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Supplementary Materials for

High-throughput peptide-MHC complex generation and kinetic screenings of TCRs with peptide-receptive HLA-A*02:01 molecules

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Other Supplementary Material for this manuscript includes the following:

(available at immunology.sciencemag.org/cgi/content/full/4/37/eaav0860/DC1)

Table S5. Raw data (Excel file).

Methods

Peptide binding measured by fluorescence anisotropy

Peptide binding was evaluated in fluorescence anisotropy assay with 300 nM of purified refolded DS-A*02:0. 100 nM of the fluorescently labeled high-affinity peptide NLVPK$_{FITC}$VATV (Genecast) was added to the folded DS-A*02:01 and kinetic measurements were performed with Tecan Infinite M1000 PRO (Tecan, Crailsheim, Germany) multimode plate reader measuring anisotropy (FITC λ_{ex} = 494 nm, λ_{em} = 517 nm). DS-A*02:01 were either used directly after refolding or preserved at -80°C in storage buffer (10% Glycerol, 50 mM Tris-HCL, pH 8.0) for the indicated amount of time before measurement. The kinetic measurements were performed at room temperature (22-24°C) in 50 mM HEPES buffer, pH 7.5. Data was plotted using GraphPad Prism v7.

Anti-beta-2 microglobulin ELISA

Streptavidin (Molecular Probes, Cat. Nr. S888) at a final concentration of 2 µg/ml in PBS was added to Nunc MAXIsorp plates (Thermo Fisher, Cat. Nr. 439454) and sealed plates incubated over night at room temperature in a damp environment. The following day plates were washed 4 times with washing buffer (PBS, 0.05% TWEEN-20) using a ELx405 plate washer (Biotek). 300 µl blocking buffer (PBS with 2% BSA) was added to each well and sealed plates incubated at 37°C for 1 hour. Blocking buffer was discarded before adding 100 µl of a 1:100 dilution in blocking buffer of the respective UV exchange pMHC preparation. A standard series ranging from 500 ng/ml to 15.6 ng/ml based on a conventionally refolded pMHC monomer was included on each plate. Edge wells were filled with 300 µl blocking buffer to reduce edge effects and sealed plates were incubated at 37°C for 1 hour. Plates were again washed 4 times before adding 100 µl anti-beta 2 microglobulin HRP conjugated

secondary antibody (Acris, Cat. Nr. R1065HRP) at a final concentration of 1 µg/ml to each well. Sealed plates were incubated at 37°C for 1 hour. Plates were washed again 4 times with washing buffer before adding 100 µl of room temperature TMB substrate (Sigma, Cat. Nr. T0440) to each well. Plates were incubated for 5 minutes at room temperature before stopping by adding 50 µl 1N H_2SO_4 to each well. Plates were immediately analyzed by reading absorbance at 450 nm for 5 seconds using a Synergy2 plate reader. pMHC concentration was calculated based on standard curve fitting ($Log(Y)=A*Log(X)+B$) using the Synergy2 software. Data was plotted using GraphPad Prism v7.

Flow cytometric T2 peptide binding assay

The TAP-deficient HLA-A*02:01-expressing cell line T2 was procured from ATCC (CRL-1992) and cultured in RPMI Medium 1640 GlutaMAX^{TM} (Thermo Fisher, Cat. Nr. 61870010) supplemented with 10% heat inactivated FCS (Life Technologies, Cat. Nr. 10270106) and the antibiotics penicillin and streptomycin (Biozym, Cat. Nr. 882082, 100 µg ml^{-1} each) up until passage number 16 if necessary. T2 cells were harvested from continuous cell culture, washed and resuspended in T2 culture medium at a concentration of 3.3×10^6 cells ml^{-1} and transferred to 96 well round bottom plates (Corning Costar®, Cat. Nr. 3799). Peptide in DMSO, 0.5% TFA was added to a final concentration of 10 µM and the suspension incubated for 2 hours 37°C, 5% CO_2. Plates were washed twice with PFEA (PBS, 2% FCS, 2 mM EDTA, 0.01% sodium azide) before addition of 50 µl PE labelled anti-human HLA-A2 (Biolegend, Cat. Nr. 343305) per well diluted 1:250 with PFEA to a final concentration of 0.8 µg/ml. Plates were incubated at 4°C for 30 minutes before being washed twice with PFEA. Finally, cells were resuspended in fixation solution (PFEA, 1% formaldehyde) and kept at 4°C before analysis using an iQue Screener (Intellicyt). T2 cells were gated based on the FSC-A/SSC-A signal and doublets removed using an FSC-H/FSC-A

doublet exclusion. The PE channel positive gate coordinates were based on an unstained control. Data was plotted using GraphPad Prism v7.

Figure S1
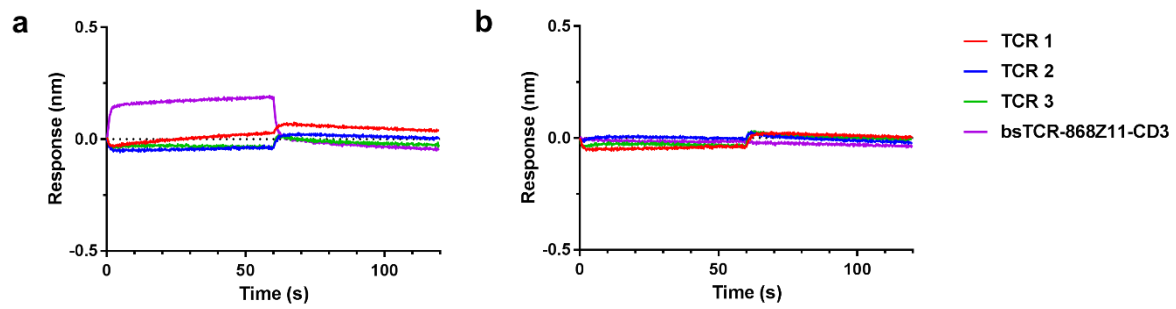
Fig. S1. Binding of multiple different soluble TCRs and bsTCR bs-868Z11-CD3 to nonloaded DS-A*02:01 or DS-A*02:01 loaded with an irrelevant peptide. (a) Binding of three different HLA-A*02:01 restricted soluble TCRs as well as bs-868Z11-CD3 to functionally-empty DS-A*02:01. DS-A*02:01 was immobilized onto a streptavidin sensor, each TCR supplied at 1 mg/ml (20 μM for soluble TCRs, 13.3 μM for bsTCR). (b) Binding of the same TCRs to DS-A*02:01 loaded with an irrelevant peptide.

Figure S2
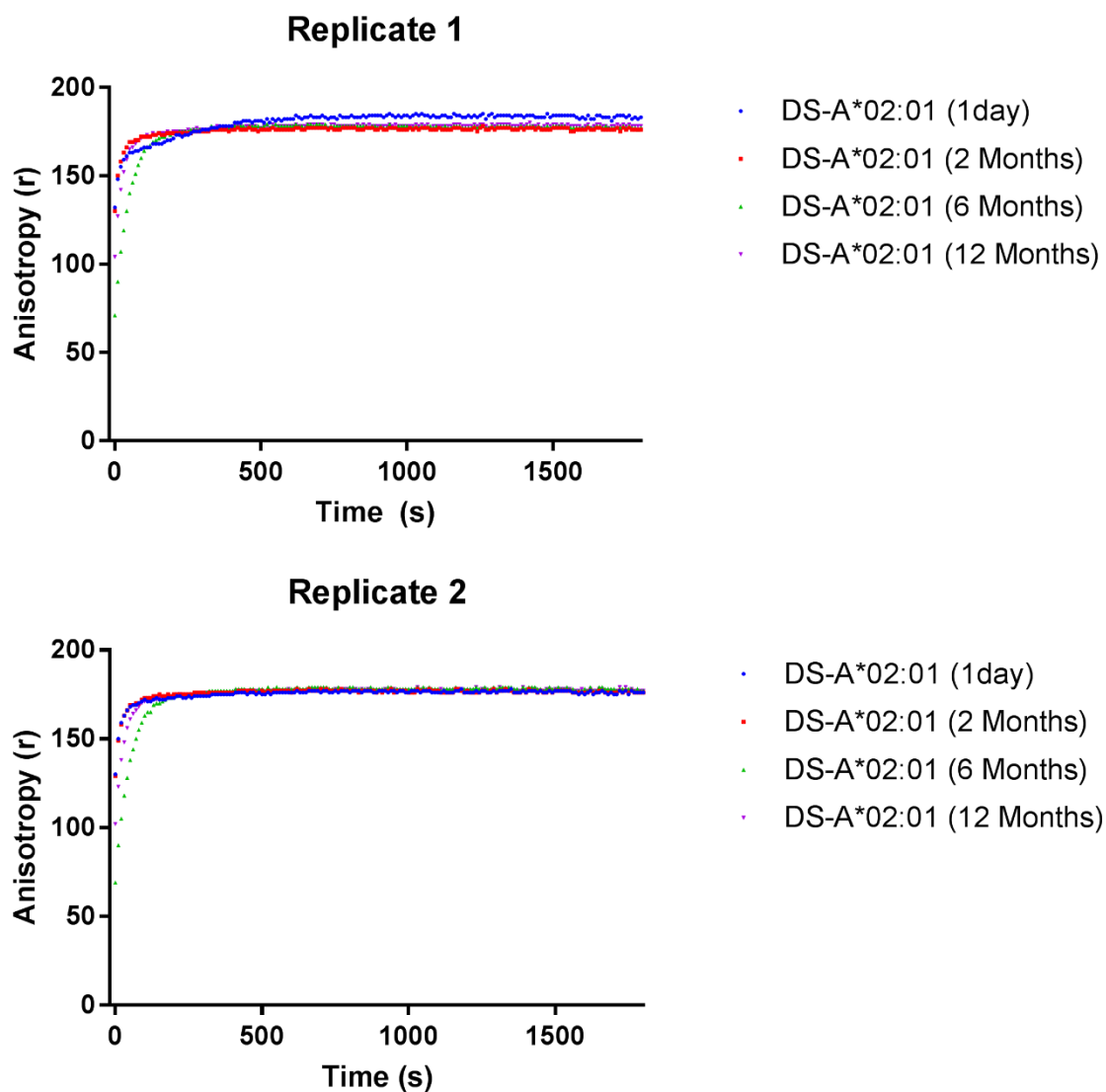
Fig. S2. Analysis of DS-A*02:01 peptide receptiveness after different storage durations at −80°C measured by fluorescence anisotropy. Binding of 100 nM $NVLPK_{FITC}VATV$ to 300 nM DS-A*02:01 diluted based on determined concentration before storage. Binding was measured in duplicates and is plotted individually.

