

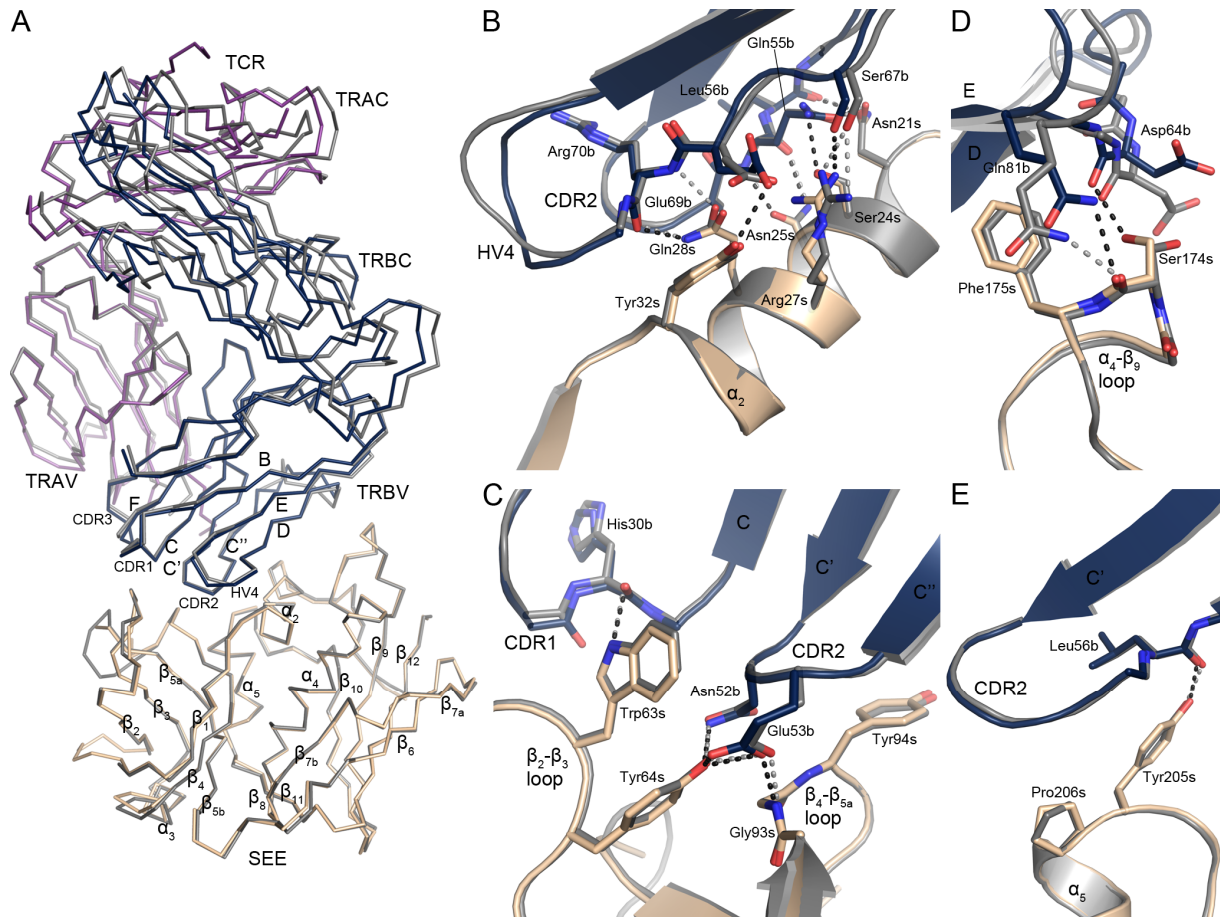
## Supplementary Information

Two common structural motifs for TCR recognition by staphylococcal enterotoxins

