Memorial Sloan Kettering Cancer Center

A primer to early T cell development for cancer biologists

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Cancer Biology GSK course
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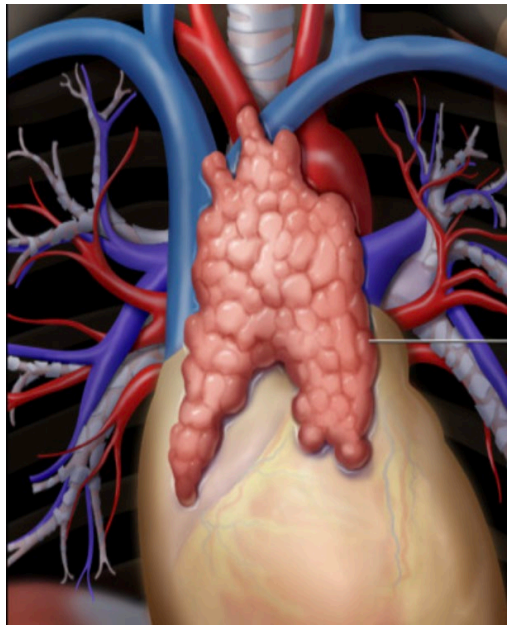
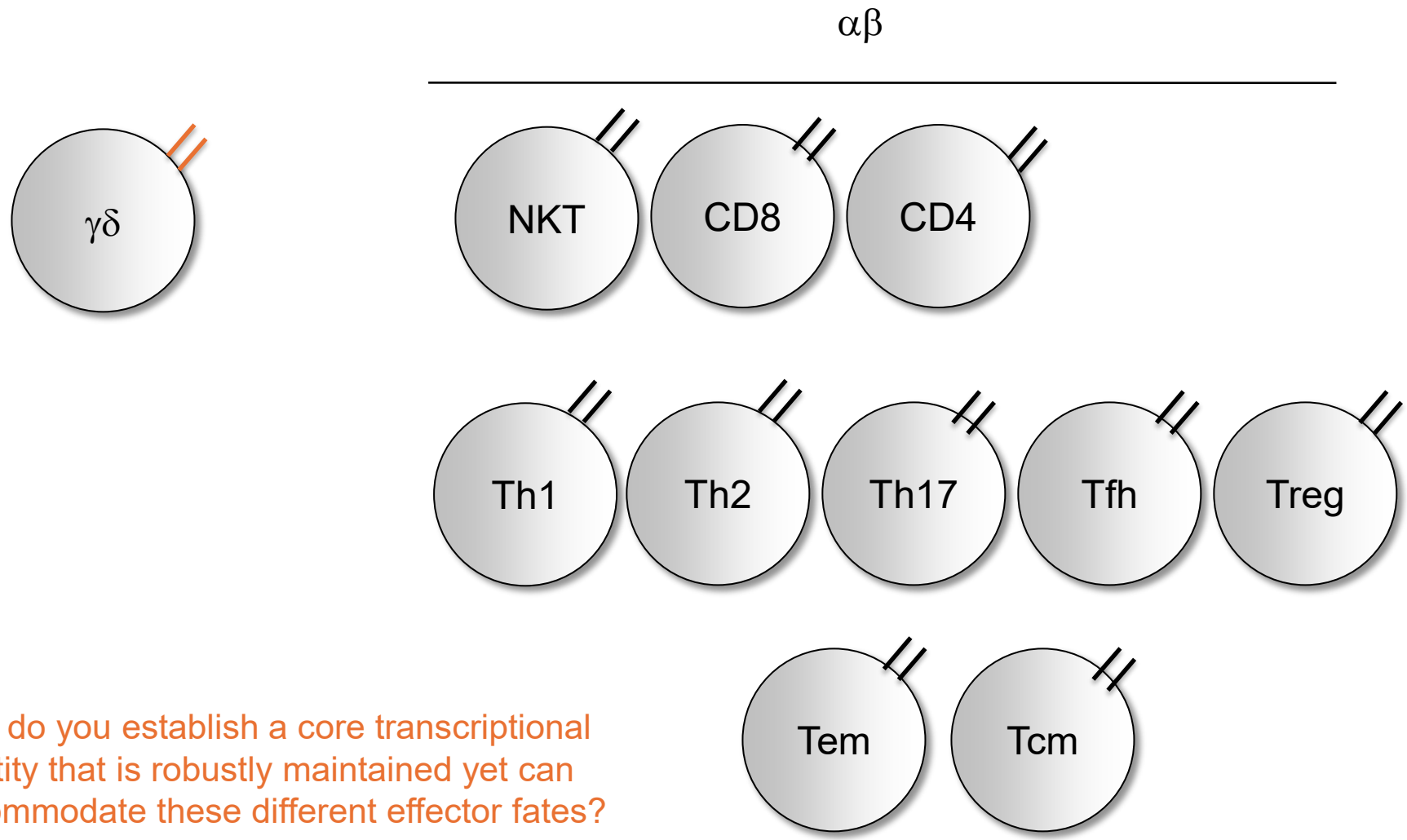
Unlike all other blood cell types that develop in the bone marrow …

(nearly all) T cells develop in the thymus



The T cell family

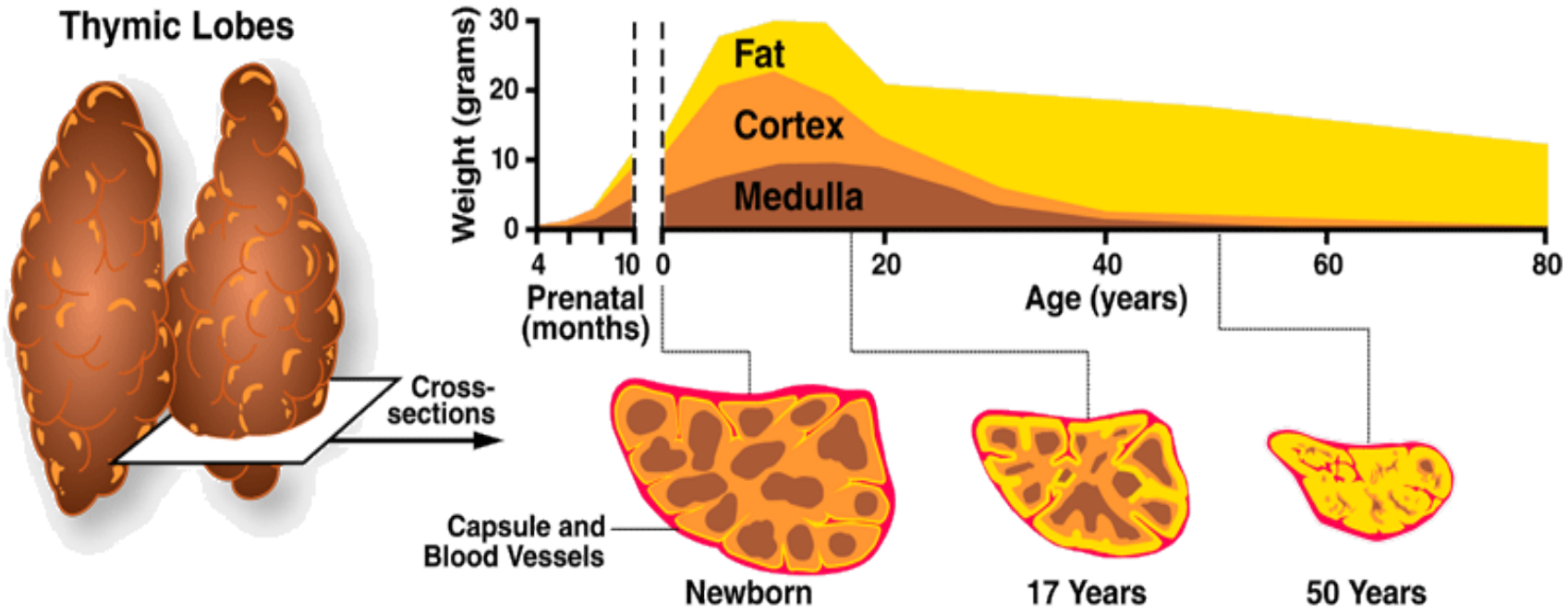


How do you establish a core transcriptional identity that is robustly maintained yet can accommodate these different effector fates?

The thymus is necessary for T cell development

Athymic “Nude mice”
Foxn1$^{nu/nu}$

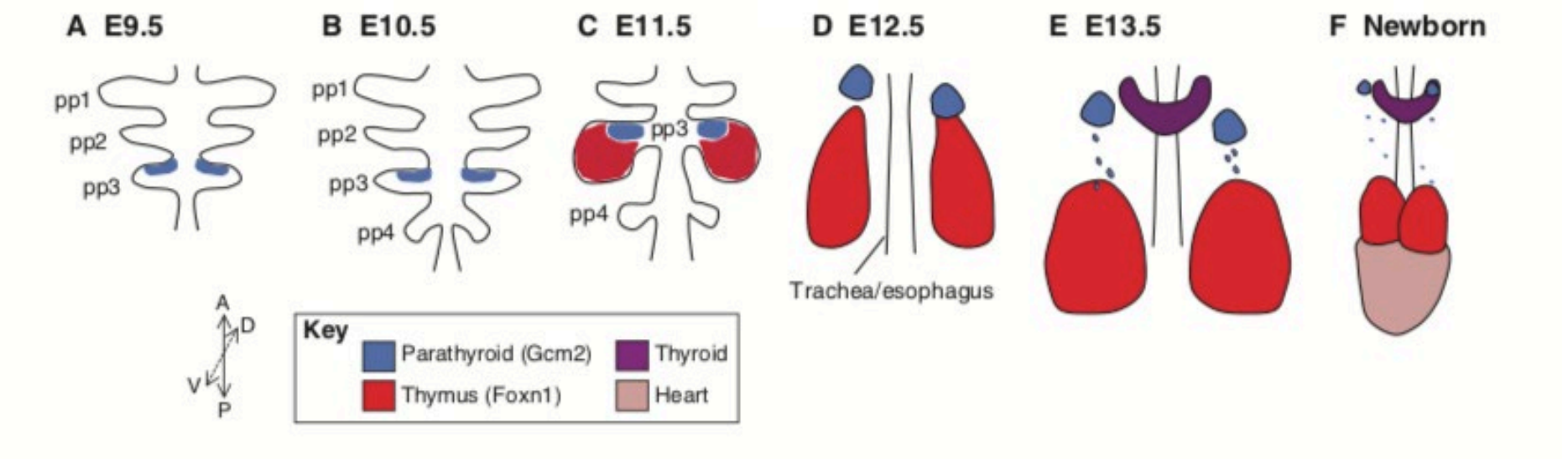
The thymus involutes with age



The causes are unknown.
The consequences can be surmised but also remain largely unknown.

