

Supplementary material

Polymeric IgA with unique glycans protects from necrotoxicogenic E. coli O55 infection in an animal model

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Supplementary Methods

Peptide mass spectrometry: Instrument setup and data processing

MALDI-TOF mass spectrometry was performed on a Microflex LRF20 instrument (Bruker Daltonik, Bremen, Germany) equipped with a 60-Hz nitrogen laser ($\lambda=337$ nm). Mass spectra in the m/z range of 700 – 10,000 were acquired in the reflector positive ion mode using the following instrumental setup: an acceleration voltage (IS1) of 19.0 kV, an extraction voltage (IS2) of 16 kV, a lens voltage of 9.0 kV, a reflector voltage of 20.0 kV, a detector voltage of 1672 V, and a pulsed ion extraction delay time of 200 ns. Peptide Calibration Standard II and Protein Calibration Standard I (Bruker Daltonik) were applied for external calibration (average $[M+H]^+$ masses between 757.9 and 8565.8 Da). The acquisition and evaluation software were flexControl 3.4 and flexAnalysis 3.4, respectively (Bruker Daltonik). The obtained peptide spectral data were processed in GlycoMod (<https://web.expasy.org/glycomod/>) to identify the *N*-glycopeptides and deduce *N*-glycan structures by combining experimental masses and the corresponding protein amino acid sequences (Uniprot accession numbers IGHA1_HUMAN and IGHA2_HUMAN). The average mass tolerance was set at 2 Da (optionally 3 Da), up to 2 tryptic missed cleavages were allowed, iodoacetamide and acrylamide adducts of Cys as well as oxidized Met and Trp were selected as considered modifications.

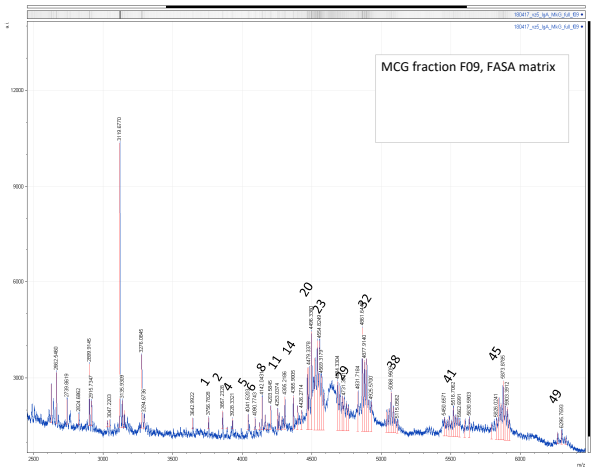
Supplementary Table 1a. plgA1(G) glycan structure

plgA1(G) Glycan structure assignments calculated in GlycoMod using experimental m/z values of glycopeptides

Cys_CAM = carbamidomethylation of cysteine; Cys_PAM = acrylamide adduct of cysteine; MSO = oxidized methionine; TPO = oxidized tryptophan

red m/z values: possible sialylated structures; blue m/z values: less expectable peptide modifications

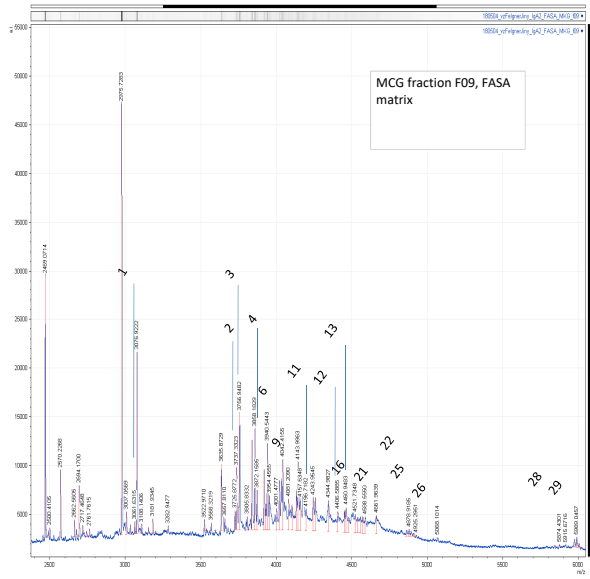
Microgradient fraction number	m/z	Peak number	Glycoform m mass	ΔMass (Dalton)	Deduced glycoform (in GlycoMod)	Peptide modification	Peptide mass	Peptide sequence	Theoretical glycopeptide mass
F09	3756.7828	1	892.817	-0.294	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2863.253	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	3757.078
F09	3857.2327	2	892.817	-1.023	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2964.43	127-153 LSUHRPALEDLLGSEANLT CTLTGLR	3858.254
F09	3857.2327	2	1549.413	0.178	(Hex) ₁ (HexNAc) ₂	1aMSO	2306.634	332-353 LAGKPTHHVNVSVMAEVDGTCY	3857.054
F09	3890.6442	3	1582.439	0.564	(Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ (GlcNAc) ₂	1xCys_CAM, 1aMSO	2792.175	332-353 LAGKPTHHVNVSVMAEVDGTCY	3890.08
F09	3890.6442	3	1096.012	1.45	(HexNAc) ₂ + (Man) ₃ (GlcNAc) ₂	1aMSO	2792.175	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	3889.194
F09	3928.3320	4	1096.012	-1.914	(HexNAc) ₂ + (Man) ₃ (GlcNAc) ₂	1xCys_CAM	2833.227	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	3930.247
F09	3928.3320	4	1549.413	0.199	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2377.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	3928.133
F09	4041.6203	5	1664.545	-1.644	(Hex) ₁ (HexNAc) ₂	1aMSO	2377.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4041.197
F09	4041.6203	5	1752.608	-2.629	(HexNAc) ₂ + (Man) ₃ (GlcNAc) ₂	No modification	2290.635	332-353 LAGKPTHHVNVSVMAEVDGTCY	4044.25
F09	4090.7742	6	1299.207	-1.614	(HexNAc) ₂ + (Deoxyhexose) ₁	1aMSO	2792.175	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4092.389
F09	4090.7742	6	1242.155	0.358	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2847.254	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4090.416
F09	4123.8448	7	1258.155	1.429	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2863.253	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4122.416
F09	4142.0431	8	1342.245	1.615	(HexNAc) ₂ + (Deoxyhexose) ₁	No modification	2776.175	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4140.428
F09	4163.0646	9	1299.207	-0.402	(HexNAc) ₂ + (Deoxyhexose) ₁	1xCys_CAM, 1aMSO	2863.253	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4163.468
F09	4182.6234	10	1404.298	1.143	(Hex) ₁ (HexNAc) ₂	No modification	2776.175	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4181.481
F09	4203.5844	11	1826.687	-1.822	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2377.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4205.407
F09	4253.0374	12	1461.35	-1.494	(Hex) ₁ (HexNAc) ₂	1aMSO	2792.175	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4254.532
F09	4253.0374	12	1404.298	0.478	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2847.254	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4252.559
F09	4264.0243	13	1299.207	-0.619	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2964.43	127-153 LSUHRPALEDLLGSEANLT CTLTGLR	4264.644
F09	4264.0243	13	1898.751	2.553	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2361.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4261.472
F09	4305.2185	14	1340.26	0.478	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2964.43	127-153 LSUHRPALEDLLGSEANLT CTLTGLR	4305.697
F09	4305.2185	14	1955.803	0.722	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2347.687	332-353 LAGKPTHHVNVSVMAEVDGTCY	4304.497
F09	4365.9004	15	1502.402	-0.761	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2863.253	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4366.662
F09	4365.9004	15	2060.893	-2.632	(Hex) ₁ (HexNAc) ₂	1aMSO	2306.634	332-353 LAGKPTHHVNVSVMAEVDGTCY	4368.534
F09	4396.9106	16	1549.413	-0.763	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2847.254	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4397.674
F09	4426.2714	17	1461.35	-0.515	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2964.43	127-153 LSUHRPALEDLLGSEANLT CTLTGLR	4426.787
F09	4426.2714	17	1445.35	1.457	(HexNAc) ₂ + (Deoxyhexose) ₁	1aMSO	2978.457	127-153 LSUHRPALEDLLGSEANLT CTLTGLR	4424.814
F09	4466.8830	18	1502.402	-0.955	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2964.43	127-153 LSUHRPALEDLLGSEANLT CTLTGLR	4467.839
F09	4466.8830	18	2101.946	2.216	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2361.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4464.667
F09	4479.3378	19	1703.529	-1.373	(Hex) ₁ (HexNAc) ₂	No modification	2776.175	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4480.712
F09	4479.3378	19	2117.945	-1.327	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2361.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4480.666
F09	4496.3360	20	2189.56	-1.26	(Hex) ₁ (HexNAc) ₂	1aMSO	2306.634	332-353 LAGKPTHHVNVSVMAEVDGTCY	4497.597
F09	4496.3360	20	2117.945	-0.328	(HexNAc) ₂ + (Deoxyhexose) ₁	1xCys_CAM, 1aMSO	2377.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4496.665
F09	4521.9752	21	1687.53	0.211	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2833.227	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4521.765
F09	4537.9587	22	1687.53	2.167	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2847.254	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4535.791
F09	4554.8248	23	1703.529	1.062	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2849.227	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4553.763
F09	4554.8248	23	2206.008	0.123	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2347.687	332-353 LAGKPTHHVNVSVMAEVDGTCY	4554.702
F09	4569.3179	24	1663.608	-0.865	(Hex) ₁ (HexNAc) ₂	No modification	2905.569	127-153 LSUHRPALEDLLGSEANLT CTLTGLR	4570.184
F09	4569.3179	24	2206.008	0.589	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2361.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4568.729
F09	4585.0200	25	1703.529	1.528	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2863.253	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4567.79
F09	4585.0200	25	2206.008	0.292	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2377.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4584.728
F09	4684.3303	26	1849.672	0.424	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2833.227	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4683.907
F09	4700.5851	27	1865.672	0.679	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2833.227	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4699.907
F09	4700.5851	27	2409.203	-0.259	(Hex) ₁ (HexNAc) ₂	No modification	2290.635	332-353 LAGKPTHHVNVSVMAEVDGTCY	4700.845
F09	4717.0886	28	1865.672	1.183	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2849.227	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4715.906
F09	4717.0886	28	2352.151	2.217	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2361.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4714.871
F09	4731.3030	29	1865.672	1.37	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2863.253	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4729.933
F09	4731.3030	29	2352.151	0.432	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2377.713	332-353 LAGKPTHHVNVSVMAEVDGTCY	4730.871
F09	4747.4916	30	1769.635	-1.606	(Hex) ₁ (HexNAc) ₂	1xCys_CAM	2978.457	127-153 LSUHRPALEDLLGSEANLT CTLTGLR	4749.099
F09	4761.0475	31	1898.751	-1.963	(Hex) ₁ (HexNAc) ₂	1xCys_CAM, 1aMSO	2863.253	328-353 TIDRLAGKPTHHVNVSVMAE VDGTCY (1 missed cleavage)	4763.012



