Supplementary Information for Identification and Characterization of Complex Glycosylated Peptides Presented by the MHC Class II Processing Pathway in Melanoma

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Source Protein	Peptide	Glycoform	1102mel	1363mel	2048mel	2048EBV	IEDB
Apolipoprotein B	KSS**VITLNTNVG**LYnQS	11	nd	nd	nd	+++	0.4
Apolipoprotein D	VINKE**LRADGTVNQ**IEGEATPEn	4	nd	nd	++	nd	0.5
	INKE**LRADGTVNQ**IEGEATPEn	4	nd	nd	+++	nd	0.5
hemopexin	GnHT**WFWDFSTKT**IK	7	+++	nd	nd	nd	2.4
IgG Heavy Chain Constant region	FnST**YRVVSALRI**QH	8	nd	++	nd	nd	0.1
	FnST**YRVVSALRI**QHQ	8	nd	++	+++	nd	0.1
IgG3	TKP**REQQYnSTL**R	*	nd	nd	+	nd	40
	VHTAQTKP**REQQYnSTL**R	*	nd	nd	+	nd	40
	IDVHTAQTKP**REQQYnSTL**R	*	nd	nd	+	nd	40
IgG4	E**WMGVIsPRD**GSTSSA	4	nd	+	nd	nd	47.8
Inter-alpha-trypsin inhibitor heavy chain H2	NPsPAP**VLPMPAVGA**QVL	2	nd	+	nd	nd	0.9

Supplementary Information Table 1. Bovine glycopeptides presented by MHC class II melanoma and EBV cell lines. Eleven glycopeptides originating from seven bovine proteins presumably originating from the fetal bovine serum used in cell culture medium. Source proteins were determined by *de novo* sequencing and BLAST. Six glycoforms were found on these peptides, which can be referenced from figure 1. One type of glycoform was not found on human proteins, GlcNAc2-Fucose-Mannose3-GlcNAc2-Galactose, indicated by an asterisk. Relative abundance key: nd, not detected, + 1-5 copies/cell, ++ 6-50 copies/cell, +++ 50-150 copies/cell, and ++++ >150 copies per cell.

	Uniprot ID	Source Protein	Location	IEDB Score	Allele
1	P01023	Alpha-2-macroglobulin	S		
2	P11717	Cation-independent mannose-6-phosphate receptor	LM	7.7	
3	Q6YHK3	CD109 antigen	M	10.8	
.4-7	P48960	CD97 antigen	M	8.6	
.10-13	P06276	Cholinesterase	S	1.4	B1*0404
14	P39060	Collagen alpha-1(XVIII) chain	S/EM	1.9	
15	O75071	EF-hand calcium-binding domain-containing protein 14	UK	8.5	B4*0103
16	P02751	Fibronectin	S/EM	2	B1*0404
17-27	P07093	Glia-derived nexin	S/ES	7.2	
28-33	P20036	HLA class II DP alpha 1 chain	M	3.5	
34-37	P05362	Intercellular adhesion molecule 1	M	0.3	
38	P40189	Interleukin 6 receptor subunit beta	M	4.8	B1*0401
39-41	P11047	Laminin subunit gamma-1	S/EM	4.2	
42	P40126	L-dopachrome tautomerase	MM	7.4*	
43-47	P40126	L-dopachrome tautomerase	MM	12.7	
48-49	Q14108	Lysosome membrane protein 2	LM		
50-53	P11279	Lysosome-associated membrane glycoprotein 1	M	2.8	B1*0404
54-61	P15586	N-acetylglucosamine-6-sulfatase	LM	1.6	B1*0401
62	O00533	Neural cell adhesion molecule L1-like protein	M	3.9*	
63-64	O15240	Neurosecretory protein VGF	S		B1*0404
65	P16671	Platelet glycoprotein 4	M	13.2	B1*0404
66-75	P07602	Prosaposin	S	4.5	
76	Q8WTV0	Scavenger receptor class B member 1	M	2.8	
77	Q04900	Sialomucin core protein 24 (MUC24)	LM	*	
78-80	P17948	Vascular endothelial growth factor receptor 1	M	0.8	

Supplementary Information Table 2. Additional MHC class II glycopeptide information.
Peptide numbers correspond to those seen in Table 1. Uniprot ID found on uniprot.org, where annotated cellular location was also determined. Key: S secreted, LM lysosome membrane, M cell membrane, EM extracellular matrix, ES extracellular space, and UK unknown. To determine best 9-residue binding motif, IEDB (http://tools.iedb.org/mhcii/) MHC II binding prediction tool was used. The assumed allele is HLA-DRβ1*0101 if found in 1363mel (as it is homozygous). For other cell lines, the lowest scoring allele and motif were used, as indicated in the last column of the table.