Supporting Information

Holland et al. 10.1073/pnas.1210882109



Fig. S1. Amino acid use within germ-line complementarity determining regions (CDRs) of mouse and human T-cell receptor (TCR) and Ig variable gene segments. CDR1 (*A*) and CDR2 (*B*) are shown. The percentage use of each amino acid at each position is represented by color intensity. Amino acids are grouped according to their chemical properties as indicated on the *y* axis. Variable (V)-segments that encode CDRs of the most common length out of the total number present in the genome were analyzed. (C) Diversity (H) values for each CDR (using the most common length) were calculated using Shannon entropy analysis in conjunction with the Protein Variability Server for each position. Error bars represent SEM. Sequences are from ImMunoGeneTics (www.imgt.org).



Fig. 52. In vitro and in vivo expression of mutant C6 T-cell receptor (TCR)- β chains. (*A*) Schematic depicts the eight mutations introduced into the TCR- β chain. The seven amino acid substitutions made in the TCR- β germ-line complementarity determining region (CDR) 1 and CDR2 and the deleted framework tyrosine residue (Y54 based on ImMunoGeneTics nomenclature) adjacent to CDR2 are highlighted in the aligned $\Delta\beta$ CDR3 and $\Delta\beta$ CDR1/2/3 sequences. Both $\Delta\beta$ CDR3 and $\Delta\beta$ CDR1/2/3 contain a minimal triple-glycine CDR3 region flanked by framework variable- and joining-segment residues (1). (*B*) Flow cytometric analysis of mutated TCR- β chain constructs transduced into Con A-activated splenocytes. Plots demonstrate expression of both retrogenic TCR chains detected with anti-TCR V β 11 antibody on gated GFP⁺CD4⁺CD8⁻ cells (blue line) relative to GFP⁺CD4⁻CD8⁻ cells (filled plot). The same gating applied to the empty vector control (pMigR1) is also shown. (*C*) (*Left*) Flow cytometric detection of GFP⁺ T cells expressing the exogenous TCR- β chains in blood. CD4 T cells, CD8 T cells, and $\Delta\beta$ CDR1/2/3 (*n* = 8) chains. Error bars represent SEM. NS, not significant. (*D*) Representative plots (*Left*) and summary (*Right*) of flow cytometric assessment of CD44 expression on splenic GFP⁺ retrogenic CD4 and CD8 T cells. ****P* < 0.0001. Data shown are collated from several independent batches of retrogenic mice.

1. Bartok I, et al. (2010) T cell receptor CDR3 loops influence alphabeta pairing. Mol Immunol 47(7-8):1613-1618.



Fig. S3. In vitro and in vivo expression of mutant C6 T-cell receptor (TCR)- α chains. (*A*) (*Upper*) Schematic highlighting the 10 amino acid substitutions introduced into the C6 TCR- α germ-line complementarity determining regions (CDRs) in the aligned α WT and $\Delta\alpha$ CDR1/2 sequences. (*Lower*) Flow cytometry of WT and mutant TCR- α chain constructs transduced into Con A-activated splenocytes is shown. Plots demonstrate expression of the α WT but not the $\Delta\alpha$ CDR1/2 retrogenic TCR chain as detected with anti-TCR V α 8.3 antibody on gated GFP⁺CD4⁺CD8⁻ T cells (blue line) relative to GFP⁺CD4⁻CD8⁻ cells (filled plot). The same gating applied to the empty vector control (pMigR1) is also shown. Failure to detect the $\Delta\alpha$ CDR1/2 chain may be due to loss of the epitope recognized by the V α 8.3 (B21.14) antibody, which is known to involve the germ-line CDRs (1). The construct was therefore used to produce retrogenic mice because selection of Legend continued on following page

