Supporting Information

Reference glycan structure libraries of primary human cardiomyocytes and pluripotent stem cell-derived cardiomyocytes reveal cell-type and culture stage-specific glycan phenotypes

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Supplemental Methods

Table S1. Donor information

Anonymized Donor Identification Number	Sex	Age	Cause of Death
10960	F	75	Stroke
11225	M	58	Gunshot wound to head
11414	M	57	Intracranial hemorrhage

Table S2. Chromatography and MS Instrument Acquisition Settings

	***N*-glycan analysis**	***O*-glycan analysis**
Sample Amount for glycan release	64 µg of protein	
Sample Volume Prepared, Injected	59 µL dried glycans dissolved in solvent A + 1 µL Dextran Ladder ISTD, 20 µL	
Injection Mode	Full Loop Direct Inject	
Sample Loop	20 µL	
Stationary Phase	Thermo Scientific Hypercarb PGC 250 Å, 180 µm x 10 cm, 3 µm	
LC Solvent A	100% H_2O 10 mM Ammonium Bicarbonate	
LC Solvent B	90% MeCN, 10 mM Ammonium Bicarbonate	
LC Gradient	0-25% B in 70 min 100% B for 10 min 0% B for 10 min	
LC Flow Rate	2 µL/min	
Column Temperature	80 ^{o}C	40 ^{o}C
Make-up Solvent	100% MeOH	
Make-up Flow Rate	2 uL/min for 75 min 1 uL/min for 15 min	
Mass Spectrometer	Thermo Orbitrap Velos	
Method Type	Top 9 Data Dependent MS2	
Spray Voltage	2 kV	
MS^1 Detector	Orbitrap	
MS^1 Scan Range	570-2000 *m/z*	500-2000 *m/z*
MS^1 Resolution	15,000 @ 200 m/z	
MS^1 AGC Target	1e6	
MS^1 Maximum IT	100 ms	
MS^2 Detector	IonTrap	
MS^2 Scan Range	Auto Normal	
MS^2 Resolution	Normal (0.5 *m/z* FWHM)	
Isolation Window	2 *m/z*	
MS^2 AGC Target	1e5	
MS^2 Maximum IT	150 ms	
Activation Type / Collision Energy	CID 33%, Wideband Activation	
Minimum Signal Req.	50	
Dynamic Exclusion	30 s,10 Repeats, 5 s Exclusion Duration	

Table S3. Flow cytometry sample preparation and data acquisition details.

Sample Information	
Cell type(s)	hiPSC-CM
Cell line(s)	DF6-9-9T
Passage #	
Dissociation Conditions:	1 mL of 0.5U/mL Liberase-TH (Sigma #5401135001), 50U/mL DNase I (Sigma #10104159001) in RPMI (Thermofisher #11875-093) for 30 min at 37 °C, followed by addition of 1 mL of TrypLE (TrypLE Thermofisher #12605-010) for 5 min at 37 °C
Total Cell Counts	6.5 – 13 x 10^6
# of Cells per Tube	1 x 10^6

Protocol Steps	**Time**	**Reagents**	**Recipe, Catalog #s**
1. Fixation	20 min	**Wash Solution**	DPBS-/- (Sigma #D8537)
2. Wash	two x 3 mL	**Fixation Solution**	2% Formaldehyde (w/v) (ThermoFisher #28906) in 1X DPBS-/-
3. Permeabilization	Performed as one 15 min incubation	**Block Solution**	0.5% w/v BSA (Sigma #A7906) in DPBS-/-
4. Wash		**Permeabilization**	0.5% Saponin (w/v) (Sigma #47036) in Block Solution
5. Block		**Resuspension Solution**	0.5% w/v BSA in DPBS-/-
6. 1°Antibody	45 min		
7. Wash	two x 3 mL		
8. Resuspension	500 µL		

Antibodies				
Target	**Clone**	**Vendor**	**Catalog #**	**Lot #**
Troponin T2	1C11	Abcam	ab105439	various
Isotype control	-	eBiosciences	11-4714	various

Instrument Configuration	
Instrument	BD LSR II
Laser line	488nm (50mw)
Emission filter	525/50
Fluorochrome	FITC

Table S4. Glycopeptides identified from Mills et al. which feature glycan compositions that were positively correlated to time of hiPSC-CM differentiation in our study.

Protein	Peptide	Starting AA	Glycan Composition	Sample
CERU_HUMAN	K.EN[+1914.697]LTAPGSDSAVFFEQGTTR.I	396	HexNAc(4)Hex(5)Fuc(2)	**Heart tissue**
HPT_HUMAN	K.VVLHPN[+1914.697]YSQVDIGLIK.L	236	HexNAc(4)Hex(5)Fuc(2)	**Heart tissue**
A1AT_HUMAN	K.YLGN[+1914.697]ATAIFFLPDEGK.L	268	HexNAc(4)Hex(5)Fuc(2)	**Heart tissue**
FETUA_HUMAN	K.AALAAFNAQNN[+1914.697]GSNFQLEEISR.A	166	HexNAc(4)Hex(5)Fuc(2)	**Heart tissue**
HRG_HUMAN	R.VIDFN[+1914.697]C[+57.021]TTSSVSSALANTK.D	121	HexNAc(4)Hex(5)Fuc(2)	**Heart tissue**
PGS2_HUMAN	K.LGLSFNSISAVDN[+2100.761]GSLANTPHLR.E	250	HexNAc(5)Hex(4)Fuc(1)NeuAc(1)	**3D Organoid**
CO6A2_HUMAN	R.GTFTDC[+57.021]ALAN[+1914.697]MTEQIR.Q	131	HexNAc(4)Hex(5)Fuc(2)	**Heart tissue**
CSPG2_HUMAN	R.FEN[+1914.697]QTGFPPPDSR.F	328	HexNAc(4)Hex(5)Fuc(2)	**3D Organoid**
LAMA2_HUMAN	R.YMQN[+1914.697]LTVEQPIEVK.K	2645	HexNAc(4)Hex(5)Fuc(2)	**3D Organoid**
LAMA2_HUMAN	K.N[+1914.697]ESGIILLGSGGTPAPPR.R	2558	HexNAc(4)Hex(5)Fuc(2)	**3D Organoid**
BCAM_HUMAN	R.TQN[+1914.697]FTLLVQGSPELK.T	437	HexNAc(4)Hex(5)Fuc(2)	**Heart tissue**
PGBM_HUMAN	R.SLTQGSLIVGDLAPVN[+1914.697]GTSQGK.F	3765	HexNAc(4)Hex(5)Fuc(2)	**3D Organoid**
PGBM_HUMAN	R.NQELEDNVHISPN[+1752.645]GSIITIVGTRPSNHGTYR.C	3060	HexNAc(4)Hex(5)Fuc(2)	**3D Organoid**
COEA1_HUMAN	R.SFMVN[+1914.697]WTHAPGNVEK.Y	368	HexNAc(4)Hex(5)Fuc(2)	**Heart tissue**

