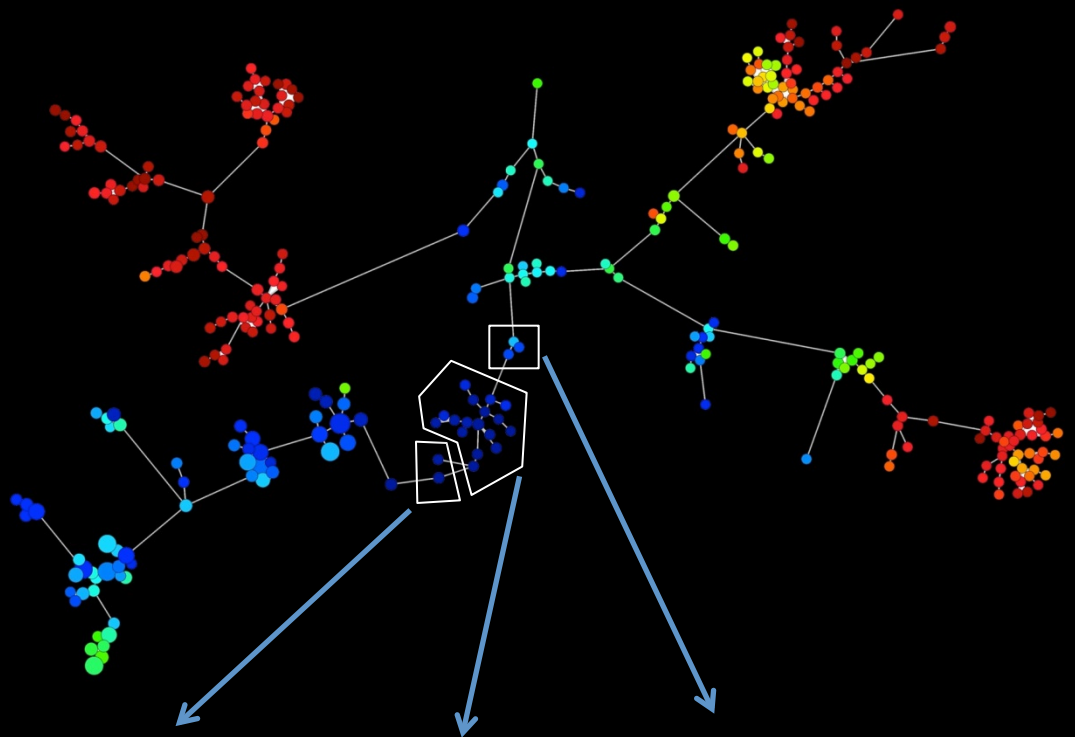
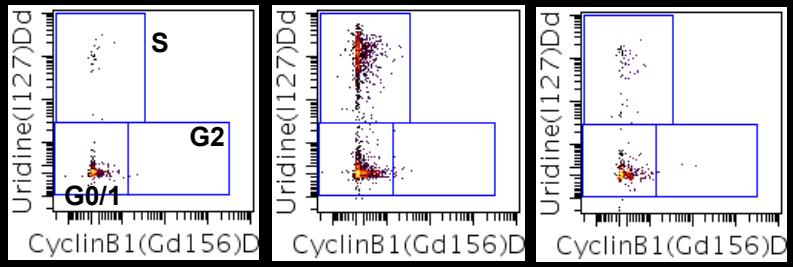
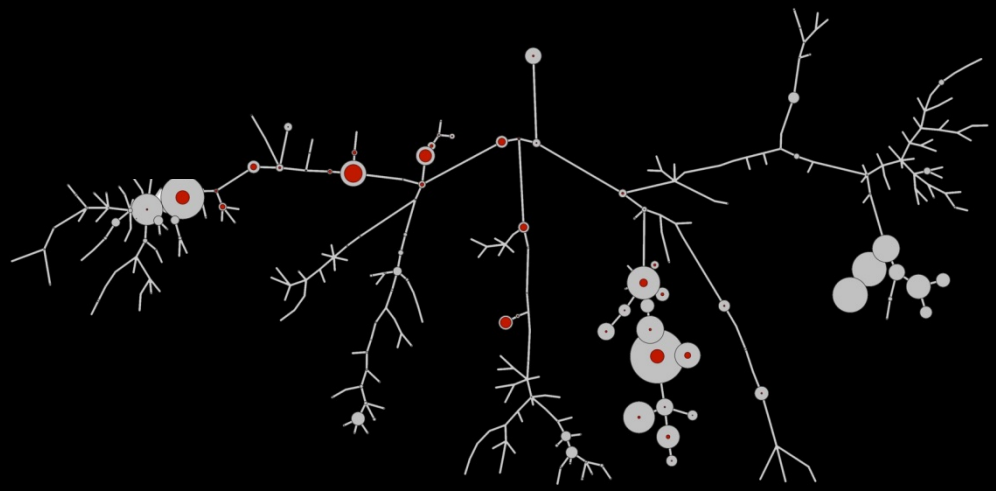
Single-cell mass cytometry adapted to measurements of the cell cycle







www.isac-net.org July 2012 | Volume 81A | Issue 7

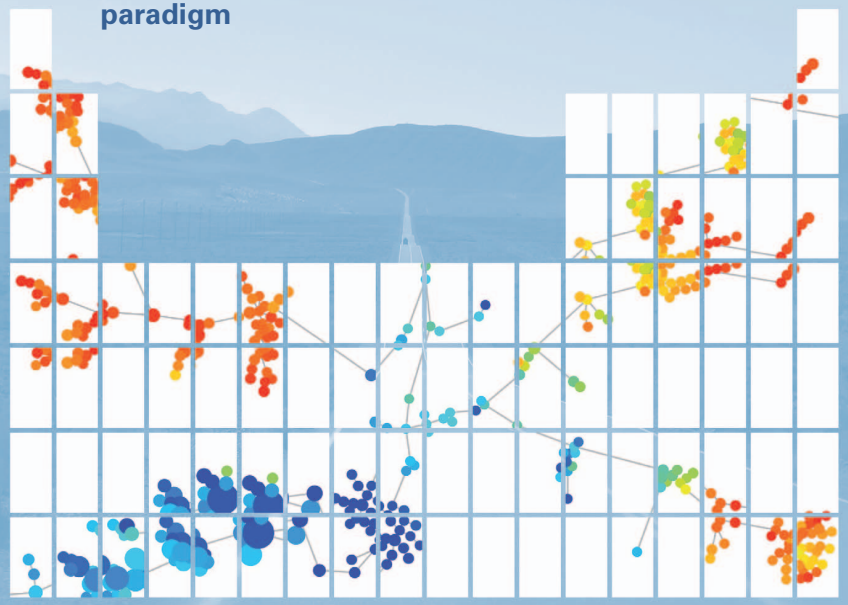
Cytometry
PART A

Journal of the International Society for Advancement of Cytometry

Illuminate: a single light source

Ultimate goal: quantification

Mass cytometry: creating a new paradigm



57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Re	69 Tm	70 Yb	71 Lu
89 Ac	90 Th	91 Ph	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

ISAC International Society for Advancement of Cytometry
WILEY-BLACKWELL
ISSN 1552-4922

Cell cycle analysis by mass cytometry

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  - 35 parameter analysis of normal bone marrow cell cycle
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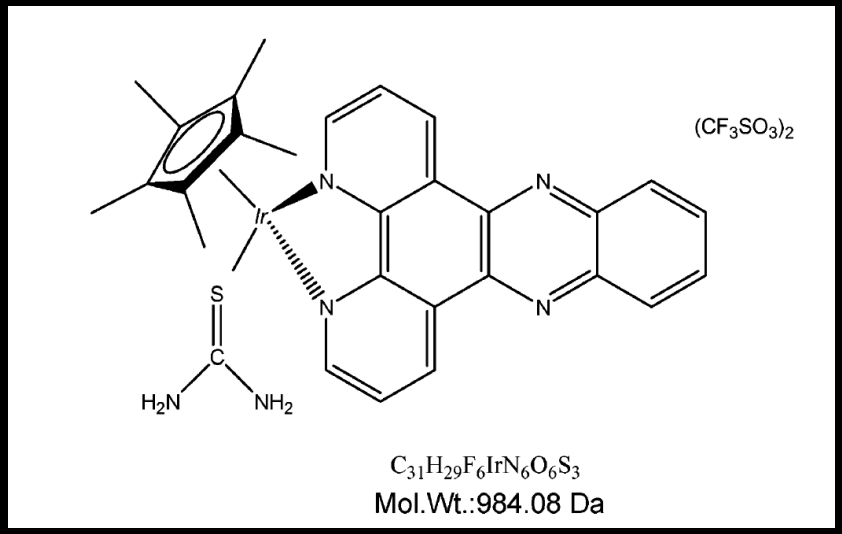
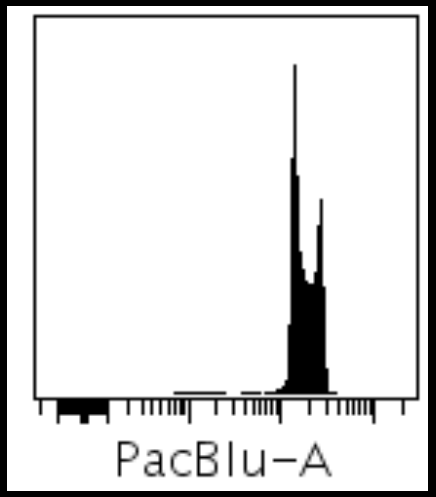
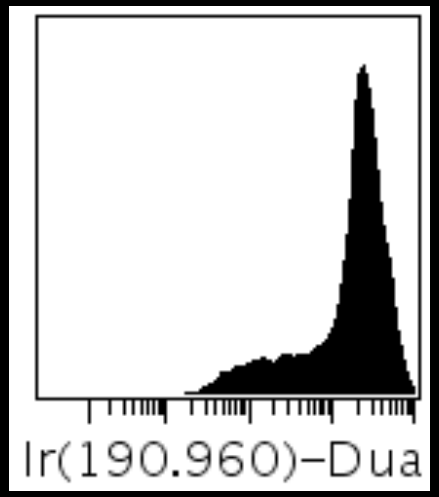
Mass cytometry DNA staining



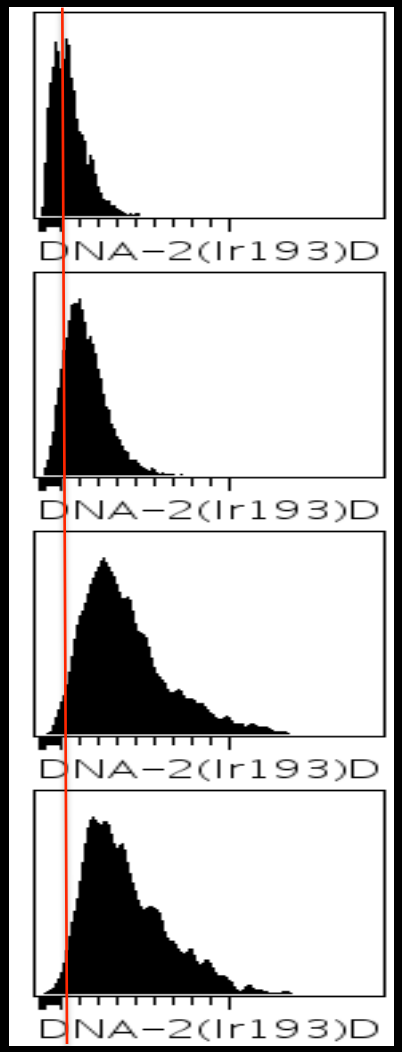
(pentamethylcyclopentadienyl)-Ir(III)-dipyridophenazine



Hoechst



Ir Intercalator



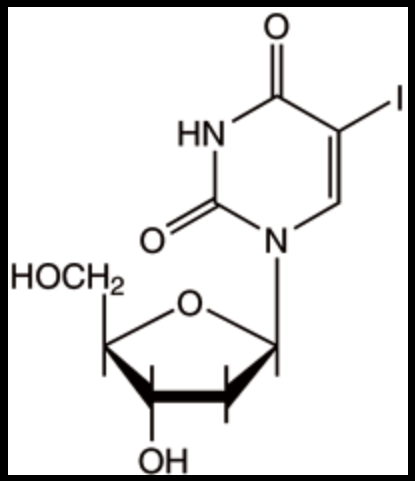
G0

G1

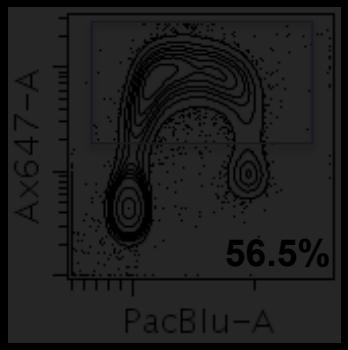
S

G2

IdU incorporates rapidly into S-phase cells



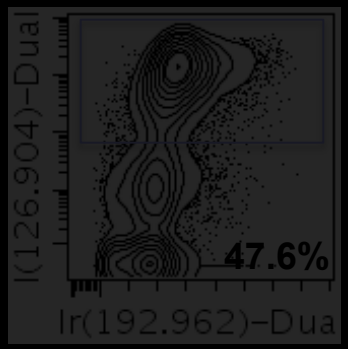
Fluorescent Cytometry



Uridine

Hoechst

Mass Cytometry



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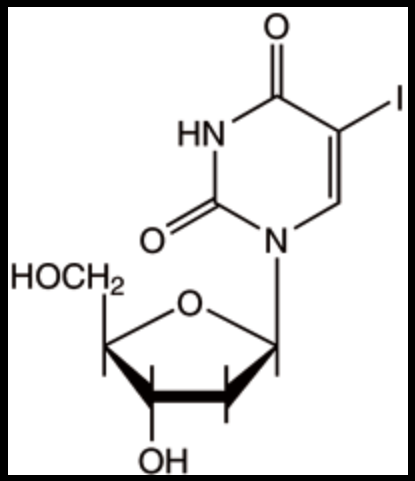

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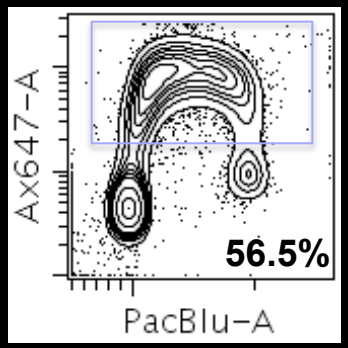

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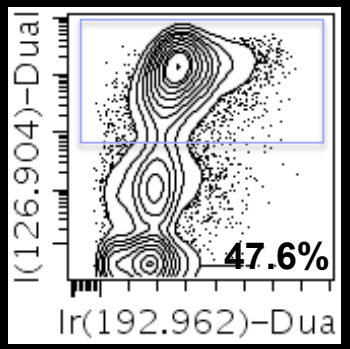