T cells on a TCR- $\alpha^{-/-}$ background would infer expression of the $\Delta \alpha$ CDR1/2 chain. (*B*) GFP⁺ CD4 and CD8 T cells were detected in blood by staining the α WT chain with V α 8.3 and TCRs containing the mutant $\Delta \alpha$ CDR1/2 chain with a pan-TCR β antibody. (*Left*) Red and blue lines and filled plots represent CD4 T cells, CD8 T cells, and CD4⁻CD8⁻ cells, respectively. (*Right*) Total GFP⁺ T cells in the spleen of α WT and $\Delta \alpha$ CDR1/2 retrogenic mice were not significantly different (*n* = 4 for α WT, *n* = 5 for $\Delta \alpha$ CDR1/2). Error bars represent SEM. NS, not significant. (*C*) GFP⁺ retrogenic CD4 and CD8 T-cell representation in spleen and lymph nodes of mice expressing α WT and $\Delta \alpha$ CDR1/2 chains selected on H2^q and H2^k haplotypes. Representative plots (*Lower*) and summary dot plots (*Upper*) show the percentage of GFP⁺ CD4 T cells within the total T-cell population. Mice were analyzed 10–12 wk post-hematopoietic stem cell transfer. **P* < 0.05. Data shown are combined from several independent batches of retrogenic mice.

1. Brodnicki TC, Holman PO, Kranz DM (1996) Reactivity and epitope mapping of single-chain T cell receptors with monoclonal antibodies. Mol Immunol 33(3):253-263.



Fig. S4. Mutated T-cell receptor (TCR) chains direct development of T cells with a regulatory phenotype. Flow cytometric analysis of splenocytes from mice expressing the TCR α WT, $\Delta\alpha$ CDR1/2, $\Delta\beta$ CDR3, or $\Delta\beta$ CDR1/2/3 chain is shown. Lymphocytes were gated on CD4⁺ cells and analyzed for FoxP3 and CD25 expression.

