

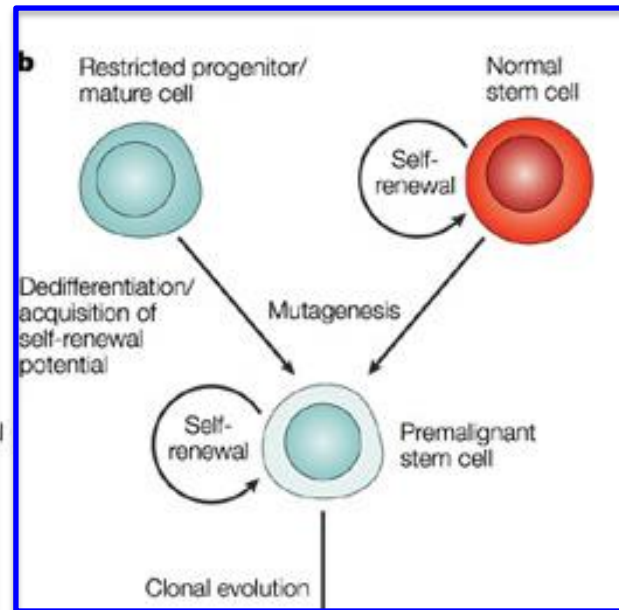
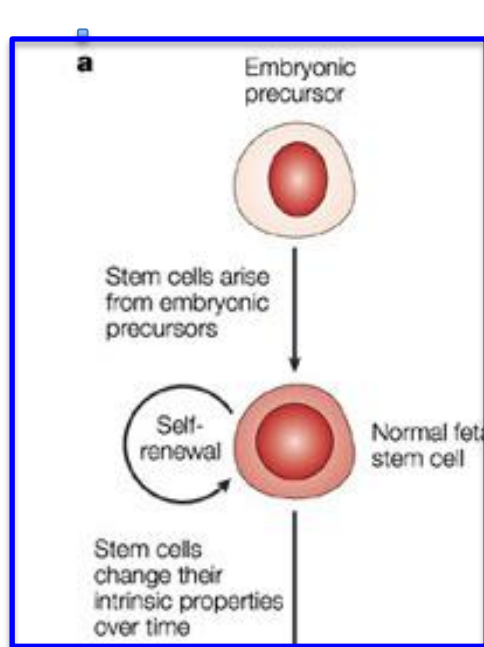
Defining the origin of tumor heterogeneity in epithelial cancers

A fluorescence microscopy image of a tumor section. The image shows a dense population of cells with blue nuclei. A prominent green signal highlights a specific region, likely representing a particular cell lineage or marker. Red signal outlines the overall tissue structure. The background is black.

Cédric Blanpain, MD, PhD
WELBIO, Université Libre de Bruxelles

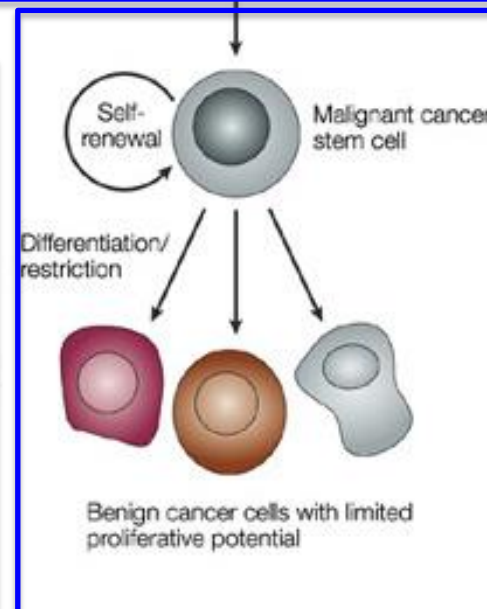
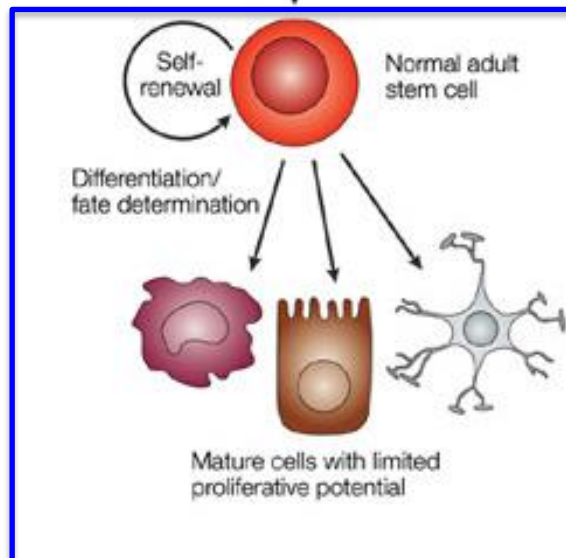
Our lab is studying the role of stem cell during development, homeostasis and cancer

Development



Cancer initiation

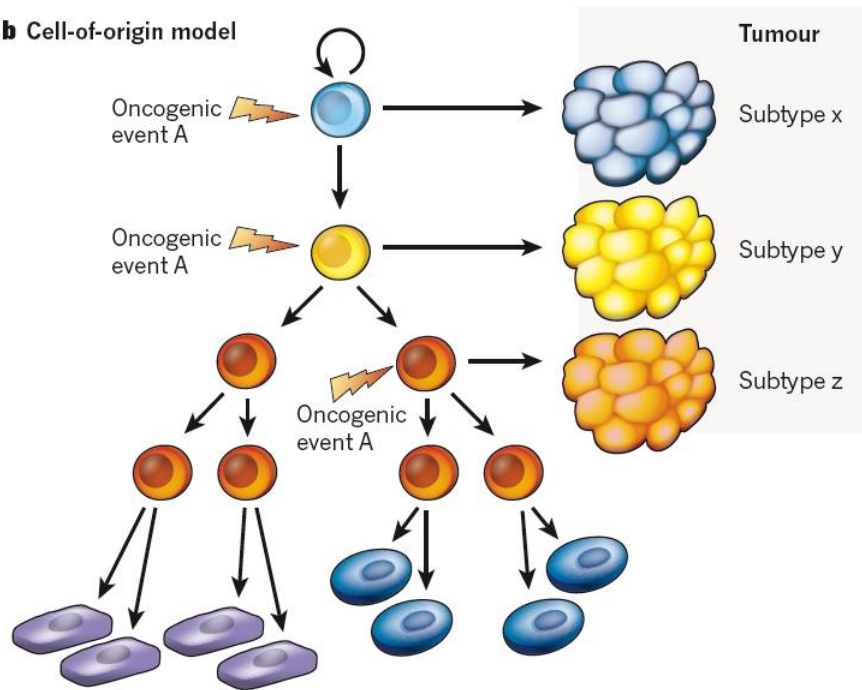
Homeostasis



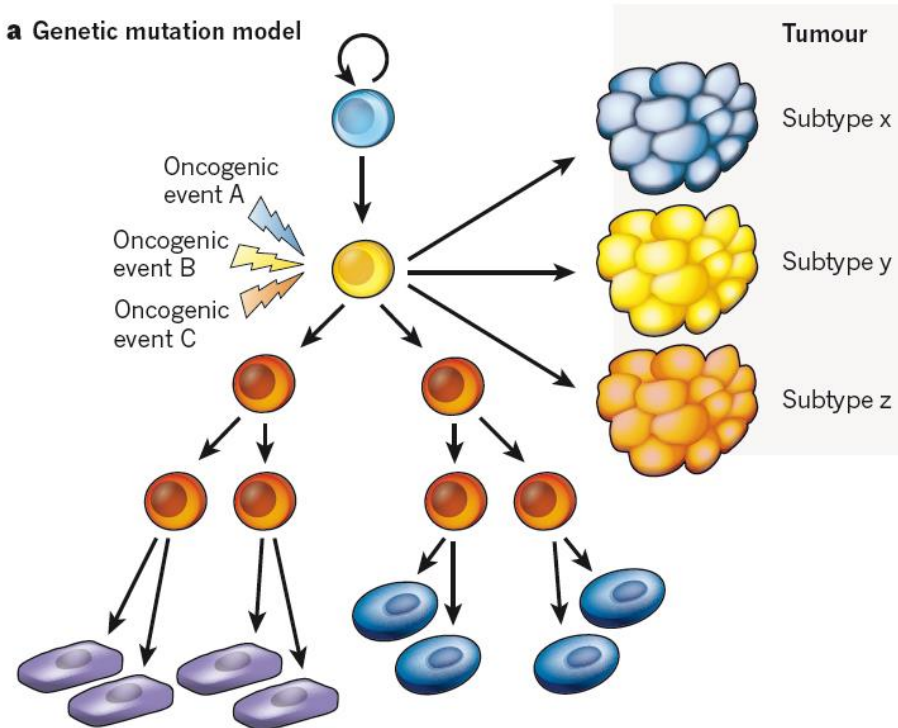
Cancer growth

Mechanisms regulating tumor heterogeneity

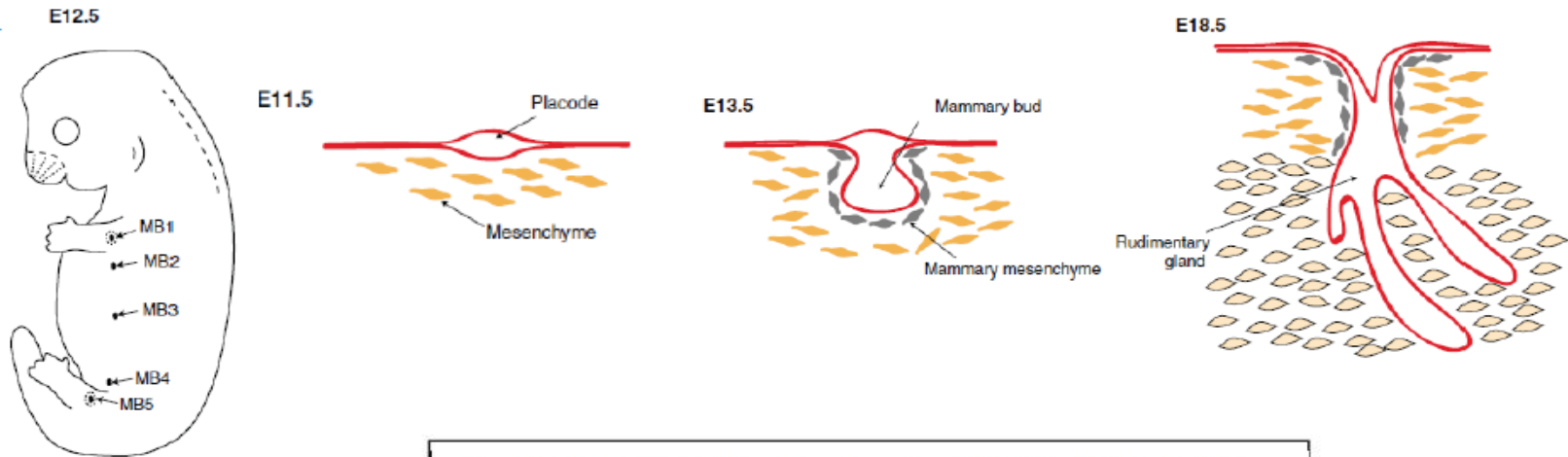
b Cell-of-origin model



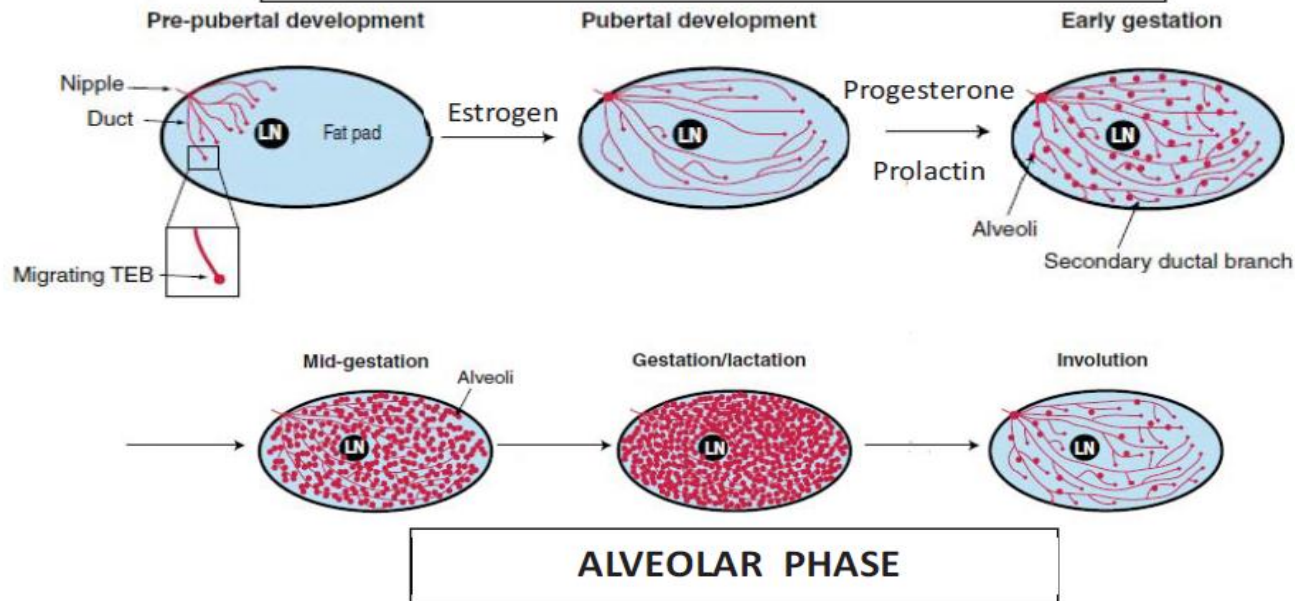
a Genetic mutation model



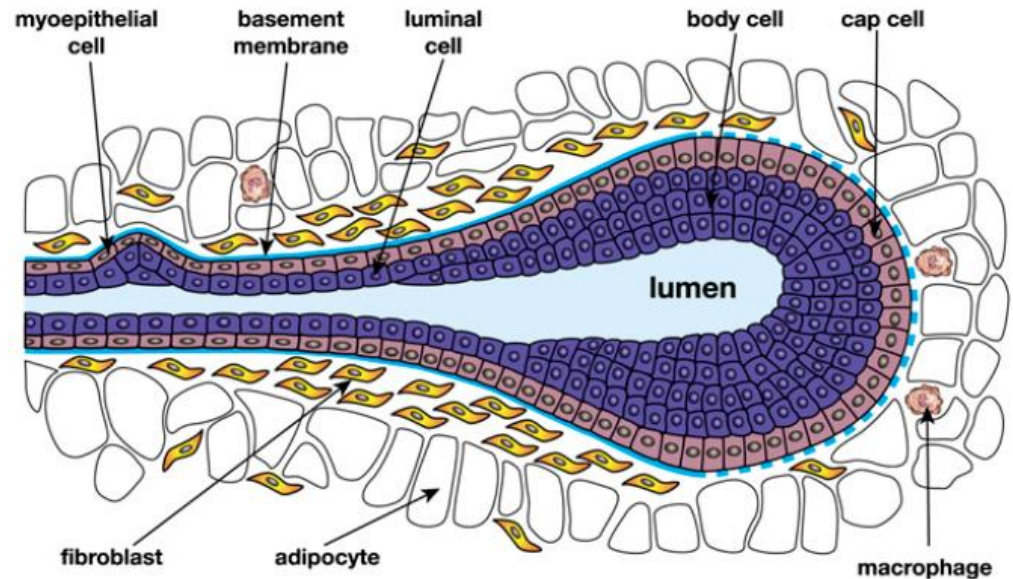
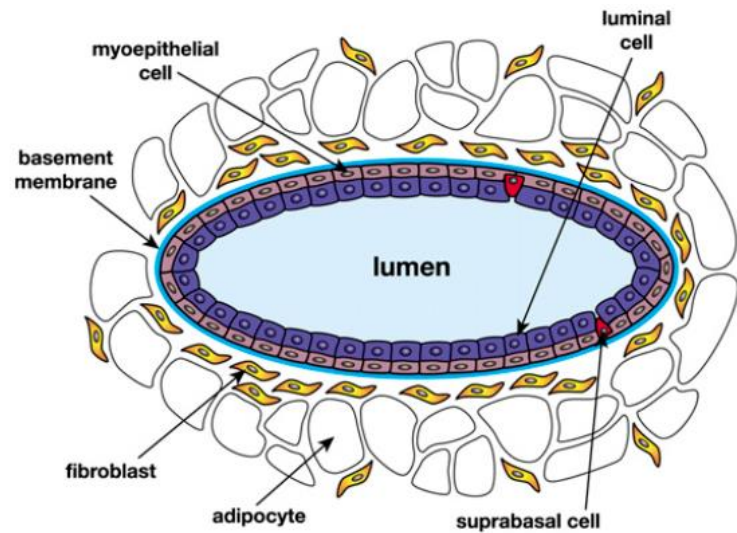
Development and homeostasis of the mammary gland



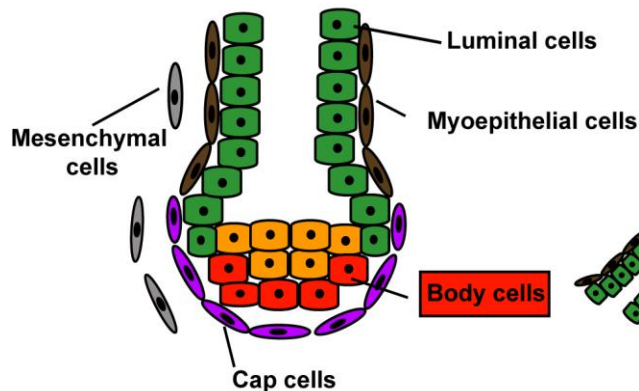
Invasion of the fat pad – DUCTAL PHASE



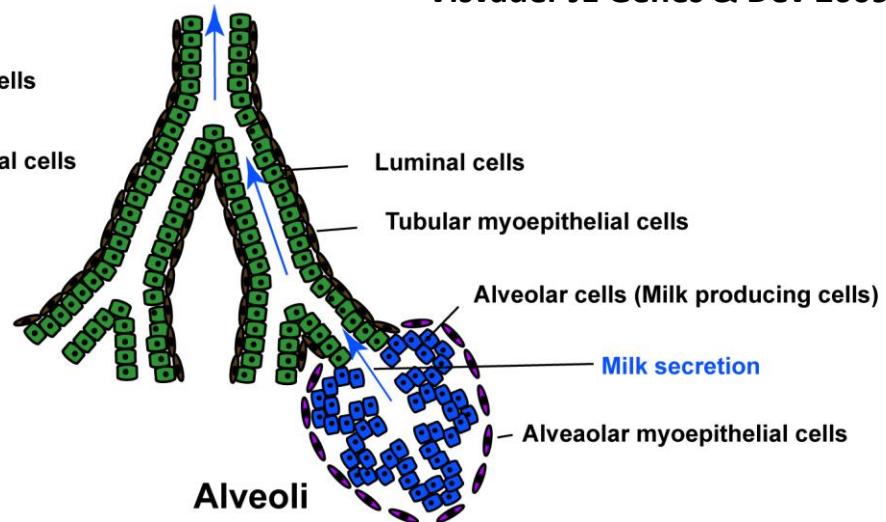
Different cellular lineages compose the mammary epithelium



Visvader JE Genes & Dev 2009



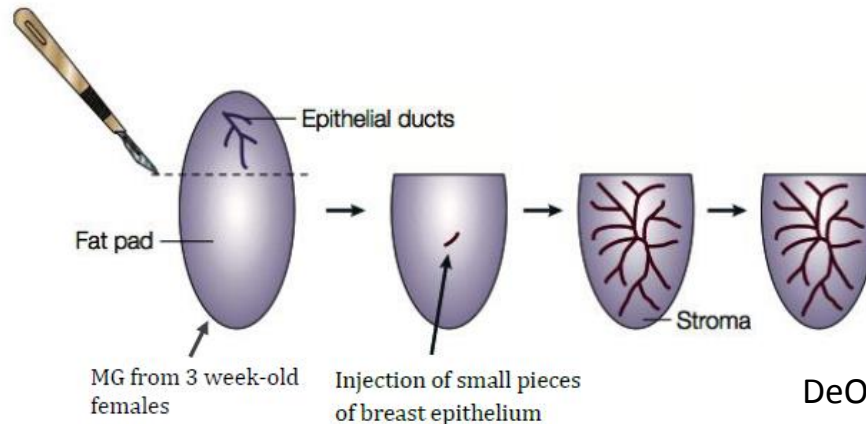
Terminal end bud (TEB)



Alveoli

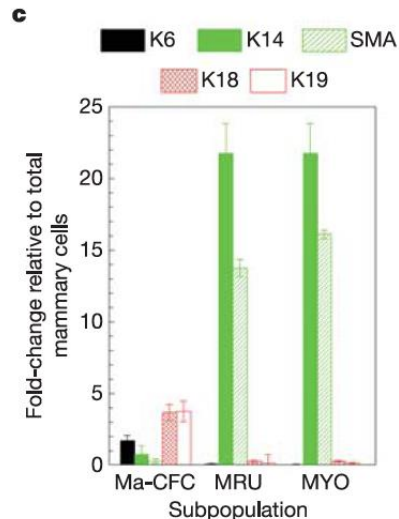
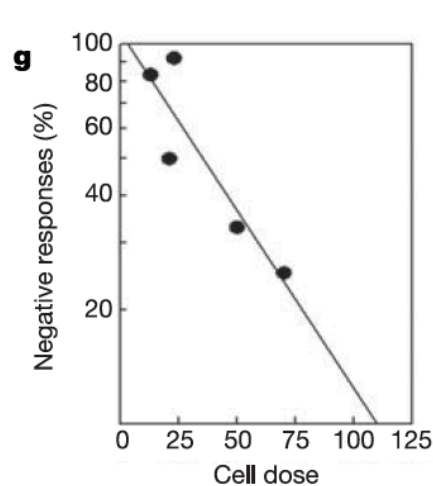
Blanpain et al. Cell 2007

Basal cells are multipotent in transplantation assays



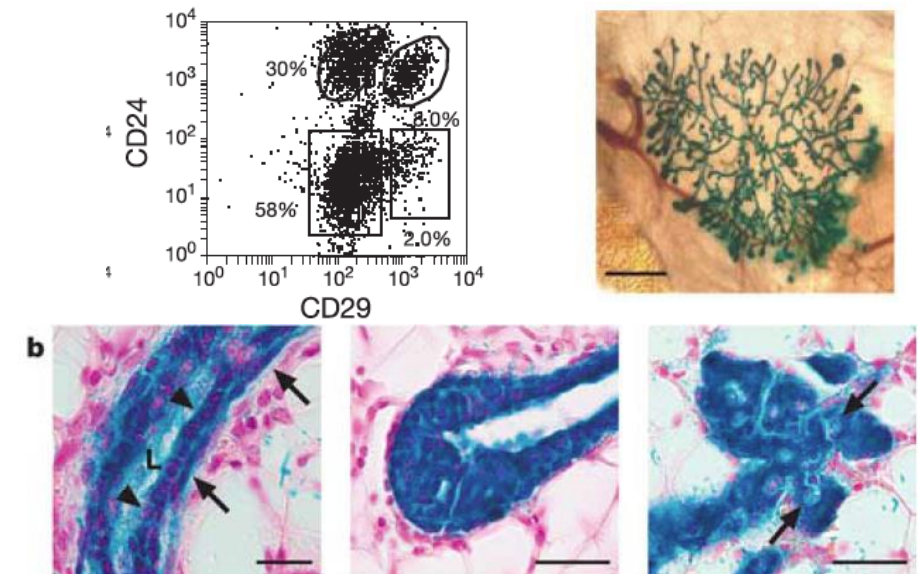
Purification and unique properties of mammary epithelial stem cells

John Stingl^{1,3}, Peter Eirew¹, Ian Ricketson¹, Mark Shackleton⁴, François Vaillant⁴, David Choi¹, Haiyan I. Li² & Connie J. Eaves^{1,5}



