

Supplementary Information

Essential roles of FUT8 SH3 domain in core fucosylation

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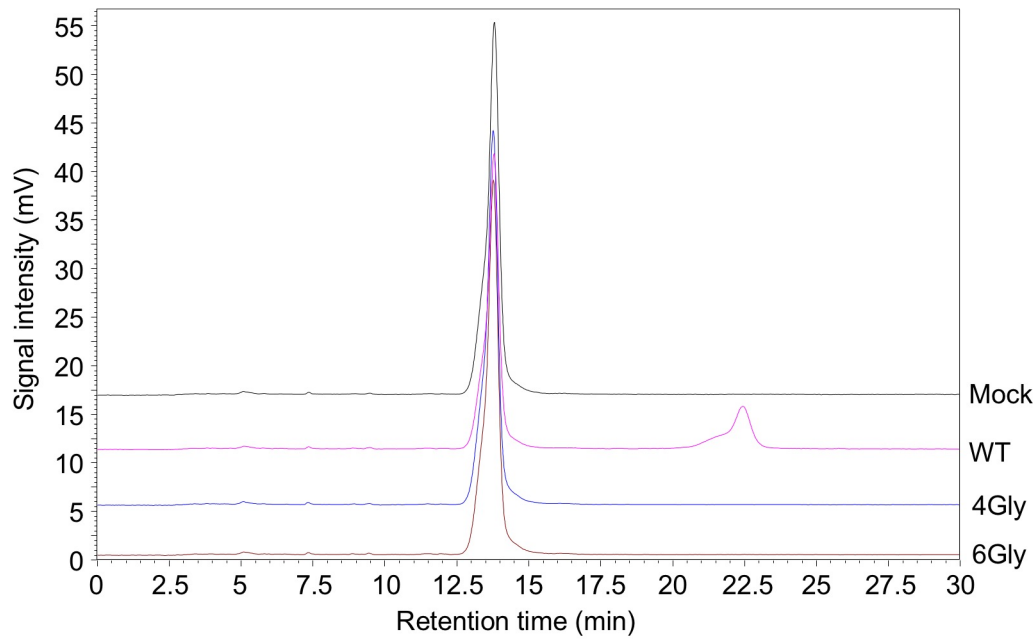
Email: kizuka@gifu-u.ac.jp

This Supplemental information includes:

Fig. S1-6 (included in this PDF)

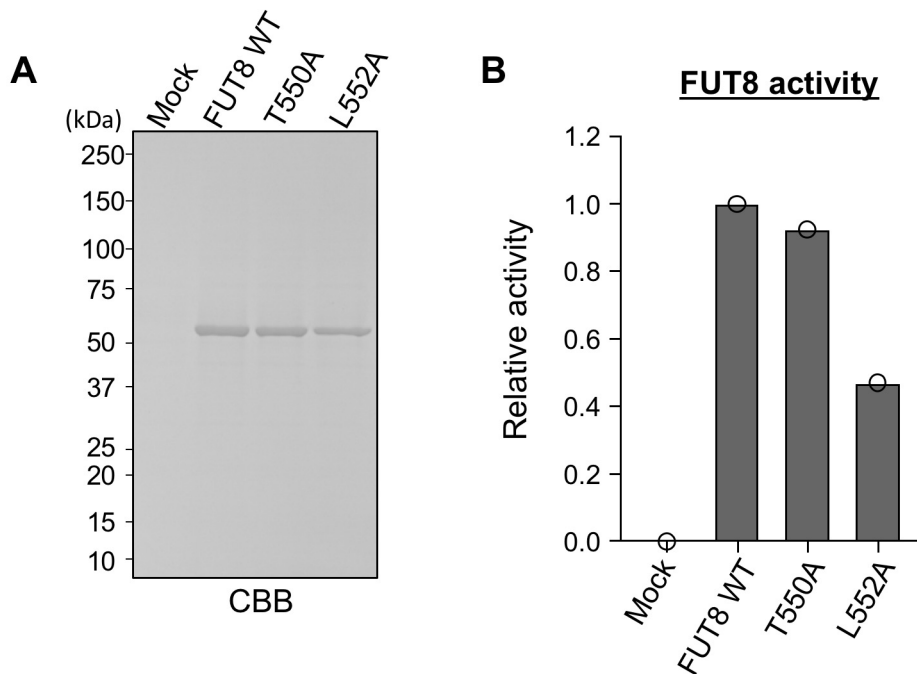
Tables S1, S2 (included in this PDF)

Figure S1



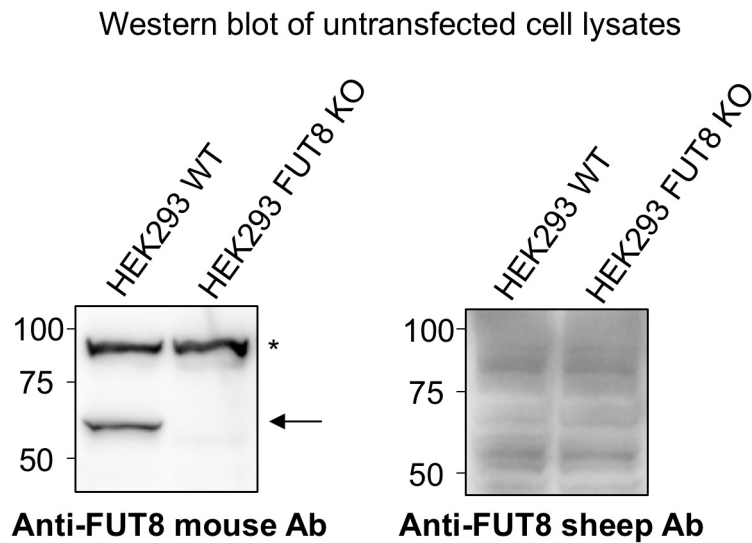
Supplemental Fig. S1. Wider view of Fig. 1F chromatogram.

