Transcriptional and epigenetic mechanisms in cancer and cancer immunotherapy

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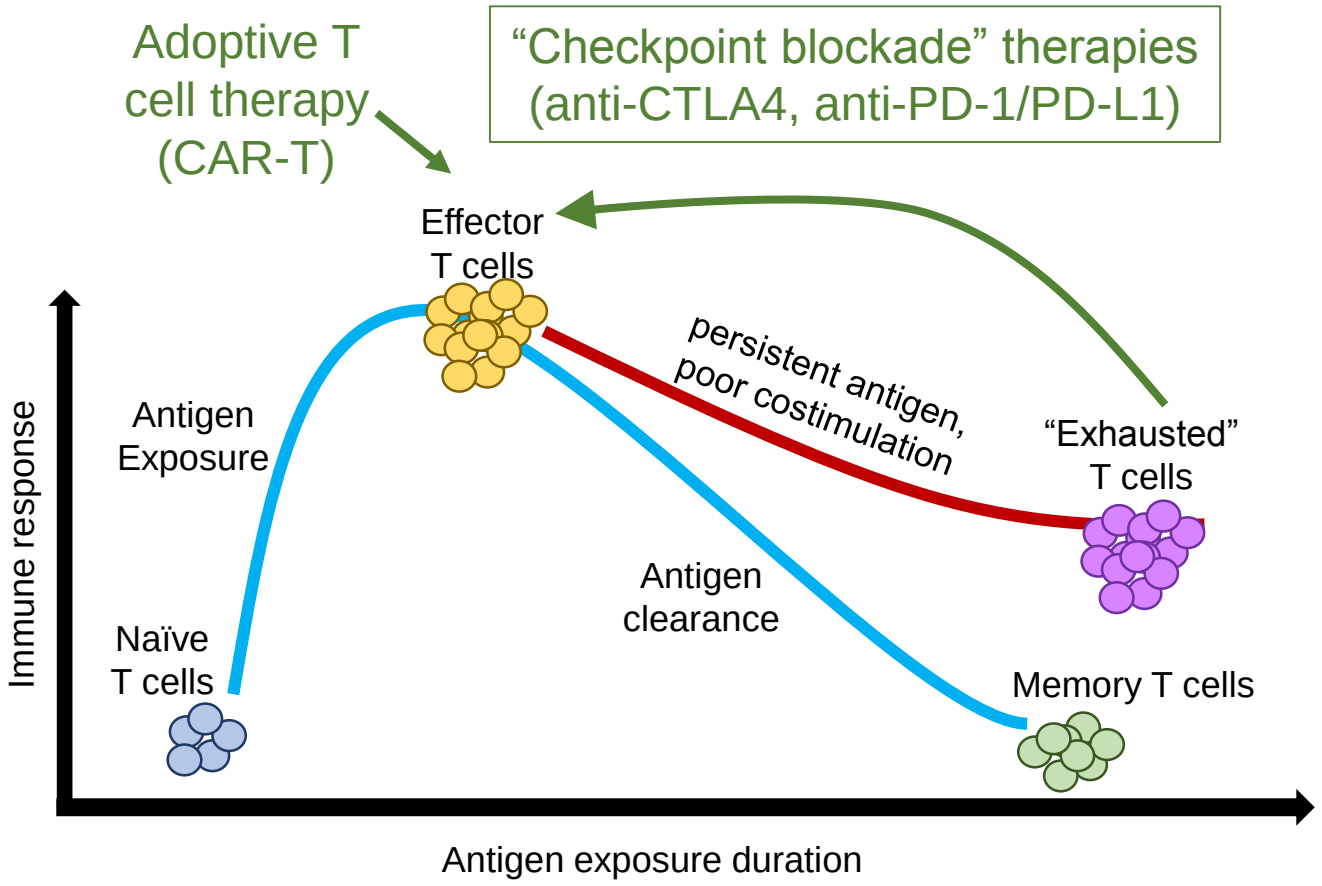
Transcriptional states of CD8+ T cells during immune responses



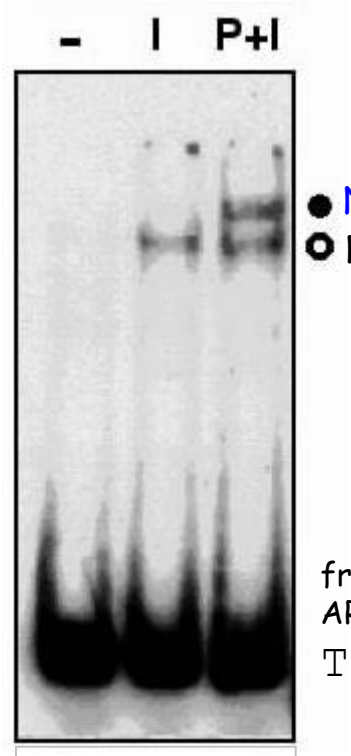
Hallmarks of "exhausted" (hyporesponsive) T cells:

- Expression of inhibitory surface receptors (PD-1, LAG3, TIM3, CTLA-4 etc)
- Decreased cytokine production (IL-2, TNF, IFNγ)
- Altered expression and use of key transcription factors

What transcription factors contribute to T cell "exhaustion"?

The cooperative NFAT:AP-1 complex

T cell nuclear extracts



● NFAT:Fos:Jun:DNA (1 nM Kd)
○ NFAT:DNA (20 nM Kd)

free DNA probe
ARRE site of mouse IL-2 promoter
TGGAAAAACTGTTTCA

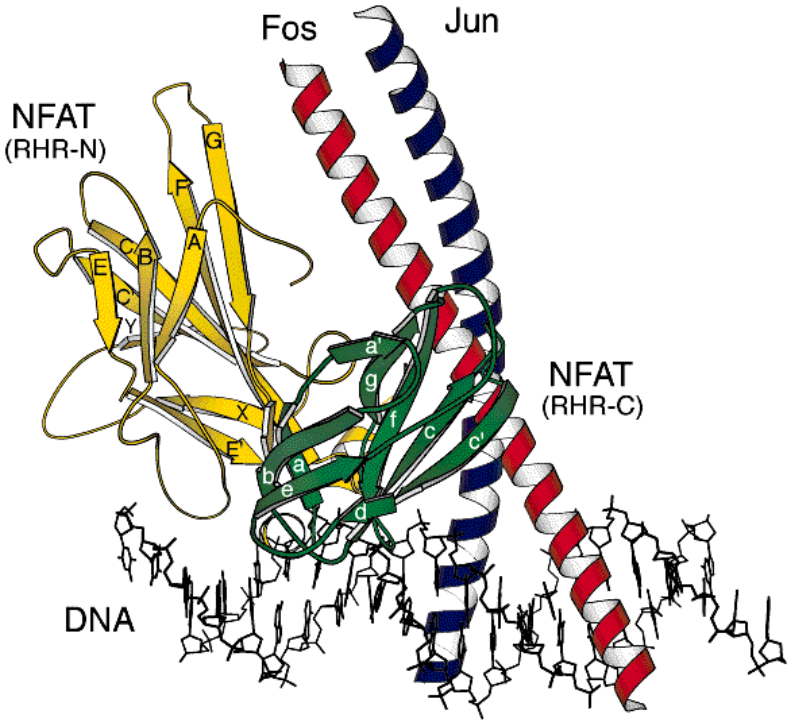
Jugnu Jain
Pat McCaffrey

Patrick Hogan

Jain et al., *Nature* 1993; McCaffrey et al., *Science* 1993
Rao, Luo and Hogan, *Annu Rev Immunol* 1997; Hogan et al., G&D 2003

Steve Harrison
Lin Chen

Patrick Hogan



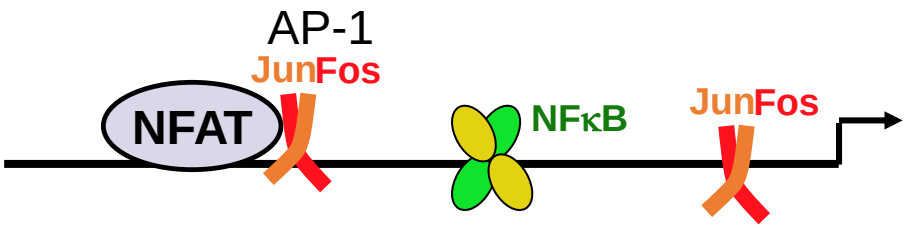
GGAAAAACTGTTTCA

L Chen et al., *Nature* 1998

Does NFAT have different biological functions with and without AP-1?

microarrays

PMA + ionomycin (surrogate for TCR + costimulation)



Genes characteristic of T cell activation (cytokines, chemokines Cell-surface receptors)

