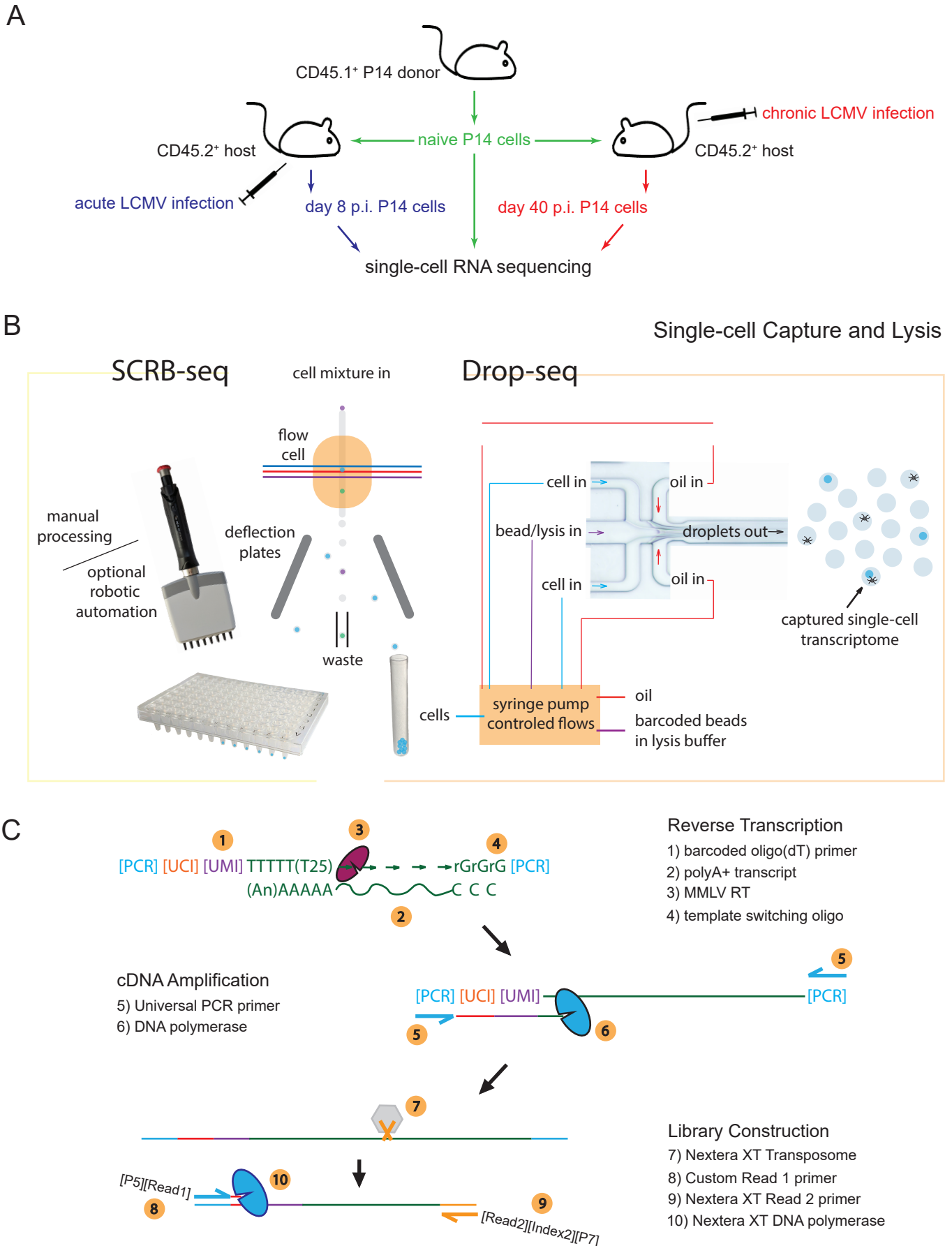


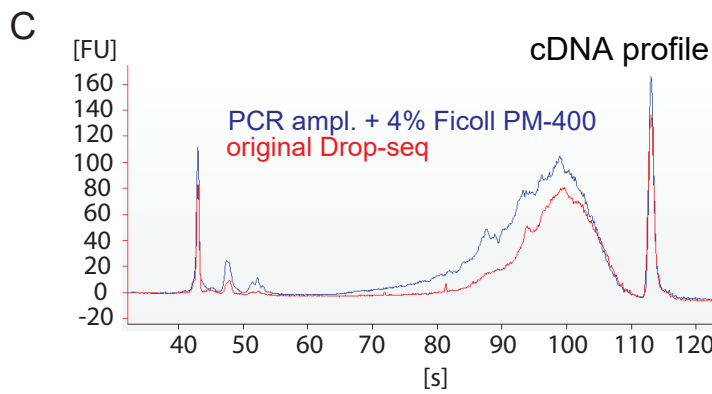
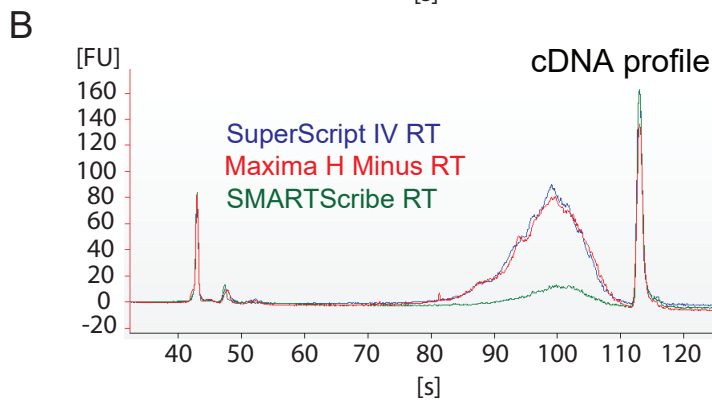
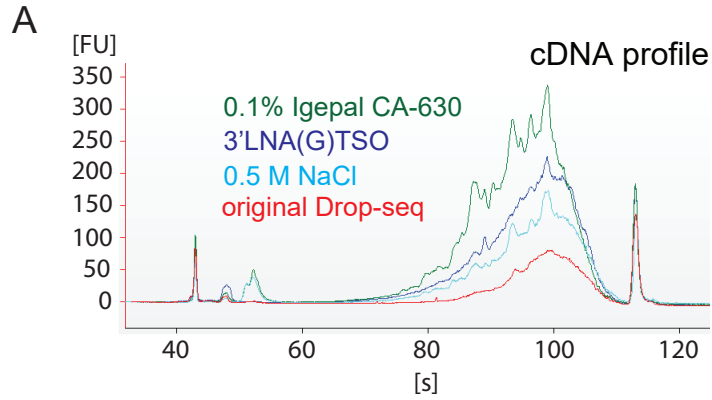
Supplementary Figure 1. Schematic protocol representation of SCRB-seq and Drop-seq.

(A) Schematic representation of the P14 experimental system. The green, blue and red colour denote naïve P14 T cells, P14 T cells recovered at day 8 post LCMV Armstrong infection and P14 T cells recovered at day 40 post LCMV clone-13 infection respectively. (B) Illustrates the difference in the single-cell capturing strategy of SCRB-seq versus Drop-seq. (C) Representation of the common chemistry downstream of single-cell capturing.



