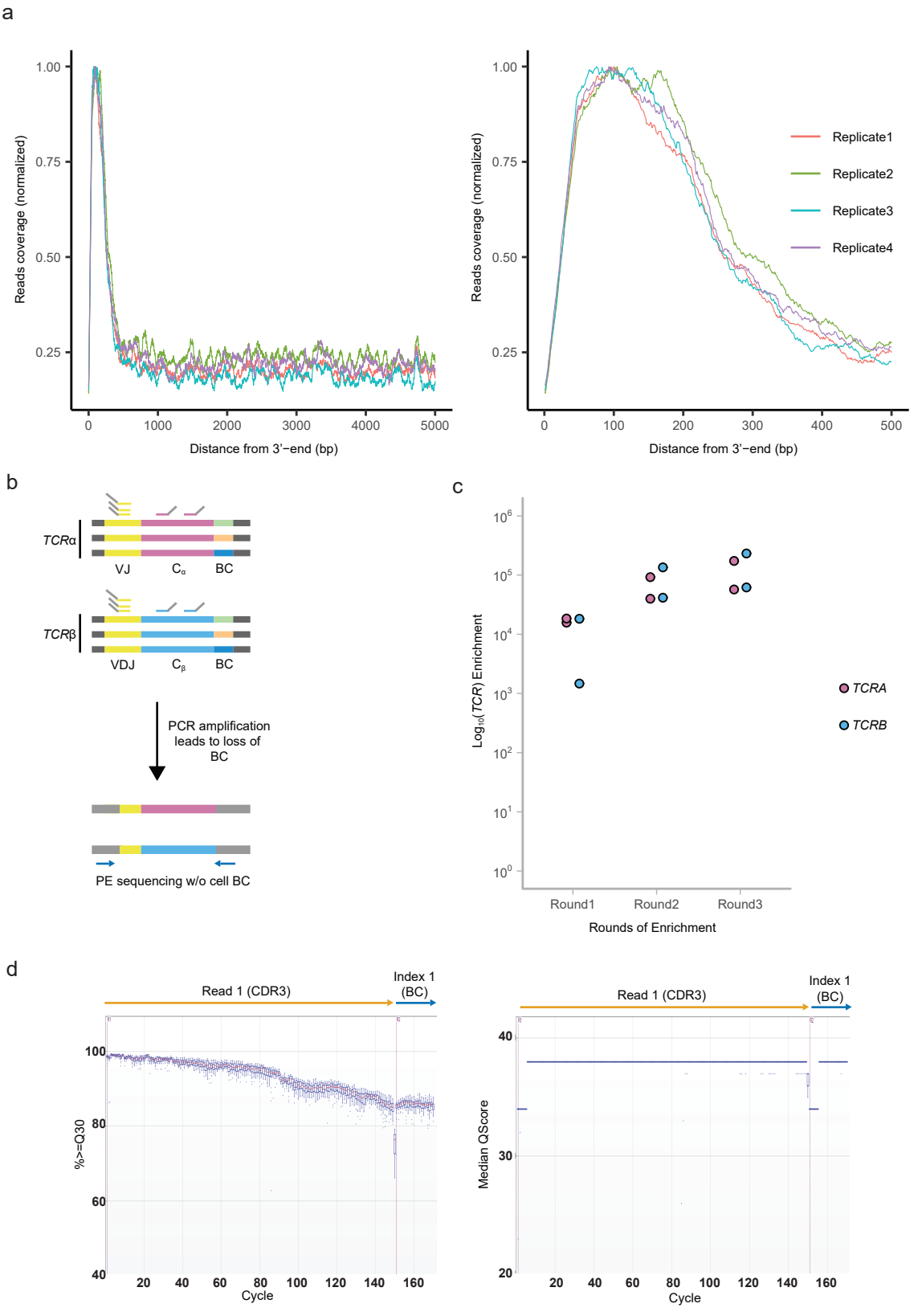
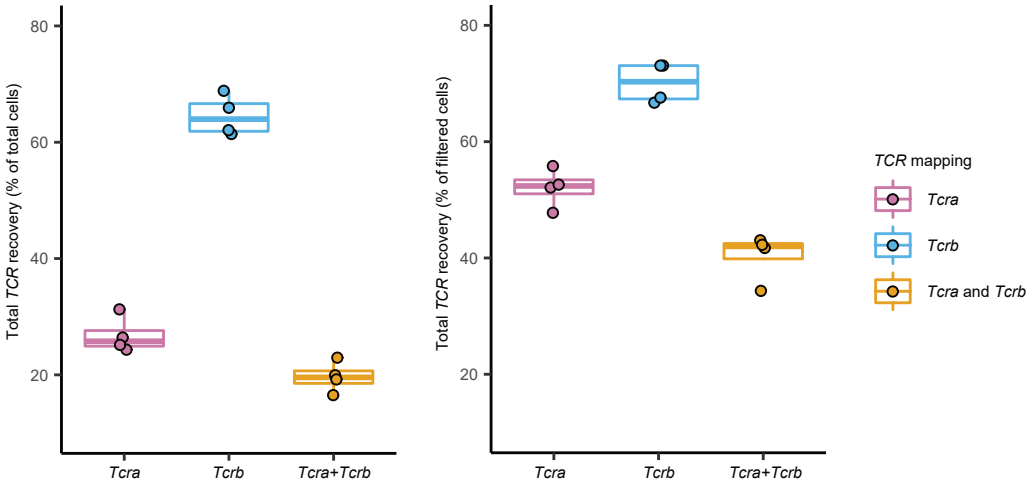
Supplementary Figure 1



Supplementary Fig. 1| a, Average insert size of 3' sequencing libraries. Results of scRNA-Seq of OT-I spiked-in libraries were analyzed for insert size. Genes with ENSEMBL defined 3' end were used, and insert size is shown as distance from the defined 3' end for up to 5kbp (left), or 500bp (right). Reads coverage normalized by maximum of each replicate is shown to aid comparison across samples. **b**, Schematic of application of conventional *CDR3* amplification to 3' barcoded libraries. The use of primers specific to the constant regions results in efficient amplification of the *TCR CDR3* region but leads to loss of single-cell barcodes. **c**, qPCR assessment of *TCR* transcript enrichment by affinity pull-down. Successive rounds of affinity pull-down on whole transcriptome libraries from T cells are shown. See **Methods** for more details. Data represent two independent experiments for each set of enrichment. **d**, Examples of quality score plots from sequencing of *TCR* enriched libraries. A total of 170 cycles were used to sequenced the *CDR3* (1-150) and cellular barcode (151-170). (left) Cumulative percentages of reads with overall QScore >=30 by cycle number. (right) Median QScore of each cycle.

