# Asymmetric cancer cell division regulated by AKT

*Journal Club, January 2012*

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**Dey-Guha, I. et al. Asymmetric cancer cell division regulated by AKT. *Proceedings of the National Academy of Sciences* 108, 12845–12850 (2011).**

# The premise:

Cells with identical

- genome
- phenotype
- environment
- history of environments

can display functionally heterogeneos behavior

**Today: we start with cancer stem cells**

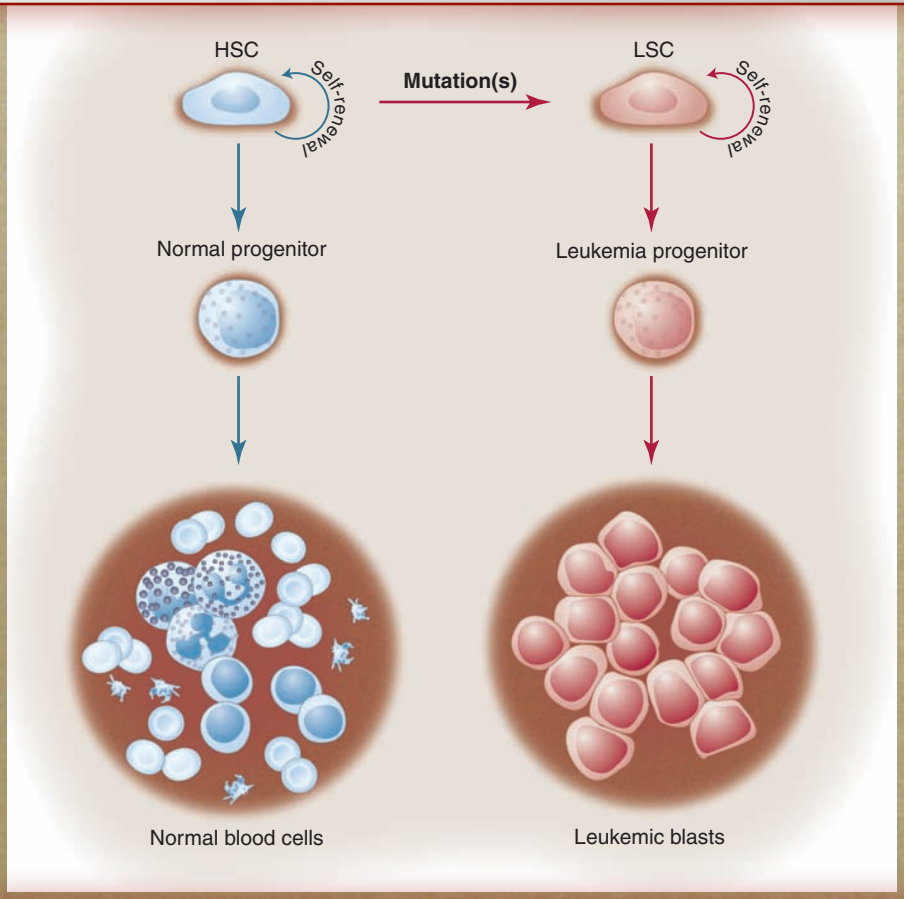
# Introduction: It takes many cancer cells to seed a new tumor

- Most tumors are clonal, the progeny of a single cell
- BUT: new tumors can only be (experimentally) seeded if > $10^6$ cells are injected
  - many types of cancer cells have limited proliferative capacity and tumorigenic potential (e.g. AML)
  - small pool of "stem" cells? — intrinsic hierarchy
  - small probability of re-entry into cell cycle? — homogeneity
  - variations in microenvironment, subclones, independent somatic mutations? — heterogeneity but not a hierarchy

Dick, J. E. Breast cancer stem cells revealed. Proc Natl Acad Sci U S A 100, 3547–3549 (2003).
Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. Stem cells, cancer, and cancer stem cells. *Nature* 414, 105–111 (2001).
Lobo, N. A., Shimono, Y., Qian, D. & Clarke, M. F. The biology of cancer stem cells. *Annu Rev Cell Dev Biol* 23, 675–699 (2007).

# A single cancer cell can initiate leukemia. (Not all, select few.)

Lapidot, T. et al. A Cell Initiating Human Acute Myeloid-Leukemia After Transplantation Into Scid Mice. *Nature* 367, 645–648 (1994).




Rosen, J. M. & Jordan, C. T. The increasing complexity of the cancer stem cell paradigm. *Science* 324, 1670–1673 (2009).

- **Hematopoietic malignancies retain remnants of normal differentiation programs**
- **Idea extends to solid tumors**
  - **breast cancer: CD44+/CD24-**

    Al-Hajj, M., Wicha, M., Benito-Hernandez, A., Morrison, S. & Clarke, M. Prospective identification of tumorigenic breast cancer cells. Proc *Natl Acad Sci U S A* 100, 3983–3988 (2003).
  - **brain tumors**

    Singh, S. K. et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 63, 5821–5828 (2003).
  - **prostate**
  - **...**

# Cancer stem cell hypothesis takes the lead

## Stem cell

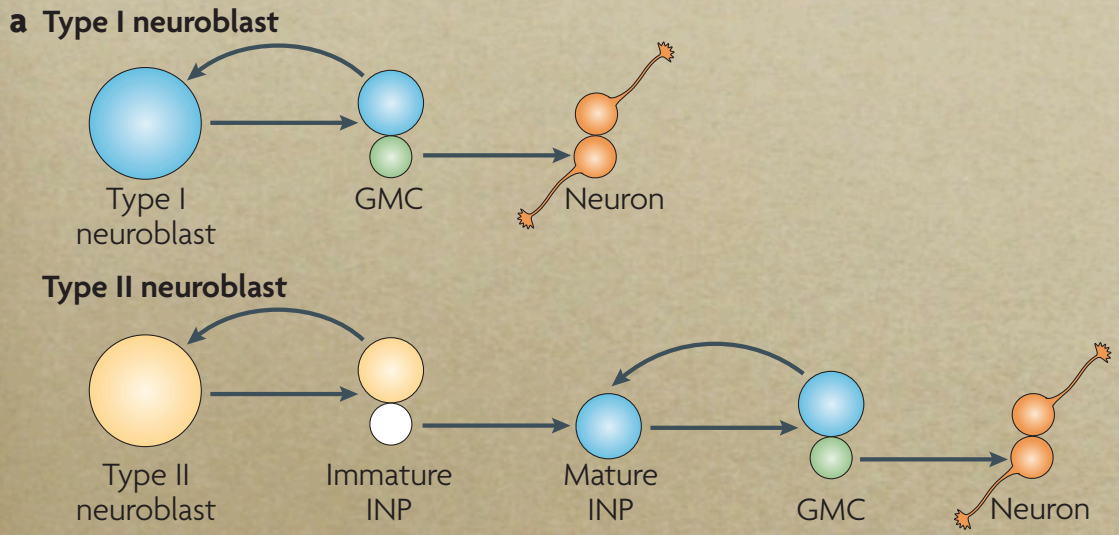
- *differentiation* - heterogeneous progeny -> diversifies in a hierarchical process
- *self-renewal* - form new stem cell with identical potential
- *homeostatic control* - modulate and balance the first two

## Cancer Stem Cell

- *tumorigenic potential* - heterogeneous progeny -> all subpopulations of the tumor
- *self-renewal* - form new cancer stem cell with identical potential
- *not good with homeostasis*

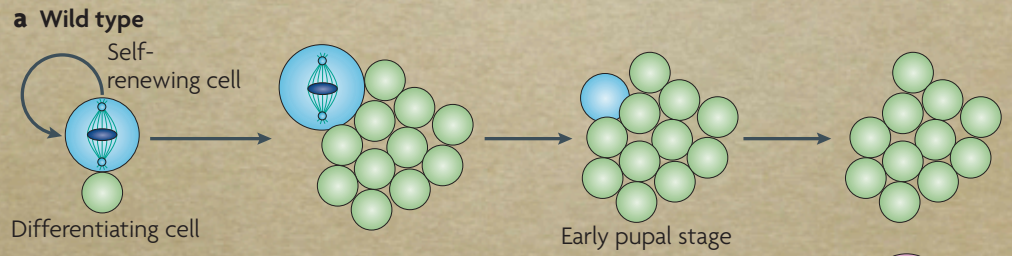
Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. Stem cells, cancer, and cancer stem cells. Nature 414, 105–111 (2001).

# Stem cells and some CSCs divide assymetrically

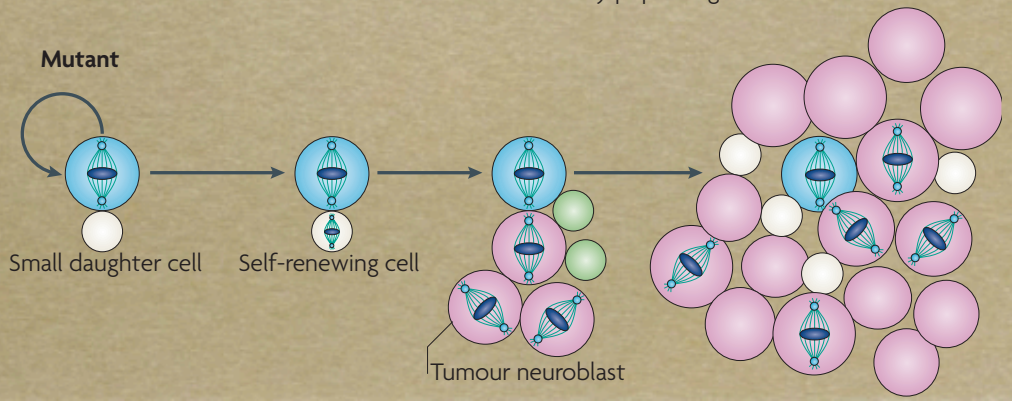



Knoblich, J. A. Asymmetric cell division: recent developments and their implications for tumour biology. *Nat Rev Mol Cell Bio* 11, 849–860 (2010).

