ERAP1 allotypes shape the epitope repertoire of virus-specific CD8$^+$ T cell responses in acute hepatitis C virus infection

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Supplementary methods

CD8 selection and polyclonal expansion of PBMCs

Procedures were carried out as described previously [1]. Briefly, $CD8^+$ T cells were isolated from PBMCs via magnetic separation using anti-CD8-loaded Dynabeads (Invitrogen, Germany), stimulated once with 0.04 µg/mL anti-CD3 (Immunotech, France), and fed every 3 days with complete medium containing 100 U/mL recombinant IL-2 (Stemcell Technologies, Germany). Cells were used for experimental purposes after 2–3 weeks.

Peptide-specific T cell lines

PBMCs were activated with peptides as described previously [2]. Briefly, 4 x 10^6 PBMCs were stimulated once with 10 µg/mL peptide and 0.5 µg/mL anti-CD28 (BD Biosciences, Germany) and fed every 3 days with complete medium containing 20 U/mL recombinant IL-2 (Stemcell Technologies, Germany). Peptide-specific T cell lines were used for experimental purposes after 14 days.

Intracellular IFN-γ staining

Procedures were carried out as described previously [3]. Briefly, expanded $CD8^+$ T cells or peptide-specific T cell lines (0.2 x 10^6 cells per well in a 96-well plate) were stimulated with peptides (10 µg/mL) or peptide-loaded autologous or allogeneic Epstein-Barr virus (EBV)-immortalized B cells in the presence of 50 U/mL recombinant IL-2 and 1 µL/mL brefeldin A (BD Biosciences, Germany). After 5 hours, cells were blocked with IgG1 antibodies, stained with 7-AAD and anti-CD8,

fixed/permeabilized with Cytofix/Cytoperm, stained with anti-IFN-γ, and fixed in 100 µL 2% PFA in PBS (all reagents from BD PharMingen, Germany). Data were acquired using a FACS Canto flow cytometer (BD Biosciences, Germany) and analyzed with FlowJo software version 10 (Tree Star, OR, USA).

HLA/peptide multimer staining

Procedures were carried out as described previously [4]. Briefly, 1 x 10^6 cells per well in a 96-well plate were incubated with HLA/peptide multimer for 15 minutes at 37 °C. Cells were then washed three times with phosphate-buffered saline containing 1% fetal calf serum, blocked with IgG1 antibodies, stained with anti-CD8, washed again three times, and fixed in 100 µL 2% PFA in PBS (all reagents from BD Pharmingen, Germany). Data were acquired using a FACSCanto flow cytometer (BD Biosciences, Germany) and analyzed with FlowJo software version 10 (Tree Star, OR, USA).

Viral sequence analysis

Viral RNA was extracted from patient sera using the QIAmp Viral RNA Mini Kit according to the manufacturer's instructions and subsequently transcribed into cDNA by SuperScript™ III Reverse Transcriptase (Invitrogen, Germany) using specific reverse primers (see CTAT Table for primer sequences). Nested PCR (see CTAT Table for primer sequences) was performed using the GoTaq® Flexi DNA Polymerase Kit (Promega, USA) to amplify sequences which were sent to GATC Biotech (Konstanz, Germany) for Sanger Sequencing. Bulk sequences were analyzed using Geneious 11.0.5 (Biomatters Ltd., NZ).

References

[1] Thimme R, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, et al. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. Proceedings of the National Academy of Sciences of the United States of America 2002;99:15661-15668.

[2] Neumann-Haefelin C, McKiernan S, Ward S, Viazov S, Spangenberg HC, Killinger T, et al. Dominant influence of an HLA-B27 restricted CD8+ T cell response in mediating HCV clearance and evolution. Hepatology 2006;43:563-572.

[3] Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. The Journal of experimental medicine 2001;194:1395-1406.

[4] Bengsch B, Spangenberg HC, Kersting N, Neumann-Haefelin C, Panther E, von Weizsacker F, et al. Analysis of CD127 and KLRG1 expression on hepatitis C virus-specific CD8+ T cells reveals the existence of different memory T-cell subsets in the peripheral blood and liver. Journal of virology 2007;81:945-953.

