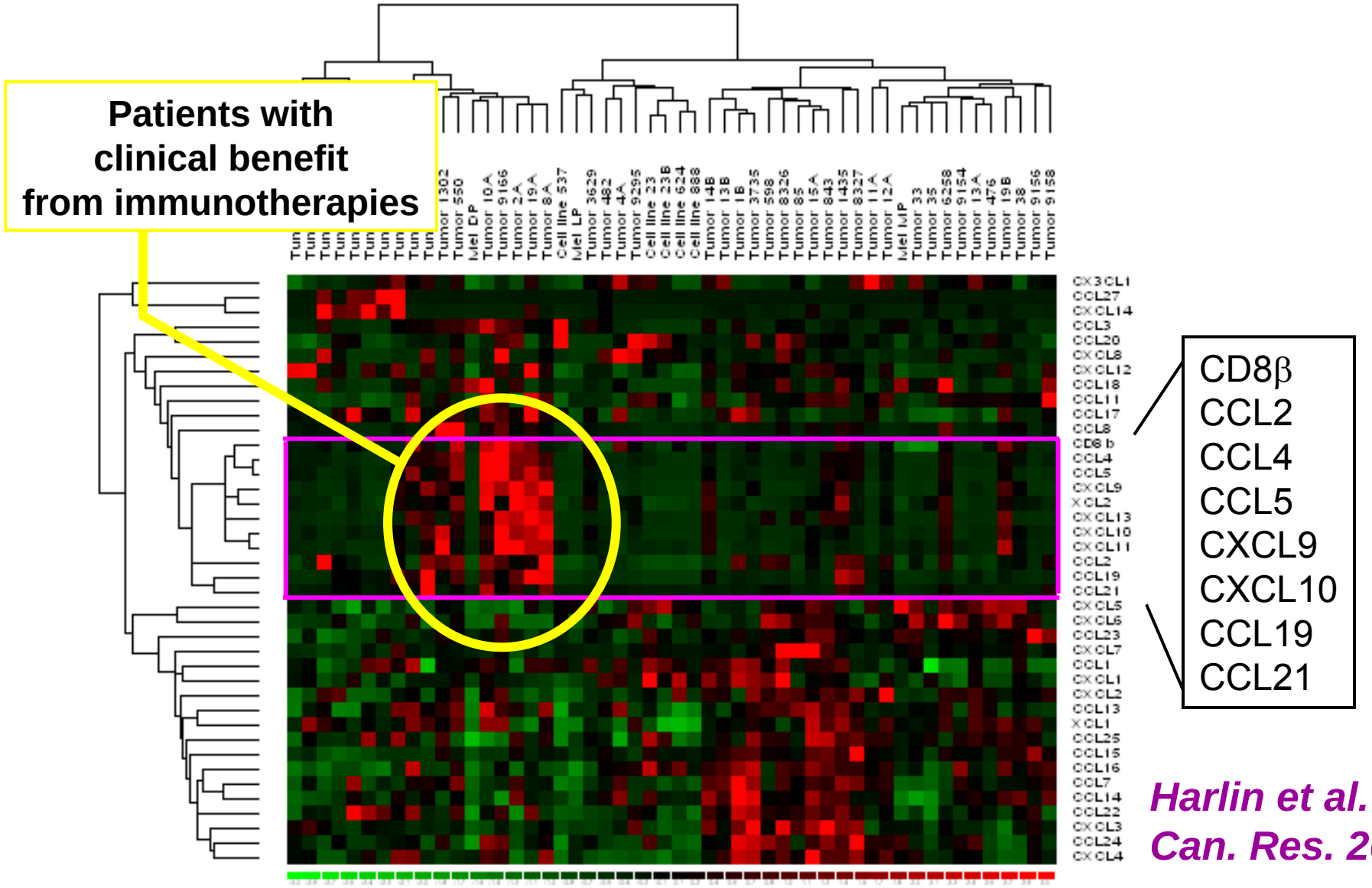
Innate and adaptive immunity regulated from within the tumor microenvironment

Thomas F. Gajewski, M.D., Ph.D.

Professor, Departments of Pathology and Medicine
Program Leader, Immunology and Cancer Program of the
University of Chicago Comprehensive Cancer Center

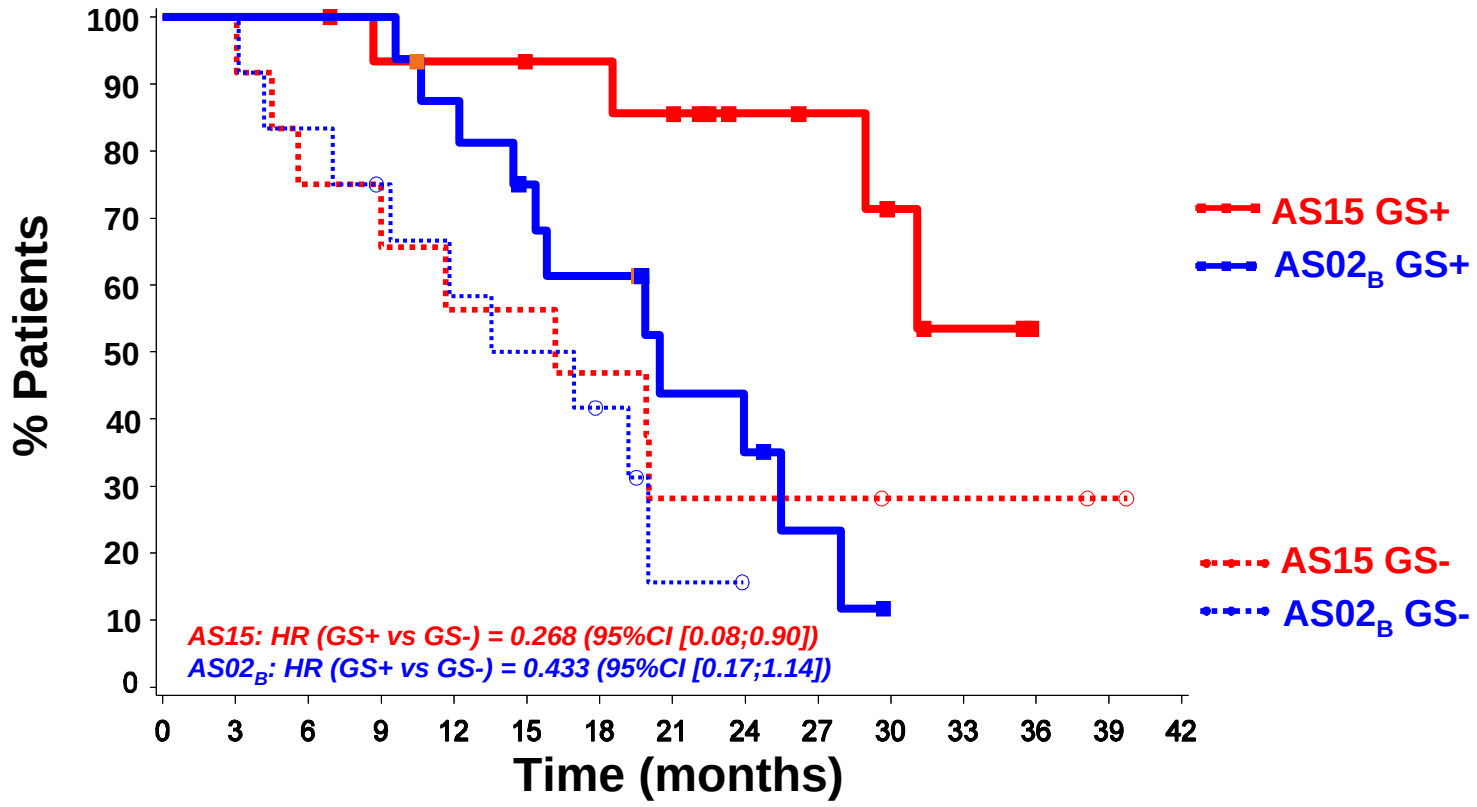
President, Society for Immunotherapy of Cancer (SITC)

Expression of a subset of chemokine genes is associated with presence of CD8+ T cells in melanoma metastases



Harlin et al. Can. Res. 2009

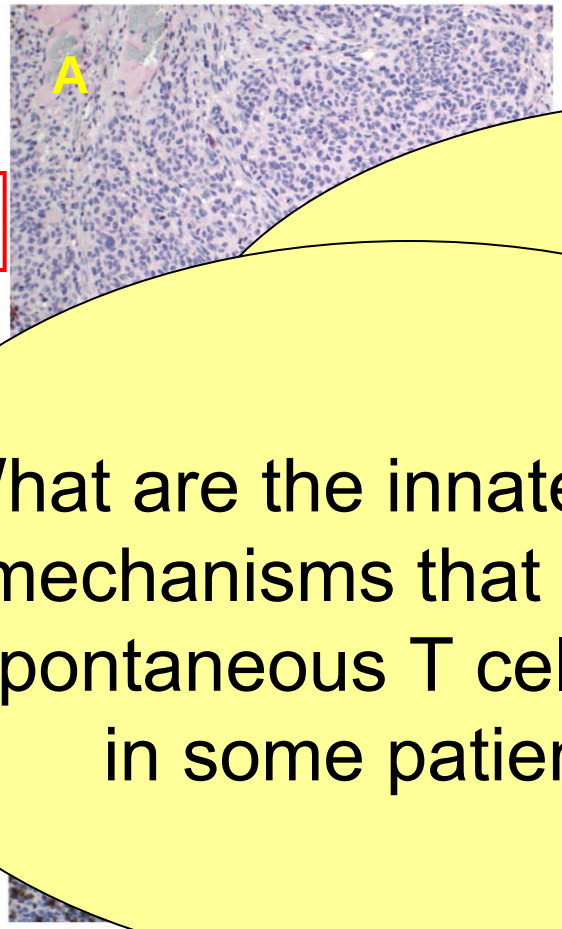
Chemokine/T cell gene expression signature is associated with survival following GSK MAGE3 protein vaccine



Louahed et al., EORTC-NCI-AACR 2009

Two broad categories of melanoma metastases defined by gene expression profiling and confirmatory assays

