Computational dissection of T cell dysfunction in tumors

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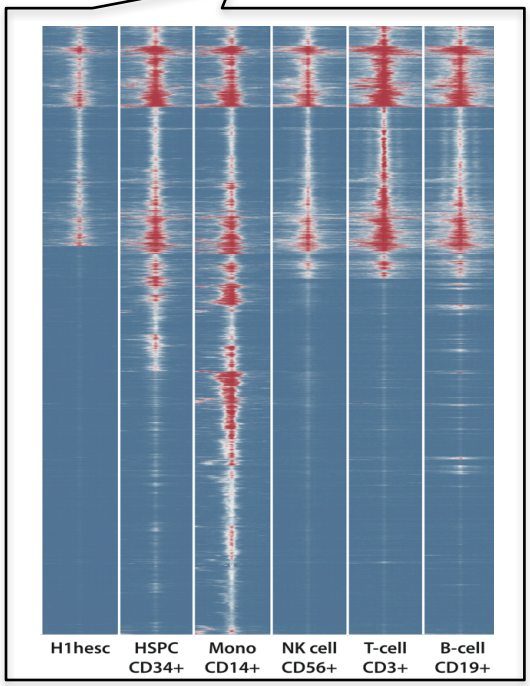
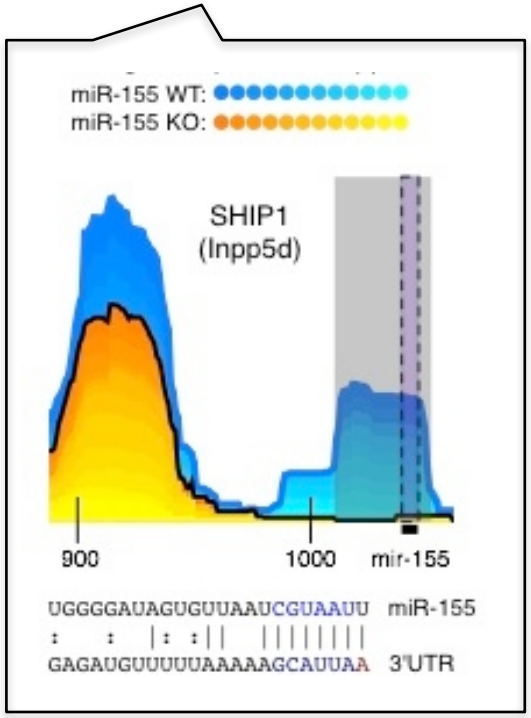
Lab for regulatory genomics and transcriptomics

Development of machine learning and statistical approaches to dissect the regulation of gene expression and the dysregulation of expression programs in cancer

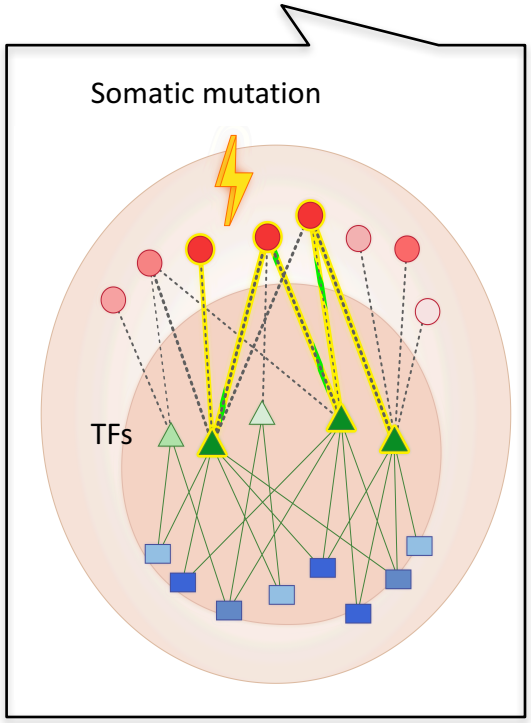
Cell-type specific epigenomic and transcriptional programs



Co- and post-transcriptional gene regulation



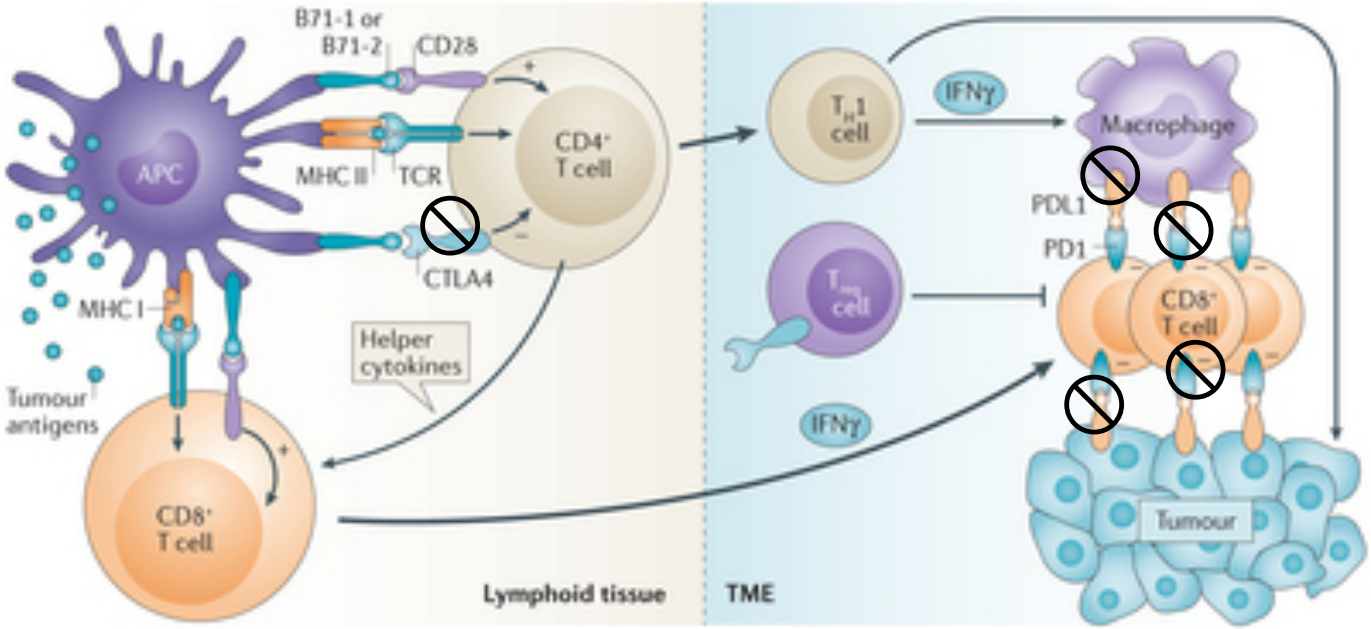
Cancer systems biology



Epigenetics of tumor-specific T cell dysfunction

- Human solid tumors often express mutant neoantigens, but tumor-specific T cells do not mount a functional response ("Hellstrom paradox")
- **Checkpoint blockade***:* promote functional immune response via blocking antibody against inhibitory molecules expressed by T cells (CTLA-4, PD1) or tumor/antigen presenting cells (PDL1)
- **Hypothesis**: *epigenetically encoded dysfunctional state in tumor-specific CD8 T cells as a barrier to checkpoint blockade*

Successful only in some tumor types, for some patients

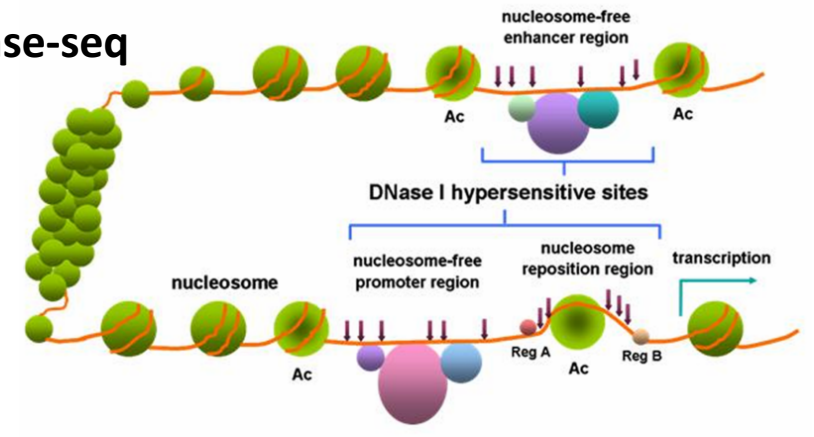


Nature Reviews | Cancer

Mapping the chromatin accessibility landscape

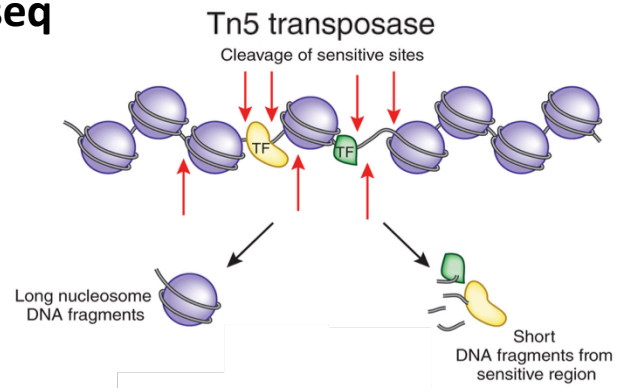
- DNase-seq: DNaseI hypersensitive site (DHS) mapping (Stamatoyannopoulos)
- ATAC-seq: transposase accessible chromatin (Greenleaf)
- Map regions of open chromatin = held open by DNA-binding proteins