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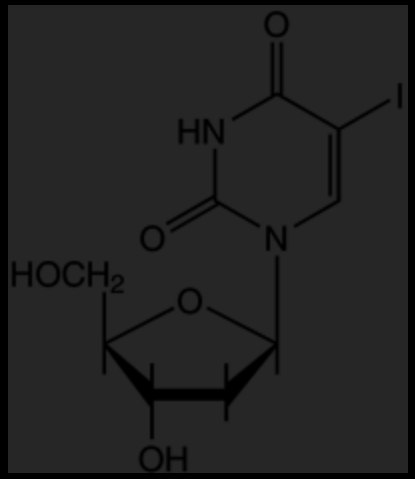
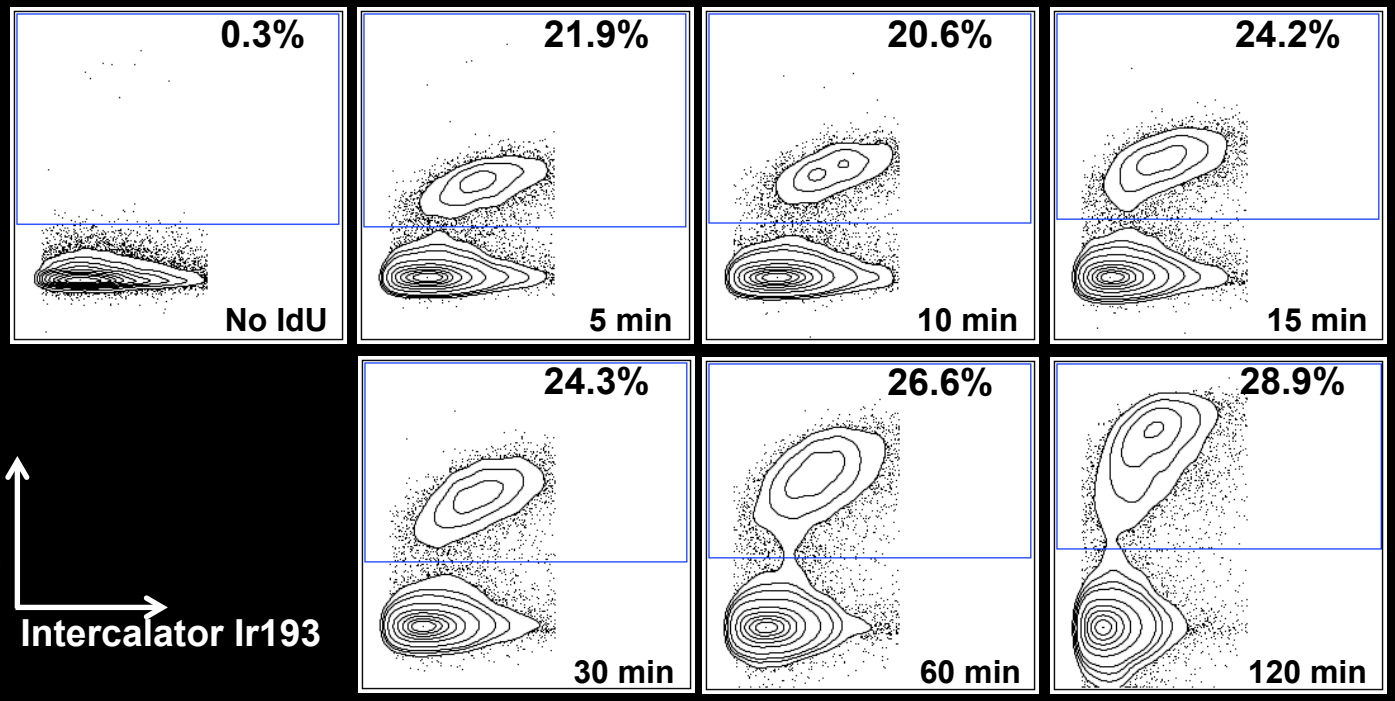
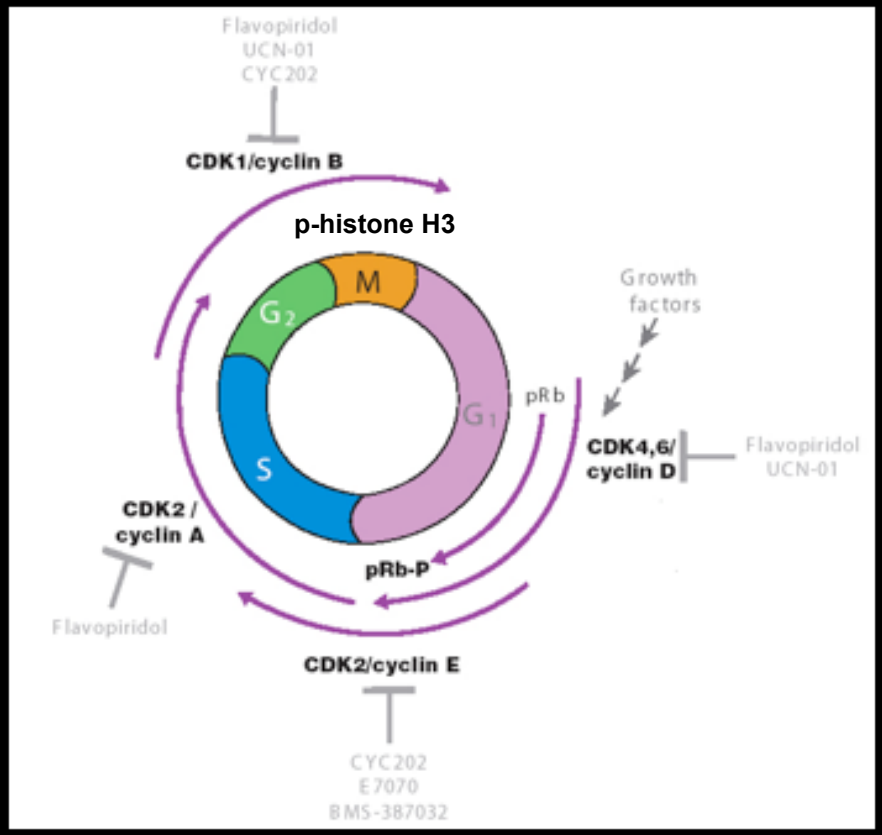




Additional markers allow for complete cell cycle state assignment



-Andrew Hughes, Gene Ther Mol Biol., 2006; 10:41

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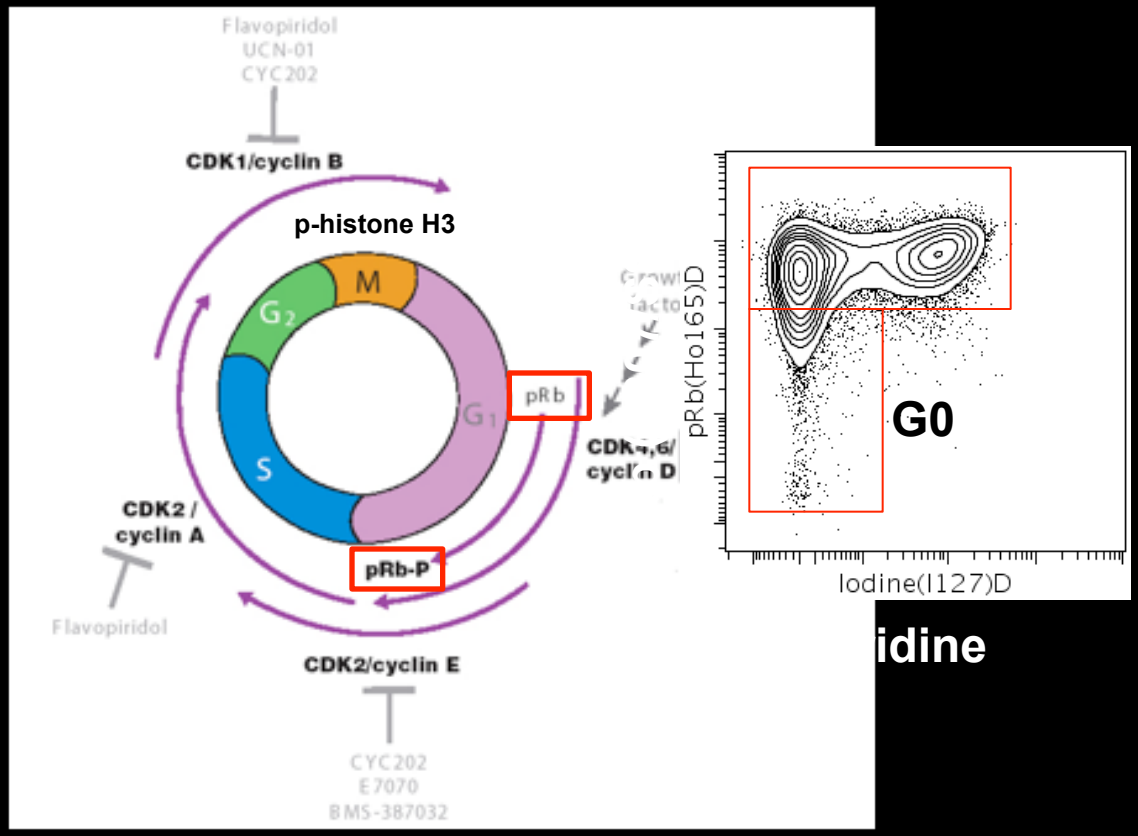

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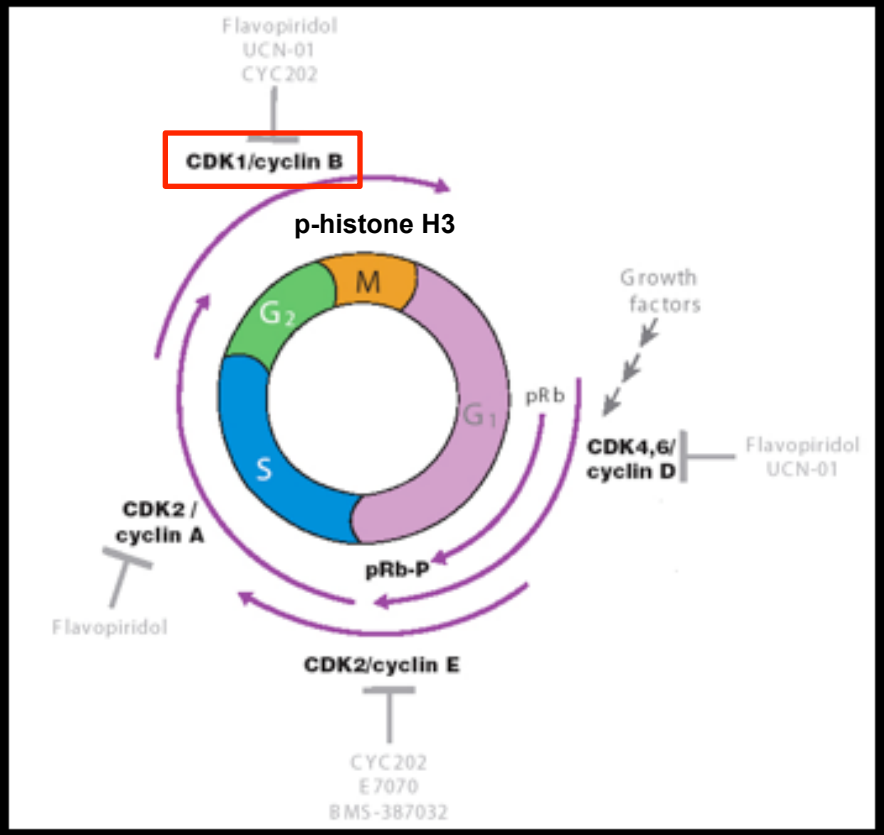

