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# T cells: Ontogeny and Maintenance

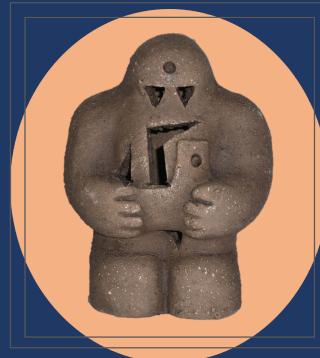
Sanket Rane

IICD & Dept of Pathology and Cell Biology

2021-01-02

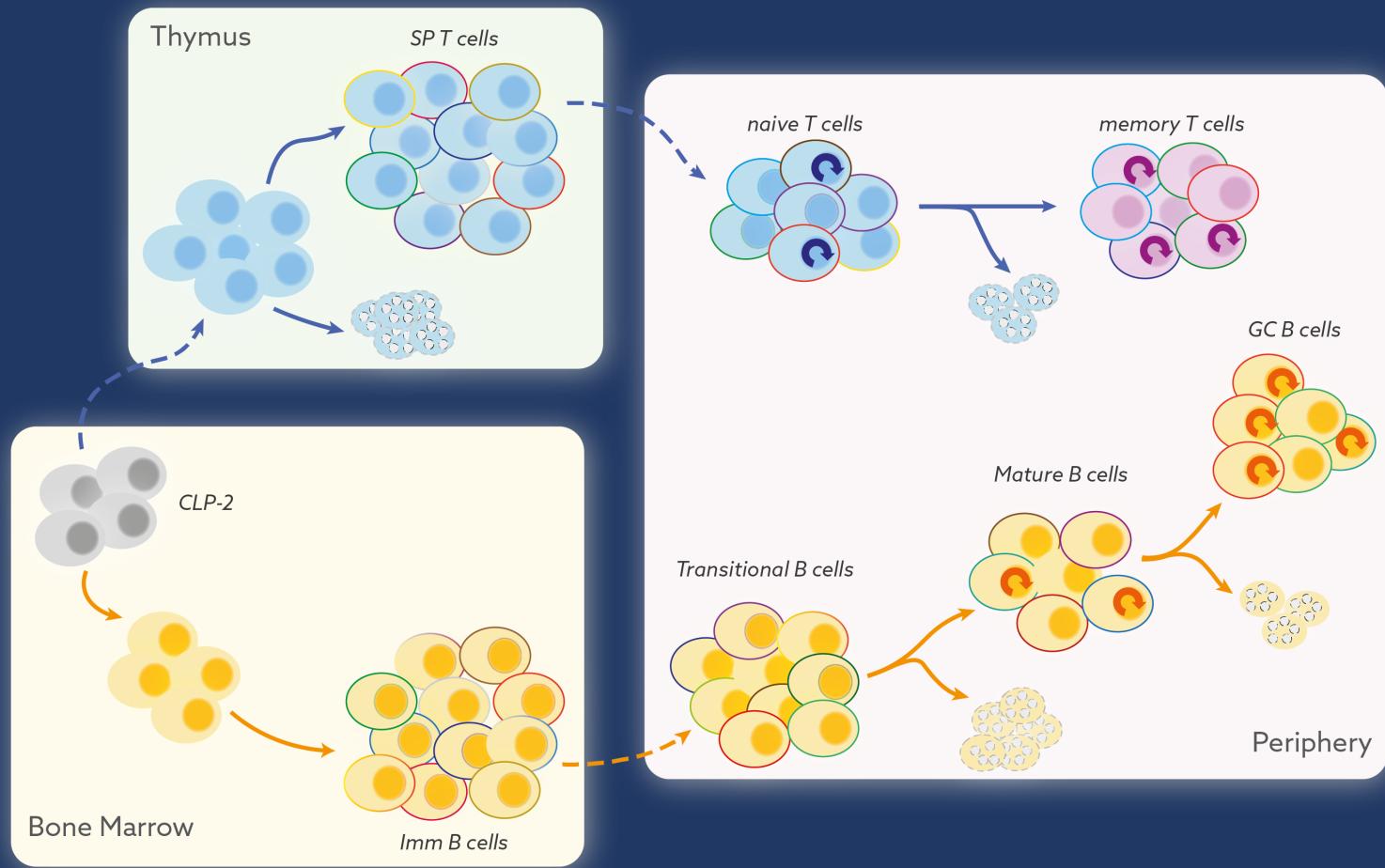
# The Emperor and the Golem

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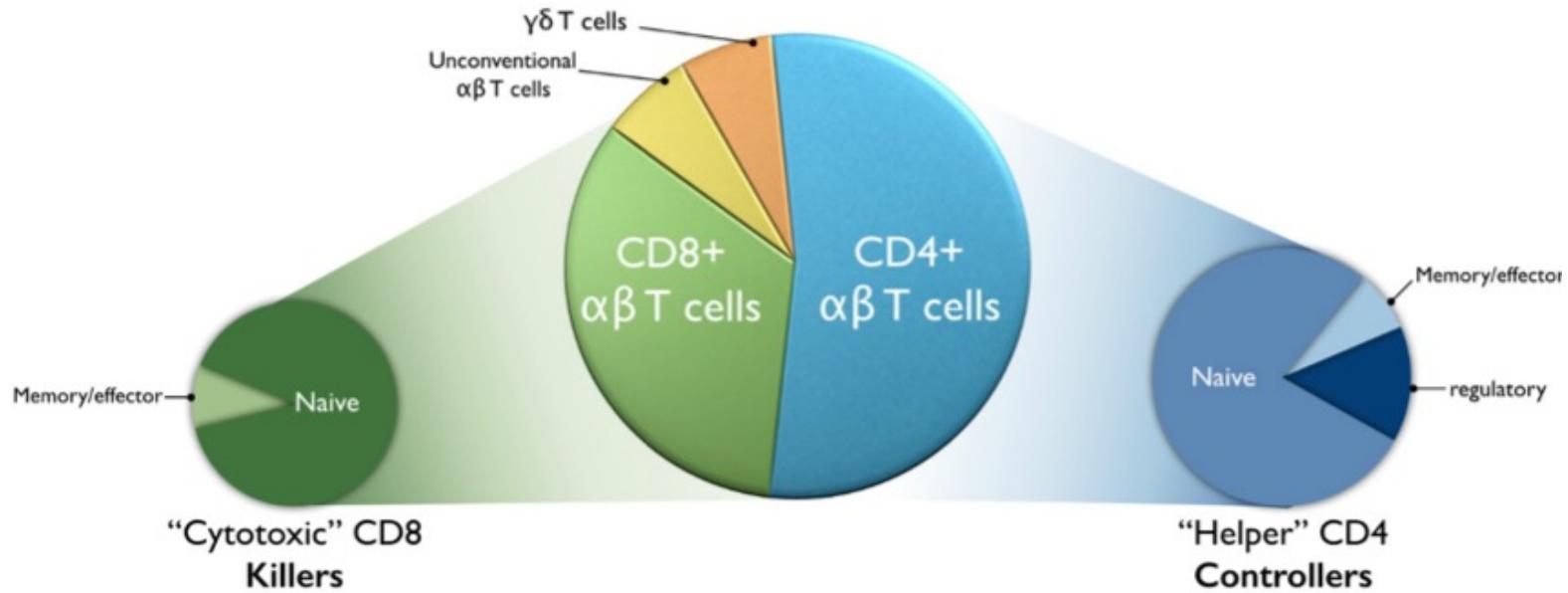
- **Specifically identify and destroy 'foreign'.**  
→ Large army for countless possibilities.
- **Recognize our own.**  
→ Remove that pose threat to 'self'.
- **Remember and respond faster next time.**

# The Golems of our immune system



## Subset composition of T cells

T cells are primarily categorized into  $CD4^+$  and  $CD8^+$  cells.



**Naive T cells make up a large fraction of these subsets.**

# Naive T cells are cornerstones of adaptive immunity

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## \* Naive T lymphocytes --

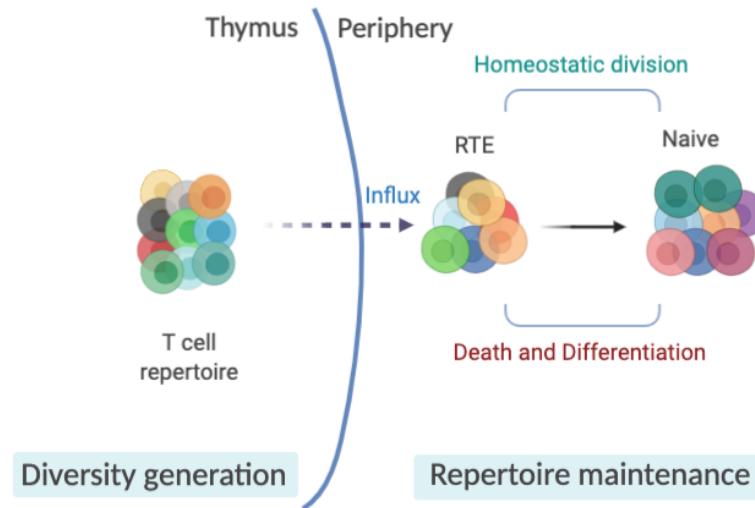
- Identify 'specific antigens' on pathogens and tumors.
- Continuously circulate within lymphatic system in search of antigens.
- Differentiate into memory and effector T cells.