DNase-seq



20M cells/experiment

ATAC-seq



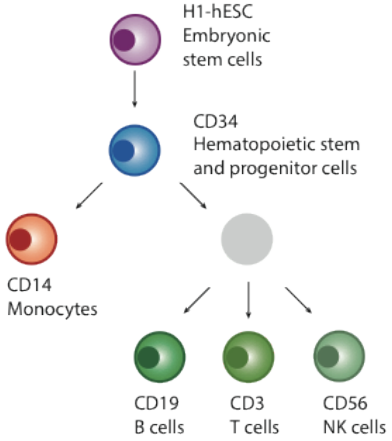
50K cells/experiment

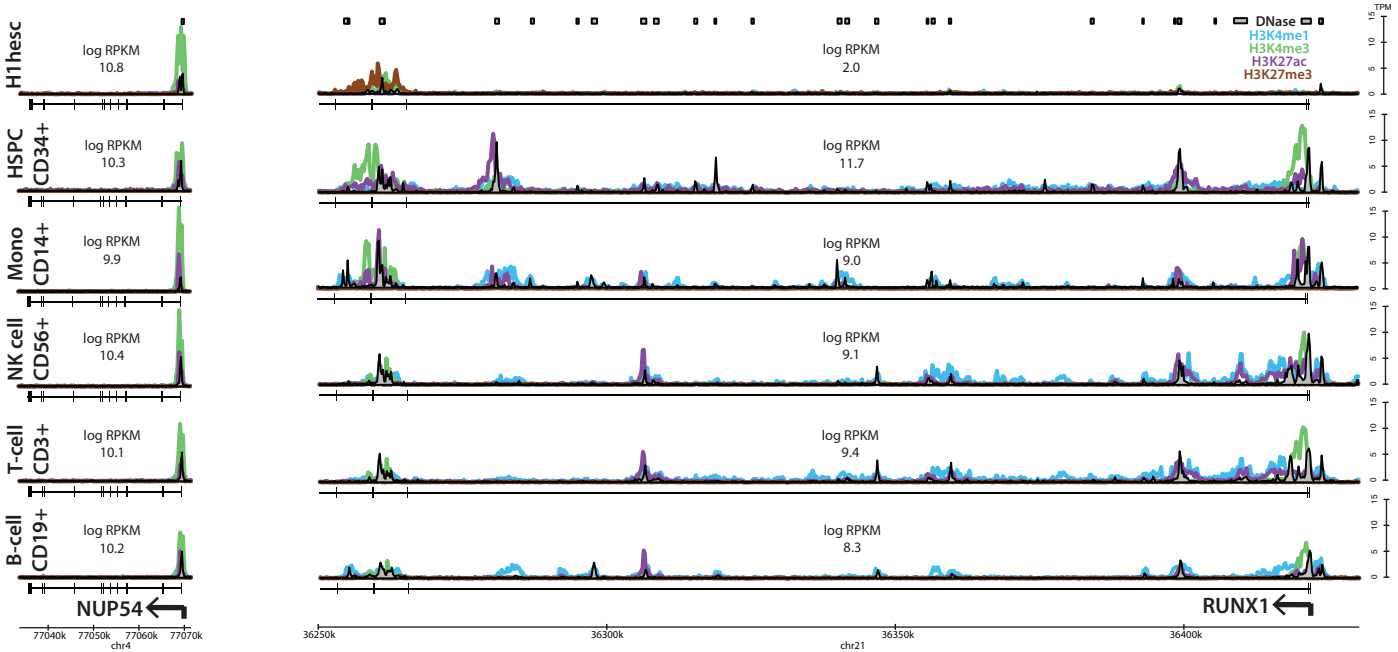
Goal: use ATAC-seq to dissect chromatin accessibility states underlying CD8 T cell dysfunction in tumors

Dynamics and spatial complexity of chromatin accessibility

- Atlas of ~120K DHSs (“peaks”) across hESC and 5 hematopoietic cell types, assigned to nearest genes
- Developmentally important genes are “high complexity” = many peaks, dynamics of accessibility and expression

low complexity *high complexity*

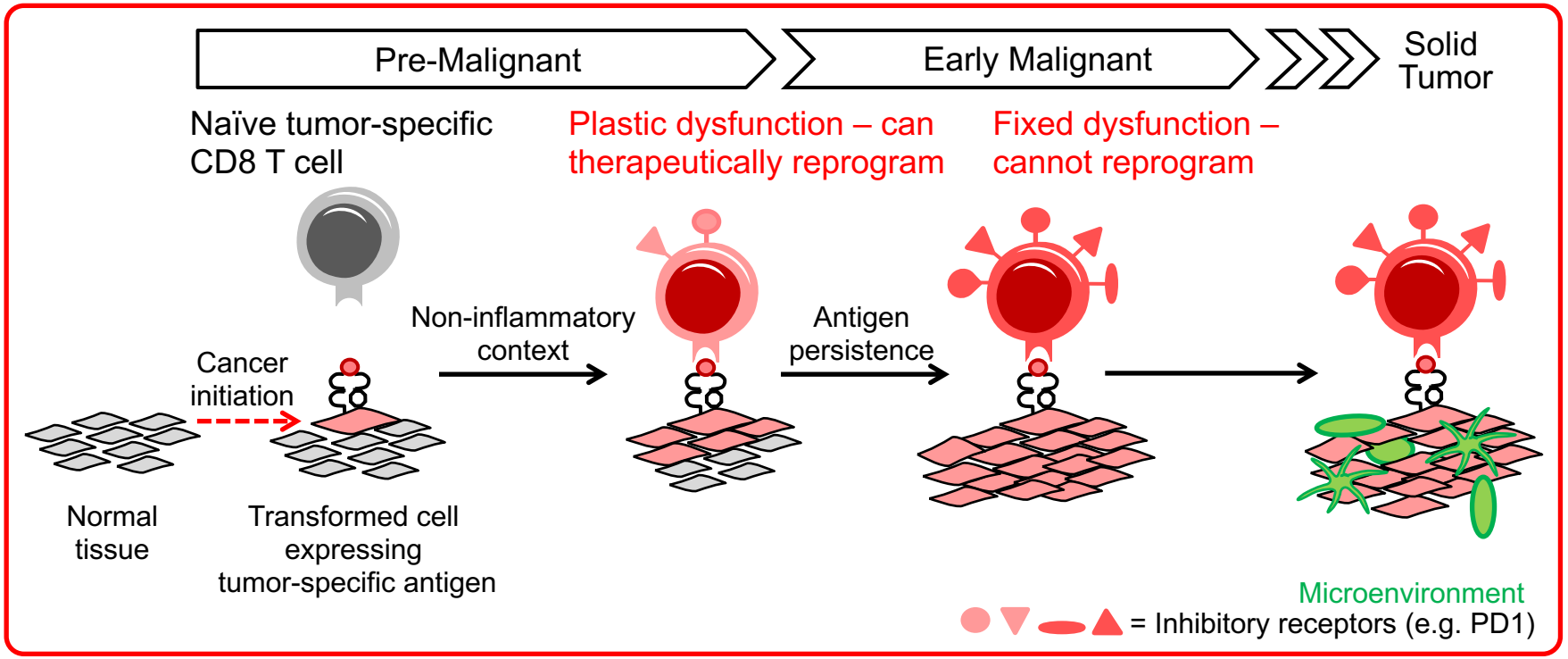




1 peak *43 peaks*

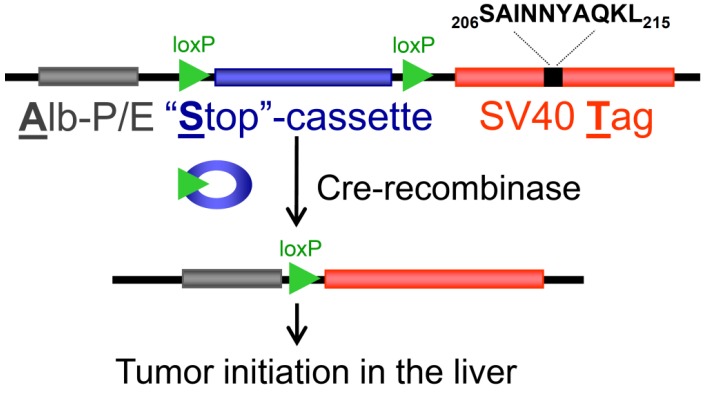
González, Setty*, Leslie, Nature Genetics 2015*

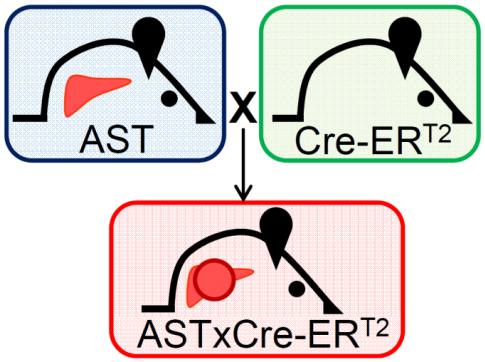
T cell differentiation in tumorigenesis



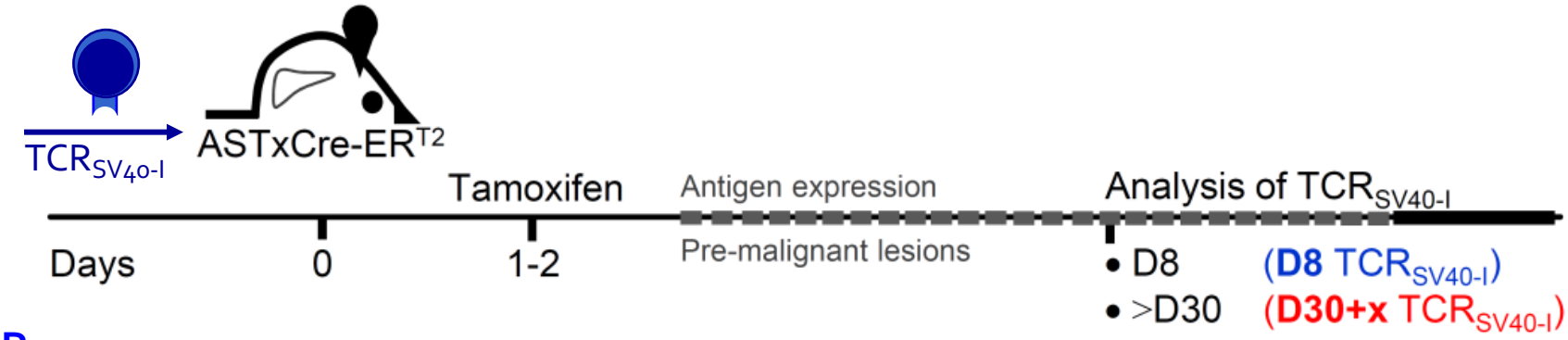
Schietinger lab, MKSCC

Mouse model:

