T cell stimuli integration at the level of clones

(work with Phil Hodgkin's Lab at the
Walter and Elisa Hall Institute of Medical Research)

Ken Duffy

Hamilton Institute,
National University of Ireland Maynooth

KITP, February 2016

Acknowledgments

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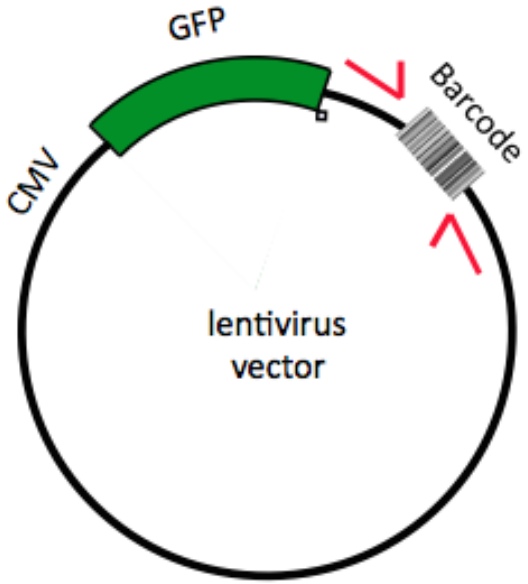
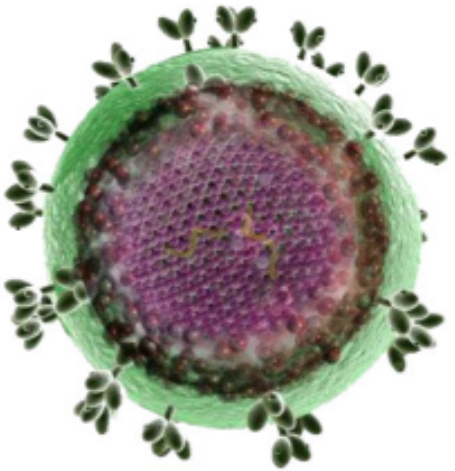
Gabrielle Belz (WEHI)
Andrew Lew (WEHI)
Mark Dowling (WEHI)

HUMAN FRONTIER SCIENCE PROGRAM
FUNDING FRONTIER RESEARCH INTO COMPLEX BIOLOGICAL SYSTEMS

What I'm not going to tell you about

Lineage tracing in vivo: cellular barcoding



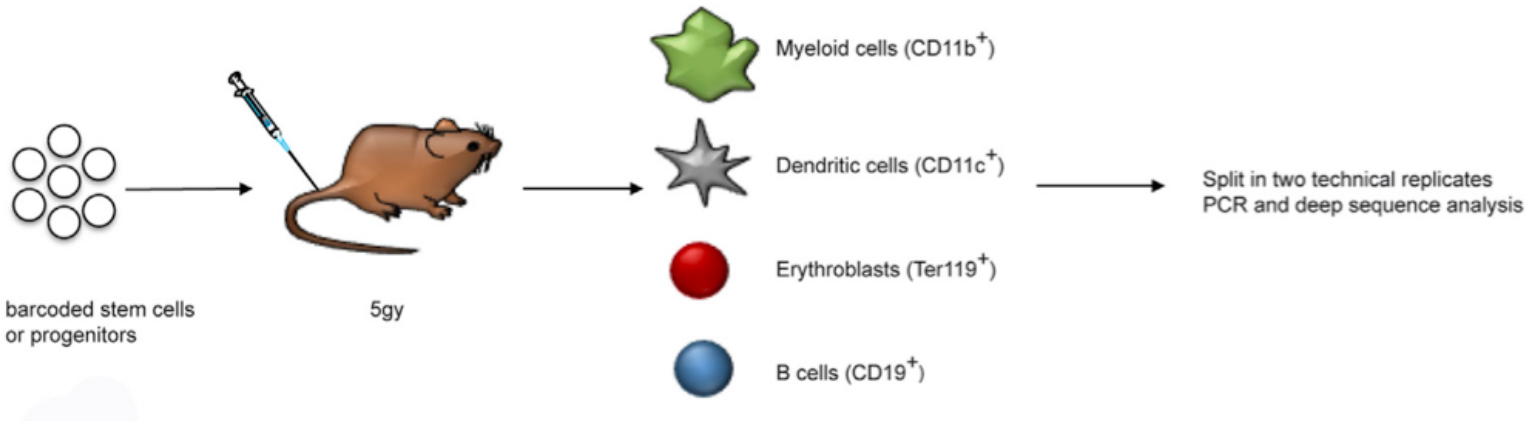


Plasmid vector **Barcode Lentivirus**

Schumacher lab.: library of 2,500 unique 100-nucleotide stretches (millions of unique 20-nucleotide stretches plus Thy-1.1 or GFP)

Golden, et al., *PNAS*, 1995. Schepers et al., *JEM*, 2008. J. van Heijst et al., *Science*, 2009. Lu et al., *Nature Biotech.*, 2011. Kreso et al., *Science*, 2013. Gerlach et al., *Science*, 2013. Naik et al., *Nature*, 2013. Perié et al., *Cell*, 2015.

A typical cellular barcoding experiment

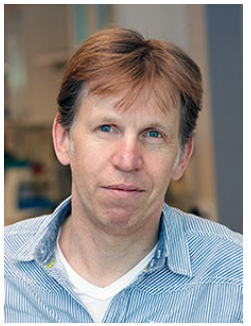


- Usually based on adoptive transfer, which oft requires perturbation.
- No dynamic information.

Perié, Duffy, Kok, De Boer & Schumacher, *Cell*, 2015.

Randomized algorithms to circumvent these drawbacks


Shalin Naik
& Stefan Glaser (WEHI)




Ton Schumacher (NKI)
& Leïla Períe (Institut Curie)

Weber, Dukes, Glaser, Naik & Duffy, "Site-specific recombinatorics: *in situ* cellular barcoding with the Cre Lox system", *arXiv:1602.02190.*

Weber, Perié & Duffy, "Inferring average generation via division-linked labeling", *J. Math. Bio.*, 2016.

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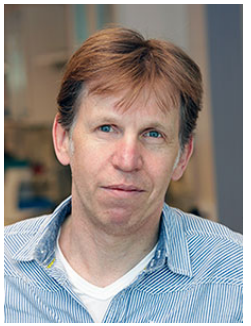


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Tom Weber

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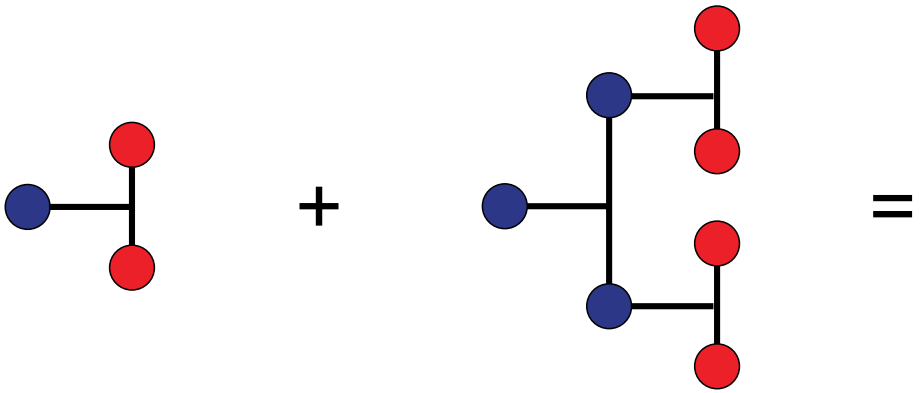
Tree addition / appending / concatenation

Concordant trees

- quiescent cells
- dividing cells



X Y

Tree addition / appending / concatenation

Concordant trees

- quiescent cells
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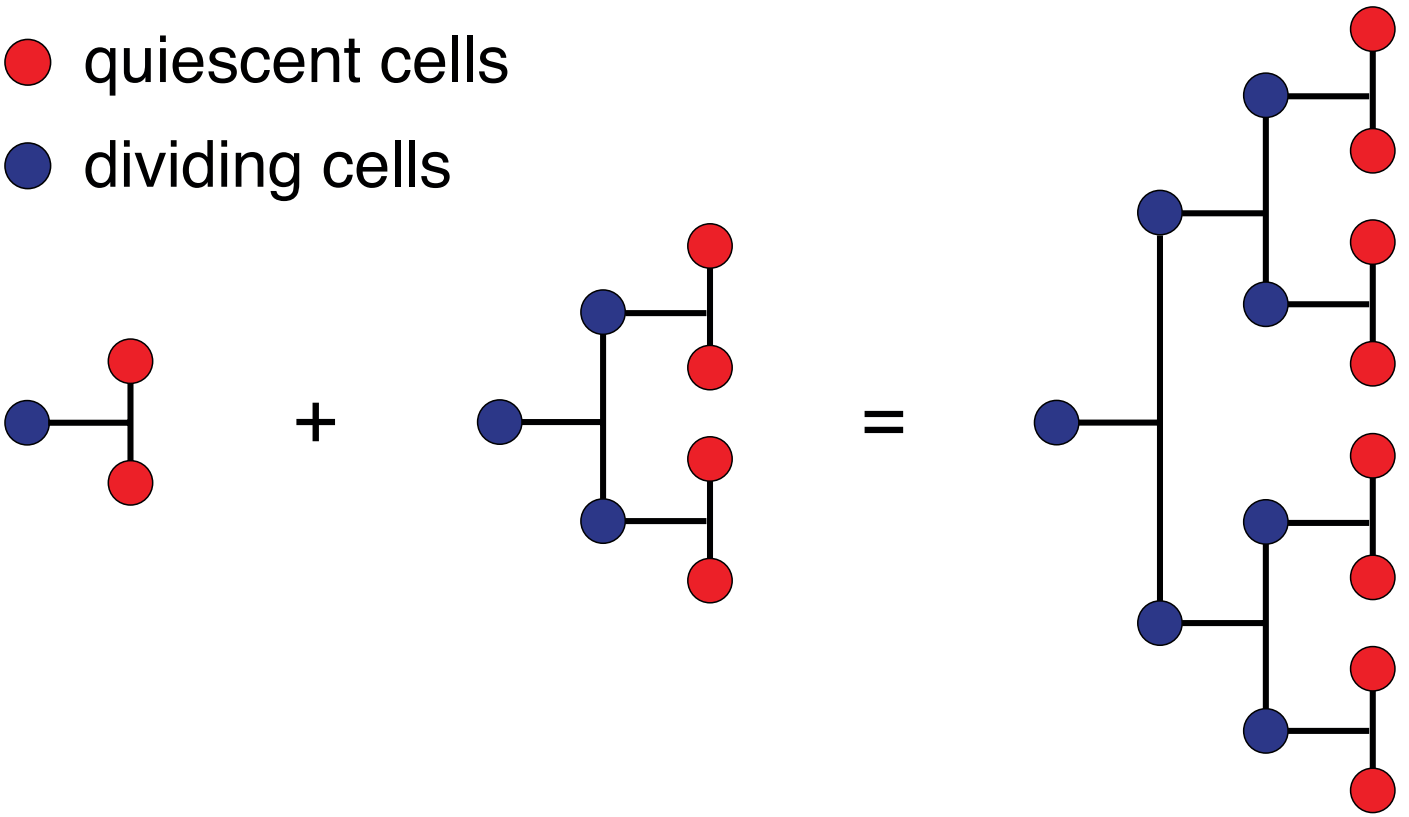
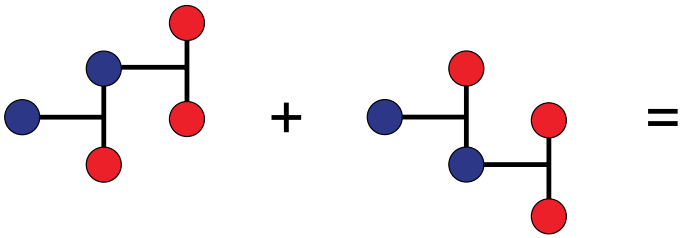


