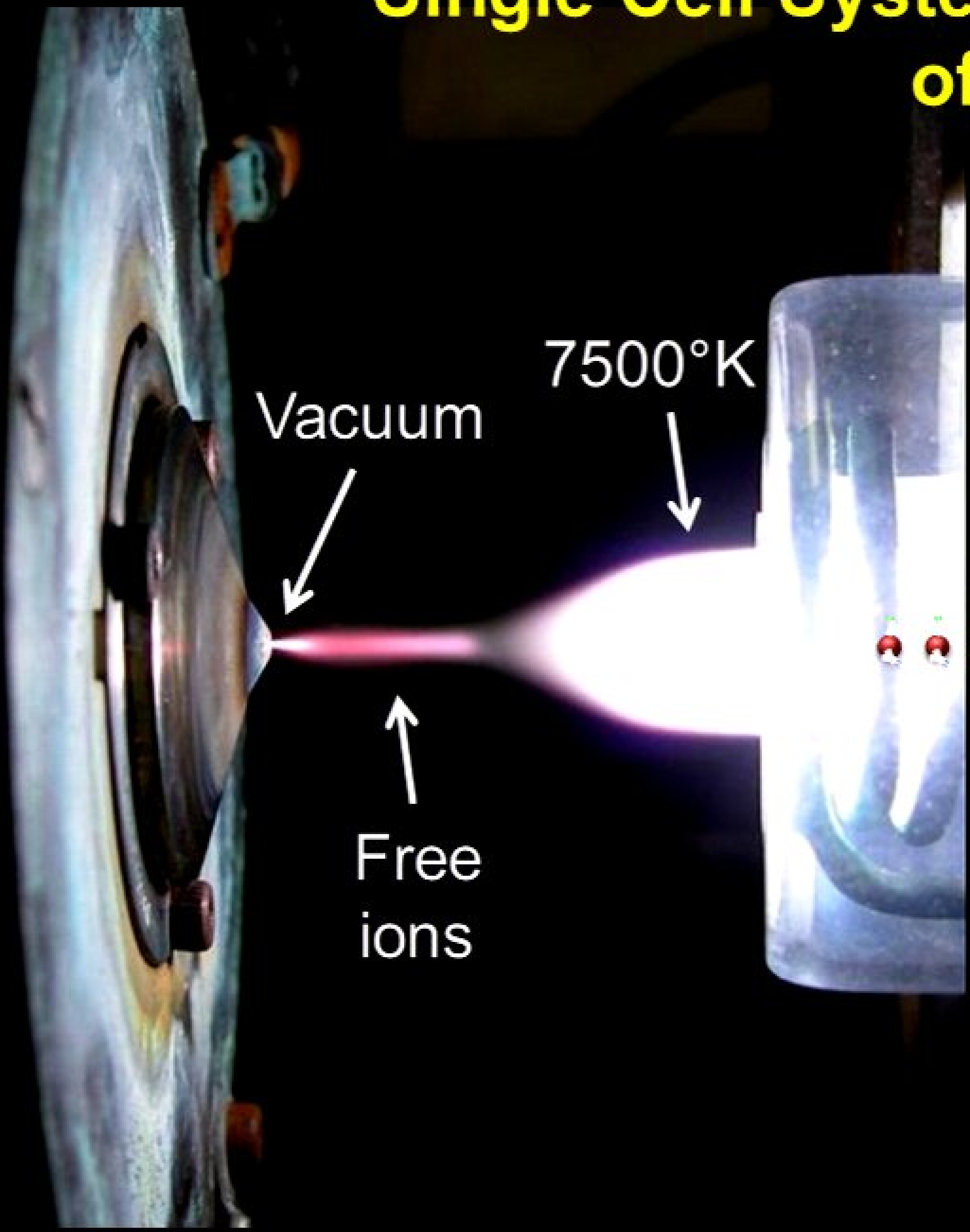
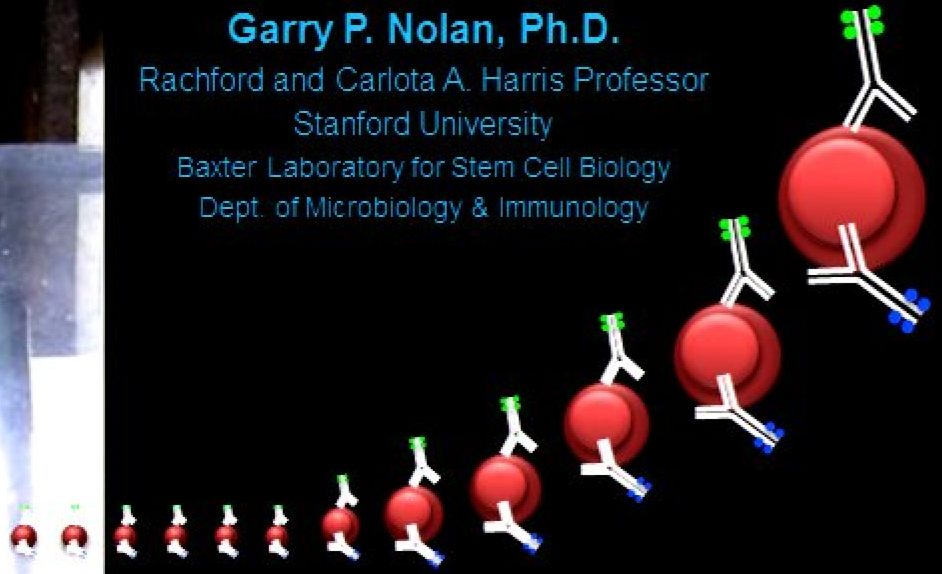
Single Cell Systems-Structured View of Immunity & Cancer



Garry P. Nolan, Ph.D.
Rachford and Carlota A. Harris Professor
Stanford University
Baxter Laboratory for Stem Cell Biology
Dept. of Microbiology & Immunology



Disclosures:

- Nodality: Founder & Chair SAB, Equity
- DVS: Chair SAB, Equity

CyTOF

- ~45 parameters right now.
- New encoding system → 100-200 parameters per cell.
- mRNA measured along with Abs

- One main goal is to develop an Immune System Reference Map
- Allows powerful models and hypotheses about immune function– much like is accomplished with genomics datasets.

- 4 CyTOFs on campus
  - 2 in Nolan lab
  - 2 in Human Immune Monitoring Core (HIMC) –
    Mark Davis & Holden Maeker
  - 5th to be placed in Shared FACS Facility.

- 30 CyTOFs in world now– 4 already in pharma.

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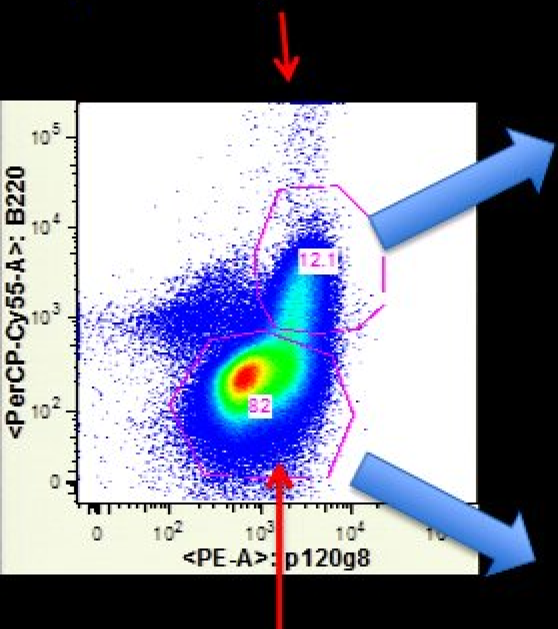
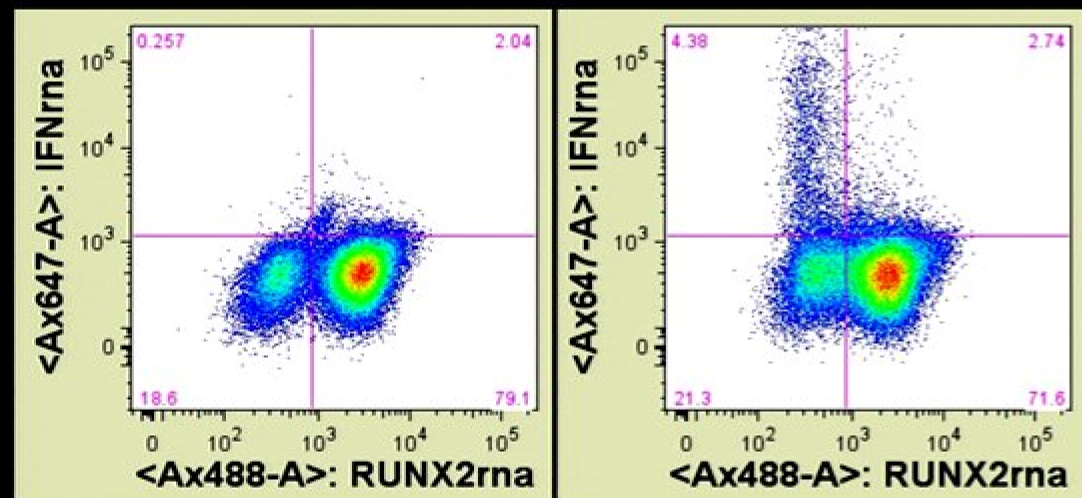
Rflow can be used for cytokine detection

plasmacytoid DC



conventional DC

UNstim stim



Rflow can be used for cytokine detection

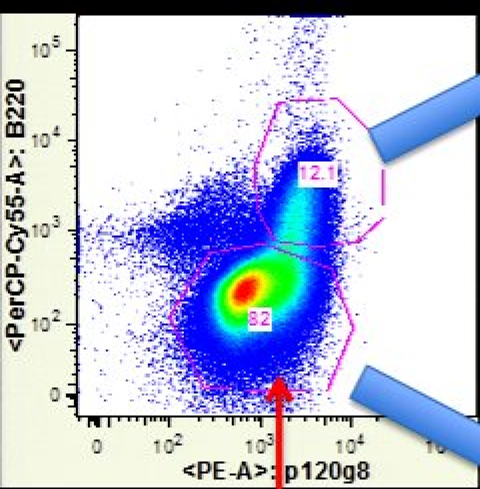
dendritic cells

CpG 11h

unstim

stim

plasmacytoid DC



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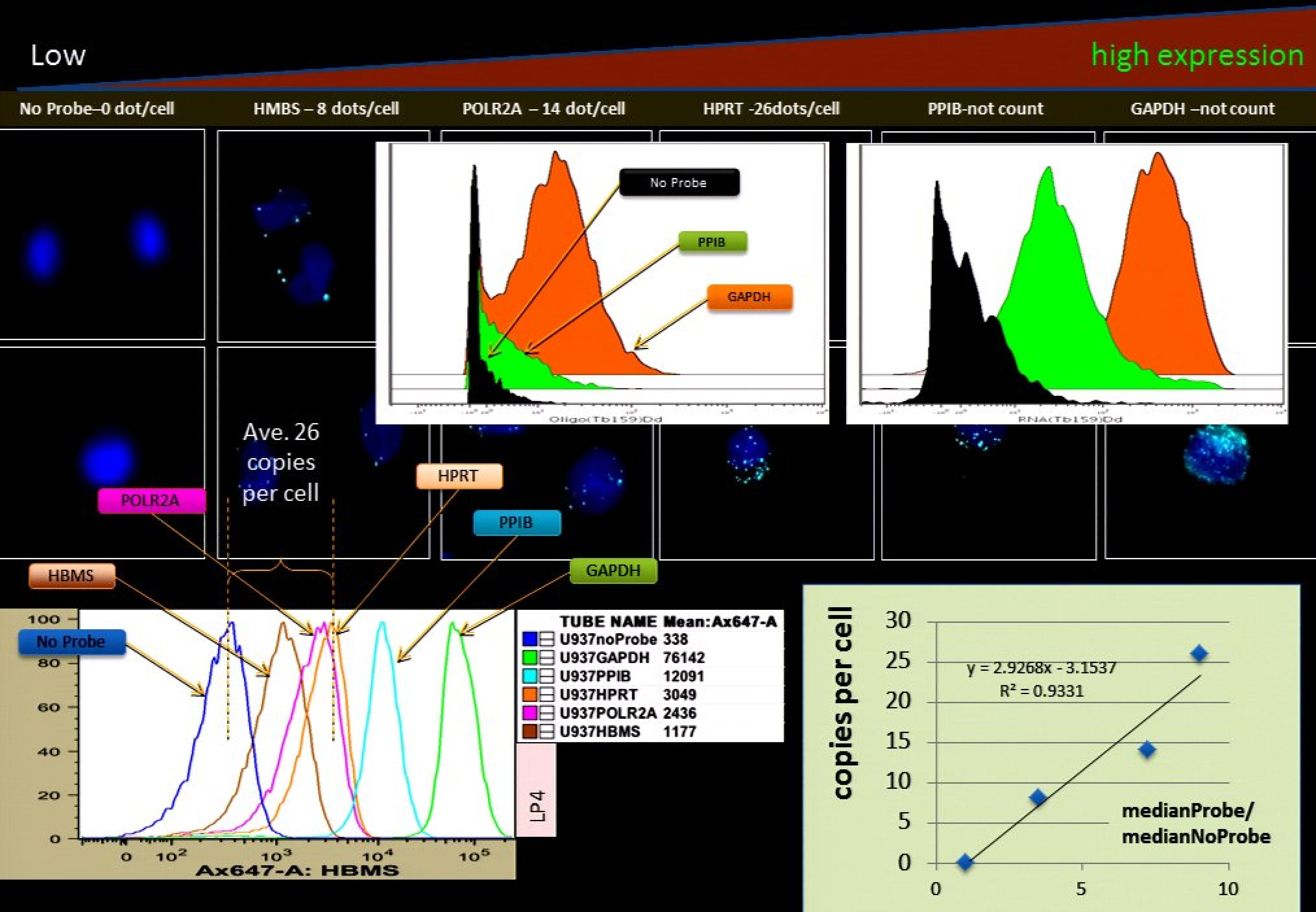
RNA flow can detect gene expressed as low as 5 copies per cell



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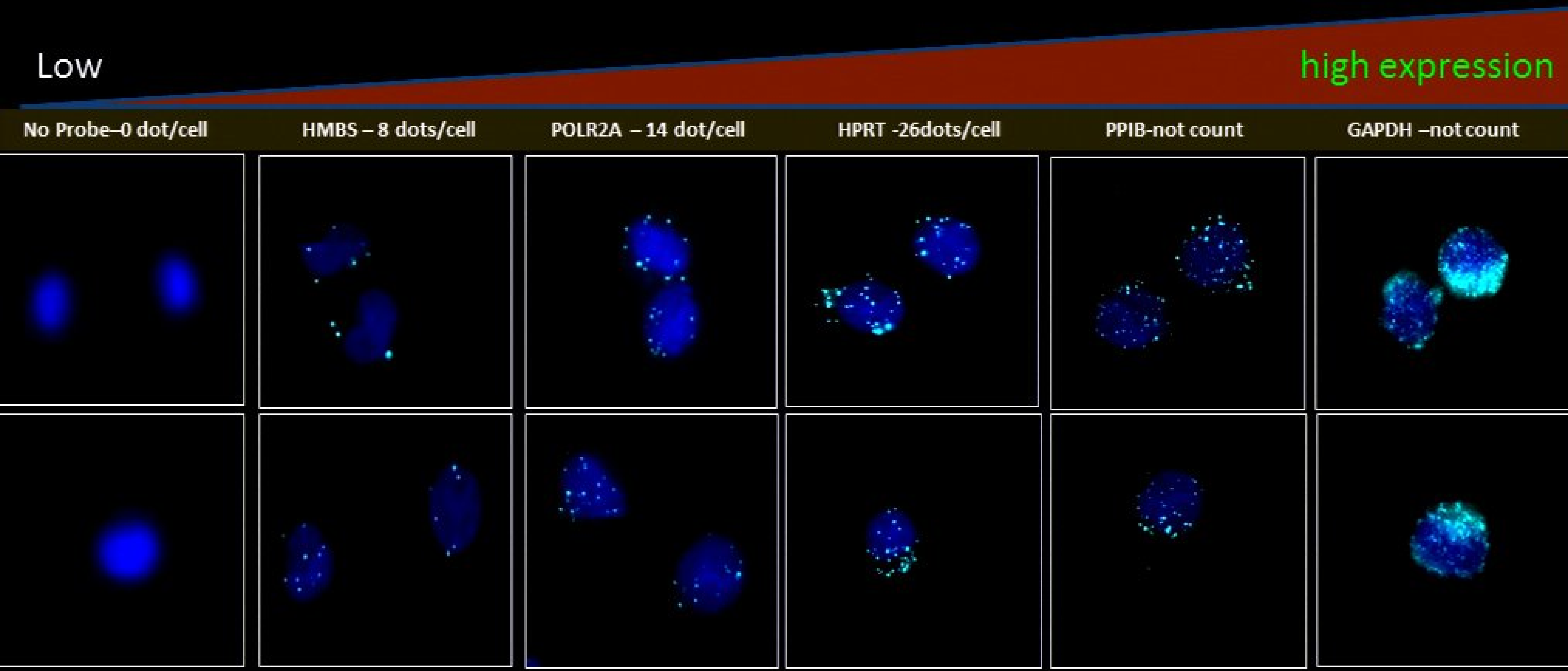
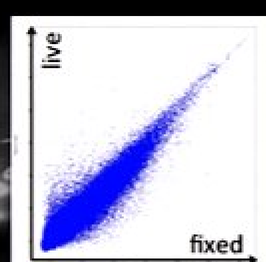
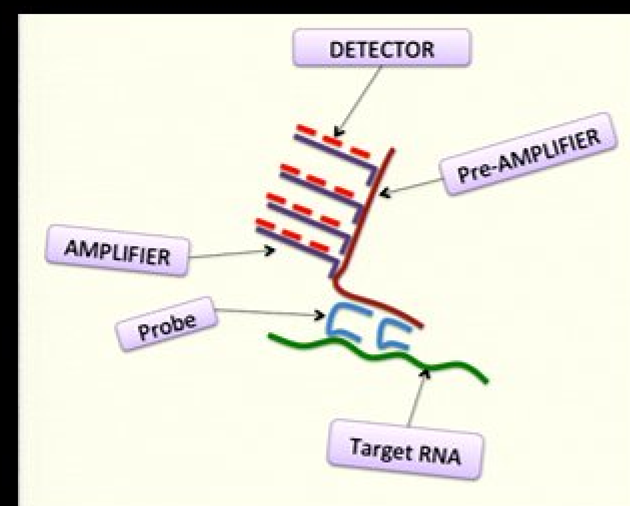
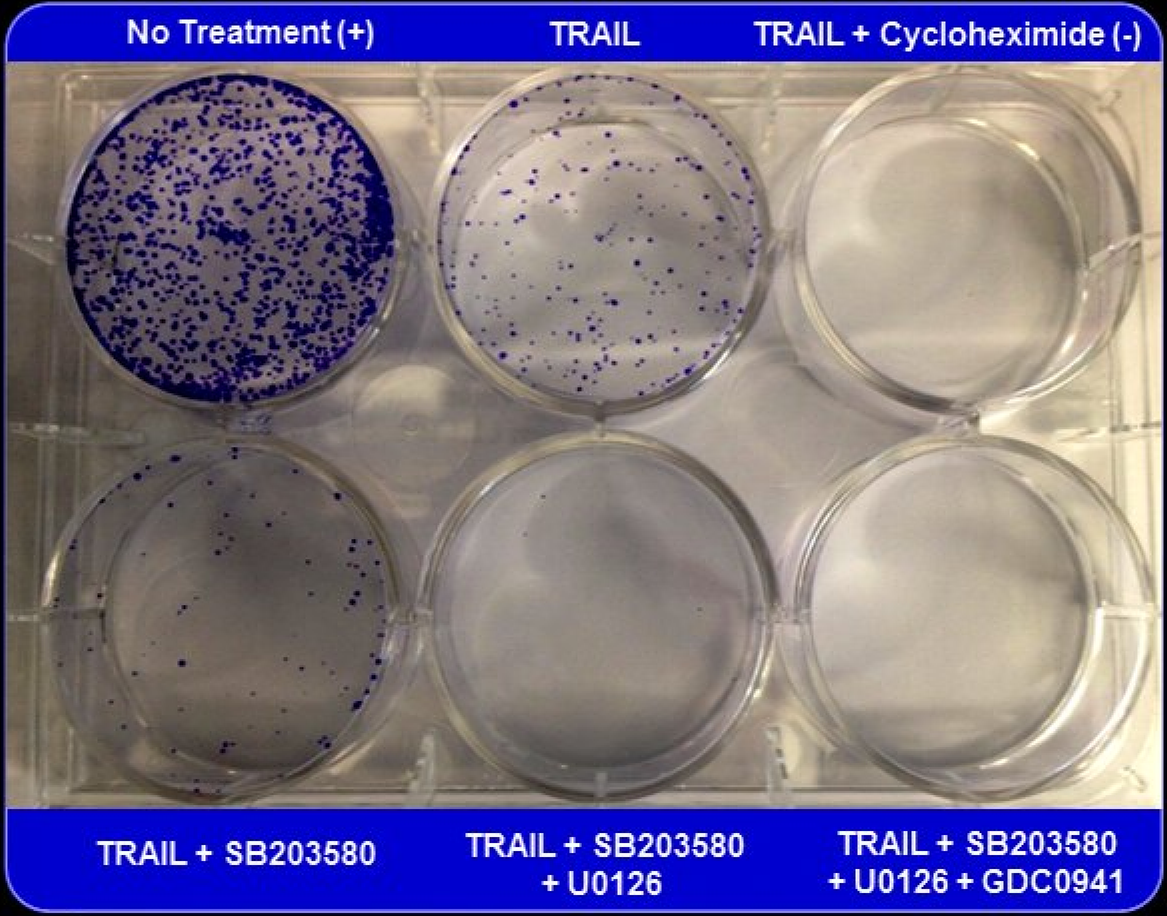
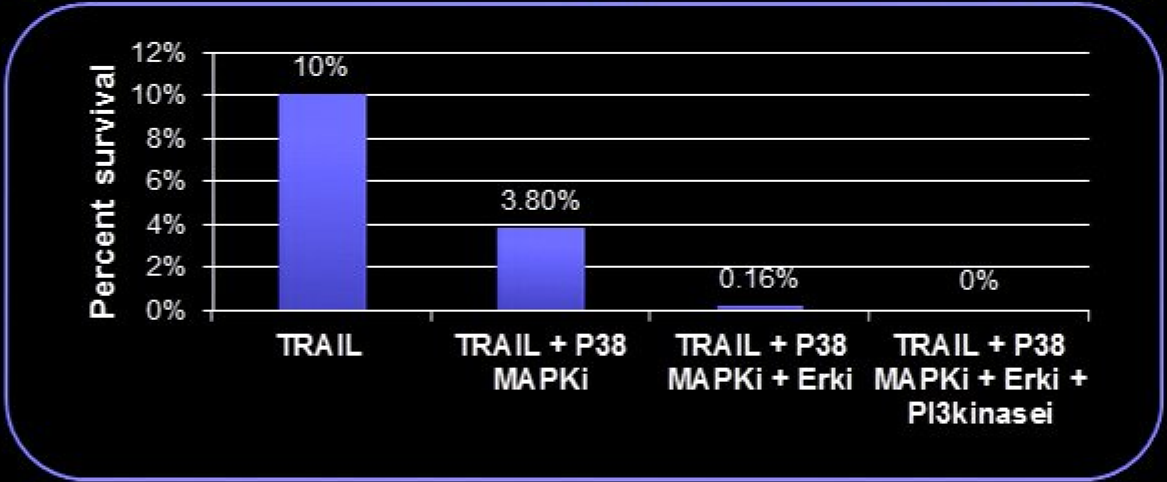




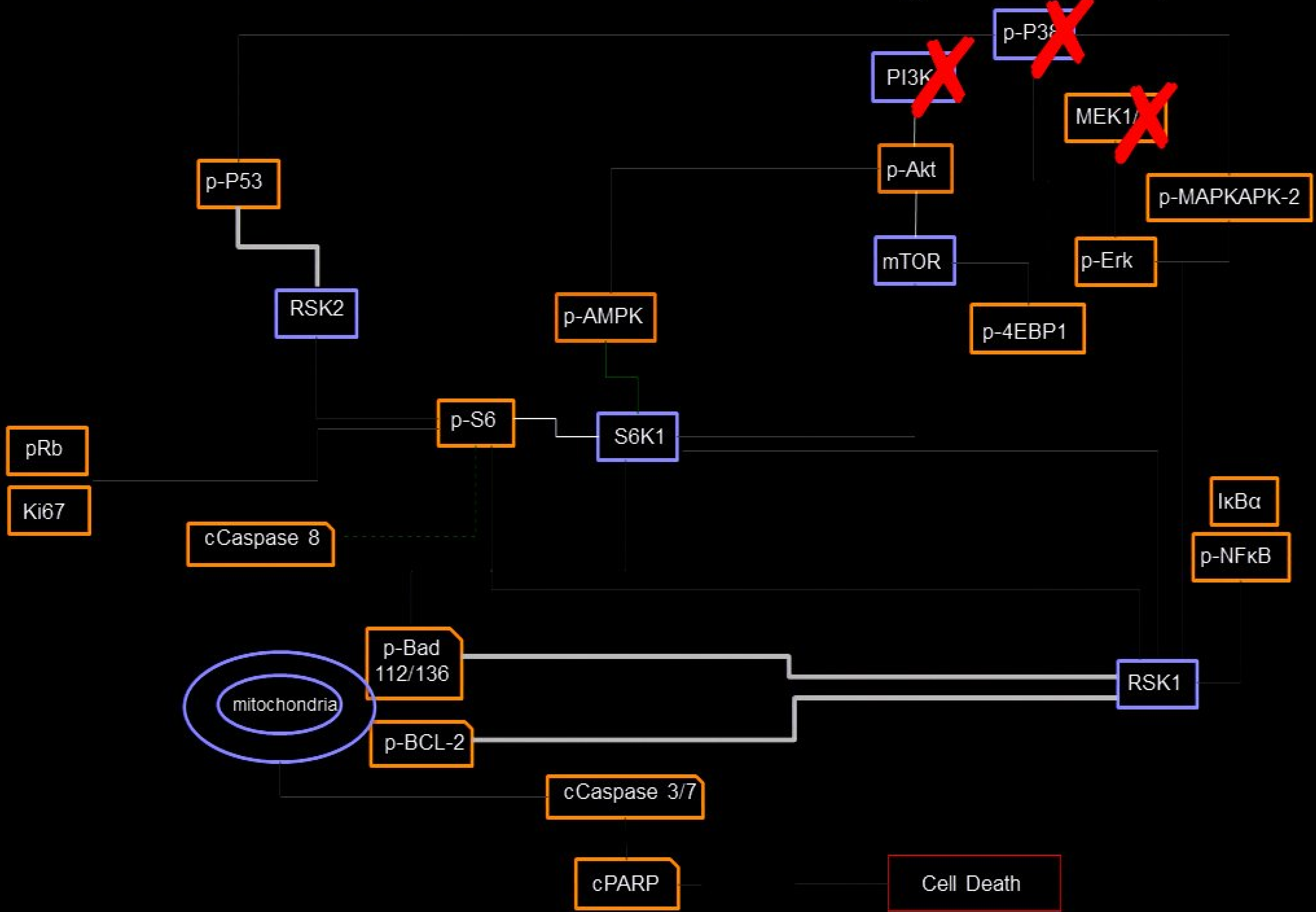


Compensatory signaling off w/ targeted kinase inhibition





Survival factor/TRAIL interaction signaling map



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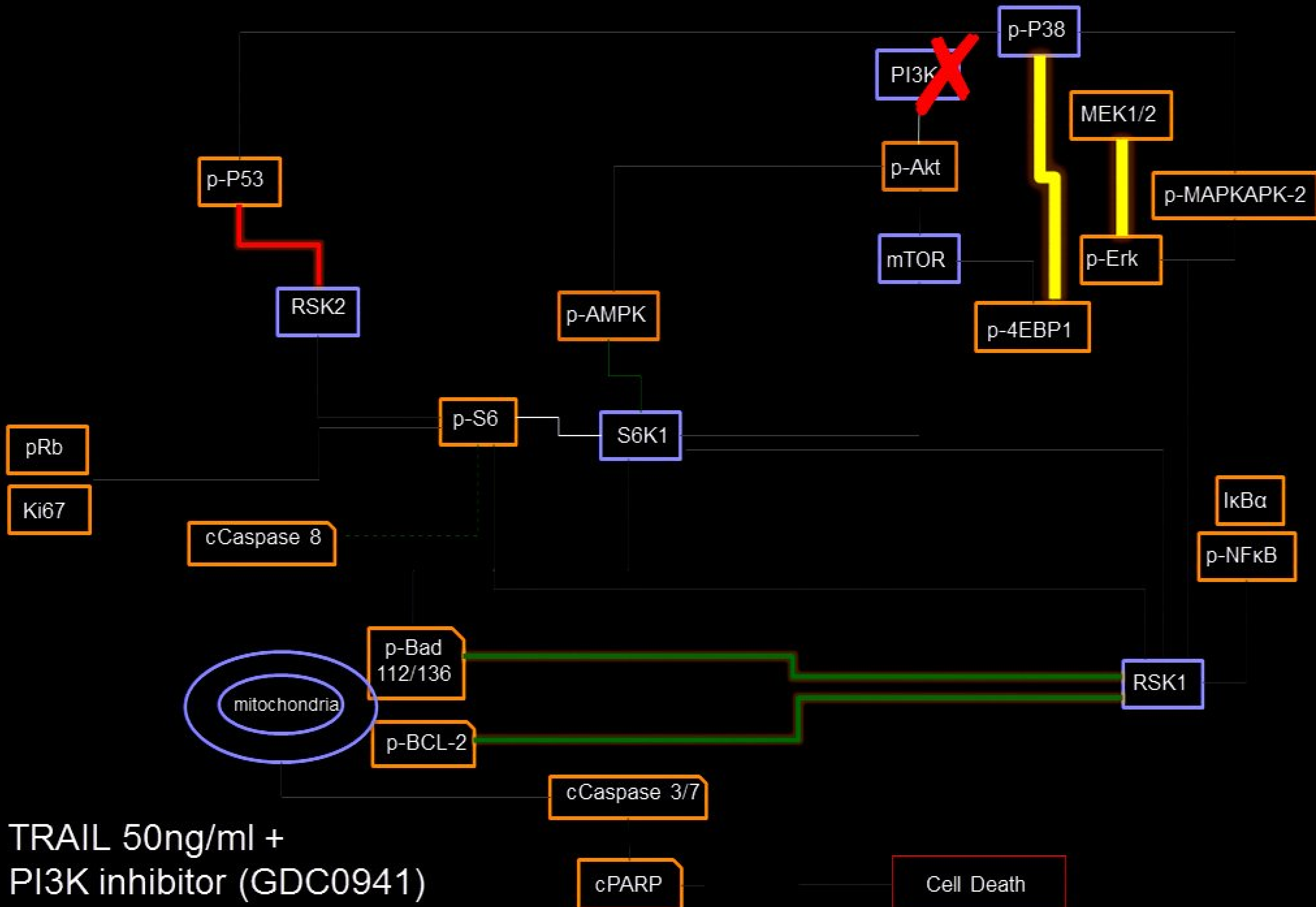

TRAIL 50ng/ml +
PI3K inhibitor (GDC0941)

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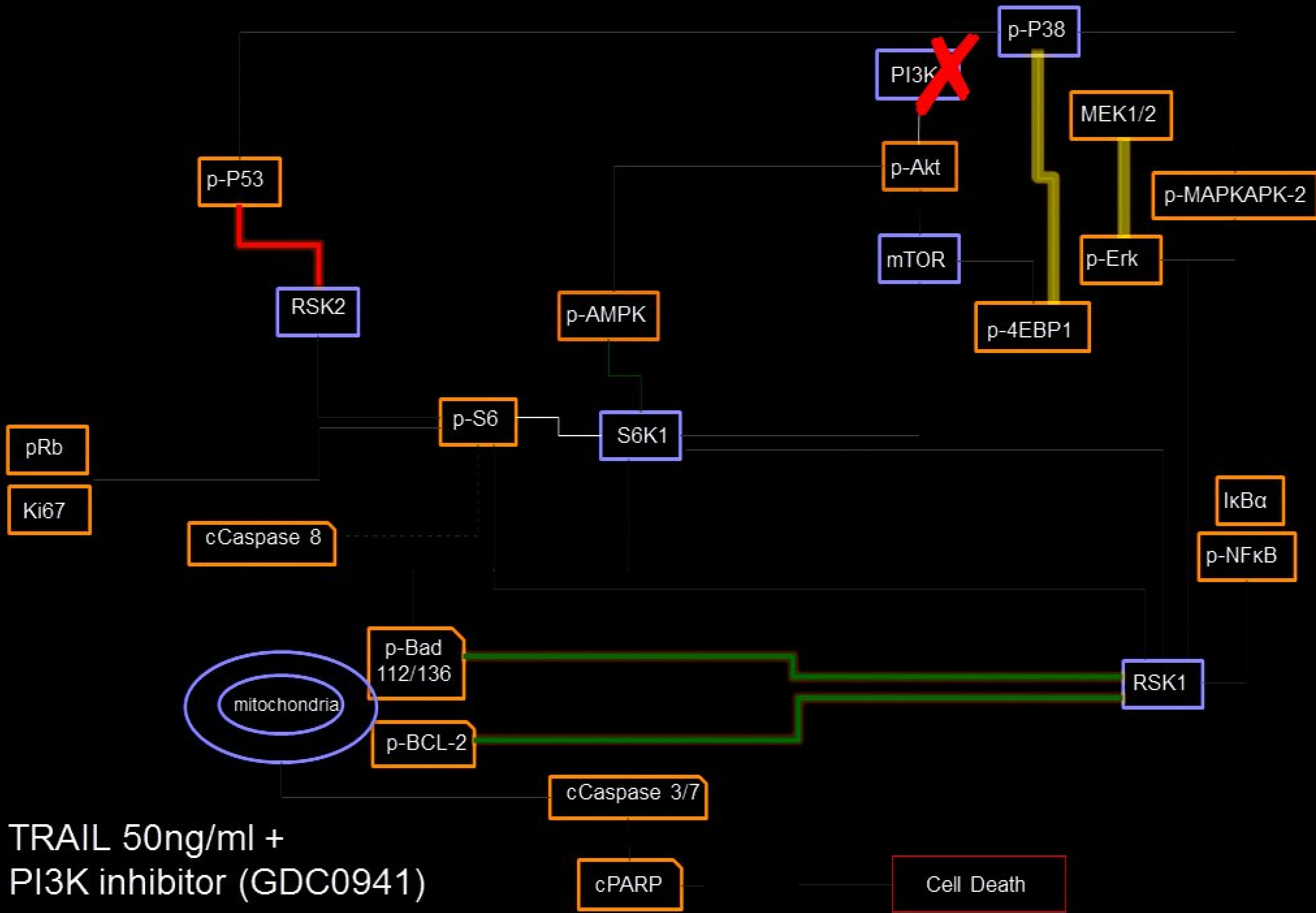

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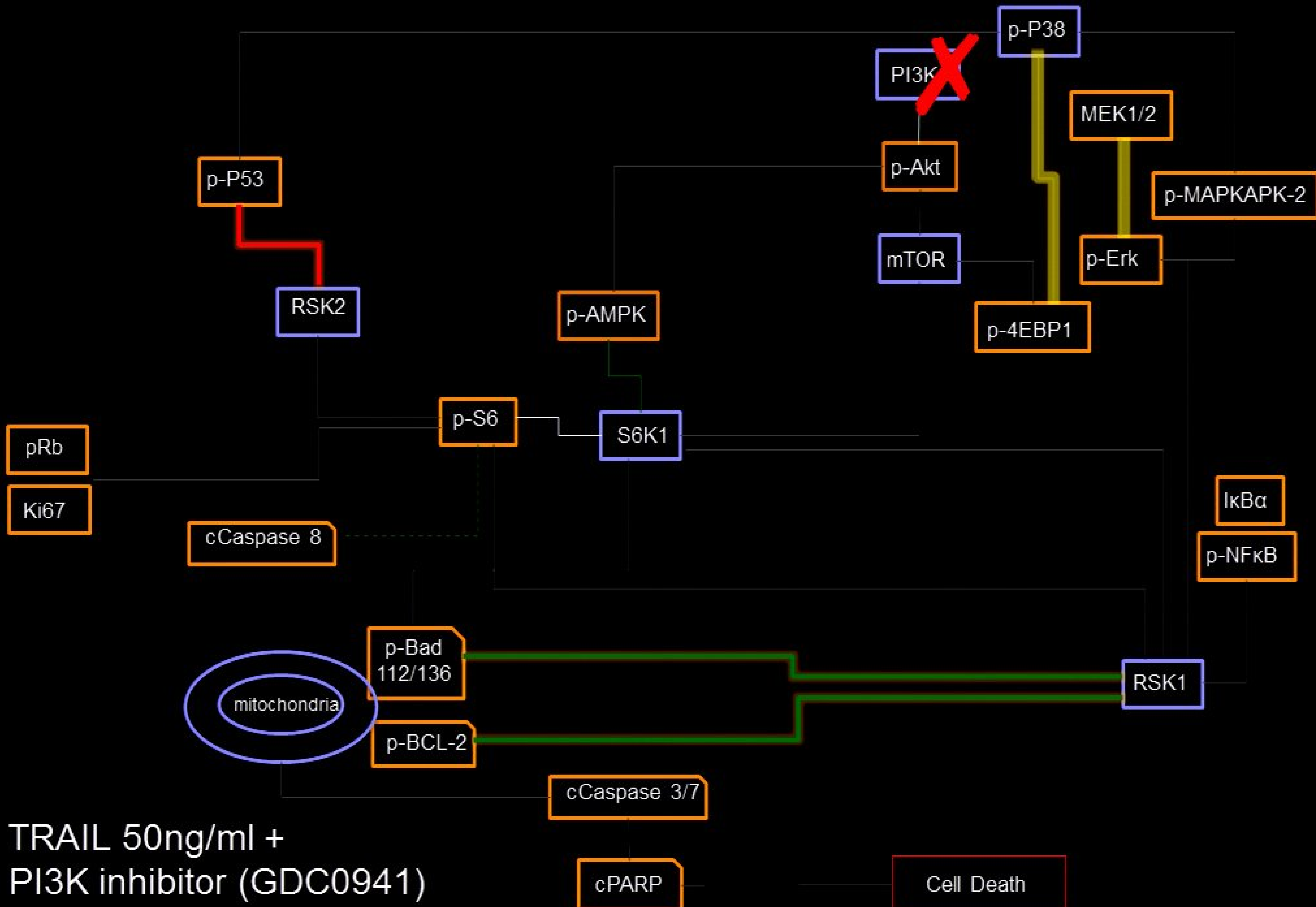

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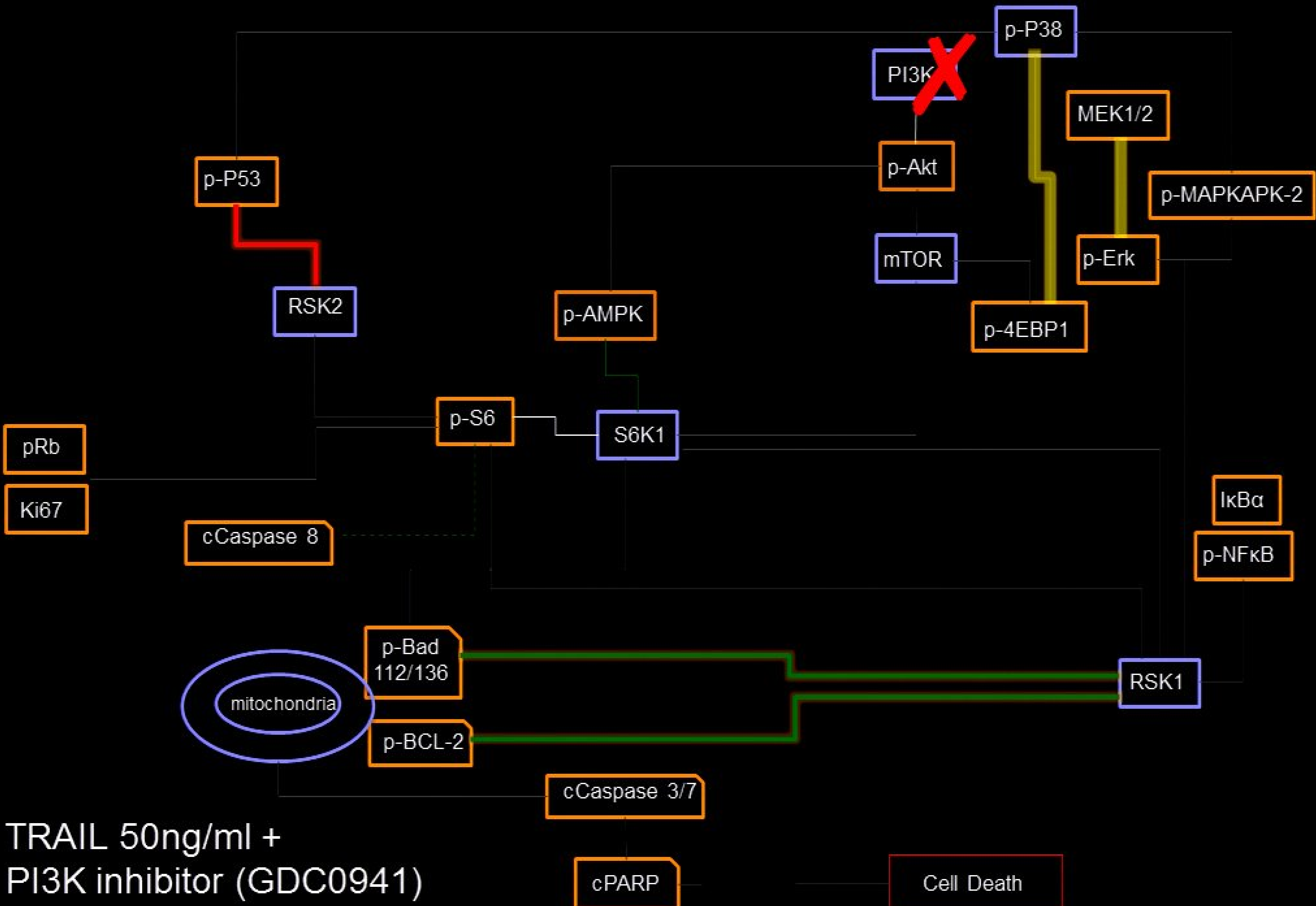

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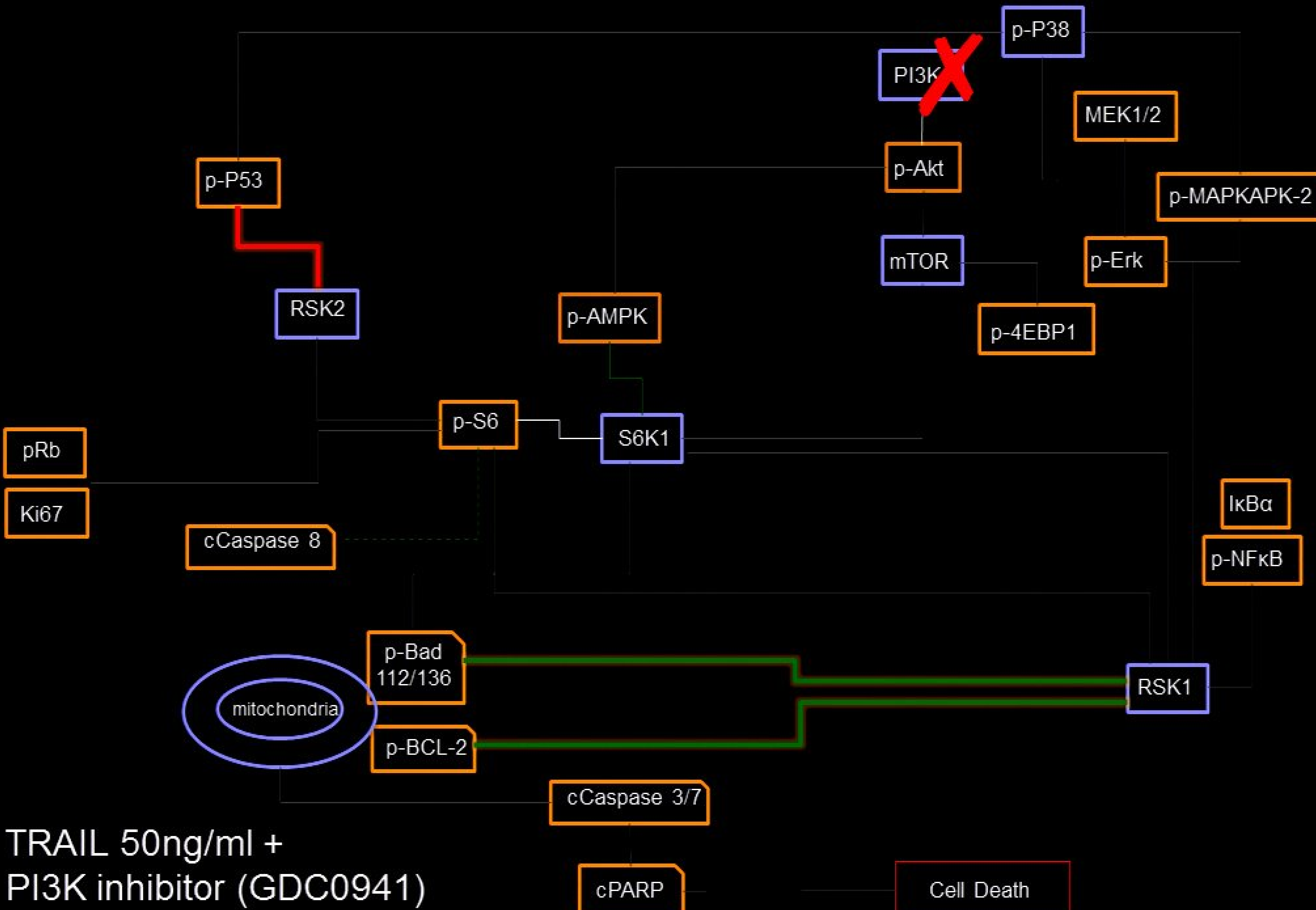

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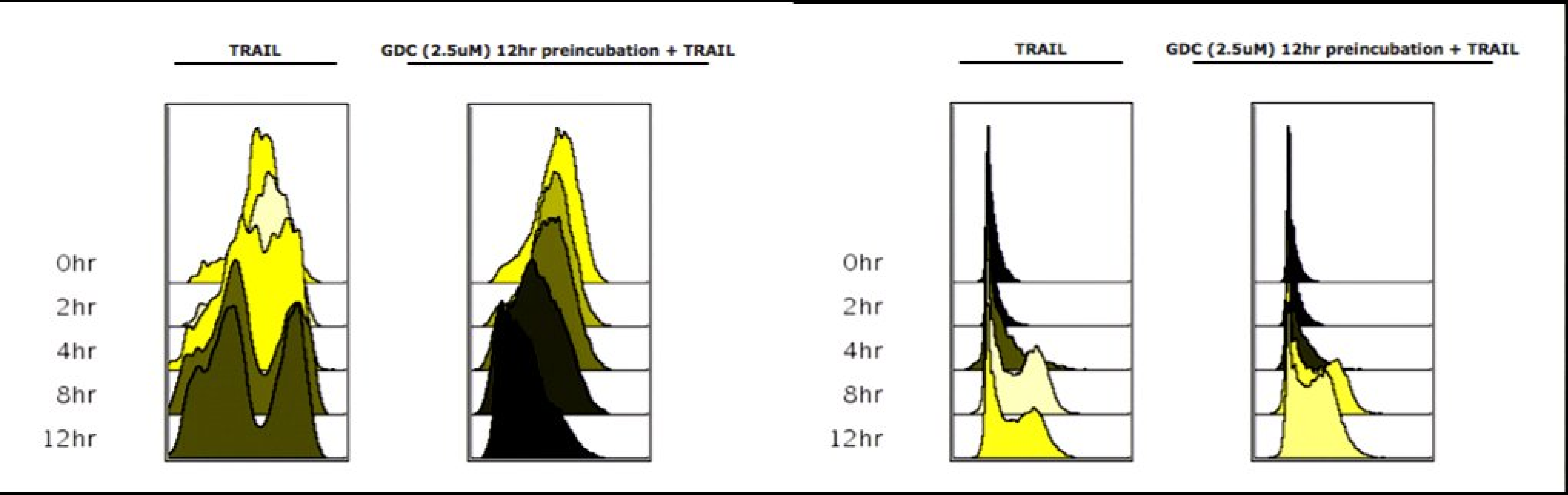
Inhibiting S6 phosphorylation via a PI3K inhibitor does not have a significant effect on survival

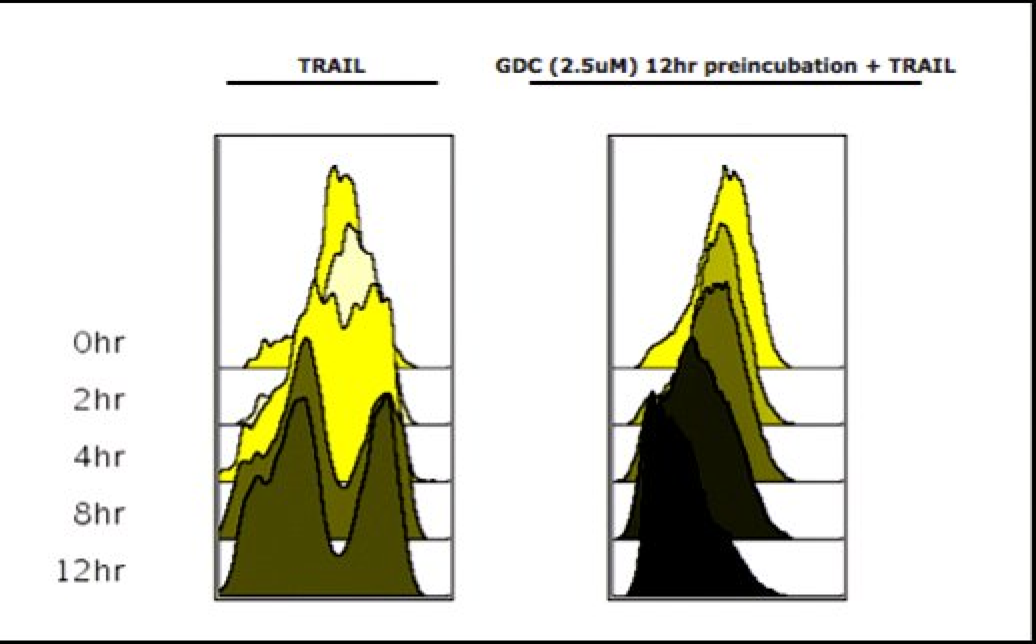
phospho-S6

Viability



HeLas treated with TRAIL 50ng/ or TRAIL with 12hr pre-incubation with GDC0941

phospho-S6



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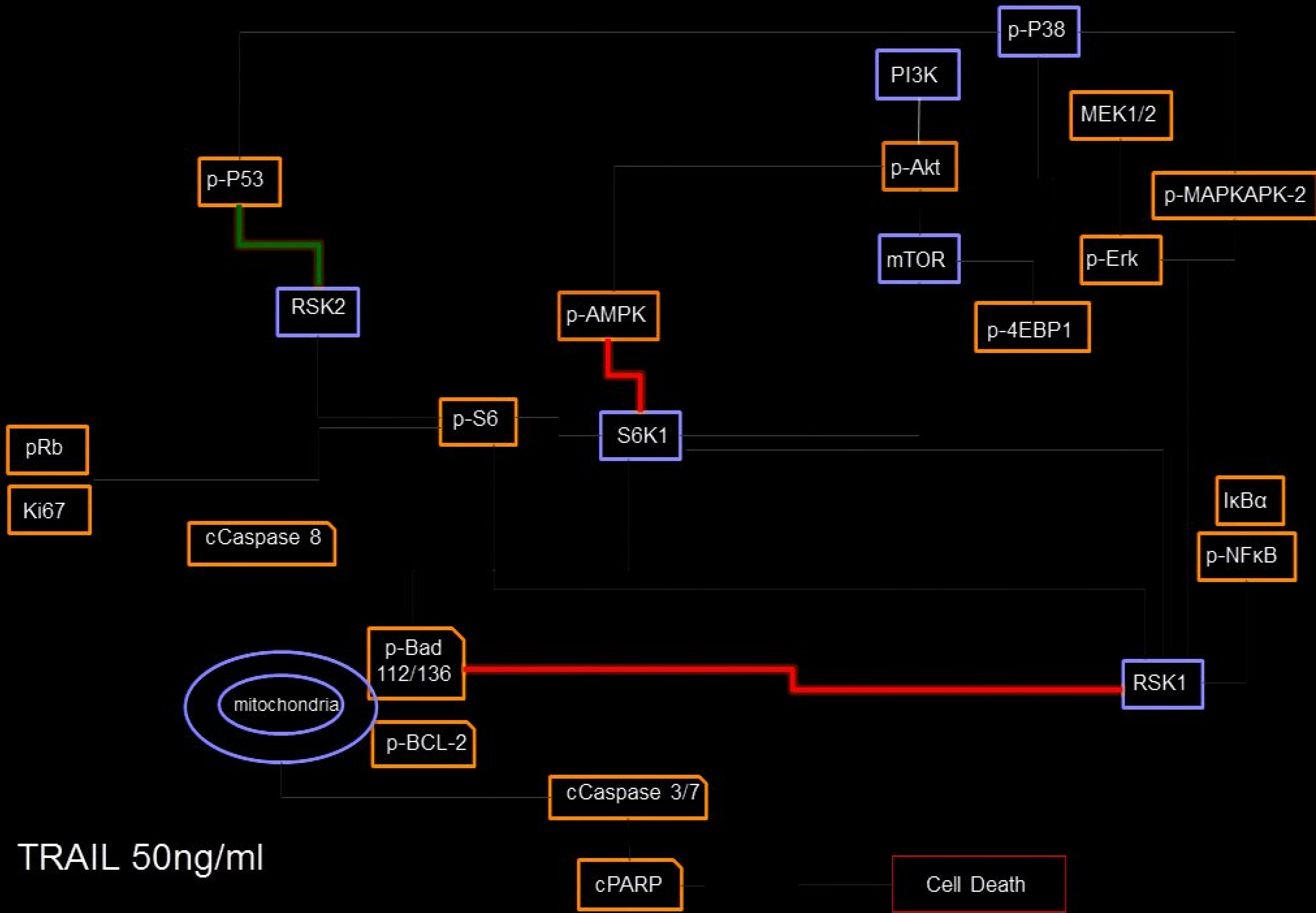

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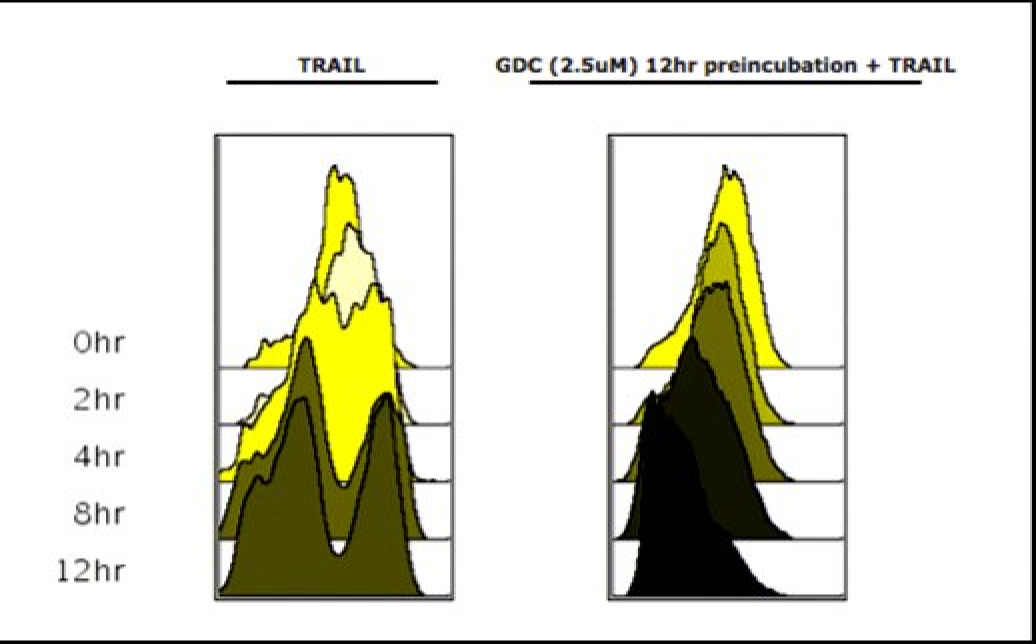

