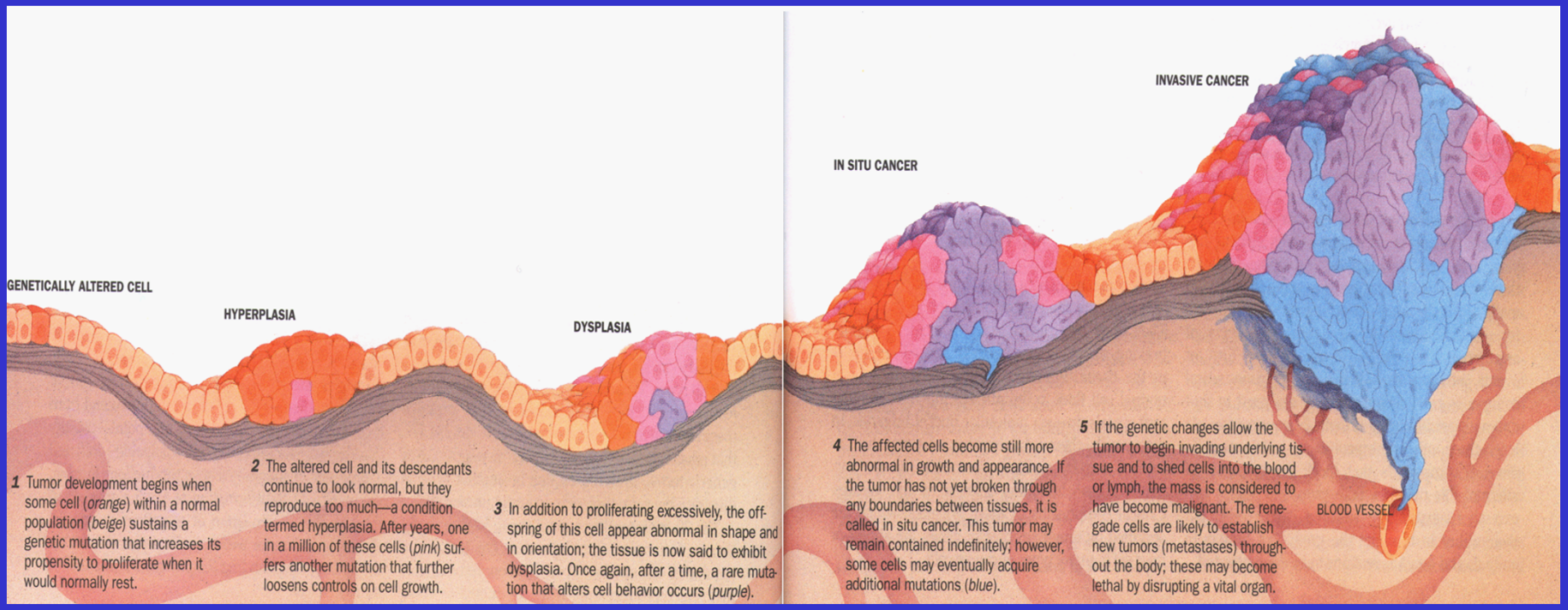
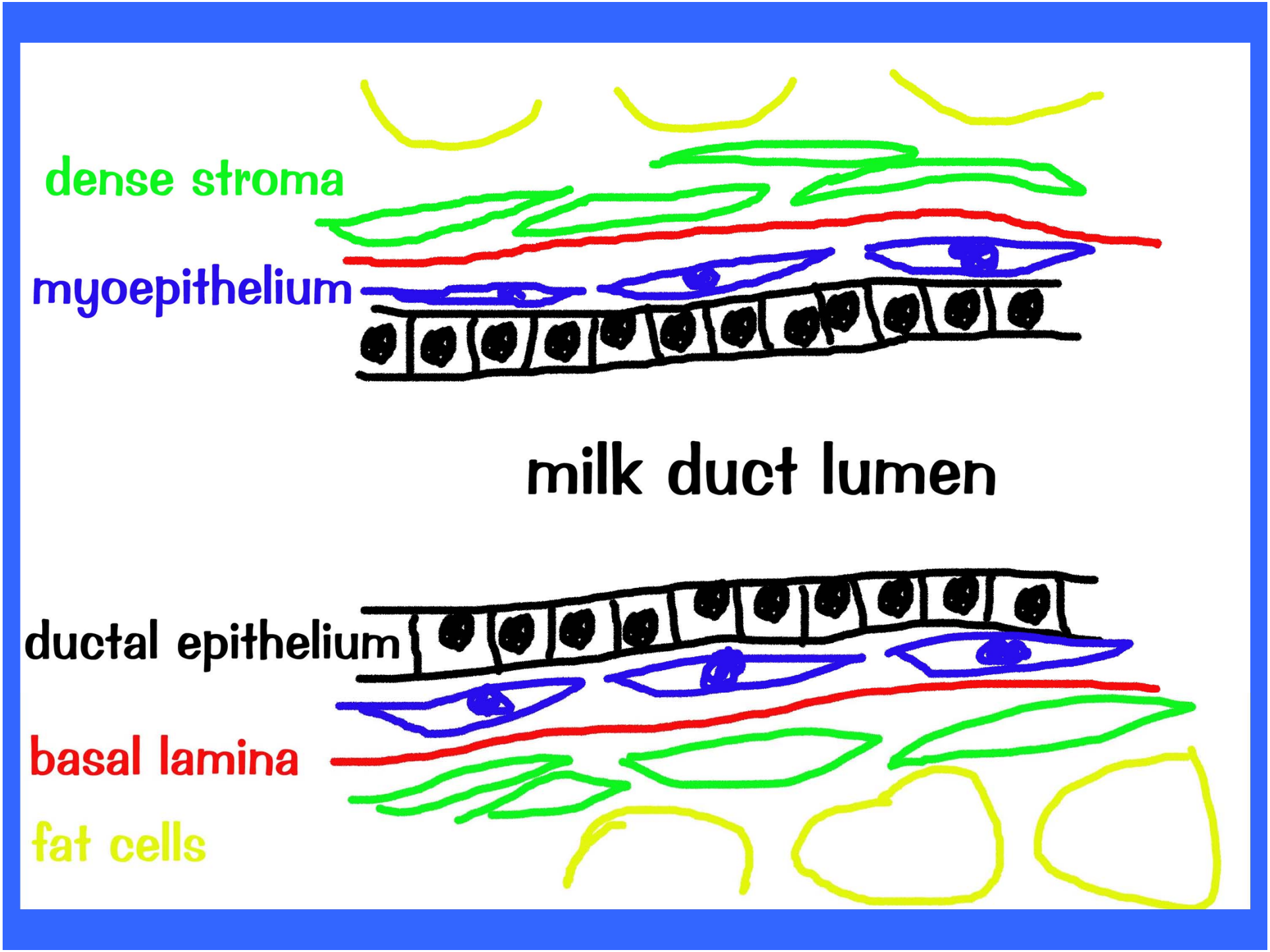
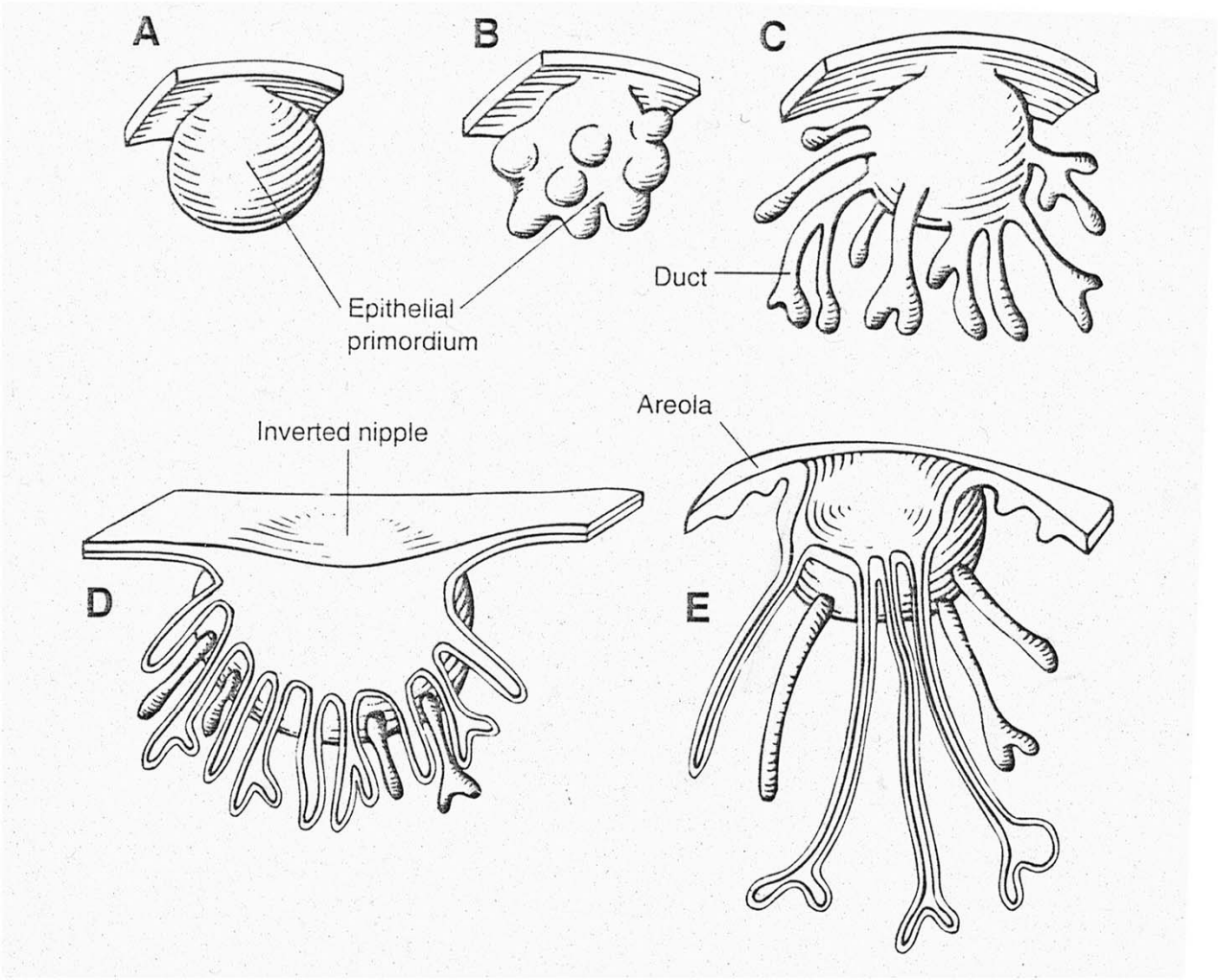
Tumor Progression

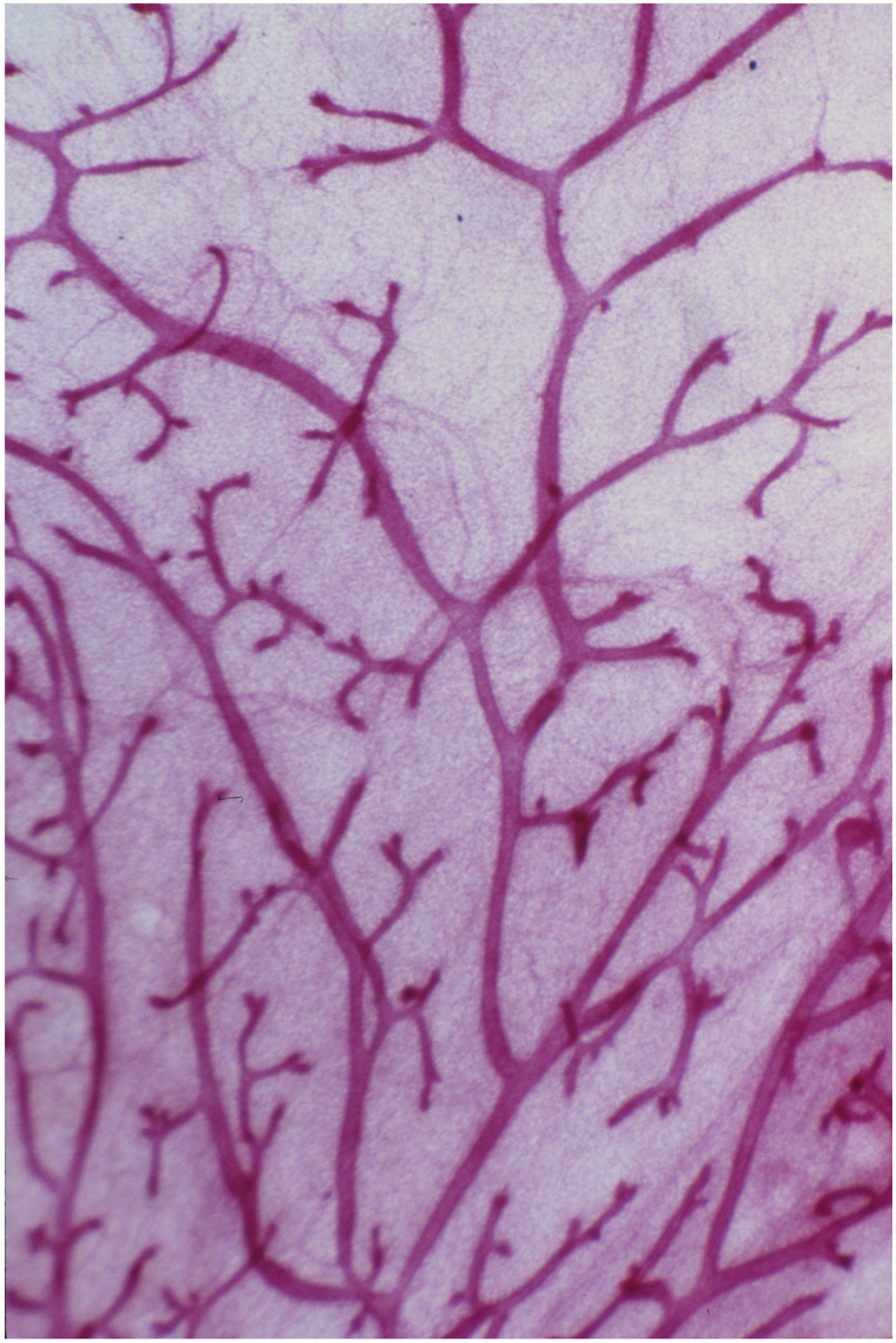


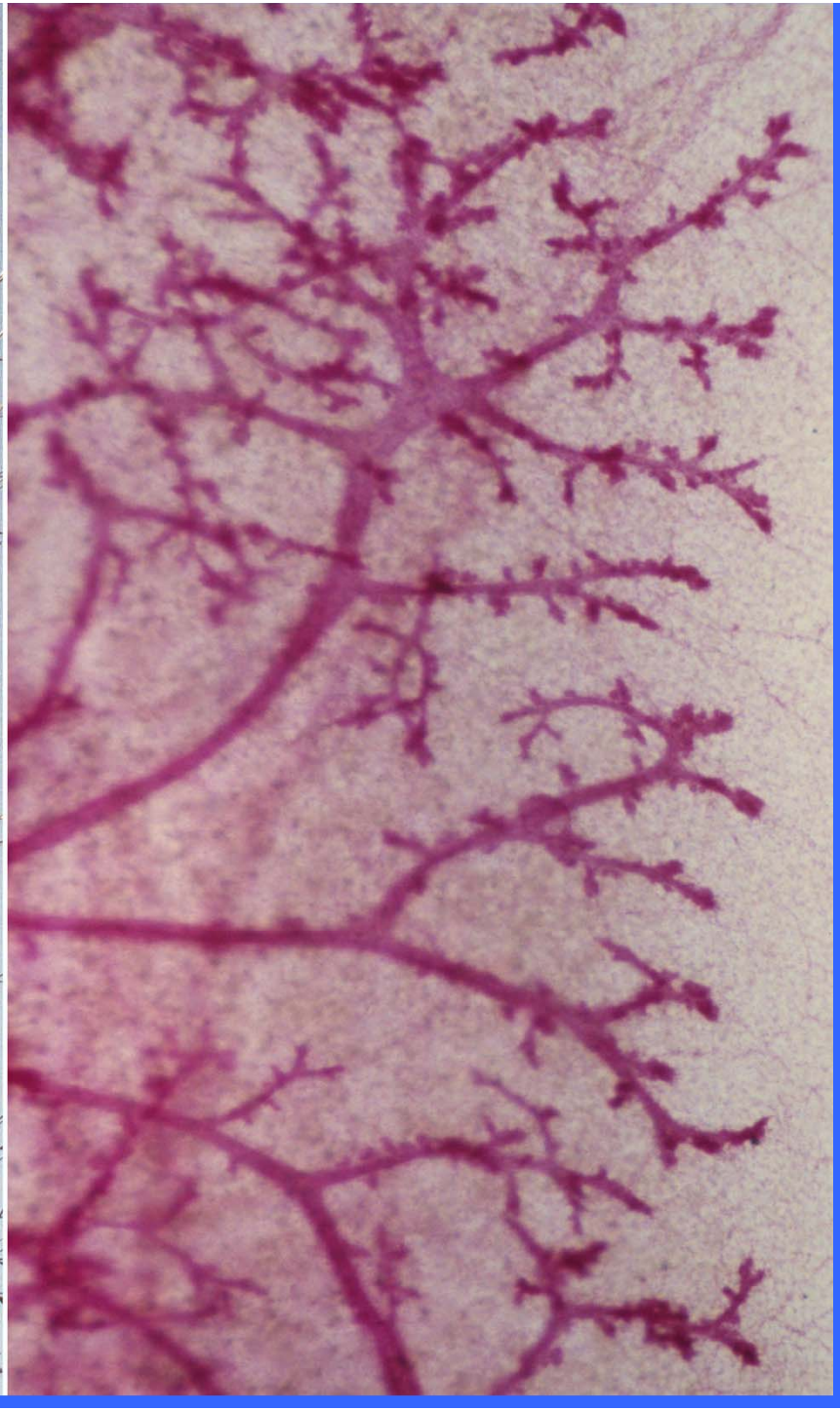
Scientific American

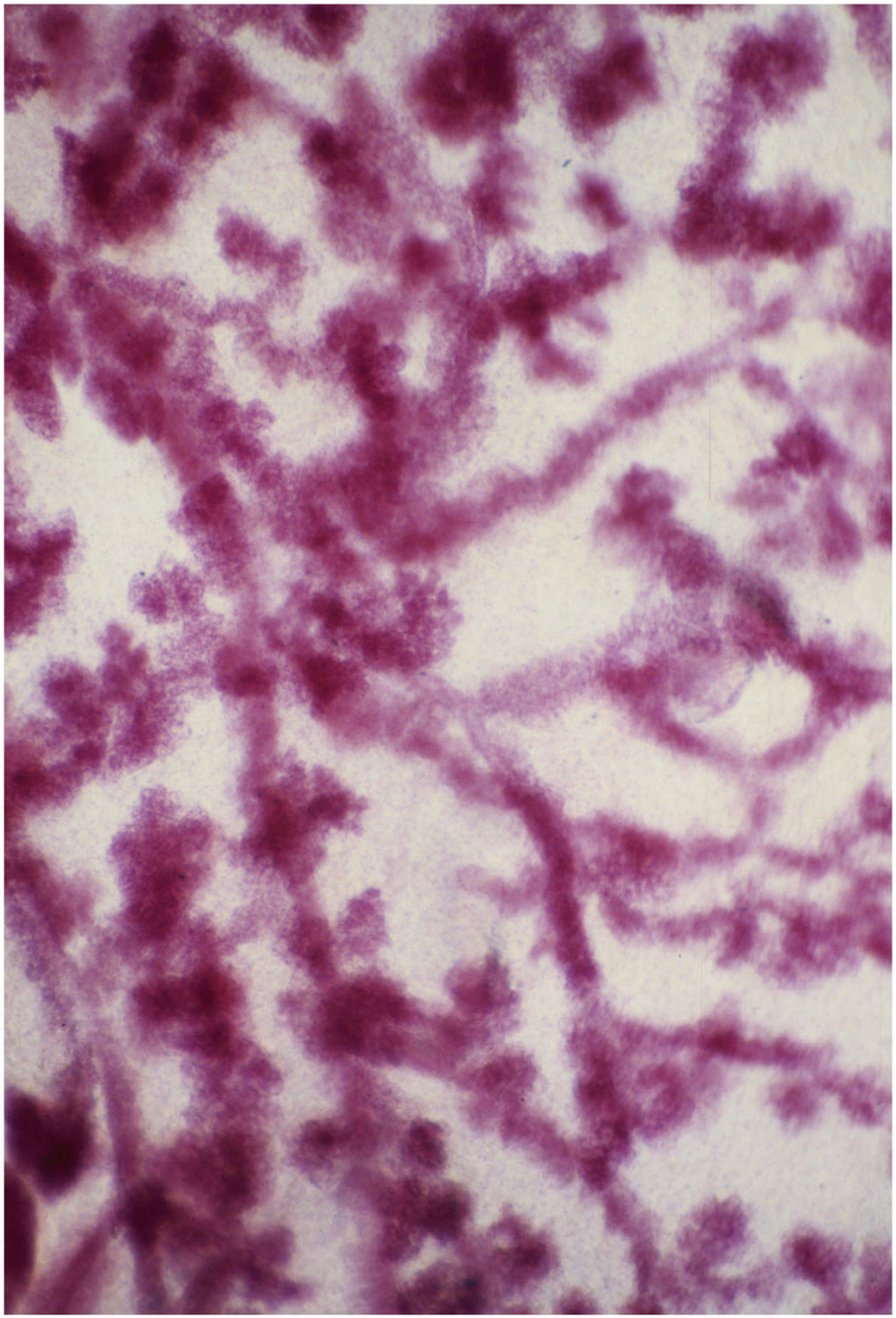

dense stroma
myoepithelium
milk duct lumen
ductal epithelium
basal lamina
fat cells