Tree addition / appending / concatenation

Non-concordant trees



X Y

Tree addition / appending / concatenation

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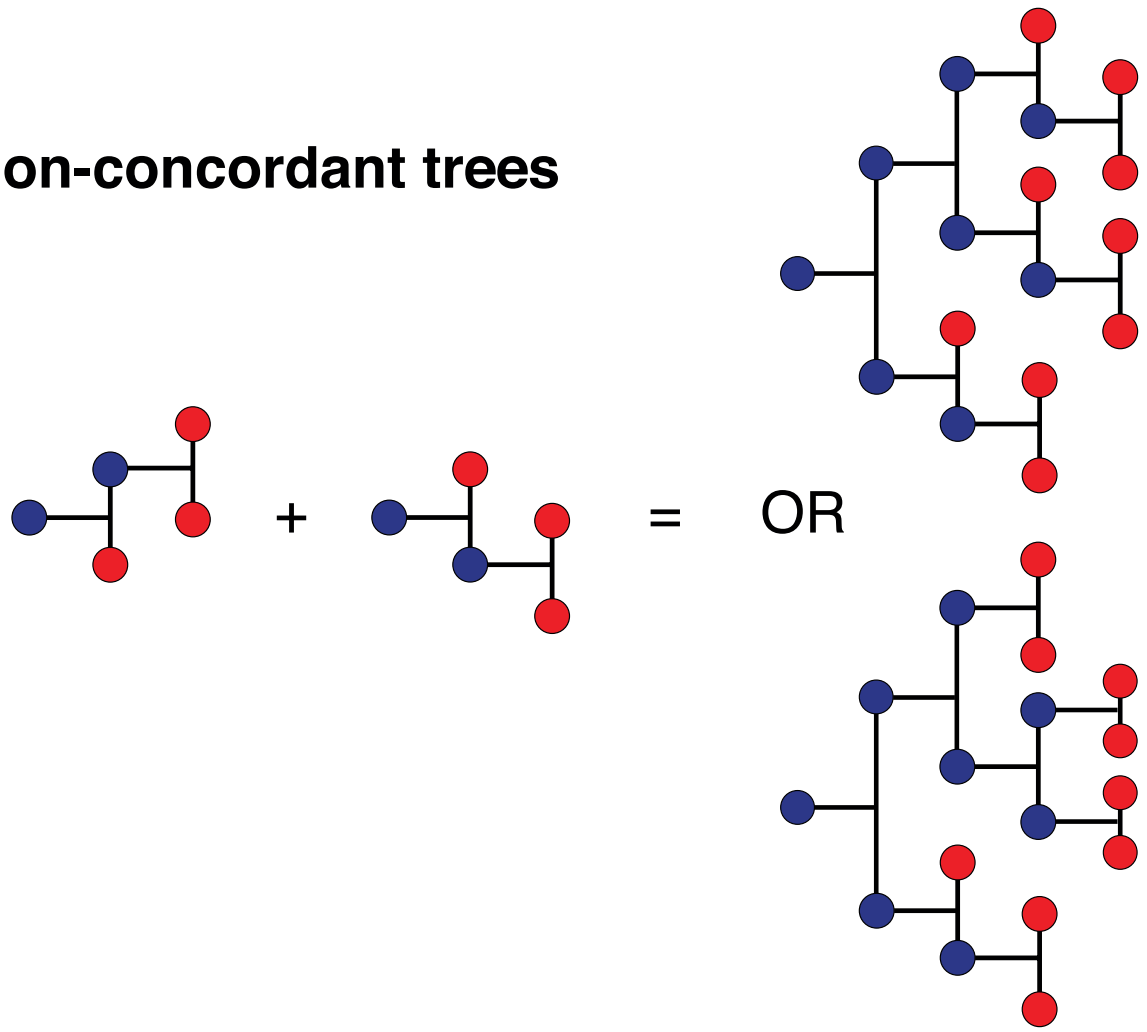
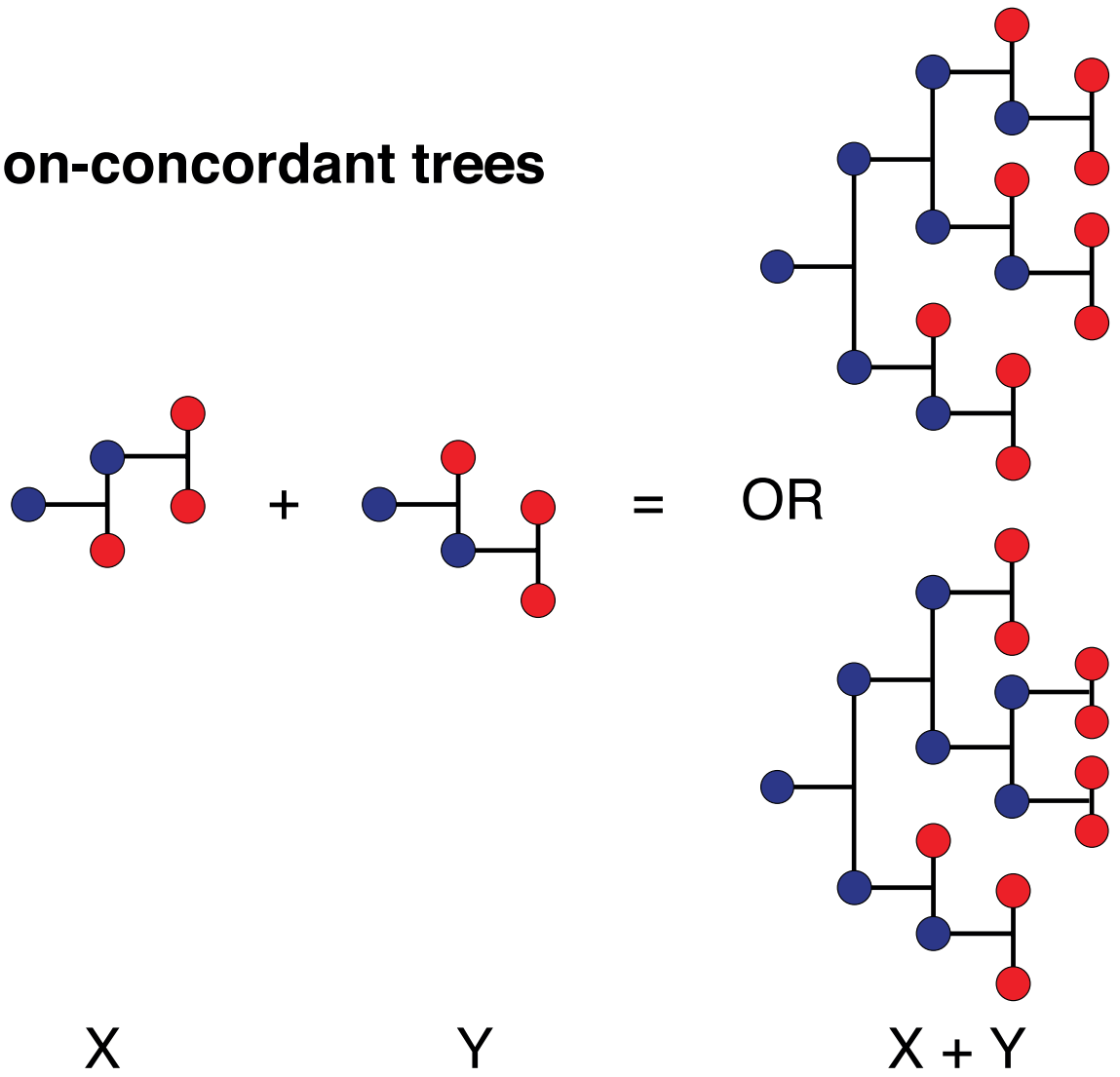


Tree addition / appending / concatenation

Non-concordant trees



Number of quiescent cells in each generation of summed tree?

(0 0 1 4 4).

Coincidence?

Two signal theory

Activation rules: the two-signal theories of immune activation

Alan G. Baxter and Philip D. Hodgkin

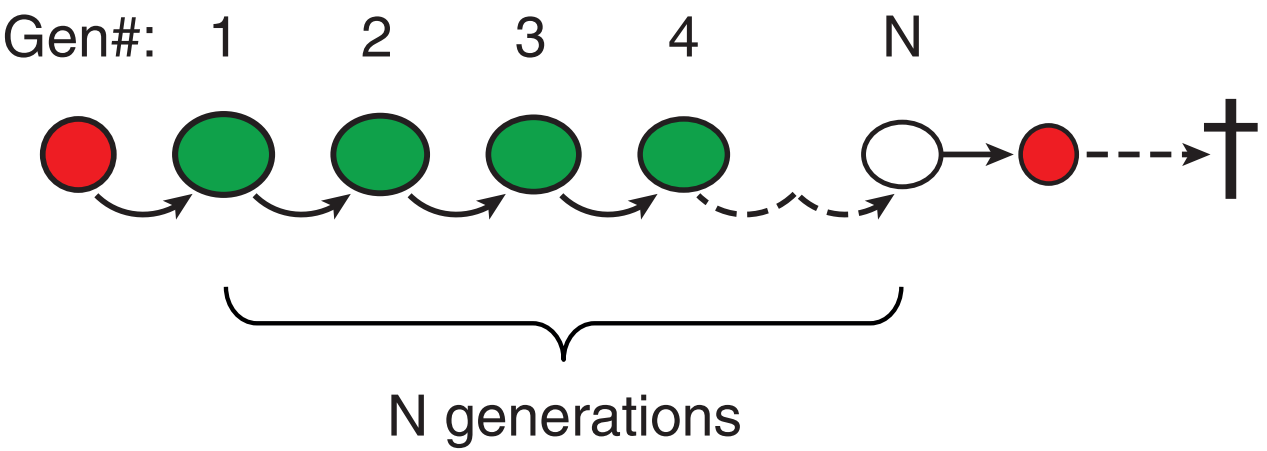
Baxter & Hodgkin, *Nature Reviews Immunology*, 2002.

Two signal theory

Two-signal theories of lymphocyte activation have evolved considerably over the past 35 years. In this article, we examine the contemporary experimental observations and theoretical concerns that have helped to forge the most influential variants of the theory. We also propose that more-rigorous quantitative methods are required to sustain theoretical development in the future.

Baxter & Hodgkin, *Nature Reviews Immunology*, 2002.

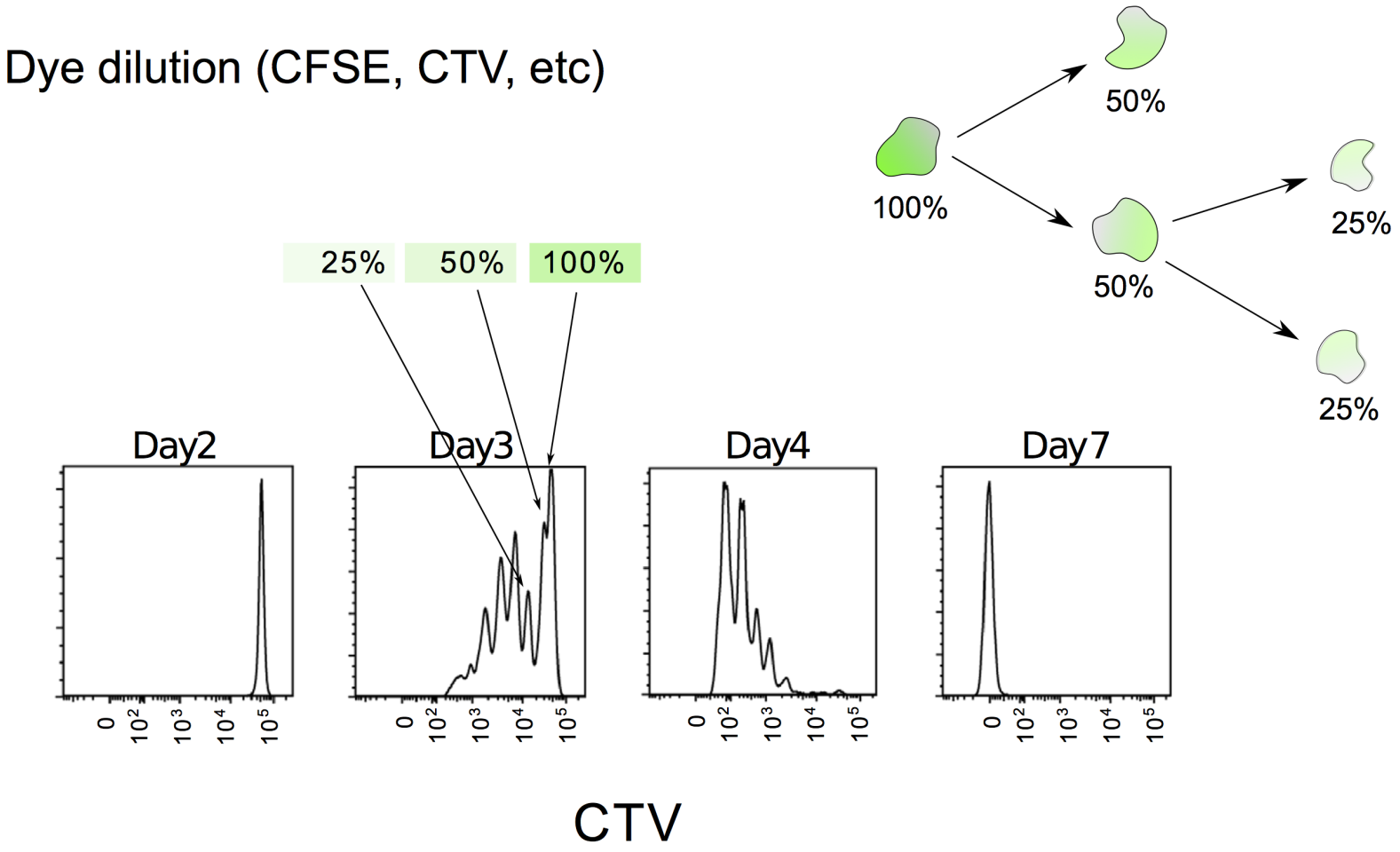
A quantitative look at signals



N = TCR (1) + costimulation (2) + cytokines (3)

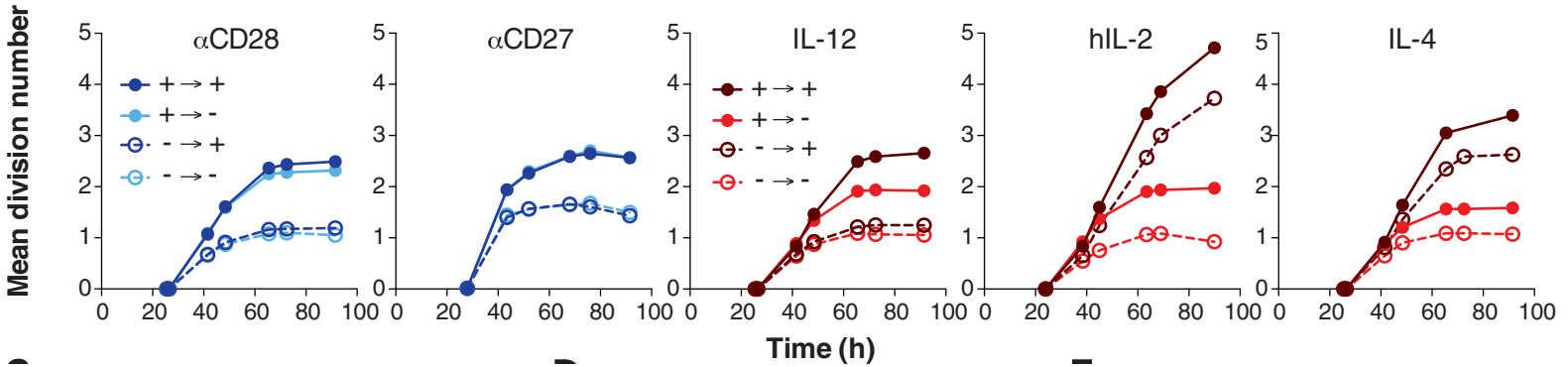
Marchingo, Kan, Sutherland, Duffy, Wellard, Belz, Lew, Dowling, Heinzel & Hodgkin, *Science*, 2014.