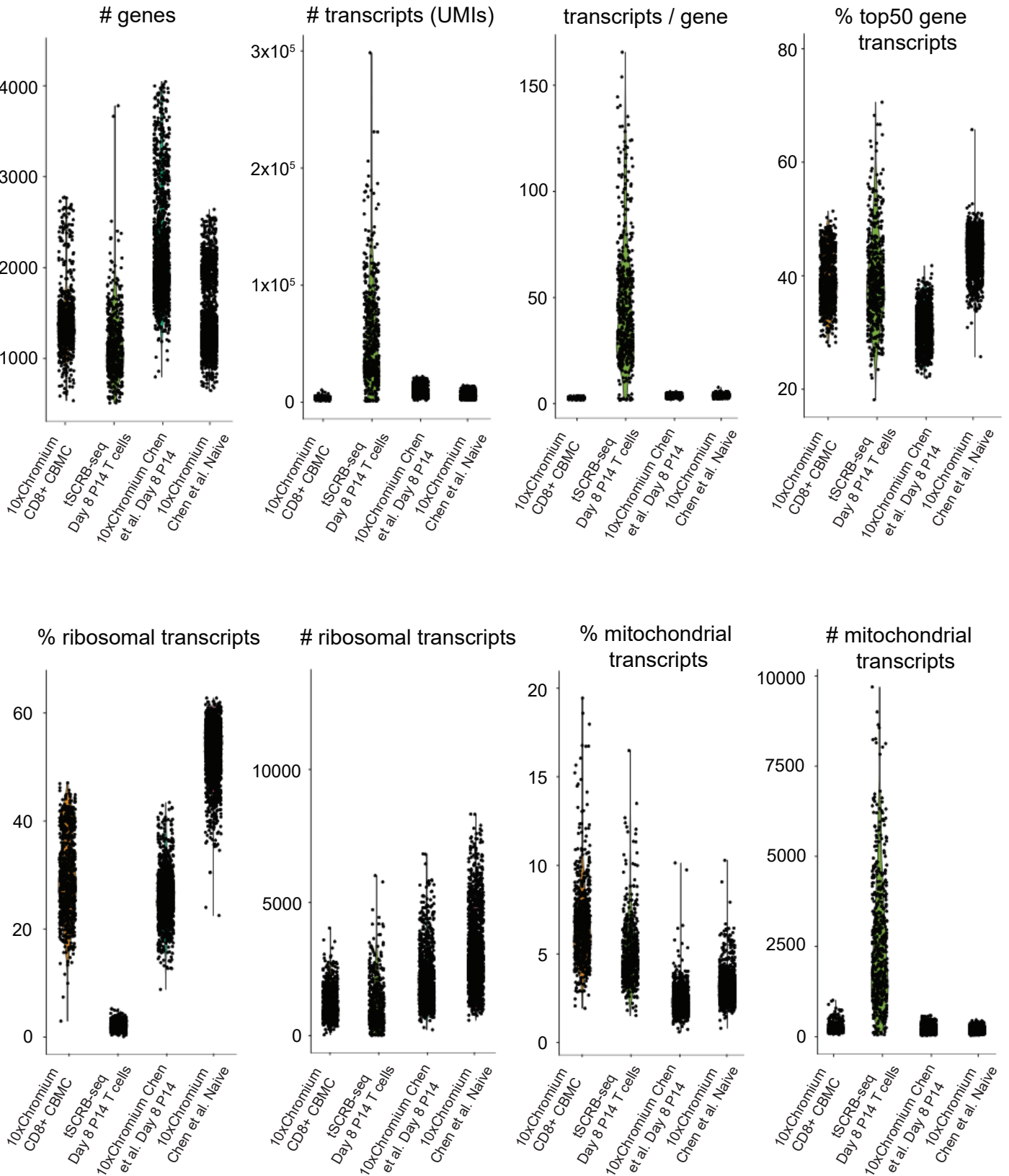
Supplementary Figure 2. Comparison of the cDNA yield among different Drop-seq optimizations.

(A-C) Bioanalyzer electropherograms comparing the amplified cDNA yield of Drop-seq between the original protocol and various optimizations.



Supplementary Figure 3. Technical characteristics of libraries generated with tSCRB-seq and 10xGenomics Chromium from different cell types and states.

Comparative analysis of major technical parameters among a library generated with tSCRB-seq (P14 T cells recovered at day 8 post LCMV Armstrong infection) and published libraries generated with 10xGenomics Chromium (naïve or recovered at day 8 post LCMV Armstrong P14 T cells²² and cord blood mononuclear cells available on the manufacturer's website).

