Published in final edited form as:

Immunogenetics. 2016 March ; 68(3): 231–236. doi:10.1007/s00251-015-0898-2.

Characterization of the peptide binding specificity of the HLA class I alleles B*38:01 and B*39:06

John Sidney¹, Jennifer Schloss², Carrie Moore¹, Mikaela Lindvall¹, Amanda Wriston³, Donald F. Hunt⁴, Jeffrey Shabanowitz³, Teresa P. DiLorenzo^{2,5}, and Alessandro Sette¹

¹La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037

²Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461

³University of Virginia, Department of Chemistry, Charlottesville, VA 222904

⁴University of Virginia, Departments of Chemistry and Pathology, Charlottesville, VA 22904

⁵Department of Medicine (Division of Endocrinology), Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461

Abstract

B*38:01 and B*39:06 are present with phenotypic frequencies <2% in the general population, but are of interest as B*39:06 is the B allele most associated with type 1 diabetes susceptibility and 38:01 is most protective. A previous study derived putative main anchor motifs for both alleles based on peptide elution data. The present study has utilized panels of single amino acid substitution peptide libraries to derive detailed quantitative motifs accounting for both primary and secondary influences on peptide binding. From these analyses, both alleles were confirmed to utilize the canonical position 2/C-terminus main anchor spacing. B*38:01 preferentially bound peptides with the positively charged or polar residues H, R and Q in position 2, and the large hydrophobic residues I, F, L, W and M at the C-terminus. B*39:06 had a similar preference for R in position two, but also well tolerated M, Q and K. A more dramatic contrast between the two alleles was noted at the C-terminus, where the specificity of B*39:06 was clearly for small residues, with A as most preferred, followed by G, V, S, T, and I. Detailed position-by-position and residue-by-residue coefficient values were generated from the panels to provide detailed quantitative B*38:01 and B*39:06 motifs. It is hoped that these detailed motifs will facilitate the identification of T cell epitopes recognized in the context of two class I alleles associated with dramatically different dispositions towards type 1 diabetes, offering potential avenues for investigation of the role of CD8 T cells in this disease.

Keywords

Major histocompatibility complex; epitopes; antigen presentation; type 1 diabetes

Type 1 diabetes is characterized by the T cell-mediated destruction of the insulin-producing beta cells (Bettini and Vignali 2011; Walter and Santamaria 2005). In both humans and non-obese diabetic mice, CD8 T cell recognition of specific peptide-class I MHC complexes is essential to this process (Coppieters et al. 2012; Knight et al. 2013; Serreze et al. 1994). In an inflammatory context, autoreactive CD8 T cells recognize beta cell epitopes presented both by professional antigen presenting cells and by the beta cells themselves, resulting in the destruction of the beta cells (Walter and Santamaria 2005). Alternatively, under non-inflammatory conditions, potential autoreactive CD8 T cells may be eliminated either centrally or peripherally following recognition of beta cell peptides; this elimination contributes to the prevention of type 1 diabetes (Brims et al. 2010; Rosmalen et al. 2002; Srinivasan and Frauwirth 2009; Workman et al. 2009). For example, beta cell epitopes may be presented to CD8 T cells by dendritic cells in a tolerogenic manner, resulting in T cell deletion and/or anergy (Steinman et al. 2003).

As CD8 T cell recognition of specific epitopes is important for both type 1 diabetes pathogenesis and prevention of disease, it is unsurprising that a number of type 1 diabetes-relevant class I HLA alleles have been identified. Of these, HLA-B*39:06 is the HLA-B allele most associated with disease progression; it is also associated with an early age of disease onset (Howson et al. 2009; Nejentsev et al. 2007; Noble et al. 2010). HLA-B*38:01 is the HLA-B allele most associated with protection from type 1 diabetes (Howson et al. 2009; Nejentsev et al. 2007). The vastly different disease association of these two alleles is surprising as both are members of the B27-supertype and, as such, are predicted to have similar peptide repertoires (Eichmann et al. 2014; Falk et al. 1995; Sidney et al. 2008b). However, while HLA-B*39:06 and HLA-B*38:01 share 97.6% amino acid sequence identity, their sequence differences appear at residues predicted to be involved in peptide binding pockets. The pocket most affected by these differences appears to be the F pocket, involved in binding to the C-terminal position of the peptide, though other pockets along the peptide-binding groove appear to be affected as well (Matsumura et al. 1992; Saper et al. 1991). These changes in sequence can significantly affect the preferred anchor residues for these MHCs, thereby changing their peptide binding repertoires. This in turn can result in HLA-B*39:06 and HLA-B*38:01 presenting different disease-relevant beta cell epitopes, which will impact the resultant islet CD8 T cell population.