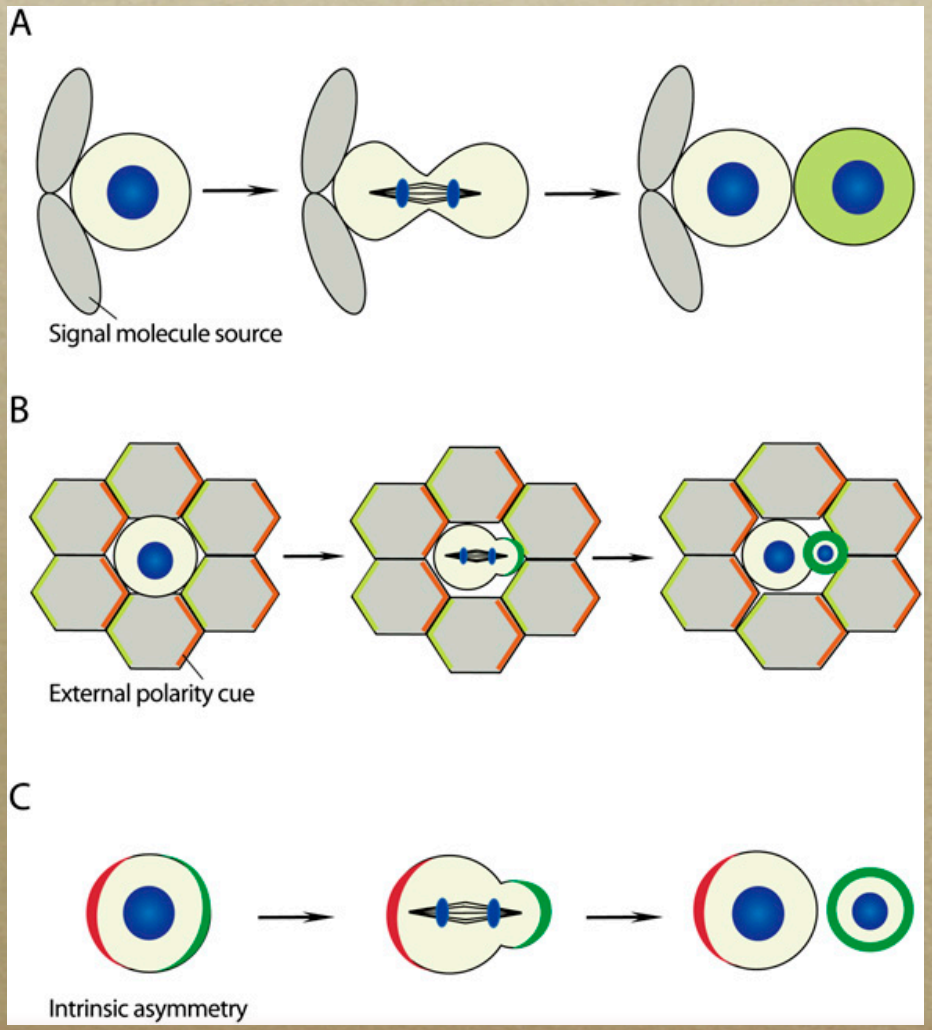
**Stem cells**



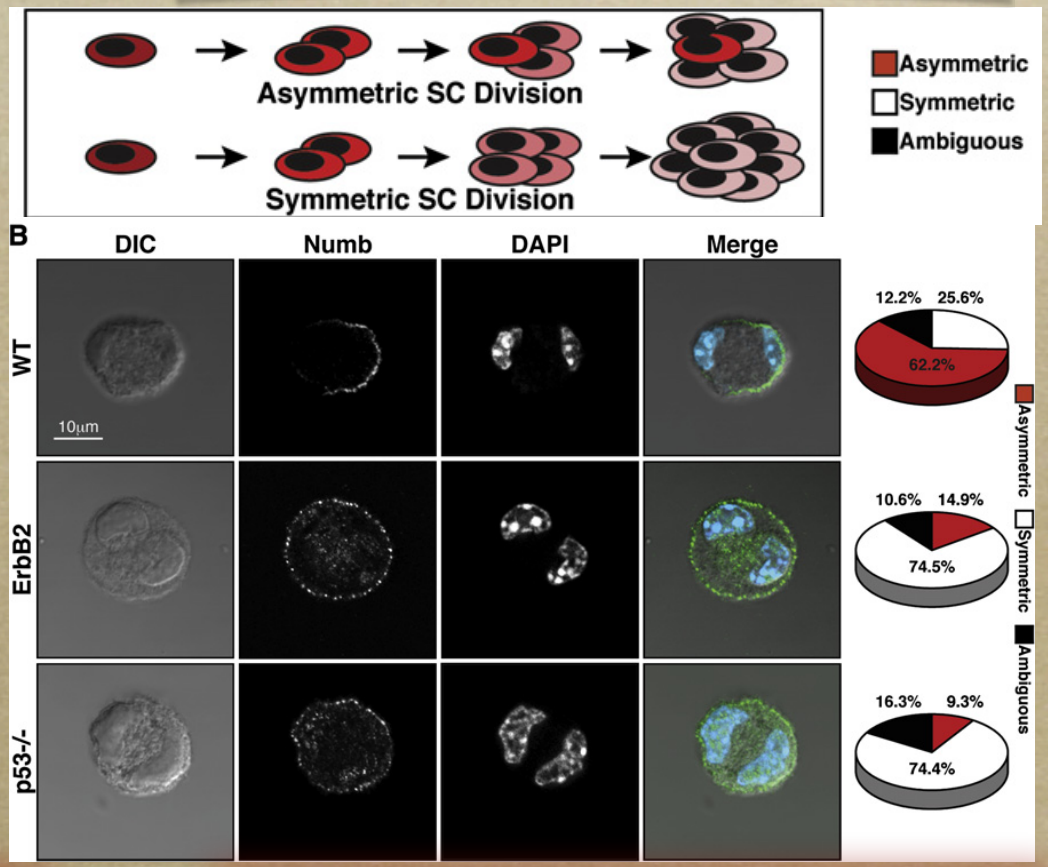

**Brain tumor in drosophila**



# Stem cells establish polarity before division to ensure assymetry




## Broken polarity in breast CSCs

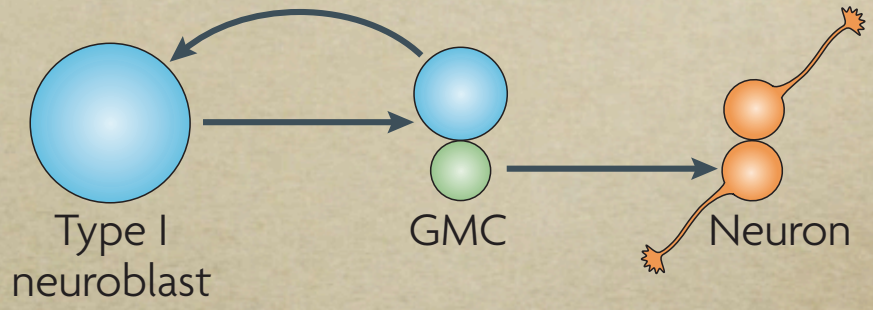



Neumueller, R. A. & Knoblich, J. A. Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer. *Genes & Development* 23, 2675–2699 (2009).

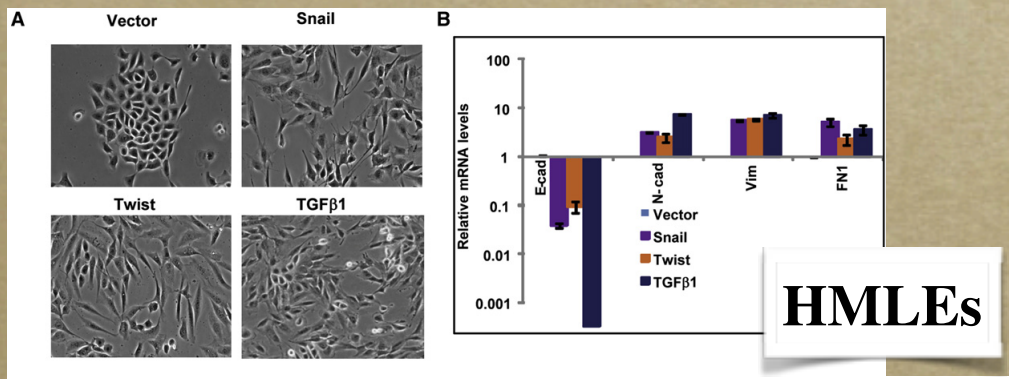
Cicalese, A. et al. The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell 138, 1083–1095 (2009).

# Induced EMT is a pathological "way back" from differentiation

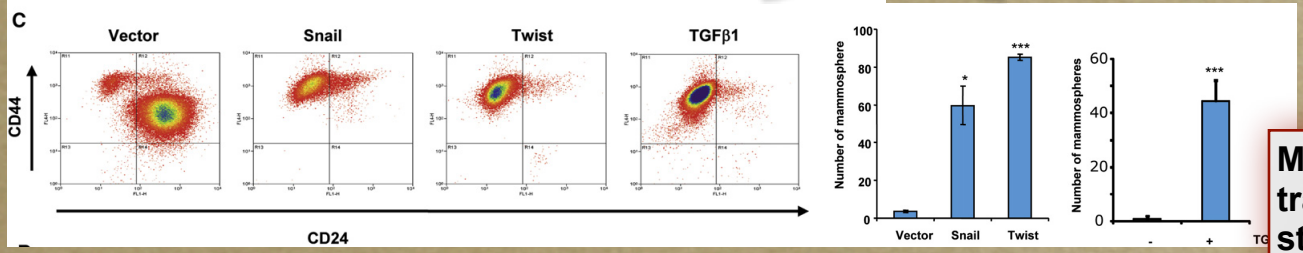
- **Tacit assumption: assymetric division & differentiation are one-way**
  - **two daughters -> different fates**




- **Epithelial Mesencymal Transition generates stem cell-like cells**




**HMLEs**




- **EMT -> CD44+/CD24-**
- **mammospheres -> number & structure**
- **markers: Vimentin & FN1 (also in normal mammary SCs)**
  - **BUT: induced EMT -> Cancerous SCs**

Mani, S. A. et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133, 704–715 (2008).