F09	4861.6446	32	2027.814	-0.403	(Hex) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_CAM	2833.227	328-353 TIDRLAGKPTHVNVSVVMAE VDGTCY (1 missed cleavage)	4862.049
F09	4861.6446	32	2555.346	-1.342	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xMSO	2306.634	332-353 LAGKPTHVNVSVVMAEVDGT CY	4862.987
F09	4877.9140	33	2027.814	-0.133	(Hex) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_CAM, 1xMSO	2849.227	328-353 TIDRLAGKPTHVNVSVVMAE VDGTCY (1 missed cleavage)	4878.048
F09	4877.9140	33	1898.751	-0.3	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_PAM	2978.457	127-153 (LSHRPALEDLLGSEANLT CTLTLGLR 127-168 (LSHRPALEDLLGSEANLT CTLTLGLRDASGVFTFTWTPSS GK (1 missed cleavage)	4878.215
F09	4892.0363	34	406.39	-2.424	(HexNAc) ₁ (Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_CAM	4487.064	127-153 (LSHRPALEDLLGSEANLT CTLTLGLR	4894.462
F09	4892.0363	34	1914.75	-2.177	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_PAM	2978.457	127-153 (LSHRPALEDLLGSEANLT CTLTLGLR	4894.214
F09	4908.3733	35	2060.893	-0.78	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_PAM	2847.254	328-353 TIDRLAGKPTHVNVSVVMAE VDGTCY (1 missed cleavage)	4909.154
F09	5038.7009	36	2060.893	-1.656	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_PAM	2978.457	127-153 (LSHRPALEDLLGSEANLT CTLTLGLR	5040.357
F09	5054.0488	37	2189.956	-0.167	(Hex) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_PAM, 1xMSO	2863.253	328-353 TIDRLAGKPTHVNVSVVMAE VDGTCY (1 missed cleavage)	5054.217
F09	5068.9509	38	2189.956	-1.489	(Hex) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_CAM, 1xMSO	2849.227	328-353 TIDRLAGKPTHVNVSVVMAE VDGTCY (1 missed cleavage)	5040.19
F09	5068.9509	38	2101.946	1.568	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_CAM	2964.43	127-153 (LSHRPALEDLLGSEANLT CTLTLGLR	5067.383
F09	5085.2450	39	2117.945	1.863	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_CAM	2964.43	127-153 (LSHRPALEDLLGSEANLT CTLTLGLR	5083.382
F09	5098.6210	40	2134.972	-1.787	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_CAM	2964.43	127-153 (LSHRPALEDLLGSEANLT CTLTLGLR	5100.409
F09	5098.6210	40	2117.945	1.212	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_PAM	2978.457	127-153 (LSHRPALEDLLGSEANLT CTLTLGLR	5097.409
F09	5115.0561	41	2336.099	1.774	(Hex) ₁ (Deoxyhexose) ₁ + (Man) ₁ (GlcNAc) ₁	No modification	2776.175	328-353 TIDRLAGKPTHVNVSVVMAE VDGTCY (1 missed cleavage)	5113.282
F09	5115.0561	41	2206.008	0.663	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₁ (GlcNAc) ₁	No modification	2907.378	127-153 (LSHRPALEDLLGSEANLT CTLTLGLR	5114.393
F09	5470.0616	42	2676.384	0.496	(Hex) ₁ + (Man) ₁ (GlcNAc) ₁	1xMSO	2792.175	328-353 TIDRLAGKPTHVNVSVVMAE VDGTCY (1 missed cleavage)	5469.566
F09	5470.0616	42	1038.96	0.082	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁	No modification	4430.012	127-168 (LSHRPALEDLLGSEANLT CTLTLGLRDASGVFTFTWTPSS GK (1 missed cleavage)	5469.98
F09	5486.3785	43	1038.96	0.399	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁	1xTPO	4446.012	127-168 (LSHRPALEDLLGSEANLT CTLTLGLRDASGVFTFTWTPSS GK (1 missed cleavage)	5485.979
F09	5600.7997	44	1096.01	0.717	(HexNAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_CAM, 1xTPO	4503.064	127-168 (LSHRPALEDLLGSEANLT CTLTLGLRDASGVFTFTWTPSS GK (1 missed cleavage)	5600.083
F09	5600.7997	44	1096.012	2.689	(HexNAc) ₁ + (Man) ₁ (GlcNAc) ₁	1xCys_PAM	4501.091	127-168 (LSHRPALEDLLGSEANLT CTLTLGLRDASGVFTFTWTPSS GK (1 missed cleavage)	5598.11
F09	5873.8785	45	892.817	-1.651	(Hex) ₁ (HexNAc) ₁	1xCys_PAM	4981.707	308-353 GDTFSCMVGHREALPLAFTQK TIDRLAGKPTHVNVSVVMAE VDGTCY (2 missed cleavages)	5875.531
F09	5889.5906	46	892.817	-1.939	(Hex) ₁ (HexNAc) ₁	1xCys_PAM, 1xMSO	4997.706	308-353 GDTFSCMVGHREALPLAFTQK TIDRLAGKPTHVNVSVVMAE VDGTCY (2 missed cleavages)	5891.53
F09	5919.7093	47	892.817	1.153	(Hex) ₁ (HexNAc) ₁	2xCys_CAM	5024.732	308-353 GDTFSCMVGHREALPLAFTQK TIDRLAGKPTHVNVSVVMAE VDGTCY (2 missed cleavages)	5918.556
F09	6266.4839	48	1096.012	0.559	(HexNAc) ₁ + (Man) ₁ (GlcNAc) ₁	2xCys_CAM, 1xMSO	5168.905	307-353 GDTFSCMVGHREALPLAFTQ KTIDRLAGKPTHVNVSVVMAE VDGTCY (3 missed cleavages)	6265.925
F09	6295.7592	49	1865.672	-0.931	(Hex) ₁ + (Man) ₁ (GlcNAc) ₁	No modification	4430.012	127-168 (LSHRPALEDLLGSEANLT CTLTLGLRDASGVFTFTWTPSS GK (1 missed cleavage)	6296.692
F09	6295.7592	49	1096.012	1.781	(HexNAc) ₁ + (Man) ₁ (GlcNAc) ₁	2xCys_PAM, 1xMSO	5196.959	307-353 GDTFSCMVGHREALPLAFTQ KTIDRLAGKPTHVNVSVVMAE VDGTCY (3 missed cleavages)	6293.978
F09	6326.3164	50	1299.207	1.37	(HexNAc) ₁ + (Man) ₁ (GlcNAc) ₁	2xCys_CAM	5024.732	308-353 GDTFSCMVGHREALPLAFTQK TIDRLAGKPTHVNVSVVMAE VDGTCY (2 missed cleavages)	6324.946

