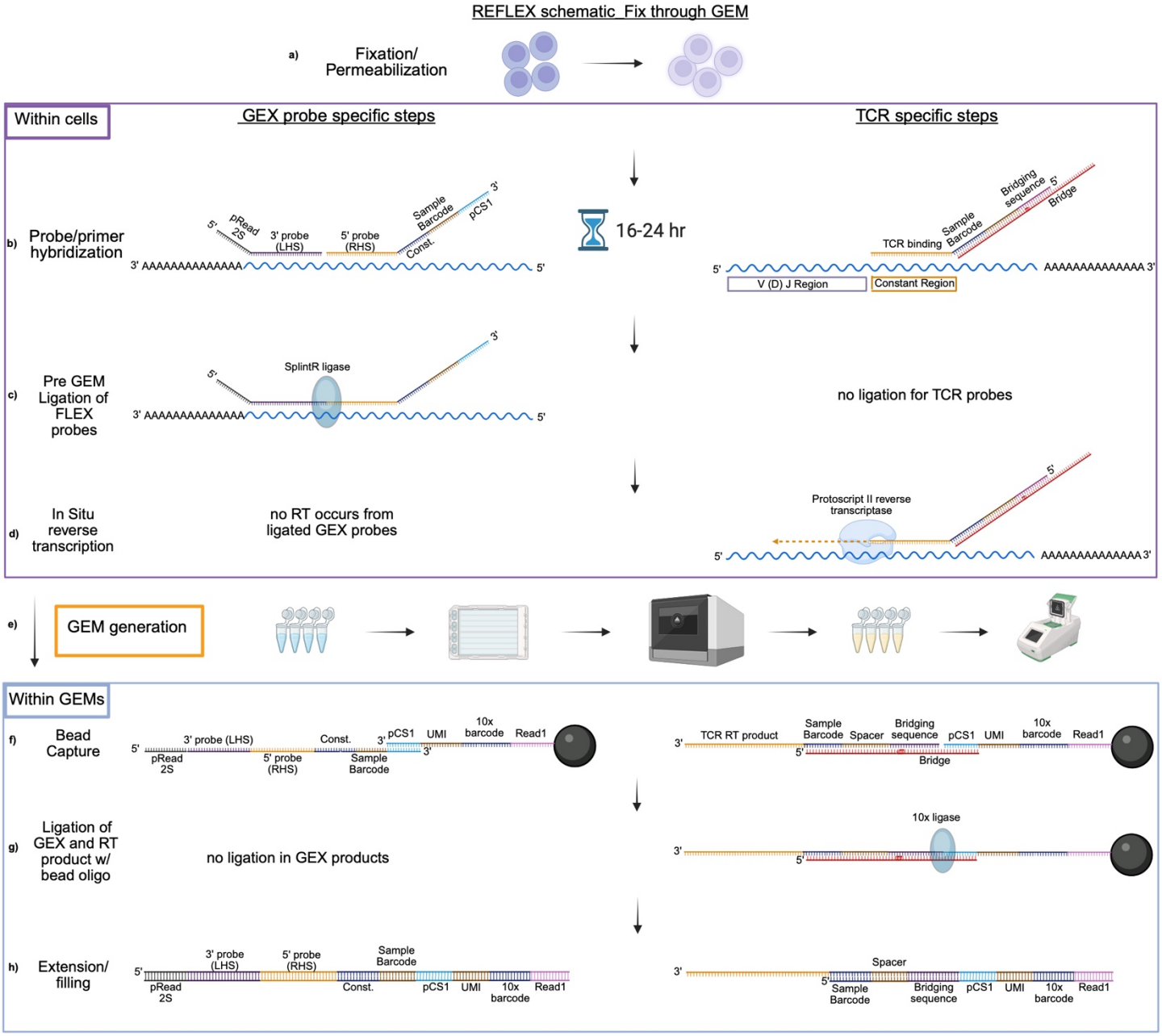
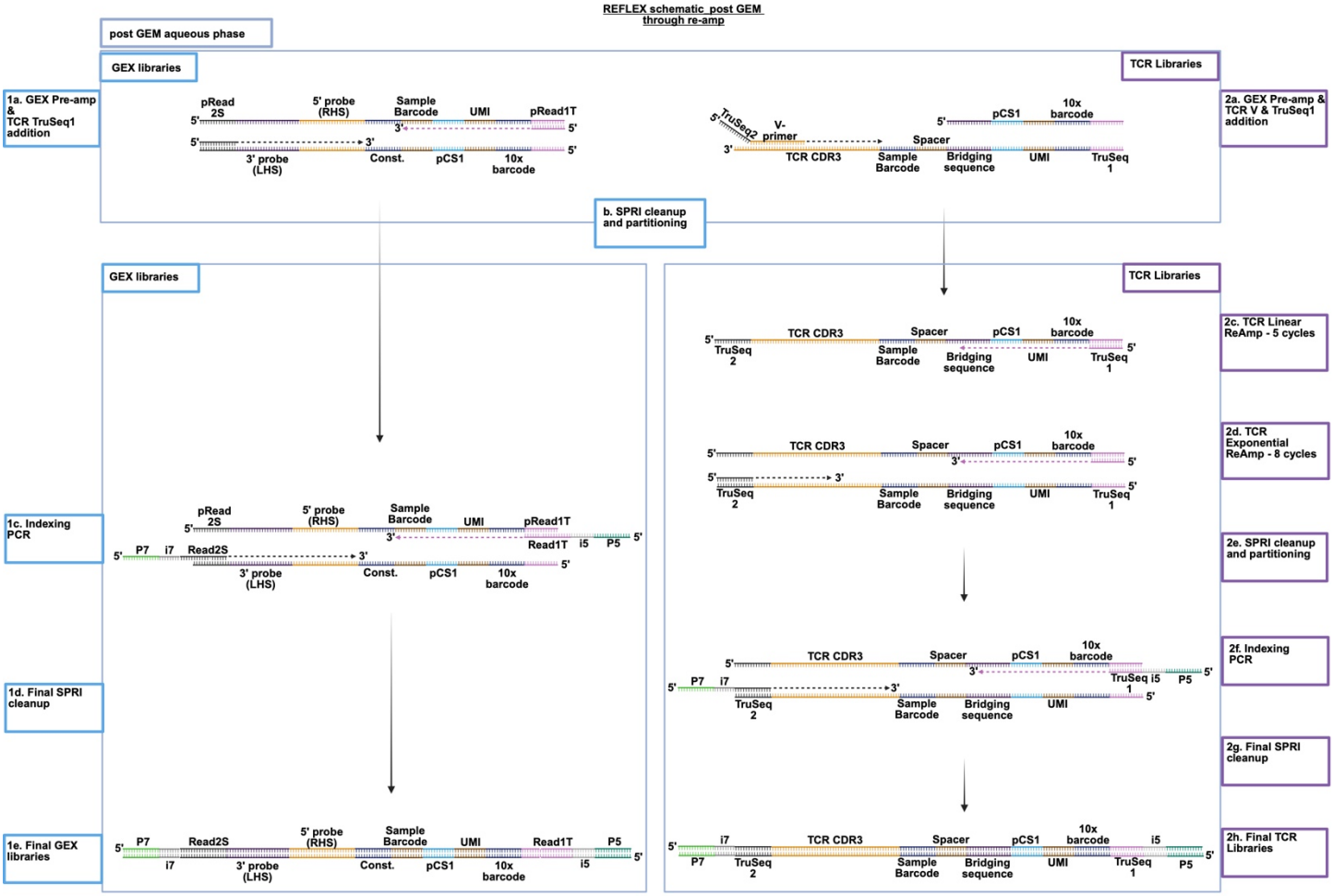
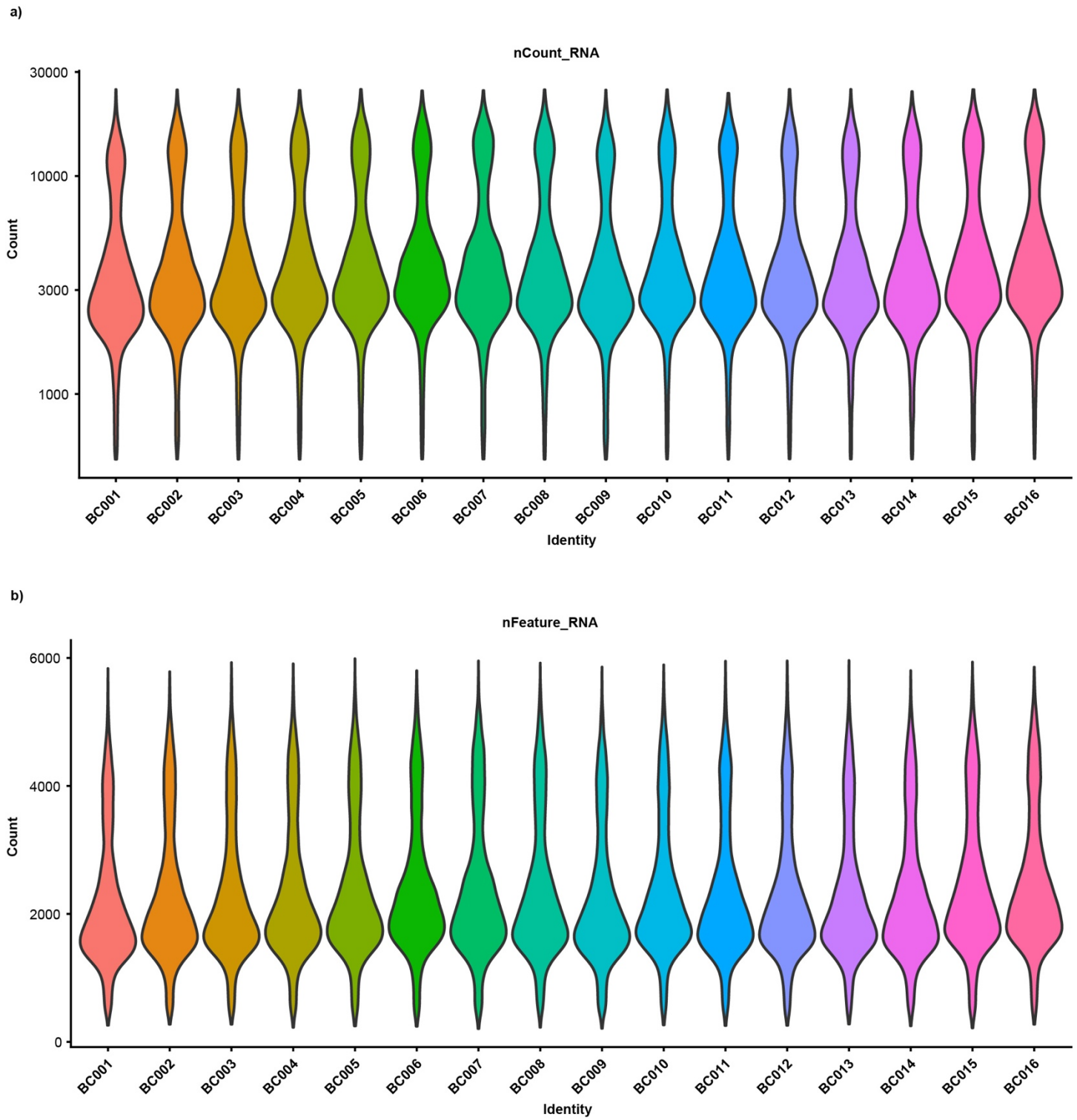
Supplemental Figures/Data