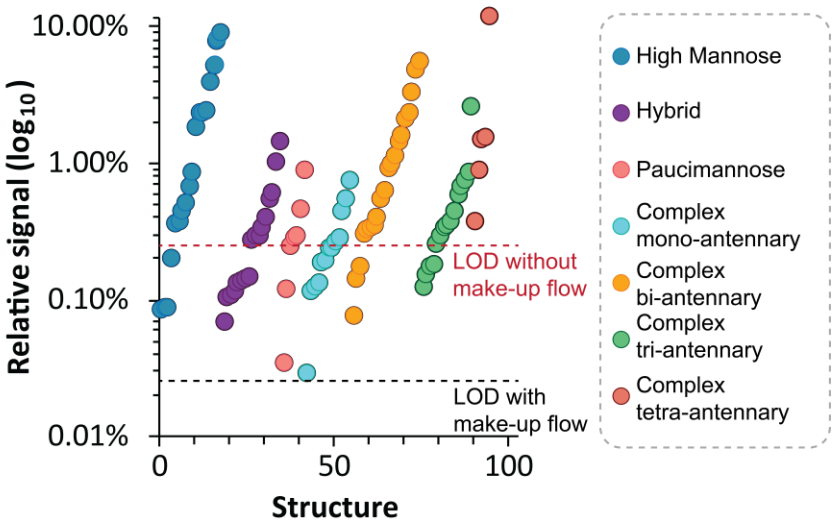


Figure S0. Effect of make-up flow on glycan structure limit of detection

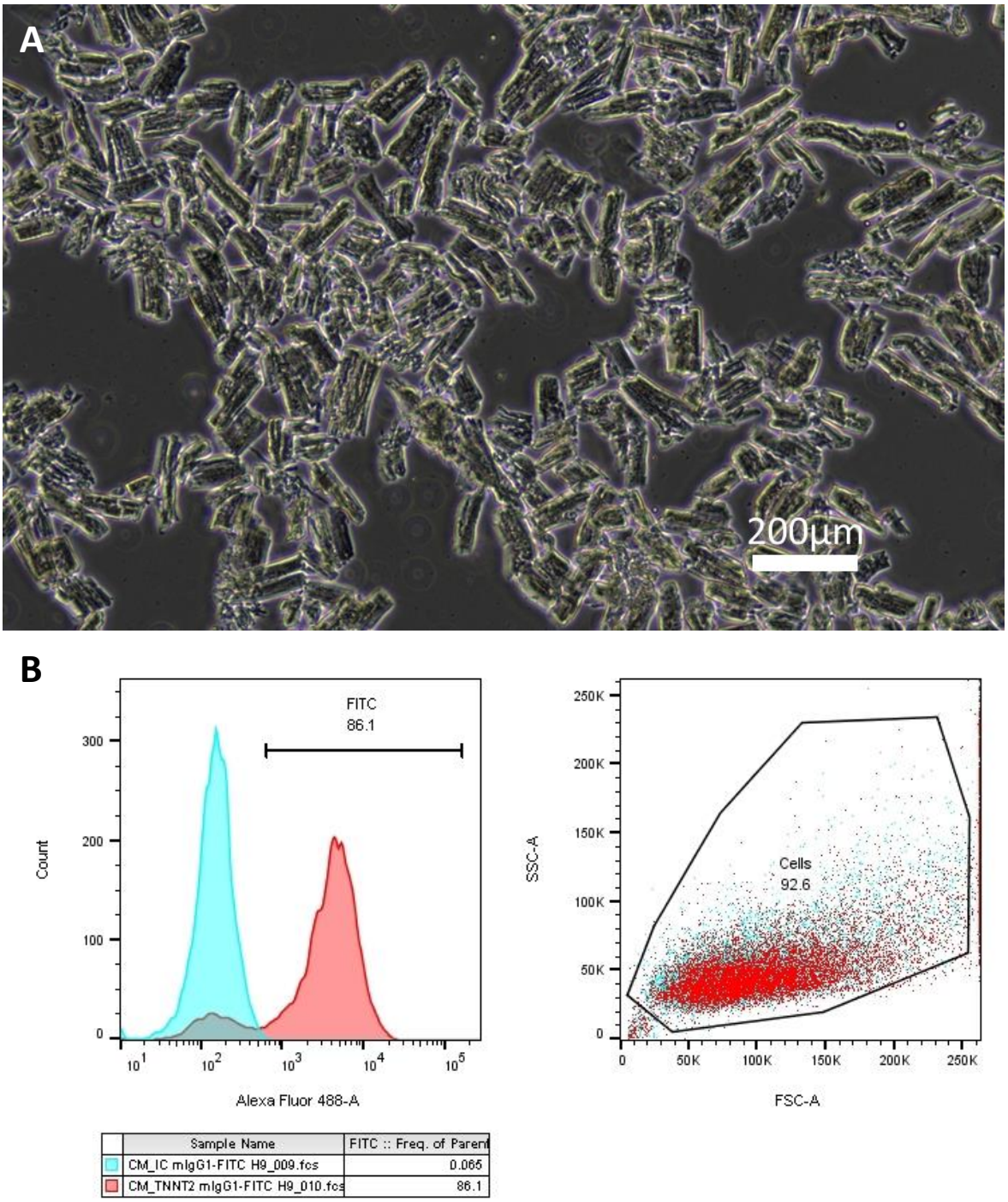


Figure S1. Examples of quality assessment for enriched primary CM and hiPSC-CM. **A** Bright-field image of cardiomyocyte-enriched samples from human heart tissue. **B** Representative example of percent troponin positivity in hiPSC-CM as determined by flow cytometry (isotope control is represented in blue, antibody for troponin is represented in red)