~1100 activation-associated genes: IL2, IL3, IL4, IL5, IFNγ, GMCSF, CD40L, etc

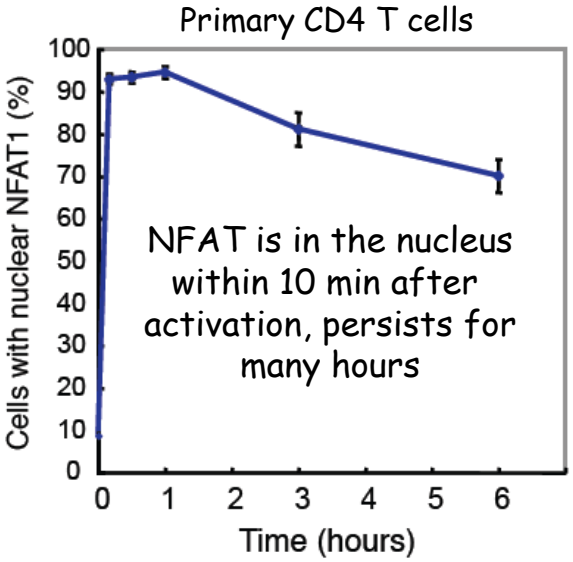
ionomycin alone (surrogate for TCR without costimulation)

Genes involved in negative signalling (T cell anergy, tolerance)

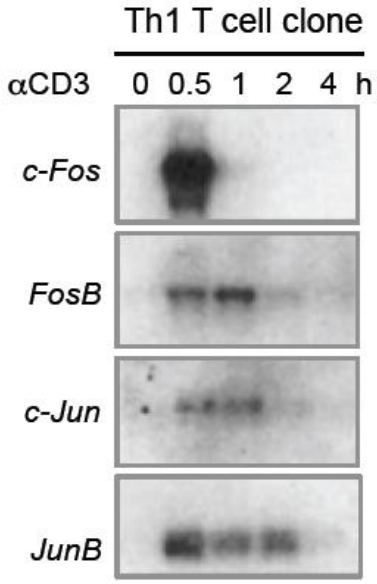
~150 anergy-associated genes (negative regulators): inhibitory cell-surface receptors
tyrosine phosphatases, diacylglycerol kinase, E3 ubiquitin ligases: Itch, Cbl-b, Grail
transcriptional repressors: Ikaros, Jumonji
LAT palmitoyl transferase

Macián et al., *Cell* 2002; Heissmeyer et al., *Nat Immunol* 2004; Koretzy; Fathman; Macián; Altman

Time course of NFAT and AP-1 (Fos-Jun) activation after stimulation

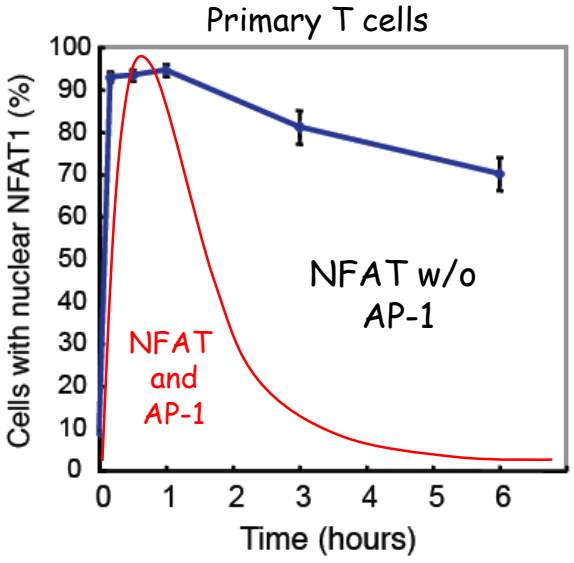


Oh-hora et al., *Nature Immuno*/2008



AP-1 components (Fos and Jun family members) are transiently activated

Jain et al., *J Immuno*/1993



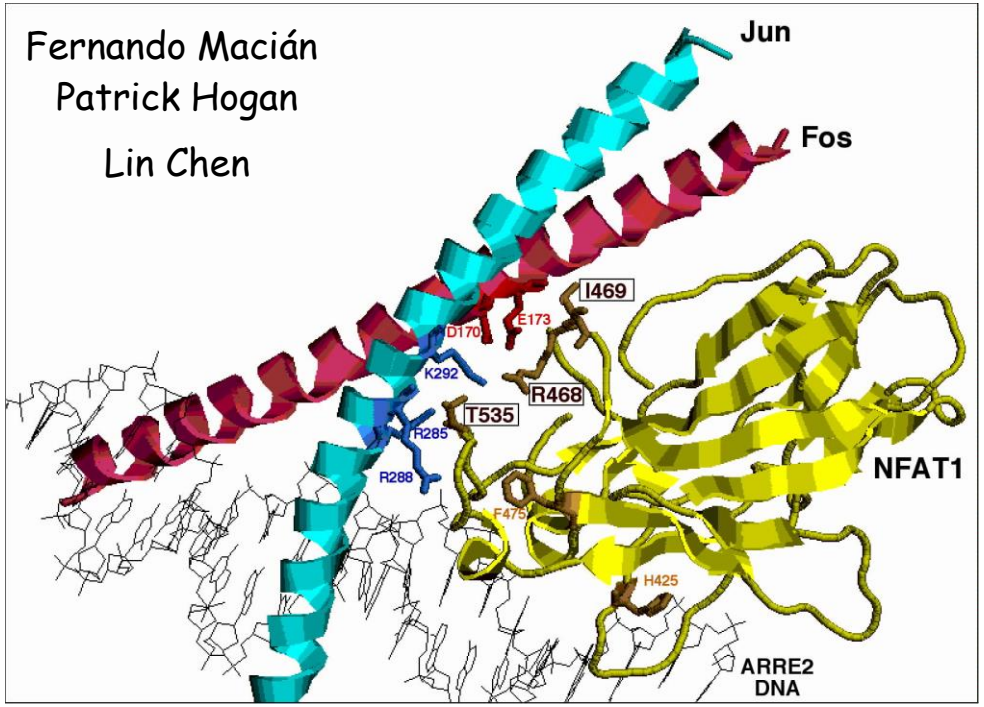
A negative feedback mechanism is turned on at the later stages of T cell activation by NFAT without AP-1, and initiates the transcriptional program of T cell hyporesponsiveness ("tolerance/ anergy/ exhaustion/ dysfunction")

This represents the end stage of a physiological (esp. chronic) immune response: hyporesponsiveness to further stimulation through the TCR (minimal Ca influx, diminished cytokine production)

Later reinforced by epigenetic mechanisms that stabilise the "exhausted" state

NFAT without AP-1 → decreased IL-2 production, upregulation of inhibitory receptors

Fernando Macián



Gustavo Martinez
Renata Pereira

CA-RIT-NFAT1 (R>A, I>A, T>G) = constitutively active NFAT1 that cannot cooperate with AP-1

Retrovirally introduce CA-RIT-NFAT1 into $CD4^+$ and $CD8^+$ T cells (i.e. initiate the inhibitory program before the activation program):

- decreased cytokine production, increased expression of inhibitory receptors
- decreased tumour rejection in a mouse model in vivo
- decreased ability to combat *Listeria* infection in vivo
- resemble “exhausted” $CD8^+$ T cells by transcriptional profiling and ATAC-seq

Macián et al., EMBO J 2000; Macián et al., *Cell* 2002; Martinez et al., *Immunity* 2015

- The same transcription factor, NFAT, induces different transcriptional programs in the same cells: activation when AP-1 is present, hyporesponsiveness later when AP-1 has died away

- a constitutively active NFAT that cannot cooperate with AP-1 induces the hyporesponsive programme in transduced $CD4^+$ as well as $CD8^+$ T cells (decreased cytokine production, expression of several inhibitory surface receptors, attenuated immune responses)

Macián et al., EMBO J 2000; Macián et al., *Cell* 2002; Martinez et al., *Immunity* 2015

