

## Supplementary Materials for

### How C-terminal additions to insulin B-chain fragments create superagonists for T cells in mouse and human type 1 diabetes

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#### The PDF file includes:

Fig. S1. Mutations to prevent I.29 and 8F10 TCR dimerization.

Fig. S2. Extension of the mutational analysis of the I.29 TCR shown in Fig. 2B in the main manuscript.

Table S1. Crystallography statistics.

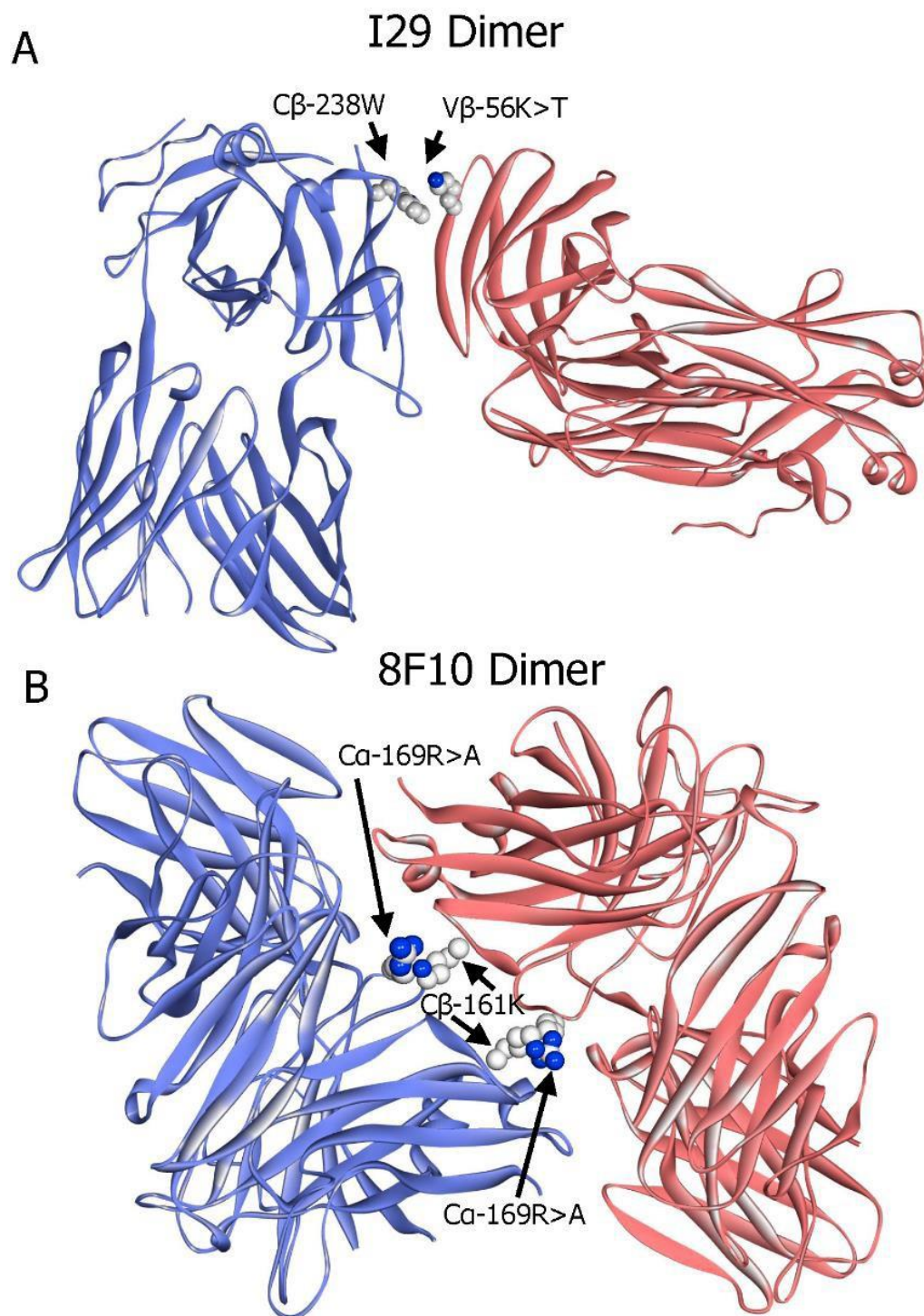
#### Other Supplementary Material for this manuscript includes the following:

(available at [immunology.sciencemag.org/cgi/content/full/4/34/aav7517/DC1](http://immunology.sciencemag.org/cgi/content/full/4/34/aav7517/DC1))

Table S2 (Microsoft Excel format). Supplementary spreadsheet listing TCR- to MHC-peptide contacts.

