To Sleep or Die:
Cell fate determination after genotoxic therapy

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Fields Institute – Mathematical Oncology
March 19th, 2010

American Cancer Society and NIH/NCI Integrative Cancer Biology Program

What are we trying to accomplish in this meeting? (Stating the obvious!)

1. Educate mathematicians about work at the interfaces between mathematics and medicine and mathematics and biology.

2. Motivate mathematicians and biologists to engage with each other to create NOVEL approaches to BOTH disciplines.

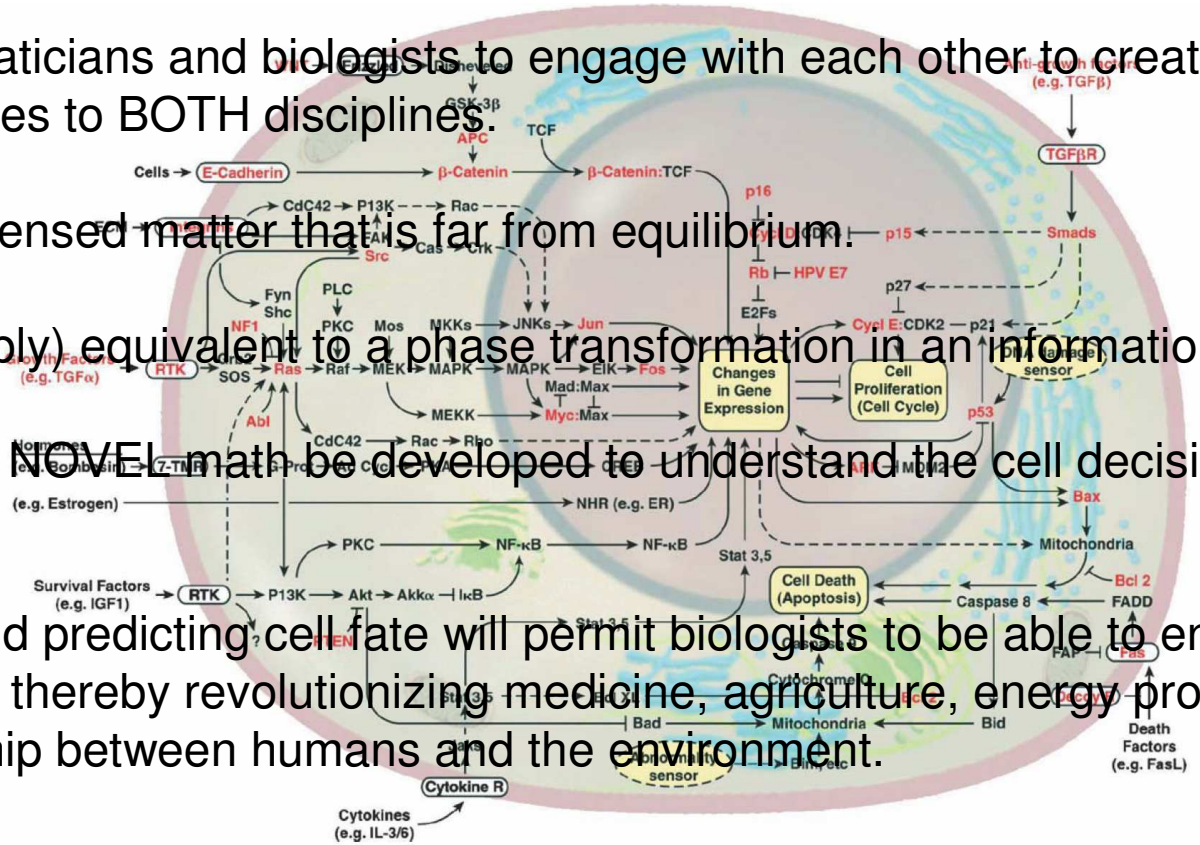
3. A cell is soft condensed matter that is far from equilibrium.

4. Cell fate is (possibly) equivalent to a phase transformation in an information field.

5. It is essential that NOVEL math be developed to understand the cell decision process.

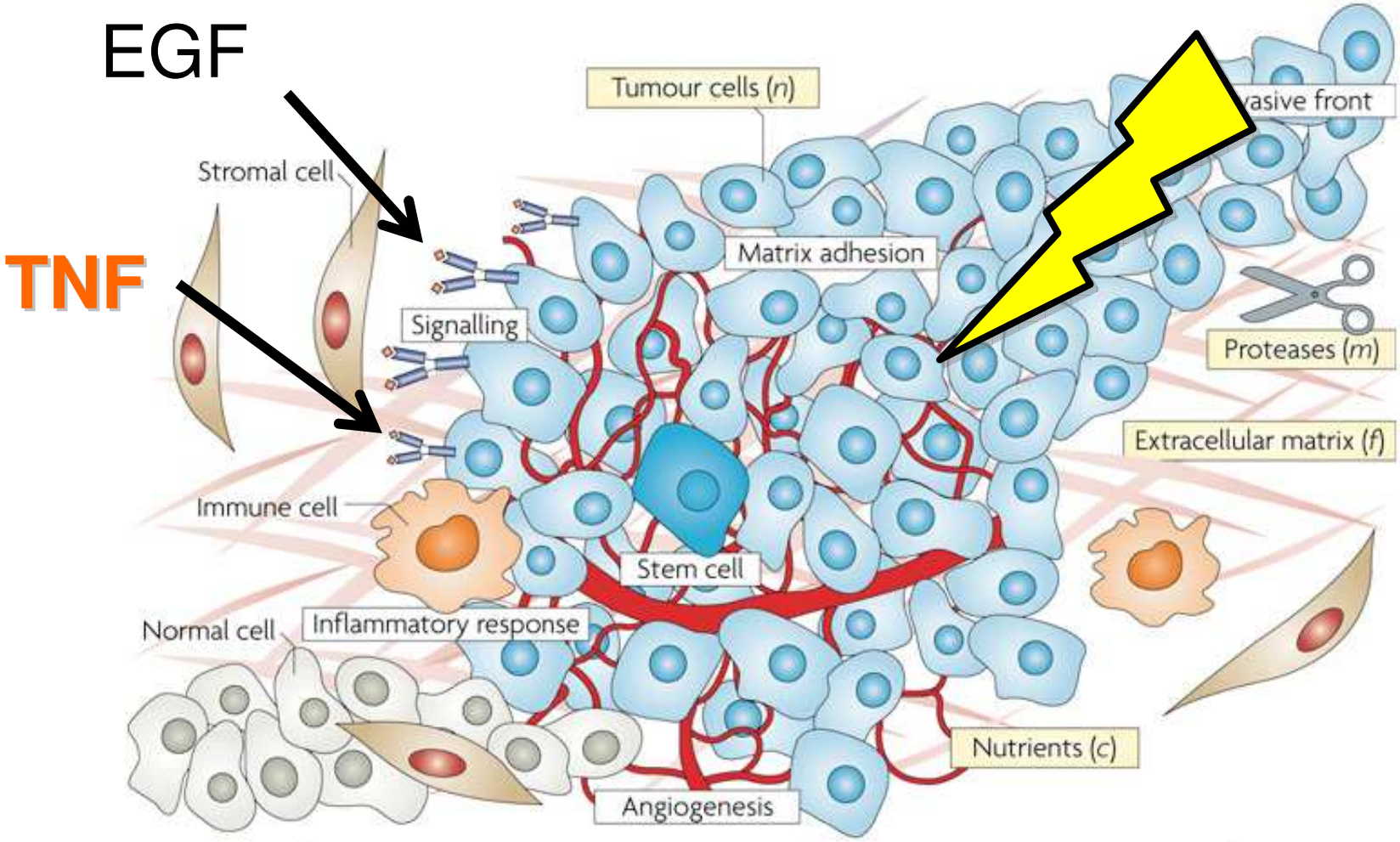
6. Understanding and predicting cell fate will permit biologists to be able to engineer cellular outcomes thereby revolutionizing medicine, agriculture, energy production and the relationship between humans and the environment.

7. Issues such as ENTROPY and INFORMATION are covert in molecular/systems biology. Can we bring them into the light?



D. Hanahan and R.A. Weinberg. *Cell* (2000)

We aim to develop quantitative models of how cancer cells respond to clinically relevant genotoxic stress.



Alexander Anderson and Vito Quaranta

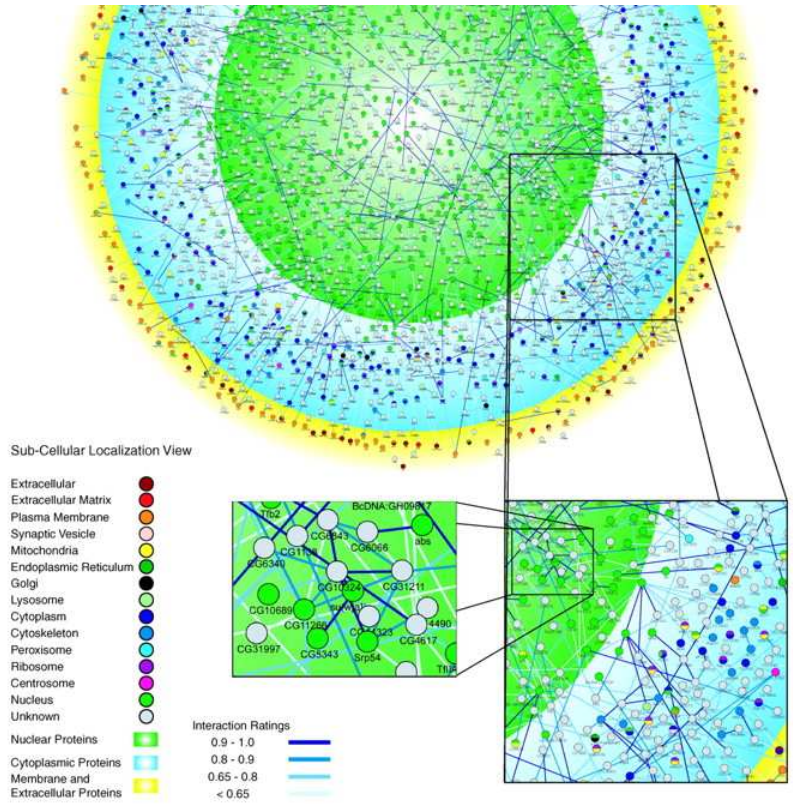
Nature Reviews | Cancer

Systems biology seeks to characterize the underlying principles and function of biomolecular networks

Experimental systems biology has two limits:

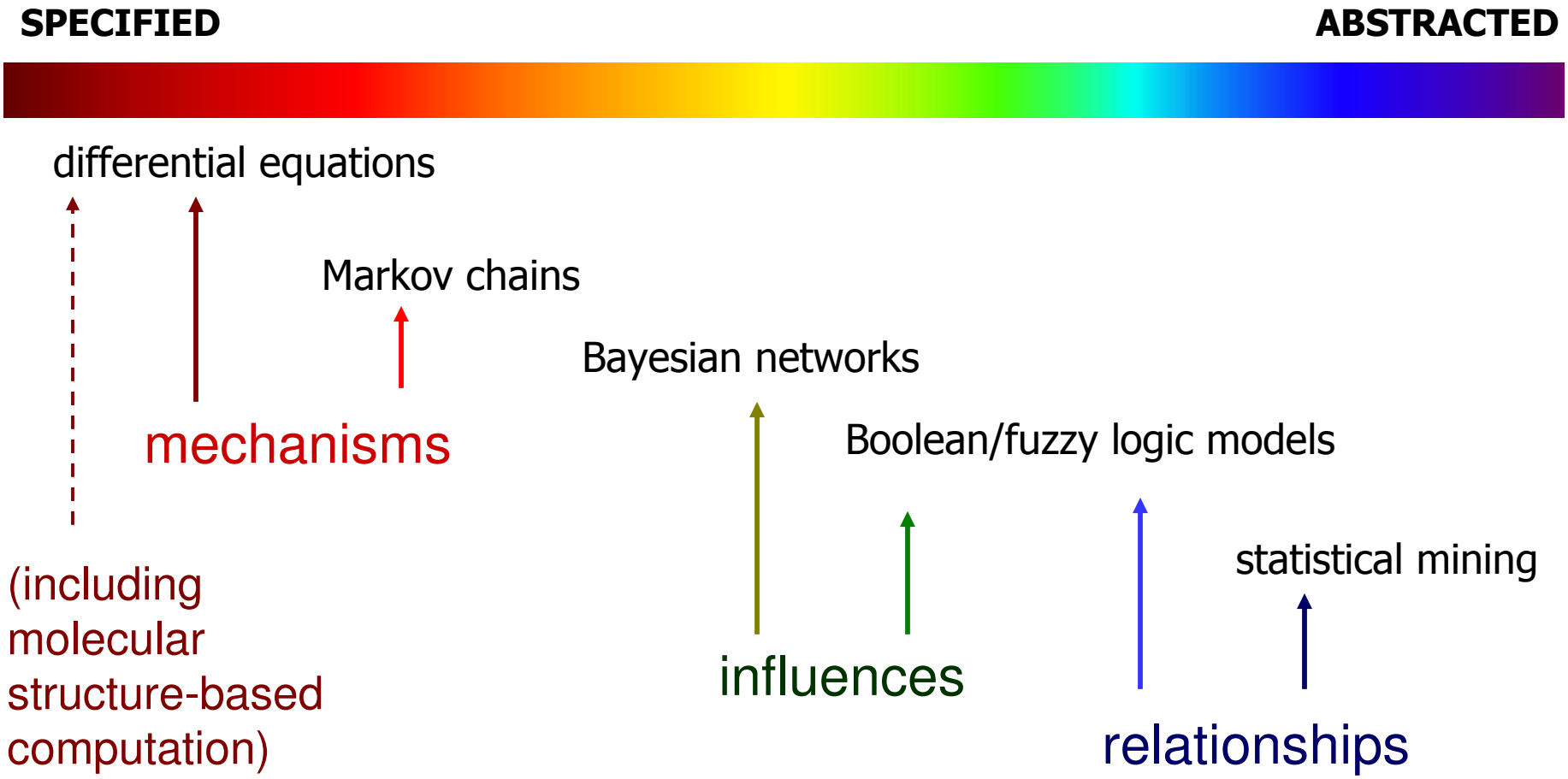
Quantifying the edges

Finding new nodes



Giot et al. Science 2003

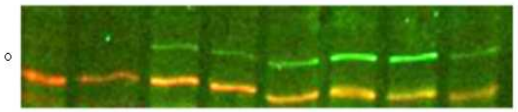
Computational cell biology seeks to create quantitative, predictive models of biomolecular networks