# Asymmetric cancer cell division regulated by AKT

# Why are cultured cancer cells heterogeneous in proliferative potential?

**In vivo tumors**

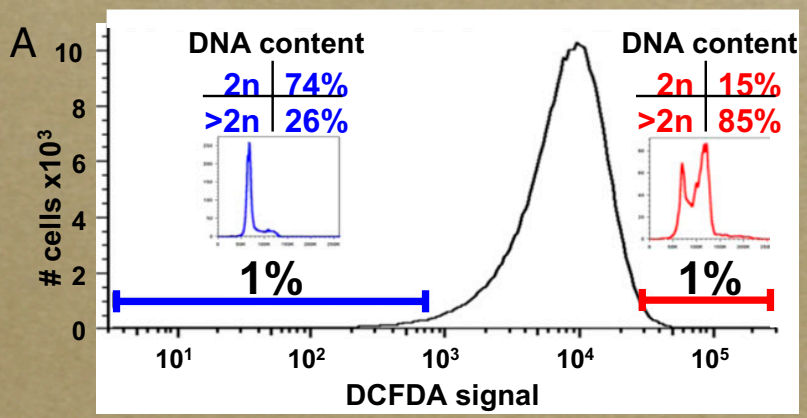
- *typically have a slowly proliferating pool of cells*
  - ≃ ***CSCs***
- *proliferative heterogeneity correlates with*
  - *time to detection*
  - *growth*
  - *metastasis*
  - *treatment response*

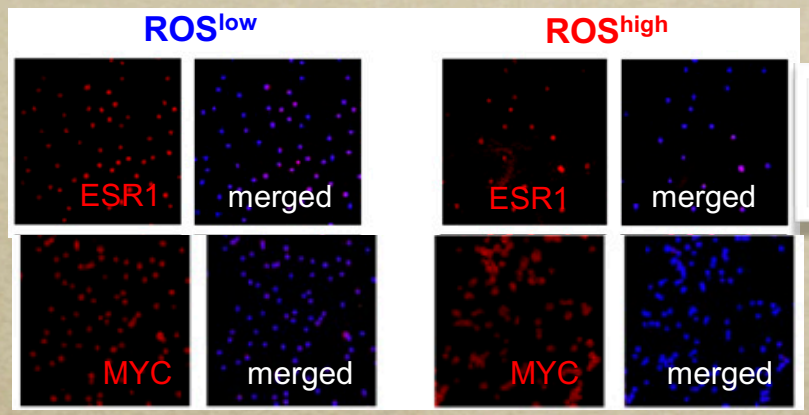
**In vitro tumor cell lines**

- *aquired mutations that drive proliferation*
- *many lines also have slowly proliferating populations*
- **how can these remain in competition in spite of selection for fast growing cells?**

# Slowly proliferating MCF7 cells have low levels of ROS

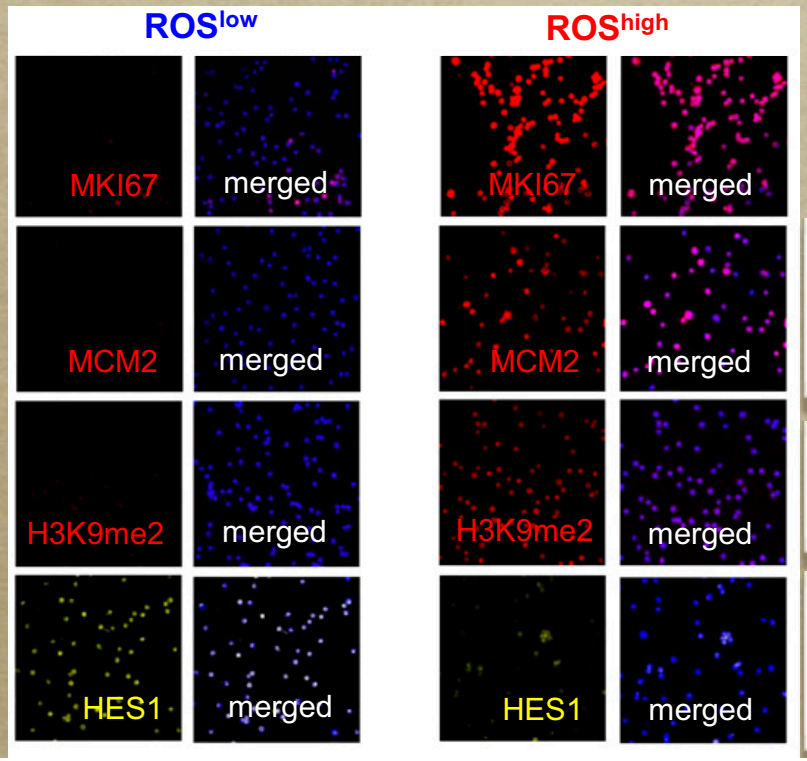
- **MCF7s: highly proliferative cancer line (ER+/ERBB2-)**
  - ***synergy between mutations in CDKN2A & PIK3CA***
- **Looking for CSCs:**
  - ***slowly cycling hematopoietic, neural and breast adult and cancer SCs have low ROS***




Estrogen receptor

Myc



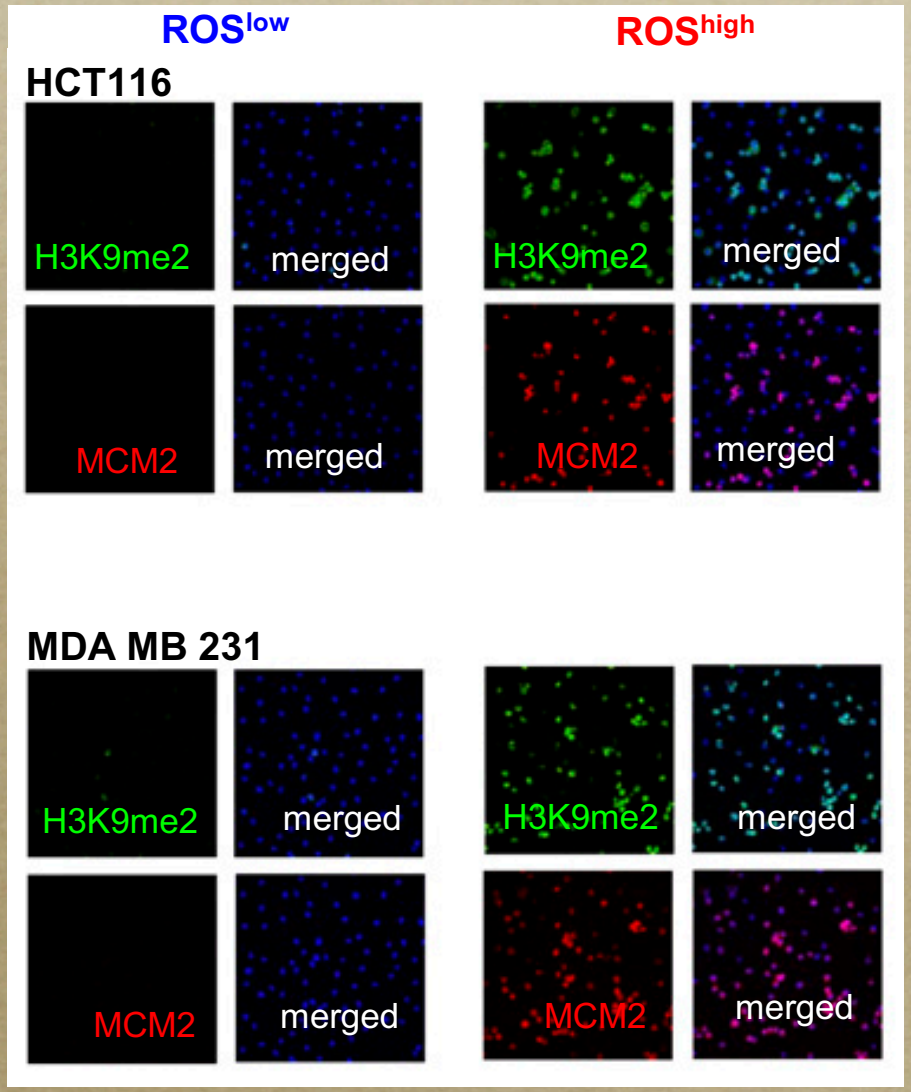

Proliferation & ribosomal RNA transcription

Key component of pre-replication complex

High in proliferating cells

Robust $G_0$ marker in fibroblasts

# Two other cancer cell lines mimic heterogeneity of MFC7 cells

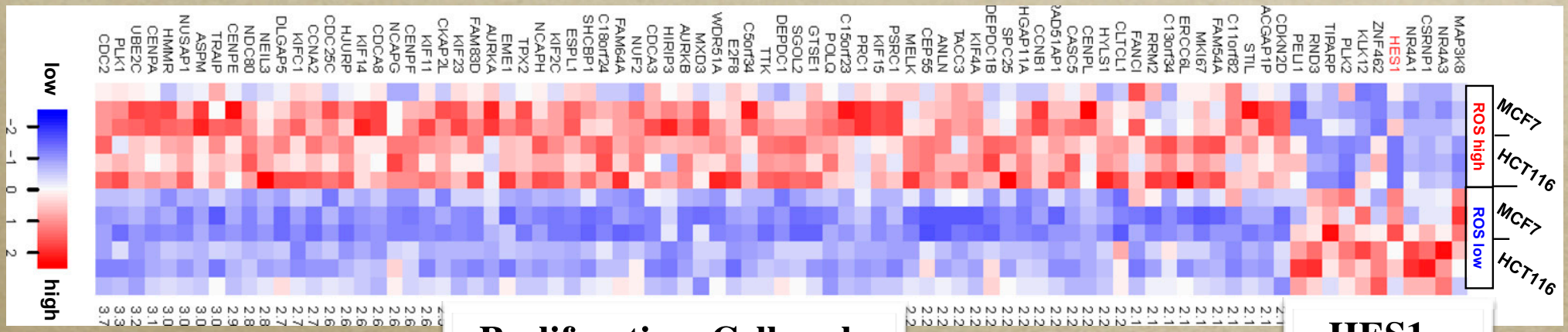



**HCT116 - human colon cancer**

**MDA MB 231 - breast cancer**

# Microarray profiling of ROS$^{low}$ cells points to $G_0$ phenotype

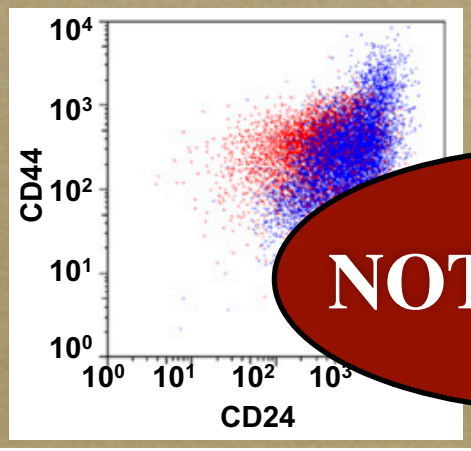
- **Differential expression in low ROS versus high ROS cells**