Heterogeneous response in homogeneous conditions



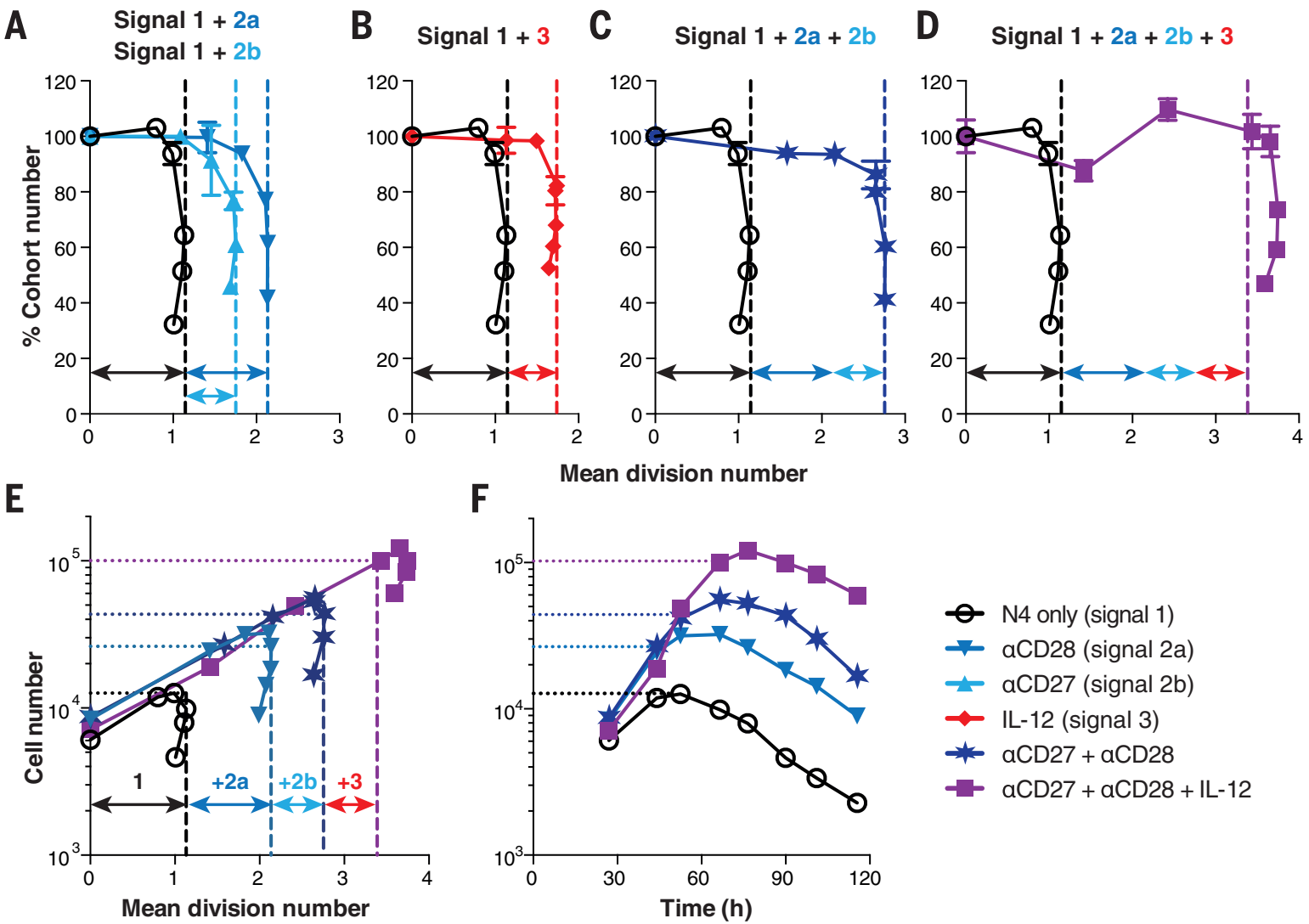
Adapted from Kinjyo et al., *Nature Communications*, 2015.

Signal impact



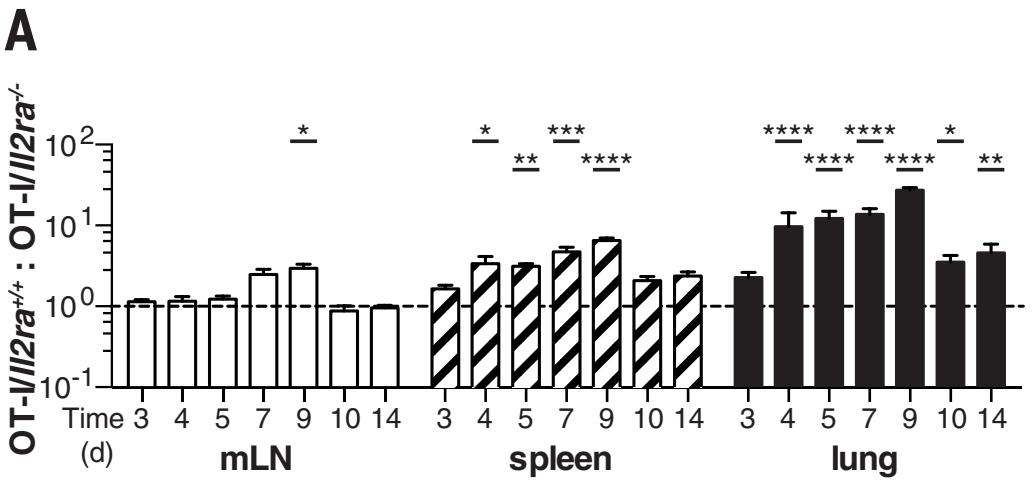
Marchingo et al., *Science*, 2014.

A quantitative look at signal integration



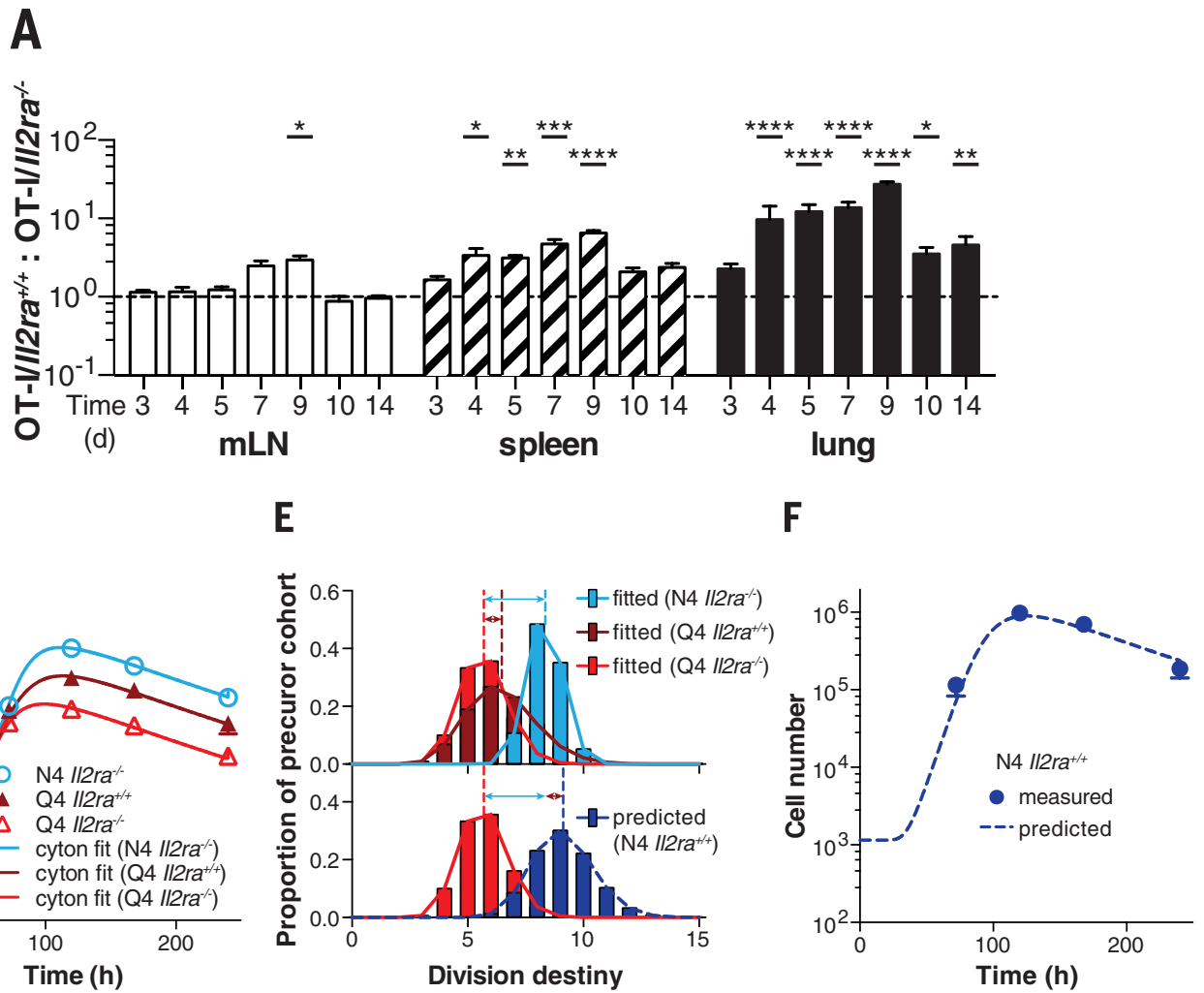
Marchingo et al., *Science*, 2014.

A quantitative look at signal theory in vivo



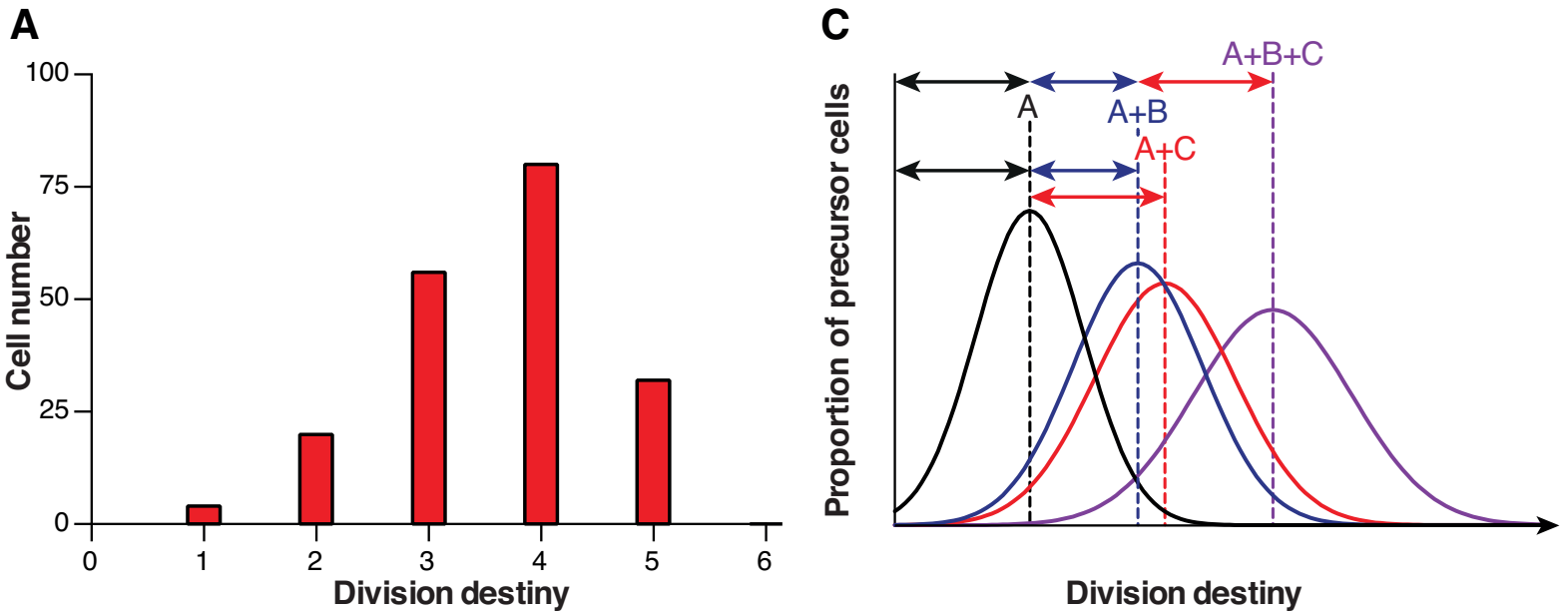
Marchingo et al., *Science*, 2014.

A quantitative look at signal theory in vivo



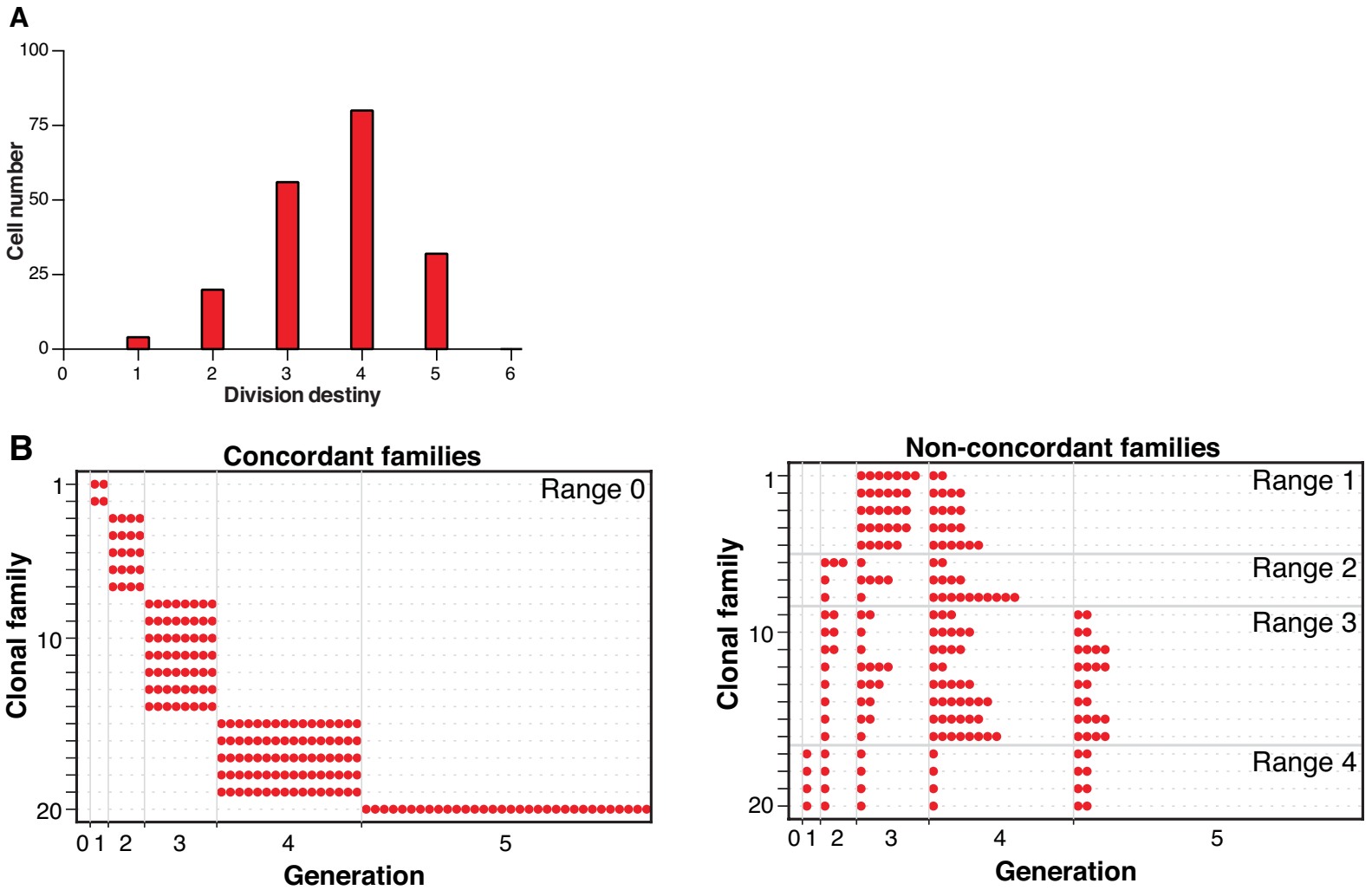
Marchingo et al., *Science*, 2014.