CD8 IHC



2/3

- **T cell "poor"**

Lack chemokines for
ont
s of
olecul

What are the innate immune mechanisms that promote spontaneous T cell priming in some patients?

mors that contain
d CD8+ T cells
ed spontaneously?

several
immunotherapies

Gajewski, Brichard; Cancer J. 2010

1. Hypothetical mechanisms that could explain spontaneous T cell-based inflammation in tumor microenvironment in a subset of patients

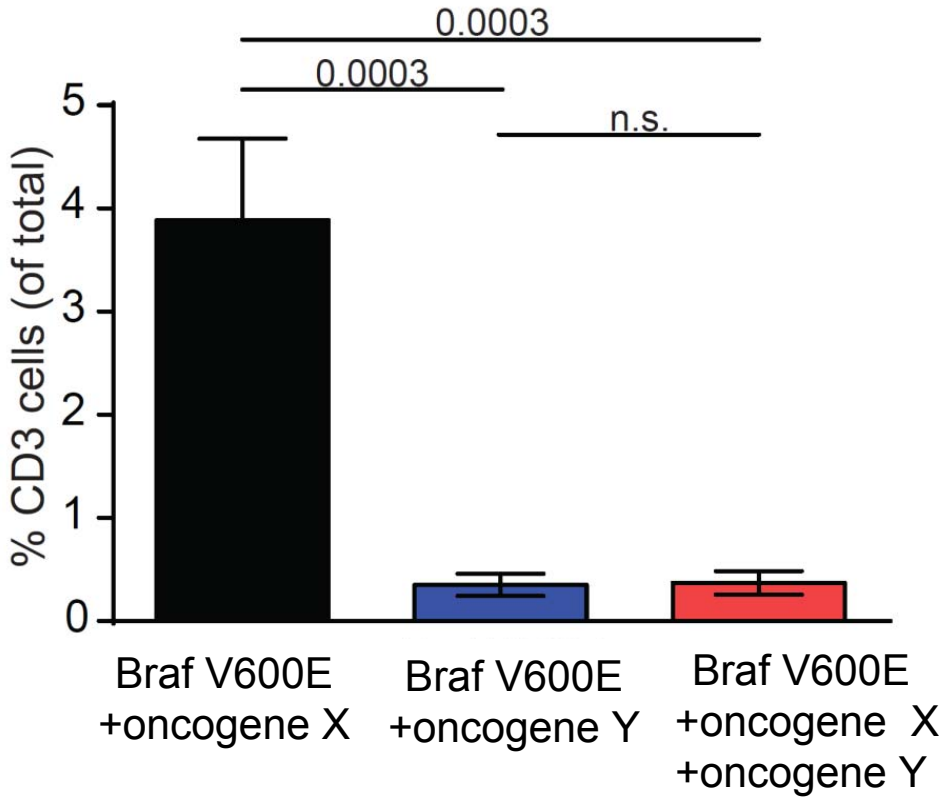
A. Somatic differences at the level of tumor cells
   - Oncogene pathways differentially activated
   - Mutational landscape

B. Germline genetic differences at the level of the host
   - Polymorphisms in immune regulatory genes

C. Environmental differences
   - Intestinal microbiome
   - Immunologic exposure history of patients

A. T cell infiltrate in mouse melanoma can be excluded by expression of accessory oncogene in a genetic tumor model

Oncogene combinations that give melanomas with Tyr-CreER and topical 4-HO Tam:



B. Loss of inflamed gene expression pattern in B16 tumors grown in type I IFNR$^{-/-}$ mice

- **Diminished expression of chemokines and T cell markers that recapitulates human subsets**
- **Implies that genetic variability in type I IFN pathway is one hypothetical mechanism that could explain differential immune phenotypes**

Selected transcripts downregulated:

IFN-induced genes

IFI27
IFI44
IFI202B
IFI203
IFI35
IFN-induced p30
IRF7

T cell markers

TCRα
TCRβ
CD3γ
Itk
Fyb
Granzyme B
Perforin
CD28

Chemokines

CCL2
CCL5
CXCL9
CXCL10
CXCL13

Other immune genes

CD40
CD83
CD86
FcγR1
Complement C1q
Complement factor B
IL-18
CD68
Class II MHC

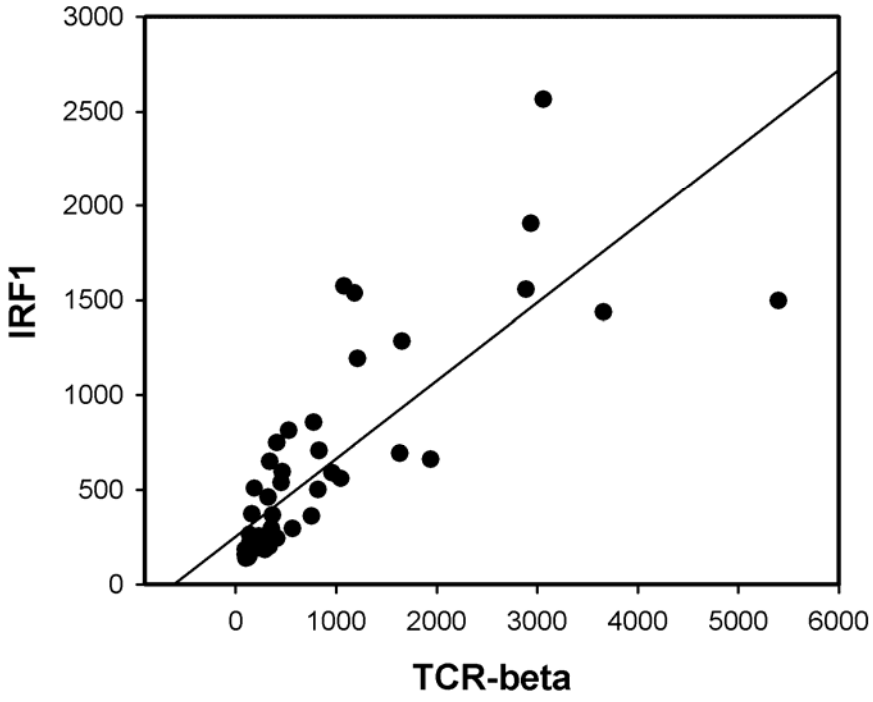
2. Innate immune signals—type I IFNs and tumor sensing

How are anti-tumor T cells sometimes becoming spontaneously primed?
What is the innate immune sensing mechanism that drives adaptive immunity against tumors?

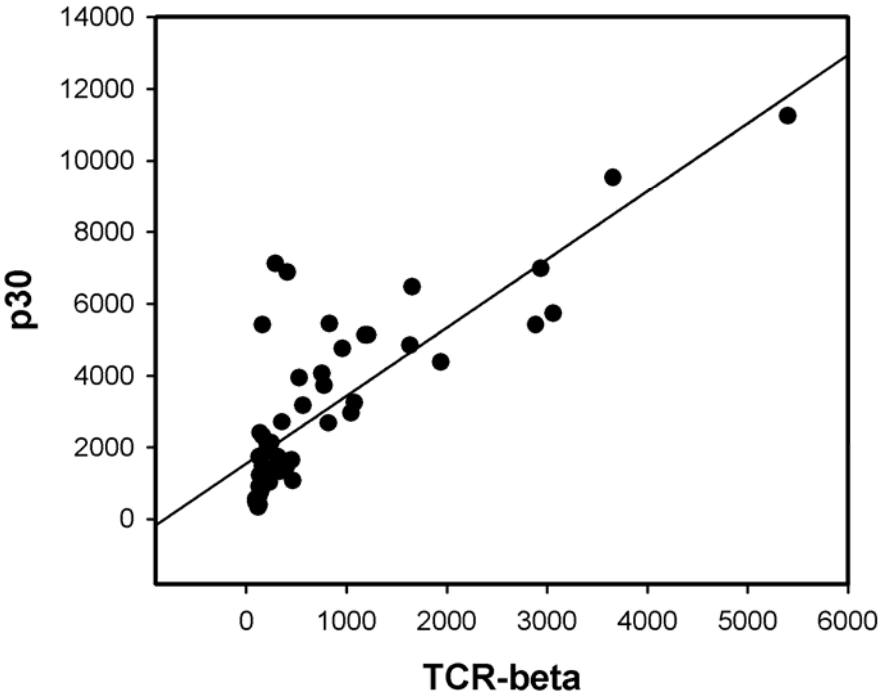
What initiates spontaneous T cell priming and recruitment in a subset of melanomas?

Melanoma metastases that contain T cell transcripts also contain transcripts known to be induced by type I IFNs

