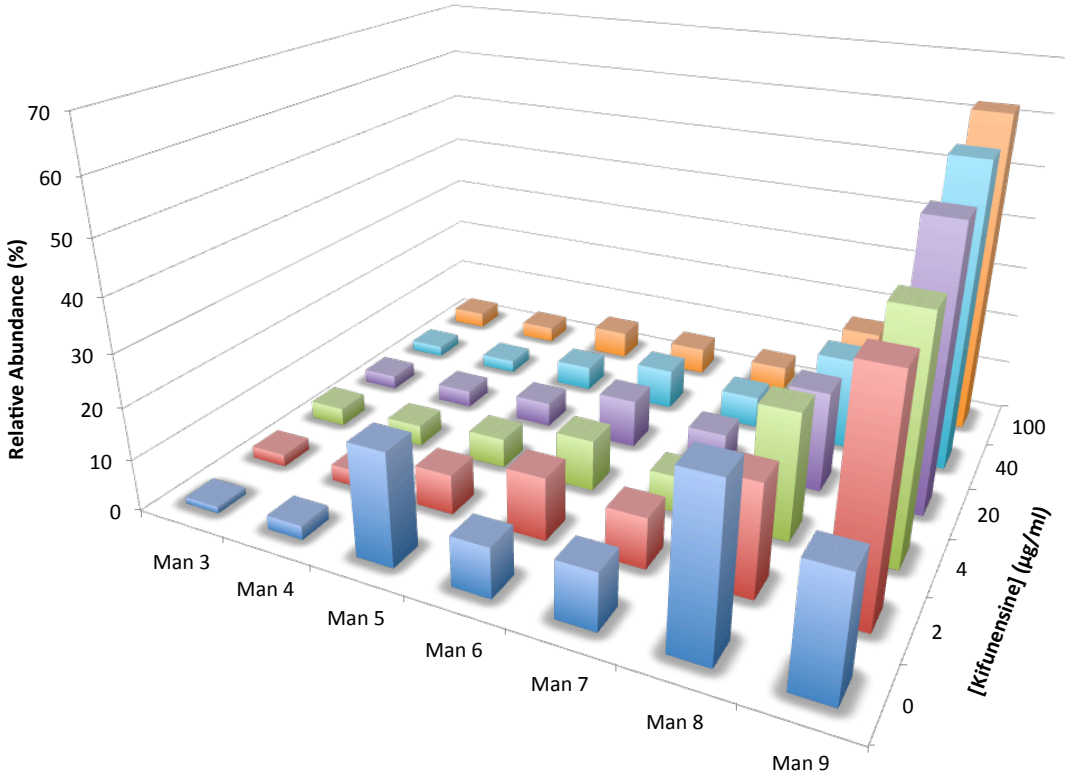


Figure S5.


Relative Abundance (%)
70
60
50
40
30
20
10
0
Man 3
Man 4
Man 5
Man 6
Man 7
Man 8
Man 9
100
40
20
4
2
0
[kifunensine] (µg/ml)