



The development of particular T cells at a specific time prevents autoimmunity

Siyoung Yang

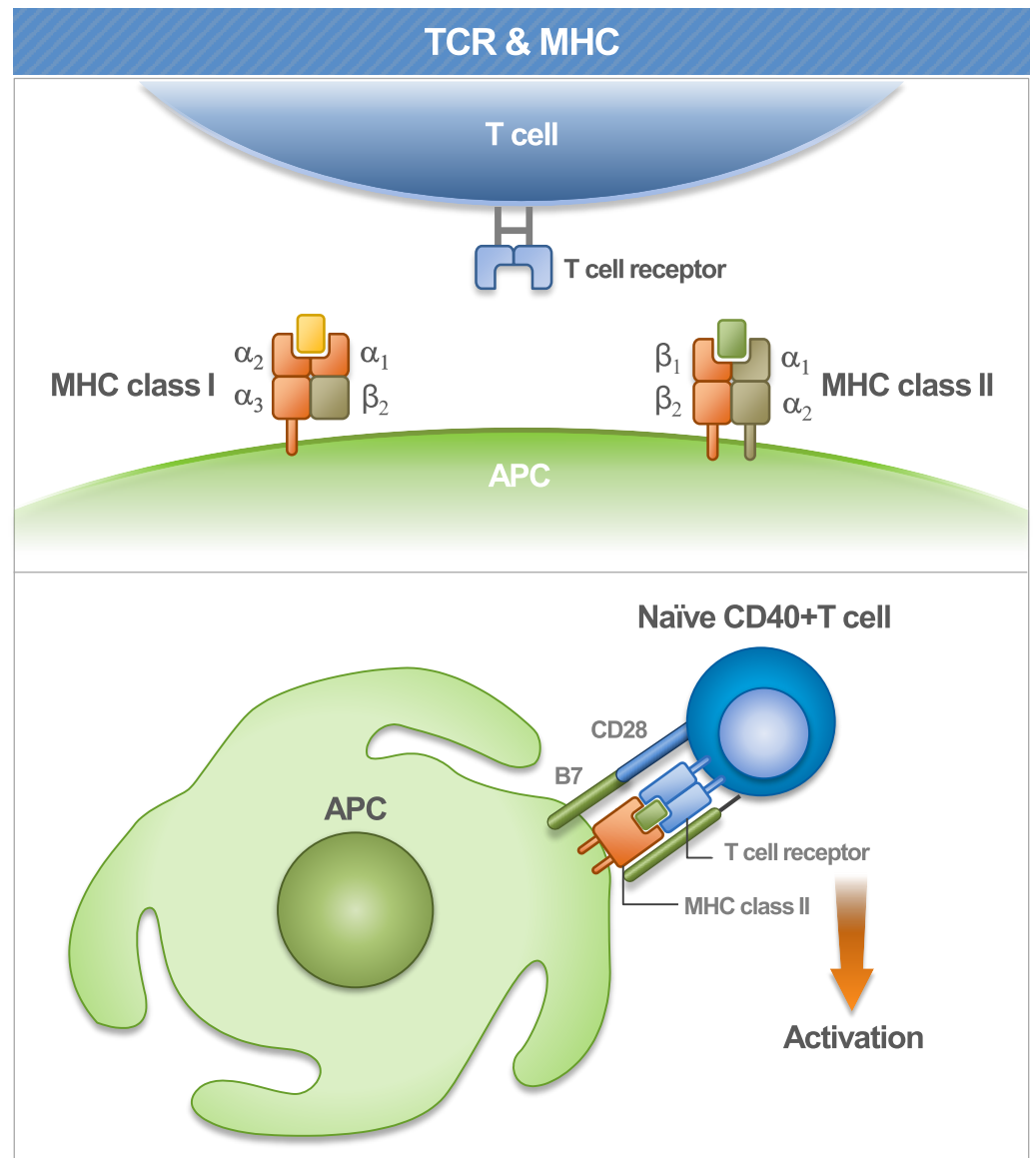
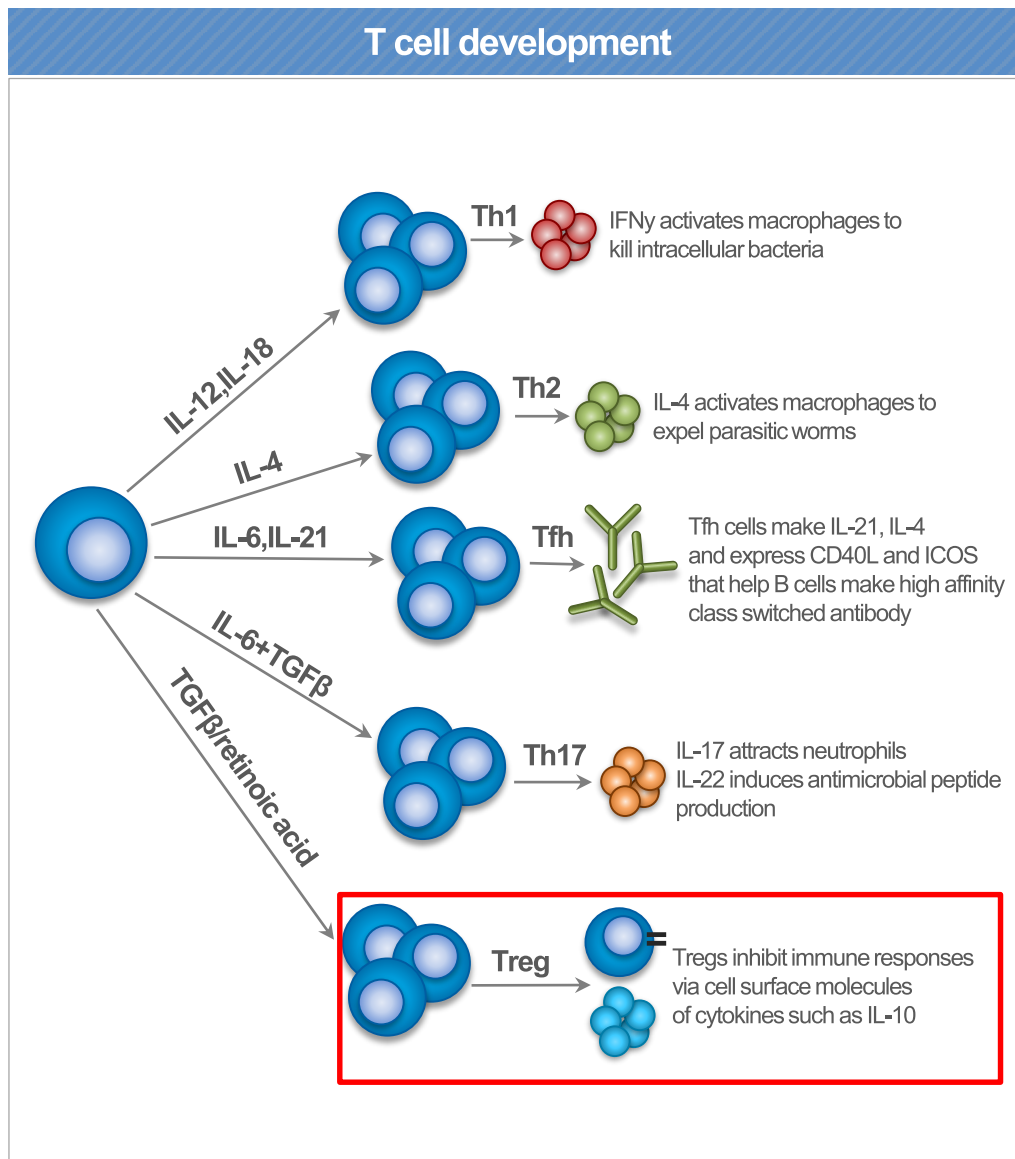
Department of Pharmacology
Ajou university School of Medicine

April 29, 2016

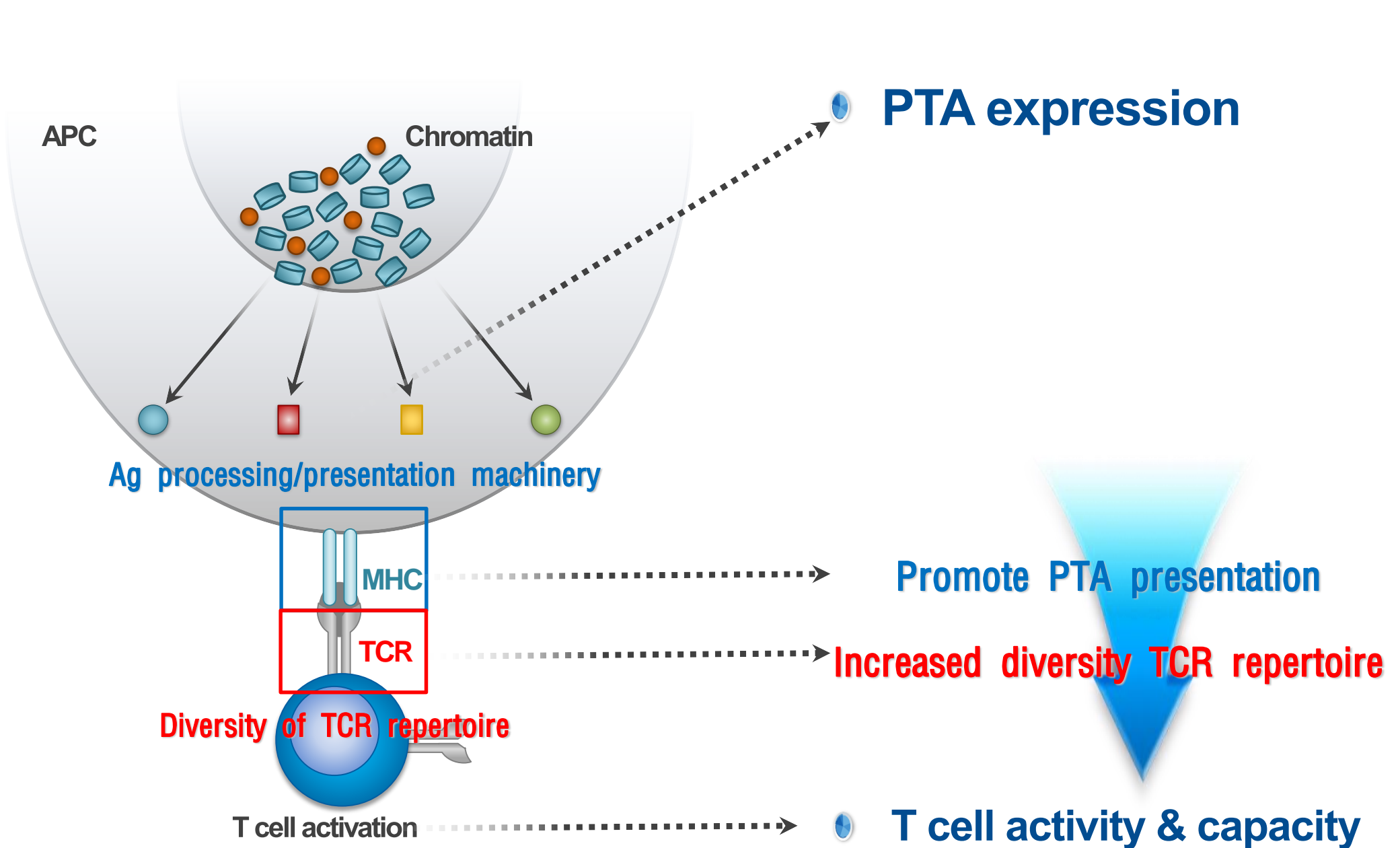
T cell development

T cell

- T cells mature in the thymus
- several subsets of T cells, each with a distinct function
- a type of lymphocyte that play central role in cell-mediated immunity

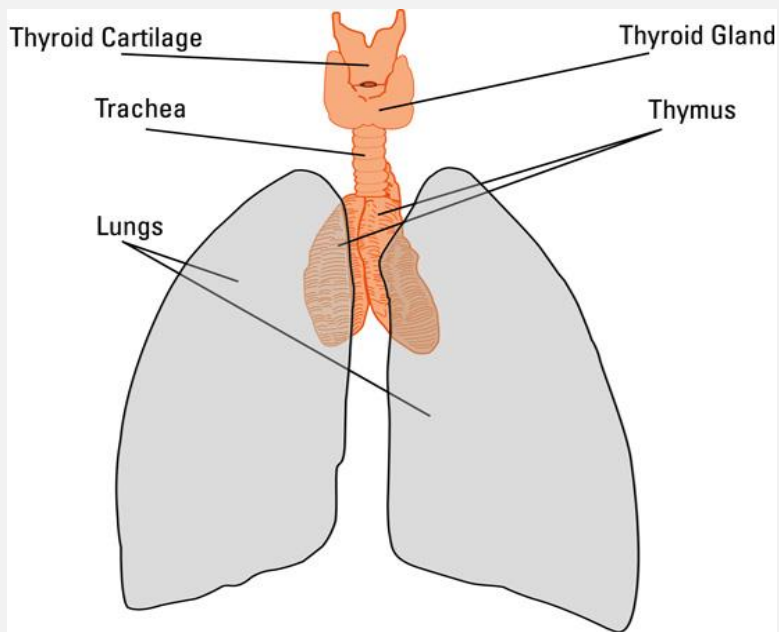
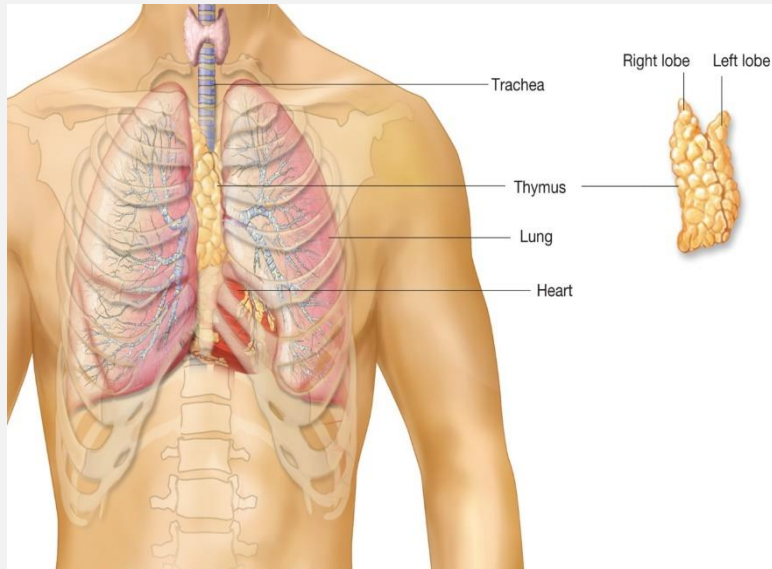


T cell activation by APC stimulation

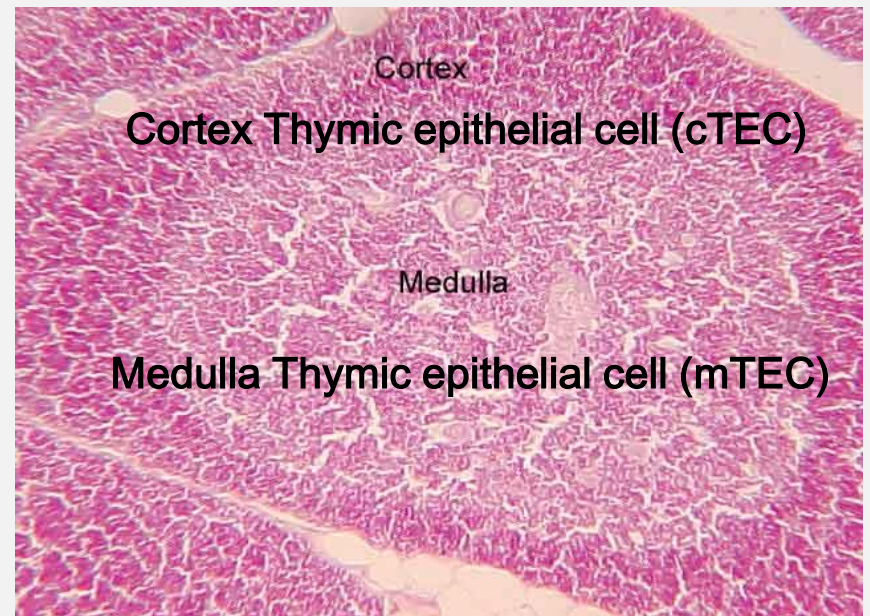
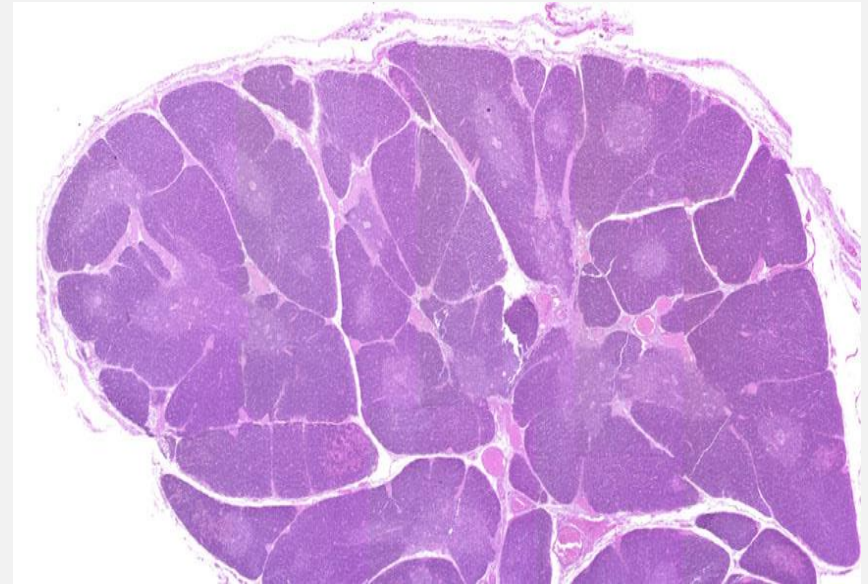


