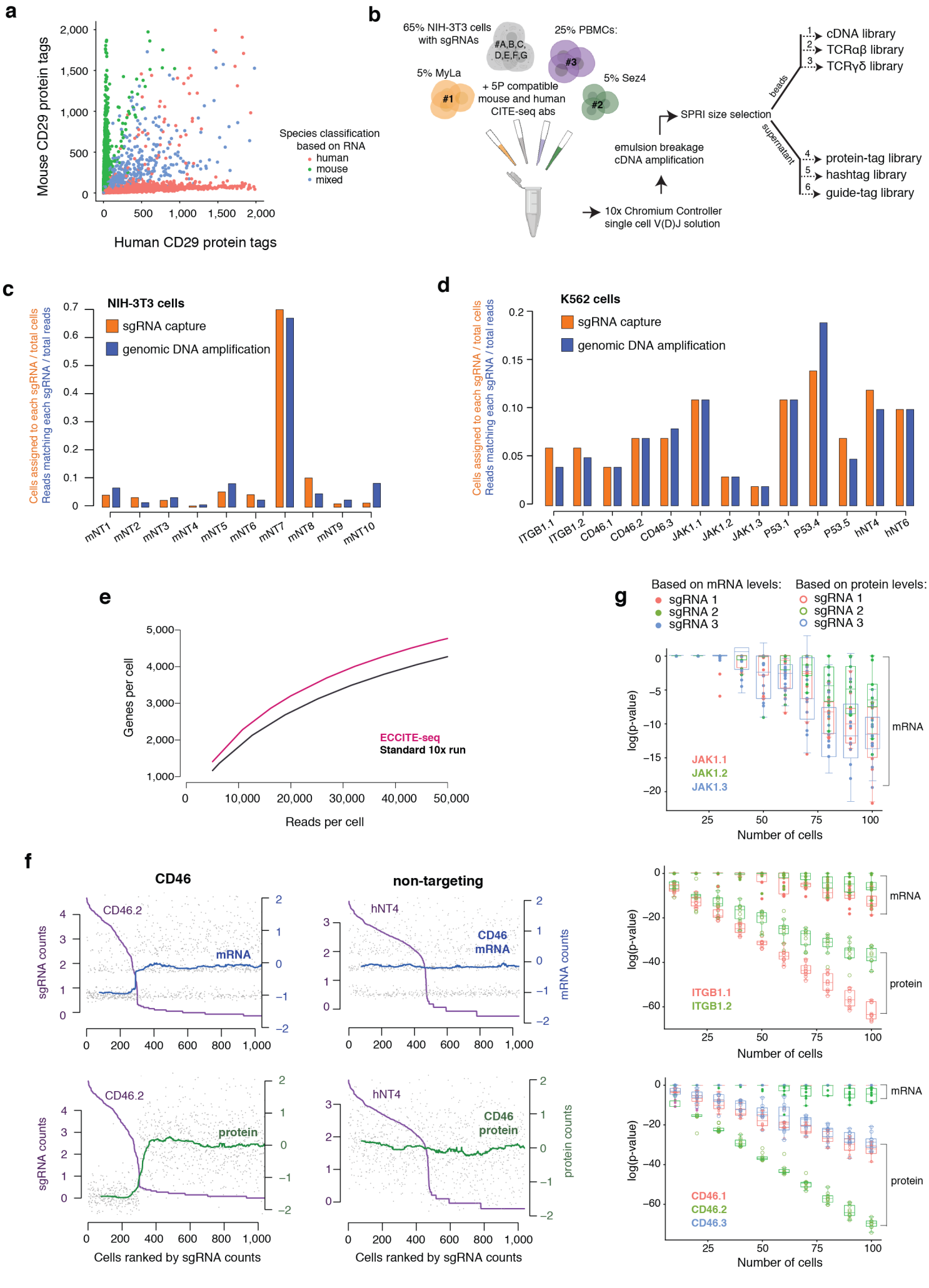
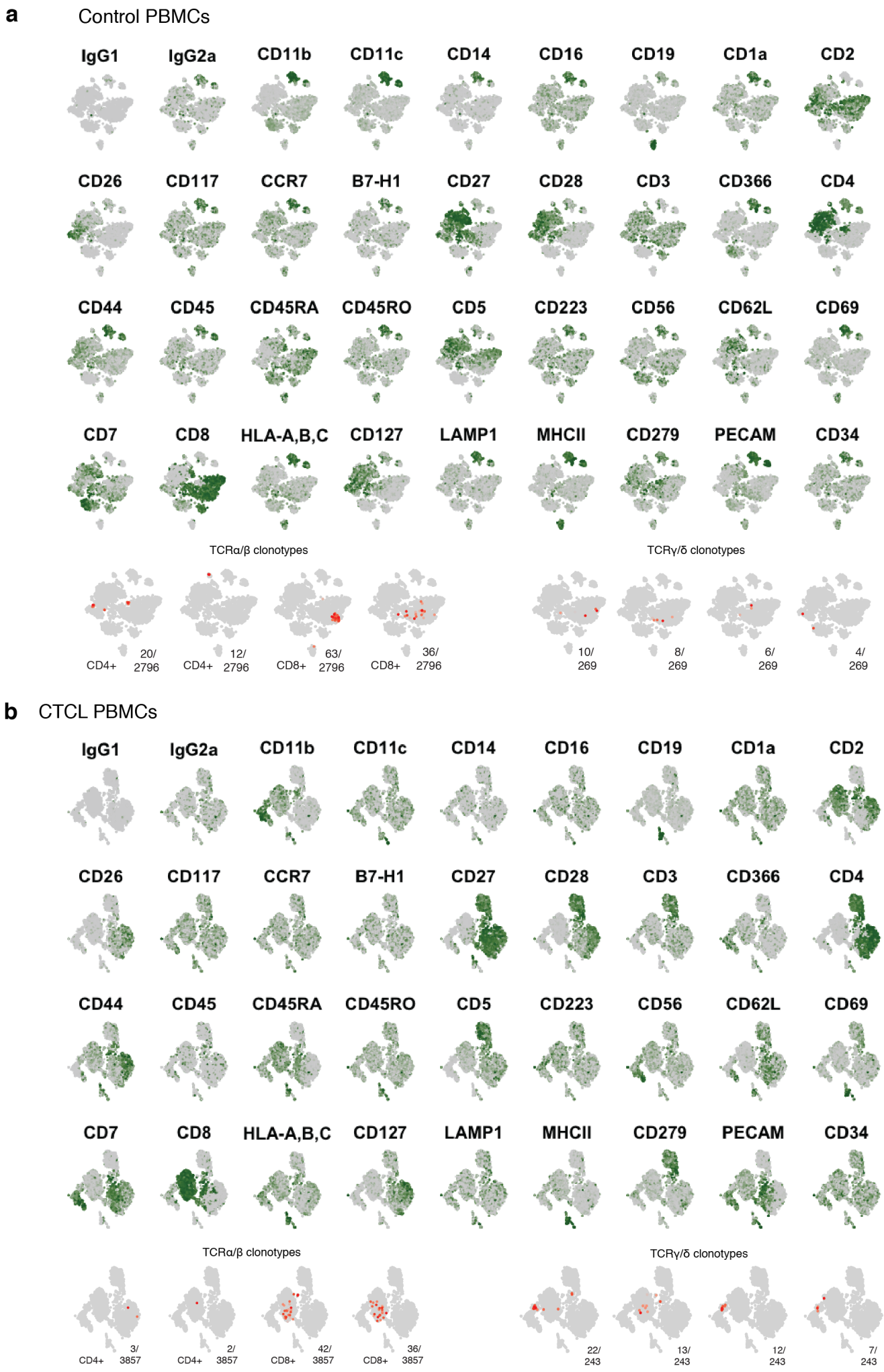
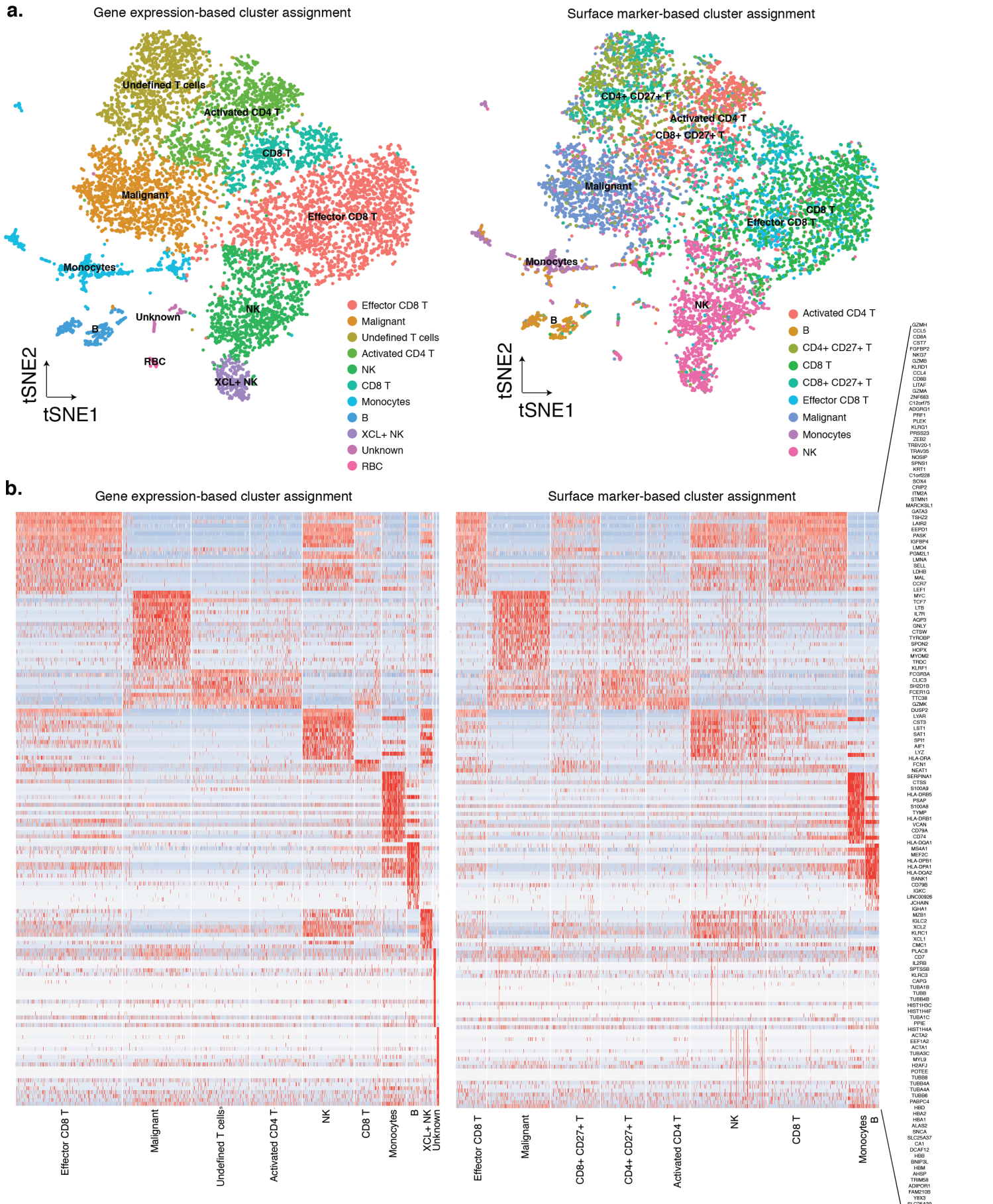

