Mass spectrometry-based identification of a naturally presented receptor tyrosine kinase-like orphan receptor 1-derived epitope recognized by CD8+ cytotoxic T cells

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Supplemental Tables

Supplemental Table 1. Mass spectrometry-based identification of ROR1-derived peptide NPRYPNYMF.

Sample	HLA type	cell count	DDA method with 20% share for first sample validation					NPRYNYMF identification		mass spectrometer
			peptide IDs	PSMs	proteins	binders IDs	purity (% binders)	DDA method	PRM method	
CLL_1	A*01:01; A*03:01; B*07:02; B*44:02	1.8 x 10^8	2664	5137	2266	2336	88%	Yes	not used	LTQ Orbitrap Fusion Lumos
CLL_2	A*03:01; A*30:01; B*07:02; B*13:02; C*06:02; C*07:02	64.0 x 10^8	1183	1261	1229	902	91%	Yes	not used	LTQ Orbitrap XL
786-0	A*03:01; B*07:02; B*44:02; C*05:01; C*07:02	2.3 x 10^8	2915	7125	2364	2652	91%	Yes	Yes	LTQ Orbitrap Fusion Lumos
NIH: OVCAR3	A*02:01; A*29:02; B*07:02; B*58:01; C*07:02	2.0 x 10^8	1546	4103	1476	1031	67%	No	Yes	LTQ Orbitrap Fusion Lumos

DDA: data-dependent acquisition (a top speed CID fragmentation method). PRM: parallel reaction monitoring. PSM: peptide-spectrum match.

Supplemental Table 2. NPRYPNYMF-peptide binding scores for HLA-B*07:02 determined by four different binding prediction software tools.

Name of database	Score	Binding attribute	URL
SYFPEITHI	Score: 21	64% of max. score	http://syfpeithi.de/
Rankpep	Score: 21.301	'predicted binder'	http://imed.med.ucm.es/Tools/rankpep.html
NetMHC 4.0	%rank: 0.15	'strong binding peptide'	http://www.cbs.dtu.dk/services/NetMHC/
IEDB	Percentile_rank 0.5	'good binder'	http://tools.iedb.org/mhci/

The top score for the NPRYPNYMF-peptide was designated to HLA-B*07:02 in all four tested online-prediction tools.