Construct	Peptide	CDR length	Nucleotide (no. of sequences)
CDR2 WT	WTP	3	TGGACTCCT (1)
	AWAP	4	GCGTGGGCTCCT (4)
	FPAP	4	TTTCCCGCTCCT (1)
	FRAP	4	TTTCGTGCTCCT(1)
	VLPP	4	GTACTTCCTCCT (1)
	FRKAP	5	TTTCGGAAAGCTCCT (1)
	LKQAP	5	TTAAAGCAAGCTCCT (9)
	LSLAP	5	TTATCTCTAGCTCCT (2)
	LSQAP	5	TTATCTCAAGCTCCT (1)
	SRQAP	5	TCCCGTCAAGCTCCT (2)
	FLTGAP	6	TTCCTGACCGGGGCTCCT (1)
	FRAQAP	6	TTTCGTGCCCAAGCTCCT (6)
	FRDQAP	6	TTTCGAGACCAAGCTCCT (3)
	FRFQAP	6	TTTCGTTTTCAAGCTCCT (12)
			TTTCGTTTTCAGGCTCCT (1)
	FRGQAP	6	TTTCGGGGGCAAGCTCCT (1)
		6	TTTCGTGGTCAAGCTCCT (1)
	FRHQAP	6	TTTCGGCATCAAGCTCCT (3)
	FRIQAP	6	TTTCGAATTCAAGCTCCT (1)
	FRIKAP	6	TTTTCGAATTCCGAGCTCCT (1)
	FKKGAP	6	TTTCGAAAGGGAGCTCCT (3)
		c	TTTTCGAAAGGGGGCTCCT (6)
	FRKKAP	6	TTTTCGAAAGAAAGCTCCT (8)
	FRKQAP	6	TTTTCGAAAACAAGCTCCT (3)
	FKKKAP	6	TTTCGAAAGAGGGCTCCT (1)
		c	TITUGAAAGUGAGUTUUT (1)
		6	TTTCGAAAGTGGGGGTCCT (1)
	FREQAF	0	mmmcchcmmchhccmccm (2)
			TITCGACITCAAGCICCI (3)
	FRMAAP	6	TITCGGCIGCAAGCICCI (1)
	FRMLAP	6	TTTCGAATGGCGGCTCCT (1)
	FRMOAP	6	TTTCGAATGCIGGCICCI (3)
	FRNFAP	6	TTTCGAAATGAAGCTCCT (3)
		· ·	TTTCGAAATGAGGCTCCT (1)
	FRNGAP	6	TTTCGAAACGGGGCTCCT (2)
			TTTCGAAATGGAGCTCCT (11)
			TTTCGAAATGGCGCTCCT (1)
			TTTCGAAATGGGGCTCCT (36)
I	FRNKAP	6	TTTCGAAATAAAGCTCCT (7)
	FRNKGP	6	TTTCGAAATAAAGGCCCT (1)
	FRNLAP	6	TTTCGAAATCTGGCTCCT (1)
			TTTCGAAATTTGGCTCCT (3)
	FRNMAP	6	TTTCGAAATATGGCTCCT (1)
	FRNQAP	6	TTTCGAAATCAAGCTCCT (11)
	FRNRAP	6	TTTCGAAACCGGGCTCCT (4)
			TTTCGAAATAGAGCTCCT (2)
			TTTCGAAATAGGGCTCCT (6)
			TTTCGAAATCGAGCTCCT (5)
			TTTCGAAATCGCGCTCCT (4)
			TTTCGAAATCGGGCTCCT (4)
	FRNRTP	6	TTTCGAAATCGGACTCCT (1)
	FRNSAP	6	TTTCGAAATAGCGCTCCT (2)
		-	TTTCGAAATTCGGCTCCT (2)
	FRNTAP	6	TTTCGAAATACGGCTCCT (2)
	FRNWAP	6	TTTCGAAATTGGGCTCCT (7)
	FRQQAP	6	TTTCGACAGCAAGCTCCT (1)
	EDDC (D	~	TTTCGGCAACAAGCTCCT (1)
	FKKGAP	6	TTTTCGAAGGGGAGCTCCT (2)
	FKRQAP	6	TTTTCGAAGACAAGCTCCT (4)
			TTTTCGAAGGCAAGCTCCT (1)
		C	TTTCGGCGGCAAGCTCCT (1)
		6	
	LUDAAL	Ö	IIIUGGAGUGUAGUIUUT (I)