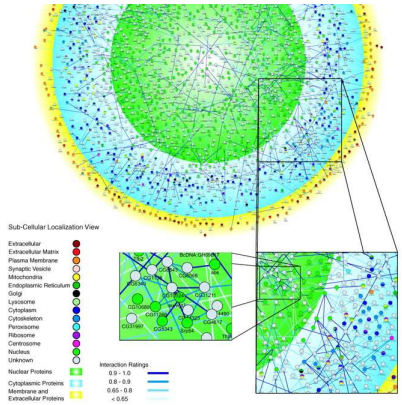


Modern biology consists of both discovery science and hypothesis driven research

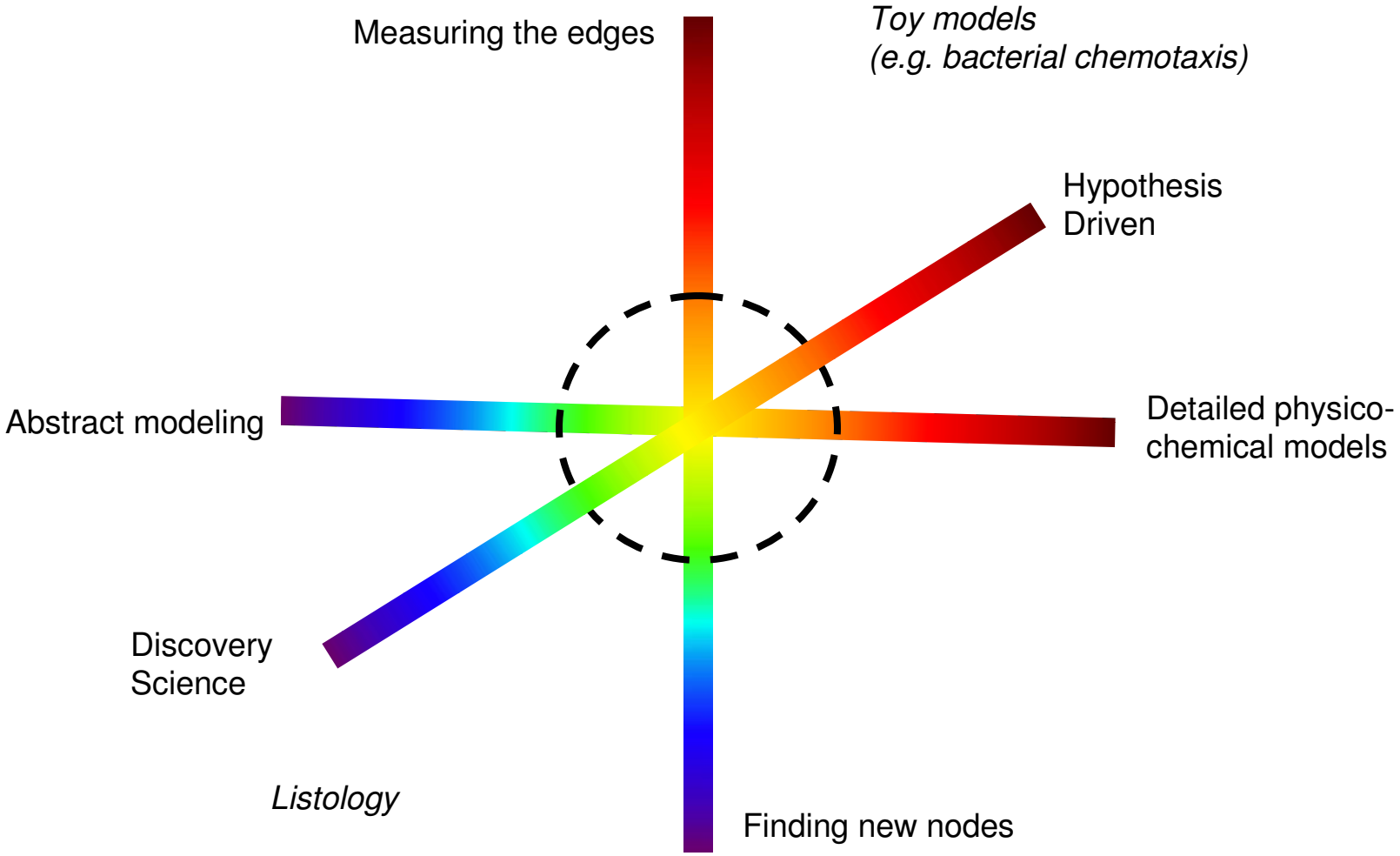
Hypothesis Driven

Discovery Science

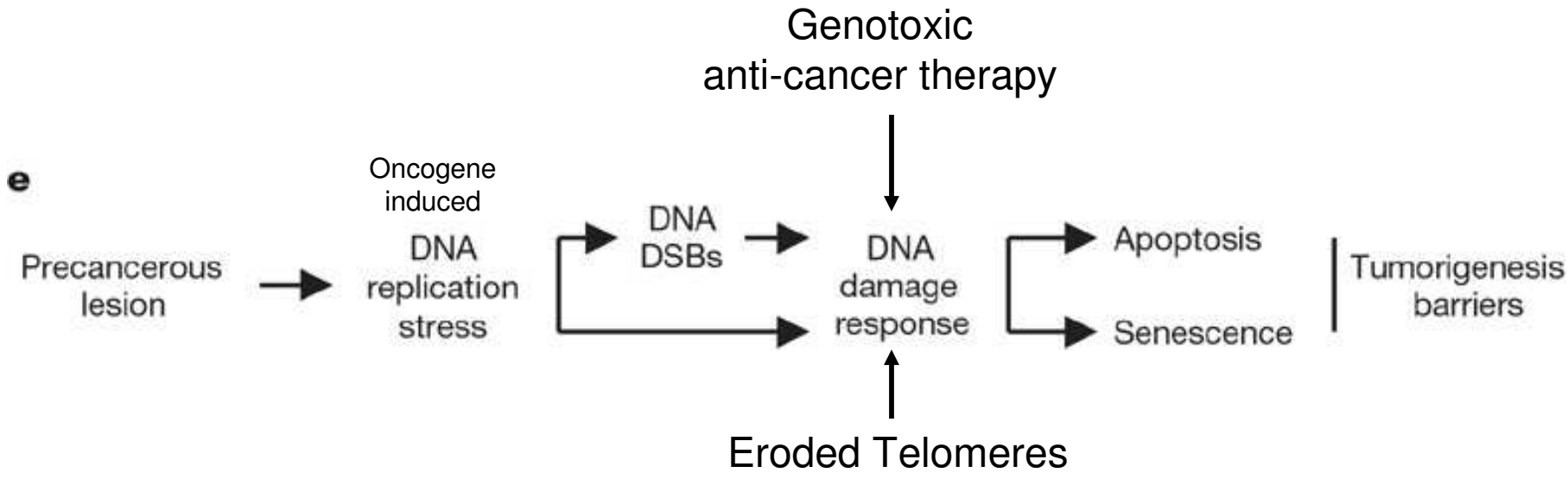


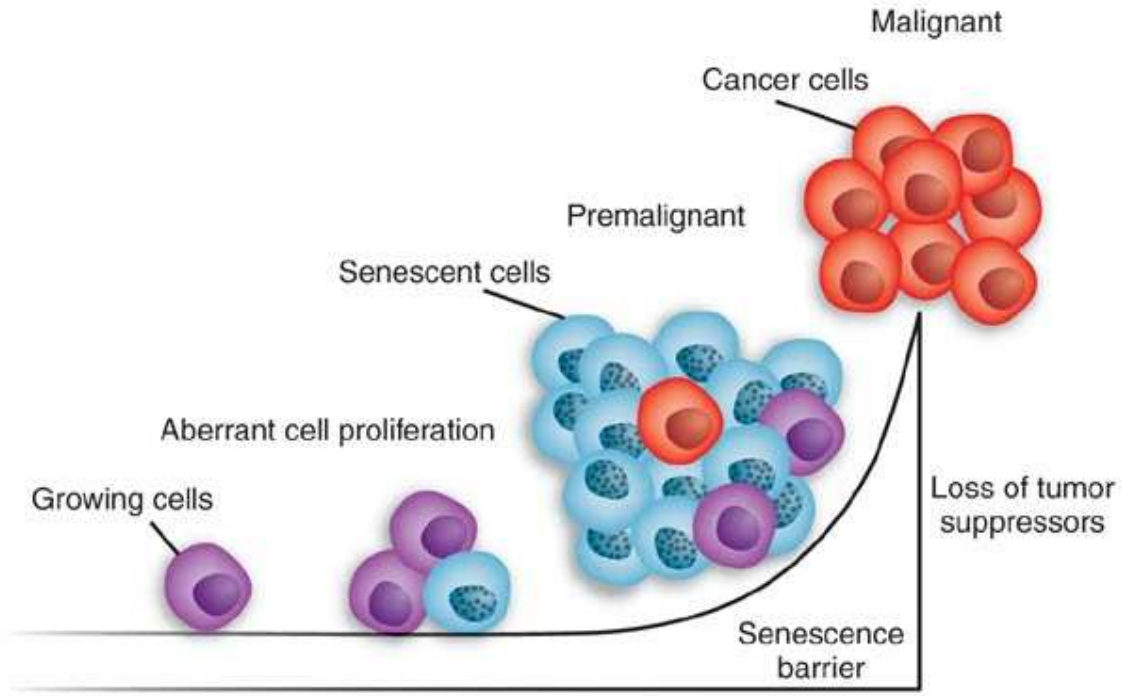


We seek the middle ground in order to best produce "New Biology"

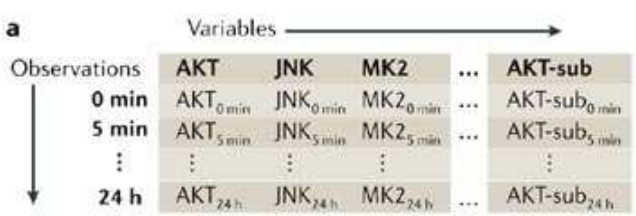


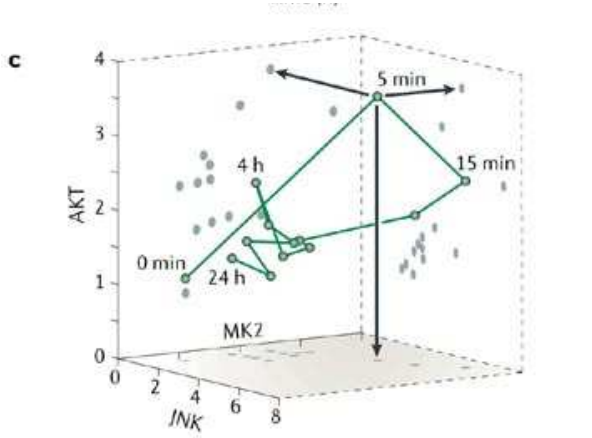
The DNA damage response is integral to preventing to tumorigenesis.

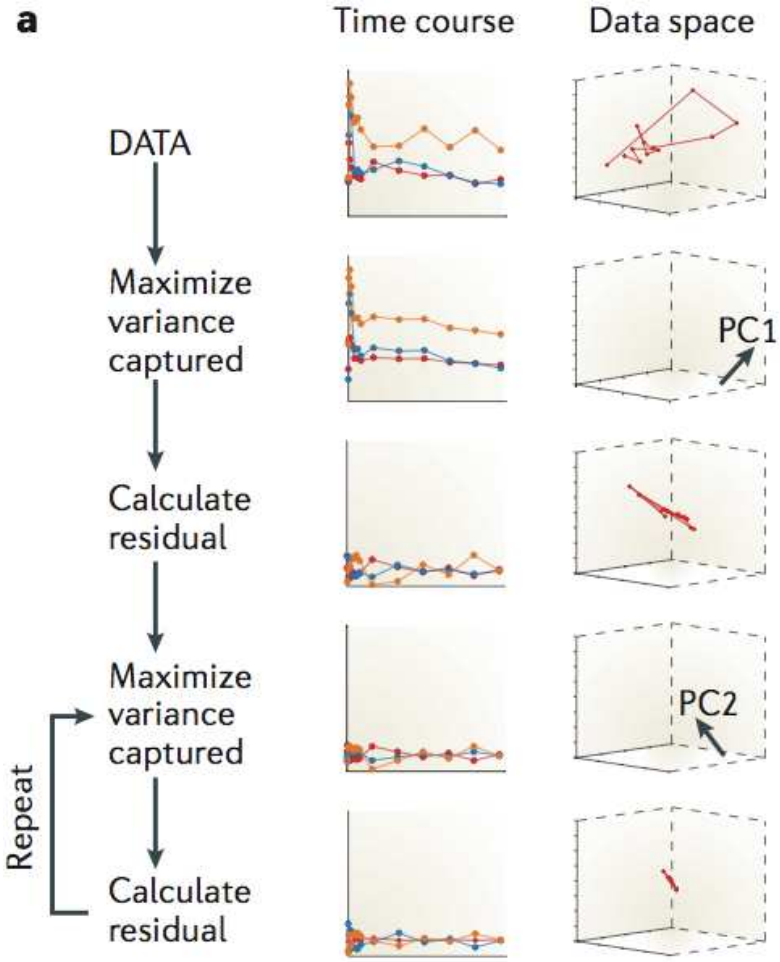




Principle Component Analysis and PLSR reduce multi-dimensional data sets into a set of vectors along orthogonal axes.

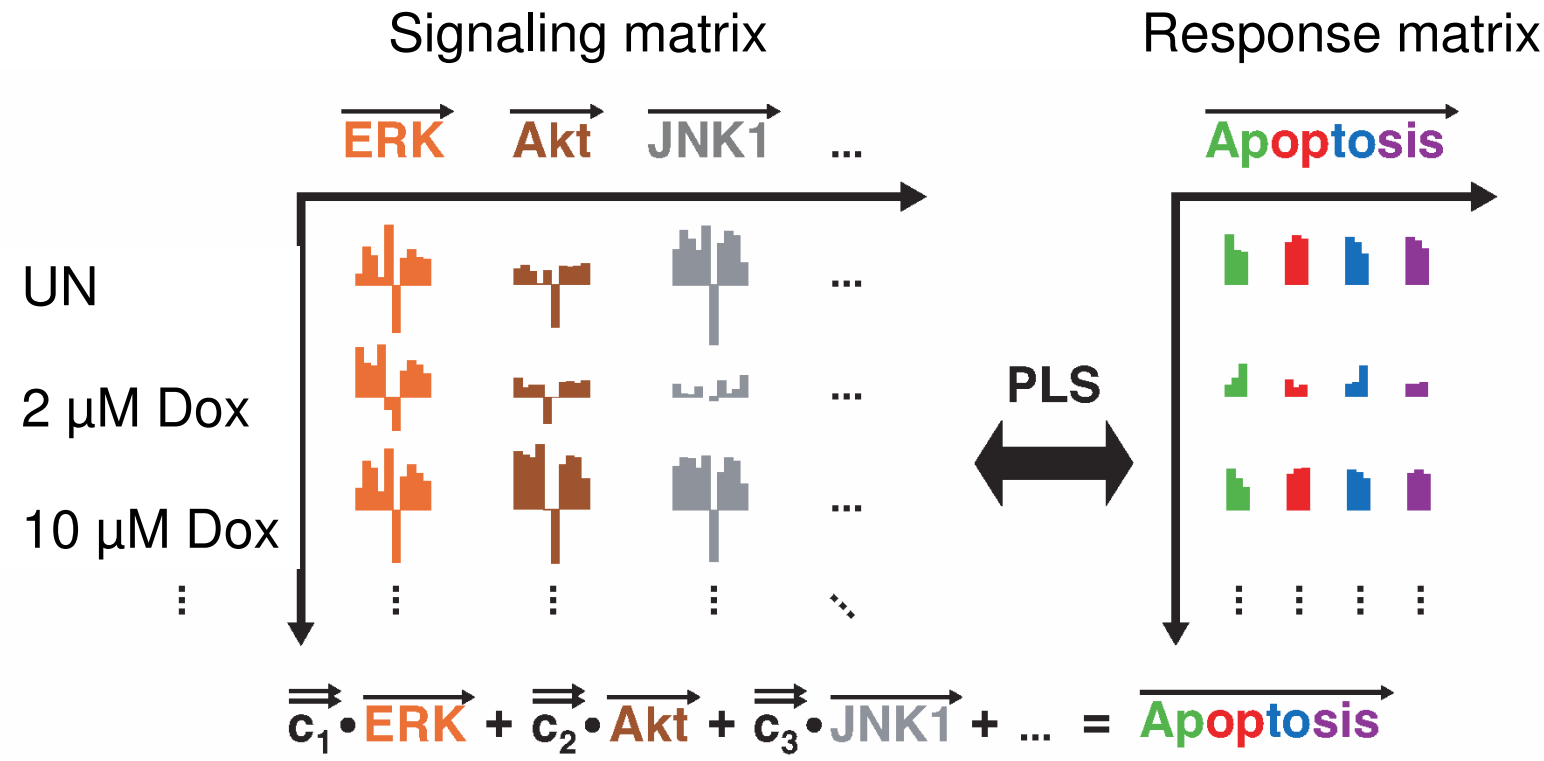






Janes and Yaffe, 2007

We use Partial Least Squares Regression to establish quantitative relations between signaling and cellular responses



+ Cell Cycle Arrest

+ Senescence

The modeling is unsupervised, but the selection of measurements is guided by prior biological knowledge.

