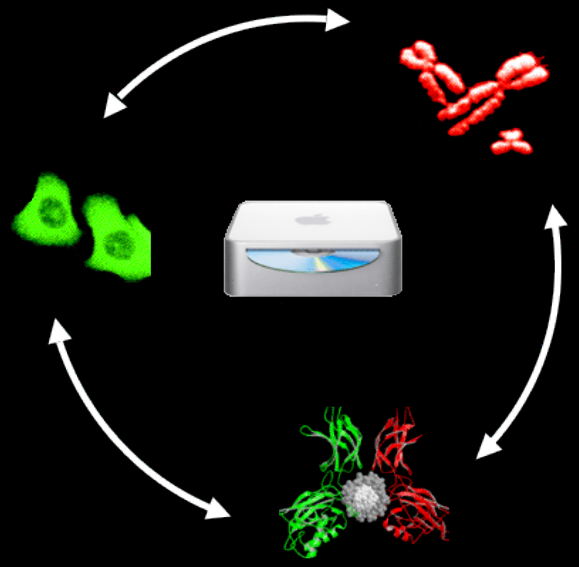
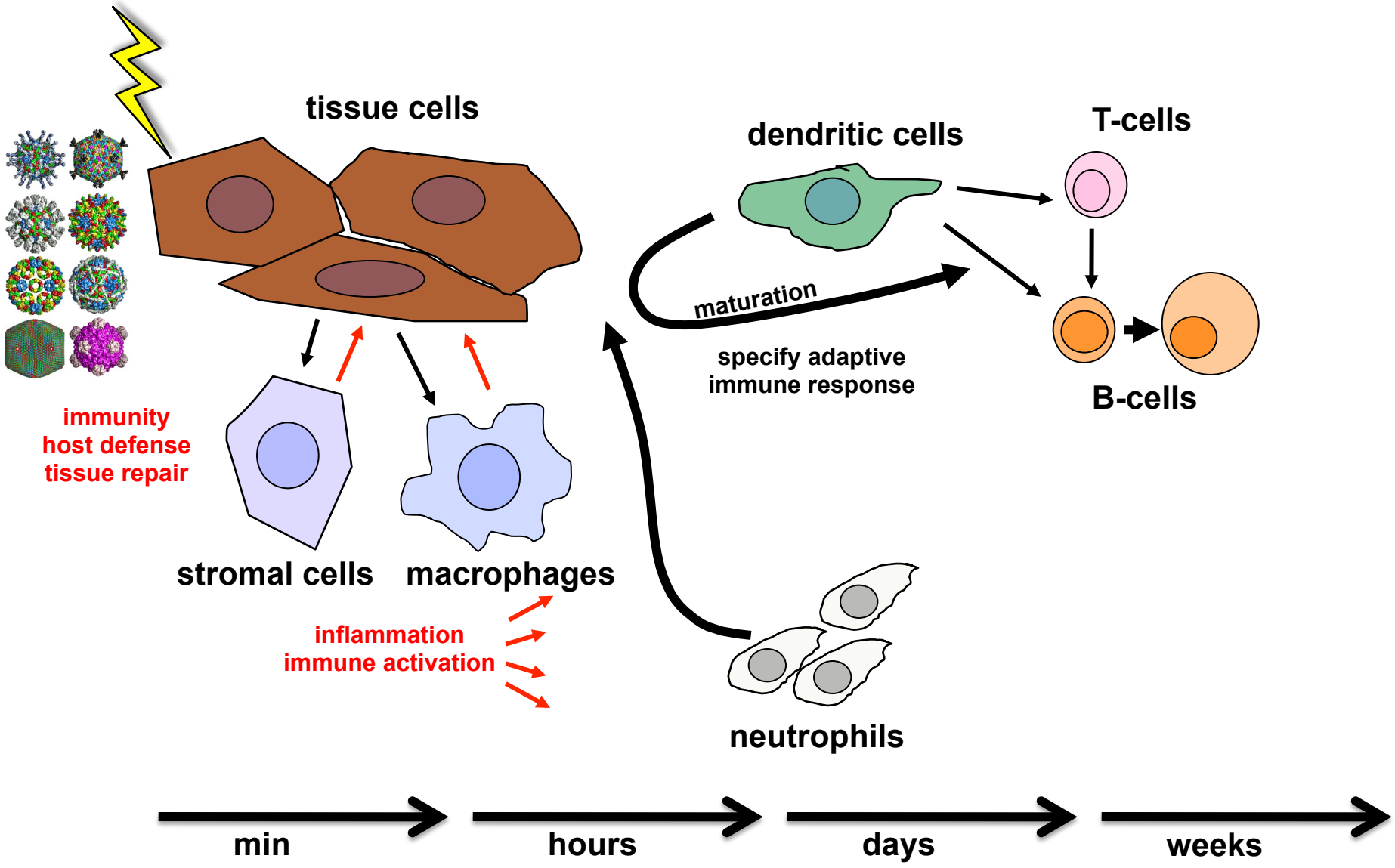
Towards a quantitative understanding of how intra-cellular signaling networks control B-cell proliferation



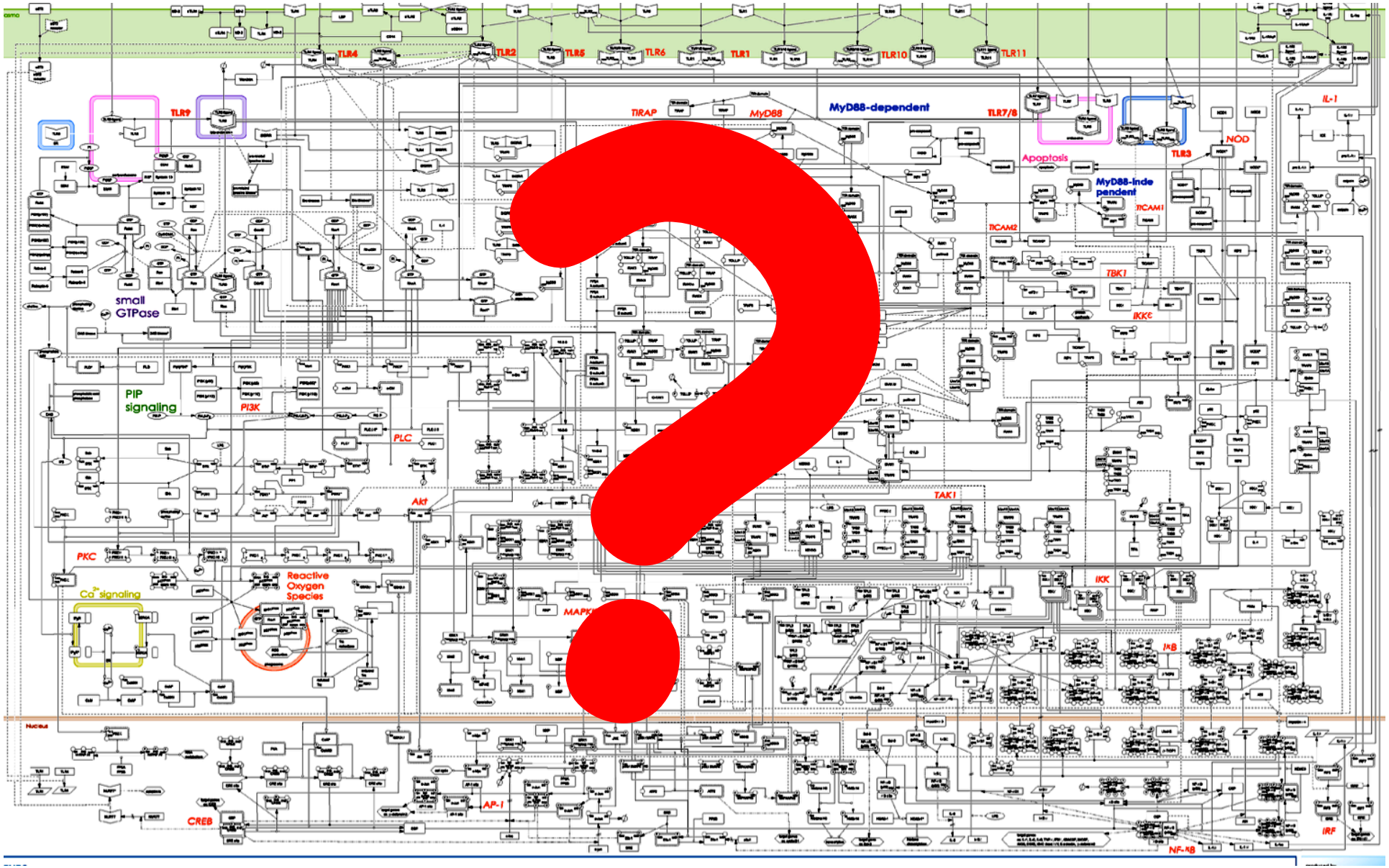
Alexander Hoffmann
Signaling Systems Laboratory
UCLA

