Immunity
Article

An Inherently Bifunctional Subset of Foxp3$^+$ T Helper Cells Is Controlled by the Transcription Factor Eos

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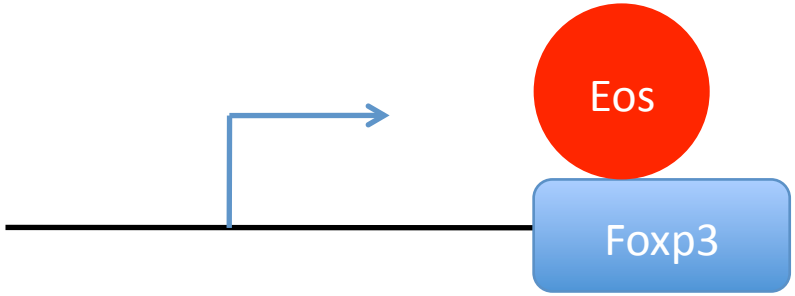
Introduction

- Foxp3+ regulatory T cells (Treg) are essential to maintain self-tolerance and under most circumstances, the role of Treg is clearly suppressive.
- However, in certain settings such as vaccination, graft rejection, Treg may also adopt a “helper-like” role.
- These “helper-like” Treg appeared to have lost Foxp3 expression.
- Previous studies have suggested that Foxp3 expression is highly stable in Treg cells once established (Miyao *et al;* Immunity 2012).

1. $Foxp3^+$ T cells contain a minor nonregulatory population
2. Th cells can be generated from $Foxp3^+$ non-Treg cells but not Treg cells
3. A few Treg cells transiently lose Foxp3 expression but retain its memory
4. The committed state of Treg cells is ensured by TSDR demethylation

- Thus, there has been some controversy over whether fully-committed Foxp3-lineage Treg cells ever transform into helper T cells.
- **Hypothesis: inducible transformation into helper cells might be a natural property of certain Treg and not a loss of lineage fidelity.**

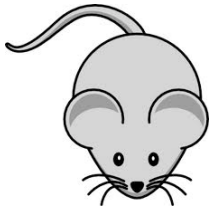
Eos: Foxp3 corepressor



1. Eos is a member of the Ikaros family of transcription factors that also include Aiolos and Helios.
2. Eos forms a complex with Foxp3 and is required for Foxp3 to inhibit its downstream target genes and maintain Treg suppressive phenotype.

Foxp3 is stable in reprogrammed Treg but Eos is downregulated.

OT-I CD8+ T cells (specific for OVA)
+
Whole OVA protein in incomplete Freund's adjuvant and CpG



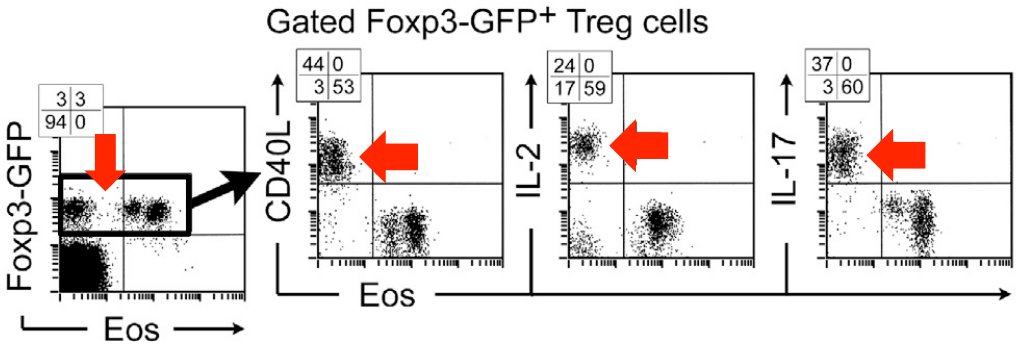
Foxp3^{GFP}

4 days

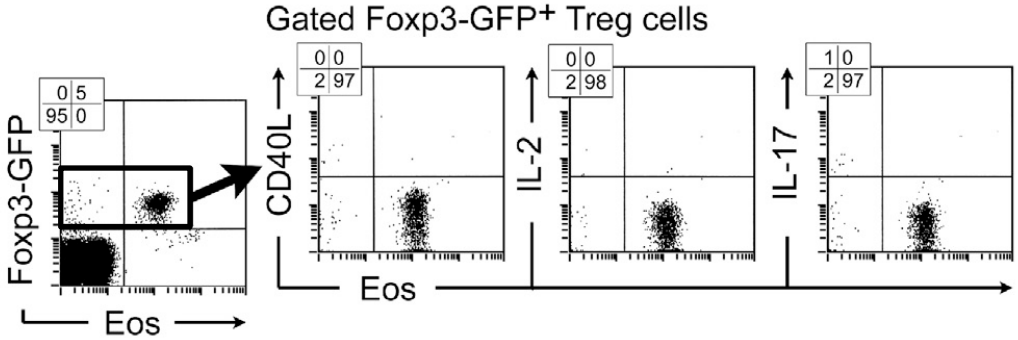
Treg stained for IL-2, IL-17 and CD40L

A

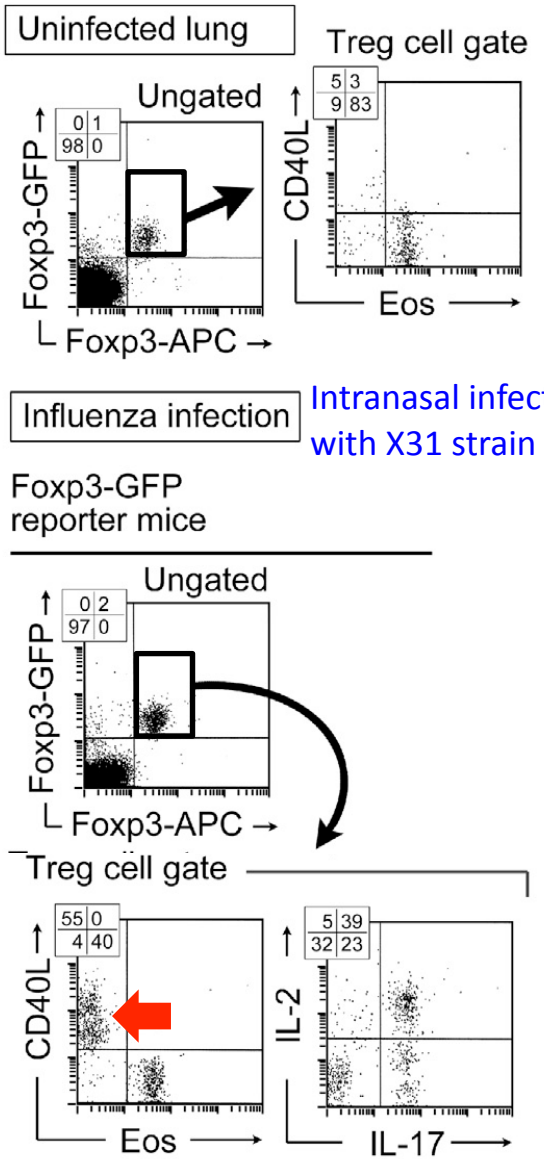
Vaccine-draining LN (VDLN)



Non-draining LNs (control)

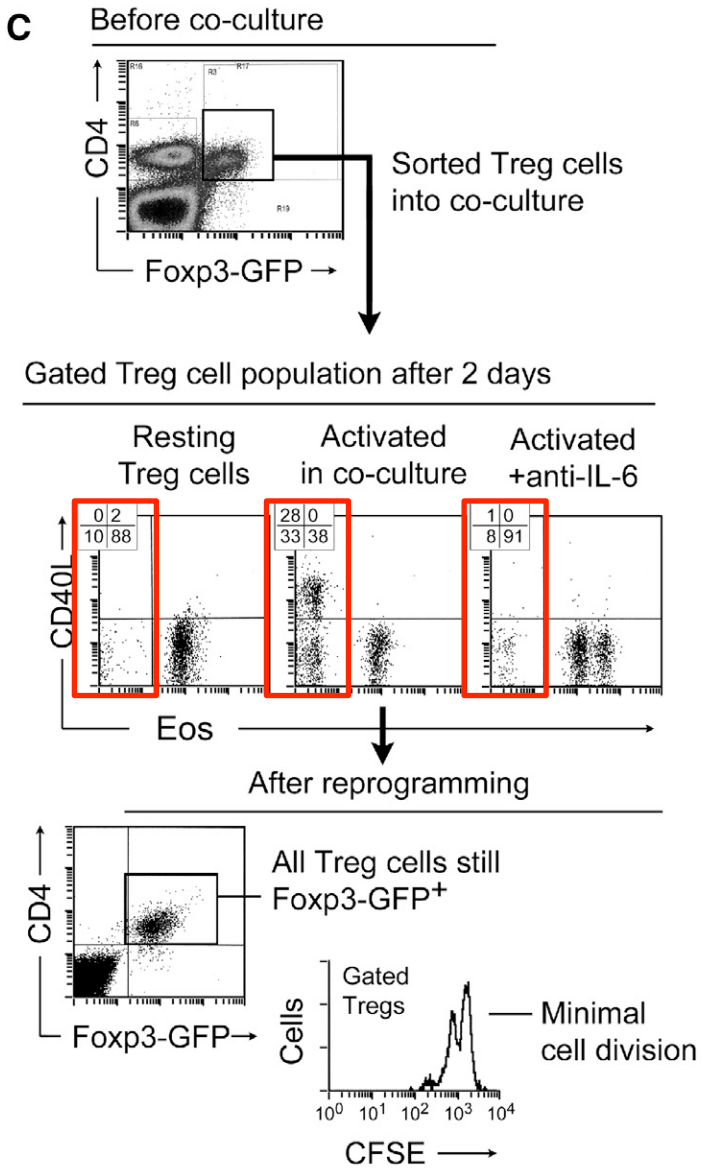


B



Downregulation of Eos requires IL-6

Splenic Treg co-cultured with DC from VDLN in the presence of OT-I effector T cells and cognate SIINFEKL antigen



