Supplemental Table 1: Manually identified glycoforms of ^{313}pyrQIHATPTPVR^{322} derived from αDG373(T322R)-Fc and their calculated relative abundance[1]

Composition	Theoretical $[M+H]^+$	Measured m/z	z	Accuracy (ppm)	Elution Time (min)	HEK293 WT	HCT116 WT	HCT116 ΔISPD	HCT116 ΔFKTN	HCT116 ΔFKRP	HCT116 ΔTMEM5
Mucin/Core M1/Core M2											
$HexNAc_3$	1711.8385	856.4243	2	1.3	23.1	2.98E+05					
$Hex_1HexNAc_1NeuAc_1$	1758.8279	879.9188	2	1.1	27.2	1.66E+07	4.30E+07	8.59E+07	9.54E+07	3.42E+07	3.95E+07
$Hex_3HexNAc_1$	1791.8381	896.4244	2	1.6	23.0	7.66E+05	4.13E+05	1.03E+06	1.60E+05	1.11E+06	1.05E+06
$Hex_2HexNAc_2$	1832.8647	916.9369	2	0.7	20.8	6.81E+07	1.16E+07	3.08E+07	2.45E+07	2.23E+07	2.14E+07
$Hex_1HexNAc_3$	1873.8913	937.4496	2	0.0	21.7	4.25E+06	1.47E+06	2.32E+06	3.00E+06	4.75E+06	2.30E+06
$Hex_2HexNAc_3$	2035.9441	1018.4753	2	0.7	20.3	7.77E+05		3.31E+05	3.63E+05	5.75E+05	4.77E+05
$Hex_1HexNAc_1NeuAc_2$	2049.9233	1025.4678	2	2.2	32.9	1.32E+07	3.02E+07	2.94E+07	1.53E+07	4.81E+06	1.58E+07
$Hex_2HexNAc_2NeuAc_1$	2123.9601	1062.4857	2	1.6	25.6	1.26E+07	6.56E+06	1.12E+07	1.91E+07	1.06E+07	7.62E+06
$Hex_4HexNAc_2$	2156.9703	1078.9910	2	1.8	24.2	7.82E+05	3.71E+05	8.33E+05	2.93E+05	5.81E+05	5.73E+05
$Hex_1HexNAc_3NeuAc_1$	2164.9867	1082.9991	2	1.7	26.9	1.40E+06	1.47E+06	8.36E+05	1.43E+06	1.32E+06	7.08E+05
$Hex_3HexNAc_3$	2197.9969	1099.5023	2	0.1	19.3	3.47E+06	4.64E+06	5.71E+06	1.43E+07	8.57E+06	5.86E+06
$Hex_2HexNAc_4$	2239.0235	1120.0148	2	0.8	20.4	1.41E+06	3.31E+05	3.08E+05	1.13E+06	1.03E+06	5.92E+05
$Hex_2HexNAc_2NeuAc_2$	2415.0555	805.6923	3	2.4	28.8	1.07E+07	5.07E+06	3.50E+06	4.52E+06	2.89E+06	2.24E+06
$Hex_3HexNAc_3NeuAc_1$	2489.0923	830.3703	3	1.2	24.2	2.22E+06	5.52E+06	3.45E+06	6.69E+06	3.17E+06	3.28E+06
$Hex_2HexNAc_4NeuAc_1$	2530.1189	844.0464	3	1.8	23.5	5.80E+05	2.84E+05	1.37E+05	4.95E+05	1.79E+05	
$Hex_4HexNAc_4$	2563.1291	855.0499	3	1.9	19.7		3.35E+06	2.88E+06	1.19E+07	6.18E+06	6.43E+06
$Hex_3HexNAc_5$	2604.1557	868.7255	3	2.0	20.0		2.31E+05	2.31E+05	1.37E+06	7.24E+05	3.10E+05
$Hex_4HexNAc_4NeuAc_1$	2854.2245	952.0809	3	0.9	23.1	3.73E+05	3.31E+06	2.33E+06	5.62E+06	3.42E+06	2.54E+06
$Hex_3HexNAc_3NeuAc_3$	3071.2831	1024.4330	3	0.1	33.1		2.66E+06	4.77E+05	6.23E+05	2.05E+05	2.14E+05
$Hex_4HexNAc_4NeuAc_2$	3145.3199	1049.1144	3	2.4	27.6	8.78E+04	1.26E+06	9.89E+05	1.07E+06	6.12E+05	5.74E+05
$Hex_4HexNAc_4NeuAc_3$	3436.4153	1146.1439	3	0.2	32.7		9.90E+05	1.62E+05	2.08E+05		
Core M3											
$Hex_1HexNAc_2P_1$	1750.7784	875.8930	2	0.1	30.3	1.88E+06	1.99E+06	1.78E+06	2.12E+06	1.67E+06	1.21E+06
$Hex_2HexNAc_1P_2$	1789.7179	895.3634	2	0.6	34.2	2.74E+05					