Naive T cells play a central role in fights against novel and mutating pathogens.



A broad and long-lasting naive repertoire is crucial to protect against new infections throughout our lifetime.

# Complexity & Dynamism in naive T cell homeostasis:



Multiple processes regulate the numbers and diversity of naive T cells.

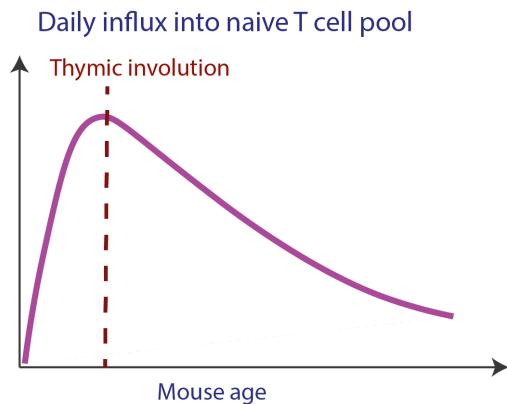
Contribution of these processes to naive T cell maintenance may vary with time.

**Understanding the development and maintenance of our T cell repertoires requires quantifying their ecology.**

# Influx into the naive pool

- Thymic involution:

Thymus starts shrinking from around 8 weeks of age in mice (halves in size every ~5 months) and from puberty in humans.



→ Fewer and fewer new cells enter the naive compartment.

→ Diminished inflow of new specificities.

Thymic involution strongly impacts naive T cell numbers and repertoire diversity.

# Big Questions

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 **Is the majority of T cell repertoire established early on in life (pre-involution)?**

 **What are the dynamics of its establishment?**

- Is there clonal proliferation in periphery → forming large clonal families?  
    ↳ **Skews clone size distribution and lowers diversity.**
- Is it all new specificities from the thymus?  
    ↳ **Enhances diversity.**

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## How are naive T cells maintained after thymic involution?

- Quorum sensing to preserve the numbers?  
↳ Compensatory increase in cell division or decrease in turnover or both?

⇒ Can this explain the collapse in repertoire diversity in old ages?



## What are the rules of replacement within naive pool?

- Is it purely stochastic?
- Do new immigrants displace pre-existing cells?
- Homeostatic advantage for relatively older cells?

⇒ Consequences on how diversity is maintained over lifetime.

# What we know and don't know

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Most of our understanding of naive T cell homeostasis comes from studies in adult mice.

↪ Relatively little is known about their neonatal dynamics.

## 1. Heterogeneity in naive T cell pool

→ We have shown that naive T cells' ability to persist in the circulation increases with their cell-age (**first-in-last-out rule**).

- Shown in diverse experimental settings in adult mice.

Hogan *et. al.*, PNAS 2015. Rane *et. al.* Plos Bio 2018

- With the use of  $C^{14}$  dating in humans (age: 20-65 years).

Mold *et. al.* Plos Bio 2019

## Can cell-age dependence explain naive T cell development in neonatal niches?

## 2. Quorum sensing in naive T cell pool

- Naive T cells have shown to undergo rapid division in profoundly lymphopenic conditions (>90% depletion).

Bourgeois and Stockinger, JI 2006

↳ Reduced competition for resources (IL-7 and self-MHC derived tonic signals).

- Does it manifest under normal conditions early in life?
- Is there reduced competition in neonates?

## 3. Dynamics of Recent Thymic Emigrants

- RTE within naive CD4 T cell pool are short-lived as compared to pre-existing naive T cells.
- No kinetic heterogeneity within naive CD8 T cell pool.

van Hoeven Front. Imm. 2017

# A unified model of naive T cell life-histories

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A single model that explains it all –

⇒ Dynamism in neonates and stability in adults.

⇒ How and why does our immunity wanes with age?

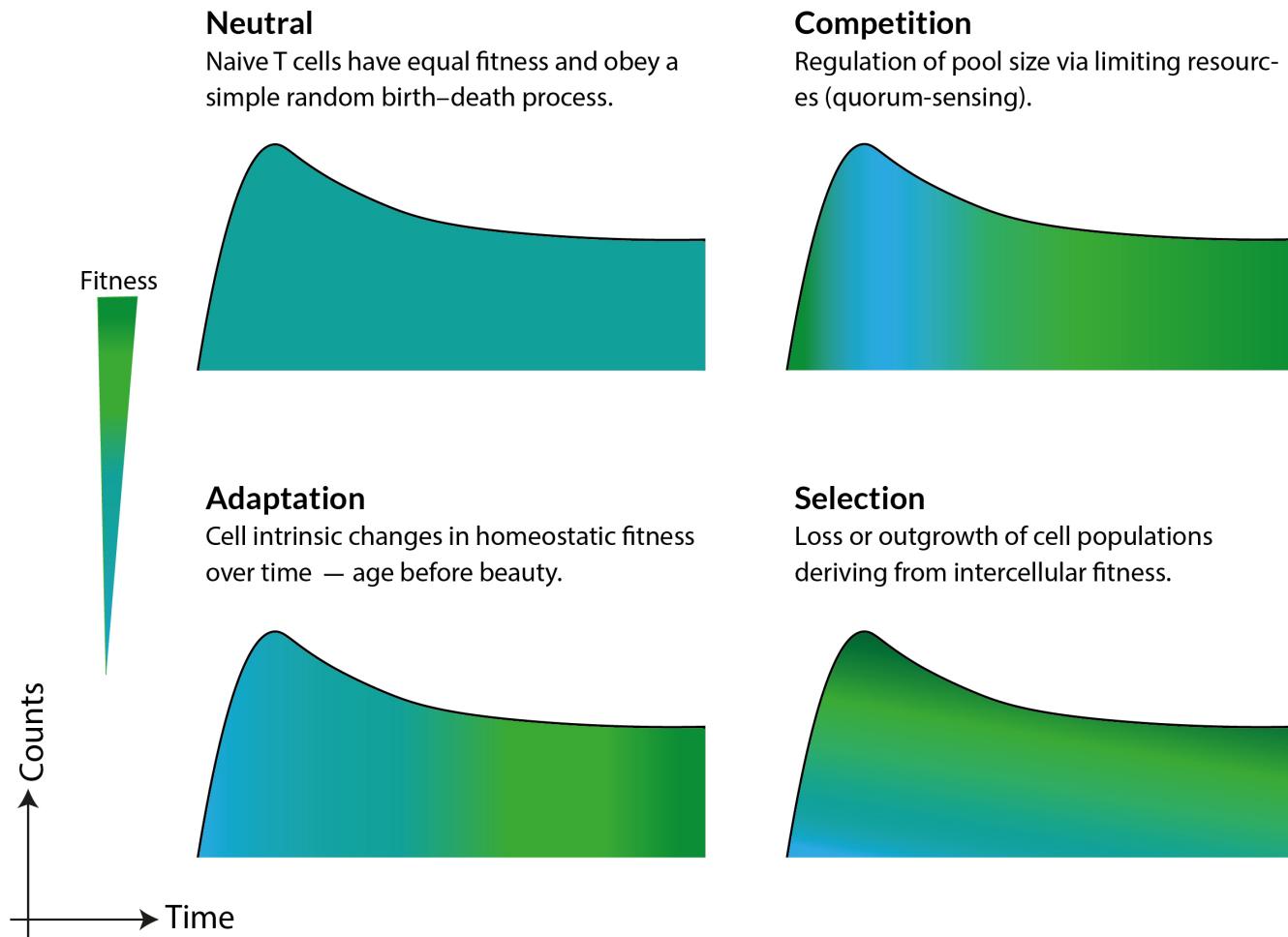
⇒ Evolution of CD4 and CD8 repertoires and differences between them.

- More skewing in CD8 repertoires than in CD4 repertoires.

⇒ Prediction of T cell recovery upon depletion.

- Reconstitution in HSC Transplants and in HIV patients.