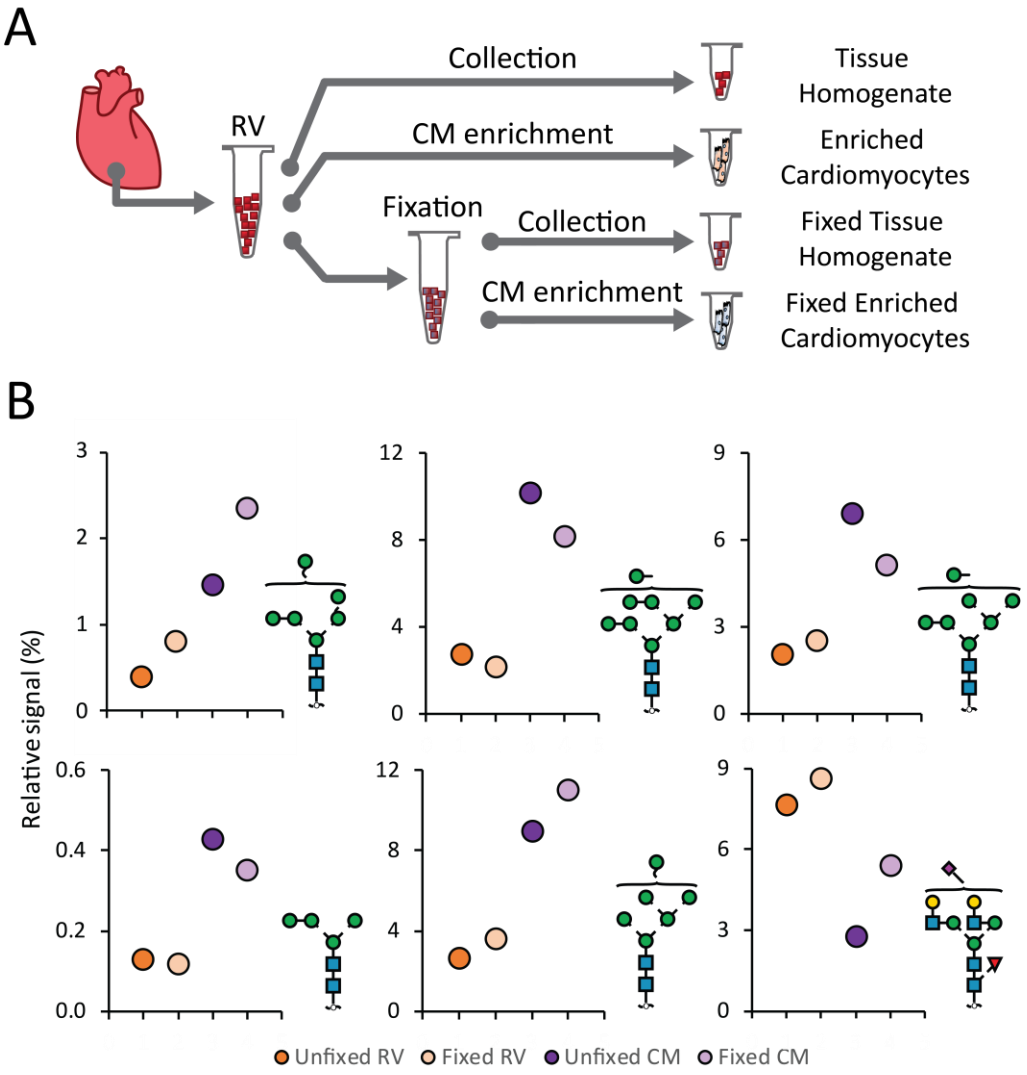


Figure S2. Evaluation of the possibility of artifacts owing to residual enzymatic activity during CM enrichment. **A** Experimental design to compare fixed and unfixed heart tissue homogenate and enriched CM. **B** Quantitative analysis of enriched CM glycans compared to cardiac tissue homogenate. For each sample type, no major differences are observed between unfixed and fixed samples.