a
Mouse CD29 protein tags
Human CD29 protein tags
Species classification based on RNA
human
mouse
mixed
b
65% NIH-3T3 cells with sgRNAs
#A,B,C,D,E,F,G
25% PBMCs:
#3
5% MyLa
#1
+ 5P compatible mouse and human CITE-seq abs
5% Sez4
#2
emulsion breakage cDNA amplification
SPRI size selection
beads
supernatant
cDNA library
TCRαβ library
TCRγδ library
protein-tag library
hashtag library
guide-tag library
10x Chromium Controller single cell V(D)J solution
c
NIH-3T3 cells
sgRNA capture
genomic DNA amplification
Cells assigned to each sgRNA / total cells
Reads matching each sgRNA / total reads
mNT1
mNT2
mNT3
mNT4
mNT5
mNT6
mNT7
mNT8
mNT9
mNT10
d
K562 cells
sgRNA capture
genomic DNA amplification
Cells assigned to each sgRNA / total cells
Reads matching each sgRNA / total reads
ITGB1.1
ITGB1.2
CD46.1
CD46.2
CD46.3
JAK1.1
JAK1.2
JAK1.3
P53.1
P53.4
P53.5
hNT4
hNT6
e
Genes per cell
Reads per cell
ECCITE-seq
Standard 10x run
g
Based on mRNA levels:
sgRNA 1
sgRNA 2
sgRNA 3
Based on protein levels:
sgRNA 1
sgRNA 2
sgRNA 3
log(p-value)
JAK1.1
JAK1.2
JAK1.3
mRNA
Number of cells
f
CD46
non-targeting
CD46.2
hNT4
mRNA
CD46 mRNA
sgRNA counts
mRNA counts
protein
CD46 protein
protein counts
Cells ranked by sgRNA counts
ITGB1.1
ITGB1.2
CD46.1
CD46.2
CD46.3

