Cancer plasticity and cancer stem cells

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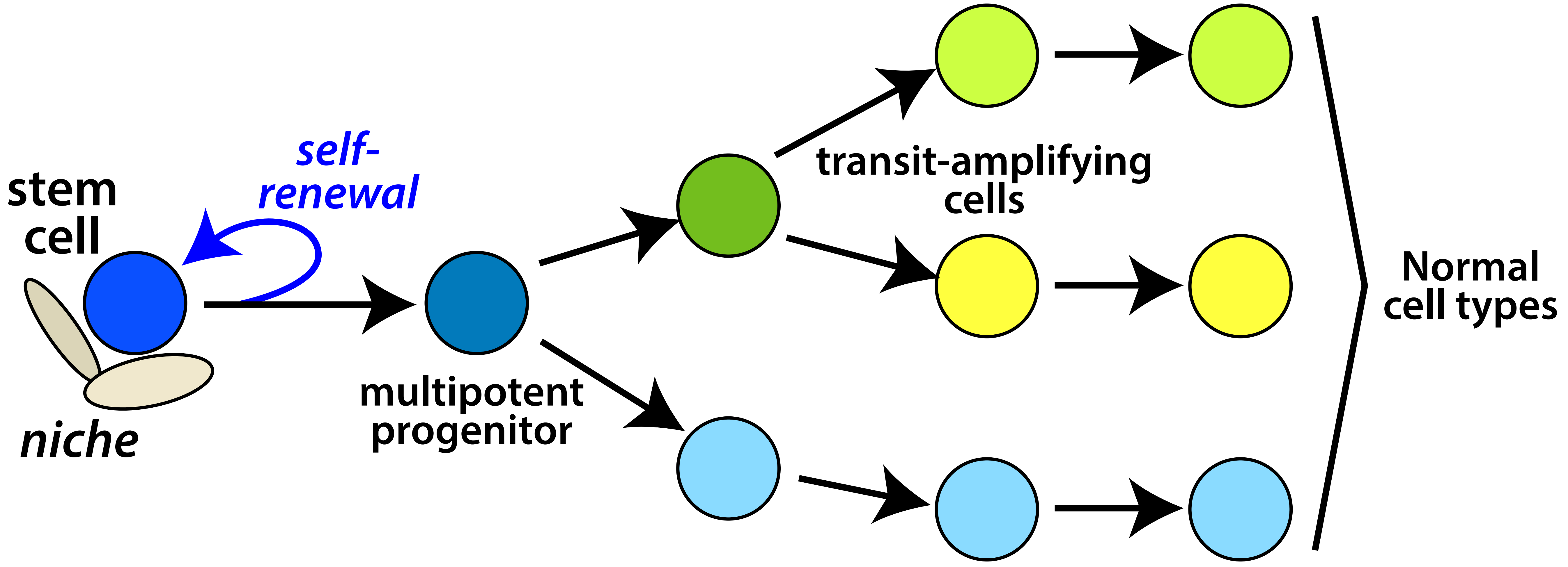
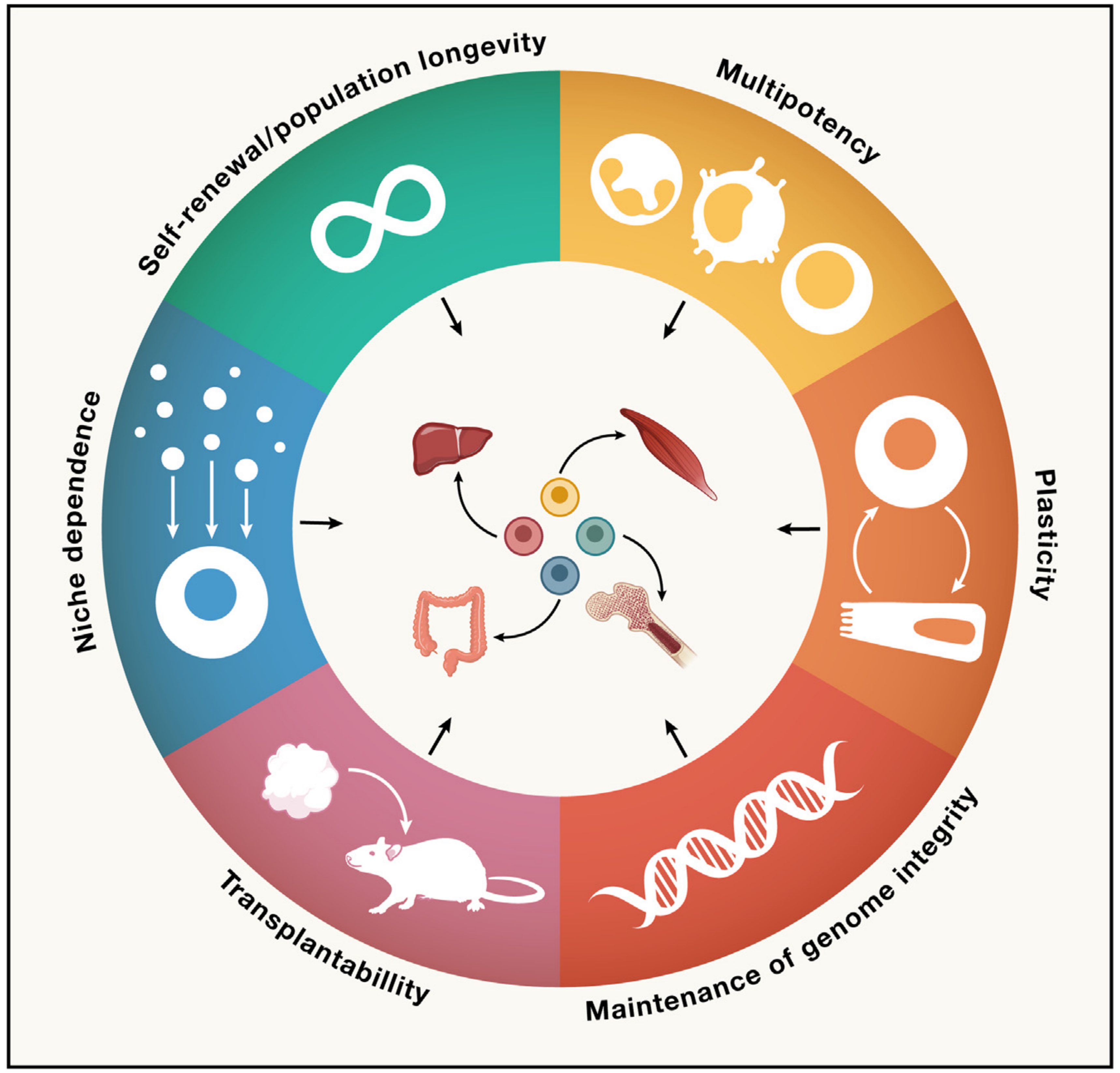
Lecture objectives

- Identify key properties of stem cells and cancer stem cells
- Describe methods for assaying stemness in cancer and their limitations
- Understand the cell of origin hypothesis
- Understand lineage plasticity and its relationship to stemness

Properties of mammalian stem cells





Shibata and Shen (2013) *Bioassays* 35: 253-260

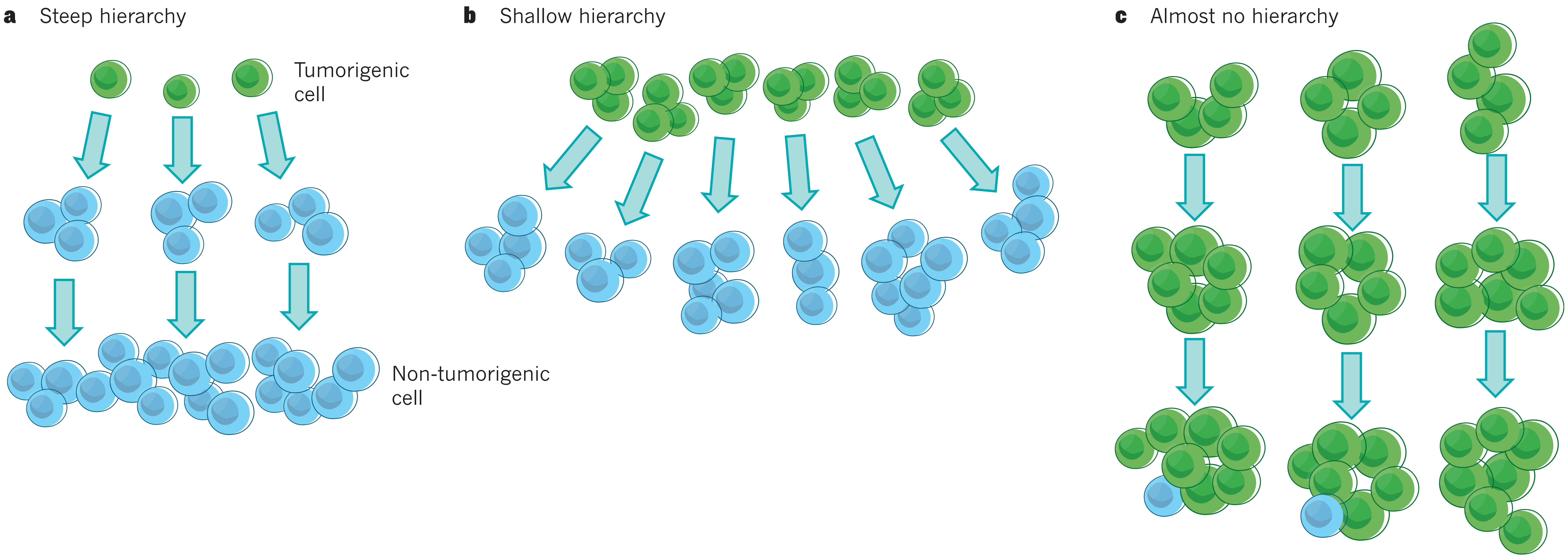
Beumer and Clevers (2024) *Cell Stem Cell* 31: 7-24

Properties of a cancer stem cell

- Self-renewal
- Differentiation into non-tumorigenic cells
- Relatively rare
- Basis for metastasis
- Origin from a stem cell or progenitor?

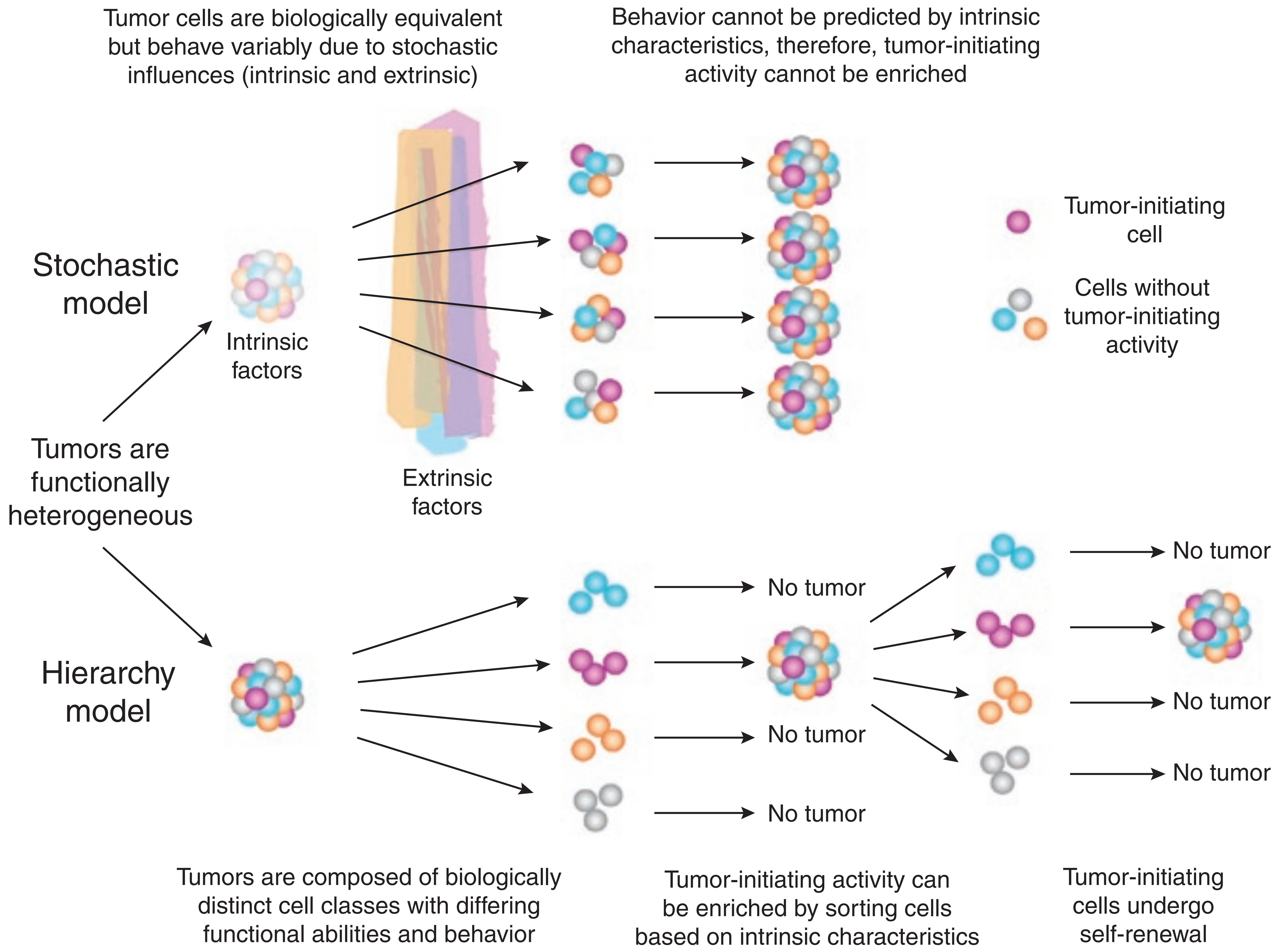
- *Cell of origin*
- *Cancer stem cell*
- *Tumor-initiating cell*
- *Tumor-propagating cell*

Hierarchical organization of tumors



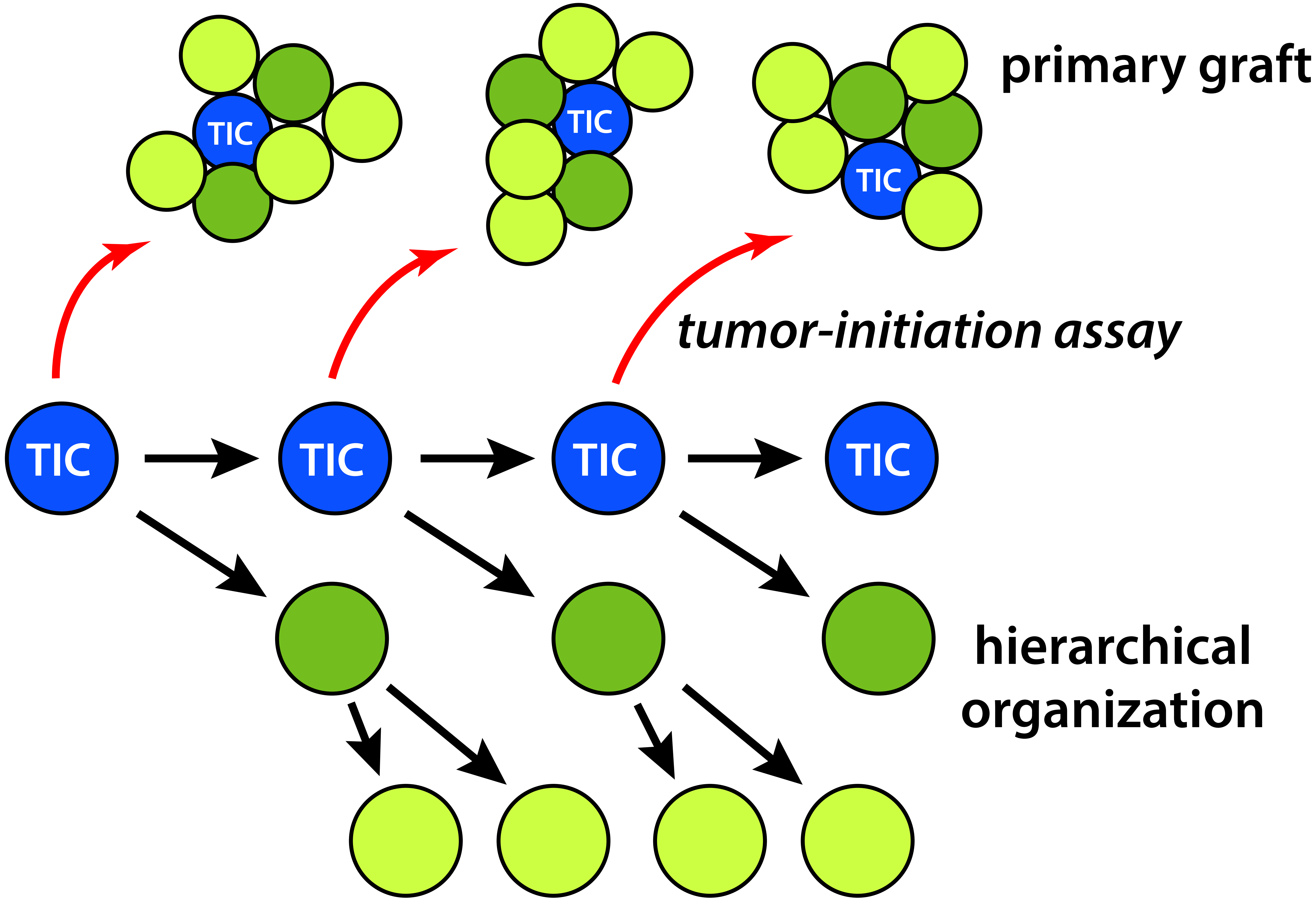
Meacham and Morrison (2013) *Nature* 501: 328-337

Models for tumor heterogeneity



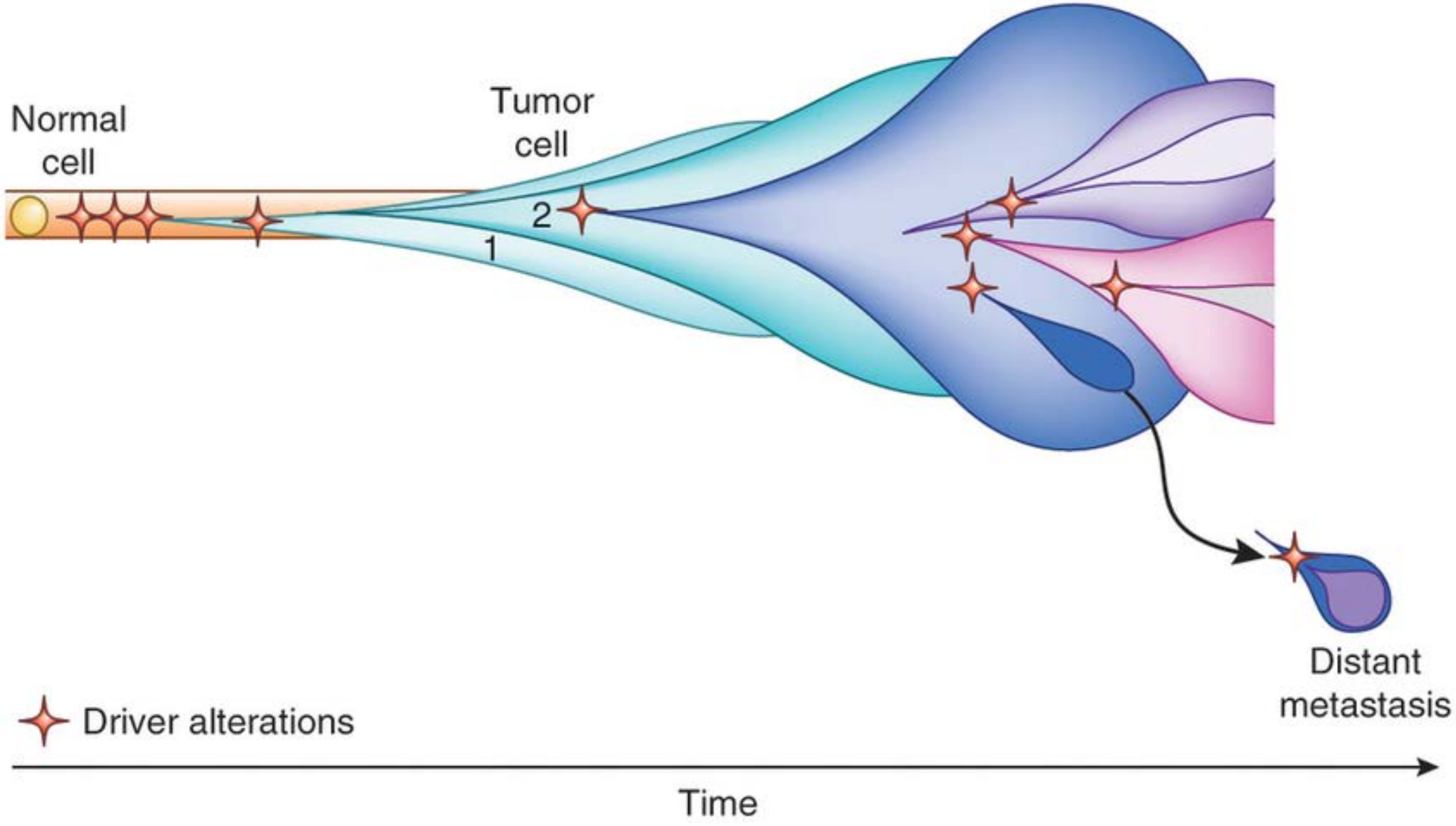
Dick (2009) *Nat. Biotech.* 27: 44-46

Classical cancer stem cell model



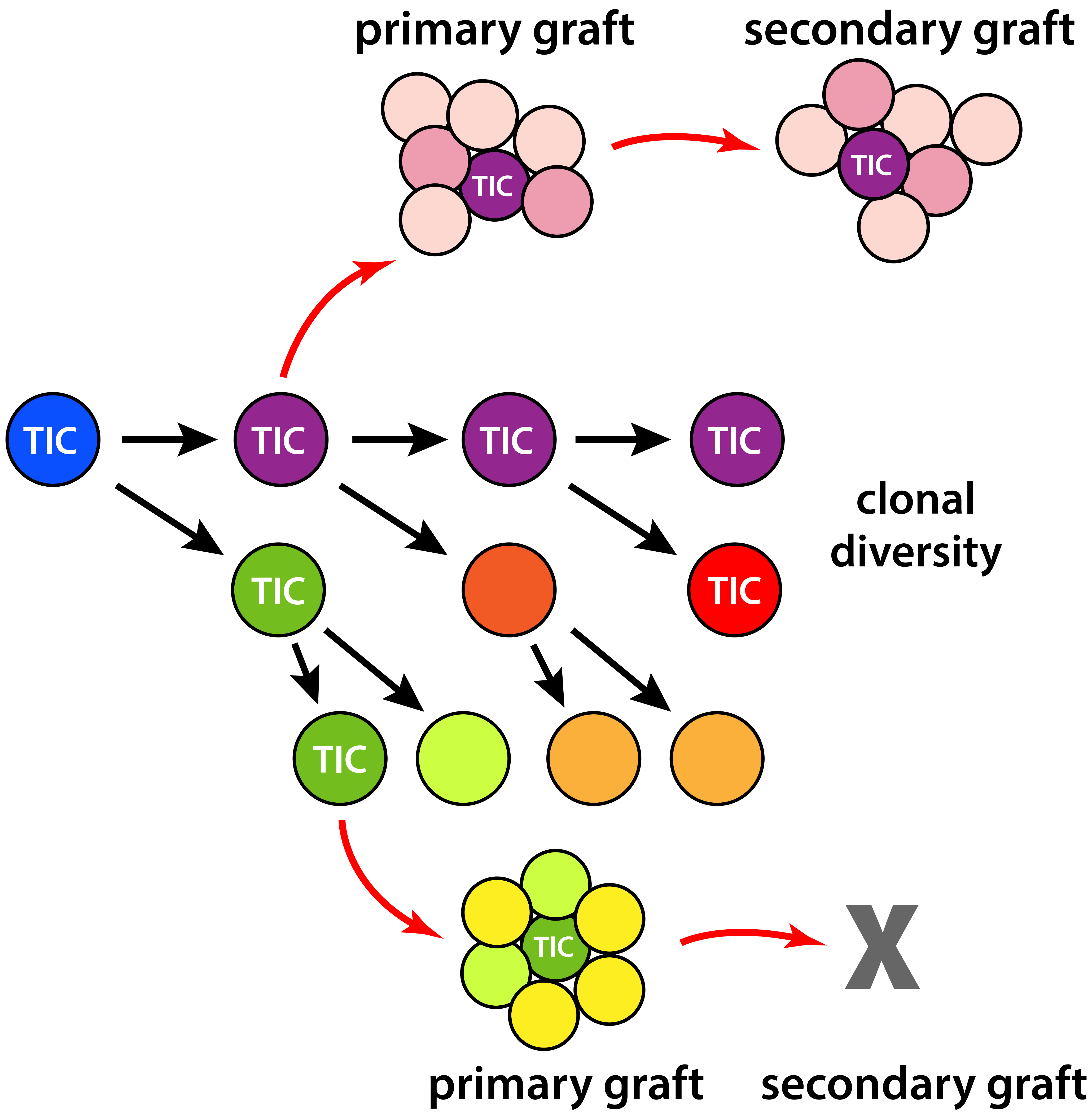
Shibata and Shen (2013) *Bioassays* 35: 253-260