Figure S2



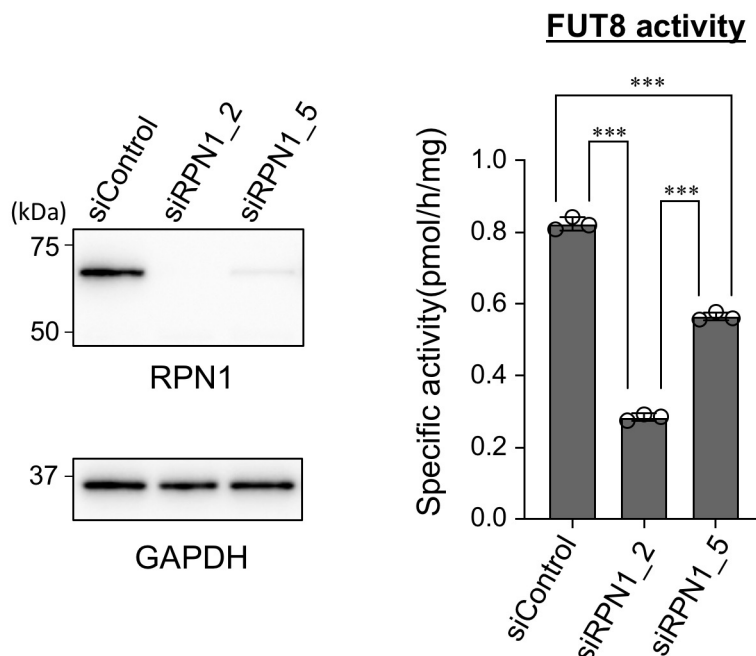
Supplemental Fig. S2. Activity of T550A and L552A single mutants. (A) Soluble forms of two FUT8 point mutants (T550A and L552A) were expressed and purified from the COS7 culture media, and then subjected to CBB staining. (B) Purified point mutants were mixed with the FUT8 substrates, and the reaction mixtures were separated by HPLC. The activity relative to that of FUT8 WT was calculated by the peak area.

Figure S3



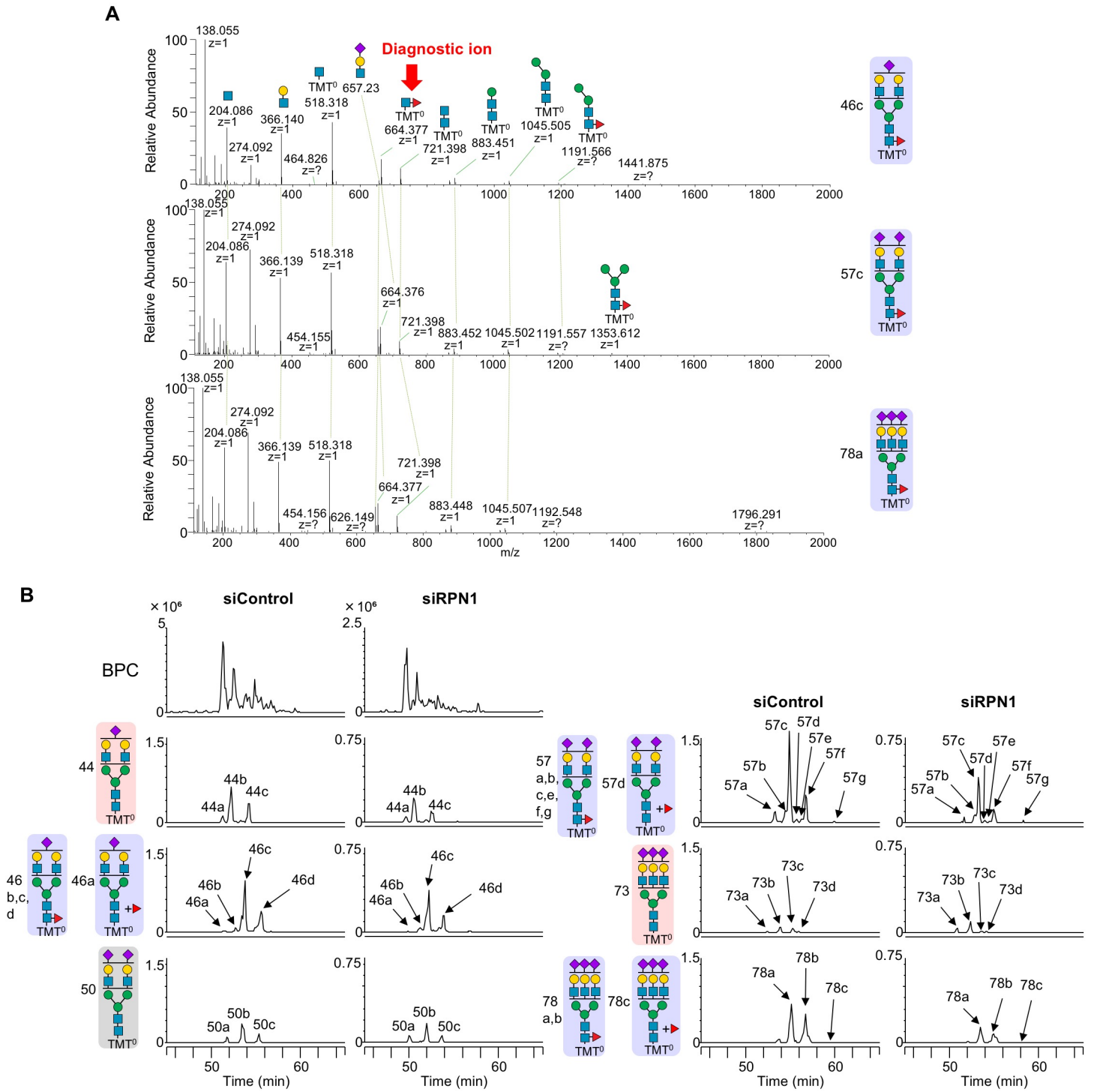
Supplemental Fig. S3. Validation of the antibody specificities for detection of endogenous FUT8. HEK293 WT or FUT8 KO cell lysates were analyzed by western blotting with mouse or sheep anti-FUT8 Ab. Asterisk and arrow indicates non-specific binding and FUT8, respectively.