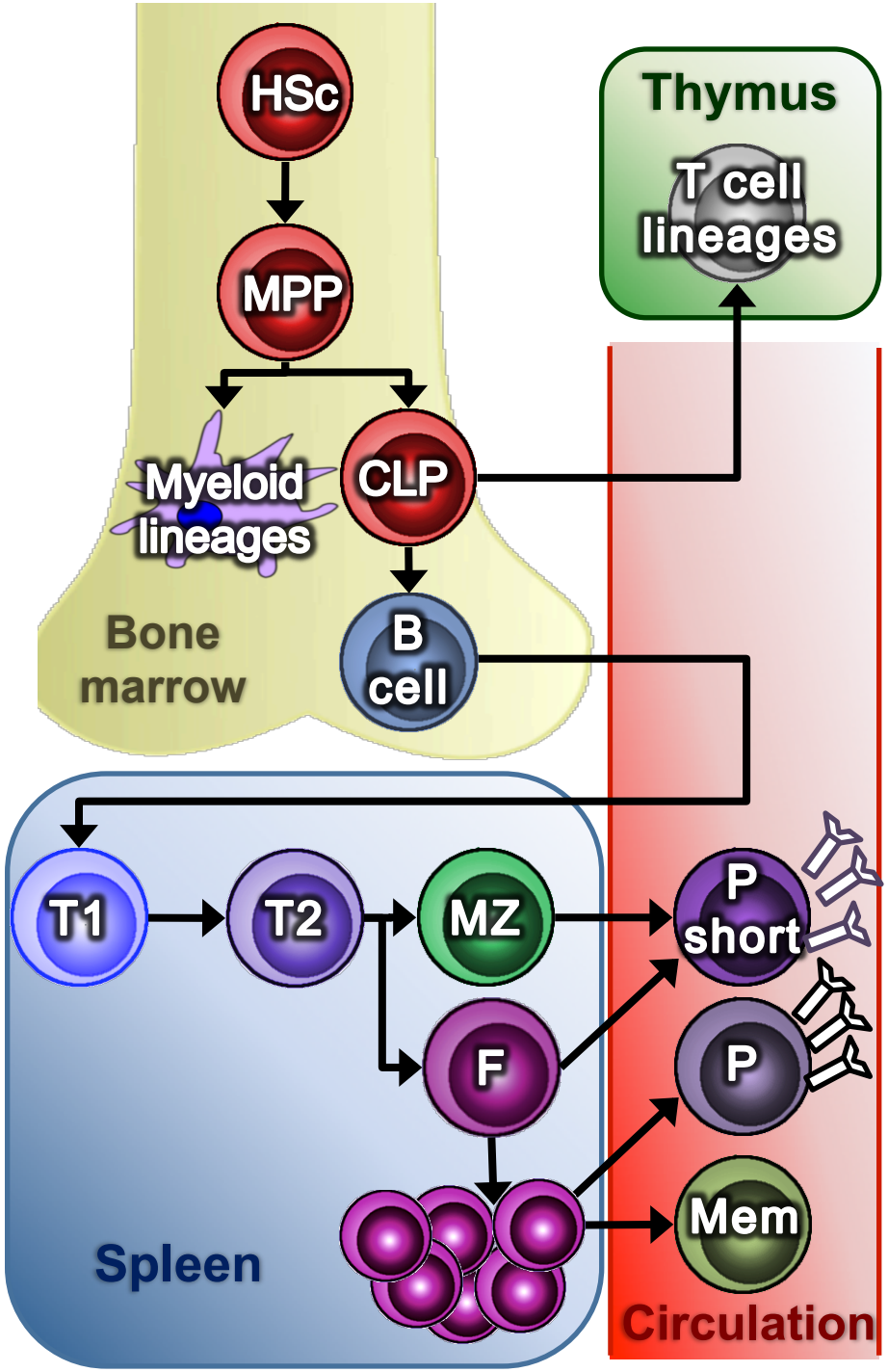
UCLA QCBio

Immune Responses: multiple tiers, cells, timescales



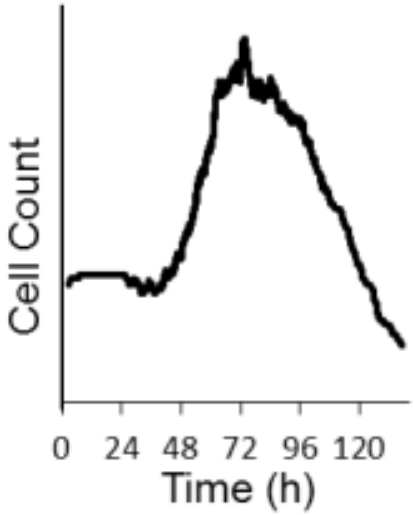
The Immune Response Signaling Network

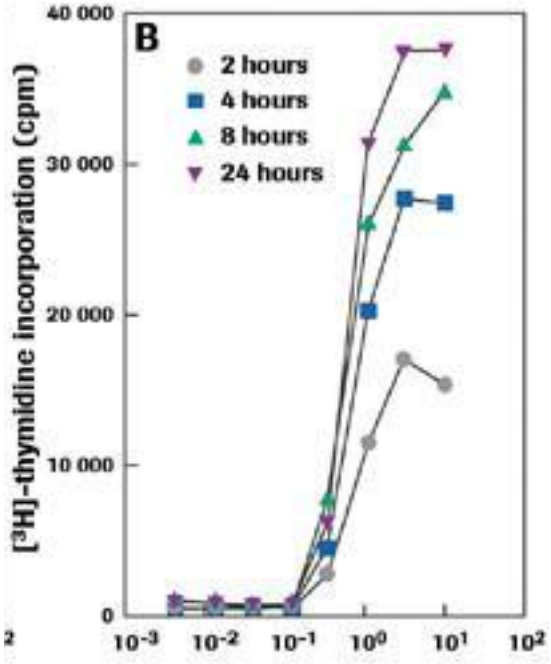


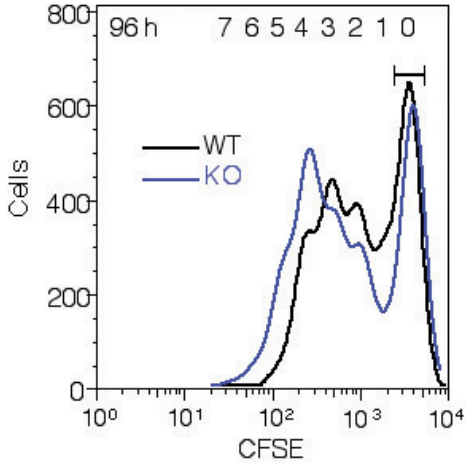


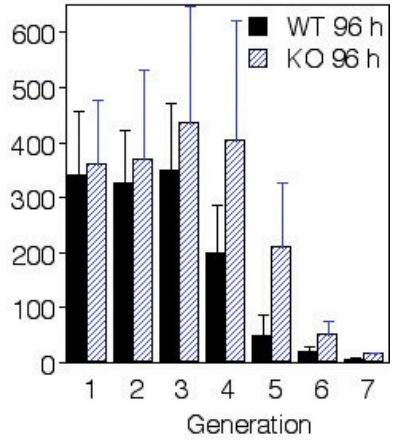
Immune Responses are a function of lymphocyte dynamics

How is lymphocyte proliferation studied?





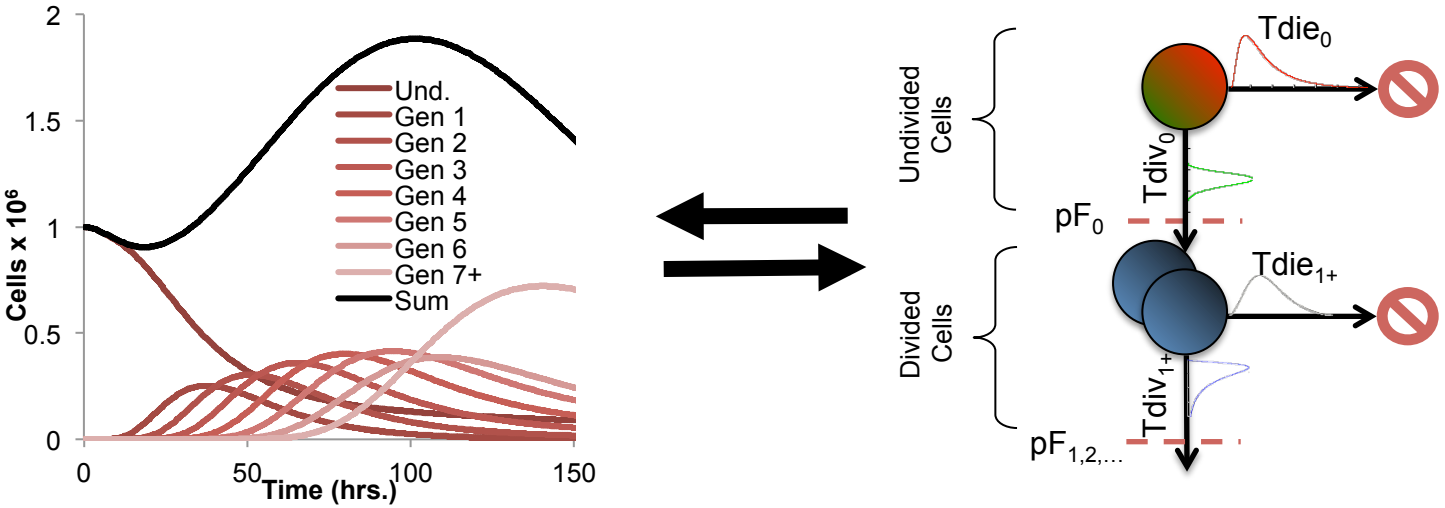




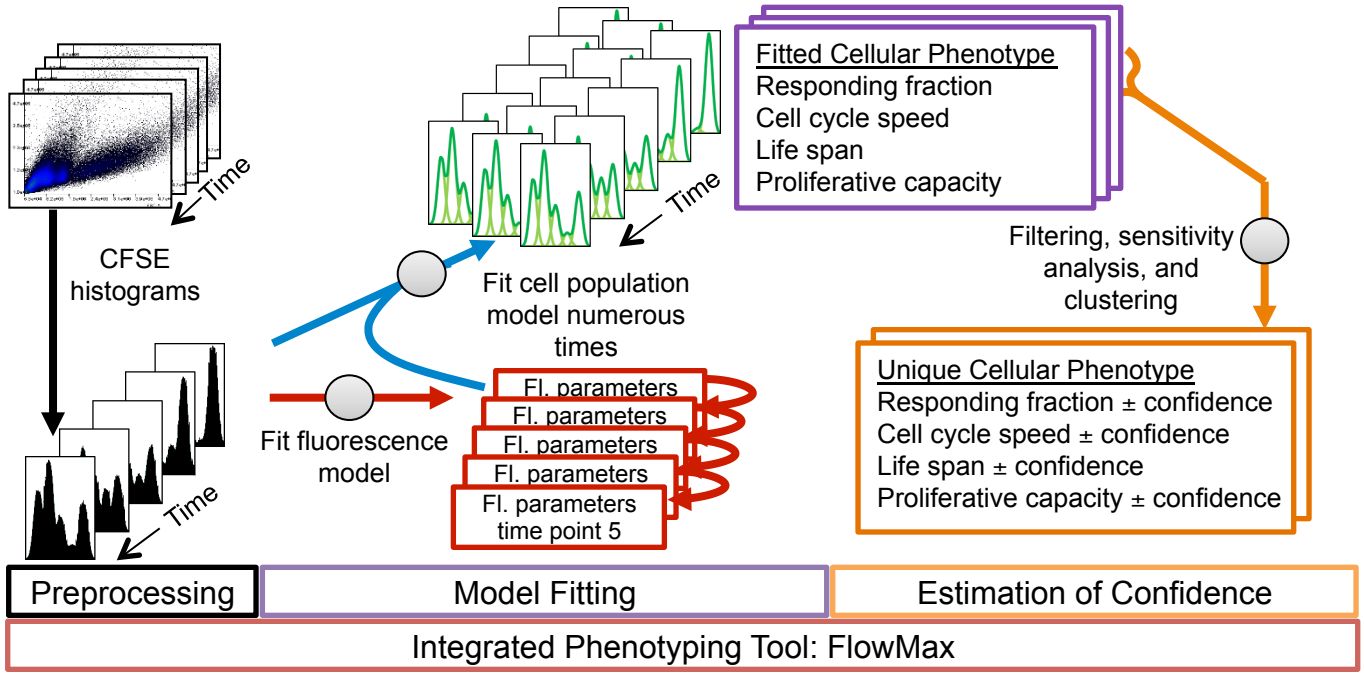
Can we extract quantitative information about what lymphocytes are actually doing?

NUMBERS: how many cells respond, how quickly, cell cycle time, survival time, number of divisions, etc

Understand population dynamics in terms of the cell biology of single cells.

