Supplemental information for 'A cloning and expression system to probe T cell receptor specificity and assess functional avidity to neoantigens.'

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A. Supplemental Methods

Human PBMC samples, cell lines and cell culture

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque and cryopreserved with 10% dimethylsulfoxide (DMSO) in fetal bovine serum (FBS) until the time of analysis. HEK 293T cells (ATCC, Manassas, VA) were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Waltham, MA) containing 10% FBS and 1% penicillin-streptomycin (P/S, Gibco). Jurkat $\Delta \alpha \beta$ with stable CD28, CD8 $\alpha\beta$ +/- CD4 expression (Jurkat $\Delta\alpha\beta$) and Jurkat $\Delta\alpha\beta$ reporter cells were cultured in 'complete RPMI media': RPMI-1640 media supplemented with L-glutamine (Gibco), 10% FBS and 1% P/S. Mono-allelic B cells generated by transduction of B721.221 cells with a retroviral vector coding a single class I HLA allele were used (cells expressing HLA-A*24:02 were purchased from the Fred Hutchinson Research Cell Bank, University of Washington; cells expressing HLA-A*03:01 were a gift from Dr. Marcus Altfeld and Dr. Wilfredo F. Garcia-Beltran, Ragon Institute; others were a gift from Dr. E.L. Reinherz, DFCI). K562 cells expressing HLA-A*02:01 (K562-A2)^{1,2} and B721.221 cells stably expressing HLA-A*24:02 were cultured in complete RPMI with 400 µg/ml G418. The B721.221 cell lines stably expressing HLA-B*27:05³ and HLA-A*03:01⁴ were cultured in complete RPMI with 0.3 µg/ml puromycin. PBMCs were cultured in RPMI-1640 supplemented with L-glutamine with 10% human serum AB (Gemini Bioproduct, West Sacramento, CA; heat-inactivated 30 mins, 56°C), 1% P/S, 1% MEM sodium pyruvate, 1% MEM non-essential amino acids (NEAA, Gibco), 10mM HEPES buffer and 50uM 2-mercaptoethanol (Gibco). All cells were cultured at 37°C, 5% CO₂.

HLA typing of healthy donor samples

PBMC DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) for HLA class I and class II molecular typing (Tissue Typing Laboratory, Brigham and Women's Hospital, MA). Typing was determined by PCR-rSSO (reverse sequence specific oligonucleotide probe), with

ambiguities resolved by PCR-SSP (sequence specific primer) techniques (One Lambda Inc, Canoga Park, CA).

Peptides

Lyophilized CEF peptides were purchased as a pool from AnaSpec (Fremont, CA). Individual CEF and neoantigen peptides were synthesized from either JPT Peptide Technologies (Berlin, Germany) or RS Synthesis (Louisville, KY) (>80% purity). Sequences of the peptides are provided in Supplemental Table 3.

IFNγ enzyme-linked immunospot (ELISPOT) assay

IFNy ELISPOT assays were performed using 96-well MultiScreen Filter Plates (Millipore, Billerica, MA), coated with 2 µg/ml anti-human IFNy monoclonal antibody (mAb) in PBS overnight (1-D1K, Mabtech, Nacka Strand, Sweden). Plates were washed with PBS and blocked with complete RPMI for 1h before use. 5×10^3 T cells (for Figure 3B) or 3×10^4 (for Supplemental Figure 3) were co-cultured with 1x10⁴ autologous CD4⁺ and CD8⁺ T cell-depleted PBMC, used as antigen presenting cells (APCs). For assessment of immunogenicity of predicted neoantigens in CLL, 5x10⁴ T cells were co-cultured with $2.5x10^4$ K562-A2 cells, used as APCs. APCs were pulsed with peptides (10 µg/ml) or peptides were directly added to the ELISPOT wells with APCs and incubated with T cells overnight in complete RPMI at 37°C. Plates were rinsed with PBS containing 0.05% Tween-20 and then 1 µg/ml anti-human IFNy mAb (7-B6-1-Biotin, Mabtech) was added, followed by Streptavidin-ALP (Mabtech). After rinsing, SIGMA FAST 5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium (Sigma-Aldrich, St Louis, MO) was used to develop the immunospots, and spots were imaged and enumerated with ELISPOT plate reader (Cellular Technology Ltd, Shaker Heights, OH). Statistical analysis was performed with the square root of spot counts from each peptide condition, compared to negative control DMSO, with a one-sided two-sample t-test and significance level of 0.05.

Flow cytometry and IFN_γ catch assay analyses

Antibodies used for cell staining included anti-CD4 antibody (BV510, OKT-4, eBioscience, San Diego, CA, Alexa Fluor488, OKT-4, Biolegend, San Diego, CA), anti-CD8 (PE-Cy7, SK1, eBioscience), anti-CD3 (APC-Cy7, HIT3a, and APC, UCHT-1, Biolegend, San Diego, CA), anti-CD69 (Pacific Blue, FN50, Biolegend), anti-Vβ5.1 (APC, LC4, eBioscience), anti-CD28 (APC, T44, Biolegend), anti-CD27 (PE-Cy5, O323, eBioscience), anti-LFA1 (APC, m24, Biolegend), anti-ICOS (APC, ISA-3, eBioscience), anti-OX40 (APC, Ber-ACT35, Biolegend) and anti-4-1BB (APC, 4B4-1, Biolegend). EBNA3A-specific tetramer was obtained from MBL (Woburn, MA). All flow cytometry analysis was performed with BD FACSCanto II High Throughput Sampler (HTS) instrument.

For *ex vivo* IFN γ catch experiments, PBMCs were stimulated with 5 µg/ml peptide in complete RPMI at 37°C overnight. For detection of cytokines from pre-stimulated CD8⁺ T cells, 2x10⁶ T cells were restimulated overnight with 1x10⁶ T cell-depleted PBMCs pulsed with 5 µg/ml peptide in complete RPMI at 37 °C. Subsequently, reactive cells were tagged using the IFN γ secretion assay. Following T cell stimulation culture, cells were washed, tagged with IFN γ Catch Reagent (Miltenyi, Bergisch Gladbach, Germany), and incubated in 10 ml complete RPMI at 37°C for 45 minutes. PE-conjugated IFN γ Detection Reagent was used to stain cells secreting cytokine. Cells were then stained with anti-CD4, CD3, and CD8 antibodies for 20 min at 4°C, followed by staining with 7AAD (BD-Bioscience, Franklin Lakes, NJ). IFN γ^+ single cells were then sorted into 384 well PCR plates using the FACSAria II SORP UV instrument (DFCI Flow Cytometry Core).

For characterization of costimulatory marker expression on Jurkat $\Delta\alpha\beta$ reporter cells, Jurkat $\Delta\alpha\beta$ reporter cells transduced with M1-specific TCR (P1.7) and healthy donor PBMC were stained with antibodies either resting or after stimulation with anti-CD3 antibody (functional grade, OKT3, Miltenyi). For activation, 6 well plates were coated with anti-CD3 (5 µg/ml) at 4°C overnight, and washed before

adding the cells (reporter cells at $5x10^5$ cells/ml and PBMCs at $1.5x10^6$ /ml). After overnight culture, cells were cultured with Human TruStain FcX (Biolegend) for 20 min at 4°C for 20 min, followed by staining with costimulatory marker antibodies or isotype control antibodies at 4°C for 20 min, and analyzed by flow cytometry.

Paired TCRαβ chain single-cell sequencing

Linked TCR α /TCR β Illumina libraries from single cells were made using a multi-primer based approach. One-step reverse transcription-PCR reactions were performed using gene specific TCR α /TCR β -V region and -C region primers (Supplemental Table 4). The first strand complementary DNA (cDNA) was primed using TRAC and TRBC primers. V region PCR amplification used 41 TRAV-specific, 38 TRBV-specific, TRAC and TRBC primers. Amplicons were generated following a second PCR using nested V and C primers: 39 TRAV-specific, 38 TRBV-specific, TRAC and TRBC primers. The C primers were barcoded, and both the C and V primers were tailed using partial Illumina adapter sequences. Final library amplification using Illumina primers introduced two additional barcodes. The barcoding system served a dual purpose – identifying plates and identifying individual wells within a plate, thus permitting parallel sequencing of multiple plates. Illumina sequencing was performed and sequences were aligned with the IMGT database for TCR α and TCR β sequences.

The raw fastq files from sequencing were first demultiplexed by P7, P5 and inline barcodes into each well in the plates; TCR α and TCR β were also separated by inline barcodes in this step. Wells with TCR α or TCR β read counts less than 10 were removed from further analysis. The reads were then aligned to IMGT TCR reference sequences and a list of clonotypes was assembled in each well (MiXCR-2.1.5).⁵ The most abundant productive alpha and beta clonotypes were selected and paired for each well. If the fraction of the most abundant productive TCR α or TCR β in a well was less than 20% or 60% respectively, the wells were removed.

For 4 of 5 runs, targeted expression data of 5 housekeeping (HK) genes was available for quality control (*ACTB, B2M, PPPIA, RPS3* and *UBB*), and in these instances, pre-filtering based on the presence of detectable expression of these genes was performed before TCR alignment (86-98% of wells in the run had all housekeeping genes expressed. The raw fastq files of HK genes were first aligned to the HK primers (BLAST-2.2.30+).⁶ Cutoffs for positive expression were determined by density distribution of the read count per well, for each HK gene in each run. The wells with absent expression of all 5 HK genes were removed from further analysis.

TCR cloning from variable chain plasmid library and TCR expression

The variable chain plasmid library included two types of vectors, encoding the variable segments of the TCR: 46 variable α with constant β (V α -C β) and 52 variable β with constant α (V β -C α). All constructs were synthesized in pUC57-Kanamycin backbones (Genscript, Piscataway, NJ, example sequence in Supplemental Figure 1B, complete list of segment sequences in Supplemental Table 1). For each TCR, double-stranded oligonucleotides encoding CDR3 α and CDR3 β , flanked by BsaI restriction sites designed to be compatible with the variable chain plasmid library, were custom synthesized on demand (Integrated DNA Technologies [IDT], Coralville, IA) (example sequence in Supplemental Figure 1C, all CDR3 oligonucleotide sequences in Supplemental Table 2). Two library plasmids and a CDR3 oligonucleotide, all digested with BsaI, were assembled using Golden Gate Assembly mix (New England Biolabs [NEB], Ipswich, MA) to produce a single vector encoding both TCR α and TCR β , separated by a furin, SGSG, and F2A-peptide sequence. The Golden Gate reaction mix was used to transform competent cells (NEB), that were then plated on kanamycin and grown overnight. Colony PCR was performed to select clones. The PCR product and lentiviral backbone PEW were digested with restriction enzymes AgeI and SalI, and ligated using T4 DNA ligase (NEB). The ligation product was used to transform competent cells that were then plated on ampicillin, and grown overnight. Colony PCR was performed to select clones to expand overnight, and the TCR plasmid was isolated by Midi prep (Qiagen).

TCRs were expressed in reporter cells by lentiviral transduction as follows. HEK293T cells were plated in 6 well plate in antibiotic-free DMEM (DMEM + 10% FBS), and cultured overnight at 37°C, 5% CO₂. HEK293T cells were transfected with the TCR vector, psPAX2 (Addgene, Cambridge, MA) and VSV (Addgene) at a ratio of 10:10:1 using Lipofectamine 2000 (Thermo Fisher, Waltham, MA). Media was replaced 16h after transfection. Supernatant was harvested after 72h, filtered using a 0.45 µm syringe filter and concentrated using size-exclusion columns (VIVASPIN20 [30,000MW], Sartorius, Goettingen, Germany) by centrifugation at 4°C for 60 min at 2000 rpm. For transduction, Jurkat $\Delta\alpha\beta$ reporter cells were plated with 8 µg/ml polybrene (Santa Cruz Biotech, Dallas, TX) in complete RPMI, and transduced with concentrated virus by spin infection (90 min, 2000 rpm, 37°C). After 16h, media was replaced with complete RPMI. All TCRs were expressed in Jurkat $\Delta\alpha\beta$ reporter cells with stable CD8 $\alpha\beta$ expression, except TCRs from CD4⁺ T cells from melanoma study Patient 1, which were expressed in Jurkat $\Delta\alpha\beta$ reporter cells with stable CD8 $\alpha\beta$ and CD4 expression. Expression of the TCR was confirmed after 72h by measuring CD3 expression in transduced reporter cells by flow cytometry

A TCR specific for EBNA3A was cloned and expressed using published sequence information: TRAV8-1, TRAJ23, TRBV5-1, TRBD2, TRBJ2-7, CDR3 α (CAGRLVDQGGKLIF) and CDR3 β (CASSIGLAGYEQYF).⁷ The library components for TRAV8-1 and TRBV5-1 were assembled with oligonucleotide encoding CDR3 α and CDR3 β (Supplemental Figure 1B-C).

TCR activation assays

Autologous antigen-presenting cells (APCs) (derived from CD4/CD8 depleted PBMCs) and HLAexpressing cell lines were pulsed with candidate peptide (10 µg/ml unless specified otherwise) for 2 hours in complete RPMI. 5x10⁵ TCR-expressing reporter cells were co-cultured with pulsed APCs (5x10⁵ autologous APC or 2.5x10⁵ HLA-expressing cell line) in 96-well U-bottom plate overnight. The addition of PMA (50 ng/ml) and ionomycin (500 ng/ml) to TCR-expressing reporter cells was used as a positive control. TCR activation was measured by IL-2 ELISA, luciferase activity or CD69-expression. Supernatant was harvested from co-culture and diluted 1:2 (unless otherwise specified) with ELISA Assay Diluent (Biolegend) and IL-2 production was measured using the Human IL-2 ELISA Kit II (BD Bioscience) or ELISA MAX Deluxe Kit (Biolegend) according to manufacturer's instructions.

Luciferase production was measured using the Luciferase Assay System with Reporter Lysis Buffer (Promega, Madison, WI), according to manufacturer's instructions. Co-cultured TCR-expressing reporter cells and K562-A2 cells were washed twice with PBS, then lysed in 20 µl/well of 1X Reporter Lysis Buffer, followed by a single freeze-thaw cycle in liquid nitrogen. Within one hour of lysis, Light production (RLU) was measured (three seconds per well) using a Luminoskan Ascent Microplate Luminometer (ThermoFisher Scientific, Waltham, MA).

CD69 expression was measured by staining co-cultured TCR-expressing reporter cells with anti-CD69 antibody for 20 min at 4°C and analyzing by flow cytometry.

To measure functional avidity of antigen-specific TCRs, TCR-expressing reporter cells were co-cultured as described above with APCs pulsed with a range of peptide concentrations from 10 pg/ml to 10 μ g/ml. IL-2 production was measured by ELISA as described. Comparison of IL-2 secretion between mutant and wildtype forms of the peptide was assessed at each concentration using a two-sample t-test with Welch's correction, with p-values considered significant at the 0.05 level.

Accession numbers

The dbGaP accession numbers for the WES data reported previously and used in this paper are phs000435.v1.p1 and phs000922.v1.p1^{8,9}.

B. Supplemental Tables

<u>Table S1. Sequences of variable and constant TCR segments used in plasmid library</u> **Table S1A.** TRAV and TRBC sequences used in V α -C β library plasmids

The TRBC sequence used to construct our library is 6 bp shorter on the 5' end than the sequence listed in IMGT. All TRAV sequences used to construct our library are approximately 16 bp shorter on the 3' end from those listed in IMGT. The absent regions are included in the CDR3 oligonucleotides. Single nucleotide changes (synonymous changes) were introduced to remove potential cut sites by restriction enzymes (highlighted). Sequences are from IMGT database, accessed in January 2015.