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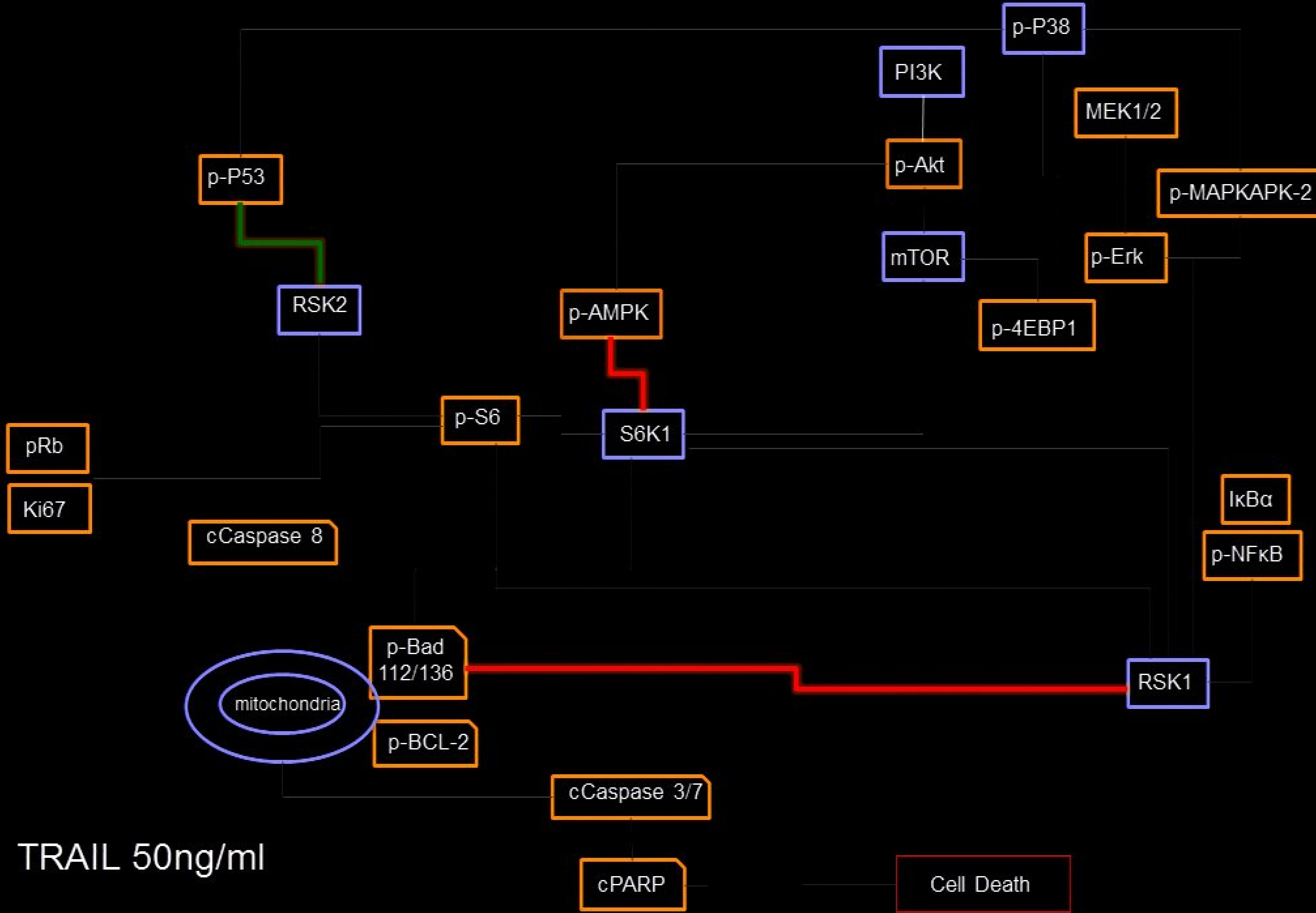
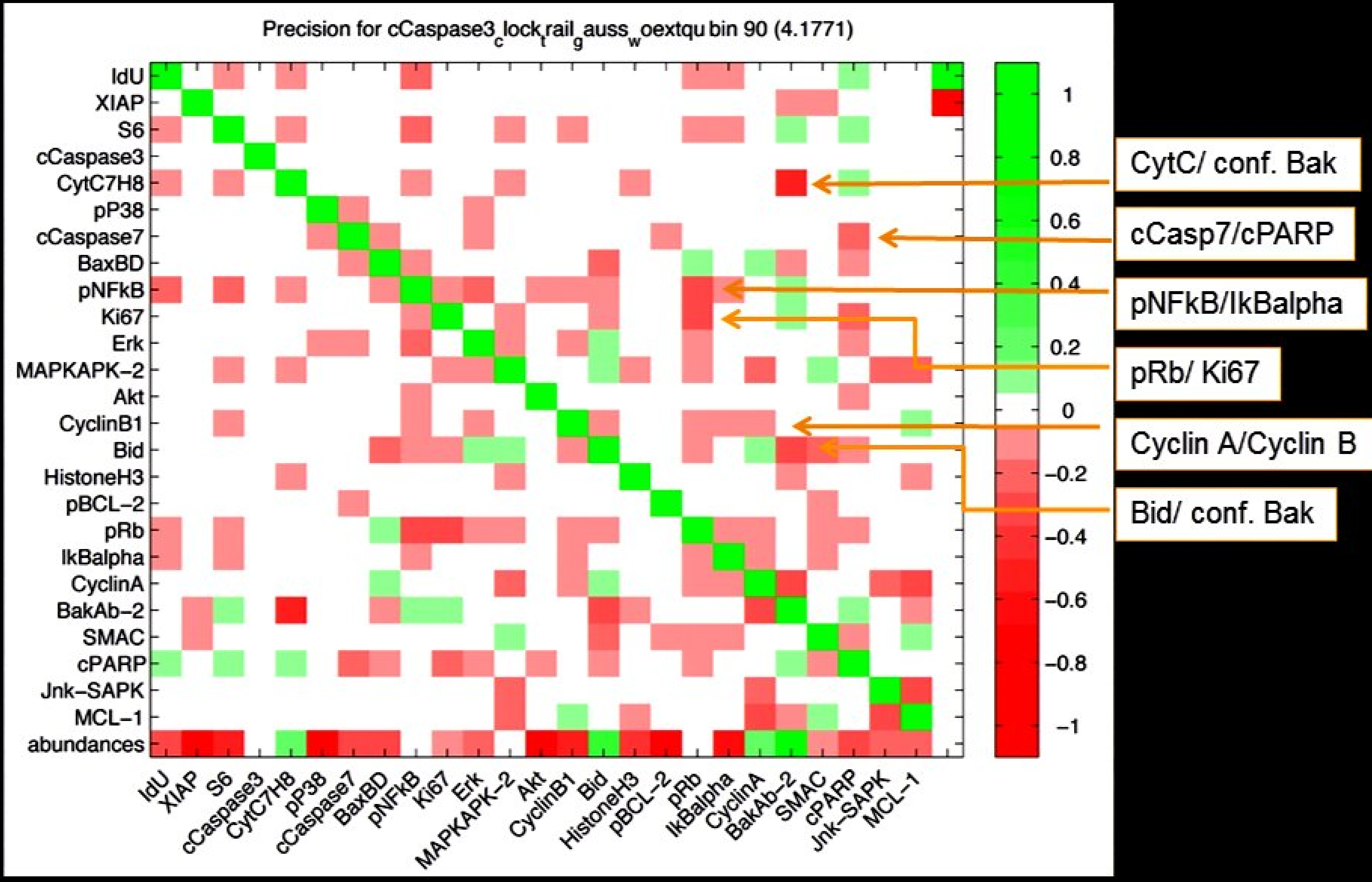
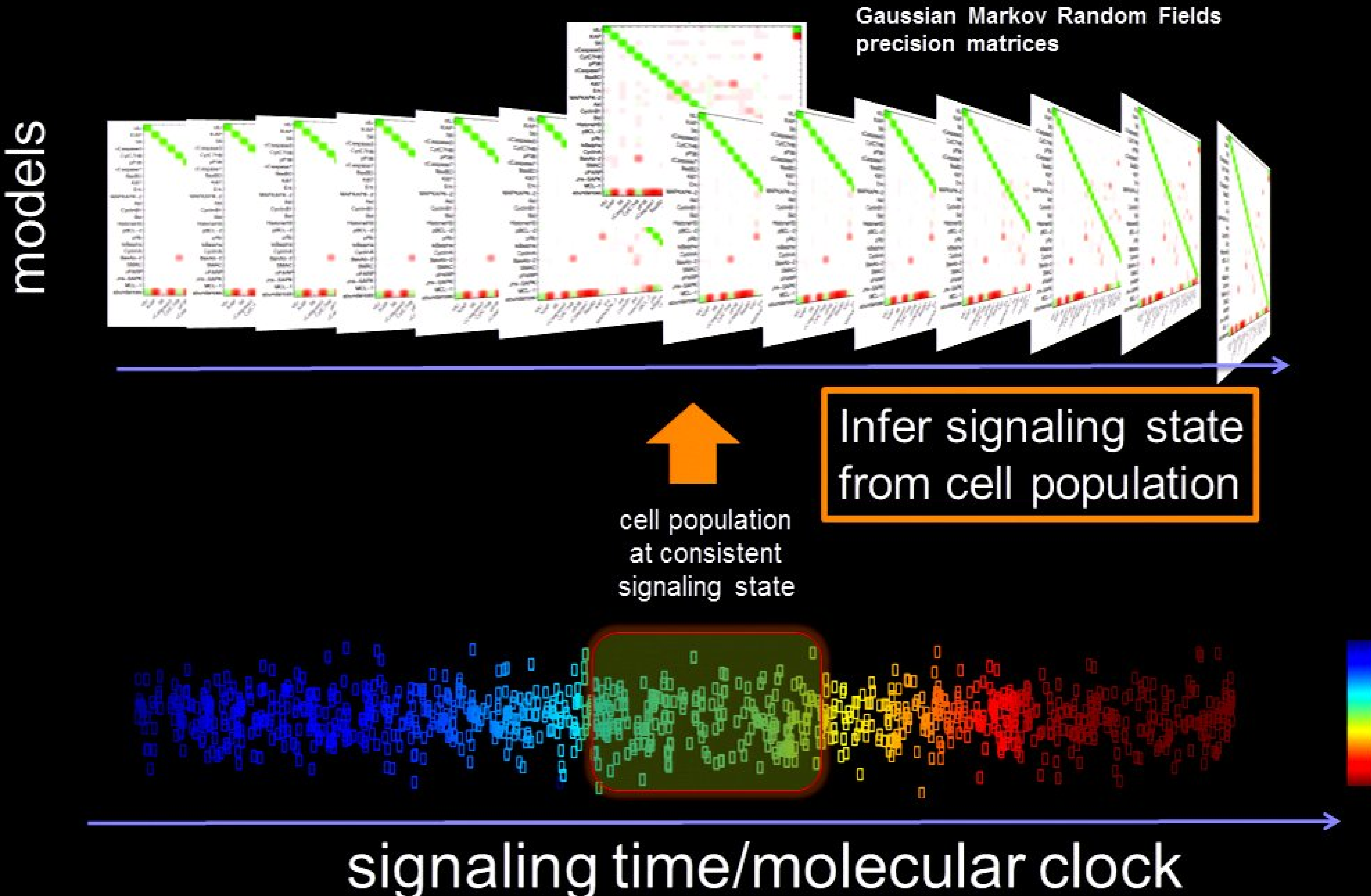


TRAIL 50ng/ml

Precision matrices recapitulate expected signaling relationships



Signaling Time with a Molecular Clock



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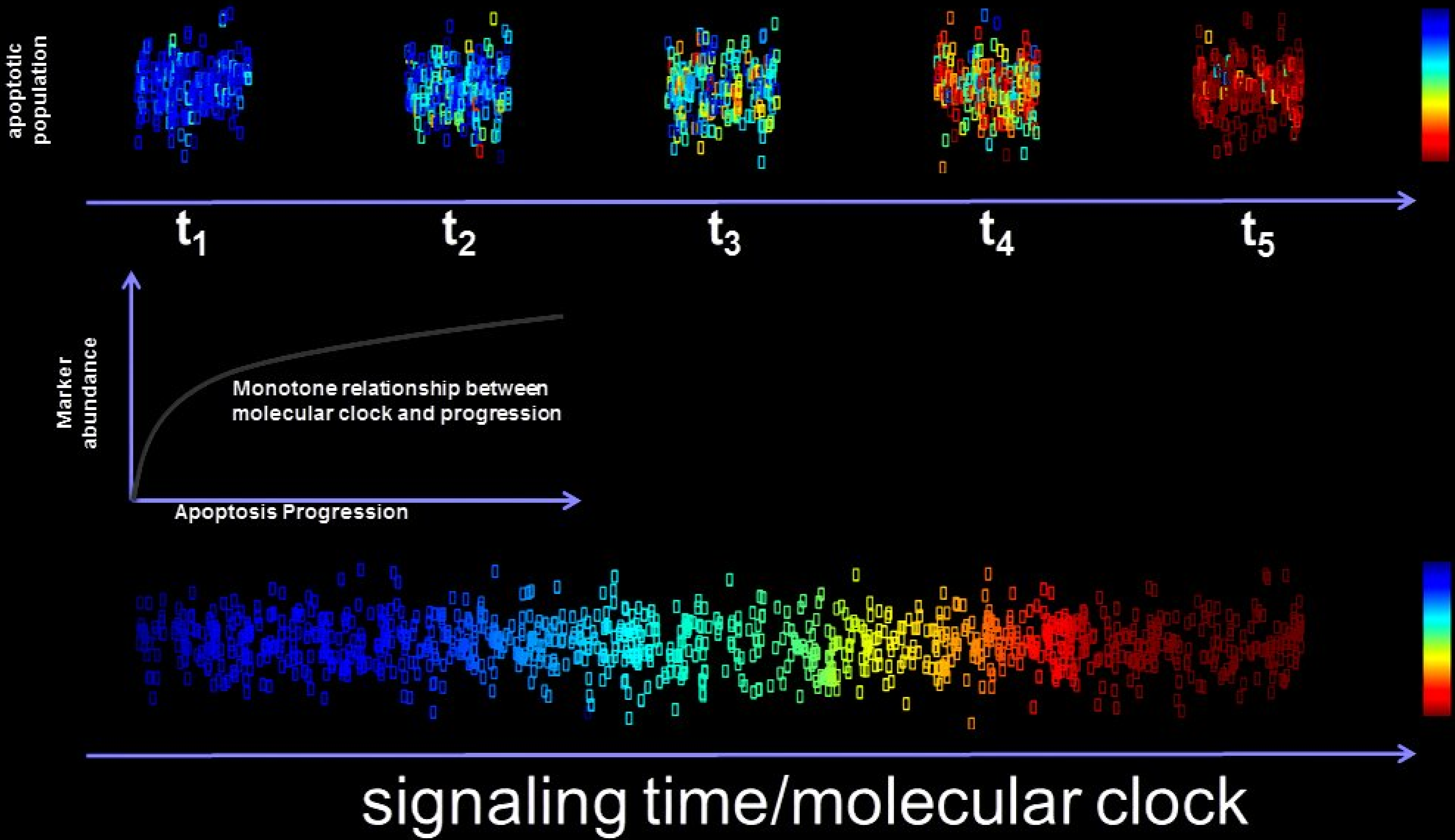

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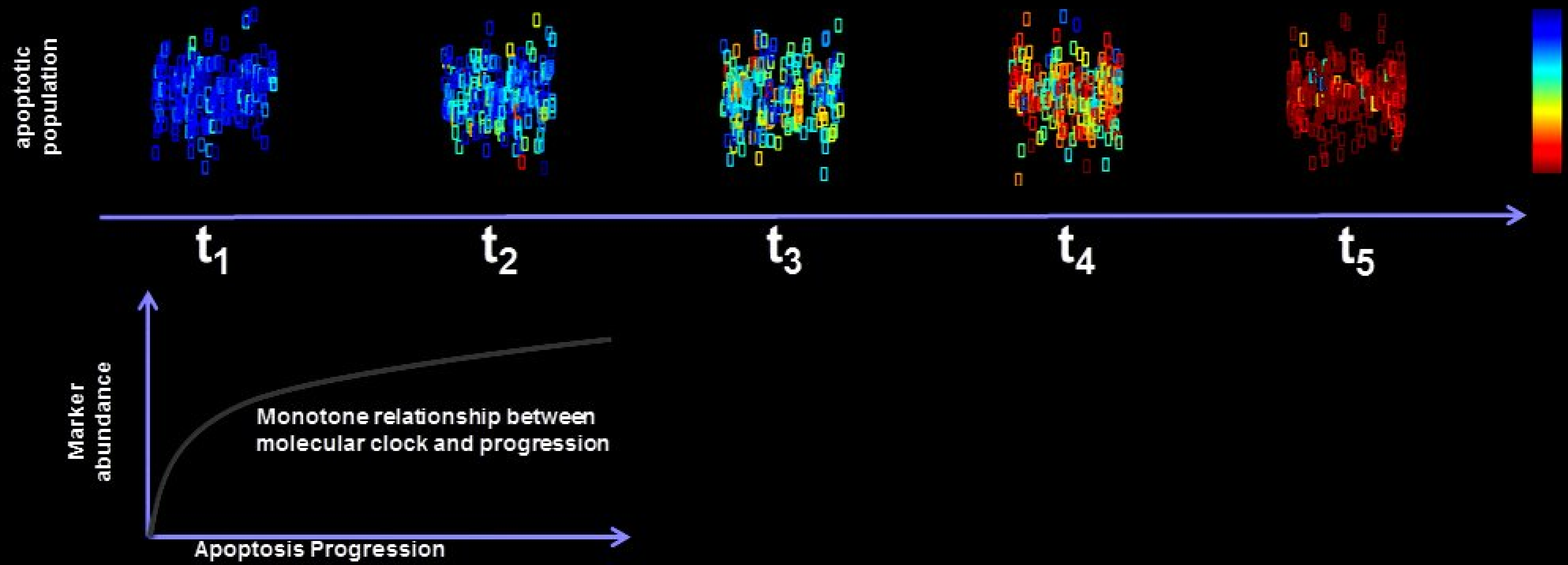

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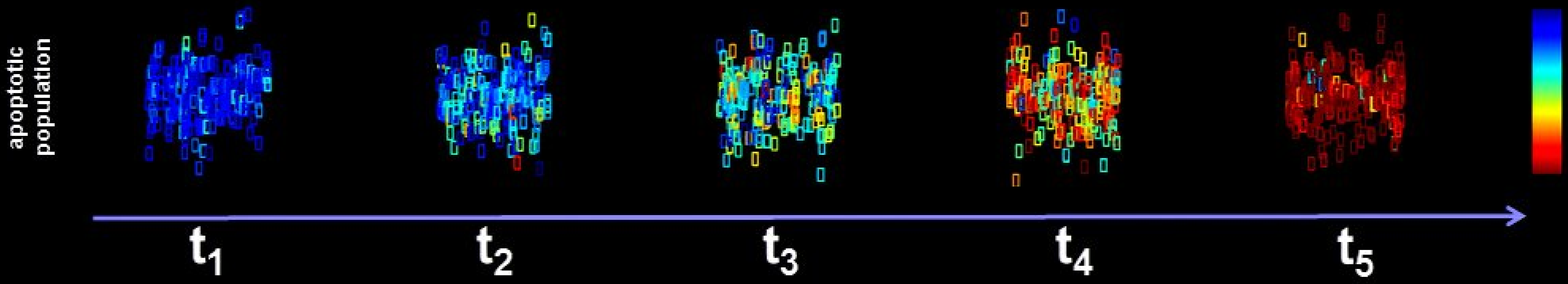
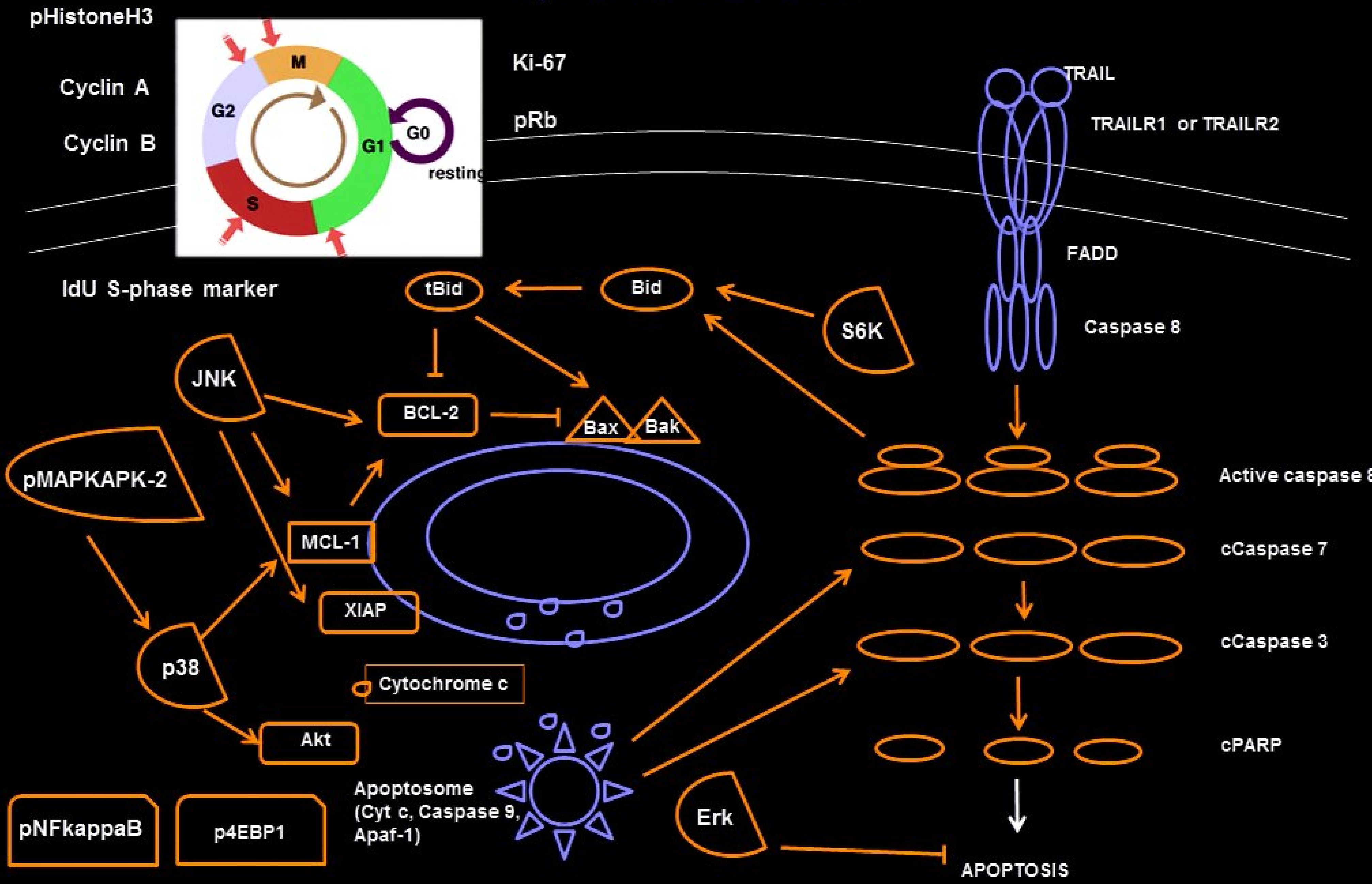


Apoptosis, cell cycle, transcriptional and metabolic system context



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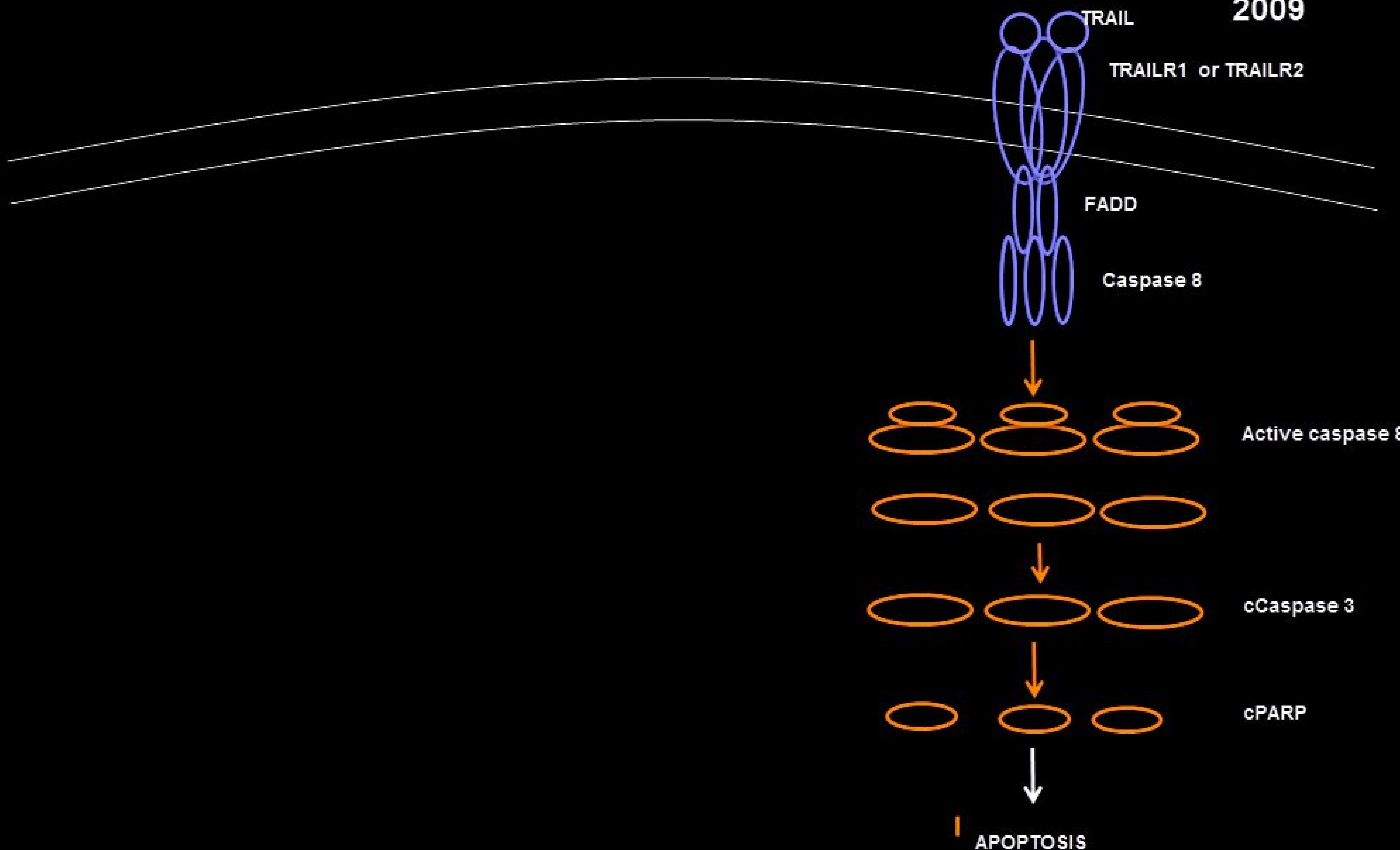
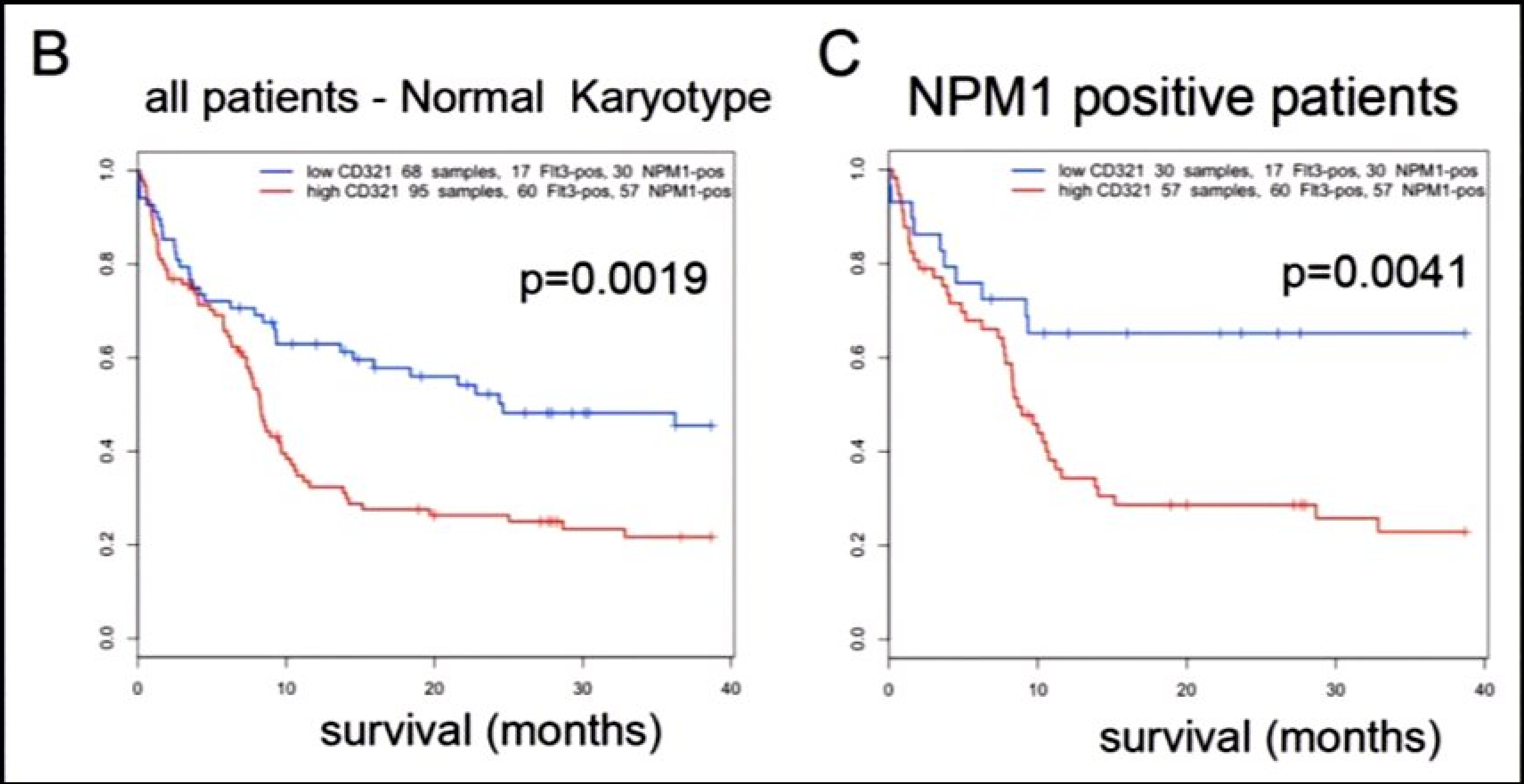
Sorger et al Nature 2009

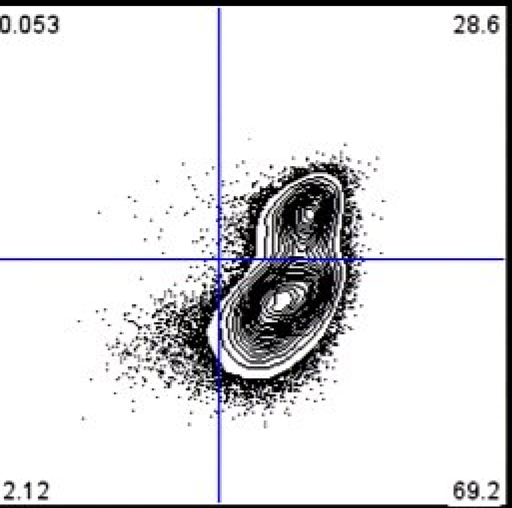


CD321 segregates outcome & better with NPM1

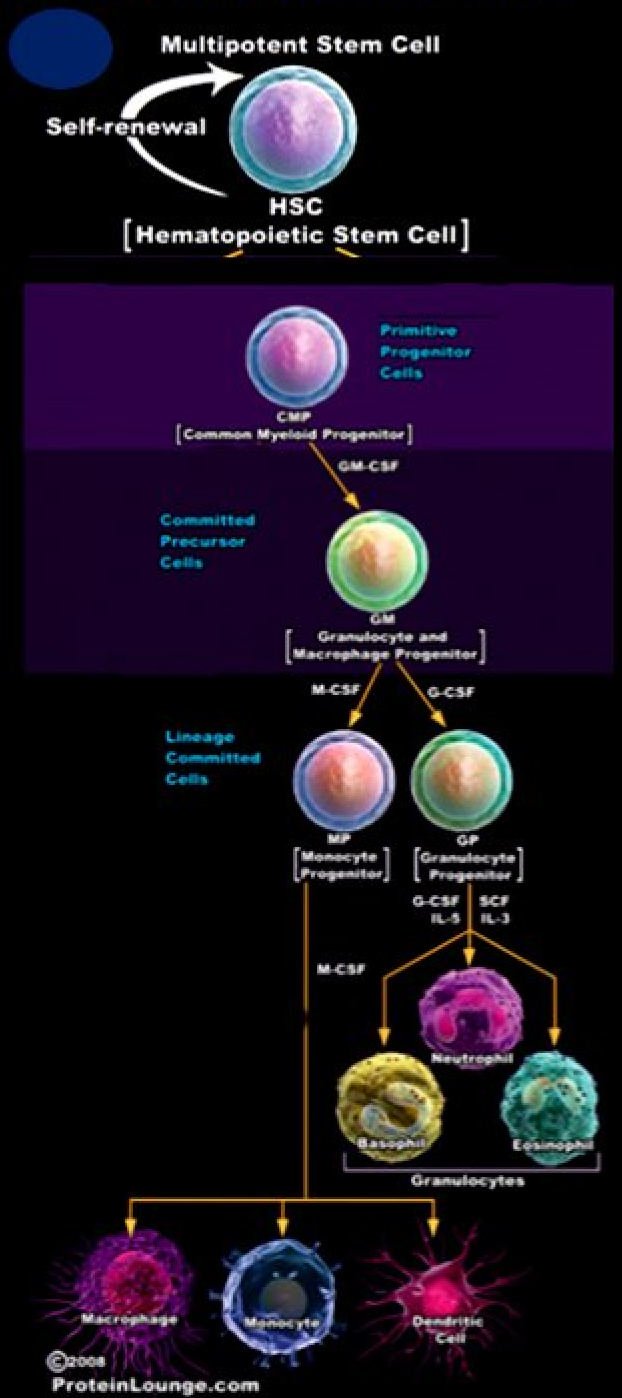


Nucleophosmin (NPM), also known as **nucleolar phosphoprotein B23** or **numatrin**

Responding cells are primitive … Non-responders are mature



Cytokine Feedback



Yury Goltsev,
Faye Hsu

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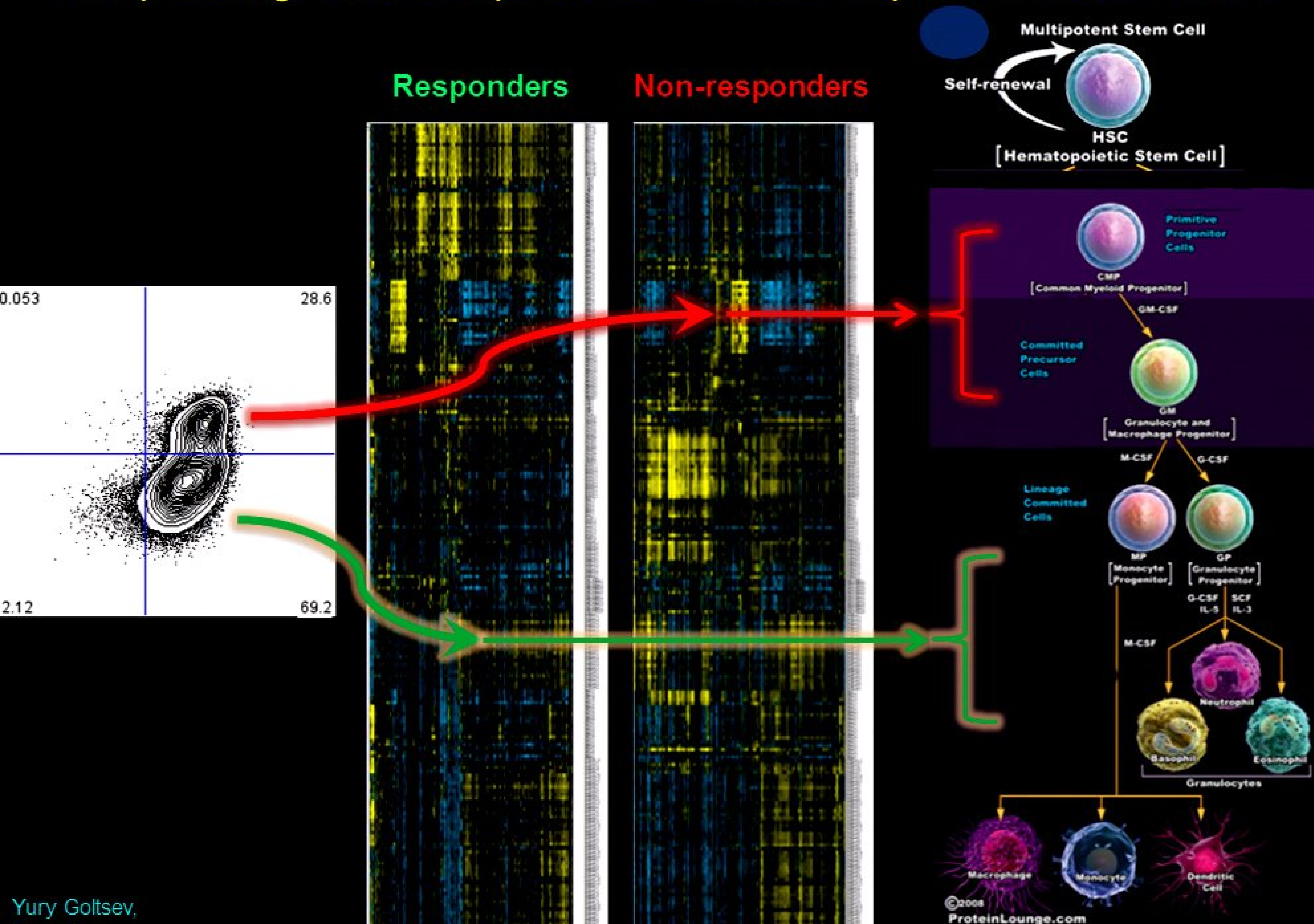

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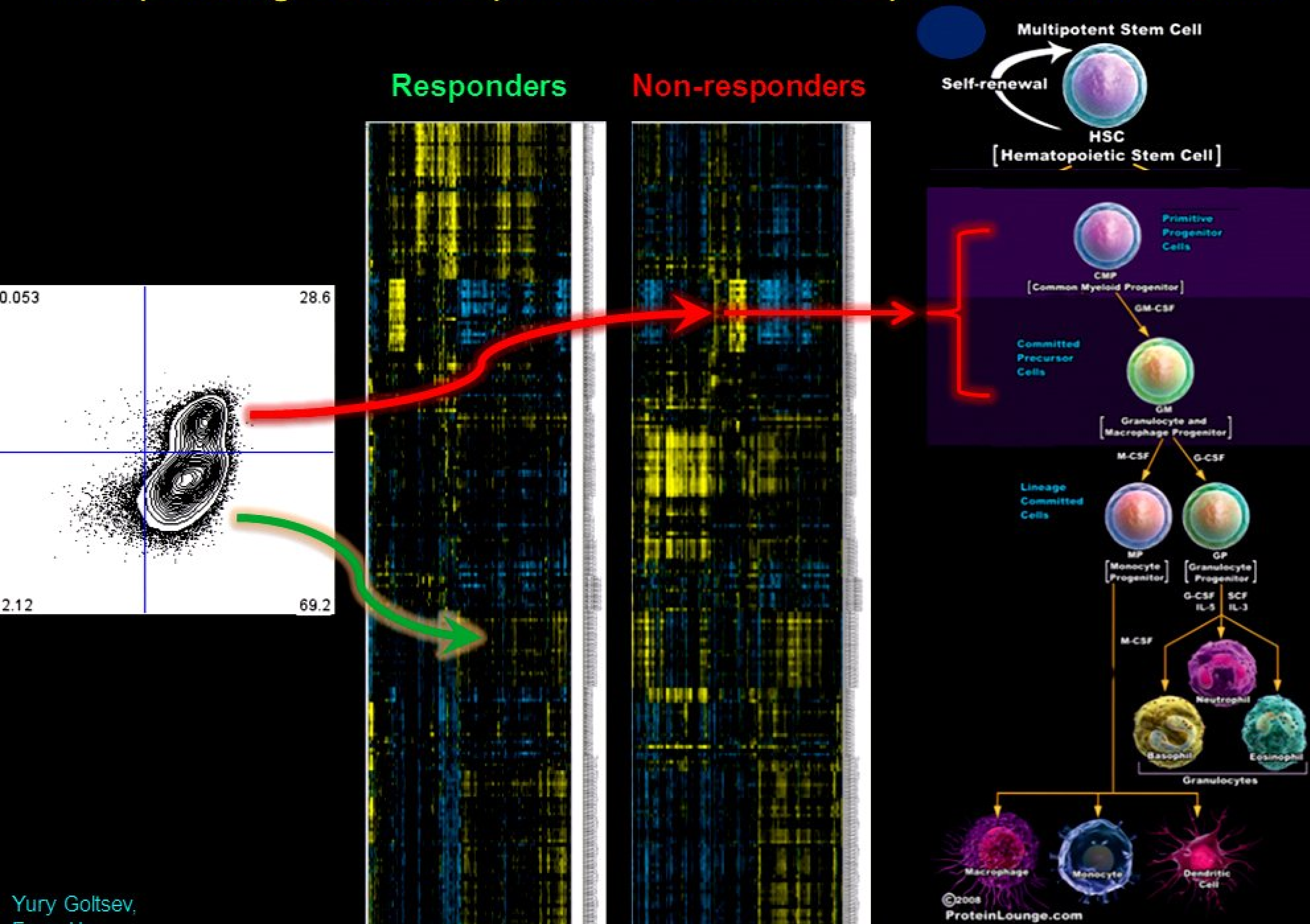

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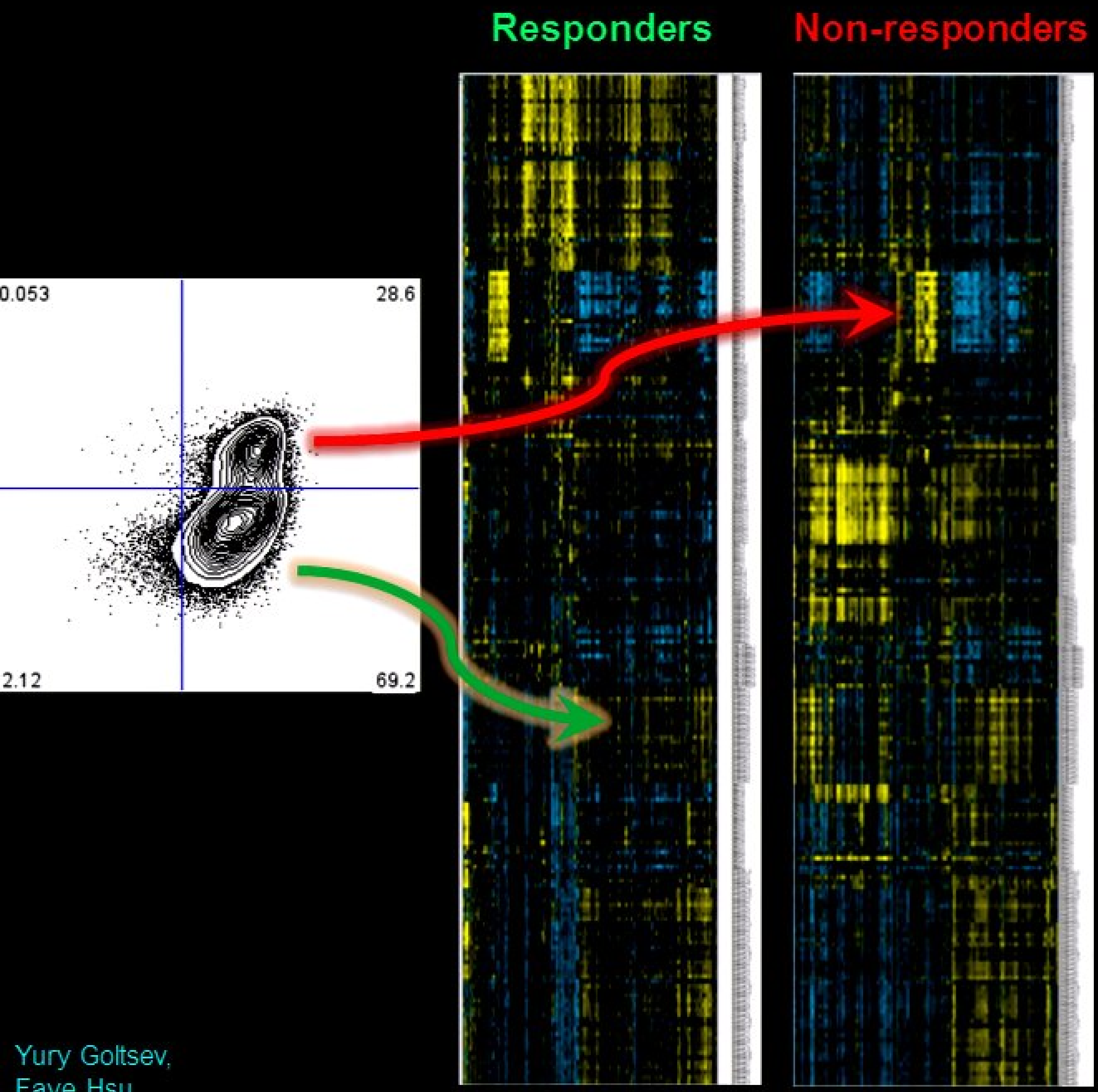

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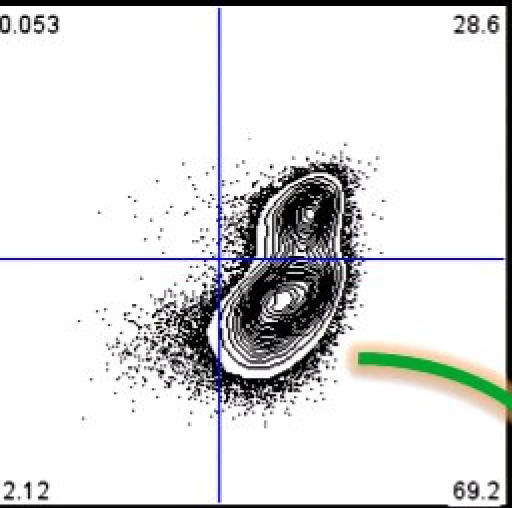

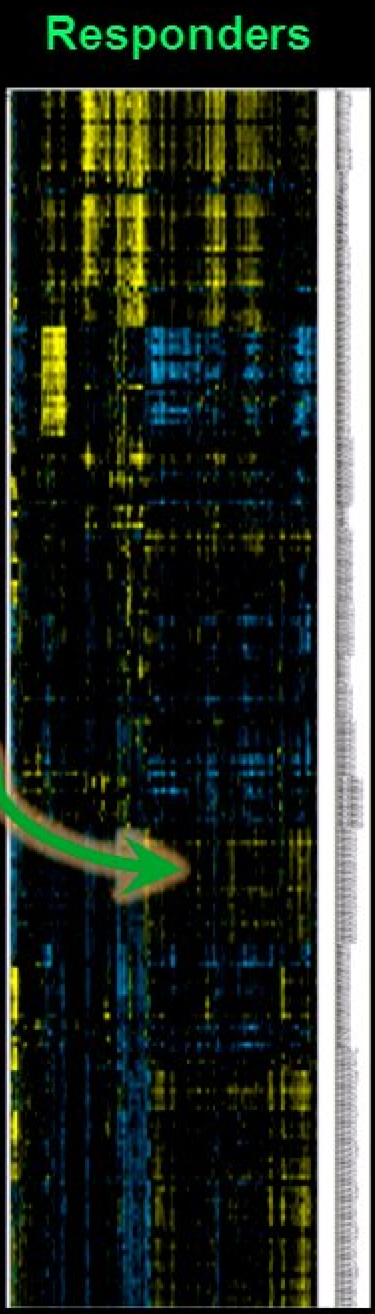

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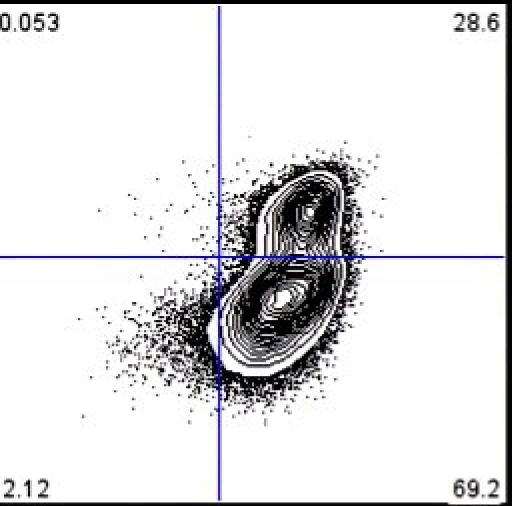

Yury Goltsev,
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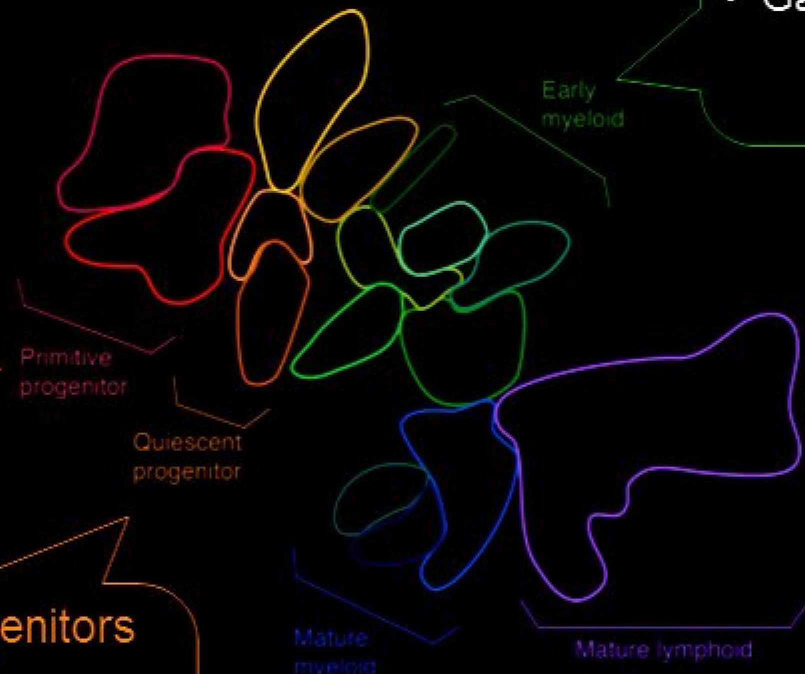
Summary of differential signaling in phenotype groups

Early myeloids

- Constitutive pAkt / 4EBP1
- Gaining cytokine signaling

Primitive progenitors

- Constitutive pSTAT3
- Low basal pAkt / 4EBP1

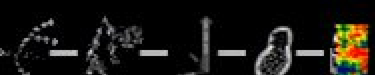


Quiescent progenitors

- Low basal pRb
- Low basal Akt / 4EBP1
- Weak cytokine signaling
- Akt-driven pCREB

Mature myeloids

- Constitutive pAkt / 4EBP1
- Constitutive pSTAT1 / pS6
- Constitutive c-Cbl
- Strong cytokine signaling



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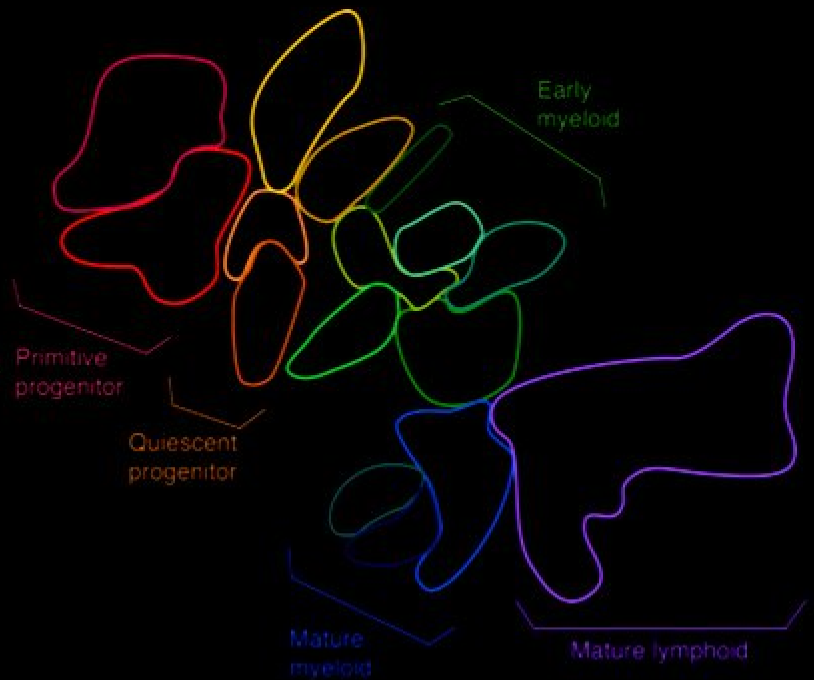
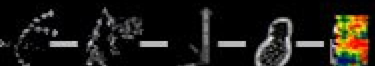
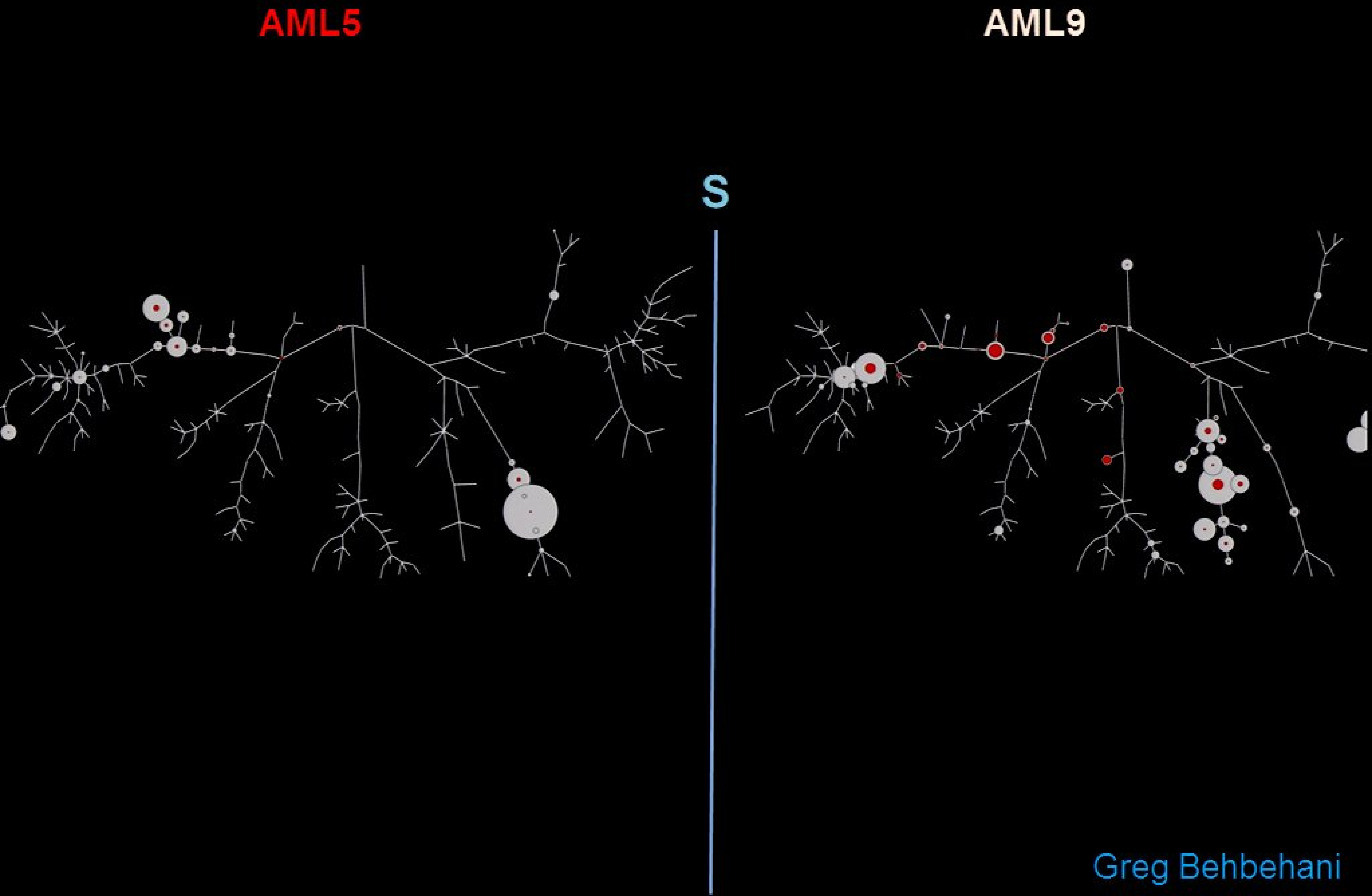