Tumor heterogeneity and evolution



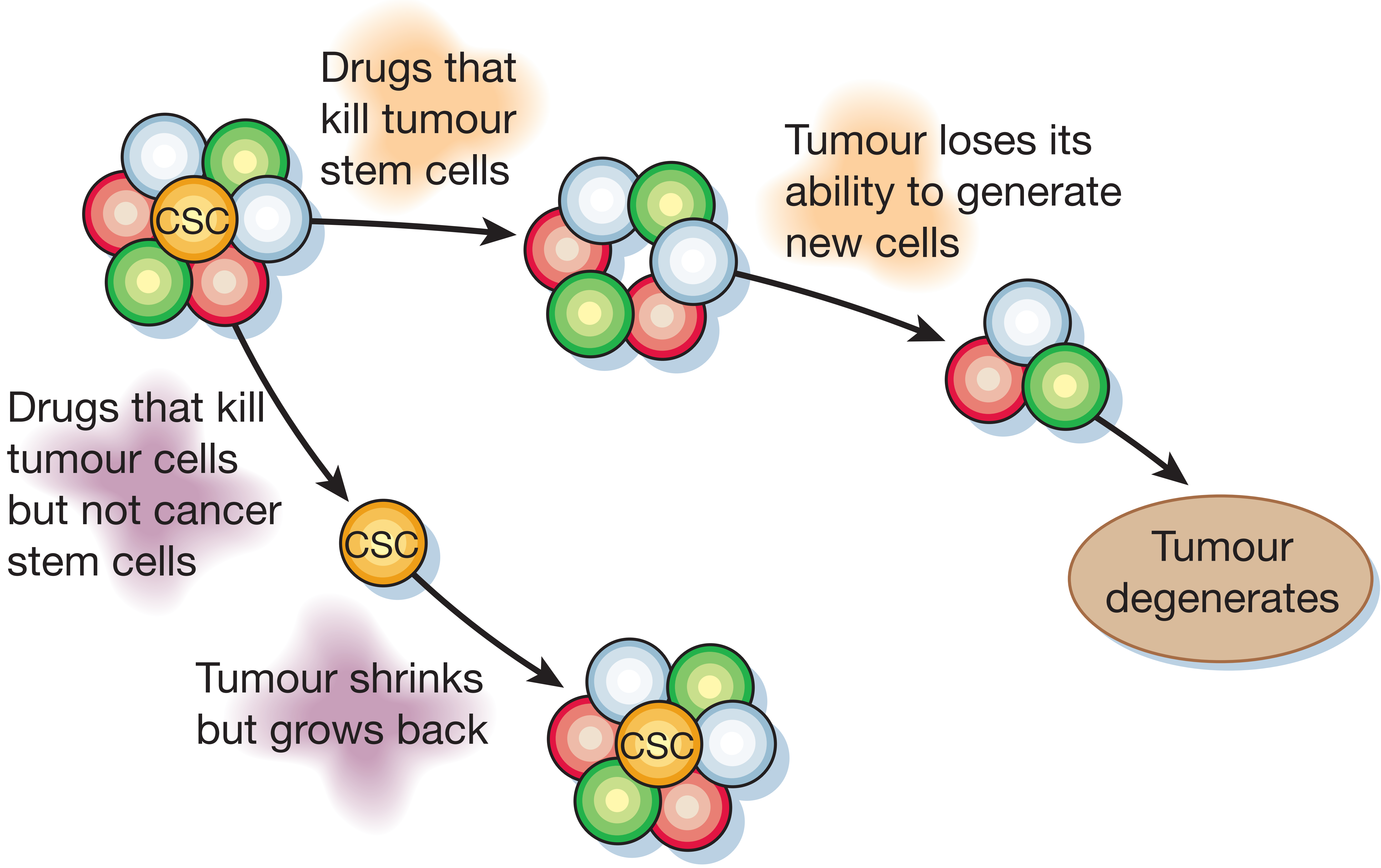
Alizadeh *et al.* (2015) *Nat. Med.* 21: 846-853

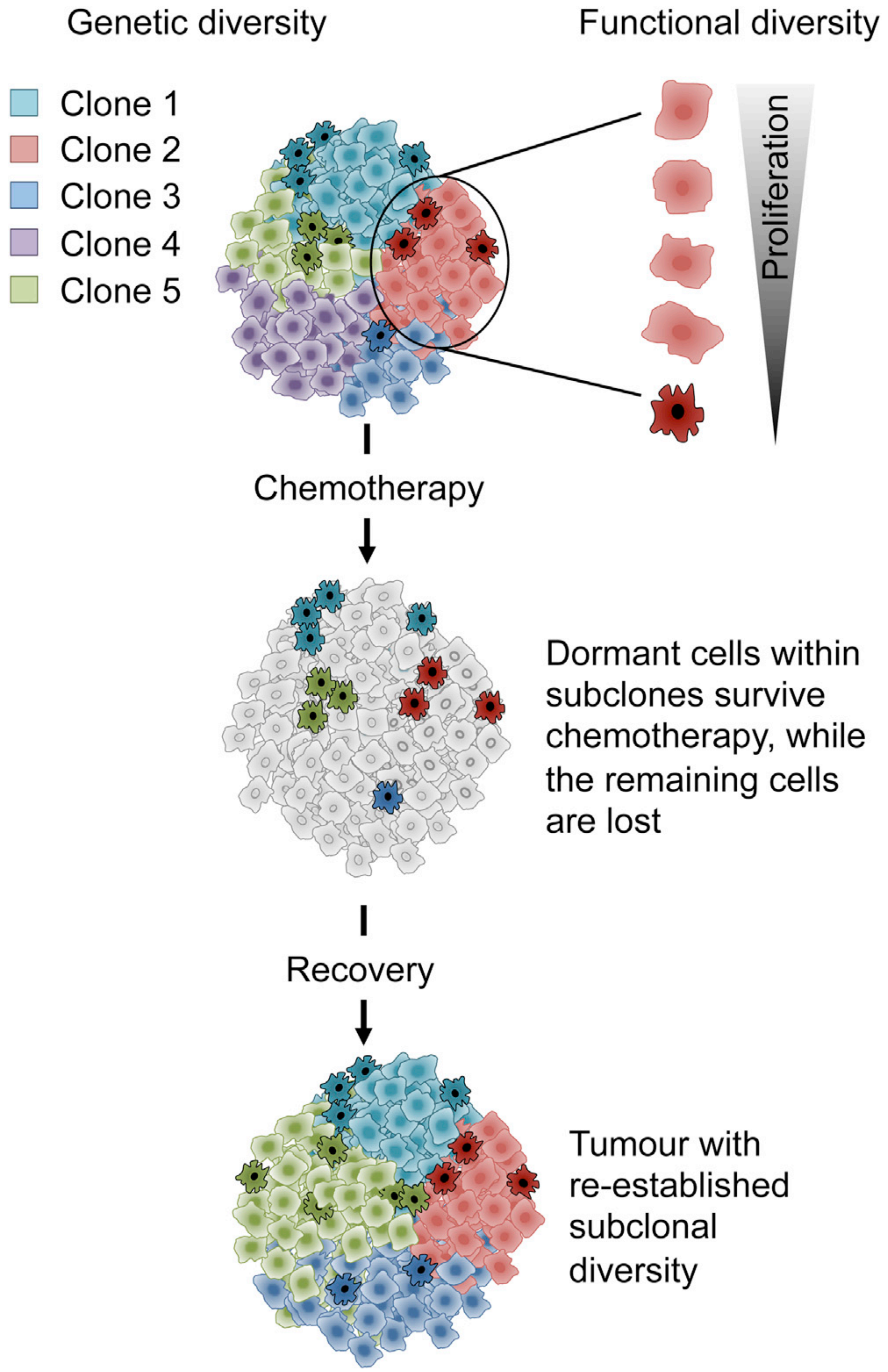
Clonal evolution and tumor initiation



Shibata and Shen (2013) *Bioassays* 35: 253-260

Therapeutic targeting and heterogeneity

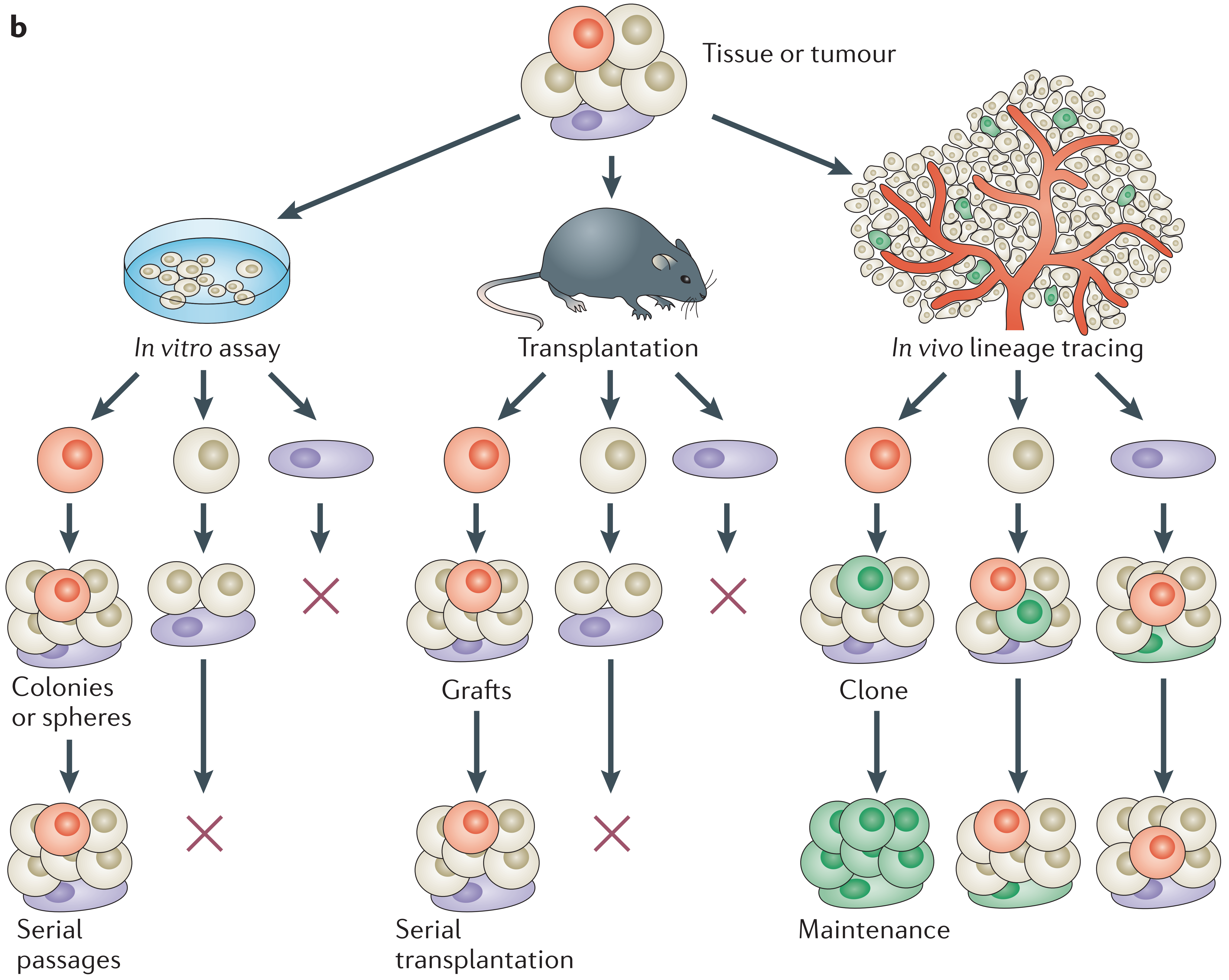




Reya *et al.* (2001) *Nature* 414: 105-111

Kreso and Dick (2014) *Cell Stem Cell* 14: 275-291

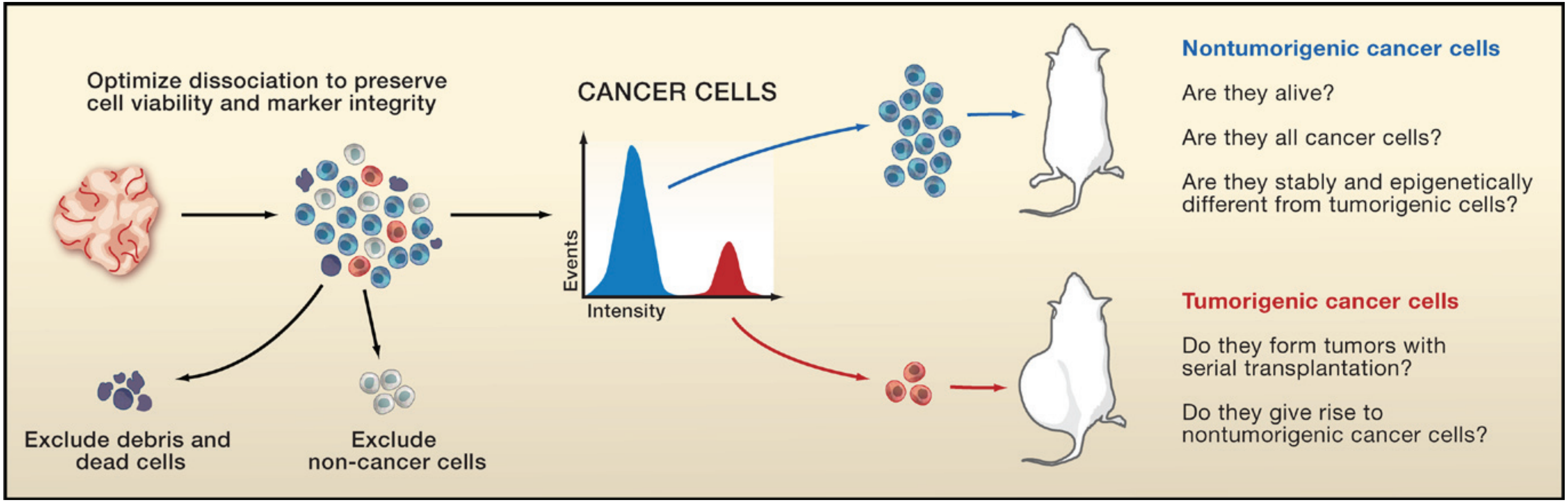
Distinct assays for progenitor activity



Do these assays identify the same cell populations?

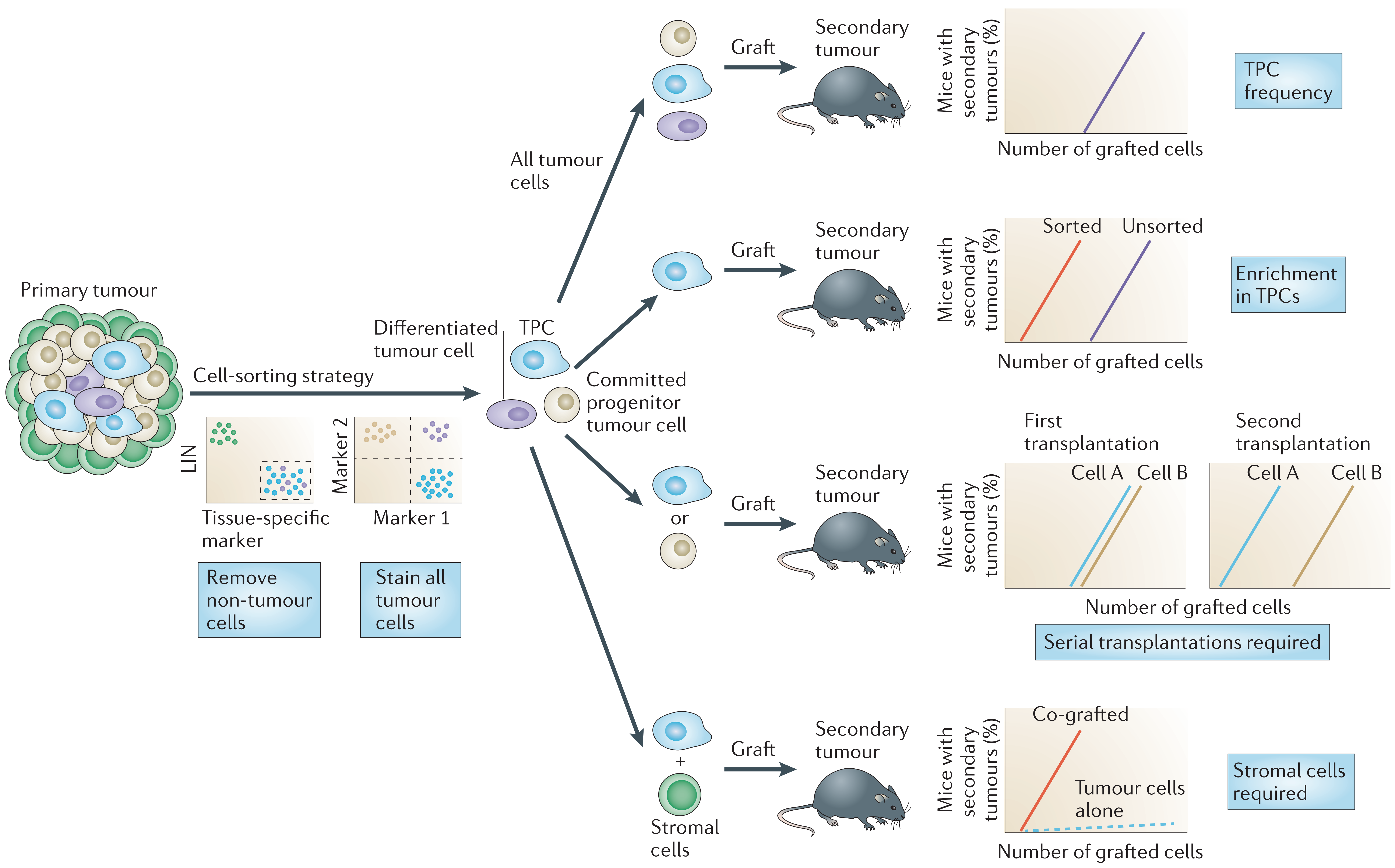
Beck and Blanpain (2013) *Nat. Rev. Cancer* 13: 727-738

Common pitfalls in assaying tumor initiation



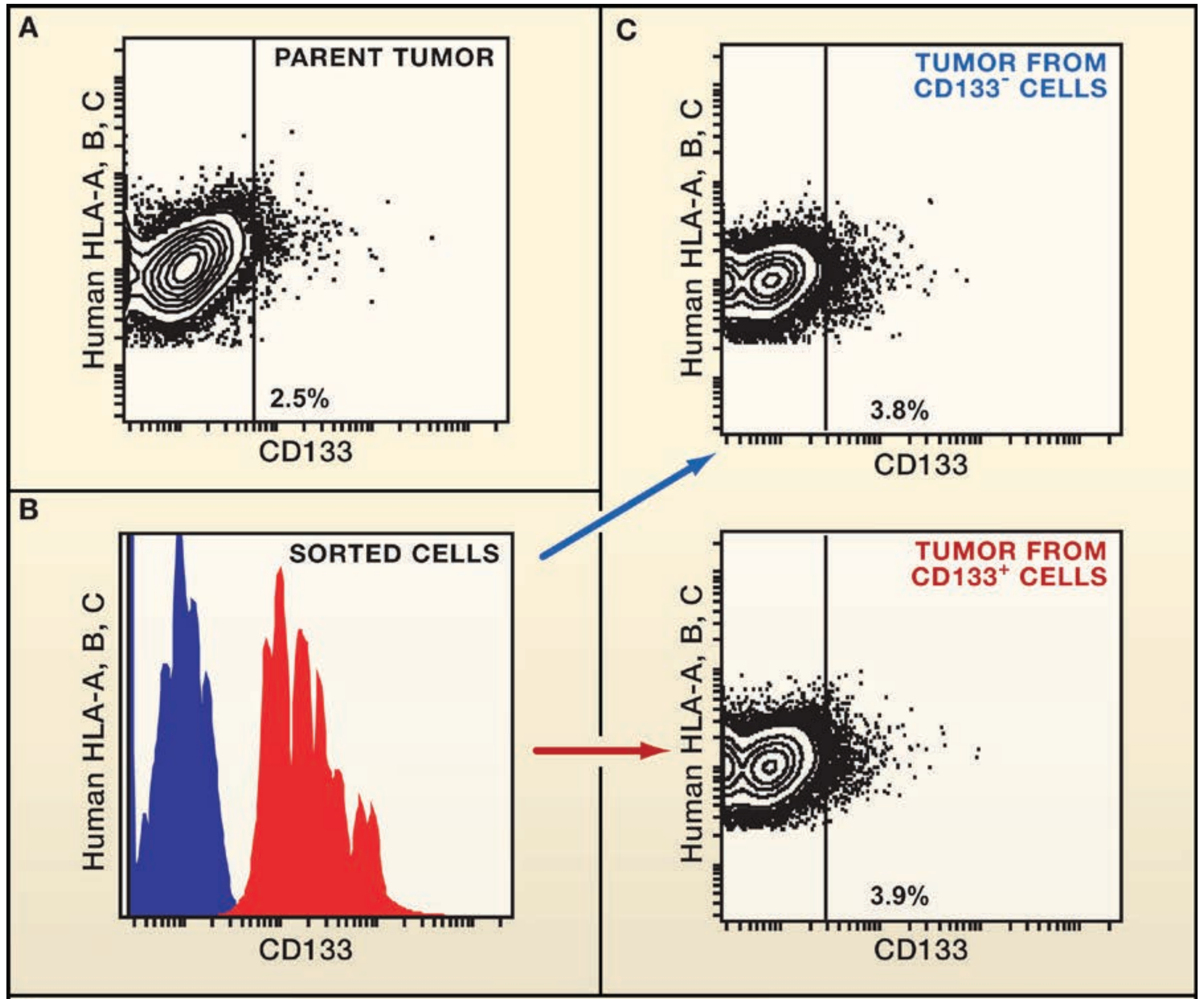
Shackleton *et al.* (2009) *Cell* 138: 822-829

