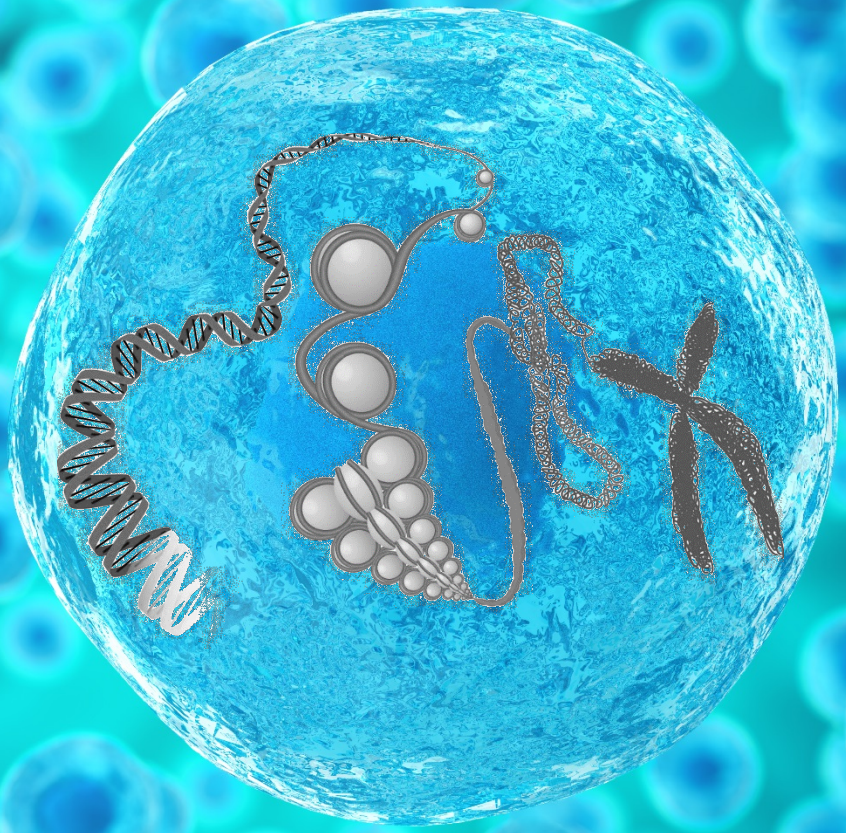
# Transcript-indexed ATAC-seq for immune profiling



**Technical Journal Club 22nd of May 2018**

**Christina Müller**

nature methods

# Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position

Jason D Buenrostro[1–3], Paul G Giresi[2,3], Lisa C Zaba[2,3], Howard Y Chang[2,3] & William J Greenleaf[1]

Nature Methods, Vol.10 No.12, 2013

nature biotechnology

# Linking T-cell receptor sequence to functional phenotype at the single-cell level

Arnold Han[1,2], Jacob Glanville[3], Leo Hansmann[2] & Mark M Davis[2–4]

Nature Biotechnology, Vol.32 No.7, 2014

LETTERS
https://doi.org/10.1038/s41591-018-0008-8
nature medicine

# Transcript-indexed ATAC-seq for precision immune profiling

Ansuman T. Satpathy[1,2,14], Naresha Saligrama[3,14], Jason D. Buenrostro[4,5,14], Yuning Wei[1,6], Beijing Wu[7], Adam J. Rubin[6], Jeffrey M. Granja[1,7,8], Caleb A. Lareau[4], Rui Li[1,6], Yanyan Qi[1,6], Kevin R. Parker[1,6], Maxwell R. Mumbach[1,7], William S. Serratelli[3], David G. Gennert[1,7], Alicia N. Schep[1,7], M. Ryan Corces[1,6], Michael S. Khodadoust[9], Youn H. Kim[6], Paul A. Khavari[6], William J. Greenleaf[7,10,11], Mark M. Davis[3,12,13,15*] and Howard Y. Chang[1,6,7,15*]

Nature Medicine, Vol.24, May 2018

nature methods

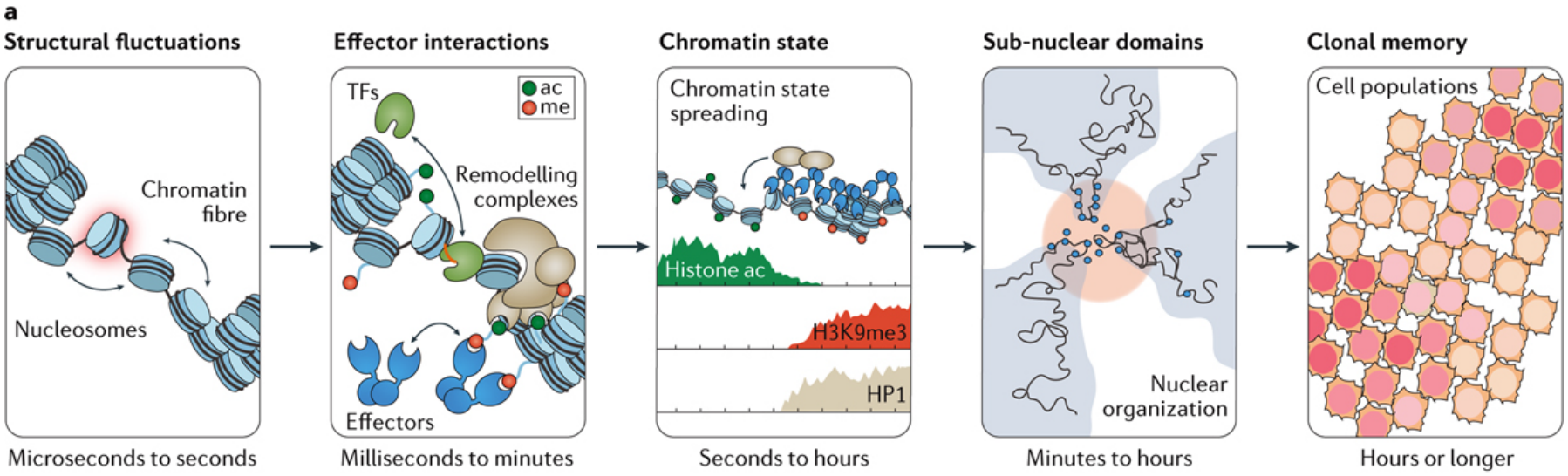
# Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position

Jason D Buenrostro[1–3], Paul G Giresi[2,3], Lisa C Zaba[2,3], Howard Y Chang[2,3] & William J Greenleaf[1]

**Levels of chromatin structures:**

1. positioning and composition of nucleosomes are controlled in part by the underlying DNA sequence
2. interlocking systems of transcription factors (TFs) with ATP-dependent chromatin remodelers and histone chaperones
3. Histone post-translational modifications (PTMs**)** → provide a complex interaction landscape for effector proteins
4. large-scale chromatin organization in the 3D nuclear space

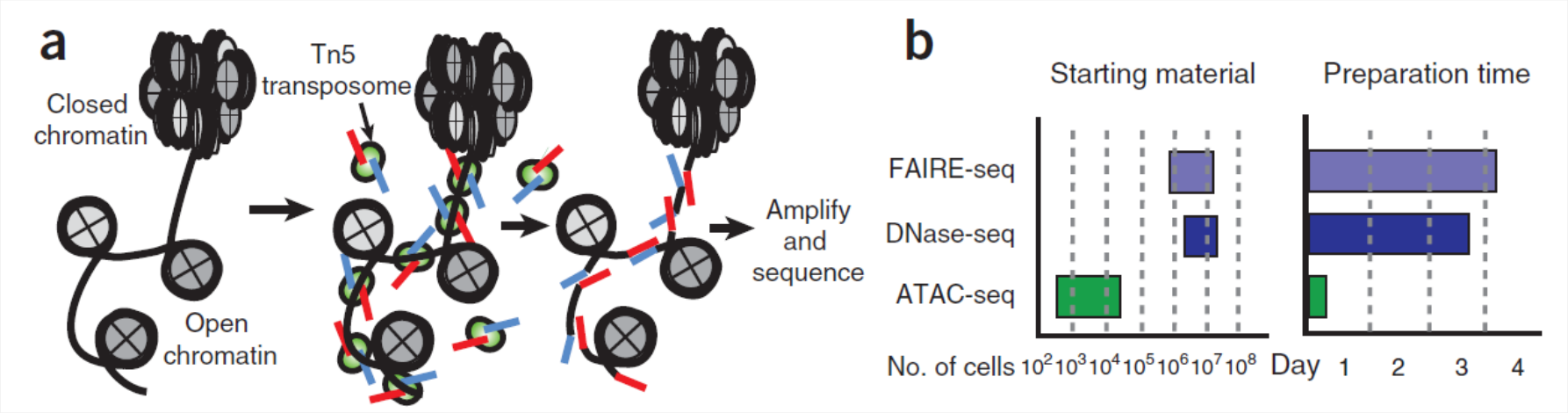
- **chromatin states are stable over time and can be transmitted over the cell cycle to form cellular memory**




*Cuvier and Fierz, Nature Reviews Genetics;18, p. 457–472 (2017)*

**Limitations of published protocols:**

- millions of cells required as starting material
- time consuming
- can average over or “drown out” heterogeneity in cellular populations
- *ex vivo* expansion of cells to obtain efficient input material
- input requirements often prevent application of these assays to well defined clinical samples

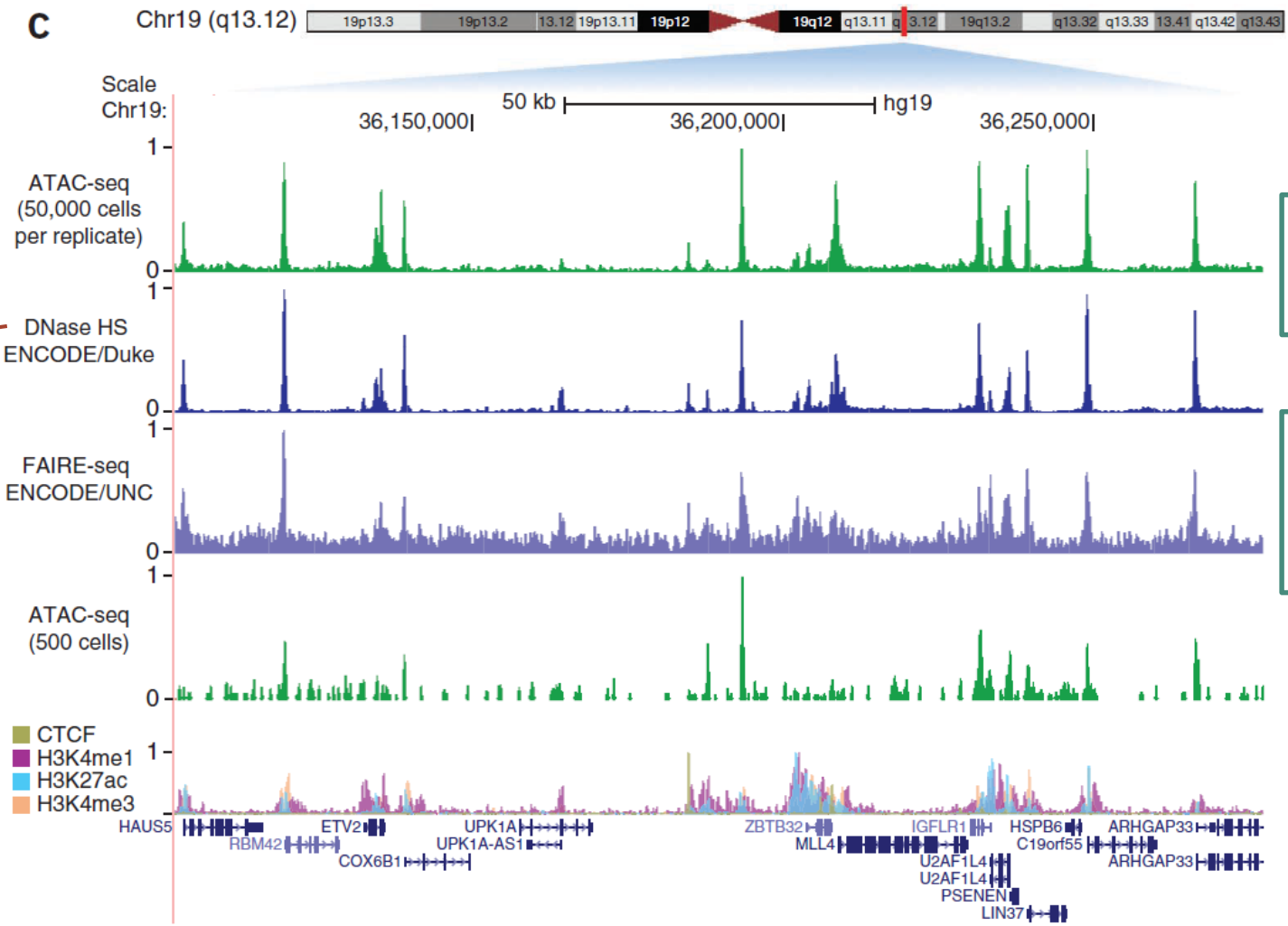



**Assay for transposase-accessible chromatin using sequencing (ATAC-seq):**

- Fast and robust
- Based on tagmentation using the hyperactive Tn5 transposase and its *in vitro* loaded adaptor payloads
- Requires only 500 cells as starting material