Figure S3
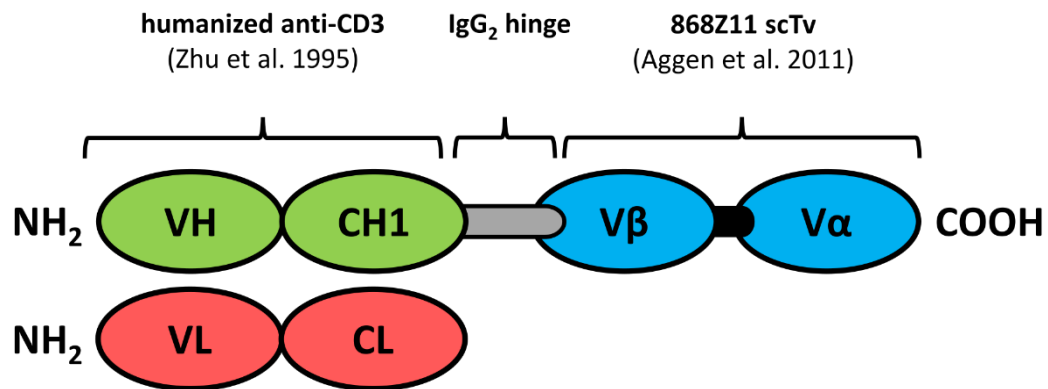
Fig. S3. Illustration of bsTCR bs-868Z11-CD3 construct. The 868Z11 domain is based on the SLYNTVATL-reactive TCR 868 and incorporates affinity enhancing mutations in the CDR2β (YYEEEE to YVRGEE) and CDR3α region (CAVRTNSGYALN to CAVRGAHDYALN) identified by Varela-Rohena et al.(*8*). The Vβ and Vα domains of the affinity enhanced TCR were linked through a single chain linker (GSADDAKKDAAKKDGKS) and further modified with a surface stability conferring mutation in the Vα2 region (F49S) to allow for soluble expression by Aggen et al.(*22*). To create the bs-868Z11-CD3 molecule, this 868Z11 scTv domain was fused to the F(ab') heavy chain portion of a humanized anti-CD3 antibody through an IgG2 derived CH2 hinge domain (APPVAG) with two cysteine-knock-outs ($C_{226}S$ and $C_{229}S$), incorporated to prevent the formation of F(ab')$_2$ homodimers on expression.
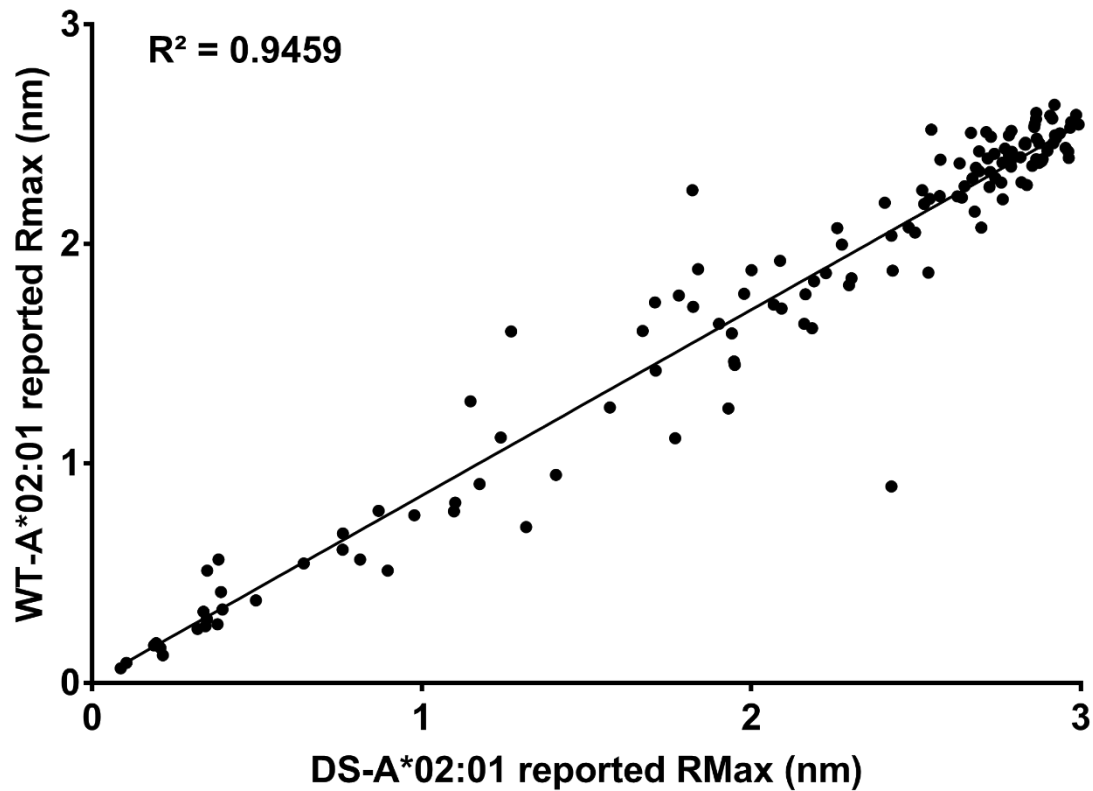
Fig. S4. R_{max} values reported for immobilized DS-A*02:01 and UV exchange generated WT-A*02:01 pMHCs. Rmax is plotted for 140 different peptide ligands from the positional scanning library analyzed with soluble bs-868Z11-CD3. Rmax reported after fitting using 500 nM and 158 nM analyte concentrations. The in-picture R^2 is the calculated correlation coefficient.