Generation of a functional mammary gland from a single stem cell

Mark Shackleton^{1,2}, François Vaillant^{1,2}, Kaylene J. Simpson^{3†}, John Stingl^{4,5}, Gordon K. Smyth¹, Marie-Liesse Asselin-Labat^{1,2}, Li Wu¹, Geoffrey J. Lindeman^{1,2} & Jane E. Visvader^{1,2}



Defining the cellular hierarchy of mammary gland using genetic lineage tracing experiments

Alexandra Van Keymeulen, PhD

Marielle Ousset, PhD

Gaelle Bouvencourt



Anna Sofia Rocha, PhD

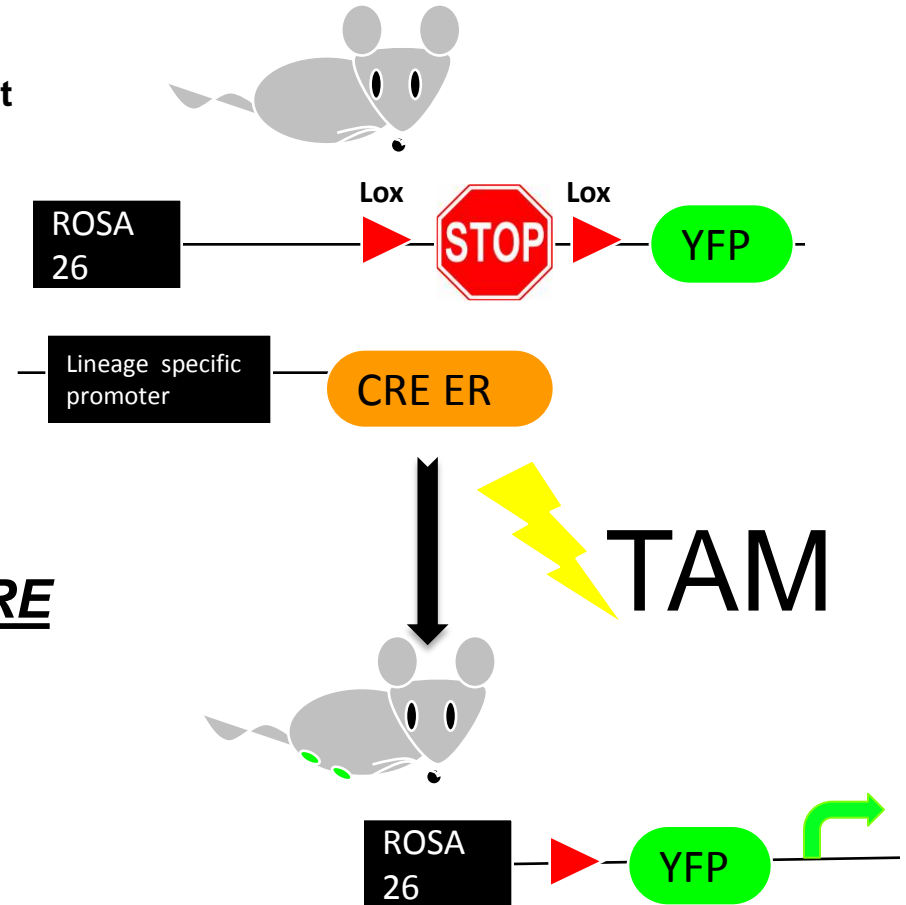
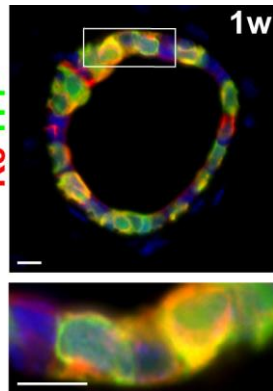
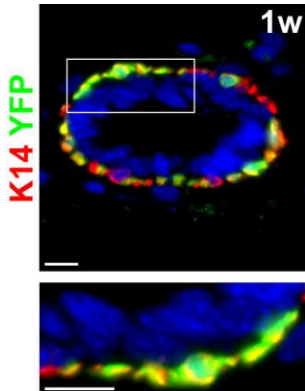
Lineages specific inducible CRE

Myoepithelial:

K14, K5, Lgr5 CREER

Luminal:

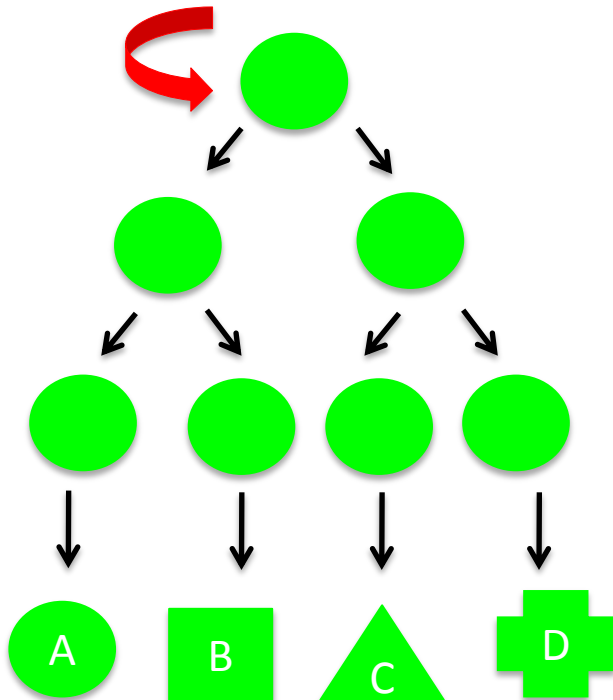
K8, K18, K19 CREER



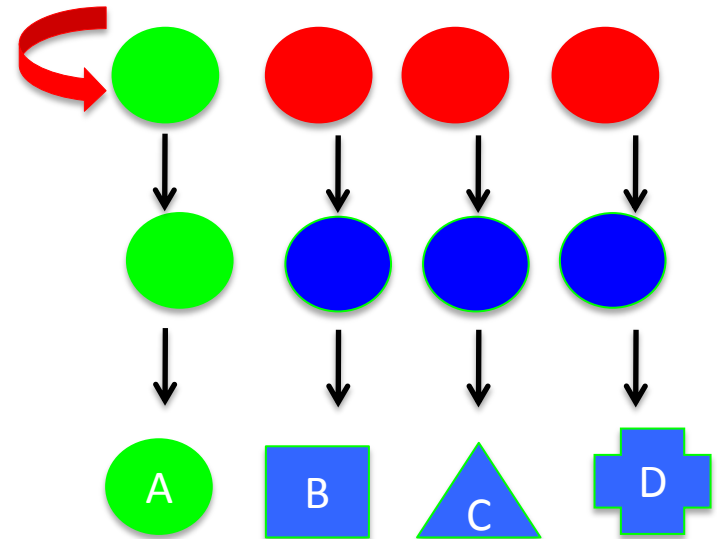
→ **Genetic** and **irreversible** labelling of the cells
→ **Spatial** and **temporal** control of Cre activation

Lineage tracing SC and their progeny

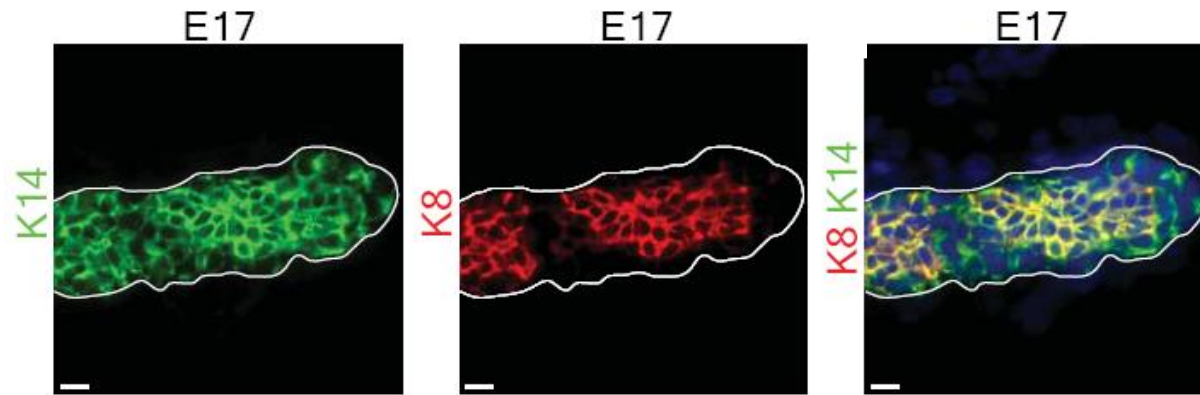
Tracing multipotent SC



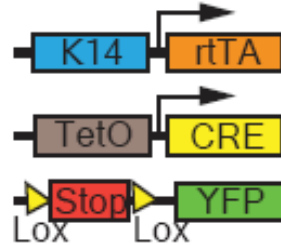
Tracing unipotent SC



All mammary epithelial lineages derived from K14 expressing cells during morphogenesis



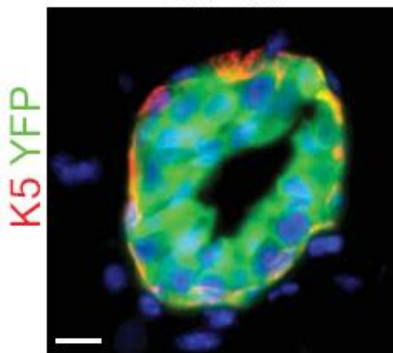
K14rtTA/TetOCRE/
RosaYFP



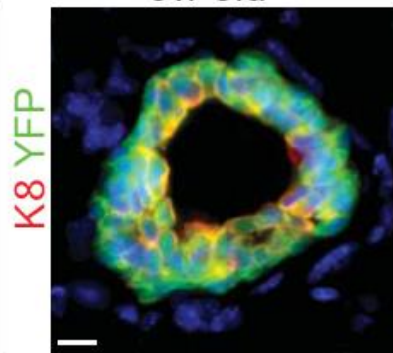
Contribution of K14 derived cells
during development



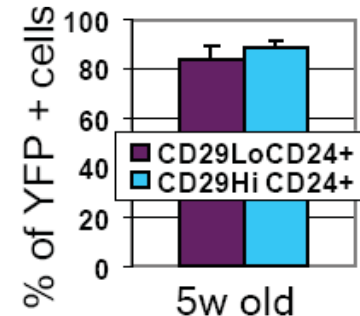
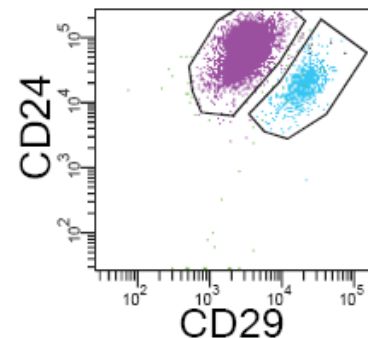
5w old



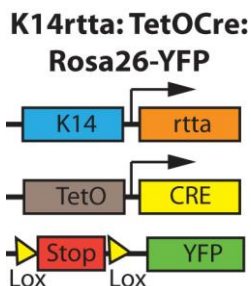
5w old



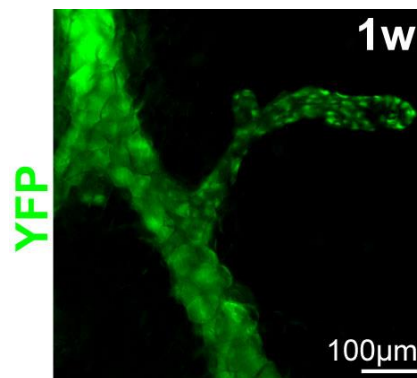
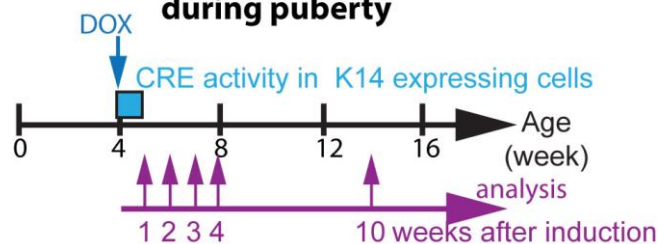
In YFP+ cells



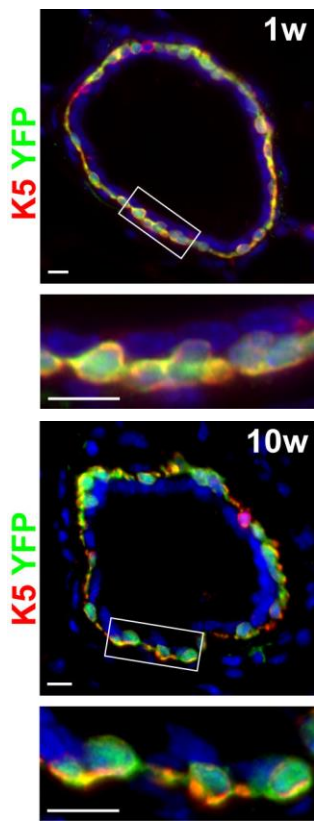
K14⁺ unipotent stem cells ensure mammary myoepithelial lineage expansion during puberty



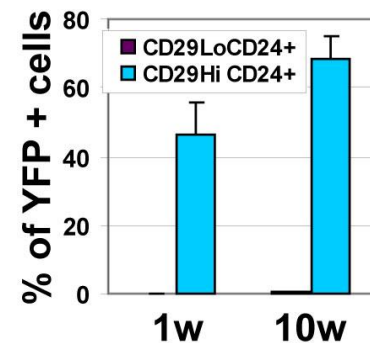
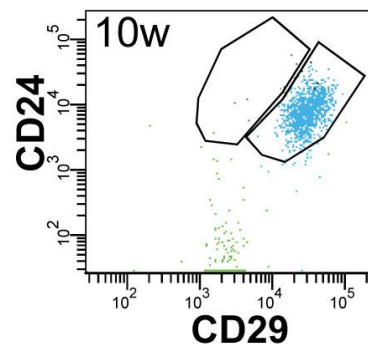
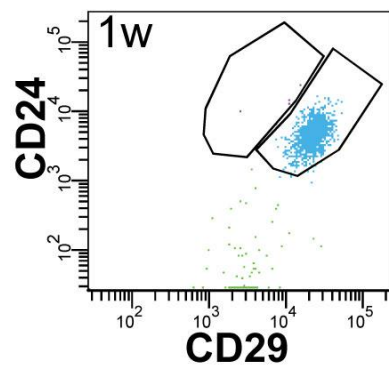
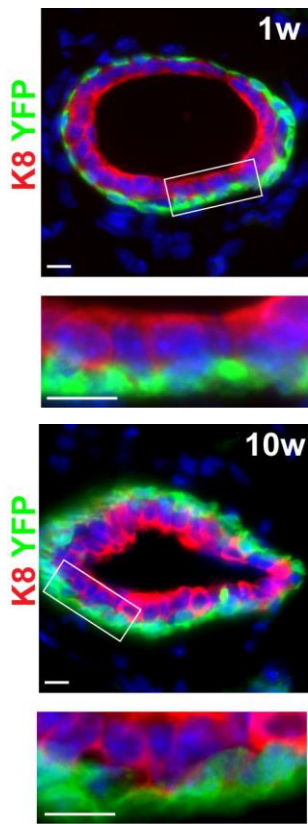
Contribution of K14 derived cells during puberty



Myoepithelial lineage

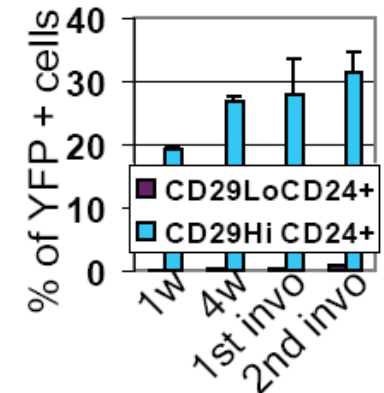
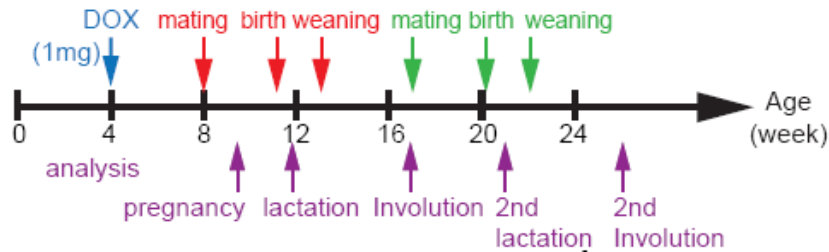


Luminal lineage



K14+ SCs ensure mammary myoepithelial lineage expansion during pregnancy

Contribution of K14 derived cells during pregnancy



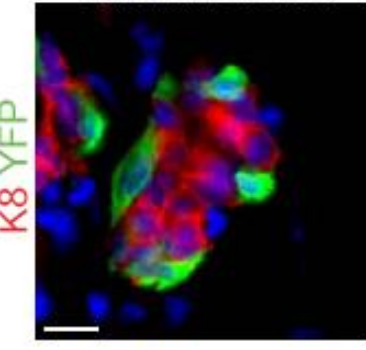
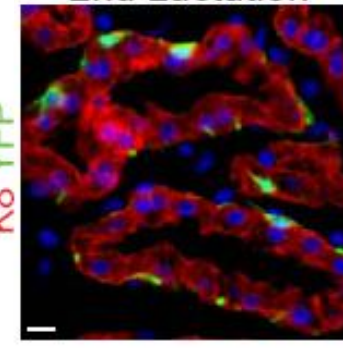
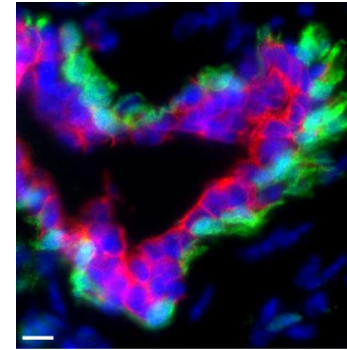
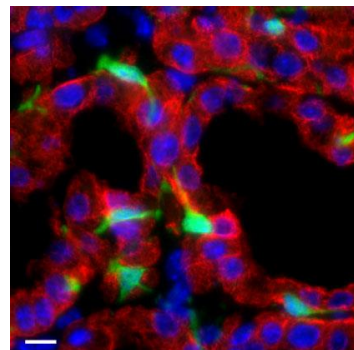
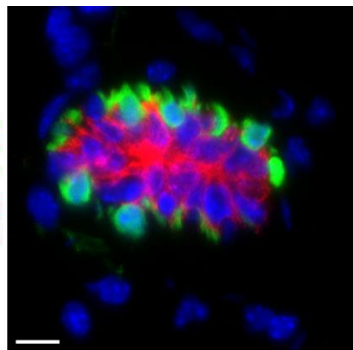
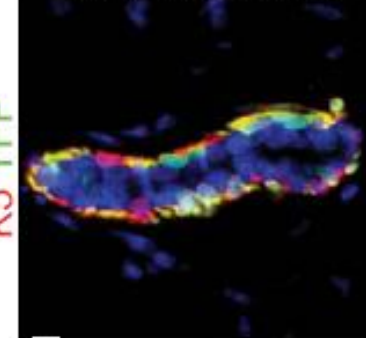
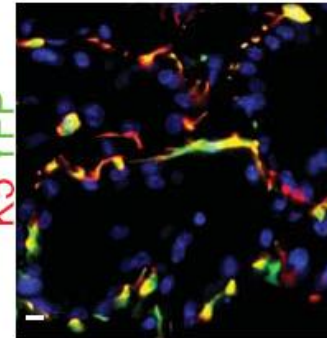
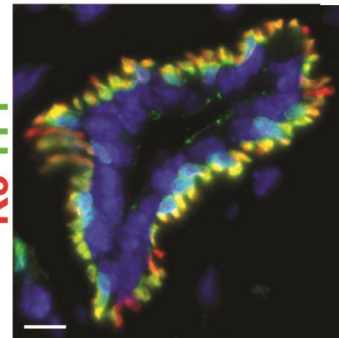
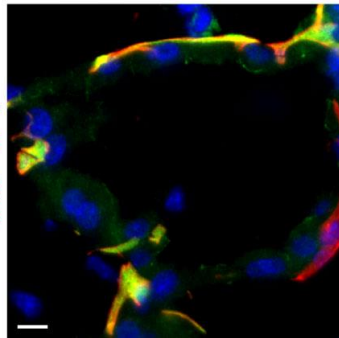
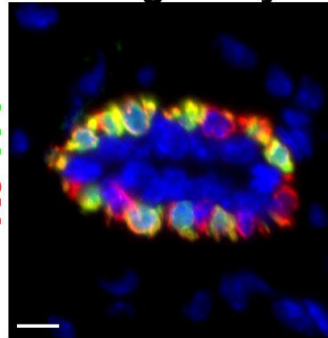
Pregnancy

Lactation

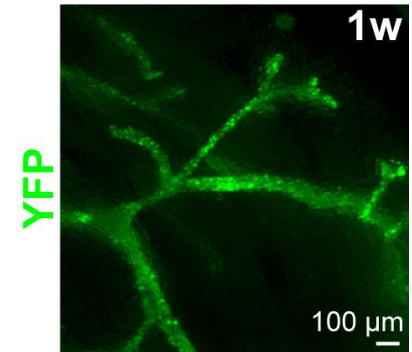
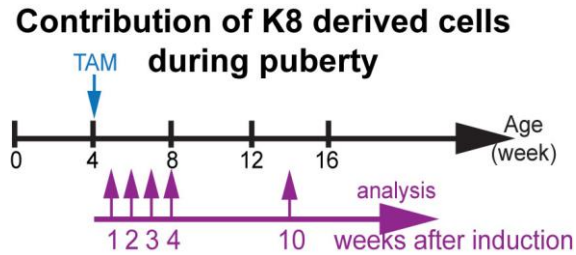
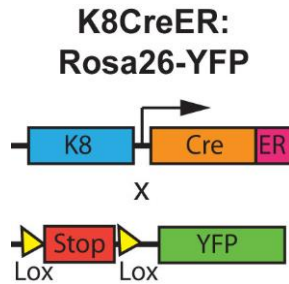
Involution

2nd Lactation

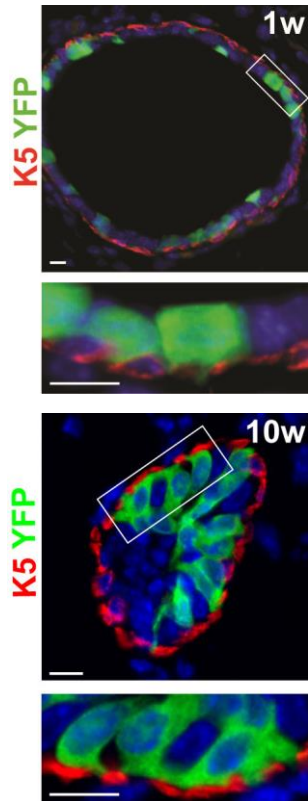
2nd Involution



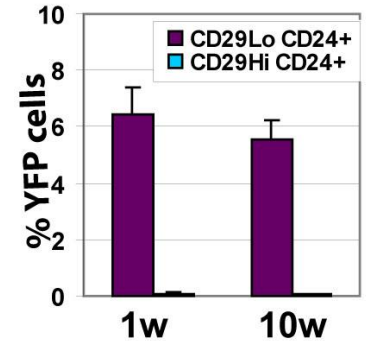
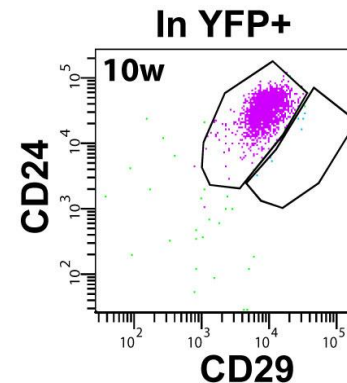
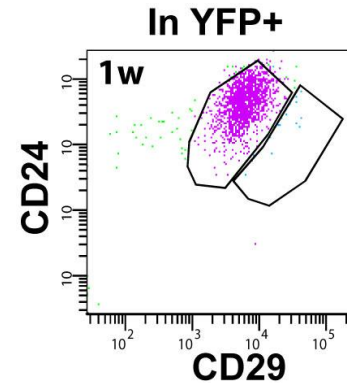
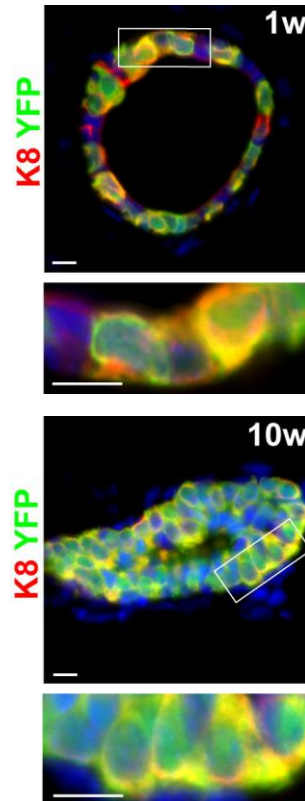
K8+ unipotent SCs ensure mammary luminal lineage expansion during puberty



