



Modeling T lymphocyte dynamics: From physiology to perturbations of immune system

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ImmunoComplexiT network

<http://www.immunocomplexit.net/>

Differentiation & selection of T cells

Quantify T cell processes and dynamics

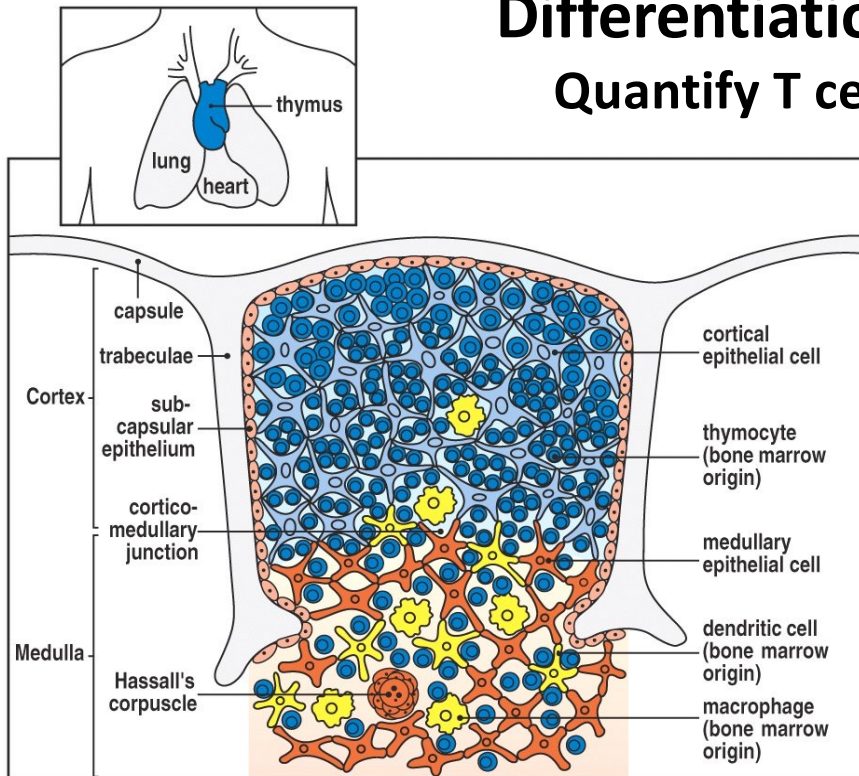
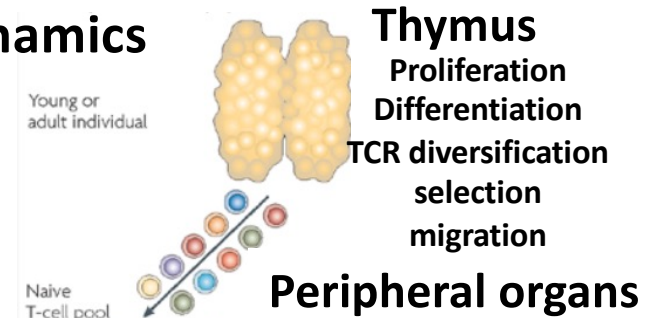
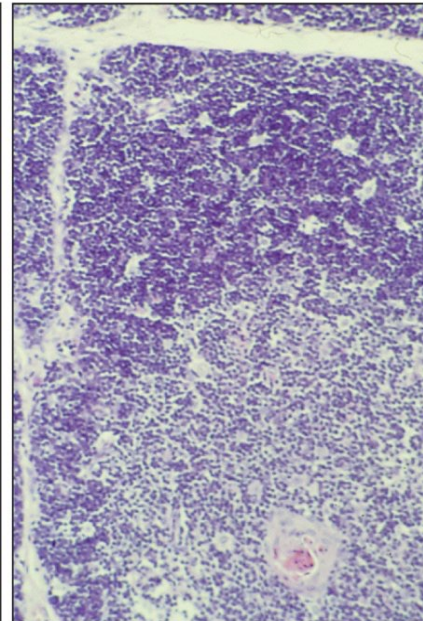


Figure 7-8 Immunobiology, 6/e. (© Garland Science 2005)



Lymphocyte population diversity

Dynamics through aging



Repertoire diversity
Proliferation/death

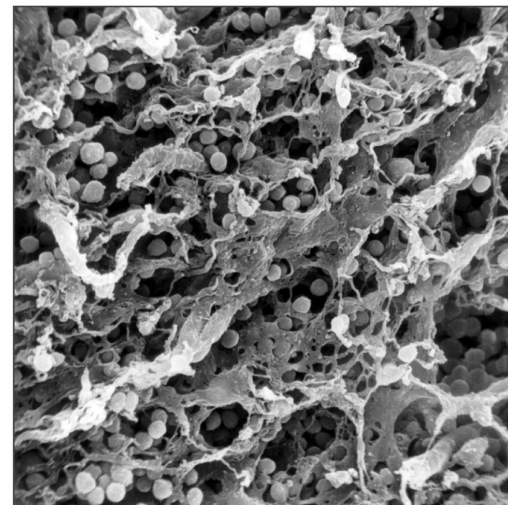
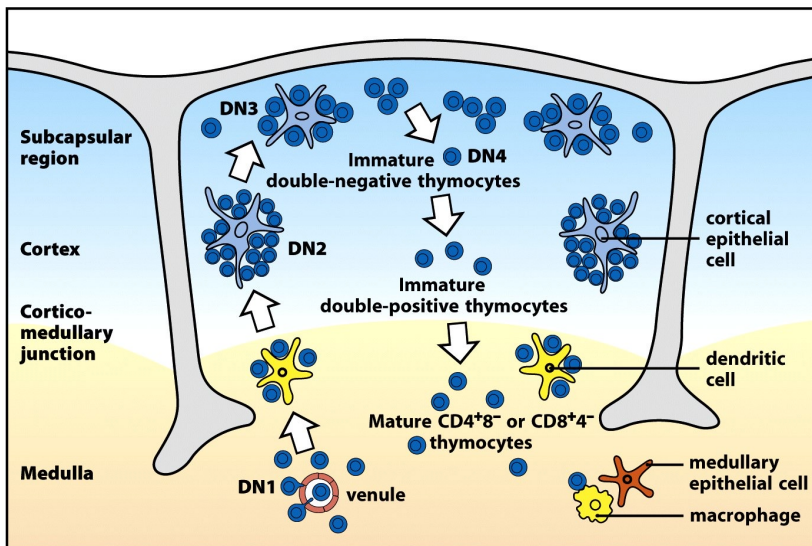
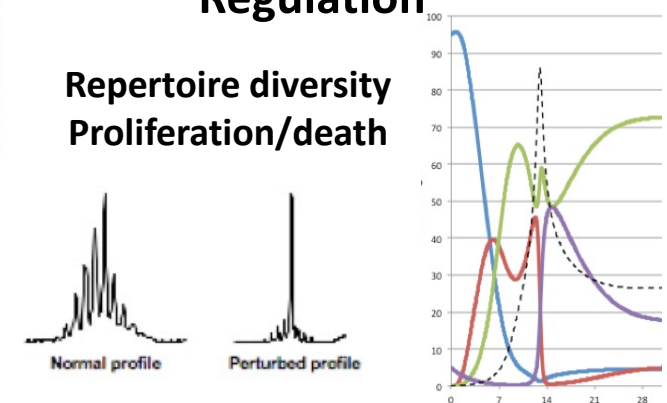
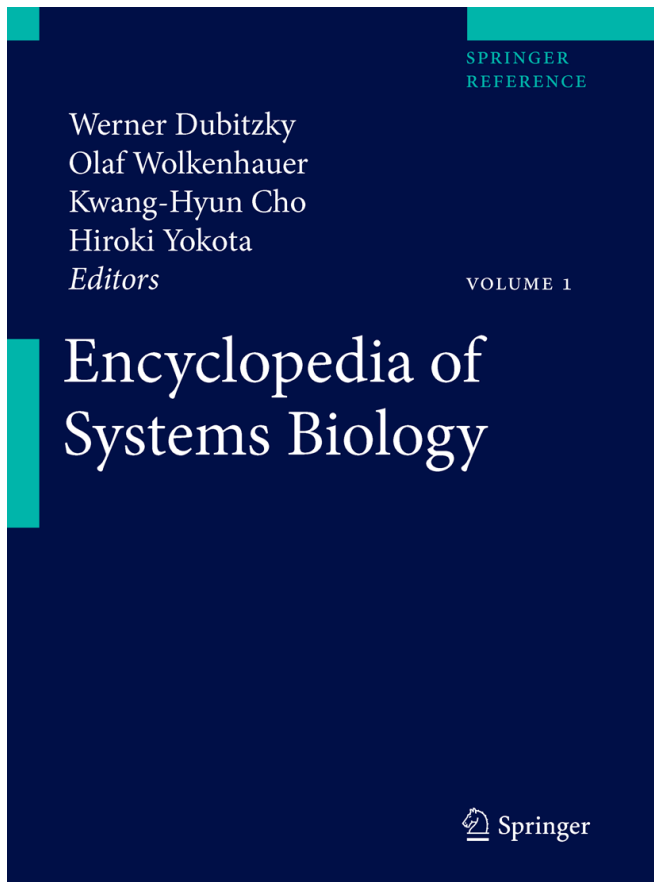


Figure 7-9 Immunobiology, 6/e. (© Garland Science 2005)

Figure 7-21 Immunobiology, 7ed. (© Garland Science 2008)



Encyclopedia of Systems Biology

2013, pp 1145-1149

Lymphocyte Dynamics and Repertoires, Biological Methods

Dr. Véronique Thomas-Vaslin, Dr. Adrien Six, Bertrand Bellier, Dr. David Klatzmann

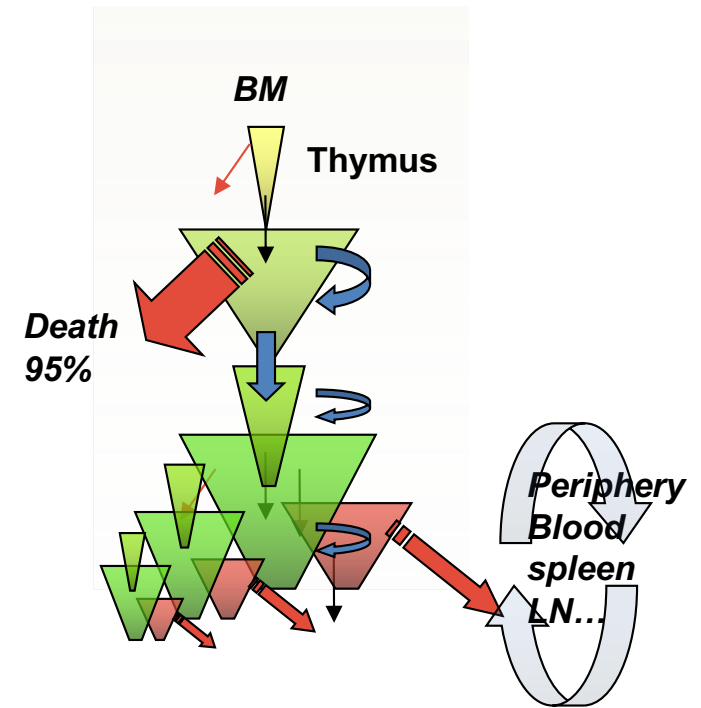
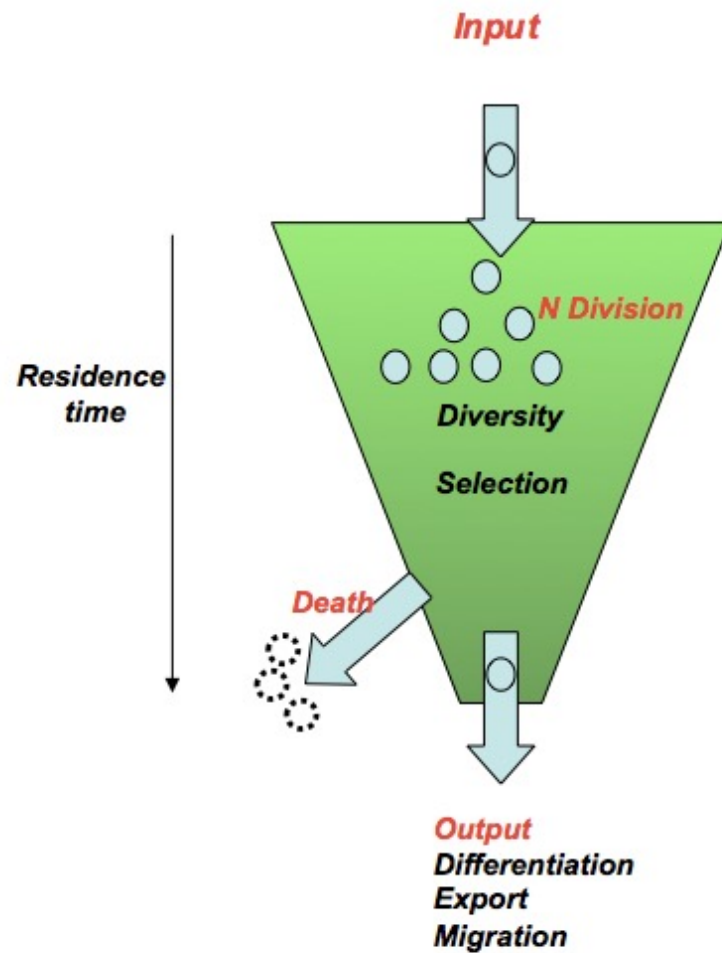
Encyclopedia of Systems Biology

2013, pp 1149-1152

Lymphocyte Dynamics and Repertoires, Modeling

Dr. Véronique Thomas-Vaslin, Dr. Adrien Six, Bertrand Bellier, Dr. David Klatzmann

Dynamics and selection of lymphocytes



Multiplicity of T cell populations

- Naive/memory
- Function
- Repertoire
- Regulation

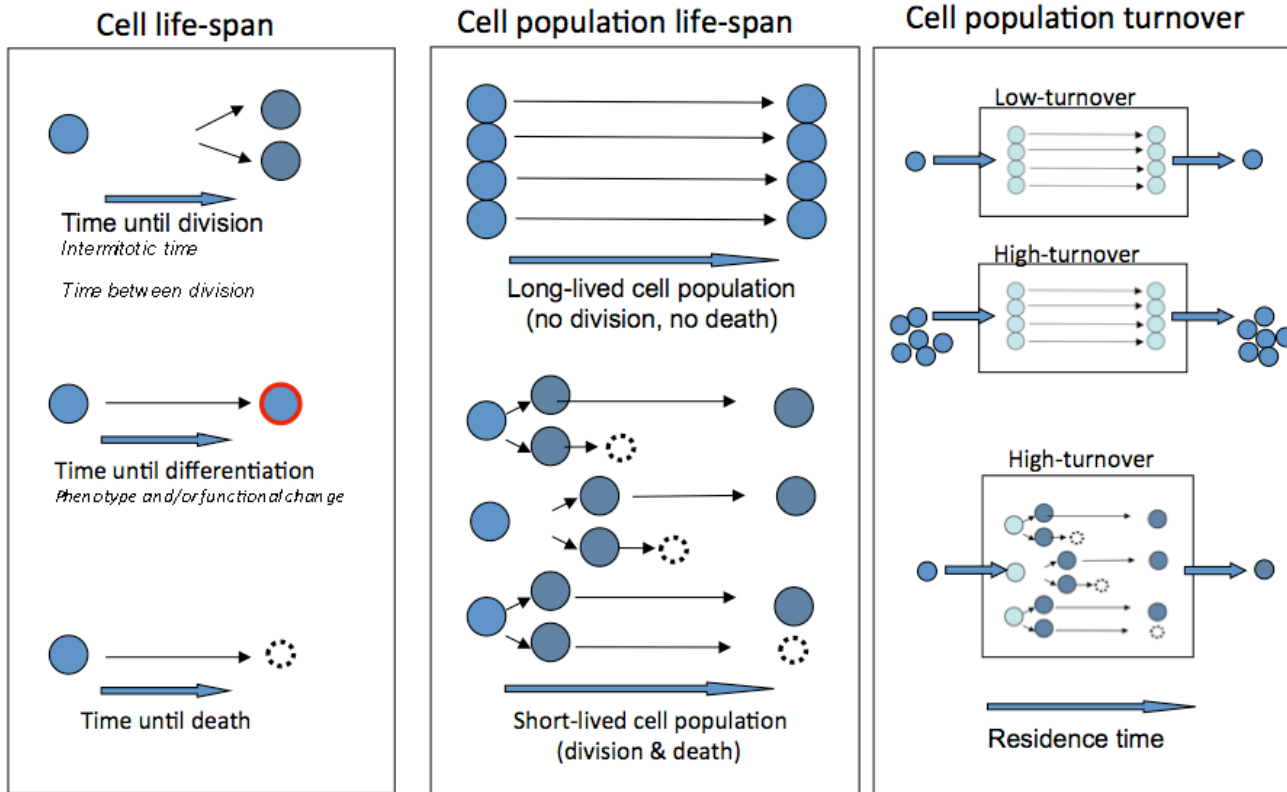
Perturbations

- Aging
- Diseases
- Treatments
- ...

Define components, rules,
granularity...

Thomas-Vaslin Immunosuppression Inteck book 2012

Provide definitions



Estimation of cell population dynamics

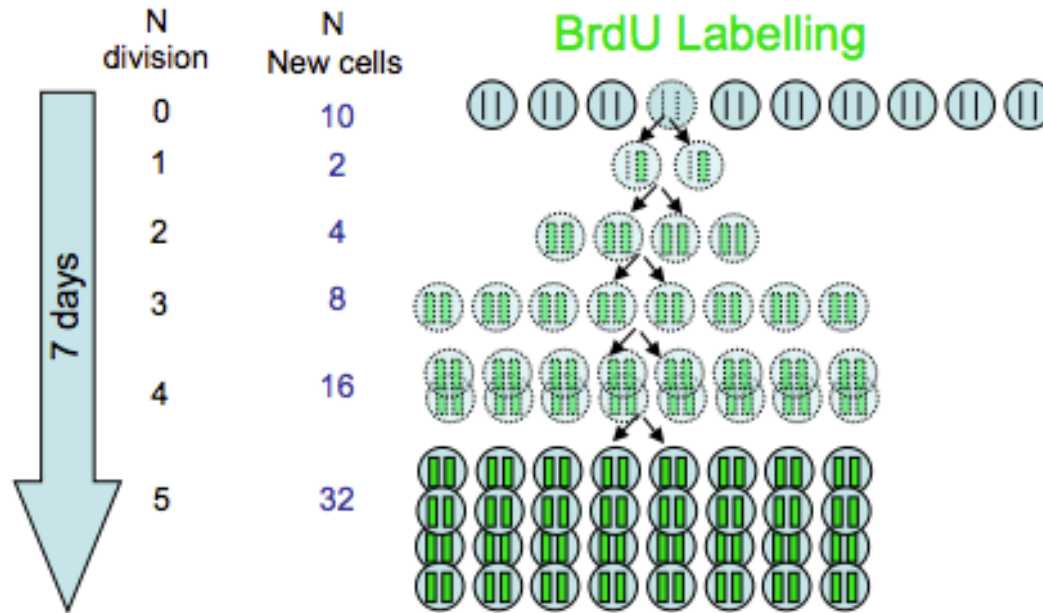
- Input/output in a compartment
- Number of cell generation
- Production rates
- Death rate
- Selection rates
- Migration rates
- Time of residence

Estimation of cell division in a population

- Proliferation rate/day (p) = percentage of cells entering division per day
- % of cell in division (at a t time)
- Time between two divisions = intermitotic time = division time = $1/p$
- Doubling time: time to double the cell number.