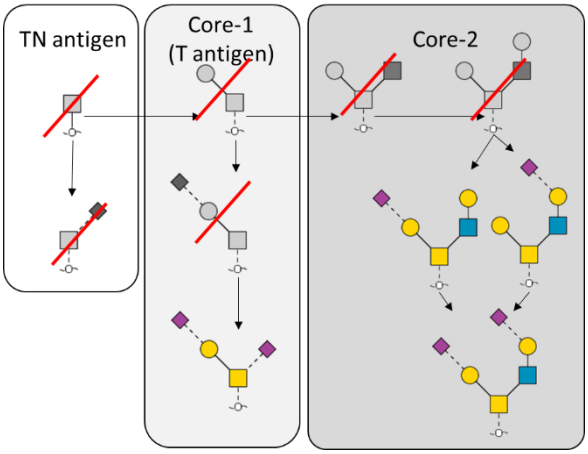


Figure S3. Identified *O*-glycan structures in primary CM and whole heart tissue

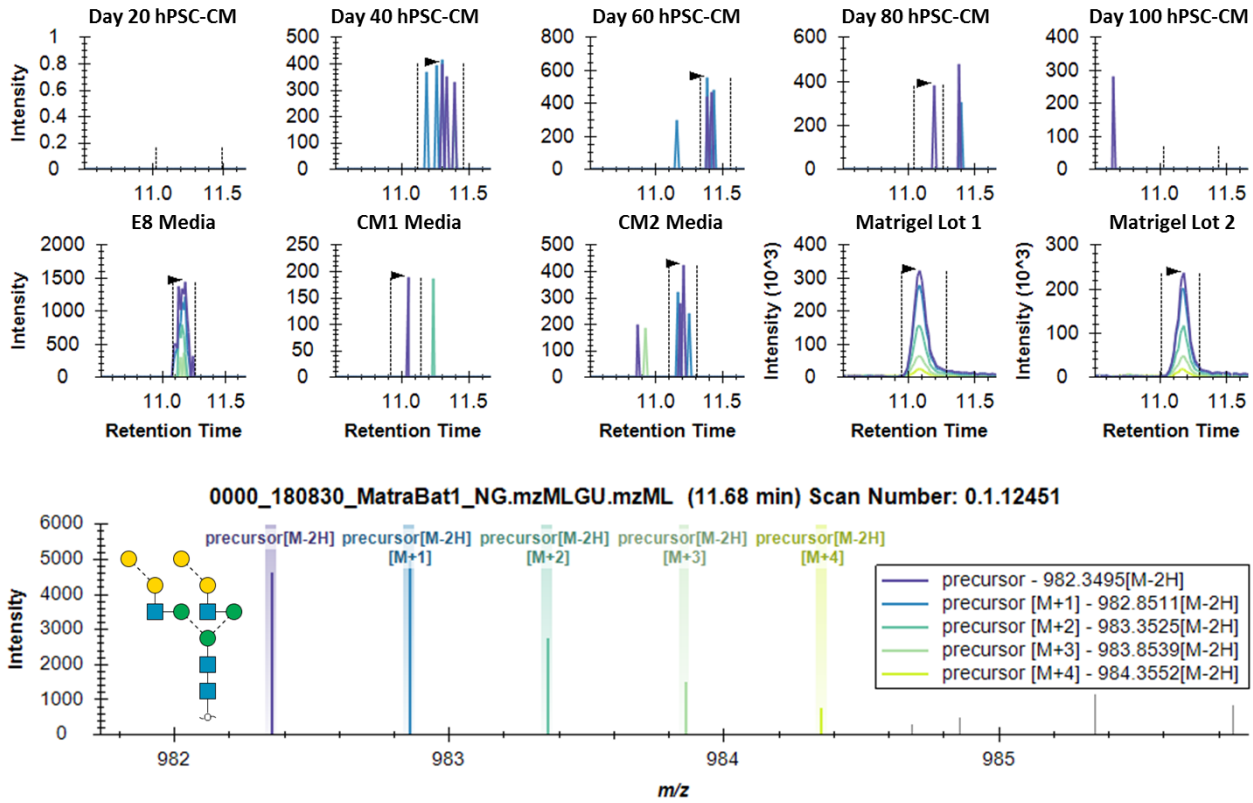


Figure S4. Targeted data analysis evaluating the presence of a culture component non-human *N*-glycan structure (di-gal motif) in hiPSC-CM samples

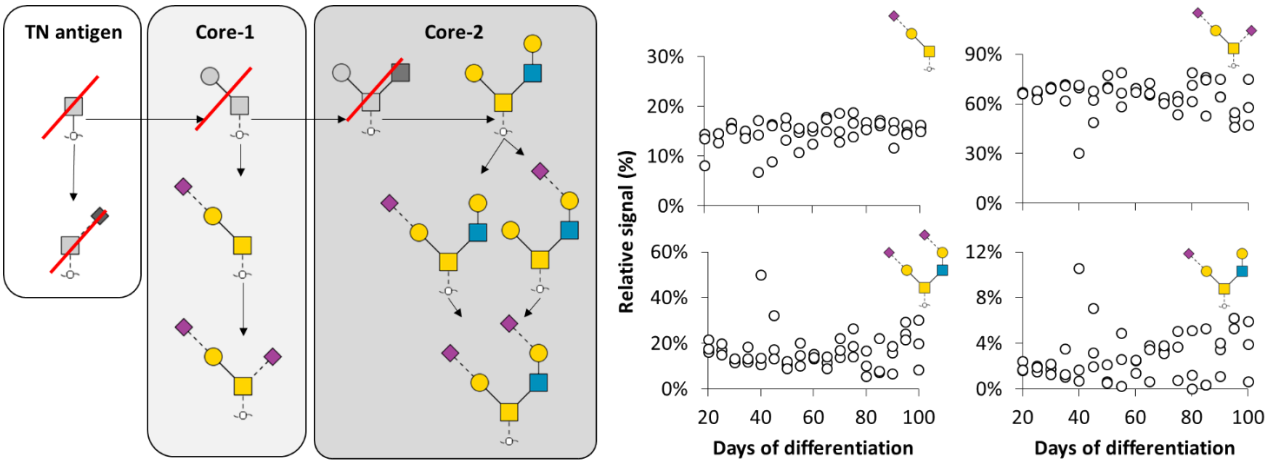


Figure S5. Identified *O*-glycan structures in hiPSC-CM and relative signal of quantitative glycan structures over the differentiation period

