Supplementary Materials

Beryllium-specific $CD4^+$ T cells induced by chemokine neoantigens perpetuate inflammation

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Supplementary Methods

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Table S4. Mean EC50 values (nM) of two experiments for 4 hybridomas to CCL3 length variant peptides.

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Supplementary Methods

Generation of hybridomas expressing TCRs from human T cell clones.

Complete αβTCRs were introduced into the human $CD4^+$ mouse recipient hybridoma cell line 54ζ (1). Three DNA fragments were generated for each TCR: two synthesized fragments (IDT DNA) encoding the variable domains of the TCR α- and β-chains of each T cell clone, and a purified PCR product encoding the murine Cα domain connected to the porcine teschovirus-1 2A peptide. These fragments possessed homologous overlapping nucleotide sequences allowing cloning in the proper orientation into a murine stem cell virus (MSCV)-based retroviral vector using a Gibson Assembly method (New England Biolabs). MSCV plasmids encoding full-length chimeric *TRA* and *TRB* genes separated by the 2A peptide cleavage site were packaged as retrovirus by transient transfection of Phoenix 293T cells. Retroviral transduction with viral supernatants, flow-sorting for TCR and CD4 expression, and maintenance of cell lines in culture were as previously described (2).

T cell hybridoma activation assays.

T cell hybridomas and either HLA-DP2 transfected fibroblasts (3) or B cells harvested from HLA-DP2 Tg mice (4) were incubated overnight with $BeSO_4$ (75-200 μM) and positional scanning library (PSL) mixtures (20-200 μg/ml) or peptides as previously described (2, 3). All assays were done in OptiPRO serum-free medium (Gibco, ThermoFisher) supplemented with 0.5% FBS (Hyclone). Testing of individual crude peptides was performed at 0.5, 5, and 50 μg/ml, and for dose response curves, peptide concentrations ranged from 0.1 nM to 10 μM. Recombinant CCL3 and CCL4 proteins (Peprotech) were tested in triplicate at 0.3 - 20 μg/ml in wells containing DP8302 fibroblasts.

For all experiments, supernatants were harvested after 22-24 hours incubation, and mouse IL-2 was measured by ELISA (eBioscience). In dosing experiments, the concentration of peptide that generated 50% of the maximum IL-2 response (EC50) for each hybridoma was determined using nonlinear regression (sigmoidal-fit; Prism, GraphPad Software).

Positional scanning libraries and peptides.

T cell hybridomas expressing selected TCRs were first screened for responses against an unbiased decapeptide PSL (3, 5, 6). PSLs are comprised of 200 mixtures synthesized in an OX9 format, where O represents a specific amino acid at a defined position and X represents an equimolar mixture of 19 natural amino acids (except cysteine) in each of the remaining 9 positions. A biased decapeptide PSL was also designed such that all peptides in each mixture were composed of a D at position 5 and an E at position 8 of the peptide (D5E8 PSL). Individual peptides were synthesized using the PEPScreen 96-well array (Sigma-Aldrich). Peptides chosen for further study were synthesized at 95% purity (CPC Scientific).

Scoring matrices and database searches.

Multiple scoring matrices were generated by assigning numerical values to the stimulatory potency of defined amino acids at each position of the decapeptide D5E8 PSL. For each hybridoma, two matrices were generated using the value of IL-2 (pg/ml) in the presence of peptide mixtures/Be and the logarithm of that value. For the two defined positions (D5E8), the minimum value of each matrix was assigned to all amino acids except for the amino acid fixed at that position (i.e., D at position 5, E at position 8). The value for these amino acids was assigned

the maximum stimulatory potency measured among all the mixtures at all positions. For hybridomas tested at multiple dose points (8845-c3 and 8133-c4r), each dose was used independently, and interpolated ED300 (the dose to reach 300 pg/ml) values were generated. A third matrix consisting of $\frac{200}{ED300}$ values was also used. Finally, the four hybridoma matrices generated from testing at 50 μg/ml were normalized to a maximum value of 1000 pg/ml and added together to create composite activity matrices using these values and the logarithm of these values. The predicted stimulatory potential of a peptide, or score, was calculated by summing the matrix values associated with each amino acid in each position of the peptide. The sum of the maximum values at each position was defined as the maximum matrix score. The scoring matrix was applied to rank, according to their stimulatory score, all of the overlapping peptides within each protein sequence of a human Uniprot protein database (downloaded 7/2/2018), as previously described (5, 7).

ELISA for CCL3 and CCL4 secretion by CBD BAL cells

BAL cells (1 x 10^6 cells/ml) from CBD patients were in placed in culture in 96 well U-bottomed plates (5 wells/condition) in medium alone or 100 μM $BeSO_4$. After 48 hours incubation, supernatants were pooled, cleared of cellular debris and stored at -80°C. Human CCL3 and CCL4 chemokines were assessed by ELISA (Invitrogen), and results are presented as the average of duplicate wells.

Tetramer staining and dual intracellular interferon-γ/tetramer assay.

Beryllium-saturated MHCII tetramers with covalently attached peptides were made using a baculovirus expression system (3, 8), and an HLA-DP2-CLIP tetramer was provided by the NIH

Tetramer Core Facility at Emory University (Atlanta, GA). Hybridoma cells matched for expression of high levels of TCR were stained with HLA-DP2–CCL3/Be, DP2–CCL4/Be, DP2-PLXNA4/Be or DP2-CLIP tetramers (20 μg/ml) as previously described (3).

For dual assessment of IFN-γ expression and HLA-DP2-tetramer binding, BAL cells were stimulated with medium or $BeSO_4$ (100 μM) for 6 h prior to tetramer staining. Cells were stained for surface markers and then fixed, permeabilized, and stained with anti–IFN-γ–PE-Cy7 (B27; BD Biosciences) mAb for 30 min. Cell staining was evaluated on a FACSCanto II flow cytometer (BD Biosciences), and data were analyzed with FlowJo software (Tree Star). $CD3^+$, $CD4^+$ T cells were analyzed for tetramer binding and cytokine expression using no stimulation and HLA-DP2-CLIP tetramer staining to set gates.

HLA-DP2 Tg and LKGGG CDR3β TCR retrogenic HLA-DP2 Tg mice.

HLA-DP2 Tg C57BL/6 mice were housed and bred at the University of Colorado Biological Resource Center. C57BL/6 $RAG^{-/-}$ mice were purchased from The Jackson Laboratory and bred to express HLA-DP2. Mice were used at 6-8 weeks of age.

To generate HLA-DP2 Tg C57BL/6 mice TCR retrogenic mice, Phoenix cells were cotransfected with TCR-encoding MSCV vectors and the pCL-Eco packaging plasmid using Lipofectamine 2000 (Invitrogen) to produce replication-incompetent retroviruses encoding TCR genes. High-titer viral supernatants were collected after 24 hours from large-scale transfections and stored at -80°C for use in multiple experiments. For retroviral-mediated transfer of TCR genes (9, 10), bone marrow cells were extracted from femurs of HLA-DP2 Tg $RAG^{-/-}$ B6 mice.

Purified hematopoietic progenitor cells (Stemcell) were placed in culture for 48 hours in DMEM supplemented with 20% FBS (Hyclone) and a cytokine cocktail (all from Peprotech) containing IL-3 (20 ng/ml), IL-6 (50 ng/ml) and mouse stem cell factor (50 ng/ml). Stem cells were transduced with viral supernatant on successive days by spinfection at 37°C for 2 hours at 2500 rpm with retroviral supernatant, polybrene (7.5 µg/ml) and freshly added cytokines. Cells were expanded in culture 72 hours to maximize the yield and percentage of GFP^+ (i.e., virally-transduced) cells. Mice received 15-20 x 10^6 cells by intravenous injection and were bled for TCR reconstitution starting week 5 post-transplantation. Mice typically began the standard protocol of Be exposure at 6 weeks after injection of bone marrow-transduced cells.

Mice were exposed to BeO oropharyngeal aspiration using a sensitization/boost protocol as previously described (4, 11). At sacrifice on day 21, single cell suspensions of lung cells, BAL cells and fluid were collected for analysis. Flow cytometry, IFN-γ ELISPOTs, lung injury assessment, and immunohistochemistry of paraffin-embedded lung tissue were performed as described (11). BAL was completed using 1 ml of sterile PBS, and CCL4 and CCL3 chemokines were assessed in fluid by ELISA (R & D Systems).

For LPS exposure experiments, HLA-DP2 Tg FVB/N mice were exposed to BeO using our standard sensitization/boost protocol with and without a single dose of LPS (10 µg; ENZO Life Sciences, USA) by oropharyngeal aspiration on day 14. BAL fluid was obtained from sacrificed mice after 24 hours to measure CCL4 and CCL3 in BAL fluid, and additional mice were sacrificed at day 21 to assess other parameters of disease progression as described above (11). To quantitate mononuclear cell infiltrates, whole slide imaging was performed on H&E stained lung

sections cut from formalin fixed paraffin embedded tissue. Pyramidal tiff files were analyzed using OuPath software (v.0.2.3). Briefly, stain vectors values were automatically determined and cells were counted by adjusting the cell detection threshold to maximize the difference between areas containing perivascular mononuclear infiltrates and unaffected areas.