Cell Cycle Distribution Varies Within & Across AML Cell Subsets



Greg Behbehani

Cell Cycle Distribution Varies Within & Across AML Cell Subsets

AML5

AML9

Greg Behbehani

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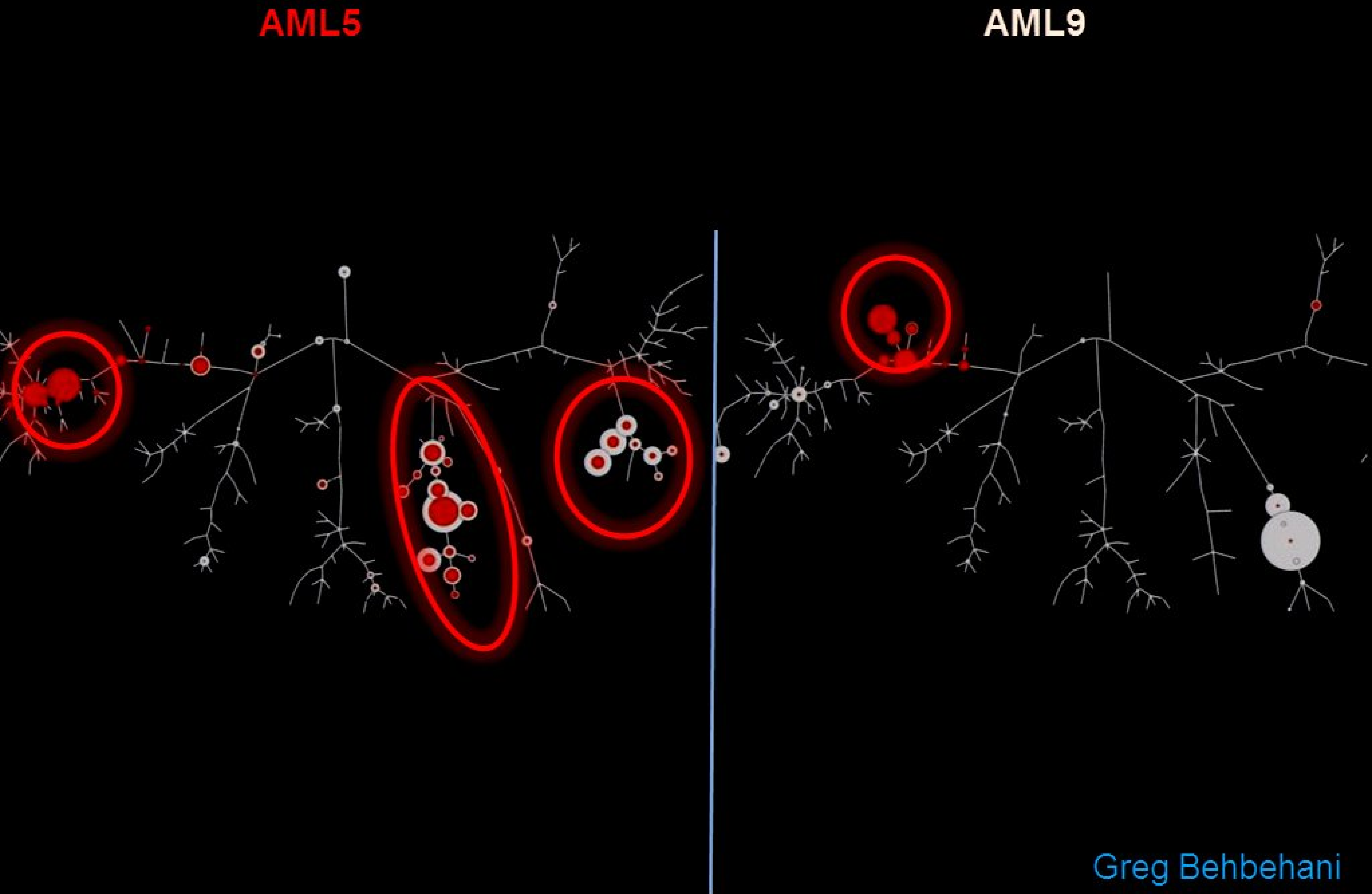

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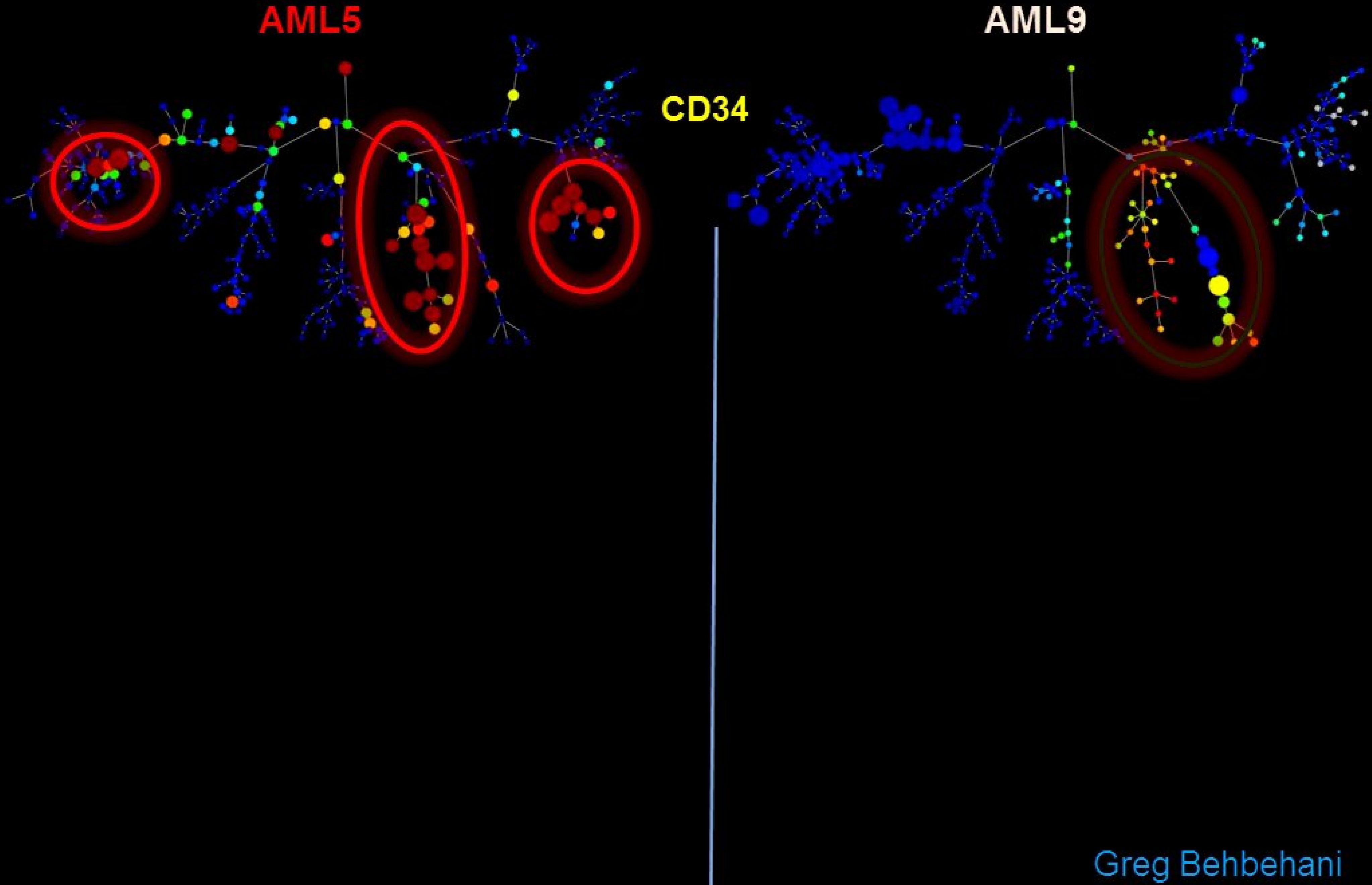

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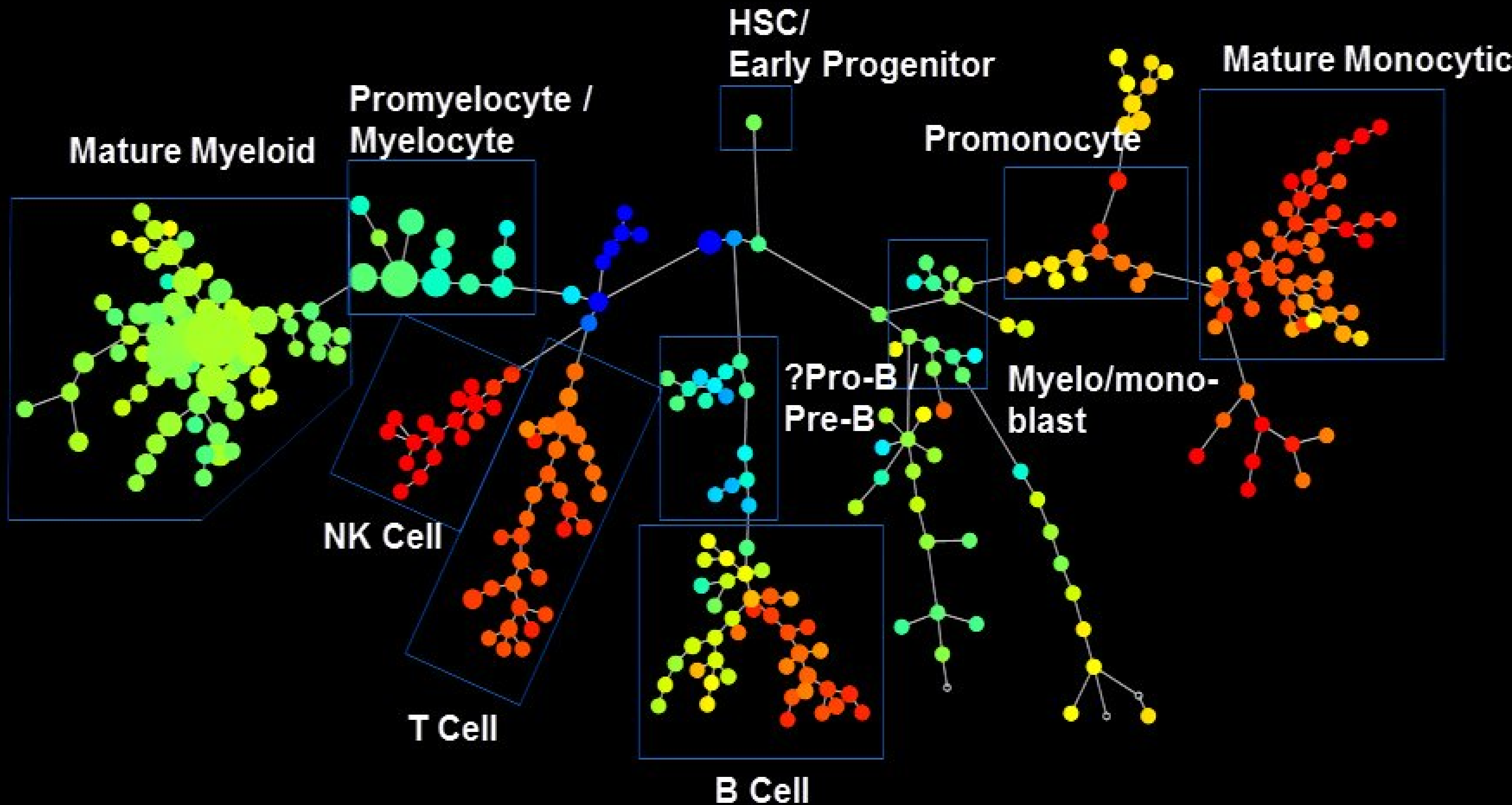
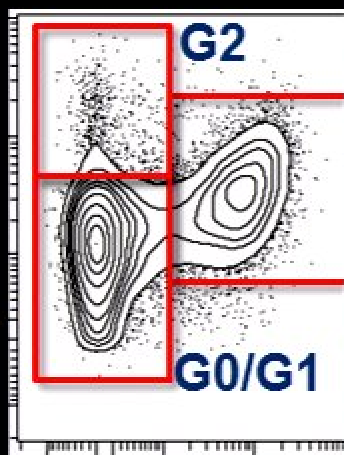
Distinct AML Immuno-phenotypes

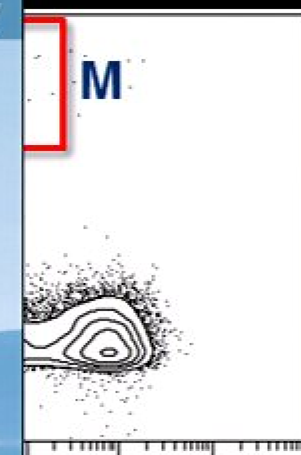


Normal human bone marrow
Clustered with AML samples
Colored for CD45

Greg Behbehani

Additional Markers Allow for Complete Cell
C





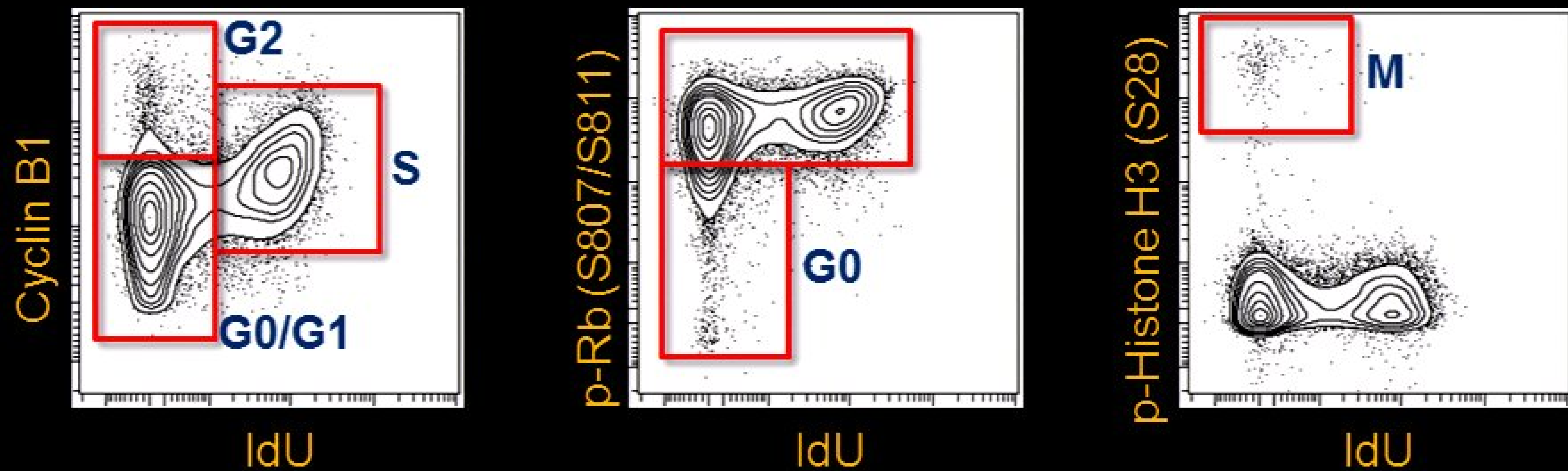
p16
p21
Ki-67
Cyclin A
Cyclin B1
pAMPK

p
C
C
c
p
p

1
2
C2D
RP
P1
oneH3(S28)

Greg Behbehani, et al, Cytometry A

Additional Markers Allow for Complete Cell Cycle State Assignment



p16
p21
Ki-67
Cyclin A
Cyclin B1
pAMPK

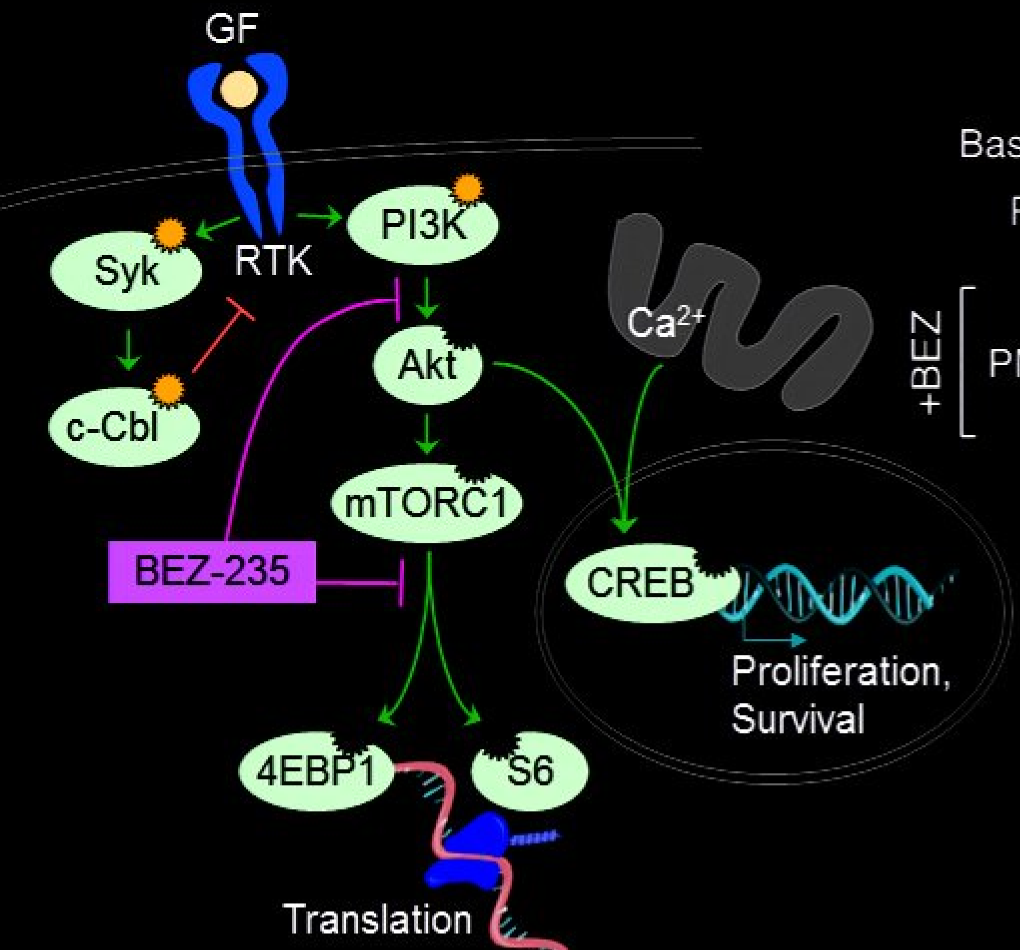
p-pRb (S807/811)
Cyclin D1/2/3
Cyclin E
c-Caspase3
prpS6
pCDK1

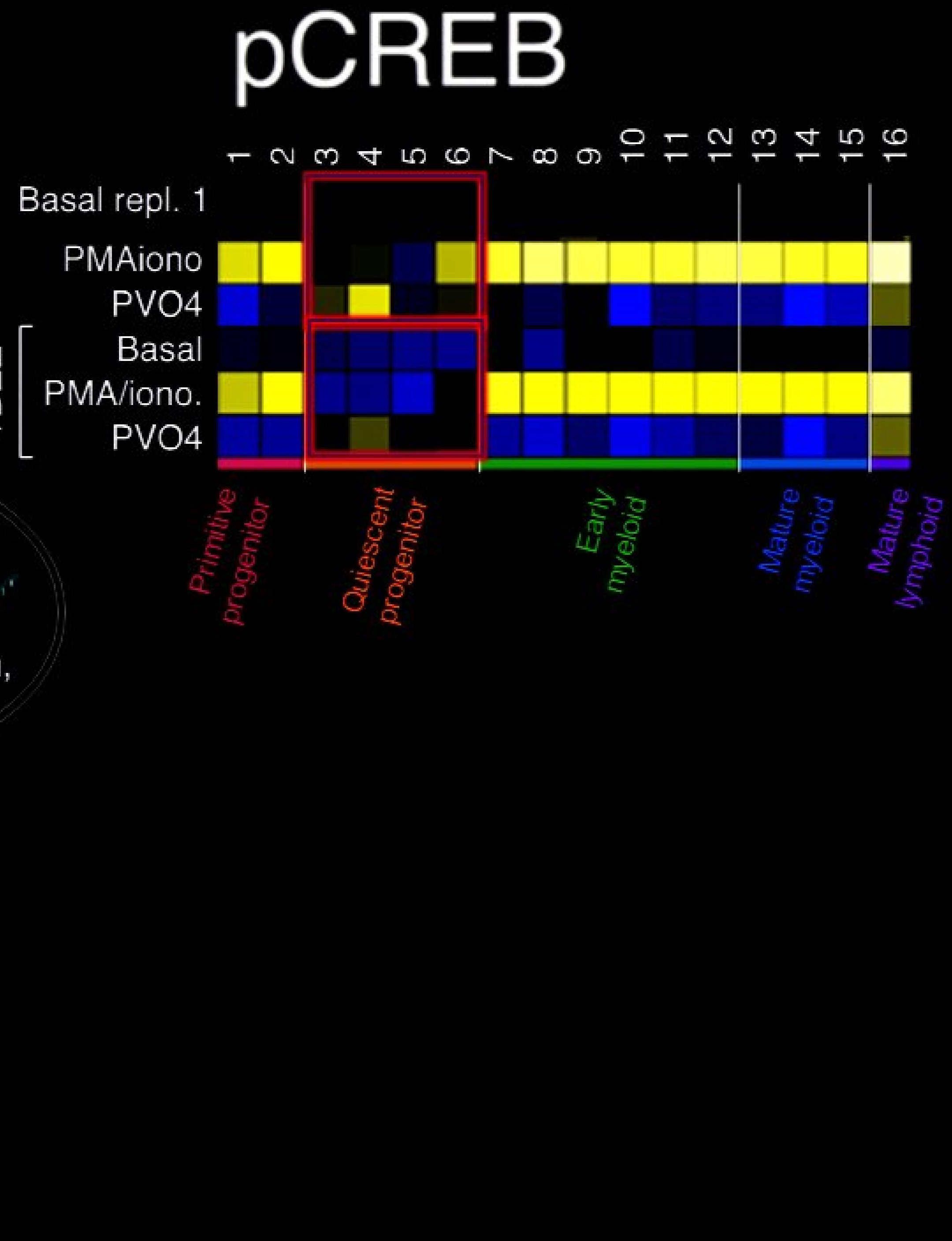
p27
p53 (pS15)
p53 (pS20)
pH2AX
pATM
pATR

pCHK1
pCHK2
pFANC2D
c-PARP
p53BP1
pHistoneH3(S28)

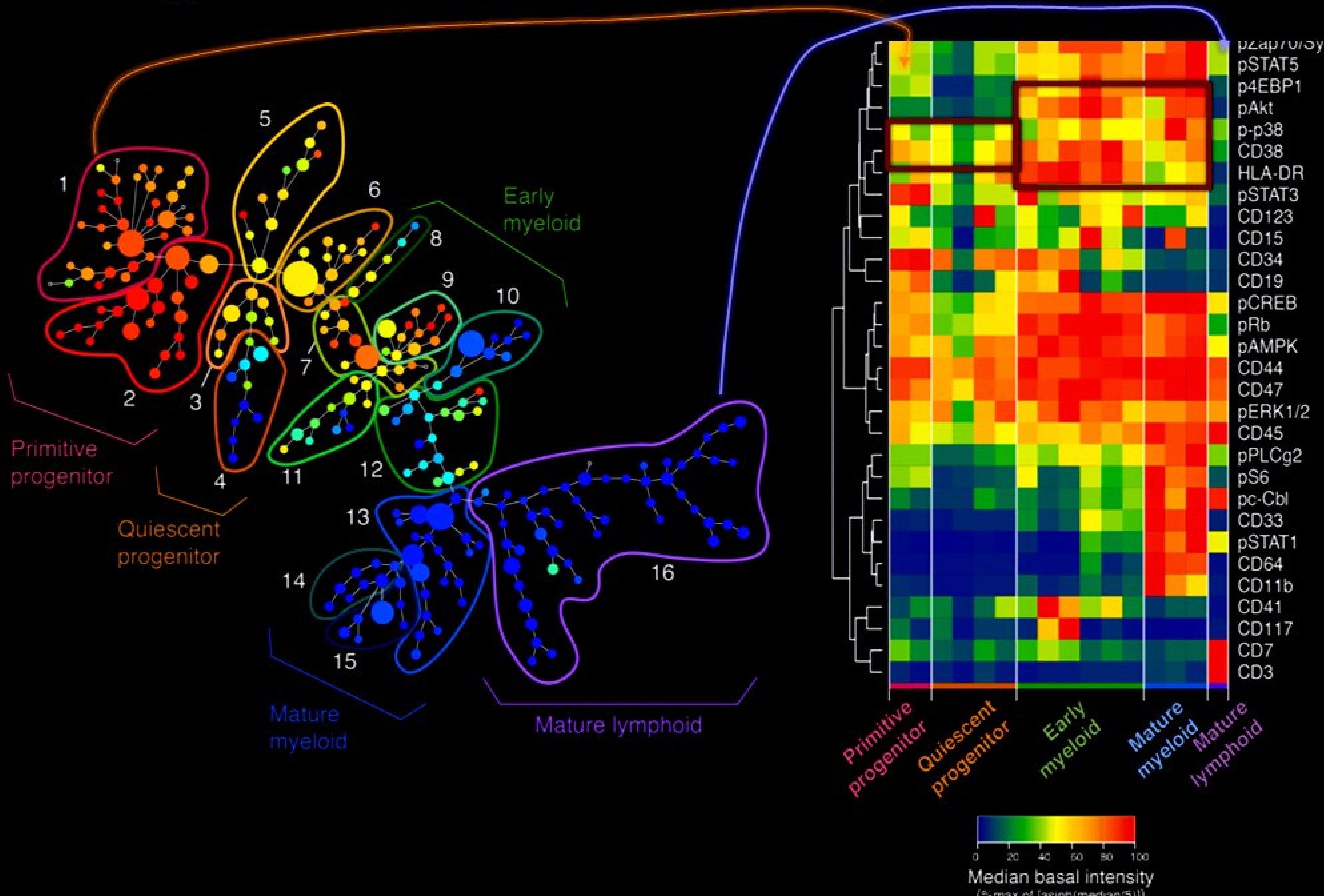
Greg Behbehani, et al, Cytometry A

Quiescent Cells can still be inhibited





Cell types exhibit differential constitutive pathway activation



with I Radtke, A Gedman, J Downing, S Bendall

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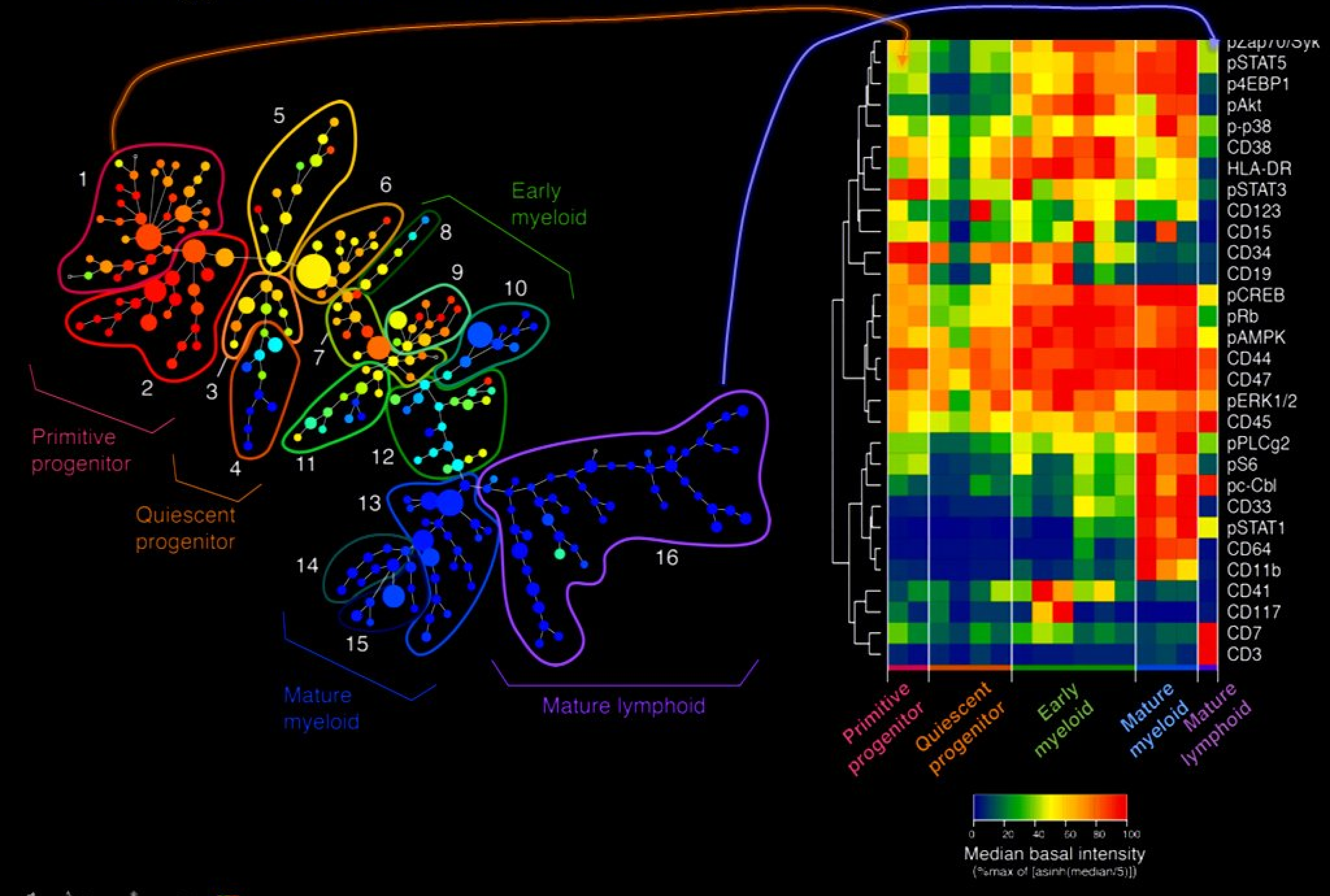

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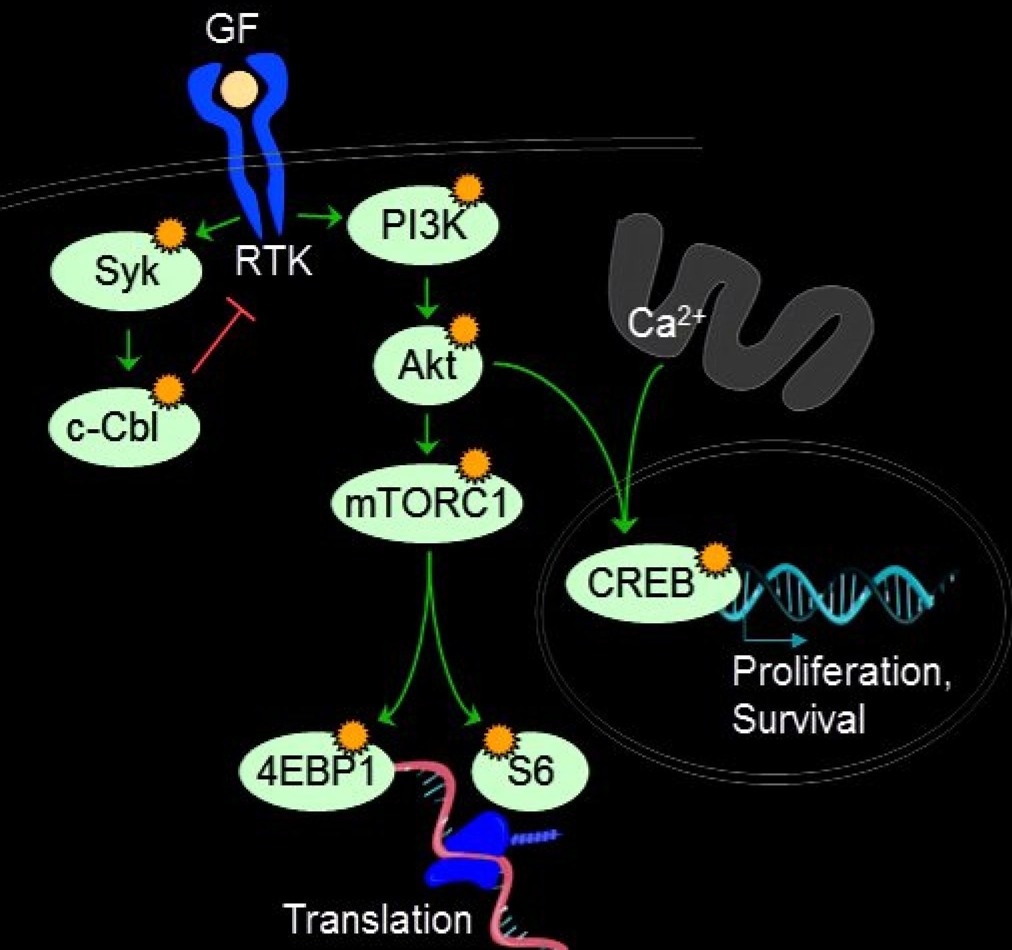
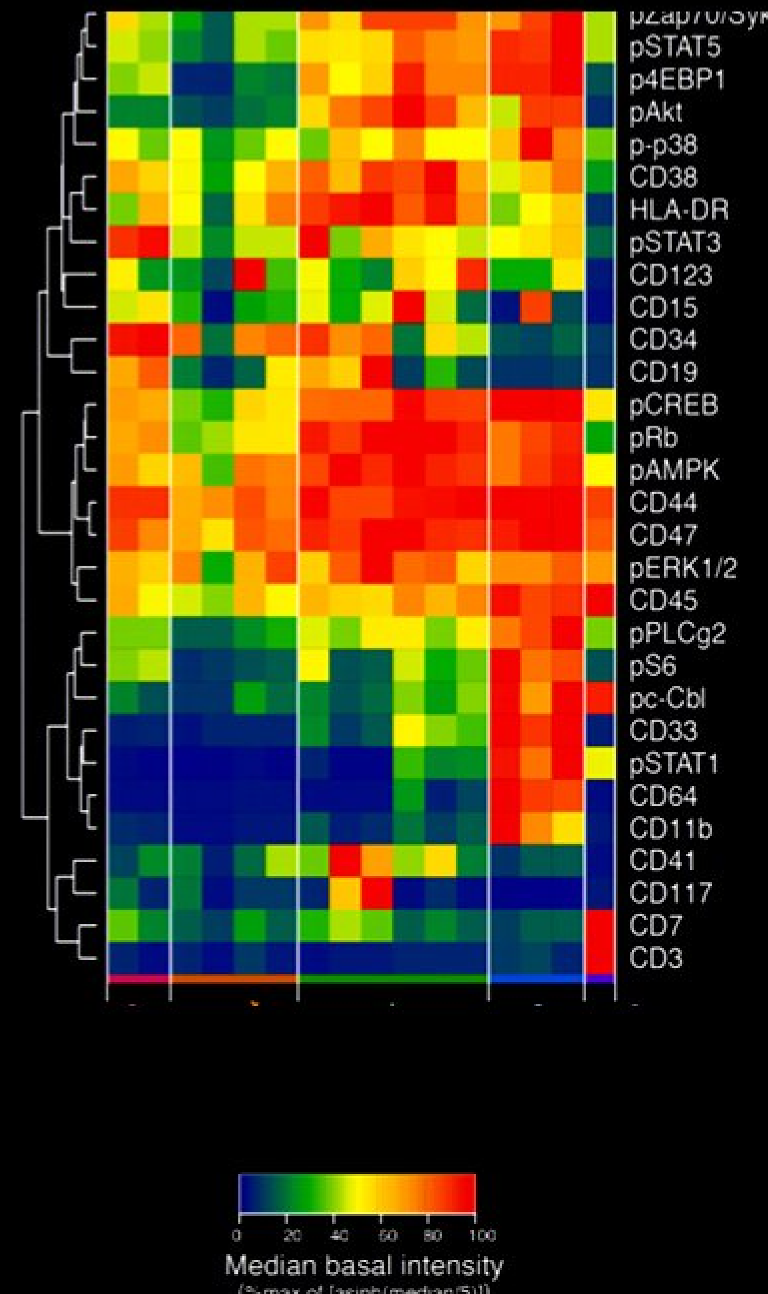
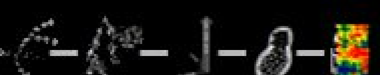
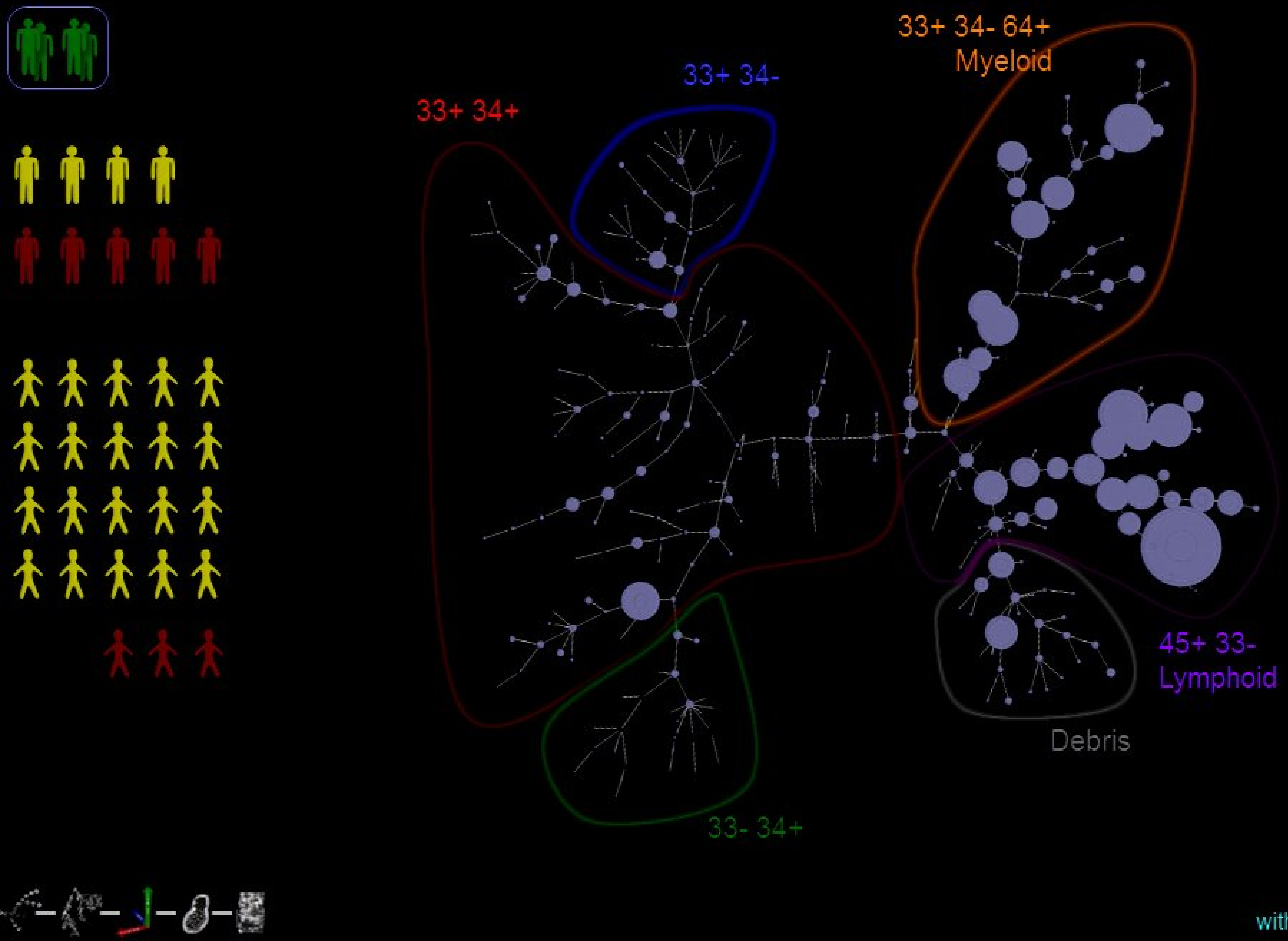






with I Radtke, A Gedman, J Downing, S Bendall

Phenotypic landscape of AML is diverse, but constrained...



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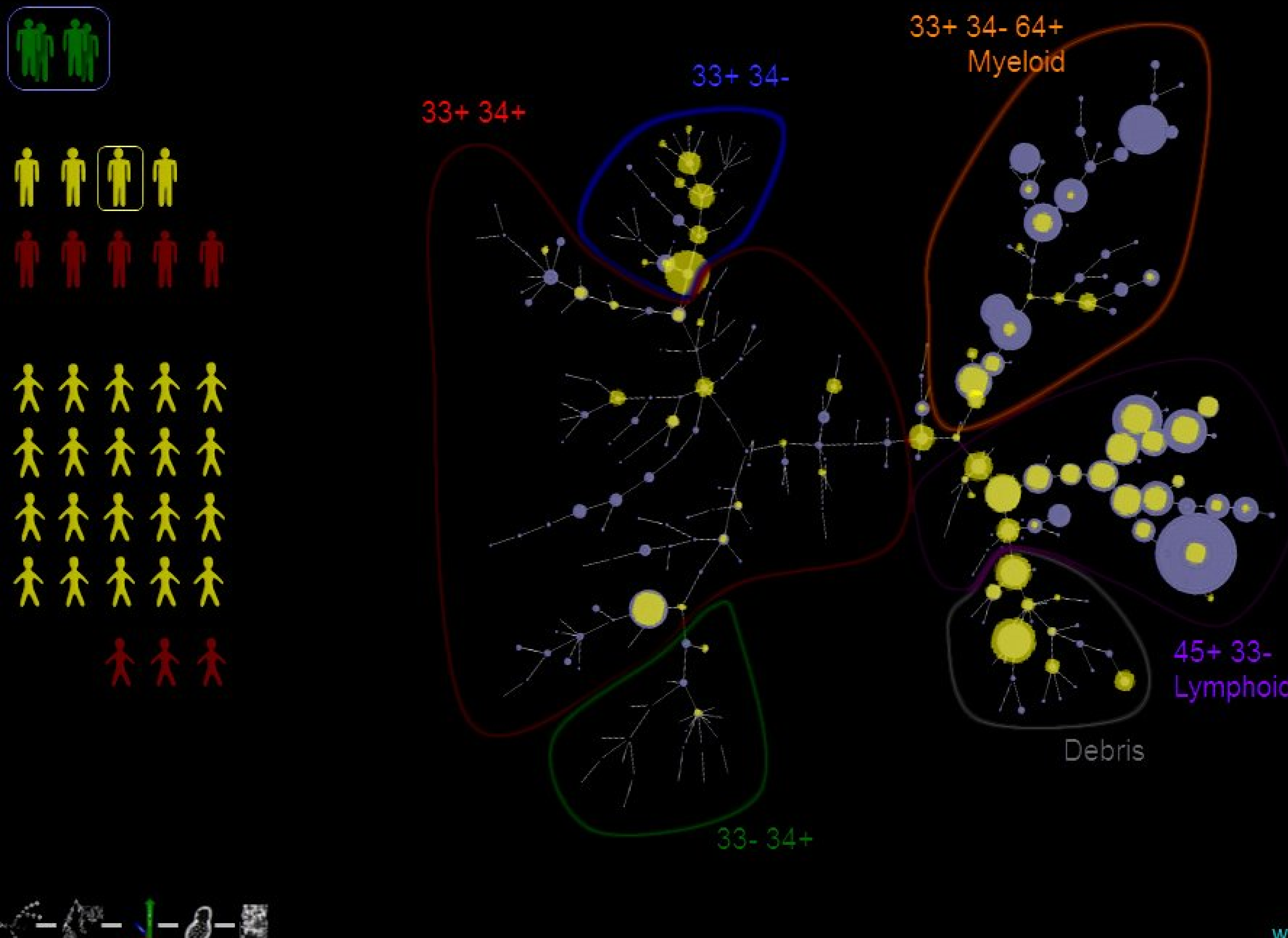

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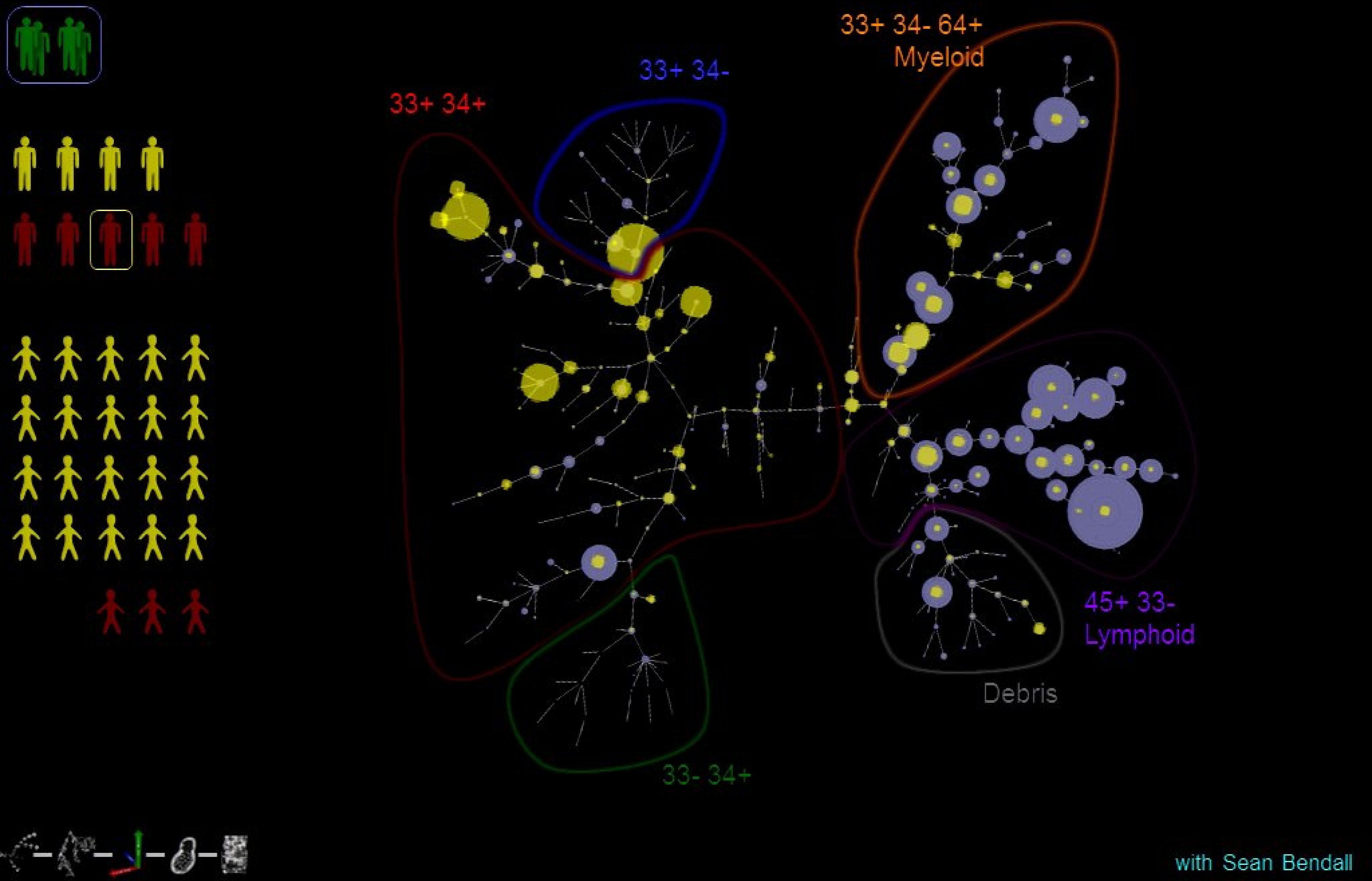

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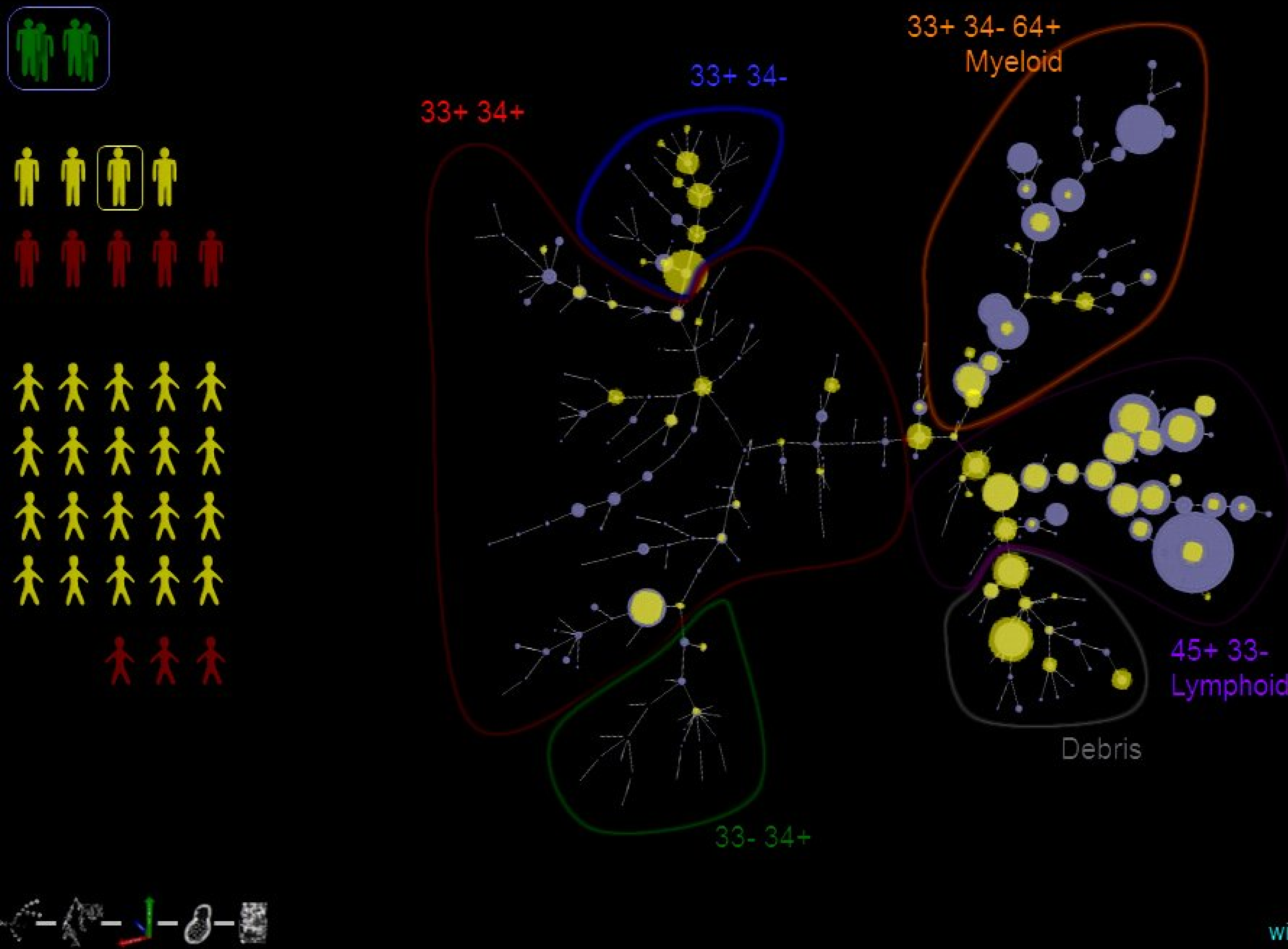

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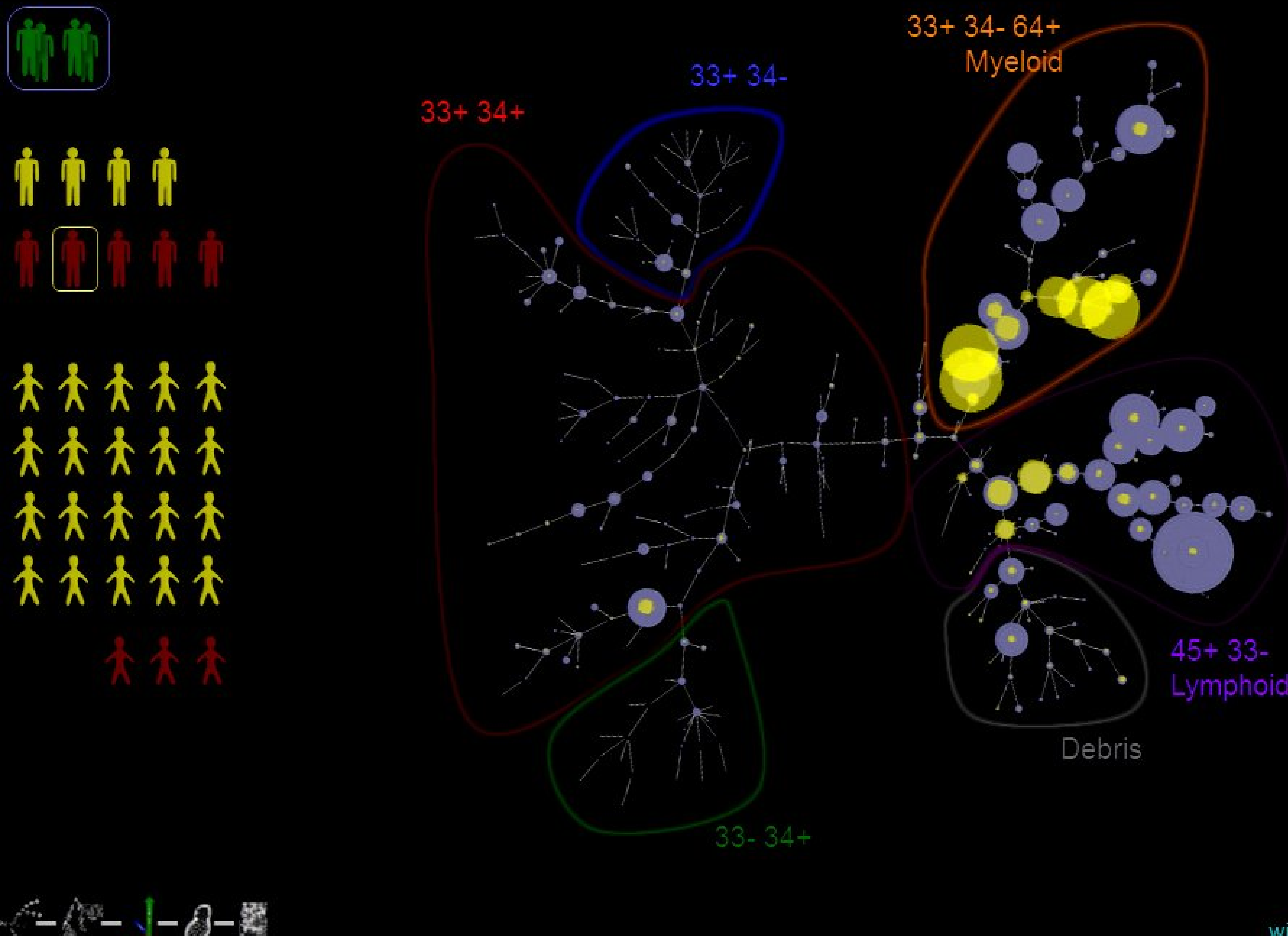