Supplementary Figure 2

a



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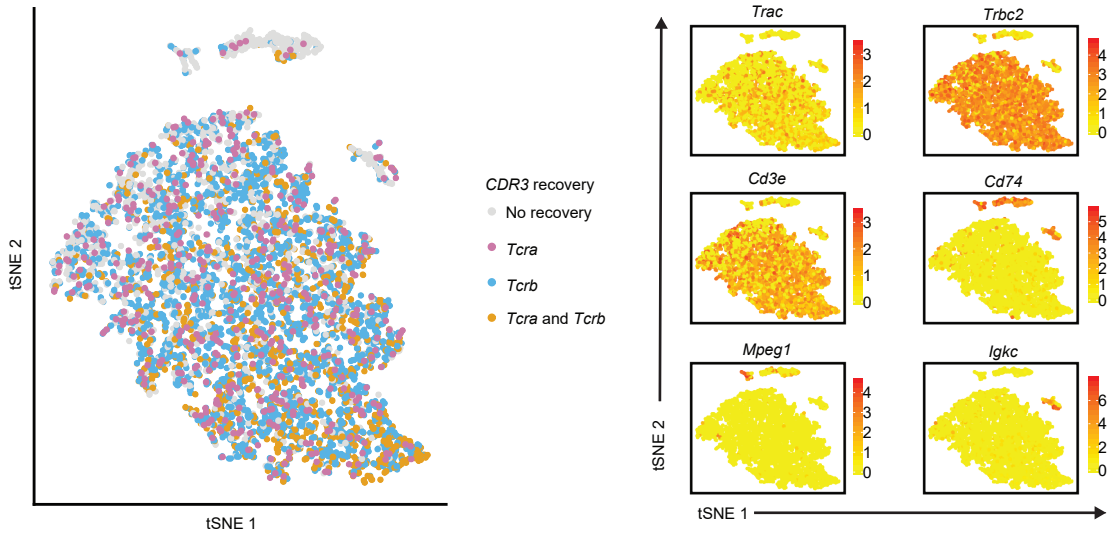

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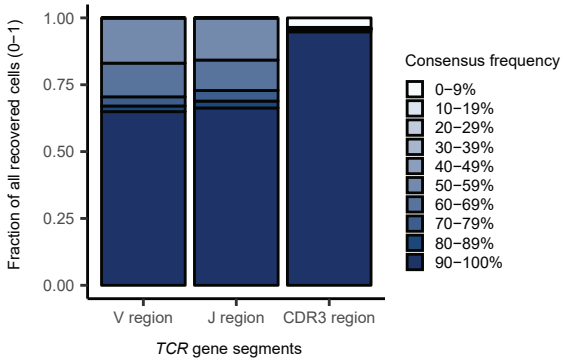
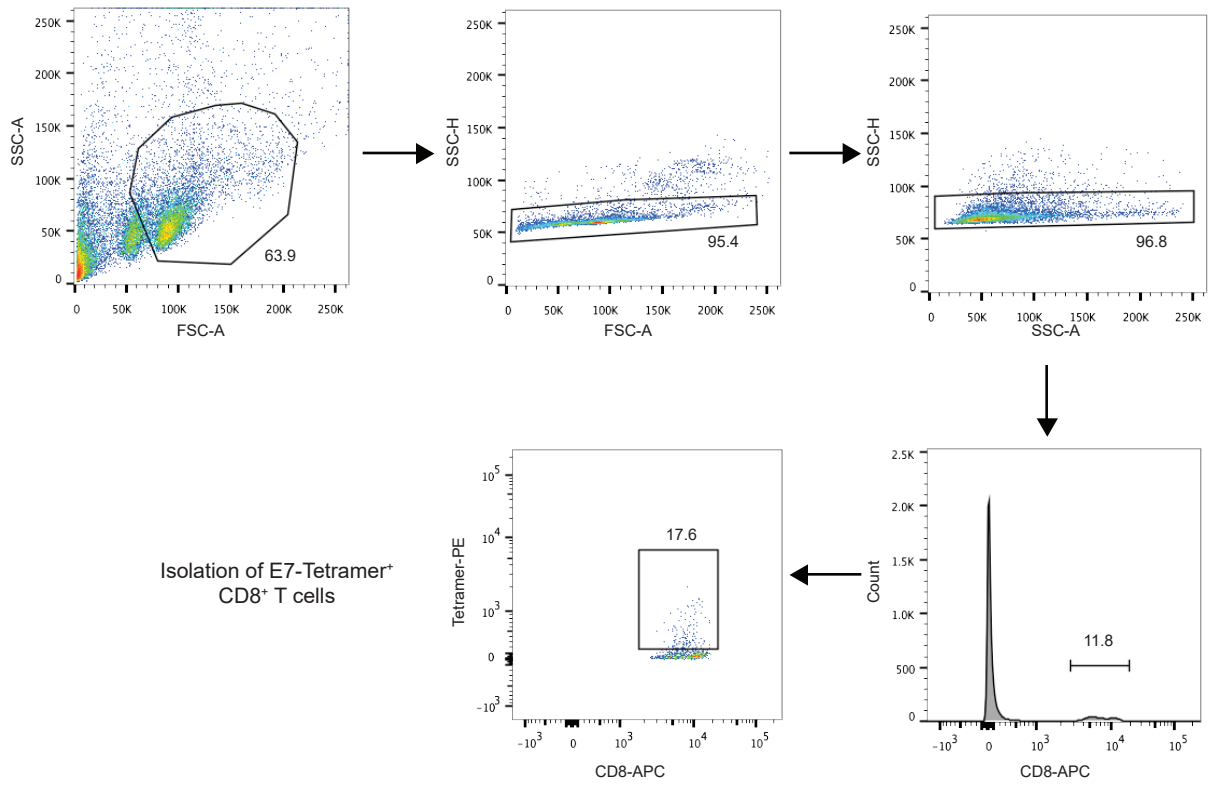


Supplementary Fig. 2| a, Total *TCR* recovery across four OT-I Spiked-in libraries (n = 4 samples). (left) Overall *CDR3* recovery rates for all cells. (right) *CDR3* recovery rates after removing cells without mapped *TCR* transcripts in whole transcriptome data. Box and whisker plots indicate the (box) 25^{th} and 75^{th} percentile along with (whisker) +/- 1.5*interquartile range. **b**, (left) *CDR3* recovery mapped on tSNE visualization of whole transcriptomes, and (right) key cell type surface markers (n = 6,620 cells). (right) Color indicates log-normalized gene expression (yellow to red). T cells were marked by expression of *Cd3e*, *Trac* and *Trbc*. Small numbers of other cell types were present due to incomplete magnetic enrichment. These included B cells (*Igkc*), macrophages (*Mpeg1*), and myeloid cells (*Cd74*). A small number of *TCR* sequences was also recovered from these clusters, correlating with trace amount of *Cd3e* expression in these clusters. These clusters were removed in subsequent analysis. **c**, Ratios of most frequent V,J, and CDR3 call for each UMI relative to either the second most frequent call (for V and J segments, resulting in "consensus frequency" between 0.5-1) or to the total number of reads (for CDR3, resulting in consensus frequency between 0-1). See **Methods** for more details.