Supplementary Table 1b. plgA1(F1) glycan structure

plgA2(F1) Glycan structure assignments calculated in GlycoMod using experimental m/z values of glycopeptides									
Microgradient fraction number	m/z	Peak number	Glycoform mass	ΔMass (Dalton)	Deduced glycoform (in glycoMod)	Peptide modification	Peptide mass	Peptide sequence	Theoretical glycopeptide mass
F09	3061.6314	1	2101.946	0.555	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	958.123	200-208 TPLTANITK	3061.076
F09	3725.8771	2	1363.245	-0.007	(Hex) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2361.633	82-102 SVTCHVQHTYSSQDVTVP R (1 missed cleavage)	3725.885
F09	3725.8771	2	2060.893	0.195	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	1663.782	89-102 HYTSSQDVTVP R	3725.683
F09	3737.3322	3	1258.155	2.433	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	2xCys_CAM	2475.737	82-102 SVTCHVQHTYSSQDVTVP R (1 missed cleavage)	3734.899
F09	3737.3322	3	2060.893	-2.376	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_PAM	1677.809	89-102 HYTSSQDVTVP R	3739.709
F09	3872.1585	4	1502.402	1.057	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 1xMSO	2367.692	MAGKPTHINVVVMAEADGT CY	3871.102
F09	3872.1585	4	2264.088	0.333	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	1606.73	89-102 HYTSSQDVTVP R	3871.826
F09	3930.1044	5	1566.44	1.024	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2361.633	82-102 SVTCHVQHTYSSQDVTVP R (1 missed cleavage)	3929.08
F09	3930.1044	5	2264.088	1.227	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	1663.782	89-102 HYTSSQDVTVP R	3928.878
F09	3940.5443	6	1461.35	2.45	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	2xCys_CAM	2475.737	82-102 SVTCHVQHTYSSQDVTVP R (1 missed cleavage)	3938.094
F09	3940.5443	6	2264.088	-2.359	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_PAM	1677.809	89-102 HYTSSQDVTVP R	3942.904
F09	4020.3955	7	1541.387	2.264	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	2xCys_CAM	2475.737	82-102 SVTCHVQHTYSSQDVTVP R (1 missed cleavage)	4018.111
F09	4020.3955	7	1695.556	-2.807	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	2xMSO	2326.64	MAGKPTHINVVVMAEADGT CY	4023.203
F10	4033.5618	8	1242.155	2.23	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2788.169	89-113 HYTSSQDVTVP RPPPP CCHPR (1 missed cleavage)	4031.332
F10	4033.5618	8	1664.545	0.317	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 1xMSO	2367.692	319-340 MAGKPTHINVVVMAEADGT CY	4033.244
F10	4033.5618	8	1648.545	0.317	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2383.692	319-340 MAGKPTHINVVVMAEADGT CY	4033.244
F10	4033.5618	8	2352.151	2.594	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_PAM	1677.809	89-102 HYTSSQDVTVP R	4030.967
F09	4081.2090	9	1242.155	0.813	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2837.233	315-340 TIDRMAGKPTHINVVVMAEADGT CY (1 missed cleavage)	4080.396
F09	4081.2090	9	1695.556	0.954	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2383.692	319-340 MAGKPTHINVVVMAEADGT CY	4080.255
F10	4092.3096	10	1728.582	1.087	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2361.633	82-102 SVTCHVQHTYSSQDVTVP R (1 missed cleavage)	4091.222
F10	4092.3096	10	1695.556	-1.971	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_PAM, 2xMSO	2397.718	319-340 MAGKPTHINVVVMAEADGT CY	4094.281
F09	4196.7181	11	1826.687	1.332	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 1xMSO	2367.692	319-340 MAGKPTHINVVVMAEADGT CY	4195.387
F09	4196.7181	11	1810.688	1.331	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2383.692	319-340 MAGKPTHINVVVMAEADGT CY	4195.387
F09	4196.7181	11	1898.751	2.319	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2294.641	319-340 MAGKPTHINVVVMAEADGT CY	4194.399
F10	4405.9619	12	1769.635	-1.384	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2837.233	315-340 TIDRMAGKPTHINVVVMAEADGT CY (1 missed cleavage)	4407.875
F10	4405.9619	12	1648.545	-2.386	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	3xCys_CAM	2959.325	89-113 HYTSSQDVTVP RPPPP CCHPR (1 missed cleavage)	4408.877
F10	4405.9619	12	1898.751	2.413	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_PAM	2503.79	82-102 SVTCHVQHTYSSQDVTVP R (1 missed cleavage)	4403.549
F09	4460.9483	13	2093.92	-1.67	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 1xMSO	2367.692	319-340 MAGKPTHINVVVMAEADGT CY	4462.619
F09	4460.9483	13	2134.972	-1.67	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 1xMSO	2853.233	315-340 TIDRMAGKPTHINVVVMAEADGT CY (1 missed cleavage)	4461.733
F09	4460.9483	13	2060.893	1.329	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_PAM, 2xMSO	2397.718	319-340 MAGKPTHINVVVMAEADGT CY	4459.619
F09	4479.0187	14	1623.492	1.287	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 1xMSO	2853.233	315-340 TIDRMAGKPTHINVVVMAEADGT CY (1 missed cleavage)	4477.732
F09	4479.0187	14	1607.493	1.286	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2869.232	315-340 TIDRMAGKPTHINVVVMAEADGT CY (1 missed cleavage)	4477.733
F09	4479.0187	14	2060.893	-1.565	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2418.685	82-102 SVTCHVQHTYSSQDVTVP R (1 missed cleavage)	4480.585
F10	4497.9852	15	406.39	1.771	(HexNAc) ₂	No modification	4088.817	103-140 VPPPPCCHPRLSHRPALE DLLGSEANLTCTLTGLR (1 missed cleavage)	4496.214
F10	4497.9852	15	2101.946	-2.686	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_PAM, 2xMSO	2397.718	319-340 MAGKPTHINVVVMAEADGT CY	4500.672
F09	4521.7347	16	2150.972	2.063	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 1xMSO	2367.692	319-340 MAGKPTHINVVVMAEADGT CY	4519.672