Figure S5
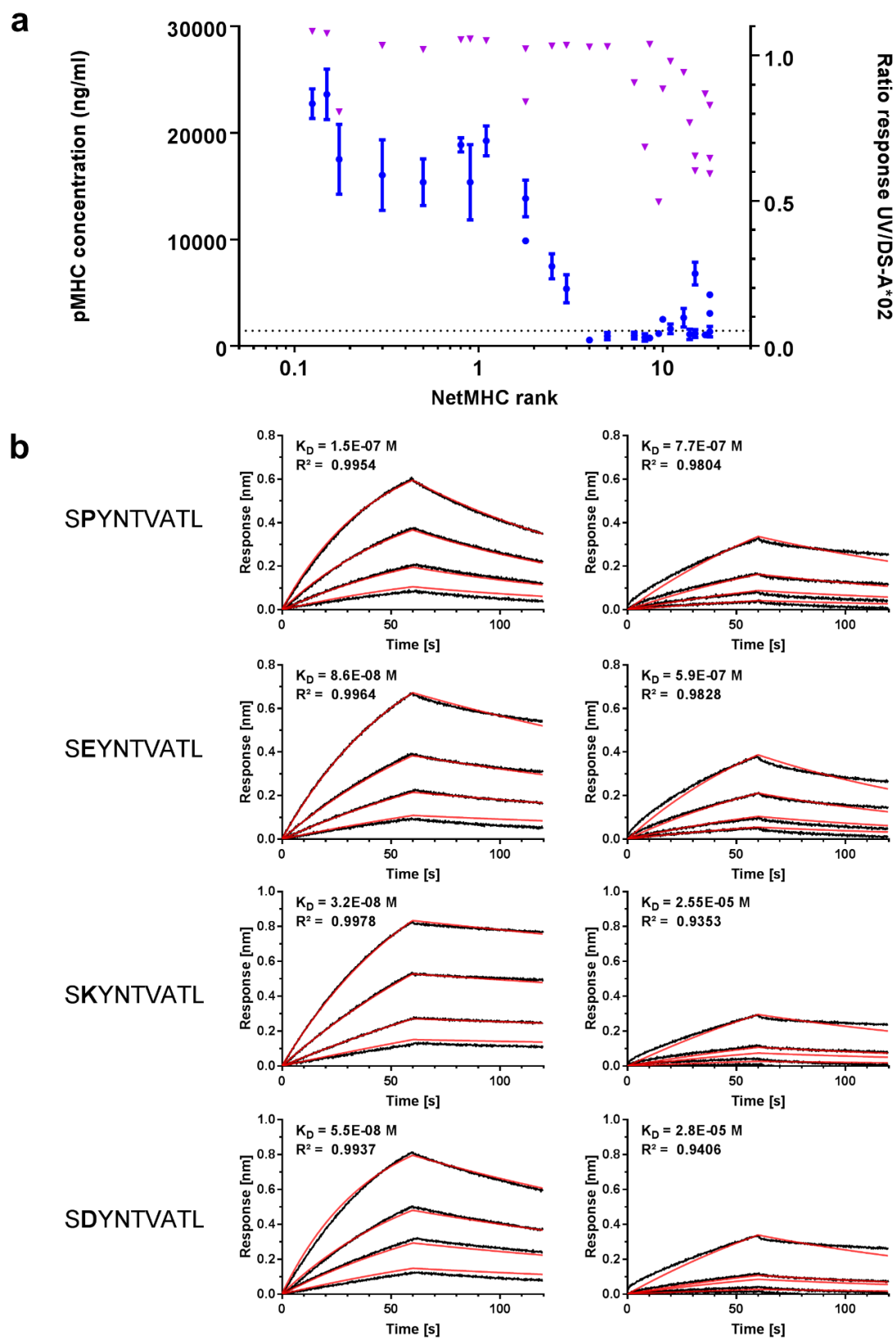
a

b

SPYNTVATL

SEYNTVATL

SKYNTVATL

SDYNTVATL

Fig. S5. Analysis of UV exchange efficiency and Octet measurement results for 28 different peptides selected from SLYNTVATL-based positional scanning library. (a) Left axis: pMHC concentration after UV exchange with 25,000 ng/ml of UV-sensitive pMHC monomer determined using an anti-β2m ELISA. Dotted line represents ELISA/UV exchange background signal based on an UV exchange without peptide. Error bars represent technical triplicates. Right axis: Ratio of binding responses of soluble pMHC analytes to immobilized bs-868Z11-CD3 on Octet RED384 system. pMHCs were either prepared using UV exchange or by DS-A*02:01 peptide loading. Ratios calculated by dividing UV-A*02:01 response by the DS-A*02:01 response after 60s of association with similarly loaded anti-F(ab) biosensors. (b) Detailed curve fittings for four peptides with NetMHC ranks 15 and larger. DS-A*02:01 complexes left, WT-A*02:01 complexes right. All measurements were performed using 1:2 analyte dilution series starting at 500 nM. Raw data is displayed in black, curve fittings in red.

Figure S6
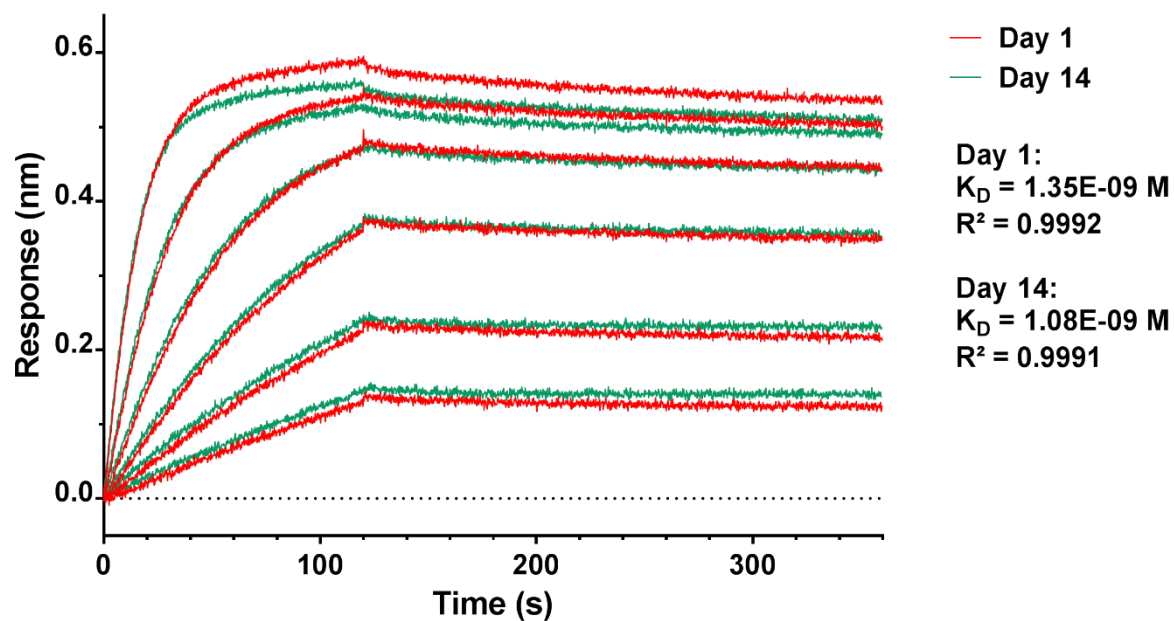
Fig. S6. Octet binding kinetics measurements for DS-A*02:01 SLYNTVATL pMHC with immobilized bs-868Z11-CD3 directly after exchange and after 2 weeks of storage at 4°C. Both measurements were performed using 1:2 analyte dilution series starting at 277.8 nM.

Figure S7
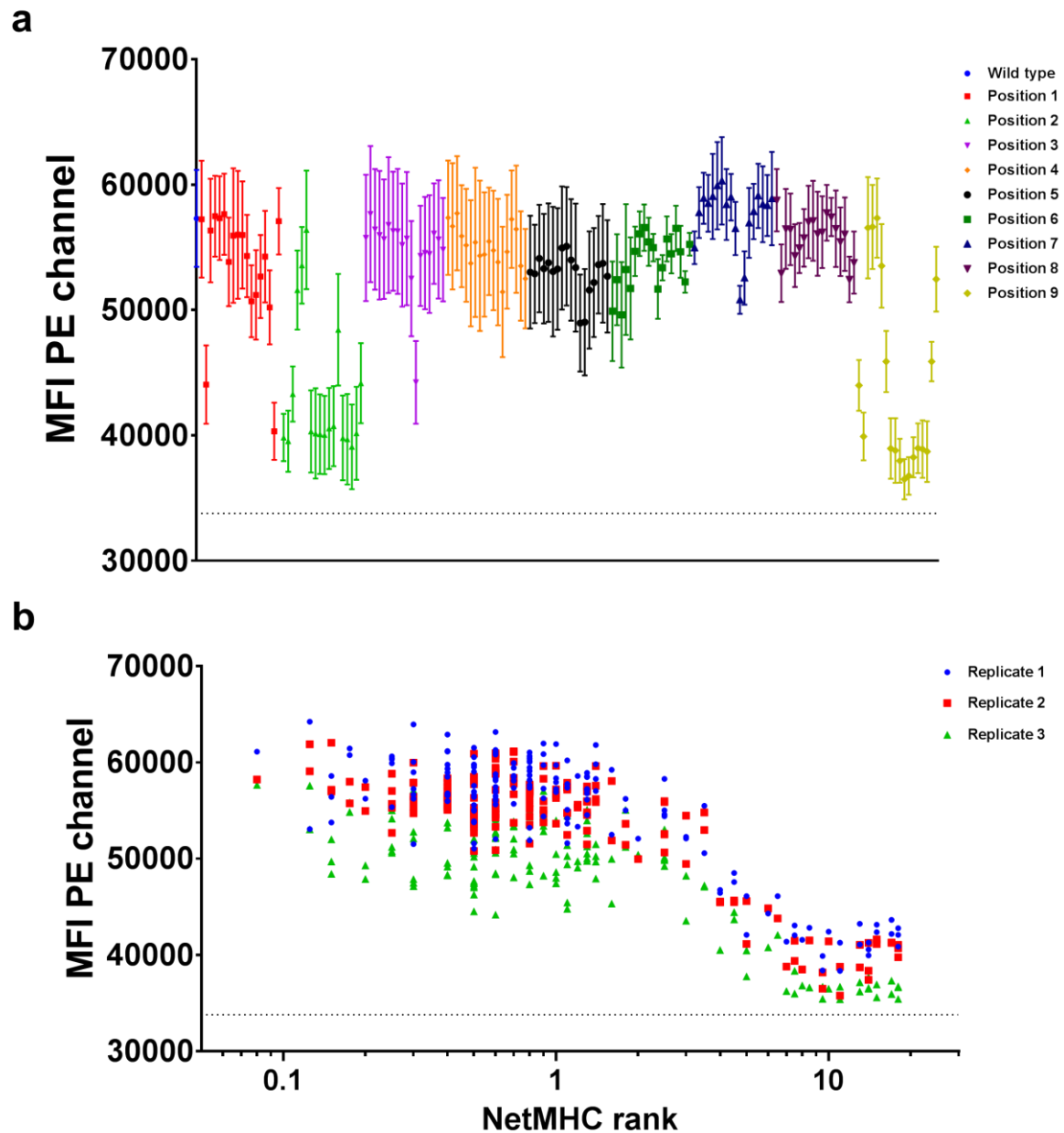
Fig. S7. Flow cytometric peptide binding assay with an anti-human HLA-A2 antibody staining of T2 cells after exogenous peptide loading. (a) PE channel MFI of T2 cells loaded individually with one specific peptide from the SLYNTVATL positional scanning library for 2 hours and stained afterwards using a PE-labeled anti-human HLA-A2 antibody. Results are colored based on the position of the substitution compared to the wild type sequence. Error