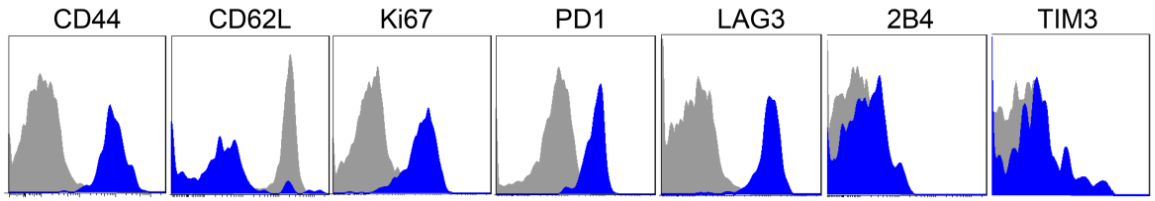


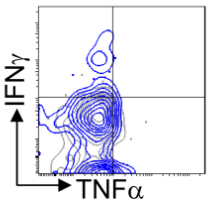


Tumor-specific T cells enter dysfunctional state in pre-malignancy



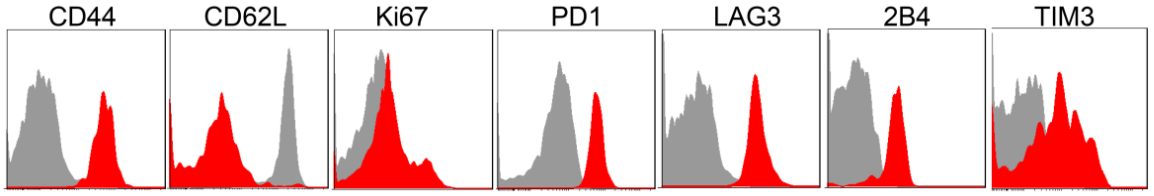
D8 TCR$_{SV40-I}$

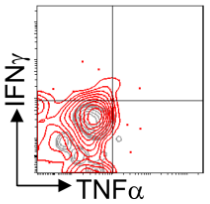




Plastic, susceptible to therapeutic reprogramming

D34 TCR$_{SV40-I}$



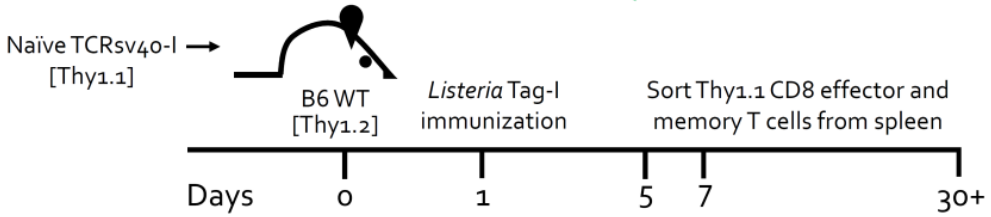


Fixed, resistant to therapeutic reprogramming

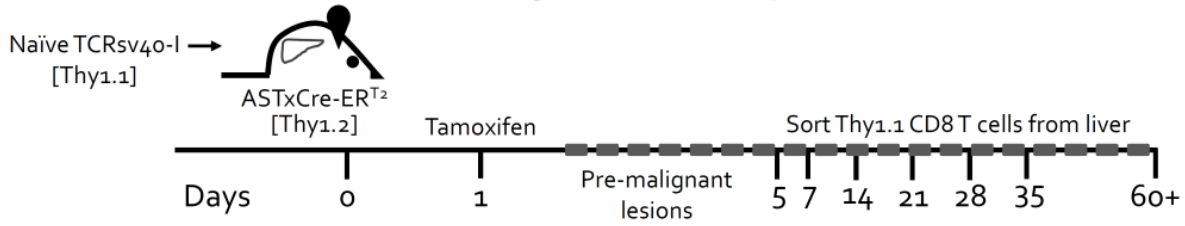
- At D30, no tumors are present, but antigen-specific T cells are in fixed dysfunctional state

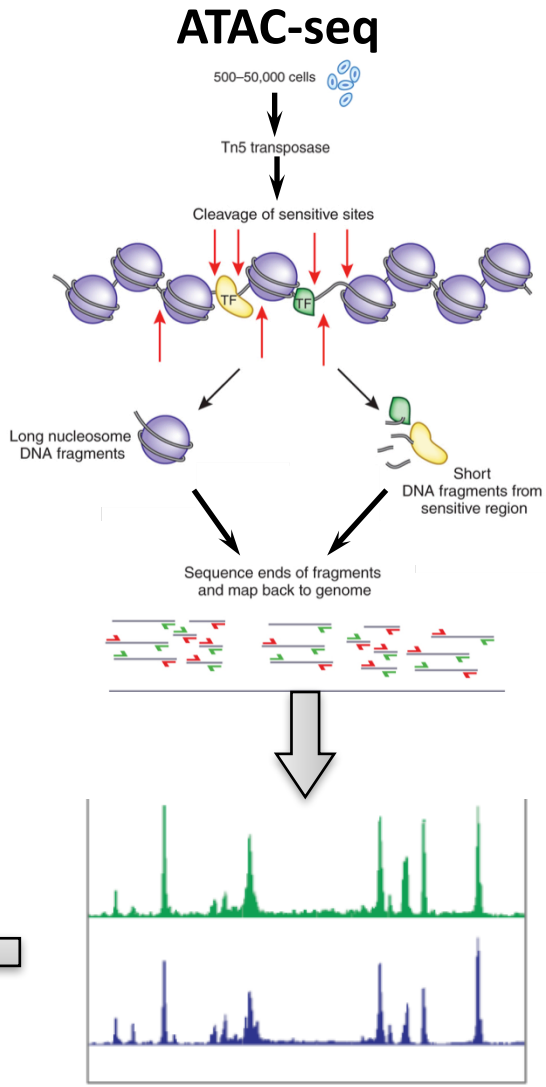
Chromatin dynamics in normal and dysfunctional T cell differentiation

A. Functional CD8 T cell differentiation (Naïve → Effector → Memory)



B. CD8 T cell differentiation during tumor development



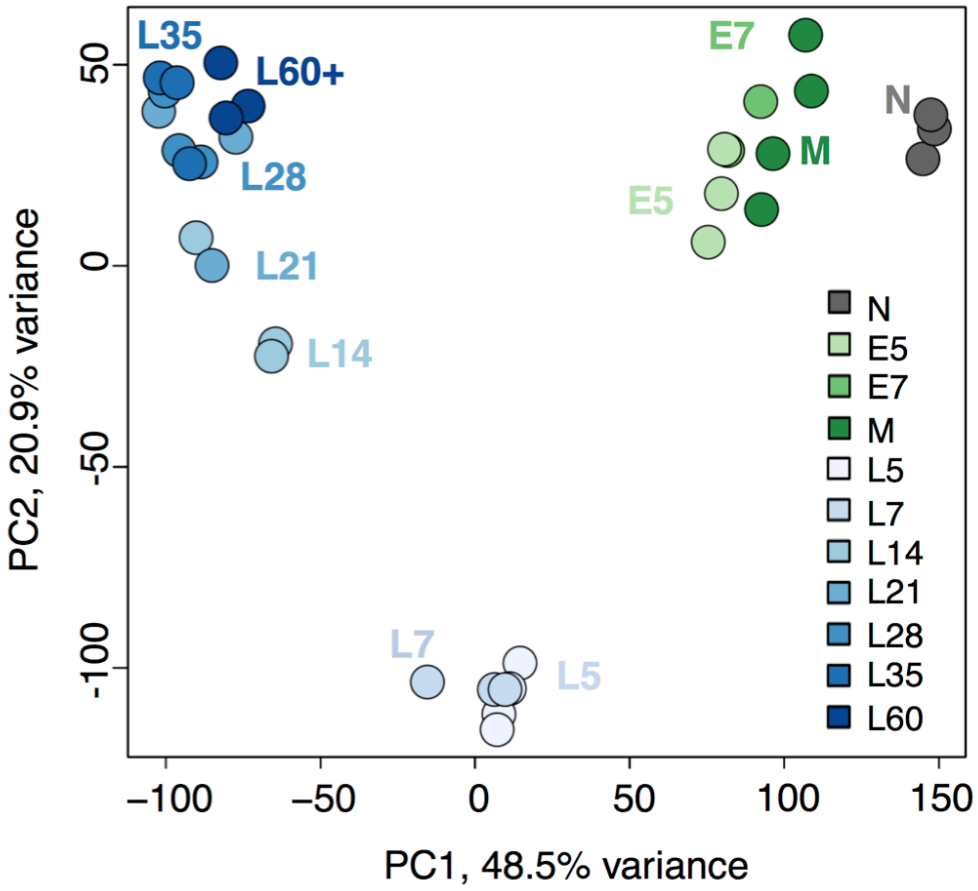


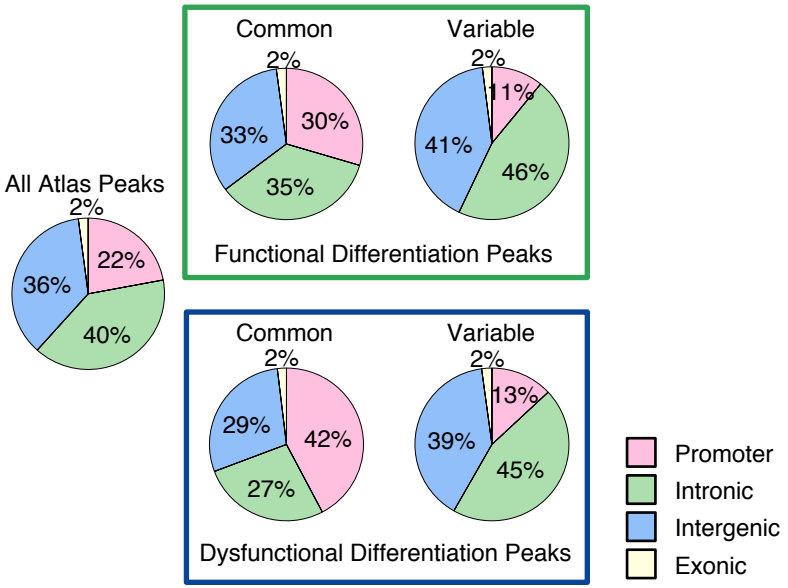
- ATAC-seq atlas of **~74.5K peaks**
  - Reproducible between biological replicates in at least one cell type
- RNA-seq in same cell types