Assaying tumor propagation in grafts



Beck and Blanpain (2013) *Nat. Rev. Cancer* 13: 727-738

Heterogeneity without hierarchical organization



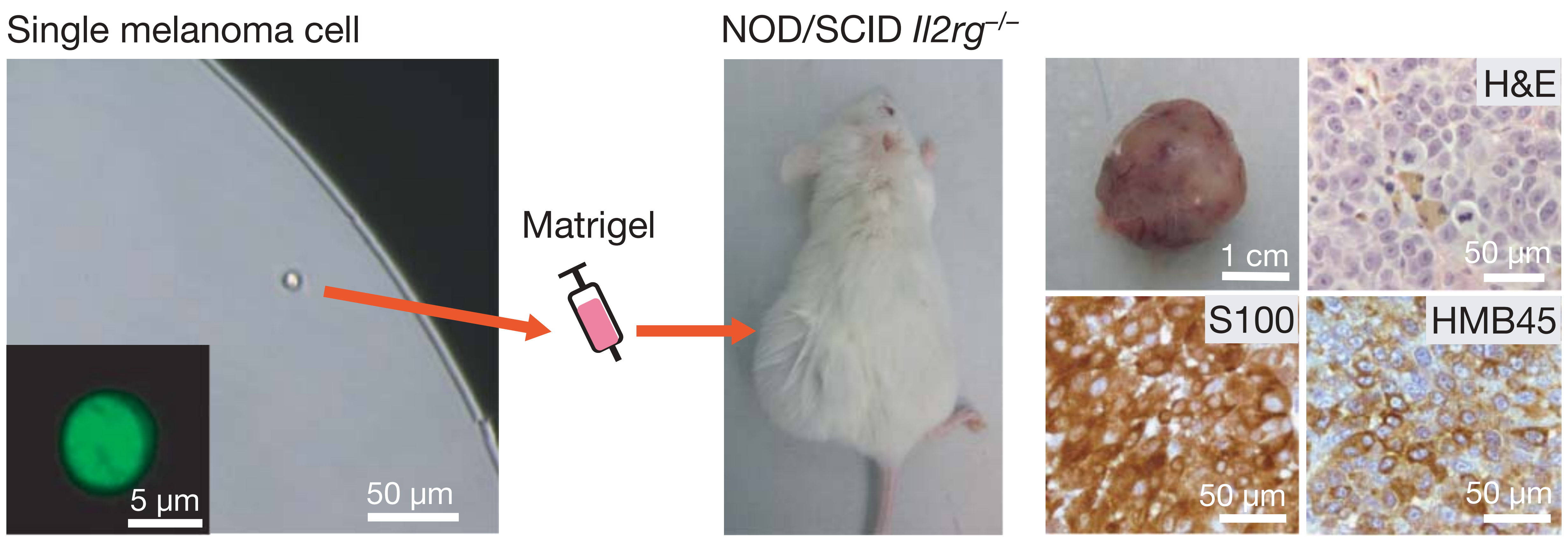
D

Sorted fraction	# of tumors/# of injections Cells/injections 50	 10	Melanoma-initiating cell frequency (95%CI)	
Unfractionated	18/18	14/18	1/7	(1/4-1/12)
$CD133^-$	17/17	12/18	1/9	(1/5-1/15)
$CD133^+$	14/18	9/18	1/25	(1/15-1/40)

Shackleton *et al.* (2009) *Cell* 138: 822-829

Efficient tumor initiation by single melanoma cells

a

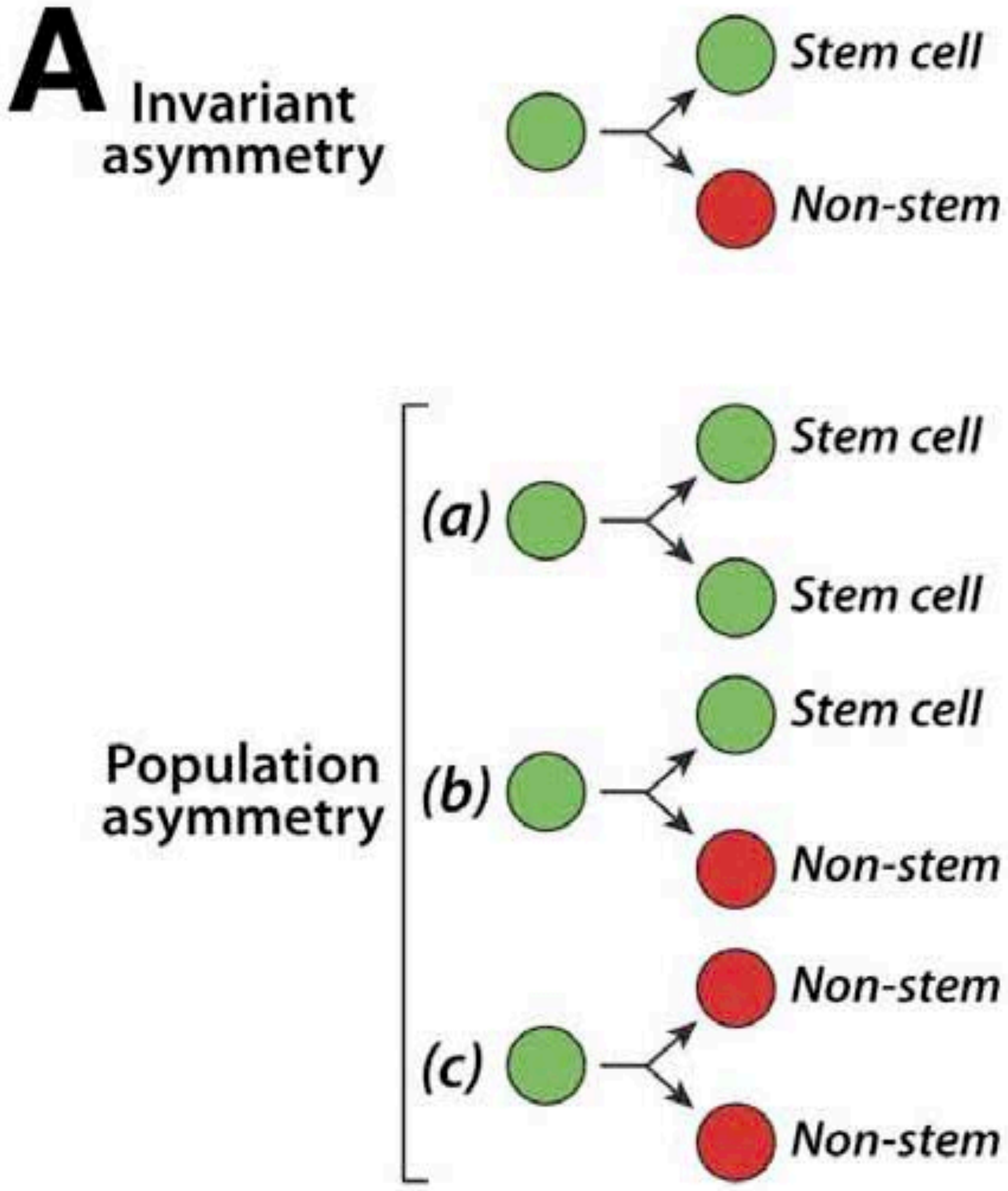


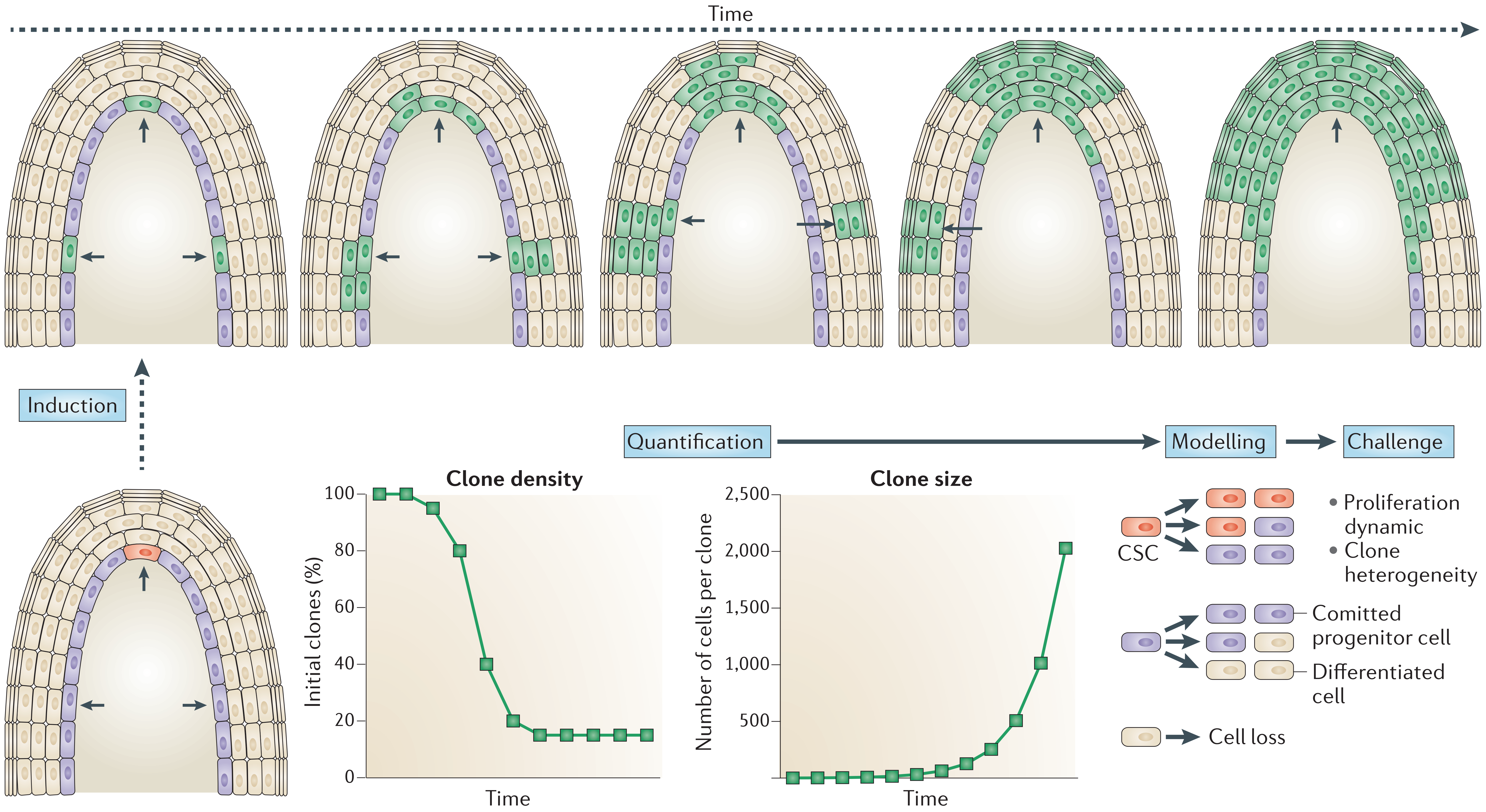
b

Patient	Engraftment rate tumours/injections (%)		Melanoma-initiating cell frequency (95% confidence interval)		Weeks to first palpability
205	11/89	(12%)	1/8	(1/5–1/14)	7 ± 2
214	12/73	(16%)	1/6	(1/4–1/10)	10 ± 4
481	40/62	(65%)	1/2	(1/1–1/2)	12 ± 3
487	6/30	(20%)	1/5	(1/3–1/11)	10 ± 1
All	69/254	(27%)	1/4	(1/3–1/5)	11 ± 3

Quintana *et al.* (2008) *Nature* 456: 593-598

Clonal analysis of tumor growth

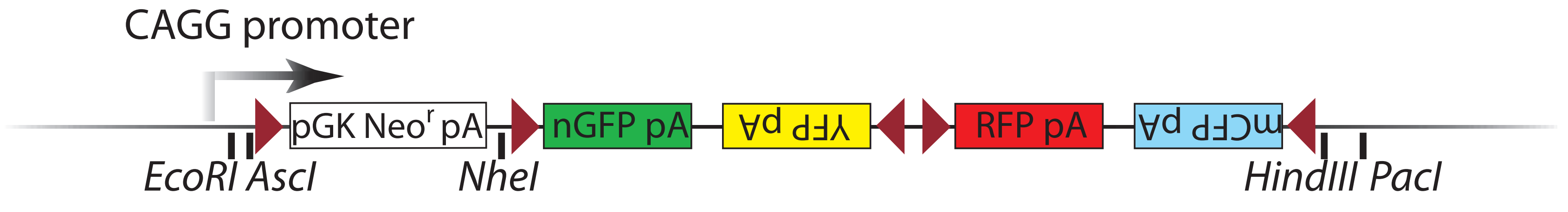


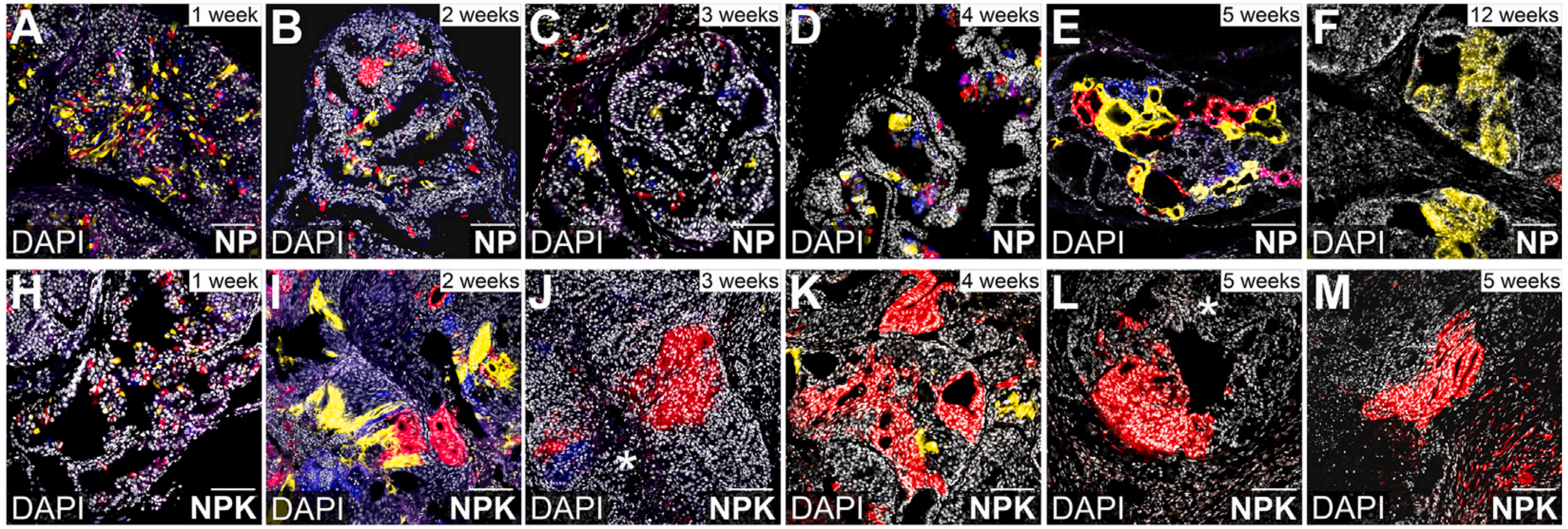


Beck and Blanpain (2013) *Nat. Rev. Cancer* 13: 727-738

Lineage tracing of clonal evolution in prostate cancer

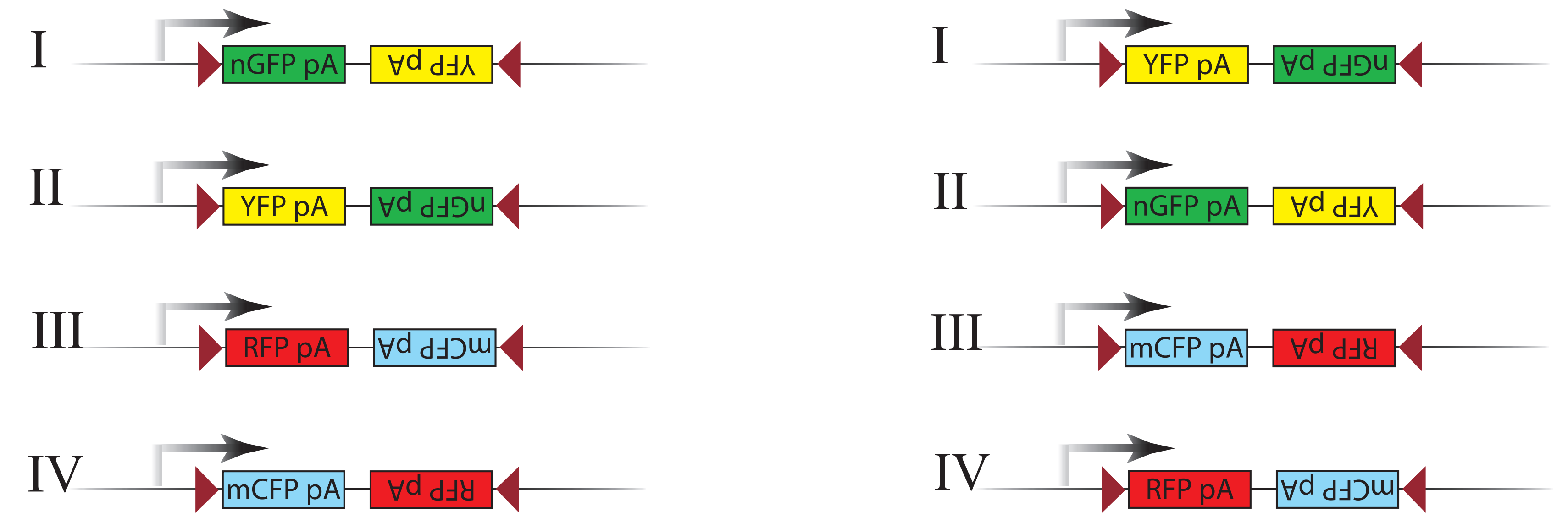
Rosa26-Confetti locus

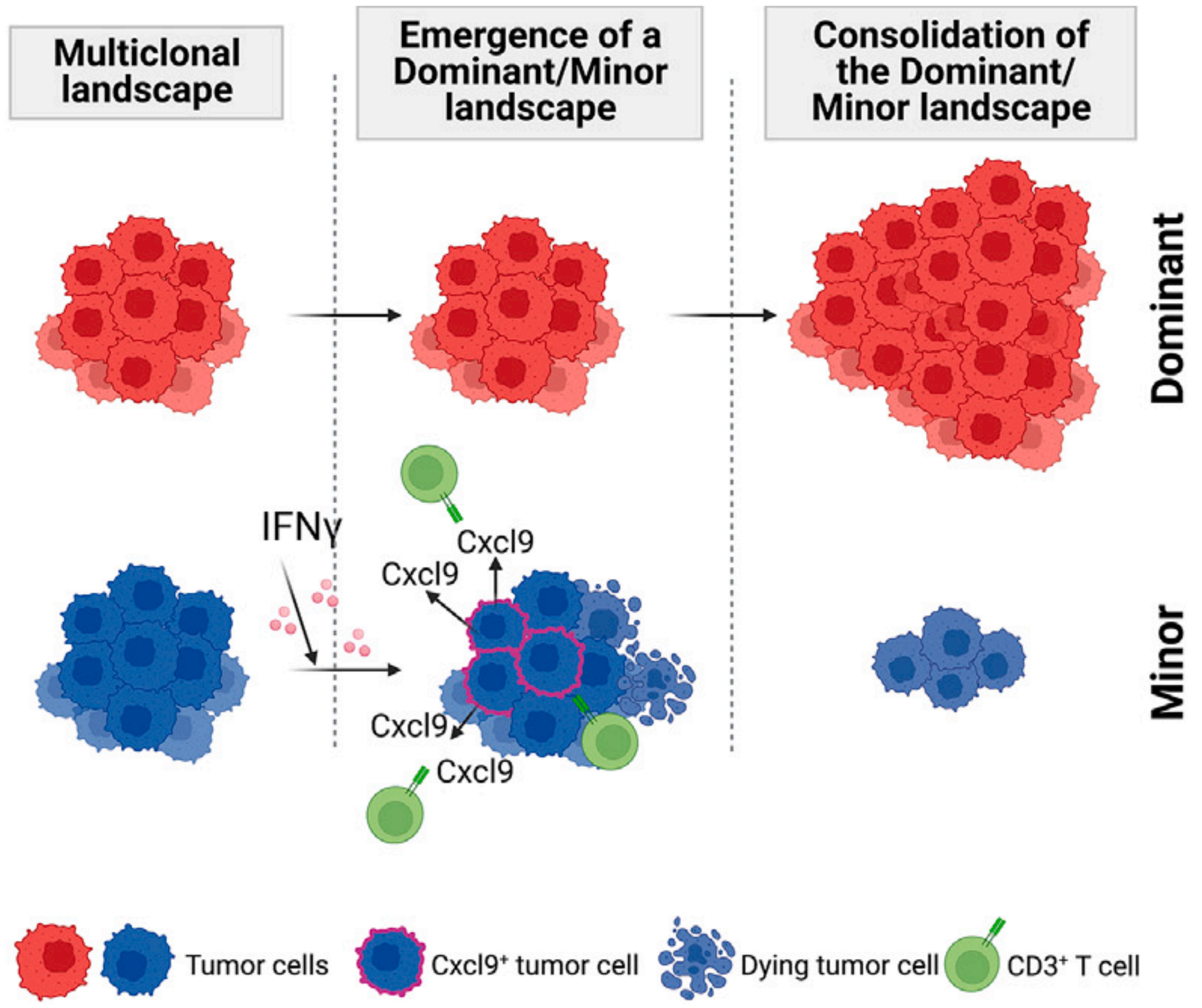




Transient Cre recombination
'Tracing'

Color conversion
'Re-tracing'