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Figure S1

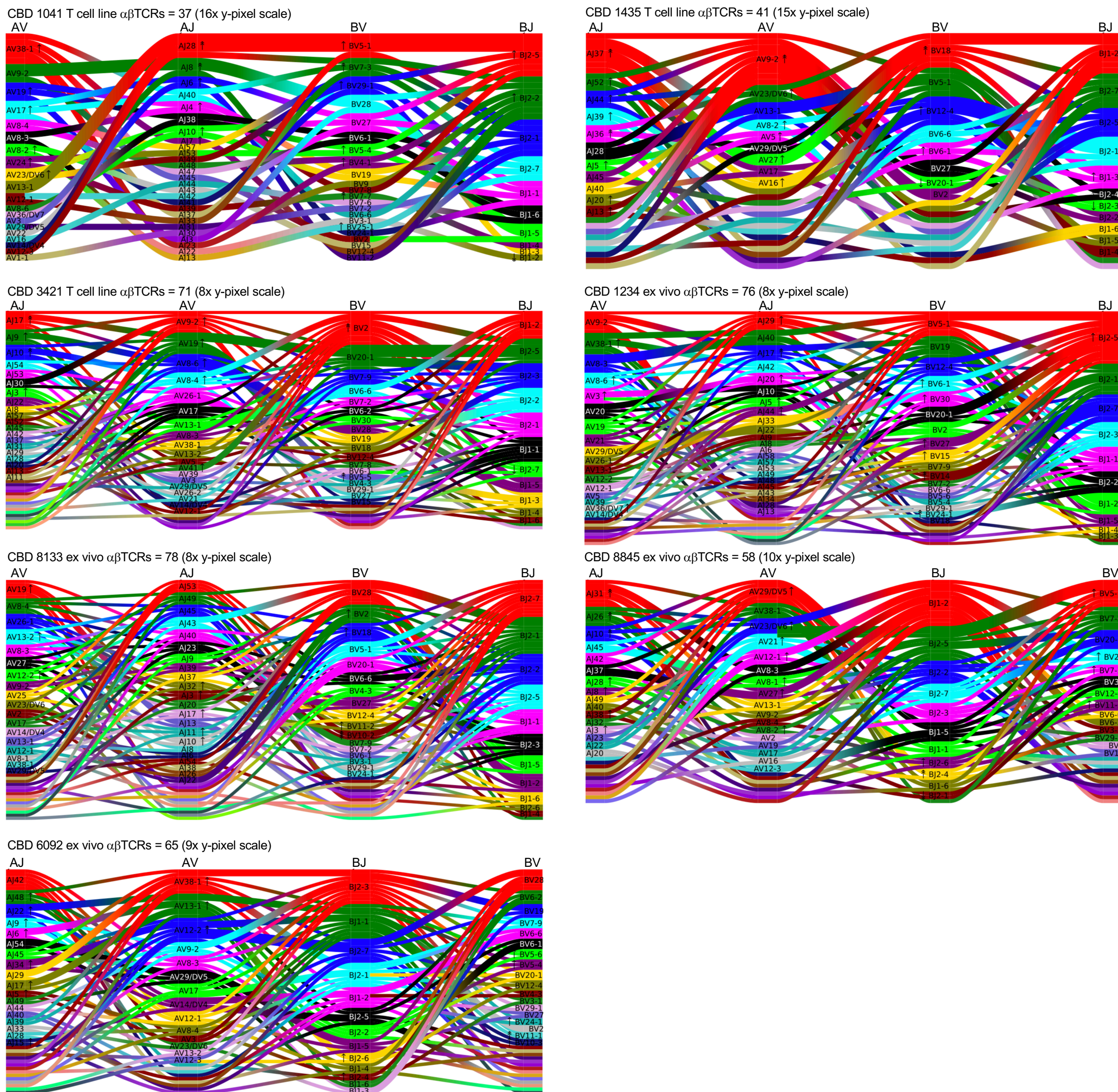


Figure S1. Cord diagrams of *TR* gene segment usage of individual CBD patient's T cells. Cord diagram of gene segment usage of T cells from all CBD patients combined (n = 426 T cells). Each individual T cell's *TR* gene segment usage (*BV*, *BJ*, *AV*, *AJ*) is connected by a curved line whose thickness is proportional to the number of T cells with the respective gene pairing. Genes are color-coded based on frequency of usage, and observed enrichment of some gene segments relative to a background naïve repertoire is indicated by arrows. The number of complete αβTCRs obtained for each patient is indicated at the top of each plot.

Figure S2

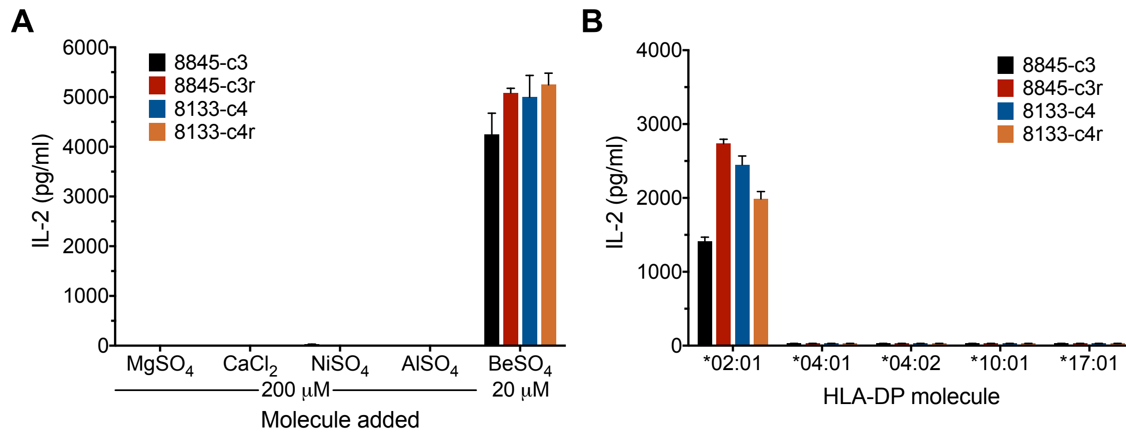


Figure S2. Beryllium and HLA-DP2 specificity of LKGGG CDR3β-expressing T cell hybridomas. (A) Equal numbers of hybridoma cells (from CBD patients 8845 and 8133) and HLA-DP2-expressing fibroblasts were mixed with solutions of metal cations (0.2, 2.0, 20 and 200 μM). IL-2 secretion by hybridomas was measured by ELISA after 22 hours of culture. Data are presented as IL-2 release (mean ± SD pg/ml) for the single concentration of cation providing a maximal response. **(B)** DAP3.L fibroblast cells transfected with the indicated HLA-DP molecule were mixed with equal numbers of hybridoma cells and placed in culture in the presence of $BeSO_4$ (100 μM). IL-2 secretion was measured by ELISA after 22 hours of culture, and data are presented as mean IL-2 ± SD (pg/ml) release. Both **(A)** and **(B)** are representative of two experiments done in triplicate.

Figure S3

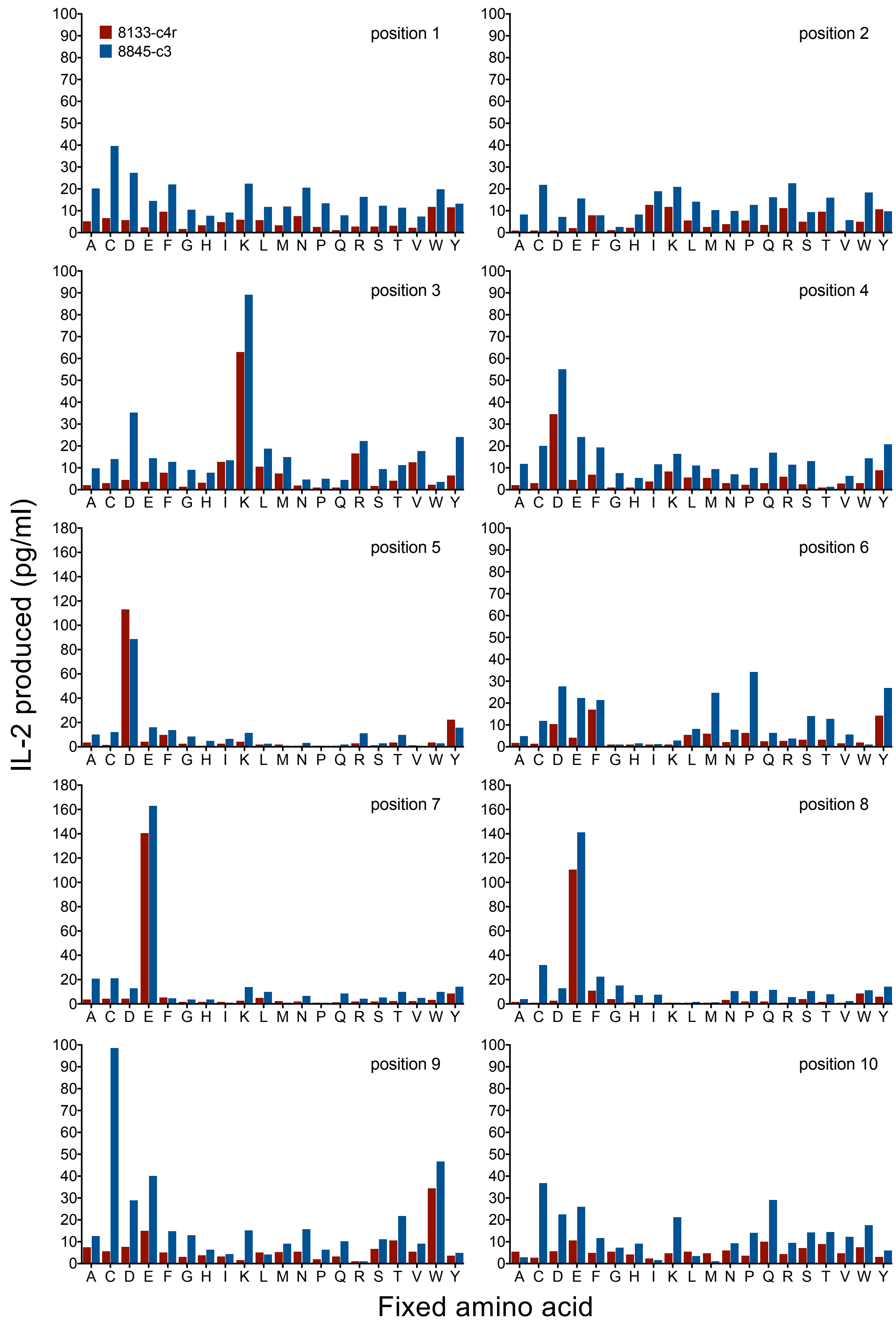


Figure S3. IL-2 response of BAL-derived LKGGG CDR3β TCRs against an unbiased decapeptide PSL. Equal numbers of hybridoma cells (8133-c4r and 8845-c3) and DP2.21 antigen-presenting cells were mixed with $BeSO_4$ (75 μM) and peptide mixtures (200 μg/ml) from an unbiased PSL. IL-2 secretion was measured by ELISA after 22 hours of culture. Each panel shows results from a scan of an individual peptide position with the x-axis denoting the amino acid (single letter code) fixed at each defined position. Data are representative of two experiments for each hybridoma performed in duplicate.