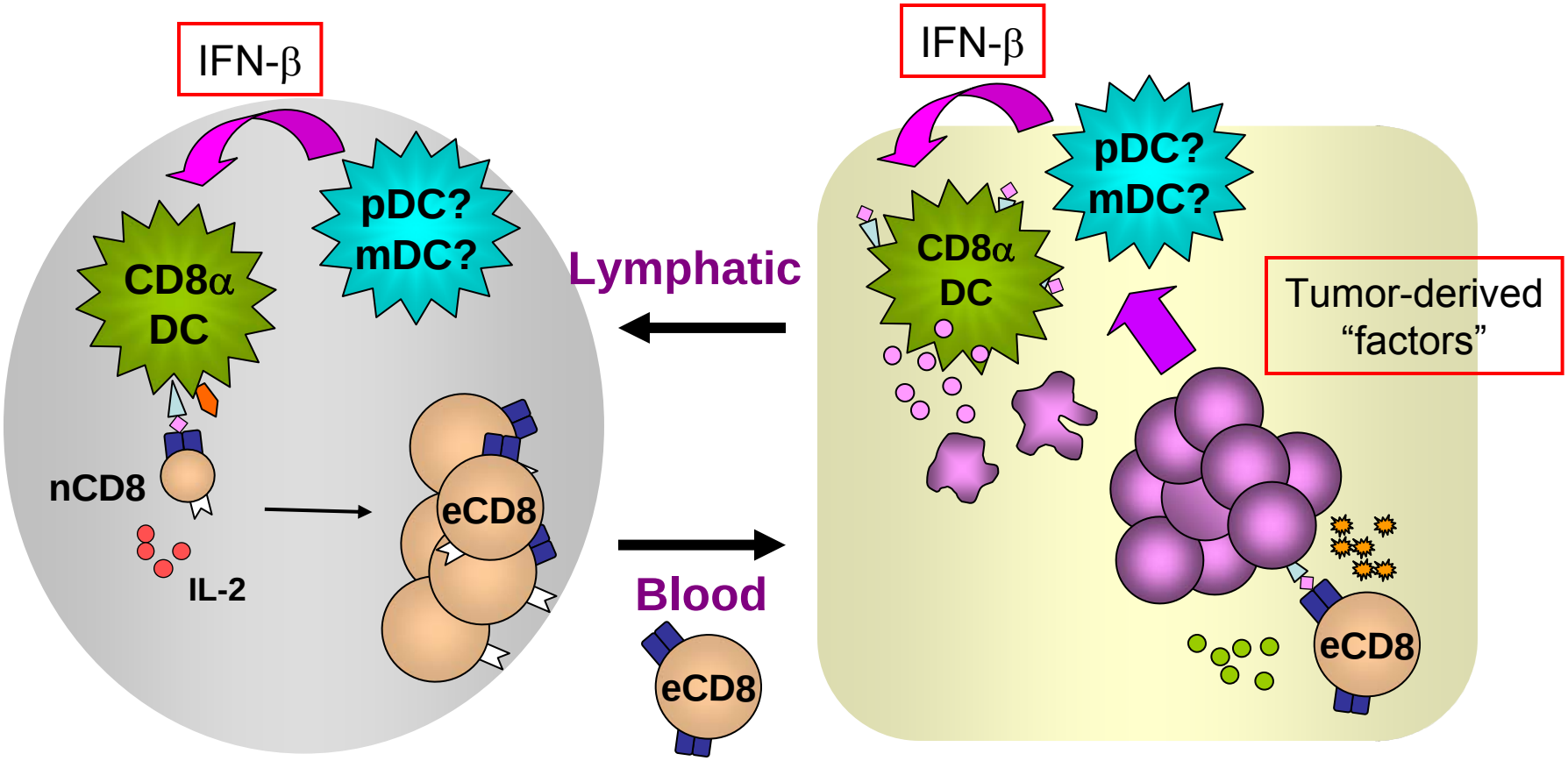
A: IRF1



B: IFN-induced p30

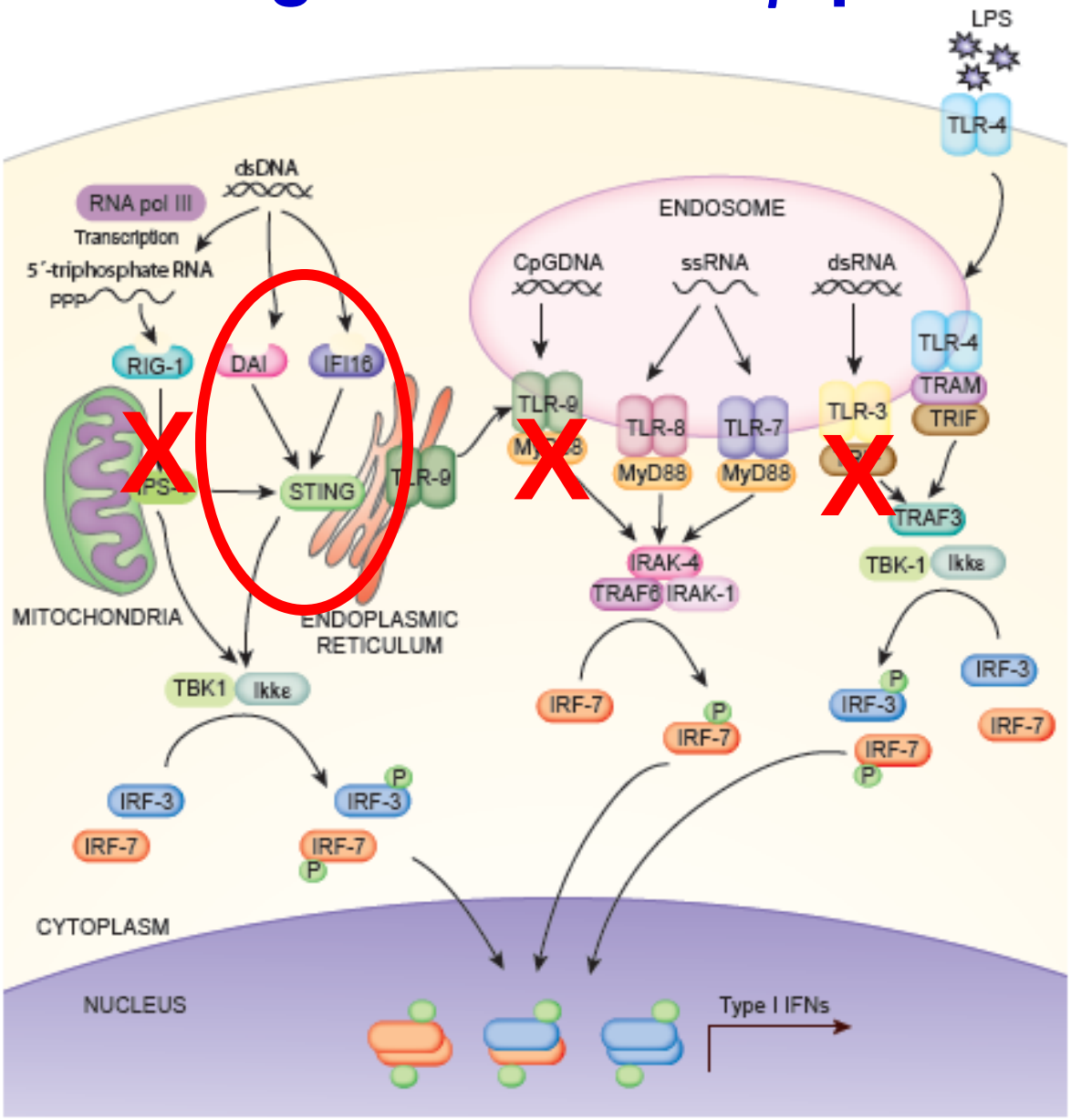


Innate immune sensing of tumors drives host type I IFN production and cross-priming of CD8$^+$ T cells via CD8α DCs



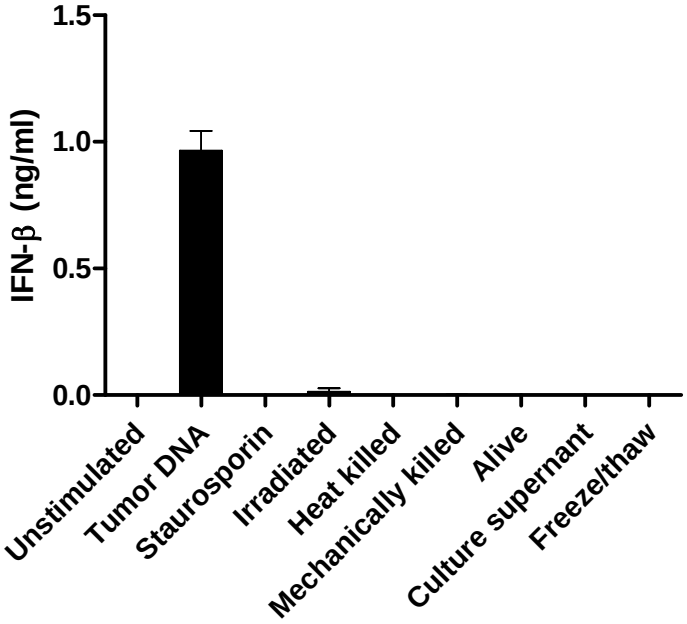
Fuertes et al; J. Exp. Med. 2011

Innate sensing toward IFN-β production

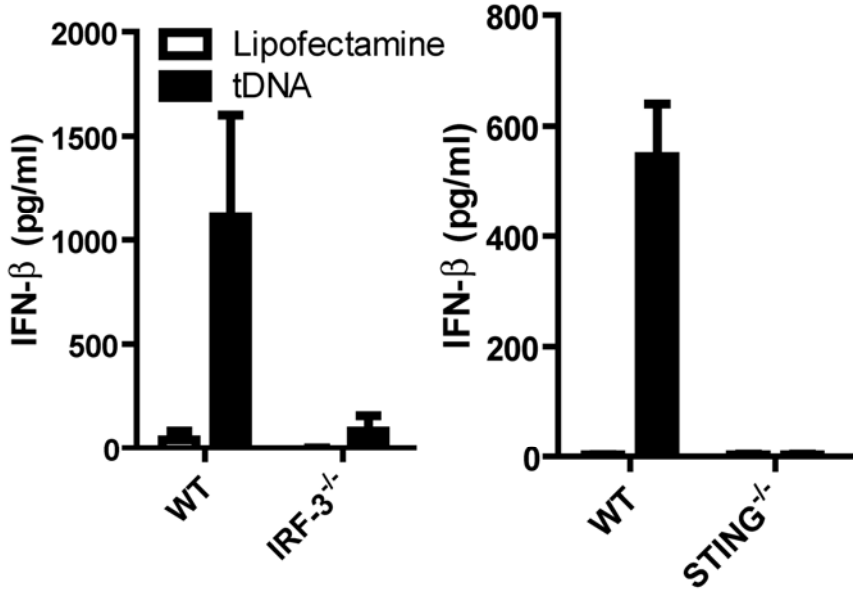


Tumor-derived DNA induces IFN-β production from DCs in an IRF3- and STING-dependent fashion