**Proliferation, Cell cycle**

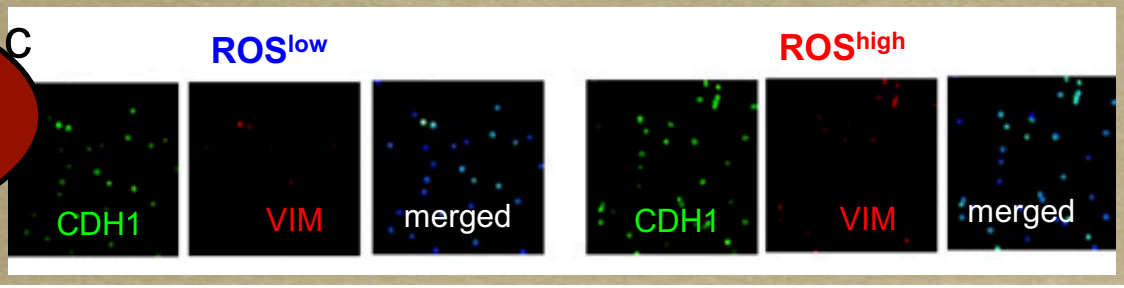
**HES1 - $G_0$ marker**

- **No enrichment in CSC markers**
  - **CD44$^+$/CD24$^-$**




**NOT CSC-like**

- **No EMT markers**

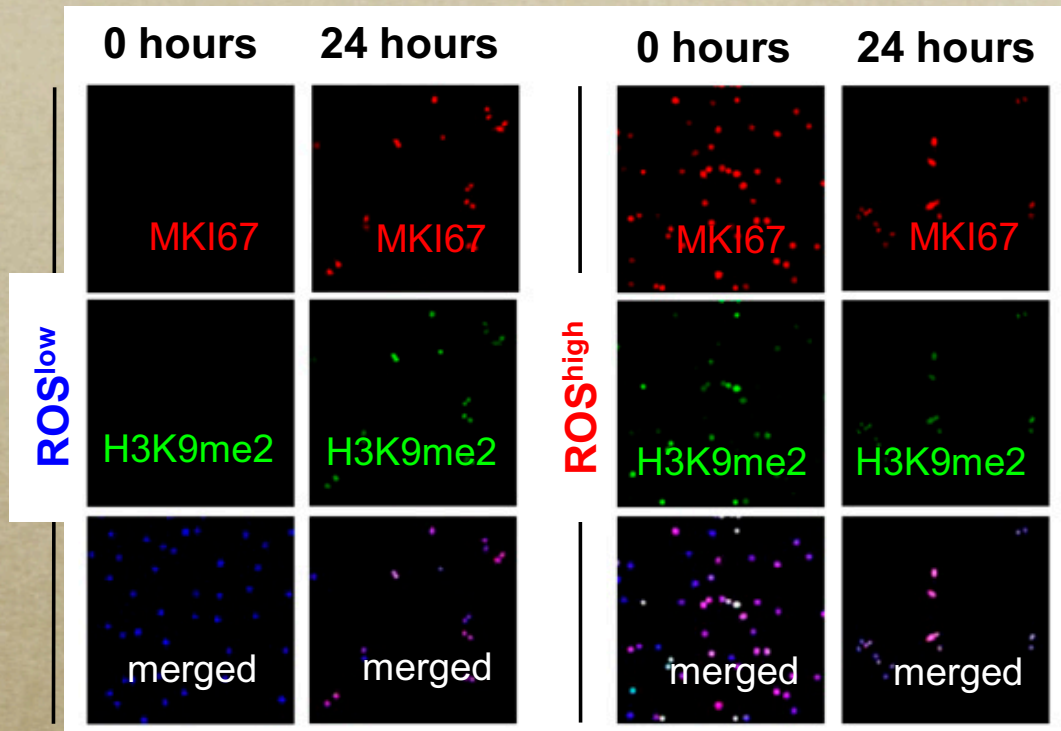



Coller, H. A., Sang, L. & Roberts, J. M. A new description of cellular quiescence. *PLoS Biol* 4, e83 (2006).

# The ROS$^{LOW}$ population does not remain quiescent

- After 24h in identical culture -> convergence to similar average
- Colony forming potentian *THE SAME*
- *Heterogeneity of potential: larger in ROS$^{high}$ cells*

H

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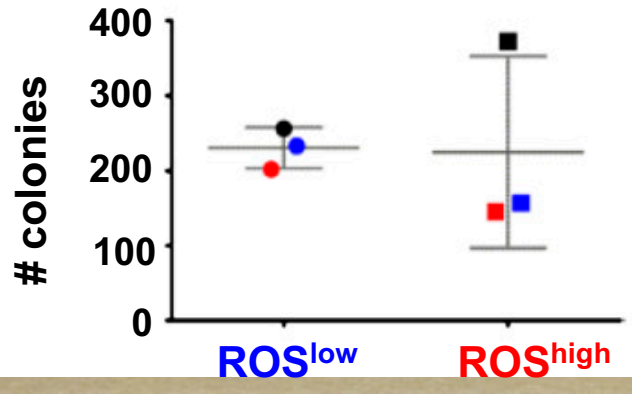
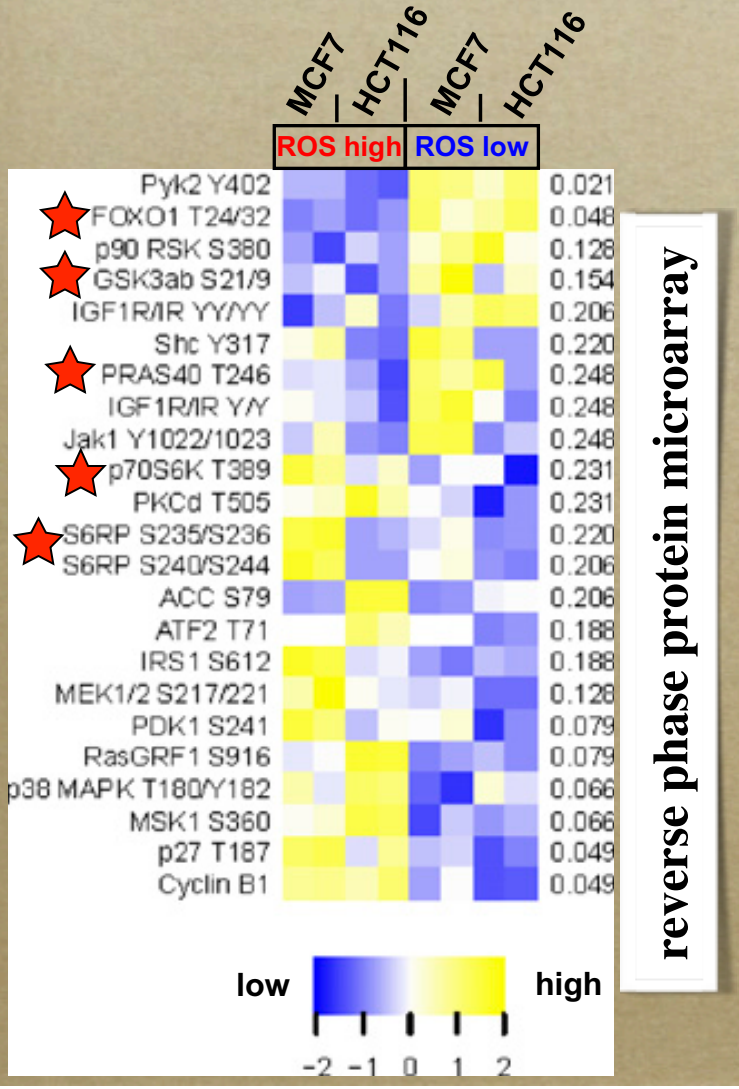
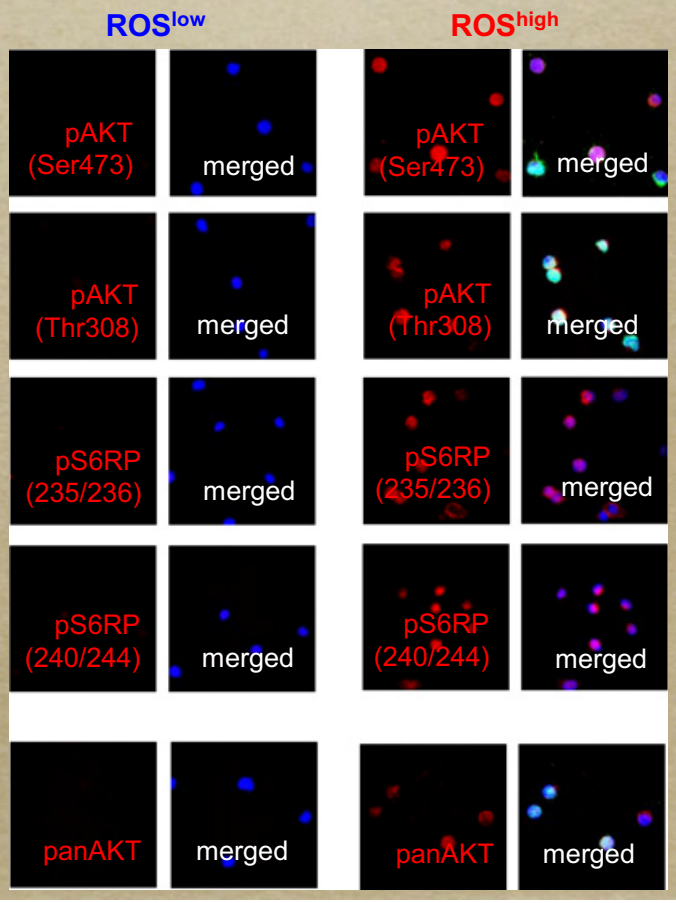


# Protein profile of ROS$^{LOW}$ cells point to diminished AKT signaling

- **Differential protein levels in low ROS versus high ROS cells**

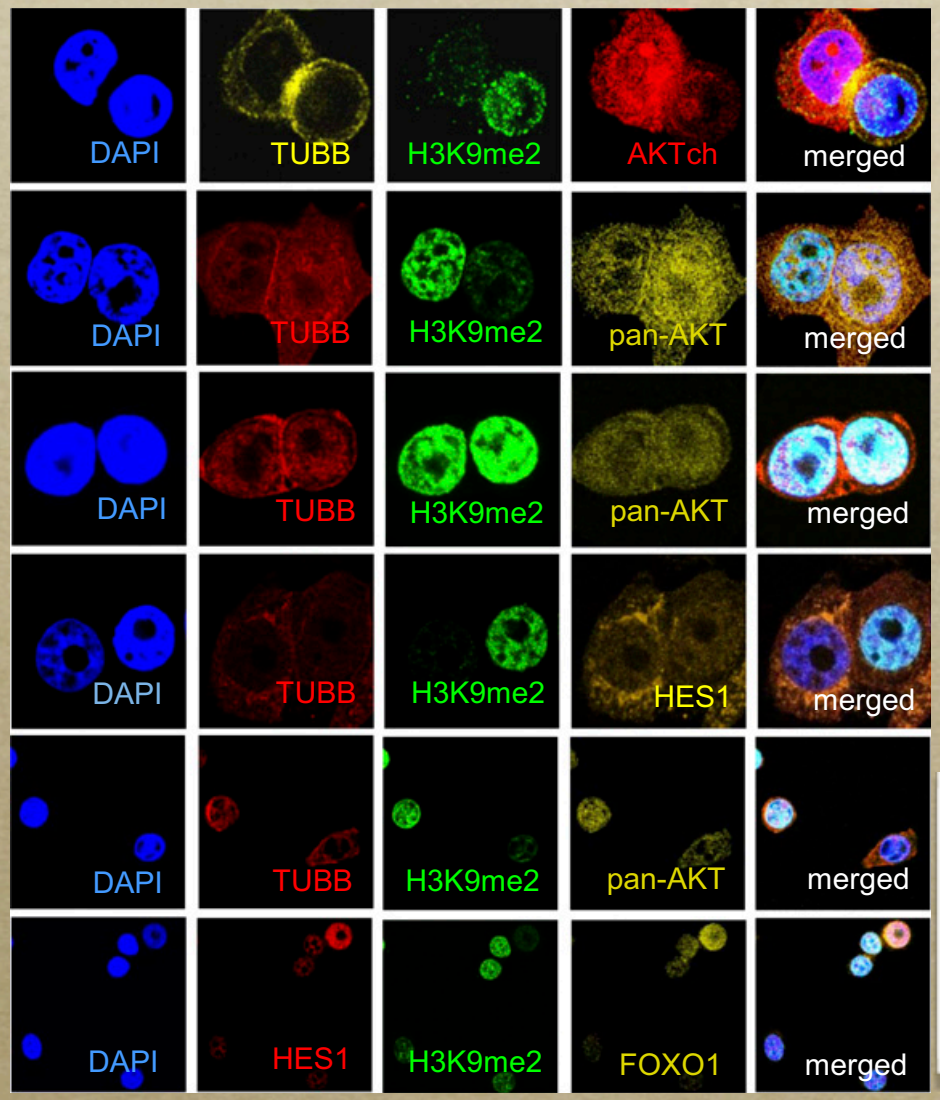



- **Akt protein level and activity is low in ROS$^{LOW}$ cells**

# $G_0$-like cancer cells arise through infequent assymetric division

- **Forced Akt expression by AKT1-mCherry fusion protein**
  - ***did not change % of Akt1 low cells***
  - ***these downregulated both endogenous and fusion Akt1***
- **Some, but not most divisions were assymetric**

