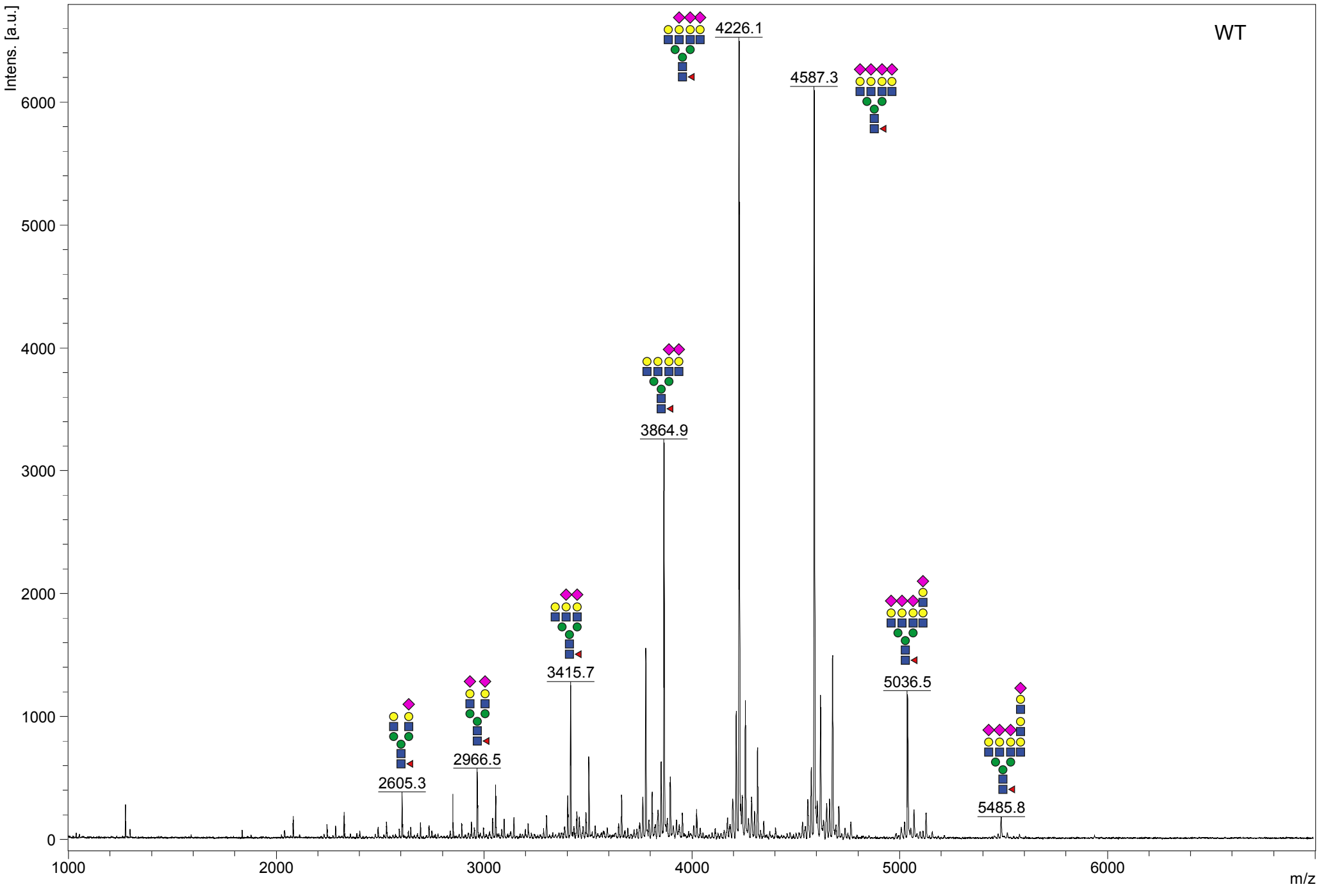
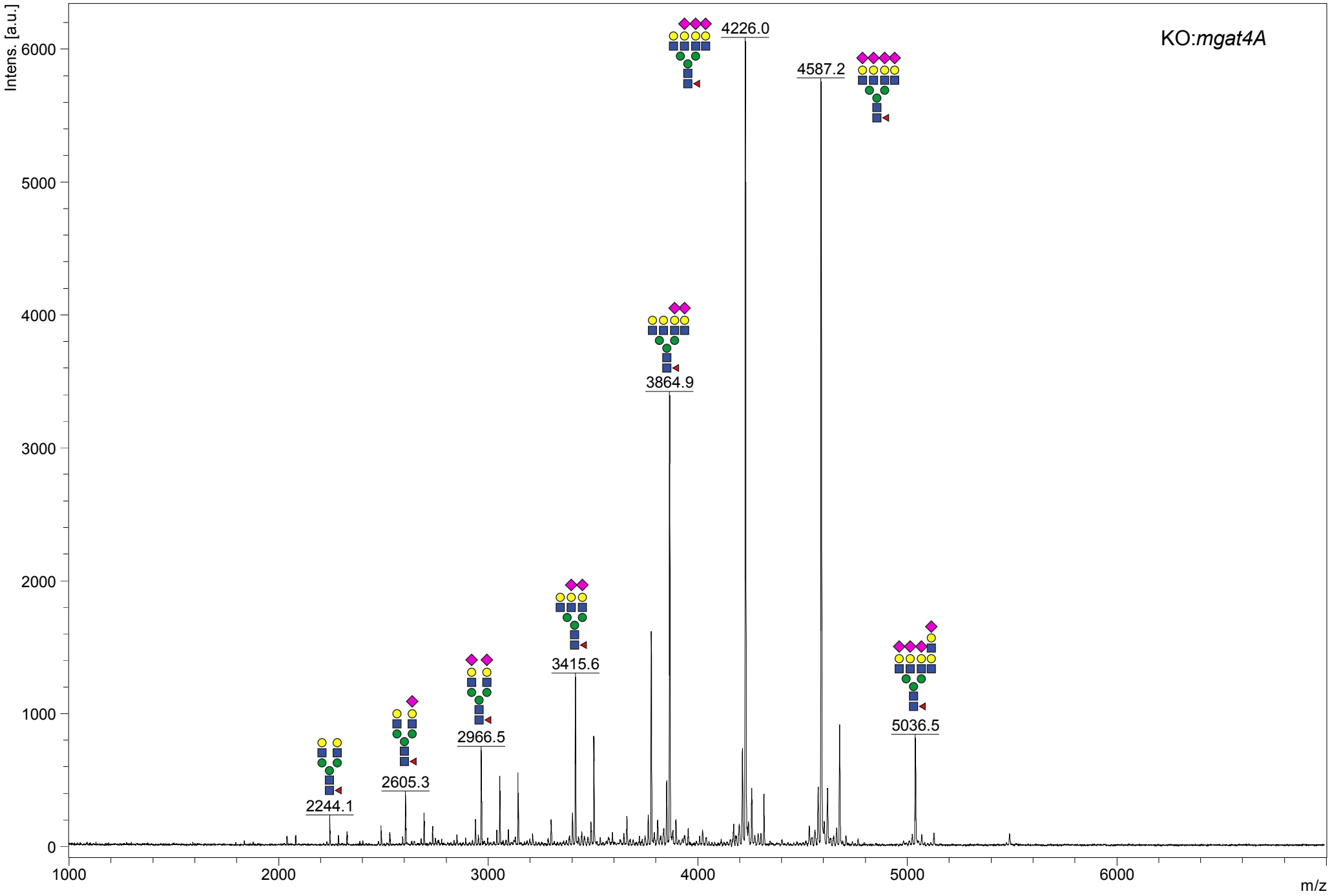
Supplementary Spectra

MALDI-TOF spectra of PNGase F released permethylated N-glycans with predicted structures for EPO (spectra #1-34) and IgG (spectra #35-38) samples.

#1

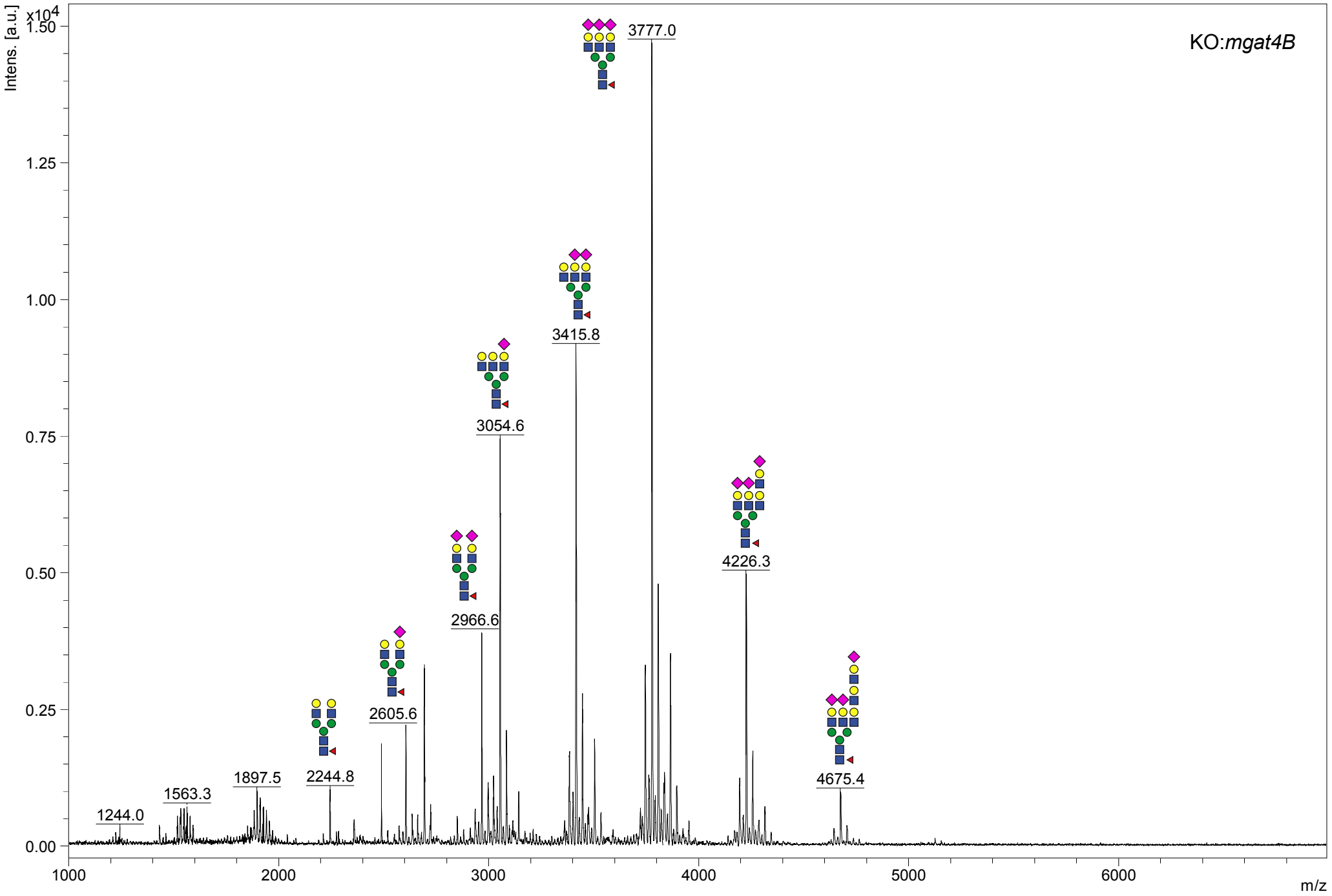


Supplementary Spectra
#2



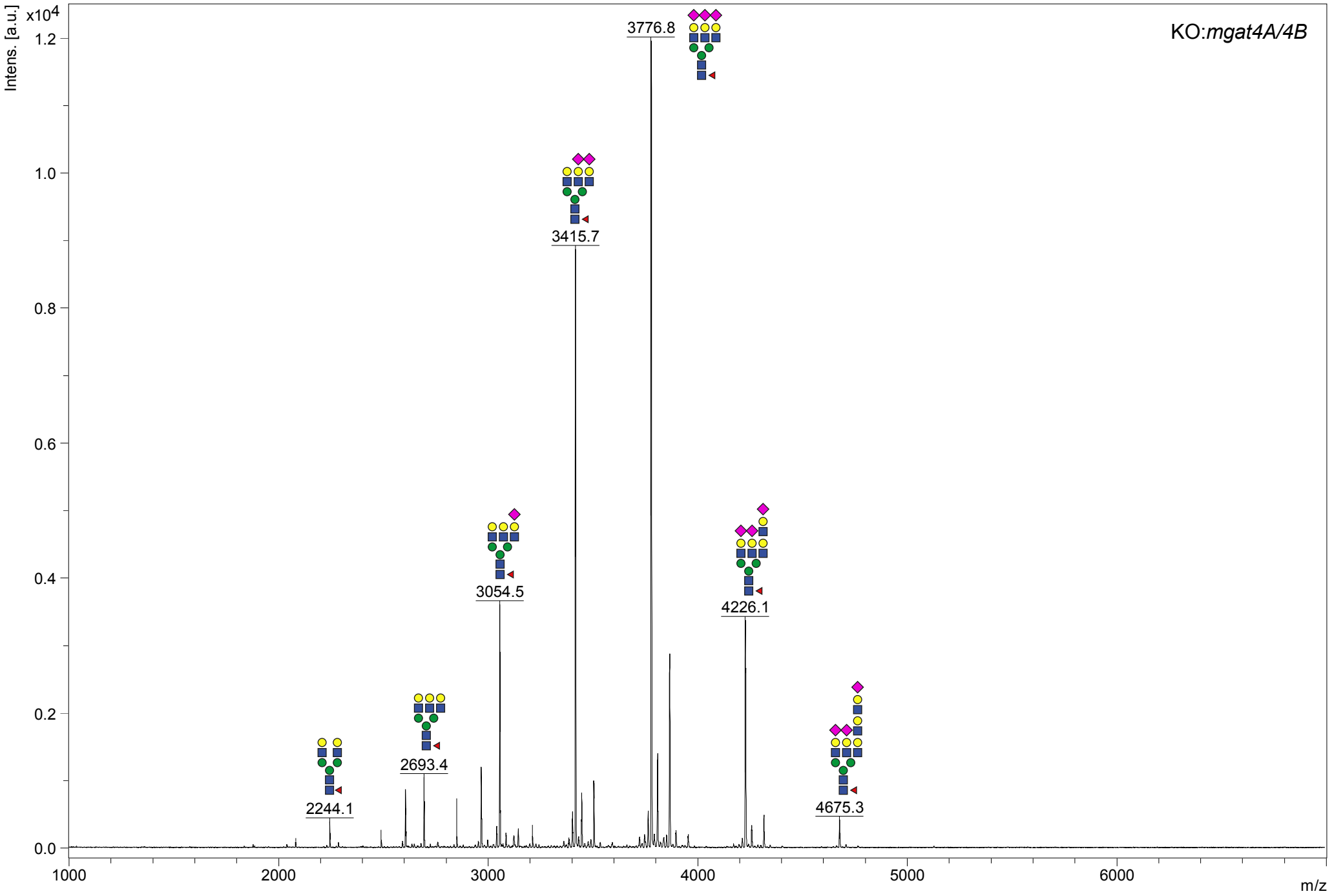
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#3



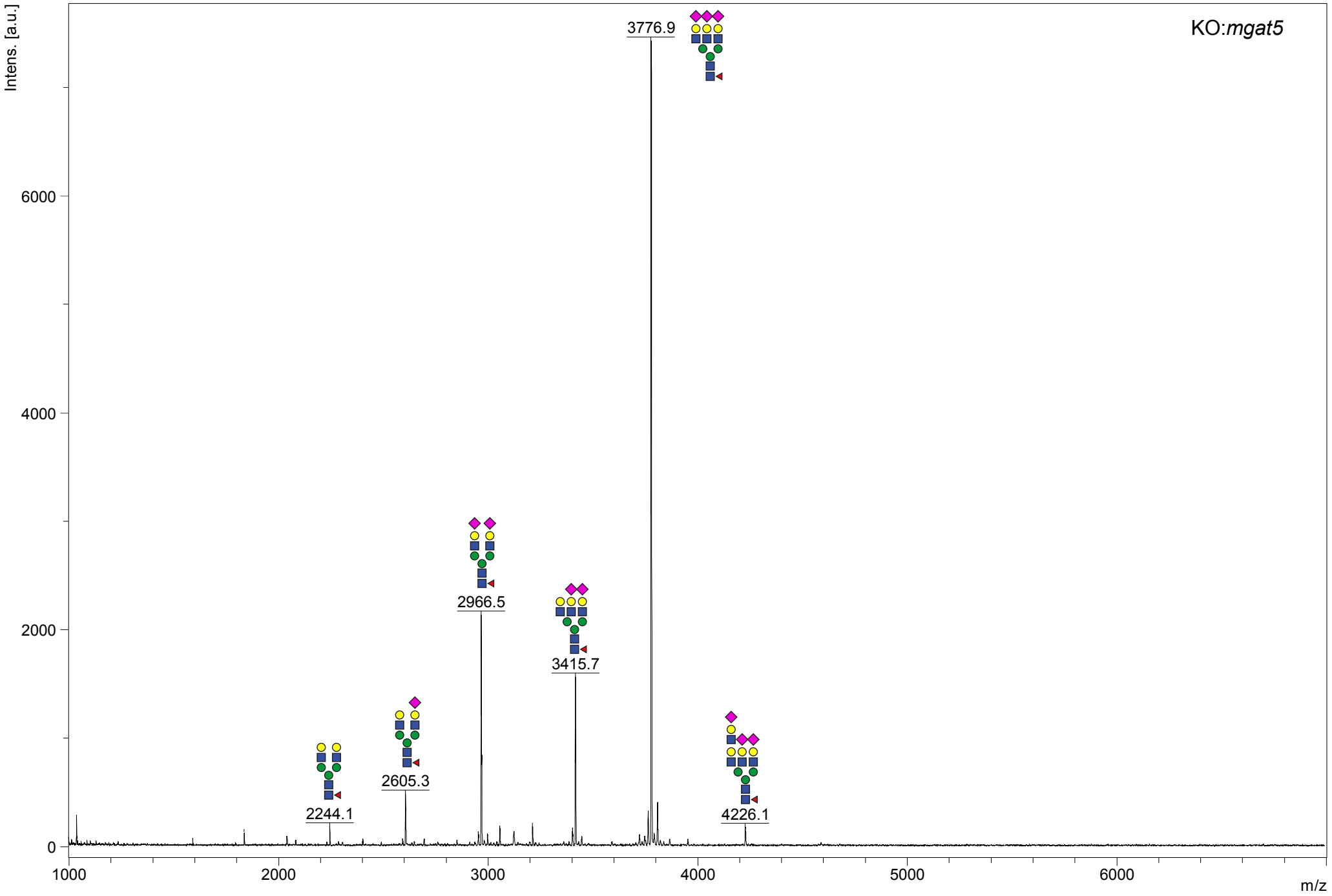
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#4



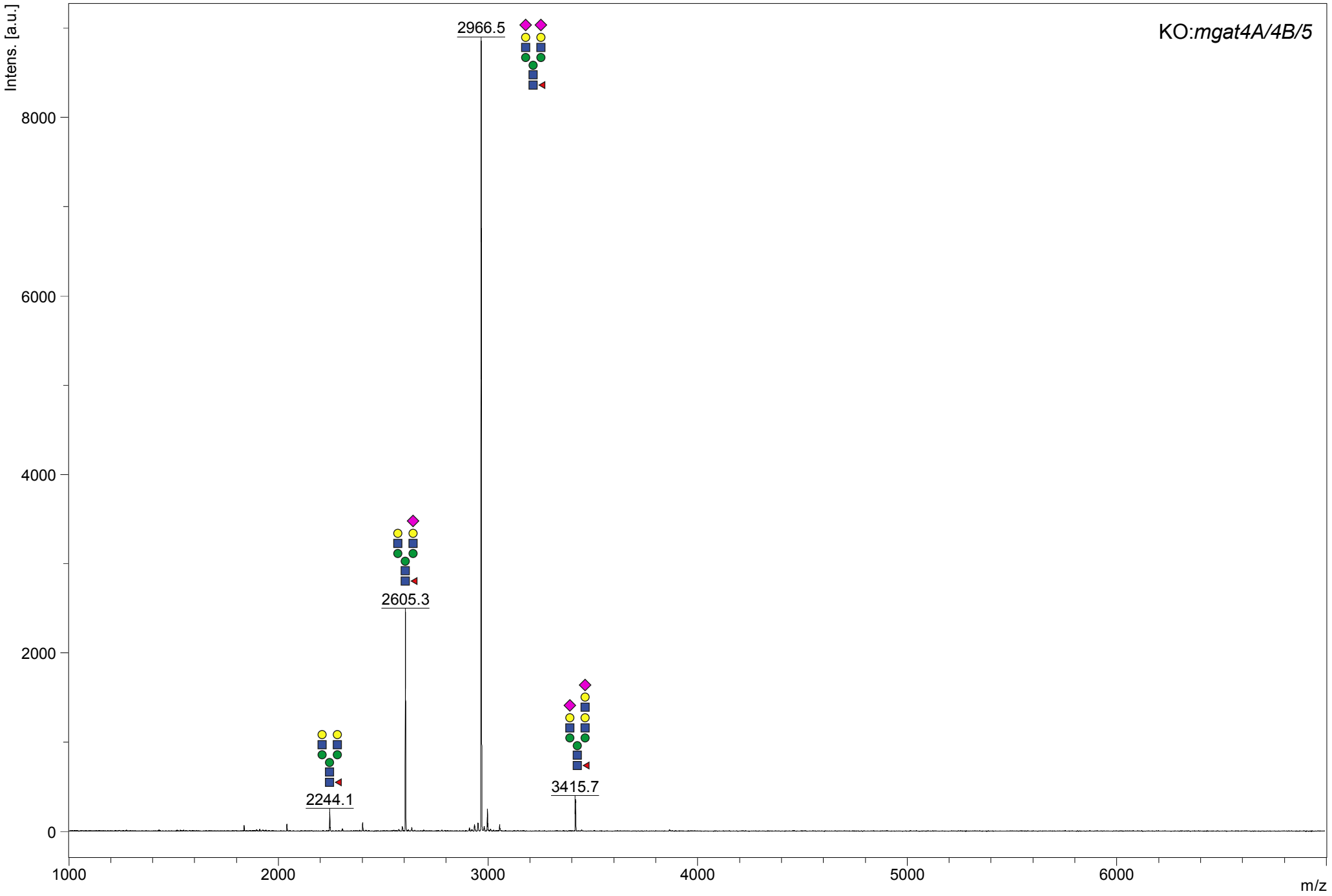
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#5



