

Supplementary information

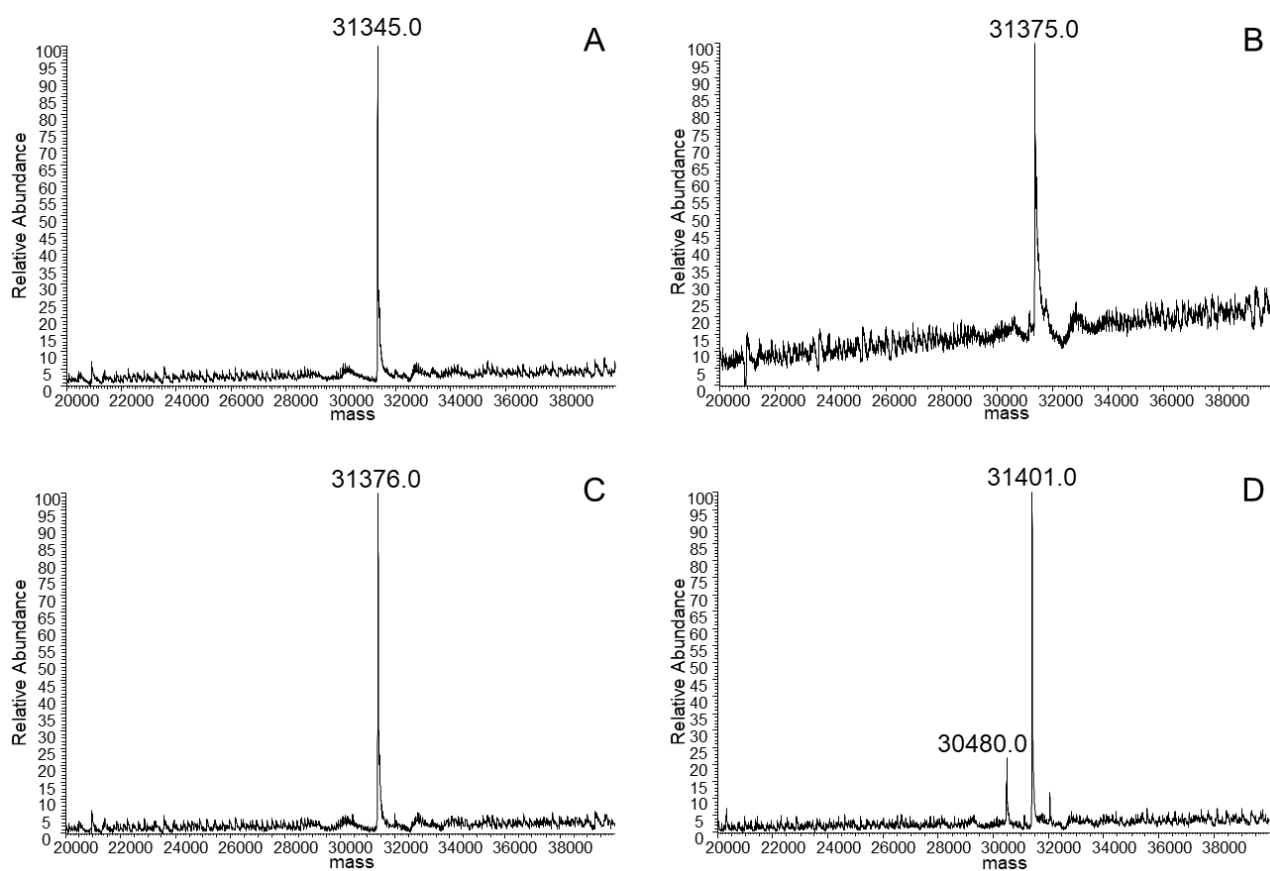


Fig. S1 Deconvoluted ESI-MS spectra of purified rAg85B (A), rAg85B-K30R (B), rAg85B-K282R (C) and rAg85B-K30R/K282R (D).

Table S1 Identified peptides in the mappings of rAg85B and its variants. Peptides including R30 are highlighted in yellow, those with R282 are in violet, while peptides comprising the unmodified K30 and K282 residues are in blue.