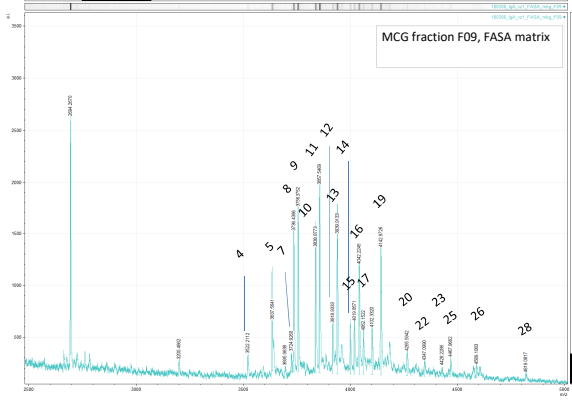
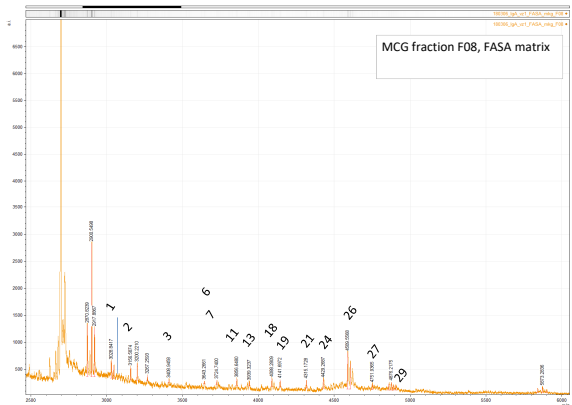


F09	4521.7347	16	2134.972	2.064	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM, 2xMSO	2383.692	319-340 MAGKPTHINVVVMAEADGT CY	4519.671
F09	4521.7347	16	2101.946	0.097	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2418.685	82-102 SVTCHVHHTNSSQDVTVP C R (1 missed cleavage)	4521.638
F09	4539.2184	17	1728.582	-2.55	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	2xMSO	2812.18	315-340 TIDRMAGKPTHINVVVMAE ADGTCY (1 missed cleavage)	4541.77
F09	4539.2184	17	1687.53	-2.551	(Hex) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM, 1xMSO	2853.233	315-340 TIDRMAGKPTHINVVVMAE ADGTCY (1 missed cleavage)	4541.77
F09	4539.2184	17	2060.893	1.581	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	2xCyT_CAM	2475.737	82-102 SVTCHVHHTNSSQDVTVP C R (1 missed cleavage)	4537.637
F09	4554.8351	18	2134.972	0.171	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM	2418.685	82-102 SVTCHVHHTNSSQDVTVP C R (1 missed cleavage)	4554.664
F09	4554.8351	18	1648.545	-2.094	(HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2907.378	114-140 LSLHRPALEDLLLGSEANLT CTLTGLR	4556.93
F09	4554.8351	18	1695.556	-0.975	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_PAM	2859.248	89-113 HYTNSQDVTVP C R V P P P P P CCHPR (1 missed cleavage)	4555.811
F10	4590.2897	19	1744.582	-0.52	(Hex) ₁ (HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM	2845.221	89-113 HYTNSQDVTVP C R V P P P P P CCHPR (1 missed cleavage)	4590.811
F10	4590.2897	19	1687.53	-0.52	(Hex) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	2xCyT_CAM	2902.273	89-113 HYTNSQDVTVP C R V P P P P P CCHPR (1 missed cleavage)	4590.811
F10	4590.2897	19	1623.492	1.36	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM	2964.43	114-140 LSLHRPALEDLLLGSEANLT CTLTGLR	4588.929
F10	4590.2897	19	2264.088	-1.445	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	2xMSO	2326.64	319-340 MAGKPTHINVVVMAEADGT CY	4591.735
F10	4606.4908	20	1769.635	-1.384	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM	2837.233	315-340 TIDRMAGKPTHINVVVMAE ADGTCY (1 missed cleavage)	4607.875
F10	4606.4908	20	1648.545	-2.386	(HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	3xCyT_CAM	2959.325	89-113 HYTNSQDVTVP C R V P P P P P CCHPR (1 missed cleavage)	4608.877
F10	4606.4908	20	1695.556	2.549	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2907.378	114-140 LSLHRPALEDLLLGSEANLT CTLTGLR	4603.941
F09	4661.9639	21	1703.529	-1.897	(Hex) ₁ + (Man) ₂ (GlcNAc) ₂	3xCyT_CAM	2959.325	89-113 HYTNSQDVTVP C R V P P P P P CCHPR (1 missed cleavage)	4663.862
F09	4661.9639	21	1695.556	0.971	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2964.43	114-140 LSLHRPALEDLLLGSEANLT CTLTGLR	4660.993
F09	4878.9184	22	1972.83	-2.296	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2907.378	114-140 LSLHRPALEDLLLGSEANLT CTLTGLR	4881.215
F09	4878.9184	22	1898.751	0.703	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_PAM	2978.457	114-140 LSLHRPALEDLLLGSEANLT CTLTGLR	4878.215
F09	4891.2896	23	1988.829	-0.819	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	2xCyT_CAM	2902.273	89-113 HYTNSQDVTVP C R V P P P P P CCHPR (1 missed cleavage)	4892.11
F09	4891.2896	23	1931.777	-0.819	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	3xCyT_CAM	2959.325	89-113 HYTNSQDVTVP C R V P P P P P CCHPR (1 missed cleavage)	4892.11
F09	4891.2896	23	2101.946	0.167	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2788.169	89-113 HYTNSQDVTVP C R V P P P P P CCHPR (1 missed cleavage)	4891.123
F09	4909.0061	24	1947.777	0.897	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	3xCyT_CAM	2959.325	89-113 HYTNSQDVTVP C R V P P P P P CCHPR (1 missed cleavage)	4908.11
F09	4909.0061	24	2052.867	1.899	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM, 1xMSO	2853.233	315-340 TIDRMAGKPTHINVVVMAE ADGTCY (1 missed cleavage)	4907.107
F09	4909.0061	24	2555.346	0.96	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM	2351.693	319-340 MAGKPTHINVVVMAEADGT CY	4908.046
F09	4925.2950	25	2555.294	1.301	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM, 1xMSO	2367.692	319-340 MAGKPTHINVVVMAEADGT CY	4923.994
F09	4925.2950	25	2068.867	2.188	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM, 1xMSO	2853.233	315-340 TIDRMAGKPTHINVVVMAE ADGTCY (1 missed cleavage)	4923.107
F09	4925.2950	25	2555.346	1.25	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM, 1xMSO	2367.692	319-340 MAGKPTHINVVVMAEADGT CY	4924.046
F09	5068.1014	26	2701.437	-2.034	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM, 1xMSO	2367.692	319-340 MAGKPTHINVVVMAEADGT CY	5070.137
F09	5068.1014	26	2215.01	-1.148	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM, 1xMSO	2853.233	315-340 TIDRMAGKPTHINVVVMAE ADGTCY (1 missed cleavage)	5069.25
F09	5068.1014	26	2101.946	0.718	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM	2964.43	114-140 LSLHRPALEDLLLGSEANLT CTLTGLR	5067.383
F09	5842.8787	27	2879.579	-2.137	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM	2964.43	114-140 LSLHRPALEDLLLGSEANLT CTLTGLR	5845.016
F09	5842.8787	27	1582.439	-0.54	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	3xCyT_CAM	4259.973	103-140 VPPPPPCCHPRLSHRPALE DLLLGSEANLTCTLTGLR (1 missed cleavage)	5843.419
F09	5842.8787	27	1695.556	0.446	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM	4145.869	103-140 VPPPPPCCHPRLSHRPALE DLLLGSEANLTCTLTGLR (1 missed cleavage)	5842.432
F09	5874.4301	28	2215.01	1.237	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	2xCyT_CAM	3657.176	82-113 SVTCHVHHTNSSQDVTVP C R V P P P P P CCHPR (2 missed cleavage)	5873.193
F09	5874.4301	28	1728.582	-1.027	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCyT_CAM	4145.869	103-140 VPPPPPCCHPRLSHRPALE DLLLGSEANLTCTLTGLR (1 missed cleavage)	5875.458
F09	5874.4301	28	2101.946	0.197	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	4xCyT_CAM	3771.28	82-113 SVTCHVHHTNSSQDVTVP C R V P P P P P CCHPR (2 missed cleavage)	5874.233
F09	5989.0456	29	3512.149	0.153	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	2xCyT_CAM	2475.737	82-102 SVTCHVHHTNSSQDVTVP C R (1 missed cleavage)	5988.893
F09	5989.0456	29	1096.012	0.529	(HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	4xCyT_CAM	4891.498	165-208 DLGGCYYSVSLPGCAQPNW HGFTTCTTAHPELKTPLTA NITK (1 missed cleavage)	5988.517