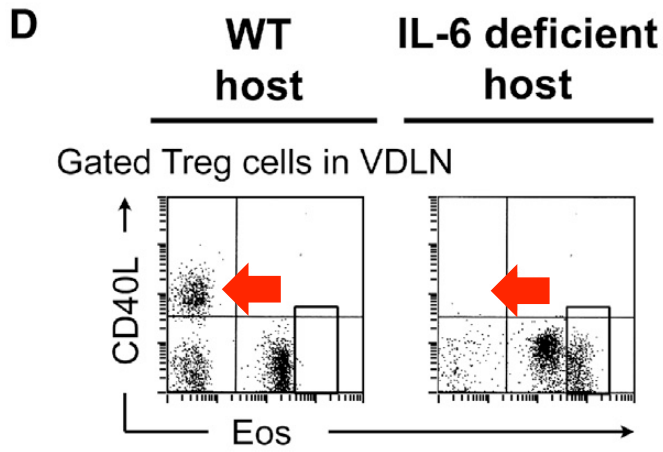
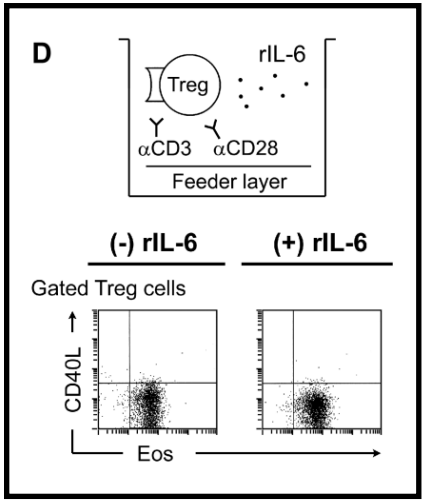
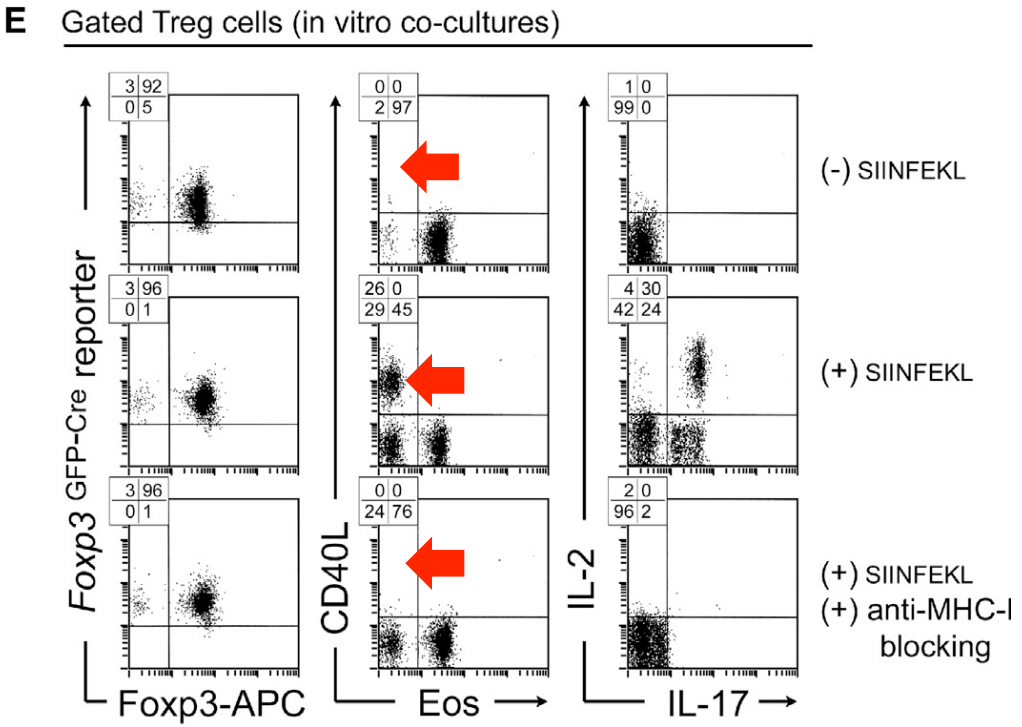
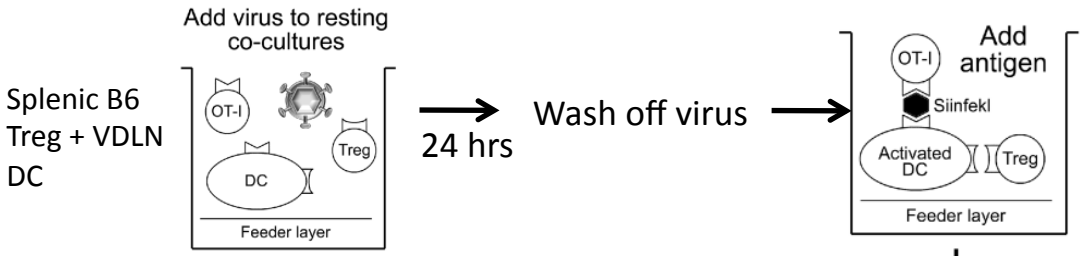