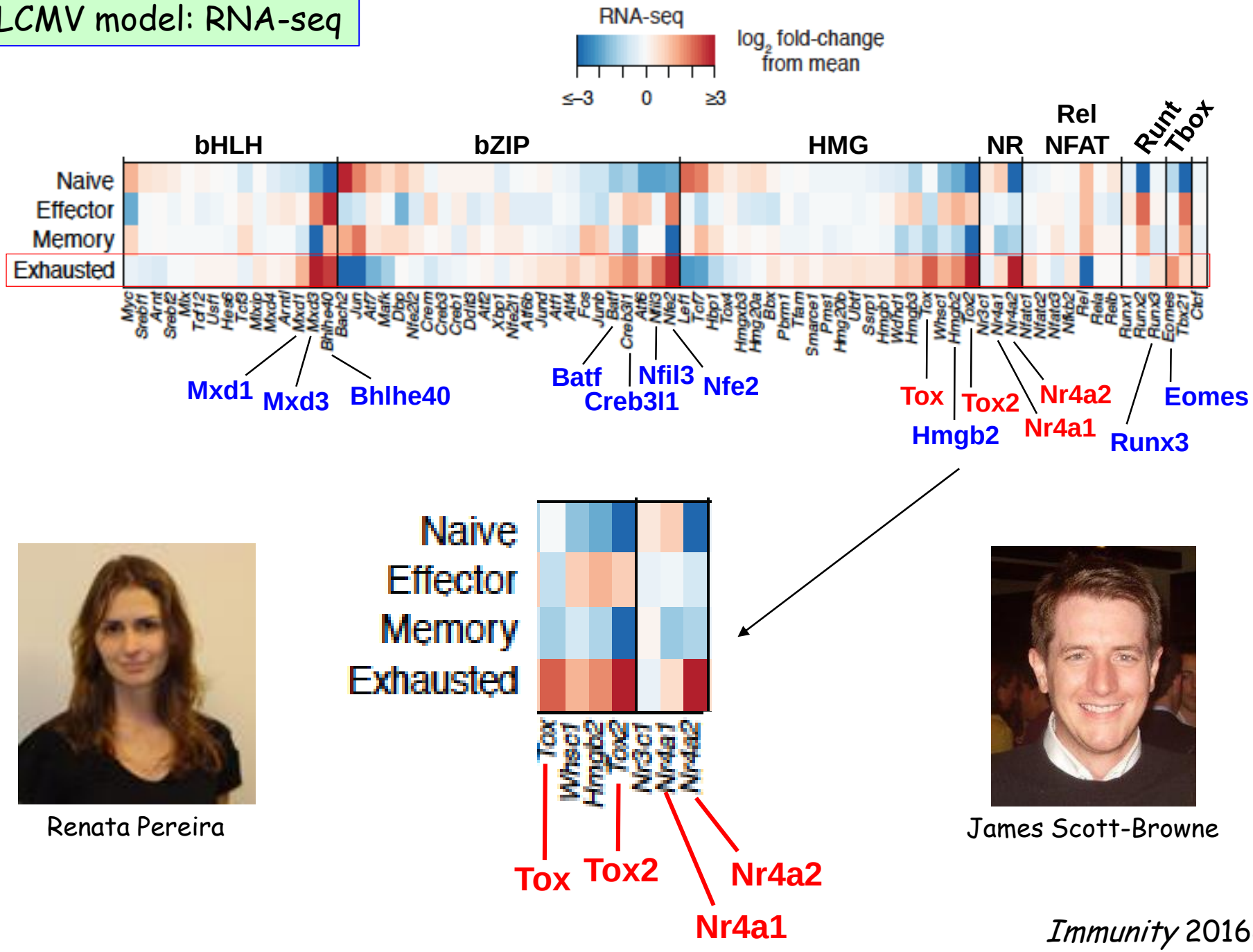
Does endogenous NFAT function in the same way?

Three model systems to study $CD8^+$ T cell exhaustion:

1. **LCMV chronic infection model**: James Scott-Browne, Renata Pereira
2. **B16-OVA and OT-I anti-tumor response model**: Sara Trifari, Giuliana Mognol
3. **CAR-T cell model to examine effects of complex gene deletions**: Joyce Chen

Scott-Browne et al., *Immunity* 2016; Mognol et al., *PNAS* 2017; Chen et al., *Nature* 2019

LCMV model: RNA-seq

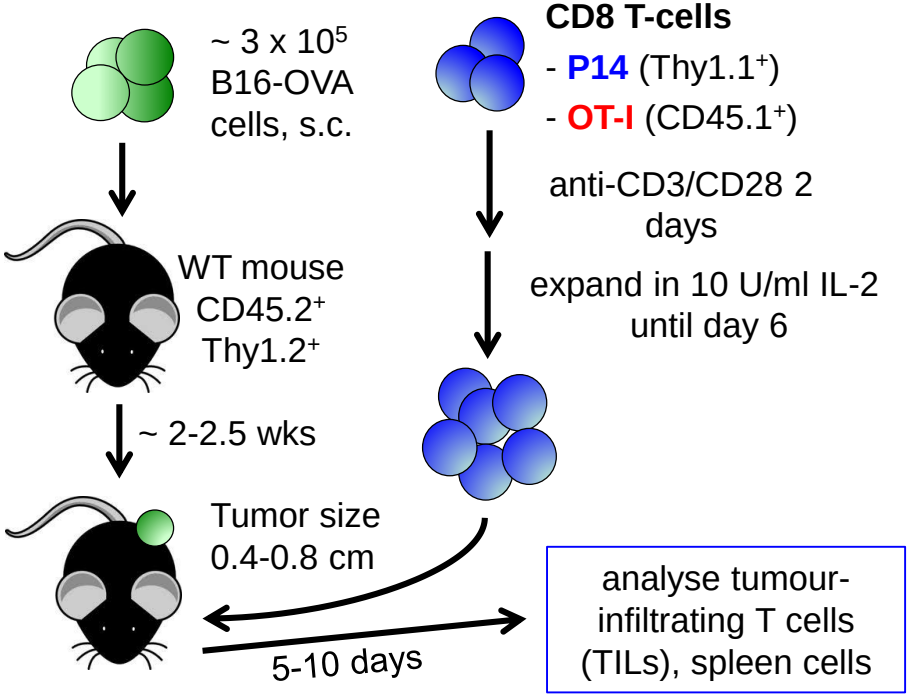


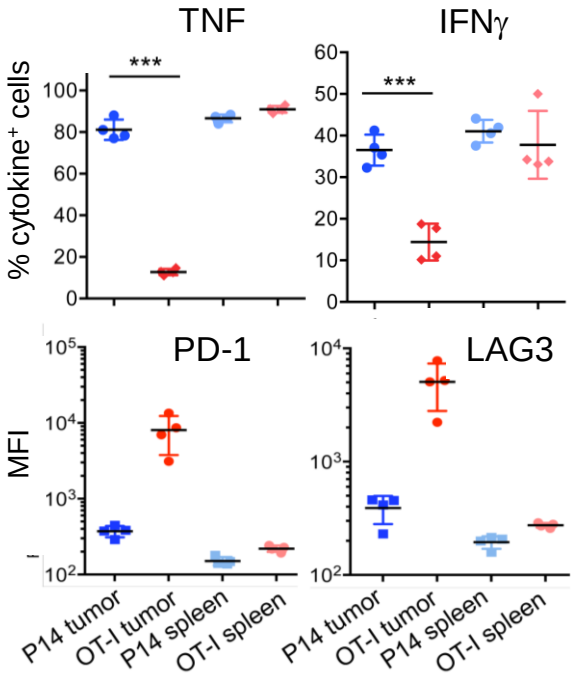
Renata Pereira

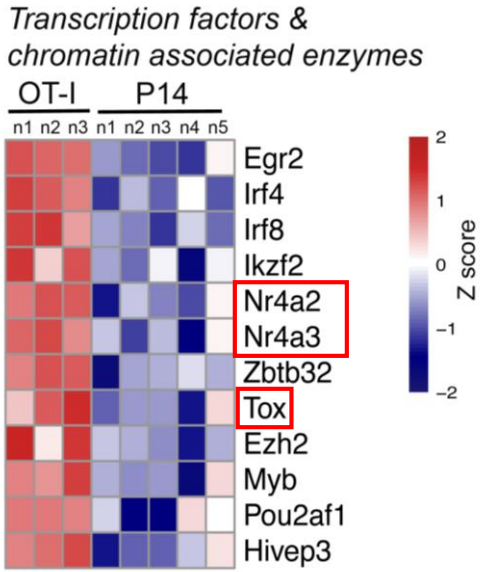
James Scott-Browne

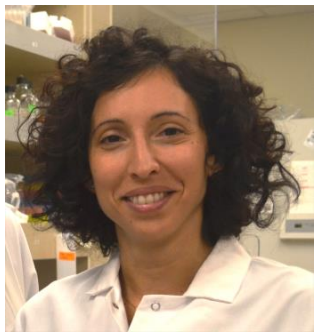
Immunity 2016c

B16-OVA model of anti-tumor response









Sara Trifari

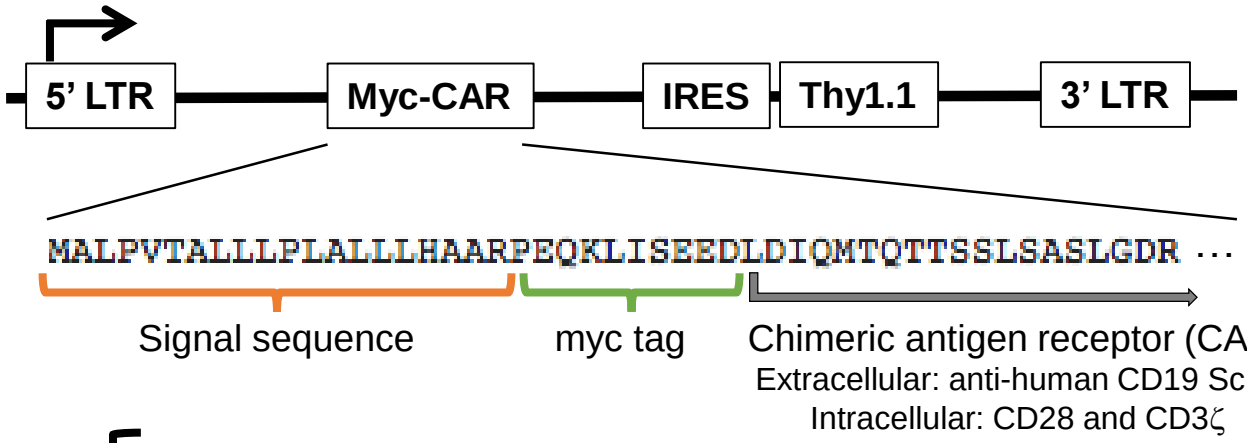
Giuliana Mognol (Patrick Hogan's lab)