F09	5989.0456	29	1728.582	0.516	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	3xCys_CAM	4259.973	103-140 VPPPPPCCHPRLSLHRPALE DLLLGSEANLCTLTGLR (1 missed cleavage)	5989.562
F09	5989.0456	29	1898.751	0.47	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	4088.817	103-140 VPPPPPCCHPRLSLHRPALE DLLLGSEANLCTLTGLR (1 missed cleavage)	5988.575
F09	6021.4984	30	1299.207	0.942	(HexNAc) ₂ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	4720.342	165-208 DLGCGYSVSVLPGCAQPWN HGETFTCTAAHPELKTPLTA NITK (1 missed cleavage)	6020.556
F09	6021.4984	30	1242.155	0.942	(HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	2xCys_CAM	4777.394	165-208 DLGCGYSVSVLPGCAQPWN HGETFTCTAAHPELKTPLTA NITK (1 missed cleavage)	6020.556
F09	6021.4984	30	1242.155	0.009	(HexNAc) ₂ (Deoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	4778.327	8-51 VFPLSLDSTPDQGNVVVACL VGFFFGQEFLSVTWSESGQV VYAR	6021.49
F09	6021.4984	30	1873.697	0.925	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	4145.869	103-140 VPPPPPCCHPRLSLHRPALE DLLLGSEANLCTLTGLR (1 missed cleavage)	6020.573
F09	6021.4984	30	2264.088	0.095	(Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	3xCys_PAM	3756.308	82-113 SVTCNKKHYTNLSQDVTVPC RVPPPPPCCHPE (2 missed cleavage)	6021.404

Supplementary Table 1c. plgA1(F2) glycan structure

Microgradient fraction number	m/z	Peak number	Glycoform mass	ΔMass (Da)	Deduced glycoform (in GlycoMod)	Glycan type	Peptide modification	Peptide mass	Peptide sequence	Theoretical glycopeptide mass
F08		3045.8276	1	1379.244	1.794 (Hex) ₁ + (Man) ₃ [GlcNAc] ₂ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂	HM	1xCy ₂ _CAM	1663.782	89-102 HYTNSSQDVTVPCK	3044.034
F08	3156.5874	2	1549.413	-0.562	(Hex) ₁ [GlcNAc] ₂ (Man) ₃ [GlcNAc] ₂ + (Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂	H/C	No modification	1606.73	89-102 HYTNSSQDVTVPCK	3157.151
F08	3408.9457	3	1744.582	-0.425	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁	H/C	1xCy ₂ _CAM 1xCy ₂ _CAM, 1xMSO	1663.782 2367.692	89-102 HYTNSSQDVTVPCK MAGKPTIHVSVVMAEADGT CY	3409.372 3407.66
F09	3522.2112	4	1914.75	-0.275	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂ (Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂	H/C	No modification	1606.73	89-102 HYTNSSQDVTVPCK	3522.488
F09	3637.5640	5	1972.83	-0.054	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂ + (Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂	H/C	1xCy ₂ _CAM 1xCy ₂ _CAM, 2xMSO	1663.782 2383.692	89-102 HYTNSSQDVTVPCK MAGKPTIHVSVVMAEADGT CY	3637.62 3642.854
F08	3724.7400	7	1363.245	-1.144	(Hex) ₁ (Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂	HM	No modification	2361.633	82-102 SVTCHVHYTNSSQDVTVPCK (1 missed cleavage)	3725.885
F08	3724.7400	7	2060.893	-0.941	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂	H/C	1xCy ₂ _CAM	1663.782	89-102 HYTNSSQDVTVPCK	3725.683
F09	3736.4399	8	1258.155	1.54	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂	H/C	2xCy ₂ _CAM	2475.737	82-102 SVTCHVHYTNSSQDVTVPCK (1 missed cleavage)	3734.899
F09	3756.3751	9	1404.298	-0.622	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂	H/C	1xCy ₂ _CAM	2351.693	319-340 MAGKPTIHVSVVMAEADGT CY	3756.998
F09	3756.3751	9	1404.298	-0.622	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂	H/C	1xCy ₂ _CAM	2351.693	319-340 MAGKPTIHVSVVMAEADGT CY	3756.998
F09	3837.8773	10	1420.297	-2.111	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (HexNAc) ₁ + (Man) ₃ [GlcNAc] ₂	H/C	1xCy ₂ _CAM	2418.685	82-102 SVTCHVHYTNSSQDVTVPCK (1 missed cleavage)	3839.989
F09	3857.5468	11	1502.402	2.445	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂	H/C	1xCy ₂ _CAM	2351.693	319-340 MAGKPTIHVSVVMAEADGT CY	3855.102
F09	3918.9330	12	1566.44	-0.206	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2351.693	319-340 MAGKPTIHVSVVMAEADGT CY	3919.14
F09	3918.9330	12	1549.413	0.821	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM, 1xMSO	2367.692	319-340 MAGKPTIHVSVVMAEADGT CY	3918.113
F09	3939.9130	13	1461.35	1.819	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		2xCy ₂ _CAM	2475.737	82-102 SVTCHVHYTNSSQDVTVPCK (1 missed cleavage)	3938.094
F09	4001.3220	14	1648.545	0.077	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2351.693	319-340 MAGKPTIHVSVVMAEADGT CY	4001.245
F09	4019.8571	15	1541.387	1.726	(Hex) ₁ + (Man) ₃ [GlcNAc] ₂		2xCy ₂ _CAM	2475.737	82-102 SVTCHVHYTNSSQDVTVPCK (1 missed cleavage)	4018.131
F09	4042.2244	16	1623.492	-0.959	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (HexNAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2418.685	82-102 SVTCHVHYTNSSQDVTVPCK (1 missed cleavage)	4043.184
F09	4062.1522	17	1096.012	0.703	(HexNAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2964.43	114-140 LSLHRALEDLLGSEANLT CTLTLGR	4061.449
F08	4088.2808	18	1242.155	-0.102	(HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2845.221	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4088.384
F09	4142.9725	19	1664.545	1.683	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (HexNAc) ₁ + (Man) ₃ [GlcNAc] ₂		2xCy ₂ _CAM	2475.737	82-102 SVTCHVHYTNSSQDVTVPCK (1 missed cleavage)	4141.289
F09	4265.5942	20	1299.207	0.95	(HexNAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2964.43	114-140 LSLHRALEDLLGSEANLT CTLTLGR	4264.644
F08	4315.1727	21	1461.35	-0.417	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (HexNAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM, 1xMSO	2853.233	315-340 TIDRMAGKPTIHVSVVMAE ADGTCY (1 missed cleavage)	4315.59
F09	4347.0990	22	1502.402	-1.53	(HexNAc) ₁ + (Man) ₃ [GlcNAc] ₂ (HexNAc) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2845.221	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4348.63
F09	4347.0990	22	1445.35	-1.53	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂		2xCy ₂ _CAM	2902.273	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4348.63
F09	4347.0990	22	1549.413	0.498	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xMSO	2796.181	315-340 TIDRMAGKPTIHVSVVMAE ADGTCY (1 missed cleavage)	4346.601
F08	4428.2286	23	1582.439	-0.438	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2845.221	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4428.668
F09	4428.2286	23	1525.387	-0.438	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		2xCy ₂ _CAM	2902.273	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4428.668
F09	4428.2286	23	1461.35	1.441	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (HexNAc) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2964.43	114-140 LSLHRALEDLLGSEANLT CTLTLGR	4426.787
F08	4429.2686	24	2060.893	-1.363	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM, 1xMSO	2367.692	319-340 MAGKPTIHVSVVMAEADGT CY	4425.593
F09	4467.9662	25	1623.492	-1.753	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2845.221	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4469.72
F09	4467.9662	25	1566.44	-1.753	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		2xCy ₂ _CAM	2902.273	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4469.721
F09	4467.9662	25	1502.402	0.127	(HexNAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2964.43	114-140 LSLHRALEDLLGSEANLT CTLTLGR	4467.839
F08	4589.5568	26	1744.582	-1.253	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2845.221	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4590.811
F08	4589.5568	26	1687.53	-1.253	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		2xCy ₂ _CAM	2902.273	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4590.811
F08	4589.5568	26	1623.492	0.627	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2964.43	114-140 LSLHRALEDLLGSEANLT CTLTLGR	4588.929
F08	4751.9365	27	1849.672	-1.015	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		2xCy ₂ _CAM	2902.273	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4752.953
F08	4751.9365	27	1785.634	0.865	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2964.43	114-140 LSLHRALEDLLGSEANLT CTLTLGR	4751.071
F08	4751.9365	27	1898.751	-1.054	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM, 1xMSO	2853.233	315-340 TIDRMAGKPTIHVSVVMAE ADGTCY (1 missed cleavage)	4752.991
F09	4819.0816	28	1972.83	0.023	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2845.221	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4819.059
F09	4819.0816	28	1914.75	1.051	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ + (Man) ₃ [GlcNAc] ₂		2xCy ₂ _CAM	2902.273	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4818.031
F08	4906.3232	29	1947.77	-1.785	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂		3xCy ₂ _CAM	2959.32528	89-113 HYTNSSQDVTVPCKVPPPPP CCHPR (1 missed cleavage)	4908.109
F08	4906.3232	29	2555.346	-1.722	(Hex) ₁ (HexNAc) ₁ (Hex) ₁ [GlcNAc] ₂ (Hex) ₁ (Deoxyhexose) ₁ (NeuAc) ₁ + (Man) ₃ [GlcNAc] ₂		1xCy ₂ _CAM	2351.693	319-340 MAGKPTIHVSVVMAEADGT CY	4908.046