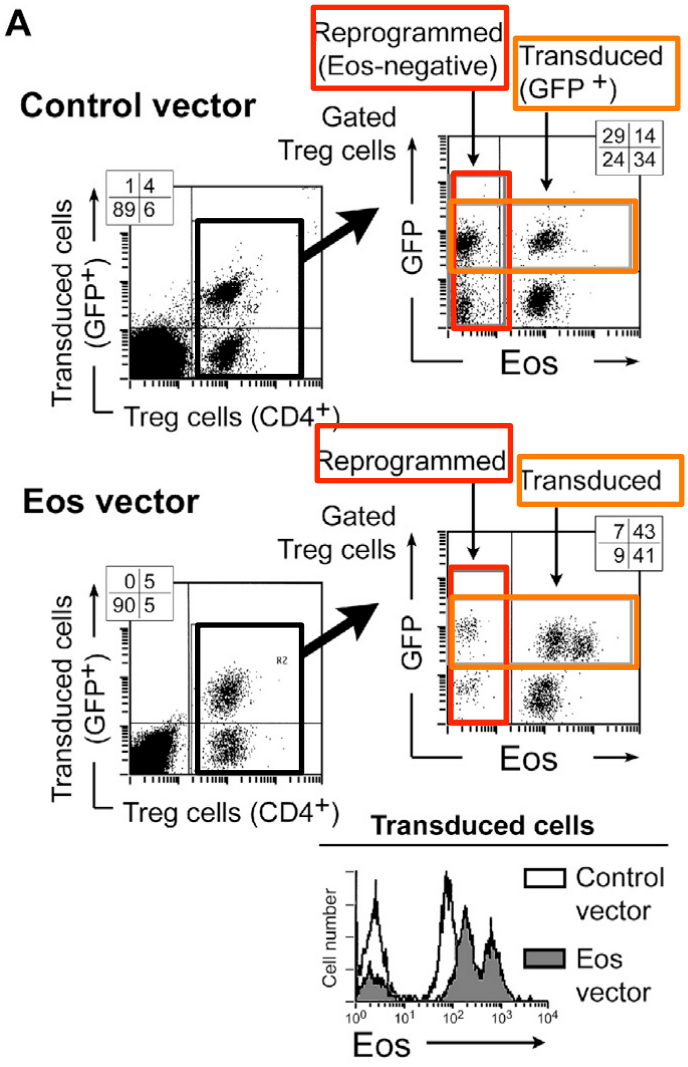
Fig S1D

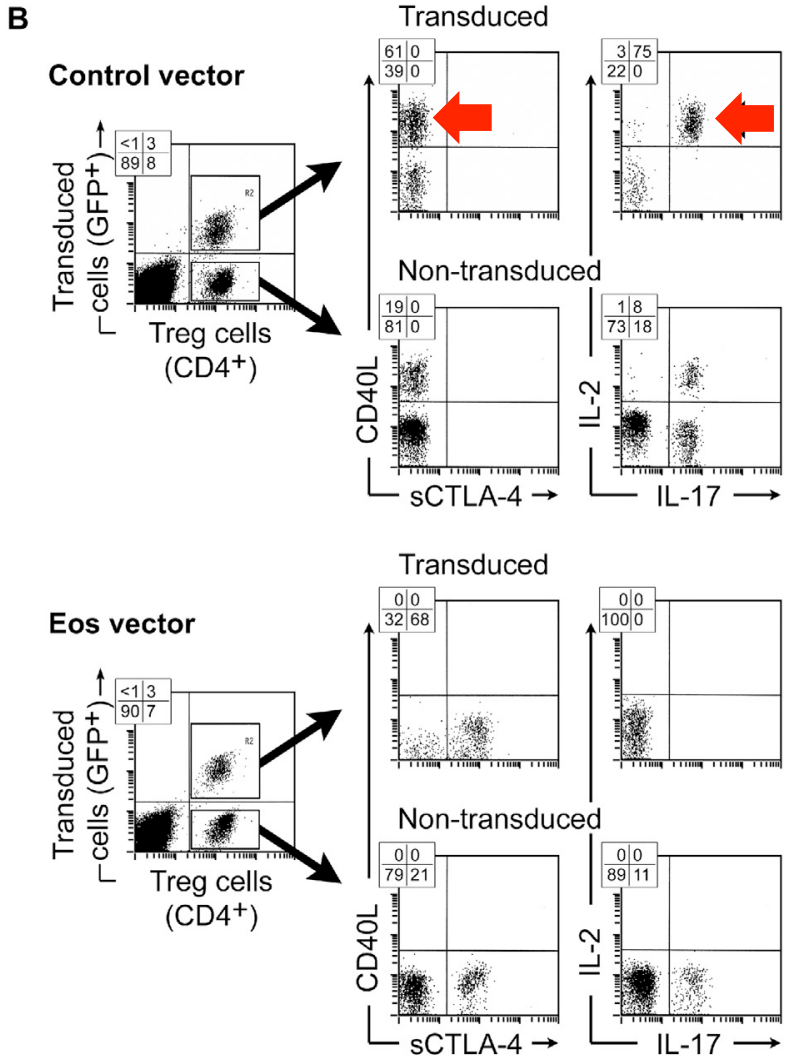




Forced overexpression of Eos in Treg prevents reprogramming





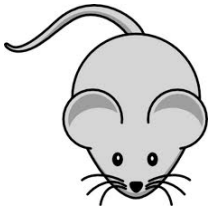


Forced overexpression of Eos abrogates the functional helper activity of reprogrammed Treg

CD40L sufficient *Foxp3*^{GFP} Thy1.1 Treg
or
non regulatory CD4^{+} T cells

+

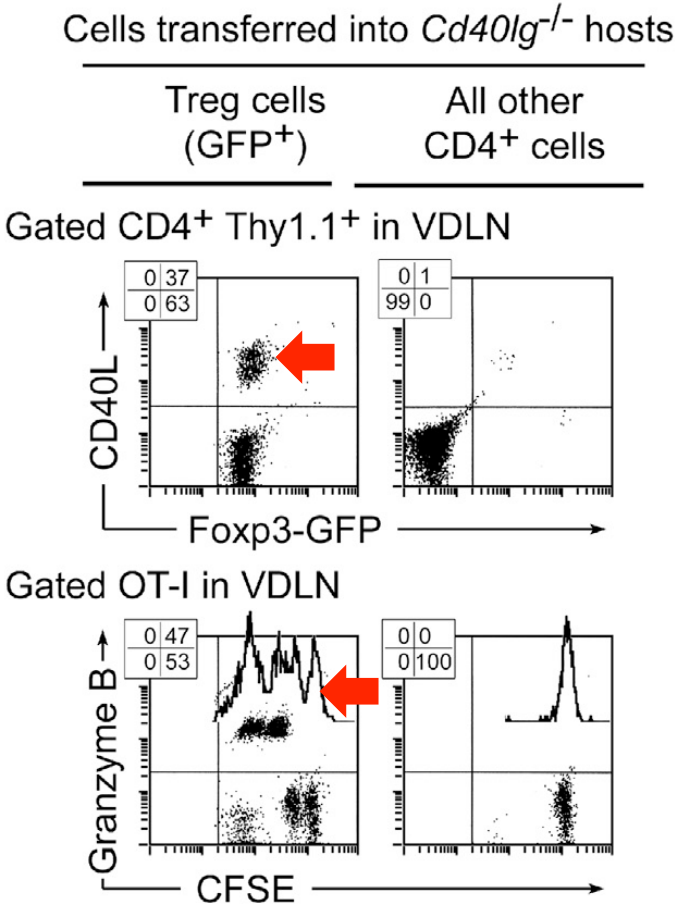
CFSE labeled OT-I cells plus
OVA vaccine



Cd40lg$^{-/-}$ mice

CD40L is critical in order to license DC and CD40lg-/- mice are unable to prime naïve CD8+ T cells to OVA protein

D

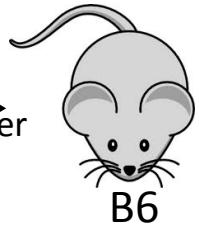


Eos-labile phenotype identifies the subset of Treg capable of functional reprogramming

Identification of a specific subset of Treg with labile Eos expression

Sort thymic Treg according to CD38 and CD103

Cell transfer

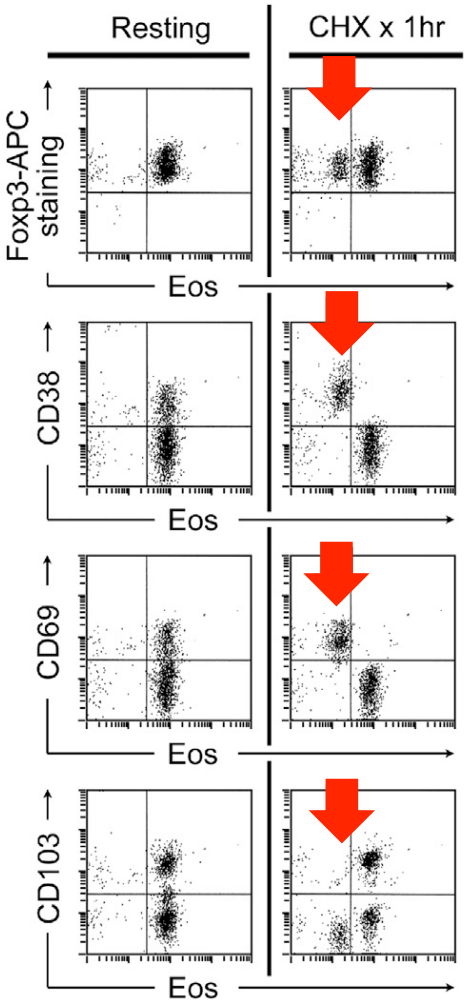


Challenge mice with OT-1 and vaccine

A

Thymic Treg cells (gated Foxp3-GFP$^+$)

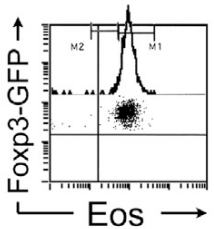
CHX: blocks new protein synthesis



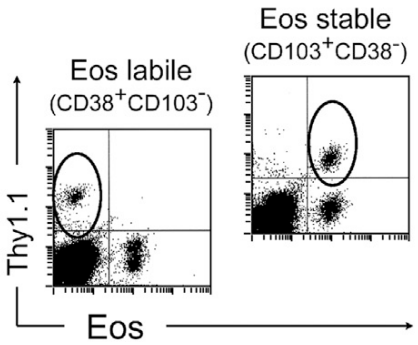
Same level of Foxp3

C

Thymic Treg cells before sorting



Treg cell subsets after sorting → transfer → vaccination



4 days after transfer
Transferred Treg cells in VDLN (gated Thy1.1$^+$GFP$^+$)