**Validation of ATAC-Seq identified open chromatin states by direct comparison with DNAse-Seq and FAIRE-Seq results:**

- unfixed nuclei isolated from a human lymphoblastoid cell line GM12878




**Input:**
1-50
Mio cells

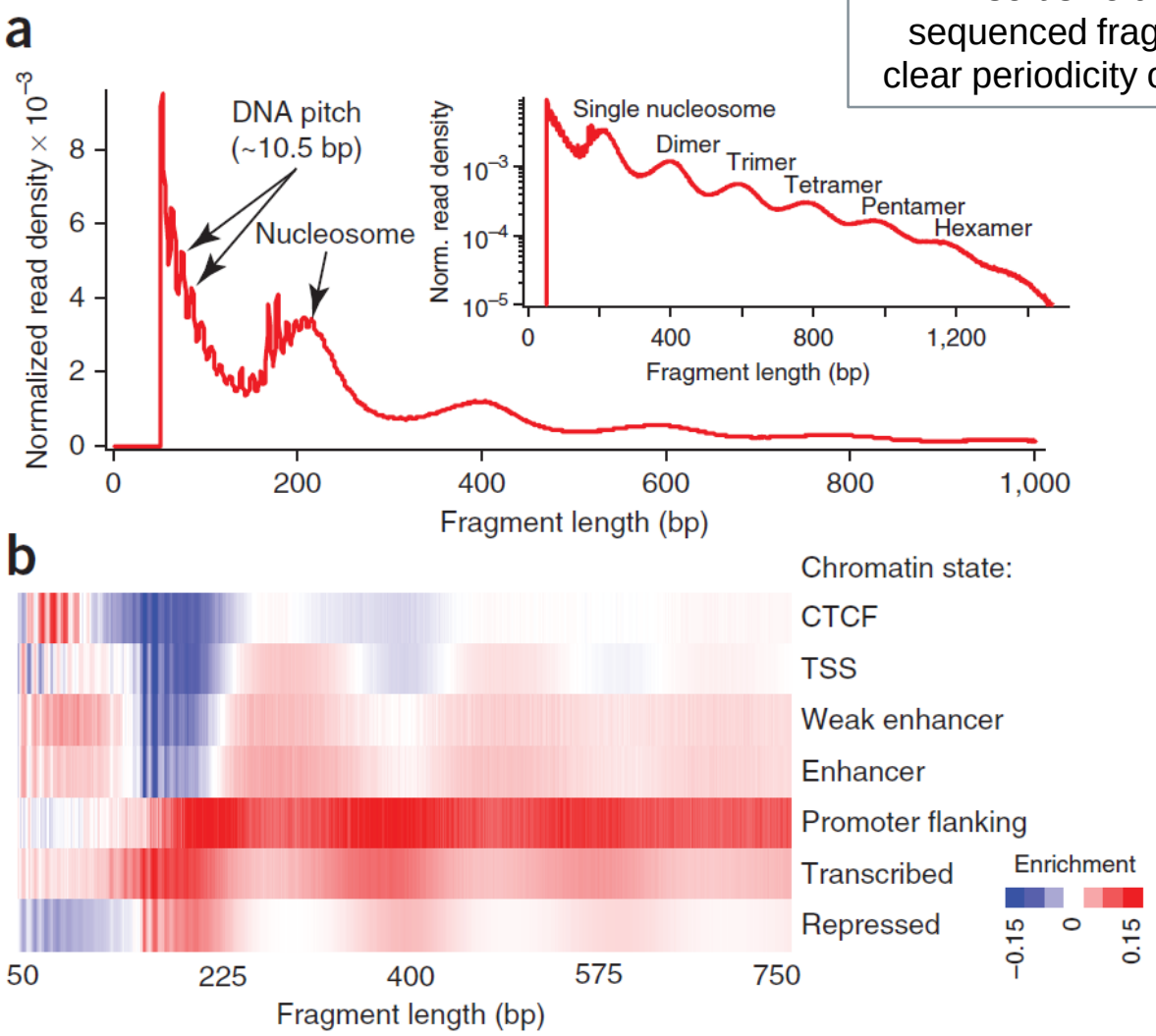
Similar signal to noise ratio

Peak intensities highly correlated

Highly reproducible between technical replicates (*R*=0.98)

# ATAC-Seq provides genome-wide information on chromatin compaction

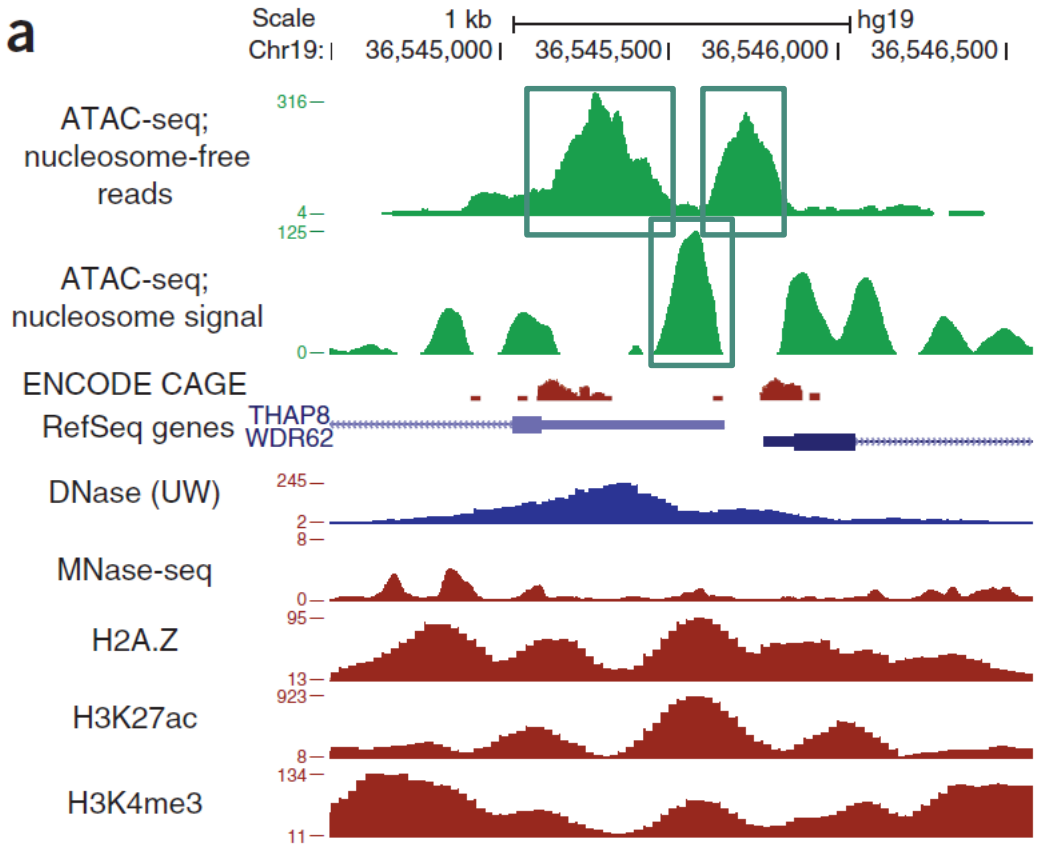
Insert size distribution of sequenced fragments showed clear periodicity of approx. 200bp




ATAC-seq is able to reveal differentially accessible forms of chromatin

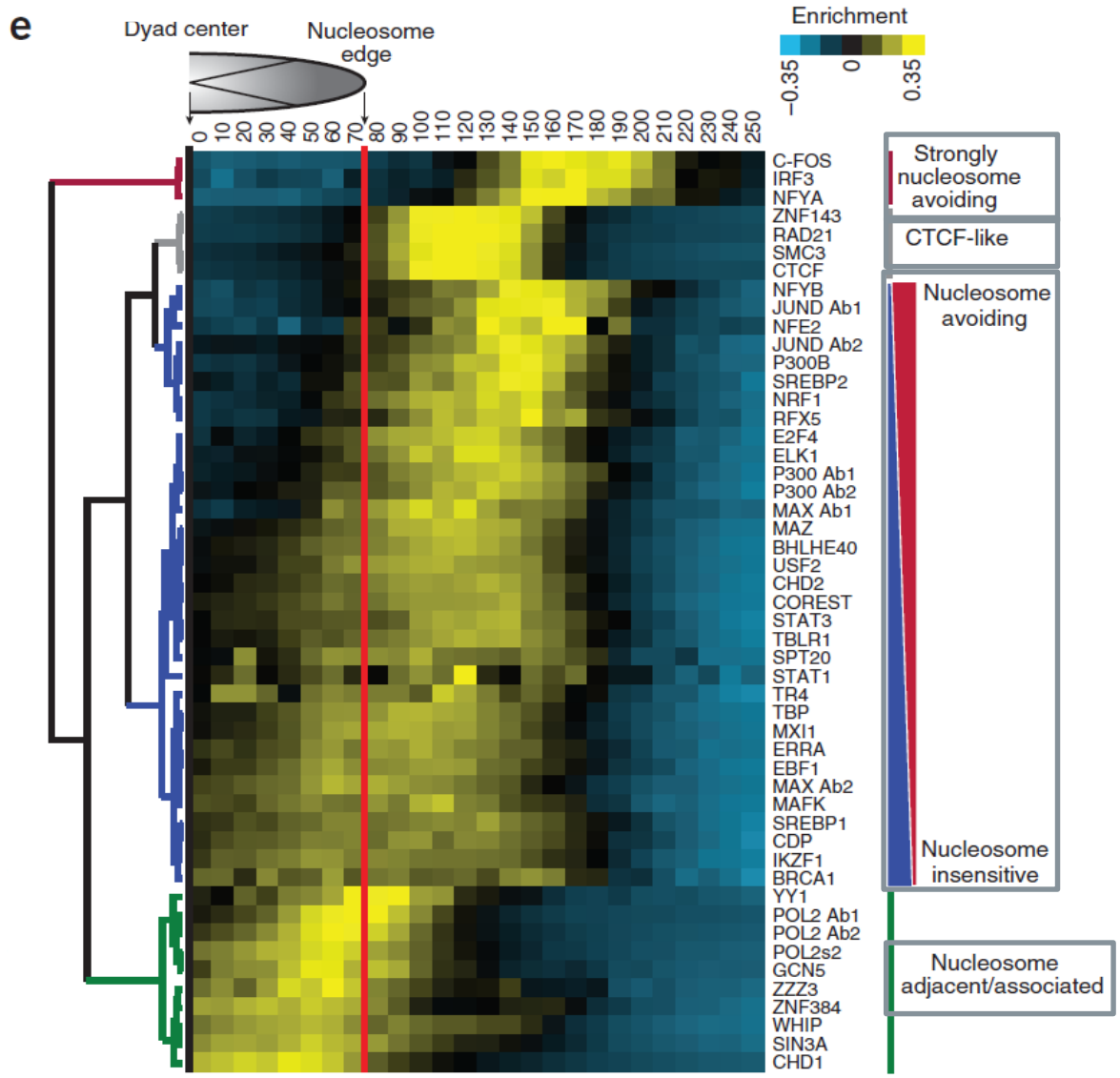
**ATAC-Seq provides information on nucleosome positioning in regulatory regions**

- Portioning of ATAC-Seq data into reads generated from putative nucleosome free regions of DNA and reads likely derived from nucleosome-associated DNA
- Example locus containing a putative bidirectional promoter with TSSs separated by 700bp (TSS sides identified by CAGE)



