Nonstochastic Reprogramming from a Privileged Somatic Cell State

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Yale Universiry

Journal Club, 2015

Erzsébet Ravasz Regan

Biological noise themed Journal Club (#5 - last)

The premise:

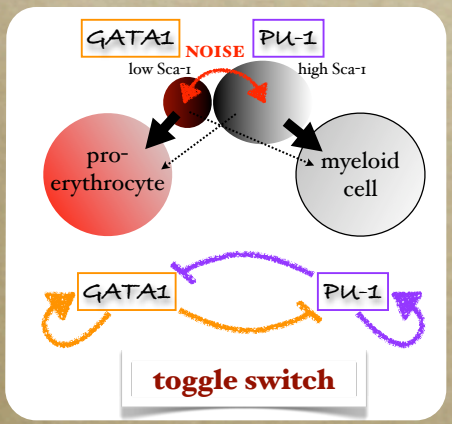
Cells with identical

- ***genome***
- ***present phenotype***
- ***environment***
- ***history of environments***
- ***history of phenotypes***

can display functionally heterogeneous behavior

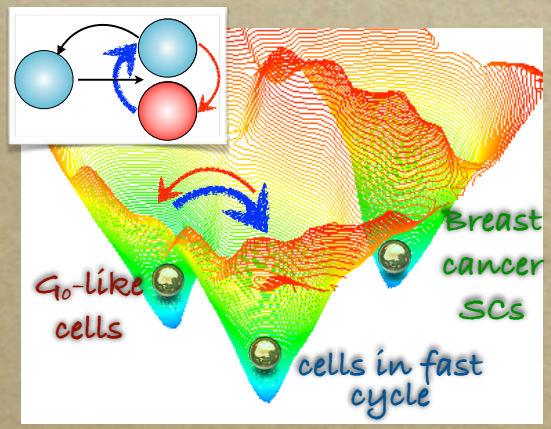
When does cellular noise impact on biology?

Differentiation



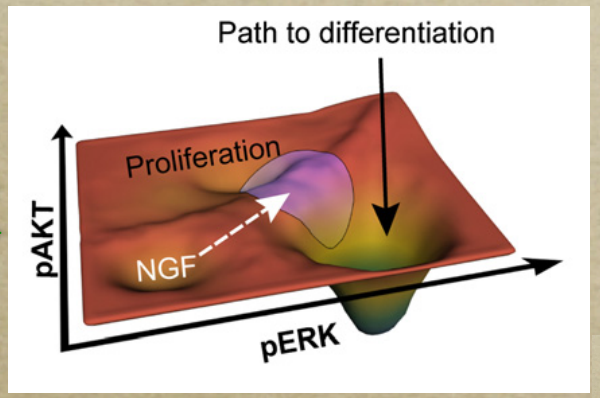
2010

Asymmetric division



2012

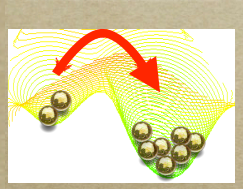
Growth signaling

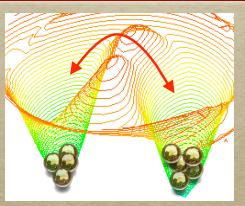


2013

Epigenetics

Histone-code alone bistable	Histone-code + DNA methylation strongly bistable





2014

Today: we look at iPS cell reprogramming

4 transcription factors can reprogram a somatic cell into an embryonic state

- **Yamanaka factors (2006)**

Oct4 Sox2 Klf4 Myc

fibroblasts

stomach, liver, skin, blood, prostate, urinary tract cells

→ **iPS cells** → **mouse**

Takahashi, K; Yamanaka, S *Cell* **126** (4): 663–76, 2006

Okita K. et al, *Nature* **448:** 260–262, 2007

- Can be achieved with recombinant protein (no genomic change)

Zhou H, Wu S, Joo JY et al. *Cell Stem Cell* **4**(5): 381–4, 2009

- Can be done without Myc - no cancer in iPS-derived mice!

Nakagawa, M. et al, *Nature biotechnology* 26(1):101-106, 2008

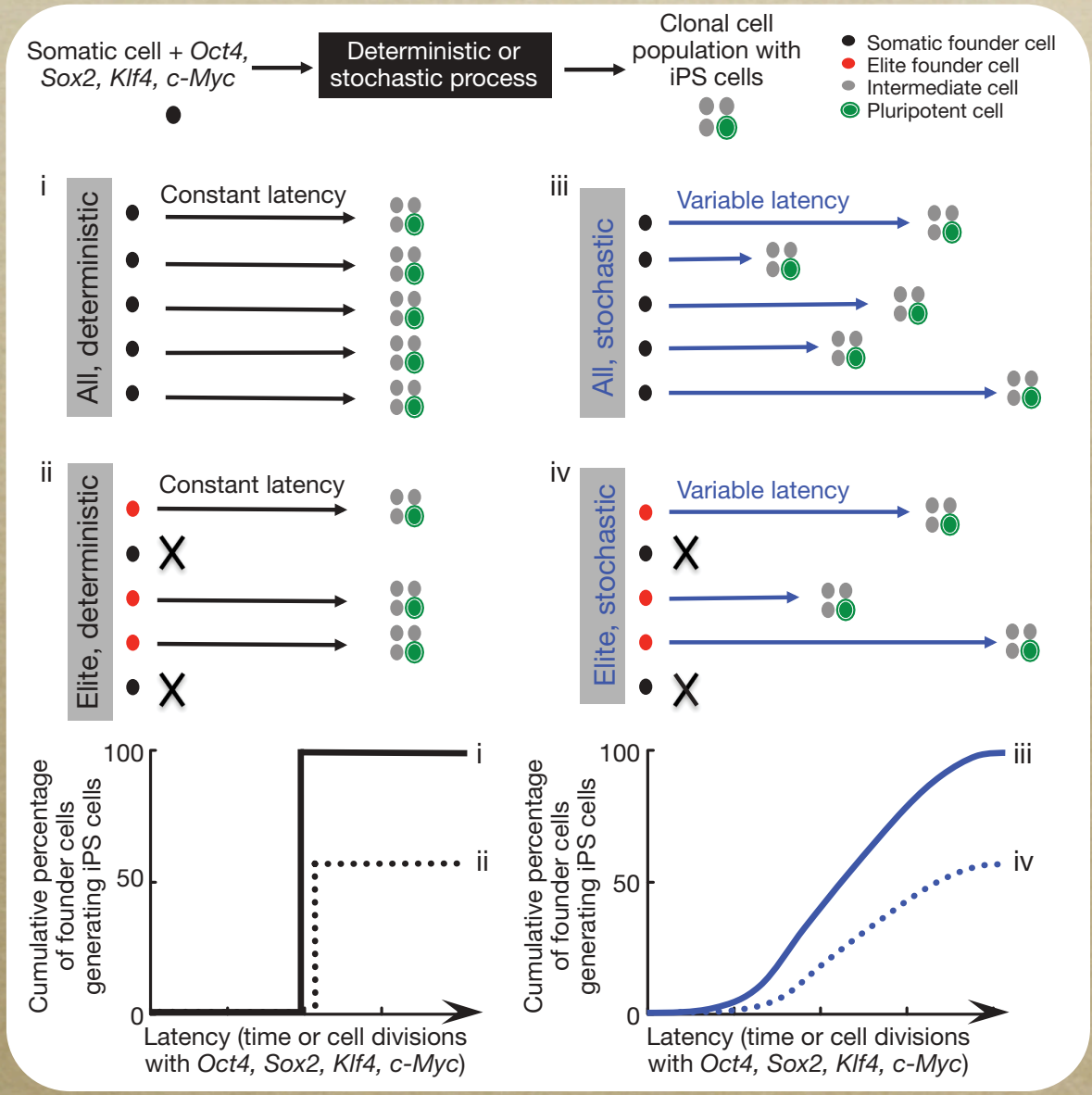
However, only a few cells become iPS cells, very slowly

Hanna, J. et al, *Nature* **462**, 595–601, 2009

- **Aside from annoying people and limiting applications**

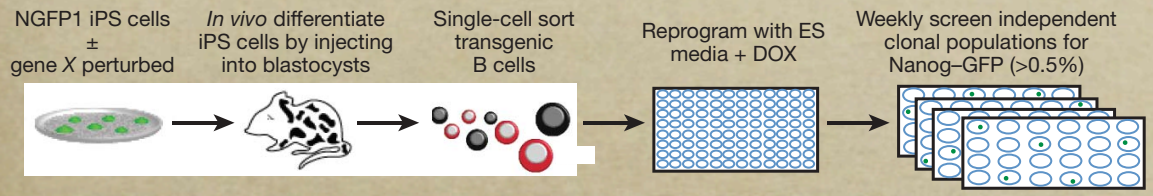
WHY?

- **deterministic for an elite set of cells?**
- **stochastic for all?**
- **stochastic for elite?**

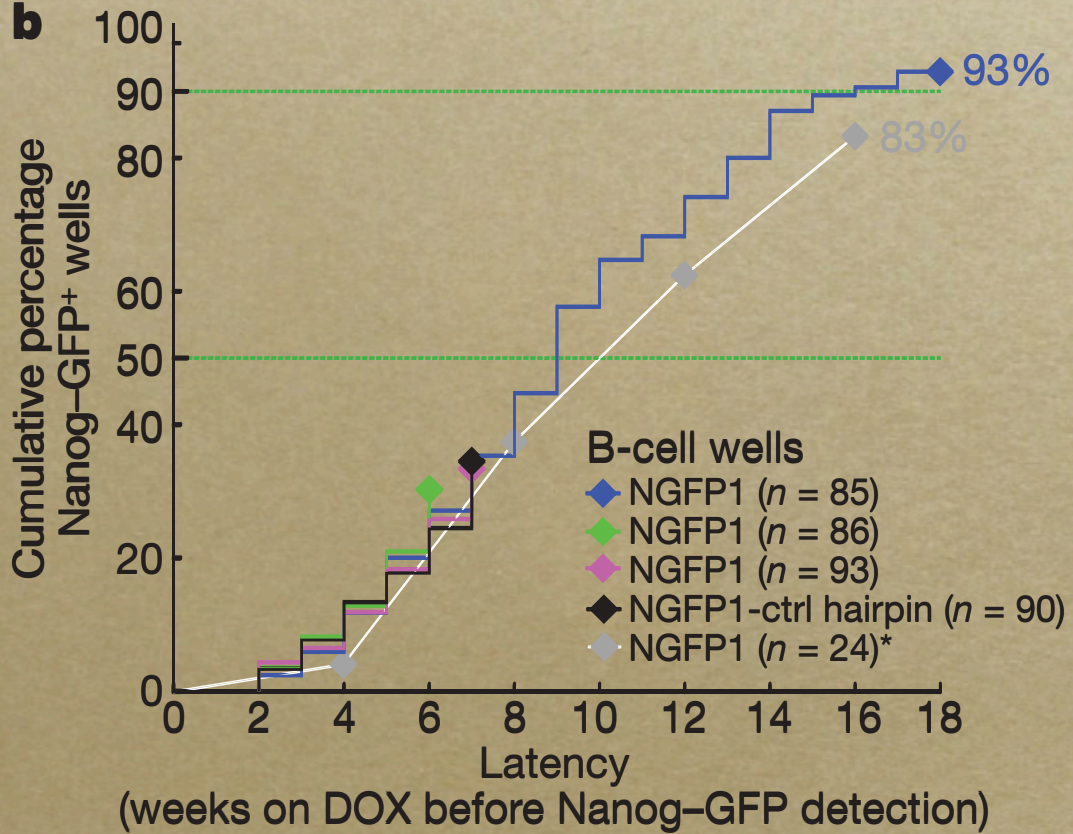


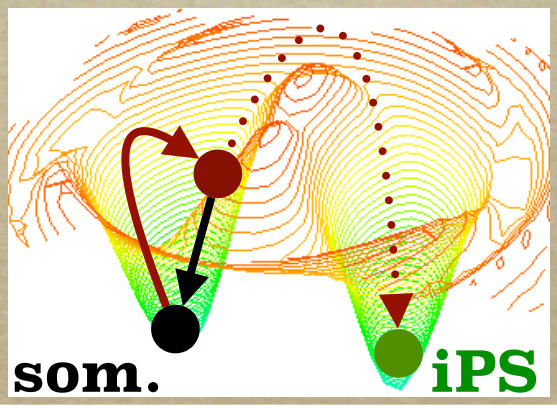
iPS reprogramming is a stochastic process, accessible to every cell

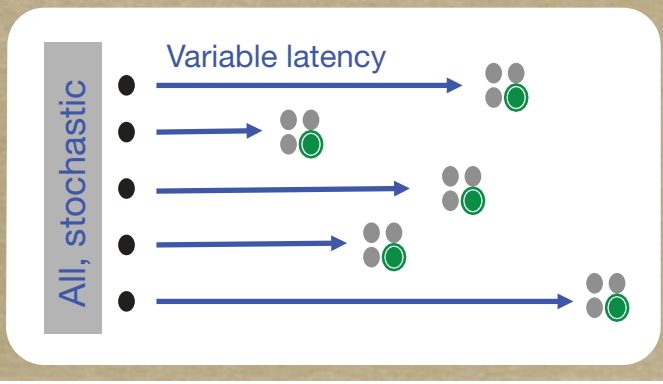
Hanna, J. et al, *Nature* **462**, 595–601, 2009



- **NGFP1 iPS cell line**
  - ➡ **Nanog–GFP fibroblasts**
  - ➡ **dox-inducible lentiviral vector (Oct4, Sox2, Klf4, c-Myc)**
  - ➡ **injected into host blastocysts**
  - ➡ **secondary chimaeras**



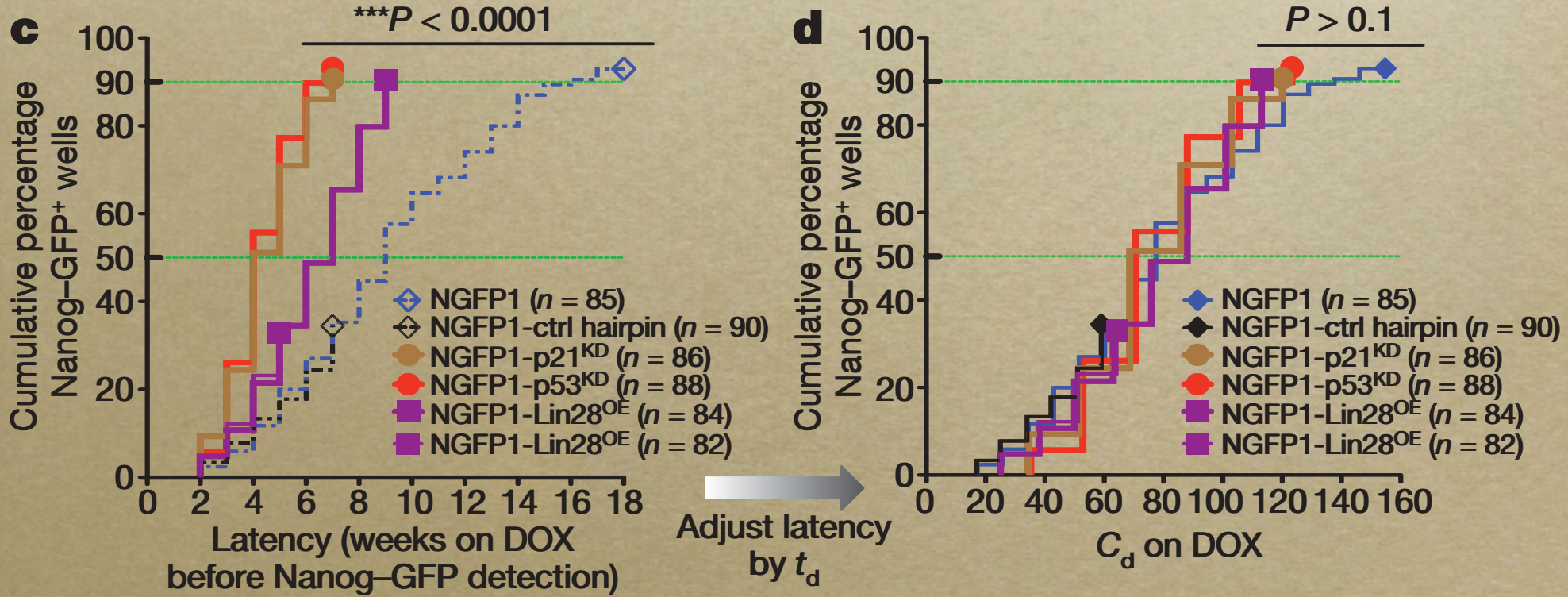




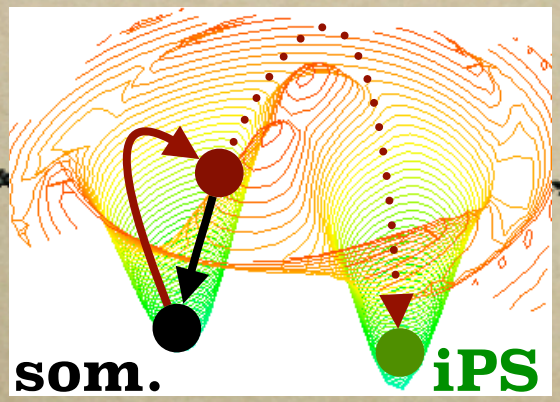
Reprogrammed cells emerge more often if cells proliferate more often

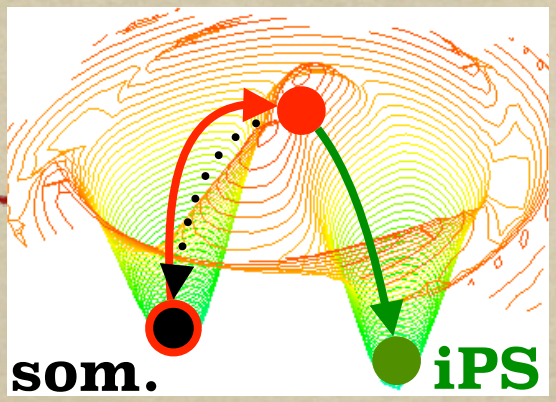
Hanna, J. et al, *Nature* **462**, 595–601, 2009

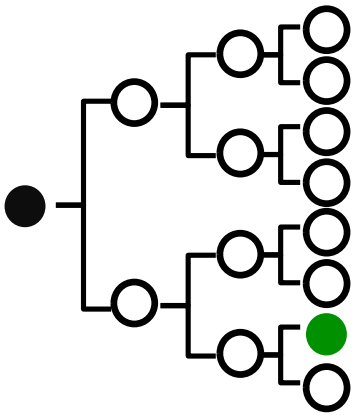
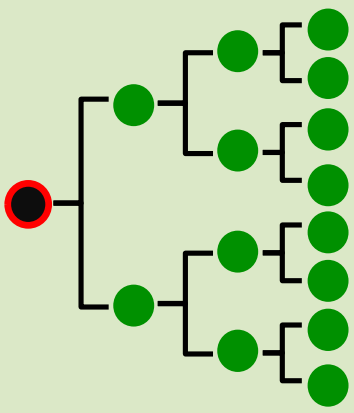
- **p21 or p53 knockdown**



Stochastic versus privileged reprogramming





Reprogramming behavior	Stochastic reprogramming	Privileged reprogramming
Progeny resulted in pluripotency	Few	Most or all
Latency	Long	Short
Kinetics	Asynchronous	Largely synchronous
Prevalence	Common	Rare
Hypothetic lineage scheme		