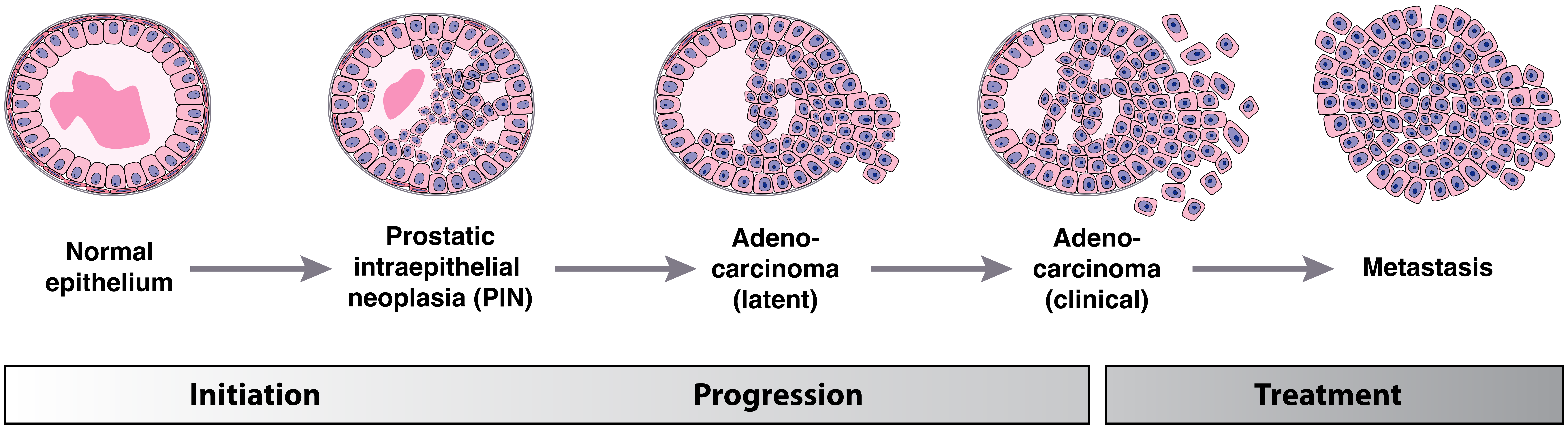




Schepers *et al.* (2012) *Science* 337: 730-735

Tshering *et al.* (2023) *Dev. Cell* 58: 1071-1086

Pathway for prostate cancer progression

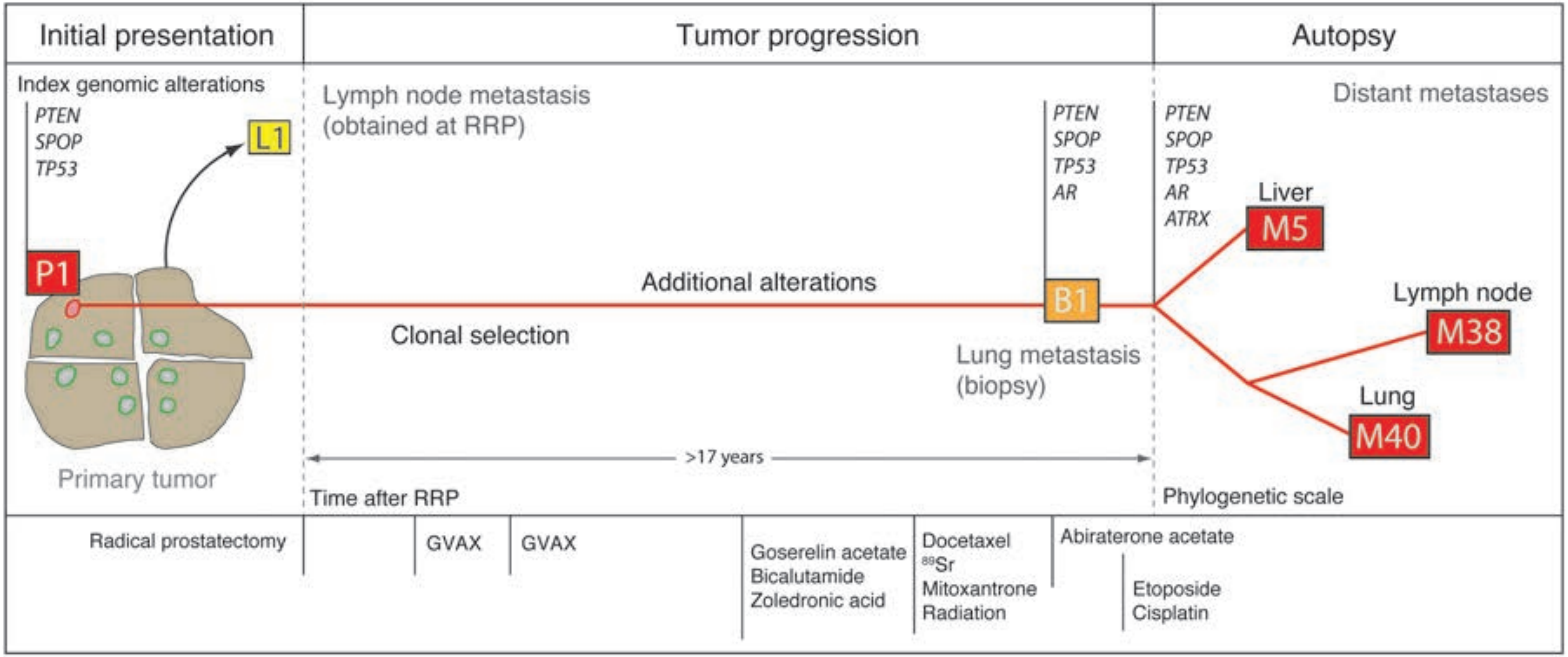


Loss of basal cells

Castration-resistance

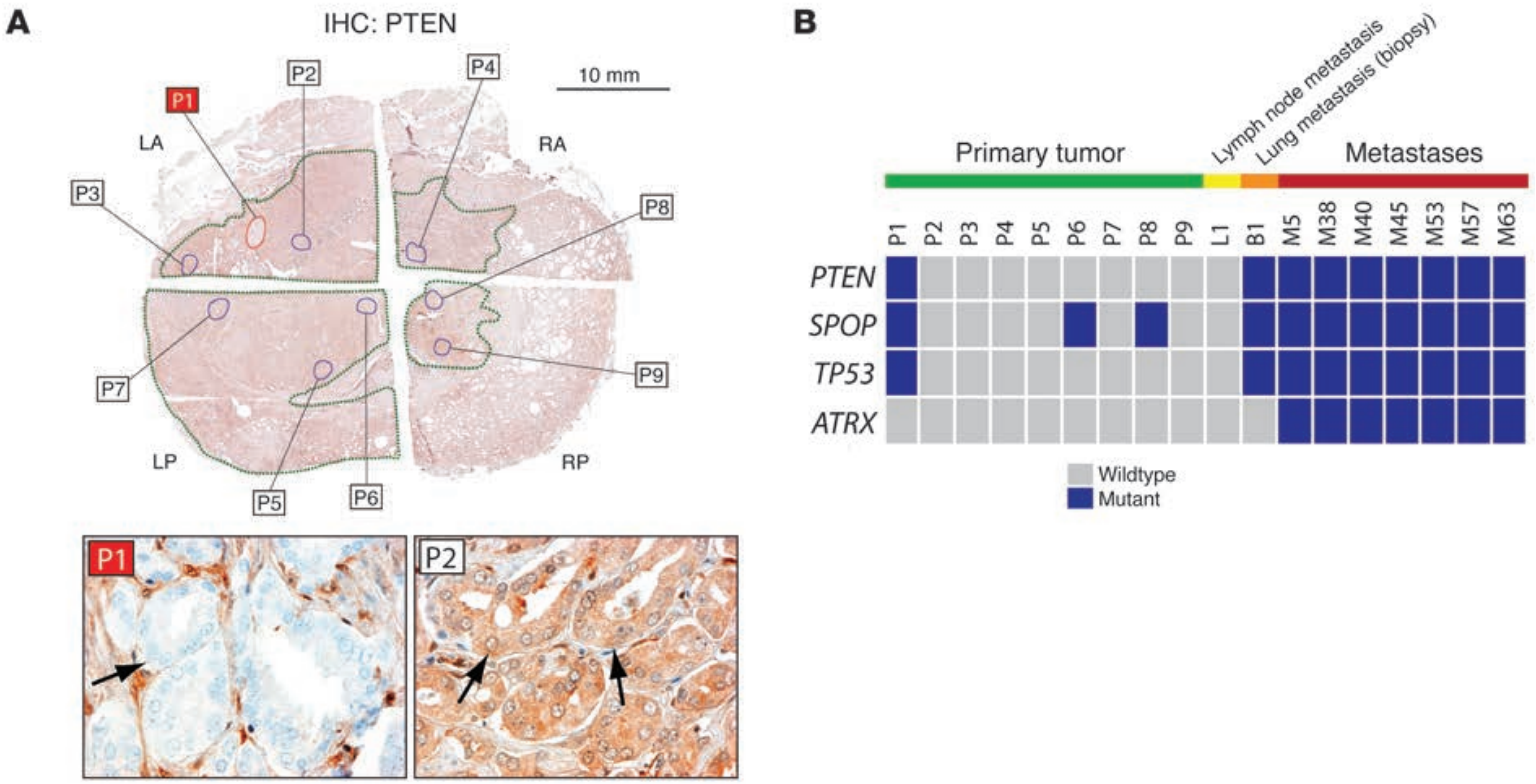
Shen and Abate-Shen (2010) *Genes Dev.* 24: 1967-2000

Evolutionary history of a lethal prostate cancer



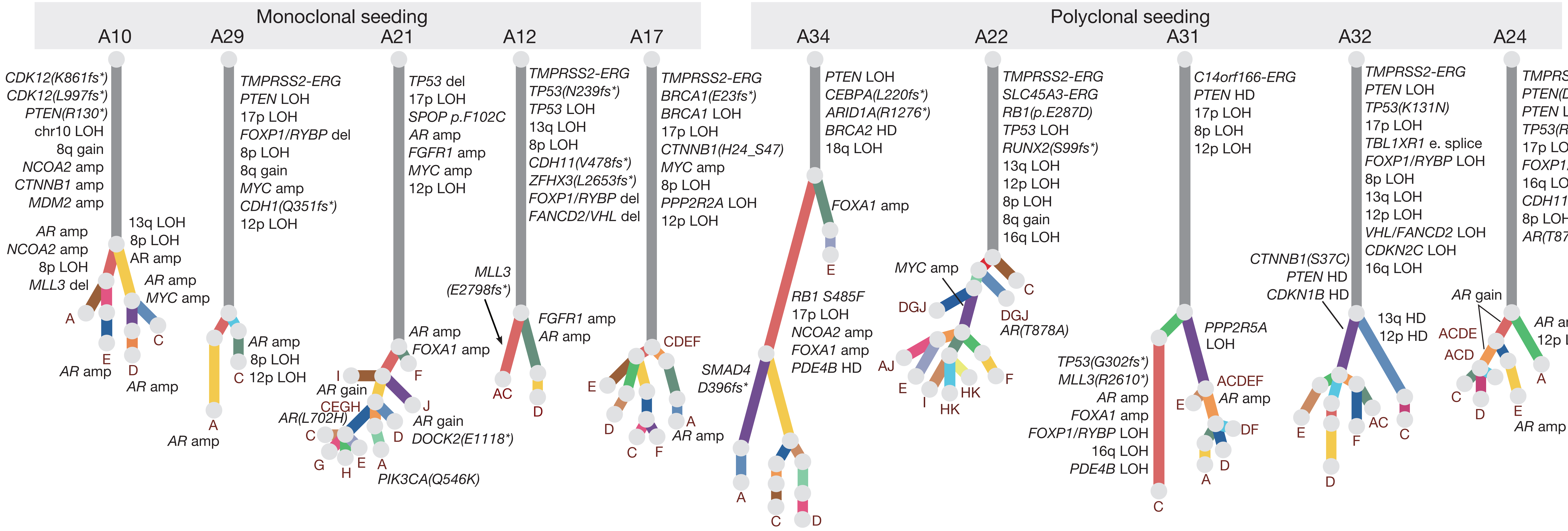
Haffner *et al.* (2013) *J. Clin. Invest.* 123: 4918-4922

Clonal analysis of a lethal prostate cancer



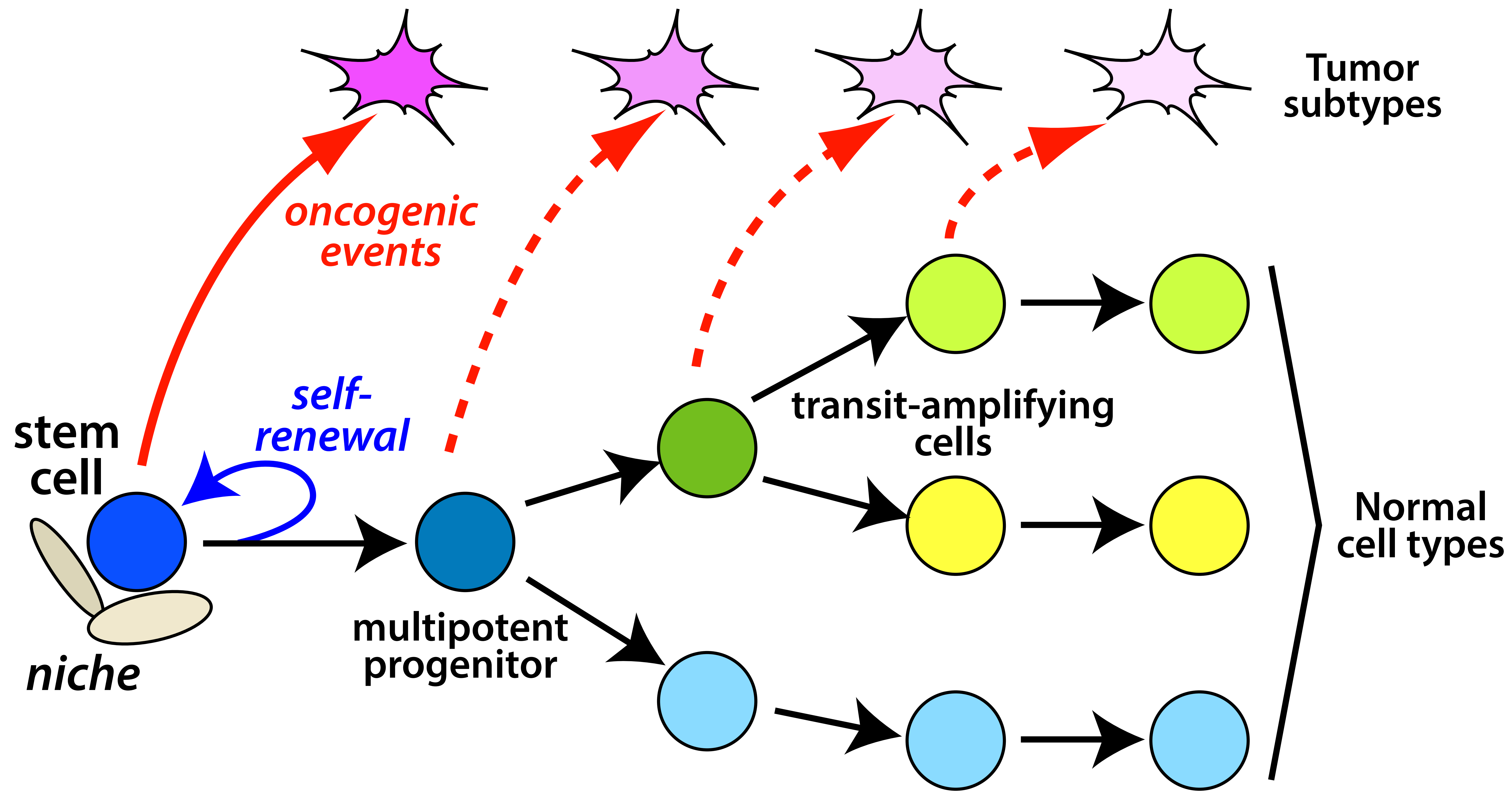
Haffner *et al.* (2013) *J. Clin. Invest.* 123: 4918-4922

Complex heterogeneity in metastatic prostate cancer



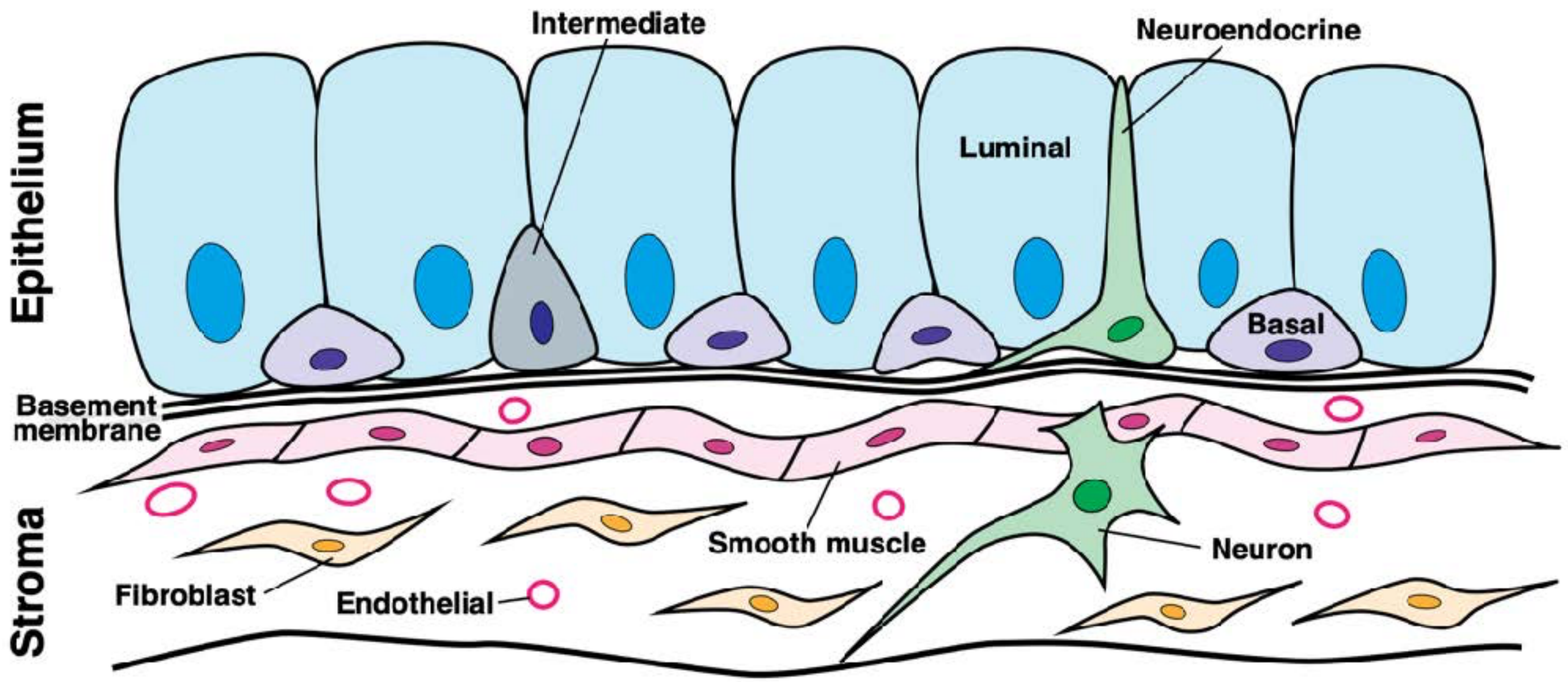
Gundem *et al.* (2015) *Nature* 520: 353-357

Progenitor cells and the origin of cancer



Shibata and Shen (2013) *Bioassays* 35: 253-260

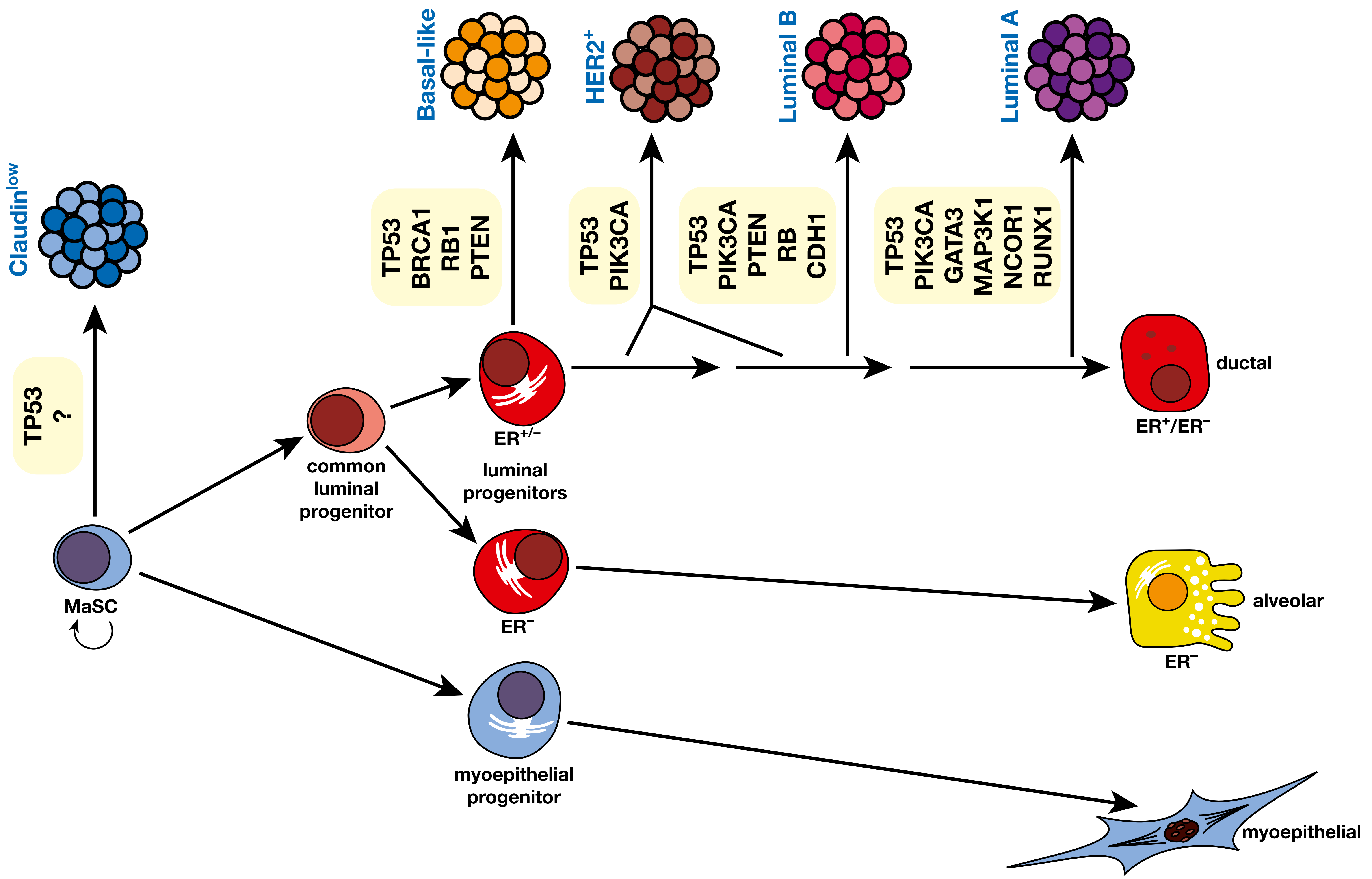
Cell types of the adult prostate



- *Luminal:* AR$^+$, CK18$^+$
- *Basal:* AR$^-$, p63$^+$, CK5$^+$
- *Neuroendocrine:* Syn$^+$

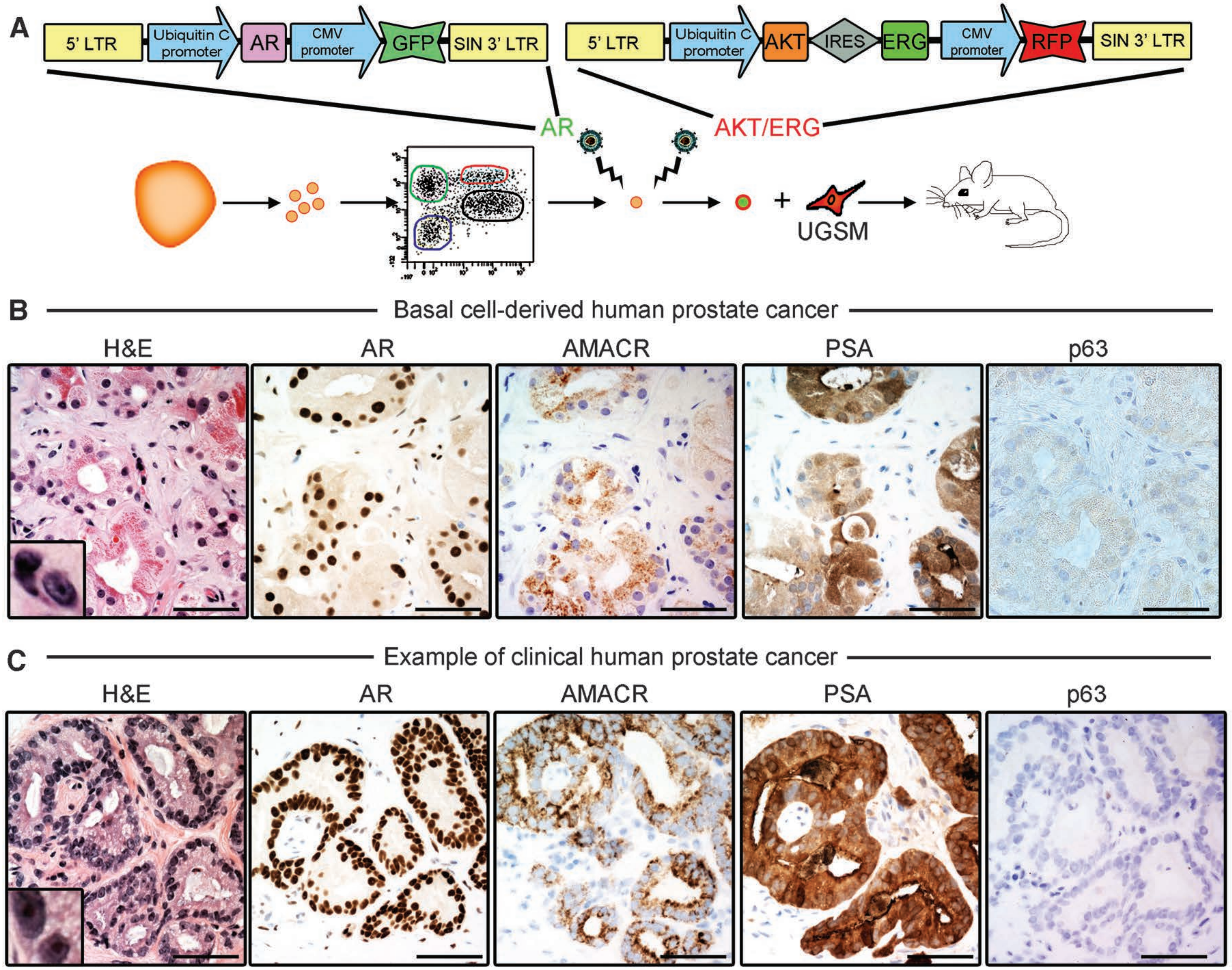
Toivanen and Shen (2017) *Development* 144: 1382-1398