Table S3 (Microsoft Excel format). Supplementary spreadsheet with raw data used to construct graphs in Figs. 2 and 5.

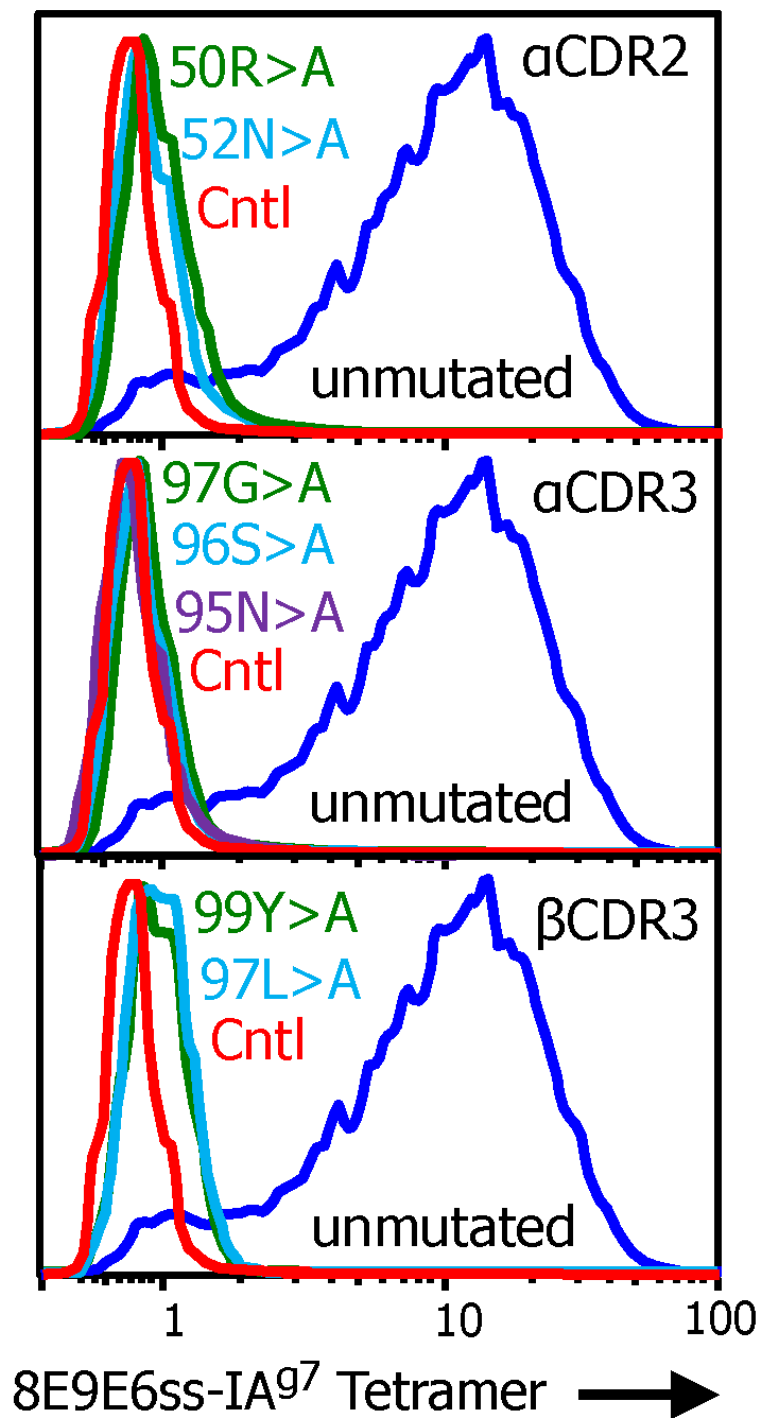
# Figure S1



**Fig. S1. Mutations to prevent I.29 and 8F10 TCR dimerization.**

Dimers of the free I.29 (A) and 8F10 (B) TCRs in the crystals described in Table S1 are shown as ribbon representations. Pairs of interacting amino acids (CPK representation) predicted to be essential for dimer formation are shown along with the mutation that was made to one of the amino acids to disrupt the dimer.