In order to better understand the differential effects of these two highly similar MHCs on disease pathogenesis, deeper knowledge of their peptide-binding characteristics is essential. For example, identification of each MHC's preferred anchor residues will be useful as a guide towards rationally designed beta cell epitope identification strategies. This is necessary as epitope identification is essential for designing novel therapies and diagnostic techniques. Finally, while the comparison between HLA-B*39:06 and HLA-B*38:01 is important in the study of type 1 diabetes, the relevance of the work presented here is not limited to this disease. Both MHCs have been linked to a variety of other autoimmune diseases. HLA-B*39:06 has been associated with a risk for Takayasu arteritis (Rodriguez-Reyna et al. 1998; Salazar et al. 2000; Vargas-Alarcon et al. 2005). Preliminary studies have linked HLA-B*38:01 to multiple sclerosis and to protection from pemphigus vulgaris (Khankhanian et al. 2015; Mortazavi et al. 2013).

To characterize the specificity of these alleles, high-throughput peptide-binding assays, based on the use of purified MHC molecules and high affinity radiolabeled ligands, were developed and performed for both alleles following previously detailed methodologies (Sidney et al. 2013). B*38:01 MHC molecules were purified from the EBV transformed homozygous line TEM (IHW 9057), and peptide YHIPGDTLF (Variola virus RNA-helicase 346–354; IC50 1.4 nM) was used as the radiolabeled probe. B*39:06 molecules were purified (Sidney et al. 2013) from a single allele transfected C1R line, and peptide FRYQGHVGA (*Homo sapiens* proteasome subunit 127–135; IC50 24 nM), endogenously bound and eluted from B*39:06 molecules (data not shown), was used as the radiolabeled probe.

To probe the specificity of B*38:01, a panel of single amino acid substitution analogs (SAAS) of the human ribosomal protein L9 39–47 peptide (sequence NHINVELSL; IC50 68 nM) was synthesized. The panel represented a complete scan of each position with substitution to each of the 20 naturally occurring amino acids. The measured IC50 nM values for each individual peptide are shown in Supplemental Table 1. The IC50 nM value for each substituted peptide was standardized as a ratio to the IC50 nM of the wild type peptide (Table 1). Finally, and as done previously with similar data (Reed et al. 2011; Sidney et al. 2008a), we calculated for each position a ratio of the (geometric) average relative binding (ARB) of all substitutions at a given position to the affinity of the wild type peptide. This ratio, denominated the specificity factor (SF), identifies positions where the majority of residues are associated with significant decreases in binding capacity; that is, positions with the highest specificity will have the highest SF value.

With these considerations, B*38:01 was found to utilize the residues in position 2 and at the C-terminus, associated with a SF of 22.5 and 12.5, respectively, as primary anchors. No other position was found to have a SF >1. At position 2, 12 different substitutions were associated with >50-fold decreases in B*38:01 binding capacity, and H and R, with ARB of 1 and 0.93, respectively, were the most preferred residues. Q, with an ARB of 0.13, was also tolerated. All other substitutions at position 2 were associated with >10-fold decreases in binding affinity.

At the C-terminus, 10 residues were associated with >50-fold changes in B*38:01 affinity. Here, the large hydrophobic residues I, F and L were the most preferred, with ARB of 13, 1.4 and 1, respectively. Two other large hydrophobic residues, W and M, were also tolerated, with ARB in the 0.1–1 range.

At both positions 1 and 3, four different residues had >10-fold effects on binding, suggesting that these may be weak secondary anchors. Only a few substitutions at other positions were associated with >10-fold increases or decreases in B*38:01 binding affinity, suggesting that B*38:01 binding capacity is not generally highly dependent upon secondary anchor interactions. A summary B*38:01 motif, highlighting the most prominent influences on binding capacity, is shown in Figure 1 (upper panel).