Network Measurements of the DNA Damage Response

Untreated

2 µM Dox

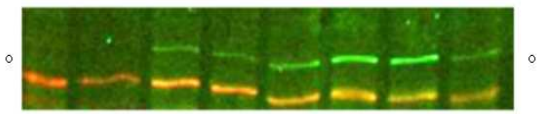
10 µM Dox

+ Dox/TNF

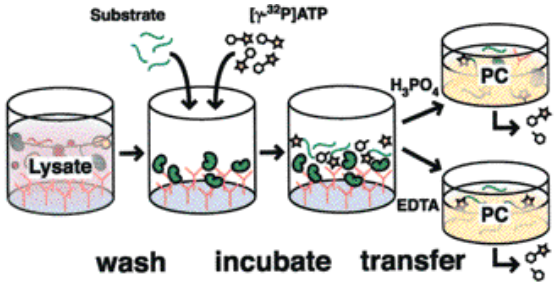
Replace with
1% Serum Media

1 2 4 8 12 16 24 48 72 96

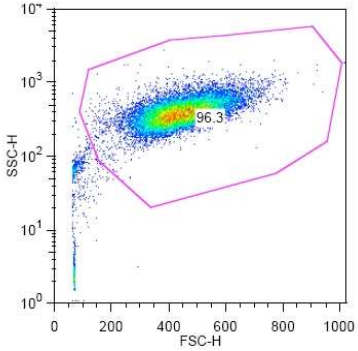
Western Blots



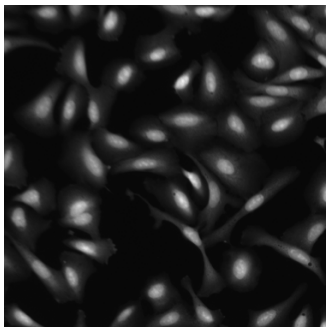
Kinase Assays



Flow Cytometry

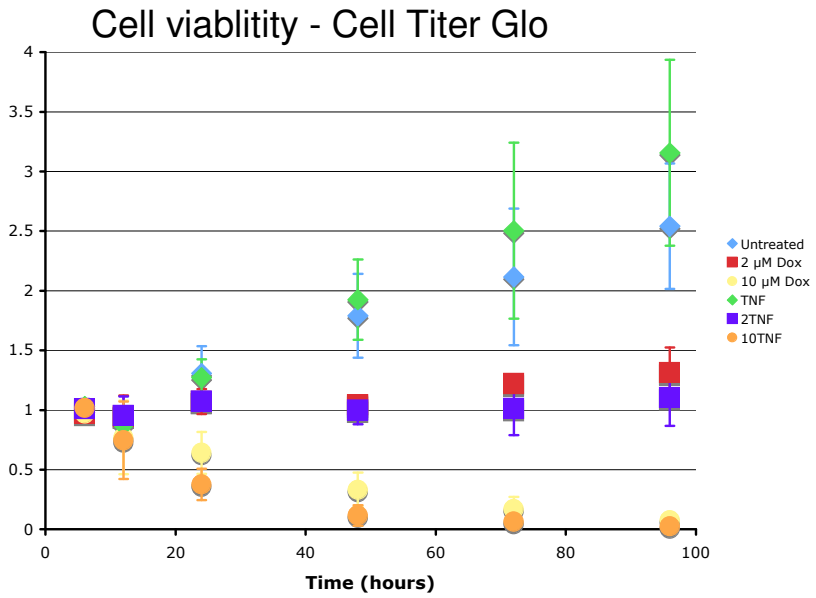


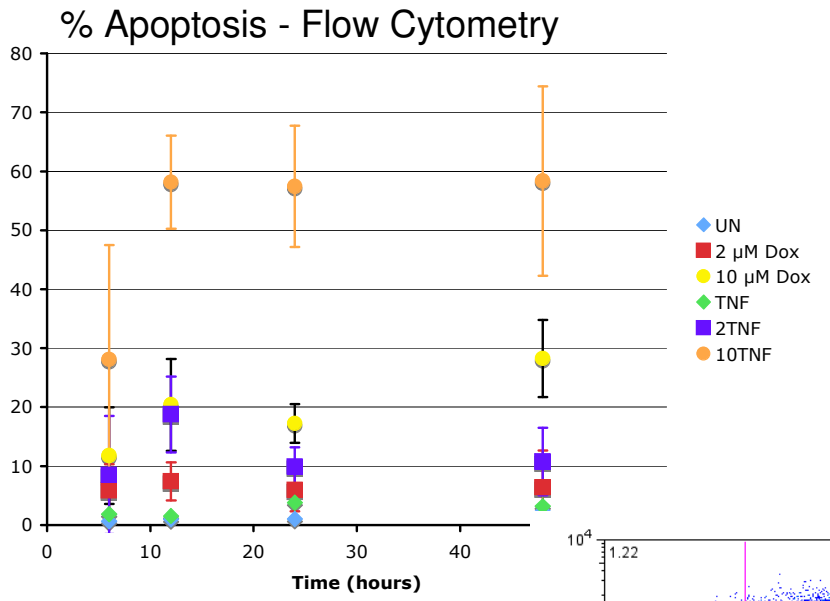
Microscopy

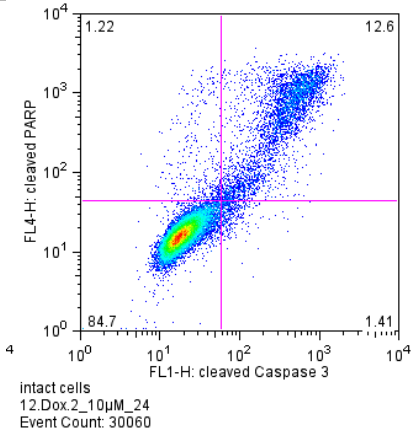


The first step in our “New Biology” discovery algorithm is quantifying the cellular response of interest

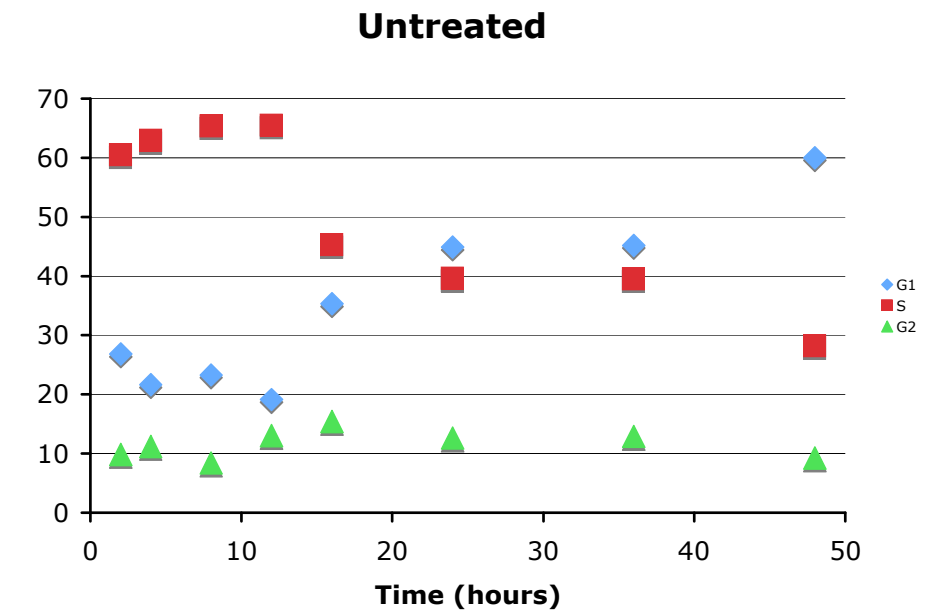
2 µM doxorubicin arrests cell growth and 10 µM doxorubicin induces apoptosis

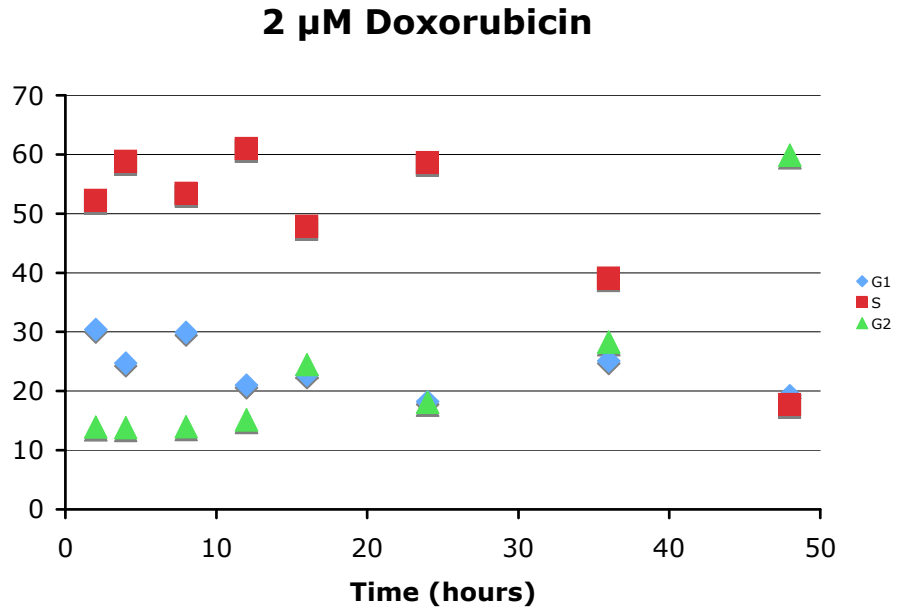




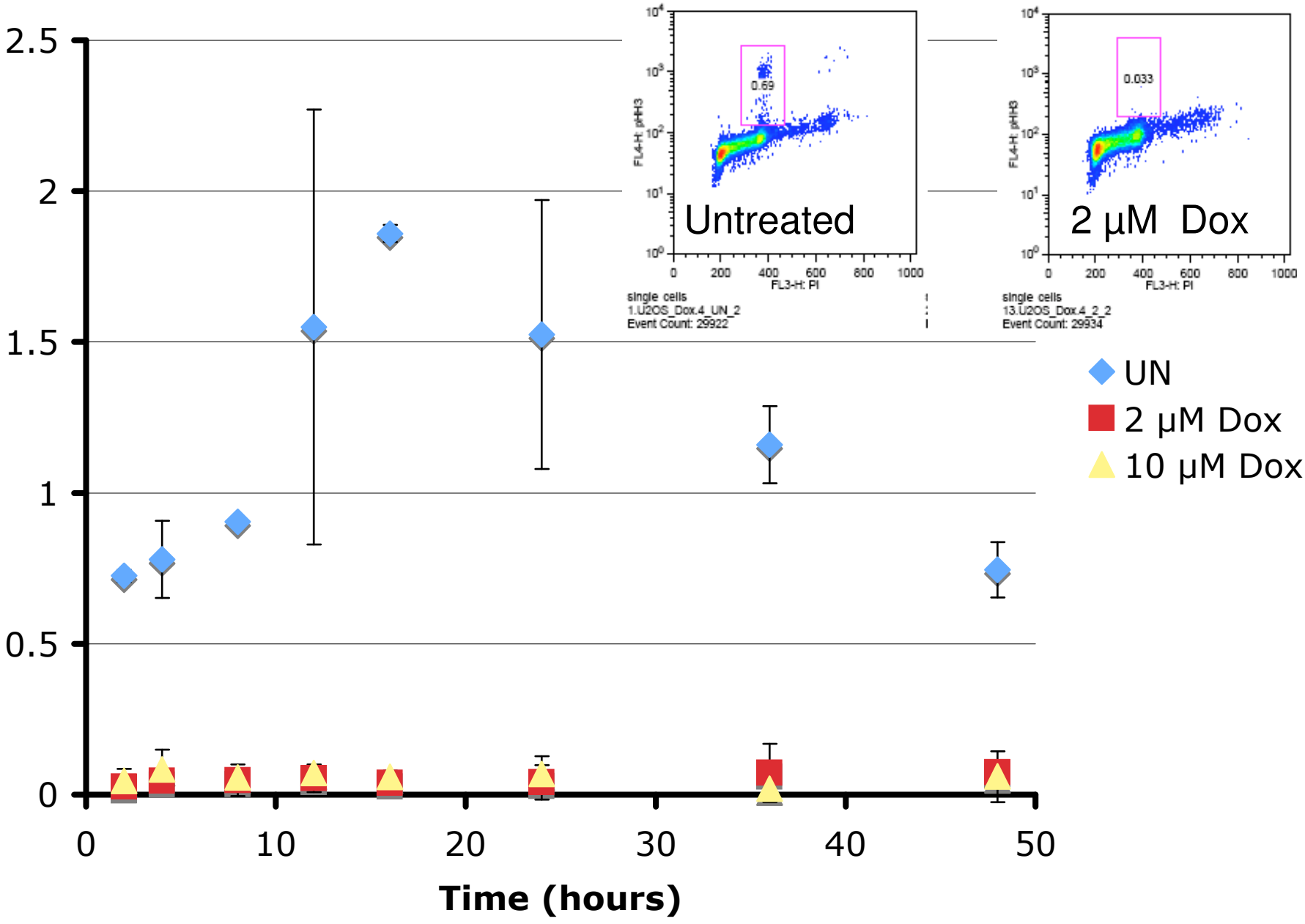


2 µM Doxorubicin leads to a G2 arrest

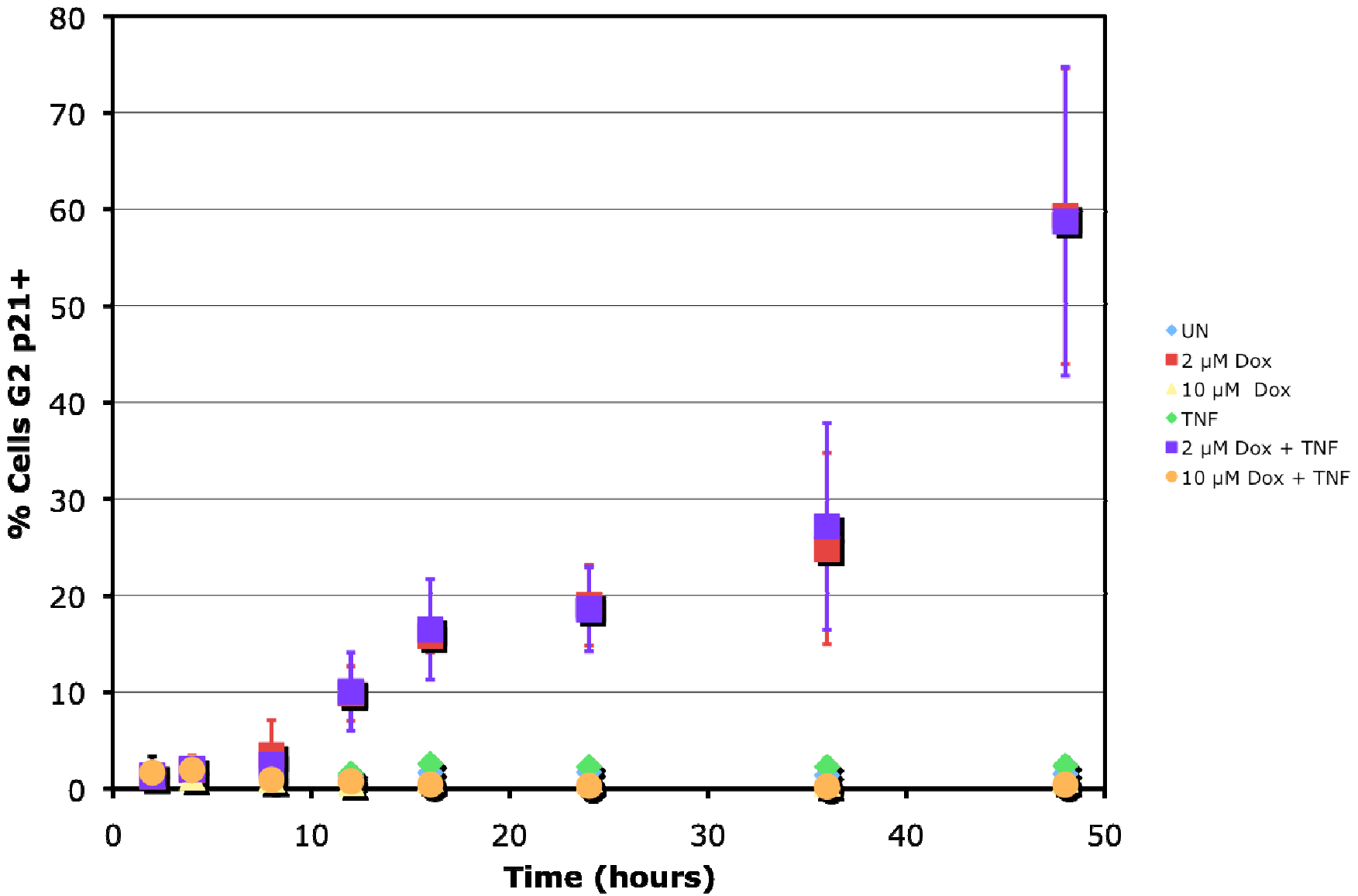




2 µM and 10 µM Doxorubicin treatment blocks mitosis

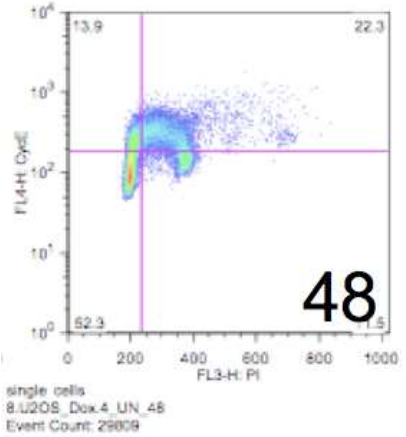


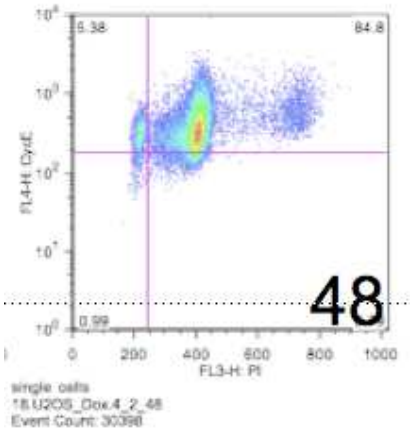
2 µM Dox treatments lead to a G2 population that is p21+



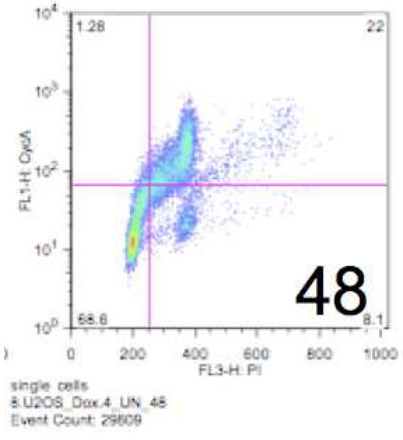
2 µM Doxorubicin induces a 4N, G1/S-like permanent arrest.