Image from L. Napolitano (UCSF)

The thymus derives from pharyngeal endoderm



N.B. Di George syndrome
Hypoplastic thymus + parathyroid hypoplasia and other abnormalities
Defective pharyngeal arch development
Del22q11.2 (including TBX1 loss)

Image from Gordon and Manley, *Development* 2011

Foxn1 is a master regulator in the development, maintenance and function of thymic epithelial cells

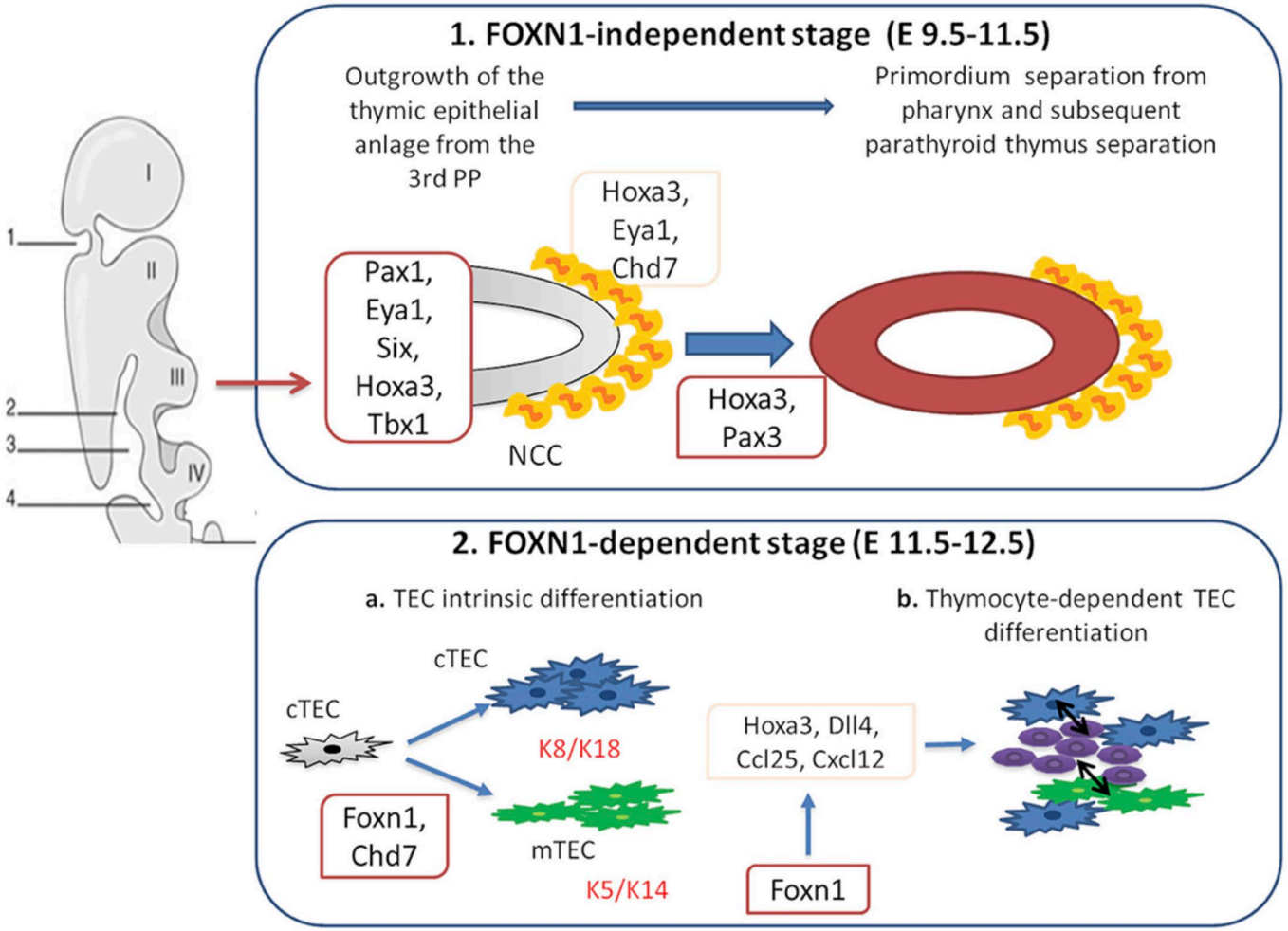
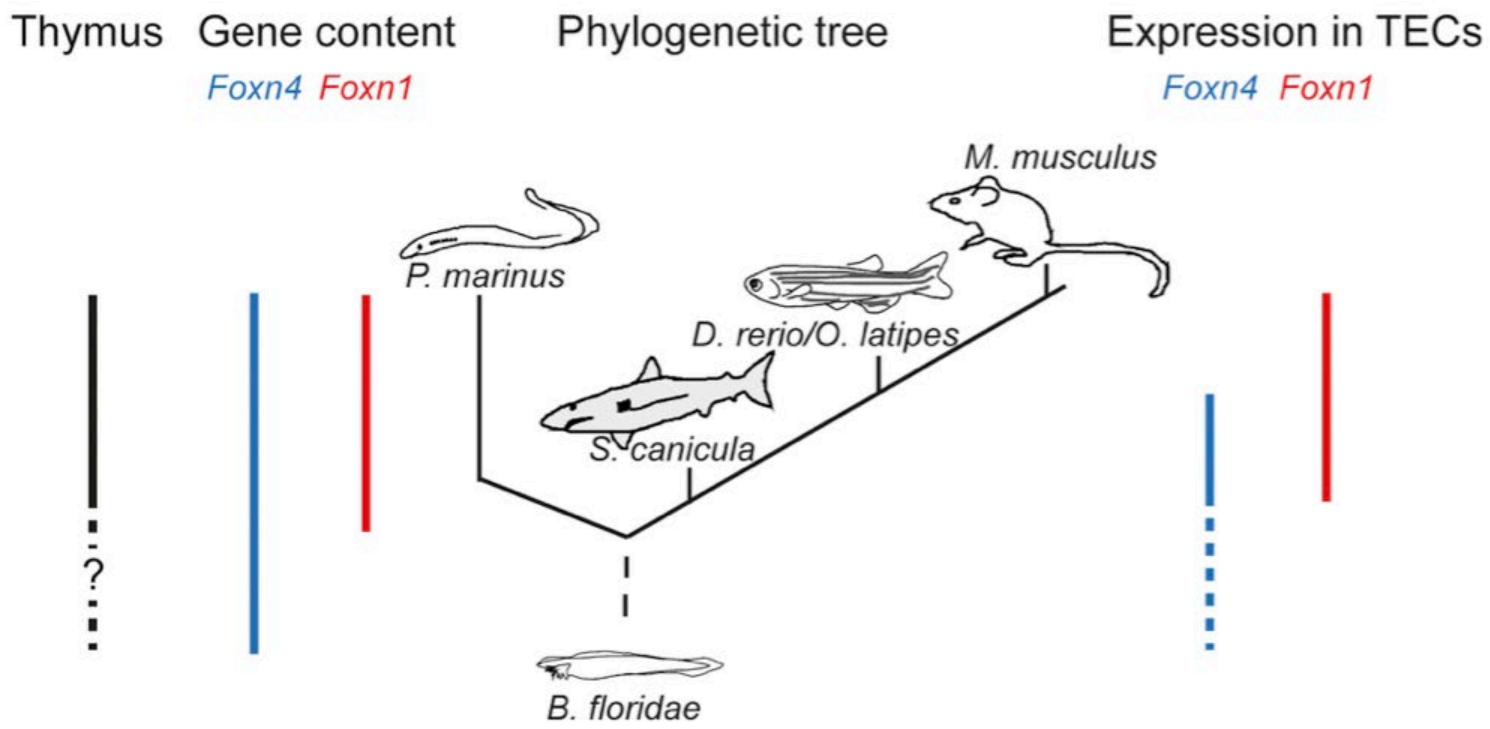


Image from Giardino et al., *Front Immunol* 2020

The Foxn1 TEC program has an ancient evolutionary history



Swann et al., *Cell Reports* 2014

T Cell Development and the Thymus

Some important questions

How is the thymus necessary for T cell development?
How does the thymus imprint MHC restriction?
What is thymic education and selection?
Why is positive selection necessary?
How is negative selection effected?
How is efficient and specific negative selection even possible?

Why is T cell development geographically isolated?

Key milestones in T cell development

T lineage commitment	*Irreversible engagement into T lineage differentiation*
β selection	*Selection of thymocytes that have successfully rearranged a functional TCR beta chain*
Positive selection	*Selection of thymocytes that have successfully rearranged functional TCR alpha and beta chain capable of interacting with MHC*
Negative selection	*Elimination of thymocytes with autoreactive TCRs (vs. diversion into the regulatory T cell lineage)*