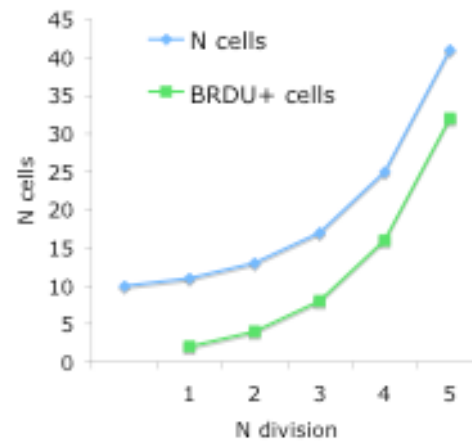
Quantify cell division with various methods:

Consider 1 of 10 cells start to divide with symetric clonal proliferation (5 division cycles)



exponential cell expansion +400% in 7 days

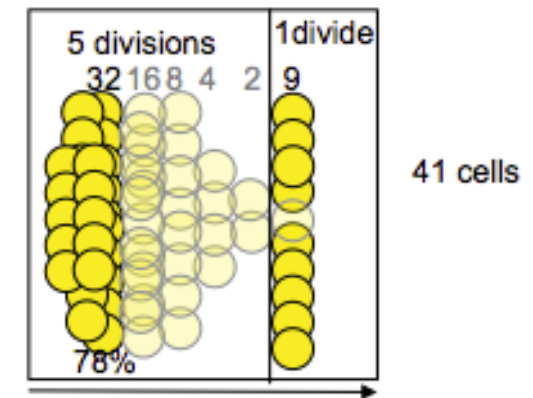
Continuous BrdU labeling for 7 days
32 new BRDU+ cells on 41 cells (78% are BRDU+)



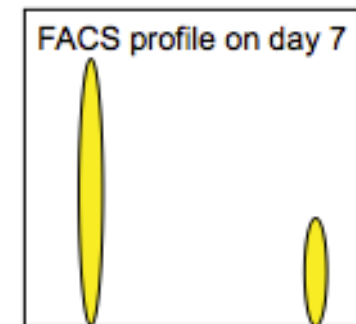
% cells in division on day 0
(DNA labelling or cyclin) : 10%

Intermitotic time: 1.4 days

CFSE de-labeling

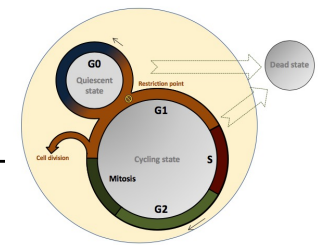


CFSE intensity
(2 times dilution with 1 division)



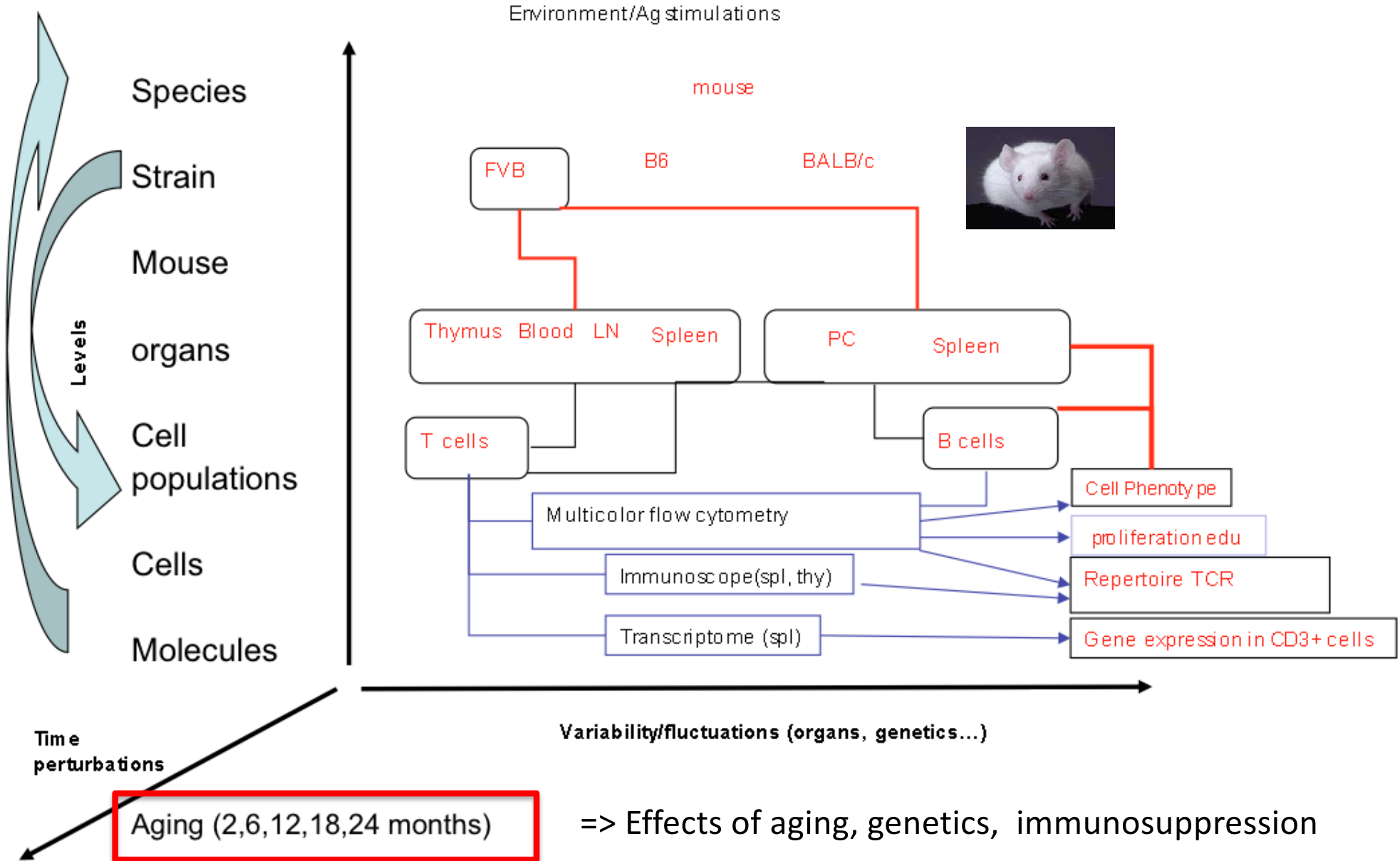
Cell division and turnover methods

Thomas-Vaslin & al (2013). Lymphocyte Dynamics and Repertoires, Biological Methods Encyclopedia of Systems Biology .



Methodology	Molecule	Level of detection	Administration	Time		Investigation level	Method of detection	Result
Active labelling during cell division	Isotopes			Pulse	Chase			Rate of division
	3H-thymidine	DNA	In vitro/ in vivo	Hours	Hours, days	Cell population	β counter	cpm/time
	2D-Glucose	DNA	In vivo	Hours	Days	Cell population	Spectromass	Fraction DNA labeled/time
	2H2O	DNA	In vivo	Days	Days	Cell population	Spectromass	Fraction DNA labeled/time
	14-Carbone	DNA	In vivo 1960-1967	Days	Years	Cell population	Spectromass	Decrease/time
	Nucleotide analogue							
	BrDU EDU	DNA	In vivo/ in vitro	minutes to days	Days	Cell	Flow cytometry Histology	% cell in division/time
Passive labeling DNA content	Fluorescent dye (e.g. PI, 7AAD...)	DNA	In vitro	instant		Cell	Flow cytometry	% cell in G0/G1 S, G2/ M at a time point
Passive labeling Dilution of dye with division	Fluorescent dye (e.g. CFSE)	cytoplasm	In vitro In vivo	Days		Cell	Flow cytometry	% cells that have divided in a given time and number of cell cycles
Detection of Cyclin	e.g. KI67	Nucleus	In vitro	instant		Cell	Flow cytometry Histology	% cell in division at a given time
Depletion of dividing cells	e.g. Ganciclovir in transgenic cells expressing HSV-1 TK; Hydroxyurea	DNA	In vivo	Days		Cell	Flow cytometry	% cell depletion

Multi-level organisation & approach



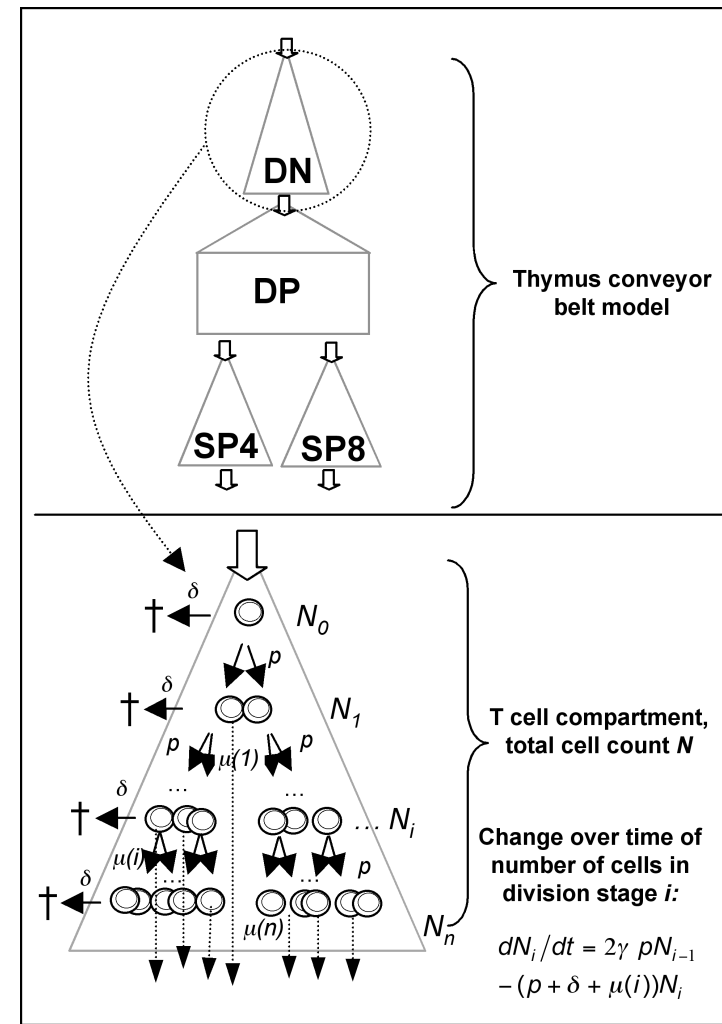
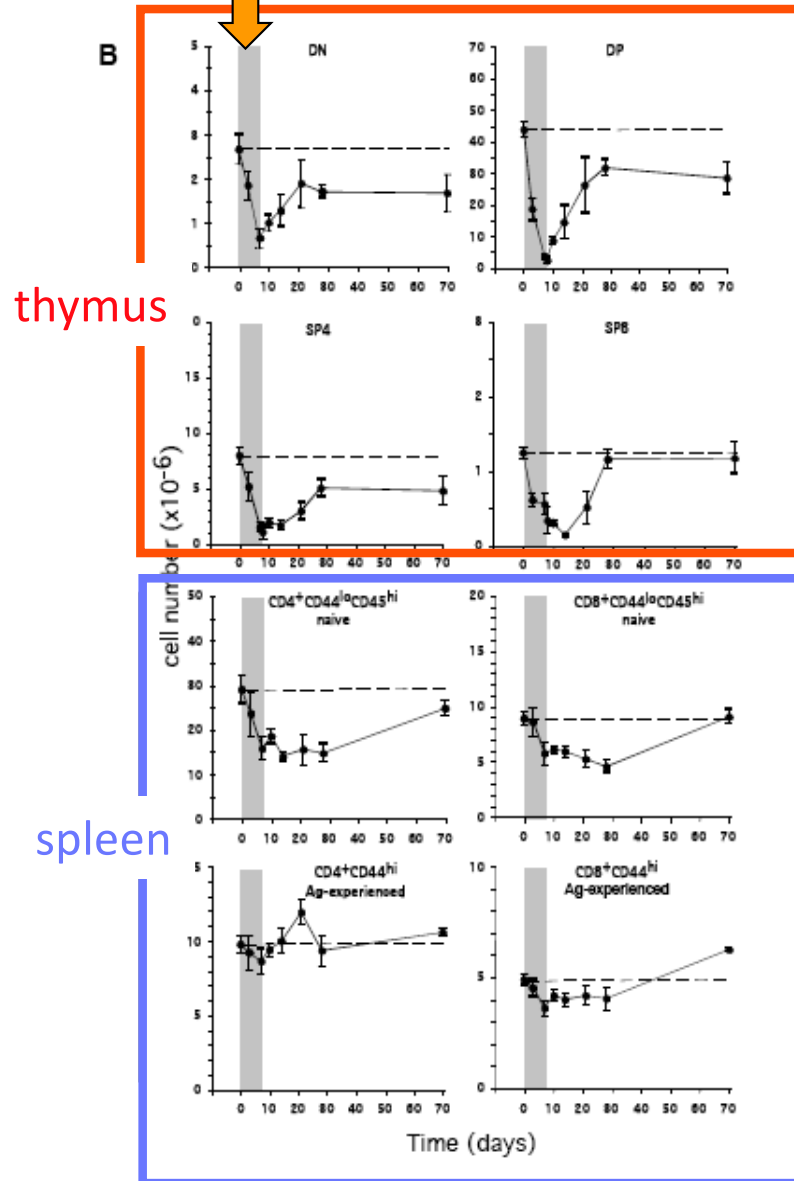
Reconstructing lymphocyte proliferation, differentiation,
selection

FROM POPULATION ODE TO STATE TRANSITION DIAGRAMS

Modeling T cell dynamics & homeostasis in young FVB adult mice

Perturbation: transient depletion of dividing T cells for 7 days

Pharmaco-genetic conditional immunosuppression (Thomas-Vaslin Intech 2012)



Mathematical ODE model

Thomas-Vaslin J. Immunol 2008

Modeling T cell dynamics & homeostasis in young FVB mice

Thomas-Vaslin & al. JI 2008

ODE



Fit exp data

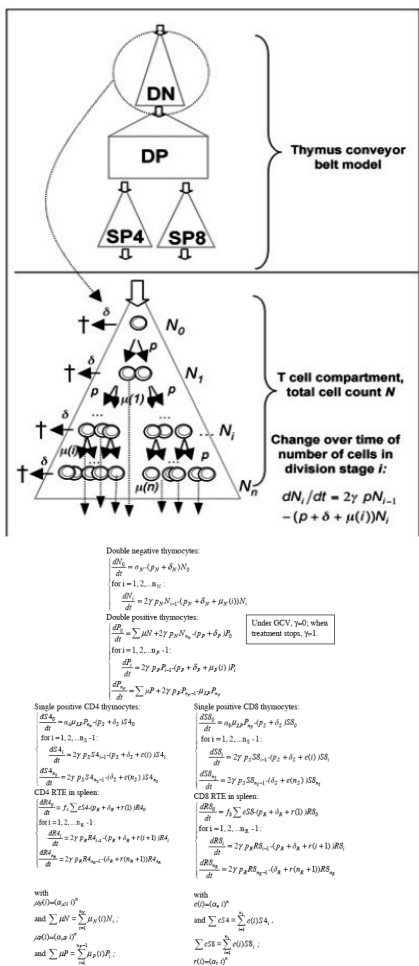


Deduce parameter values
Biological model

Conveyor belt model

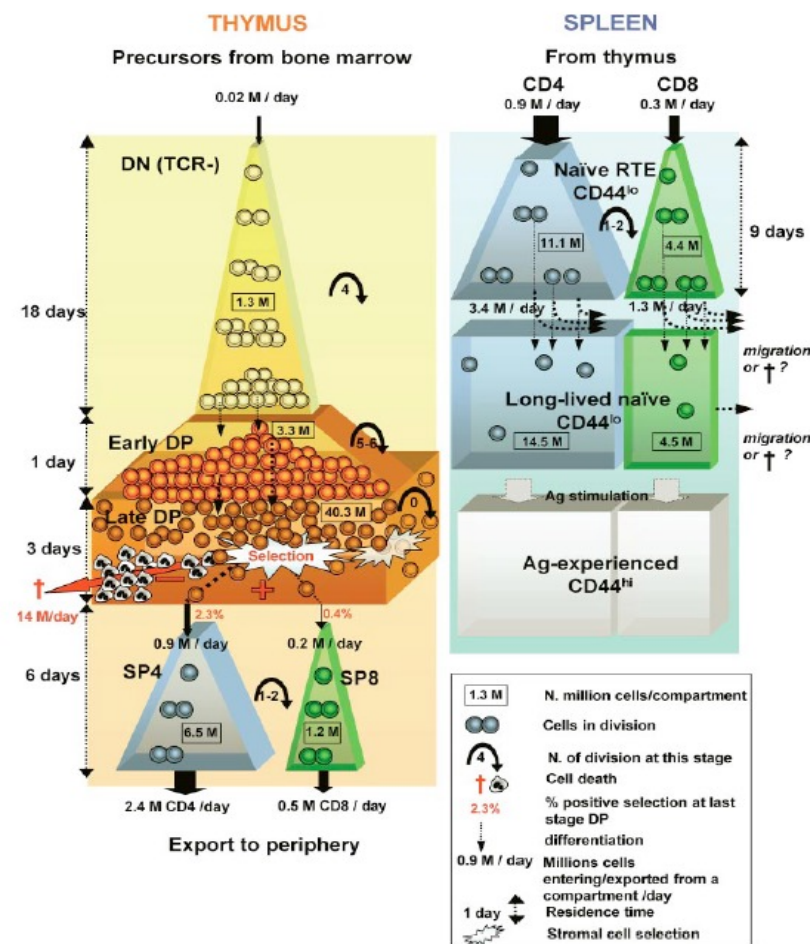
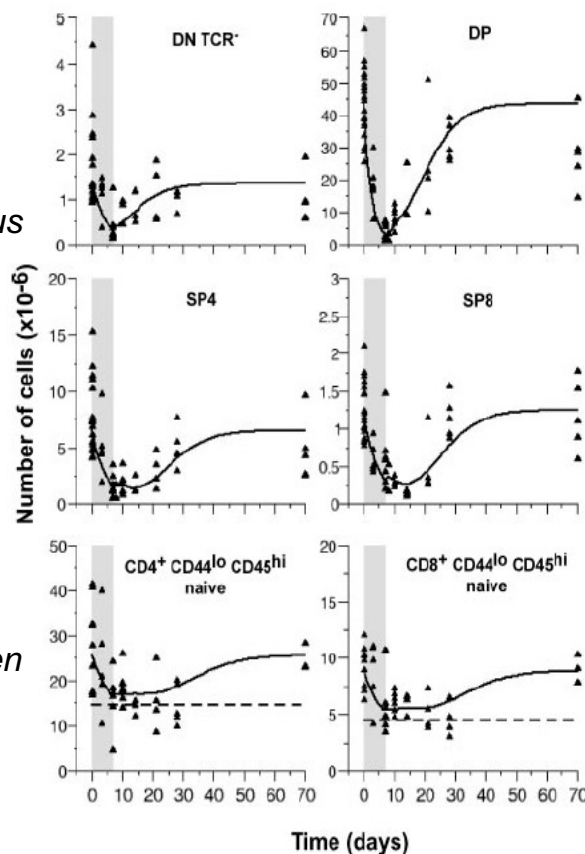
Perturbation: depletion /repletion

Resilience of the system



thymus

spleen



Modeling of T cell population dynamics at steady-state in young FVB mice

Number of cells at steady state
and differentiating, at each division stage

Cell compartment	Division stage	Number of cells (millions) ¹	Number of cells differentiating to next compartment
Thymus			
DN	N ₀	0.09	0.00
	N ₁	0.18	0.00
	N ₂	0.36	0.00
	N ₃	0.72	0.00
	N ₄	3×10 ⁻³	0.32
Total DN		1.34	
Early DP	P ₀	0.07	0.00 ²
	P ₁	0.14	0.00 ^b
	P ₂	0.29	0.00 ^b
	P ₃	0.58	0.00 ^b
	P ₄	1.16	0.00 ^b
	P ₅	0.94	6.18 ^b
	P ₆	0.08	8.14 ^b
Last stage DP	P ₇ ³	40.29	15.05 ⁴
Total DP		43.57	
SP4	S4 ₀	4.02	0.00
	S4 ₁	2.49	1.26
	S4 ₂	0.01	1.13
Total SP4		6.53	
SP8	S8 ₀	0.77	0.00
	S8 ₁	0.48	0.24
	S8 ₂	2×10 ⁻³	0.22
Total SP8		1.25	
Total thymocytes		52.69	

Naive spleen cells

CD4 RTE	R4 ₀	3.79	0.00 ⁵
	R4 ₁	7.27	0.07*
	R4 ₂	0.03	3.30*
Total CD4 RTE		11.09	
Long-lived naïve CD4		14.50	
Total CD4 CD44^{low}		25.59	
CD8 RTE	R8 ₀	1.49	0.00*
	R8 ₁	2.86	0.03*
	R8 ₂	0.01	1.30*
Total CD8 RTE		4.36	
Long-lived naïve CD8		4.50	
Total CD8 CD44^{low}		8.86	