Lineage hierarchy and origin of breast cancer



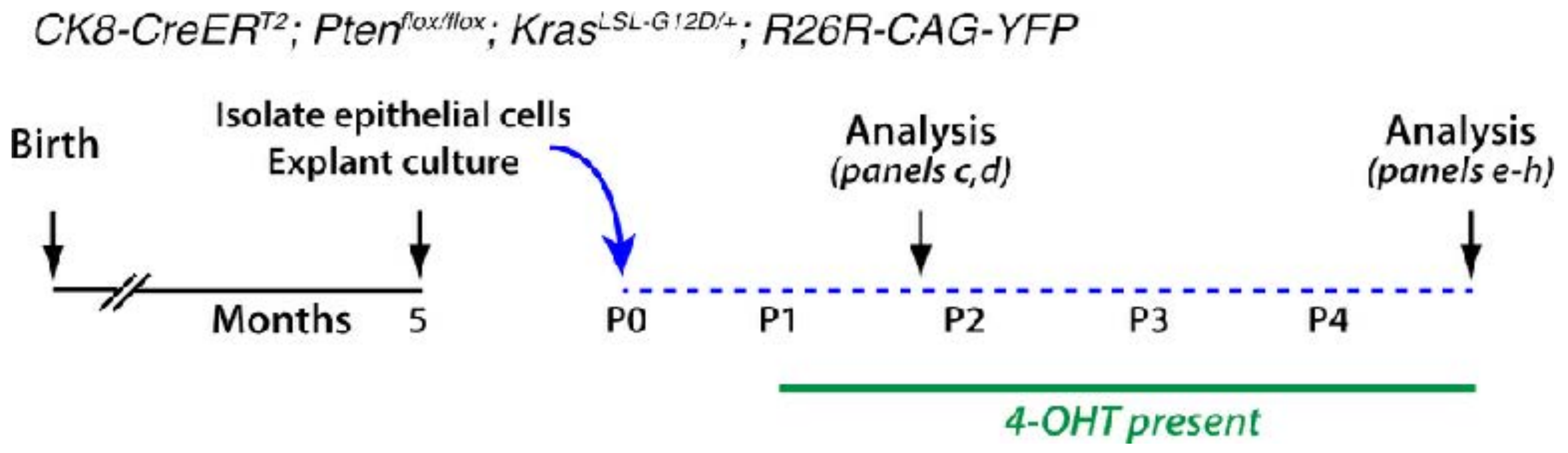
Visvader and Stingl (2014) *Genes Dev.* 28: 1143-1158

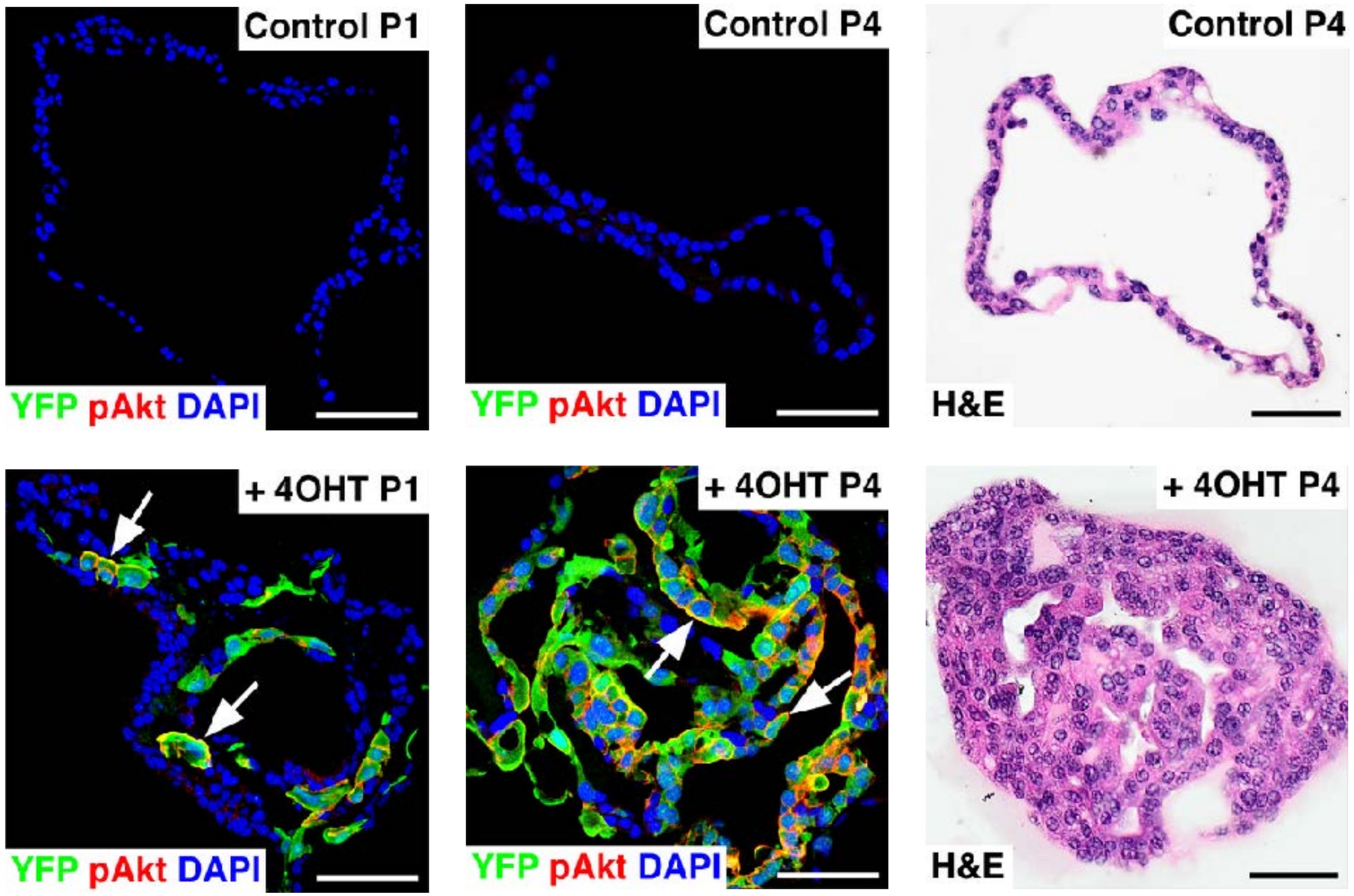
Basal cell of origin for human prostate cancer?



Goldstein et al. (2010) *Science* 329: 568-571

Luminal cell of origin in organoid culture

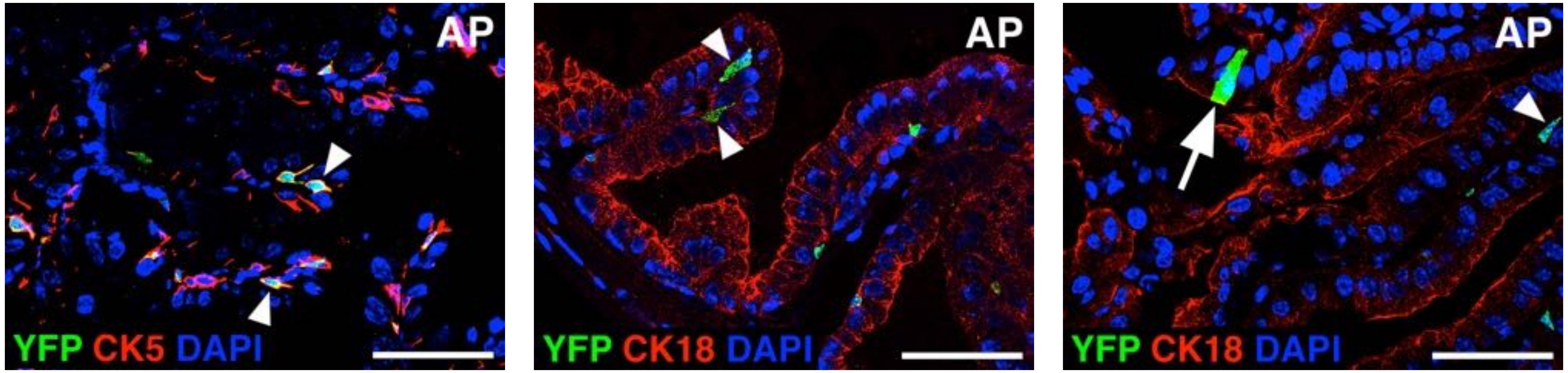




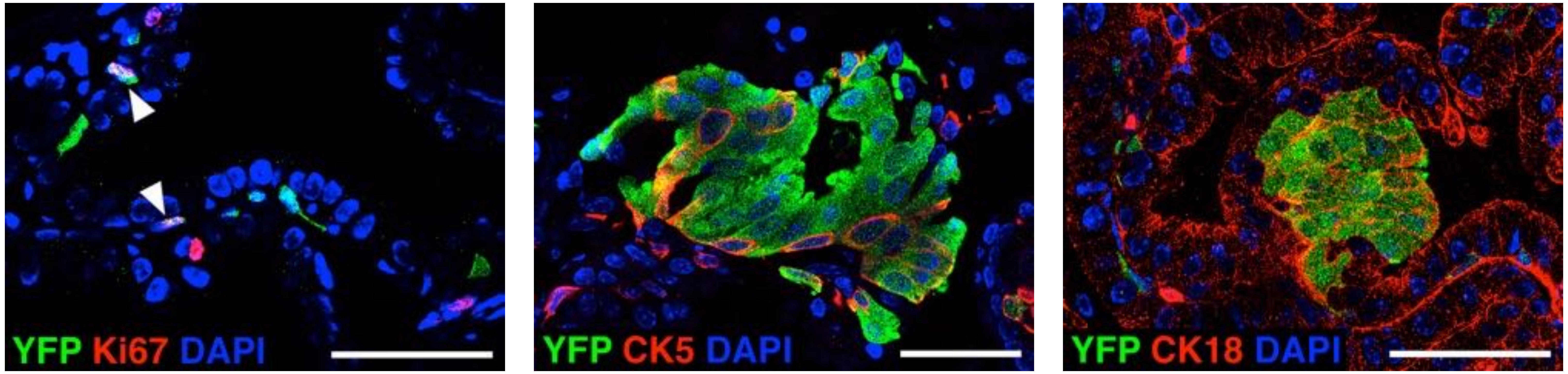
Chua *et al.* (2014) *Nat. Cell Biol.* 16: 951-961

Plasticity of basal cells during tumor initiation

Homeostasis and regeneration: ***CK5-CreER^{T2}/+; R26R-YFP/+***

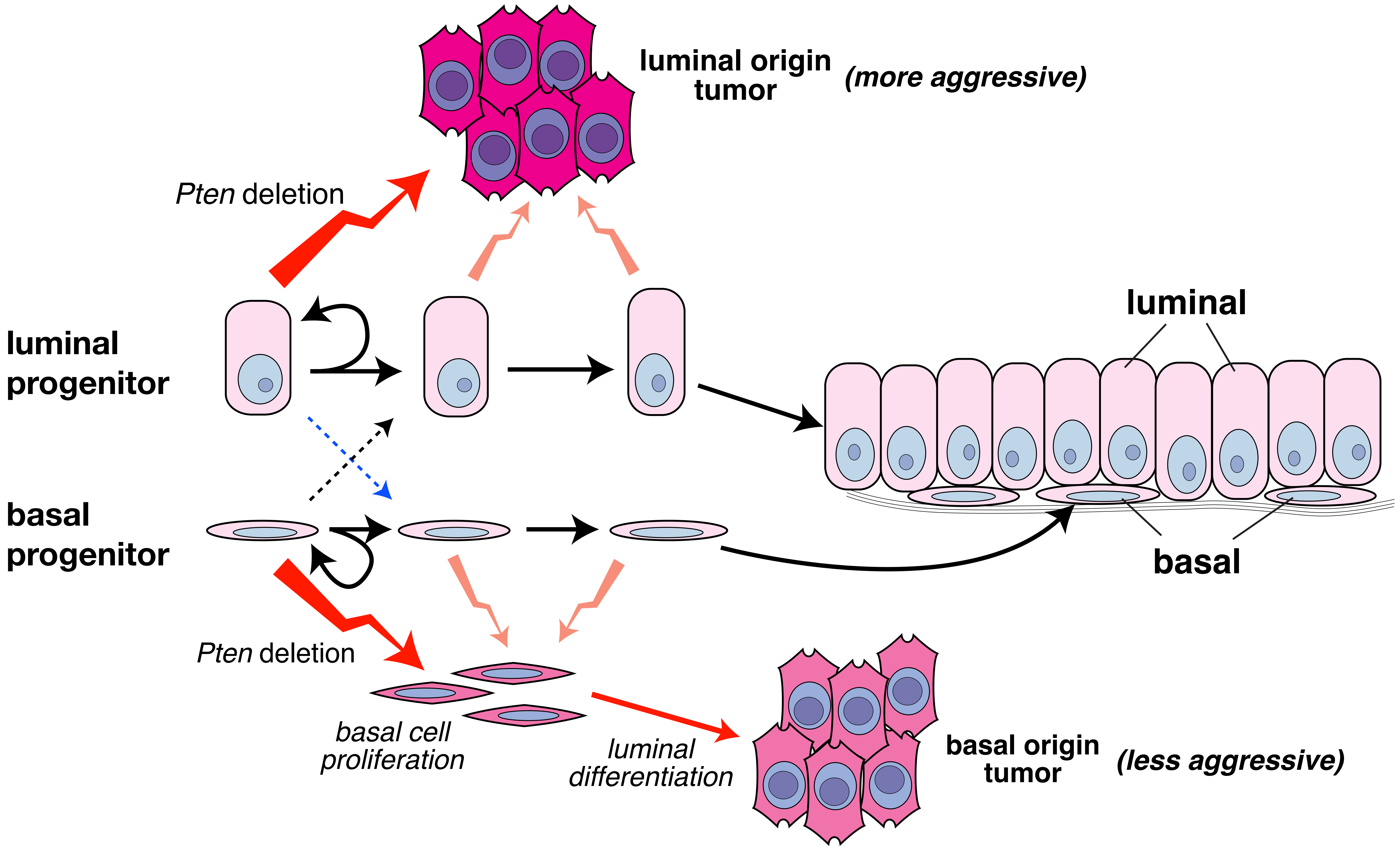


Tumor initiation: ***CK5-CreER^{T2}; Pten$^{flox/flox}$; R26R-YFP/+***



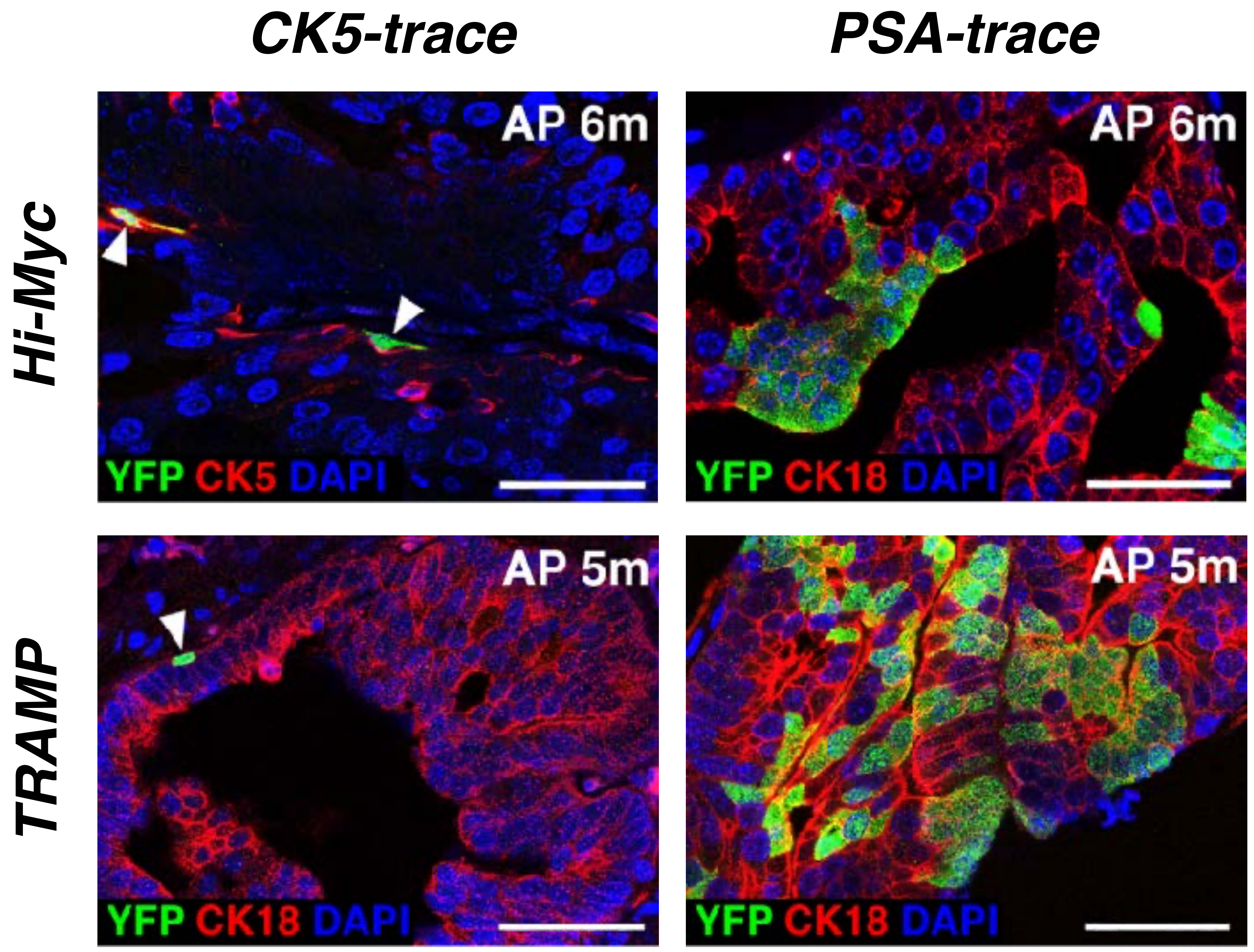
Wang *et al.* (2013) *Nat. Cell Biol.* 15: 274-283

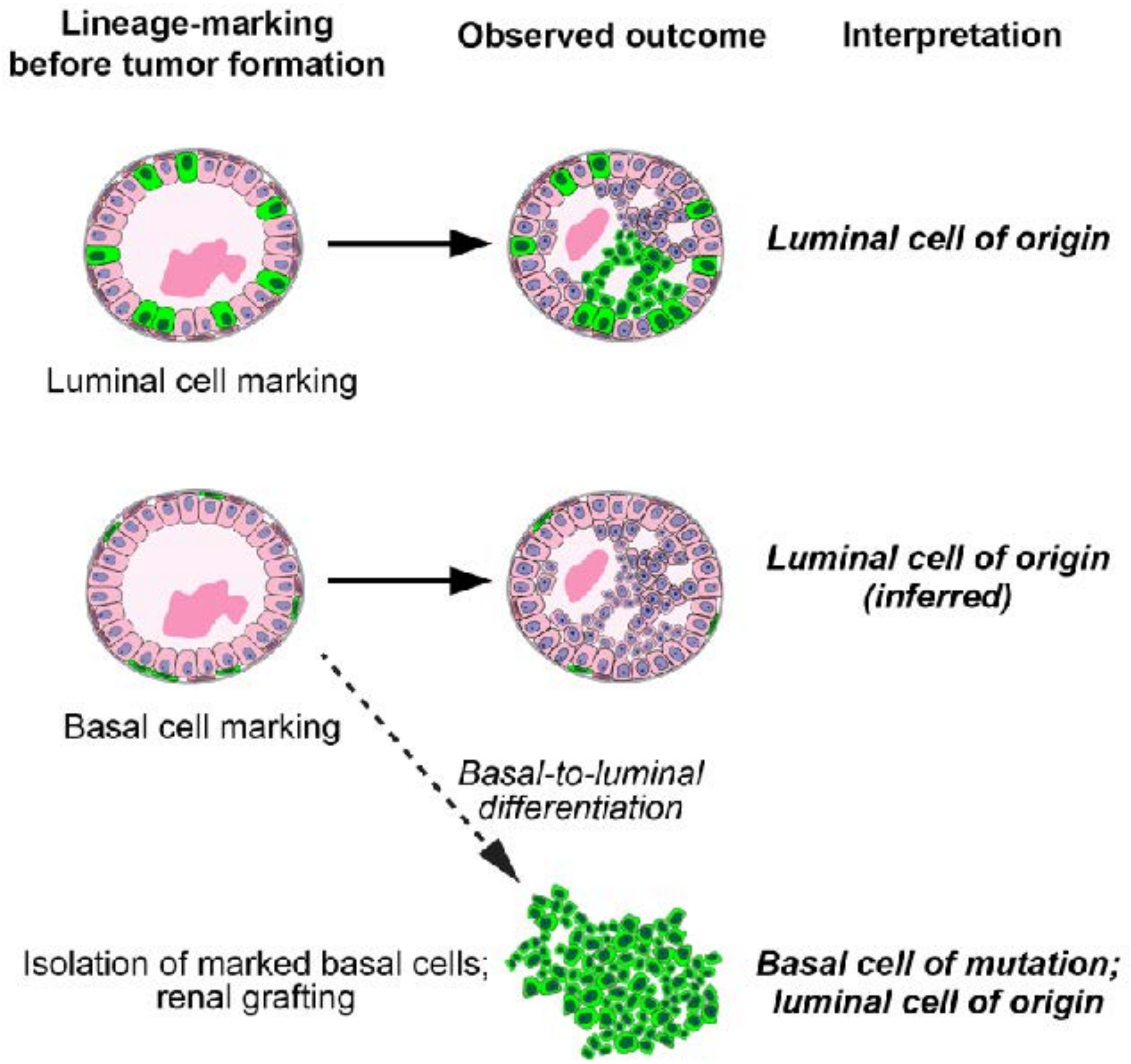
Cell lineages and origin of prostate cancer