CD8$^+$ and CD4$^+$ $\alpha\beta$ T cells

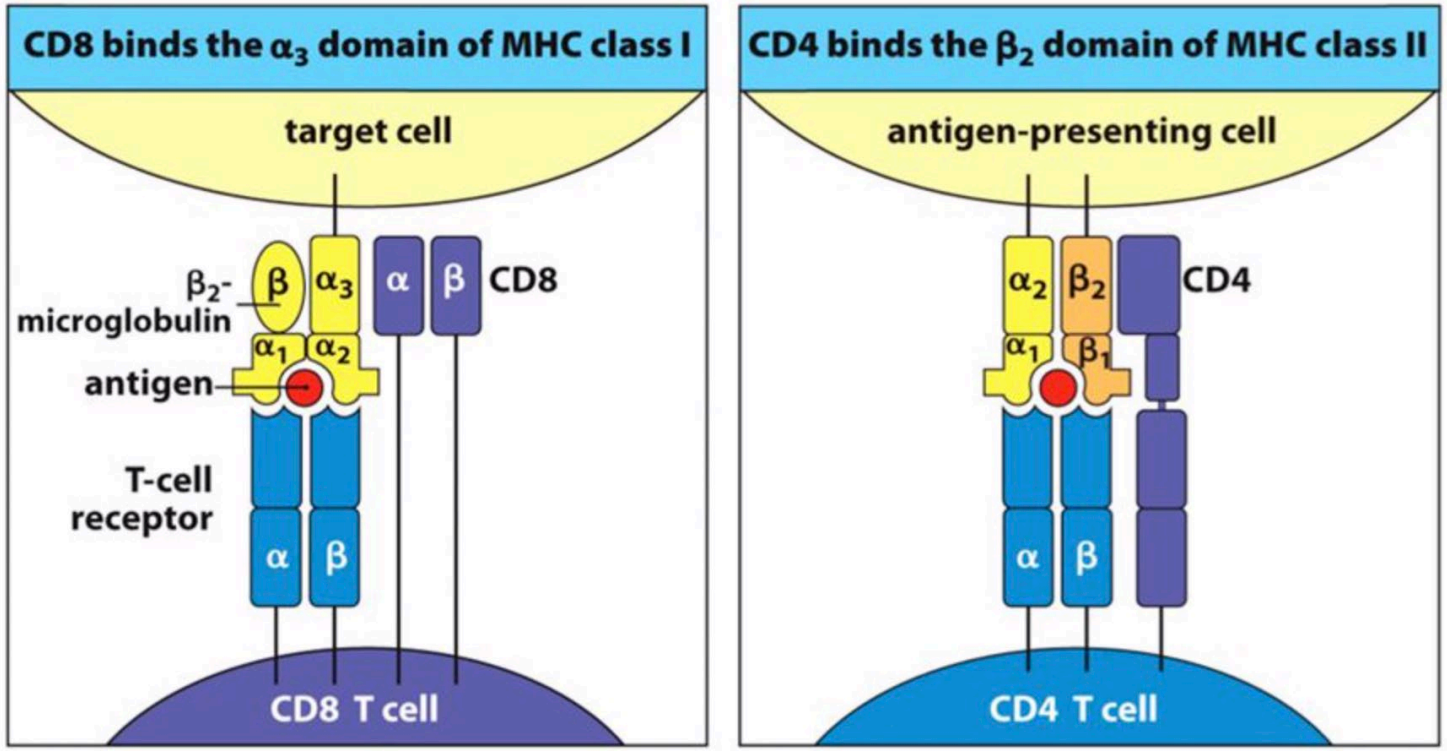
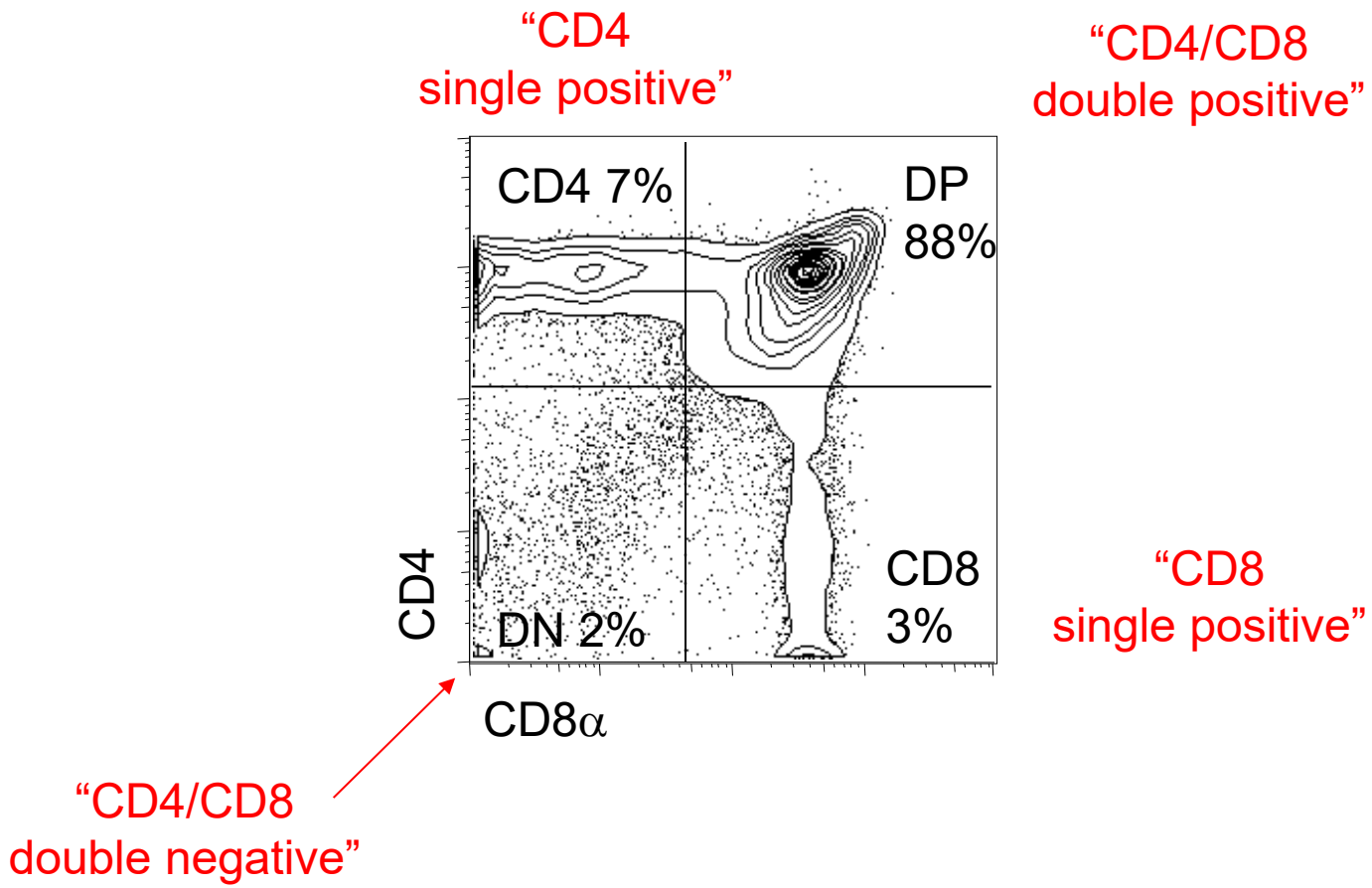
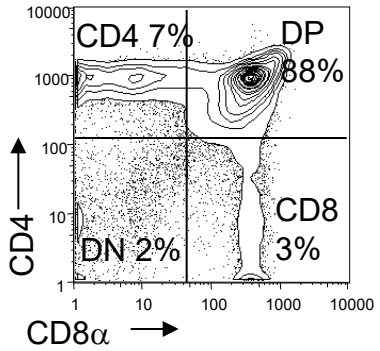


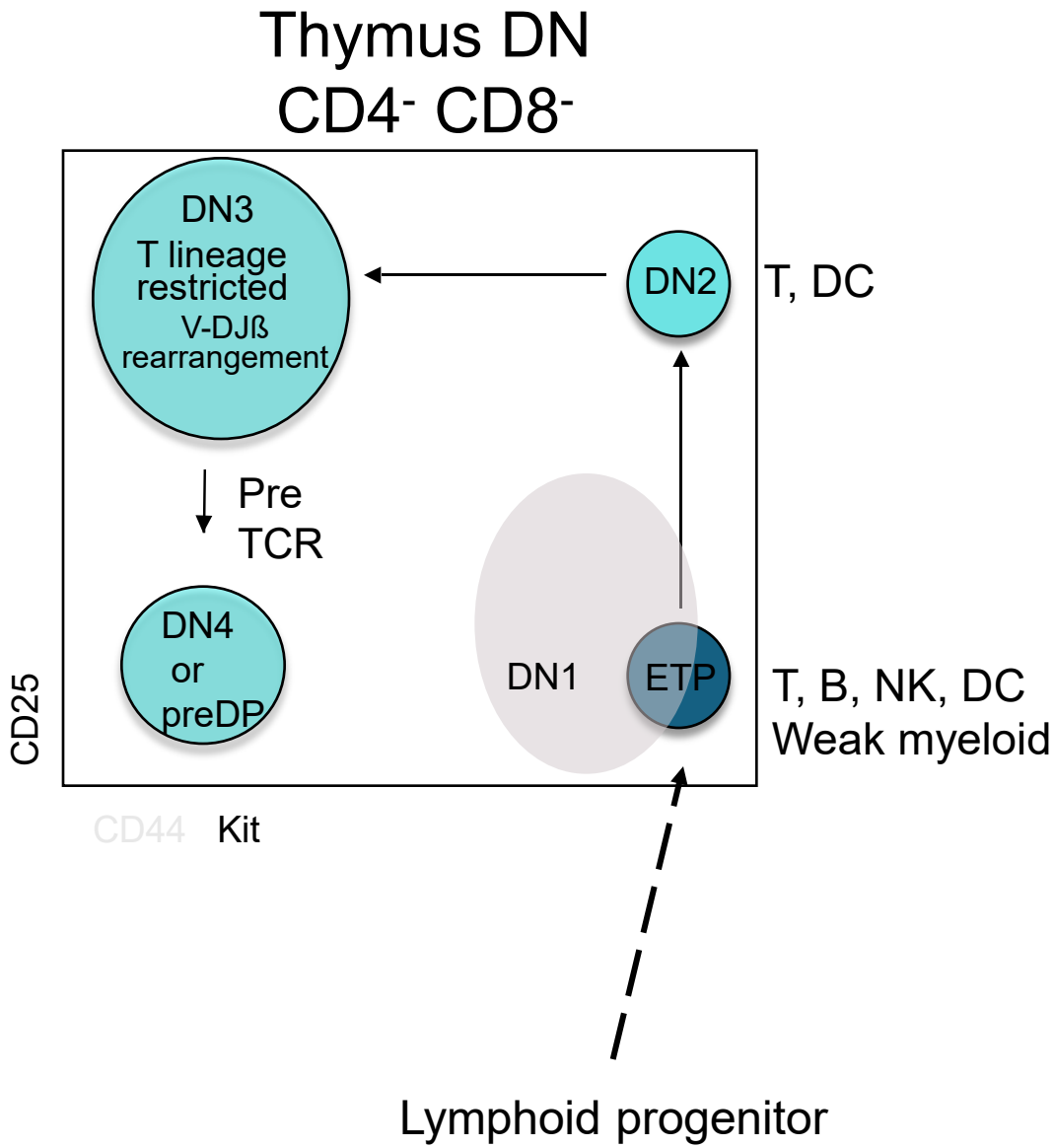
Figure 5.14 The Immune System, 3ed. (© Garland Science 2009)

Representative flow cytometry plot from a single cell suspension of mouse thymocytes