Signal integration & families

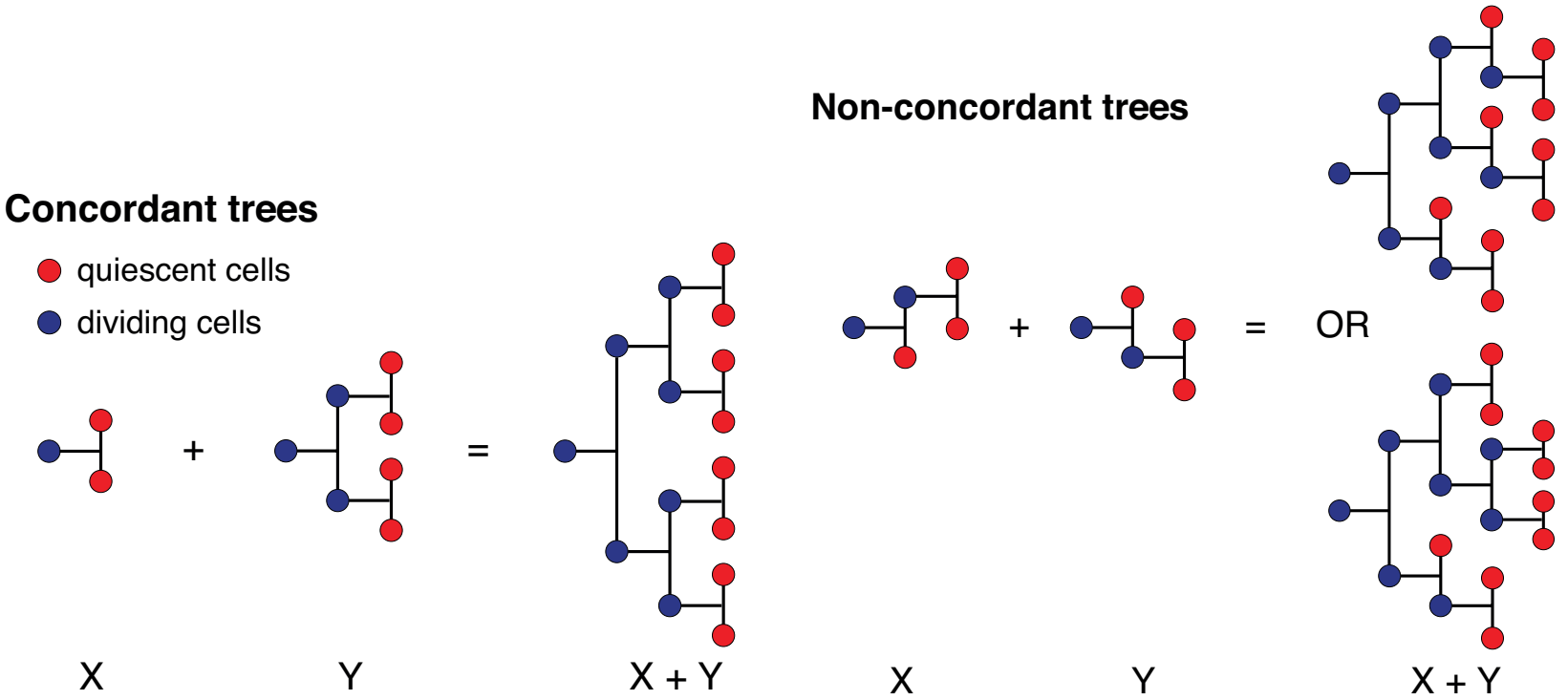


Marchingo, Prevedello, Kan, Heinzel, Hodgkin & Duffy, *in prep.*, 2016.

Concordance or discordance



Tree addition / appending / concatenation



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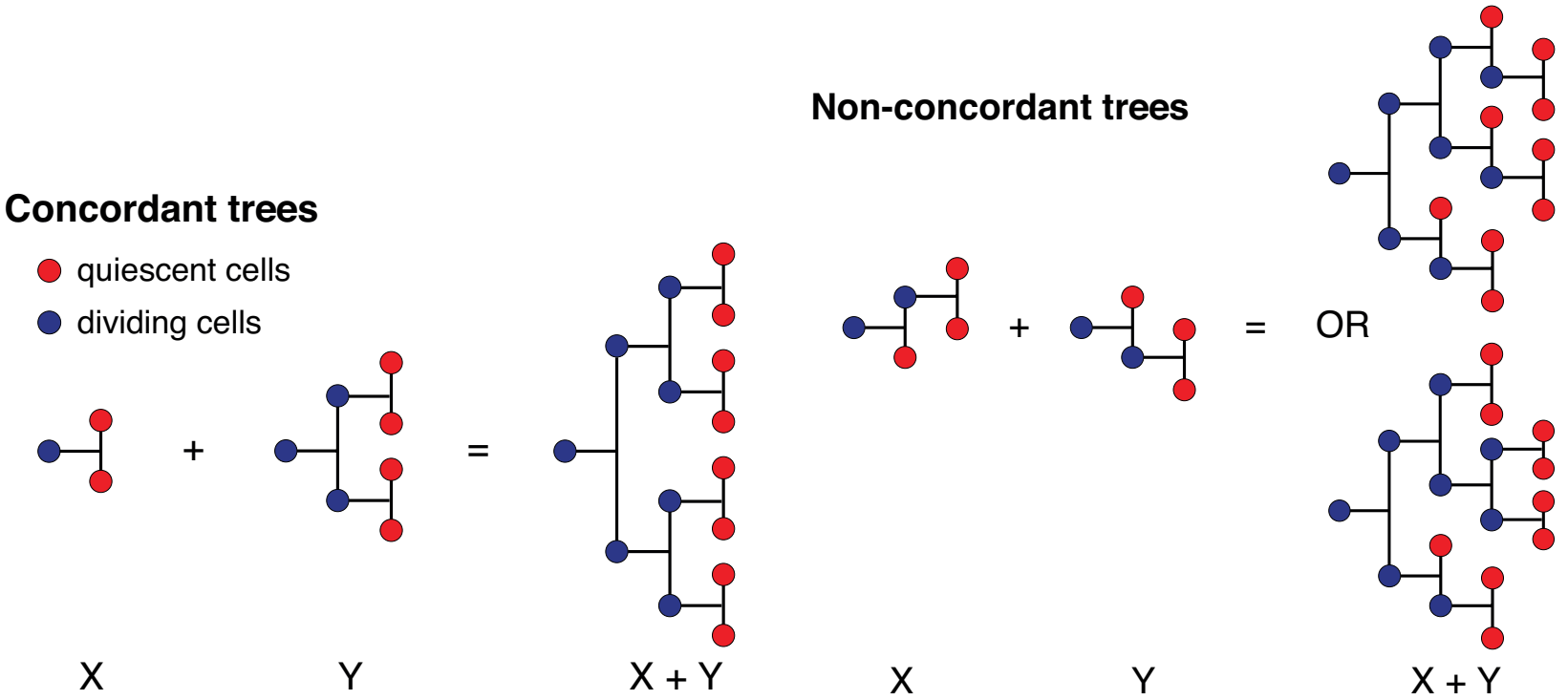


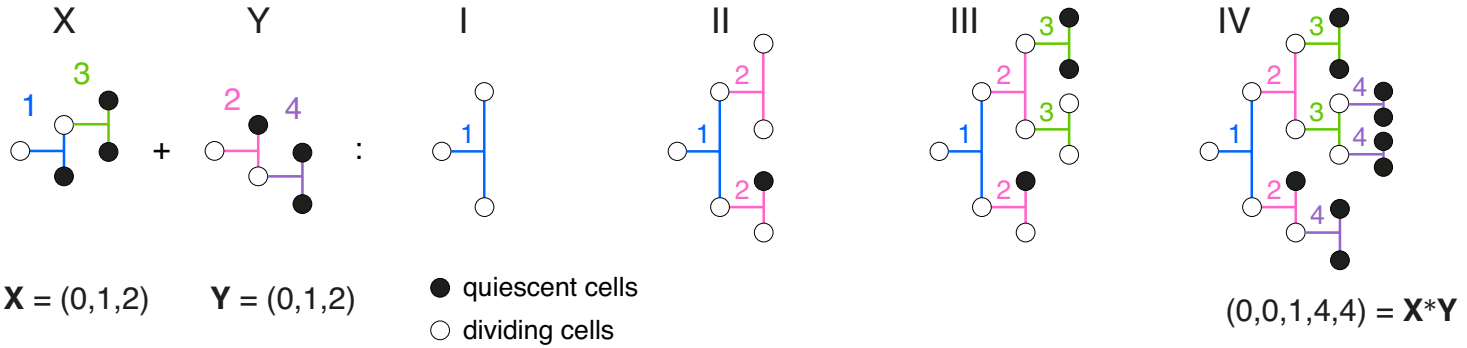
If X_i = number of quiescent cells in gen i, then the number of quiescent cells in each generation is

$$(X * Y)_i := \sum_j X_j Y_{i-j}.$$

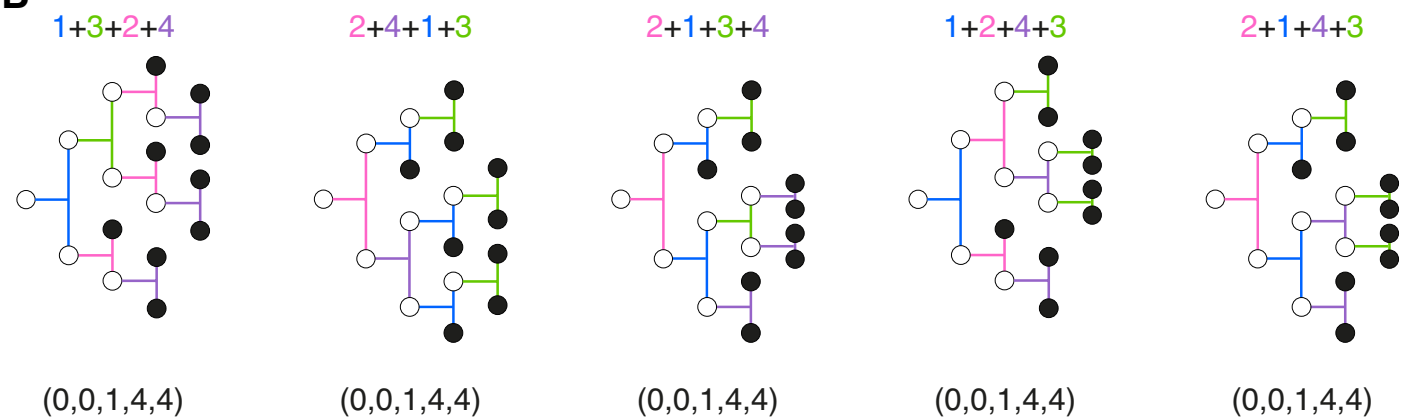
Tree addition / appending / concatenation

A

Example of adding procedure: 1+2+3+4



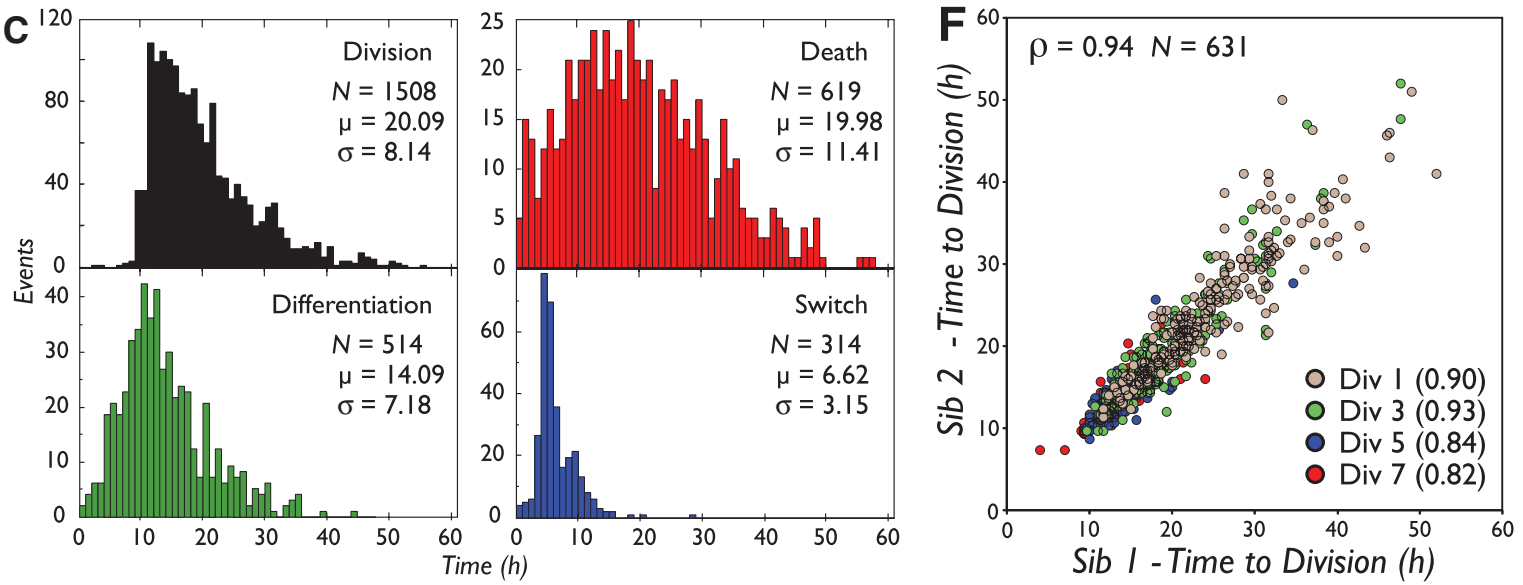
B



Number of cells quiescent cells in each generation of summed tree:

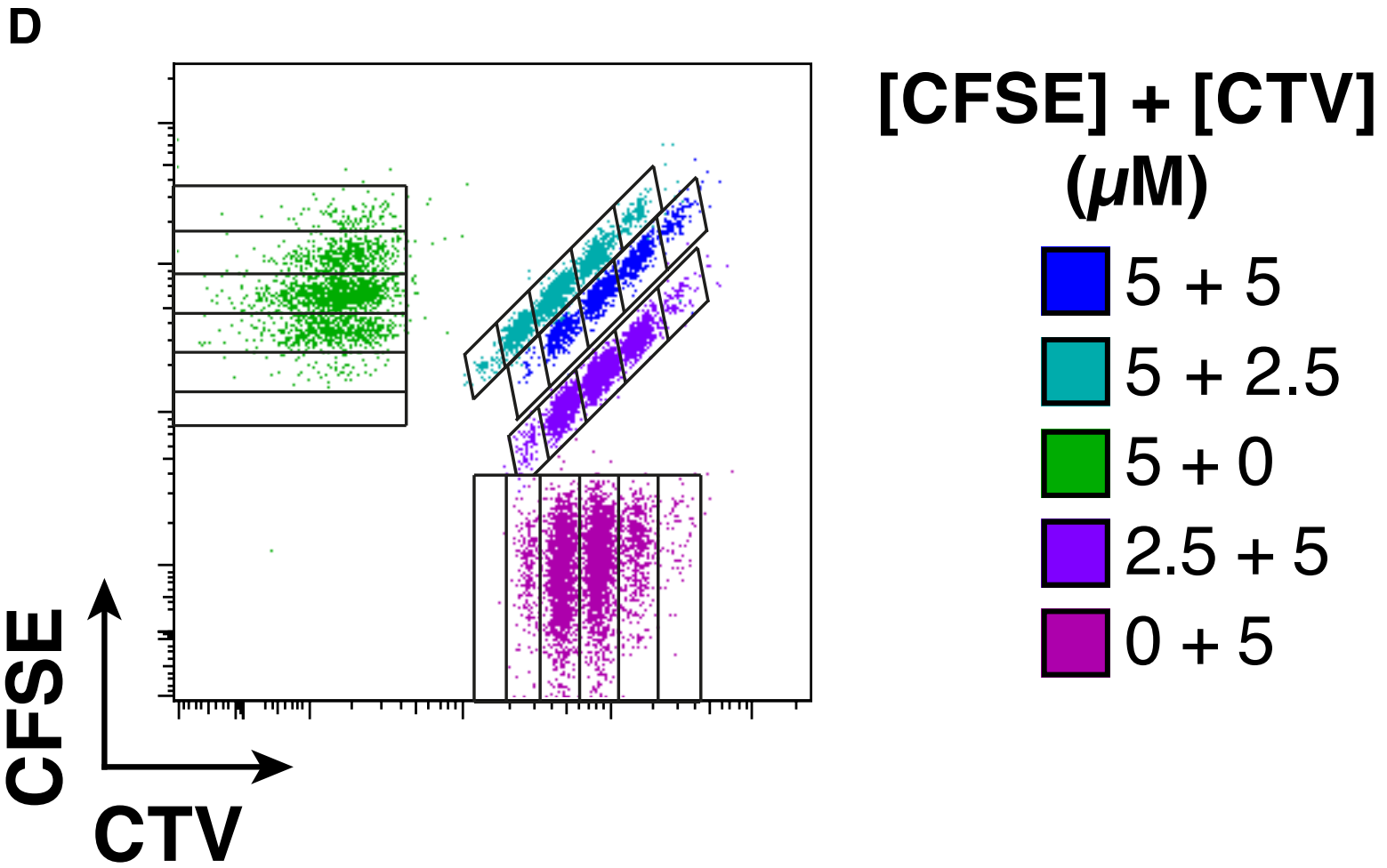
$$(X * Y)_i := \sum_j X_j Y_{i-j}.$$

Microscopy, naïve B cells, anti-CD40, IL4 & IL5



Duffy, Wellard, Markham, Zhou, Holmberg, Hawkins, Hasbold, Dowling & Hodgkin, *Science*, 2012.

A new proliferation multiplex CFSE/CTV/CPD assay



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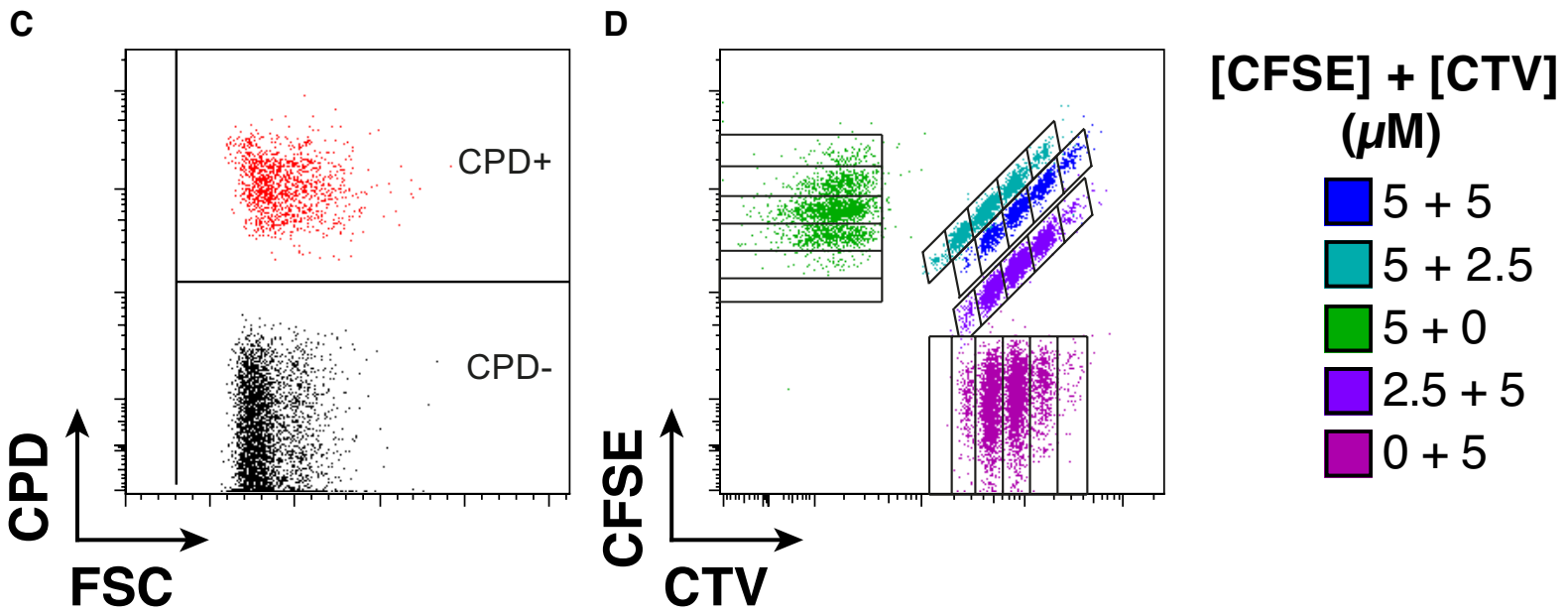

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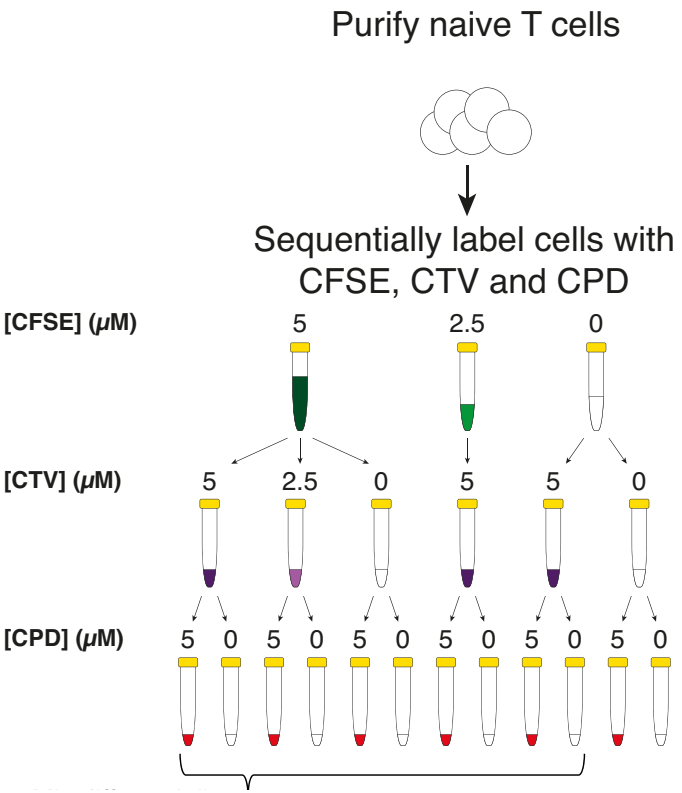

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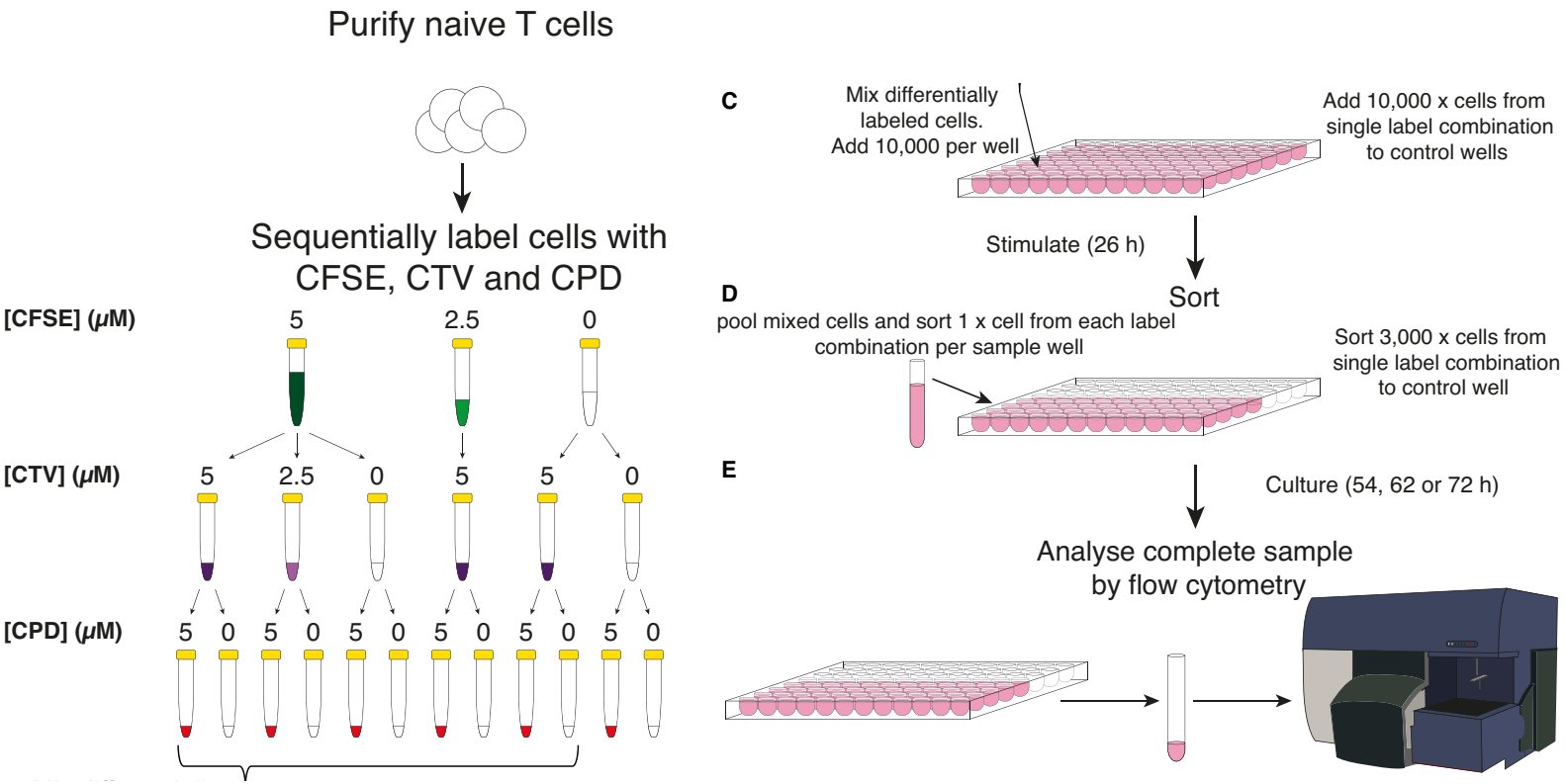
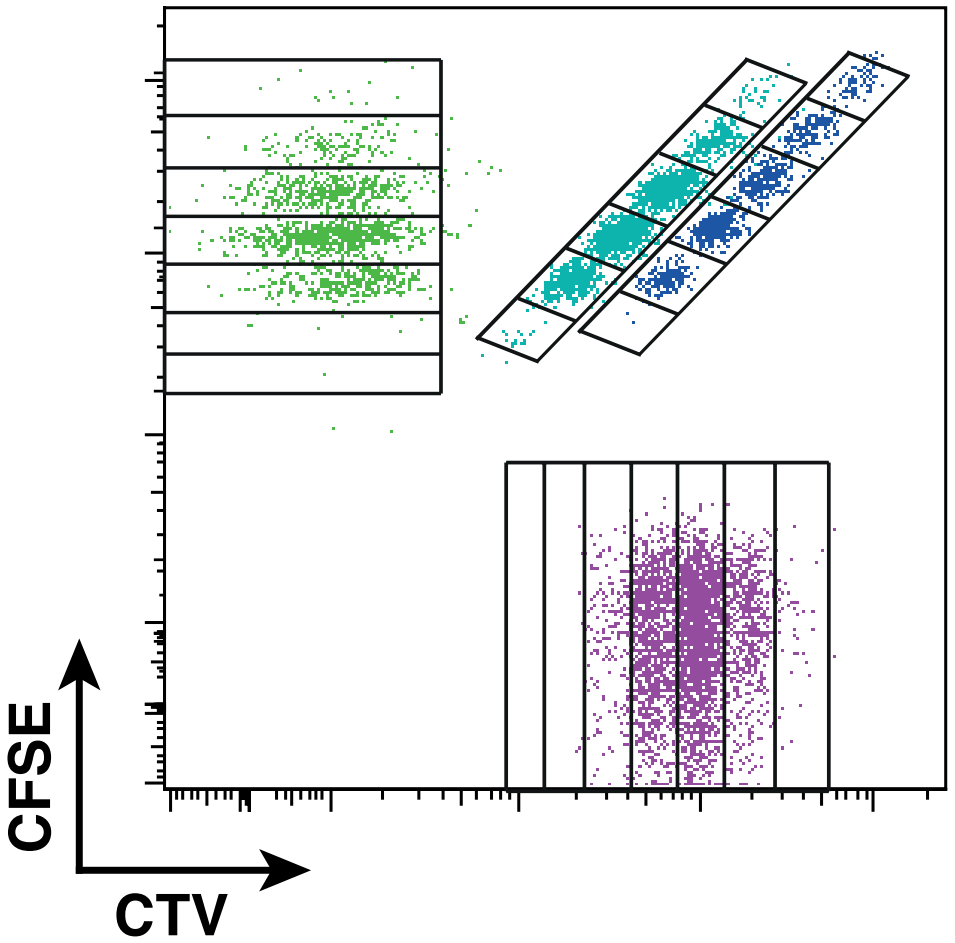