Thymus: Cortex & Medulla

Anatomy of the Thymus Gland



Structure of thymus



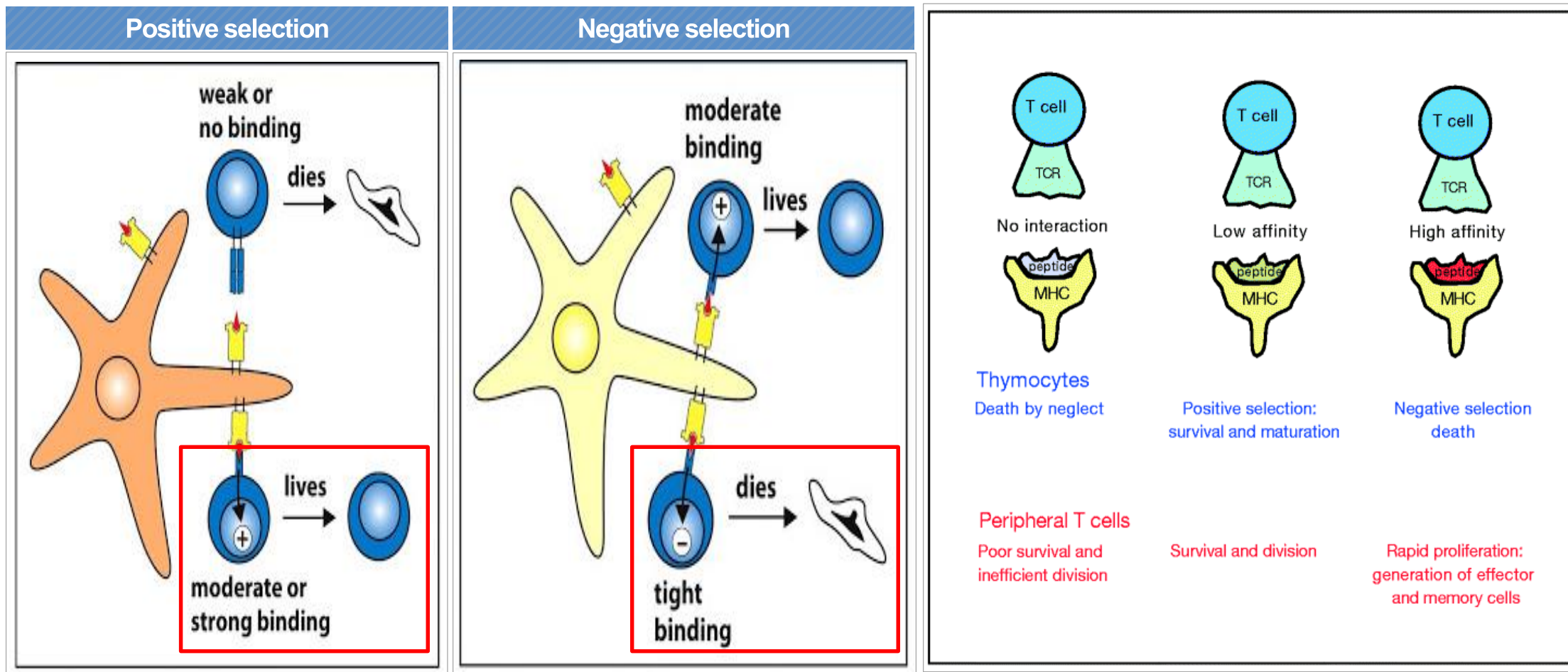
Positive & Negative selection

Positive selection; cTEC

- ✓ self-antigens are expressed by thymic cortical epithelial cells on MHC molecules in cortex
- ✓ Thymocytes that interact with MHC-I or II (i.e., not too strongly or too weakly) will receive survival signal

Negative selection; mTEC

- ✓ T cells that recognizes self MHC molecules and peptides with high affinity are deleted in medulla

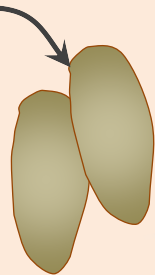
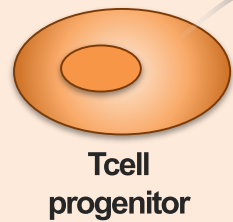


Central tolerance & Peripheral tolerance

Central tolerance

Bone marrow

Thymus



Peripheral tolerance

Periphery

T_{reg} cell

Foxp3+

Foxp3+

Naive Tcell

TGF-β

Foxp3+

T_H1
T_H2
T_H17

Autoimmune disease

Allergy

Graft rejection

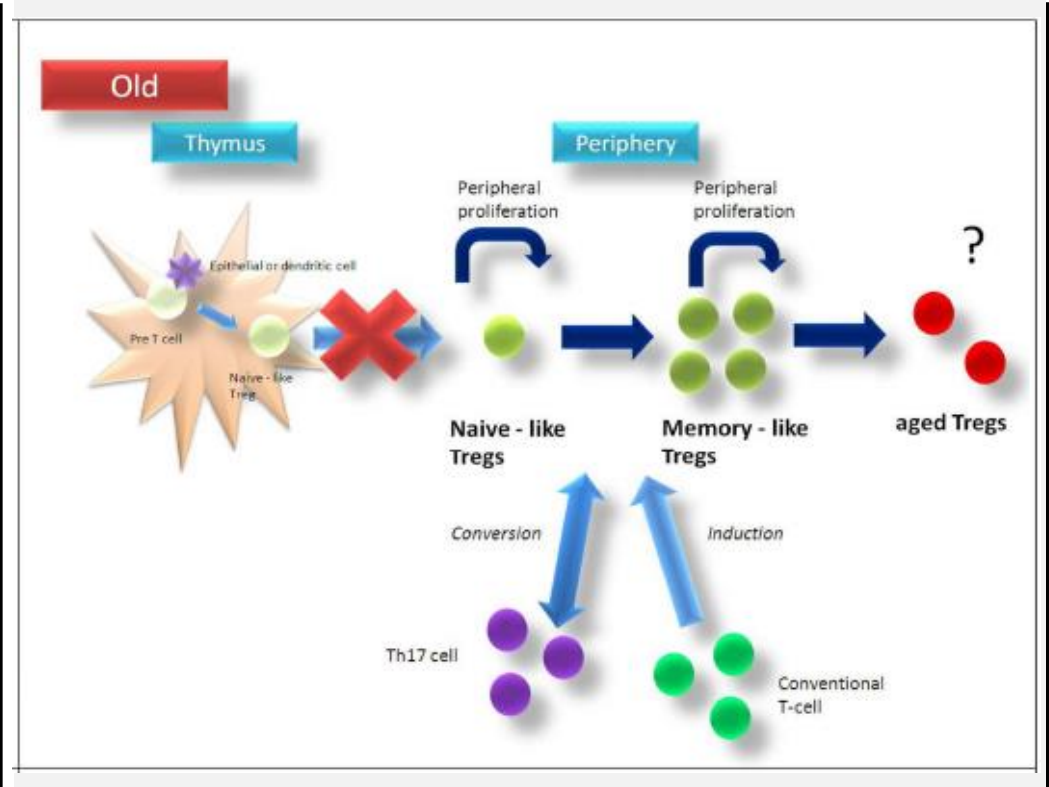
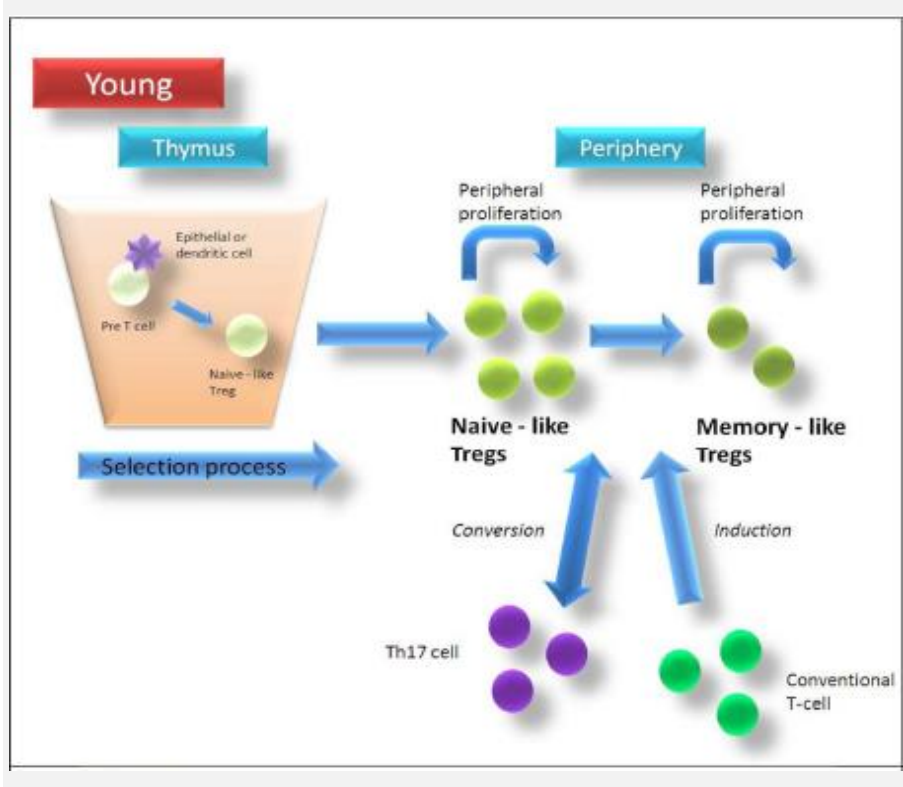
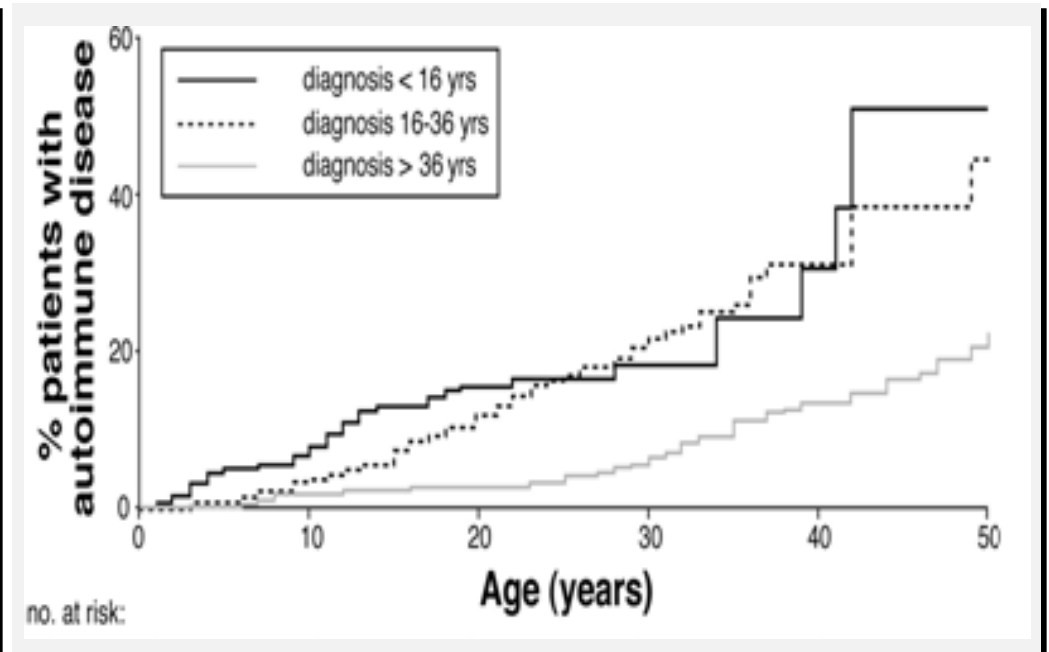
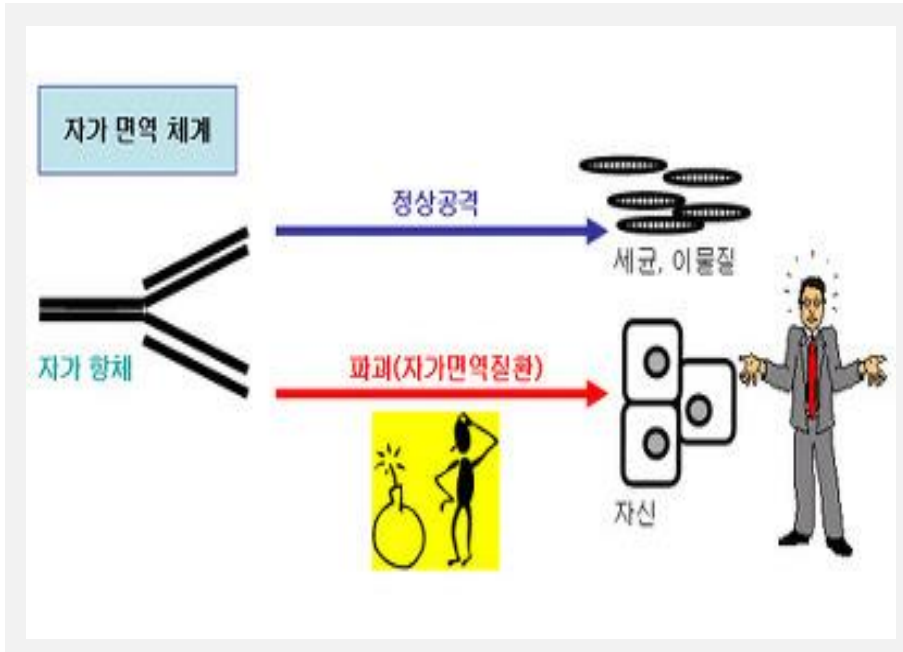
Inflammatory bowel disease

Cancer

Infectious disease

- T_{reg} cells are a small subset of CD4⁺ T lymphocytes (5-20%)
- T_{reg} cells function to maintain immune homeostasis
- T_{reg} cell characteristics
 - Expression of forkhead-winged-helix transcription factor (Foxp3)

Autoimmune disease in Aging



Aire & Aire Function

Aire (Autoimmune regulator)

- ✓ mutation of AIRE in humans results in APECED/APS-1
- ✓ classified as a transcription factor due to a number of encoded transcription-associated domains
- ✓ regulation of PTA expression in medullar thymic epithelial cells (MTECs)
- ✓ ensures efficient negative selection of autoreactive T cells during differentiation



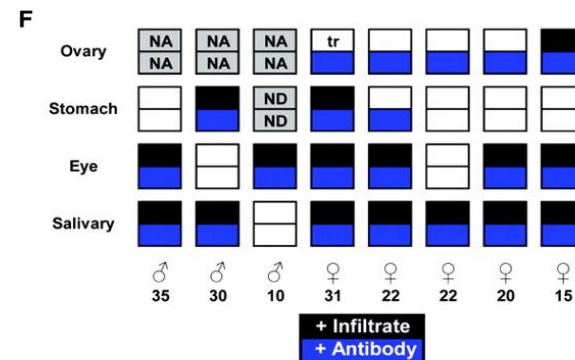
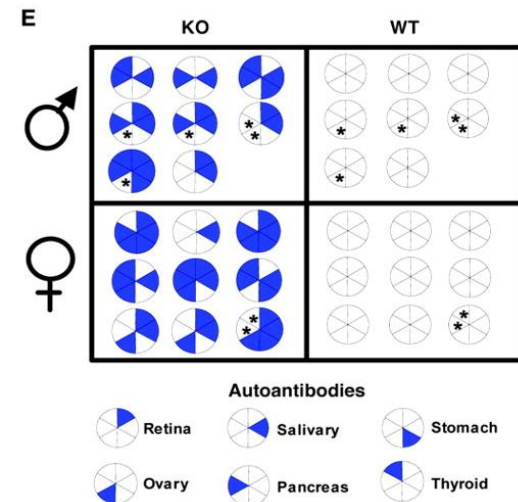
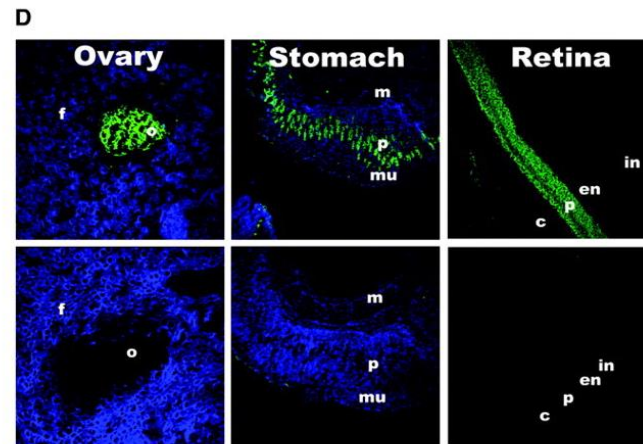
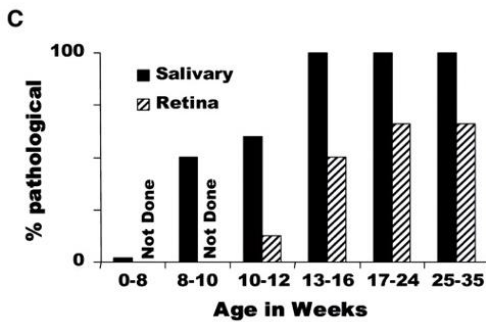
Aire-deficient mice develop autoimmunity

Science, 2002 Nov 15;298(5597):1395-401. Epub 2002 Oct 10.