Position	[M+H] ⁺	Sequence	rAg85B	rAg85B-K30R	rAg85B-K282R	rAg85B-K30R/K282R
[1-8]	851.99	-.AMAISDPF.S	✓	✓	✓	✓
[1-17]	1851.12	-.AMAISDPFSRPGLPVEY.L			✓	✓
[3-8]	649.72	M.AISDPF.S	✓	✓		
[3-17]	1648.84	M.AISDPFSRPGLPVEY.L			✓	✓
[9-17]	1018.15	F.SRPGLPVEY.L	✓	✓	✓	✓
[18-24]	727.83	Y.LQVSPS.M		✓		
[18-30]	1456.70	Y.LQVSPSMGRDIR.V		✓		
[18-33]	1803.12	Y.LQVSPSMGRDIKVQF.Q	✓		✓	
[18-33]	1831.13	Y.LQVSPSMGRDIRVQF.Q		✓		✓
[18-44]	2878.21	Y.LQVSPSMGRDIKVQFQSGGNNSPAVY.L			✓	
[19-33]	1689.96	L.QVSPSMGRDIKVQF.Q	✓		✓	
[19-44]	2765.06	L.QVSPSMGRDIKVQFQSGGNNSPAVY.L			✓	
[20-33]	1561.83	Q.VSPSMGRDIKVQF.Q	✓		✓	
[25-33]	1094.31	S.MGRDIKVQF.Q	✓			
[34-44]	1094.12	F.QSGGNNSPAVY.L		✓		
[44-58]	1799.92	V.YLLDGLRAQDDYNGW.D		✓		
[45-55]	1279.38	Y.LLDGLRAQDDY.N		✓	✓	
[45-58]	1636.75	Y.LLDGLRAQDDYNGW.D	✓	✓	✓	✓
[46-58]	1523.59	L.LDGLRAQDDYNGW.D	✓	✓	✓	✓
[45-68]	2874.07	Y.LLDGLRAQDDYNGWDINTPAFEWY.Y			✓	
[46-65]	2282.41	L.LDGLRAQDDYNGWDINTPAF.E			✓	
[47-58]	1410.43	L.DGLRAQDDYNGW.D			✓	
[50-58]	1125.13	L.RAQDDYNGW.D		✓	✓	
[56-68]	1613.71	Y.NGWDINTPAFEWY.Y			✓	
[58-68]	1256.35	W.DINTPAFEWY.Y				✓
[59-67]	1093.17	W.DINTPAFEWY.Y	✓	✓	✓	✓
[59-68]	1256.35	W.DINTPAFEWY.Y	✓	✓	✓	
[59-73]	1804.94	W.DINTPAFEWYYQSGL.S			✓	
[68-86]	2084.34	W.YYQSGLSIVMPVGGQSSFY.S			✓	
[69-85]	1757.99	Y.YQSGLSIVMPVGGQSSF.Y			✓	✓
[69-86]	1921.16	Y.YQSGLSIVMPVGGQSSFY.S			✓	✓
[74-85]	1209.40	L.SIVMPVGGQSSF.Y		✓		
[74-86]	1372.57	L.SIVMPVGGQSSFY.S	✓	✓	✓	✓
[74-90]	1924.12	L.SIVMPVGGQSSFYSDWY.S			✓	
[78-86]	942.01	M.PVGGQSSFY.S			✓	✓
[86-102]	1901.07	F.YSDWYSPACGKAGCQTY.K			✓	
[87-102]	1737.90	Y.SDWYSPACGKAGCQTY.K	✓	✓	✓	✓
[90-102]	1349.52	W.YSPACGKAGCQTY.K			✓	
[91-102]	1186.35	Y.SPACGKAGCQTY.K		✓	✓	
[92-102]	1099.27	S.PACGKAGCQTY.K			✓	
[103-107]	710.80	Y.KWETF.L		✓		✓
[103-115]	1665.87	Y.KWETF.LTSELQWL.L			✓	
[103-116]	1779.03	Y.KWETF.LTSELQWL.S	✓		✓	✓
[104-107]	710.80	Y.KWETF.L	✓			
[108-116]	1087.25	F.LTSELQWL.S	✓	✓		
[117-132]	1513.73	L.SANRAVKPTGSAAIL.S	✓	✓	✓	✓
[120-130]	1071.26	N.RAVKPTGSAAIL.G		✓		
[120-132]	1241.47	N.RAVKPTGSAAIL.S	✓	✓	✓	✓
[121-132]	1085.28	R.AVKPTGSAAIL.S		✓		
[122-132]	1014.20	A.VKPTGSAAIL.S		✓		
[141-150]	1188.36	M.ILAAAYHPQQF.I	✓		✓	✓
[143-150]	962.04	L.AAYHPQQF.I	✓	✓	✓	✓
[151-156]	623.72	F.IYAGSL.S		✓		
[151-173]	2261.62	F.IYAGSLSALLDPSQGMGPSLIGL.A			✓	✓
[151-181]	2984.40	F.IYAGSLSALLDPSQGMGPSLIGLAMGDAGGY.K			✓	
[153-173]	1985.29	Y.AGSL.SALLDPSQGMGPSLIGL.A			✓	✓
[153-181]	2708.06	Y.AGSL.SALLDPSQGMGPSLIGLAMGDAGGY.K			✓	
[153-184]	2978.39	Y.AGSL.SALLDPSQGMGPSLIGLAMGDAGGYKAA.D			✓	