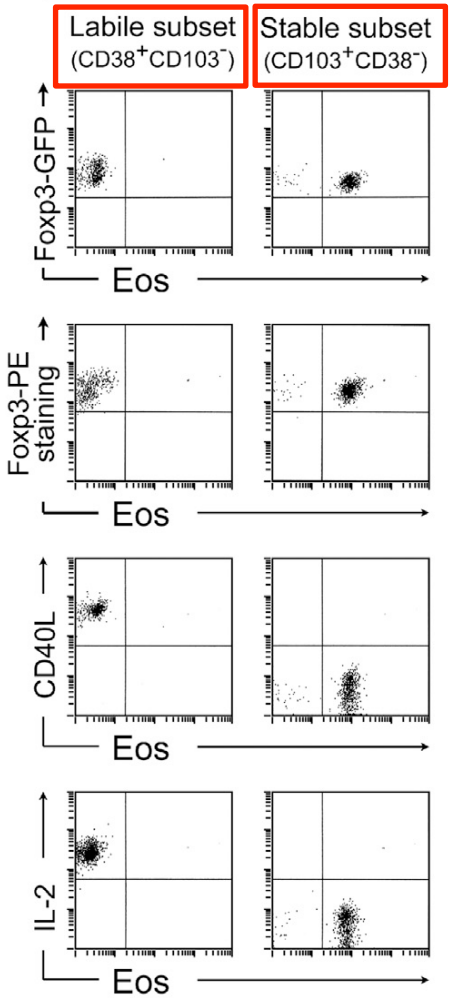
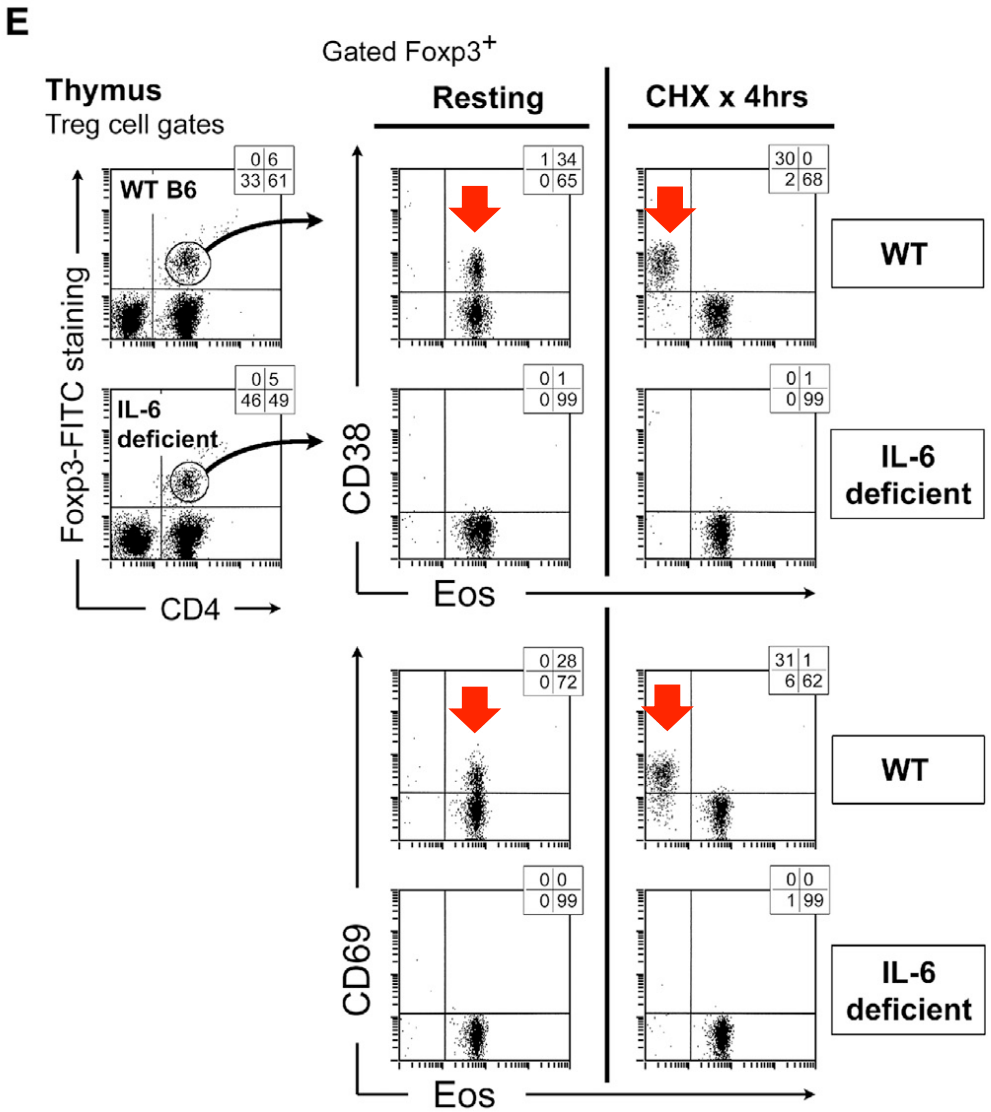


Fig 3D: Eos labile Treg functionally rescued OT-I priming but not Eos stable Treg

Eos-labile subset requires IL-6 for intrathymic development

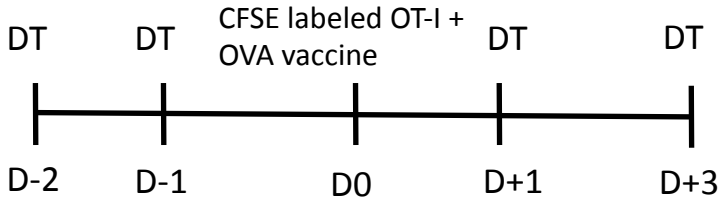
From Fig 1C, IL-6 is an important driver of functional reprogramming



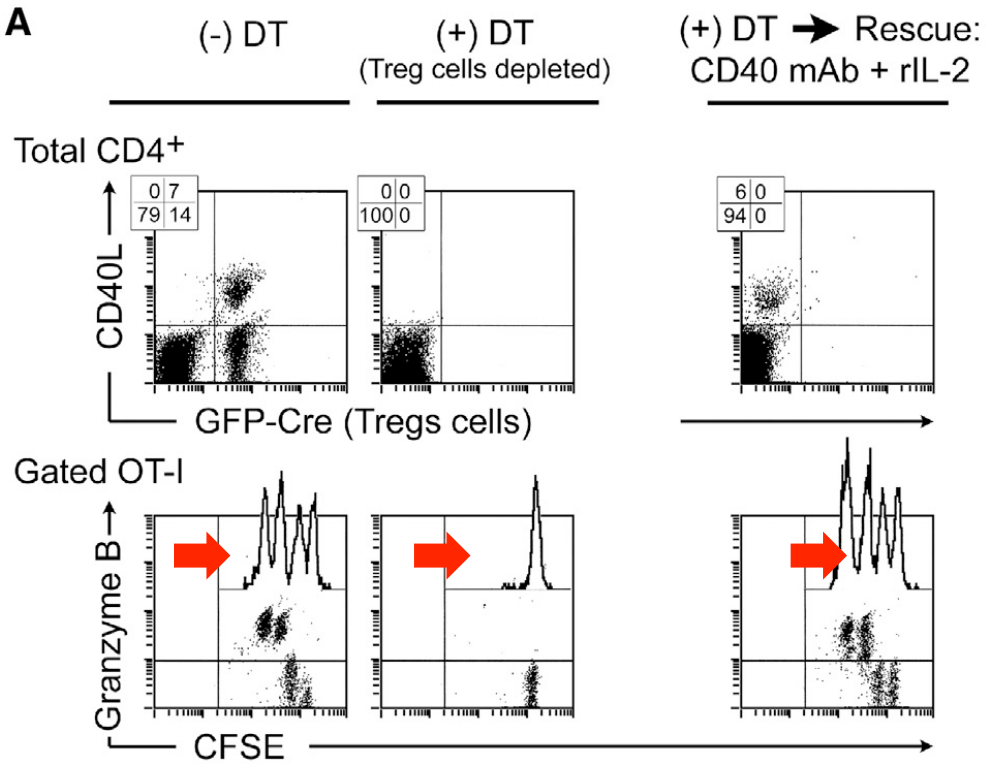
Help from Eos-labile Treg is required during priming of naïve mice to new antigen

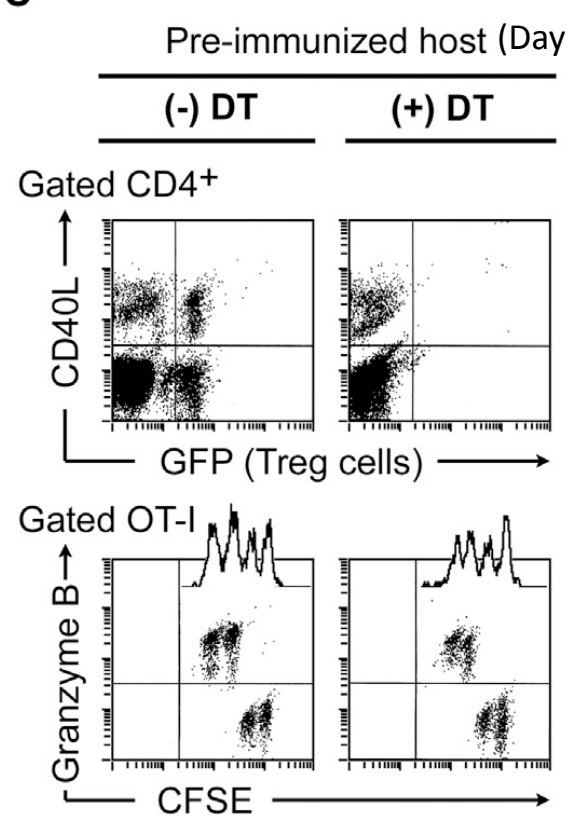
*Foxp3*GFP-Cre x ROSA26$^{lox\text{-}stop\text{-}lox}$ *DTR*