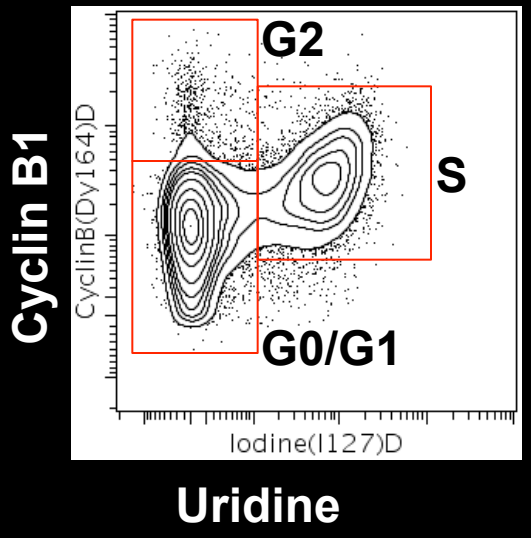

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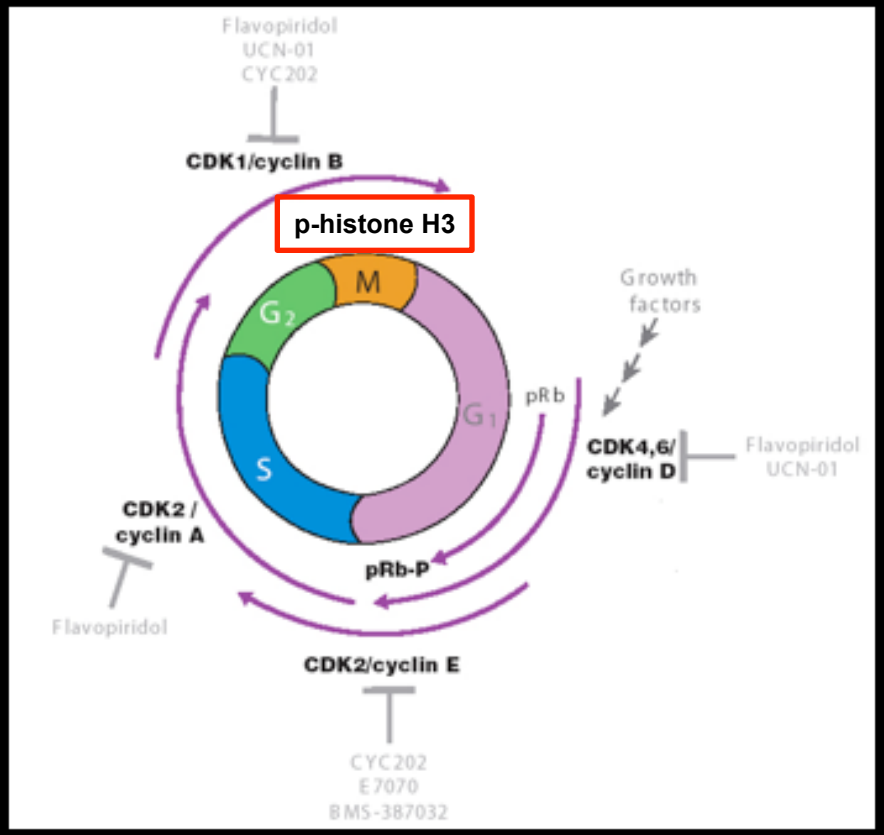
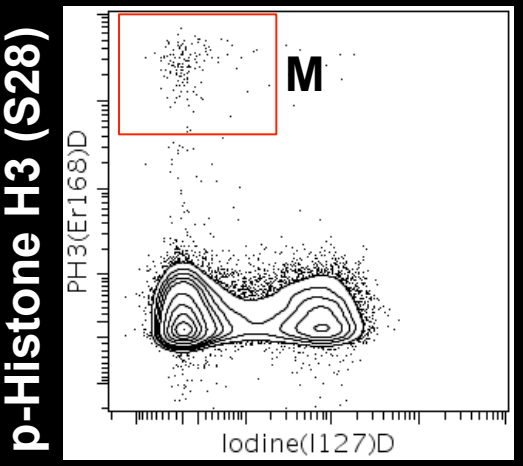
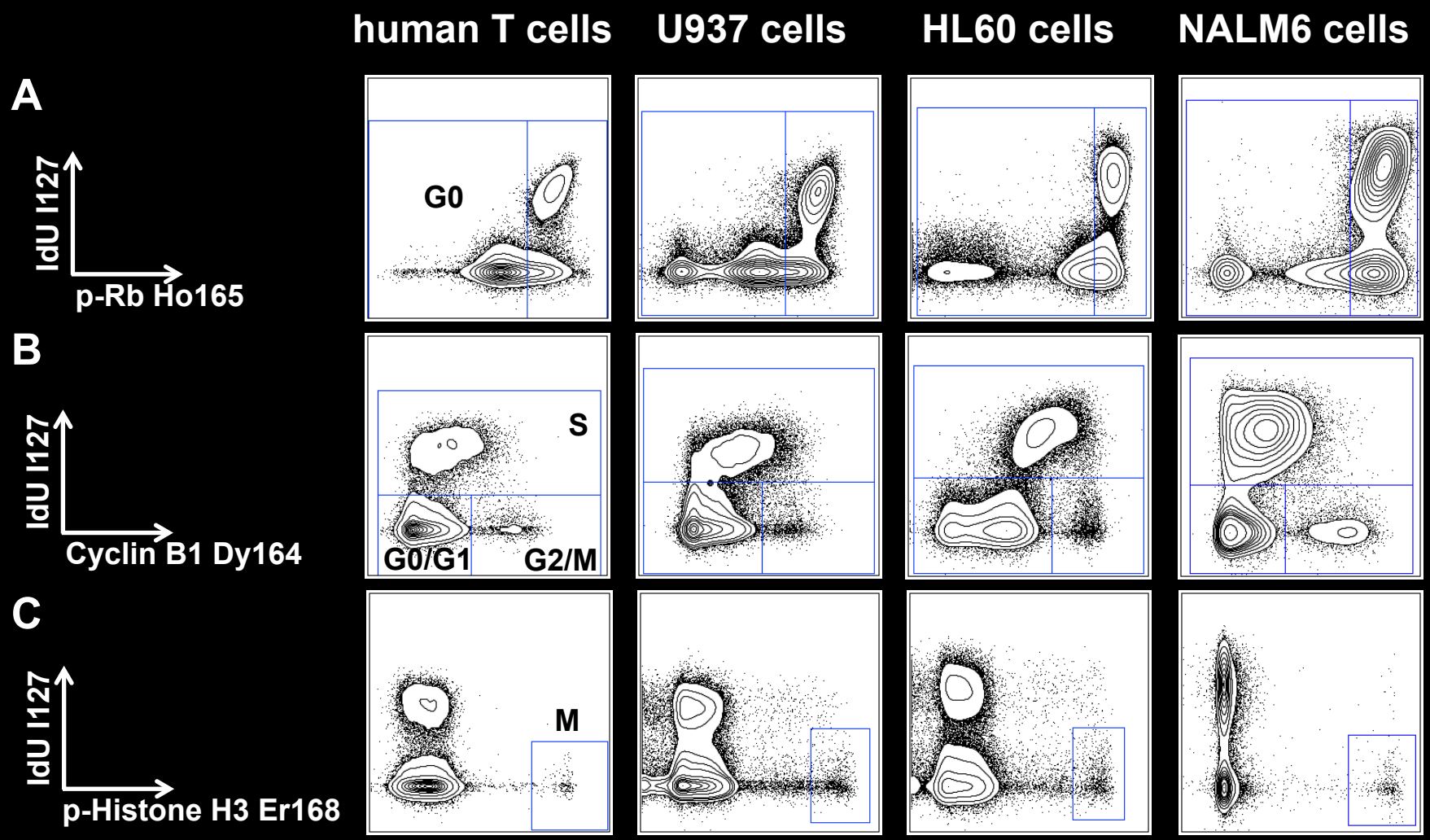




Cell cycle assessment is robust and consistent across multiple cell types



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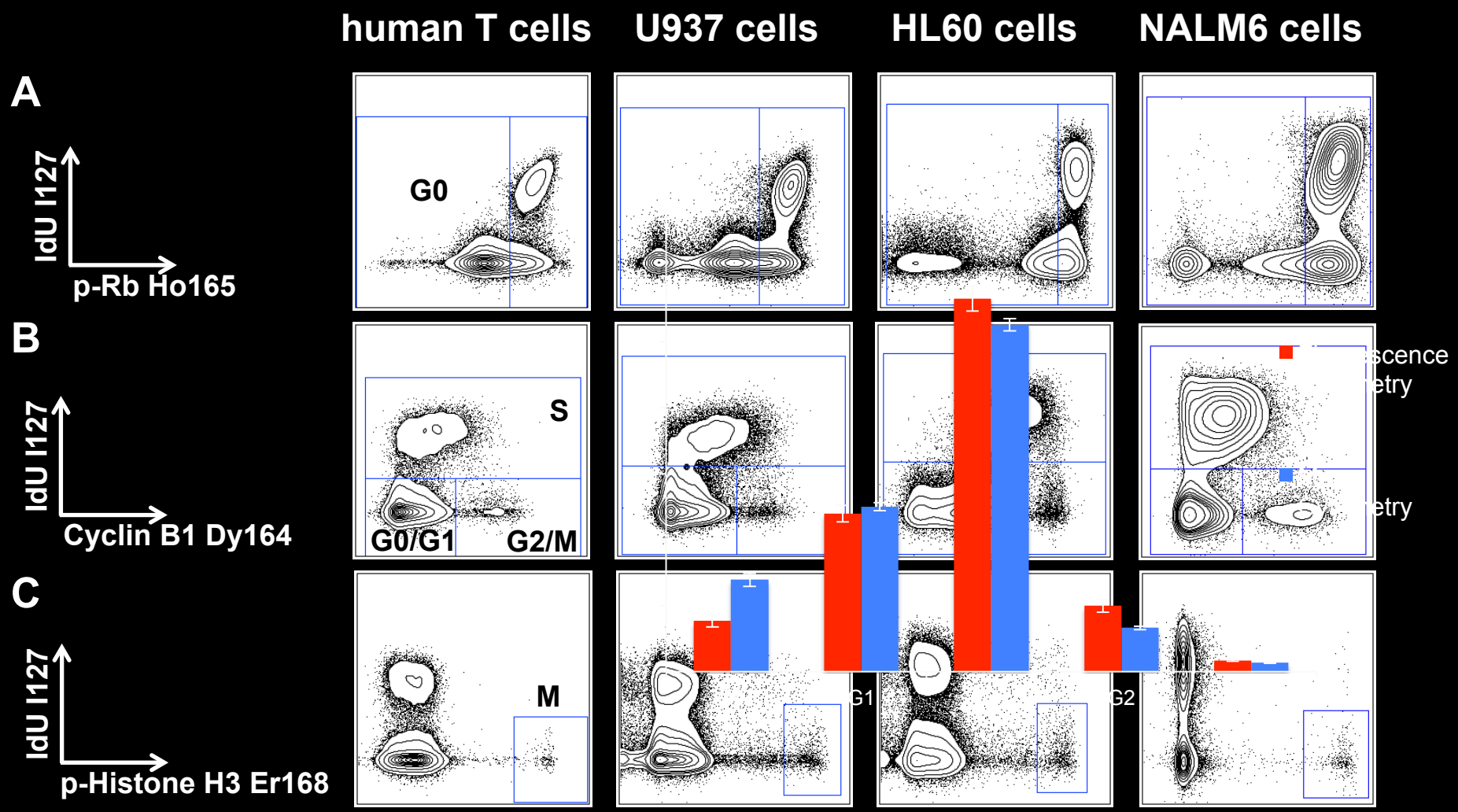
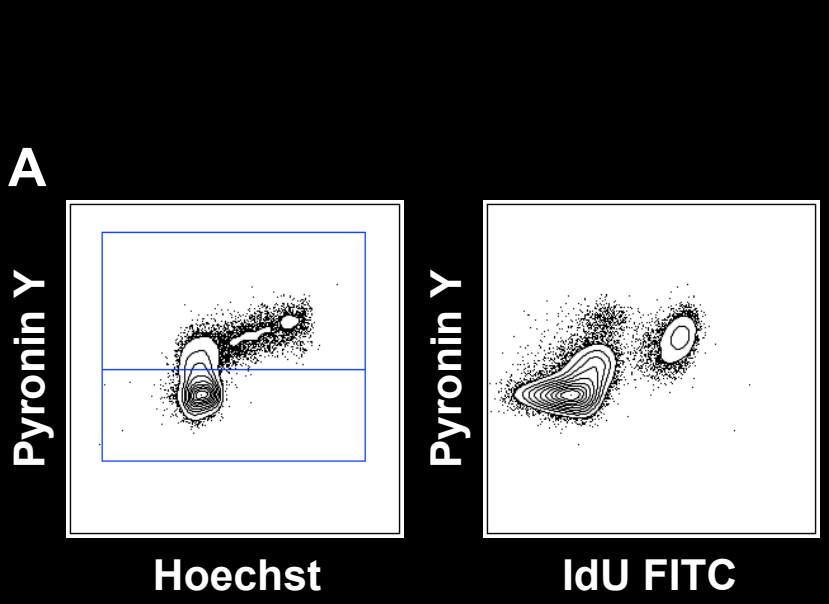
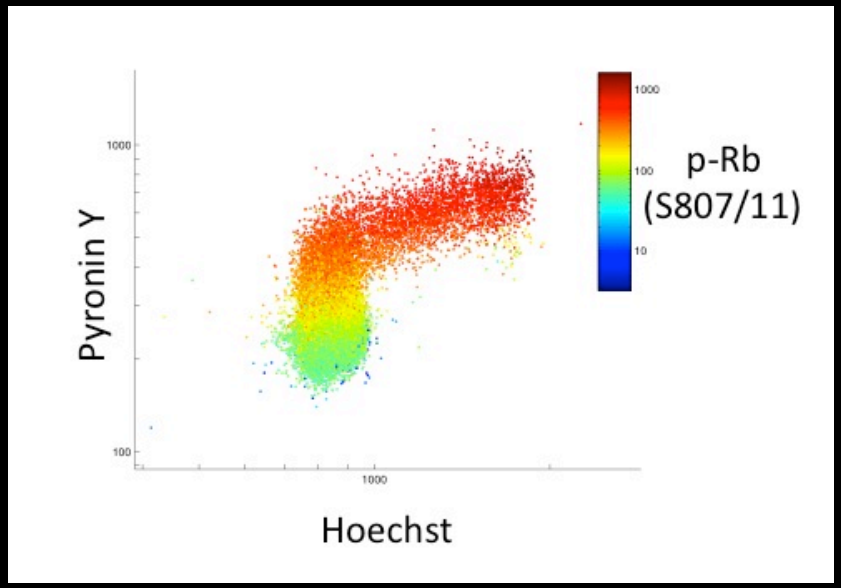
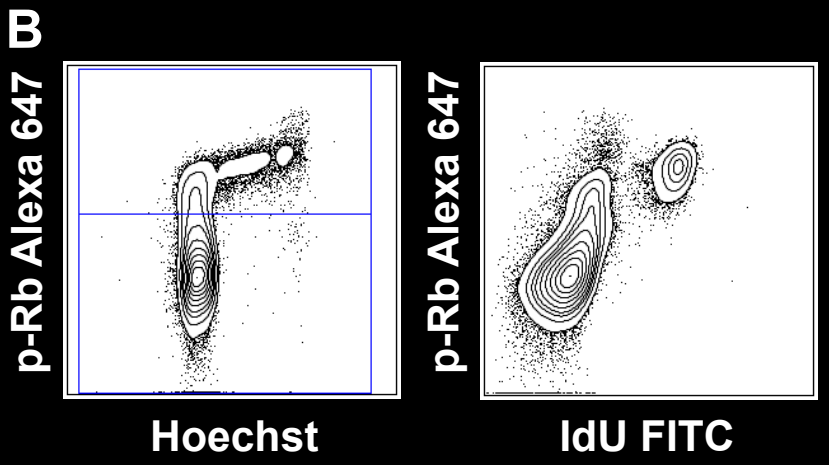
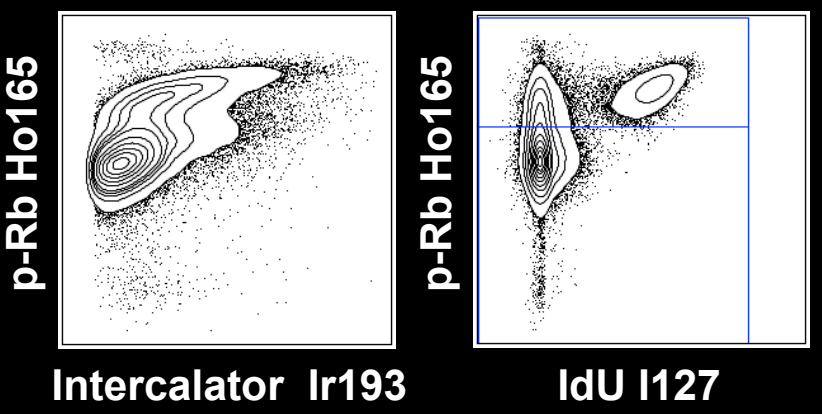