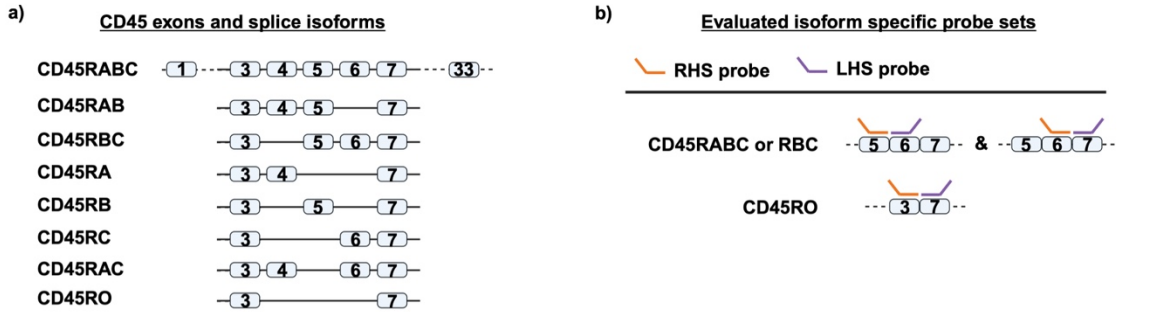
Supplemental Figure 1. Detailed step-by-step schematic of REFLEX chemistry leading up to emulsion. Showing representative primer/probe oligos and relative orientations. GEX and TCR pictured separately but with various steps occurring in parallel. a) Cell fixation and permeabilization. b) Overnight hybridization of 10X FLEX probes and pre-annealed bridge/TCR constant region binding RT primers. c) Ligation of left and right hand 10X probes to prevent off-target/strand displacing RT. d) Reverse transcription of TCR CDR3 variable region. e) Pooling, filtering, counting, and 10X GEM generation on Chromium platform. f) Bead capture of FLEX probes and TCR RT products by GEM beads in emulsion. g) Ligation of GEX and RT products to GEM beads, ligation of RT product eliminating bridging sequence dependency, h) extension and gap-filling of GEX and RT products.