Philip et al., Nature 2017

ATAC-seq analysis reveals distinct dysfunctional states

- Chromatin accessibility states coincide with functional states





ATAC-seq analysis reveals distinct dysfunctional states

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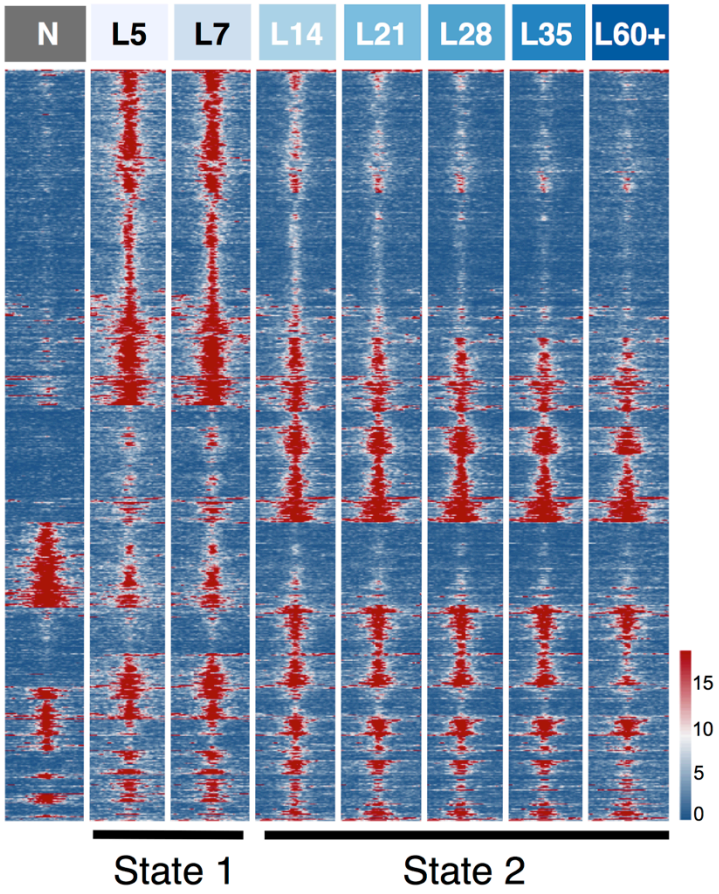
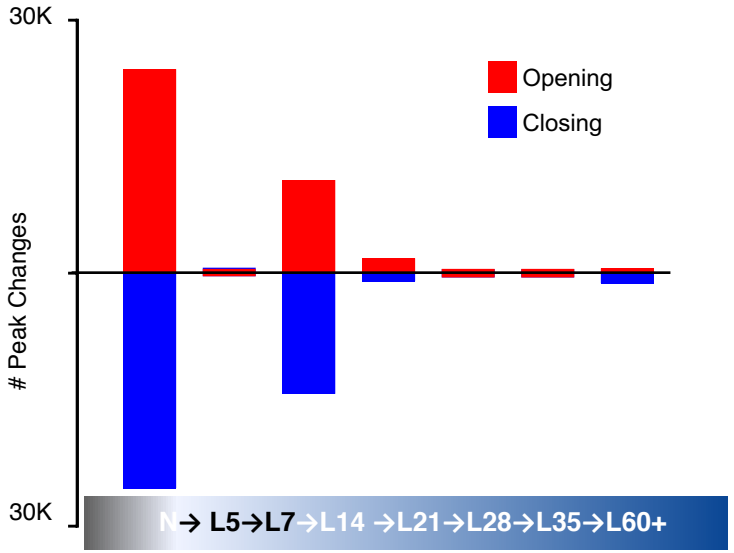
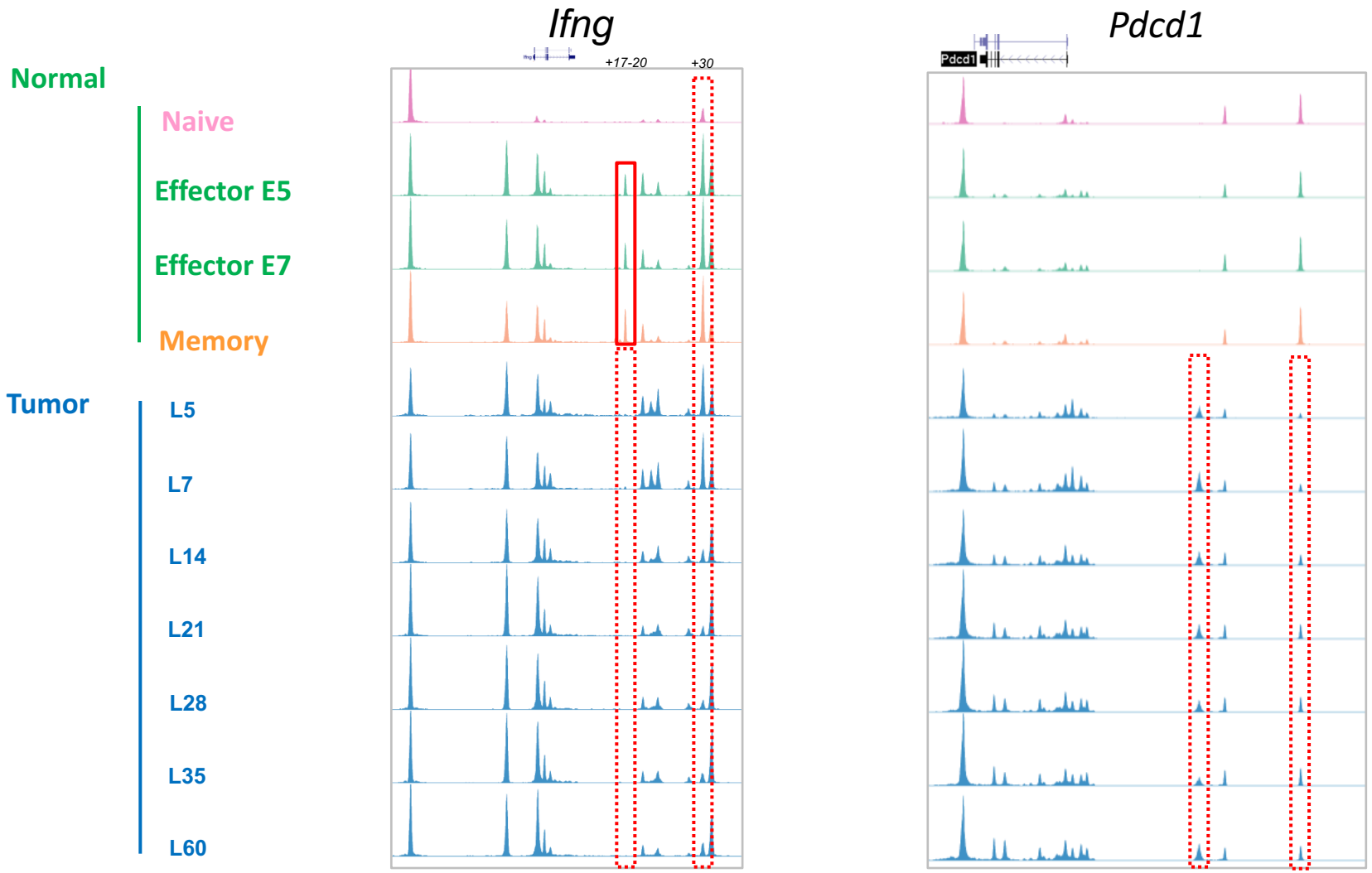