Supplementary Figure 3

a



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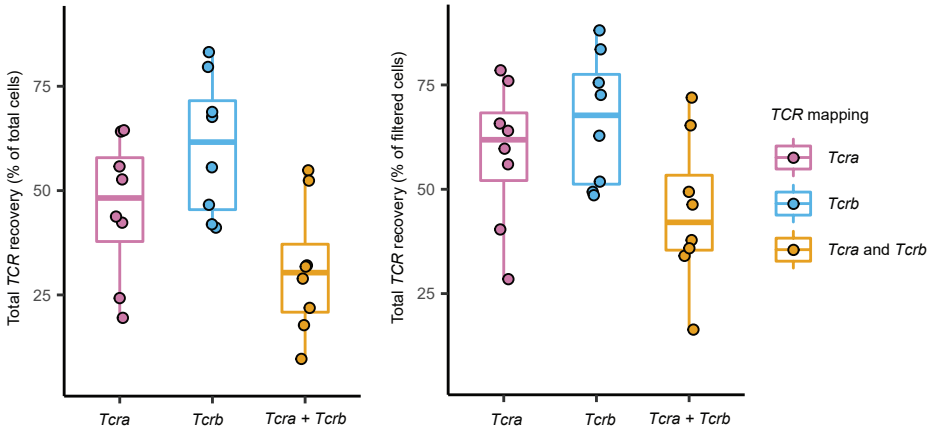
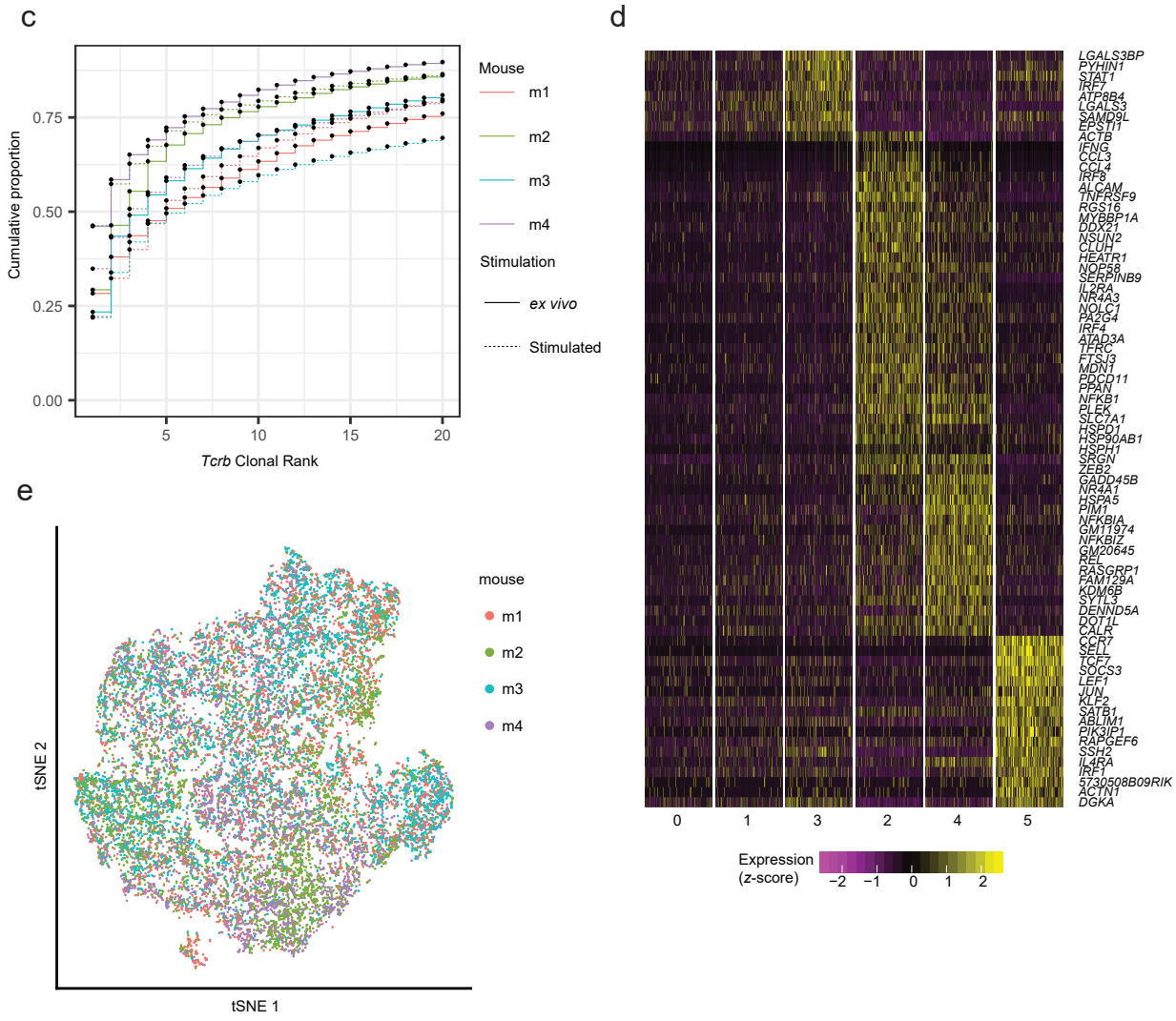
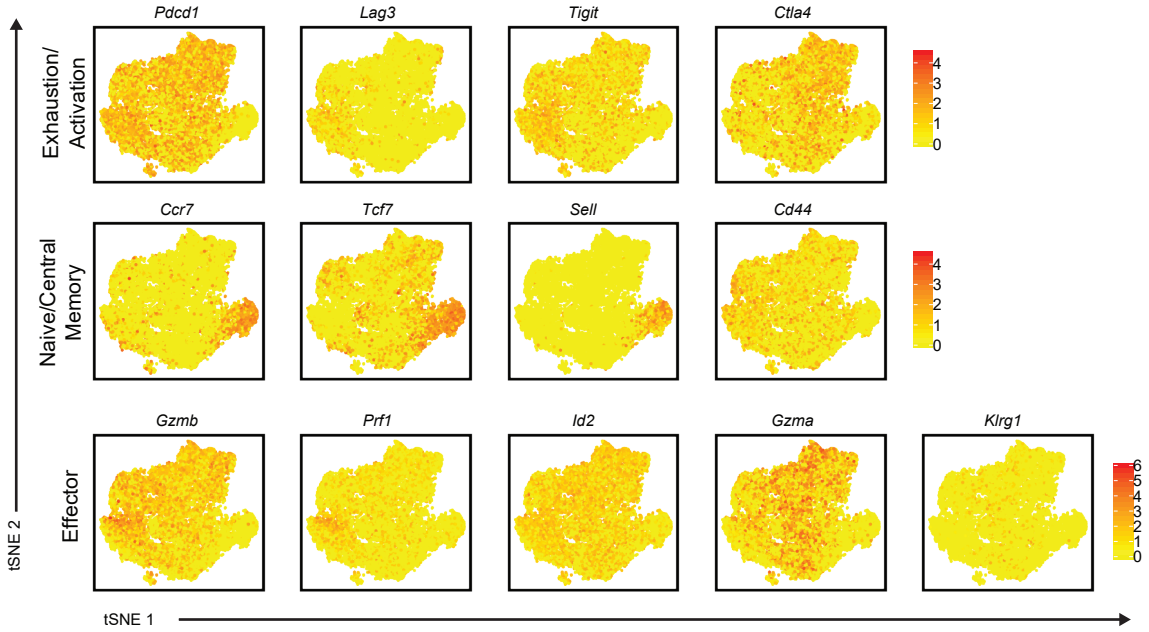