Supplemental Figure 2. Detailed schematic of REFLEX chemistry post GEM. Showing representative primer/probe oligos and relative orientations. GEX portion shown with 1a) combined preamplification after GEM emulsion disruption, including pre-amp primer group B (or C if library contains ADTs) and addition of TruSeq 1 primers, b) 1.8X SPRI clean up and partitioning of pre-amp product, 1c) indexing PCR, addition of Illumina-compatible p5/p7 primers from 10X Dual Index TS kit (or TN for ADT's), 1d) 1X SPRI clean up, 1e) fully indexed GEX (or ADT) product structure, including sequencing primers, 10X barcode, and UMI for sample identification. TCR portion shown with 2a) combined reamplification after GEM emulsion disruption, TCR-variable pool and TruSeq1 spike-in primers for TCR capture and amplification, 2b) 1.8X SPRI clean-up and partitioning of pre-amp product, 2c) linear reamplification phase of TCR products, using only TruSeq1 primer to preferentially amplify TCR libraries for 5 cycles, 2d) TruSeq2 primer added to re-amplification for 8-cycle exponential amplification of TCR libraries, 2e) pooling of reamplification products and 0.8X SPRI clean up, 2f) indexing PCR, addition of Illumina-sequencing compatible p5/p7 primers from 10X Dual Index TT kit, 2g) 0.7X SPRI clean up, 2h) fully indexed TCR product structure, including sequencing primers, 10X barcode, and UMI for sample identification.



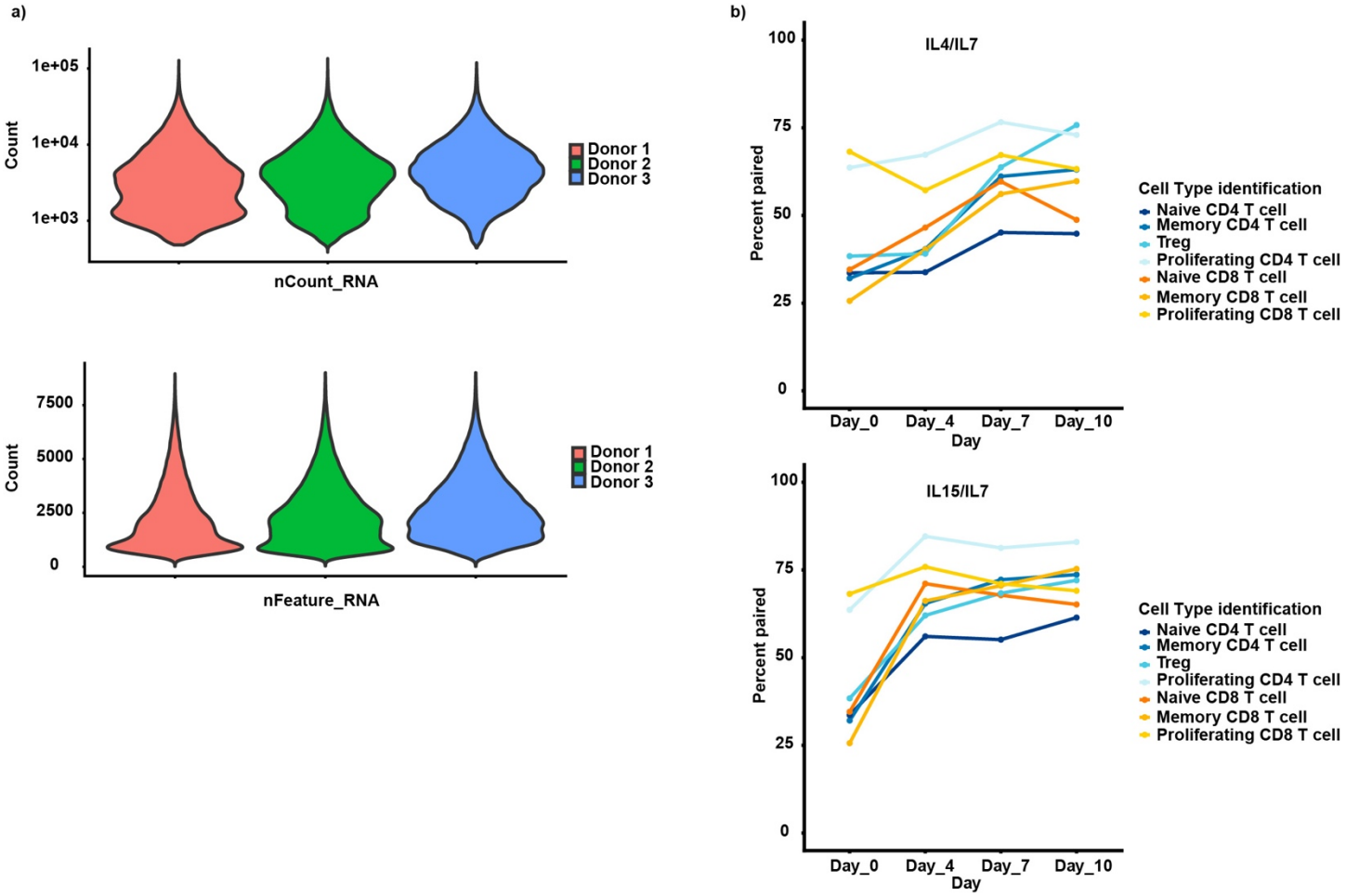
Supplemental Figure 3. REFLEX gives consistent performance across multiplex barcodes. a) UMI and b) features detected across 16 multiplexed barcodes in PBMCs.