Table Shi Hon gennine este and este matant sequences generated in the	Table S1.	Non-germ-line CDR	1 and CDR2 mutant se	equences generated in vive
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Construct	Peptide	CDR length	Nucleotide (no. of sequences)
	FRSGAP	6	TTTCGATCGGGAGCTCCT (1)
	FRSQAP	6	TTTCGAAGCCAAGCTCCT (1)
			TTTCGAAGTCAAGCTCCT (3)
			TTTCGATCACAAGCTCCT (1)
			TTTCGATCTCAAGCTCCT (1)
			TTTCGTAGTCAAGCTCCT (1)
	FRTGAP	6	TTTCGAACGGGGGGCTCCT (1)
	FRVQAP	6	TTTCGGGTGCAAGCTCCT (13)
	FRVRAP	6	TTTCGGGTCCGGGCTCCT (1)
	FRYQAP	6	TTTCGGTATCAAGCTCCT (3)
	FTWQAP	6	TTTACGTGGCAAGCTCCT (1)
	HLRGAP	6	CACCTCAGGGGCGCTCCT (1)
		6	CTTCGAAATCGGGCTCCT (1)
	SILQAP	6	TCGACCTTGCAAGCTCCT (1)
	FQTQAP	7	TTCCAAACGCAAGCTCCT (1)
	FRGMQAP	7	TTTCGAGGGATGCAAGCTCCT (1)
	FRKGQAP	7	TTTCGAAAGGGGCAAGCTCCT (1)
		7	TTTCGAAAAAAAAAAGCTCCT (1)
		7	TTTCGAAATATAGGGGCTCCT (1)
		7	TTTCGTAACTCTCAAGCTCCT (2)
		7	
		7	
	FRIDGAP	7	TITCGAAGGGGGGAAAGCICCI (3)
	FRIHPOAP	8	
	FRIPGGAP	8	TITCGAATCCACCECCAAGCICCI (1)
		8	
	FRNIPGAP	8	TTTCGAAATATCCCACCCCCTCCT (1)
	FRNIRGAP	8	TTTCGAAATATAAGGGGAGCTCCT (2)
	FRNISKAP	8	TTTCGAAATATTTCCAAAGCTCCT (2)
	FRNIVGAP	8	TTTCGAAATATCGTAGGGGCTCCT (3)
	FRNPI RAP	8	TTTCGAAATCCCTTGCGAGCTCCT (1)
	FRTPLOAP	8	TTTCGAACCCCTTTGCAAGCTCCT (2)
	FRTSLOAP	8	TTTCGAACCTCCCTCCAAGCTCCT (2)
	FLTSTLOAP	9	TTCCTGACTTCGACCTTGCAAGCTCCT (1)
	FRKRPLOOAP	10	TTTCGAAAGAGACCCCTCCAACAAGCTCCT (6
CDR1 WT	SGHHV	5	TCAGGACACCACGTT (5)
	SGHKV	5	TCAGGACATAAGGTT (2)
	SGHNV	5	TCAGGACATAATGTT (2)
	SGHRV	5	TCAGGACATAGAGTT (3)
			TCAGGACATAGGGTT (6)
	SGHSR	5	TCAGGACATAGTCGG (1)
	SGHSV	5	TCAGGACATAGTGTT (5)
	SGHTV	5	TCAGGACATACCGTT (1)
	SGLHV	5	TCAGGACTCCACGTT (1)
	SGQAV	5	TCAGGACAAGCTGTT (2)
			TCAGGACAGGCTGTT (1)
	SGQNV	5	TCAGGACAAAATGTT (2)
	SGRHA	5	TCAGGACGCCATGCT (1)
	SRRAV	5	TCACGTCGAGCTGTT (1)
	CIIVAV	6	TGTATCATTGTGGCTGTT (1)
	CNIVAV	6	TGTAACATTGTGGCTGTT (1)
	SGHFAV	6	AGTGGACATTTTGCTGTT (1)
	SGHHAV	6	TCAGGACACCACGCTGTT (3)
	SGHNGV	6	TCAGGACATAACGGTGTT (2)
	SGHNHV	6	TCAGGACATAACCATGTT (1)
	SGHPAV	6	TCAGGACATCCCGCTGTT (1)
	SGHRAV	6	TCAGGACATAGAGCTGTT (1)
			TCAGGACATAGAGCTGTT (22)
			TCAGGACATAGGGCTGTT (6)
	SGHRDV	6	TCAGGACATAGAGATGTT (1)
			TCAGGACATAGGGATGTT (1)
	SGHRGV	6	TCAGGACATAGAGGTGTT (2)
	SGHRSV	6	TCAGGACATAGAAGTGTT (2)