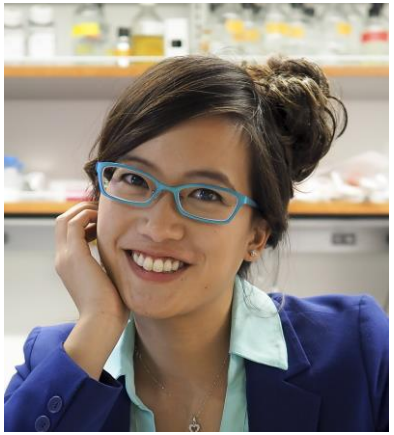
Victor Wong

Bioinformatics: Roberto Spreafico, Alex Hoffmann (UCLA)

Mognol et al., *PNAS* 2017

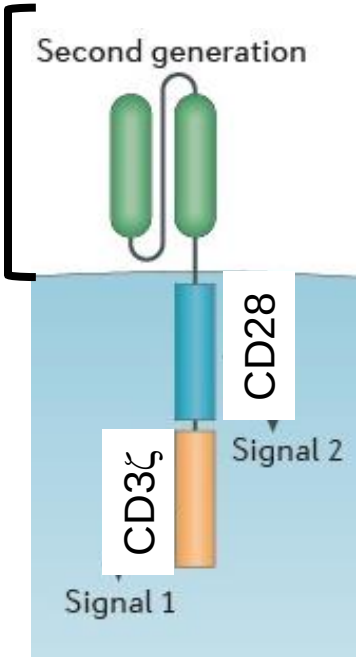
Experimental system to define the roles of candidate proteins in CD8⁺ T cell exhaustion



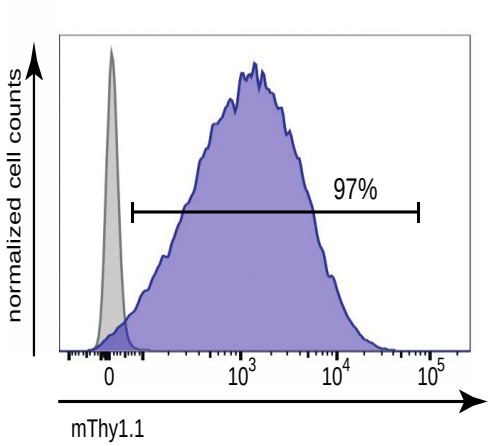


Joyce Chen

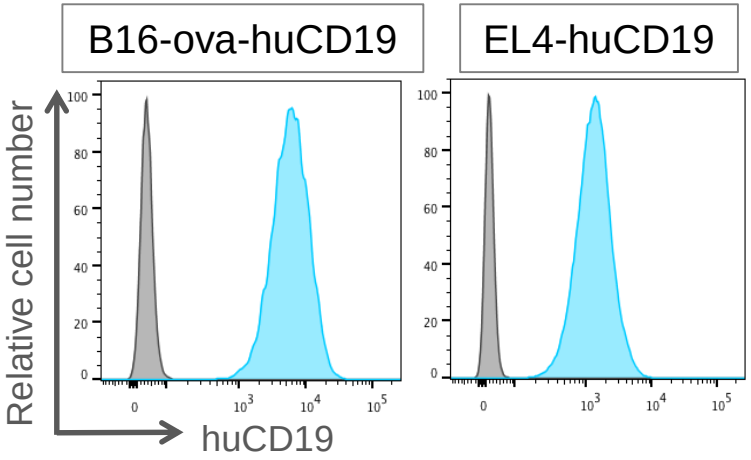
scFv against human CD19



~97% transduction efficiency of CAR-2A-Thy1.1 into CD8⁺ T cells

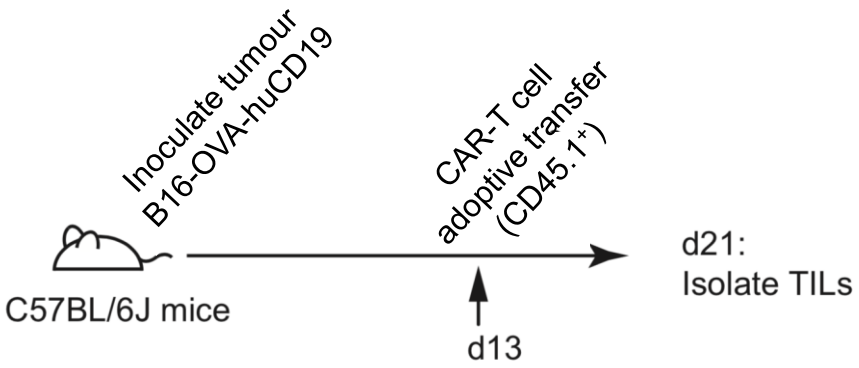


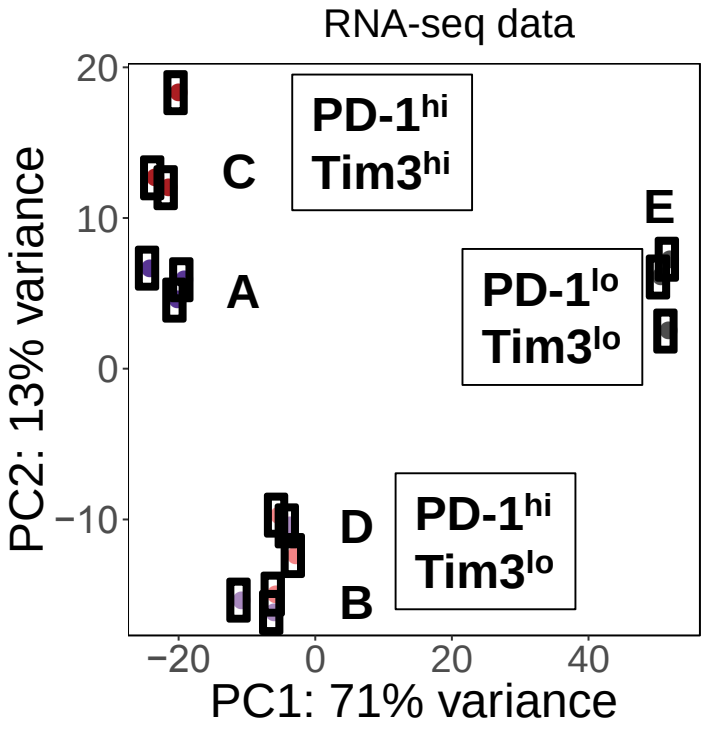
Generation of huCD19-expressing target cells

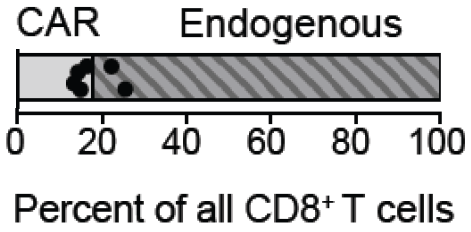


Joyce Chen, James Scott-Browne

Schematic of CAR model and isolation of tumor-infiltrating lymphocytes (TILs)