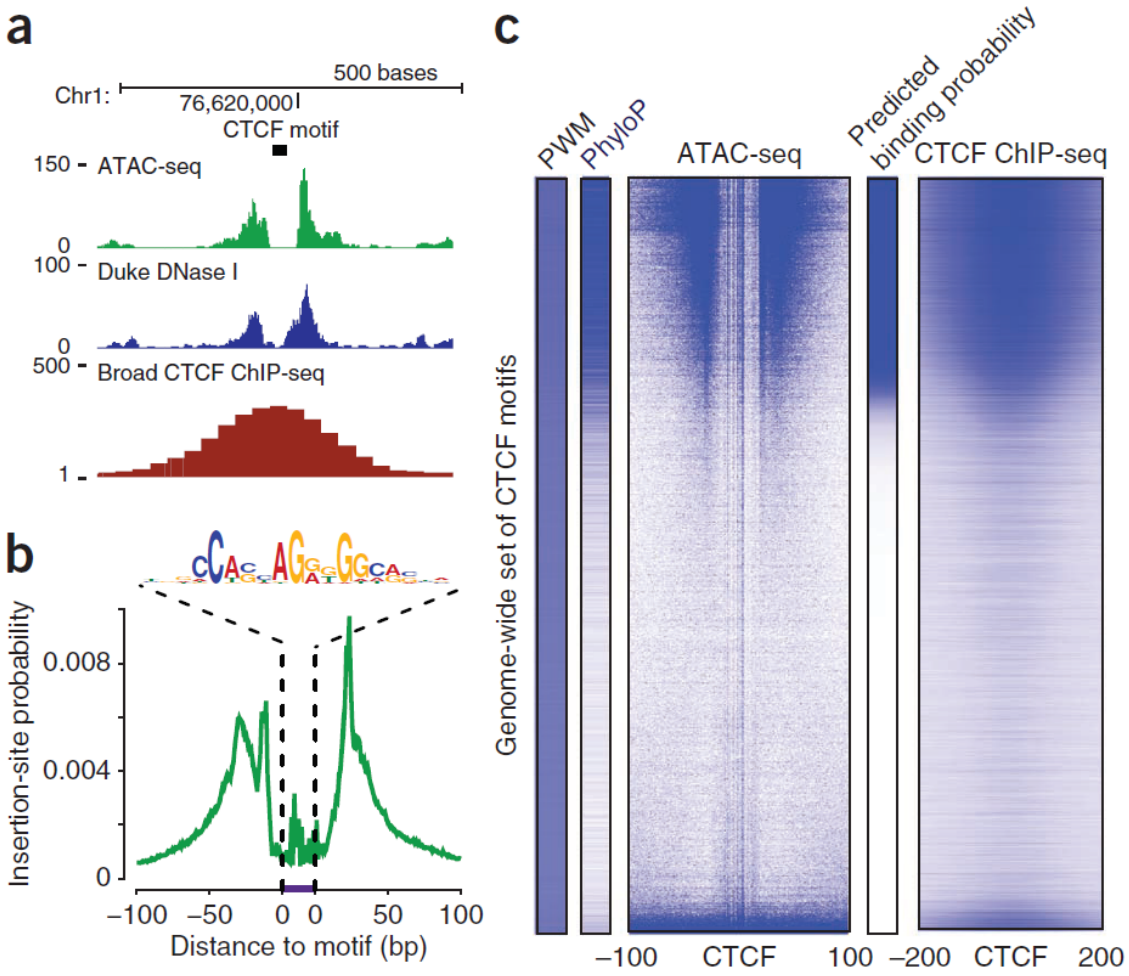
## ATAC-Seq reveals patterns of nucleosome-TF spacing

- Hierarchical clustering of DNA-binding factor positions (CHIP-Seq data) with respect to the nearest nucleosome dyad within accessible chromatin



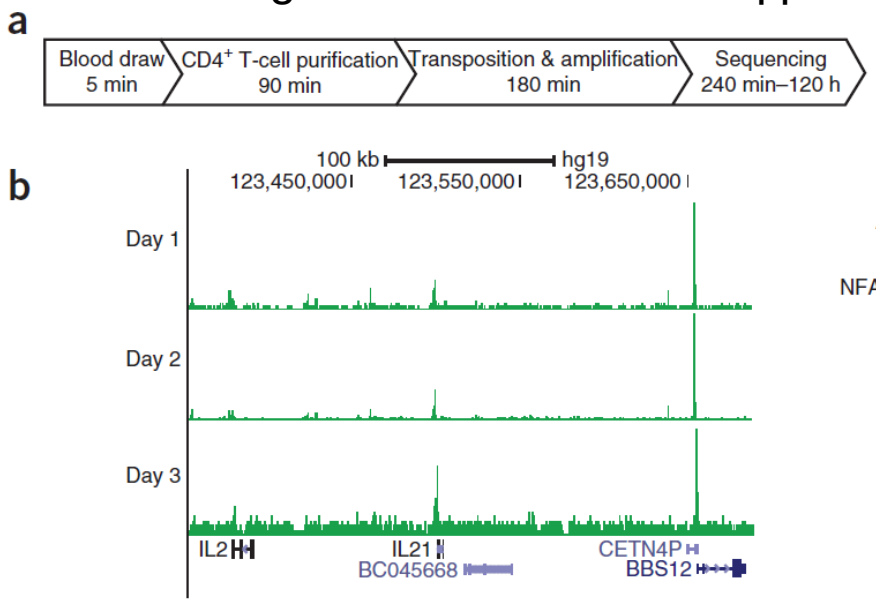
## ATAC-Seq assays genome-wide factor occupancy

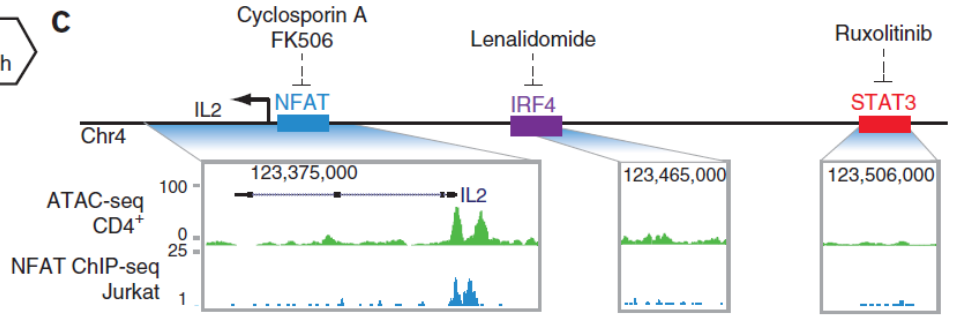
- DNA sequences directly occupied by DNA-binding proteins are protected from transposition → sequence “footprint” reveals presence of DNA-binding protein

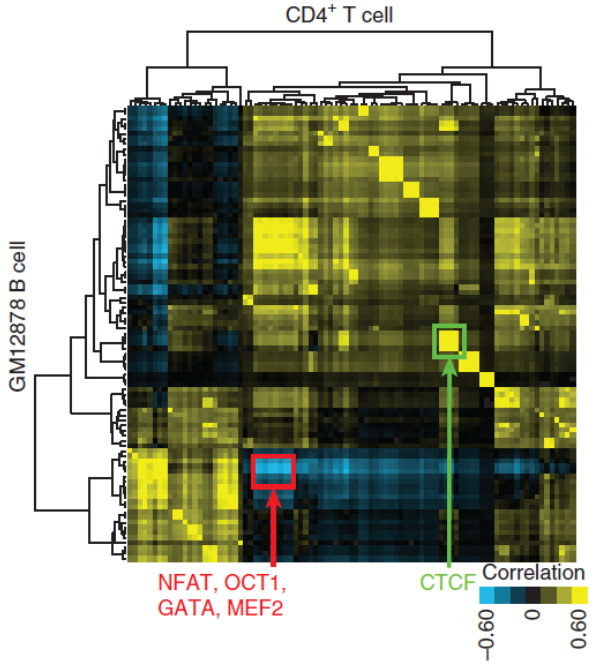


## Epigenomic analysis on clinical timescales

- ATAC-seq protocol was applied on T cells from healthy volunteer on three consecutive days
- Investigation of the ATAC-Seq profile of the *IL2* locus




- ATAC-Seq allows the simultaneous interrogation of factor occupancy, nucleosome positions in regulatory sites, and genome-wide chromatin accessibility
- Requires smaller input and less time

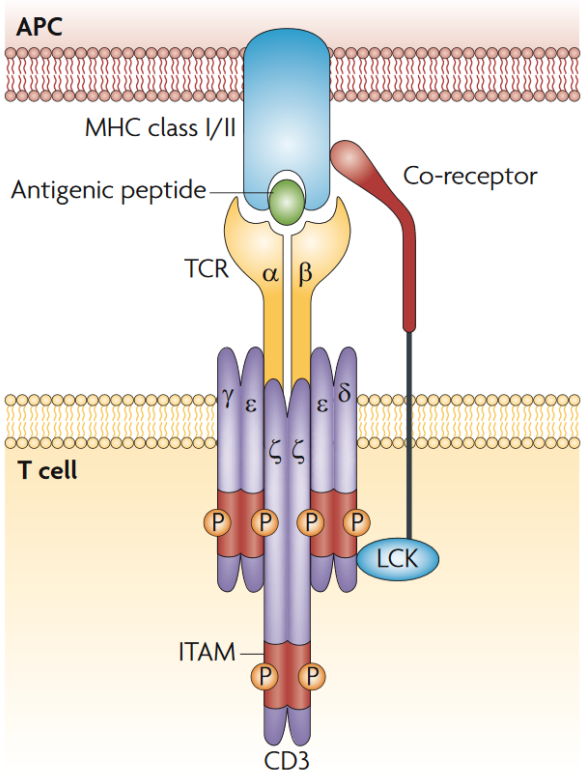
nature biotechnology

# Linking T-cell receptor sequence to functional phenotype at the single-cell level

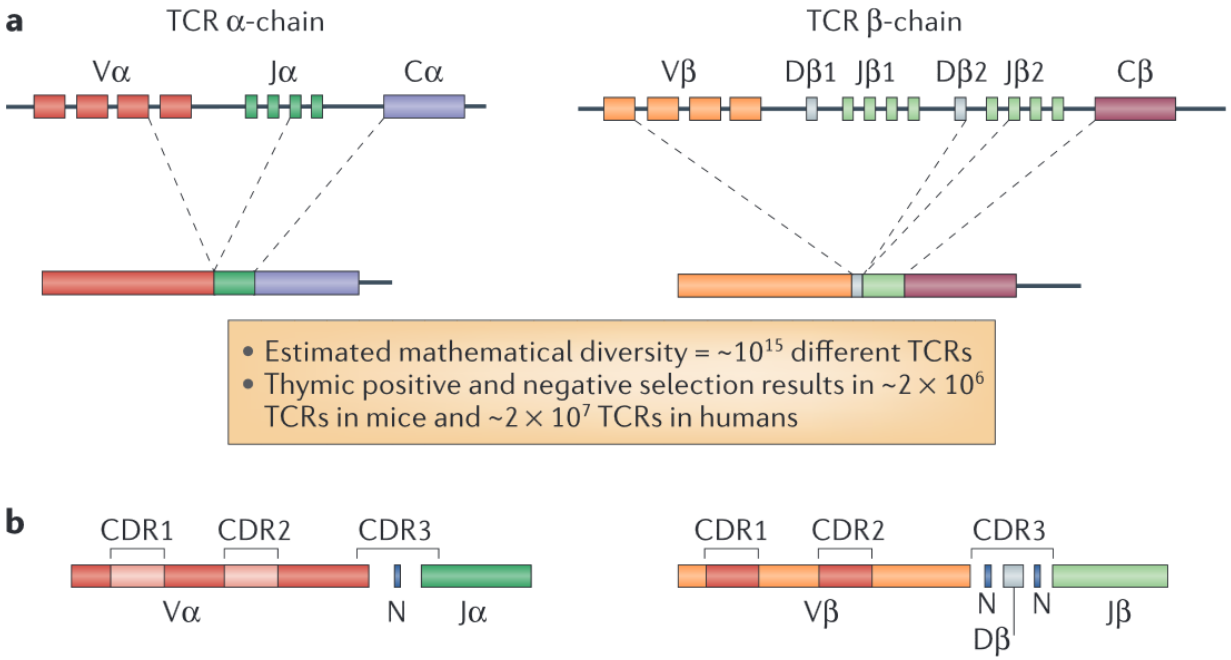
Arnold Han[1,2], Jacob Glanville[3], Leo Hansmann[2] & Mark M Davis[2–4]

# T cell receptor

- Determines to which antigenic peptide MHC complexes T cells respond
- Plays a major role in controlling T cell selection, function and activation
- The major TCR species composed of α- and β- subunits encoded by genes derived by somatic V(D)J recombination