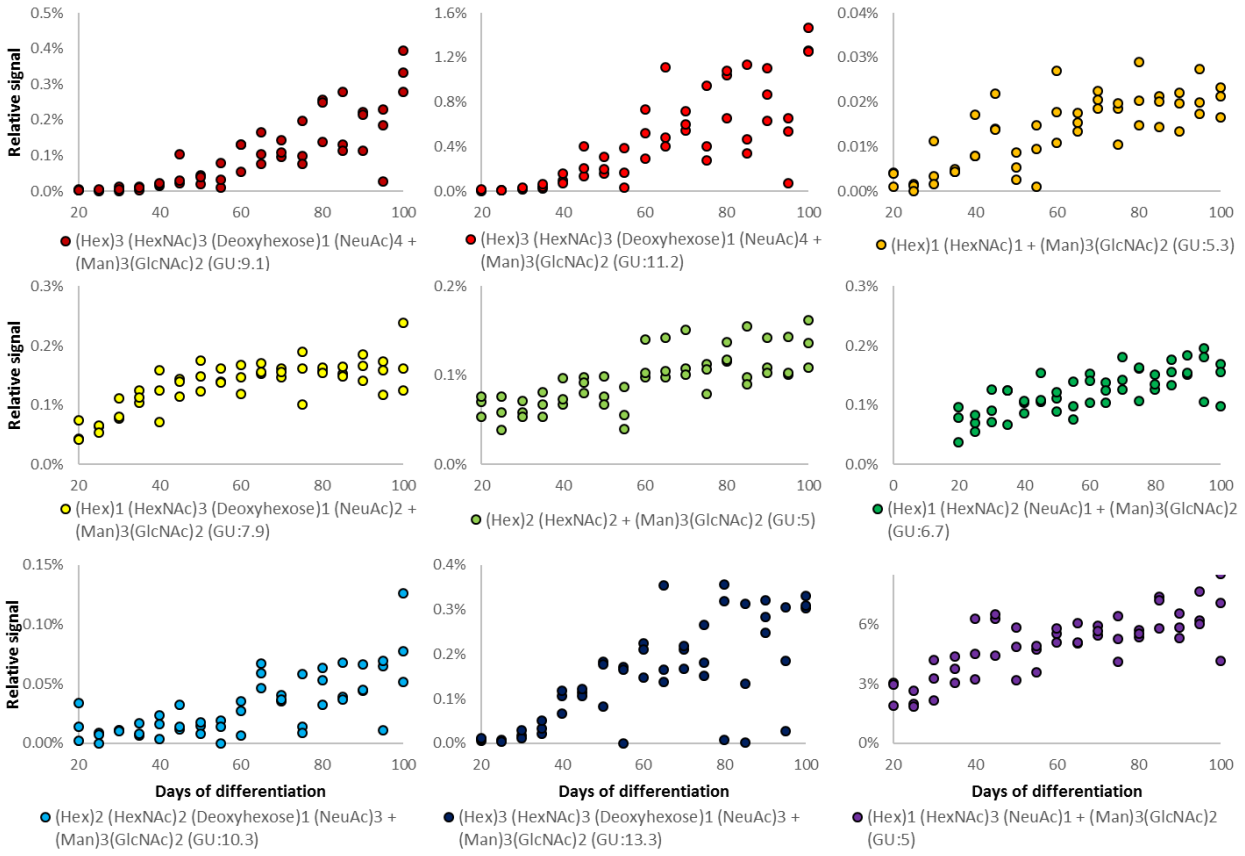


Figure S6. Scatter plot of relative signal for *N*-glycans from hiPSC-CM significantly positively correlated (>0.7) to days of differentiation

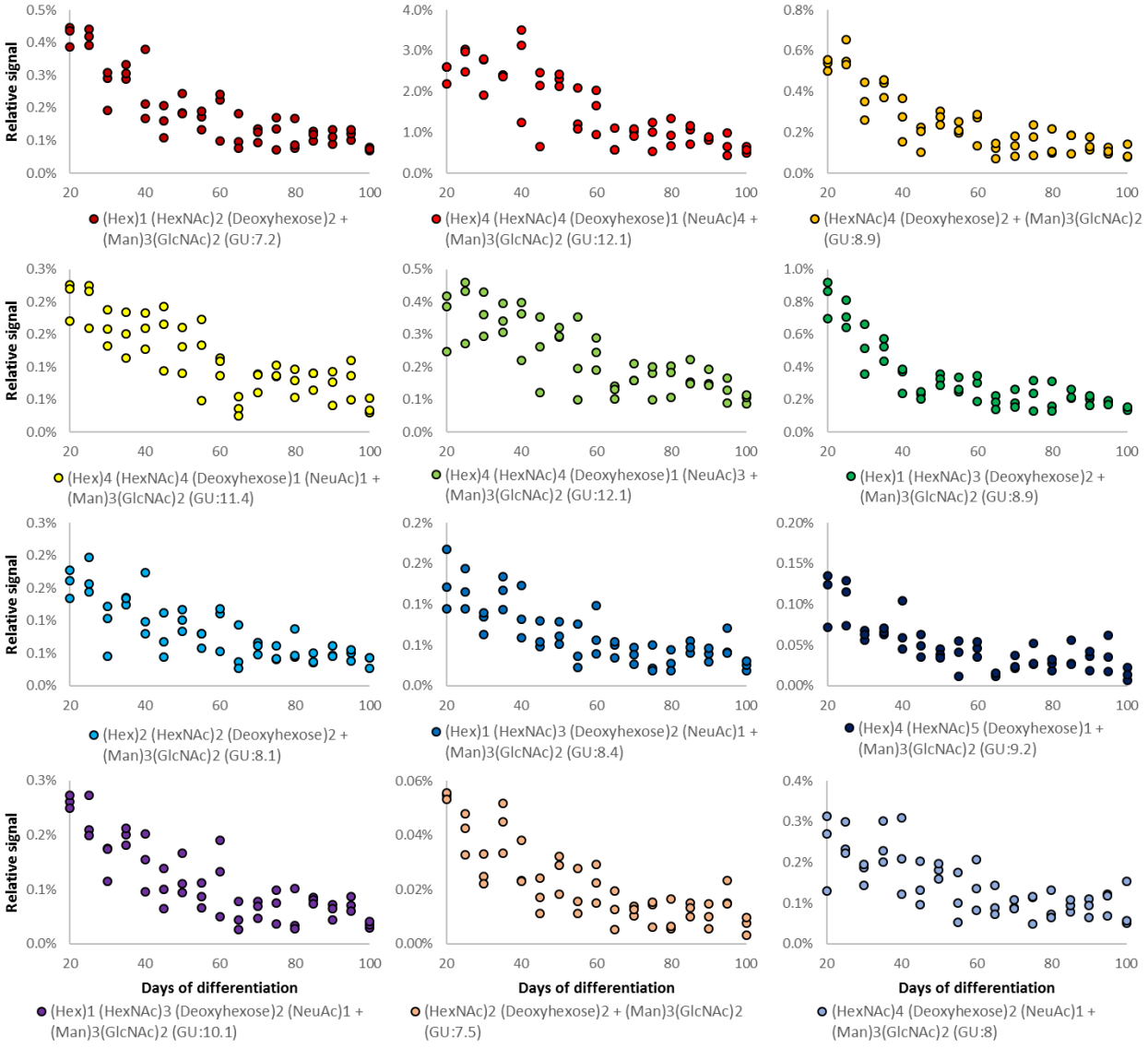


Figure S7. Scatter plot of relative signal for *N*-glycans from hiPSC-CM significantly negatively correlated (<-0.7) to days of differentiation