DT receptor on all cells that have activated the *Foxp3* promoter



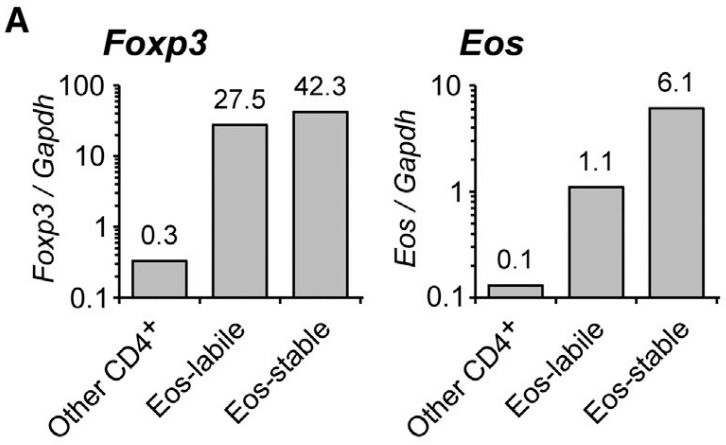
With OVA vaccine (prime + boost x2), then rechallenged with CFSE labeled OT-I cells + OVA vaccine

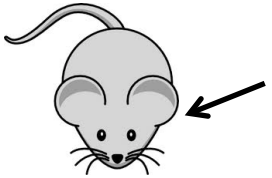




Tregs no longer needed for priming when memory T cells present

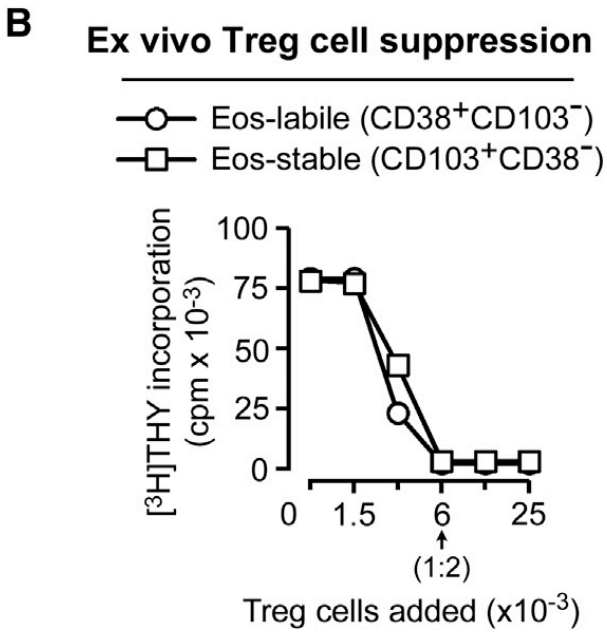
Eos labile cells are functionally suppressive Treg

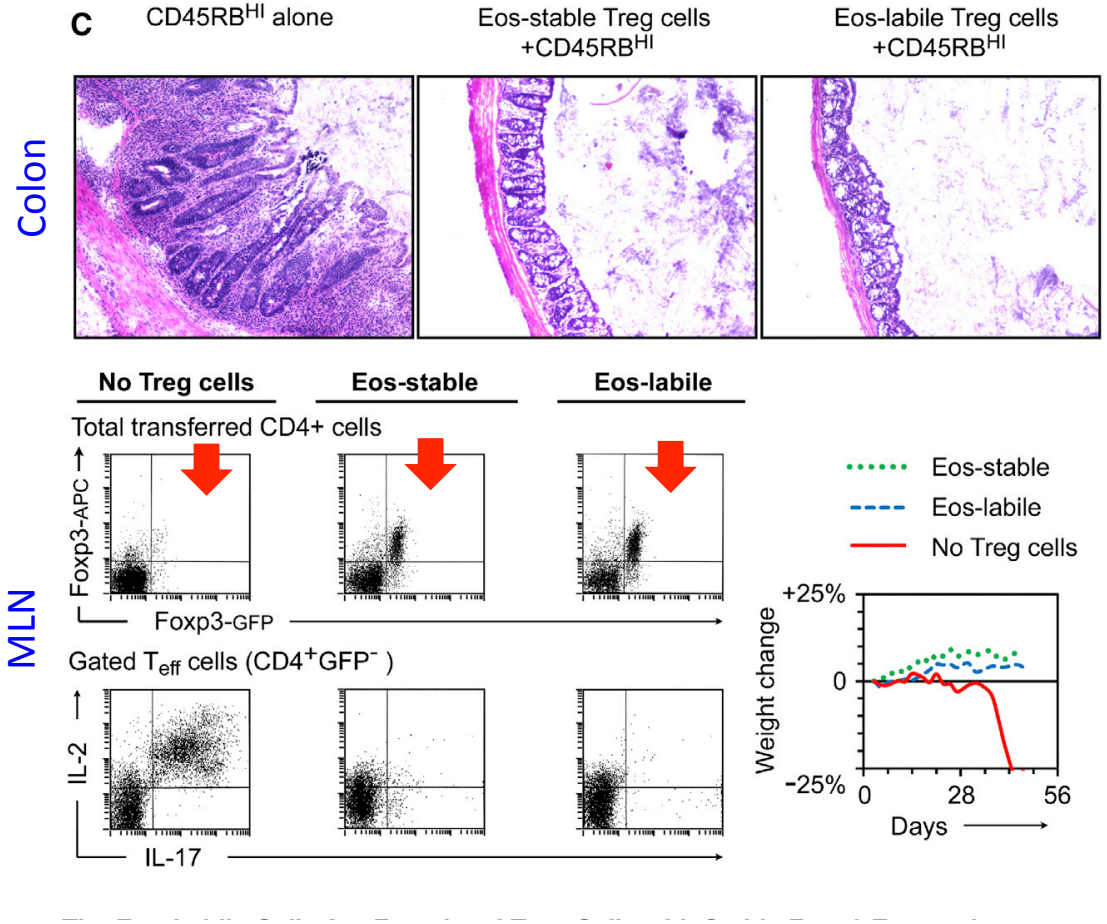




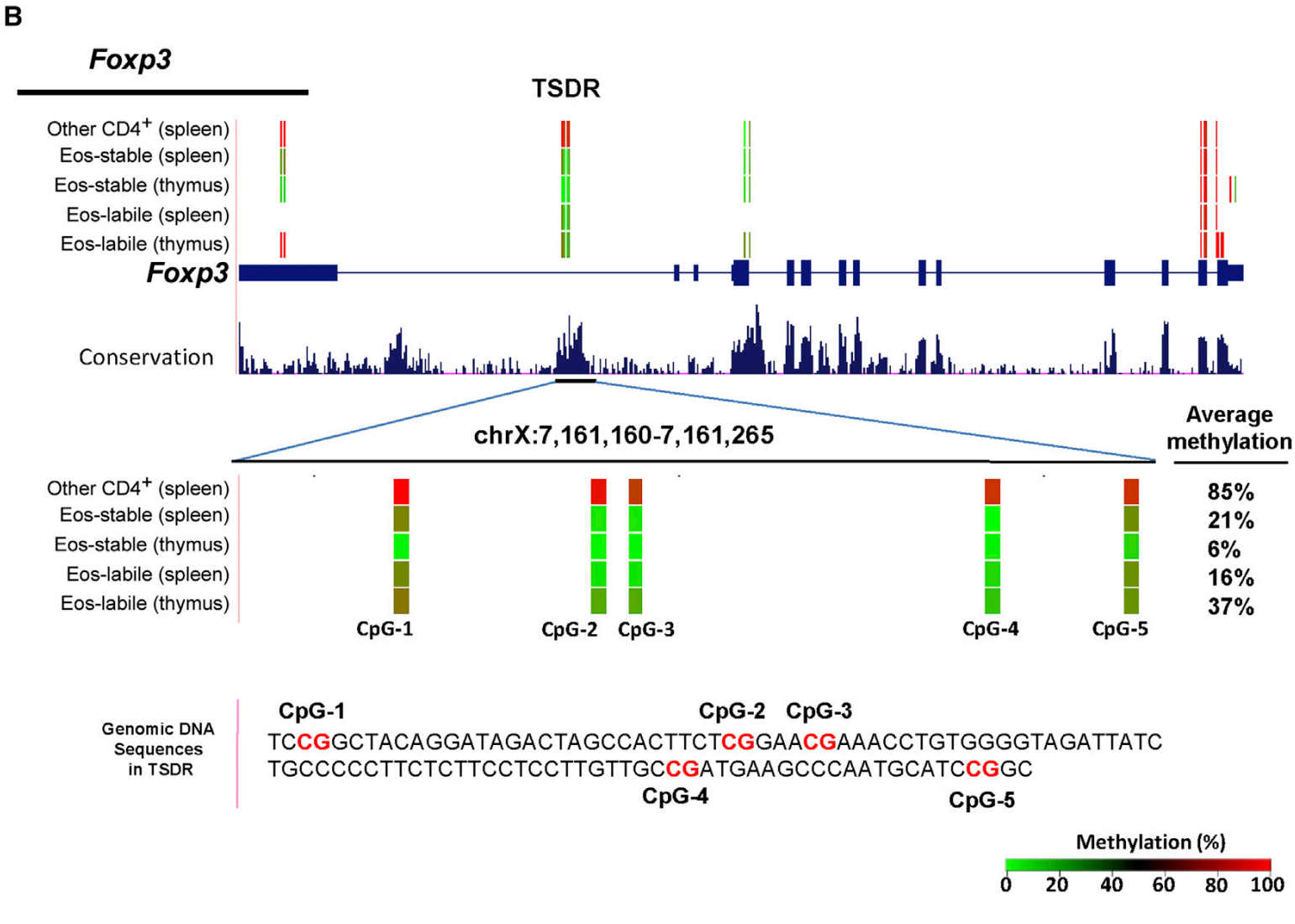
Rag2-/- mice

CD4^{+}CD45RB^{hi} with or without sorted Treg





Eos-labile Treg are distinct from Eos-stable Treg by epigenetic analysis



Eos-labile are less demethylated at the TSDR than Eos-stable Treg but both showed greater demethylation than non-Treg