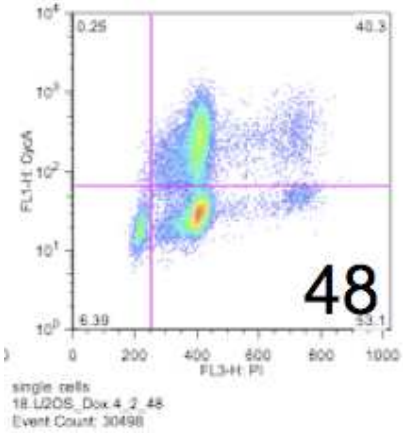
Cyclin E



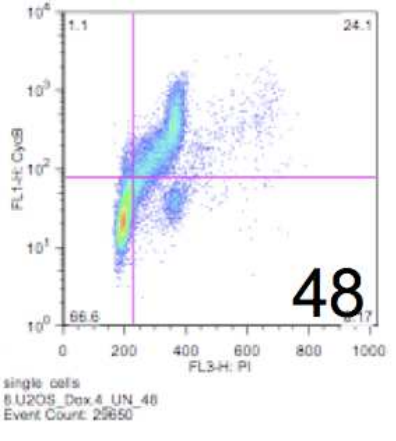


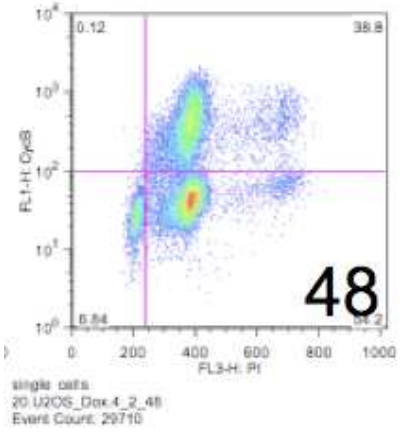
Cyclin A



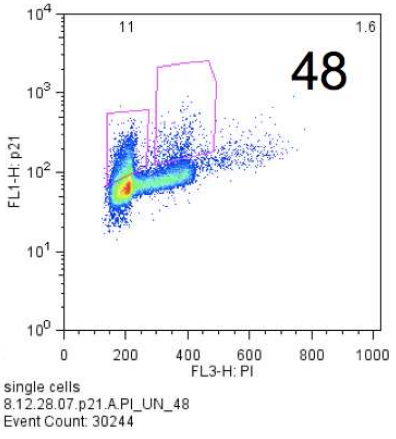


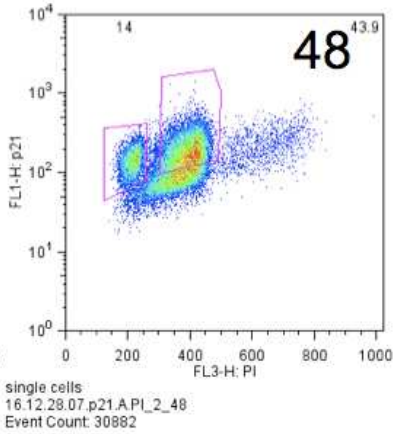
Cyclin B





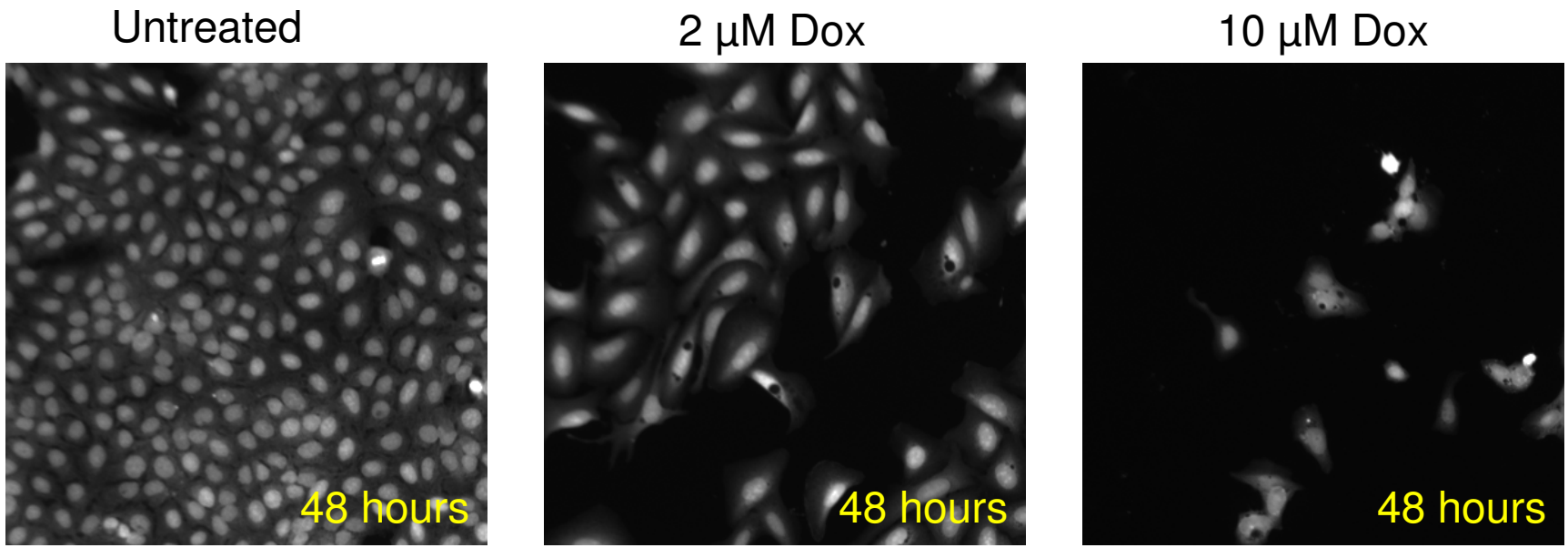
p21



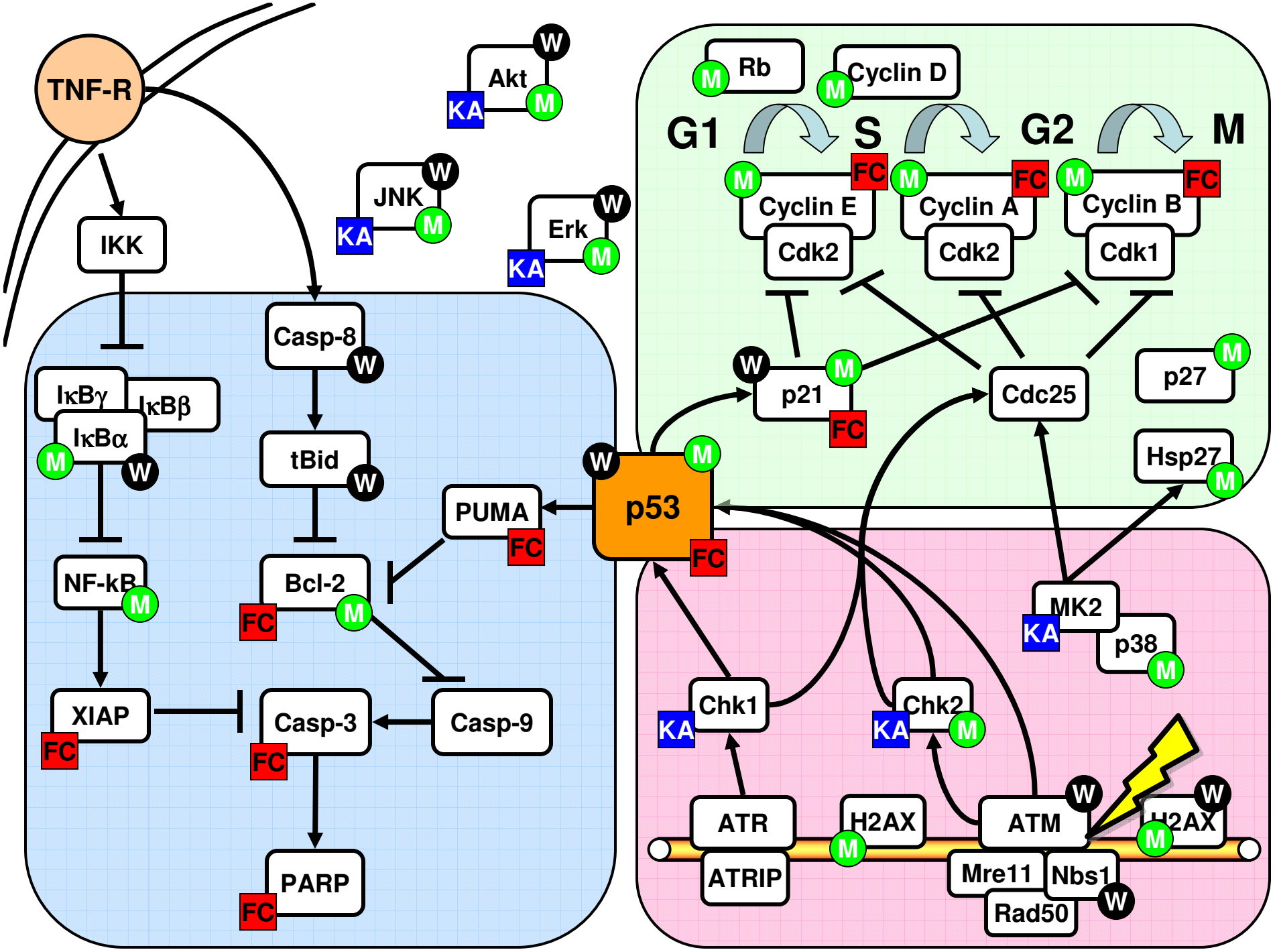


2 µM Doxorubicin induces senescence

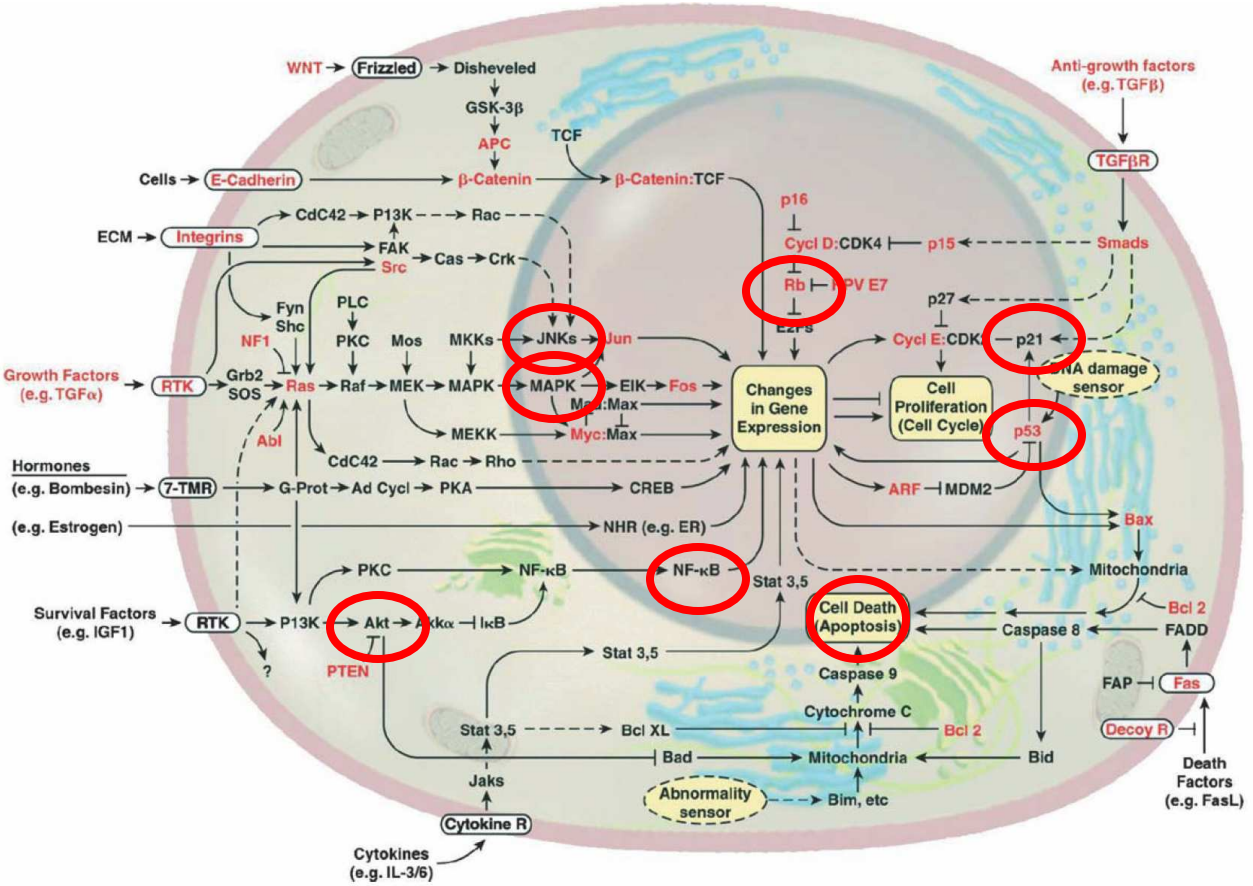
10 µM Doxorubicin induces apoptosis



We can now study of the cell commits to either of these fates.

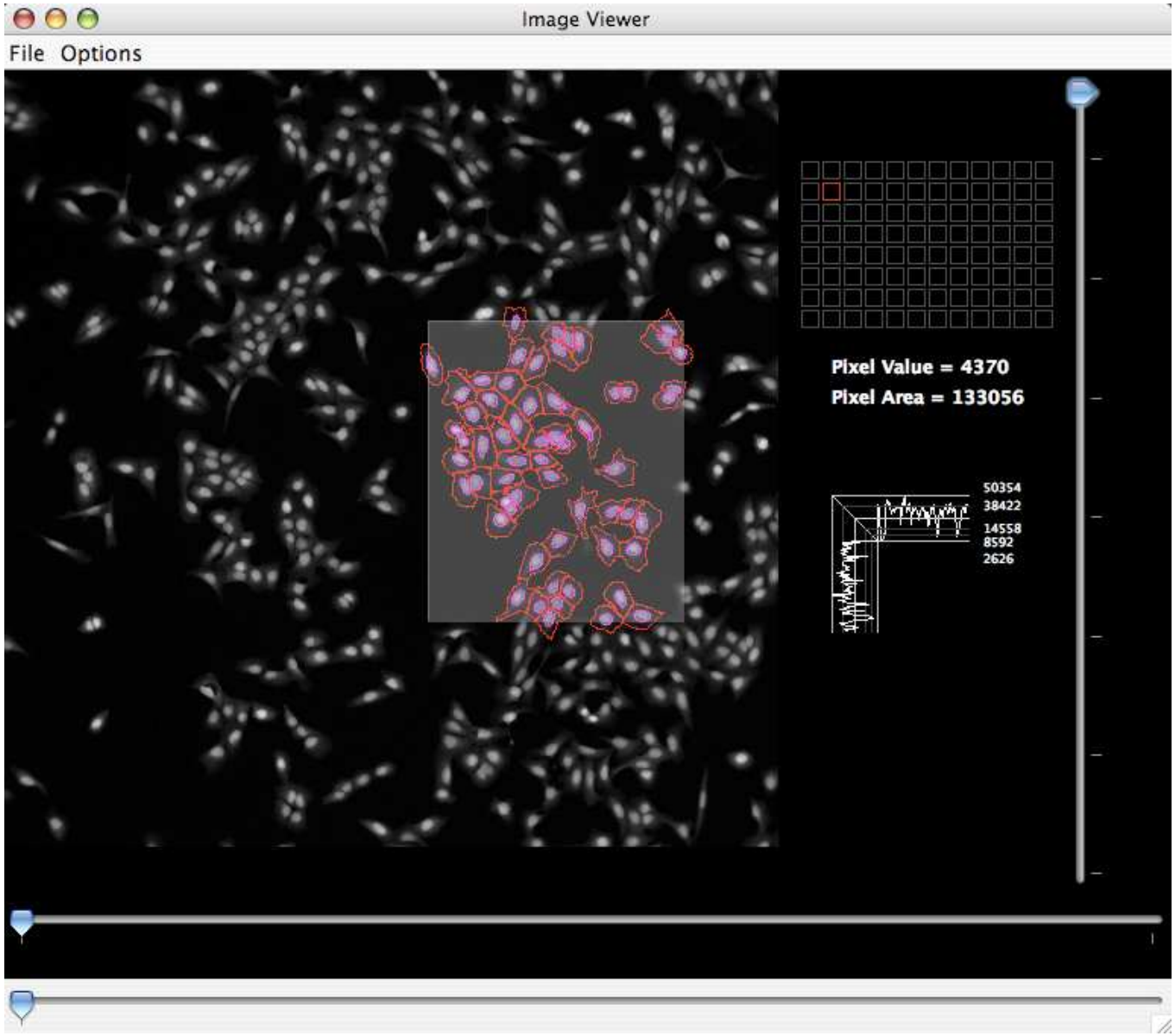

TNF-R
IKK
Akt
W
KA
M
JNK
W
KA
M
Erk
W
KA
M
Casp-8
W
IκBγ
IκBβ
IκBα
M
W
tBid
W
NF-kB
M
Bcl-2
FC
M
PUMA
FC
XIAP
FC
Casp-3
FC
Casp-9
PARP
FC
W
p53
M
FC
Rb
M
Cyclin D
M
G1
S
G2
M
M
Cyclin E
FC
M
Cyclin A
FC
M
Cyclin B
FC
Cdk2
Cdk2
Cdk1
W
p21
M
FC
Cdc25
p27
M
Hsp27
M
MK2
KA
p38
M
Chk1
KA
Chk2
KA
M
ATR
ATRIP
H2AX
M
ATM
W
H2AX
M
W
Mre11
Nbs1
Rad50
W

Based on prior biological knowledge we select key nodes to monitor the information flow through the cell.