Plastic – amenable to therapeutic reprogramming

Fixed – resistant to therapeutic reprogramming

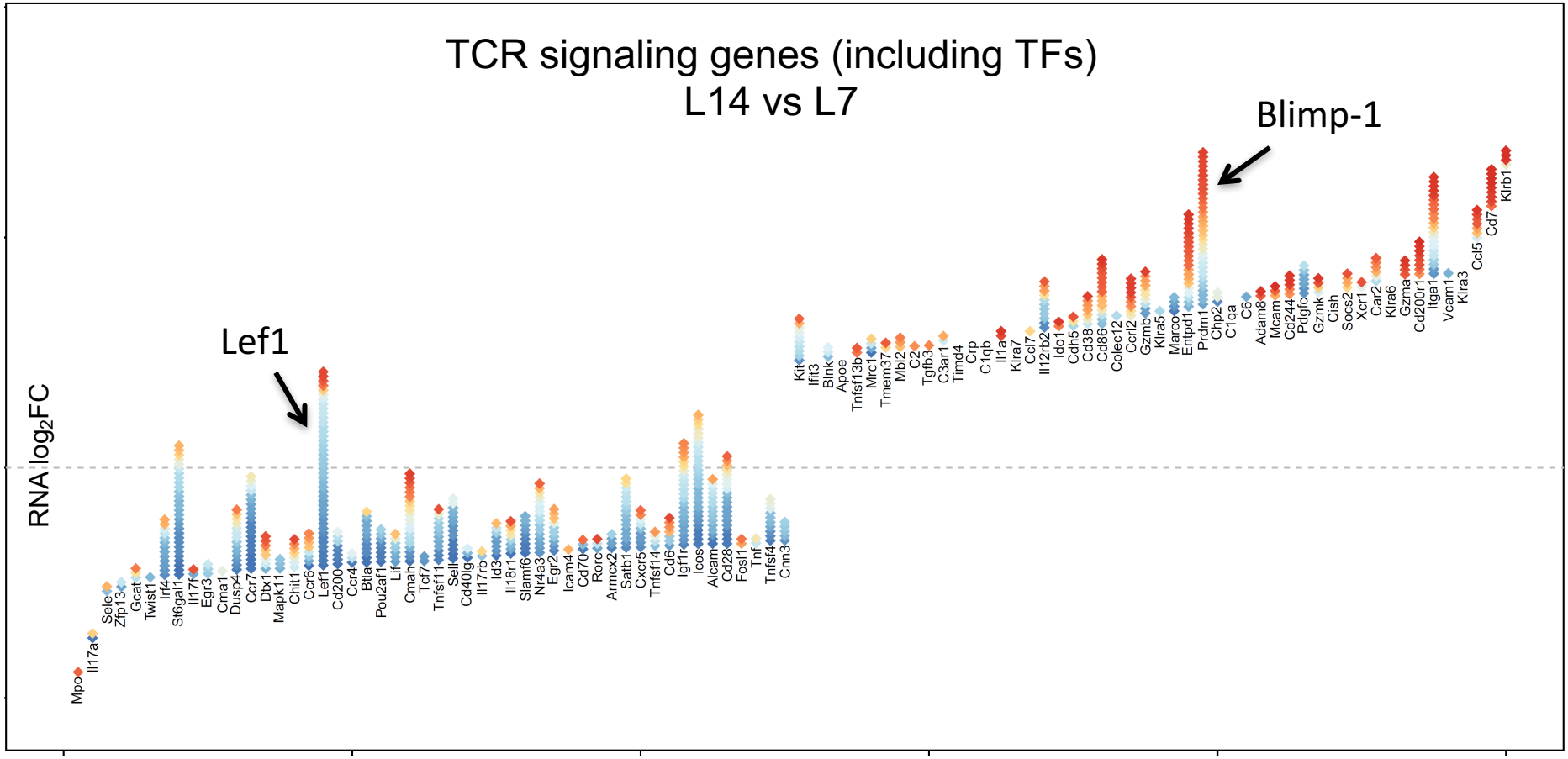


ATAC-seq peaks associated to distinct differentiation states



Large gene expression changes accompanied by gain/loss of peaks

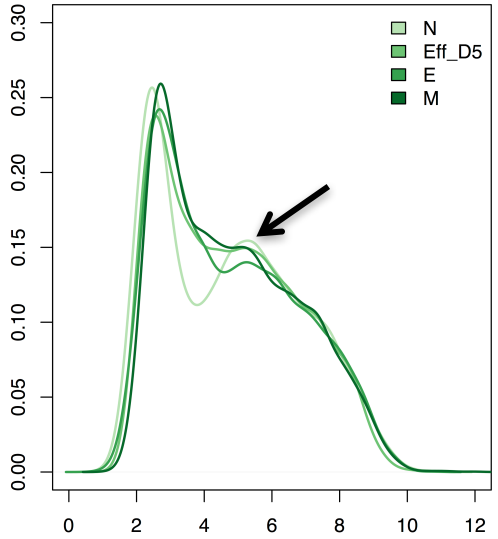
- Red = increased accessibility; blue = decreased accessibility



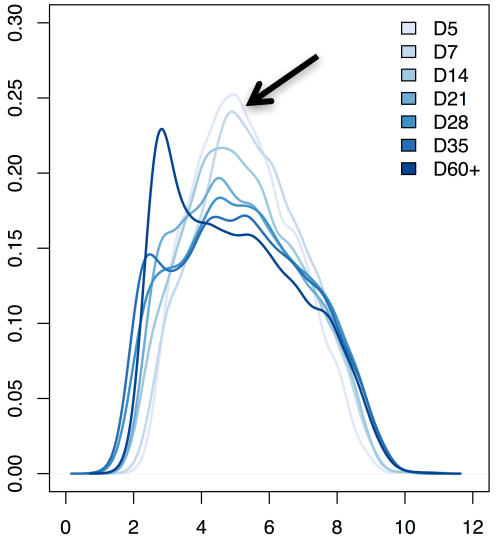
Linking TFs to global accessibility changes in dysfunctional T cells