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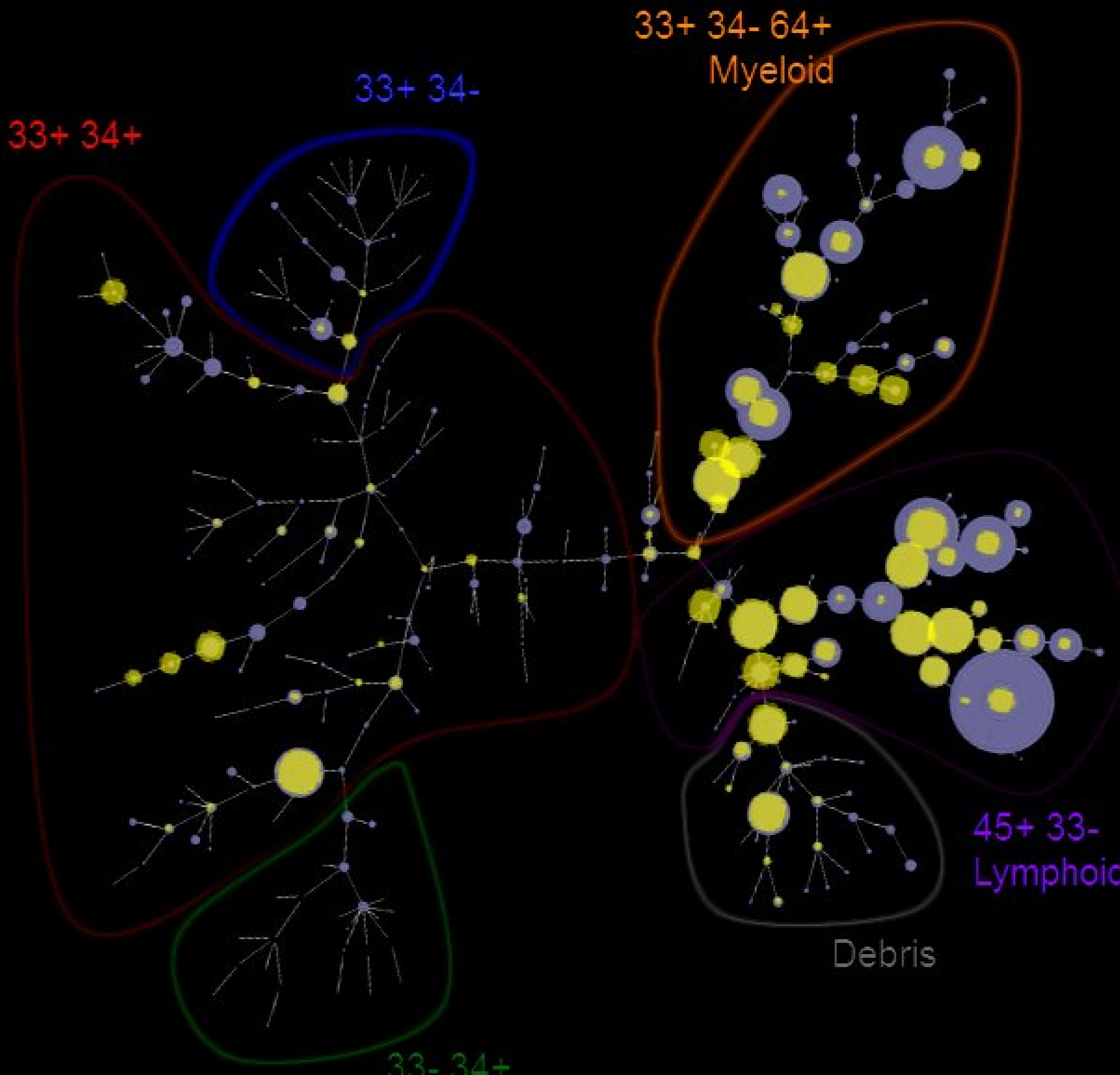

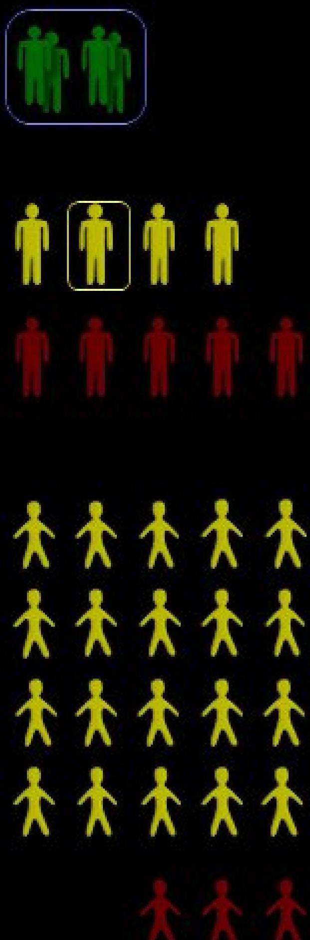

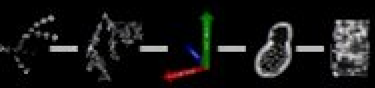

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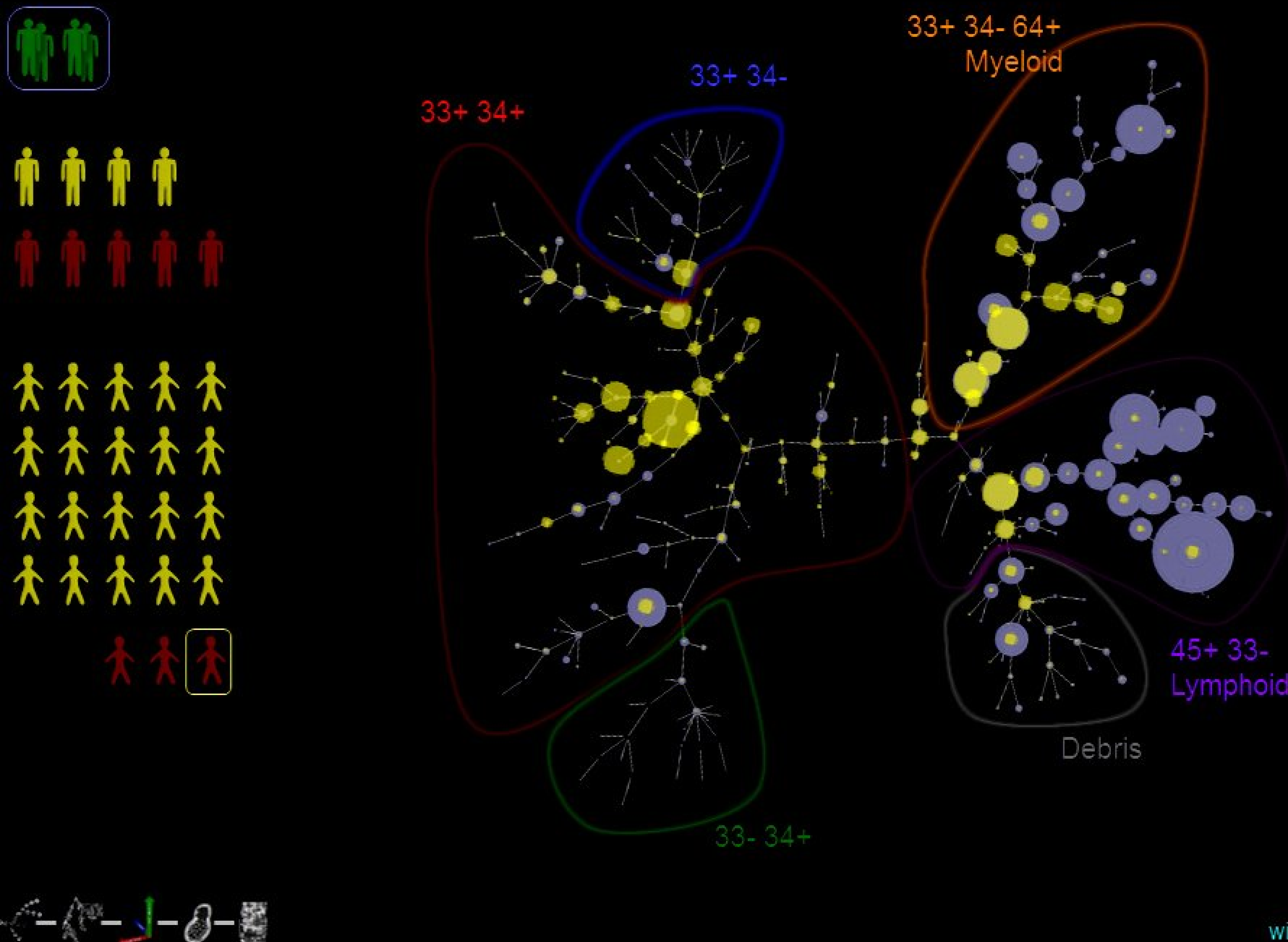

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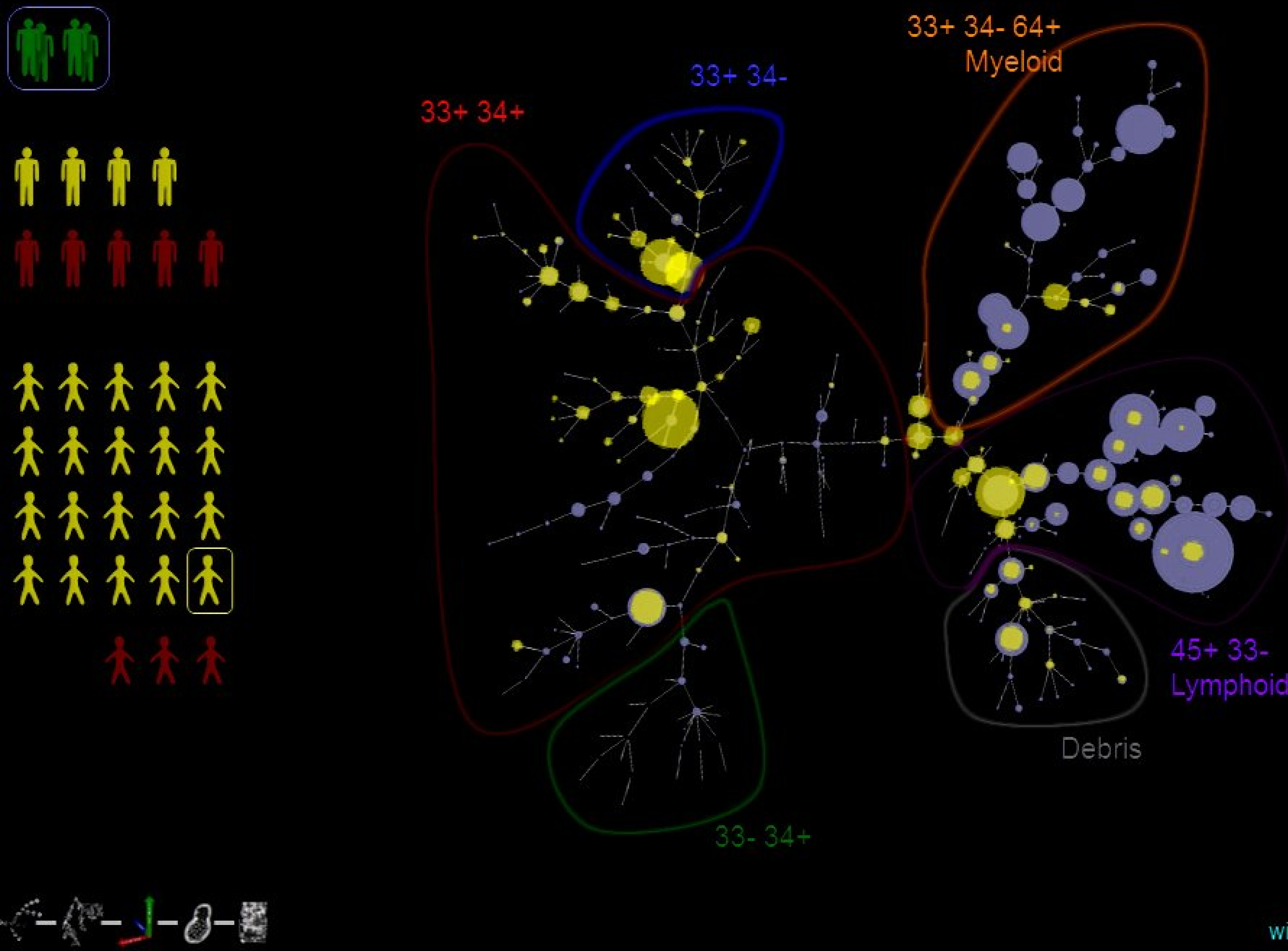

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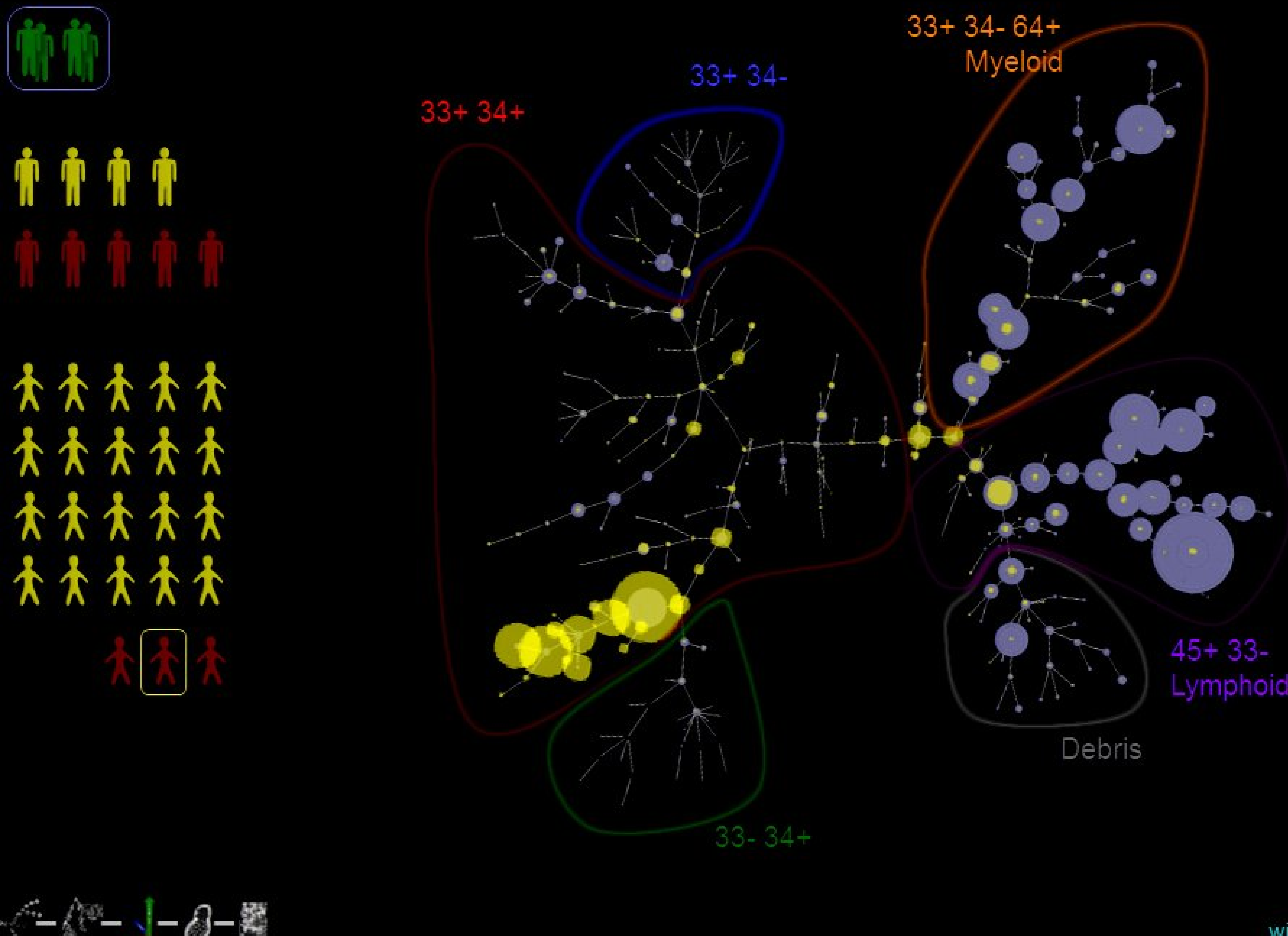

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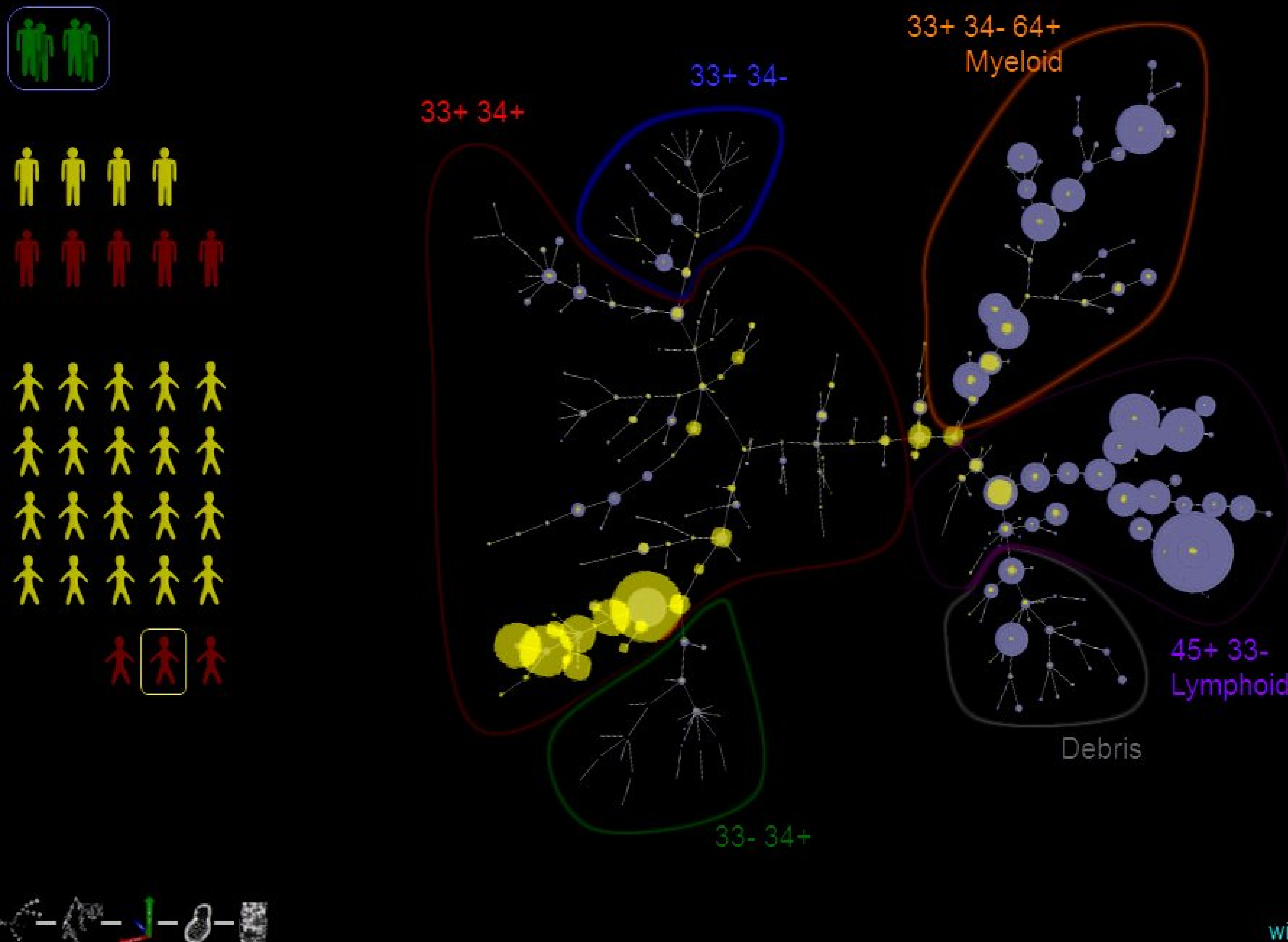

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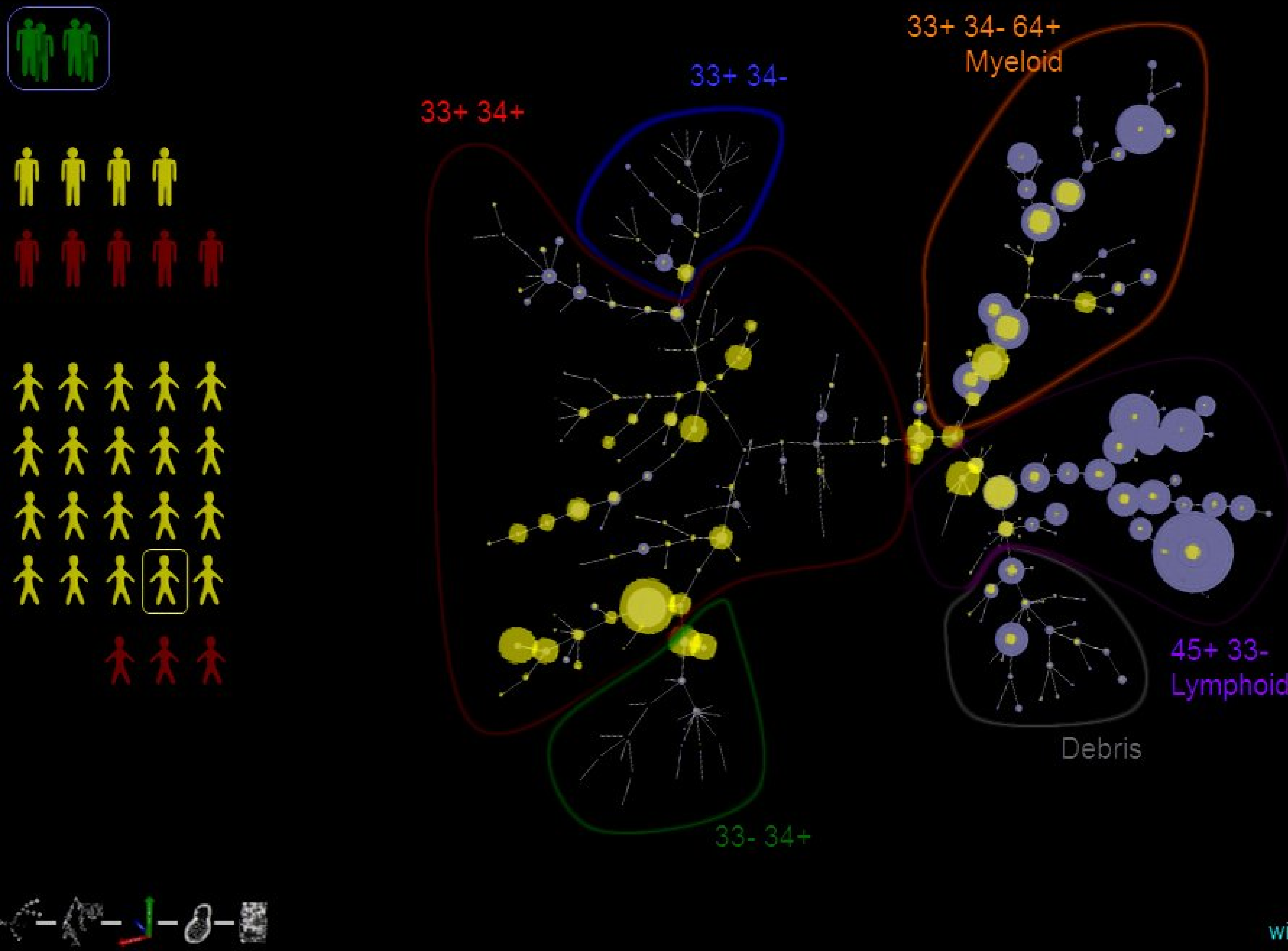

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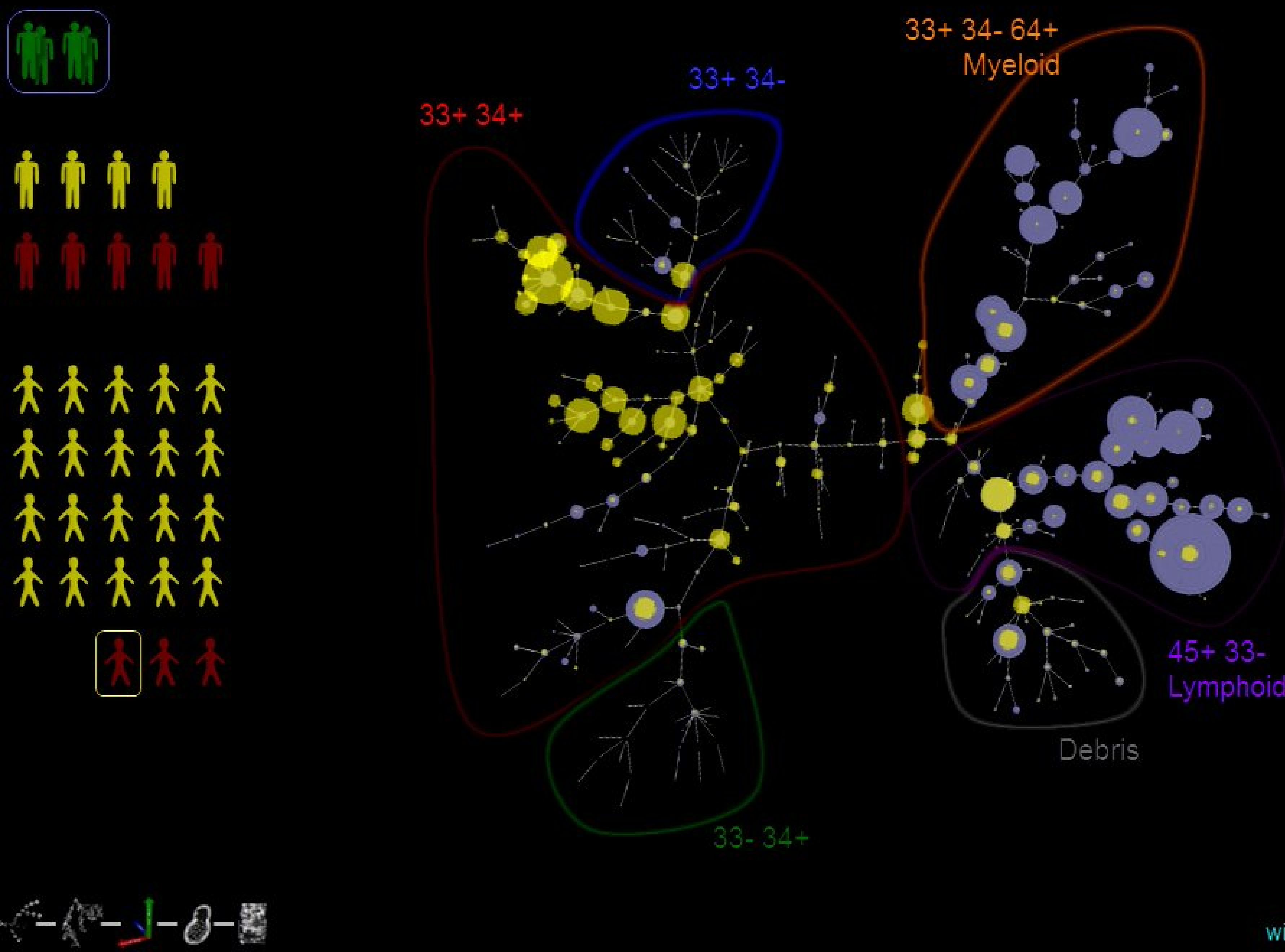

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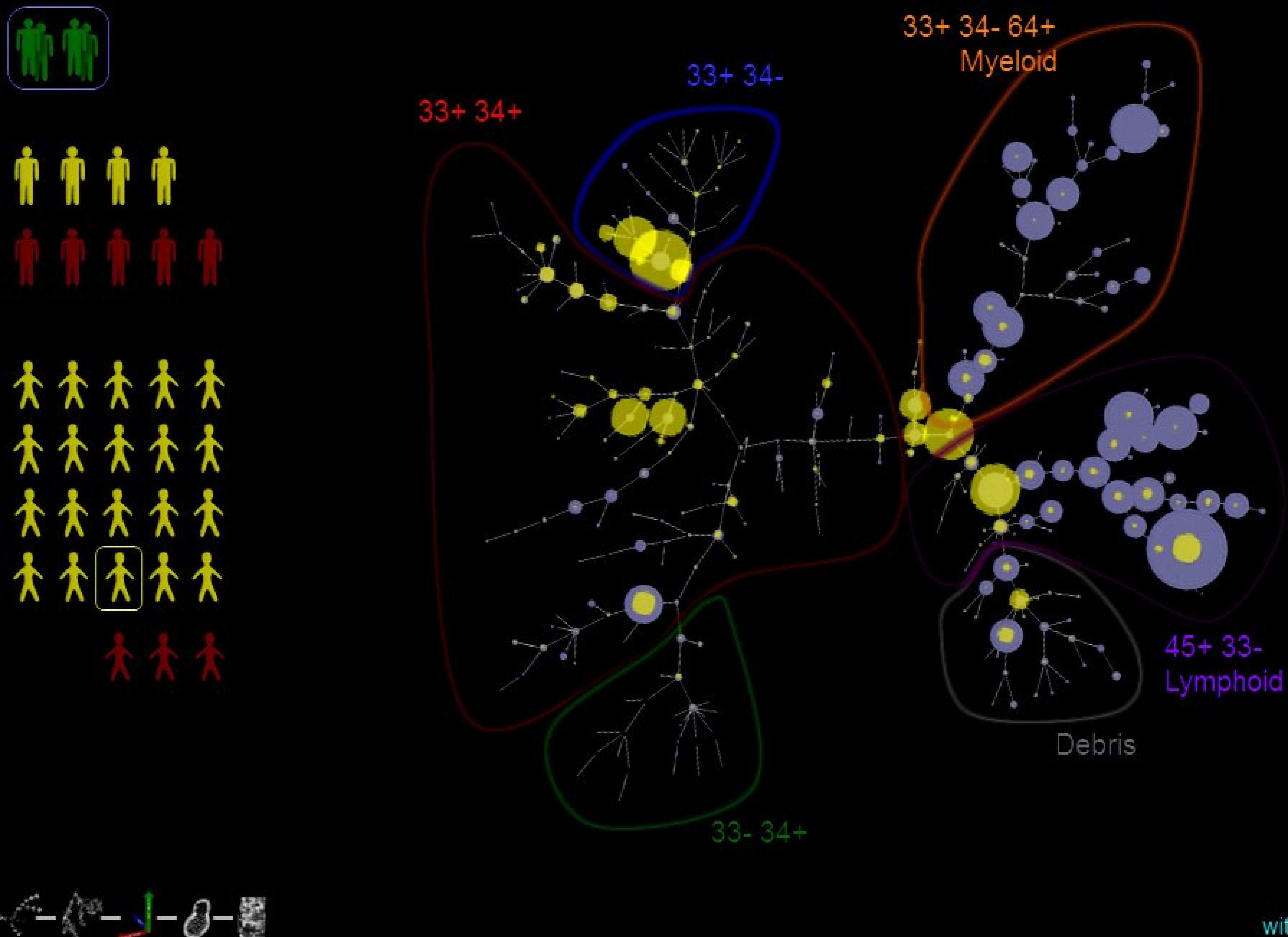

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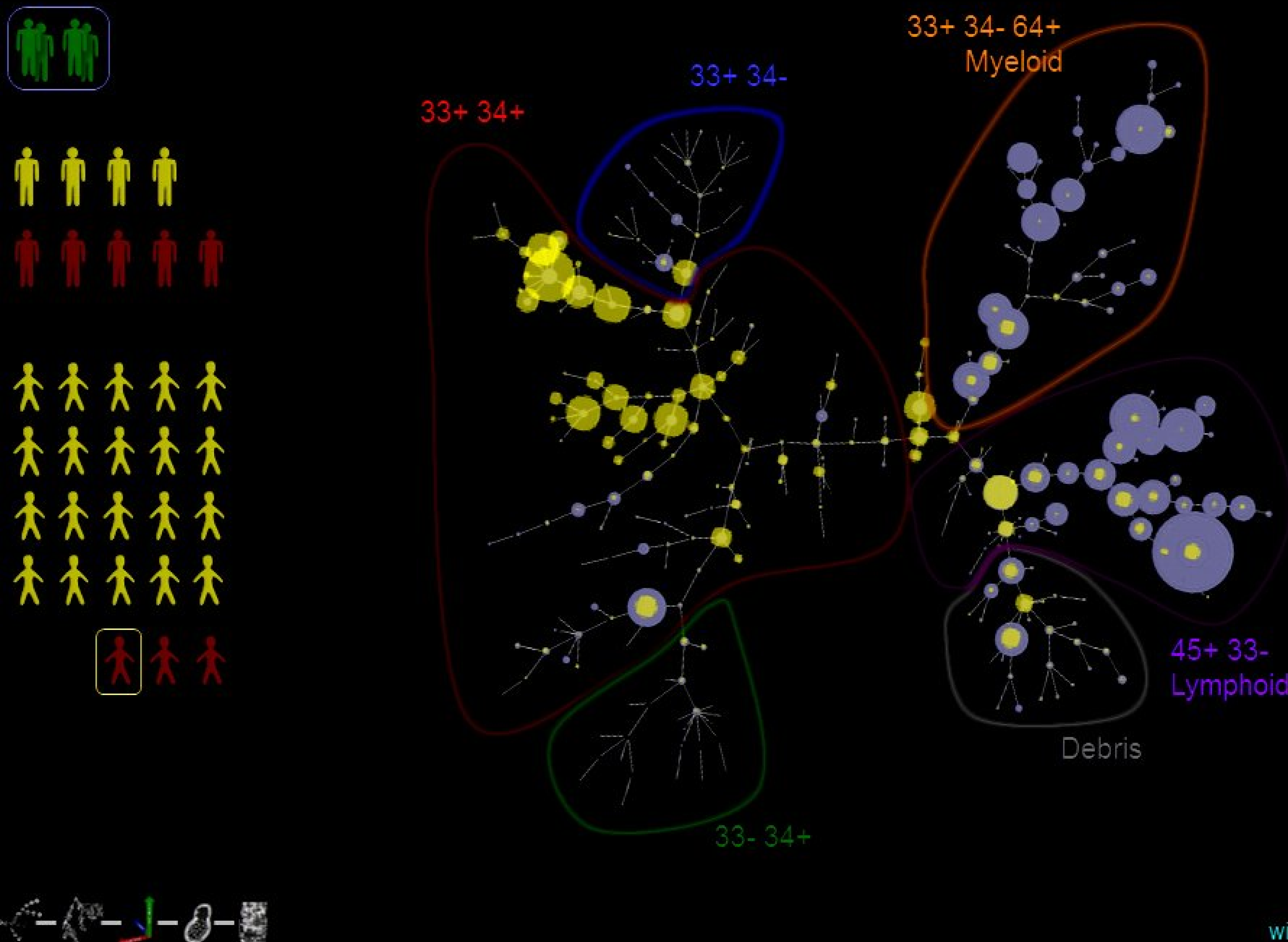

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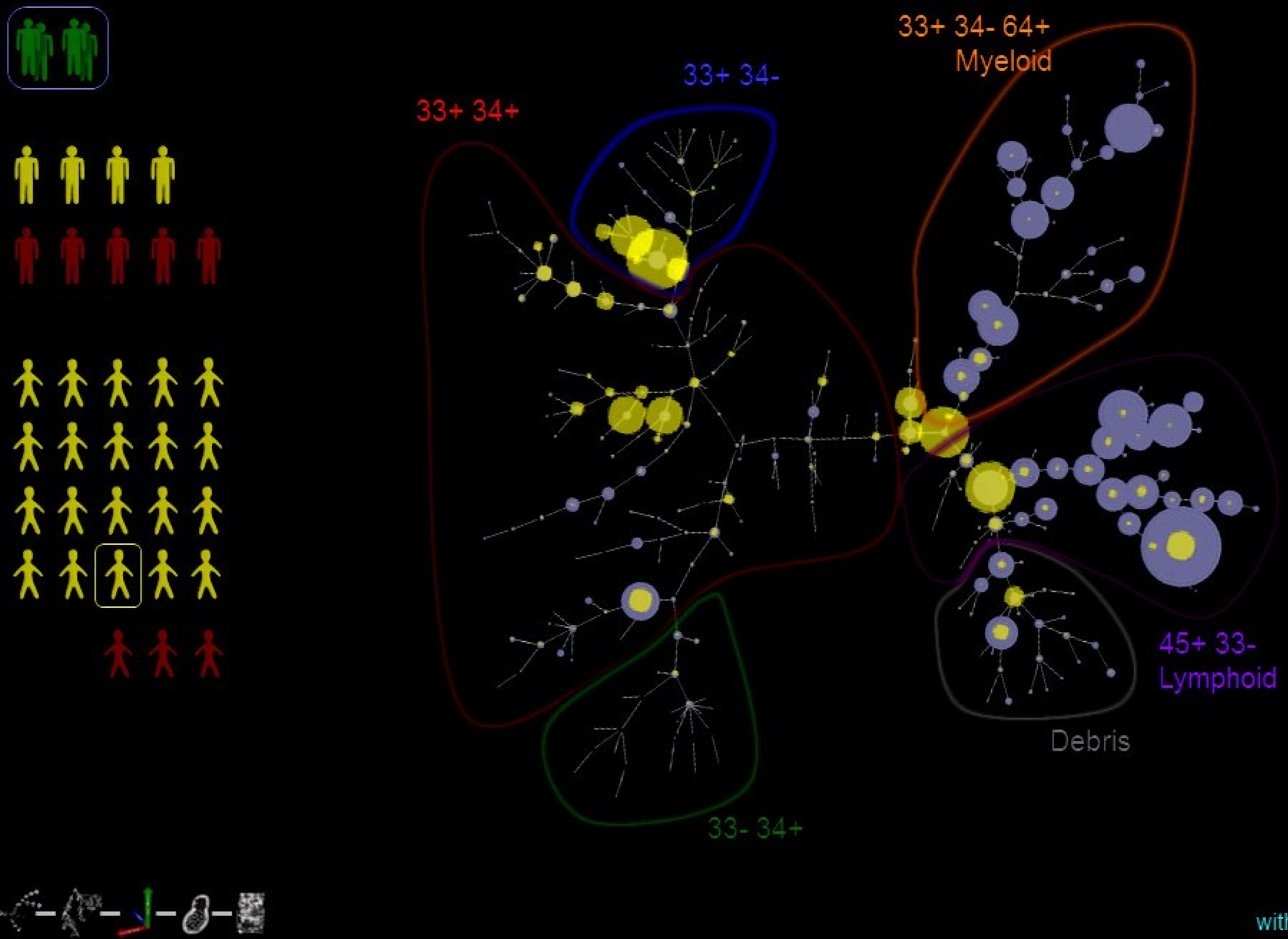

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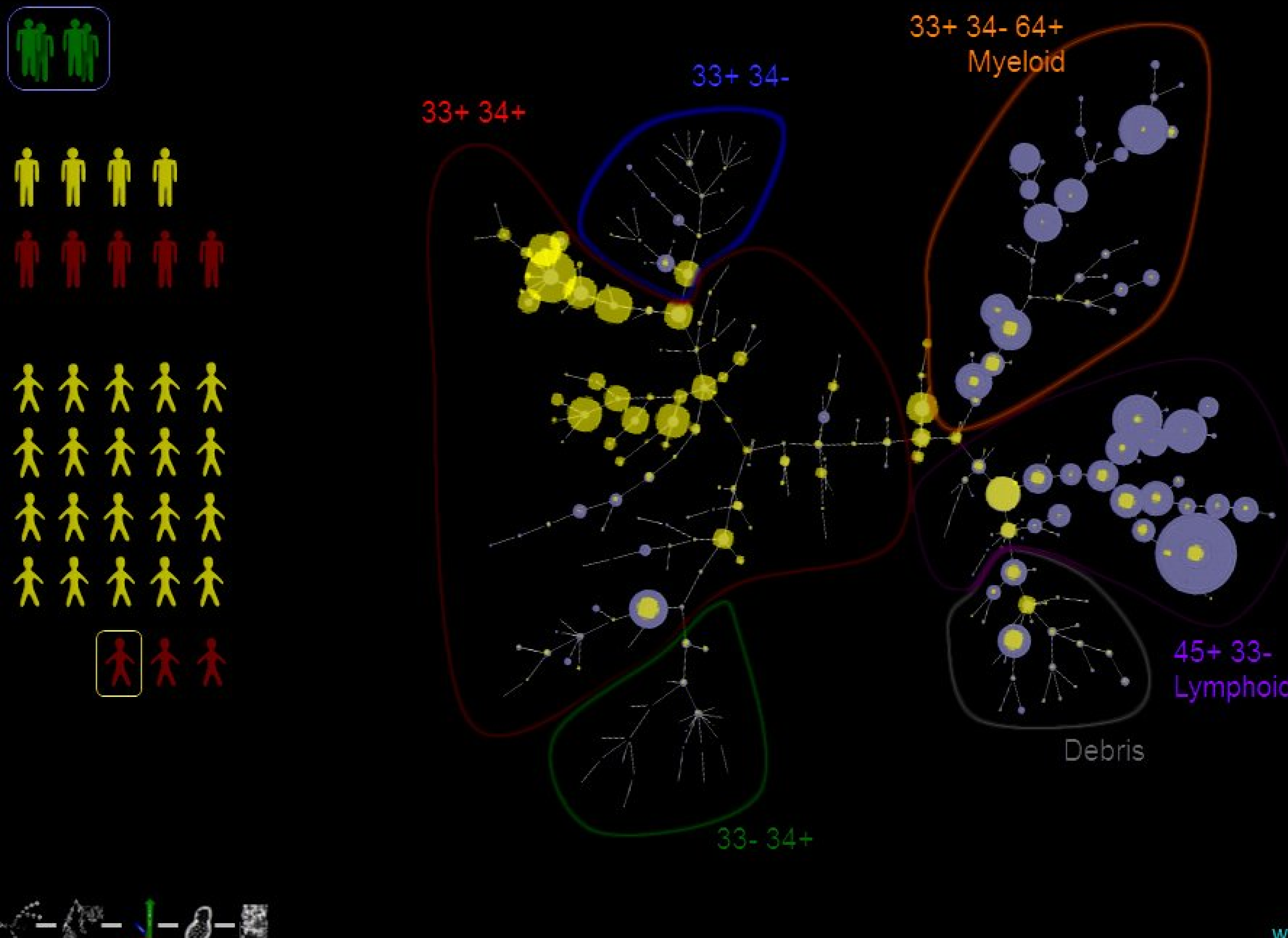

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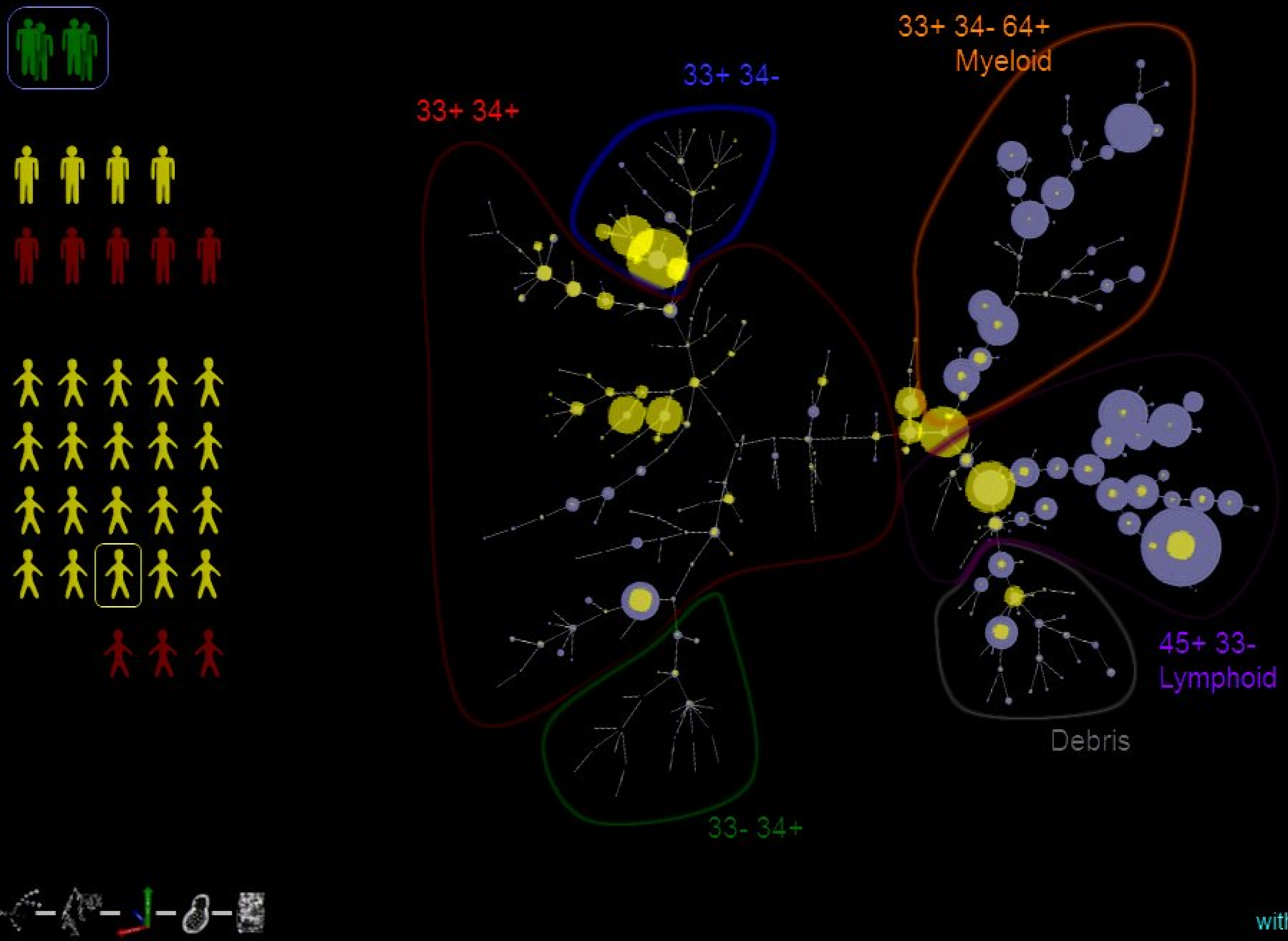

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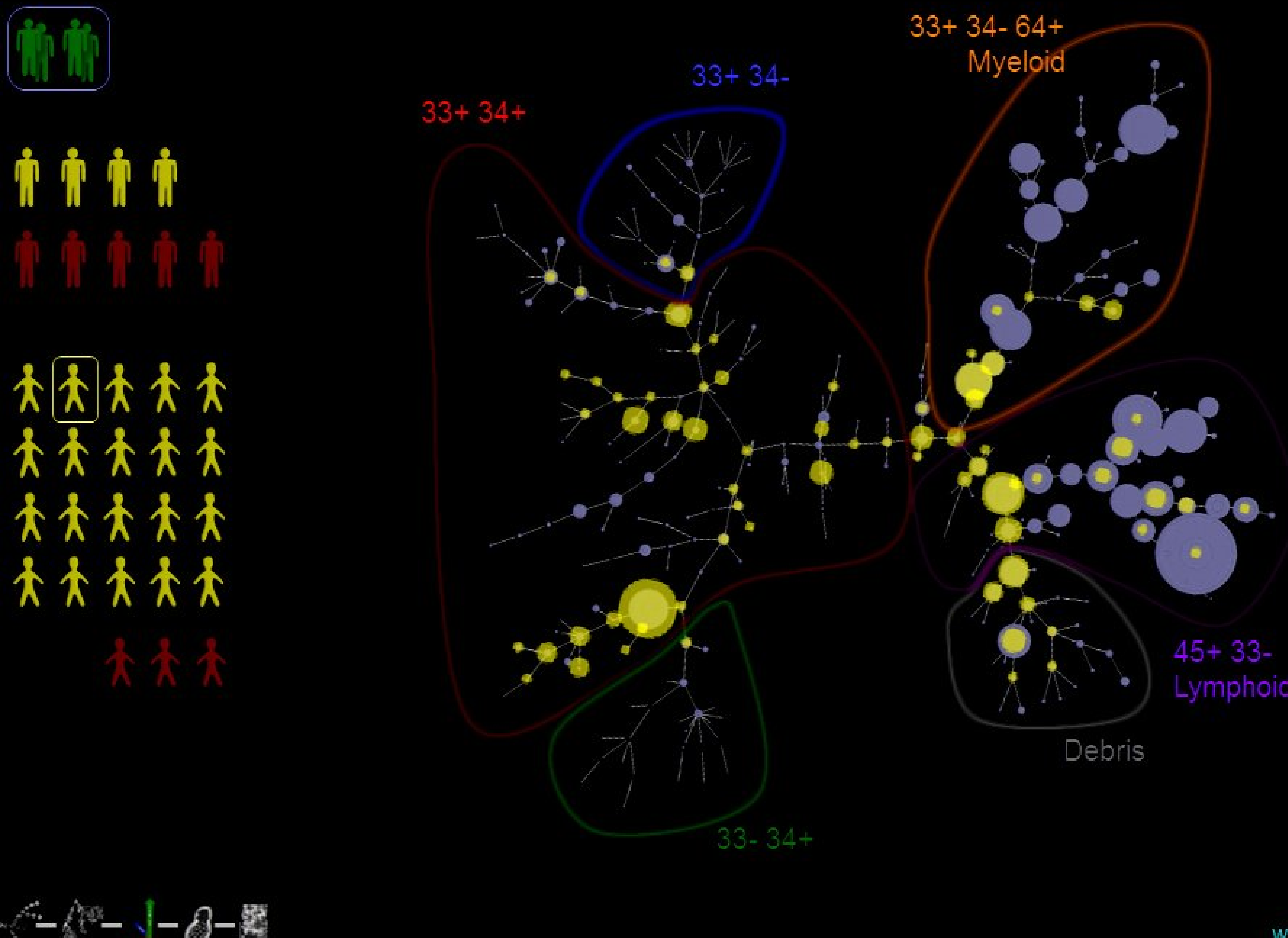

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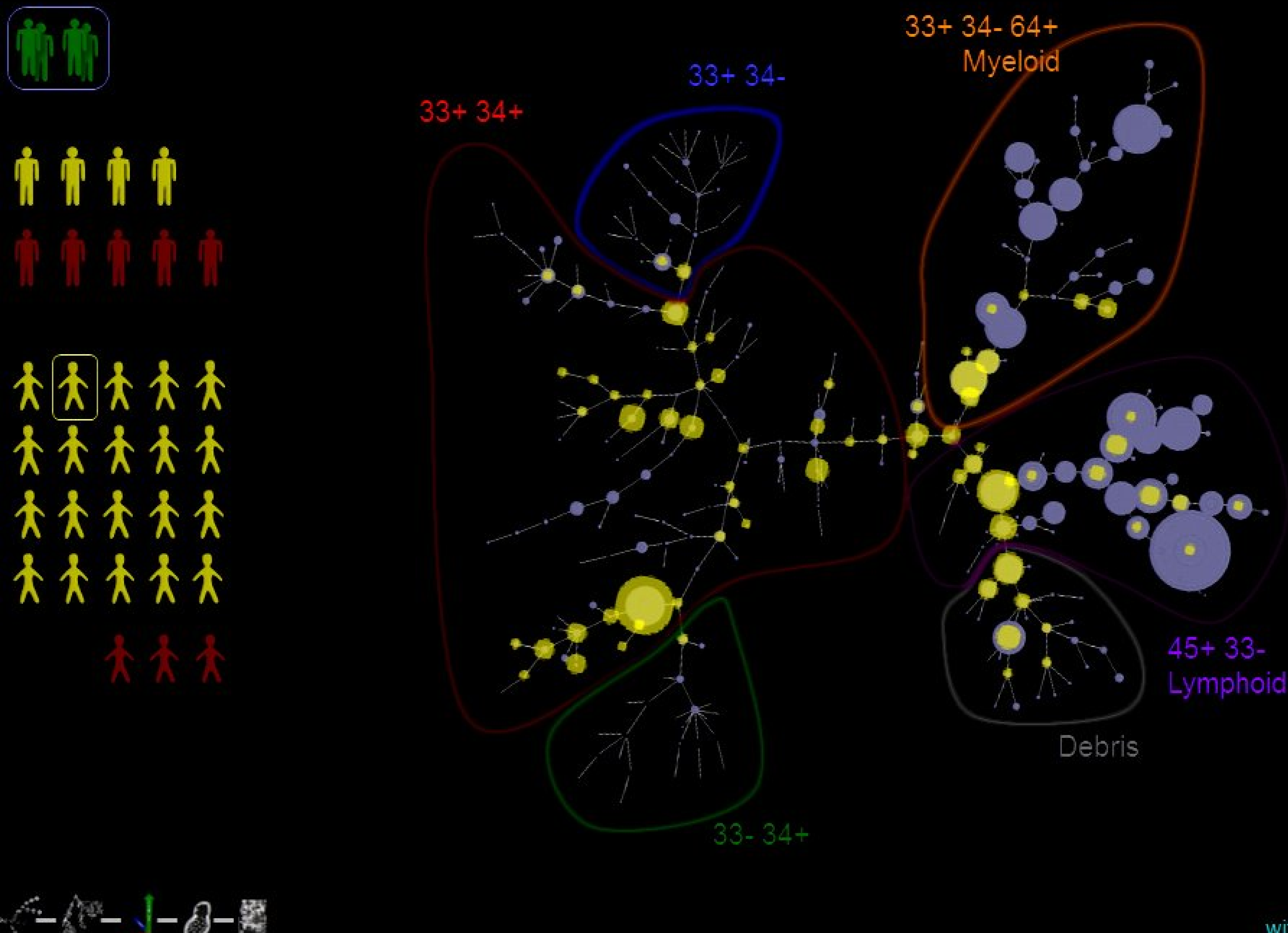