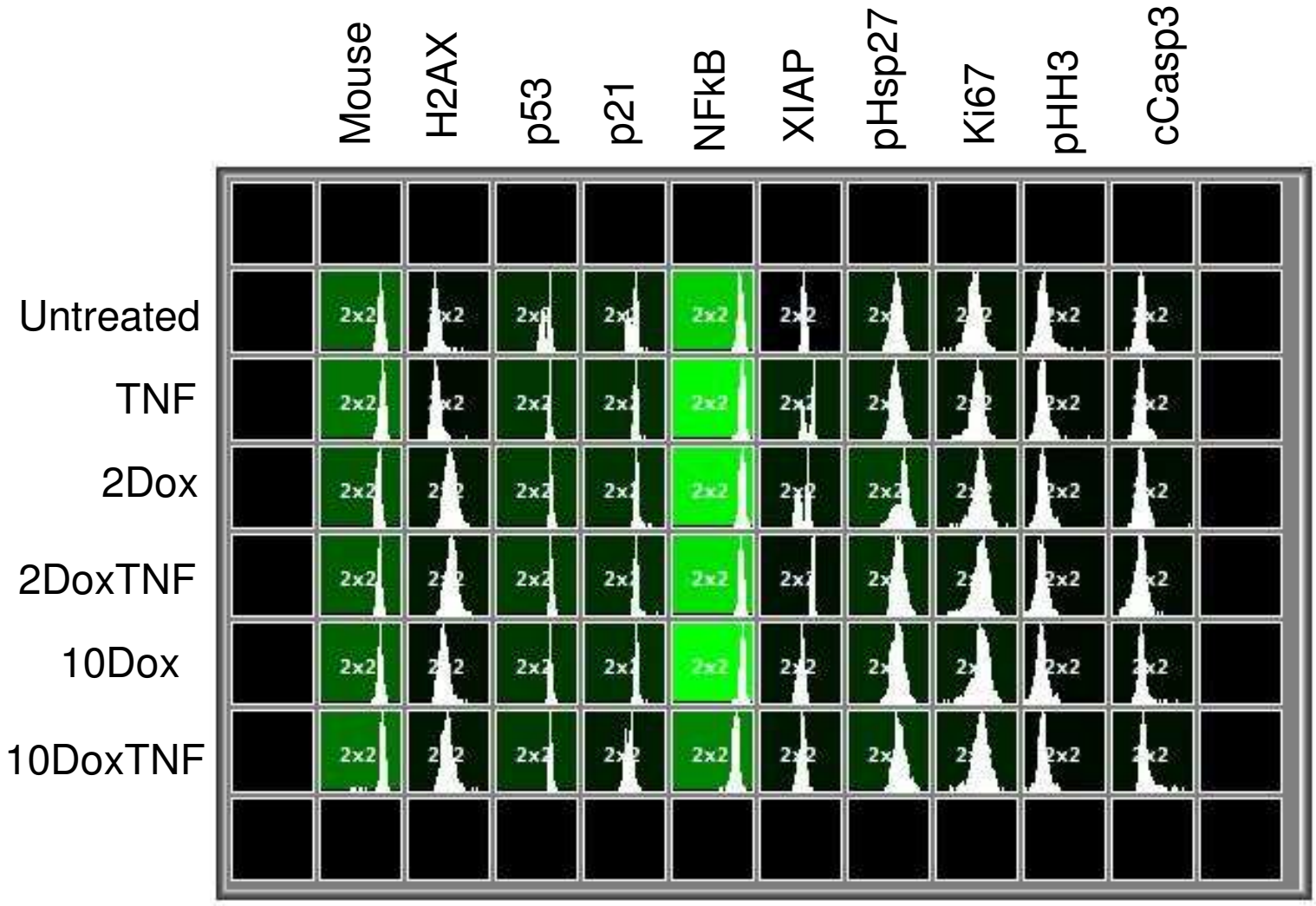


D. Hanahan and R.A. Weinberg. *Cell* (2000)

Quantitative Immuno-fluorescence is used to quantify signaling.

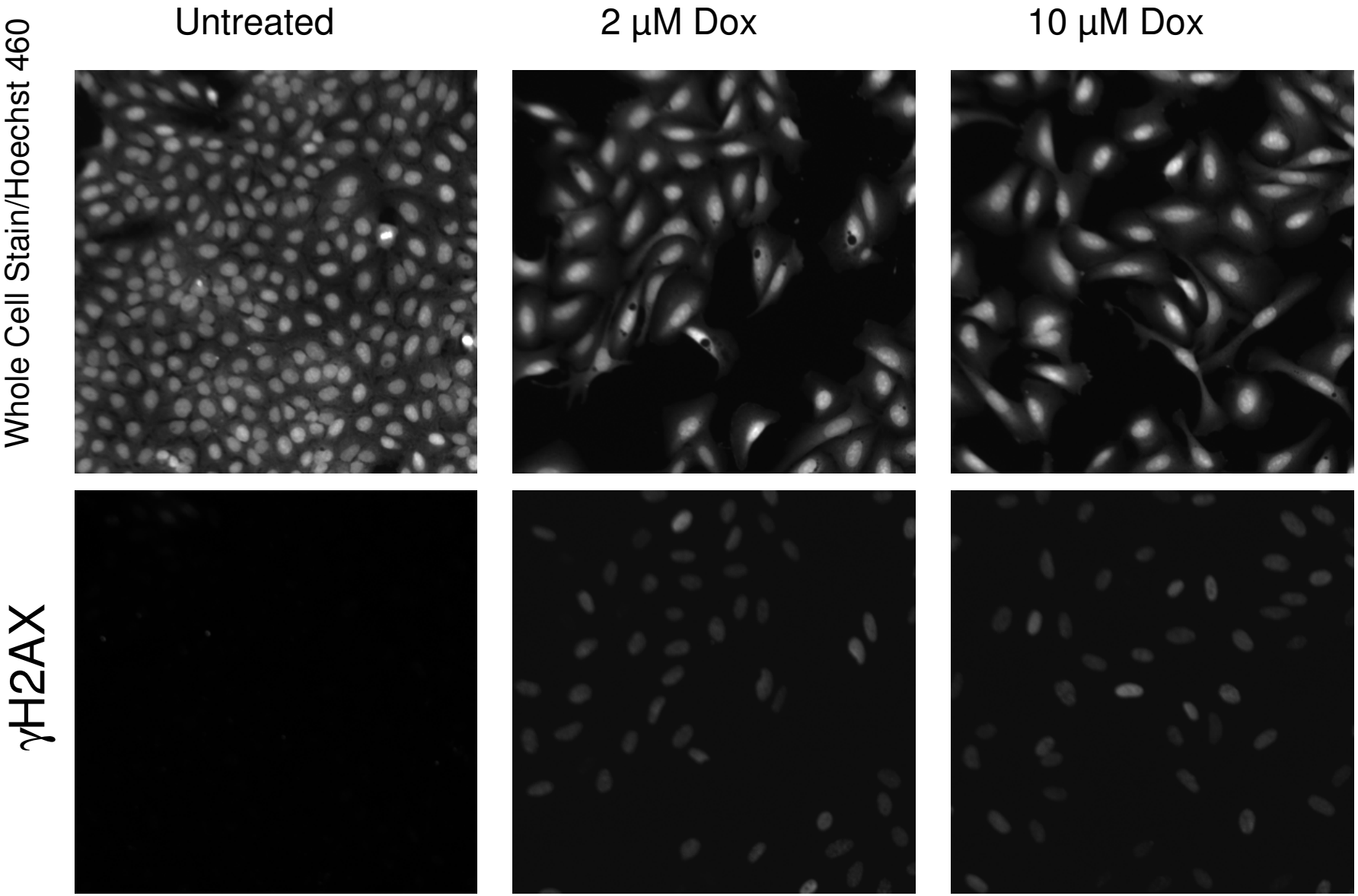


5. The single cell data is analogous to flow cytometry data.

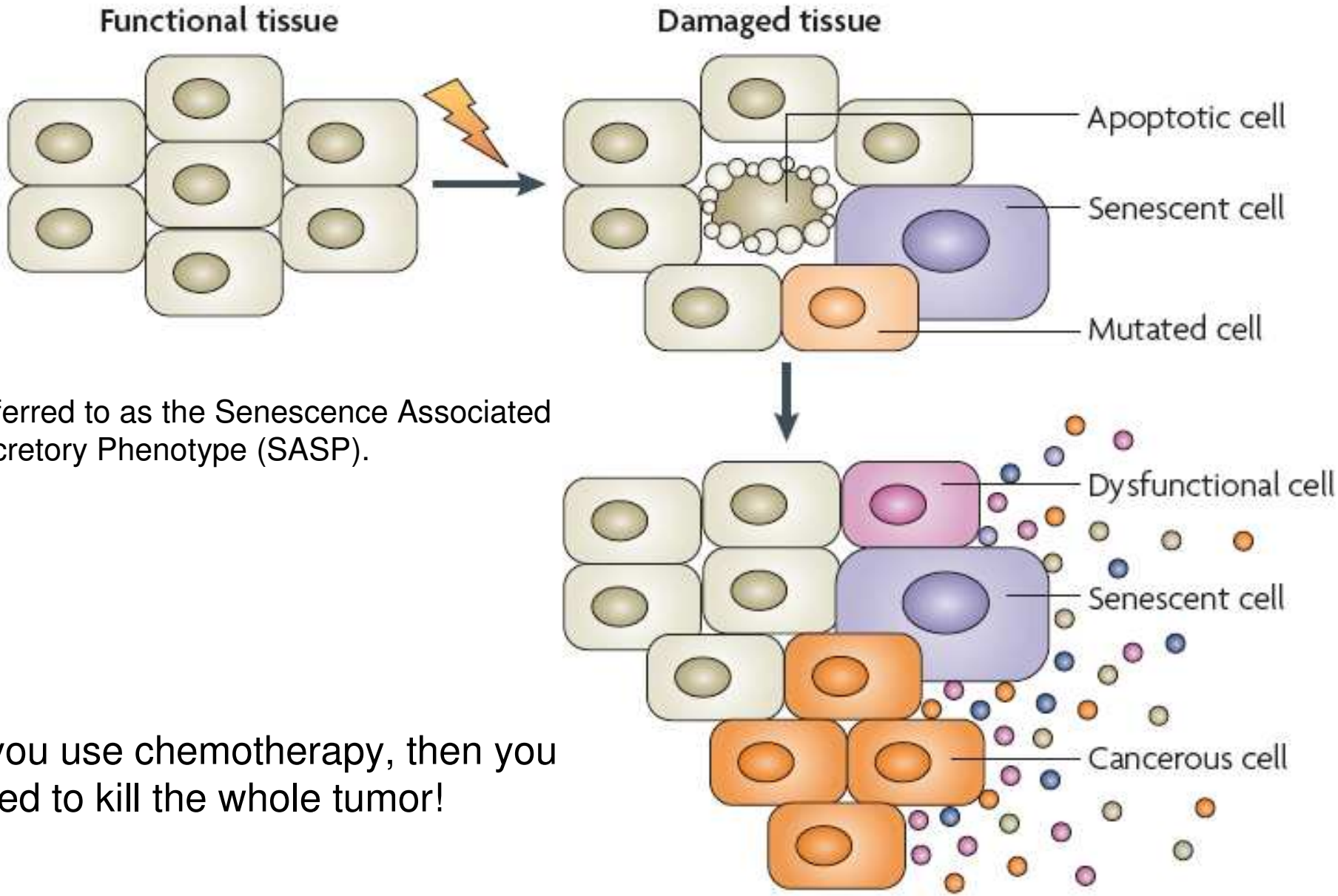


One gets information on signal strength and cell population dynamics.

High-content microscopy is a powerful means of monitoring signaling.



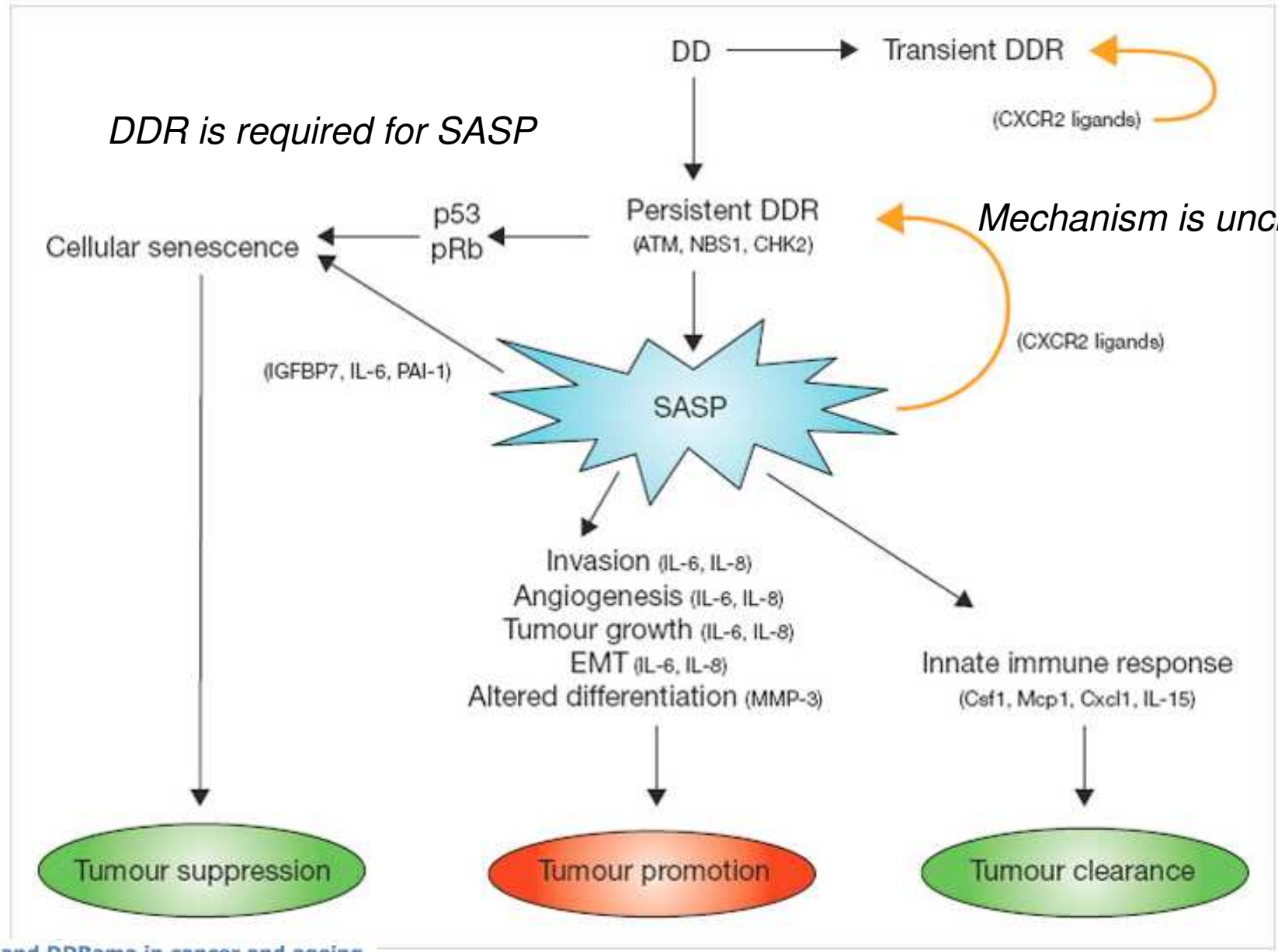
Senescent cells release factors that encourage transformation.



Referred to as the Senescence Associated Secretory Phenotype (SASP).