Supplementary Figures

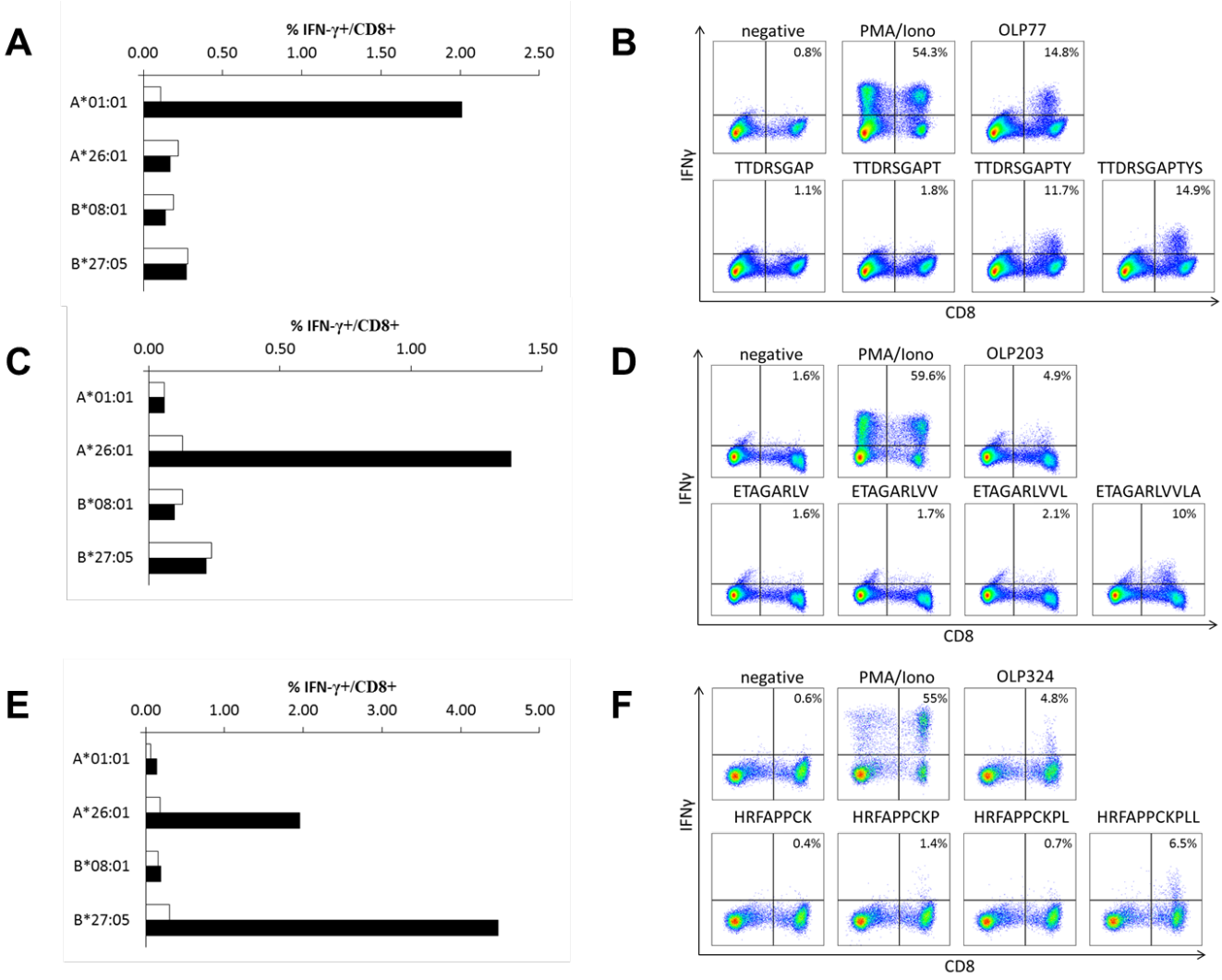

Fig. S1. HLA class I restriction and fine mapping of HCV-derived CD8⁺ T cell epitopes identified in donor MM. (**A**, **C**, **E**) HLA class I restriction analysis for CD8⁺ T cells targeting olp-77 (A), olp-203 (C), and olp-323/324 (E). CD8⁺ T cell lines specific for each olp were tested for IFN-γ production after incubation with EBV-immortalized B cell lines partially matched for the indicated HLA class I alleles, either unloaded (white bars) or loaded with the relevant olp (black bars). (**B**, **D**, **F**) Fine mapping experiments for CD8⁺ T cell epitopes in olp-77 (B), olp-203 (D), and olp-323/324 (F). CD8⁺ T cell lines specific for each olp were incubated with the indicated 8–11mer peptides and tested for IFN-γ production.