Supplementary Figure 1: ECCITE-seq enables CRISPR screens with single cell multimodal readout. **a.** Species-mixing proof-of-principle experiment: protein tag reads associated with each cell barcode. Points are colored based on species classification using transcripts as shown in Fig. 1b. About 1.2% of either human or mouse cells show cross-reactivity with mouse or human antibodies respectively. **b.** Mixed-species ECCITE-seq experiment demonstrating measurement of six cellular modalities. Cells of different origin were stained with ECCITE-seq and hashing antibodies, washed and combined to a single 10x run. All cells were stained with a mix of anti-human CD29 and anti-mouse CD29 antibodies. NIH-3T3 cells were split into 7 tubes and stained with 7 barcoded hashing antibodies (Hashtag-A to Hashtag-G), followed by washing and pooling. MyLa, Sez4 and PBMCs were stained with Hashtag_1, Hashtag_2 and Hashtag_3 respectively. Eventually, a cell mix comprising 65% NIH-3T3 cells, 25% PBMCs, and 5% each MyLa and Sez4 cells was loaded into the 10x Chromium for droplet formation and reverse transcription. After emulsion breakage and cDNA amplification, the distinctly sized products were separated with size selection capture beads and amplified separately. A pool consisting 88% cDNA, 7% guide-tag, 3% hashtag and 2% protein-tag library was submitted for NGS sequencing. **c,d**. sgRNA representation as measured by direct sgRNA capture or genomic DNA amplification of the guide variable region in two different cell lines. To assess the direct capture, we assigned a guide to each cell with a single sgRNA and quantified their proportion in the total cell population (orange). This ratio is compared to the ratio of genomic reads matching each sgRNA to the total genomic sgRNA reads. **e.** scRNA-seq saturation curves for the ECCITE-seq versus standard 10x V(D)J run using the K562 cells expressing the targeting sgRNA library. The same cell suspension was used into two parallel experiments; one underwent a standard 10x V(D)J run while the other was preceded by staining with CITE-seq and hashing antibodies, as well as spike-in of guide RT primer in the reverse transcription reaction. For this analysis, data were downsampled to the same total reads per cell after correcting for the different fraction of reads in cell. **f.** Levels of target mRNA or protein (moving median smoothing window = 151) in 1,000 cells ranked on decreasing sgRNA counts for CD46 sgRNA 2 or non-targeting sgRNA 4. **g.** Adjusted p-values of detecting the indicated gene expression or protein abundance change as a function of cell number. Different-size, randomized cell samples assigned to unique targeting sgRNAs were used against equal size, randomized cell samples from the non-targeting sgRNAs groups.