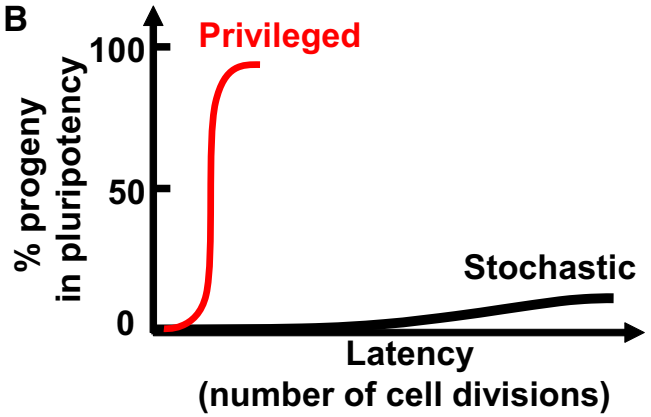
= Somatic founder cell

= Privileged somatic founder cell

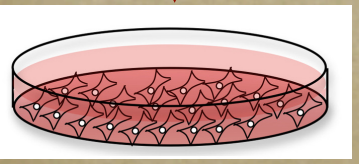
= Progeny failed to reprogram

= Pluripotent progeny

B



Approach: Oct4:GFP cells + virus with inducible Yamanaka factors



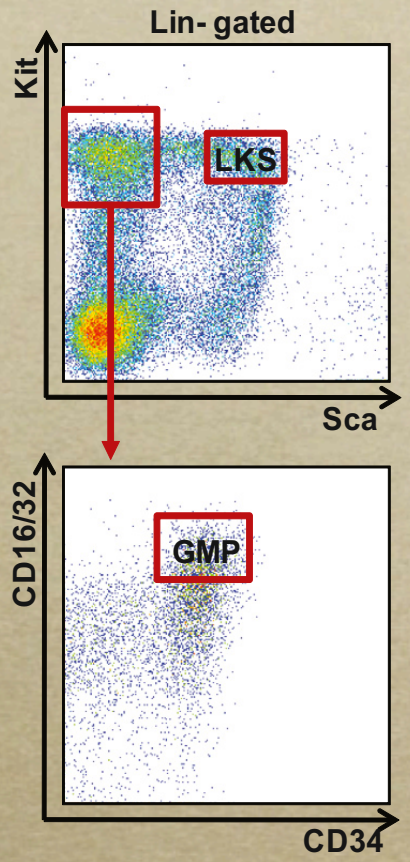
Oct4:GFP x Rosa26:rtTA

X

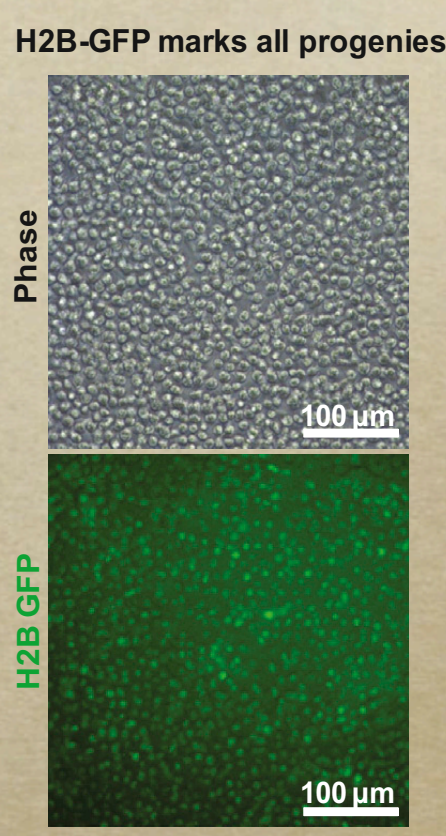
H2B-GFP transgenic line
marking ALL cells

+

lentivirus with
dox-inducible
Yamanaka factors:
Oct4, Sox2, Klf4, c-Myc



C



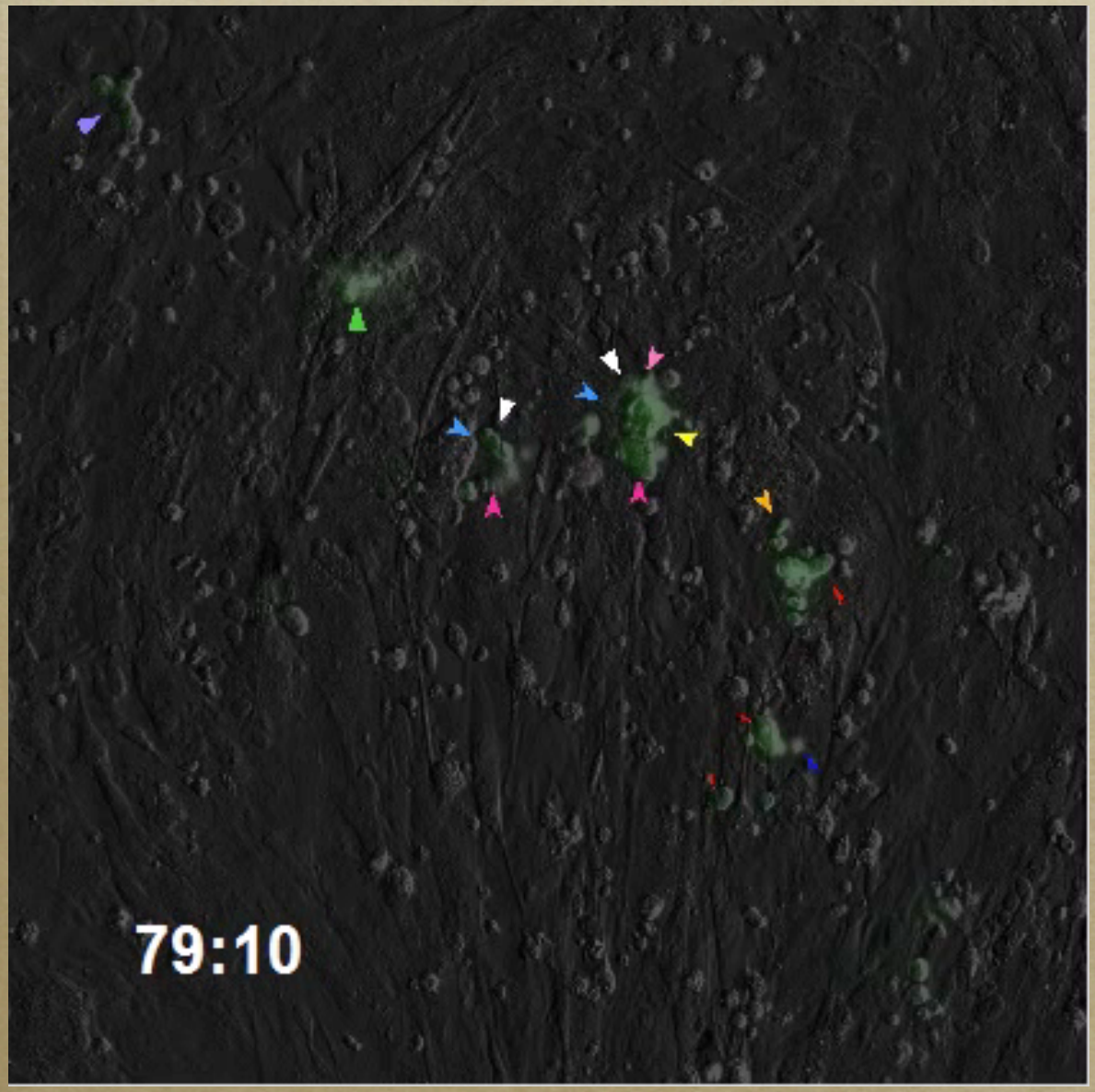
GMP

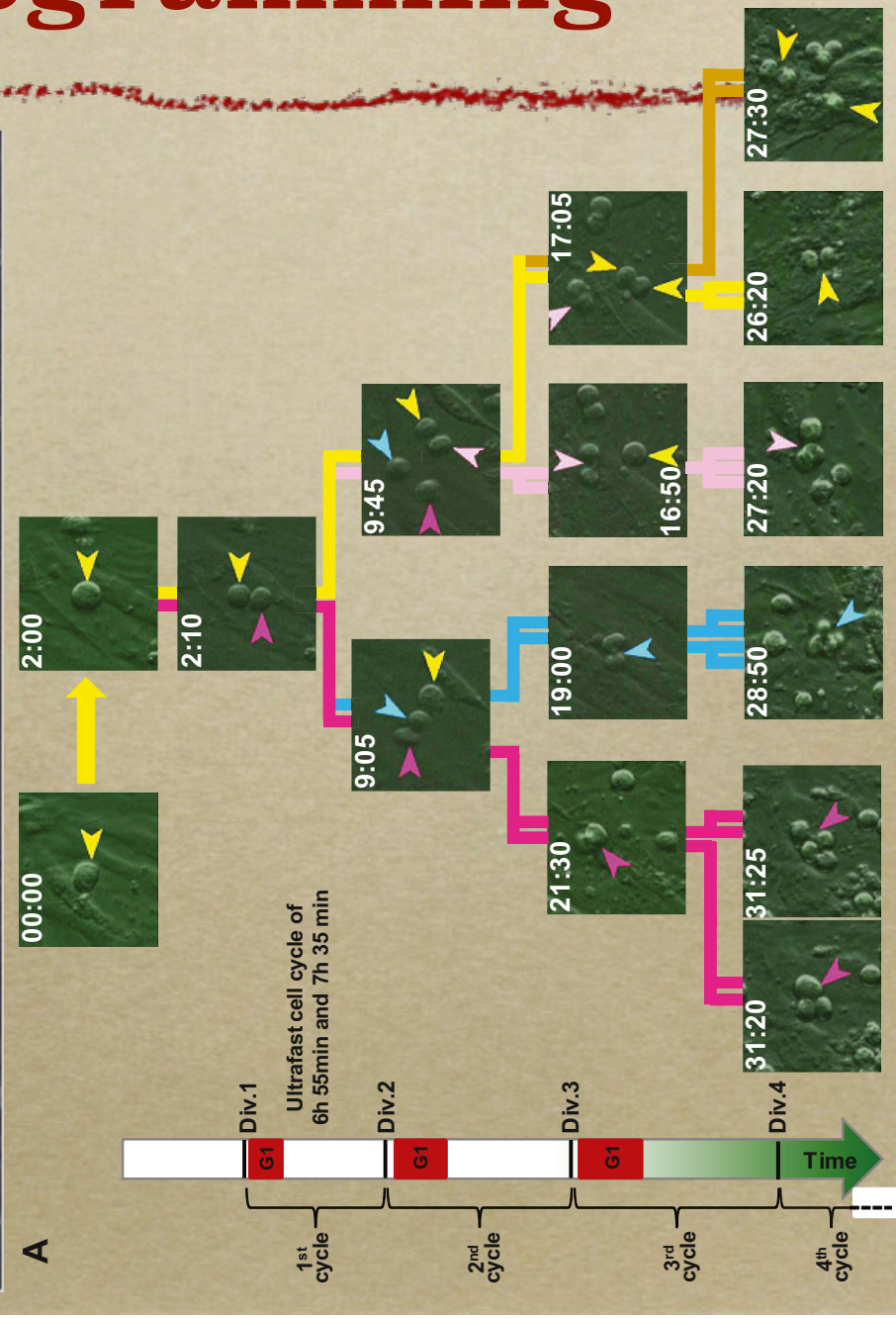
granulocyte monocyte progenitors

LKS

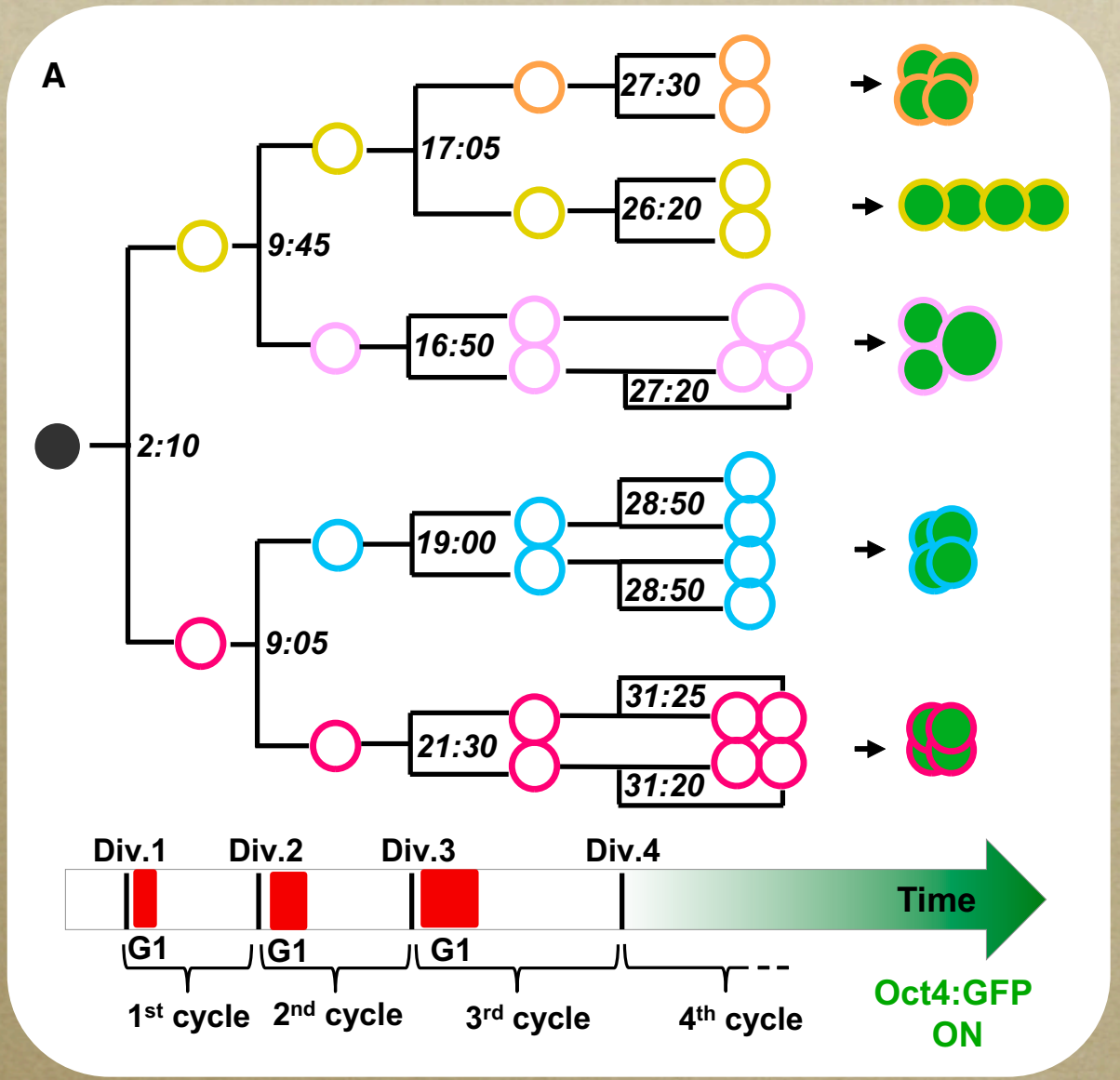
single lineage-negative-Kit+Sca+ HSPCs

A subset bone marrow GMPs show non-stochastic reprogramming

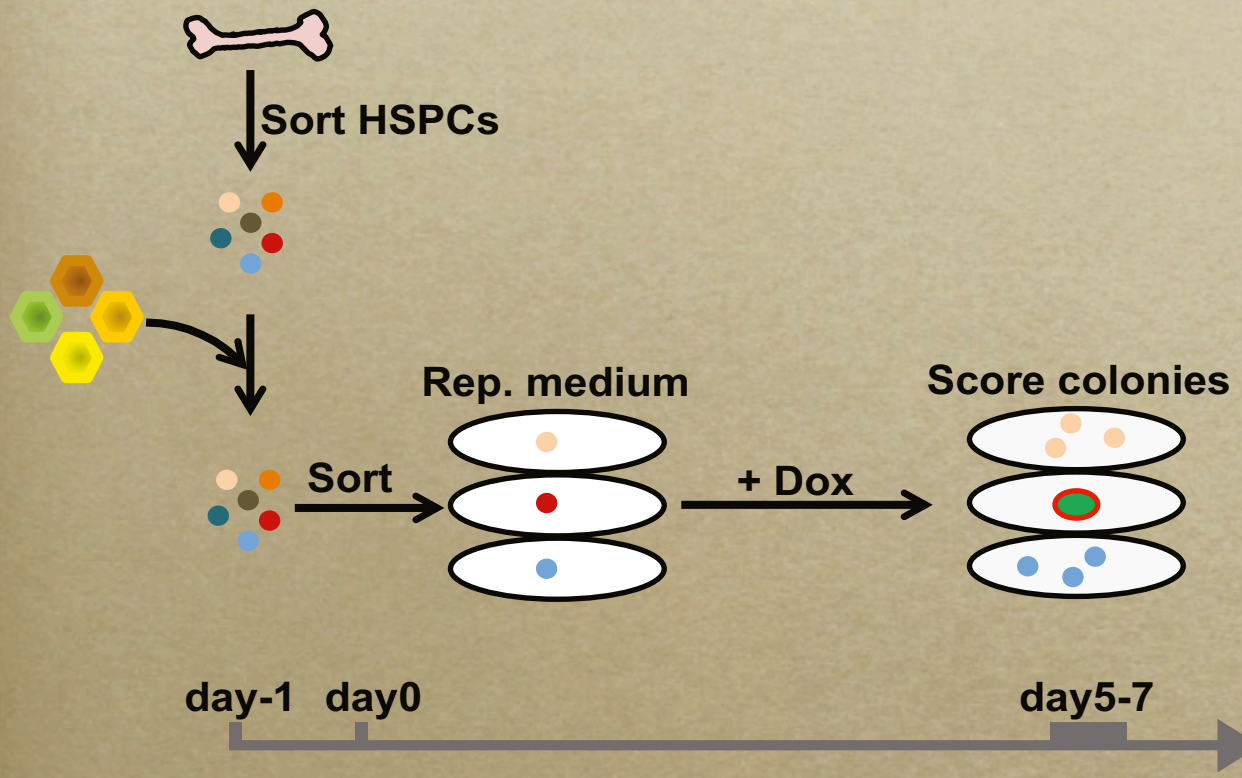


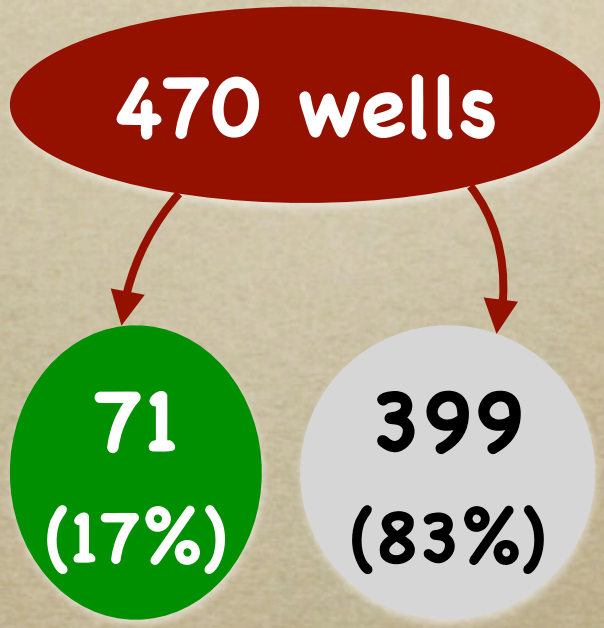


For certain parent cells, every descendant was reprogrammed!