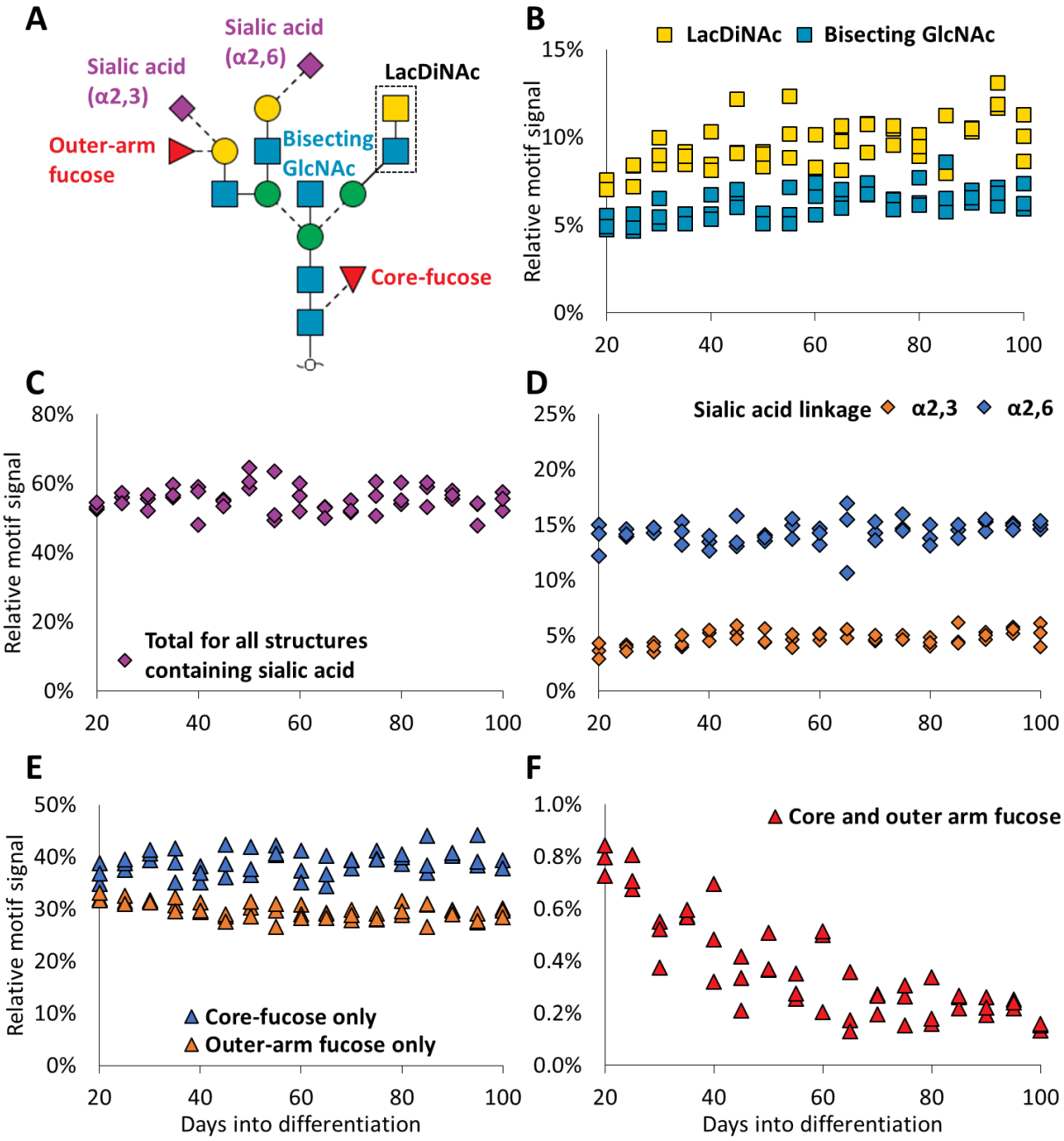


Figure S8. A majority of glycan structural motifs do not significantly change among hiPSC-CM collected throughout 100 days of differentiation. **A** Model *N*-glycan structure with motifs mapped to structure. **B-F** Scatter plots of relative signal for *N*-glycan structural motifs shown in A.