a
Control PBMCs
IgG1
IgG2a
CD11b
CD11c
CD14
CD16
CD19
CD1a
CD2
CD26
CD117
CCR7
B7-H1
CD27
CD28
CD3
CD366
CD4
CD44
CD45
CD45RA
CD45RO
CD5
CD223
CD56
CD62L
CD69
CD7
CD8
HLA-A,B,C
CD127
LAMP1
MHCII
CD279
PECAM
CD34
TCRα/β clonotypes
TCRγ/δ clonotypes
CD4+ 20/ 2796
CD4+ 12/ 2796
CD8+ 63/ 2796
CD8+ 36/ 2796
10/ 269
8/ 269
6/ 269
4/ 269
b
CTCL PBMCs
IgG1
IgG2a
CD11b
CD11c
CD14
CD16
CD19
CD1a
CD2
CD26
CD117
CCR7
B7-H1
CD27
CD28
CD3
CD366
CD4
CD44
CD45
CD45RA
CD45RO
CD5
CD223
CD56
CD62L
CD69
CD7
CD8
HLA-A,B,C
CD127
LAMP1
MHCII
CD279
PECAM
CD34
TCRα/β clonotypes
TCRγ/δ clonotypes
CD4+ 3/ 3857
CD4+ 2/ 3857
CD8+ 42/ 3857
CD8+ 36/ 3857
22/ 243
13/ 243
12/ 243
7/ 243

Supplementary Figure 2: Surface protein and clonotype detection on PBMCs from a healthy donor and a CTCL patient. a. Transcriptome-based clustering of PBMCs from healthy donor after removing cell doublets. Projected is the protein signal for 36 out of 49 antibodies used to stain the cells, as well as the 2nd and 3rd most abundant CD4+ or CD8+ TCRα/β or the 3rd, 4th, 5th and 6th most abundant TCRγ/δ clonotype (red). **b.** Same analysis as panel a, using data from CTCL patient PBMCs.



Supplementary Figure 3: Gene expression and surface marker-based clustering of the combined dataset. a. Unsupervised clustering of PBMCs from both healthy and CTCL donors (n=9,816) after removing cell duplicates, merging, depth-normalization, and cell alignment28. Each cell was colored and labelled based on unsupervised clustering information on gene expression (left) or surface marker (right). b. Heatmap of genes differentially expressed across gene expression-based (left) or surface marker-based (right) cluster assignments.

Supplementary Table 1: Human sgRNA sequences
[a]HEK cells for mixed-species experiment
*K562 cells

target	name	sequence
-	hNT1[a]	ACGGAGGCTAAGCGTCGCAA
-	hNT2[a]	CGCTTCCGCGGCCCGTTCAA
-	hNT3[a]	ATCGTTTCCGCTTAACGGCG
-	hNT4[a]*	TAGGCGCGCCGCTCTCTAC
-	hNT5[a]	CCATATCGGGGCGAGACATG
-	hNT6[a]*	TACTAACGCCGCTCCTACAG
-	hNT7[a]	TGAGGATCATGTCGAGCGCC
-	hNT8[a]	GGCCCGCATAGGATATCGC
-	hNT9[a]	TAGACAACCGCGGAGAATGC
-	hNT10[a]	ACGGGCGGCTATCGCTGACT
ITGB1	ITGB1.1*	TAGGCCTCTGGGCTTTACGG
ITGB1	ITGB1.2*	AGTACTTGTGAAGCCAGCAA
CD46	CD46.1*	CGTACAGATATCTTCAAAGG
CD46	CD46.2*	CCGATCACAAATAGTATGGG
CD46	CD46.3*	CTGTCAAGTATTCCTTCCTC
JAK1	JAK1.1*	GGACTCCAAGAACCTGAGTG
JAK1	JAK1.2*	TATTCCTTAAGGCTTCCCGA
JAK1	JAK1.3*	TCTCGTCATACAGGGCAAAG
TP53	TP53.1*	GCAGTCACAGCACATGACGG
TP53	TP53.4*	CATGTAGTTGTAGTGGATGG
TP53	TP53.5*	TCCTCAGCATCTTATCCGAG

Supplementary Table 2: Mouse non-targeting sgRNA sequences

target	name	sequence
-	mNT1	CGAGGTATTCGGCTCCGCG
-	mNT2	CTTTCACGGAGGTTCGACG
-	mNT3	TAGTTGCAGTTCGGCTCGAT
-	mNT4	ACGTGTAAGGCGAACGCCTT
-	mNT5	ACTCCGGGTACTAAATGTC
-	mNT6	CCGCGCCGTTAGGGAACGAG
-	mNT7	ATTGTTCGACCGTCTACGGG
-	mNT8	ACCCATCGGGTGCGATATGG
-	mNT9	CGGGCGTCACCTGCTAGTAA
-	mNT10	CTTCTACTCGCAACGTATT