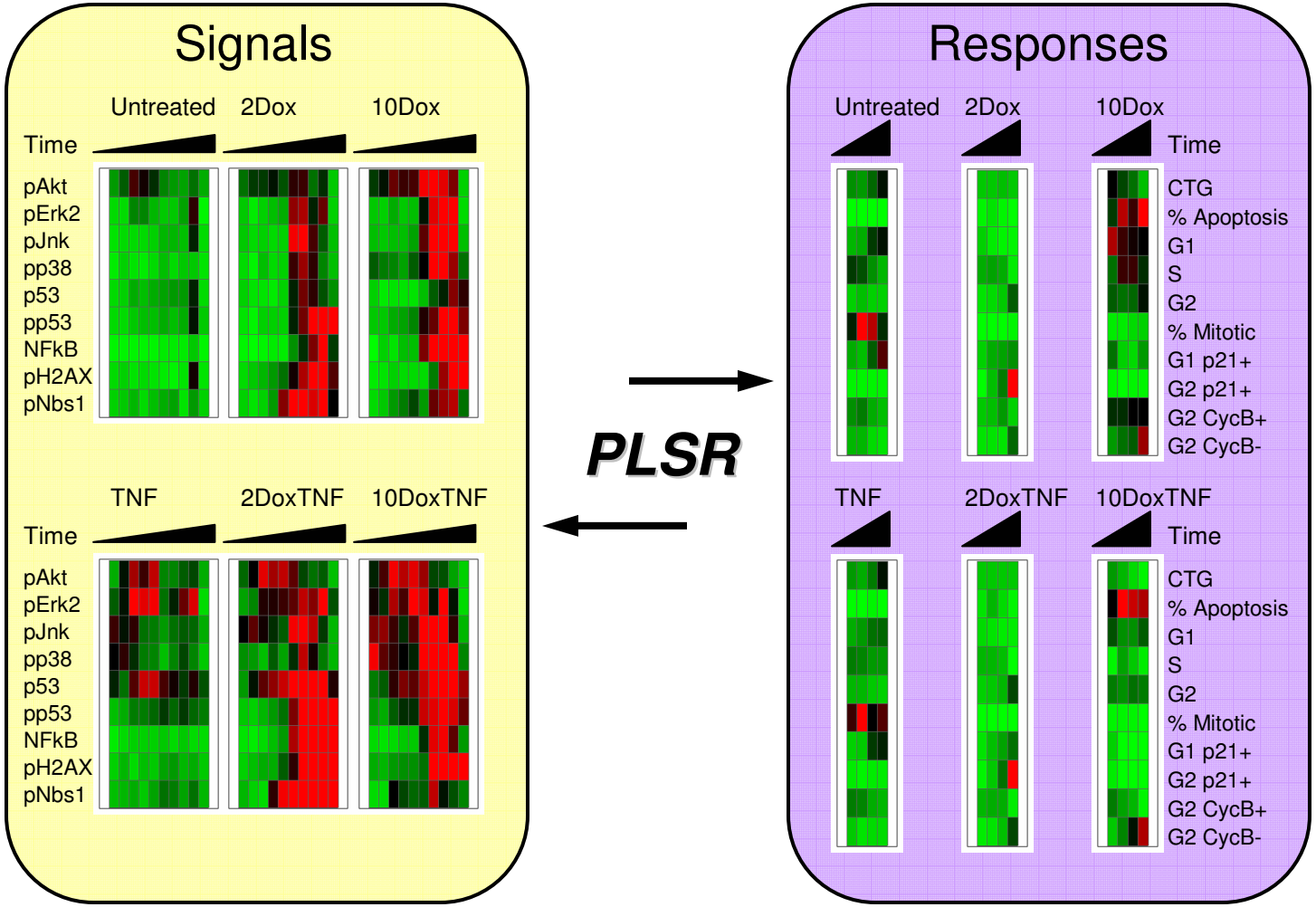
If you use chemotherapy, then you need to kill the whole tumor!

A persistent DDR drives the Senenesence Associated Secretory Phenotype



SASPense and DDRama in cancer and ageing
Marzia Fumagalli & Fabrizio d'Adda di Fagagna
Nature Cell Biology **11**, 921 - 923 (2009)

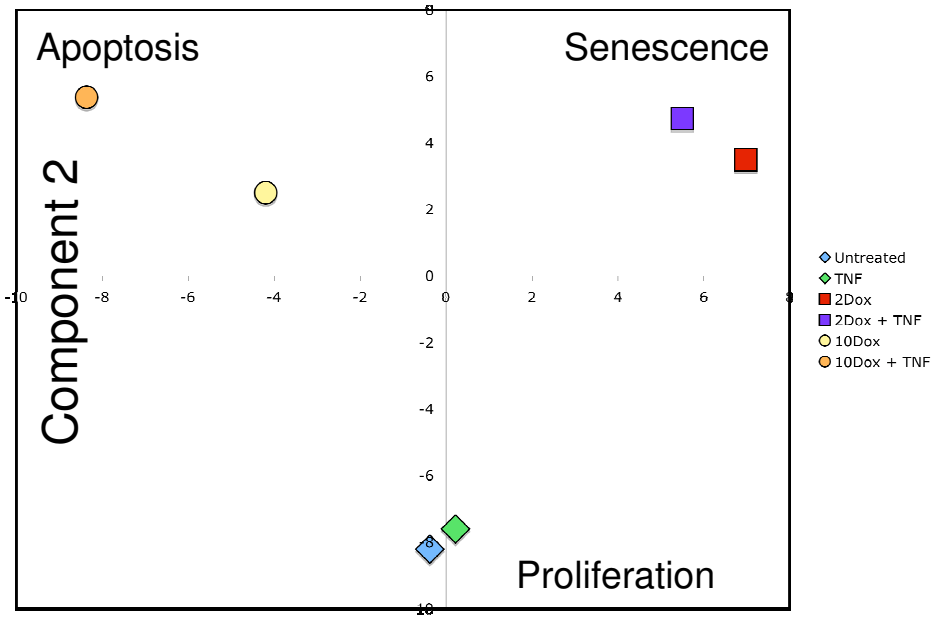
Regression modeling can quantify the contribution of signaling events to a given outcome.



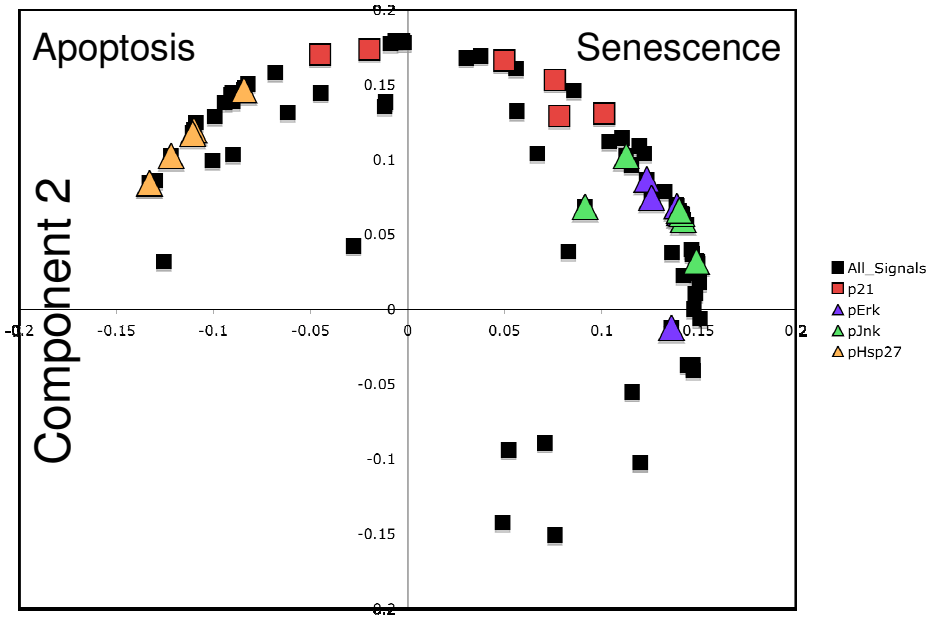
DataRail facilitates data handling and modeling.

Partial Least Squares Rregression suggests a role for pJnk is senescence.

Response Loadings

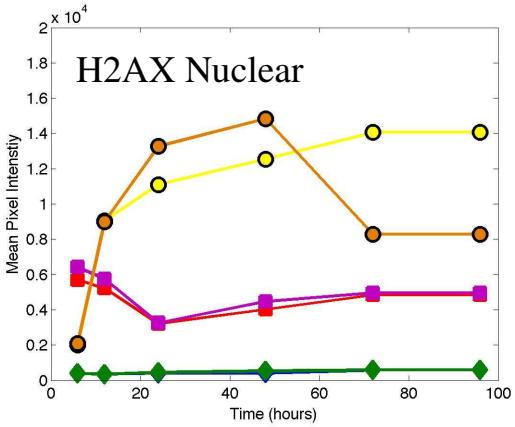


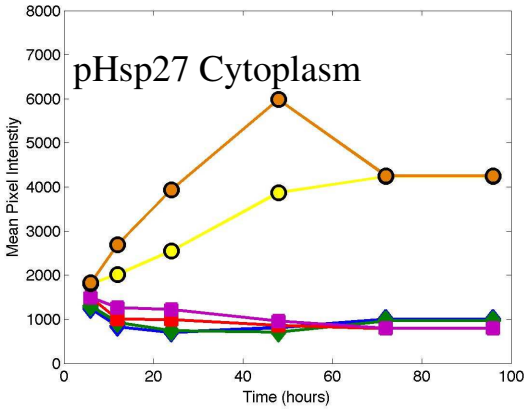
Signal Loadings



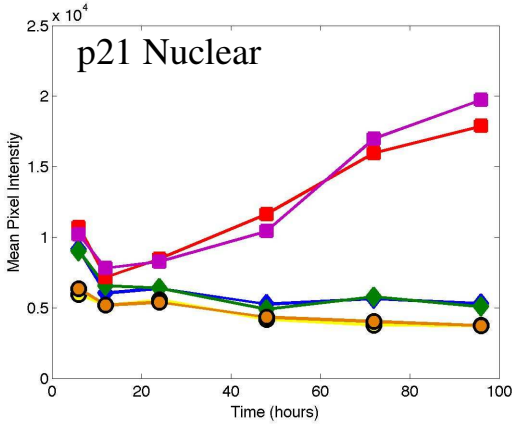
Timecourses of interest

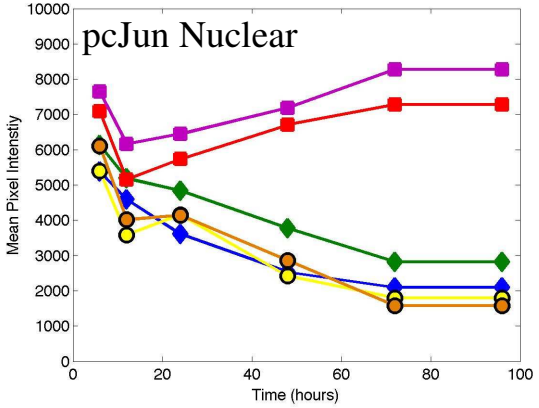
Apoptosis Signals

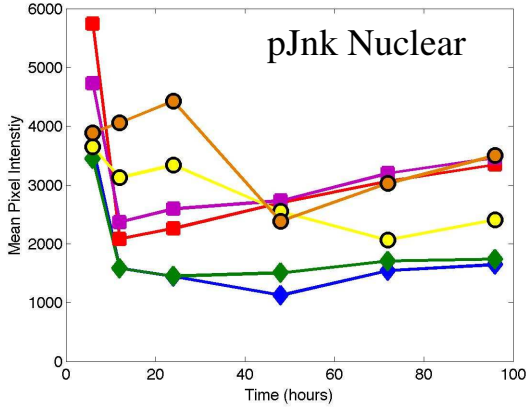




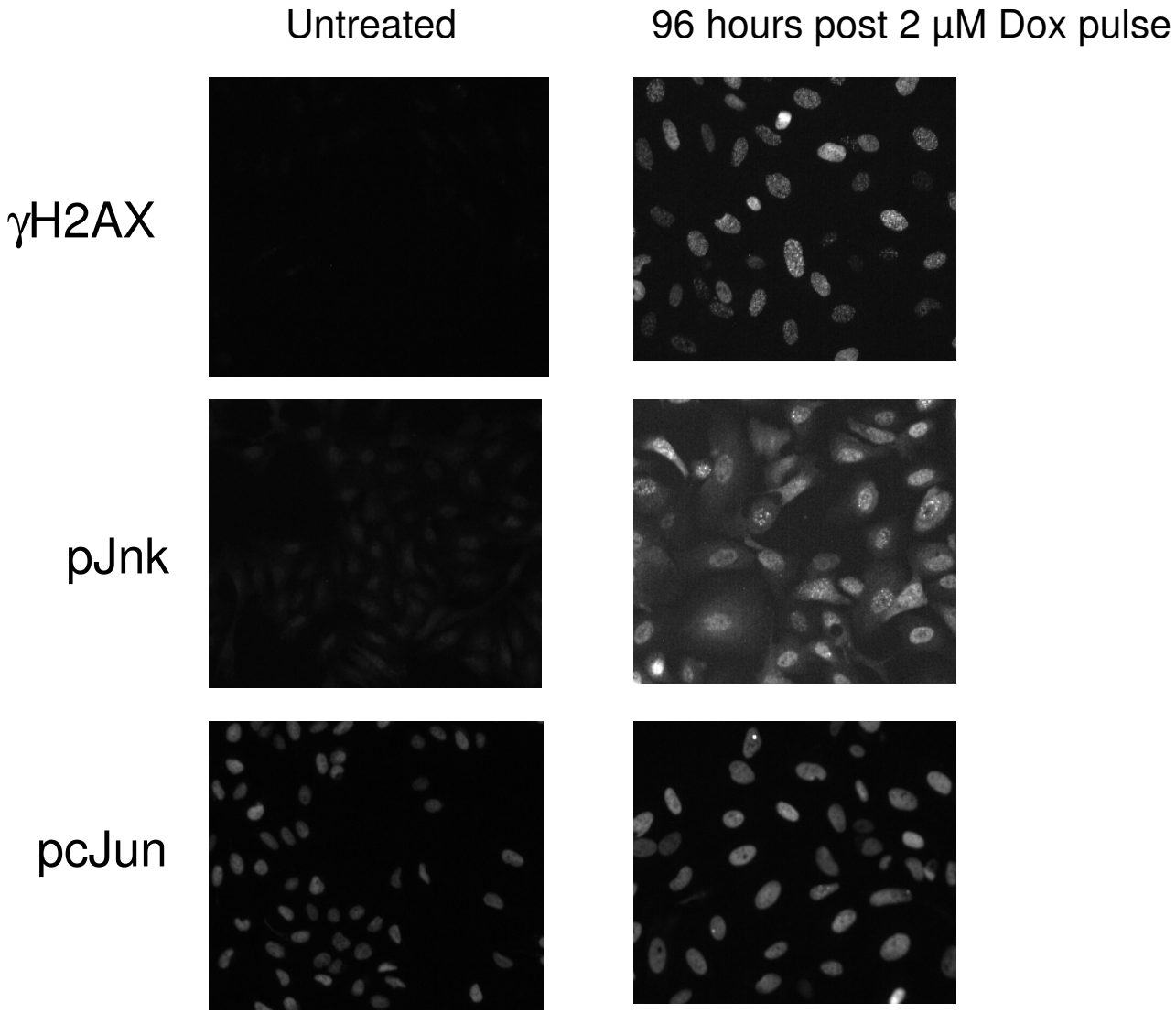
Senescence Signals







Senescent U2OS cells exhibit nuclear pJnk foci.



Bartek et al. showed that JNK is recruited to the sites of DNA Damage by H2AX this April.

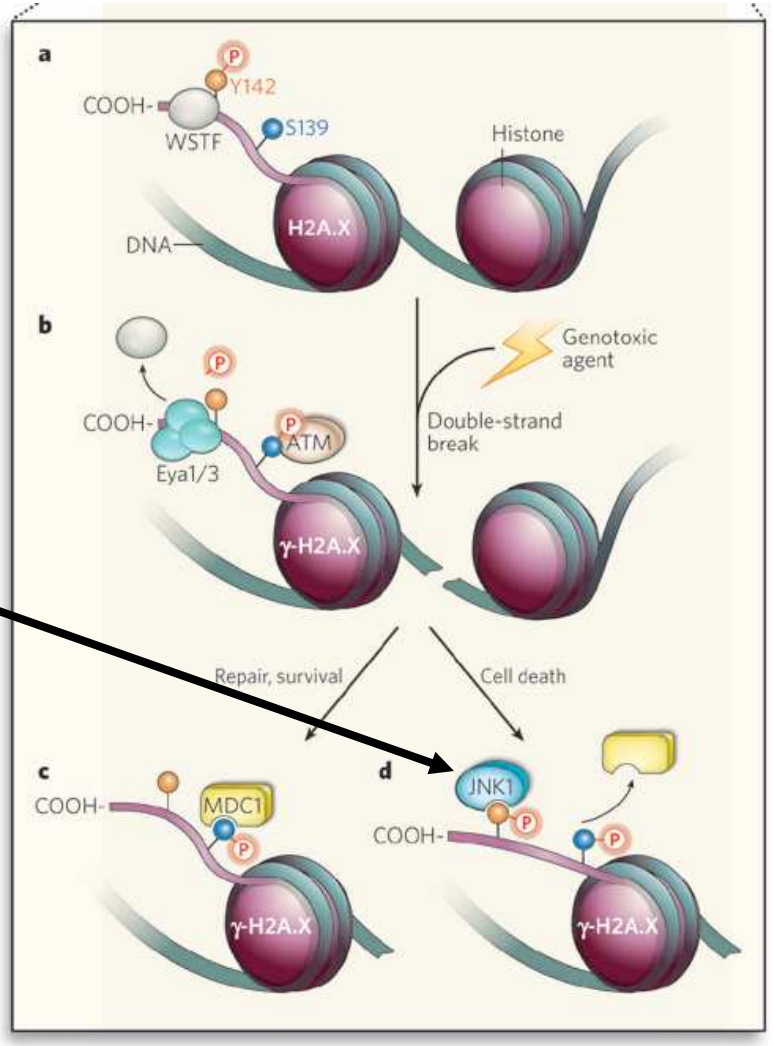
a) WSTF kinase associates with the C-terminus of H2A.X and phosphorylates Y142.

b) Eya1/3 phosphatases dephosphorylate Y142 facilitating S139 phosphorylation.

c) If repair is possible, then phosphorylated S139 recruits MDC1.

d) If it is not repairable, then Y142 is phosphorylated and recruits JNK1, which promotes apoptosis.