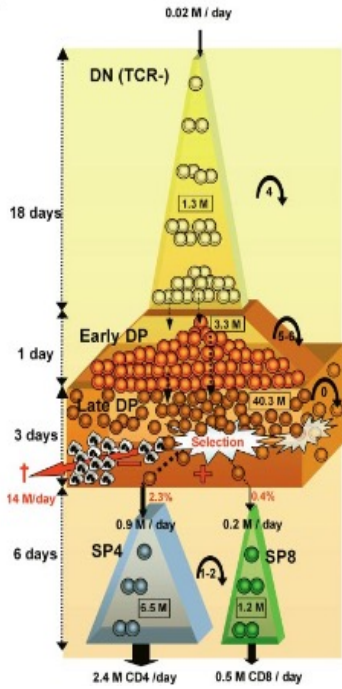
➤ quantitative parameter values at steady state

- Cell fluxes
- Residence times
- Rates of import, export, proliferation, death
- Time /space
- Quantification of selection and post-selection peripheral expansion

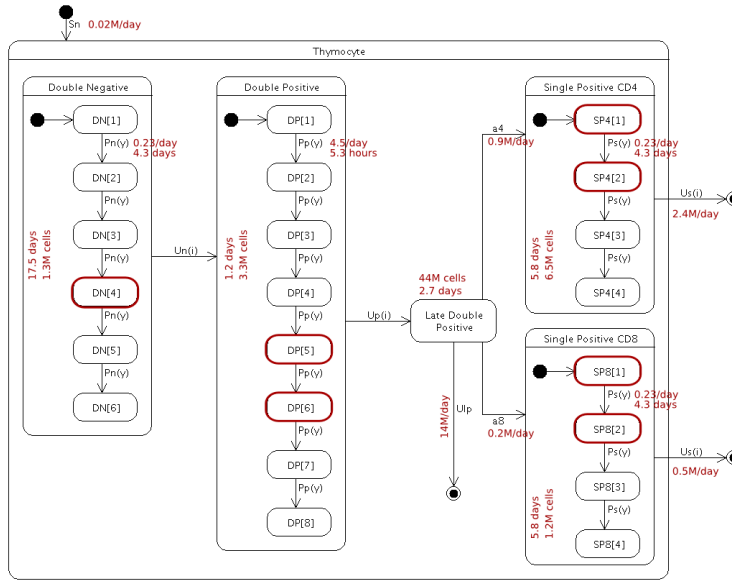
Production and death of cells ⁱ		
Thymocytes dying ⁱⁱ (/day)	14.0 million	
Thymocytes exported (/day)	2.9 million	
Thymocytes produced by division (/day)	16.8 million	
Cells produced by division before SP stage (/day)	15.0 million	
Percentage cells produced in thymus dying (/day)	83.1 %	
Percentage cells produced before SP stage dying in the thymus (/day)	92.9 %	
Splenic RTEs produced by division (/day)	3.5 million	
Thymocytes exported to spleen (/day)	1.2 million	
Contribution thymus versus division splenic RTE	25.5%	
Percentage thymocytes exported to periphery	5.4%	
Percentage positive selection last stage DP to SP (/day)		
	CD4 2.3%	
	CD8 0.4%	
	Cell turnover (/day)	50% replacement time (days)
DN	0.24	2.1
DP	0.35	1.4
SP4	0.37	1.3
SP8	0.37	1.3
CD4 RTE	0.30	1.7
CD8 RTE	0.30	1.7

Overcoming the limitations of traditional ODE modeling using state-transition diagrams

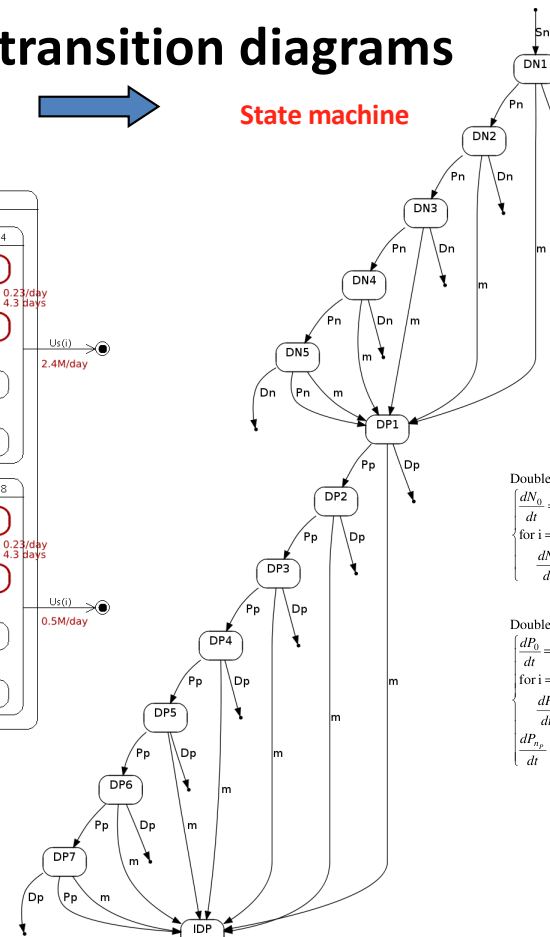
Biologist's schematic



Executable Statechart Model (UML)



State machine



ODE

Double negative thymocytes:

$$\frac{dN_{D_i}}{dt} = \sigma_N \cdot (p_N + \delta_N) N_{D_i}$$

for $i = 1, 2, \dots, n_N$:

$$\frac{dN_{D_i}}{dt} = 2\gamma p_N N_{i-1} \cdot (p_N + \delta_N + \mu_N(i)) N_{D_i}$$

Double positive thymocytes:

$$\frac{dP_0}{dt} = \sum \mu N + 2\gamma p_N N_{n-1} \cdot (p_P + \delta_P) P_0$$

for $i = 1, 2, \dots, n_P - 1$:

$$\frac{dP_i}{dt} = 2\gamma p_P P_{i-1} \cdot (p_P + \delta_P + \mu_P(i)) P_i$$

$$\frac{dP_{n_P}}{dt} = \sum \mu P + 2\gamma p_P P_{n_P-1} \cdot \mu_{LP} P_{n_P}$$

Single positive CD4 thymocytes:

$$\frac{dS4_0}{dt} = \alpha_4 \mu_{LP} P_{n_P} \cdot (p_S + \delta_S) S4_0$$

for $i = 1, 2, \dots, n_S - 1$:

$$\frac{dS4_i}{dt} = 2\gamma p_S S4_{i-1} \cdot (p_S + \delta_S + e(i)) S4_i$$

$$\frac{dS4_{n_S}}{dt} = 2\gamma p_S S4_{n_S-1} \cdot (\delta_S + e(n_S)) S4_{n_S}$$

Single positive CD8 thymocytes:

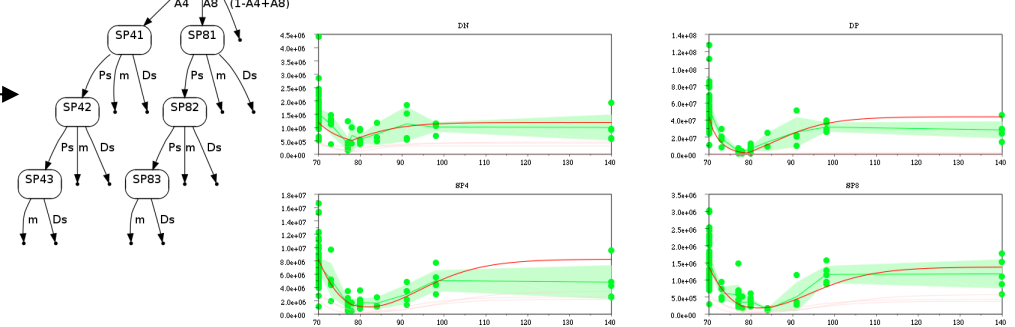
$$\frac{dS8_0}{dt} = \alpha_8 \mu_{LP} P_{n_P} \cdot (p_S + \delta_S) S8_0$$

for $i = 1, 2, \dots, n_S - 1$:

$$\frac{dS8_i}{dt} = 2\gamma p_S S8_{i-1} \cdot (p_S + \delta_S + e(i)) S8_i$$

$$\frac{dS8_{n_S}}{dt} = 2\gamma p_S S8_{n_S-1} \cdot (\delta_S + e(n_S)) S8_{n_S}$$

Fit/ simulations



xml

```

<!-- treatment on/off -->
<parameter label="N" value="1"/>
<parameter label="Np" value="1.27"/>
<parameter label="Sn" value="20000"/>
<parameter label="Ddp" value="0.37" min="0" max="1.0"/>
<parameter label="A4" value="0.06" min="0" max="0.1"/>
<parameter label="A8" value="0.01" min="0" max="0.1"/>
<!-- same prolifer. rate shared by SP and SP -->
<parameter label="Pns" value="0.23" min="0" max="1.0"/>
<!--
<parameter label="Pn" value="0.23" min="0" max="1.0"/>
-->
<parameter label="Pn" ref="Pns"/>
<parameter label="Dn" value="0.0"/>
<parameter label="As" value="0.29" min="0" max="1.0"/>
<parameter label="Pp" value="4.5" min="0" max="4.5"/>
<parameter label="Dp" value="0.0"/>
<parameter label="Ap" value="0.2" min="0" max="1.0"/>
<!--
<parameter label="Ps" value="0.23" min="0" max="1.0"/>
-->
<parameter label="Ps" ref="Pns"/>
<parameter label="Ds" value="0.0"/>
<parameter label="As" value="0.99" min="0" max="1.0"/>
<!--
The model structure
-->
<state class="goaml.HierarchicalState" label="Main">
  <!-- Double negative compartment -->
  <state class="goaml.HierarchicalState" label="DN">
    <state class="goaml.State" label="DN1" start="true"/>
    <state class="goaml.State" label="DN2"/>
    <state class="goaml.State" label="DN3"/>
    <state class="goaml.State" label="DN4"/>
    <state class="goaml.State" label="DN5"/>
    <!-- proliferation transitions -->
    <transition class="upmc.deboer.GCVProliferation" label="Pn" guard="" source="DN1" target="DN2">
      <parameter label="Y" ref="Y"/>
    </transition>
    <transition class="upmc.deboer.GCVProliferation" label="Pn" guard="" source="DN2" target="DN3">
      <parameter label="Y" ref="Y"/>
    </transition>
    <transition class="upmc.deboer.GCVProliferation" label="Pn" guard="" source="DN3" target="DN4">
      <parameter label="Y" ref="Y"/>
    </transition>
  
```

Thymocyte differentiation, interactions, migration

AGENT BASED MODEL: DISCRETE TIME

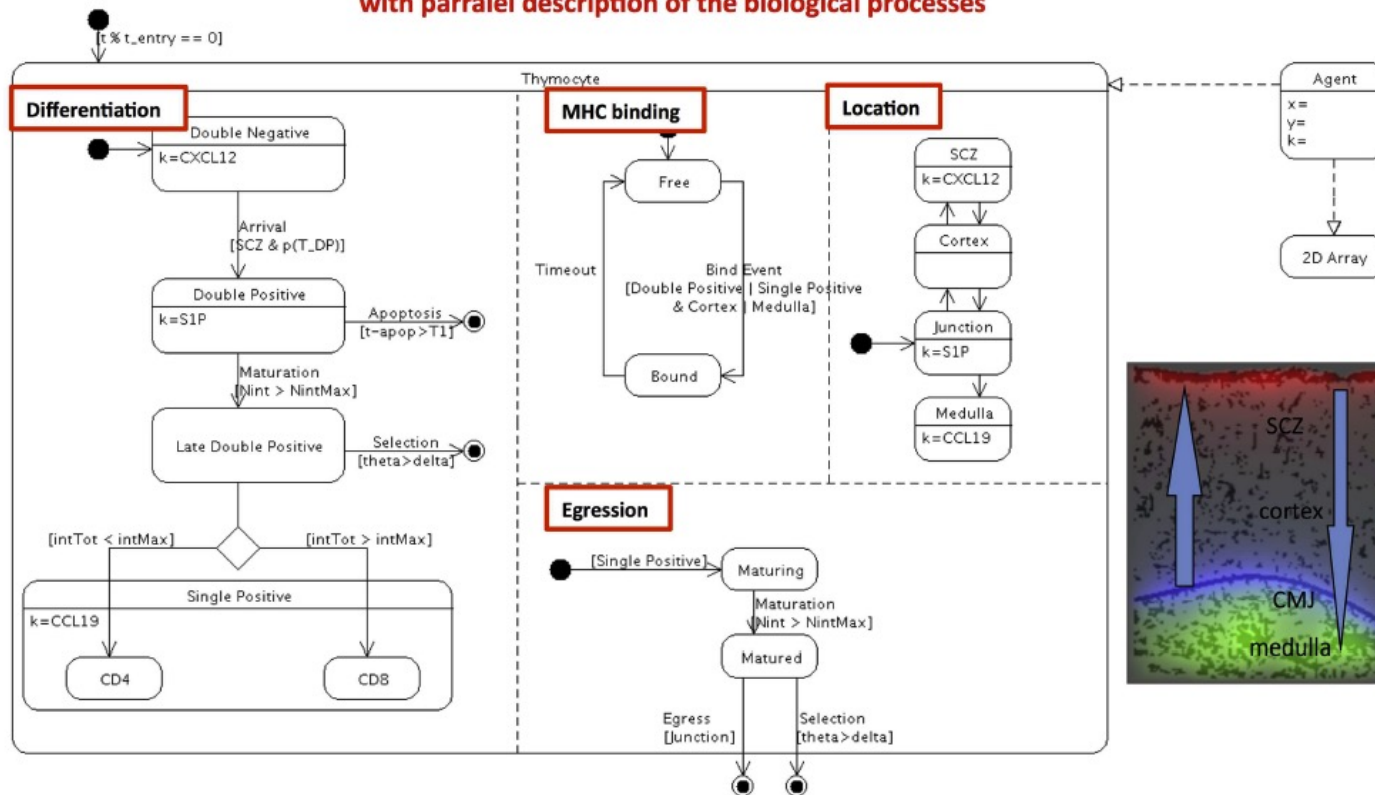


Dynamical and mechanistic reconstructive approaches of T lymphocyte dynamics: using visual modeling languages to bridge the gap between immunologists, theoreticians, and programmers

Véronique Thomas-Vaslin^{1,2*}, Adrien Six^{1,2}, Jean-Gabriel Ganascia^{3,4} and Hugues Bersini⁵

Single cell-based model– Discrete-Stochastic

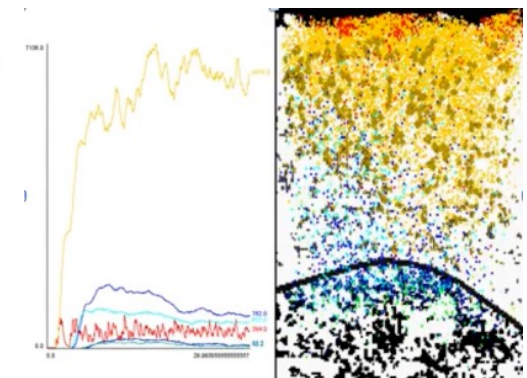
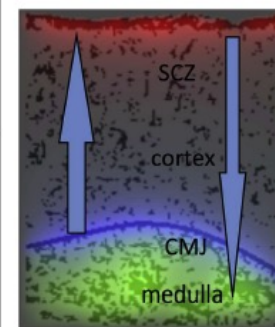
Executable Statechart Model (UML)
with parallel description of the biological processes



DP
CD4
CD8
DN
egress



Thymic epithelial network
=pattern organization



Perturbations: Oriented versus stochastic T cell migration in thymus

Organization in empty thymus

State shift

Permanent alteration followed by slow re-organization

1
Growth
Exploitation
Ressources
space available

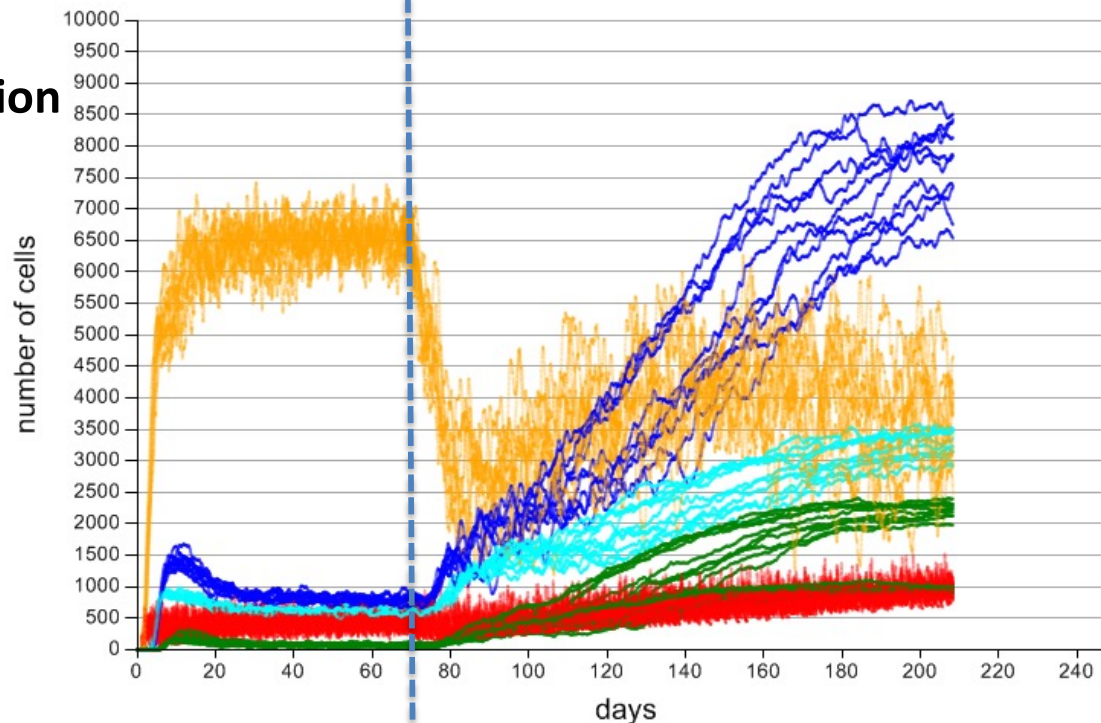
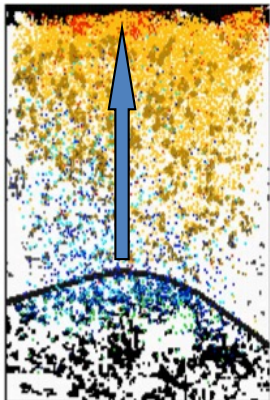
2
Conservation:
Permanent
renewal
Steady state
equilibrium

3
Rapid collapse
failure CXCL12 gradient

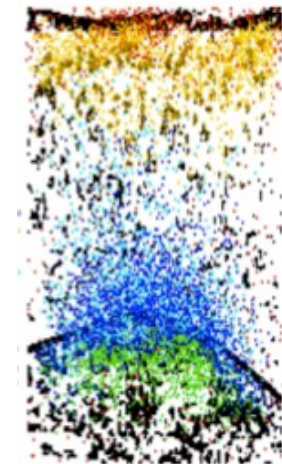
Differentiation

4
Reorganisation is slow
different steady state equilibrium, if reached ???
Longer to establish
Amplitude of oscillations is higher

Oriented migration
Chemokine



Remove CXCL12
=> Stochastic
migration



Thomas-Vaslin 2015 , le vivant critique et chaotique, Ed Matériologiques

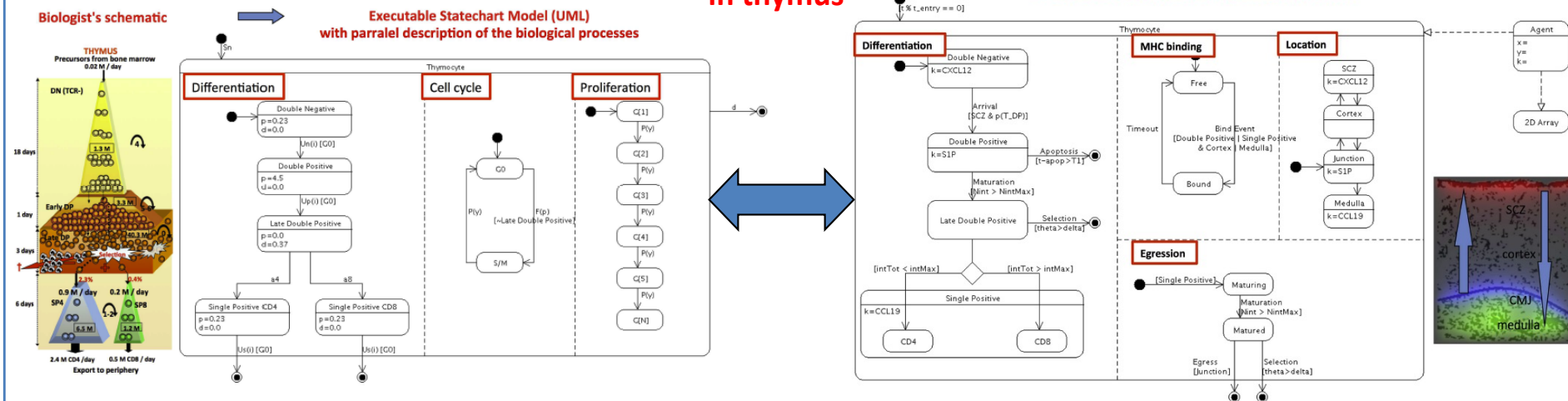
Modelling T cell dynamics with state transition diagram

Cell population-based model
Continuous ODE

Single cell-based model
Discrete/Population

Description of
parallel processes
in thymus

Executable Statechart Model (UML)
with parallel description of the biological processes



Thomas-Vaslin, V., A. Six, J. G. Ganascia and H. Bersini (2013). "Dynamical and mechanistic reconstructive approaches of T lymphocyte dynamics: Using visual modelling languages to bridge the gap between immunologists, theoreticians and programmers." **Frontiers in Immunology**

Bersini, H., D. Klatzmann, A. Six and V. Thomas-Vaslin (2012). "State-Transition Diagrams for Biologists." [PloS one 7 \(7\), e41165](#).