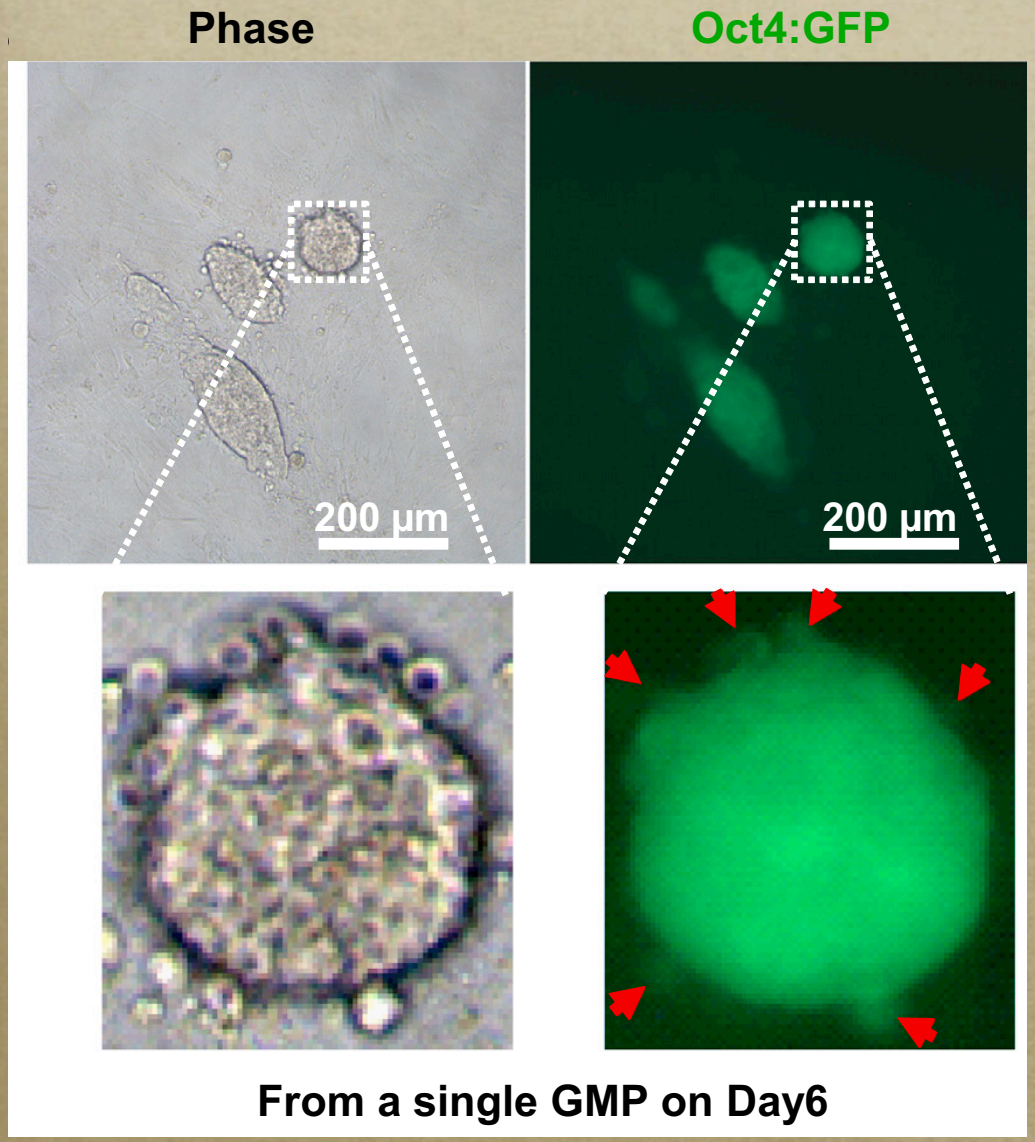
Really? — Single-cell approach



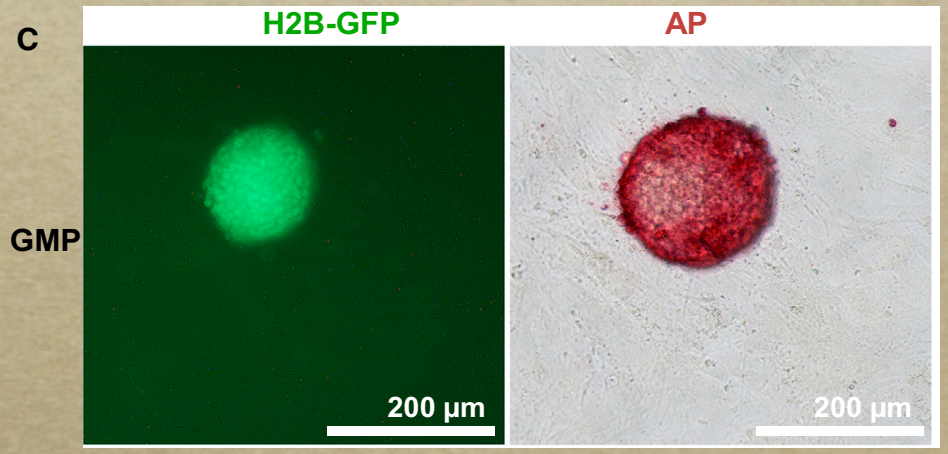


d-1: Sort from BM and transduce with 4F
d0: Single cell sort into 96 wells containing reprogramming medium
d5-7: Score wells containing Oct4 GFP+ colonies

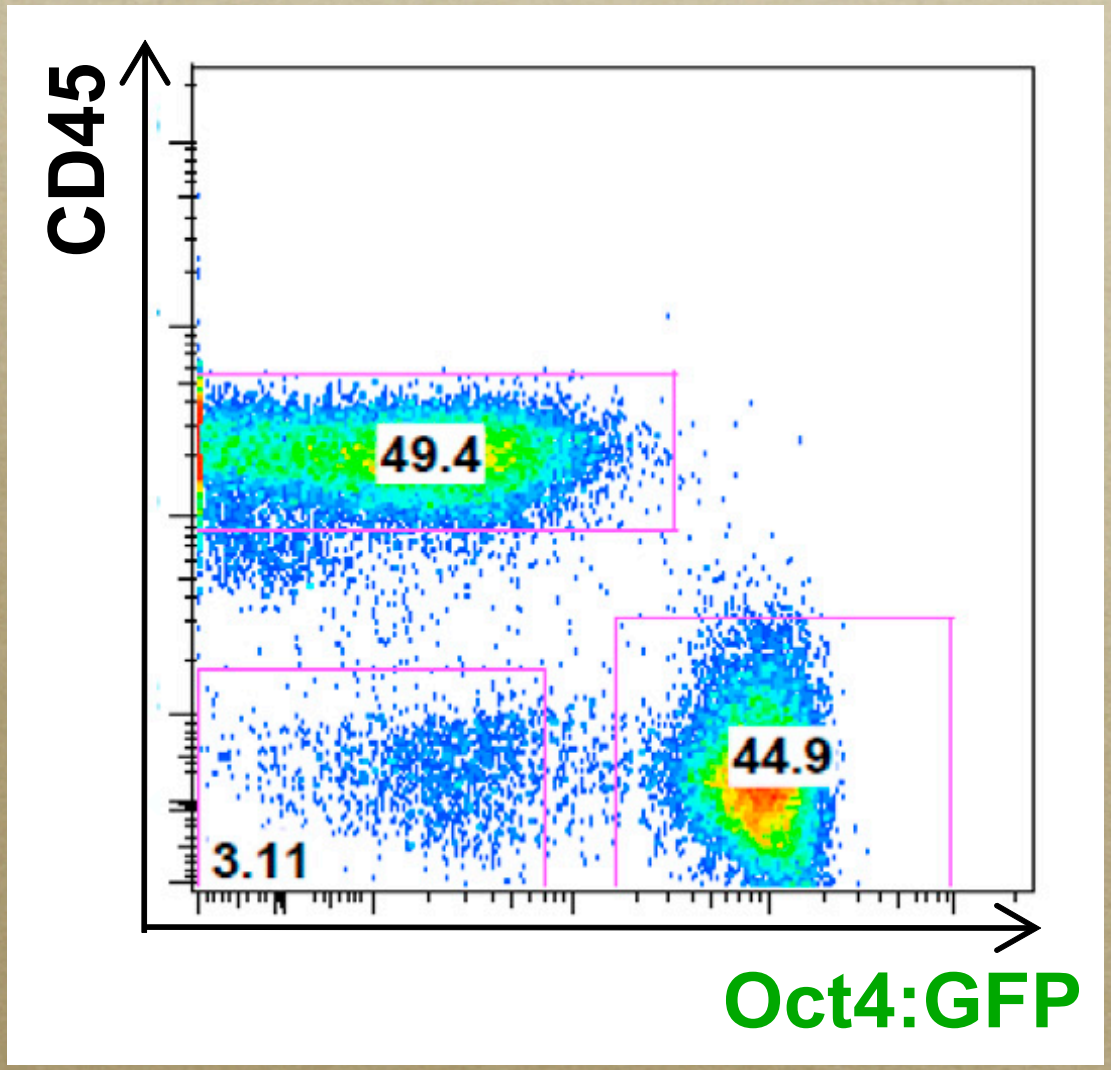
97% of GFP (Oct4)+ colonies had no hematopoietic cell left!



- **no alkaline-phosphatase (AP) negative cells left!**



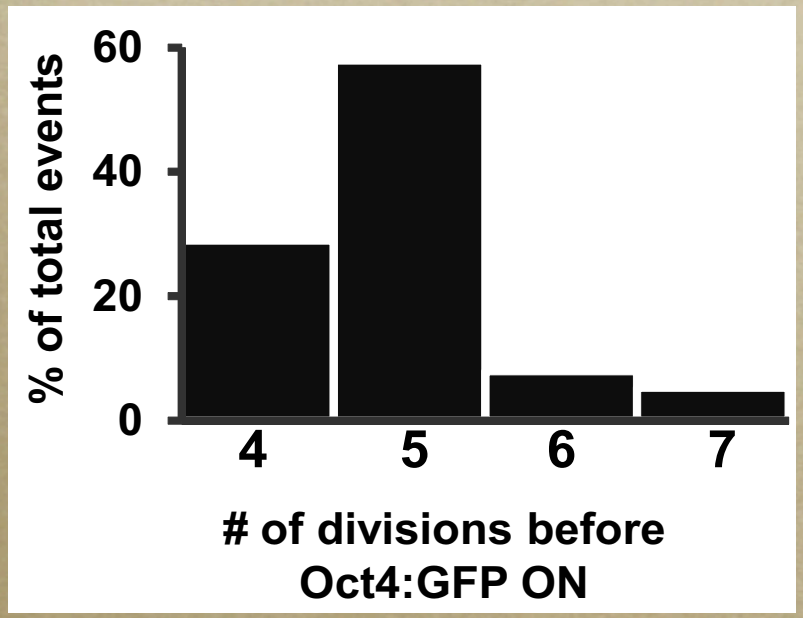
GMP or iPS - there is no third option



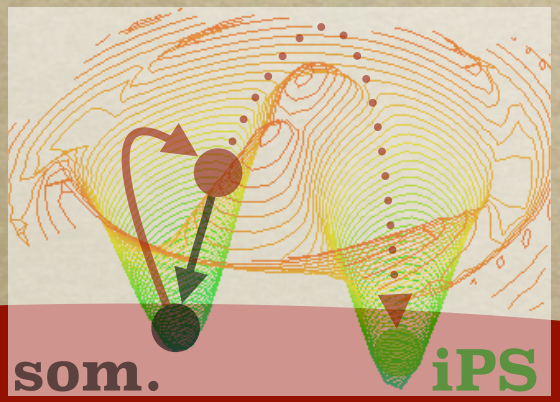
GMP XOR iPS

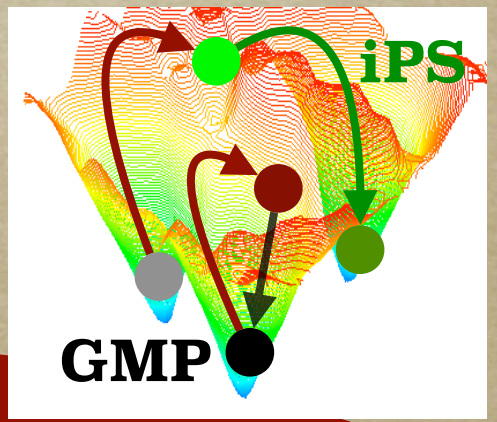
Day 6

Reprogramming from privileged state => short, uniform latency



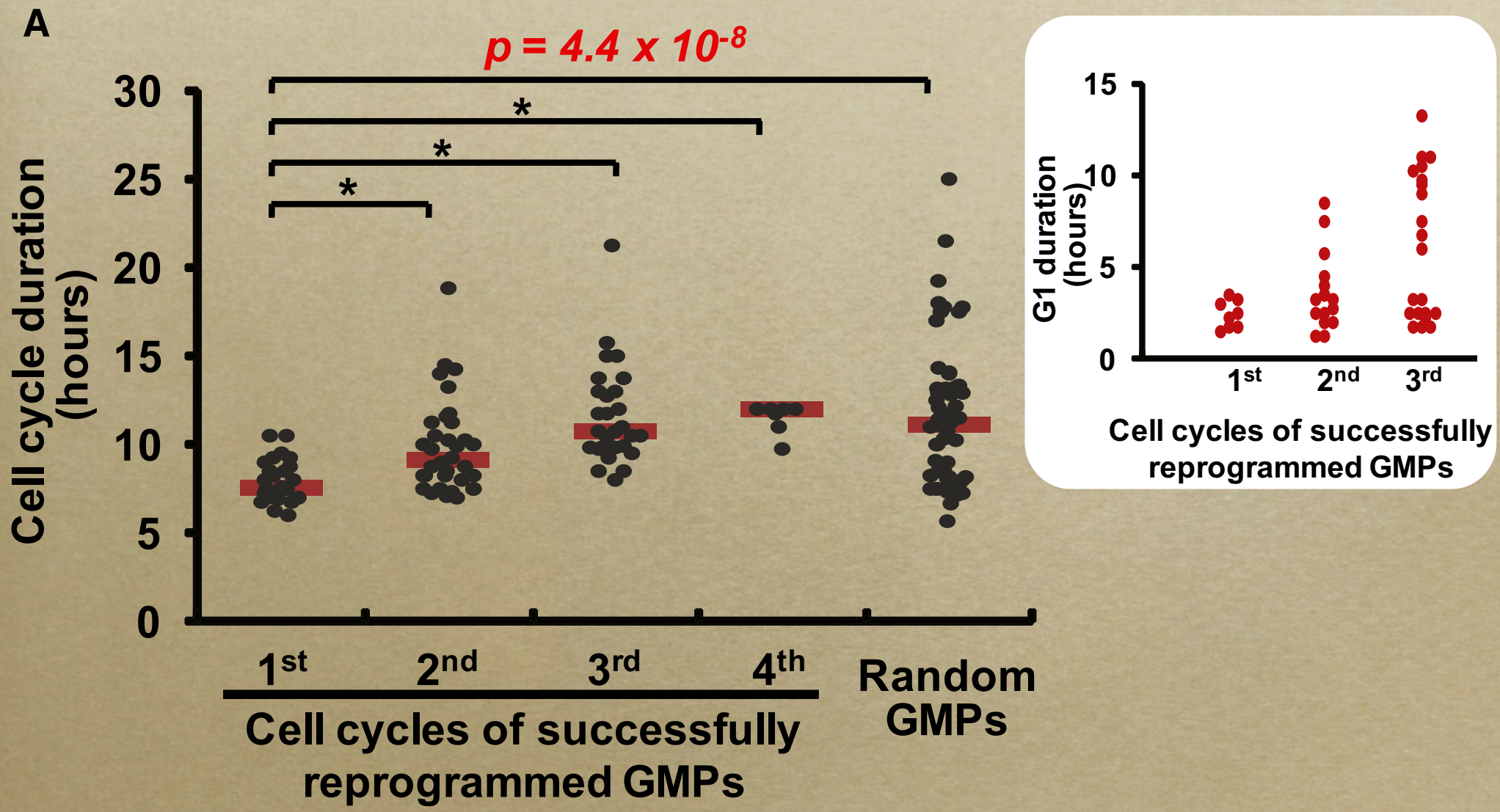
- all progeny -> Oct4:GFP+ within 46.0 ± 6.8 hr (n = 38)
- highly consistent among the 14 GMP lineages across five experiments





Evidence for a privileged somatic state => deterministic reprogramming!