Wang *et al.* (2013) *Nat. Cell Biol.* 15: 274-283

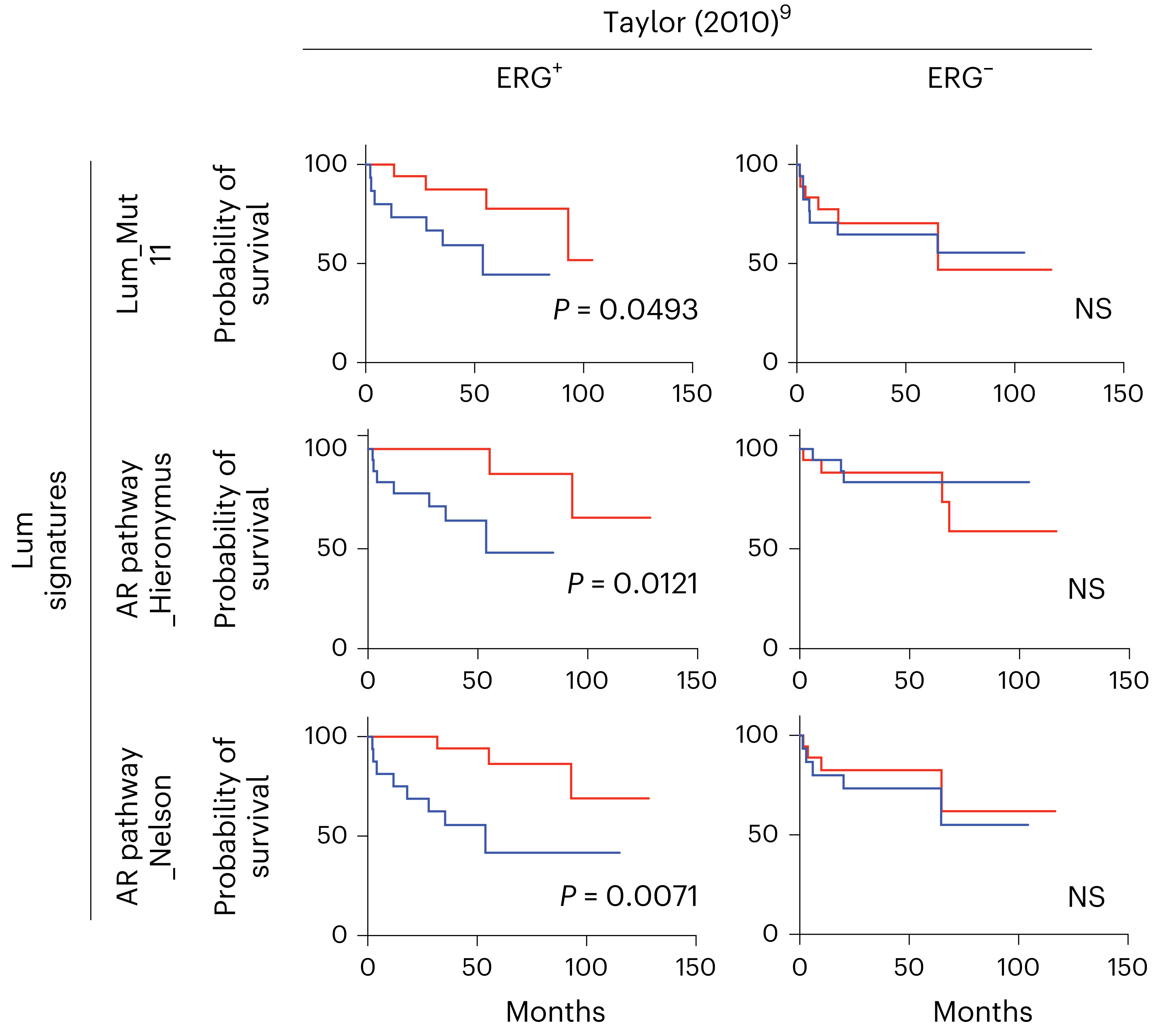
Luminal cells are favored cells of origin for prostate cancer

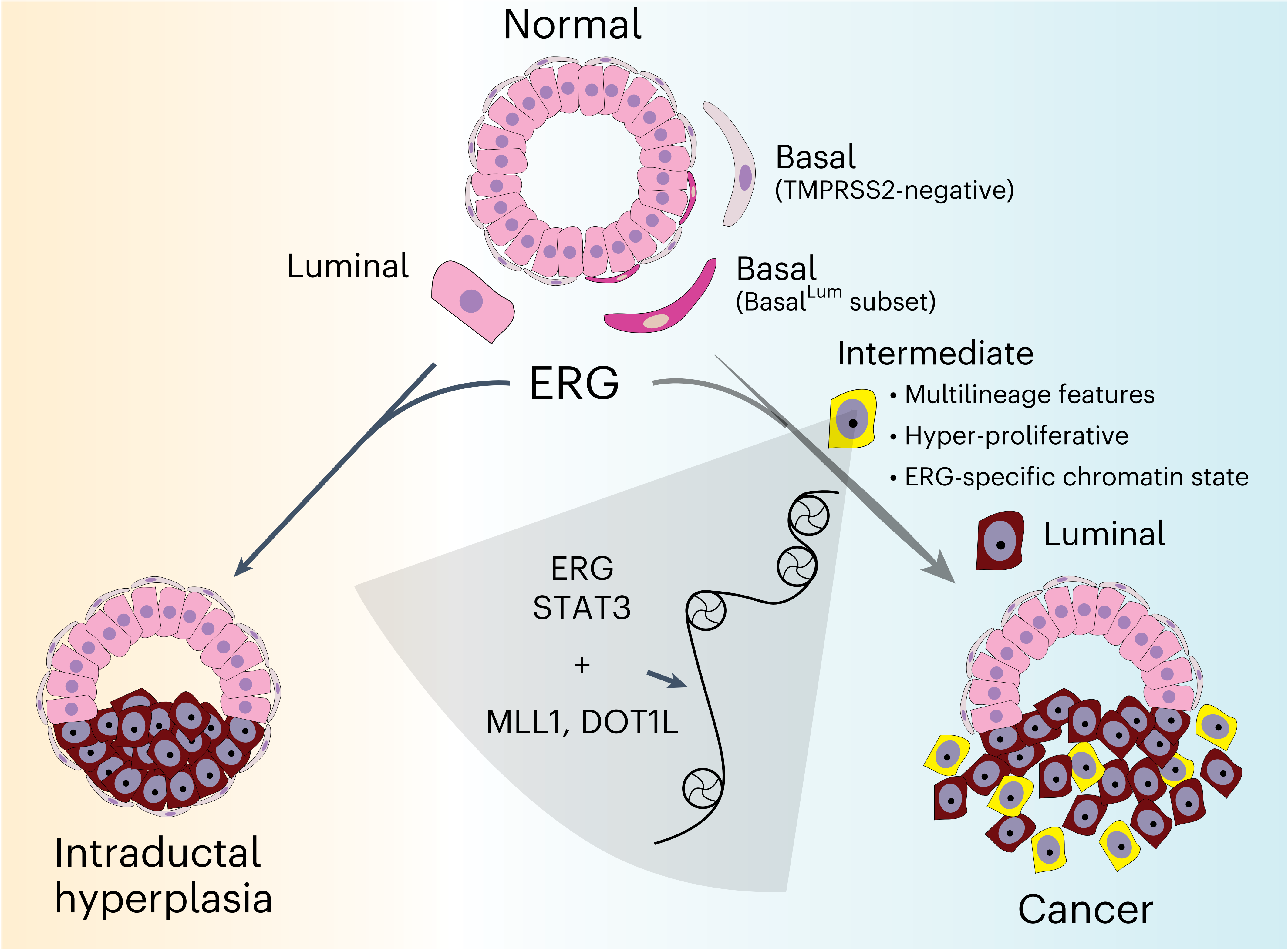




Wang *et al.* (2014) *Cell Rep.* 8: 1339-1346

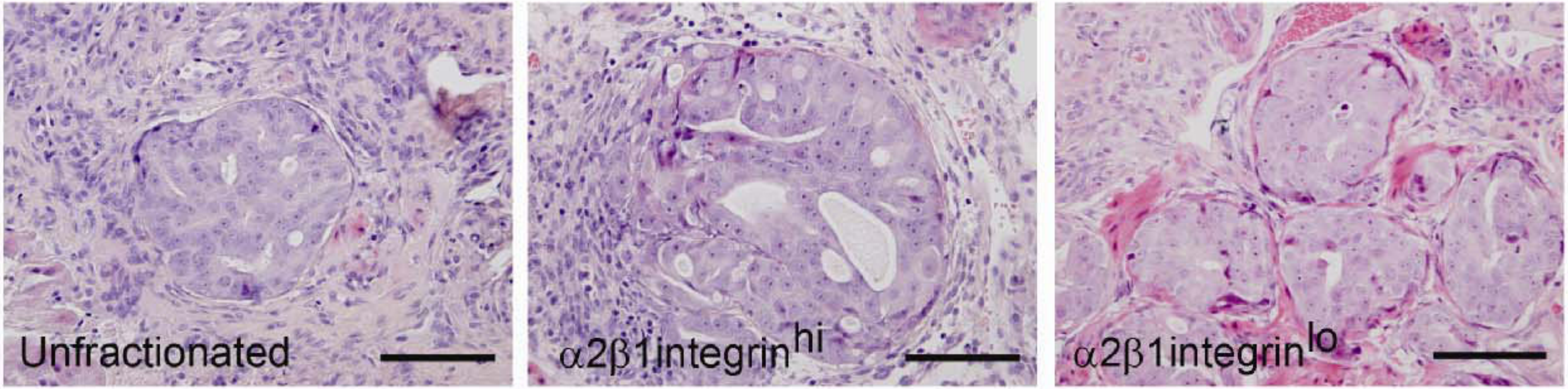
Initiation of ERG-positive tumors from hybrid basal-luminal cells





Feng *et al.* (2025) *Nature Genet.* 57: 2177-2191

Human prostate tumor-repopulating cells

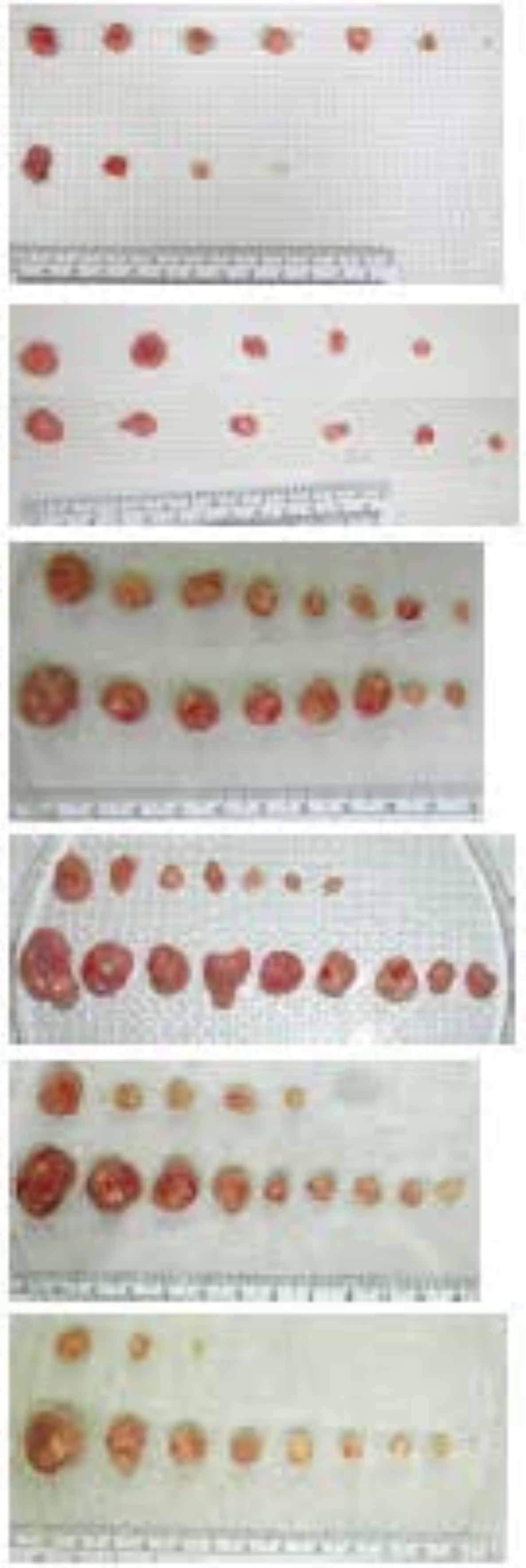


Toivanen *et al.* (2011) *Stem Cells* 29: 1310-1314

Two types of tumor-initiating cells in prostate xenografts

PSA^hi AR+

PSA^lo AR−

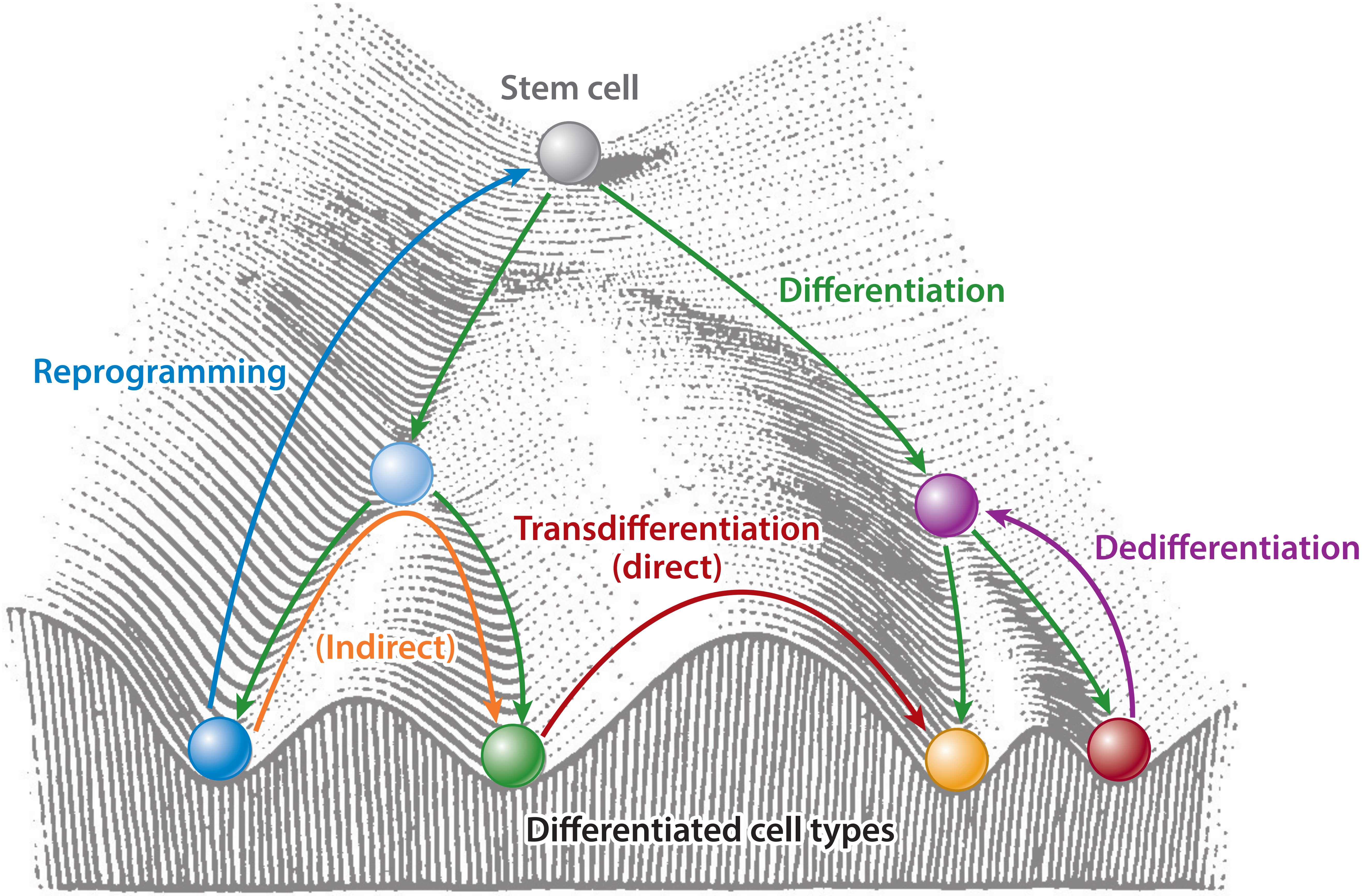


		Incidence	*Weight (g)*	*P value*
1°	+10K	8/10 (80%)	0.35 ± 0.22	
	-10K	6/6 (100%)	0.17 ± 0.22	0.038
2°	+10K	6/8 (75%)	0.60 ± 0.45	
	-10K	7/8 (88%)	0.64 ± 0.56	0.44
3°	+10K	8/9 (89%)	0.20 ± 0.17	
	-10K	8/9 (89%)	0.39 ± 0.30	0.04
4°	+10K	7/10 (70%)	0.16 ± 0.17	
	-10K	9/10 (90%)	0.75 ± 0.41	0.001
5°	+10K	5/10 (50%)	0.24 ± 0.26	
	-10K	9/10 (90%)*	0.52 ± 0.52	0.03
6°	+10K	3/10 (30%)	0.18 ± 0.18	
	-10K	9/10 (90%)**	0.39 ± 0.48	

Qin *et al.* (2012) *Cell Stem Cell* 10: 556-569

Lineage plasticity in development and cancer

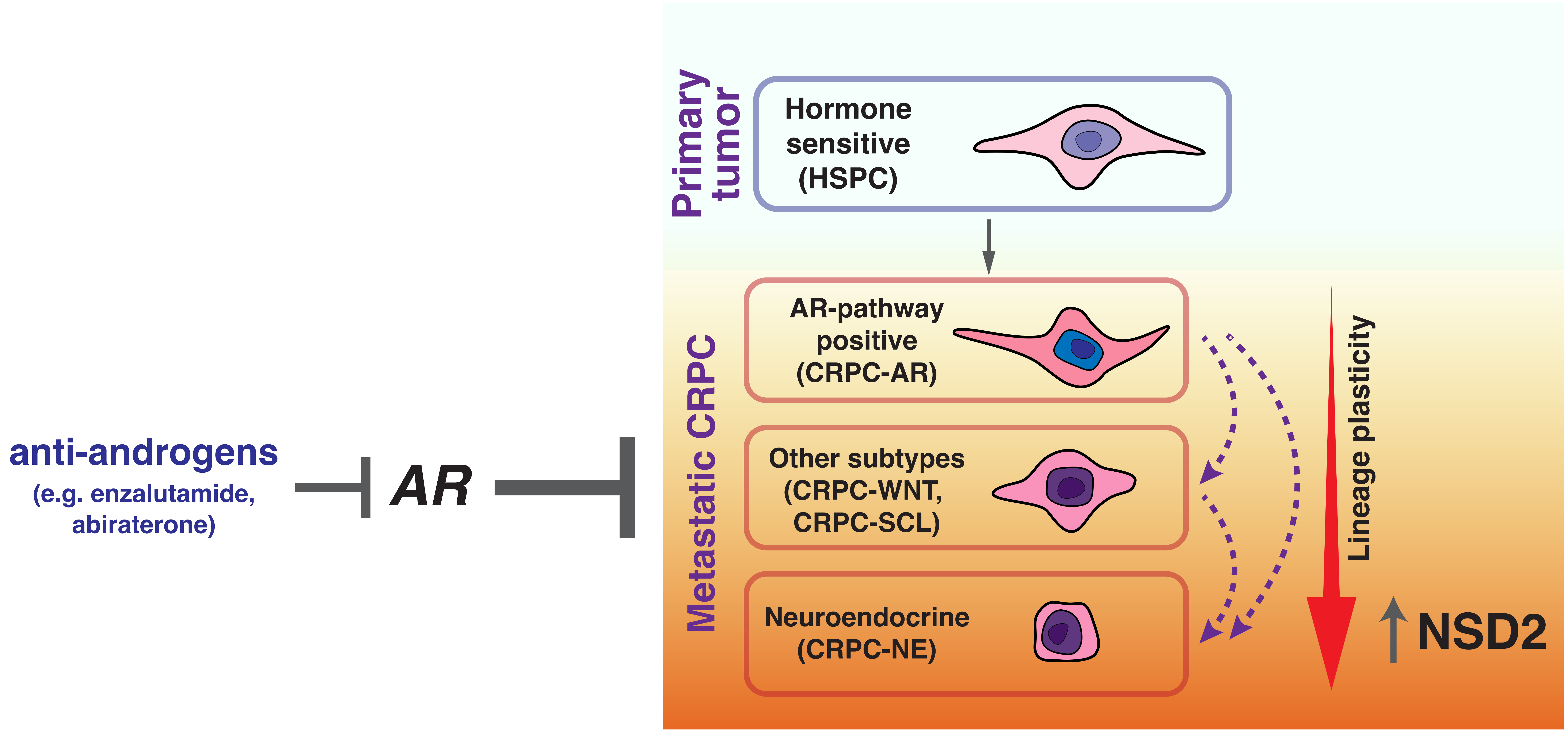
"ability of a cell to change from one identity to another "



- A phenotypic change in cellular state at the single-cell level, often in response to microenvironmental signals or drug treatment
- Can occur through alterations at the genomic, epigenetic, transcriptional, or post-transcriptional level
- Can be reversible or irreversible
- Can be difficult to distinguish from clonal selection at the population level

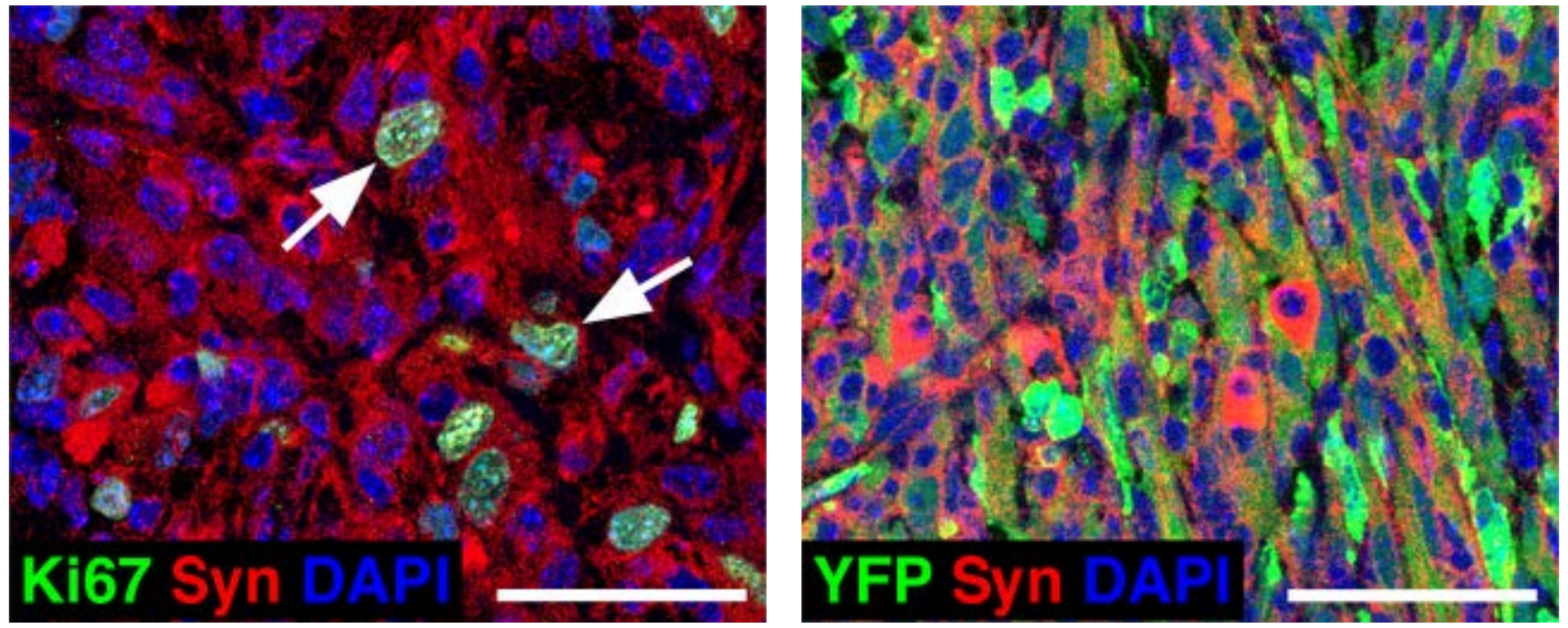
Le Magnen *et al.* (2018) *Annu. Rev. Cancer Biol.* 2: 271-289