Segment	Leader Sequence	Sequence	
Constant beta (Cβ)	N/A	CTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTC CCACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTTCCCCGACCACGTGG AGCTGAGCTG	
TRAV1-1	ATGTGGGGGAGCTTT CCTTCTCTATGTTTC CATGAAGATGGGAG GCACTGCA	GGACAAAGCCTTGAGCAGCCCTCTGAAGTGACAGCTGTGGAAGGAGCCATTGTCCAGAT AAACTGCACGTACCAGACATCTGGGTTTTATGGGCTGTCCTGGTACCAGCAACATGATG GCGGAGCACCCACATTTCTTTCTTACAATGCTCTGGATGGTTTGGAGGAGACAGGTCGTT TTTCTTCATTCCTTAGTCGCTCTGATAGTTATGGTTACCTCCTTCTACAGGAGCTCCAGAT GAAAGACTCTGCCTCTTAC	
TRAV1-2	ATGTGGGGAGTTTT CCTTCTTTATGTTTC CATGAAGATGGGAG GCACTACA	GGACAAAACATTGACCAGCCCACTGAGATGACAGCTACGGAAGGTGCCATTGTCCAGAT CAACTGCACGTACCAGACATCTGGGTTCAACGGGCTGTTCTGGTACCAGCAACATGCTG GCGAAGCACCCACATTTCTGTCTTACAATGTTCTGGATGGTTTGGAGGAGAAAGGTCGTT TTTCTTCATTCCTTAGTCGGTCTAAAGGGTACAGTTACCTCCTTTTGAAGGAGCTCCAGAT GAAAGACTCTGCCTCTTAC	
TRAV2	ATGGCTTTGCAGAG CACTCTGGGGGGCGG TGTGGCTAGGGCTT CTCCTCAACTCTCTC TGGAAGGTTGCAGA AAGC	AAGGACCAAGTGTTTCAGCCTTCCACAGTGGCATCTTCAGAGGGAGCTGTGGGGAAAT CTTCTGTAATCACTCTGTGTCCAATGCTTACAACTTCTTCTGGTACCTTCACTTCCCGGGA TGTGCACCAAGACTCCTTGTTAAAGGCTCAAAGCCTTCTCAGCAGGGACGATACAACAT GACCTATGAACGGTTCTCTTCATCGCTGCTCATCCTCCAGGTGCGGGAGGCAGATGCTGC TGTTTAC	
TRAV3	ATGGCCTCTGCACC CATCTCGATGCTTG CGATGCTCTTCACA TTGAGTGGGGCTGAG A	GCTCAGTCAGTGGCTCAGCCGGAAGATCAGGTCAACGTTGCTGAAGGGAATCCTCTGAC TGTGAAATGCACCTATTCAGTCTCTGGAAACCCTTATCTTTTTTGGTATGTTCAATACCCC AACCGAGGCCTCCAGTTCCTTCTGAAATACATCACAGGGGATAACCTGGTTAAAGGCAG CTATGGCTTTGAAGCTGAATTTAACAAGAGCCAAACCTCCTTCCACCTGAAGAAACCATC TGCCCTTGTGAGCGACTCCGCTTTGTAC	
TRAV4	ATGAGGCAAGTGGC GAGAGTGATCGTGT TCCTGACCCTGAGT ACTTTGAGC	CTTGCTAAGACCACCCAGCCCATCTCCATGGACTCATATGAAGGACAAGAAGTGAACAT AACCTGTAGCCACAACAACATTGCTACAAATGATTATATCACGTGGTACCAACAGTTTCC CAGCCAAGGACCACGATTTATTATTCAAGGATACAAGACAAAAGTTACAAAACGAAGTGG CCTCCCTGTTTATCCCTGCCGACAGAAAGTCCAGCACTCTGAGCCTGCCCCGGGTTTCCC TGAGCGACACTGCTGTGTAC	
TRAV5	ATGAAGACATTTGC TGGATTTTCGTTCCT GTTTTTGTGGCTGC AGCTGGACTGTATG AGTAGA	GGAGAGGATGTGGAGCAGAGTCTTTTCCTGAGTGTCCGAGAGGGAGACAGCTCCGTTAT AAACTGCACTTACACAGACAGCTCCTCCACCTACTTATACTGGTATAAGCAAGAACCTG GAGCAGG <mark>A</mark> CTCCAGTTGCTGACGTATATTTTTTCAAATATGGACATGAAACAAGACCAA AGACTCACTGTTCTATTGAATAAAAAGGATAAACATCTGTCTCTGCGCATTGCAGACACC CAGACTGGGGACTCAGCTATCTAC Bsal site (T to A)	

TRAV6	ATGGAGTCATTCCT GGGAGGTGTTTTGC TGATTTTGTGGCTTC AAGTGGACTGGGTG AAG	AGCCAAAAGATAGAACAGAATTCCGAGGCCCTGAACATTCAGGAGGGTAAAACGGCCA CCCTGACCTGCAACTATACAAACTATTCCCCAGCATACTTACAGTGGTACCGACAAGATC CAGGAAGAGGCCCTGTTTTCTTGCTACTCATACGTGAAAATGAGAAAGAA
TRAV7	ATGGAGAAGATGCG GAGGCCTGTCCTAA TTATATTTGTCTAT GTCTTGGCTGGGCA AATGGA	GAAAACCAGGTGGAGCACAGCCCTCATTTTCTGGGACCCCAGCAGGGAGACGTTGCCTC CATGAGCTGCACGTACTCTGTCAGTCGTTTTAACAATTTGCAGTGGTACAGGCAAAATAC AGGGATGGGTCCCAAACACCTATTATCCATGTATTCAGCTGGATATGAGAAGCAGAAAG GAAGACTAAATGCTACATTACTGAAGAATGGAAGCAGCTTGTACATTACAGCCGTGCAG CCTGAAGATTCAGCCACCTAT
	Bsal site (A to G)	
TRAV8-1	ATGCTCCTGTTGCTCGCCCAGTCTGTGAGCCAGCATAACCACCACGTAATTCTCTCTGAAGCAGCATACCAGTGCTGGGGTTGGGATGCAACTATTCCTATGGTGGAACTGTTAATCTCTTCTGGTATGTGATGATTTTTGCCCTTGGTCAACACCTTCAGCTTCTCCTCAAGTACTTTTCAGGGGATCCACTGGTGAGAGATGCCAGACAAGGGCTTTGAGGCTGAATTTATAAAGAGTAAATTCTCCTTTAATCTGATGTGCAGTGGAGTGACACACGTGAGTACTGTGCAGTGGAGTGACACAGCTGAGTAC	
TRAV8-2	ATGCTCCTGCTGCT CGTCCCAGTGCTCG AGGTGATTTTACT CTGGGAAGGAACCAG A	GCCCAGTCGGTGACCCAGCTTGACAGCCACGTCTCTGTCTCTGAAGGAACCCCGGTGCTG CTGAGGTGCAACTACTCATCTTCTTATTCACCATCTCTCTGGTATGTGCAACACCCCA ACAAAGGACTCCAGCTTCTCCTGAAGTACACATCAGCGGCCACCCTGGTTAAAGGCATC AACGGTTTTGAGGCTGAATTTAAGAAGAGTGAAACCTCCTTCCACCTGACGAAACCCTC AGCCCATATGAGCGACGCGGCTGAGTAC
TRAV8-3	ATGCTCCTGGAGCT TATCCCACTGCTGG GGATACATTTTGTC CTGAGAACTGCCAG A	GCCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTCACTGGA GTTGAGATGTAACTATTCCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCC CGGCCAAGGCCTCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCAT TAAAGGCTTTGAGGCTGAATTTAAGAGGAGTCAATCTTCCTTC
TRAV8-4	ATGCTCCTGCTGCT CGTCCCAGTGCTCG AGGTGATTTTTACC CTGGGAGGAACCAG A	GCCCAGTCGGTGACCCAGCTTGGCAGCCACGTCTCTGTCTCTGAAGGAGCCCTGGTTCTG CTGAGGTGCAACTACTCATCGTCTGTTCCACCATATCTCTTCTGGTATGTGCAATACCCC AACCAAGGACTCCAGCTTCTCCTGAAGTACACATCAGCGGCCACCCTGGTTAAAGGCAT CAACGGTTTTGAGGCTGAATTTAAGAAGAGTGAAACCTCCTTCCACCTGACGAAACCCT CAGCCCATATGAGCGACGCGGCTGAGTAC
TRAV8-6	ATGCTCCTGCTGCT CGTCCCAGCGTTCC AGGTGATTTTTACC CTGGGAGGAACCAG A	GCCCAGTCTGTGACCCAGCTTGACAGCCAAGTCCCTGTCTTTGAAGAAGCCCCTGTGGAG CTGAGGTGCAACTACTCATCGTCTGTTTCAGTGTATCTCTTCTGGTATGTGCAATACCCCA ACCAAGGACTCCAGCTTCTCCTGAAGTATTTATCAGGATCCACCCTGGTTGAAAGCATCA ACGGTTTTGAGGCTGAATTTAACAAGAGTCAAACTTCCTTC
TRAV8-7	ATGCTCTTAGTGGT CATTCTGCTGCTTG GAATGTTCTTCACA CTGAGAACCAGA	ACCCAGTCGGTGACCCAGCTTGATGGCCACATCACTGTCTCTGAAGAAGCCCCTCTGGA ACTGAAGTGCAACTATTCCTATAGTGGAGTTCCTTCTCTCTTCTGGTATGTCCAATACTCT AGCCAAAGCCTCCAGCTTCTCCTCAAAGACCTAACAGAGGCCACCCAGGTTAAAGGCAT CAGAGGTTTTGAGGCTGAATTTAAGAAGAGCGAAACCTCCTTCTACCTGAGGAAACCAT CAACCCATGTGAGTGATGCTGCTGCTGAGTAC
TRAV9-1	ATGAATTCTTCTCC AGGACCAGCGATTG CACTATTCTTAATGT TTGGGGGGAATCAAT	GGAGATTCAGTGGTCCAGACAGAAGGCCAAGTGCTCCCCTCTGAAGGGGATTCCCTGAT TGTGAACTGCTCCTATGAAACCACACAGTACCCTTCCCTTTTTTGGTATGTCCAATATCCT GGAGAAGGTCCACAGCTCCACCTGAAAGCCATGAAGGCCAATGACAAGGGAAGGAA
TRAV9-2	ATGAACTATTCTCC AGGCTTAGTATCTC TGATACTCTTACTG CTTGGAAGAACCCG T	GGAAATTCAGTGACCCAGATGGAAGGGCCAGTGACTCTCTCAGAAGAGGGCCTTCCTGAC TATAAACTGCACGTACACAGCCACAGGATACCCTTCCCTTTTCTGGTATGTCCAATATCC TGGAGAAGGTCTACAGCTCCTCCTGAAAGCCACGAAGGCTGATGACAAGGGAAGCAAC AAAGGTTTTGAAGCCACATACCGTAAAGAAACCACTTCTTTCCACTTGGAGAAAGGCTC AGTTCAAGTGTCAGACTCAGCGGTGTAC
TRAV10	ATGAAAAAGCATCT GACGACCTTCTTGG TGATTTTGTGGCTTT	AAAAACCAAGTGGAGCAGAGTCCTCAGTCCCTGATCATCCTGGAGGGAAAGAACTGCAC TCTTCAATGCAATTATACAGTGAGCCCCTTCAGCAACTTAAGGTGGTATAAGCAAGATAC TGGGAGAGGTCCTGTTTCCCTGACAATCATGACTTTCAGTGAGAACACAAAGTCGAACG

	ATTTTTATAGGGGG AATGGC	GAAGATATACAGCAACTCTGGATGCAGACACAAAGCAAAGCTCTCTGCACATCACAGCC TCCCAGCTCAGCGATTCAGCCTCCTAC	
TRAV12-1	ATGATATCCTTGAG AGTTTTACTGGTGA TCCTGTGGCTTCAG TTAAGCTGGGTTTG GAGCCAA	CGGAAGGAGGTGGAGCAGGATCCTGGACCCTTCAATGTTCCAGAGGGAGCCACTGTCGC TTTCAACTGTACTTACAGCAACAGTGCTTCTCAGTCTTTCTT	
TRAV12-2	ATGAAATCCTTGAG AGTTTTACTAGTGA TCCTGTGGCTTCAG TTGAGCTGGGTTTG GAGCCAA	CAGAAGGAGGTGGAGCAGAATTCTGGACCCCTCAGTGTTCCAGAGGGAGCCATTGCCTC TCTCAACTGCACTTACAGTGACCGAGGTTCCCAGTCCTTCTTCTGGTACAGACAATATTC TGGGAAAAGCCCTGAGTTGATAATGTTCATATACTCCAATGGTGACAAAGAAGATGGAA GGTTTACAGCACAGC	
TRAV12-3	ATGATGAAAATCCTT GAGAGTTTTACTGG TGATCCTGTGGGCTT CAGTTAAGCTGGGT TTGGAGCCAA	CAGAAGGAGGTGGAGCAGGATCCTGGACCACTCAGTGTTCCAGAGGGAGCCATTGTTTC TCTCAACTGCACTTACAGCAACAGTGCTTTTCAATACTTCATGTGGTACAGACAG	
TRAV13-1	ATGACATCCATTCG AGCTGTATTTATATT CCTGTGGCTGCAGC TGGACTTGGTGAAT	GGAGAAATGTGGAGCAGCATCCTTCAACCCTGAGTGTCCAGGAGGGAG	
TRAV13-2	ATGGCAGGCATTCG AGCTTTATTTATGTA CTTGTGGCTGCAGC TGGACTGGGTGAGC AGA	GGAGAGAGTGTGGGGCTGCATCTTCCTACCCTGAGTGTCCAGGAGGGTGACAACTCTAT TATCAACTGTGCTTATTCAAACAGCGCCTCAGACTACTTCATTTGGTACAAGCAAG	
TRAV14/DV4	ATGTCACTTTCTAG CCTGCTGAAGGTGG TCACAGCTTCACTG TGGCTAGGACCTGG CATT	GCCCAGAAGATAACTCAAACCCAACCAGGAATGTTCGTGCAGGAAAAGGAGGCTGTG, CTCTGGACTGCACATATGACACCAGTGATCCAAGTTATGGTCTATTCTGGTACAAGCAC CCAGCAGTGGGGGAAATGATTTTTCTTATTTATCAGGGGTCTTATGACCAGCAAAATGC/ CAGAAGGTCGCTACTCATTGAATTTCCAGAAGGCAAGAAAATCCGCCAACCTTGTCAT TCCGCTTCACAACTGGGGGGACTCAGCAATGTAC	
TRAV16	ATGAAGCCCACCCT CATCTCAGTGCTTG TGATAATATTTATA CTCAGAGGAACAAG A	GCCCAGAGAGTGACTCAGCCCGAGAAGCTCCTCTCTGTCTTTAAAGGGGGCCCCAGTGGA GCTGAAGTGCAACTATTCCTATTCTGGGAGTCCTGAACTCTTCTGGTATGTCCAGTACTC CAGACAACGCCTCCAGTTACTCTTGAGACACATCTCTAGAGAGAG	
TRAV17	ATGGAAACTCTCCT GGGAGTGTCTTTGG TGATTCTATGGCTTC AACTGGCTAGGGTG AAC	AGTCAACAGGGAGAAGAGGATCCTCAGGCCTTGAGCATCCAGGAGGGTGAAAATGCCA CCATGAACTGCAGTTACAAAACTAGTATAAACAATTTACAGTGGTATAGACAAAATTCA GGTAGAGGCCTTGTCCACCTAATTTTAATACGTTCAAATGAAAGAGAGAAAACACAGTGG AAGATTAAGAGTCACGCTTGACACTTCCAAGAAAAGCAGTTCCTTGTTGATCACGGCTTC CCGGGCAGCAGACACTGCTTCTTAC	
TRAV18	ATGCTGTCTGCTTCC TGCTCAGGACTTGT GATCTTGTTGATATT CAGAAGGACCAGT	GGAGACTCGGTTACCCAGACAGAAGGCCCAGTTACCCTCCCT	
TRAV19	ATGCTGACTGCCAG CCTGTTGAGGGCAG TCATAGCCTCCATC TGTGTTGTATCCAG CATG	GCTCAGAAGGTAACTCAAGCGCAGACTGAAATTTCTGTGGTGGAGAAGGAGGATGTGAC CTTGGACTGTGTATGAAACCCGTGATACTACTTATTACTTATTCTGGTACAAGCAACC ACCAAGTGGAGAATTGGTTTTCCTTATTCGTCGGAACTCTTTTGATGAGCAAAATGAAAT AAGTGGTCGGTATTCTTGGAACTTCCAGAAATCCACCAGTTCCTTCAACTTCACCATCAC AGCCTCACAAGTCGTGGACTCAGCAGTATAC	
TRAV20	ATGGAGAAAATGTT GGAGTGTGCATTCA	GAAGACCAGGTGACGCAGAGTCCCGAGGCCCTGAGACTCCAGGAGGGGAGAGAGTAGCA GTCTTAACTGCAGTTACACAGTCAGCGGTTTAAGAGGGCTGTTCTGGTATAGGCAAGATC	