A: Tumor-derived preparations



B: IRF3 and STING$^{-/-}$

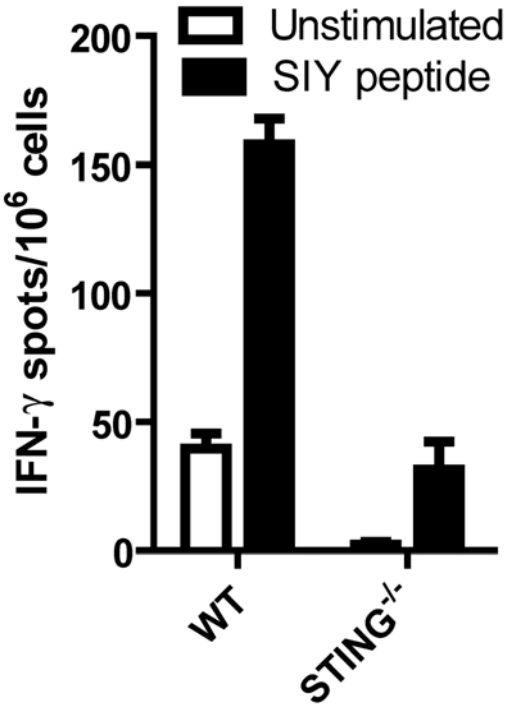


DNA includes lipofectamine;
RNA not effective

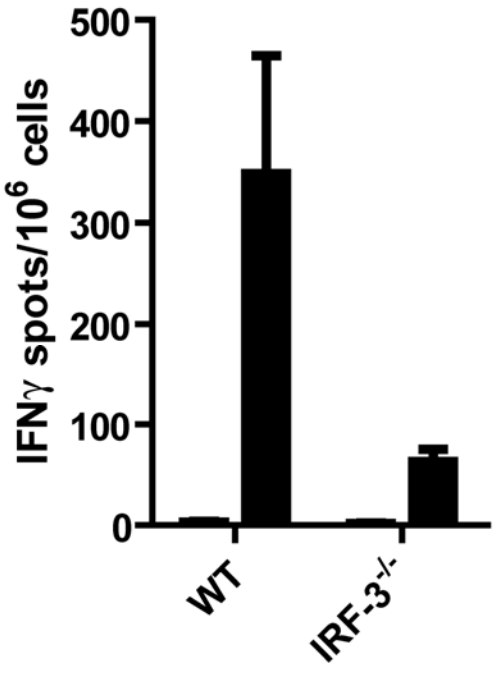
In vivo verification:

Host STING and IRF3 are required for spontaneous induction of CD8^{+} T cell responses against tumor-derived antigen in vivo

A: STING$^{-/-}$

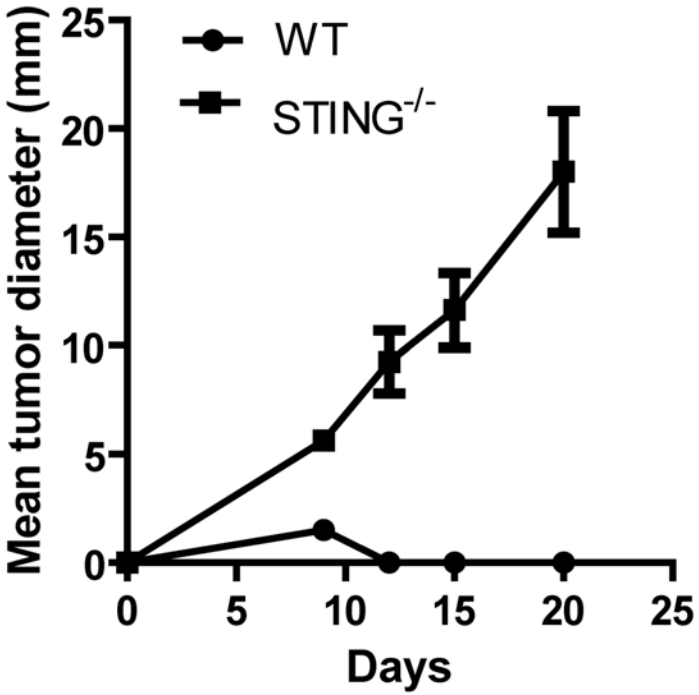


B: IRF3$^{-/-}$



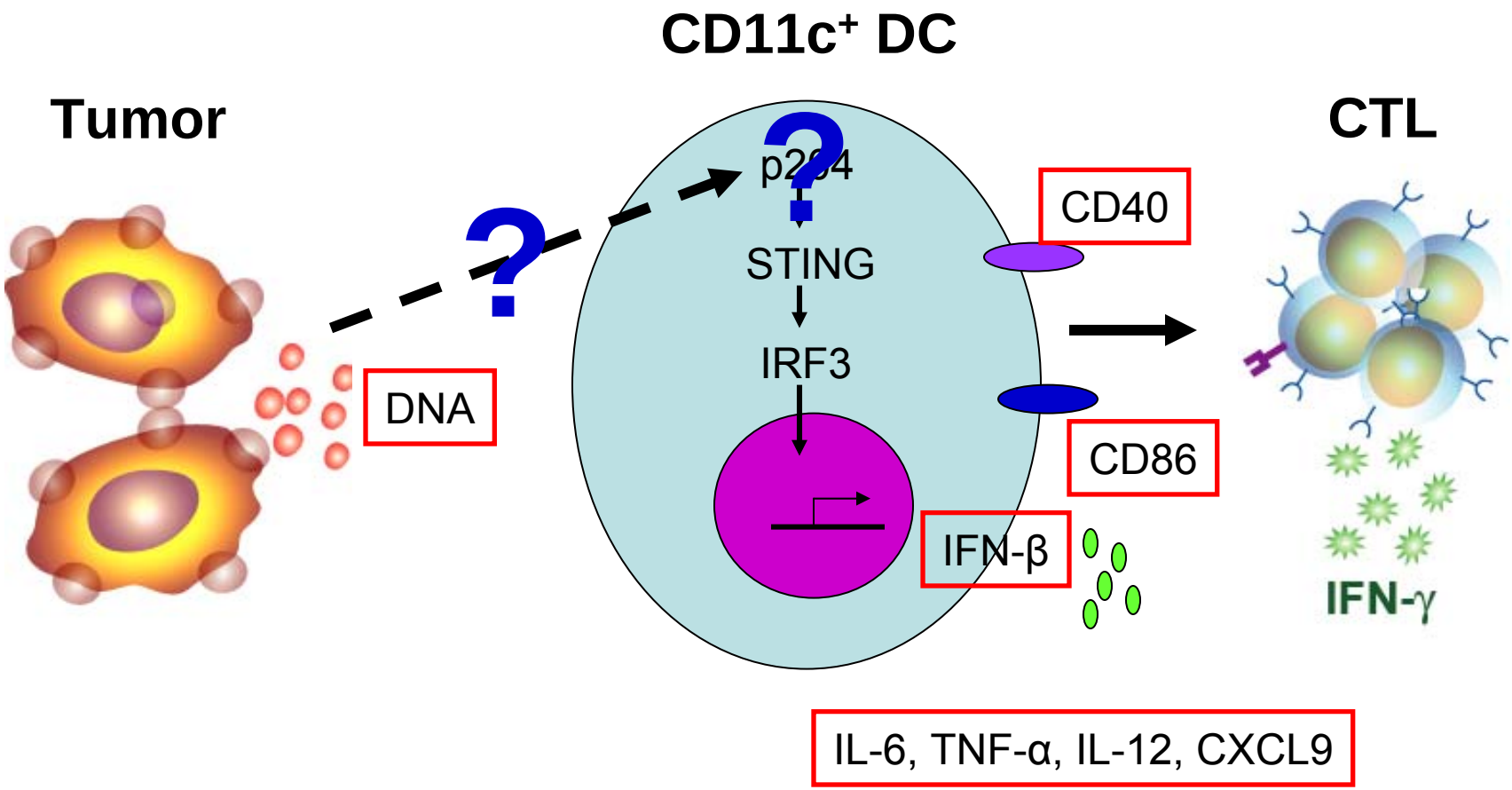
Rejection of immunogenic tumors is ablated in STING$^{-/-}$ mice

B16.SIY melanoma in 129 mice



Similar results in 3 immunogenic mouse tumor models

Model for tumor-induced DC activation and subsequent T cell priming

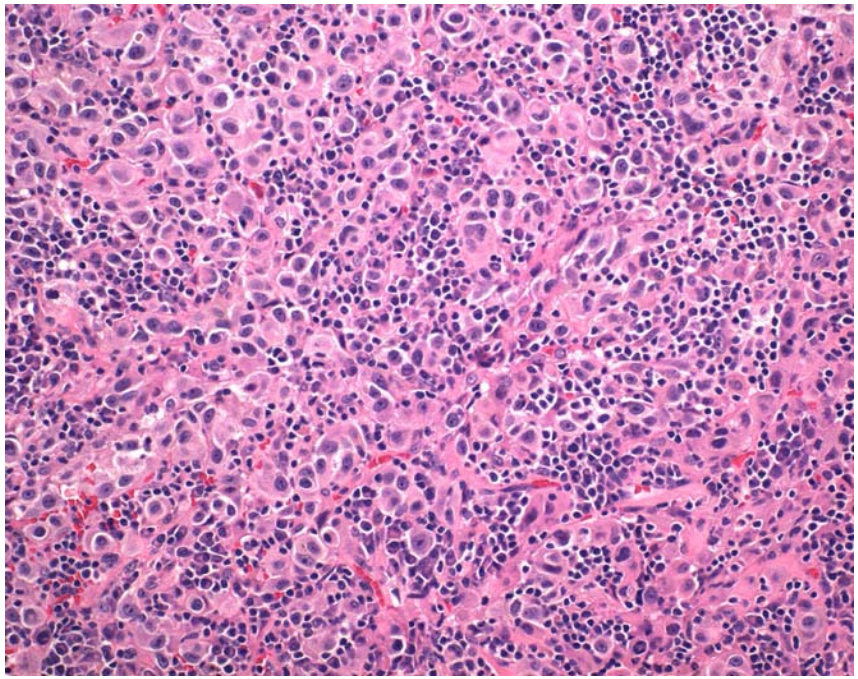


Woo et al; manuscript in preparation

3. T cell suppressive mechanisms

Why are TIL not eliminating the tumor cells they are infiltrating? Can we overcome this defect and restore tumor rejection?