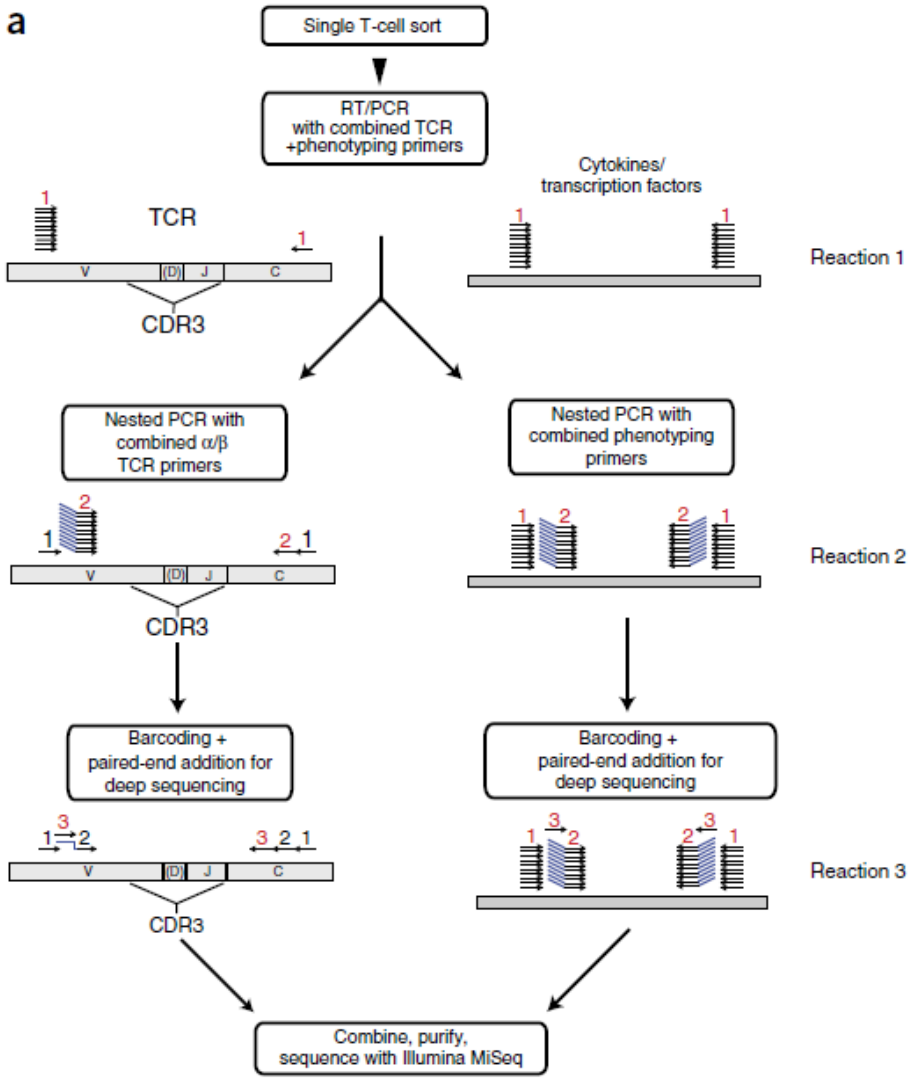

*Gascoigne, N., Nature Reviews Immunology vol. 8, p. 895–900 (2008)*

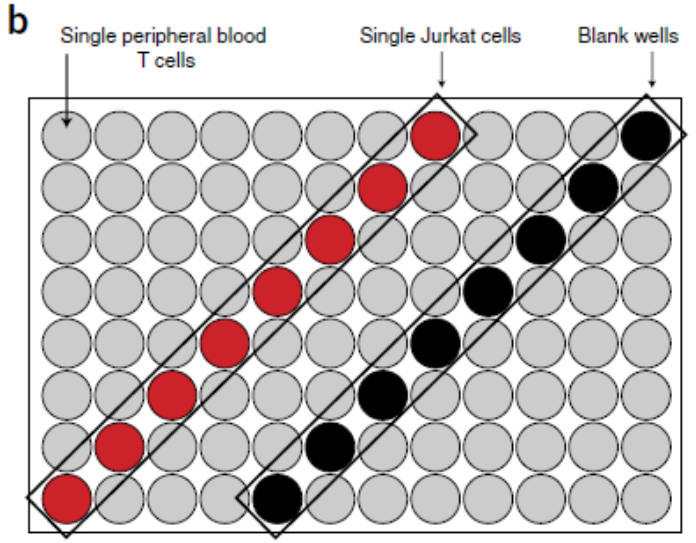



*Turner et al., Nature Reviews Immunology, vol. 6, p. 883–894 (2006)*

# Strategy for single cell TCR sequencing and phenotyping - Validation

- Comparison of 80 single CD45RA+CD4+TCRαβ+ T cells (plate 1), CD8+TCRαβ+ T cells (plate 2) isolated from the same donor




c

| | TCR-α efficiency | TCR-β efficiency |
|---|---|---|
| Plate 1 (split) | 79/88 (90%) | 80/88 (91%) |
| Plate 2 (split) | 76/88 (86%) | 83/88 (94%) |
| Split plates | 155/176 (88%) | 163/176 (93%) |
| Plate 1 (combined) | 74/88 (84%) | 77/88 (88%) |
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| Jurkat wells | 16/16 (100%) | 16/16 (100%) |
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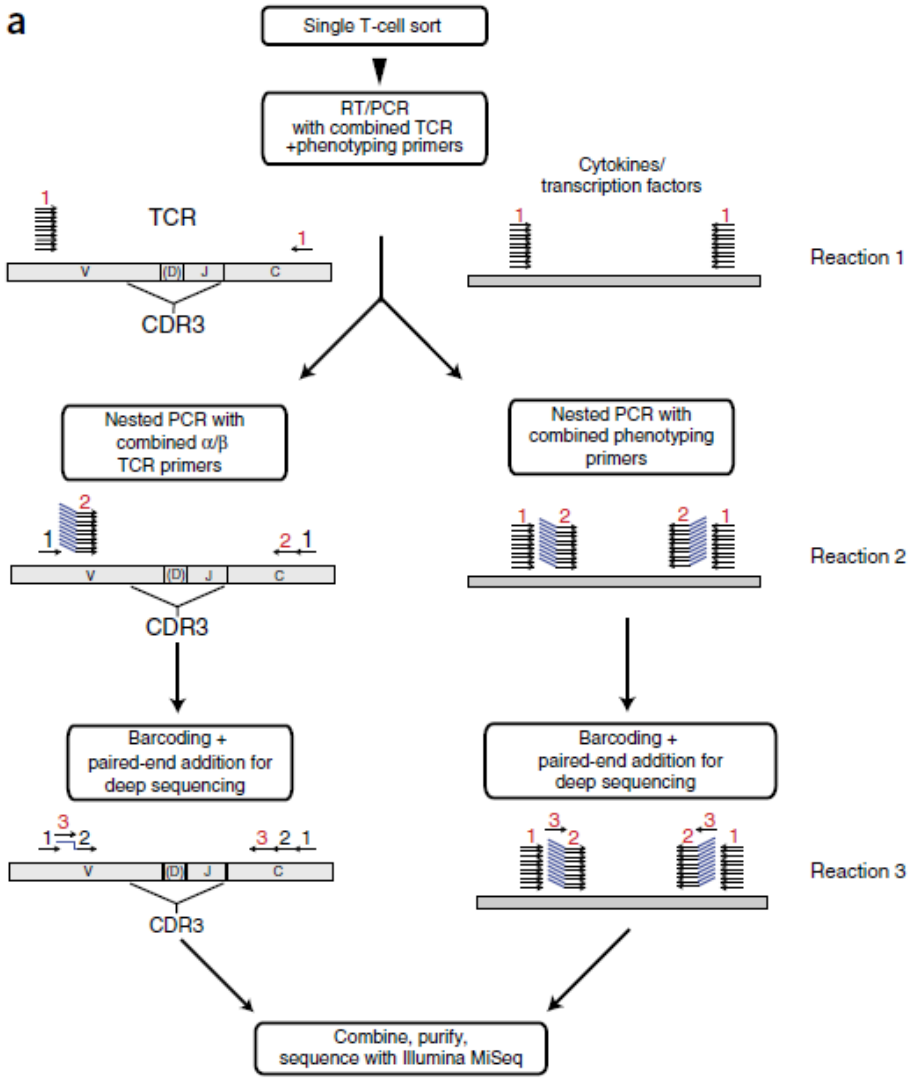


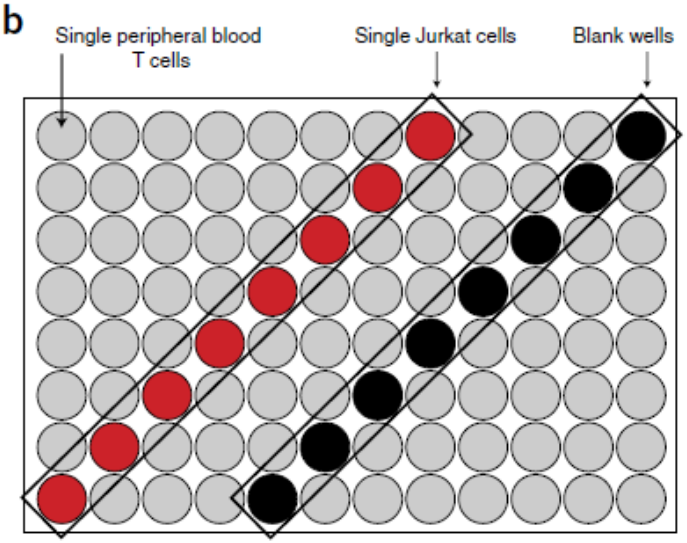


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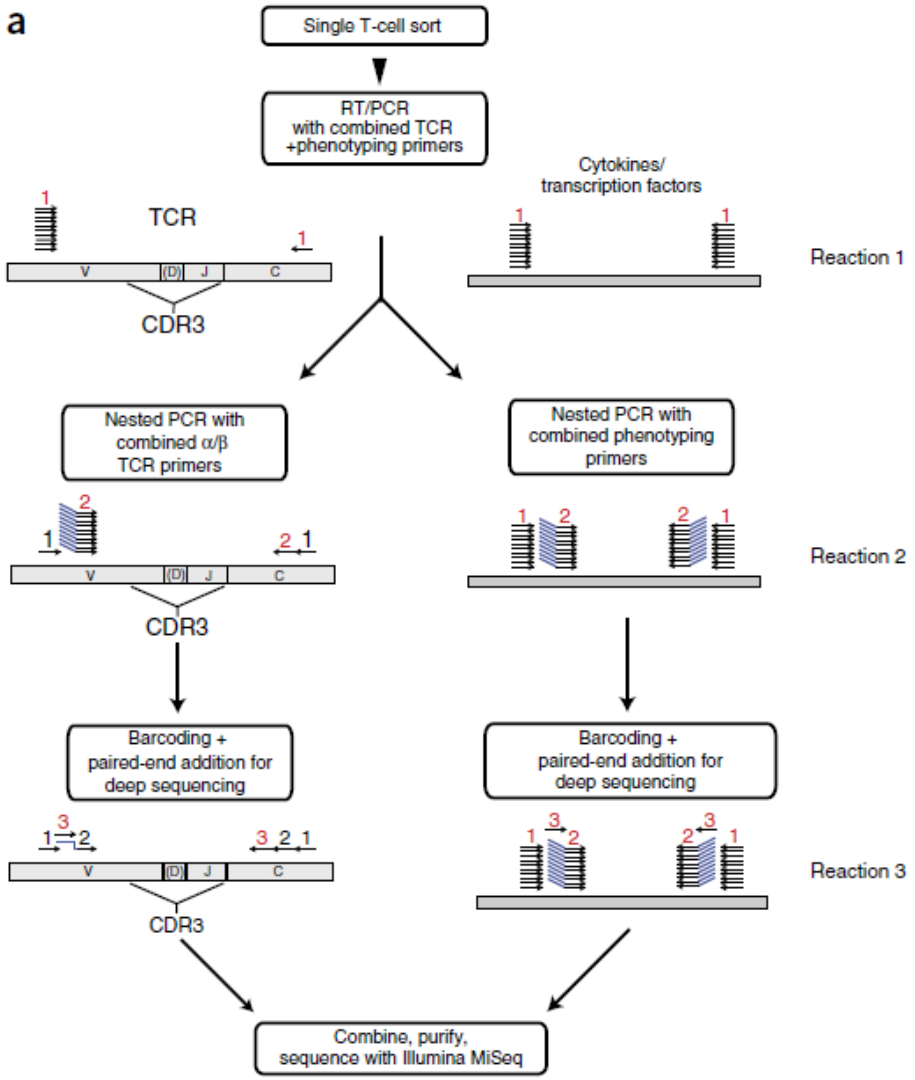