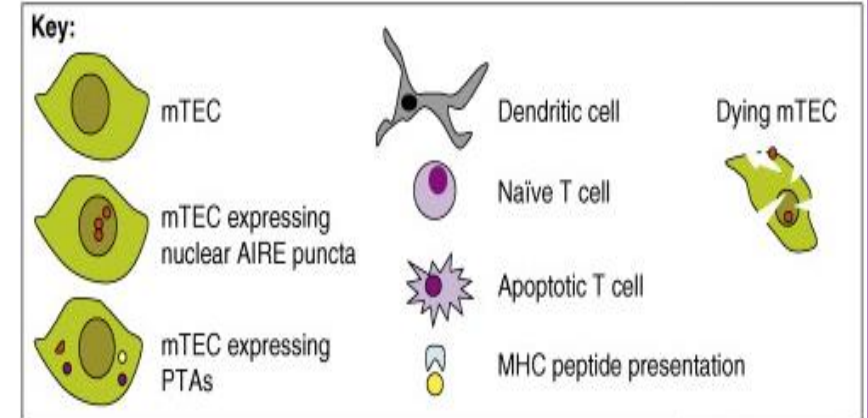
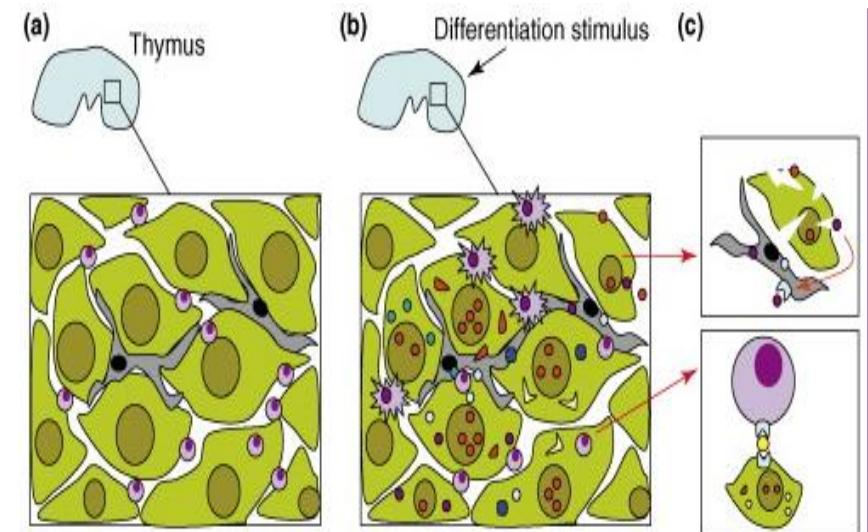
Projection of an immunological self shadow within the thymus by the aire protein.

Anderson MS¹, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D.

Author information



Medullary thymic epithelium maturation



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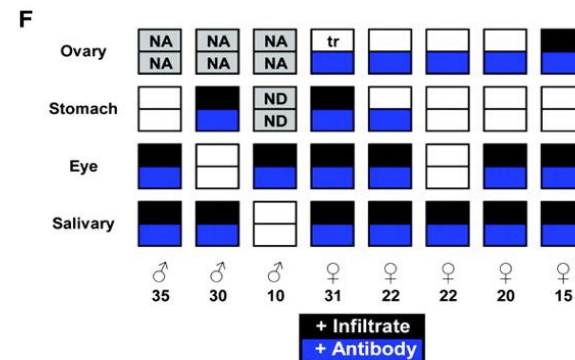
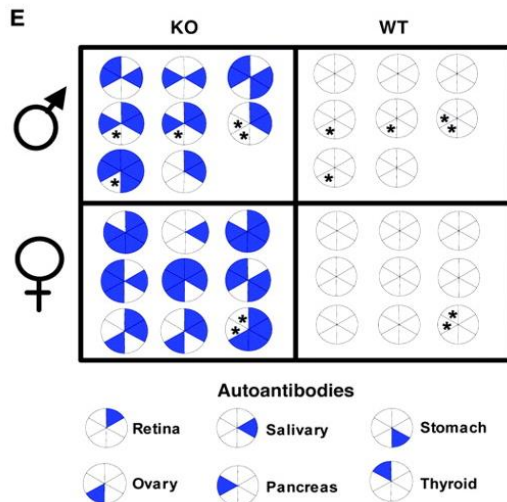
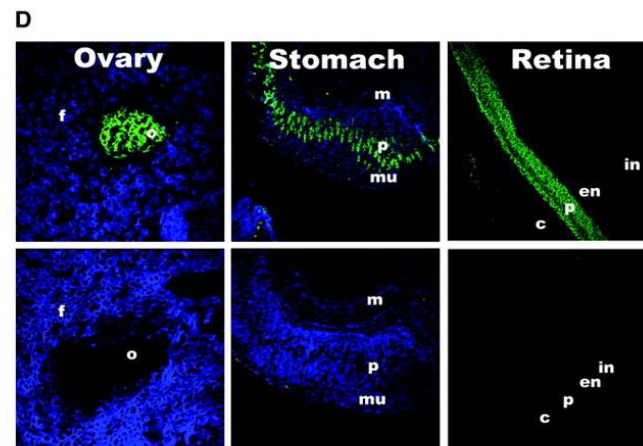
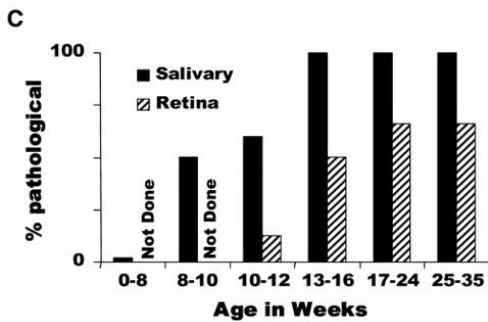
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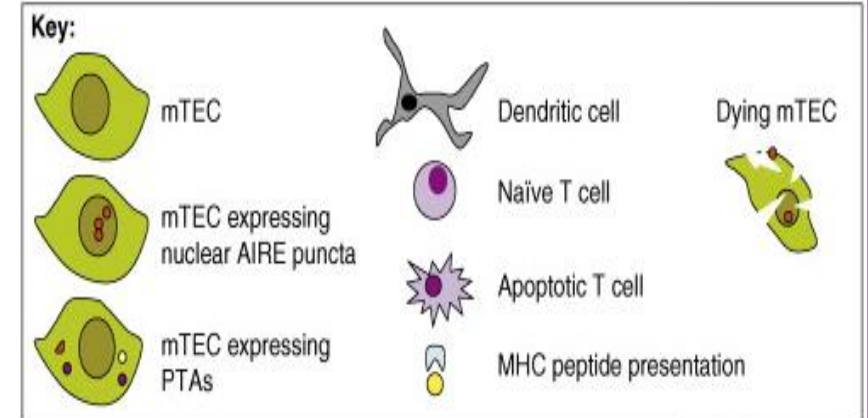
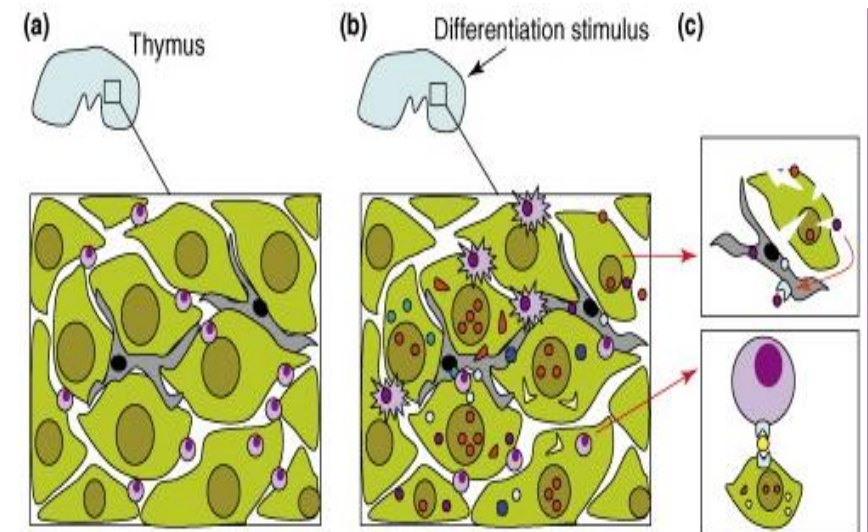
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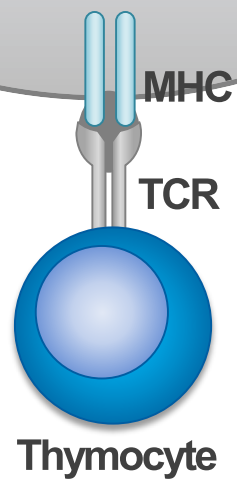
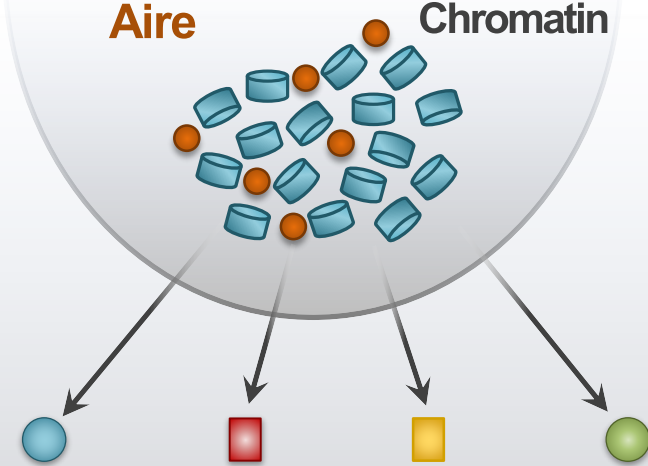


PTA expression by Aire regulation

MEC

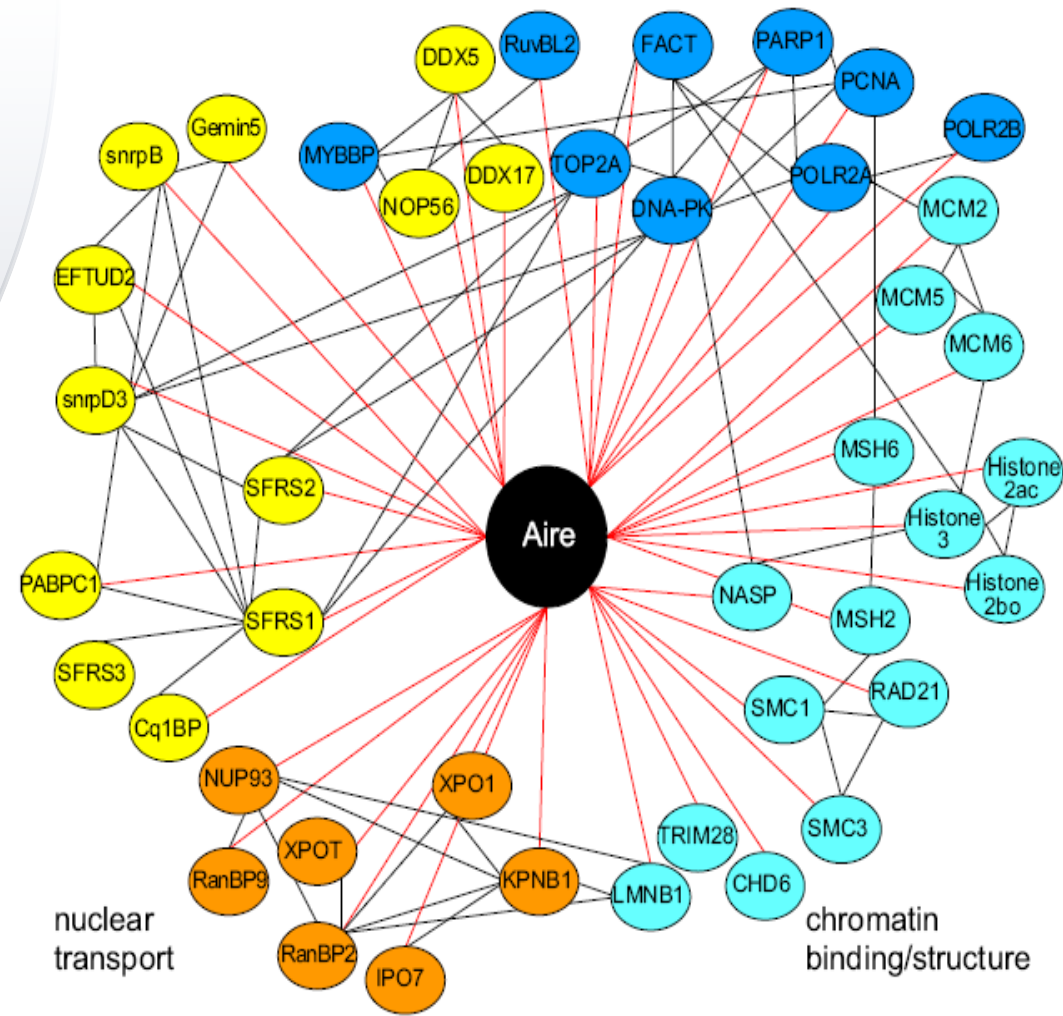
Aire

Chromatin



pre-mRNA processing

transcription



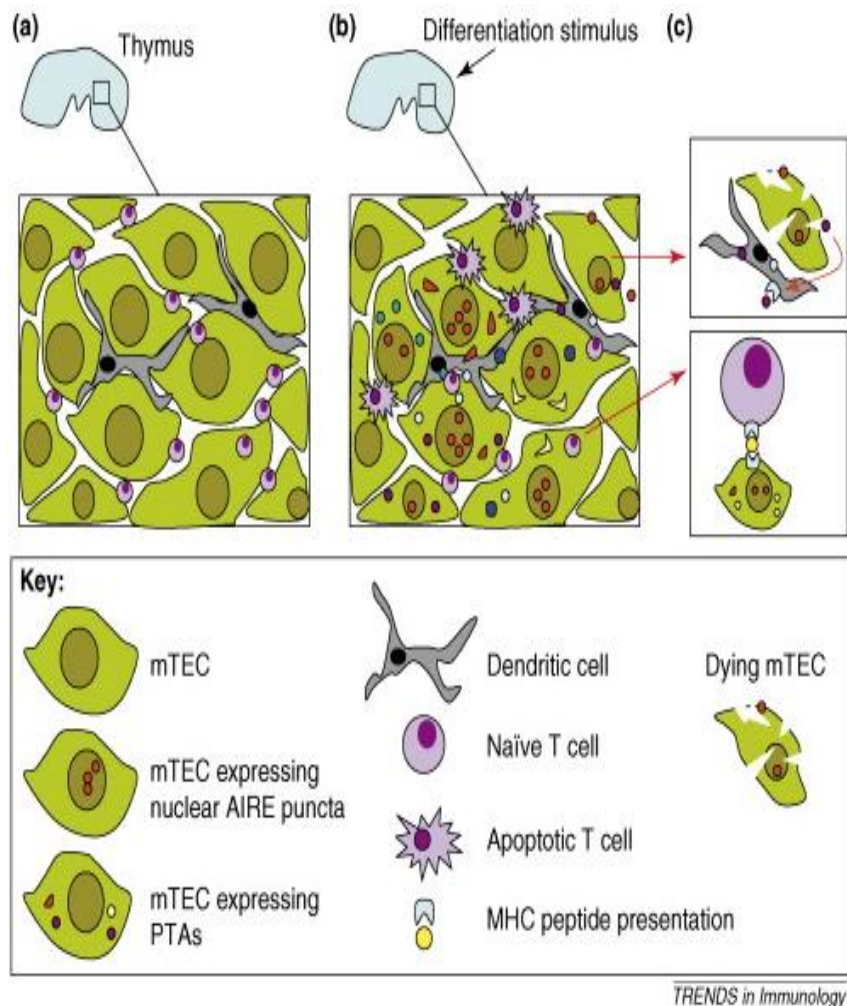
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□ Aire (Autoimmune regulator)