Following the same approach, a SAAS panel of the *Mus musculus* proteasome subunit 117–125 peptide (IC50 24 nM) was synthesized, tested for binding to B*39:06, and the data

analyzed as above (Table 2 and Supplemental Table 2). Like B*38:01, B*39:06 was found to utilize position 2 and the C-terminus as its main anchors. Position 2, where 13 residues were associated with >50-fold decreases in binding capacity, had a SF of 9.85. Here, R and M with ARB of 1 and 0.45, respectively, were the most preferred residues, and K and Q (ARB in the 0.1–0.15 range) were tolerated.

The C-terminus had a SF of 4.39, and 9 residues caused >50-fold decreases in binding. The small residue A (ARB =1) was the most preferred at the C-terminus, followed by other small residues G and V, with ARB of 0.46 and 0.25, respectively. The small polar residues S and T, and the aliphatic residue I, were also tolerated, with ARB in the 0.1–0.2 range.

Position 3 was found to have a SF of 3.49, and 6 substitutions were associated with >50-fold decreases on B*39:06 binding capacity, suggesting that position 3 is a dominant secondary anchor. Here, the aromatic/large hydrophobic residues W, Y, M and F, and P and C, were the most preferred. At position 1 there were 6 additional >10-fold deleterious effects on binding, suggesting this position may function as a weak secondary anchor; the aromatic/large hydrophobic residues W, Y, M and F were the most preferred here also. A summary B*39:06 motif, highlighting the most prominent influences on binding capacity, is shown in Figure 1 (lower panel).

A previous study by Eichmann et al (Eichmann et al. 2014) reported basic primary anchor motifs for B*38:01 and B*39:06 based on sequence analyses of eluted peptides. In that study, B*38:01 was found to preferentially bind ligands with H (and secondarily, Q) in position 2 and L (and secondarily I and F) at the C-terminus, a motif that is largely in agreement with one defined by Falk et al. (Falk et al. 1995), also based on peptide elution. B*39:06 was reported as preferring ligands with R and H in position 2, and A and V at the C-terminus. These motifs are largely in agreement with the respective motifs delineated herein.

In conclusion, the present study has characterized the peptide binding specificity of the HLA class I alleles B*38:01 and B*39:06. Confirming previous predictions, both alleles were found to recognize a motif that largely overlaps with the HLA B27-supermotif (Sette and Sidney 1999). The present study also confirms for both alleles previously defined main anchor specificity, and extends their utility by providing a more detailed quantitative motif, inclusive of influences of all 20 naturally occurring residues at each position. It is hoped that these more detailed motifs for two alleles with diametrically opposed associations with type 1 diabetes will facilitate future efforts to elucidate the T cell mechanisms involved in disease. Encouragingly, validation of predictions with small pilot sets of about 30 peptides each (Supplemental Table 3) returned area-under-the-curve (AUC) values from receiver-operating-curve (ROC) analyses of 0.852 and 0.833 for B*38:01 and B39:06, respectively. These performances compare very favorably with predictions on extensively characterized alleles done with more advanced bioinformatic approaches (Peters et al. 2006; Trolle et al. 2015). Whether predicted binding peptides will be efficiently generated by natural processing is unknown. However, it has been shown in the context of many pathogens, including HCV, HIV, SIV, *P. falciparum*, and vaccinia, that CTL epitopes can be identified using a motif/algorithm-based approach (see, e.g., (Doolan et al. 2003; Sette 2000; Sette and

Fikes 2003; Sette et al. 2001; Sette and Peters 2007; Sette and Rappuoli 2010)), as binding to MHC is a necessary requirement for a peptide to elicit a T cell response. We also note previous data from our group indicating that, in the case of infectious diseases, the majority of epitopes can be identified by selecting peptides scoring in the top 1–2% of all peptides (Moutafsi et al. 2006). We have also identified epitopes targeted by autoreactive CD8 T cells based on predicted binding affinities (Mukherjee et al. 2015). Thus, our report of quantitative peptide-binding motifs for B*38:01 and B*39:06 should facilitate the identification of T cell epitopes derived either from autoantigens or from microbes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The experiments described herein comply with the current laws of the United States of America. This work was supported by the National Institutes of Health (National Institutes for Allergy and Infectious Diseases) contracts and grants HHSN272201400045C (A.S.), R01 DK094327, R01 DK064315, and R03 AI119225 to T.P.D.; T32 GM007288 and F30 DK103368, which supported J.S.; and P60 DK020541, which supports the Diabetes Research Center of the Albert Einstein College of Medicine. T.P.D. is the Diane Belfer, Cyprus and Endelson Families Faculty Scholar in Diabetes Research.