# Potential mechanisms of naive T cell maintenance



# Story so far...

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Table: Summary of previous results

Dataset	Neutral	Competition	Selection	Adaptation
Cell counts in Wild Type mice	✓	✓	✓	✓
Cell counts in Thymectomised mice	✗	✓	✓	✓
Donor fractions in Busulfan BM chimeras	✗	✗	✓	✓
Kinetics of adoptively transferred cells	—	—	✗	✓

Rane *et. al.* Plos Bio 2018

- The adaptation model captured the naive T cell dynamics across **multiple datasets** generated in **diverse experimental setups** in adult mice.
- Rest of the models when tested in isolation failed with one or more datasets.

## Different combinations of models?

continued...

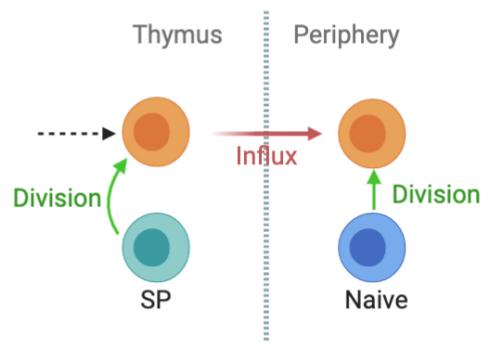
## Untangling division and loss:

- Previous studies estimated **Net Growth (division - loss)** rate of naive T cells.

⇒ Separating the effects of division and loss is crucial for studying lymphocyte dynamics.

Verheijen & Rane *et. al.* Cell Reports 2020

- Ki67 – a nuclear protein expressed during cell-division.  
↳ Independent handle on the rate of cell division.



- T cells continue expressing Ki67 protein for ~ 4 days after the division.

Hogan *et. al.*, PNAS 2015

Thymic Inheritance or division in periphery?

**Modeling division-history is crucial for understanding how repertoires evolve.**

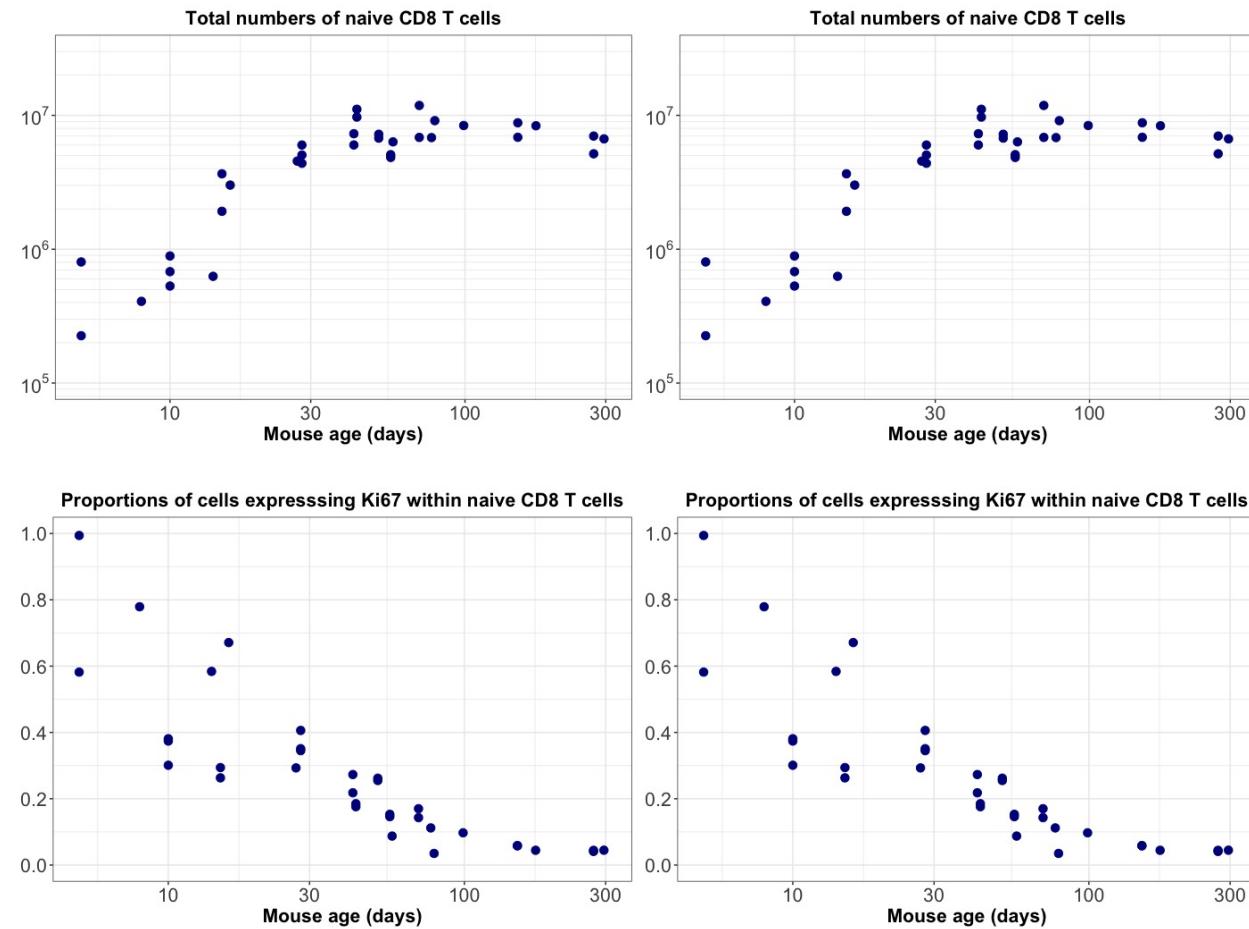
## 1. Ontogeny dataset

Total counts and proportions of  $\text{Ki67}^{\text{high}}$  cells from d5 to d300 in mice.

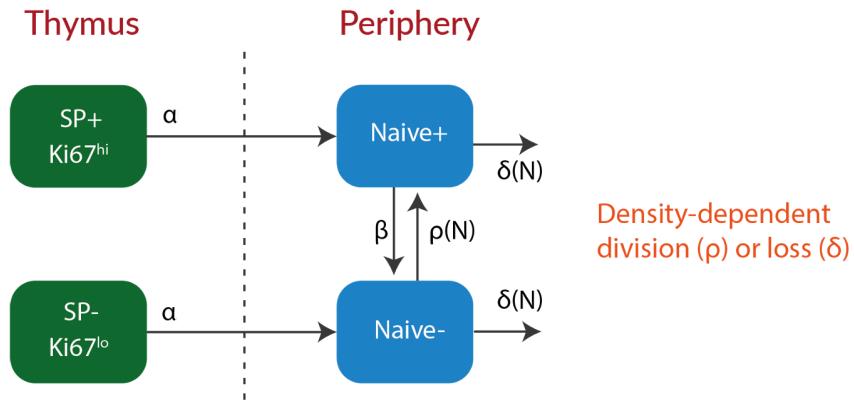
## 2. Timestamp dataset

Data from a mouse system that allows us to track cohorts of naive T cells as they leave the thymus.

# Dynamics of Naive T cell pool size and Ki67 expression



# Neutral and Competition Models



$$N(t) = N^+(t) + N^-(t); \quad \kappa(t) = \frac{N^+(t)}{N(t)}$$

Models are represented as systems of ordinary differential equations (ODEs).

$$\begin{aligned}\dot{N}^+ &= \alpha + \rho(N) (2N^- + N^+) - (\beta + \delta(N)) N^+ \\ \dot{N}^- &= \alpha + \beta N^+ + \delta(N) N^-\end{aligned}$$

# Model validation

$$y_i \sim \text{normal}(\mu_i, \sigma)$$

[likelihood]

$$\mu_i = f(\text{time}_i, \theta)$$

[model]

$\theta$  : Vector of parameters  $[\alpha, \beta, \delta, \sigma]$  within the model.

$$\alpha \sim \text{uniform}(0.0, 1.0)$$

[\mathbf{\alpha} \text{ prior}]

$$\rho \sim \text{normal}(0.005, 0.01)$$

[\mathbf{\rho} \text{ prior}]

$$\delta \sim \text{normal}(0.05, 0.05)$$

[\mathbf{\delta} \text{ prior}]

$$\sigma \sim \text{uniform}(0, 5)$$

[\mathbf{\sigma} \text{ prior}]

Models are fitted simultaneously to the total counts and the proportions of Ki67+ cells.

$$\log(\text{Total counts}) \Rightarrow$$

$$y_1 \sim \text{normal}(\mu_1, \sigma_1)$$

$$\text{logit}(\text{Ki67}^+ \text{ proportions}) \Rightarrow$$

$$y_2 \sim \text{normal}(\mu_2, \sigma_2)$$

... continued

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## Bayesian approach:

$$P(\hat{\theta}|y_1, y_2) = \frac{P(y_1|\hat{\theta}) \cdot P(y_2|\hat{\theta}) \cdot P(\theta)}{P(y_1) \cdot P(y_2)}$$

## Hamiltonian Monte Carlo (H-MC):

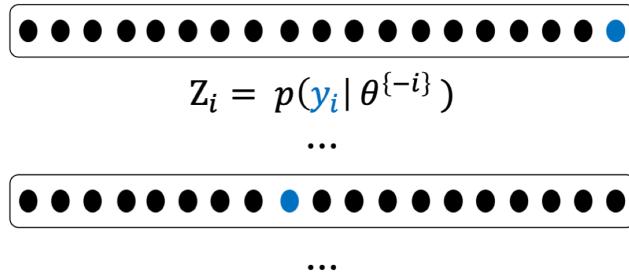
Parameters are sampled from the joint prior distribution  $P(\theta)$  to estimate the posterior distribution  $P(\hat{\theta})$ .