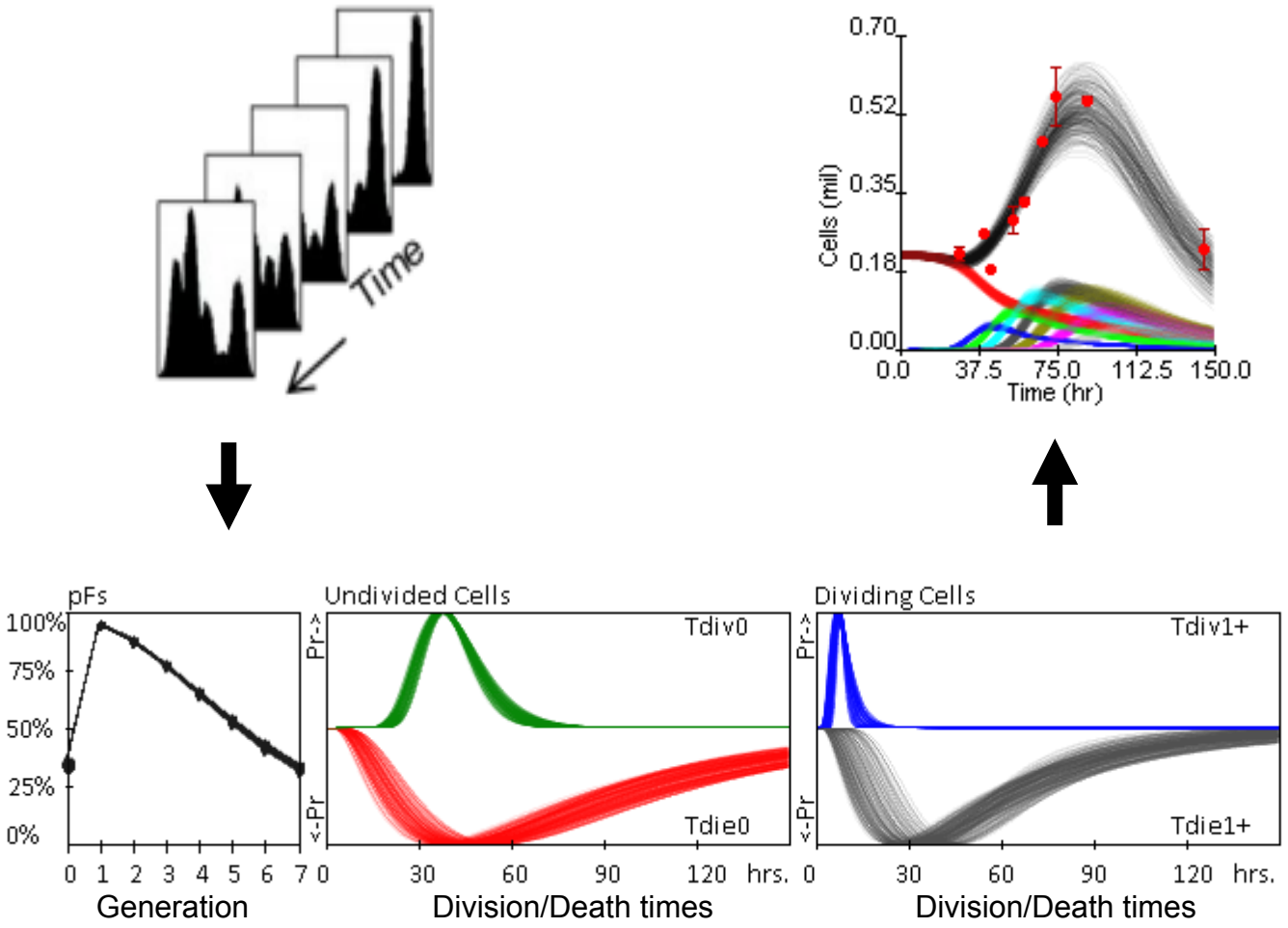


FlowMax: a software tool for Maximum likelihood interpretation of CFSE data

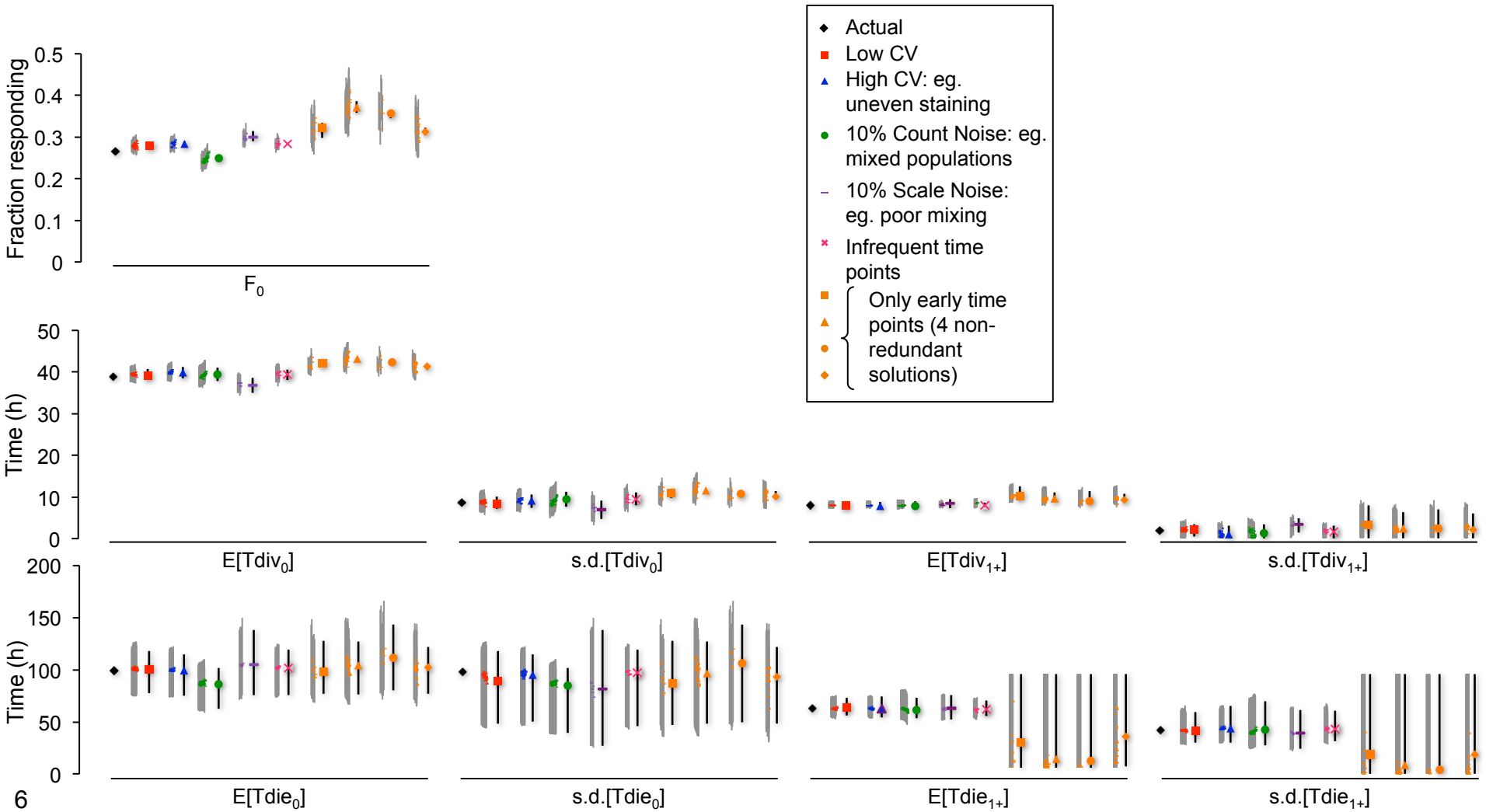


Max Shokhirev

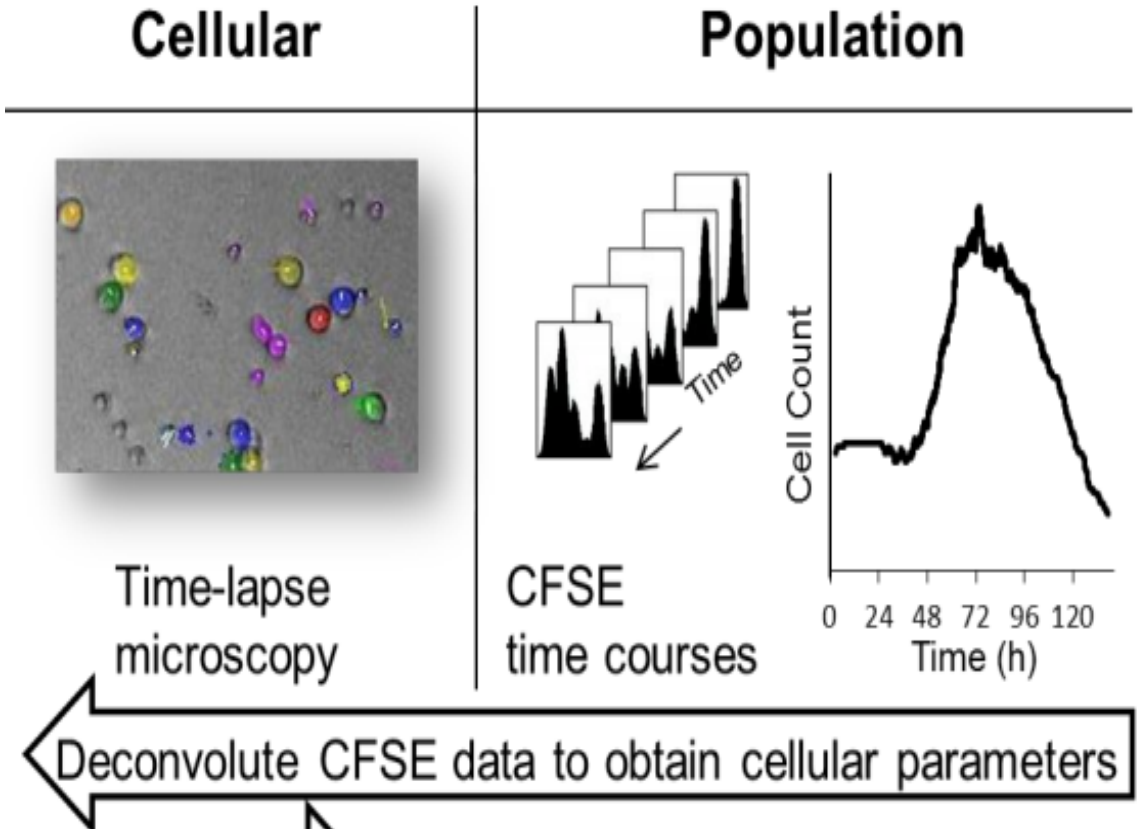
FlowMax allows quantitative interpretation of CFSE data



Quality of maximum likelihood interpretation as a function of imperfections in the data



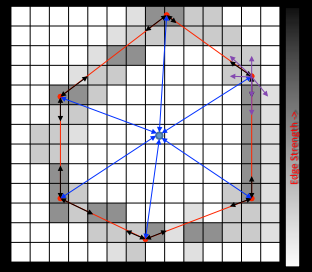
Is the FlowMax interpretation of CFSE data correct?



The BigData problem of live cell microscopy

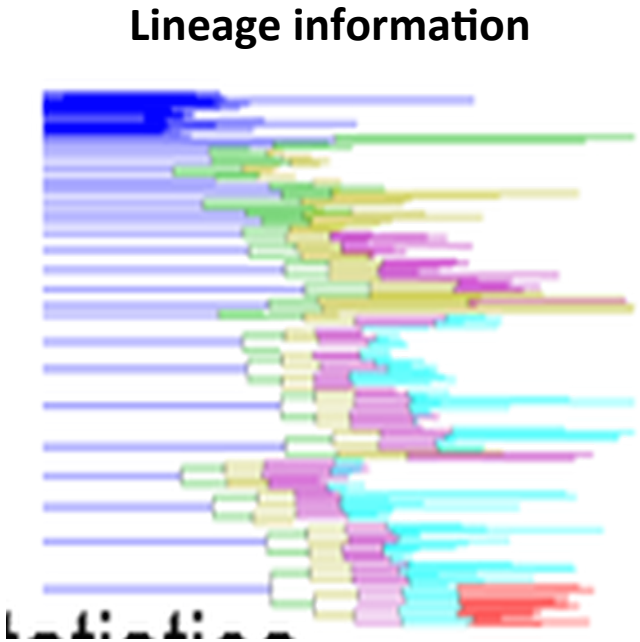
~ 10,000 images with ~ 1,000 cells/image
Cells move, deform, divide, die, enter, leave, aggregate

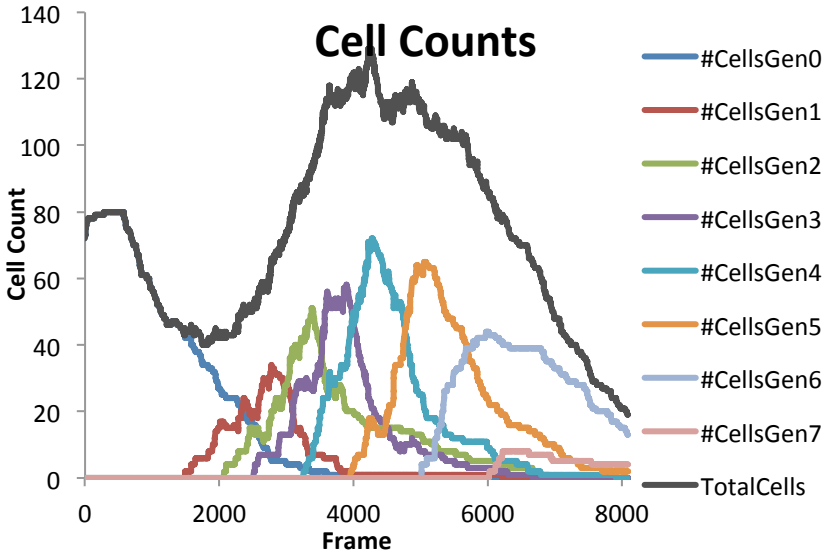
Identify each cell in each frame



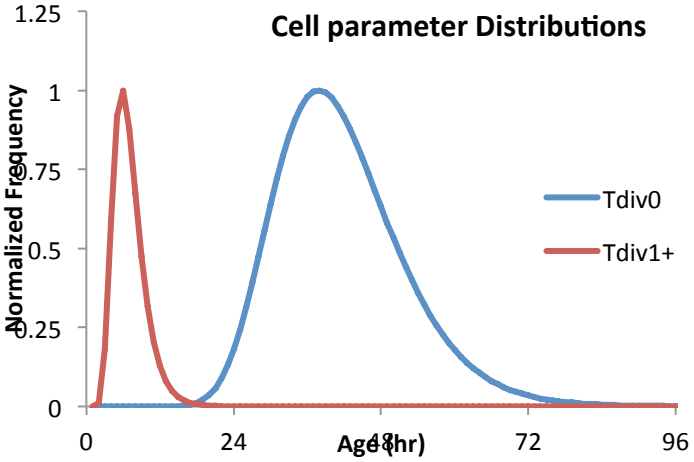
Run Physics Optimization
Polygon “Blobs”