Phosphorylated Rb (S807/S811) discriminates G0 and G1 phase cells



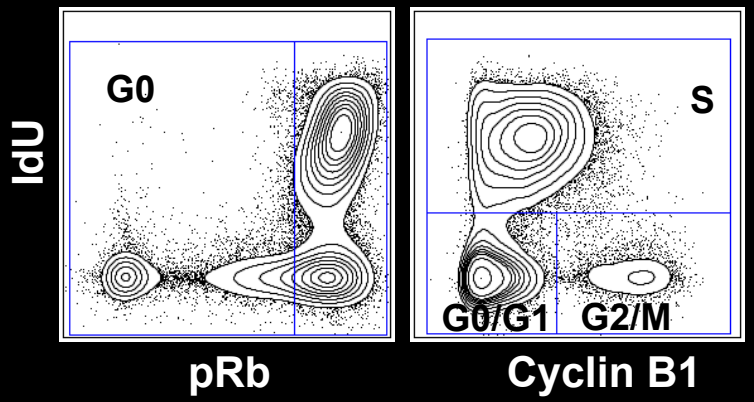






The same cell cycle markers can be used for fluorescence cytometry

Mass Cytometry



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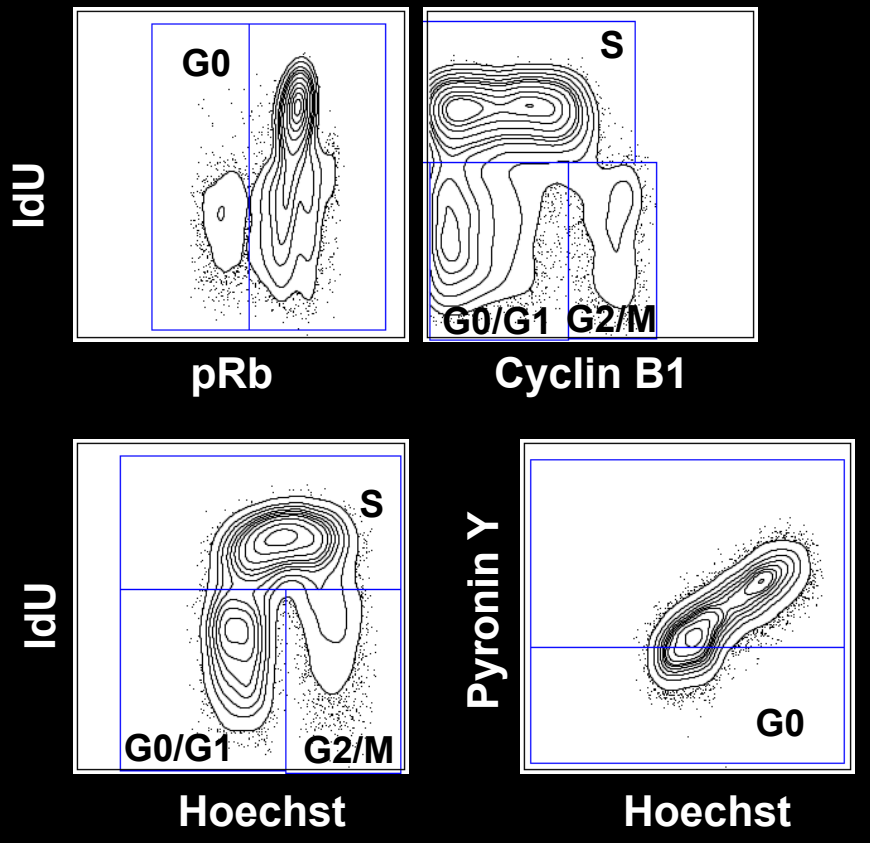
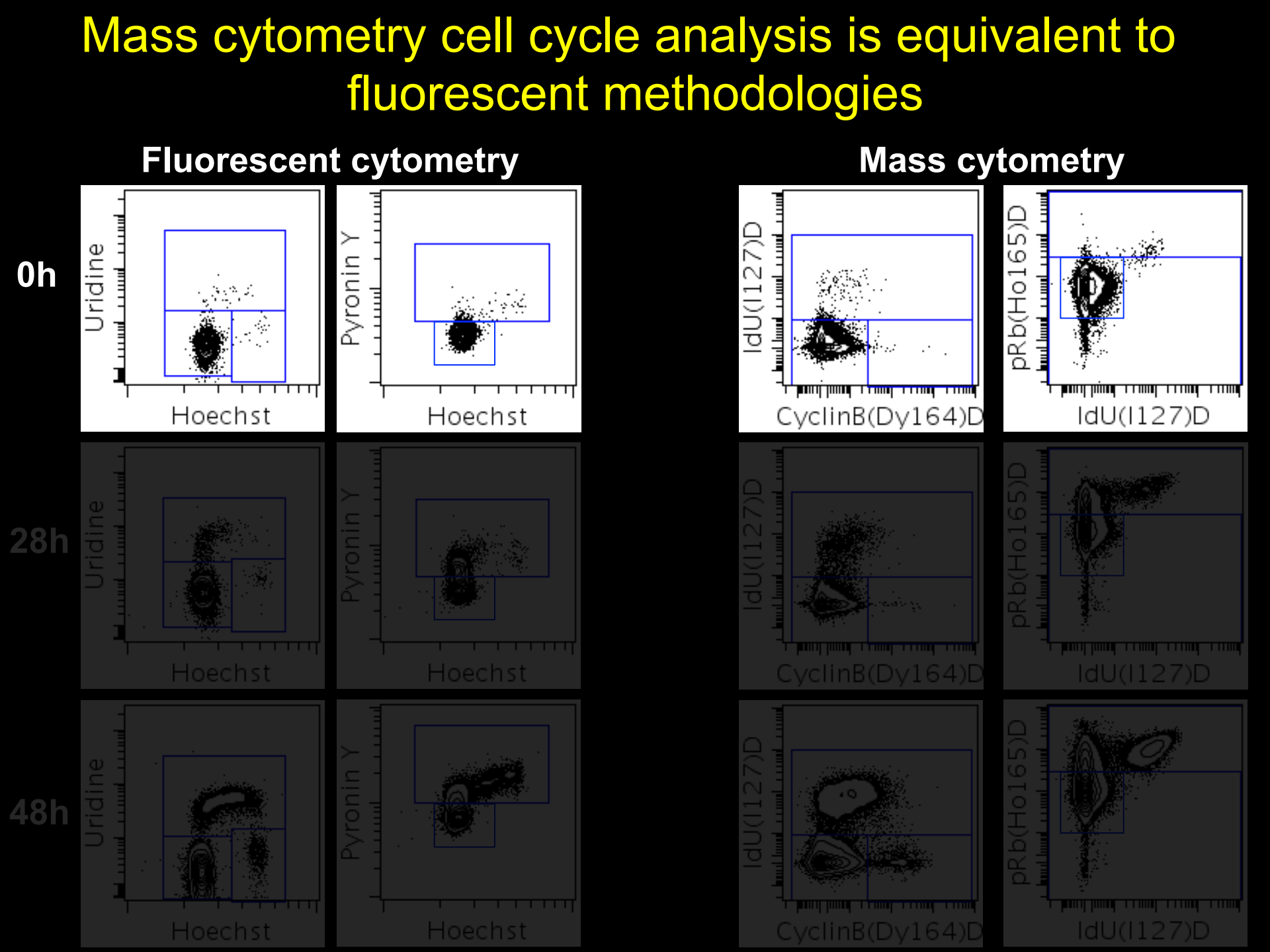


Mass cytometry cell cycle analysis is equivalent to fluorescent methodologies



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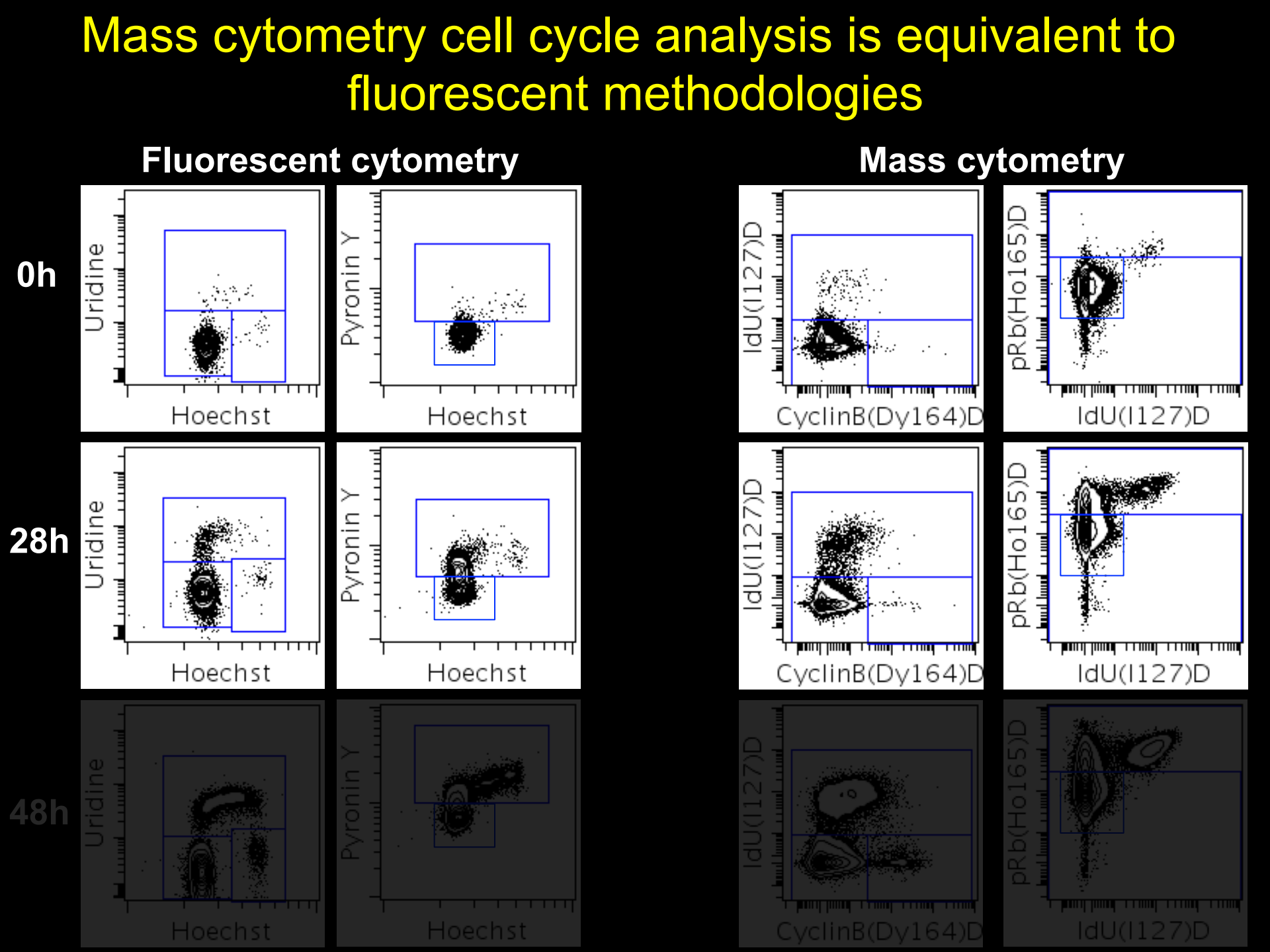

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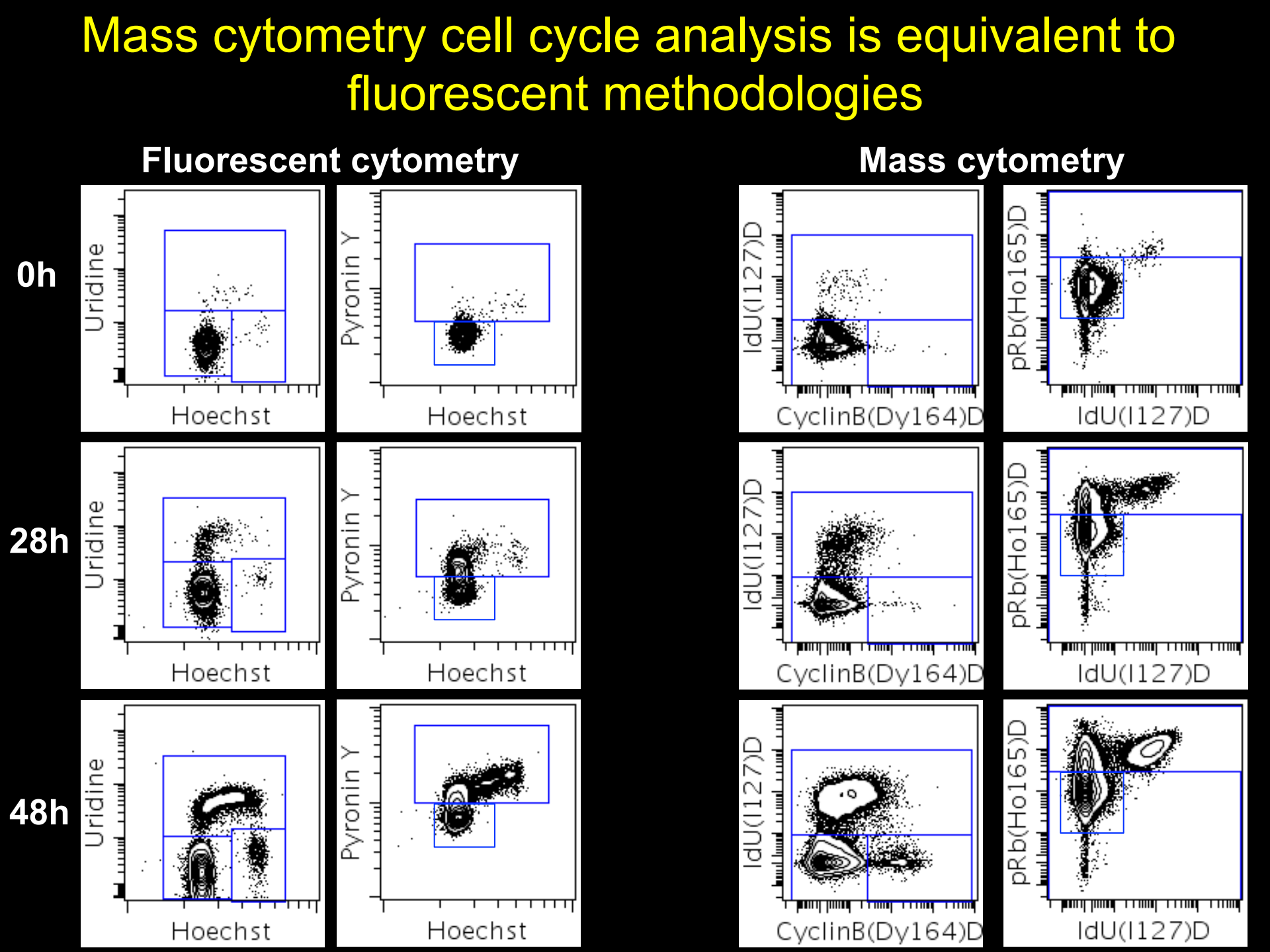