[157-173]	1656.93	L.SALLDPSQGMGPSLIGL.A	✓	✓	✓	✓
[157-181]	2379.70	L.SALLDPSQGMGPSLIGLAMGDAGGY.K			✓	✓
[158-173]	1569.85	S.ALLDPSQGMGPSLIGL.A		✓		
[160-173]	1385.61	L.LDPSQGMGPSLIGL.A	✓	✓		
[160-181]	2108.38	L.LDPSQGMGPSLIGLAMGDAGGY.K			✓	
[161-173]	1272.45	L.DPSQGMGPSLIGL.A		✓		
[161-181]	1995.22	L.DPSQGMGPSLIGLAMGDAGGY.K				
[182-187]	721.85	Y.KAADMW.G	✓	✓		
[182-206]	2827.08	Y.KAADMWGPSSDPAWERNDPTQQIPK.L	✓	✓	✓	✓
[182-207]	2940.24	Y.KAADMWGPSSDPAWERNDPTQQIPK.L.V	✓	✓		✓
[182-210]	3224.55	Y.KAADMWGPSSDPAWERNDPTQQIPKLVAN.N	✓		✓	
[182-211]	3338.66	Y.KAADMWGPSSDPAWERNDPTQQIPKLVANN.T	✓			✓
[182-212]	3439.76	Y.KAADMWGPSSDPAWERNDPTQQIPKLVANN.T.R	✓		✓	✓
[182-215]	3895.32	Y.KAADMWGPSSDPAWERNDPTQQIPKLVANN.T.RL.W.V			✓	✓
[188-206]	2124.26	W.GPSSDPAWERNDPTQQIPK.L	✓	✓		
[188-207]	2237.42	W.GPSSDPAWERNDPTQQIPK.L.V	✓	✓		
[188-208]	2336.55	W.GPSSDPAWERNDPTQQIPK.L.V.A	✓	✓		
[188-210]	2521.73	W.GPSSDPAWERNDPTQQIPKLVAN.N	✓	✓		
[188-211]	2635.83	W.GPSSDPAWERNDPTQQIPKLVANN.T	✓	✓		
[188-212]	2736.94	W.GPSSDPAWERNDPTQQIPKLVANN.T.R	✓	✓		✓
[188-215]	3192.49	W.GPSSDPAWERNDPTQQIPKLVANN.T.RL.W.V	✓		✓	✓
[196-206]	1326.44	W.ERNPTQQIPK.L		✓		
[196-215]	2394.68	W.ERNPTQQIPKLVANN.T.RL.W.V			✓	✓
[207-212]	631.70	K.LVANNT.R		✓		
[207-215]	1087.26	K.LVANNT.RL.W.V	✓		✓	
[216-235]	2024.20	W.VYCGNGTPNELGGANIPAEF.L			✓	
[216-239]	2527.75	W.VYCGNGTPNELGGANIPAEFLENF.V			✓	
[216-245]	3184.49	W.VYCGNGTPNELGGANIPAEFLENFVRRSSNL.K			✓	
[218-235]	1761.90	Y.CGNGTPNELGGANIPAEF.L			✓	
[218-239]	2265.45	Y.CGNGTPNELGGANIPAEFLENF.V			✓	
[236-245]	1179.31	F.LENFVRRSSNL.K	✓		✓	✓
[238-245]	937.04	E.NFVRRSSNL.K	✓			
[246-251]	771.84	L.KFQDAY.N		✓		
[246-261]	1710.83	L.KFQDAYNAAGGHNAVF.N	✓		✓	✓
[252-261]	958.01	Y.NAAGGHNAVF.N	✓	✓	✓	✓
[262-274]	1635.72	F.NFPPNGTHSWEYW.G			✓	
[264-271]	895.94	F.PPNGTHSW.E	✓		✓	
[272-278]	866.94	W.EYWGAQL.N	✓		✓	
[275-289]	1561.75	W.GAQLNAMRGDLQSSL.G			✓	
[275-292]	1746.93	W.GAQLNAMRGDLQSSLGAG.-			✓	✓
[279-289]	1192.33	L.NAMRGDLQSSL.G			✓	✓
[279-291]	1164.32	L.NAMKGDQSSL.G		✓		
[279-292]	1349.50	L.NAMKGDQSSLGAG.-	✓	✓		
[279-292]	1377.51	L.NAMRGDLQSSLGAG.-			✓	✓
[280-291]	1050.21	N.AMKGDQSSL.G		✓		
[280-292]	1235.39	N.AMKGDQSSLGAG.-	✓	✓		
[280-292]	1263.41	N.AMRGDLQSSLGAG.-			✓	✓
[282-292]	1033.12	M.KGDQSSLGAG.-		✓		