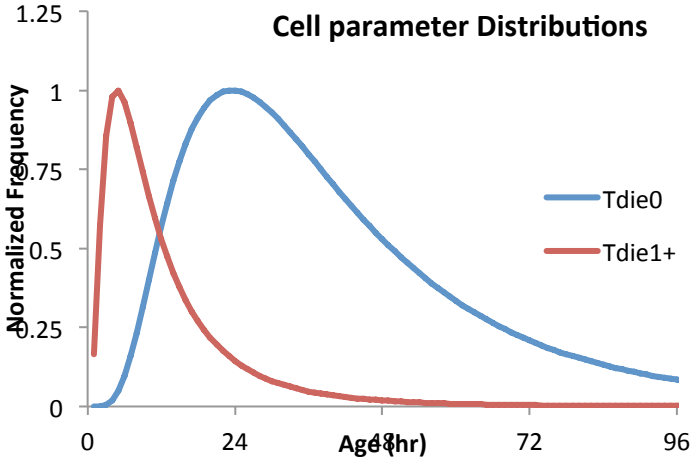
Population Dynamics as a result of single cell microscopy

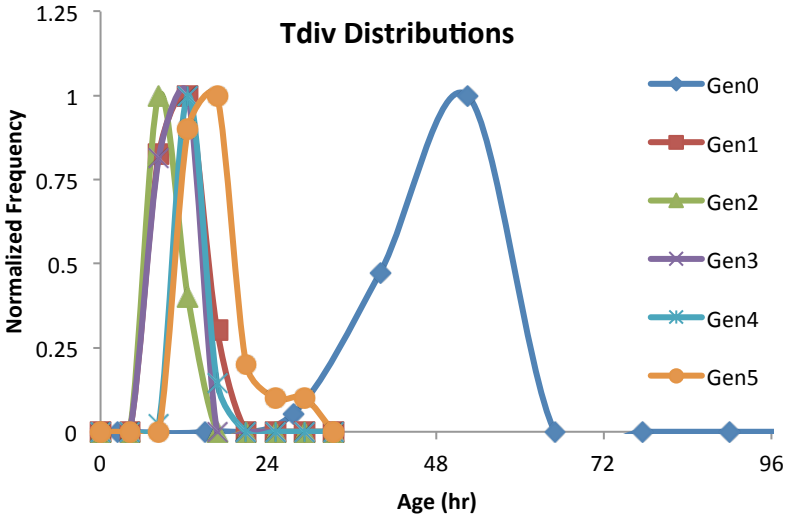


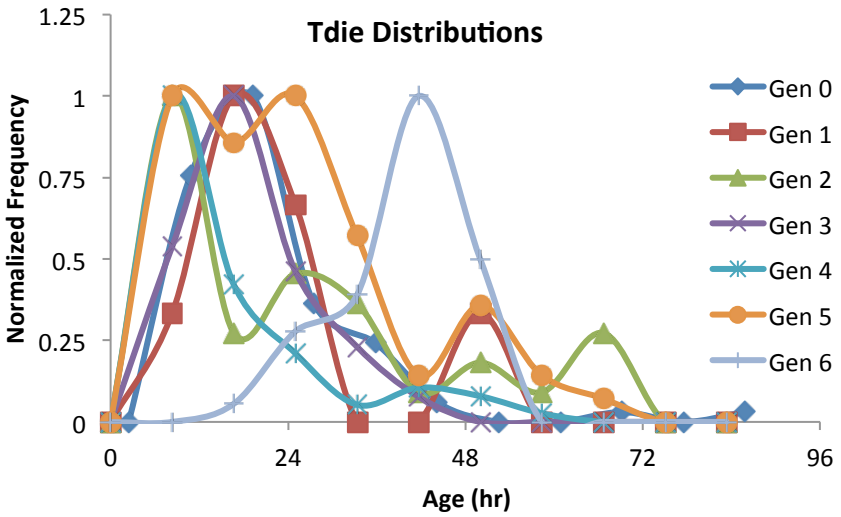


Comparing cellular parameters derived from CFSE and from single cell microscopy

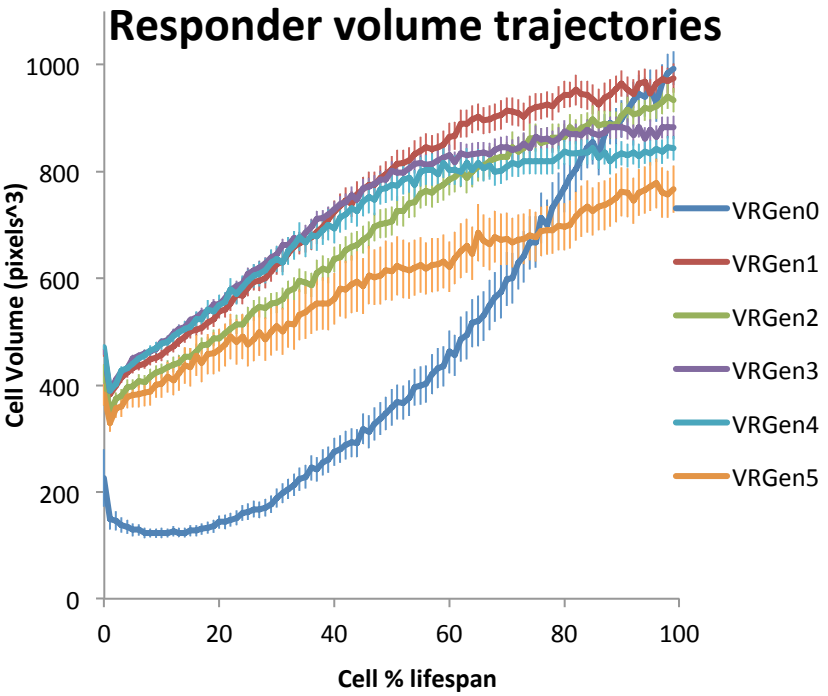


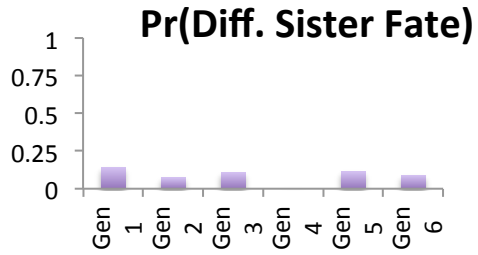


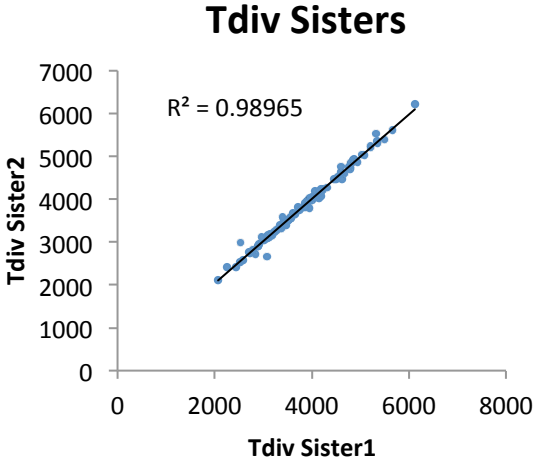


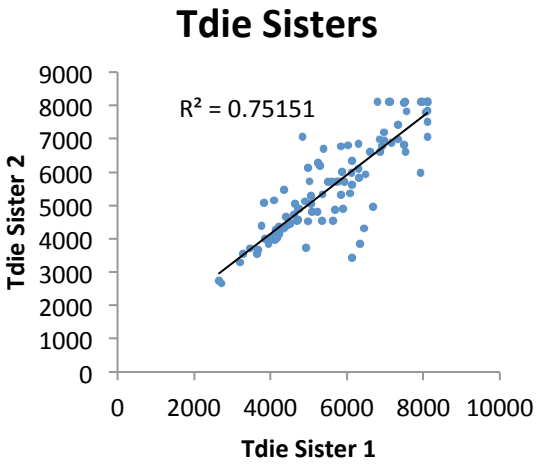


Single cell microscopy provides additional information

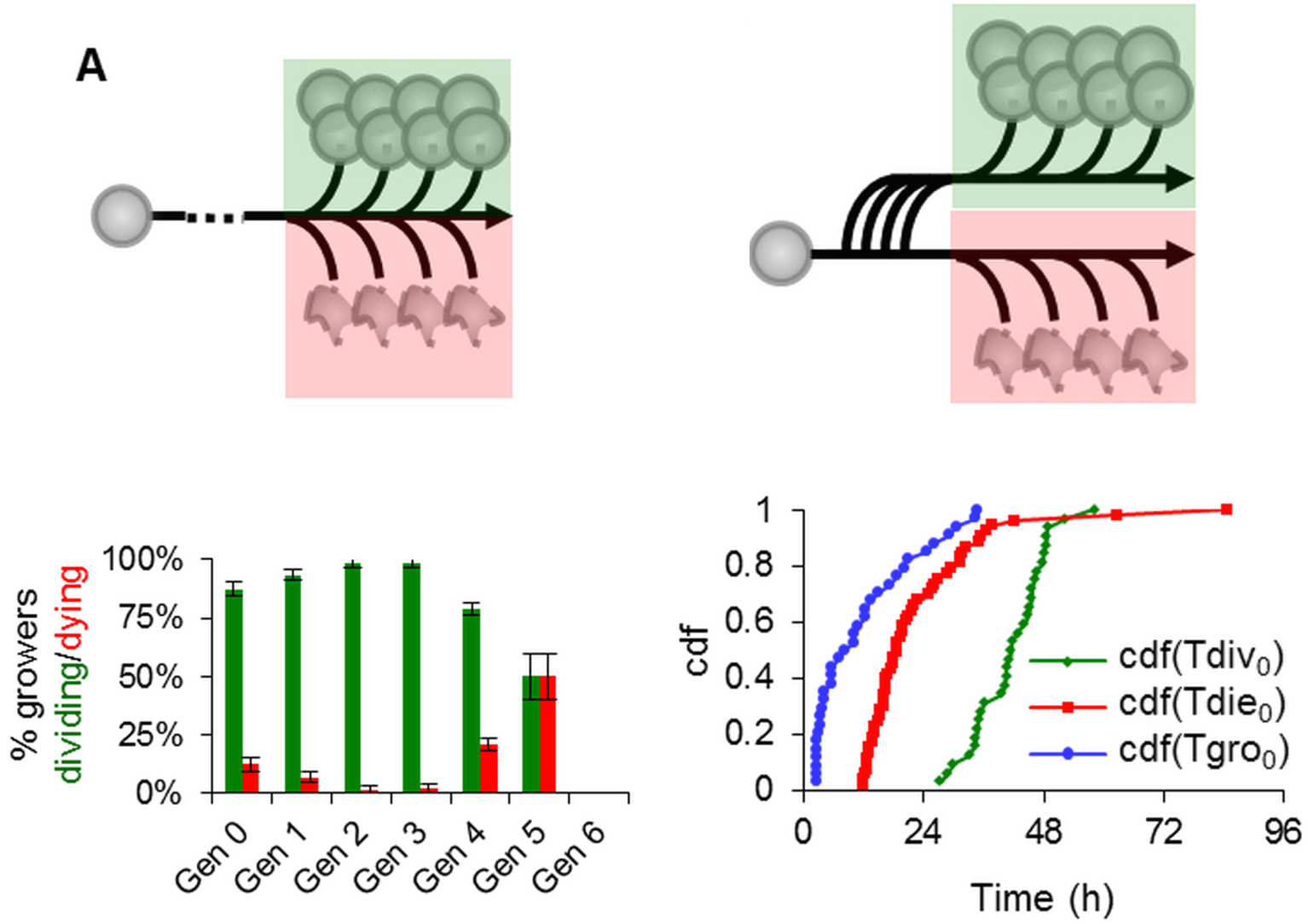




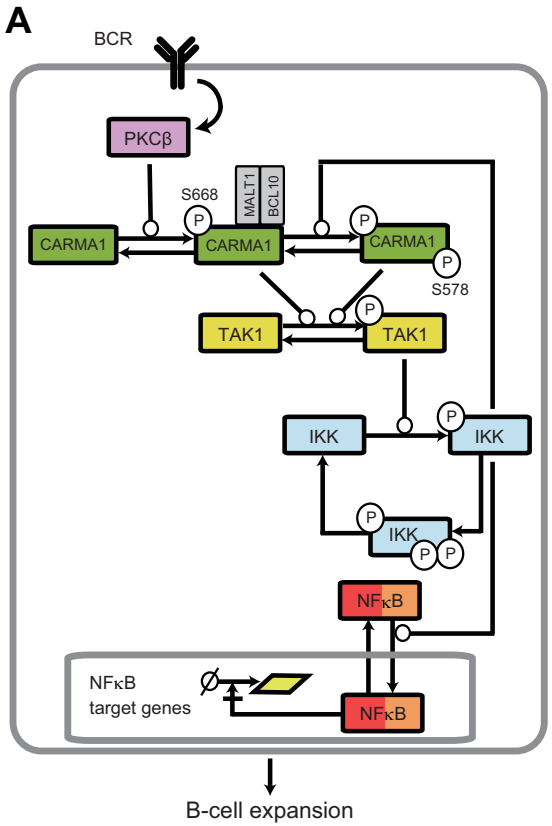


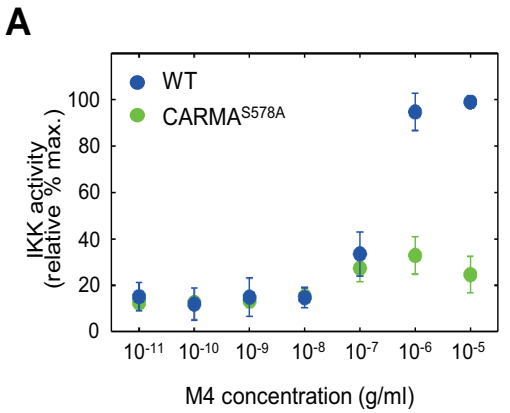


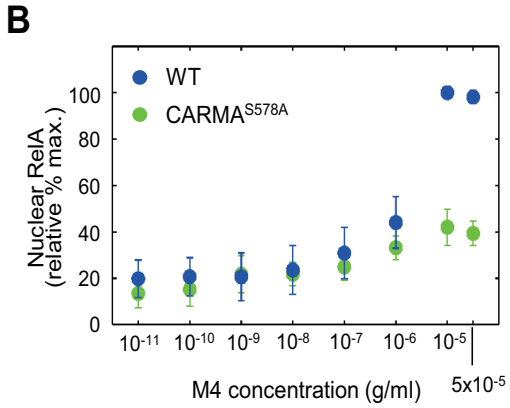
A key question:
Are B-cell fates determined by a race or by a decision?



The B-cell fate decision is in part mediated by a positive feedback switch within the IKK-TAK1-CBM signaling complex

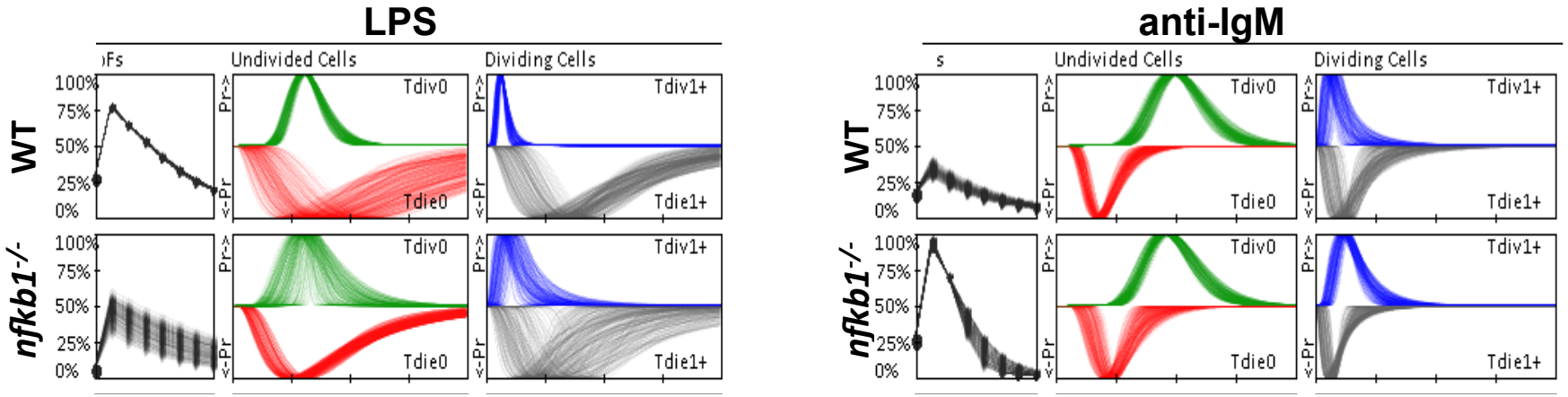






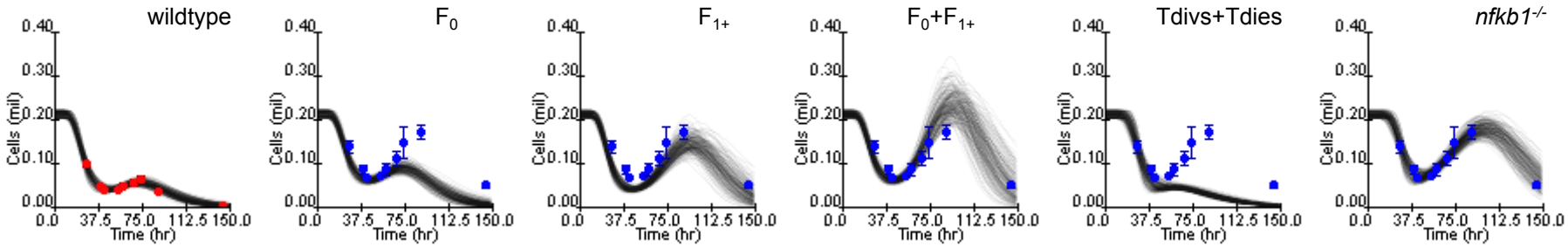
Shinohara, H.*, Behar, M.* et al … Hoffmann, A., Okada-Hatakeyama, M. 2014
Positive feedback within a kinase signaling complex functions as a switch mechanism for NFκB activation.
Science, **344**, pp.760-764.

Putting quantitative CFSE phenotyping to a test: nfkb1$^{-/-}$



anti-IgM

nfkb1$^{-/-}$ params added to WT models



WT cells nfkb1$^{-/-}$ cell measurements

nfkb1 controls the proliferative capacity past generation 1.

FLowMax has proven useful

89. Shokhirev, M.N., **Hoffmann, A.** 2013. FlowMax: a computational tool for maximum likelihood deconvolution of CFSE time courses. PLOS ONE, **8,** e67620. PMID:2382639, PMC3694893