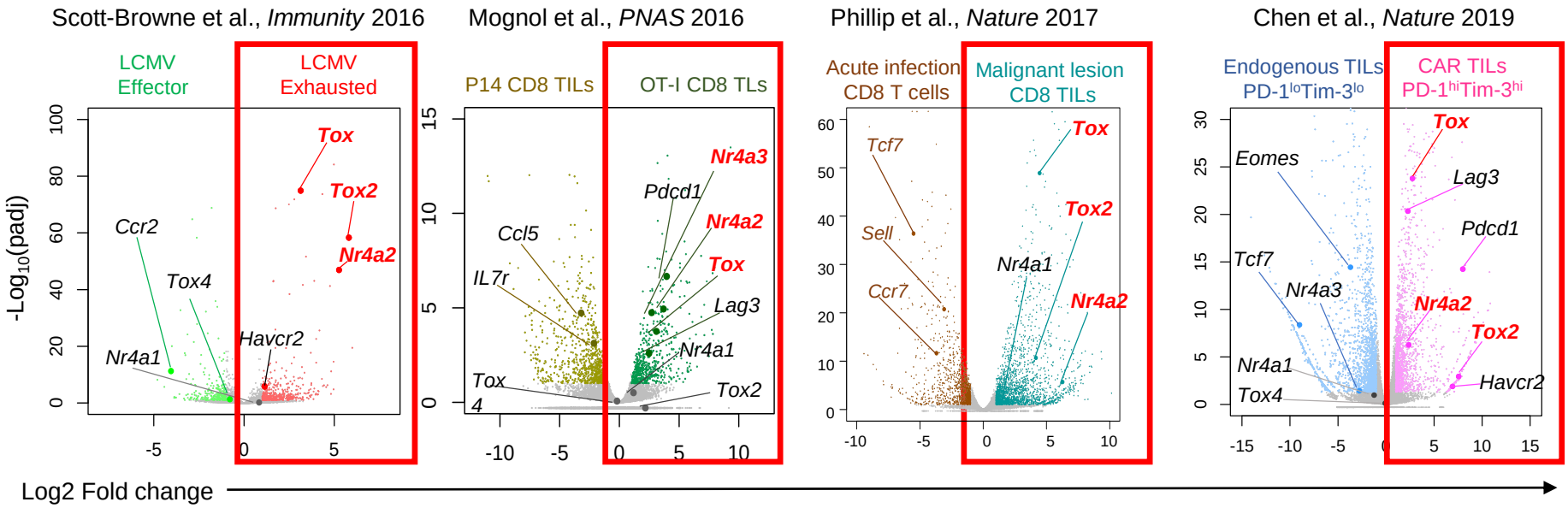




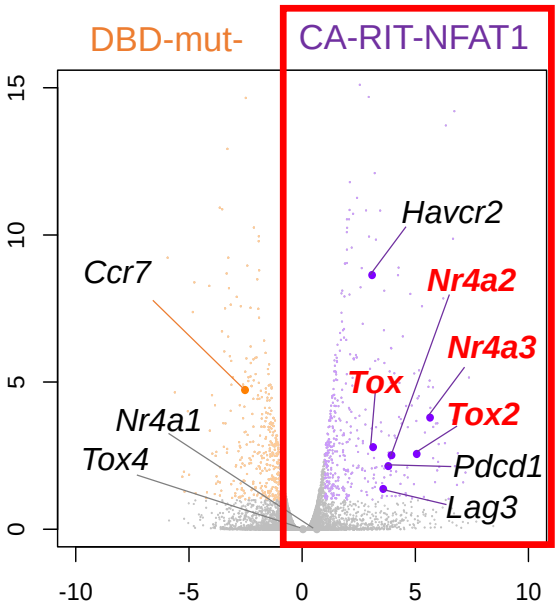


Joyce Chen

Volcano plots of RNA-seq data: differentially expressed genes



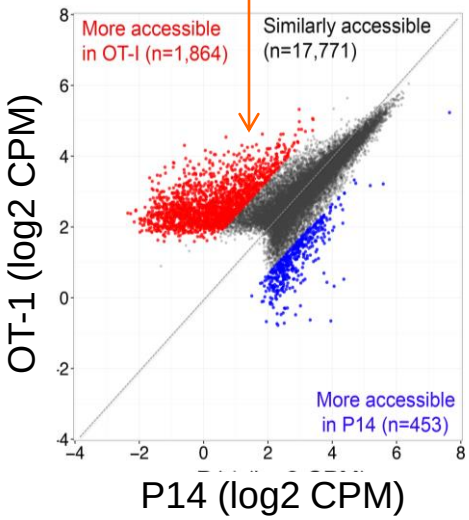
Martinez et al., *Immunity* 2015

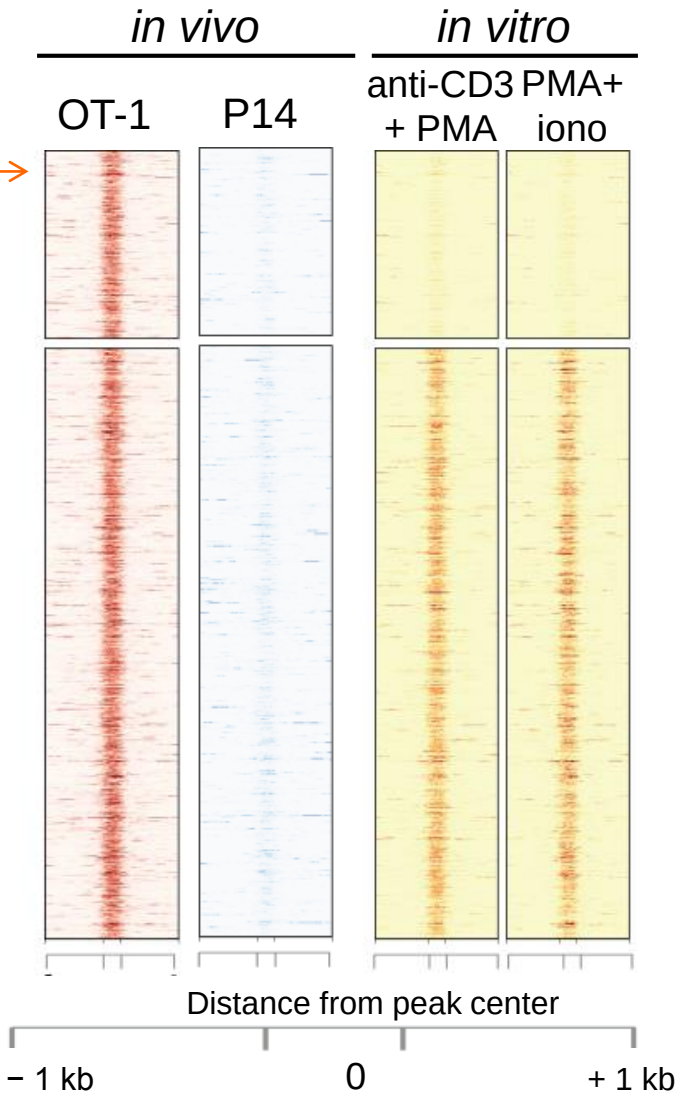


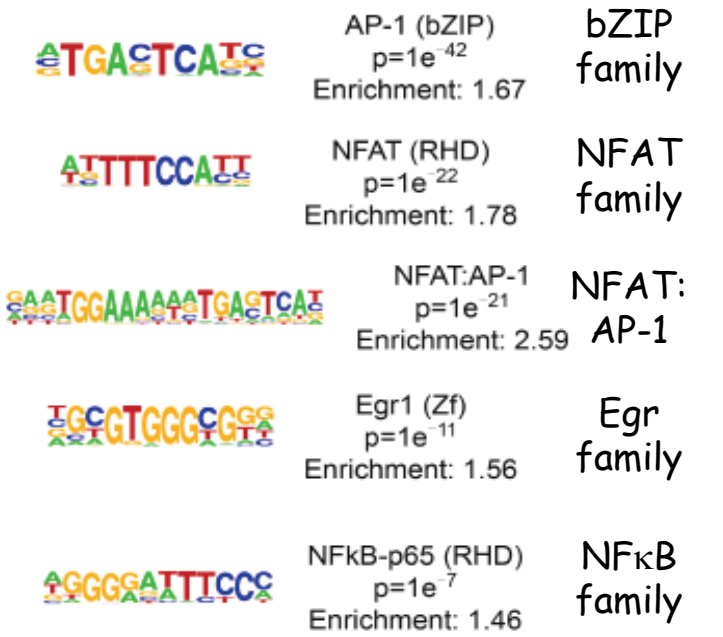
Reason to examine TOX and TOX2:
Nr4a, *Tox* and *Tox2* gene loci display "exhaustion"-specific accessible regions that contain "NFAT without AP-1" motifs

Reason to examine NR4A:
Differentially-accessible regions in each of these "exhausted" cell types are enriched for consensus binding motifs for nuclear receptors including NFAT and NR4A

ATAC-seq (B16-OVA model):
Nr4a2 binds acccessible chromatin regions specific to "exhausted" cells (B16-OVA model)