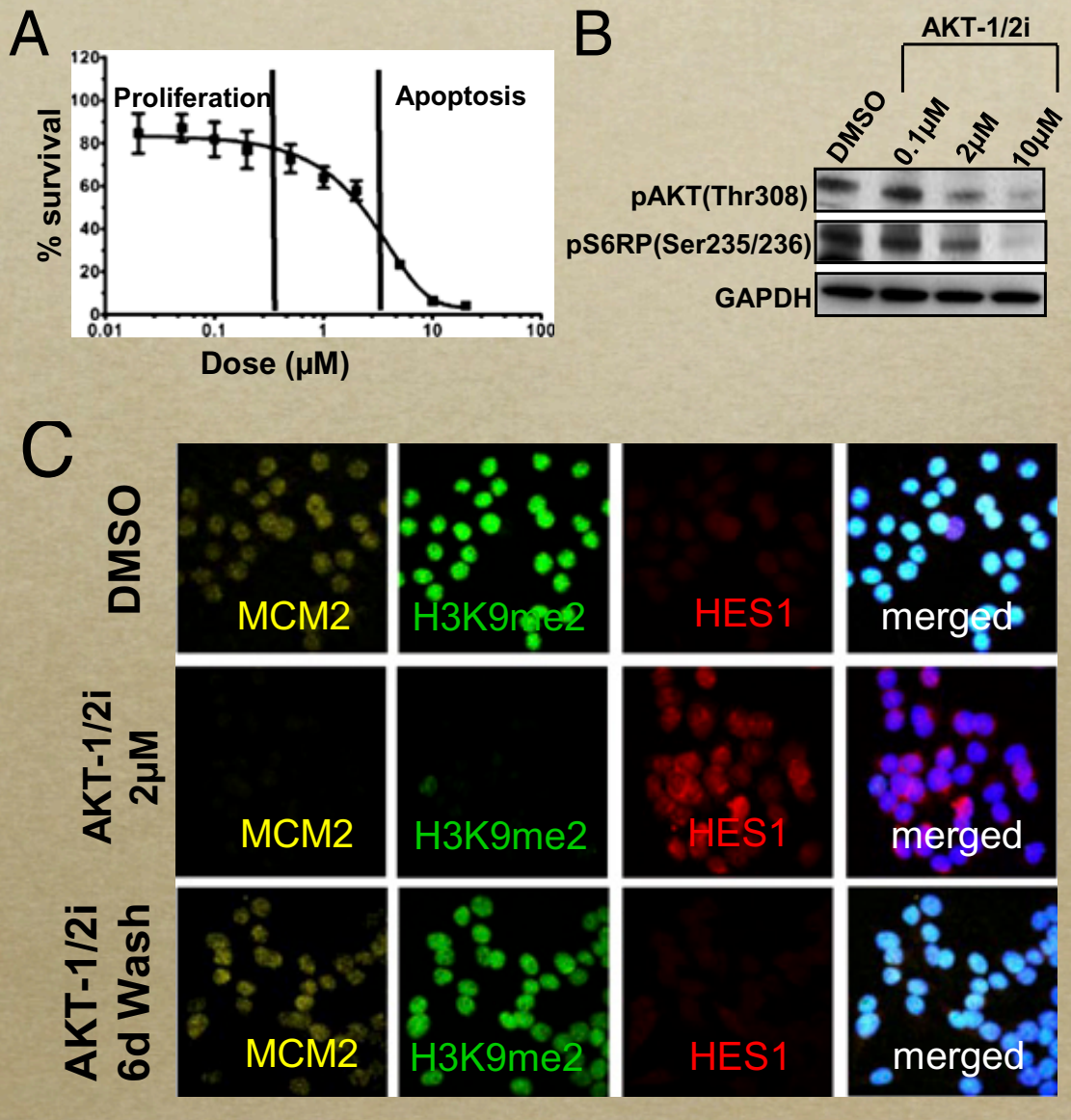



**Telophase: low proliferation mark with high NUCLEAR Akt**

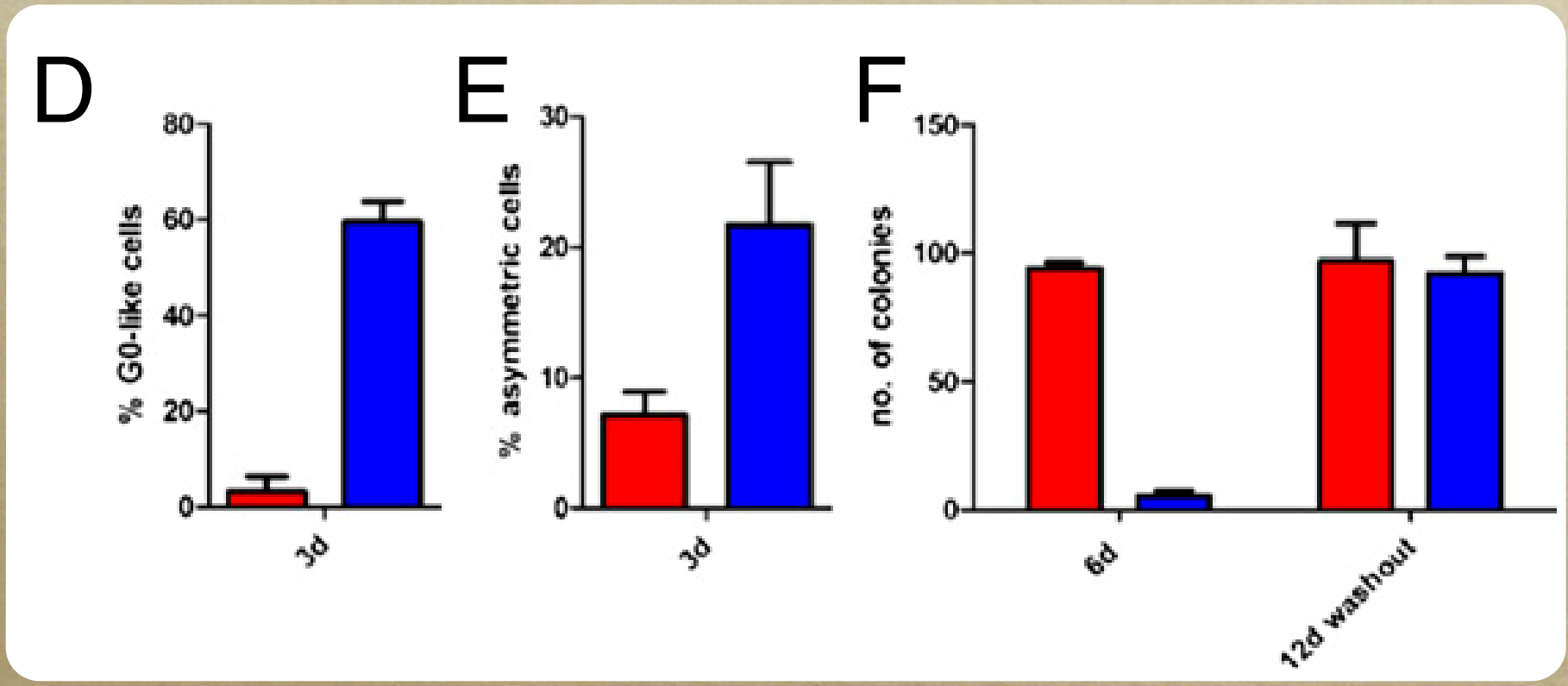
**Interphase: low overall Akt, high nuclear Foxo1**