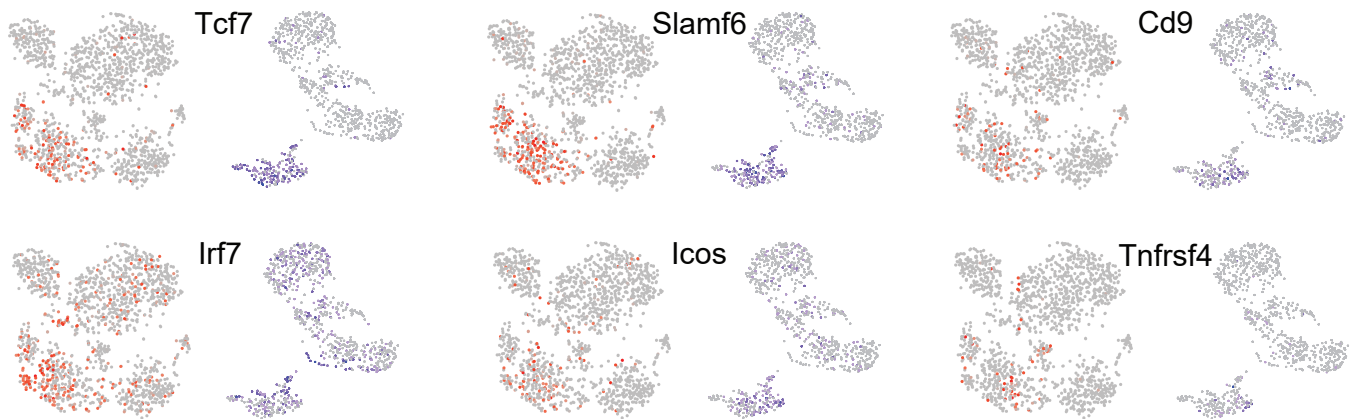


Supplementary Figure 4. The higher dynamic range of gene expression detected with tSCBR-seq results in improved compartment-resolved expression of key immune genes in chronic infection.

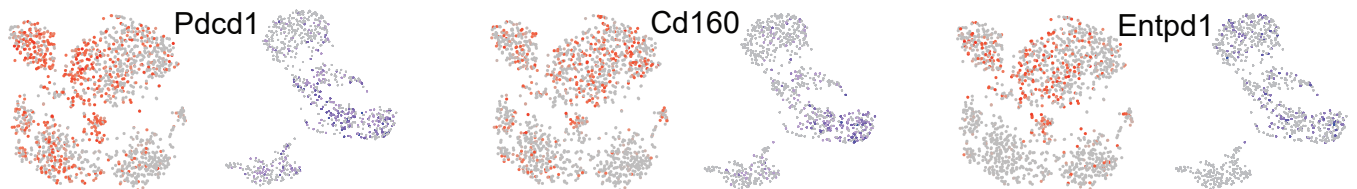
Comparative analysis of single-cell P14 T-cell transcriptomes generated with tSCBR-seq¹² or 10xChromium¹³ and recovered from established LCMV clone-13 infections (on day 40 and day 30 respectively). Each circle represents a single-cell. The plots represent the expression of key for CD8 T cell differentiation and function immune genes and regulatory receptors depicted over tSNE. The expression level is illustrate using the red scale in plots based on the tSCBR-seq data and violet scale in plots using the 10xChromium data.