References

- Bettini M, Vignali DA. T cell-driven initiation and propagation of autoimmune diabetes. *Curr Opin Immunol.* 2011; 23:754–60. [PubMed: 22056379]
- Brims DR, Qian J, Jarchum I, Mikesh L, Palmieri E, Ramagopal UA, Malashkevich VN, Chaparro RJ, Lund T, Hattori M, Shabanowitz J, Hunt DF, Nathenson SG, Almo SC, D'Irenzo TP. Predominant occupation of the class I MHC molecule H-2Kwm7 with a single self-peptide suggests a mechanism for its diabetes-protective effect. *Int Immunol.* 2010; 22:191–203. [PubMed: 20093428]
- Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, Roep BO, von Herrath MG. Demonstration of islet-autoreactive CD8 T cells in insulinitic lesions from recent onset and long-term type 1 diabetes patients. *J Exp Med.* 2012; 209:51–60. [PubMed: 22213807]
- Doolan DL, Southwood S, Freilich DA, Sidney J, Graber NL, Shatney L, Bebris L, Florens L, Dobano C, Witney AA, Appella E, Hoffman SL, Yates JR 3rd, Carucci DJ, Sette A. Identification of *Plasmodium falciparum* antigens by antigenic analysis of genomic and proteomic data. *Proc Natl Acad Sci U S A.* 2003; 100:9952–7. [PubMed: 12886016]
- Eichmann M, de Ru A, van Veelen PA, Peakman M, Kronenberg-Versteeg D. Identification and characterisation of peptide binding motifs of six autoimmune disease-associated human leukocyte antigen-class I molecules including HLA-B*39:06. *Tissue Antigens.* 2014; 84:378–88. [PubMed: 25154780]
- Falk K, Rotzschke O, Takiguchi M, Gnau V, Stevanovic S, Jung G, Rammensee HG. Peptide motifs of HLA-B38 and B39 molecules. *Immunogenetics.* 1995; 41:162–4. [PubMed: 7806291]
- Howson JM, Walker NM, Clayton D, Todd JA. Confirmation of HLA class II independent type 1 diabetes associations in the major histocompatibility complex including HLA-B and HLA-A. *Diabetes Obes Metab.* 2009; 11(Suppl 1):31–45. [PubMed: 19143813]
- Khankhanian P, Matsushita T, Madireddy L, Lizee A, Din L, More JM, Gourraud PA, Hauser SL, Baranzini SE, Oksenberg JR. Genetic contribution to multiple sclerosis risk among Ashkenazi Jews. *BMC Med Genet.* 2015; 16:55. [PubMed: 26212423]
- Knight RR, Kronenberg D, Zhao M, Huang GC, Eichmann M, Bulek A, Wooldridge L, Cole DK, Sewell AK, Peakman M, Skowera A. Human beta-cell killing by autoreactive preproinsulin-specific CD8 T cells is predominantly granule-mediated with the potency dependent upon T-cell receptor avidity. *Diabetes.* 2013; 62:205–13. [PubMed: 22936177]

