Early fate decisions and inheritance in T cell responses informed by *in vitro* continuous imaging

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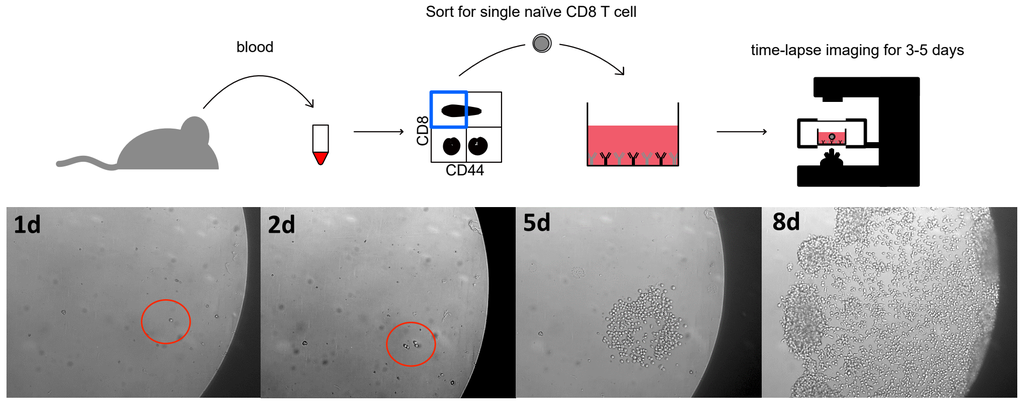
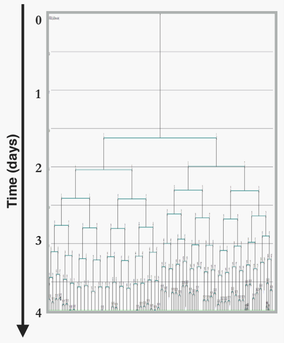
A **Experimental setup: Continuous stimulation of T cells *in vitro***



Experiments done by Marten Plambeck

B **Sample tree**



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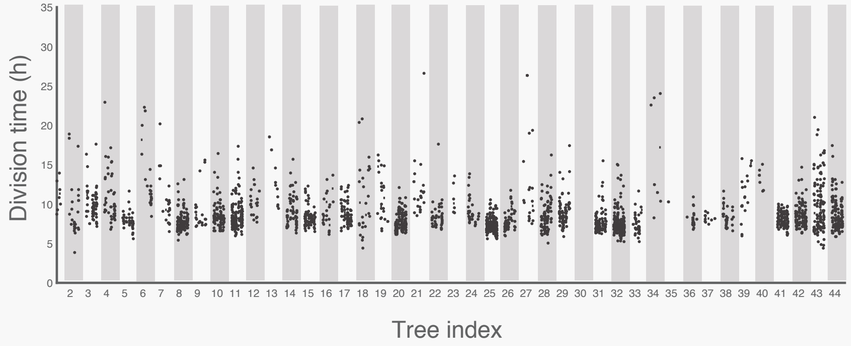

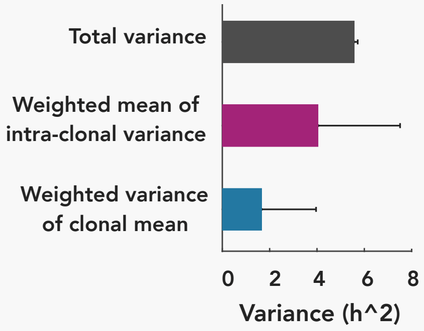

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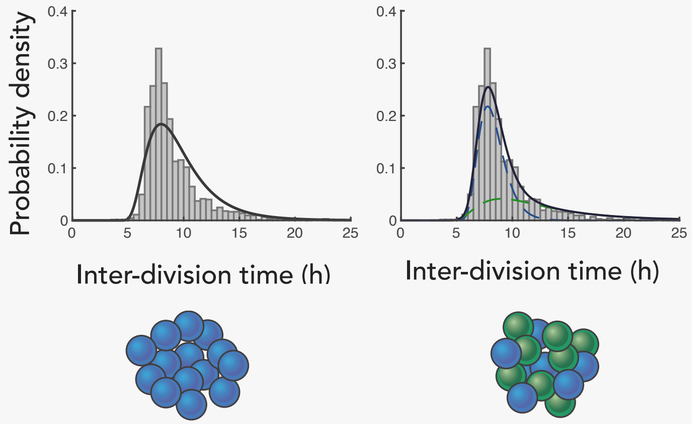