- Models are coded in *Stan* language and fitted using the **NUTS** sampler.
  - Posterior distributions and model fits are analyzed using *R* package **rstan**.
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# Model selection

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## Leave-One-Out cross validation:



Leave-One-Out information criterion:  $\text{LooIC} = -2 \sum_{i=1}^n Z_i$

⇒ LooIC measures the model's ability to predict new data.

... continued

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## Scaling the models:

$$\Delta\text{LooIC} = \text{LooIC}_i - \min(\text{LooIC})$$

- $\Delta\text{LooIC} = 0 \Rightarrow$  Best-fit model.
- Higher values suggest poorer support from the data.

## Relative support:

Analog of Akaike weight

$$\omega_i = \frac{\exp(-\frac{1}{2} [\Delta\text{LooIC}]_i)}{\sum_m^M \exp(-\frac{1}{2} [\Delta\text{LooIC}]_m)}$$

- Proportional statistical support among the models under consideration.

Table: Statistical analysis for CD4 data

Model	$\Delta\text{LooIC}$	Akaike weight %
Neutral	5.2	6.71
LIP	6.8	3.01
Density-dependent loss	0.0	90.28

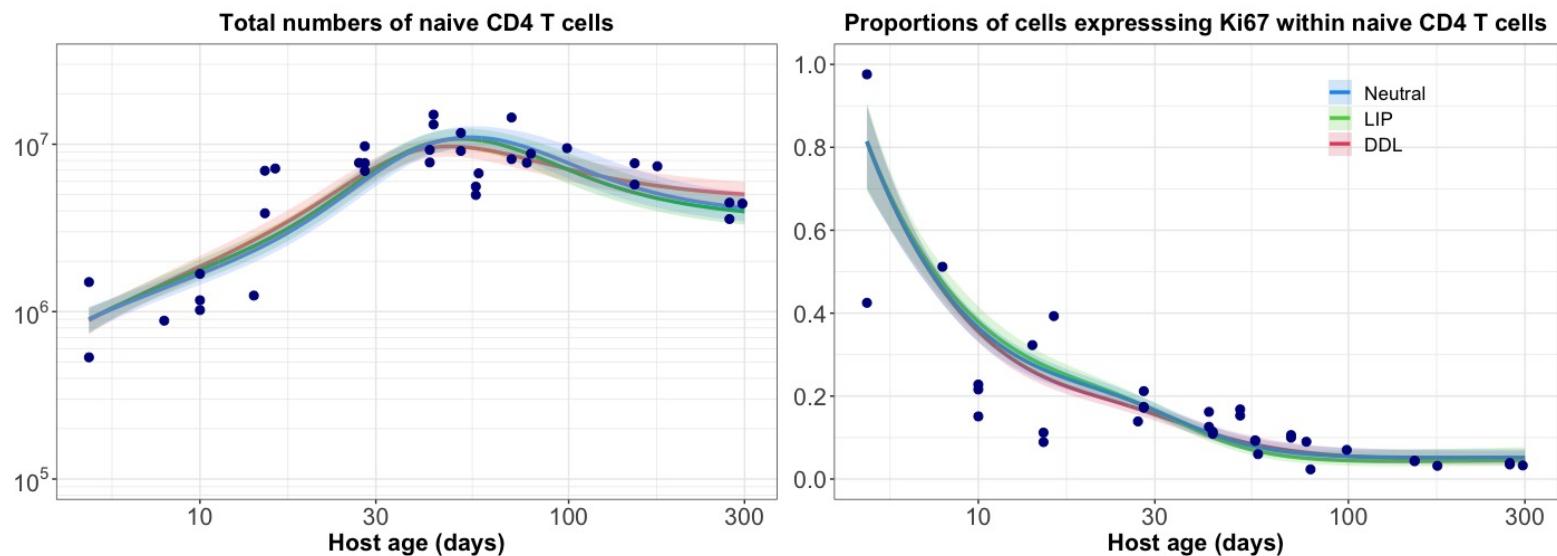
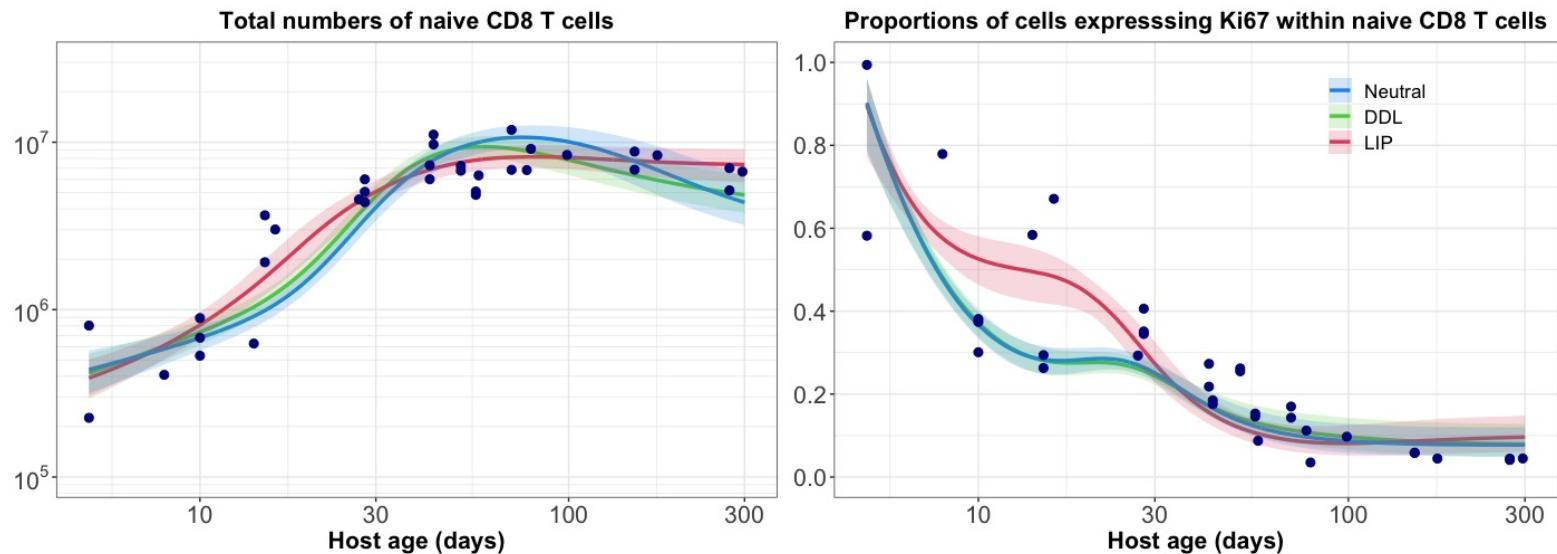


Table: Statistical analysis for CD8 data

Model	$\Delta\text{LooIC}$	Akaike weight %
Neutral	13.14	0.13
LIP	0.00	93.80
Density-dependent loss	5.48	6.07