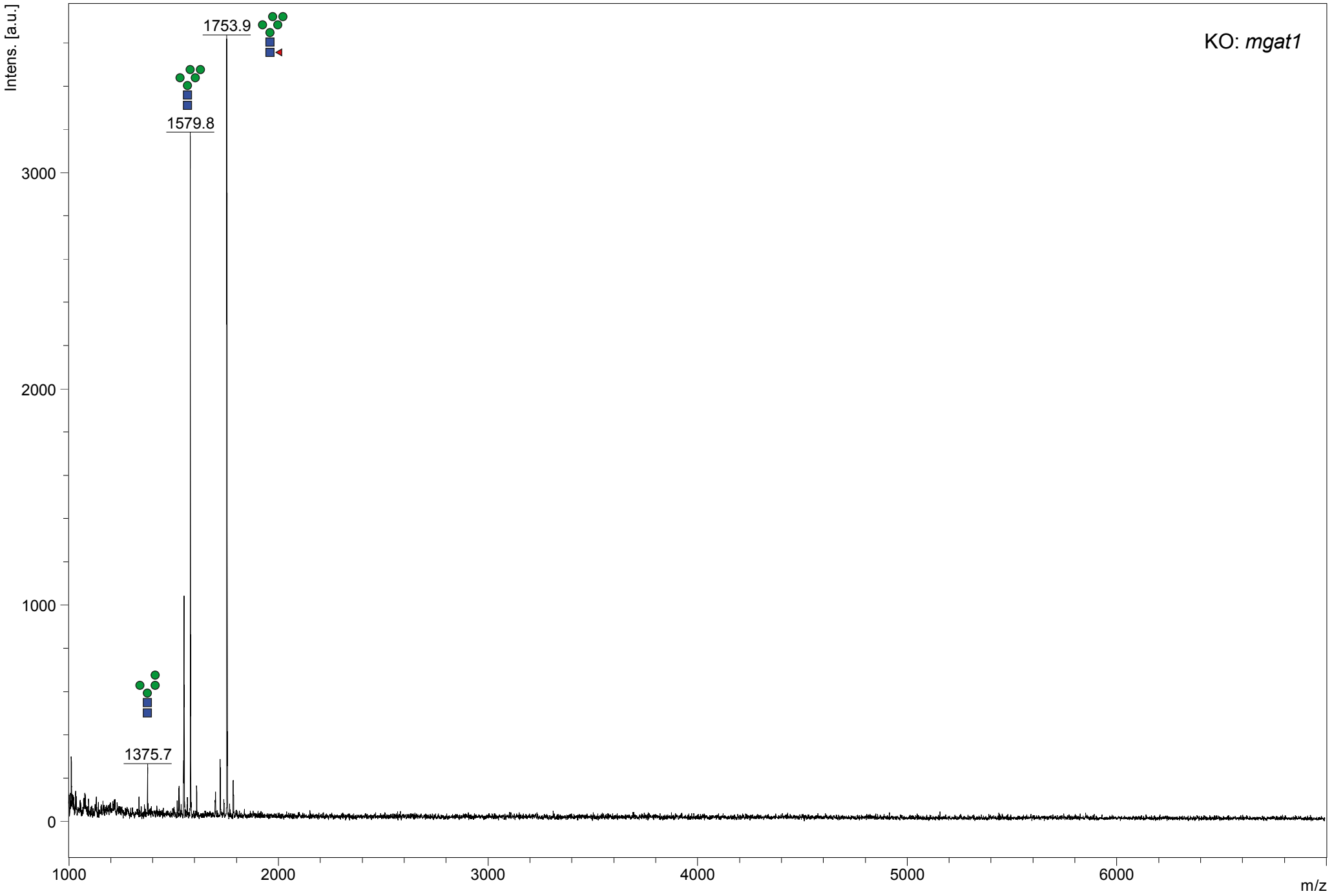
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#6



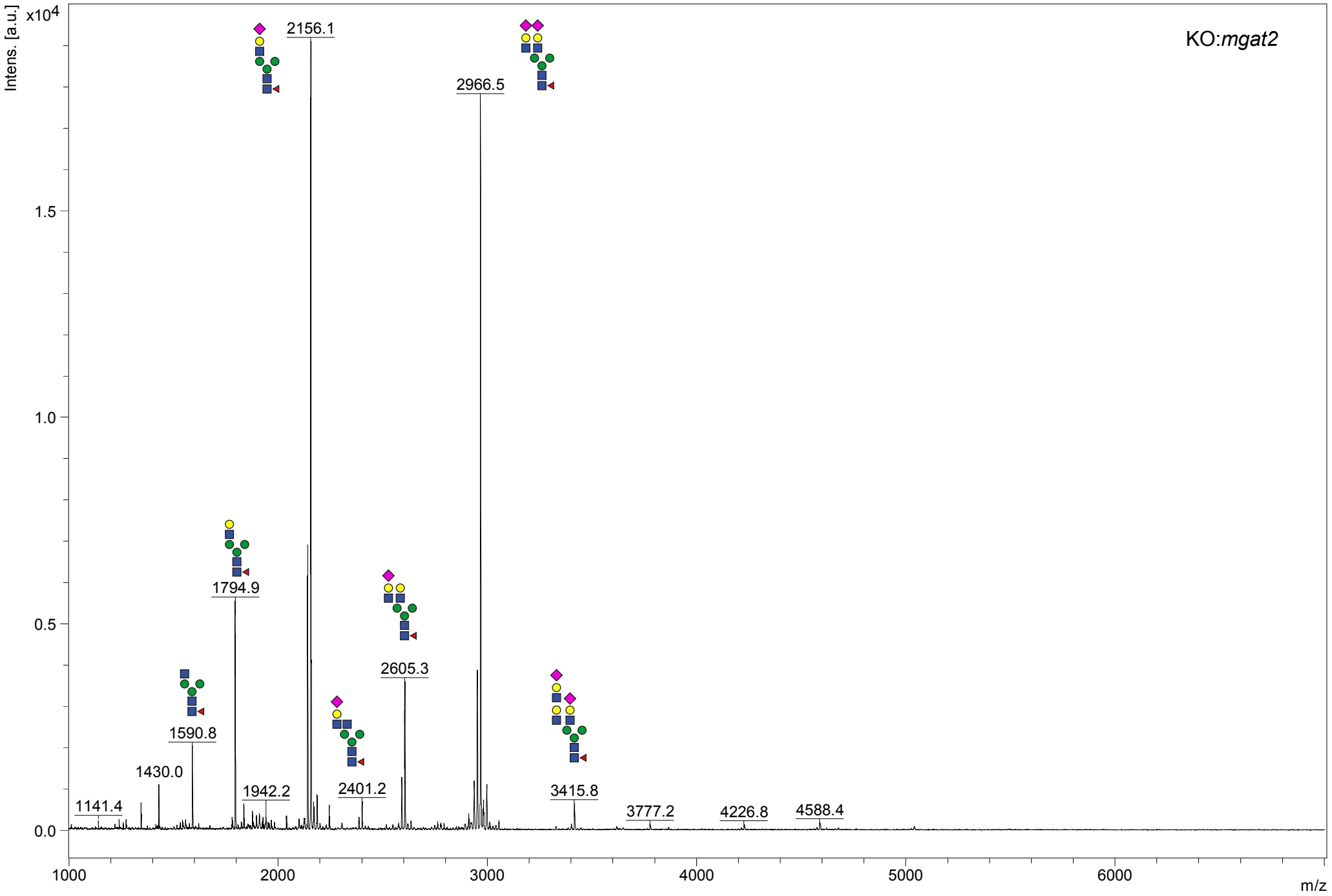
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#7



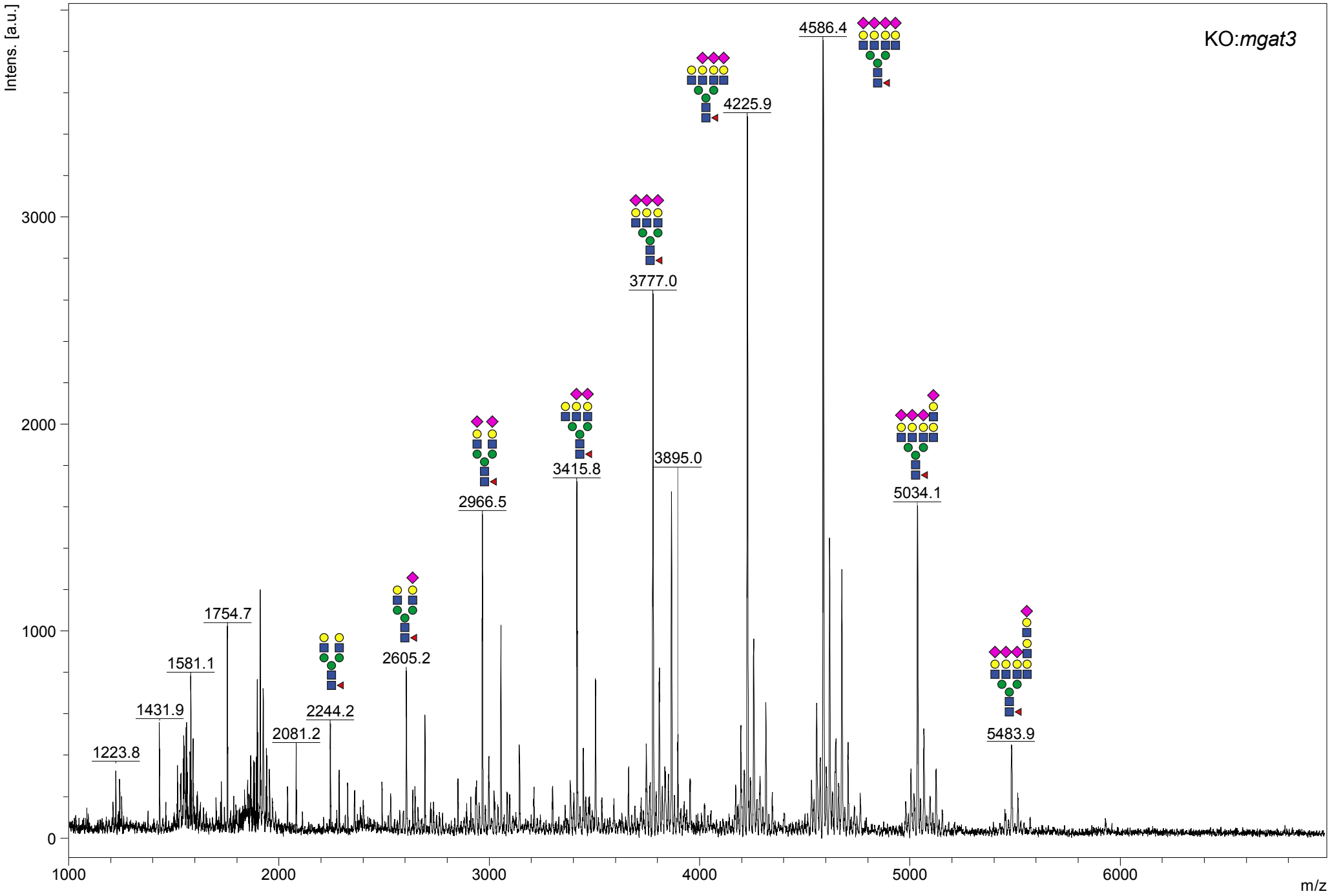
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#8



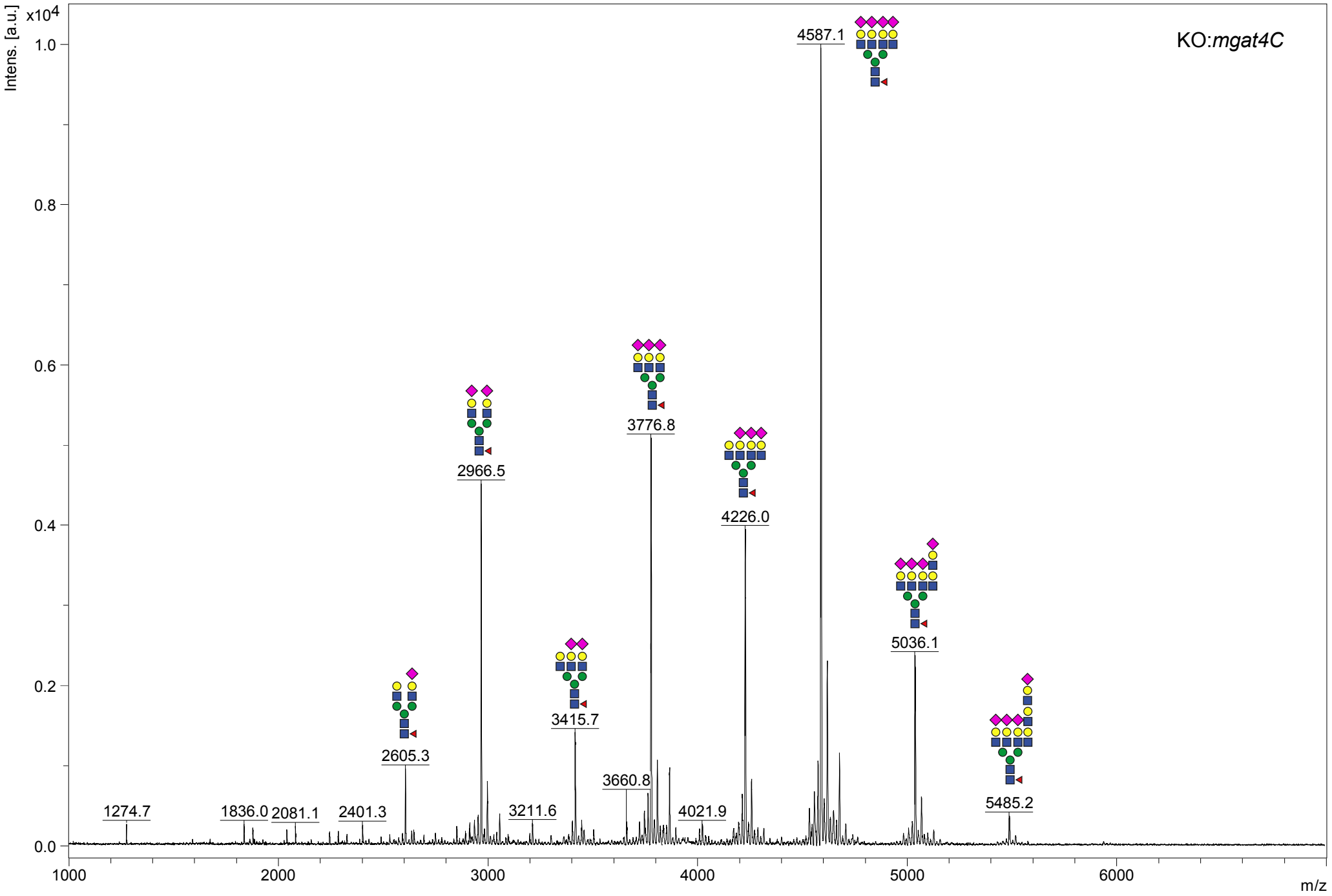
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#9



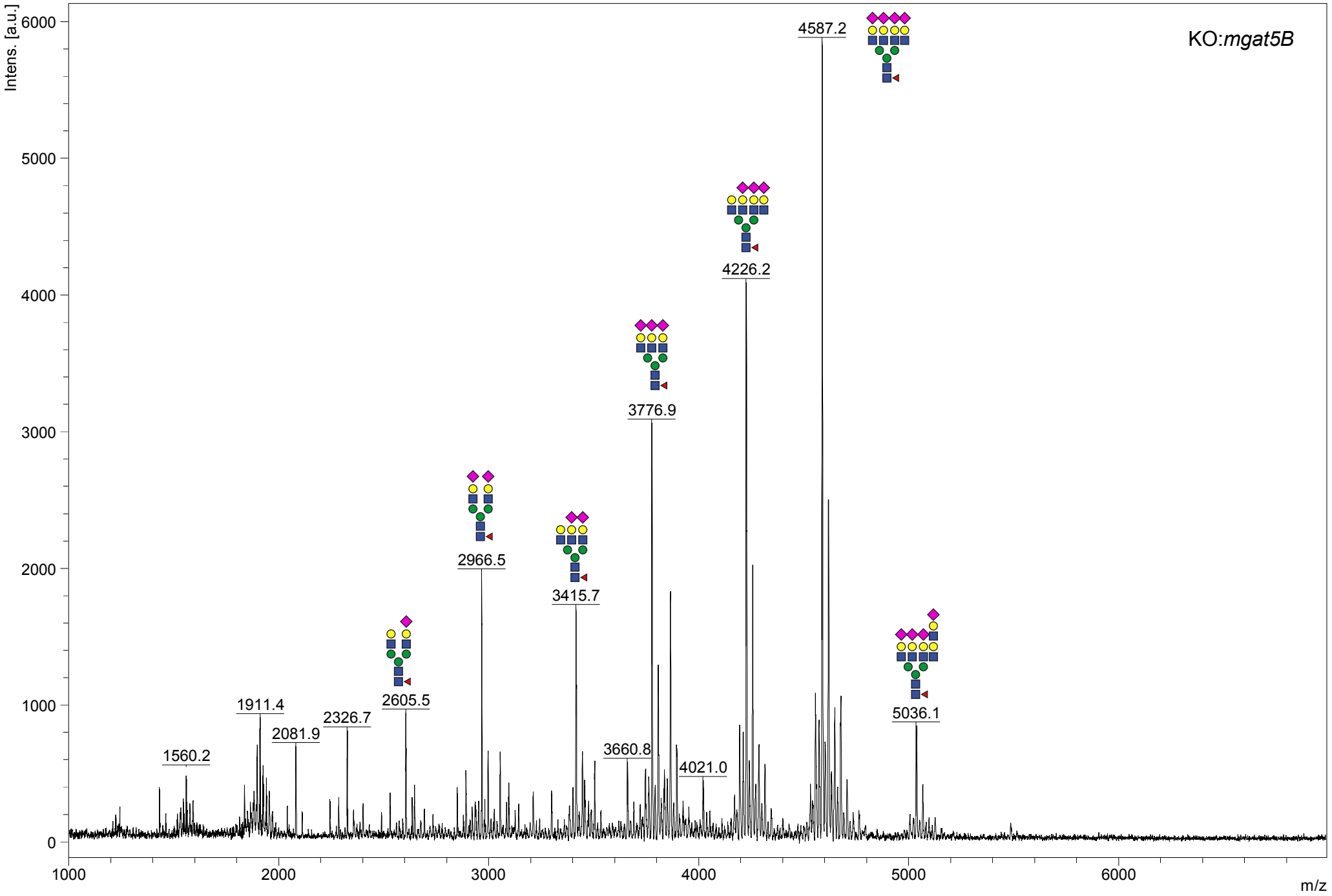
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#10



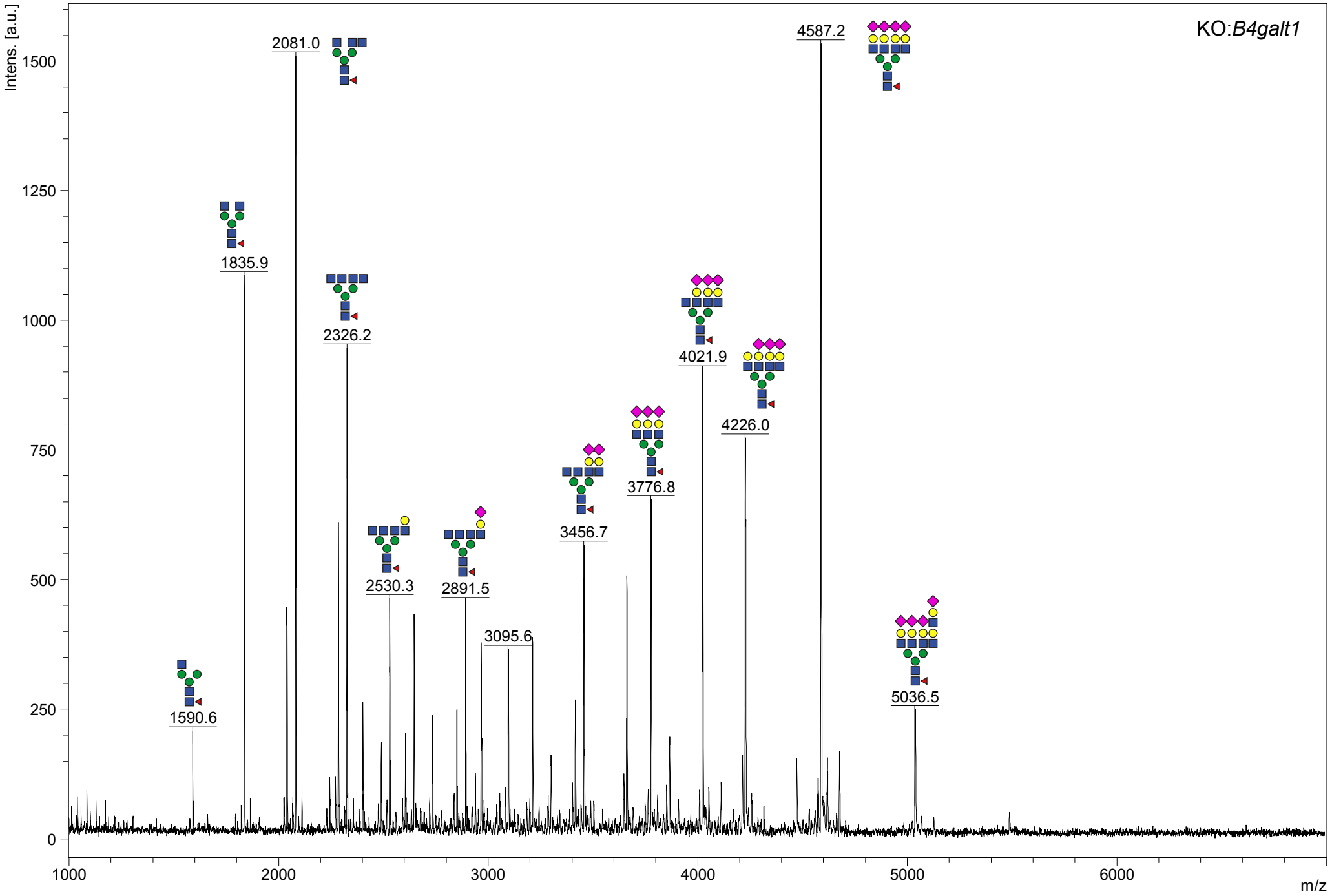
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#11