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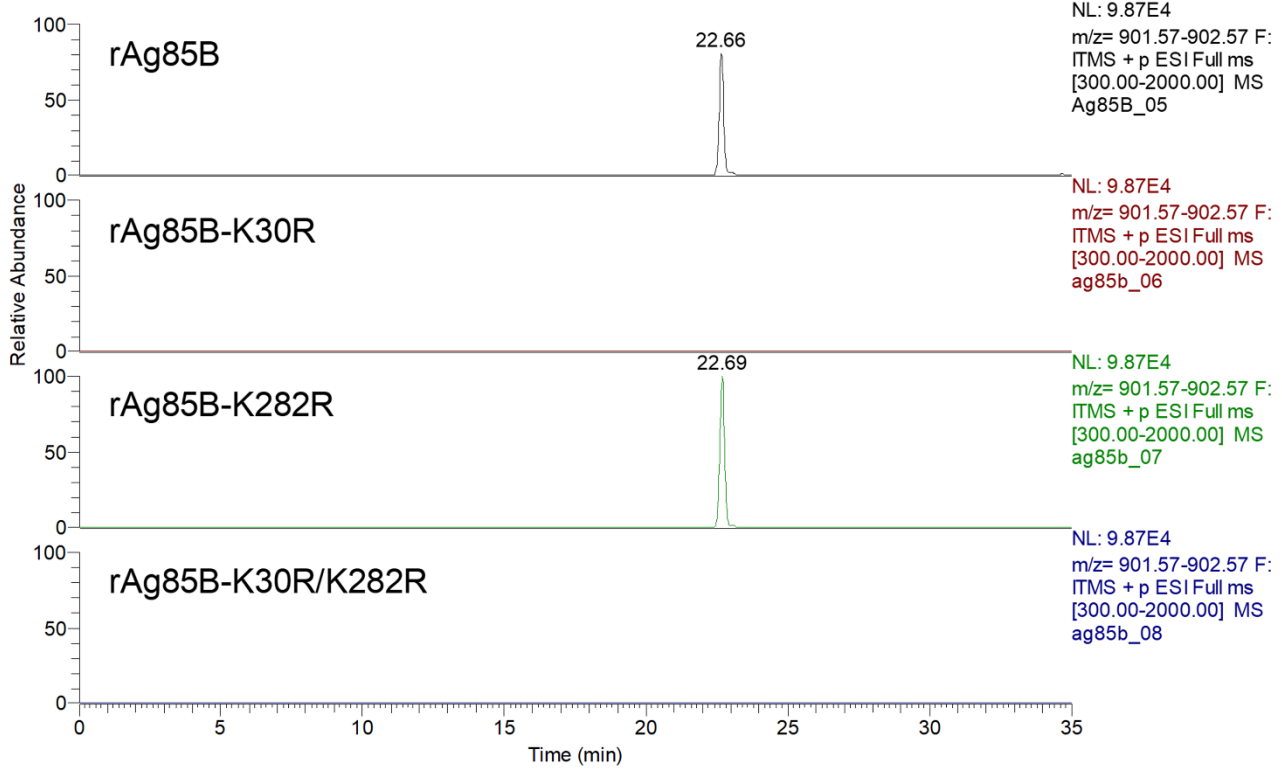