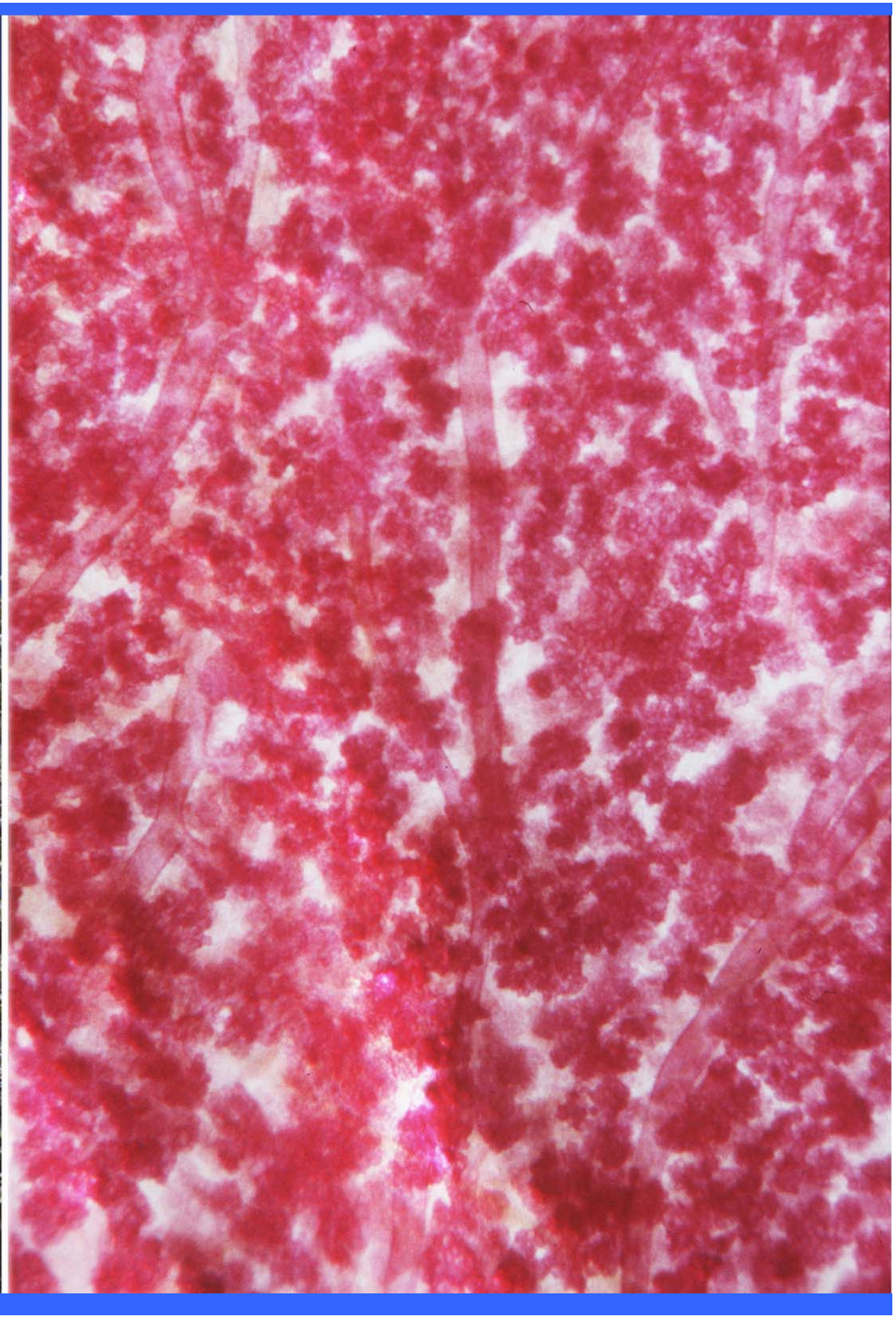
Mammary Gland
Early Development

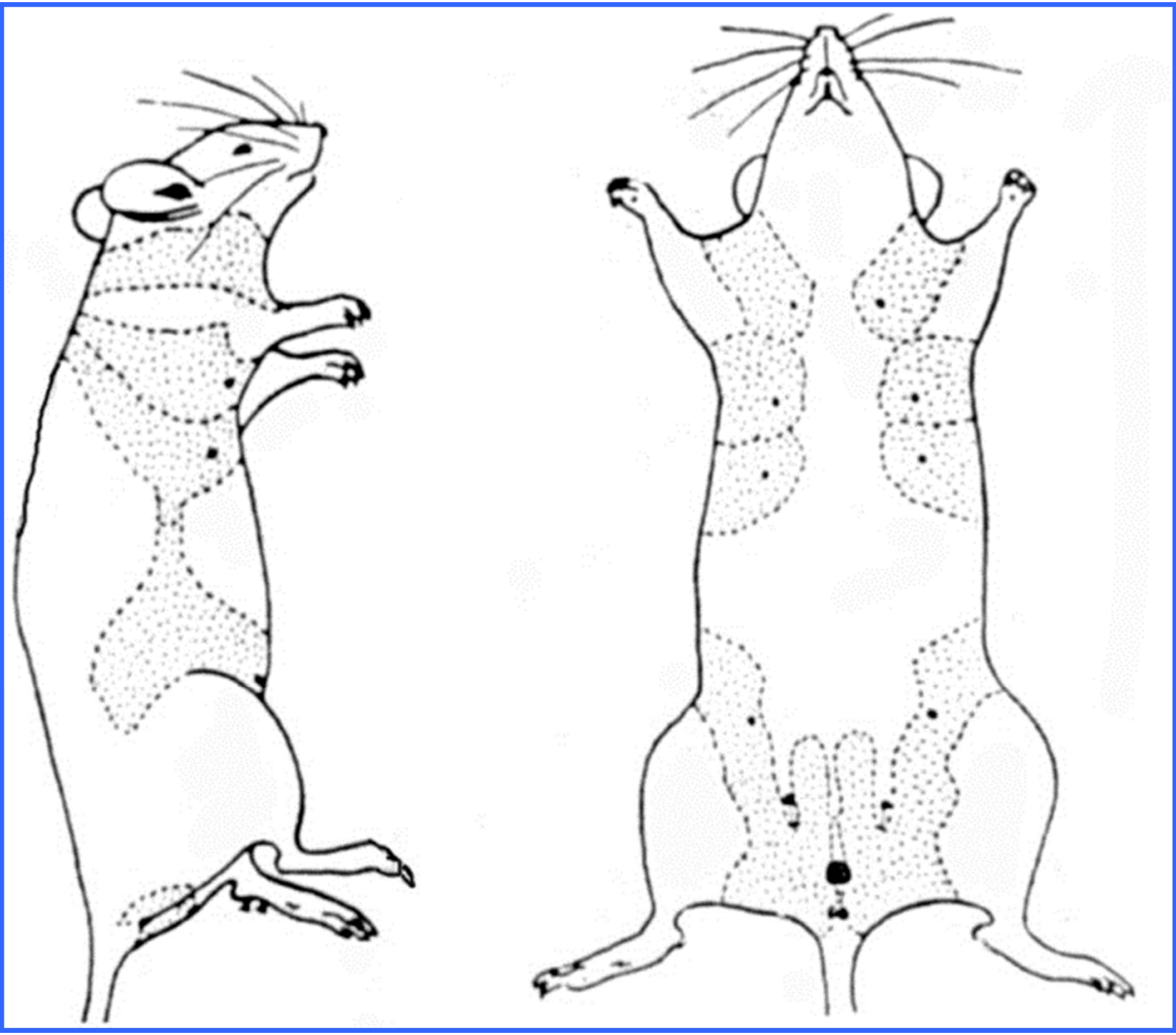






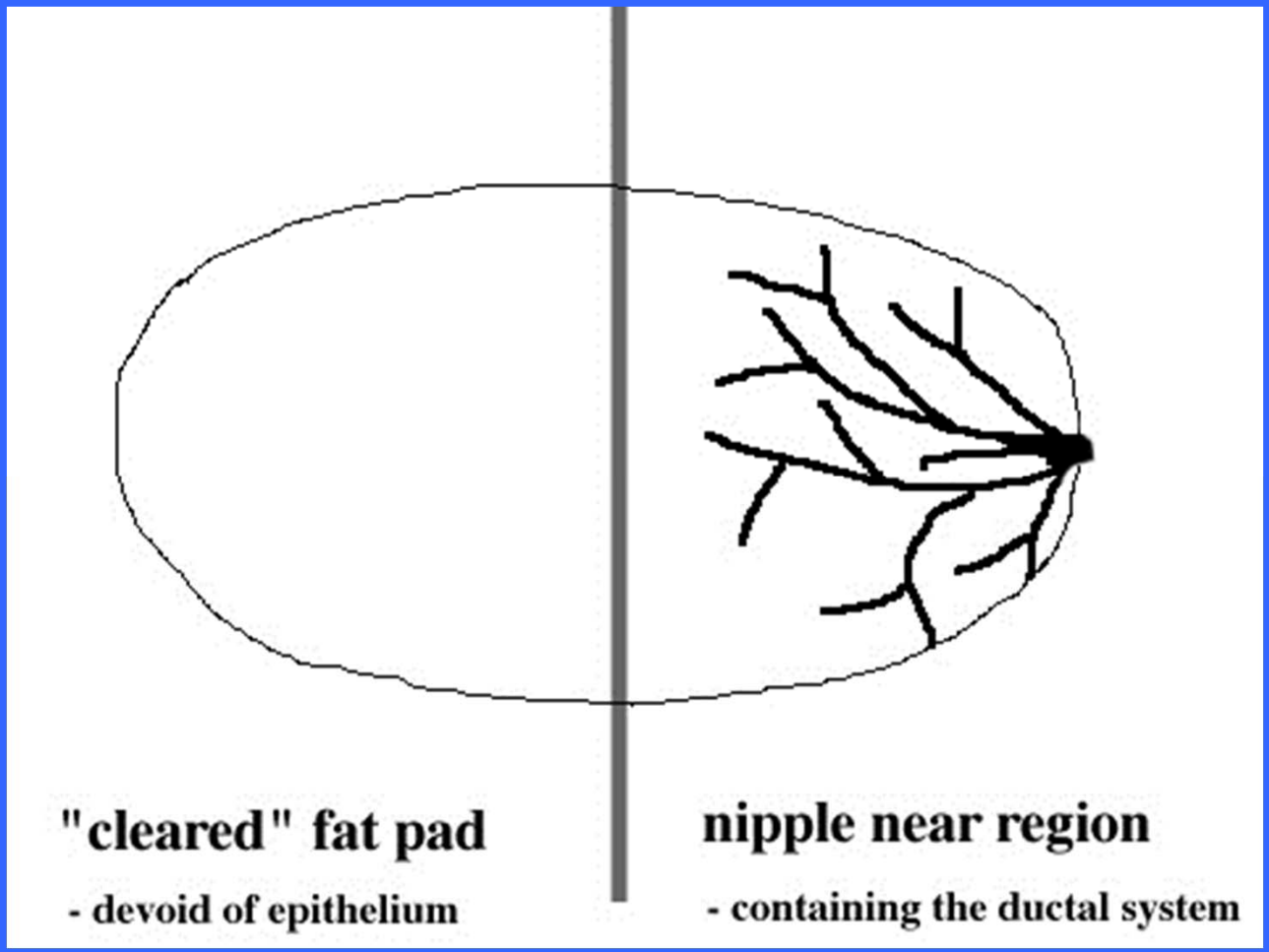






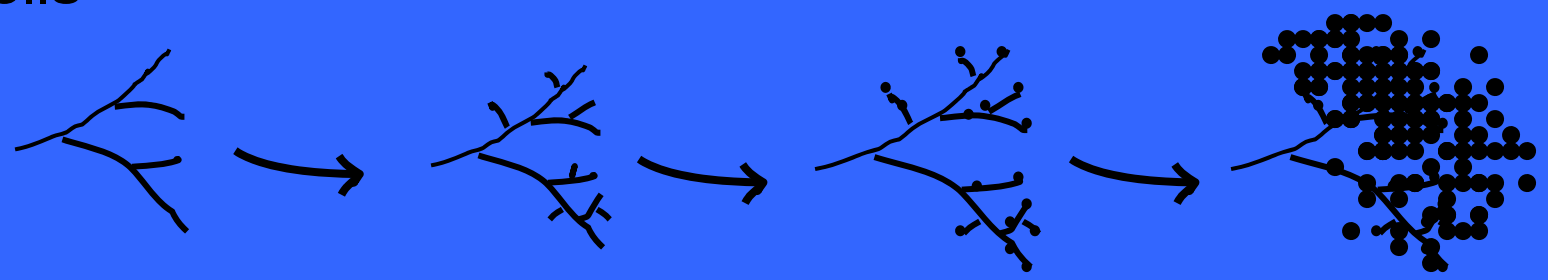
“Fat pad clearing”