# Akt inhibition boosts frequency of assymetric division

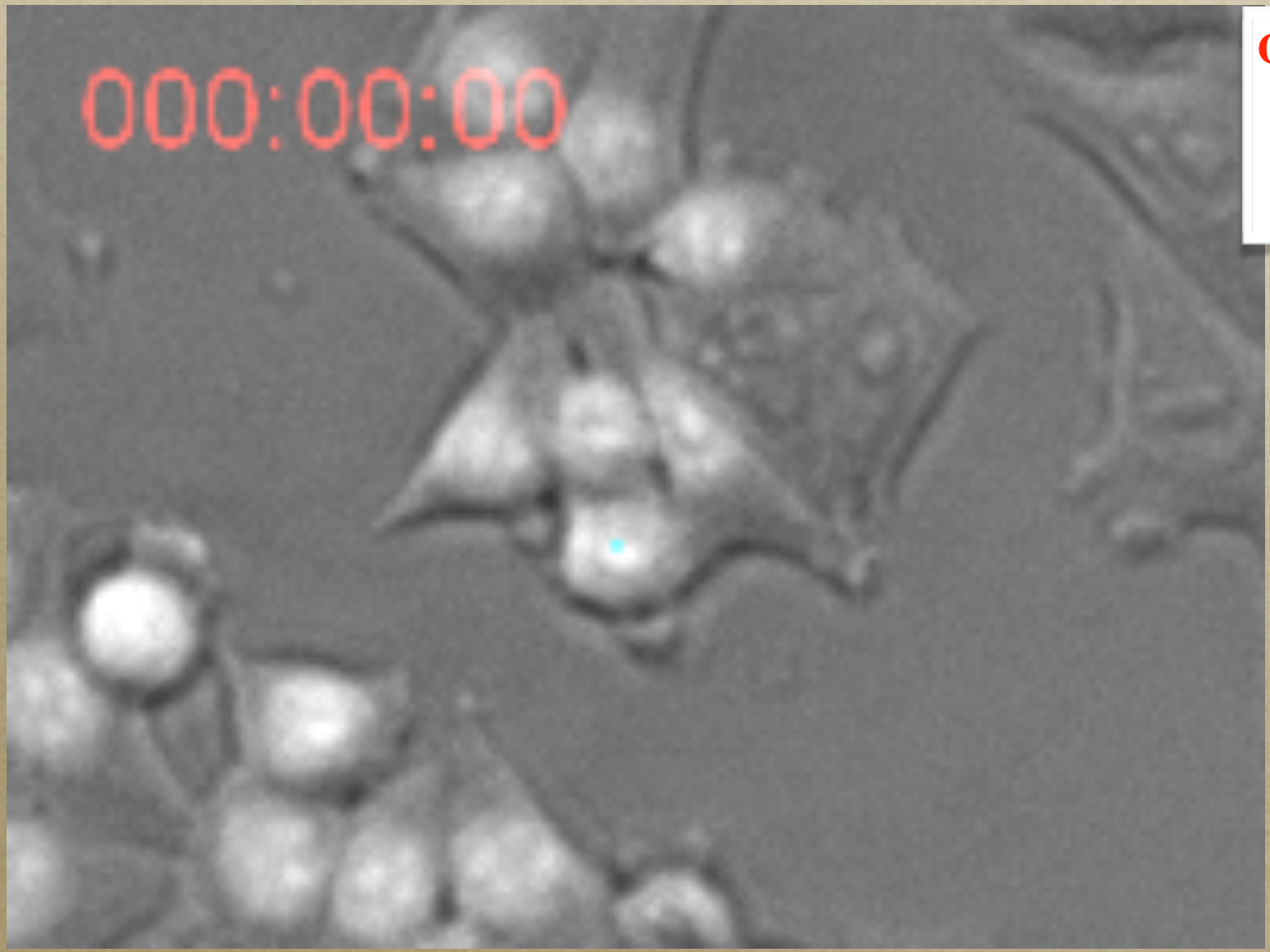
- **Full Akt inhibition triggers apoptosis**
  - ***PIK3CA mutation -> constitutive AKT signaling -> survival***
- **Intermediate Akt inhibition *increased the precentage* of slow-proliferating cells**
- **rapamycin, DAPT and a general protein kinase inhibitor fail to affect assymetric proliferation**



# Effect of Akt inhibition on overall proliferative potential is reversible



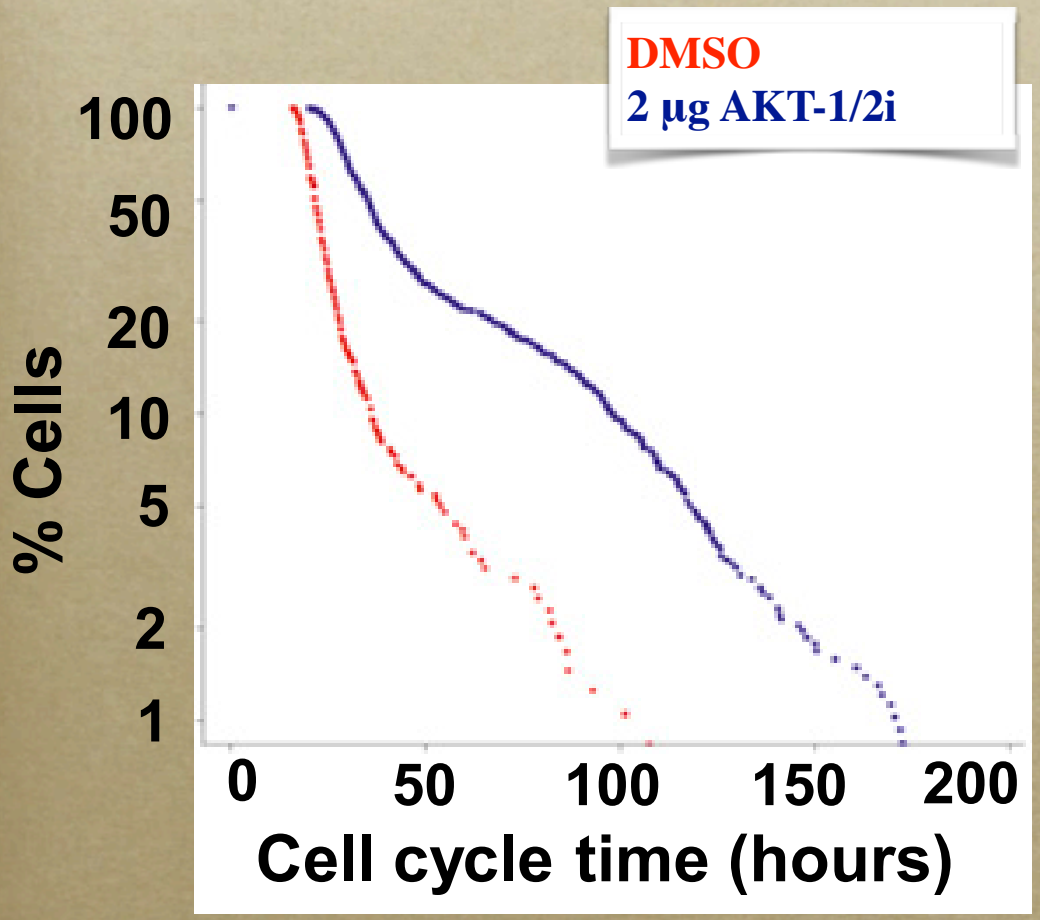
# Two daughter cells with inter-mitotic times