## Two-compartment models

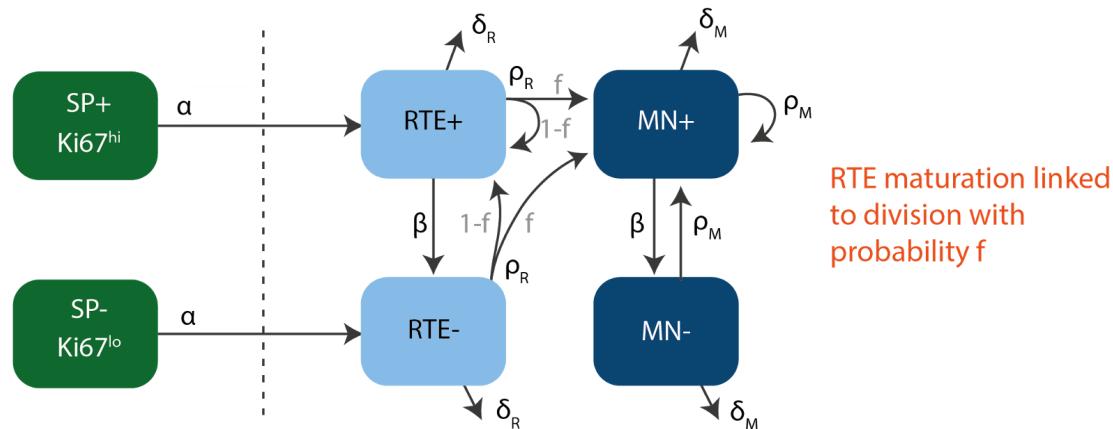
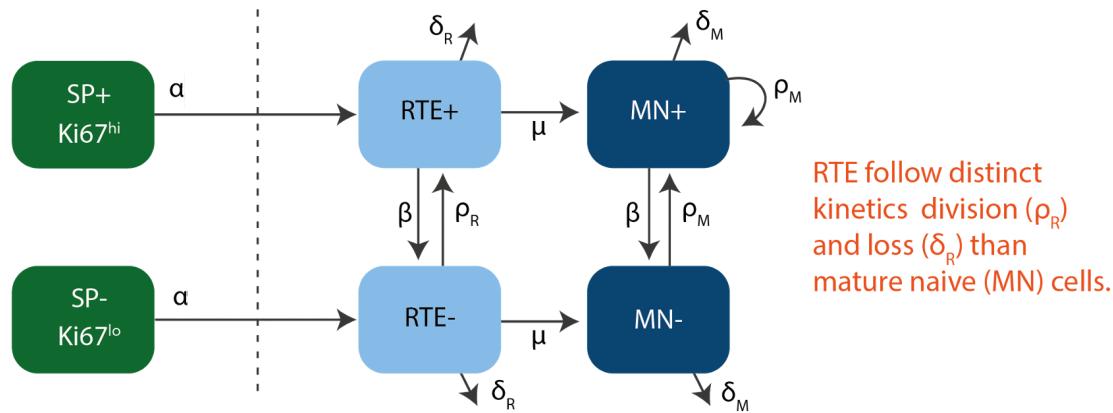


Table: Statistical analysis for CD4 data

Model	$\Delta\text{LooIC}$	Akaike weight %
Neutral	5.20	5.74
LIP	6.80	2.58
Density-dependent loss	0.00	77.23
RTE	6.87	2.49
RTE maturation linked division	3.73	11.96

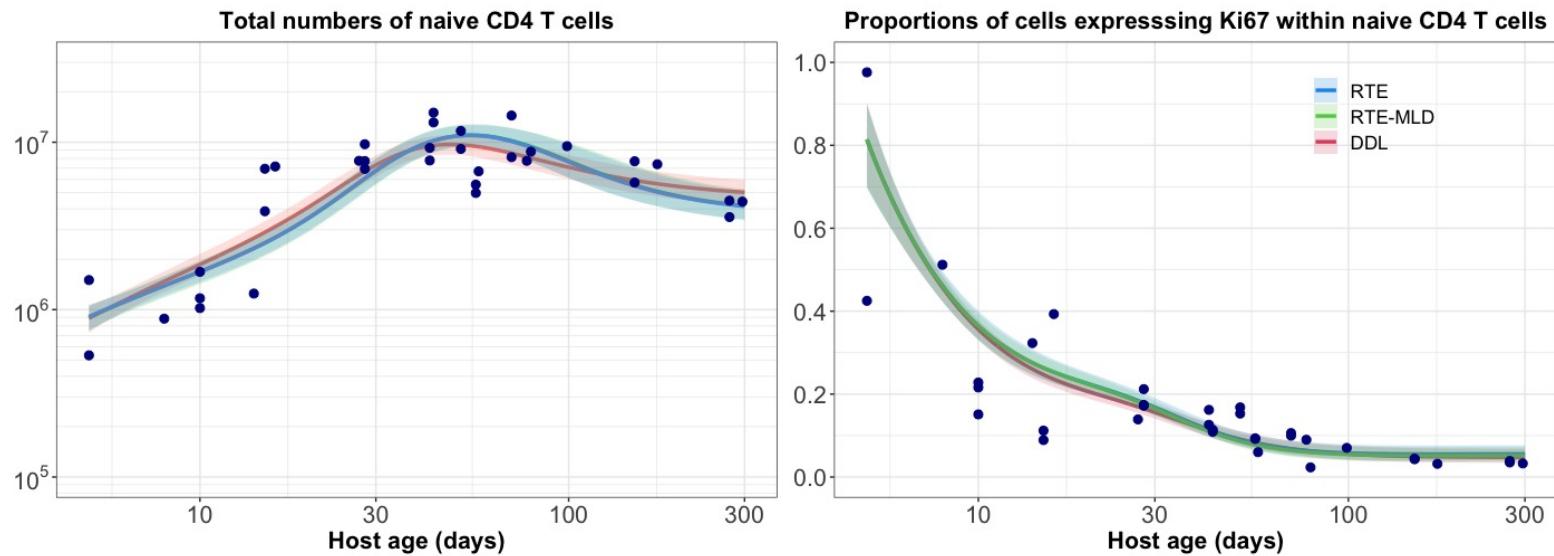
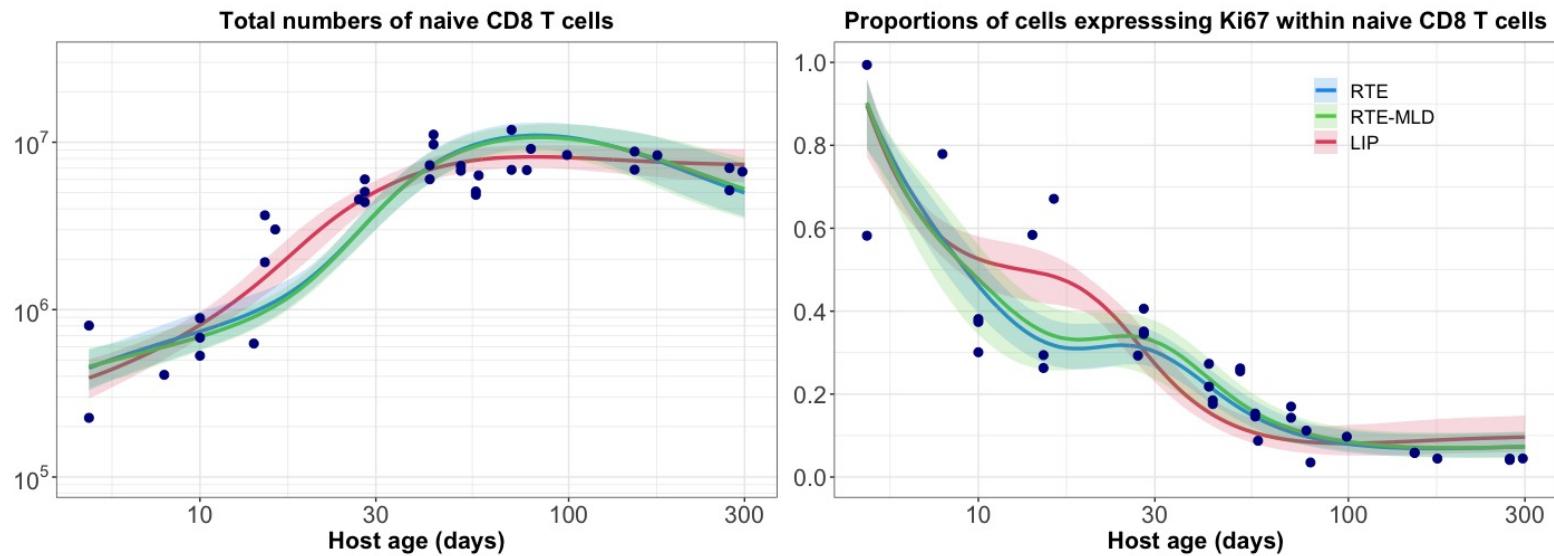
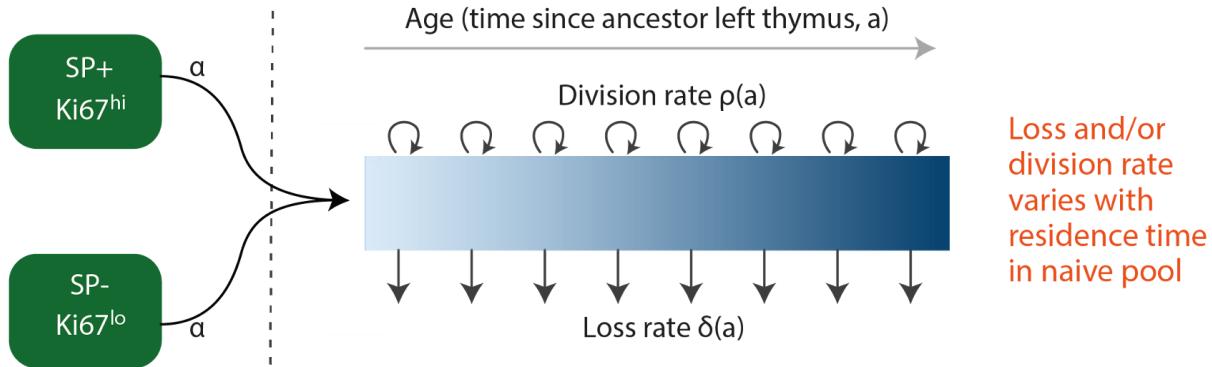