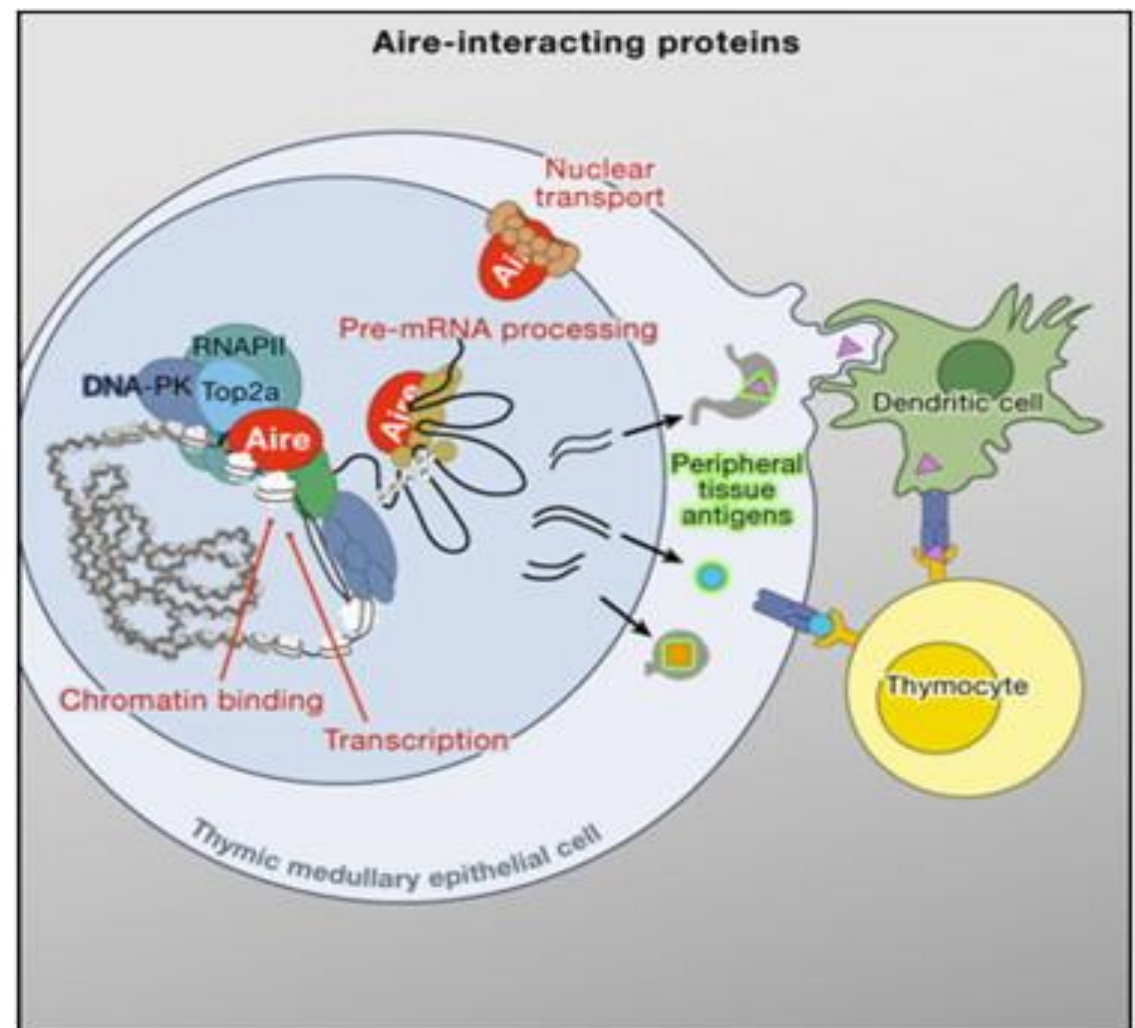
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Medullary thymic epithelium maturation

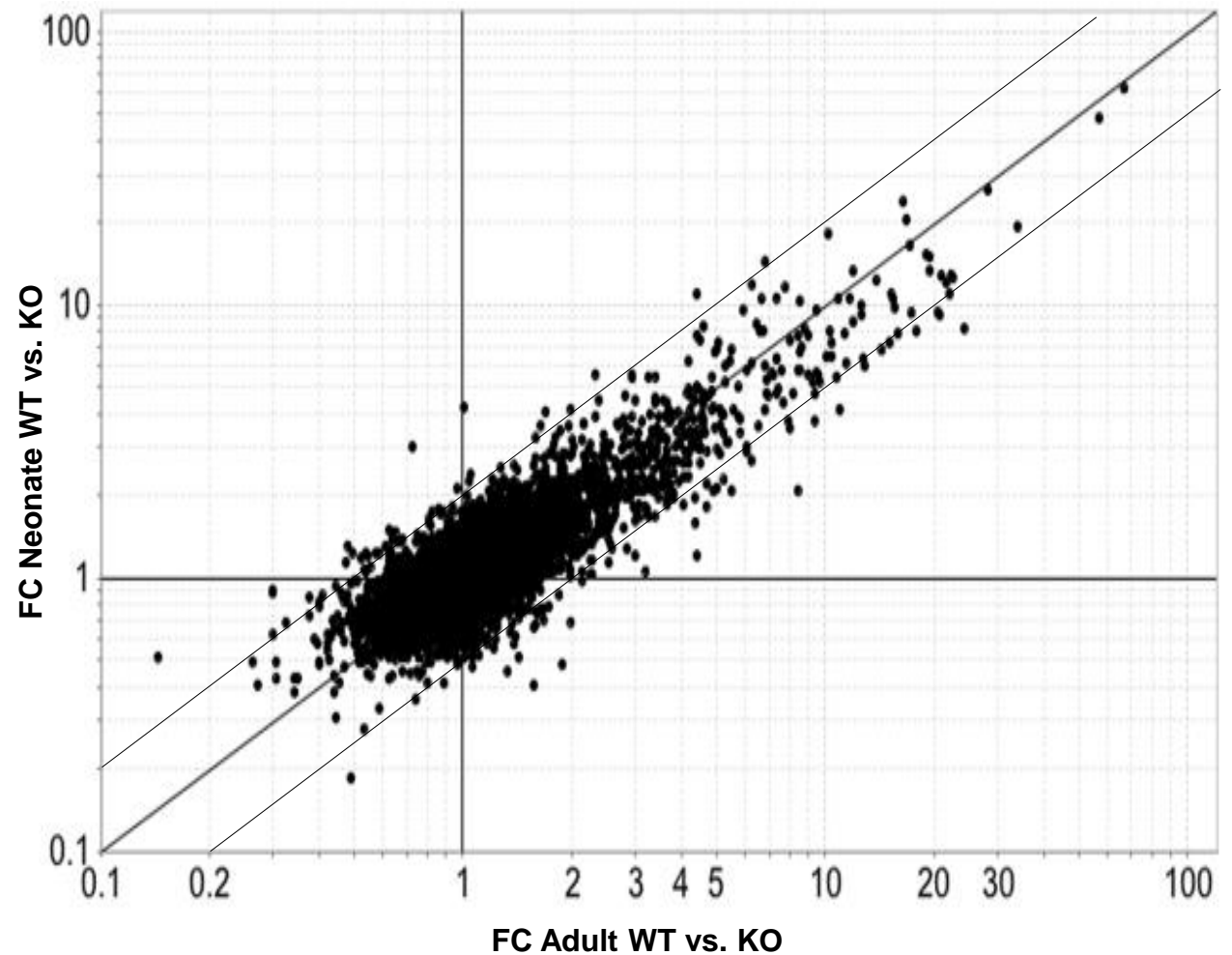
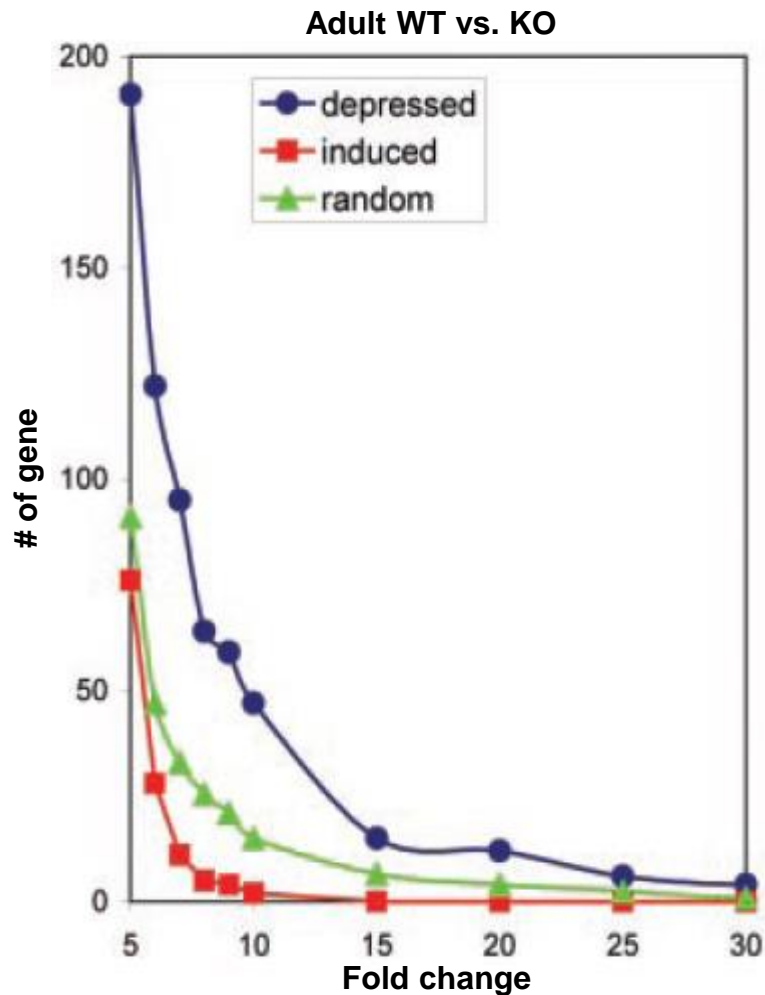


PTA expression by Aire regulation



Is PTA expression different from neonatal and adult window?

MEC cell isolation from adult and neonate → sorting → microarray



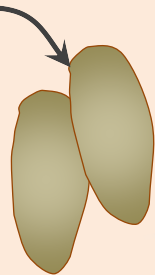
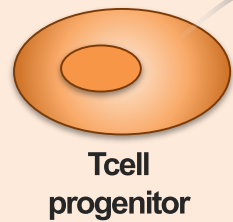
There are no substantial difference in the Aire-dependent PTA repertoires of adult and neonates

Central tolerance & Peripheral tolerance

Central tolerance

Bone marrow

Thymus



Peripheral tolerance

Periphery

T_{reg} cell

Foxp3+

Foxp3+

Naive Tcell

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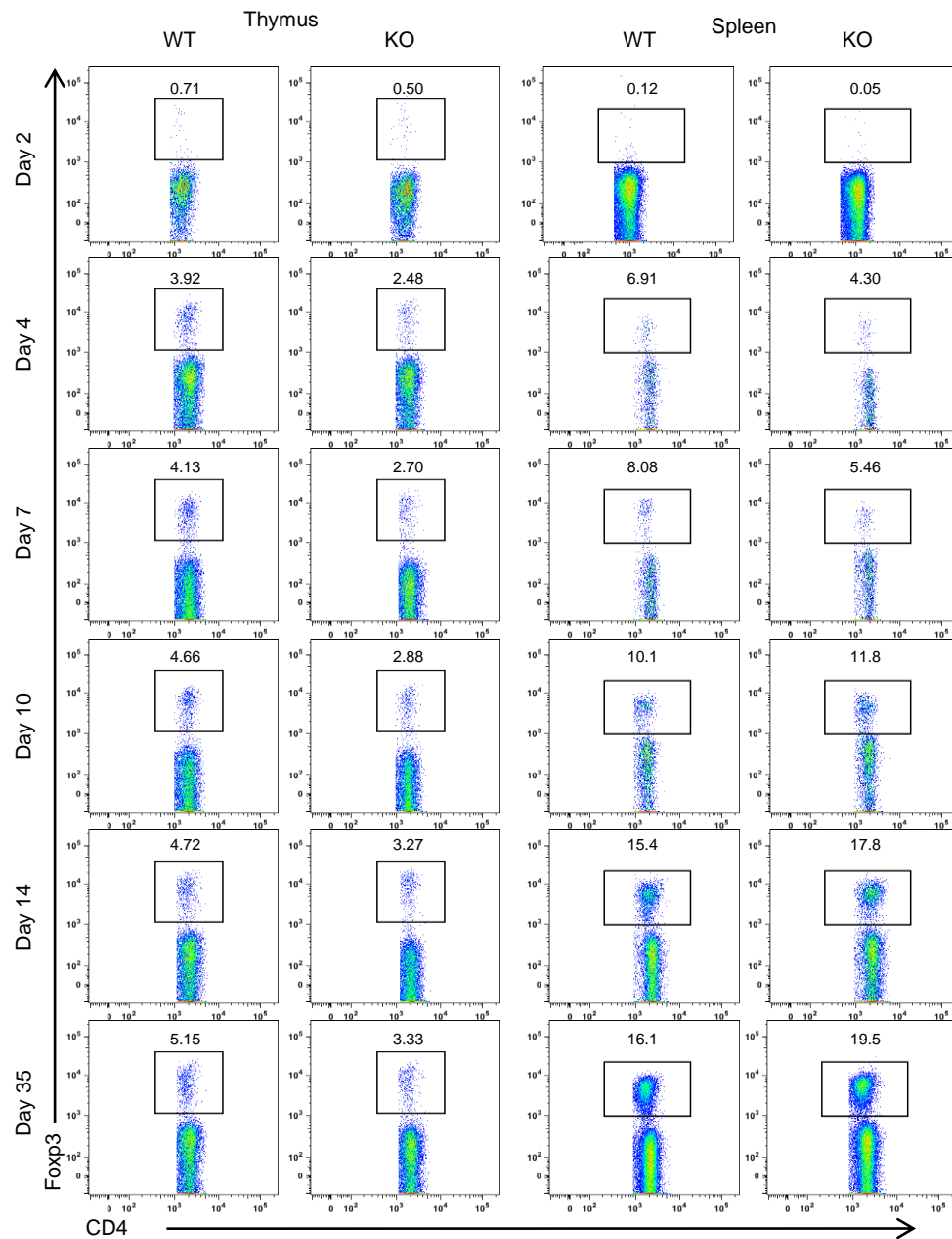
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Infectious disease

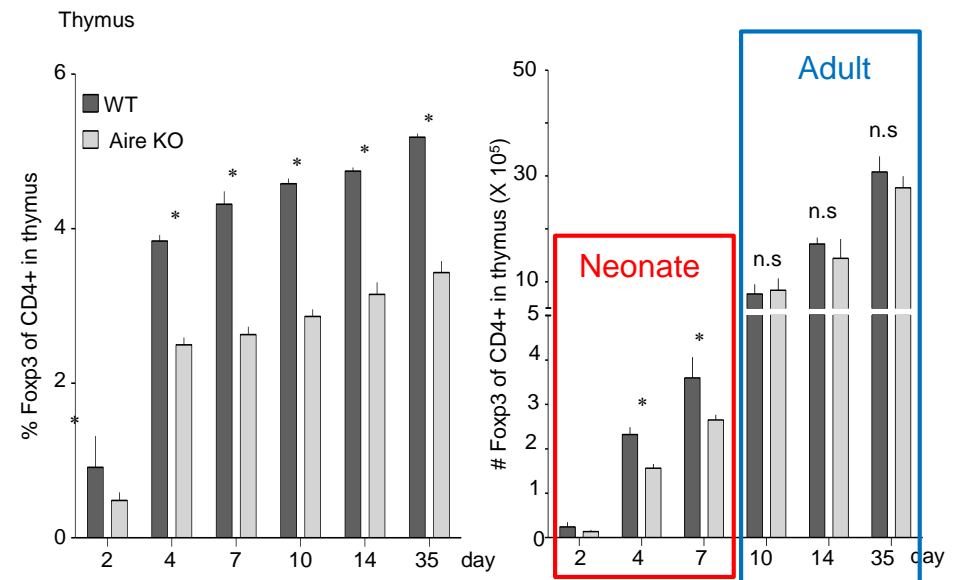
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Relationship between Treg and Aire in perinatal window