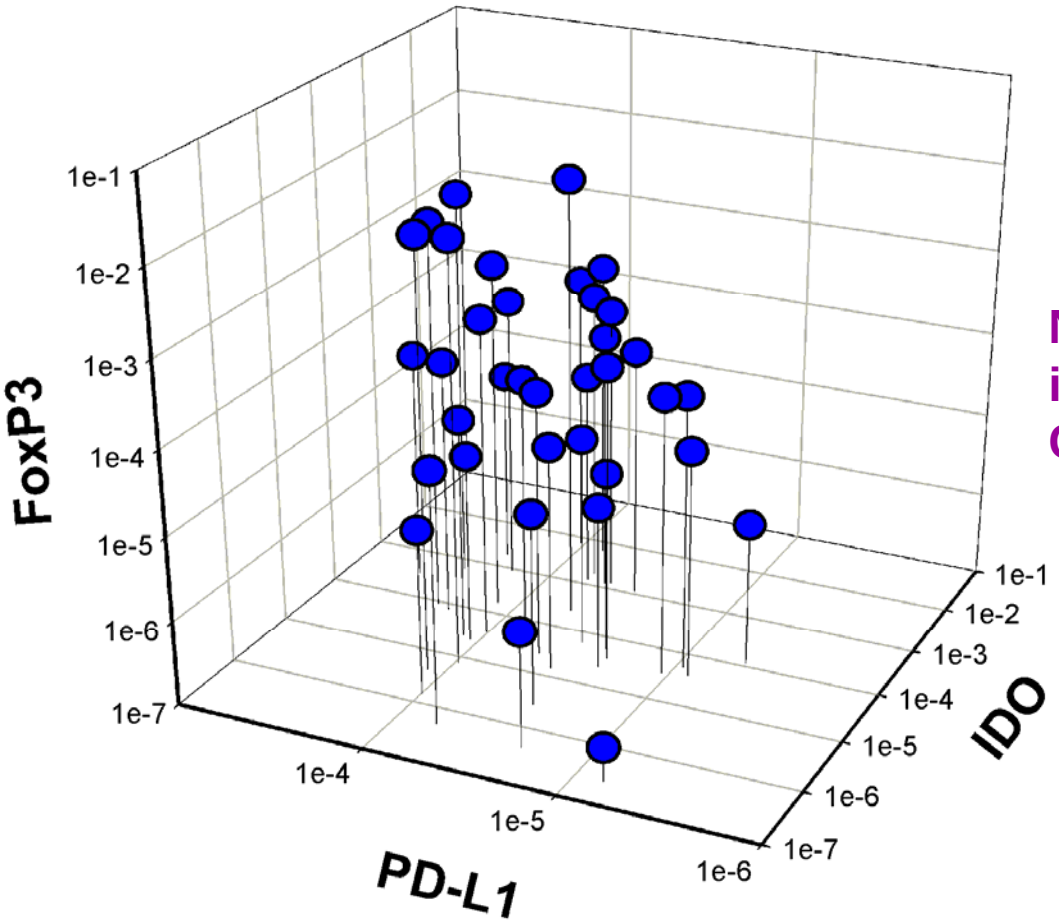
Why are melanomas that do attract $CD8^+$ T cell not rejected spontaneously?



- **IDO** (indoleamine-2,3-dioxygenase)
- **PD-L1** (engages PD-1)
- $CD4^+CD25^+FoxP3^+$**Tregs**
- T cell **anergy** (B7-poor)

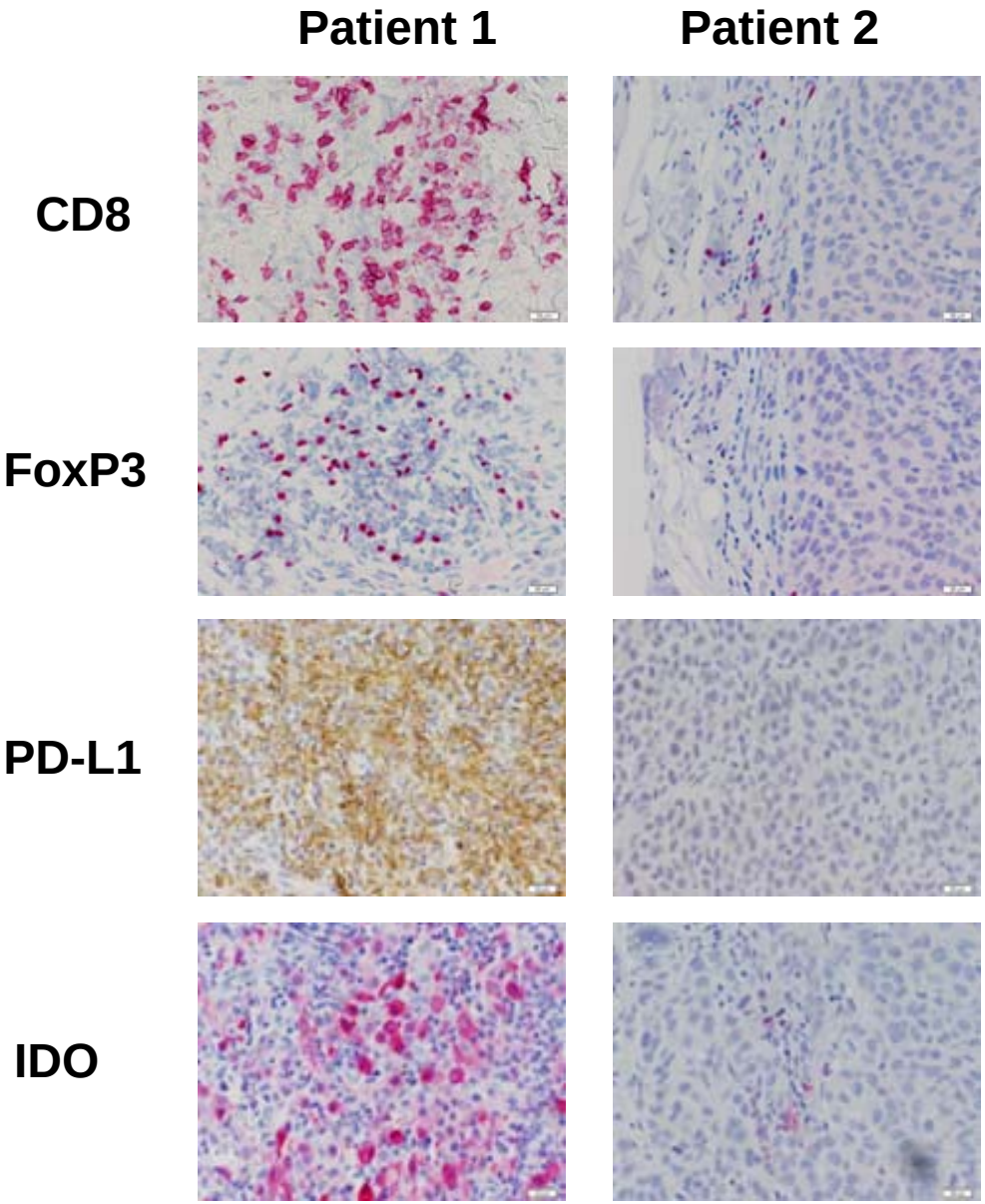
Immunol. Rev. 2006,
Clin. Can. Res. 2007

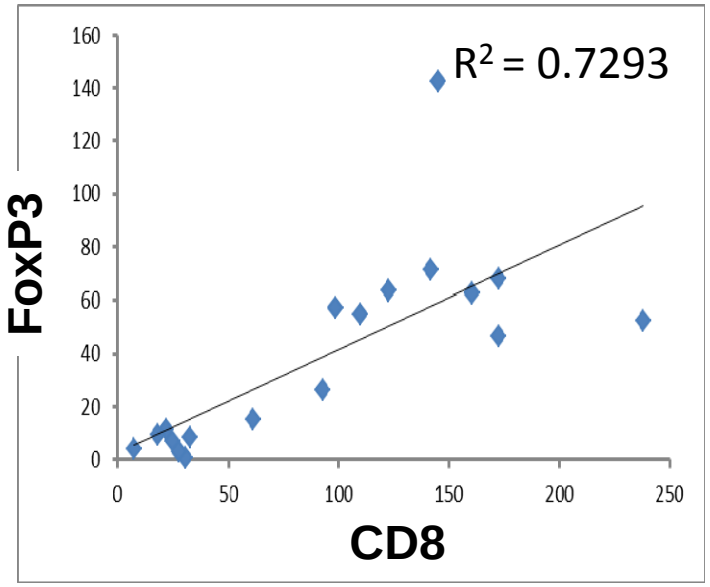
Correlated expression of IDO, FoxP3, and PD-L1 transcripts in individual tumors



Note: these are highest in tumors that contain $CD8^+$ T cells

Presence of Tregs and expression of PD-L1 and IDO are associated with a CD8+ T cell infiltrate

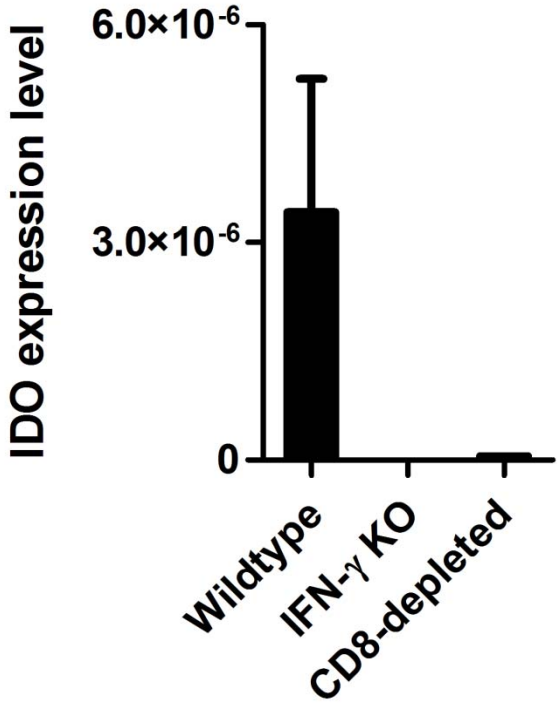




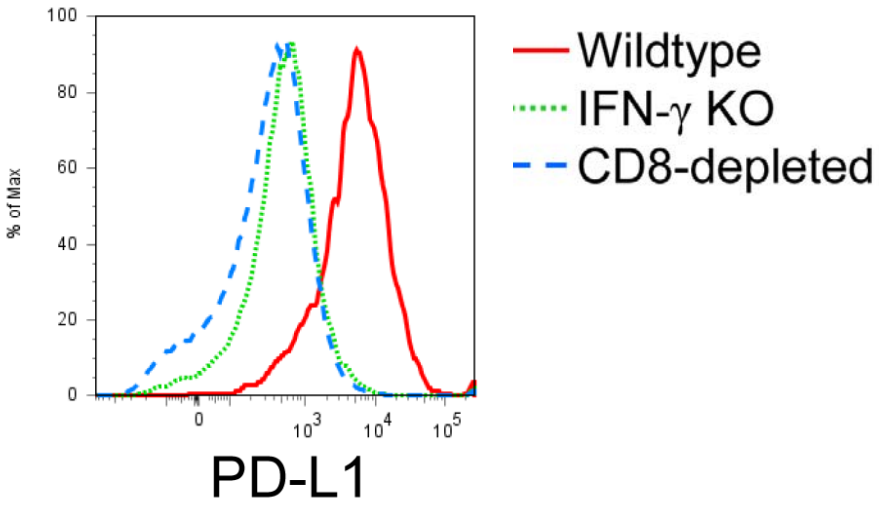
Correlations also between CD8+ T cells and PD-L1, IDO