Myoepithelial lineage

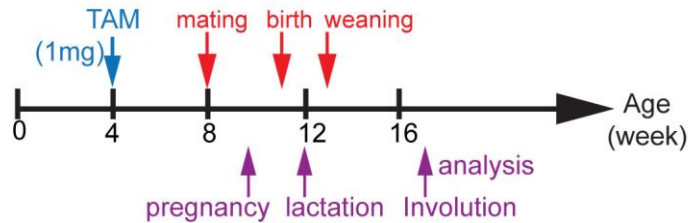


Luminal lineage

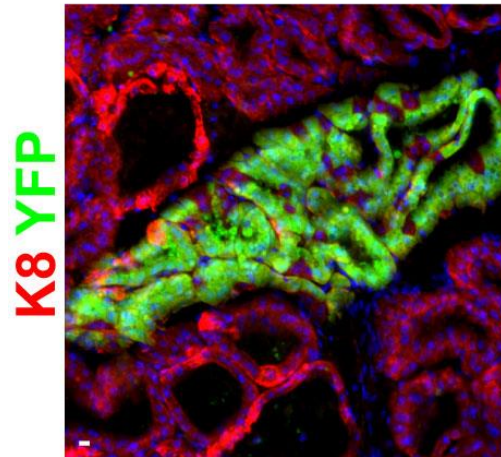


K8+ unipotent SCs ensure mammary luminal lineage expansion during pregnancy

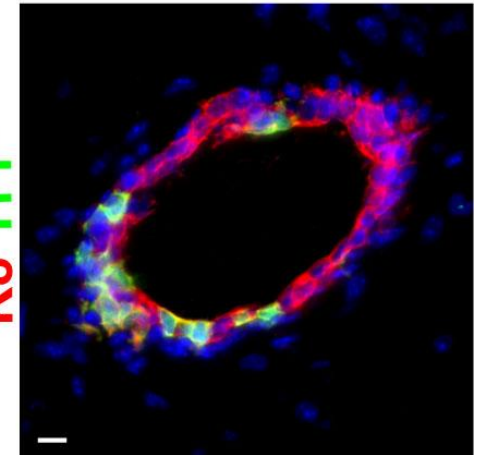
Contribution of K8 derived cells during pregnancy



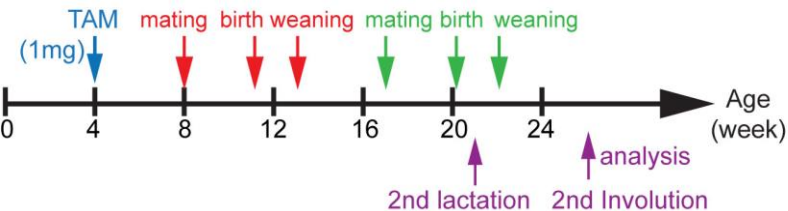
Lactation



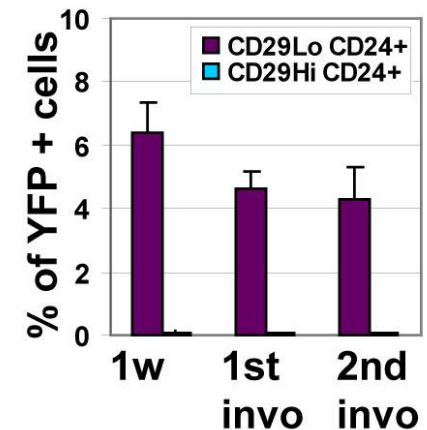
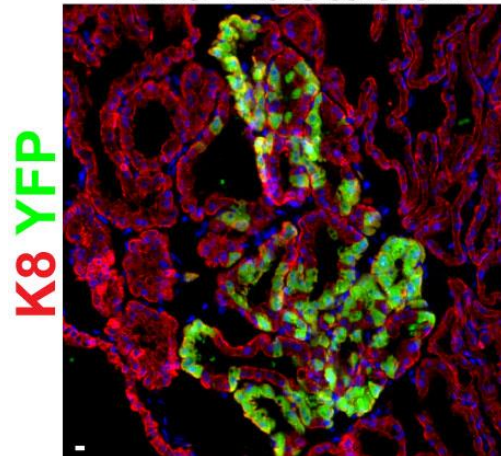
Involution



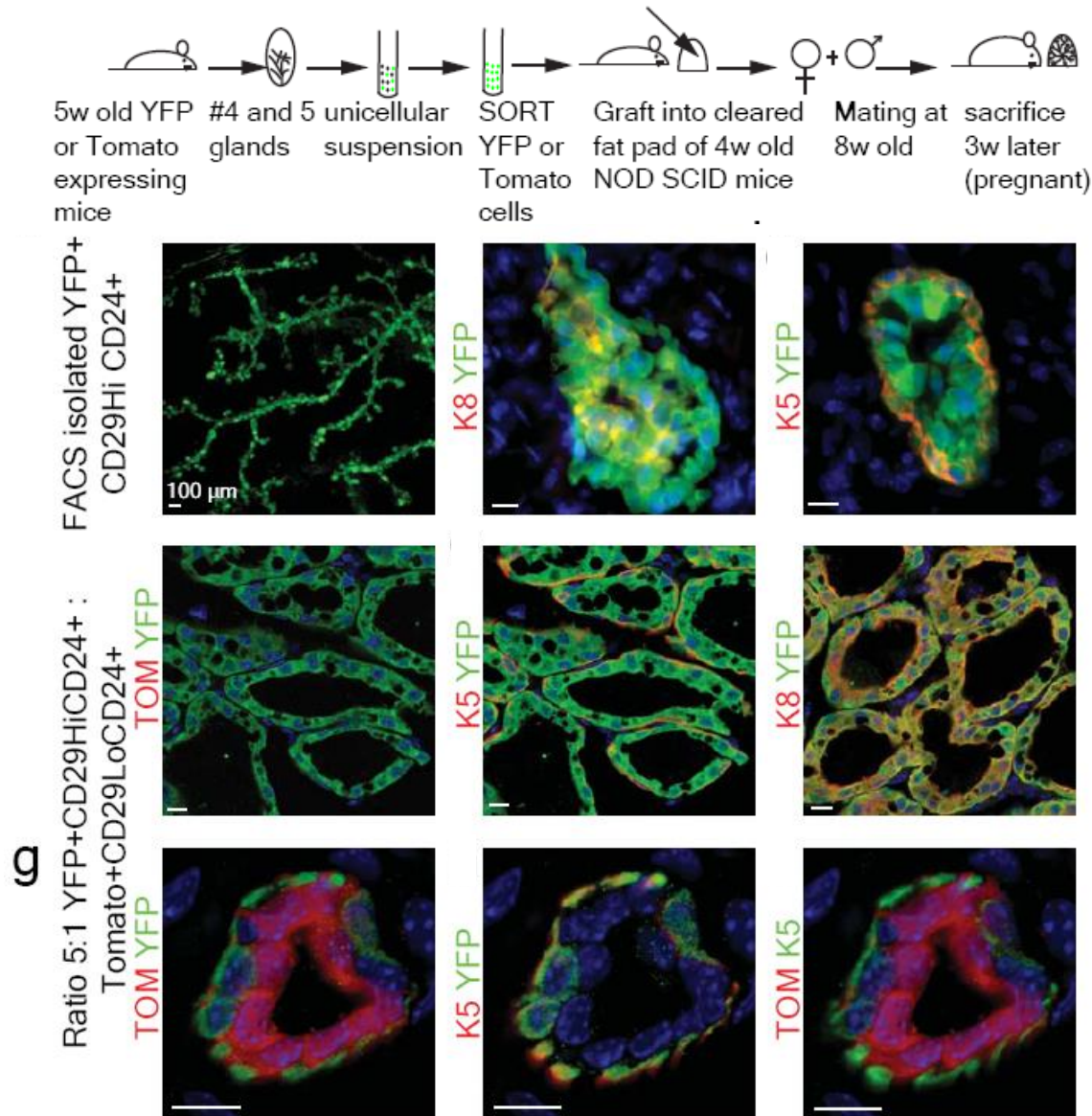
Contribution of K8 derived cells after a second pregnancy



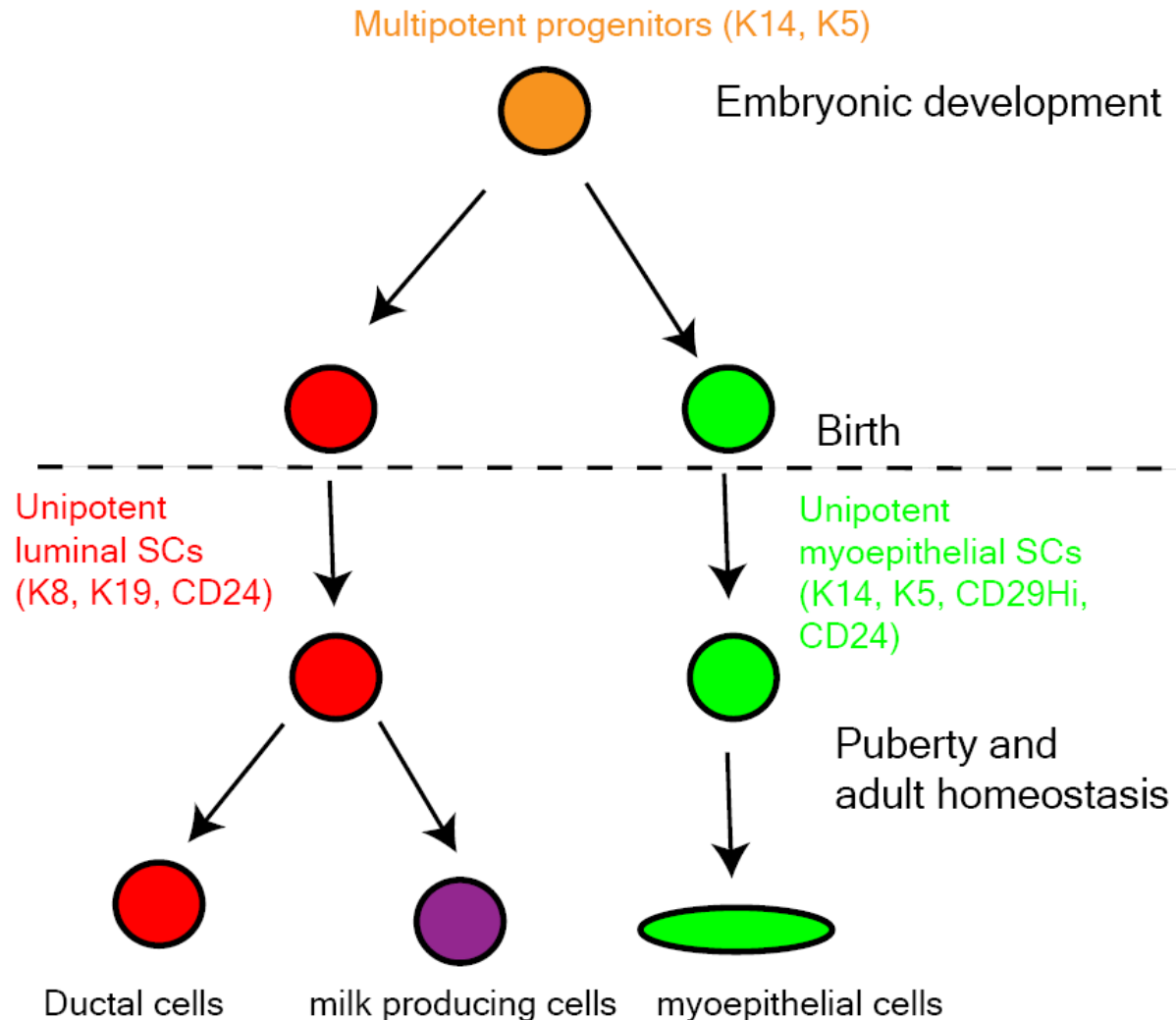
2nd Lactation



Unipotent basal stem cells become multipotent in transplantation assay



Lineage hierarchy in the mammary gland under physiological conditions



Notch2 genetic fate mapping reveals two previously unrecognized mammary epithelial lineages

Sanja Šale¹, Daniel Lafkas² and Spyros Artavanis-Tsakonas^{1,3}

Notch3 marks clonogenic mammary luminal progenitor cells in vivo

Daniel Lafkas,^{1,2,3,4} Veronica Rodilla,^{1,2,3} Mathilde Huyghe,^{1,2,3} Larissa Mourao,^{1,2,3} Hippokratis Kiaris,⁴ and Silvia Fre^{1,2,3}

Lineage Tracing of Mammary Epithelial Cells Using Cell-Type-Specific Cre-Expressing Adenoviruses

Luwei Tao,^{1,2} Maaike P.A. van Bragt,^{1,2} Elizabeth Laudadio,^{1,3} and Zhe Li^{1,2,*}

Luminal Progenitors Restrict Their Lineage Potential during Mammary Gland Development

Veronica Rodilla^{1,2,3}, Alessandro Dasti^{1,2,3}, Mathilde Huyghe^{1,2,3}, Daniel Lafkas^{1,2,3*},
Cécile Laurent⁴, Fabien Reyat^{4,5}, Silvia Fre^{1,2,3*}

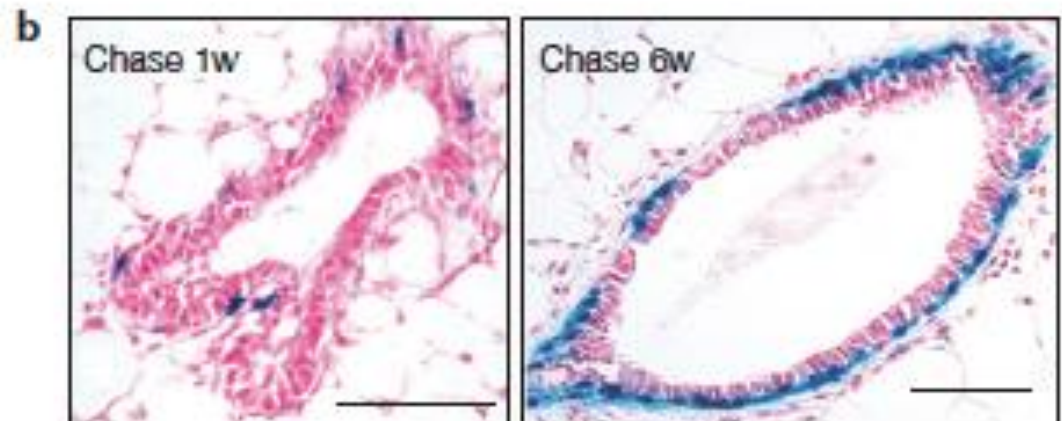
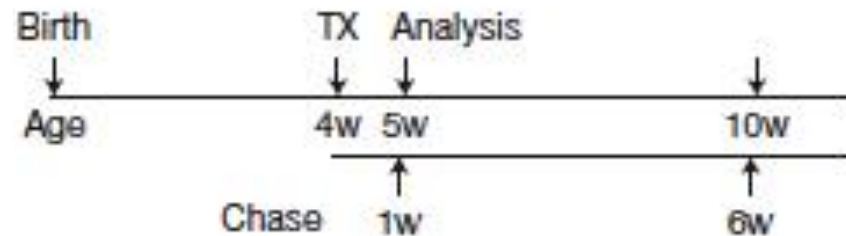
Mammary stem cells have myoepithelial cell properties

Michael D. Prater¹, Valérie Petit^{2,3}, I. Alasdair Russell¹, Rajshekhar R. Giraddi¹, Mona Shehata¹, Suraj Menon¹, Reiner Schulte¹, Ivo Kalajzic⁴, Nicola Rath⁵, Michael F. Olson⁵, Daniel Metzger⁶, Marisa M. Faraldo^{2,3}, Marie-Ange Deugnier^{2,3}, Marina A. Glukhova^{2,3,7} and John Stingl^{1,7}

nature
cell biology

d

Cell population	Number of cells injected per fat pad	Number of engrafts	MRU frequency (95% CI)
Basal α SMA ⁺	50	1/5	1 in 93 (1/54 to 1/160)
	100	6/8	
	200	8/9	
	500	5/5	
Basal α SMA ⁻	10	0/3	NA (1/287 to 1/ ∞)
	20	0/4	
	25	0/10	
	50	0/10	

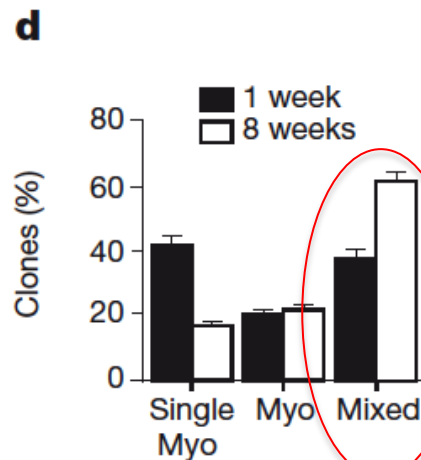
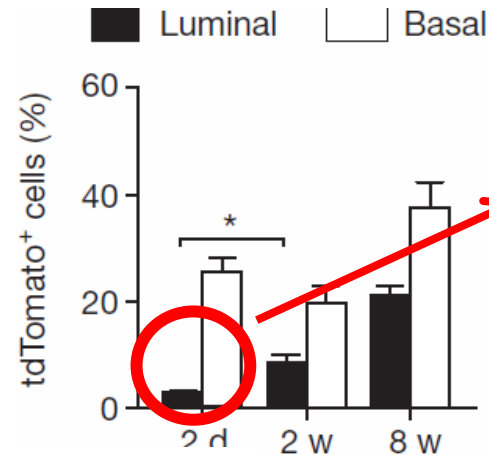
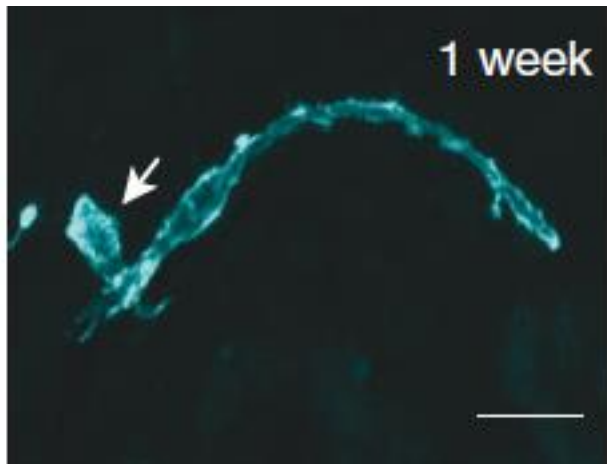
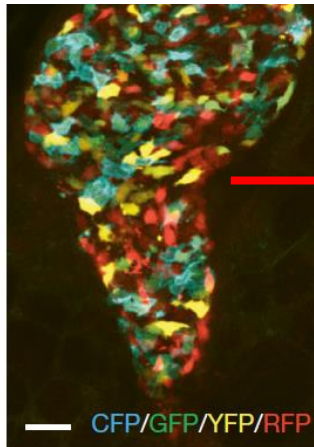


In situ identification of bipotent stem cells in the mammary gland

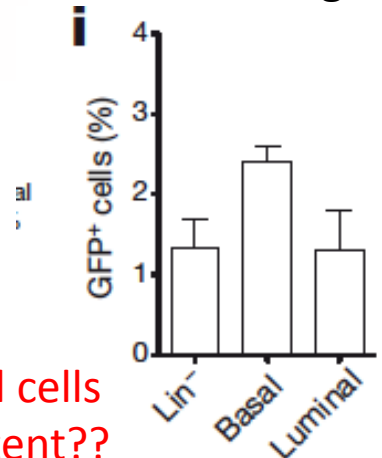
Anne C. Rios^{1,2*}, Nai Yang Fu^{1,2*}, Geoffrey J. Lindeman^{1,3,4} & Jane E. Visvader^{1,2}

Identification of multipotent mammary stem cells by protein C receptor expression

Daisong Wang^{1*}, Cheguo Cai^{1*}, Xiaobing Dong¹, Qing Cissy Yu¹, Xiao-Ou Zhang², Li Yang² & Yi Ariel Zeng¹



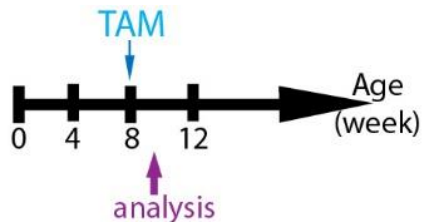
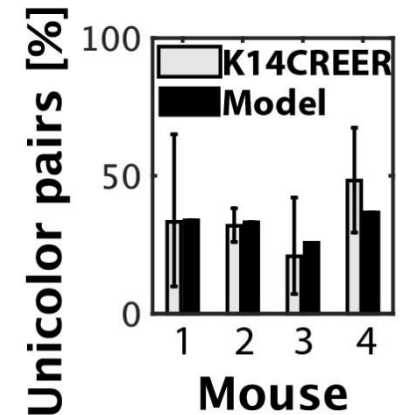
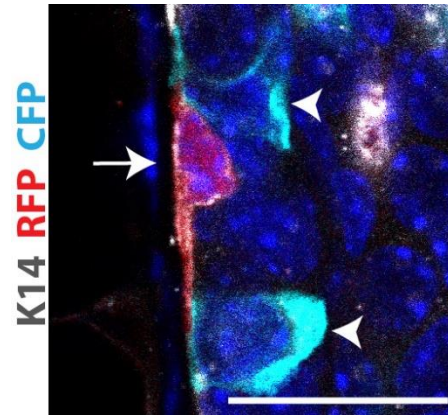
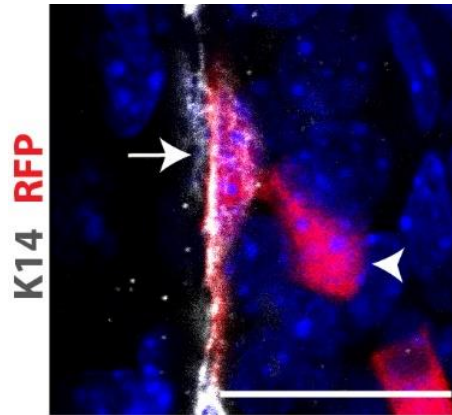
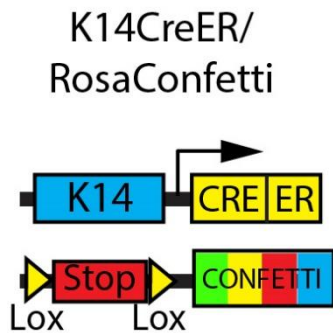
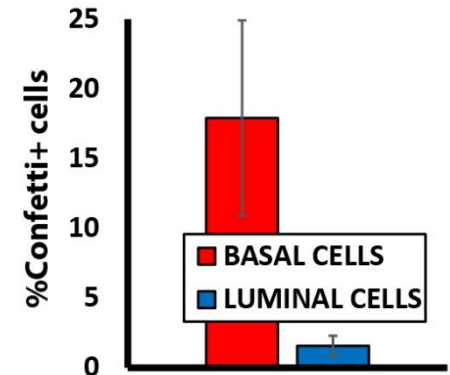
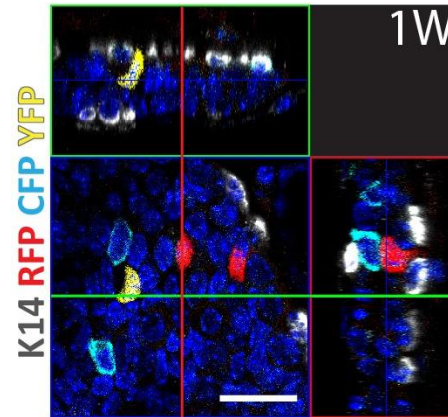
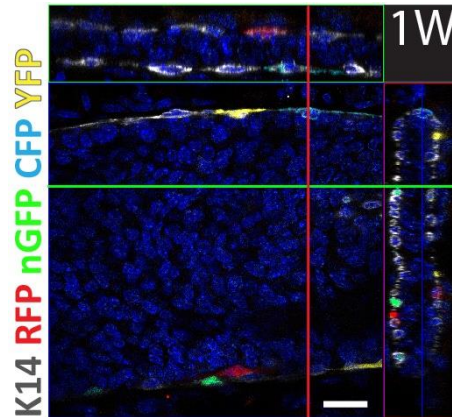
Procr tracing