-3-week-old mammary gland-



sidebranch

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells



growth hormone

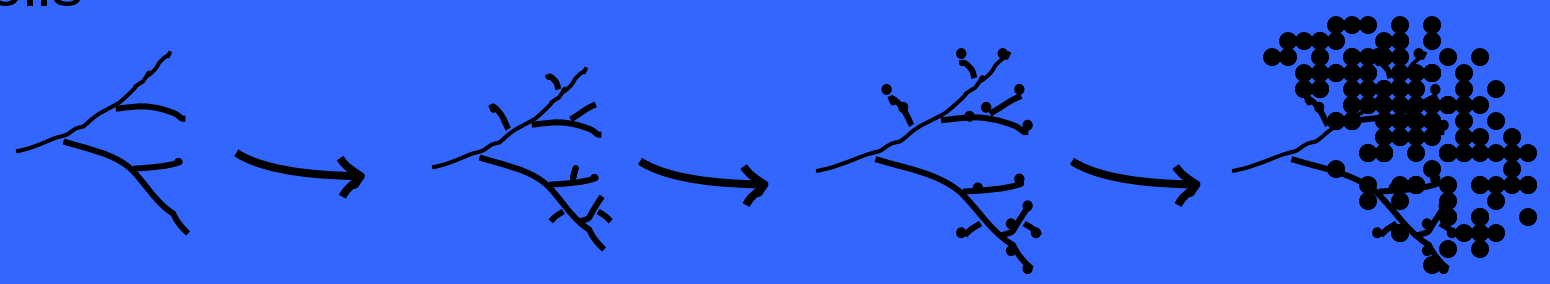
estrogen

progesterone

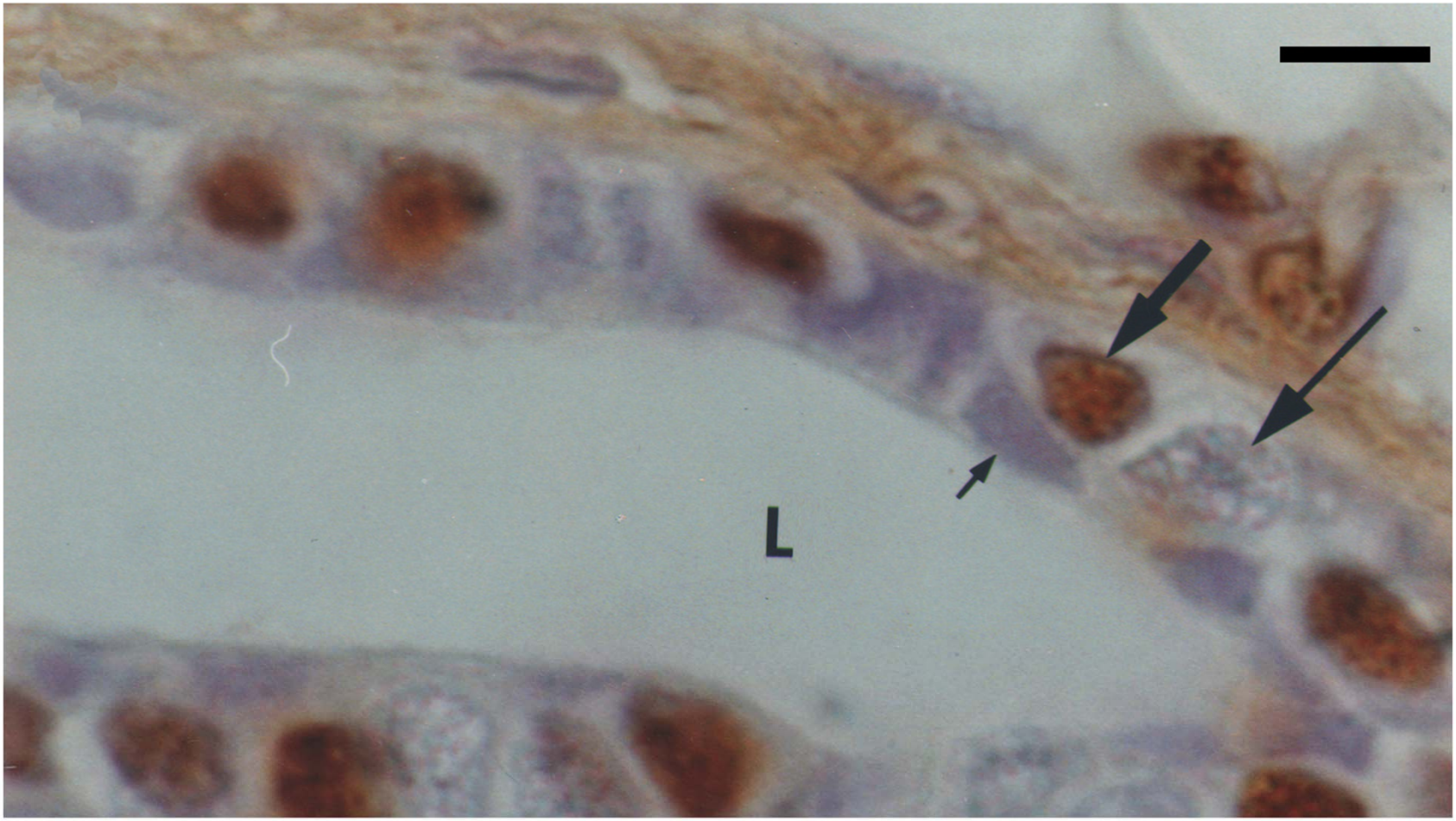
prolactin

sidebranch

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells



Distribution of the progesterone receptor in the ductal epithelium

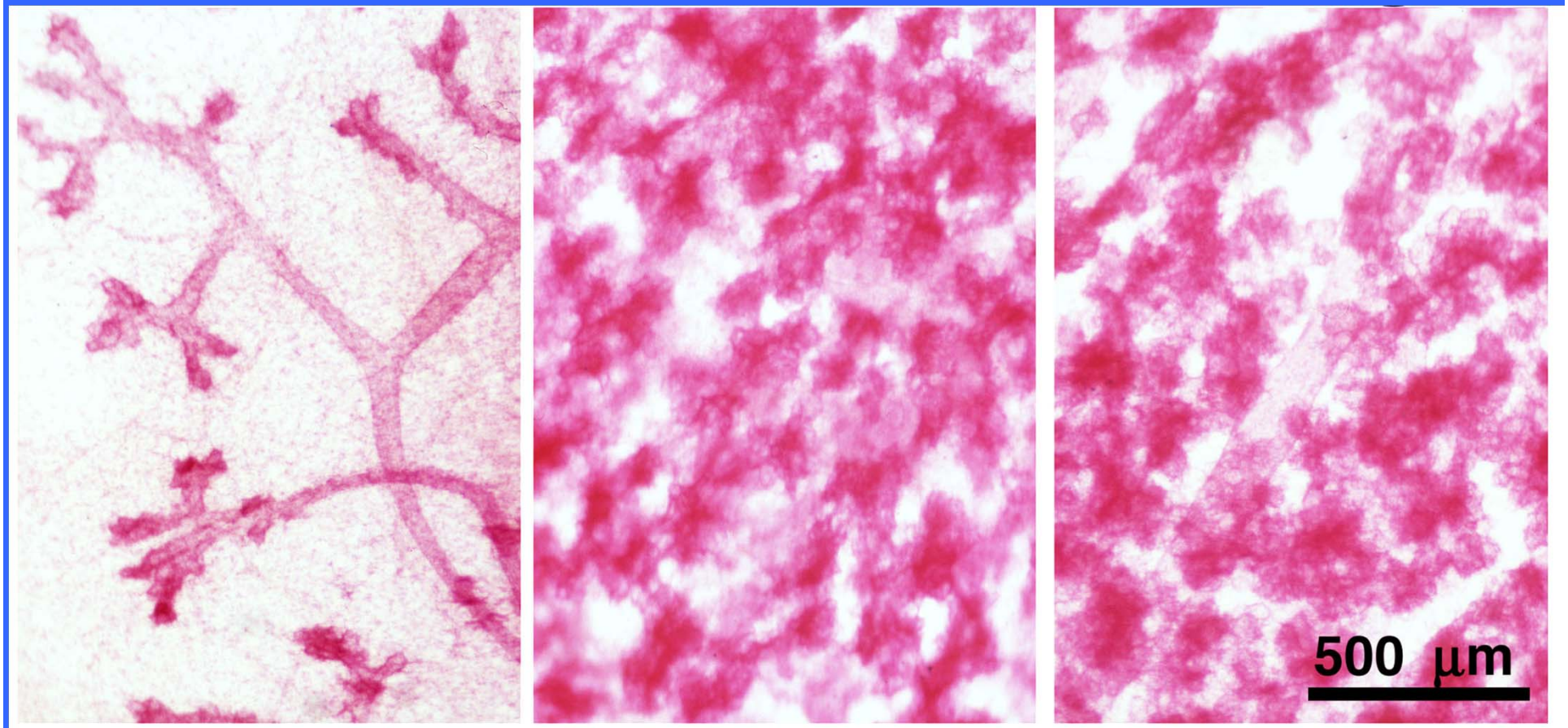


courtesy of Gary B. Silberstein

scale bar : 8μm

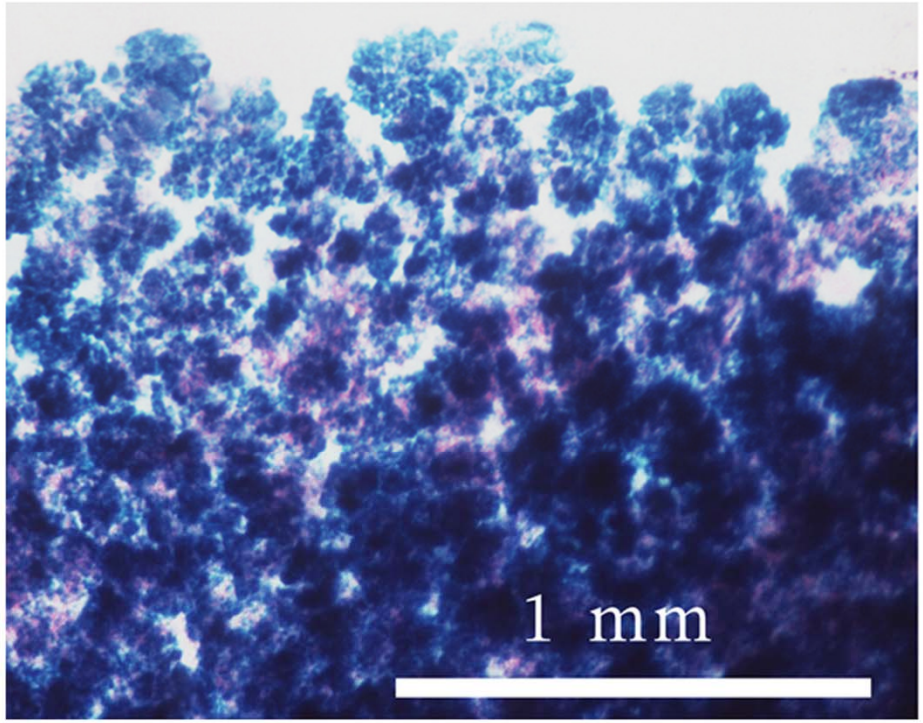
Whole breast transplant

PRKO **PRWT** **control**

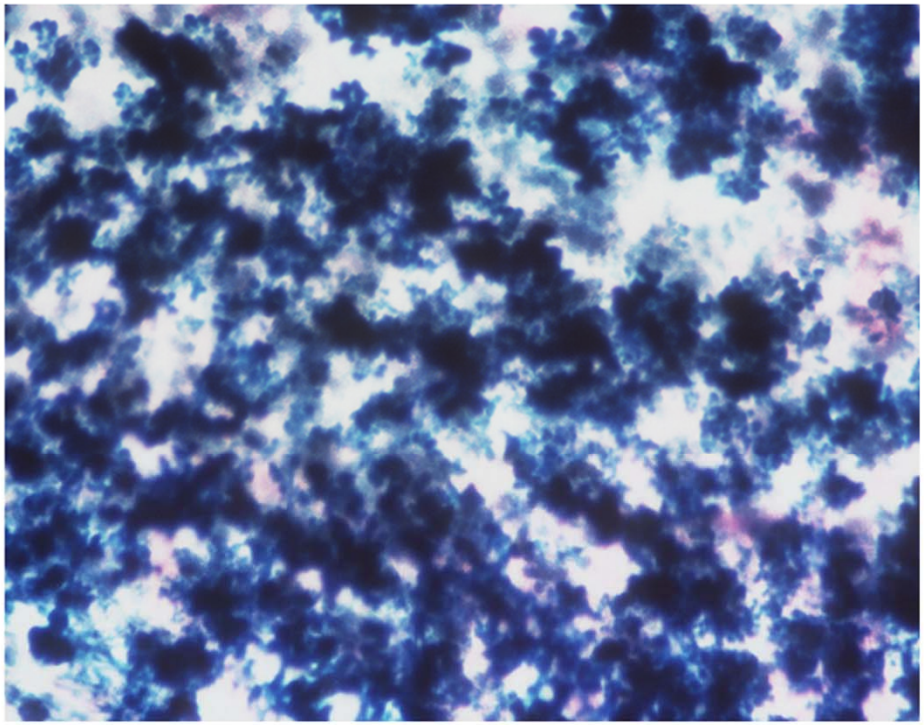


Fat Pad Transplant

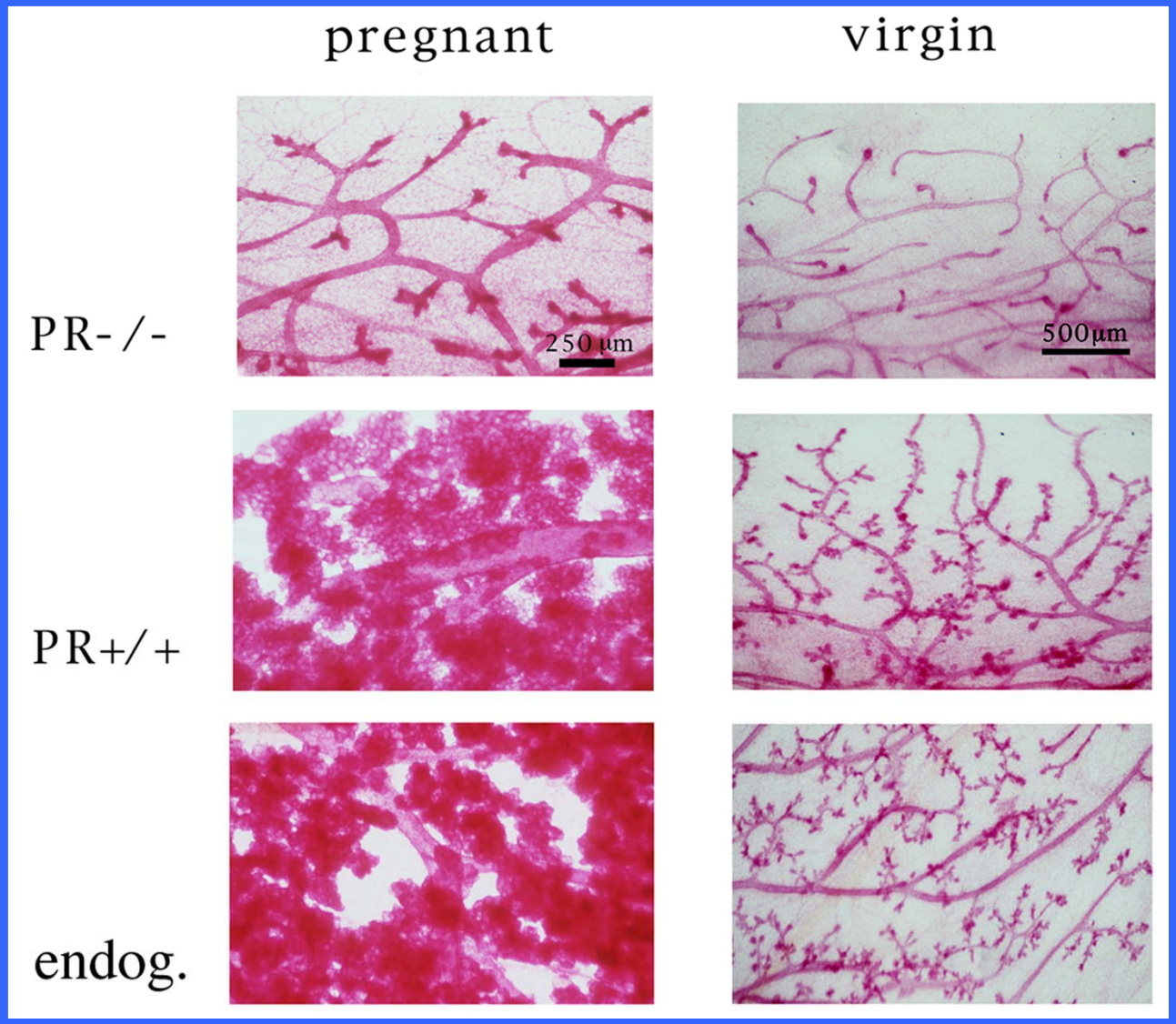
PR-/-



PR+/+



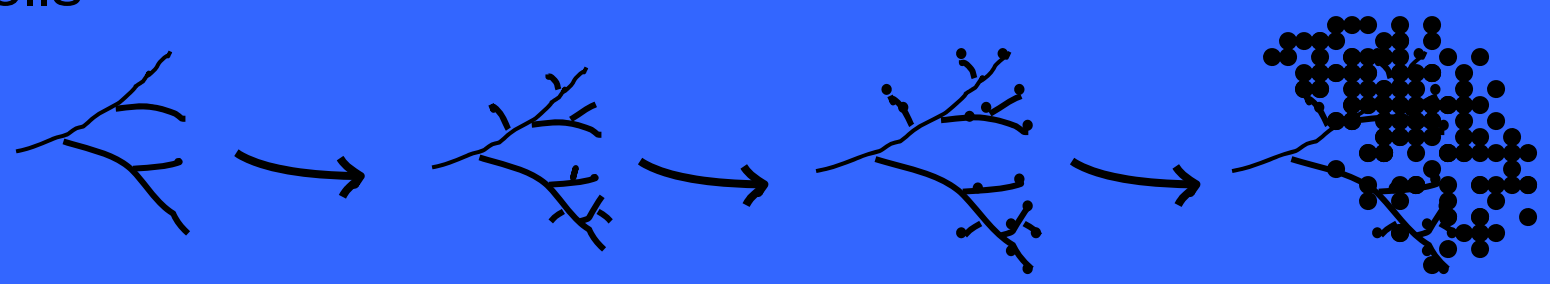
Epithelial Transplant



progesterone

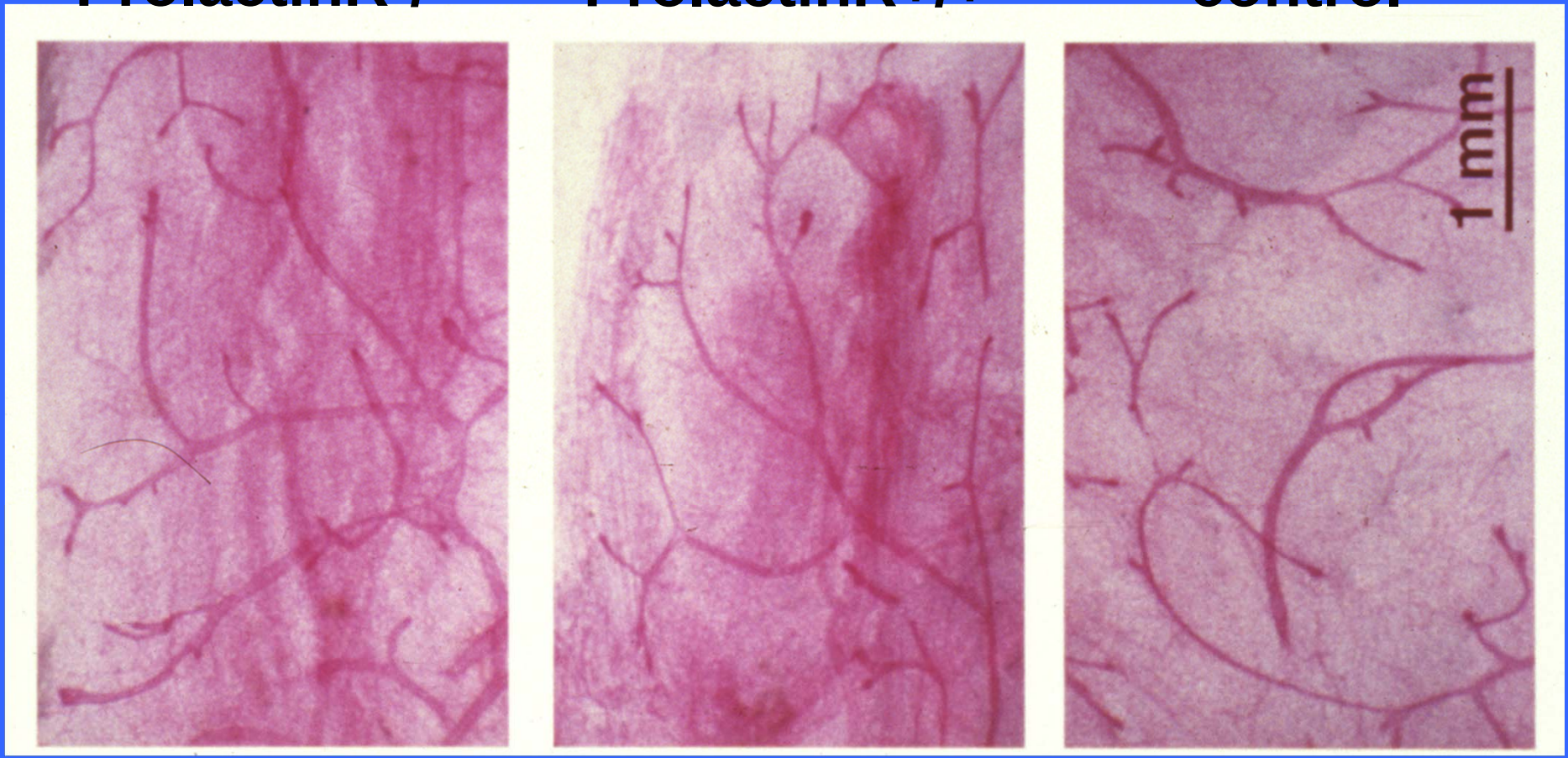
sidebranch

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells



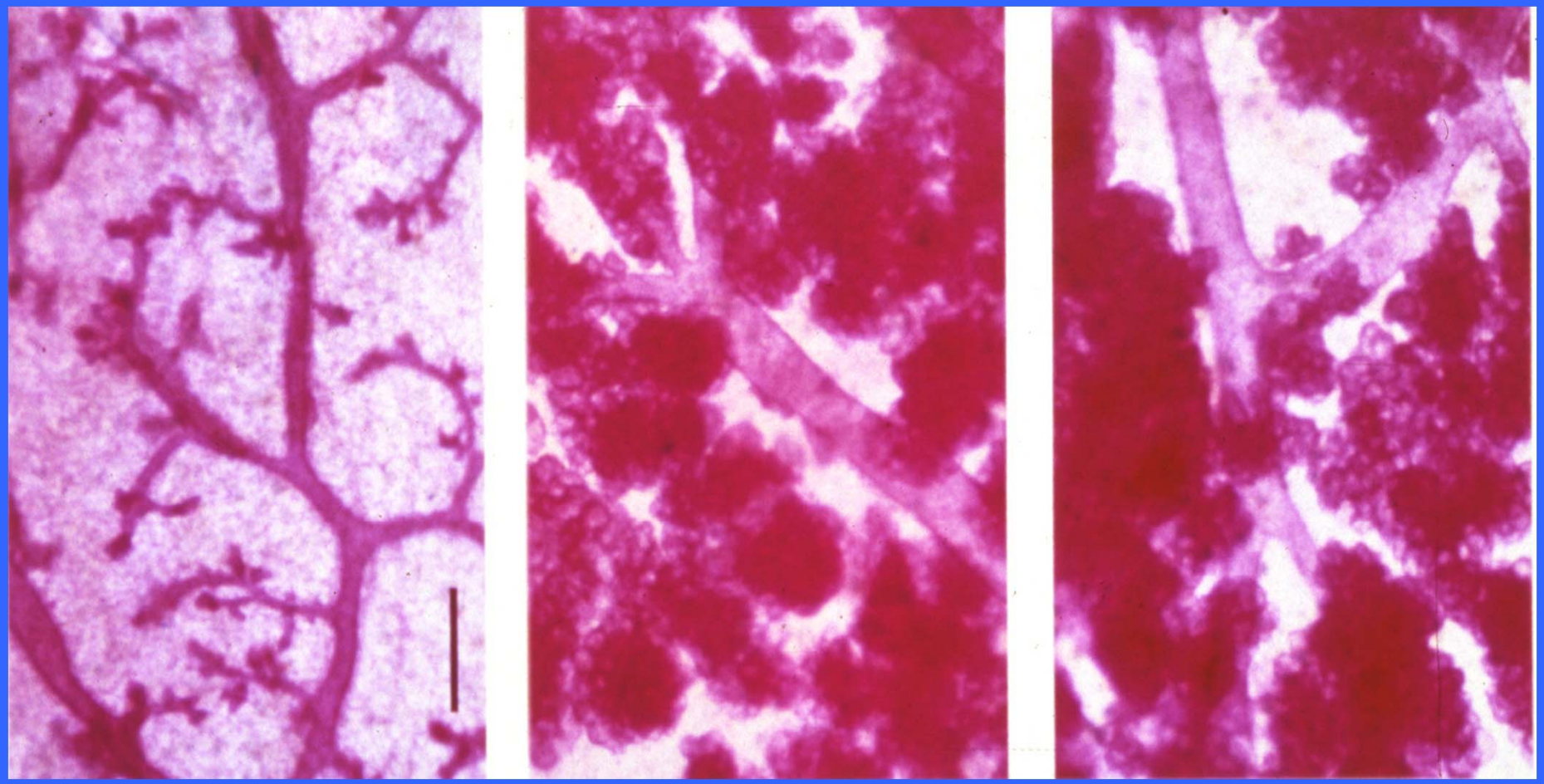
Epithelial transplant (virgin)

ProlactinR-/- **ProlactinR+/+** **control**



Epithelial transplant (end of pregnancy)