tSCBR-seq 10xChromium

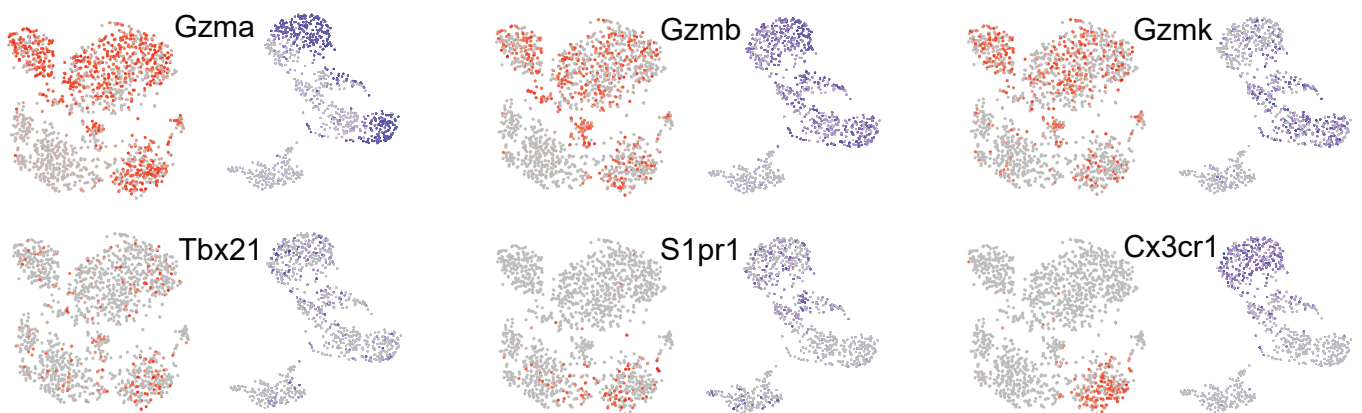
Progenitor markers



Exhaustion markers

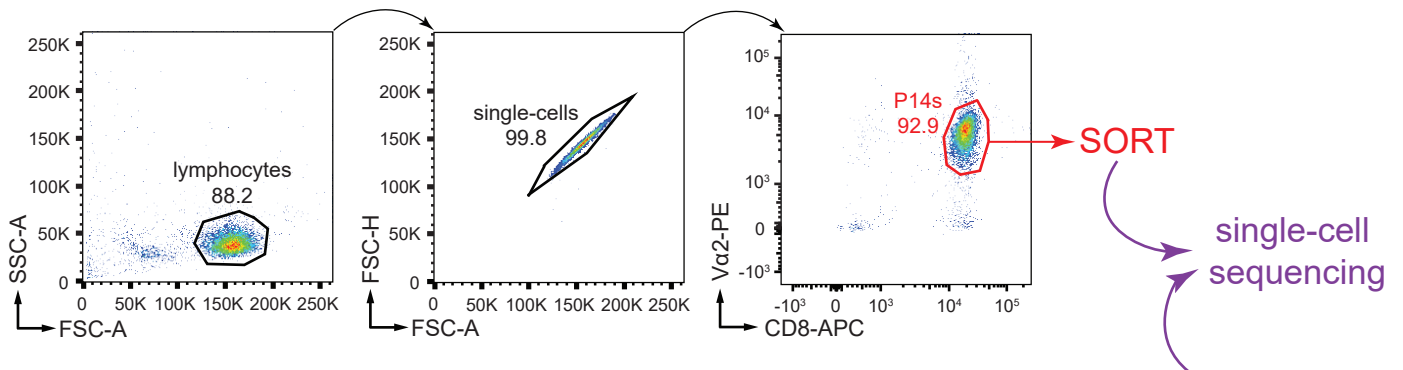


Effector markers

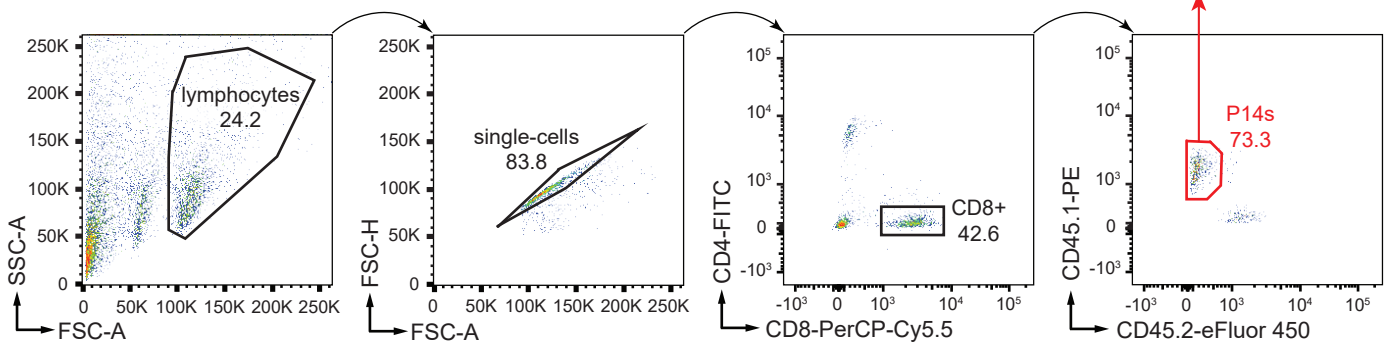


Supplementary Figure 5. Single-cell sort gating strategy.