Figure S2



**Fig. S2. Extension of the mutational analysis of the I.29 TCR shown in Fig. 2B in the main manuscript.** Individual mutations to alanine were introduced into I.29 TCR amino acids within V $\alpha$ CDR2, V $\alpha$ CDR3 and V $\beta$ CDR3 making the most contact with the IAg7-8E9E6ss ligand (Table 2). The mutated TCRs were introduced into 5KC to construct avatars expressing the TCR on the surface, which was confirmed by anti-C $\beta$  staining. Each resulting T cell was tested for binding of the IAg7-8E9E6ss fluorescent tetramer. The positive control was the 5KC avatar expressing the unmutated TCR and the negative control was the 5KC recipient, which lacks functional TCR genes.

**Table S1. Crystallography statistics.**

	I29 TCR	IA <sup>97</sup> 8E9E6ss I29 TCR Complex	8F10 TCR	IA <sup>97</sup> 8G9E 8F10 TCR Complex	DQ8 8E9E11ss T1D3 TCR Complex
PDB No.	6DFQ	6DFS	6DFV	6DFW	6DFX
Space Group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	P6 <sub>3</sub>	P1	P2 <sub>1</sub>	P2 <sub>1</sub>
<b>Data collection</b>					
Dimensions (Å)	a 95.11 b 117.63 c 234.77	269.52	62.02	93.10	111.71
Angles (°) (Å)	α 90.00 β 90.00 γ 90.00	90.00	111.66	90.00	90.00
Resol. Range (Å)	50-2.60	50-3.10	50-1.71	50-3.20	50-2.03
$R_{\text{sym}}$ or $R_{\text{merge}}^{*†}$	0.05(0.73)	0.03(0.66)	0.04(0.86)	0.07(0.86)	0.05(0.26)
$I/\sigma I^*$	12.4(1.2)	24.4(1.4)	11.6(1.2)	7.6(1.0)	14.4(2.1)
Completeness (%) <sup>*</sup>	98.1(99.5)	99.7(100)	91.5(91.1)	96.9(97.3)	98.1(88.3)
Redundancy <sup>*</sup>	3.7 (3.7)	6.3(6.8)	4.1(4.1)	3.4(3.4)	4.5(3.3)
<b>Refinement</b>					
Resol. Range (Å)	47.6-2.60	46.73-3.10	48.21-1.71	46.56-3.20	47.78-2.03
No. reflections	76175	32259	91564	36910	135207
Rwork‡	22.0	24.0	17.1	23.3	18.1
Rfree‡	25.3	28.5	23.1	29.7	21.7
No. atoms	13644	6254	7097	12189	13693
Protein	13467	6221	6770	12189	12872
Ligand/ion	9	14	8	N/A	46
Water	168	19	319	N/A	775
<b>B-factors</b>					
Protein	74.98	69.59	41.64	132.2	49.56
Ligand/ion	69.97	72.10	45.95	N/A	54.61
Water	52.85	18.14	38.65	N/A	46.85
<b>R.m.s deviations</b>					
Bond lengths (Å)	0.014	0.019	0.015	0.019	0.019
Bond angles (°)	1.746	1.939	1.747	1.934	1.937

\*All data (outer shell).

†  $R_{\text{merge}} = \sum(|I - \langle I \rangle|) / \sum(I)$ .

‡  $R_{\text{work}}$  or  $R_{\text{free}} = \sum||F_o| - |F_c|| / \sum|F_o|$ ,  $R_{\text{free}}$  is calculated from a set of ~5% of the total reflections randomly chosen and set aside.

Diffraction data collection, indexing and merging, model building and refinement statistics are shown for the 5 structures reported in this paper.