Many CREER target initially and independently basal and luminal cells in adult mice



Aline Wuidart

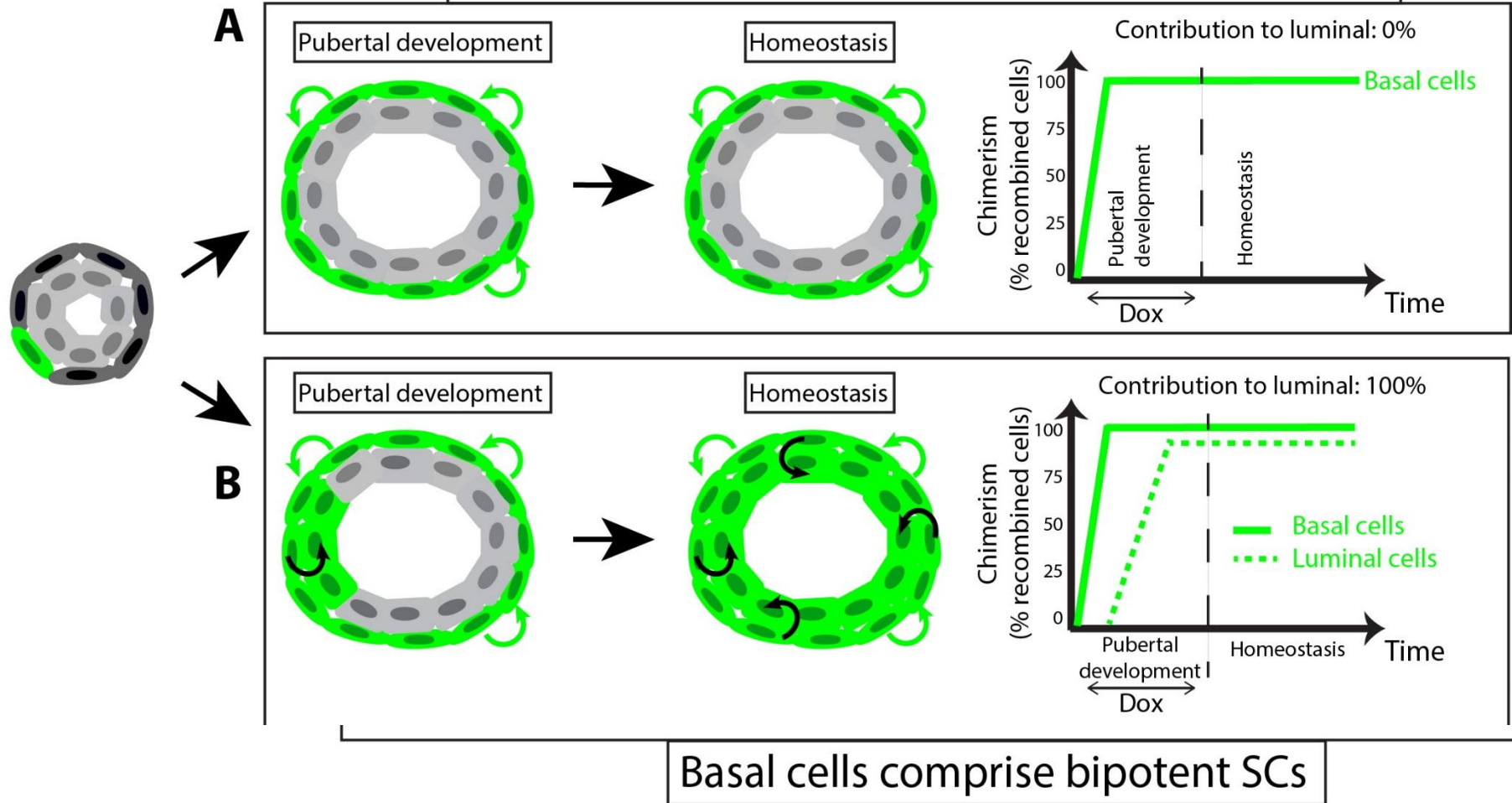


Statistical analysis demonstrates that doublets of BCs and LCs labelled with the same colour occur by chance

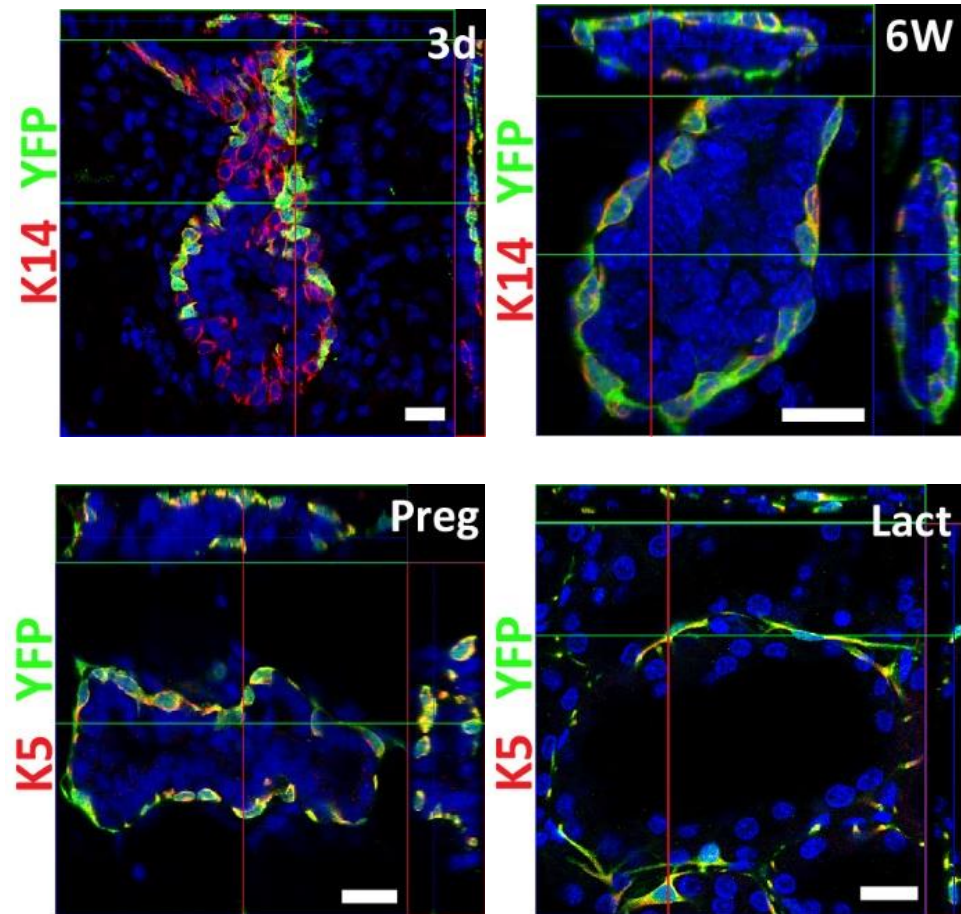
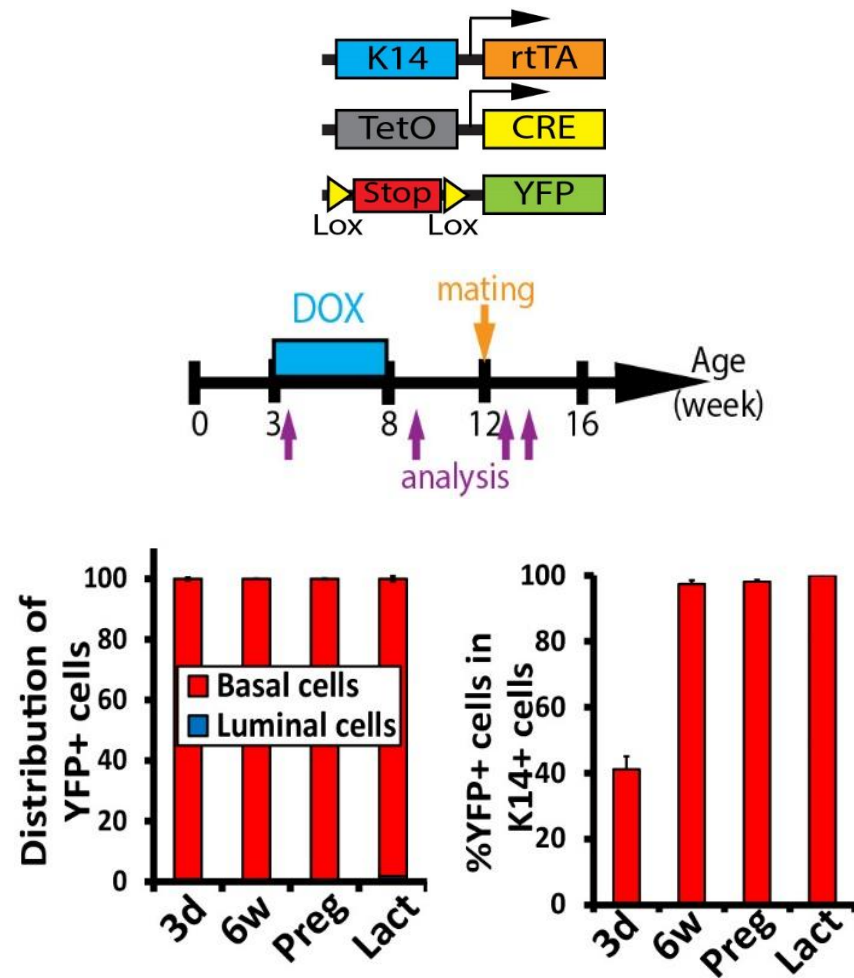
Statistical analysis in collaboration with Steffen Rulands and Ben Simons, Cambridge University

Theoretical outcomes of lineage tracing basal cells at saturation

Basal cells are a self-sustained unipotent lineage

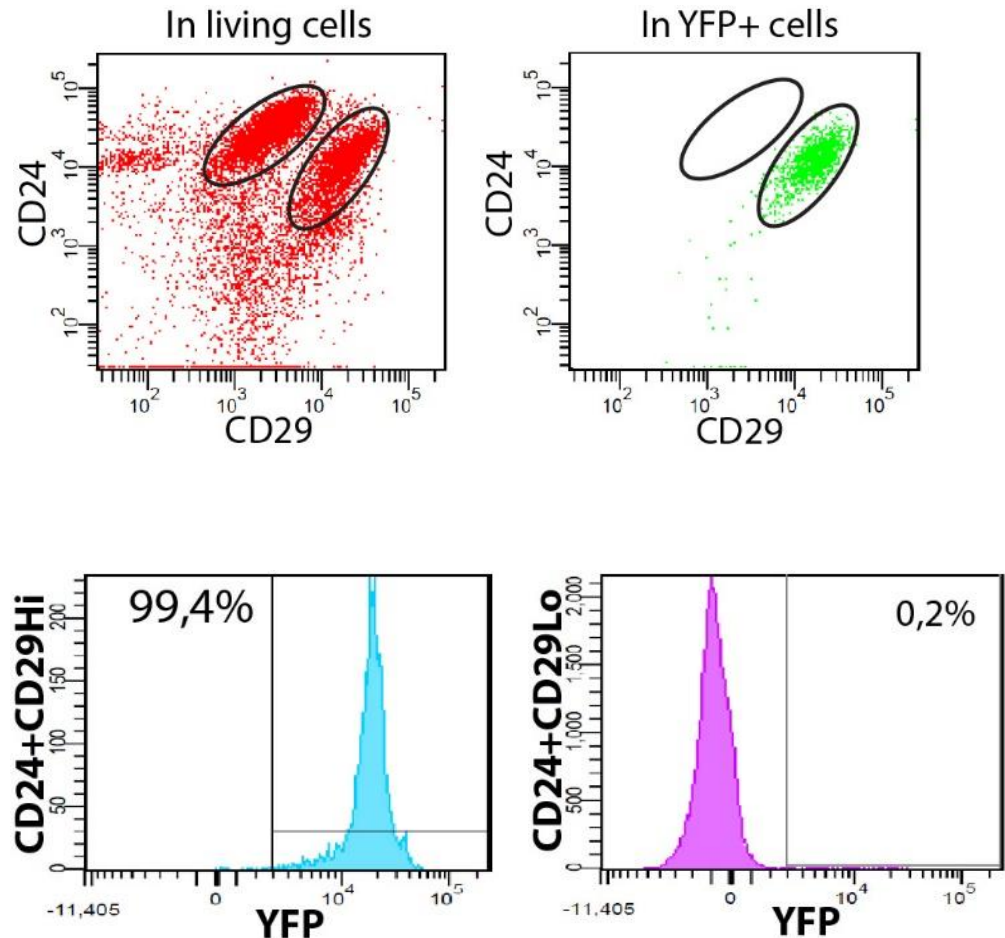
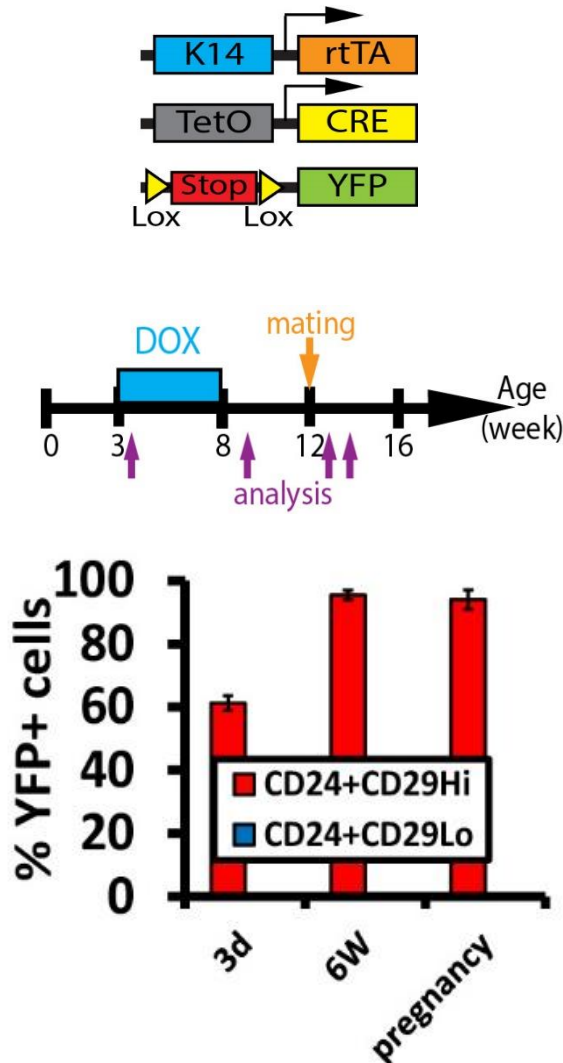


Basal cells are a self-sustained unipotent lineage in adult mice

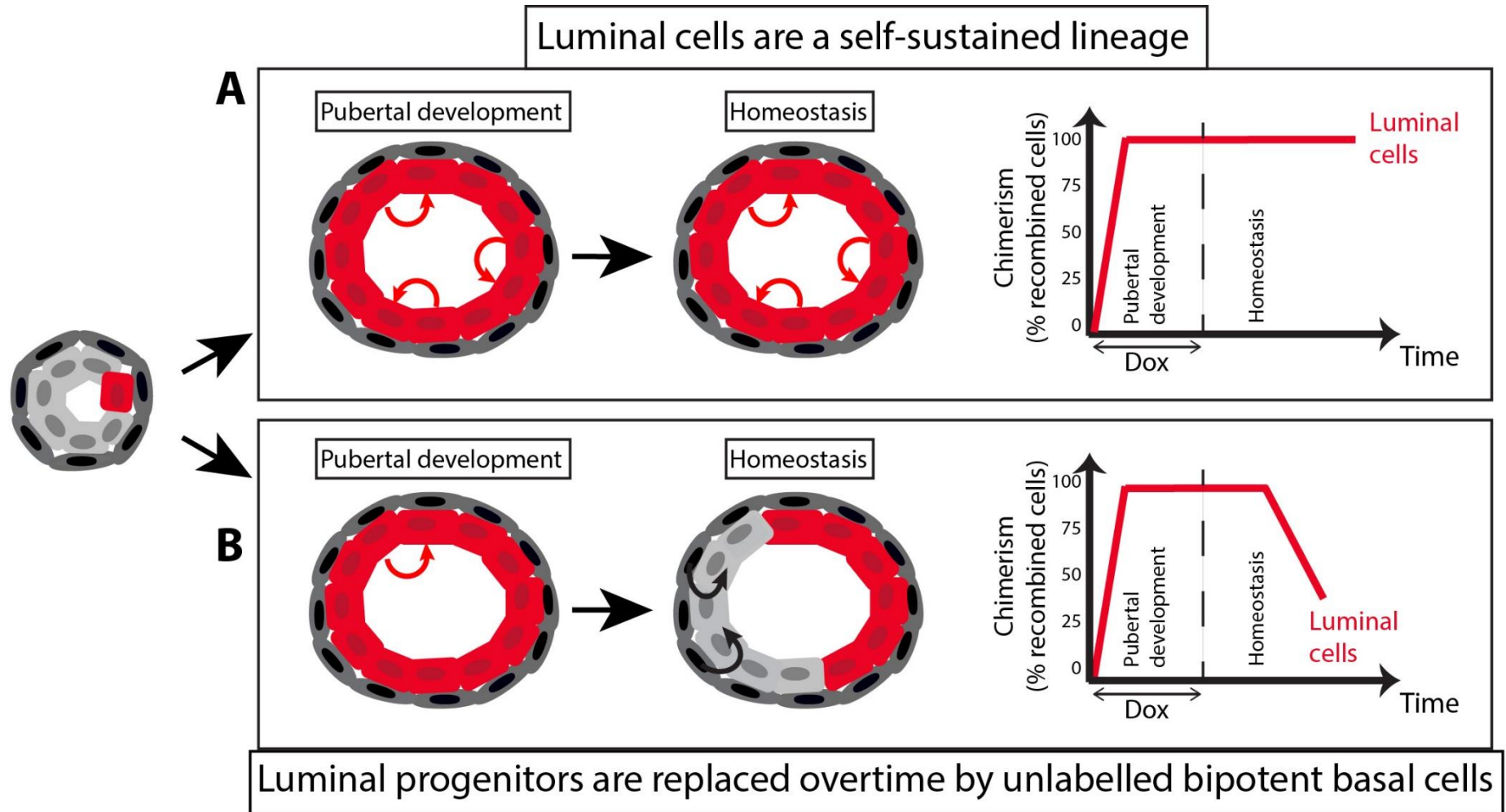


Immunofluorescence quantification on 3D whole-mounts of mammary gland

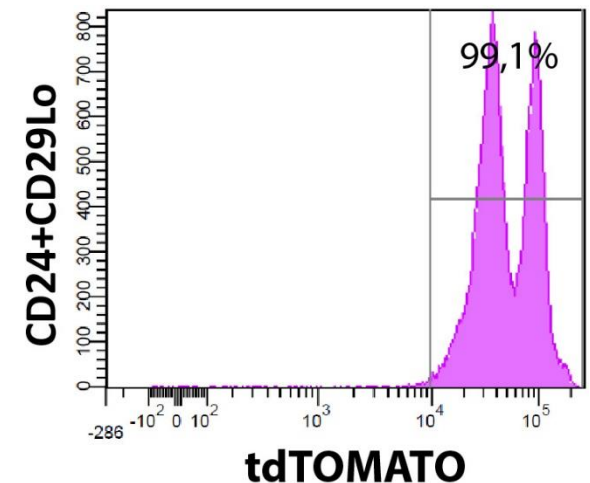
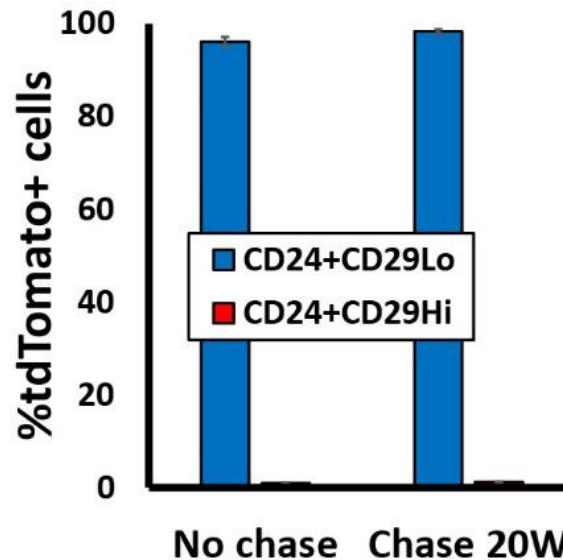
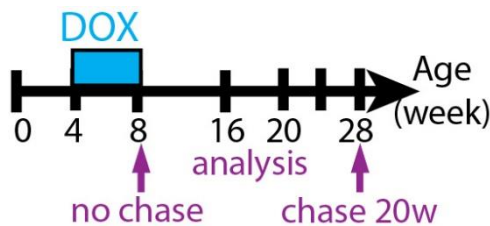
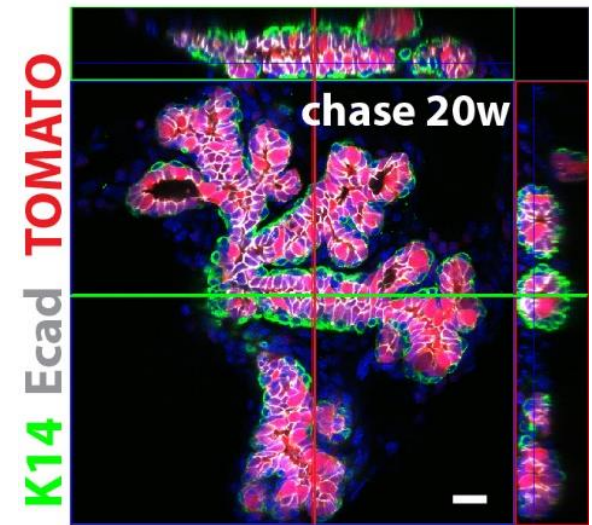
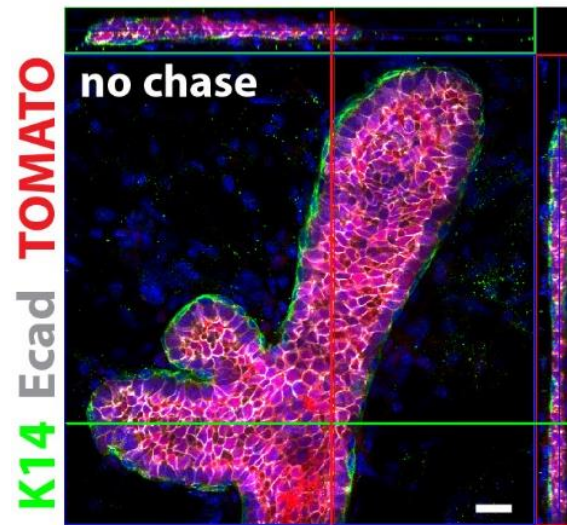
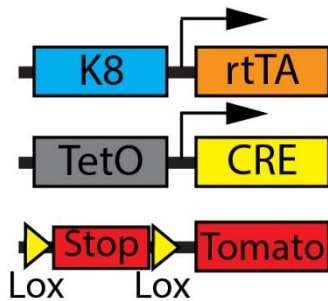
Basal cells are a self-sustained unipotent lineage in adult mice