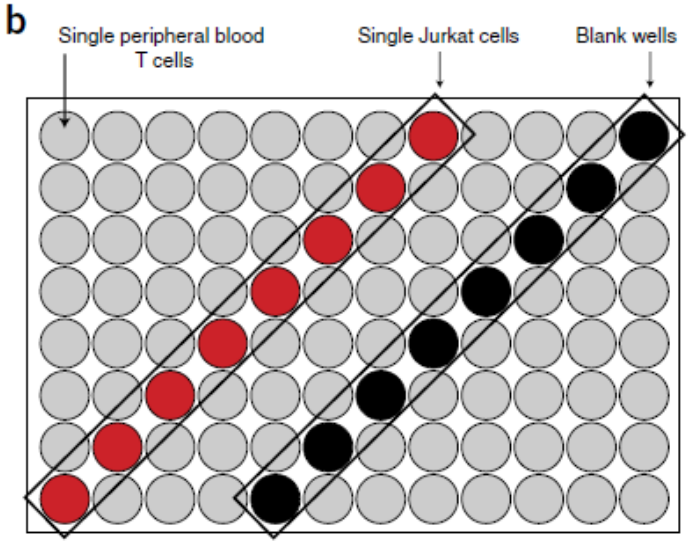


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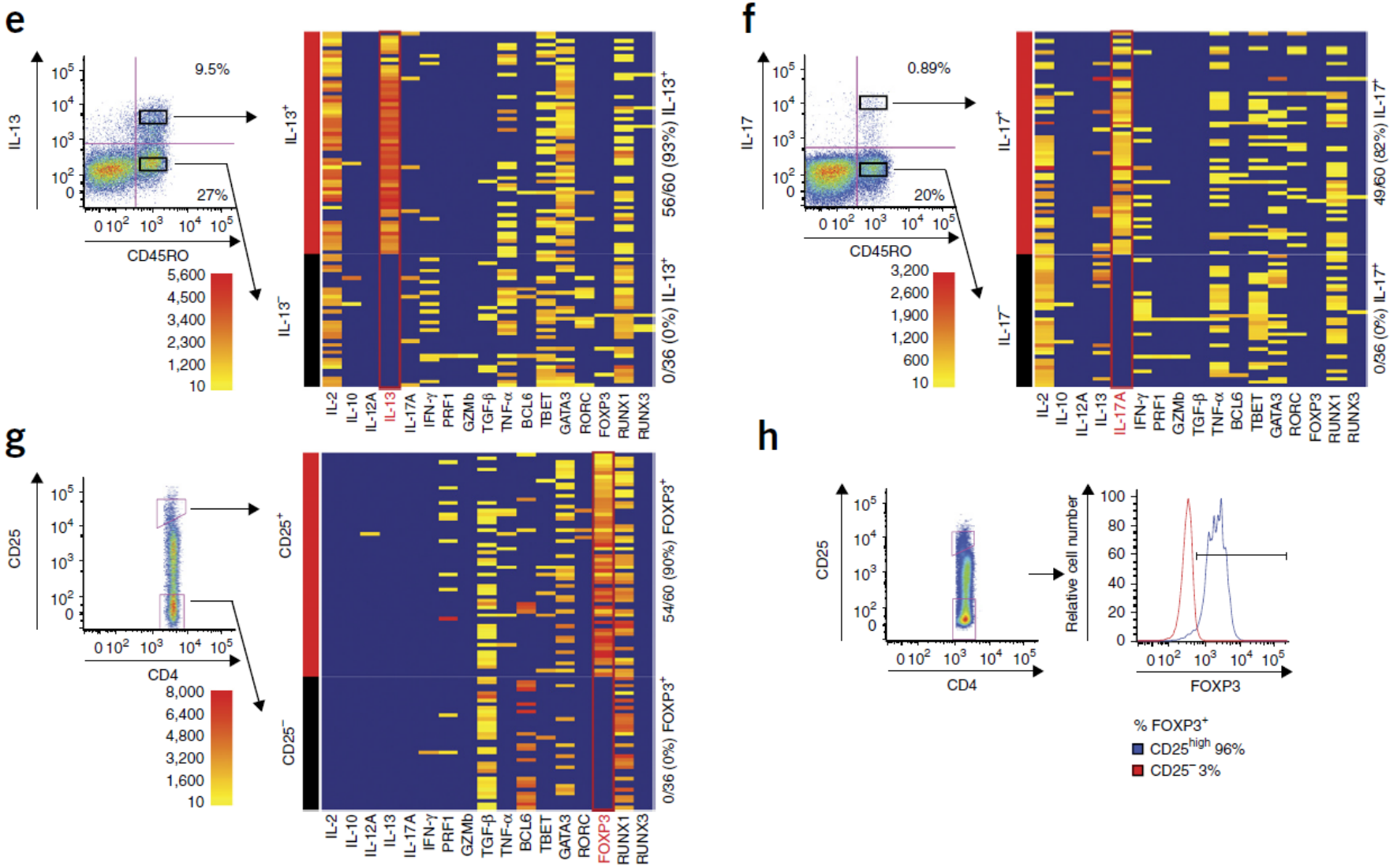
# Strategy for single cell TCR sequencing and phenotyping - Validation

- Validation based on flow cytometry based cytokine capture assay and single cell sorting for subsequent TCR-Seq protocol



## Analysis of tumor – infiltrating lymphocytes (TILs) by TCR-Seq

- 736 sorted CD4+ TILs, 372 CD4+ T cells derived resected adjacent colon tissue and peripheral blood T cells from a human colorectal-cancer patient were analyzed
  - TCRβ sequences were successfully obtained from 81% of CD4+ T cells
  - 68% of the total sequences obtained were assigned to productive, paired TCRαβ sequences
  - Detection of marked T cell clonal expansion; most frequent TCRβ sequence was detected in 52/597 cells; 10 most frequent TCRβ sequences accounted for 36% of the cells
  - Cells obtained from resected adjacent colon tissue showed only minimal clonal expansion (only 4 clones were detected twice within the population)
  - No single TCRαβ sequence was shared between the T cells in the tumor and the adjacent colon tissue

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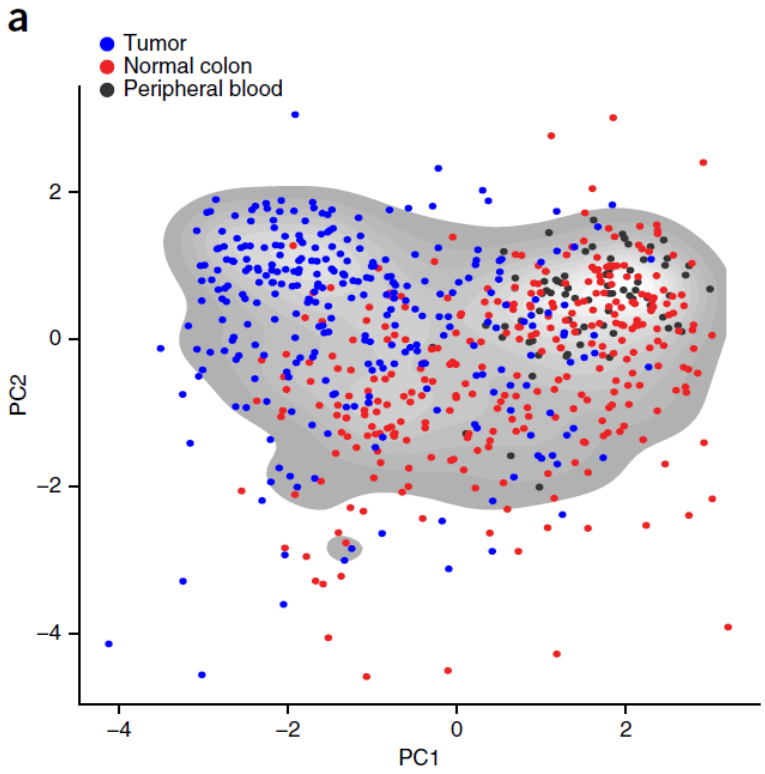
- For phenotypic analysis half of the cells were stimulated for 3hrs wit PMA and ionomycin




PCA for depicted phenotypic diversity between stimulated TILs and stimulated cells obtained from the adjacent tissue or peripheral blood

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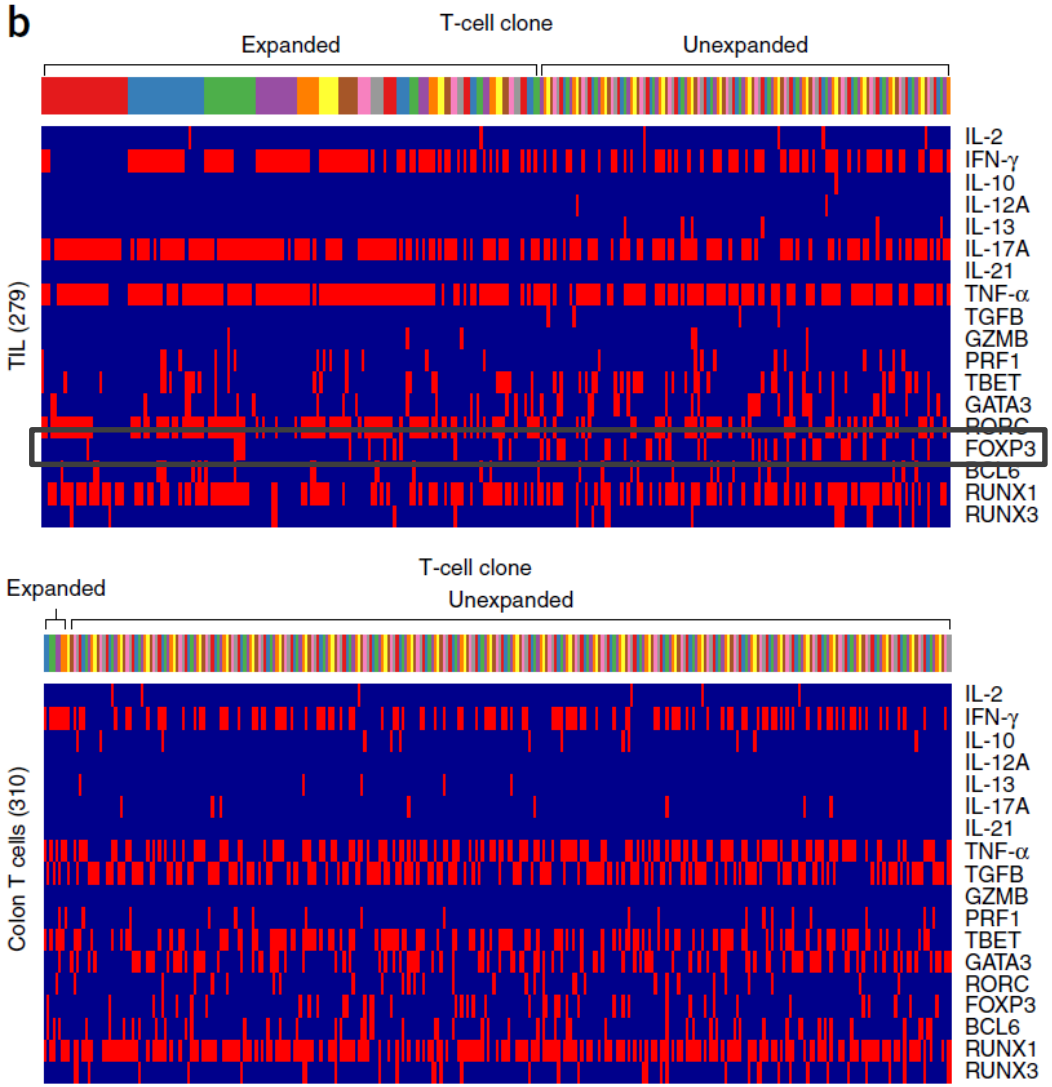


TILs can be distinguished by their co-expression of INFγ, TNFα and IL17

Notable heterogeneity within each T cell population

Individual cells co-express multiple, different, master regulator transcription factors

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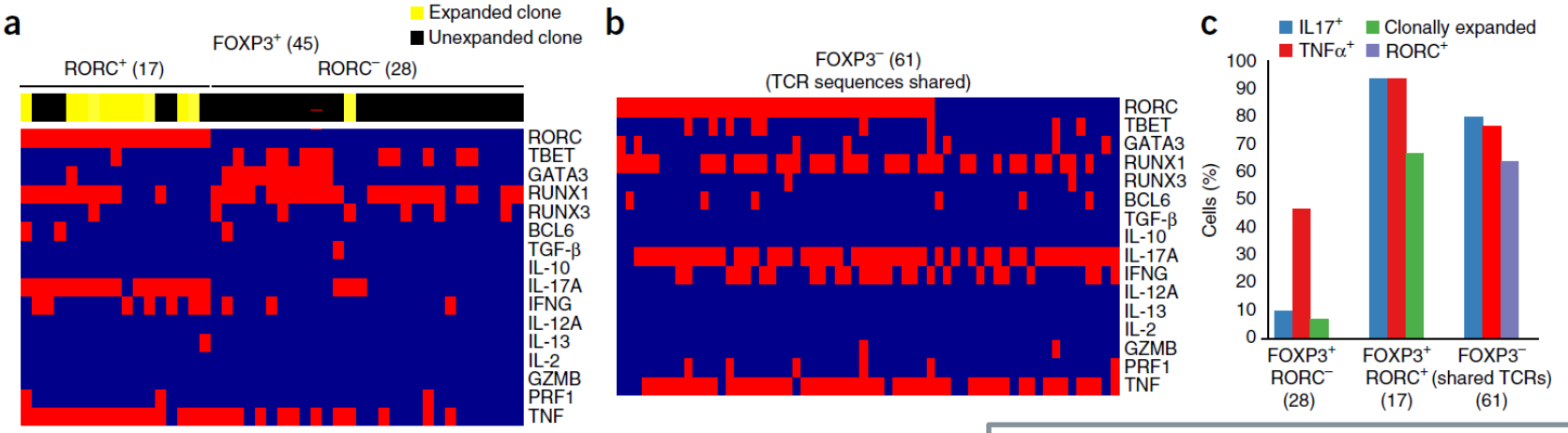


Identification of 2 distinct subsets based on differential RORC expression

RORC+ FOXP3+ cell population was further characterized by the co-expression of IL17