bars represent technical triplicates. (b) Correlation between PE channel MFI measured after peptide loading and staining and NetMHC predicted peptide affinity for the MHC.

Table S1. Data collection and refinement statistics 1G4/DS-A*02:01/ESO 9V.

	1G4/DS-A*02:01/ESO 9V
Data collection	
Space group	$P2_1$
Cell dimensions	
a, b, c (Å)	75.44, 53.67, 121.74
α, β, γ (°)	90.0 98.0 90.0
Resolution (Å)	2.50 (2.60 – 2.50) *
R_{pim}	0.037 (0.69)
$I / \sigma I$	9.6 (1.1)
CC (1/2)	100.0 (0.68)
Completeness (%)	99.3 (99.1)
Redundancy	4.8 (5.0)
Refinement	
Resolution (Å)	30 – 2.50
No. reflections	33552
R_{work} / R_{free}	0.229 (0.273)
No. atoms	
Protein	3180
Ligand/ion	19
Water	589
B-factors	
Protein	98.1
Ligand/ion	97.8
Water	66.2
R.m.s. deviations	
Bond lengths (Å)	0.002
Bond angles (°)	0.47

*Values in parentheses are for highest-resolution shell.

Table S2. bs-868Z11-CD3 binding affinity against SV9 peptide SLYNTVATL and peptides from positional scanning library. Table includes K_D, k_{on} and k_{off} values determined by curve fittings following a 1:1 Langmuir binding model using the Fortébio Data Analysis HT 10.0.3.7 software. Respective errors are reported as well as accuracy of the fit according to the model. Peptides reported as "No fit" had no evaluable curves reaching at least a peak signal of 0.05 nm at any concentration.

Peptide	KD (M)	KD Error	k_{on} ($M^{-1}s^{-1}$)	k_{on} Error	k_{off} (s^{-1})	k_{off} Error	Full X²	Full R²
SLYNTVATL	3.81E-09	1.49E-10	1.03E+05	1.45E+02	3.91E-04	1.53E-05	0.262	0.9993
GLYNTVATL	3.05E-08	3.55E-10	1.04E+05	3.42E+02	3.19E-03	3.56E-05	1.0915	0.9966
PLYNTVATL	8.54E-09	3.46E-10	9.65E+04	3.03E+02	8.24E-04	3.33E-05	1.2363	0.9969
ALYNTVATL	5.82E-09	3.18E-10	1.04E+05	3.14E+02	6.04E-04	3.29E-05	1.2791	0.9969
VLYNTVATL	5.74E-09	2.24E-10	1.05E+05	2.27E+02	6.04E-04	2.35E-05	0.6719	0.9984
LLYNTVATL	4.99E-08	2.99E-10	1.04E+05	2.67E+02	5.17E-03	2.80E-05	0.5623	0.9981
ILYNTVATL	1.35E-08	2.35E-10	1.06E+05	2.40E+02	1.43E-03	2.47E-05	0.6748	0.9982
MLYNTVATL	4.19E-08	2.95E-10	1.09E+05	2.93E+02	4.56E-03	2.96E-05	0.6922	0.9978
FLYNTVATL	5.22E-08	3.07E-10	1.15E+05	3.20E+02	6.02E-03	3.13E-05	0.6452	0.9976
YLYNTVATL	1.24E-07	5.65E-10	1.15E+05	4.01E+02	1.43E-02	4.21E-05	0.4931	0.9972
WLYNTVATL	4.62E-07	4.57E-09	1.66E+05	1.48E+03	7.66E-02	3.27E-04	0.1216	0.9955
HLYNTVATL	3.43E-07	2.50E-09	1.34E+05	8.87E+02	4.60E-02	1.43E-04	0.1908	0.9961
KLYNTVATL	1.91E-08	2.14E-10	9.03E+04	1.67E+02	1.73E-03	1.91E-05	0.3239	0.999
RLYNTVATL	4.42E-09	5.15E-10	8.86E+04	3.93E+02	3.92E-04	4.56E-05	2.0699	0.9944
QLYNTVATL	1.38E-07	5.55E-10	9.85E+04	3.05E+02	1.36E-02	3.50E-05	0.3534	0.9981
NLYNTVATL	3.13E-08	3.42E-10	9.84E+04	2.98E+02	3.08E-03	3.23E-05	0.9466	0.9973
ELYNTVATL	4.85E-07	4.84E-09	9.29E+04	8.66E+02	4.50E-02	1.63E-04	0.4525	0.9948
DLYNTVATL	4.49E-08	3.46E-10	9.55E+04	2.77E+02	4.28E-03	3.06E-05	0.7816	0.9977
TLYNTVATL	6.94E-09	2.07E-10	1.02E+05	1.98E+02	7.07E-04	2.10E-05	0.544	0.9988
SGYNTVATL	1.86E-08	4.56E-10	8.40E+04	3.18E+02	1.56E-03	3.79E-05	1.3876	0.9964
SPYNTVATL	1.65E-07	2.27E-09	6.80E+04	7.29E+02	1.12E-02	9.66E-05	2.2025	0.9852
SAYNTVATL	1.00E-08	1.28E-10	1.02E+05	1.23E+02	1.02E-03	1.30E-05	0.2052	0.9995
SVYNTVATL	8.47E-09	1.64E-10	1.01E+05	1.55E+02	8.57E-04	1.65E-05	0.3327	0.9992
SIYNTVATL	8.68E-09	9.77E-11	1.02E+05	9.42E+01	8.89E-04	9.97E-06	0.1192	0.9997
SMYNTVATL	6.55E-09	2.07E-10	1.01E+05	1.95E+02	6.61E-04	2.08E-05	0.4808	0.9987
SFYNTVATL	8.52E-09	3.97E-10	9.54E+04	3.41E+02	8.13E-04	3.77E-05	1.5251	0.996
SYYNTVATL	3.26E-08	3.90E-10	5.83E+04	1.62E+02	1.90E-03	2.21E-05	0.23	0.9989
SWYNTVATL	8.16E-08	1.74E-09	4.46E+04	4.66E+02	3.64E-03	6.77E-05	0.9827	0.991
SHYNTVATL	2.73E-08	8.86E-10	6.92E+04	4.66E+02	1.89E-03	5.99E-05	2.1947	0.9915
SKYNTVATL	7.43E-08	1.57E-09	5.08E+04	5.00E+02	3.77E-03	7.06E-05	2.0162	0.9899
SRYNTVATL	1.02E-07	2.33E-09	4.84E+04	6.42E+02	4.95E-03	9.17E-05	0.6946	0.9837
SQYNTVATL	9.41E-09	2.19E-10	1.09E+05	2.35E+02	1.03E-03	2.37E-05	0.6976	0.9984