Privileged GMPs have a very short cell cycle, especially G1



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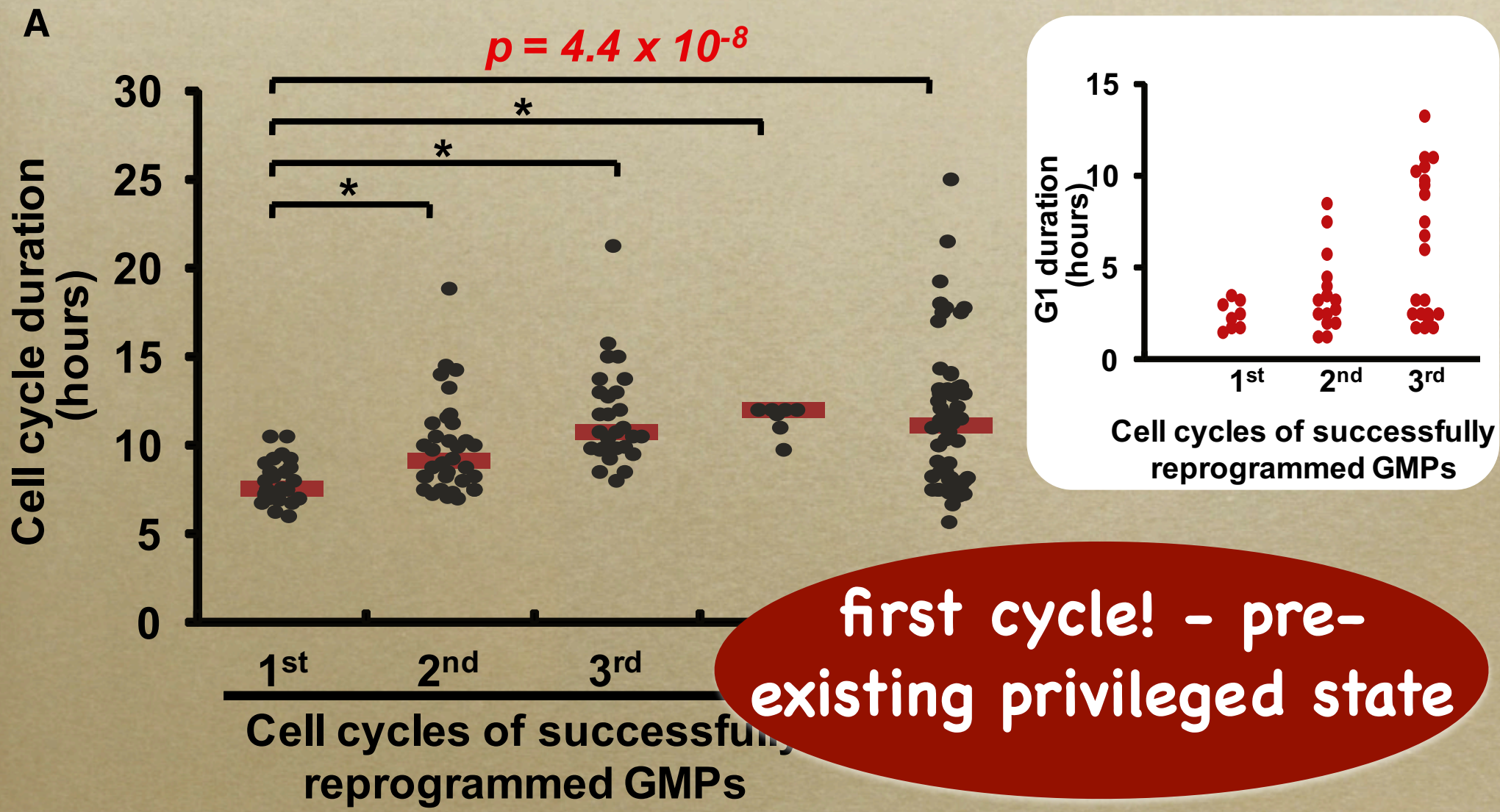
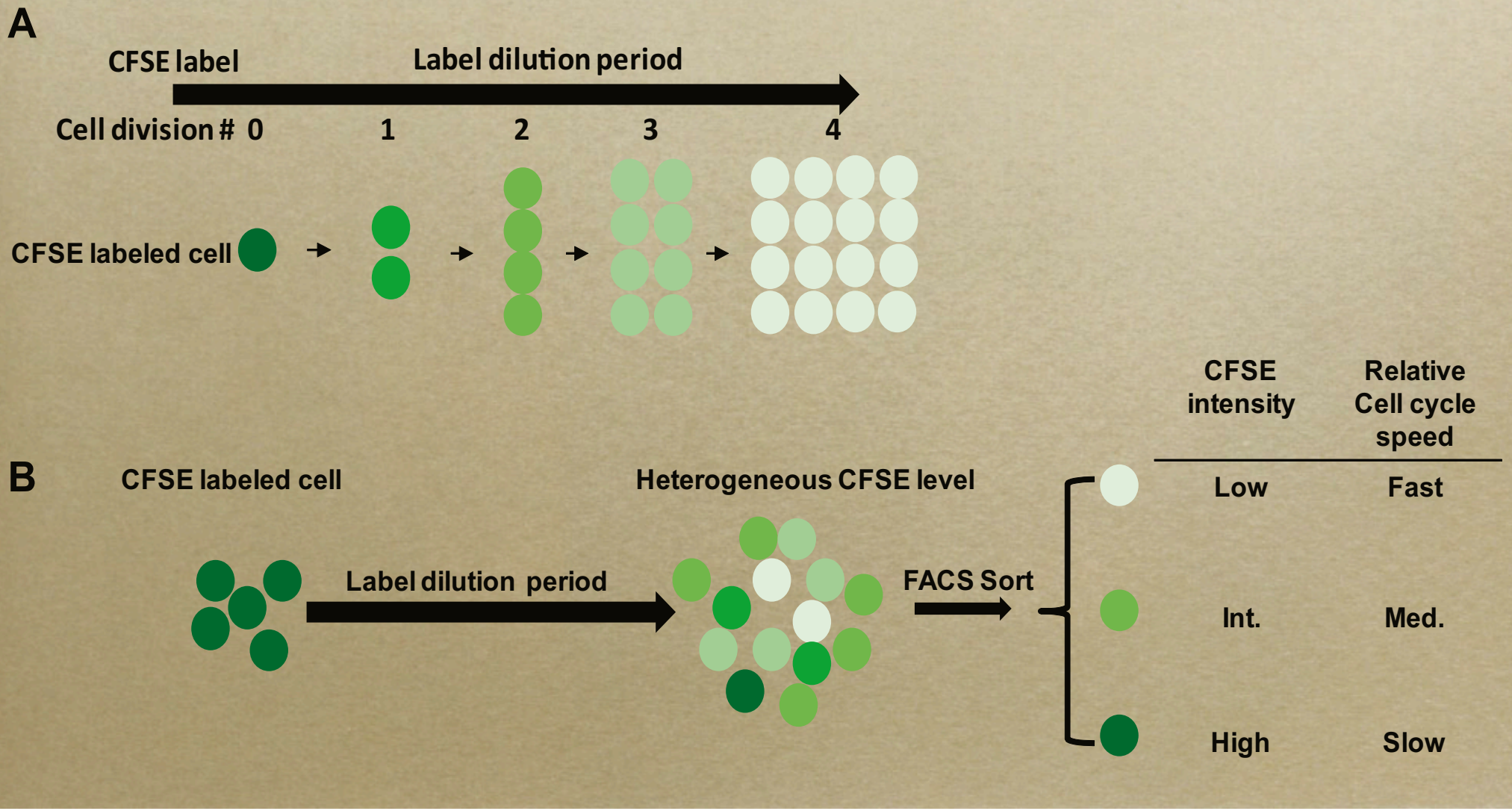


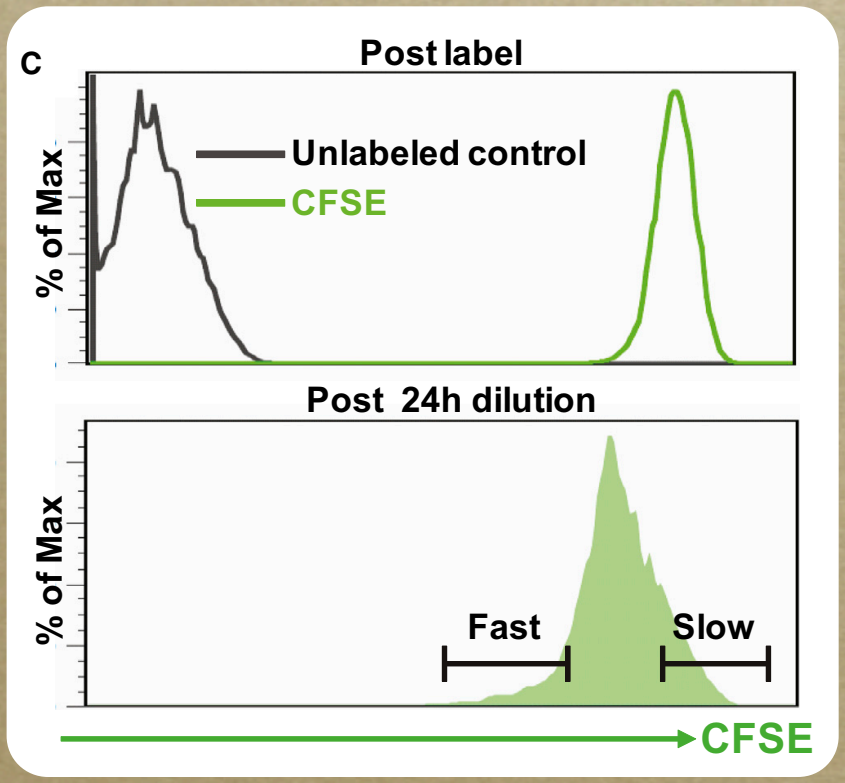
Conversely: are GMPs with short cell cycle privileged?

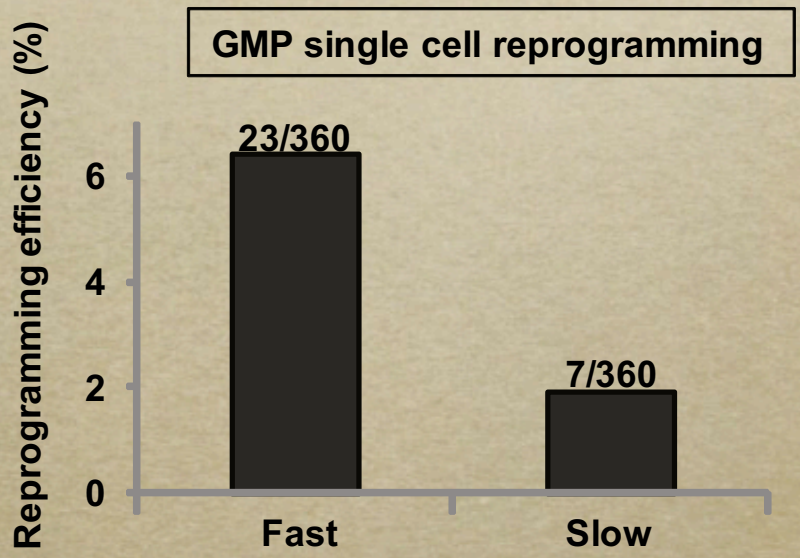
- **die dilution experiment!**



Indeed, faster cycling cells reprogram more often

- **GMPs labeled with stable die CFSE**
- **24h dilution => fast cycle = less die**





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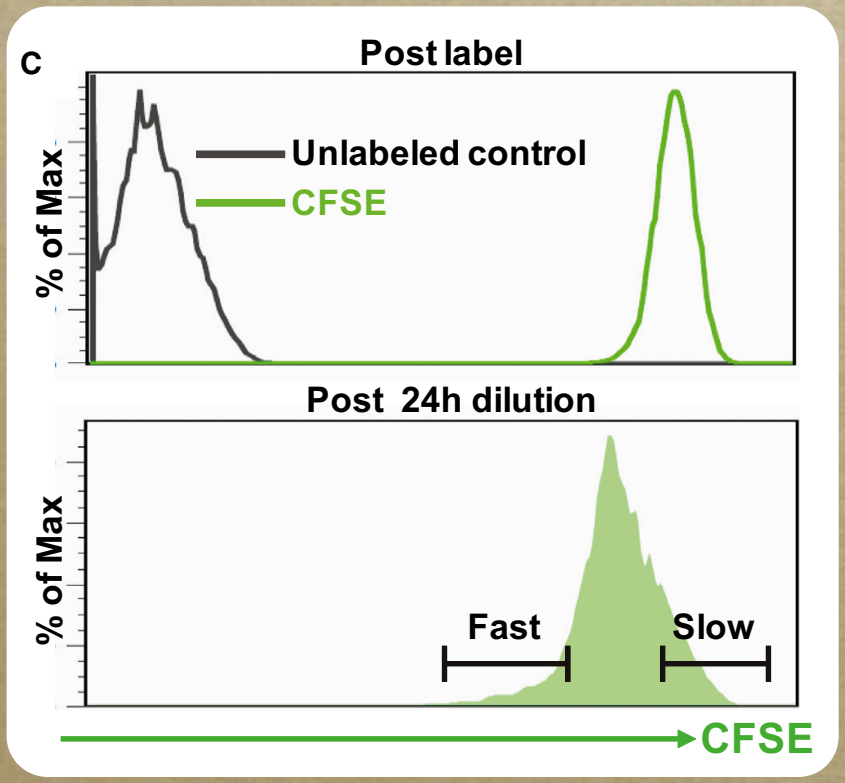
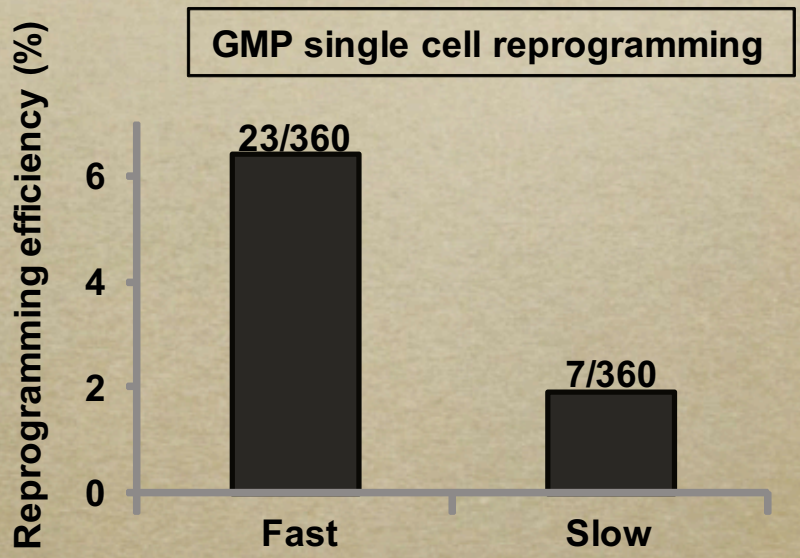
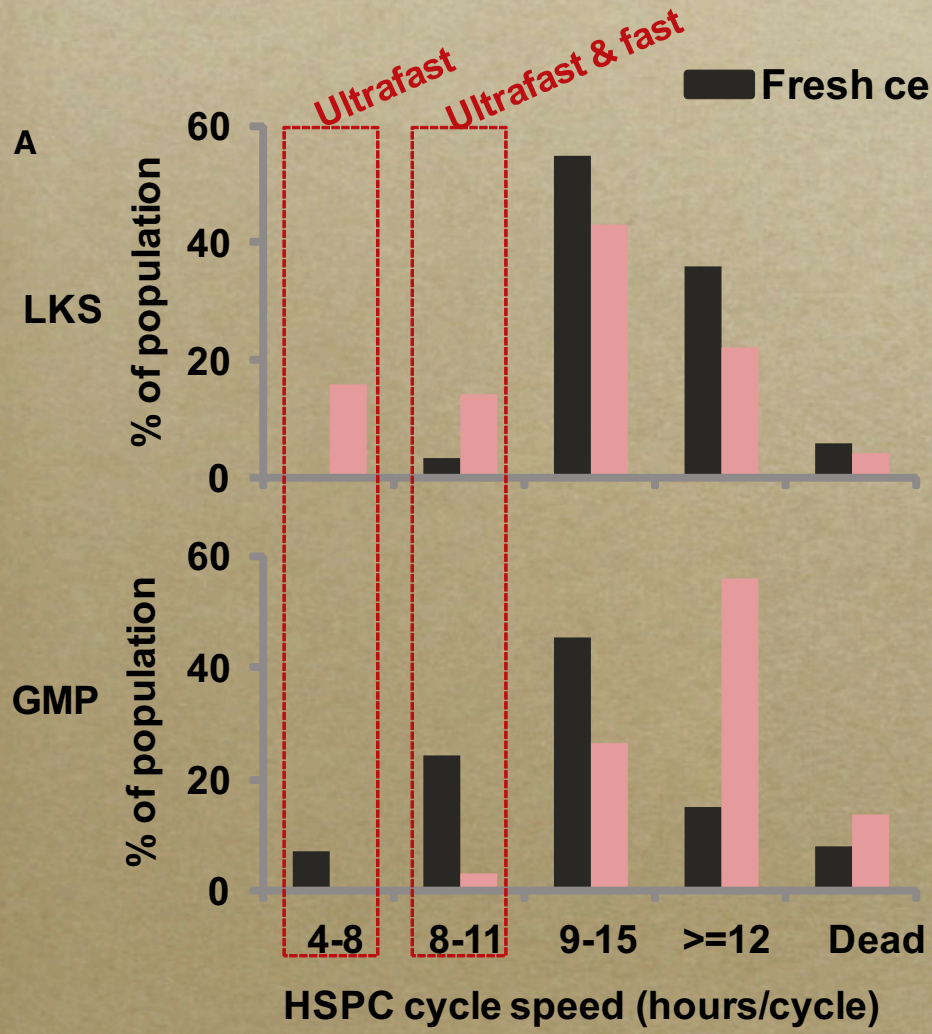


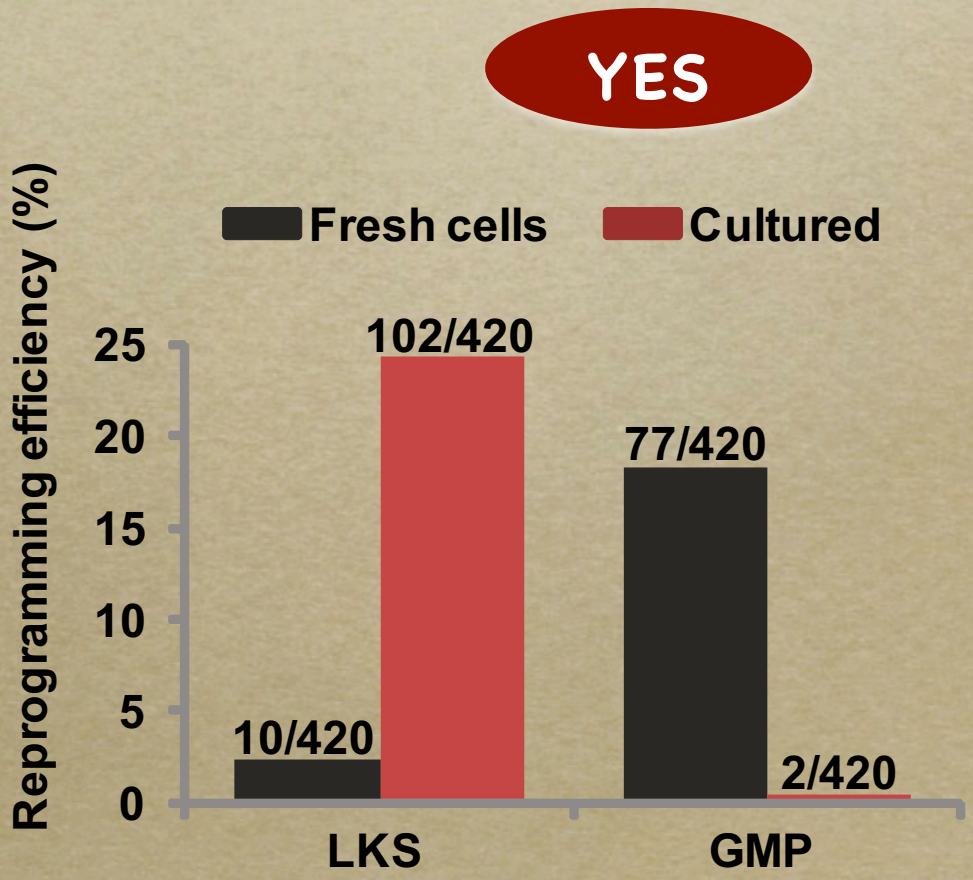


Low %!
FACS likely disturbs the fast-cycling state

So, can we speed up the cell cycle to help reprogramming?

- **HSPCs after 5 days in culture (GF + cytokines)**

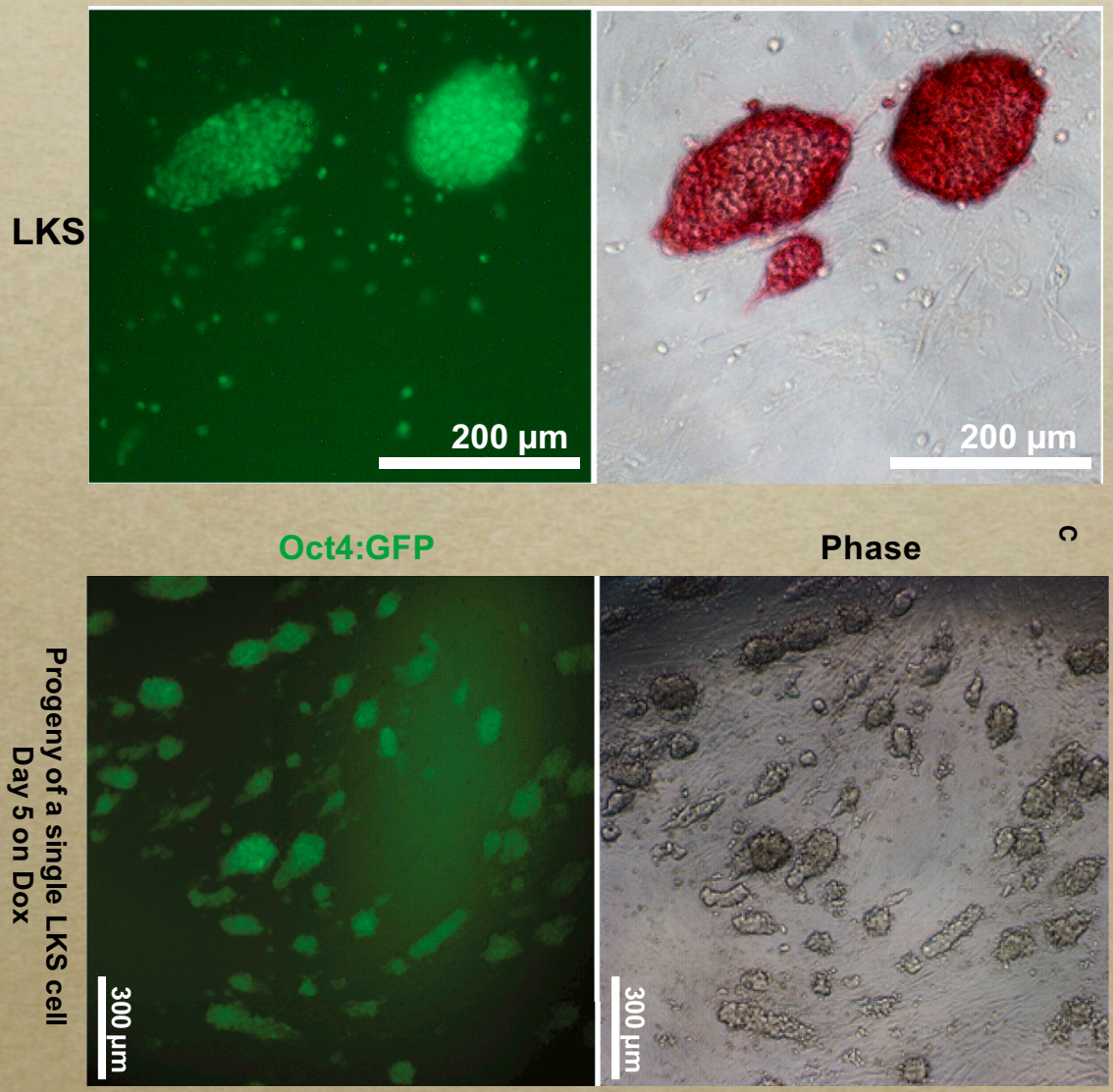




Privileged reprogramming emerges among LKS cells!