Expression of IDO and PD-L1 in B16 melanoma tumors growing in vivo depends on host $CD8^+$ T cells and IFN-γ

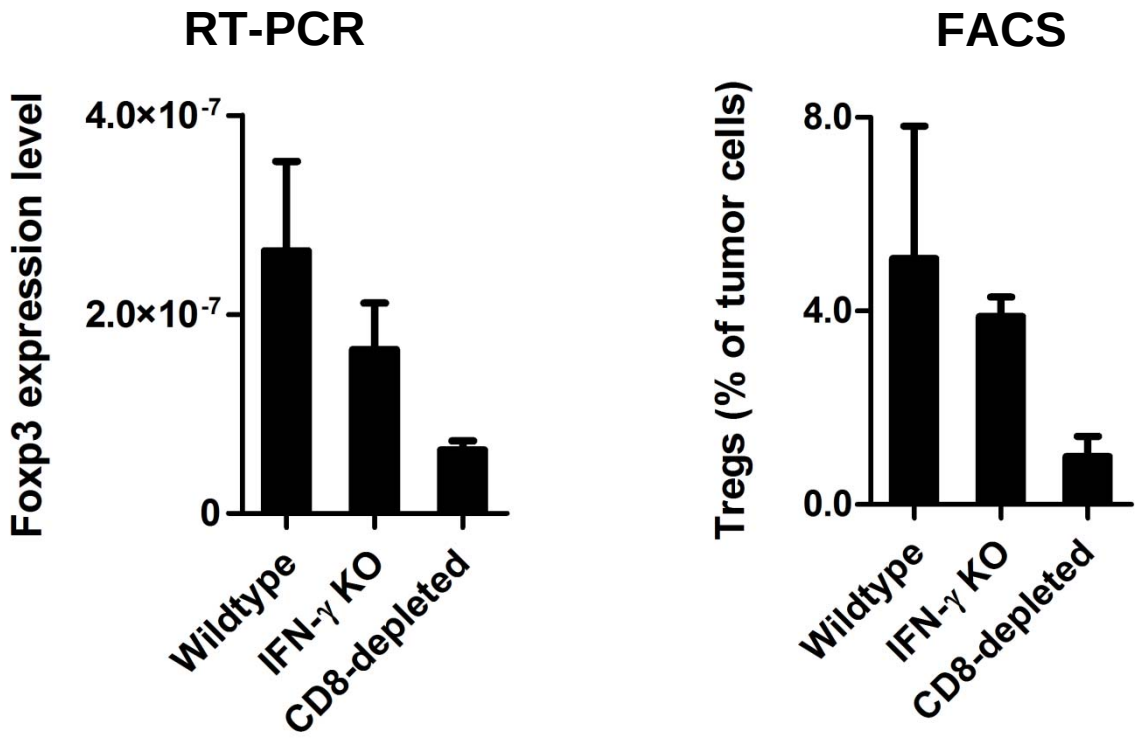
A: IDO



B: PD-L1



Treg accumulation in B16 melanoma depends upon CD8$^+$ T cells but <u>not</u> IFN-γ



- **Treg recruitment appears to be regulated by chemokines (CCL22/CCR4)**
- **Also, no evidence for CD8s promoting migration or conversion**

Spaapen et al; manuscript submitted

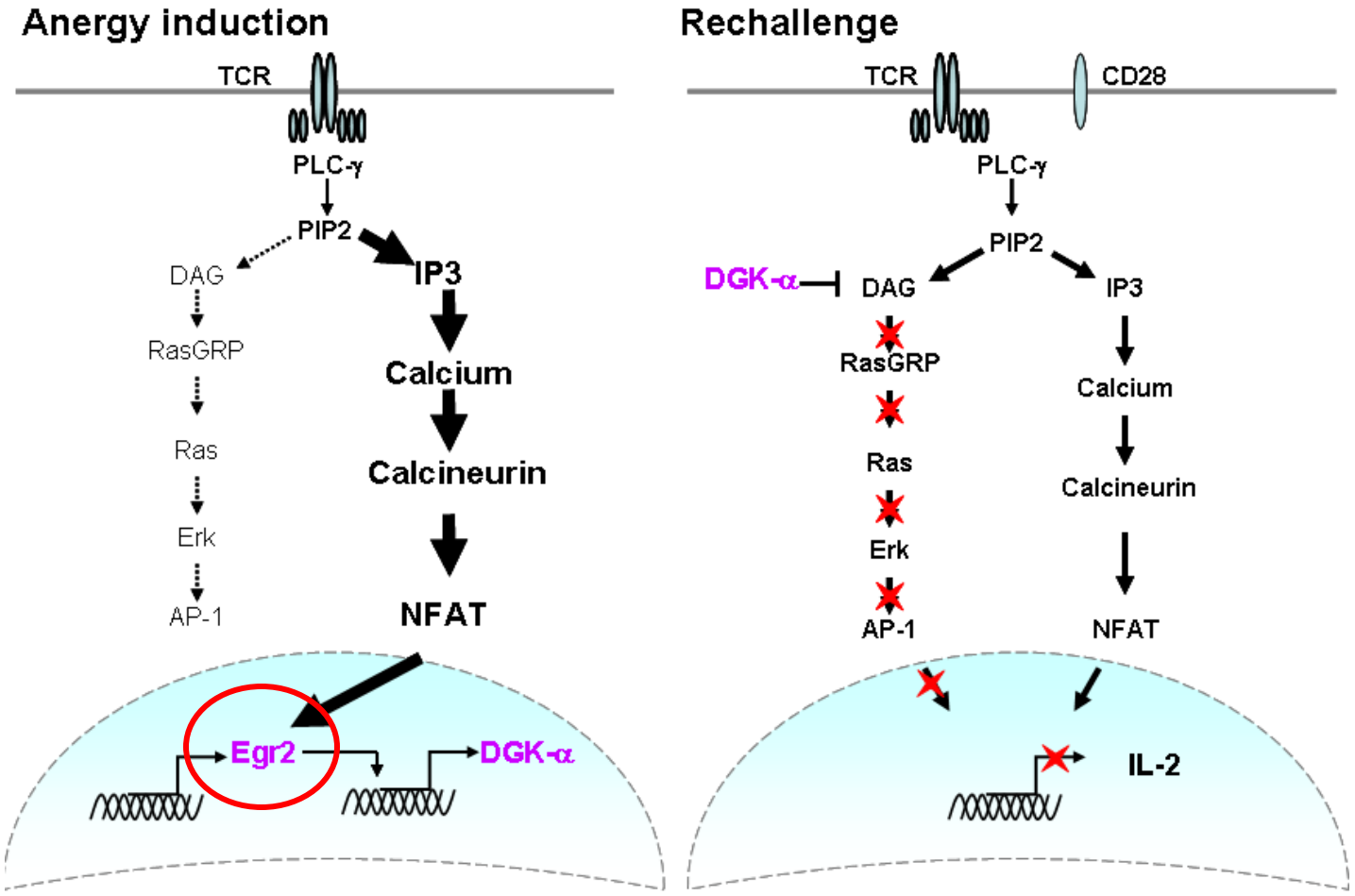
Summary of regulation of immune suppressive mechanisms in the tumor microenvironment

- The three major immune inhibitory mechanisms confirmed to be present in the melanoma tumor microenvironment appear to be immune-intrinsic, driven by $CD8^+$ T cells
- For IDO and PD-L1, IFN-γ is the major mediator in vivo
- For Tregs, CCL22 production by $CD8^+$ effector cells is the major mediator, via CCR4 on Tregs (no evidence for Treg conversion or proliferation driven by CD8s)
- Blockade of these mechanisms represents attractive strategy to restore anti-tumor T cell function and promote tumor rejection in patients, and because these are intrinsic to the host they may be less mutable
- Clinical studies ongoing with anti-PD-1, IDO inhibitors, Treg targeting via CD25, and anergy reversal with homeostatic cytokines: already showing promise

Focusing in on T cell anergy

- A hyporesponsive state induced by TCR engagement in the absence of B7 costimulation
- Indirect evidence for involvement in tumor escape
- Functional overlap with “exhaustion”
- After anergy induction
  - T cells show defective TCR/CD28-induced Ras pathway activation (*Fields et al. Science 1996*) and blunted IL-2 production and proliferation
- Mechanism of anergy induction
  - Unbalanced activation of NFAT over AP-1 pathway: induction blocked by CsA, therefore is NFAT-dependent
  - Depends on new protein synthesis➔ induction of negative regulators
  - Recently identified diacylglycerol kinases (DGKs) as key inhibitors of Ras-mediated signalinig in anergic cells (*Zha et al Nature Immunol. 2006*)