Figure S4



Supplemental Fig. S4. Reduced FUT8 activity of RPN1-knockdown. (A) HEK293 WT cells were treated with two kinds of siRPN1 or siControl for 96 h. The cell lysates were analyzed by western blotting with anti-RPN1, and anti-GAPDH Abs. (B) The lysates of HEK293 WT cells treated with two kinds of siRPN1 or siControl (96 h) were incubated with the FUT8 acceptor substrate and GDP-Fuc, and the acceptor substrates and products were separated by HPLC. The FUT8 specific activities were calculated by the peak areas and shown as the mean \pm SD ($n = 3$, triplicates from one sample, *** $p < 0.001$, Tukey-Kramer test).

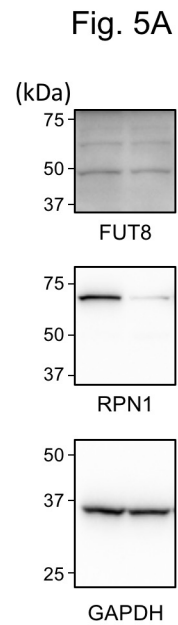
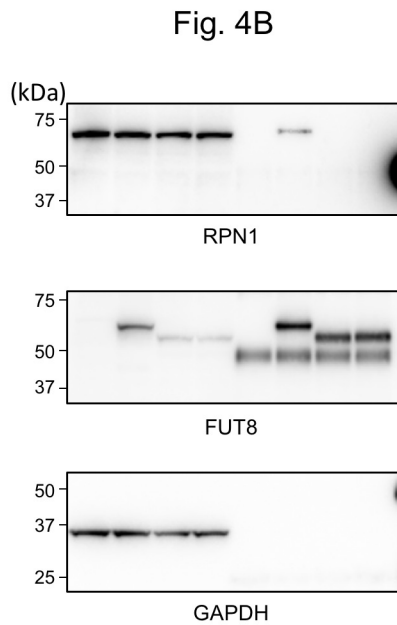
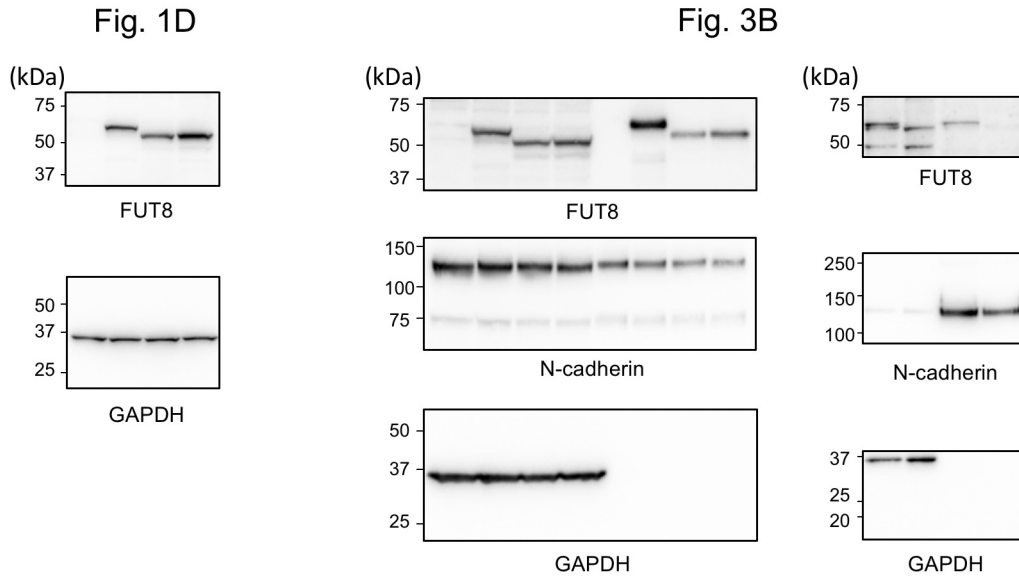
Figure S5



Supplemental Fig. S5. LC-MS analysis of *N*-glycans from siRPN1-treated HEK293 cells. (A) MS/MS analysis of the representative core-fucosylated *N*-glycans from HEK293 cells treated with siControl. The diagnostic ions for core fucose structure are indicated. (B) Extracted ion chromatograms (EICs) of the major non-fucosylated and fucosylated *N*-glycans shown in Fig. 6C.