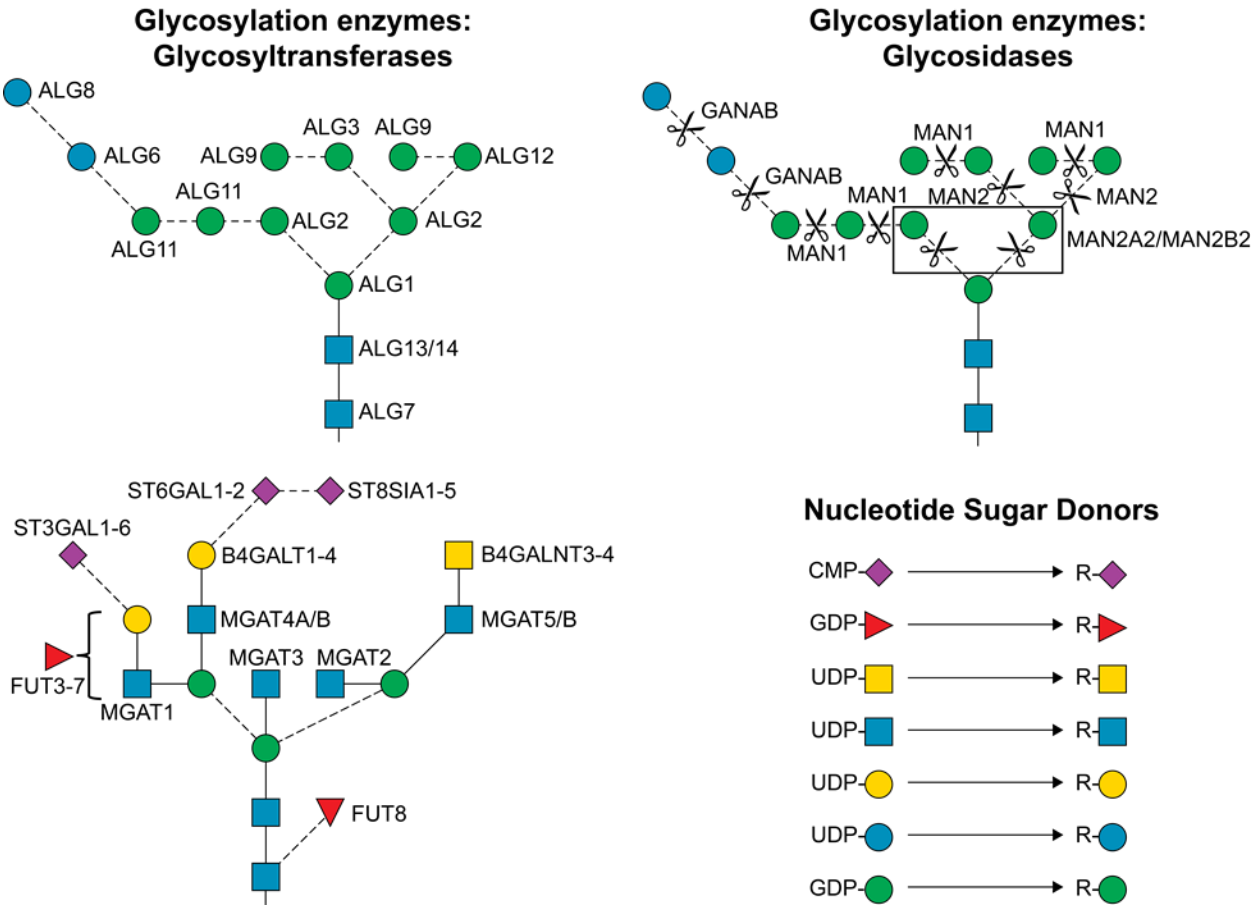
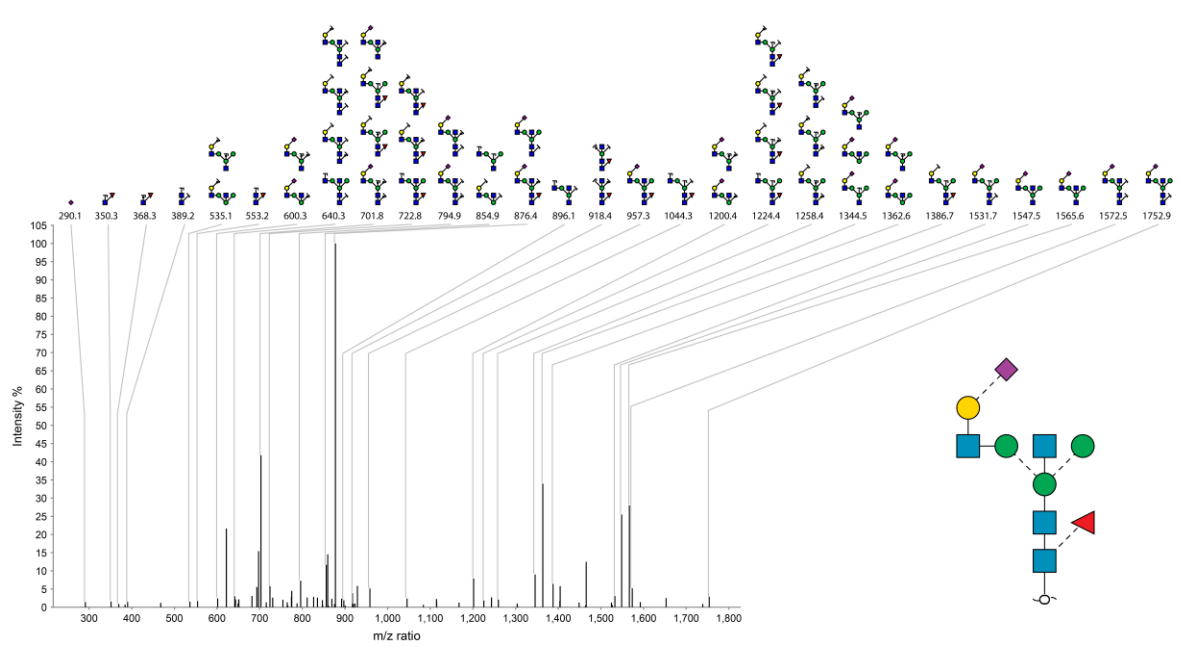
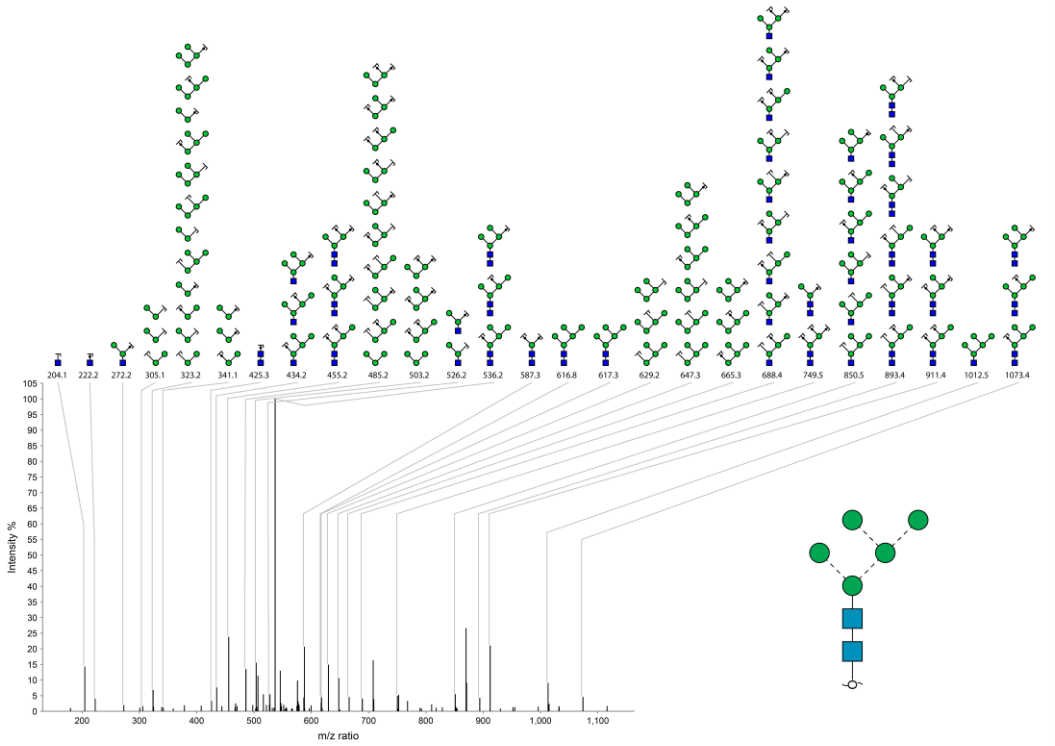