Thomas-Vaslin, V., Altes, H. K., de Boer, R. J., and Klatzmann, D. (2008). *Comprehensive assessment and mathematical modeling of T cell population dynamics and homeostasis*. [J Immunol](#)

Immune response in peripheral tissues

POSITIVE AND NEGATIVE FEEDBACK LOOPS CONTROL TOLERANCE OR IMMUNITY

Mathematical and computer modeling of T cell dynamics

Production of T cells

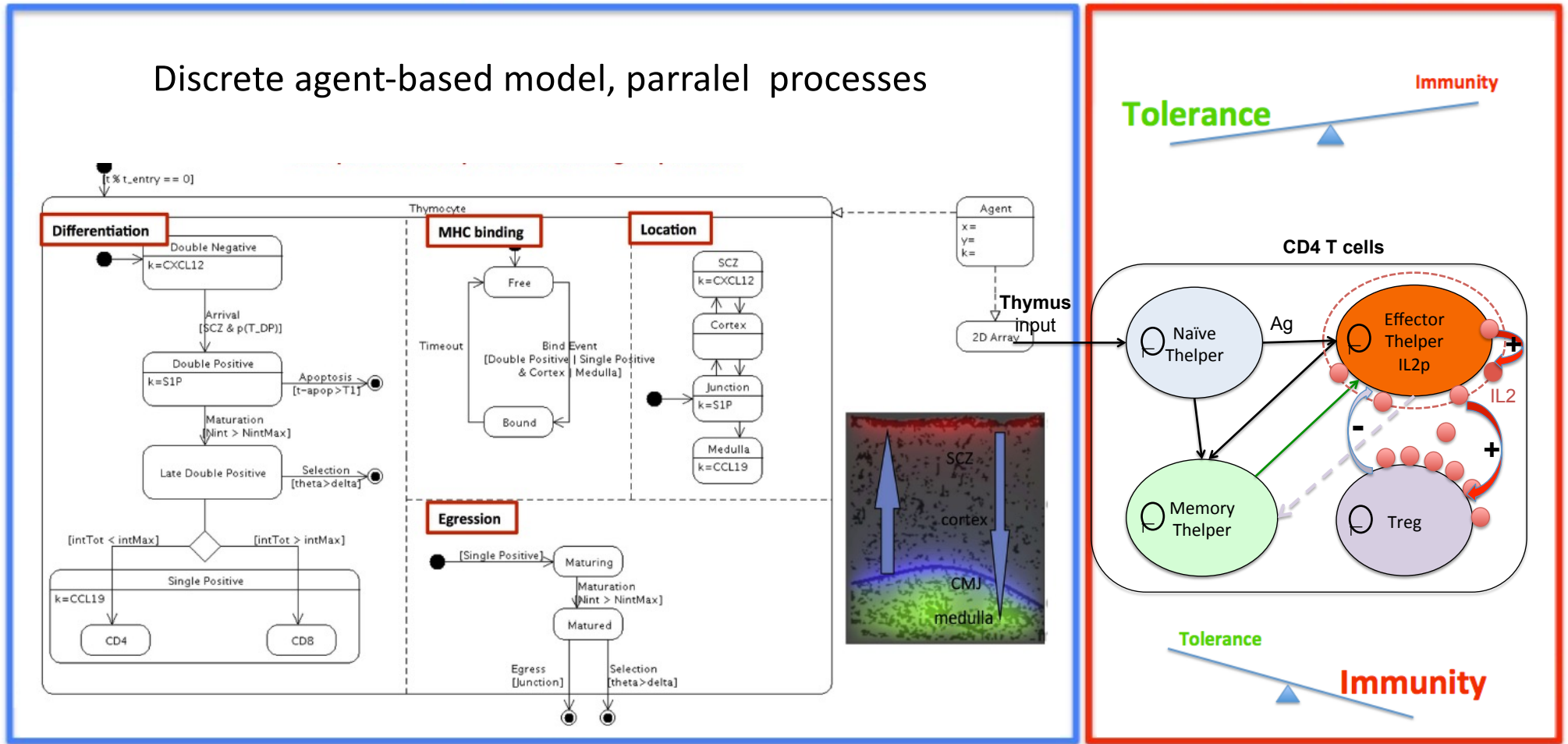
Circulation/distribution

Immune activities

Thymus

Spleen/lymph node

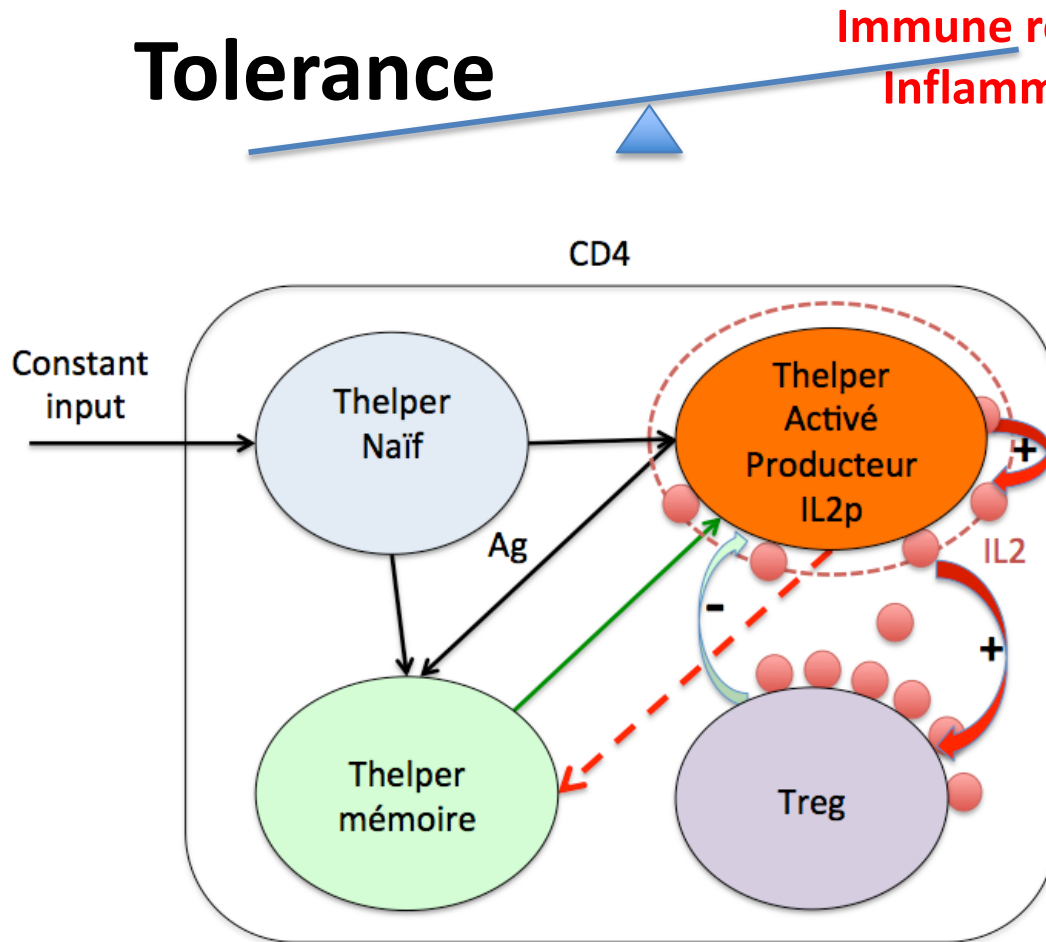
Discrete agent-based model, parallel processes



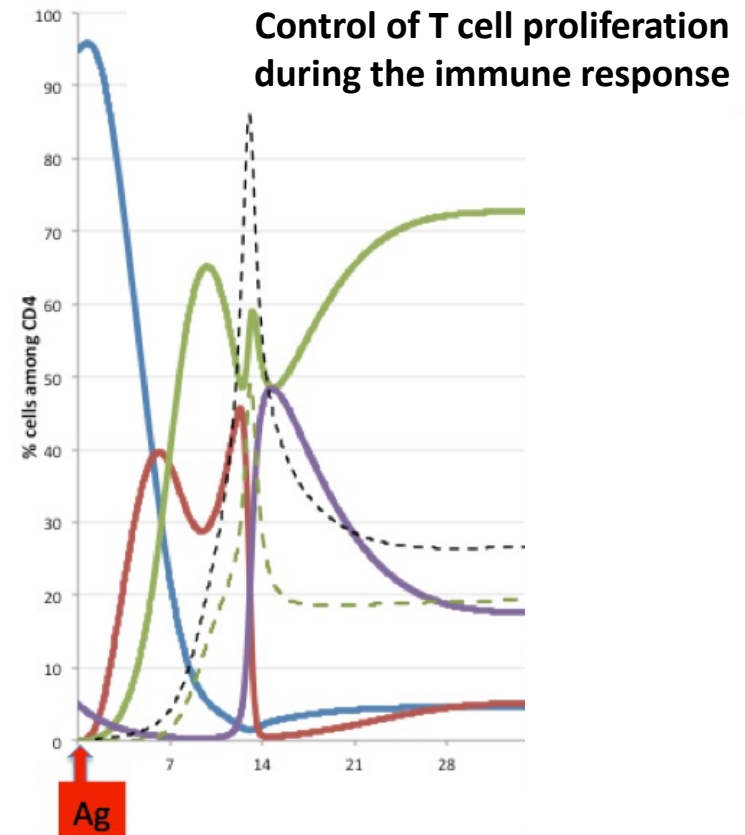
Computer simulation of the immune response: positive and negative feedback loops to control T cell proliferation

Tolerance

Immune response Inflammation



- Naive/CD4
- Activated/CD4
- Memory/CD4
- Treg/CD4
- - - CD4
- - - Memory



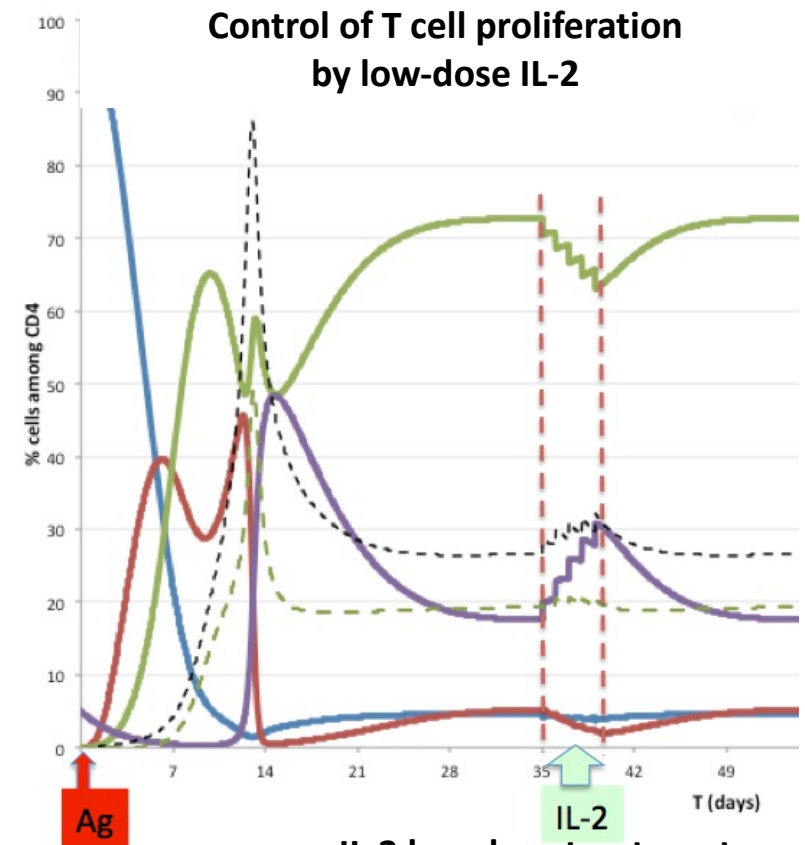
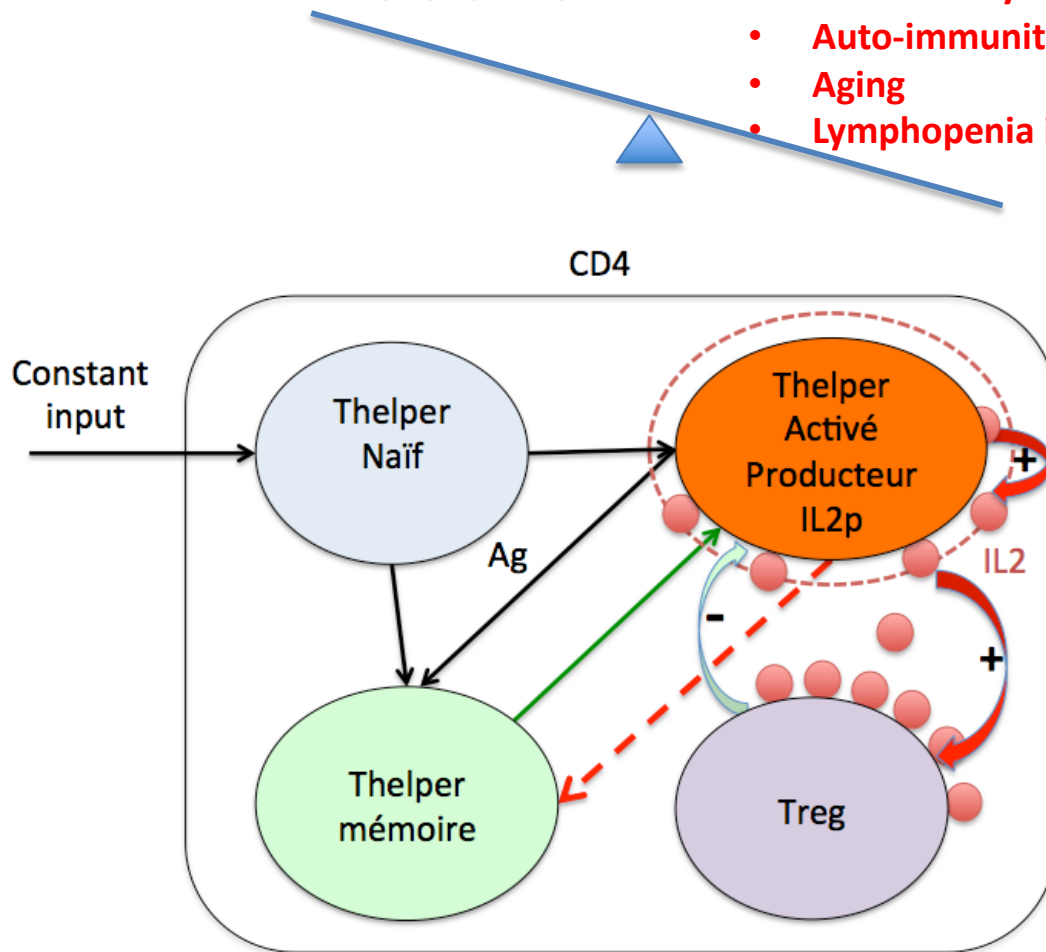
Microbe/ self antigen/allergen ...