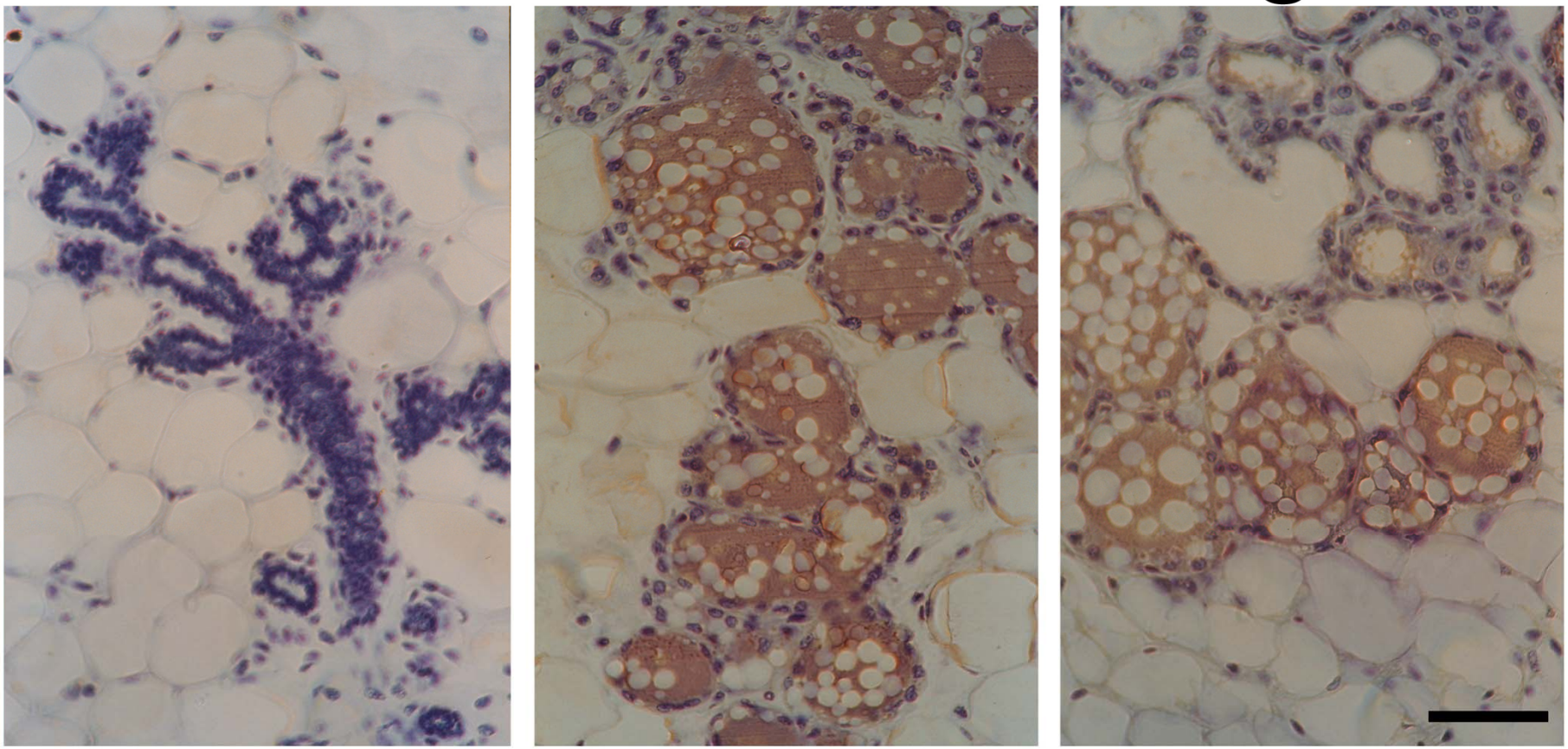
ProlactinR-/- **ProlactinR+/+** **control**



scalebar = 200μm

PrlR-/-

PrlR+/+ endogenous

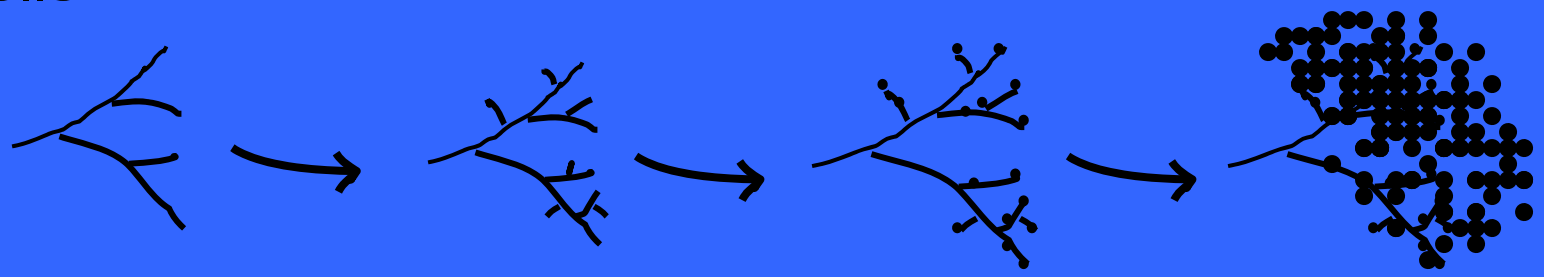


scale bar: 25 um

prolactin

sidebranch

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells

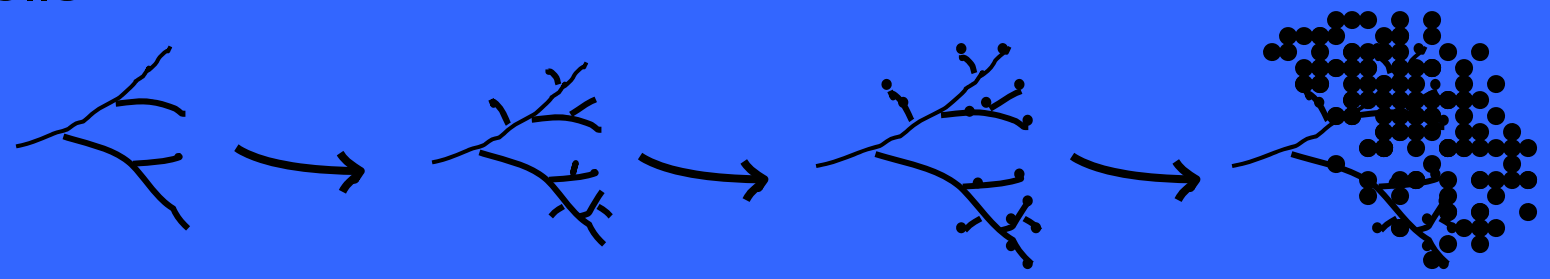


progesterone

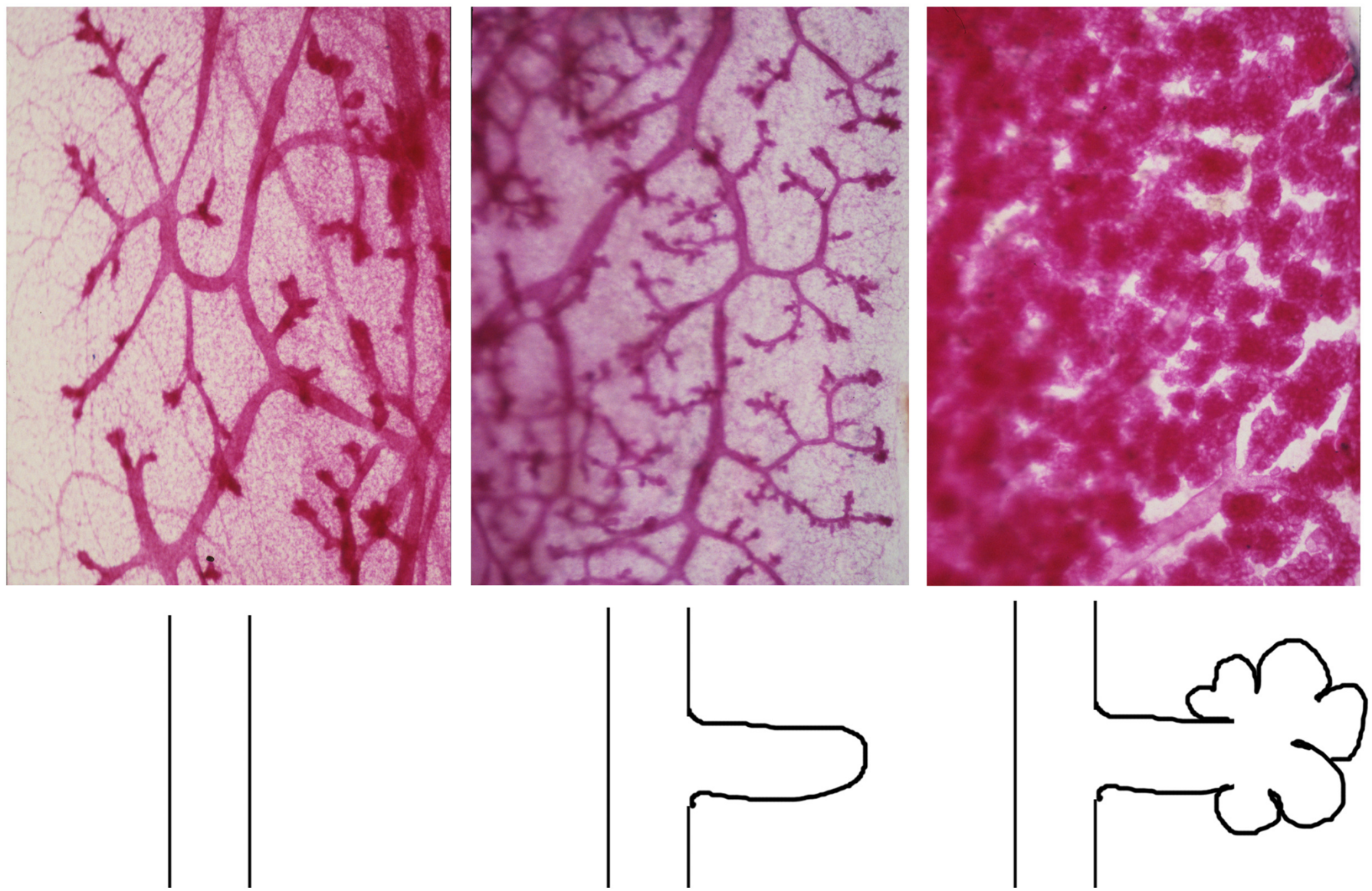
prolactin

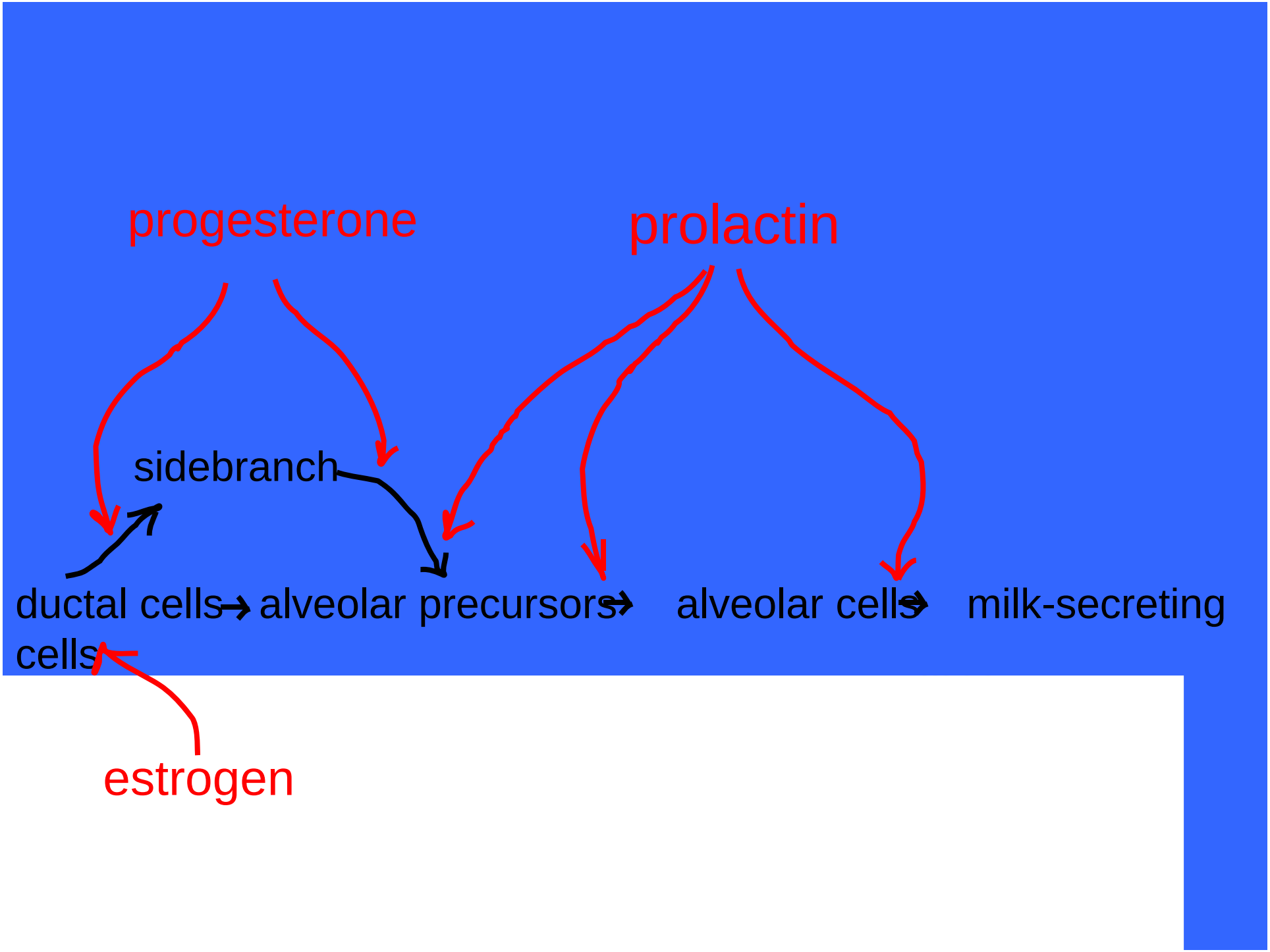
sidebranch

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells



Progesterone R-/- **Prolactin R-/-** **wild type**




progesterone
prolactin
sidebranch
ductal cells → alveolar precursors → alveolar cells → milk-secreting cells
estrogen