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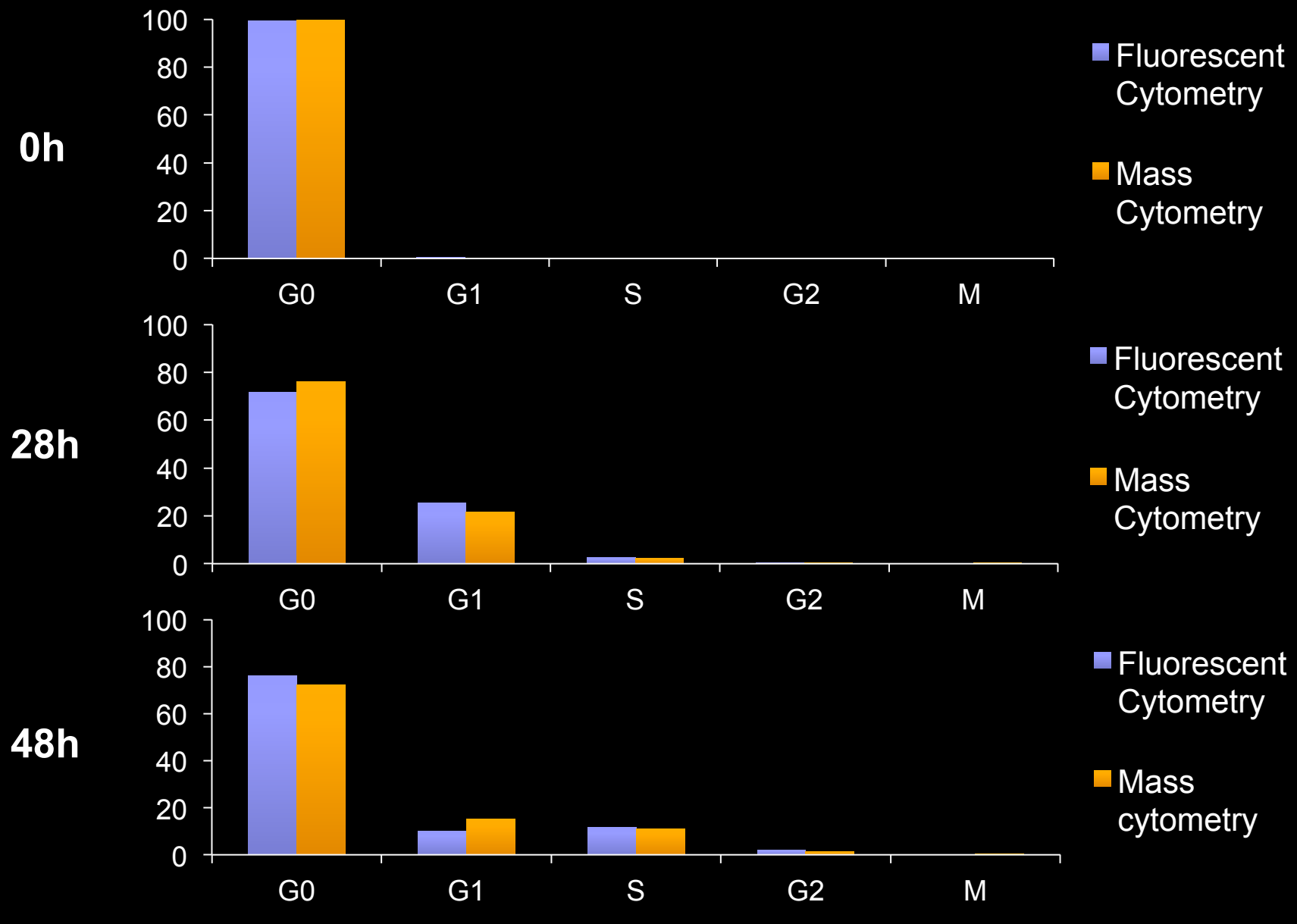

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  - 35 parameter analysis of normal bone marrow cell cycle
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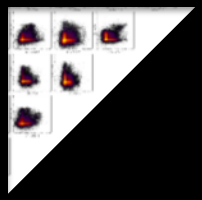
Panel for analysis of cell cycle in human marrow

ANTIGEN	CONJUGATE	CLONE	CONCENTRATION	MANUFACTURER
Mass cytometry				
CD3	In-113	UCHT1	1.5 μg/mL	Biolegend
CD45	In-115	Hl30	1.5 μg/mL	Biolegend
CD45RA	La-139	Hl100	1 μg/mL	Biolegend
CD133	Pr-141	AC133	2 μg/mL	Militeney
CD19	Nd-142	H1B19	1 μg/mL	BD Biosciences
CD71	Nd-143	R17217	2 μg/mL	eBiosciences
CD11b	Nd-144	ICRF44	2 μg/mL	Biolegend
CD4	Nd-145	RPA-T4	2 μg/mL	Biolegend
CD8	Nd-146	RPA-T8	1 μg/mL	Biolegend
CD20	Sm-147	2H7	1 μg/mL	BD Biosciences
CD34	Nd-148	8G12	2 μg/mL	BD Biosciences
CD90	Sm-149	5E10	2 μg/mL	Biolegend
CD117	Nd-150	104D2	0.5 μg/mL	Biolegend
CD123	Eu-151	9f5	2 μg/mL	BD Biosciences
CD235	Sm-152	HIR2	0.5 μg/mL	Biolegend
HLA-DR	Eu-153	L243	2 μg/mL	Biolegend
Cyclin A	Sm-154	BF683	2 μg/mL	BD Biosciences
Cyclin B1	Gd-156 Dy-164	GNS-1	2 μg/mL	BD Biosciences
CD33	Gd-158	WM53	1.5 μg/mL	Biolegend
CD38	Tb-159	HIT2	1 μg/mL	Biolegend
CD14	Gd-160	M5E2	2 μg/mL	Biolegend
CD7	Dy-162	M-T701	0.5 μg/mL	BD Biosciences
CD15	Dy-164	W6D3	0.5 μg/mL	Biolegend
p-pRb (S807/811)	Ho-165	J112-906	0.5 μg/mL	BD Biosciences
Ki-67	Er-167	B56	1 μg/mL	BD Biosciences
CD13	Er-168	L138	2 μg/mL	BD Biosciences
p-CDK1(Y15)	Tm-169	10A11	2 μg/mL	Cell Signaling Technology
CD56	Er-170	HCD56	2 μg/mL	Biolegend
cleaved-PARP(D214)	Yb-171	F21-852	1 μg/mL	BD Biosciences
p-RPS6(S235/36)	Yb-172	N7-548	1 μg/mL	BD Biosciences
CD10	Yb-174	Hl10a	4 μg/mL	Biolegend
CD16	Lu-175	3G8	2 μg/mL	Biolegend
p-Histone H3(S28)	Yb-176 Er-168	HTA28	0.5 μg/mL	Biolegend

Biaxial plots are not a scalable solution

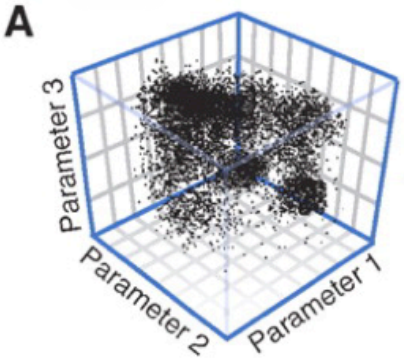


Parameters: 32

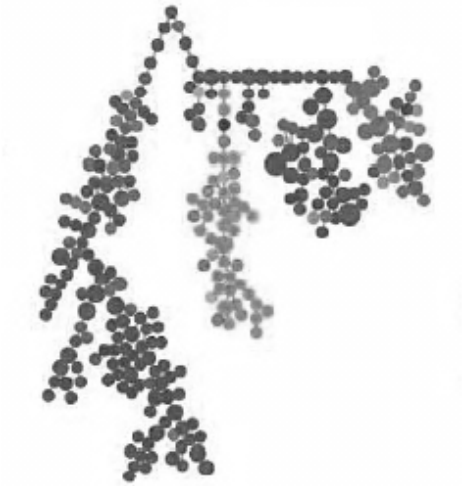
Plots: 496

Sean Bendall, Erin Simonds. *Science,* May 2011

SPADE: Spanning-tree Progression Analysis of Density-normalized Events – Peng Qiu

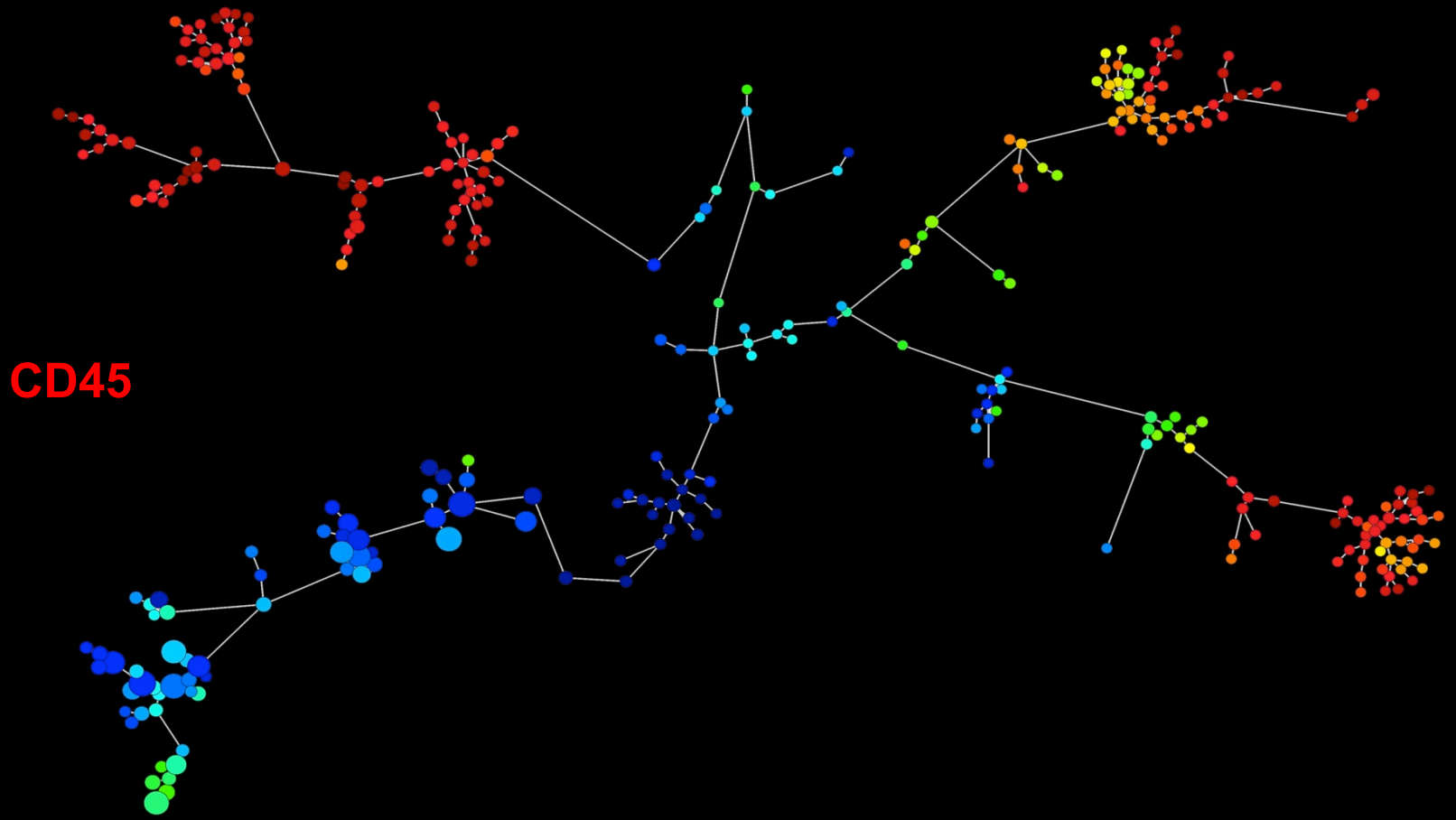


1. **Determine Tree Structure**



2. **Overlay regions with surface marker expression levels**

SPADE clustering of normal bone marrow mirrors immunophenotypic differentiation.



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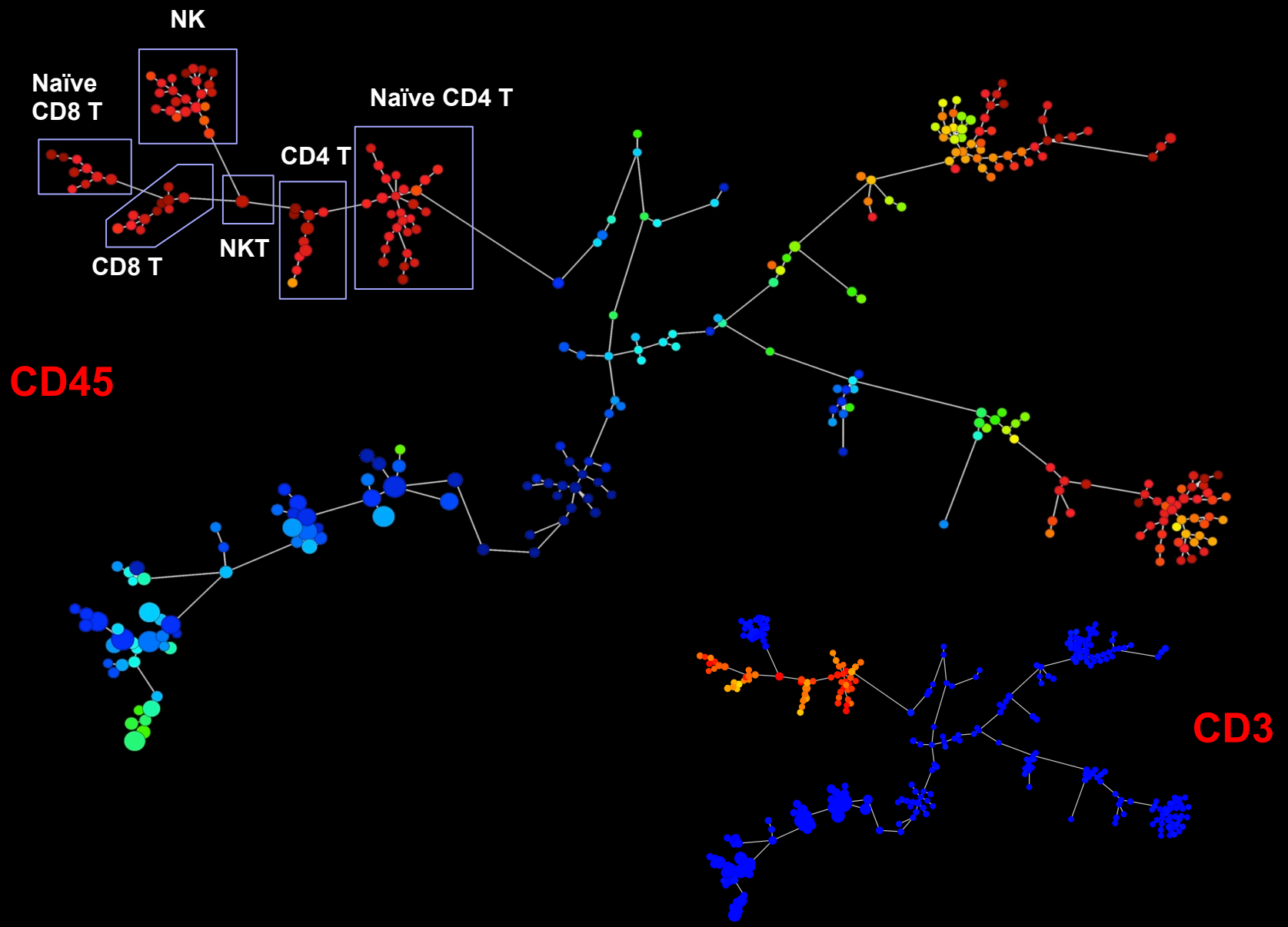