Figure S6

Image data of Western blotting used in the figures



Supplemental Table S1

Precursor ions with a diagnostic ion for core fucose were indicated in yellow.

No.	glycoform mass	calculated mass (Δ 10 ppm)				structure	Charge	190110608	19110609	190110608	19110609	
		[M+H]	[M+2H] ²	[M+3H] ³	[M+4H] ⁴			HEK293_	HEK293_	HEK293_	HEK293_	
								siControl_	siRPN1_	siControl_	siRPN1_	
							TMT0	TMT0	TMT0	TMT0		
							peak intensity in EIC		relative abundance(%)			
1	a	1216.423	1531.663	766.335	511.226	383.672	(Hex)2 + (Man)3(GlcNAc)2	1	39795	16823	0.13	0.10
	b								22245	5710	0.07	0.03
	c								48658	35764	0.16	0.21
2	a	1378.476	1693.716	847.362	565.244	424.185	(Hex)3 + (Man)3(GlcNAc)2	2	279454	133512	0.90	0.79
	b								2661525	1197631	8.57	7.07
	c								50365	96731	0.16	0.57
3	a	1540.528	1855.768	928.388	619.261	464.698	(Hex)4 + (Man)3(GlcNAc)2	2	46035	18010	0.15	0.11
	b								1454631	577197	4.68	3.41
	c								832863	370020	2.68	2.18
4	a	1702.581	2017.821	1009.414	673.279	505.211	(Hex)5 + (Man)3(GlcNAc)2	2	118225	59198	0.38	0.35
	b								4210995	1405139	13.56	8.29
	c								92626	54187	0.30	0.32
5	a	1864.634	2179.874	1090.441	727.296	545.724	(Hex)6 + (Man)3(GlcNAc)2	2	143221	91173	0.46	0.54
	b								3831980	1897655	12.34	11.20
	c								23618	14562	0.08	0.09
6	a	2026.687	2341.927	1171.467	781.314	586.238	(Hex)7 + (Man)3(GlcNAc)2	2	388514	285632	1.25	1.69
	b								0	25816	0.00	0.15
	c								0	54574	0.00	0.32
7	a	892.317	1207.557	604.282	403.191	302.645	(Hex)3(HexNAc)2	1	0	56681	0.00	0.33
	b								28586	16082	0.09	0.09
	c								18913	10233	0.06	0.06
	d								33723	14329	0.11	0.08
	e								21158	6779	0.07	0.04
	f								23497	32461	0.08	0.19
	g								33052	62341	0.11	0.37
8	a	1038.375	1353.615	677.311	451.877	339.160	(Hex)3 (HexNAc)2 (Deoxyhexose)1	1	17002	7808	0.05	0.05
	b								15868	10121	0.05	0.06
	c								23272	7191	0.07	0.04
9	a	1257.449	1572.689	786.848	524.901	393.928	(Hex)1 (HexNAc)1 + (Man)3(GlcNAc)2	1	49614	122000	0.16	0.72
	b								0	23015	0.00	0.14
	c								0	29653	0.00	0.17
10	a	1298.476	1613.716	807.362	538.577	404.185	(HexNAc)2 + (Man)3(GlcNAc)2	2	6001	3609	0.02	0.02
	b								439888	388284	1.42	2.29
	c								46766	11819	0.15	0.07
11	a	1419.502	1734.742	867.875	578.919	434.441	(Hex)2 (HexNAc)1 + (Man)3(GlcNAc)2	2	18535	5829	0.06	0.03
	b								39015	20628	0.13	0.12
	c								0	117090	0.00	0.69
	d								0	14922	0.00	0.09
	e								4090	18917	0.01	0.11
	f								2993	11935	0.01	0.07
12	a	1460.529	1775.769	888.388	592.595	444.698	(Hex)1 (HexNAc)2 + (Man)3(GlcNAc)2	2	6548	52091	0.02	0.31
	b								11748	20033	0.04	0.12
	c								0	16299	0.00	0.10
13	a	1501.555	1816.795	908.901	606.270	454.955	(HexNAc)3 + (Man)3(GlcNAc)2	2	33326	15078	0.11	0.09
	b								1074270	360583	3.46	2.13
	c								36433	9910	0.12	0.06
14	a	1622.582	1937.822	969.415	646.612	485.211	(Hex)2 (HexNAc)2 + (Man)3(GlcNAc)2	2	19695	5564	0.06	0.03
	b								44838	27830	0.14	0.16
	c								433215	219533	1.39	1.30
	d								49003	19065	0.16	0.11
	e								13180	25253	0.04	0.15
	f								6721	12083	0.02	0.07
15	a	1663.608	1978.848	989.928	660.288	495.468	(Hex)1 (HexNAc)3 + (Man)3(GlcNAc)2	2	44156	19959	0.14	0.12
	b								0	35622	0.00	0.21
	c								26688	14476	0.09	0.09
	d								0	18606	0.00	0.11
	e								0	4912	0.00	0.03
	f								0	7809	0.00	0.05
16	a	1825.661	2140.901	1070.954	714.305	535.981	(Hex)2 (HexNAc)3 + (Man)3(GlcNAc)2	2	66517	0	0.21	0.00
	b								7065	20411	0.02	0.12
	c								0	22626	0.00	0.13
17	a	2190.793	2506.033	1253.520	836.016	627.264	(Hex)3 (HexNAc)4 + (Man)3(GlcNAc)2	3	10107	5864	0.03	0.03
	b								28407	4751	0.09	0.03
	c								14608	72256	0.05	0.43
18	a	1241.455	1556.695	778.851	519.570	389.930	(HexNAc)1 (Deoxyhexose)1 + (Man)3(GlcNAc)2	1	19505	14737	0.06	0.09
	b								14616	8823	0.05	0.05
	c								25295	19584	0.08	0.12
	d								18146	8456	0.06	0.05
	e								5798	4522	0.02	0.03
	f								8216	16495	0.03	0.10
19	a	1362.481	1677.721	839.364	559.912	420.186	(Hex)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	6896	9934	0.02	0.06
	b								32531	39844	0.10	0.24
	c								2675	8573	0.01	0.05
20	a	1403.507	1718.747	859.877	573.587	430.443	(Hex)1 (HexNAc)1 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	111182	86633	0.36	0.51
	b								131502	67508	0.42	0.40
	c								102459	30893	0.33	0.18
	d								43142	50626	0.14	0.30
	e								267215	268366	0.86	1.58
	f								31890	34798	0.10	0.21
21	a	1444.534	1759.774	880.391	587.263	440.699	(HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	13536	17707	0.04	0.10
	b								286414	229030	0.92	1.35
	c								18038	40930	0.06	0.24
	d								18714	49719	0.06	0.29