growth hormone

wnt 5a
wnt 5b
wnt 6

TGFβ2

EGF

estrogen

amphiregulin

TGFβ3

wnt 4

TGFβ1

progesterone

RANKL

prolactin

TGFα

CSF-1

sidebranch

heregulin

FGF-4

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells

EGF

HGF

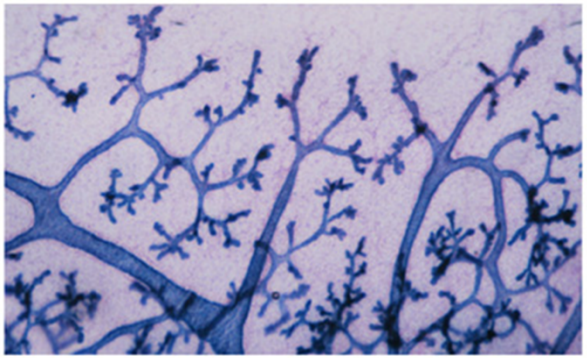
wnt 10b

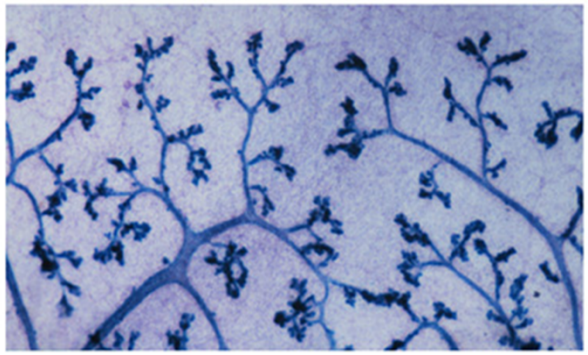
activin

IGF-1

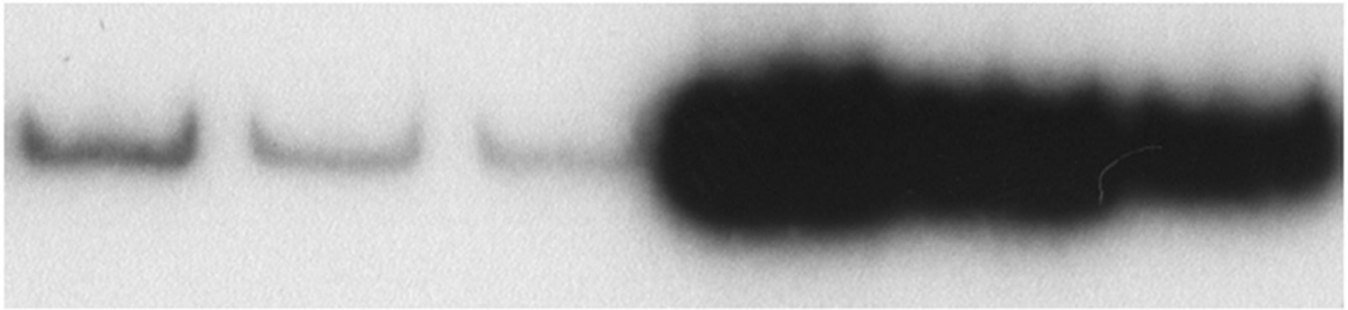
prolactin R -/-

cyclin D1 -/-

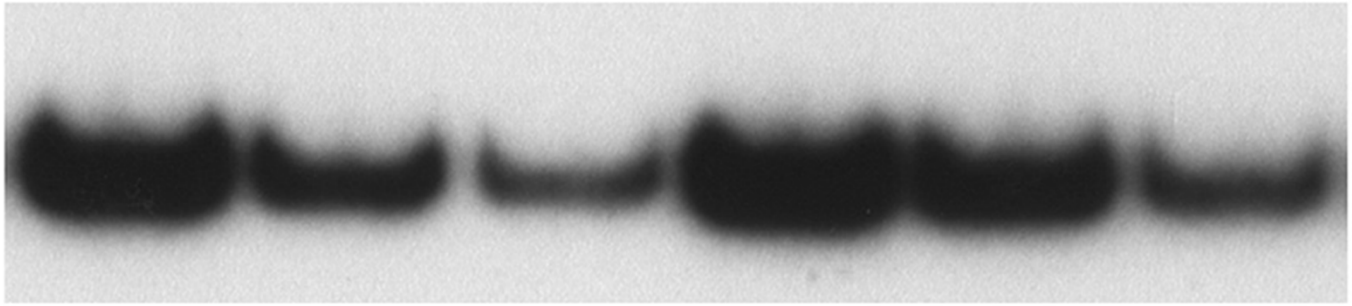




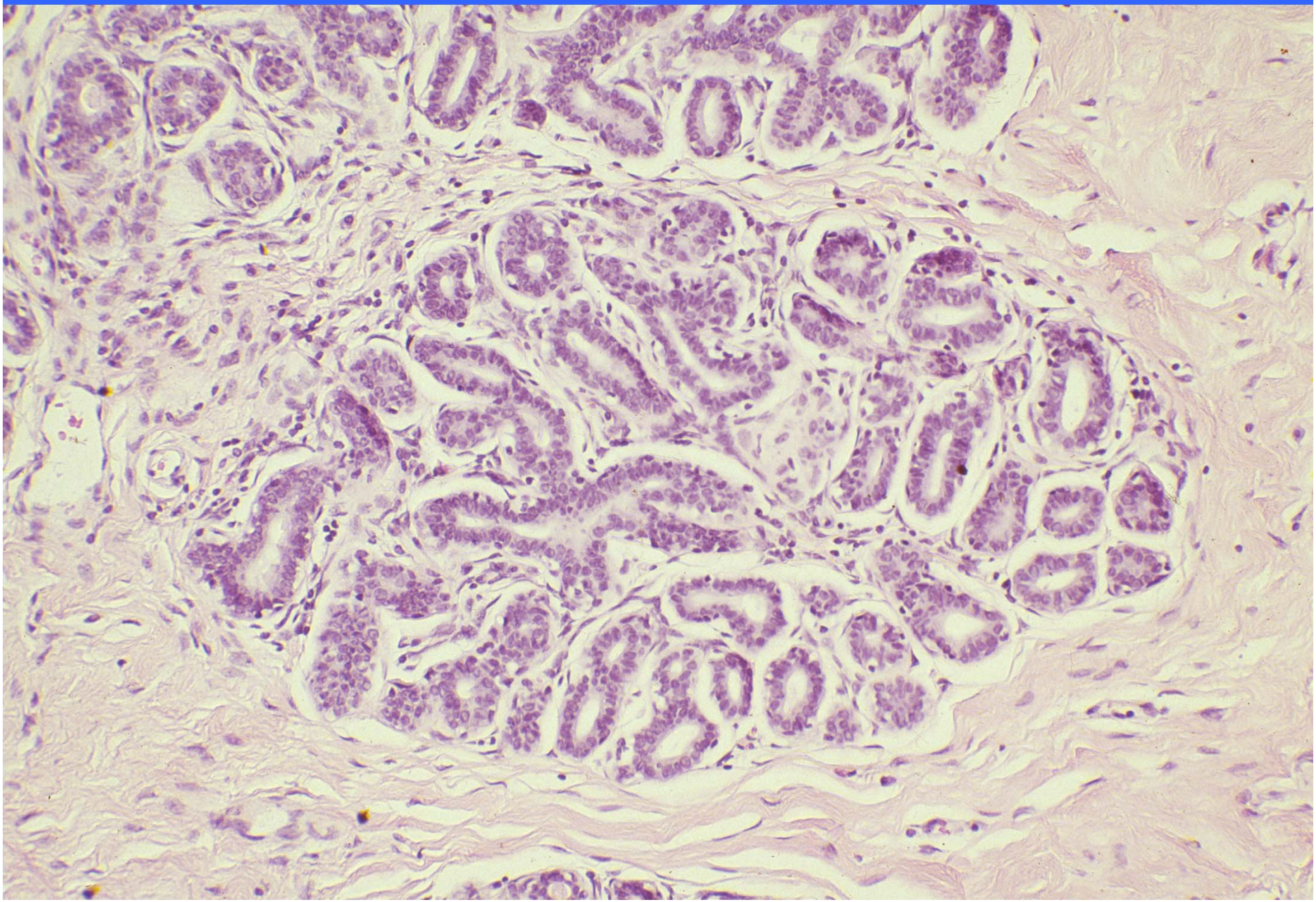
IGF-2



LacZ



Terminal Ductule Lobular Unit



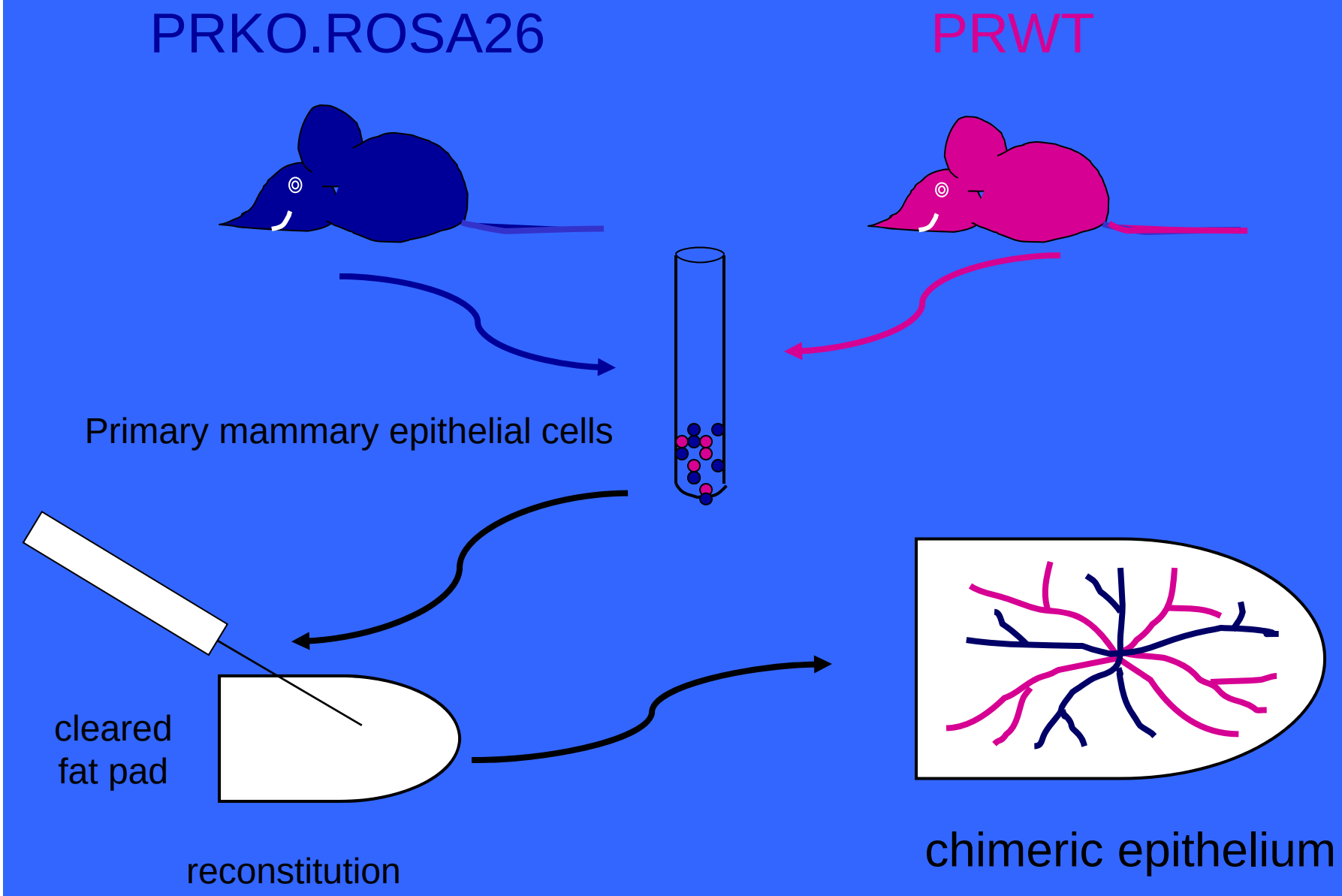
TDLU

terminal ductal lobular unit

=

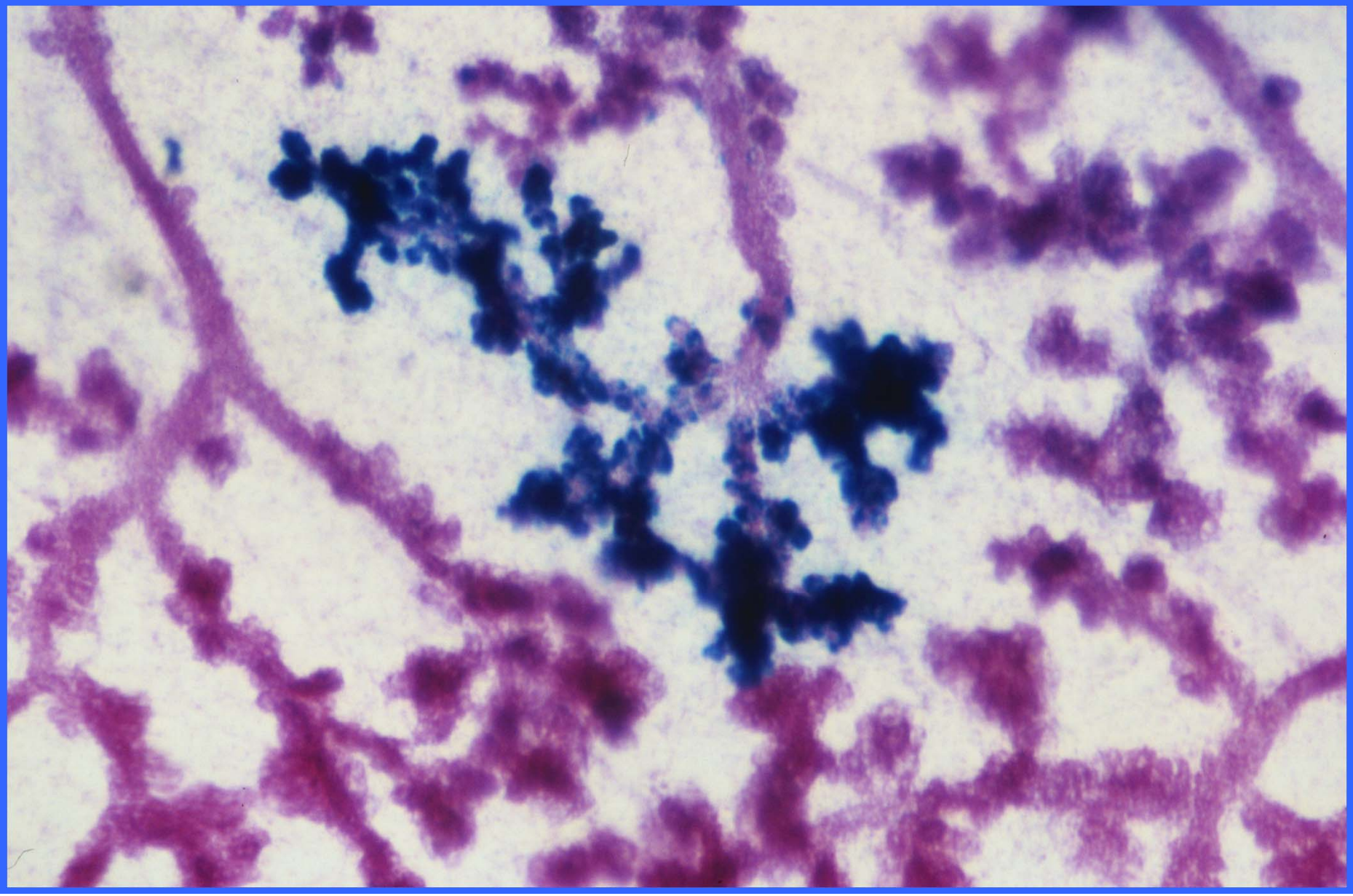
sidebranch

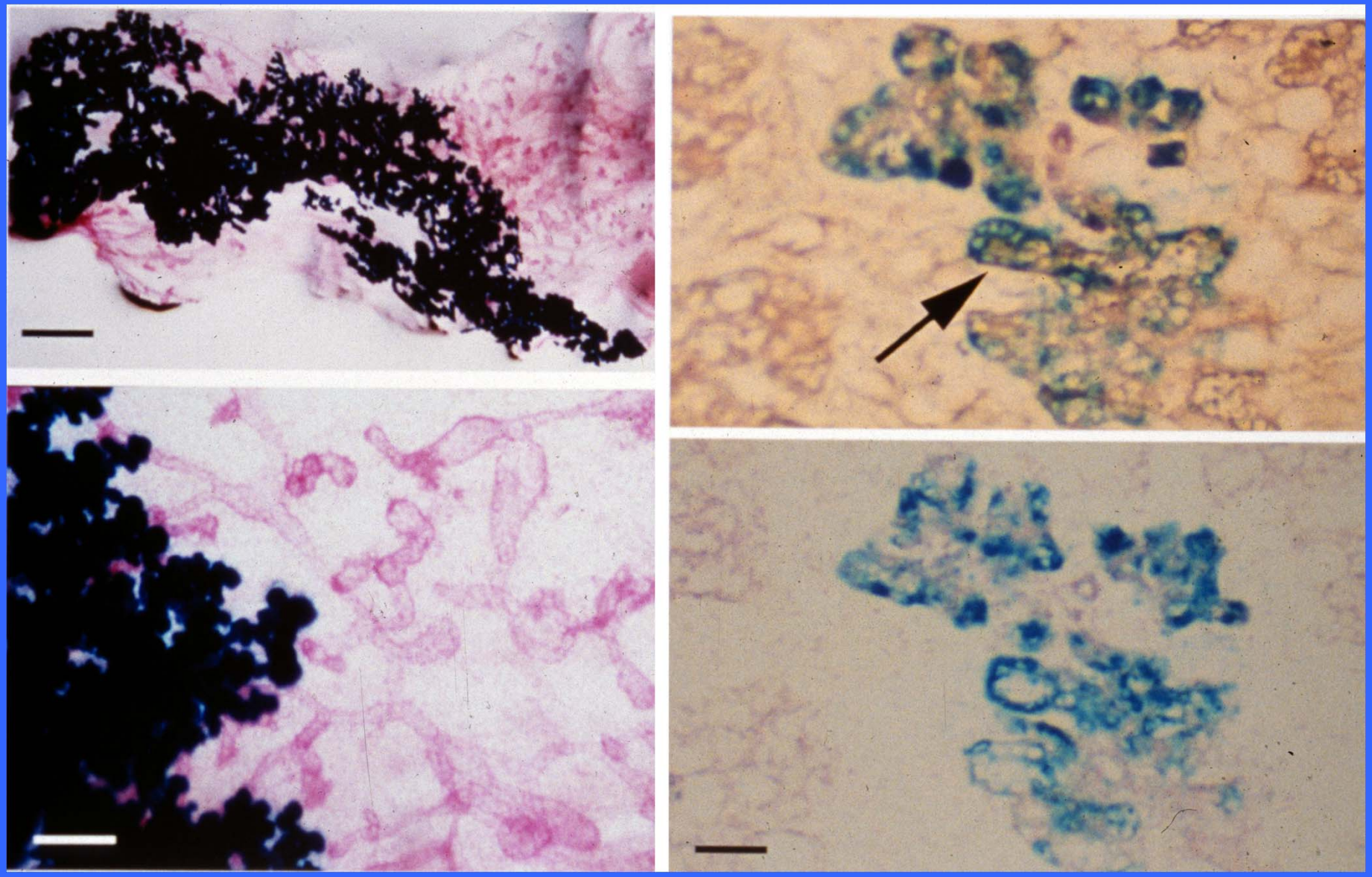
CREATING CHIMERIC EPITHELIA



PRKO.ROSA&PRWT chimeric epithelium

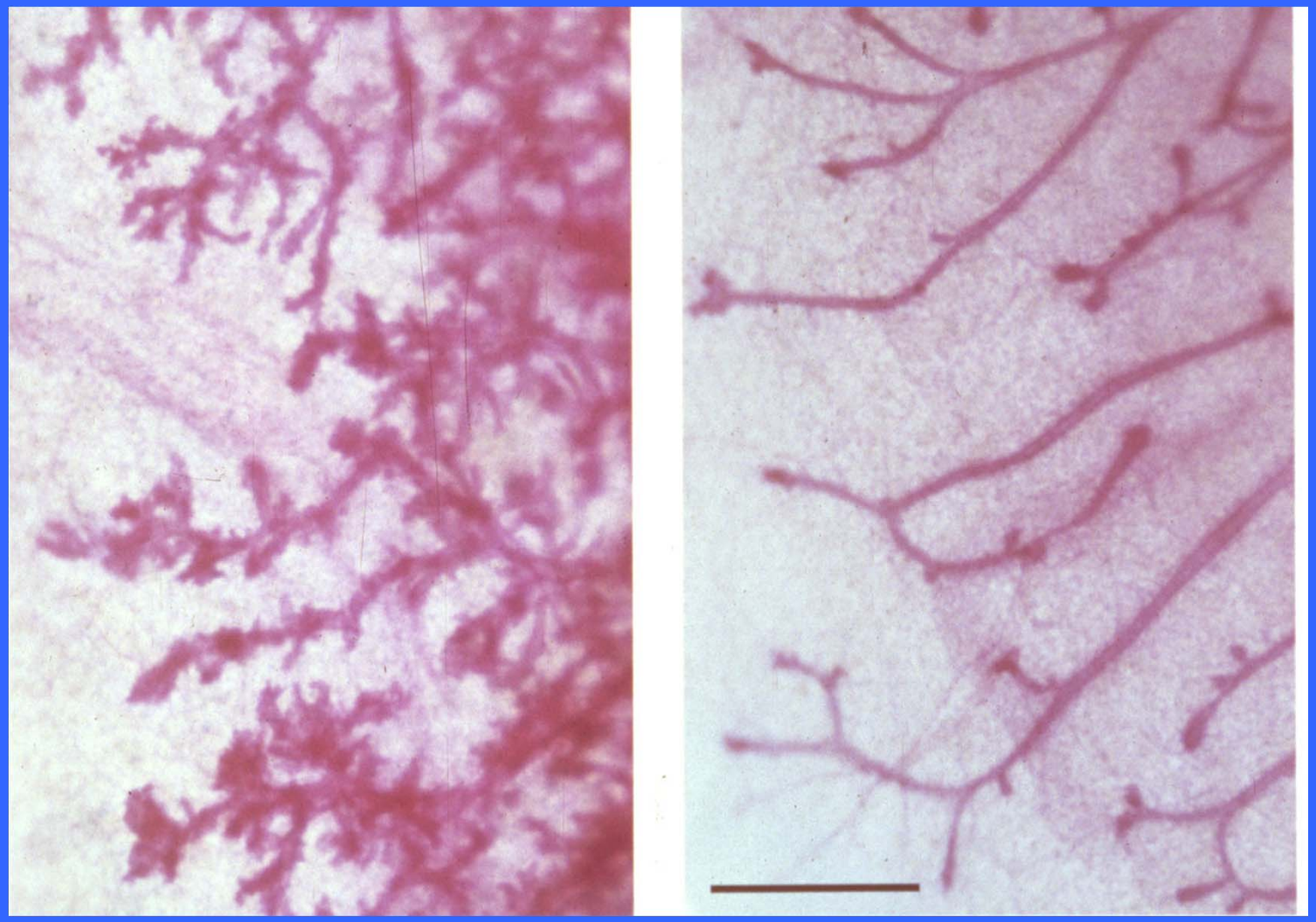
(blue) **(magenta)**





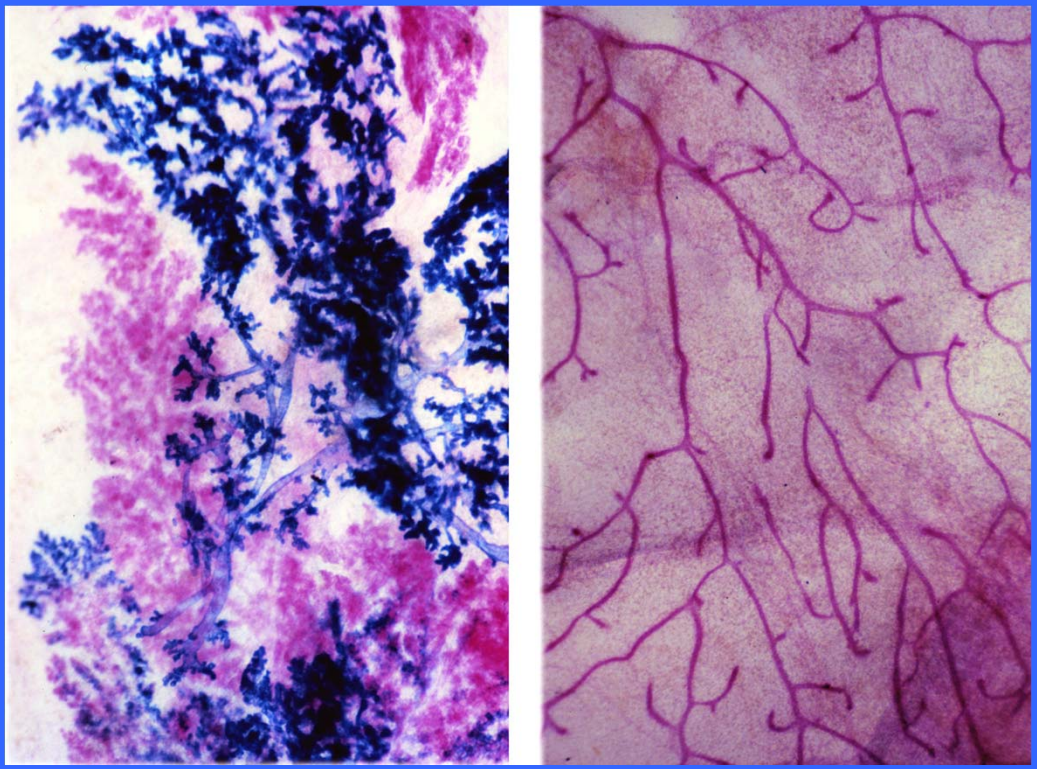
Epithelial transplants (virgin)

MMTV-wnt-1 wild type



PR-/-.MMTVwnt1
PR+/+. MMTVwnt1
control

wt/MMTV-wnt1 chimeric epithelium



wnt 4

wnt 5a

wnt 7b

wnt 5b

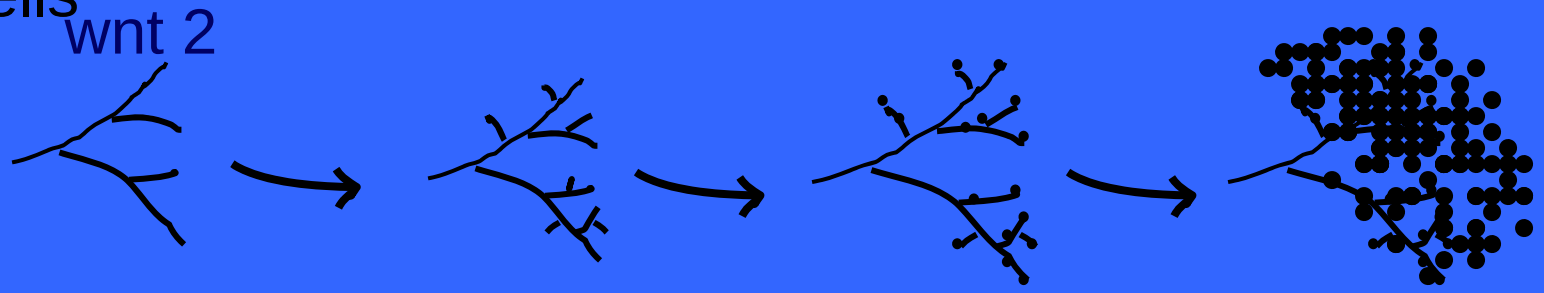
wnt 6

wnt 10b

sidebranch

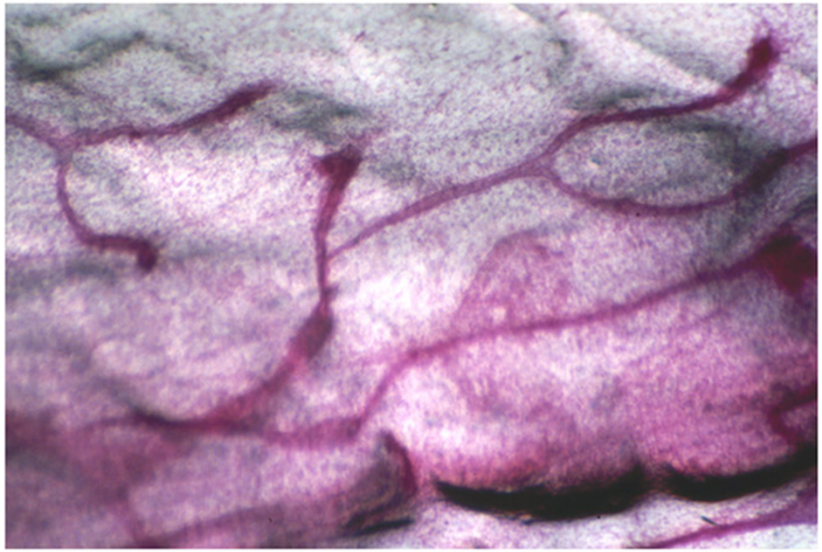
ductal cells → alveolar precursors → alveolar cells → milk-secreting cells