	TAGTCTTGTGGCTTC AGCTTGGCTGGTTG AGTGGA	CTGGGAAAGGCCCTGAATTCCTCTTCACCCTGTATTCAGCTGGGGAAGAAAAGGAGAAA GAAAGGCTAAAAGCCACATTAACAAAGAAGGAAAGCTTTCTGCACATCACAGCCCCTAA ACCTGAAGACTCAGCCACTTAT	
TRAV21	ATGGAGAC <mark>A</mark> CTCTT GGGCCTGCTTATCC TTTGGCTGCAGCTG CAATGGGTGAGCAG C	AAACAGGAGGTGACGCAGATTCCTGCAGCTCTGAGTGTCCCAGAAGGAGAAAACTTGGT TCTCAACTGCAGTTTCACTGATAGCGCTATTTACAACCTCCAGTGGTTTAGGCAGGACCC TGGGAAAGG <mark>A</mark> CTCACATCTCTGTTGCTTATTCAGTCAAGTCAGAGAGAGA	
	Bsal site (C to A)	BsaI site (T to A)	
TRAV22	V22 ATGAAGAGGATATT GGAATACAAGTGGAGCAGAGTCCTCCAGACCTGATTCTCCAGGAGG GGGAGCTCTGCTGG GCTGCGGTGCAATTTTTCTGACTCTGTGAACAATTTGCAGTGGTTTC GGCTCTTGAGTGCC GGGACAGCTCATCAACCTGTTTACATTCCCTCAGGGACAAAACAG CAGGTTTGCTGTGT GCGCCACGACTGTCGCTACGGAACGCTACAGCTTATTGTACATTCCC GAGA CAGACTCAGCGTTTAC		
TRAV23/DV6	ATGGACAAGATCTT AGGAGCATCATTTT TAGTTCTGTGGGCTTC AACTATGCTGGGTG AGTGGCCAACAGAA GGAGAAAAGTGAC	ATGGACAAGATCTTCAGCAGCAGGTGAAACAAAGTCCTCAATCTTTGATAGTCCAGAAAGGAGGGGATTTAGGAGCATCATTTTTATAAACTGTGCTTATGAGAACACTGCGTTTGACTACTTTCCATGGTACCAACAATTAGTTCTGTGGCTTCTGGGAAAGGCCCTGCATTATTGATAGCCATACGTCCAGATGTGAGTGA	
TRAV24	ATGGAGAAGAATCC TTTGGCAGCCCCAT TACTAATCCTCTGG TTTCATCTTGACTGC GTGAGCAGC	ATACTGAACGTGGAACAAAGTCCTCAGTCACTGCATGTTCAGGAGGGAG	
TRAV25	ATGCTACTCATCAC ATCAATGTTGGTCT TATGGATGCAATTG TCACAGGTGAAT	GGACAACAGGTAATGCAAATTCCTCAGTACCAGCATGTACAAGAAGGAGGAGGACTTCAC CACGTACTGCAATTCCTCAACTACTTTAAGCAATATACAGTGGTATAAGCAAAGGCCTG GTGGACATCCCGTTTTTTTGATACAGTTAGTGAAGAAGTGGAGAAGTGAAGAAGCAGAAA AGACTGACATTTCAGTTTGGAGAAGCAAAAAAGAACAGCTCCCTGCACATCACAGCCAC CCAGACTACAGATGTAGGAACCTAC	
TRAV26-1	ATGAGGCTGGTGGC AAGAGTAACTGTGT TTCTGACCTTTGGA ACTATAATT GATGCTAAGACCACCCAGCCCCCCTCCATGGATTGCGCTGAAGGAAG		
TRAV26-2	ATGAAGTTGGTGAC AAGCATTACTGTAC TCCTATCTTTGGGTA TTATGGGT AGGAGAGTGCTAAGACCACACAGCCAAATTCAATGGAGAGTAACGAAGAAGAGCCTG GCCTTGTAACCACTCCACAATCAGTGGAACTGATTACATACA		
TRAV27	ATGGTCCTGAAATT CTCCGTGTCCATTCT TTGGATTCAGTTGG CATGGGTGAGC	ACCCAGCTGCTGGAGCAGAGCCCTCAGTTTCTAAGCATCCAAGAGGGAGAAAATCTCAC TGTGTACTGCAACTCCTCAAGTGTTTTTTCCAGCTTACAATGGTACAGACAG	
TRAV29/DV5	ATGGCCATGCTCCT GGGGGCATCAGTGC TGATTCTGTGGGCTTC AGCCAGACTGGGTA AACAGTCAACAGAA GAATGAT	GACCAGCAAGTTAAGCAAAATTCACCATCCCTGAGCGTCCAGGAAGGA	
TRAV30	ATGGAGACTCTCCT GAAAGTGCTTTCAG GCACCTTGTTGTGG CAGTTGACCTGGGT GAGAAGC	CAACAACCAGTGCAGAGTCCTCAAGCCGTGATCCTCCGAGAAGGGGAAGATGCTGTCAT CAACTGCAGTTCCTCCAAGGCTTTATATTCTGTACACTGGTACAGGCAGAAGCATGGTGA AGCACCCGTCTTCCTGATGATATTACTGAAGGGTGGAGAACAGAAGGGTCATGAAAAAA TATCTGCTTCATTTAATGAAAAAAAGCAGCAAAGCTCCCTGTACCTTACGGCCTCCCAGC TCAGTTACTCAGGAACCTAC	

TRAV34	ATGGAGACTGTTCT GCAAGTACTCCTAG GGATATTGGGGTTC CAAGCAGCCTGGGT CAGT	AGCCAAGAACTGGAGCAGAGTCCTCAGTCCTTGATCGTCCAAGAGGGAAAGAATCTCAG CATAAACTGCACGTCATCAAAGACGTTATATGGCTTATACTGGTATAAGCAAAAGTATG GTGAAGGTCTTATCTTCTTGATGATGCTACAGAAAGGTGGGGAAGAGAAAAGTCATGAA AAGATAACTGCCAAGTTGGATGAGAAAAAGCAGCAAAGTTCCCTGCATATCACAGCCTC CCAGCCCAGC	
TRAV35	ATGCTCCTTGAACA TTTATTAATAATCTT GTGGATGCAGCTGA CATGGGTCAGT	GGTCAACAGCTGAATCAGAGTCCTCAATCTATGTTTATCCAGGAAGGA	
TRAV36/DV7	ATGATGAAGTGTCC ACAGGCTTTACTAG CTATCTTTTGGCTTC TACTGAGCTGGGTG AGCAGT	GAAGACAAGGTGGTACAAAGCCCTCTATCTCTGGTTGTCCACGAGGGAGACACCGTAAC TCTCAATTGCAGTTATGAAGTGACTAACTTTCGAAGCCTACTATGGTACAAGCAGGAAA AGAAAGCTCCCACATTTCTATTTATGCTAACTTCAAGTGGAATTGAAAAGAAGTCAGGA AGACTAAGTAGCATATTAGATAAGAAAGAACTTTCCAGCATCCTGAACATCACAGCCAC CCAGACCGGAGACTCGGCCATCTAC	
TRAV38-1	ATGACACGAGTTAG CTTGCTGTGGGCAGGCCCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGCAGGAGGGCAG CCTGAGTTGCACCATATGACACCAGTGAGAATAATTATTATTGTTCTGGT TCCCAGCAGGCAGATGATTCTCGTTATTCGCCAAGAAGCTTATAAGCAA CGGAGAATCGTTTCTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAG CAGACTCACAGCTGGGGGACACTGCGATGTATBsal site (C to G)Bsal site (C to G)		
TRAV38-2/DV8	ATGGCATGCCCTGG CTTCCTGTGGGCAC TTGTGATCTCCACCT GTCTTGAATTTAGC ATG	GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCAGAGACAGTGAC CCTGAGCTGCACATATGACACCAGTGAGAGTGATTATTATTTAT	
		Bsal site (C to A)	
TRAV39	ATGAAGAAGCTACT AGCAATGATTCTGT GGCTTCAACTAGAC CG <mark>C</mark> TTAAGTGGA Agel site (G to C)	Bsal site (C to A) GAGCTGAAAGTGGAACAAAACCCTCTGTTCCTGAGCATGCAGGAGGGAAAAAACTATAC CATCTACTGCAATTATTCAACCACTTCAGACAGACTGTATTGGTACAGGCAGG	
TRAV39	ATGAAGAAGCTACT AGCAATGATTCTGT GGCTTCAACTAGAC CG <mark>C</mark> TTAAGTGGA Agel site (G to C) ATGAACTCCTCTCT GGACTTTCTAATTCT GATCTTAATGTTTG GAGGAACCAGC	Bsal site (C to A) GAGCTGAAAGTGGAACAAAACCCTCTGTTCCTGAGCATGCAGGAGGGAAAAAACTATAC CATCTACTGCAATTATTCAACCACTTCAGACAGACTGTATTGGTACAGGCAGG	

Table S1B. TRBV and TRAC sequences used in V β -C α library plasmids

The TRAC sequence used to construct our library is 14 bp shorter on the 5' end than the sequence listed in IMGT. All TRBV sequences used to construct our library are approximately 20 bp shorter on the 3' end from those listed in IMGT. The absent regions are included in the CDR3 oligonucleotides. Single nucleotide changes (synonymous changes) were introduced to remove potential cut sites by restriction enzymes (highlighted). Sequences are from IMGT database, accessed in January 2015.

Segment	Leader Sequence	Sequence	
Constant alpha (Cα)	N/A	TGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCT	
TRBV2	ATGGATACCTGGCT CGTATGCTGGGCAA TTTTTAGTCTCTTGA AAGCAGGACTCACA	GAACCTGAAGTCACCCAGACTCCCAGCCATCAGGTCACACAGATGGGACAGGAAGTGAT CTTGCGCTGTGTCCCCATCTCTAATCACTTATACTTCTATTGGTACAGACAAATCTTGGGG CAGAAAGTCGAGTTTCTGGTTTCCTTTTATAATAATGAAATCTCAGAGAAGTCTGAAATA TTCGATGATCAATTCTCAGTTGAAAGGCCTGATGGATCAAATTTCACTCTGAAGATCCGG TCCACAAAGCTGGAGGACTCAGCCATGTAC	
TRBV3-1	ATGGGCTGCAGGCT CCTCTGCTGTGTGG TCTTCTGCCTCCTCC AAGCAGGTCCCTTG	GACACAGCTGTTTCCCAGACTCCAAAATACCTGGTCACACAGATGGGAAACGACAAGTC CATTAAATGTGAACAAAATCTGGGCCATGATACTATGTATTGGTATAAACAGGACTCTA AGAAATTTCTGAAGATAATGTTTAGCTACAATAATAAGGAGCTCATTATAAATGAAACA GTTCCAAATCGCTTCTCACCTAAATCTCCAGACAAAGCTCACTTAAATCTTCACATCAAT TCCCTGGAGCTTGGTGACTCTGCTGTGTAT	
TRBV4-1	ATGGGCTGCAGGCT GCTCTGCTGTGCGG TTCTCTGTCTCCTGG GAGCAGTTCCCATA	GACACTGAAGTTACCCAGACACCAAAACACCTGGTCATGGGAATGACAAATAAGAAGTC TTTGAAATGTGAACAACATATGGGGCACAGGGCTATGTATTGGTACAAGCAGAAAGCTA AGAAGCCACCGGAGCTCATGTTTGTCTACAGCTATGAGAAACTCTCTATAAATGAAAGT GTGCCAAGTCGCTTCTCACCTGAATGCCCCAACAGCTCTCTCT	
TRBV4-2	ATGGGCTGCAGGCT GCTCTGCTGTGCGG TTCTCTGTCTCCTGG GAGCGGTCCCCATG	GAAACGGGAGTTACGCAGACACCAAGACACCTGGTCATGGGAATGACAAATAAGAAGT CTTTGAAATGTGAACAACATCTGGGGCATAACGCTATGTATTGGTACAAGCAAAGTGCT AAGAAGCCACTGGAGCTCATGTTTGTCTACAACTTTAAAGAACAGACTGAAAACAACAG TGTGCCAAGTCGCTTCTCACCTGAATGCCCCAACAGCTCTCACTTATTCCTTCACCTACAC ACCCTGCAGCCAGAAGACTCGGCCCTGTAT	
TRBV4-3	ATGGGCTGCAGGCT GCTCTGCTGTGCGG TTCTCTGTCTCCTGG GAGCGGGTGAGTTG GTCCCCATG	GAAACGGGAGTTACGCAGACACCAAGACACCTGGTCATGGGAATGACAAATAAGAAGT CTTTGAAATGTGAACAACATCTGGGTCATAACGCTATGTATTGGTACAAGCAAAGTGCT AAGAAGCCACTGGAGCTCATGTTTGTCTACAGTCTTGAAGAACGGGTTGAAAAACAACAG TGTGCCAAGTCGCTTCTCACCTGAATGCCCCAACAGCTCTCACTTATTCCTTCACCTACAC ACCCTGCAGCCAGAAGACTCGGCCCTGTAT	
TRBV5-1	ATGGGCTCCAGGCT GCTCTGTTGGGTGC TGCTTTGTCTCCTGG GAGCAGGCCCAGTA	AAGGCTGGAGTCACTCAAACTCCAAGATATCTGATCAAAACGAGAGGACAGCAAGTGA CACTGAGCTGCTCCCCTATCTCTGGGCATAGGAGTGTATCCTGGTACCAACAGACCCCAG GACAGGGCCTTCAGTTCCTCTTTGAATACTTCAGTGAGACACAGAGAAACAAAGGAAAC TTCCCTGGTCGATTCTCAGGGCGCCAGTTCTCTAACTCTCGCTCTGAGATGAATGTGAGC ACCTTGGAGCTGGGGGGACTCGGCCCTTTAT	
TRBV5-3	ATGGGCCCCGGGCT CCTCTGCTGGGAAC TGCTTTATCTCCTGG GAGCAGGCCCAGTG	GAGGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCAAGTGA CTCTGAGATGCTCTCCTATCTCTGGGCACAGCAGTGTGTCCTGGTACCAACAGGCCCCGG GTCAGGGGCCCCAGTTTATCTTTGAATATGCTAATGAGTTAAGGAGATCAGAAGGAAAC TTCCCTAATCGATTCTCAGGGCGCCAGTTCCATGACTGTTGCTCTGAGATGAATGTGAGT GCCTTGGAGCTGGGGGGACTCGGCCCTGTAT	
TRBV5-4	ATGGGCCCTGGGCT CCTCTGCTGGGTGC TGCTTTGTCTCCTGG	GAGACTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCAAGTGA CTCTGAGATGCTCTTCTCAGTCTGGGCACAACACTGTGTCCTGGTACCAACAGGCCCTGG GTCAGGGGCCCCAGTTTATCTTTCAGTATTATAGGGAGGAAGAGAAATGGCAGAGGAAAC	