SNYNTVATL	2.45E-08	6.68E-10	6.85E+04	3.45E+02	1.68E-03	4.50E-05	1.7367	0.9953
SEYNTVATL	4.09E-08	1.77E-09	5.16E+04	6.23E+02	2.11E-03	8.78E-05	4.5691	0.9843
SDYNTVATL	1.01E-07	1.68E-09	6.51E+04	6.61E+02	6.56E-03	8.69E-05	3.2507	0.9854
SSYNTVATL	8.17E-09	1.97E-10	9.64E+04	1.72E+02	7.88E-04	1.89E-05	0.4063	0.999
STYNTVATL	5.41E-09	1.49E-10	9.87E+04	1.35E+02	5.34E-04	1.47E-05	0.2427	0.9994
SLGNTVATL	No fit							
SLPNTVATL	No fit							
SLANTVATL	No fit							
SLVNTVATL	5.11E-07	5.80E-09	1.95E+05	2.01E+03	9.96E-02	4.80E-04	0.0769	0.9966
SLLNTVATL	1.32E-07	8.45E-10	1.21E+05	6.09E+02	1.60E-02	6.33E-05	1.038	0.9944
SLINTVATL	4.77E-07	5.50E-09	1.40E+05	1.48E+03	6.69E-02	3.15E-04	0.325	0.9939
SLMNTVATL	1.07E-06	5.52E-08	2.35E+05	1.12E+04	2.50E-01	5.13E-03	0.1244	0.979
SLFNTVATL	3.47E-08	1.92E-10	9.54E+04	1.59E+02	3.31E-03	1.75E-05	0.2445	0.9992
SLWNTVATL	3.36E-08	1.91E-10	9.34E+04	1.53E+02	3.14E-03	1.71E-05	0.2479	0.9992
SLHNTVATL	9.09E-08	3.31E-10	1.16E+05	2.84E+02	1.06E-02	2.85E-05	0.3676	0.9984
SLKNTVATL	No fit							
SLRNTVATL	5.55E-07	5.54E-09	9.64E+04	9.00E+02	5.35E-02	1.88E-04	0.1675	0.9957
SLQNTVATL	6.29E-07	9.45E-09	2.62E+05	3.51E+03	1.65E-01	1.11E-03	0.0384	0.9961
SLNNTVATL	4.74E-07	5.90E-09	1.81E+05	2.05E+03	8.59E-02	4.48E-04	0.1049	0.9953
SLENTVATL	No fit							
SLDNTVATL	No fit							
SLSNTVATL	No fit							
SLTNTVATL	3.01E-06	3.45E-06	1.88E+05	2.15E+05	5.66E-01	6.50E-02	0.1123	0.9199
SLYGTVATL	5.33E-07	1.20E-08	2.07E+05	4.15E+03	1.11E-01	1.12E-03	0.5678	0.9842
SLYPTVATL	5.54E-07	1.44E-08	3.62E+05	8.18E+03	2.00E-01	2.58E-03	0.0884	0.9892
SLYATVATL	1.15E-07	6.46E-10	1.40E+05	6.09E+02	1.60E-02	5.72E-05	0.9354	0.9952
SLYVTVATL	1.80E-07	9.65E-10	1.31E+05	5.98E+02	2.35E-02	6.64E-05	0.4669	0.9962
SLYLTVATL	6.70E-08	3.12E-10	1.11E+05	2.85E+02	7.44E-03	2.89E-05	0.5152	0.9981
SLYITVATL	5.25E-07	5.68E-09	1.18E+05	1.18E+03	6.19E-02	2.55E-04	0.2208	0.9949
SLYMTVATL	1.88E-06	2.10E-06	3.58E+05	3.96E+05	6.72E-01	1.11E-01	0.09	0.876
SLYFTVATL	No fit							
SLYYTVATL	No fit							
SLYWTVATL	No fit							
SLYHTVATL	8.11E-08	6.16E-10	1.38E+05	7.03E+02	1.12E-02	6.26E-05	1.8422	0.9923
SLYKTVATL	No fit							
SLYRTVATL	No fit							
SLYQTVATL	2.84E-07	3.23E-09	1.71E+05	1.73E+03	4.86E-02	2.53E-04	0.6721	0.9898
SLYETVATL	No fit							
SLYDTVATL	No fit							
SLYSTVATL	5.95E-08	2.69E-10	1.27E+05	3.13E+02	7.57E-03	2.87E-05	0.5404	0.9981
SLYTTVATL	1.61E-07	7.59E-10	1.35E+05	5.34E+02	2.18E-02	5.63E-05	0.3965	0.9968
SLYNGVATL	6.03E-07	1.50E-07	5.42E+05	1.29E+05	3.27E-01	2.34E-02	0.0452	0.9399
SLYNPVATL	No fit							
SLYNAVATL	9.66E-08	5.82E-10	1.27E+05	5.41E+02	1.22E-02	5.20E-05	0.9944	0.995
SLYNVVATL	3.07E-08	4.63E-10	9.09E+04	3.58E+02	2.79E-03	4.06E-05	1.3333	0.9958

SLYNLVATL	1.46E-08	3.05E-10	9.80E+04	2.73E+02	1.43E-03	2.97E-05	0.917	0.9976
SLYNIVATL	4.85E-08	3.37E-10	8.41E+04	2.21E+02	4.08E-03	2.62E-05	0.4314	0.9984
SLYNMVATL	4.26E-08	3.52E-10	1.20E+05	4.09E+02	5.12E-03	3.86E-05	1.2415	0.9962
SLYNFVATL	7.26E-07	3.75E-08	2.97E+05	1.36E+04	2.16E-01	5.09E-03	0.3346	0.9697
SLYNYVATL	4.60E-06	9.88E-06	1.37E+05	2.94E+05	6.31E-01	9.34E-02	0.1113	0.8904
SLYNWVATL	No fit							
SLYNHVATL	6.23E-07	4.07E-08	4.00E+05	2.26E+04	2.49E-01	8.17E-03	0.2106	0.9526
SLYNKVATL	2.24E-07	1.05E-09	1.35E+05	5.55E+02	3.02E-02	6.82E-05	0.2572	0.9973
SLYNRVATL	7.78E-07	7.24E-08	3.54E+05	3.19E+04	2.76E-01	6.54E-03	0.02	0.9899
SLYNQVATL	4.72E-07	7.25E-09	2.10E+05	2.90E+03	9.91E-02	6.64E-04	0.131	0.9936
SLYNNVATL	1.19E-07	5.68E-10	1.32E+05	4.88E+02	1.58E-02	4.76E-05	0.5956	0.9966
SLYNEVATL	No fit							
SLYNDVATL	3.91E-05	5.33E-04	1.23E+04	1.67E+05	4.79E-01	5.67E-02	0.1685	0.904
SLYNSVATL	6.91E-08	3.75E-10	1.21E+05	3.86E+02	8.39E-03	3.68E-05	0.7181	0.997
SLYNTGATL	1.34E-07	6.48E-10	1.37E+05	5.33E+02	1.84E-02	5.26E-05	0.5267	0.9965
SLYNTPATL	1.54E-08	1.56E-10	1.19E+05	1.93E+02	1.83E-03	1.82E-05	0.3561	0.999
SLYNTAATL	5.48E-08	3.74E-10	1.10E+05	3.59E+02	6.05E-03	3.62E-05	0.8843	0.9967
SLYNTLATL	9.08E-09	1.01E-10	1.12E+05	1.15E+02	1.02E-03	1.13E-05	0.15	0.9996
SLYNTIATL	8.74E-09	1.86E-10	9.97E+04	1.72E+02	8.71E-04	1.85E-05	0.3788	0.999
SLYNTMATL	2.72E-08	3.66E-10	9.75E+04	3.17E+02	2.65E-03	3.46E-05	0.9551	0.9966
SLYNTFATL	5.79E-07	6.47E-09	7.96E+04	8.39E+02	4.61E-02	1.69E-04	0.1416	0.9946
SLYNTYATL	4.43E-07	8.76E-09	4.61E+04	8.61E+02	2.04E-02	1.33E-04	0.1286	0.9831
SLYNTWATL	1.74E-05	1.28E-05	1.91E+03	1.41E+03	3.33E-02	2.40E-04	0.0063	0.9878
SLYNTHATL	1.75E-07	1.46E-09	7.39E+04	4.94E+02	1.30E-02	6.42E-05	0.3653	0.9929
SLYNTKATL	No fit							
SLYNTRATL	No fit							
SLYNTQATL	2.71E-07	1.50E-09	1.19E+05	5.86E+02	3.22E-02	7.94E-05	0.1392	0.9969
SLYNTNATL	1.79E-07	7.80E-10	1.20E+05	4.42E+02	2.15E-02	5.03E-05	0.2983	0.9974
SLYNTEATL	1.43E-06	5.11E-08	5.54E+04	1.93E+03	7.94E-02	5.83E-04	0.0772	0.99
SLYNTDATL	6.04E-07	7.08E-09	1.12E+05	1.22E+03	6.77E-02	2.89E-04	0.1139	0.995
SLYNTSATL	1.66E-07	6.43E-10	1.43E+05	4.69E+02	2.38E-02	4.93E-05	0.2673	0.9979
SLYNTTATL	3.37E-08	4.58E-10	1.07E+05	4.51E+02	3.59E-03	4.64E-05	1.7673	0.9938
SLYNTVGTL	8.29E-09	4.59E-10	8.24E+04	3.12E+02	6.83E-04	3.77E-05	1.2283	0.996
SLYNTVPTL	3.71E-09	4.42E-10	1.22E+05	5.76E+02	4.51E-04	5.36E-05	4.052	0.9904
SLYNTVVTL	5.99E-07	1.07E-08	1.44E+05	2.37E+03	8.64E-02	6.15E-04	0.1883	0.9892
SLYNTVLTL	No fit							
SLYNTVITL	No fit							
SLYNTVMTL	1.02E-07	4.28E-10	6.90E+04	1.80E+02	7.04E-03	2.31E-05	0.1333	0.9988
SLYNTVFTL	5.14E-07	1.01E-08	1.72E+05	3.07E+03	8.85E-02	7.06E-04	0.1274	0.9897
SLYNTVYTL	No fit							
SLYNTVWTL	No fit							
SLYNTVHTL	1.14E-07	2.51E-10	8.42E+04	1.27E+02	9.63E-03	1.53E-05	0.0763	0.9995
SLYNTVKTL	1.20E-06	5.58E-08	5.35E+04	2.43E+03	6.42E-02	6.41E-04	0.0792	0.9775
SLYNTVRTL	1.28E-06	2.41E-08	2.49E+04	4.61E+02	3.20E-02	9.10E-05	0.0547	0.9967
SLYNTVQTL	5.38E-08	7.00E-10	6.84E+04	3.40E+02	3.68E-03	4.43E-05	0.9296	0.9952