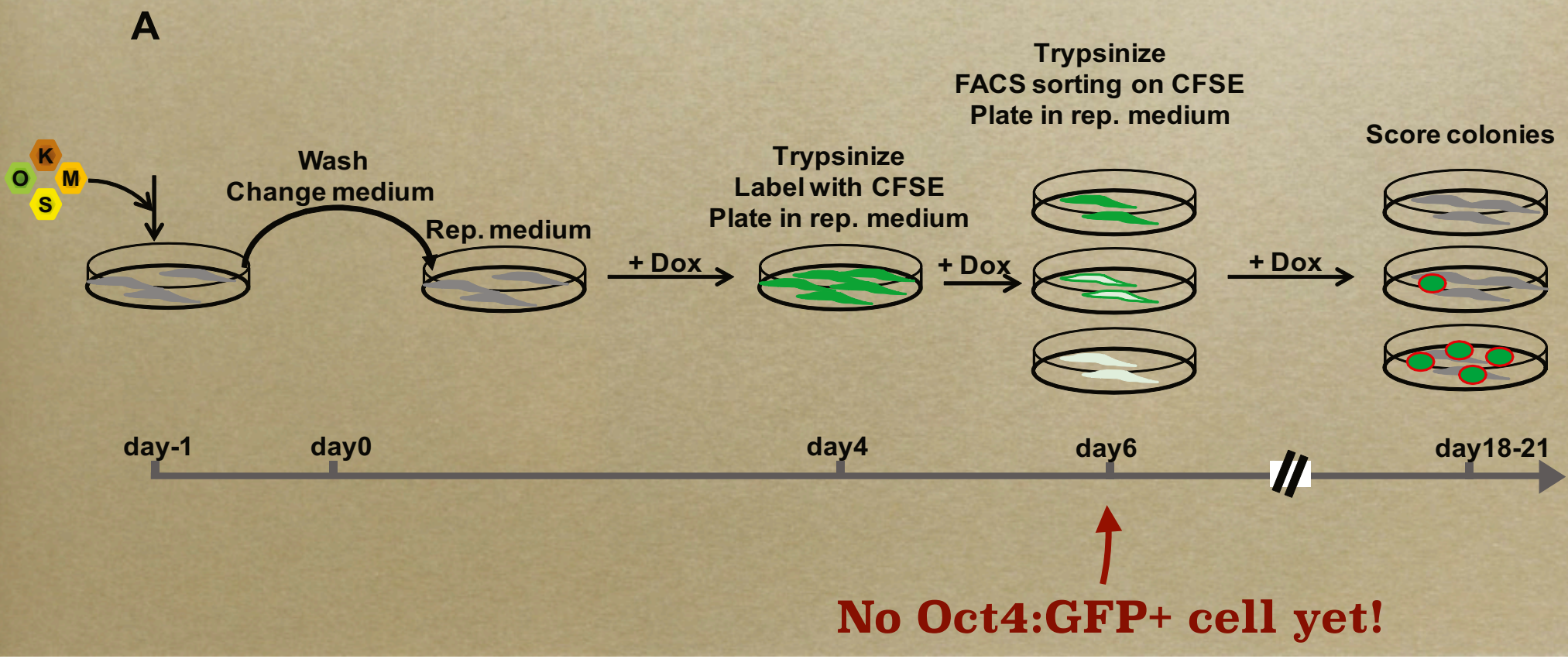
- progeny of a single **freshly isolated** LSK
- **some** reprogrammed

- progeny of a single **cultured** LSK
- **all** reprogrammed
- 15% wells with Oct4:GFP+ cells have *no* HPSC!



The Yamanaka factors include c-MYC… Can they *induce* the privileged state?

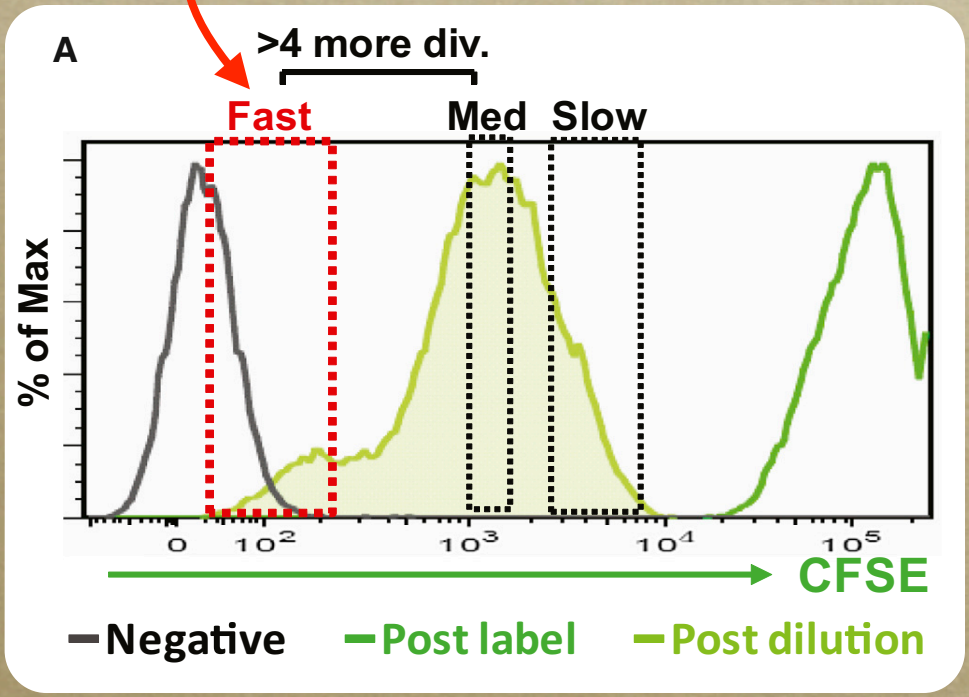
- **MEFs from E13.5 embryos**
  - ➡ **0.1% reprogramming, long latency**
  - ➡ **no fast-cycling cells**

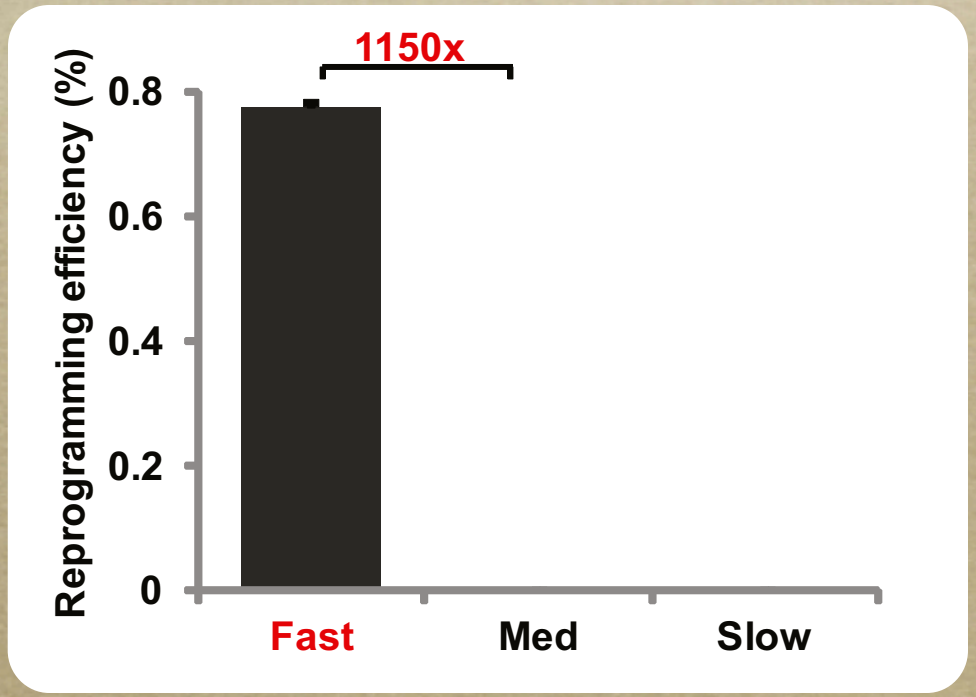


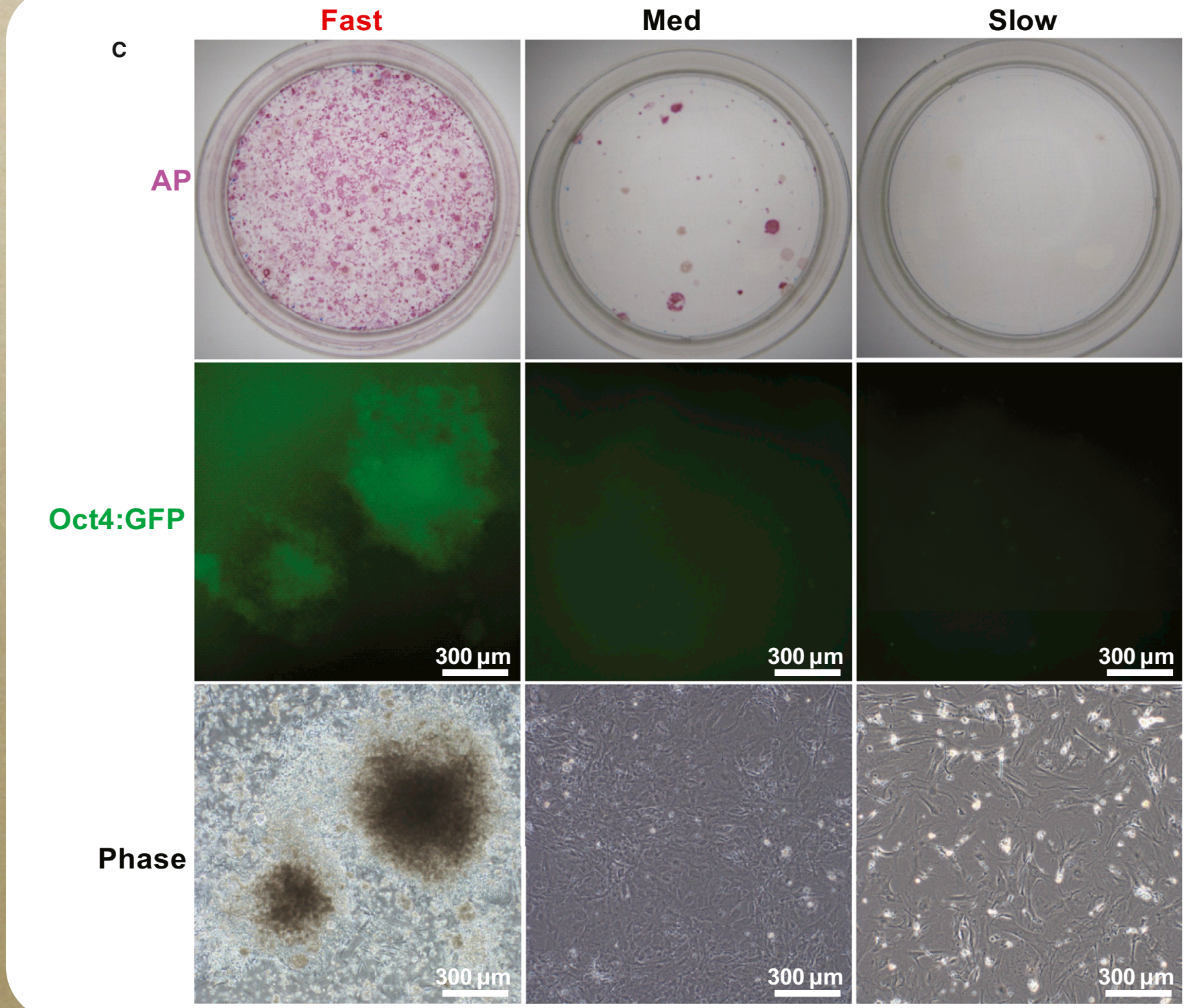
Nearly all MEF reprogramming comes from (induced) fast-cycling cells

1%-6% fast-cycling cells induced by 6 days of dox treatment

>= 4 divisions in 48h (average = 1 or 2 / 48h)



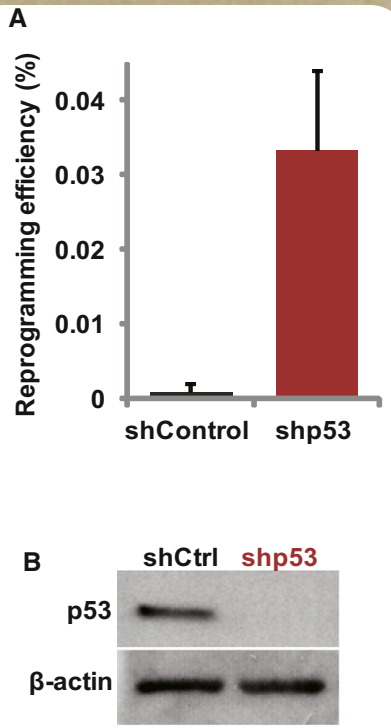



c
Fast
Med
Slow
AP
Oct4:GFP
Phase
300 μm
300 μm
300 μm
300 μm
300 μm
300 μm

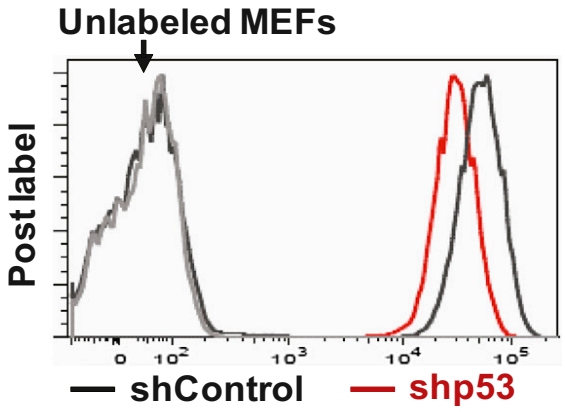
Known: increased proliferation => more reprogramming. But why?

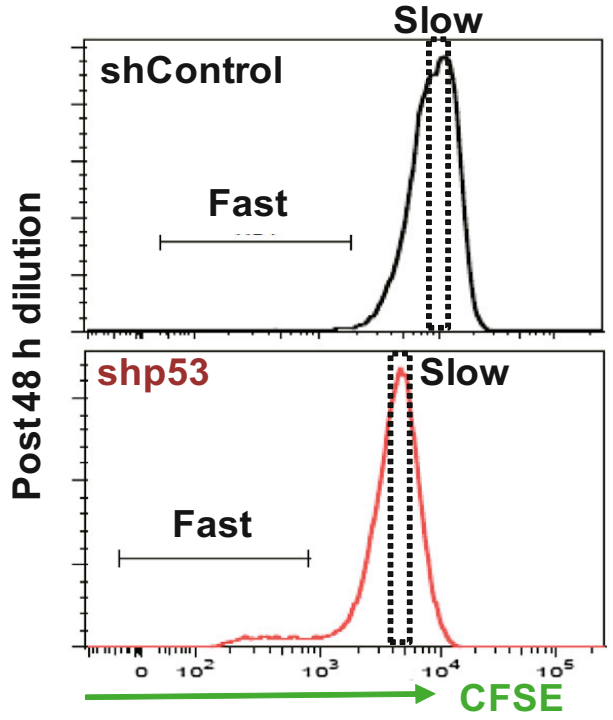
Q vs. **more cells to choose from ?**
more fast-cycling cells ?