	GAGCAGGCTCAGTG	TTCCCTCCTAGATTCTCAGG <mark>A</mark> CTCCAGTTCCCTAATTATAGCTCTGAGCTGAATGTGAAC GCCTTGGAGCTGGACGACTCGGCCCTGTAT
		BsaI site (T to A)
TRBV5-5	ATGGGCCCTGGGCT CCTCTGCTGGGTGC TGCTTTGTCTCCTGG GAGCAGGCCCAGTG	GACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCAAGTGA CTCTGAGATGCTCTCCTATCTCTGGGCACAAGAGTGTGTCCTGGTACCAACAGGTCCTGG GTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGAAAGAA
TRBV5-6	ATGGGCCCCGGGCT CCTCTGCTGGGGCAC TGCTTTGTCTCCTGG GAGCAGGCTTAGTG	GACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCAAGTGA CTCTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTCCTGGTACCAACAGGCCCTGG GTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGGAGGAAGAGAGAG
TRBV5-7	ATGGGCCCCGGGCT CCTCTGCTGGGTGC TGCTTTGTCCCCTAG GAGAAGGCCCAGTG	GACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCACGTGA CTCTGAGATGCTCTCCTATCTCTGGGCACACCAGTGTGTCCTCGTACCAACAGGCCCTGG GTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGAAAGAA
TRBV5-8	ATGGGACCCAGGCT CCTCTTCTGGGCAC TGCTTTGTCTCCTCG GAACAGGCCCAGTG	GAGGCTGGAGTCACACAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCAAGCGA CTCTGAGATGCTCTCCTATCTCTGGGCACACCAGTGTGTACTGGTACCAACAGGCCCTGG GTCTGGGCCTCCAGTTCCTCCTTTGGTATGACGAGGGTGAAGAGAGAAACAGAGGAAAC TTCCCTCCTAGATTTTCAGGTCGCCAGTTCCCTAATTATAGCTCTGAGCTGAATGTGAAC GCCTTGGAGCTGGAAGACTCGGCCCTGTAT
TRBV6-1	ATGAGCATCGGGCT CCTGTGCTGTGTGG CCTTTTCTCTCCTGT GGGCAAGTCCAGTG AAT	GCTGGTGTCACTCAGACCCCAAAATTCCAGGTCCTGAAGACAGGACAGAGCATGACACT GCAGTGTGCCCAGGATATGAACCATAACTCCATGTACTGGTATCGACAAGACCCAGGCA TGGGACTGAGGCTGATTTATTACTCAGCTTCTGAGGGTACCACTGACAAAGGAGAAGTC CCCAATGGCTACAATGTCTCCAGATTAAACAAACGGGAGTTCTCGCTCAGGCTGGAGTC GGCTGCTCCCTCCCAGACATCTGTGTAC
TRBV6-2	ATGAGCCTCGGGCT CCTGTGCTGTGGGGG CCTTTTCTCTCCTGT GGGCAGGTCCAGTG	AATGCTGGTGTCACTCAGACCCCAAAATTCCGGGTCCTGAAGACAGGACAGAGCATGAC ACTGCTGTGTGCCCAGGATATGAACCATGAATACATGTACTGGTATCGACAAGACCCAG GCATGGGGCTGAGGCTGATTCATTACTCAGTTGGTGAGGGTACAACTGCCAAAGGAGAG GTCCCTGATGGCTACAATGTCTCCAGATTAAAAAAAACAGAATTTCCTGCTGGGGTTGGA GTCGGCTGCCCCCCCCAAACATCTGTGTAC
TRBV6-3	ATGAGCCTCGGGCT CCTGTGCTGTGGGGG CCTTTTCTCTCCTGT GGGCAGGTCCAGTG	AATGCTGGTGTCACTCAGACCCCAAAATTCCGGGTCCTGAAGACAGGACAGAGCATGAC ACTGCTGTGTGCCCAGGATATGAACCATGAATACATGTACTGGTATCGACAAGACCCAG GCATGGGGCTGAGGCTGATTCATTACTCAGTTGGTGAGGGTACAACTGCCAAAGGAGAG GTCCCTGATGGCTACAATGTCTCCAGATTAAAAAAACAGAATTTCCTGCTGGGGTTGGA GTCGGCTGCTCCCTCCCAAACATCTGTGTAC
TRBV6-4	ATGAGAATCAGGCT CCTGTGCTGTGTGG CCTTTTCTCTCCTGT GGGCAGGTCCAGTG	ATTGCTGGGATCACCCAGGCACCAACATCTCAGATCCTGGCAGCAGGACGGCGCATGAC ACTGAGATGTACCCAGGATATGAGACATAATGCCATGTACTGGTATAGACAAGATCTAG GACTGGGGCTAAGGCTCATCCATTATTCAAATACTGCAGGTACCACTGGCAAAGGAGAA GTCCCTGATGGTTATAGTGTCTCCAGAGCAAACACAGAGTGATTTCCCCCTCACGTTGGCG TCTGCTGTACCCTCTCAGACATCTGTGTAC
TRBV6-5	ATGAGCATCGGCCT CCTGTGCTGTGCAG CCTTGTCTCTCCTGT GGGCAGGTCCAGTG	AATGCTGGTGTCACTCAGACCCCAAAATTCCAGGTCCTGAAGACAGGACAGAGCATGAC ACTGCAGTGTGCCCAGGATATGAACCATGAATACATGTCCTGGTATCGACAAGACCCAG GCATGGGGGCTGAGGCTGATTCATTACTCAGTTGGTGCTGGTATCACTGACCAAGGAGAA GTCCCCAATGGCTACAATGTCTCCAGATCAACCACAGAGGATTTCCCGCTCAGGCTGCTG TCGGCTGCTCCCTCCCAGACATCTGTGTAC
TRBV6-6	ATGAGCATCAGCCT CCTGTGCTGTGCAG CCTTTCCTCTCCTGT GGGCAGGTCCAGTG	AATGCTGGTGTCACTCAGACCCCAAAATTCCGCATCCTGAAGATAGGACAGAGCATGAC ACTGCAGTGTACCCAGGATATGAACCATAACTACATGTACTGGTATCGACAAGACCCAG GCATGGGGCTGAAGCTGATTTATTATTCAGTTGGTGCTGGTATCACTGATAAAGGAGAA GTCCCGAATGGCTACAACGTCTCCAGATCAACCACAGAGGATTTCCCGCTCAGGCTGGA GTTGGCTGCTCCCCCGAGACATCTGTGTAC
TRBV6-7	ATGAGCCTCGGGCT	AATGCTGGTGTCACTCAGACCCCAAAATTCCACGTCCTGAAGACAGGACAGAGCATGAC

	CCTGTGCTGTGTGG CCTTTTCTCTCCTGT GGGCAGGTCCAATG	TCTGCTGTGTGCCCAGGATATGAACCATGAATACATGTATCGGTATCGACAAGACCCAG GCAAGGGGCTGAGGCTGATTTACTACTCAGTTGCTGCTGCTGCTCCACTGACAAAGGAGAA GTTCCCAATGGCTACAATGTCTCCAGATCAAACACAGAGGATTTCCCCCTCAAGCTGGA GTCAGCTGCTCCCTCTCAGACTTCTGTTTAC
TRBV6-8	ATGAGCCTCGGGCT CCTGTGCTGTGCGG CCTTTTCTCTCCTGT GGGCAGGTCCCGTG	AATGCTGGTGTCACTCAGACCCCAAAATTCCACATCCTGAAGACAGGACAGAGCATGAC ACTGCAGTGTGCCCAGGATATGAACCATGGATACATGTCCTGGTATCGACAAGACCCAG GCATGGGGCTGAGACTGATTTACTACTCAGCTGCTGCTGGTACTACTGACAAAGAAGTC CCCAATGGCTACAATGTCTCTAGATTAAACACAGAGGATTTCCCACTCAGGCTGGTGTCG GCTGCTCCCTCCCAGACATCTGTGTAC
TRBV6-9	ATGAGCATCGGGCT CCTGTGCTGTGTGGG CCTTTTCTCTCCTGT GGGAGGGTCCAGTG	AATGCTGGTGTCACTCAGACCCCAAAATTCCACATCCTGAAGACAGGACAGAGCATGAC ACTGCAGTGTGCCCAGGATATGAACCATGGATACTTGTCCTGGTATCGACAAGACCCAG GCATGGGGCTGAGGCGCATTCATTACTCAGTTGCTGCTGGTATCACTGACAAAGGAGAA GTCCCCGATGGCTACAATGTATCCAGATCAAACACAGAGGATTTCCCGGCTCAGGCTGGA GTCAGCTGCTCCCTCCCAGACATCTGTATAC
TRBV7-1	ATGGGCACAAGGCT CCTCTGCTGGGGCAG CCATATGTCTCCTG GGGGCAGATCACAC A	GGTGCTGGAGTCTCCCAGTCCCTGAGACACAAGGTAGCAAAGAAGGGAAAGGATGTAG CTCTCAGATATGATCCAATTTCAGGTCATAATGCCCTTTATTGGTACCGACAGAGCCTGG GGCAGGGCCTGGAGTTTCCAATTTACTTCCAAGGCAAGG
TRBV7-2	ATGGGCACCAGGCT CCTCTTCTGGGTGG CCTTCTGTCTCCTGG GGGCAGATCACACA	GGAGCTGGAGTCTCCCAGTCCCCCAGTAACAAGGTCACAGAGAAGGGAAAGGATGTAG AGCTCAGGTGTGATCCAATTTCAGGTCATACTGCCCTTTACTGGTACCGACAGAGCCTGG GGCAGGGCCTGGAGTTTTTAATTTACTTCCAAGGCAACAGTGCACCAGACAAATCAGGG CTGCCCAGTGATCGCTTCTCTGCAGAGAGGACTGGGGGGATCCGTCTCCACTCTGACGATC CAGCGCACACAGCAGGAGGACTCGGCCGTGTAT
TRBV7-3	ATGGGCACCAGGCT CCTCTGCTGGGCAG CCCTGTGCCTCCTG GGGGCAGATCACAC A	GGTGCTGGAGTCTCCCAGACCCCCAGTAACAAGGTCACAGAGAAGGGAAAATATGTAG AGCTCAGGTGTGATCCAATTTCAGGTCATACTGCCCTTTACTGGTACCGACAAAGCCTGG GGCAGGGCCCAGAGTTTCTAATTTACTTCCAAGGCACGGGTGCGGCAGATGACTCAGGG CTGCCCAACGATCGGTTCTTTGCAGTCAGGCCTGAGGGATCCGTCTCTACTCTGAAGATC CAGCGCACAGAGCGGGGGGGACTCAGCCGTGTAT
TRBV7-4	ATGGGCACCAGGCT CCTCTGCTGGGTGG TCCTGGGTTTCCTA GGGACAGATCACAC A	GGTGCTGGAGTCTCCCAGTCCCCAAGGTACAAAGTCGCAAAGAGGGGACGGGATGTAGC TCTCAGGTGTGATTCAATTTCGGGTCATGTAACCCTTTATTGGTACCGACAGACCCTGGG GCAGGGCTCAGAGGTTCTGACTTACTCCCAGAGTGATGCTCAACGAGACAAATCAGGGC GGCCCAGTGGTCGGTTCTCTGCAGAGAGGCCTGAGAGATCCGTCTCCACTCTGAAGATC CAGCGCACAGAGCAGGGGGGACTCAGCTGTGTAT
TRBV7-6	ATGGGCACCAGTCT CCTATGCTGGGTGG TCCTGGGTTTCCTA GGGACAGATCACAC A	GGTGCTGGAGTCTCCCAGTCTCCCAGGTACAAAGTCACAAAGAGGGGACAGGATGTAGC TCTCAGGTGTGATCCAATTTCGGGTCATGTATCCCTTTATTGGTACCGACAGGCCCTGGG GCAGGGCCCAGAGTTTCTGACTTACTTCAATTATGAAGCCCAACAAGACAAATCAGGGC TGCCCAATGATCGGTTCTCTGCAGAGAGGCCTGAGGGATCCATCTCCACTCTGACGATCC AGCGCACAGAGCAGCGGGACTCGGCCATGTAT
TRBV7-7	ATGGGTACCAGTCT CCTATGCTGGGTGG TCCTGGGTTTCCTA GGGACAGATCACAC A	GGTGCTGGAGTCTCCCAGTCTCCCAGGTACAAAGTCACAAAGAGGGGACAGGATGTAAC TCTCAGGTGTGATCCAATTTCGAGTCATGCAACCCTTTATTGGTATCAACAGGCCCTGGG GCAGGGCCCAGAGTTTCTGACTTACTTCAATTATGAAGCTCAACCAGACAAATCAGGGC TGCCCAGTGATCGGTTCTCTGCAGAGAGGGCCTGAGGGATCCATCTCCACTCTGACGATTC AGCGCACAGAGCAGCGGGACTCAGCCATGTAT
TRBV7-8	ATGGGCACCAGGCT CCTCTGCTGGGTGG TCCTGGGTTTCCTA GGGACAGATCACAC A	GGTGCTGGAGTCTCCCAGTCCCCTAGGTACAAAGTCGCAAAGAGAGGACAGGATGTAGC TCTCAGGTGTGATCCAATTTCGGGTCATGTATCCCTTTTTTGGTACCAACAGGCCCTGGG GCAGGGGCCAGAGTTTCTGACTTATTTCCAGAATGAAGCTCAACTAGACAAATCGGGGC TGCCCAGTGATCGCTTCTTTGCAGAAAGGCCTGAGGGATCCGTCTCCACTCTGAAGATCC AGCGCACACAGCAGGAGGACTCCGCCGTGTAT
TRBV7-9	ATGGGCACCAGCCT CCTCTGCTGGATGG CCCTGTGTCTCCTG GGGGCAGATCACGC A	GATACTGGAGTCTCCCAGAACCCCAGACACAAGATCACAAAGAGGGGACAGAATGTAA CTTTCAGGTGTGATCCAATTTCTGAACACAACCGCCTTTATTGGTACCGACAGACCCTGG GGCAGGGCCCAGAGTTTCTGACTTACTTCCAGAATGAAGCTCAACTAGAAAAATCAAGG CTGCTCAGTGATCGGTTCTCTGCAGAGAGGGCCTAAGGGATCTTTCTCCACCTTGGAGATC CAGCGCACAGAGCAGGGGGGACTCGGCCATGTAT
TRBV9	ATGGGCTTCAGGCT CCTCTGCTGTGTGG	GATTCTGGAGTCACACAAACCCCAAAGCACCTGATCACAGCAACTGGACAGCGAGTGAC GCTGAGATGCTCCCCTAGGTCTGGAGA <mark>T</mark> CTCTCTGTGTACTGGTACCAACAGAGCCTGGA