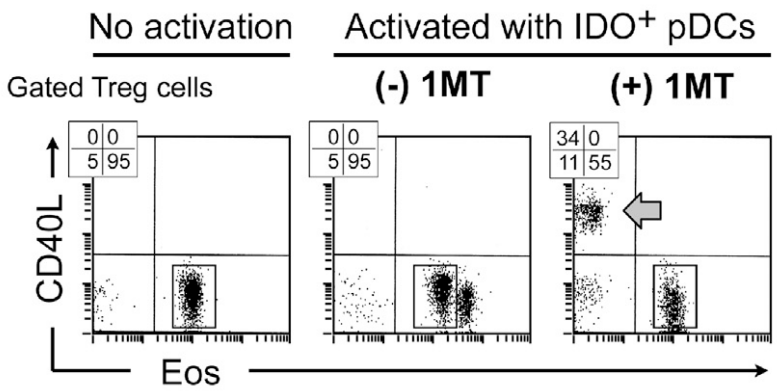
Downregulation of Eos is prevented by IDO

pDCs with high IDO expression isolated from TDLN from mice with B16F10 tumors and cocultured with *Foxp3*^{GFP} Treg in the presence of SIINFEKL

Foxp3GFP mice with tumors were treated with tumor specific CFSE labeled pmel-1 T cells + hgp100 peptide vaccine in IFA+CpG

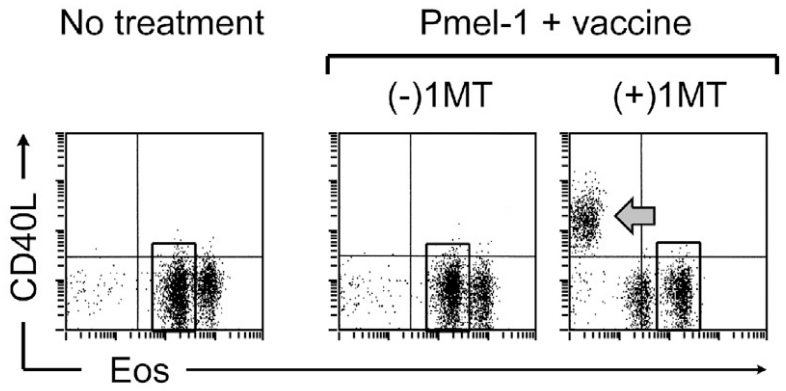
A

MT: inhibitor of the IDO pathway

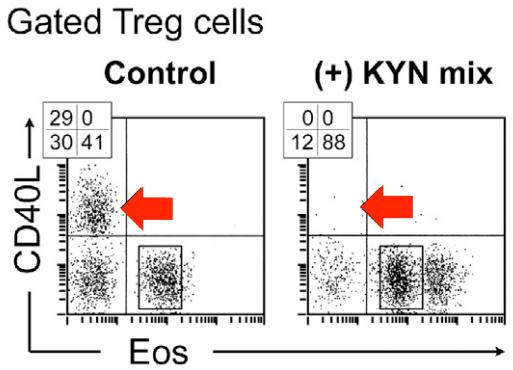


B

Treg cells in tumor-draining LN



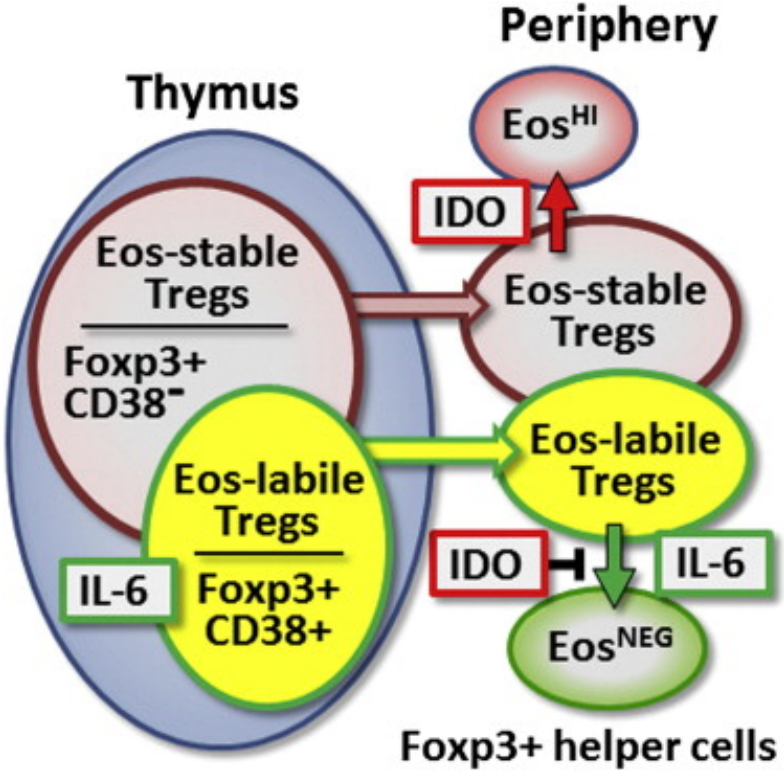
D



KYN: Kynurenine-pathway metabolites

DCs from VDLN with low IDO expression used for co-culture

Discussion and conclusions



- Current study identifies a committed subset of reprogrammable Treg cells characterized by rapid loss of corepressor Eos.
- Absence of Eos-labile Treg cell subset selectively compromised the ability of naïve mice to activate resting DCs and prime initial T cell responses.
- Reprogrammed Treg provide rapid help. This property of Foxp3+ lineage allows Eos-labile Treg to act as “first responders”, delivering help when non regulatory T cells have not yet had time to activated.

LETTER

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BACH2 represses effector programs to stabilize T_{reg}-mediated immune homeostasis

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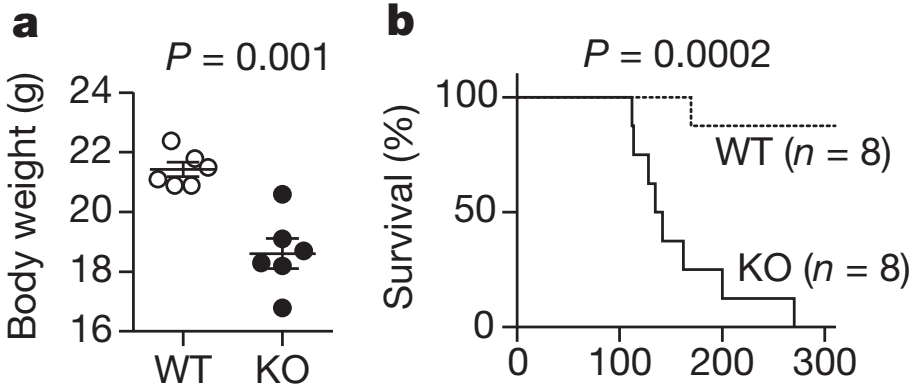
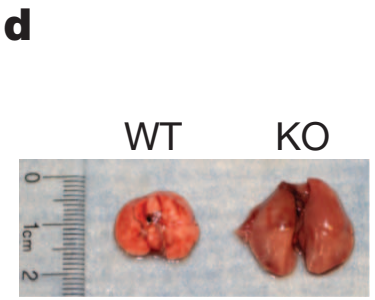
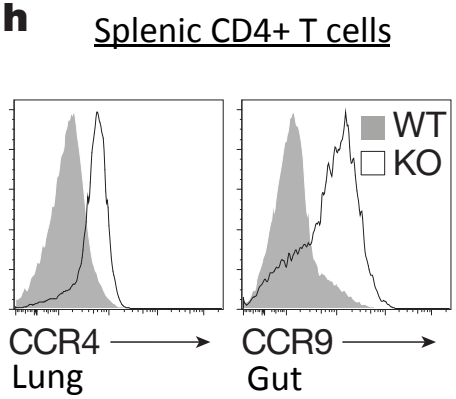
Introduction