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Table S1.	Cont.		
Construct	Peptide	CDR length	Nucleotide (no. of sequences)
			TCAGGACATAGATCTGTT (5)
			TCAGGACATAGGTCTGTT (1)
	SGHRTV	6	TCAGGACATAGAACTGTT (1)
	SGHRWV	6	TCAGGACATAGATGGGTT (1)
	SGHSAV	6	TCAGGACATAGCGCTGTT (2)
			TCAGGACATTCCGCTGTT (1)
	SGHSGV	6	TCAGGACATAGTGGGGTT (4)
	SGHSHV	6	TCAGGACATTCCCATGTT (1)
	SGHSKV	6	TCAGGACATAGTAAAGTT (1)
	SGHSNV	6	TCAGGACATAGTAATGTT (1)
	SGHSRV	6	TCAGGACATAGCCGGGTT (1)
			TCAGGACATAGTAGGGTT (4)
		_	TCAGGACATAGTCGCGTT (4)
	SGHSSV	6	TCAGGACATAGTAGTGTT (1)
	SGHSTV	6	TCAGGACATAGTACCGTT (4)
		_	TCAGGACATAGTACTGTT (6)
	SGHTAV	6	TCAGGACATACCGCTGTT (2)
	SGLCVF	6	TCAGGACTTTGTGTTTTC (1)
	SGQIRV	6	TCAGGACAAATCCGTGTT (2)
	SGQNEV	6	TCAGGACAGAACGAGGTT (1)
	SGQTSV	6	TCAGGACAAACCTCTGTT (2)
	SGSPDV	6	TCAGGATCCCCTGACGTT (3)
	SGSRAV	6	TCAGGATCTCGCGCTGTT (1)
CDR1A	SADGV	5	TCGGCAGACGGAGTT (1)
	SAFGV	5	TCAGCATTTGGAGTT (1)
	SAGRV	5	TCGGCAGGTCGAGTT (1)
	LAEGGV	6	TTGGCAGAGGGGGGGGGTT (1)
	LAGSVV	6	TTGGCAGGATCAGTAGTT (1)
	SAEGGV	6	TCGGCAGAGGGGGGGGGGTT (12)
	SAESGV	6	TCGGCAGAATCAGGAGTT (2)
	SAEVGV	6	TCGGCAGAGGTAGGAGTT (1)
	SAFFRF	6	TCGGCATTTTTCAGATTT (1)
	SAFLGV	6	TCGGCATTTTTGGGAGTT (1)
	SAFSGF	6	TCTGCATTTTCAGGATTTT (1)
	SAGAGV	6	TCGGCAGGGGCAGGAGTT (1)
	SAGFGV	6	TCGGCAGGATTTGGGGTT (2)
	SAGLKF	6	TCGGCAGGTTTAAAATTT (1)
	SAGLKV	6	TCGGCAGGTTTGAAAGTT (1)
	SAGLRV	6	TCGGCAGGATTGAGAGTT (1)
	SAGRGV	6	TCGGCAGGAAGGGGAGTT (3)
	SAGSGV	6	TCGGCAGGATCAGGAGTT (7)
			TCGGCAGGATCCGGAGTT (3)
			TCGGCAGGATCGGGAGTT (7)
			TCGGCAGGATCTGGAGTT (5)
			TCGGCAGGATCTGGGGTT (2)
			TCTGCAGGATCAGGAGTT (1)
			TCTGCAGGATCTGGAGTT (1)
	SAGSKL	6	TCGGCAGGATCAAAACTT (1)
	SAGSKV	6	TCGGCAGGATCAAAAGTT (8)
			TCGGCAGGATCGAAAGTT (3)
	SAGSRV	6	TCGGCAGGATCAAGAGTT (7)
			TCGGCAGGATCCAGAGTT (4)
			TCGGCAGGATCCCGGGTT (3)
			TCGGCAGGATCGAGAGTT (2)
			TCGGCAGGATCGAGGGTT (1)
			TCGGCAGGATCTCGAGTT (1)
	SAGSSV	6	TCGGCAGGATCAAGTGTT (2)
	SAGSVV	6	TCGGCAGGATCAGTAGTT (16)
	SALFRV	6	TCGGCATTATTCAGAGTT (1)
	SAVGGV	6	TCGGCAGTTGGAGGAGTT (1)
	SGSPGV	6	TCGGGGAGTCCGGGAGTT (1)
	VADVGV	6	GTCGCAGATGTAGGAGTT (1)

Sequences shaded in gray represent regeneration of the original complementarity determining region (CDR) template sequence.

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