Naïve P14 sort



Activated P14 sort



Supplementary Table 1. Fraction of cell positive for selected key immune genes among methods.

Analysis of libraries generated with tDrop-seq and tSCRB-seq from P14 T-cells recovered at day 8 post-acute LCMV Armstrong infection, compared to a published 10xChromium dataset with matching experimental setup²². Comparison of the fraction of cell detected to express key immune genes among methods.

P14 cells recovered on day 8 from acute infection			
GENE	tDROP-seq	tSCRB-seq	10xChromium
Gzma	87%	95%	42%
Gzmb	81%	89%	90%
Prf1	55%	64%	11%
Fasl	12%	10%	21%
Klrg1	67%	56%	55%
Id2	80%	76%	95%
Tbx21	38%	56%	47%
Klf2	72%	85%	43%
Klf3	46%	70%	54%
S1pr1	38%	41%	58%
S1pr4	40%	53%	92%
S1pr5	37%	42%	21%
Tcf7	10%	9%	24%
Slamf6	14%	12%	45%
Il7r	9%	6%	10%
Eomes	23%	6%	22%
Id3	0%	1%	5%
Bcl2	7%	9%	29%
Mki67	61%	31%	13%
Ezh2	35%	38%	29%
Cumulative detection	41%	42%	40%

Supplementary Table 2. Number of transcripts per cell of selected key immune genes detected by each method.

Comparative analysis of the number of transcripts per cell of key immune genes detected by tDrop-seq, tSCRB-seq and 10xGenomics Chromium²² in P14 T cells recovered on day 8 post LCMV Armstrong infection. All sequencing data are down-sampled to 40 000 mapped reads per cell. Source data are provided as a Source Data file. The abbreviations are as follows: md. transcripts, median number of transcripts captured per cell; SD, standard deviation

P14 CD8 T cells recovered on day 8 post LCMV Armstrong infection						
	tDrop-seq		tSCRB-seq		10xChrom	
	md. # transcripts	SD	md. # transcripts	SD	md. # transcripts	SD
Gzma	7.0	17.5	180.0	405.1	4.0	34.7
Gzmb	3.0	3.7	62.0	115.0	9.0	11.9
Prf1	1.0	1.3	35.0	70.7	2.0	1.4
Fasl	1.0	0.7	23.0	46.3	1.0	0.5
Klrg1	2.0	2.3	22.0	34.8	2.0	2.1
Id2	4.0	4.0	35.5	72.8	11.0	10.0
Tbx21	1.0	0.6	20.0	32.9	1.0	1.0
Klf2	1.0	1.1	27.0	74.3	1.0	1.0
Klf3	2.0	1.8	28.5	43.8	1.0	1.2
S1pr1	1.0	0.9	14.0	24.2	1.0	1.1
S1pr4	1.0	0.9	13.0	18.5	3.0	2.8
S1pr5	1.5	1.3	26.0	55.6	1.0	1.0
Tcf7	2.0	0.8	12.5	28.5	1.0	1.9
Slamf6	2.0	1.0	23.5	46.7	1.0	1.8
Il7r	1.0	1.3	25.0	49.0	1.0	1.0
Eomes	1.0	1.5	6.5	42.6	1.0	0.8
Id3	1.0	0.0	9.0	37.5	1.0	1.8
Bcl2	2.0	0.6	12.0	20.3	1.0	1.6
Mki67	2.0	6.4	28.0	208.5	2.0	2.1
Ezh2	2.0	1.4	44.0	76.2	1.0	1.6

*All sequencing data were down-sampled to 40 000 mapped reads/cell.