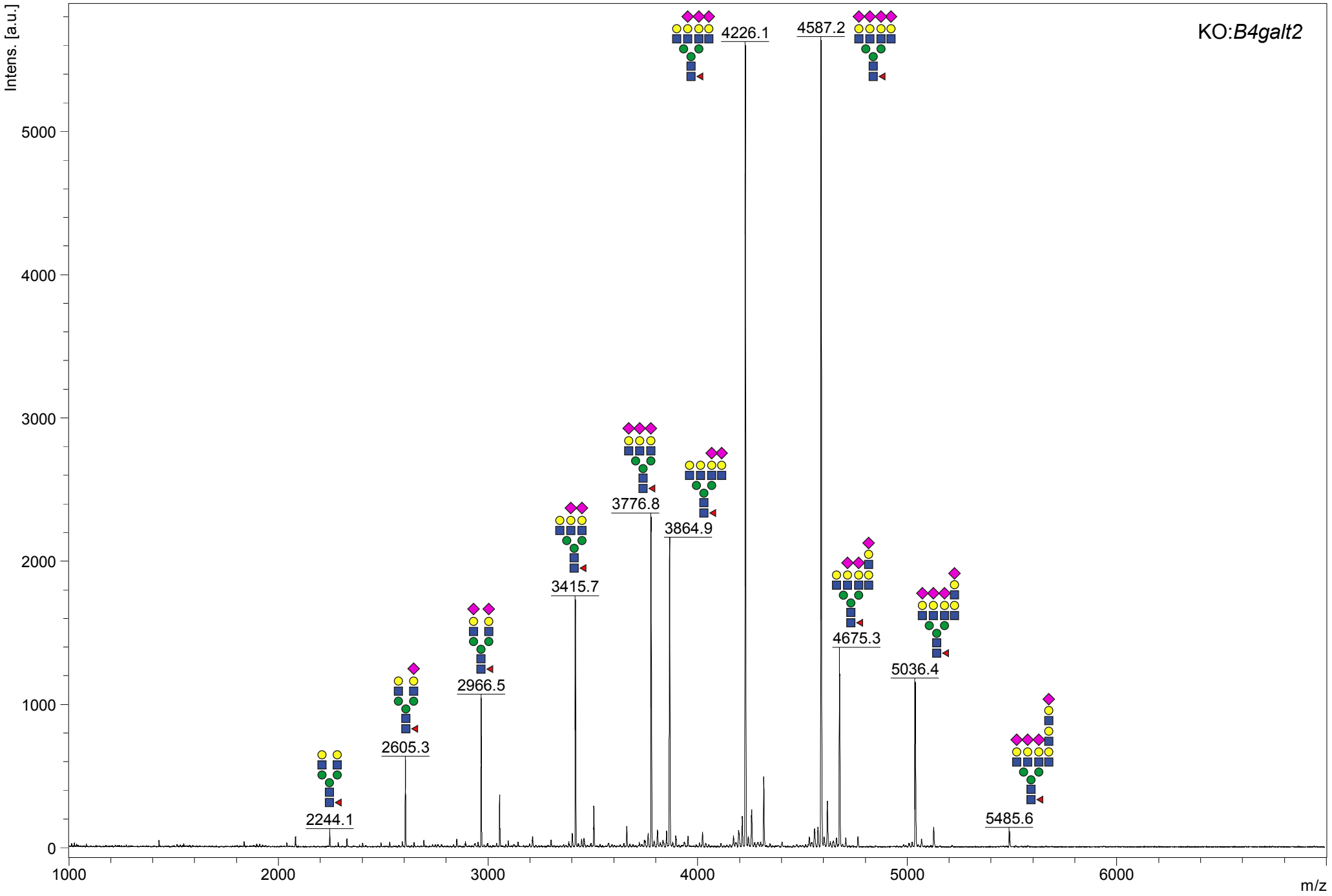


Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#12

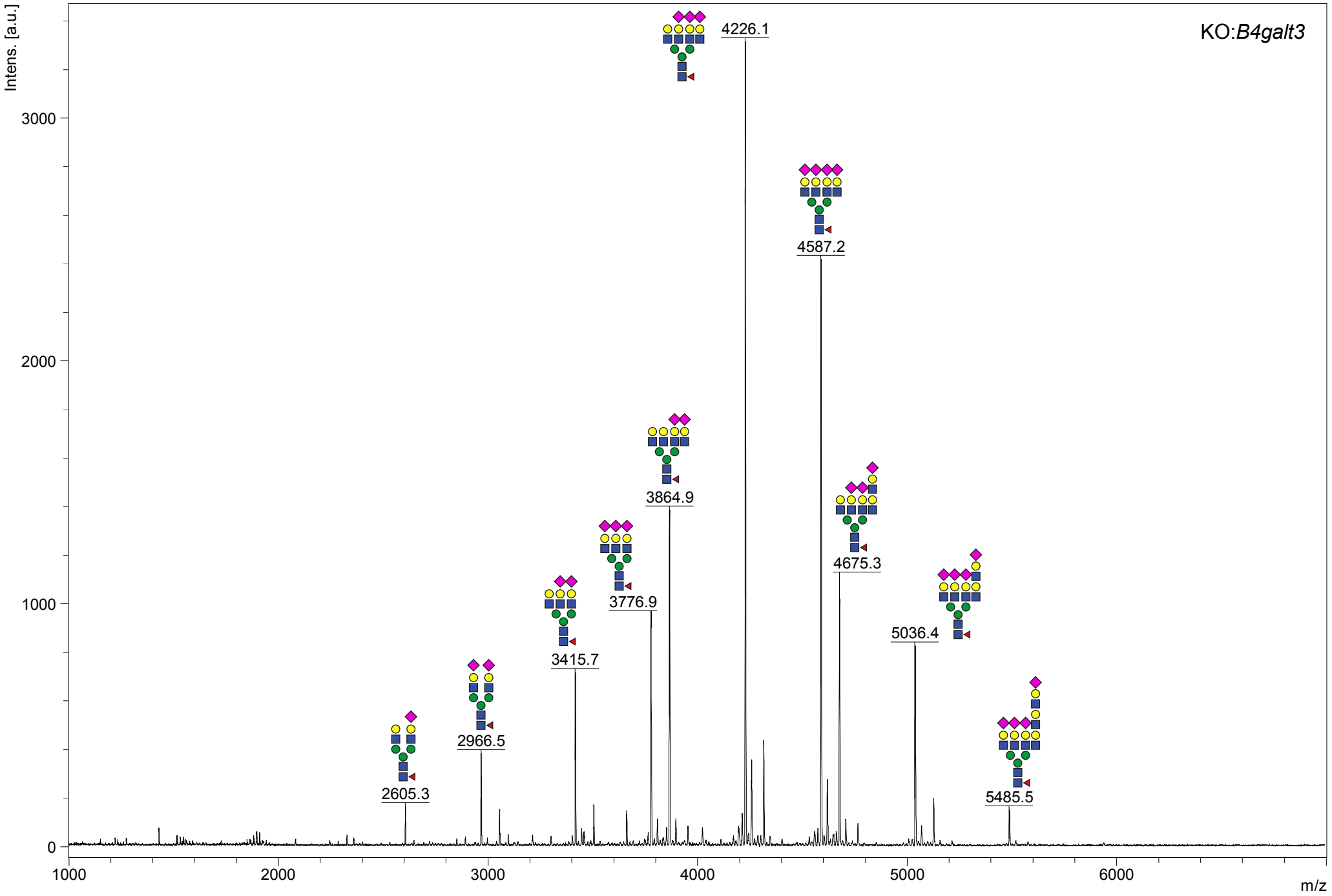


Nature Biotechnology: doi:10.1038/nbt.3280



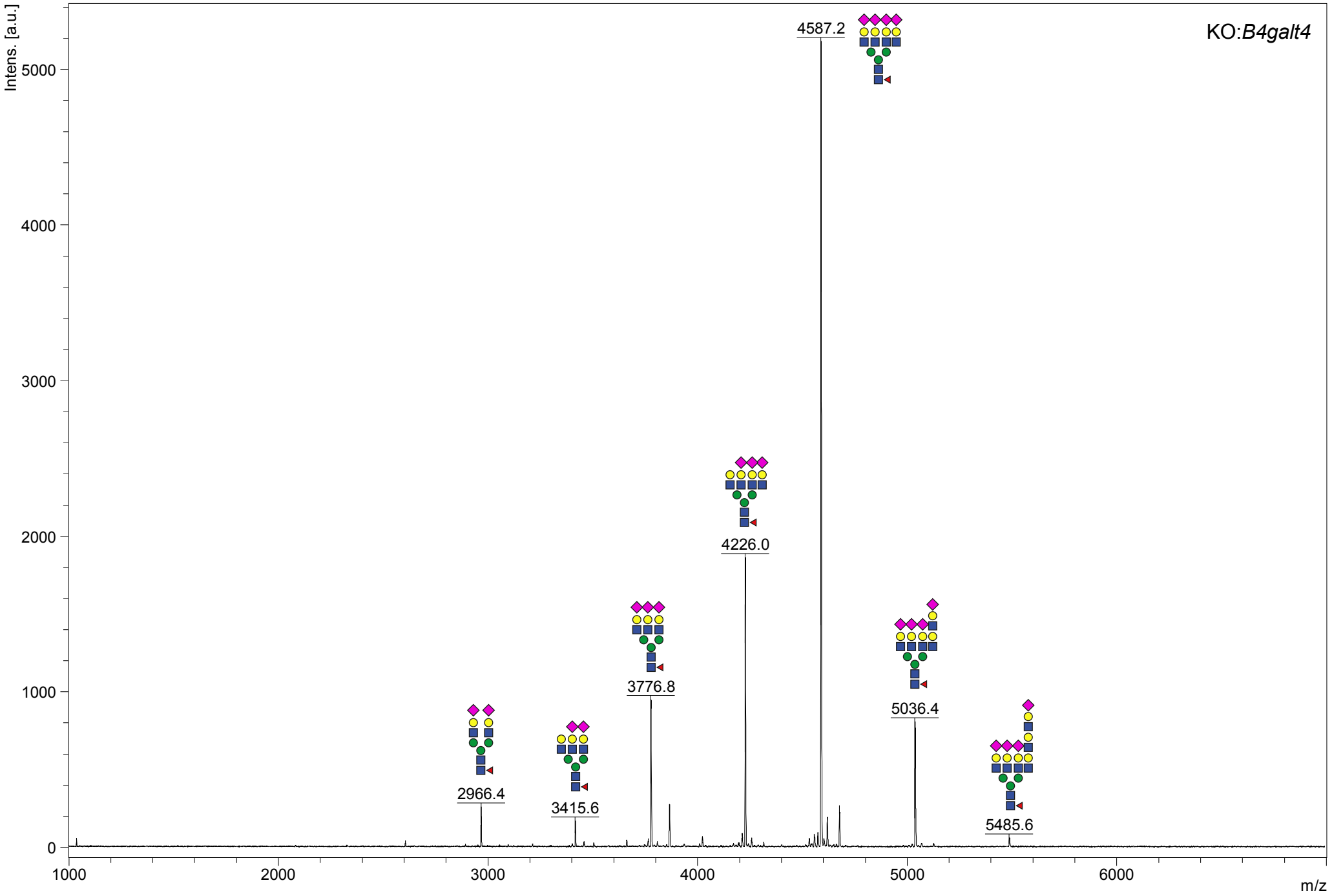
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#14



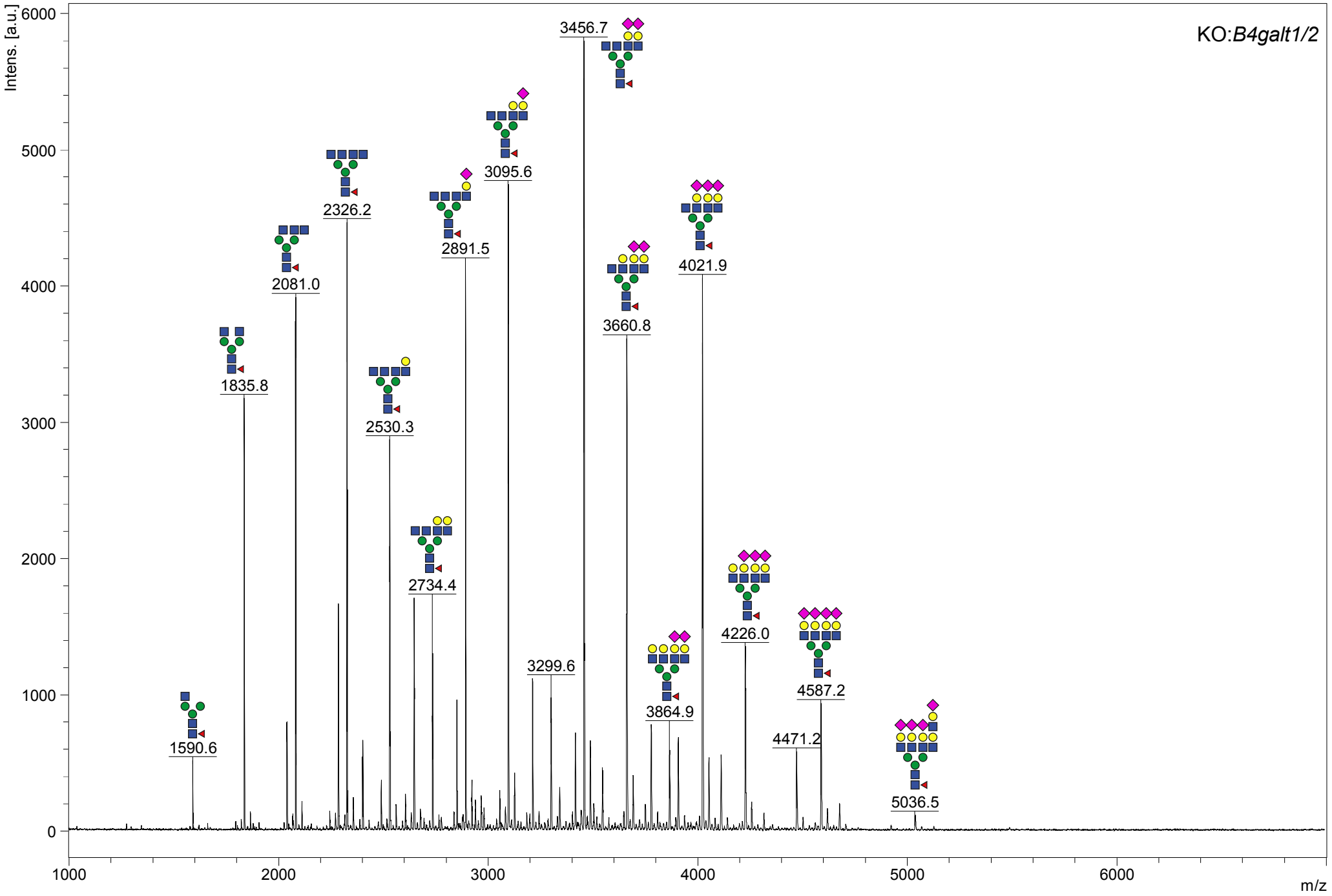
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#15



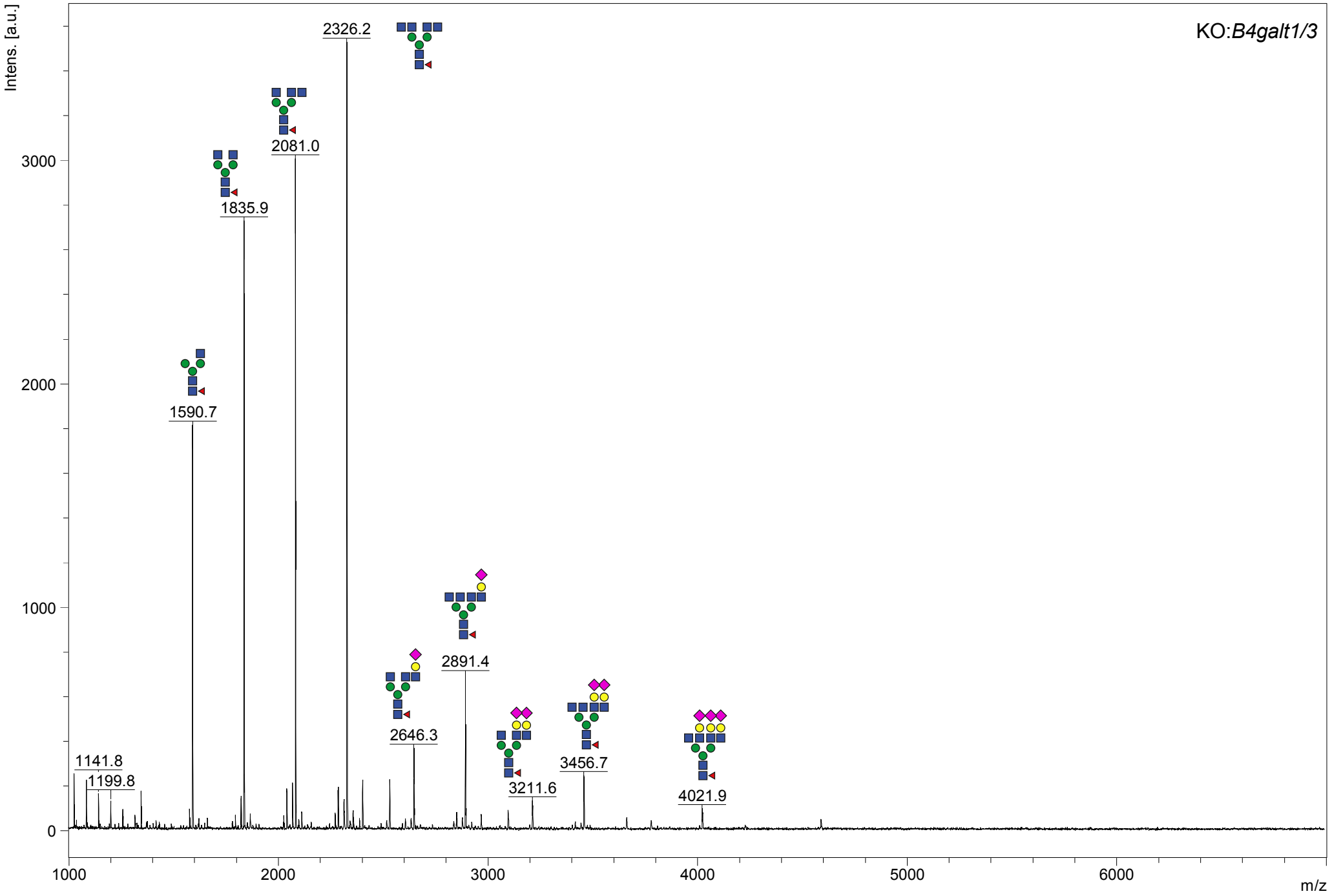
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#16



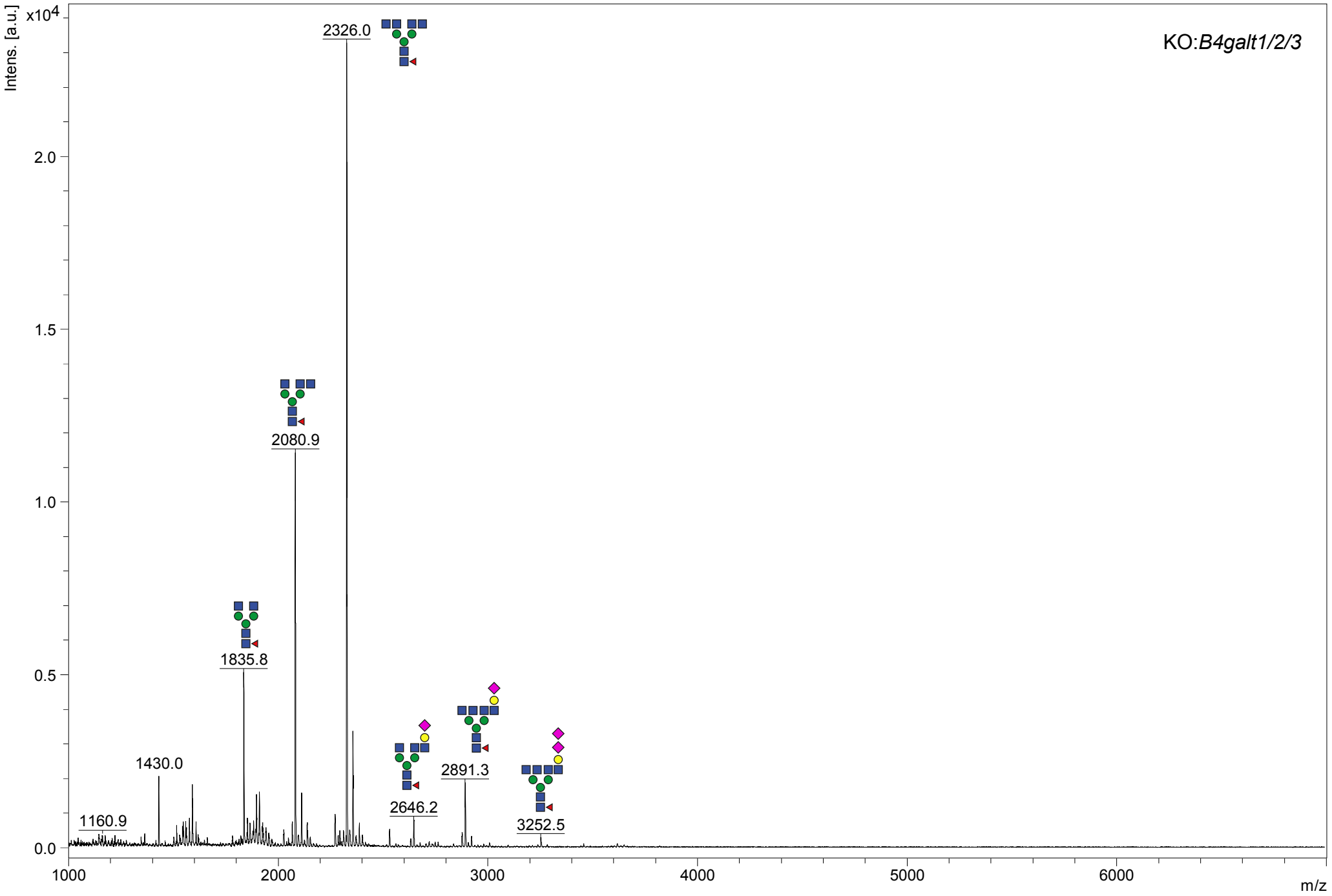
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#17