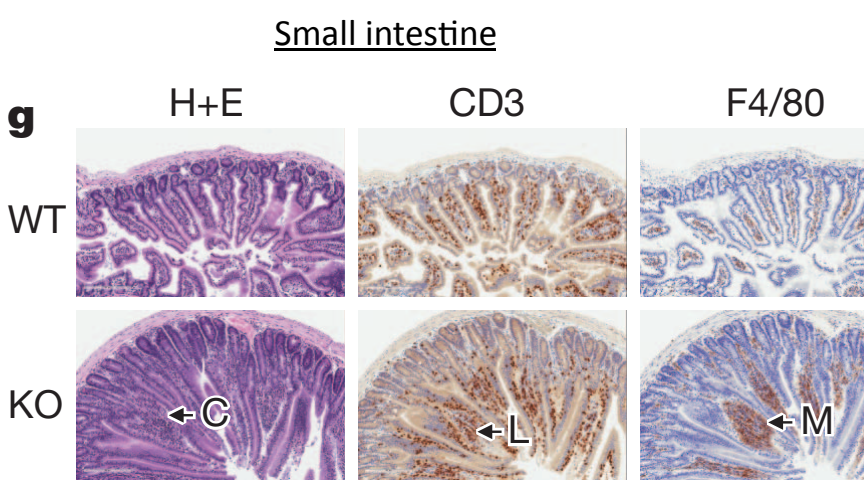
- BACH2 is expressed in activated B cells where it acts as a transcriptional regulator of Blimp-1
- It is critical for somatic hypermutation and class switch recombination, as well as for the efficient formation of germinal centres.
- Genetic polymorphisms within a single locus encoding BACH2 are associated with numerous autoimmune and allergic diseases.
- However a function of BACH2 in the maintenance of immune homeostasis has not been established.
- It is proposed that BACH2 may have a role in the prevention of inflammation.

Bach2 KO animals develop spontaneous lethal inflammation

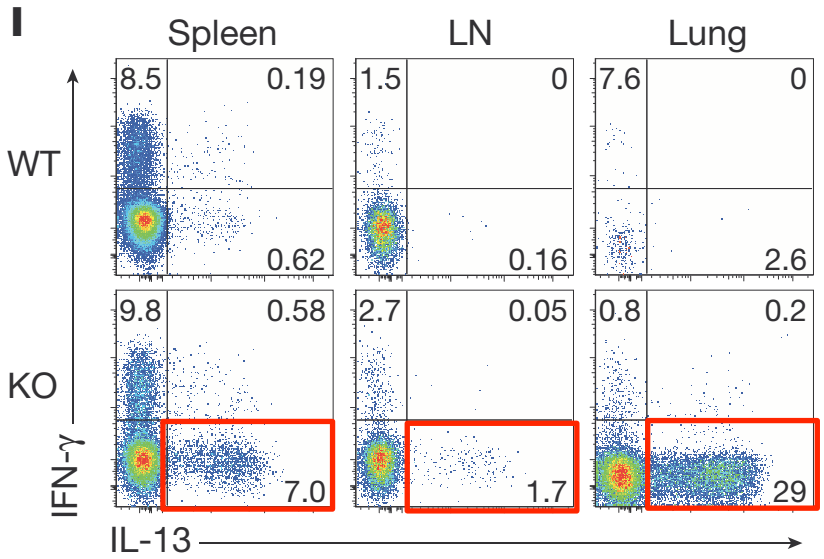




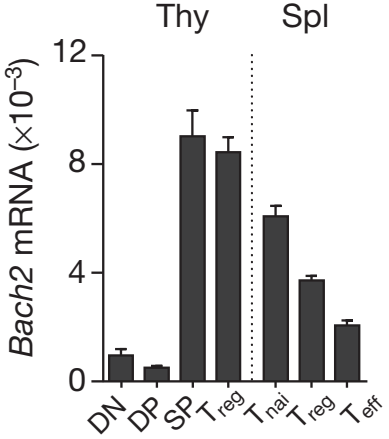




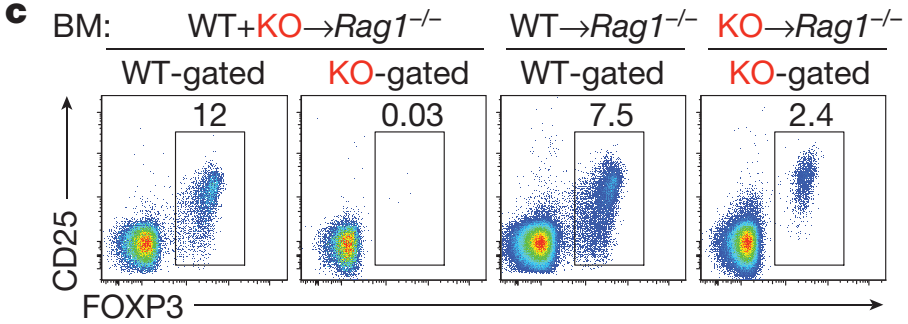
C: Hypertrophic crypts
L: lymphocytic infiltration
M: Macrophage infiltration



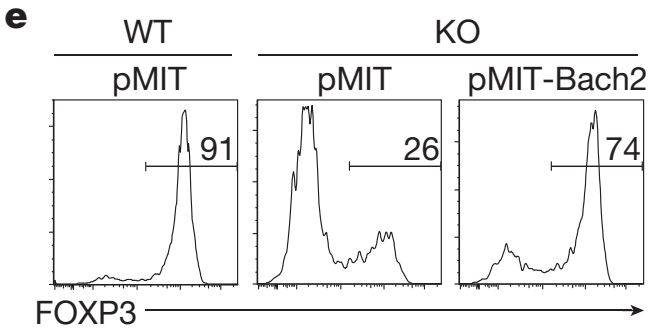
BACH2 is required for efficient formation of Treg



Foxp3 expression in CD4 SP thymocytes

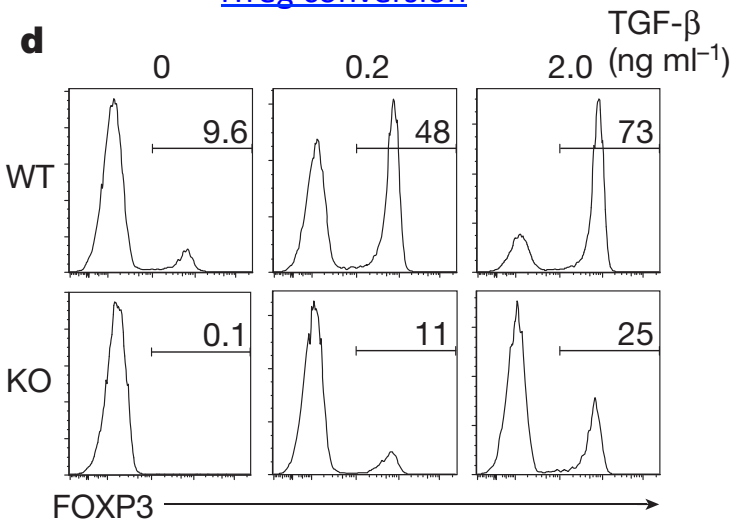


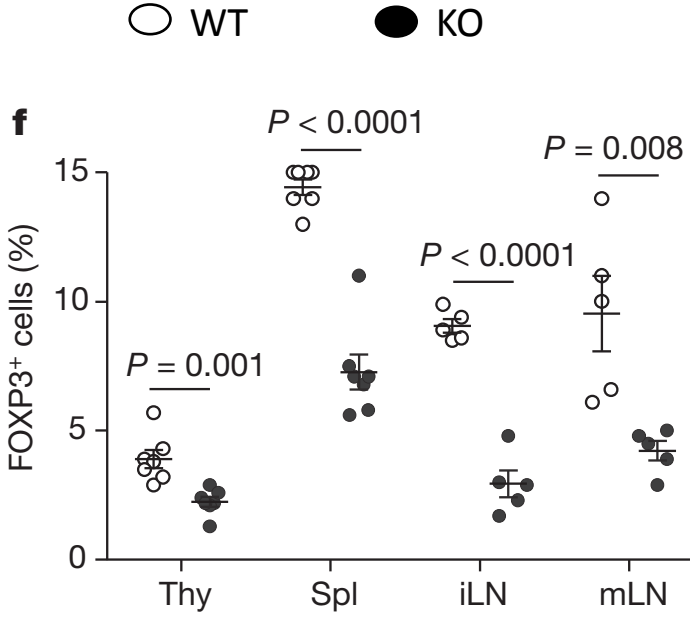
Lineage depleted BM cells: Ly5.1+ WT and Ly5.1- KO