Fig. S2 EIC for m/z 902.07 Da (z=2) corresponding to peptide 18-33 comprising K30.

RT: 0.00 - 35.00 SM: 15G

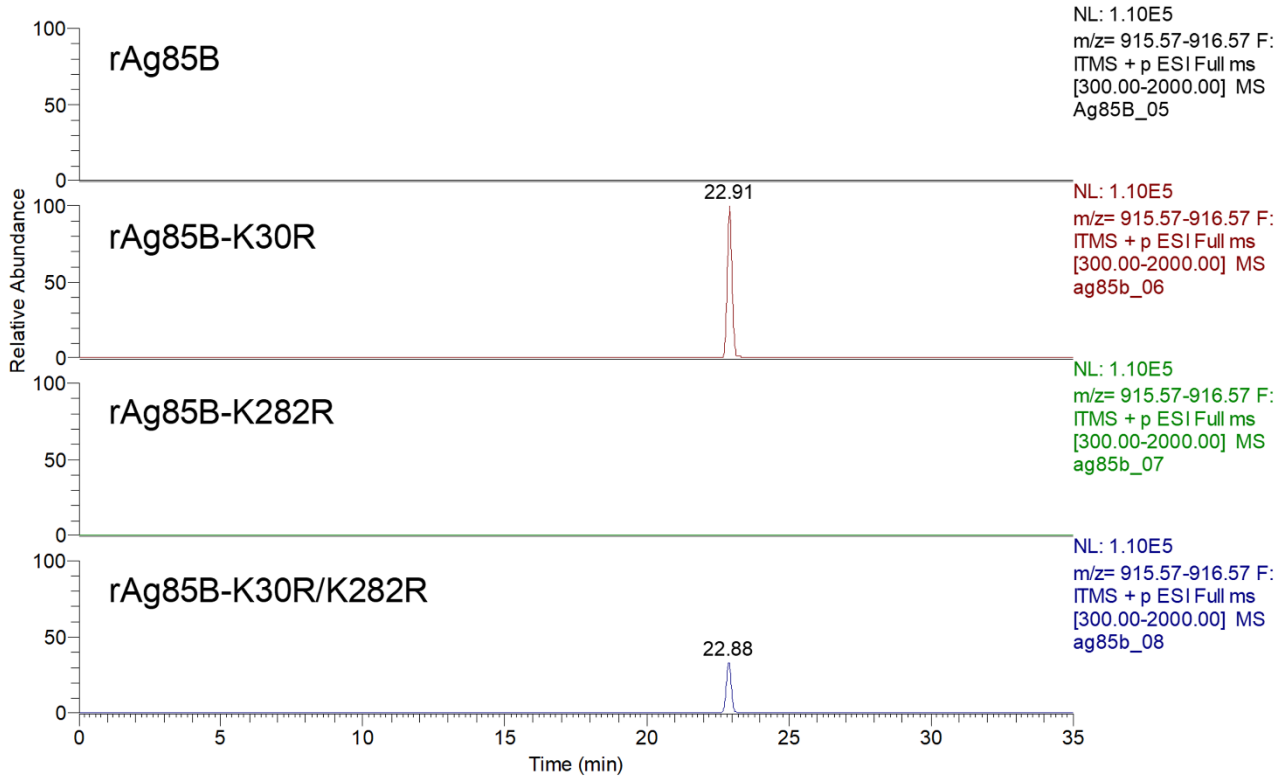


Fig. S3 EIC for m/z 916.07 Da (z=2) corresponding to peptide 18-33 comprising R30.