Figure S4

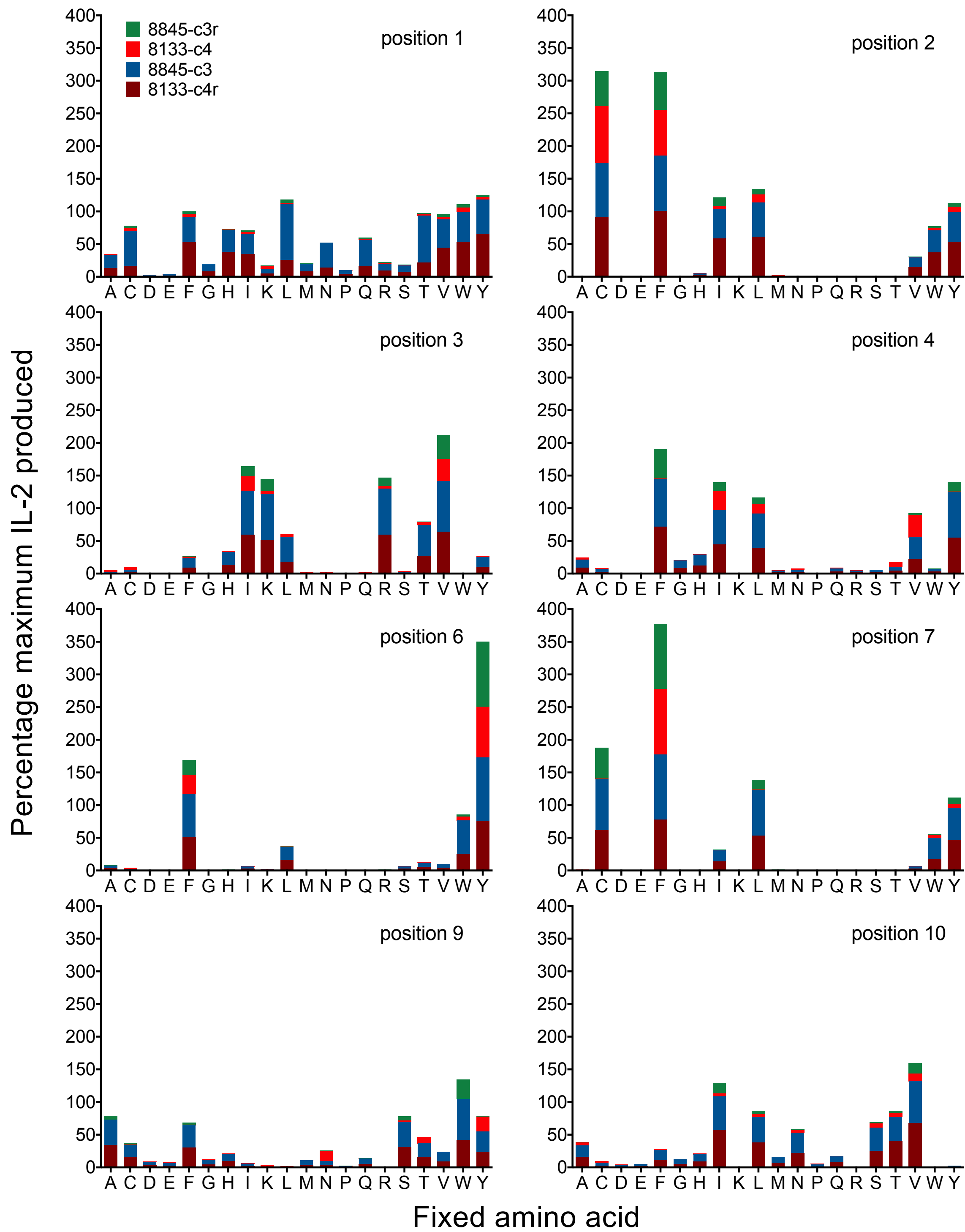


Figure S4. IL-2 response of BAL-derived LKGGG CDR3β TCRs against a biased D5E8 decapeptide PSL. Equal numbers of hybridoma cells (8133-c4r, 8845-c3, 8133-c4 and 8845-c3r) and DP2.21 antigen-presenting cells were mixed with $BeSO_4$ (75 μM) and peptide mixtures (50 μg/ml) from a biased PSL containing a fixed aspartic acid (D) at position 5 and a fixed glutamic acid (E) at position 8. IL-2 secretion was measured by ELISA after 22 hours of culture. Data were normalized for each hybridoma against the mixture which evoked the highest IL-2 release for that hybridoma (F2 for 8133-c4r; F7 for others) and presented as stacked bars. Each panel shows results of a scan of an individual peptide position. Data are representative of two experiments for each hybridoma completed in duplicate.

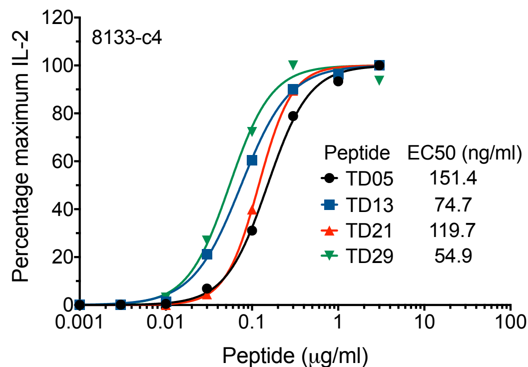
Figure S5

A

	Mimotopes										
		W V	F	R V	F I	D	Y	F L	E	S W	I

ID	10mer	Position										IL-2 (pg/ml)			
		p1	p2	p3	p4	p5	p6	p7	p8	p9	p10	8845-c3	8845-c3r	8133-c4	8133-c4r
TD01	VFRFDYFESI	V	F	R	F	D	Y	F	E	S	I	6	1074	2	1037
TD02	VFRFDYFEWI	V	F	R	F	D	Y	F	E	W	I	311	1081	0	832
TD03	VFRFDYLESI	V	F	R	F	D	Y	L	E	S	I	1	1154	2	920
TD04	VFRFDYLEWI	V	F	R	F	D	Y	L	E	W	I	708	1071	1	814
TD05	VFRIDYFESI	V	F	R	**I**	D	Y	**F**	E	**S**	I	1176	1079	1193	909
TD06	VFRIDYFEWI	V	F	R	I	D	Y	F	E	W	I	1146	1079	5	620
TD07	VFRIDYLESI	V	F	R	I	D	Y	L	E	S	I	1208	1098	2	856
TD08	VFRIDYLEWI	V	F	R	I	D	Y	L	E	W	I	1186	967	1	571
TD09	VFVFDYFESI	V	F	V	F	D	Y	F	E	S	I	1146	1138	31	976
TD10	VFVFDYFEWI	V	F	V	F	D	Y	F	E	W	I	1233	1003	1	755
TD11	VFVFDYLESI	V	F	V	F	D	Y	L	E	S	I	1216	1057	1	888
TD12	VFVFDYLEWI	V	F	V	F	D	Y	L	E	W	I	1277	932	1	804
TD13	VFVIDYFESI	V	F	V	**I**	D	Y	**F**	E	**S**	I	1120	1067	1194	841
TD14	VFVIDYFEWI	V	F	V	I	D	Y	F	E	W	I	907	956	11	532
TD15	VFVIDYLESI	V	F	V	I	D	Y	L	E	S	I	1071	1097	33	791
TD16	VFVIDYLEWI	V	F	V	I	D	Y	L	E	W	I	689	722	1	413
TD17	WFRFDYFESI	W	F	R	F	D	Y	F	E	S	I	2	1067	0	931
TD18	WFRFDYFEWI	W	F	R	F	D	Y	F	E	W	I	1119	1007	1	688
TD19	WFRFDYLESI	W	F	R	F	D	Y	L	E	S	I	1	1089	1	826
TD20	WFRFDYLEWI	W	F	R	F	D	Y	L	E	W	I	1111	894	1	460
TD21	WFRIDYFESI	W	F	R	**I**	D	Y	**F**	E	**S**	I	1188	1059	1013	821
TD22	WFRIDYFEWI	W	F	R	I	D	Y	F	E	W	I	1002	828	1	237
TD23	WFRIDYLESI	W	F	R	I	D	Y	L	E	S	I	1103	994	1	660
TD24	WFRIDYLEWI	W	F	R	I	D	Y	L	E	W	I	949	614	1	210
TD25	WFVFDYFESI	W	F	V	F	D	Y	F	E	S	I	1179	1099	59	931
TD26	WFVFDYFEWI	W	F	V	F	D	Y	F	E	W	I	1228	951	1	870
TD27	WFVFDYLESI	W	F	V	F	D	Y	L	E	S	I	1192	1088	1	867
TD28	WFVFDYLEWI	W	F	V	F	D	Y	L	E	W	I	1202	1005	1	743
TD29	WFVIDYFESI	W	F	V	**I**	D	Y	**F**	E	**S**	I	1103	1069	1213	846
TD30	WFVIDYFEWI	W	F	V	I	D	Y	F	E	W	I	880	947	84	635
TD31	WFVIDYLESI	W	F	V	I	D	Y	L	E	S	I	1045	1085	73	838
TD32	WFVIDYLEWI	W	F	V	I	D	Y	L	E	W	I	775	794	1	511

B



C

ID	Peptide	EC50 (ng/ml)			
		8845-c3	8845-c3r	8133-c4	8133-c4r
TD05	V F R I **D** Y F **E** S I	89.2	24.7	151.4	16.9
TD13	V F V I **D** Y F **E** S I	49.2	31.9	74.7	37.2
TD21	W F R I **D** Y F **E** S I	17.5	17.9	119.7	20.5
TD29	W F V I **D** Y F **E** S I	63.4	22.2	54.9	28.1

Figure S5. Identification of mimotopes that stimulate hybridomas expressing the LKGGG CDR3β motif. (**A**) List of potential mimotopes, chosen based on selection of amino acids at each peptide position (shown at top of Figure) having the most stimulatory activity in the biased D5E8 PSL in the presence of $BeSO_4$. Hybridoma response to peptides tested at 1 μg/ml with $BeSO_4$ are shown with activity depicted by color-coding (green, high; yellow, moderate; orange, negative). Red bolding (I4, F7, S9) highlights amino acids allowing hybridoma 8133-c4 recognition of peptides. (**B**) Peptide dose-response curves for hybridoma 8133-c4 evaluating peptides that induced activity in all 4 T cell hybridomas. Equal numbers of 8133-c4 hybridoma cells and DP2.21 antigen-presenting cells were mixed with $BeSO_4$ (75 μM) and peptide, and IL-2 secretion was measured by ELISA after 22 hours of culture. Data is plotted as the percentage of maximum IL-2 secretion against peptide concentration. EC50 values, defined as the concentration of peptide that induces a half-maximal response, are listed. (**C**) Summary of EC50 values for each mimotope that stimulated the 4 Be-specific hybridomas, calculated from their respective dose-response curves is shown. Data are representative of two separate experiments done in duplicate.