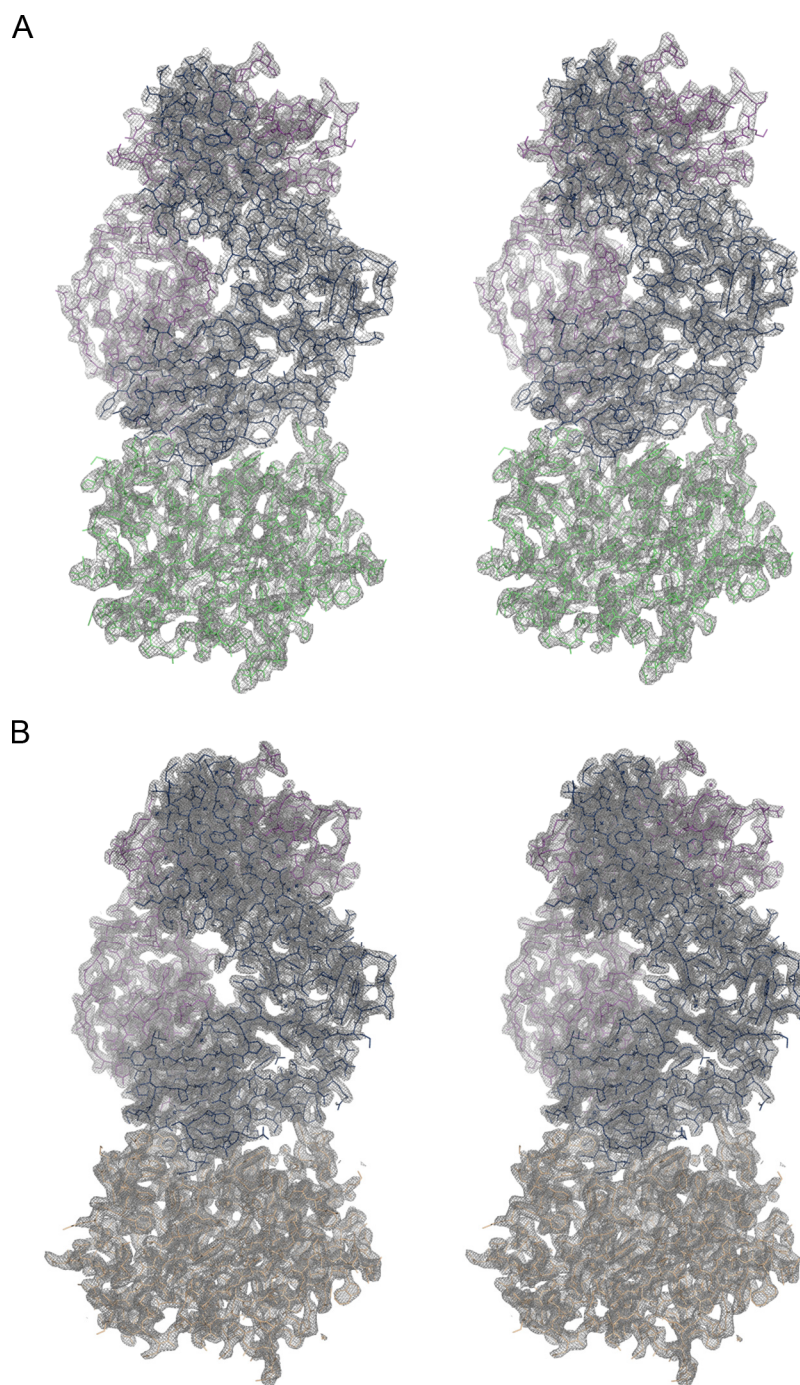
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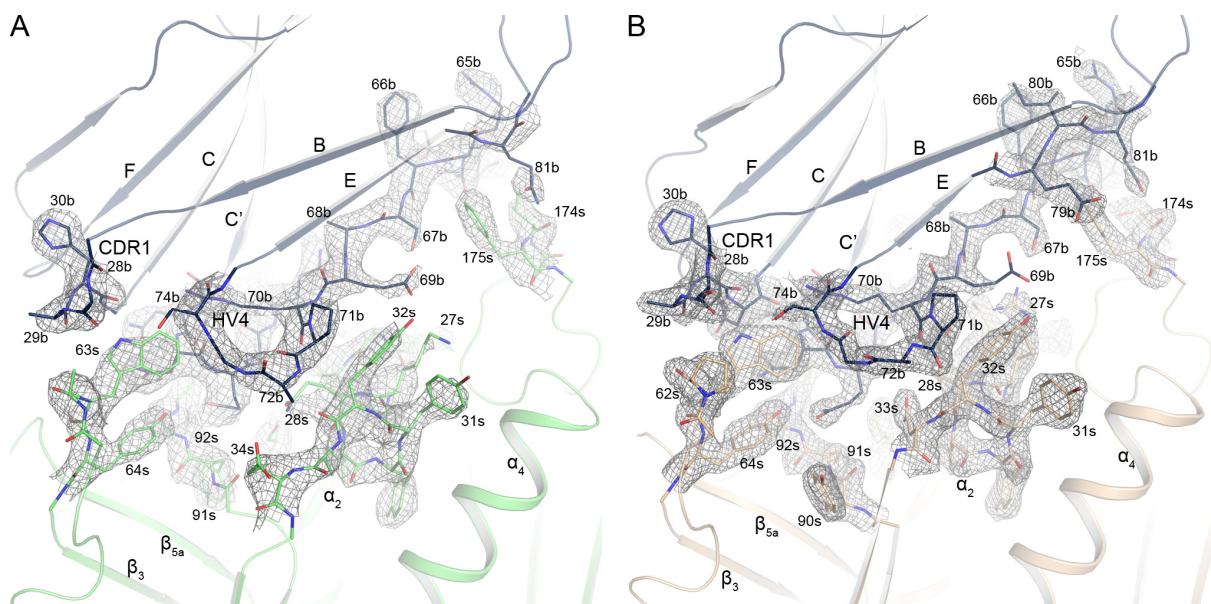
## Supplementary Figures