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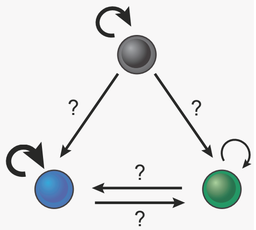
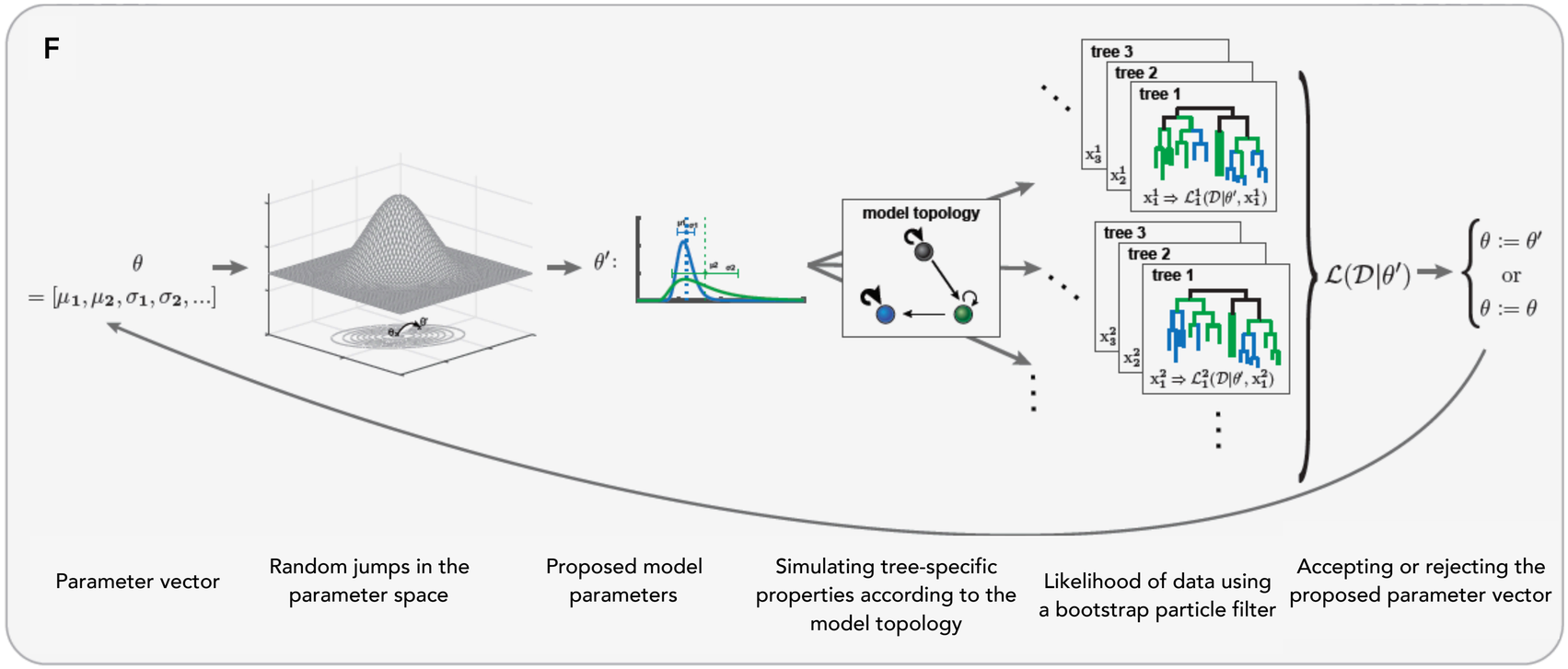
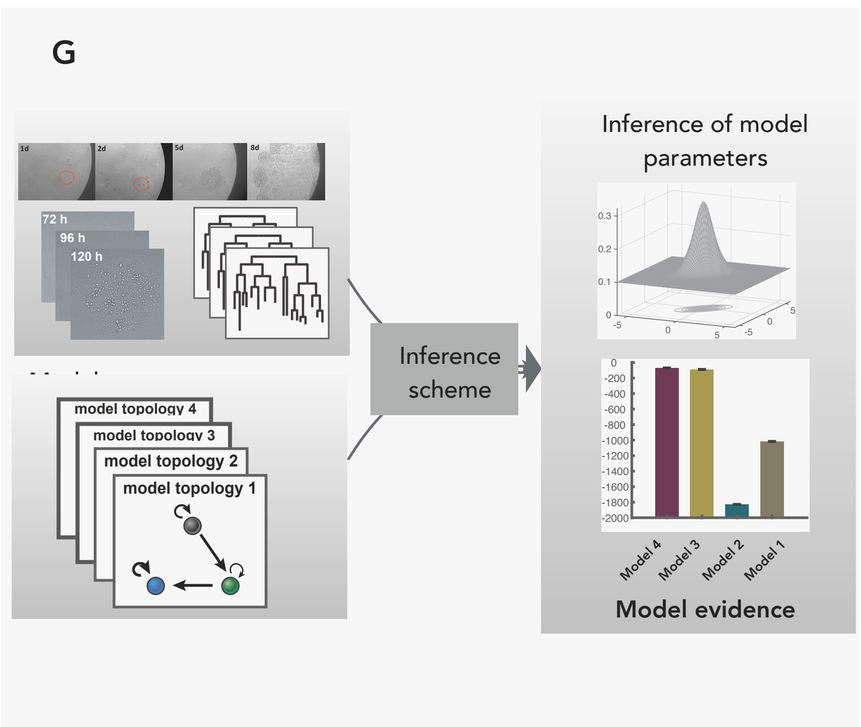