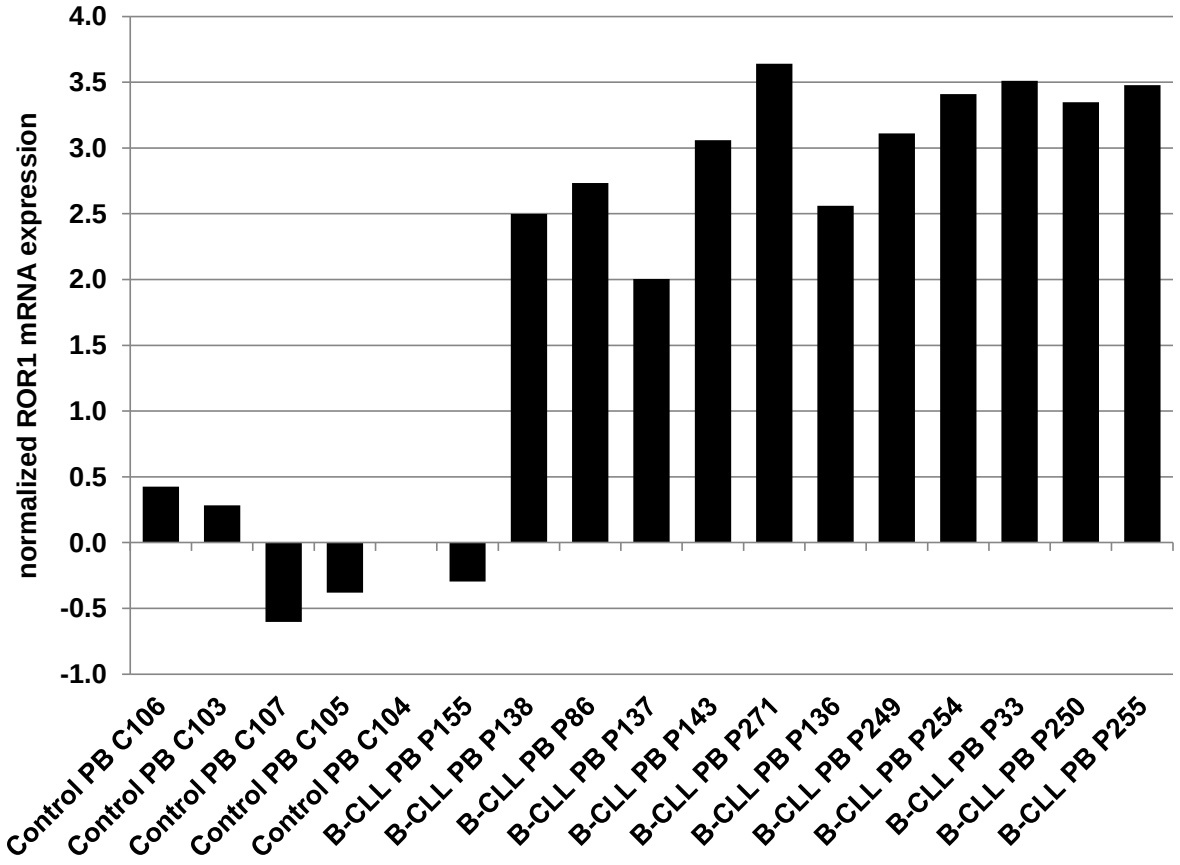
Supplemental Table 3. Amino acid sequences of TCR CDR3 α and β regions of ROR1-specific $CD8^+$ T-cell clones.

Clone	TRAV	AA sequence CDR3-α	TRAJ	
XB6	29/DV5*01	CAGPPLHGGYQKVTF	13*01	
XD8	12-2*01	CAVNAGSQGNLIF	42*01	
Clone	**TRBV**	**AA sequence CDR3-β**	**TRBD**	**TRBJ**
XB6	20-1*01	CSARTSGGYEQYF	2*01	2-7*01
XD8	6-6*01	CASSFTIGQGNSPLHF	1*01	1-6*01

CDR3 DNA sequence encoding amino acids between the last cysteine of the V- and the conserved phenylalanine in the J-segment was analyzed. Underlined amino acid sequences are encoded by the respective V- or J-gene segment.

Supplemental Figures

Supplemental Figure 1. ROR1 mRNA expression in normal B lymphocytes and CLL cells.



Supplemental Figure 2. ROR1 expression in human healthy tissues.