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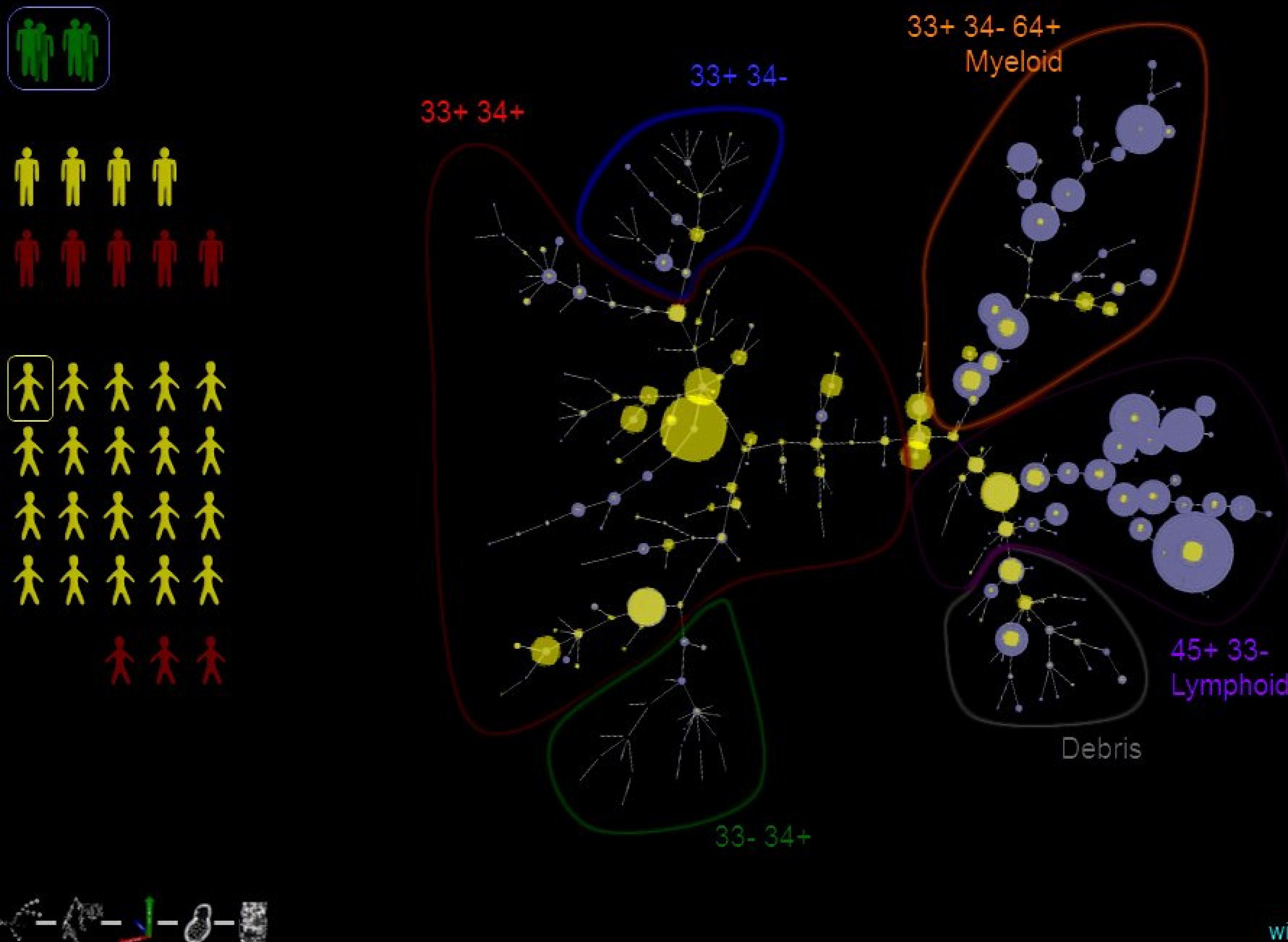

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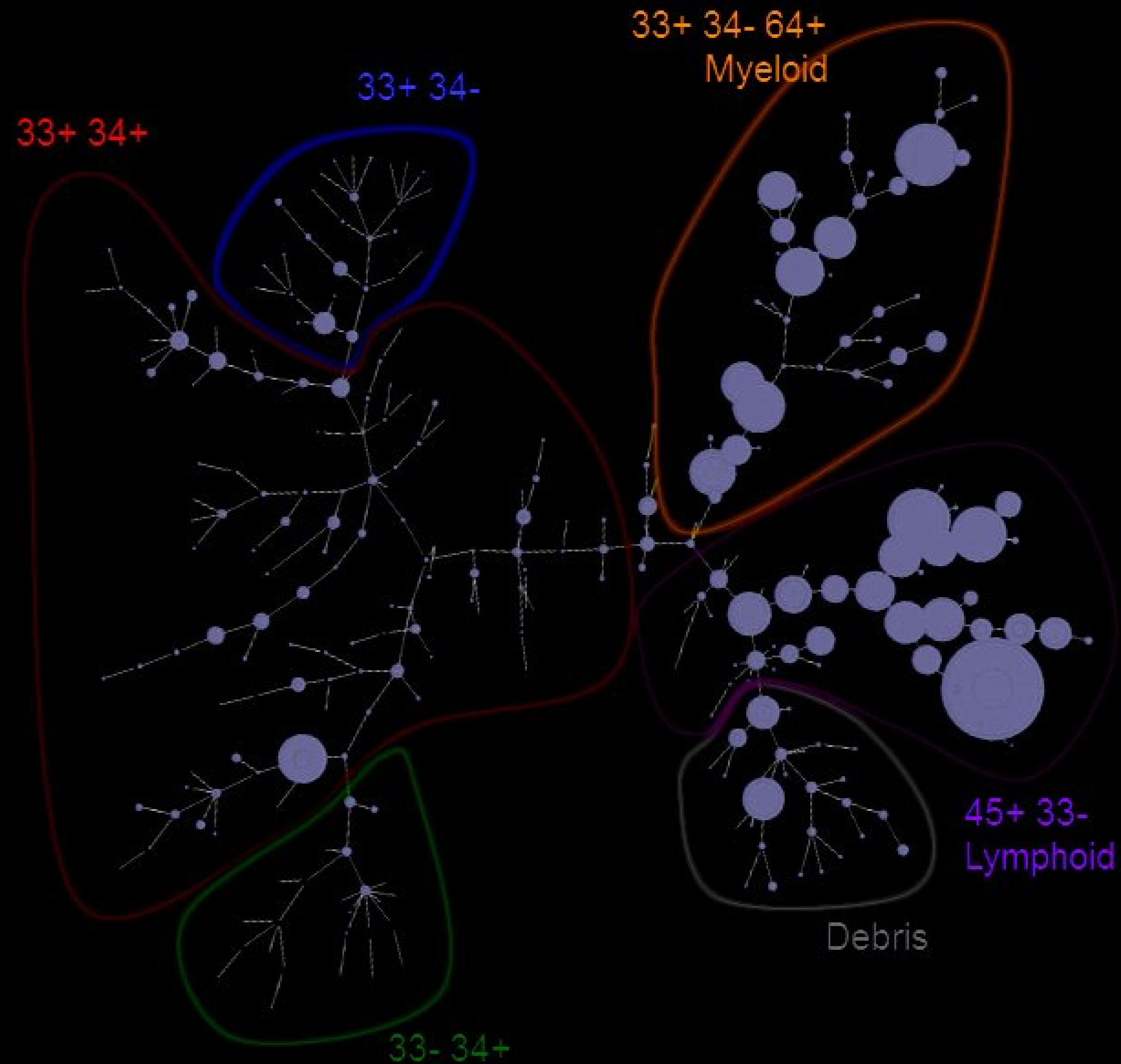

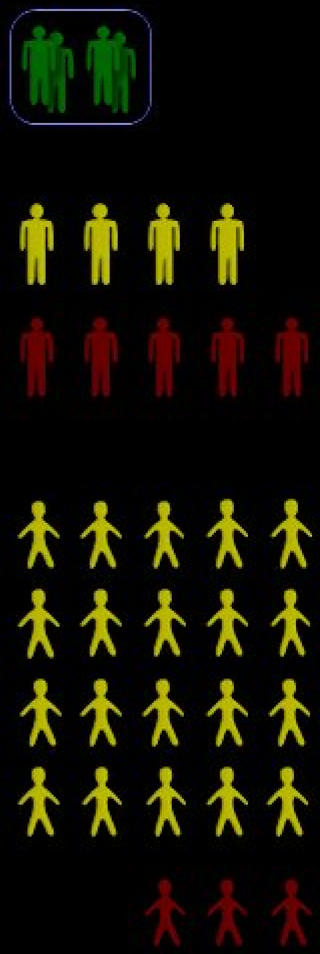

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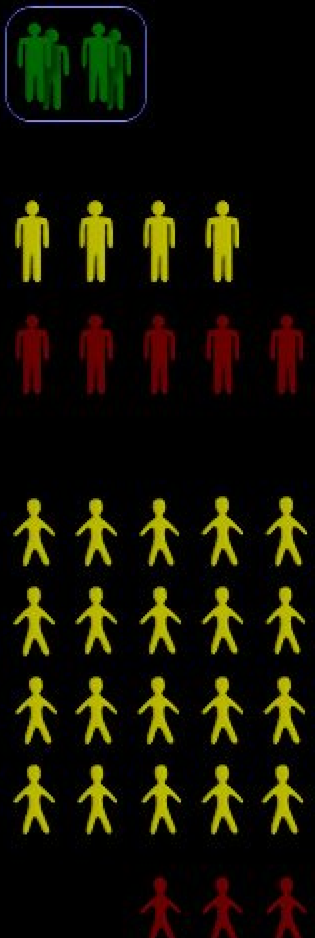
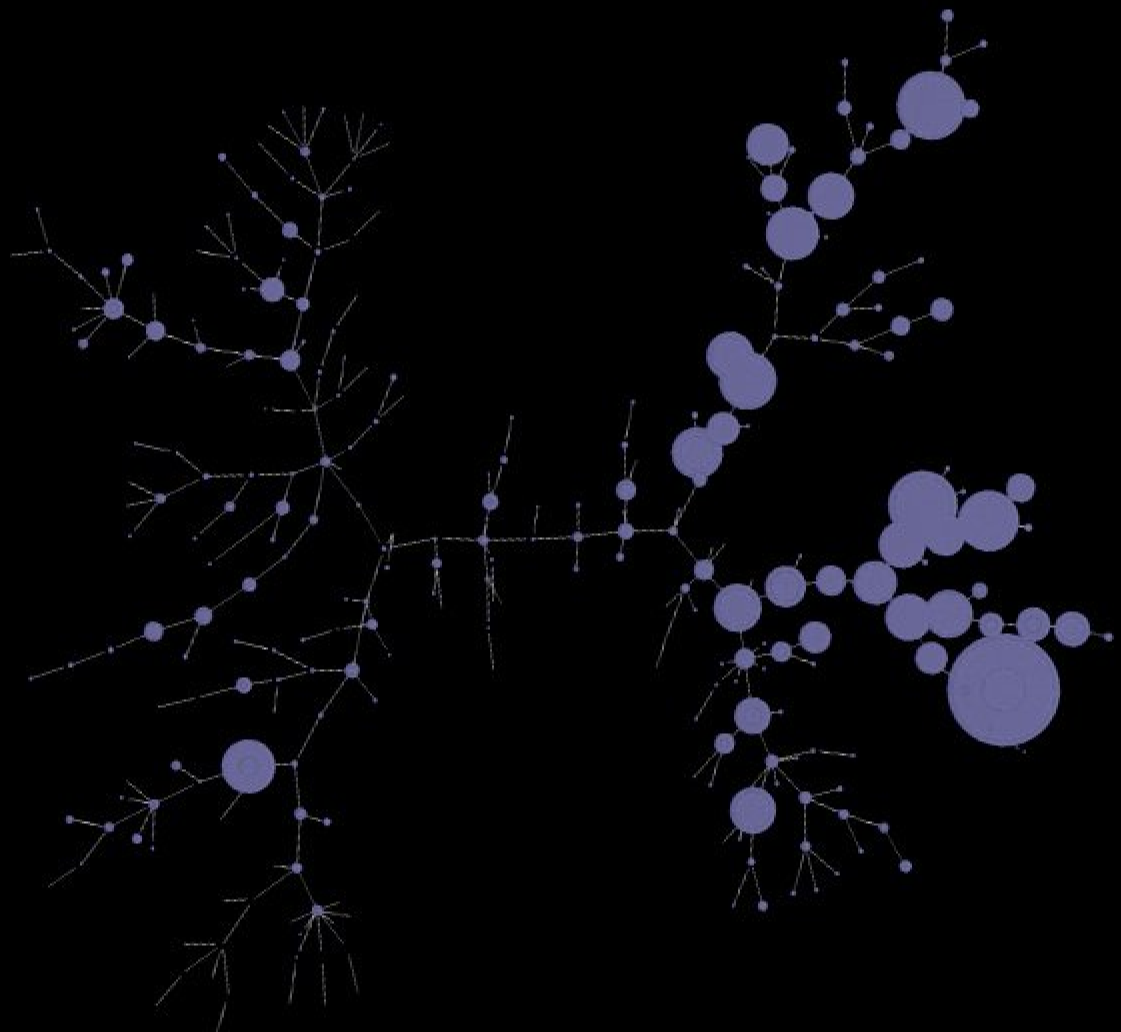
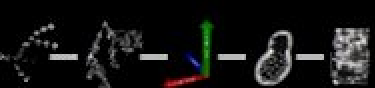
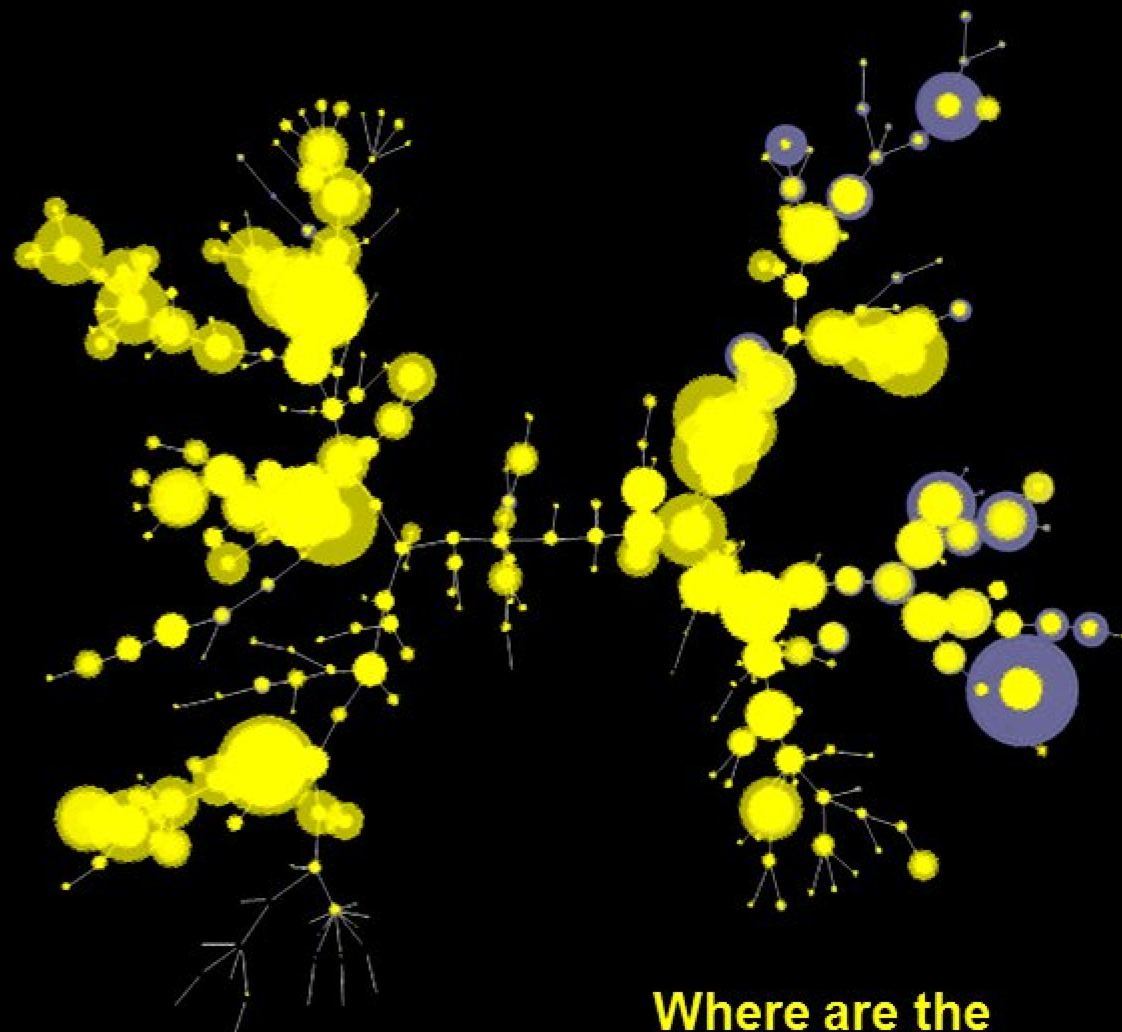




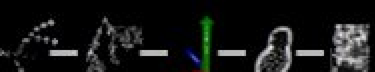


with Sean Bendall

Relandscaping by Tumor Niche Filling



Where are the “invisible” leukemia cells?



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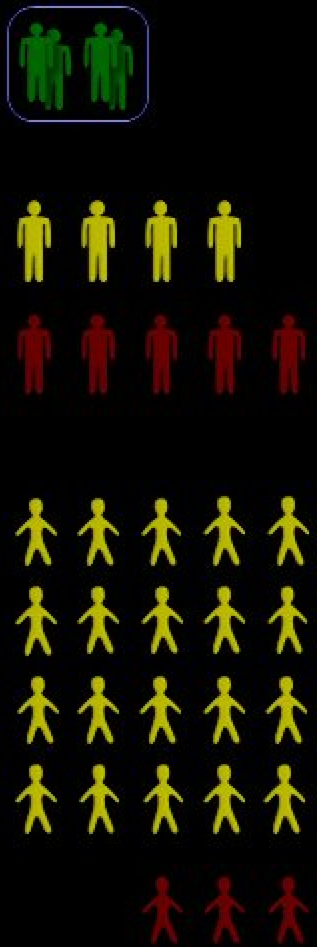

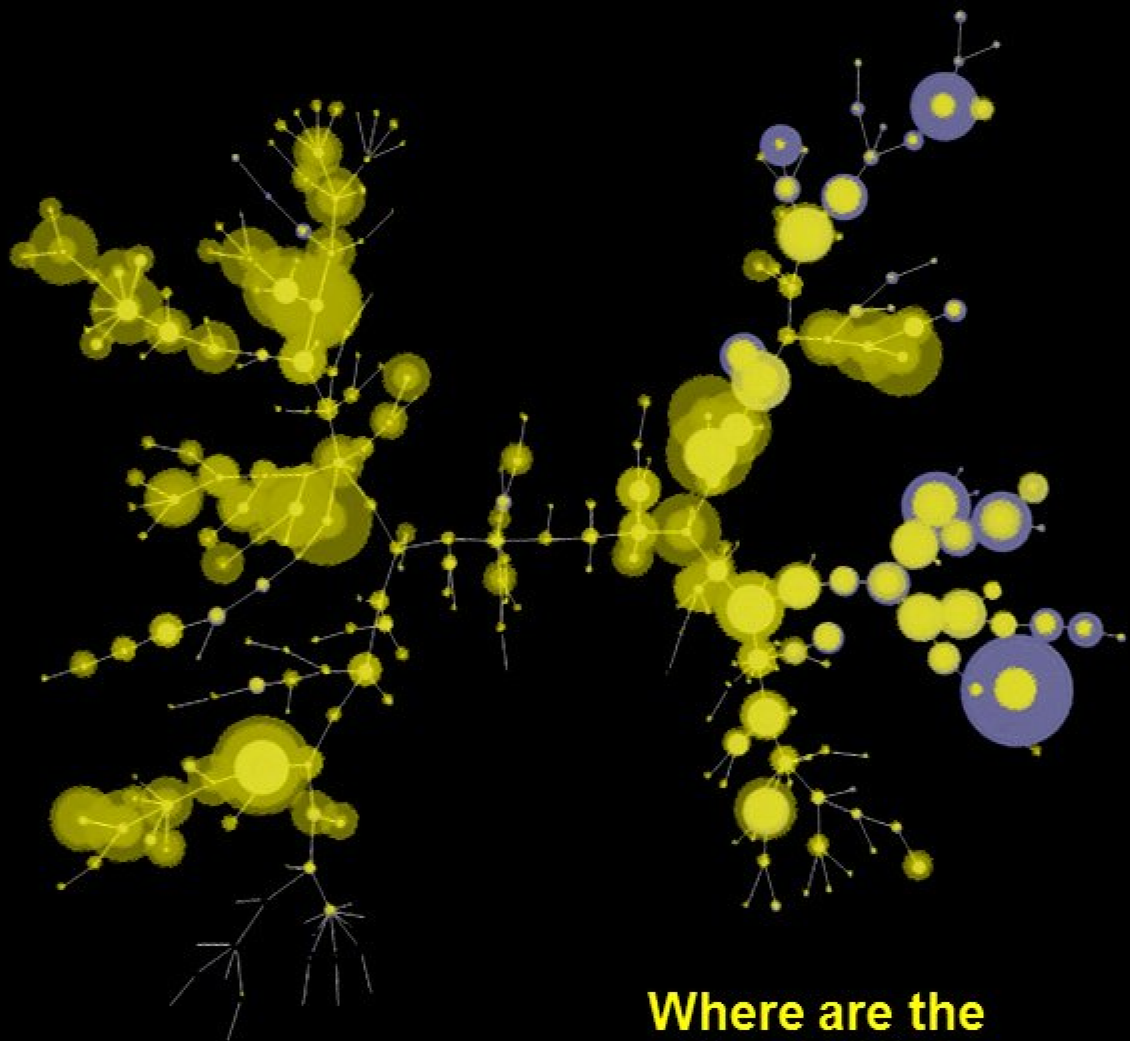

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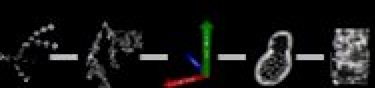

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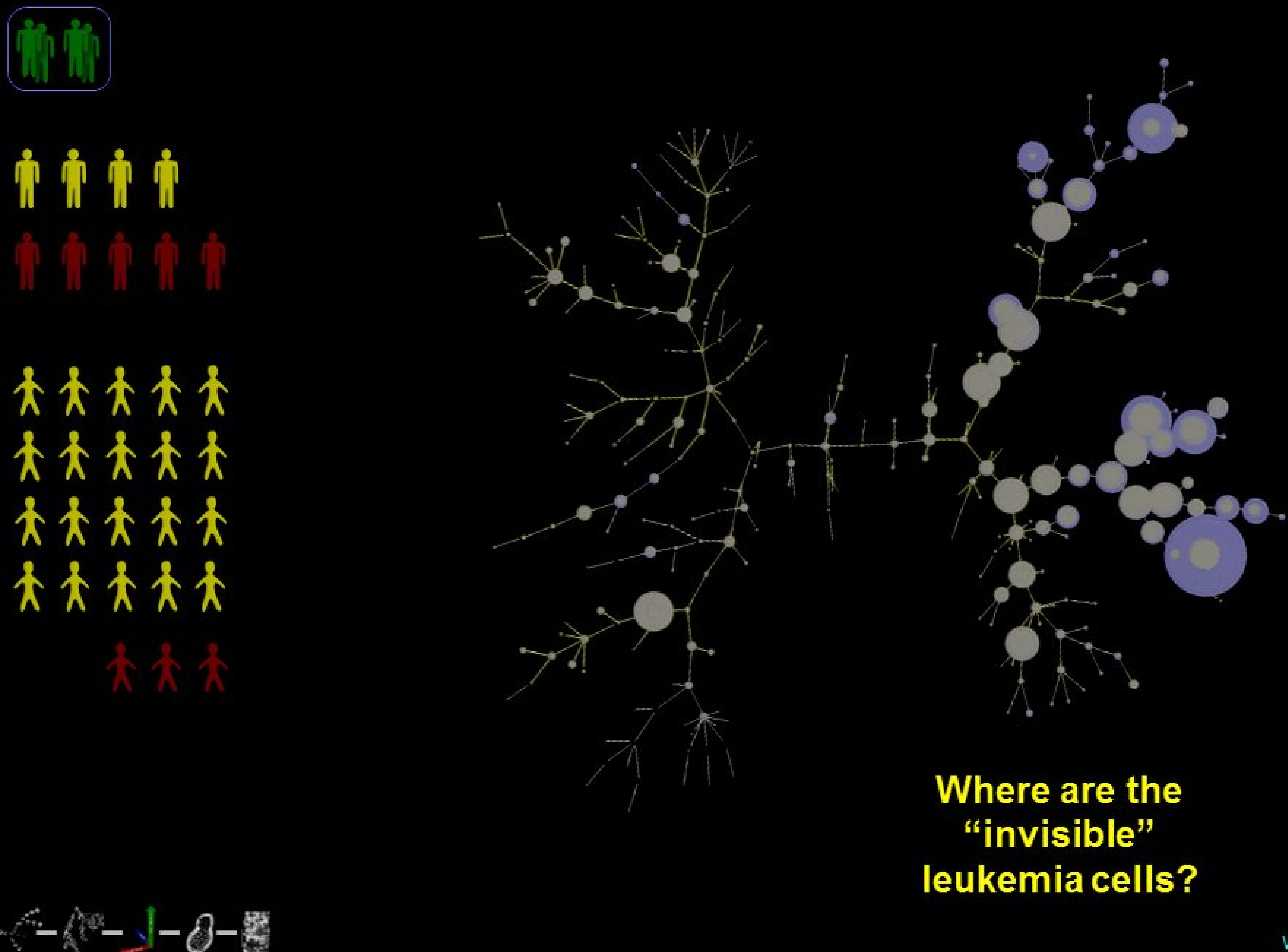

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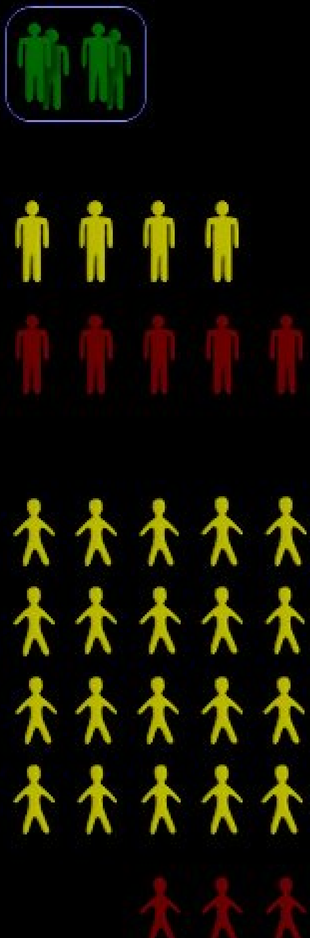

Add
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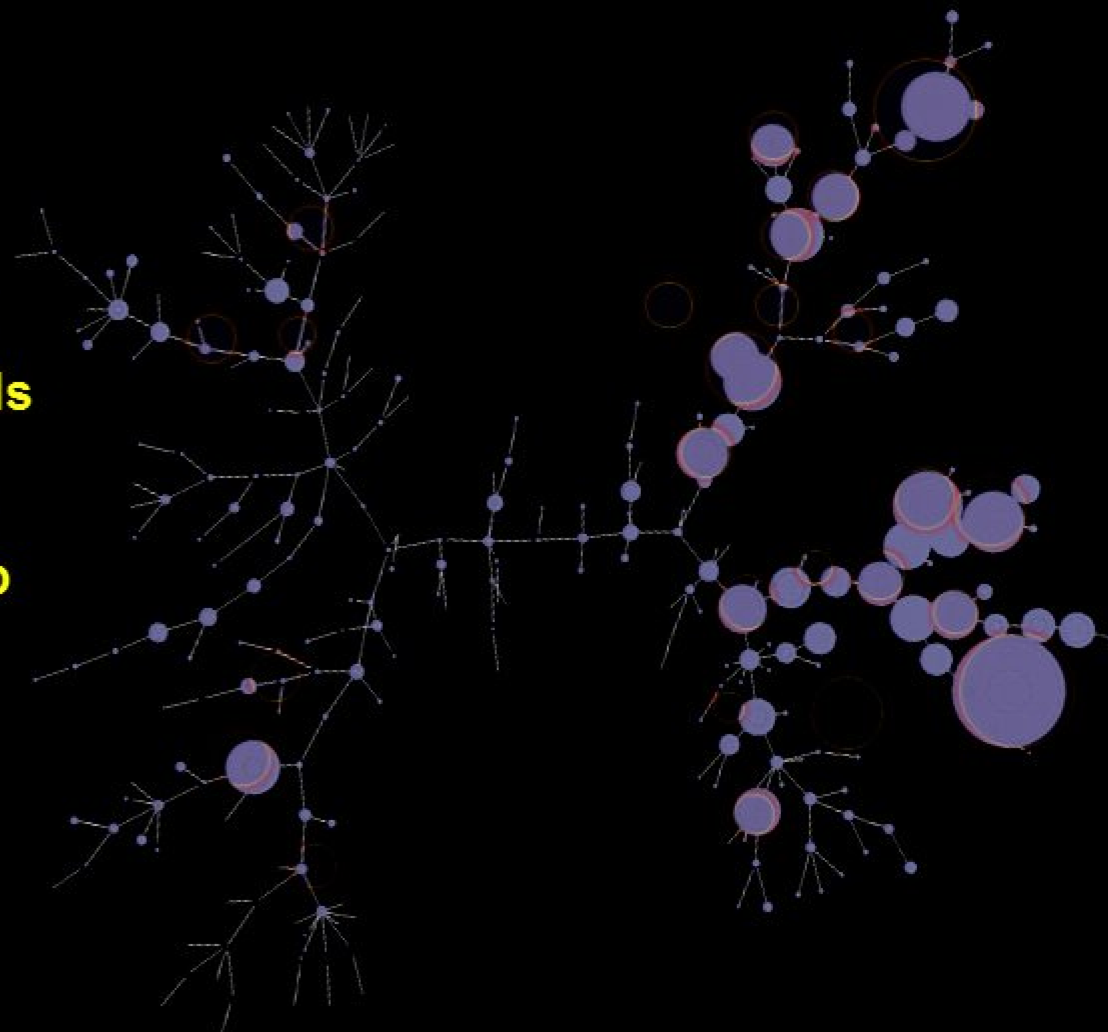
leukemia cells
from all
Patients &
redraw map



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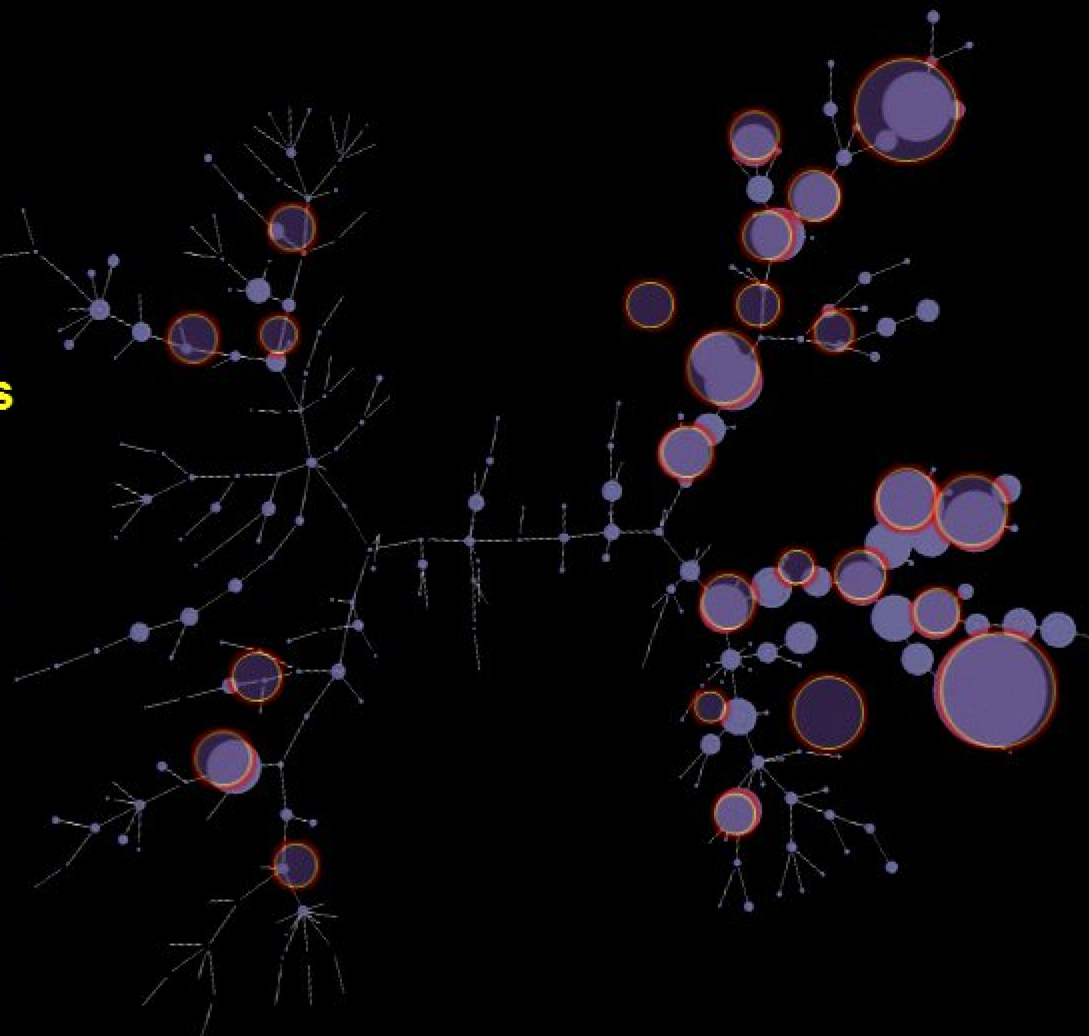

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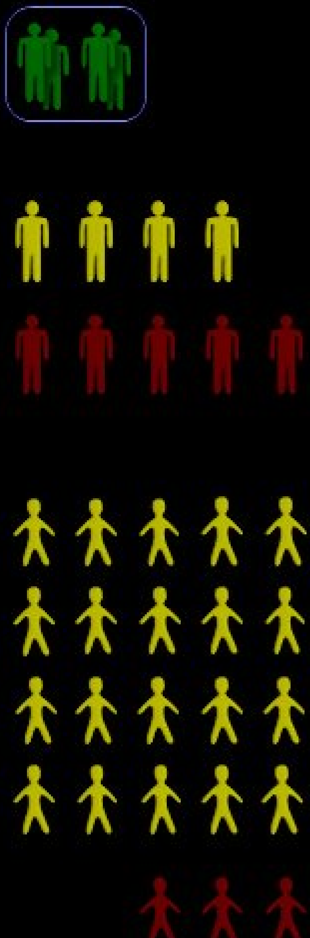

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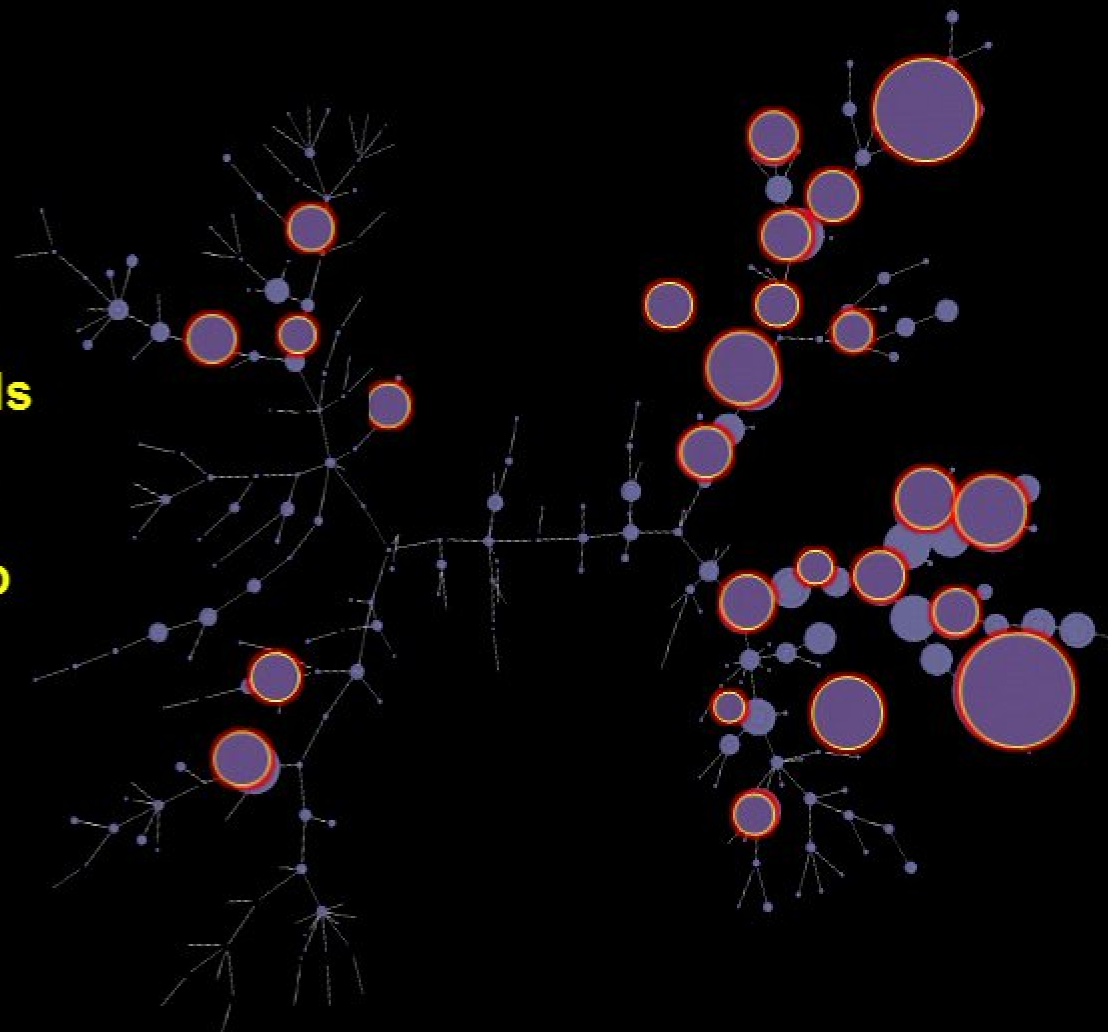

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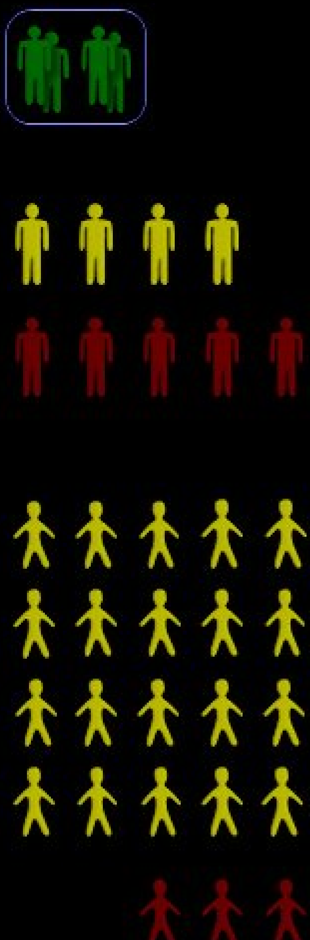
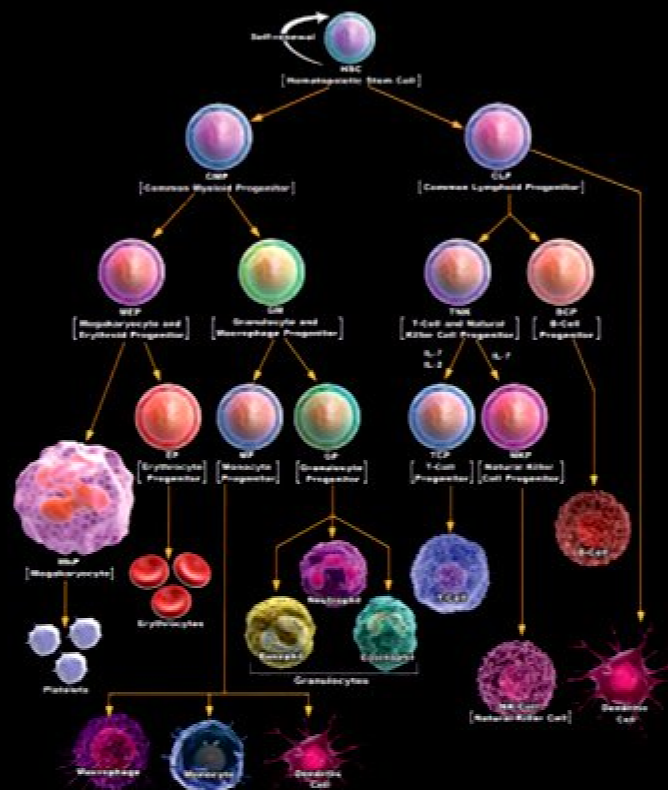


Add "invisible" leukemia cells from all Patients & redraw map



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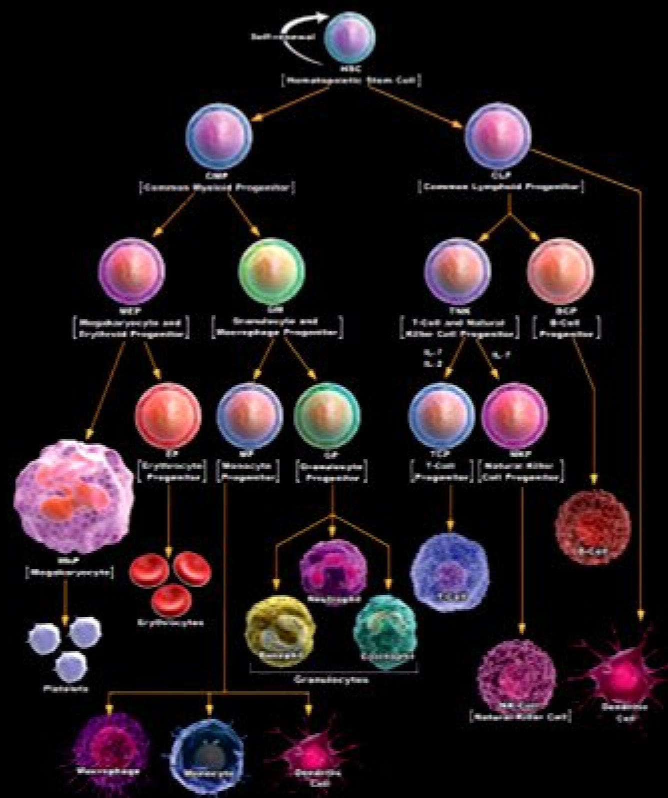


with Sean Bendall

Deep profiling of AML signaling in oligoclonal AML

Pediatric AML
diagnosis
bone marrow
(n = 1)

Perturbations (19)

No inhibitor

- Basal (unstim.)
- AICAR
- Flt3 ligand
- G-CSF
- GM-CSF
- IFNα
- IFNγ
- IL-3
- IL-6
- IL-10
- IL-27
- PMA + ionomycin
- PVO_4
- SCF
- TNFα
- TPO

PI3K/mTOR inhib.

- Inhibitor alone
- PMA/ionomycin
- PVO_4

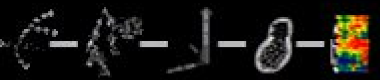
Staining panel

15 Functional Markers

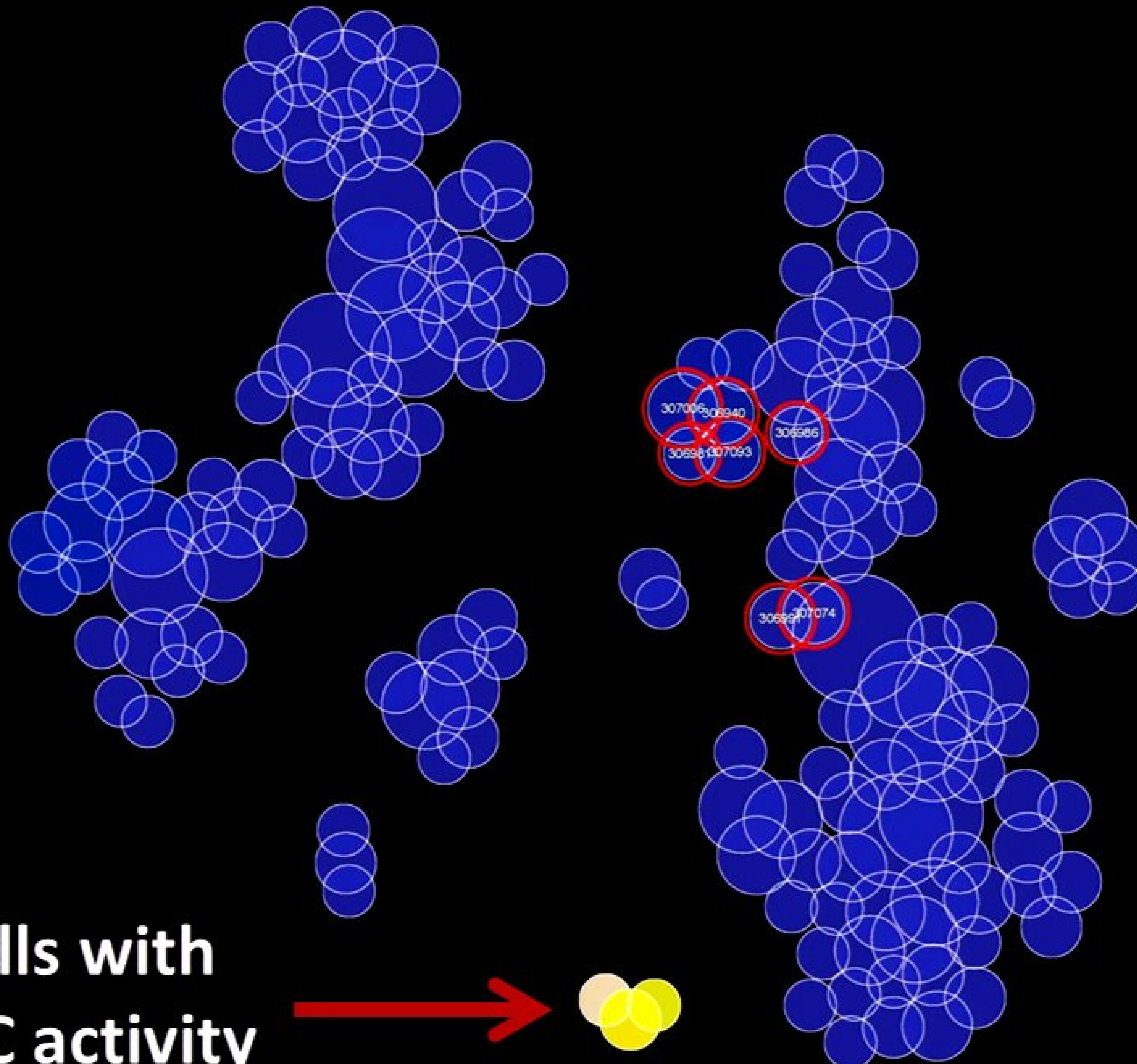
- p4EBP1
- pAkt
- pAMPK
- pCbl
- pCREB
- pERK1/2
- p-p38 MAPK
- cleaved Casp3
- pPLCgamma2
- pRb
- pS6
- pSTAT1
- pSTAT3
- pSTAT5
- pSyk

16 Surface Markers

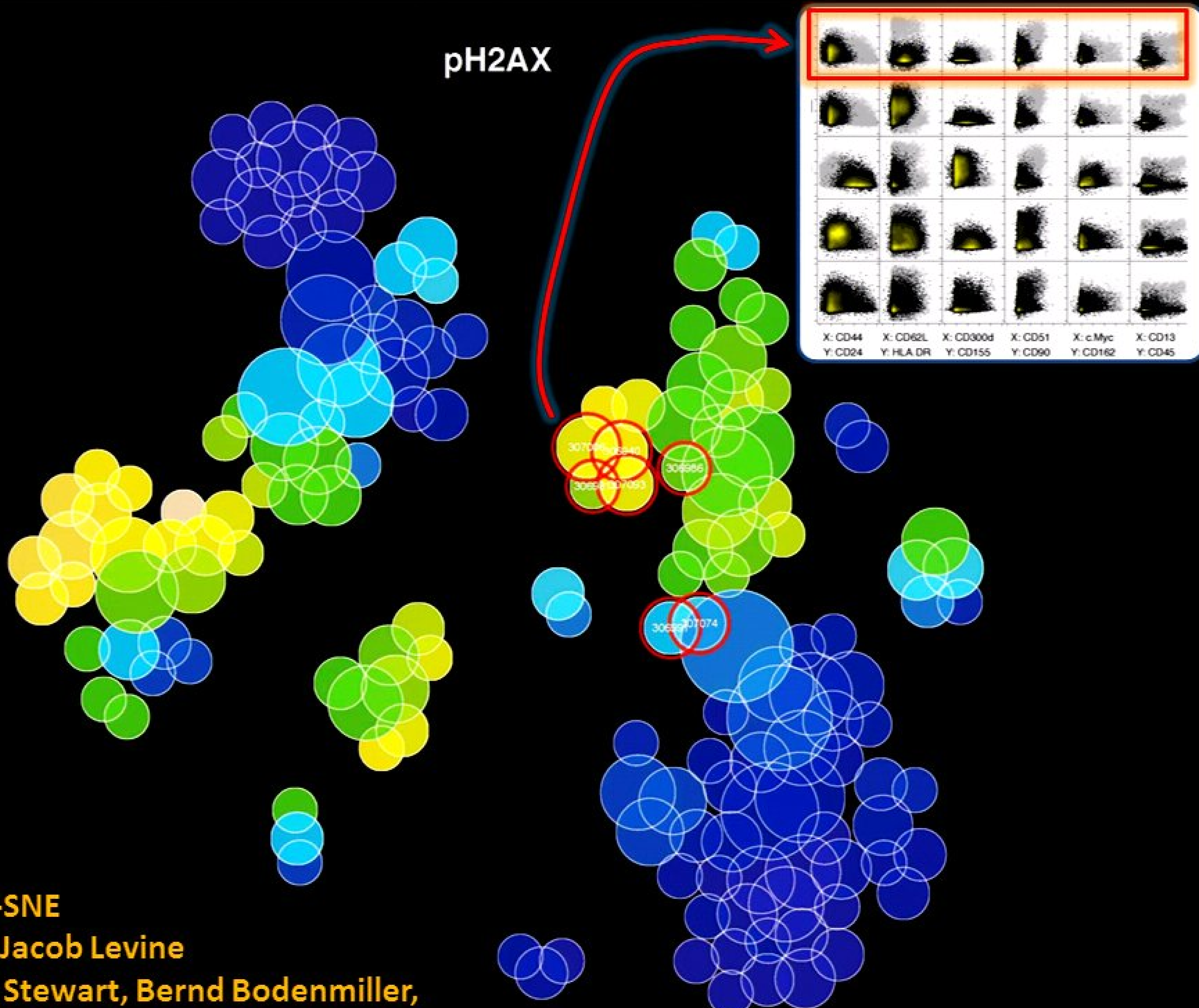
- CD3
- CD7
- CD11b
- CD15
- CD19
- CD33
- CD34
- CD38
- CD41
- CD44
- CD45
- CD47
- CD64
- CD117
- CD123
- HLADR



CD133



Cells with
TIC activity



Based on cell-SNE
Dana Pe'er & Jacob Levine
Data: Jocelyn Stewart, Bernd Bodenmiller, Rob Bruggner, Ben Neel, GPN

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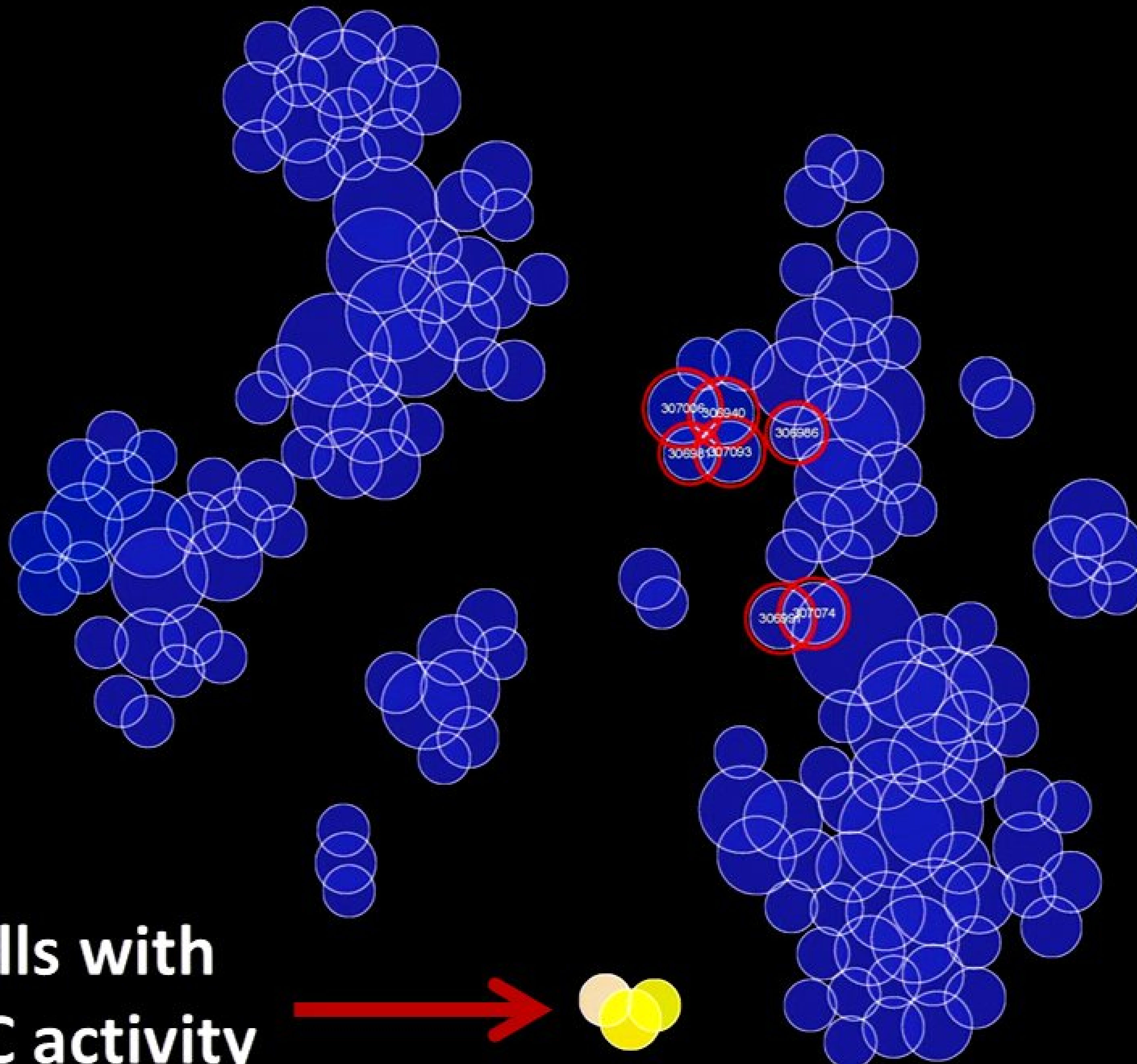

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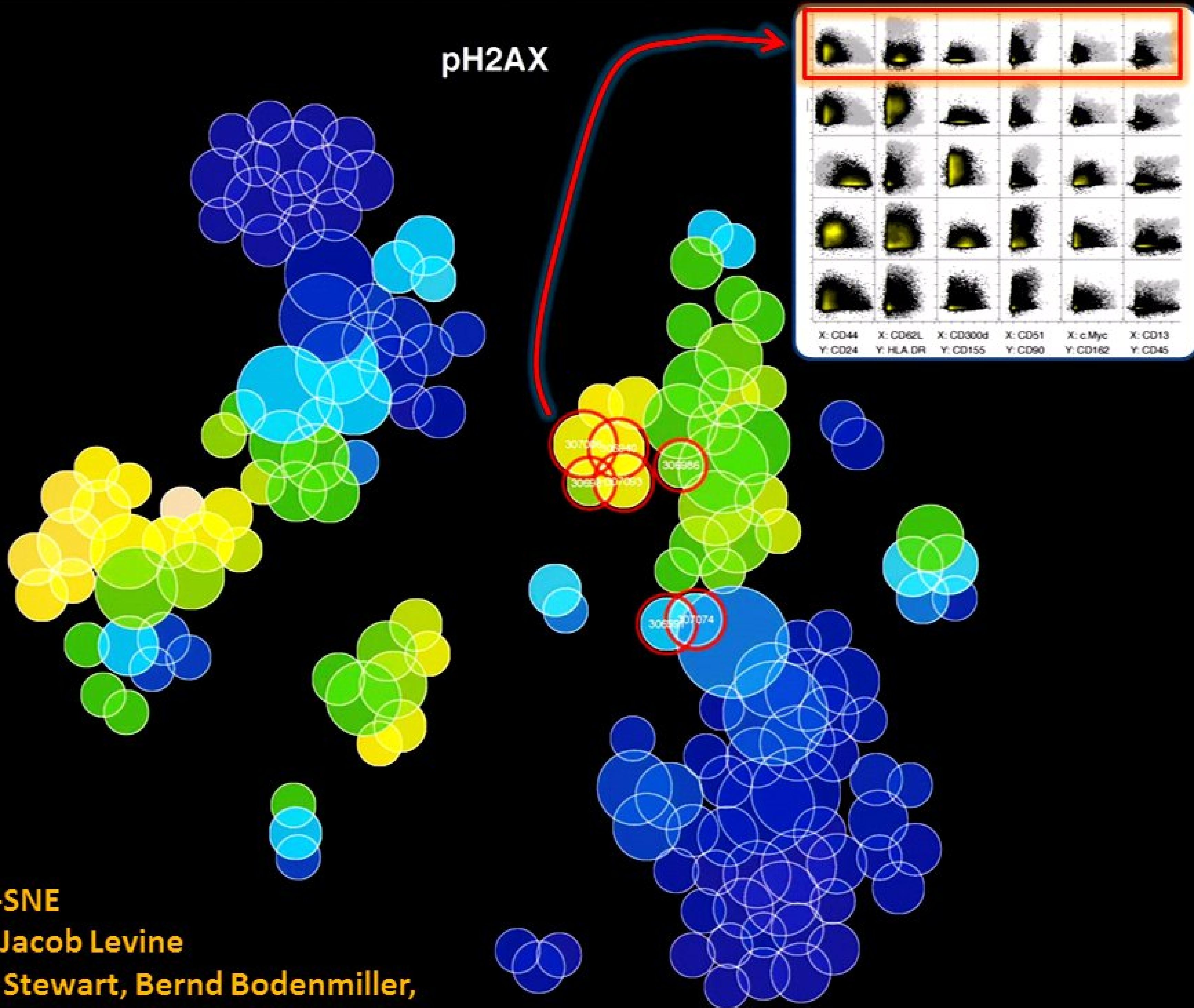