Supplementary Figure 3

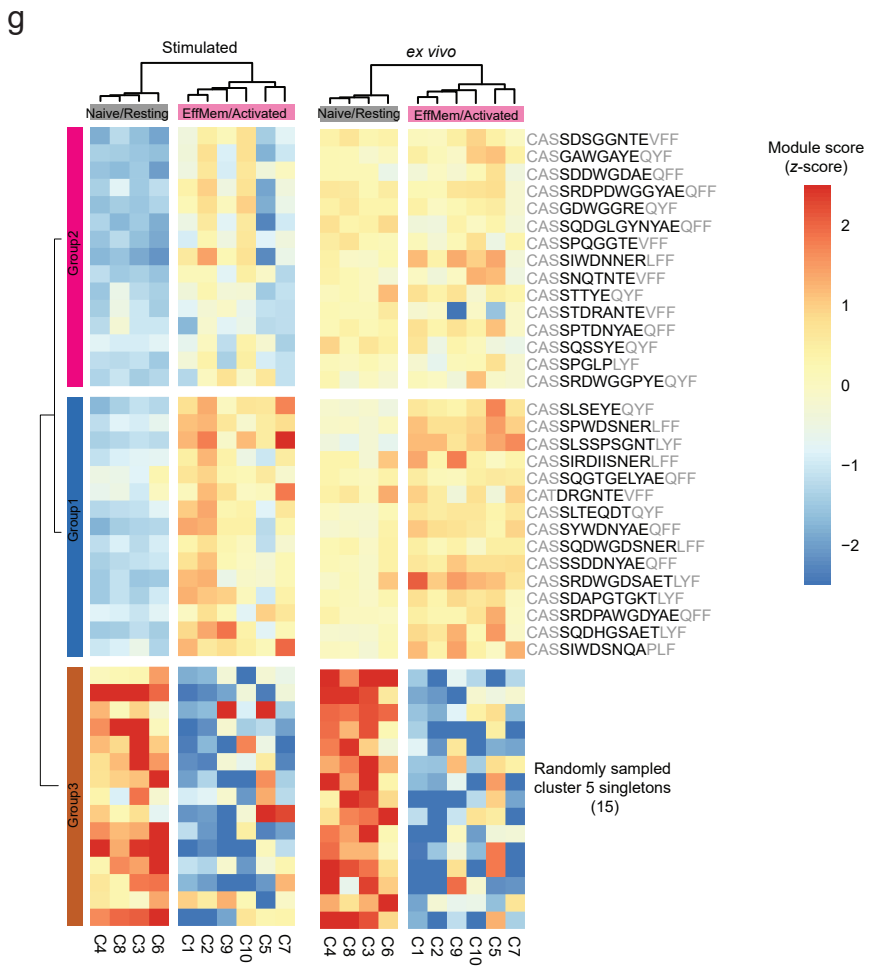


Supplementary Figure 3

f



Supplementary Figure 3



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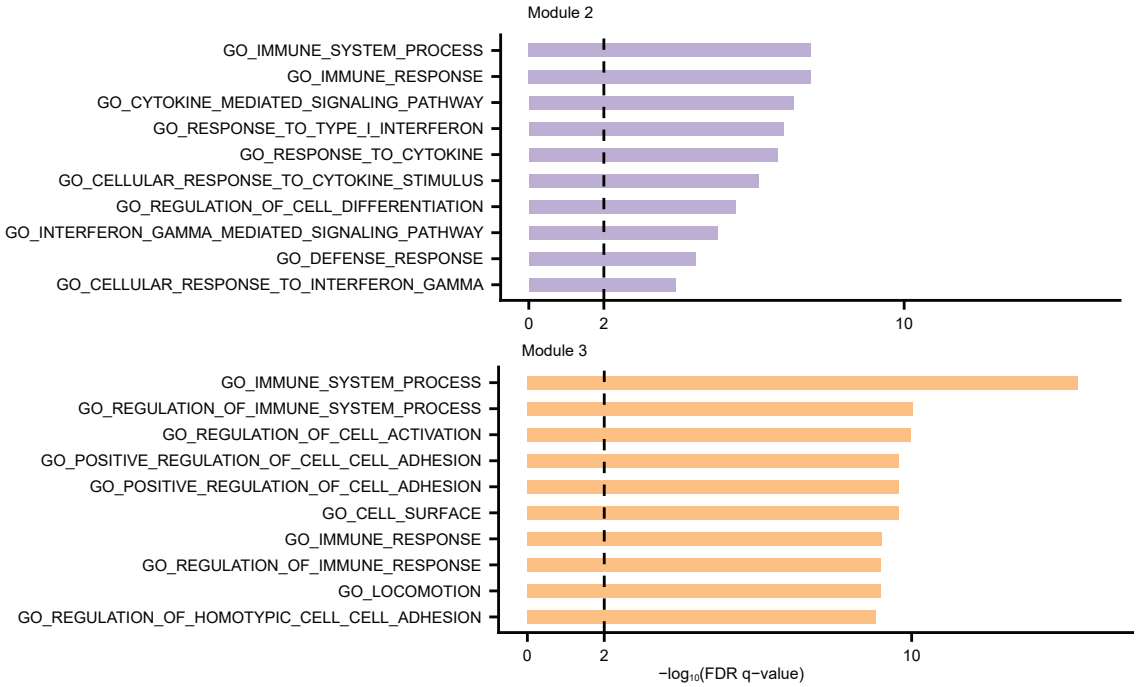
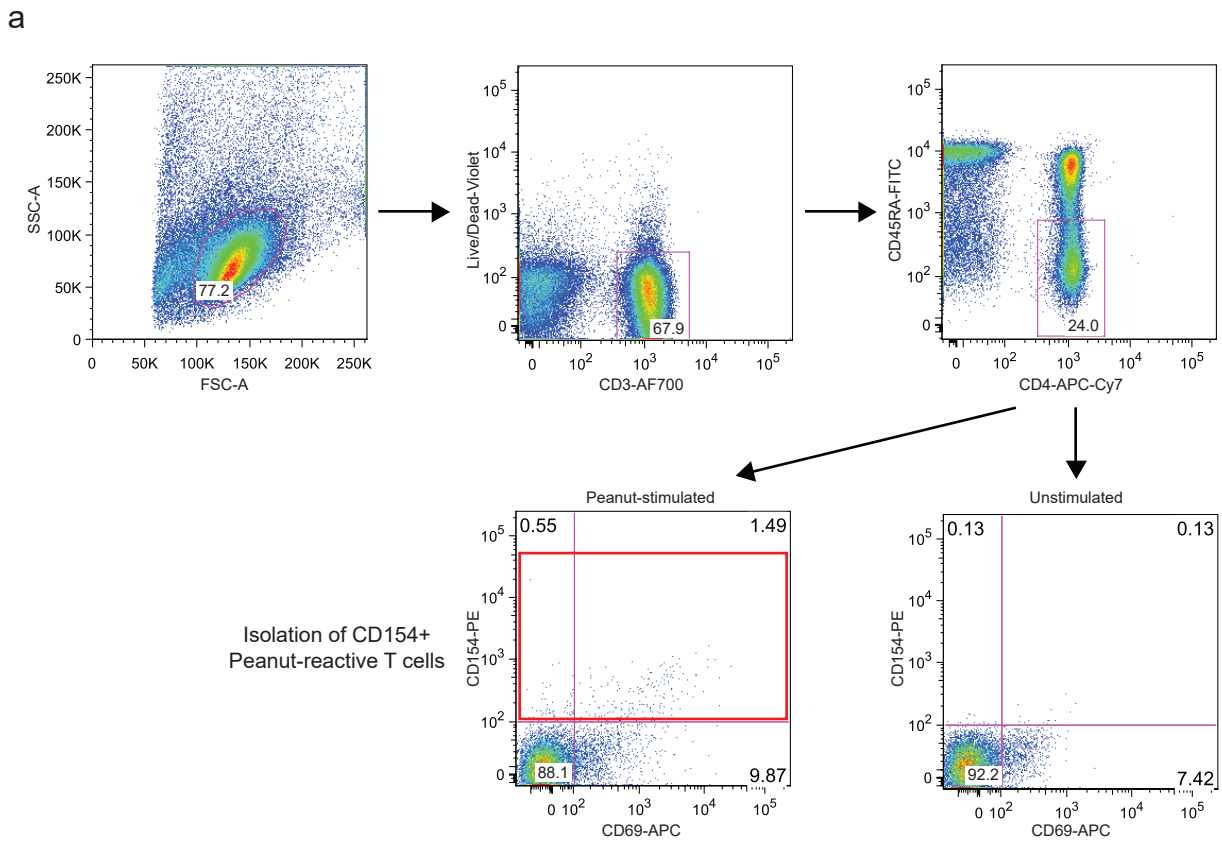
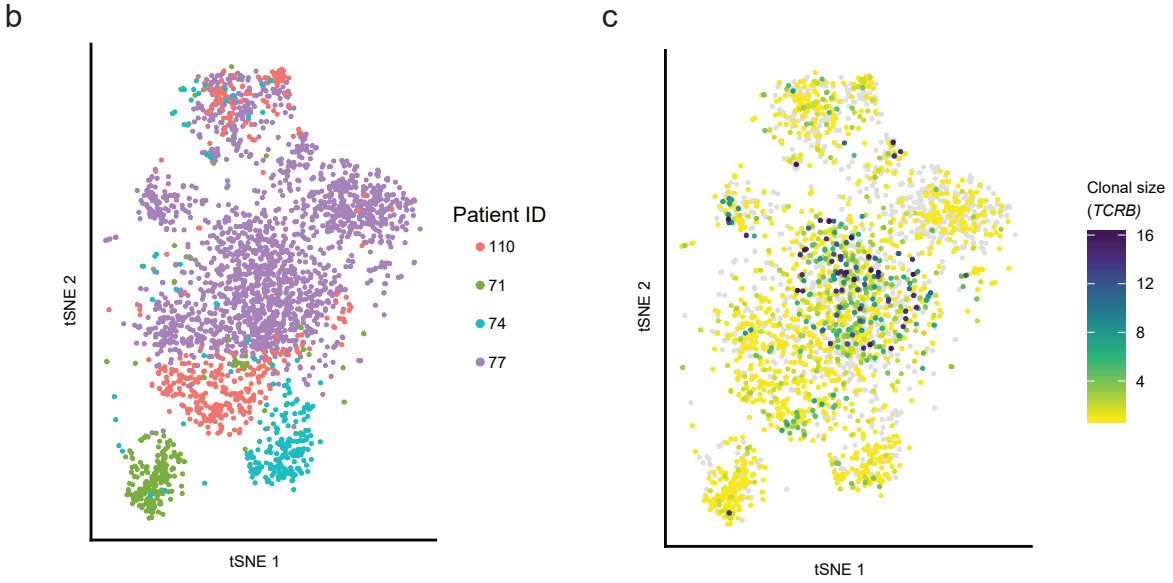

Module 2
GO_IMMUNE_SYSTEM_PROCESS
GO_IMMUNE_RESPONSE
GO_CYTOKINE_MEDIATED_SIGNALING_PATHWAY
GO_RESPONSE_TO_TYPE_I_INTERFERON
GO_RESPONSE_TO_CYTOKINE
GO_CELLULAR_RESPONSE_TO_CYTOKINE_STIMULUS
GO_REGULATION_OF_CELL_DIFFERENTIATION
GO_INTERFERON_GAMMA_MEDIATED_SIGNALING_PATHWAY
GO_DEFENSE_RESPONSE
GO_CELLULAR_RESPONSE_TO_INTERFERON_GAMMA
0
2
10
Module 3
GO_IMMUNE_SYSTEM_PROCESS
GO_REGULATION_OF_IMMUNE_SYSTEM_PROCESS
GO_REGULATION_OF_CELL_ACTIVATION
GO_POSITIVE_REGULATION_OF_CELL_CELL_ADHESION
GO_POSITIVE_REGULATION_OF_CELL_ADHESION
GO_CELL_SURFACE
GO_IMMUNE_RESPONSE
GO_REGULATION_OF_IMMUNE_RESPONSE
GO_LOCOMOTION
GO_REGULATION_OF_HOMOTYPIC_CELL_CELL_ADHESION
0
2
10
−log10(FDR q−value)