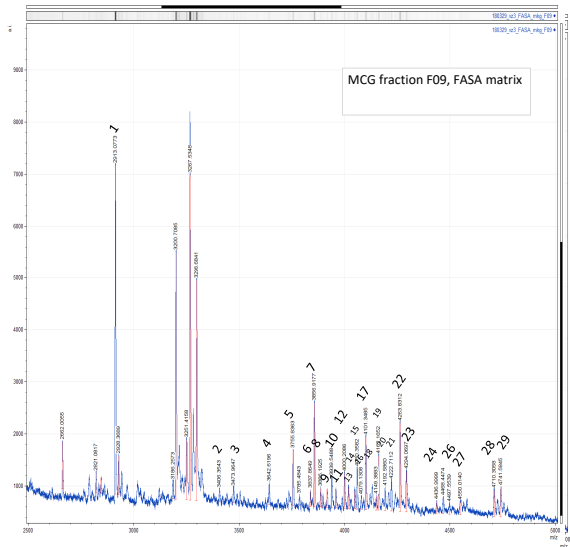
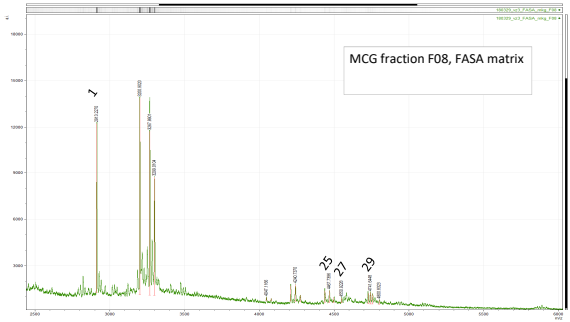
Supplementary Table 1d. plgA1(H) glycan structure

plgA2(H) Glycan structure assignments calculated in GlycoMod using experimental m/z values of glycopeptides

Cys_CAM = carbamidomethylation of cysteine; Cys_PAM = acrylamide adduct of cysteine; MSO = oxidized methionine; TPO = oxidized

red m/z values: possible sialylated structures; blue m/z values: less expectable peptide modifications

Microgradient fraction number	m/z	Peak number	Glycoform mass	ΔMass (Dalton)	Deduced glycoform (in GlycoMod)	Peptide modification	Peptide mass	Peptide sequence	Theoretical glycopeptide mass
F09	2913.0772	1	1955.803	-1.855	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	958.123	200-208 TPLTANITK	2914.933
F09	3406.3543	2	1096.012	-1.304	(HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xMSO	2310.64	MAGKPTHNIVSVVMAEADGT CY	3407.66
F09	3473.9647	3	1810.688	-1.512	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	1663.782	89-102 HYTNSQDVTVPQR	3475.478
F09	3642.6196	4	1258.155	-0.233	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2383.692	319-340 MAGKPTHNIVSVVMAEADGT CY	3642.854
F09	3755.8362	5	1404.298	-1.161	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2351.693	319-340 MAGKPTHNIVSVVMAEADGT CY	3756.998
F09	3837.8648	6	2173.957	-0.881	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	1663.782	89-102 HYTNSQDVTVPQR	3838.747
F09	3837.8648	6	1541.387	0.83	(Hex) ₁ (HexNAc) ₁	No modification	2294.641	MAGKPTHNIVSVVMAEADGT CY	3837.035
F09	3856.9177	7	892.817	-1.336	(Hex) ₁ (HexNAc) ₁	1xCys_CAM	2964.43	114-140 LSLHRPALEDLLGSEANLT CTLTGLR	3858.254
F09	3886.1925	8	1502.402	-0.908	(HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2383.692	319-340 MAGKPTHNIVSVVMAEADGT CY	3887.101
F09	3917.0678	9	1549.413	-1.044	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 1xMSO	2367.692	319-340 MAGKPTHNIVSVVMAEADGT CY	3918.113
F09	3939.5487	10	1461.35	1.455	(Hex) ₁ (HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	2xCys_CAM	2475.737	82-102 SVTCHVKHYTNSQDVTVPQR (1 missed cleavage)	3938.094
F09	3959.1353	11	1541.387	-1.943	(Hex) ₁ (HexNAc) ₁	1xCys_CAM	2418.685	82-102 SVTCHVKHYTNSQDVTVPQR (1 missed cleavage)	3961.079
F09	3959.1353	11	1664.545	-1.057	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2294.641	319-340 MAGKPTHNIVSVVMAEADGT CY	3960.193
F09	4000.2086	12	1648.545	-1.036	(HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2351.693	319-340 MAGKPTHNIVSVVMAEADGT CY	4001.245
F09	4019.1179	13	1664.545	1.873	(Hex) ₁ (HexNAc) ₁	1xCys_CAM	2351.693	319-340 MAGKPTHNIVSVVMAEADGT CY	4017.245
F09	4048.9401	14	1664.545	-0.303	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 1xMSO	2383.692	319-340 MAGKPTHNIVSVVMAEADGT CY	4049.344
F09	4060.3581	15	1582.439	1.175	(Hex) ₁ (HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	2xCys_CAM	2475.737	82-102 SVTCHVKHYTNSQDVTVPQR (1 missed cleavage)	4059.183
F09	4060.3581	15	1096.012	-1.09	(HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2964.43	114-140 LSLHRPALEDLLGSEANLT CTLTGLR	4061.449
F09	4079.1308	16	1242.155	-1.264	(HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2837.233	315-340 TIDRMAGKPTHNIVSVVMAEADGT CY (1 missed cleavage)	4080.396
F09	4101.3484	17	1623.492	1.112	(Hex) ₁ (HexNAc) ₁	2xCys_CAM	2475.737	82-102 SVTCHVKHYTNSQDVTVPQR (1 missed cleavage)	4100.236
F09	4101.3484	17	1217.102	1.918	(Hex) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2780.181	315-340 TIDRMAGKPTHNIVSVVMAEADGT CY (1 missed cleavage)	3998.291
F09	4149.3882	18	1728.582	1.114	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2418.685	82-102 SVTCHVKHYTNSQDVTVPQR (1 missed cleavage)	4148.274
F09	4149.3882	18	1242.155	-1.151	(HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2907.378	114-140 LSLHRPALEDLLGSEANLT CTLTGLR	4150.54
F09	4162.1652	19	1258.155	0.73	(Hex) ₁ (HexNAc) ₁	2xCys_CAM	2902.273	89-113 HYTNSQDVTVPQRVPPPP CCHPR (1 missed cleavage)	4161.436
F09	4162.1652	19	2555.346	-0.917	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	1606.73	89-102 HYTNSQDVTVPQR	4163.083
F09	4192.5858	20	1865.672	-0.732	(Hex) ₁ + (Man) ₂ (GlcNAc) ₂	2xMSO	2326.64	319-340 MAGKPTHNIVSVVMAEADGT CY	4193.319
F09	4192.5858	20	1898.751	-1.812	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2294.641	319-340 MAGKPTHNIVSVVMAEADGT CY	4194.399
F09	4222.7111	21	1258.155	-0.88	(Hex) ₁ (HexNAc) ₁	1xCys_CAM	2964.43	114-140 LSLHRPALEDLLGSEANLT CTLTGLR	4223.592
F09	4263.8312	22	1299.207	-0.812	(HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2964.43	114-140 LSLHRPALEDLLGSEANLT CTLTGLR	4264.644
F09	4294.0697	23	2052.867	0.963	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2869.232	315-340 TIDRMAGKPTHNIVSVVMAEADGT CY (1 missed cleavage)	4923.107
F09	4436.9999	24	1566.44	0.32	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2869.232	315-340 TIDRMAGKPTHNIVSVVMAEADGT CY (1 missed cleavage)	4436.68
F09	4436.9999	24	1648.545	-0.721	(HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2788.169	89-113 HYTNSQDVTVPQRVPPPP CCHPR (1 missed cleavage)	4437.722
F09	4468.4474	25	1566.44	-1.272	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	2xCys_CAM	2902.273	89-113 HYTNSQDVTVPQRVPPPP CCHPR (1 missed cleavage)	4469.721
F09	4497.5539	26	2111.987	0.868	(HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM, 2xMSO	2383.692	319-340 MAGKPTHNIVSVVMAEADGT CY	4496.686
F09	4550.0140	27	892.817	-0.985	(Hex) ₁ (HexNAc) ₁	2xCys_CAM	3657.176	82-113 SVTCHVKHYTNSQDVTVPQR RVPVPVPCCHPR (2 missed cleavage)	4551
F09	4710.3057	28	1744.582	0.286	(Hex) ₁ (HexNAc) ₁	1xCys_CAM	2964.43	114-140 LSLHRPALEDLLGSEANLT CTLTGLR	4710.019
F09	4710.3057	28	1914.75	-1.631	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xMSO	2796.181	315-340 TIDRMAGKPTHNIVSVVMAEADGT CY (1 missed cleavage)	4711.938
F09	4741.5848	29	2379.22	-0.275	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	No modification	2361.633	82-102 SVTCHVKHYTNSQDVTVPQR (1 missed cleavage)	4741.86
F09	4741.5848	29	2264.088	0.752	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	2xCys_CAM	2475.737	82-102 SVTCHVKHYTNSQDVTVPQR (1 missed cleavage)	4740.832
F09	4819.0816	30	1972.83	0.023	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2845.221	89-113 HYTNSQDVTVPQRVPPPP CCHPR (1 missed cleavage)	4819.059
F09	4819.0816	30	1914.75	1.051	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ + (Man) ₂ (GlcNAc) ₂	2xCys_CAM	2902.273	89-113 HYTNSQDVTVPQRVPPPP CCHPR (1 missed cleavage)	4818.031
F08	4906.3232	31	2555.294	2.335	(Hex) ₁ (HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2347.687	332-353 LAGKPTHNIVSVVMAEVDGT CY	4903.988
F08	4906.3232	31	1947.777	-1.785	(Hex) ₁ (HexNAc) ₁ + (Man) ₂ (GlcNAc) ₂	3xCys_CAM	2959.325	89-113 HYTNSQDVTVPQRVPPPP CCHPR (1 missed cleavage)	4908.109
F08	4906.3232	31	2555.346	-1.722	(Hex) ₁ (HexNAc) ₁ (Decoxyhexose) ₁ + (Man) ₂ (GlcNAc) ₂	1xCys_CAM	2351.693	319-340 MAGKPTHNIVSVVMAEADGT CY	4908.046