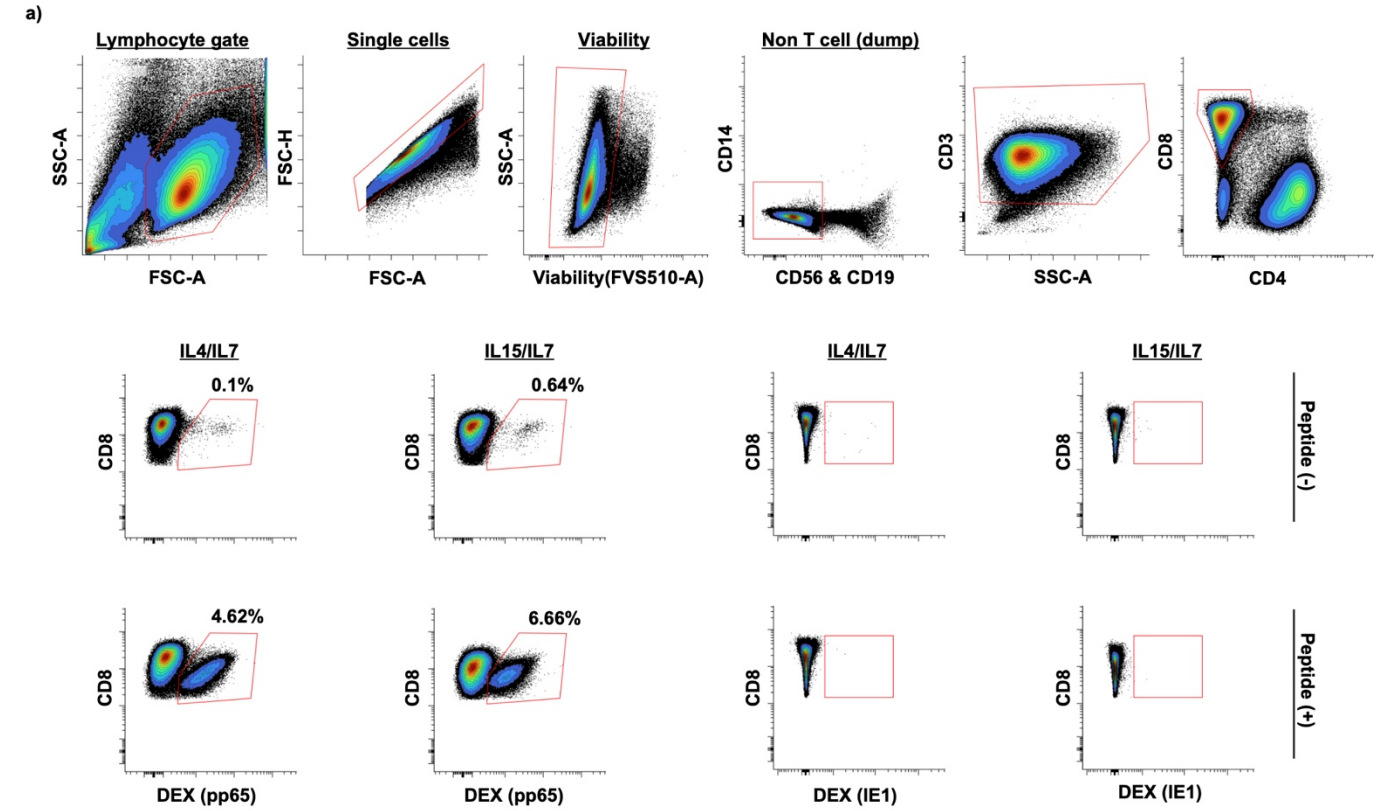
c)

gene_id	probe_seq	probe_id	included	region	gene_name
ENSG00000081237	GTTTCAGAGGCATTAAGGTAGGCATCAGTGGGGGAAGGTGTTGGGCTTTG	ENSG00000081237|PTPRC|E3_7jxn	TRUE	spliced	PTPRC
ENSG00000081237	GCTGTACTCCTCTCTCCTGGGACATCTGAGATAGCATTGCTGCCTGGGGT	ENSG00000081237|PTPRC|E5_6jxn	TRUE	spliced	PTPRC
ENSG00000081237	GTTTCAGAGGCATTAAGGTAGGCATCTGAGGTGTTCGCTGTGATGGTGGT	ENSG00000081237|PTPRC|E6_7jxn	TRUE	spliced	PTPRC

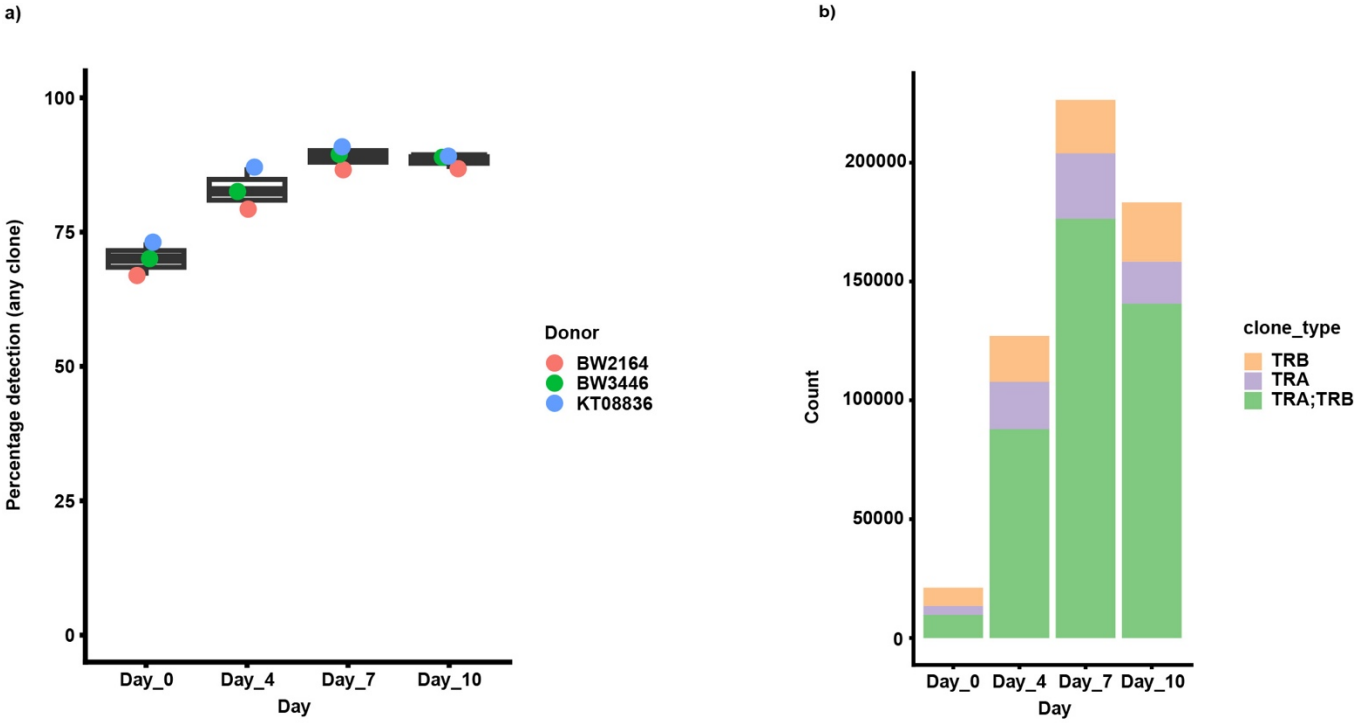
Supplemental Figure 4. REFLEX is compatible with custom FLEX probes for CD45 isoforms. a) CD45 isoform exon inclusion/exclusion, b) Schematic design for tested isoform specific probe pairs and c) target sequence information for combined left and right-hand side probes (not inclusive of overhangs).