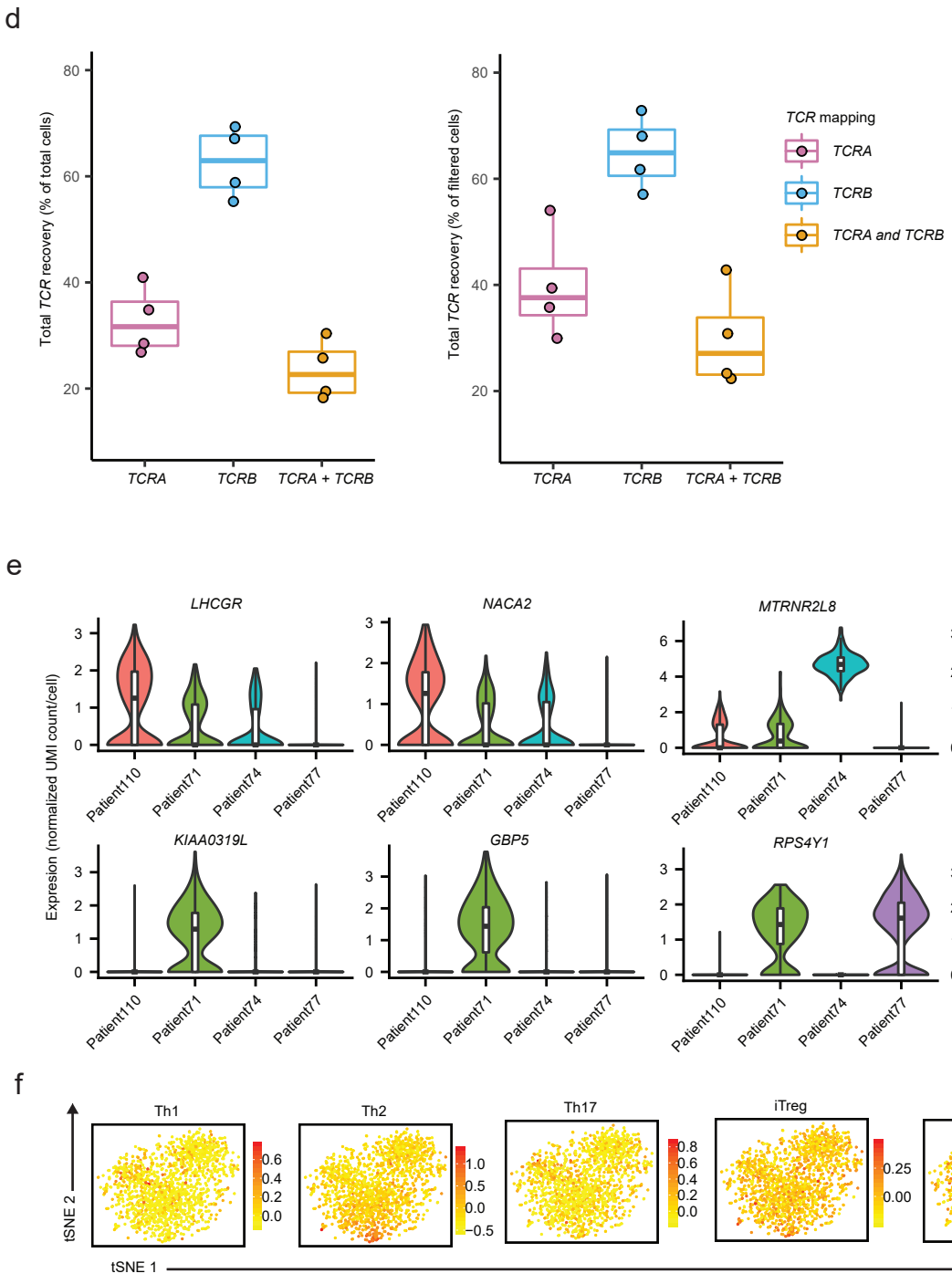
Supplementary Fig. 3| a, Gating strategy for flow cytometry sorting of E7-tetramer$^+$ CD8$^+$ T cells. **b**, Total *TCR* recovery across four HPV-E7 immunized mice (n = 4 animals). (left) Overall *CDR3* recovery rates for all cells. (right) *CDR3* recovery rates after removing cells without mapped *TCR* transcripts in whole transcriptome data. Box and whisker plots indicate the (box) 25th and 75th percentile along with (whisker) +/- 1.5*interquartile range. **c**, Cumulative proportion of the top 20 most expanded TCRβ clonotypes in each animal and stimulation condition. **d**, Heatmap of genes most upregulated in each of the clusters shown in **Figure 3a**. **e**, tSNE visualization of single cells colored by the animal identity. **f**, Expression of canonical markers associated with naïve/CM, effector, and T cell activation/exhaustion phenotypes. Color indicates log-normalized gene expression (yellow to red). Color scales apply to each respective row separately. **g**, Average scores of CD8$^+$ T cell modules identified by *Singer, M. et al.*[29] (C1-10, labeled on bottom of heatmap) in Group 1, 2, and 3 clonotypes shown in **Figure 3d**. In *Singer, M. et al.,* C3, C4, C5, C6, C8 were upregulated in sorted naïve or *TIM3*$^-$ *PD1*$^-$ *CD8*$^+$ T cells (naïve/resting). C1, C2, C7, C9, C10 were upregulated in sorted effector/effector memory, *TIM3*$^+$ *PD1*$^-$, or *TIM3*$^+$ *PD1*$^+$ *CD8*$^+$ T cells (Effmem/Activated). See *Singer, M. et al.* and **Supplementary Table 11** for more details. **h**, Gene Ontology (GO; C5) term enrichment of genes in Module 2 and 3. FDR q-values represent Benjamini and Hochberg-corrected hypergeometric *P* values. 49 and 35 genes are used from Module 2 and 3, respectively. See **Supplementary Table 12** for more details. Data represent combined data from four independent experiments of four mice total, with a total of 14,424 cells (**b-h**).