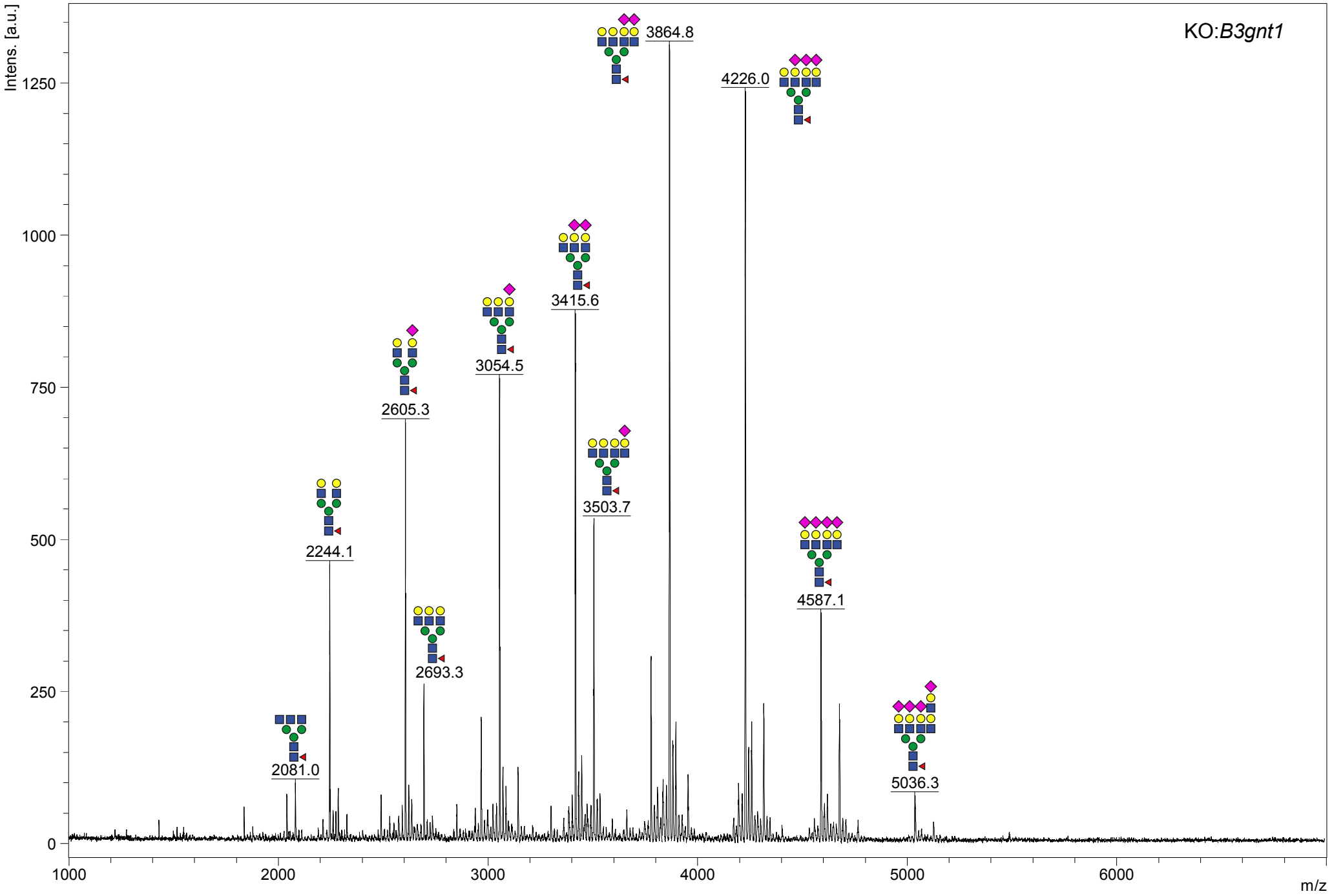
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#18

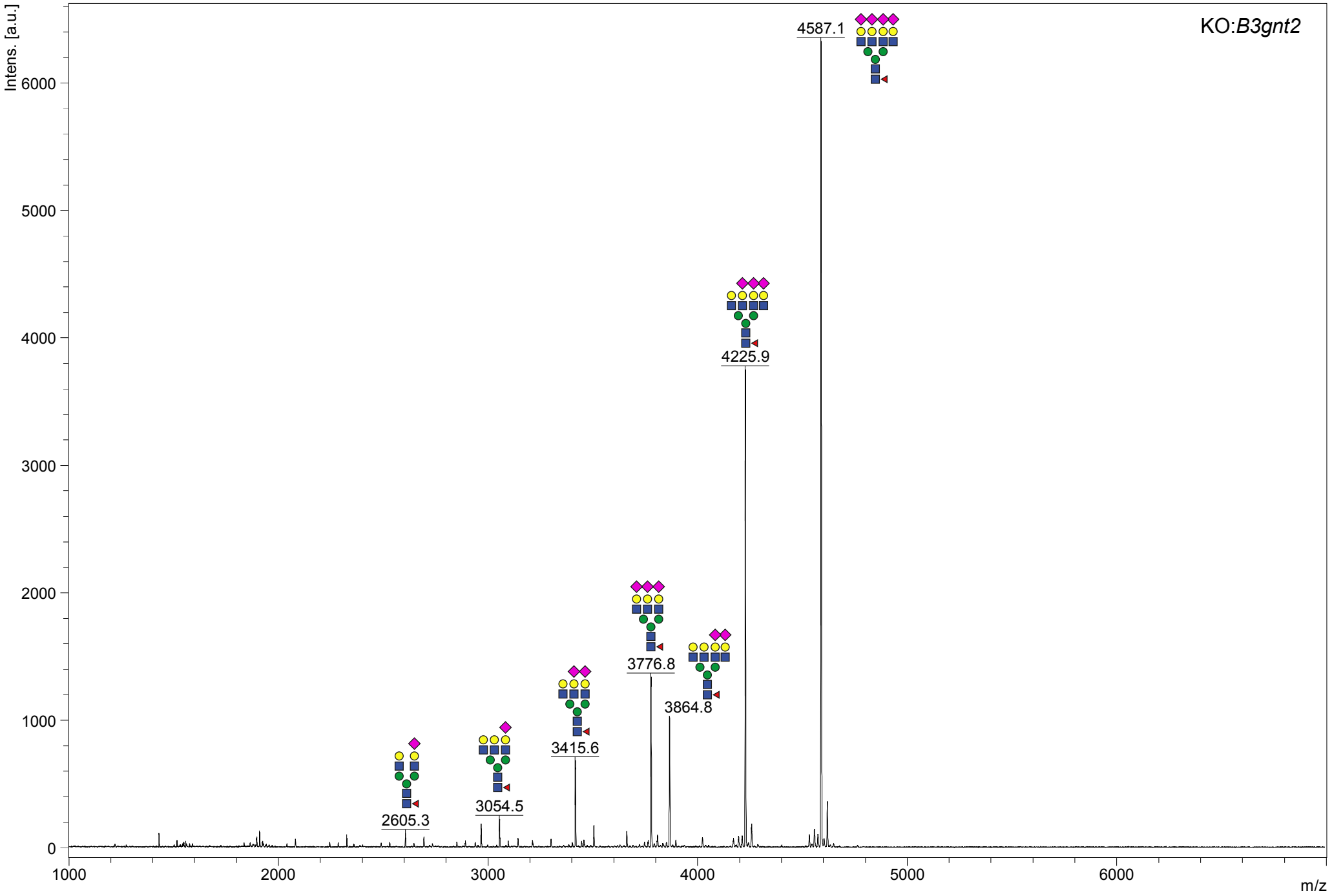


Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#19

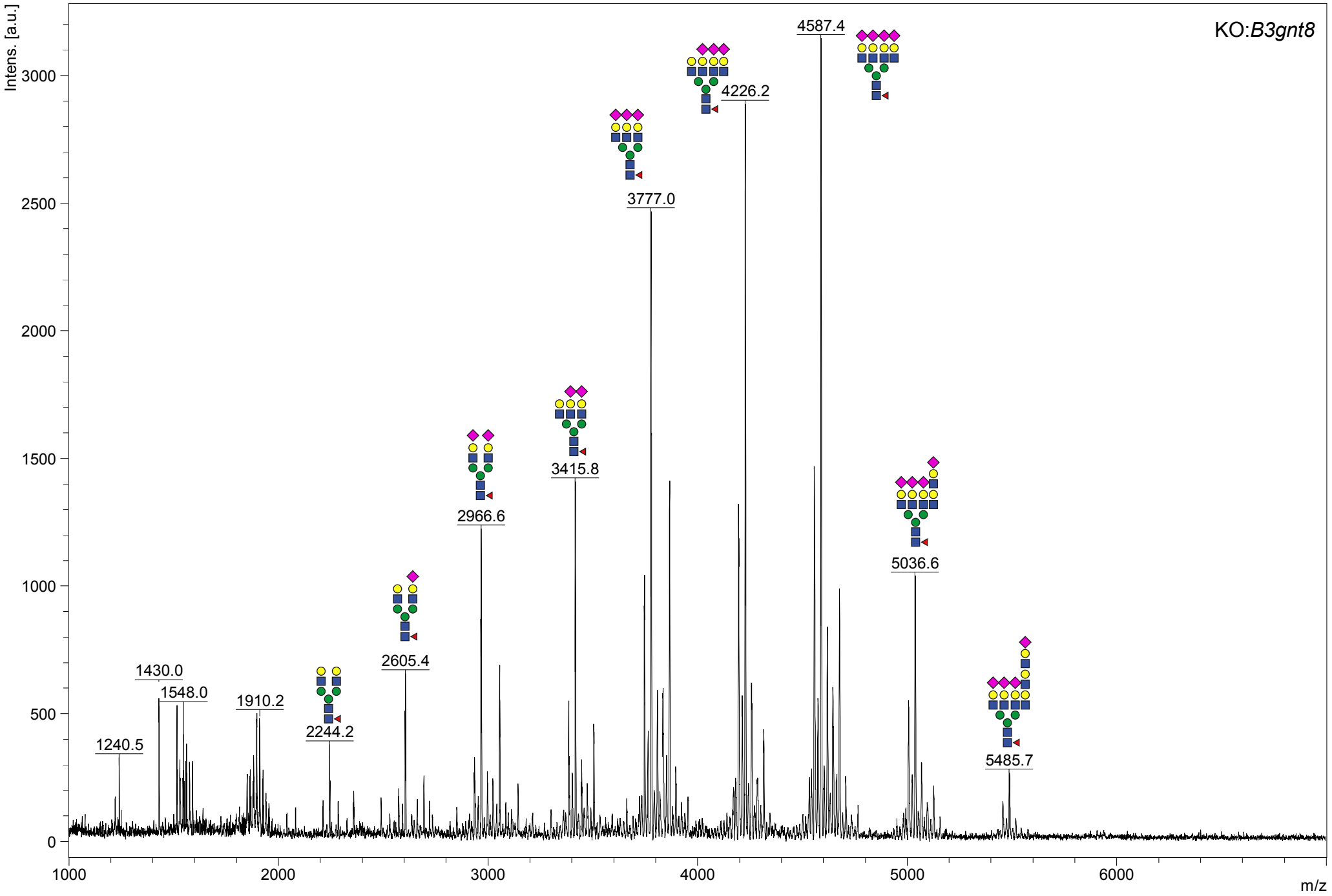


Nature Biotechnology: doi:10.1038/nbt.3280



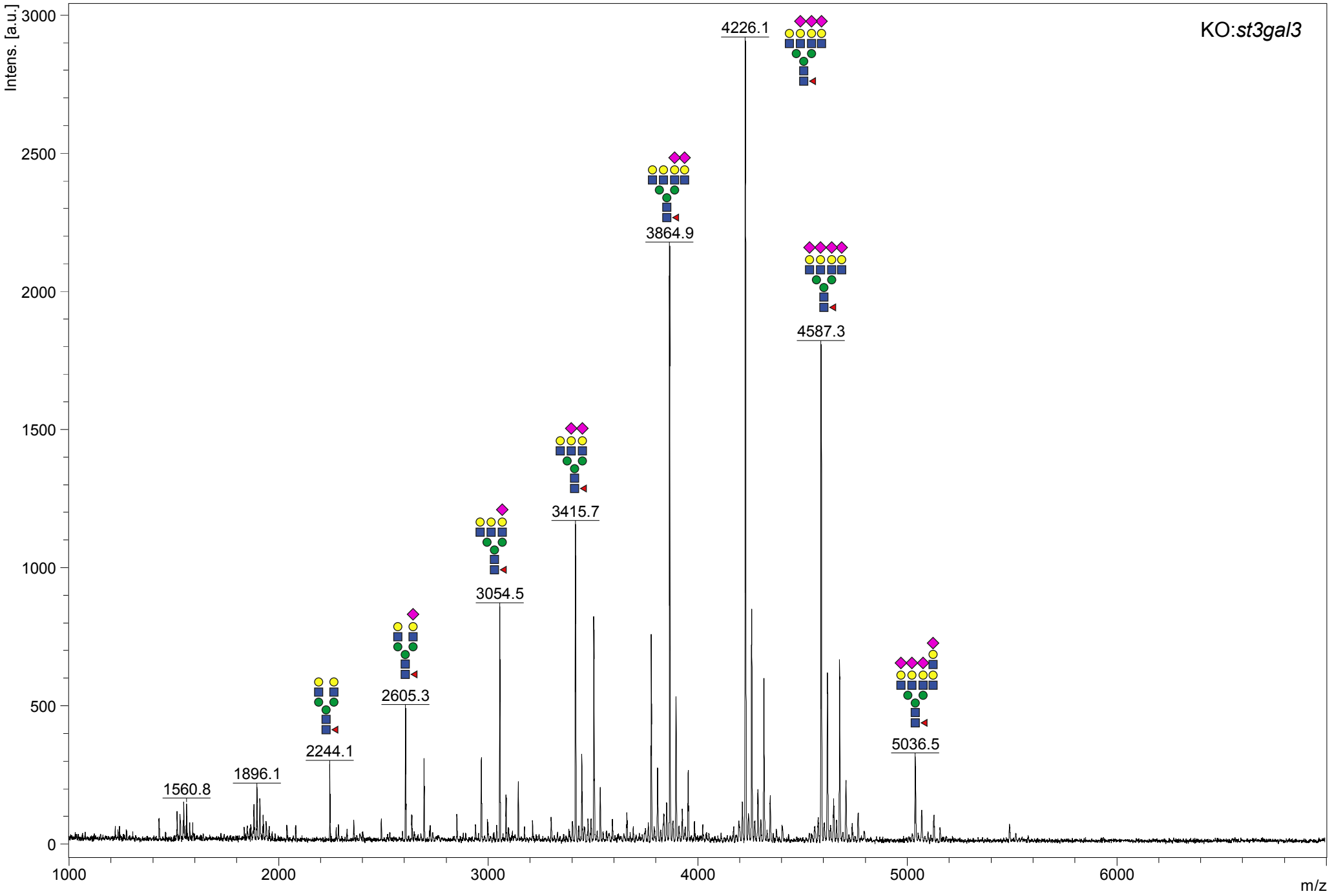
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#21



Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#22



Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
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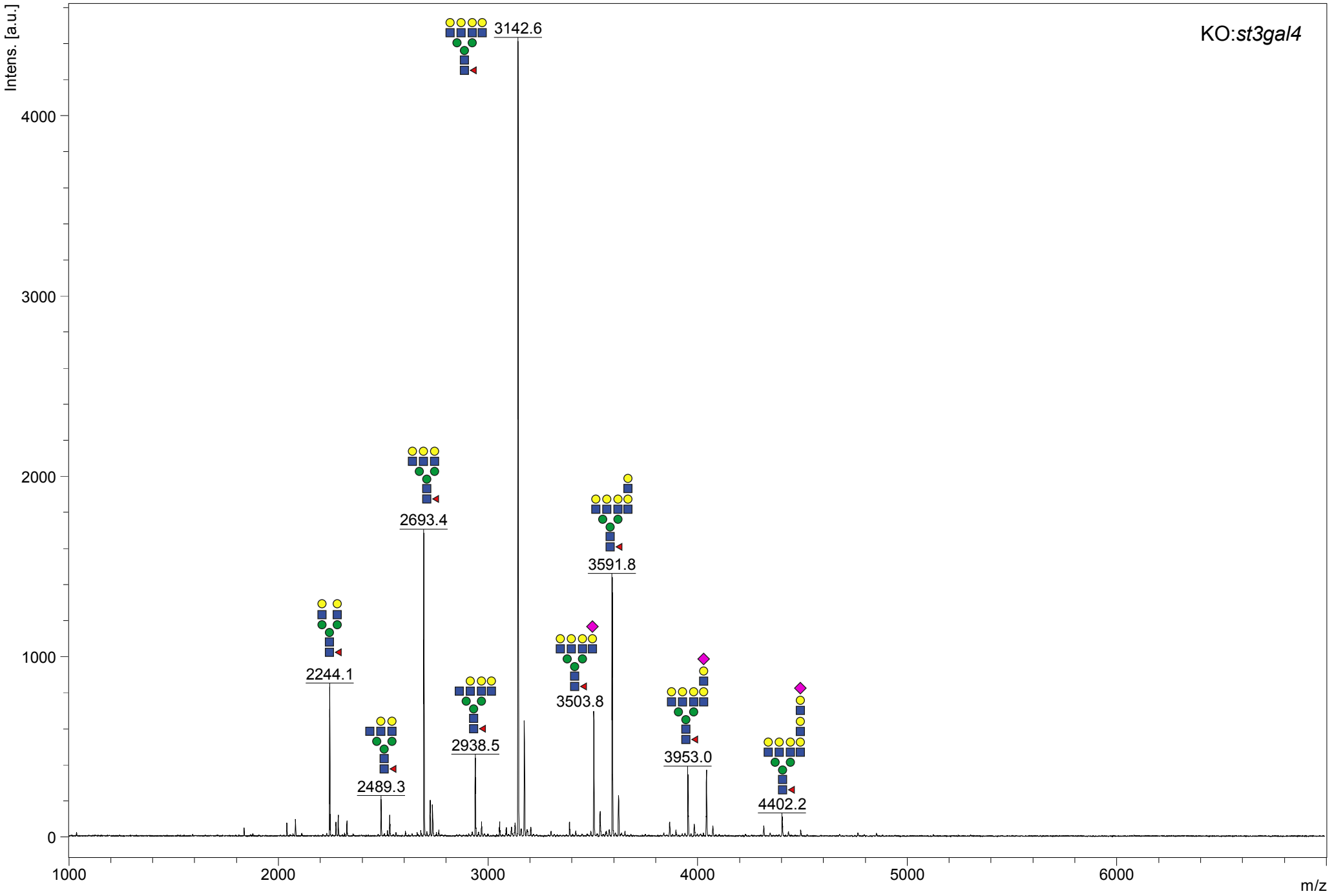
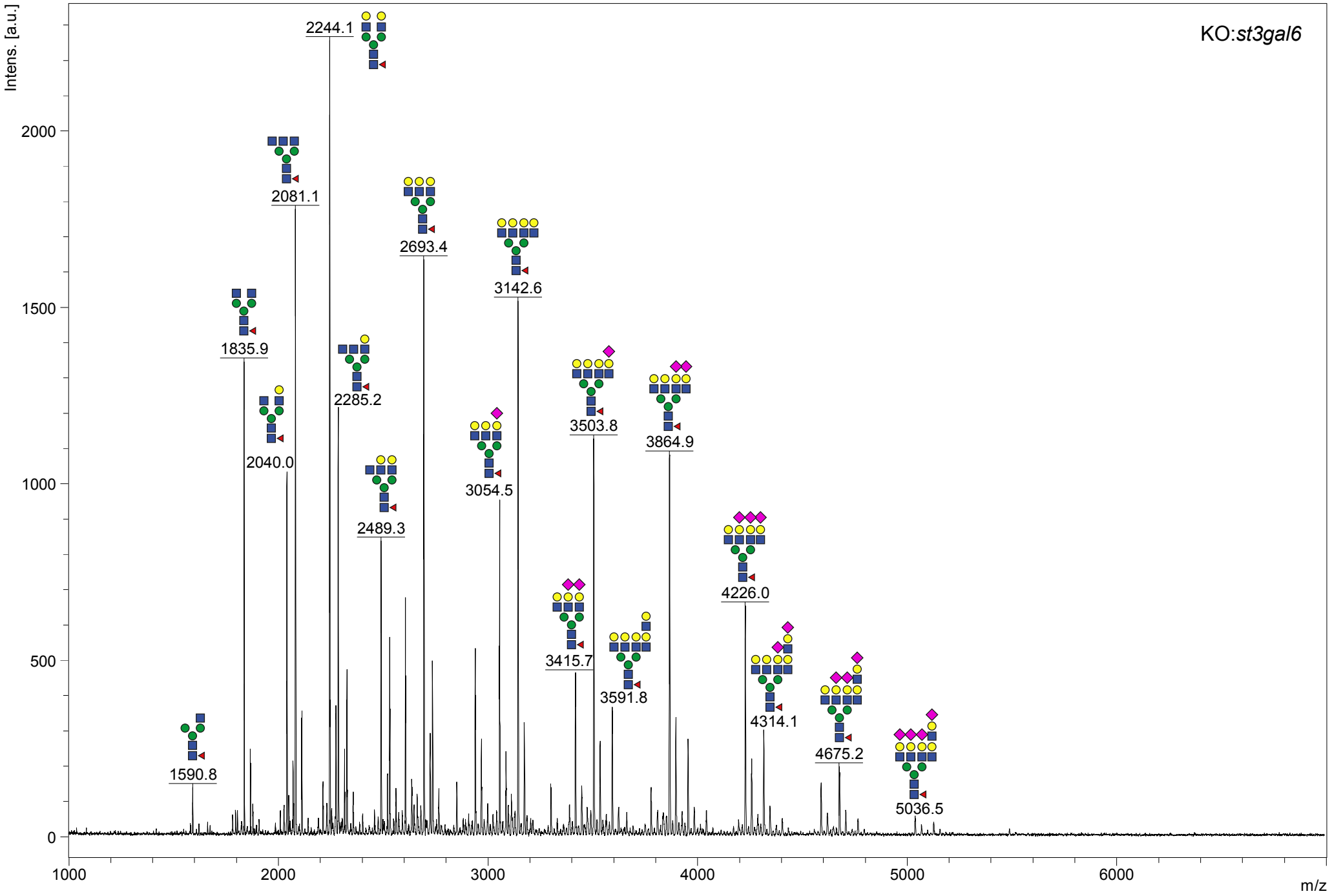


Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#24



Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
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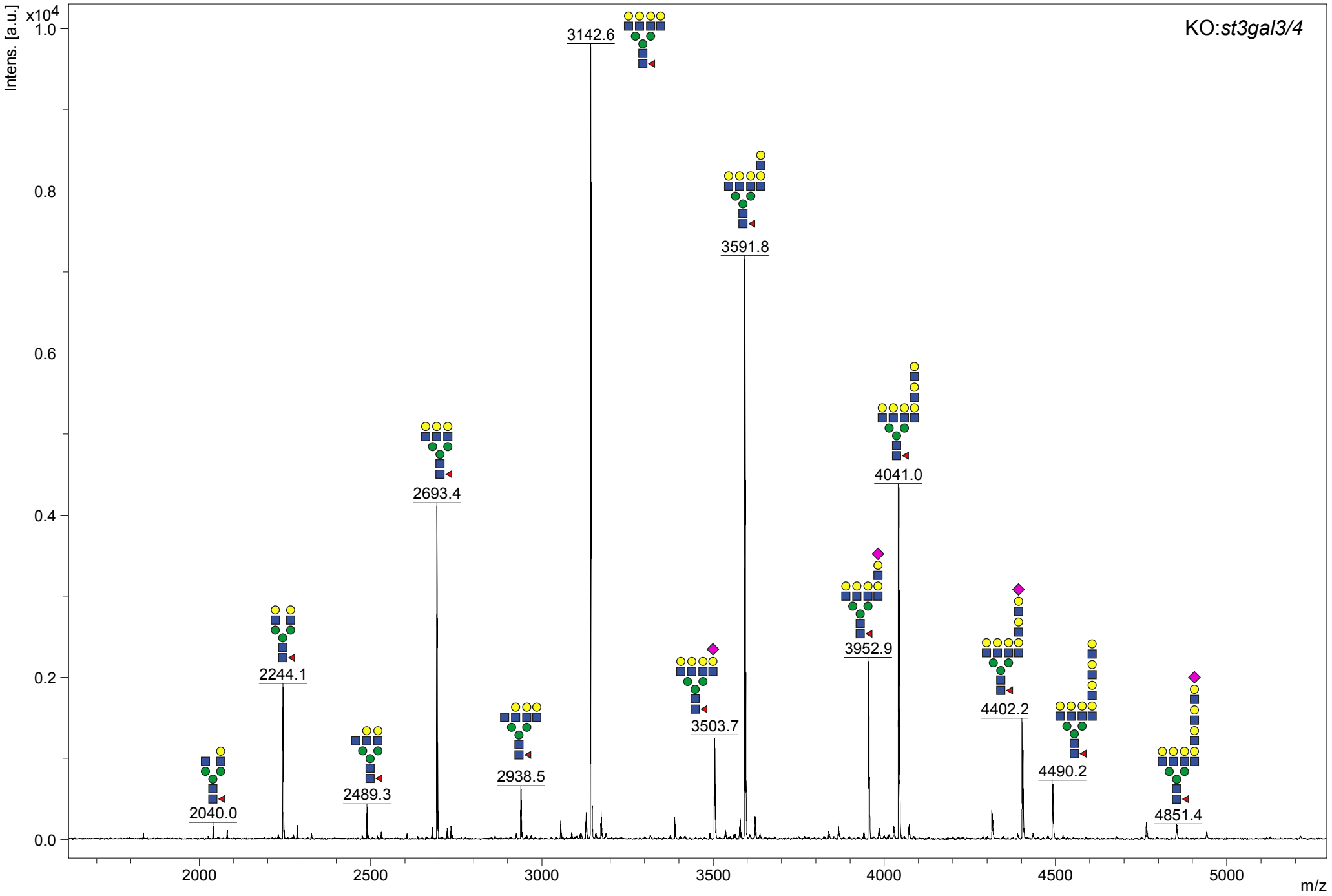

Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
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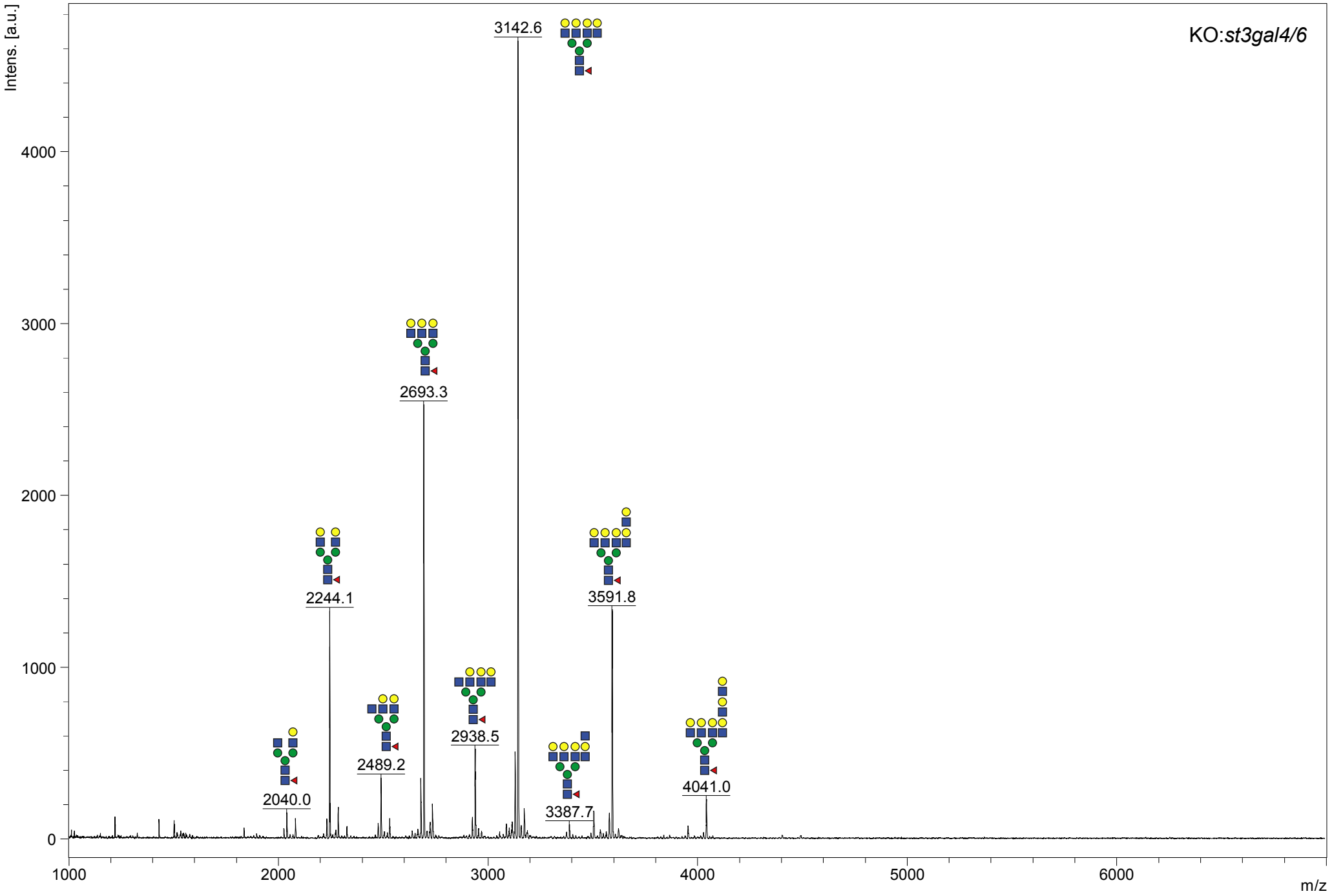
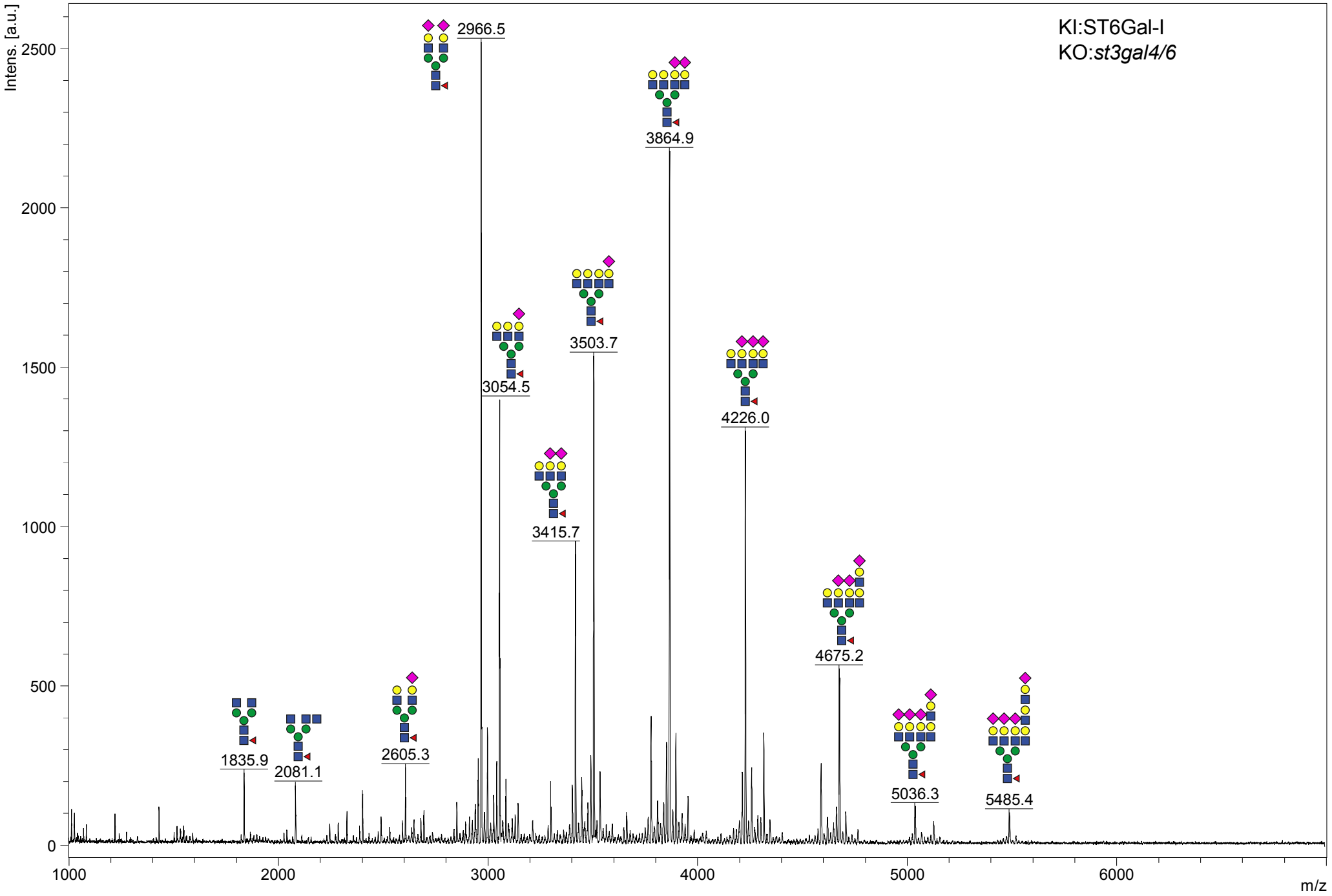


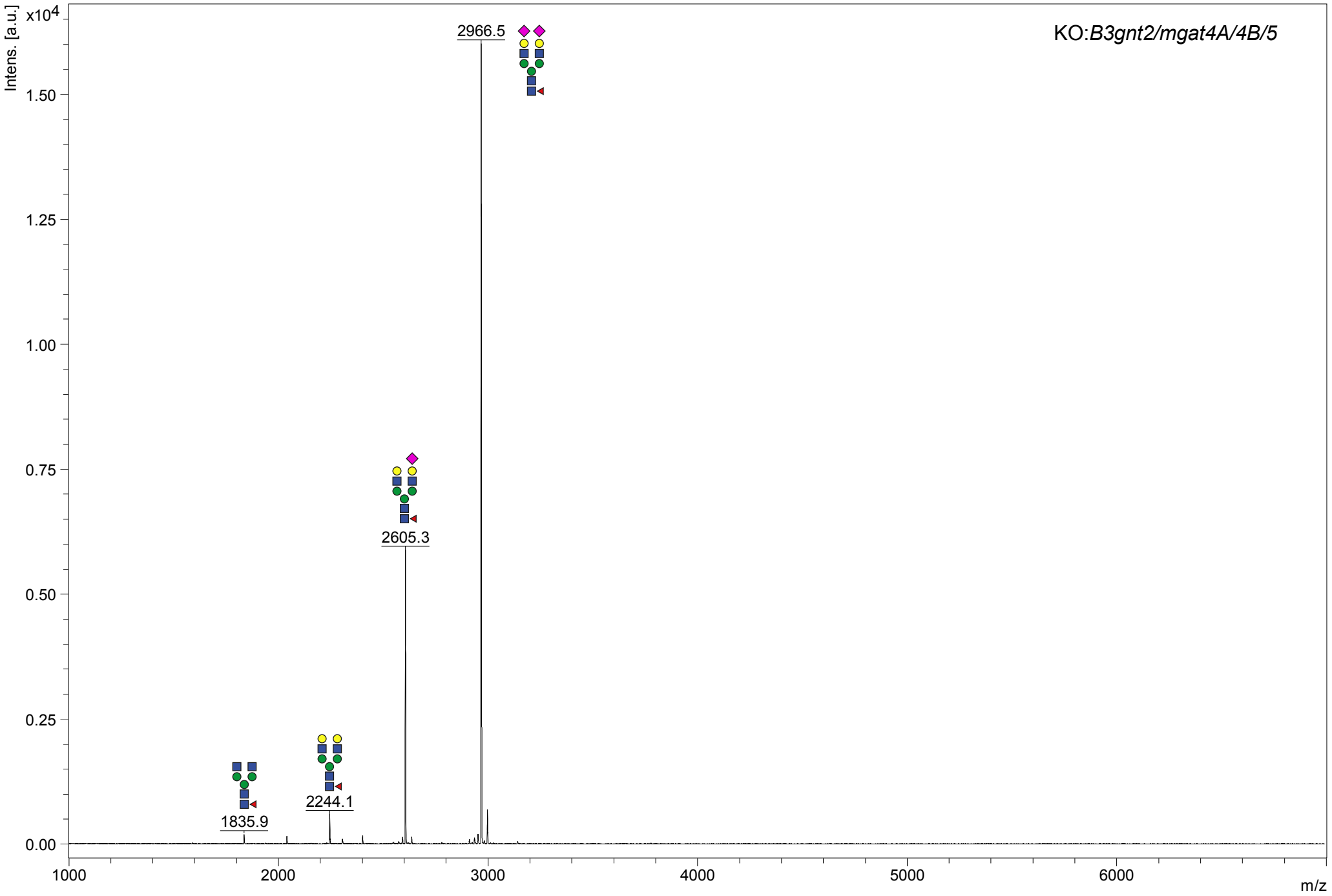
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#27



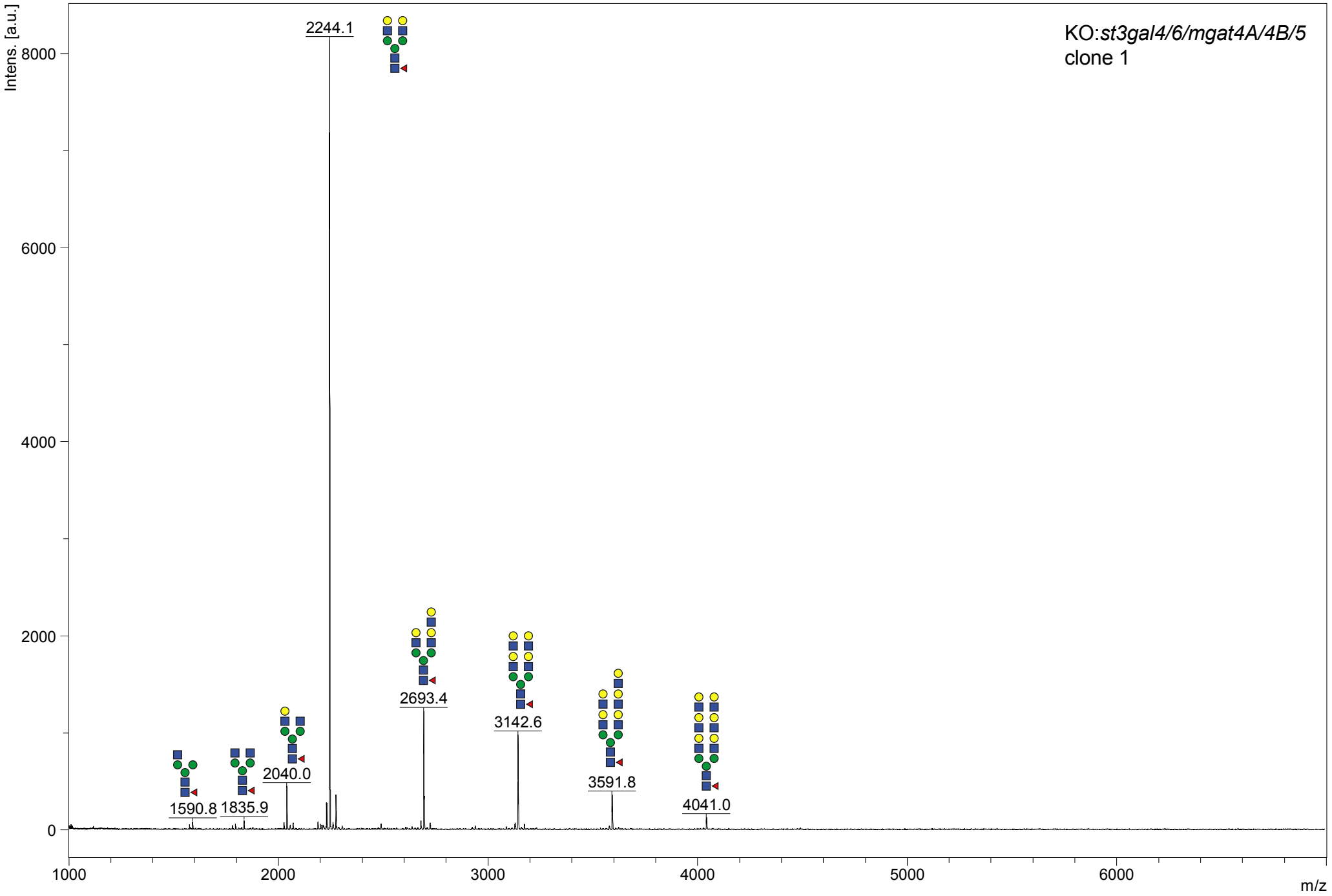
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#28



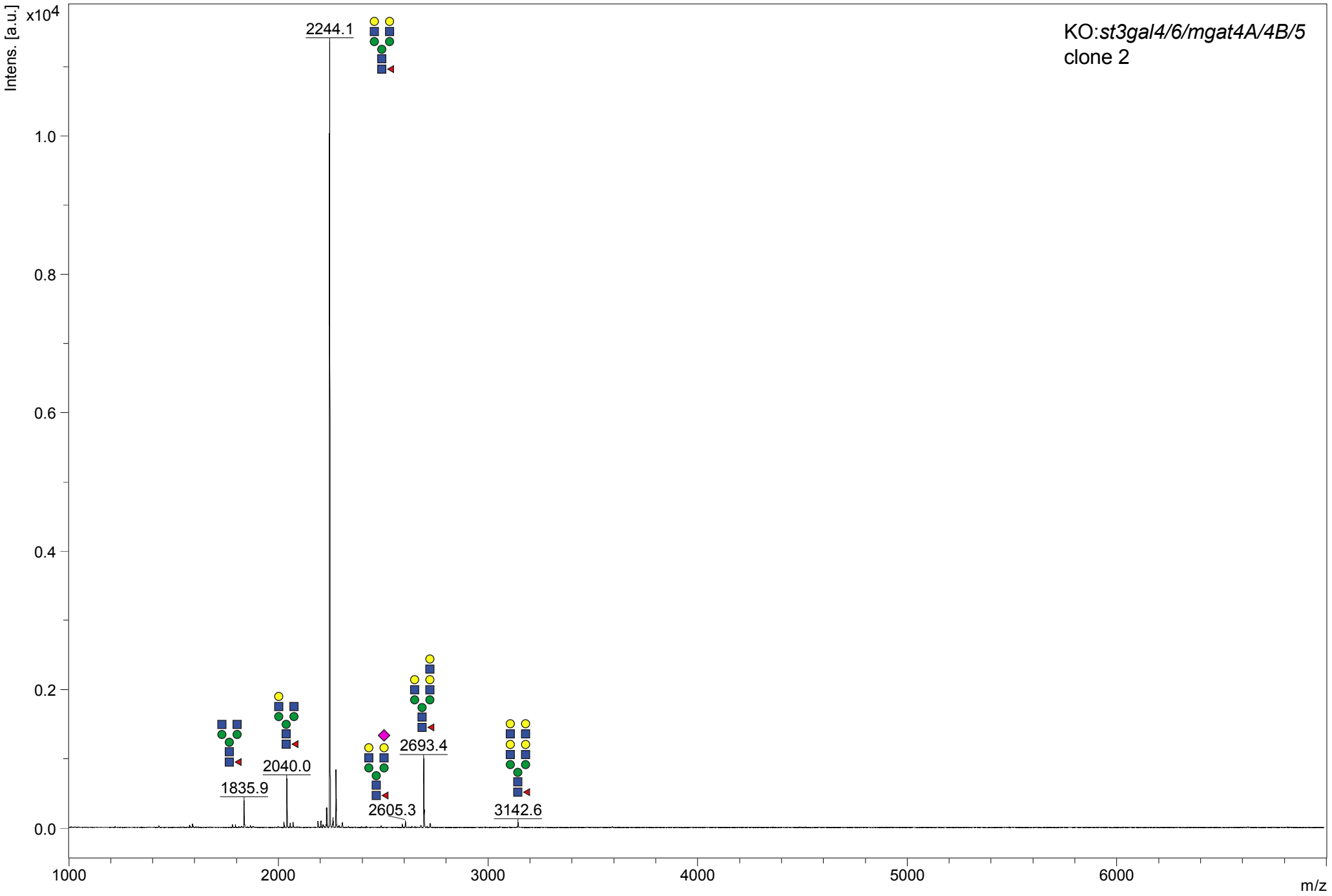
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#29a