But how is Jnk activated?

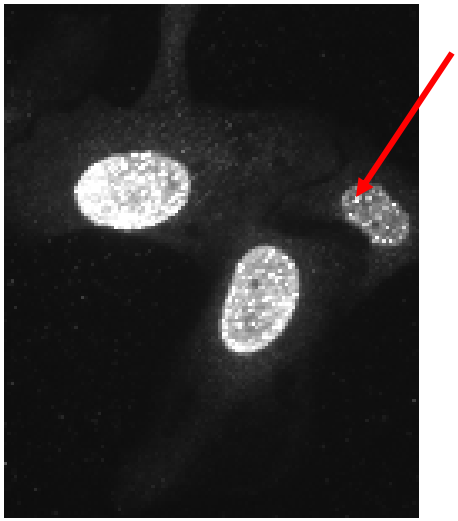


DNA repair: New tales of an old tail
Jiri Lukas & Jiri Bartek
Nature 458, 581-583(2 April 2009)

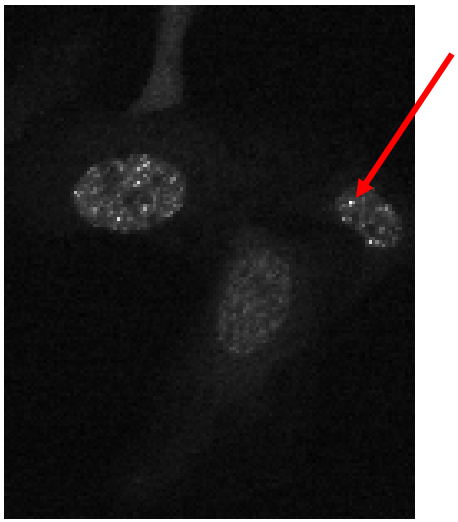
Xiao, A. et al. Nature 457, 57–62 (2009).
Cook, P. J. et al. Nature 458, 591–596 (2009).

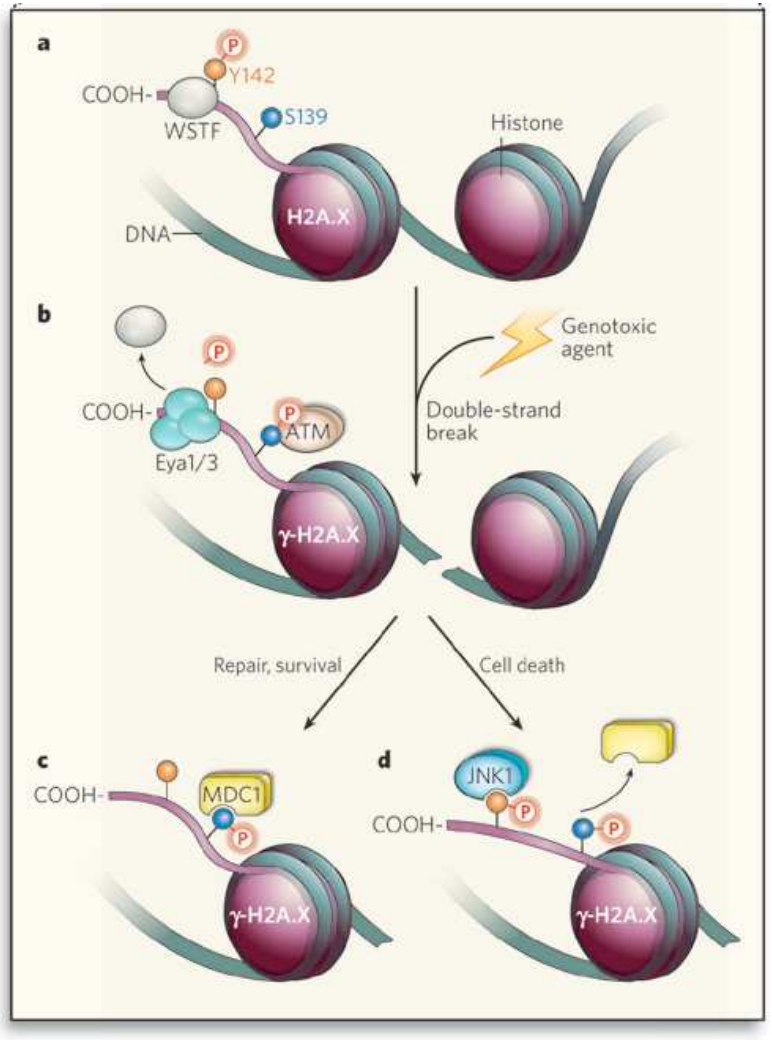
Co-localization of pJnk and γH2AX in DNA damage induced senescent U2OS cells suggests that Jnk signaling is not definitively coupled to cell death.

γH2AX



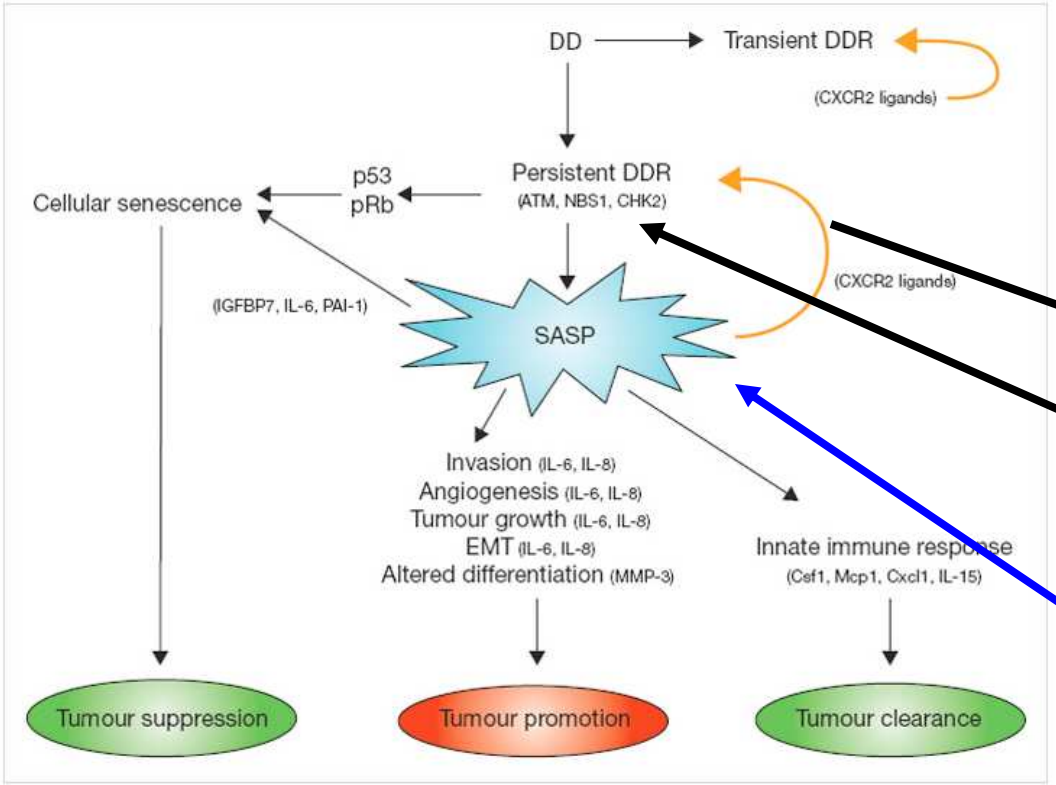
pJnk

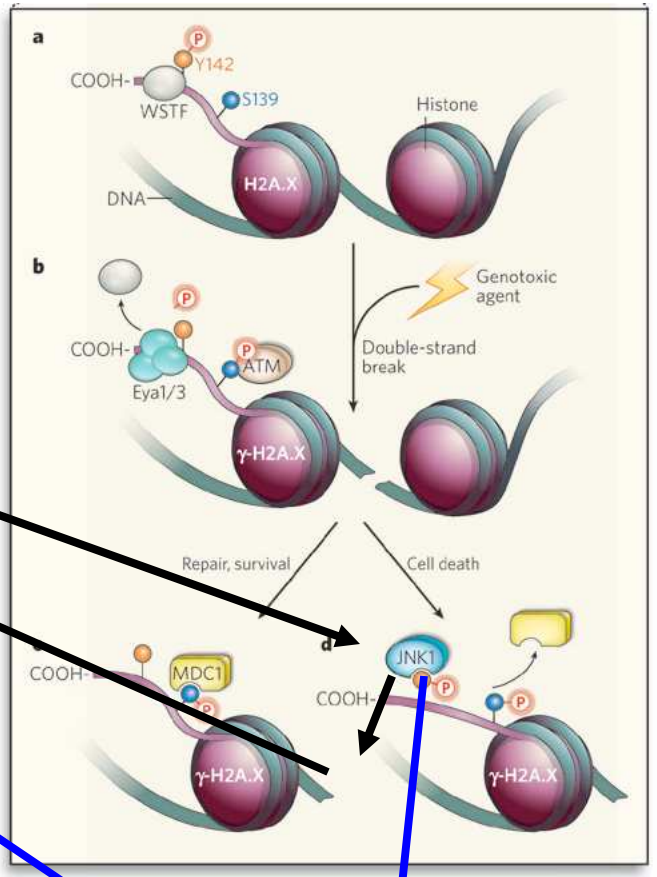




Does the SASP activate Jnk?

Are the DNA damage foci maintained intentionallyin order to maintain the permanent arrest?

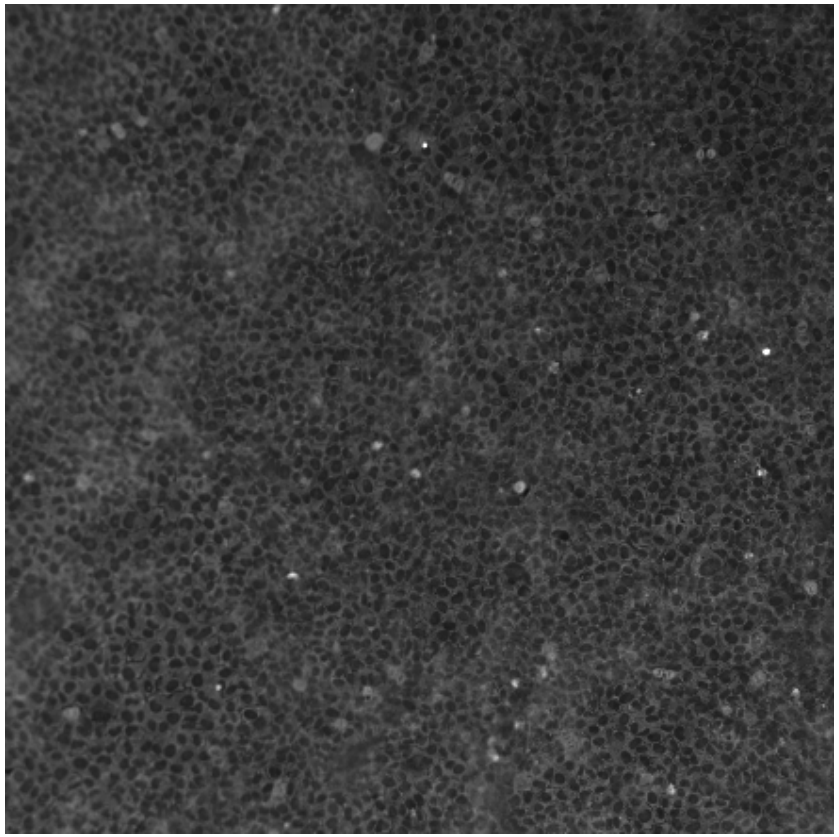




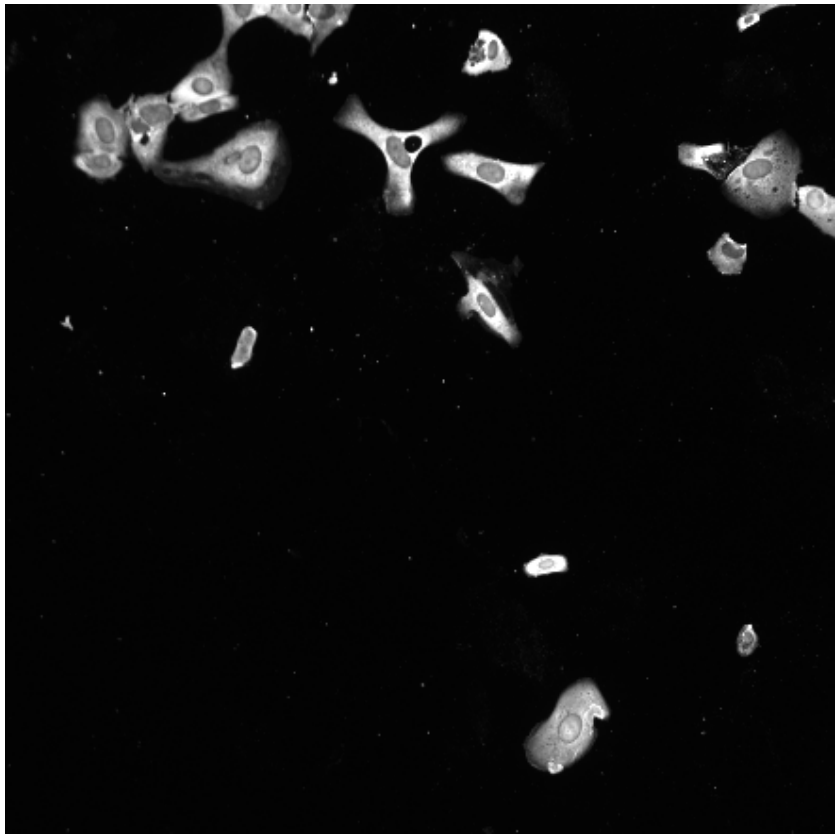
cJun

Senescent U2OS cells up-regulate IL-6

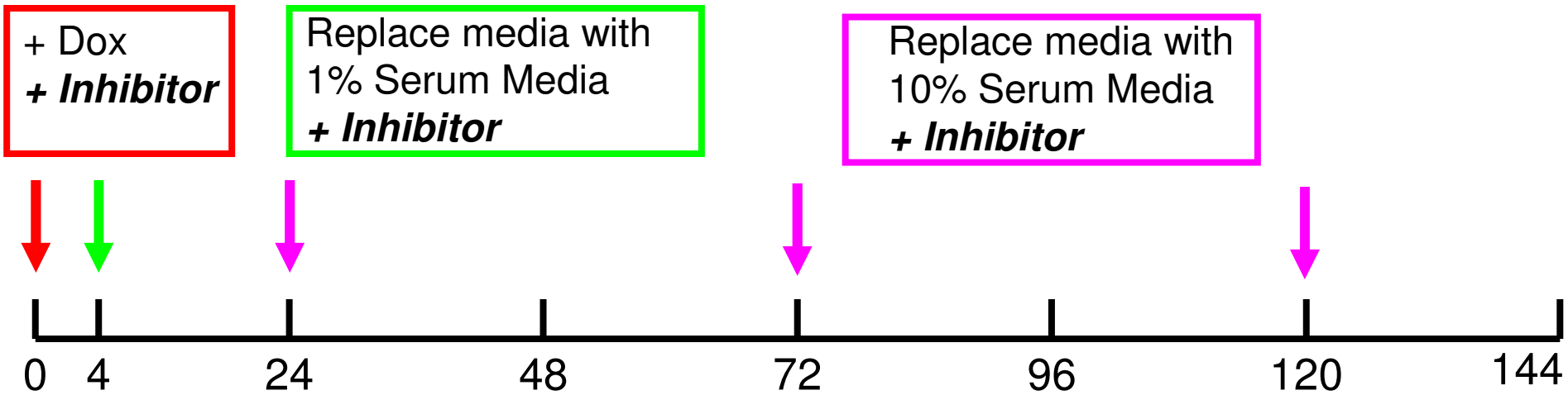
Untreated U2OS cells Day 6.