wnt 2

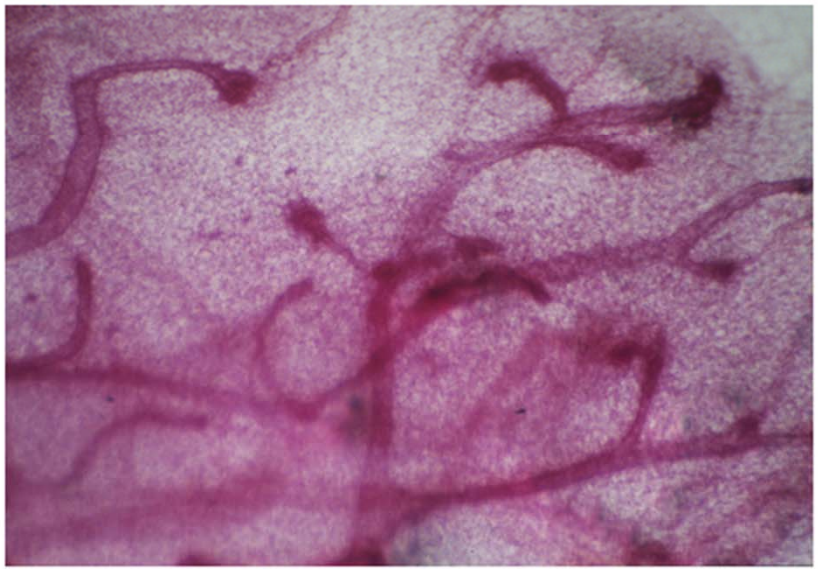


virgin

wnt4-/-

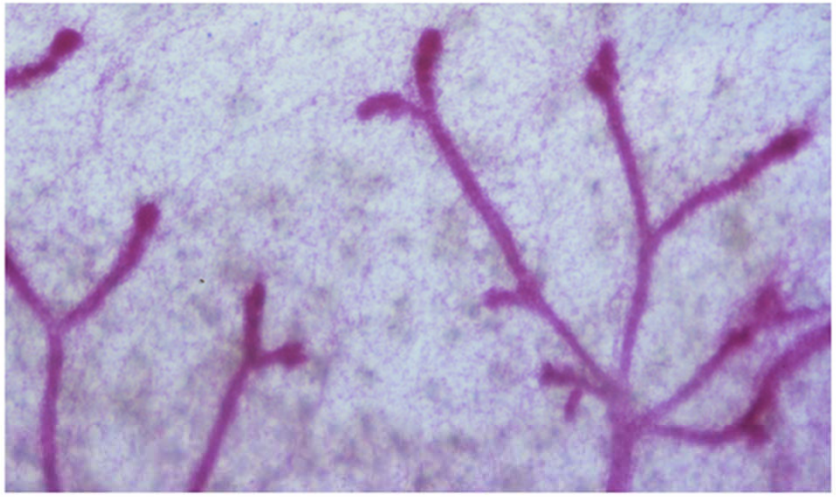


wnt4+/+

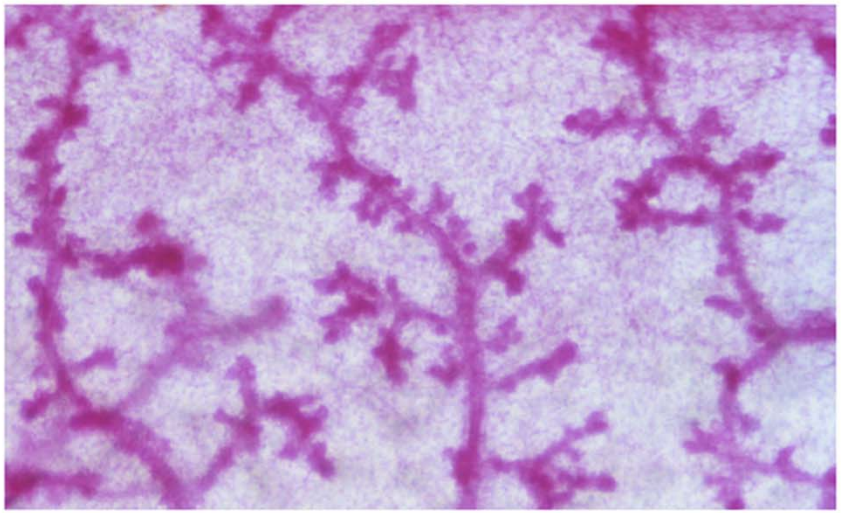


d12 pregnant

wnt4-/-

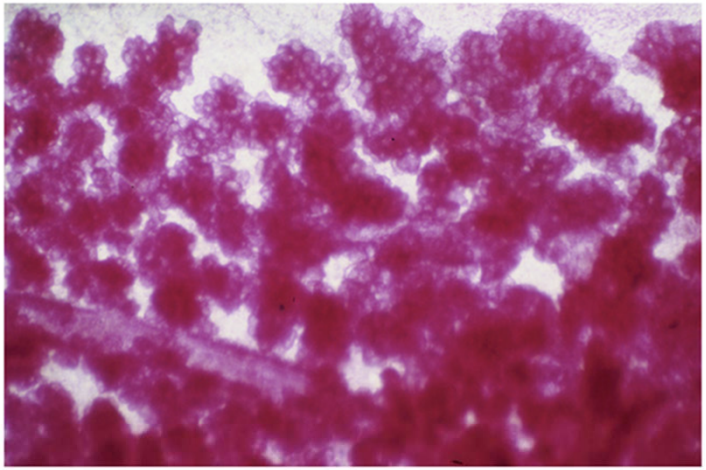


wnt4+/+

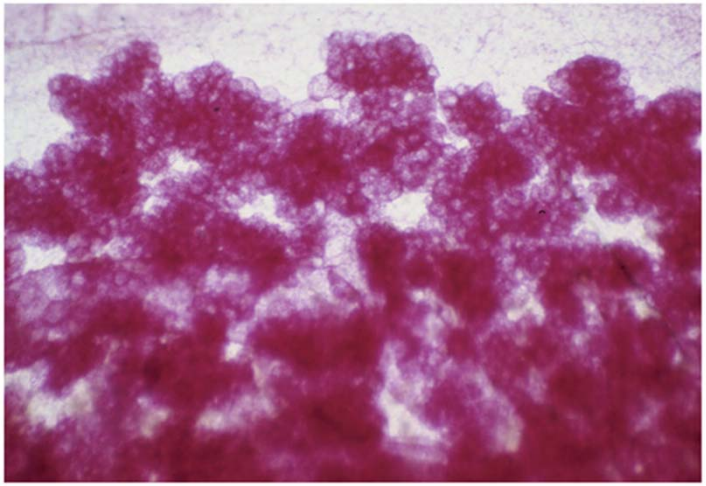


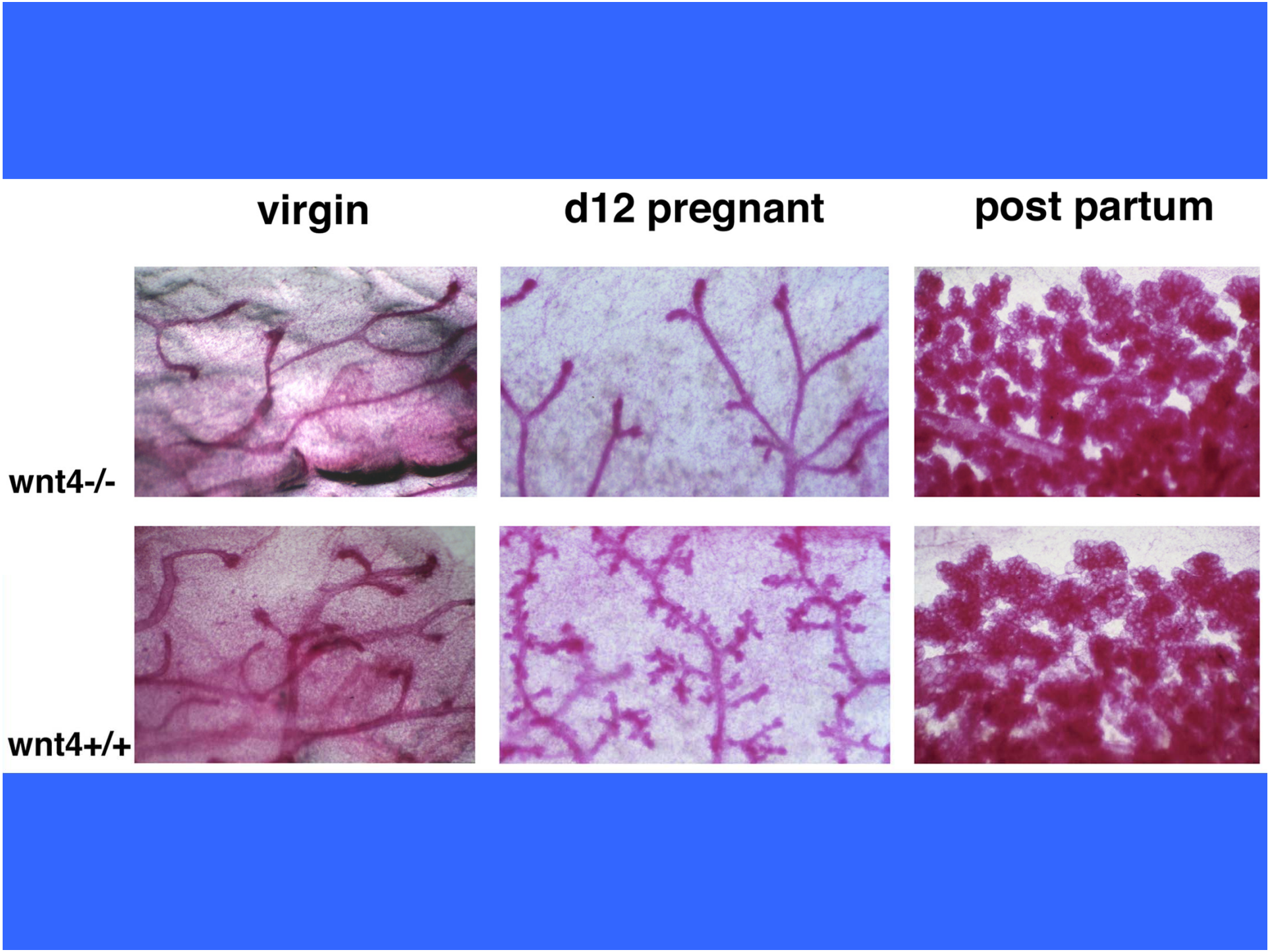
post partum

wnt4-/-



wnt4+/+

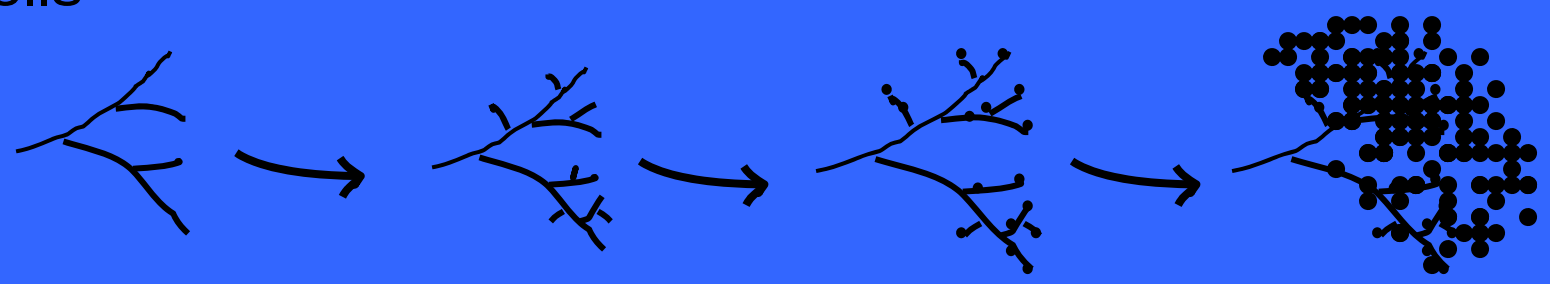



virgin
d12 pregnant
post partum
wnt4-/-
wnt4+/+

wnt-4

sidebranch

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells

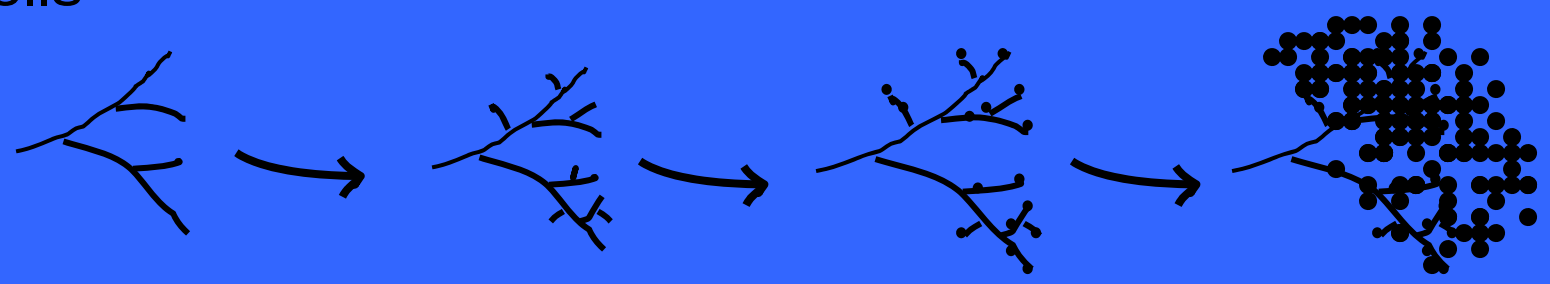


progesterone

wnt-4

sidebranch

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells



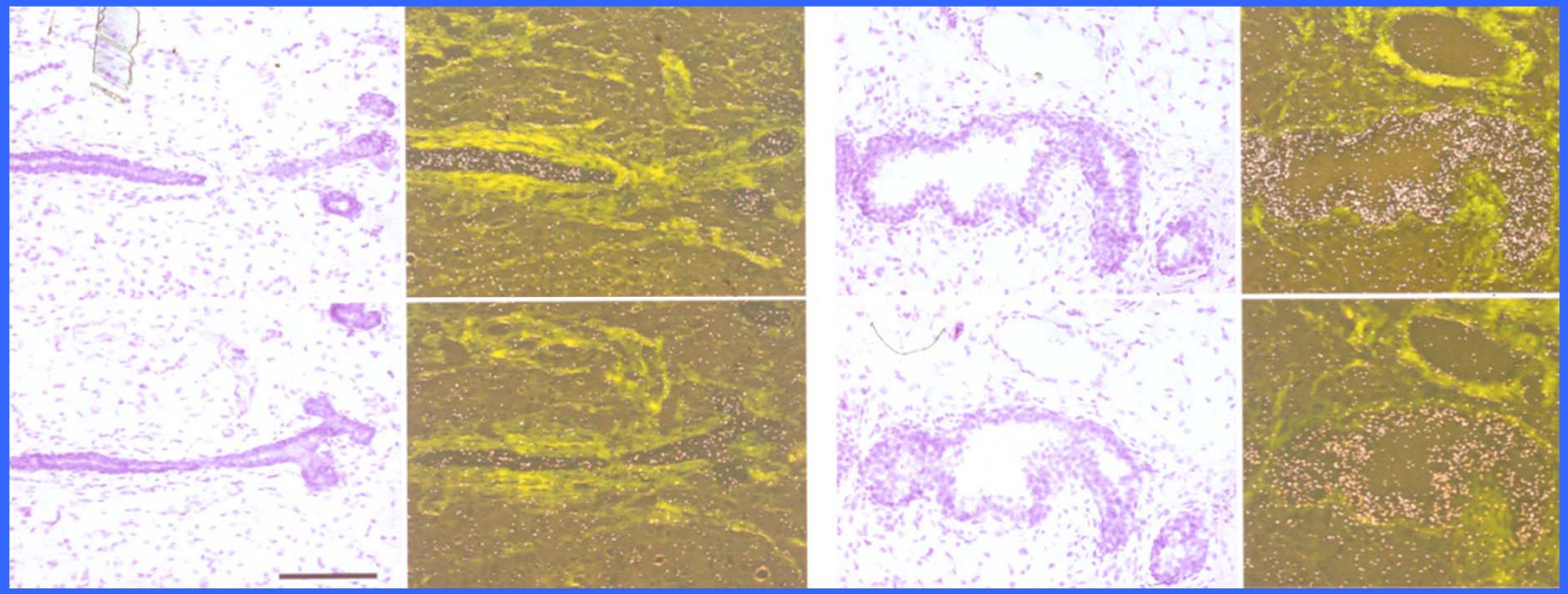
PR and Wnt-4 mRNA expression

virgin

d8

Wnt-4

PR



Wnt-4 mRNA induced by progesterone *in vivo*

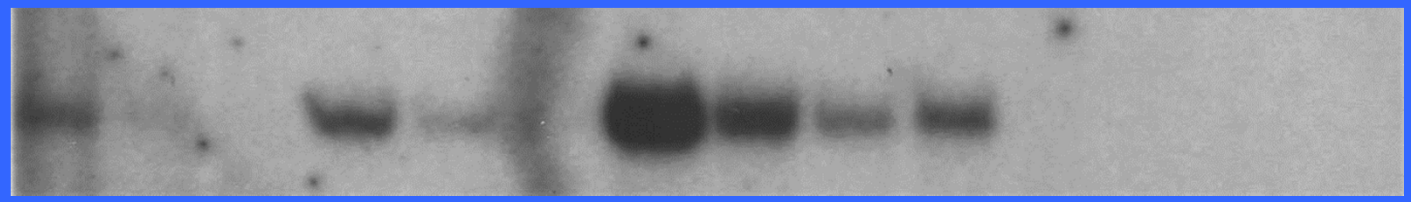
(20-day treatment)

+RT **-RT**

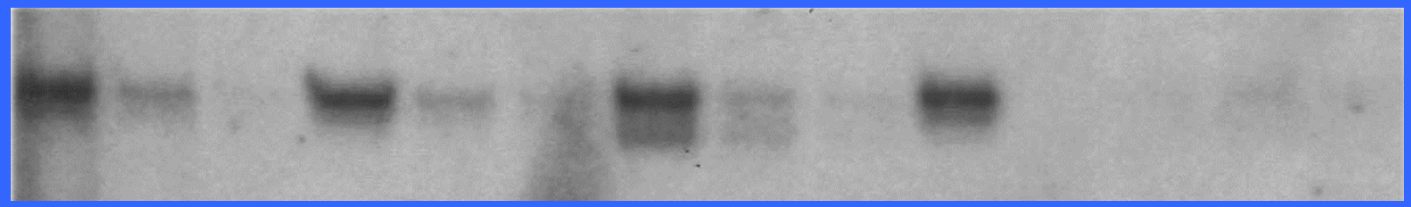
control estradiol E2 & prog.