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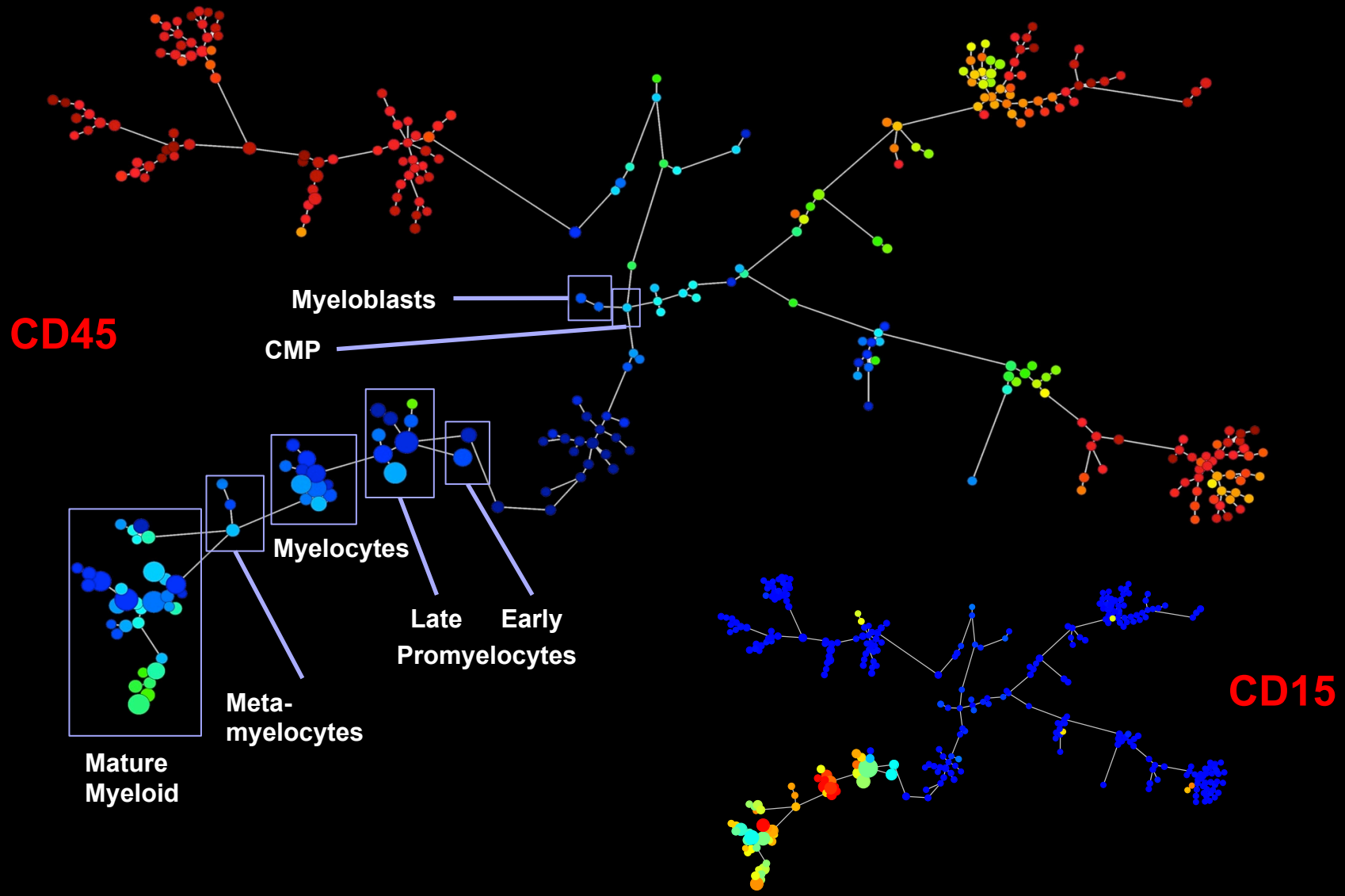

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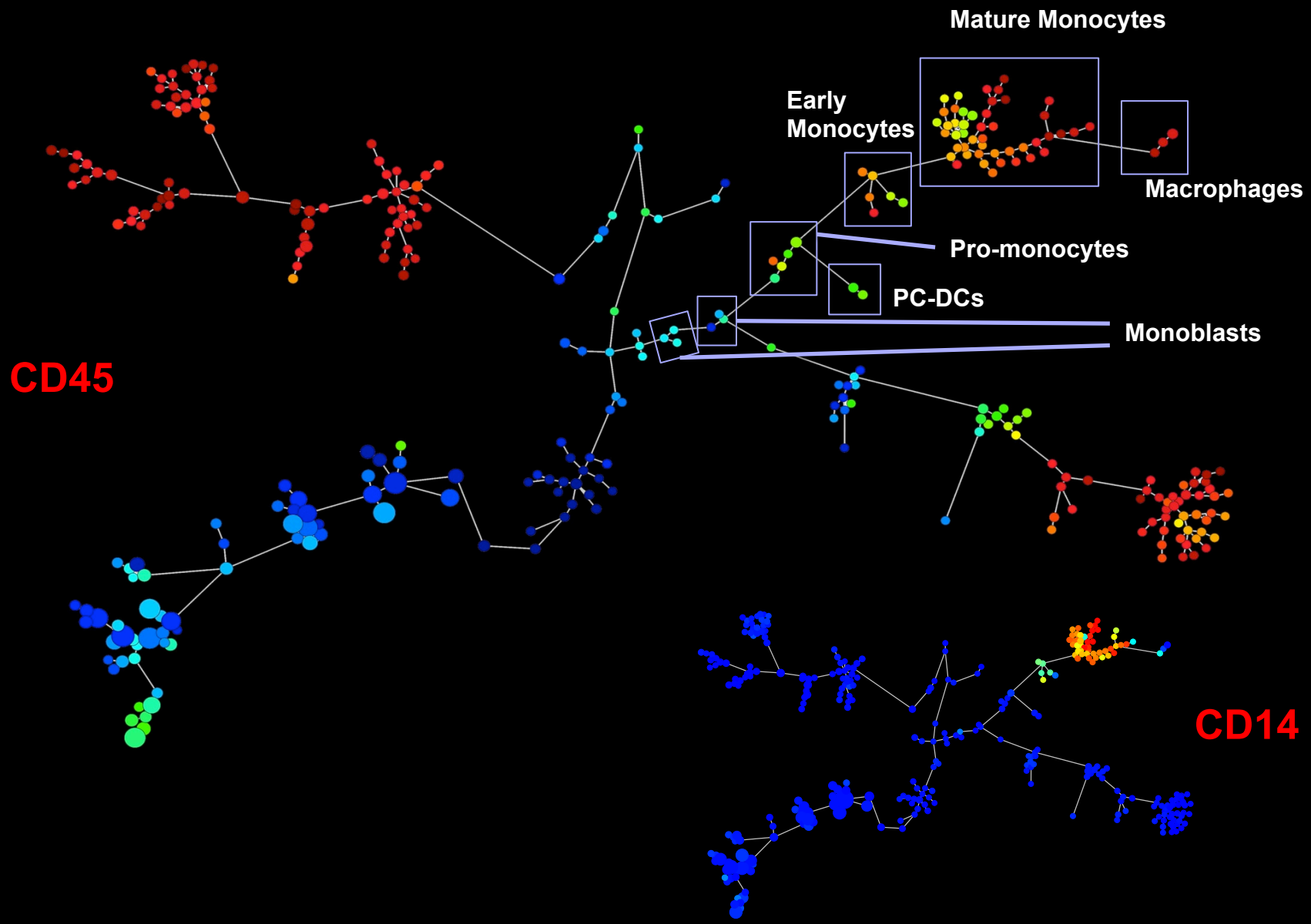

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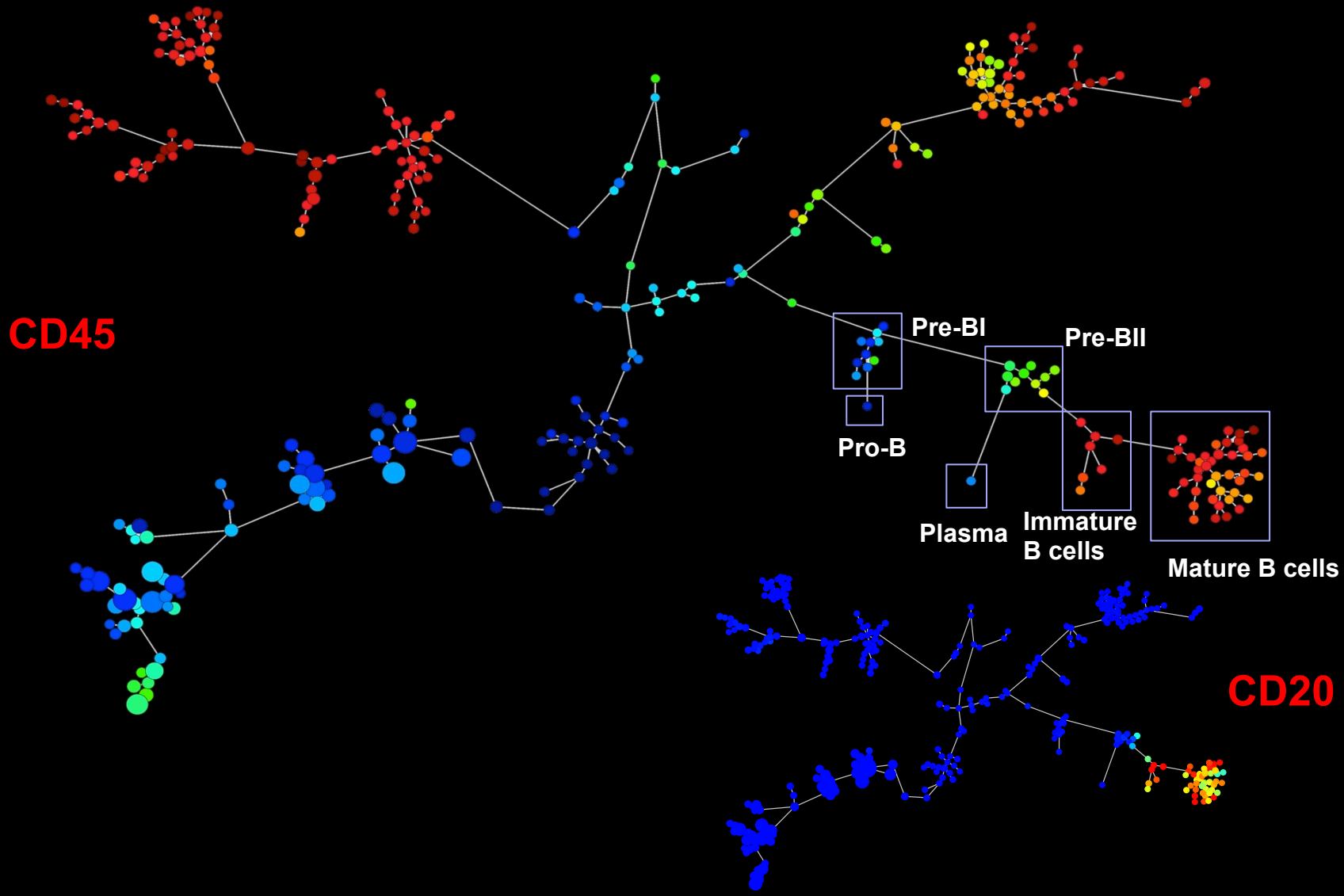

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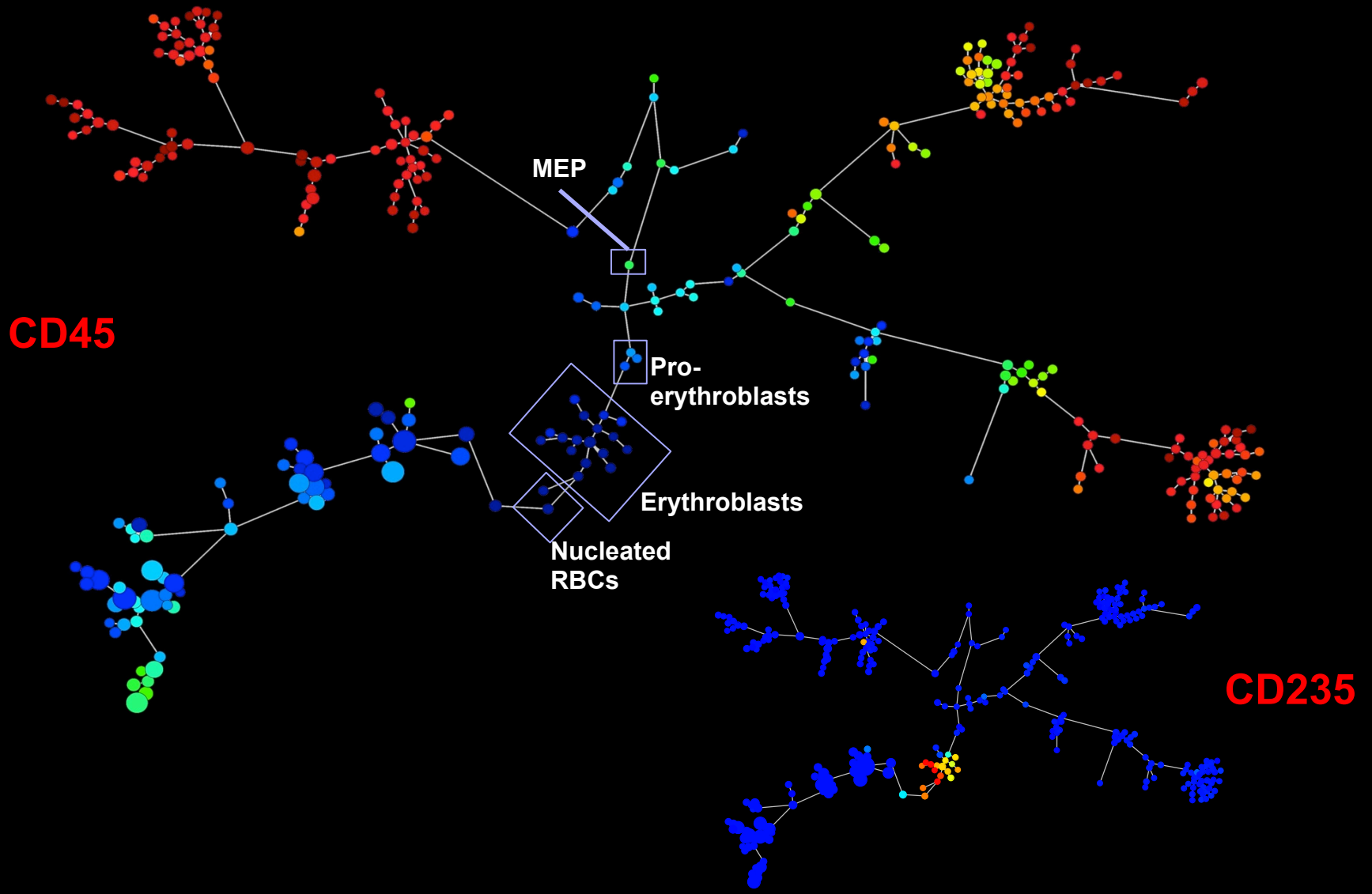