Supplementary Figure 4



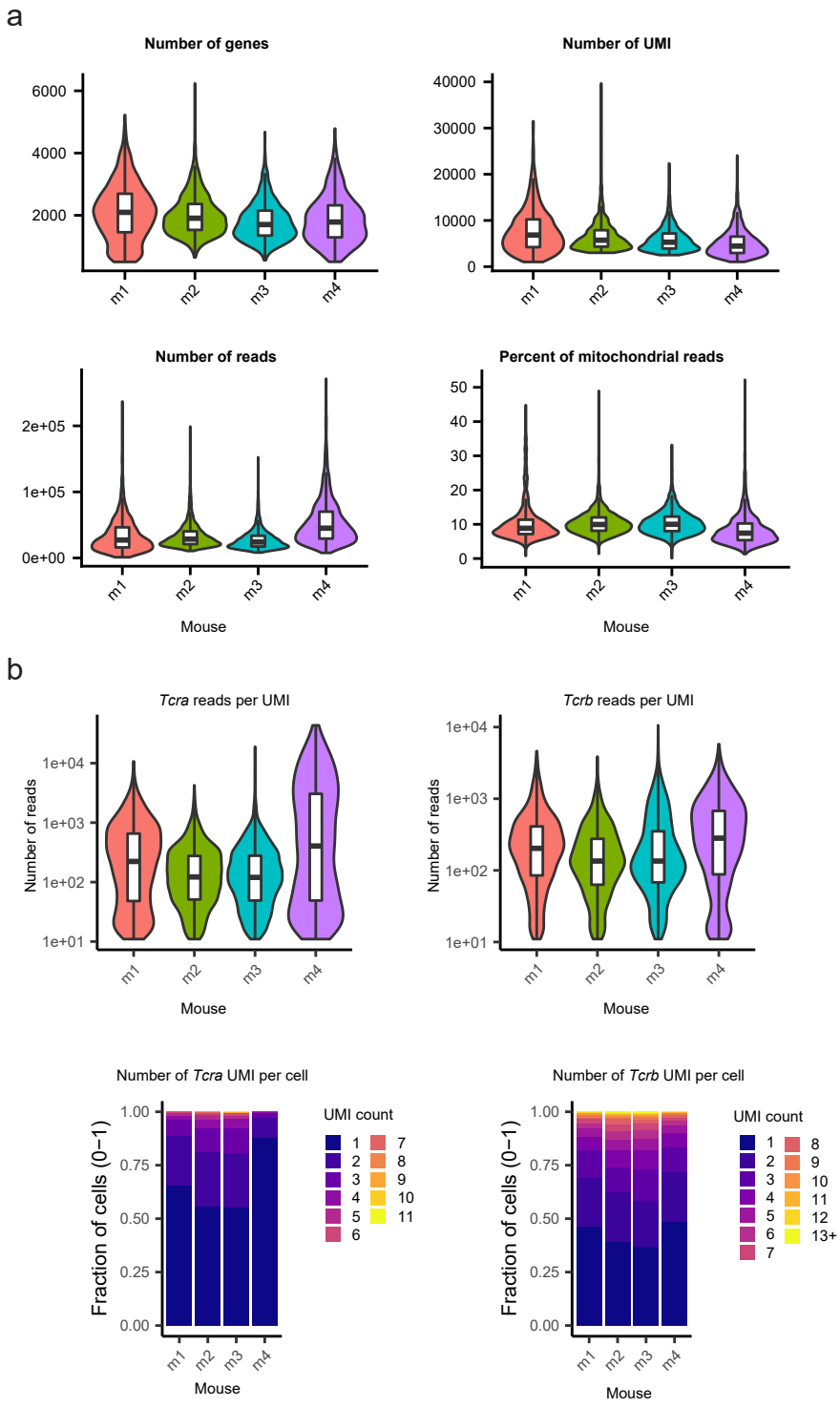


Supplementary Figure 4

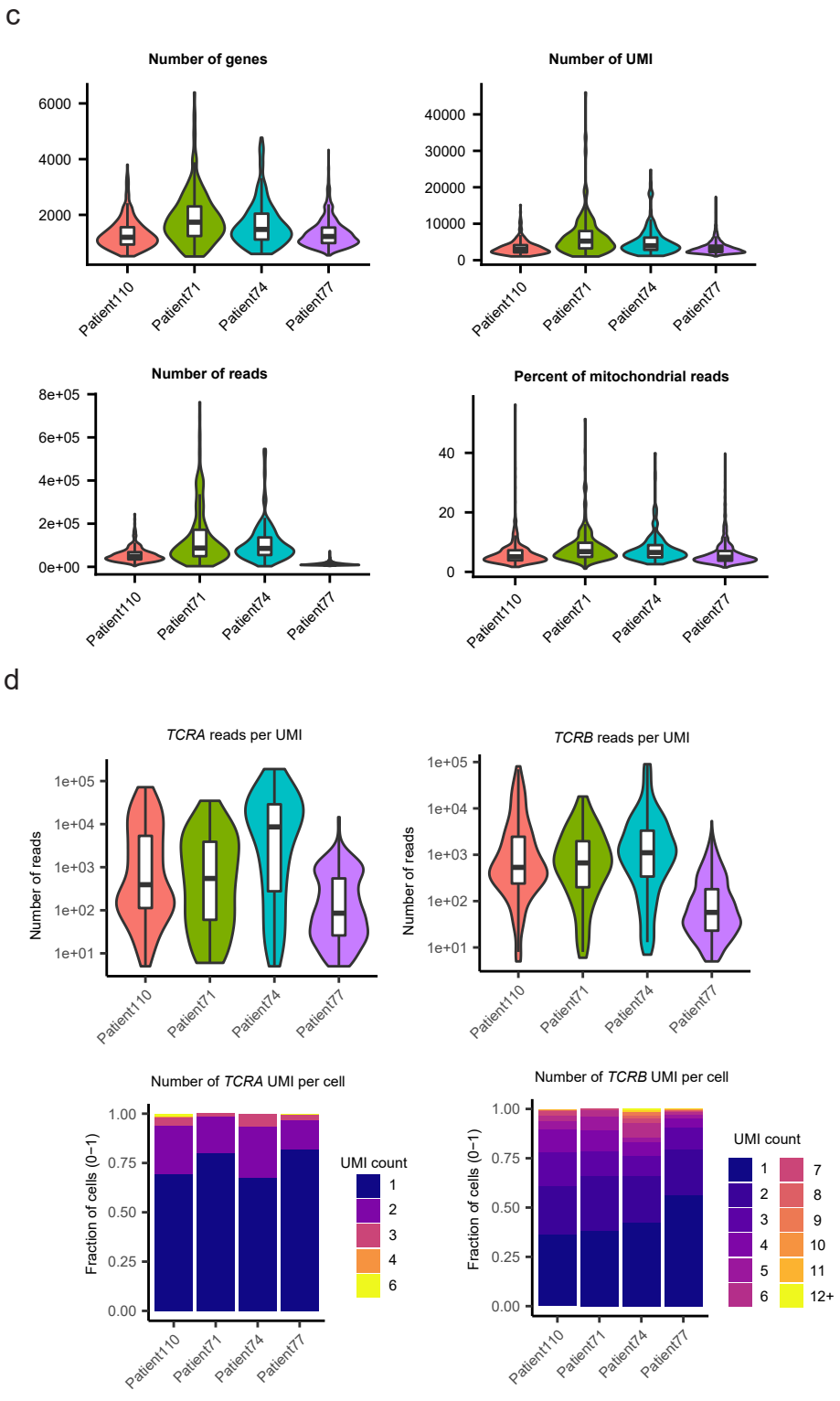


Supplementary Fig. 4| a, Gating strategy for flow cytometry sorting of $CD154^+$ CD4+ T cells after *ex vivo* stimulation with peanut antigens. **b**, tSNE visualization of $CD154^+$ T cells from four peanut-allergic patients, colored by patient identity (n = 2,712 cells). **c**, Clonal size of TCRβ mapped on the tSNE visualization (n = 2,712 cells). **d**, (left) *CDR3* recovery rates of all cells, and (right) *CDR3* recovery rates after removing cells without mapped TCR transcript in the whole transcriptome data. **e**, Expression of selected genes that most differentiated the four patients. Violin plots represent estimated density of cells (n = 398 cells for Patient110; 246 cells for Patient71; 221 cells for Patient74; and 1847 cells for Patient 77). **f**, Module scores (yellow to red) of CD4 effector T cell signatures outlined by *Wei, G., et al.*[38] mapped on the tSNE visualization of cells from Patient 77 (n = 1847 cells). See **Supplementary Table 11** and **Methods** for more information. All box and whisker plots indicate the (box) 25^{th} and 75^{th} percentile along with (whisker) +/- 1.5*interquartile range (**d**,**e**). Data represent combined data of four independent experiments with a total of four patients (**b**-**e**), or of an individual experiment with one patient (patient 77, **f**).