23	a	1565.560	1880.800	940.904	627.605	470.956	(Hex)2 (HexNAc)1 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	20009	5381	0.06	0.03
	b								969104	243126	3.12	1.43
24	a	1606.587	1921.827	961.417	641.281	481.213	(Hex)1 (HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	34421	16526	0.11	0.10
	b								117979	344626	0.38	2.03
	c								33232	28922	0.11	0.17
	d								26488	57068	0.09	0.34
	e								20132	29270	0.06	0.17
	f								16458	20264	0.05	0.12
25	a	1647.613	1962.853	981.930	654.956	491.469	(HexNAc)3 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	34917	56789	0.11	0.34
	b								21712	24851	0.07	0.15
26	a	1727.613	2042.853	1021.930	681.623	511.469	(Hex)3 (HexNAc)1 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	28880	13451	0.09	0.08
	b								16577	6461	0.05	0.04
27	c	1768.640	2083.880	1042.444	695.298	521.726	(Hex)2 (HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	348416	117892	1.12	0.70
	d								23265	13535	0.07	0.08
	e								18741	0	0.06	0.00
	f								366104	291987	1.18	1.72
	g								51119	28702	0.16	0.17
	h								33760	42457	0.11	0.25
28	a	1809.666	2124.906	1062.957	708.974	531.982	(Hex)1 (HexNAc)3 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	20769	22994	0.07	0.14
	b								14044	13155	0.05	0.08
	c								10689	58695	0.03	0.35
	d								0	19478	0.00	0.11
	e								69719	42664	0.22	0.25
	f								0	33743	0.00	0.20
	g								0	7613	0.00	0.04
29	a	1850.693	2165.933	1083.470	722.649	542.239	(HexNAc)4 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	0	9078	0.00	0.05
	b								35782	57053	0.12	0.34
30	a	1930.692	2245.932	1123.470	749.316	562.239	(Hex)3 (HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	102136	36091	0.33	0.21
31	a	1971.719	2286.959	1143.983	762.991	572.496	(Hex)2 (HexNAc)3 (Deoxyhexose)1 + (Man)3(GlcNAc)2	2	214893	35250	0.69	0.21
	b								22078	45435	0.07	0.27
	c								0	36569	0.00	0.22
	d								40060	60892	0.13	0.36
32	a	1987.714	2302.954	1151.981	768.323	576.494	(Hex)3 (HexNAc)3 + (Man)3(GlcNAc)2	2	21837	19294	0.07	0.11
	b								41295	26837	0.13	0.16
	c								26820	17391	0.09	0.10
	d								19490	13598	0.06	0.08
	e								8941	4247	0.03	0.03
33	a	2133.772	2449.012	1225.010	817.009	613.009	(Hex)3 (HexNAc)3 (Deoxyhexose)1 + (Man)3(GlcNAc)2	3	188706	215501	0.61	1.27
	b								237730	109579	0.77	0.65
	c								38826	26153	0.13	0.15
34	a	2174.798	2490.038	1245.523	830.684	623.265	(Hex)2 (HexNAc)4 (Deoxyhexose)1 + (Man)3(GlcNAc)2	3	16805	12256	0.05	0.07
	b								13145	11823	0.04	0.07
	c								28619	83128	0.09	0.49
	d								16341	167498	0.05	0.99
35	a	2336.851	2652.091	1326.549	884.702	663.779	(Hex)3 (HexNAc)4 (Deoxyhexose)1 + (Man)3(GlcNAc)2	3	40910	14302	0.13	0.08
	b								65619	15556	0.21	0.09
	c								101175	158504	0.33	0.94
36	a	2498.904	2814.144	1407.576	938.720	704.292	(Hex)4 (HexNAc)4 (Deoxyhexose)1 + (Man)3(GlcNAc)2	3	420602	253828	1.35	1.50
	b								13822	6072	0.04	0.04
37	a	1548.545	1863.785	932.396	621.933	466.702	(Hex)1 (HexNAc)1 (NeuAc)1 + (Man)3(GlcNAc)2	2	77722	72142	0.25	0.43
	b								18529	9697	0.06	0.06
	c								76315	31657	0.25	0.19
	d								0	12746	0.00	0.08
	e								13765	17374	0.04	0.10
38	a	1694.603	2009.843	1005.425	670.619	503.217	(Hex)1 (HexNAc)1 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	2	22422	4973	0.07	0.03
	b								231734	161612	0.75	0.95
	c								72277	40616	0.23	0.24
	d								33264	56351	0.11	0.33
	e								48319	53509	0.16	0.32
39	a	1710.598	2025.838	1013.423	675.951	507.215	(Hex)2 (HexNAc)1 (NeuAc)1 + (Man)3(GlcNAc)2	2	6626	5334	0.02	0.03
	b								337635	99358	1.09	0.59
	c								284327	80037	0.92	0.47
40	a	1751.624	2066.864	1033.936	689.626	517.472	(Hex)1 (HexNAc)2 (NeuAc)1 + (Man)3(GlcNAc)2	2	0	14528	0.00	0.09
	b								33294	86081	0.11	0.51
	c								0	13474	0.00	0.08
41	a	1856.656	2171.896	1086.452	724.637	543.730	(Hex)2 (HexNAc)1 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	2	246731	124566	0.79	0.73
	b								274943	74212	0.89	0.44
42	a	1872.650	2187.890	1094.449	729.968	547.728	(Hex)3 (HexNAc)1 (NeuAc)1 + (Man)3(GlcNAc)2	2	11939	9743	0.04	0.06
	b								394009	191951	1.27	1.13
	c								424980	133987	1.37	0.79
43	a	1897.682	2212.922	1106.965	738.312	553.986	(Hex)1 (HexNAc)2 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	2	20819	22442	0.07	0.13
	b								93409	150606	0.30	0.89
	c								14267	17660	0.05	0.10
	d								40084	77265	0.13	0.46
44	a	1913.677	2228.917	1114.962	743.644	557.985	(Hex)2 (HexNAc)2 (NeuAc)1 + (Man)3(GlcNAc)2	2	99752	53129	0.32	0.31
	b								640255	217667	2.06	1.28
	c								345954	100159	1.11	0.59
45	a	2018.708	2333.948	1167.478	778.654	584.243	(Hex)3 (HexNAc)1 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	2	127021	53809	0.41	0.32
	b								125875	32500	0.41	0.19
	c								38386	13888	0.12	0.08
46	a	2059.735	2374.975	1187.991	792.330	594.500	(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	2	79566	47006	0.26	0.28
	b								927949	371282	2.99	2.19
	c								375456	155371	1.21	0.92
	d								5560	6904	0.02	0.04
47	a	2100.762	2416.002	1208.505	806.006	604.756	(Hex)1 (HexNAc)3 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	3	27175	12091	0.09	0.07
	b								38769	82267	0.12	0.49
	c								194230	127726	0.63	0.75
	d								81173	41433	0.26	0.24
	e											