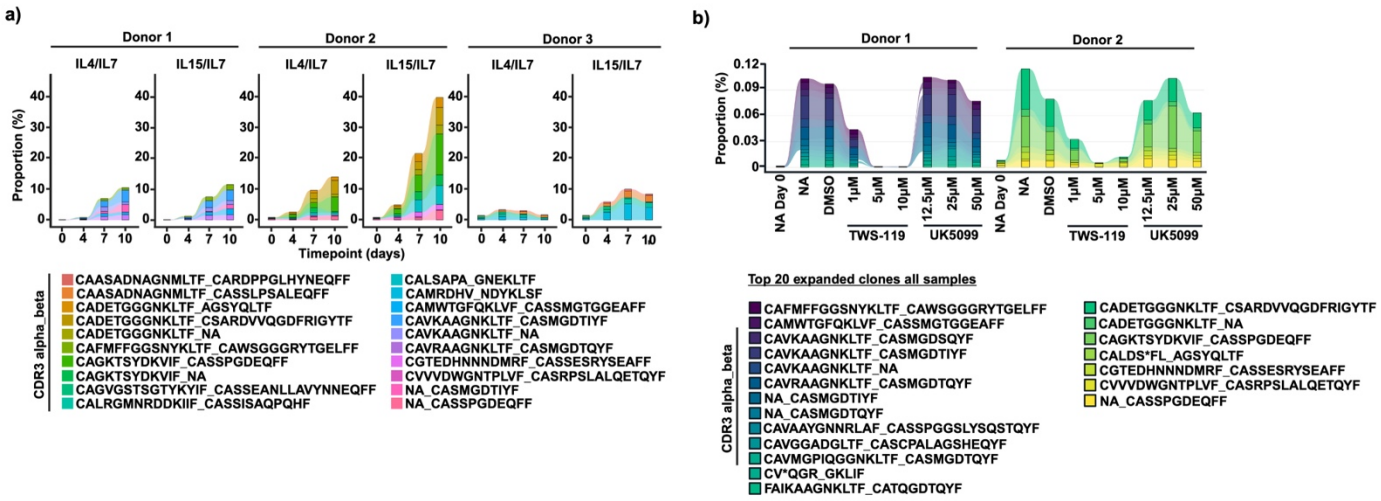
Supplemental Figure 5. REFLEX allows for gene expression profiling from fixed/stored samples. a) GEX performance by donor and b) TCR performance over time and by T cell subset ID (donors combined).



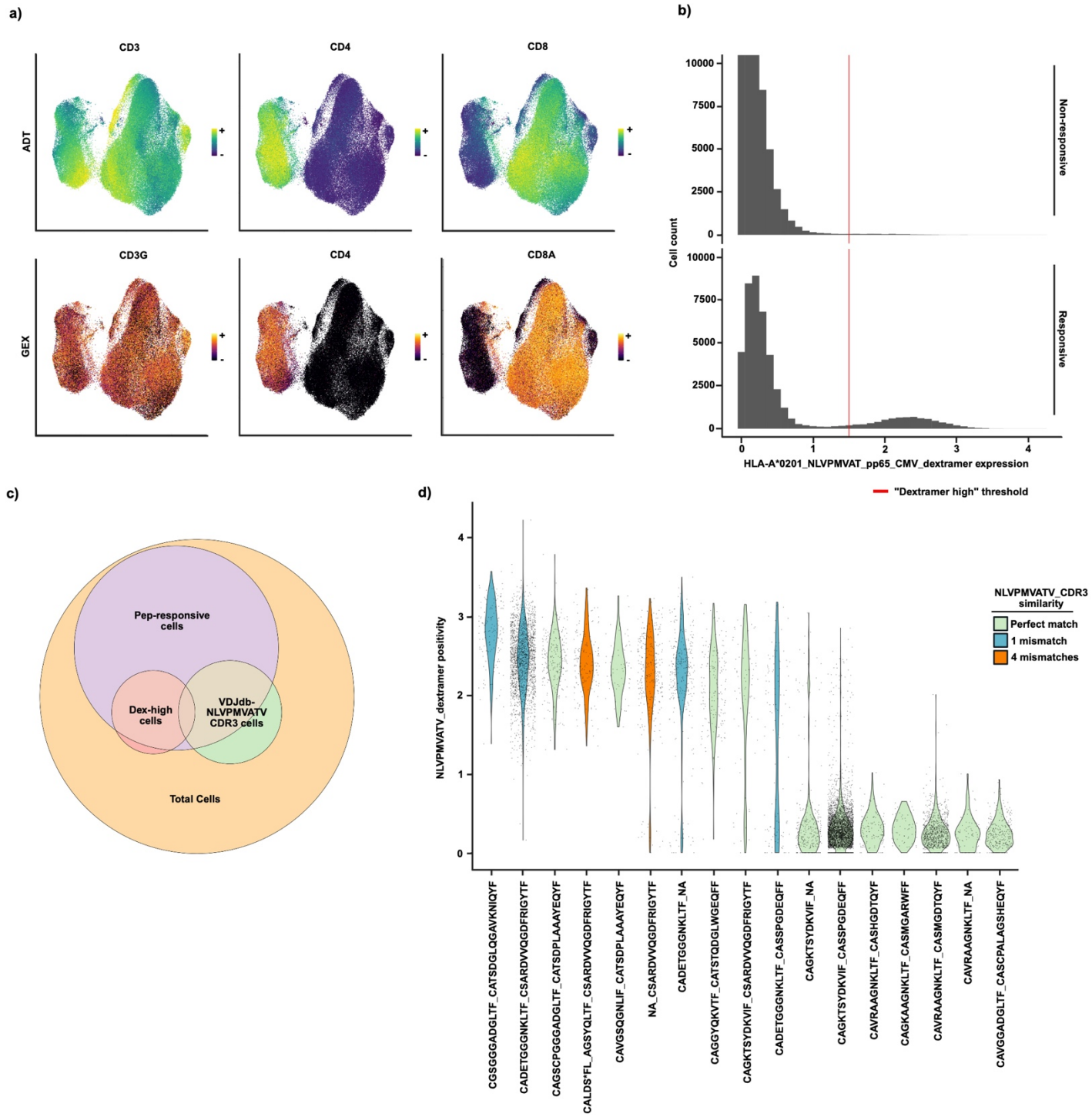
Supplemental Figure 6. CMV peptide pulse and rapid expansion successfully generated VSTs. a) Gating scheme and flow cytometry confirmation of CMV reactive T cell expansion by fluorophore conjugated dextramer stain in a single representative donor.