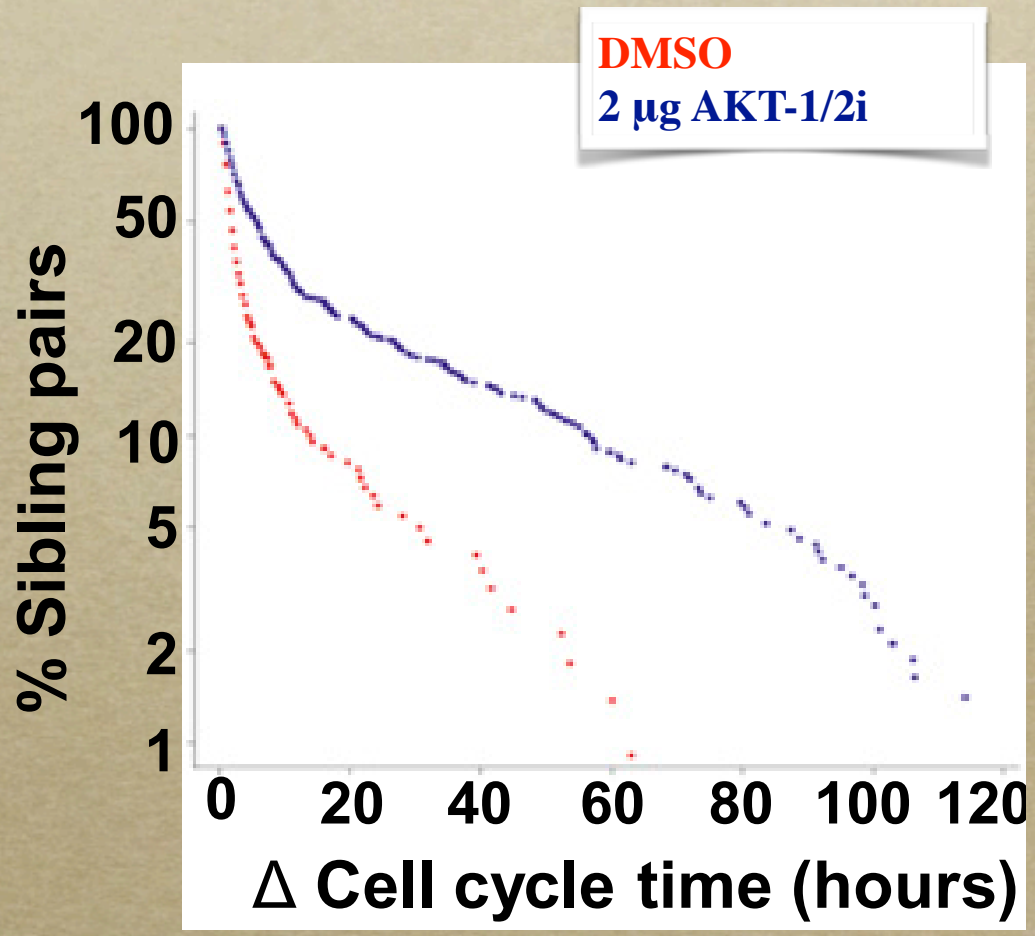

**Original: @ 14h**
**D1: @ 60h**
**D1-D1 @170h**
**D2:@175h**

**No difference in size or morphology**

# Proliferative heterogeneity increases with Akt inhibition

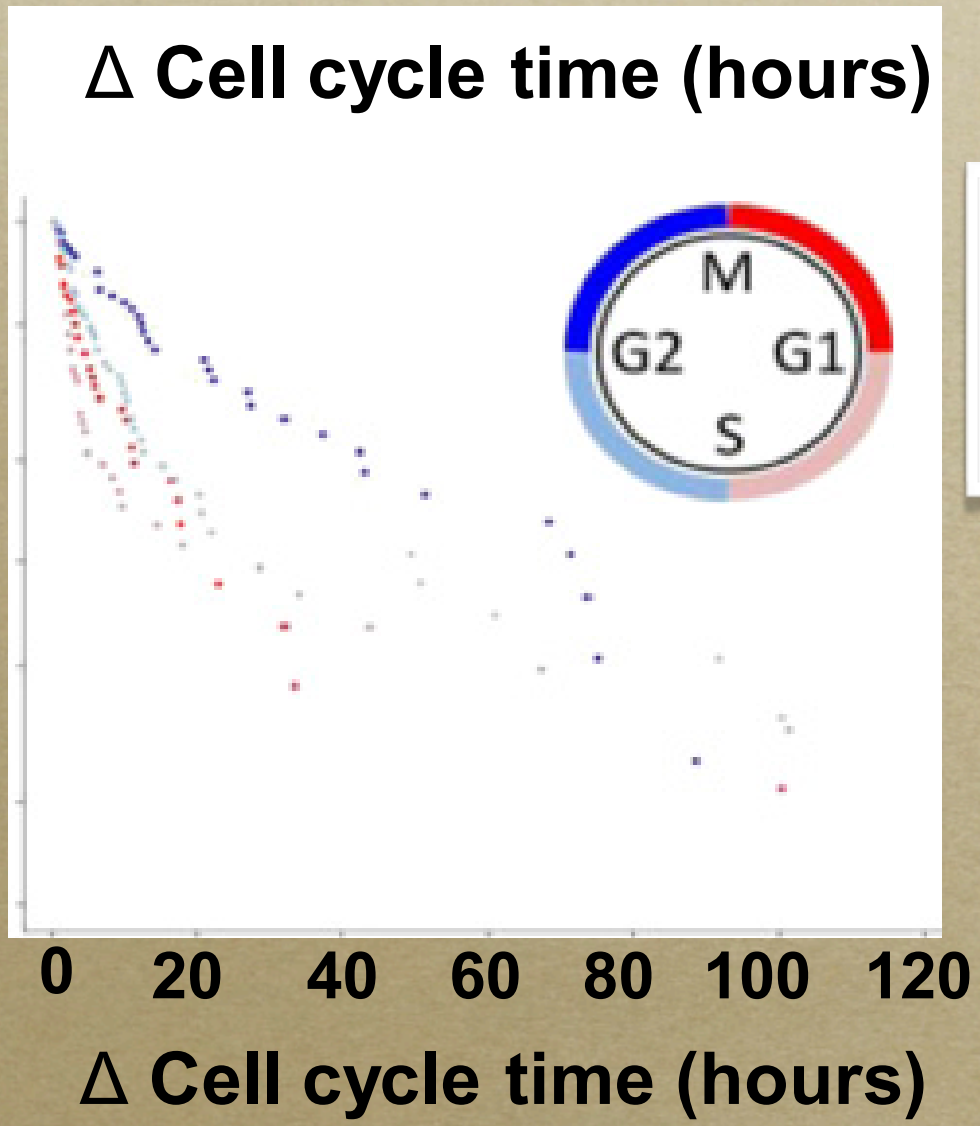



- Average: 26h -> 49h




- Average: 6.2h -> 16.9h

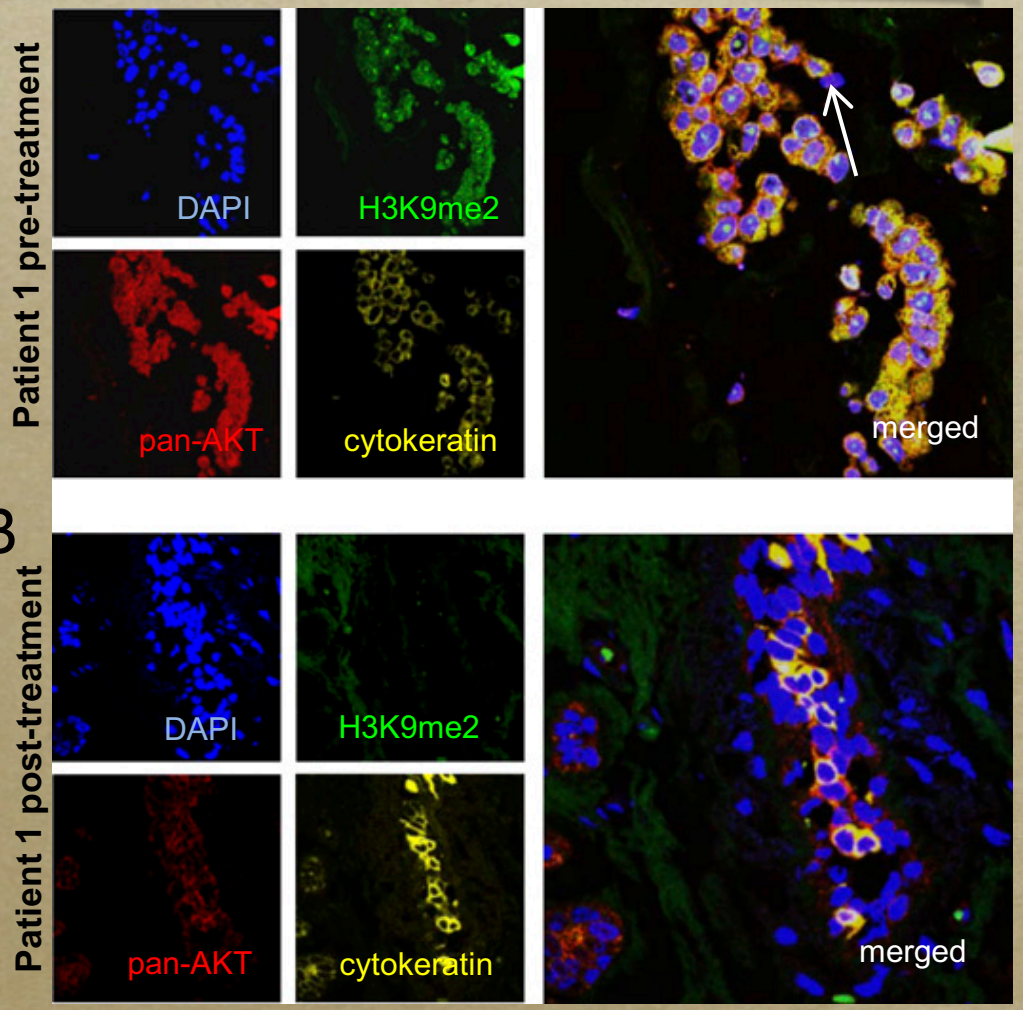
# Akt inhibition immediately before mitosis has biggest effect




within 6h after mitosis
6-12h after mitosis
6-12h before mitosis
within 6h before mitosis

# $G_0$-like cells are enriched after cytotoxic treatment in vivo

**Present in ER$^+$, ERBB2$^+$ and ER$^-$/ERBB2$^-$ tumors**

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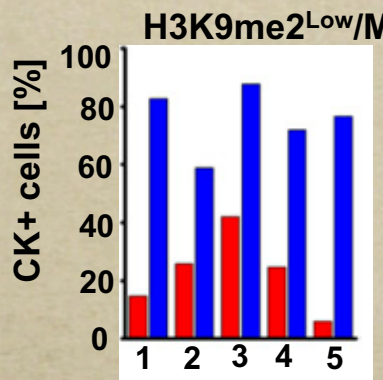


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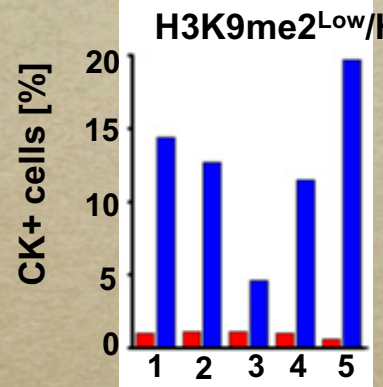


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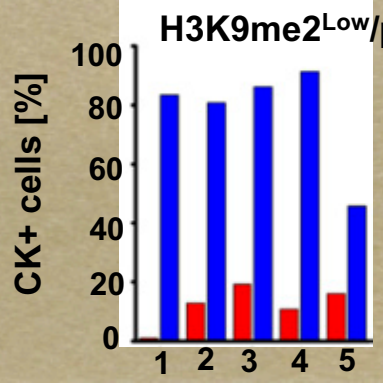
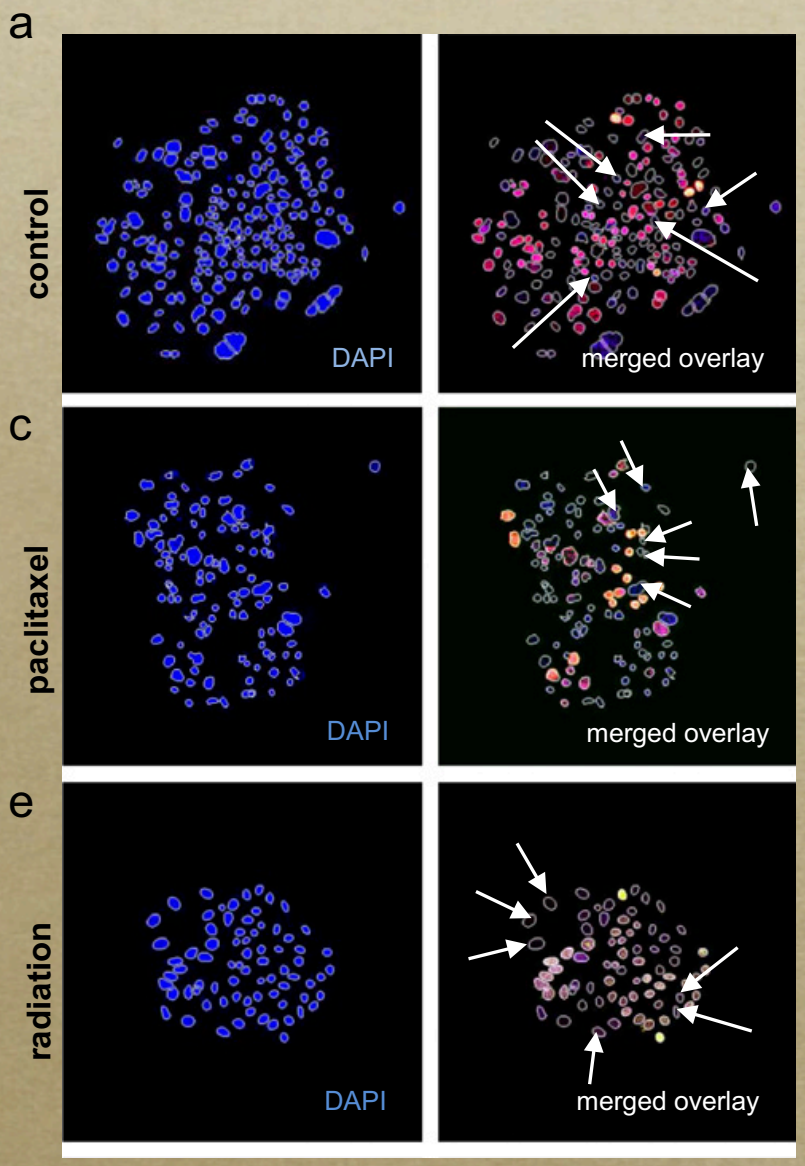
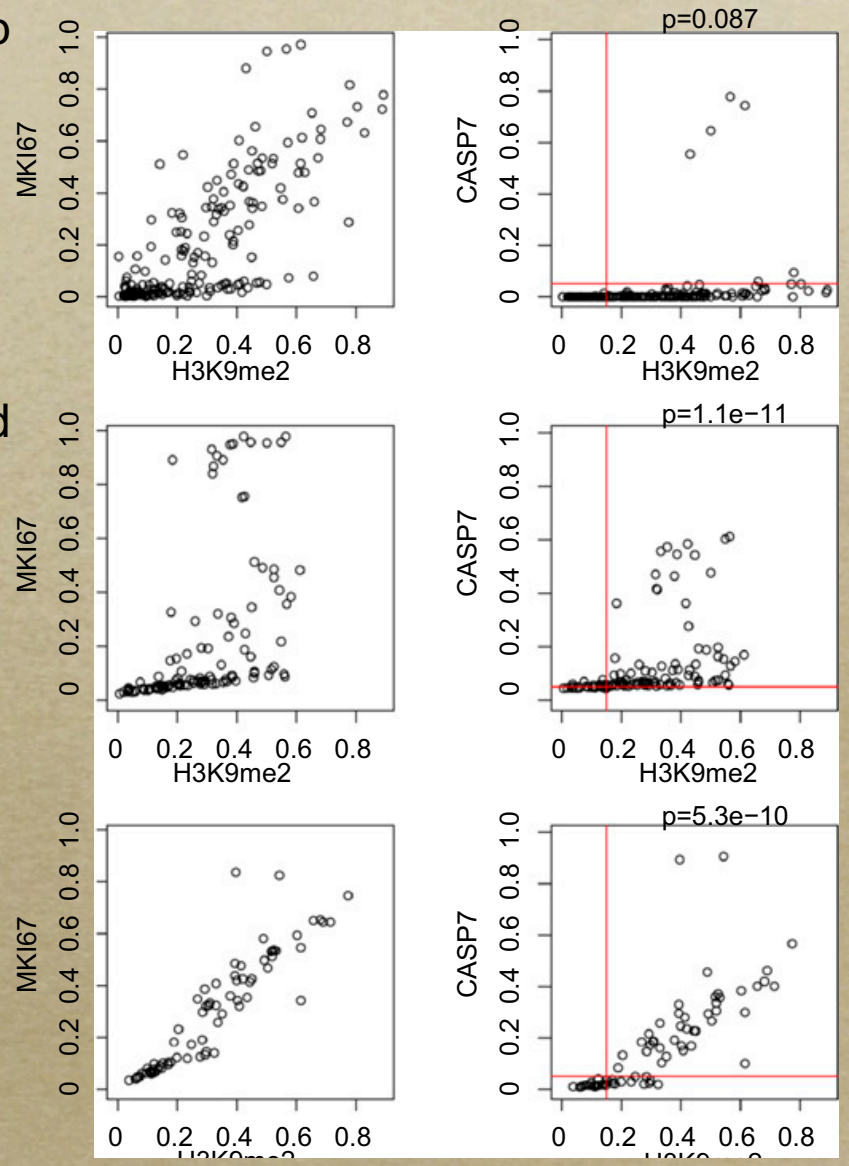