- **MEFs, p53 knockdown => more reprogramming (expected)**



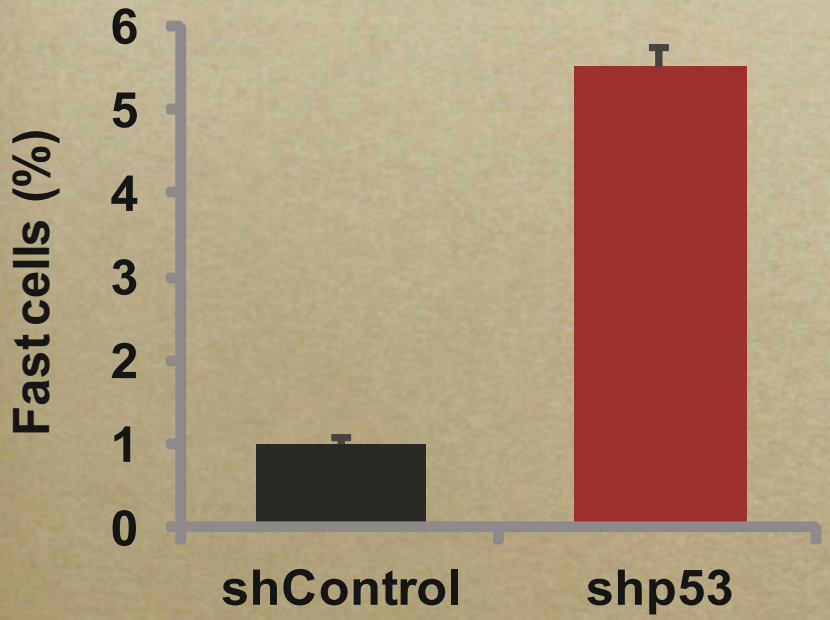
- **fast-cycling cells do emerge**



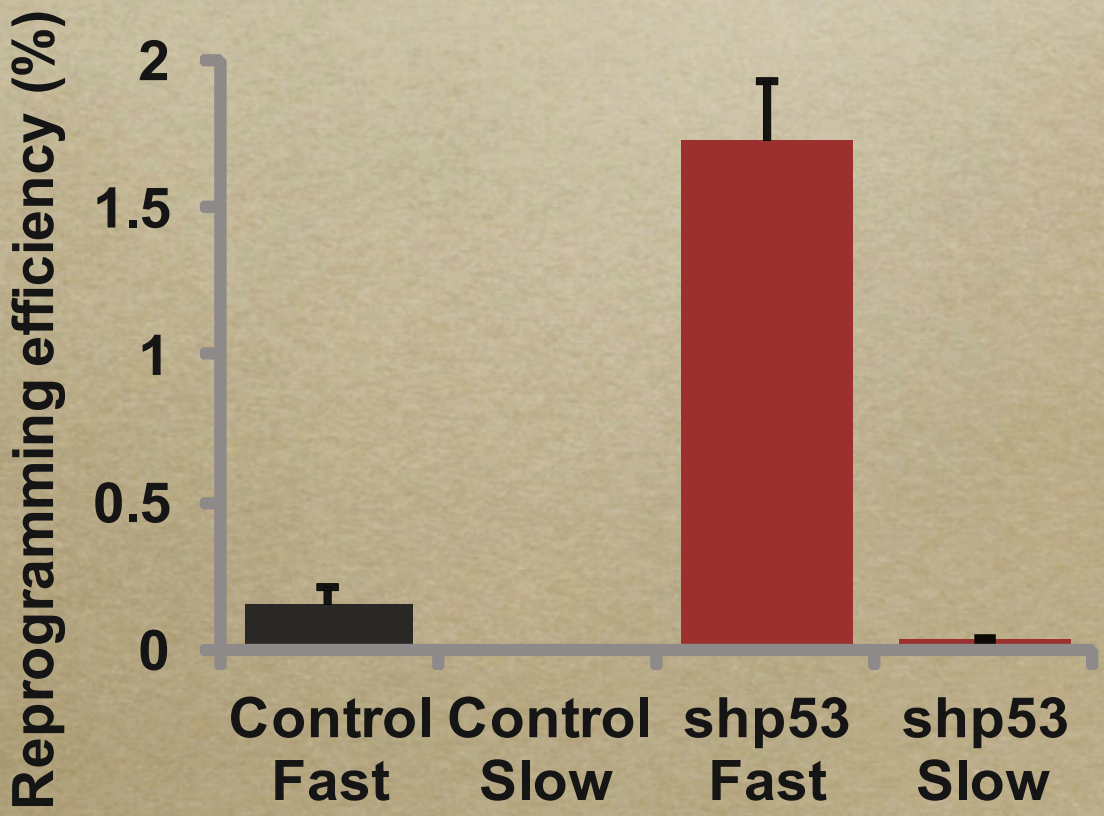


Again, nearly all iPS cells came from fast-cycling cells!

more fast-cycling cells

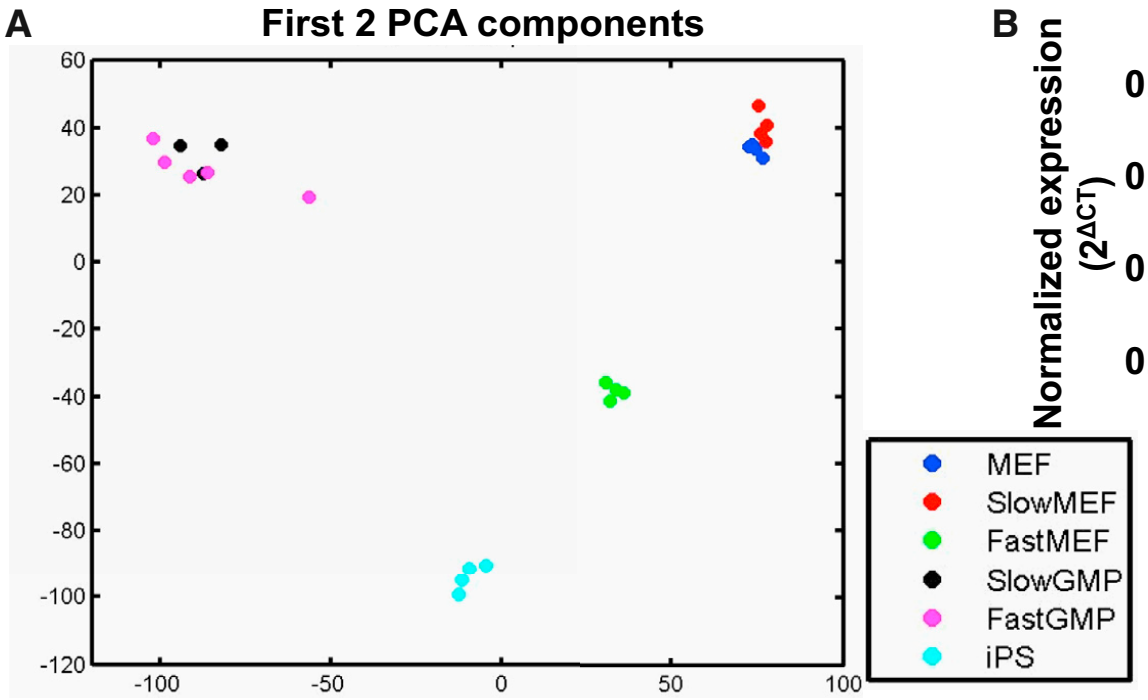


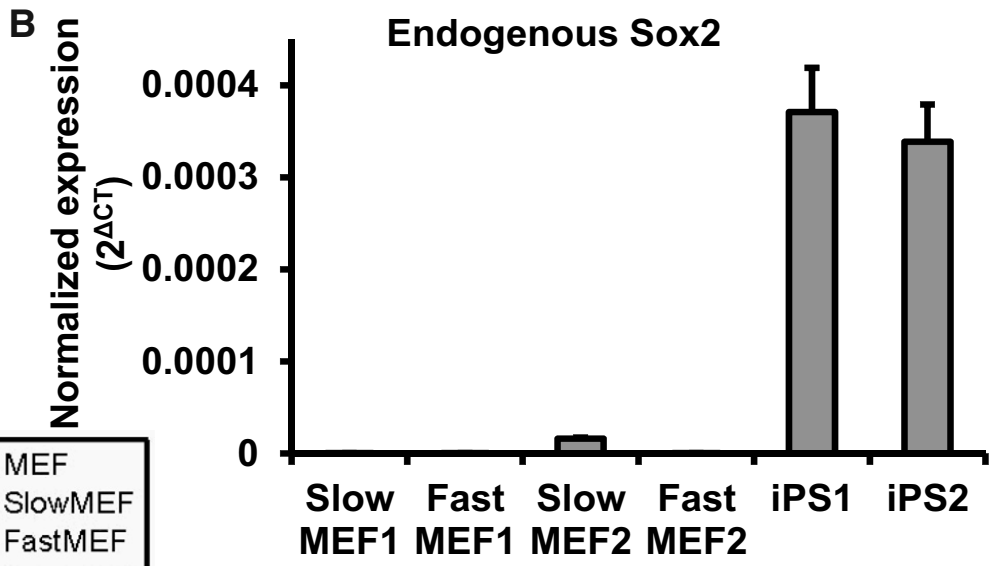
only fast-cycling cells become iPS cells!



How different are fast-cycling cells?

- **RNA-seq on fast vs. slow subpopulations**

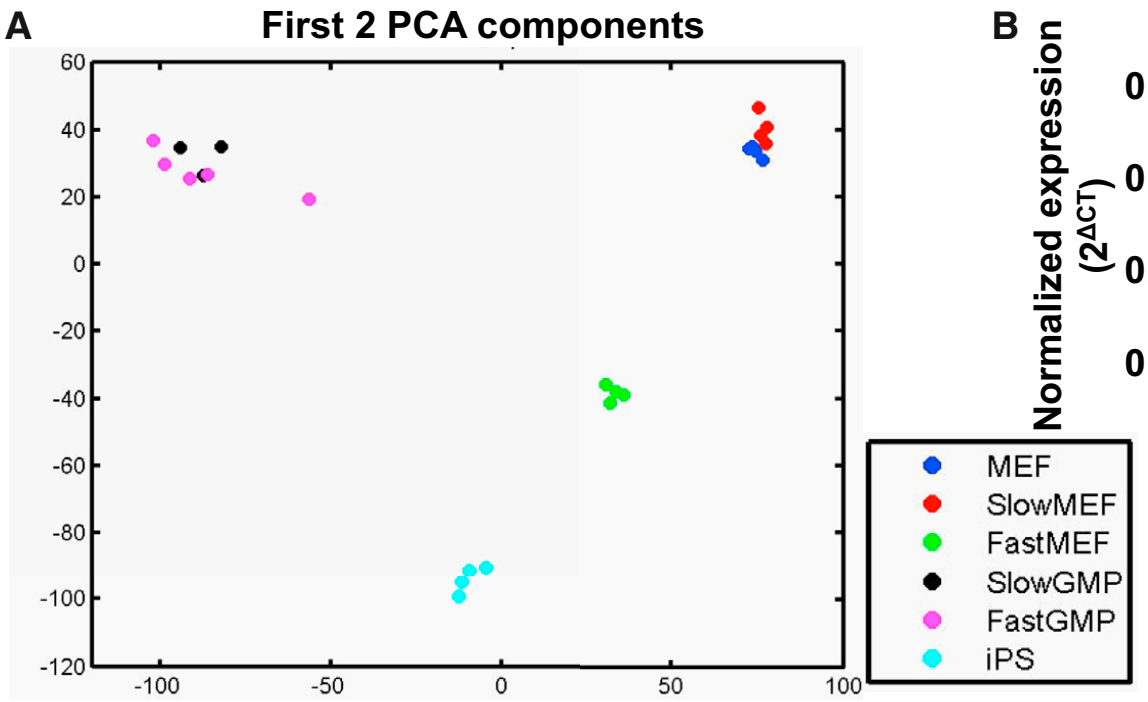


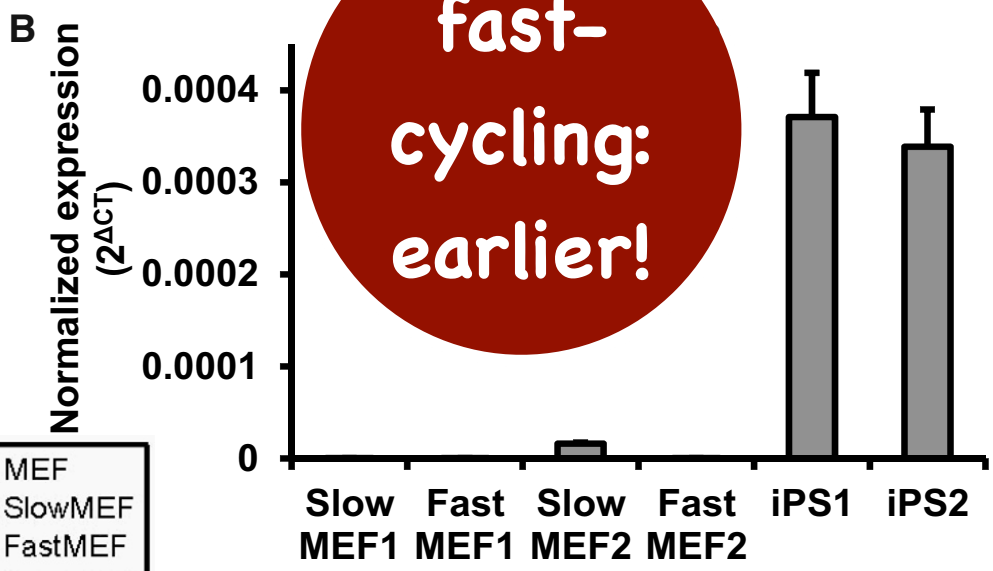


Sox2 activation: earliest known point of commitment

How different are fast-cycling cells?

- **RNA-seq on fast vs. slow subpopulations**





fast-cycling: earlier!

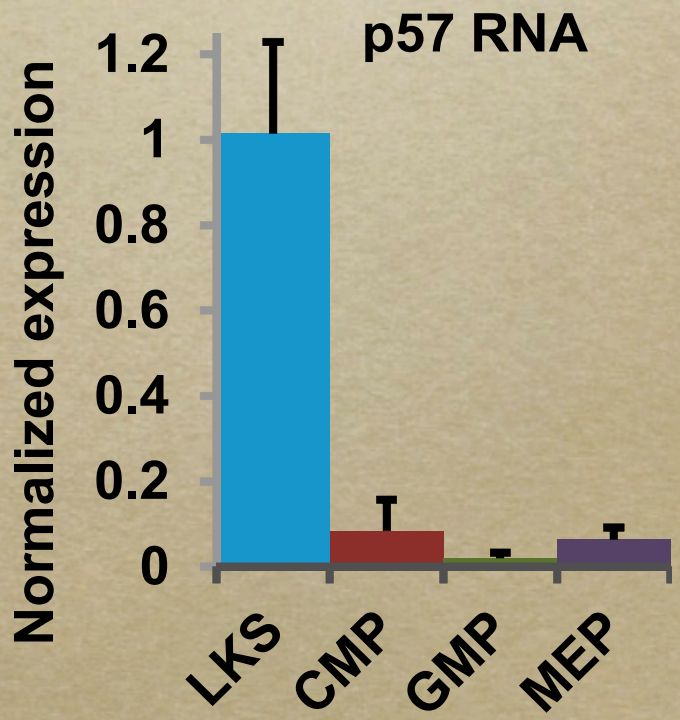
Sox2 activation: earliest known point of commitment

- **slow vs. fast MEFs (dox-induced) — quite different**
- **slow vs. fast GMPs — not so different!**

Naturally, the cell cycle machinery *is* different…

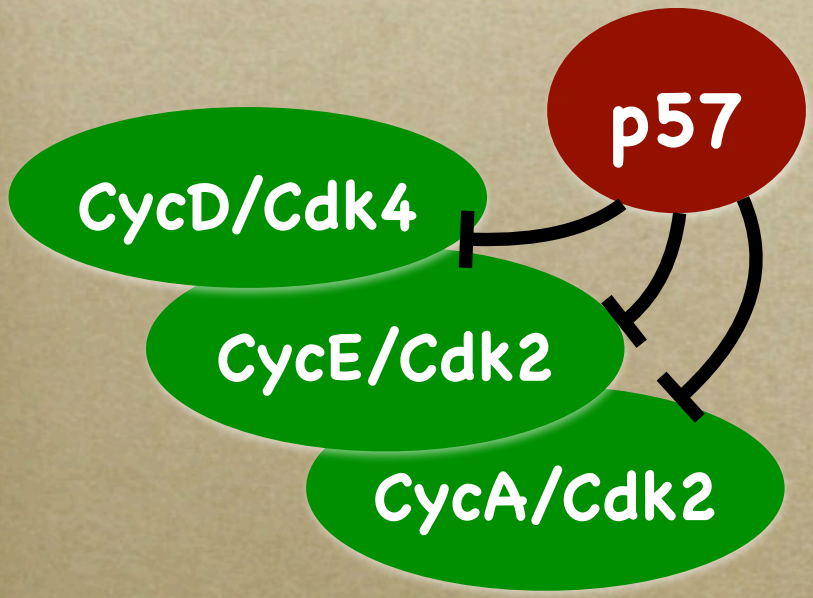
GO Category Name	Related Cellular Process
DNA_REPLICATION	Cell Cycle
CHROMOSOME	Cell Cycle
DNA_DEPENDENT_DNA_REPLICATION	Cell Cycle
M_PHASE	Cell Cycle
CHROMOSOMAL_PART	Cell Cycle
MITOSIS	Cell Cycle
M_PHASE_OF_MITOTIC_CELL_CYCLE	Cell Cycle
REPLICATION_FORK	Cell Cycle
DNA_PACKAGING	Cell Cycle
CELL_CYCLE_PROCESS	Cell Cycle
CELL_CYCLE_PHASE	Cell Cycle
DNA_METABOLIC_PROCESS	Cell Cycle
CONDENSED_CHROMOSOME	Cell Cycle
SPINDLE	Cell Cycle
SPLICEOSOME	RNA Processing
DNA_REPAIR	Cell Cycle
CHROMOSOMEPERICENTRIC_REGION	Cell Cycle
RNA_PROCESSING	RNA Processing
RIBONUCLEOPROTEIN_COMPLEX	RNA Processing
SPINDLE_POLE	Cell Cycle
SMALL_NUCLEAR_RIBONUCLEOPROTEIN_COMPLEX	RNA Processing
MITOTIC_CELL_CYCLE	Cell Cycle
STRUCTURAL_CONSTITUENT_OF_RIBOSOME	Protein Translation
CHROMOSOME_SEGREGATION	Cell Cycle
NUCLEAR_PART	Cell Cycle
CHROMATIN_BINDING	RNA Transcription