	CCTTTTGTCTCCTGG GAGCAGGCCCAGTG	CCAGGGCCTCCAGTTCCTCATTCAGTATTATAATGGAGAAGAGAGAG
		Bsal site (C to T)
TRBV10-1	ATGGGCACGAGGCT CTTCTTCTATGTGGC CCTTTGTCTGCTGTG GGCAGGACACAGG	GATGCTGAAATCACCCAGAGCCCAAGACACAAGATCACAGAGACAGGAAGGCAGGTGA CCTTGGCGTGTCACCAGACTTGGAACCACAACAATATGTTCTGGTATCGACAAGACCTG GGACATGGGCTGAGGCTGATCCATTACTCATATGGTGTTCAAGACACTAACAAAGGAGA AGTCTCAGATGGCTACAGTGTCTCTAGATCAAACACAGAGGACCTCCCCCTCACTCTGGA GTCTGCTGCCTCCCCAGACATCTGTATAT
TRBV10-2	ATGGGCACCAGGCT CTTCTTCTATGTGGC CCTTTGTCTGCTGTG GGCAGGACACAGG	GATGCTGGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGA CCTTGATGTGTCACCAGACTTGGAGCCACAGCTATATGTTCTGGTATCGACAAGACCTGG GACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGATATTACAGATAAAGGAGAA GTCCCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGGAG TCAGCTACCCGCTCCCAGACATCTGTGTAT
TRBV10-3	ATGGGCACAAGGTT GTTCTTCTATGTGGC CCTTTGTCTCCTGTG GACAGGACACATG GACAGGACACATG GACAGGACACATG GCCCCCCAGGCTGAGGCTGATCCATTACTCATATGGTGTTAAAGATACTGACAA AGTCTCAGATGGCTATAGTGTCTCTAGATCAAAGACAGAGGATTTCCTCCTCA GTCCGCTACCAGCTCCCAGACATCTGTAC	
TRBV11-1	ATGAGCACCAGGCT TCTCTGCTGGATGG CCCTCTGCTCCCGG GGGCAGAACTCTCA GCCTAGGATCGATCCTATTTCTGGCCAGGCCA	
TRBV11-2	ATGGGCACCAGGCT CCTCTGCTGGCGGG CCCTCTGTCTCCTGG GAGCAGAACTCACA	GAAGCTGGAGTTGCCCAGTCTCCCAGATATAAGATTATAGAGAAAAGGCAGAGTGTGGC TTTTTGGTGCAATCCTATATCTGGCCATGCTACCCTTTACTGGTACCAGCAGATCCTGGG ACAGGGCCCAAAGCTTCTGATTCAGTTCAG
TRBV11-3	ATGGGTACCAGGCT CCTCTGCTGGGTGG CCTTCTGTCTCCTGG TGGAAGAACTCATA	GAAGCTGGAGTGGTTCAGTCTCCCAGATATAAGATTATAGAGAAAAAAACAGCCTGTGGC TTTTTGGTGCAATCCTATTTCTGGCCACAATACCCTTTACTGGTACCTGCAGAACTTGGG ACAGGGCCCGGAGCTTCTGATTCGATATGAGAATGAGGAAGCAGTAGACGATTCACAGT TGCCTAAGGATCGATTTTCTGCAGAGAGGCTCAAAGGAGTAGACTCCACTCTCAAGATC CAGCCTGCAGAGCTTGGGGACTCGGCCGTGTAT
TRBV12-3	ATGGACTCCTGGAC CTTCTGCTGTGTGTGTC CCTTTGCATCCTGGT AGCGAAGCATACA	GATGCTGGAGTTATCCAGTCACCCCGCCATGAGGTGACAGAGATGGGACAAGAAGTGAC TCTGAGATGTAAACCAATTTCAGGCCACAACTCCCTTTTCTGGTACAGACAG
TRBV12-4	ATGGGCTCCTGGAC CCTCTGCTGTGTGTC CCTTTGCATCCTGGT AGCAAAGCACACA	GATGCTGGAGTTATCCAGTCACCCCGGCACGAGGTGACAGAGATGGGACAAGAAGTGA CTCTGAGATGTAAACCAATTTCAGGACACGACTACCTTTTCTGGTACAGACAG
TRBV12-5	ATGGCCACCAGGCT CCTCTGCTGTGTGG TTCTTTGTCTCCTGG GAGAAGAGCTTATA	GATGCTAGAGTCACCCAGACACCAAGGCACAAGGTGACAGAGATGGGACAAGAAGTAA CAATGAGATGTCAGCCAATTTTAGGCCACAATACTGTTTTCTGGTACAGACAG
TRBV13	ATGCTTAGTCCTGA CCTGCCTGACTCTG CCTGGAACACCAGG CTCCTCTGCCATGTC ATGCTTTGTCTCCTG GGAGCAGTTTCAGT G	GCTGCTGGAGTCATCCAGTCCCCAAGACATCTGATCAAAGAAAAGAGGGAAACAGCCAC TCTGAAATGCTATCCTATC

TRBV14	ATGGTTTCCAGGCT TCTCAGTTTAGTGTC CCTTTGTCTCCTGGG AGCAAAGCACATA	GAAGCTGGAGTTACTCAGTTCCCCAGCCACAGCGTAATAGAGAAGGGCCAGACTGTGAC TCTGAGATGTGACCCAATTTCTGGACATGATAATCTTTATTGGTATCGACGTGTTATGGG AAAAGAAATAAAATTTCTGTTACATTTTGTGAAAGAGTCTAAACAGGATGAGTCCGGTA TGCCCAACAATCGATTCTTAGCTGAAAGGACTGGAGGGACGTATTCTACTCTGAAGGTG CAGCCTGCAGAACTGGAGGATTCTGGAGTTTAT
TRBV15	ATGGGTCCTGGGCT TCTCCACTGGATGG CCCTTTGTCTCCTTG GAACAGGTCATGGG	GATGCCATGGTCATCCAGAACCCAAGATACCAGGTTACCCAGTTTGGAAAGCCAGTGAC CCTGAGTTGTTCTCAGACTTTGAACCATAACGTCATGTACTGGTACCAGCAGAAGTCAAG TCAGGCCCCAAAGCTGCTGTTCCACTACTATGACAAAGATTTTAACAATGAAGCAGACA CCCCTGATAACTTCCAATCCAGGAGGCCGAACACTTCTTTCT
TRBV16	ATGAGCCCAATATT CACCTGCATCACAA TCCTTTGTCTGCTGG CTGCAGGTTCTCCT	GGTGAAGAAGTCGCCCAGACTCCAAAACATCTTGTCAGAGGGGAAGGACAGAAAGCAA AATTATATTGTGCCCCCAATAAAAGGACACAGTTATGTTTTTTGGTACCAACAGGTCCTGA AAAACGAGTTCAAGTTCTTGATTTCCTTCCAGAATGAAAATGTCTTTGATGAAACAGGTA TGCCCAAGGAAAGATTTTCAGCTAAGTGCCTCCCAAATTCACCCTGTAGCCTTGAGATCC AGGCTACGAAGCTTGAGGATTCAGCAGTGTAT
TRBV18	ATGGACACCAGAGT ACTCTGCTGTGCGG TCATCTGTCTTCTGG GGGCAGG <mark>A</mark> CTCTCA Bsal (T to A)	AATGCCGGCGTCATGCAGAACCCAAGACACCTGGTCAGGAGGAGGGGACAGGAGGCAA GACTGAGATGCAGCCCAATGAAAGGACACAGTCATGTTTACTGGTATCGGCAGCTCCCA GAGGAAGGTCTGAAATTCATGGTTTATCTCCAGAAAGAAA
TRBV19	ATGAGCAACCAGGT GCTCTGCTGTGGG GCTCTGCTGTGGG TCCTTTGTTTCCTGG GAGCAAACACCGTG GAGCAAACACCGTG ATAGCTGAAGGGCTGAGATTGATCTACTACTACAGAAAGGAATGACTTTCAC ATAGCTGAAGGGTACAGCGTCTCTCGGGAGAAGAAGGAATCCTTTCCTC ATCGGCCCAAAAGAACCCGACAGCTTTCTAT	
TRBV20-1	ATGCTGCTGCTTCT GCTGCTTCTGGGGC CAGGTATAAGCCTC CTTCTACCTGGGAG CTTGGCAGGCTCCG GGCTT	GGTGCTGTCGTCTCTCAACATCCGAGCTGGGTTATCTGTAAGAGTGGAACCTCTGTGAAG ATCGAGTGCCGTTCCCTGGACTTTCAGGCCACAACTATGTTTTGGTATCGTCAGTTCCCG AAACAGAGTCTCATGCTGATGGCAACTTCCAATGAGGGCTCCAAGGCCACATACGAGCA AGGCGTCGAGAAGGACAAGTTTCTCATCAACCATGCAAGCCTGACCTTGTCCACTCTGA CAGTGACCAGTGCCCATCCTGAAGACAGCAGCTTCTAC
TRBV24-1	ATGGCCTCCCTGCT CTTCTTCTGTGGGG CCTTTTATCTCCTGG GAACAGGGTCCATG	GATGCTGATGTTACCCAGACCCCAAGGAATAGGATCACAAAGACAGGAAAGAGGATTA TGCTGGAATGTTCTCAGACTAAGGGTCATGATAGAATGTACTGGTATCGACAAGACCCA GGACTGGGCCTACGGTTGATCTATTACTCCTTTGATGTCAAAGATATAAACAAAGGAGA GATCTCTGATGGATACAGTGTCTCTCGACAGGCACAGGCTAAATTCTCCCTGTCCCTAGA GTCTGCCATCCCCAACCAGACAGCTCTTTAC
TRBV25-1	ATGACTATCAGGCT CCTCTGCTACATGG GCTTTTATTTTCTGG GGGCAGGCCTCATG	GAAGCTGACATCTACCAGACCCCAAGATACCTTGTTATAGGGACAGGAAAGAAGATCAC TCTGGAATGTTCTCAAACCATGGGCCATGACAAAATGTACTGGTATCAACAAGATCCAG GAATGGAACTACACCTCATCCACTATTCCTATGGAGTTAATTCCACAGAGAAGGGAGAT CTTTCCTCTGAGTCAACAGTCTCCAGAATAAGGACGGAGCATTTTCCCCTGACCCTGGAG TCTGCCAGGCCCTCACATACCTCTCAGTAC
TRBV27	ATGGGCCCCCAGCT CCTTGGCTATGTGG TCCTTTGCCTTCTAG GAGCAGGCCCCCTG	GAAGCCCAAGTGACCCAGAACCCAAGATACCTCATCACAGTGACTGGAAAGAAGTTAAC AGTGACTTGTTCTCAGAATATGAACCATGAGTATATGTCCTGGTATCGACAAGACCCAG GGCTGGGCTTAAGGCAGATCTACTATTCAATGAATGTTGAGGTGACTGATAAGGGAGAT GTTCCTGAAGGGTACAAAGTCTCTCGAAAAGAGAAGAAGAGGAATTTCCCCCTGATCCTGGA GTCGCCCAGCCCCAACCAGACCTCTCTGTAC
TRBV28	ATGGGAATCAGGCT CCTGTGTCGTGTGG CCTTTTGTTTCCTGG CTGTAGGCCTCGTA	GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGGAGAGAAAGTTTT TCTGGAATGTGTCCAGGATATGGACCATGAAAATATGTTCTGGTATCGACAAGACCCAG GTCTGGGGCTACGGCTGATCTATTTCTCATATGATGTTAAAATGAAAGAAA
TRBV29-1	ATGCTGAGTCTTCT GCTCCTTCTCCTGG GACTAGGCTCTGTG TTC	AGTGCTGTCATCTCTCAAAAGCCAAGCAGGGATATCTGTCAACGTGGAACCTCCCTGAC GATCCAGTGTCAAGTCGATAGCCAAGTCACCATGATGTTCTGGTACCGTCAGCAACCTG GACAGAGCCTGACACTGATCGCAACTGCAAATCAGGGCTCTGAGGCCACATATGAGAGT GGATTTGTCATTGACAAGTTTCCCATCAGCCGCCCAAACCTAACATTCTCAACTCTGACT

		GTGAGCAACATGAGCCCTGAAGACAGCAGCATATAT
TRBV30	ATGCTCTGCTCTCTC CTTGCCCTTCTCCTG GGCACTTTCTTTGG GGTCAGA	TCTCAGACTATTCATCAATGGCCAGCGACCCTGGTGCAGCCTGTGGGCAGCCCGCTCTCT CTGGAGTGCACTGTGGAGGGAACATCAAACCCCAACCTATACTGGTACCGACAGGCTGC AGGCAGGGGCCTCCAGCTGCTCTTCTACTCCGTTGGTATTGGCCAGATCAGCTCTGAGGT GCCCCAGAATCTCTCAGCCTCCAGACCCCAGGACCGGCAGTTCATCCTGAGTTCTAAGA AGCTCCTTCTCAGTGACTCTGGCTTCTAT

TCR	Vα-Cβ Library	Vβ-Cα Library	Oligo Sequence
EBNA3A	TRAV8-1	TRBV5-1	TCTAGATGGGGATCCGGTCTCTGTACTTCTGTGCTGGTCGCCTCGTTGATCAG GGCGGTAAACTGATCTTCGGACAGGGAACGGAGTTATCTGTGAAACCCAATA TCCAGAACCCTGACTGAGACCATGCGGTGGTGGTGGTTCTGGTTCTGGTTCTG GTGGTGGTTCTGGTGGTGGTCTCTTTTATCTTTGCGCTTCTAGTATCGGCCTGG CCGGCTATGAGCAGTACTTCGGGCCGGGC
D1.1	TRAV38-2/DV8	TRBV7-9	TCTAGATGGGGATCCGGTCTCTGTATTTCTGTGCTTATATTCAGGGAGCCCAG AAGCTGGTATTTGGCCAAGGAACCAGGCTGACTATCAACCCAAATATCCAGA ACCCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTTCTGGTTCTGGTGGTG GTTCTGGTGGTGGTCTCTTGTATCTCTGTGCCAGCAGCTTAGGTGGGGGGGG
D1.2	TRAV21	TRBV10-2	TCTAGATGGGGATCCGGTCTCTCTACCTCTGTGCTGTCCTCATGGATAGCAAC TATCAGTTAATCTGGGGCGCTGGGACCAAGCTAATTATAAAGCCAGATATCC AGAACCCTGACAGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTGGTG GTGGTTCTGGTGGTGGTGGTCTCTTGTATTTCTGCGCCAGCAGTTCGGACGGA
D1.3	TRAV5	TRBV29-1	TCTAGATGGGGATCCGGTCTCTCTACTTCTGTGCAGAGAGTACAGGCAAACTA ATCTTTGGGCAAGGGACAACTTTACAAGTAAAACCAGATATCCAGAACCCTG ACAGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTGGTGGTGGTGGTG
D1.4	TRAV12-2	TRBV19	TCTAGATGGGGATCCGGTCTCTCTACCTCTGTGCCGGCGACTTTGAAGGCTCT GGCAACACAGGCAAACTAATCTTTGGGCAAGGGACAACTTTACAAGTAAAAC CAGATATCCAGAACCCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTTCTG GTTCTGGTGGTGGTTCTGGTGGTGGTCTCTTCTATCTCTGTGCCAGTAGCATCA GGTCCGCCTACGAGCAGTACTTCGGGCCGGGC
D1.5	TRAV4	TRBV12-3	TCTAGATGGGGATCCGGTCTCTGTACTACTGCCTCGTGCCCCGGAGAGATGAC AAGATCATCTTTGGAAAAGGGACACGACTTCATATTCTCCCCAATATCCAGAA CCCTGACAGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTGGTGGTGGTGG TTCTGGTGGTGGTCTCTTGTACTTCTGTGCCAGCAGTCCTACCAGGGGCGGGG GACTTGAAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCTGTCTTGGAGGAC CTAGAGACCAATCAAATCGGATCC
D1.6	TRAV19	TRBV19	TCTAGATGGGGATCCGGTCTCTATACTTCTGTGCTCTGAGTGAG
D1.7	TRAV3	TRBV19	TCTAGATGGGGATCCGGTCTCTGTACTTCTGTGCTGTGAGAGAGA
D1.8	TRAV27	TRBV19	TCTAGATGGGGATCCGGTCTCTCTACCTCTGTGGGTATGGAGGAAGCCAAGG AAATCTCATCTTTGGAAAAGGCACTAAACTCTCTGTTAAACCAAATATCCAGA ACCCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTTCTGGTTCTGGTGGTG GTTCTGGTGGTGGTCTCTTCTATCTCTGTGCCAGTAGTATTCGTTCG
D1.9	TRAV13-1	TRBV19	TCTAGATGGGGATCCGGTCTCTCTACTTCTGTGCAGCAAGCGGGGGAGGAAG CCAAGGAAATCTCATCTTTGGAAAAGGCACTAAACTCTCTGTTAAACCAAATA TCCAGAACCCTGACAGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTG GTGGTGGTTCTGGTGGTGGTCTCTTCTATCTCTGTGCCAGTAGTCCCCGGTCGG GTATCGAGCAGTACTTCGGGCCGGGC
D2.1	TRAV21	TRBV10-2	TCTAGATGGGGATCCGGTCTCTCTACCTCTGTGCTGTCCTCATGGATAGCAAC TATCAGTTAATCTGGGGCGCTGGGACCAAGCTAATTATAAAGCCAGATATCC AGAACCCTGACTGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTGGTTCTGGTG GTGGTTCTGGTGGTGGTCTCTTGTATTTCTGCGCCAGCCA

Table S2.	CDR3	oligonucleotide	sequences	(CDR3α,	CDR3 _β)) used to	clone	TCRs
		-	-					

			AACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTAGAGGACC
D2.2		TDD1/11.1	TCTAGATGGGGATCCGGTCTCTGTATTCTGTGCTTATAGGAGTATGTAT
D2.2	1KAV38-2/DV8	IKBVII-I	GGAGGAGGTGCTGACGGACTCACCTTTGGCAAAGGGACTCATCTAATCATCC
			AGCCCTATATCCAGAACCCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTT
			CTGGTTCTGGTGGTGGTTCTGGTGGTGGTCTCTTGTATCTCTGTGCCAGCAGCC
			TAGGGTACGGCAATCAGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATC
			CTAGAGGACCTAGAGACCAATCAAATCGGATCC
D2.3	TRAV2	TRBV9	TCTAGATGGGGATCCGGTCTCTTTACTACTGTGCTGTGGACAACCAGGCAGG
			ACTGCTCTGATCTTTGGGAAGGGAACCACCTTATCAGTGAGTTCCAATATCCA
			GAACCCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTTCTGGTTCTGGTGG
			TGGTTCTGGTGGTGGTCTCTTGTATTTCTGTGCCAGCAGCGTAGAAGGGACGT
D2.4	TRAV12-1	TRBV27	TACAAGCTCAGCTTTGGAGCCGGAACCACAGTAACTGTAAGAGCAAAACCIAACGAC
			AGA ACCCTGACTGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTGGTG
			GTGGTTCTGGTGGTGGTCTCTTGTACTTCTGTGCCAGCAGCAGTGGGACTAGC
			GGGTATTACAATGAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTGCTAG
			AGGACCTTGAGACCAATCAAATCGGATCC
D2.5	TRAV38-1	TRBV19	TCTAGATGGGGATCCGGTCTCTGTATTTCTGTGCTTTCATGACGAATGCTGGT
22.0	11011001	1112 (1)	GGTACTAGCTATGGAAAGCTGACATTTGGACAAGGGACCATCTTGACTGTCC
			ATCCAAATATCCAGAACCCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTT
			CTGGTTCTGGTGGTGGTTCTGGTGGTGGTGGTCTCTTCTATCTCTGTGCCAGTAGTG
			CCGGAAGCTATGGCTACACCTTCGGTTCGGGGACCAGGTTAACCGTTGTAGA
			GGACCTAGAGACCAATCAAATCGGATCC
D2.6	TRAV12-3	TRBV29-1	
			GTGGTTCTGGTGGTGGTCTCTTATATCTCTGCAGCGTCCCAGGCCTTTTGAACA
			CTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTAGAGGACCTAGA
			GACCAATCAAATCGGATCC
P1 1	TRAV20	TRBV5-1	TCTAGATGGGGATCCGGTCTCTTTATCTCTGTGCTGGTGCTGGTGGTACTAGCT
1 1.1	1101120	1100 1 2	ATGGAAAGCTGACATTTGGACAAGGGACCATCTTGACTGTCCATCCA
			CAGAACCCTGACAGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTGGTTCTGGT
			GGTGGTTCTGGTGGTGGTCTCTTTTATCTTTGCGCCAGCAGCTCCGGGACAGG
			GGGAAACACCGGGGAGCTGTTTTTTGGAGAAGGCTCTAGGCTGACCGTACTG
P1.2	TRAV22	TRBV29-1	
			TGGTGGTTCTGGTGGTGGTGGTCTCTTATATCTCTGCAGCGCTCGACAGGGCCTCG
			ATCAGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATCCTAGAGGACCTT
			GAGACCAATCAAATCGGATCC
P1 3		TRBV29-1	TCTAGATGGGGATCCGGTCTCTGTACTTCTGTGCAGCAAGCGCTAATGCTGGT
11.5	1101125/1015	11(1)(2) 1	GGTACTAGCTATGGAAAGCTGACATTTGGACAAGGGACCATCTTGACTGTCC
			ATCCAAATATCCAGAACCCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTT
			CTGGTTCTGGTGGTGGTTCTGGTGGTGGTCTCTTATATCTCTGCAGCACATCCG
			GGACAGGGTTCCCCGGGGAGCTGTTTTTTGGAGAAGGCTCTAGGCTGACCGT
			ACTGGAGGACCTAGAGACCAATCAAATCGGATCC
P1.4	TRAV8-4	TRBV11-1	
			GTGGTTCTGGTGGTGGTCTCTTTGTATCTCTGTGCCAGCAGCTTAGTTCCGGAA
			ACCTACGAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGGACC
			TAGAGACCAATCAAATCGGATCC
P1 5	TRAV8-6	TRBV10-3	TCTAGATGGGGATCCGGTCTCTGTACTTCTGTGCTGTGATCCCCACCTCAGGA
11.5	11111 0-0	TKD V 10-5	ACCTACAAATACATCTTTGGAACAGGCACCAGGCTGAAGGTTTTAGCAAATA
		1	TCCAGAACCCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTTCTGGTTCTG
		1	GTGGTGGTTCTGGTGGTGGTCTCTTGTACTTCTGTGCCATCAGGGGAAAGACG
		1	GGCACCTACGAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGG
		l	
P1.6	TRAV9-2	TRBV6-6	TUTAGATGGGGATCCGGTCTUTGTACTTCTGTGCTCTGAGAGTCCCTTCTGGTT
		1	
		1	
		1	GGCGGCGAACACCGGGGAGCTGTTTTTTGGAGAAGGCTCTAGGCTGACCGTA
		1	CTGGAGGACCTAGAGACCAATCAAATCGGATCC
P3 1	TRAV21	TRBV20 1	TCTAGATGGGGATCCGGTCTCTCTACCTCTCTACTACCAACACACCC
1.5.1	11111121	110 127-1	

			AAACTAATCTTTGGGCAAGGGACAACTTTACAAGTAAAACCAGATATCCAGA ACCCTGACAGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTGGTGGTGGTG
			GTTCTGGTGGTGGTCTCTTATATCTCTGCAGCGTTTTTGGCGGTGGCCTCTTCT
			AGAGACCAATCAGATCGGATCC
P3.2	TRAV12-1	TRBV27	TCTAGATGGGGATCCGGTCTCTCTACCTCTATCATAACCAGGGAGGAAAGCTT
			ATCTTCGGACAGGGAACGGAGTTATCTGTGAAACCCAATATCCAGAACCCTG
			GTGGTGGTCTCTTGTACTTCTGTGCCAGCAGTCCCTCGTTGGCCGGGGAGCTG
			TTTTTTGGAGAAGGCTCTAGGCTGACCGTACTGGAGGACCTAGAGACCAATC
			AAATCGGATCC
P3.3	TRAV38-1	TRBV18	TCTAGATGGGGATCCGGTCTCTGTATTTCTGTGCTTTCATGAAGCCCCCTACA
			TGGTGGTTCTGGTGGTGGTCGTCTCTTTTTTCTGTGCCAGCTCACCGTACGGACA
			GAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTAGAGGAC
			CTAGAGACCAATCAAATCGGATCC
P3.4	TRAV14/DV4	TRBV5-4	TCTAGATGGGGATCCGGTCTCTGTACTTCTGTGCAATGAGAGAGGGTAATAAT
			CTGGTGGTGGTTCTGGTGGTGGTGGTCTCTTGTATCTCTGTGCCAGCAGCTTGGGT
			GGAGGCCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATCCTAGAGGACCT
			AGAGACCAATCAAATCGGATCC
P3.5	TRAV8-2	TRBV12-3	TCTAGATGGGGATCCGGTCTCTGTACTTCTGTGTTGTGAGTGA
			GTTCTGGTGGTGGTCTCTTGTACTTCTGTGCCAGCAGTACCGCTAGAGGGGGC
			GTACGTCAGCCCCAGCATTTTGGTGATGGGACTCGACTC
			CCTAGAGACCAATCAAATCGGATCC
P3.6	TRAV38-1	TRBV6-2	TCTAGATGGGGATCCGGTCTCTGTATTTCTGTGCTTTCATGAAGCCGGGCAAC
			CCAGAACCCTGACAGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTGGTTCTGG
			TGGTGGTTCTGGTGGTGGTCTCTTGTACTTCTGTGCCAGCACCTTGACAGGGC
			ATTCTAGTGAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTGCTAGAGGA
			CCTAGAGACCAATCAAATCGGATCC
P3.7	TRAV8-2	TRBV15	
			AGAACCCTGACAGAGACCATGCGGTGGTGGTGGTGGTTCTGGTTCTGGTTCTGGTG
			GTGGTTCTGGTGGTGGTCTCTTGTACCTGTGTGCCACCAGCAGCCCGCTGGGC
			GGGACTAGGGGGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATCCTAGA
			GGACCTAGAGACCAATCAAATCGGATCC
C-1	TRAV5	TRBV5-1	
			TCCAGAACCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTTCTGGTTCTG
			GTGGTGGTTCTGGTGGTGGTCTCTTTTATCTTTGCGCCAGCAGCCCCCTAGTCG
			CCACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTAGAGGACCTA
C-2	TRAV26-1	TRBV27	
			CTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTTCTGGTGCTGGTGGTGGTT
			CTGGTGGTGGTCTCTTGTACTTCTGTGCCAGCAGTTTAGACAGCTACAATGAG
			CAGTTCTTCGGGCCAGGGACACGGCTCACCGTGCTAGAGGACCTAGAGACCA
		TDDIVIS	
C-3	TRAV12-1	TRBV12-3	GGTCAGAATTTTGTCTTTGGTCCCGGAACCAGATTGTCCGTGCCCTATAT
			CCAGAACCCTGACAGAGACCATGCGGTGGTGGTGGTTCTGGTTCTGGTTCTGG
			TGGTGGTTCTGGTGGTGGTCTCTTGTACTTCTGTGCCAGCAGTTCTGTGACAGC
			GTCCGACATGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTG
1		1	TAUAUUAUTAUAUAUAATUAAATUUUATUU

Table S3. Peptide sequences

HLA Allele	Virus/Antigen Name	Peptide Sequence
A*24:02	EBV EBNA3A246-254	RYSIFFDYM
A*24:02	EBV BRLF-1 ₁₉₈₋₂₀₆	TYPVLEEMF

Table S3A. EBNA3A and BRLF1 peptide sequences

 Table S3B. CEF peptide sequences

HLA Allele	Virus/Antigen Name	Peptide Sequence
A*01	Influenza A	VSDGGPNLY
	Influenza A	CTELKLSDY
A*02	Influenza M	GILGFVFTL
	Influenza A	FMYSDFHFI
	EBV LMP2A	CLGGLLTMV
	EBV BMLF1259-267	GLCTLVAML
A*02:01	HCMV pp65	NLVPMVATV
A*68	Influenza NP	KTGGPIYKR
A*03	Influenza NP	RVLSFIKGTK
	Influenza A	ILRGSVAHK
	EBV	RVRAYTYSK
	EBV	RLRAEAQVK
A*03, A*11, A*06	Influenza M	SIIPSGPLK
A*11	EBV EBNA 4NP	AVFDRKSDAK
	EBV	IVTDFSVIK
	EBV	ATIGTAMYK
A*24	EBV RTA	DYCNVLNKEF
B*07	Influenza NP	LPFDKTTVM
	EBV	RPPIFIRRL
B*08	Influenza NP	ELRSRYWAI
	EBV BZLF-1	RAKFKQLL
	EBV EBNA 3A	FLRGRAYGL
	EBV EBNA 3	QAKWRLQTL
B*18	HCMV	SDEEEAIVAYTL
B*27	Influenza NP	SRYWAIRTR
	Influenza M	ASCMGLIY

	EBV EBNA 3C	RRIYDLIEL	
B*35	EBV EBNA3A	YPLHEQHGM	
	CMV pp65	IPSINVHHY	
B*44	EBV	EENLLDFVRF	
	HCMV	EFFWDANDIY	
B*07:02	HCMV	TPRVTGGGAM	

 Table S3C. Melanoma neoantigen peptide sequences

Patient	Antigen Name	Peptide Sequence (predicted HLA class I binding)
Patient 1		IMP: LCPREEFLRLCKKIMMRSIQ
		ASP CASP5-1: LCPREEFLRLCKKIM
	Mut-CASP5	ASP CASP5-2: EEFLRLCKKIMMRSIQ
		IMP: SGSPPLRVSVGDFSQEFSPIQEAQQD
		ASP RUSC2-1: SGSPPLRVSVGDFSQ
		ASP RUSC2-2: PLRVSVGDFSQEFSP
		ASP RUSC2-3: SVGDFSQEFSPIQEA
	Mut-RUSC2	ASP RUSC2-4: DFSQEFSPIQEAQQD
		IMP: SHNELADSGIPENSFNVSSLVE
		ASP LUM-1: SHNELADSGIPENSF
		ASP LUM-1: LADSGIPENSFNVSS
	Mut-LUM	ASP LUM-1: SGIPENSFNVSSLVE
Patient 3		IMP: RGRLPAGAVRTLLSQVNKVWDQSS
		EPT4A: TLLSQVNKV (HLA-A*02:01) (a)
	Mut-CIT	EPT4C: VRTLLSQVNK (HLA-B*27:05) (b)
		IMP: ERFWRNILLLSLHKGSLYPRIPGLGKE
		EPT27A: WRNILLLSLH (HLA-B*27:05) (a)
	Mut-CASP1	EPT27C: SLHKGSLYPR (HLA-A*03:01) (b)
		IMP: GHEHQPDMQKSLLRAAFFGKCFLDR
		EPT17A: SLLRAAFFGK (HLA-A*03:01) (a)
	Mut-VPS16	EPT17C: LRAAFFGKCF (HLA-B*27:05) (b)
		IMP: SPGPRTAPRPGSQKQAGKDWQ
	Mut-ENDOV	EPT7A: RTAPRPGSQK (HLA-A*03:01)
		IMP: HASHLQEHQRIYTGEKPFKCDT
	Mut-ZNF234	EPT14A: RIYTGEKPFK (HLA-A*03:01)
		IMP: EDLDANLRKLNFRLFVIRGQPAD
		EPT26A: KLNFRLFVI (HLA-A*02:01) (a)
		EPT26C: RKLNFRLFVI (HLA-B*27:05) (b)
	Mut-CRY1	EPT26D: KLNFRLFVIR (HLA-A*03:01) (c)
		IMP: ELQYRGRELRFNLIANQHLLAPGFVSETR
	Mut-ADAMTS7	EPT30A: LRFNLIANQH (HLA-B*27:05)

Predicted HLA class I binding	Antigen Name	Peptide Sequence
A*02:01	Mut- <i>MGA_106</i>	NLDQRLLMV
A*02:01	Mut-MGA _94	RLLMVHCPL
A*02:01	Mut-ITPKB	GLLHGLLLI
A*02:01	Mut- <i>RBBP6</i>	LLHHPQYHL
A*02:01	Mut-ZNF449	FTNSGSFAV

 Table S3D. CLL neoantigen peptide sequences

Table S4. TCR and housekeeping gene primer sequences

Table S4A. Sequences of TRAC and TRAV primers used in RT-PCR1. Obtained from Han et al, 2014¹⁰, with additional TRAV primers that we designed highlighted in blue.