SLYNTVNTL	4.11E-08	8.02E-10	7.22E+04	4.32E+02	2.97E-03	5.51E-05	1.5878	0.9921
SLYNTVETL	1.61E-06	2.46E-07	5.74E+03	8.80E+02	9.22E-03	1.00E-04	0.007	0.989
SLYNTVDTL	No fit							
SLYNTVSTL	1.04E-08	4.47E-10	9.80E+04	4.00E+02	1.02E-03	4.36E-05	1.9634	0.9944
SLYNTVTTL	6.90E-08	2.99E-10	9.23E+04	2.09E+02	6.37E-03	2.36E-05	0.2893	0.9987
SLYNTVAGL	1.14E-08	1.42E-10	1.14E+05	1.65E+02	1.30E-03	1.61E-05	0.302	0.9992
SLYNTVAPL	2.34E-07	1.20E-09	1.35E+05	6.11E+02	3.16E-02	7.71E-05	0.3155	0.9969
SLYNTVAAL	8.50E-09	1.51E-10	1.14E+05	1.75E+02	9.69E-04	1.72E-05	0.3504	0.9991
SLYNTVAVL	6.98E-09	1.19E-10	1.05E+05	1.20E+02	7.31E-04	1.25E-05	0.1881	0.9995
SLYNTVALL	1.58E-08	1.20E-10	9.58E+04	1.03E+02	1.51E-03	1.14E-05	0.1259	0.9996
SLYNTVAIL	4.16E-09	7.48E-10	9.74E+04	6.62E+02	4.05E-04	7.28E-05	5.8607	0.9834
SLYNTVAML	7.69E-09	5.22E-10	9.75E+04	4.63E+02	7.50E-04	5.08E-05	2.7181	0.9922
SLYNTVAFL	1.93E-07	1.68E-09	9.29E+04	6.77E+02	1.80E-02	8.45E-05	0.9456	0.9906
SLYNTVAYL	4.00E-07	3.75E-09	9.61E+04	8.32E+02	3.85E-02	1.39E-04	0.2451	0.994
SLYNTVAWL	2.09E-07	1.94E-09	9.65E+04	7.69E+02	2.01E-02	9.70E-05	1.0358	0.9893
SLYNTVAHL	1.09E-08	5.55E-10	9.19E+04	4.47E+02	1.00E-03	5.07E-05	2.6388	0.9925
SLYNTVAKL	1.73E-08	2.87E-10	1.02E+05	2.76E+02	1.77E-03	2.90E-05	0.9054	0.9975
SLYNTVARL	7.93E-09	3.98E-10	1.06E+05	4.06E+02	8.37E-04	4.19E-05	2.1201	0.9946
SLYNTVAQL	1.59E-08	6.14E-10	1.01E+05	5.74E+02	1.61E-03	6.13E-05	4.0059	0.9888
SLYNTVANL	1.08E-08	6.43E-10	1.01E+05	6.03E+02	1.09E-03	6.46E-05	4.5943	0.9874
SLYNTVAEL	4.73E-08	2.37E-10	9.22E+04	1.79E+02	4.36E-03	2.02E-05	0.291	0.999
SLYNTVADL	2.12E-08	3.17E-10	8.90E+04	2.40E+02	1.88E-03	2.77E-05	0.6889	0.9979
SLYNTVASL	4.68E-09	2.55E-10	1.09E+05	2.71E+02	5.08E-04	2.76E-05	0.918	0.9977
SLYNTVATG	7.71E-09	4.30E-10	1.01E+05	4.05E+02	7.79E-04	4.34E-05	2.1199	0.9943
SLYNTVATP	5.03E-08	1.57E-09	3.41E+04	3.34E+02	1.72E-03	5.08E-05	0.6961	0.9945
SLYNTVATA	6.74E-09	4.88E-10	1.13E+05	5.56E+02	7.61E-04	5.49E-05	3.6905	0.9904
SLYNTVATV	8.41E-09	6.00E-10	1.04E+05	5.95E+02	8.76E-04	6.23E-05	4.752	0.988
SLYNTVATI	6.70E-09	2.87E-10	1.13E+05	3.25E+02	7.53E-04	3.22E-05	1.2712	0.9968
SLYNTVATM	7.45E-09	2.48E-10	9.88E+04	2.26E+02	7.36E-04	2.44E-05	0.6922	0.9982
SLYNTVATF	1.19E-08	2.46E-10	7.18E+04	1.37E+02	8.51E-04	1.76E-05	0.2228	0.9992
SLYNTVATY	1.02E-08	3.37E-10	7.11E+04	1.85E+02	7.24E-04	2.39E-05	0.4625	0.9985
SLYNTVATW	3.32E-08	5.59E-10	3.70E+04	1.34E+02	1.23E-03	2.02E-05	0.0824	0.9991
SLYNTVATH	1.37E-08	3.64E-10	4.75E+04	1.19E+02	6.51E-04	1.72E-05	0.089	0.9993
SLYNTVATK	4.57E-08	1.20E-09	2.70E+04	2.00E+02	1.23E-03	3.11E-05	0.0929	0.9982
SLYNTVATR	5.71E-09	2.30E-10	9.59E+04	1.99E+02	5.48E-04	2.20E-05	0.5532	0.9986
SLYNTVATQ	5.88E-09	3.12E-10	8.96E+04	2.41E+02	5.27E-04	2.79E-05	0.7397	0.9978
SLYNTVATN	9.10E-09	3.77E-10	9.76E+04	3.36E+02	8.88E-04	3.67E-05	1.5971	0.9961
SLYNTVATE	6.96E-06	9.43E-06	3.79E+02	5.13E+02	2.64E-03	8.02E-05	0.1997	0.9908
SLYNTVATD	7.18E-06	8.48E-06	3.95E+02	4.67E+02	2.83E-03	7.30E-05	0.1137	0.9924
SLYNTVATS	7.19E-09	2.13E-10	1.16E+05	2.54E+02	8.33E-04	2.46E-05	0.7637	0.9981
SLYNTVATT	5.66E-09	1.27E-10	1.12E+05	1.42E+02	6.32E-04	1.41E-05	0.2627	0.9994

Table S3. Cross-reactive peptide ligand search motif for bs-868Z11-CD3 based on the binding affinities measured using the positional scanning library. All amino acids of the 19 proteinogenic amino acids investigated at each position that increased the respective affinity of the bsTCR above 50 nM were removed to reach the search motif.

Peptide Position	Permitted Amino Acids
1	GPAVLIMKRNDST
2	GAVLIMFYHQNEST
3	FYW
4	N
5	VLIMT
6	PVLIMT
7	GPANS
8	GAVLIMHKRQNEDST
9	GAVLIMFYWHKRQNST

Table S4. bs-868Z11-CD3 binding affinity for selected peptide ligands identified on the basis of the bs-868Z11-CD3 binding motif. Peptide sequences and associated genes according to the NCBI data base are reported and peptides are sorted by decreasing K_Ds. Table includes K_D, k_{on} and k_{off} values determined by curve fittings following a 1:1 Langmuir binding model using the Fortébio Data Analysis HT 10.0.3.7 software. Respective errors are reported as well as accuracy of the fit according to the model. Peptides reported as "No fit" had no evaluable curves reaching at least a peak signal of 0.05 nm at any concentration.