- **A**: Individual T cells under continuous stimulation expand heterogeneously *in vitro* and give rise to single-cell-derived progenies.
- **B**: Live cell imaging yield such tree-structured data where every branch in the tree represents a cell in the developmental history of the progeny.
- **C**: The inter-division times of individual cells are variable within a tree as well as across different family trees.
- **D**: The overall distribution of inter-division times (grey histograms) does not reveal whether all cells follow the same dynamics or whether there are sub-populations with distinct inter-division time statistics.
- **E**: To infer the existence of distinct sub-populations and the corresponding diversification pathways mathematical modelling is needed.

Bayesian framework for model inference based on tree-structured data

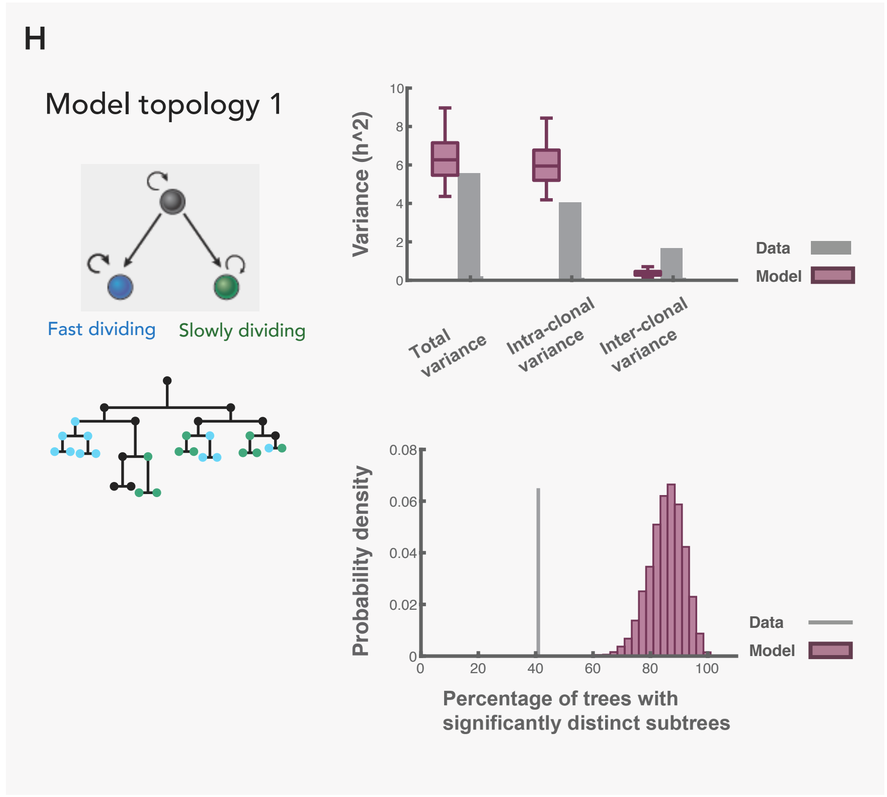


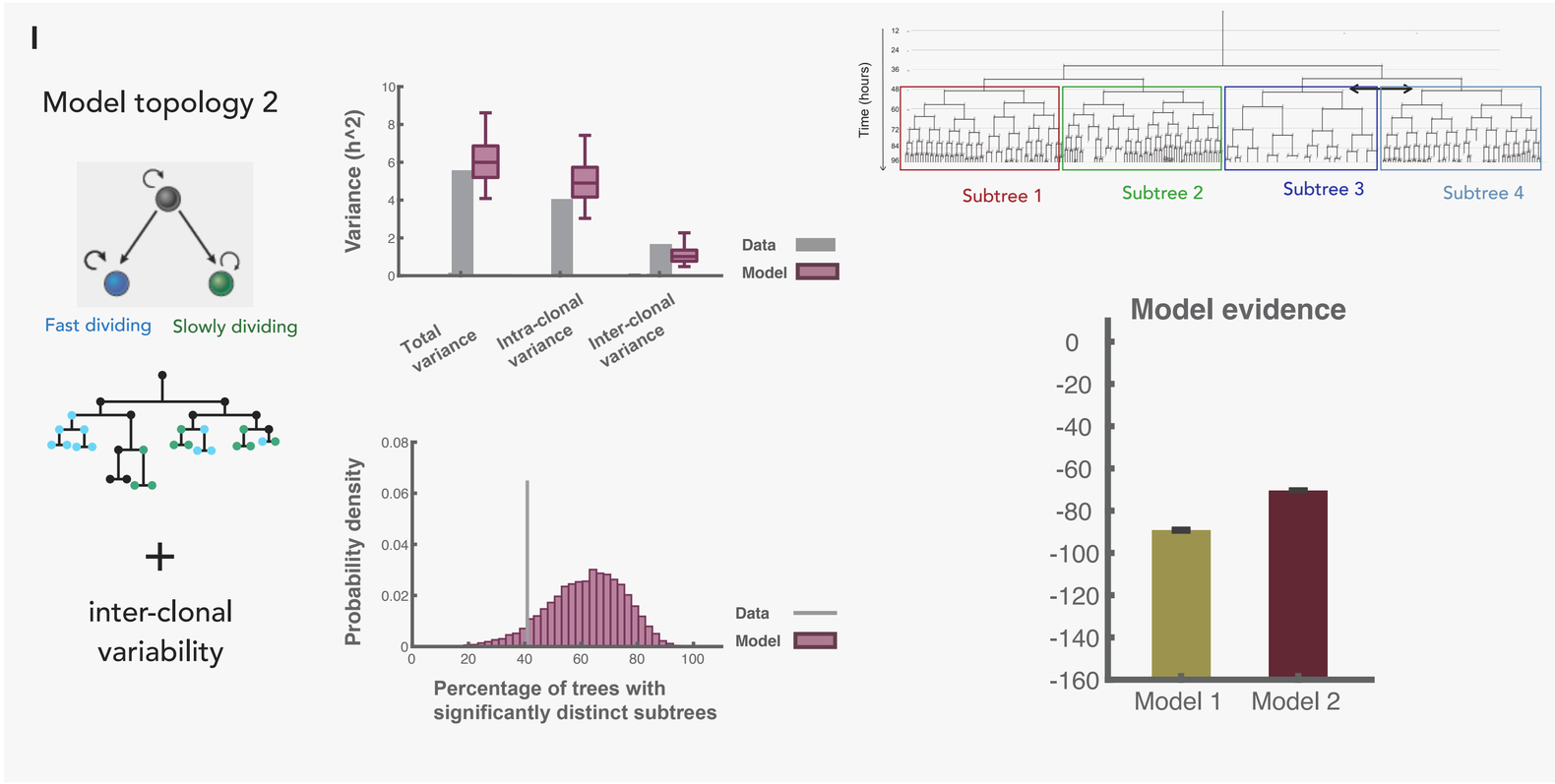


- Live cell imaging provides a rich source of information as the history of temporal evolution of the single cell-derived progenies is recorded. In addition, the tree structure is preserved which is especially important if certain properties, e.g. inter-division times, are inherited along the tree.
- **F**: We developed a Bayesian inference framework tailored for tree-structured data to analyse the data provided by live cell imaging.
- **G**: With this inference scheme, we can compare various hypotheses about the existence of subsets with distinct inter-division times, and infer the model parameters associated with different model hypotheses/topologies.