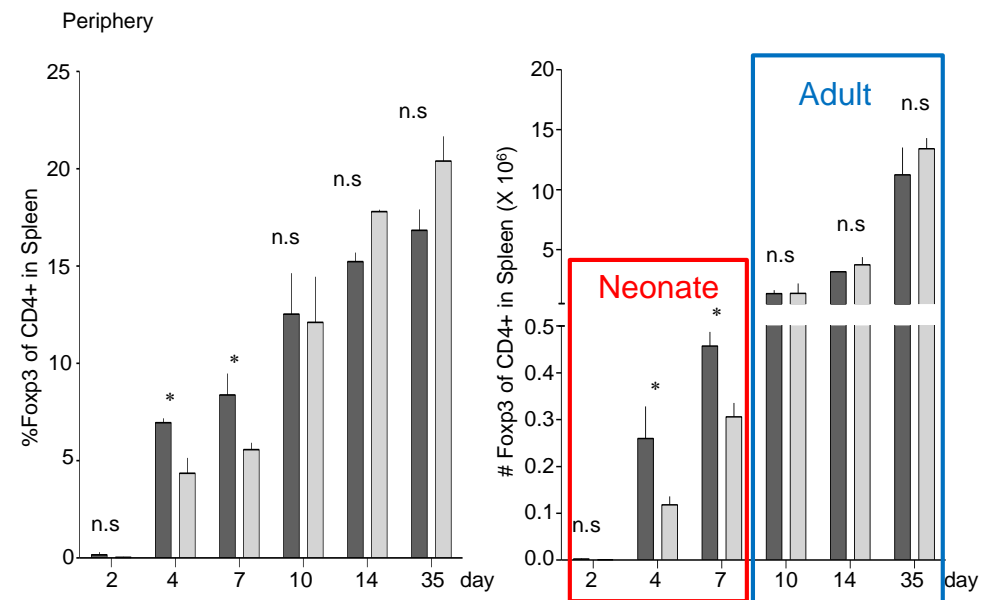
A



B

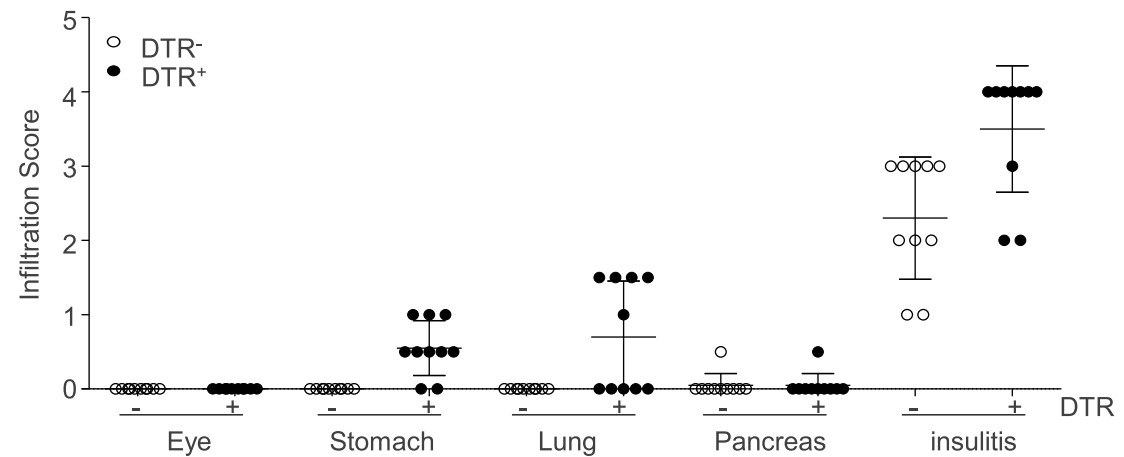
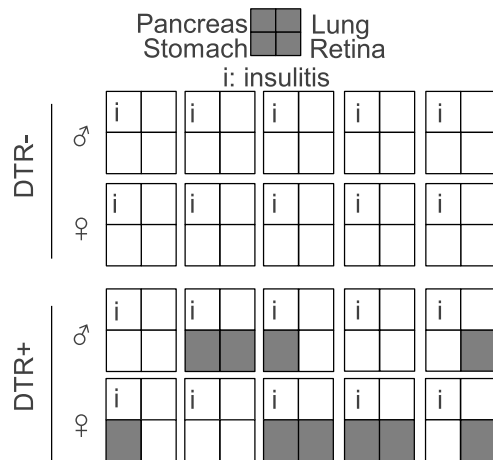
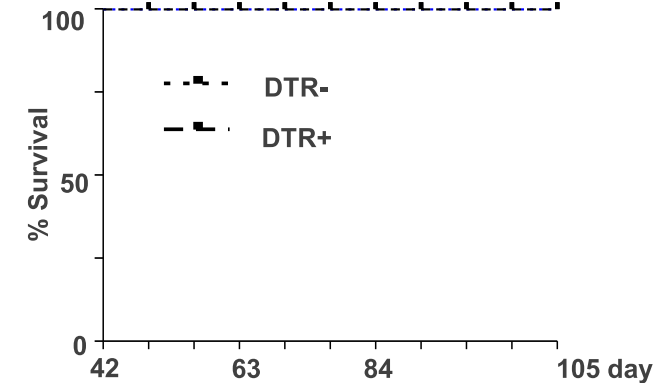
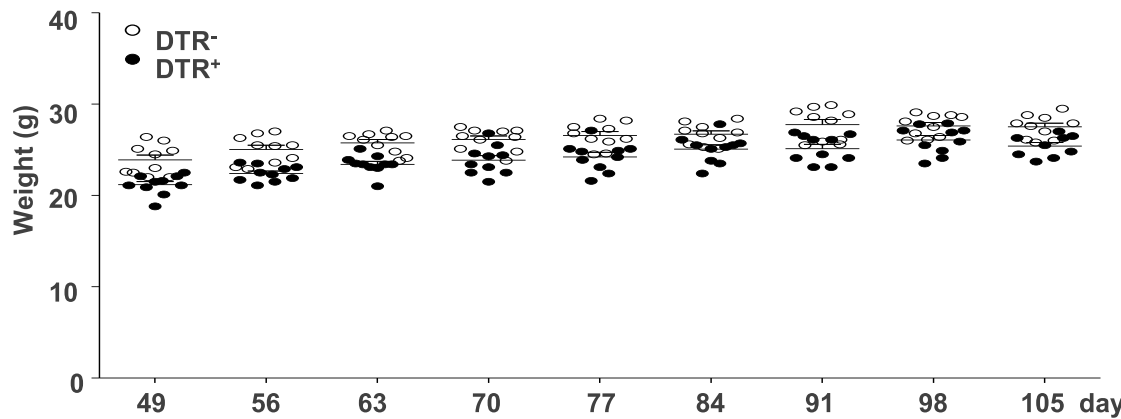
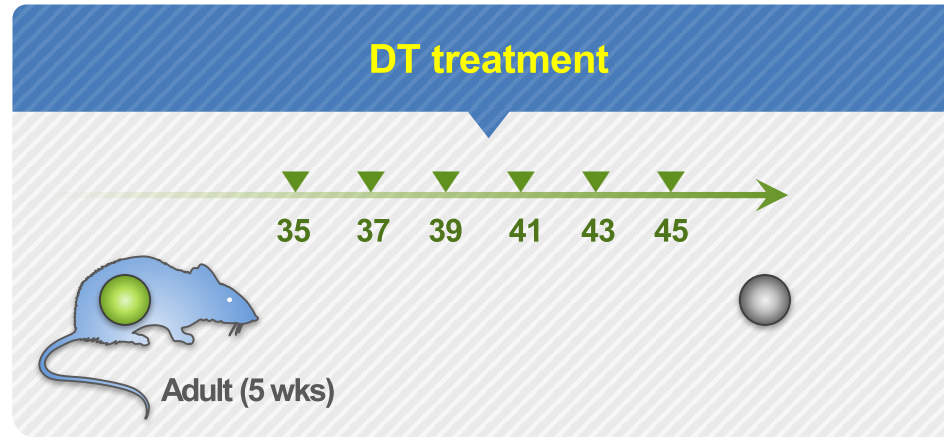


C



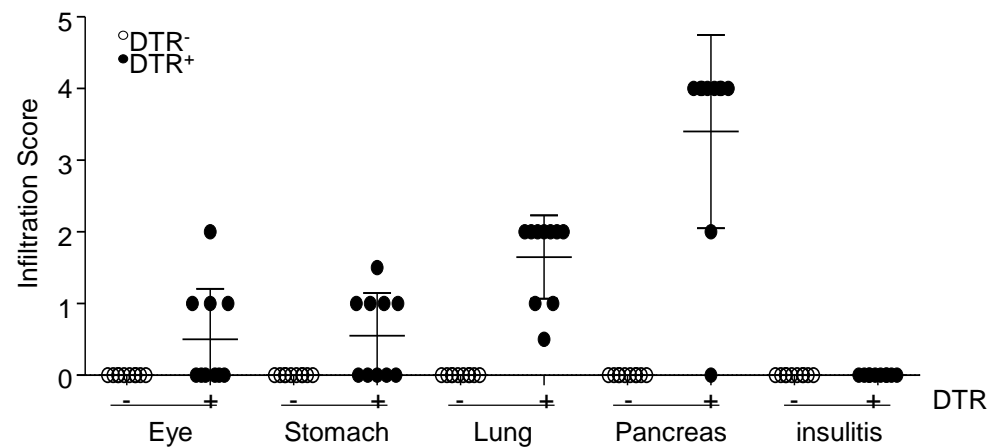
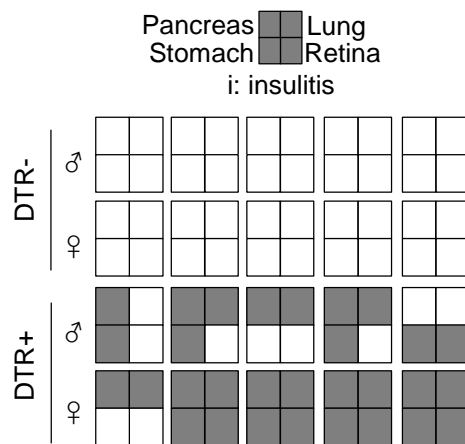
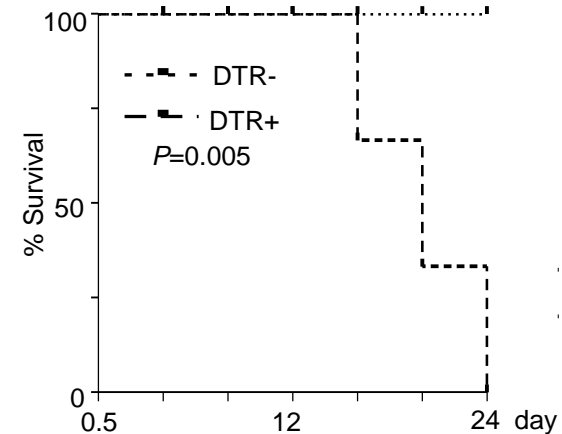
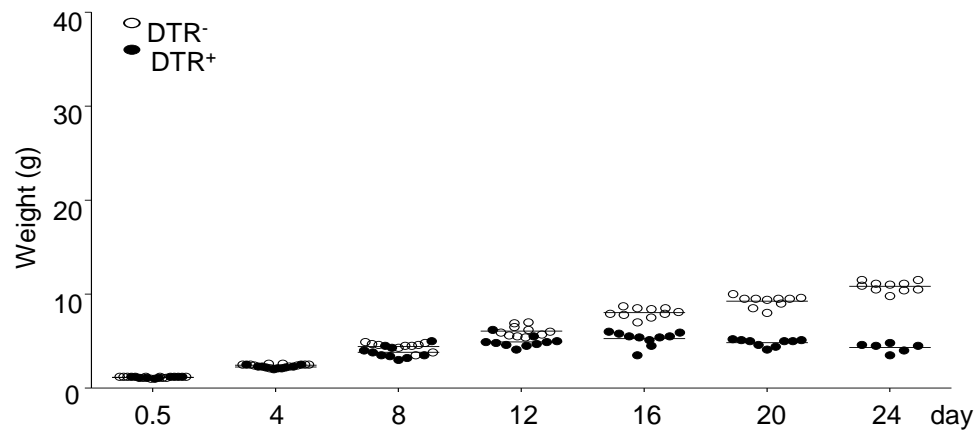
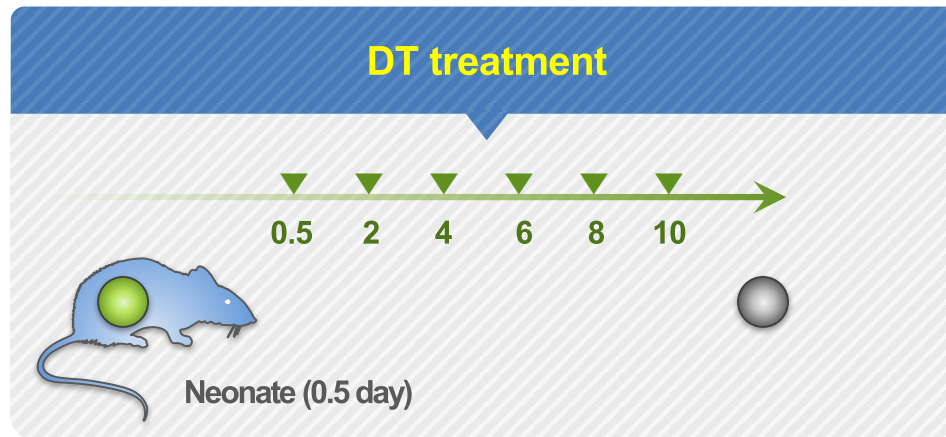
Aire turn off in perinatal stage may influence down regulation of the special repertoire of Treg cells.

Treg depletion in adult



Treg depletion during the adult stage had no significant effect on inducing multiorgan autoimmunity

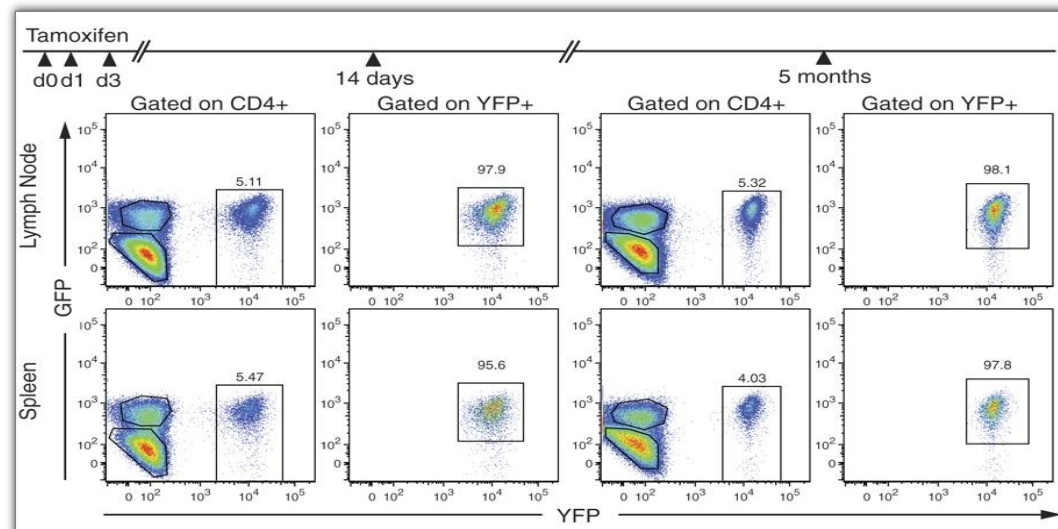
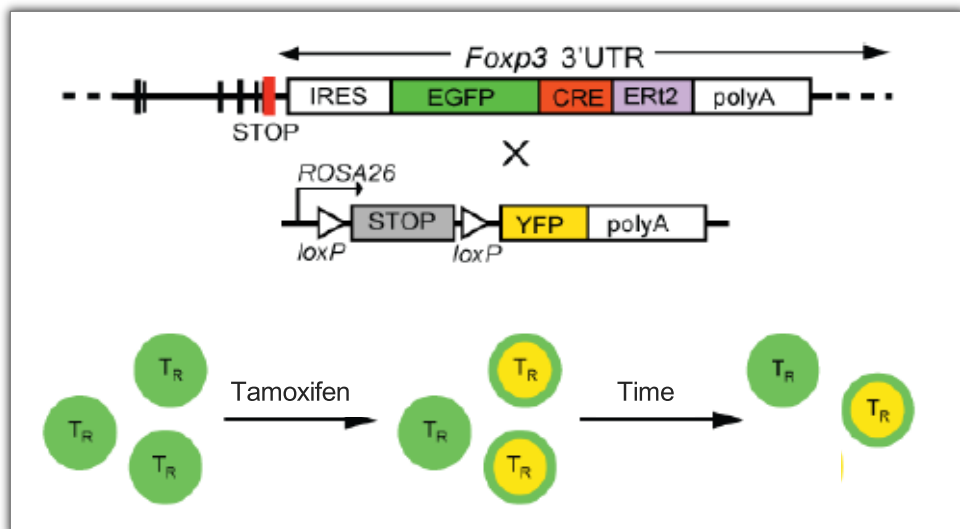
Treg depletion on neonate stage