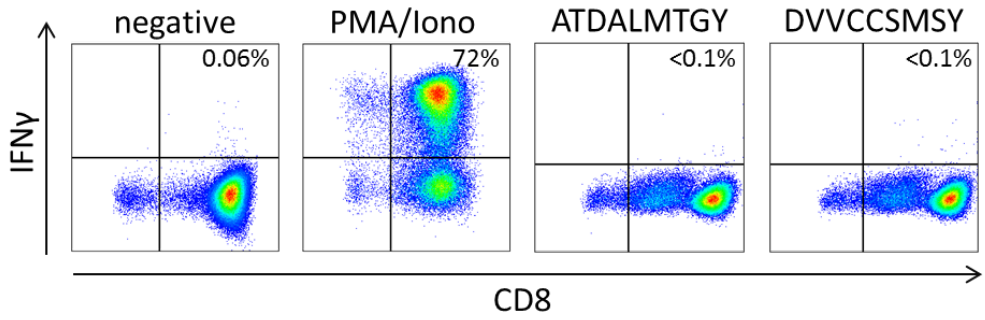

Fig. S2. Absence of CD8$^+$ T cell responses to classically immunodominant HCV-derived epitopes restricted by HLA-A*01 or HLA-A*26 in donor MM. CD8$^+$ T cells from donor MM were tested for IFN-γ production after stimulation with the epitopes HLA-A*26:01/NS3$_{1436\text{-}1444}$ (ATDALMTGY) or HLA-B*27:05/NS5A/B$_{2416\text{-}2424}$ (DVVCCSMSY). Medium alone served as the negative control; medium supplemented with PMA and ionomycin served as the positive control.

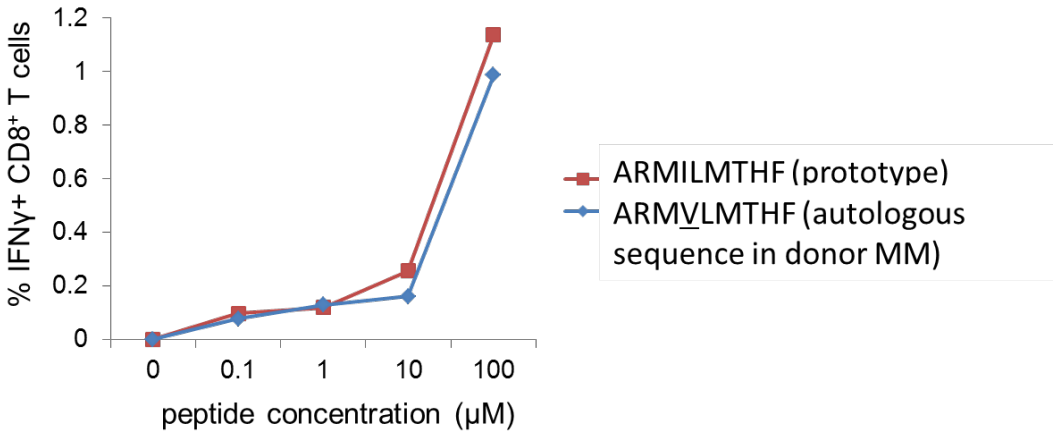

Fig. S3. Cross-recognition of the prototype and autologous variant HLA-B*27:05-restricted NS5B$_{2841\text{-}2849}$ epitopes by CD8$^+$ T cells from donor MM. CD8$^+$ T cells from donor MM were tested for IFN-γ production after stimulation with serial dilutions of ARMILMTHF (prototype epitope) or ARMVLMTHF (autologous variant).