CFSE/CTV/CPD Multiplex

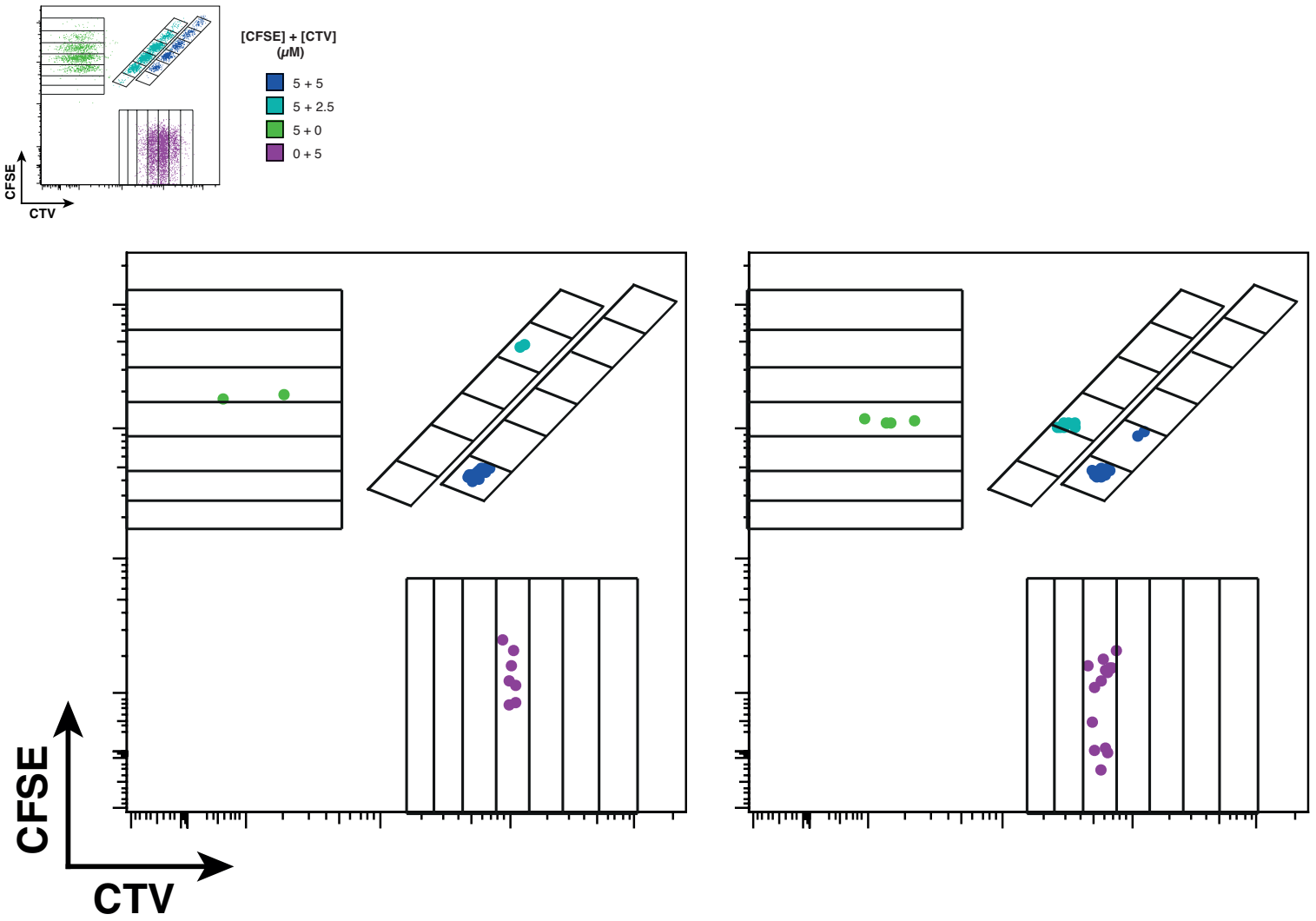


[CFSE] + [CTV] (μM)

- 5 + 5
- 5 + 2.5
- 5 + 0
- 0 + 5

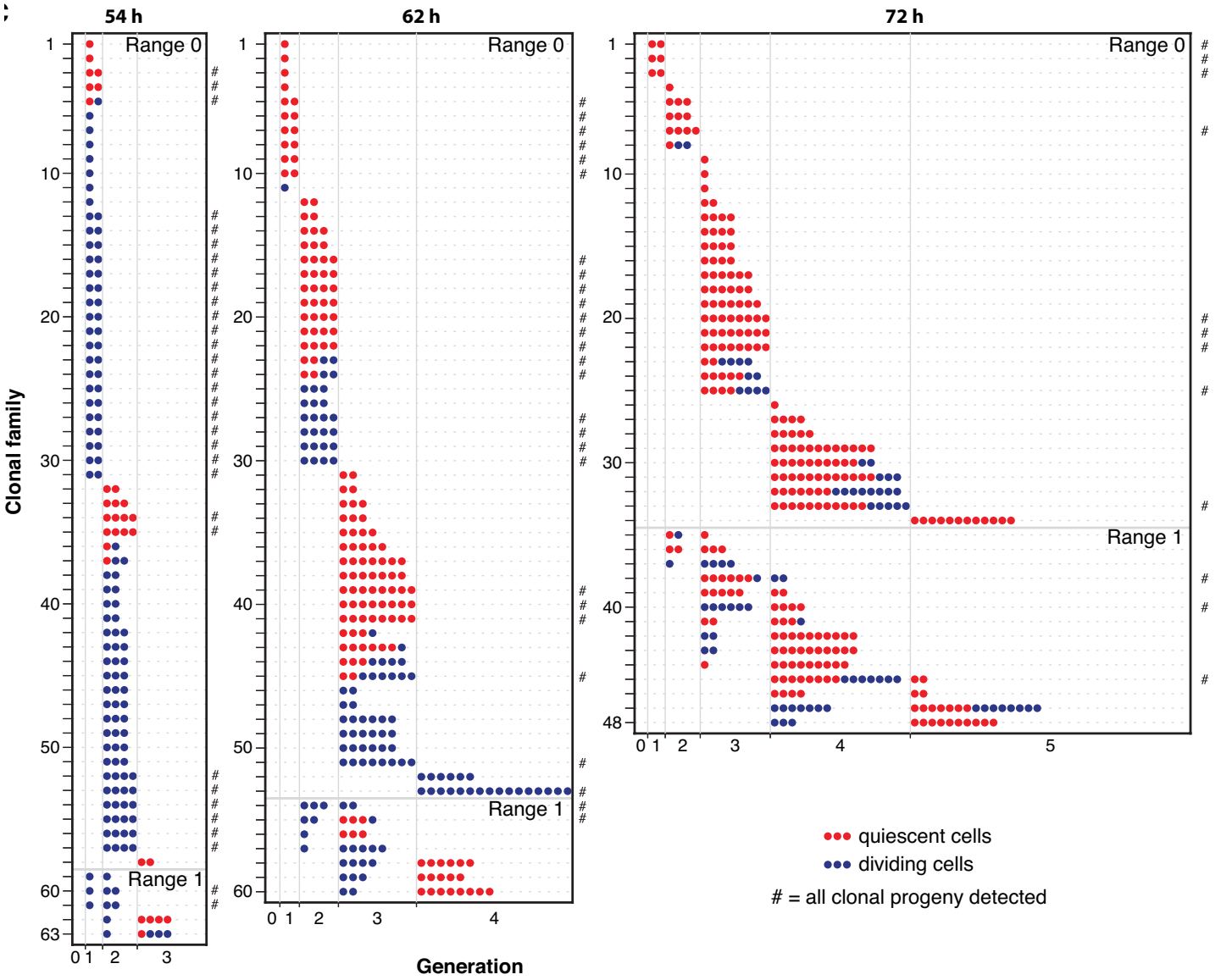
OT1/Bcl211$^{-/-}$ CD8+ T cells, N4 peptide & anti-CD28.

Individual families

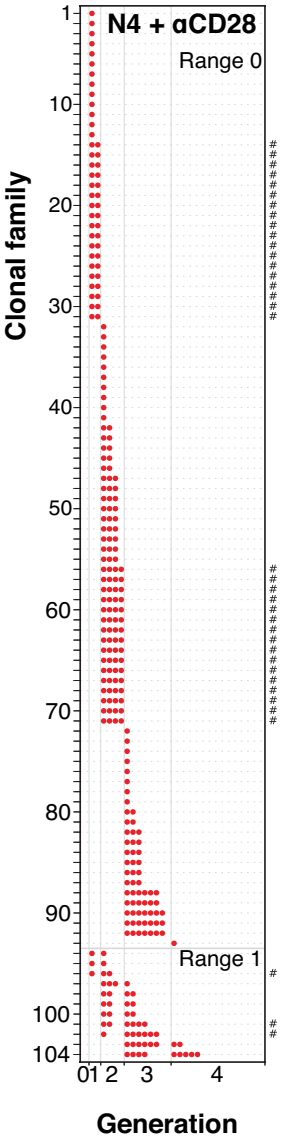


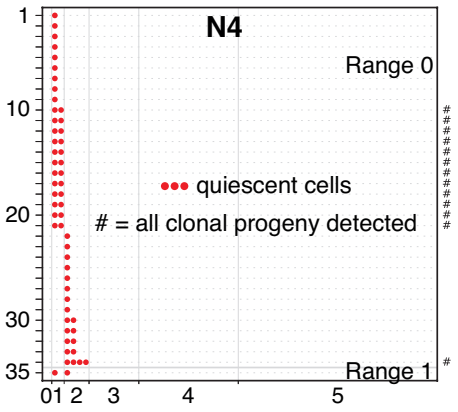
OT1/Bcl211$^{-/-}$ CD8+ T cells, N4 peptide & anti-CD28.

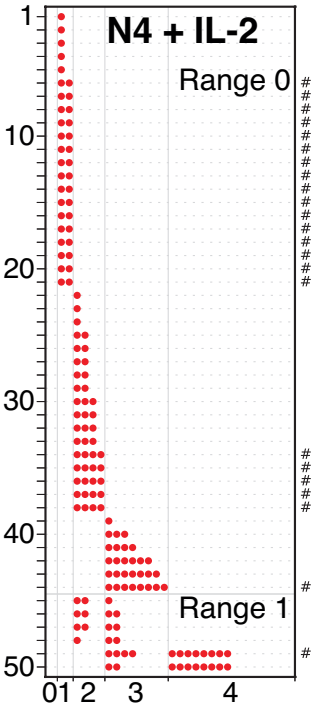
Concordance: CD8+ T cells, N4 peptide & anti-CD28

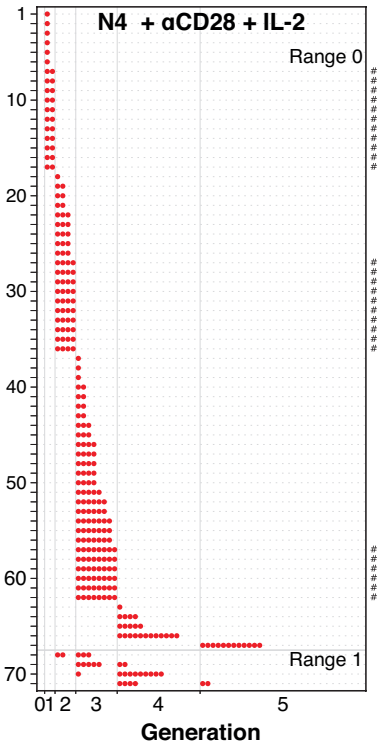


Signal combinations: N4, anti-CD28, IL-2









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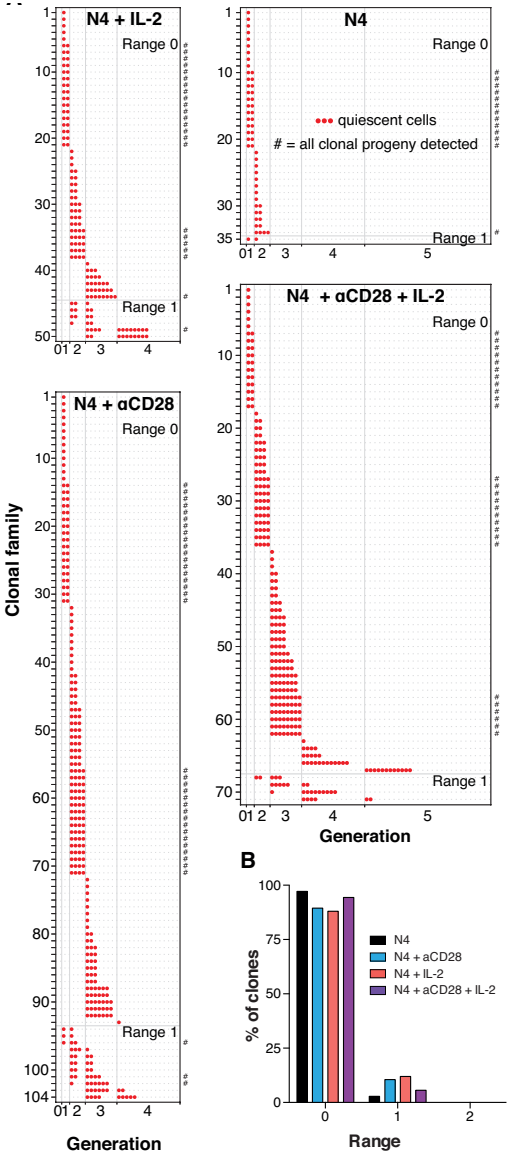


Number of cells quiescent cells in each generation:

$$(X * Y)_i := \sum_j X_j Y_{i-j}.$$

The depth of trees is a linear operator. Defining

$$\mathrm{maxDD}(X) := \max\{i : X_i > 0\},$$

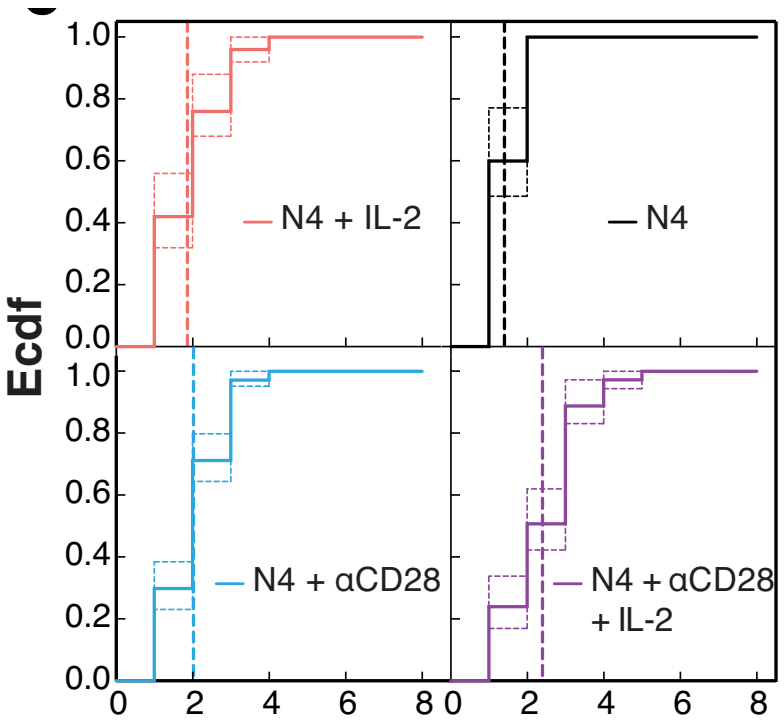
we have

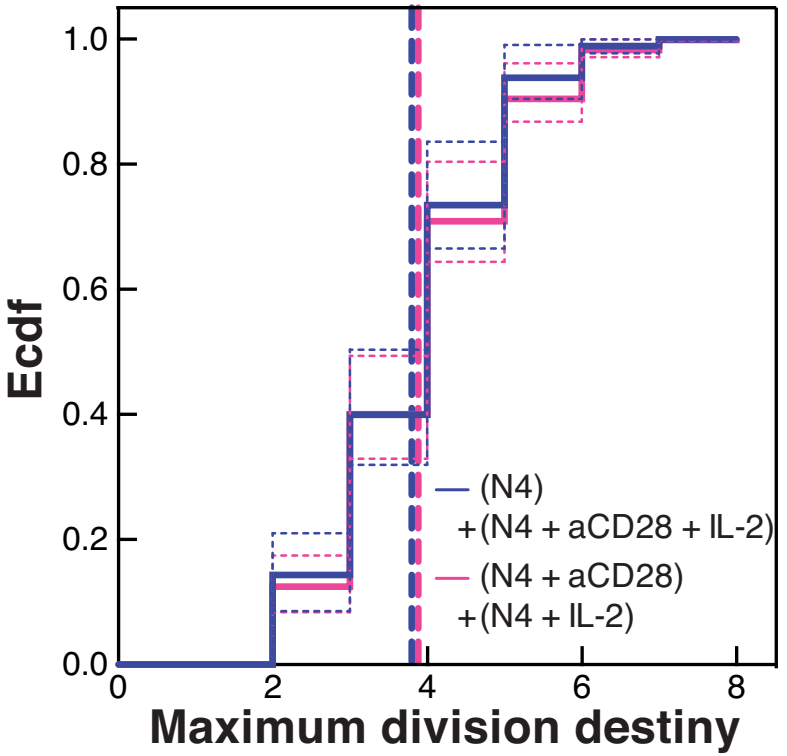
$$\mathrm{maxDD}(X * Y) = \mathrm{maxDD}(X) + \mathrm{maxDD}(Y).$$

Is signal additivity probabilistically independent?