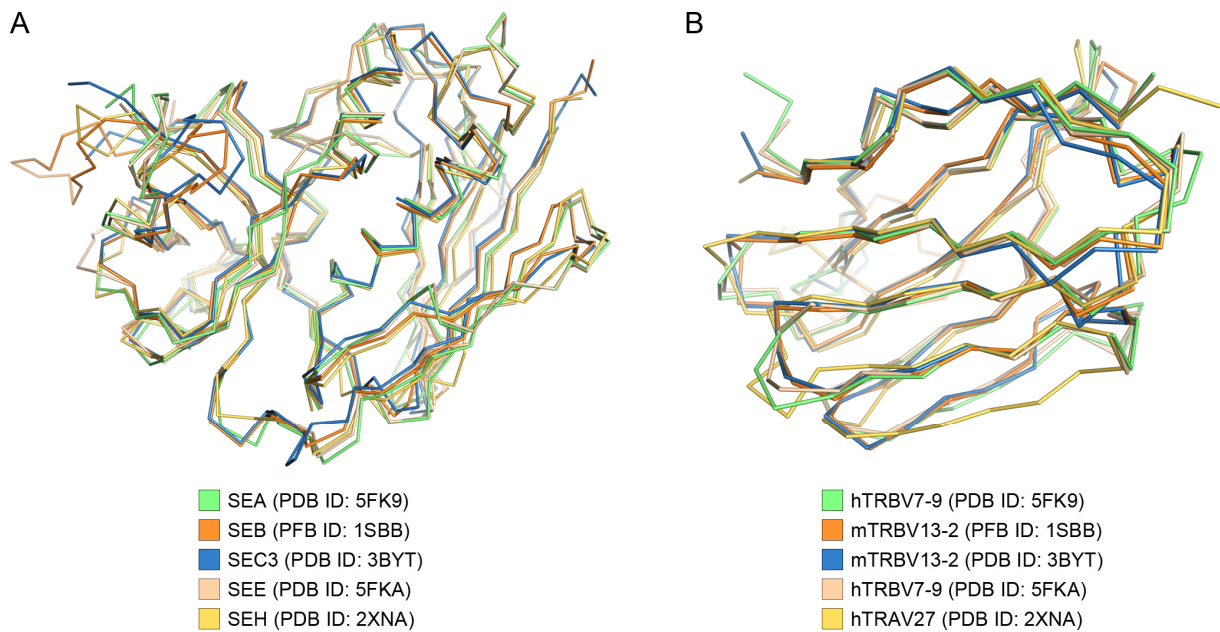
**Figure S1. Structure of the SEE-TCR Complex.** The present structure (PDB ID: 5FKA) (coloured) compared to the previously published SEE-TCR structure (PDB ID: 4UDU) (grey) (A) Overall structure of SEE-TCR, with SEE shown in beige, and TCR in purple and blue for the  $\alpha$ - and  $\beta$ -chain, respectively. Close-up of the SEE  $\alpha_2$ -helix (B) and contacting residues in TCR, the hydrophobic patch, consisting of the  $\beta_2$ - $\beta_3$  and  $\beta_4$ - $\beta_{5a}$  loops (C), the  $\alpha_4$ - $\beta_9$  loop (D), (E) and the upper part of the  $\alpha_5$ -helix. Hydrogen bonds are marked as dotted lines and residues designated as “s” for SAg and “b” for TRBV7-9.