RNA µg **.1 .03 .01 .1 .03 .01 .1 .03 .01 .1 .1 .1 .1 .1**

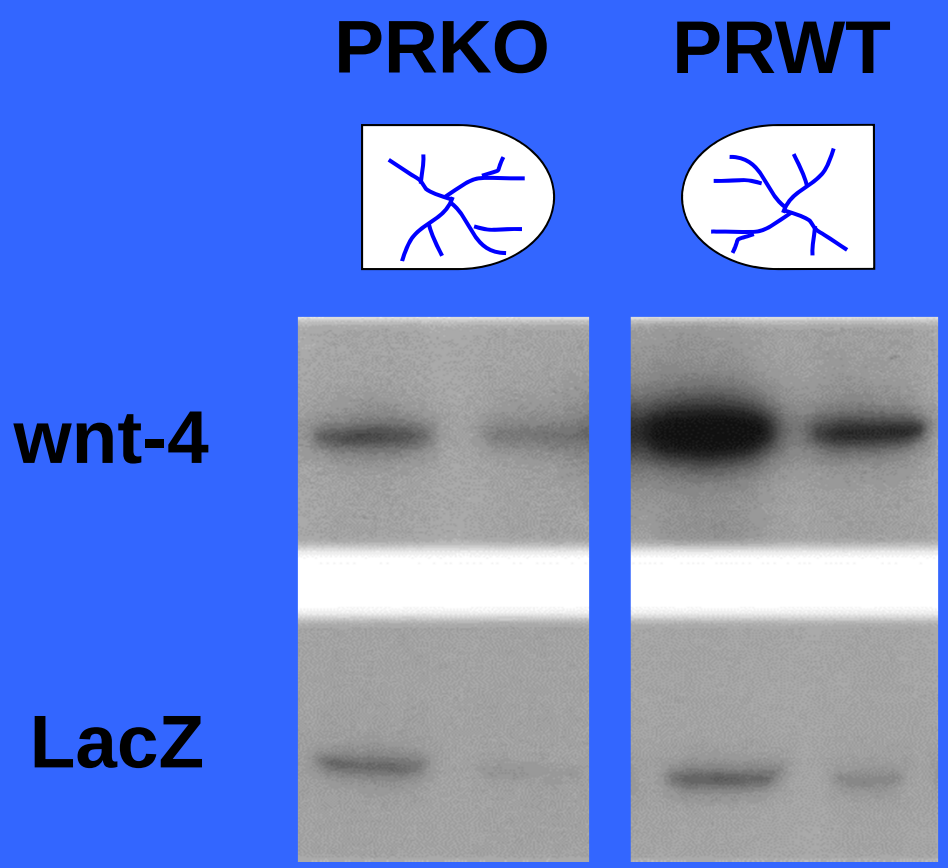
wnt-4



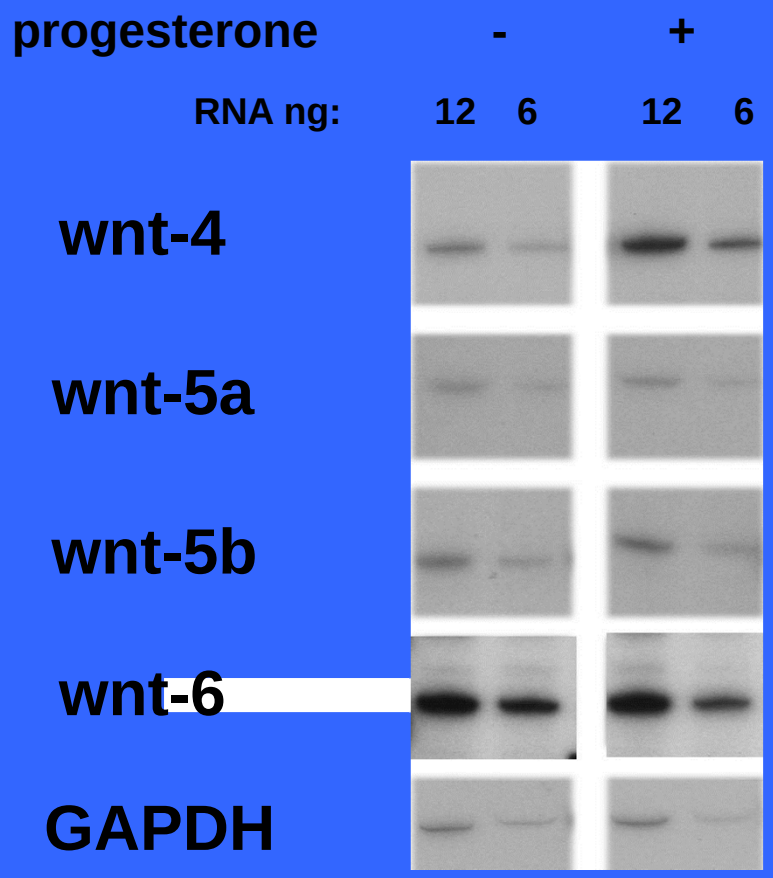
GAPDH



Wnt-4 expression depends on the epithelial progesterone receptor



Wnt-4 mRNA induction by progesterone in primary mammary epithelial cells

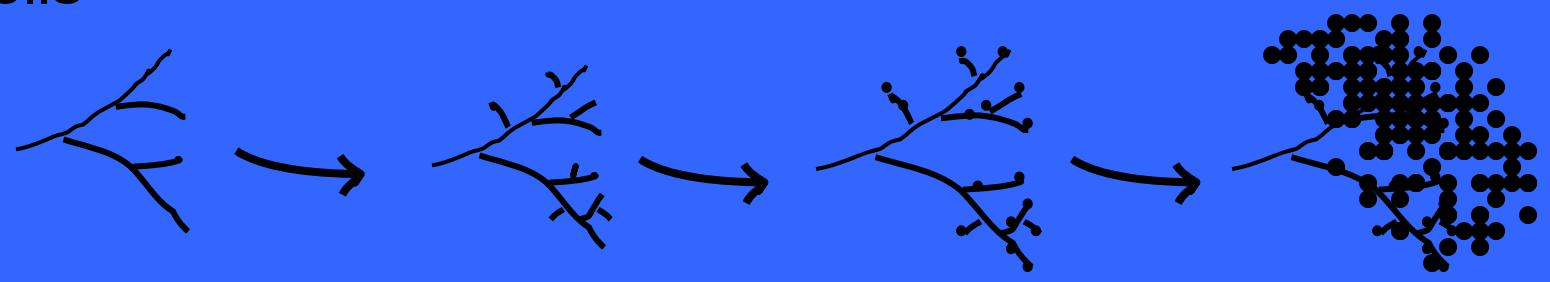


progesterone

wnt-4

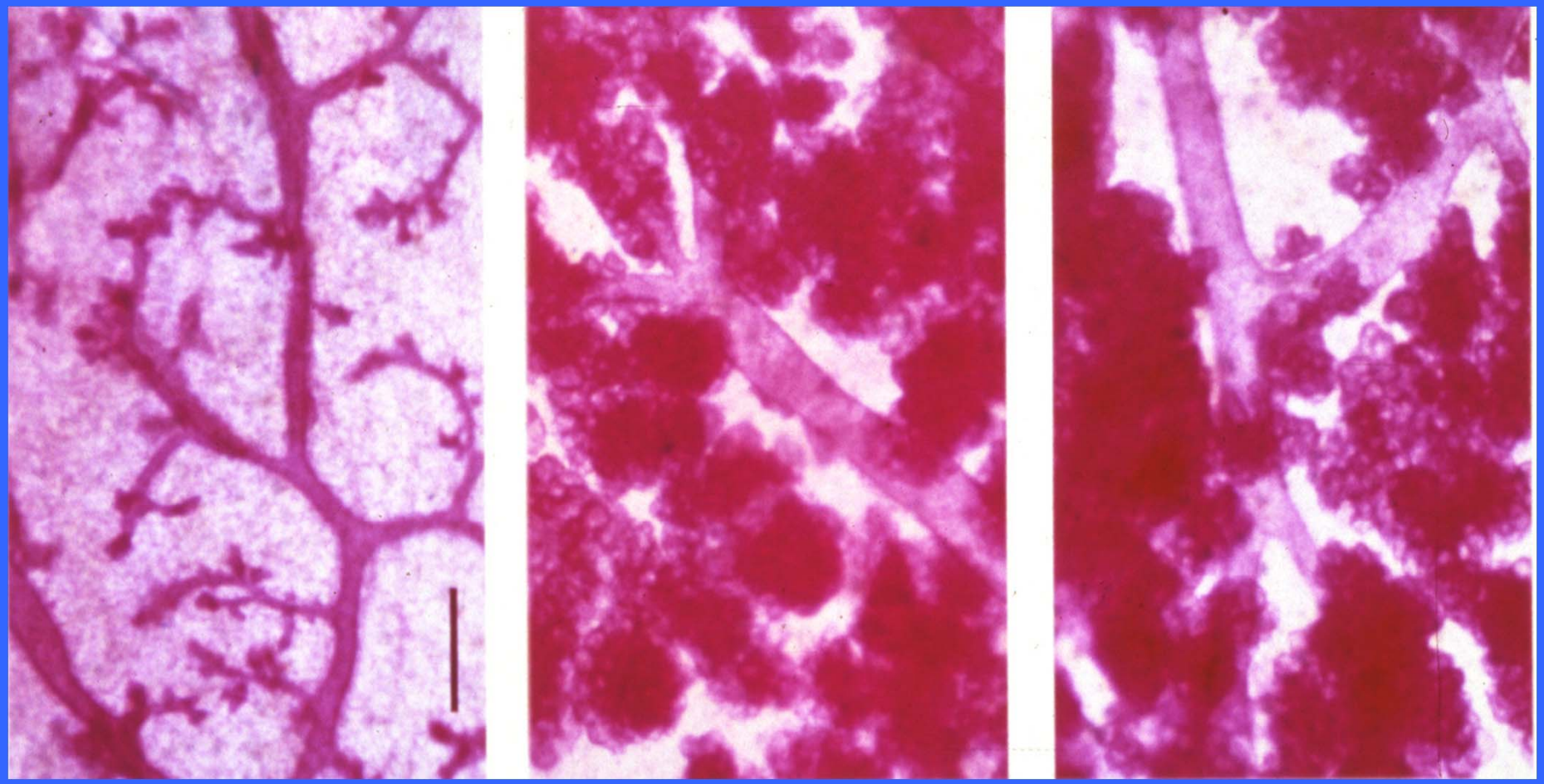
sidebranch

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells



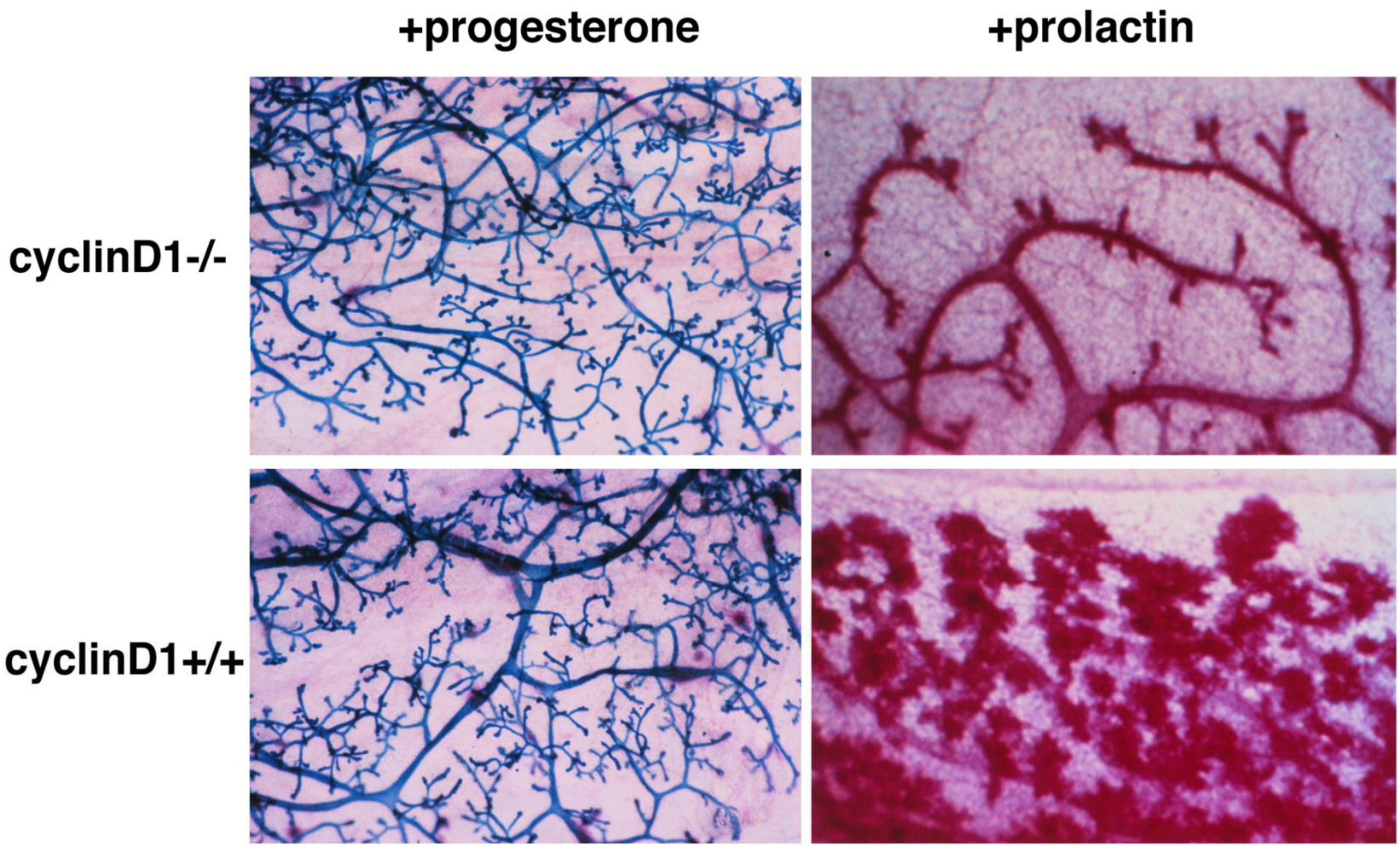
Epithelial transplant (end of pregnancy)

ProlactinR-/- **ProlactinR+/+** **control**

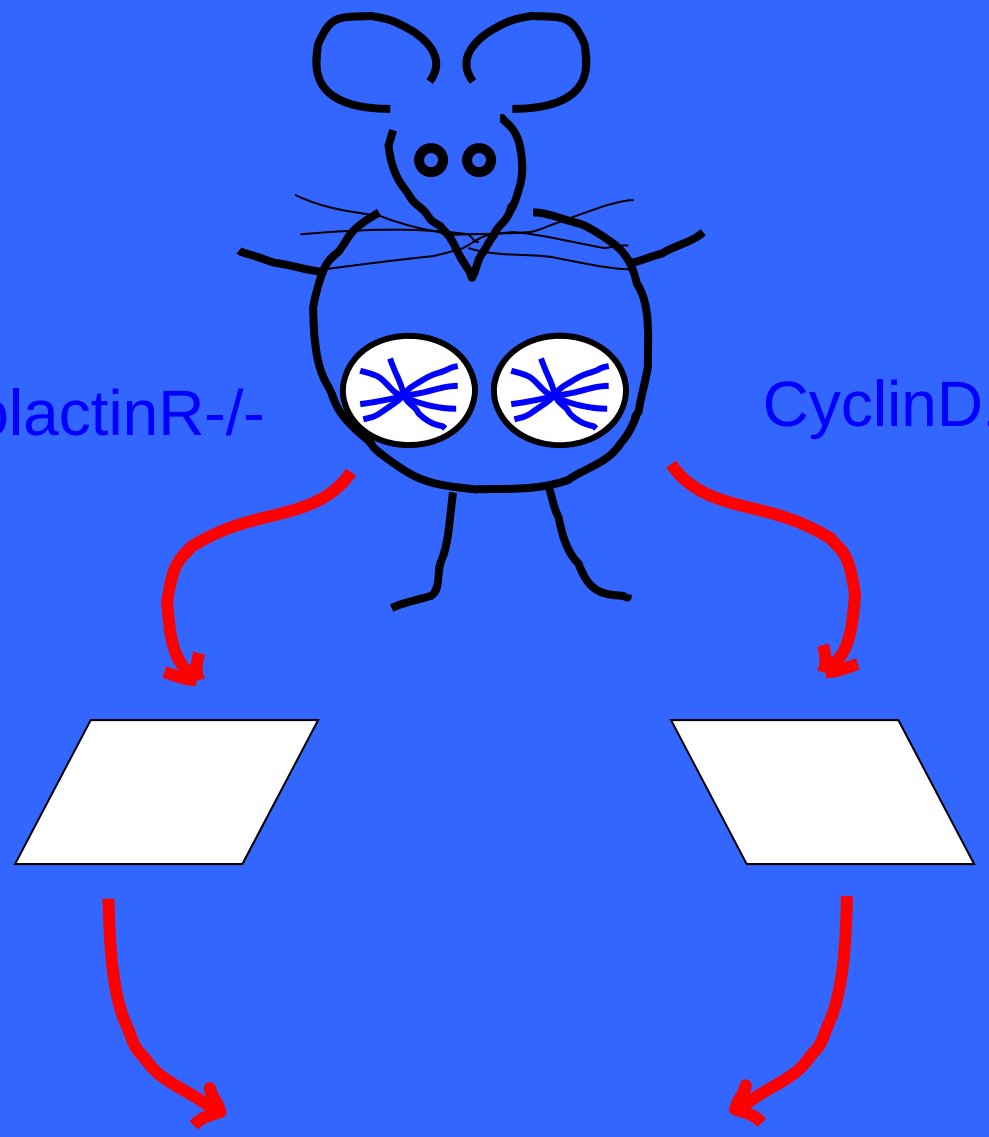


scalebar = 200μm

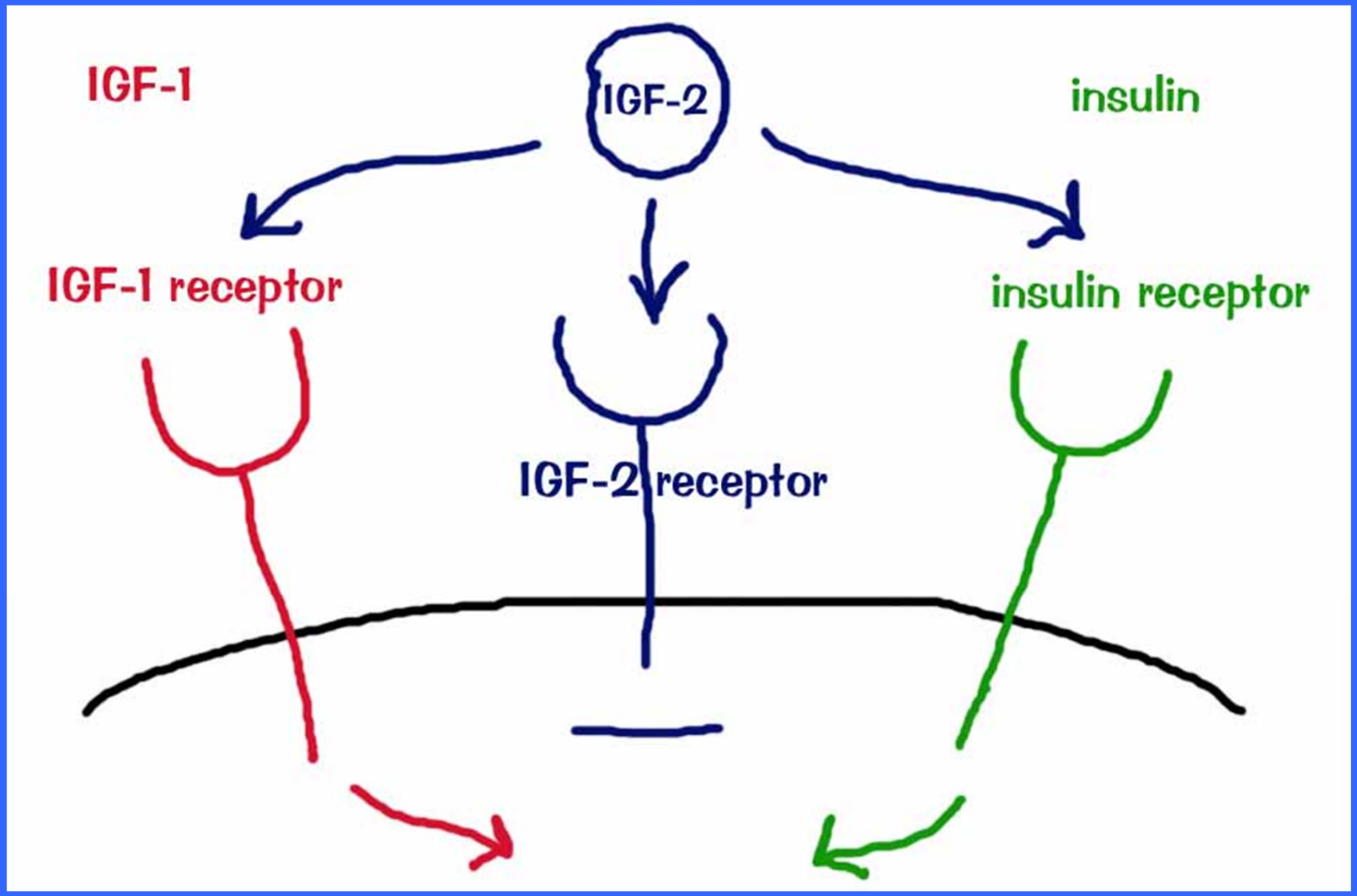
Response of cyclin D1$^{-/-}$ mammary epithelium to pregnancy hormones