Figure S6

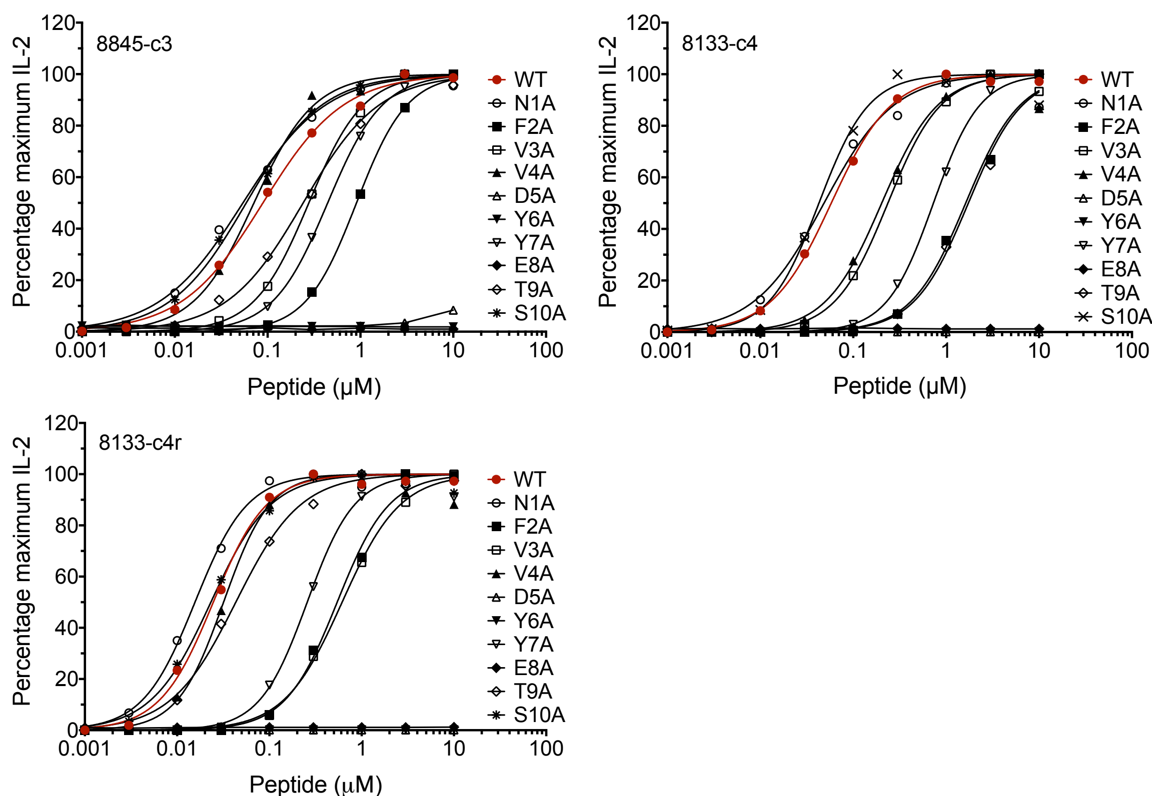


Figure S6. CCL4 peptide dose-response curves. Dose-response curves to pure CCL4 peptides with single alanine substitutions are shown for hybridomas 8845-c3 (top, left), 8133-c4 (top, right) and 8133-c4r (bottom, left). Equal numbers of hybridoma cells and DP2.21 antigen-presenting cells were mixed with $BeSO_4$ (75 µM) and highly-purified CCL4 peptides with single alanine substitutions. IL-2 secretion was measured by ELISA after 22 hours of culture, and data are plotted as the percentage of maximum IL-2 secretion against peptide concentration in the presence of $BeSO_4$. The natural CCL4 peptide (WT) curve is drawn in red. EC50 values (nM) for each peptide are displayed in Figure 4B, and data are representative of two experiments.

Figure S7

A

Hyb ID	AV	Deduced CDR3α sequence	AJ	BV	Deduced CDR3β sequence	BJ	Freq
8845-c1	21	CAV**N**DRGSTLGRLYFG	18	7-2	CASSL**KLAGGIVV**TGELFF	2-2	6/78
8845-c2	38-1	CAFM**T**EYGNKLVFG	47	7-2	CASS**PGGGGKI**YEQYFG	2-7	5/78
1435-c1r	27	CAGG**A**SSNTGKLIFG	37	18	CASSP**SGDA**YGYTF	1-2	1/103
3421-c2r	13-1	CAAS**Q**LTGGGNKLTFG	10	6-5	CASS**QDRER**SYEQYF	2-7	1/106

B

ID	Peptide 10mer	1	2	3	4	5	6	7	8	9	10	IL-2 (pg/ml) 8845-c1	8845-c2	1435-c1r	3421-c2r	Human UniProt ID and protein name
BA001	NFVVDYYETS	N	F	V	V	D	Y	Y	E	T	S	745.9	-	-	5.3	Q8NHW4|CC4L C-C motif chemokine 4-like
BA002	NFIADYFETS	N	F	I	A	D	Y	F	E	T	S	755.4	-	-	0.4	P10147|CCL3 C-C motif chemokine 3
BA003	FFRYDFFERI	F	F	R	Y	D	F	F	E	R	I	7.0	2.5	1.0	2.2	
BA004	LFVIDSFEEL	L	F	V	I	D	S	F	E	E	L	1.1	-	0.4	4.5	
BA005	YFRVDFYEAM	Y	F	R	V	D	F	Y	E	A	M	618.4	-	1.0	-	Q5I0X7|TTC32 Tetratricopeptide repeat protein 32
BA006	ARVFDYFEGA	A	R	V	F	D	Y	F	E	G	A	244.6	0.2	4.5	0.4	Q9UPN6|SCAF8 Protein SCAF8
BA007	KFVDDLFETI	K	F	V	D	D	L	F	E	T	I	736.3	-	-	-	Q9HCM2|PLXA4 Plexin-A4
BA008	QLVVDWLESI	Q	L	V	V	D	W	L	E	S	I	756.8	-	-	-	P57740|NU107 Nuclear pore complex protein Nup10
BA009	HFILDFYEKV	H	F	I	L	D	F	Y	E	K	V	0.4	-	-	16.8	
BA010	NLVDDYFELV	N	L	V	D	D	Y	F	E	L	V	-	-	-	-	
BA014	DFIYDLFEHV	D	F	I	Y	D	L	F	E	H	V	761.2	0.2	-	-	Q9HD67|MYO10 Unconventional myosin-X
BA016	FFRNDFLEVV	F	F	R	N	D	F	L	E	V	V	1.9	-	-	-	
BA017	LFTFDLIESV	L	F	T	F	D	L	I	E	S	V	790.1	-	-	-	Q92990|GLMN Glomulin
BA019	LFIIDGFEEI	L	F	I	I	D	G	F	E	E	I	1.7	-	-	-	
BA020	YLVFDFCEHD	Y	L	V	F	D	F	C	E	H	D	3.5	0.8	-	0.7	
BA022	LFVLDYREAH	L	F	V	L	D	Y	R	E	A	H	0.4	0.2	-	-	
BA023	YLVADYLEFQ	Y	L	V	A	D	Y	L	E	F	Q	-	-	-	-	
BA027	TYRLDVLEAV	T	Y	R	L	D	V	L	E	A	V	-	-	0.4	-	
BA029	VFIVDDFESF	V	F	I	V	D	D	F	E	S	F	8.6	0.6	1.3	1.6	
BA030	WFVYDYSEPA	W	F	V	Y	D	Y	S	E	P	A	-	-	-	-	
BA031	WFIGDWLECS	W	F	I	G	D	W	L	E	C	S	198.5	-	-	-	Q9UKP5|ATS6 A disintegrin and metalloproteinase with thrombospondin motifs 6
BA032	QCIADFLEYM	Q	C	I	A	D	F	L	E	Y	M	1.5	1.9	2.5	1.0	
BA034	LCLIDYYESK	L	C	L	I	D	Y	Y	E	S	K	24.7	-	3.4	-	
BA035	QLGFDFFEAS	Q	L	G	F	D	F	F	E	A	S	0.2	-	-	-	
BA037	YFVLDTSESV	Y	F	V	L	D	T	S	E	S	V	1.1	1.7	3.9	2.8	
BA038	FIKDDYLETI	F	I	K	D	D	Y	L	E	T	I	93.0	3.9	2.8	6.9	
BA039	QCKFDLLEEL	Q	C	K	F	D	L	L	E	E	L	-	9.7	-	21.1	
BA044	TFPIDFFEHN	T	F	P	I	D	F	F	E	H	N	0.4	-	1.9	-	
BA045	ICVADPFEVT	I	C	V	A	D	P	F	E	V	T	-	2.6	1.6	14.6	
BA048	NYIYDLLEEV	N	Y	I	Y	D	L	L	E	E	V	393.3	-	-	-	Q02241|KIF23 Kinesin-like protein KIF23
BA051	FFVLDTSESV	F	F	V	L	D	T	S	E	S	V	-	-	-	9.6	
BA053	ELIFDFFEED	E	L	I	F	D	F	F	E	E	D	-	-	-	9.9	
BA058	LTVLDFFEGS	L	T	V	L	D	F	F	E	G	S	3.7	0.8	-	-	
BA060	FLVFDLWEDT	F	L	V	F	D	L	W	E	D	T	785.7	-	-	7.8	Q16816-2|PHKG1 Isoform 2 of Phosphorylase b kinase gamma catalytic chain, skeletal muscle/heart isoform
BA061	FTRHDFFESL	F	T	R	H	D	F	F	E	S	L	481.7	7.9	-	1.3	Q5THJ4|VP13D Vacuolar protein sorting-associated protein 13D
BA064	VLVADFLEQN	V	L	V	A	D	F	L	E	Q	N	772.7	21.5	3.7	1.0	Q9H9S4|CB39L Calcium-binding protein 39-like
BA066	VFVIDSSESI	V	F	V	I	D	S	S	E	S	I	3.2	6.7	0.4	-	
BA070	VFVIDSSESV	V	F	V	I	D	S	S	E	S	V	-	-	-	-	
BA084	DYLFDFFEHL	D	Y	L	F	D	F	F	E	H	L	-	-	-	3.1	
BA087	LLVLDIFEDL	L	L	V	L	D	I	F	E	D	L	22.9	-	-	-	