2 populations of IL17 expressing T cells that share common ancestry

LETTERS
https://doi.org/10.1038/s41591-018-0008-8
nature medicine

# Transcript-indexed ATAC-seq for precision immune profiling

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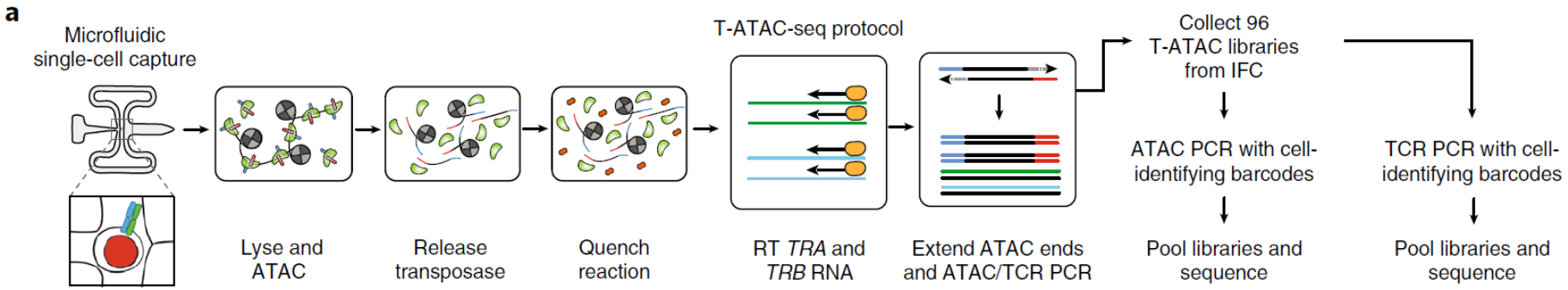
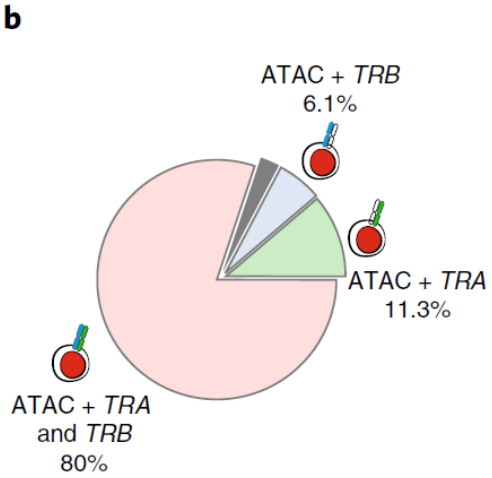
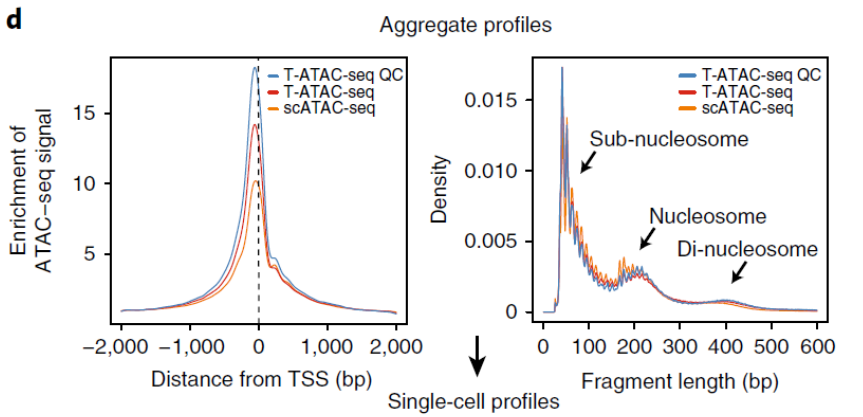
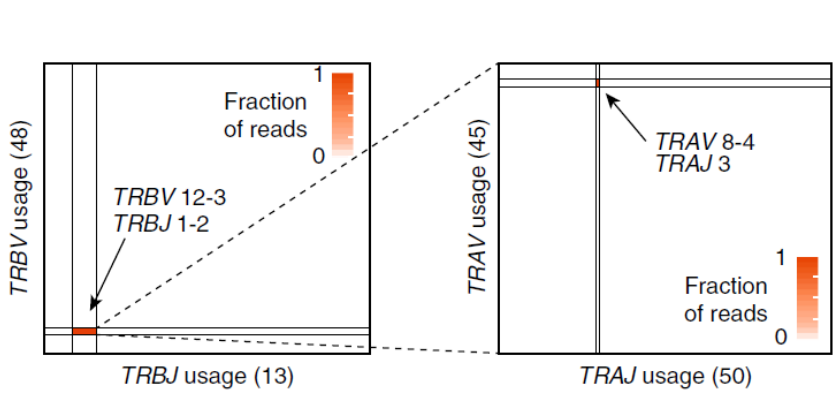


## Performance of T-ATAC-Seq in human immortalized T cells

- 288 single human Jurkat leukemia cells
  - ➢ Combined ATAC-Seq and TCR-Seq were obtained in 93.9% of the cells
  - ➢ 80% of the cells produced ATAC-Seq and a paired *TRA* and *TRB* sequence
  - ➢ T-ATAC-seq data recapitulated fragment-length periodicity and enrichment of fragments at TSSs, with similar quality as observed for ATAC-Seq
  - ➢ *TRA* and *TRB* sequences correctly identified the Jurkat TCR heterodimer

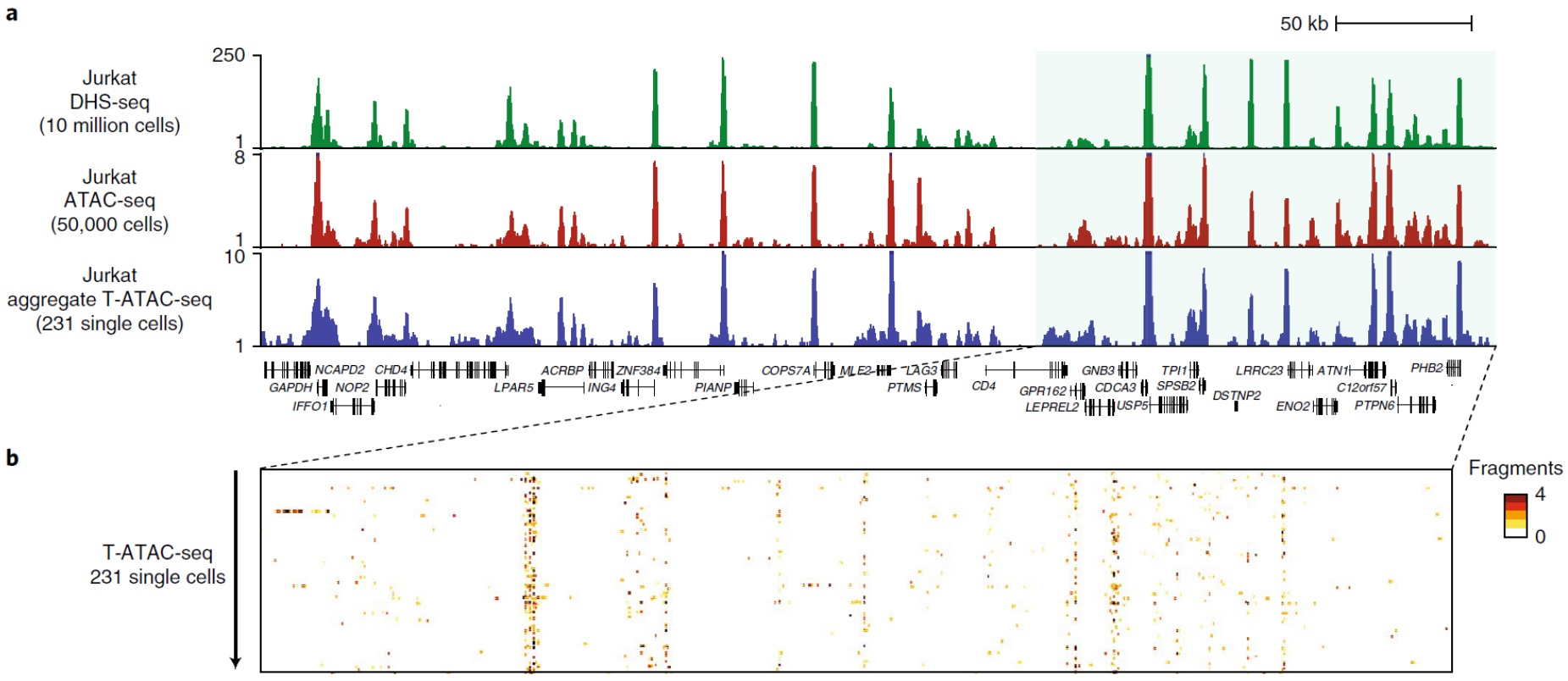


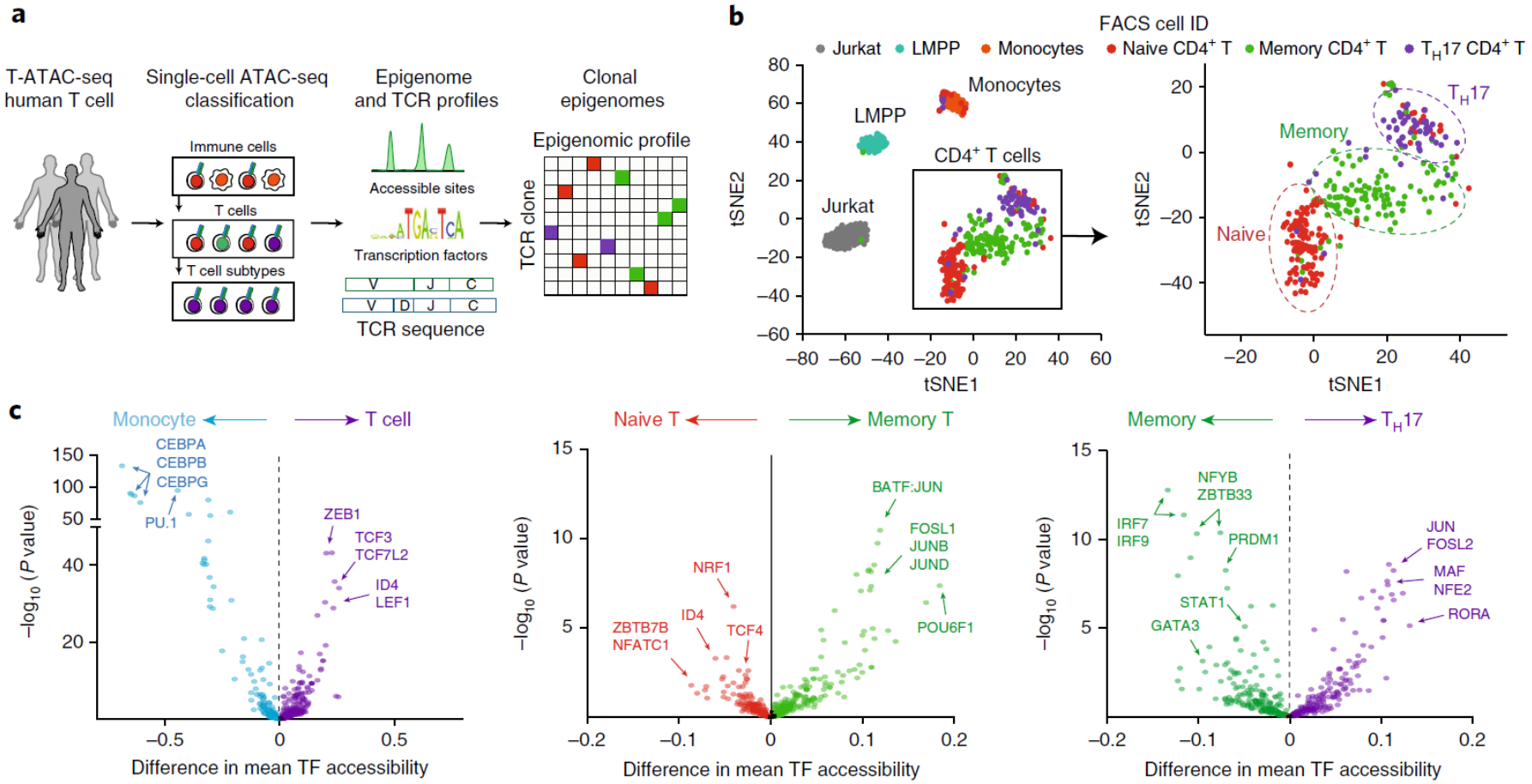
# Performance of T-ATAC-Seq in human immortalized T cells

- Level of regulatory elements in Jurkat cells
  - ➢ T-ATAC-Seq data reproduced population measurement profiles obtained by Jurkat DHS-Seq or ATAC-Seq
  - ➢ Single-cell profiles were enriched for fragments within open chromatin sites