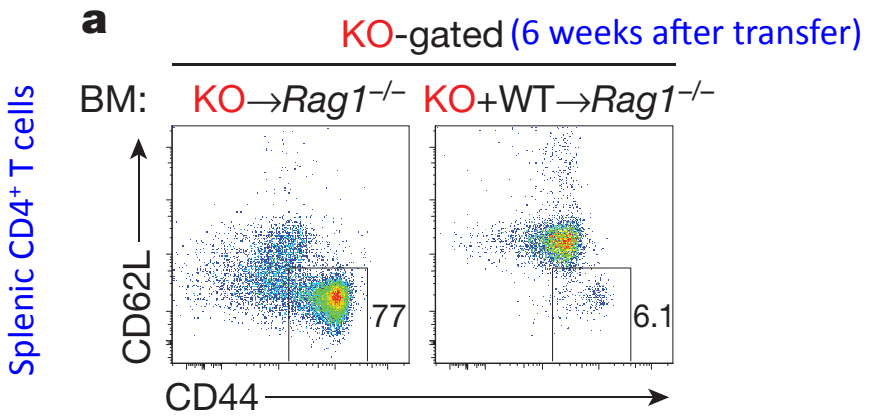
Thy1.1+ (transduced) naïve splenic CD4+ T cells stimulated with 2 ng ml^{-1} TGF-β and transduced with indicated retroviruses

iTreg conversion

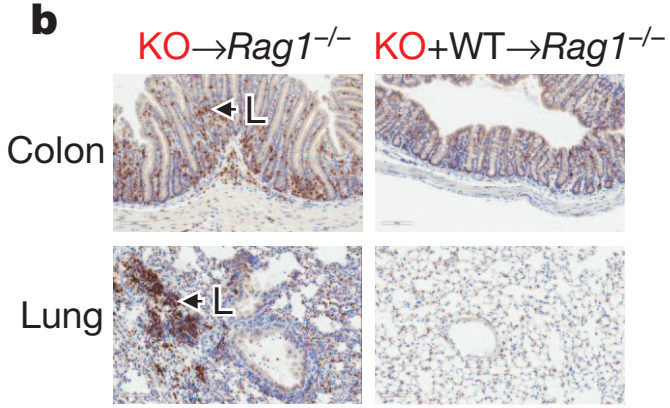




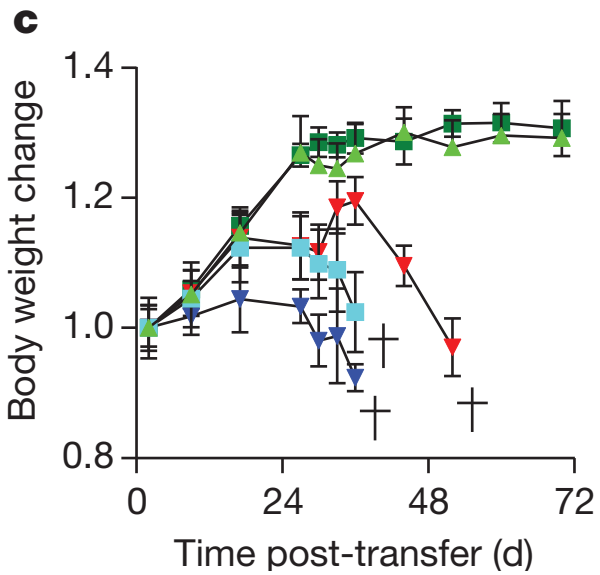
BACH2 is required for suppression of lethal inflammation in a Treg dependent manner



CD3 staining 6 weeks following BM reconstitution



L indicate KO T cells

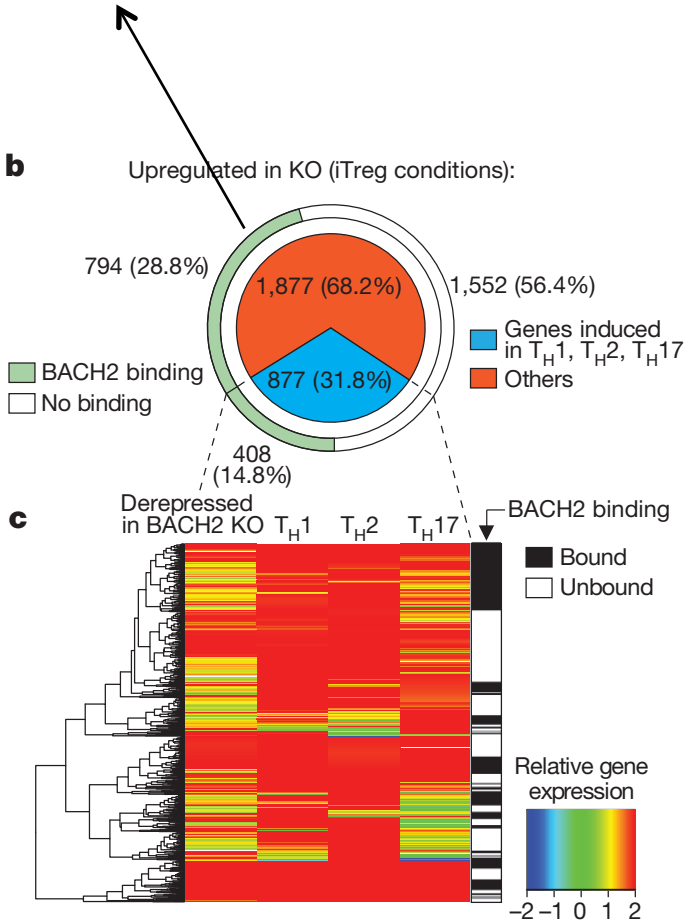


	BM			T_{reg}
	KO	WT	Sf	WT
▼	●			
■	●	●		
■	●		●	
▲	●			●
▼			●	

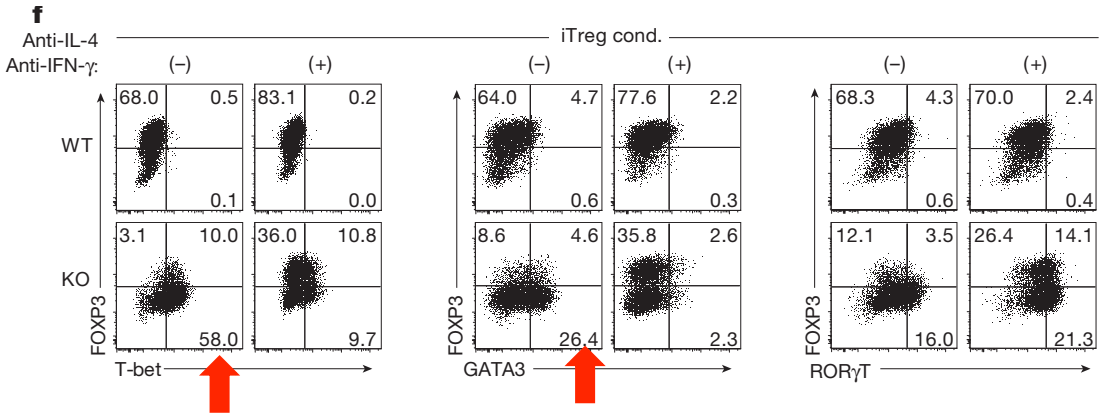
Immunoregulatory effect exerted by Bach2-sufficient BM was dependent upon Foxp3

BACH2 represses effector programs to stabilize iTreg cell development

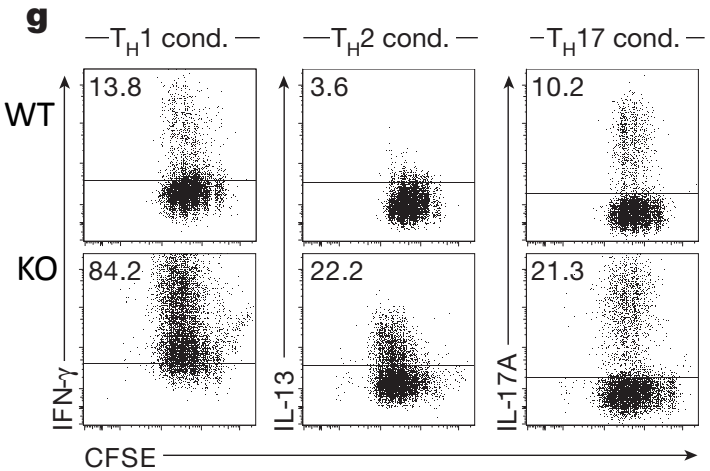
Outer arc: genes that are directly bound by BACH2 in iTreg



Heat map indicating expression of effector-lineage associated transcript derepressed in KO cells



KO cells stimulated under iTreg conditions preferentially differentiated into FOXP3- cells expressing T-bet, GATA3 or RORγt



Discussion and conclusions

- BACH2 represses the differentiation programs of multiple effector lineages in CD4+ T cells.
- By doing so, BACH2 stabilizes the development of Treg while limiting full effector differentiation in conventional T cell lineages.
- Thus BACH2 functions to constrain immune activation, enabling it to play a critical role in the maintenance of immune homeostasis.