Critical stages of early T cell development





Pearse et al. 1989. PNAS 86:1614
Godfrey et al., J Immunol 1993

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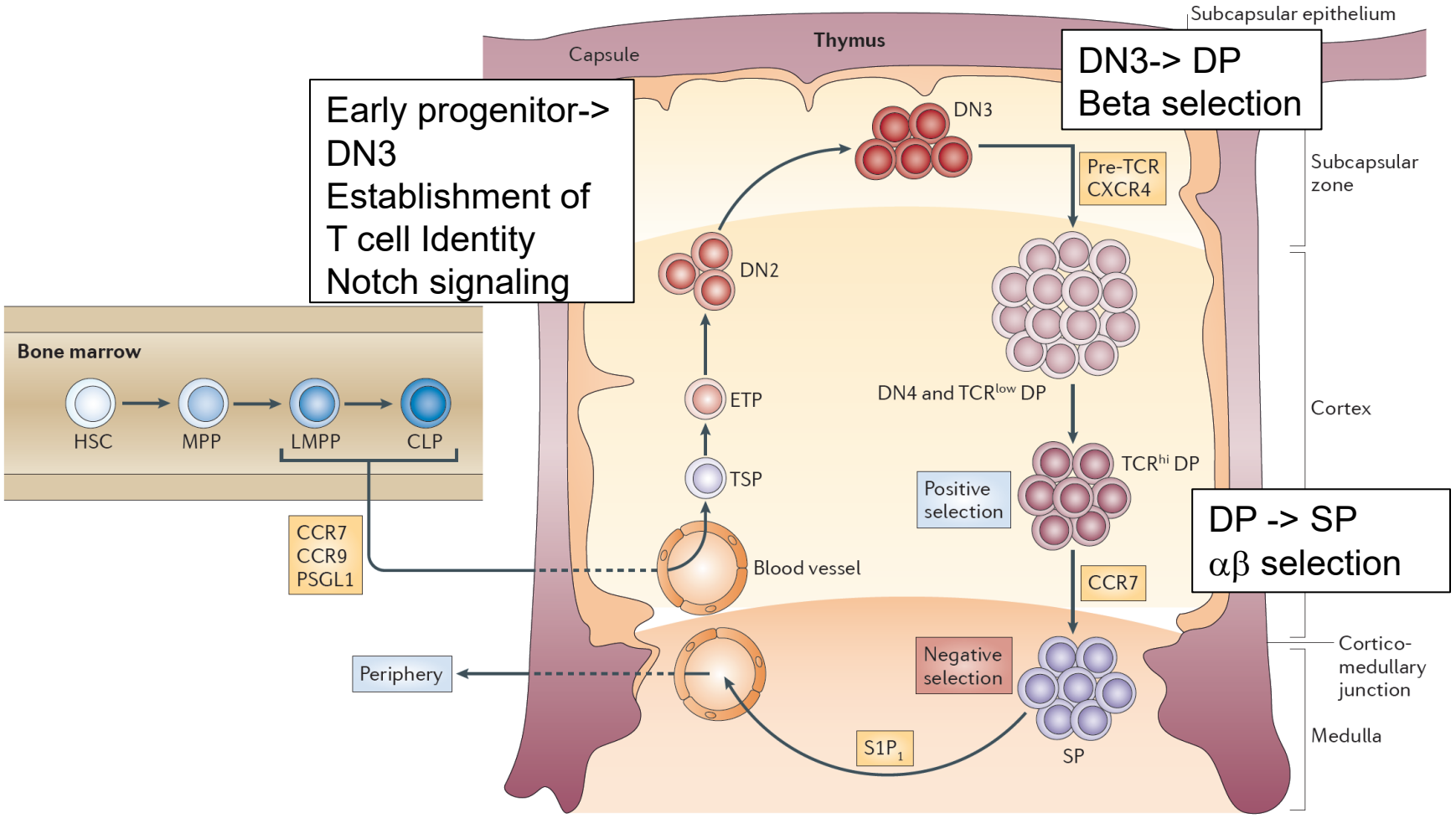
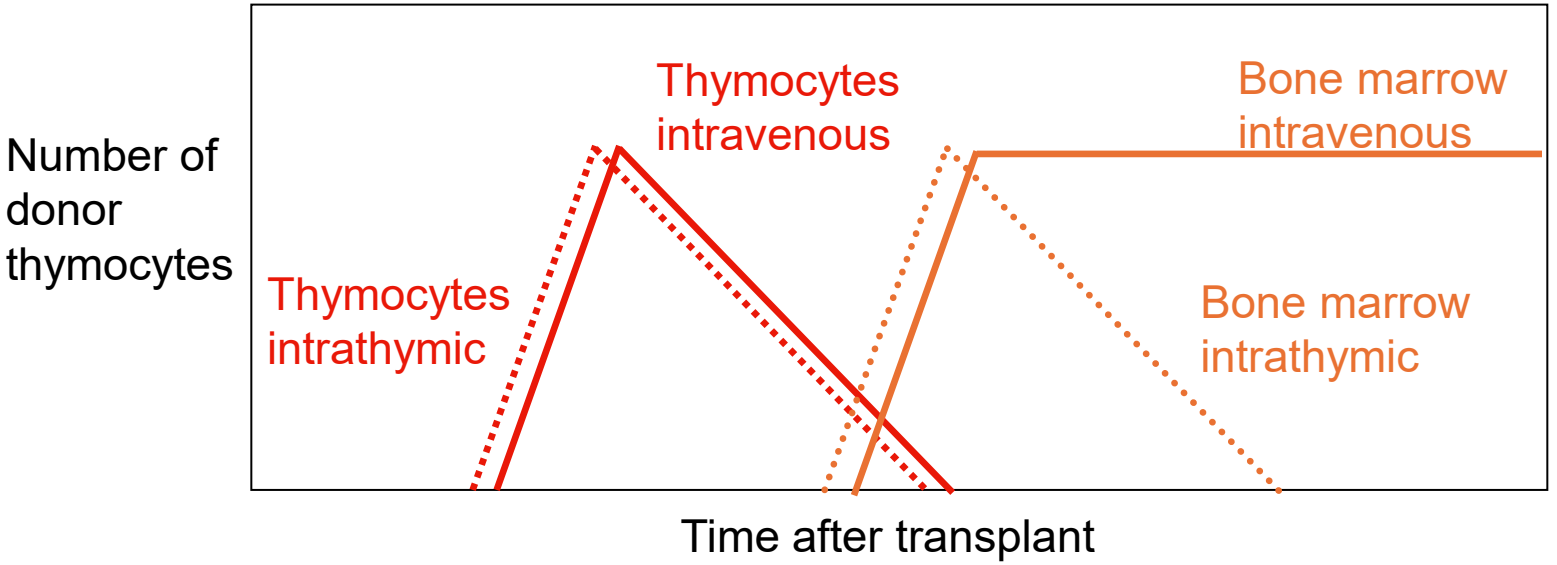
Critical stages of T cell development



Love and Bhandoola, *Nature Reviews Immunology*, 2011
Mingueneau et al., *Nat. Immunol.*, 2013

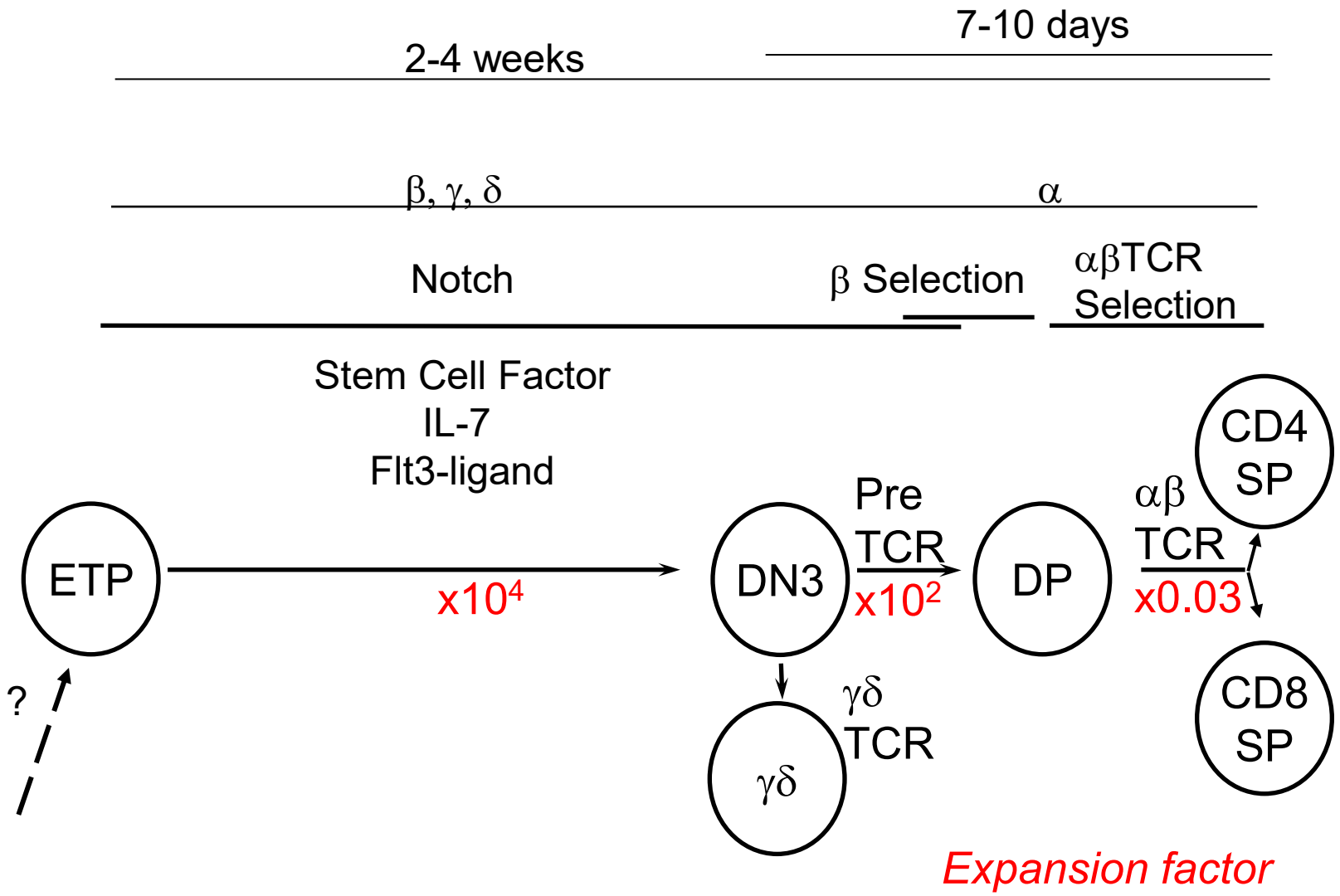
From hematopoietic stem cells to committed T cell progenitors in the thymus

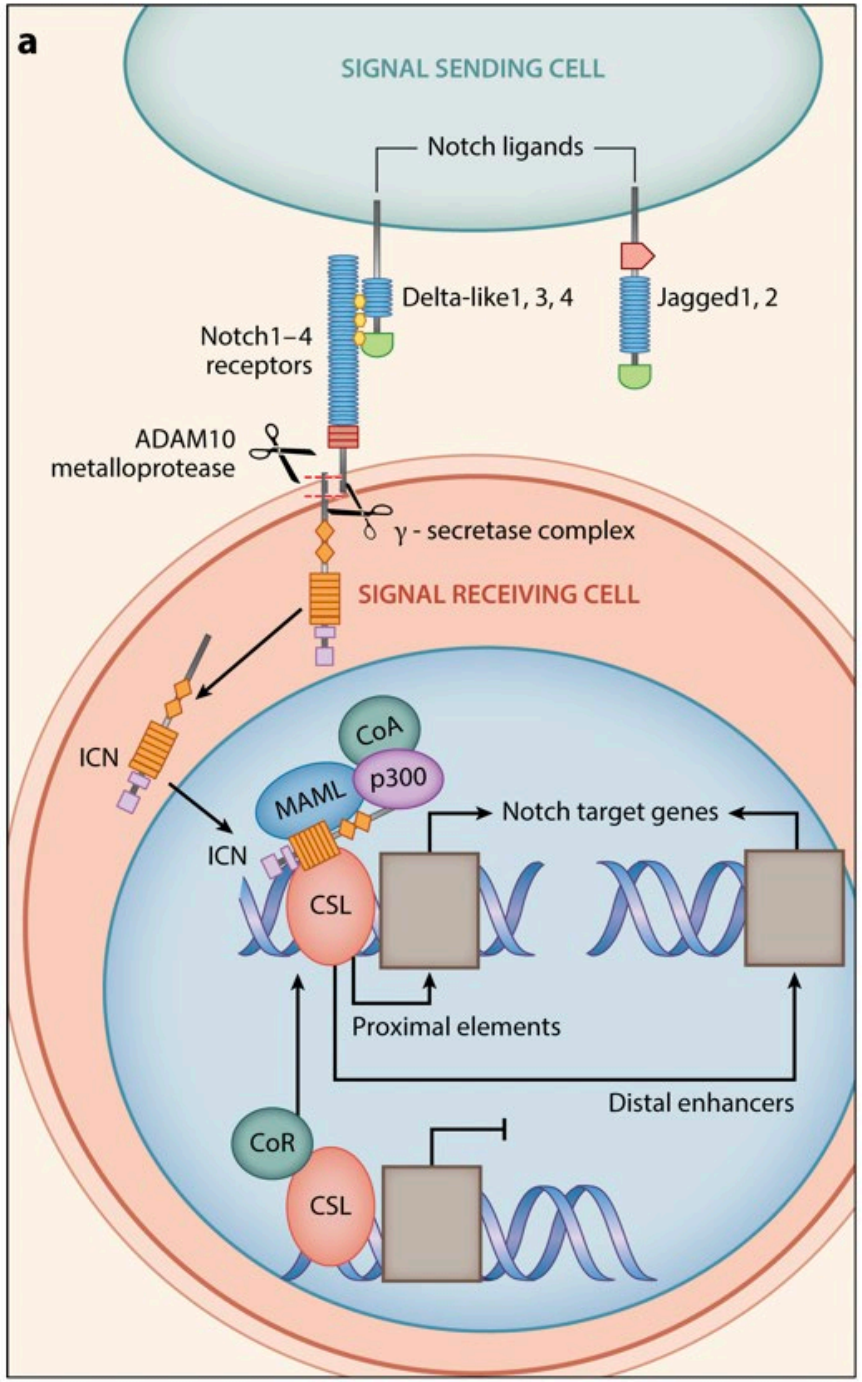
There are no "thymic stem cells"