2 µM Dox U2OS cells Day 6.



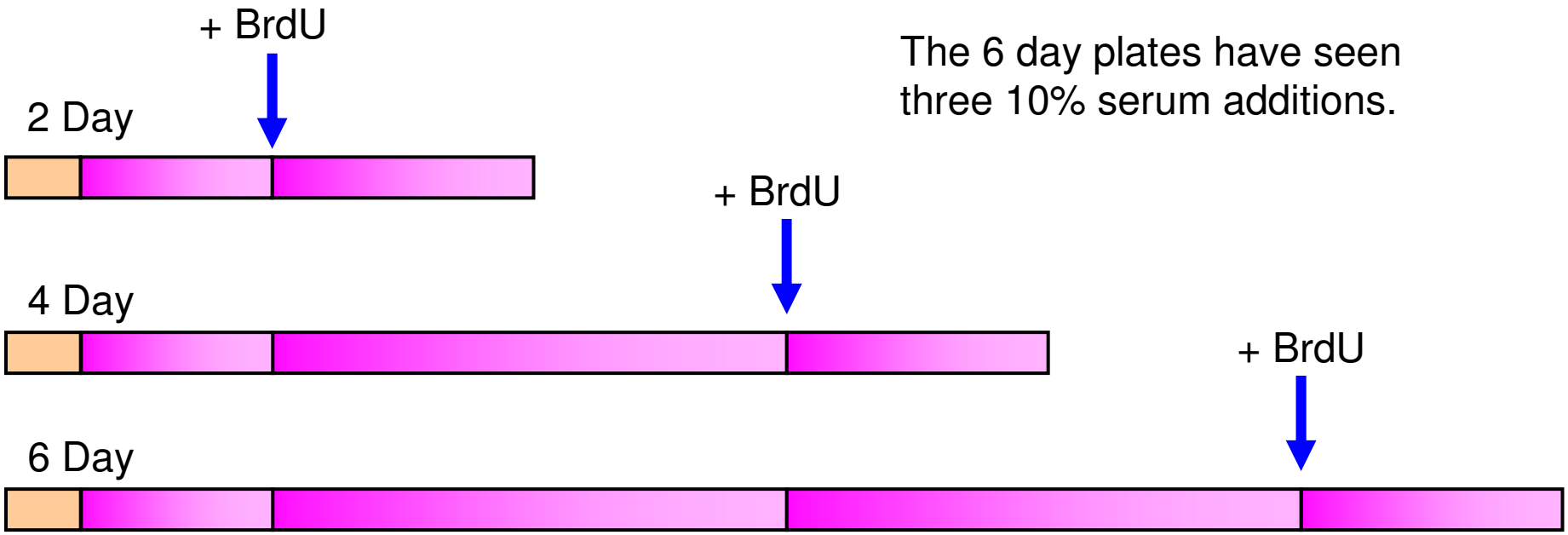
Timecourse of the BrdU addition experiments



Timepoint

BrdU is added for 24 hours.

The 6 day plates have seen three 10% serum additions.

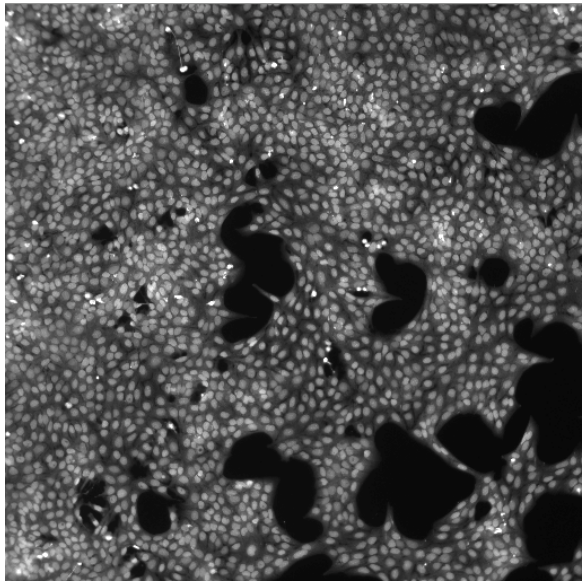


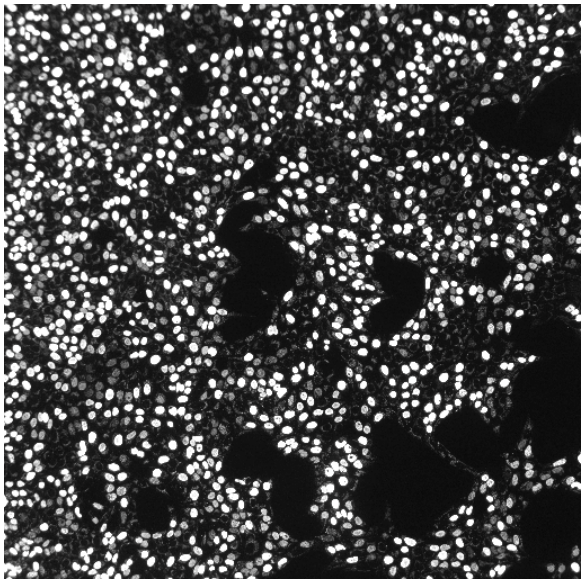
Untreated U2OS cells without Serum addition proliferate.
The Jnk inhibitor slows proliferation and induces a morphology change.

Whole Cell Blue / Hoechst

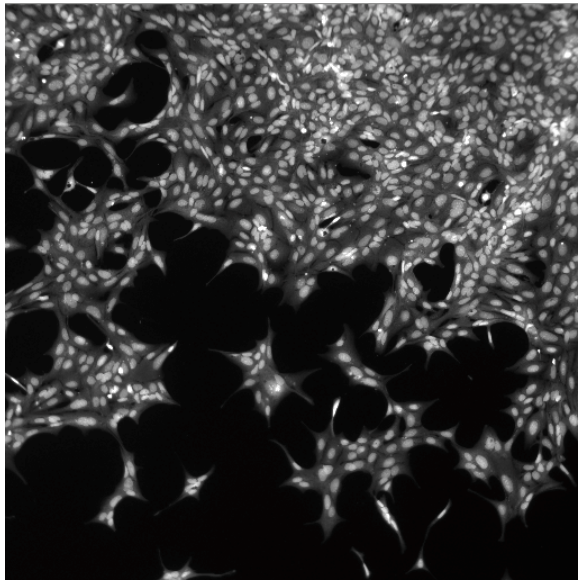
BrdU

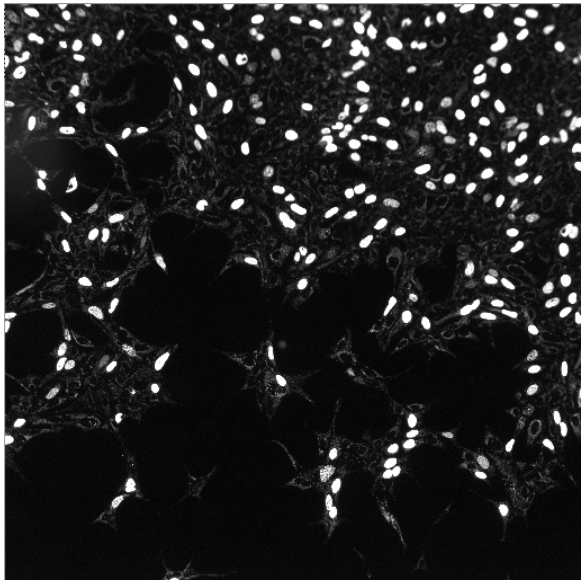
- Doxorubicin
- Serum
- Jnk Inhibitor





- Doxorubicin
- Serum
+ Jnk Inhibitor



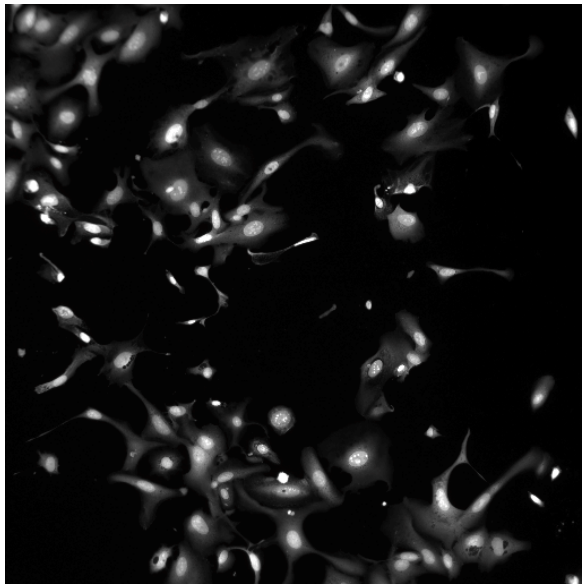


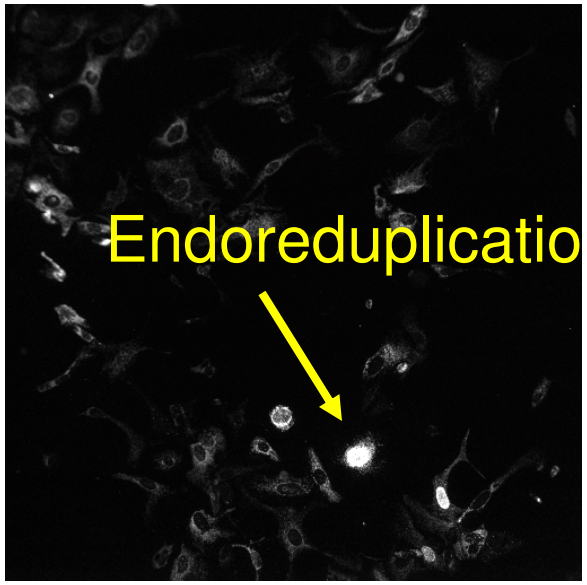
U2OS cells treated with 2 μM Dox (4 hours) do not have a sub-population of proliferating cells even after serum addition.

Whole Cell Blue / Hoechst

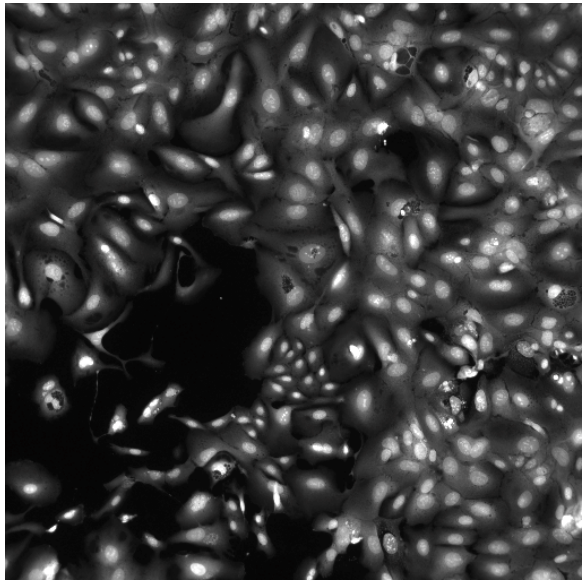
BrdU

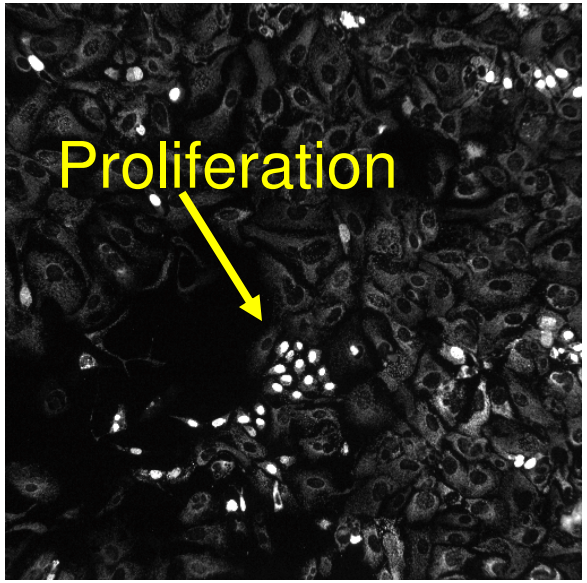
+ 2 μM Doxorubicin
+ Serum
- Jnk Inhibitor





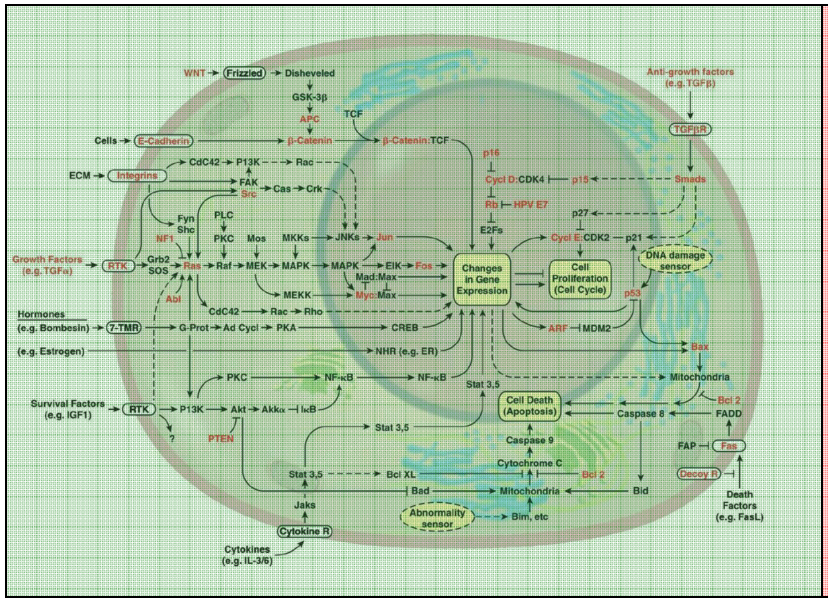
+ 2 μM Doxorubicin
+ Serum
+ Jnk Inhibitor



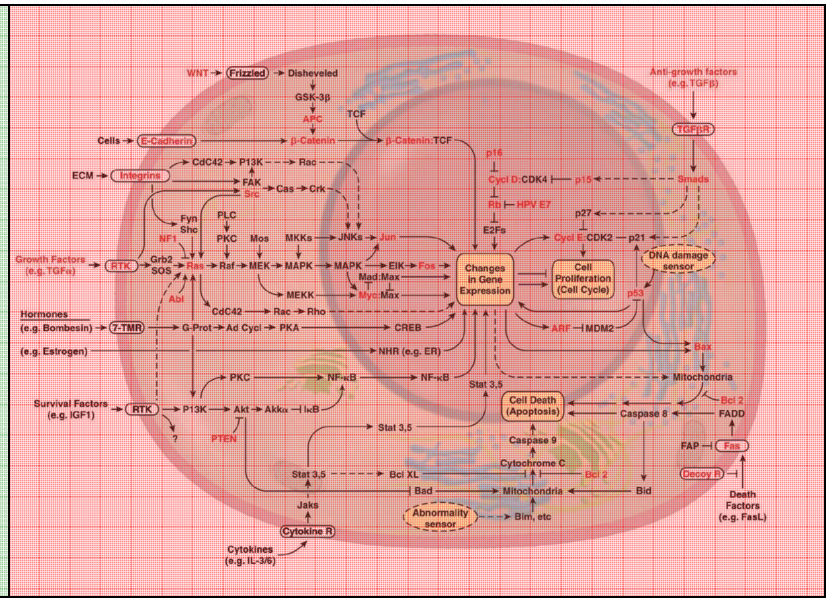


Genotoxic chemotherapy drives a cell into a novel signaling state in which the effect of a given molecule is drastically altered.

Untreated



DNA Damage



Jnk activity drives proliferation.

Jnk activity drives arrest.

Particular thanks to

- Doug Lauffenburger
  - Arthur Goldsipe
  - Ben Cosgrove
  - Matt Lazarra
  - Megan Palmer
  - Pam Kreeger
  - Shannon Alvord
- Leona Samson
  - Ericka Noonan
  - Dharini Shah
  - Jennifer Calvo
  - Lisiane Meira
- Mike Yaffe
  - Alexandra Gardino
  - Andrea Tentner
  - Marcel ATM van Vugt
  - Mary Stewart
  - Scott Floyd and Emily Blake
  - Scott Clarke and Anna Fydrych
  - Elena Garza
- Glenn Paradis
- ***Your Name Here***
  -
- Peter Sorger
  - Julio Saez-Rodriguez
  - Bjorn Millard
  - Mario Niepel
- Ken Loparo (CWR)
  - Evren Gurkan-Cavosglu
- John Tyson (VaTech)
  - Tongli Zhang
- Vito Quaranta (Vanderbilt)
- Alissa Weaver (Vanderbilt)
  - Lourdes Estrada
  - Mohamed Hassanein
- Bruce Tidor (MIT)
- Galit Lahav (Harvard)
  - Jared Toettcher
- Rune Linding (Imperial Cancer Research)
- Riet Hielhorst (PAMGENE)