Theoretical outcomes of tracing luminal cells at saturation

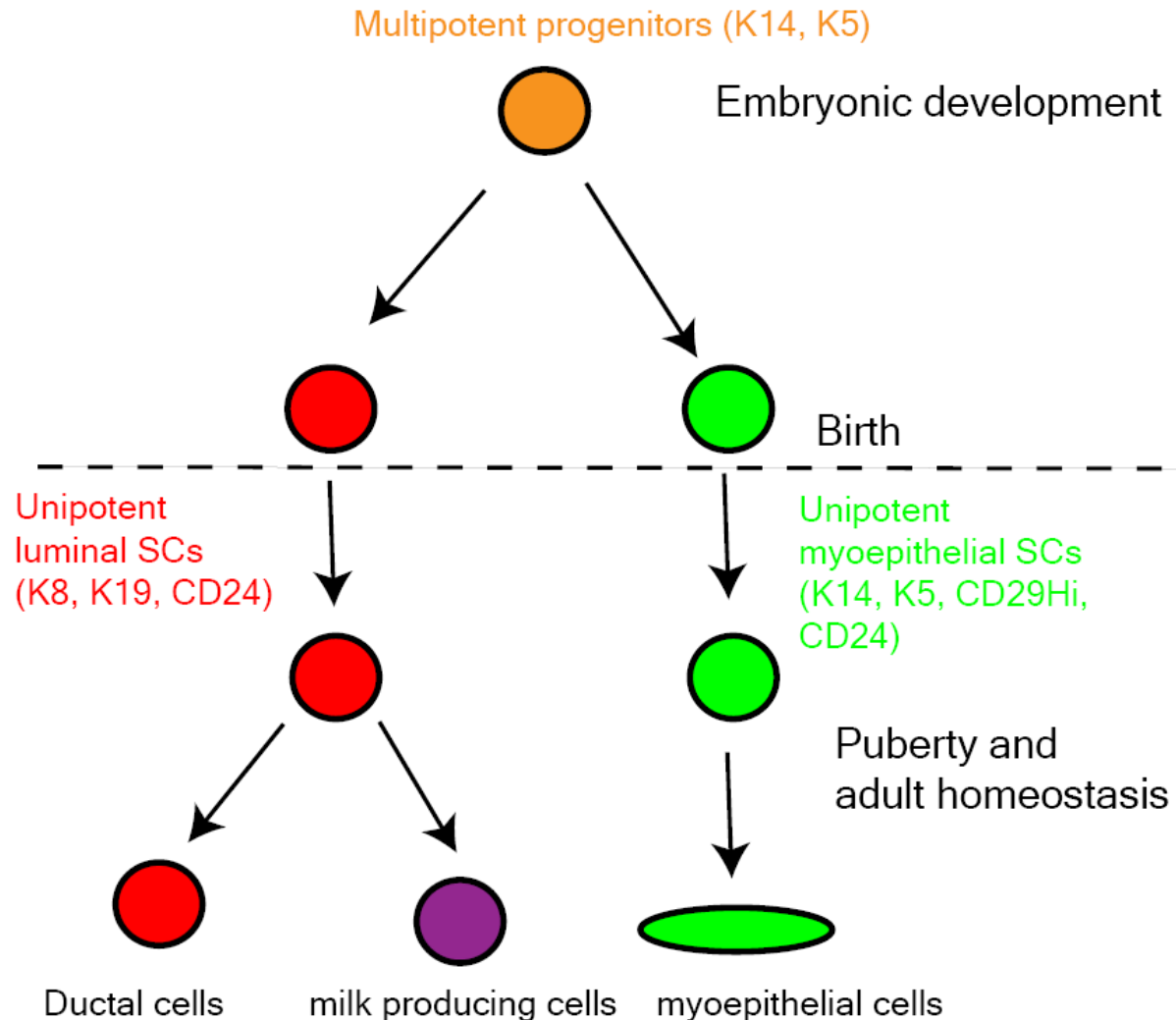


Luminal cells are a self-sustained lineage in adult mice



Wuidart et al. 2015 unpublished

Lineage hierarchy in the mammary gland under physiological conditions



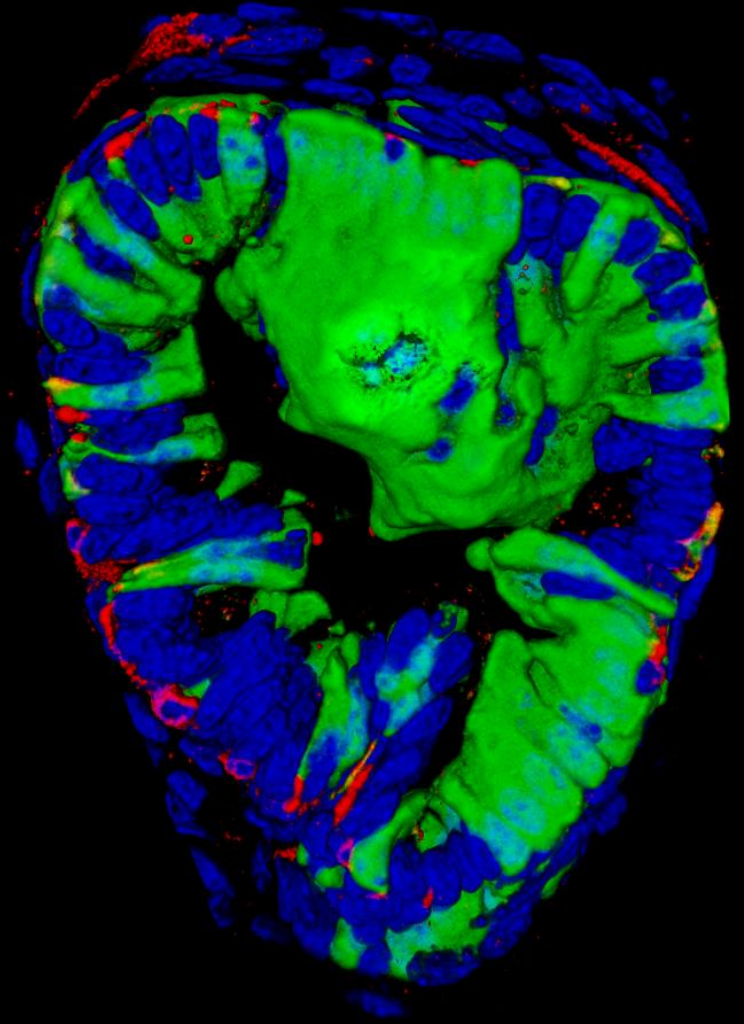
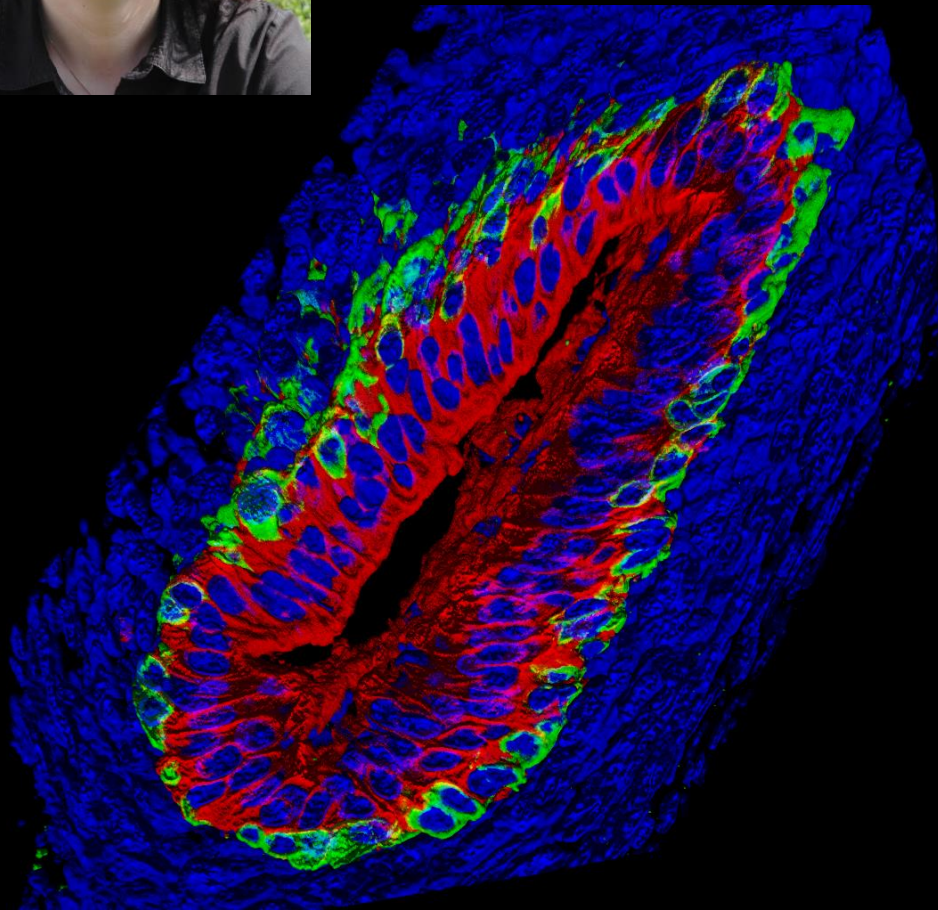
Van Keymeulen et al. Nature 2011

Wuidart et al. unpublished 2015

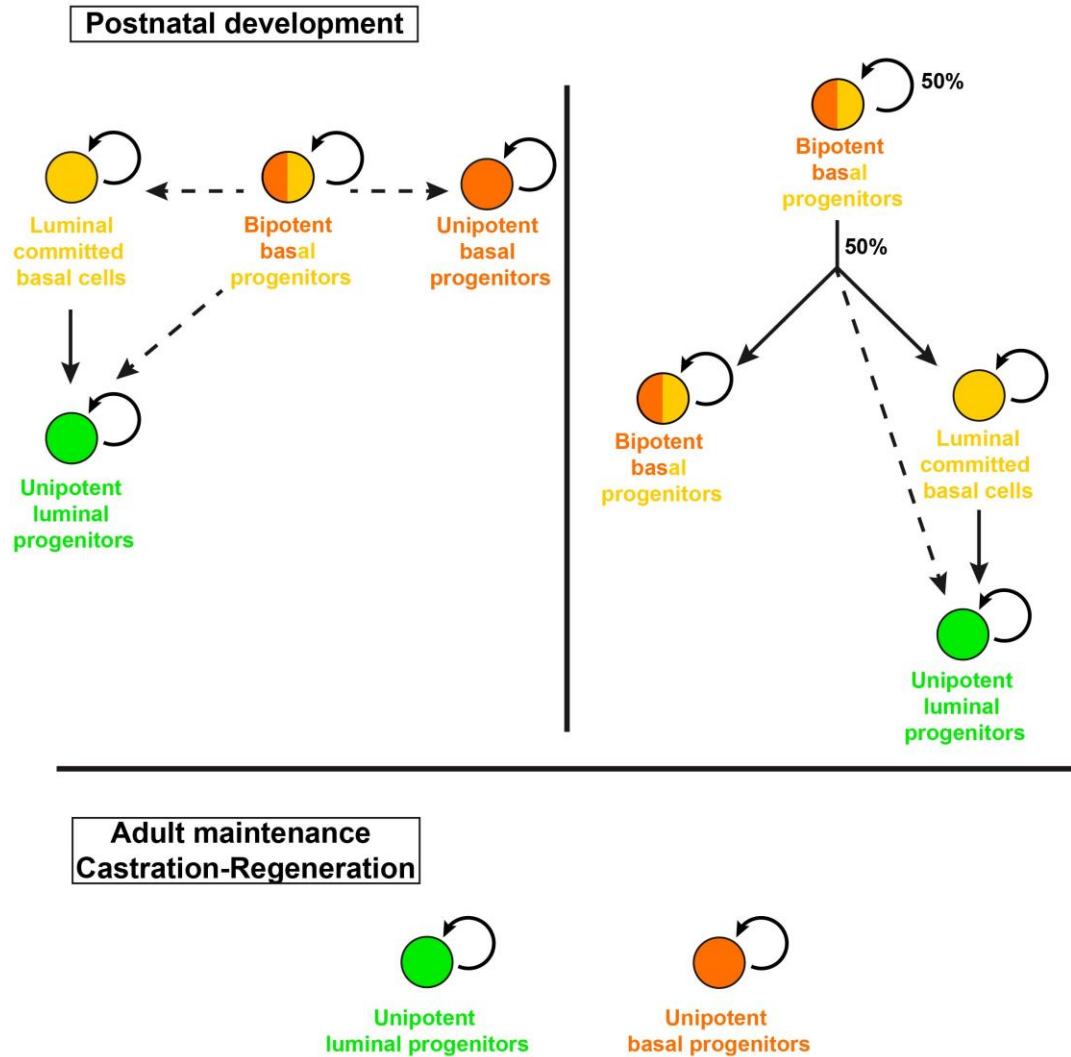
Deciphering the cellular hierarchy of prostate epithelium during development and adult regeneration



Marielle Ousset, PhD



Model for prostate postnatal development and homeostasis



Identification of Stem Cell Populations in Sweat Glands and Ducts Reveals Roles in Homeostasis and Wound Repair

Catherine P. Lu,² Lisa Polak,² Ana Sofia Rocha,³ H. Amalia Pasolli,² Shann-Ching Chen,⁴ Neha Sharma,³ Cedric Blanpain,³ and Elaine Fuchs^{1,2,*}

¹Howard Hughes Medical Institute

²Laboratory of Mammalian Cell Biology & Development

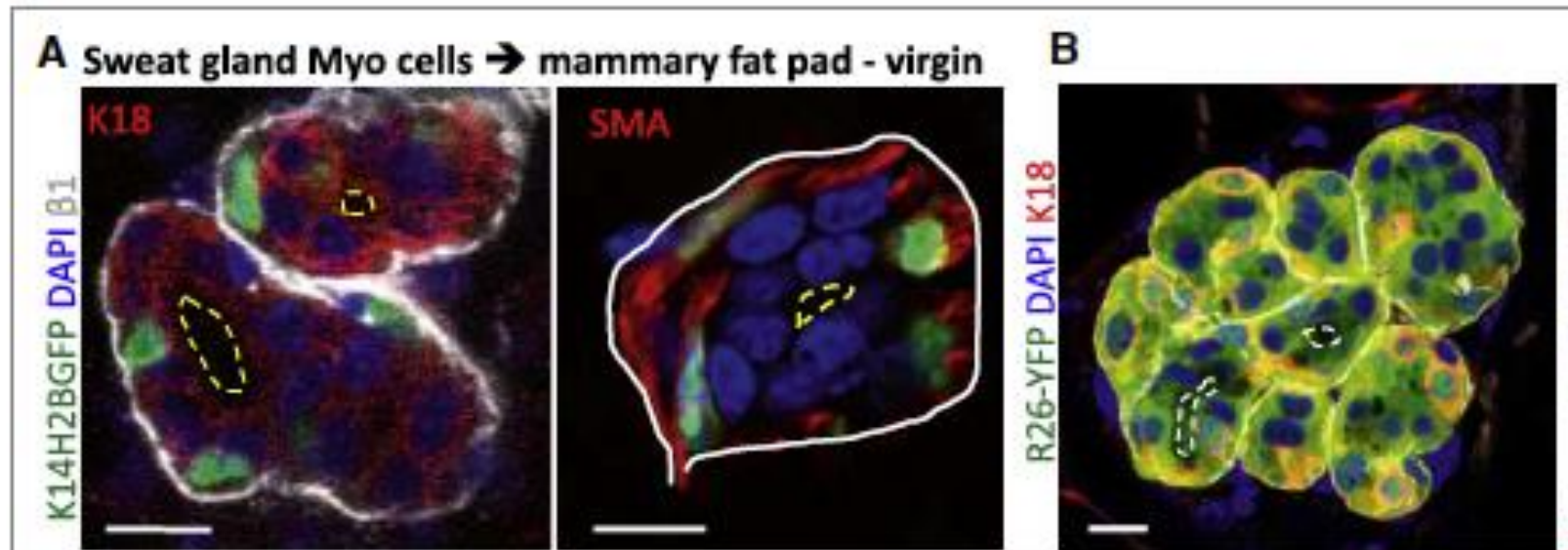
The Rockefeller University, New York, NY 10065, USA