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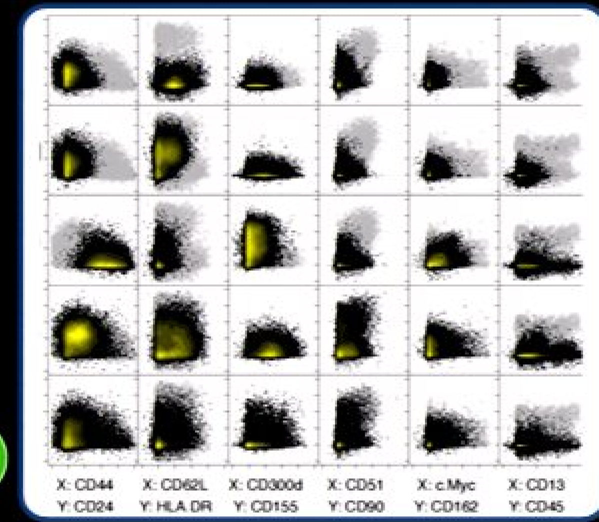
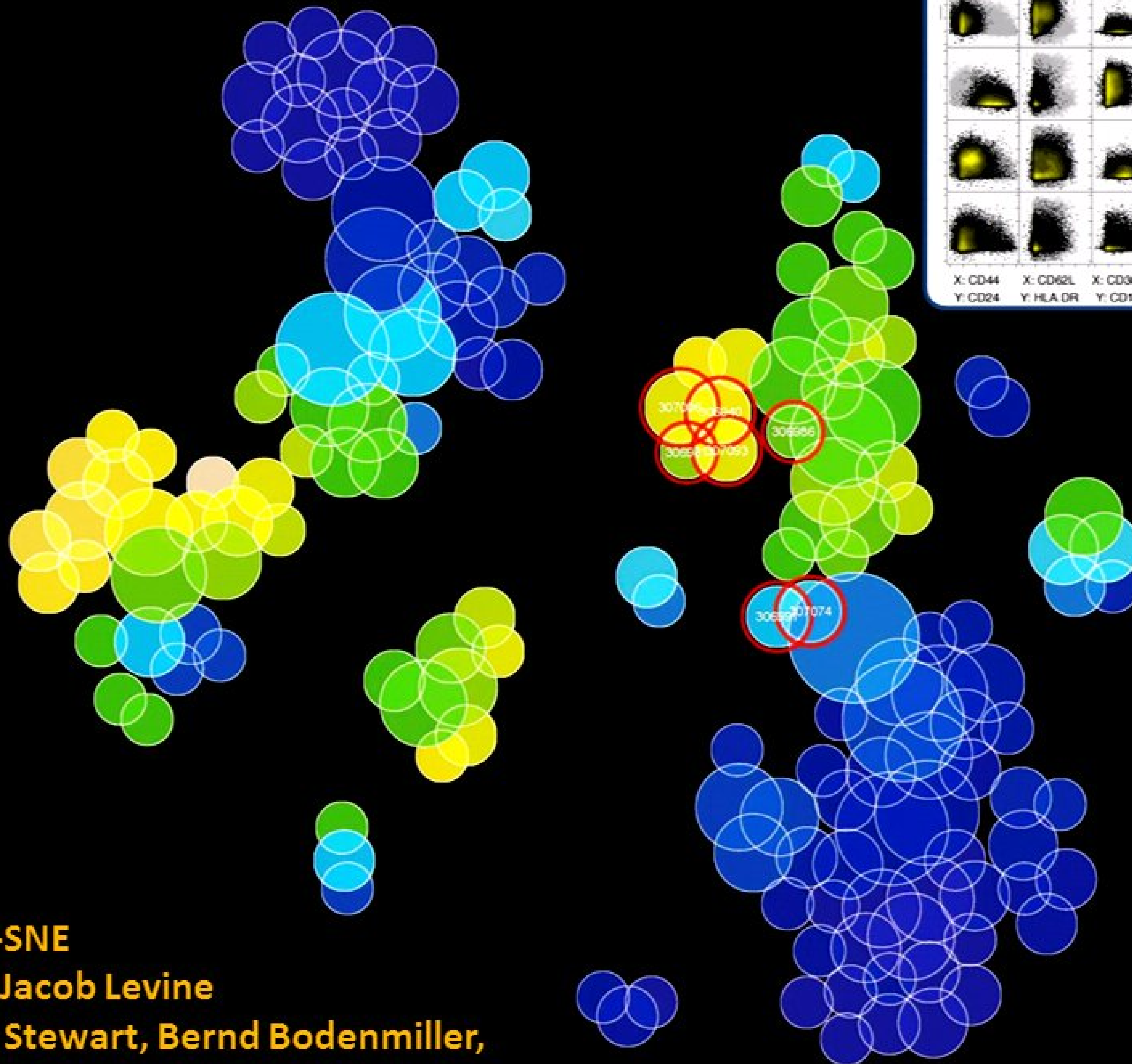
pH2AX





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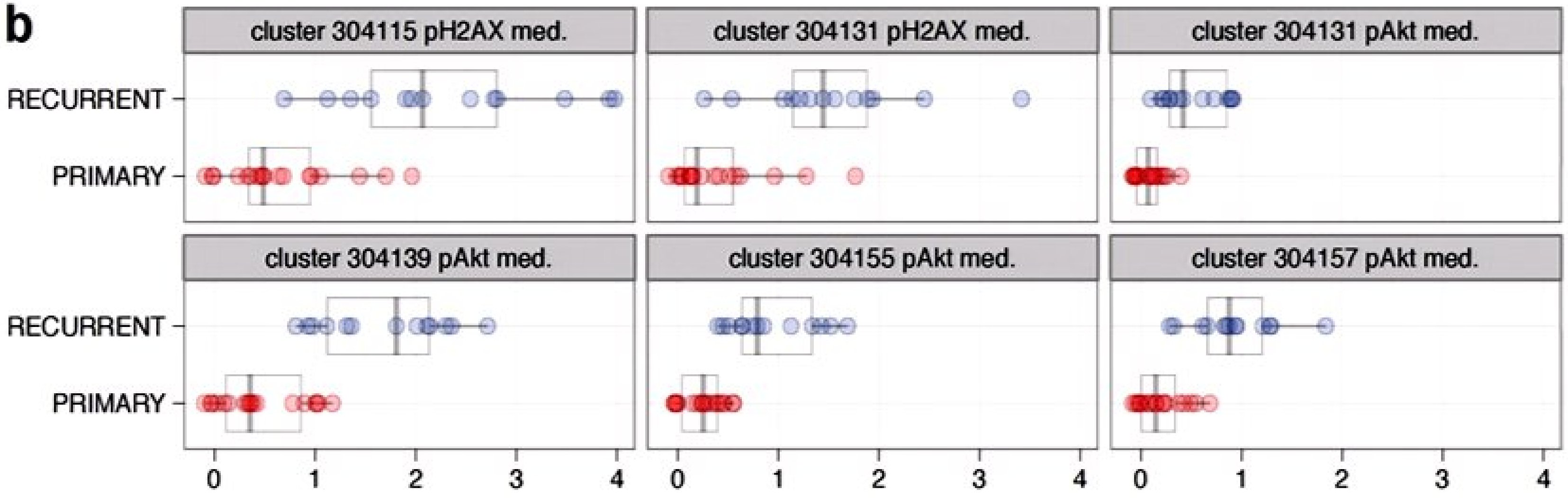
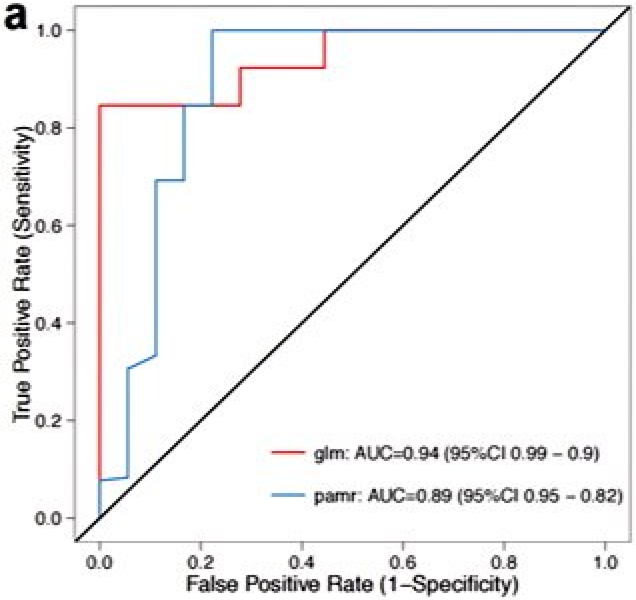
Cellular clusters stratify outcome for primary and recurrent forms of ovarian cancer

b



a



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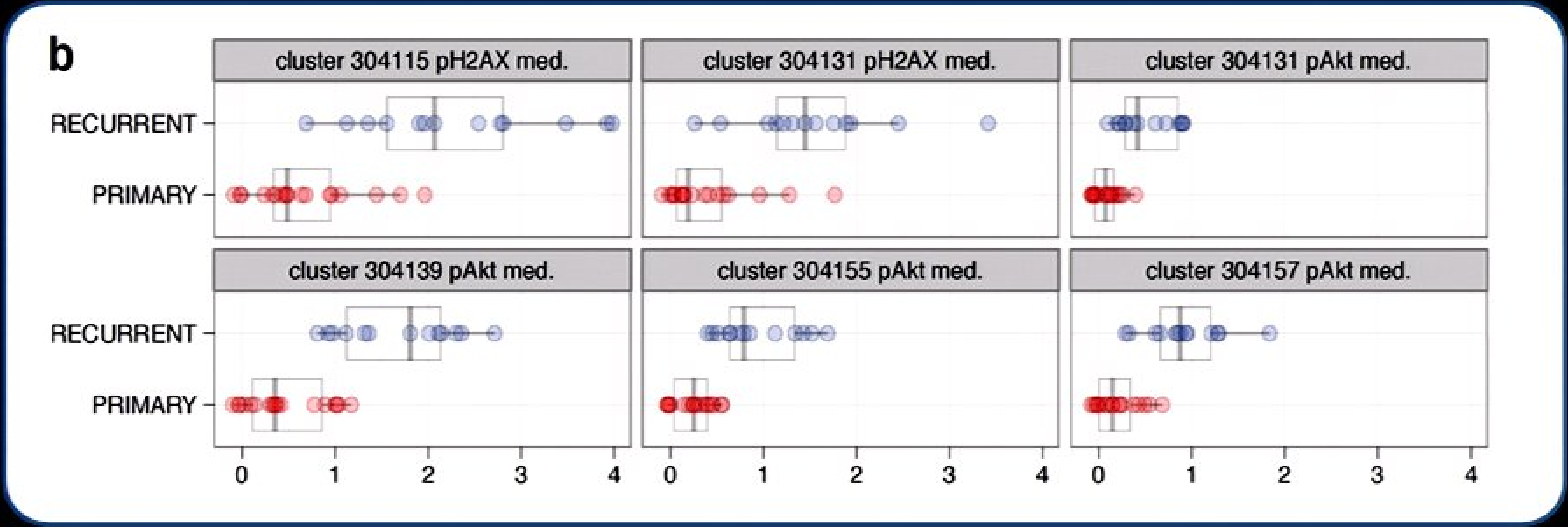
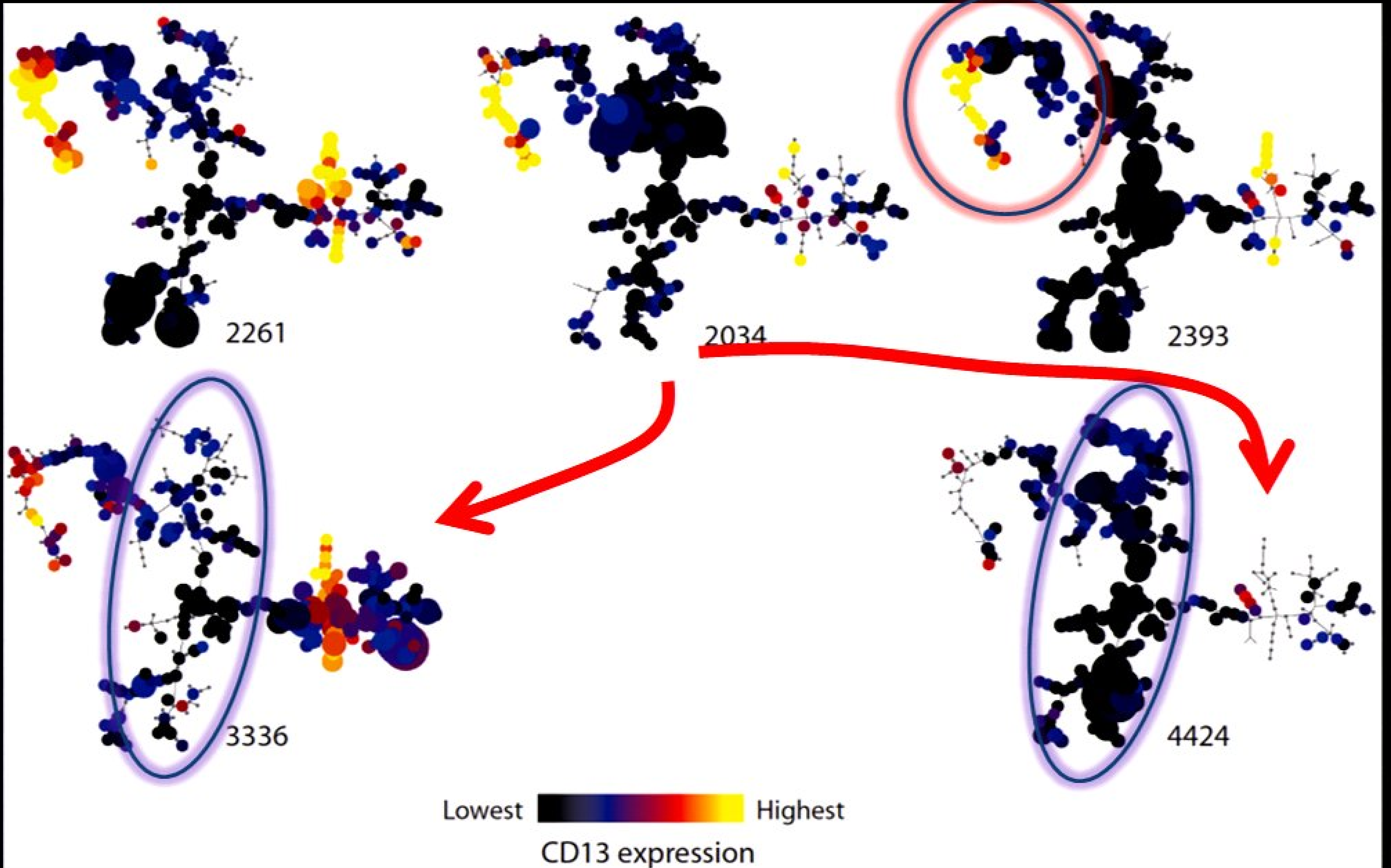


SOC Cell Subset Differences Within & Across Patients – Surface Markers CD13



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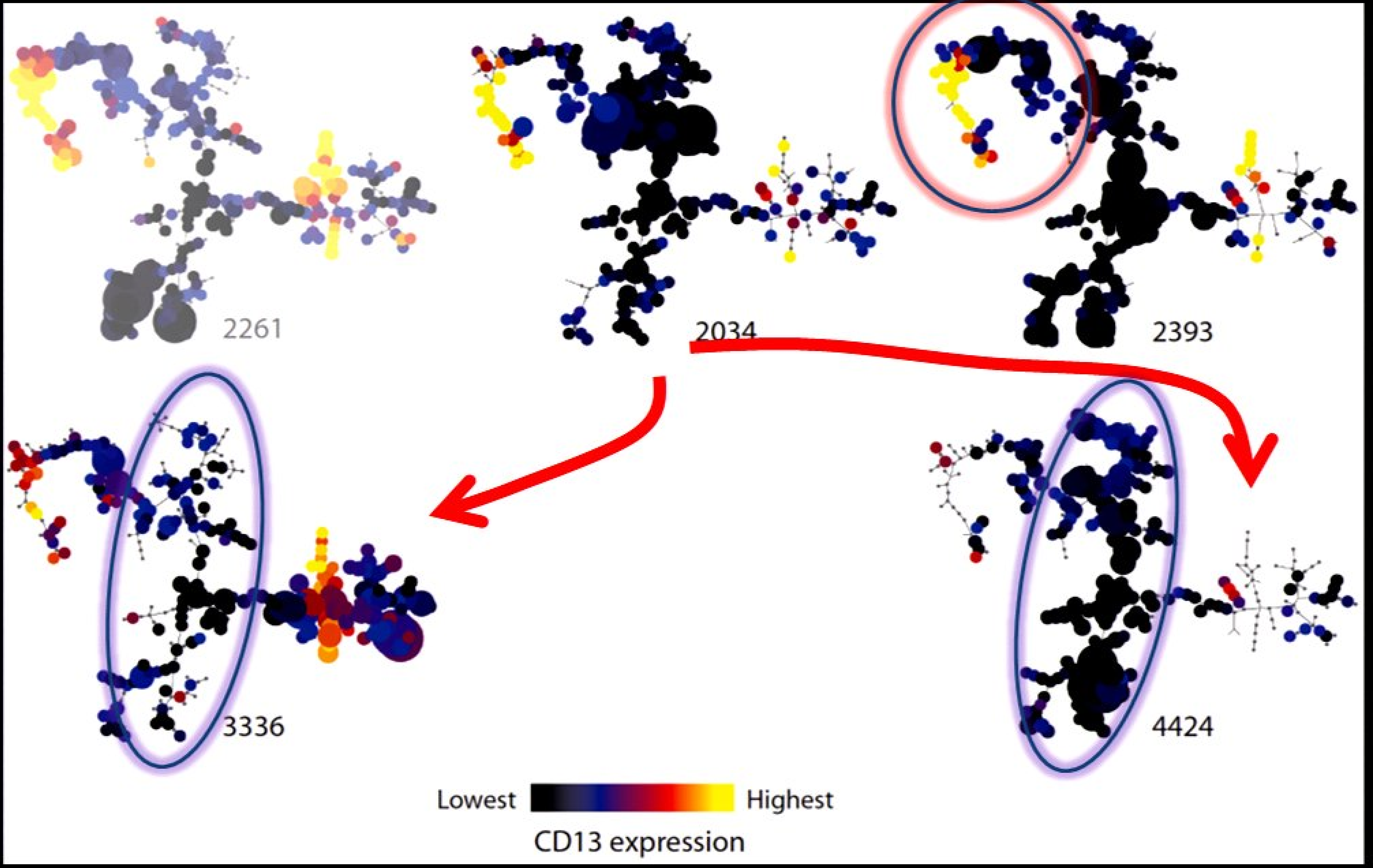

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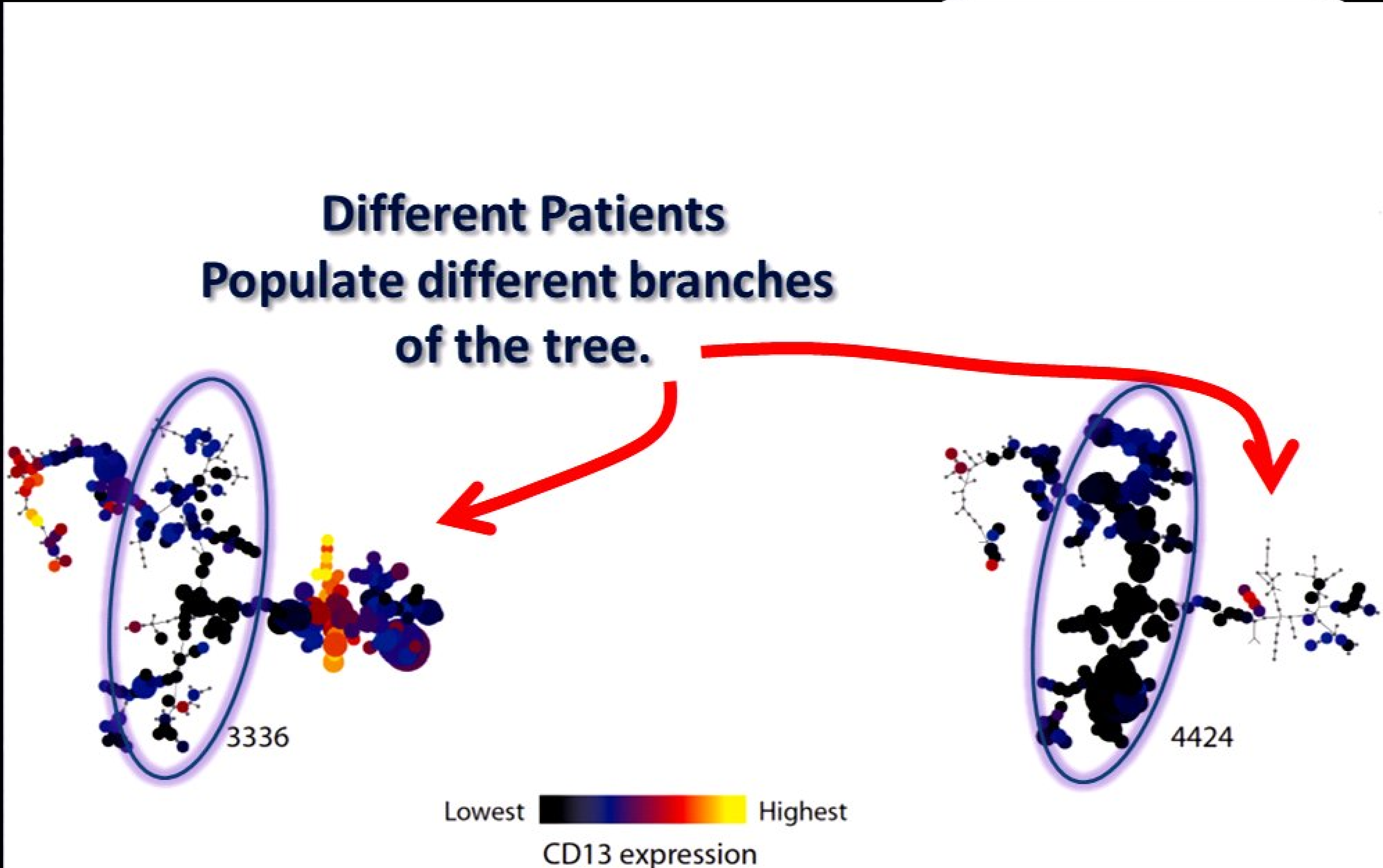
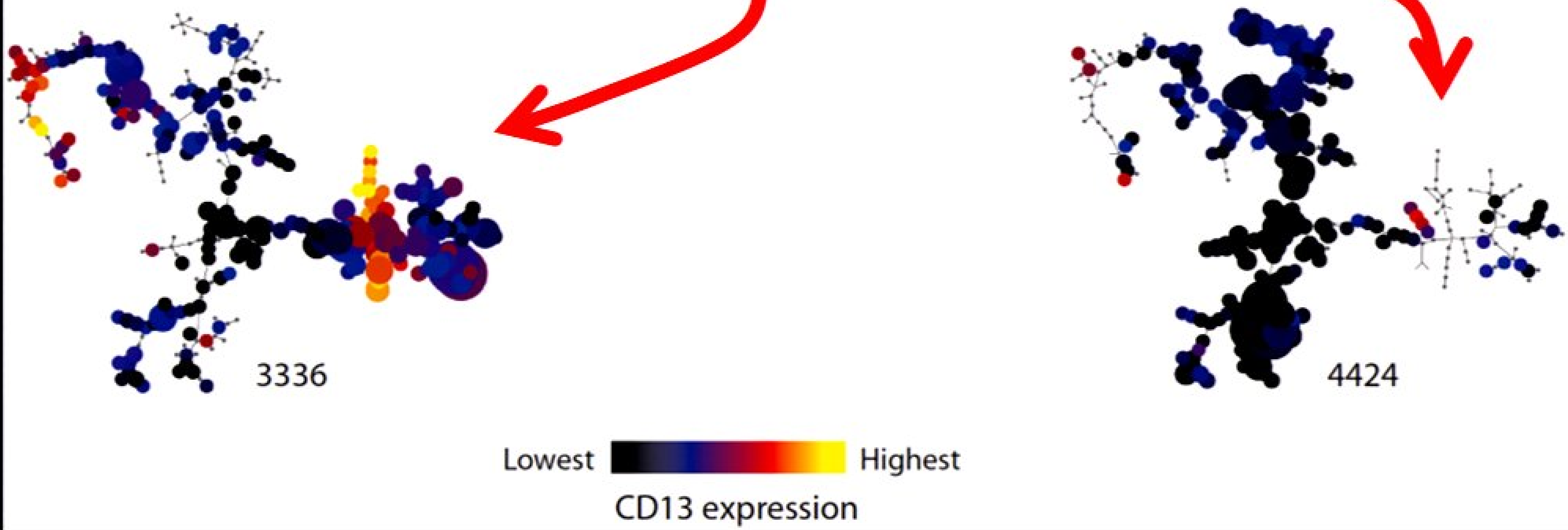
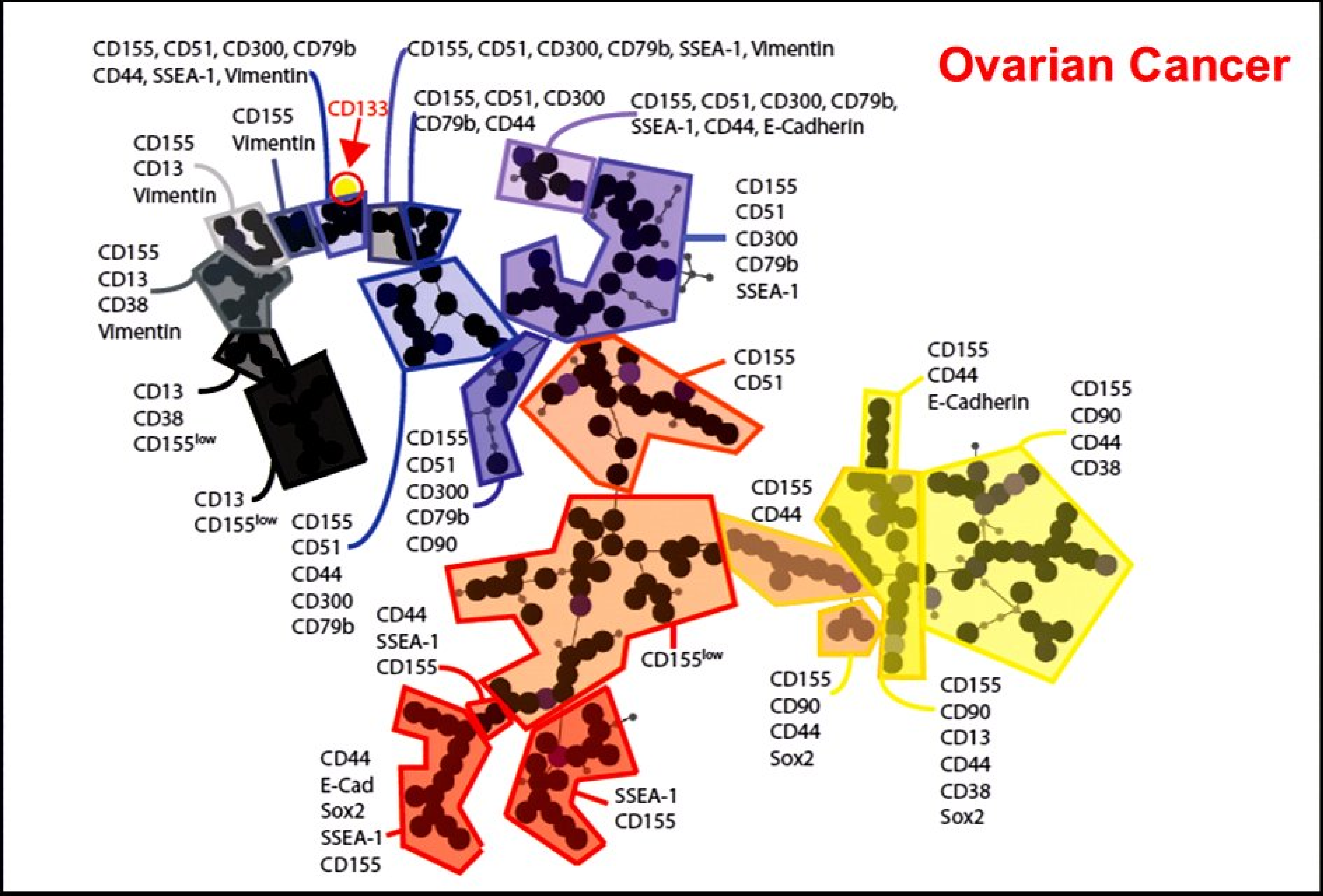


SOC Cell Subset Differences Within & Across Patients – Surface Markers CD13

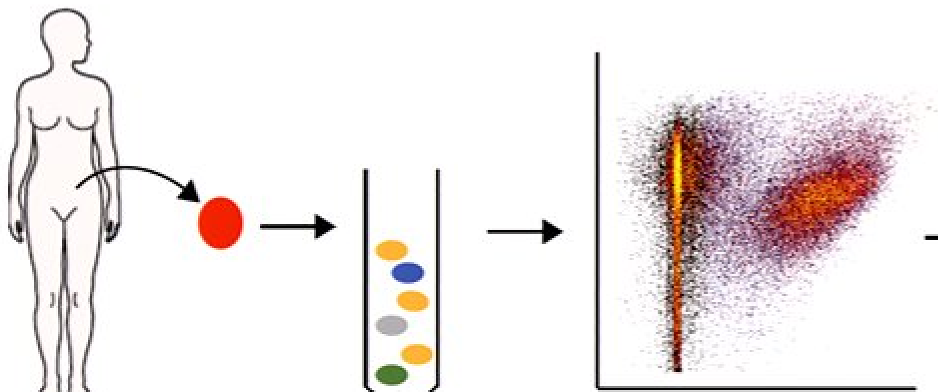
Different Patients Populate different branches of the tree.



Meta-clusters Based on Expression of Surface & Stem Cell Markers in Ovarian Cancer

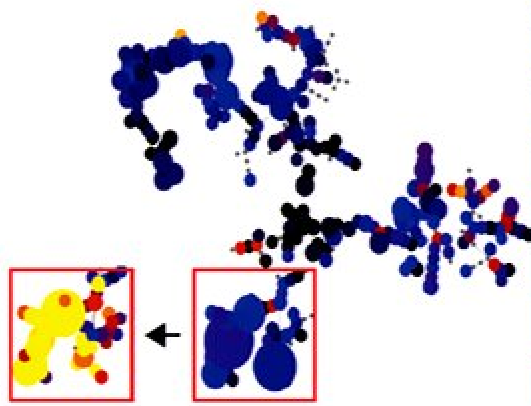


Extending Approach to Disease

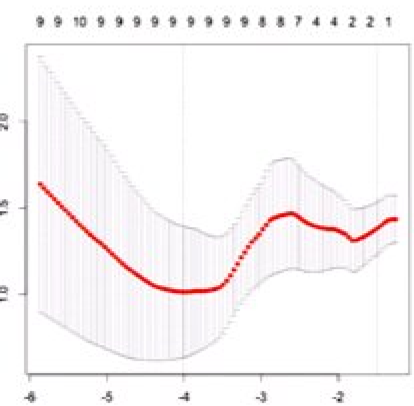


Ovarian cancer samples → Single cells → 365 cell surface markers screened by FACS

Isotope Channel	Marker	Isotope Channel	Marker
115	CD45	158	E-Cadherin
139	p-S6	159	p-Akt
141	CD133	160	Sox2
142	CD51	162	SSEA-1
143	p-H2AX	164	p-Stat5
144	CD90	165	CD162
145	CD79B	166	CD44
146	p-ATM	167	CD38
147	c-Myc	168	CD13
148	CD34	169	p-p53
149	p-NFkb	170	CD49A
150	CD117	171	CD24
151	p-Erk	172	c-Casp 3
152	CD155	174	HLA-DR
153	CD62L	175	CD300d
154	Vimentin	176	p-HH3
156	CD10		

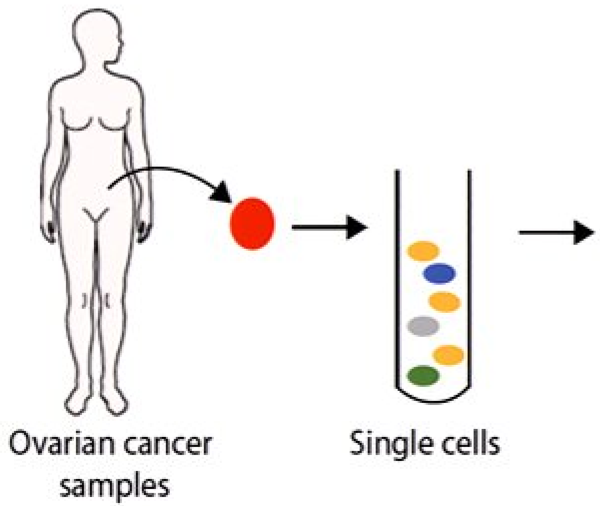


Cell subpopulation signaling response analysis



Patient stratification

Extending Approach to Disease



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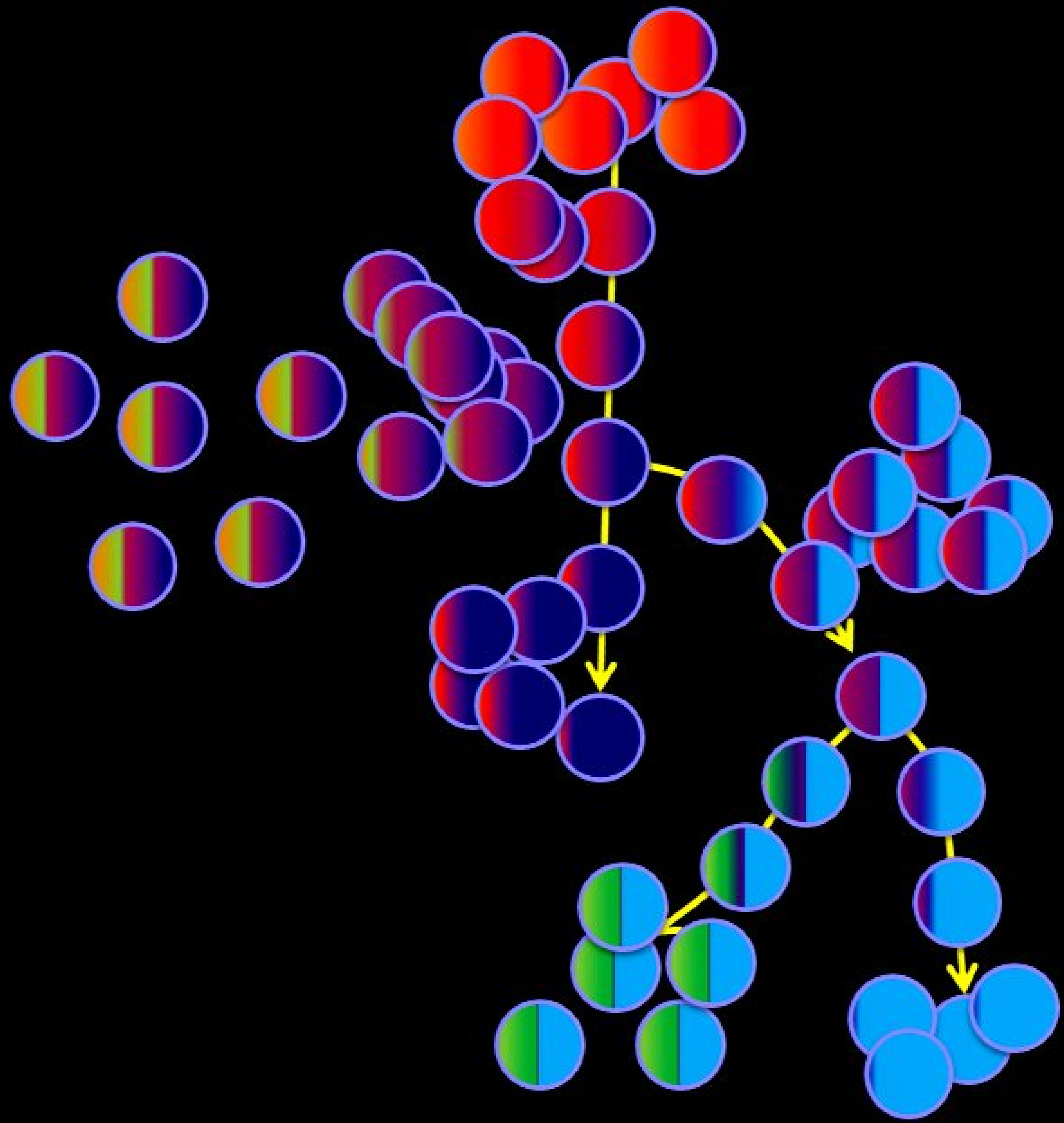
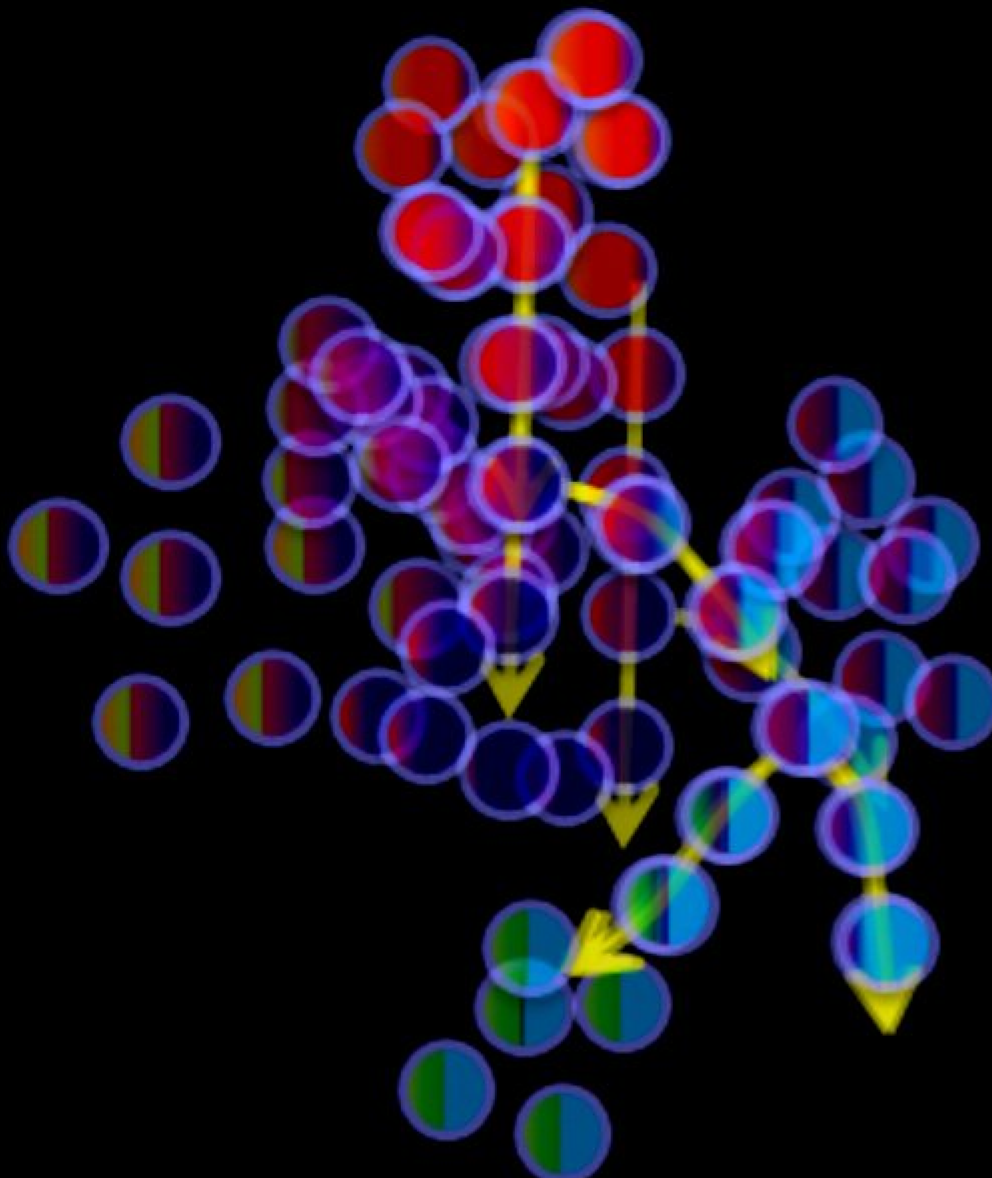
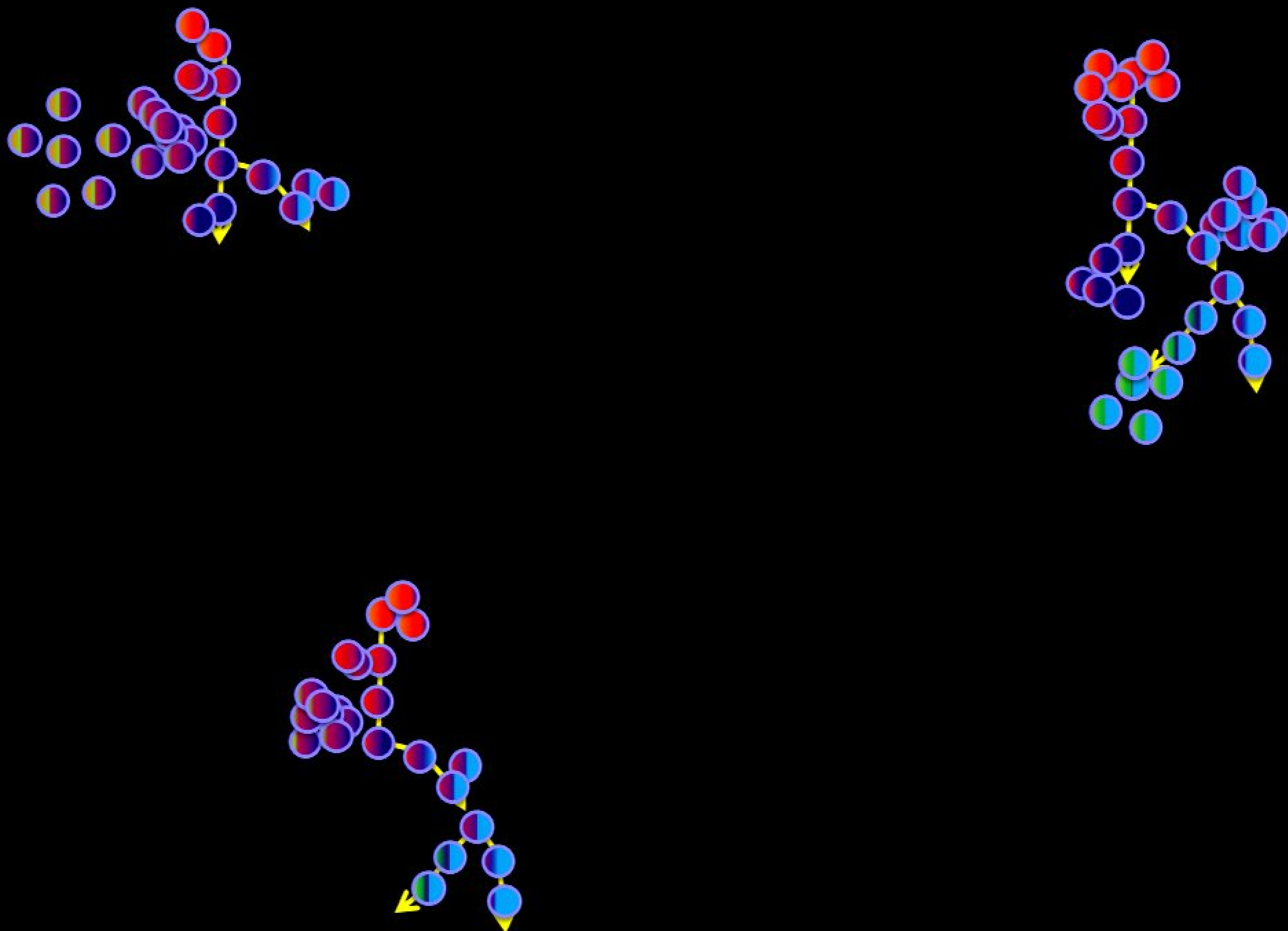
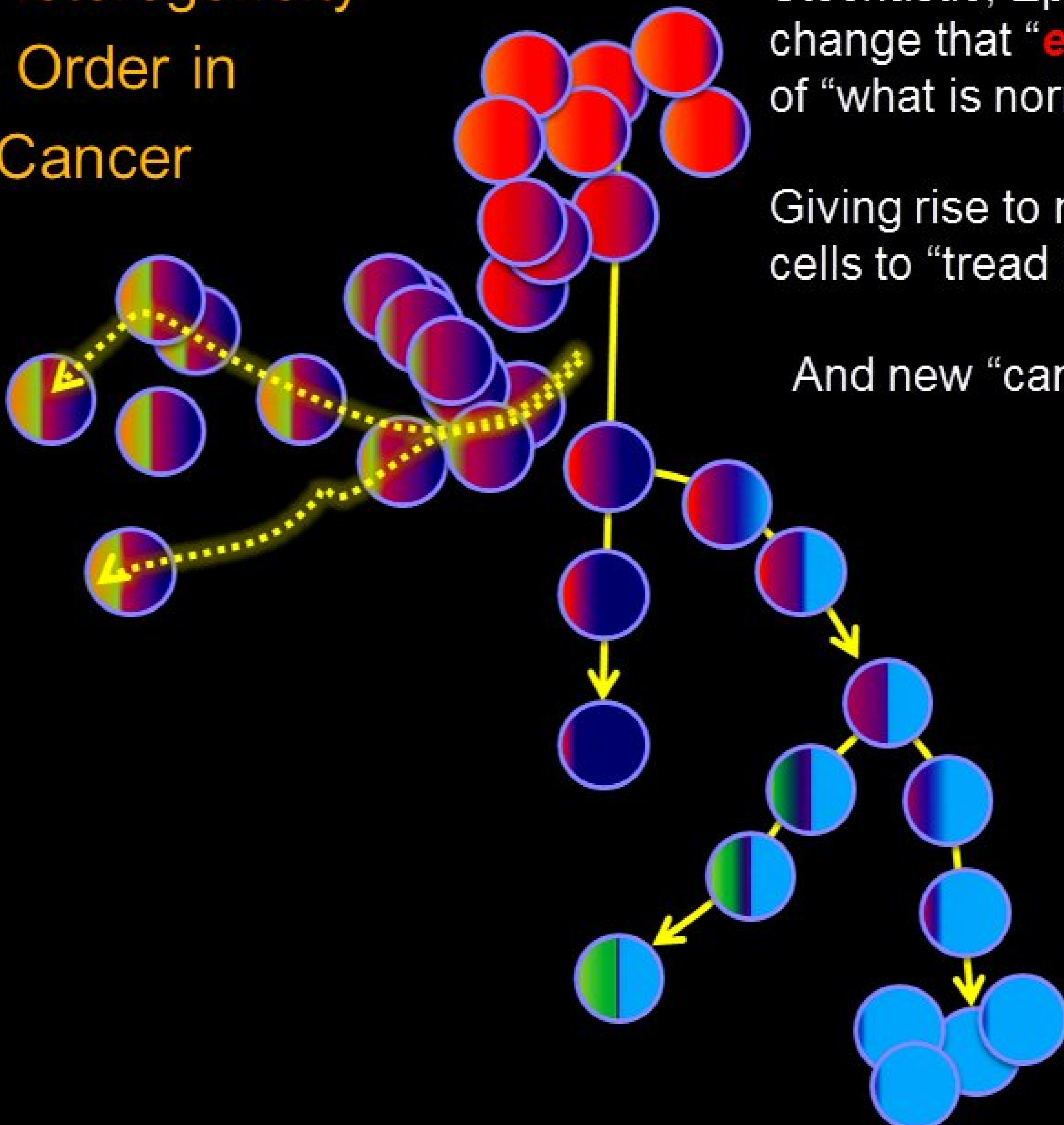
154	Vimentin	176	p-HH3
156	CD10		







From Heterogeneity to Order in Cancer



Stochastic, Epigenetic, or Genetic change that “***expands the boundary***” of “what is normal”…

Giving rise to new opportunities for cells to “tread into” new regulatory terrain.

And new “cancer” lineages...

From Heterogeneity to Order in Cancer

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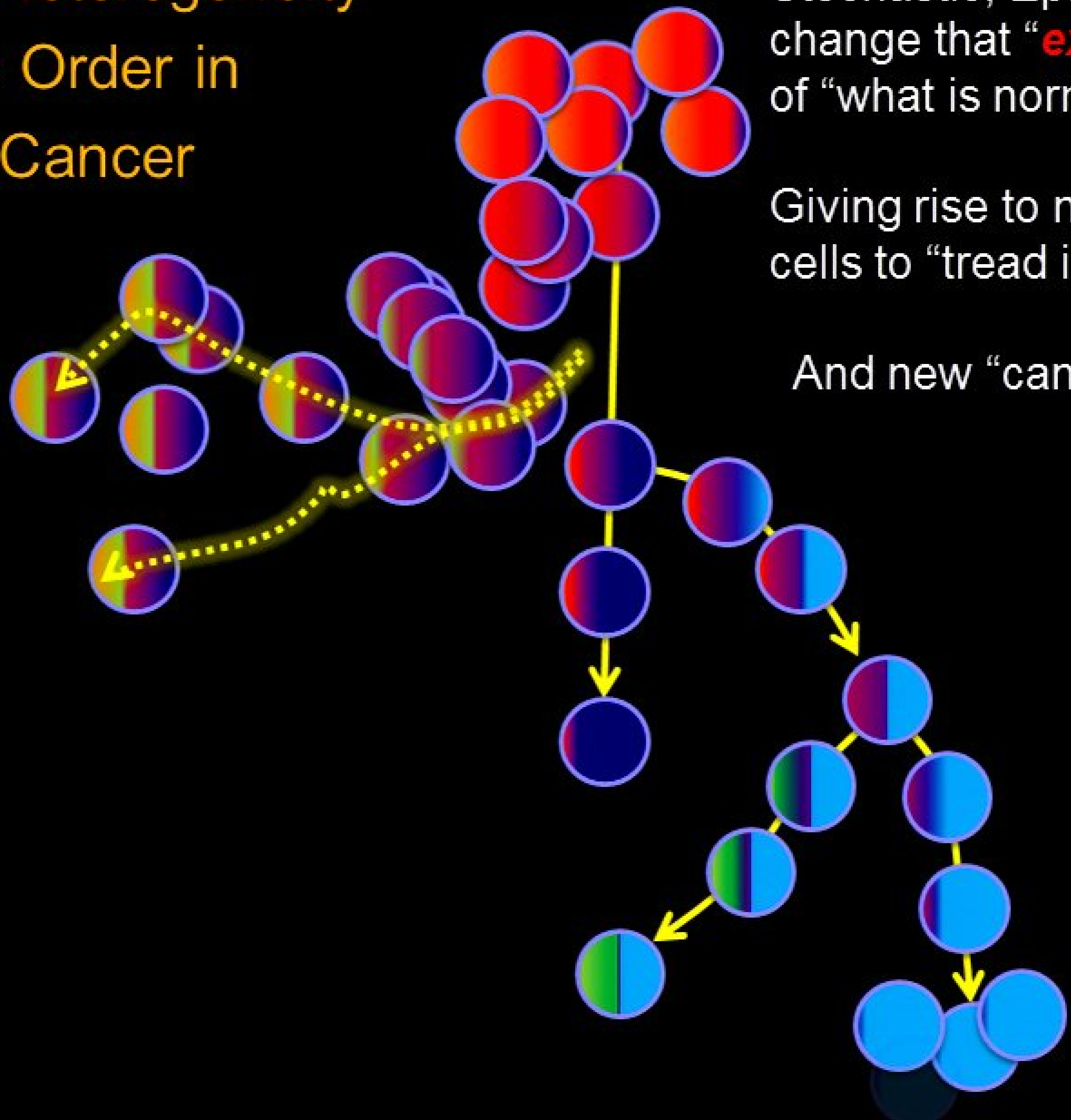
Giving rise to new opportunities for cells to “tread into” new regulatory terrain.

And new “cancer” lineages...