Supplementary Table 3: ECCITE-seq antibody-oligo conjugates

Antibody	clone	barcode
CD3E	UCHT1	TAGAACCGTATCCGT
CD4	RPA-T4	TGTGGTAGCCCTTGT
CD8A	RPA-T8	TATCCCTGCCTTGCA
TCRb	IP26	TAGCAGTCACTCCTA
TCRg	B1	CTATCGTTTGATGCA
CD44	BJ18	ATCTGTATGTCACAG
CD62L	DREG-56	AACCGCGCTTCAGAT
Ox40 (CD134)	Ber-ACT35	GCGATTCATGTCACG
PD1 (CD279)	EH12.2H7	GAGGCACTTAACATA
PD-L1	29E.2A3	GTTGTCCGACAATAC
EpCAM (CD326)	9C4	AAACTCAGGTCCTTC
CD66b	6/40C	GCGAGAAATCTGCAT
MHCII (HLA-DR)	L243	GCCTAGTTTGAACGC
CD45	H130	CGATTTGATCTGCCT
CD19	H1B19	TCACGCAGTCCTCAA
B220 (CD45R)	RA3-6B2	GTATAGACCAAAGCC
CD11c	3.9	CGTATGCCCTTTAGA
CD14	M5E2	GCTTGGGCAATTAAG
CD34	581	TGGCTTAGCTTTACA
CD56	5.1H11	CGTATCTGTCATTAG
CD16	B73.1	TATATCCCTCAACGC
CD2	TS1/8	AATTCCGTCAGATGA
CD5	UCHT2	ATTCCTCATTCCTGA
CD45RA	HI100	ACCCGTCCCTAAGAA
CD45RO	UCHL1	ACGTAGTCACCTAGT
CCR7	GO43H7	ACTGCCATATCCCTA
CD11b	ICRF44	CCATTCATTTGTGGC
CD1a	HI149	CAACTTGGCCGAATC
CD27	M-T271	ACGCAGTATTTCCGA
CD69	FN50	CATTAGAGTCTGCCA
PECAM (CD31)	WM59	CTCAGCCCTAGTATA
CD138	DL-101	CTCGTTTGTAGCAAT
CD24	ML5	GATTCCTTTACGAGC
Siglec-8	7C9	GAACCGTACCCATGA
LAMP1	H4A3	GAGAAATCAACCAAG
C-kit (CD117)	104D2	CTTACCTAGTCATTC
IL7Ralpha (CD127)	A019D5	TTGTTGTATCCGATC
CTLA4	BNI3	CAGAGCACCCATTAA
HLA-A,B,C	W6/32	GGATGTACCGCGTAT
CD77	5B5	GTCATTGTATGTCTG
CD366 (tim3)	F38-2E2	GGGCAATTAGCGAGT
CD223 (lag3)	11C3C65	GCCATTCCTGCCTAA
CD28	CD28.2	GACAGTCGATGCAAC
CD7	CD7-6B7	CTCCCTAGTTCCTTT
CD26 (Adenosine)	BA5b	TTCCTGCACGAGGAT
PD-1 (CD279)	NAT105	GTTGGATGGTAGACT
PD-L1 (CD274)	MIH1	GCAGTTGTCCGATTC
IgG1	MOPC-21	TCCTTTCCTGATAGG
IgG2a	MOPC-173	TCCCTTGCGATTTAC
CD29 (human)	TS2/16	AATAGCGGAGCC
CD46 (human)	TRA-2-10	GCCAATTGCACT
CD29 (mouse)	HMβ1-1	CGAAGACCAAGA

Supplementary Table 4: Mouse hashing antibody-oligo conjugates

ANTIBODY COCKTAIL	HASHTAG	BARCODE
CD29 (HMB1-1) CD45 (30-F11) B2M (A16041A)	Hashtag_A	AGGACCATCCAA
	Hashtag_B	TCGATAATGCGA
	Hashtag_C	GAGGCTGAGCTA
	Hashtag_D	GTGTGACGTATT
	Hashtag_E	ACTGTCTAACGG
	Hashtag_F	CACATAATGACG
	Hashtag_G	TAACGACGTGGT