³IRIBHM, Université Libre de Bruxelles, Brussels 1050, Belgium

⁴Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA

*Correspondence: fuchslb@rockefeller.edu

<http://dx.doi.org/10.1016/j.cell.2012.04.045>



Tracing the cancer cell of origin

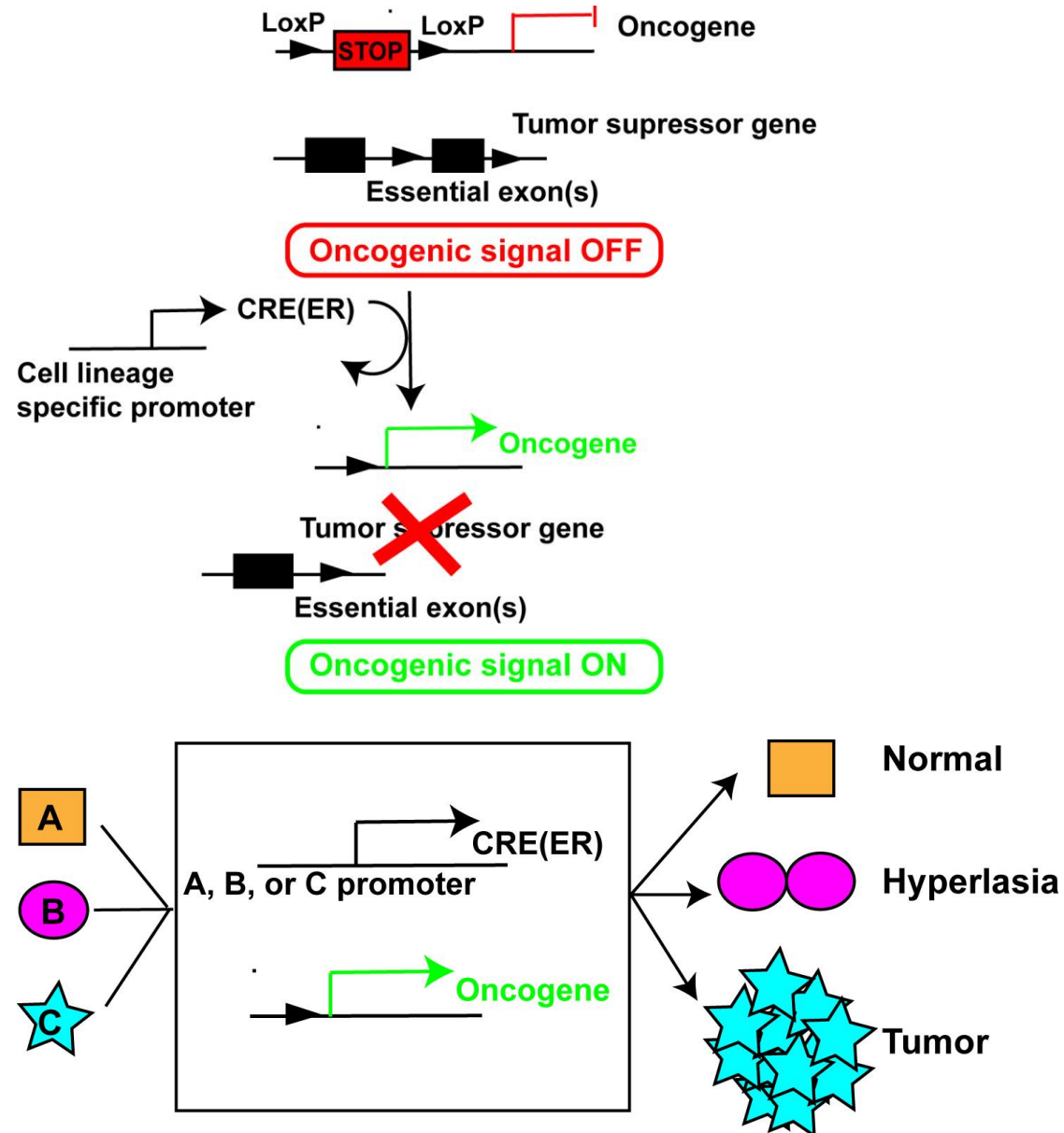
Khalil Kass Youseff



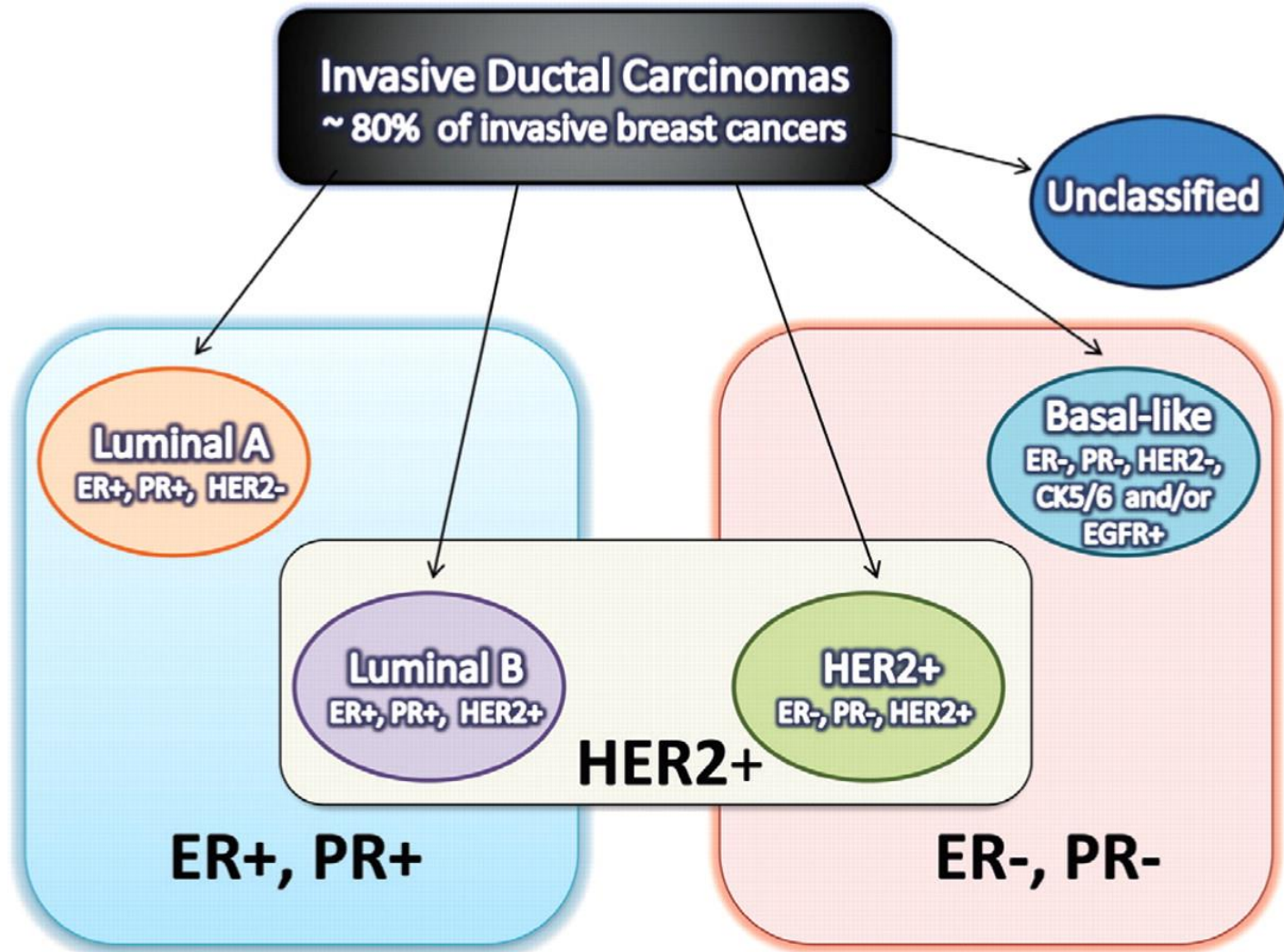
Gaelle Lapouge, PhD



Blanpain C. Nature Cell Biology 2013

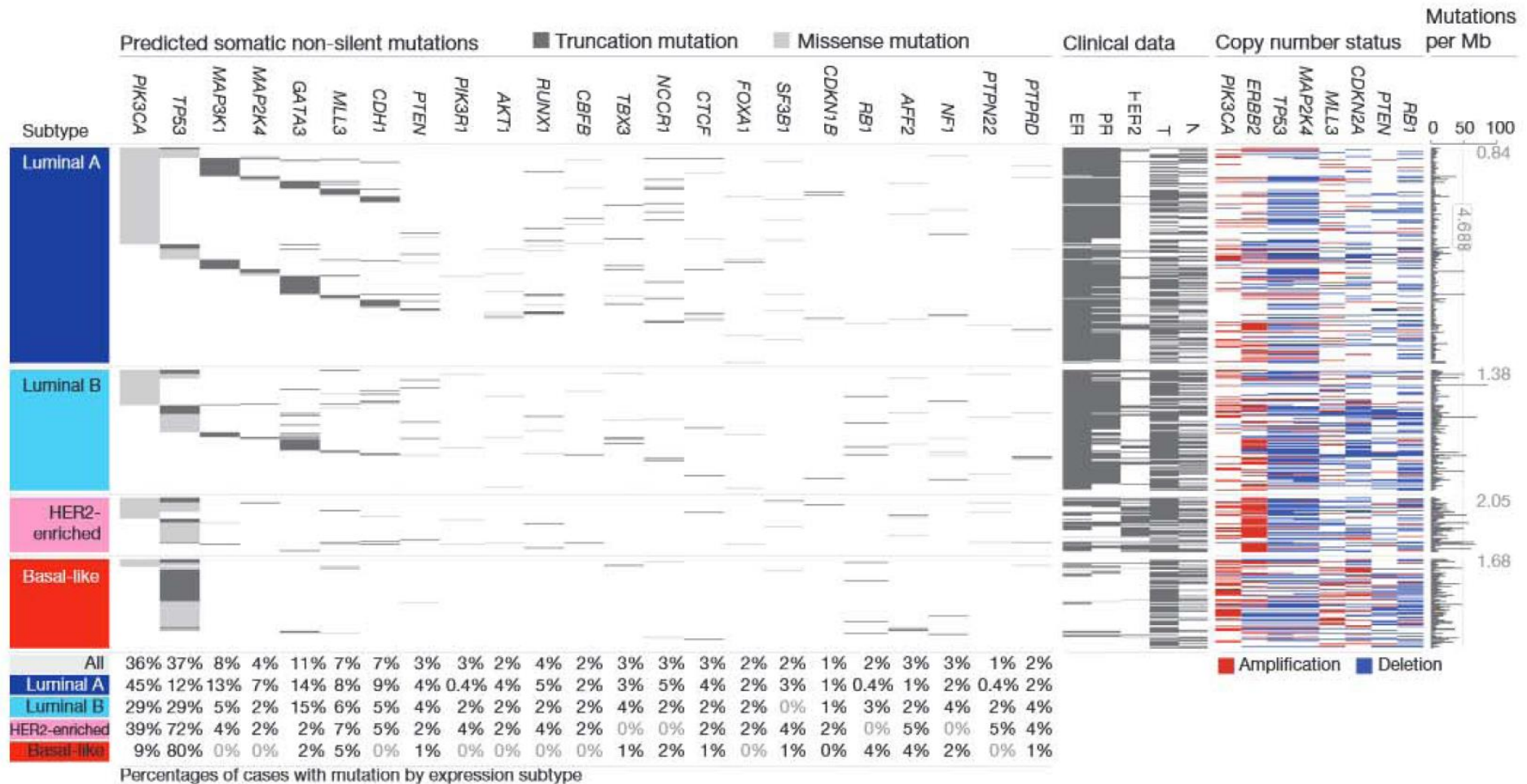


Breast cancers are heterogeneous



Comprehensive molecular portraits of human breast tumours

4 OCTOBER 2012 | VOL 490 | NATURE | 61



***BRCA1* Basal-like Breast Cancers Originate from Luminal Epithelial Progenitors and Not from Basal Stem Cells**

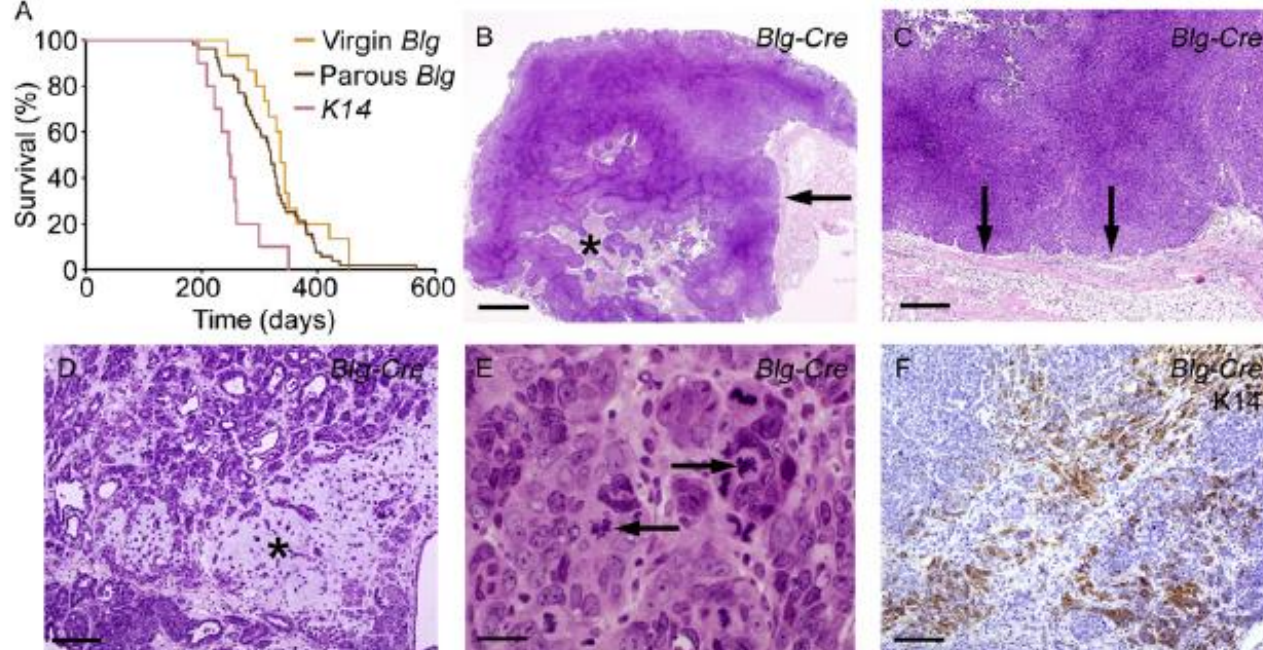
Gemma Molyneux,¹ Felipe C. Geyer,¹ Fiona-Ann Magnay,¹ Afshan McCarthy,¹ Howard Kendrick,¹ Rachael Natrajan,¹ Alan MacKay,¹ Anita Grigoriadis,² Andrew Tutt,² Alan Ashworth,¹ Jorge S. Reis-Filho,¹ and Matthew J. Smalley^{1,*}

¹The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK

²Breakthrough Breast Cancer Research Unit, Guy's Hospital, King's Health Partners AHSC, London SE1 9RT, UK

*Correspondence: matthew.smalley@icr.ac.uk

DOI 10.1016/j.stem.2010.07.010

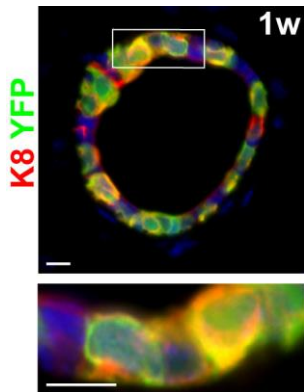
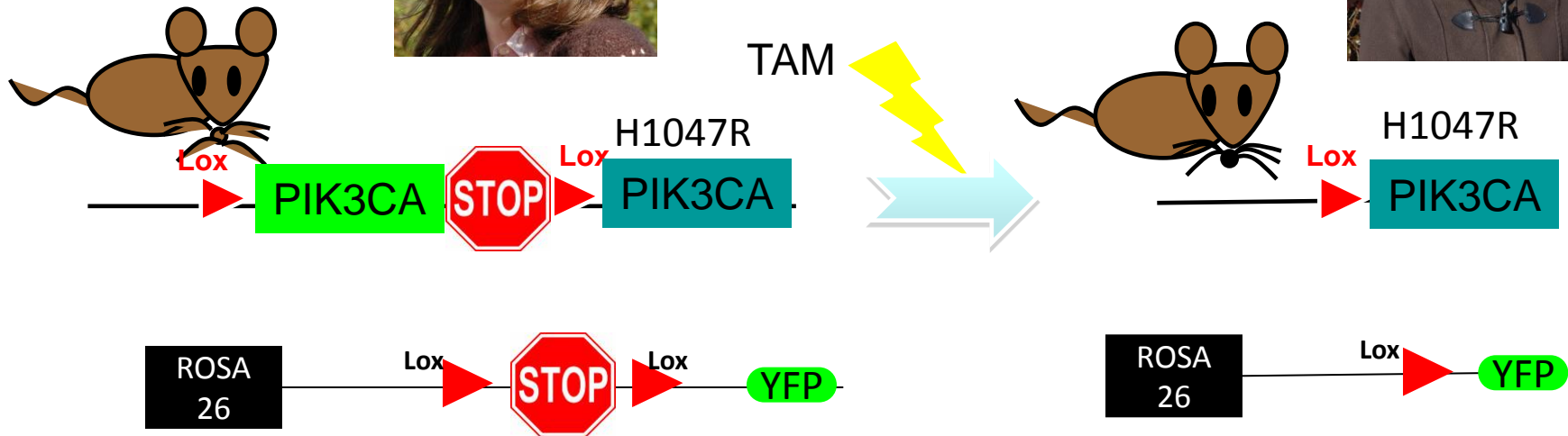


Defining the cellular origin of PIK3CA Induced mammary tumours

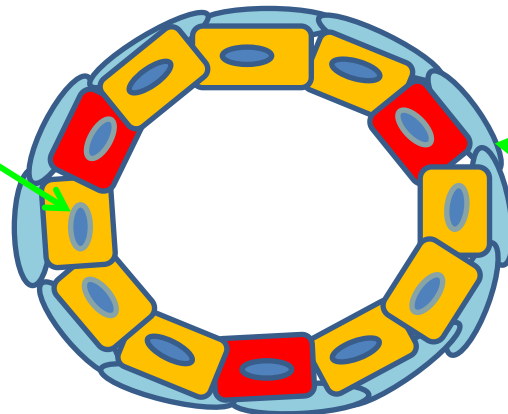
Alexandra Van Keymeulen, PhD



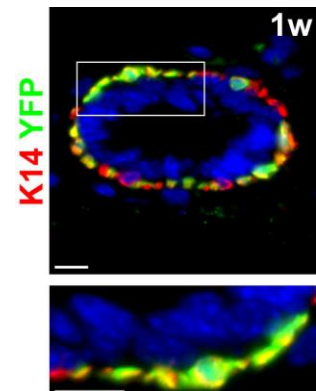
May Yin Lee, PhD



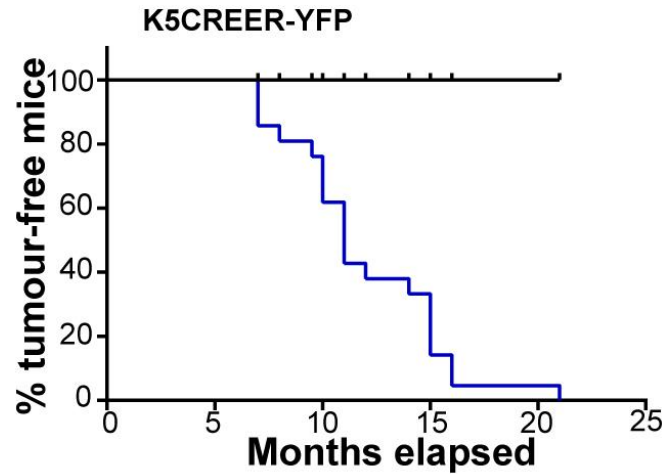
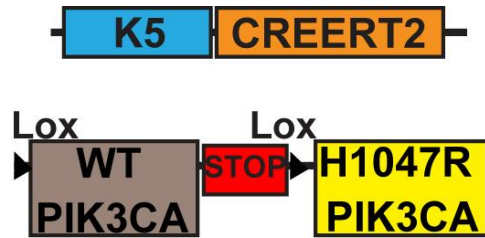
Luminal cells
K8CREER



Basal cells
K5CREER

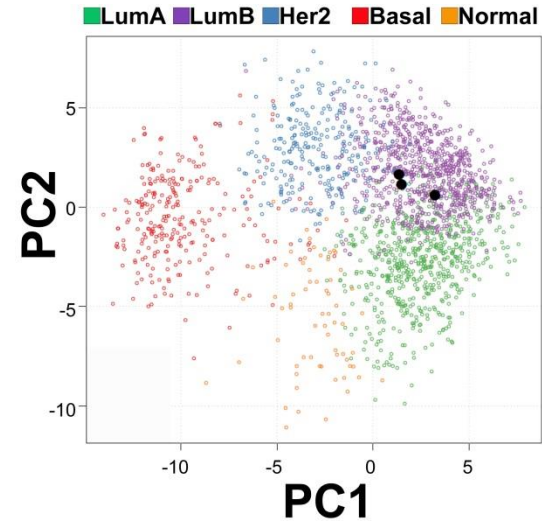
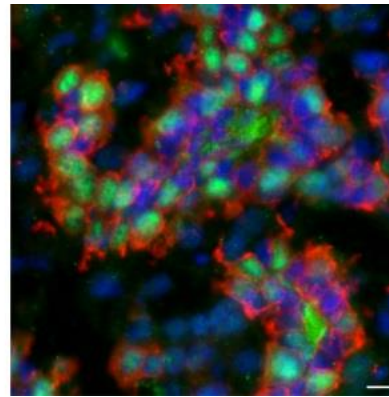
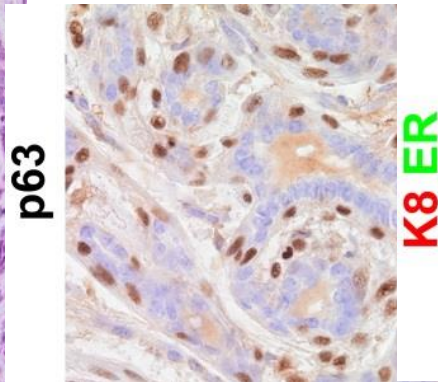
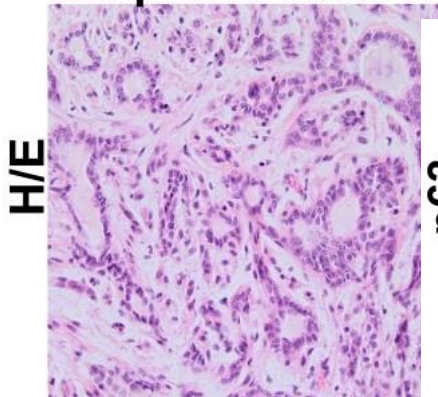


Basal cells give rise to luminal-like mammary tumors upon expression of oncogenic PIK3CA

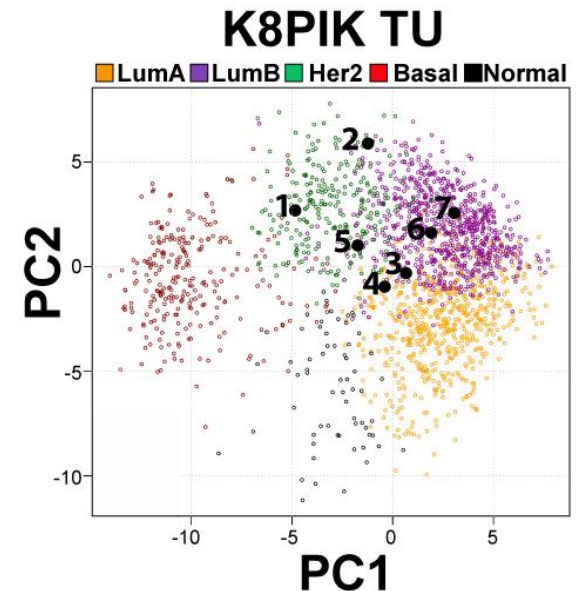
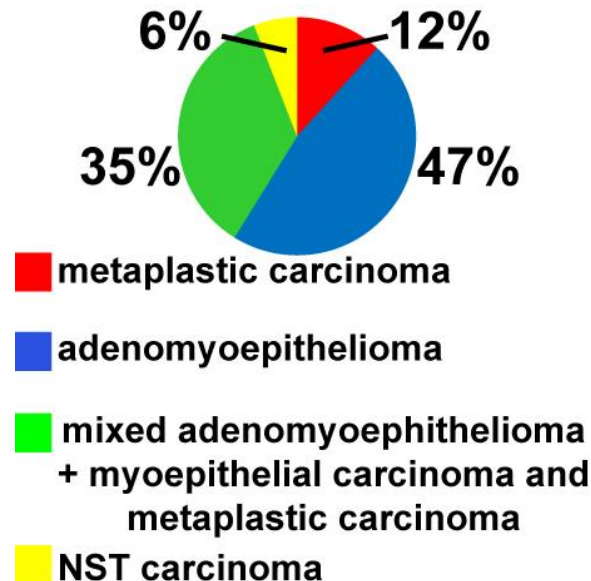
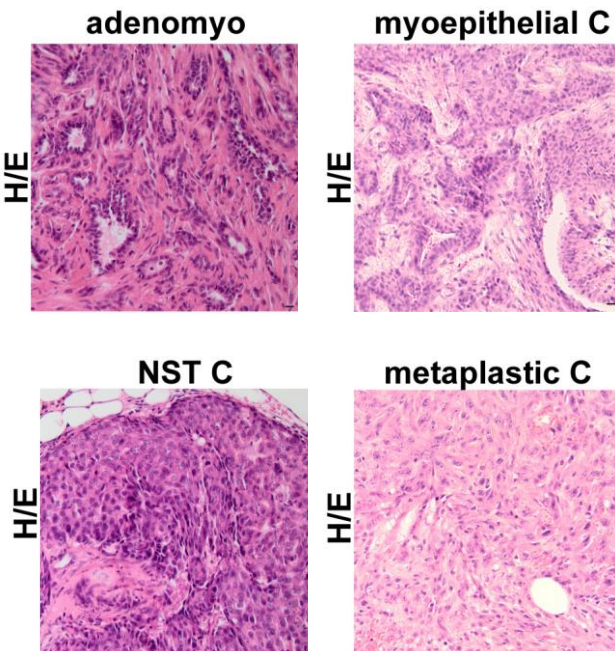
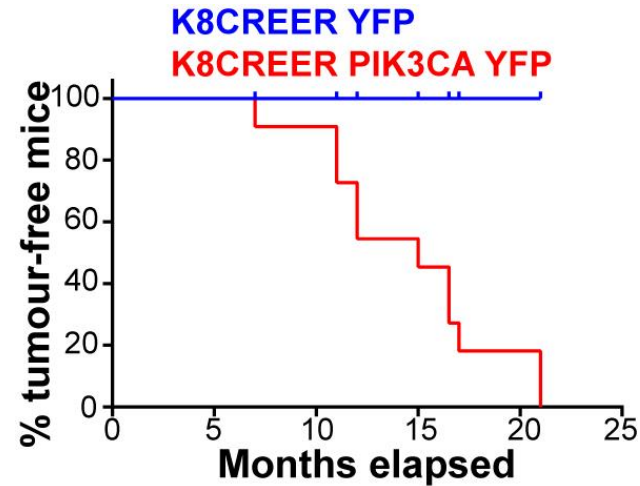
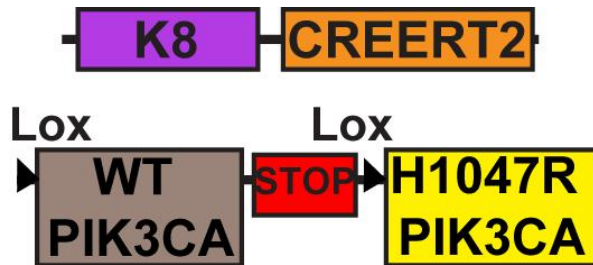


100%
Adenomyo-
epithelioma

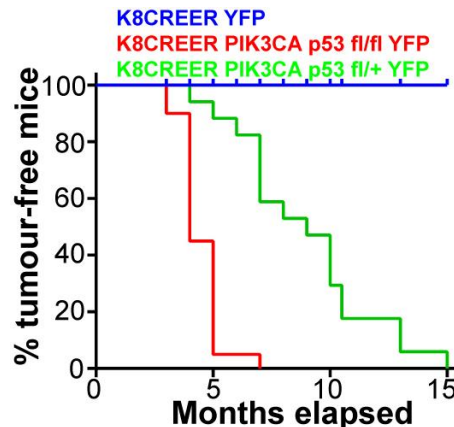
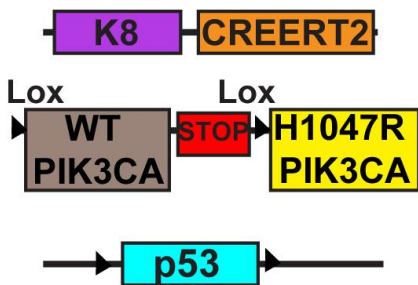
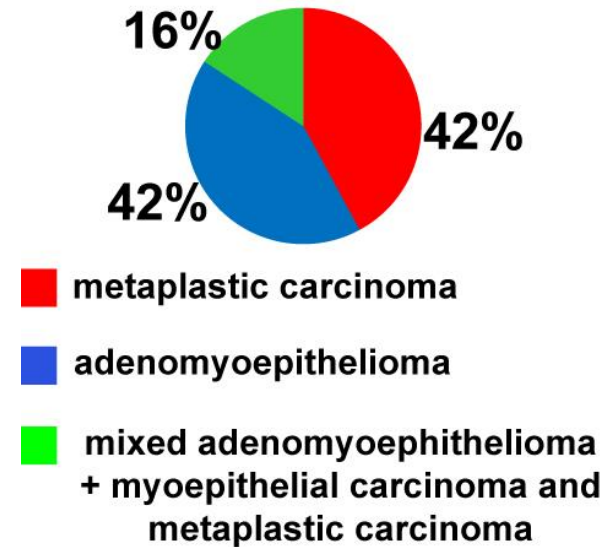
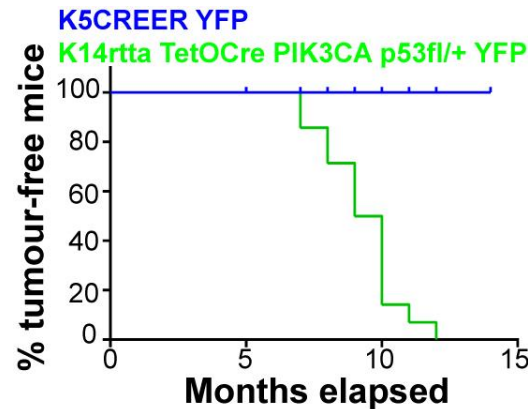
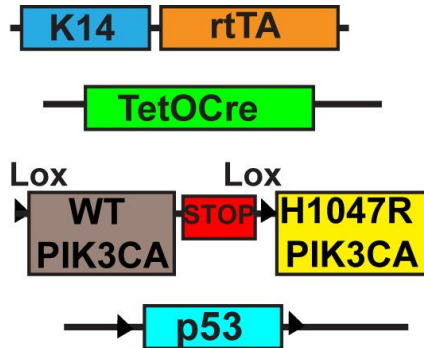
adenomyo-
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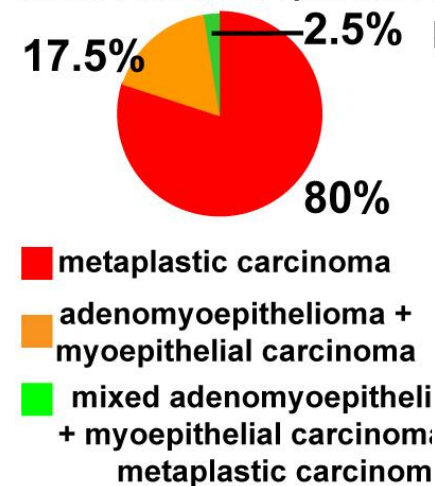
Luminal cells give rise to heterogeneous mammary tumors upon expression of oncogenic PIK3CA