Computer simulation of the immune response: positive and negative feedback loops to control T cell proliferation

Tolerance

Defective IL-2/Treg

- Auto-immunity
- Aging
- Lymphopenia induced proliferation



**IL-2 low dose treatment
5 injections**

Thomas-Vaslin 2015 , le vivant critique et chaotique, Ed Matériologiques

Evolution of lymphocytes in mice with aging, phenotype, repertoire

QUALITATIVE AND QUANTITATIVE DESCRIPTION OF CELL POPULATIONS THROUGH TIME

Alterations at various levels contribute to immune system aging

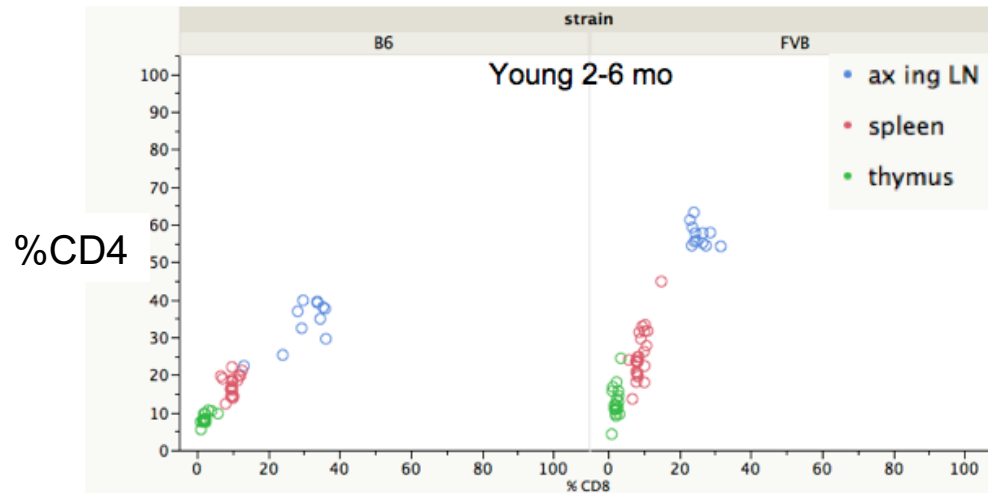
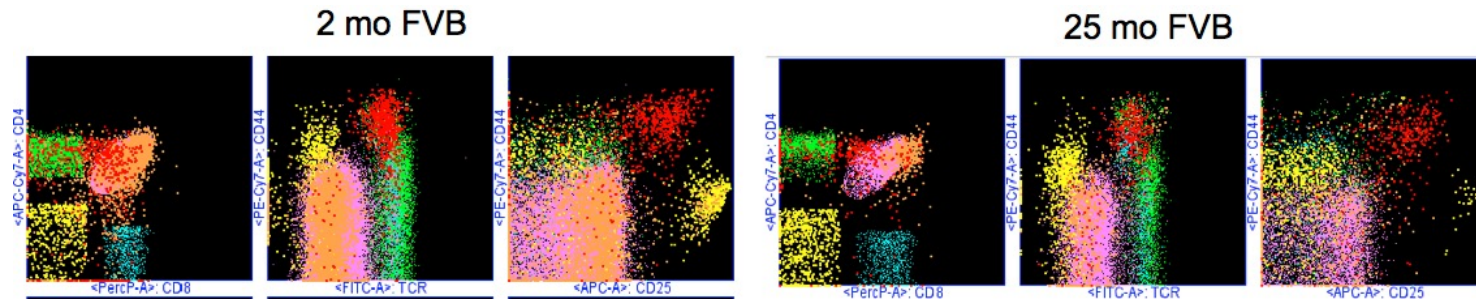
- **Organism level**
 - Increase susceptibility to infection, tumors, **autoimmunity**
 - Decrease vaccine response and protection
- **Organ level**
 - **Involution of primary lymphoid organs (bone marrow, thymus)**
 - **Desorganisation of thymic architecture**
 - Alteration of germinal centers
- **Cell population level**
 - Decrease of precursor differentiation
 - **Decrease of lymphoid repertoire diversity**
 - Chronic or repeated stimulation of lymphoid cell by pathogen.
 - **Accumulation of effector and memory cells**
 - **Decrease of naive /memory cell ratio**
 - **Decrease of CD4/ CD8 ratio**
 - **Increase of Treg cell numbers**
- **Cell level**
 - **Decrease of cell activation (defect of presentation or migration of APC, TLR Activation, Ag presentation)**
 - Decrease of Antibody production
 - **Decrease of IL-2 production**
 - Alteration of stimulation signaling through the TCR
 - **Decreased proliferation**
 - Accumulation of non functional senescent T cells
- **Molecular level**
 - Shortening of telomere in lymphoid naive and memory T cells
 - Alteration in lipid raft in lymphoid cells
 - **Altered gene and protein expression**
 - **Altered apoptosis and proliferation**
 - Increased SHP-1 expression



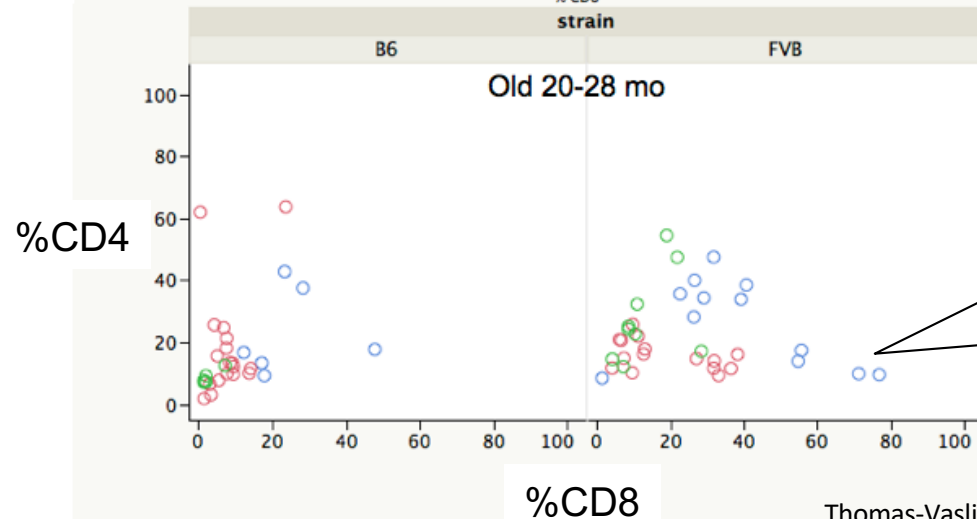
INTECH
OPEN ACCESS PUBLISHER

Thomas-Vaslin Immunosuppression Inteck book 2012

Variation in T cells according to tissues, age, genetics



Signatures of strains
in young mice
High CD4/CD8 ratio in FVB



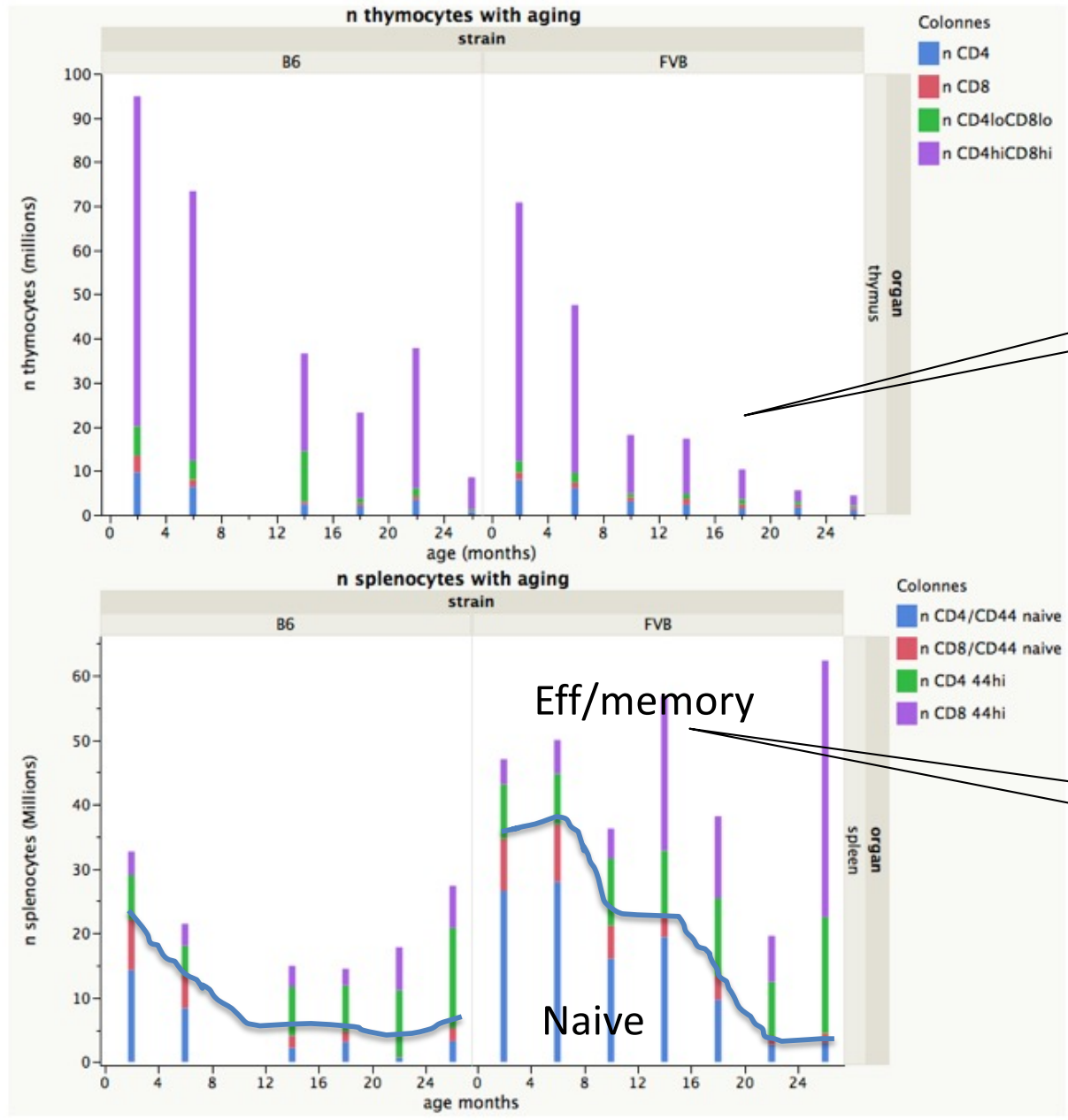
High variability of
CD4/CD8 ratio

CD8 expansions

INTECH
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Thomas-Vaslin Immunosuppression Inteck book 2012

Variation in T cells according to tissues, age, genetics



Accelerated thymic involution in FVB mice

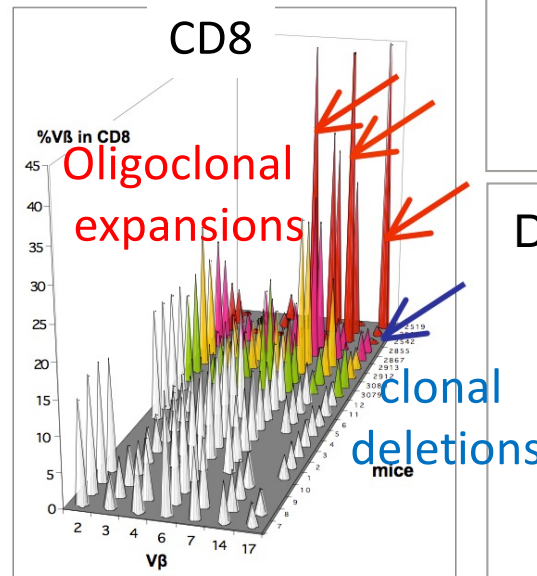
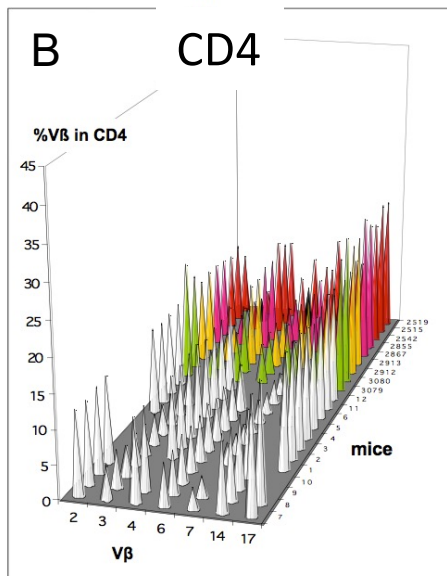
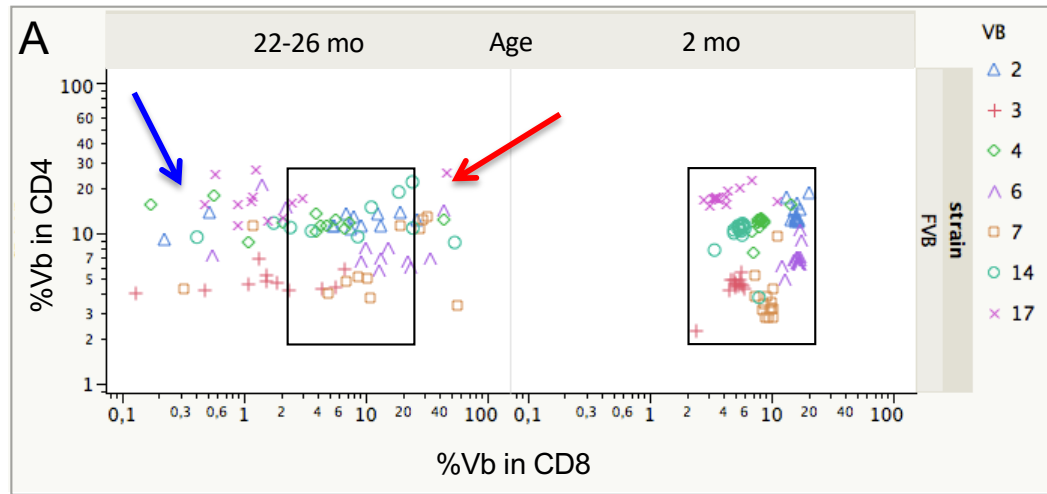
Accumulation of effector/memory T cells in FVB mice

Thomas-Vaslin Immunosuppression Inteck book 2012

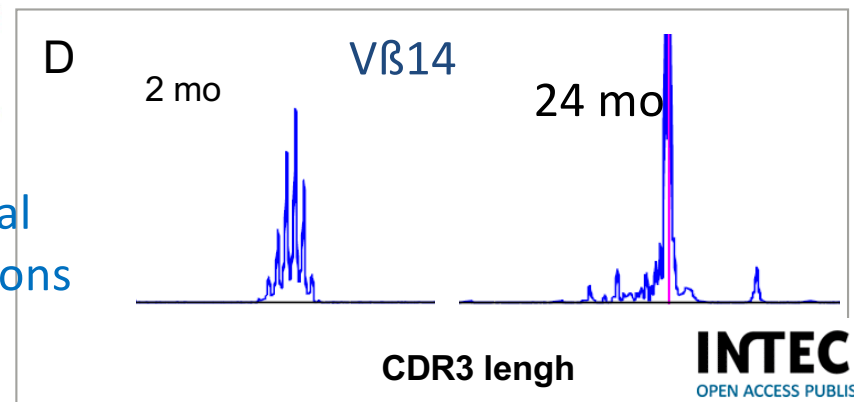
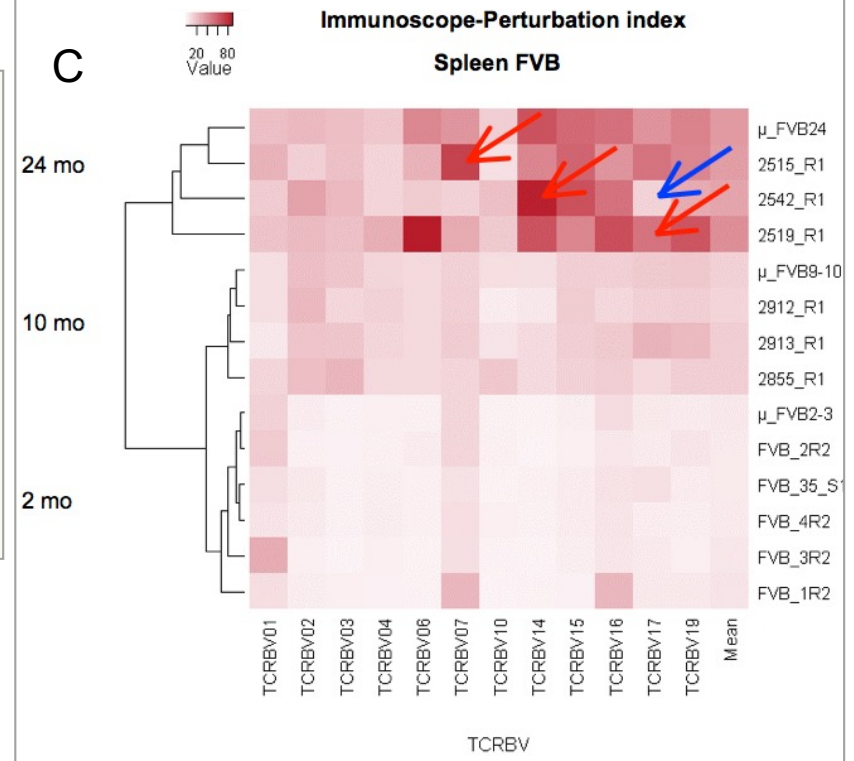
Decrease of lymphocyte repertoire diversity with aging, in FVB mice

Alteration of repertoire diversity landscape with aging

Cell repertoire -FACS



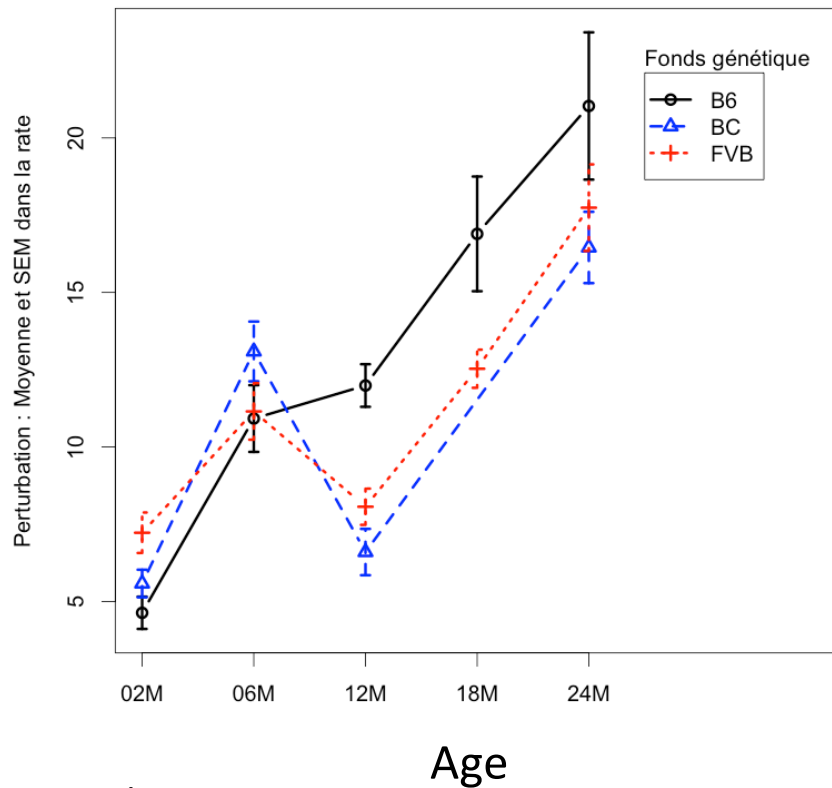
CDR3 repertoire- Immunoscope



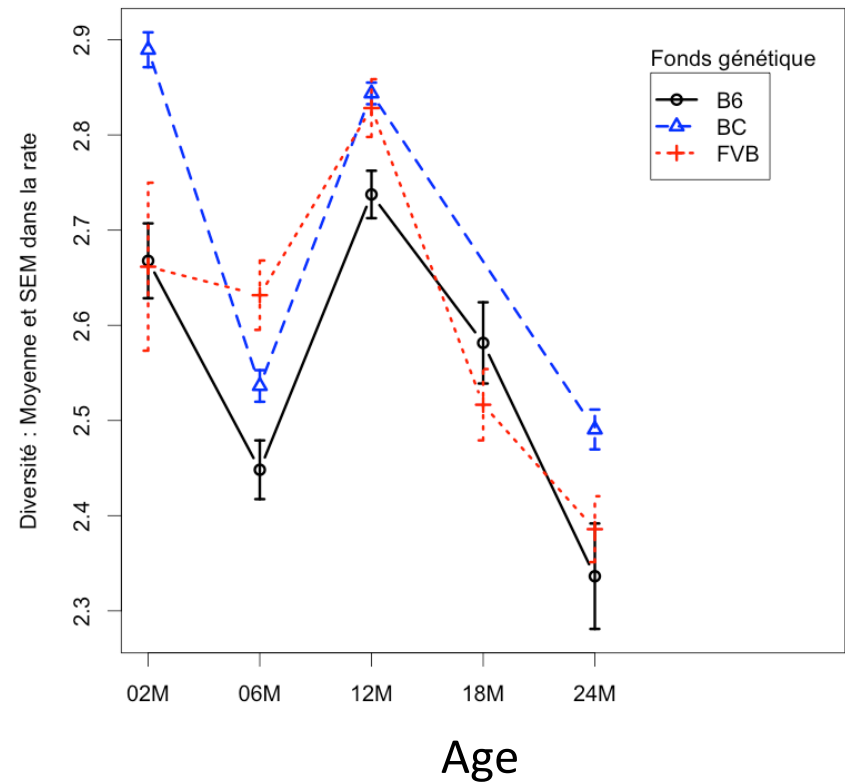
Thomas-Vaslin, Immunosuppression Inteck book 2012

Aging increases perturbations and decreases repertoire diversity in B6, BALB/c and FVB mice

Perturbation score



Diversity index

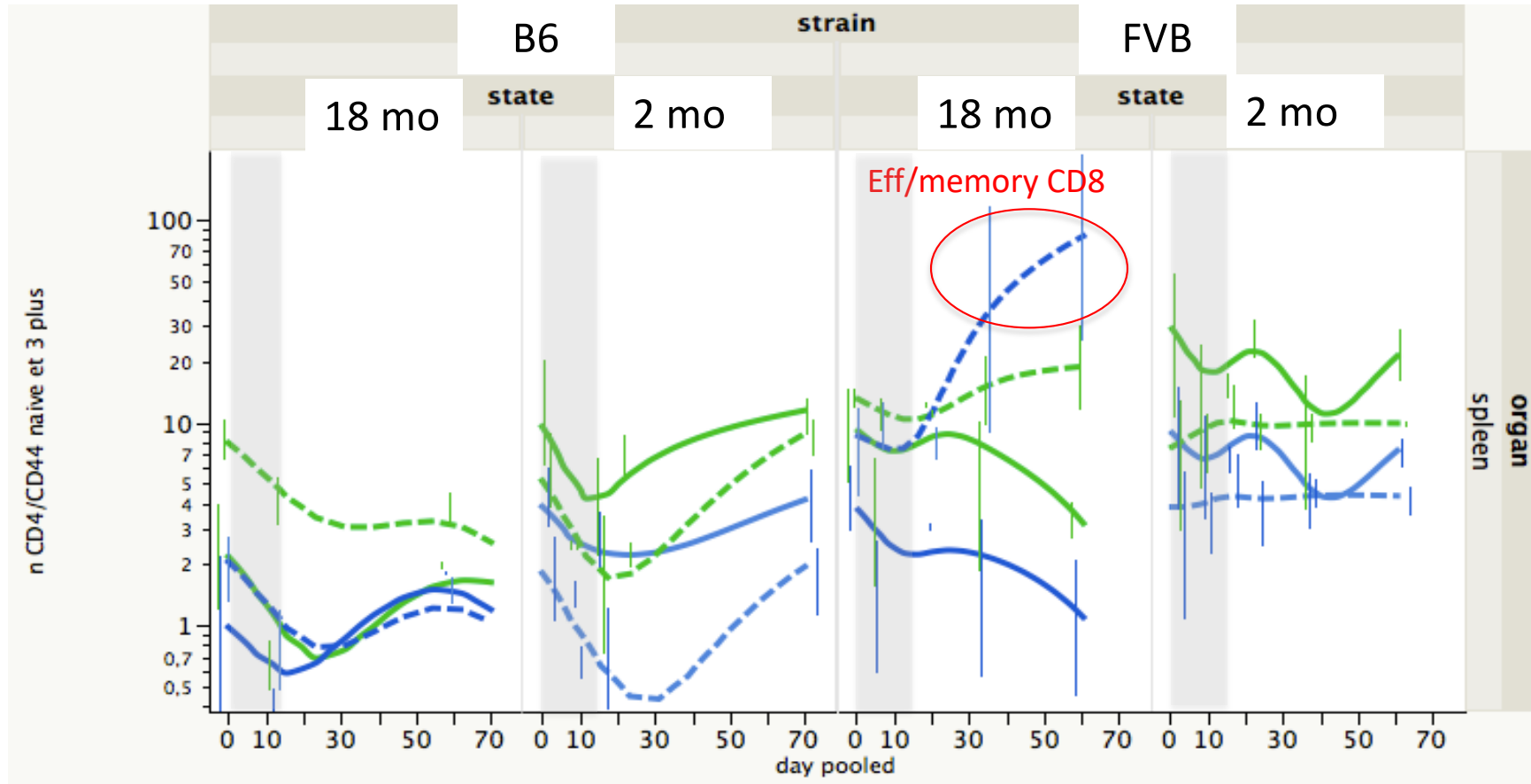


Pham et al.