Figure S7. Response of Be-specific non-LKGGG CDR3β motif TCRs to biometrical analysis naturally-occurring peptides. (**A**) *TR* gene segment usage and CDR3 amino acid sequence of Be-specific T cell hybridomas expressing TCRs derived from CBD patient BAL CD4+ T cells. Amino acids encoded by the *TRBD* gene and non-germline nucleotides are indicated in red bold. The 8845-c1 TCR expresses the LKGGG CDR3β motif with an extended CDR3β length. The frequency of these αβTCR pairs relative to the total number of αβ pairs obtained is shown. (**B**) Hybridoma 8845-c1 and 3 non-LKGGG CDR3β T cell hybridoma's responses to a subset of human natural peptides identified from the biometrical analyses of the D5E8 PSL results. Peptides were tested at 5 µg/ml in the presence of $BeSO_4$ (75 µM). Green color-coding indicates a positive response, and UniProt protein sources of peptides are indicated.

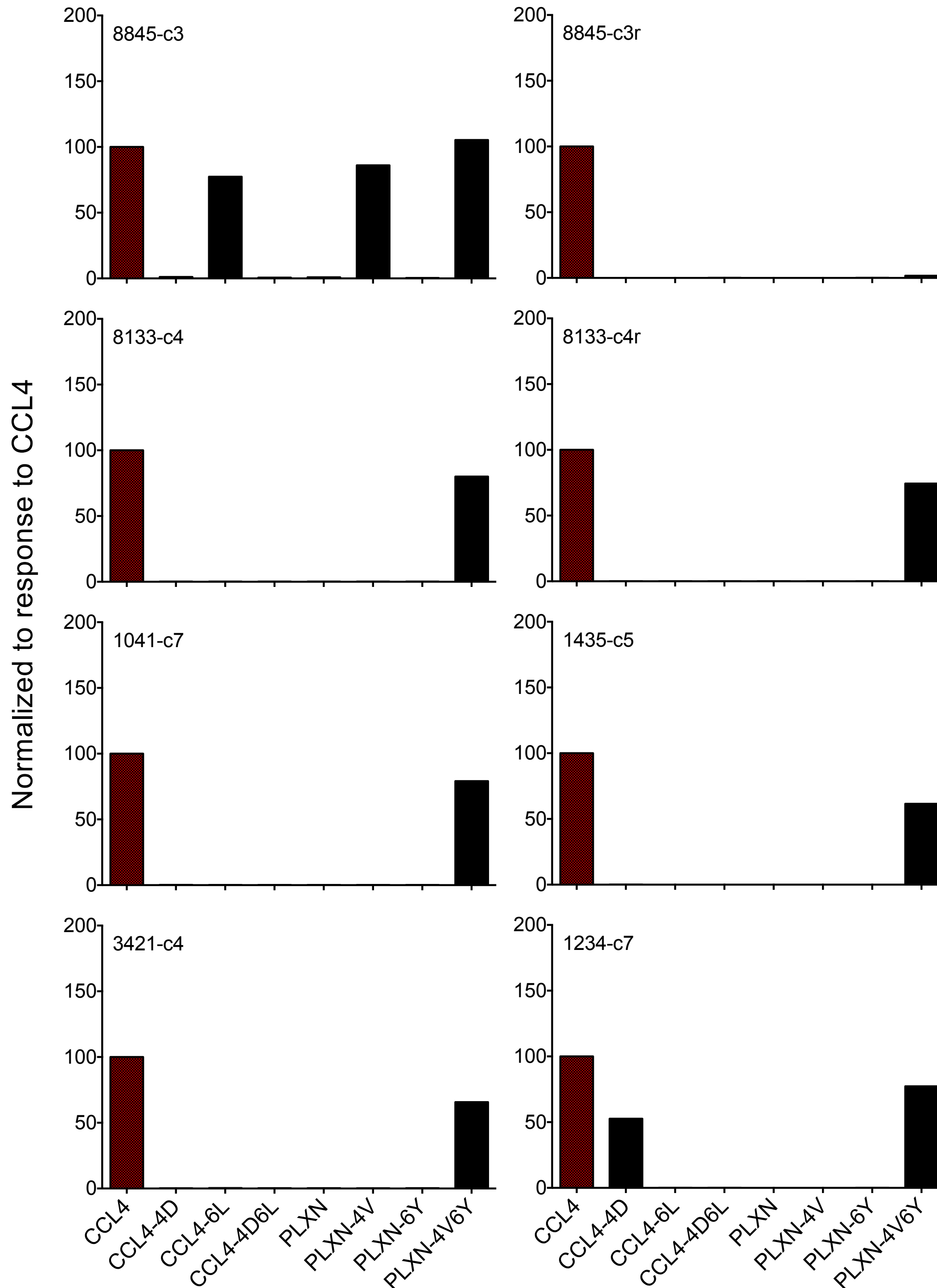


Figure S8. Investigation of chemokine/Be-specific TCRs potentially cross-reactive to plexin A/Be ligands. Eight T cell hybridomas expressing LKGGG CDR3β TCRs specific to CCL4/Be were tested for their ability to recognize PLXNA4 and variant CCL4 and PLXNA4 peptides that differ at the p4 and p6 positions. Peptides (300 ng/ml) were presented by HLA-DP2 transfected fibroblasts in the presence of $BeSO_4$ (75 μM). All data were normalized to hybridoma responses to the wild-type CCL4 peptide.

Table S1. *TRA* genes used by T cells expressing the LKGGG CDR3β motif.

Patient	TRAV	Deduced CDR3α sequence	TRAJ	Freq	Hyb ID
1041	17	C A **K L K P H H** A S G G S Y I P T F	6	5/103	1041-c7
1435	23/DV6	C A A S **T P D E K S** T A S K L T F	44	1/99	**1435-c5**
3421	14/DV4	C A M R E G **H Q D** S S A S K I I F	3	2/105	3421-c4
1234	8-6	C A V **D P T F G** G G S Q G N L I F	42	3/93	1234-c7
	3	C A V R D **G** N S G G Y Q K V T F	13	2/93	nt[1]
8133	12-2	C A V **K G S D K** Y S S A S K I I F	3	1/94	8133-c4
	17	C A T **A T P A T D** N A G N M L T F	39	1/94	nt
	13-2	C A E **K D P D R** Y S S A S K I I F	3	1/94	8133-c4r
8845	12-1	C V V **K T P V P L** N T G N Q F Y F	49	4/80	8845-c3
	13-1	C A A **S N P D K G** S S A S K I I F	3	4/80	8845-c3r
	21	C A V **N** D R G S T L G R L Y F	18	6/80	8845-c1
	10	C V V **I R** S N D Y K L S F	20	1/80	nt
	8-6	C A V **S P V** N N A R L M F	31	1/80	nt
	26-2	C I L **L S** S G T Y K Y I F	40	1/80	nt
	29/DV5	C A **V F** N A G N N R K L I W F	38	1/80	nt

[1] Hybridoma not made.

Table S2. Biometrical analysis peptides, their protein source and hybridoma IL-2 responses to peptide plus Be.