Goldschneider et al., JEM, 1986
Scollay et al., Immunol Rev, 1986

Early T cell development in the thymus
Critical cells, factors and timeline





Principles of Notch signaling

- Highly conserved cell-cell communication pathway
- Array of five ligands and four receptors
- Ligand-mediated proteolytic receptor activation
- Intracellular receptor release leads to context-dependent transcriptional activation

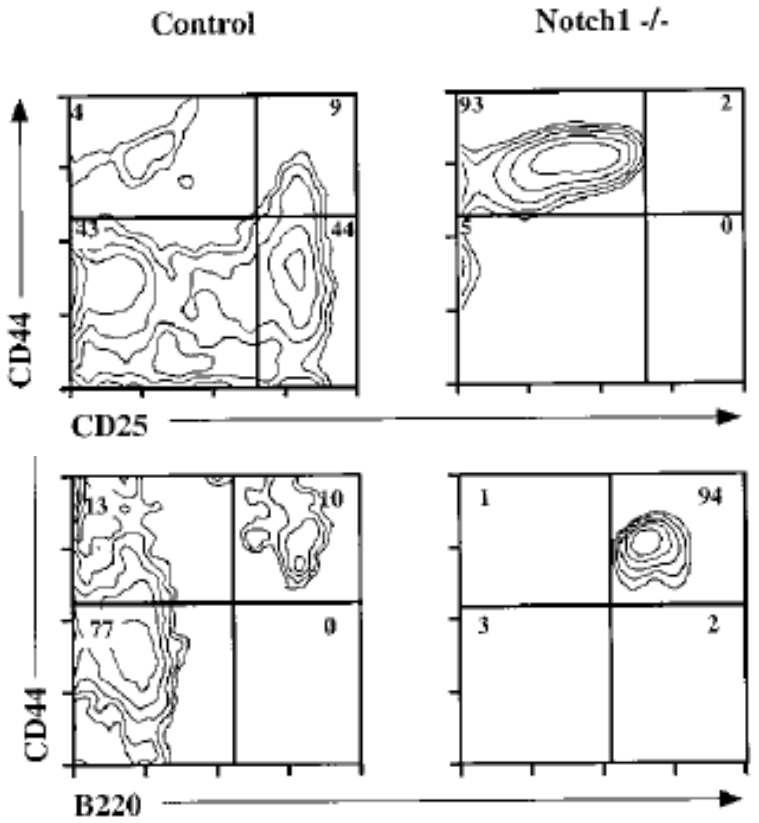
Perkey and Maillard, *Ann Rev Pathol* 2018

A critical role for Notch1 in early T cell development

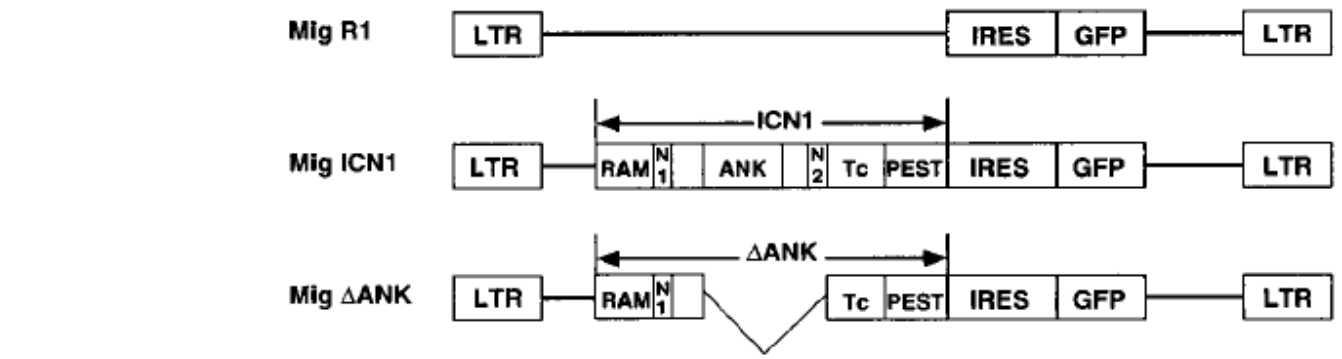
Immunity, Vol. 10, 547–558, May, 1999, Copyright ©1999 by Cell Press

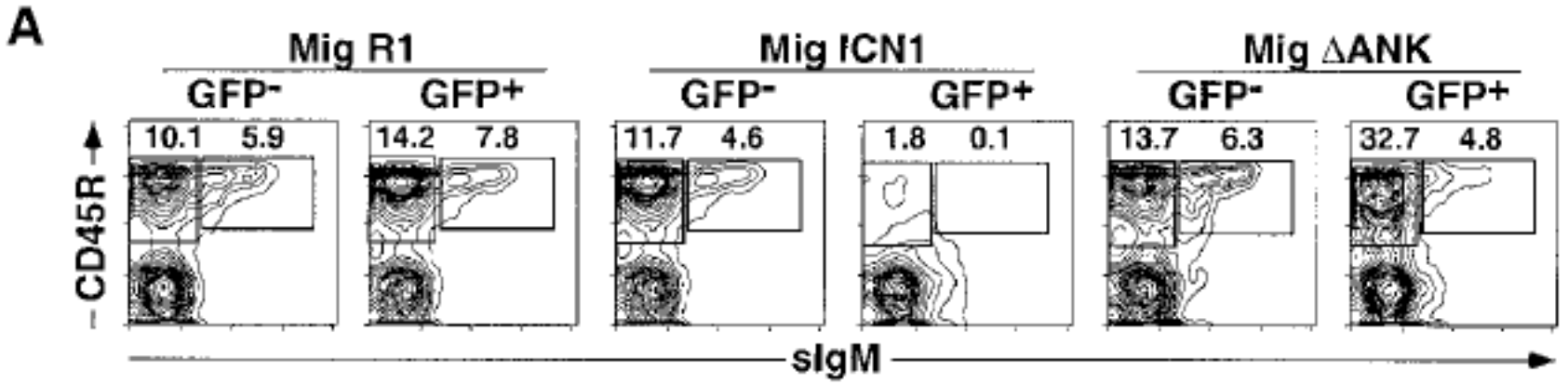
Deficient T Cell Fate Specification in Mice with an Induced Inactivation of *Notch1*

Freddy Radtke,* Anne Wilson,†
Gerlinde Stark,* Michelle Bauer,‡
Joost van Meerwijk,†‖ H. Robson MacDonald,†
and Michel Aguet*§



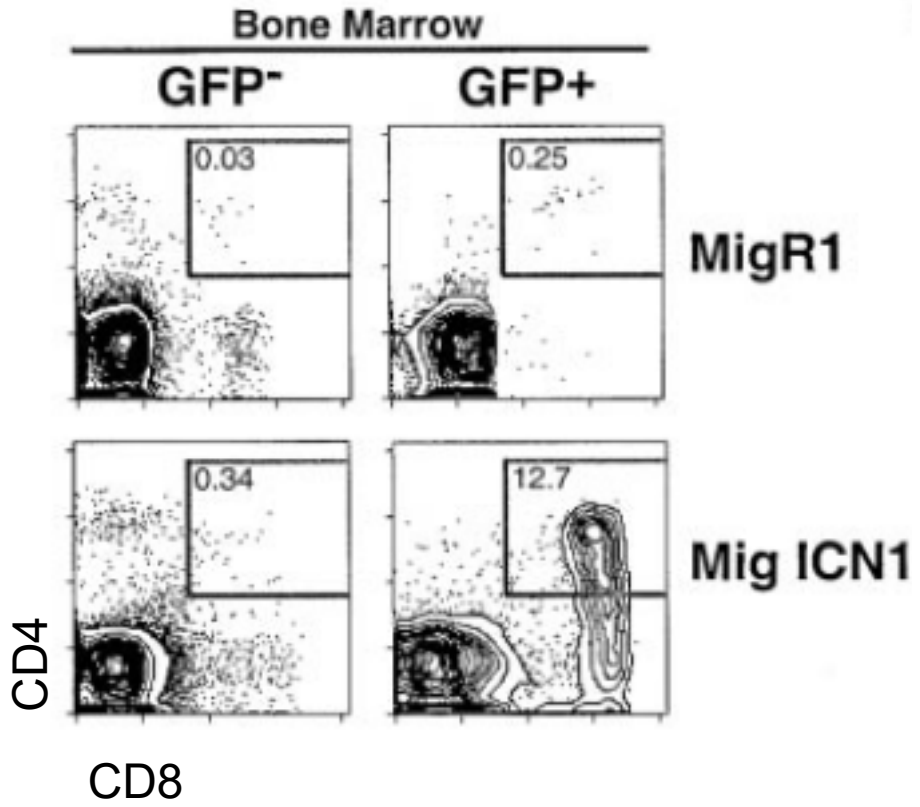
Activated Notch inhibits B cell development





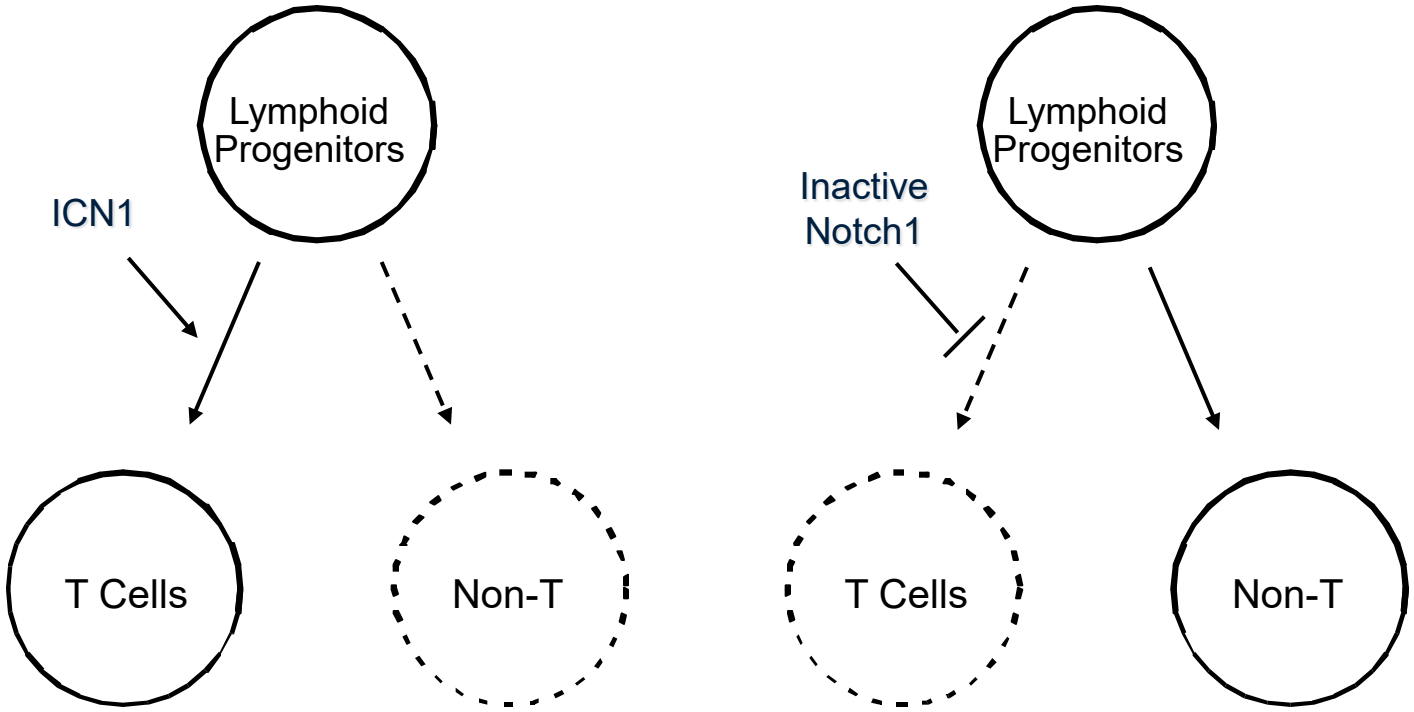
Pui et al, Immunity 11 (1999): 299

Constitutively active Notch1 (ICN1) induces ectopic T cell development in the Bone Marrow