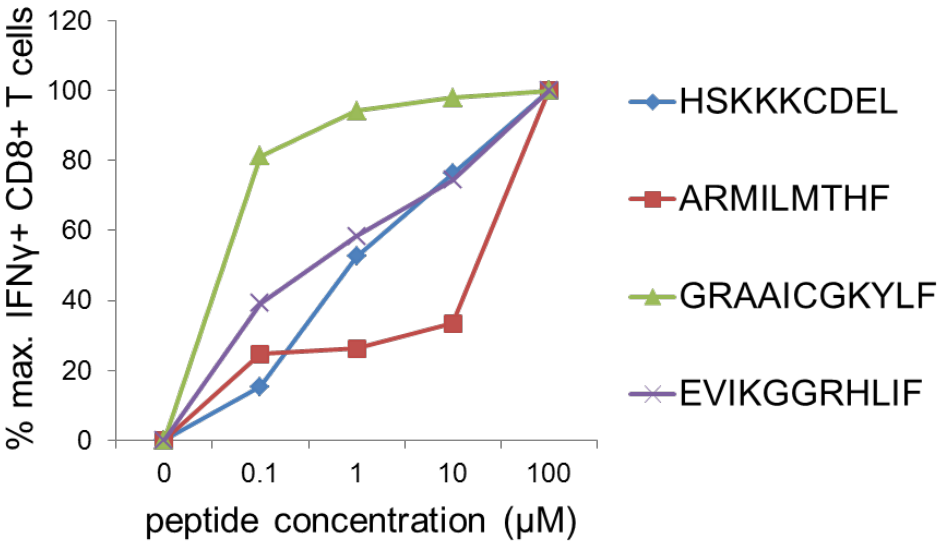

Fig. S4. Functional avidity of four HCV-specific CD8+ T cell epitopes in donor MM. Epitope-specific CD8+ T cell responses were tested on polyclonally expanded CD8+ T cells from donor MM using intracellular IFN-γ staining with peptides in serial dilution as read-out. The greatest response per peptide was set to 100%.