Supplementary Figure 5



Supplementary Figure 5



Supplementary Fig. 5| a, Number of genes, unique molecular identifiers (UMIs), sequencing reads, and percent of reads mapped to mitochondrial genes for each cell of the E7-tetramer^{+} sorted T cell samples shown in **Figure 3** (n = 3231 cells for mouse 1; 3932 cells for mouse 2; 3937 cells for mouse 3; 3324 cells for mouse 3). **b**, (top) Per-UMI Read-depth of *Tcra* (n = 2164 UMIs for mouse 1; 4159 UMIs for mouse 2; 4141 UMIs for mouse 3; 847 UMIs for mouse 4) and *Tcrb* (n =4453 UMIs for mouse 1; 7389 UMIs for mouse2; 7986 UMIs for mouse 3; 4118 UMIs for mouse 4) as well as number of UMIs for each recovered *CDR3* in E7-tetramer^{+} sorted T cell samples shown in **Figure 3** (n = 3231 cells for mouse 1; 3932 cells for mouse 2; 3937 cells for mouse 3; 3324 cells for mouse 3). **c**, Number of genes, UMIs, sequencing reads, and percent of reads mapped to mitochondrial genes for each cell (n = 398 cells for Patient 110; 246 cells for Patient 71; 221 cells for Patient 74; and 1847 cells for Patient 77) from the peanut allergic patients shown in **Figure 4**. **d**, (top) Per-UMI Read-depth of *TCRA* (n = 230 UMIs for Patient 110; 80 UMIs for Patient 71; 107 for Patient 74; 642 for Patient 77) and *TCRB* (n = 681 UMIs for Patient 110; 383 UMIs for Patient 71; 347 UMIs for Patient 74; 1884 UMIs for Patient 77). (bottom) number of UMIs for each recovered *CDR3* in T cells (n = 398 cells for Patient 110; 246 cells for Patient 71; 221 cells for Patient 74; and 1847 cells for Patient 77) from peanut allergic patients shown in **Figure** 4. All box and whisker plots indicate the (box) 25th and 75th percentile along with (whisker) +/- 1.5*interquartile range (**a**-**d**). Violin plots represent estimated density of cells (**a**-**d**).

Supplementary Table 1. Biotinylated oligonucleotide probes for TCR enrichment

Name	Sequence	Vendor/Service
Human TRBC-1	/5BiosG/GTGTTCCCACCCRAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGC	IDT Ultramer/Standard Desalting
Human TRAC-1	/5BiosG/CTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAG	IDT Ultramer/Standard Desalting
Mouse TRBC-1	/5BIOSG/AGGATCTGAGAAATGTGACTCCACCCAAGGTCTCCTTGTTTGAGCCATCAAAAGCAGAGATTGCAAACAAACAAAAGGCTACCCTCGTGT	IDT Ultramer/Standard Desalting
Mouse TRAC-1	/5BIOSG/ACATCCAGAACCCAGAACCTGCTGTGTACCAGTTAAAAGATCCTCGGTCTCAGGACAGCACCCTCTGCCTGTTCACCGACTTTGACTCCC	IDT Ultramer/Standard Desalting

Supplementary Table 2. PCR primers

Name	Sequence	Vendor/Service
UPS	AAGCAGTGGTATCAACGCAGAGT	IDT/Standard Desalting
UPS-mod-N50x	AATGATACGGCGACCACCGAGATCTACACGCCTGTCCGCGGAAGCAGTGGTATCAACGCAGAGT*A*C	IDT/HPLC
UPS2-N70x	CAAGCAGAAGACGGCATACGAGATGTCTCGTGGGCTCGG	IDT/HPLC

Supplementary Table 4. Custom sequencing primers

Name	Sequence	Vendor/Service
Human Alpha Sequencing Primer	AGAGTCTCTCAGCTGGTACACGGCAGGGTCAGGITTCTGGATAT	IDT/HPLC
Human Beta Sequencing Primer	CAAACACAGCGACCTCGGGTGGGAACACSTTKTTCAGGTCCT	IDT/HPLC
Mouse Alpha Sequencing Primer	GTCCTGAGACCGAGGATCTTTTAACTGGTACACAGCAGGTTCTGGGTTCTGGATGT	IDT/HPLC
Mouse Beta Sequencing Primer	TGCTTTTGATGGCTCAAACAAGGAGACCTTGGGTGGAGTCACATTTCTCAGATCCT	IDT/HPLC
Seq-Well Sequencing Primer	GCCTGTCCGCGGAAGCAGTGGTATCAACGCAGAGTAC	IDT/HPLC

Supplementary Table 5. Repeatability statistics from OT-1 spiked-in samples technical duplicates

OT-1 Technical Duplicates	Duplicate 1	Duplicate 2
Total reads	7,646,012	12,281,202
% reads on-target	91.92	93.80
number of cells w/ TCR call	4,452	4,511
Jaccard index of overlap cells	0.94	
number of UMI	13,862	14,396
Jaccard index of overlap UMI	0.84	
% identical clonotype call	99.74	

Supplementary Table 8. E7-tetramer sorted CD8+ T cells with dual functional TCRα transcripts

TRB_CDR3	TRA_CDR3	TRA.2_CDR3	AnimalID	TRAV	TRAJ	TRBV	TRBJ	TRAV.2	TRAJ.2	#cells
CASSQDLGNYAEQFF	CAMREGLMATGGNNKLTF	CAMREGLMATGGNNKLTF	m2	TRAV16D	TRAJ56	TRBV2	TRBJ2-1	TRAV16N	TRAJ56	1
CASSQDLGNYAEQFF	CAMREGLMATGGNNKLTF	CAMREGLMATGGNNKLTF	m2	TRAV16N	TRAJ56	TRBV2	TRBJ2-1	TRAV16D	TRAJ56	2
CASSQDLGNYAEQFF	CAMREGLMATGGNNKLTF	CAVSNSGGSNYKLTF	m1	TRAV16D	TRAJ56	TRBV2	TRBJ2-1	TRAV7D-5	TRAJ53	2
CASSQDLGNYAEQFF	CAMREGLMATGGNNKLTF	CAVSNSGGSNYKLTF	m2	TRAV16D	TRAJ56	TRBV2	TRBJ2-1	TRAV7D-5	TRAJ53	143
CASSQDLGNYAEQFF	CAMREGLMATGGNNKLTF	CAVSNSGGSNYKLTF	m3	TRAV16D	TRAJ56	TRBV2	TRBJ2-1	TRAV7D-5	TRAJ53	11
CASSQDLGNYAEQFF	CAVSNSGGSNYKLTF	CAMREGLMATGGNNKLTF	m2	TRAV7D-5	TRAJ53	TRBV2	TRBJ2-1	TRAV16D	TRAJ56	60
CASSQDLGNYAEQFF	CAVSNSGGSNYKLTF	CAMREGLMATGGNNKLTF	m3	TRAV7D-5	TRAJ53	TRBV2	TRBJ2-1	TRAV16D	TRAJ56	6

Supplementary Table 9. Peanut allergy subject information

Patient ID	Age	Gender	Skin prick test wheal (mm)	Peanut-specific IgE (kU/L)	Ara h 2-specific IgE (kU/L)	Total IgE (kU/L)
110	10	Female	6	247	168	1437
71	13	Male	4	6.34	1.26	516
74	14	Female	8	4.65	1.39	249
77	17	Male	13	240	74.5	1595