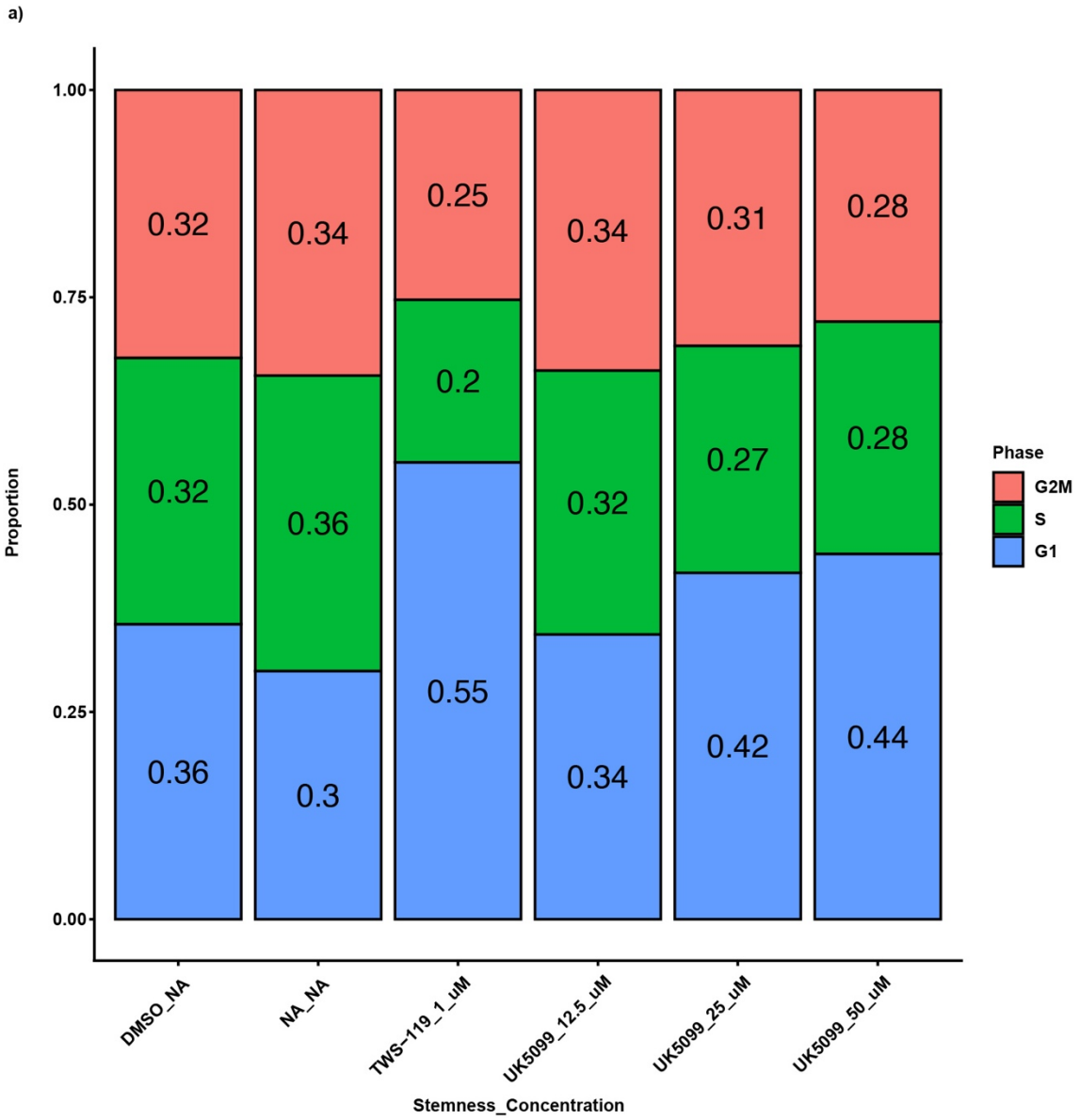
Supplemental Figure 7. REFLEX shows strong detection of TCR clones across donors and over time. a) TCR “any” clone detection frequency per cell by time point and b) total number of paired or alpha/beta single clones detected by timepoint.



Supplemental Figure 8. REFLEX tracks clonal expansion across culture timepoints with and without metabolic perturbation. a) CDR3 amino acid sequences from Figure 2d and b) Figure 2g.