100. Alves, B.N., Tsui, R., Almaden, J., Shokhirev, M.N., Davis-Turak, J., Fujimoto, J., Birnbaum, H., Ponomarenko, J., **Hoffmann, A.** 2014 IκBε is a key regulator of B-cell expansion by providing negative feedback on cRel and RelA in a stimulus-specific manner. J. Immunol., **192**, pp.3121-32. PMID: 24591377, PMC3965642

101. Shinohara, H.*, Behar, M.*, Inoue, K., Hiroshima, M., Yasuda, T., Nagashima, T., Kmura, S., Sanjo, H., Maeda, S., Yumoto, N., Ki, S., Sako, Y., **Hoffmann , A.**$^{+}$, Kurosaki, T.$^{+}$, Okada-Hatakeyama, M.$^{+}$ 2014 Positive feedback within a kinase signaling complex functions as a switch mechanism for NF-κB activation. Science, **344**, pp. 760-764. PMID: 24833394

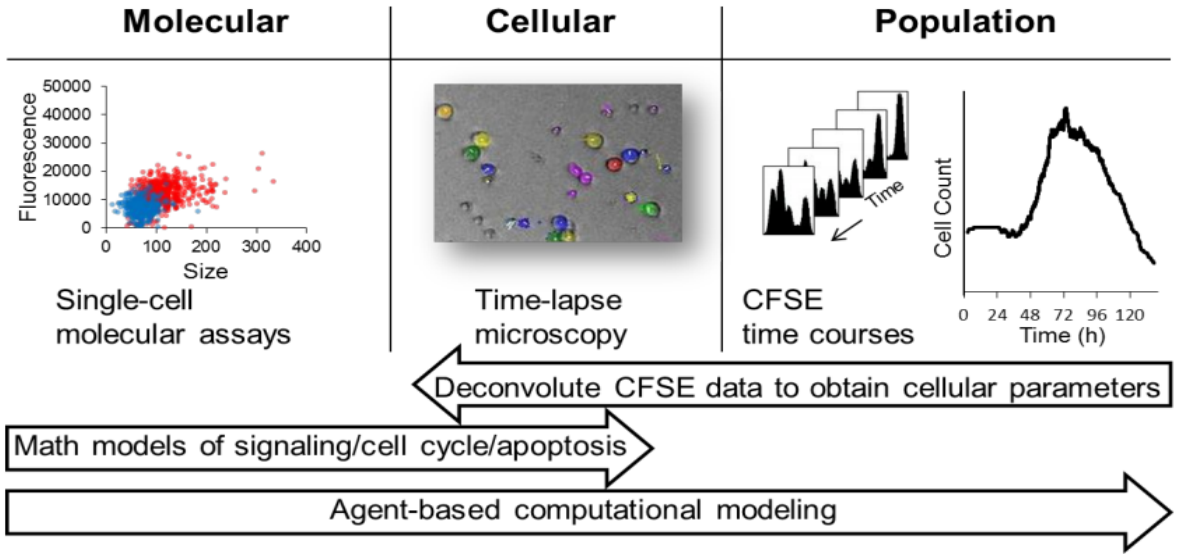
104. Almaden, J., Tsui. J., Liu, Y.C., Birnbaum, H., Shokhirev, M.N., Ngo, K.A., Davis-Turak, J., Otero, D., Basak, S., Rickert, R., **Hoffmann, A.**, 2014 A pathway switch directs BAFF signaling to distinct NFκB transcription factors in maturing and proliferating B cells. Cell Reports, **9**, pp.2098-111. PMID: 25497099

108. Shokhirev, M.N., Almaden, J., Davis-Turak, J., Birnbaum, H.A., Russell, T.M., Vargas, J.A., **Hoffmann, A.** 2015 A multi-scale approach reveals that NFκB cRel enforces a B-cell decision to divide. Molecular Systems Biology, **11**, pp.783-96. PMID: 25680807

Do we have enough information to construct a model of B-cell proliferation ?

- how molecular mechanisms in each cell determine the cell biological parameters of that cell

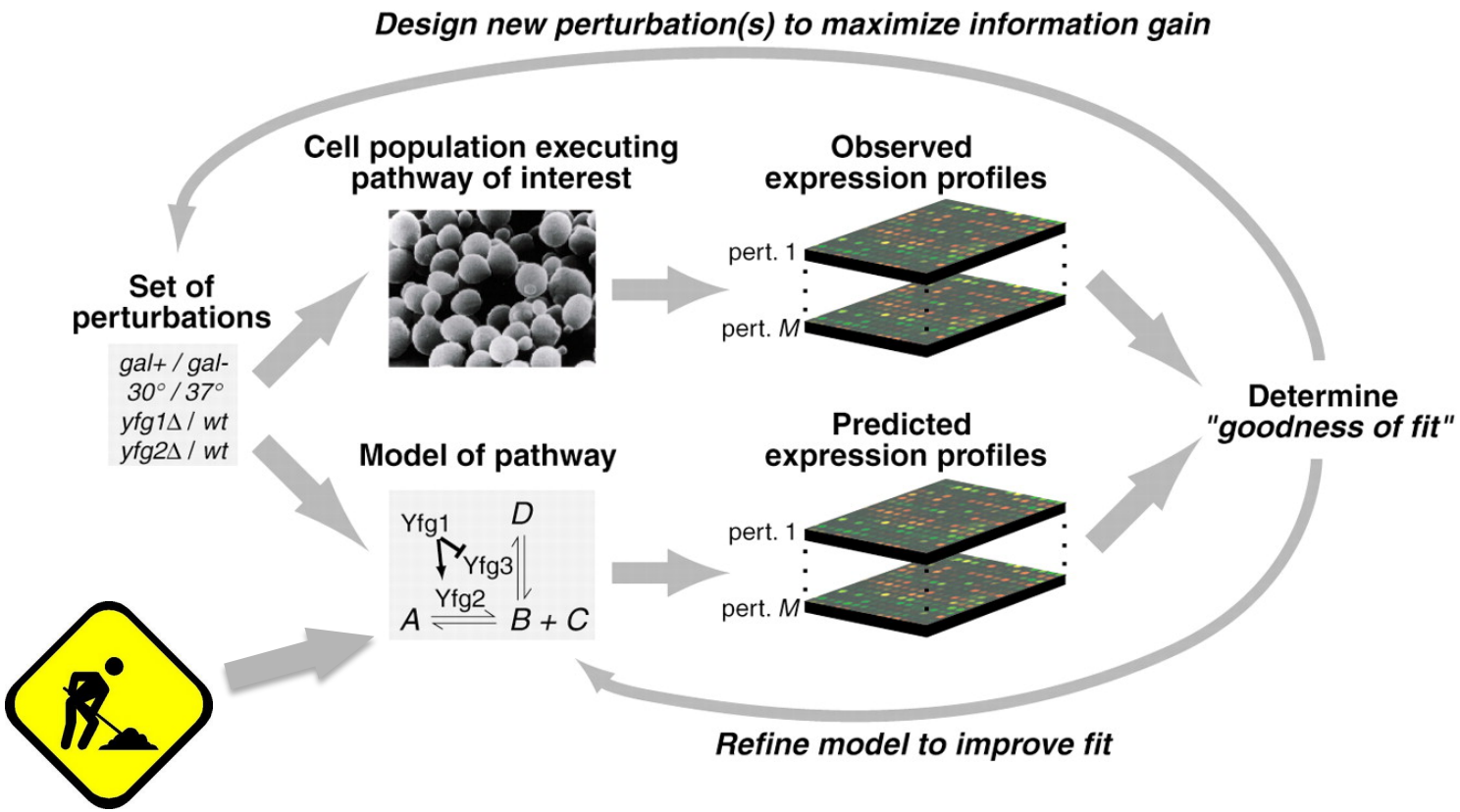
- and thereby determine the overall population dynamics



Why would such a model be useful?