If independent:

$$\text{maxDD(N4)} + \text{maxDD(N4+IL2+antiCD28)}$$
$$\stackrel{\partial}{=} \text{maxDD(N4+IL2)} + \text{maxDD(N4+antiCD28)}$$





p-values?

Pearson: X is categorical, with $P(X = i) = p_i$, and one is given n independent obs., with n_i being the number in category i, then

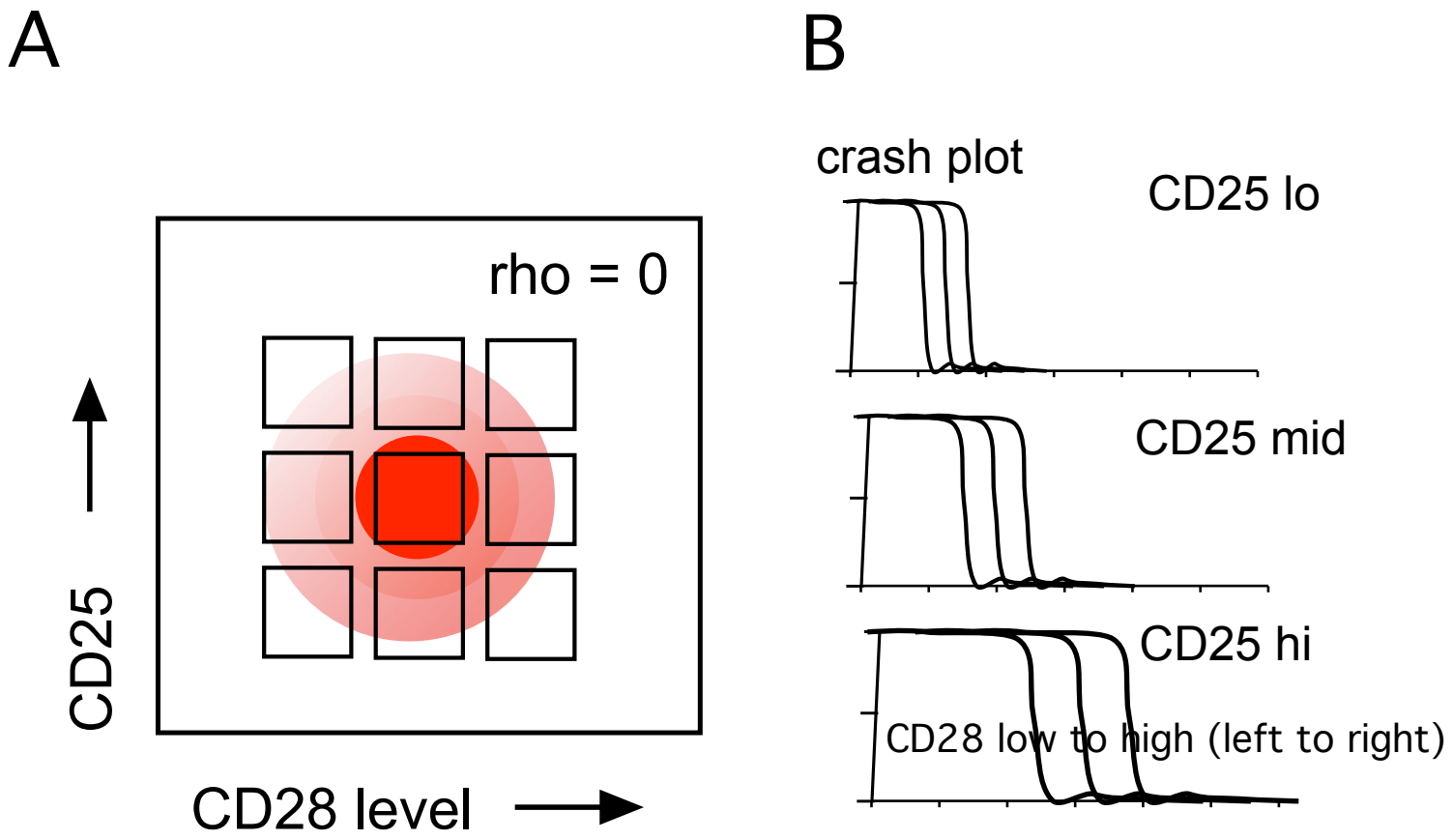
$$\sqrt{n}\left(\left(\frac{n_1}{n}, \cdots, \frac{n_K}{n}\right) - (p_1, \ldots, p_K)\right)$$

is Asymptotically Normal with mean 0 and covariance structure

$$\Sigma = \begin{pmatrix} (1-p_1)p_1 & -p_1p_2 & \cdots & -p_1p_K \\ -p_2p_1 & (1-p_2)p_2 & \cdots & -p_2p_K \\ \vdots & \vdots & \ddots & \vdots \\ -p_Kp_1 & -p_Kp_2 & \cdots & (1-p_K)p_K \end{pmatrix}; C = \text{diag}\left(\frac{1}{p_1}, \ldots, \frac{1}{p_K}\right)$$

$$(\Sigma C) = (\Sigma C)^2 \text{and DoF} = \text{trace}(\Sigma C).$$

Concordance & independent additivity suggests lineage priming



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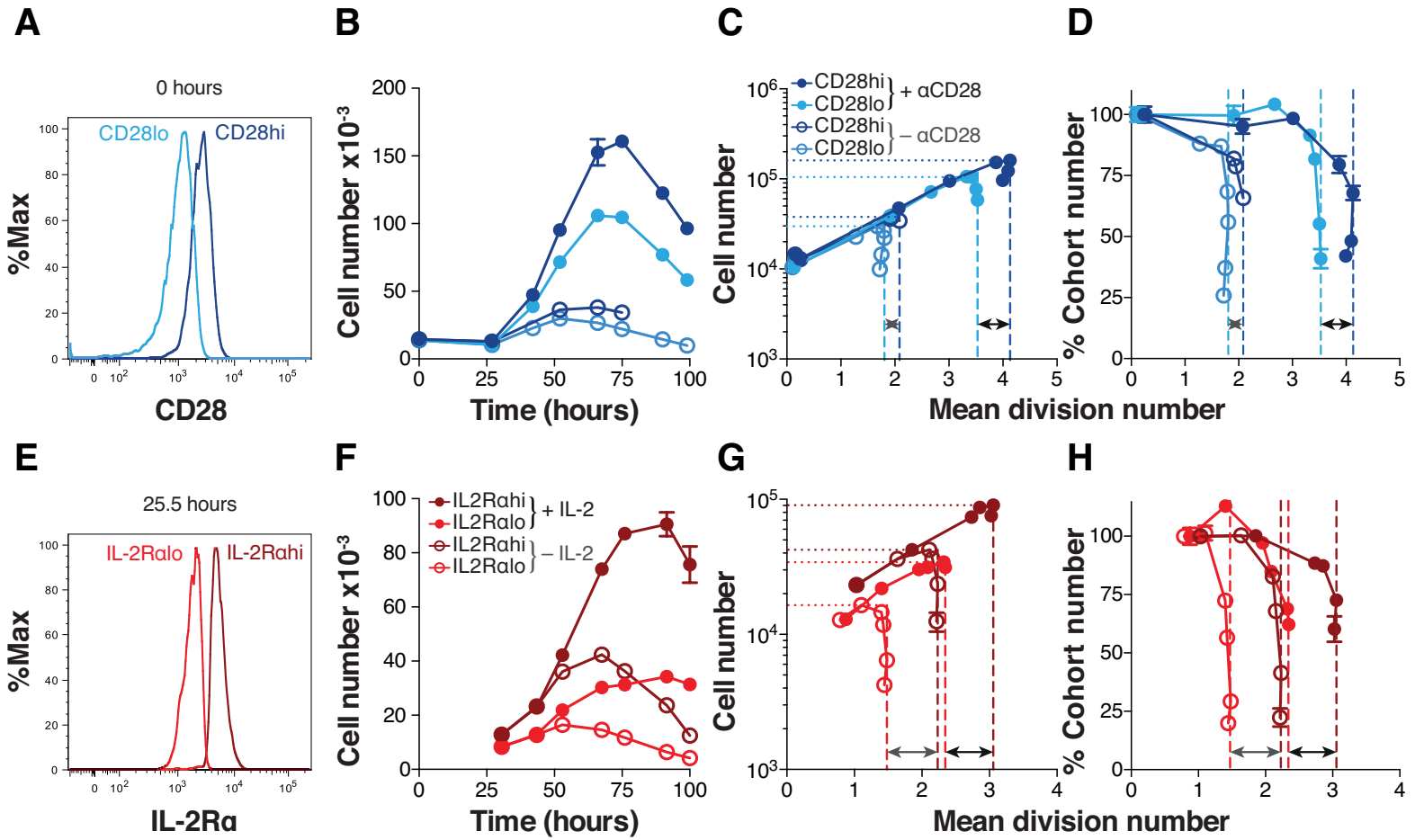
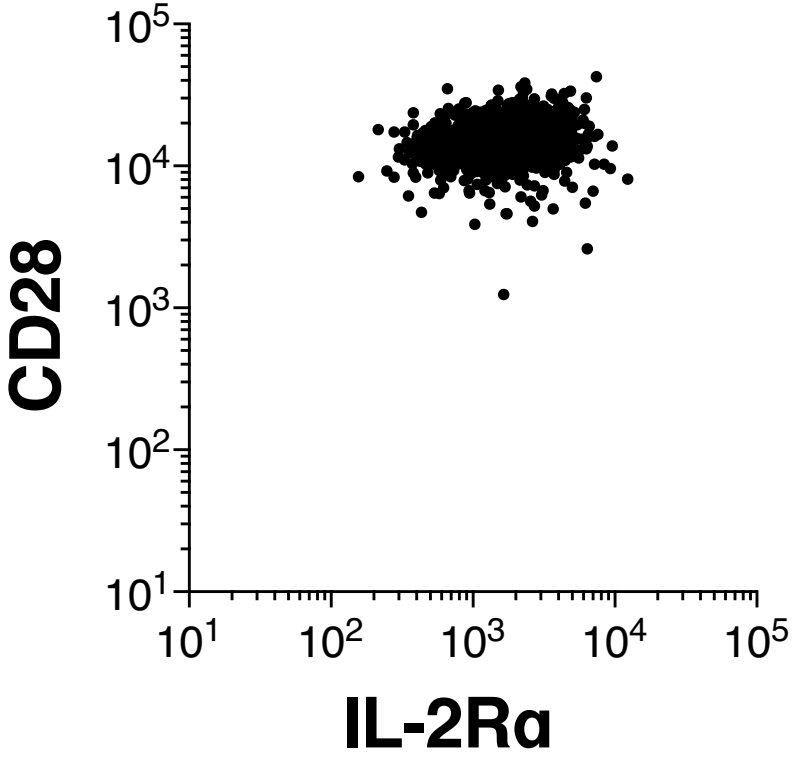
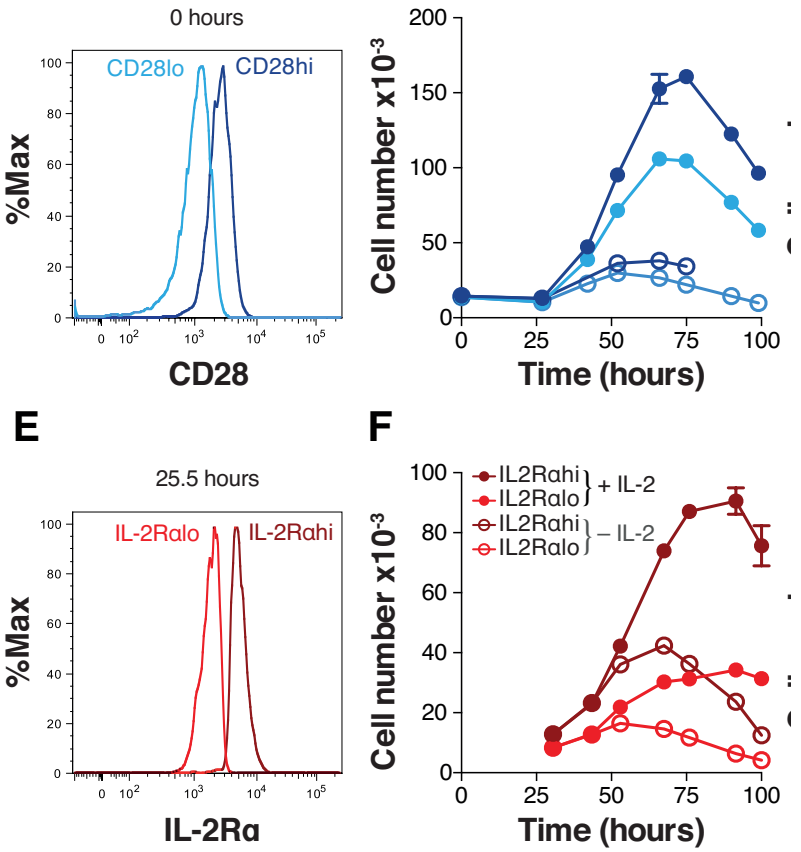


Lineage priming





Marchingo, Prevedello, Kan, Heinzel, Hodgkin & Duffy, *in prep.*, 2016.

What is the mechanism?

Attend Phil's talk.