Pui et al, *Immunity*, 1999

Notch signaling leads to T cell development at the expense of non-T cell development in the thymus



Notch1 Gain of Function
Bone Marrow T cell development
Lack of B cells

Notch1 Conditional Knockout
Lack of T cells
B cells/DC in the thymus

In vitro T cell development in coculture with stromal cells expressing Notch ligands

Immunity, Vol. 17, 749–756, December, 2002, Copyright ©2002 by Cell Press

Induction of T Cell Development from Hematopoietic Progenitor Cells by Delta-like-1 In Vitro

Thomas M. Schmitt and Juan Carlos Zúñiga-Pflücker[1] the development

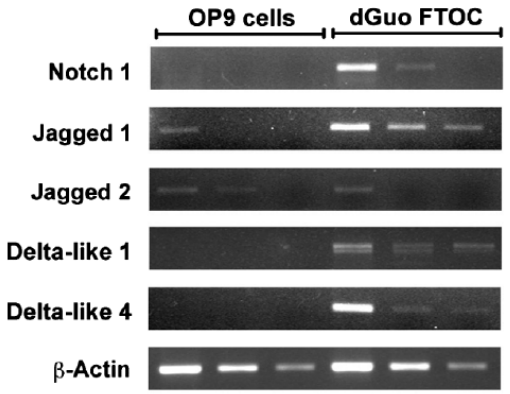
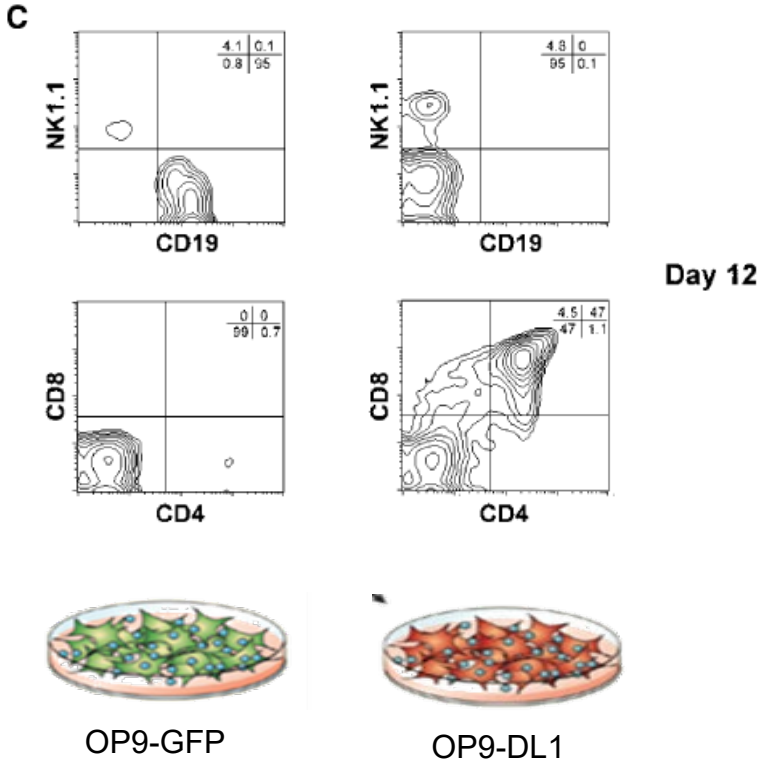
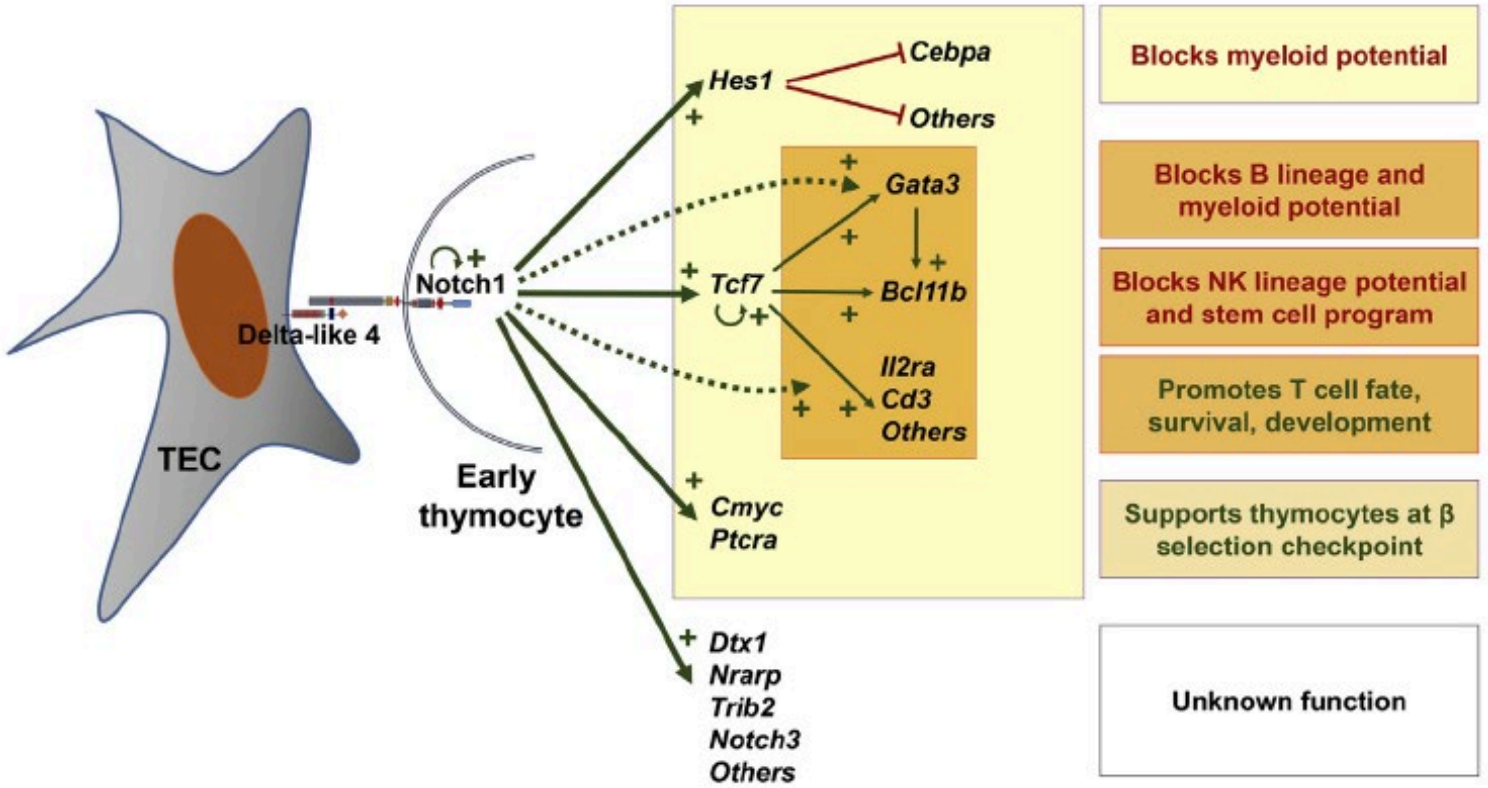


Figure 1. Analysis of Notch Ligand Expression by OP9 Cells

RT-PCR was performed for the indicated transcripts from OP9 cells and thymic stroma-enriched d14 fetal thymic lobes. Three serial dilutions (3-fold) of template cDNA are shown for each primer pair.