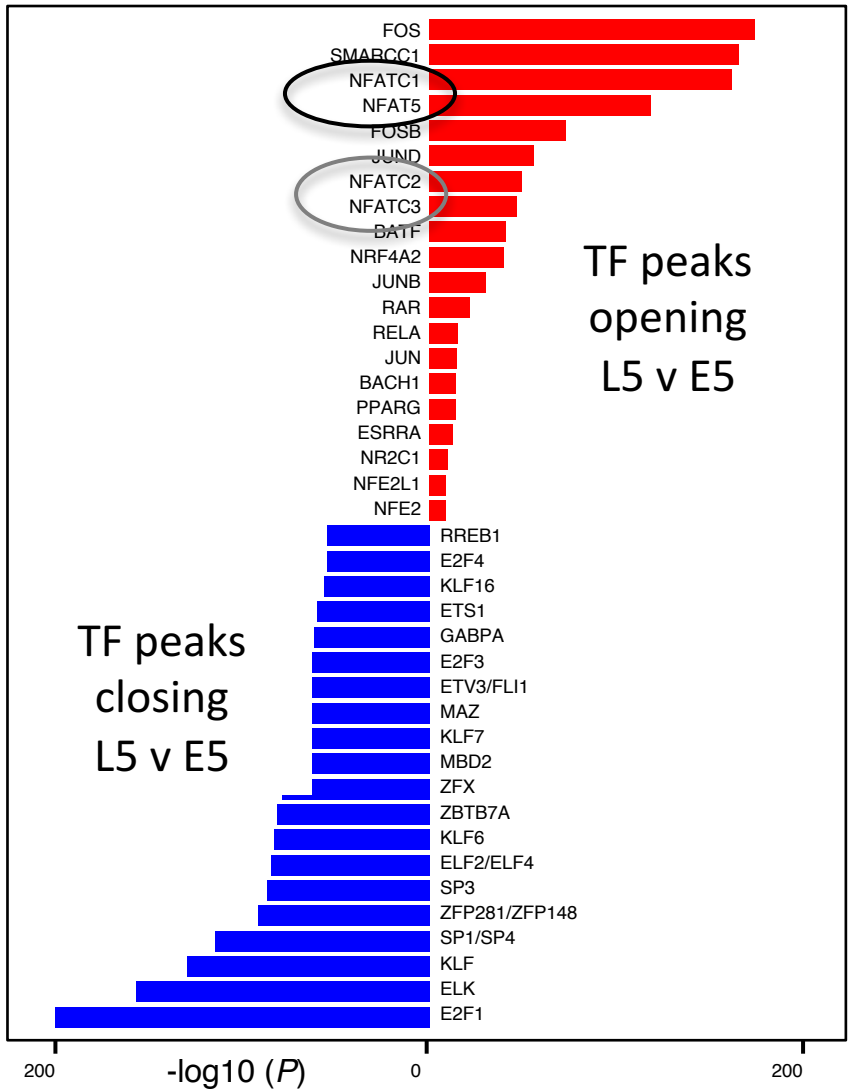
Nfatc1 peaks

Normal

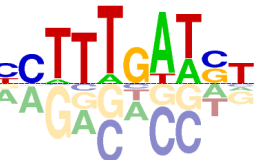


Tumor



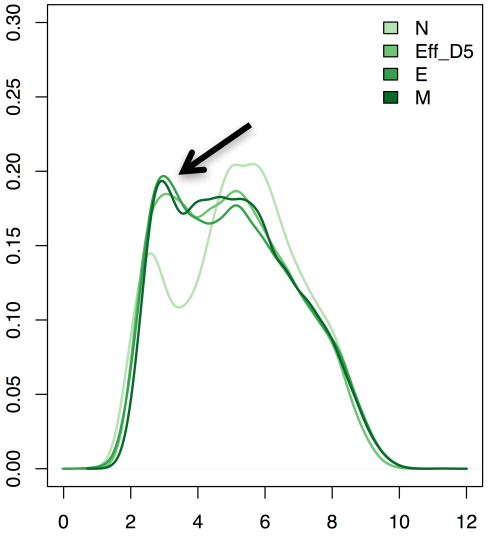


Linking TFs to global accessibility changes in dysfunctional T cells

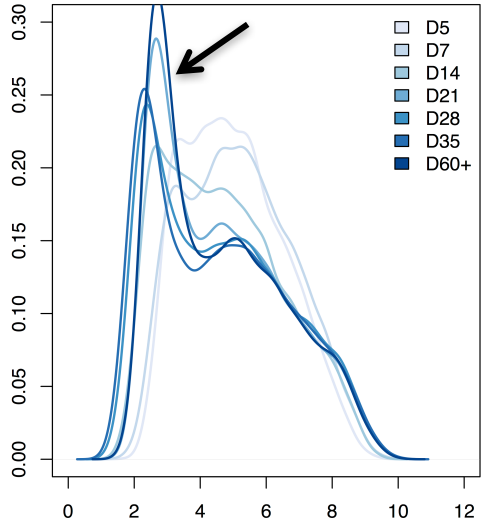


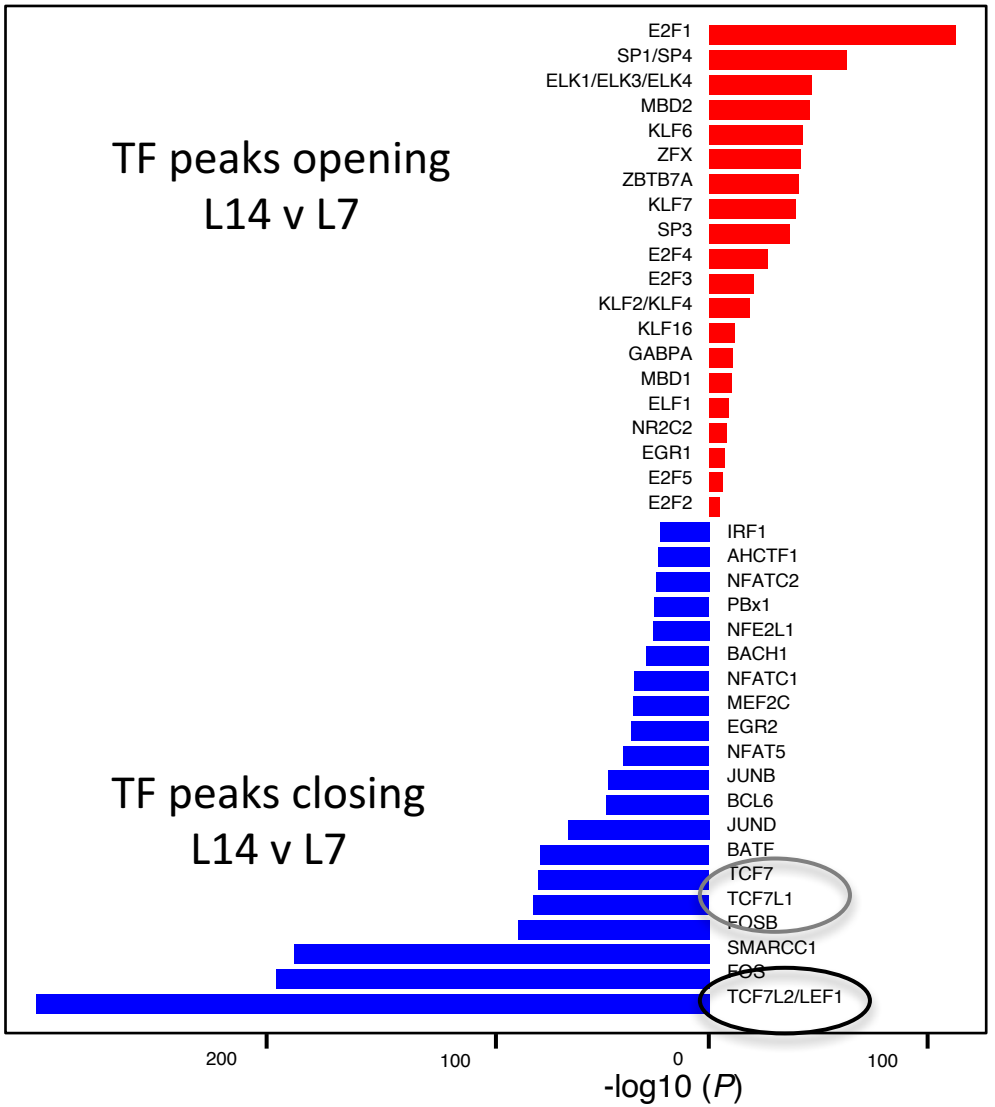
Lef1 peaks

Normal



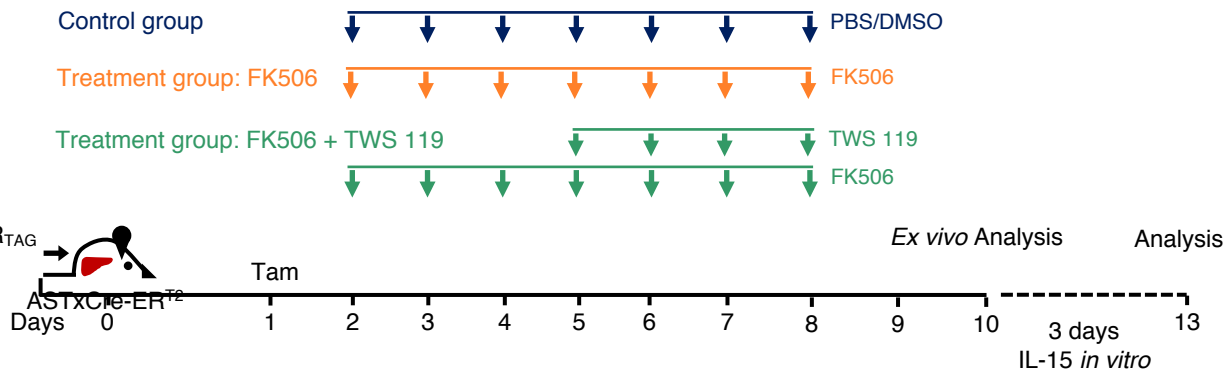
Tumor

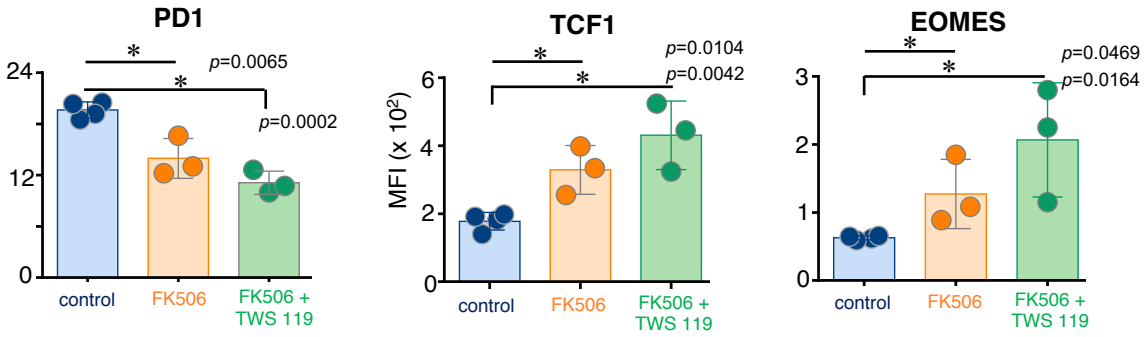


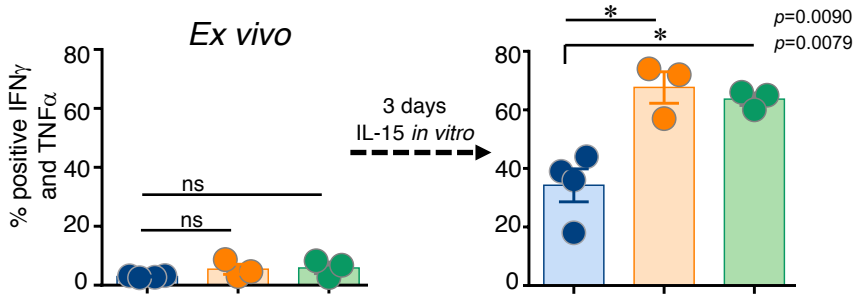


In vivo pharmacological modulation of TFs delays/decreases dysfunction

- Used calcineurin inhibitor FK506 to inhibit nuclear translocation of NFAT
- Also tried combination with GSK3β inhibitor TWS119 to activate Wnt/β-catenin signaling and increase TCF1 activity
- Improved ability to reprogram with IL15 after TF modulation

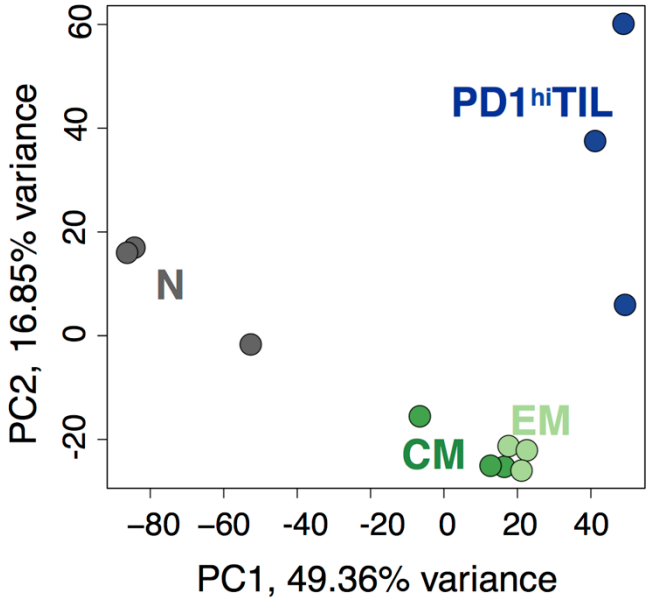


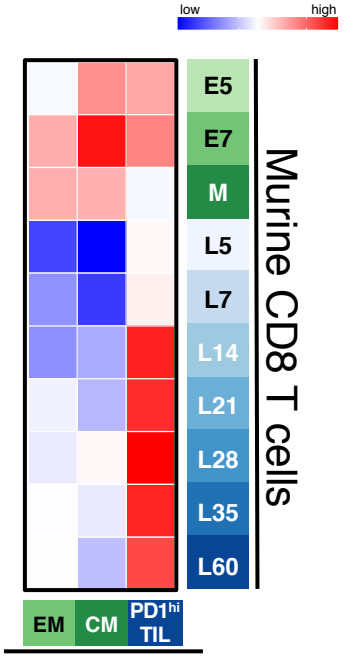


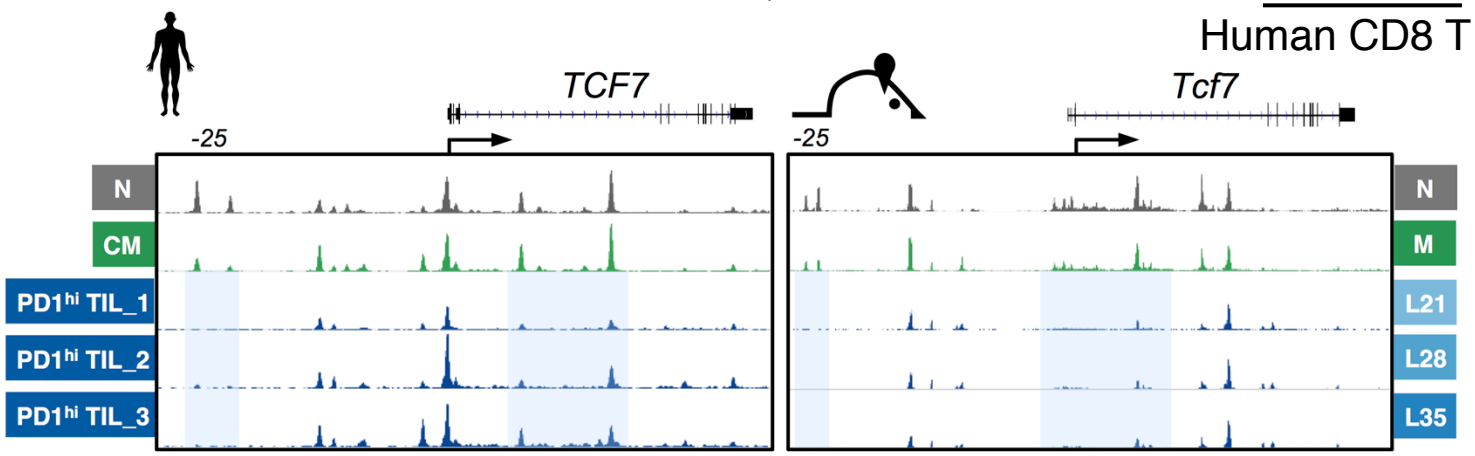


Fixed dysfunction signature recovered in T cells in human tumors

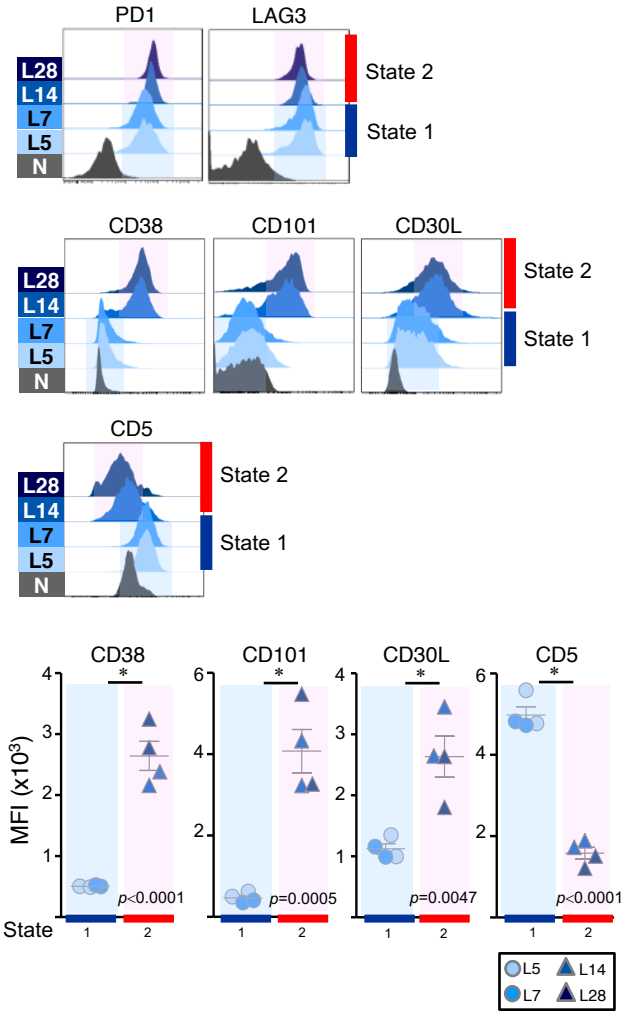
- ATAC-seq from healthy human donors, PD1 high CD8 T cells from melanoma, NSCLC patients
- Genomic liftover to compare accessibility signatures (relative to naïve T cells)