Peptide	Associated Gene	KD (M)	KD Error	k_{on} ($M^{-1}s^{-1}$)	k_{on} Error	k_{off} (s^{-1})	k_{off} Error	Full X^2	Full R^2
RVYNTVPLV	HIPK3	1.32E-08	1.76E-10	9.50E+04	1.69E+02	1.26E-03	1.66E-05	0.2908	0.9993
RMYNLVSRI	CUL1	1.91E-08	2.52E-10	9.25E+04	2.28E+02	1.76E-03	2.29E-05	0.5429	0.9986
SLYNMVPSI	OVOS	1.97E-08	1.71E-10	1.31E+05	2.89E+02	2.57E-03	2.16E-05	0.586	0.9987
TVYNMVPSI	OVOS	2.07E-08	1.54E-10	1.28E+05	2.50E+02	2.66E-03	1.91E-05	0.4228	0.999
ALYNVIAMA	SECISBP2L	2.11E-08	1.51E-10	9.28E+04	2.25E+02	1.96E-03	1.32E-05	0.114	0.9997
AIYNLLPDI	NCAPD2	2.33E-08	1.82E-10	1.01E+05	1.90E+02	2.36E-03	1.79E-05	0.3299	0.9992
STYNLVSTS	KIAA2018	2.47E-08	2.16E-10	7.07E+04	1.28E+02	1.75E-03	1.49E-05	0.1772	0.9995
SVYNMVPSI	OVOS2	2.68E-08	1.96E-10	1.32E+05	3.26E+02	3.53E-03	2.43E-05	0.6471	0.9984
RTYNVLAIL	ATP8B1	3.11E-08	1.55E-10	7.54E+04	9.93E+01	2.34E-03	1.12E-05	0.0937	0.9997
SVYNLVSIA	KPTN	3.65E-08	2.01E-10	7.97E+04	2.13E+02	2.91E-03	1.40E-05	0.0926	0.9997
RAYNLIGTV	LOC100128501	3.72E-08	1.71E-10	8.94E+04	1.40E+02	3.33E-03	1.43E-05	0.1721	0.9995
ALFNLIPVG	FGF12	3.83E-08	3.22E-10	6.64E+04	1.71E+02	2.54E-03	2.04E-05	0.266	0.999
RIYNVIGTL	FOLH1,FOLH1B	4.53E-08	3.00E-10	5.75E+04	1.28E+02	2.61E-03	1.62E-05	0.1095	0.9994
RIYNVVGTI	NAALAD2	5.15E-08	4.13E-10	5.94E+04	1.81E+02	3.06E-03	2.27E-05	0.2723	0.9989
TLFNLVPNS	CLASP2	5.40E-08	3.31E-10	9.76E+04	2.90E+02	5.28E-03	2.82E-05	0.4822	0.9981
SLFNVISIL	KCNK12,KCNK13	5.83E-08	3.11E-10	6.94E+04	1.64E+02	4.05E-03	1.94E-05	0.2068	0.9992
STFNLVAIS	CCKAR	6.06E-08	2.91E-10	4.96E+04	9.98E+01	3.01E-03	1.31E-05	0.0436	0.9996
TLFNLIPVG	FGF12,FGF13,FGF14	6.32E-08	3.97E-10	6.72E+04	1.98E+02	4.25E-03	2.36E-05	0.3044	0.9988
TIFNLIPNS	CLASP1	6.41E-08	2.67E-10	8.97E+04	1.99E+02	5.75E-03	2.03E-05	0.2655	0.999
ALYNVLAKV	IFFO1,IFFO2	6.59E-08	2.96E-10	1.02E+05	2.63E+02	6.75E-03	2.49E-05	0.3602	0.9986
AVFNLLPHT	SMYD4	7.11E-08	2.72E-10	8.53E+04	1.82E+02	6.07E-03	1.93E-05	0.2344	0.9991
RMYNLLGHM	ZNF710	8.71E-08	7.55E-10	5.30E+04	2.55E+02	4.62E-03	3.33E-05	0.2278	0.9977
STWNTPPNM	KIAA0922	8.98E-08	3.45E-10	9.05E+04	2.28E+02	8.13E-03	2.36E-05	0.2591	0.9989
NIYNLIAII	BICD2	9.32E-08	3.91E-10	1.06E+05	3.10E+02	9.84E-03	2.95E-05	0.3894	0.9983
RIYNLPPEL	WRAP53	9.95E-08	3.78E-10	9.47E+04	2.51E+02	9.42E-03	2.56E-05	0.3026	0.9988
TTFNLPSAA	WDR17	1.02E-07	6.71E-10	7.87E+04	3.47E+02	8.04E-03	3.91E-05	0.579	0.997

MFFNVIAIV	UGGT2	1.06E-07	1.29E-09	5.21E+04	3.95E+02	5.52E-03	5.23E-05	0.0772	0.9934
SLWNTVSGI	HHLA1	1.08E-07	5.04E-10	8.88E+04	2.94E+02	9.60E-03	3.14E-05	0.3873	0.9982
MLWNLLALR	COX7A2	1.17E-07	1.03E-08	1.26E+06	1.05E+05	1.47E-01	4.25E-03	0.0448	0.9675
VFWNLLPTV	C12orf74	1.20E-07	7.20E-10	1.24E+05	5.97E+02	1.49E-02	5.29E-05	0.9116	0.9955
STFNTTSNG	QSER1	1.52E-07	6.60E-09	1.76E+05	7.05E+03	2.67E-02	4.39E-04	0.0311	0.9225
GFFNLLSHV	PCP2	1.59E-07	9.88E-09	6.24E+05	3.64E+04	9.94E-02	2.08E-03	0.0321	0.9717
LLYNVPAVA	APP	1.67E-07	1.27E-08	7.41E+05	5.29E+04	1.24E-01	3.21E-03	0.0164	0.9712
ALFNTISQG	VTA1	1.83E-07	8.26E-10	7.15E+04	2.68E+02	1.31E-02	3.28E-05	0.2214	0.9984
TTFNTLAGS	MUC16	1.97E-07	8.70E-10	9.03E+04	3.44E+02	1.78E-02	3.98E-05	0.1556	0.9981
SLWNLLGNA	LMAN2L	2.14E-07	3.02E-08	1.02E+06	1.35E+05	2.19E-01	1.07E-02	0.0324	0.952
SLYNLLNLT	SLC4A5	2.19E-07	8.40E-10	6.75E+04	2.24E+02	1.48E-02	2.85E-05	0.094	0.9988
GVWNLLSIV	ZSWIM8	2.52E-07	4.59E-08	1.09E+06	1.86E+05	2.74E-01	1.73E-02	0.0545	0.9403
ALFNVVNSI	SLC38A11	2.55E-07	2.10E-09	6.83E+04	4.99E+02	1.74E-02	6.59E-05	0.2897	0.9956
VIYNLLGLA	SH3TC2	2.64E-07	1.88E-09	1.07E+05	7.25E+02	2.83E-02	5.92E-05	0.2115	0.9983
SIFNIVAIA	GPR50	2.84E-07	1.60E-09	4.44E+04	2.37E+02	1.26E-02	2.25E-05	0.0369	0.9995
TVYNTVSEG	SLC39A6	3.04E-07	2.50E-09	4.71E+04	3.50E+02	1.43E-02	4.99E-05	0.2538	0.997
DLWNTLSSL	EFCAB13,ITGB3	3.39E-07	2.60E-08	4.15E+05	3.02E+04	1.41E-01	3.55E-03	0.0238	0.9752
IFFNLLAVL	POMT1	3.50E-07	4.75E-08	8.47E+05	1.08E+05	2.97E-01	1.35E-02	0.023	0.9688
DLFNLLPDV	PSMD7	3.60E-07	1.08E-08	7.69E+04	2.15E+03	2.77E-02	3.14E-04	0.0881	0.9367
LSWNVVPNA	SPCS3	3.67E-07	2.91E-08	4.13E+05	3.09E+04	1.52E-01	3.89E-03	0.0234	0.9734
MLWNLLALH	COX7A2P2	3.67E-07	2.04E-08	1.07E+06	4.75E+04	3.94E-01	1.33E-02	0.1159	0.9595
TIFNTVNTS	TIMMDC1	3.87E-07	2.77E-09	4.20E+04	2.80E+02	1.63E-02	4.19E-05	0.0304	0.9978
KTFNLIPAV	MRPL4	4.13E-07	2.59E-09	1.12E+05	6.52E+02	4.62E-02	1.07E-04	0.1185	0.9979
NLFNVTPLI	ZNF66P	4.28E-07	1.38E-07	1.05E+06	3.19E+05	4.49E-01	4.68E-02	0.0447	0.9139
SYWNIISTV	OR2D3	4.39E-07	4.84E-09	4.56E+04	4.74E+02	2.00E-02	7.23E-05	0.1373	0.9952
GVFNLIAVL	AC002365.5, LOC100288814	4.59E-07	4.94E-09	7.59E+04	7.70E+02	3.48E-02	1.26E-04	0.3268	0.9946
RLFNITSSA	IFITM10	4.74E-07	4.12E-08	2.51E+05	2.08E+04	1.19E-01	3.08E-03	0.0167	0.9706
NLWNLVAVI	WDR17	4.97E-07	1.16E-08	2.07E+05	4.34E+03	1.03E-01	1.04E-03	0.256	0.9836
RIFNLIGMM	HCN1,HCN3	4.98E-07	1.26E-08	2.29E+04	5.51E+02	1.14E-02	8.55E-05	0.0712	0.9889
RLFNVVSRG	TRPV2	5.02E-07	6.55E-09	6.50E+04	8.05E+02	3.26E-02	1.34E-04	0.1875	0.9931
LVFNVIPTL	ABCB6	5.35E-07	3.99E-09	1.33E+05	9.21E+02	7.13E-02	2.00E-04	0.0445	0.9982
TTWNILSSA	COX1	5.36E-07	4.08E-08	2.26E+05	1.65E+04	1.21E-01	2.63E-03	0.0214	0.9794
KLFNVLSTL	NUP210P2	5.76E-07	3.35E-08	2.97E+05	1.65E+04	1.71E-01	2.83E-03	0.0131	0.9912
RVYNLTAKS	VWA3B	5.95E-07	4.57E-09	4.53E+04	3.35E+02	2.69E-02	5.65E-05	0.0219	0.9981
LTFNTISLS	ENTHD1	7.09E-07	2.12E-07	4.78E+05	1.37E+05	3.39E-01	2.72E-02	0.0387	0.929
AQFNLLSST	TP73	7.13E-07	9.97E-09	8.59E+04	1.15E+03	6.12E-02	2.58E-04	0.1658	0.9947
VVYNVLSEL	SP100,SP140L	7.35E-07	6.29E-08	1.84E+05	1.52E+04	1.35E-01	2.89E-03	0.0255	0.9785
KVYNTPSTS	AEBP2	7.51E-07	1.39E-08	1.71E+05	2.91E+03	1.28E-01	9.13E-04	0.0718	0.9945
GIFNIIPST	CAPN7	7.90E-07	8.67E-09	1.32E+05	1.36E+03	1.04E-01	4.00E-04	0.0364	0.9979
NIYNTLSGL	UBR4	8.73E-07	1.71E-08	1.59E+05	2.89E+03	1.38E-01	9.89E-04	0.0564	0.9947
RLFNLTSTF	FLJ44715,FUT11	9.32E-07	2.82E-08	1.72E+05	4.83E+03	1.60E-01	1.79E-03	0.0525	0.9894