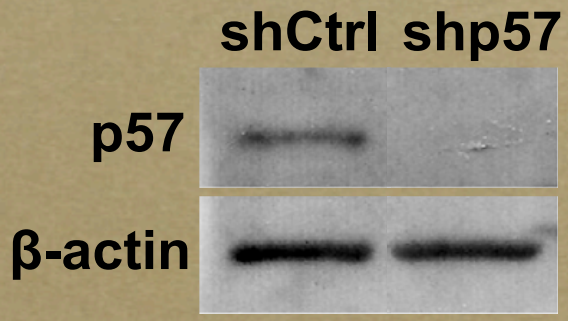
- **in LKS vs. GMP: p57**

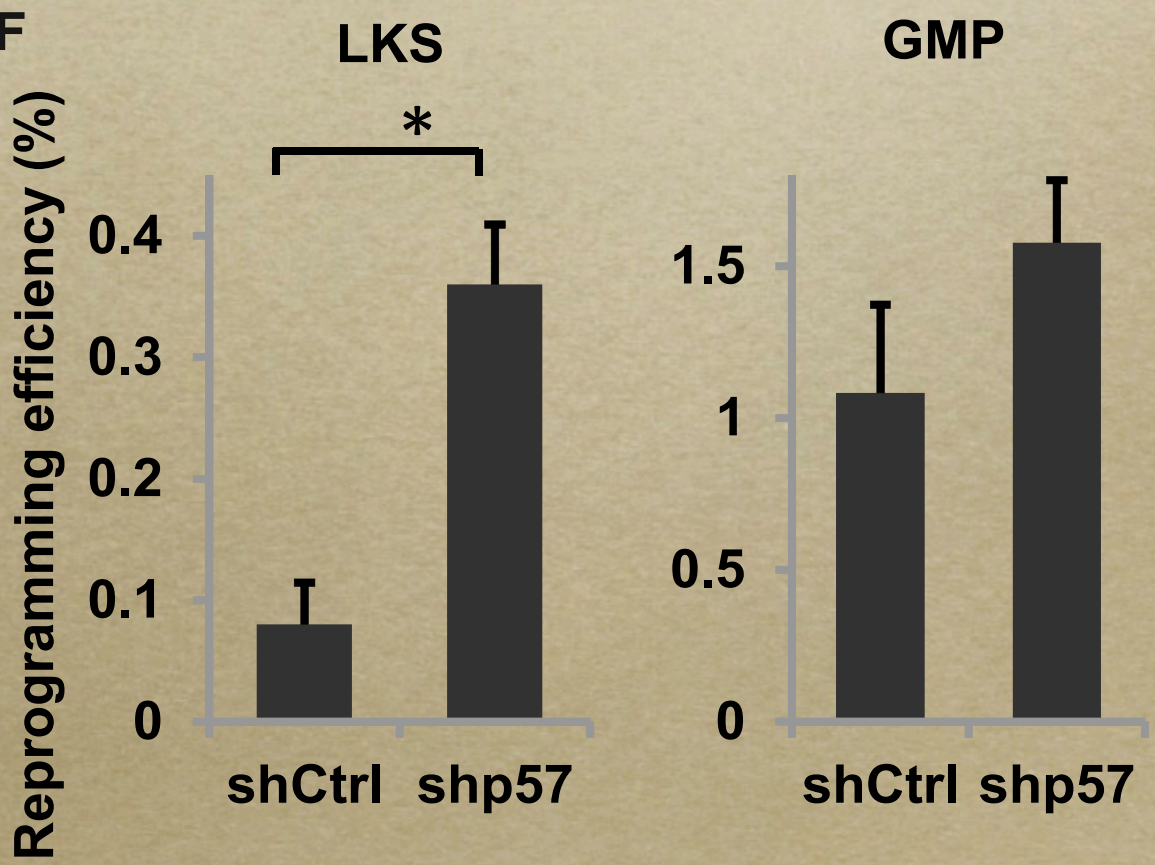


p57 helps block LKS reprogramming

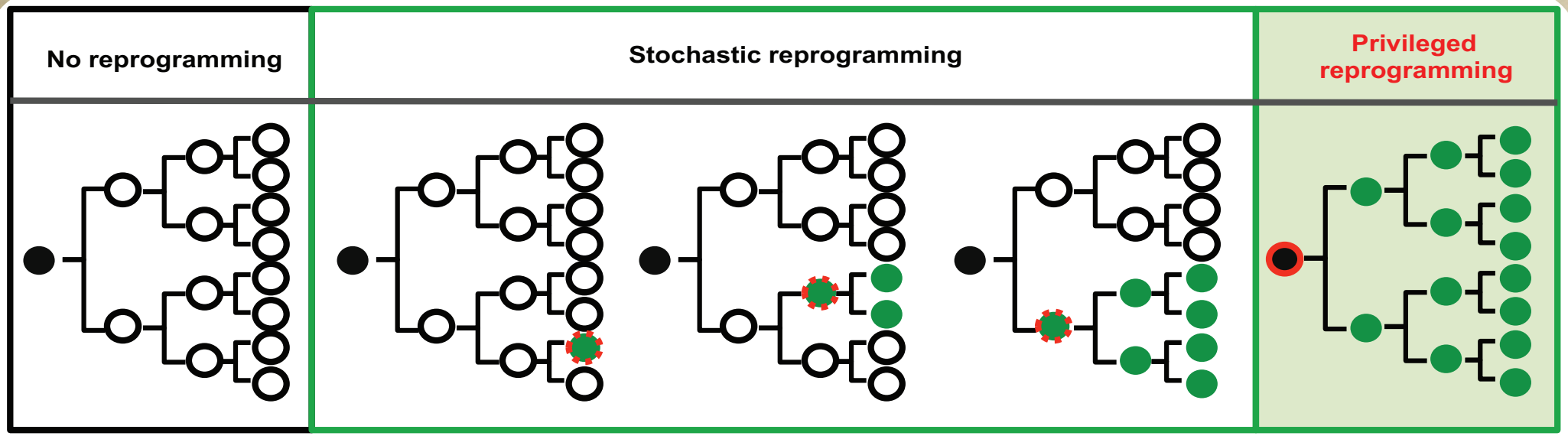
- p57 is know to slow HSC cycling





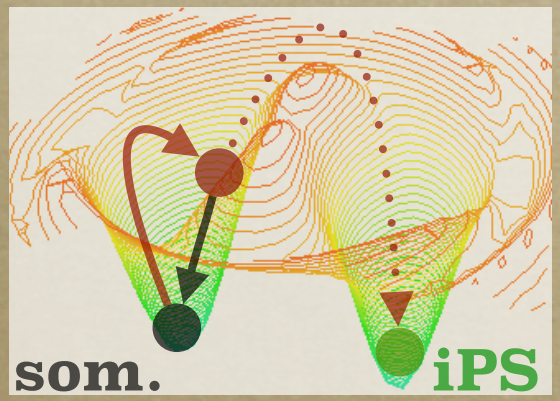


Between stochastic and elite reprogramming: a dynamic privileged state

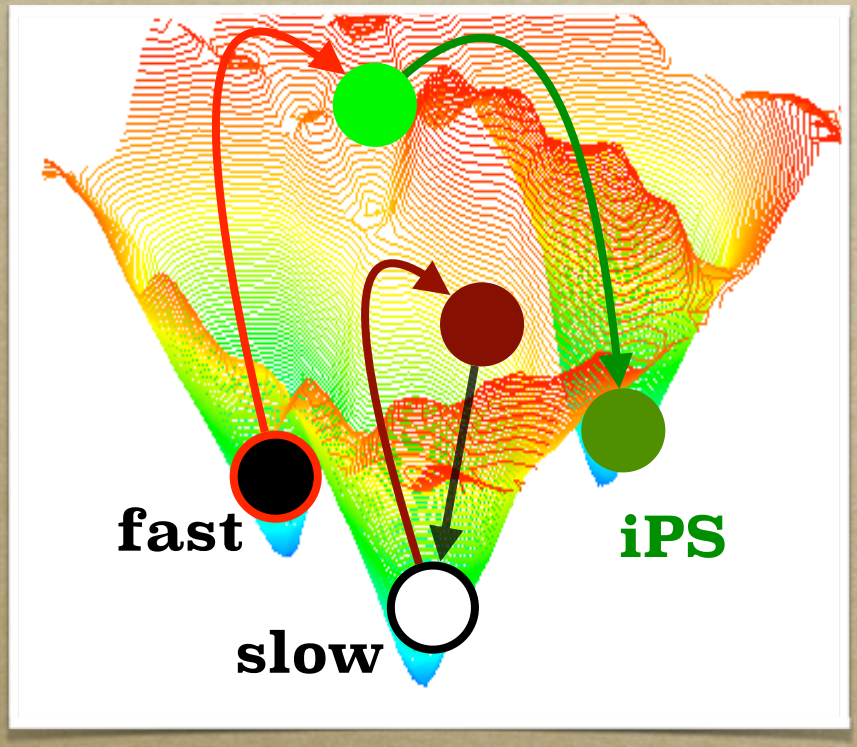


Between stochastic and elite reprogramming: a dynamic privileged state

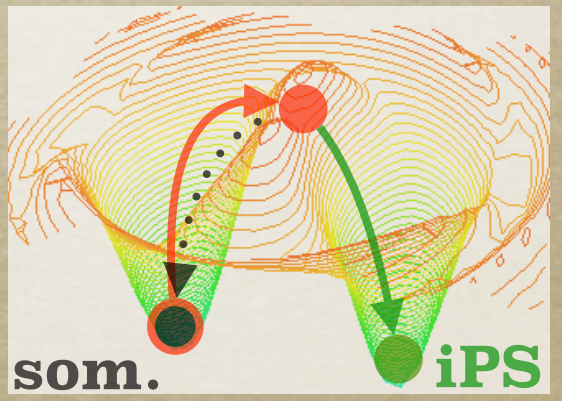
stochastic



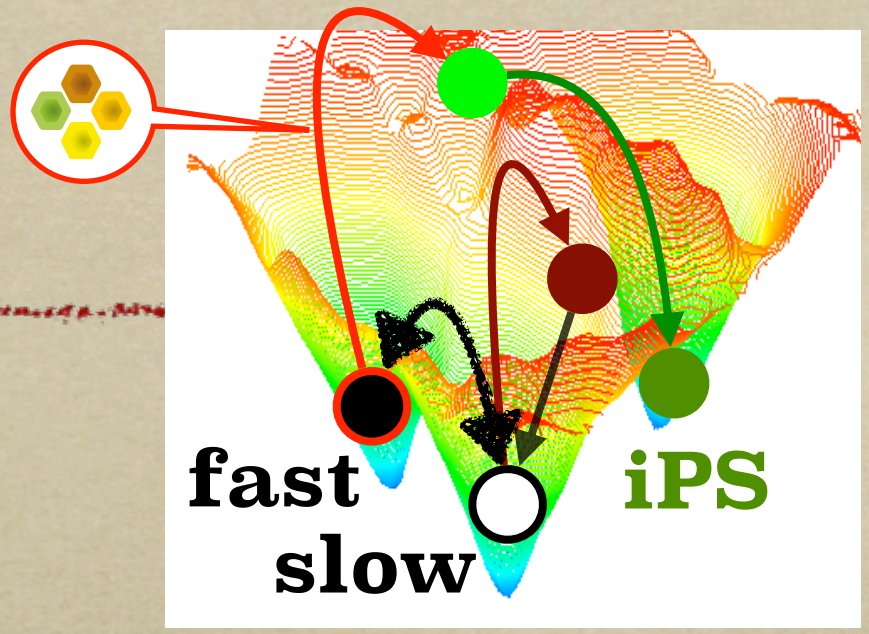
dynamic privileged state



elite



Strengths



- **Conceptual elegance**
  - ➡ does not seek black/white answers in place of

- **A substantial advance to how we see reprogramming**
  - ➡ before: large, hard-to-breach <u>epigenetic barrier</u> the 4 factors need to overcome by accident

    => slow, random reprogramming

  - ➡ now: something about a very short cell cycle (especially G1!) obliterates this <u>epigenetic barrier!</u>

    => My hypothesis: barrier in uncommitted G1 cells

    - cell-wide state of chromatin?
    - cross-talk between cell cycle and iPS switch?
    - metabolic state of the cell?

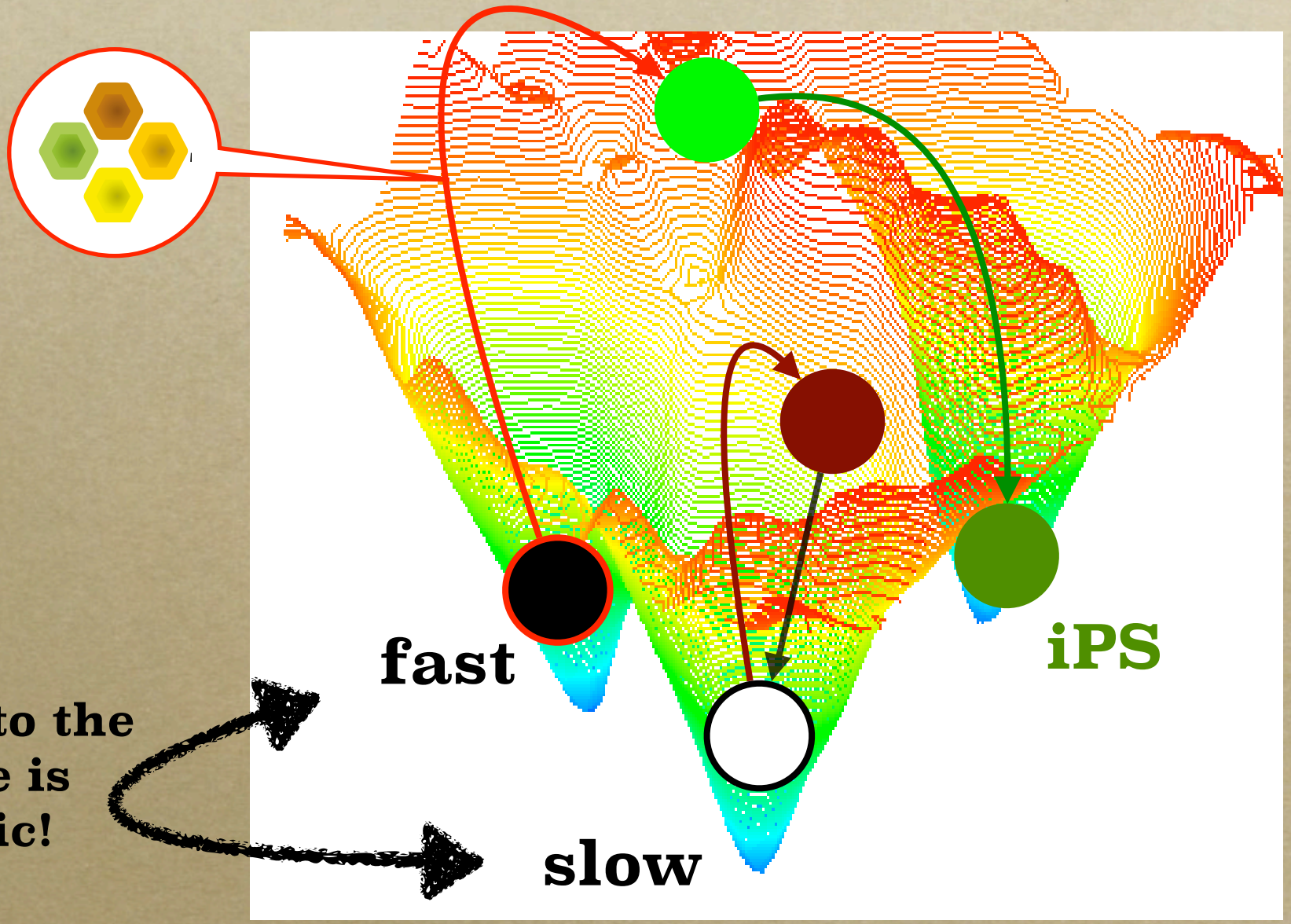
Drawbacks

- **No connection made to the stochastic cell cycle entry literature in the discussion**
  - ➡ focus on specific molecules that stop certain cells from cycling fast - a limiting trend
  - ➡ a key unifying feature of fast-cycling cells, ***commitment BEFORE cytokinesis***, is missed!

- **Experimental drawbacks?**

HELP!

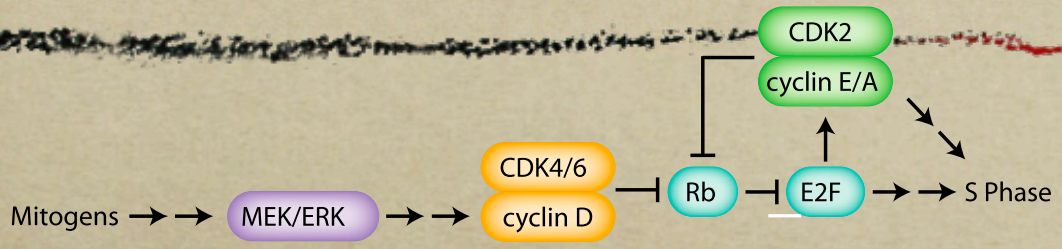
So… where does the original stochasticity come from?

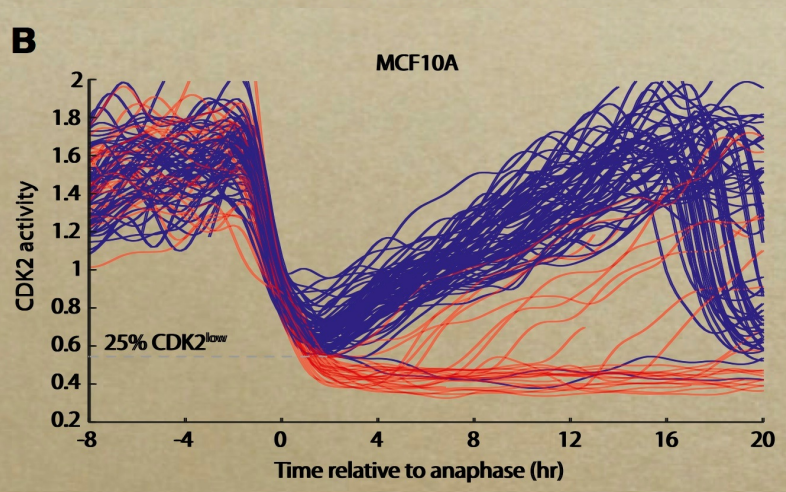


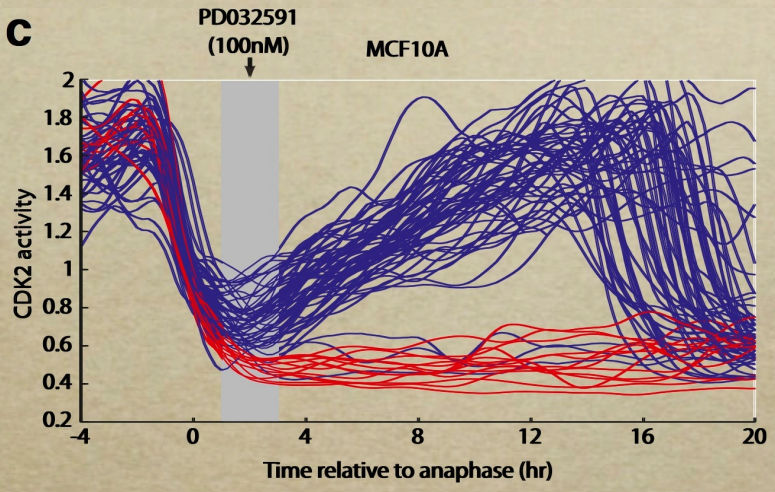
- entry into the cell cycle is stochastic!

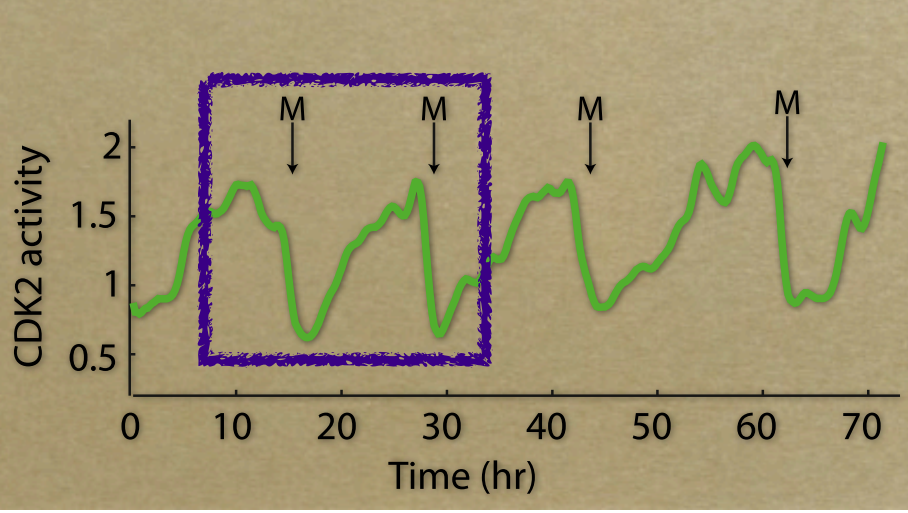
“The proliferation-quiescence decision is controlled by a bifurcation in CDK2 activity at mitotic exit”

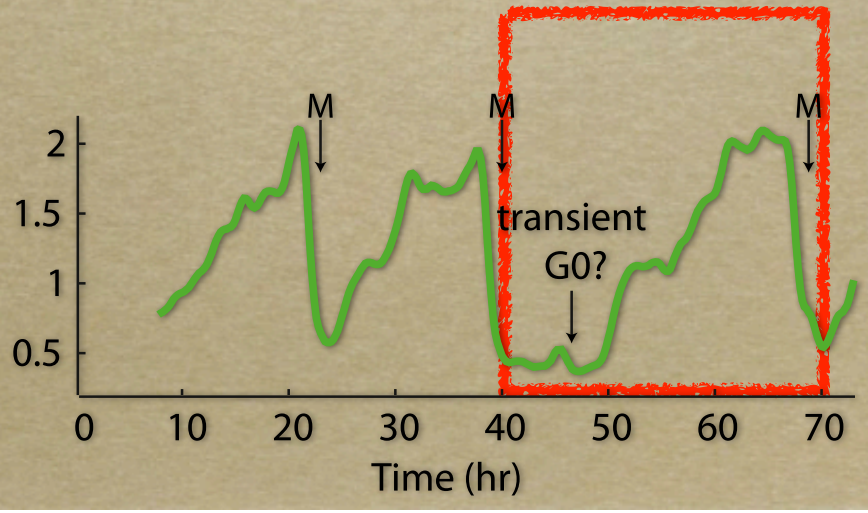
Spencer SL et al. *Cell* **155**(2):369–83, 2013











"The proliferation-quiescence decision is controlled by a bifurcation in CDK2 activity at mitotic exit"