- Matsumura M, Fremont DH, Peterson PA, Wilson IA. Emerging principles for the recognition of peptide antigens by MHC class I molecules. *Science*. 1992; 257:927–34. [PubMed: 1323878]
- Mortazavi H, Amirzargar AA, Esmaili N, Toofan H, Ehsani AH, Hosseini SH, Rezaei N. Association of human leukocyte antigen class I antigens in Iranian patients with pemphigus vulgaris. *J Dermatol*. 2013; 40:244–8. [PubMed: 23330836]
- Moutaftsi M, Peters B, Pasquetto V, Tschärke DC, Sidney J, Bui HH, Grey H, Sette A. A consensus epitope prediction approach identifies the breadth of murine T(CD8+)-cell responses to vaccinia virus. *Nat Biotechnol*. 2006; 24:817–9. [PubMed: 16767078]
- Mukherjee G, Chaparro RJ, Schloss J, Smith C, Bando CD, DiLorenzo TP. Glucagon-reactive islet-infiltrating CD8 T cells in NOD mice. *Immunology*. 2015; 144:631–40. [PubMed: 25333865]
- Nejentsev S, Howson JM, Walker NM, Szeszko J, Field SF, Stevens HE, Reynolds P, Hardy M, King E, Masters J, Hulme J, Maier LM, Smyth D, Bailey R, Cooper JD, Ribas G, Campbell RD, Clayton DG, Todd JA. Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. *Nature*. 2007; 450:887–92. [PubMed: 18004301]
- Noble JA, Valdes AM, Varney MD, Carlson JA, Moonsamy P, Fear AL, Lane JA, Lavant E, Rappner R, Louey A, Concannon P, Mychaleckyj JC, Erlich HA. HLA class I and genetic susceptibility to type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium. *Diabetes*. 2010; 59:2972–9. [PubMed: 20798335]
- Peters B, Bui H-H, Frankild S, Nielsen M, Lundegaard C, Kostem E, Basch D, Lamberth K, Harndahl M, Fleri W, Wilson SS, Sidney J, Lund O, Buus S, Sette A. A Community Resource Benchmarking Predictions of Peptide Binding to MHC-I Molecules. *PLoS Comput Biol*. 2006; 2:e65. [PubMed: 16789818]
- Reed JS, Sidney J, Piaskowski SM, Glidden CE, Leon EJ, Burwitz BJ, Kolar HL, Eernisse CM, Furlott JR, Maness NJ, Walsh AD, Rudersdorf RA, Bardet W, McMurtrey CP, O'Connor DH, Hildebrand WH, Sette A, Watkins DI, Wilson NA. The role of MHC class I allele Mamu-A*07 during SIV(mac)239 infection. *Immunogenetics*. 2011; 63:789–807. [PubMed: 21732180]
- Rodriguez-Reyna TS, Zuniga-Ramos J, Salgado N, Hernandez-Martinez B, Vargas-Alarcon G, Reyes-Lopez PA, Granados J. Intron 2 and exon 3 sequences may be involved in the susceptibility to develop Takayasu arteritis. *Int J Cardiol*. 1998; 66(Suppl 1):S135–8. discussion S139. [PubMed: 9951813]
- Rosmalen JG, van Ewijk W, Leenen PJ. T-cell education in autoimmune diabetes: teachers and students. *Trends Immunol*. 2002; 23:40–6. [PubMed: 11801453]
- Salazar M, Varela A, Ramirez LA, Uribe O, Vasquez G, Egea E, Yunis EJ, Iglesias-Gamarra A. Association of HLA-DRB1*1602 and DRB1*1001 with Takayasu arteritis in Colombian mestizos as markers of Amerindian ancestry. *Int J Cardiol*. 2000; 75(Suppl 1):S113–6. [PubMed: 10980348]
- Saper MA, Bjorkman PJ, Wiley DC. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. *J Mol Biol*. 1991; 219:277–319. [PubMed: 2038058]
- Serreze DV, Leiter EH, Christianson GJ, Greiner D, Roopenian DC. Major histocompatibility complex class I-deficient NOD-B2mnull mice are diabetes and insulinitis resistant. *Diabetes*. 1994; 43:505–9. [PubMed: 8314025]
- Sette A. Tools of the trade in vaccine design. *Science*. 2000; 290:2074–5. [PubMed: 11187832]
- Sette A, Fikes J. Epitope-based vaccines: an update on epitope identification, vaccine design and delivery. *Curr Opin Immunol*. 2003; 15:461–70. [PubMed: 12900280]
- Sette A, Livingston B, McKinney D, Appella E, Fikes J, Sidney J, Newman M, Chesnut R. The development of multi-epitope vaccines: epitope identification, vaccine design and clinical evaluation. *Biologicals*. 2001; 29:271–6. [PubMed: 11851327]
- Sette A, Peters B. Immune epitope mapping in the post-genomic era: lessons for vaccine development. *Current Opinion in Immunology*. 2007; 19:106–110. [PubMed: 17113275]
- Sette A, Rappuoli R. Reverse Vaccinology: Developing Vaccines in the Era of Genomics. *Immunity*. 2010; 33:530–541. [PubMed: 21029963]
- Sette A, Sidney J. Nine major HLA class I supertypes account for the vast preponderance of HLA-A and -B polymorphism. *Immunogenetics*. 1999; 50:201–12. [PubMed: 10602880]