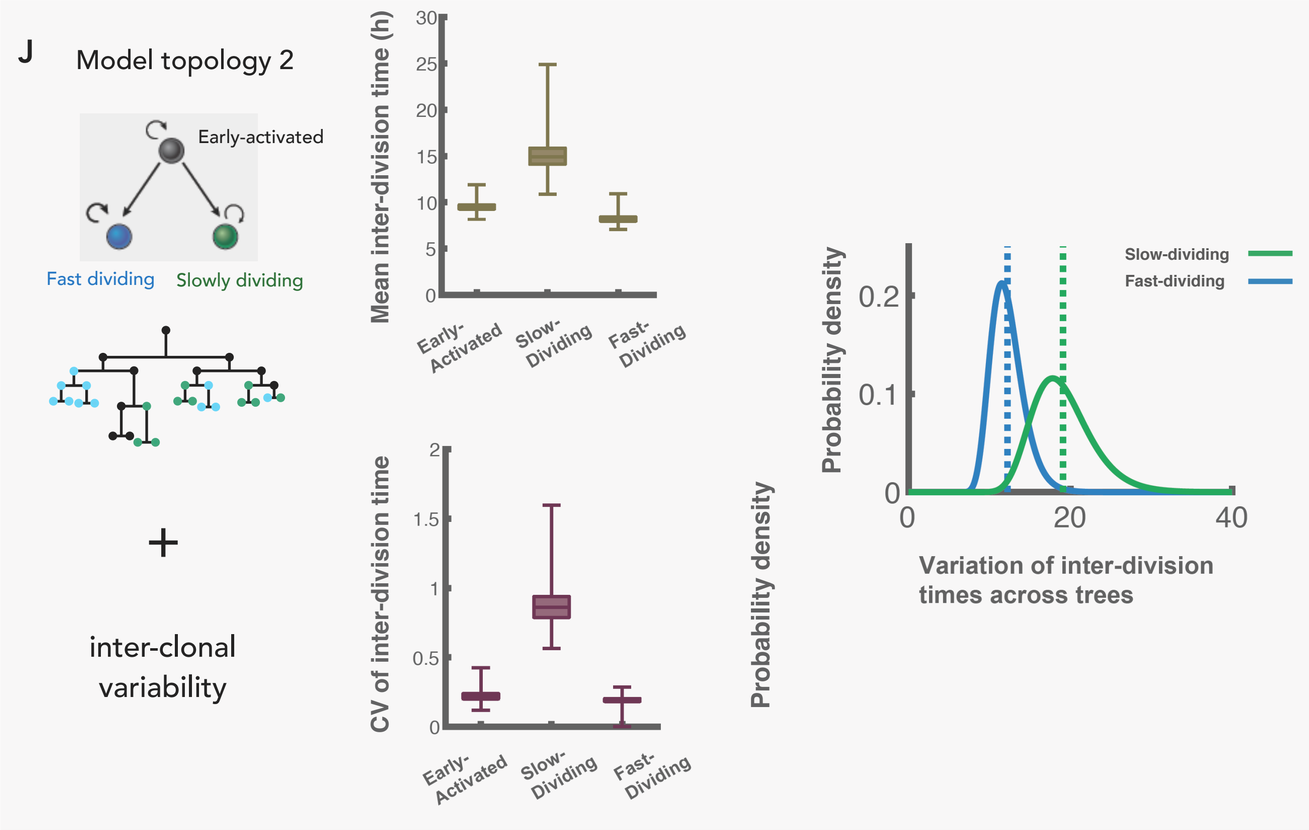
Two distinct inter-division times are inherited in the *in-vitro* expansion of T-cells

Inter-clonal variability is needed to explain the observed statistics of the data





- **H**: Extensive model comparison supports the existence of two subsets with distinct inter-division times. The cells diversify into these two subsets after an initial fast dividing state.
- **I**: In addition, variability across family trees is required to explain the statistical features of the data. Correspondingly, the model including inter-clonal variability is superior in terms of model evidence.



- **J**: mathematical modelling predicts:
  - In the early expansion phase of T cells *in vitro* a bifurcating diversification pathway emerges after transitioning through an early state of fast and semi-synchronous divisions.
  - The two emerging subsets possess distinct inter-division time statistics with an average difference of approximately 5 hours.
  - The slow-dividing cells are more variable in terms of their inter-division times compared to the fast-dividing cells.

Summary and outlook:

- Mathematical modelling based on live cell imaging helps to reveal distinct subsets in populations of T cells without any direct phenotype observation
- In the study of T-cells expanded *in vitro*, we could show:
  - the existence of two subsets with distinct inter-division times
  - inheritability of division times along the family tree
  - distinct inter-division times co-exist within a tree
- Under continuous stimulation, a difference of ~ 5 hours is predicted between the inter-division times of the two distinct T cell subsets
- Variability across trees (inter-clonal variability) is required to explain the statistics of the T cell expansion *in vitro*
- Further diversification pathways, e.g. progressive model of differentiation, should be investigated
- The developed inference framework could be further extended to incorporate potential direct measurements of phenotype