Heinzel, Giang, Kan, Marchingo, Lye, Corcoran & Hodgkin, *in prep.*, 2016.

Walter+Eliza Hall
Institute of Medical Research

DISCOVERIES FOR HUMANITY

Finding the mechanism of familial division destiny

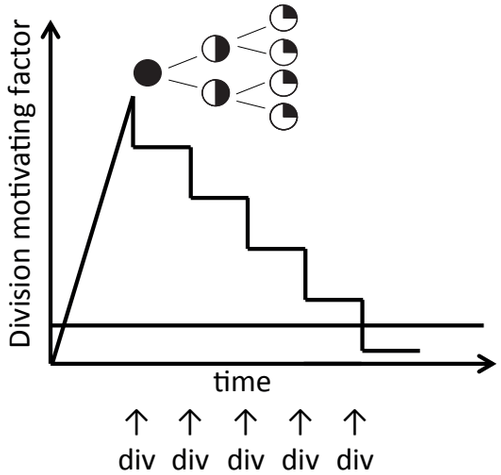
Phil Hodgkin

For KITP Quantitative Immunology Workshop, 2016

Su Heinzel
Lynn Corcoran
Andrew Giang
Julia Marchingo
Andrey Kan

Properties of putative unknown ‘x’

x = induced division motivating factor



x = expression changes inherited symmetrically

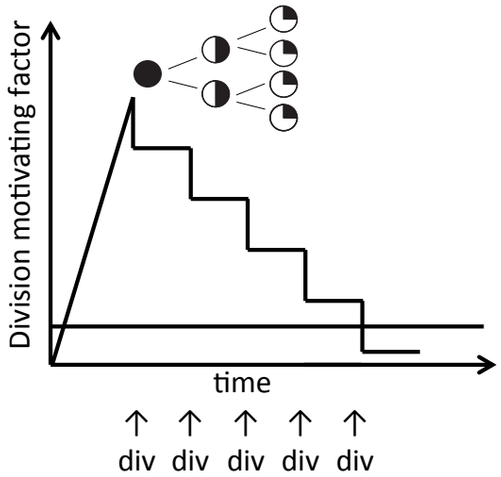
x = lost in cells as they stop division

x = Quantitative ‘addition’ with multiple stimuli

x = likely ‘oncogene’ known to alter division

Properties of putative unknown ‘x’

x = induced division motivating factor



x = expression changes inherited symmetrically

x = lost in cells as they stop division

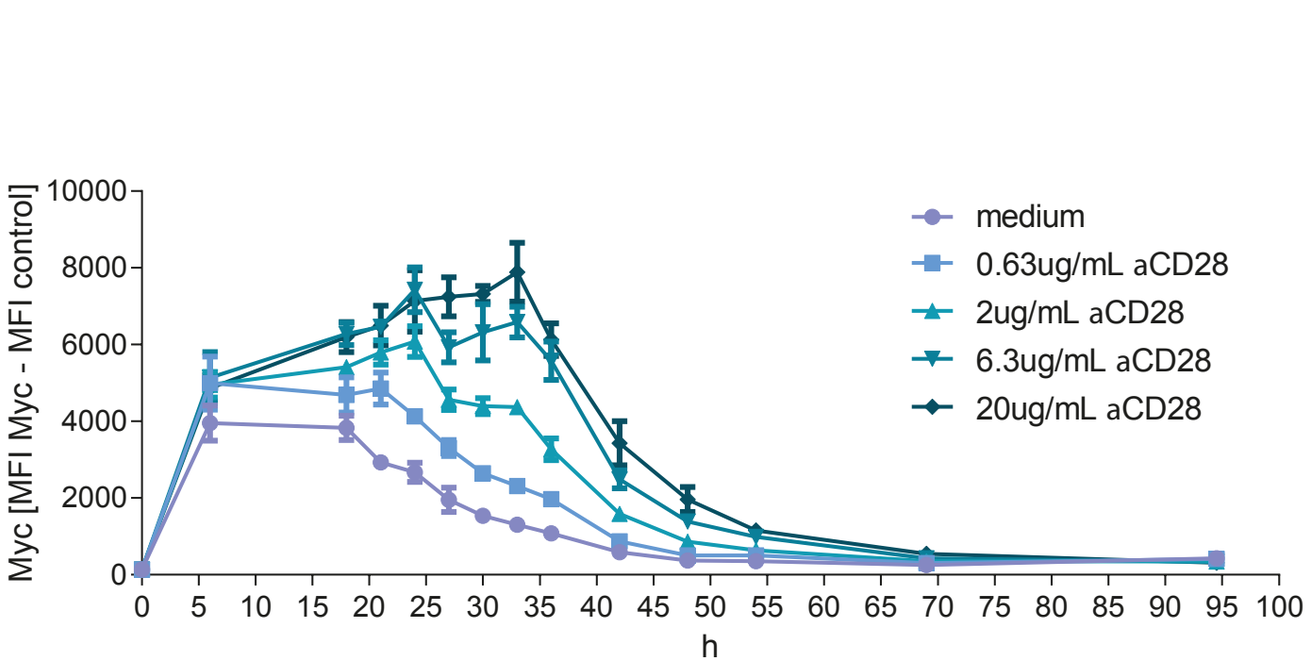
x = Quantitative ‘addition’ with multiple stimuli

x = likely ‘oncogene’ known to alter division

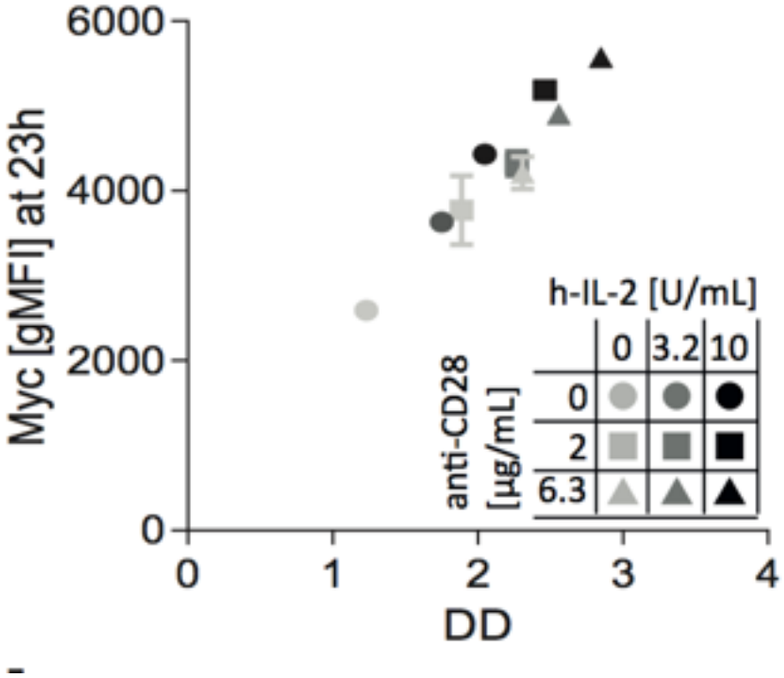
Can x be Myc?

✓ - Forced expression of Myc in dividing B and T cells prevents division cessation (must also keep alive*)

Myc levels change with stimulation strength and signal addition



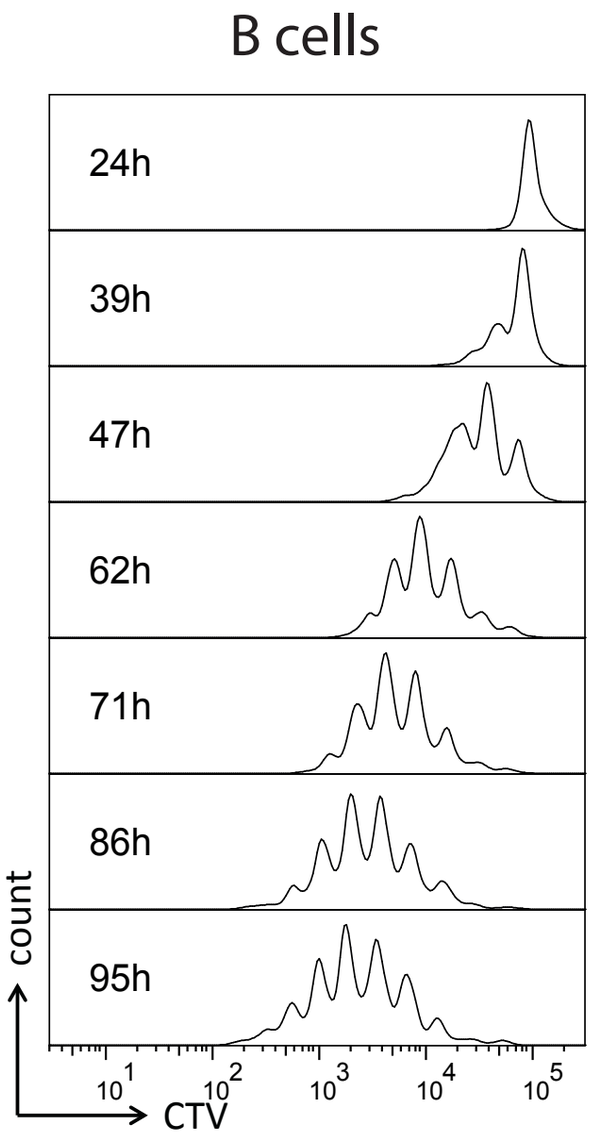
Anti-CD28 titration

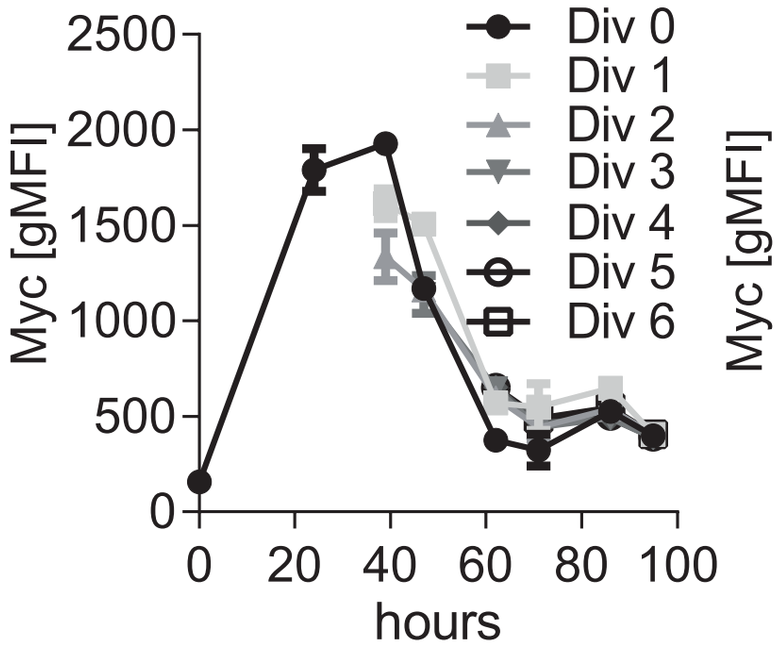


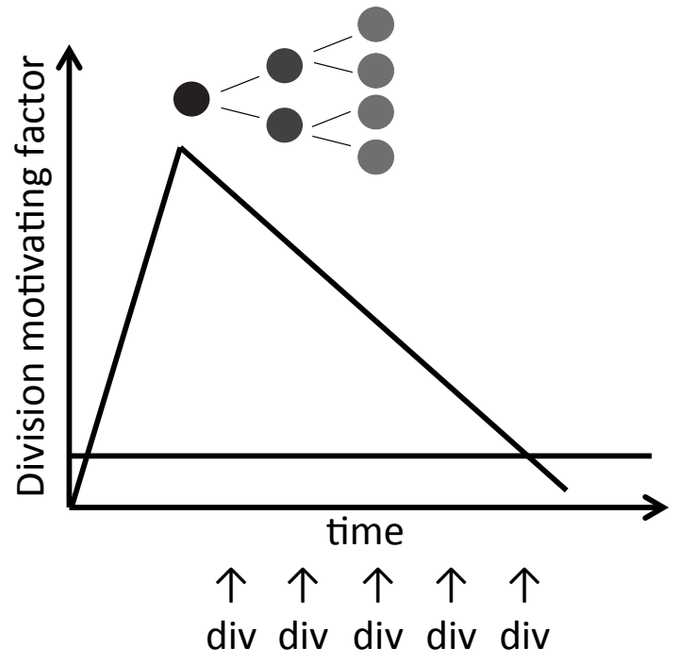
Correlating 23 h Myc with Division Destiny

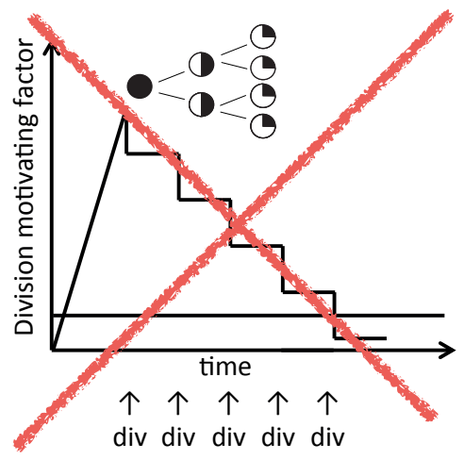
Different combinations IL-2 and aCD28

Myc kinetic changes not affected by division



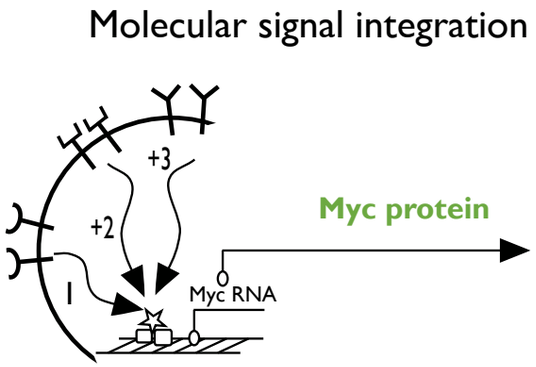


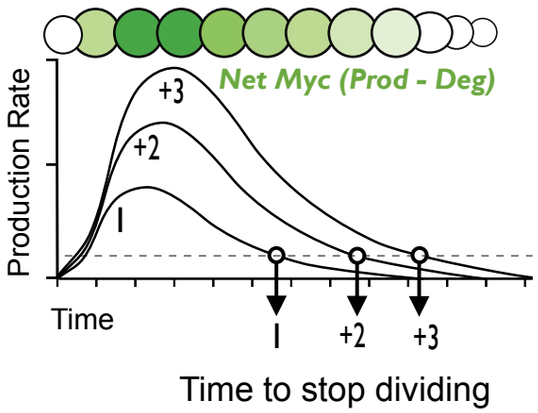




Myc loss is ‘timed’ (not diluted) and machinery for loss heritable and division independent
A new cellular machine
(working on details including high rate of turnover - production rate is main change with time)

Reconciling molecular machine with tree formation





Integrated signals modify production rate
Net levels change over time