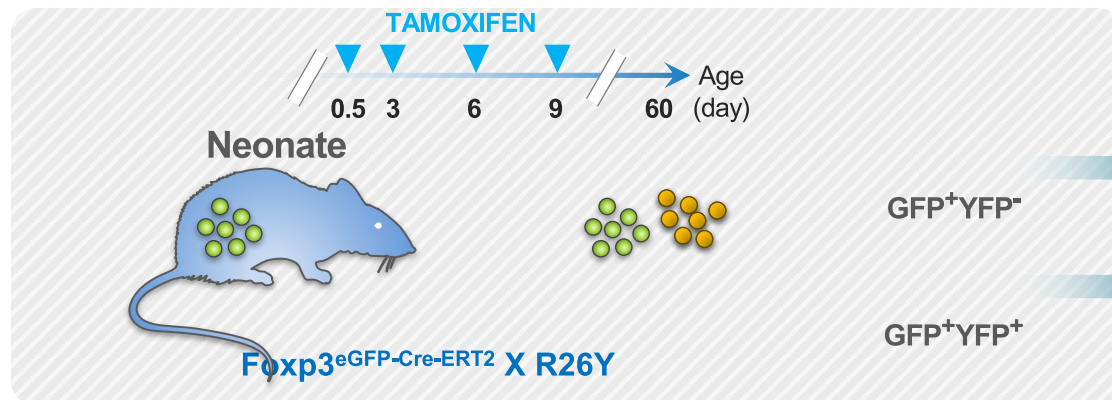
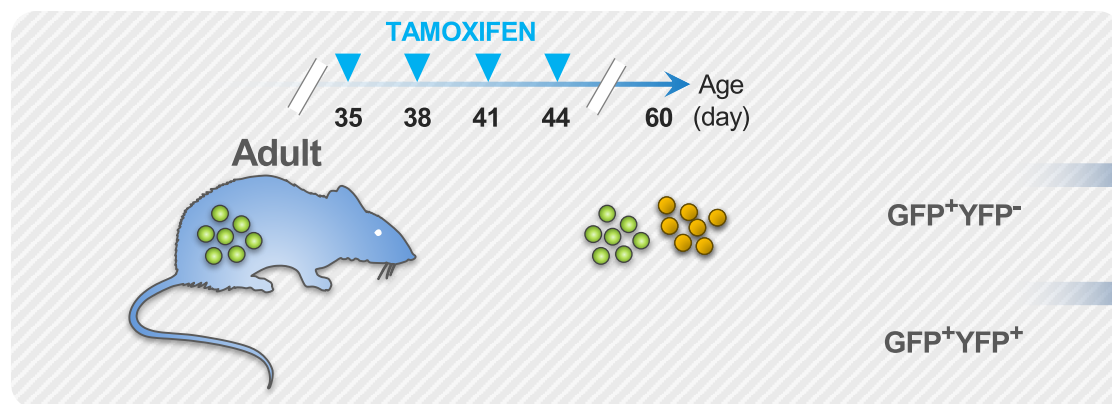
Treg depletion during the neonate stage induced severe multiorgan autoimmunity

Foxp3 tracking system

Foxp3^{eGFP-Cre-ERT2} X R26Y mice: Foxp3⁺-lineage tracking



Rubtsov et al., Science. 2010 Sep 24;329(5999):1667-71



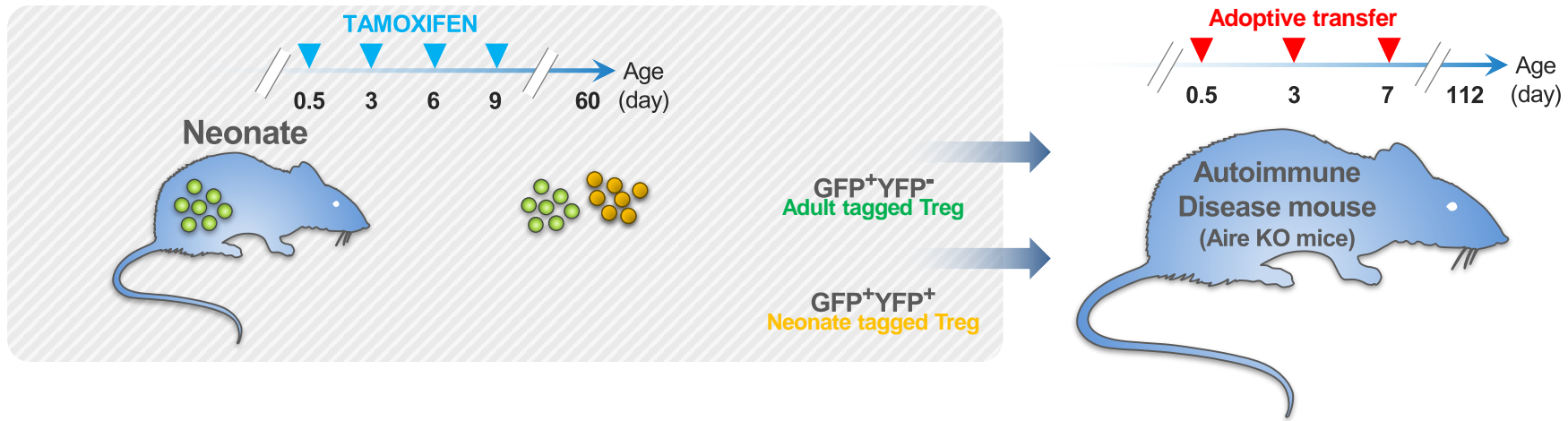
in vivo analysis

- Adoptive transfer

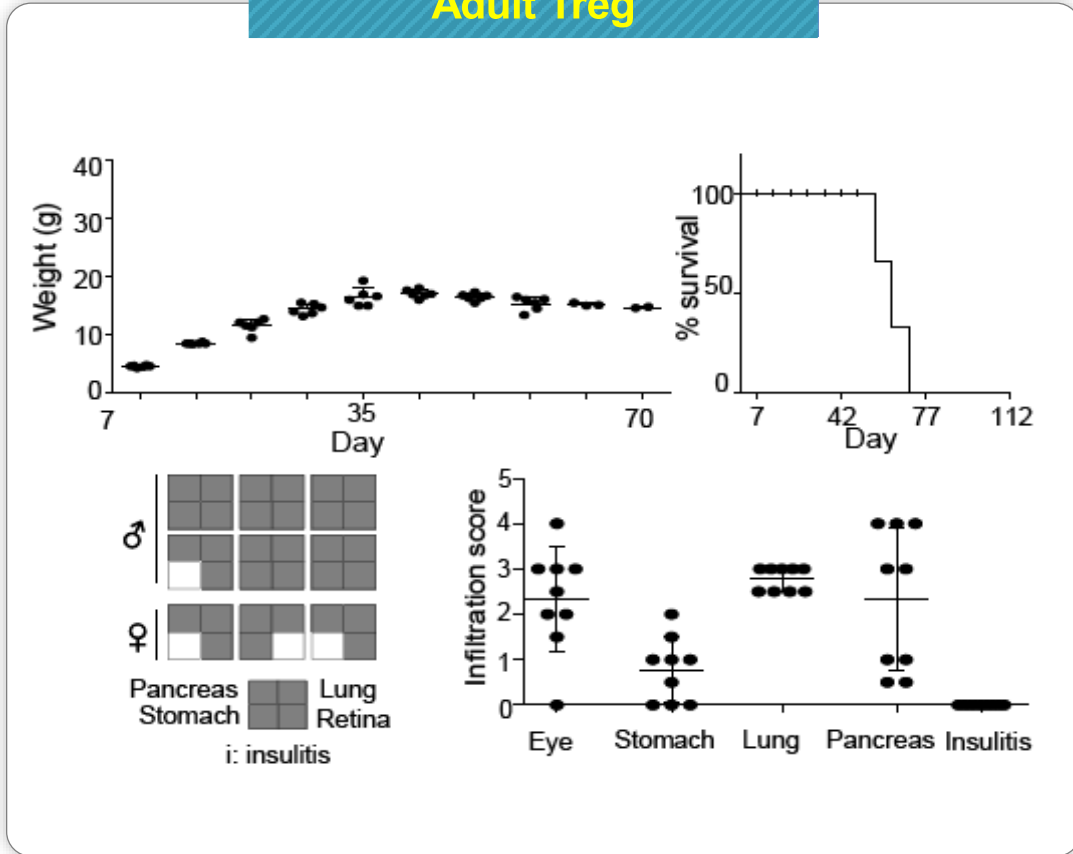
in vitro analysis

- Microarray
- TCR sequencing

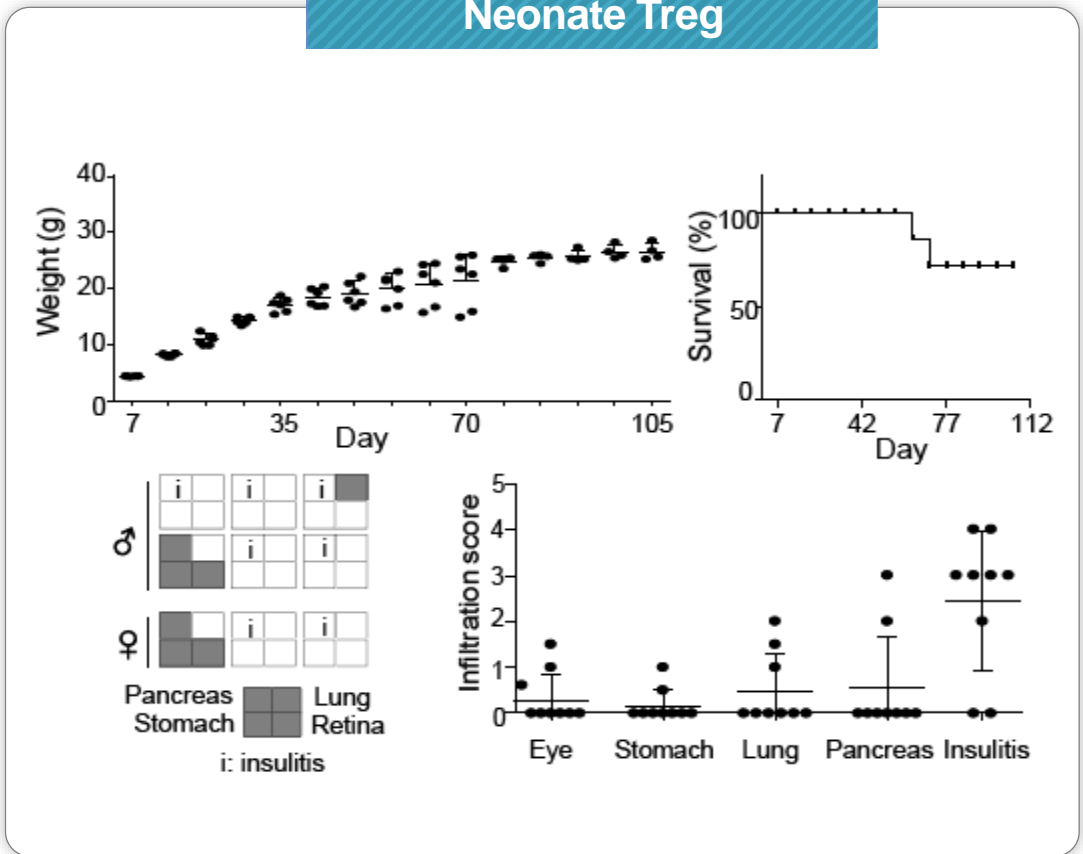
Adult Treg vs neonate Treg



Adult Treg



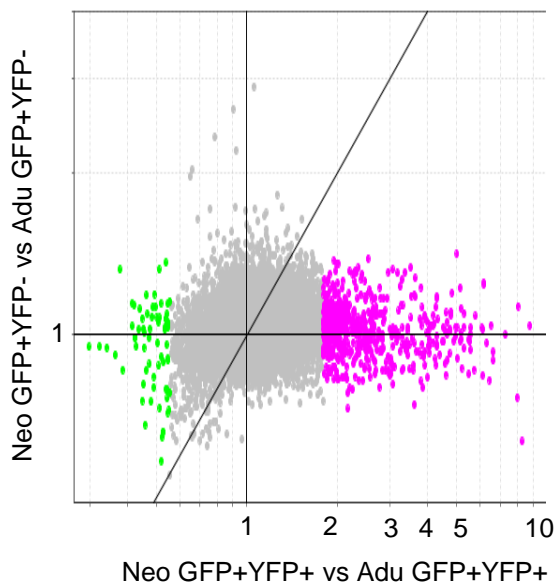
Neonate Treg



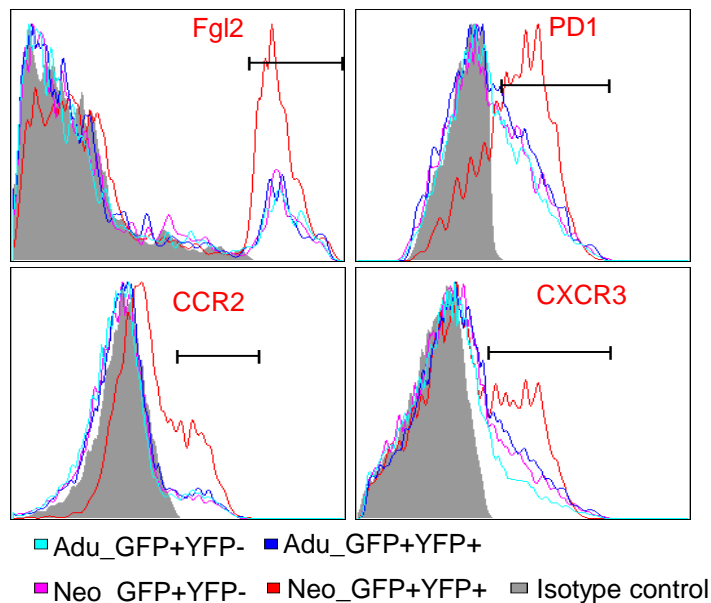
Neonate Treg had strong capacity for rescuing multiorgan autoimmunity

Neonate tagged Treg have more functional activity

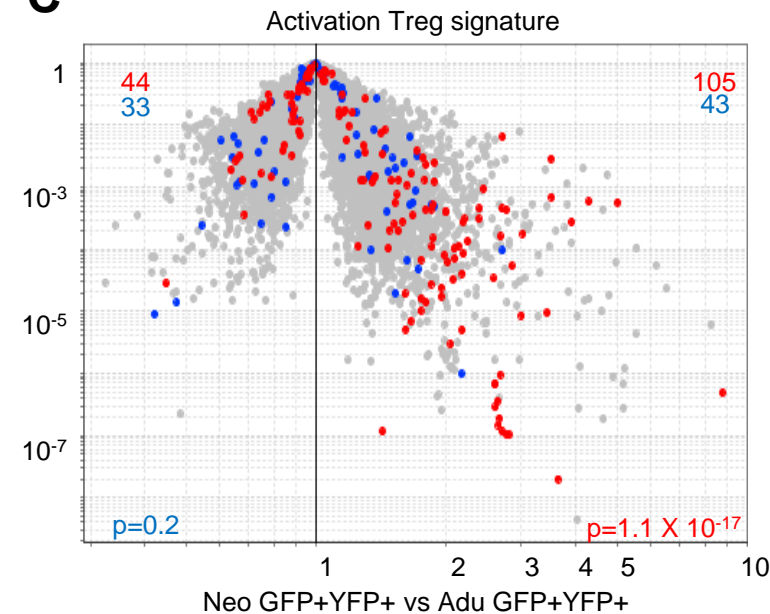
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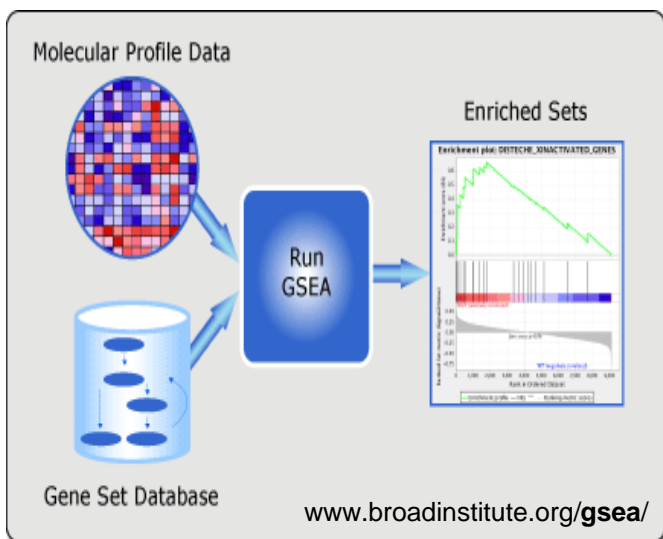
B



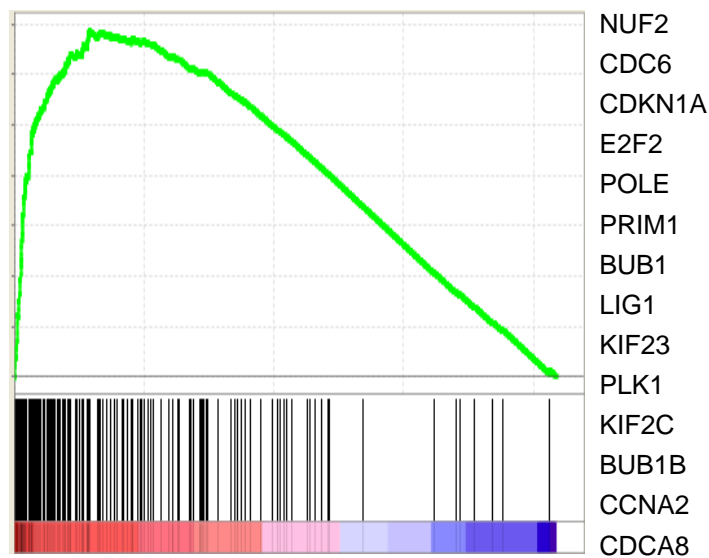
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D