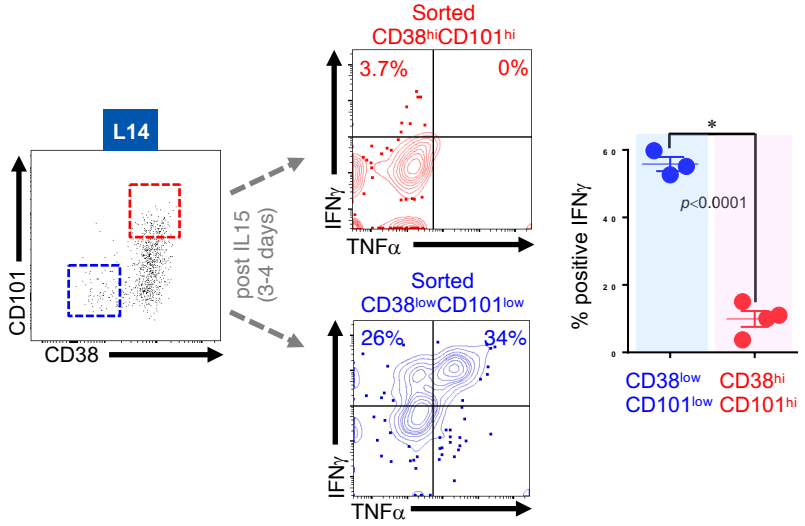




Cell surface markers identify T cells amenable to reprogramming

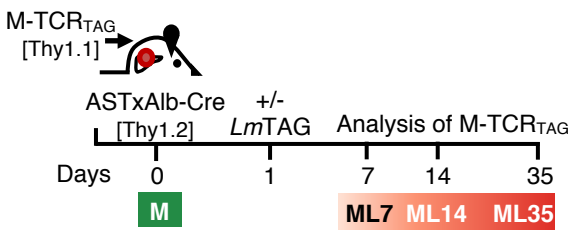


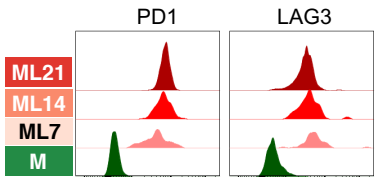
- Tumor-specific T cells is plastic and fixed state are both PD1^{hi}, but other markers can discriminate between them
- CD38^{hi}/CD101^{hi} population from L14 cannot be therapeutically programmed, while CD38^{low}/CD101^{low} are rescued

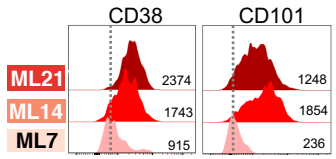


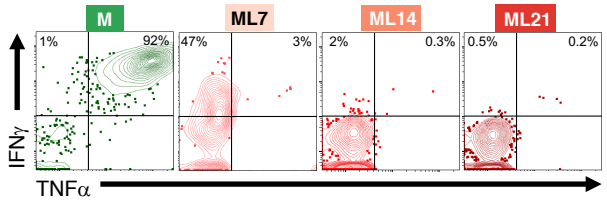
Neoantigen-specific memory cells also differentiate to dysfunction in tumor

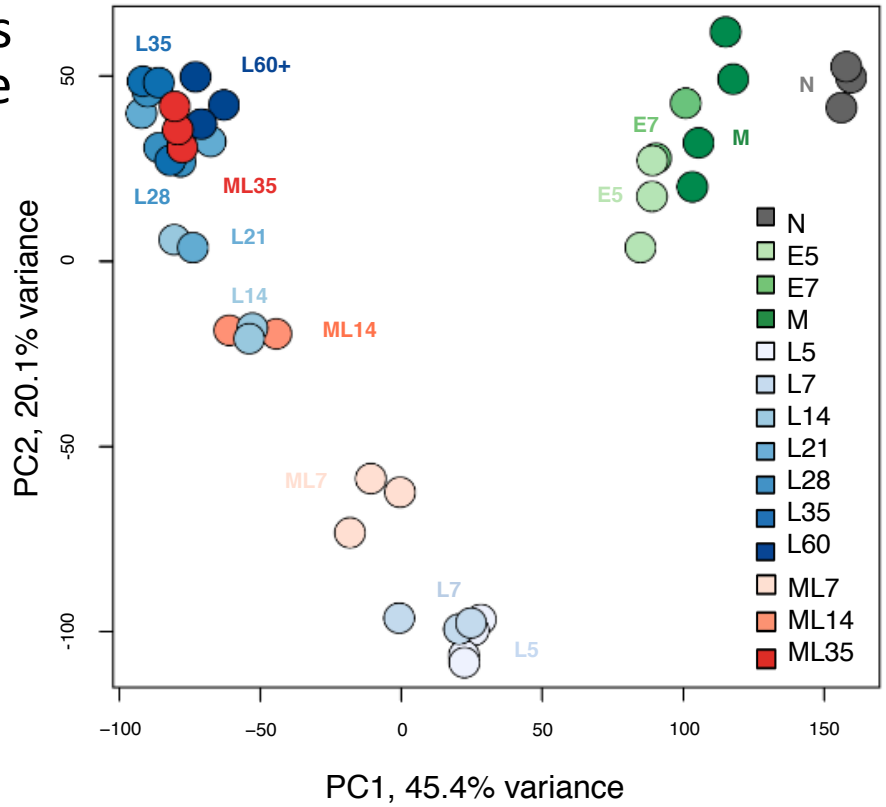
- Memory tumor-specific T cells transferred into established HCCs underwent same chromatin state changes









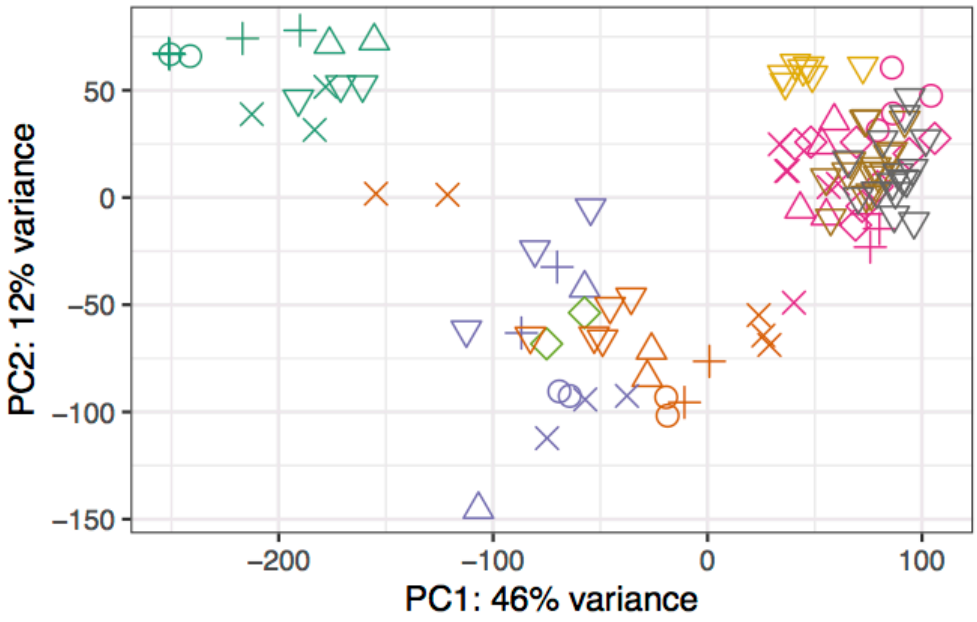


Key points from dysfunctional T cell analysis

- Epigenetic states of tumor-specific T cells defined by chromatin accessibility (ATAC-seq) coincide with plastic and fixed functional states, i.e. amenability to therapeutic reprogramming
- TF binding site accessibility across the ATAC-seq atlas identifies potential TF drivers of dysfunction
- *In vivo* pharmacological modulation of TFs identified in the analysis delays/decreases dysfunction
- Patient-derived $PD1^{hi}$ CD8 T cells display an epigenetic signature of fixed dysfunction
- Cell surface markers can discriminate between plastic and fixed states, potentially relevant in the clinic for identifying patients more likely to respond to immunotherapy
- Memory T cells specific to neoantigen also differentiate to fixed dysfunction