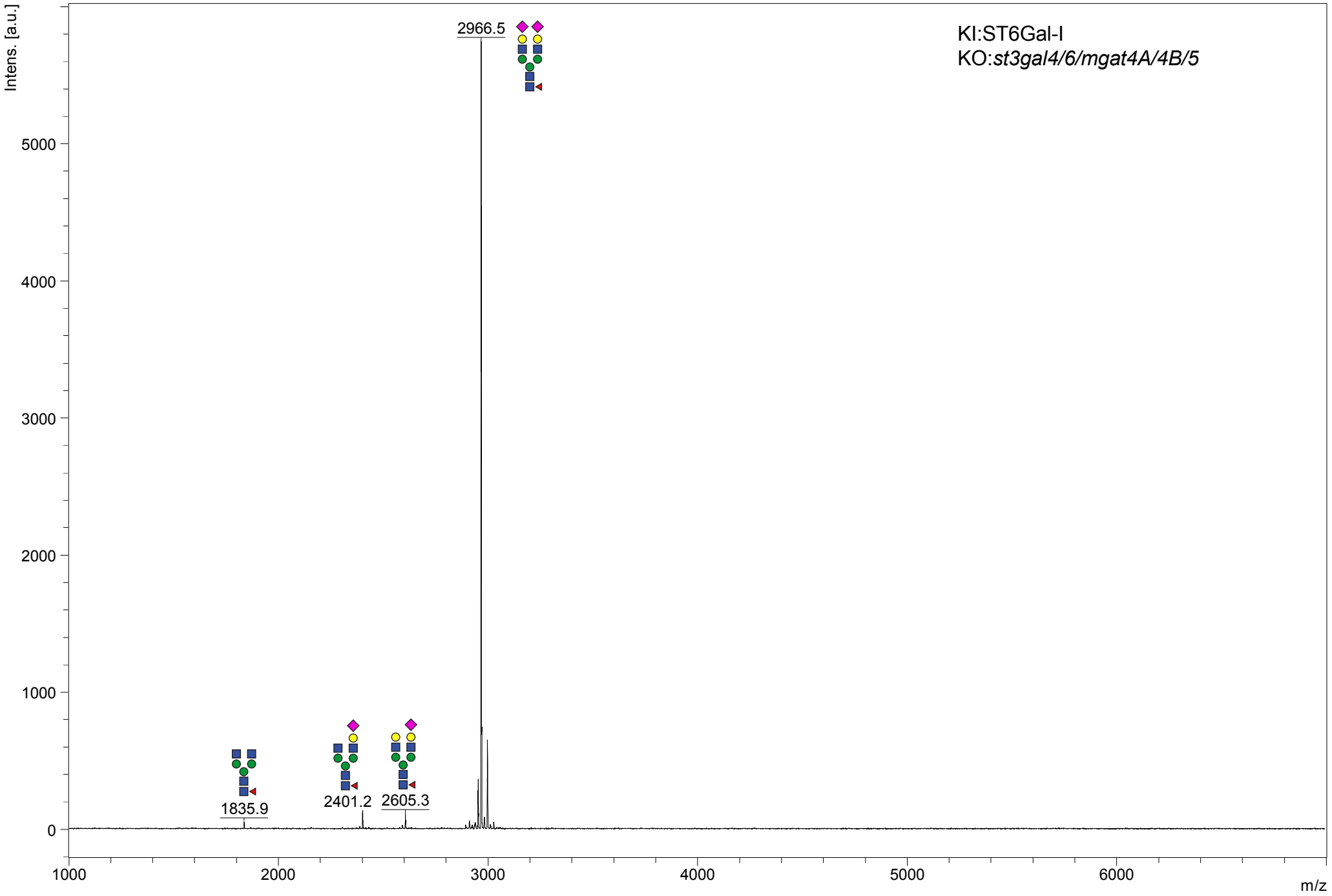
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#29b



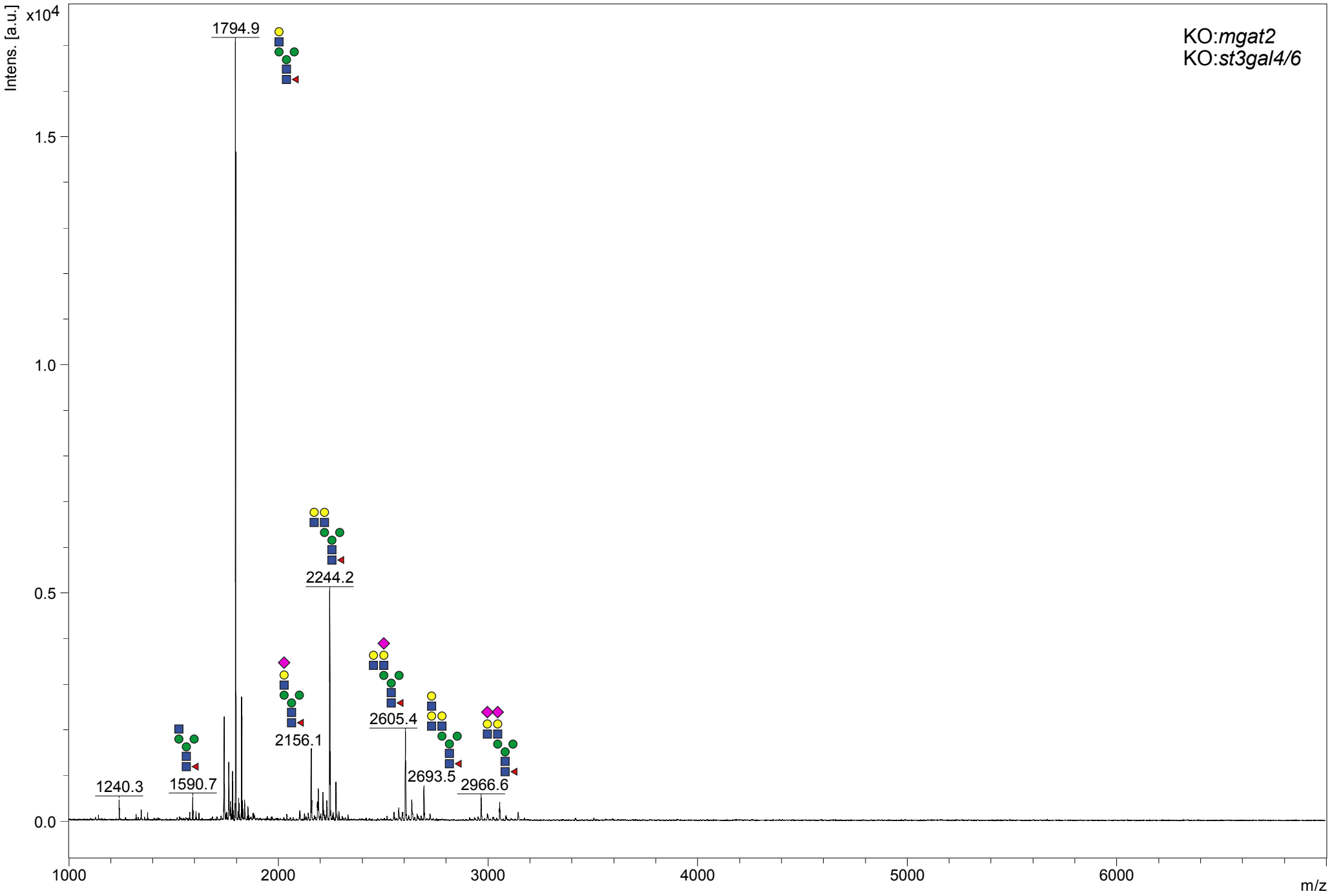
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#30



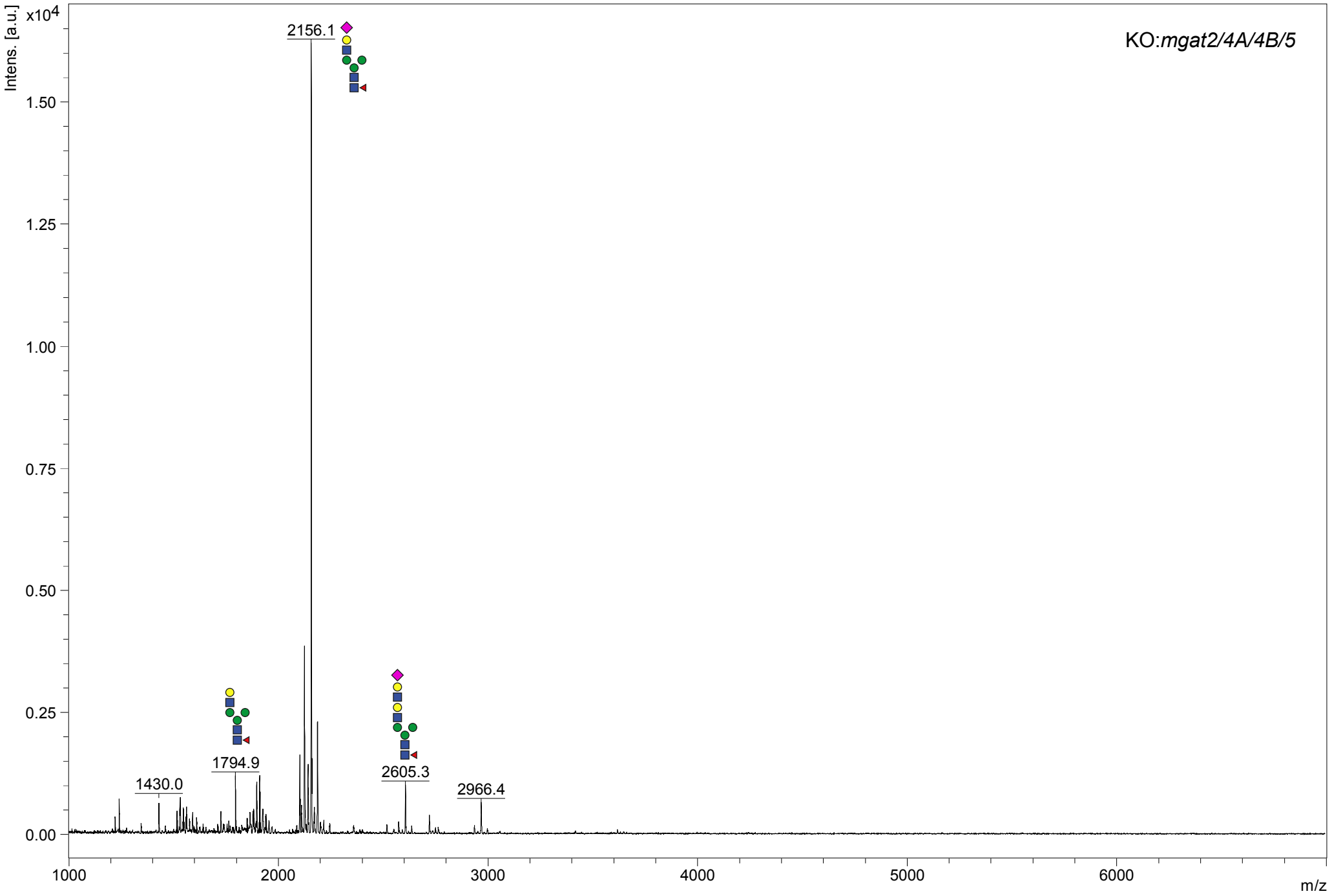
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#31



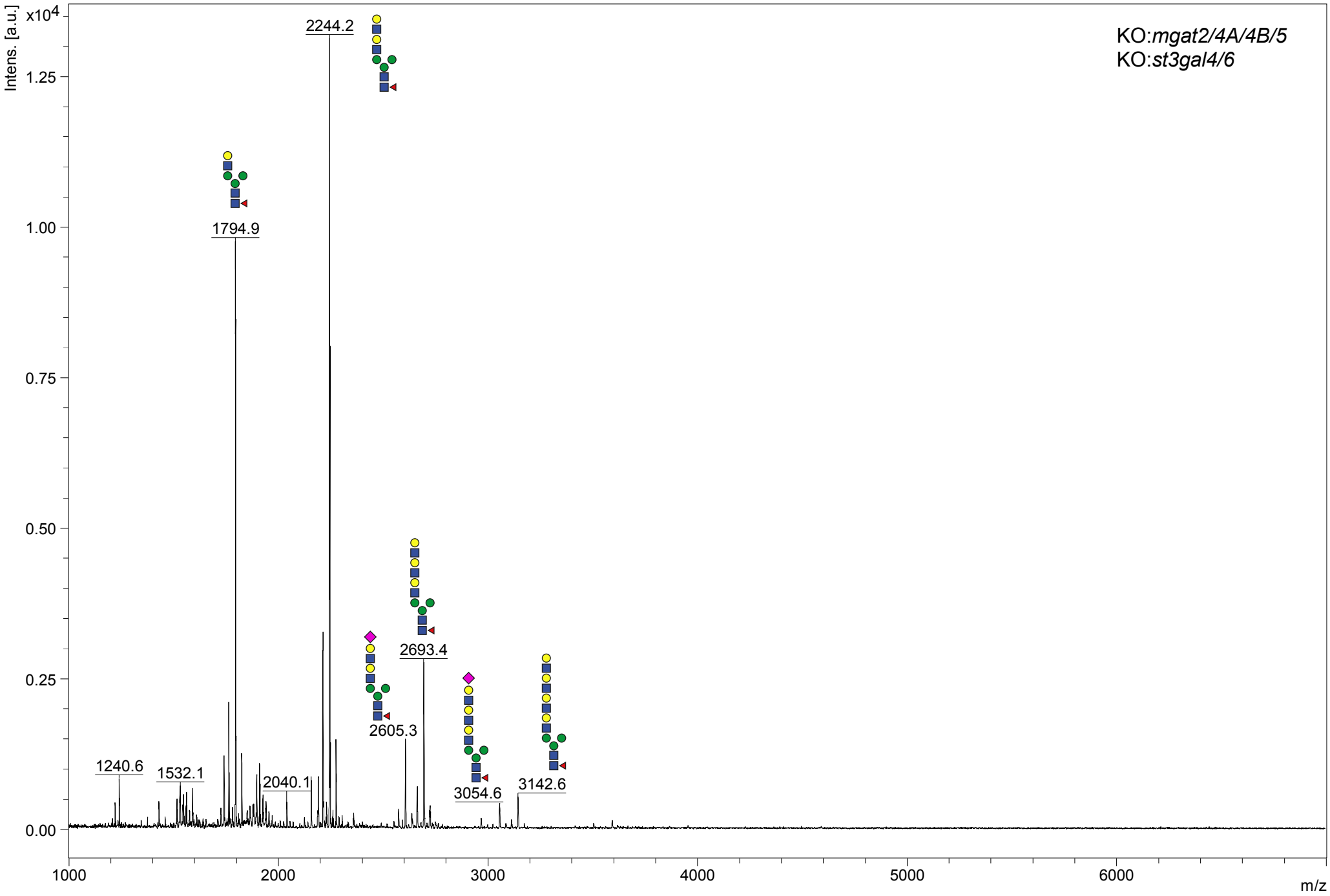
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#32



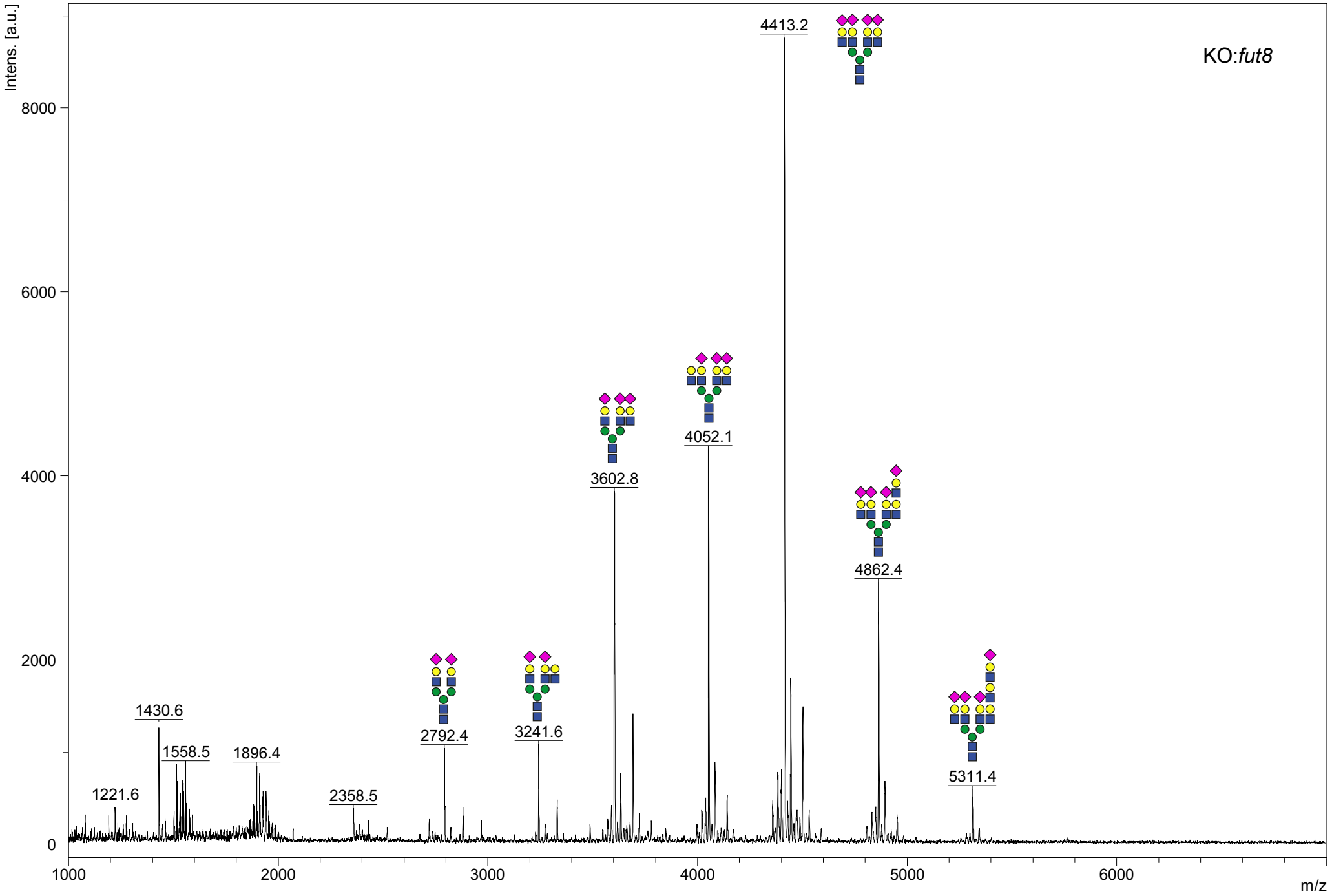
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#33



Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#34



Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
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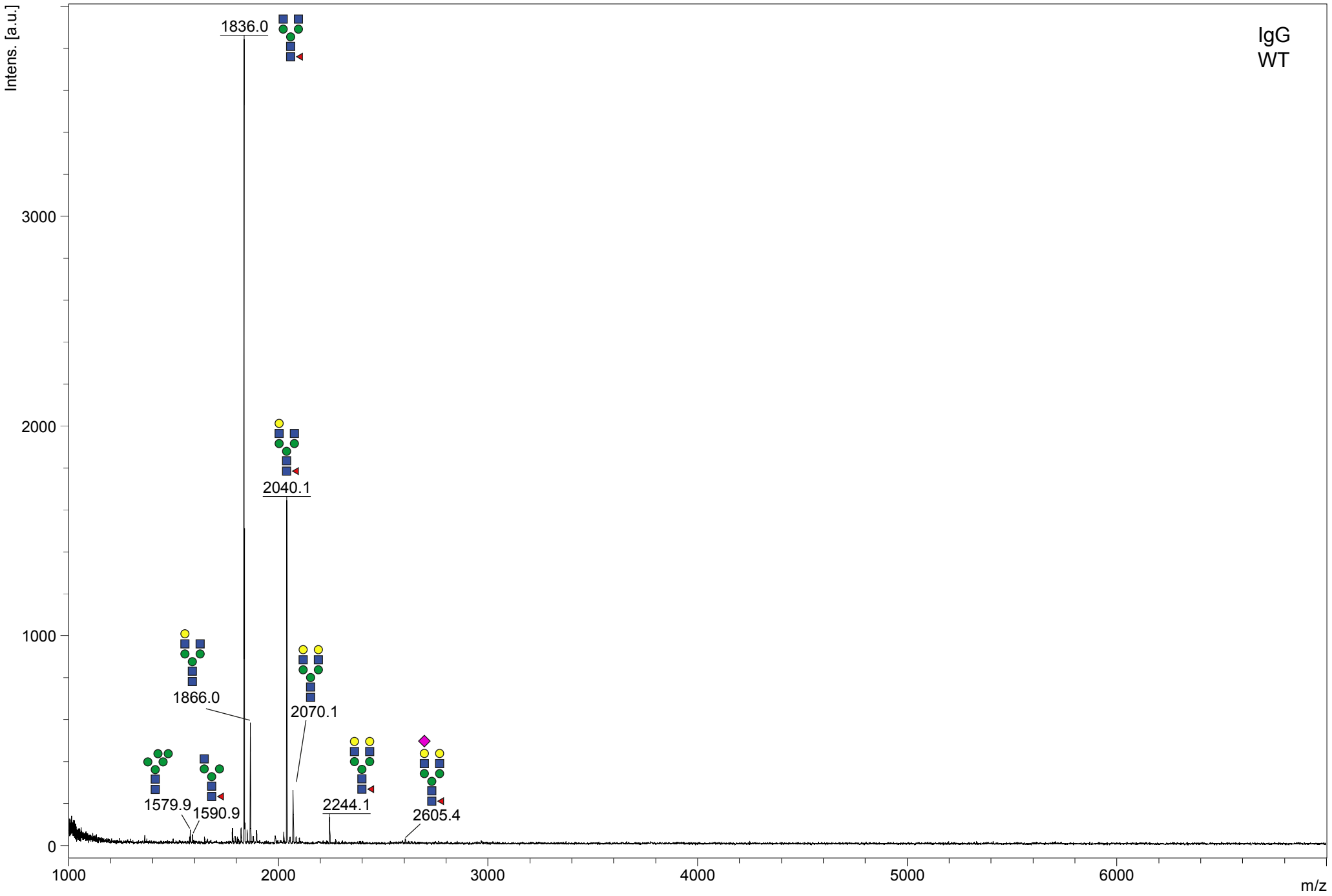
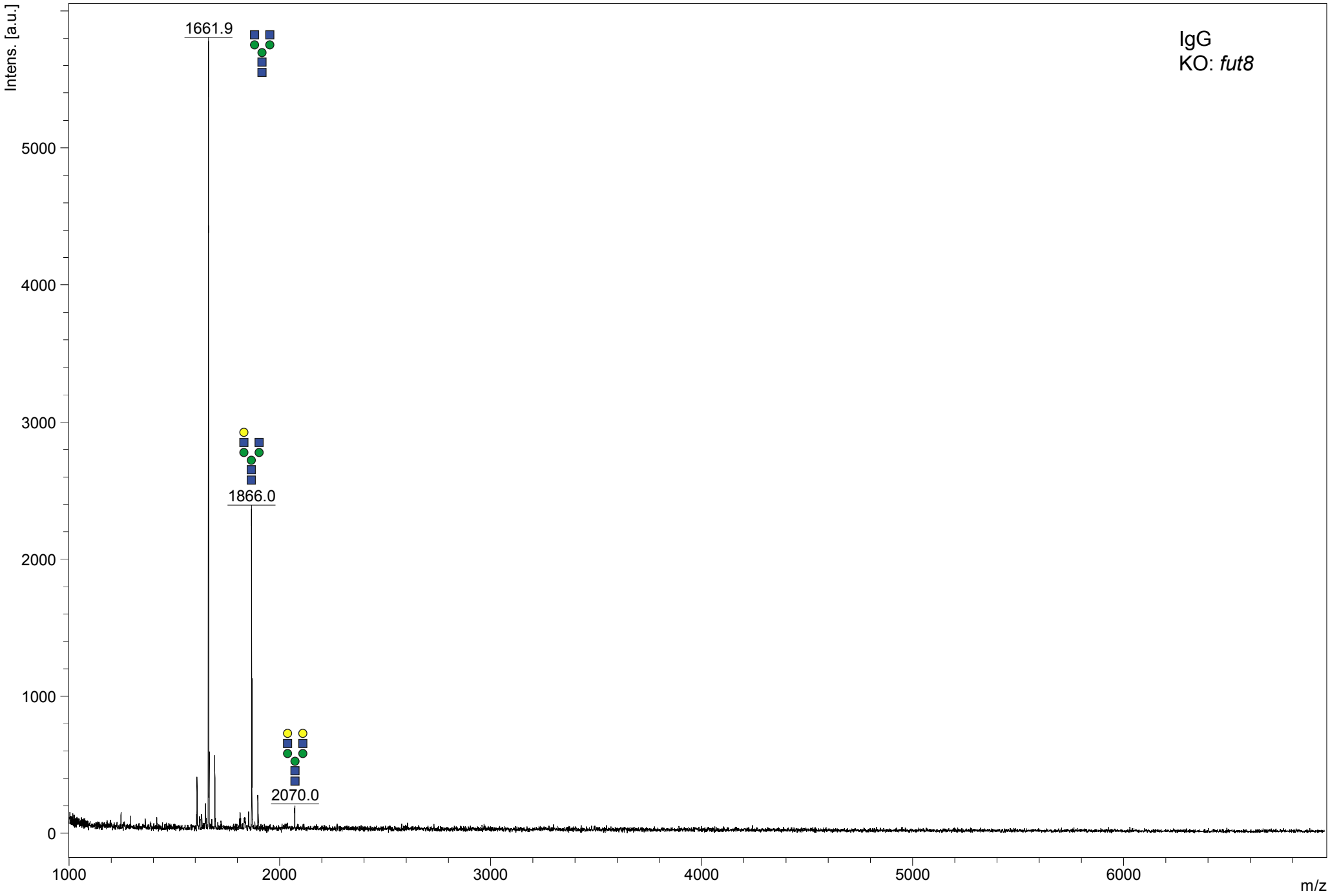


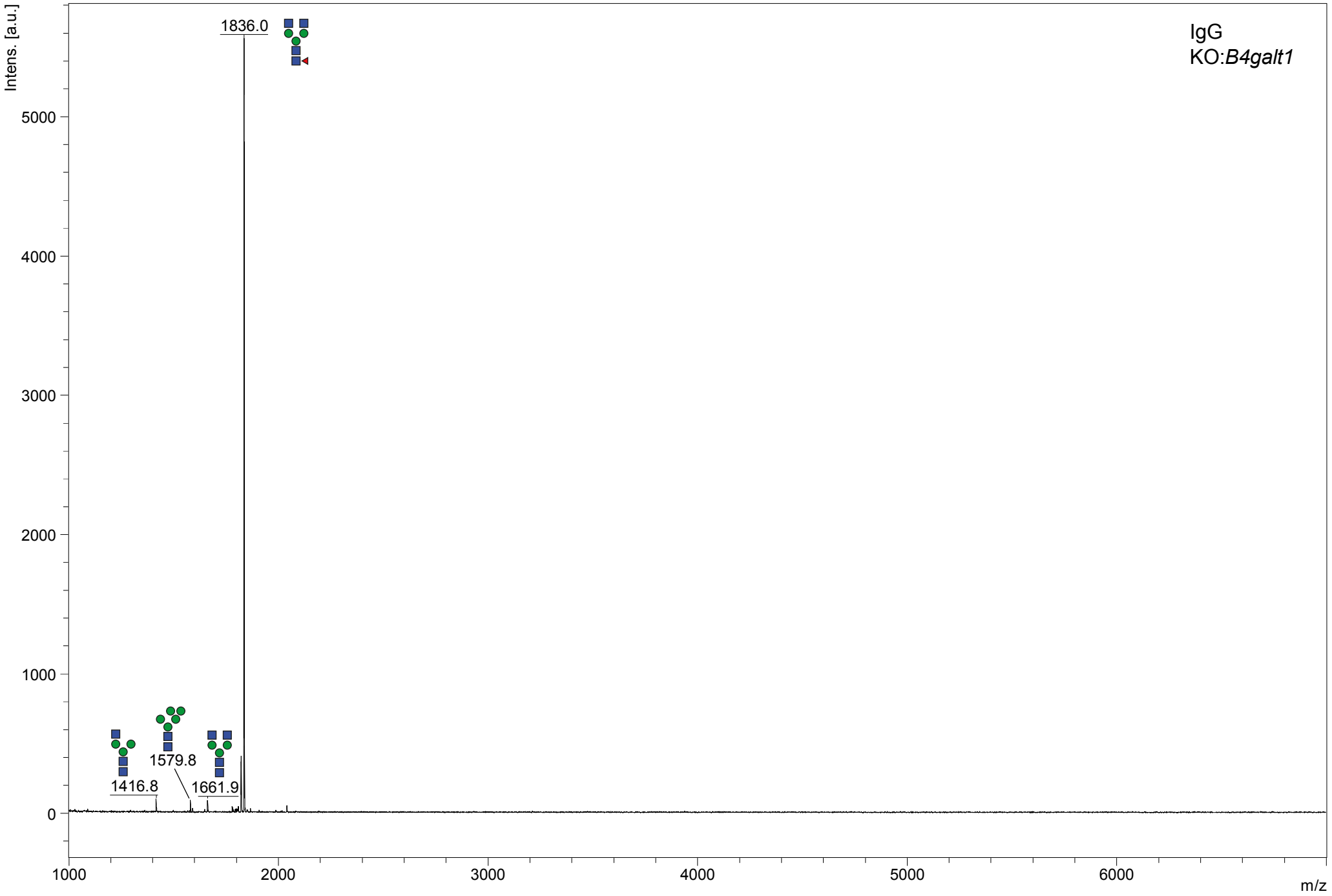
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#36



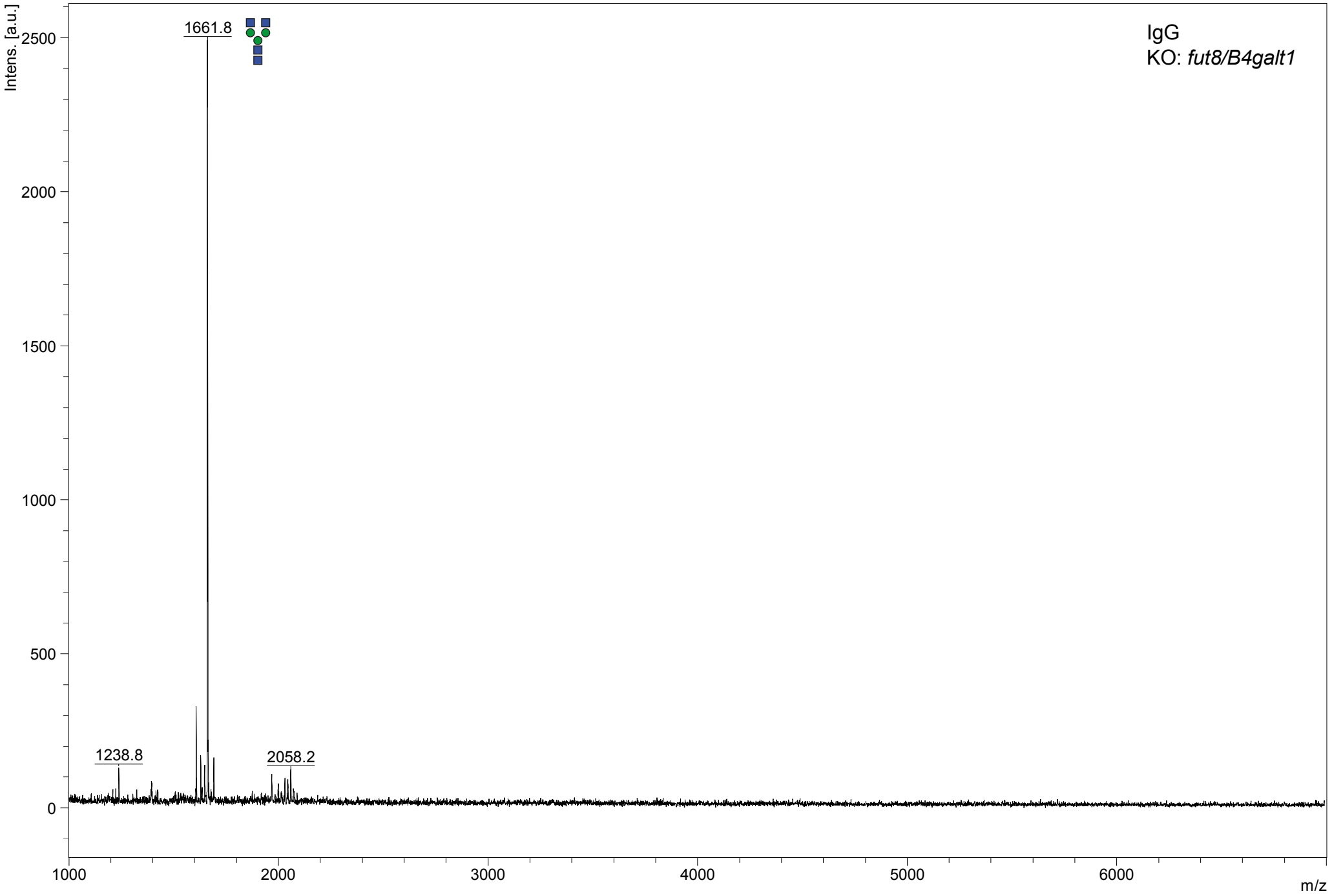
Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#37



Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Spectra
#38



Nature Biotechnology: doi:10.1038/nbt.3280

Supplementary Table 1. Glycoengineered CHO clones and stacking ancestry.

Project number	Targeted genes	Parental CHO line	Glycoprofiled
ZFN127	KO:*mgat1*	WT	x
ZFN174	KO:*mgat2*	WT	x
ZFN048	KO:*mgat3*	WT	x
ZFN001	KO:*mgat4A*	WT	x
ZFN078	KO:*mgat4B*	WT	x
ZFN143	KO:*mgat4C*	WT	x
ZFN004	KO:*mgat5*	WT	x
ZFN104	KO:*mgat5B*	WT	x
ZFN056	KO:*mgat3/5*	ZFN048	
ZFN067	KO:*mgat4A/5*	ZFN001	x
ZFN229	KO:*mgat4B/5*	ZFN078	x
ZFN150	KO:*mgat5/5B*	ZFN104	
ZFN077	KO:*mgat4A/4B*	ZFN001	x
ZFN091	KO:*mgat4A/4B/5*	ZFN077	x
ZFN115	KO:*mgat3/4A/4B/5*	ZFN091	x
ZFN144	KO:*mgat4A/4B/4C*	ZFN077	x
ZFN173	KO:*mgat4A/4B/4C/5*	ZFN144	
ZFN110	KO:*B4galt1*	WT	x
ZFN111	KO:*B4galt2*	WT	x
ZFN112	KO:*B4galt3*	WT	x
ZFN113	KO:*B4galt4*	WT	x
ZFN120	KO:*B4galt1/2*	ZFN110	x
ZFN121	KO:*B4galt1/3*	ZFN110	x
ZFN200	KO:*B4galt1/2/3*	ZFN121	x
ZFN141	KO:*B4galt1/2/4*	ZFN120	
ZFN164	KO:*B4galt1/2/3/4*	ZFN141	
ZFN224	KO:*B4galt1/2/3/4*	ZFN200	x
ZFN084	KO:*B3gnt2*	WT	x
ZFN085	KO:*B3gnt1*	WT	x
ZFN275	KO:*B3gnt8*	WT	x
ZFN096	KO:*B3gnt1/2*	ZFN084	x
ZFN072	KO:*st3gal3*	WT	x
ZFN082	KO:*st3gal6*	WT	x

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ZFN083	KO:*st3gal4*	WT	x
ZFN090	KO:*st3gal3/4*	ZFN072	x
ZFN095	KO:*st3gal3/6*	ZFN082	
ZFN098	KO:*st3gal4/6*	ZFN082	x
ZFN099	KO:*st3gal3/4/6*	ZFN090	x
ZFN136	KO:*B4galt1/mgat4A/4B/5*	ZFN091	x
ZFN137	KO:*B4galt2/mgat4A/4B/5*	ZFN091	
ZFN161	KO:*B4galt3/mgat4A/4B/5*	ZFN091	x
ZFN162	KO:*B4galt4/mgat4A/4B/5*	ZFN091	
ZFN119	KO:*B4galt1/st3gal4/6*	ZFN098	
ZFN138	KO:*B4galt2/stgal4/6*	ZFN098	
ZFN118	KO:*B3gnt2/st3gal4/6*	ZFN098	
ZFN167	KO:*B3gnt2/mgat4A/4B/5*	ZFN091	x
ZFN117	KO:*st3gal4/mgat4A/4B/5*	ZFN091	
ZFN163	KO:*st3gal4/6/mgat4A/4B/5*	ZFN117	x
ZFN225	KO:*st3gal3/4/6/mgat4A/4B/5*	ZFN163	
ZFN170	KO:*B3gnt2/st3gal4/6/mgat4A/4B/5*	ZFN163	x
ZFN134	KI:ST6GAL-I/KO:*st3gal4/6*	ZFN098	x
ZFN168	KI:ST6GAL-I/KO:*B3gnt2/st3gal4/6*	ZFN118	x
ZFN169	KI:ST6GAL-I/KO:*st3gal4/6/mgat4A/4B/5*	ZFN163	x
ZFN184	KI:ST6GAL-I/KO:*B3gnt2/st3gal4/6/mgat4A/4B/5*	ZFN170	x
ZFN226	KI:ST6GAL-I/KO:*st3gal3/4/6/mgat4A/4B/5*	ZFN169	x
ZFN174	KO:*mgat2*	WT	x
ZFN259	KO:*mgat2/stgal4/6*	ZFN98	x
ZFN261	KO:*mgat2/mgat4A/4B/5*	ZFN91	x
ZFN263	KO:*mgat2/st3gal4/6/mgat4A/4B/5*	ZFN163	x
ZFN281	KO:*B3gnt2/mgat2/st3gal4/6/mgat4A/4B/5*	ZFN263	x
ZFN266	KO:*fut8*	WT	x
ZFN268	KO:*fut8/B4galt1*	ZFN110	x

Supplementary Table 2. ZFN design and sequence analysis of CHO glycoengineered clones.

Clone	Inser & Del	Alignment¤
KO:mgat4A		
mgat4A-WT	ZFN	TTCTGAGTTGAATGCCATTGTCCAACAgtt----------tcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle1	-5bp	TTCTGAGTTGAATGCCATTGTCCAACAg----------CCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle2	+13bp	TTCTGAGTTGAATGCCATTGTCCAACAgttGAATTCTAGATGAtcGCCGTGCAGGAGCAGCAACTAACGGAAG
KO:mgat4A/4B		
mgat4A-WT	ZFN	TTCTGAGTTGAATGCCATTGTCCAACAgtt----------tcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle1	-5bp	TTCTGAGTTGAATGCCATTGTCCAACAg----------CCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle2	+13bp	TTCTGAGTTGAATGCCATTGTCCAACAgttGAATTCTAGATGAtcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4B-WT	ZFN	GCCCTCCAGCAGCCCTCTgaggaCTGGATGATCCTGGAGTT
mgat4B-alle1	-7bp	GCCCTCCAGCAGCCCTCTg-------GATGATCCTGGAGTT
KO:mgat5		
mgat5-WT	ZFN	GGGGGATGATGCTTCTGCACTTCACCATCCAg----------cagcgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle1	+14bp	GGGGGATGATGCTTCTGCACTTCACCATCCAgcaTGAATTCTAGATGAgcgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle2	-1bp	GGGGGATGATGCTTCTGCACTTCACCATCCAg----------agcgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle3	+61bp	GGGGGATGATGCTTCTGCACTTCACCATCCAgcag-----(+61bp)--cgGACTCAGCCTGAGAGCAGCTCCATGTT
KO:mgat4A/4B/5		
mgat4A-WT	ZFN	TTCTGAGTTGAATGCCATTGTCCAACAgtt----------tcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle1	-5bp	TTCTGAGTTGAATGCCATTGTCCAACAg----------CCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle2	+13bp	TTCTGAGTTGAATGCCATTGTCCAACAgttGAATTCTAGATGAtcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4B-WT	ZFN	GCCCTCCAGCAGCCCTCTgaggaCTGGATGATCCTGGAGTT
mgat4B-alle1	-7bp	GCCCTCCAGCAGCCCTCTg-------GATGATCCTGGAGTT
mgat5-WT	ZFN	GGGGGATGATGCTTCTGCACTTCACCATCCAg----cagcgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle1	+4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAgcagcCAGCgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle2	-4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAg--------gGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle3	-16bp	GGGGGATGATGCTTCTGCACTTC----------CTCAGCCTGAGAGCAGCTCCATGTT
KO:B3gnt1		
B3gnt1-WT	ZFN	TGCAGCTGCTCTACCTGTC----------gctgcTCTCCGGACTGCACG
B3gnt1-alle1	+11bp	TGCAGCTGCTCTACCTGTCgcagcTGCTCTACCTGTCTCCGGACTGCACG
B3gnt1-alle2	-5bp	TGCAGCTGCTCTACCTGTC----------TCTCCGGACTGCACG
KO:B3gnt2		
B3gnt2-WT	ZFN	TTCAGCCCTTCCcgggcGTACTGGAACAGAGAGCA
B3gnt2-alle1	-1bp	TTCAGCCCTTCC-gggcGTACTGGAACAGAGAGCA
B3gnt2-alle2	-4bp	TTCAGCCCTTCCcg----TACTGGAACAGAGAGCA
KO:B4galt1		
B4galt1-WT	ZFN	TGCATCCGGTCCTACAGCgccagc----AACTGGACTATGGTA