Further insight into T cell anergy: regulation by Egr2 driving DGK-α/ζ



Fields et al, Science 1996
Zha et al, Nature Immunol. 2006

Zha et al, EMBO Reports. 2008
Zheng et al, JEM In Press

Egr2 deletion leads to resistance to anergy induction *in vitro* and *in vivo*

CAR Tg x Egr2$^{fl/fl}$ clones
EV and Cre

↓

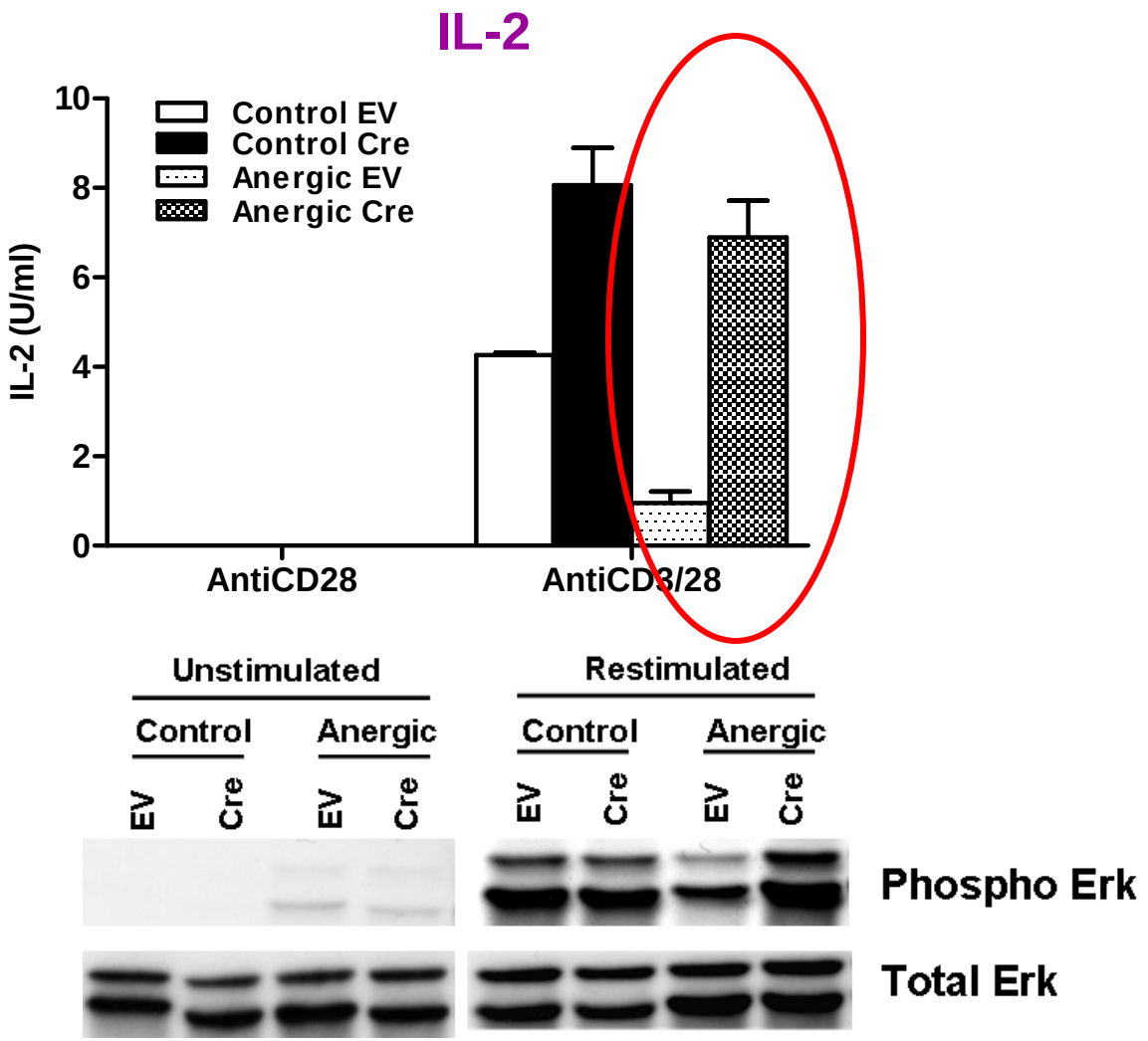
Treat cells
Control
plate-bound anti-CD3 (Anergic)

↓

Rest 1-2 days
In
medium

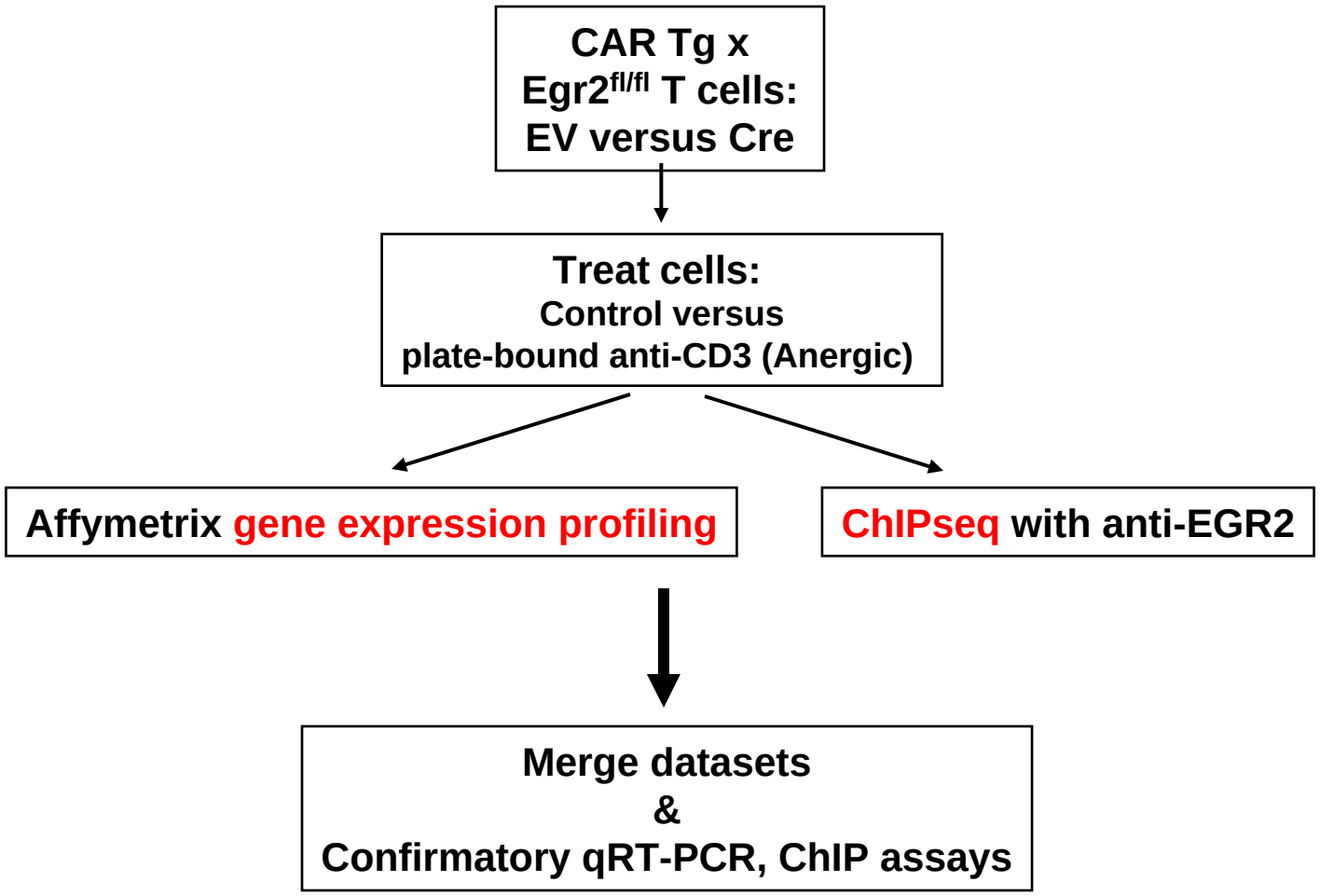
↓

Rechallenge with plate-bound anti-CD3+anti-CD28



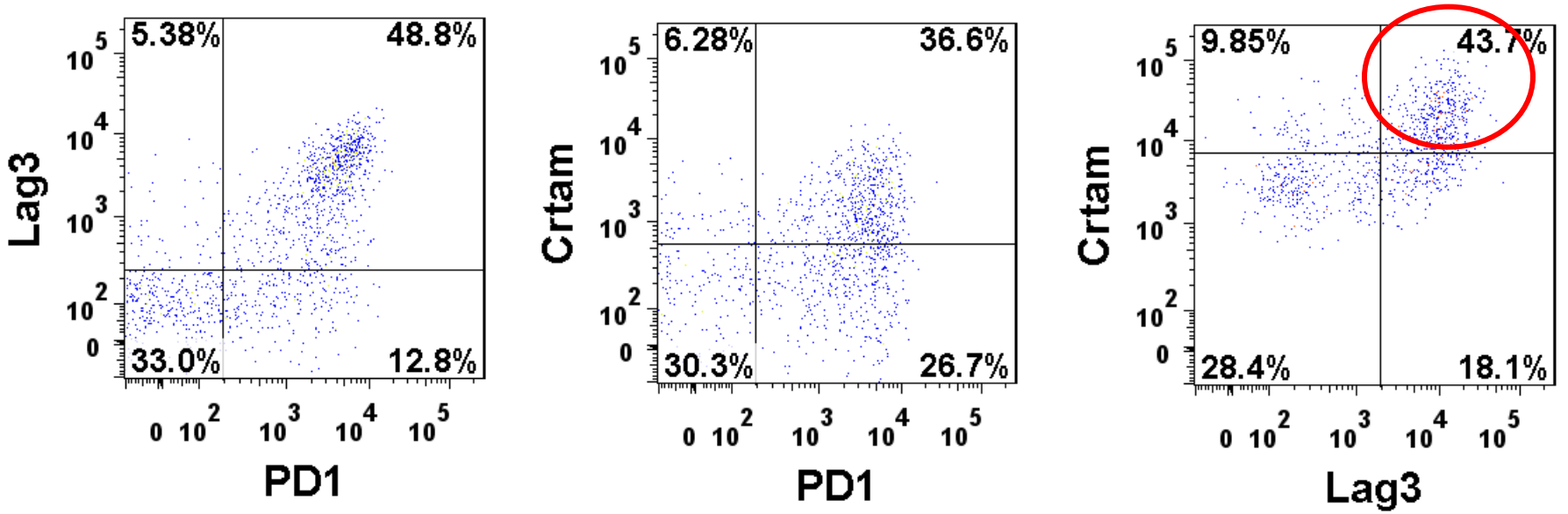
Zheng et al, JEM In Press

T cell-intrinsic dysfunction (anergy):
Strategy to determine global Egr2-driven transcriptional program in anergic T cells