Supplementary Table 5: Human hashing antibody-oligo conjugates

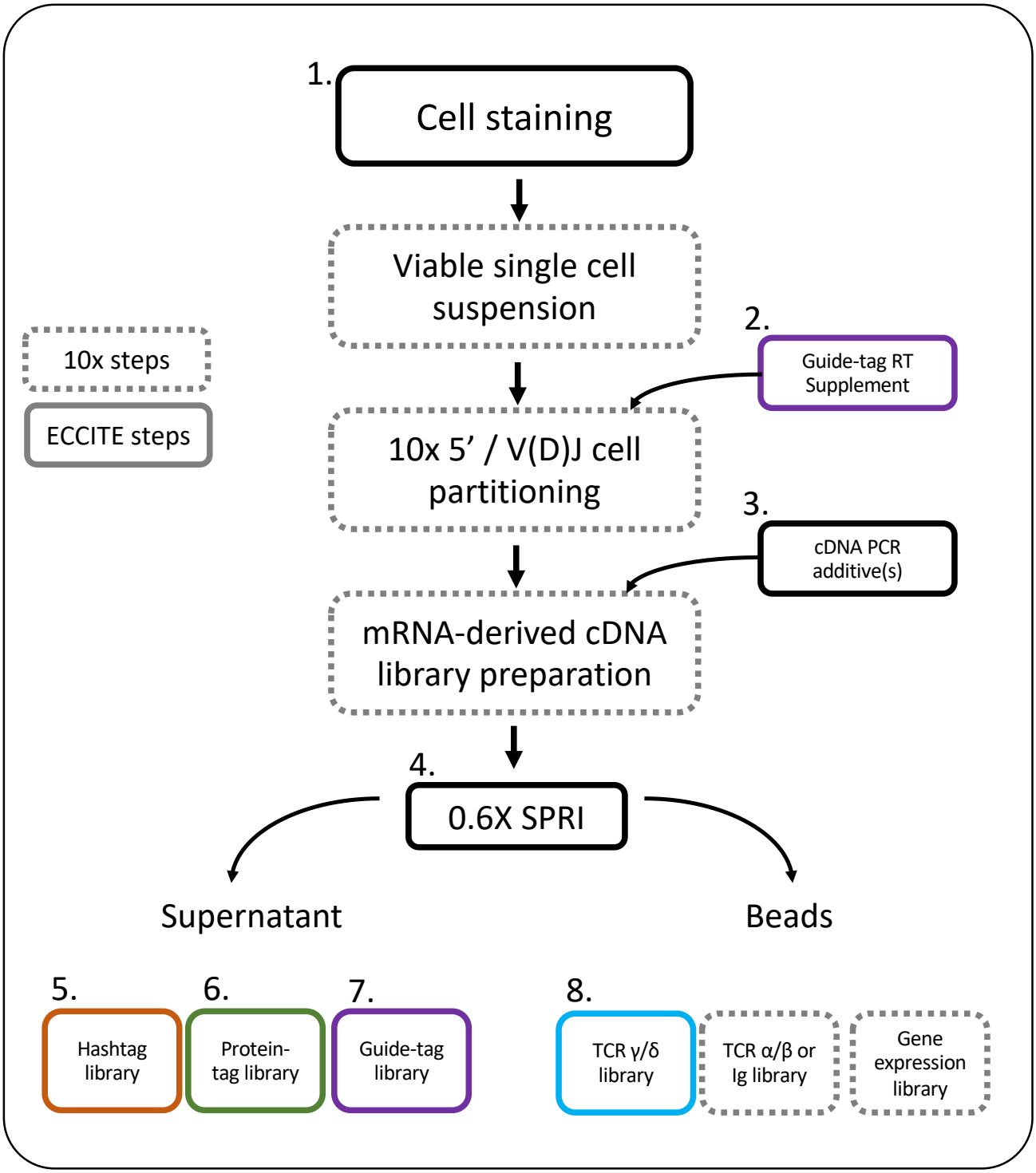
ANTIBODY COCKTAIL	HASHTAG	BARCODE
CD298 (LNH-94) B2M (2M2)	Hashtag_1	ACATGTTACCGT
	Hashtag_2	AGCTTACTATCC
	Hashtag_3	TATCACATCGGT
	Hashtag_4	ACTGTCTAACGG
	Hashtag_5	CACATAATGACG
	Hashtag_6	TAACGACGTGGT

ECCITE-seq protocol

Preparation of CITE-seq oligos and antibodies:

As described in Stoeckius et al 2018 cell hashing manuscript: https://doi.org/10.1186/s13059-018-1603-1

Sections of protocol that differ from the 10x Genomics V(D)J kit are highlighted in flowchart below



CITE-seq run:

1. **Cell staining**
   - Obtain all single cell suspensions from different samples/conditions that will be multiplexed in the run. Keep samples in separate tubes until after cell hashing and shortly before loading cells into the single cell RNA-seq instrument. When aiming to super-load the same sample into one run, divide the sample up into equal proportions before staining with distinct cell hashing antibodies. Keep cell suspensions on ice (unless otherwise stated) at all times.
   - Carefully count all cells to ensure accurate quantitation.
     - Make note of cell viability (>95%) and also include dead cells in the total cell count!
     - If you observe many dead cells, live cell enrichment (*e.g.* Dead Cell Removal kit) is recommended!
   - Resuspend ~1-2 million cells in 100 µl Staining buffer (2%BSA, 0.01%Tween in PBS).
   - Add 10 µl Fc Blocking reagent (FcX, BioLegend).
   - Incubate for 10 minutes at 4˚C.
   - While cells are incubating in Fc Block, prepare antibody-pool using 1-1.5 µg (or titrated amounts) of each CITE-seq antibody and 1 µg of single cell hashing antibody (pool).
   - Add antibody-oligo pool to cells.
   - Incubate for 30 minutes at 4˚C.
   - Wash cells 3 times with 1 mL Staining buffer spin 5 minutes 300*g* at 4˚C.
   - Resuspend cells in PBS and filter through 40 µm strainers (*e.g.* Flowmi cell strainer).
   - Verify cell concentration by counting on hemocytometer after filtration.
   - Pool all different samples/conditions at desired proportions and immediately proceed to next step.

Run 10x Genomics single cell V(D)J assay according to manufacturer's instructions with the following modifications:

2. **Guide-tag RT supplement**
   At reverse transcription step: spike-in the guide RT primer
   - Add 5.9 µl of 2 µM gd_RT_v4 primer in the RT reaction (0.12 µM final concentration). Subtract that volume from the water added to the cells.

3. **cDNA PCR additive(s)**
   At cDNA amplification step: spike-in additive primers to increase yield of applicable tag products
   - Add 1 µl of 2 µM protein-tag additive,
   - Add 1 µl of 1 µM hashtag additive
   - Add 1 µl of 1 µM guide-tag additive

4. **Separation of tag and cDNA libraries**
   After cDNA amplification: separate protein tags and guide tags (~180-200bp) from cDNAs (>300bp)
   - Perform 0.6x SPRI selection to separate protein and guide tags from full-length cDNAs.
   - **DO NOT DISCARD SUPERNATANT FROM 0.6x SPRI. THIS CONTAINS THE ADTs and hashtags!**
     - Add 0.6x SPRI to cDNA reaction as described in 10x Genomics or Drop-seq protocol.
     - Incubate 5 minutes and place on magnet.
     - Supernatant contains ADTs and hashtags.
     - Beads contain full length mRNA-derived cDNAs.