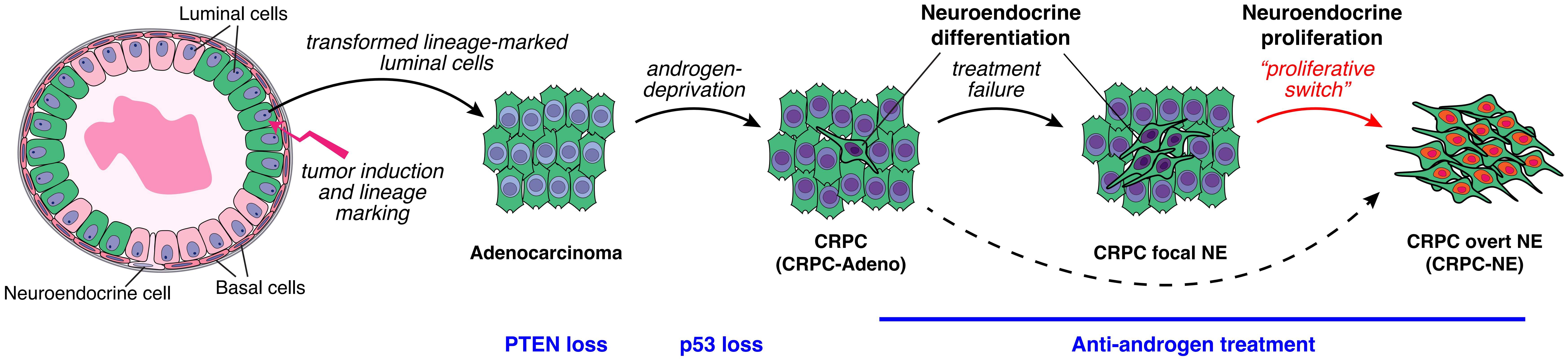
Lineage plasticity in castration-resistant prostate cancer



Transdifferentiation of luminal to neuroendocrine cells

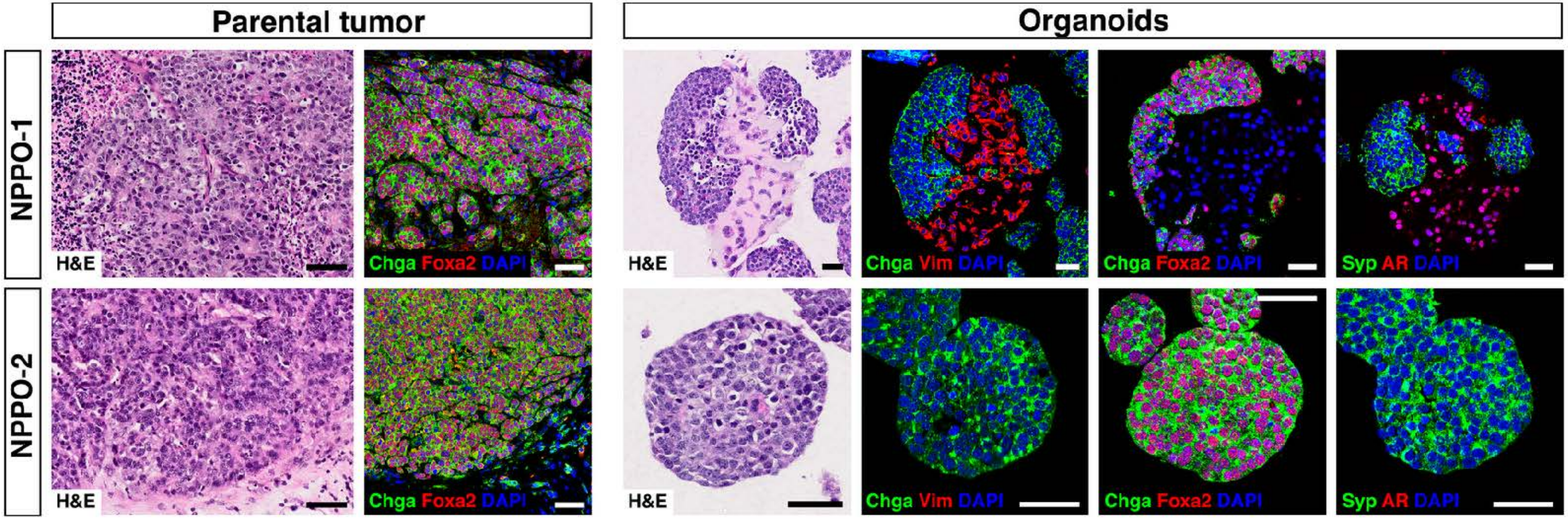
Nkx3.1**$^{CreERT2/+}$**; Pten**$^{flox/flox}$**; Trp53**$^{flox/flox}$**; R26R-YFP (NPp53)





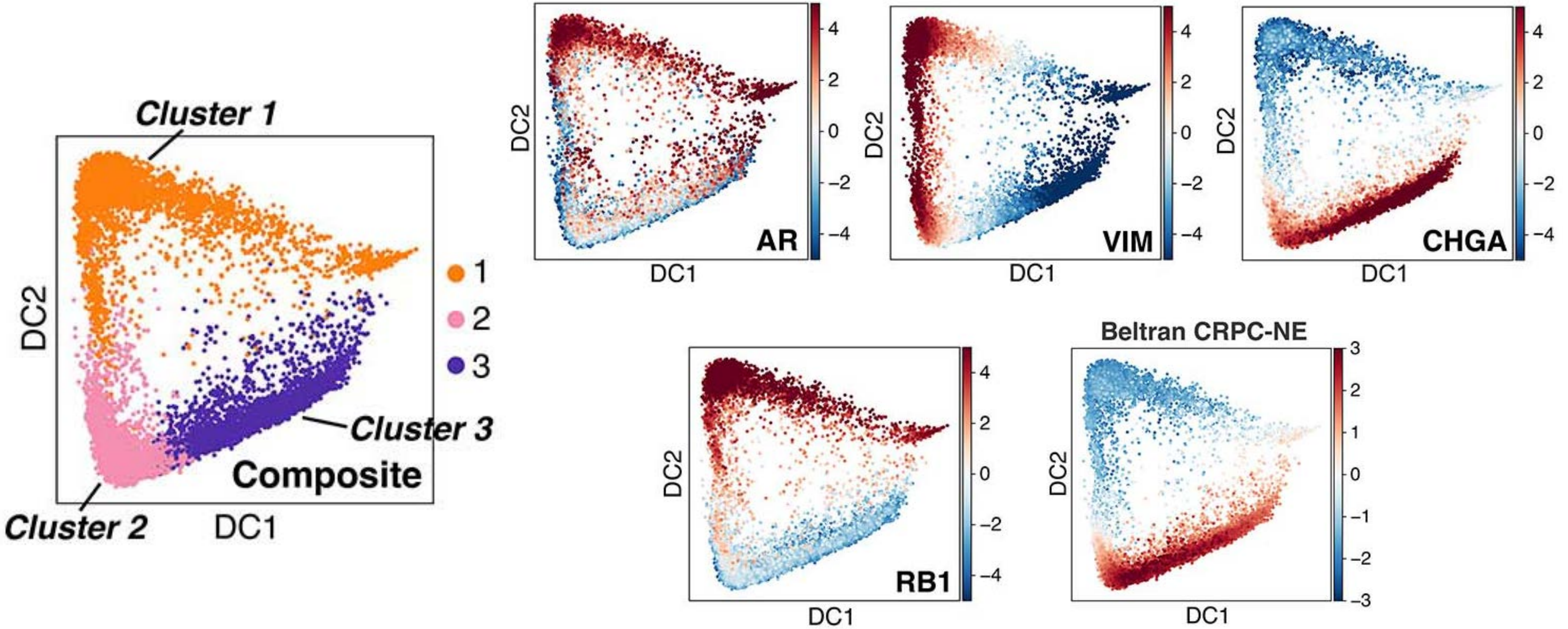
Zou *et al.* (2017) *Cancer Disc.* 7: 736-749

Neuroendocrine organoid lines from *NPp53* mice

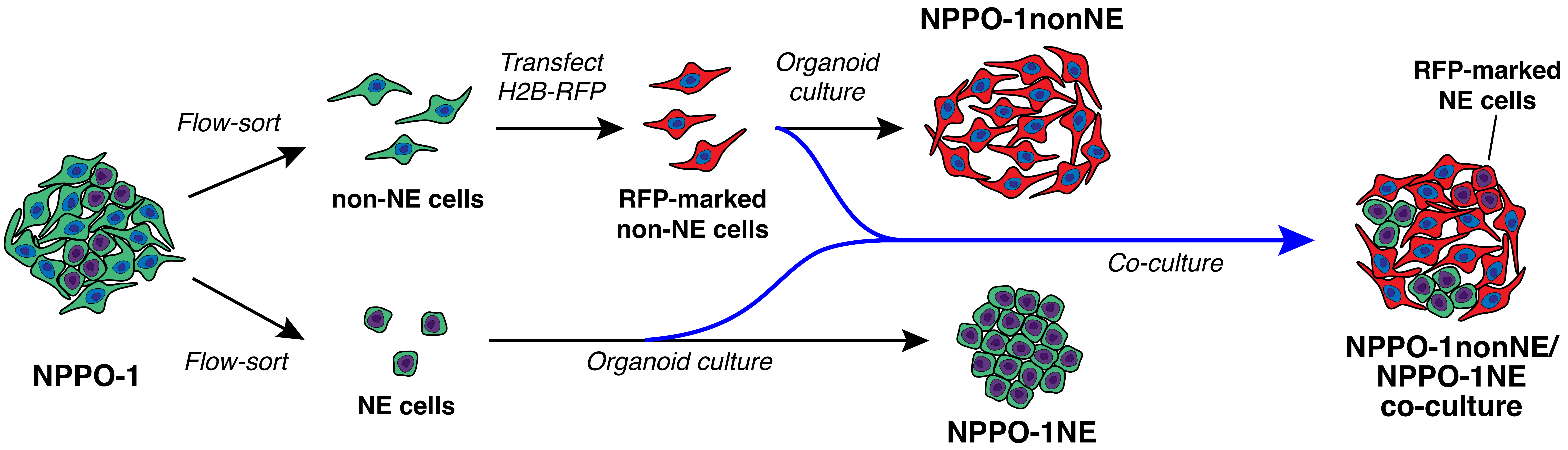


Li *et al.* (2025) *Nature,* in press

Three distinct cell clusters in neuroendocrine organoids

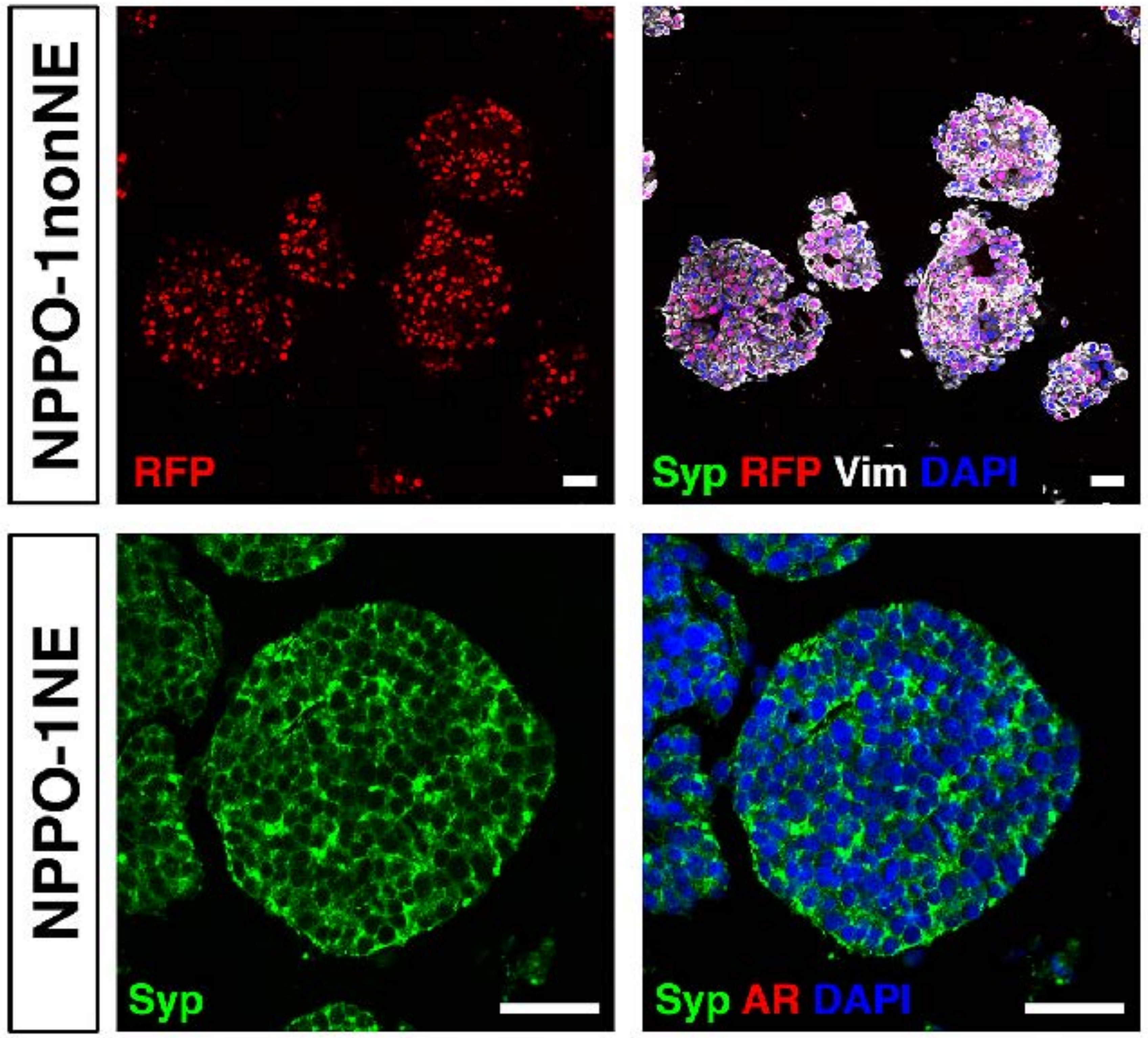


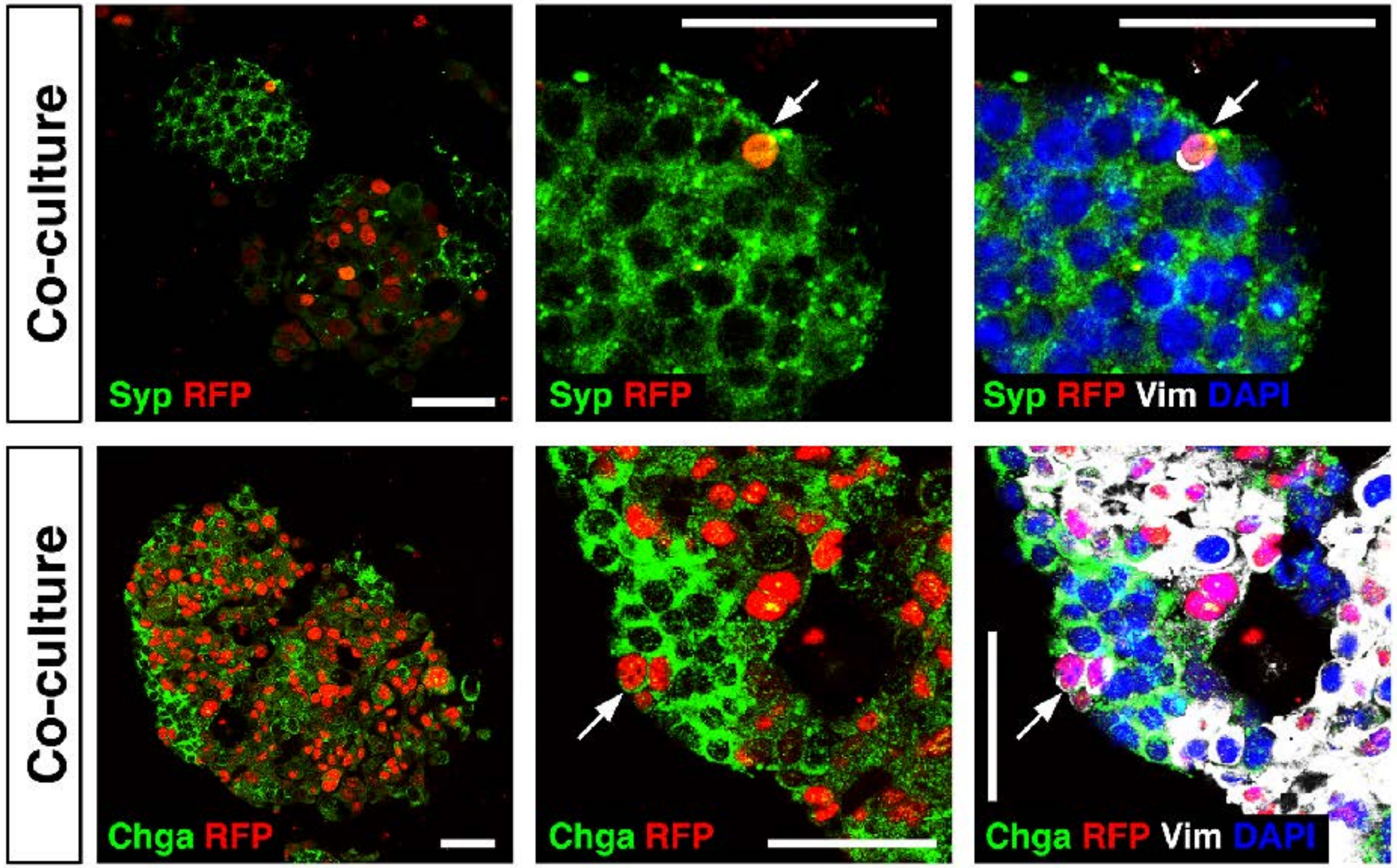
Lineage-tracing analysis of transdifferentiation



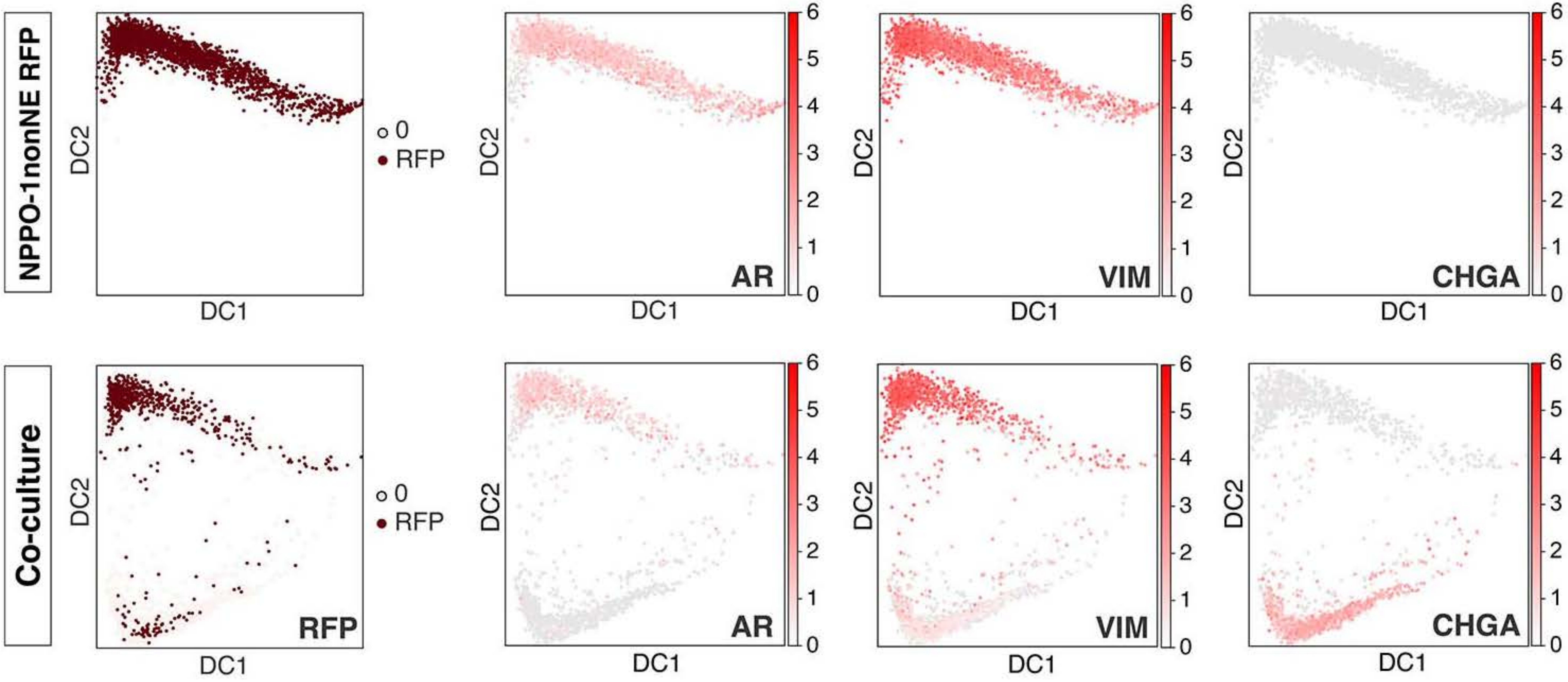
Neuroendocrine transdifferentiation in culture

Separate NPPO-1 NE and nonNE cells by flow sorting, mark nonNE cells with H2B-RFP and co-culture