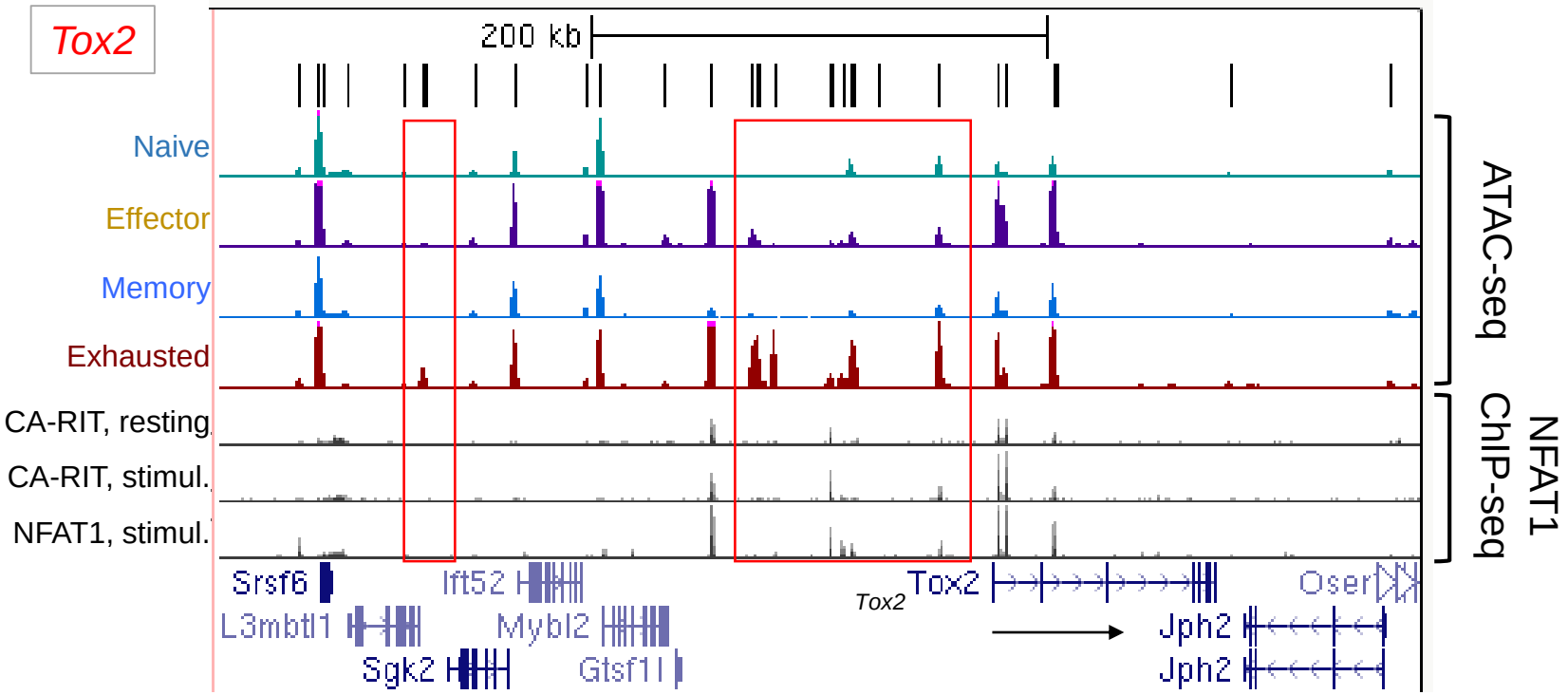


Activation-related Regions (n= 1,414)

Are the tumour-infiltrating cells a uniform population or a mixture of activated and "exhausted" cells? Single-cell analyses ….

Mognol et al., *PNAS* 2017

Exhaustion-specific accessible regions in the *Tox2* gene



NR4A: a family of orphan nuclear receptors

NR4A1 = Nur77, NGFI-β NR4A2 = Nurr1 NR4A3 = Nor1

NR4A family members exhibit functional redundancy

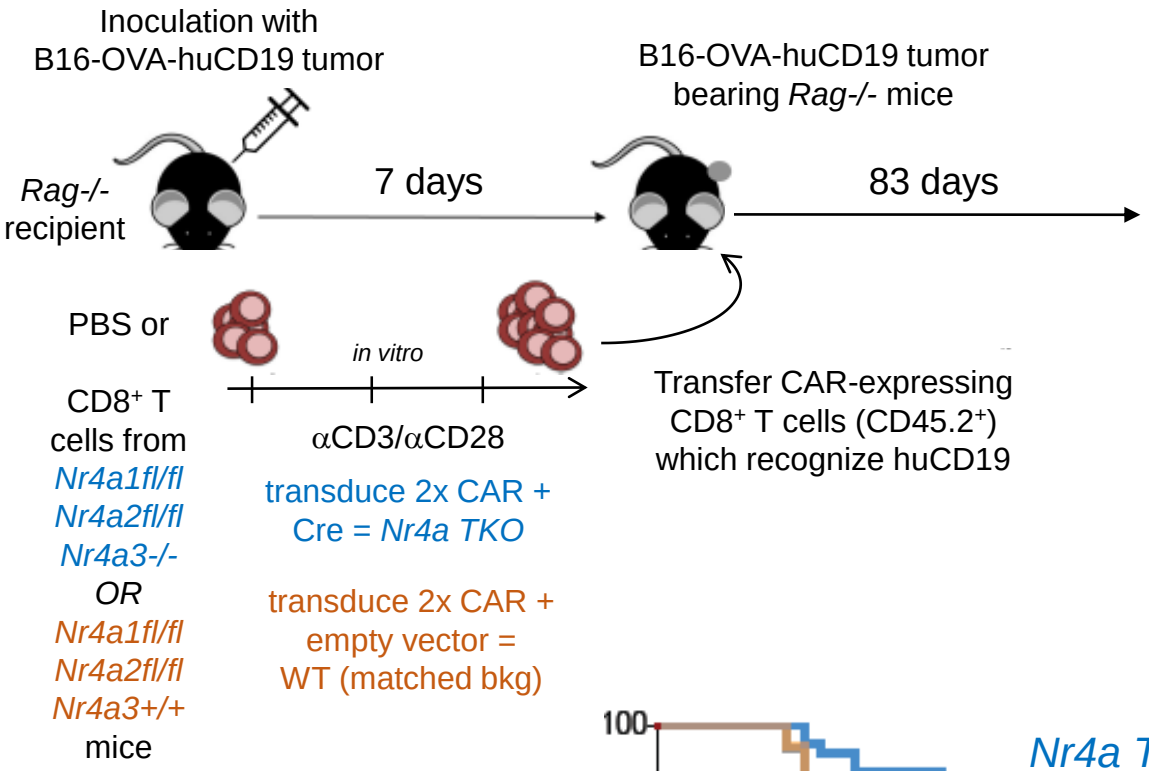
- NR4A triple knockout mice have severe defects in T regulatory cell development but mice lacking just two NR4A family members are less affected or not affected at all

Sekiya T, et al. *Nat. Commun.* 2011; Saijo K, et al. *Cell*, 2009; Sekiya T, et al. *Nat. Immunol.* 2013

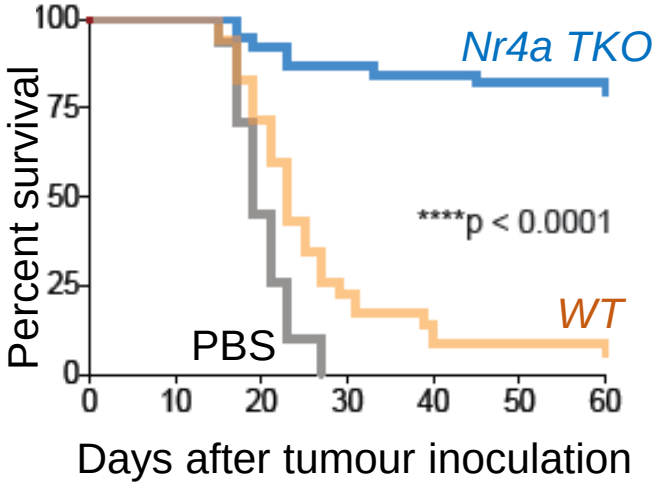
Donor	Genotype	Retrovirus 1	Retrovirus 2
PBS	n/a	none	none
WT	*Nr4a1 fl/fl Nr4a2 fl/fl Nr4a3 +/+*	CAR	empty vector
Nr4a TKO	*Nr4a1 fl/fl Nr4a2 fl/fl Nr4a3 -/-*	CAR	Cre
Nr4a1 KO	*Nr4a1 fl/fl Nr4a2 +/+ Nr4a3 +/+*	CAR	Cre
Nr4a2 KO	*Nr4a1 +/+ Nr4a2 fl/fl Nr4a3 +/+*	CAR	Cre
Nr4a3 -/-	*Nr4a1 fl/fl Nr4a2 fl/fl Nr4a3 -/-*	CAR	empty vector

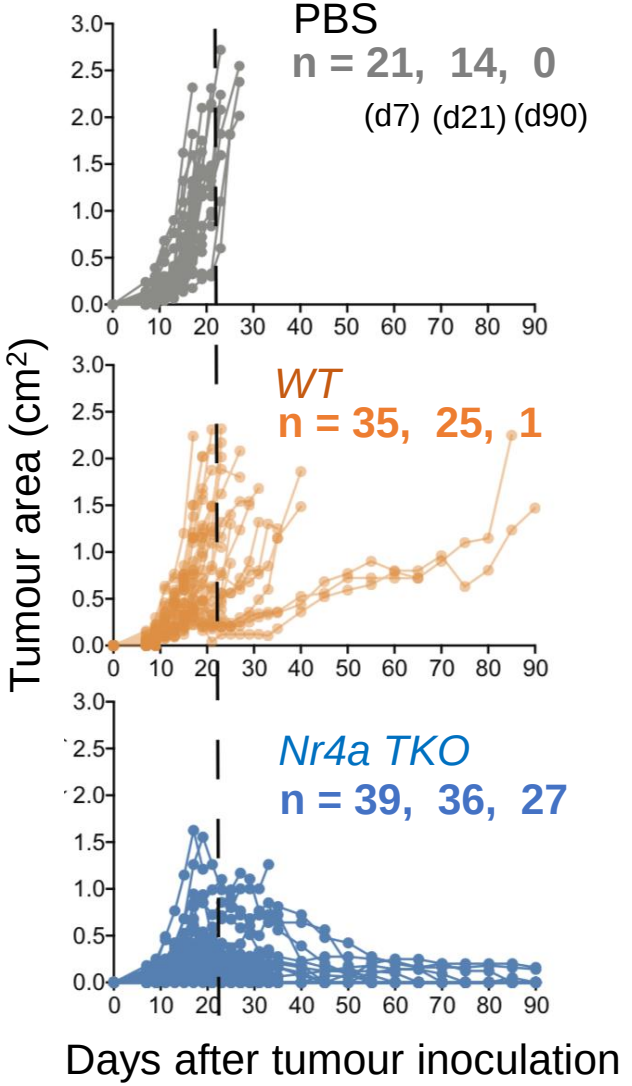
Collaboration with Dr. Takashi Sekiya and Dr. Akihiko Yoshimura, Keio University, Japan