Thomas-Vaslin 2015 , le vivant critique et chaotique, Ed Matériologiques

Transient depletion of dividing T cells at 18 months (middle life) accelerates aging, with oligoclonal CD8 T cell expansions in FVB mice

kinetics of depletion/repletion in the spleen



CD4 ——— Naive
 CD8 - - - - Effector/memory

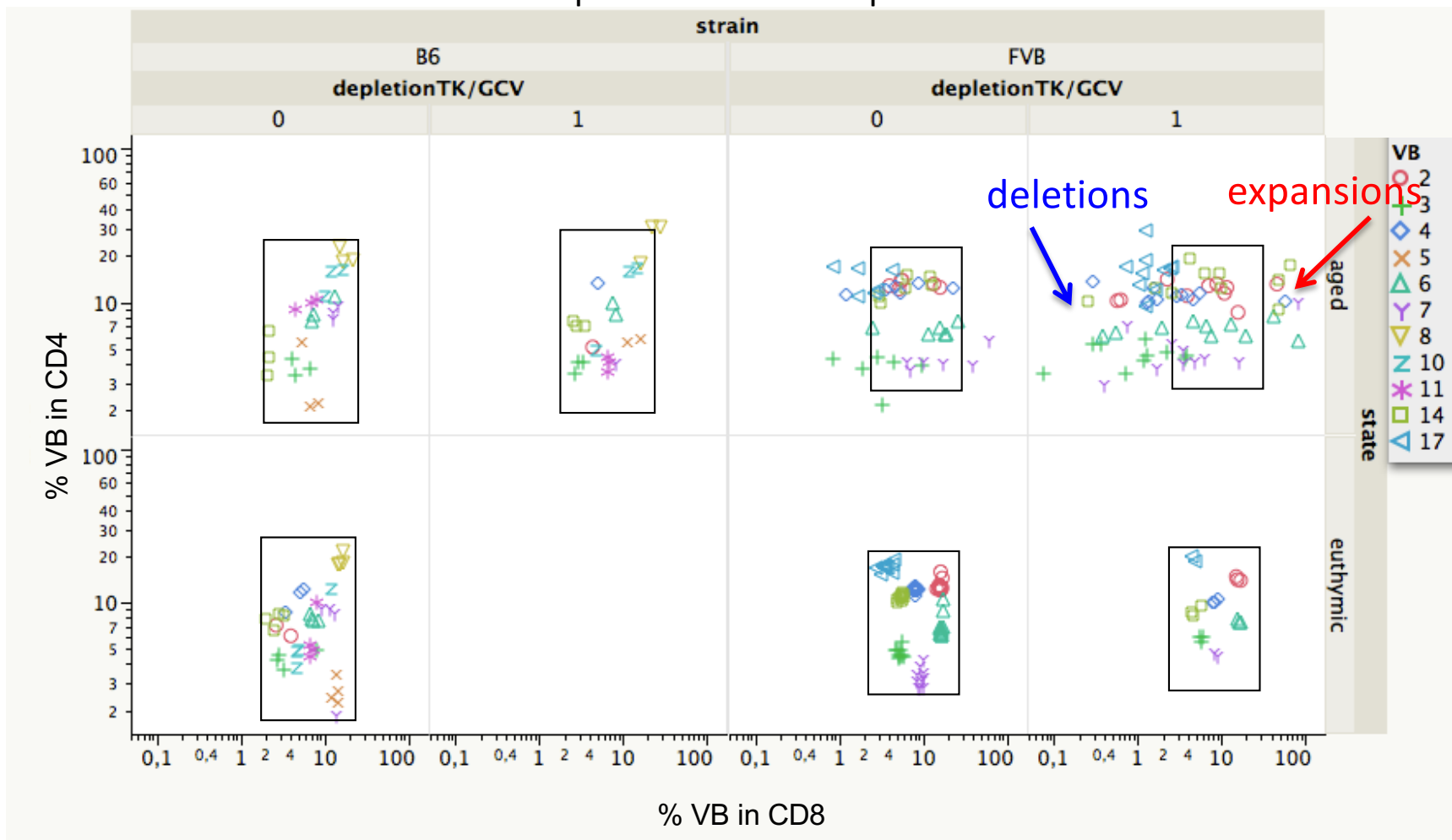
INTECH
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Thomas-Vaslin Immunosuppression Inteck book 2012

Transient depletion of dividing T cells at middle life accelerates aging, with oligoclonal CD8 T cell expansions in FVB mice

Immunodepression alter the TCR repertoire diversity landscape
accelerates immunological aging

T cell repertoire: % Vb in spleen cells



Heterogeneity and adaptation of cell proliferation/death

QUANTIFY CELL DIVISION, DEATH

ESTIMATE PROLIFERATION RATES, CELL CYCLE PHASES DURATION

Proliferating cell populations and heterogeneity

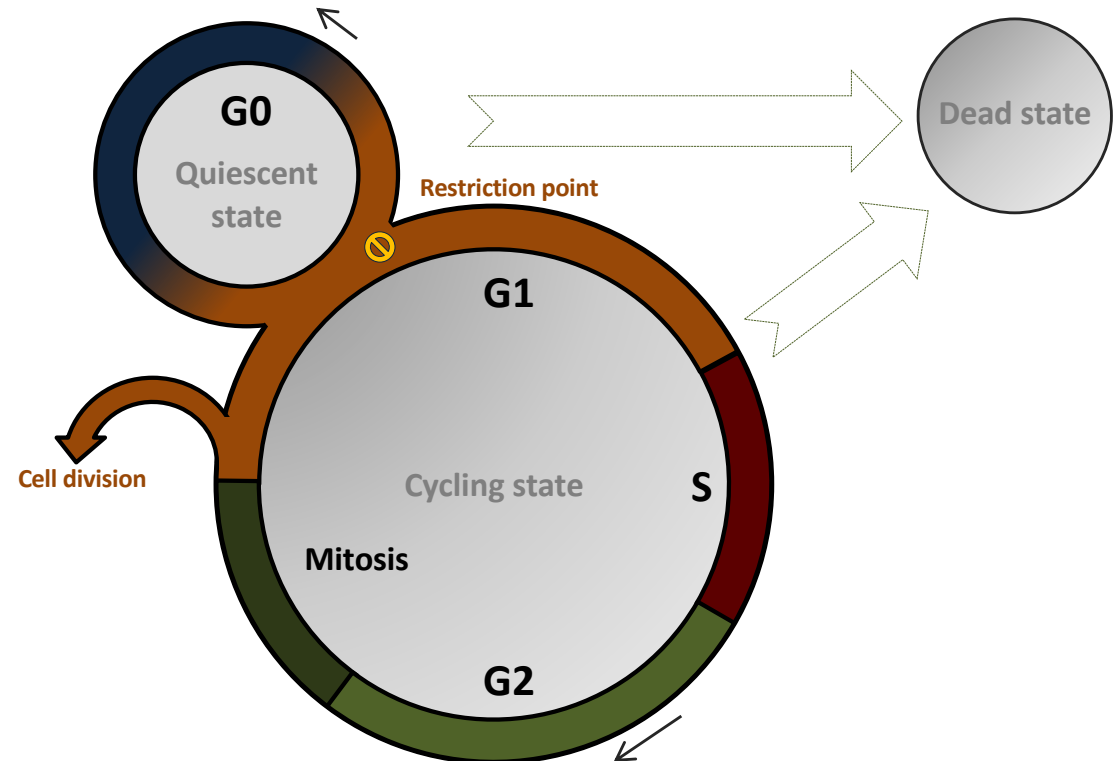
Cycling cells

- G1
- S
- G2/M

Quiescent cells G0

Dead cells

Different cell generations



How to identify and quantify these cell states?

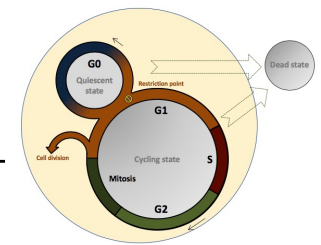
Compare the dynamics of heterogeneous proliferating cell populations ?

Compare tissues, effet of age and genetic origin of the organims ?

=> estimate rates/day

Cell division and turnover methods

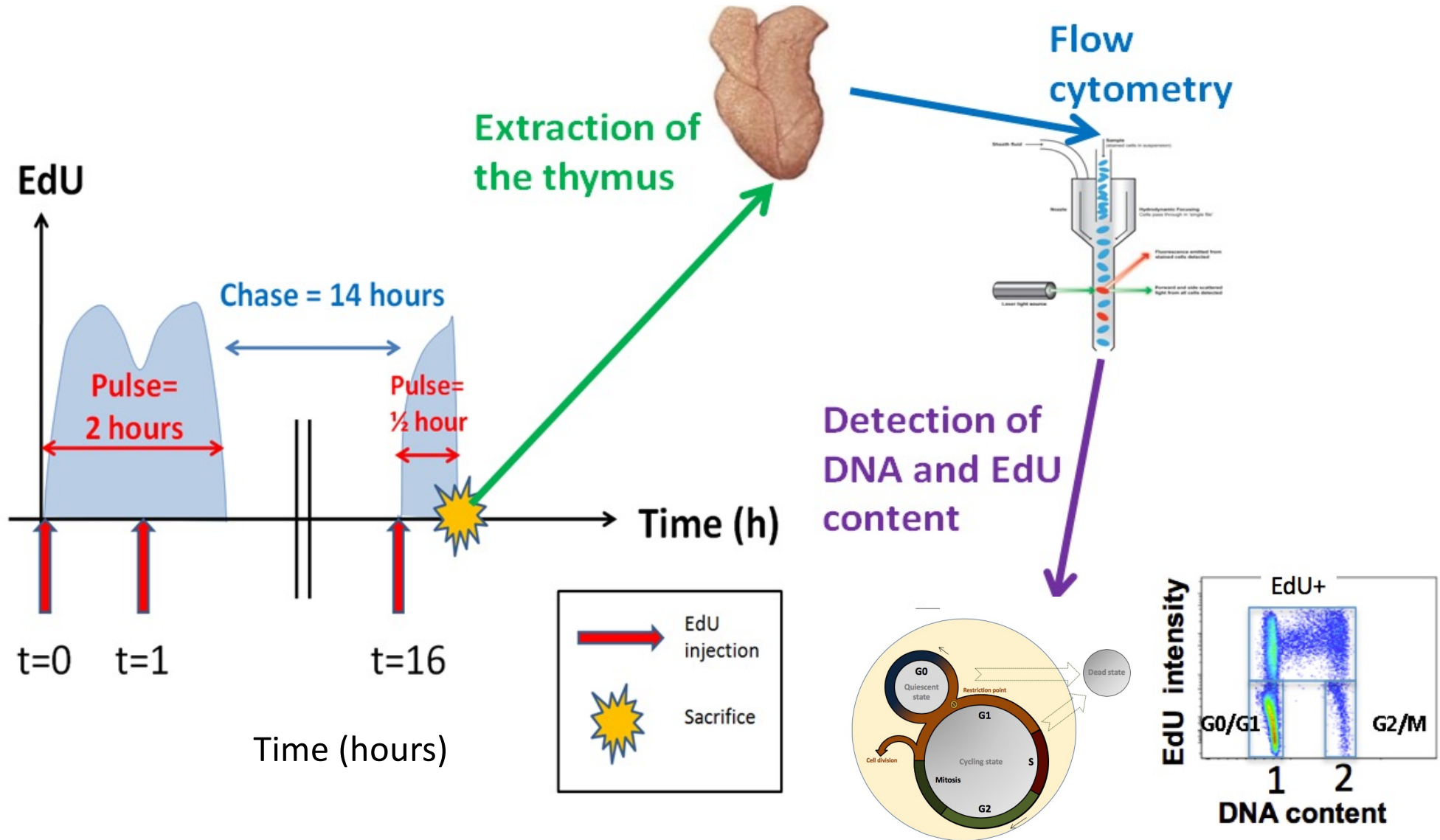
Thomas-Vaslin & al (2013). Lymphocyte Dynamics and Repertoires, Biological Methods Encyclopedia of Systems Biology .



Methodology	Molecule	Level of detection	Administration	Time		Investigation level	Method of detection	Result
Active labelling during cell division	Isotopes			Pulse	Chase			Rate of division
	3H-thymidine	DNA	In vitro/ in vivo	Hours	Hours, days	Cell population	β counter	cpm/time
	2D-Glucose	DNA	In vivo	Hours	Days	Cell population	Spectromass	Fraction DNA labeled/time
	2H2O	DNA	In vivo	Days	Days	Cell population	Spectromass	Fraction DNA labeled/time
	14-Carbone	DNA	In vivo 1960-1967	Days	Years	Cell population	Spectromass	Decrease/time
	Nucleotide analogue							
	BrDU EDU	DNA	In vivo/ in vitro	minutes to days	Days	Cell	Flow cytometry Histology	% cell in division/time
Passive labeling DNA content	Fluorescent dye (e.g. PI, 7AAD...)	DNA	In vitro	instant		Cell	Flow cytometry	% cell in G0/G1 S, G2/ M at a time point
Passive labeling Dilution of dye with division	Fluorescent dye (e.g. CFSE)	cytoplasm	In vitro In vivo	Days		Cell	Flow cytometry	% cells that have divided in a given time and number of cell cycles
Detection of Cyclin	e.g. KI67	Nucleus	In vitro	instant		Cell	Flow cytometry Histology	% cell in division at a given time
Depletion of dividing cells	e.g. Ganciclovir in transgenic cells expressing HSV-1 TK; Hydroxyurea	DNA	In vivo	Days		Cell	Flow cytometry	% cell depletion

Quantification of cell proliferation in vivo

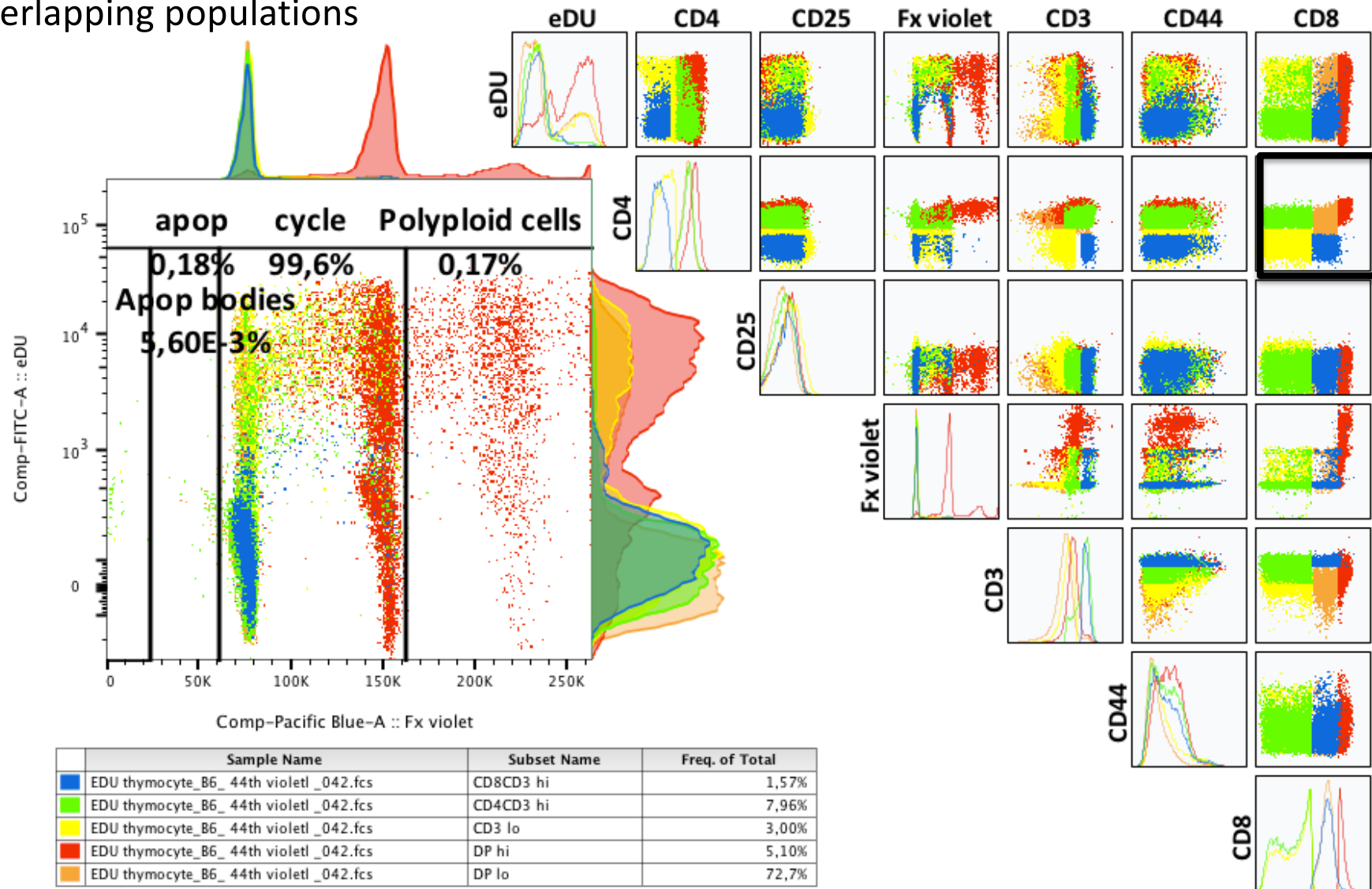
Nucleotide analog (EdU) pulse chase incorporation in DNA of dividing cells
Single cell multi-parameter analysis