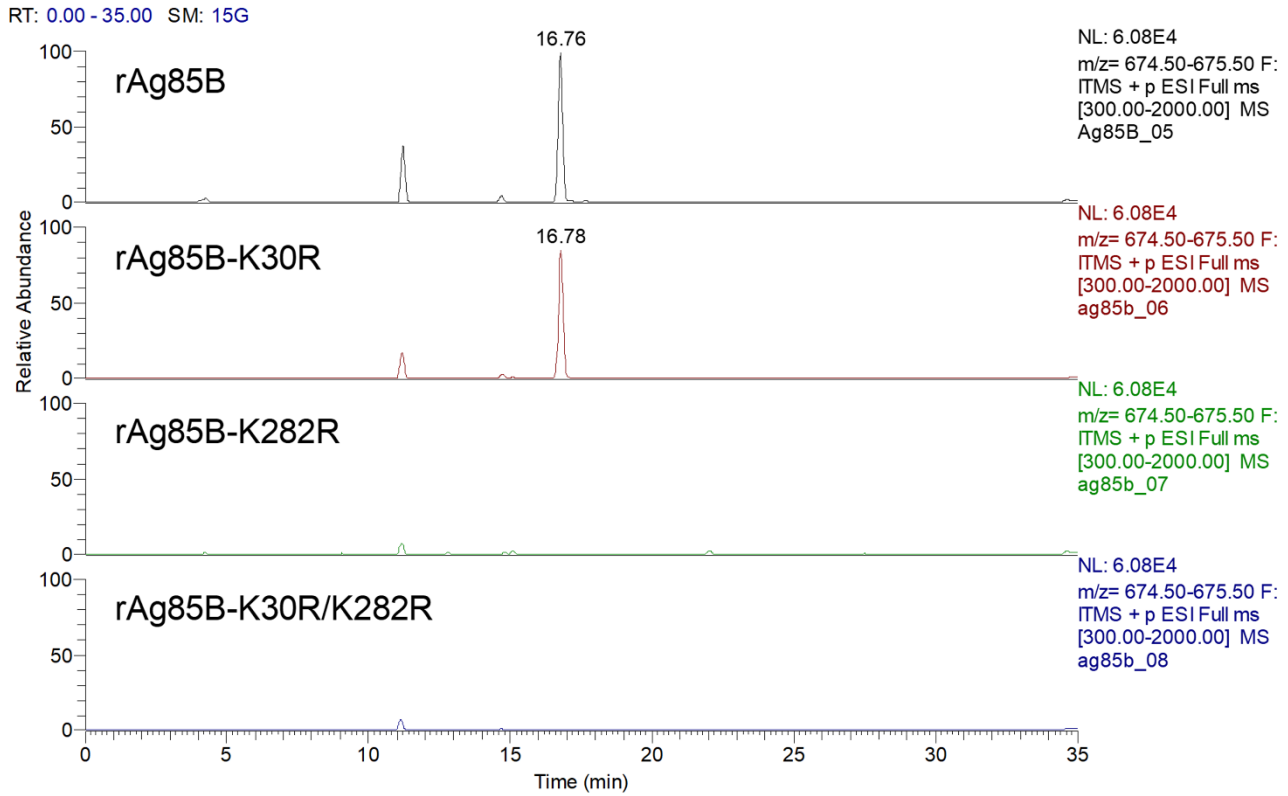


Fig. S4 EIC for m/z 675.00 Da ($z=2$) corresponding to peptide 279-292 comprising K282.