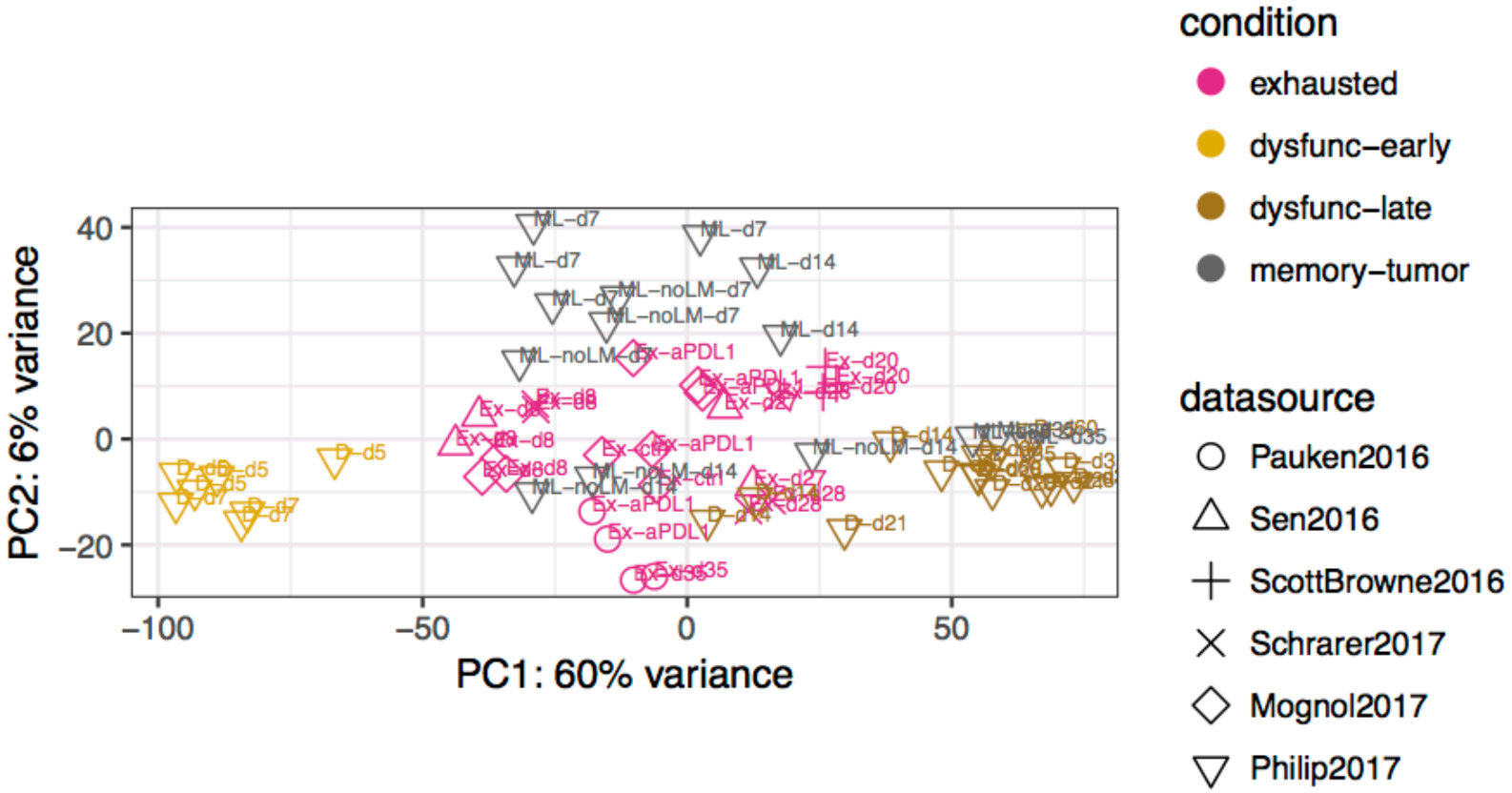
Pan-dysfunction analysis

- Pull down ATAC-seq from published T cell exhaustion and tumor-specific dysfunction studies to compare dysfunctional states
- GLM normalization to correct for batch effects



Pritykin, Fairchild et al., in preparation

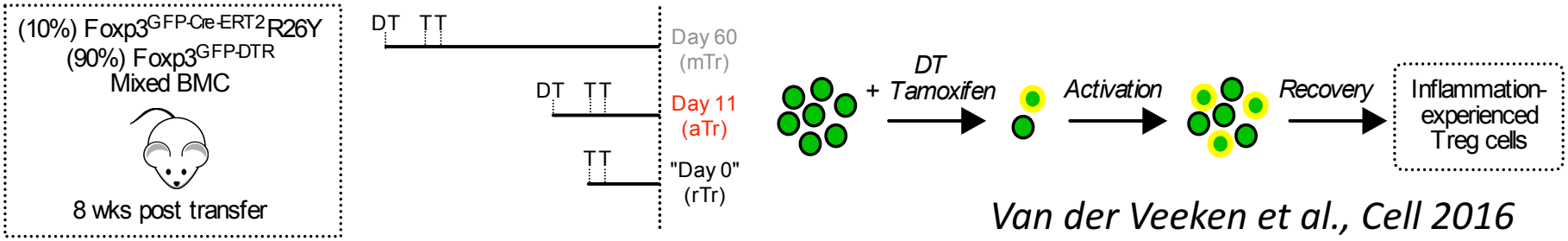
T cell exhaustion vs. dysfunction



- Is there an “axis of dysfunction” that we can quantitatively characterize by chromatin accessibility and link to expression programs?

Recent/ongoing epigenomics projects

- Memory of inflammation in Treg cells; genetics of T cell response using hybrid mice (with A. Rudensky lab)



Van der Veeken et al., Cell 2016

- Dysfunctional neoantigen-specific CD8 T cells in early malignancies; epigenomics of self-tolerance (with A. Schietinger lab)

Philip et al., Nature 2017

Pritykin, Philip et al., in preparation

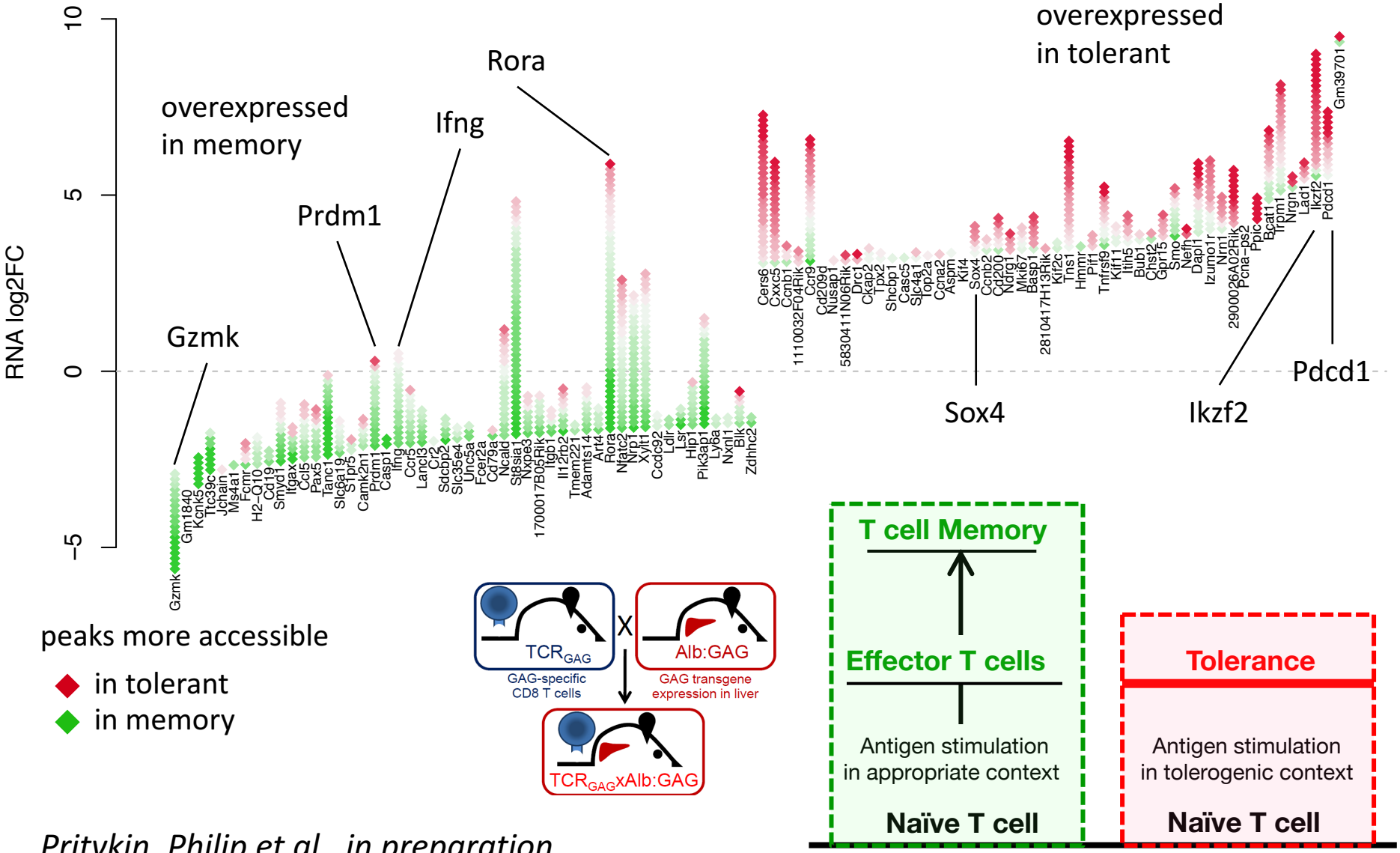
- Enhancer landscape in innate lymphoid cells and innate-like T cells (with M. Li lab)

Dadi et al., Cell 2016

- Role of ILC1s in viral infection; epigenomics of NK response (with J. Sun lab)

Weizman et al., Cell, in press

Next: epigenetics of self-tolerance



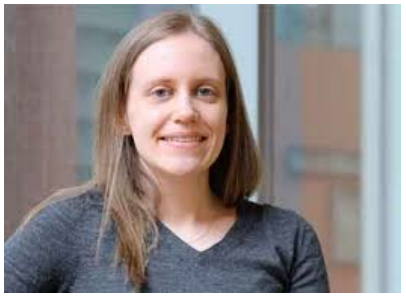
Pritykin, Philip et al., in preparation

Acknowledgements

- Leslie lab:
  - **Lauren Fairchild**
  - **Yuri Pritykin**
  - Hatice Osmanbeyoglu
  - Lee Zamparo
  - Yi Zhong
  - Meghana Kshirsagar
  - Yuheng Lu
  - Hyunwoo Cho
  - Han Yuan
  - Alex Perez
  - Sagar Chhangawala
  - Merve Sahin
  - Chirag Krishna

collaborators:

- Andrea Schietinger
- Mary Philip



NATIONAL INSTITUTES OF HEALTH

ENCODE

STARR CANCER CONSORTIUM

- Dysfunctional T cell

Epigenetic memory in T lymphocytes

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