Name	Sequence 5' to 3'
TRAC-PCR1	CGGTGAATAGGCAGACAGACTTGT
TRAV1-1,2-PCR1	CTGCACGTACCAGACATCTGGGTT
TRAV2-PCR1	GGCTCAAAGCCTTCTCAGCAGG
TRAV3-PCR1	GGATAACCTGGTTAAAGGCAGCTA
TRAV4-PCR1	GGATACAAGACAAAAGTTACAAACGA
TRAV5-PCR1	GCTGACGTATATTTTTTCAAATATGGA
TRAV6-PCR1	GGAAGAGGCCCTGTTTTCTTGCT
TRAV7-PCR1	GCTGGATATGAGAAGCAGAAAGGA
TRAV8-2, 4, 6-PCR1	AGGACTCCAGCTTCTCCTGAAGTA
TRAV8-1-PCR1	GTATGTCCAGTACCCTGGTCAA
TRAV8-3-PCR1	CAGGAGACACTCTGGTTCAAG
TRAV9-1, 2-PCR1	GTATGTCCAATATCCTGGAGAAGGT
TRAV10-PCR1	CAGTGAGAACACAAAGTCGAACGG
TRAV12-1-PCR1	CCTAAGTTGCTGATGTCCGTATAC
TRAV12-2-PCR1	GGGAAAAGCCCTGAGTTGATAATGT
TRAV12-3-PCR1	GCTGATGTACACATACTCCAGTGG
TRAV13-1-PCR1	CCCTTGGTATAAGCAAGAACTTGG
TRAV13-2-PCR1	CCTCAATTCATTATAGACATTCGTTC
TRAV14/DV4-PCR1	GCAAAATGCAACAGAAGGTCGCTA
TRAV16-PCR1	TAGAGAGAGCATCAAAGGCTTCAC
TRAV17-PCR1	CGTTCAAATGAAAGAGAGAAACACAG
TRAV18-PCR1	CCTGAAAAGTTCAGAAAACCAGGAG
TRAV19-PCR1	GGTCGGTATTCTTGGAACTTCCAG
TRAV20-PCR1	GCTGGGGAAGAAAGGAGAAAGAAA
TRAV21-PCR1	GTCAGAGAGAGCAAACAAGTGGAA
TRAV22-PCR1	GGACAAAACAGAATGGAAGATTAAGC
TRAV23/DV6-PCR1	CCAGATGTGAGTGAAAAGAAAGAAG
TRAV24-PCR1	GACTTTAAATGGGGATGAAAAGAAGA
TRAV25-PCR1	GGAGAAGTGAAGAAGCAGAAAAGAC
TRAV26-1-PCR1	CCAATGAAATGGCCTCTCTGATCA
TRAV26-2-PCR1	GCAATGTGAACAACAGAATGGCCT
TRAV27-PCR1	GGTGGAGAAGTGAAGAAGCTGAAG
TRAV29/DV5-PCR1	GGATAAAAATGAAGATGGAAGATTCAC
TRAV30-PCR1	CCTGATGATATTACTGAAGGGTGGA

TRAV34-PCR1	GGTGGGGAAGAGAAAAGTCATGAA
TRAV35-PCR1	GGTGAATTGACCTCAAATGGAAGAC
TRAV36/DV7-PCR1	GCTAACTTCAAGTGGAATTGAAAAGA
TRAV36/DV7-PCR1.2	ACGGCAGGAAAAGAAAGCTCCCA
TRAV38-1,2-PCR1	GAAGCTTATAAGCAACAGAATGCAAC
TRAV39-PCR1	GGAGCAGTGAAGCAGGAGGGAC
TRAV40-PCR1	GAGAGACAATGGAAAACAGCAAAAAC
TRAV41-PCR1	GCTGAGCTCAGGGAAGAAGAAGC

Table S4B. Sequences of TRBC and TRBV primers used in RT-PCR1. Obtained from Han et al, 2014¹⁰, with additional TRBV primers that we designed highlighted in blue.

Name	Sequence 5' to 3'	
TRBC-PCR1	ACCAGTGTGGCCTTTTGGGTGTG	
TRBV2-PCR1	CTGAAATATTCGATGATCAATTCTCAG	
TRBV3-1-PCR1	TCATTATAAATGAAACAGTTCCAAATCG	
TRBV4-1, 2, 3-PCR1	AGTGTGCCAAGTCGCTTCTCAC	
TRBV5-1-PCR1	GAGACACAGAGAAACAAAGGAAACTTC	
TRBV5-4,8-PCR1	CAGAGGAAACTYCCCTCCTAGATT	
TRBV5-5,6,7-PCR1	CCAGTTCCCTAACTATAGCTCTGA	
TRBV6-1-PCR1	GGTACCACTGACAAAGGAGAAGTCC	
TRBV6-2,3-PCR1	GAGGGTACAACTGCCAAAGGAGAGGT	
TRBV6-4-PCR1	GGCAAAGGAGAAGTCCCTGATGGTT	
TRBV6-5,6-PCR1	AAGGAGAAGTCCCSAATGGCTACAA	
TRBV6-8-PCR1	CTGACAAAGAAGTCCCCAATGGCTAC	
TRBV6-9-PCR1	CACTGACAAAGGAGAAGTCCCCGAT	
TRBV6-9-PCR1.2	TCGTGCTGCTGGTATCACTGACAA	
TRBV7-2-PCR1	AGACAAATCAGGGCTGCCCAGTGA	
TRBV7-3-PCR1	GACTCAGGGCTGCCCAACGAT	
TRBV7-8-PCR1	CCAGAATGAAGCTCAACTAGACAA	
TRBV7-4,6-PCR1	GGTTCTCTGCAGAGAGGCCTGAG	
TRBV7-7-PCR1	GGCTGCCCAGTGATCGGTTCTC	
TRBV7-9-PCR1	GACTTACTTCCAGAATGAAGCTCAACT	
TRBV9-PCR1	GAGCAAAAGGAAACATTCTTGAACGATT	
TRBV10-1,3-PCR1	GGCTRATCCATTACTCATATGGTGTT	
TRBV10-2-PCR1	GATAAAGGAGAAGTCCCCGATGGCT	
TRBV11-1, 2, 3-PCR1	GATTCACAGTTGCCTAAGGATCGAT	
TRBV12-3,4-PCR1	GATTCAGGGATGCCCGAGGATCG	
TRBV12-5-PCR1	GATTCGGGGATGCCGAAGGATCG	
TRBV13-PCR1	GCAGAGCGATAAAGGAAGCATCCCT	
TRBV14-PCR1	TCCGGTATGCCCAACAATCGATTCT	
TRBV15-PCR1	GATTTTAACAATGAAGCAGACACCCCT	
TRBV16-PCR1	GATGAAACAGGTATGCCCAAGGAAAG	
TRBV18-PCR1	TATCATAGATGAGTCAGGAATGCCAAAG	
TRBV19-PCR1	GACTTTCAGAAAGGAGATATAGCTGAA	
TRBV20-1-PCR1	CAAGGCCACATACGAGCAAGGCGTC	
TRBV24-1-PCR1	CAAAGATATAAACAAAGGAGAGAGATCTCT	
TRBV25-1-PCR1	AGAGAAGGGAGATCTTTCCTCTGAGT	
TRBV27-PCR1	GACTGATAAGGGAGATGTTCCTGAAG	

TRBV28-PCR1	GGCTGATCTATTTCTCATATGATGTTAA
TRBV29-1-PCR1	GCCACATATGAGAGTGGATTTGTCATT
TRBV30-PCR1	GGTGCCCCAGAATCTCTCAGCCT

Table S4C. Sequences of housekeeping gene primers used in RT-PCR1.

Gene	Sequence 5' to 3'
ACTB-f	cgaAAGGCCAACCGCGAGAA
B2M-f	cgaTAGCTGTGCTCGCGCTAC
PPIA-f	cgaACCGCCGAGGAAAACC
RPS3-f	cgaGAGTCTCTGCGTTACAAACTCC
UBB-f	cgaTGCCCAGTGATGGCATT
ACTB-r	cgtCATCACGATGCCAGTGGTAC
B2M-r	cgtTCGGATGGATGAAACCCAGAC
PPIA-r	cgtCTGTCTTTGGGACCTTGTCT
RPS3-r	cgtGAGTTTCCCAGACACCACAAC
UBB-r	cgtTACCATGCAACGAAACCTTTATT

Table S4D. TRAV primers used in PCR2 (black). Obtained from Han et al, 2014¹⁰, with additional TRAV primers that we designed highlighted in blue. The TRAV primers are tailed with Illumina SBS3 sequence (green).

Name	Sequence 5' to 3'
TRAV1-1,2-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGTCGTTTTTCTTCATTCCTTAGTC
TRAV2-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGATACAACATGACCTATGAACGG
TRAV3-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTTGAAGCTGAATTTAACAAGAGCC
TRAV4-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCCCTGTTTATCCCTGCCGAC
TRAV5-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAACAAGACCAAAGACTCACTGTTC
TRAV6-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGACTGAAGGTCACCTTTGATACC
TRAV7-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTAAATGCTACATTACTGAAGAATGG
TRAV8-2,4,6-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCATCAACGGTTTTGAGGCTGAATTTAA
TRAV8-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCAGCTTCTCCTCAAGTACTTTTCA
TRAV8-3-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGCTTTGAGGCTGAATTTAAGAGG
TRAV9-1,2-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAAACCACTTCTTTCCACTTGGAGAA
TRAV10-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACAGCAACTCTGGATGCAGACAC
TRAV12-1, 2, 3-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAAGATGGAAGGTTTACAGCACA
TRAV13-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGACATTCGTTCAAATGTGGGCGAA
TRAV13-2-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCAAGGCCAAAGAGTCACCGT
TRAV14/DV4-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCAGAAGGCAAGAAAATCCGCCA
TRAV16-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTGACCTTAACAAAGGCGAGACA
TRAV17-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTAAGAGTCACGCTTGACACTTCCA
TRAV18-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGAGGTTTTCAGGCCAGTCCT
TRAV19-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCACCAGTTCCTTCAACTTCACC
TRAV20-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCACATTAACAAAGAAGGAAAGCT
TRAV21-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCTCGCTGGATAAATCATCAGGA
TRAV22-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGACTGTCGCTACGGAACGCTA
TRAV23/DV6-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACAATCTCCTTCAATAAAAGTGCCA
TRAV24-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGAATAAGTGCCACTCTTAATACCA
TRAV25-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTTGGAGAAGCAAAAAAGAACAGCT
TRAV26.1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGAAGACAGAAAGTCCAGCACCT
TRAV26.2-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCGCTGAAGACAGAAAGTCCAGT
TRAV27-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTAACCTTTCAGTTTGGTGATGCAA
TRAV29/DV5-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTAAACAAAAGTGCCAAGCACCTC
TRAV30-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATATCTGCTTCATTTAATGAAAAAAAGC
TRAV34-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCAAGTTGGATGAGAAAAAGCAGCA
TRAV35-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCAGTTTGGTATAACCAGAAAGGA
TRAV36/DV7-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAAGACTAAGTAGCATATTAGATAAG
TRAV36/DV7-PCR2.2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAGCATATTAGATAAGAAAGA
TRAV38-1,2-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGTGAACTTCCAGAAAGCAGCCA
TRAV39-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTCACTTGATACCAAAGCCCGT
TRAV40-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGCGGAAATATTAAAGACAAAAACTC
TRAV41-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATTAATTGCCACAATAAACATACAGG

Table S4E. TRBV primers used in PCR2 (black). Obtained from Han et al, 2014¹⁰, with additional TRBV primers that we designed highlighted in blue. The TRBV primers are tailed with Illumina SBS3 sequence (green).

Name	Sequence 5' to 3'
TRBV2-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCTGATGGATCAAATTTCACTCTG
TRBV3-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCACCTAAATCTCCAGACAAAGCT
TRBV4-1,2,3-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTGAATGCCCCAACAGCTCTC
TRBV5-4,8-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGAGCTGAATGTGAACGCCT
TRBV5-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATTCTCAGGGCGCCAGTTCTCT
TRBV5-5,6,7-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTGAATGTGAACGCCTTGTT
TRBV6-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGCTACAATGTCTCCAGATTAAACAA
TRBV6-2,3-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCTGATGGCTACAATGTCTCCAGA
TRBV6-4-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGTCTCCAGAGCAAACACAGATGATT
TRBV6-5,6-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCTCCAGATCAACCACAGAGGAT
TRBV6-8-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCTCTAGATTAAACACAGAGGATTTC
TRBV6-9-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCTACAATGTATCCAGATCAAACA
TRBV6-9-PCR2.2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATGGCTACAATGTATCCAGATCAA
TRBV7-2-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGCTTCTCTGCAGAGAGGACTGG
TRBV7-3-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGTTCTTTGCAGTCAGGCCTGA
TRBV7-8-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCAGTGATCGCTTCTTTGCAGAAA
TRBV7-4,6-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCCACTCTGAMGATCCAGCGCA
TRBV7-7-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGAGAGGCCTGAGGGATCCAT
TRBV7-9-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCAGAGAGGCCTAAGGGATCT
TRBV9-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCGCACAACAGTTCCCTGACTT
TRBV10-1,3-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGATGGCTAYAGTGTCTCTAGATCAAA
TRBV10-2-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTGTCTCCAGATCCAAGACAGAGAA
TRBV11-1,2,3-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCAGAGAGGCTCAAAGGAGTAGACT
TRBV12-3,4-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTAAGATGCCTAATGCATCATTCTC
TRBV12-5-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCAGCAGAGATGCCTGATGCAACT
TRBV13-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCAGCTCAACAGTTCAGTGACTA
TRBV14-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTGAAAGGACTGGAGGGACGTAT
TRBV15-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATAACTTCCAATCCAGGAGGCCG
TRBV16-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTAAGTGCCTCCCAAATTCACCC
TRBV18-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAACGATTTTCTGCTGAATTTCCCA
TRBV19-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTACAGCGTCTCTCGGGAGAAGA
TRBV20-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGACAAGTTTCTCATCAACCATGCAA
TRBV24-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGATACAGTGTCTCTCGACAGGC
TRBV25-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAACAGTCTCCAGAATAAGGACGGA
TRBV27-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACAAAGTCTCTCGAAAAGAGAAGAGGA
TRBV28-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGGGTACAGTGTCTCTAGAGAGA
TRBV29-1-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTTCCCATCAGCCGCCCAAACCTA
TRBV30-PCR2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGACCCCAGGACCGGCAGTTCAT

Table S4F. TRAC primers used in PCR2. TRAC nested primer sequences (purple) were obtained from Han et al, 2014¹⁰. An inline barcode (blue) identifies the column on the 384 well plate. The primers are tailed with Illumina SBS12 sequence (black).

Name	Sequence 5' to 3'
TRAC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTXXXXXXCAGACAGACTTGTCACTGGATTTAG
TRAC-R2-1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATGCGTTCAGACAGA
TRAC-R2-2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTTAGTCAGACAGA
TRAC-R2-3	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGAGTTGCAGACAGA
TRAC-R2-4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAGGCGCAGACAGA
TRAC-R2-5	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATTACAGCAGACAGA
TRAC-R2-6	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTAGGCCAGACAGA
TRAC-R2-7	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCTTACAGACAG
TRAC-R2-8	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCAGCACAGACAG
TRAC-R2-9	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAACATTCAGACAGA
TRAC-R2-10	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTACCTCAGACAGA
TRAC-R2-11	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACCGCTGCAGACAGA
TRAC-R2-12	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGAGTTAGCAGACAGA
TRAC-R2-13	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTGGTCCAGACAGA
TRAC-R2-14	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTTACCAGACAGA
TRAC-R2-15	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGGATACAGACAG
TRAC-R2-16	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATTACACAGACAG
TRAC-R2-17	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATACCTGTCAGACAGA
TRAC-R2-18	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTGATAATCAGACAGA
TRAC-R2-19	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATGTTGGCAGACAGA
TRAC-R2-20	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGCATAGCAGACAGA
TRAC-R2-21	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCGACGGCCAGACAGA
TRAC-R2-22	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGGACCAGACAGA
TRAC-R2-23	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAGATACAGACAG
TRAC-R2-24	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATAGACAACAGACAG

Table S4G. TRBC primers used in PCR2. TRBC nested primer sequences (purple) were obtained from Han et al, 2014¹⁰. An inline barcode (blue) identifies the column on the 384 well plate. One TRBC oligo is added per column of the 384 well plate. The primers are tailed with Illumina SBS12 sequence (black).

Name	Sequence 5' to 3'
TRBC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTXXXXXXXCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATTGTTCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCATGCTCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-3	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGGAATCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATAGCGCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-5	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTCCT
TRBC-R2-6	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGATATCCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-7	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTAGCGACTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-8	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTTACACTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-9	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAACTGTCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-10	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCGACCTCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-11	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTATTGCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-12	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCCGCAGCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-13	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACGGTCCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-14	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGGCTGCCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-15	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTGCAGACTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-16	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGATTAACTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-17	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATACAGTCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-18	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCGCACCTCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-19	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGCTGGCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-20	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCCACAGCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-21	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTAGGTCCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-22	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTGCGGCCTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-23	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACTTGCACTTTTGGGTGTGGGAGATCTCTG
TRBC-R2-24	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGATATAACTTTTGGGTGTGGGAGATCTCTG

Table S4H. Housekeeping gene primers used in PCR2 (black). The forward primers are tailed with SBS3 sequence (blue). The reverse primers are tailed with SBS12 sequence (green).