Overview of Notch signaling in the thymus



Radojcic and Maillard, *Encyclopedia of Immunology 2016*

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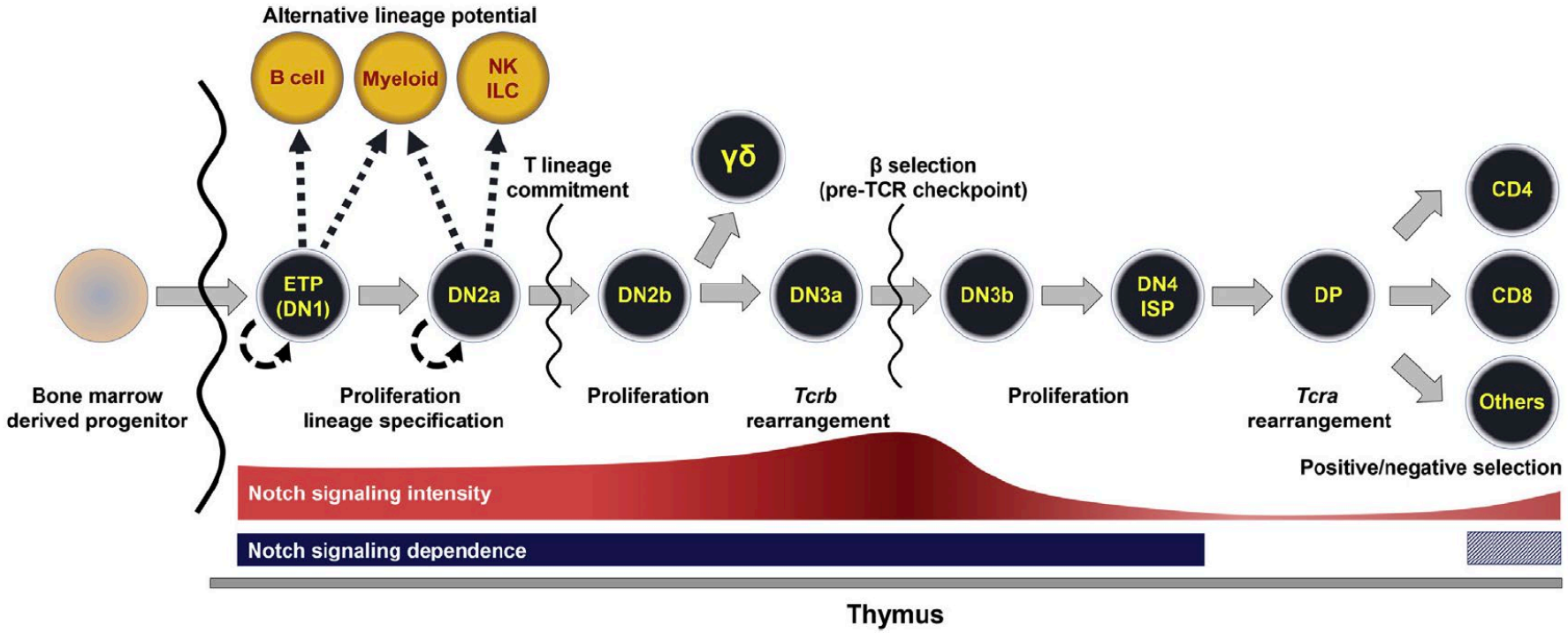
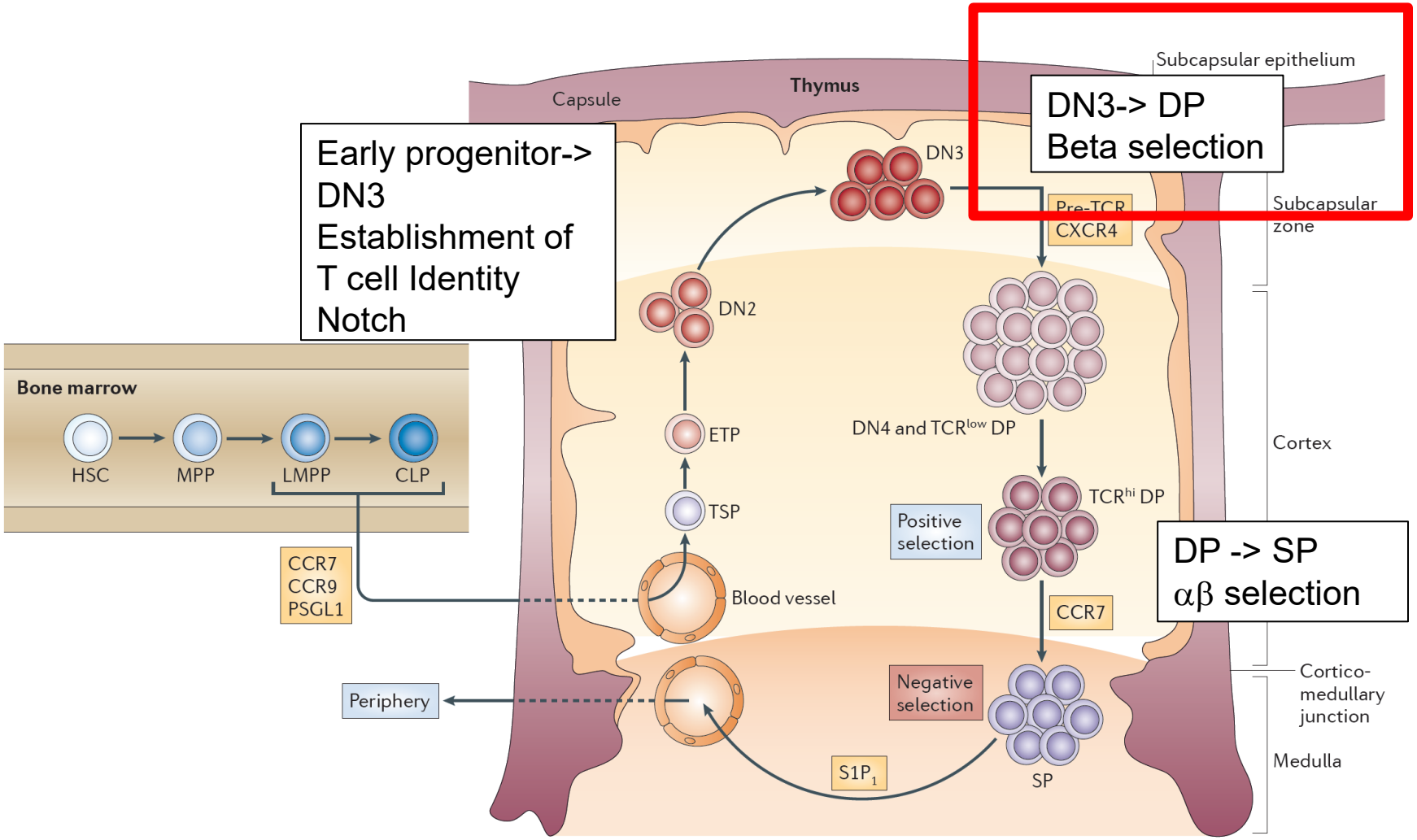


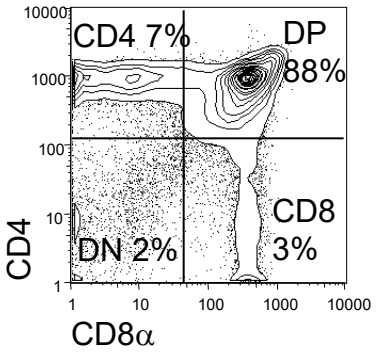
Radojcic and Maillard, *Encyclopedia of Immunology 2016*

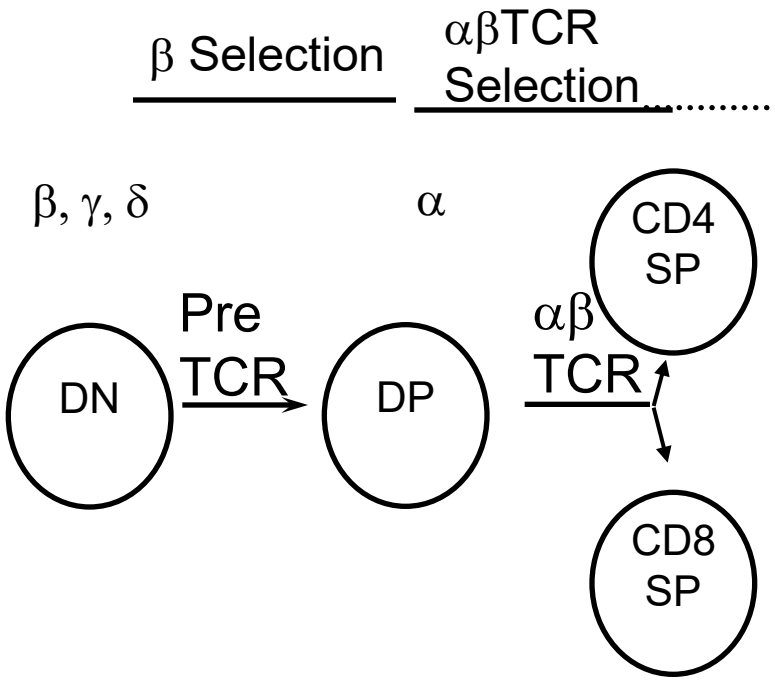
Critical stages of T cell development

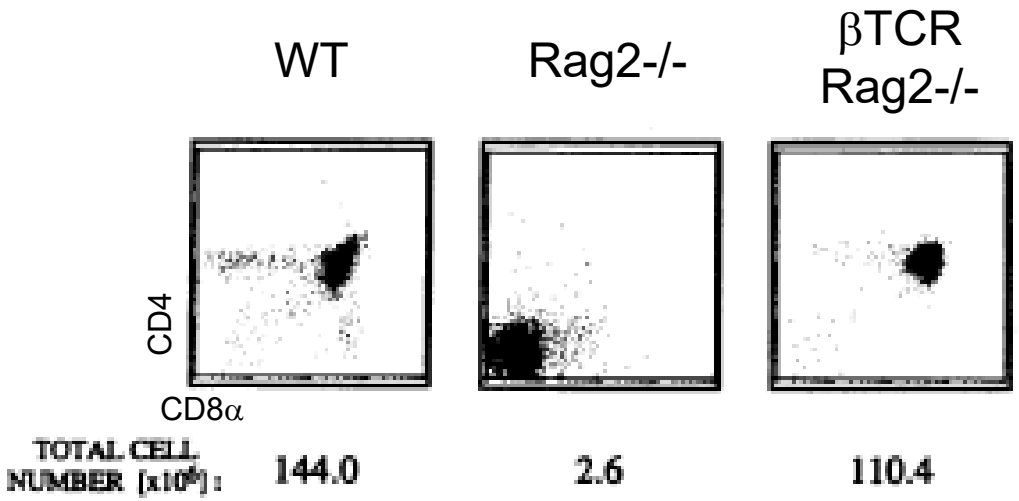


Love and Bhandoola, *Nature Reviews Immunology*, 2011
Mingueneau et al., *Nat. Immunol.*, 2013