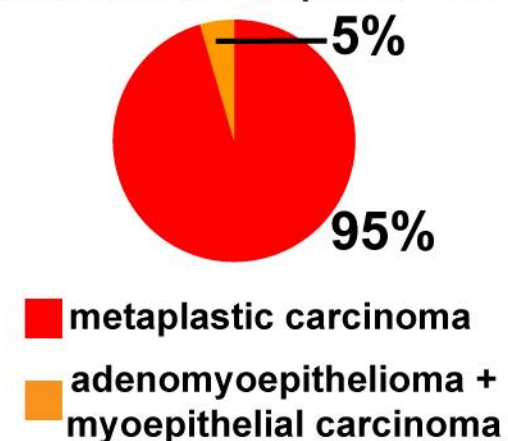
Luminal cells give rise to more aggressive breast tumors following oncogenic PIK3CA and p53 deletion



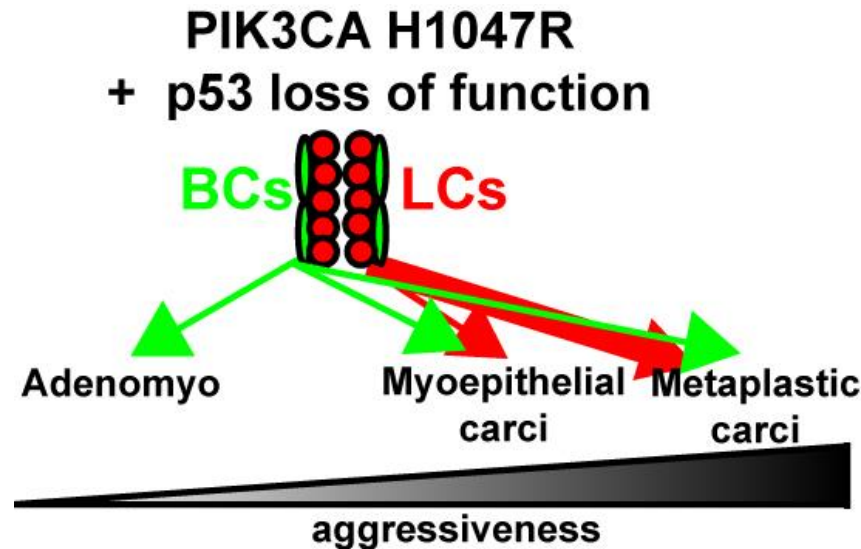
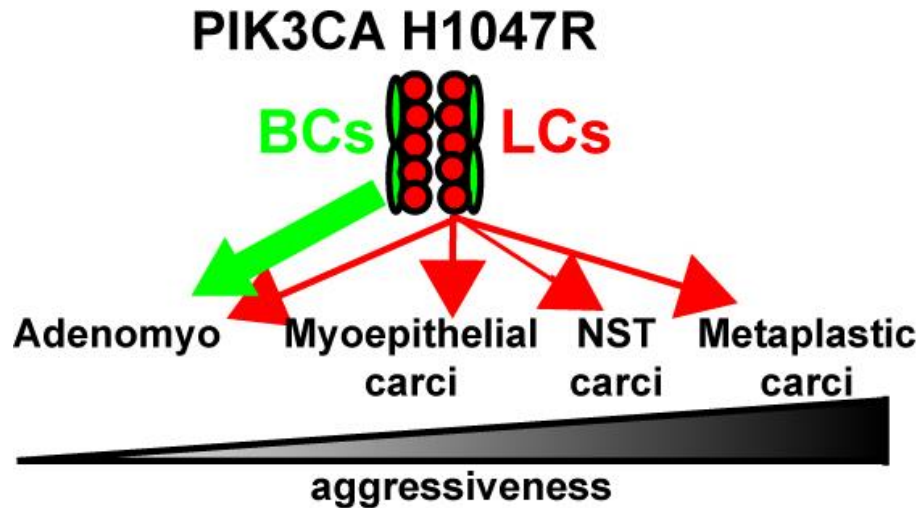
K8CREER PIK3CA p53 fl/fl YFP



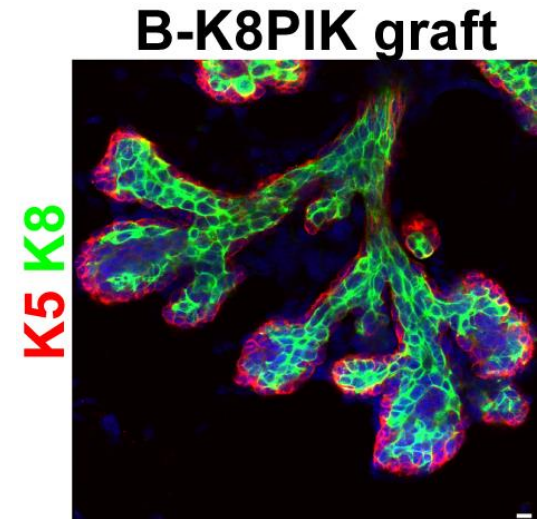
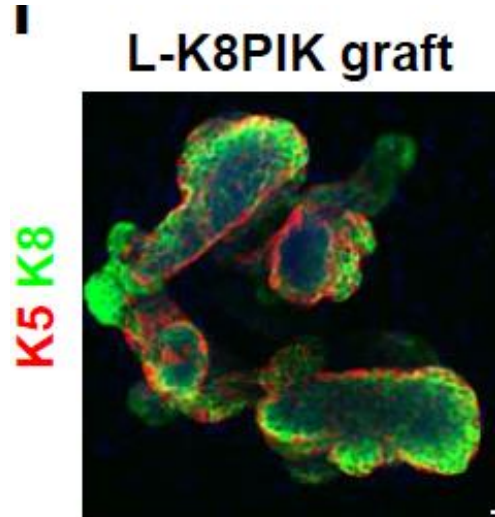
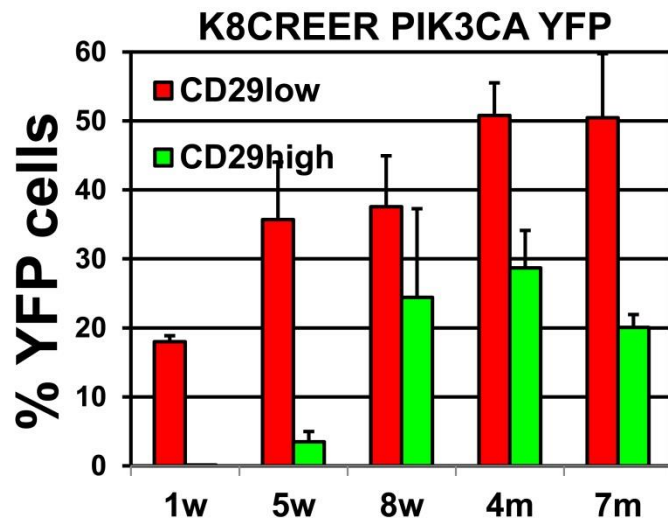
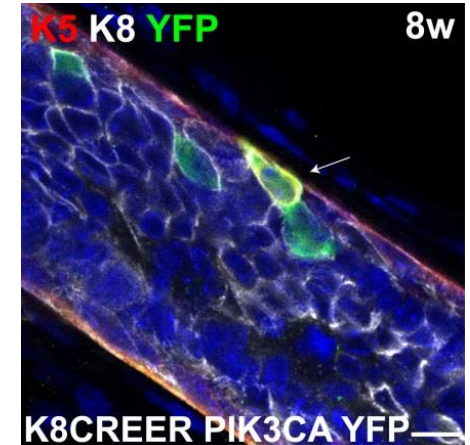
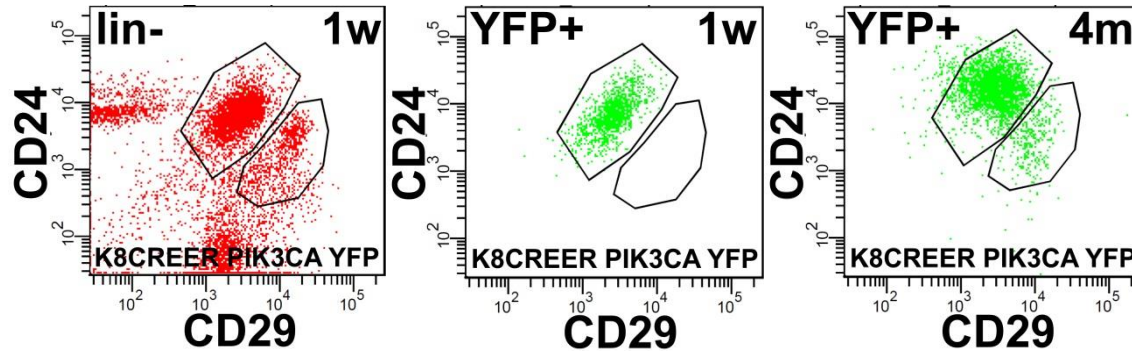
K8CREER PIK3CA p53 fl/+ YFP



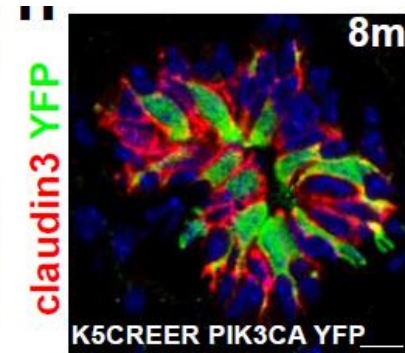
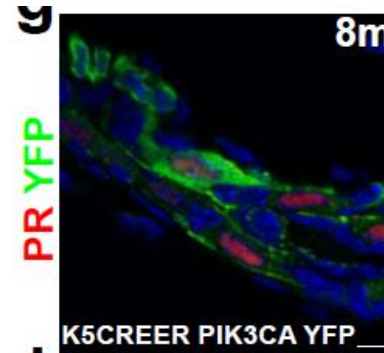
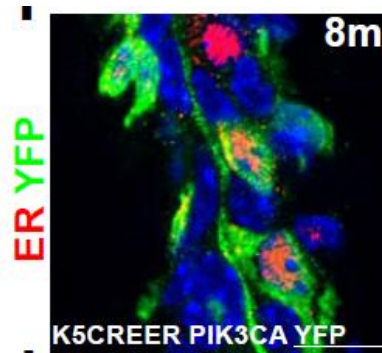
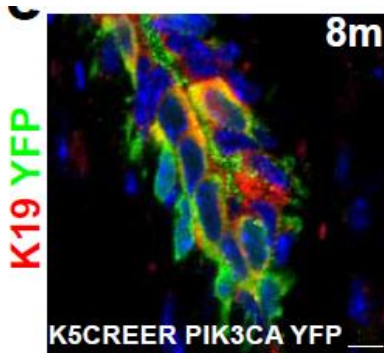
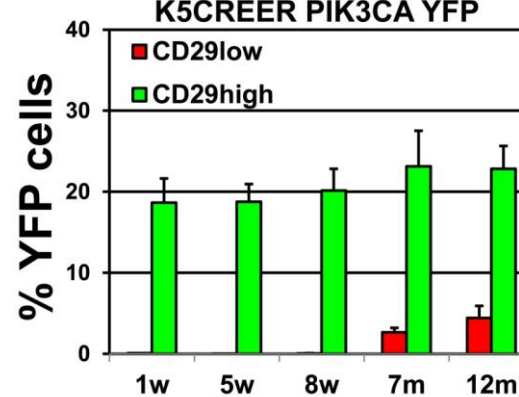
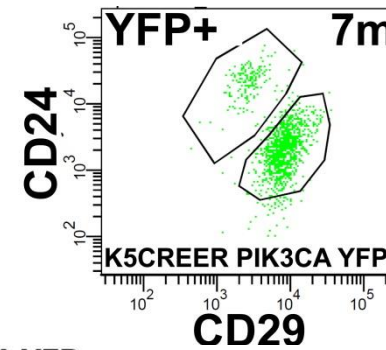
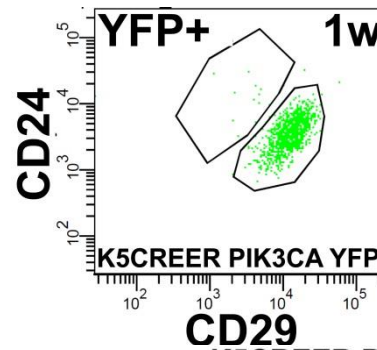
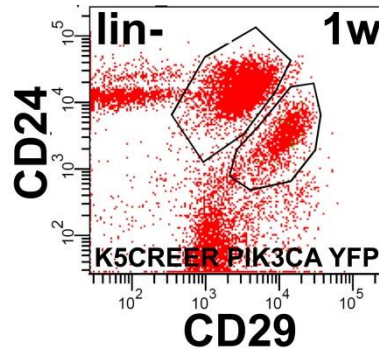
Cancer cell of origin controls tumor heterogeneity in breast tumors



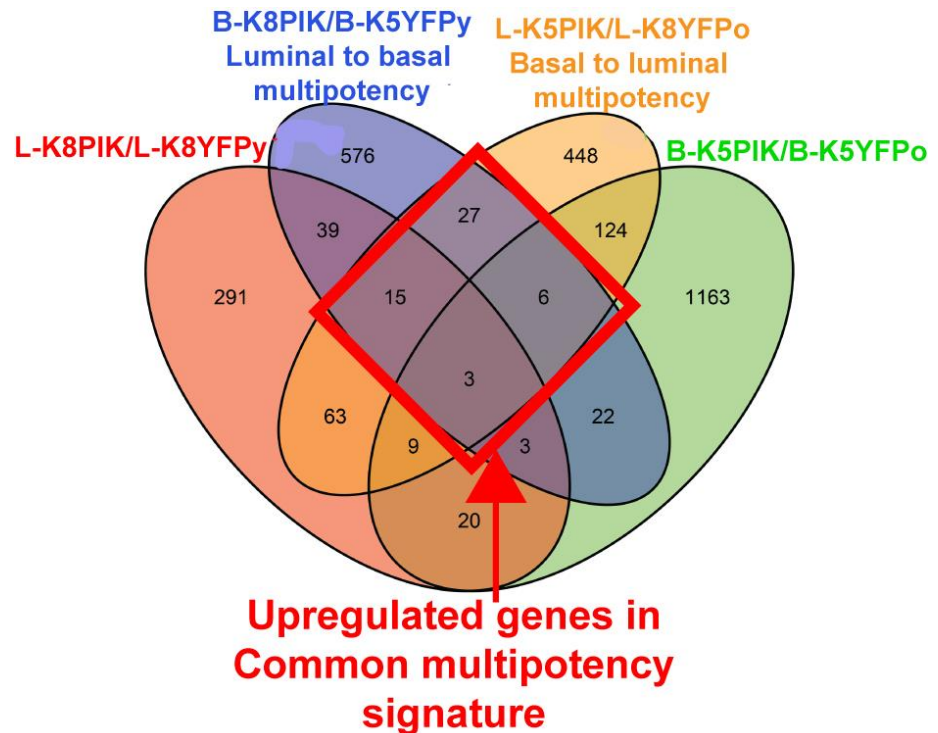
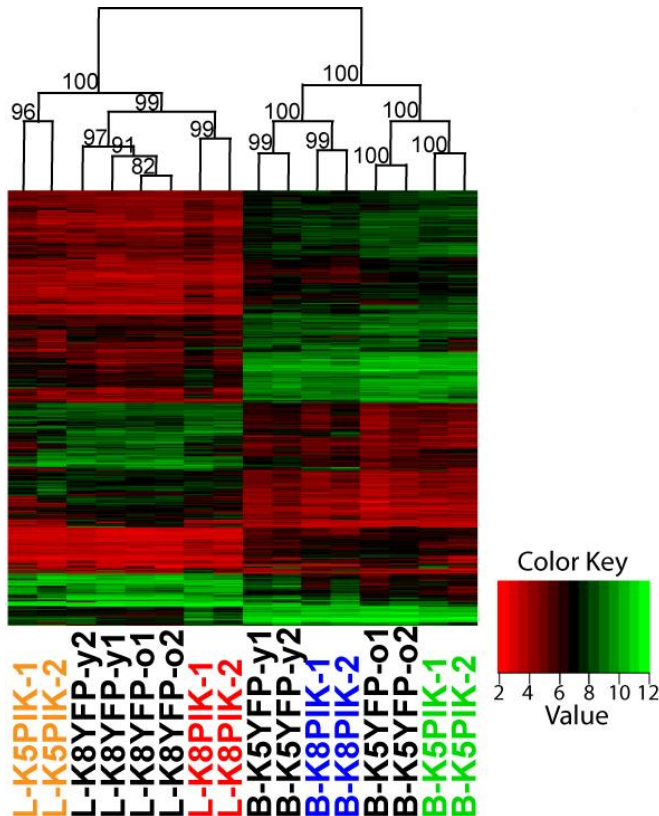
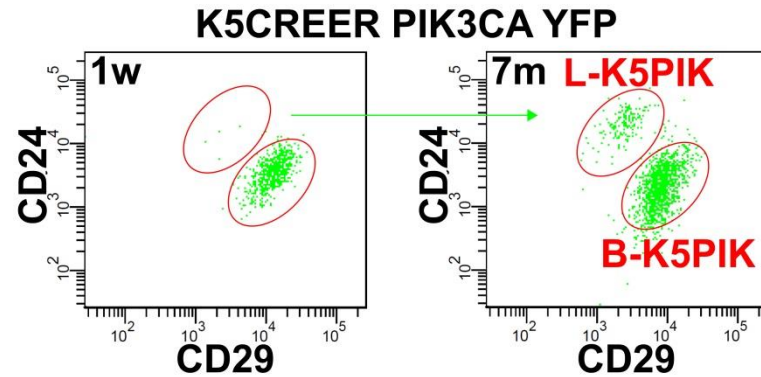
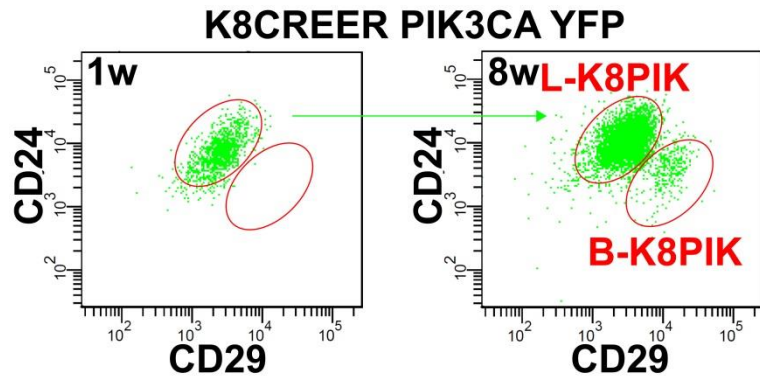
Oncogenic PIK3CA induces multipotency in unipotent luminal progenitors



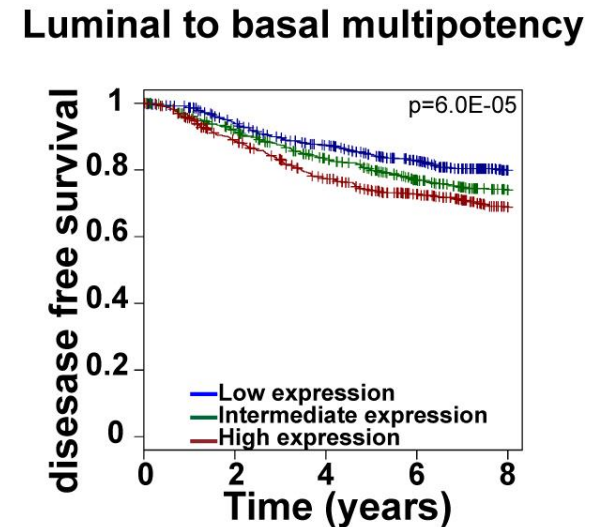
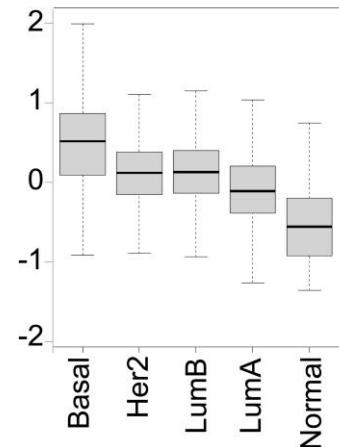
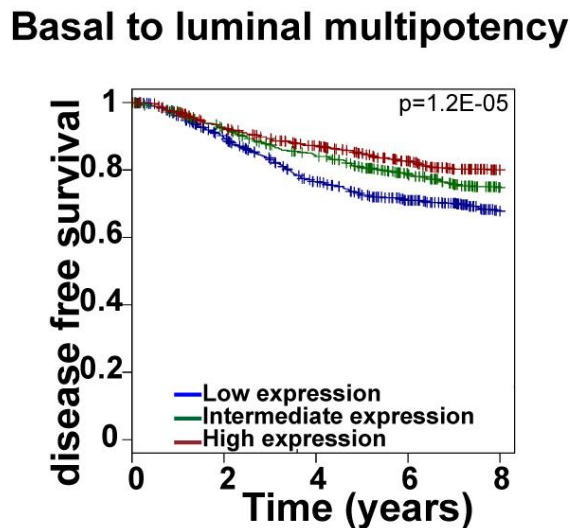
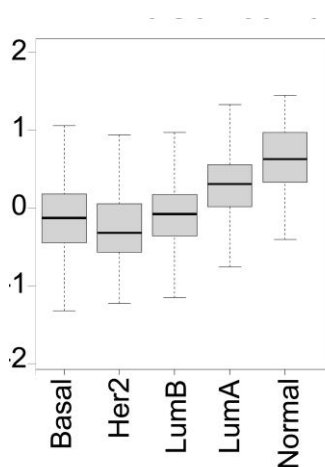
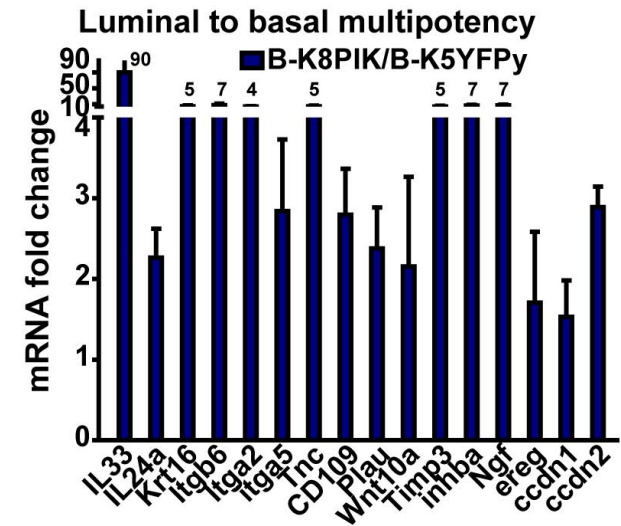
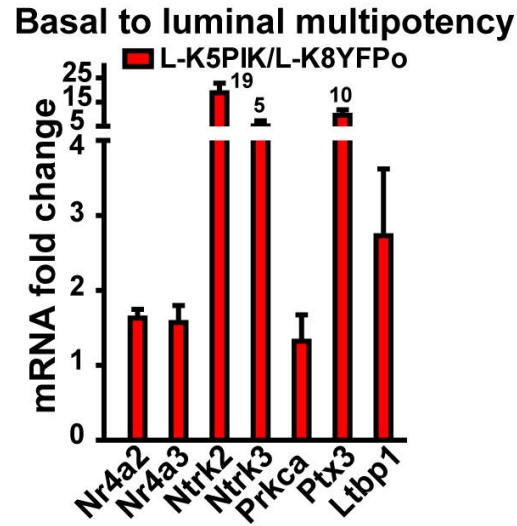
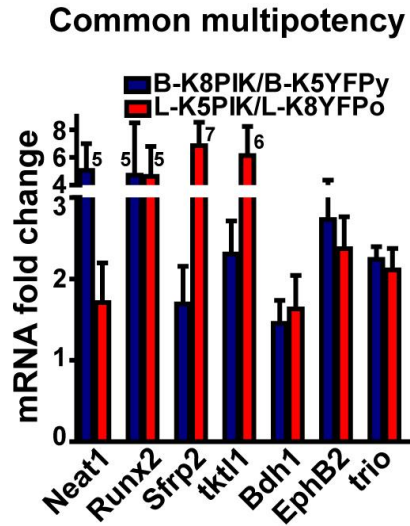
Oncogenic PIK3CA induces multipotency in unipotent basal progenitors