**Figure S2. Stereo view of the  $2F_o-F_c$  electron density maps of the structures. (A) SEA<sup>F47A</sup>-TCR with SEA in green, TCR $\alpha$  in purple and TCR $\beta$  in blue. (B) SEE-TCR, with SEE in beige, TCR $\alpha$  in purple and TCR $\beta$  in blue. Structures are shown in stick representation, with the  $2F_o-F_c$  maps contoured at  $1.0\sigma$ .**



**Figure S3. View of the SAg-TCR interfaces.** The  $2F_o-F_c$  electron density maps contoured at  $1.0\sigma$  are shown around interface residues in the (A) SEA<sup>F47A</sup>-TCR and (B) SEE-TCR structures. Residues designated as “s” for SAg and “b” for TRBV7-9.



**Figure S4. Alignment of the SAgS and TRBV/TRAVs investigated.** (A) Superantigens SEB, SEC3, SEE, and SEH are aligned with respect to SEA (B) The hTRBV7-9, mTRBV 13-3 and hTRAV27 aligned with respect to hTRBV7-9 in the SEA<sup>F47A</sup>-TCR structure. Alignments were performed using secondary structure matching and RMSD values are shown in Supplementary Table S3.

## Supplementary Tables

**Table S1. Intermolecular Hydrogen Bonds in the SEE-TCR Structure.**

Residue SEE	Atom	Residue TRBV	Atom	Distance [Å]
Asn21	Oδ1	Gln55	Oε1	3.0
Asn21	Nδ2	Leu56	O	3.2
Ser24	Oγ	Gln55	Nε2	2.9
<u>Asn25</u>	Oδ1	Gln55	N	2.8
<u>Asn25</u>	Nδ2	Gln55	O	3.1
Arg27	Nη2	Ser67	Oγ	3.1
Gln28	Nε2	Arg70	O	3.0
Tyr32	Oη	Glu69	Oε2	2.9
Trp63	Nε1	His30	O	3.1
Tyr64	Oη	Asn52	Nδ2	2.9
Tyr64	Oη	Glu53	Oε1	3.0
Tyr64	Oη	Glu53	Oε2	2.9
Gly93	N	Glu53	Oε1	3.0
Ser174	O	Gln81	Nε2	3.1
Ser174	Oγ	Asp64	O	2.7
Tyr205	Oη	Leu56	O	2.6

**Table S2. Intermolecular Van der Waals Contacts (distances less than 4 Å) in the SEA<sup>F47A</sup>-TCR and SEE-TCR Complexes.**

SEA	TCR	SEE	TCR
Thr21	Ser28	Arg20	Ser28
<u>Asn25</u>	Glu29	Asn21	Glu29
Lys27	His30	Ser24	His30
Gln28	Gln51	<u>Asn25</u>	Asn31
Tyr32	Asn52	Arg27	Gln51
Glu34	Glu53	Gln28	Asn52
Ser62	Ala54	Tyr32	Glu53
Trp63	Gln55	Asn33	Ala54
Tyr64	Leu56	Pro62	Gln55
Tyr92	Asp64	Trp63	Leu56
Gly93	Arg65	Tyr64	Glu57
<u>Tyr94</u>	Ser67	Tyr91	Lys58
Val174	Glu69	Tyr92	Asp64
Phe175	Arg70	Gly93	Arg65
Tyr205	Pro71	<u>Tyr94</u>	Phe66
Ser206	Lys72	Ser174	Ser67
	Gly73	Phe175	Ala68
	Ser74	Tyr205	Glu69
	Gln81	Pro206	Arg70
			Pro71
			Ser74
			Glu79
			Gln81

**Table S3. Pairwise RMSD values (Å) between SAg (chain IDs B or C) and variable TCR domains (chain IDs A or B), calculated using secondary structure matching.**