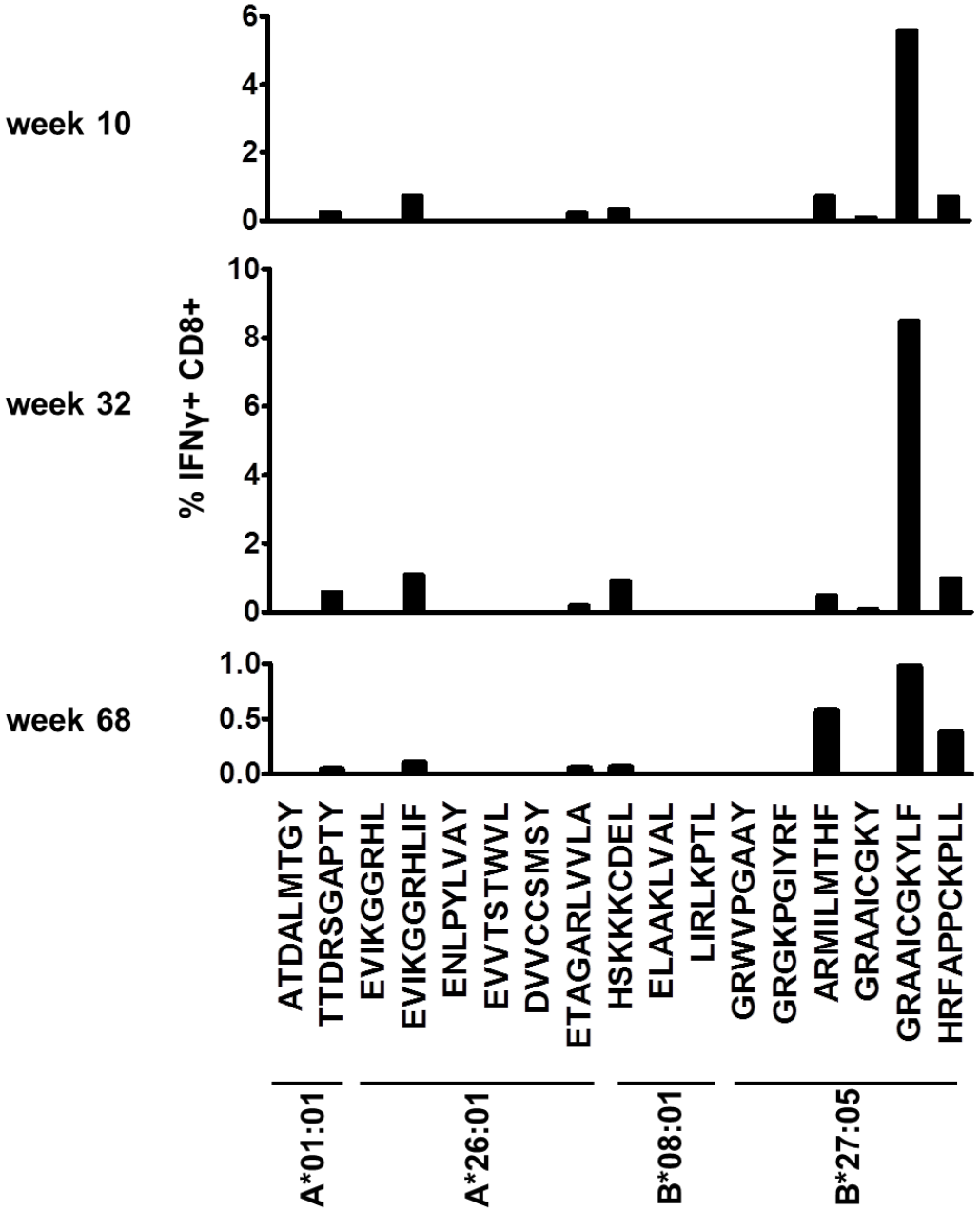

Fig. S5. Immunodominance pattern in donor MM over time. Epitope-specific CD8+ T cell responses were tested on polyclonally expanded CD8+ T cells from donor MM using intracellular IFN-γ staining as read-out at the time-points indicated.

Supplementary Tables

Table S1. Viral sequences corresponding to epitopes targeted in donor MM and previously described HCV-specific CD8+ T cell epitopes restricted by the HLA class I alleles present in donor MM. Prototype genotype 1a sequences, sequences from the infection source at the day of transmission as well as viral sequences from donor MM 5 weeks after infection are displayed. A dash ("-") indicates amino acid identity to the prototype.

(please find the table on the next page)

Supplementary Table S1

HLA allele	Described HCV-specific epitopes (immunodominant epitopes in bold)			Epitopes targeted in donor MM (dominant responses in bold)		
A*01:01	**NS3$_{1436-1444}$**	*wild-type:*	**ATDALMTGY**			
		source:	**--------F**			
		donor MM:	**--------F**			
				E2$_{518-527}$	*wild-type:*	**TTDRSGAPTY**
					source:	**---K------**
					donor MM:	**---K------**
A*26:01	NS3$_{1383-1391}$	*wild-type:*	EVIKGGRHL	**NS3$_{1383-1393}$**		**EVIKGGRHLIF**
		source:	-T-------			**-T---------**
		donor MM:	-T-------			**-T---------**
	NS3$_{1582-1590}$	*wild-type:*	ENLPYLVAY			
		source:	---------			
		donor MM:	---------			
	NS3/4A$_{1654-1662}$	*wild-type:*	EVVTSTWVL			
		source:	---------			
		donor MM:	---------			
	NS5A/B$_{2416-2424}$	*wild-type:*	**DVVCCSMSY**			
		source:	---------			
		donor MM:	---------			
				NS3$_{1337-1347}$	*wild-type:*	**ETAGARLVVLA**
					source:	**----------**
					donor MM:	**----------**
B*08:01	**NS3$_{1395-1403}$**	*wild-type:*	**HSKKKCDEL**	NS3$_{1395-1403}$	*wild-type:*	HSKKKCDEL
		source:	---------		*source:*	---------
		donor MM:	---------		*donor MM:*	---------
	NS3$_{1402-1410}$	*wild-type:*	ELAAKLVAL			
		source:	---------			
		donor MM:	---------			
	NS3$_{1611-1618}$	*wild-type:*	LIRLKPTL			
		source:	-T------			
		donor MM:	-T------			
B*27:05	P7$_{780-788}$	*wild-type:*	GRWVPGAAY			
		source:	---------			
		donor MM:	---------			
	NS3$_{1492-1501}$	*wild-type:*	GRGKPGIYRF			
		source:	----------			
		donor MM:	----------			
	NS5B$_{2841-2849}$	*wild-type:*	**ARMILMTHF**	NS5B$_{2841-2849}$	*wild-type:*	ARMILMTHF
		source:	**---V-----**		*source:*	---V-----
		donor MM:	**---V-----**		*donor MM:*	---V-----
	NS5B$_{2936-2944}$	*wild-type:*	GRAAICGKY	**NS5B$_{2936-2946}$**	*wild-type:*	**GRAAICGKYLF**
		source:	---------		*source:*	**----------**
		donor MM:	---------		*donor MM:*	**----------**
				NS5A$_{2131-2141}$	*wild-type:*	**HRFAPPCKPLL**
					source:	**----------**
					donor MM:	**----------**