TVWNTLSSL	DNAH9	9.39E-07	1.22E-08	4.52E+04	5.73E+02	4.25E-02	1.20E-04	0.0344	0.9971
RLFNMLSAV	CFAP221,PCDP1	9.71E-07	3.06E-08	1.73E+05	5.09E+03	1.68E-01	1.94E-03	0.0714	0.9892
SIWNVTAIA	HTR5A	1.10E-06	5.12E-07	3.21E+05	1.45E+05	3.54E-01	3.47E-02	0.0576	0.9051
ALFNLMSGI	EGR4	1.19E-06	3.21E-08	9.57E+04	2.48E+03	1.14E-01	8.53E-04	0.0645	0.9931
IVYNLLSAM	SLC39A10	1.30E-06	1.62E-07	1.61E+05	1.98E+04	2.10E-01	4.82E-03	0.0245	0.987
ISFNMLPSI	GPR98	1.37E-06	4.65E-08	1.24E+05	4.04E+03	1.70E-01	1.70E-03	0.0581	0.991
NTYNILPGS	C9orf173	1.38E-06	1.17E-07	1.14E+05	9.57E+03	1.57E-01	2.30E-03	0.025	0.9925
RLWNMVNVT	IL12RB2	1.39E-06	2.57E-07	1.52E+05	2.77E+04	2.11E-01	6.87E-03	0.049	0.9763
SAFNITSLI	WAC	1.41E-06	3.21E-07	1.65E+05	3.70E+04	2.32E-01	9.29E-03	0.0314	0.9682
NIFNLPNIV	OMD	1.48E-06	6.62E-07	4.19E+05	1.85E+05	6.20E-01	4.90E-02	0.0905	0.9596
GVYNLPGAS	GPX2	1.58E-06	3.07E-07	1.17E+05	2.23E+04	1.84E-01	5.65E-03	0.0488	0.9756
GTYNVISLV	TRPC4,TRPC5	1.64E-06	4.18E-07	1.23E+05	3.10E+04	2.02E-01	8.00E-03	0.0666	0.965
SIFNTLSDI	SGSM3	1.97E-06	5.86E-08	4.07E+04	1.20E+03	8.01E-02	3.78E-04	0.0856	0.9957
TIFNILSGI	ABCA3	2.66E-06	2.37E-07	4.68E+04	4.13E+03	1.24E-01	1.66E-03	0.1728	0.9807
LLFNLISSS	MON1A	2.79E-06	2.57E-06	1.51E+05	1.38E+05	4.20E-01	4.10E-02	0.0599	0.9183
RTFNLTAGS	PDXDC1	2.85E-06	5.89E-07	4.63E+04	9.54E+03	1.32E-01	2.56E-03	0.0356	0.9845
TVFNILPGG	PAFAH2	3.23E-06	1.06E-06	3.69E+04	1.21E+04	1.19E-01	3.22E-03	0.025	0.968
GLFNIPPAS	CYP2S1	3.91E-06	4.13E-06	8.52E+04	8.97E+04	3.33E-01	2.74E-02	0.0395	0.9216
RMFNIISDS	RASA1	3.99E-06	4.08E-07	1.51E+04	1.54E+03	6.02E-02	4.39E-04	0.0509	0.9862
TTFNIVGTT	GABRA3	6.79E-06	3.10E-06	8.68E+03	3.96E+03	5.89E-02	8.72E-04	0.0351	0.9743
ALFNLMSGV	EGR4	7.87E-06	1.14E-05	3.17E+04	4.60E+04	2.50E-01	1.45E-02	0.1115	0.9454
SVFNITAIA	MTNR1B	1.96E-05	2.39E-04	2.58E+04	3.15E+05	5.06E-01	1.06E-01	0.2805	0.7869
KIYNTPSAS	NCAM1	2.56E-05	2.88E-05	8.86E+03	9.95E+03	2.27E-01	5.62E-03	0.2474	0.9662
LLYNLLGSS	ABCC9	1.41E-04	6.82E-03	3.21E+03	1.55E+05	4.54E-01	5.27E-02	0.1153	0.9007
SLYNMMGEA	TMTC2	No fit							
SLWNLMGNA	LMAN2L	No fit							
GLYNIVGNA	SUMF1	No fit							
LTWNLTPKA	DLEC1	No fit							
LIFNVTGLA	ZDHHC23	No fit							
SIFNITGIA	MTNR1A	No fit							
LTFNLVSDA	CASP8AP2	No fit							
MQWNILAQA	CCRN4L	No fit							
LSWNLVPEA	COL7A1	No fit							
DLWNTLSEA	TRHDE	No fit							
GLFNIPPAF	CYP2S1	No fit							
LIWNILASF	TTC29	No fit							
LLFNMLPGG	EXT2	No fit							
LVYNIMSSG	FAM120B	No fit							
IIYNVPGTG	RNF133	No fit							
VIYNVTSDG	TTN	No fit							
GTFNLPSDG	BAG6	No fit							

KLWNTLNLI	ENPP5	No fit						
LMWNIISII	VTCN1	No fit						
GLFNTTSNI	SEMA3E	No fit						
LIFNTLSLI	PDCD6IP	No fit						
SVFNLMNAI	SLC38A6	No fit						
LTFNILGQI	DOCK11	No fit						
GLFNMVSSL	RRN3	No fit						
KIFNIINSL	FER1L5	No fit						
AVWNVLGNL	BAG5	No fit						
KVFNIVSDL	FSIP2	No fit						
DLWNVVSHL	DDX60L	No fit						
LQFNTVSKL	JAM2	No fit						
MSFNTVSEL	ZNF33A,ZNF33B	No fit						
ASWNIVNLL	TRPA1	No fit						
ISFNIISAL	MS4A18	No fit						
AFFNILNEL	FNBP1L	No fit						
LVFNLLPIM	ABCB7	No fit						
KIFNTVPDM	ARHGAP26	No fit						
MLFNLIGLS	OR10J1	No fit						
LLFNLPPGS	VGLL1	No fit						
MTFNLIGES	CR1,CR1L	No fit						
KVYNIPGIS	KLHL10	No fit						
GIYNIPGDS	TNS1	No fit						
GLYNLMNIT	INSR	No fit						
LTWNMINTT	LRIT3	No fit						
IVFNVLSDT	HCN3	No fit						
IVFNVVSDT	HCN2,HCN4	No fit						
LIFNITASV	SVEP1	No fit						
IVFNLTNNV	MNAT1	No fit						
KSFNVLSSV	ZNF557	No fit						
LAFNILGMV	SLC46A1	No fit						
VSWNITGTV	SEH1L	No fit						