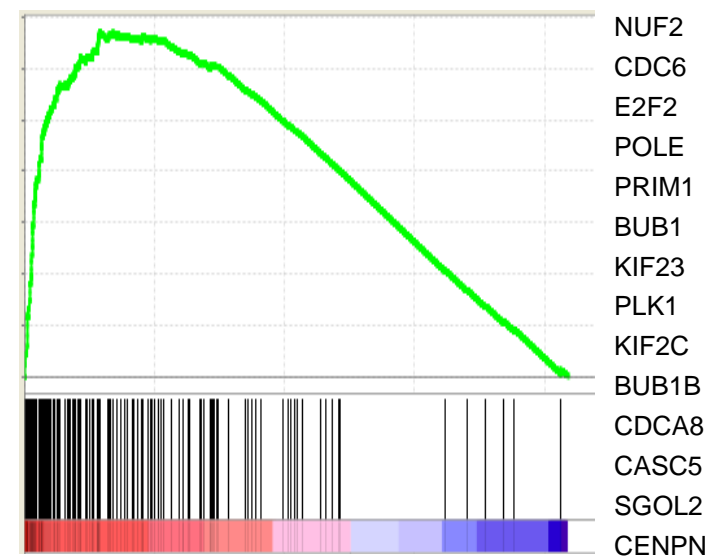


DNA REPLICATION



Neo_GFP+YFP+ Adu_GFP+YFP+
Adu_GFP+YFP-
Neo_GFP+YFP-

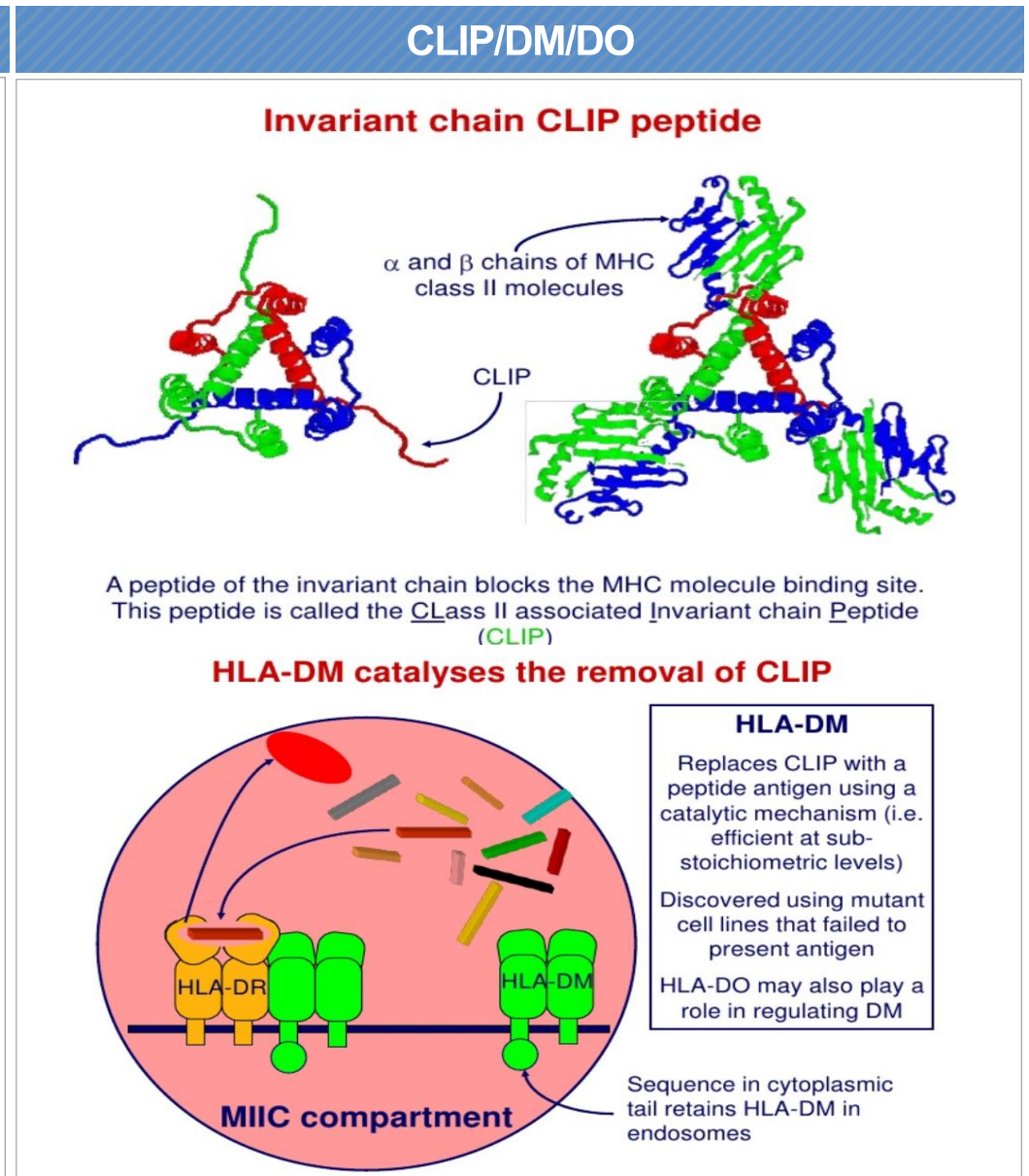
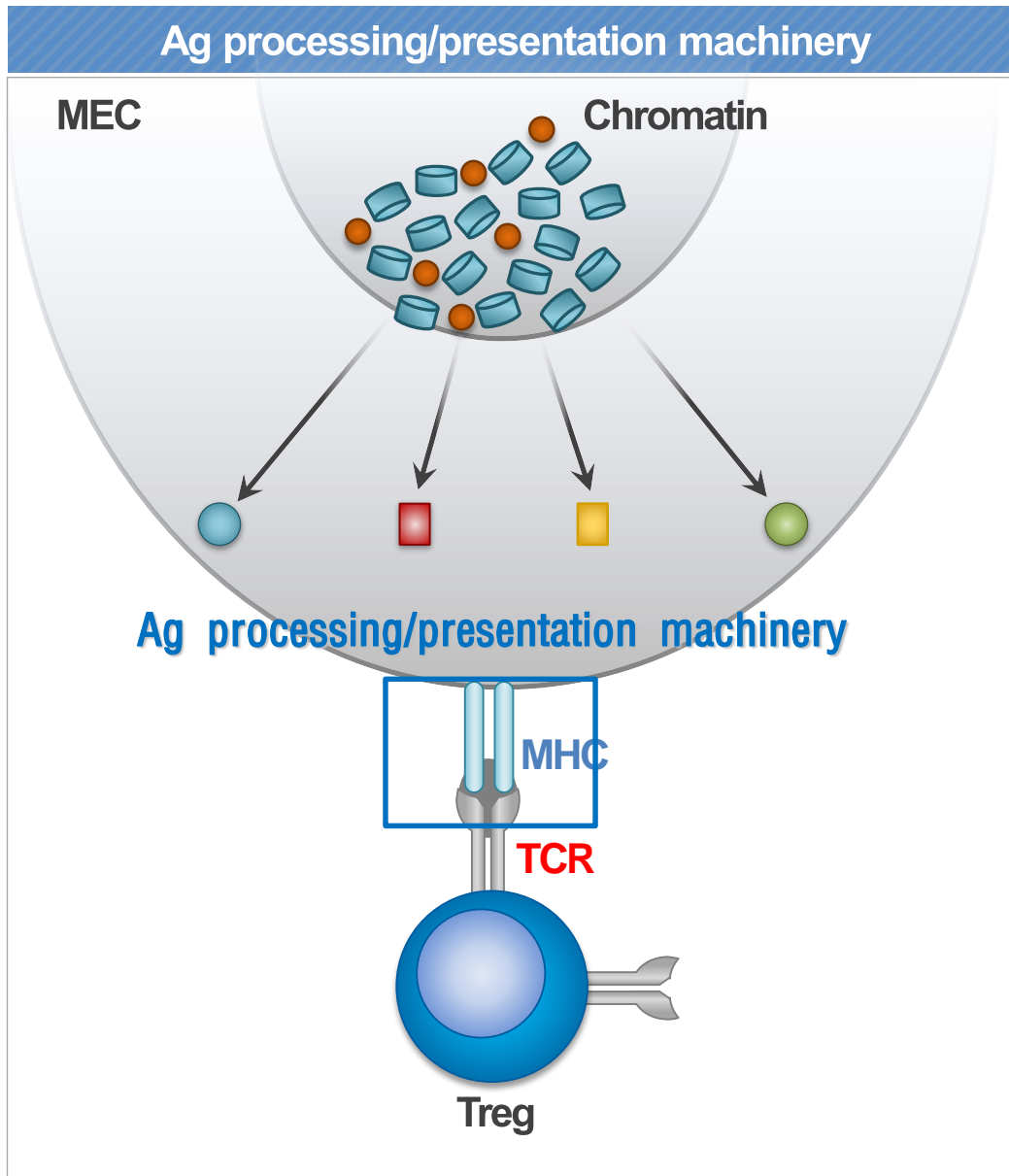
MITOTIC M M G1 PHASE



Neo_GFP+YFP+ Adu_GFP+YFP+
Adu_GFP+YFP-
Neo_GFP+YFP-

Neonate Treg have more functional activity: increased Treg activity, proliferation, etc

Ag processing/presentation machinery

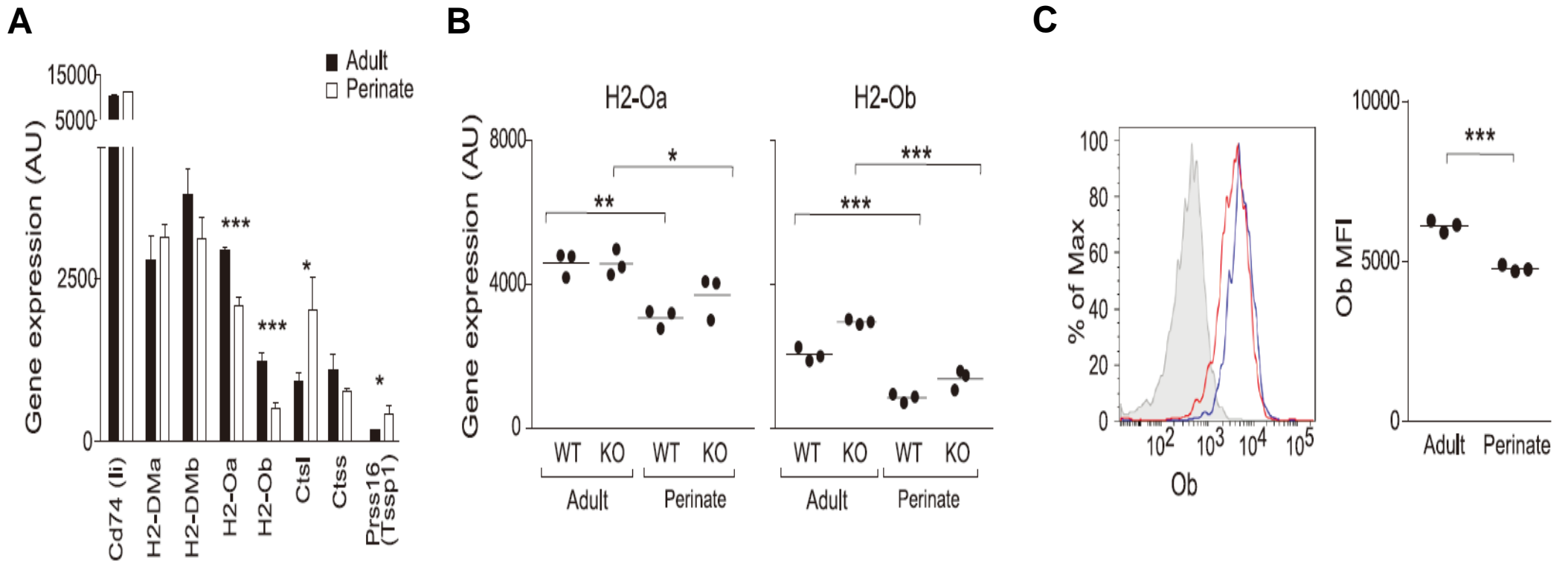


- CLIP block the MHC class binding site
- DM catalyzes the removal of CLIP
- DO inhibit the DM activity

**Ag processing/
presentation machinery**

Expression level of DO in neonate and adult

Do expression is age dependent

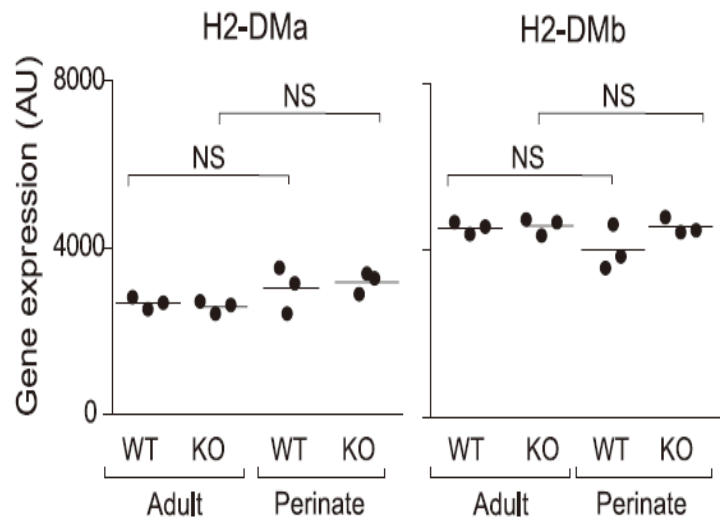


Do chains were expressed at a significantly lower level in perinatal than in adult MECs

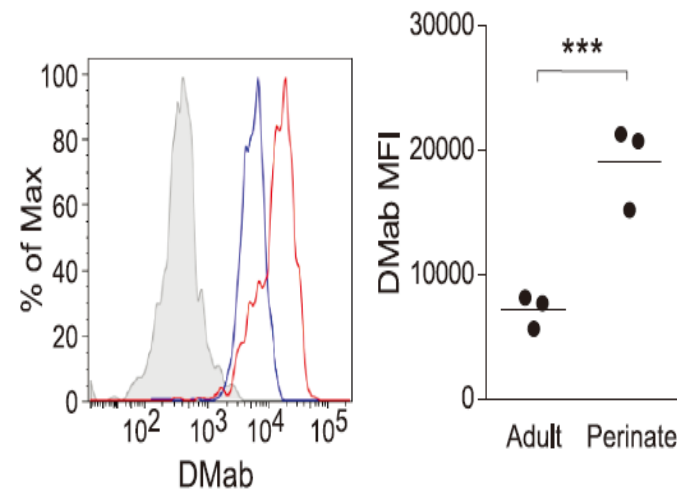
Expression level of DM in neonate and adult