RAG-dependent checkpoint – pre-TCR or beta selection

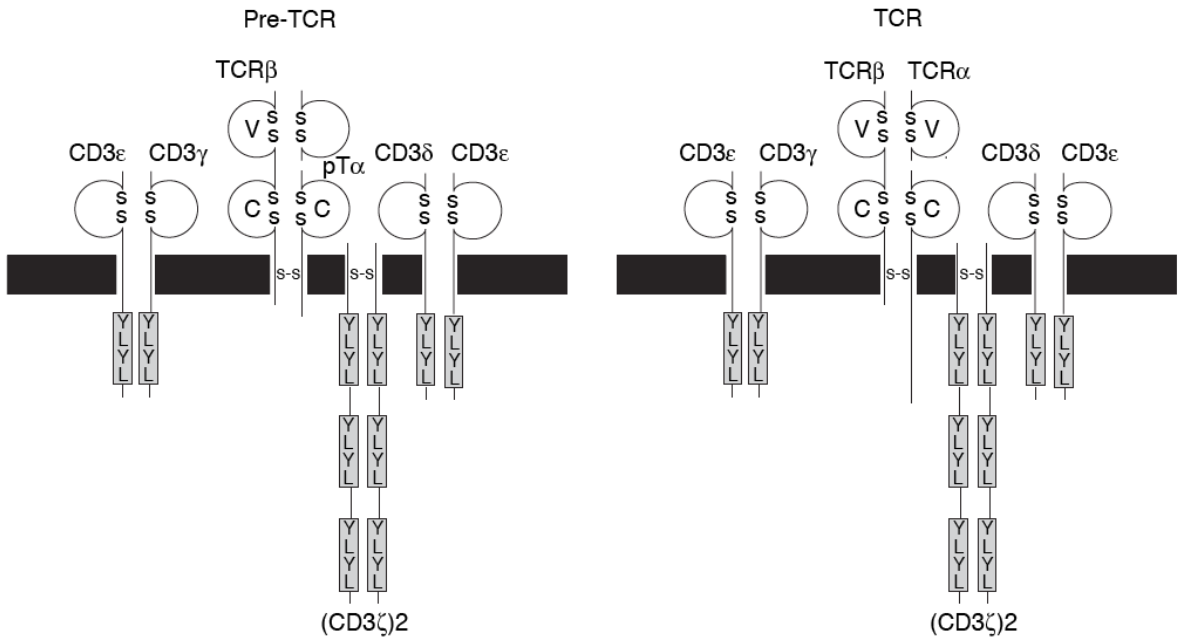






Groettrup and von Boehmer
Immunology Today, 1993

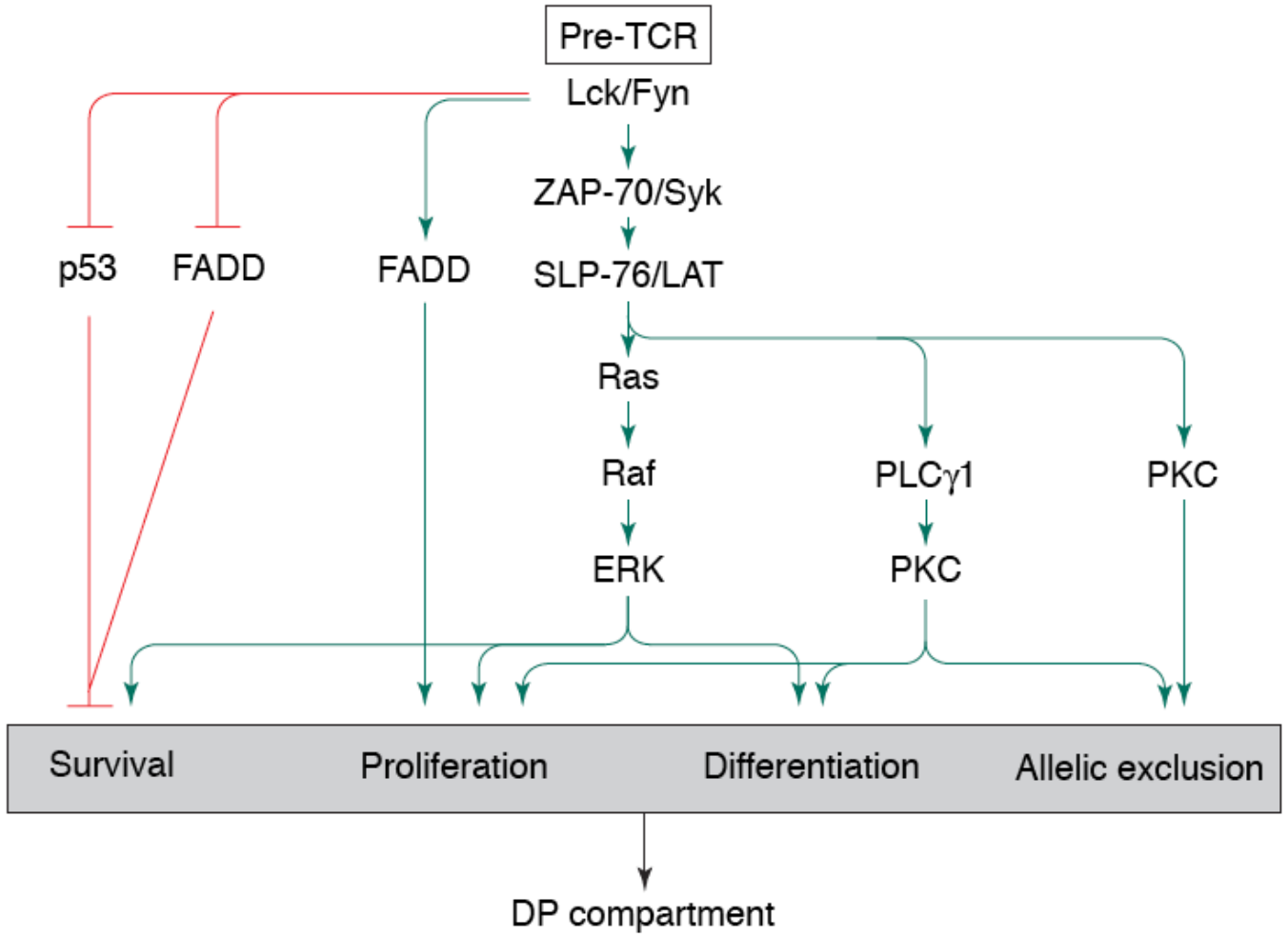
Structure of the pre-TCR



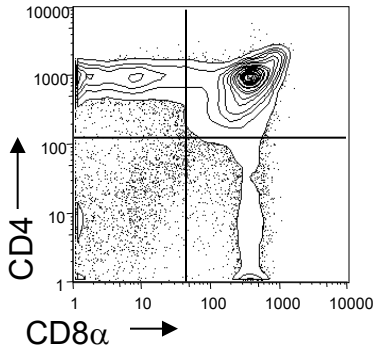
The pre-TCR
- probably has to go to the cell surface
- Unclear if it binds and needs a ligand to signal

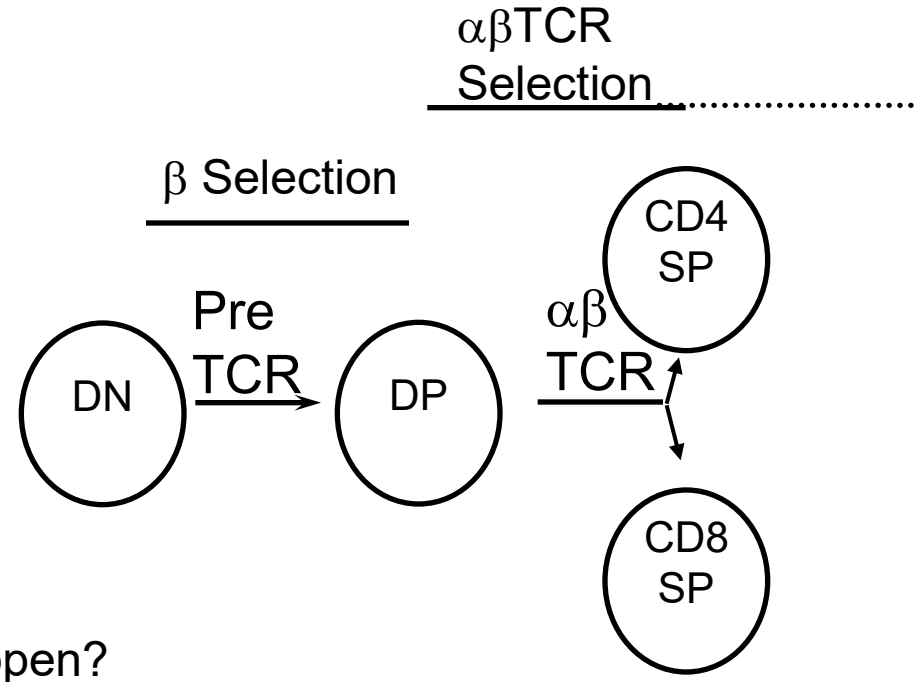
Kruisbeek et al. Immunol Today. 2000

Pre-TCR signaling



Pre-TCR or beta selection checkpoint





Beta selection - what must happen?

Survival of pre-T cells with a functional beta chain
Proliferation (~100x)
Differentiation
- CD4/CD8 expression
- Activation of TCRalpha transcription and rearrangement

Allelic exclusion of TCRbeta (rearranged TCRbeta suppresses endogenous)
Metabolic adaptation

Interaction of Notch and pre-TCR signaling

Notch promotes survival of pre–T cells at the β-selection checkpoint by regulating cellular metabolism

Maria Ciofani & Juan Carlos Zúñiga-Pflücker

The requirement for Notch signaling at the β-selection checkpoint in vivo is absolute and independent of the pre–T cell receptor

Ivan Maillard,[1,2] LiLi Tu,[2,3,4] Arivazhagan Sambandam,[3] Yumi Yashiro-Ohtani,[2,3,4] John Millholland,[2,3,4] Karen Keeshan,[2,3,4] Olga Shestova,[2,3,4] Lanwei Xu,[2,3,4] Avinash Bhandoola,[3] and Warren S. Pear[2,3,4]

[1]Division of Hematology-Oncology, [2]Abramson Family Cancer Research Institute, [3]Department of Pathology and Laboratory Medicine, and [4]Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA 19104

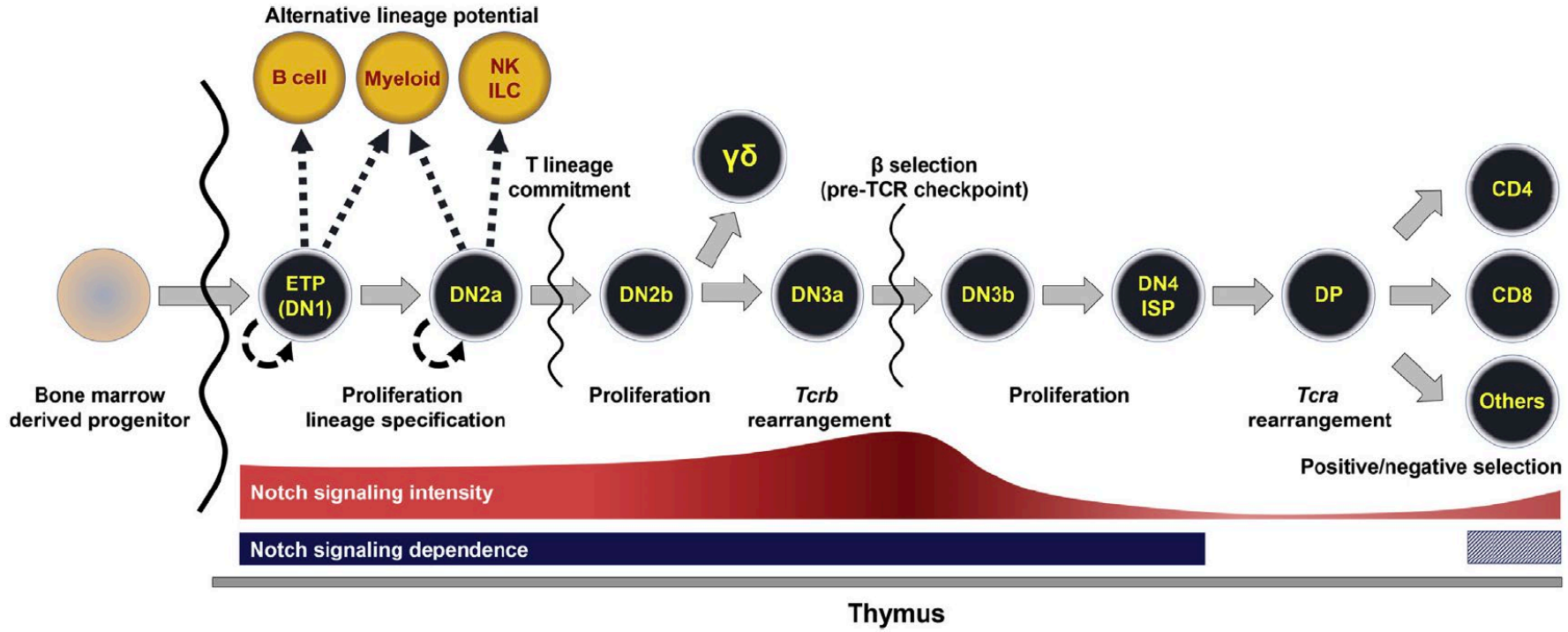
- Notch and pre-TCR signals cooperate during beta selection
- Notch is turned off once the beta selection checkpoint has been cleared

Pre-TCR signaling inactivates Notch1 transcription by antagonizing E2A

Yumi Yashiro-Ohtani,[1] Yiping He,[1] Takuya Ohtani,[1] Mary E. Jones,[2] Olga Shestova,[1] Lanwei Xu,[1] Terry C. Fang,[1] Mark Y. Chiang,[1] Andrew M. Intlekofer,[1] Stephen C. Blacklow,[3] Yuan Zhuang,[2] and Warren S. Pear[1,4]

Ciofani and Zuniga-Pflucker, Nat Immunol 2004
Maillard et al., J Exp Med 2006
Yashiro-Ohtani et al., Genes and Dev 2009

Overview of Notch signaling in the thymus



Radojcic and Maillard, *Encyclopedia of Immunology 2016*

Summary

- T lymphopoiesis requires input of progenitors from the bone marrow.
- Bone marrow progenitors migrate to the thymus via the blood.
- Thymus settling progenitors receive strong Notch signals that drive T cell development and discourage non-T cell development.
- The early steps of T cell development can be replicated in vitro using stromal cell lines that express Notch ligands (OP9-DL1 or OP9-DL4).
- Notch signaling is active and essential at the pre-TCR checkpoint, but downregulated at subsequent stages of T cell development.