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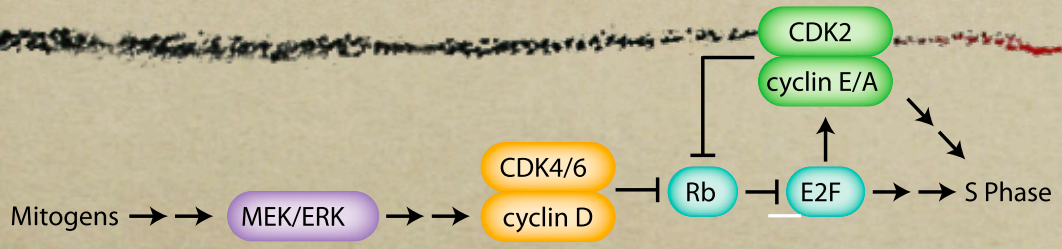
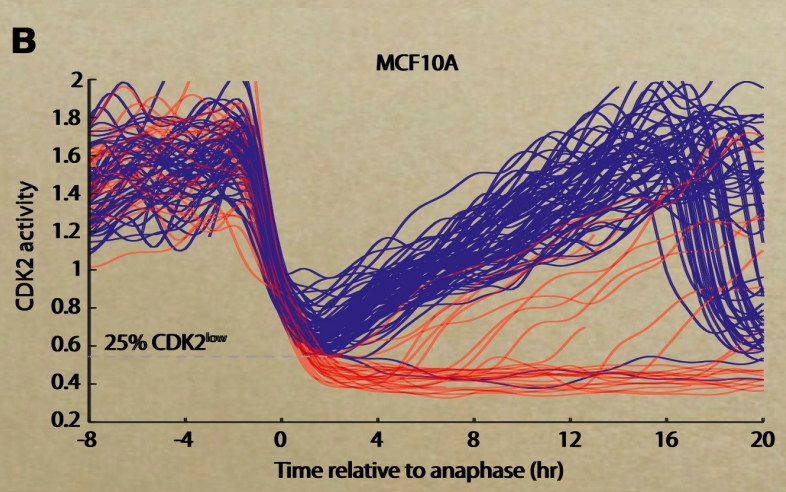
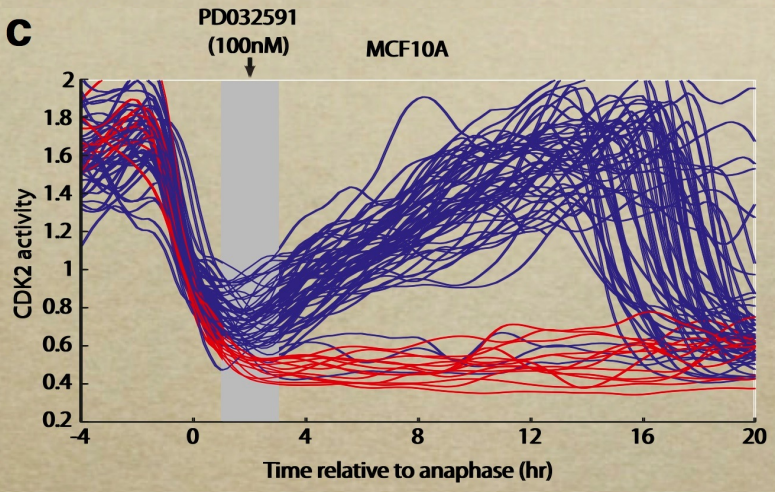
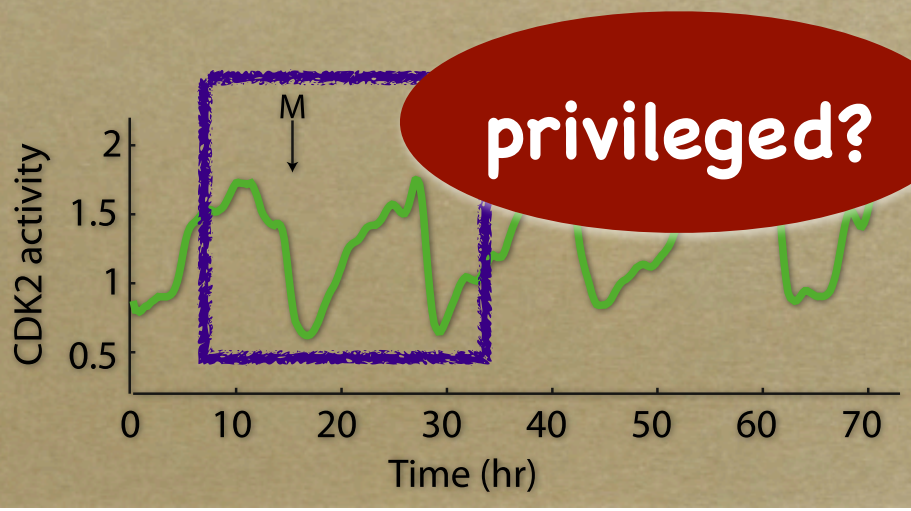
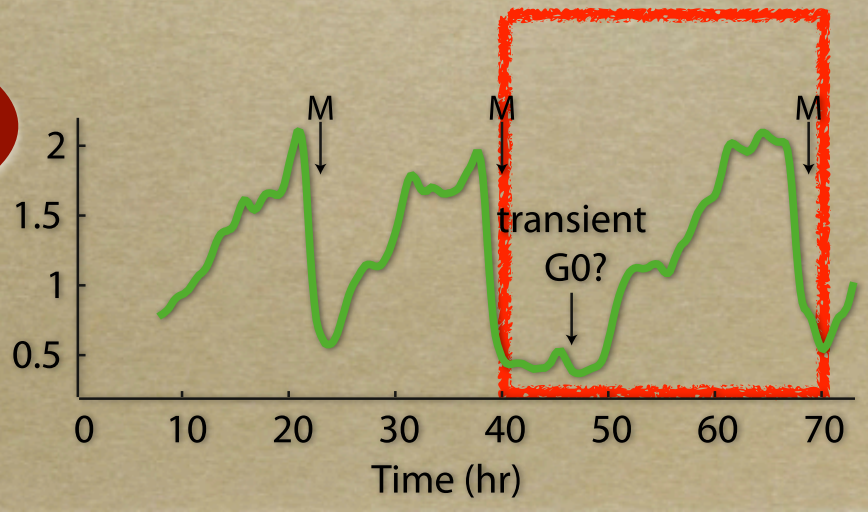






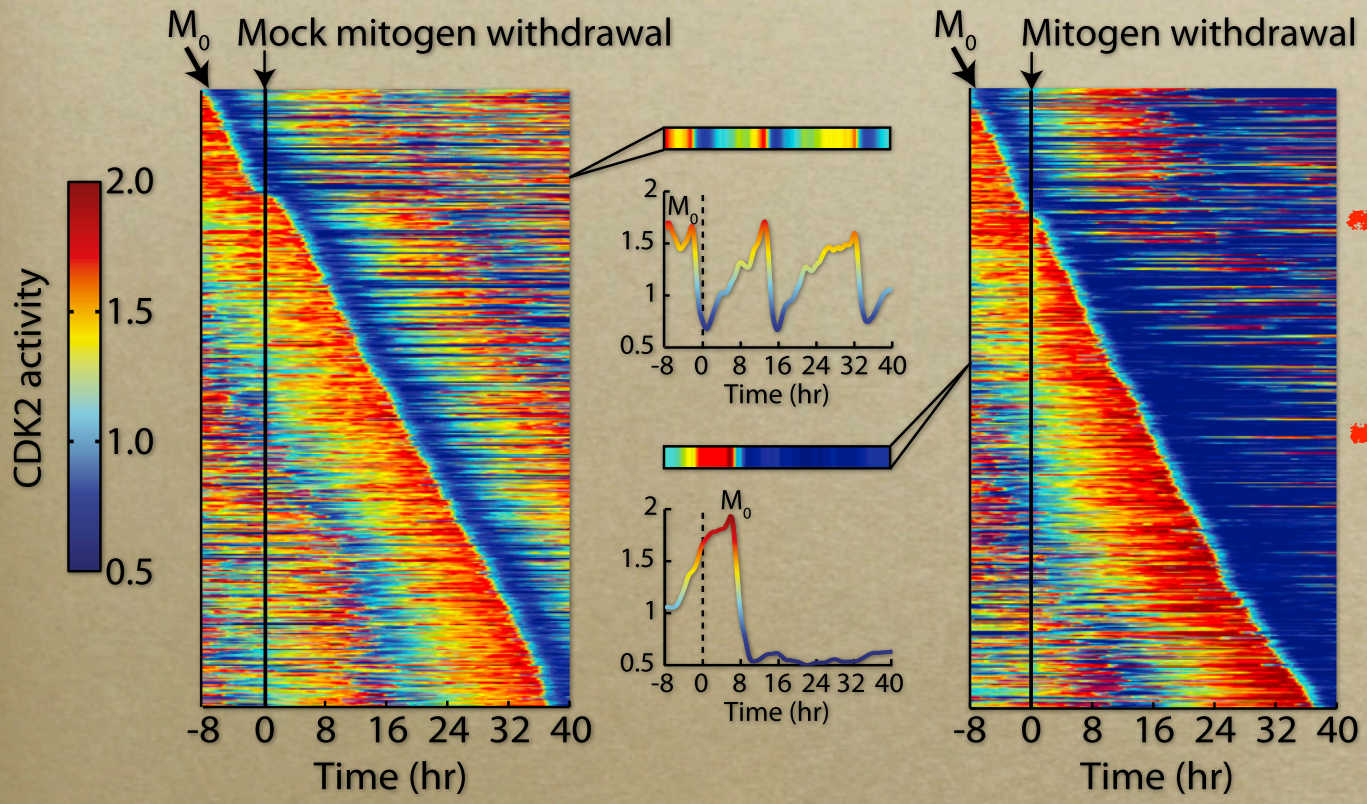
privileged?



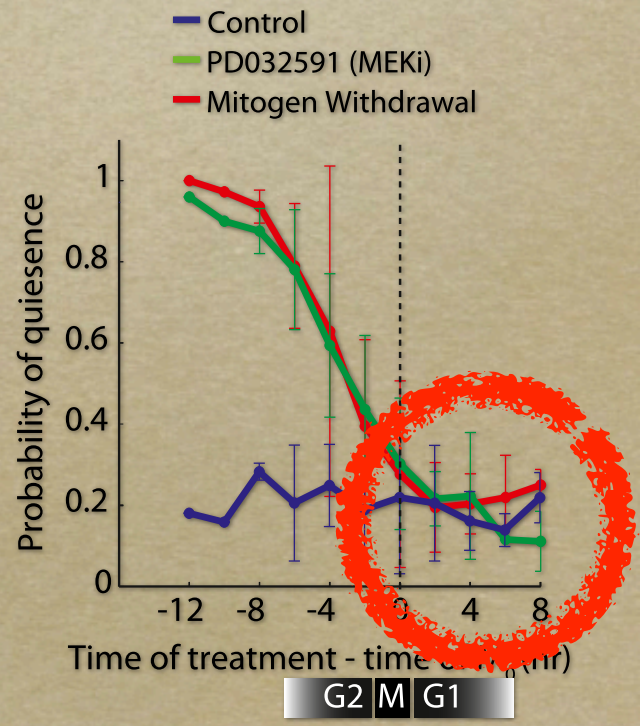


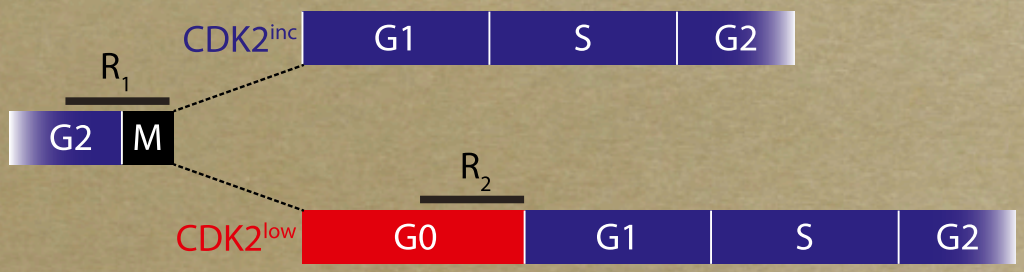
Fast-cycling cells commit to the next cycle *before* the finish Mitosis

Spencer SL et al. *Cell* **155**(2):369–83, 2013

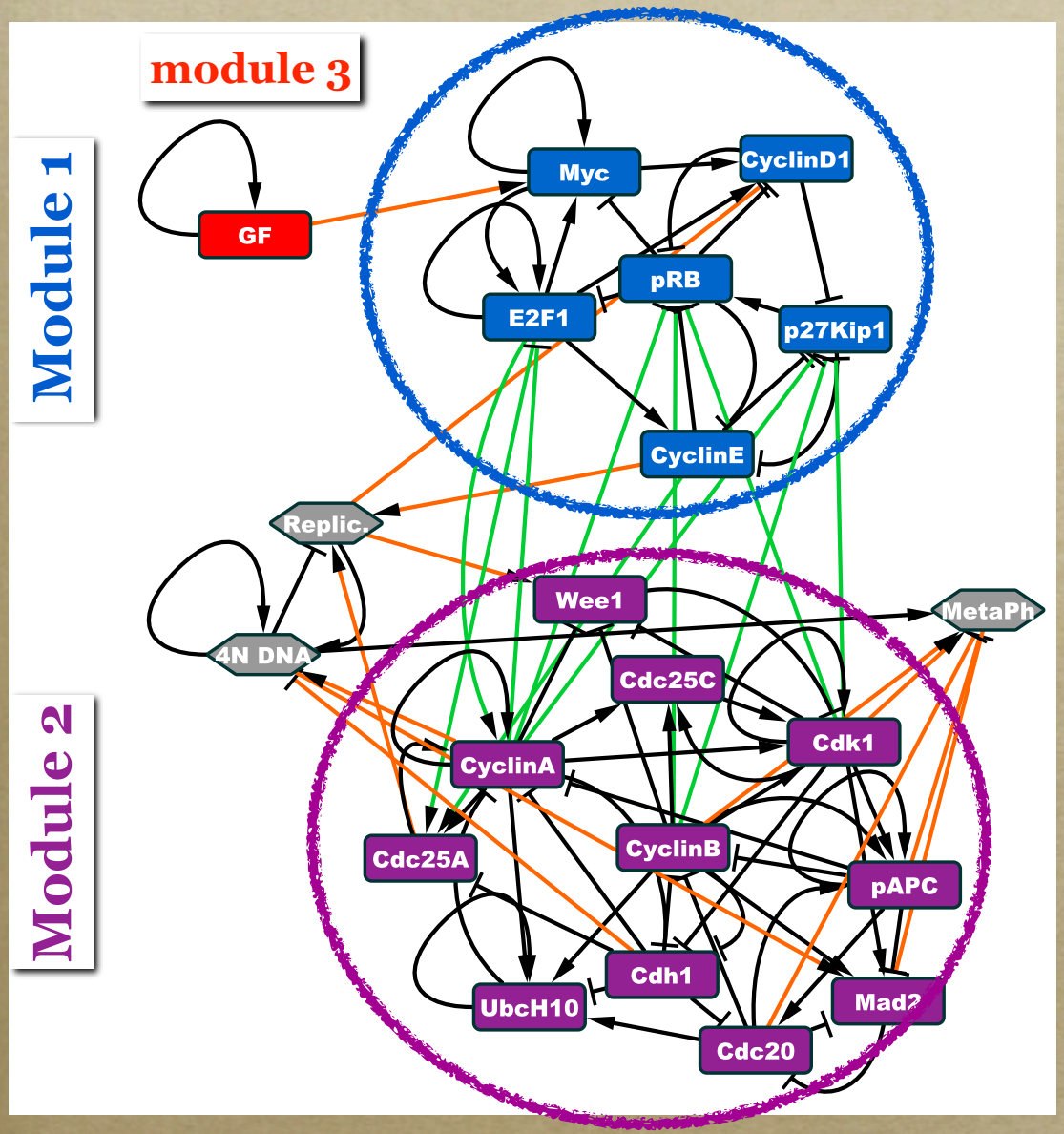


- ***Cdk2* peaks after withdrawal**





A modular view

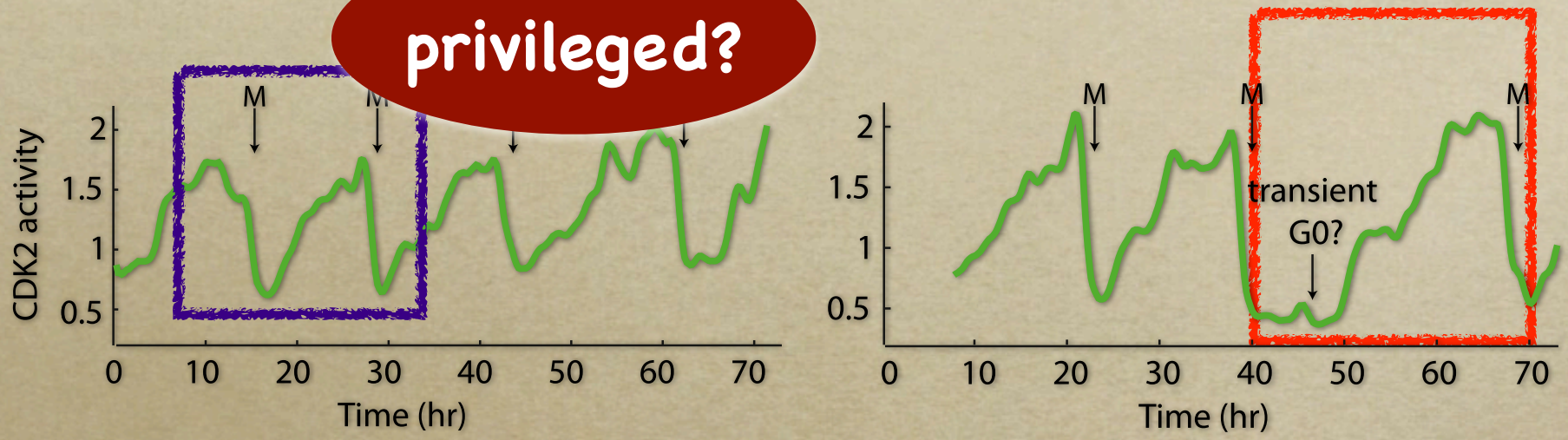


- **Restriction Switch**
  - committed (past RP)
  - not committed (before RP)
- **Phase Switch**
  - G0/G1
  - G2
  - Spindle Assembly Checkpoint

Restriction point in G1 represents a large barrier!

Spencer SL et al. *Cell* **155**(2):369–83, 2013

privileged?



- **Restriction Switch**
  - **committed (past RP)**
  - **not committed (before RP)**
- **Phase Switch**
  - **G0/G1**
  - **G2**
  - **SAC**

Is this combination forbidding to reprogramming?

Outlook

➡ **Something about a very short cell cycle (especially G1!) obliterates the epigenetic barrier to reprogramming**

Barrier in uncommitted G1 cells ?

- cell-wide state of chromatin?
- cross-talk between cell cycle and iPS switch?
- metabolic state of the cell?

Outlook

- **Could the concept be extended to (de)differentiation in general?**

  ➡ are fast-cycling cells more susceptible to large, difficult-to-induce cell-state changes?

  ➡ cancer cells:

  - is there a possible connection to the emergence of embyonic-looking "cancer stem cells"? (*thank you, Carmelo!)*

- **New insights into development / differentiation**

  ➡ critical differences between ESC and somatic cell signals for cell cycle entry (Jak/Stat vs. MAPK)

  ➡ how is the "handoff" regulated?

Cell-wide state of chromatin

Cell cycle <-> iPS switch

Thank you!

http://regan.med.harvard.edu/CVBR-JournalClub.php

Nonstochastic Reprogramming from a Privileged Somatic Cell State
S. Guo, X. Zi, V.P. Schulz, J. Cheng, M. Zhong,
S.H.J. Koochaki, C.M. Megyola, X.Pan,
K. Heydari, S.M. Weissman, P.G. Gallagher,
D.S. Krause, R.Fan, J. Lu
Cell **156**, 649–662, 2014

Journal Club, 2015

Erzsébet Ravasz Regan