Supplemental Figure 9. ADT and Dextramer staining identify cell types and potentially novel NLVPMVATV responsive TCRs through REFLEX. T cell labeling with Total Seq-C TBNK and oligo conjugated “dCode” HLA-A*0201 + NLVPMVATV peptide dextramers demonstrates REFLEX compatibility with ADT modalities and supports novel identification of NLVPMVATV peptide reactive CDR3 a/b pairs. a) UMAPs showing select T cell markers from Total Seq TBNK panel (Biolegend) and corresponding GEX. b) Selection of threshold for “Dextramer high” cells. c) Venn diagram showing overlap between total, peptide responsive, dextramer high, and CDR3 pairings associated with NLVPMVATV peptide by VDJdb. d) Violin plots showing overlap between top 10 dextramer high and top 10 expanded CDR3 from the dataset. Colors indicate similarity between sequenced CDR3 and database.



Supplemental Figure 10. REFLEX profiles effects of metabolic perturbations on cell cycle phase. Cell cycle phase proportions based on gene expression signature across treatments (showing all cells/donors).

Name	Target Region Primer Sequence	Purification
TRAC-RT-BC001-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ACTTTAGG**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC001-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ACTTTAGG**ACCAGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC002-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AACGGGAA**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC002-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AACGGGAA**ACCAGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC003-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AGTAGGCT**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC003-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AGTAGGCT**ACCAGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC004-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATGTTGAC**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC004-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATGTTGAC**ACCAGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC005-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ACAGACCT**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC005-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ACAGACCT**ACCAGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC006-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATCCCAAC**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC006-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATCCCAAC**ACCAGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC007-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AAGTAGAG**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC007-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AAGTAGAG**ACCAGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC008-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AGCTGTGA**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC008-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AGCTGTGA**ACCAGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC009-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ACAGTCTG**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC009-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ACAGTCTG**ACCAGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC010-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AGTGAGTG**CGGTGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC010-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AGTGAGTG**ACC	HPLC

	AGTGTGGCCTTTTGGGTGTGGGAG	
TRAC-RT-BC011-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AGAGGCAA**CGG TGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC011-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AGAGGCAA**ACC AGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC012-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ACTACTCA**CGGT GAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC012-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ACTACTCA**ACCA GTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC013-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATACGTCA**CGGT GAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC013-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATACGTCA**ACCA GTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC014-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATCATGTG**CGGT GAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC014-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATCATGTG**ACCA GTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC015-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AACGCCGA**CGG TGAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC015-1-P	/5phos/ctagcgtacgagcactccagattacttcca**AACGCCGA**ACC AGTGTGGCCTTTTGGGTGTGGGAG	HPLC
TRAC-RT-BC016-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATTCGGTT**CGGT GAATAGGCAGACAGACTTGTCACTGG	HPLC
TRBC-RT-BC016-1-P	/5phos/ctagcgtacgagcactccagattacttcca**ATTCGGTT**ACCA GTGTGGCCTTTTGGGTGTGGGAG	HPLC

Supplemental Table 1. Human TRAC and TRBC RT primer sequences. mRNA targeting regions shown in UPPERCASE, 10x Genomics sample barcode shown in **bold**, constant sequence and splint oligo overlapping sequence shown in lowercase.

Name	Full Primer Sequence	Purification
Bdg-RT-BC001-38nt	CCTAAAGTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC002-38nt	TTCCCGTTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC003-38nt	AGCCTACTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC004-38nt	GTCAACATTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC005-38nt	AGGTCTGTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC006-38nt	GTTGGGATTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC007-38nt	CTCTACTTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC008-38nt	TCACAGCTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC009-38nt	CAGACTGTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC010-38nt	CACTCACTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC011-38nt	TTGCCTCTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC012-38nt	TGAGTAGTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC013-38nt	TGACGTATTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC014-38nt	CACATGATTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC015-38nt	TCGGCGTTTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC
Bdg-RT-BC016-38nt	AACCGAATTGGAAGTAATCTGGAGTGCTCGTACGCTAGCGGTCCTAGCAA	HPLC

Supplemental Table 2. hTRAC and hTRBC bridging sequences used for mediating splinting and ligation of first strand cDNA onto 10X Genomics GEM Beads for scRNAseq capture

Name	Full Primer Sequence
TRAV1	AGGTCGTTTTTCTTCATTCCTTAGTC
TRAV2	ACGATACAACATGACCTATGAACGG
TRAV3.1	CTTTGAAGCTGAATTTAACAAGAGCC
TRAV4.1	CTCCCTGTTTATCCCTGCCGAC
TRAV5.1	AAACAAGACCAAAGACTCACTGTTC
TRAV6	AAGACTGAAGGTCACCTTTGATACC
TRAV7	ACTAAATGCTACATTACTGAAGAATGG
TRAV8	GCATCAACGGTTTTGAGGCTGAATTTAA
TRAV8.1	GCATCAAGGGCTTTGAGGCTGAATTTAT
TRAV8.3	GCATTAAAGGCTTTGAGGCTGAATTTAA
TRAV9	GAAACCACTTCTTTCCACTTGGAGAA
TRAV10	TACAGCAACTCTGGATGCAGACAC
TRAV12	GAAGATGGAAGGTTTACAGCACA
TRAV13.1	GACATTCGTTCAAATGTGGGCGAA
TRAV13.2	GGCAAGGCCAAAGAGTCACCGT
TRAV14	TCCAGAAGGCAAGAAAATCCGCCA
TRAV16	GCTGACCTTAACAAAGGCGAGACA
TRAV17	TTAAGAGTCACGCTTGACACTTCCA
TRAV18	GCAGAGGTTTTCAGGCCAGTCCT
TRAV19	TCCACCAGTTCCTTCAACTTCACC
TRAV20	GCCACATTAACAAAGAAGGAAAGCT
TRAV21	GCCTCGCTGGATAAATCATCAGGA
TRAV22	ACGACTGTCGCTACGGAACGCTA
TRAV23	CACAATCTCCTTCAATAAAAGTGCCA
TRAV24	ACGAATAAGTGCCACTCTTAATACCA
TRAV25	GTTTGGAGAAGCAAAAAAGAACAGCT
TRAV26.1	CAGAAGACAGAAAGTCCAGCACCT
TRAV26.2	ATCGCTGAAGACAGAAAGTCCAGT
TRAV27	ACTAACCTTTCAGTTTGGTGATGCAA
TRAV29	CTTAAACAAAAGTGCCAAGCACCTC
TRAV30	AATATCTGCTTCATTTAATGAAAAAAAGC
TRAV34	CCAAGTTGGATGAGAAAAAGCAGCA
TRAV35	CTCAGTTTGGTATAACCAGAAAGGA
TRAV36	GGAAGACTAAGTAGCATATTAGATAAG