From Heterogeneity to Order in Cancer

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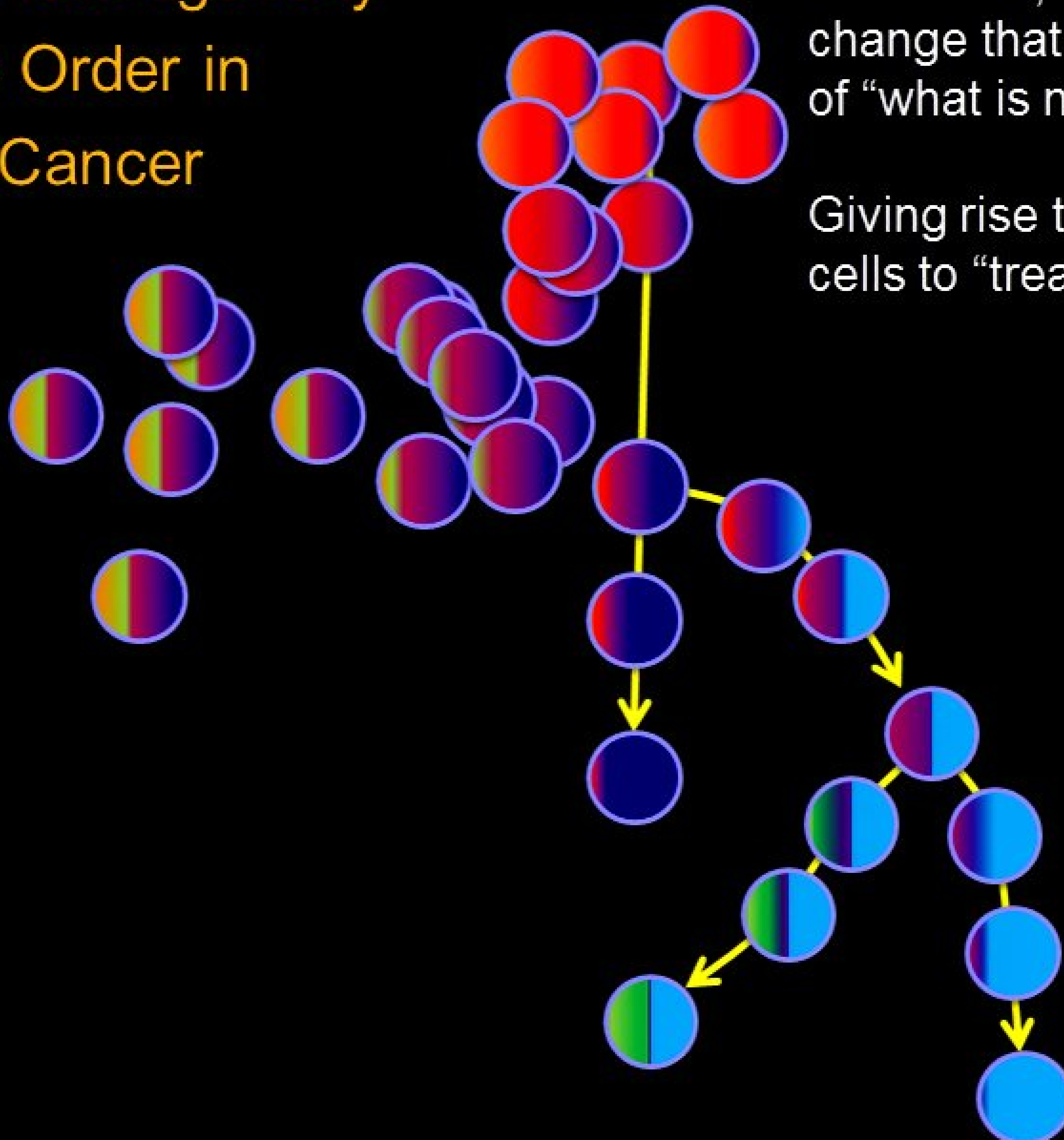

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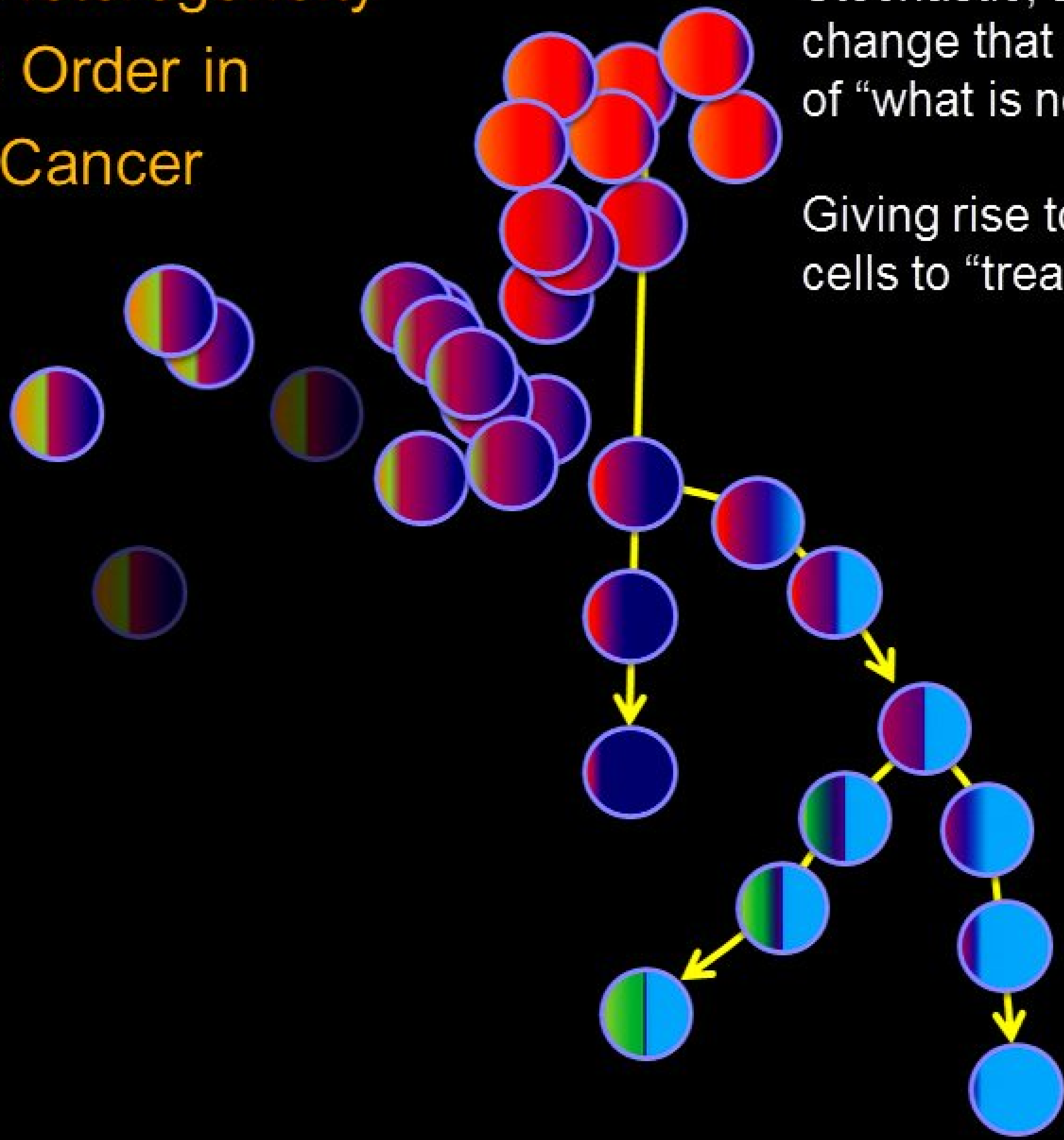

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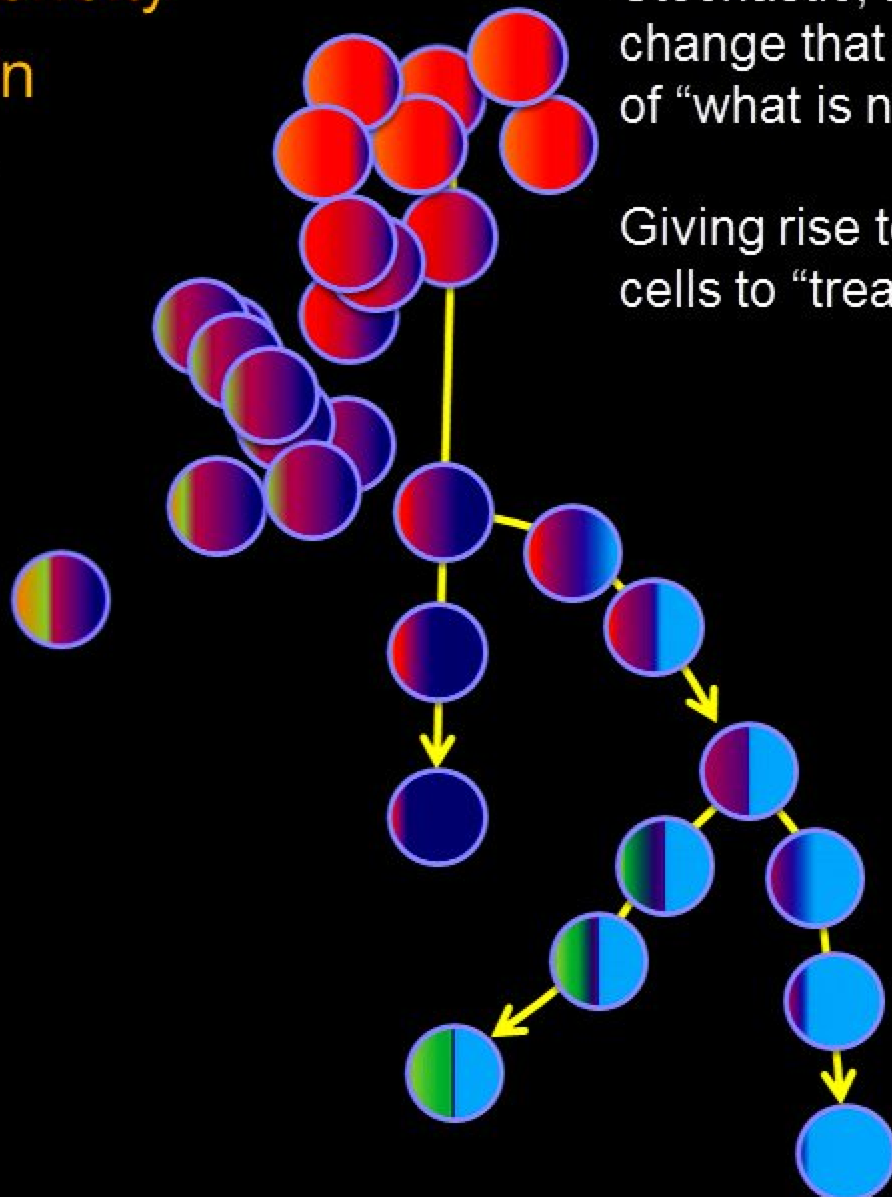

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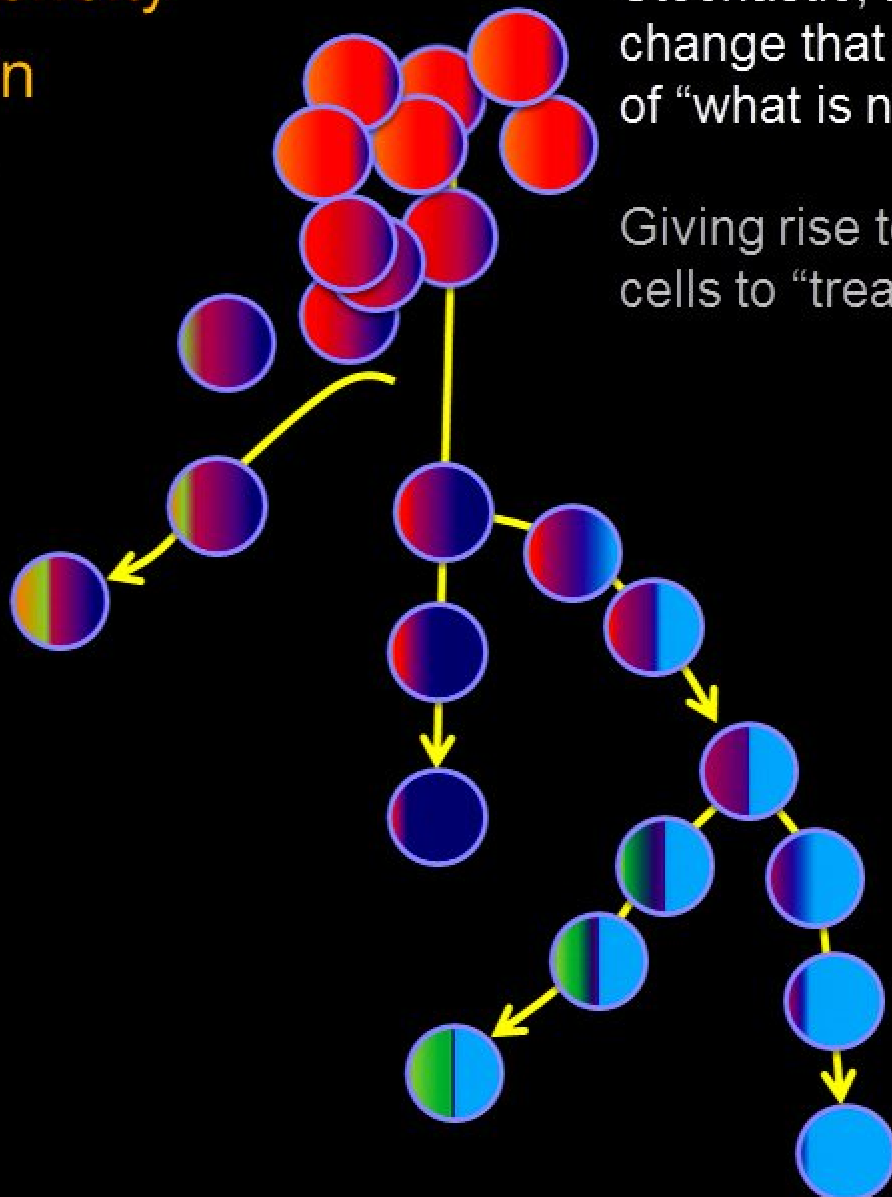

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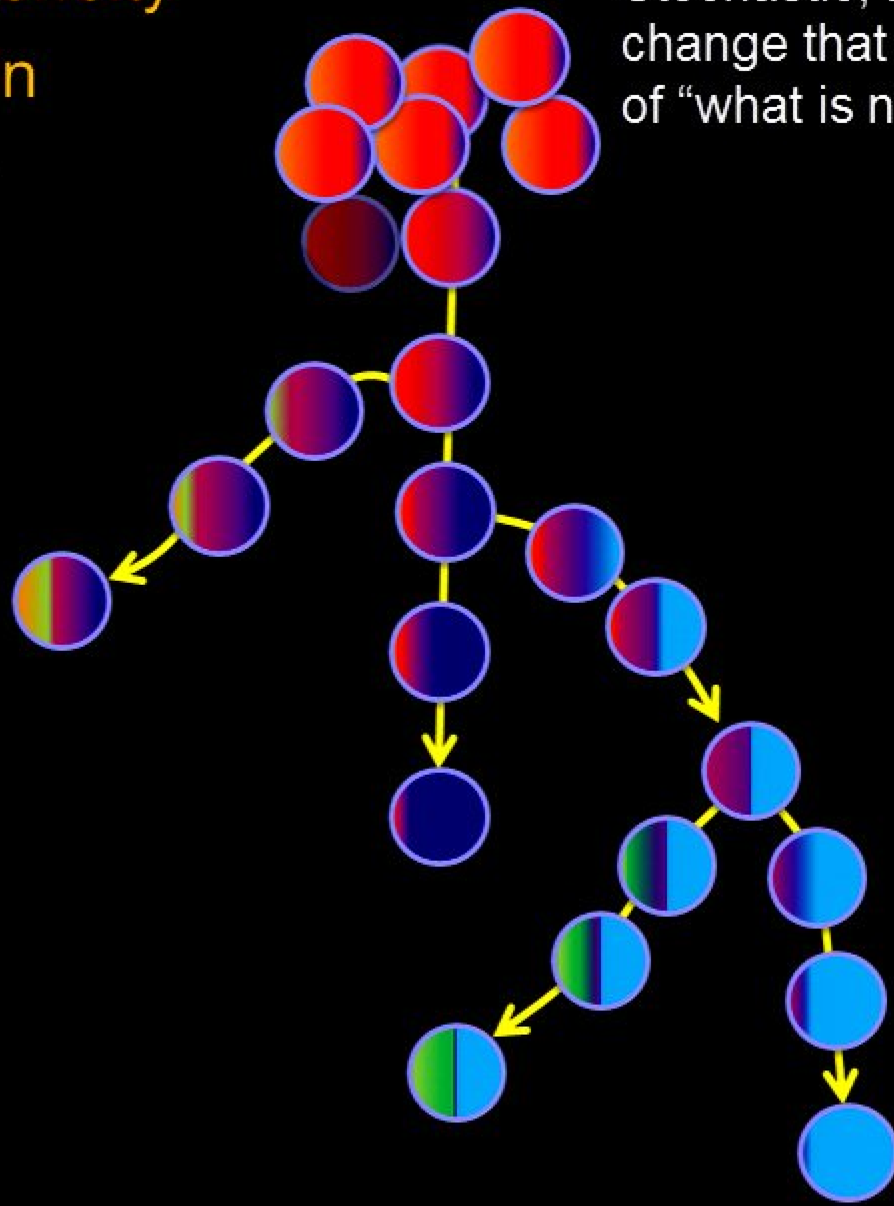

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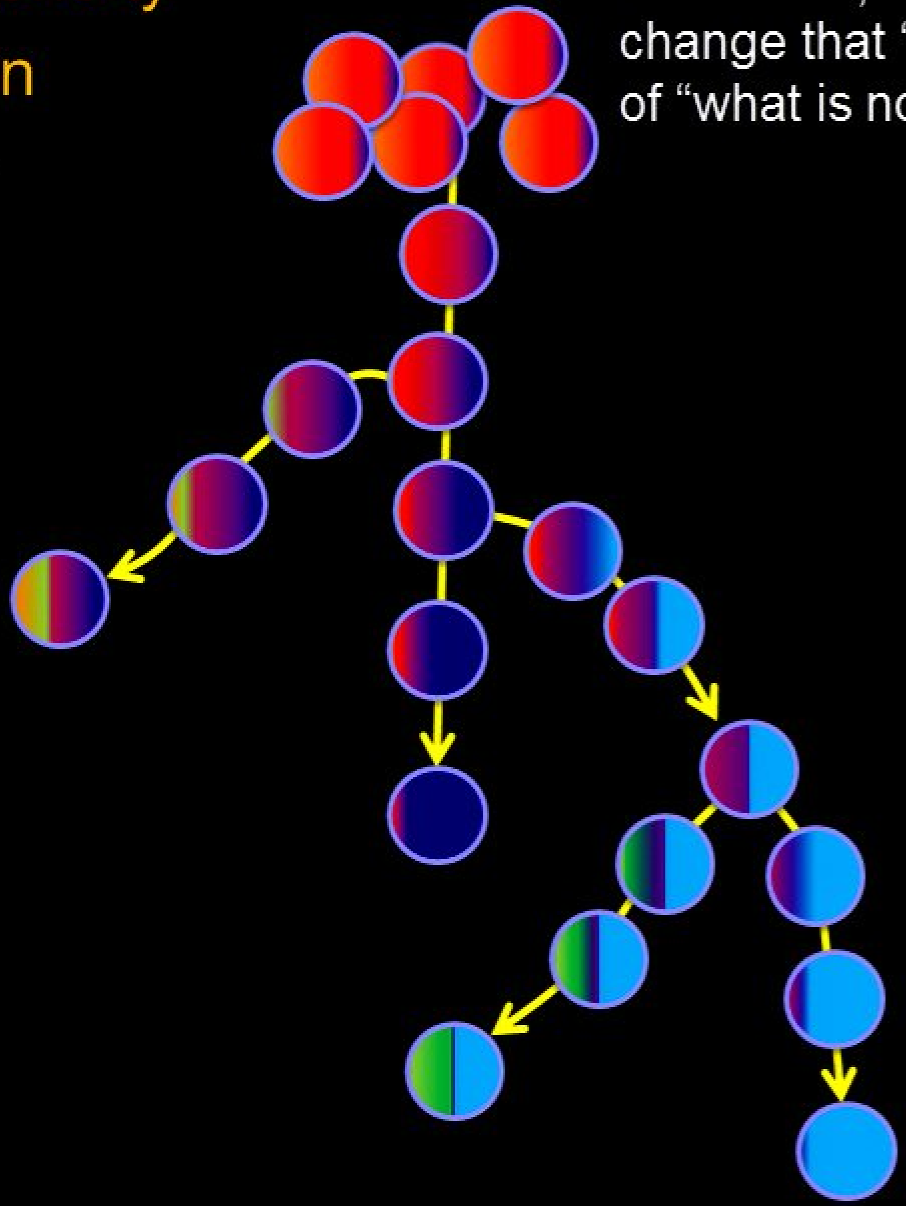

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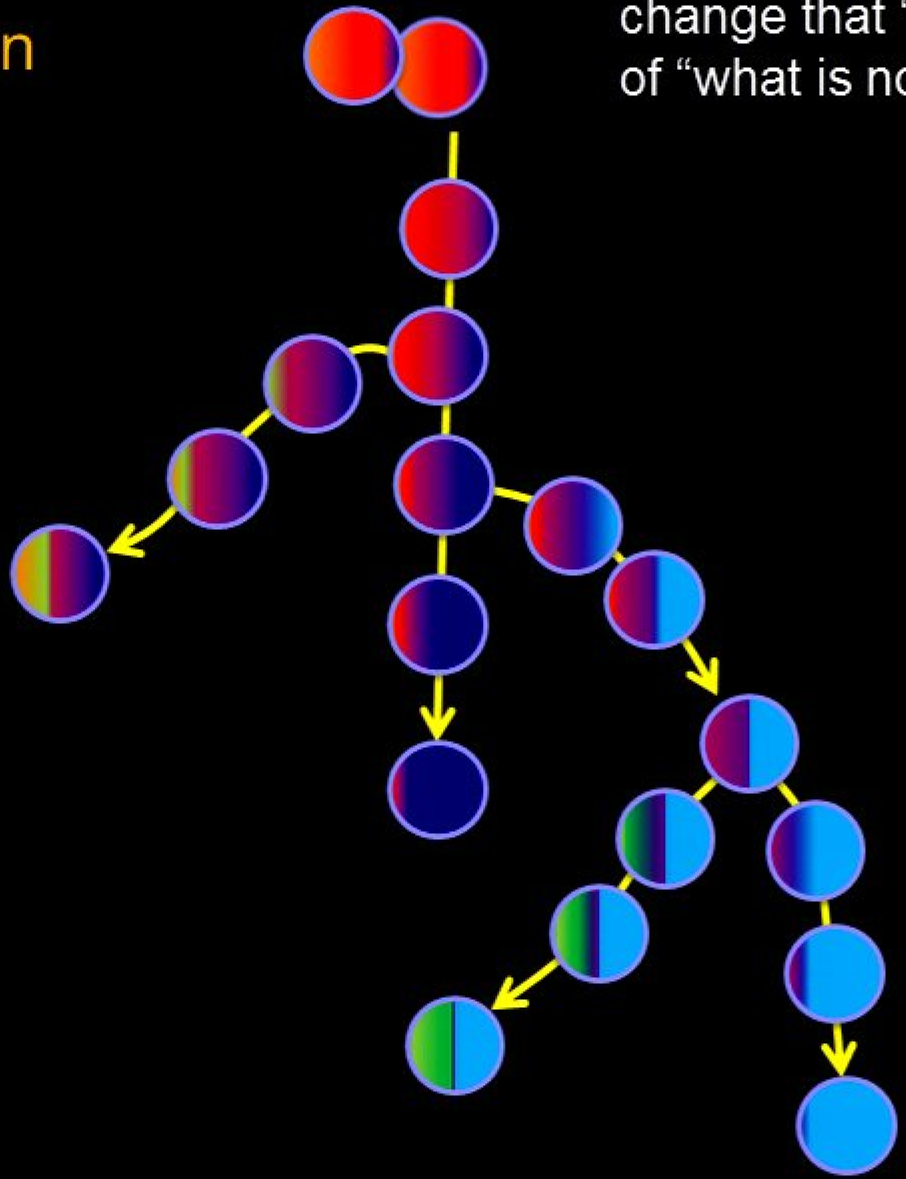

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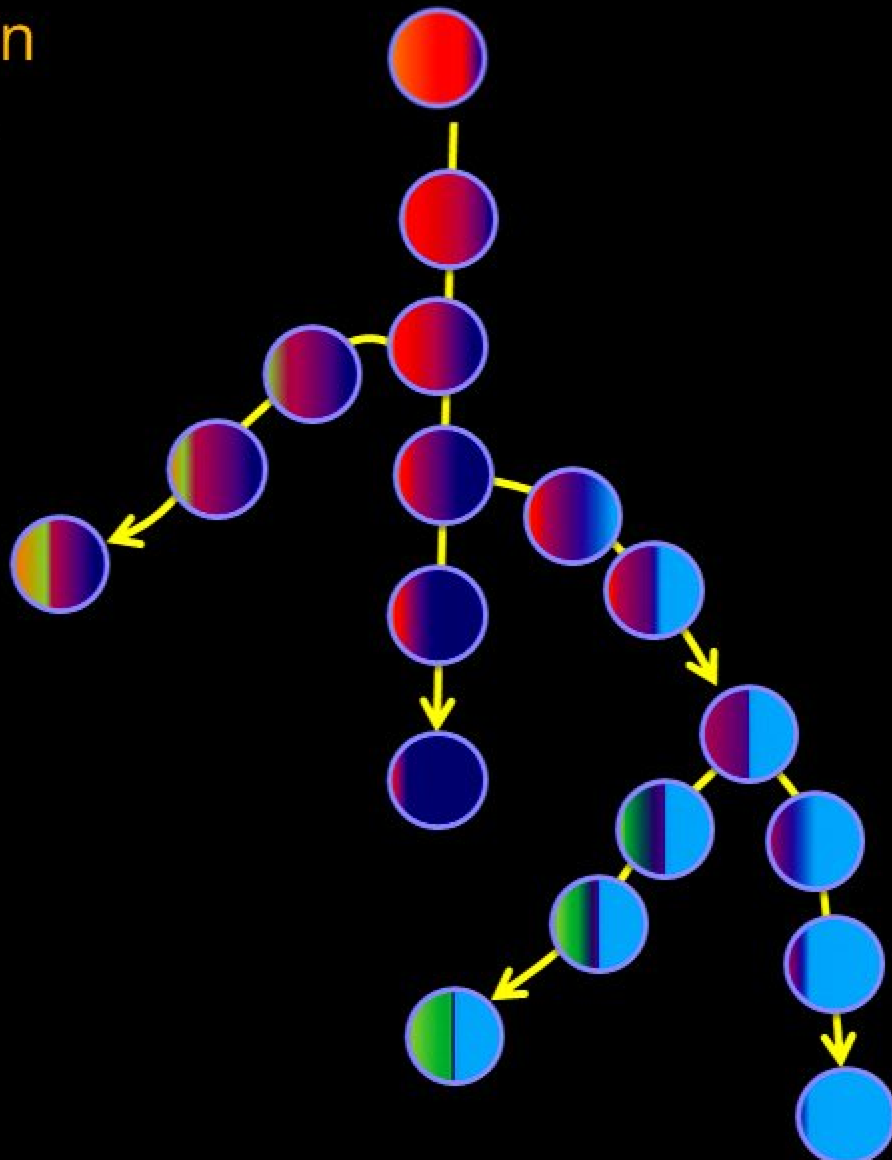

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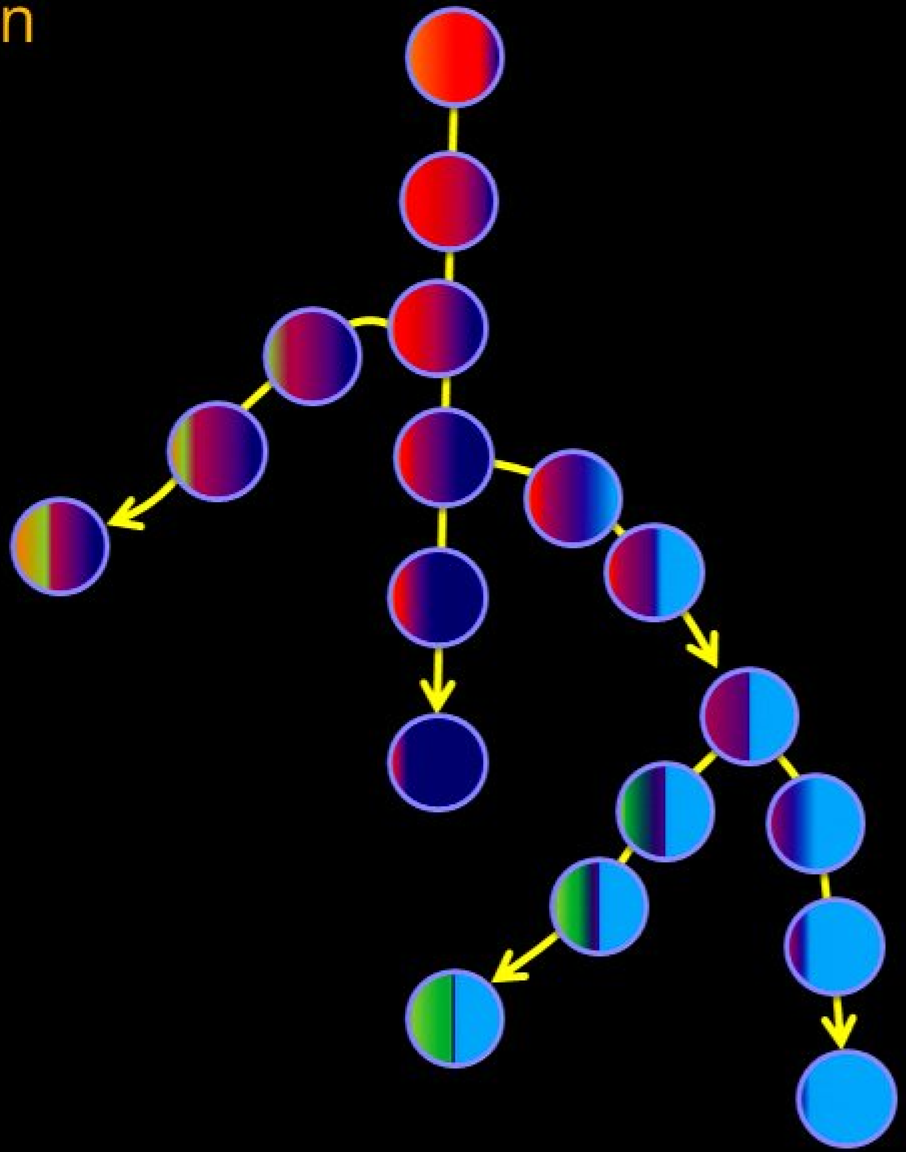
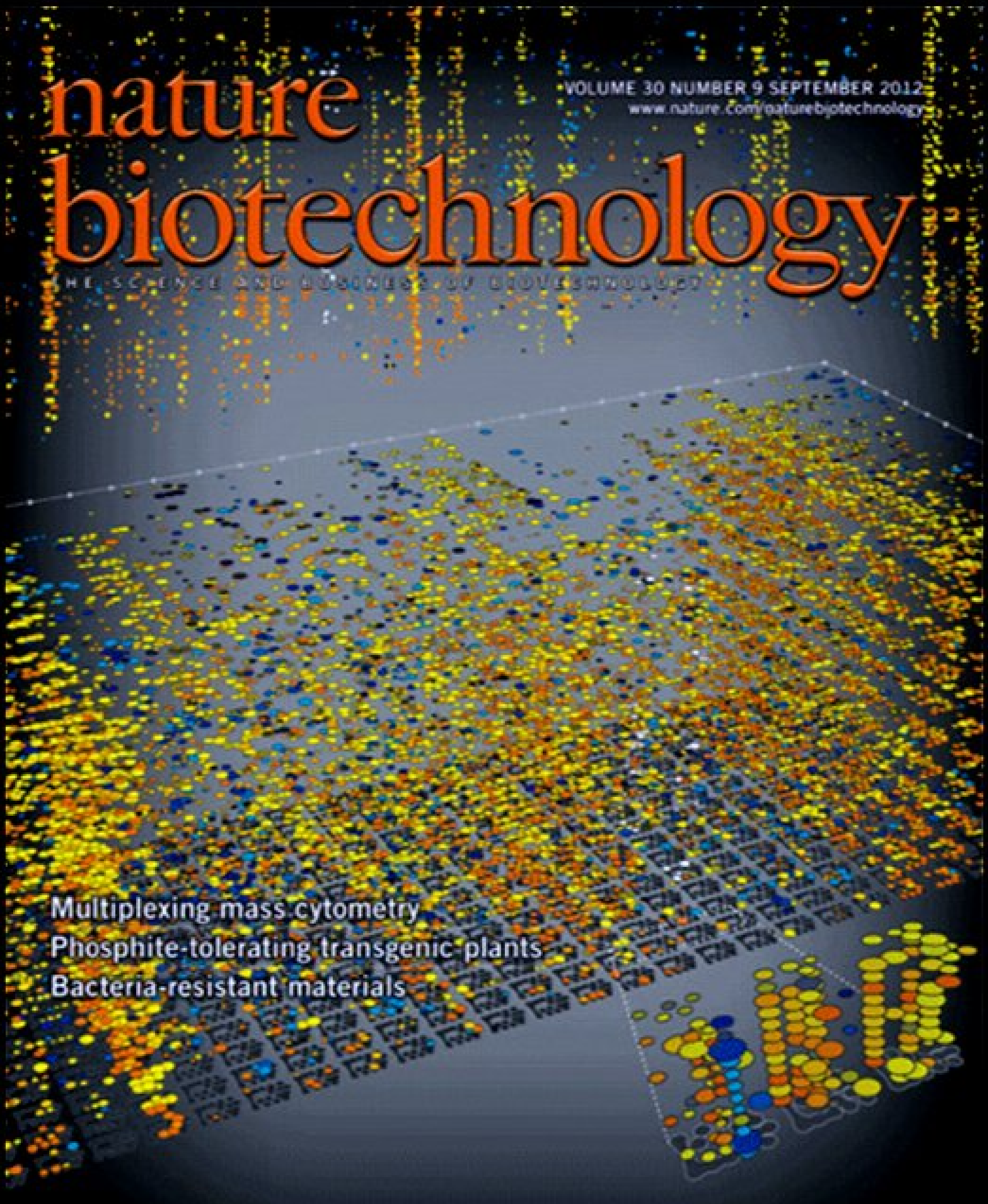
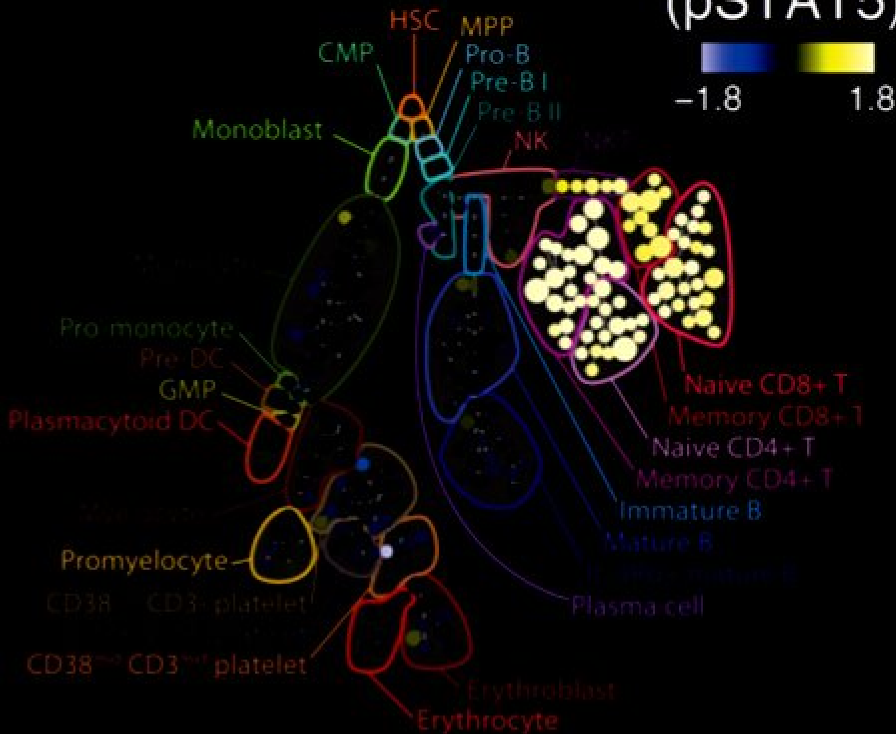


Bodenmiller et al
Nature Biotechnology, August, 2012



Single cell signaling on an immune system wide basis

IL-7
(pSTAT5)

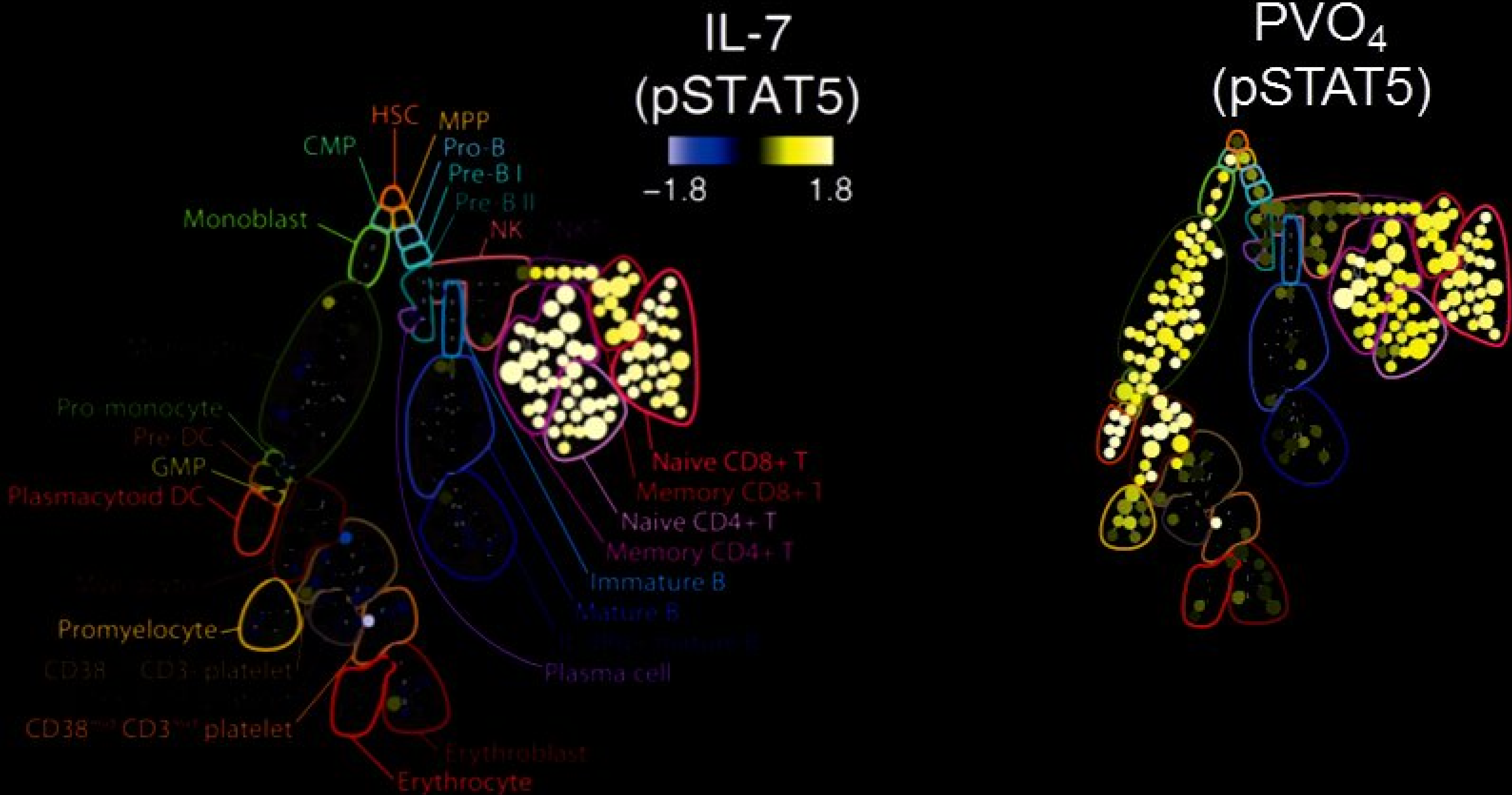


PVO_4
(pSTAT5)

+ Dasatinib
Abl / Src-family
kinase inhibitor

Bendall, Simonds, et al. *Science*, May 2011

Single cell signaling on an immune system wide basis



Bendall, Simonds, et al. *Science*, May 2011

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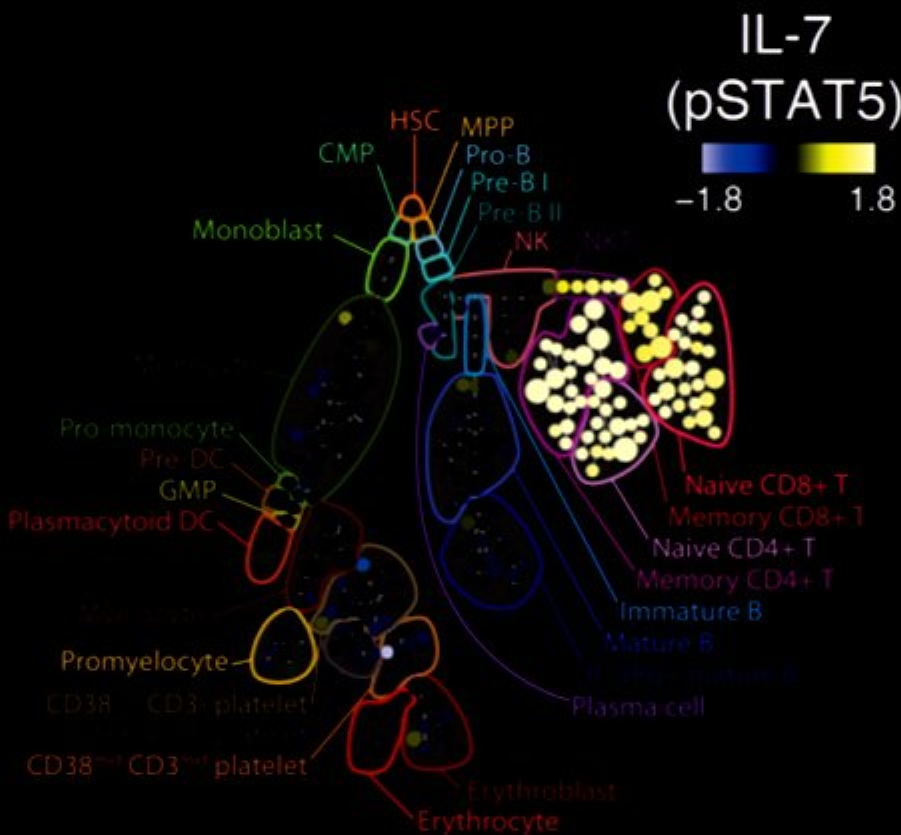

Bendall, Simonds, et al. *Science*, May 2011

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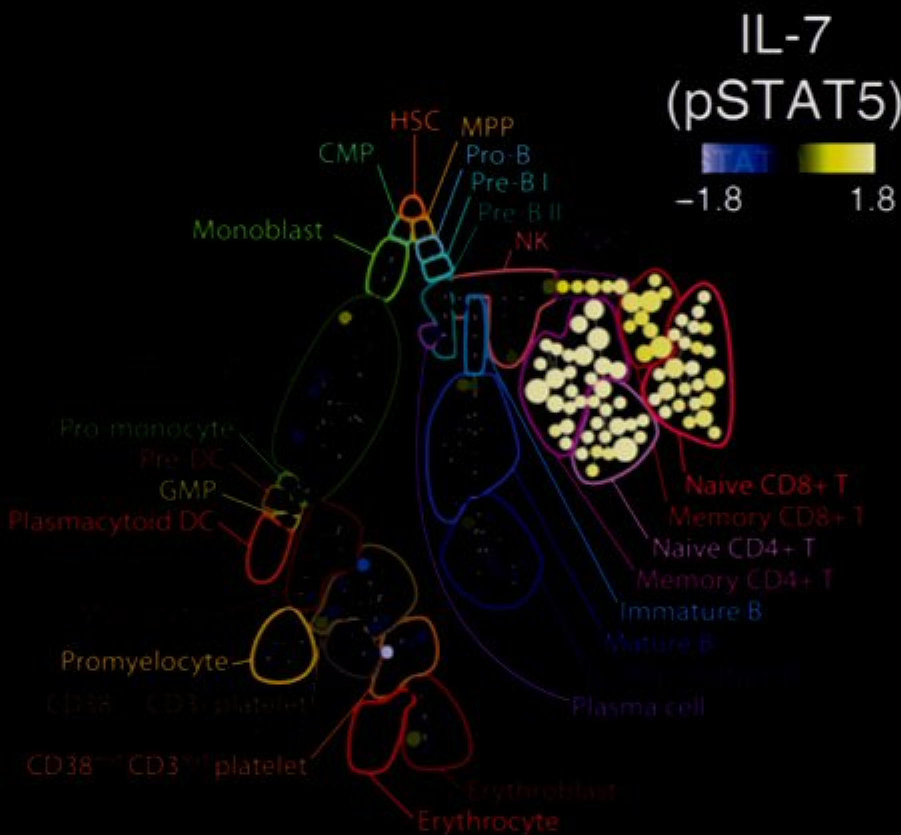

Bendall, Simonds, et al. *Science*, May 2011

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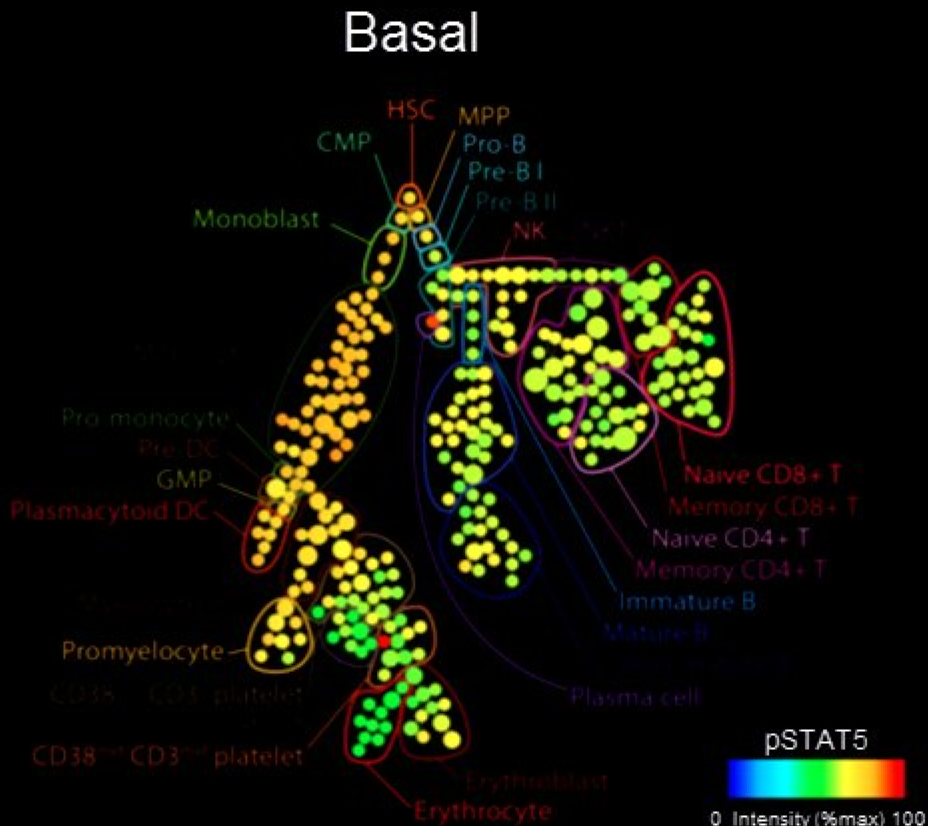
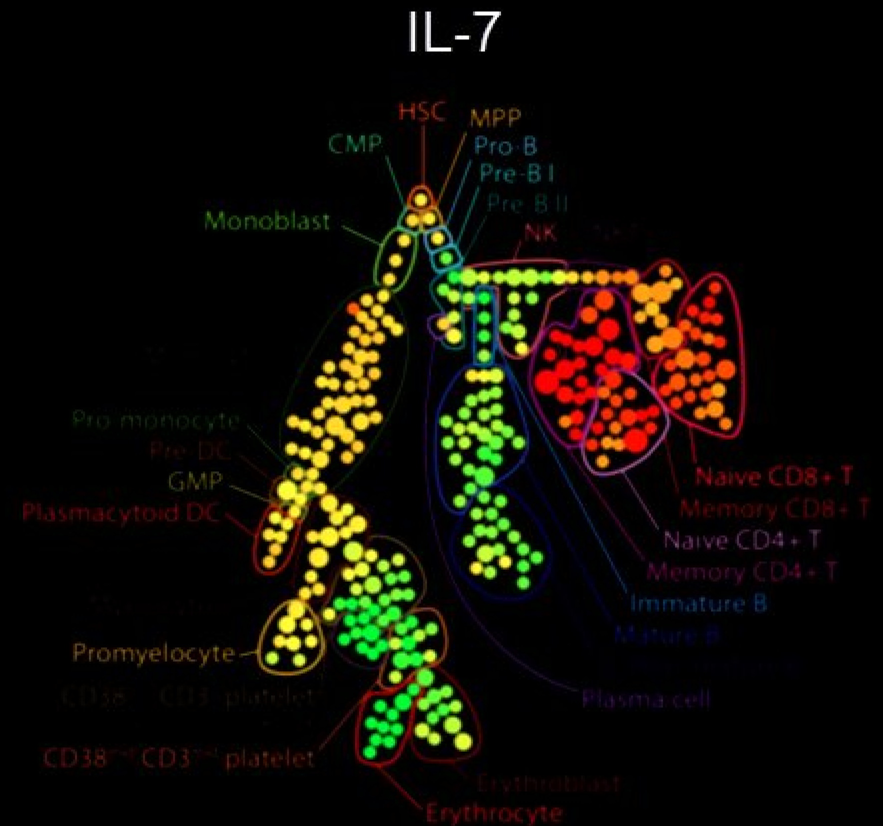
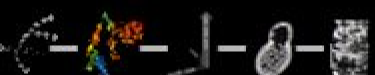


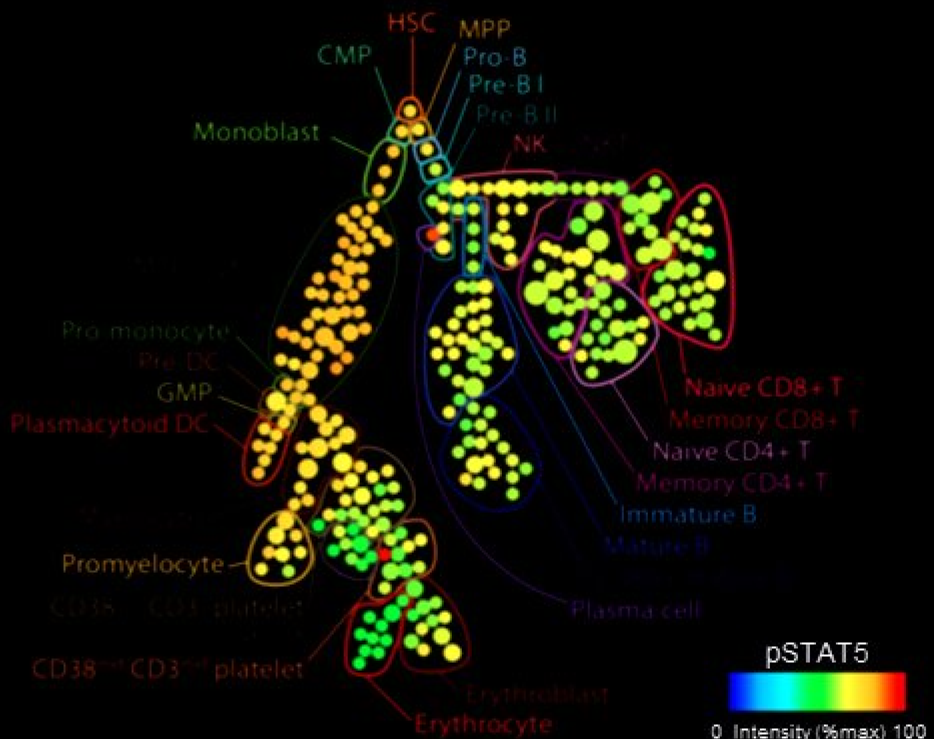




Bendall, Simonds, et al. *Science*, May 2011

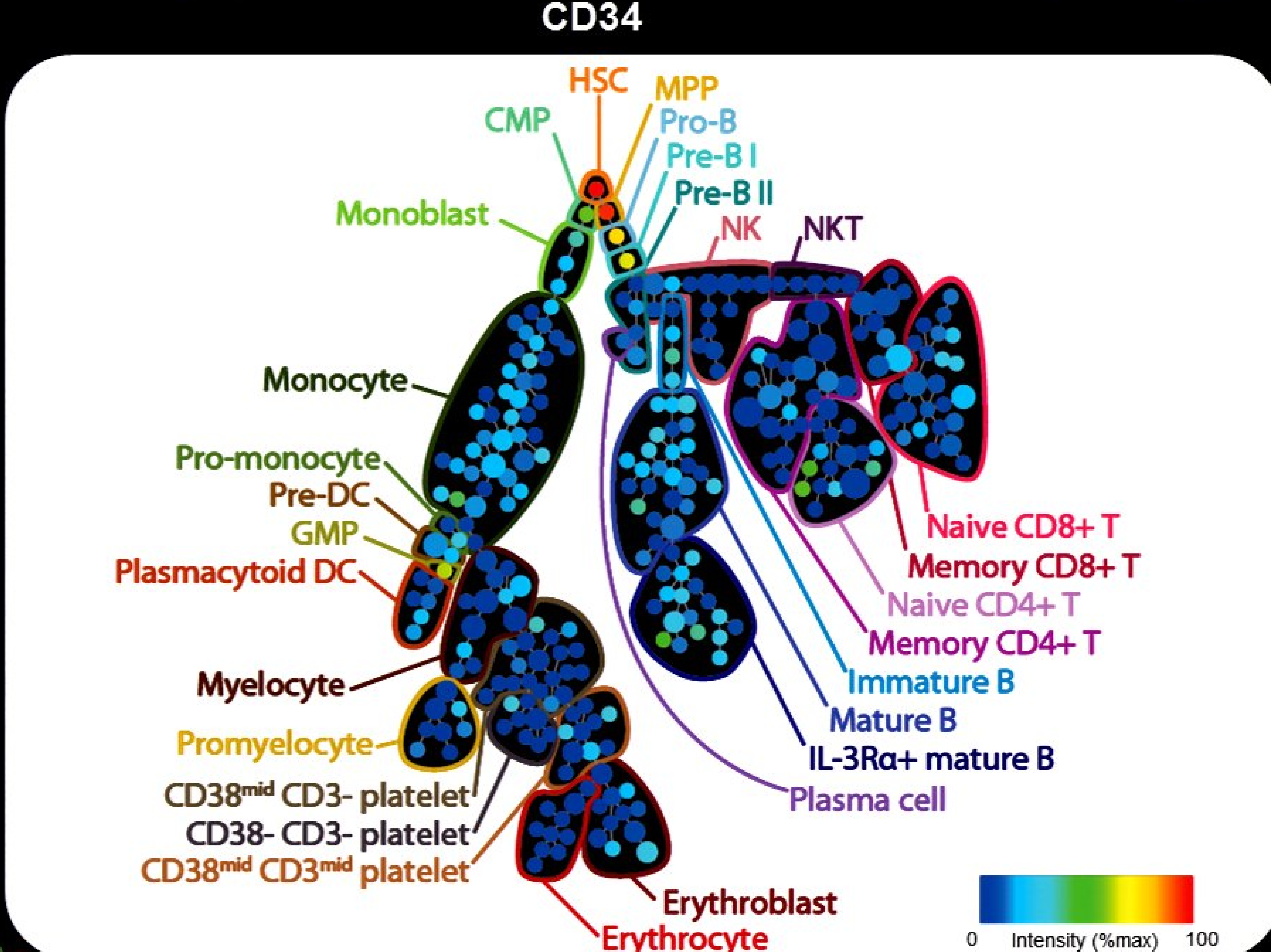
Single cell signaling on an immune system wide basis

Basal



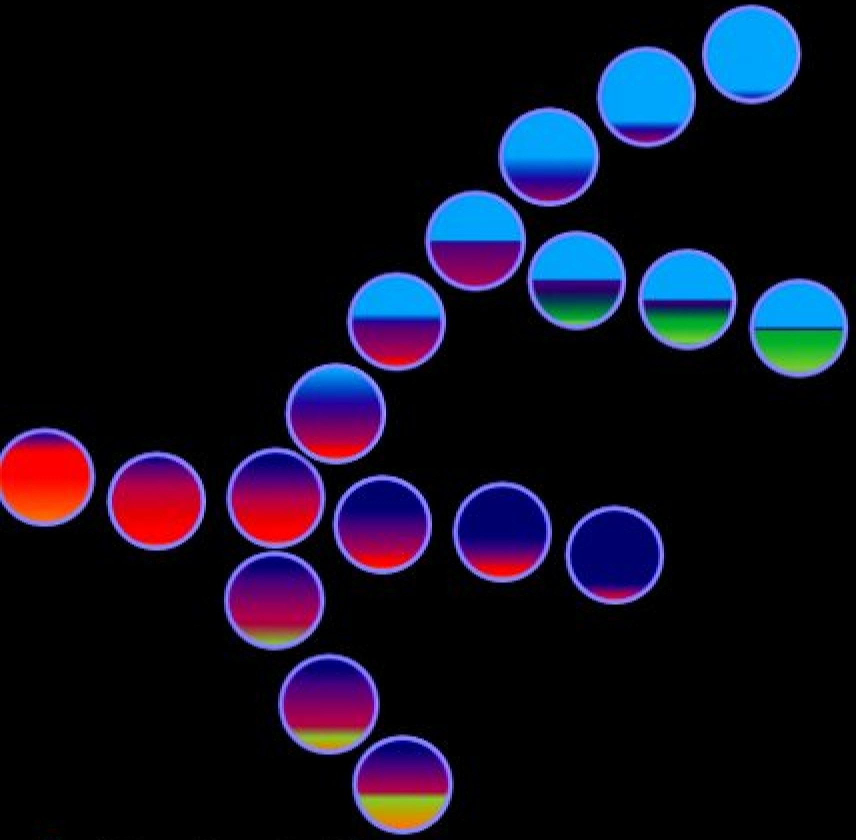
Bendall, Simonds, et al. *Science*, May 2011

SPADE projects bone marrow as a continuum of phenotypes



Bendall, Simonds, et al. *Science*, May 2011

Can we reconstruct the lineages by aligning "Like near Like"?