66	b	2569.905	2885.145	1443.076	962.387	722.042	(Hex)3 (HexNAc)3 (NeuAc)2 + (Man)3(GlcNAc)2	3	16852	6390	0.05	0.04
	c								387579	79351	1.25	0.47
	d								105918	36274	0.34	0.21
	e								403630	124944	1.30	0.74
67	a	2586.920	2902.160	1451.584	968.058	726.296	(Hex)4 (HexNAc)3 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	3	10239	6139	0.03	0.04
	b								3391	3602	0.01	0.02
68	a	2627.947	2943.187	1472.097	981.734	736.553	(Hex)3 (HexNAc)4 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	3	17405	10454	0.06	0.06
	b								176894	88961	0.57	0.52
	c								40404	82164	0.13	0.48
	d								26387	35583	0.08	0.21
69	a	2643.941	2959.181	1480.094	987.065	740.551	(Hex)4 (HexNAc)4 (NeuAc)1 + (Man)3(GlcNAc)2	3	137455	68749	0.44	0.41
	b								49141	57840	0.16	0.34
	c								81686	61087	0.26	0.36
	d								22072	18298	0.07	0.11
	e								7196	7956	0.02	0.05
70	a	2715.963	3031.203	1516.105	1011.073	758.557	(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuAc)2 + (Man)3(GlcNAc)2	3	793752	271653	2.56	1.60
	b								148544	74866	0.48	0.44
	c								642751	198671	2.07	1.17
	d								82187	22858	0.26	0.13
	e								26301	9038	0.08	0.05
	f								8215	6461	0.03	0.04
71	a	2756.989	3072.229	1536.618	1024.748	768.813	(Hex)2 (HexNAc)4 (Deoxyhexose)1 (NeuAc)2 + (Man)3(GlcNAc)2	3	5099	4959	0.02	0.03
	b								16302	32275	0.05	0.19
	c								21788	31654	0.07	0.19
72	a	2789.999	3105.239	1553.123	1035.751	777.066	(Hex)4 (HexNAc)4 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	3	5697	13800	0.02	0.08
	b								248240	149958	0.80	0.88
	c								282815	176876	0.91	1.04
	d								347546	148913	1.12	0.88
	e								82337	49523	0.27	0.29
	f								21198	14274	0.07	0.08
73	a	2861.000	3176.240	1588.624	1059.418	794.816	(Hex)3 (HexNAc)3 (NeuAc)3 + (Man)3(GlcNAc)2	3	33211	39875	0.11	0.24
	b								110043	97429	0.35	0.57
	c								78732	18072	0.25	0.11
	d								36779	137751	0.12	0.81
74	a	2878.015	3193.255	1597.131	1065.090	799.070	(Hex)4 (HexNAc)3 (Deoxyhexose)1 (NeuAc)2 + (Man)3(GlcNAc)2	3	10665	10790	0.03	0.06
	b								28213	5299	0.09	0.03
	c								13659	3795	0.04	0.02
75	a	2919.042	3234.282	1617.645	1078.766	809.326	(Hex)3 (HexNAc)4 (Deoxyhexose)1 (NeuAc)2 + (Man)3(GlcNAc)2	3	80790	10922	0.26	0.06
	b								68417	65483	0.22	0.39
	c								56313	54320	0.18	0.32
	d								10219	10754	0.03	0.06
	e								14108	20269	0.05	0.12
	f								13573	14333	0.04	0.08
	g								7427	8913	0.02	0.05
76	a	2935.037	3250.277	1625.642	1084.097	813.325	(Hex)4 (HexNAc)4 (NeuAc)2 + (Man)3(GlcNAc)2	3	108531	61466	0.35	0.36
	b								115423	69819	0.37	0.41
	c								43710	22711	0.14	0.13
	d								25854	20431	0.08	0.12
	e								23306	9965	0.08	0.06
77	a	2952.052	3267.292	1634.150	1089.769	817.579	(Hex)5 (HexNAc)4 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	3	6004	1800	0.02	0.01
	b								48498	25443	0.16	0.15
	c								71702	39808	0.23	0.23
	d								14754	7912	0.05	0.05
	e								7074	5655	0.02	0.03
	f								10169	10928	0.03	0.06
78	a	3007.058	3322.298	1661.653	1108.104	831.330	(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuAc)3 + (Man)3(GlcNAc)2	3	5827	4898	0.02	0.03
	b								696712	136899	2.24	0.81
	c								514268	79734	1.66	0.47
79	a	3081.095	3396.335	1698.671	1132.783	849.840	(Hex)4 (HexNAc)4 (Deoxyhexose)1 (NeuAc)2 + (Man)3(GlcNAc)2	3	12084	4765	0.04	0.03
	b								500512	237776	1.61	1.40
	c								558936	184365	1.80	1.09
	d								161787	184365	0.52	1.09
	e								161787	69825	0.52	0.41
80	a	3155.132	3470.372	1735.690	1157.462	868.349	(Hex)5 (HexNAc)5 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2	3	95413	25195	0.31	0.15
	b								0	1918	0.00	0.01
	c								16693	25043	0.05	0.15
	d								20781	20082	0.07	0.12
	e								21992	23963	0.07	0.14
	f								5505	4379	0.02	0.03
	g								11145	7527	0.04	0.04
81	a	3210.137	3525.377	1763.192	1175.797	882.100	(Hex)3 (HexNAc)4 (Deoxyhexose)1 (NeuAc)3 + (Man)3(GlcNAc)2	3	8719	3738	0.03	0.02
	b								14568	0	0.05	0.00
82	a	3226.132	3541.372	1771.190	1181.129	886.099	(Hex)4 (HexNAc)4 (NeuAc)3 + (Man)3(GlcNAc)2	3	24089	12634	0.08	0.07
	b								7842	8767	0.03	0.05
	c								67381	37011	0.22	0.22
	d								28725	11947	0.09	0.07
	e								86730	40498	0.28	0.24
83	a	3284.174	3599.414	1800.211	1200.476	900.609	(Hex)4 (HexNAc)5 (Deoxyhexose)1 (NeuAc)2 + (Man)3(GlcNAc)2	3	8987	3148	0.03	0.02
	b								8157	0	0.03	0.00
	c								14844	3737	0.05	0.02
	d								32036	9429	0.10	0.06
	e								0	2378	0.00	0.01
	f								11911	4167	0.04	0.02
	g								0	3690	0.00	0.02
	a								0	6792	0.00	0.04
	b								67612	29147	0.22	0.17
	c								312769	79496	1.01	0.47
									186692	84410	0.60	0.50