Do inhibit activity of DM

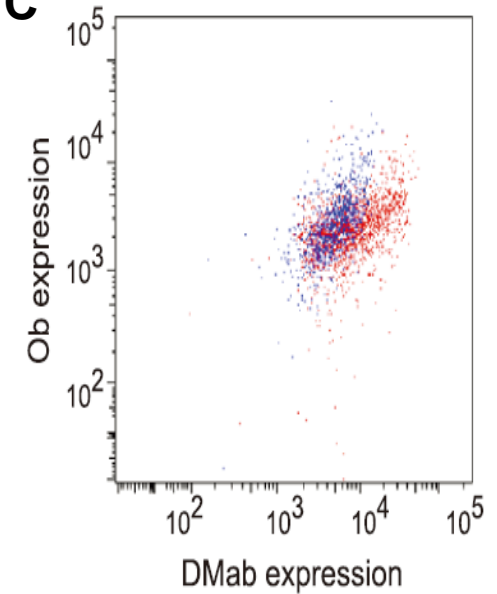
A



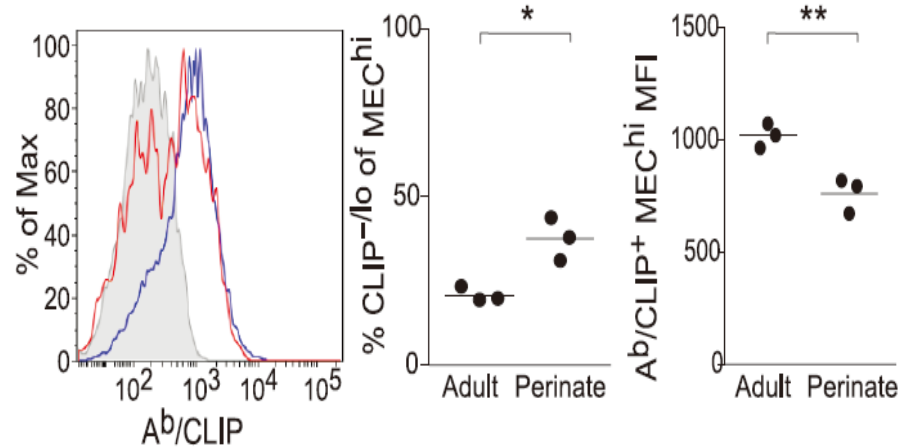
B



C

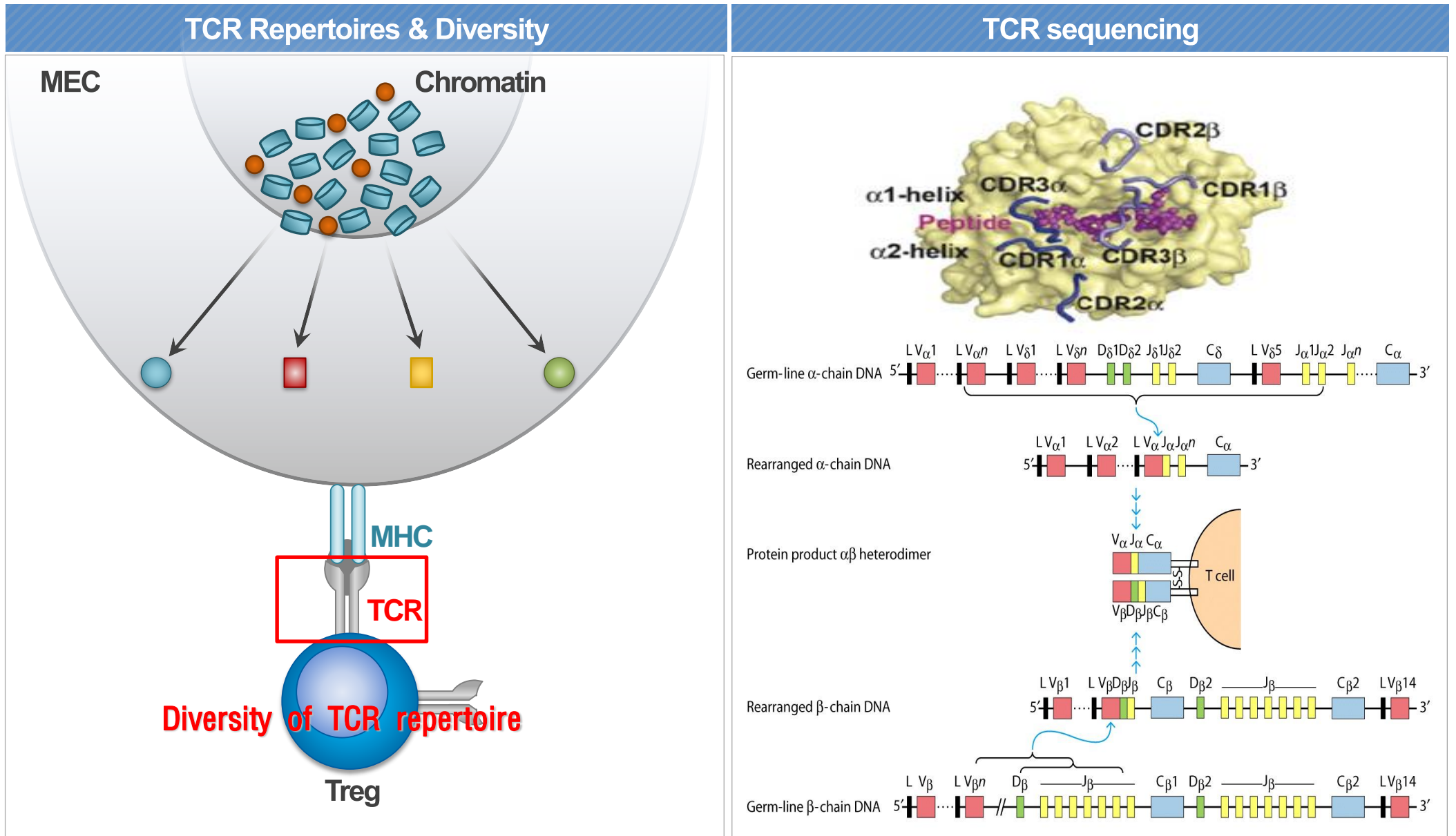


D



Low DO:DM ratio should promote more effective antigen presentation in perinatal than adult MECs

Diversity of neonate vs adult Treg TCR repertoires



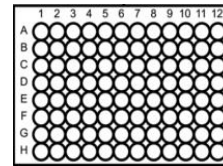
Ag processing and presentation machinery MECs promotes more diversity of neonate Treg TCR repertoires than adult Treg TCR repertoires

Single-cell TCR sequencing strategy



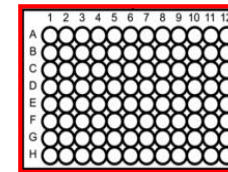
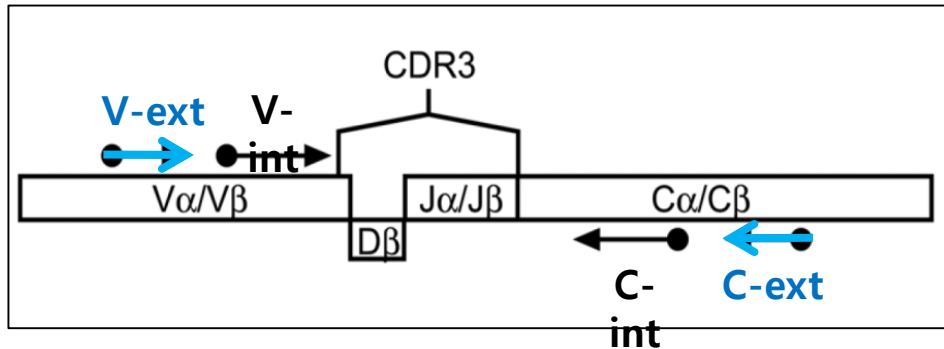
Isolate cells
Spleen

Single cell sort

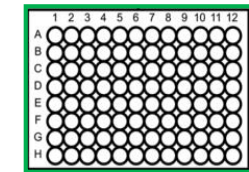


2 columns blank
2 wells have 15
cells/well

Reverse
Transcription

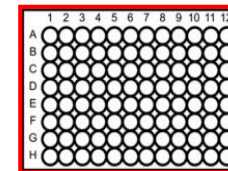


1st rnd TCRα PCR
20 Vα forward primers &
1 Cα reverse primers

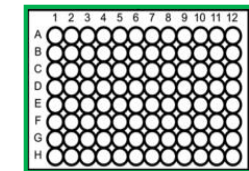


1st rnd TCRβ PCR
19 Vβ forward primers &
1 Cβ reverse primers

Nested PCR with
internal primers



2nd rnd TCRα PCR
20 Vα forward primers &
1 Cα reverse primers

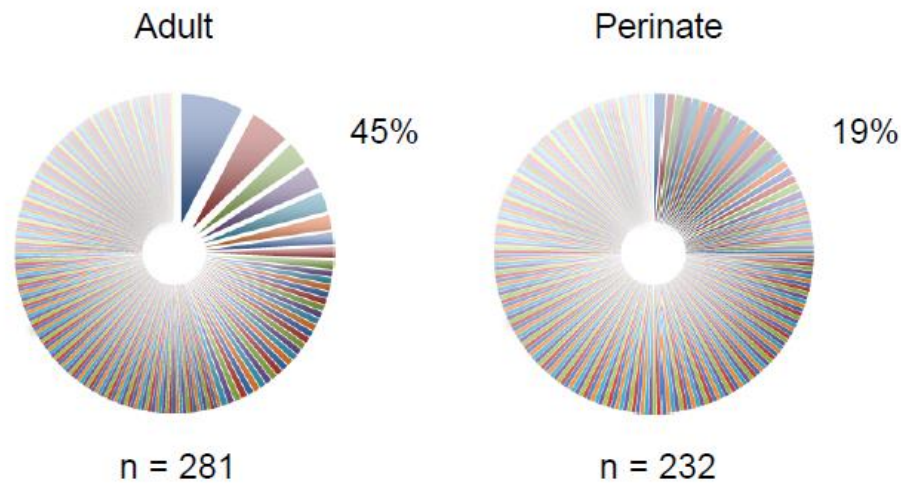


2nd rnd TCRβ PCR
19 Vβ forward primers &
1 Cβ reverse primers

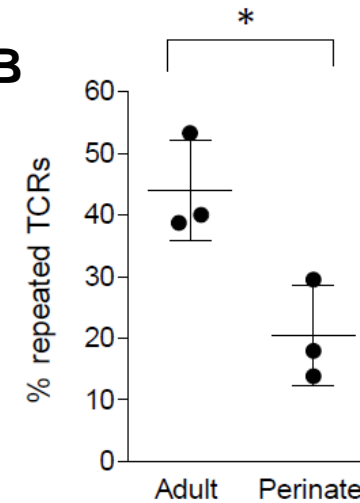
Sequencing cells which had both
TCRα and TCRβ PCR products

Different TCR repertoires in perinatal and adult mice

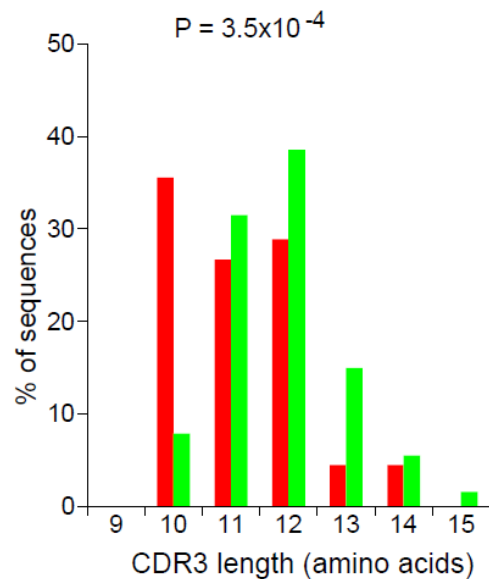
A



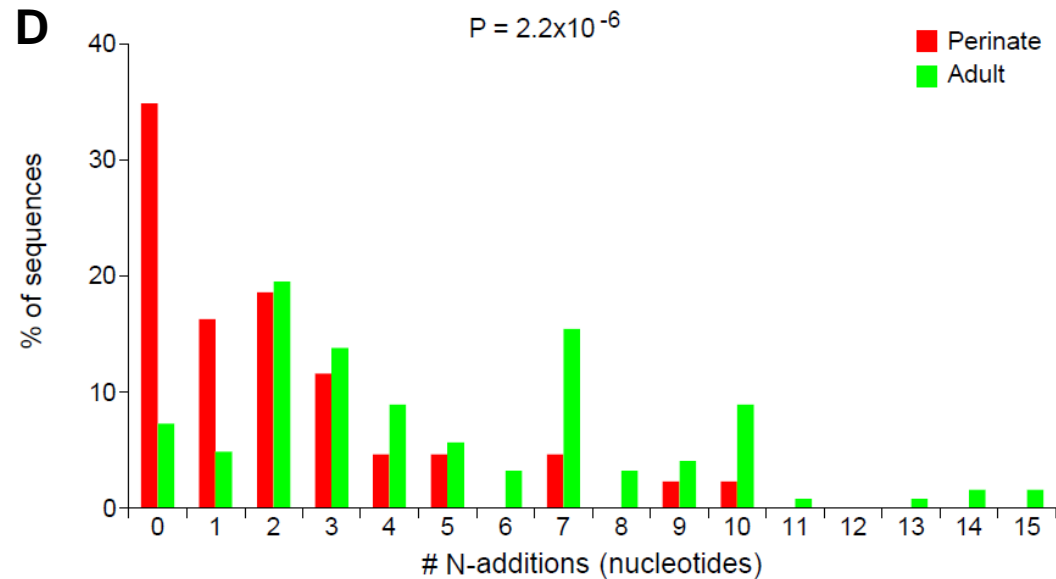
B



C



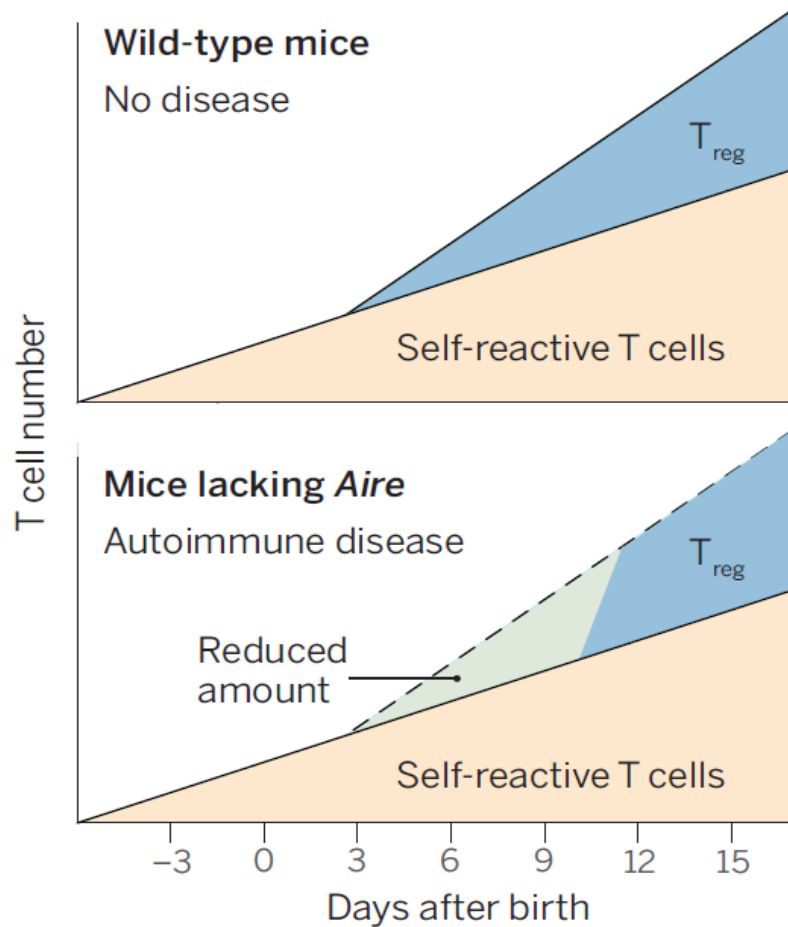
D



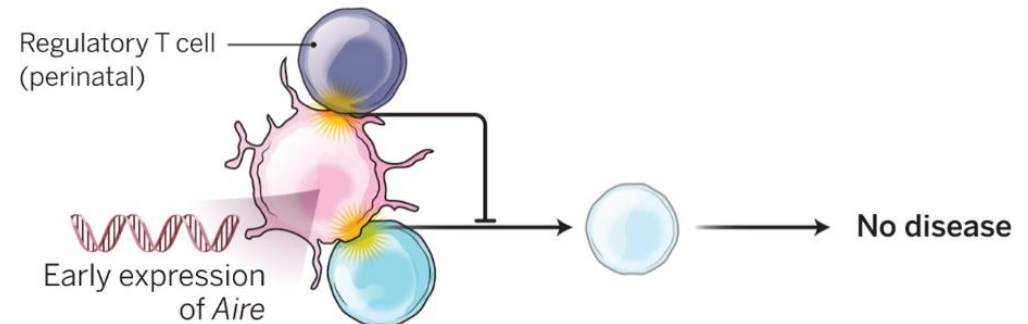
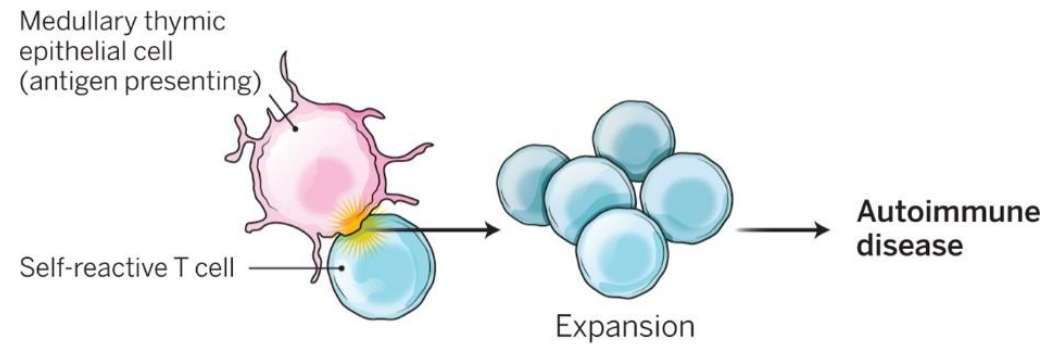
Age dependent Ag processing/presentation machinery generated more diverse TCR repertoires of neonatal Treg

Summary

Perinatal window



Age dependent Treg cells produced early in life prevent autoimmunity



- *Developmental anomalies of thymic negative selection or Treg cell generation, and the resulting imbalance between Treg cells and autoimmune T cells, can be causative of, and predispose to, autoimmune disease*
- *Anomalies or variations of not only aire but also other genes controlling T cell development might exert distinct or stronger effects early in life than in adults*