ID/Rank	Peptide	Position										TPI 2610 D5E8 Biased Library						IL-2 (pg/ml) at 1 μg/ml peptide									First human UniProt number and protein name appearing
												Overall (/18)↓		Overall 50 μg/ml (/10)		Four LKGGG Sum (/2)											
	10mer	1	2	3	4	5	6	7	8	9	10	Count	Best Rank	Count	Best Rank	Count	Best Rank	#>200	8845-c3	8845-c3r	8133-c4	8133-c4r	1041-c7	1435-c5	3421-c4	1234-c7	
BA001	NFVVDYYETS	N	F	V	V	D	Y	Y	E	T	S	18	1	10	1	2	1	8	388	420	426	365	717	694	651	602	Q8NHW4|CC4L C-C motif chemokine 4-like
BA002	NFIADYFETS	N	F	I	A	D	Y	F	E	T	S	18	1	10	1	2	1	7	431	0	422	393	730	512	665	608	P10147|CCL3 C-C motif chemokine 3
BA003	FFRYDFFERI	F	F	R	Y	D	F	F	E	R	I	18	1	10	1	2	2	2	0	0	0	283	0	12	413	0	A0A126LAV1|U7
BA004	LFVIDSFEEL	L	F	V	I	D	S	F	E	E	L	18	1	10	4	2	4	0	0	0	0	0	0	0	0	0	Q96MN2|NALP4 NACHT, LRR and PYD domains-containing protein 4
BA005	YFRVDFYEAM	Y	F	R	V	D	F	Y	E	A	M	12	1	7	1	2	4	6	434	428	0	379	702	733	687	0	Q5I0X7|TTC32 Tetratricopeptide repeat protein 32
BA006	ARVFDYFEGA	A	R	V	F	D	Y	F	E	G	A	12	1	6	1	1	11	0	0	0	0	3	0	0	0	7	Q9UPN6|SCAF8 Protein SCAF8
BA007	**KFVDDLFETI**	K	F	V	D	D	L	F	E	T	I	12	1	6	25	1	25	0	0	0	0	0	0	3	0	0	Q9HCM2|PLXA4 Plexin-A4
BA008	QLVVDWLESI	Q	L	V	V	D	W	L	E	S	I	10	1	6	1	1	3	6	451	302	0	213	621	591	271	0	P57740|NU107 Nuclear pore complex protein Nup10
BA009	HFILDFYEKV	H	F	I	L	D	F	Y	E	K	V	16	2	8	2	2	8	1	400	0	0	7	0	0	0	0	Q92674|CENPI Centromere protein I
> 1 Cys	DCIFDKFECV	D	C	I	F	D	K	F	E	C	V	15	2	8	5	2	7	0									Q00005|2ABB Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B beta isoform
BA10	NLVDDYFELV	N	L	V	D	D	Y	F	E	L	V	13	2	7	3	1	3	0	0	0	0	0	0	0	0	0	P21580|TNAP3 Tumor necrosis factor alpha-induced protein 3
No D5	LFKFPFFEAI	L	F	K	F	P	F	F	E	A	I	12	2	7	2	2	28	0									Q6ZMT4|KDM7A Lysine-specific demethylase 7A
BA011	**KFVDDLFETV**	K	F	V	D	D	L	F	E	T	V	12	2	6	14	1	18	1	7	0	0	1	1	0	330	0	P51805|PLXA3 Plexin-A3
BA012	KSVFDYFEEY	K	S	V	F	D	Y	F	E	E	Y	7	2	3	2	1	20	0	0	0	6	2	0	0	0	0	Q07075|AMPE Glutamyl aminopeptidase
BA013	ACLKDYFEIQ	A	C	L	K	D	Y	F	E	I	Q	5	2	3	2	1	19	0	8	0	0	0	0	0	0	0	Q9UBS8|RNF14 E3 ubiquitin-protein ligase
BA014	DFIYDLFEHV	D	F	I	Y	D	L	F	E	H	V	17	3	9	4	2	10	4	424	0	0	388	5	488	429	0	Q9HD67|MYO10 Unconventional myosin-X
BA015	RFTTDYFEVS	R	F	T	T	D	Y	F	E	V	S	14	3	9	3	2	5	1	6	0	0	4	2	3	620	0	Q96PH6|DB118 Beta-defensin 118
BA016	FFRNDFLEVV	F	F	R	N	D	F	L	E	V	V	13	3	7	3	2	11	0	0	0	0	148	0	0	73	0	Q8IZE3|PACE1 Protein-associating with the carboxyl-terminal domain of ezrin
BA017	LFTFDLIESV	L	F	T	F	D	L	I	E	S	V	10	3	6	3	1	5	0	141	0	0	0	120	9	0	0	Q92990|GLMN Glomulin
No E8	LCRFDYLTVV	L	C	R	F	D	Y	L	T	V	V	9	3	5	3	2	67	0									Q6PFW1|VIP1 Inositol hexakisphosphate- diphosphoinositol-pentakisphosphate kinase 1
No E8	FLIFDFLLSL	F	L	I	F	D	F	L	L	S	L	7	3	3	36	0	NA	0									B0QY84|B0QY84 Phosphatase and actin regulator
BA018	KFIVDYSETS	K	F	I	V	D	Y	S	E	T	S	4	3	3	3	1	68	6	476	0	197	271	590	278	541	549	P55774|CCL18 C-C motif chemokine 18
BA019	LFIIDGFEEI	L	F	I	I	D	G	F	E	E	I	15	4	8	6	2	6	0	0	0	0	0	0	0	0	0	Q86W25|NAL13 NACHT, LRR and PYD domains-containing protein 13
BA020	YLVFDFCEHD	Y	L	V	F	D	F	C	E	H	D	15	4	7	7	2	16	0	0	0	0	0	0	0	0	0	P50750|CDK9 Cyclin-dependent kinase 9
BA021	NFMADYFETS	N	F	M	A	D	Y	F	E	T	S	11	4	7	4	2	9	5	115	0	769	642	702	0	617	663	Q14745|Q14745 C-C motif chemokine (Fragment)
BA022	LFVLDYREAH	L	F	V	L	D	Y	R	E	A	H	13	5	7	5	2	12	0	0	0	0	0	0	0	0	0	Q8TAV6|Q8TAV6 C6orf134 protein
BA023	YLVADYLEFQ	Y	L	V	A	D	Y	L	E	F	Q	12	5	6	5	2	6	0	0	0	0	0	4	1	5	0	Q9H013|ADA19 Disintegrin and metalloproteinase domain-containing protein 19
BA024	NLKLDLLEAN	N	L	K	L	D	L	L	E	A	N	7	5	4	5	1	12	0	0	0	0	0	0	0	0	0	Q8IVI9|NOSTN Nostrin
> 1 Cys	CYVQDYLECV	C	Y	V	Q	D	Y	L	E	C	V	13	6	7	6	2	7	0									Q9H160|ING2 Inhibitor of growth protein 2
BA025	KFIVDYCEKH	K	F	I	V	D	Y	C	E	K	H	11	6	8	6	2	22	0	0	0	0	0	0	0	0	0	Q96EK5|KBP KIF1-binding protein
BA026	**KFVDDLFETL**	K	F	V	D	D	L	F	E	T	L	10	6	4	24	1	38	0	8	0	0	0	0	0	0	0	O75051|PLXA2 Plexin-A2
BA027	TYRLDVLEAV	T	Y	R	L	D	V	L	E	A	V	9	6	6	6	1	10	0	0	4	0	2	0	5	0	0	O75800|ZMY10 Zinc finger MYND domain-containing protein 10
BA028	FRVSDYFEYM	F	R	V	S	D	Y	F	E	Y	M	9	6	4	6	1	24	0	0	0	0	26	74	3	5	15	Q96NH3|BROMI Protein broad-minded
BA029	VFIVDDFESF	V	F	I	V	D	D	F	E	S	F	16	7	8	7	2	23	0	0	0	0	0	0	0	3	0	Q99715|COCA1 Collagen alpha-1(XII) chain
BA030	WFVYDYSEPA	W	F	V	Y	D	Y	S	E	P	A	11	7	5	15	1	17	0	0	0	0	9	0	5	0	0	Q5GH72|XKR7 XK-related protein 7
BA031	WFIGDWLECS	W	F	I	G	D	W	L	E	C	S	10	7	6	7	1	9	0	0	0	0	0	0	0	0	0	Q9UKP5|ATS6 A disintegrin and metalloproteinase with thrombospondin motifs 6
BA032	QCIADFLEYM	Q	C	I	A	D	F	L	E	Y	M	9	7	4	13	1	14	0	111	5	0	71	153	91	7	0	P52789|HXK2 Hexokinase-2
BA033	SFVTDIFERI	S	F	V	T	D	I	F	E	R	I	9	7	4	36	1	50	0	0	0	0	0	0	0	0	0	Q96A08|H2B1A Histone H2B type 1-A
BA034	LCLIDYYESK	L	C	L	I	D	Y	Y	E	S	K	14	8	9	8	2	16	0	0	0	0	15	11	0	0	0	Q8NHQ1|CEP70 Centrosomal protein of 70 kDa
BA035	QLGFDFFEAS	Q	L	G	F	D	F	F	E	A	S	13	8	7	8	2	34	0	0	0	0	0	0	0	0	0	P20337|RAB3B Ras-related protein Rab-3B
BA036	SFVNDIFERI	S	F	V	N	D	I	F	E	R	I	7	8	3	47	1	58	0	0	0	5	0	0	0	0	0	P57053|H2BFS Histone H2B type F-S

Table S2 cont.