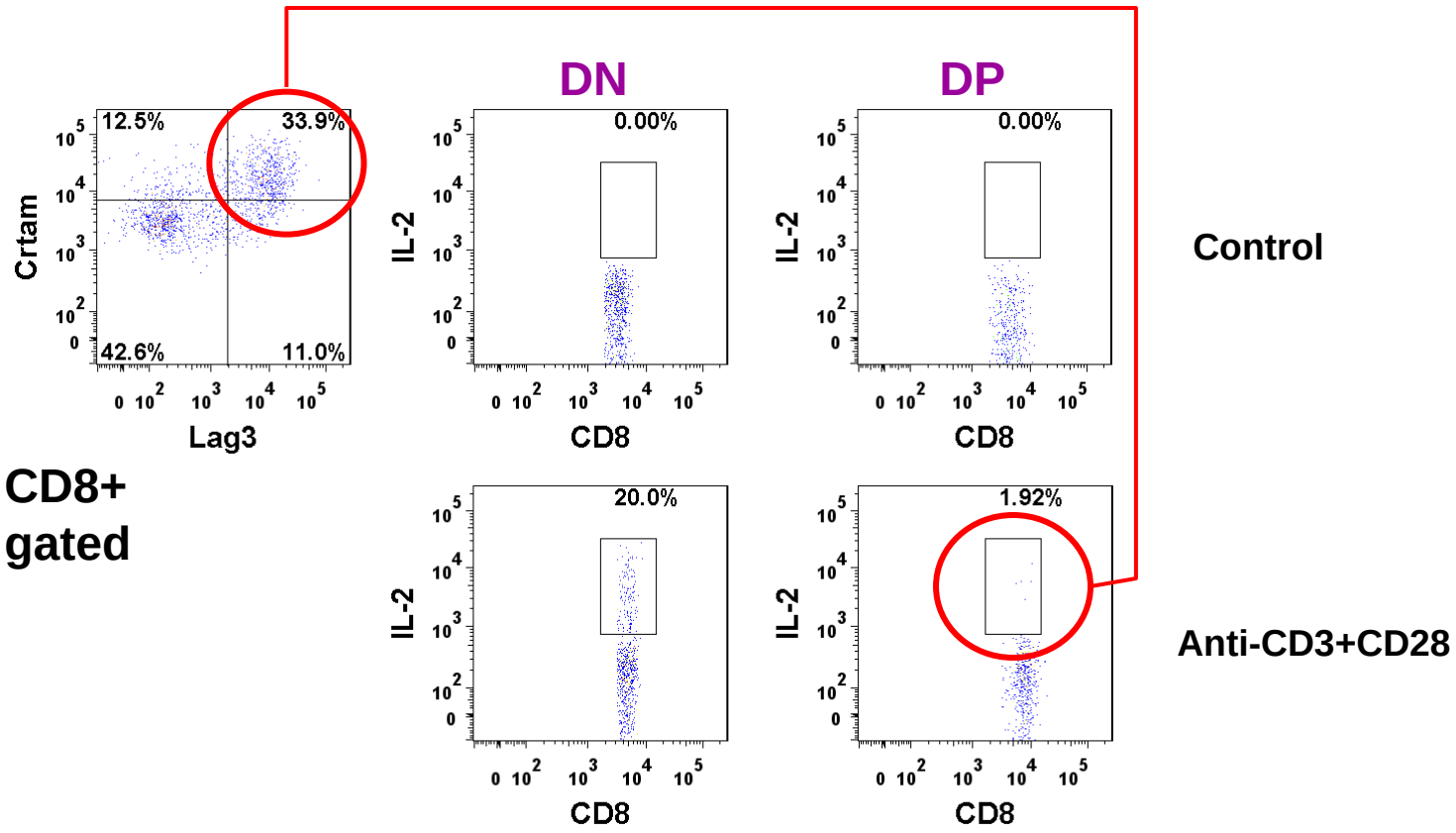


46 genes identified, including several surface proteins: LAG3 and CRTAM

Lag3 and Crtam are highly upregulated on a subset of CD8^{+}PD-1^{+} TILs in B16 melanoma



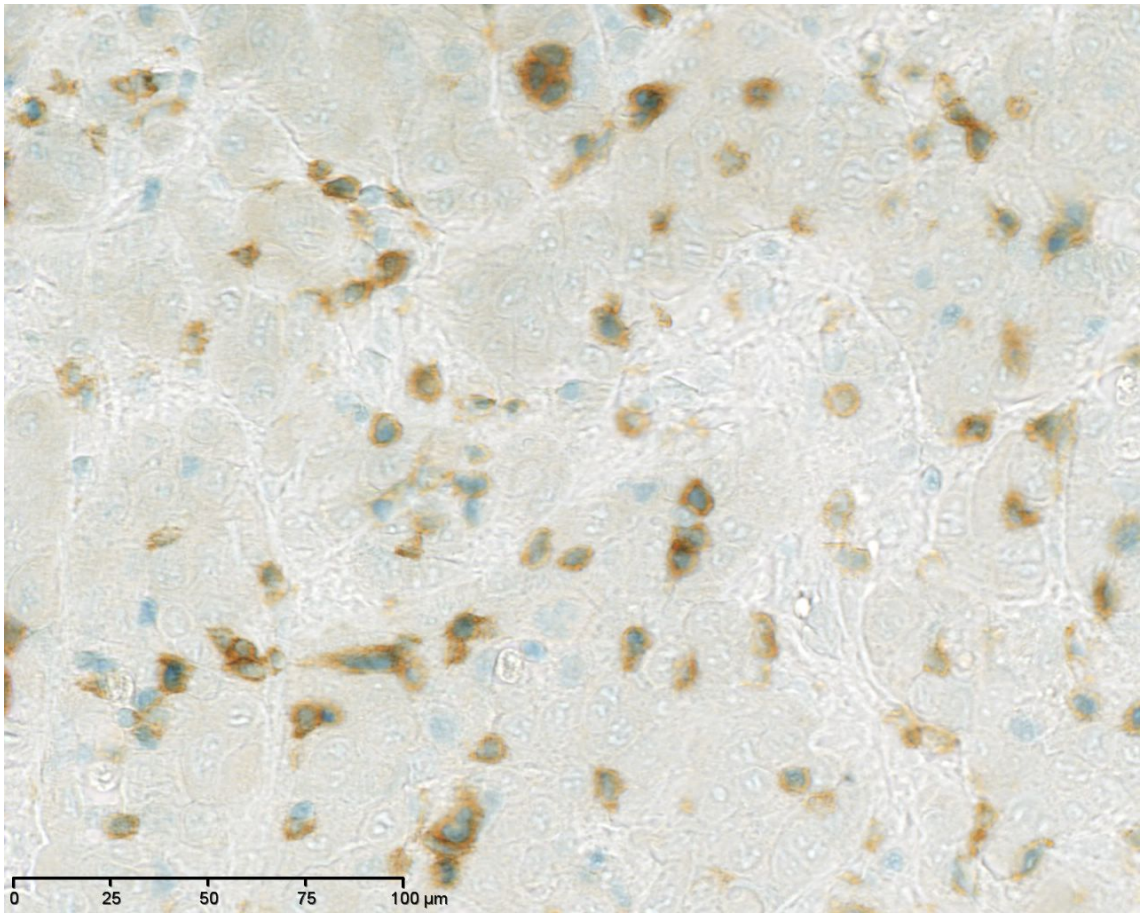
Lag3+Crtam+ CD8+ TILs are defective in IL-2 production upon ex vivo stimulation



The CRTAM+LAG3+CD8+ T cells also have blunted proliferation and express EGR2 and anergy-associated genes

Zheng et al, manuscript submitted

Tumor-infiltrating $CD8^+$ T cells (brown) in human melanoma are $EGR2^+$ (blue)



Implies that strategies to inhibit EGR2 pathway or target genes may have the potential to improve T cell function in human tumor context

Conclusions

- A T cell-inflamed tumor microenvironment may be a predictive biomarker for response to immunotherapies
  - Prospective analysis ongoing in GSK-Bio vaccine trials
- Innate immune “sensing” of tumors appears to occur via a STING-dependent pathway and host type I IFNs
- “Inflamed” tumors likely are not rejected due to dominant immune suppressive mechanisms
  - IDO, PD-L1, Tregs, Anergy: We can target these!
- Increased PD-L1, IDO, and Tregs in the tumor site are driven by $CD8^+$ T cells in the tumor microenvironment
- A new set of surface markers driven by EGR2 may provide a strategy for identifying intrinsically dysfunctional $CD8^+$ T cells from the tumor microenvironment, and may also regulate the anergic phenotype and be therapeutic targets

Acknowledgments

Melanoma gene array/ Chemokines
Helena Harlin
Yuan-yuan Zha
Amy Peterson
Mark McKee
Craig Slingluff
Functional genomics core

Type I IFNs
Mercedes Fuertes
Robbert Spaapen
Seng-Ryong Woo
Aalok Kacha
Justin Kline
David Kranz
Hans Schreiber
Ken Murphy

Genetic melanoma model
Stefani Spranger

Uncoupling negative regulation
Robbert Spaapen
Justin Kline
Stefani Spranger
Ruth Meng
Yuan-yuan Zha
Christian Blank
Ian Brown

Innate immune sensing
Seng-Ryong Woo
Leticia Corrales
Mercedes Fuertes
Kate Fitzgerald
Glen Barber

T cell anergy/ChIP-SEQ
Yan Zheng
Yuan-yuan Zha
Albert Bendelac
Harinder Singh