84	d	3372.190	3687.430	1844.219	1229.815	922.613	(Hex)4 (HexNAc)4 (Deoxyhexose)1 (NeuAc)3 + (Man)3(GlcNAc)2	3	332562	100673	1.07	0.59
	e								70751	30479	0.23	0.18
	f								21880	9828	0.07	0.06
	g								39097	6851	0.13	0.04
	h								18317	5332	0.06	0.03
	i								16439	2034	0.05	0.01
85	a	3446.227	3761.467	1881.237	1254.494	941.123	(Hex)5 (HexNAc)5 (Deoxyhexose)1 (NeuAc)2 + (Man)3(GlcNAc)2	3	16913	9019	0.05	0.05
	b								27102	15033	0.09	0.09
	c								22940	14890	0.07	0.09
	d								21249	7510	0.07	0.04
86	a	3663.286	3978.526	1989.767	1326.847	995.387	(Hex)4 (HexNAc)4 (Deoxyhexose)1 (NeuAc)4 + (Man)3(GlcNAc)2	3	94775	40097	0.31	0.24
	b								172449	67796	0.56	0.40
	c								3413	0	0.01	0.00
87	a	3737.322	4052.562	2026.785	1351.526	1013.896	(Hex)5 (HexNAc)5 (Deoxyhexose)1 (NeuAc)3 + (Man)3(GlcNAc)2	4	80645	33400	0.26	0.20
	b								16342	9487	0.05	0.06
	c								47951	19191	0.15	0.11
	d								18652	6158	0.06	0.04
	e								24180	2425	0.08	0.01
	f								16927	4451	0.05	0.03
88	a	4028.418	4343.658	2172.333	1448.558	1086.670	(Hex)5 (HexNAc)5 (Deoxyhexose)1 (NeuAc)4 + (Man)3(GlcNAc)2	4	18754	8800	0.06	0.05
	b								32755	5385	0.11	0.03
	c								2068	0	0.01	0.00
	d								65889	14404	0.21	0.08
	e								8858	1695	0.03	0.01
	f								5730	0	0.02	0.00
	g								2440	0	0.01	0.00
	h								11412	3693	0.04	0.02
89	a	4102.455	4417.695	2209.351	1473.237	1105.180	(Hex)6 (HexNAc)6 (Deoxyhexose)1 (NeuAc)3 + (Man)3(GlcNAc)2	4	8101	2343	0.03	0.01
	b								24688	4661	0.08	0.03
	c								23205	8699	0.07	0.05
	d								19939	4765	0.06	0.03
	e								6466	3046	0.02	0.02
	f								22542	6653	0.07	0.04
	g											
	h											
SUM of Hign Man									14,244,750	6,258,944	45.8659293	36.9276074
SUM without Hign Man									31,057,367	16,949,227	100	100
SUM with Hign Man (=Total)									45,302,117	23,208,171	145.865929	136.927607