Supplementary Table 2. Combinations of IgA variants used in incubation with cells
IPEC-1

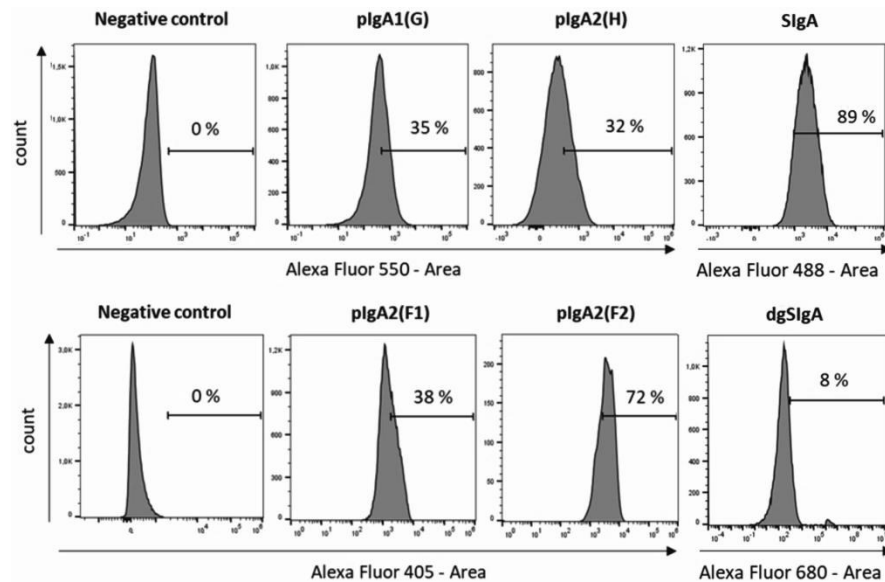
First incubation	Second incubation
SIgA-DyLight™488	-
dgSIgA-DyLight™680	-
plgA1(G)-DyLight™550	-
plgA2(F1)-DyLight™405	-
plgA2(F2)-DyLight™405	-
plgA2(H) DyLight™550	-
SIgA-DyLight™488	plgA1(G)-DyLight™550
SIgA-DyLight™488	plgA2(F1)-DyLight™405
SIgA-DyLight™488	olgA2(F2)-DyLight™405
SIgA-DyLight™488	olgA2(H) DyLight™550
plgA1(G)-DyLight™550	SIgA-DyLight™488
plgA2(F1)-DyLight™405	SIgA-DyLight™488
plgA2(F2)-DyLight™405	SIgA-DyLight™488
plgA2(H) DyLight™550	SIgA-DyLight™488

Supplementary Table 3. Sequences of primers used for Real-Time PCR

Target gene (protein)	Sequence (5' to 3')	Product size (bp)
RPL4	ACCCTGCATCATCTATAATGAGGAC TGGGAAGGTTGTAGTTACTCTTGAG	224
PPIA	CAAACGGTTCCCAGTTTTTCATTTG TTAGATTTGTCCACAGTCAGCAATG	176
GAPDH	GAGTGAACGGATTTGGCCGC ACCAGCATCACCCCATTTGA	254
IL1A	GAAGAAATCATCAAGCCCAGATCAG AACAGTCGGGTTTCTGAGATTCTTA	260
IL1B	CATGGAGAAGCGATTTGTCTTCTAC TGGGCATCGATTAGACAATAGTGAA	298
IL6	CACTGGGCACATAACTTATGTTGTT TCTGAAACAACAAGGATCTTCAAGC	231
IL8	GACCCCACTGTGAAAATTCAGAAAT CTTGTTGTTGTTACTGCTGTTGTTG	150
TNF α	ACAACAGCAACACTTAGAAATCAGG GGAAGAAAGGGAAGCCTAAAAAGTC	251
ATF3	AGGAACTACTGTGTGCAAGG CTGAGCGAAGTCACGAAGAA	103
CLDN1 (claudin 1)	GAAAGACTACGTGTGACACAGAATC AGACCATGTCAATTTTAGCAATGGG	183
OCLN (occludin)	TACAAGAGAAATTTTGACAGTGGCC CTTACTTTTGTAAATCCGCAGATCCC	195
TBP	GATATTCAGCTCTGGGAAAATGGTG TCAGACTTACCTACTGAACTGTTGG	220
HSP90	AAACATTGTTAAGAAGTGCCTGGAG GACGTACTCTGAGAGAGAAGTCATC	202
TGF β	GAAAGCGGCAACCAAATCTATGATA TTGCTGTATTTCTGGTATAGCTCCA	185
PA2	GGTTTGAGGTTTGGTTTGTAGC CTCCCTTCTTGGGCTTTATTT	83
EGR	CCTTCGGCTCCTTTCCACAT GGGAAAAGACTCTGCGGTCA	263
TFRC (TfR)	GGCTTTGAAGAACCAGATCGC TGTCGGAGAGTATCTGGGCA	124
FCAR (Fc α R)	CCCTGCACCAAATGGGAGA CTGGTTCGTGGTGTCTGTGA	164
FCAMR (Fc α / μ R)	ATCCTGACTCCCCTCTCCAC TTTCGACCTCCTGAGAGCTG	103
MBL1 (MBL)	TGGGTTGCCAGGACTAAAGG	115