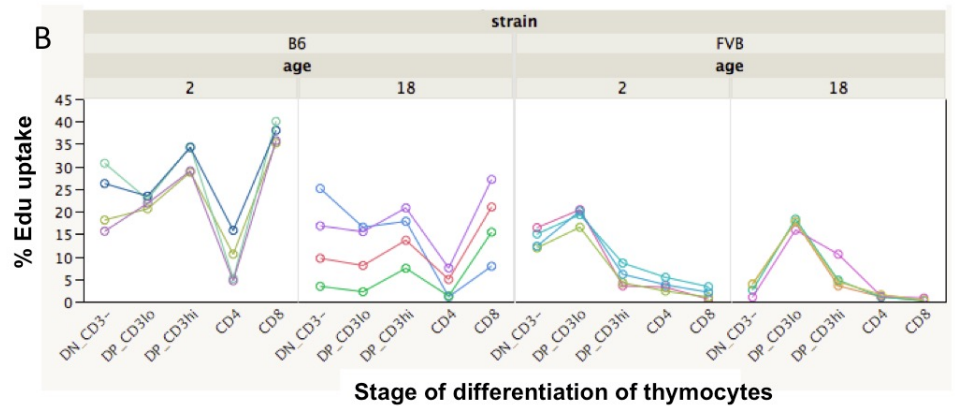
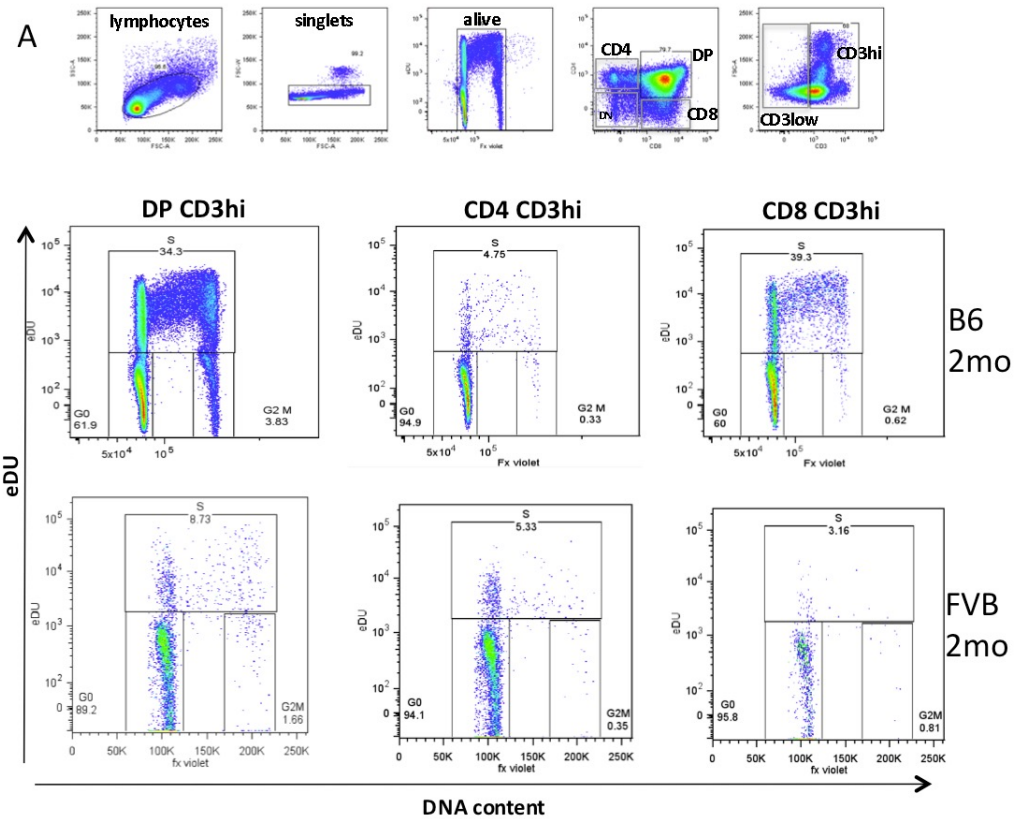
Single cell multi-parameter flow cytometry analysis

Thymus of B6 2 months old mouse

Identify manually
60 overlapping populations



Thymocyte proliferation in mice according to cell differentiation stage, age and genetic



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State Transition model of the cell cycle - EdU labeling through time

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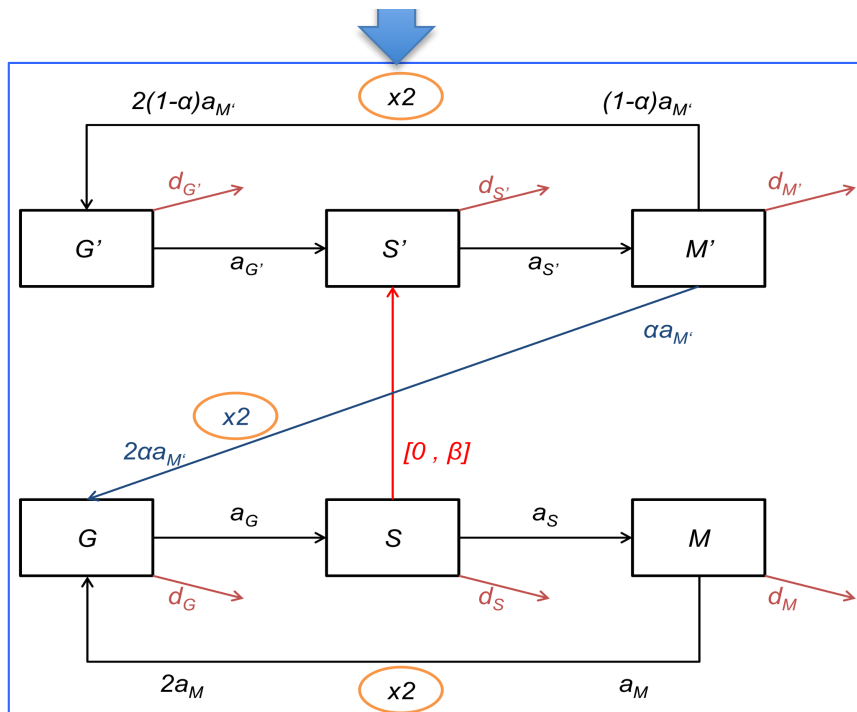
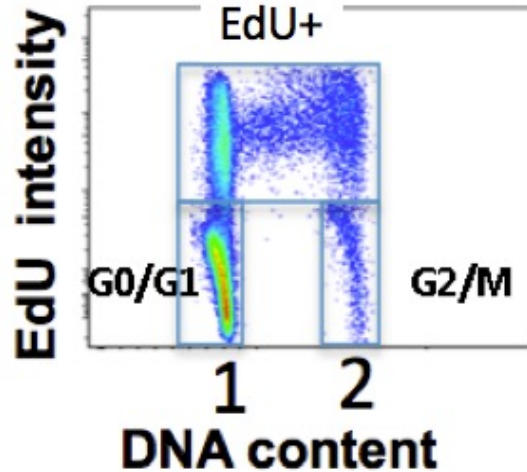


Table 1. Cell populations described in the model and initial values used in the fit according to our hypotheses.

Population name	Population description	Initial value used for the fit
G	Cells in G0 or G1 phase, unlabelled	$(G + G')_{ss} = \frac{200a_M(a_S+d_S)}{2a_M(a_S+d_S)+2a_Ma_G+(a_G+d_G)(a_S+d_S)}$
S	Cells in S phase, unlabelled	$(S + S')_{ss} = \frac{200a_Ma_G}{2a_M(a_S+d_S)+2a_Ma_G+(a_G+d_G)(a_S+d_S)}$
M	Cells in G2 or M phase, unlabelled	$(M + M')_{ss} = \frac{100(a_G+d_G)(a_S+d_S)}{2a_M(a_S+d_S)+2a_Ma_G+(a_G+d_G)(a_S+d_S)}$
G'	Cells in G0 or G1 phase, labelled	0
S'	Cells in S phase, labelled	0
M'	Cells in G2 or M phase, labelled	0

$$\begin{aligned}
 \frac{dG}{dt} &= 2a_M M + 2\alpha a_{M'} M' - (a_G + d_G)G \\
 \frac{dS}{dt} &= a_G G - (a_S + d_S)S - [0, \beta]S \\
 \frac{dM}{dt} &= a_S S - (a_M + d_M)M \\
 \frac{dG'}{dt} &= 2(1 - \alpha)a_{M'} M' - (a_{G'} + d_{G'})G' \\
 \frac{dS'}{dt} &= a_{G'} G' - (a_{S'} + d_{S'})S' + [0, \beta]S \\
 \frac{dM'}{dt} &= a_{S'} S' - (a_{M'} + d_{M'})M'
 \end{aligned} \tag{1}$$

Define parameter model constraints

Table 2. Model parameters and values used for the fit in our experimental conditions.

Parameter name	Parameter description	Value used for the fit
$a_G, a_{G'}$	Rate of entry into S phase of G1 phase unlabelled, labelled cells	$a_G = a_{G'}$ = parameter to fit
$a_S, a_{S'}$	Rate of entry into G2 phase of S phase unlabelled, labelled cells	$a_S = a_{S'}$ = 1/6.5
$a_M, a_{M'}$	Rate of entry into cell division of M phase unlabelled, labelled cells	$a_M = a_{M'}$ = parameter to fit
$d_G, d_{G'}$	Rate of cell death, differentiation or migration of G0/G1 phase unlabelled, labelled cells	$d_G = d_{G'}$ = a_G
$d_S, d_{S'}$	Rate of cell death, differentiation or migration of S phase unlabelled, labelled cells	$d_S = d_{S'} = 0$
$d_M, d_{M'}$	Rate of cell death, differentiation or migration of G2/M phase unlabelled, labelled cells	$d_M = d_{M'} = 0$
α	Proportion ($0 \leq \alpha \leq 1$) of labelled M phase cells shedding label during cell division	$\alpha = 0$ (no cell loses its label with cell division)
β	Rate of labelling of S phase cells during the pulse phase	$\beta = \infty$ (instant labelling by EdU)
p	Proliferation rate	Result of the fit

Table 3. Biological and experimental hypotheses, allowing for constraint of parameter values.

Hypothesis number	Hypothesis description	Consequence for parameters
1	EdU labelling does not affect cell-cycle kinetics	$(a_G, a_S, a_M, d_G, d_S, d_M) = (a_{G'}, a_{S'}, a_{M'}, d_{G'}, d_{S'}, d_{M'})$
2	Labelling of S phase cells is instantaneous during the pulse phase	$\beta = \infty$
3	Labelled cells do not divide enough times during the experiment to shed label	$\alpha = 0$
4	No dynamics perturbation under physiological conditions: steady-state cell number in each phase of cell cycle and in each cell population	$(G_0 = (G + G')_{SS}, S_0 = (S + S')_{SS}, M_0 = (M + M')_{SS})$ $a_M M + a_{M'} M' = d_G G + d_S S + d_M M + d_{G'} G' + d_{S'} S' + d_{M'} M'$
5	No cell death or differentiation during S phase and G2/M phase	$d_S = d_M = d_{S'} = d_{M'} = 0$
6	Bio-disponibility of EdU is one hour after an injection	None
7	S phase lasts 6.5 hours	$a_S = 1/6.5$

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Multidimension heterogeneity of proliferation rates of T lymphocytes

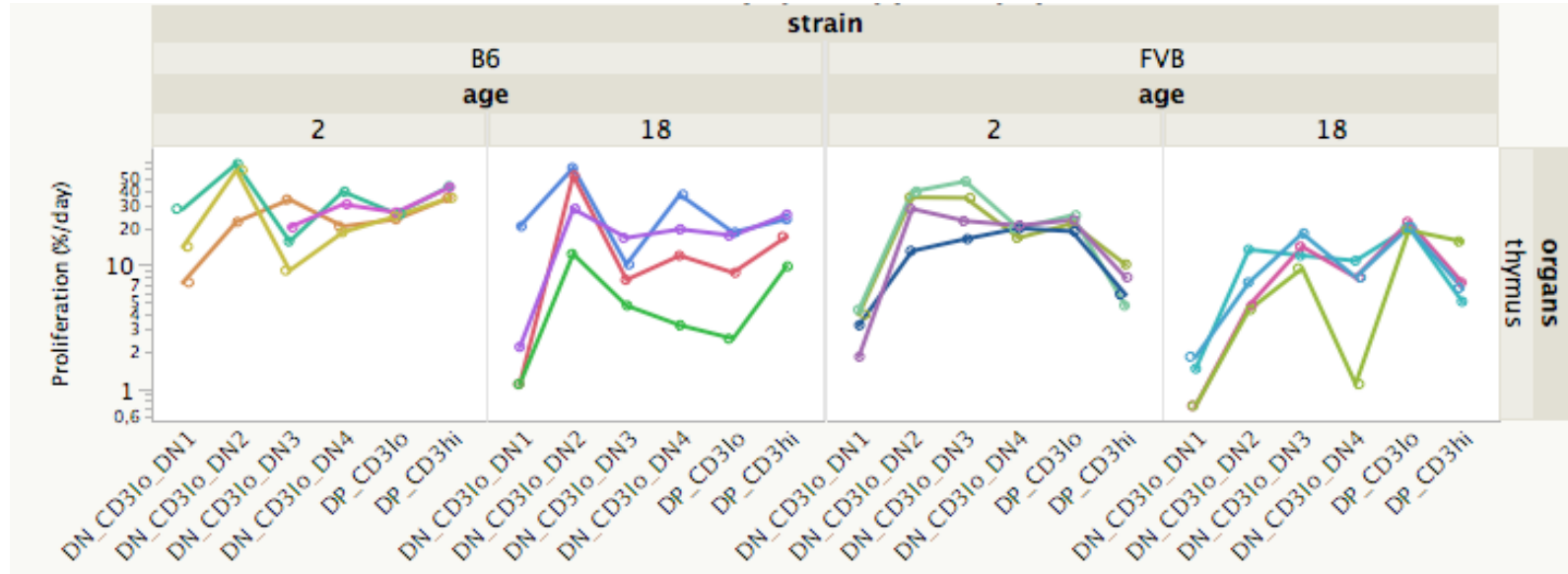
Local populations

THYMUS

Global thymus

Age

Strains

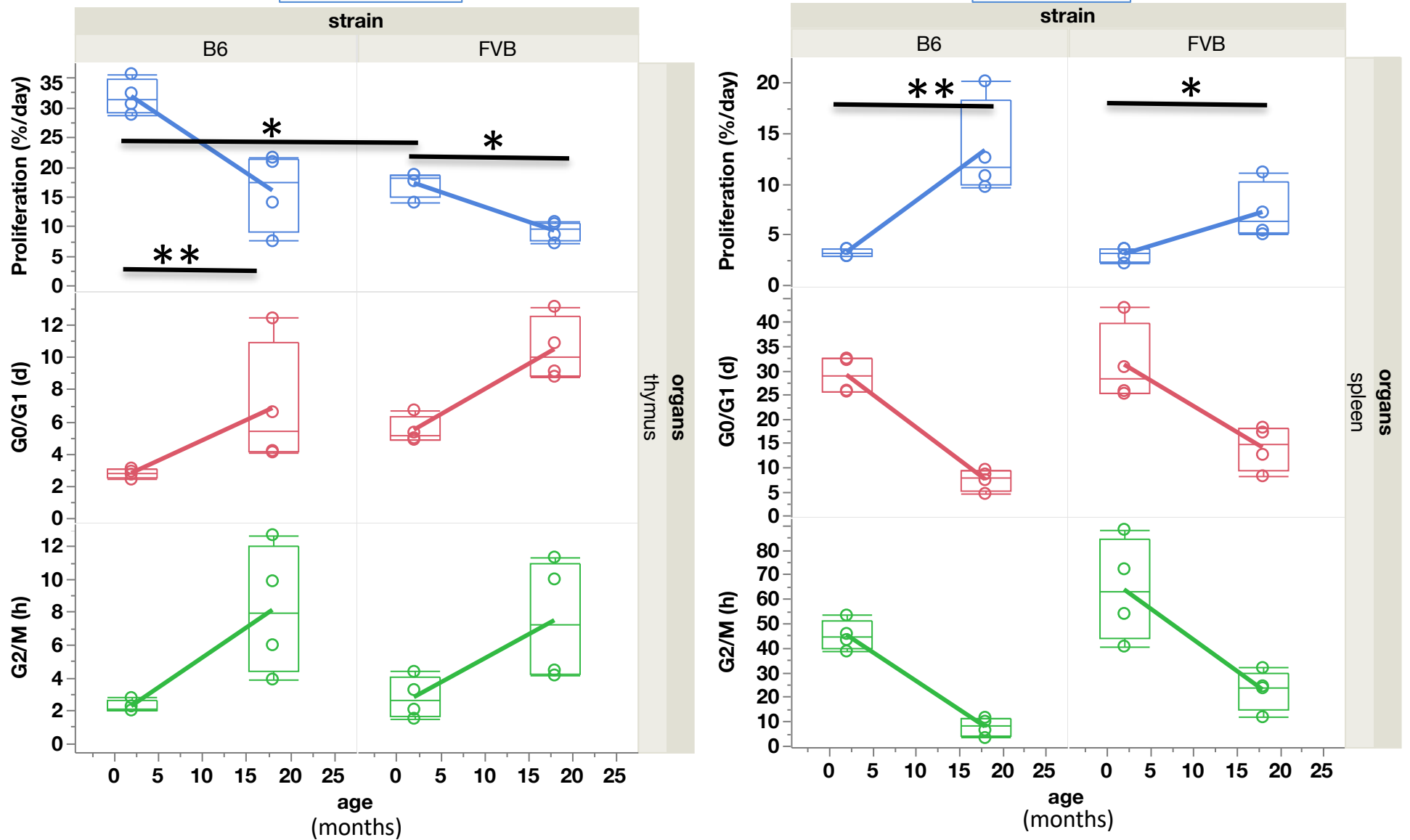


stages of differentiation

Population = total

organs	strain	age	Proliferation (%/day)		G0/G1 (d)		G2/M (h)	
			Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
spleen	B6	2	3,2	0,4	29,1	3,8	45,3	6,1
		18	13,3	4,7	7,5	2,2	7,9	3,7
	FVB	2	3,1	0,7	31,2	8,2	63,8	20,8
		18	7,2	2,8	14,1	4,6	23,0	8,3
thymus	B6	2	31,9	2,9	2,8	0,3	2,2	0,4
		18	16,0	6,6	6,8	3,9	8,1	3,9
	FVB	2	17,3	2,2	5,5	0,8	2,8	1,3
		18	9,3	1,7	10,5	2,0	7,5	3,7

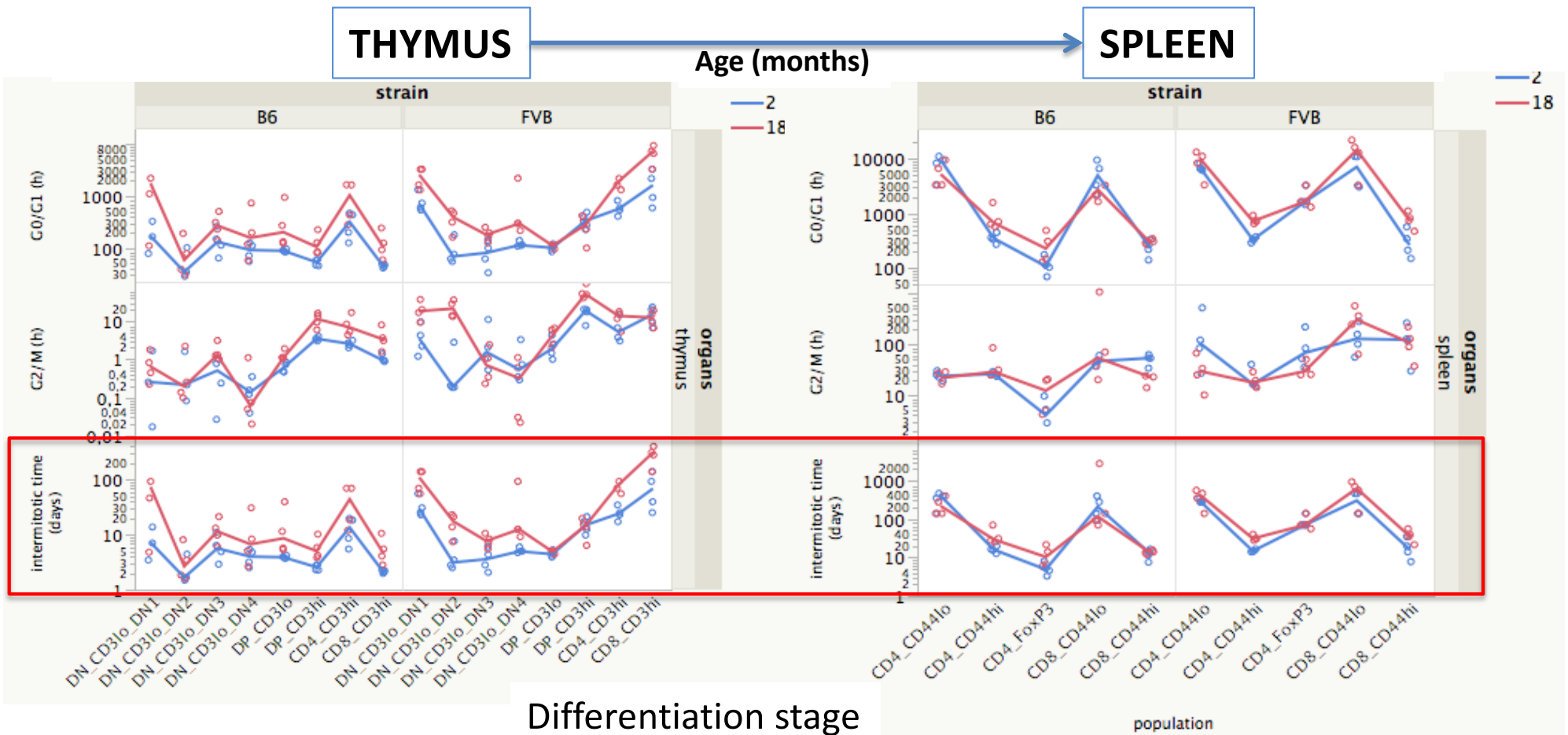
Proliferation rates and cell cycle phase durations in total thymus and spleen.