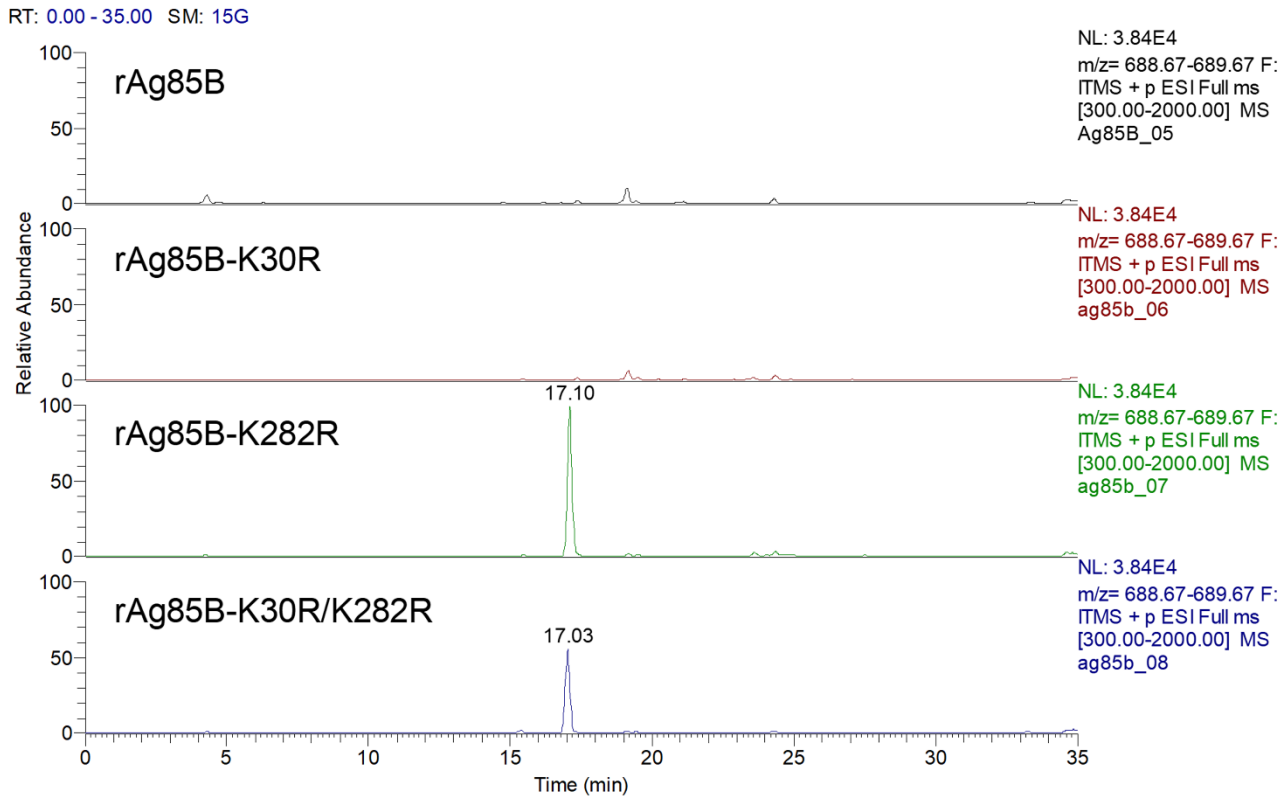


Fig. S5 EIC for m/z 689.17 Da ($z=2$) corresponding to peptide 279-292 comprising R282.