   - **mRNA-derived cDNA >300bp (beads fraction).**
     - Proceed with standard 10x V(D)J solution for cDNA sequencing library preparation.

   - **Hashtags, Protein tags and guide tags ~180-200bp (supernatant fraction)**
     - Purify tags using two 2x SPRI purifications per manufacturer protocol:
       - Add 1.4x SPRI to supernatant to obtain a final SPRI volume of 2x SPRI
       - Transfer entire volume into a low-bind 1.5mL tube and incubate 10 minutes at room temperature.

- Place tube on magnet and wait ~2 minutes until solution is clear.
- Carefully remove and discard the supernatant.
- Add 400 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds.
- Carefully remove and discard the ethanol wash.
- Centrifuge tube briefly and return it to magnet.
- Remove and discard any remaining ethanol.
- Resuspend in beads in 50 µl water.
- Perform another round of 2x SPRI purification by adding 100 µl SPRI reagent directly onto resuspended beads.
- Mix by pipetting and incubate 10 minutes at room temperature.
- Place tube on magnet and wait ~2 minutes until solution is clear.
- Carefully remove and discard the supernatant.
- Add 200 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds.
- Carefully remove and discard the ethanol wash.
- Repeat wash
- Centrifuge tube briefly and return it to magnet.
- Remove and discard any remaining ethanol and allow the beads to air dry for 2 minutes
- Resuspend beads in 45 µl water for each element captured (135 µl for example if you need to make protein-tag, hashtag- and guide-tag libraries).
- Pipette mix vigorously and incubate at room temperature for 5 minutes.
- Place tube on magnet and transfer clear supernatant into new tube. This will be your input for the following PCR reactions:

5. Hashtag library preparation

PCR in 100 µL using 25 pmol of each primer:

45 µl bead elution
50 µl 2x KAPA Hifi PCR Master Mix
2.5 µl 10 µM SI-PCR oligo
2.5 µl 10 µM TruSeq DNA D7xx_s primer (containing i7 index)

Cycling conditions:

95˚C	3 min		
95˚C	20 sec	|	
64˚C	30 sec	|	~8-12 cycles
72˚C	20 sec	|	
72˚C	5 min		

Expected product: 180bp

6. Protein-tag library preparation

PCR in 100 µL using 25 pmol of each primer:

45 µl bead elution
50 µl 2x KAPA Hifi PCR Master mix
2.5 µl 10 µM SI-PCR oligo
2.5 µl 10 µM TruSeq Small RNA RPIx primer (containing i7 index)

Cycling conditions:

95˚C	3 min		
95˚C	20 sec	|	
60˚C	30 sec	|	~6-10 cycles
72˚C	20 sec	|	
72˚C	5 min		

Expected product: 180bp

7. **Guide-tag library preparation**

1. Guide-tag library (this is an enrichment PCR to improve yield and specificity, indexing to follow in subsequent PCR):

PCR in 100 µL using 25 pmol of each primer:

45 µl bead elution
50 µl 2x KAPA Hifi Master mix
2.5 µl 10 µM SI-PCR primer
2.5 µl 10 µM gd_add_v4 primer

Cycling conditions:

95˚C	3 min		
95˚C	20 sec	|	
64˚C	30 sec	|	~8-10 cycles
72˚C	20 sec	|	
72˚C	5 min		

Expected product: 220bp

- Purify all tag libraries using 1.6x SPRI purification by adding 160 µl SPRI reagent.
  - Incubate 5 minutes at room temperature.
  - Place tube on magnet and wait 1 minute until solution is clear.
  - Carefully remove and discard the supernatant.
  - Add 200 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds (first Ethanol wash).
  - Carefully remove and discard the ethanol wash.
  - Add 200 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds (second Ethanol wash).
  - Carefully remove and discard the ethanol wash.
  - Centrifuge tube briefly and return it to magnet.
  - Remove and discard any remaining ethanol and allow the beads to air dry for 2 minutes.
  - Resuspend beads in 20 µl water.
  - Pipette mix vigorously and incubate at room temperature for 5 minutes.
  - Place tube on magnet and transfer clear supernatant to PCR tube.
  - Protein-tag and hashtag libraries are ready to be sequenced.
- Proceed with indexing PCR for the guide-tag library

PCR in 100 µL using 25 pmol of each primer:

x µl bead elution (carry over 5-10 ng of the 1st PCR product)
50 µl 2x KAPA Hifi Master mix
2.5 µl 10 µM SI-PCR primer
2.5 µl 10 µM Next_nst_x primer (containing i7 index)
100 µl total

Cycling conditions:

95˚C	3 min		
95˚C	20 sec	|	
54˚C	30 sec	|	~6-10 cycles
72˚C	20 sec	|	
72˚C	5 min		

Expected product: 210bp

  - Purify library using 1.6x SPRI as described above.
  - Elute in 20 µl water
- Quantify all tag libraries by standard methods (QuBit, BioAnalyzer, qPCR)