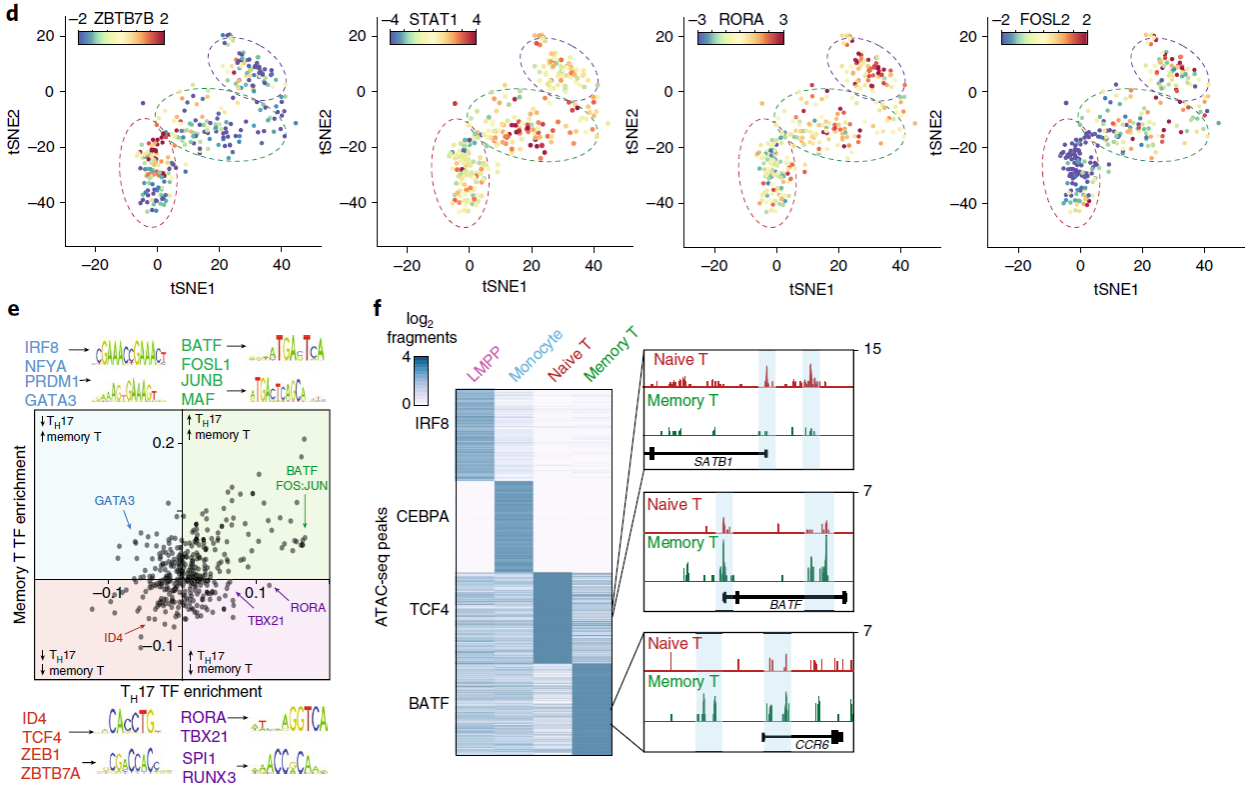
# Performance of T-ATAC-Seq in human primary T cells

- T-ATAC-Seq profiles of cell surface marker defined CD4+ naïve and memory T cells compared to those of Jurkat cells, Monocytes and LMPPs
  - ➢ t-SNE projection of epigenomic profiles revealed clustering of single cells largely according to cell type
  - ➢ Identification of cell type specific TFs in aggregated single cell profiles



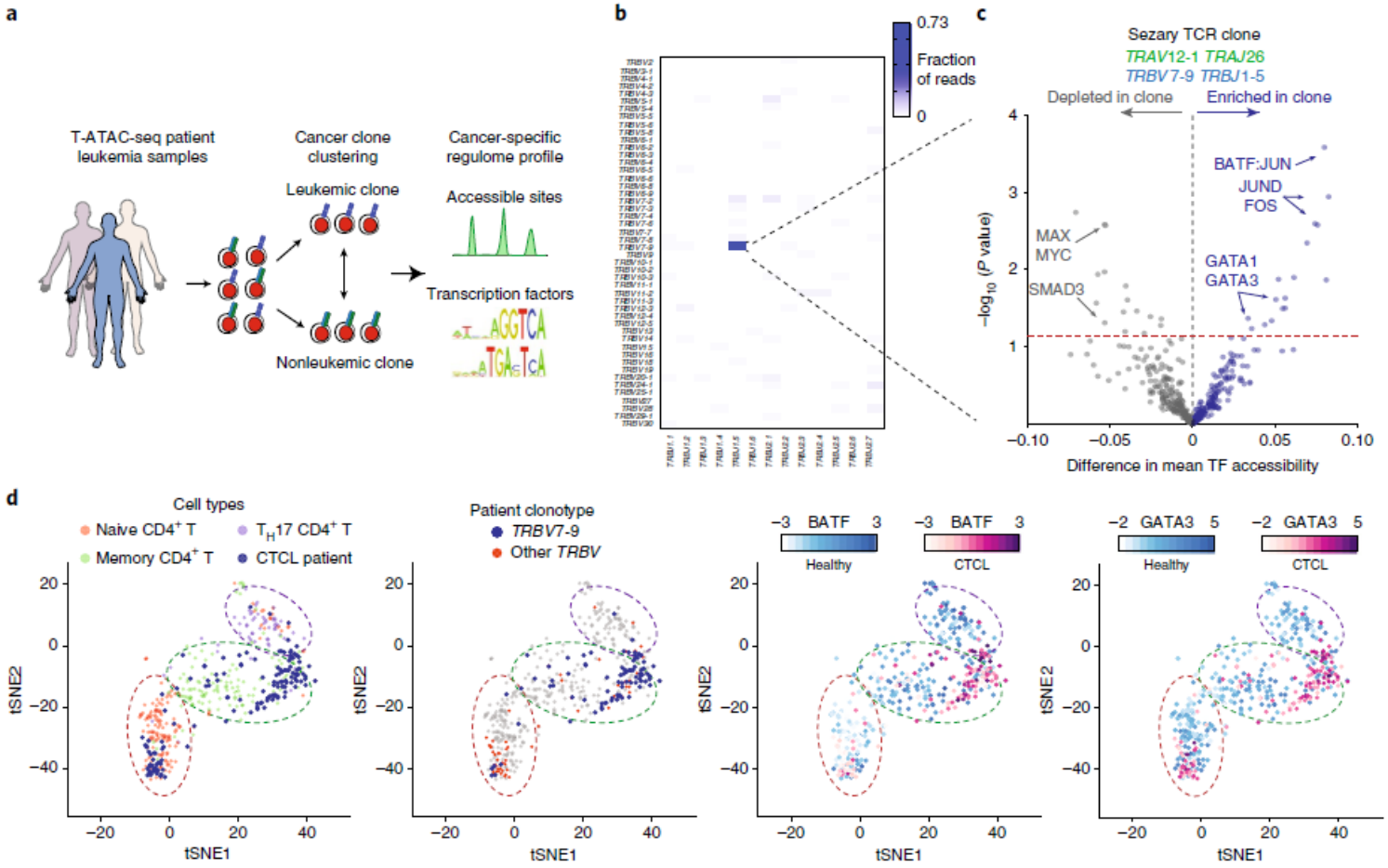
# Performance of T-ATAC-Seq in human primary T cells

- T-ATAC-Seq profiles of cell surface marker defined CD4+ naïve and memory T cells compared to those of Jurkat cells, Monocytes and LMPPs
  - ➢ CD45RA+ T cells showed substantial TF heterogeneity
  - ➢ Identification of cell type specific TFs in aggregated single cell profiles
  - ➢ Integration of TCR-Seq results with single-cell epigenomic profiles revealed two clonal populations characterized by high deviation for GATA factors, consistent with a Th2 phenotype



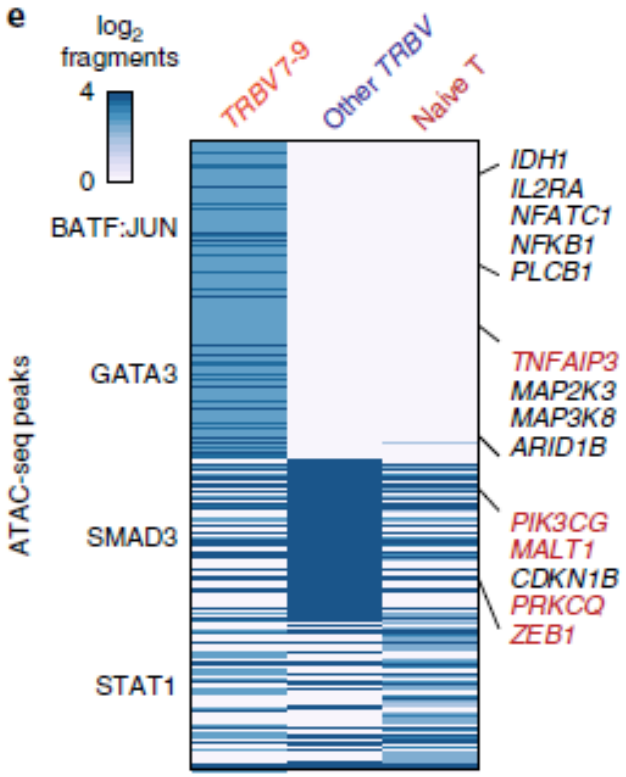
# Performance of T-ATAC-Seq in human primary T cells

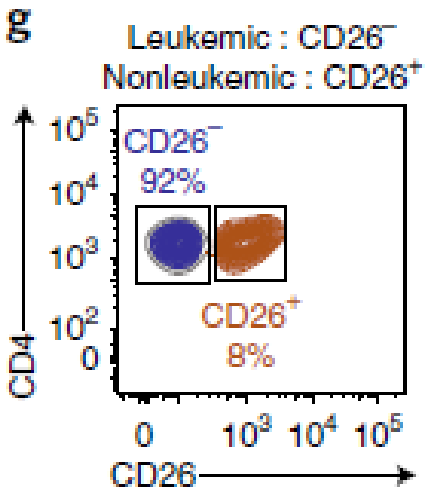
- T cells were isolated from patients with a leukemic form of cutaneous T cell lymphoma
  - ➢ 73% of all CD4 T cells expressed a single *TCRB* sequence
  - ➢ Almost all memory T cells are replaced by the Th2 cells → possible cause of systemic immunodeficiency

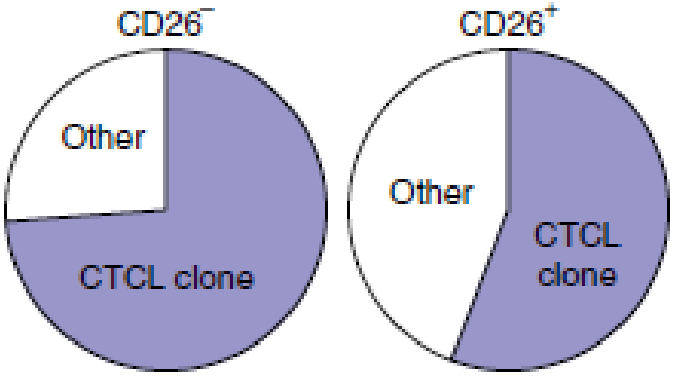


## Performance of T-ATAC-Seq in human primary T cells

- T cells were isolated from patients with a leukemic form of cutaneous T cell lymphoma
  - ➢ Nonmalignant T cell clones showed SMAD3 associated chromatin accessibility which may reflect an immunosuppressive TGF beta pathway
  - ➢ T-ATAC-Seq analysis shows superiority of TCR clonotyping over CD26 immunophenotyping



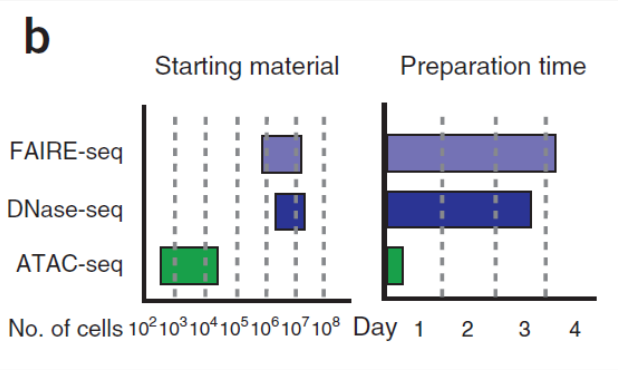



## Summary

### ATAC-Seq:

- Simultaneous interrogation of factor occupancy, nucleosome positions in regulatory sites and chromatin accessibility genome wide
- Assay compatible with low cell numbers/ single cells
- Low preparation time
- Compatible with FACS