Triple Nr4a-deficient CAR TILs → tumour regression & prolonged survival



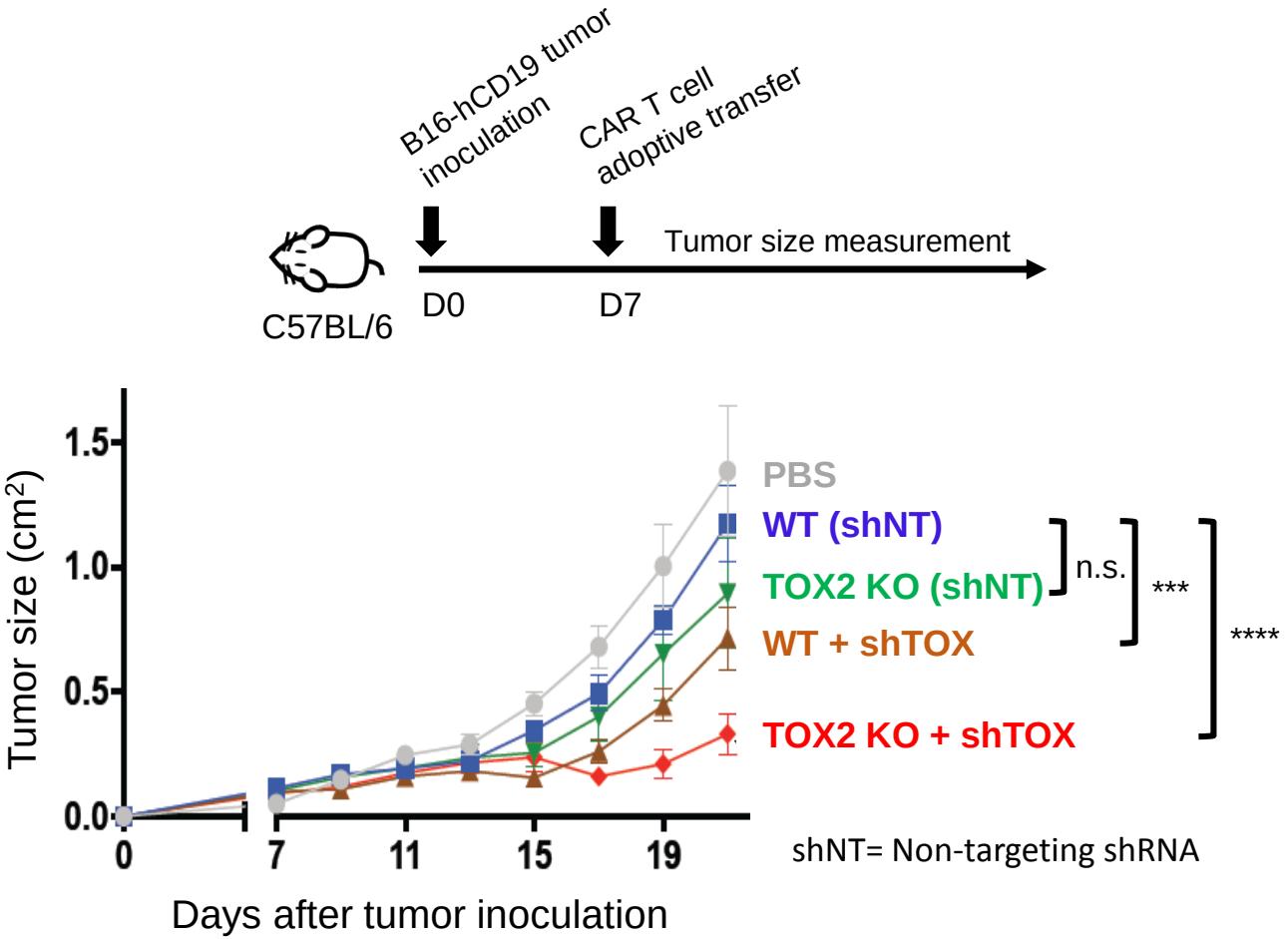
Conclusion: Nr4a has an important role in the transcriptional program of CD8⁺ T cell exhaustion





Joyce Chen

CAR TILs deficient in both TOX and TOX2 potentiate tumour regression more effectively than CAR TILs deficient in either TOX family member alone

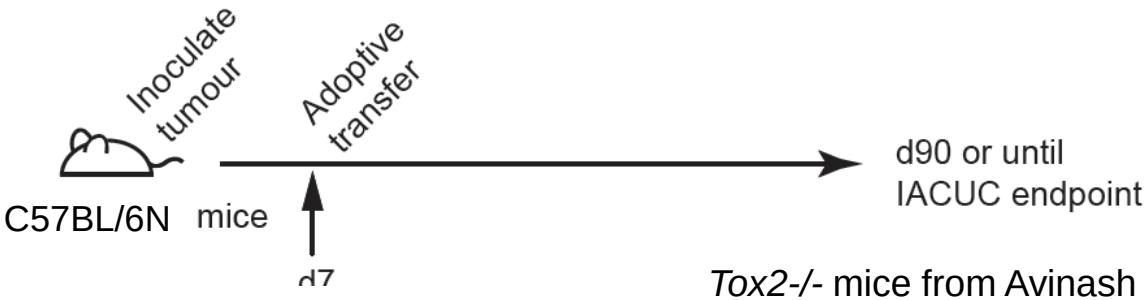


Hyungseok Seo

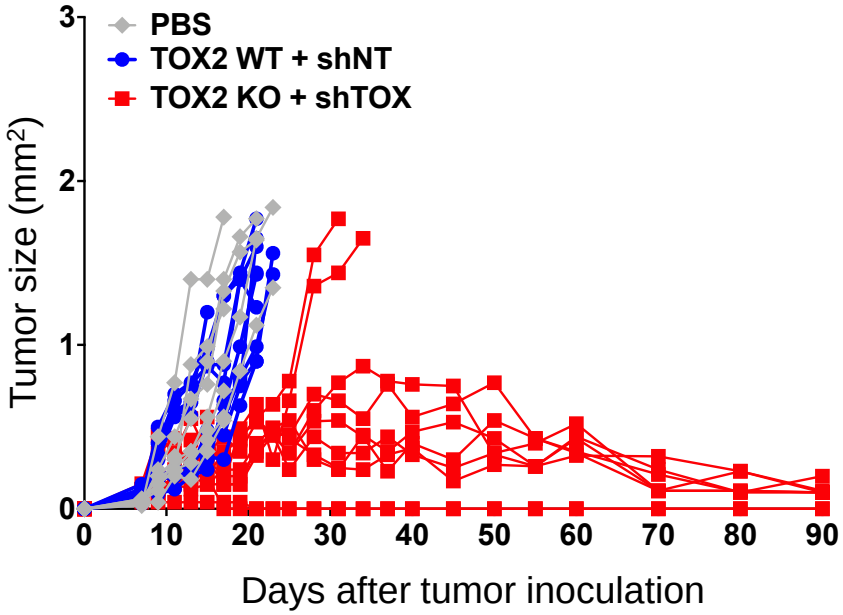
Avinash Bhandoola, Patrick Hogan

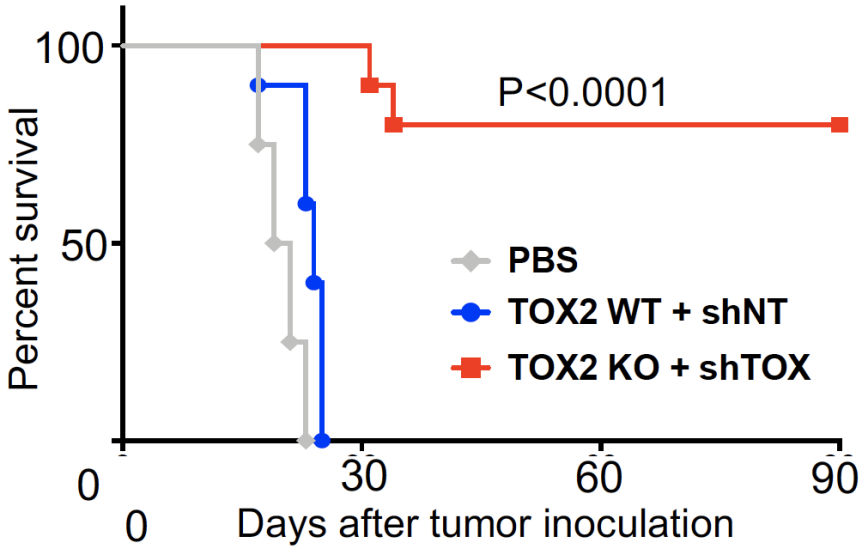
H Seo et al., *PNAS* 2019

CAR TILs deficient in both TOX and TOX2 potentiate tumour regression more effectively than CAR TILs deficient in either TOX family member alone