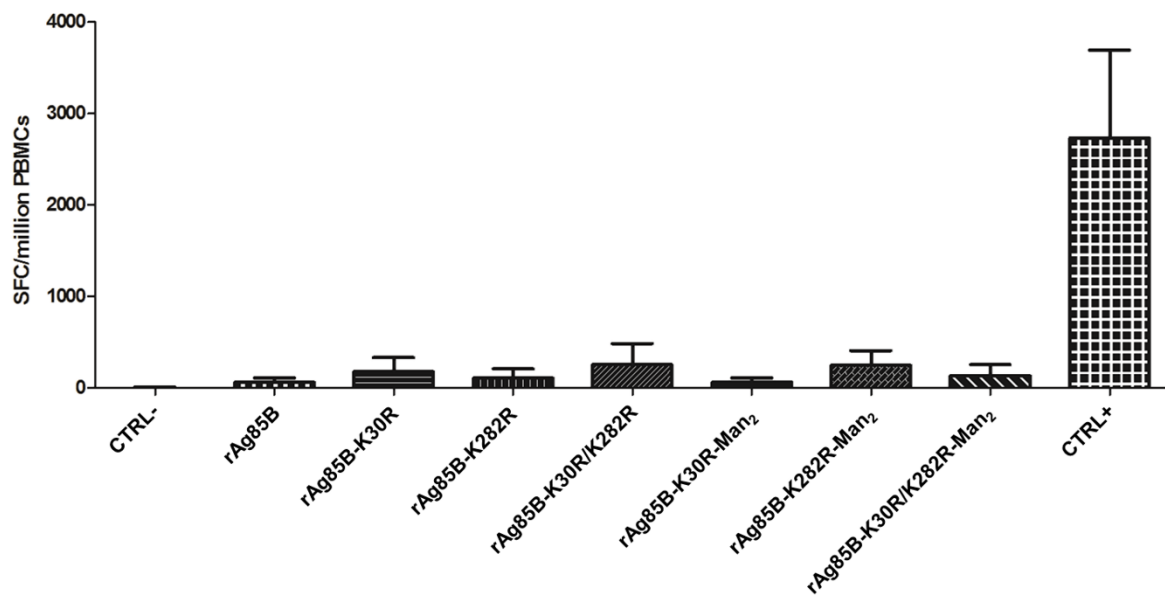


Fig. S6 T-cell responses to rAg85B and glycoconjugated rAg85B antigens. Data are presented as mean±standard deviation of the SFC per million PBMCs obtained by ELISPOT in non-BCG-vaccinated subjects. PBMC were stimulated with (CTRL+) or without (CTRL-) phytohemagglutinin.

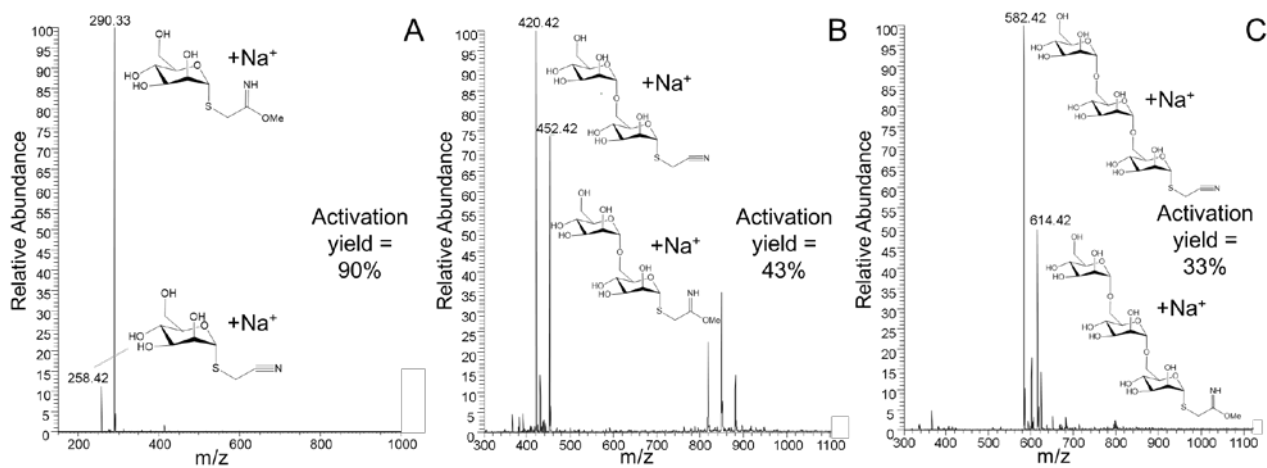


Fig. S7 ESI-MS spectra of Man-IME (A), Man₂-IME (B) and Man₃-IME (C) glycosides employed in this study.

Table S2 Glycoform distribution (relative abundance %), glycosylation yield (%) and glycoside/protein ratio (mol mol⁻¹) calculated for glycoconjugates assayed by ELISPOT.

Glycoconjugate	Number of incorporated glycosides (%)									Y (%)	Glycoside/protein (mol mol ⁻¹)
	0	1	2	3	4	5	6	7	8		
rAg85B-K30R-Man ₂	-	9.6	20.4	30.0	25.2	14.8	-	-	-	100.0	3.1
rAg85B-K282R-Man ₂	-	9.2	18.0	28.7	28.4	15.7	-	-	-	100.0	3.2
rAg85B-K30R/K282R-Man ₂	8.5	25.5	32.8	22.3	11.0	-	-	-	-	91.5	2.0