$Hex_3HexNAc_1P_1$	1871.8044	936.4070	2	0.9	27.3	5.20E+05					
$Hex_2HexNAc_2P_1$	1912.8310	956.9213	2	2.0	28.6	2.46E+06	5.08E+05	6.38E+05	3.04E+05		
$Hex_2HexNAc_2P_2$	1992.7934	996.9041	2	3.5	33.0	3.81E+06					
$Hex_3HexNAc_2P_1$	2074.8838	1037.9473	2	1.4	28.1	2.47E+06	2.55E+05	6.27E+05	2.67E+05	3.32E+05	4.08E+05
$Hex_2HexNAc_3P_1$	2115.9104	1058.4630	2	3.7	27.4	9.57E+06	9.48E+05	1.72E+06	2.08E+06	1.29E+06	2.34E+06
$Hex_2HexNAc_3P_2$	2195.8767	1098.4422	2	0.1	33.9	1.51E+07	1.06E+06	8.81E+05	1.65E+06	6.30E+05	9.58E+05
$Hex_2HexNAc_2NeuAc_1P_1$	2203.9264	1102.4678	2	0.6	32.3	4.12E+05					
$Hex_3HexNAc_3P_1$	2277.9632	1139.4877	2	1.9	28.3	3.48E+07	2.63E+06	5.73E+06	7.12E+06	2.37E+06	5.89E+06
$Hex_2HexNAc_4P_1$	2318.9898	1160.0016	2	2.4	28.2	6.06E+06	2.59E+05	3.84E+05	5.73E+05	1.03E+05	3.46E+05
$Hex_3HexNAc_3P_2$	2357.9295	1179.4707	2	1.7	33.5	1.11E+06			4.23E+05	2.55E+05	
$Hex_3HexNAc_2NeuAc_1P_1$	2365.9792	1183.4945	2	0.8	33.7	1.04E+06					

$Hex_2HexNAc_4P_2$	2398.9561	800.3239	3	0.0	33.8	8.30E+07	7.14E+06	5.14E+06	1.16E+07	2.93E+06	4.01E+06
$Hex_2HexNAc_3NeuAc_1P_1$	2407.0058	1204.0075	2	0.6	32.3	6.54E+06	7.71E+05	9.27E+05	1.62E+06	2.63E+05	7.38E+05
$Hex_3HexNAc_3Fuc_1P_1$	2424.0211	1212.5168	2	1.9	27.7	3.90E+06					
$Hex_2HexNAc_4Fuc_1P_1$	2465.0477	1233.0302	2	2.0	27.6	3.17E+06					
$Hex_2HexNAc_4P_3$	2478.9224	1239.9669	2	1.4	41.2	3.30E+06					
$Hex_3HexNAc_4P_1$	2481.0426	1241.0286	2	2.7	26.7	1.37E+06	3.67E+05	1.96E+05	6.30E+05	2.79E+05	3.10E+05
$Hex_2HexNAc_5P_1$	2522.0692	841.3625	3	1.0	26.7	3.85E+05					
$Hex_3HexNAc_3NeuAc_1P_1$	2569.0586	857.0248	3	0.1	33.7	7.66E+06	2.51E+06	3.50E+06	3.49E+06	8.26E+05	2.05E+06
$Hex_4HexNAc_4P_1$	2643.0954	1322.0543	2	2.0	27.8			3.73E+05	4.91E+05	1.09E+05	3.80E+05
$Hex_2HexNAc_3NeuAc_2P_1$	2698.1012	900.0403	3	1.5	37.0	1.45E+06					
$Hex_4HexNAc_4NeuAc_1P_1$	2934.1908	978.7375	3	2.1	33.0		4.01E+05	7.40E+05	6.09E+05	1.21E+05	3.03E+05
Core M3 with RboP											
$Hex_1HexNAc_2P_1RboP_1$	1964.8025	982.9086	2	3.5	36.8	2.35E+05	6.09E+05			4.60E+06	
$Hex_2HexNAc_2P_1RboP_1$	2126.8553	1063.9316	2	0.0	33.7					4.94E+05	
$Hex_2HexNAc_3P_1RboP_1$	2329.9347	1165.4716	2	0.3	31.4	5.02E+05				7.80E+05	
$Hex_2HexNAc_3P_2RboP_1$	2409.9010	1205.4568	2	2.0	41.1	7.29E+05					
$Hex_1HexNA_2P1RboP_2Pent_1HexA_1$	2486.9010	1243.9546	2	0.2	49.0	3.40E+05					
$Hex_3HexNAc_3P_1RboP_1$	2491.9875	831.3351	3	0.9	32.4	1.19E+06	4.70E+05			2.90E+06	1.80E+05
$Hex_2HexNAc_4P_2RboP_1$	2612.9804	871.6663	3	1.1	40.6	6.17E+06	9.69E+05			2.45E+06	
$Hex_2HexNAc_3NeuAc_1P_1RboP_1$	2621.0301	874.3494	3	0.9	38.7					2.61E+05	