Table: Statistical analysis for CD8 data

Model	$\Delta\text{LooIC}$	Akaike weight %
Neutral	13.14	0.13
LIP	0.00	93.49
Density-dependent loss	5.48	6.05
RTE	11.76	0.26
RTE maturation linked division	14.43	0.07



## Adaptation: age-structured model



Naive T cells of age 'a' and Ki67 expression 'k' are tracked across time 't', by analytically solving the 3-dimensional PDE,

$$\frac{\partial N}{\partial t} + \frac{\partial N}{\partial a} - \beta k \frac{\partial N}{\partial k} = -(\rho(a) + \delta(a)) N(t, a, k)$$

Table: Statistical analysis for CD4 data

Model	$\Delta\text{LooIC}$	Akaike weight %
Neutral	5.20	4.42
LIP	6.80	1.99
<b>Density-dependent loss</b>	<b>0.00</b>	<b>59.56</b>
RTE	6.87	1.92
RTE maturation linked division	3.73	9.23
ASM $\delta$ -varying with cell-age	5.98	2.99
<b>ASM <math>\rho</math>-varying with cell-age</b>	<b>2.19</b>	<b>19.89</b>

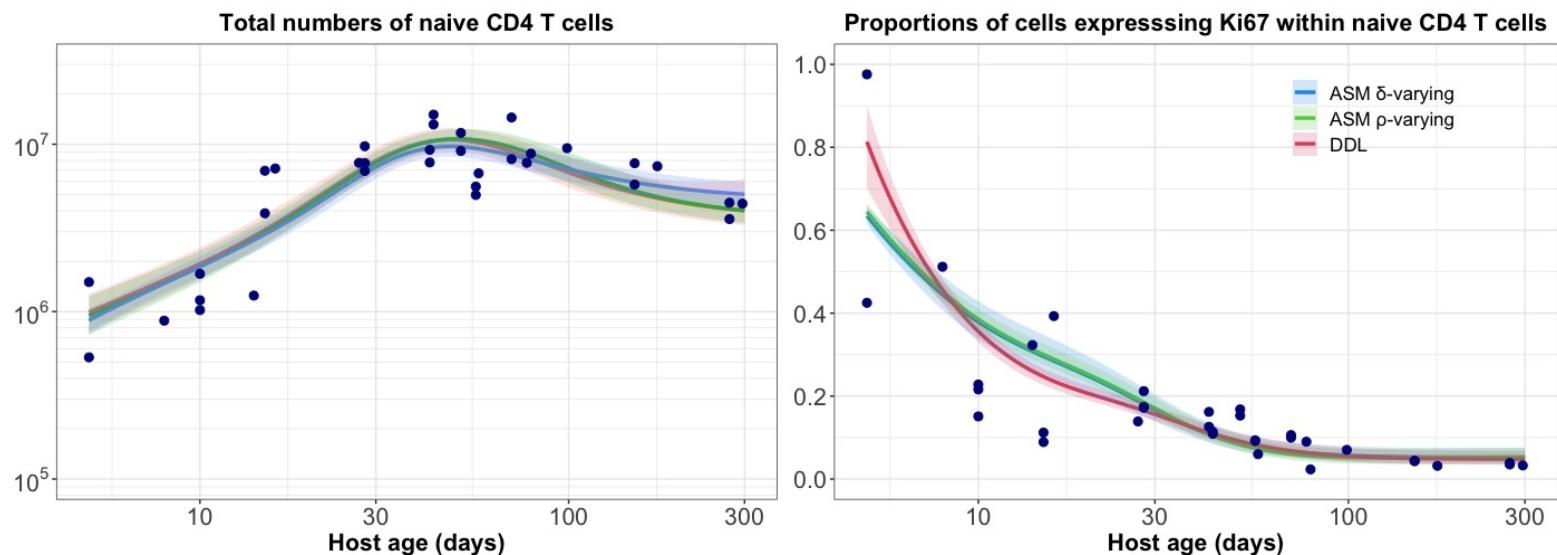
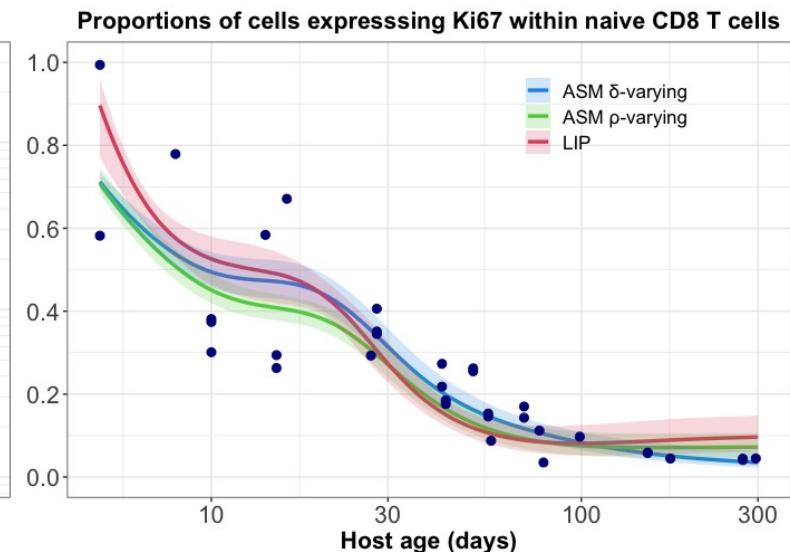
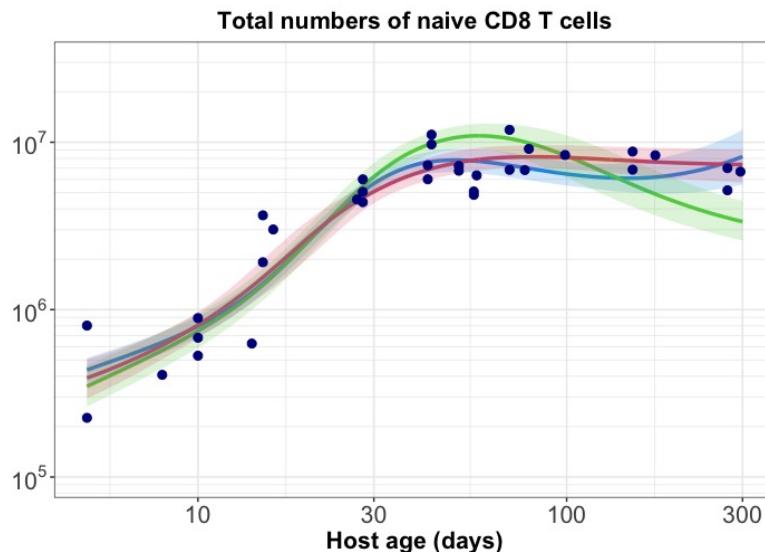


Table: Statistical analysis for CD8 data

Model	$\Delta\text{LooIC}$	Akaike weight %
Neutral	13.14	0.10
<b>LIP</b>	<b>0.00</b>	<b>70.67</b>
Density-dependent loss	5.48	4.57
RTE	11.76	0.20
RTE maturation linked division	14.43	0.05
<b>ASM <math>\delta</math>-varying with cell-age</b>	<b>2.14</b>	<b>24.25</b>
ASM $\rho$ -varying with cell-age	12.22	0.16



## Summary from the ontogeny data

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- Naive CD4 and CD8 T cells follow distinct dynamics early on in life.  
↳ Agrees with the [thymic export profile](#) of CD4 and CD8 RTE.
- Equivocal support for age-structured and density-dependence models.