TRAV38	CTGTGAACTTCCAGAAAGCAGCCA
TRAV39	CCTCACTTGATACCAAAGCCCGT
TRAV40	AGGCGGAAATATTAAAGACAAAAACTC
TRAV41	GATTAATTGCCACAATAAACATACAGG
TRBV2	GCCTGATGGATCAAATTTCACTCTG
TRBV3-1	TCTCACCTAAATCTCCAGACAAAGCT
TRBV4	CCTGAATGCCCCAACAGCTCTC
TRBV5-1	CGATTCTCAGGGCGCCAGTTCTCT
TRBV5-4,8	CTCTGAGCTGAATGTGAACGCCT
TRBV6-1	TGGCTACAATGTCTCCAGATTAAACAA
TRBV6-2,3	CCCTGATGGCTACAATGTCTCCAGA
TRBV6-4	GTGTCTCCAGAGCAAACACAGATGATT
TRBV6-5,6	GTCTCCAGATCAACCACAGAGGAT
TRBV6-8	GTCTCTAGATTAAACACAGAGGATTTC
TRBV6-9	GGCTACAATGTATCCAGATCAAACA
TRBV7-2	TCGCTTCTCTGCAGAGAGGACTGG
TRBV7-3	CGGTTCTTTGCAGTCAGGCCTGA
TRBV7-4,6	TCTCCACTCTGAAGATCCAGCGCA
TRBV7-4,6	TCTCCACTCTGACGATCCAGCGCA
TRBV7-7	GCAGAGAGGCCTGAGGGATCCAT
TRBV7-8	CCAGTGATCGCTTCTTTGCAGAAA
TRBV7-9	CTGCAGAGAGGCCTAAGGGATCT
TRBV9	CTCCGCACAACAGTTCCCTGACTT
TRBV10-1,3	CAGATGGCTACAGTGTCTCTAGATCAAA
TRBV10-1,3	CAGATGGCTATAGTGTCTCTAGATCAAA
TRBV10-2	GTTGTCTCCAGATCCAAGACAGAGAA
TRBV11	GCAGAGAGGCTCAAAGGAGTAGACT
TRBV12-3,4	GCTAAGATGCCTAATGCATCATTCTC
TRBV12-5	CTCAGCAGAGATGCCTGATGCAACT
TRBV13	TCTCAGCTCAACAGTTCAGTGACTA
TRBV14	GCTGAAAGGACTGGAGGGACGTAT
TRBV15	GATAACTTCCAATCCAGGAGGCCG
TRBV16	GCTAAGTGCCTCCCAAATTCACCC
TRBV18	GGAACGATTTTCTGCTGAATTTCCCA
TRBV19	GGTACAGCGTCTCTCGGGAGAAGA

TRBV20-1	GGACAAGTTTCTCATCAACCATGCAA
TRBV24-1	TGGATACAGTGTCTCTCGACAGGC
TRBV25-1	CAACAGTCTCCAGAATAAGGACGGA
TRBV27-1	TACAAAGTCTCTCGAAAAGAGAAGAGGA
TRBV28	GGGGTACAGTGTCTCTAGAGAGA
TRBV29	GTTTCCCATCAGCCGCCCAAACCTA
TRBV30	CAGACCCCAGGACCGGCAGTTCAT

Supplemental Table 3. hTRAV sequences used during the pre-amplification stage of library prep for enriching full length TCRalpha and beta CDR3.

Donor	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
Donor 1	HLA-A*02:01	HLA-A*24:02	HLA-DRB1*15:01	HLA-DRB1*15:02
Donor 2	HLA-A*02:01	HLA-A*11:02	HLA-DRB1*04:03:01	HLA-DRB1*15:02
Donor 3	HLA-A*01:01	HLA-A*02:03	DRB1*07:01	DRB1*08:03

Supplemental Table 4. Donor HLA typing

id	name	read	pattern	sequence	feature_type
ADT_C0053	Hu.CD11c	R2	5PNNNNNNNNNN(BC)	TACGCCTATAACTTG	Antibody Capture
ADT_C0081	Hu.CD14_M5E2	R2	5PNNNNNNNNNN(BC)	TCTCAGACCTCCGTA	Antibody Capture
ADT_C0083	Hu.CD16	R2	5PNNNNNNNNNN(BC)	AAGTTCACTCTTTGC	Antibody Capture
ADT_C0050	Hu.CD19	R2	5PNNNNNNNNNN(BC)	CTGGGCAATTACTCG	Antibody Capture
ADT_C0034	Hu.CD3_UCHT1	R2	5PNNNNNNNNNN(BC)	CTCATTGTAACTCCT	Antibody Capture
ADT_C0048	Hu.CD45_2D1	R2	5PNNNNNNNNNN(BC)	TCCCTTGCGATTTAC	Antibody Capture
ADT_C0072	Hu.CD4_RPA.T4	R2	5PNNNNNNNNNN(BC)	TGTTCCCGCTCAACT	Antibody Capture
ADT_C0047	Hu.CD56	R2	5PNNNNNNNNNN(BC)	TCCTTTCCTGATAGG	Antibody Capture
ADT_C0046	Hu.CD8	R2	5PNNNNNNNNNN(BC)	GCGCAACTTGATGAT	Antibody Capture
DEX_01	HLA-A*0201+NLVPMVATV+pp65+CMV	R2	5PNNNNNNNNNN(BC)	GCCGCGCGGTAGCGC	Antibody Capture

Supplemental Table 5. Antibody Derived Tag CellRanger Multi Table, including ADT probe name, protein target, read, tag pattern, sequence, and feature type.

Target	Conjugate	Clone	Purpose
CD3	BUV395	UCHT1	Pan T cells
CD56	BUV615	NCAM16.2	NK cell Exclusion
CD19	BUV615	HIB19	B Cell Exclusion
CD8	BUV737	RPA-T8	CD8 T cells
pp65+	PE	N/A	Dextramer
Live/Dead	FVS510	N/A	Viability
CD4	BB700	SK3	CD4 T cells
CD14	SparkYG 593	S18004B	Monocyte Exclusion

Supplemental Table 6. Antibody panel used in dextramer flow analysis