Supplementary Table 3. Primer sequences.

Primer	Primer full name	Primer sequence	Producer	Purity	Note
DS-BEADS	ChemGenes Barcoded Oligo dT primer ON Beads	5'-Bead-Linker--TTTTTTTAAGCAGTGGTATCAAC GCAGAGTACJJJJJJJJJJNNNNNNNN TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT--3'	ChemGenes Corporation	-	J denotes a nucleic acid part of the cellular barcode, which is identical in all primers attached to one bead; N denotes a random nucleic acid part of the unique molecular identifier, which is a unique sequence in each primer attached to one bead.
DS-TSO	Drop-seq Template Switching Oligo	AAGCAGTGGTATCAACGCAGAGTGAATrGrGrG	Eurogentec	HPLC	rG denotes a riboguanine
DS-TSO-LNA	Drop-seq Template Switching Oligo with Locked Nucleic Acid	AAGCAGTGGTATCAACGCAGAGTGAATrGrG+G	Eurogentec	HPLC	rG denotes a riboguanine; +G denotes a locked nucleic acid guanine
DS-SMART PCR	Drop-seq SMART PCR Primer	AAGCAGTGGTATCAACGCAGAGT	Integrated DNA Technologies	HPLC	
DS-P5	Drop-seq N5 primer	AATGATACGGCGACCACCGAGATCTACACGCCTGTCCGC GGAAGCAGTGGTATCAACGCAGAGT*A*C	Integrated DNA Technologies	HPLC	* denotes a phosphorothioate bond
DS-CR1	Drop-seq Custom Read 1 Primer	GCCTGTCCGCGGAAGCAGTGGTATCAACGCAGAGTAC	Integrated DNA Technologies	HPLC	
SCRB-dT plate v1	tSCRB Barcoded Oligo-dT Primer Plate v1	/5Biosg/ACACTCTTCCCTACACGACGCTCTCCGATCTJ(6)N(10)T(30)VN	Integrated DNA Technologies	Ultramer Plate; Standard desalting	/5Biosg/ denotes a 5' biotin; J denotes a nucleic acid part of the cellular barcode; N denotes a random nucleic acid part of the unique molecular identifier; V denotes A or C or G
SCRB-dT plate v2	tSCRB Barcoded Oligo-dT Primer Plate v2 and v3	/5Biosg/ACACTCTTCCCTACACGACGCTCTCCGATCTJ(7)N(9)T(30)VN	Integrated DNA Technologies	Ultramer Plate; Standard desalting	/5Biosg/ denotes a 5' biotin; J denotes a nucleic acid part of the cellular barcode; N denotes a random nucleic acid part of the unique molecular identifier; V denotes A or C or G
SCRB-TSO	tSCRB-seq Template Switching Oligo	iC-iG-iCACACTCTTCCCTACACGACGrGrGrG	Eurogentec	HPLC	iC denotes a iso-dC; iG denotes a iso-dG; rG denotes a riboguanine
SCRB-TSO-LNA	SCRB-seq Template Switching Oligo with Locked Nucleic Acid	iC-iG-iCACACTCTTCCCTACACGACGrGrG+G	Eurogentec	HPLC	iC denotes a iso-dC; iG denotes a iso-dG; rG denotes a riboguanine; +G denotes a locked nucleic acid guanine
SCRB-SMART-PCR	SCRB-seq SMART PCR Primer	/5Biosg/ACACTCTTCCCTACACGACGC	Integrated DNA Technologies	HPLC	/5Biosg/ denotes a 5' biotin
SMART-P5	SCRB-seq N5 primer	AATGATACGGCGACCACCGAGATCTACACTCTTCCCTACACGACGC TCTCCG*A*T*C*T*	Integrated DNA Technologies	HPLC	* denotes a phosphorothioate bond
SCRB-CR1	SCRB-seq Custom Read 1 Primer	TCTTCCCTACACGACGCTCTCCGATCT	Integrated DNA Technologies	HPLC	