## Whats Next?

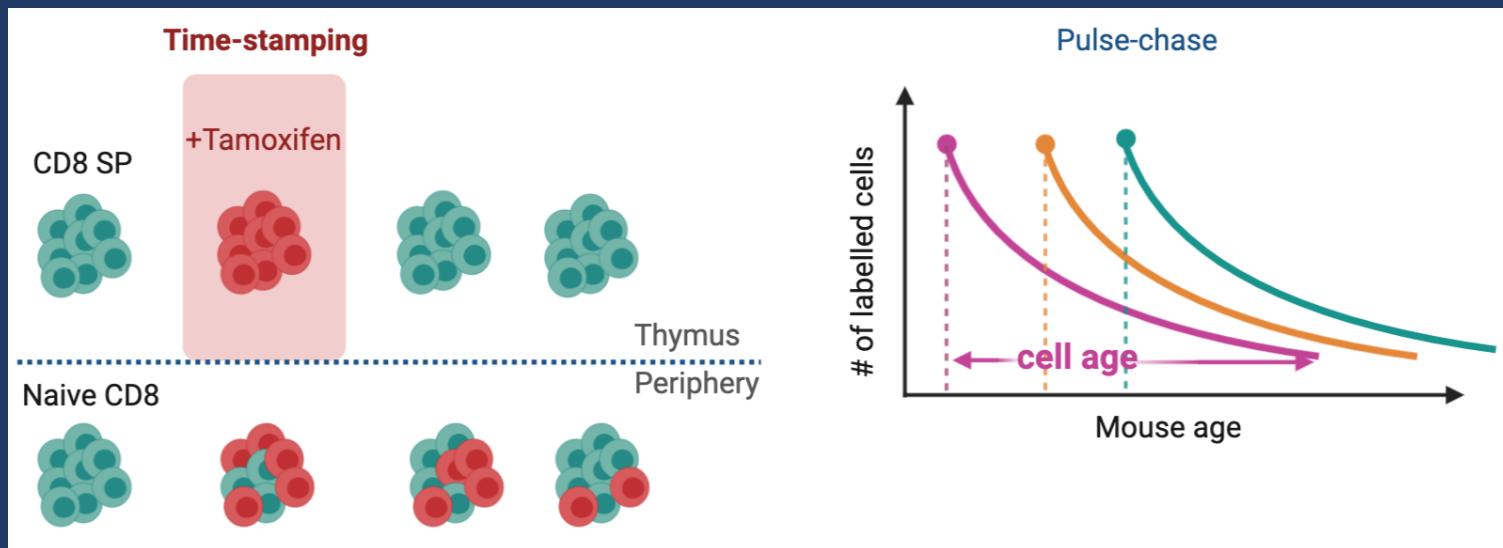
- Measuring Ki67 expression in [RAG-GFP](#) neonatal mice.  
↳ Information on the extent of cell division in young naive T cells early on in life.
- Stochastic models (Agent-based) to predict [RAG-GFP](#) data.  
↳ Simulating TCR repertoire dynamics using the parameter estimates from the best-fit model.

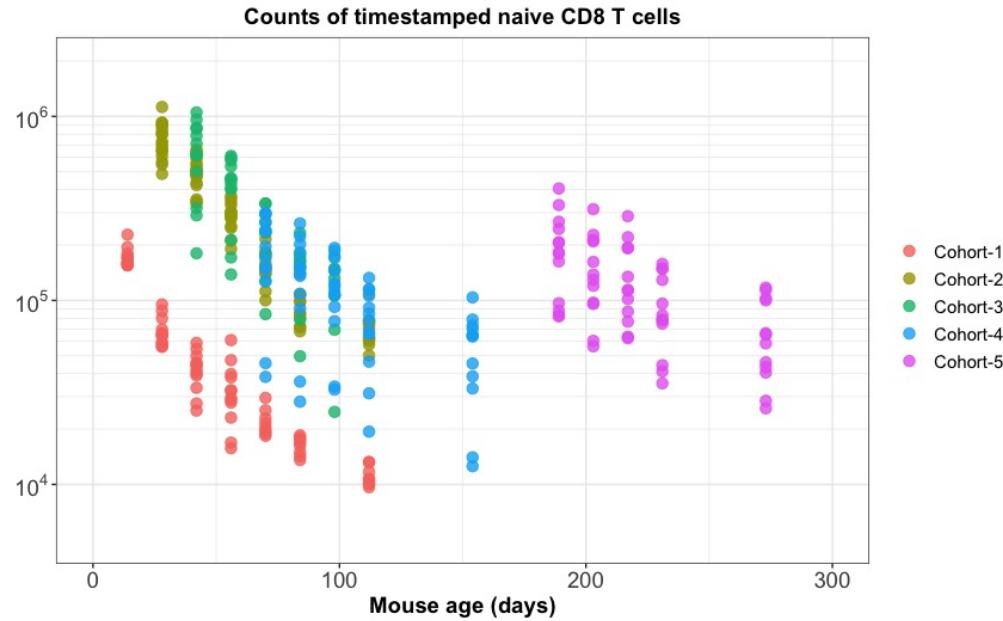
# Fate mapping reveals the age structure of the peripheral T cell compartment

Arnold Reynaldi<sup>a</sup>, Norah L. Smith<sup>b</sup>, Timothy E. Schlub<sup>c</sup>, Cybelle Tabilas<sup>b</sup>, Vanessa Venturi<sup>a</sup>, Brian D. Rudd<sup>b,1</sup>, and Miles P. Davenport<sup>a,1</sup>

<sup>a</sup>Kirby Institute for Infection and Immunity, University of New South Wales, Sydney, NSW 2052, Australia; <sup>b</sup>Department of Microbiology and Immunology, Cornell University, Ithaca, NY 14853; and <sup>c</sup>Sydney School of Public Health, Sydney University, Sydney, NSW 2006, Australia

Edited by Rafi Ahmed, Emory University, Atlanta, GA, and approved January 18, 2019 (received for review July 12, 2018)





## Need for Hierarchical modelling:

- Pooling information among cohorts while fitting models.
- Within cohorts repeat sampling on individual mice — Pooling information among mice.

# Hierarchical age-structured model

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No information on cell division in this system

↪ We track cell-age in this system using,

$$\frac{\partial N}{\partial t} + \frac{\partial N}{\partial a} = -\lambda(a) N(t, a),$$

where, net loss rate  $\rightarrow \lambda = \delta - \rho \Rightarrow \lambda(a) = \lambda_0 e^{-\gamma a}$

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## Model:

$$y_i \sim \text{normal}(\mu_i, \sigma) \quad [\text{likelihood}]$$

$$\mu_i = f(\text{time}_i, N_0, \lambda_0, \gamma) \quad [\text{model}]$$

$$\gamma \sim \text{normal}(0.05, 0.01) \quad [\gamma \text{ prior}]$$

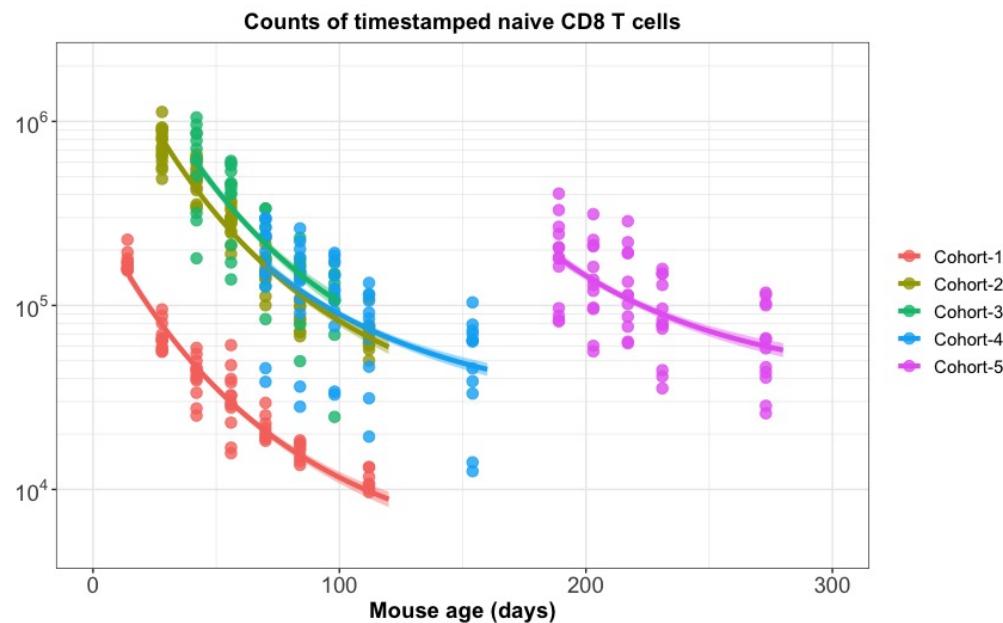
Hyper parameters

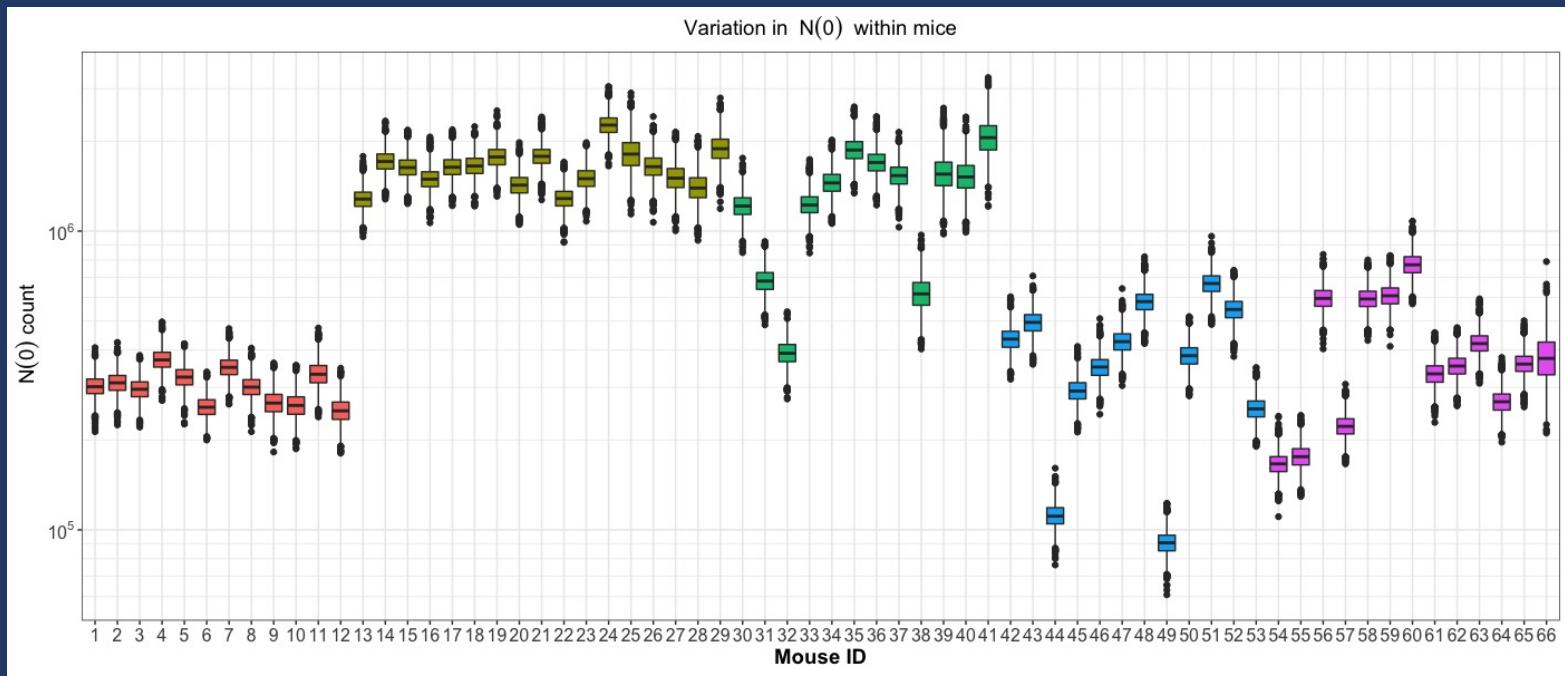
$$N_0 \sim \text{normal}(\mu_N, \sigma_N) \quad [\text{Initial counts}]$$

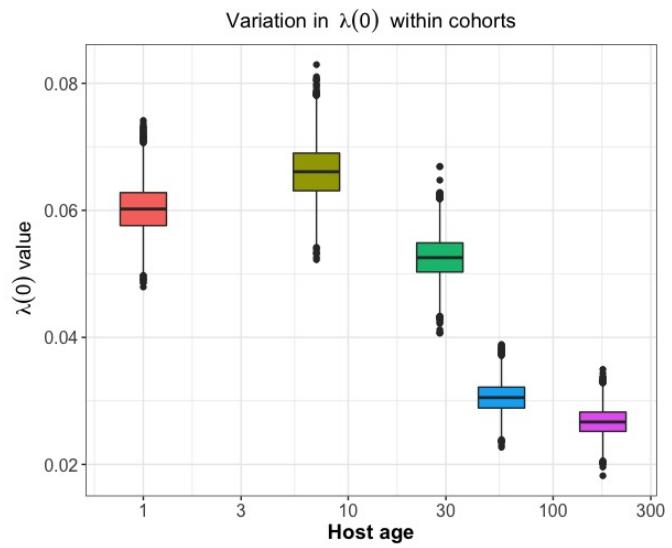
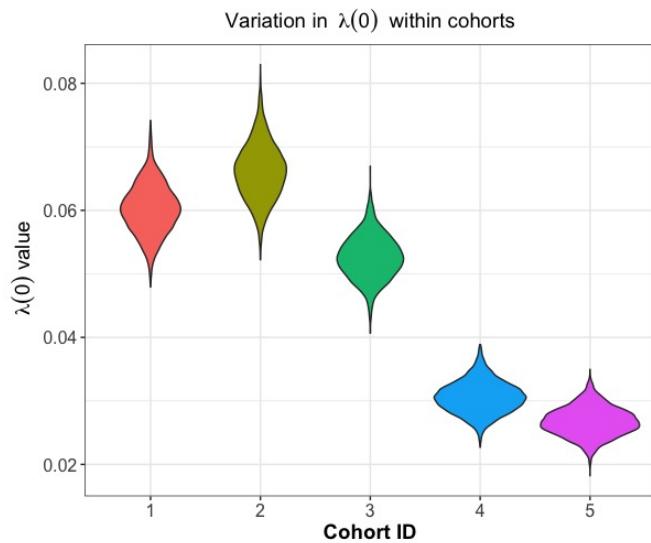
$$\lambda_0 \sim \text{normal}(\mu_\lambda, \sigma_\lambda) \quad [\text{Loss rate at age=0}]$$

## Comparison of the ASM hierarchical models

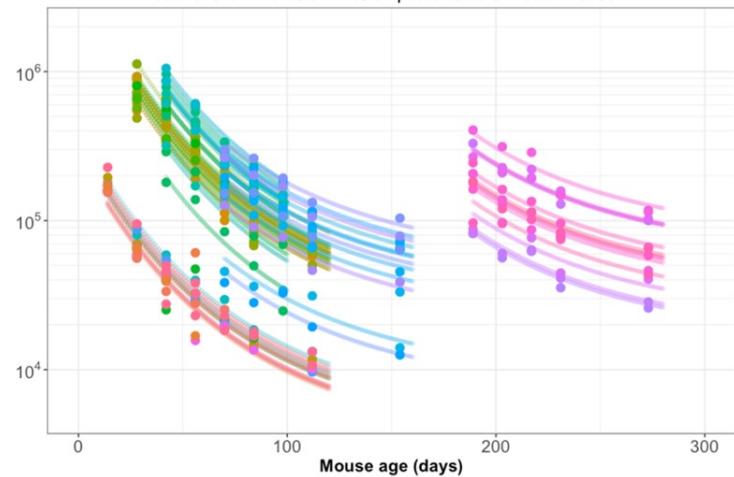
	$\Delta\text{LooIC}$	Akaike weight %
N0: mice and $\lambda$ : cohort	0.00	100
N0: mice and $\lambda$ : mice	73.06	0
N0: cohort and $\lambda$ : mice	313.94	0
N0: mice	316.71	0







Predictions for kinetics of timestamped CD8 cells in each mouse



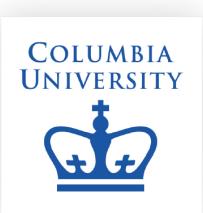
## Summary from the timestamp data

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- The hierarchical model reveals cohort-specific effects on the net loss rate  $\lambda$  of naive T cells.
- The major distinction in cohorts is the **age of the host** when naive T cells were stamped.  
↳ Possible host-age effects in naive T cell maintenance?

## Whats Next?

- Adopt density-dependence model to fit on the timestamp data.
- Include host-age dependence in ASM and refit on the ontogeny data.



**Andrew Yates**  
Sinead Morris

Maria Nowicka  
Chloé Pasin

**Benedict Seddon**  
Thea Hogan

Melissa Verheijen



# Thanks!



The models are coded in **Stan** programming language, are compiled using **CMDSTAN** interface and analysed in R using **Rstan** package.