ID/Rank	Peptide	Position										TPI 2610 D5E8 Biased Library						IL-2 (pg/ml) at 1 μg/ml peptide									First human UniProt number and protein name appearing
												Overall (/18)↓		Overall 50 μg/ml (/10)		Four LKGGG Sum (/2)											
	10mer	1	2	3	4	5	6	7	8	9	10	Count	Best Rank	Count	Best Rank	Count	Best Rank	#>200	8845-c3	8845-c3r	8133-c4	8133-c4r	1041-c7	1435-c5	3421-c4	1234-c7	
BA037	YFVLDTSESV	Y	F	V	L	D	T	S	E	S	V	15	9	7	9	2	98	0	1	0	0	3	1	0	0	1	P12110|CO6A2 Collagen alpha-2(VI) chain
BA038	FIKDDYLETI	F	I	K	D	D	Y	L	E	T	I	10	9	6	14	2	52	0	0	0	0	0	0	1	1	0	Q9BQI5|SGIP1 SH3-containing GRB2-like protein 3-interacting protein 1
BA039	QCKFDLLEEL	Q	C	K	F	D	L	L	E	E	L	8	9	6	9	1	18	0	0	0	0	0	0	0	0	0	Q8WWC4|MAIP1 m-AAA protease-interacting protein 1, mitochondrial
BA040	DNVKDYFECS	D	N	V	K	D	Y	F	E	C	S	7	9	3	9	1	72	0	15	0	0	5	5	4	3	3	Q07343|PDE4B cAMP-specific 3',5'-cyclic phosphodiesterase 4B
BA041	KIIADIFEYT	K	I	I	A	D	I	F	E	Y	T	4	9	3	9	1	83	1	5	0	666	0	0	4	2	0	P22033|MUTA Methylmalonyl-CoA mutase, mitochondrial
No E8	SCVVDYFLGH	S	C	V	V	D	Y	F	L	G	H	2	9	2	9	0	NA	0									Q9H857|NT5D2 5'-nucleotidase domain-containing protein 2
> 1 Cys	ICCFDSFEYV	I	C	C	F	D	S	F	E	Y	V	18	10	10	10	2	13	0									Q96S79|RSLAB Ras-like protein family member 10B
No E8	LCVLDYFIKL	L	C	V	L	D	Y	F	I	K	L	12	10	6	10	1	14	0									P06400|RB Retinoblastoma-associated protein
BA042	LCVSDPFELT	L	C	V	S	D	P	F	E	L	T	8	10	4	15	1	29	0	0	0	0	0	0	0	0	0	Q6ZMU1|C3P1 Putative protein C3P1
BA043	SVVRDYFEGS	S	V	V	R	D	Y	F	E	G	S	7	10	3	10	1	53	0	15	0	0	1	2	3	0	0	Q9NRD9|DUOX1 Dual oxidase 1
No D5	TIIYSYLESL	T	I	I	Y	S	Y	L	E	S	L	5	10	2	31	0	NA	0									Q7Z5P4|DHB13 17-beta-hydroxysteroid dehydrogenase 13
BA044	TFPIDFFEHN	T	F	P	I	D	F	F	E	H	N	14	11	8	11	2	15	0	44	0	0	0	0	2	0	0	Q5T4S7|UBR4 E3 ubiquitin-protein ligase UBR4
BA045	ICVADPFEVT	I	C	V	A	D	P	F	E	V	T	10	11	5	12	1	32	0	0	0	0	0	0	0	0	0	P01024|CO3 Complement C3
BA046	IIDIDYFEGL	I	I	D	I	D	Y	F	E	G	L	9	11	6	11	1	21	0	13	0	0	0	0	0	0	0	O95196|CSPG5 Chondroitin sulfate proteoglycan 5
No D5	FFIFYYLEGT	F	F	I	F	Y	Y	L	E	G	T	6	11	2	19	0	NA	0									O75175|CNOT3 CCR4-NOT transcription complex subunit 3
BA047	KLSLDYFEKQ	K	L	S	L	D	Y	F	E	K	Q	4	11	4	11	1	96	0	10	0	0	0	0	2	0	0	Q9NQZ6|ZC4H2 Zinc finger C4H2 domain-containing protein
BA048	NYIYDLLEEV	N	Y	I	Y	D	L	L	E	E	V	9	12	5	12	1	22	1	406	0	0	86	0	11	25	0	Q02241|KIF23 Kinesin-like protein KIF23
No D5	FLVFFFFERV	F	L	V	F	F	F	F	E	R	V	7	12	2	29	0	NA	0									Q8N7Y7|Q8N7Y7 cDNA FLJ40209 fis, clone TESTI2020999
BA049	SFVNDVFEQL	S	F	V	N	D	V	F	E	Q	L	7	12	3	49	1	81	0	0	0	4	0	0	0	0	0	L0R4T3|L0R4T3 Histone H2B
BA050	GVIYDLLECL	G	V	I	Y	D	L	L	E	C	L	5	12	3	29	1	57	0	0	0	0	0	0	0	0	0	Q9NV88|INT9 Integrator complex subunit 9
BA051	FFVLDTSESV	F	F	V	L	D	T	S	E	S	V	12	13	4	17	0	NA	0	0	0	0	0	0	0	0	0	P12109|CO6A1 Collagen alpha-1(VI) chain
BA052	GFVIDYTENP	G	F	V	I	D	Y	T	E	N	P	8	13	4	13	1	56	0	12	0	0	0	2	0	0	0	Q7Z408|CSMD2 CUB and sushi domain-containing protein 2
No E8	SLVFDYYNSV	S	L	V	F	D	Y	Y	N	S	V	7	13	2	22	0	NA	0									Q7Z3S7|CA2D4 Voltage-dependent calcium channel subunit alpha-2/delta-
BA053	ELIFDFFEED	E	L	I	F	D	F	F	E	E	D	7	13	2	66	1	70	1	34	0	0	142	603	0	0	0	Q9NPB8|GPCP1 Glycerophosphocholine phosphodiesterase GPCPD1
BA054	EKKIDYFERA	E	K	K	I	D	Y	F	E	R	A	3	13	3	13	1	64	0	15	0	0	0	0	0	2	0	Q14152|EIF3A Eukaryotic translation initiation factor 3 subunit A
BA055	NCVTDEFEEG	N	C	V	T	D	E	F	E	E	G	3	13	1	13	0	NA	0	14	0	3	4	4	4	3	0	Q9UL15|BAG5 BAG family molecular chaperone regulator 5
BA056	LDILDYYEAS	L	D	I	L	D	Y	Y	E	A	S	7	14	4	14	1	59	0	0	0	0	0	0	0	0	0	P78352-2|DLG4 Isoform 2 of disks large homolog 4
No E8	TYVLDYLKST	T	Y	V	L	D	Y	L	K	S	T	4	14	2	51	0	NA	0									Q6IE37|OVOS1 Ovostatin homolog 1
No D5	STVFLYFESV	S	T	V	F	L	Y	F	E	S	V	4	14	2	76	0	NA	0									Q9HC24|LFG4 Protein lifeguard 4
BA057	KIKEDYFEKH	K	I	K	E	D	Y	F	E	K	H	3	14	3	14	1	83	0	9	0	0	0	0	2	2	0	Q9H3M9|ATX3L Ataxin-3-like protein
> 1 Cys	DCIFDKFECA	D	C	I	F	D	K	F	E	C	A	11	15	5	18	1	26	0									Q9Y2T4|2ABG Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B gamma isoform
BA058	LTVLDFFEGS	L	T	V	L	D	F	F	E	G	S	9	15	4	15	1	52	0	0	0	0	0	0	0	0	0	Q9NRD8|DUOX2 Dual oxidase 2
BA059	QLTADYFEKT	Q	L	T	A	D	Y	F	E	K	T	8	15	8	15	2	30	0	11	0	0	0	0	0	0	0	Q9Y6B7|AP4B1 AP-4 complex subunit beta-1
BA060	FLVFDLWEDT	F	L	V	F	D	L	W	E	D	T	7	15	4	15	1	23	3	432	0	0	398	73	673	0	0	Q16816-2|PHKG1 Isoform 2 of Phosphorylase b kinase γ catalytic chain, skeletal muscle/heart isoform
BA061	FTRHDFFESL	F	T	R	H	D	F	F	E	S	L	7	15	2	76	0	NA	0	0	0	0	82	0	1	156	0	Q5THJ4|VP13D Vacuolar protein sorting-associated protein 13D
BA062	TLLFDFLEVC	T	L	L	F	D	F	L	E	V	C	5	15	4	15	1	24	0	0	0	3	0	3	0	0	0	Q53GS7|GLE1 Nucleoporin GLE1
BA063	PPHIDYFEEI	P	P	H	I	D	Y	F	E	E	I	5	15	3	15	1	74	0	10	0	0	0	0	0	0	0	Q9UBE8|NLK Serine/threonine-protein kinase NLK
> 1 Cys	DCIFDKFECC	D	C	I	F	D	K	F	E	C	C	9	16	5	35	1	35	0									Q66LE6|2ABD Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B delta isoform
BA064	VLVADFLEQN	V	L	V	A	D	F	L	E	Q	N	7	16	3	16	1	19	0	198	0	0	1	2	3	0	0	Q9H9S4|CB39L Calcium-binding protein 39-like
BA065	FLLTDYFEED	F	L	L	T	D	Y	F	E	E	D	4	16	4	16	1	71	2	8	0	28	17	478	4	0	683	Q8IVI9|NOSTN Nostrin
BA066	VFVIDSSESI	V	F	V	I	D	S	S	E	S	I	10	17	2	33	0	NA	0	0	0	0	0	0	0	0	0	P12110|CO6A2 Collagen alpha-2(VI) chain
No D5	LFIFALFETI	L	F	I	F	A	L	F	E	T	I	6	17	3	34	0	NA	0									Q8NHS3|MFSD8 Major facilitator super-family domain-containing protein 8

Table S2 cont.