# $G_0$-like cells in vitro survive cytotoxic insult

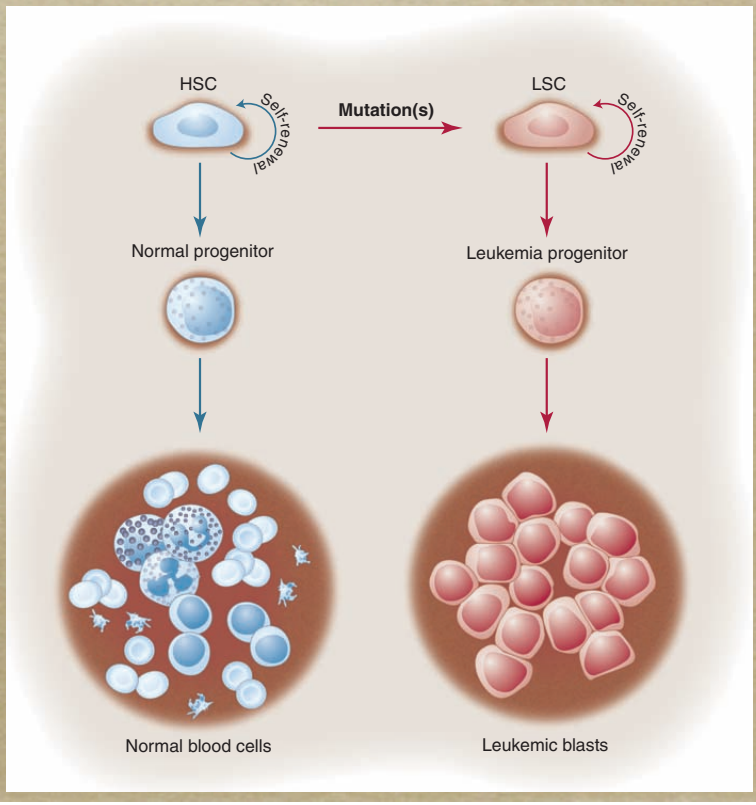
MKI67
CASP7
H3K9me2



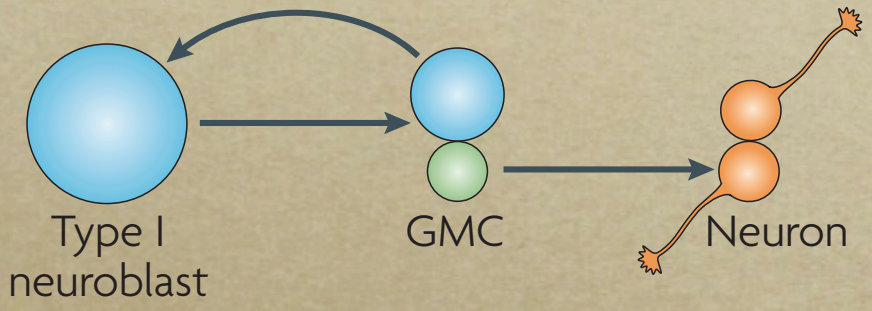
# Discussion & Drawbacks

- **Akt supressing cancer therapy could backfire by enriching the resistant, slow growing population**
- **Authors speculate: "cancer cells divide assymetrically like normal stem cells", but the $G_0$-like daughter fails to exit cell cycle**
  - ***turns original paradigm around***
- **Background very very weak**
  - ***may not be the author's fault***
- **No *in vivo* model they can manipulate**
  - ***show environmental modulation of slow cycling cell pool***

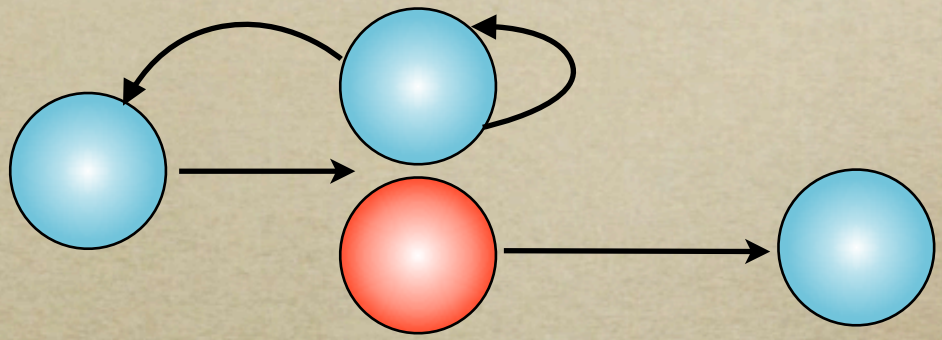


# Discussion: Assymetric division need not be a one-way street!

- **Assymetric division need not be a one-way street**




**Cell fate (terminal differentiation)**

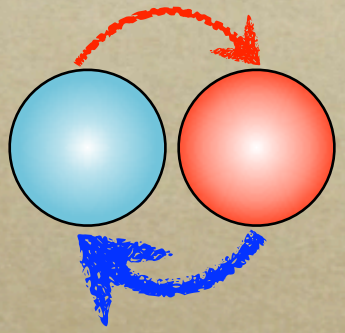


**Back and forth flux between phenotypes**

- **A new dimension to heterogeneity in cancer**
  - ***implications to remission, resistance***
  - ***does not appear to be a stem-cell like mechanism***
  - ***does not correlate with EMT***

# Outlook

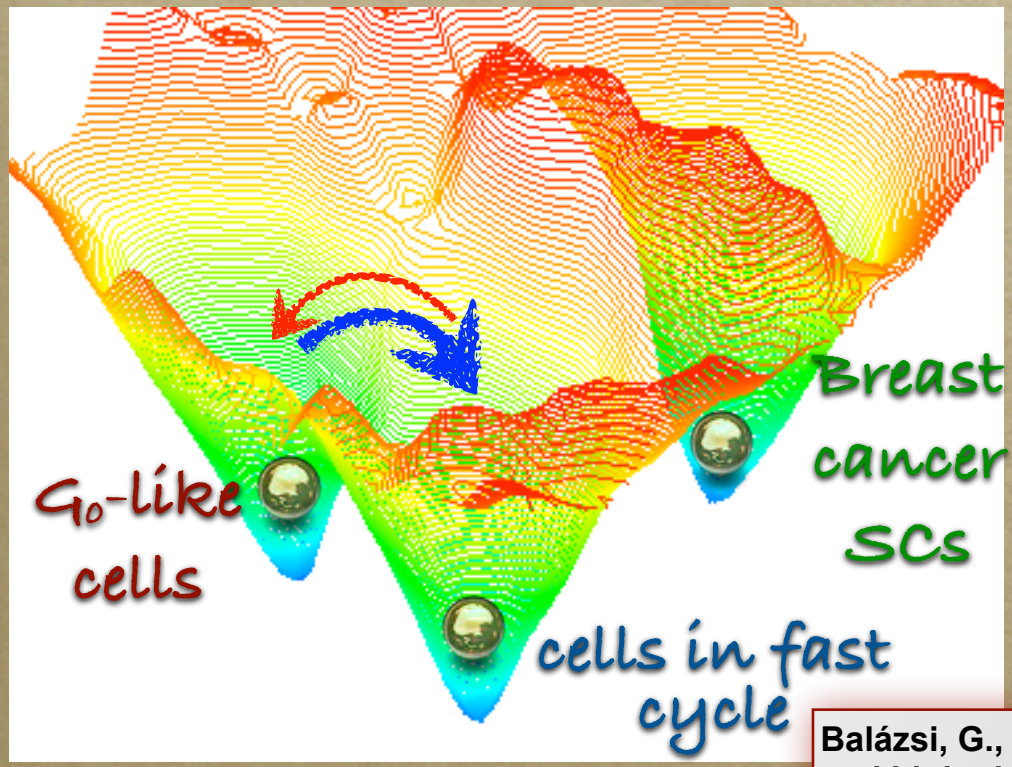
- **Multi-stablity in the regulatory system**



**(latent) property of all cancer cells (*nature*)**

**&**

**modulated by environmentally regulated signaling pathways (*nurture*)**




- **Stochastic transitions**
  - ***A cell population can hedge its bets by adopting a mixture of opposing strategies***
  - ***General phenomenon in viruses to mammals***

Balázsi, G., van Oudenaarden, A. & Collins, J. J. Cellular decision making and biological noise: from microbes to mammals. Cell 144, 910–925 (2011).

# Thank you!

The premise:

Cells with identical

- genome
- phenotype
- environment
- history of environments

can display functionally heterogeneos behavior