Figure S9. Map of the enzymes and metabolites responsible for identified and quantified glycan structures of homogenized heart tissue, primary CM and hiPSC-CM


290.1 350.3 368.3 389.2 535.1 553.2 600.3 640.3 701.8 722.8 794.9 854.9 876.4 896.1 918.4 957.3 1044.3 1200.4 1224.4 1258.4 1344.5 1362.6 1386.7 1531.7 1547.5 1565.6 1572.5 1752.9
Intensity %
m/z ratio


204.1 222.2 272.2 305.1 323.2 341.1 425.3 434.2 455.2 485.2 503.2 526.2 536.2 587.3 616.8 617.3 629.2 647.3 665.3 688.4 749.5 850.5 893.4 911.4 1012.5 1073.4
Intensity %
m/z ratio

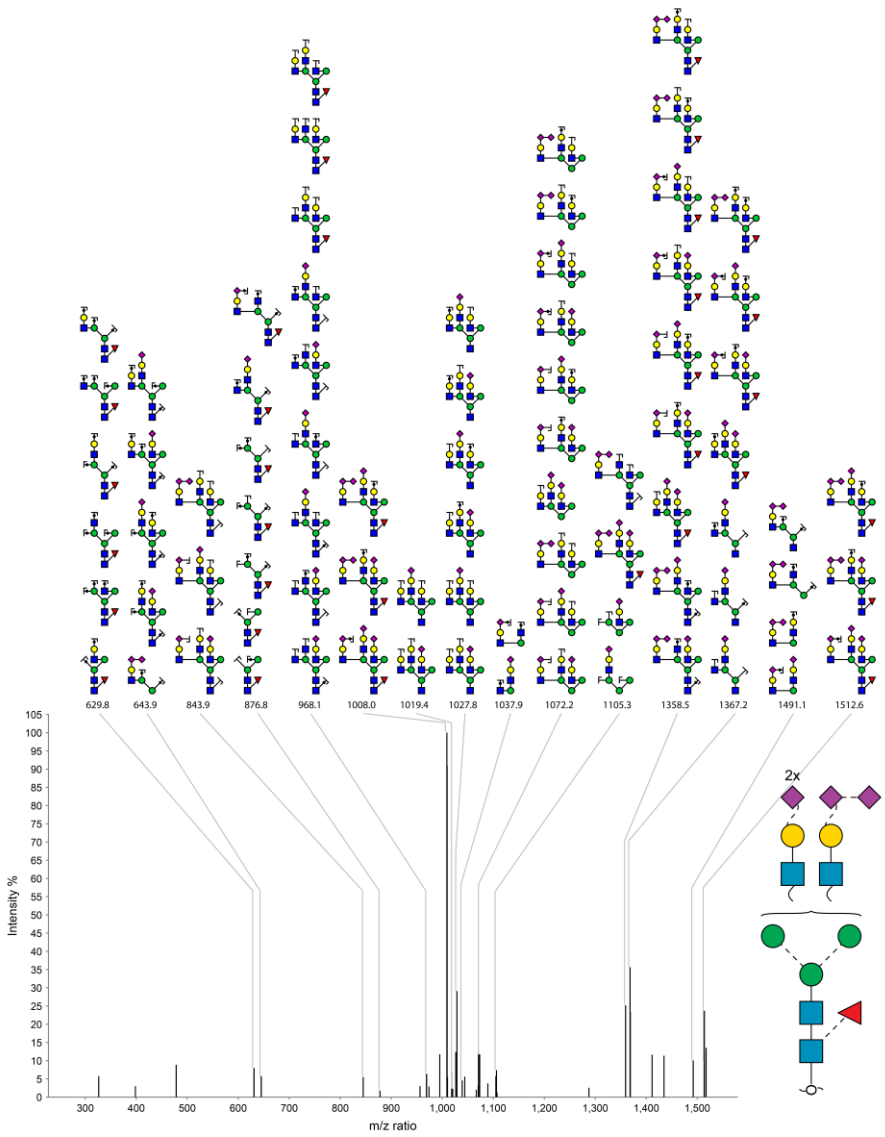


Figure S10. Example annotated MS2 spectra for several glycan structures in Figures 4 and 5.