$Hex_2HexNA_4P_2GroP_1RboP_1$	2766.9834	923.0001	3	0.5	64.5					7.64E+05	
$Hex_3HexNA_3NeuAc_1P_1RboP_1$	2783.0829	928.3659	3	0.3	40.7	9.57E+05				1.98E+06	
$Hex_3HexNA_3P_1RboP_2Pent_1HexA_1$	3014.0860	1005.3695	3	2.3	42.9	5.90E+05					
$Hex_2HexNA_4P_2RboP_2Pent_1HexA_1$	3135.0789	1045.7007	3	2.4	56.5	1.39E+06					
$Hex_3HexNA_3NeuAc_1P_1RboP_2Pent_1HexA_1$	3305.1814	1102.3987	3	0.3	55.4	3.81E+05					
Core M3 with GroP											
$Hex_1HexNAc_2P_1GroP_1$	1904.7820	952.8949	2	0.0	36.8		7.71E+05	1.25E+06		4.41E+05	5.01E+05
$Hex_2HexNAc_2P_1GroP_1$	2066.8341	1033.9222	2	1.2	33.6			2.47E+05			
$Hex_2HexNAc_3P_1GroP_1$	2269.9135	1135.4620	2	1.2	32.3			3.08E+05			1.94E+05
$Hex_3HexNAc_3P_1GroP_1$	2431.9663	811.3284	3	1.3	33.5	1.64E+05	8.33E+05	2.35E+06		4.22E+05	1.10E+06
$Hex_2HexNAc_4P_2GroP_1$	2552.9592	851.6586	3	0.4	41.1	1.39E+06	2.64E+06	1.92E+06		4.63E+05	8.99E+05
$Hex_3HexNAc_3NeuAc_1P_1GroP_1$	2723.0617	908.3601	3	1.1	40.7		1.44E+06	2.50E+06		2.90E+05	6.43E+05
$Hex_4HexNAc_4P_1GroP_1$	2797.0985	933.0386	3	0.6	32.8			5.71E+05			

[1]The normalized intensity for each glycopeptide was calculated based on the base peak intensity of its extracted ion chromatogram divided by the sample-dependent normalization factor. The normalization factor for each wild type and mutant sample was calculated based on the average intensity of the three most abundant peptides derived from the Fc region, namely FNWYVDGVEVHNAK (m/z 559.9403, z=3), EPQVYTLPPSR (m/z 643.8424, z=2), TTPPVLDSDGSFFLYSK (m/z 937.4650, z=2), common to all αDG373(T322R)-Fc proteins. This factor was used to correct for different yields of the protein from each sample to allow the abundance of each glycopeptide to be compared across different samples. The normalized intensity for each glycopeptide was calculated based on the base peak intensity of its extracted ion chromatogram divided by the sample-dependent normalization factor. The normalization factor for each wild type and mutant sample was calculated based on the average intensity of the three most abundant

peptides derived from the Fc region, namely FNWYVDGVEVHNAK (m/z 559.9403, z=3), EPQVYTLPPSR (m/z 643.8424, z=2), TTPPVLDSDGSFFLYSK (m/z 937.4650, z=2), common to all αDG373(T322R)-Fc proteins. This factor was used to correct for different yields of the protein from each sample to allow the abundance of each glycopeptide to be compared across different samples. A representative set of annotated mass labeled HCD/CID spectra for each of the precursors listed here can be found in supplemental Fig. 5.