1. **Sufficiency test**
2. **Quantify the contributions of different molecular mechanisms**
3. **Develop hypotheses about drug targets, mechanisms, etc**
4. **Assist in design or interpretation of experiments**
5. **Virtual experimentation can be very high-throughput, and can address questions intractable by available experimental tools**

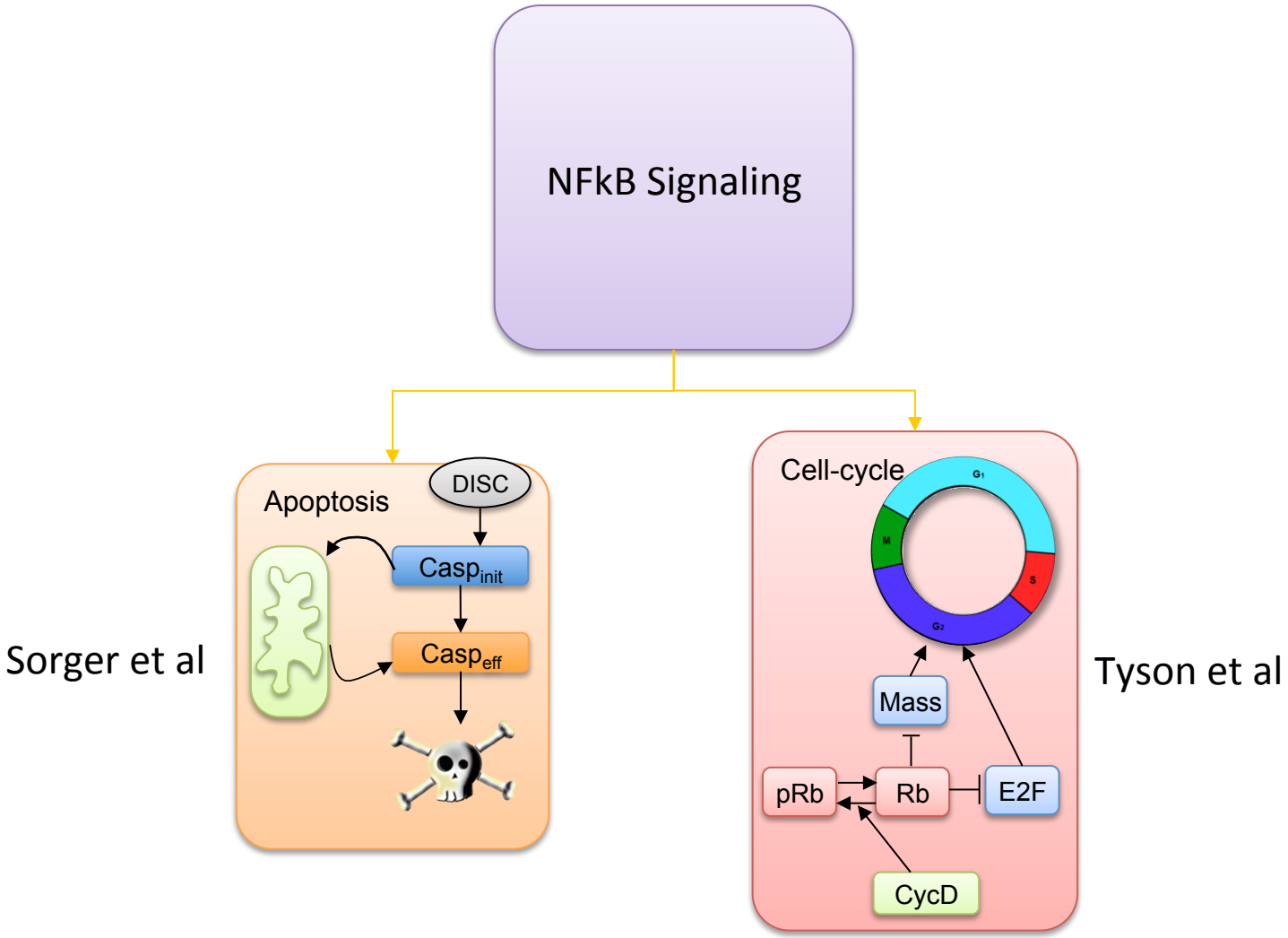
A math model enables the Systems Biology approach



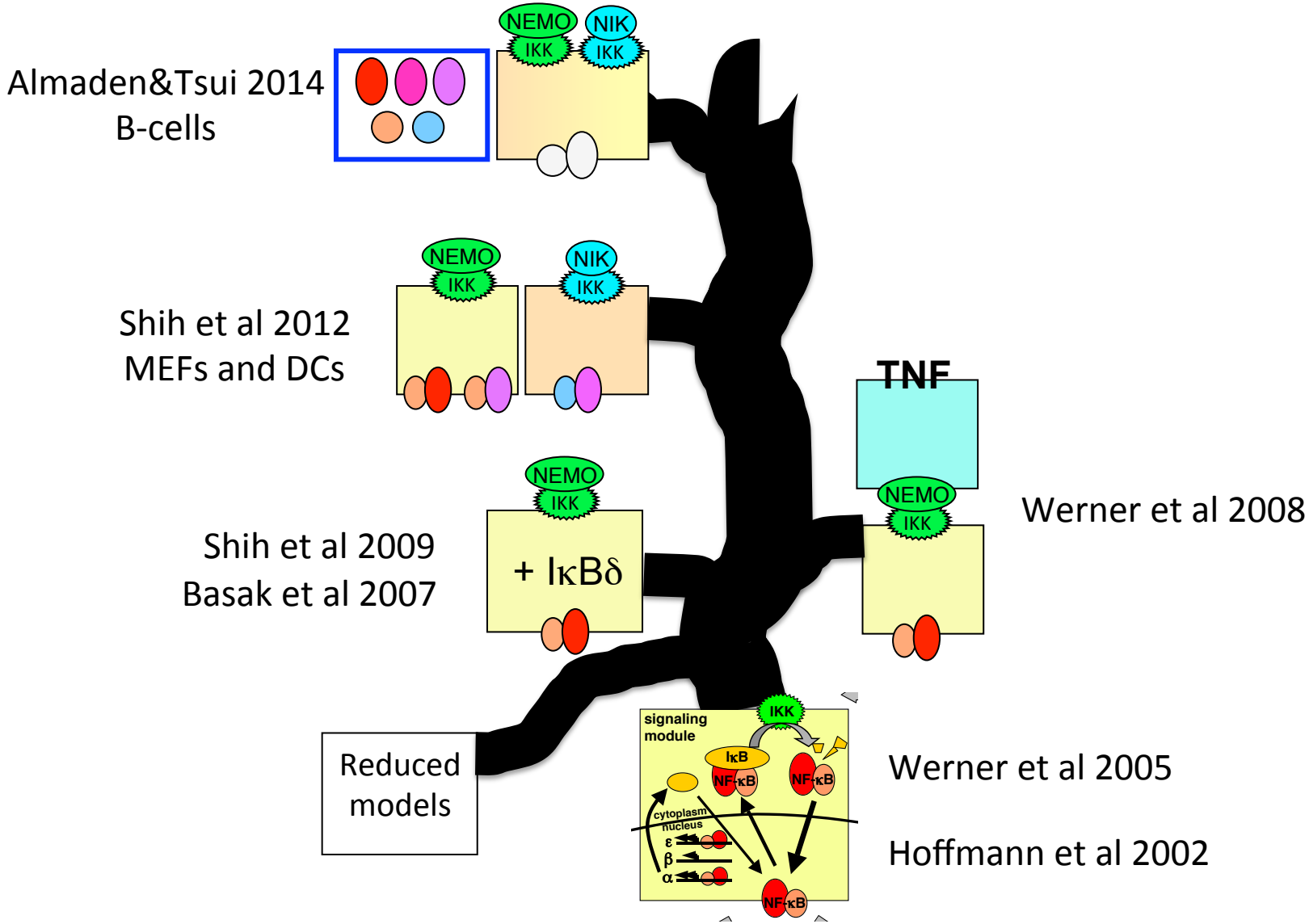
Model construction

Ideker, Galitski, Hood 2001 "A new approach to decoding Life: Systems Biology" Annual Review of Genomics and Genetics vol 2, pp.343-372

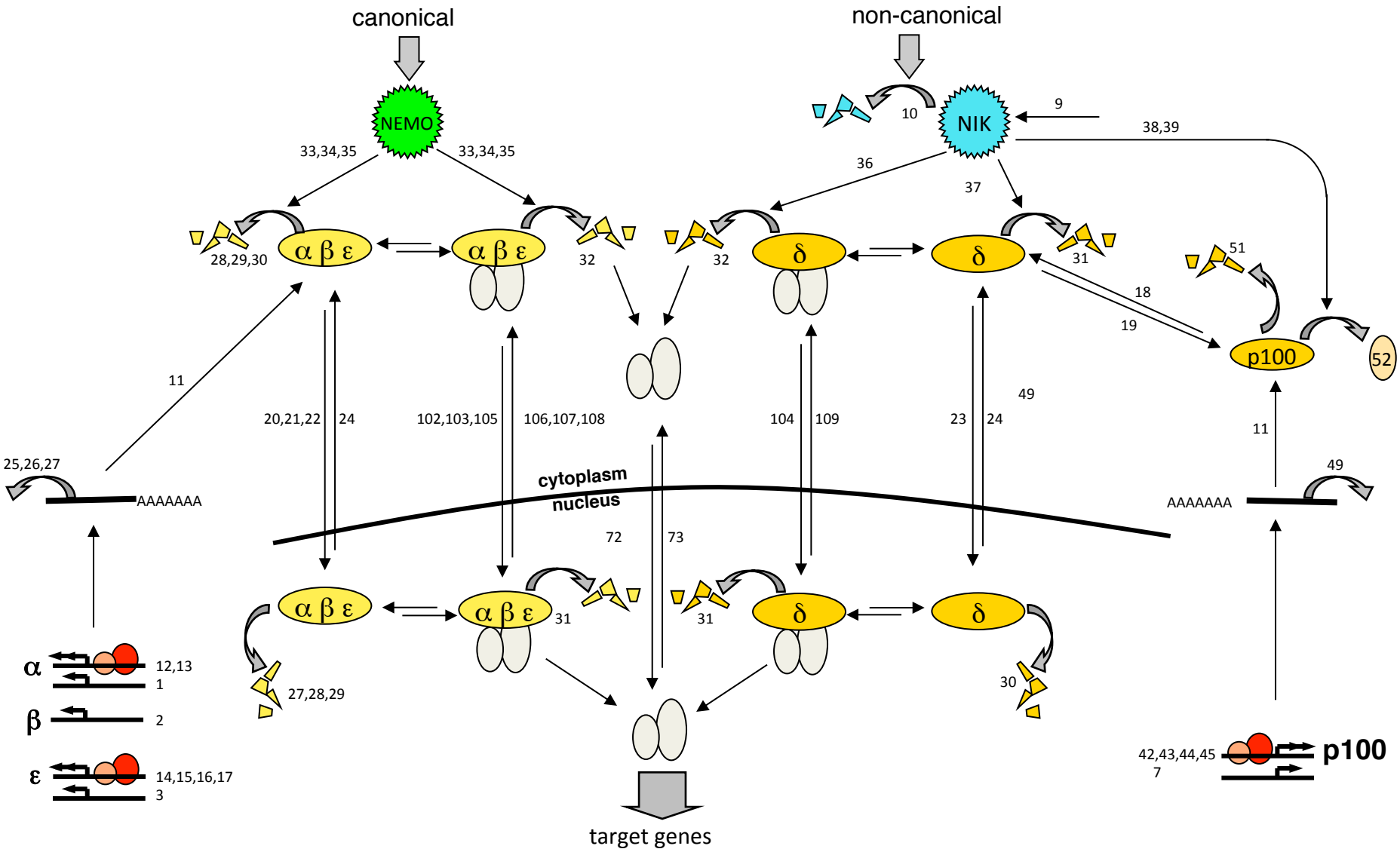
An outline of the model in each cellular agent