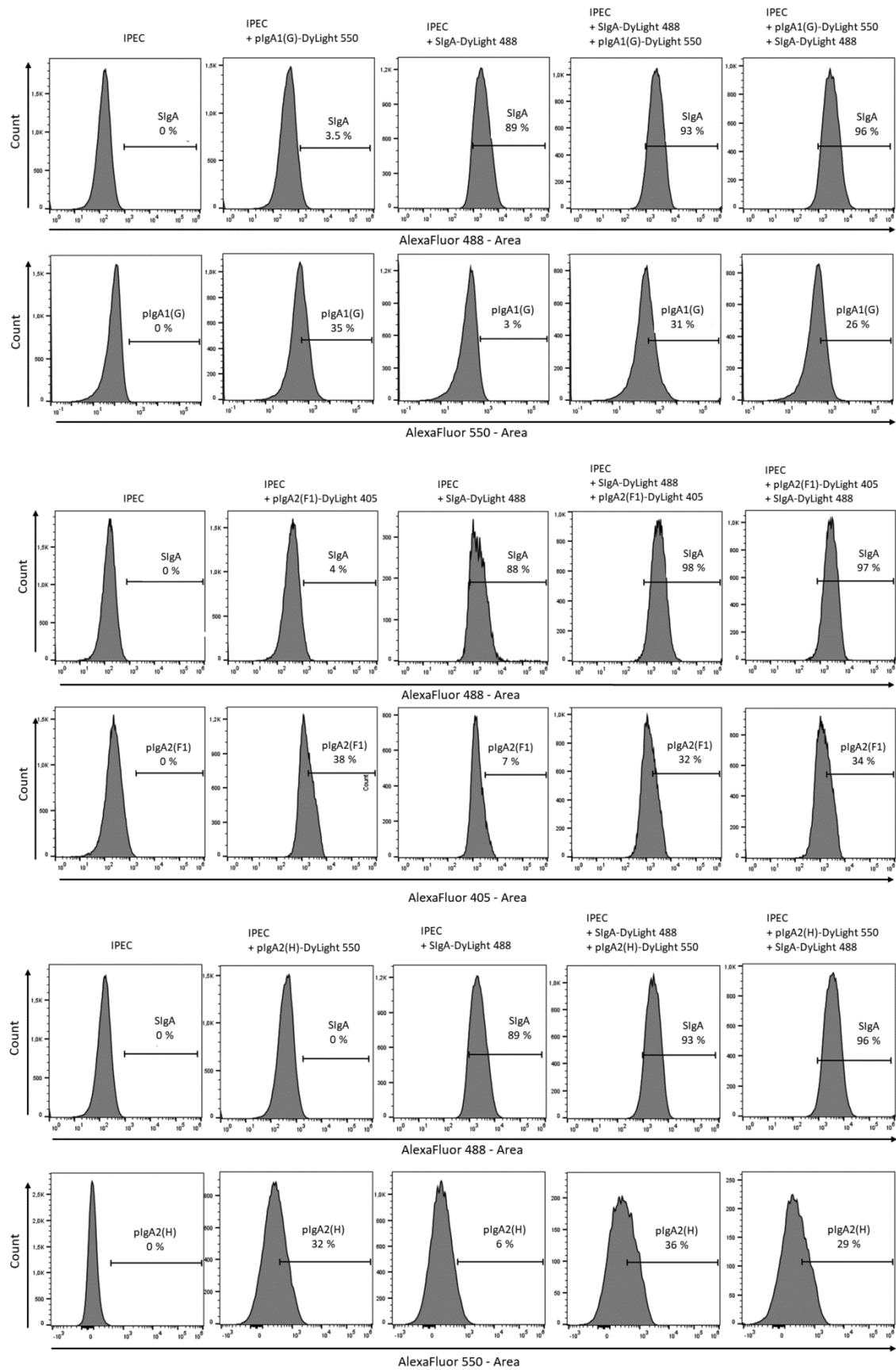
PIGR (pIgR)	CTGAGCGAAGATAAGCCACTTT	171
	AGTGTGGTCTGGGCATTAGC	
	ACACGGATTTCGGCTTCTGA	
SIGLEC5	TGACACGGAAAGAAGGATCTGAA	95
	CCAGCTTATCAGGGCAGGAG	
REG3G (REGIII γ)	CTAGCCTGTCCTGGATGCTG	70
	GCTGGGGAATCTTCACCTTG	

Supplementary Figure 1. *N*-glycan removal from SIgA reduces binding to IPEC-1 cells



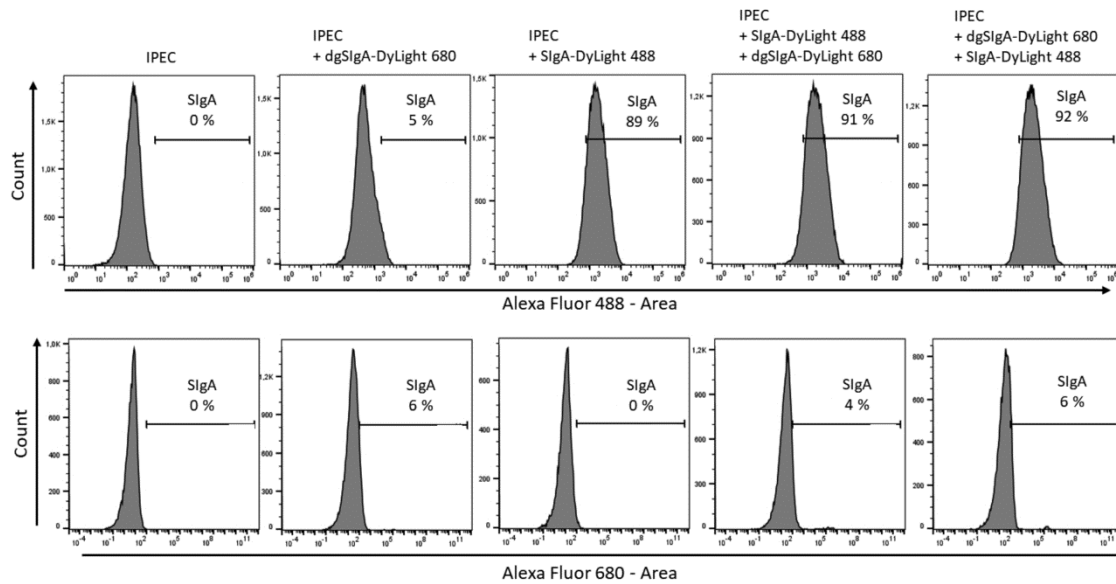
Cells were developed with plgA1(G), plgA2(H), plgA2(F1), plgA2(F2), SIgA, and dgSIgA, labeled with distinct fluorophores and IPEC-1 populations stained with labeled IgA were measured with Flow cytometry. Results are expressed as the number of positive cell in shown gates.

Supplementary Figure 2. Competition of individual IgA and SIgA for binding to IPEC-1



Fluorophores-label IgA preparations were incubated with IPEC-1 cells in depicted order and subsequently bound IgA were measured by Flow cytometry. Results are expressed as the number of positive cell in shown gates.

Supplementary Figure 3. Binding of IgAs and dgSIgA to IPEC-1 cells analysed by flow cytometry



Fluorophores-label SIgA and dgSIgA preparations were incubated with IPEC-1 cells in depicted order and subsequently bound dgSIgA and SIgA were measured by Flow cytometry. Results are expressed as the number of positive cell in shown gates.