ID/Rank	Peptide	Position										TPI 2610 D5E8 Biased Library						IL-2 (pg/ml) at 1 μg/ml peptide									First human UniProt number and protein name appearing
												Overall (/18)↓		Overall 50 μg/ml (/10)		Four LKGGG Sum (/2)		#>200	8845-c3	8845-c3r	8133-c4	8133-c4r	1041-c7	1435-c5	3421-c4	1234-c7	
	10mer	1	2	3	4	5	6	7	8	9	10	Count	Best Rank	Count	Best Rank	Count	Best Rank										
BA067	VKIKDYFEKL	V	K	I	K	D	Y	F	E	K	L	5	17	3	22	1	46	0	12	0	0	0	0	2	2	2	P04114|APOB Apolipoprotein B-100
BA068	HFVCDNFEQF	H	F	V	C	D	N	F	E	Q	F	5	17	3	58	1	88	0	11	0	0	4	4	2	4	2	A0A126LAV1|A0A126LAV1 U7
BA069	PHREDYFEPI	P	H	R	E	D	Y	F	E	P	I	4	17	2	17	1	93	0	9	0	0	0	0	1	2	0	Q69YN4|VIR Protein virilizer homolog
No E8	FLKLDYFQNL	F	L	K	L	D	Y	F	Q	N	L	2	17	1	17	0	NA	0									Q5VWM4|PRAM8 PRAME family member 8
BA070	VFVIDSSESV	V	F	V	I	D	S	S	E	S	V	11	18	3	21	0	NA	0	0	0	0	0	0	0	0	0	Q2UY09|COSA1 Collagen alpha-1(XXVIII) chain
BA071	ILPNDYFEIV	I	L	P	N	D	Y	F	E	I	V	7	18	5	18	1	34	0	11	0	0	0	0	3	0	0	Q9NVV9|THAP1 THAP domain-containing protein 1
BA072	VLLHDFLEDV	V	L	L	H	D	F	L	E	D	V	4	18	3	18	1	26	0	0	0	0	0	0	0	0	0	Q6ZV50|RFX8 DNA-binding protein RFX8
No D5	LLVYSYFEKS	L	L	V	Y	S	Y	F	E	K	S	4	18	2	18	0	NA	0									M0R1H8|M0R1H8 Zinc finger protein 431 (Fragment)
BA073	VCILDVYENM	V	C	I	L	D	V	Y	E	N	M	4	18	3	51	1	51	0	0	0	0	0	0	0	0	0	Q16644|MAPK3 MAP kinase-activated protein kinase 3
BA074	KLILDIFEYE	K	L	I	L	D	I	F	E	Y	E	2	18	1	18	0	NA	0	9	0	0	0	0	0	0	0	Q8IZF2|AGRF5 Adhesion G protein-coupled receptor F5
BA075	VIIFDALEQL	V	I	I	F	D	A	L	E	Q	L	7	19	4	19	1	39	0	1	0	0	1	0	108	3	0	Q9ULI1|NWD2 NACHT and WD repeat domain-containing protein 2
BA076	PFSFDFFEDP	P	F	S	F	D	F	F	E	D	P	7	19	3	19	1	47	3	707	0	0	382	31	0	359	0	O75190|DNJB6 DnaJ homolog subfamily B member 6
BA077	FCFVDLYEAQ	F	C	F	V	D	L	Y	E	A	Q	5	19	3	26	1	40	0	0	0	5	0	0	0	0	0	V9GZL3|V9GZL3 GBP
> 1 Cys	QCCIDNFEEI	Q	C	C	I	D	N	F	E	E	I	4	19	2	19	1	80	0									Q96T49|PP16B Protein phosphatase 1 regulatory inhibitor subunit 16B
BA078	LLLIDFYEKT	L	L	L	I	D	F	Y	E	K	T	8	20	5	20	1	28	0	14	3	0	0	0	0	0	0	Q7Z6Z7|HUWE1 E3 ubiquitin-protein ligase HUWE1
BA079	TCIKDEFEKI	T	C	I	K	D	E	F	E	K	I	7	20	2	37	1	39	0	0	0	0	0	0	0	0	0	A0A126LAY5|A0A126LAY5 Glycoprotein B
No E8	LYIIDFFIAL	L	Y	I	I	D	F	F	I	A	L	6	20	3	25	1	86	0									Q9P241|AT10D Probable phospholipid-transporting ATPase VD
BA080	GLLIDYFEKK	G	L	L	I	D	Y	F	E	K	K	4	20	4	20	1	40	0	12	0	0	0	0	1	0	0	O00237|RN103 E3 ubiquitin-protein ligase RNF103
No D5E8	LFVFNFFFWV	L	F	V	F	N	F	F	F	W	V	4	20	2	20	0	NA	0									A1L157|TSN11 Tetraspanin-11
BA081	QLLVDFWEAQ	Q	L	L	V	D	F	W	E	A	Q	3	20	3	20	1	31	1	74	0	0	0	383	0	0	0	Q969F9|HPS3 Hermansky-Pudlak syndrome 3 protein
BA082	LEEGDYFEAI	L	E	E	G	D	Y	F	E	A	I	3	20	3	20	1	51	0	8	0	0	0	0	0	0	0	Q96JQ2|CLMN Calmin
BA083	SCVEDGFEGD	S	C	V	E	D	G	F	E	G	D	3	20	1	26	0	NA	0	11	0	0	0	7	0	0	112	P42684|ABL2 Abelson tyrosine-protein kinase 2
BA084	DYLFDFFEHL	D	Y	L	F	D	F	F	E	H	L	11	21	5	30	2	36	1	1	0	0	203	94	0	0	0	Q9NPP4|NLRC4 NLR family CARD domain-containing protein 4
BA085	TCPVDPFEAQ	T	C	P	V	D	P	F	E	A	Q	1	21	1	21	0	NA	0	13	0	0	2	1	1	0	2	P49757|NUMB Protein numb homolog
No D5	FFIFLLLEAV	F	F	I	F	L	L	L	E	A	V	7	22	3	22	0	NA	0									L0R8E0|L0R8E0 Alternative protein RPIA
BA086	LFYGDFLEQL	L	F	Y	G	D	F	L	E	Q	L	5	22	4	22	1	32	0	0	0	7	0	0	0	0	0	H7C1G2|H7C1G2 Cordon-bleu protein-like 1 (Fragment)
No E8	FIILDWFHAI	F	I	I	L	D	W	F	H	A	I	4	22	1	43	0	NA	0									Q53QZ3|RHG15 Rho GTPase-activating protein 15
> 1 Cys	ACLVDFFTNC	A	C	L	V	D	F	F	T	N	C	1	22	1	22	0	NA	0									P49902|5NTC Cytosolic purine 5'-nucleotidase
BA087	LLVLDIFEDL	L	L	V	L	D	I	F	E	D	L	12	23	7	23	2	25	0	145	0	0	0	0	0	0	0	Q92911|SC5A5 Sodium/iodide cotransporter
BA088	LIILDTLEIV	L	I	I	L	D	T	L	E	I	V	5	23	4	23	1	37	0	0	0	2	1	0	0	0	0	Q96N67|DOCK7 Dedicator of cytokinesis protein 7
BA089	WLYFDALECL	W	L	Y	F	D	A	L	E	C	L	5	23	3	23	1	48	0	0	0	0	0	0	0	0	0	P22314|UBA1 Ubiquitin-like modifier-activating enzyme 1
BA090	GFVDDLLEAL	G	F	V	D	D	L	L	E	A	L	4	23	0	NA	0	NA	0	0	0	9	1	0	0	2	0	Q8IWB1|IPRI Inositol 1,4,5-trisphosphate receptor-interacting protein
BA091	HKISDYFEYQ	H	K	I	S	D	Y	F	E	Y	Q	3	23	3	23	1	57	0	16	0	0	0	2	0	3	0	Q9UKI8|TLK1 Serine/threonine-protein kinase tousled-like 1
No E8	FLKLDYCRSN	F	L	K	L	D	Y	C	R	S	N	3	23	1	29	0	NA	0									A0A126GW04|A0A126GW04 Olfactory receptor
BA092	LFIMDGFEQL	L	F	I	M	D	G	F	E	Q	L	5	24	3	44	1	44	0	0	0	0	0	0	0	0	0	Q7RTR0|NALP9 NACHT, LRR and PYD domains-containing protein 9
BA093	FLLLDALEAA	F	L	L	L	D	A	L	E	A	A	4	24	3	24	1	45	0	0	0	0	0	0	0	0	0	B2RA97|B2RA97 HemK methyltransferase family member 2
BA094	ETIKDYFEAR	E	T	I	K	D	Y	F	E	A	R	2	24	2	24	0	NA	0	14	0	0	0	0	1	0	0	Q99549|MPP8 M-phase phosphoprotein 8
BA095	VFQQDCFEYF	V	F	Q	Q	D	C	F	E	Y	F	2	24	2	24	0	NA	0	14	0	0	0	0	0	0	0	O15397|IPO8 Importin-8
BA096	NWIGDYFEKA	N	W	I	G	D	Y	F	E	K	A	6	25	6	25	2	42	0	10	0	0	0	0	0	0	0	Q8NCM8|DYHC2 Cytoplasmic dynein 2 heavy chain 1
BA097	FLTYDICEVS	F	L	T	Y	D	I	C	E	V	S	5	25	3	25	1	44	0	0	0	0	0	0	0	0	0	Q8IY18|SMC5 Structural maintenance of chromosomes protein 5
BA098	HFSEDYLECV	H	F	S	E	D	Y	L	E	C	V	4	25	2	25	1	49	0	0	0	3	2	0	0	2	0	Q9Y625|GPC6 Glypican-6
BA099	LTRLDFLEWP	L	T	R	L	D	F	L	E	W	P	2	25	2	25	0	NA	0	16	0	0	1	2	2	1	0	B4DKJ8|B4DKJ8 Oxysterol-binding protein
BA100	KLLLDTFEYQ	K	L	L	L	D	T	F	E	Y	Q	1	25	1	25	0	NA	0	14	4	8	2	4	3	2	2	Q7Z7K6|CENPV Centromere protein V

Table S3. Mean EC50 values (nM) of two experiments for 4 hybridomas to CCL4 length variant peptides.

CCL4 Peptide	Length	EC50 values (nM)			
		8845-c3	8845-c3r	8133-c4	8133-c4r
RNFVVDYYETS	11-mer	180.5	173.5	198.5	61.8
NFVVDYYETSS	11-mer	318.3	85.9	160.4	56.1
NFVVDYYETS	10-mer	164.6	106.5	156.0	45.3
FVVDYYETSS	10-mer	113.5	43.1	61.4	16.3
NFVVDYYET	9-mer	352.1	171.7	376.6	100.6
FVVDYYETS	9-mer	79.1	65.7	62.7	19.1
NFVVDYYE	8-mer	448.7	573.2	468.4	180.3
FVVDYYET	8-mer	212.7	167.6	183.9	49.2
FVVDYYE	7-mer	474.2	1189.0	429.0	222.2
FVVDYY	6-mer	nd[1]	nd	nd	nd
VVDYYETS	8-mer	nd	nd	nd	nd

[1] nd: not determined due to low responses to peptides at concentrations used.

Table S4. Mean EC50 values (nM) of two experiments for 4 hybridomas to CCL3 length variant peptides.

CCL3 Peptide	Length	EC50 values (nM)			
		8845-c3	8845-c3r	8133-c4	8133-c4r
NFIADYFETSS	11-mer	74.2	neg[1]	114.4	43.1
NFIADYFETS	10-mer	99.4	neg	114.6	49.7
FIADYFETSS	10-mer	46.9	neg	58.4	30.8
FIADYFETS	9-mer	77.6	neg	76.3	52.6
FIADYFET	8-mer	118.7	neg	142.8	105.6

[1] neg: no detectable response at highest concentration of peptide tested.

Table S5. Demographics of CBD study population.

Patient[1]	Age (yrs)	HLA-DPB1 alleles	Time from diagnosis	Percentage lymphocytes[2]	BeLPT (BAL)[3]	BeLPT (PBMC)[3]
BAL T cell lines[4]						
1041	62	*02:01/*04:01	22 yrs, 9 mo	52.5	ABNL[5]	ABNL
1435	35	*02:01/*17:01	< 1 month	78.0	ABNL	NL
3421	56	*02:01/*04:01	< 1 month	58.1	ABNL	NL
Ex vivo BAL T cells[4]						
1234	54	*02:01/*13:01	3 yrs, 7 mo	2.7	ABNL	ABNL
8133	62	*02:01/*04:02	2 yrs	59.0	ABNL	NL
8845	55	*01:01/*02:01	< 1 month	14.3	ABNL	ABNL
6092	68	*02:01/*04:02	7 yrs, 3 mos	5.6	NL	NL

[1] Six nonhispanic males and one hispanic female (1234).

[2] Percentage of collected BAL cells that are lymphocytes.

[3] LPT, Lymphocyte Proliferation Test is considered abnormal if two or more of six conditions tested are greater than a mean stimulation index of 2.5.

[4] Number of $CD4^+$ T cells sorted: BAL T cell lines = 143; ex vivo BAL T cells = 191.

[5] ABNL - abnormal result; NL - normal result.