B4galt1-alle1	+4bp	TGCATCCGGTCCTACAGCgccagcCAGCAACTGGACTATGGTA
KO:B4galt2		
B4galt2-WT	ZFN	CAGCCCCGCCACTTTgcc----------atcGCCATGGACAAGTTTGGCT
B4galt2-alle1	+73bp	CAGCCCCGCCACTTTgcc--(+73bp)--atcGCCATGGACAAGTTTGGCT
KO:B4galt3		
B4galt3-WT	ZFN	CTAGCCCTCAAGTCAGGAtgt-----tgCGGAGGCTGCTGGAGAGG
B4galt3-alle1	+5bp	CTAGCCCTCAAGTCAGGAtgtCGTGTtgCGGAGGCTGCTGGAGAGG
B4galt3-alle2	+2bp	CTAGCCCTCAAGTCAGGAtgt---tgCCCGGAGGCTGCTGGAGAGG
KO:B4galt4		
B4galt4-WT	ZFN	AACTGGGACTGCTTTat----attcCACGATGTGGACCTGGTG
B4galt4-alle1	+1bp	AACTGGGACTGCTTTat---TattcCACGATGTGGACCTGGTG
B4galt4-alle2	+4bp	AACTGGGACTGCTTTatattTATTcCACGATGTGGACCTGGTG
KO:B4galt1/2		
B4galt1-WT	ZFN	TGCATCCGGTCCTACAGCgccagc----AACTGGACTATGGTA
B4galt1-alle1	+4bp	TGCATCCGGTCCTACAGCgccagcCAGCAACTGGACTATGGTA
B4galt2-WT	ZFN	CAGCCCCGCCACTTTgccatcGCCATGGACAAGTTTGGCT
B4galt2-alle1	-8bp	CAGCCCCGCCAC--------cGCCATGGACAAGTTTGGCT
B4galt2-alle2	-14bp	CAGCCC--------------cGCCATGGACAAGTTTGGCT
KO:st3Gal3		
st3Gal3-WT	ZFN	CTCTCTCTTTGTCCTTGCtggcttCAAATGGCAGGACTTCAAG
st3Gal3-alle1	-5bp	CTCTCTCTTTGTCCTTGCt-----CAAATGGCAGGACTTCAAG
st3Gal3-alle2	-1bp	CTCTCTCTTTGTCCTTGCtggct-CAAATGGCAGGACTTCAAG
KO:st3Gal4		
st3Gal4-WT	ZFN	GGCAGCCTCCAGTGTCGTCgttgt----gTTGTGGTGGGGAATGGGC
st3Gal4-alle1	+4bp	GGCAGCCTCCAGTGTCGTCgttgtTTGTgTTGTGGTGGGGAATGGGC
st3Gal4-alle2	-4bp	GGCAGCCTCCAGTGTCGTCg--------gTTGTGGTGGGGAATGGGC
KO:st3Gal6		
st3Gal6-WT	ZFN	CGGTACCTCTGATTTTGCTttgccCTATGGGACAAGGCC
st3Gal6-alle1	-4bp	CGGTACCTCTGATTTTGCTt----CTATGGGACAAGGCC
st3Gal6-alle2	-22bp	CGGTACCTCTGA----------AGGCC
KO:st3Gal3/4		
st3Gal3-WT	ZFN	CTCTCTCTTTGTCCTTGCtggcttCAAATGGCAGGACTTCAAG
st3Gal3-alle1	-5bp	CTCTCTCTTTGTCCTTGCt-----CAAATGGCAGGACTTCAAG
st3Gal3-alle2	-1bp	CTCTCTCTTTGTCCTTGCtggct-CAAATGGCAGGACTTCAAG
st3Gal4-WT	ZFN	GGCAGCCTCCAGTGTCGTCgttgt-----gTTGTGGTGGGGAATGGGC
st3Gal4-alle1	-4bp	GGCAGCCTCCAGTGTCGTCg---------gTTGTGGTGGGGAATGGGC

st3Gal4-alle2	+5bp	GGCAGCCTCCAGTGTCGTCgACACGttgtgTTGTGGTGGGGAATGGGC

KO:st3Gal4/6		
st3Gal4-WT	ZFN	GGCAGCCTCCAGTGTCGTCgttgt----gTTGTGGTGGGGAATGGGC
st3Gal4-alle1	+4bp	GGCAGCCTCCAGTGTCGTCgttgtTTGTgTTGTGGTGGGGAATGGGC
st3Gal4-alle2	-4bp	GGCAGCCTCCAGTGTCGTCg--------gTTGTGGTGGGGAATGGGC
st3Gal6-WT	ZFN	CGGTACCTCTGATTTTGCT-ttgccCTATGGGACAAGGCC
st3Gal6-alle1	+1bp	CGGTACCTCTGATTTTGCTTttgccCTATGGGACAAGGCC
st3Gal6-alle2	-7bp	CGGTACCTCTGATTTTG--------CTATGGGACAAGGCC

KO:B3gnt2/mgat4A/4B/5		
mgat4A-WT	ZFN	TTCTGAGTTGAATGCCATTGTCCAACAgtt-------------tcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle1	-5bp	TTCTGAGTTGAATGCCATTGTCCAACAg------------------CCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle2	+13bp	TTCTGAGTTGAATGCCATTGTCCAACAgttGAATTCTAGATGAtcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4B-WT	ZFN	GCCCTCCAGCAGCCCTCTgaggaCTGGATGATCCTGGAGTT
mgat4B-alle1	-7bp	GCCCTCCAGCAGCCCTCTg-------GATGATCCTGGAGTT
mgat5-WT	ZFN	GGGGGATGATGCTTCTGCACTTCACCATCCAg----cagcgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle1	+4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAgcagcCAGCgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle2	-4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAg--------gGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle3	-16bp	GGGGGATGATGCTTCTGCACTTC--------------------CTCAGCCTGAGAGCAGCTCCATGTT
B3gnt2-WT	ZFN	TTCAGCCCTTCC--cgggcGTACTGGAACAGAGAGCA
B3gnt2-alle1	+2bp	TTCAGCCCTTCCCCcgggcGTACTGGAACAGAGAGCA
B3gnt2-alle2	+1bp	TTCAGCCCTTCCC-cgggcGTACTGGAACAGAGAGCA

KO:st3Gal4/6/mgat4A/4B/5		
mgat4A-WT	ZFN	TTCTGAGTTGAATGCCATTGTCCAACAgtt-------------tcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle1	-5bp	TTCTGAGTTGAATGCCATTGTCCAACAg------------------CCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle2	+13bp	TTCTGAGTTGAATGCCATTGTCCAACAgttGAATTCTAGATGAtcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4B-WT	ZFN	GCCCTCCAGCAGCCCTCTgaggaCTGGATGATCCTGGAGTT
mgat4B-alle1	-7bp	GCCCTCCAGCAGCCCTCTg-------GATGATCCTGGAGTT
mgat5-WT	ZFN	GGGGGATGATGCTTCTGCACTTCACCATCCAg----cagcgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle1	+4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAgcagcCAGCgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle2	-4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAg--------gGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle3	-16bp	GGGGGATGATGCTTCTGCACTTC--------------------CTCAGCCTGAGAGCAGCTCCATGTT
st3Gal4-WT	ZFN	GGCAGCCTCCAGTGTCGTCgttgt----gTTGTGGTGGGGAATGGGC
st3Gal4-alle1	-5bp	GGCAGCCTCCAGTGTCGTCgttgt---------gGTGGGGAATGGGC
st3Gal4-alle2	+4bp	GGCAGCCTCCAGTGTCGTCgTTGTttgtgTTGTGGTGGGGAATGGGC
st3Gal6-WT	ZFN	CGGTACCTCTGATTTTGCTttgccCTATGGGACAAGGCC
st3Gal6-alle1	-4bp	CGGTACCTCTGATTTTGCTt----CTATGGGACAAGGCC
st3Gal6-alle2	-2bp	CGGTACCTCTGATTTTGCTttg--CTATGGGACAAGGCC

KI:ST6GAL1/KO:st3gal4/6		
st3Gal4-WT	ZFN	GGCAGCCTCCAGTGTCGTCgttgt----gTTGTGGTGGGGAATGGGC
st3Gal4-alle1	+4bp	GGCAGCCTCCAGTGTCGTCgttgtTTGTgTTGTGGTGGGGAATGGGC

st3Gal4-alle2	-4bp	GGCAGCCTCCAGTGTCGTCg--------gTTGTGGTGGGGAATGGGC
st3Gal6-WT	ZFN	CGGTACCTCTGATTTTGCT-ttgccCTATGGGACAAGGCC
st3Gal6-alle1	+1bp	CGGTACCTCTGATTTTGCTTttgccCTATGGGACAAGGCC
st3Gal6-alle2	-7bp	CGGTACCTCTGATTTTG--------CTATGGGACAAGGCC

KI:ST6GAL1/KO:st3gal4/6/mgat4A/4B/5

mgat4A-WT	ZFN	TTCTGAGTTGAATGCCATTGTCCAACAgtt-------------tcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle1	-5bp	TTCTGAGTTGAATGCCATTGTCCAACAg------------------CCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle2	+13bp	TTCTGAGTTGAATGCCATTGTCCAACAgttGAATTCTAGATGAtcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4B-WT	ZFN	GCCCTCCAGCAGCCCTCTgaggaCTGGATGATCCTGGAGTT
mgat4B-alle1	-7bp	GCCCTCCAGCAGCCCTCTg-------GATGATCCTGGAGTT
mgat5-WT	ZFN	GGGGGATGATGCTTCTGCACTTCACCATCCAg----cagcgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle1	+4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAgcagcCAGCgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle2	-4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAg--------gGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle3	-16bp	GGGGGATGATGCTTCTGCACTTC--------------------CTCAGCCTGAGAGCAGCTCCATGTT
st3Gal4-WT	ZFN	GGCAGCCTCCAGTGTCGTCgttgt----gTTGTGGTGGGGAATGGGC
st3Gal4-alle1	-5bp	GGCAGCCTCCAGTGTCGTCgttgt---------gGTGGGGAATGGGC
st3Gal4-alle2	+4bp	GGCAGCCTCCAGTGTCGTCgTTGTttgtgTTGTGGTGGGGAATGGGC
st3Gal6-WT	ZFN	CGGTACCTCTGATTTTGCTttgccCTATGGGACAAGGCC
st3Gal6-alle1	-4bp	CGGTACCTCTGATTTTGCTt----CTATGGGACAAGGCC
st3Gal6-alle2	-2bp	CGGTACCTCTGATTTTGCTttg--CTATGGGACAAGGCC

KO:mgat2

mgat2-WT	TALEN	TCCTTTGTCGCCCATTGCTgctccagaggacgaagCCGCAGGCGGCCACCACGA
mgat2-alle1	-4bp	TCCTTTGTCGCCCATTGCTgctcc----gacgaagccgcaggcggccaccacga

KO:mgat2/stgal4/6

mgat2-WT	TALEN	TCCTTTGTCGCCCATTGCTgctccagaggacgaagCCGCAGGCGGCCACCACGA
mgat2-alle1	-4bp	TCCTTTGTCGCCCATTGCTgctccag----cgaagCCGCAGGCGGCCACCACGA
st3Gal4-WT	ZFN	GGCAGCCTCCAGTGTCGTCgttgt----gTTGTGGTGGGGAATGGGC
st3Gal4-alle1	+4bp	GGCAGCCTCCAGTGTCGTCgttgtTTGTgTTGTGGTGGGGAATGGGC
st3Gal4-alle2	-4bp	GGCAGCCTCCAGTGTCGTCg--------gTTGTGGTGGGGAATGGGC
st3Gal6-WT	ZFN	CGGTACCTCTGATTTTGCT-ttgccCTATGGGACAAGGCC
st3Gal6-alle1	+1bp	CGGTACCTCTGATTTTGCTTttgccCTATGGGACAAGGCC
st3Gal6-alle2	-7bp	CGGTACCTCTGATTTTG--------CTATGGGACAAGGCC

KO:mgat2/mgat4A/4B/5

mgat2-WT	TALEN	TCCTTTGTCGCCCATTGCTgctccagaggacgaagCCGCAGGCGGCCACCACGA
mgat2-alle1	-4bp	TCCTTTGTCGCCCATTGCTgctccag----cgaagCCGCAGGCGGCCACCACGA
mgat4A-WT	ZFN	TTCTGAGTTGAATGCCATTGTCCAACAgtt-------------tcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle1	-5bp	TTCTGAGTTGAATGCCATTGTCCAACAg------------------CCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle2	+13bp	TTCTGAGTTGAATGCCATTGTCCAACAgttGAATTCTAGATGAtcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4B-WT	ZFN	GCCCTCCAGCAGCCCTCTgaggaCTGGATGATCCTGGAGTT
mgat4B-alle1	-7bp	GCCCTCCAGCAGCCCTCTg-------GATGATCCTGGAGTT

mgat5-WT	ZFN	GGGGGATGATGCTTCTGCACTTCACCATCCAg----cagcgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle1	+4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAgcagcCAGCgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle2	-4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAg--------gGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle3	-16bp	GGGGGATGATGCTTCTGCACTTC----------CTCAGCCTGAGAGCAGCTCCATGTT

KO:mgat2/st3gal4/6/mgat4A/4B/5

mgat2-WT	TALEN	TCCTTTGTCGCCCATTGCTgctccagaggacgaagCCGCAGGCGGCCACCACGA
mgat2-alle1	-5bp	TCCTTTGTCGCCCATTGCTgctcca-----cgaaGCCGCAGGCGGCCACCACGA
mgat4A-WT	ZFN	TTCTGAGTTGAATGCCATTGTCCAACAgtt----------tcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle1	-5bp	TTCTGAGTTGAATGCCATTGTCCAACAg----------CCGTGCAGGAGCAGCAACTAACGGAAG
mgat4A-alle2	+13bp	TTCTGAGTTGAATGCCATTGTCCAACAgttGAATTCTAGATGAtcGCCGTGCAGGAGCAGCAACTAACGGAAG
mgat4B-WT	ZFN	GCCCTCCAGCAGCCCTCTgaggaCTGGATGATCCTGGAGTT
mgat4B-alle1	-7bp	GCCCTCCAGCAGCCCTCTg-------GATGATCCTGGAGTT
mgat5-WT	ZFN	GGGGGATGATGCTTCTGCACTTCACCATCCAg----cagcgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle1	+4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAgcagcCAGCgGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle2	-4bp	GGGGGATGATGCTTCTGCACTTCACCATCCAg--------gGACTCAGCCTGAGAGCAGCTCCATGTT
mgat5-alle3	-16bp	GGGGGATGATGCTTCTGCACTTC----------CTCAGCCTGAGAGCAGCTCCATGTT
st3Gal4-WT	ZFN	GGCAGCCTCCAGTGTCGTCgttgt----gTTGTGGTGGGGAATGGGC
st3Gal4-alle1	-5bp	GGCAGCCTCCAGTGTCGTCgttgt---------gGTGGGGAATGGGC
st3Gal4-alle2	+4bp	GGCAGCCTCCAGTGTCGTCgTTGTttgtgTTGTGGTGGGGAATGGGC
st3Gal6-WT	ZFN	CGGTACCTCTGATTTTGCTttgccCTATGGGACAAGGCC
st3Gal6-alle1	-4bp	CGGTACCTCTGATTTTGCTt----CTATGGGACAAGGCC
st3Gal6-alle2	-2bp	CGGTACCTCTGATTTTGCTttg--CTATGGGACAAGGCC

KO:fut8

fut8-WT	CRISPR	CAAATACTTGATCCGTCCACAACCT-TGGCTGGAAAGGGAA
fut8-alle1	-1bp	CAAATACTTGATCCGTCCACAACC--TGGCTGGAAAGGGAA
fut8-alle2	+1bp	CAAATACTTGATCCGTCCACAACCTTTGGCTGGAAAGGGAA

KO:fut8/B4galt1

fut8-WT	CRISPR	CAAATACTTGATCCGTCCACAACCT-TGGCTGGAAAGGGAA
fut8-alle1	-4bp	CAAATACTTGATCCGTCCACAA-----GGCTGGAAAGGGAA
fut8-alle2	+1bp	CAAATACTTGATCCGTCCACAACCTTTGGCTGGAAAGGGAA
B4galt1-WT	ZFN	TGCATCCGGTCCTACAGCgccagc----AACTGGACTATGGTA
B4galt1-alle1	+4bp	TGCATCCGGTCCTACAGCgccagcCAGCAACTGGACTATGGTA

KO:mgat3

mgat3-WT	ZFN	TTCCTGGACCACTTCCCAcccggt----------GGCCGGCAGGATGGC
mgat3-alle1	-21bp	TTCCTGGACCACT------------------------------ATGGC
mgat3-alle2	+293bp	TTCCTGGACCACTGATACcc--+293bp--cggtGGCCGGCAGGATGGC

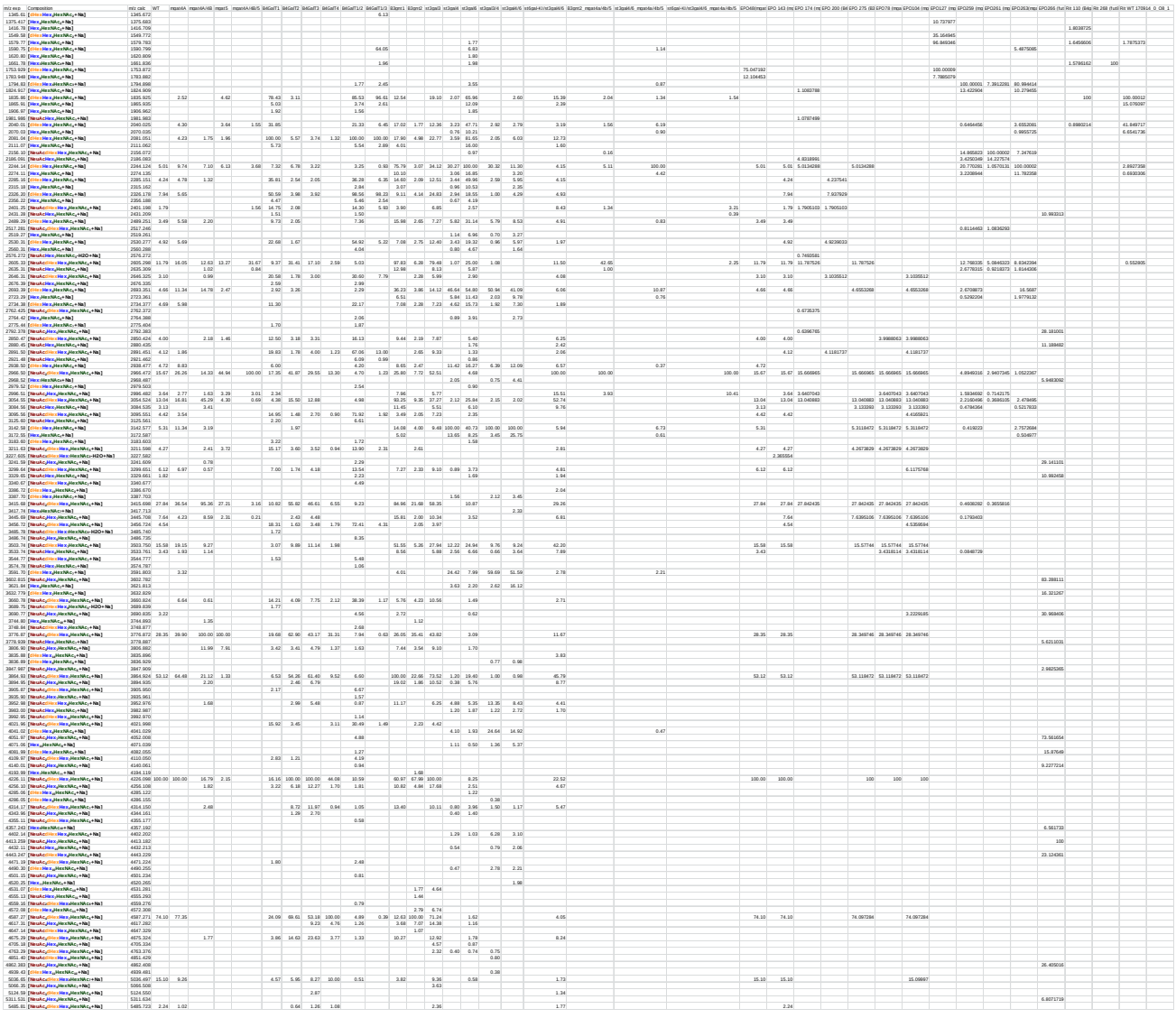
KO:mgat4C

mgat4C-WT	ZFN	ATACTTCAGACTATtatgtAATGCTCGAAGATGATGTT
mgat4C-alle1	-13bp	ATACTTCAGACT-------------CGAAGATGATGTT

mgat4C-alle2	-8bp	ATACTTCAGACTAT--------GCTCGAAGATGATGTT
KO:mgat5B		
mgat5B-WT	ZFN	CGTGGCGCCCTCCGCAAGatgagtGACCTGCTGGAGCTG
mgat5B-alle1	-7bp	CAGCTCCAGCAGGT-------CTTGCGGAGGGCGCCACG
mgat5B-alle2	-5bp	CAGCTCCAGCAGGT-----ATCTTGCGGAGGGCGCCACG
KO:B3gnt8		
B3gnt8-WT	TALEN	TGGTCCAGAGATAGCTAATgaagcttctagggtgGAGAAGCTGGGGCTGCTGA
B3gnt8-alle1	-17bp	TGGTCCAGAGG----------AGGGTGGAGAAGCTGGGGCTGCTGA
KO:mgat1		
mgat1-WT	ZFN	AACAAGTTCAAGTTCccagcaGCTGTGGTAGTGGAGGAC
mgat1-alle1	-2bp	AACAAGTTCAAGTTCc--gcaGCTGTGGTAGTGGAGGAC

¤ Gene targeting region underlined.

Supplementary Table 3. Summary of glycan composition list of permethylated N-glycans.



Supplementary Table 4. Production of recombinant human EPO and IgG in CHO clones.

EPO clones	**Final yield (mg/L)**¤
WT	3.2
KO:*B4galt1*	2.2
KO:*B4galt2*	1.7
KO:*B4galt3*	0.7
KO:*B4galt4*	1.3
KO:*B4galt1/2*	0.6
KO:*B4galt1/3*	0.2
KO:B4galt1/2/3	0.4
KO:*st3gal3*	1.5
KO:*st3gal4*	2.9
KO:*st3gal6*	0.4
KO:*st3gal3/4*	3.5
KO:*st3gal4/6*	6.2
KO:*B3gnt1*	2.3
KO:*B3gnt2*	0.5
KO:*B3gnt8*	2.4
KO:*mgat4A*	1.1
KO:*mgat4A/4B*	2.5
KO:*mgat5*	1.0
KO:*mgat4A/4B/5*	4.3
KO:*mgat4B*	6.8
KO:*mgat1*	6.3
KO:*mgat2*	5.0
KO:*mgat3*	2.1
KO:*mgat4C*	10.4
KO:*mgat5B*	3.2
KO:*B3gnt2/mgat4A/4B/5*	3.4
KO:*st3gal4/6/mgat4A/4B/5*	1.3
KO:B3gnt2/*st3gal4/6/mgat4A/4B/5*	3.7
KI: ST6GAL-I/KO:*st3gal4/6/mgat4A/4B/5*	0.2
KI: ST6GAL-I/KO:*st3gal4/6*	0.8
KO:*st3gal4/6/mgat2*	10.3
KO:*mgat2/4A/4B/5*	1.5
KO:*st3gal4/6/mgat2/4A/4B/5*	0.4
KO:*fut8*	2.3
IgG clones	**Final yield (mg/L)**¤
WT (Rituximab)	2.5
KO:*B4galt1* (Rituximab)	0.9
KO:*fut8* (Rituximab)	1.0
KO:*fut8/B4galt1* (Rituximab)	3.4
WT (SO57)	857
KO:*fut8/B4galt1* (SO57)	1,187

¤Final yields represent purified proteins after NiNTA and HPLC purification for EPO after Protein G and ultrafiltration for IgG Rituximab. The SO57 cell line (clone 27) is a CHO-GS cell line producing anti-rabies IgG1 developed by Sigma[12]. The production yield of IgG SO57 was determined by analysis of culture medium by ELISA using an internal IgG standard.

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