Qiu et al., *Nature Biotechnology*, Oct. 2011

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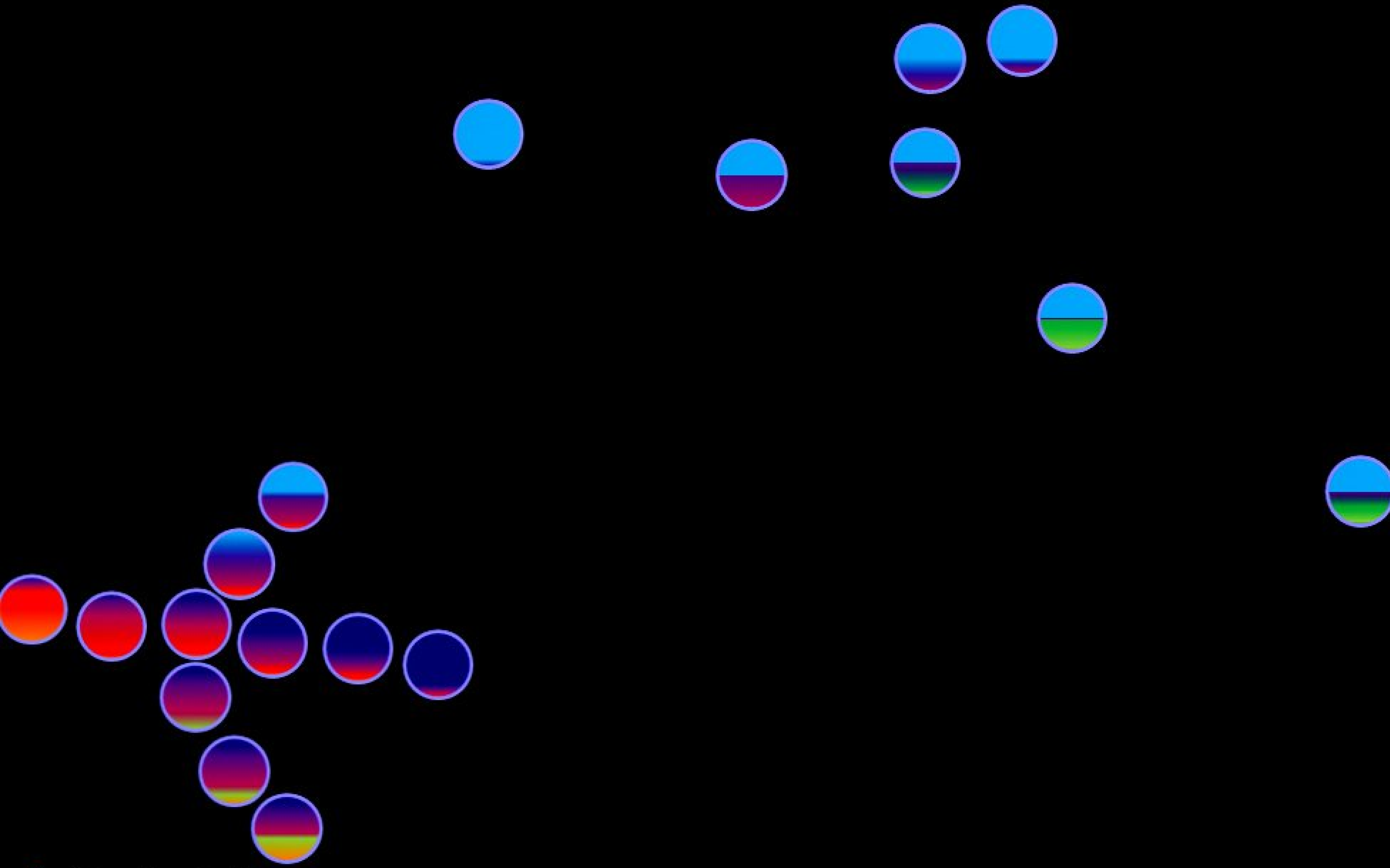

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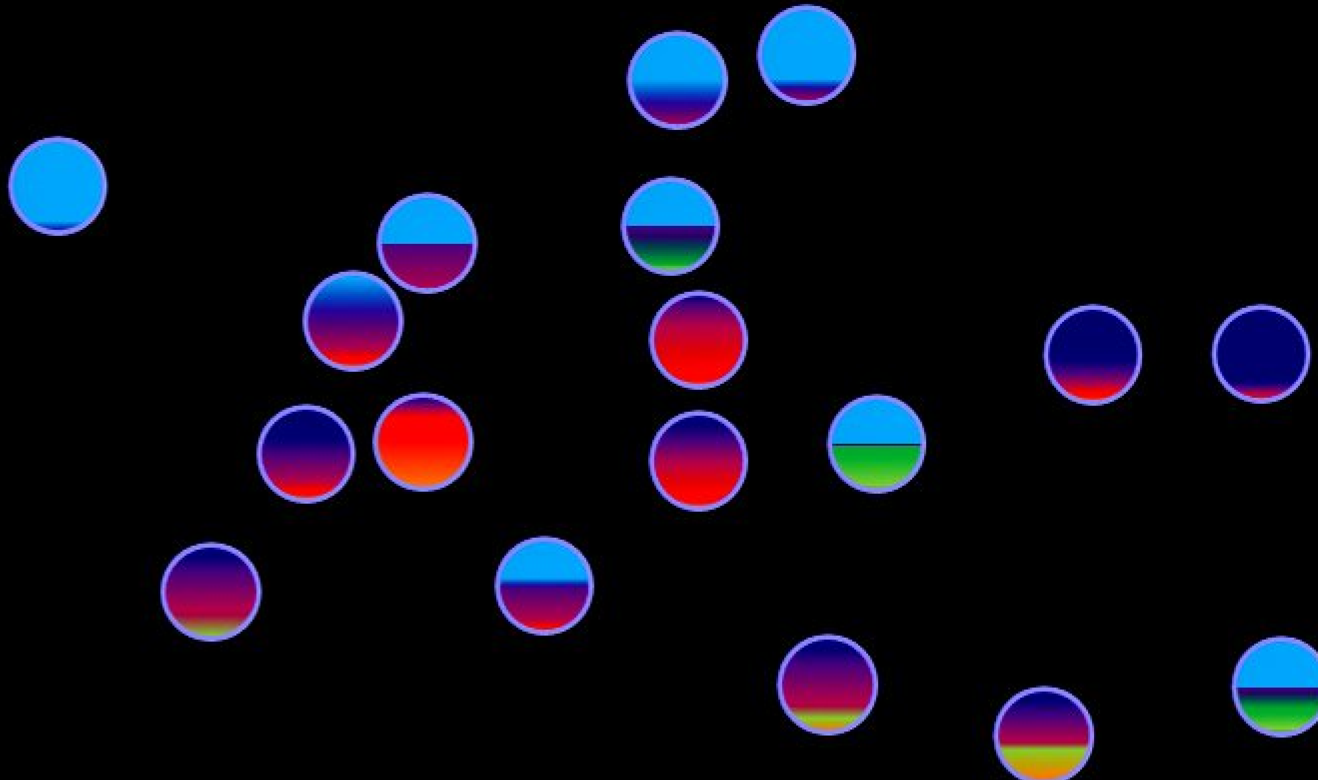
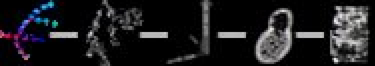
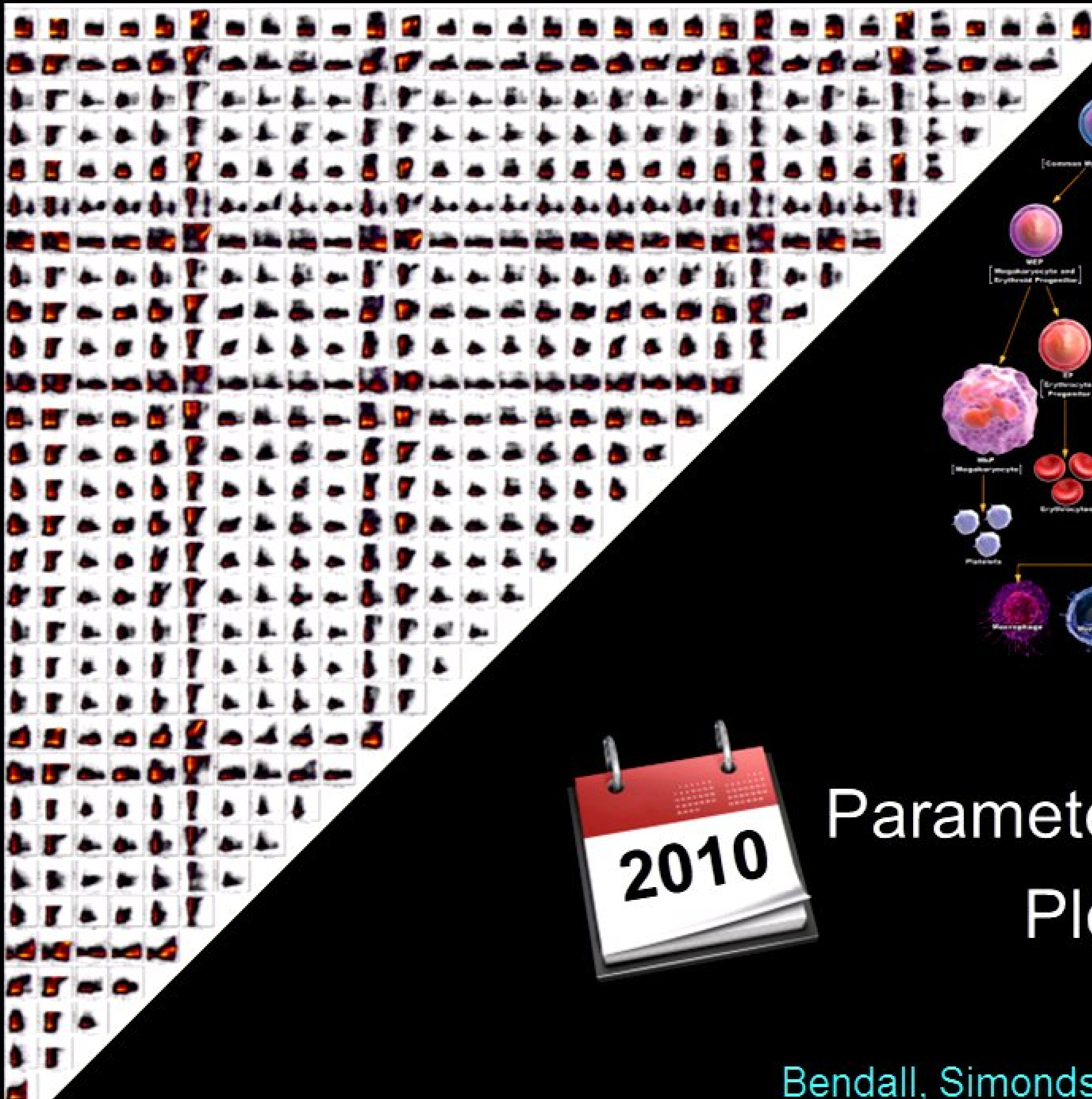
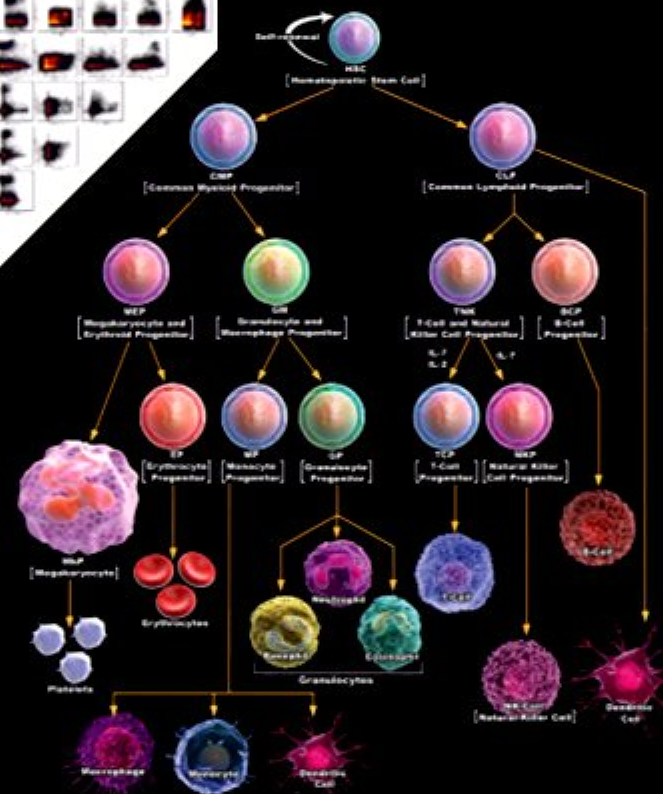




Qiu et al., *Nature Biotechnology*, Oct. 2011

Biaxial plots are not a scalable solution





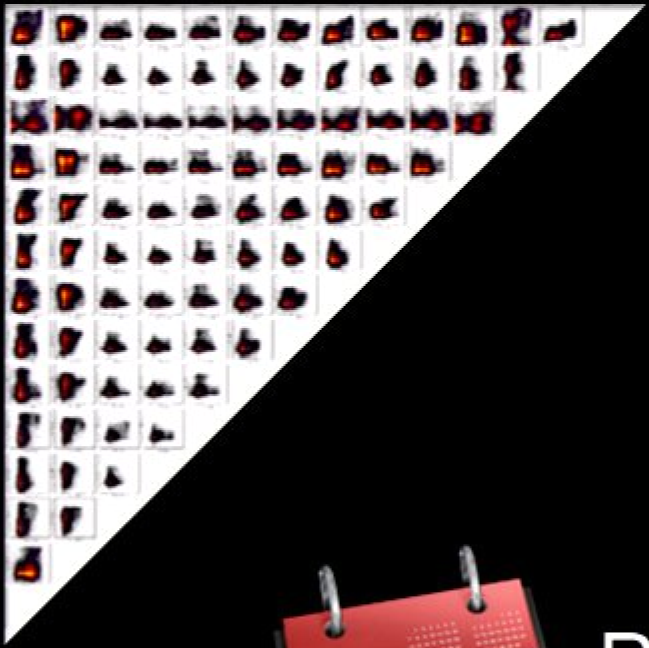
2010

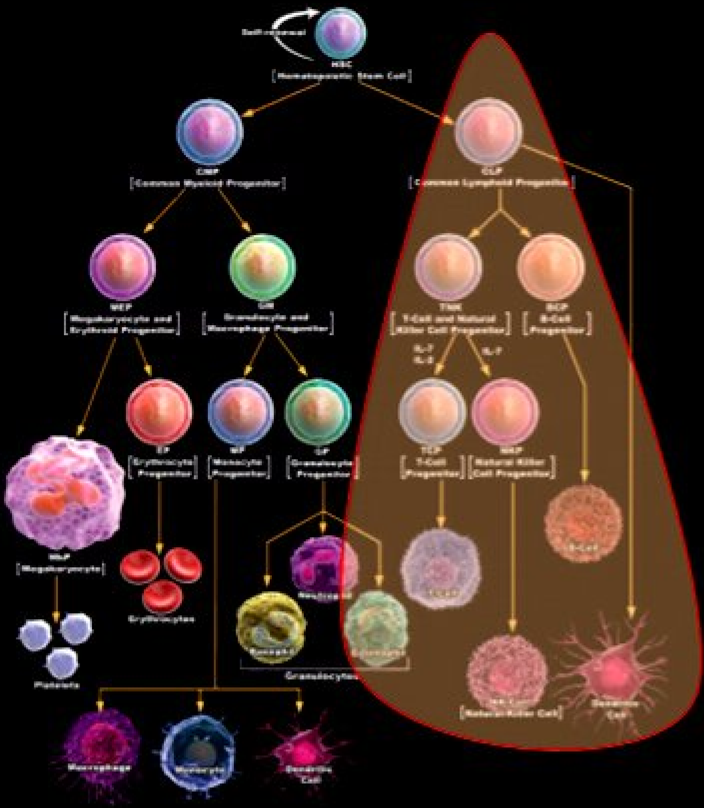
Parameters: 32

Plots: 496

Bendall, Simonds, et al. *Science*, May 2011

Biaxial plots are not a scalable solution



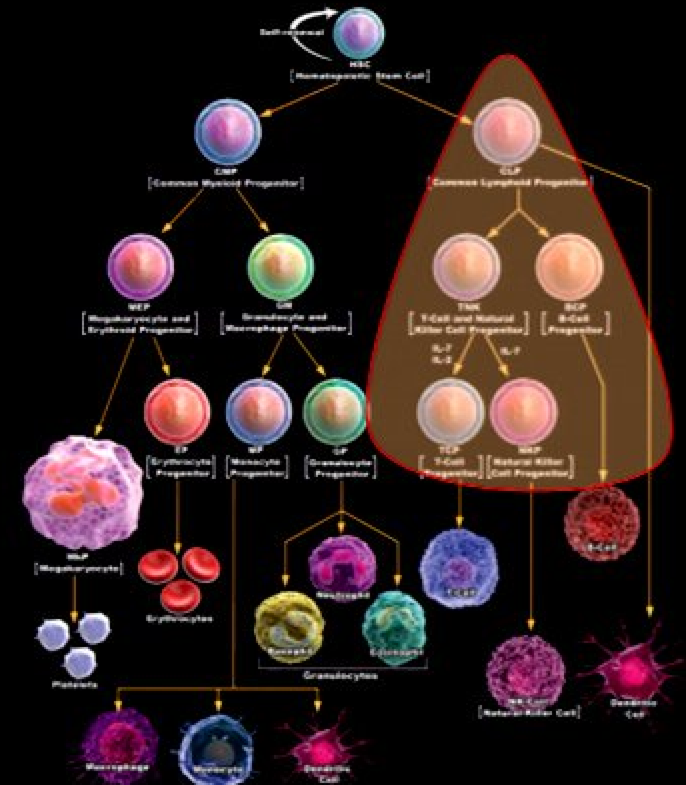


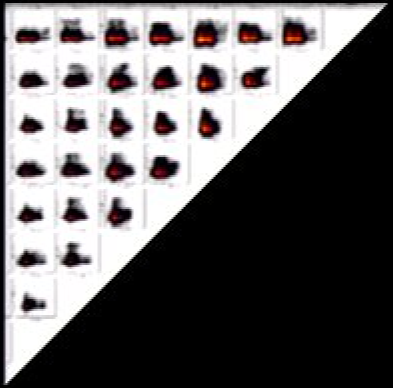
Parameters: 14

Plots: 91

Bendall, Simonds, et al. *Science*, May 2011

Biaxial plots are not a scalable solution





Parameters: 8

Plots: 28

Bendall, Simonds, et al. *Science*, May 2011

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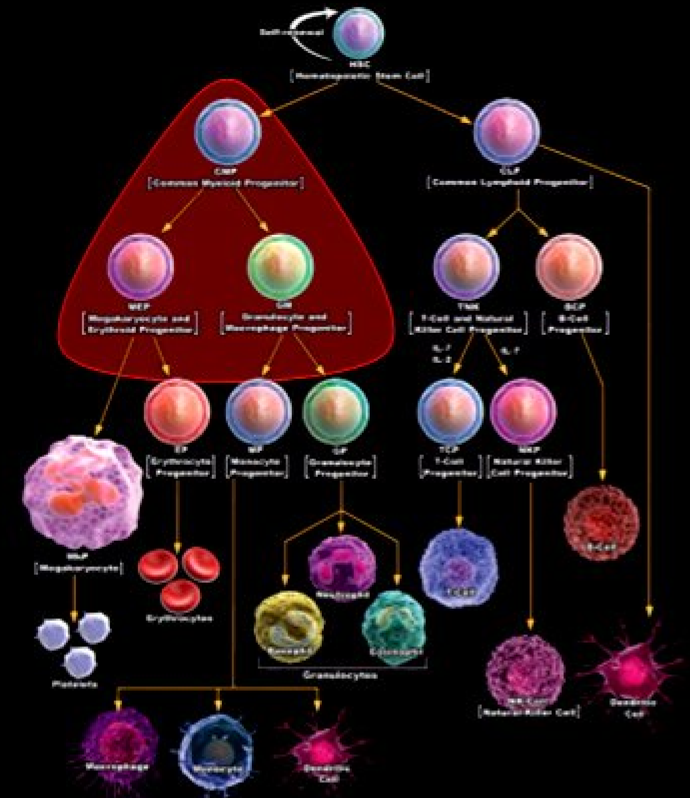
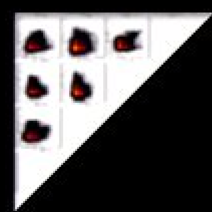




Parameters: 4

Plots: 6

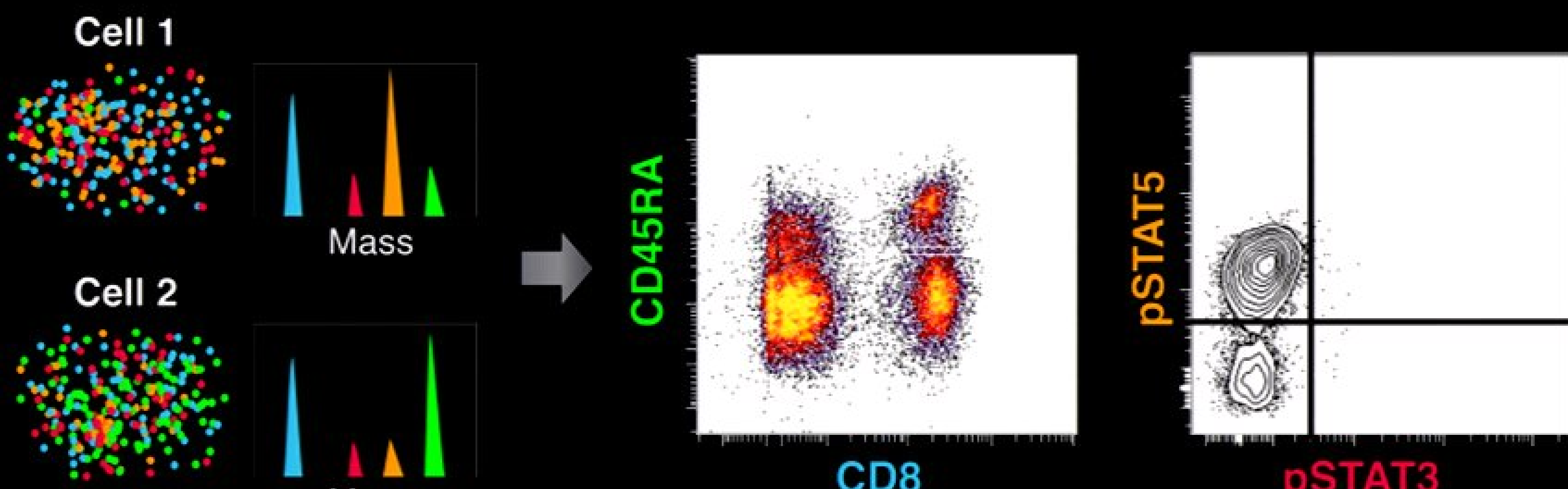
Bendall, Simonds, et al. *Science*, May 2011

CyTOF Phenotyping & Mechanism Panels

- >100 Human surface:
- > 60 Murine surface:
- > 20 murine & human cytokines

- ~ 100 intracellular mechanism targets for panels
  - **'total' protein**
  - **Phospho epitopes- kinases and targets of kinases**
  - **Apoptosis: Bad, Bax, Bcl2, Bid, Akt, CytC, caspases**
  - **Epigenetic panels: pH3, pH2AX,**
  - **DNA Damage: pATM, pRb, p53, p16, p21, ATR, pChk1, 2, etc.**
  - **Stem cell: Sox2, c-Myc, nanog, Gata, surface markers**
  - **Cell cycle: CyclinA/B/D1,2,3/E, Rb, Ki67,**
  - **Wnt, Bmp pathways panels**

Mass cytometry enables single-cell phosphoproteomics



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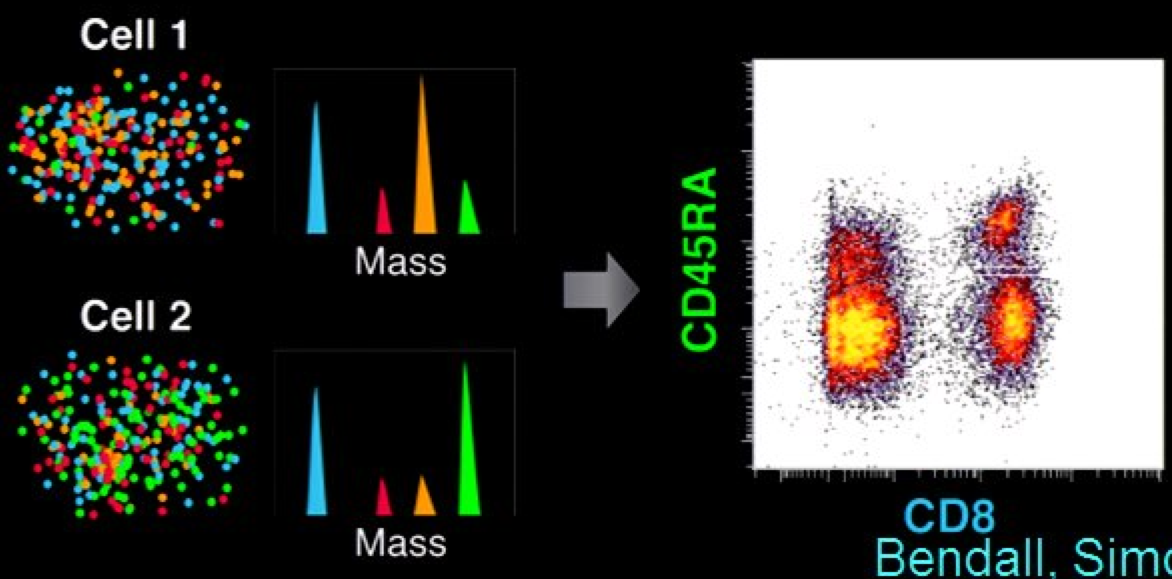

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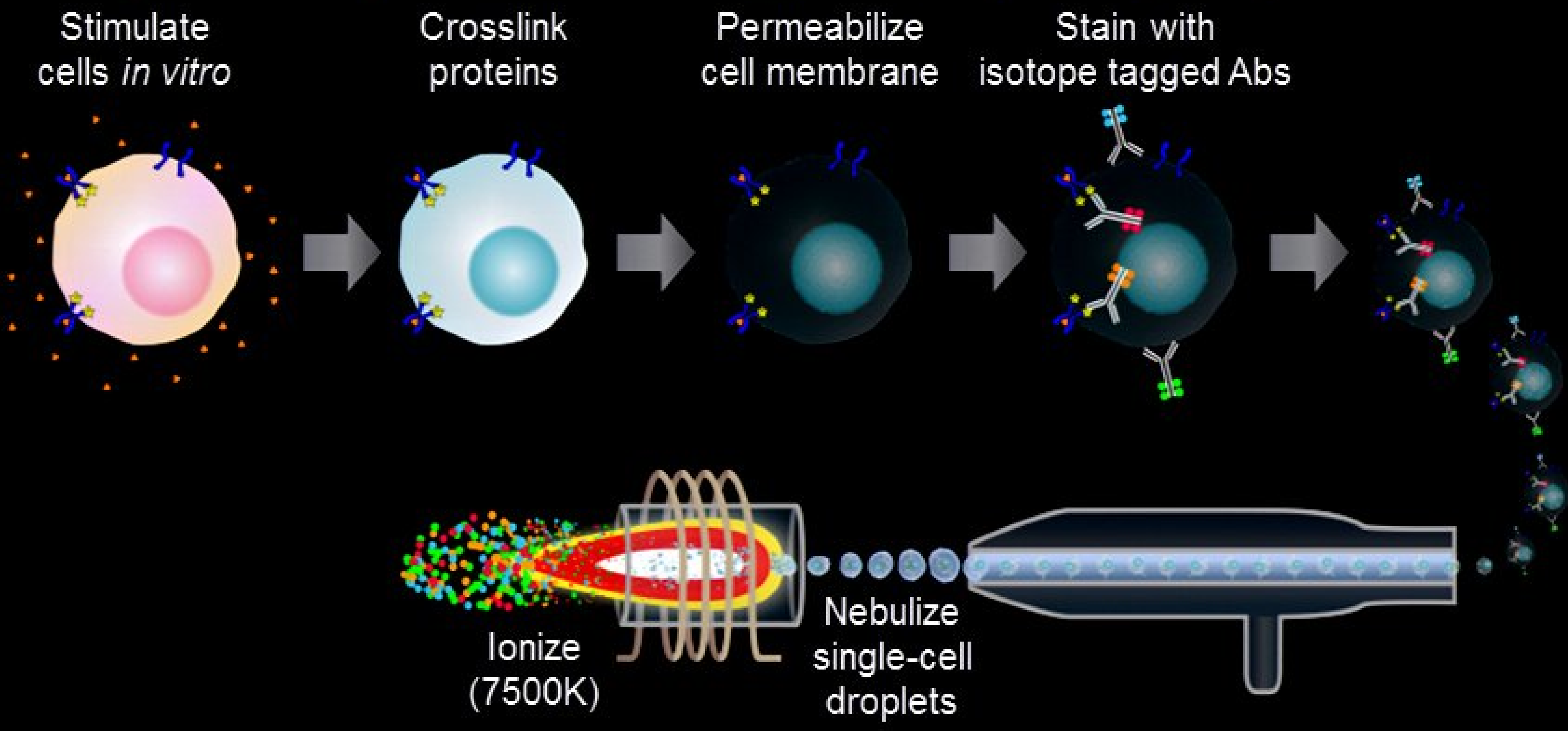

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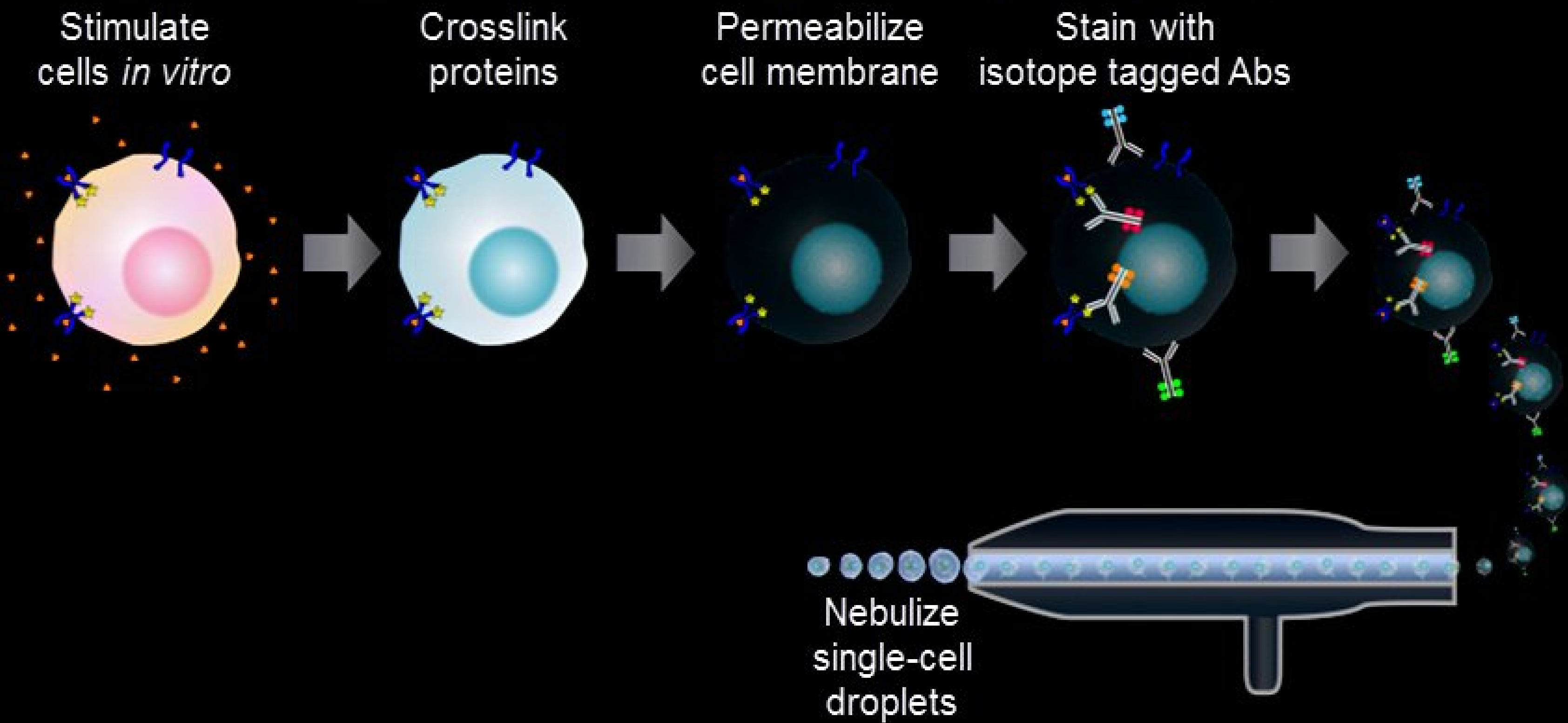

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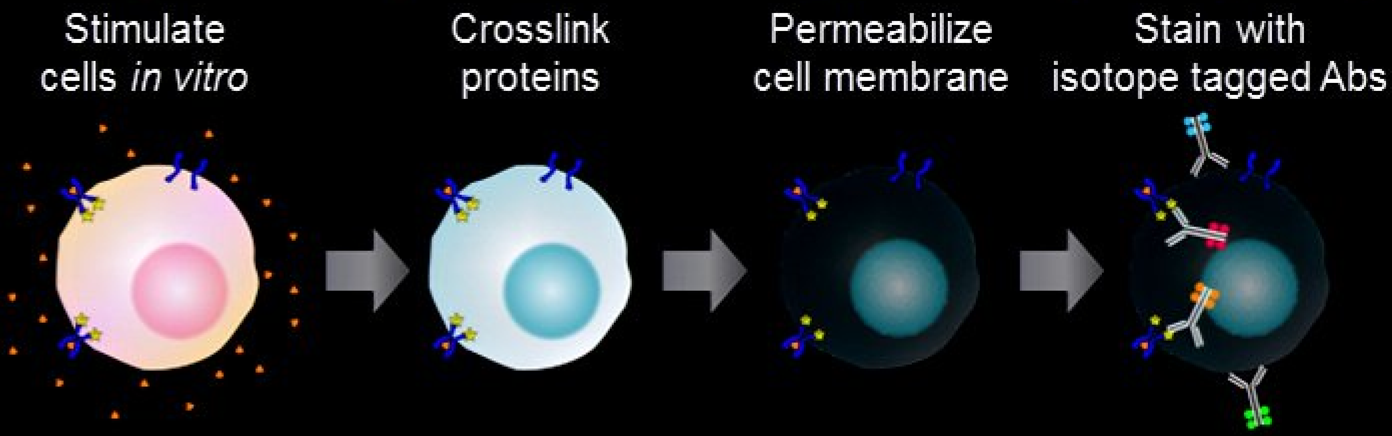

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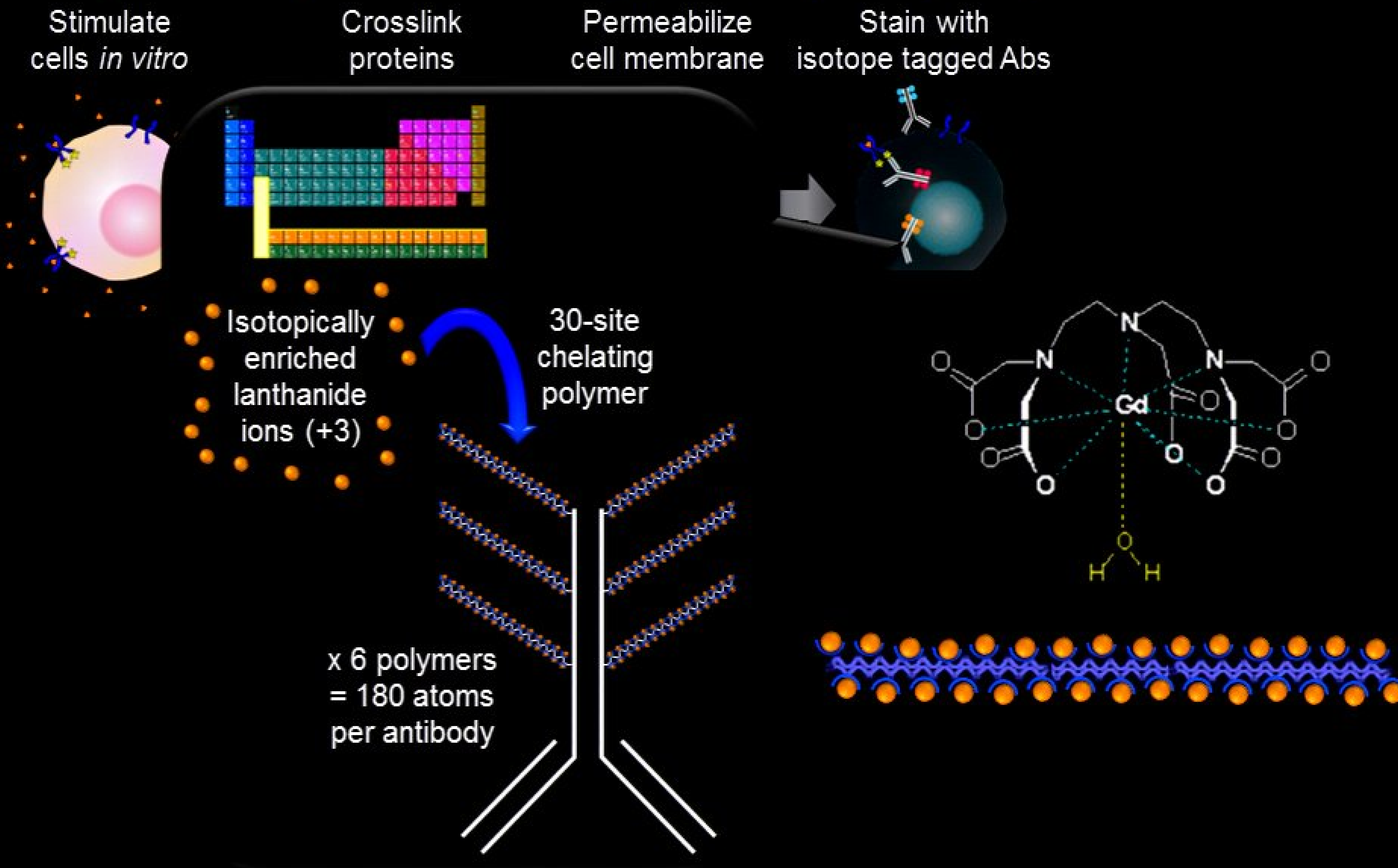

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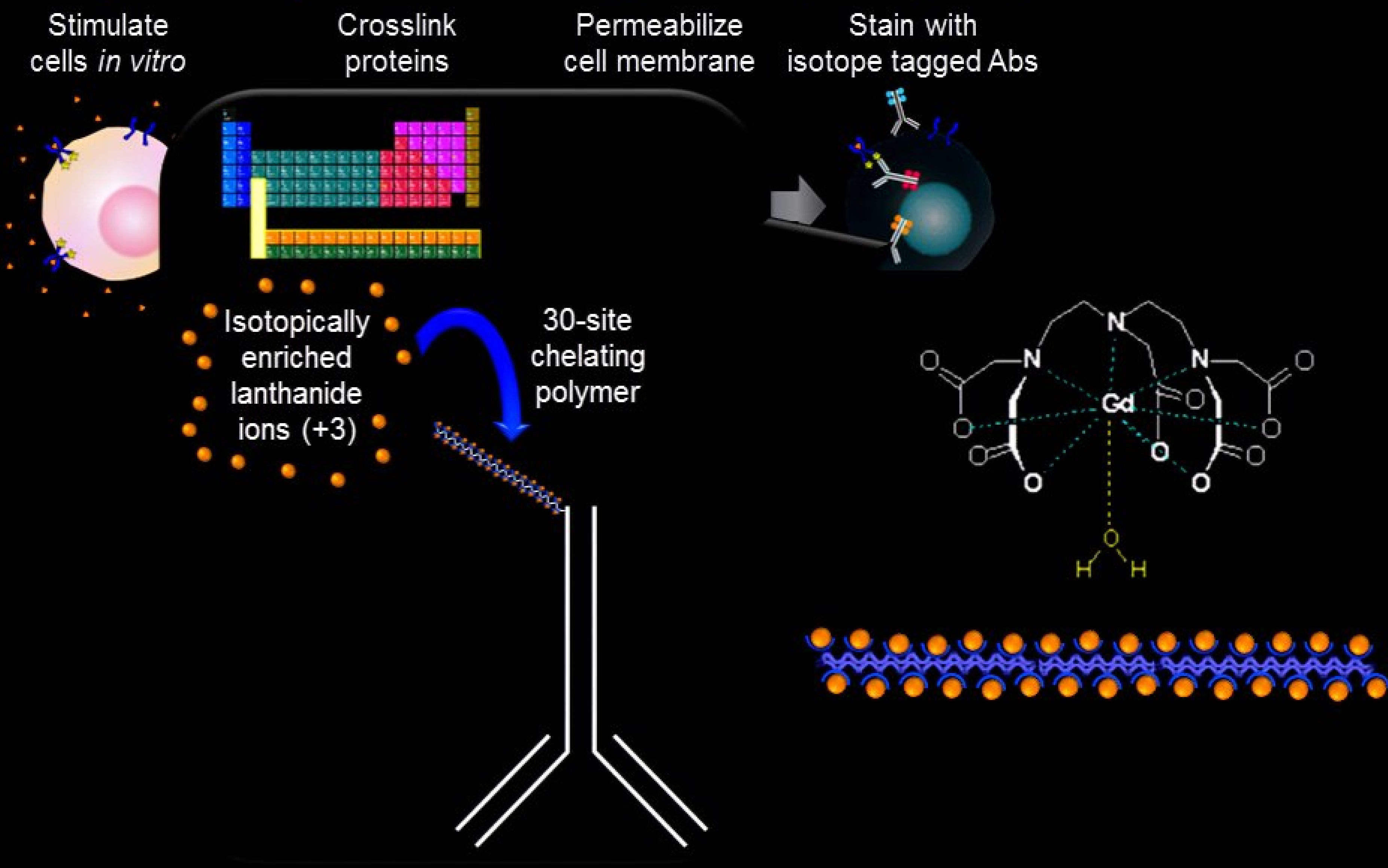

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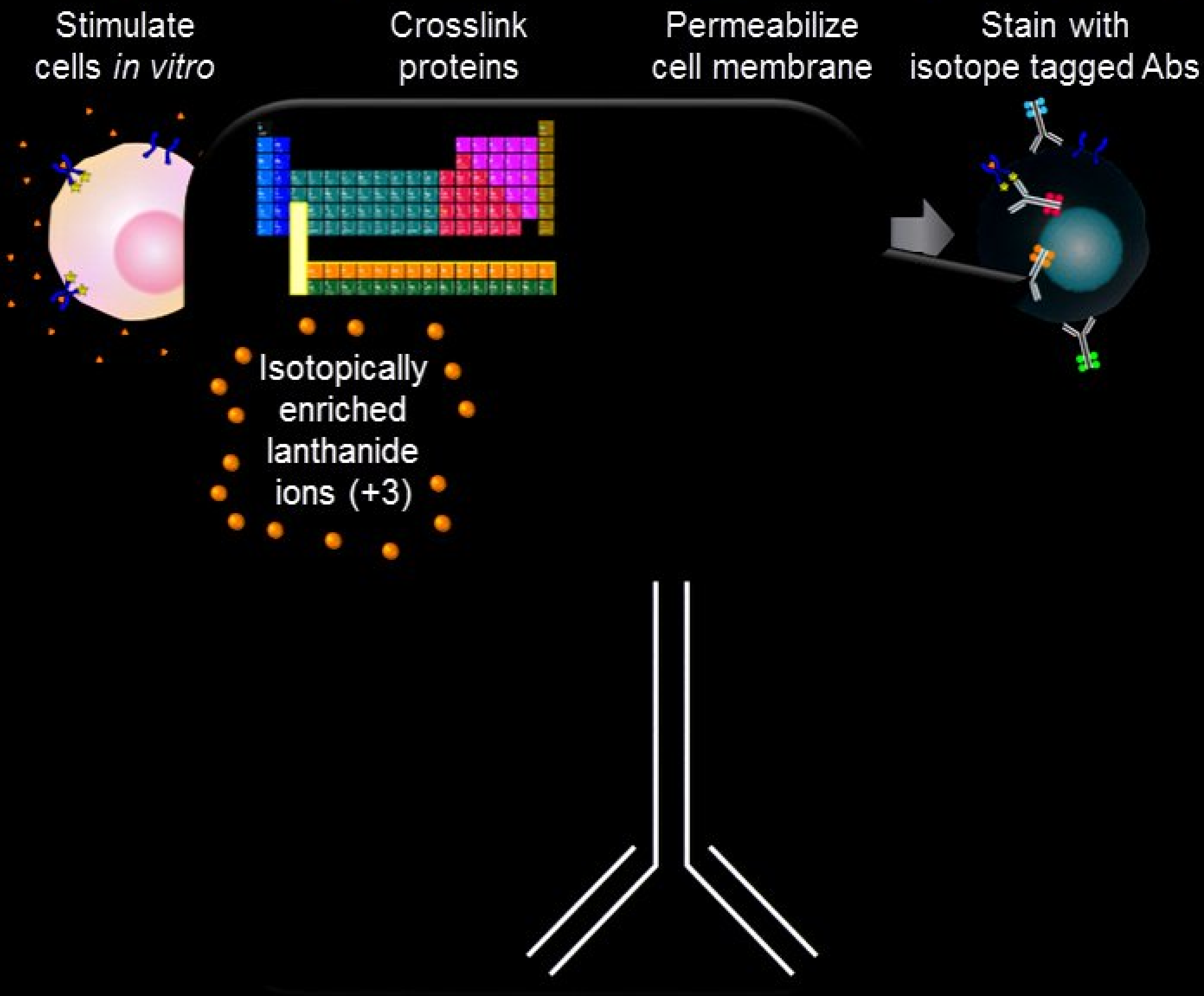

Bendall, Simonds, et al. *Science*, May 2011

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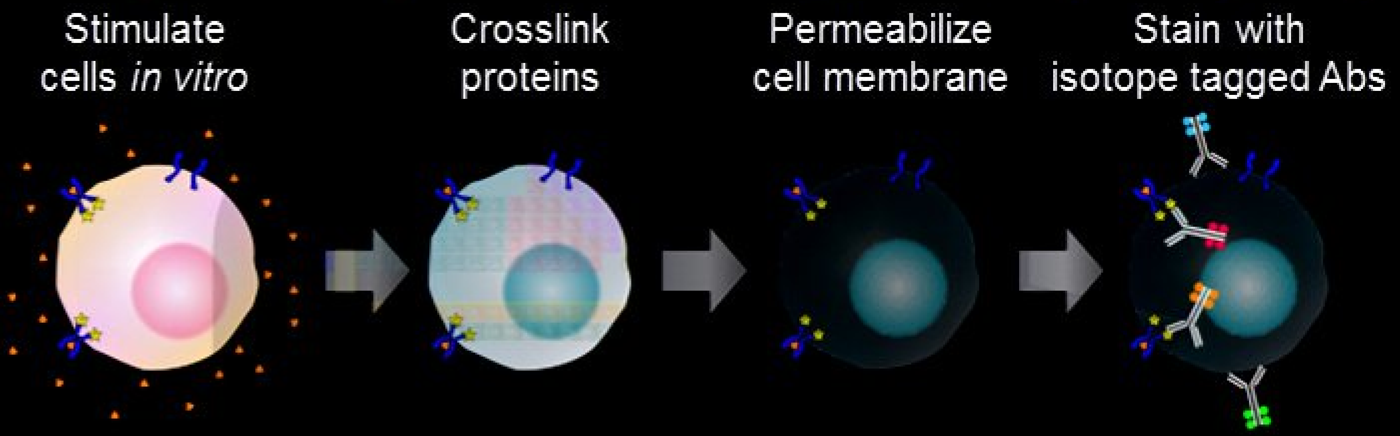

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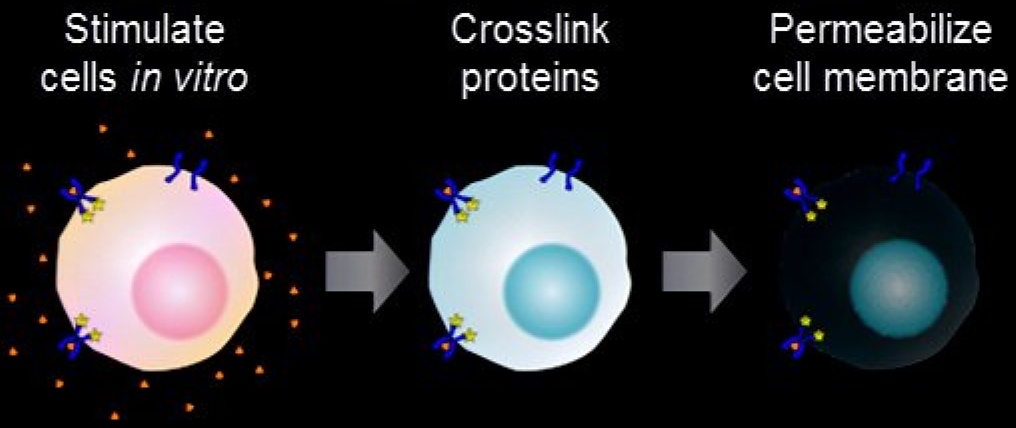

Bendall, Simonds, et al. *Science*, May 2011

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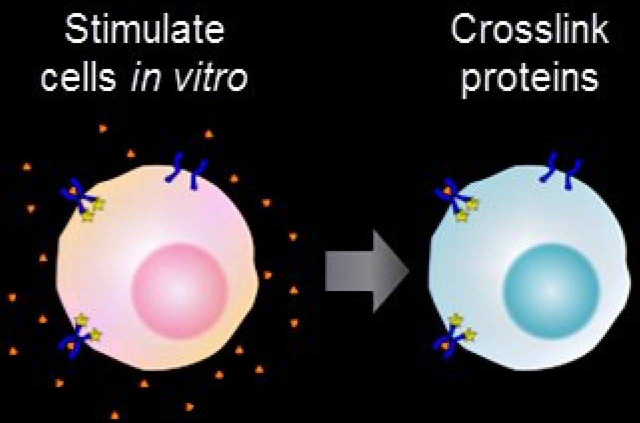
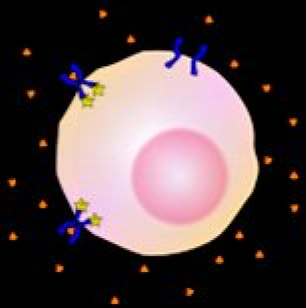


Bendall, Simonds, et al. *Science*, May 2011

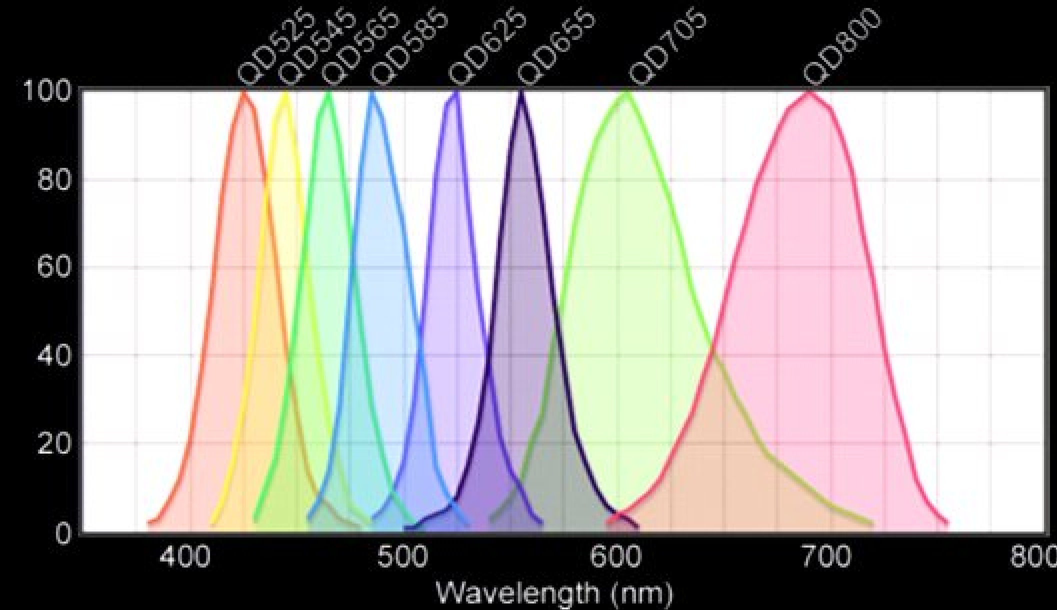
Mass cytometry enables single-cell phosphoproteomics

Stimulate
cells *in vitro*



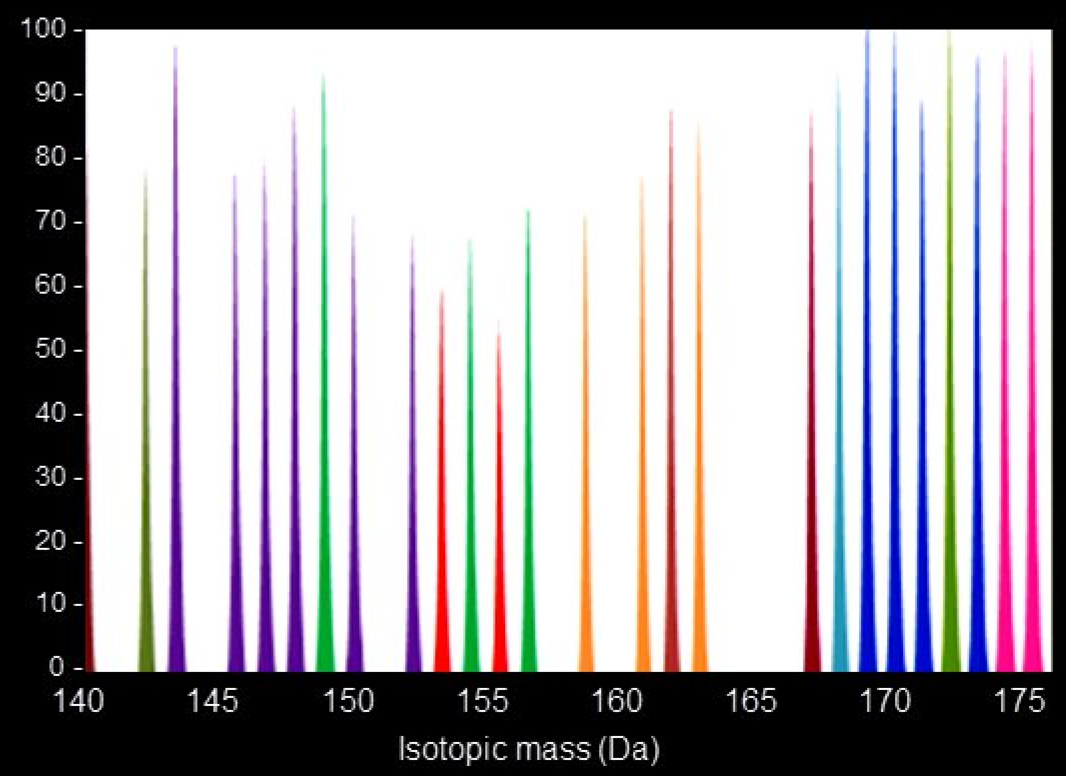
Bendall, Simonds, et al. *Science*, May 2011

The fluorescence spectrum is crowded



Fluorescent cytometry

- Up to 12 colors can be “routine”
- 17 colors have been reported
- 17? Forgedda bowdid…
- High background



Mass cytometry

- Up to 100 non-biological elemental mass channels
- No compensation required
- No autofluorescence

The fluorescence spectrum is crowded

Fluorescent cytometry

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- 17 colors have been reported
- 17? Forgedda bowdid…
- High background