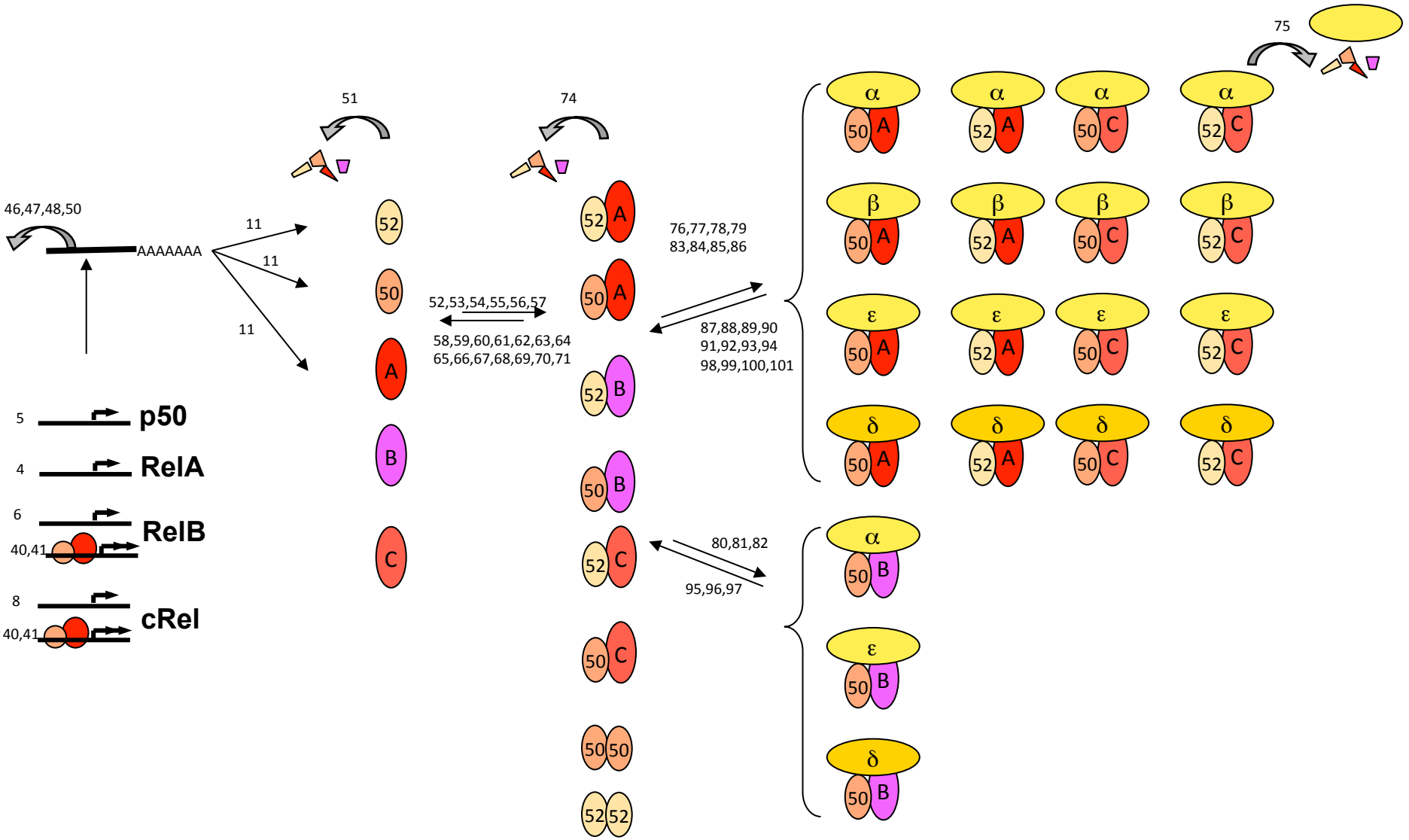
The lineage of NFκB signaling models



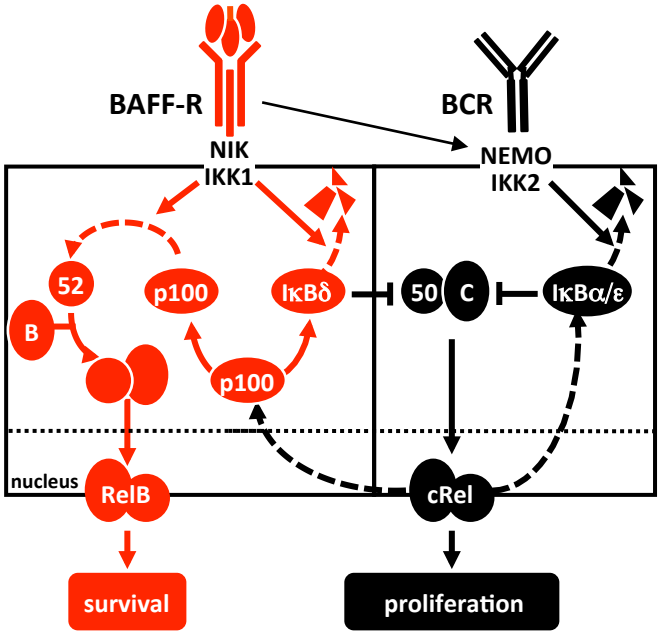
The IκB-NFκB Signaling Module

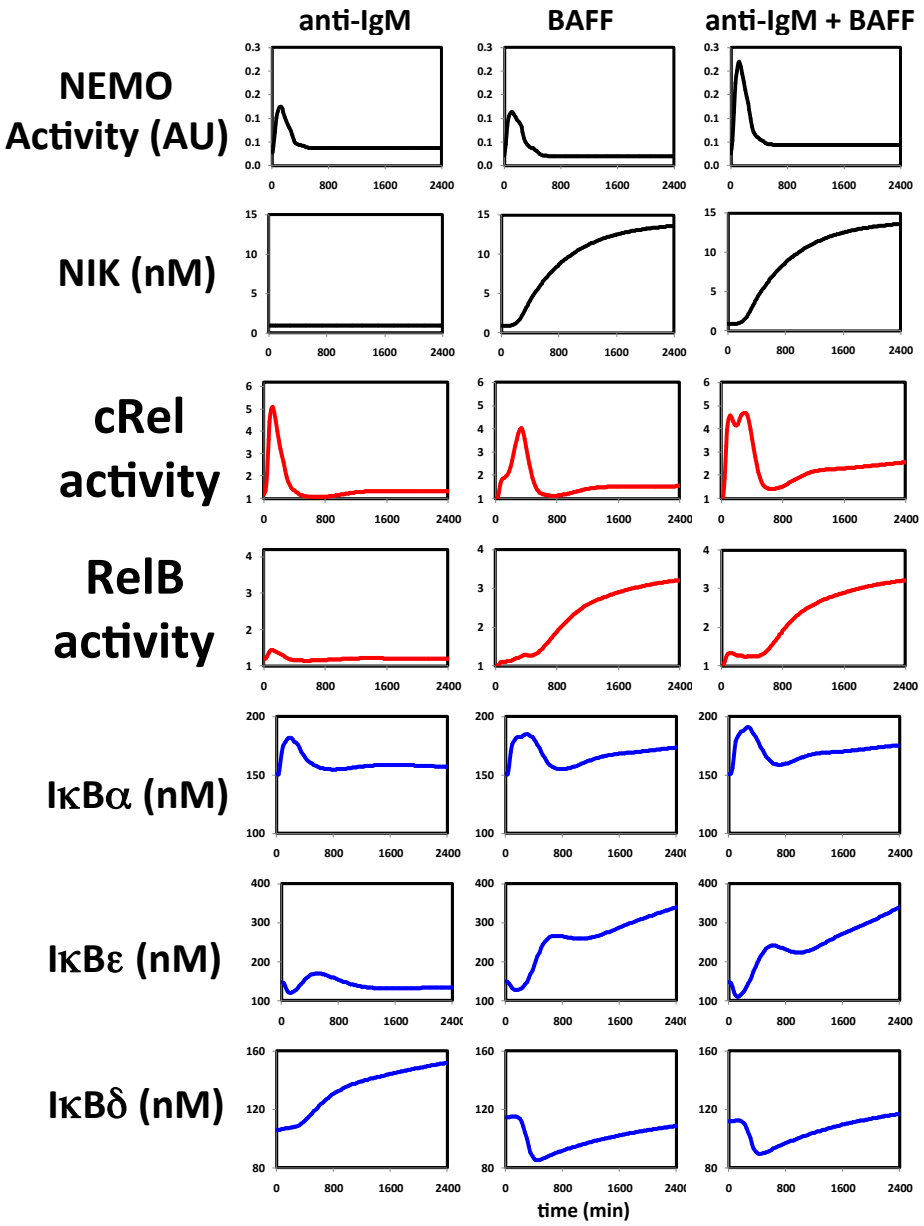


The Rel-NFκB Dimer Generation Module



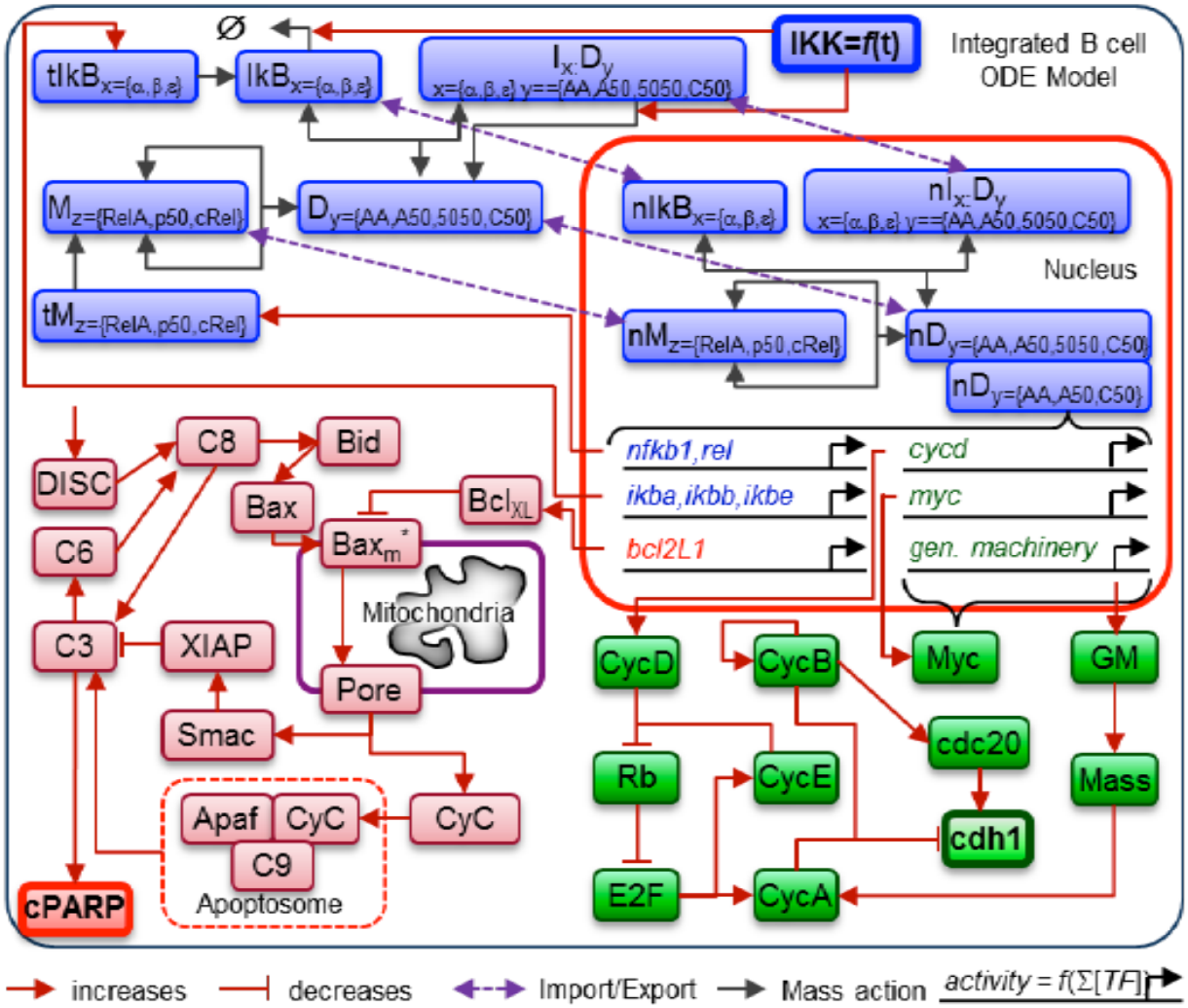
Model accounts for many NFkB dimers in response to several mitogenic and maturation stimuli