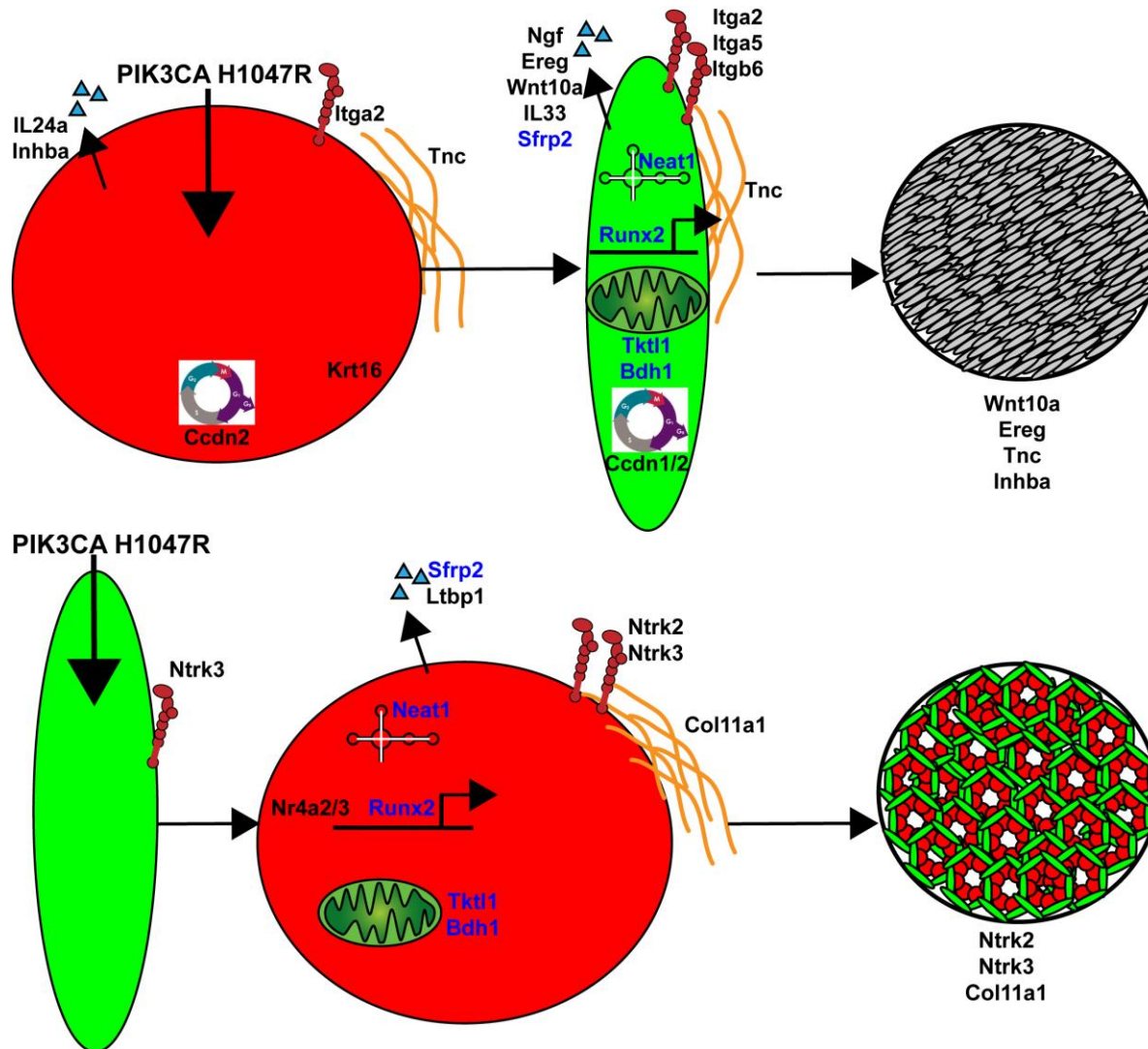
Defining the mechanisms mediating PIK3CA induced multipotency



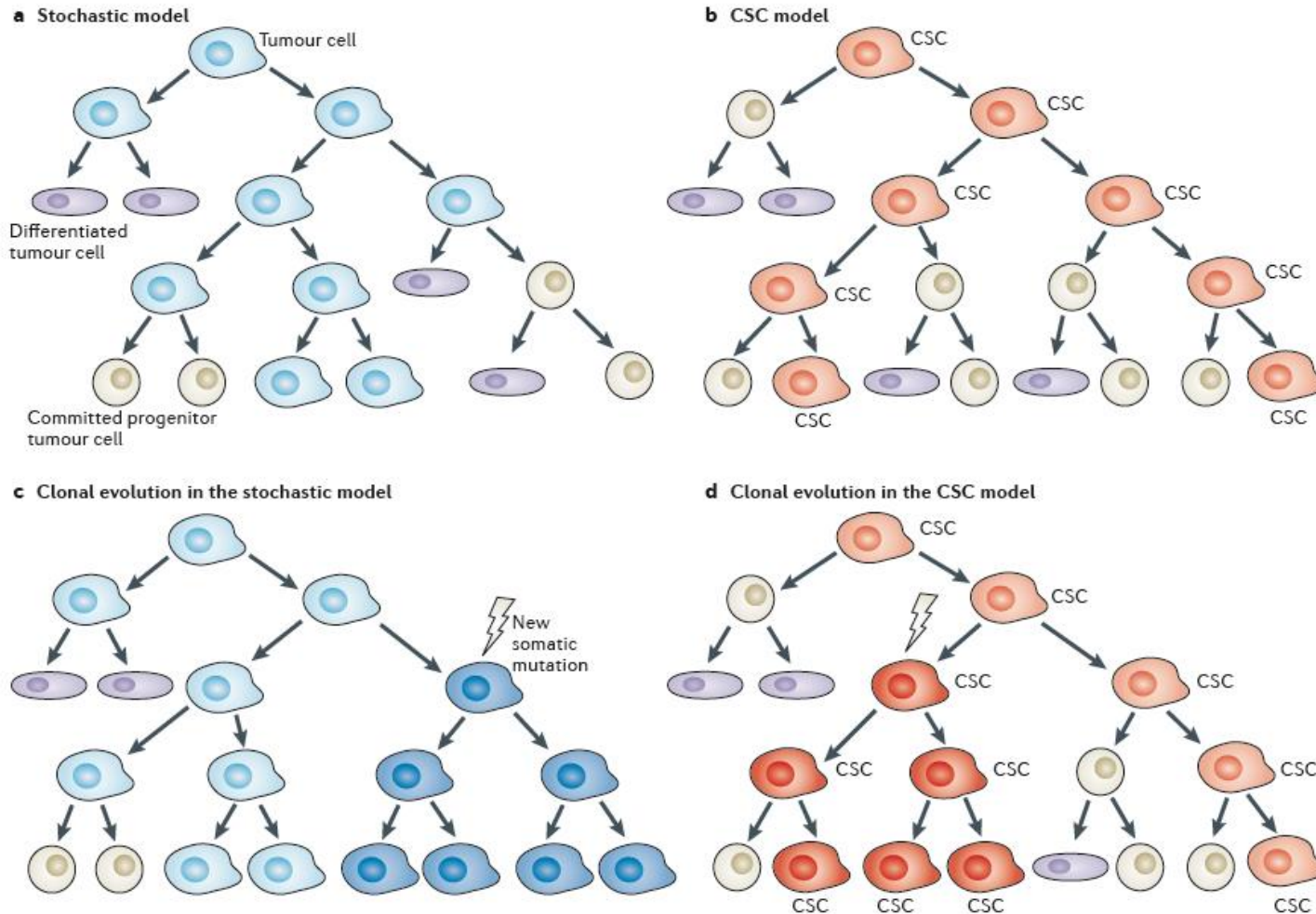
Multipotent signatures correlate with the types of breast cancers and patient outcome



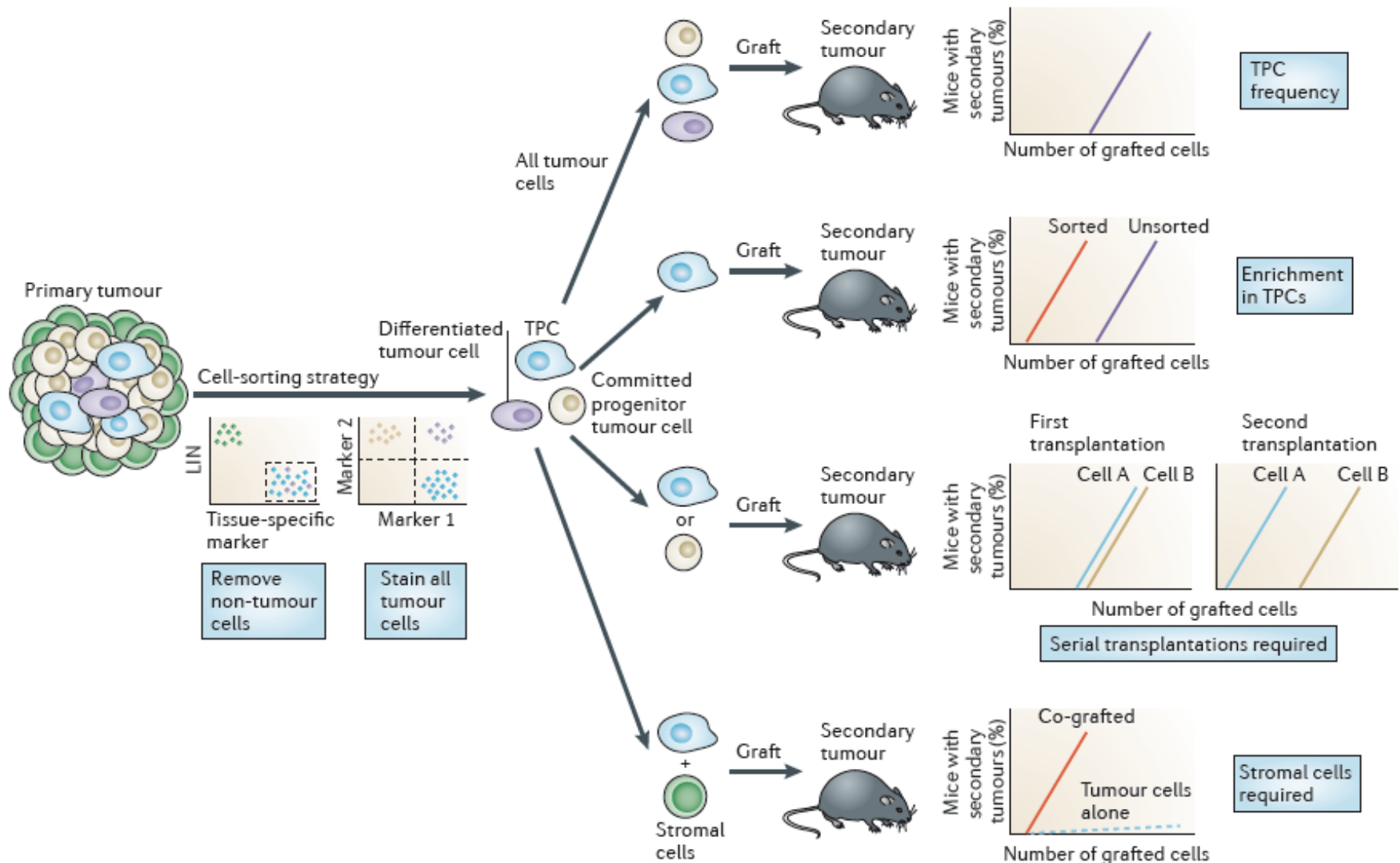
Mechanisms regulating oncogene induced multipotency in the mammary gland



Do breast cancer contain cancer stem cells?



Assessing cancer stem cell potential by transplantation assays

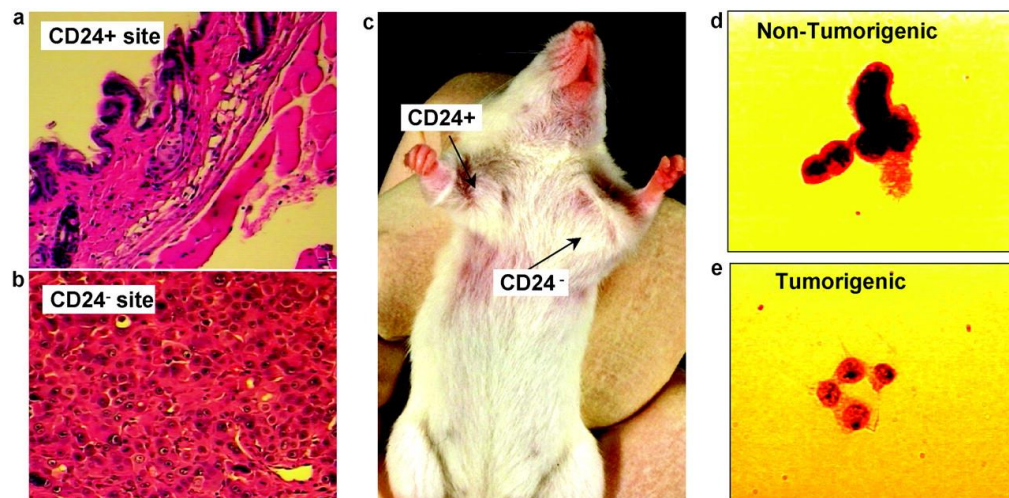


Prospective identification of tumorigenic breast cancer cells

Muhammad Al-Hajj*, Max S. Wicha*, Adalberto Benito-Hernandez[†], Sean J. Morrison^{**§}, and Michael F. Clarke^{**¶}

Departments of *Internal Medicine and [†]Pathology, Comprehensive Cancer Center, [‡]Department of Developmental Biology, and [§]Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, MI 48109

Communicated by Jack E. Dixon, University of Michigan Medical School, Ann Arbor, MI, January 16, 2003 (received for review December 18, 2002)



ALDH1 Is a Marker of Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome

Christophe Ginestier,¹ Min Hee Hur,² Emmanuelle Charafe-Jauffret,³ Florence Monville,³ Julie Dutcher,¹ Marty Brown,¹ Jocelyne Jacquemier,³ Patrice Viens,³ Celina G. Kleer,¹ Suling Liu,¹ Anne Schott,¹ Dan Hayes,¹ Daniel Birnbaum,³ Max S. Wicha,¹ and Gabriela Dontu^{1,*}

¹Department of Internal Medicine, Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI 48109, USA

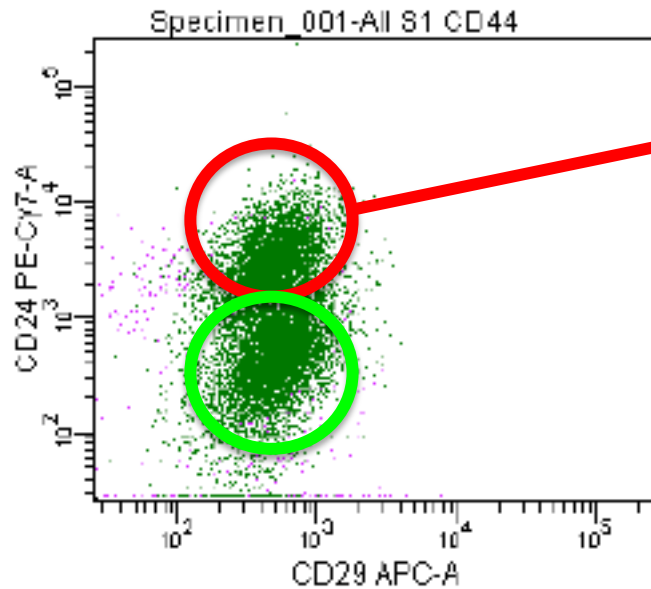
²Cheil General Hospital, Sungkyunkwan University, Seoul 100-380, South Korea

³Laboratoire d'Oncologie Moléculaire, Centre de Recherche en Cancérologie de Marseille, UMR599 Inserm/Institut Paoli-Calmettes, Marseille 13009, France

*Correspondence: gdontu@umich.edu

DOI 10.1016/j.stem.2007.08.014

CD24 marks TPCs in PI3KCA induced basal like breast tumors



CD24^{High} population

(Representative plot: CD29/CD24 profile)

**K8 PIK3CA^{het} p53^{fl/fl} Primary tumor
(n=4 tumors, 3 mice)**

CD24^{High}

Cell dose	# Transplant	Take rate	Frequency
10	6	2	1 in 45
100	6	5	(Range from 18 to 110)

CD24^{Low}

10	6	0	1 in 277
100	6	2	(Range from 70 to 1,100)

Perspectives for personalized medicine

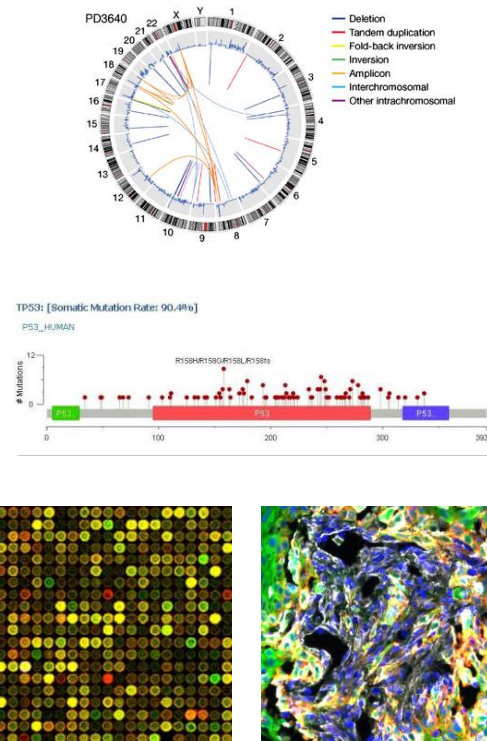
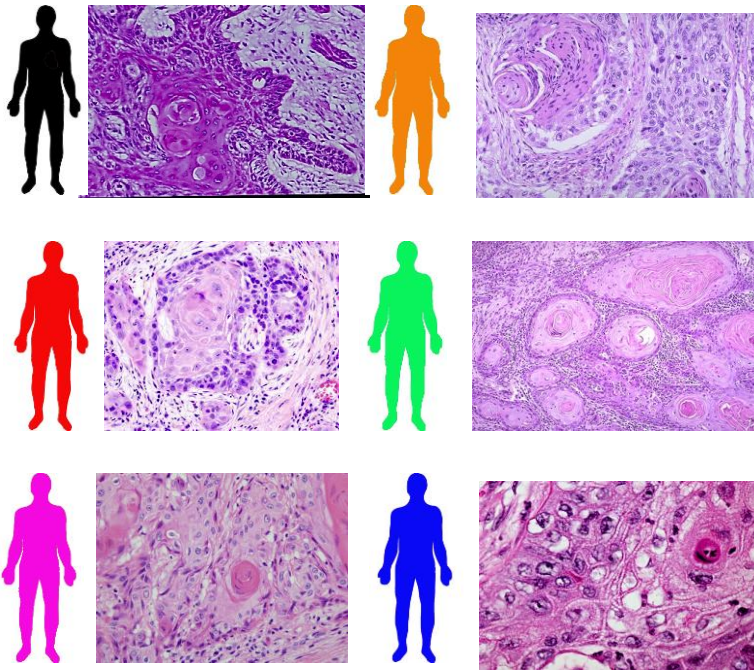
Patients primary tumors



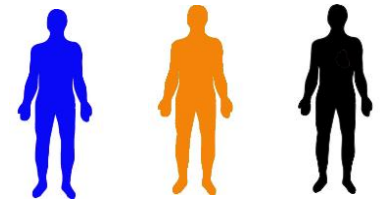
Molecular characterization
and biomarker expression



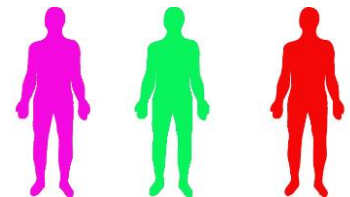
Prognosis and
patient
stratification



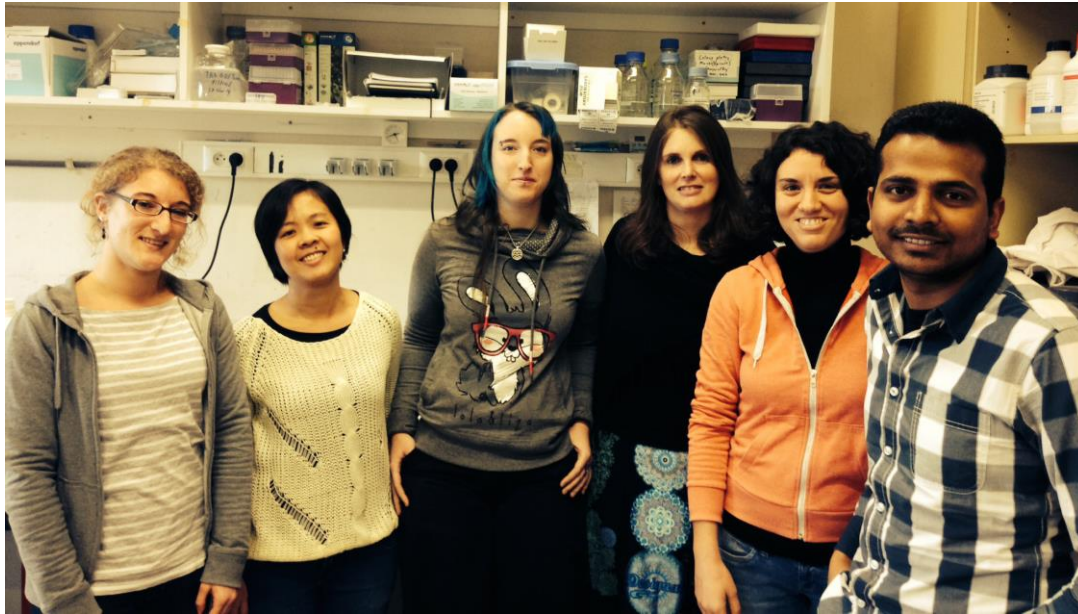
**-Good Prognosis
→ standard
therapy**



**-Bad Prognosis →
revised therapy**



Mammary stem cell group in the Blanpain Lab



Alexandra Van Keymeulen

May Yin Lee

Marielle Ousset

Gaëlle Bouvencourt

Raj Giraddi

Aline Wuidart

ULB



fnrs
LA LIBERTÉ DE CHERCHER



Collaborators

- Wayne Philipps, Peter MacCallum Cancer Centre
- Ben Simons, University of Cambridge