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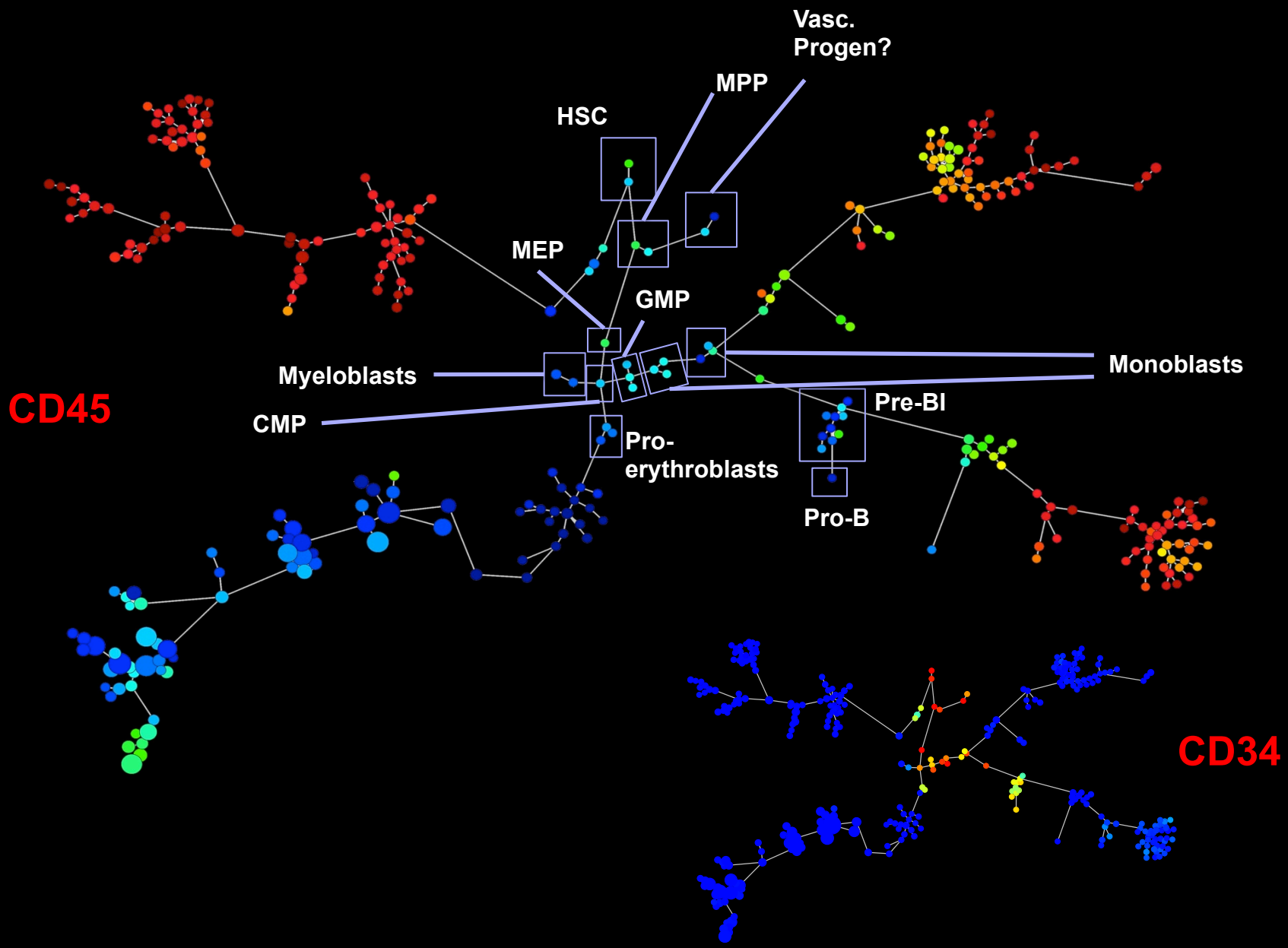

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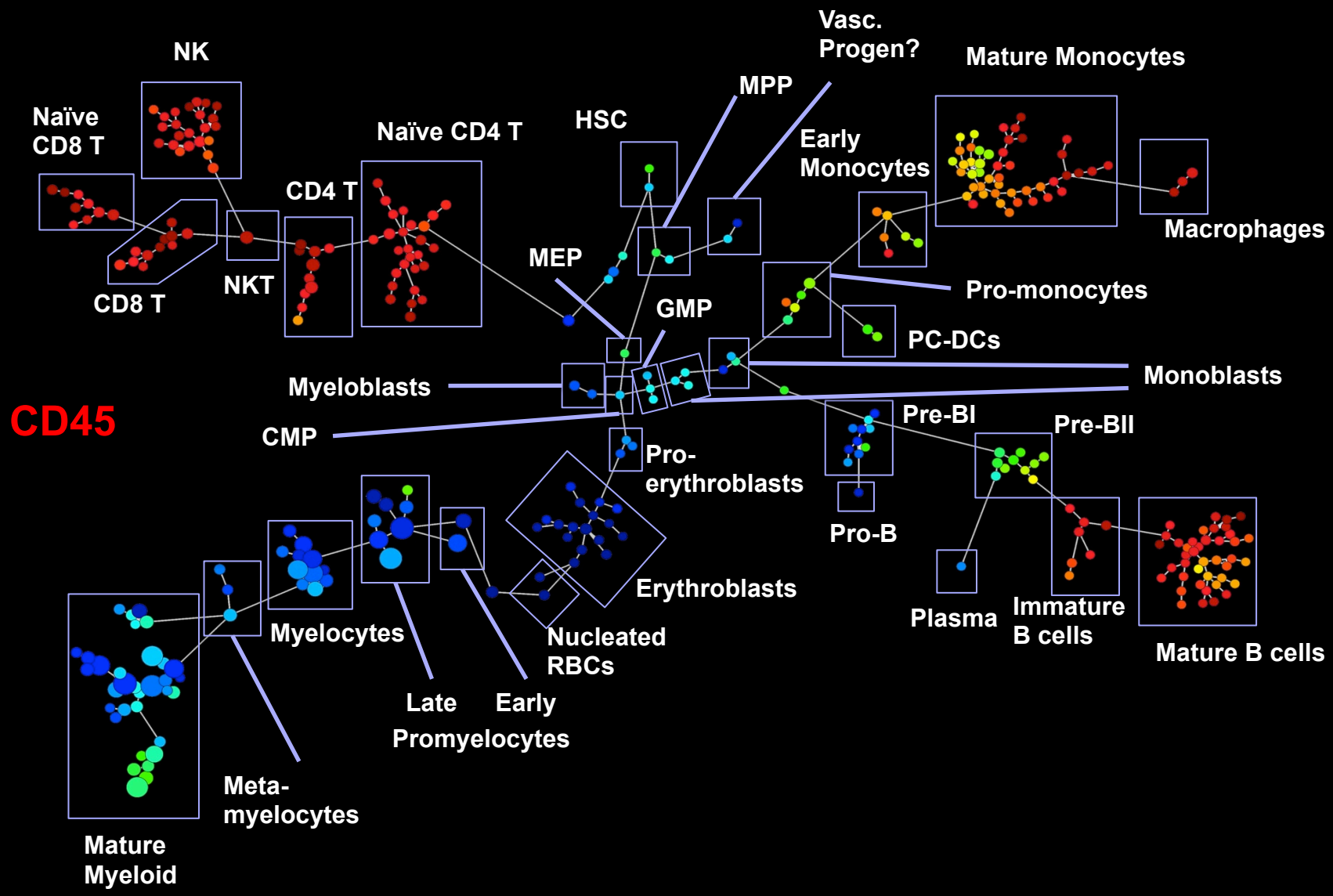
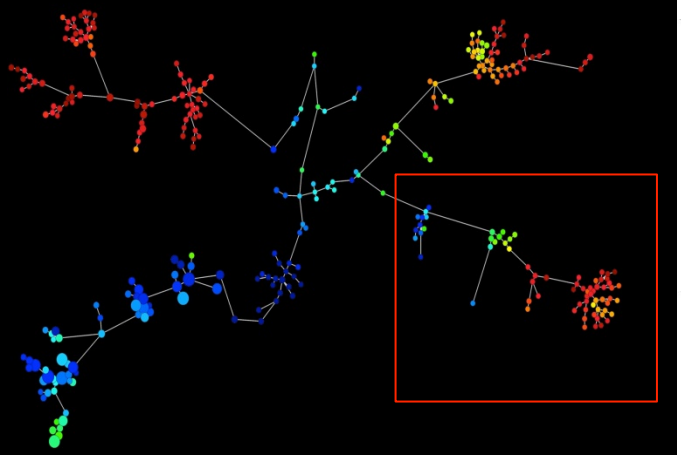


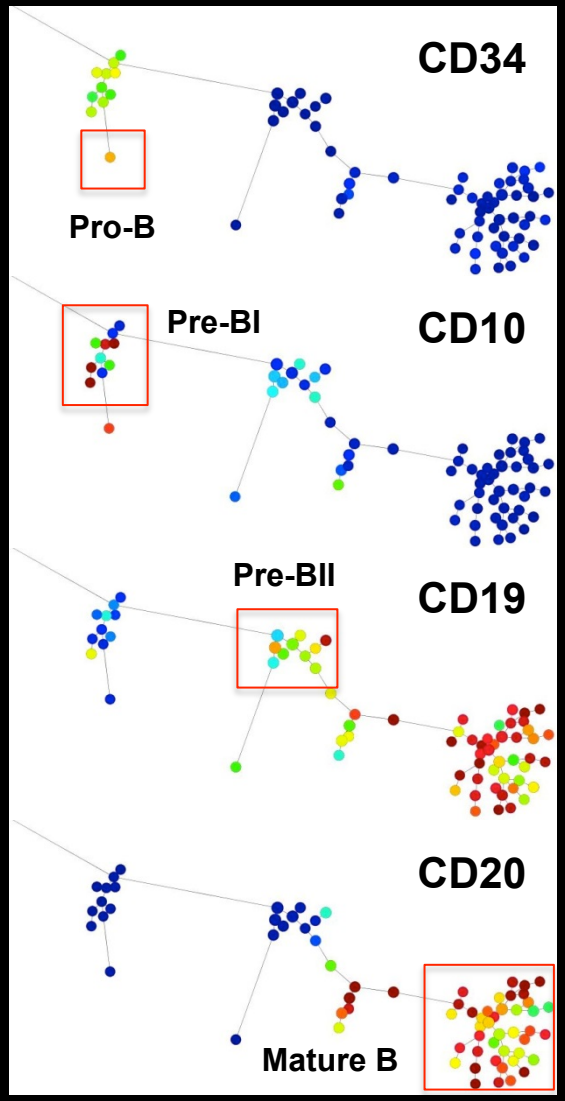
B cell proliferation is concentrated in pre-BII population

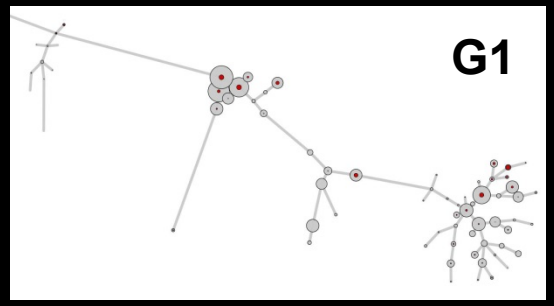


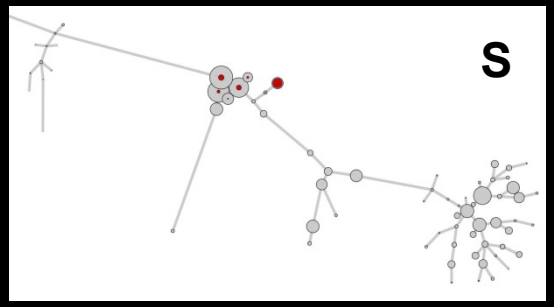
Normal Human Bone Marrow
Colored for CD45

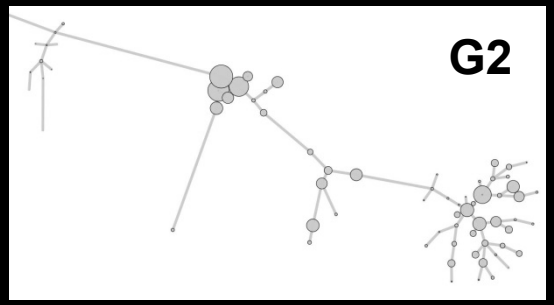
= 200 Cells

= 67%

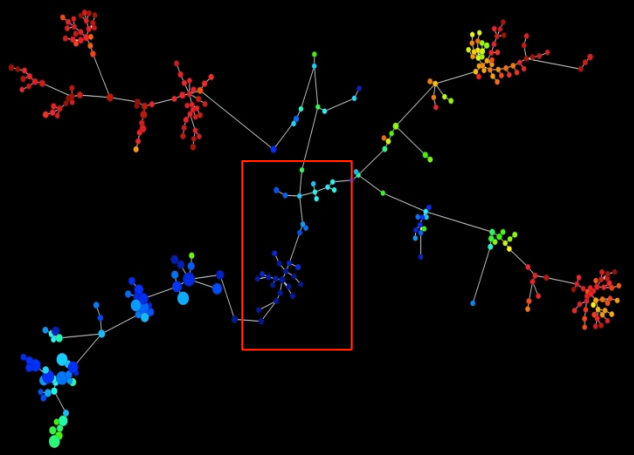






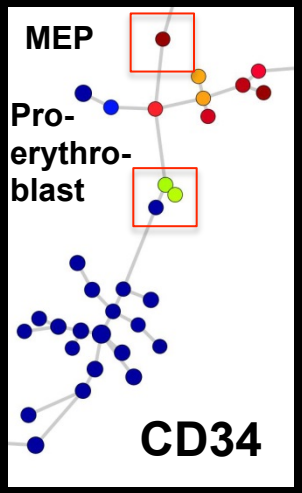


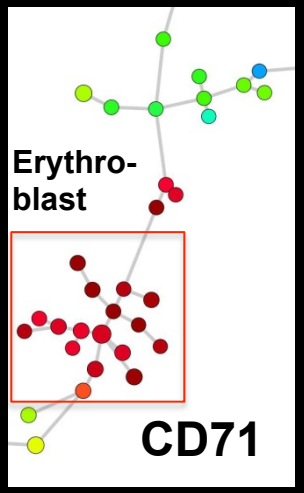
Erythroid cell proliferation is concentrated in erythroblast population