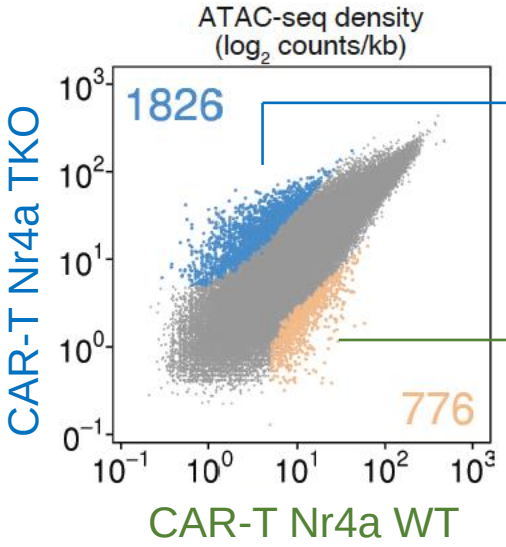
Tox2-/- mice from Avinash Bhandoola, NCI





TOX DKO CAR TILs showed decreased expression of inhibitory receptors, increased cytokine expression and increased cytolytic activity; decreased TCF1, Eomes; no change in TBET

H Seo et al., *PNAS* 2019



Nr4a TKO > WT → AP-1 (bZIP), 67%; NFκB 22%

AP-1(bZIP),1e-318, 67%

NFkB-p65(RHD),1e-22, 22%

Nr4a WT > TKO → Nr4a (NR) 34%, NFAT 11%

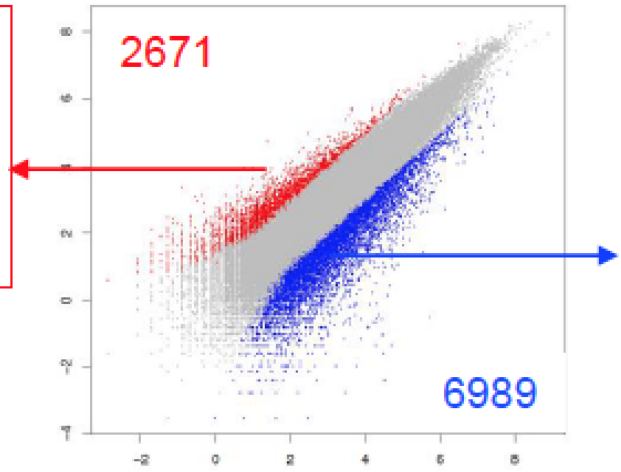
NR4A(NR),1e-128,34%

NFAT(RHD),1e-12,11%

TOX DKO > WT

NFkB-p65 (RHD),1 e-288, 21.83%

Atf3(bZIP),1e-132, 14%



WT > TOX DKO

ERG(ETS), 1e-690, 33.81%

Nur77(NR), 1e-297, 14.27%

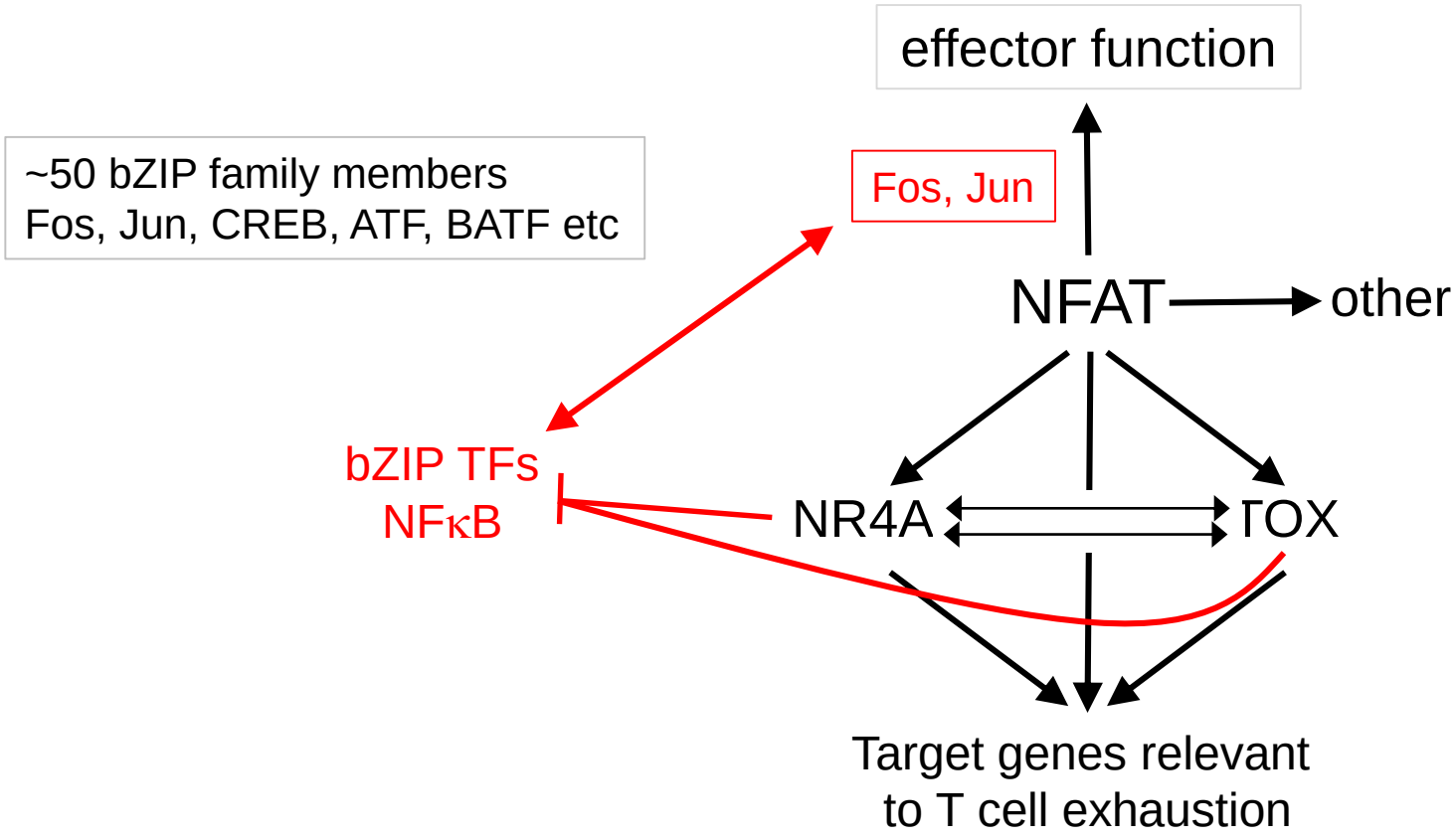
RUNX1, 1e-813, 49.68%

In both *NR4A TKO and TOX/TOX2 DKO* TILs:

Genomic regions that are more accessible in *TOX DKO* compared to WT TILs are enriched for consensus binding motifs for NFκB and bZIP transcription factors, both associated with T cell activation

Summary

- NFAT induces NR4A,TOX and other transcription factors in exhausted $CD8^+$ T cells
- Deletion of NR4A or TOX family members “reverses” exhaustion and induces tumor regression
- Deletion of NR4A or TOX induces increased accessibility of regions that bind bZIP and NFkB transcription factors relevant to T cell activation

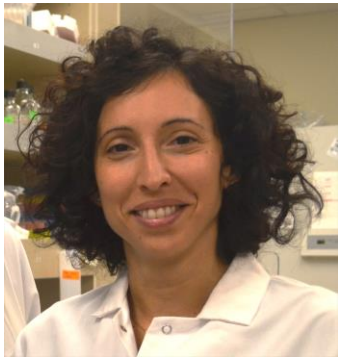


LCMV

James Scott-Browne

Renata Pereira

B16-OVA – P14 and OVA



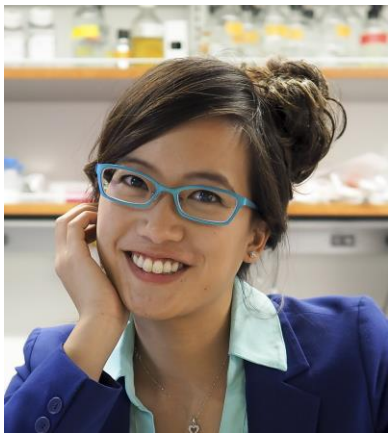
Sara Trifari

Victor Wong

Giuliana Mognol

CAR-T cells – NR4A

JSB



Joyce Chen

Isaac López Moyado

CAR-T – TOX & TOX2, TET

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