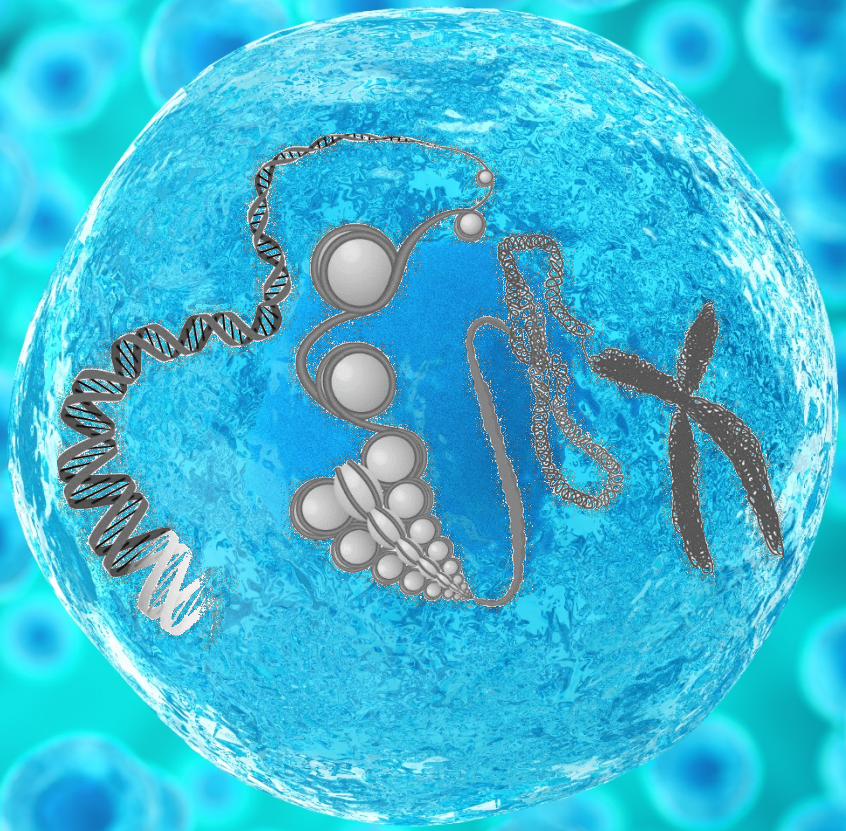

### Single cell TCR-Seq and phenotyping:

- Simultaneous sequencing of TCR genes and multiparametric analysis in single cells
- Identification of multiple TCRα genes from single cells
- TCR-Seq data adds an dimension to multiparametric phenotypic analysis by marking the ancestry of particular T cells

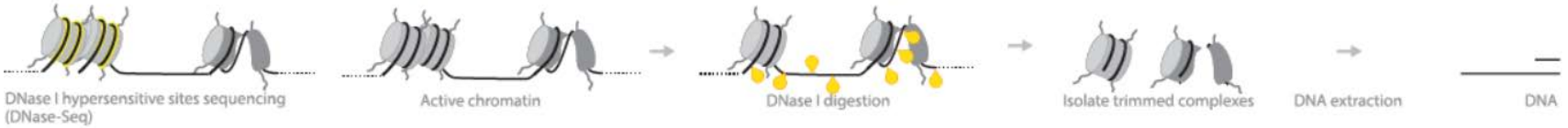
### T-ATAC-Seq:

- Strategy for pairing TCR identity to functional phenotype to investigate T cell clone dynamics, phenotypic plasticity and tumor heterogeneity
- Identification of new populations that appear similar based on surface marker profiling

# Thank you for your attention!!!



## DNAse-Seq:



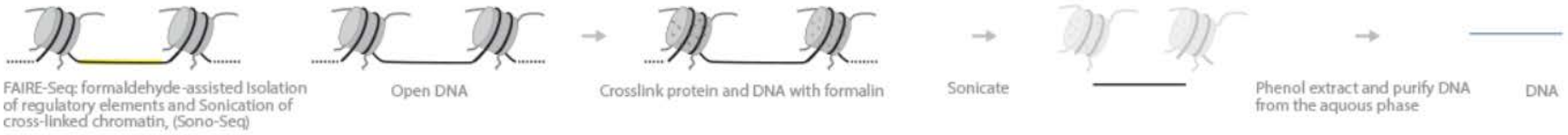

**Pros:**

- Can detect “open” chromatin
- No prior knowledge of the sequence or binding protein is required
- has greater sensitivity at promoters than FAIRE-seq

**Cons:**

- DNase I is sequence-specific and hypersensitive sites might not account for the entire genome
- DNA loss through the multiple purification steps limits sensitivity
- Integration of DNase I with ChIP data is necessary to identify and differentiate similar protein-binding sites

## FAIRE-Seq:




- Formaldehyde-assisted isolation of regulators elements with sequencing

**Pros:**

- Simple and highly reproducible protocol
- Does not require antibodies
- Does not require enzymes, such as DNase or MNase, avoiding the optimization and extra steps necessary for enzymatic processing
- Does not require a single-cell suspension or nuclear isolation, so it is easily adapted for use on tissue samples

**Cons:**

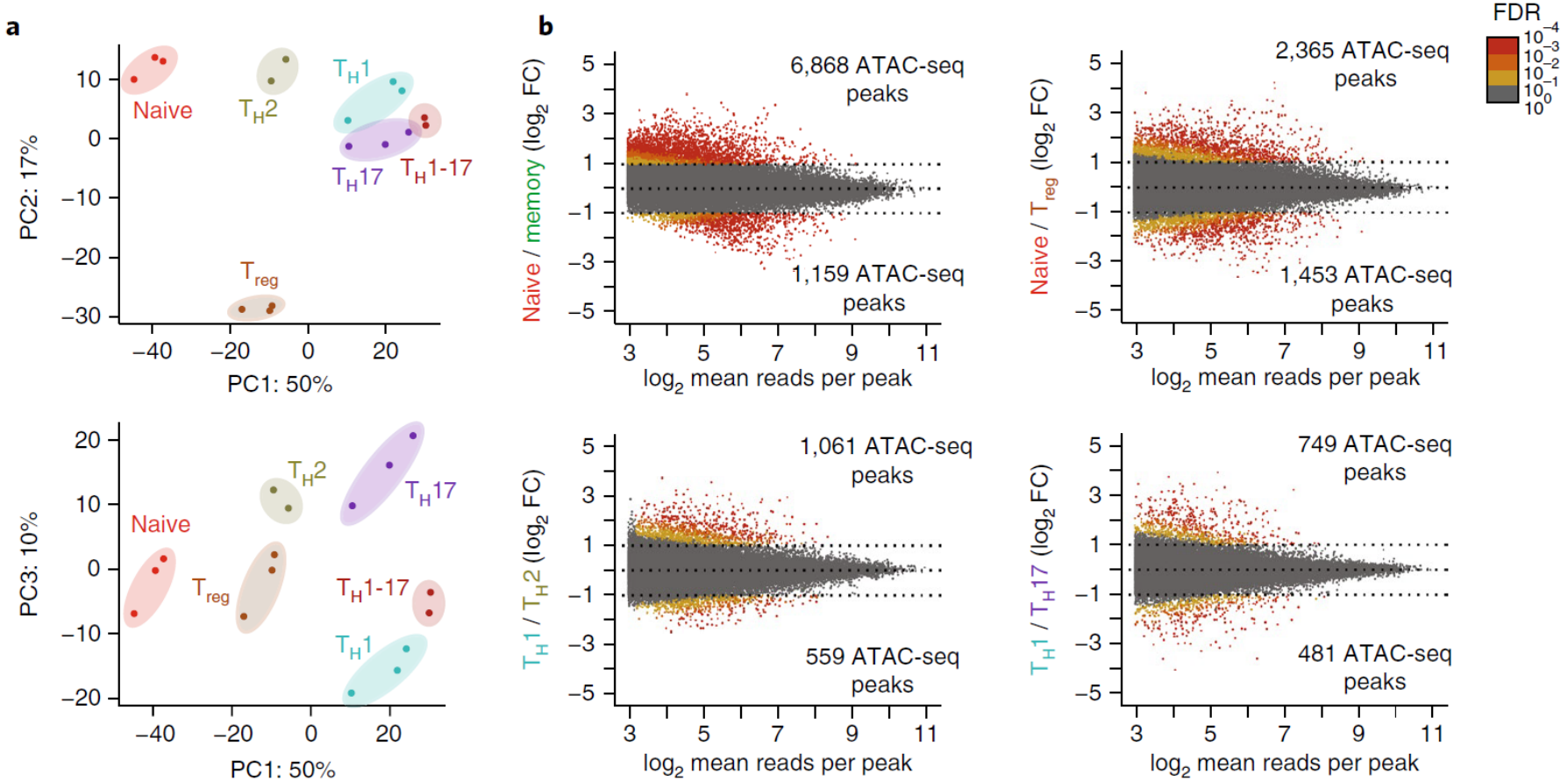
- Cannot identify regulatory proteins bound to DNA
- DNase-Seq may be better at identifying nucleosome-depleted promoters of highly expressed genes

**Fluidigm:**

https://youtu.be/Yg8yEeKoB2Q

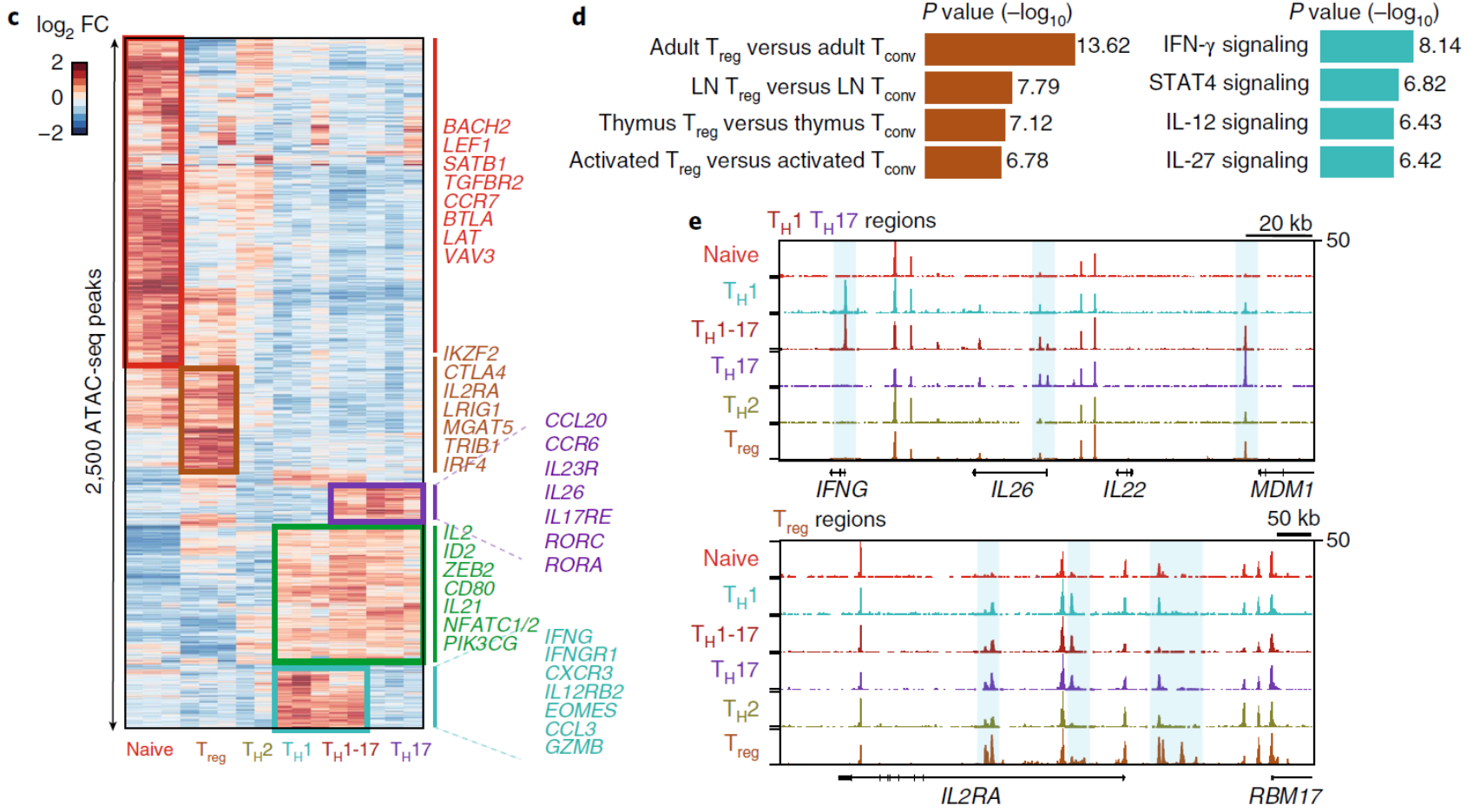
## Performance of T-ATAC-Seq in human primary T cells

- ATAC-Seq profiles of cell surface marker defined CD4+ naïve and memory subtypes
  - ➢ PCA reveals distinct chromatin profiles for each subset
  - ➢ differential ATAC-seq peaks showed that a large shift in chromatin accessibility accompanied the differentiation of naïve T cells to memory T cells



# Performance of T-ATAC-Seq in human primary T cells

- ATAC-Seq profiles of cell surface marker defined CD4+ naïve and memory subtypes
  - ➢ Tregs showed increased accessibility at the *IL2RA* locus, TH1 and TH1-17 cells at the INFG locus, TH1-17 and TH17 cells for the *IL26* and *IL22* loci



## Performance of T-ATAC-Seq in human primary T cells

- ATAC-Seq profiles of cell surface marker defined CD4+ naïve and memory subtypes
  - ➢ T cell subsets still distinguishable when down-sampled to a fragment density equivalent to that by T-ATAC-Seq data