- Sidney J, Assarsson E, Moore C, Ngo S, Pinilla C, Sette A, Peters B. Quantitative peptide binding motifs for 19 human and mouse MHC class I molecules derived using positional scanning combinatorial peptide libraries. *Immunome Res.* 2008a; 4:2. [PubMed: 18221540]
- Sidney J, Peters B, Frahm N, Brander C, Sette A. HLA class I supertypes: a revised and updated classification. *BMC Immunol.* 2008b; 9:1. [PubMed: 18211710]
- Sidney, J.; Southwood, S.; Moore, C.; Oseroff, C.; Pinilla, C.; Grey, HM.; Sette, A. Measurement of MHC/peptide interactions by gel filtration or monoclonal antibody capture. In: Coligan, John E., et al., editors. *Current protocols in immunology*. Vol. Chapter 18. 2013. p. 3
- Srinivasan M, Frauwirth KA. Peripheral tolerance in CD8+ T cells. *Cytokine.* 2009; 46:147–59. [PubMed: 19268604]
- Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol.* 2003; 21:685–711. [PubMed: 12615891]
- Trolle T, Metushi IG, Greenbaum JA, Kim Y, Sidney J, Lund O, Sette A, Peters B, Nielsen M. Automated benchmarking of peptide-MHC class I binding predictions. *Bioinformatics.* 2015
- Vargas-Alarcon G, Hernandez-Pacheco G, Soto ME, Murguia LE, Perez-Hernandez N, Granados J, Reyes PA. Comparative study of the residues 63 and 67 on the HLA-B molecule in patients with Takayasu's Arteritis. *Immunol Lett.* 2005; 96:225–9. [PubMed: 15585327]
- Walter U, Santamaria P. CD8+ T cells in autoimmunity. *Curr Opin Immunol.* 2005; 17:624–31. [PubMed: 16226438]
- Workman CJ, Szymczak-Workman AL, Collison LW, Pillai MR, Vignali DA. The development and function of regulatory T cells. *Cell Mol Life Sci.* 2009; 66:2603–22. [PubMed: 19390784]

Figure 1. Summary B*38:01 and B*39:06 peptide binding motifs

The peptide binding motifs of B*38:01 (upper panel) and B*39:01 (lower panel), determined using panels of single amino acid substituted peptides as described in the text, are summarized to show the main (red fill) and secondary (blue fill) anchor positions. The most preferred residues at the primary anchor positions are indicated by enlarged font. Tolerated anchor residues are also shown. The most preferred residues at secondary anchor positions are as highlighted in the text.

Table 1

SAAS-derived matrix describing 9-mer binding to HLA-B*38:01

Residue	Position ^a								
	1	2	3	4	5	6	7	8	9
A	0.40	0.080	6.5	0.39	0.33	0.37	1.8	0.25	0.063
C	1.3	0.042	0.97	1.3	2.2	0.37	0.77	0.45	0.068
D	0.47	0.012	11	10	7.5	0.66	0.61	0.068	0.0053
E	1.3	0.0049	0.71	2.3	1.1	1.0	0.85	0.84	0.0043
F	1.3	0.0027	8.2	0.26	6.2	0.69	17	0.50	1.4
G	0.44	0.0021	1.2	2.1	0.85	0.79	0.48	0.17	0.034
H	16	1.0	1.3	1.0	0.69	0.88	4.7	0.41	0.0063
I	0.24	0.0056	1.0	0.27	0.43	0.59	0.74	0.52	13
K	0.22	0.060	0.068	0.57	0.11	0.30	0.14	0.34	0.067
L	0.084	0.010	1.3	0.45	1.4	1.7	1.0	2.5	1.0
M	0.68	0.053	28	1.3	0.70	0.83	1.4	0.20	0.15
N	1.0	0.0025	0.63	1.0	0.57	1.0	0.50	0.42	0.013
P	0.038	0.015	0.16	0.88	0.76	0.36	1.1	0.29	0.0012
Q	6.0	0.13	0.76	1.8	0.67	2.2	0.62	0.05	0.0017
R	0.88	0.93	0.97	2.5	0.50	2.2	0.12	0.39	0.0019
S	19	0.021	47	3.8	0.37	1.1	0.72	1.0	0.0039
T	4.0	0.0041	3.0	0.31	0.31	1.4	0.31	1.2	0.0025
V	0.11	0.0040	0.38	0.15	1.0	0.56	0.42	1.1	0.058
W	2.5	0.0076	0.89	13	0.78	0.96	5.1	0.42	0.85
Y	4.9	0.0045	3.5	0.76	2.4	1.3	1.3	9.2	0.014
Geomean	0.91	0.018	1.64	1.08	0.84	0.82	0.89	0.46	0.032
SF	0.44	22.45	0.24	0.37	0.48	0.49	0.45	0.86	12.45
50-fold	0	12	0	0	0	0	0	0	10