Supplemental Table S2

Name	sequence
h-Fut8-Fw	ATAGGATCCGCCACCATGCGGCCATGGACTGGTTC
h-Fut8-GlyLinker	ATTCTGGCCCCAAAATAGT
h-FUT8-4GlyLinker-oligo-Fw	ggcggcggcggcGAAACGGTCAAGTACCCACATATCCTGAGGCTGAGAAATAAg
h-FUT8-4GlyLinker-oligo-Rv	aattcTTATTTCTCAGCCTCAGGATATGTGGGGTACTTGACCGTTTCgcccggccggcc
h-FUT8-6GlyLinker-oligo-Fw	ggcggcggcggcggcggcGAAACGGTCAAGTACCCACATATCCTGAGGCTGAGAAATAAg
h-FUT8-6GlyLinker-oligo-Rv	aattcTTATTTCTCAGCCTCAGGATATGTGGGGTACTTGACCGTTTCgcccggccggccggcc
h-Fut8-coloP	GTCTGACTGAGGTAGTATAGATCC
h-Fut8-seq1	CAGATGGAGCAGGTGATTGG
h-Fut8-seq2	ATAGAAGAAGCCACCAAGAAGC
FUT8 seq3	AATACTTGATCCGCCACAG
FUT8 seq4	AGTTATTGGAGTCCATGTCAGACG
h-Fut8-IH Fw	TCGGAATTCCGGATACCAGAAGGCCCTAT
h-Fut8-IH Rv	tatcggcggcgtTATTTCTCAGCCTCAGGAT
FUT8 Asp519Ala Fw	CTACCAACCCCCGAAGTGCAGcGAAATTCCCATGGAACCTGG
FUT8 Asp519Ala Rv	CCAGGTCCATGGGAATTTcagcTGCAGTTCGGGGTTGGTGAG
FUT8 His535Ala FW	TCATTGGTGTGGCTGGAAATgctTGGGATGGCTATTCTAAAGG
FUT8 His535Ala Rv	CCTTTAGAATAGCCATCCCAgcaATTCCAGCCACACCAATGA
FUT8 Lys541Ala FW	ATCATTTGGGATGGCTATTCTgcAGGTGTCAACAGGAAATTTGGG
FUT8 Lys541Ala Rv	CCCAATTTCTGTTGACACCTgcAGAATAGCCATCCCAATGAT
FUT8 Val543Ala Fw	GGGATGGCTATTCTAAAGGTGccAACAGGAAATTTGGGAAGGAC
FUT8 Val543Ala Rv	GTCCCTTCCCAATTTCTGTTggCACCTTTAGAATAGCCATCCC
FUT8 Arg545Ala FW	GCTATTCTAAAGGTGTCAACgcgAAATTTGGGAAGGACGGGCCT
FUT8 Arg545Ala RV	AGGCCCGTCTTCCCAATTTcgcGTTGACACCTTTAGAATAGC
FUT8 Thr550Leu552Ala Fw	ACAGGAAATTTGGGAAGGgcGGCgcaTATCCCTCCTACAAAGT
FUT8 Thr550Leu552Ala Rv	ACTTTGTAGGAGGGATatgcGCCgcCCTTCCCAATTTCTCTGT
FUT8 Thr550Ala Fw	TCAACAGGAAATTTGGGAAGGgCGGGCCTATATCCCTCCTACAA
FUT8 Thr550Ala Rv	TTGTAGGAGGGATATAGGCCCGcCCTTCCCAATTTCTCTGTGA
FUT8 Leu552Ala Fw	GGAAATTTGGGAAGGACGGGCgcATATCCCTCCTACAAAGTTCG
FUT8 Leu552Ala Rv	CGAACTTTGTAGGAGGGATatgcGCCCGTCTTCCCAATTTCC