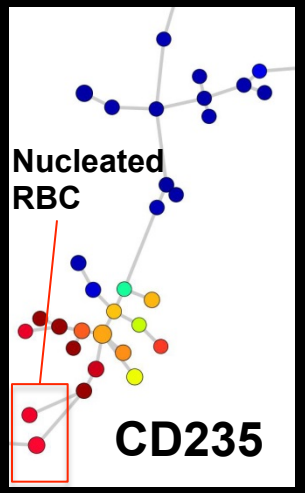


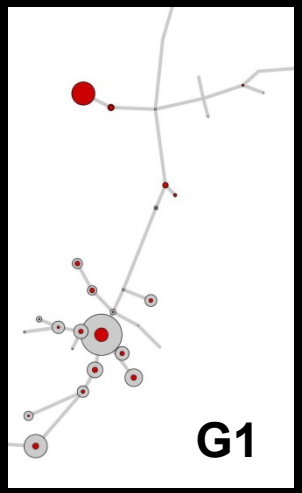
Normal Human Bone Marrow
Colored for CD45

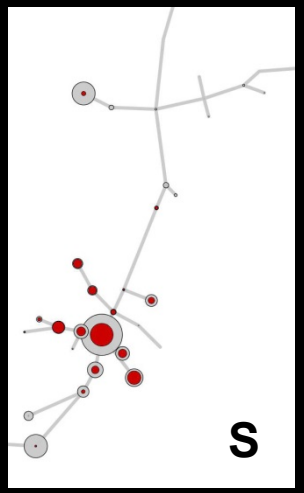
● = 200 Cells
● = 67%

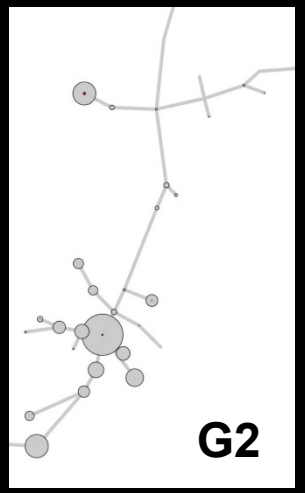




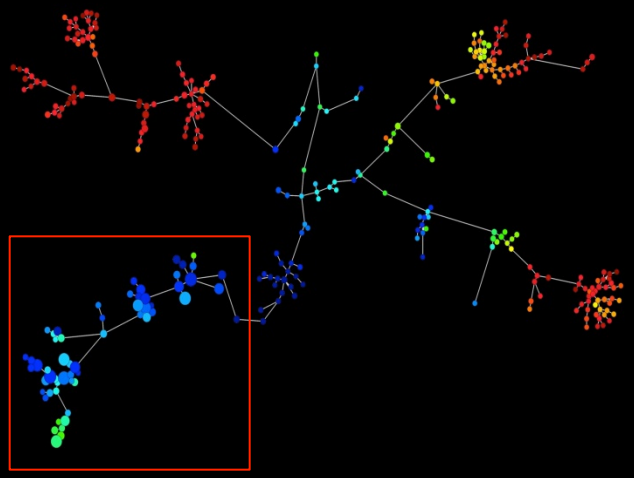






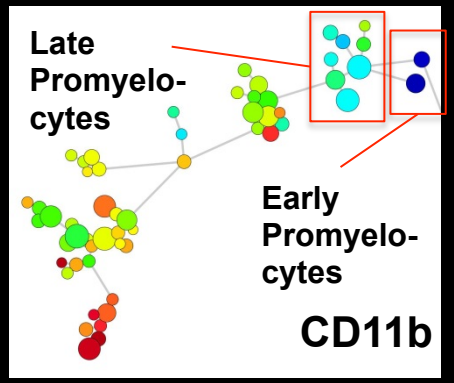


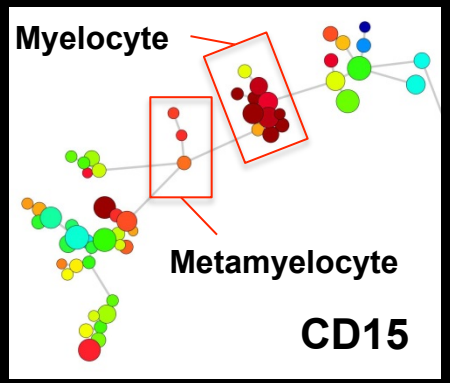
Myelocyte proliferation peaks at early promyelocyte stage

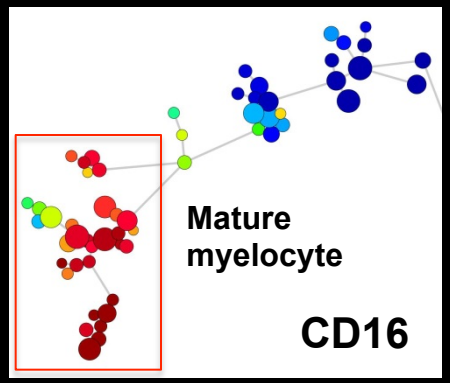


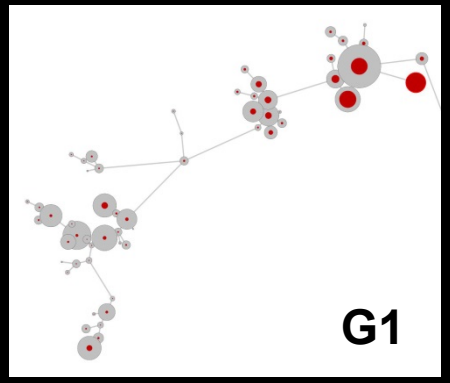
Normal Human Bone Marrow
Colored for CD45

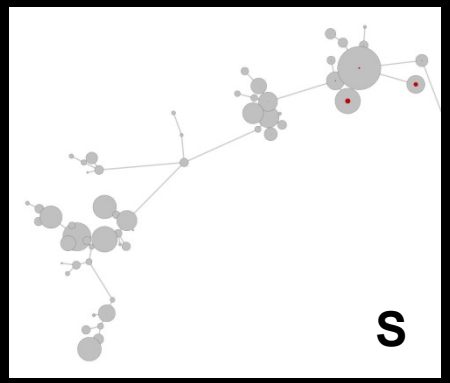
● = 3500 Cells
● = 67%

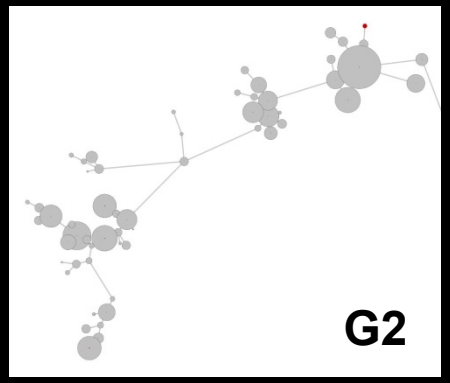




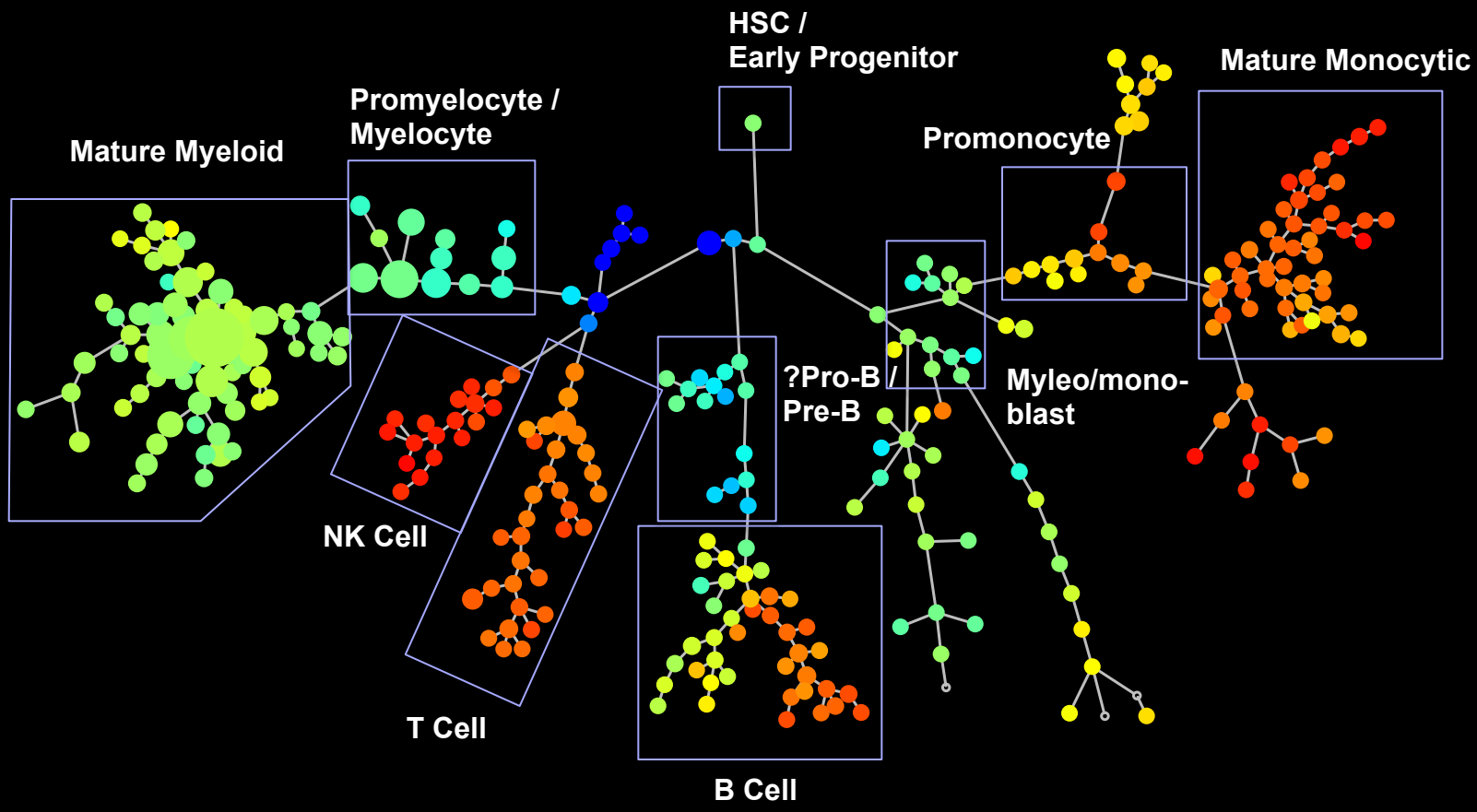






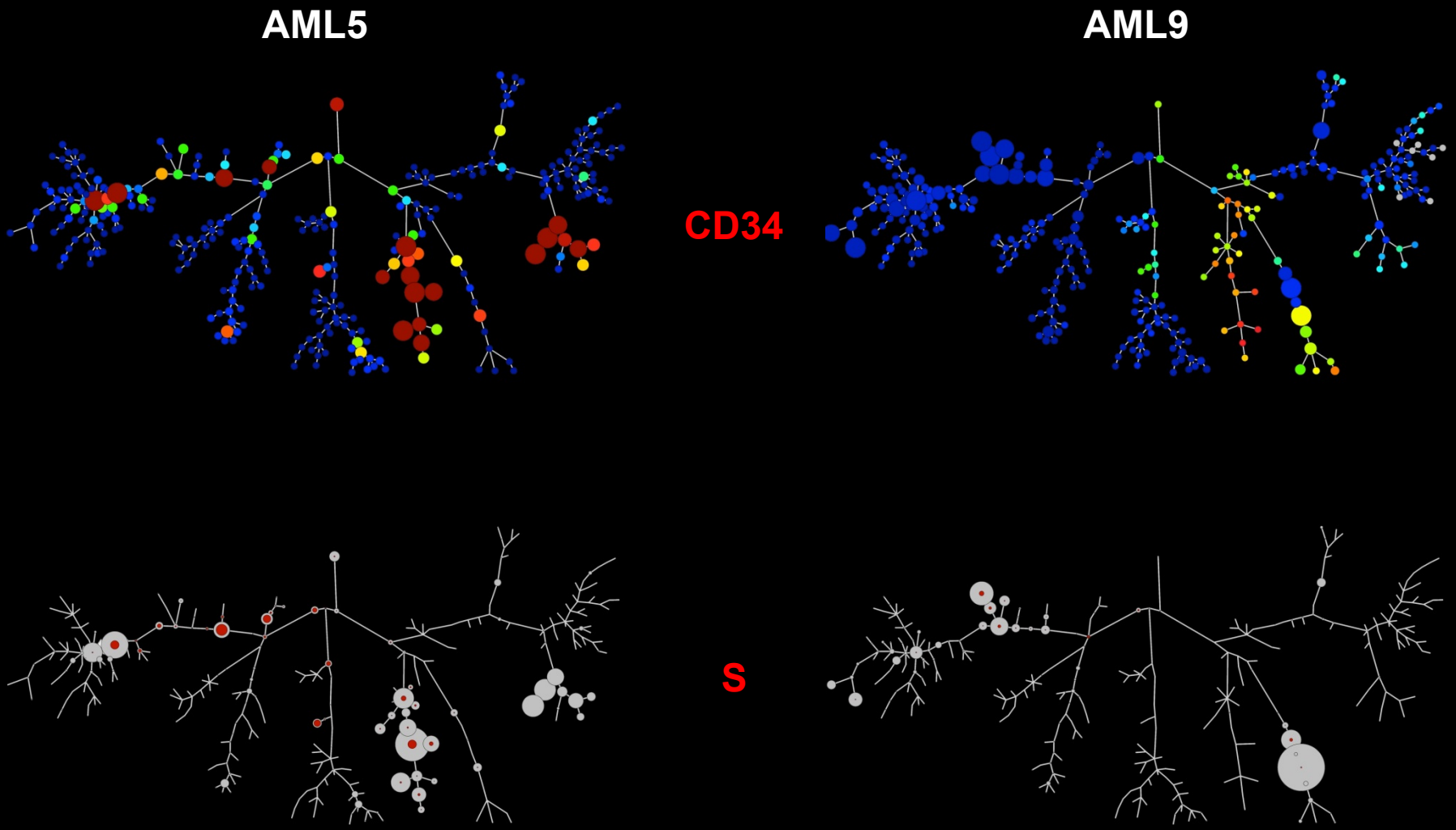


SPADE analysis allows for identification of distinct AML immunophenotypes



Normal human bone marrow
Clustered alongside AML samples
Colored for CD45

Cell cycle distribution varies across the immunophenotypic subsets within each AML sample



Conclusions

- Validated methodology for using mass cytometry to asses cell cycle state in combination with high-parameter immunophenotypic analysis
- System-wide analysis of proliferation across normal human hematopoiesis
- The ability to combine cell cycle state with multiple other variables in the monitoring of cellular responses at the single-cell level
- We intend to use this methodology to characterize the cell cycle within complex human cancer samples

Acknowledgements

- Nolan Lab
  - Garry Nolan
  - Wendy Fantl
  - Sean Bendall
  - Erin Simmonds
  - Rachel Fink
  - Matt Clutter
  - Angelica Trejo
  - Matt Hale
- Stanford
  - Michael Linderman
  - Sylvia Plevritis
- MD Anderson
  - Peng Qiu

- Hematology
  - Bruno Medeiros
  - Peter Greenberg
  - Beverly Mitchell
    - Aparna Raval
- Cytobank
  - Nikesh Kotecha