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Multi-dimension heterogeneity of cycle phase duration and intermitotic time

- According to differentiation stage of T lymphocytes
- Across aging
- Across genetics origins
- Across their migration through thymus and spleen

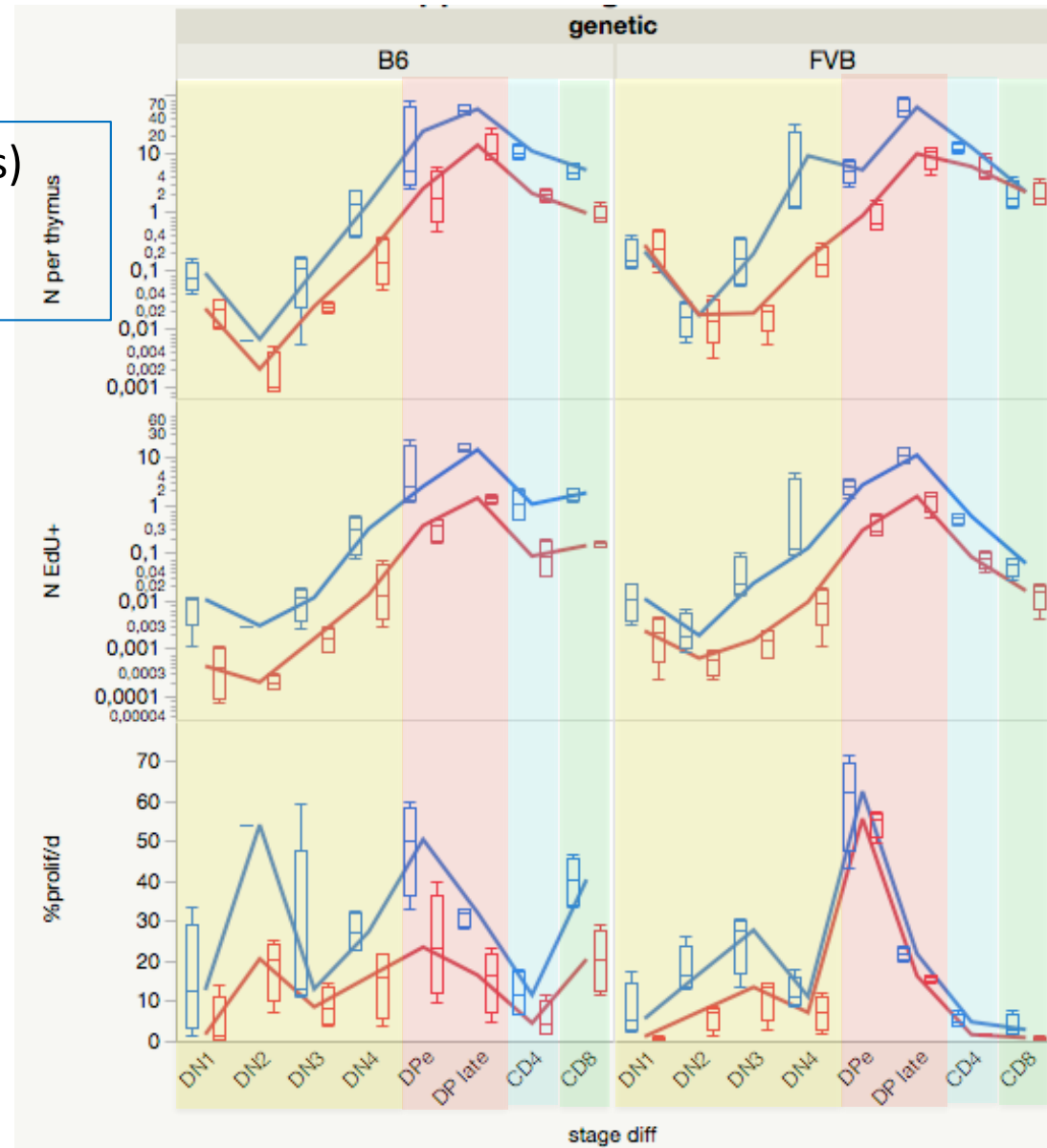


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Quantification of dividing cells during thymocyte differentiation

N cell cycles (generations)

- 13 in young/old B6
- 9 in old FVB mice



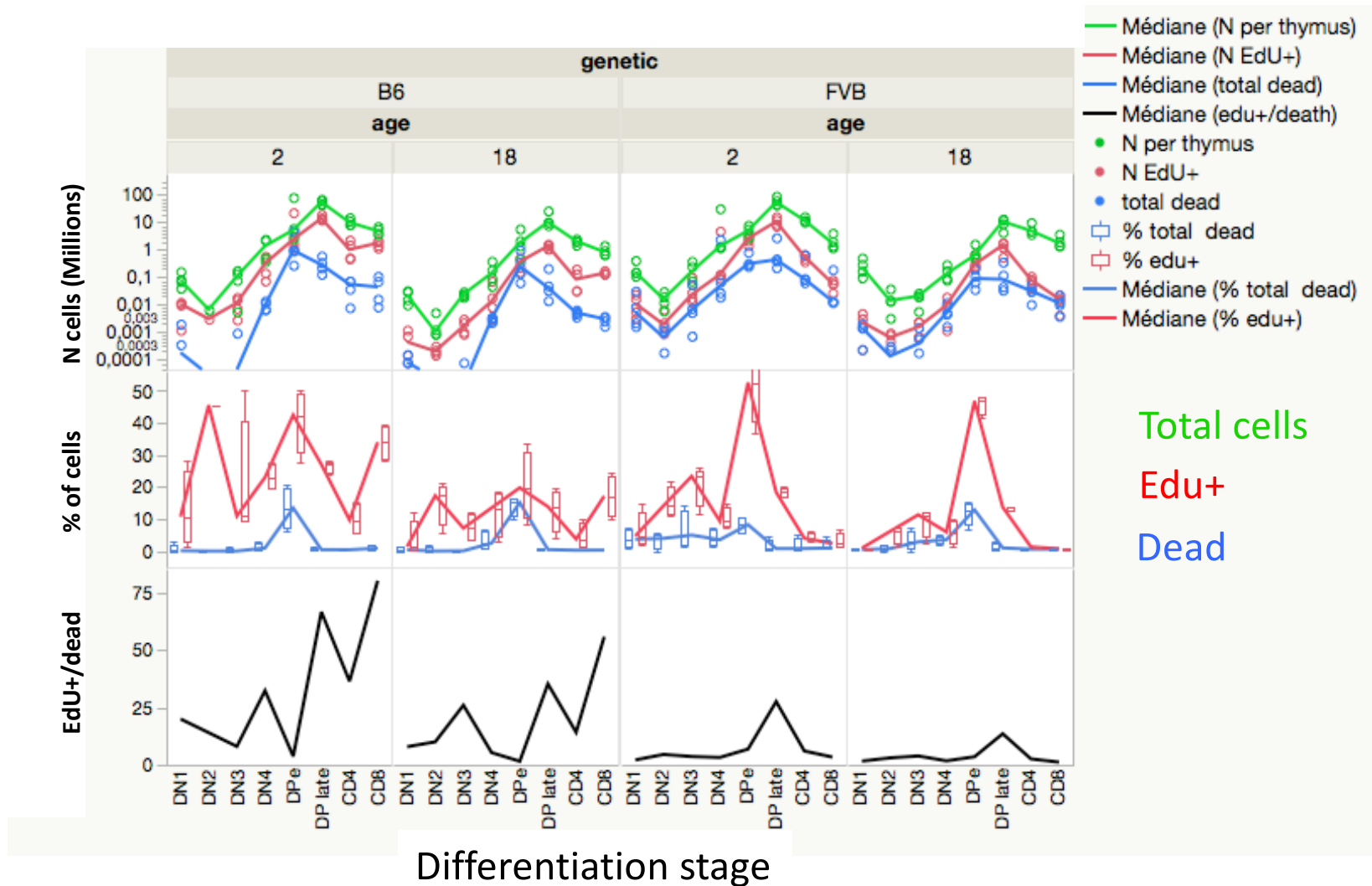
2 months

18 months

Time (3-4 weeks)

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Quantification of cell proliferation and death in thymus

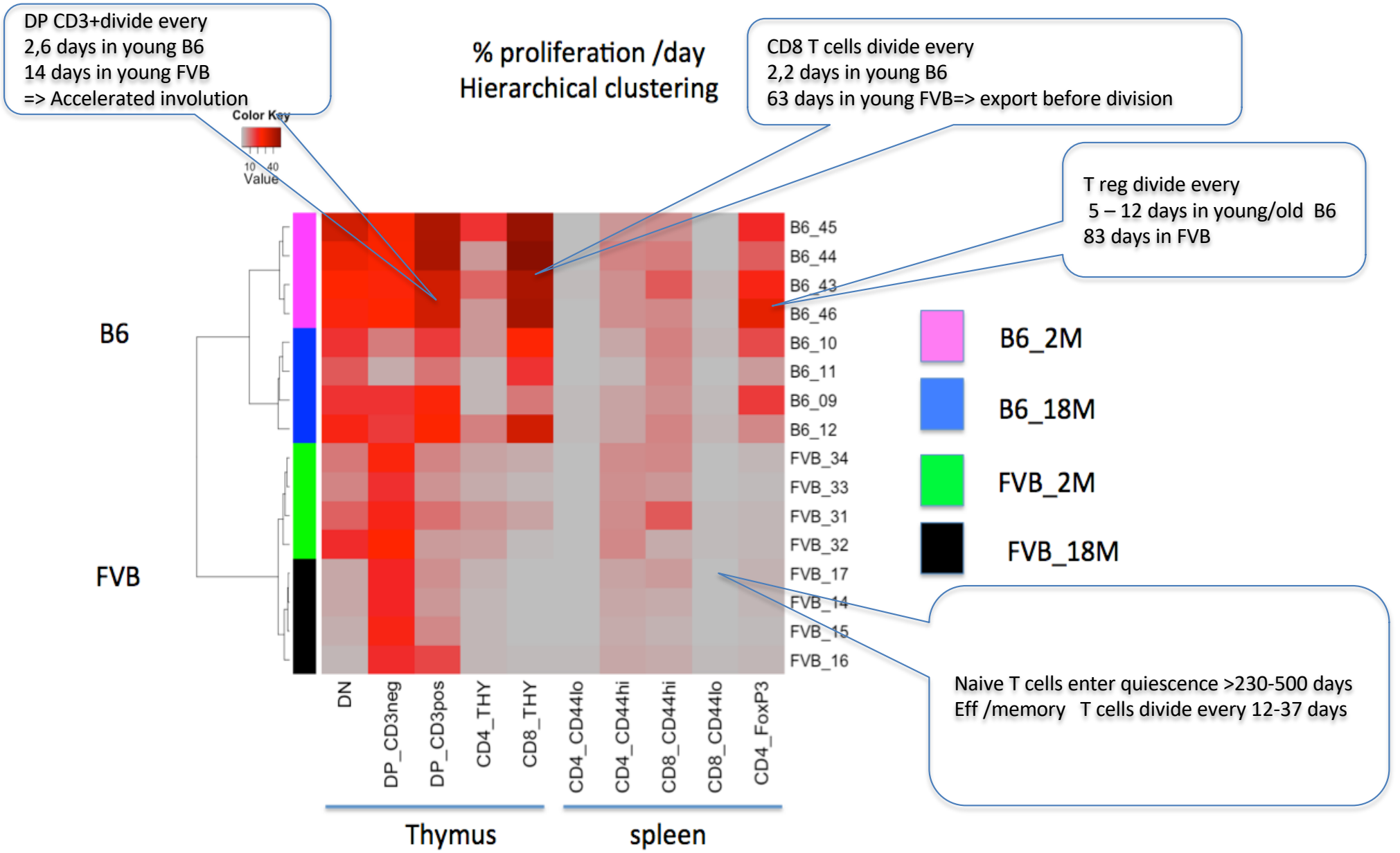


<http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1005417>

			organs							
			thymus							
			population							
	strain	age	DN_CD3lo_DN1	DN_CD3lo_DN2	DN_CD3lo_DN3	DN_CD3lo_DN4	DP_CD3lo	DP_CD3hi	CD4_CD3hi	CD8_CD3hi
			Moyenne	Moyenne	Moyenne	Moyenne	Moyenne	Moyenne	Moyenne	Moyenne
Proliferation (%/day)	B6	2	16,56	48,96	19,62	27,27	25,20	38,88	9,99	45,90
		18	6,21	38,70	9,72	17,91	11,70	18,90	4,14	21,60
	FVB	2	3,33	28,98	30,33	19,26	22,14	7,11	4,23	2,07
		18	1,17	7,38	13,32	6,93	20,43	8,55	1,35	0,41
intermitotic time (days)	B6	2	8,18	2,57	6,37	4,02	3,98	2,60	13,15	2,19
		18	59,09	3,80	12,64	11,78	15,62	6,10	42,37	5,81
	FVB	2	33,71	4,15	3,89	5,23	4,57	15,32	25,12	74,10
		18	100,69	16,48	7,94	31,78	4,91	13,91	77,55	280,53
G2/M (d)	B6	2	0,03	0,03	0,02	0,01	0,03	0,15	0,11	0,04
		18	0,03	0,03	0,07	0,01	0,05	0,47	0,36	0,17
	FVB	2	0,18	0,03	0,15	0,05	0,10	0,68	0,29	0,62
		18	0,86	0,95	0,04	0,02	0,18	2,48	0,52	0,53
G0/G1 (d)	B6	2	7,87	2,26	6,07	3,74	3,67	2,18	12,76	1,86
		18	58,78	3,50	12,29	11,49	15,29	5,35	41,73	5,37
	FVB	2	33,25	3,84	3,46	4,91	4,20	14,36	24,55	73,21
		18	99,56	15,25	7,62	31,48	4,46	11,14	76,74	279,73

			organs						
			spleen						
			population						
	strain	age	CD4	CD4_CD44lo	CD4_CD44hi	CD4_FoxP3	CD8	CD8_CD44lo	CD8_CD44hi
			Moyenne	Moyenne	Moyenne	Moyenne	Moyenne	Moyenne	Moyenne
Proliferation (%/day)	B6	2	2,25	0,37	6,39	21,15	3,87	0,60	8,82
		18	2,25	0,51	3,06	11,07	5,58	0,82	7,38
	FVB	2	1,35	0,34	6,75	1,26	2,88	0,47	7,02
		18	1,44	0,35	3,15	1,35	2,70	0,29	2,97
intermitotic time (days)	B6	2	44,64	336,47	16,06	5,23	29,97	226,52	12,33
		18	62,28	238,10	38,33	11,91	18,64	769,68	13,66
	FVB	2	75,23	295,14	14,87	86,81	47,74	300,93	19,04
		18	71,76	376,16	32,30	83,33	44,75	578,70	37,83
G2/M (d)	B6	2	1,13	1,00	1,09	0,18	2,11	1,97	2,11
		18	1,83	0,75	1,74	0,51	0,96	12,44	0,87
	FVB	2	2,61	7,81	0,91	4,05	5,79	6,16	5,57
		18	0,91	1,41	0,81	1,39	4,39	12,86	4,94
G0/G1 (d)	B6	2	43,23	335,19	14,70	4,76	27,58	224,28	9,94
		18	60,18	237,06	36,32	11,13	17,41	756,96	12,51
	FVB	2	72,34	287,05	13,68	82,48	41,67	294,49	13,19
		18	70,58	374,47	31,21	81,66	40,08	565,57	32,61

Signatures of lymphocyte proliferation according to strains and ages



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Conclusions

Modelling the biological heterogeneity, from T cell proliferation up to organisms

System biology methods

- *In vivo* transient conditional immunosuppression with T cell depletion/reconstitution
- *In vivo* Pulse chase labelling of DNA, and modeling to reveal the cell cycle transition
- Multi-parameter cell investigation by single cell flow cytometry -> T cell phenotype, cell cycle, repertoire
- Maths models using transition diagrams-> estimate cell state transition, as parallel processes
- Provide cell cycle phase duration, intermitoc time, proliferation/ death rates of various T cell populations

⇒ Organisms heterogeneity according to age, genetic origins that influence the T cell proliferation rates/death

- **Young mice**
 - At steady-state display low inter-individual variability, typical signatures, according to strains
 - Homeostasis is preserved, when the thymus is productive
- **Old mice**
 - Increased inter-individual variability, loss of strain signature
 - Thymic involution associated with decrease of cell division, and increase of death
 - In periphery decrease of TCR repertoire diversity, occurrence of CD8 cell oligoclonal expansions/extinctions

⇒ Genetic origins influence the proliferation/death of lymphocytes

- **FVB mice** as compared to B6, display
 - Accelerated thymic involution & aging
 - Lower proliferative capacities, highest mortality in thymus
 - Accumulation of CD8 oligoclonal expansions in periphery
 - Accumulation of anergic Treg, lack of feedback control on T cell proliferation
 - Low-dose IL-2 or Treg cell injections prevent aging: preserve the TCR repertoire diversity and avoid clonal expansions

⇒ Immunosuppression, accelerates immunological aging, through loss of TCR diversity and oligoclonal expansions

Immunology, Immunopathology, Immunotherapy

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Florence Giesen

Pierre Loap

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Bruno Gouritin

Christophe Huret

Pierric Parent



GOAML
Graphical ODE and
Agent-based
Modeling Language
software UPMC

Theoretical Biology and Bioinformatics,
Utrecht University

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- Programme interdisciplinaire CNRS Longévité et vieillissement
- Emergence UPMC
- Exchange South Africa NRF/ CNRS
- DIM ISC-PIF Complex systems
- PHC Tournesol
- PEPS BMI
- ERC
- Labex TransImmunoM

RNSC Réseau National des Systèmes Complexes **île de France**



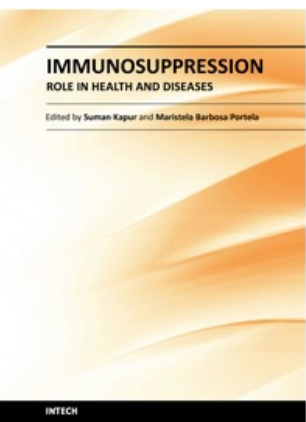
<http://www.immunocomplexit.net/>

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IFR113





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<http://www.materiologiques.com/Le-vivant-critique-et-chaotique>
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- Thomas-Vaslin, et al (2012) [Immunodepression & Immunosuppression in aging mice](#) "Immunosuppression" InTech