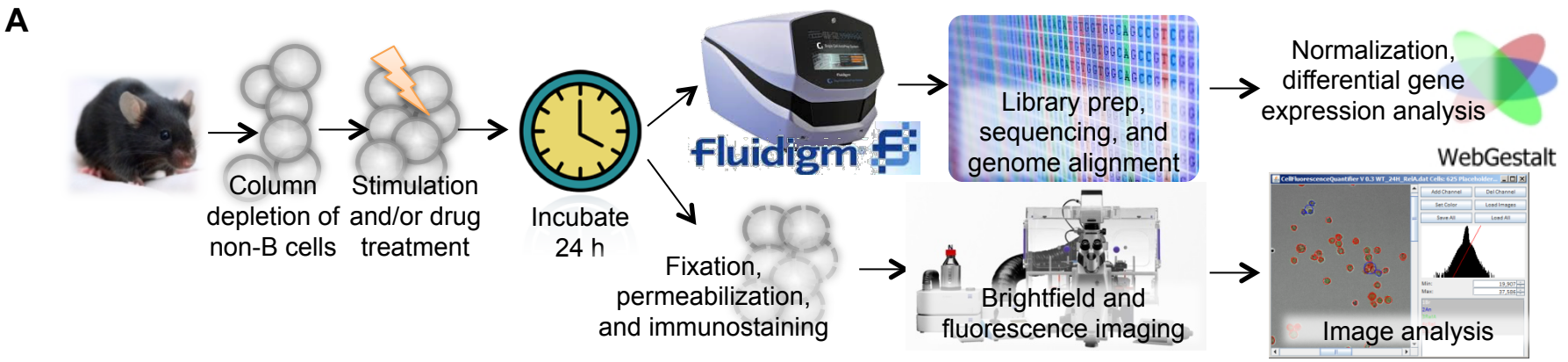


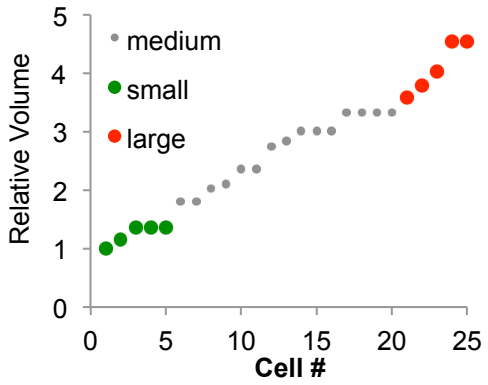
Almaden et al 2014

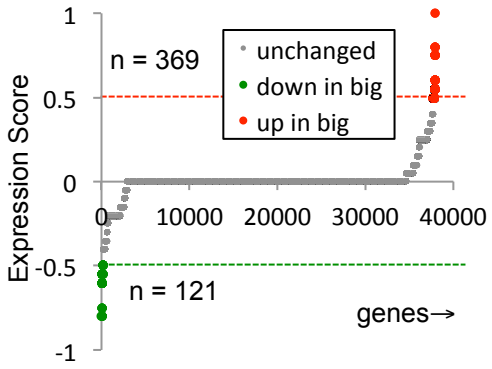
The three interconnected modules of the model



Parameterizing the model connections with single cell data







D

Metabolism
B cell R. Signaling
TNFα NFκB S.
G1 to S control
Apoptosis
TLR Signaling
Cell cycle

Myometrial
Chemikine
Integ. Cell adhesion
Sen./Autophagy

E

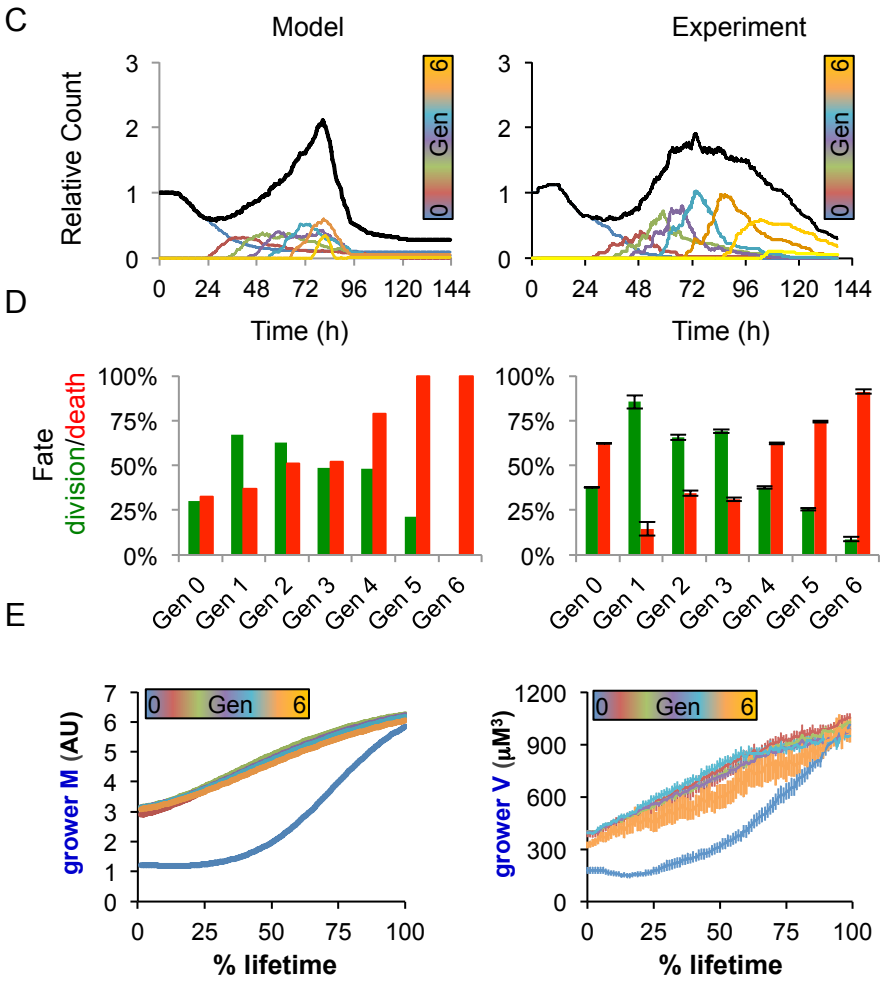
NFkB Signatures in Big Cells

NFκB TF	adj. p-value	En. Ratio
NFKB_C	*0.0263*	*2.89*
MYC	1.08E-06	2.96
CMYB	0.000005	5.49
YY1	0.0007	3.38
E2F3	0.0046	3.72
LEF1	0.0072	1.72
STAT5A	0.0096	4.5
SOX9	0.0162	3.24
CREB	0.02	3.1
EGR1	0.0309	2.79

NFkB Signatures in Small Cells

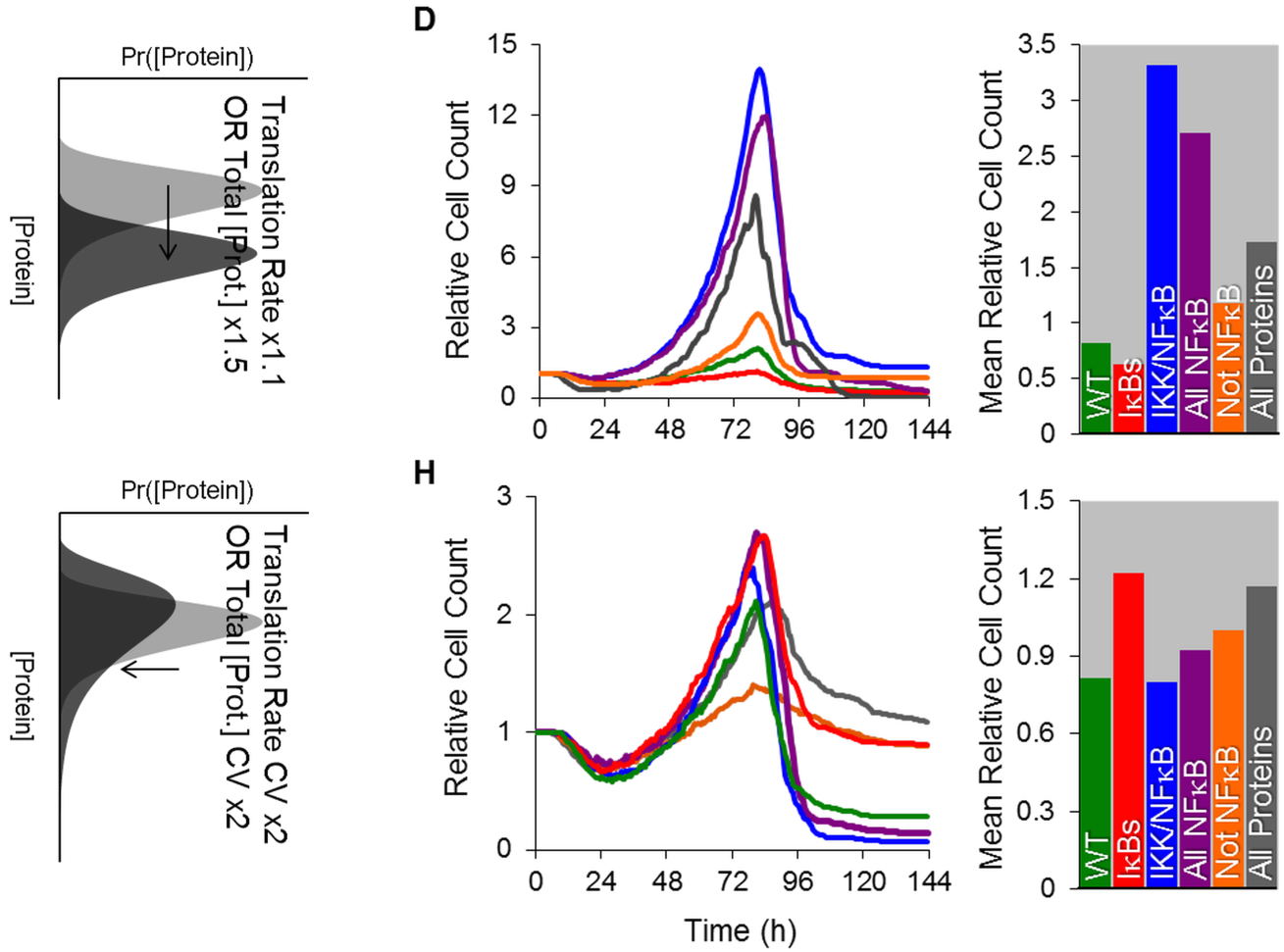
NFκB TF	adj. p-value	En. Ratio
p53	0.022	6.11

Simulations of B-lymphocyte dynamics



One question that cannot be addressed experimentally:

Is the cell-to-cell variability of gene expression important?



What can you do with the model?

- Test modulators of NFkB, mIRs, lymphoma mutations
- How signals combine
- Why different B-cell subsets respond differently
- Pharmacology
- How class switching affects BCR signaling and populations
- Add additional mechanisms: memory/plasma cell; follicular vs marginal zone cell differentiation

Dinesh Rao
UCLA

Yi Liu

Koushik Roy

Jesse Vargas