Gene	Sequence 5' to 3'
ACTB-PCR2-f	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGACCCAGATCATGTTTGAGACC
B2M-PCR2-f	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCTCTC
PPIA-PCR2-f	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCAACCCCACCGTGTT
RPS3-PCR2-f	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGGAGGGCTTGCTGTG
UBB-PCR2-f	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTGCACTATAGCCATTTGC
ACTB-PCR2-r	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGGATAGCACAGCCTGGAT
B2M-PCR2-r	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTTTCCATTCTCTGCTGGA
PPIA-PCR2-r	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAACAGCTCAAAGGAGAC
RPS3-PCR2-r	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTTGGCCCCACTCT
UBB-PCR2-r	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACATTTTGAACAGGTTCAGCTAT

Table S4I. TCR-Row barcode oligos (P5 barcode) used in PCR3. These primers consist of Illumina sequences P5 (pink)/barcode (black)/SBS3 (blue). Oligos A -P show typical barcodes. Unique row barcodes are added to every well within one row of the 384 well plate.

Name	Sequence 5' to 3'
PCR3-row	AATGATACGGCGACCACCGAGATCTCACXXXXXXACACTCTTTCCCTACACGAC
PCR3-A	AATGATACGGCGACCACCGAGATCTCACAACCTCTTACACTCTTTCCCTACACGAC
PCR3-B	AATGATACGGCGACCACCGAGATCTCACAGTCACCTACACTCTTTCCCTACACGAC
PCR3-C	AATGATACGGCGACCACCGAGATCTCACCCTCTAACACACTCTTTCCCTACACGAC
PCR3-D	AATGATACGGCGACCACCGAGATCTCACCTGAAGCTACACTCTTTCCCTACACGAC
PCR3-E	AATGATACGGCGACCACCGAGATCTCACGGCAATACACACTCTTTCCCTACACGAC
PCR3-F	AATGATACGGCGACCACCGAGATCTCACGAACGCTAACACTCTTTCCCTACACGAC
PCR3-G	AATGATACGGCGACCACCGAGATCTCACTTAGCCAGACACTCTTTCCCTACACGAC
PCR3-H	AATGATACGGCGACCACCGAGATCTCACTCTCGCGCACACTCTTTCCCTACACGAC
PCR3-I	AATGATACGGCGACCACCGAGATCTCACGCAGGTTGACACTCTTTCCCTACACGAC
PCR3-J	AATGATACGGCGACCACCGAGATCTCACATGAATTAACACTCTTTCCCTACACGAC
PCR3-K	AATGATACGGCGACCACCGAGATCTCACCGCATATTACACTCTTTCCCTACACGAC
PCR3-L	AATGATACGGCGACCACCGAGATCTCACCAACTGATACACTCTTTCCCTACACGAC
PCR3-M	AATGATACGGCGACCACCGAGATCTCACGTCTGCACACACTCTTTCCCTACACGAC
PCR3-N	AATGATACGGCGACCACCGAGATCTCACGCTAGCAGACACTCTTTCCCTACACGAC
PCR3-O	AATGATACGGCGACCACCGAGATCTCACTAATCCGGACACTCTTTCCCTACACGAC
PCR3-P	AATGATACGGCGACCACCGAGATCTCACTGGTGCATACACTCTTTCCCTACACGAC

Table S4J. TCR-Plate barcode oligos used in PCR3. These primers consist of Illumina sequences P7 (red)/barcode (black)/SBS12 (blue). Oligos A shows a typical barcode that can be included.

Name	Sequence 5' to 3'
Plate bc	CAAGCAGAAGACGGCATACGAGATXXXXXXGTGACTGGAGTTCAGACGTGT
Plate bc-A	CAAGCAGAAGACGGCATACGAGATTTGAGCCTGTGACTGGAGTTCAGACGTGT

Table S4K. Housekeeping column primers used in PCR3. These primers consist of Illumina sequences P5 (pink)/barcode (black)/SBS3 (blue). There are 24 oligos per 384 well plate and oligos 1-24 show typical barcodes.

Name	Sequence 5' to 3'
PCR3-HK-column	AATGATACGGCGACCACCGAGATCTCACXXXXXXACACTCTTTCCCTACACGAC
PCR3-HK-column1	AATGATACGGCGACCACCGAGATCTCACAACCTCTTACACTCTTTCCCTACACGAC
PCR3-HK-column2	AATGATACGGCGACCACCGAGATCTCACAGTCACCTACACTCTTTCCCTACACGAC
PCR3-HK-column3	AATGATACGGCGACCACCGAGATCTCACCCTCTAACACACTCTTTCCCTACACGAC
PCR3-HK-column4	AATGATACGGCGACCACCGAGATCTCACCTGAAGCTACACTCTTTCCCTACACGAC
PCR3-HK-column5	AATGATACGGCGACCACCGAGATCTCACGGCAATACACACTCTTTCCCTACACGAC
PCR3-HK-column6	AATGATACGGCGACCACCGAGATCTCACGAACGCTAACACTCTTTCCCTACACGAC
PCR3-HK-column7	AATGATACGGCGACCACCGAGATCTCACTTAGCCAGACACTCTTTCCCTACACGAC
PCR3-HK-column8	AATGATACGGCGACCACCGAGATCTCACTCTCGCGCACACTCTTTCCCTACACGAC
PCR3-HK-column9	AATGATACGGCGACCACCGAGATCTCACGCAGGTTGACACTCTTTCCCTACACGAC
PCR3-HK-column10	AATGATACGGCGACCACCGAGATCTCACATGAATTAACACTCTTTCCCTACACGAC
PCR3-HK-column11	AATGATACGGCGACCACCGAGATCTCACCGCATATTACACTCTTTCCCTACACGAC
PCR3-HK-column12	AATGATACGGCGACCACCGAGATCTCACCAACTGATACACTCTTTCCCTACACGAC
PCR3-HK-column13	AATGATACGGCGACCACCGAGATCTCACGTCTGCACACACTCTTTCCCTACACGAC
PCR3-HK-column14	AATGATACGGCGACCACCGAGATCTCACGCTAGCAGACACTCTTTCCCTACACGAC
PCR3-HK-column15	AATGATACGGCGACCACCGAGATCTCACTAATCCGGACACTCTTTCCCTACACGAC
PCR3-HK-column16	AATGATACGGCGACCACCGAGATCTCACTGGTGCATACACTCTTTCCCTACACGAC
PCR3-HK-column17	AATGATACGGCGACCACCGAGATCTCACAGAGTAGAACACTCTTTCCCTACACGAC
PCR3-HK-column18	AATGATACGGCGACCACCGAGATCTCACGAACTTCGACACTCTTTCCCTACACGAC
PCR3-HK-column19	AATGATACGGCGACCACCGAGATCTCACAGTAGGCAACACTCTTTCCCTACACGAC
PCR3-HK-column20	AATGATACGGCGACCACCGAGATCTCACGGAATACGACACTCTTTCCCTACACGAC
PCR3-HK-column21	AATGATACGGCGACCACCGAGATCTCACCAGCGATTACACTCTTTCCCTACACGAC
PCR3-HK-column22	AATGATACGGCGACCACCGAGATCTCACTCATGTCTACACTCTTTCCCTACACGAC
PCR3-HK-column23	AATGATACGGCGACCACCGAGATCTCACCGACTCTCACACTCTTTCCCTACACGAC
PCR3-HK-column24	AATGATACGGCGACCACCGAGATCTCACTTCACAGAACACTCTTTCCCTACACGAC

Table S4L. Housekeeping row primers used in PCR3. These primers consist of Illumina sequences P7(purple)/barcode (black)/SBS12 (green).

Name	Sequence 5' to 3'
PCR3-HK-row	CAAGCAGAAGACGGCATACGAGATXXXXXXGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowA.1	CAAGCAGAAGACGGCATACGAGATACTTCTTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowB.1	CAAGCAGAAGACGGCATACGAGATTGGTAACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowC.1	CAAGCAGAAGACGGCATACGAGATTAGATCCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowD.1	CAAGCAGAAGACGGCATACGAGATCATCAGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowE.1	CAAGCAGAAGACGGCATACGAGATTTACTGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowF.1	CAAGCAGAAGACGGCATACGAGATGTGCGTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowG.1	CAAGCAGAAGACGGCATACGAGATGGCATAGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowH.1	CAAGCAGAAGACGGCATACGAGATCTATTCAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowI.1	CAAGCAGAAGACGGCATACGAGATCAAGGCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowJ.1	CAAGCAGAAGACGGCATACGAGATCAGTTGGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowK.1	CAAGCAGAAGACGGCATACGAGATGACGCTATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowL.1	CAAGCAGAAGACGGCATACGAGATTCTGGACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowM.1	CAAGCAGAAGACGGCATACGAGATAAGGCGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowN.1	CAAGCAGAAGACGGCATACGAGATTGTTATACGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowO.1	CAAGCAGAAGACGGCATACGAGATCCTAGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT
PCR3-HK-rowP.1	CAAGCAGAAGACGGCATACGAGATTCAGCGAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGAT

Table S5. TCR sequences from paired TCRαβ sequencing (see Excel sheet)

Table S5A. TCR sequences of CEF-reactive T cells (Donor 1)

 Table S5B. TCR sequences of CEF-reactive T cells (Donor 2)

Table S5C. TCR sequences of non-CEF-reactive T cells (Donor 1 and 2)

Table S5D. TCR sequences of melanoma neoantigen pool-reactive CD4⁺ T cells (Patient 1)

Table S5E. TCR sequences of melanoma neoantigen pool-reactive CD8⁺ T cells (Patient 3)

 Table S5F. TCR sequences of mut-MGA-reactive T cells

C. Supplemental Figures

A Reporter Construct Sequence

(NFAT) _e minP	luciferase
GGAGGAAAAACTGTTT CTGTTTCATACAGAAG ATATAATGGAAGCTCC AACATAAAGGAAGGCTCC AACATAAAGAAGCTCC CAATTGCTTTTACASG GAATACAAATCACAGA AACGACATTTATAATT TGCCAAAAAAAGCTCCC ACCTCCCGGTTTTAAT AAGGGTGTCGCTCTGC GTTGTTCCAATTACACC GTTGTTCAATTACACC GATATGGCCTCACTGGAT GGTGTGGACTCTGGAT GGAGCGACCAACGCCC AGCCTCTGCAATAAGGCCAAGAA TCCCACACGCCAAGAA	ACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAA TGGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTAGATCTAGAGCTCTAGAGGGT TCCAGCTTGGCATTCCCGTAGATGTTGGTAAAAGCTTGGCAATCCGGTACGTTGGTAAAGCCACCATGAAGACGCCTAGAGAGCGAAG SCCCCATTCTATCCGCTGGAAGATGGAACCGCTGGAGACCAACTGCATCCGGTACGTTGGAAAGCACCCCTAGAACGCCAAGA SCCCCATTCTATCCGCTGGAAGATGGAACCGCTGGAGACCAACTGCATCAGGCGTTGGAAGAGATCACGCCCTGGTTCCTGGAA ACATATCGAGGTGGACATCACTTACGCGTGGGTTTCGAAAAGCCTTGGCAGAAGCTTGACAGATTGGCCGCGG SCCGGAATGCCAACAGTATGGGCATTCGAACGCCTGGTGTTCGTTACTACGGGGTTGCAAGAACATTGGGCCCGCG SCCGGAATTGCCAACAGTATGGGCATTCTAAACGGCGTTGGCGTGGTGTCCGATAGTACGGCTTCCTCAACT SCCGCATTCGCCAACGTTCTCGAAGGACAGCAATTGCACTGGTGTCCCAACAGCGCTTCCTCGACACTCCTCCTCACT SCCGCTTCGAAAATTATTACTGGATTCTAAACGGACAAGCAATTGCACTGGTCTCCCAACTACGGATCTCGCCACTTCCACCTGC SCCGCAACGTTCCGAAGGTCCTTCGAAGGACAGCAATTGCGATTTCGAGTCACAACTCCTCGTCGCCTCTCGAT SCCGCTTCGGAAGGTCCTCCGATGCCGGGGCGCGAGCCATTTTTGGCGACCTCAACTCCTCGCCT SCCGCAACGCTCCCCCCTCCTAAGGAGCCACCCCTTTCCGCCACACCCTCTAATGTATAGATTGGAAGAAGAGCCT SCCGCAACGCTGGCGGCGCGGGGGGATGATAGCGGGGATCCACTTCTCCCCCAGGTACCCGCGCGGTTGCCAAGGCCCGCGCGGTTGCCAAGCCCACCTTTTTTCGACCAGAT SCCGCAACGCCGCGCCGCGGGGGCGATGATAGCTACGGGGCGCGGCGGCGGCGGTAGCTAAGATTGCCAGGCAAG SCCGCAACGCCGCGCGCGGATGCTAGGCGAACGCTACTCTGCTCGACTGCAACGCCCGCGCG SCCGCAAGGATGGATGGCTGCCGGATCTACCGGAAGCCGAAGGGTCCCTCCACCTCCCCCGGTAAGGCCACCCCGCGCG SCCGCAAGGATGGATGGCTACCTCGGAGCCCACCCTCACCCCCACCACCTCTCCCCCCGGATGCCGCGCCGGCG SCCGCAAGGATCGCCGCCCCGCGAAGGCCAAGCCTACCGGAAGACGGAACACCCGCAGGAAAATCCGGGAAGCG SCCGCAAGGATCGCCGCCCGGTGAAGCGAAGCCGAAGGCCTTACCGGCACGCAC
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Figure S1. Examples of sequences used for reporter cell line and TCR cloning. (A) The NFATluciferase reporter construct. (B) Example of a V α -C β library construct for TRAV8-1 and a V β -C α library construct for TRB5-1, used to clone the EBNA3A-specific TCR. (C) Example of oligonucleotide encoding CDR3 α and CDR3 β , flanked with BsaI restriction sites for Golden Gate Assembly, using the published EBNA3A sequence. (D) Example of the completely assembled TCR vector, using the variable chain plasmid library components and CDR3 oligonucleotide. (E) Example of TCR in lentiviral vector, PEW.



Figure S2. Characterization of costimulation molecules expressed by the Jurkat $\Delta \alpha \beta$. Flow cytometry staining for costimulatory molecules on healthy donor PBMCs (gated on CD4⁺ T cells) and Jurkat $\Delta \alpha \beta$ reporter cells expressing M1-specific TCRs (P1.7 from Donor 1) at resting state and after overnight stimulation with anti-CD3 antibody.



Figure S3. Deconvolution of peptides by IFN γ **ELISPOT for melanoma Patient 3.** Patient 3 PBMC were stimulated with a pool of peptides for 21 days, followed by immunomagnetic isolation of CD8⁺ T cells. CD8⁺ T cells were tested by ELISPOT assay by overnight co-culture with autologous APC (CD4/CD8-depleted PBMC) and individual peptides within the pool. Three peptides (mut-*CIT*^b, mut-*VPS16*^b and mut–*CASP1*^a) were identified to be immunogenic epitopes based on detection of IFN γ secretion; testing against each peptide was performed in triplicate.



Figure S4. Identification of immunogenic neoantigens from indel mutations in CLL. (A) Summary of indels identified from 157 CLL patients analyzed by Indelocator and Strelka in which we focused on those events identified by the overlap intersection of results of these 2 algorithms. (B) Distribution of predicted binding affinities of the 76 neoantigens predicted to arise from 28 of the identified indels, 30 of which were strong binders (predicted IC₅₀ < 150 nM). (C) Two of the five predicted strong binders to HLA-A*02:01 (mut-*MGA_106*, mut-*ITPKB*) were identified by IFN_γ ELISPOT as immunogenic epitopes. SFC, spot forming cells.

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