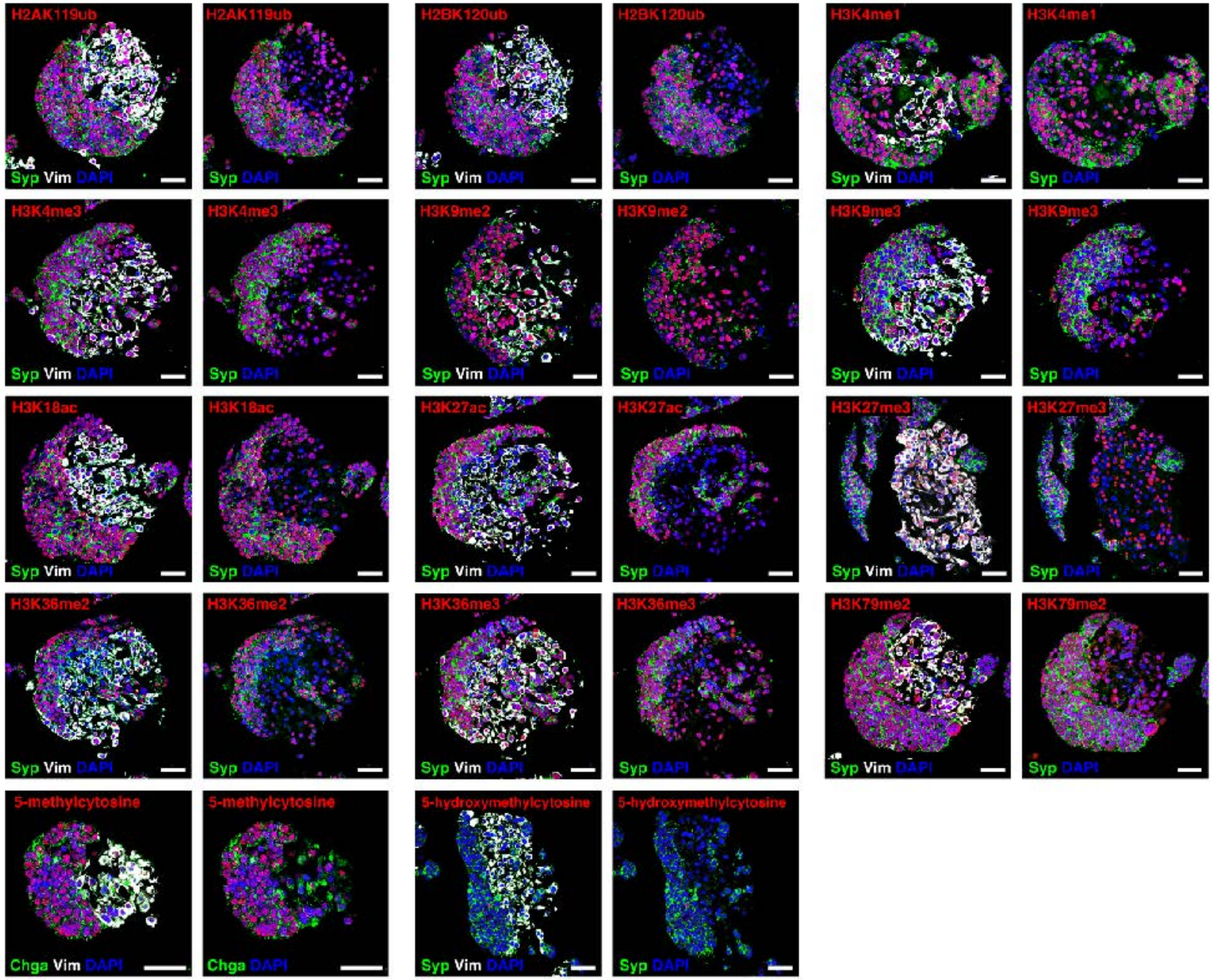


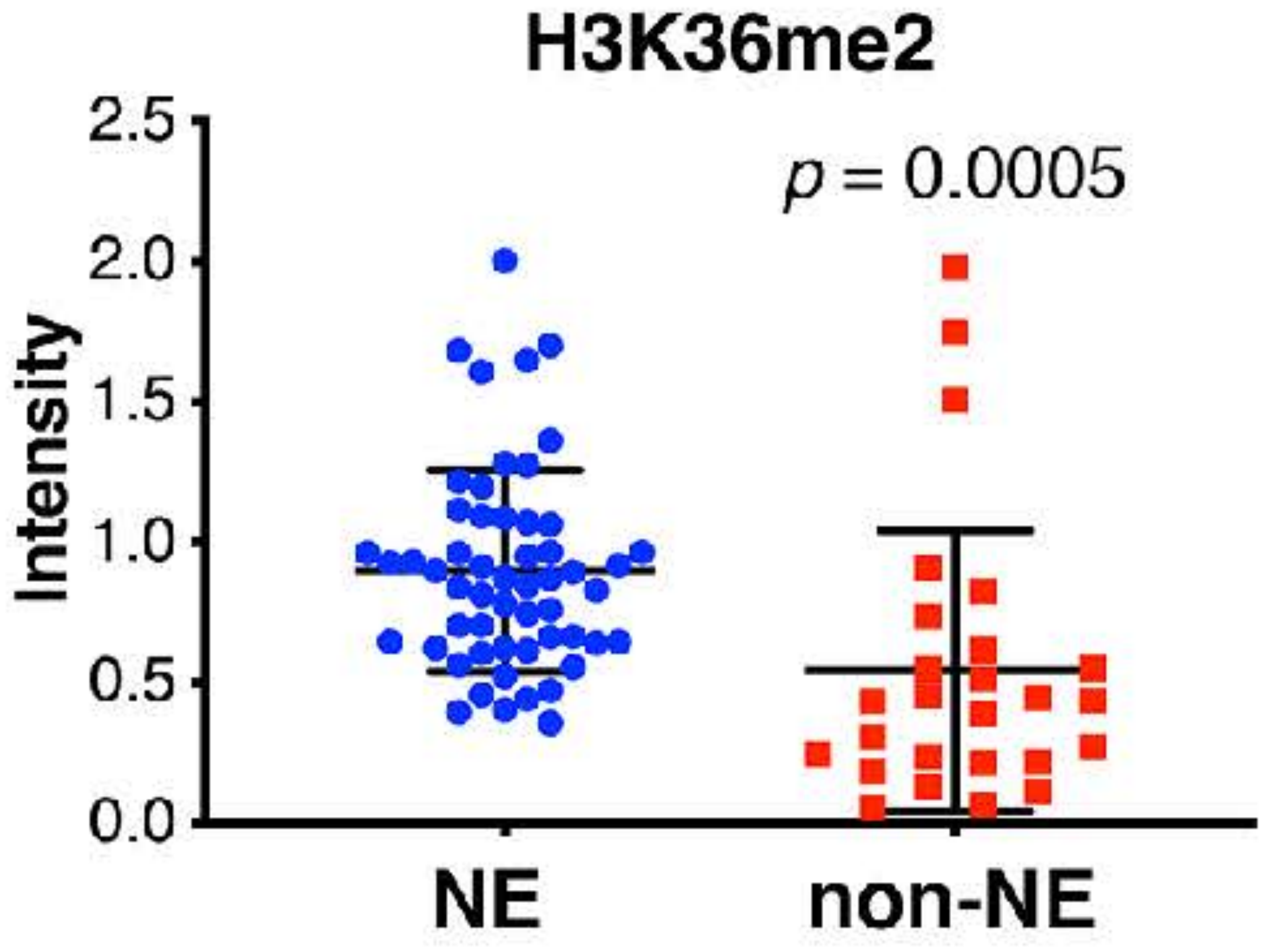


scRNA-seq analysis of transdifferentiation

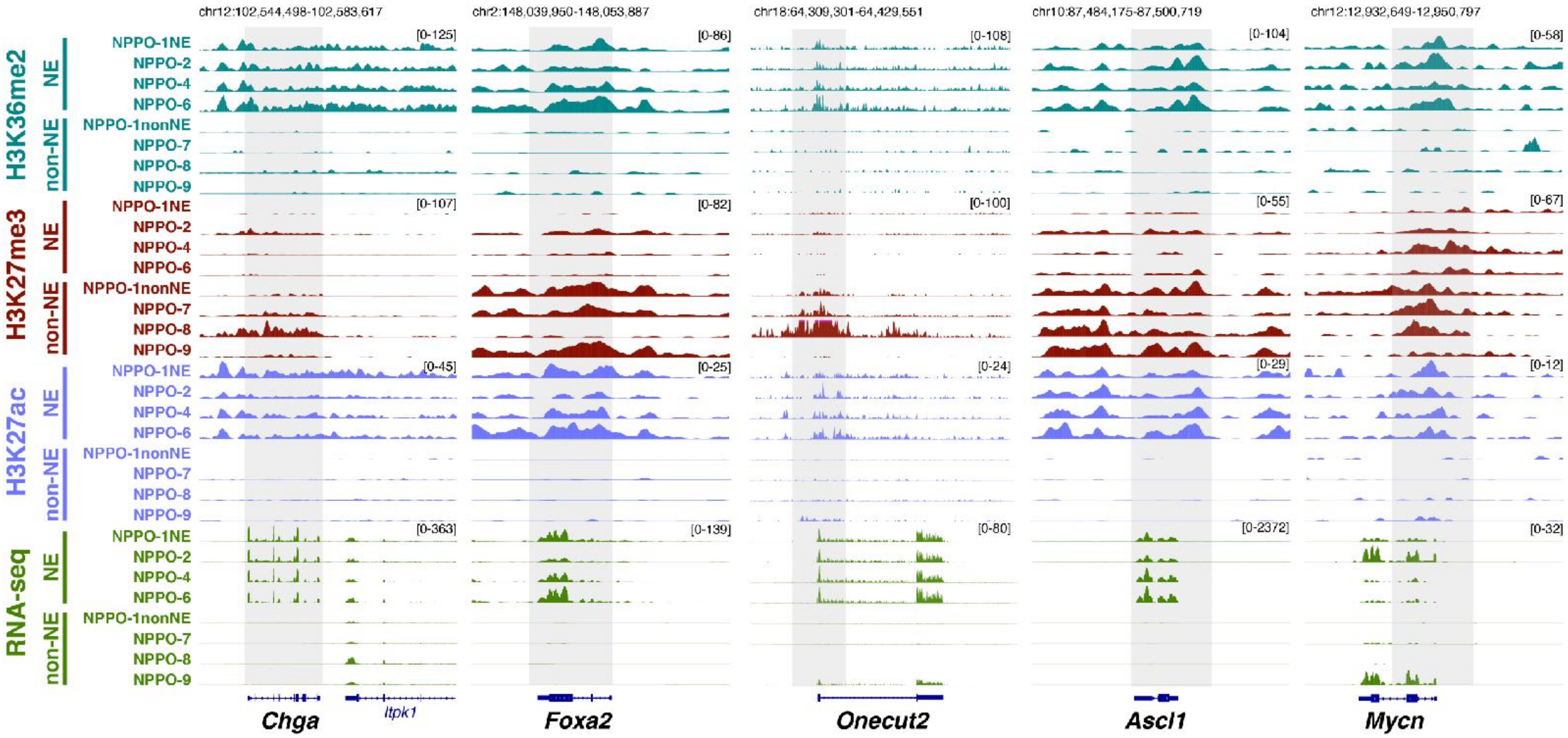


Screen for differential expression of epigenetic marks

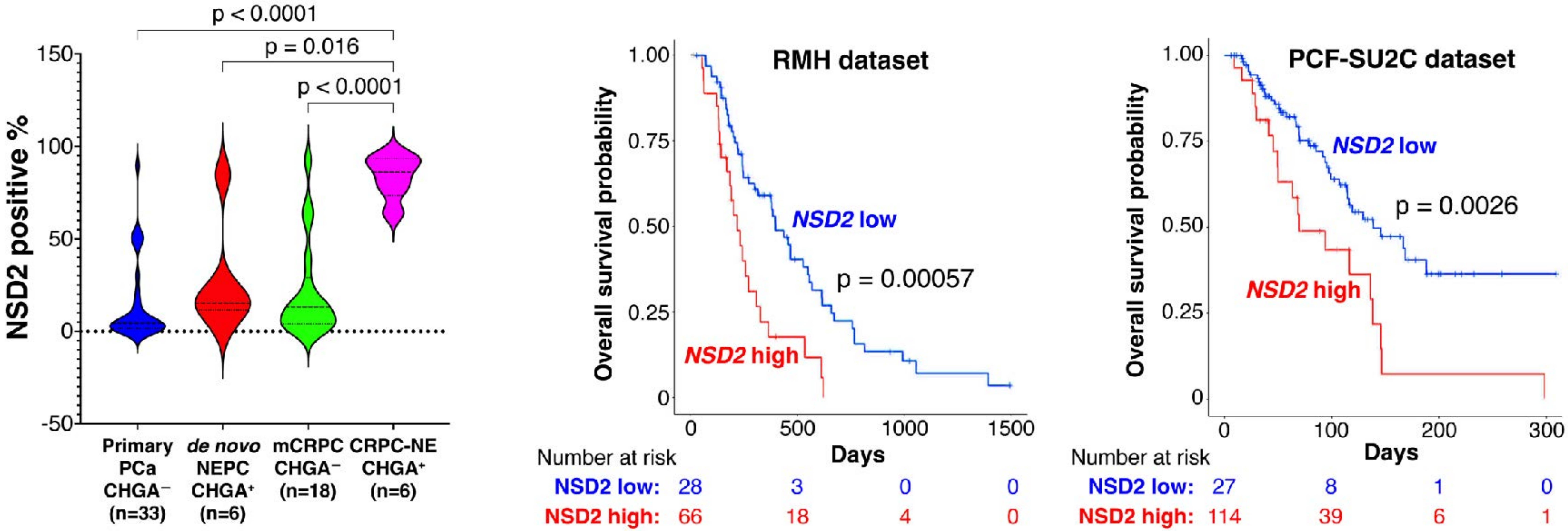




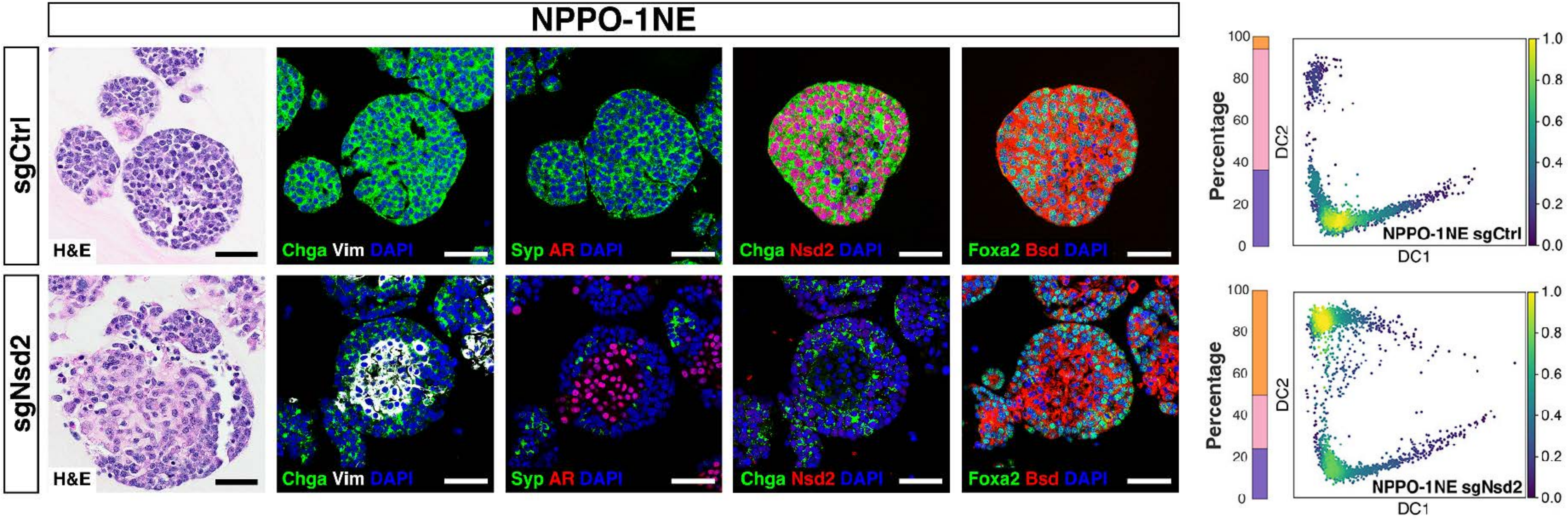
Epigenetic marks at NE gene loci



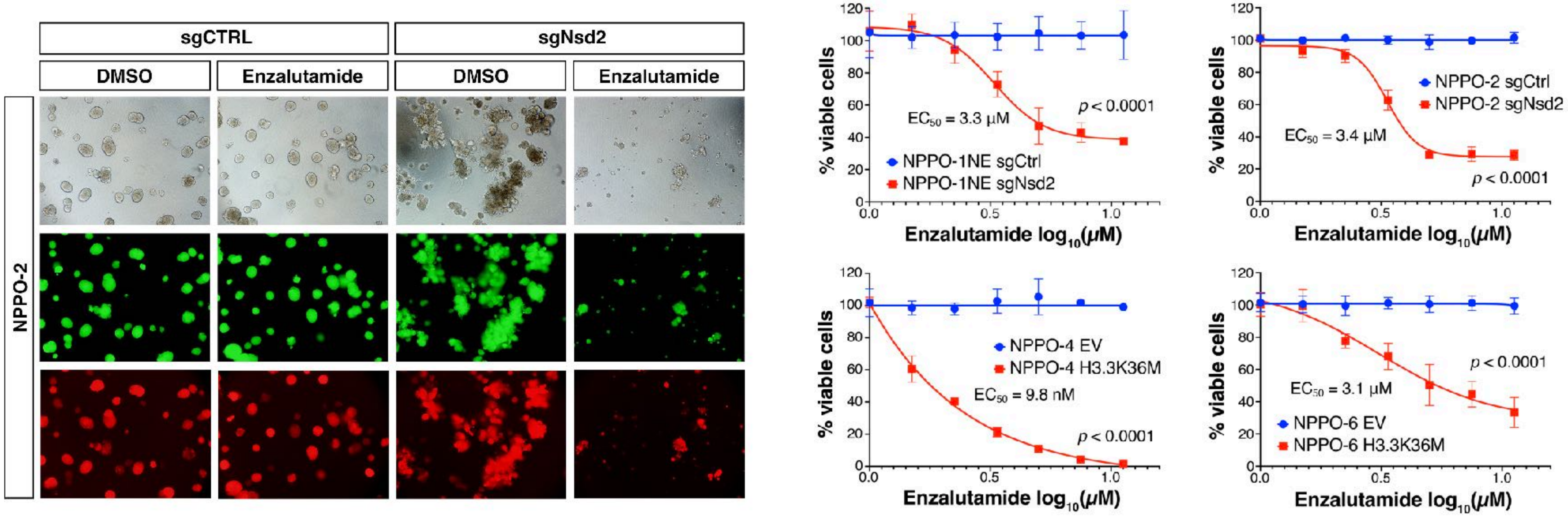
NSD2 expression is prognostic for poor survival outcomes



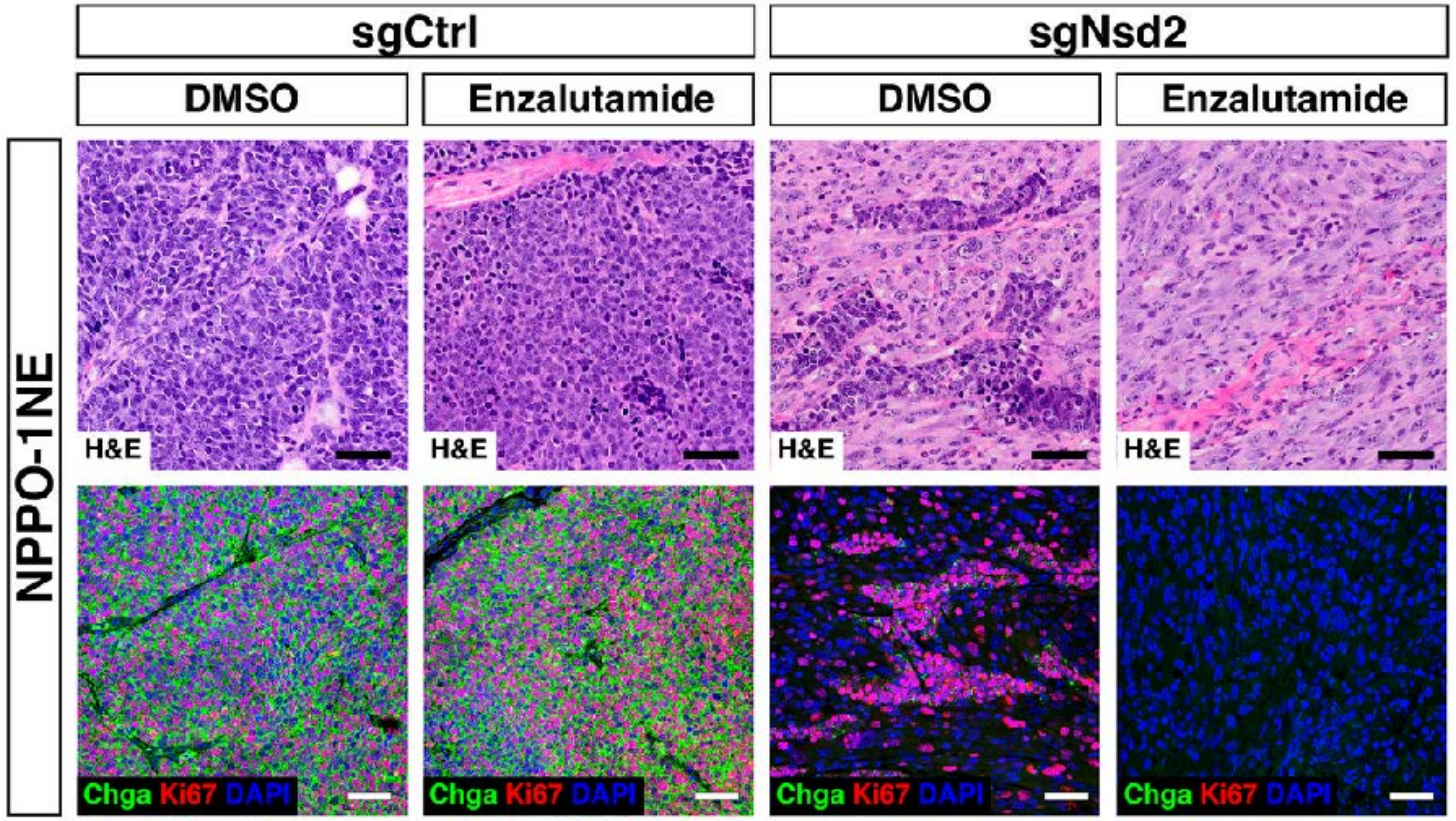
Nsd2 KO reverts neuroendocrine phenotypes

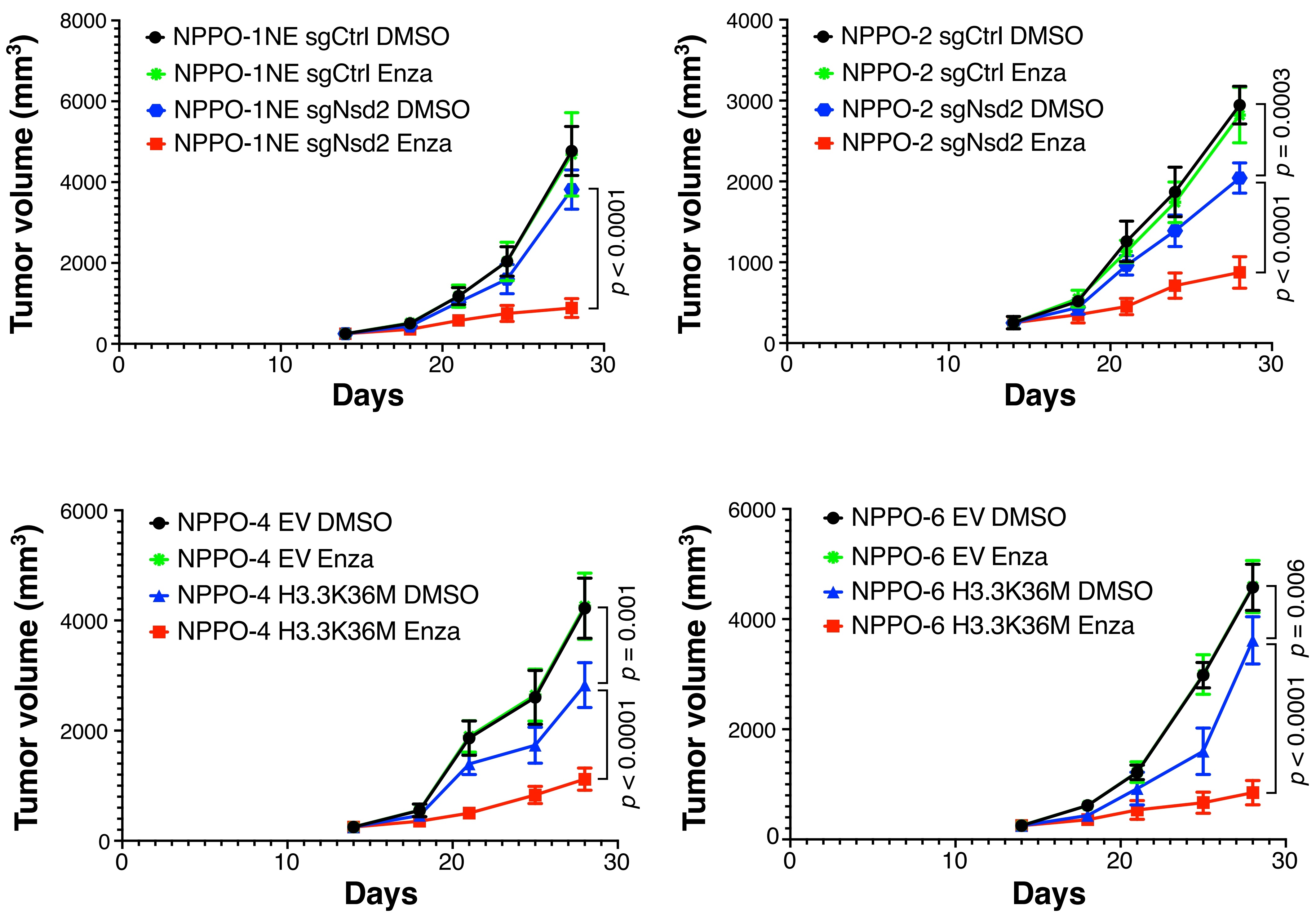


Synergy of *Nsd2* KO with enzalutamide treatment



Synergy of *Nsd2* KO with enzalutamide in grafts





Key takeaways

- “Stemness” in cancer is defined by functional assays that each have advantages and limitations
- Cancer stem cells are a useful concept but may not be readily identifiable in all cancers and/or tumor stages
- Differences in cell of origin may be relevant in some cases for determining tumor properties and patient outcomes
- Cancer stem cells may not represent a well-defined entity in “high-plasticity” tumors