SAg	SEA 5FK9 (C)	SEB 1SBB (B)	SEC3 3BYT (B)	SEE 5FKA (C)	SEH 2XNA (C)
SEA 5FK9 (C)	0	1.68	1.63	0.789	1.31
SEB 1SBB (B)		0	0.732	1.59	1.69
SEC3 3BYT (B)			0	1.60	1.66
SEE 5FKA (C)				0	1.43
SEH 2XNA (C)					0
TRBV/TRAV	hTRBV7-9 5FK9 (B)	mTRBV13-2 1SBB (A)	mTRBV13-2 3BYT (A)	hTRBV7-9 5FKA (B)	hTRAV27 2XNA (B)
hTRBV7-9 5FK9 (B)	0	1.41	1.46	0.700	1.43
mTRBV13-2 1SBB (A)		0	0.590	1.36	1.40
mTRBV13-2 3BYT (A)			0	1.44	1.46
hTRBV7-9 5FKA (B)				0	1.31
hTRAV27 2XNA (B)					0

**Table S4. Equilibrium Dissociation Constants for the Studied Staphylococcal Enterotoxins to TCR.**

SAg	TCR	K <sub>d</sub> [μM]	Reference
SEA	hTRBV7-9	0.7	Hedlund et al. (2013) <sup>1</sup>
SEE	hTRBV7-9	6	Hedlund et al. (2013) <sup>1</sup>
SEB	mTRBV13-2	140	Malchiodi et al. (1995) <sup>2</sup>
SEB	hTRBV19	40-90	Rodstrom et al. (2014) <sup>3</sup>
SEC3	mTRBV13-2	3	Leder et al. (1998) <sup>4</sup>
SEH	hTRAV27	4	Pumphrey et al. (2007) <sup>5</sup>

**Table S5. Residues in TCR Affected by *in Silico* Alanine-scanning Mutagenesis with  $\Delta\Delta G_{\text{complex}}$  values shown in kcal/mol.**

<b>SEA-hTRBV7-9</b>		<b>SEE-hTRBV7-9</b>	
<b>Residue</b>	<b><math>\Delta\Delta G_{\text{complex}}</math></b>	<b>Residue</b>	<b><math>\Delta\Delta G_{\text{complex}}</math></b>
Glu53	3.31	Asn52	1.07
Gln55	1.52	Glu53	1.83
Leu56	1.18	Leu56	1.12
Gln81	1.02	Glu57	1.30
		Gln81	1.45
<b>SEB-mTRBV13-2</b>		<b>SEC3-mTRBV13-2</b>	
<b>Residue</b>	<b><math>\Delta\Delta G_{\text{complex}}</math></b>	<b>Residue</b>	<b><math>\Delta\Delta G_{\text{complex}}</math></b>
-	-	Lys57	1.39
<b>SEB-hTRBV19</b>		<b>SEH-hTRAV27</b>	
<b>Residue</b>	<b><math>\Delta\Delta G_{\text{complex}}</math></b>	<b>Residue</b>	<b><math>\Delta\Delta G_{\text{complex}}</math></b>
Tyr49	1.11	Val28	1.07
Gln52	0.96	Arg69	4.62
Ile53	1.23		
Phe57	1.84		

## Supplementary References

- 1 Hedlund, G. *et al.* The tumor targeted superantigen ABR-217620 selectively engages TRBV7-9 and exploits TCR-pMHC affinity mimicry in mediating T cell cytotoxicity. *PLoS one* **8**, e79082, doi:10.1371/journal.pone.0079082 (2013).
- 2 Malchiodi, E. L. *et al.* Superantigen binding to a T cell receptor beta chain of known three-dimensional structure. *The Journal of experimental medicine* **182**, 1833-1845 (1995).
- 3 Rodstrom, K. E., Elbing, K. & Lindkvist-Petersson, K. Structure of the superantigen staphylococcal enterotoxin B in complex with TCR and peptide-MHC demonstrates absence of TCR-peptide contacts. *J Immunol* **193**, 1998-2004, doi:10.4049/jimmunol.1401268 (2014).
- 4 Leder, L. *et al.* A mutational analysis of the binding of staphylococcal enterotoxins B and C3 to the T cell receptor beta chain and major histocompatibility complex class II. *The Journal of experimental medicine* **187**, 823-833 (1998).
- 5 Pumphrey, N. *et al.* Cutting edge: Evidence of direct TCR alpha-chain interaction with superantigen. *J Immunol* **179**, 2700-2704, doi:10.1093/immdev/179/5/2700 [pii] (2007).