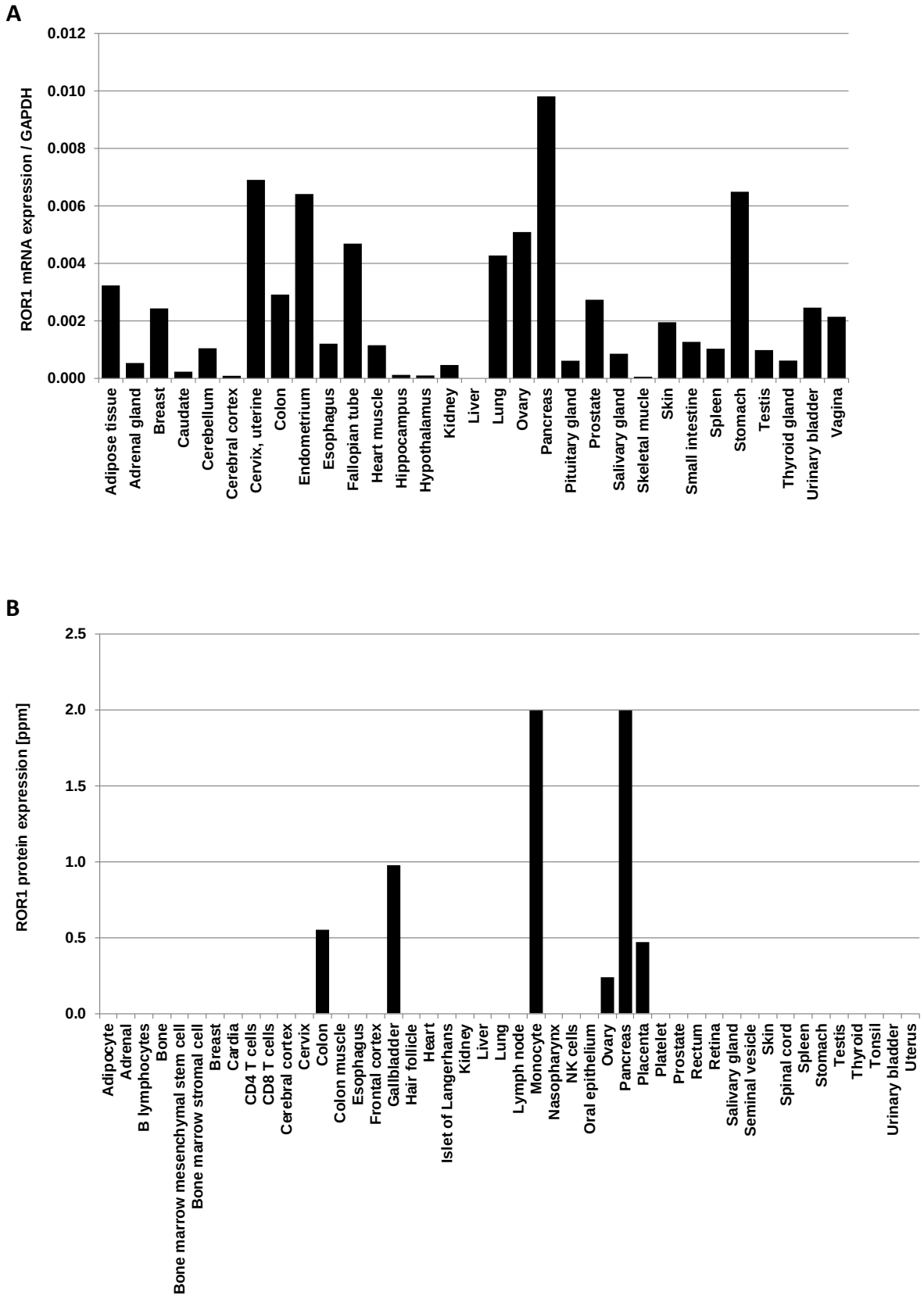


Figure Legends

Supplemental Figure 1. ROR1 mRNA expression in normal B lymphocytes and CLL cells. In the GDS3902 DataSet Record of the Gene Expression Omnibus repository, ROR1 mRNA expression in $CD19^+$ B cells (immunomagnetically isolated from PBMCs of healthy donors) was compared with peripheral blood CLL cells. Expression data were normalized on median of control samples.

Supplemental Figure 2. ROR1 expression in normal human tissues. Data for ROR1 expression in normal human tissues was obtained from public databases. A) ROR1 mRNA expression in 31 tested human tissues is displayed relative to GAPDH mRNA. Values were generated within the Genotype-Tissue Expression (GTEx) project and downloaded from www.proteinatlas.org. RNA-sequencing data was reported as median reads per kilobase per million mapped reads (RPKM). B) ROR1 protein expression in 47 tested human tissues is given. GAPDH protein expression values range from 1196 to 5474 ppm in the respective tissues. Values were obtained from the Human Integrated Protein Expression Database within GeneCards (www.genecards.org) based on ProteomicsDB as data source. For further information on data normalization see http://www.genecards.org/Guide/GeneCard#protein_expression.