8. Preparation of the TCR γ/δ library

The TCR γ/δ library is prepared by following the 10x Genomics protocol for generating TCR α/β libraries in Chapter 5 of the 10x protocol, but substituting primers as detailed below:

PCR1: Human γ/δ mix 1 (R1_hTRDC + R1_hTRGC) instead of T cell 1
PCR2: Human γ/δ mix 2 (R2_hTRDC + R2_hTRGC) instead of T cell 2

PCR1:	2-5 µl	full length cDNA
	50 µl	2x KAPA Hifi Master mix
	5 µl	cDNA additive
	2 µl	20 µM SI-PCR primer
	2 µl	20 µM human γ/δ mix 1
	Water to 100 µl	

PCR2:	35 µl	bead elution
	50 µl	2x KAPA Hifi Master mix
	5 µl	cDNA additive
	2 µl	20 µM SI-PCR or P5 primer
	2 µl	20 µM human γ/δ mix 2
	Water to 100 µl	

Cleanups and PCR conditions are identical to the 10x protocol, with the exception that γ/δ libraries often require extra cycles of amplification (~12) due to the comparative rarity of γ/δ T cells compared to α/β T cells in most cell populations.

TCR γ/δ enriched libraries are further processed according to the 10x Genomics Single Cell V(D)J protocol.

Sequencing CITE-seq libraries:

- Protein-tag, hashtag- and guide-tag libraries can be pooled with cDNA sequencing libraries at different proportions depending on the size of the antibody and guide panels. Typically, to obtain sufficient read coverage for all libraries we sequence protein-tag libraries in 3-10% of a lane, hashtag libraries in 2-5% of a lane and guide-tag libraries in 5-10% of a lane with the remainder dedicated to the cDNA library.

Oligonucleotide sequences:

Protein tags: These contain standard small TruSeq RNA read 2 sequences and can be amplified using Illumina's Truseq Small RNA primer sets (RPIx – primers, see example RPI1 below). See example below with a 12nt barcode:

example: /5AmMC12/CCTTGGCACCCGAGAATTCCA**xxxxxxxx**CCCATATAAGAAA

Hashtags: These contain standard TruSeq DNA read 2 sequences and can be amplified using truncated versions of Illumina's TruSeq DNA primer sets (see example D701_s below). See example below with a 12nt barcode:

example: /5AmMC12/GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**xxxxxxxx**CCCATATAAGAAA

Guide tags arise after reverse transcription of sgRNAs primed by the oligo:

sg_RT_v4: AGCAAGTGAGAAGCATCGTGTCAAAGCACCGACTCGGTGCCAC

Oligos required for tag library amplification and TCR γ/δ enrichment:

- 10x Genomics SI-PCR primer
  5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGC*T*C
- Protein-tag additive primer
  5'CCTTGGCACCCGAGAATT*C*C
- Hashtag additive primer
  5'GTGACTGGAGTTCAGACGTGTGC*T*C
- Guide-tag additive primer
  5'AGCAAGTGAGAAGCATCGTG*T*C
- Illumina Small RNA RPI1 primer (for protein-tag amplification; i7 index, Oligonucleotide sequences © 2015 Illumina, Inc)
  5'CAAGCAGAAGACGGCATACGAGxxxxxxxx**GTGACTGGAGTTCCTTGGCACCCGAGAATTC*C*A**
- Illumina TruSeq D701_s primer (for hashtag amplification; i7 index, shorter than the original Illumina sequence)
  5'CAAGCAGAAGACGGCATACGAGATxxxxxxxx**GTGACTGGAGTTCAGACGTGT*G*C**
- Next_nst_x: (custom primer for guide-tag amplification; i7 index, *scaffold annealing region*)
  5'CAAGCAGAAGACGGCATACGAGATxxxxxxxx**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*TATTTCTAGCTCTAAA*A*C***
- R1_hTRDC primer：(for Human γ/δ mix 1)
  5'AGCTTGACAGCATTGTACTTCC
- R1_hTRGC primer：(for Human γ/δ mix 1)
  5'TGTGTCGTTAGTCTTCATGGTGTTCC
- R2_hTRDC primer：(for Human γ/δ mix 2)
  5'TCCTTCACCAGACAAGCGAC
- R2_hTRGC primer：(for Human γ/δ mix 2)
  5'GATCCCAGAATCGTGTTGCTC

* Phosphorothioate bond
B C or G or T; not A nucleotide

Materials and Kits needed:

- FC blocking reagent (*e.g.* BioLegend FcX)
- 8-strip PCR tubes**, emulsion safe (!)** (*e.g.* TempAssure PCR 8-strips, USA Scientific)
- Bioanalyzer chips and reagents (DNA High Sensitivity kit, Agilent)
- SPRIselect reagent (GE Healthcare, B23317)
- E-gel 4% (Invitrogen, USA)
- Low-bind 1.5 mL tubes (Common lab suppliers)
- PCR Thermocycler (*e.g.* Bio-Rad, T100)
- Magnetic tube rack (*e.g.* Invitrogen, USA)
- Qubit (Invitrogen, USA)
- Hemocytometer (*e.g.* Fuchs Rosenthal)
- DMSO (Common lab suppliers).
- PBS (Common lab suppliers)
- Tween20 (Common lab suppliers)
- TE pH 8.0 (Common lab suppliers)
- BSA (DNAse, RNAse and protease free, *e.g.* VWR #0332-25G)
- Dead Cell Removal Kit (*e.g.* Miltenyi)