^a A set of single amino acid substitution (SAAS) analogs of the human ribosomal protein L9 39–47 peptide (sequence NHINVELSL; IC50 12 nM) was tested for binding to HLA B*38:01, the data analyzed, and primary and secondary anchor positions defined, as described in the text. Values shown represent the average relative binding (ARB) of the corresponding SAAS relative to the wild type peptide. SF (specificity factor), calculated as described in the text, represents the ratio of the affinity of the wild type peptide to the average of SAAS at the indicated position. Preferred residues at the position 2 and C-

terminus main anchor positions are highlighted by bold font. For each position, the number of substitutions associated with >50-fold decreases in B*38:01 binding capacity are also indicated. The average binding of the wild type peptide for HLA B*38:01 was 68 nM.

Table 2

SAAS-derived matrix describing 9-mer binding to HLA-B*39:06

Residue	Position ^a								
	1	2	3	4	5	6	7	8	9
A	0.52	0.0045	0.061	0.79	0.0038	2.0	0.34	3.7	1.0
C	0.15	0.0078	0.17	1.1	0.12	1.5	0.098	0.42	0.071
D	0.044	0.0008	0.025	0.10	0.023	0.33	0.34	2.2	0.019
E	0.10	0.014	0.014	0.0078	0.14	0.59	0.39	3.3	0.010
F	1.0	0.0020	0.12	1.2	7.8	2.2	0.18	0.53	0.0091
G	0.077	0.017	0.027	2.7	1.0	1.6	0.46	1.0	0.46
H	0.35	0.012	0.012	0.26	0.96	1.0	0.36	1.5	0.024
I	0.15	0.026	0.0027	0.55	1.7	2.4	0.29	1.3	0.10
K	0.092	0.10	0.015	0.77	0.17	0.89	0.079	0.40	0.0098
L	0.084	0.017	0.031	0.25	0.78	2.4	0.47	0.82	0.070
M	0.74	0.45	0.16	0.029	1.2	0.47	0.23	0.66	0.017
N	0.13	0.030	0.030	0.21	0.72	1.3	0.029	2.2	0.016
P	0.0044	0.014	0.18	0.76	1.8	0.42	0.21	0.69	0.0037
Q	0.058	0.15	0.088	1.0	0.51	0.44	0.086	0.27	0.022
R	0.13	1.0	0.018	0.26	0.43	0.82	0.11	0.48	0.019
S	0.14	0.076	0.076	0.021	1.8	0.29	0.23	10	0.20
T	0.25	0.017	0.015	0.53	0.047	0.17	0.20	2.3	0.14
V	0.12	0.013	0.058	0.016	1.4	0.42	1.0	0.98	0.25
W	0.58	0.0011	0.28	0.29	4.5	0.039	0.12	1.0	0.023
Y	0.69	0.0011	1.0	0.21	6.0	0.16	1.3	1.4	0.0069
Geomean	0.15	0.017	0.048	0.25	0.53	0.64	0.23	1.15	0.038
SF	1.09	9.85	3.49	0.68	0.32	0.26	0.74	0.15	4.39
50-fold	1	13	6	2	1	0	0	0	9

^a A set of SAAS of the *Homo sapiens* proteasome 127–135 peptide (sequence: FRYQGHVGA) was tested for binding to HLA-B*39:06, the data analyzed, and primary and secondary anchor positions defined, as described in the text. The average binding of the wild type peptide for HLA-B*39:06 was 24 nM. For additional details, see the legend to Table 1.