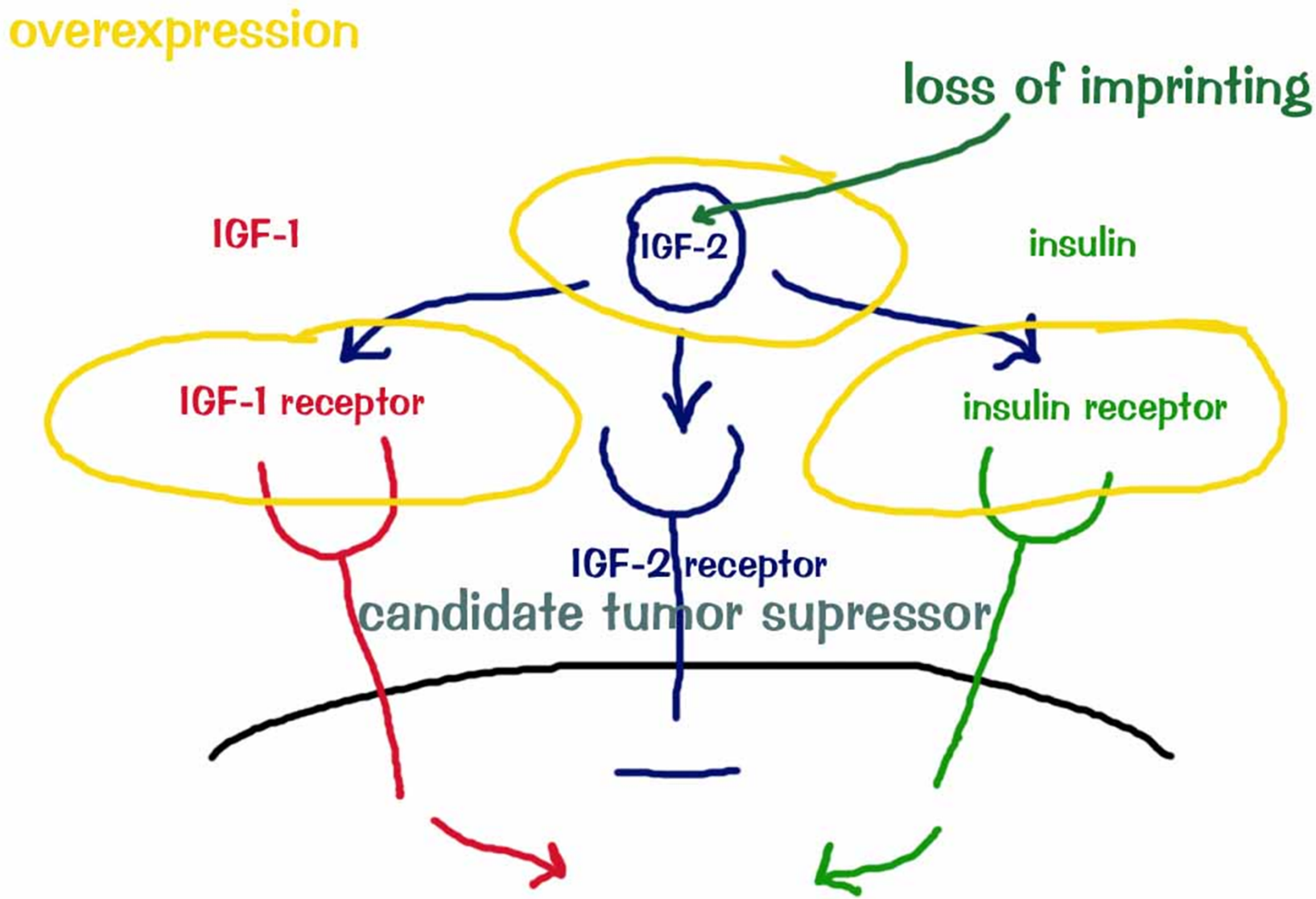
Pregnant mouse:



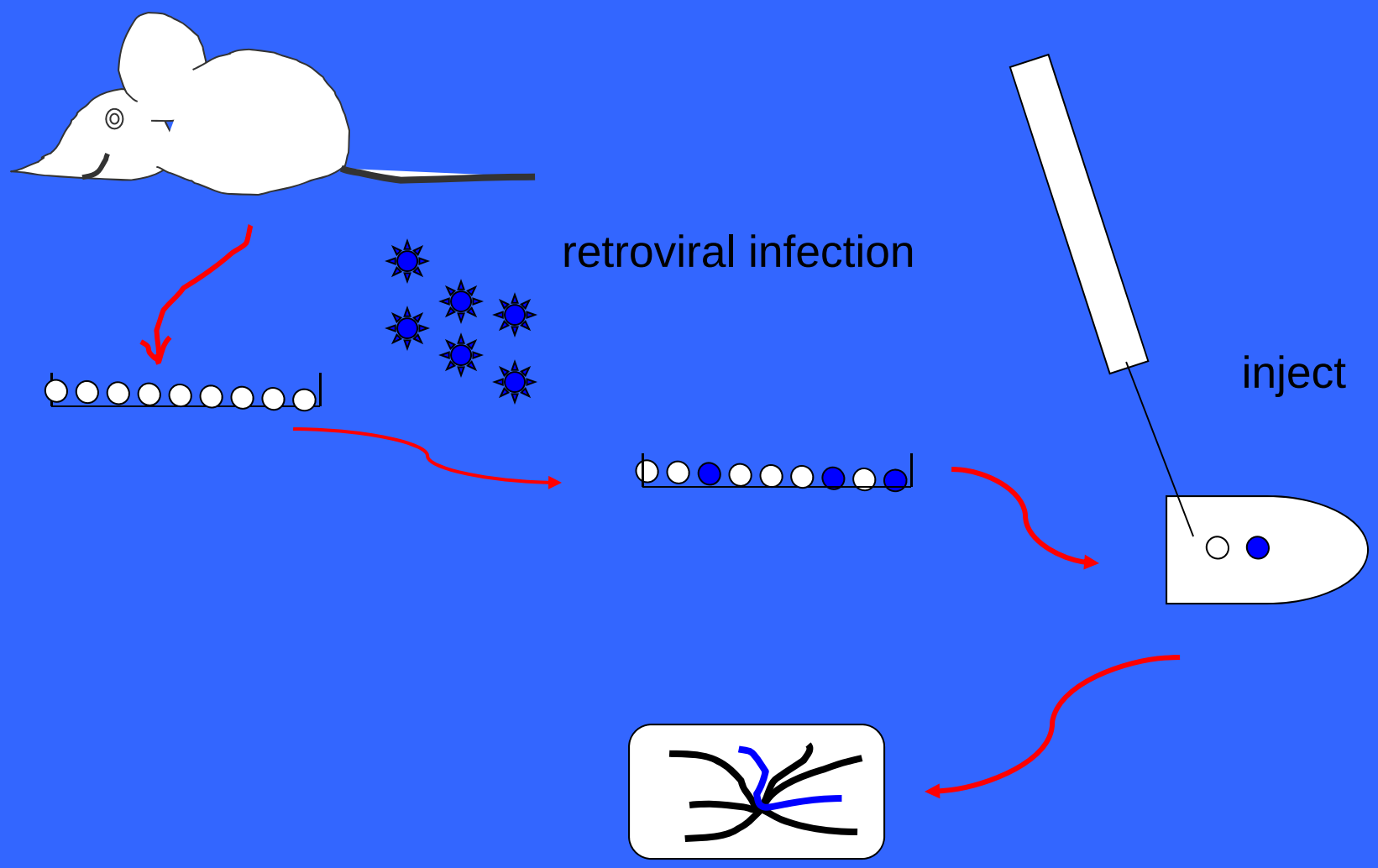
identify prolactin target genes


IGF-1
IGF-2
insulin
IGF-1 receptor
insulin receptor
IGF-2 receptor

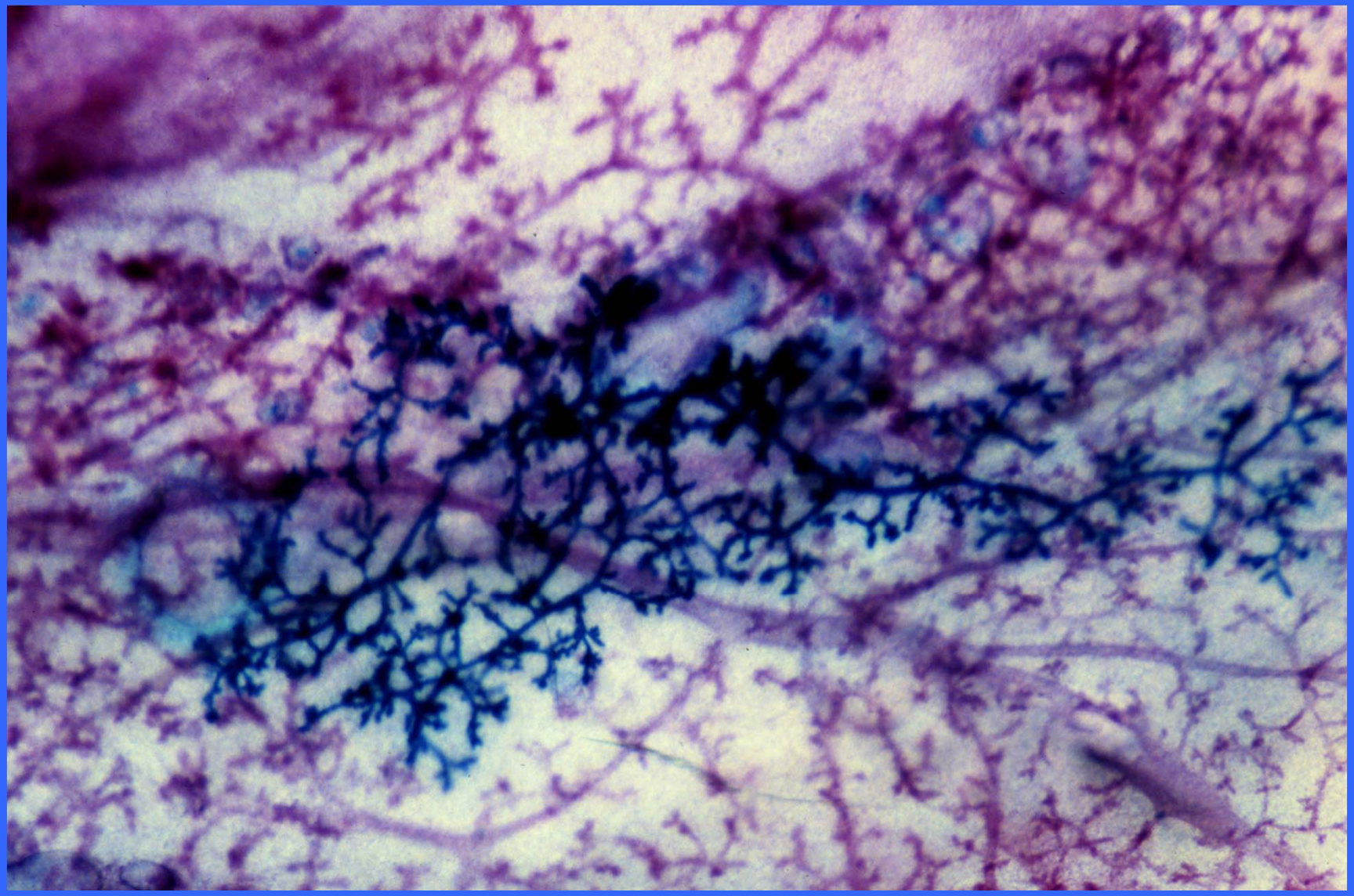
Deregulation of IGF-2 signaling in breast cancer



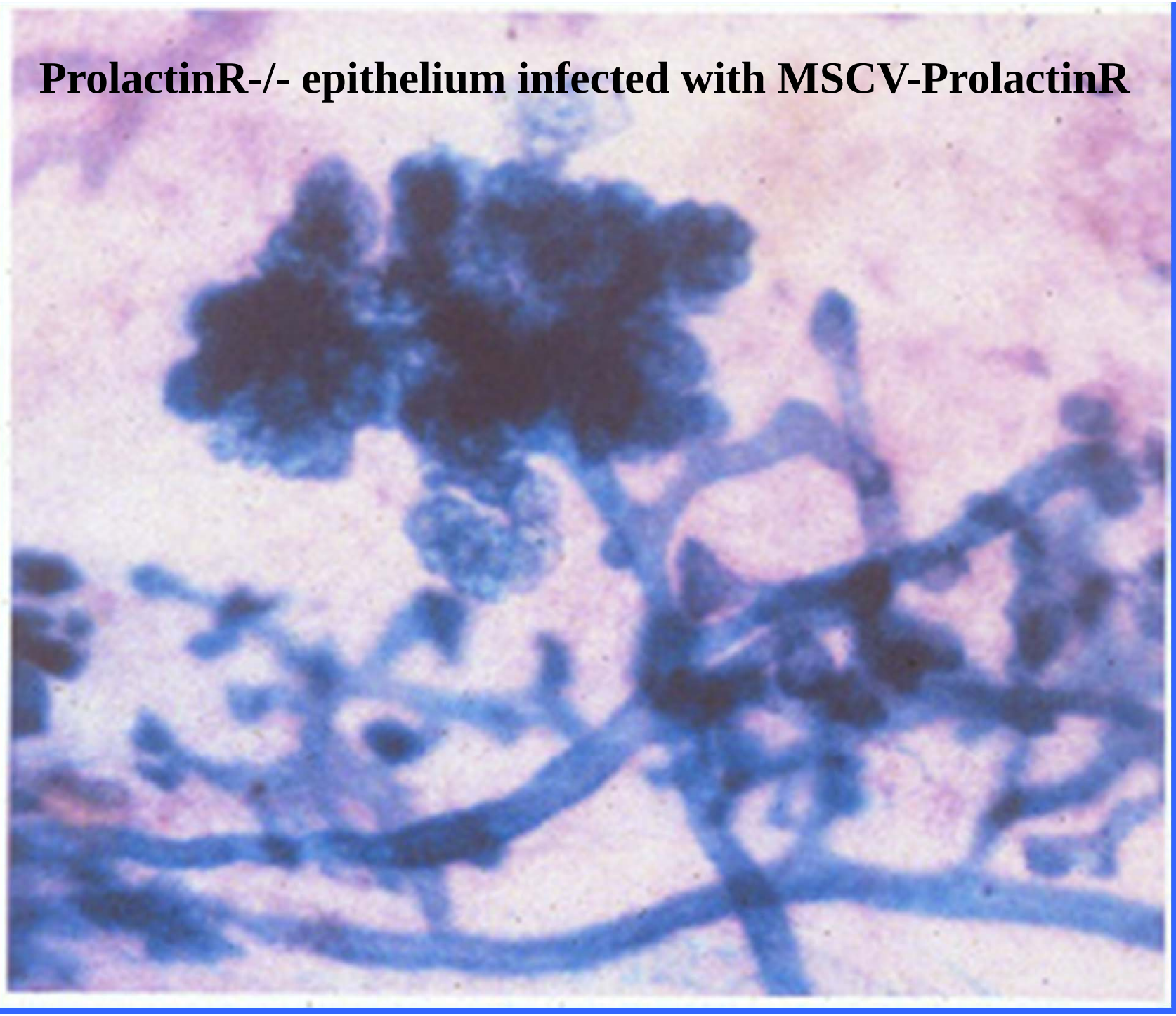
Genetic manipulation of the mammary gland



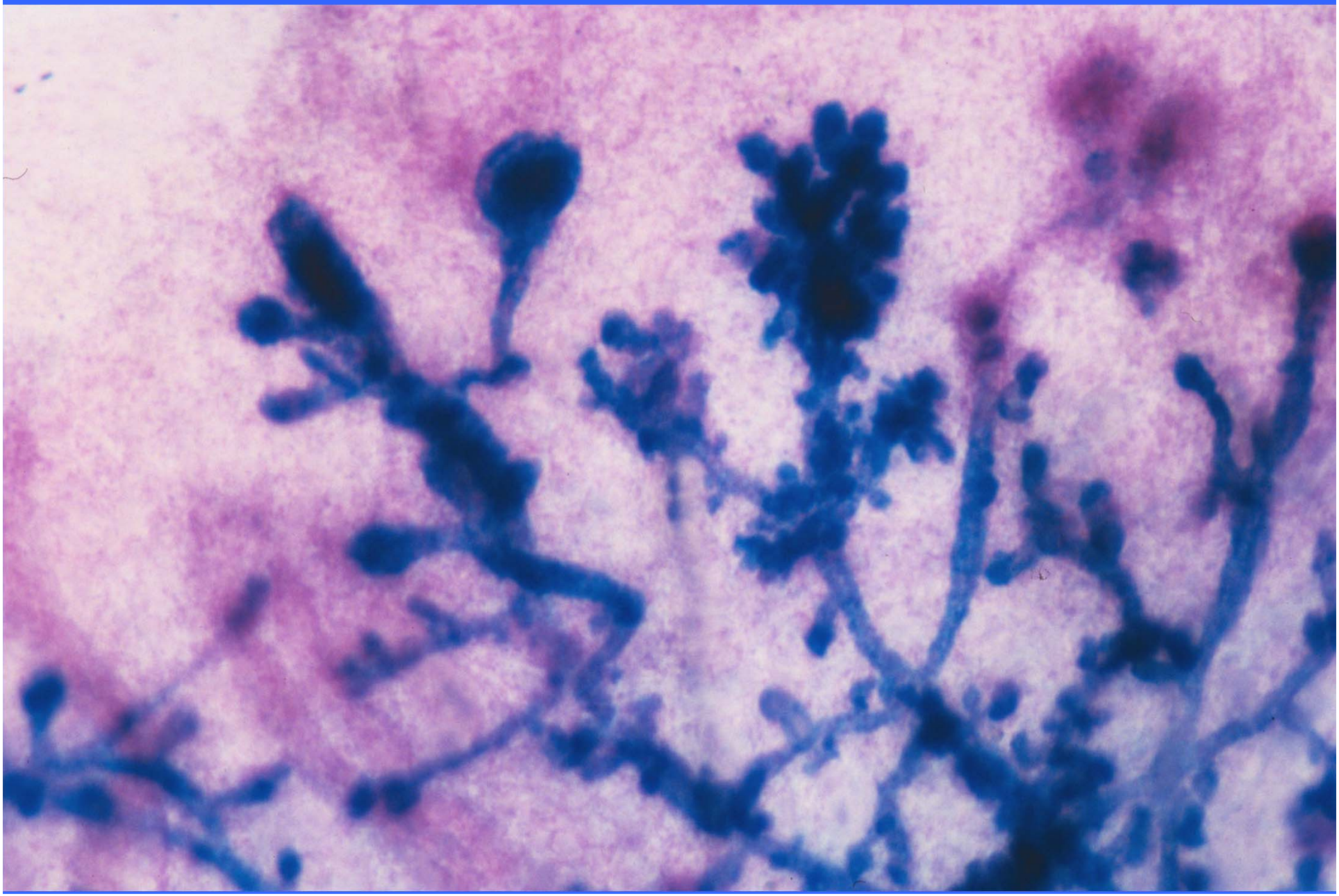
Reconstituted mammary gland: retroviral β-galactosidase



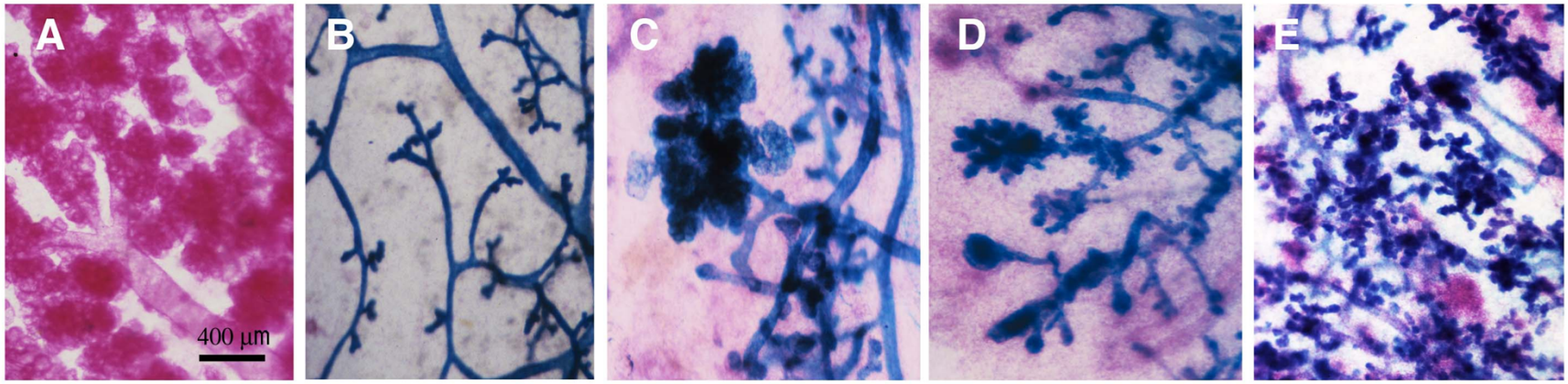
ProlactinR-/- epithelium infected with MSCV-ProlactinR



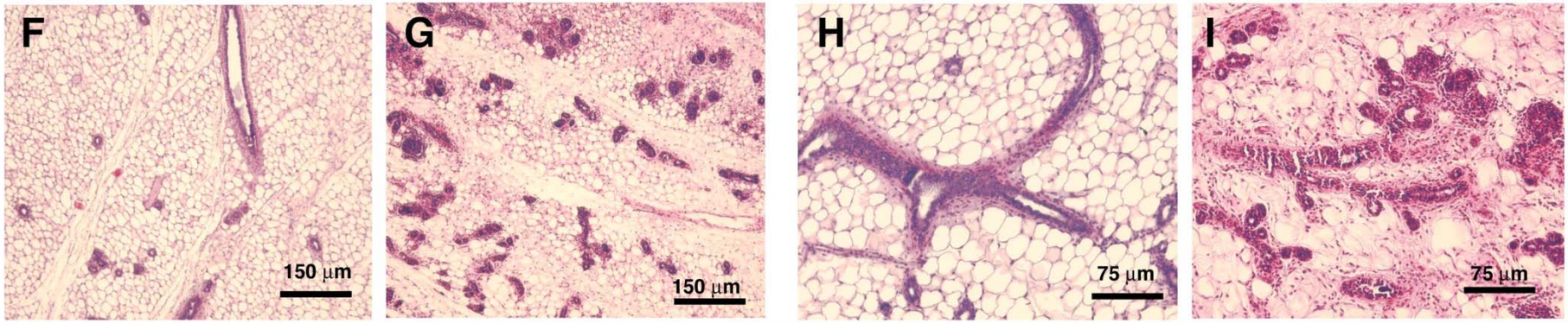
ProlactinR-/- epithelium infected with MSCV- IGF-2



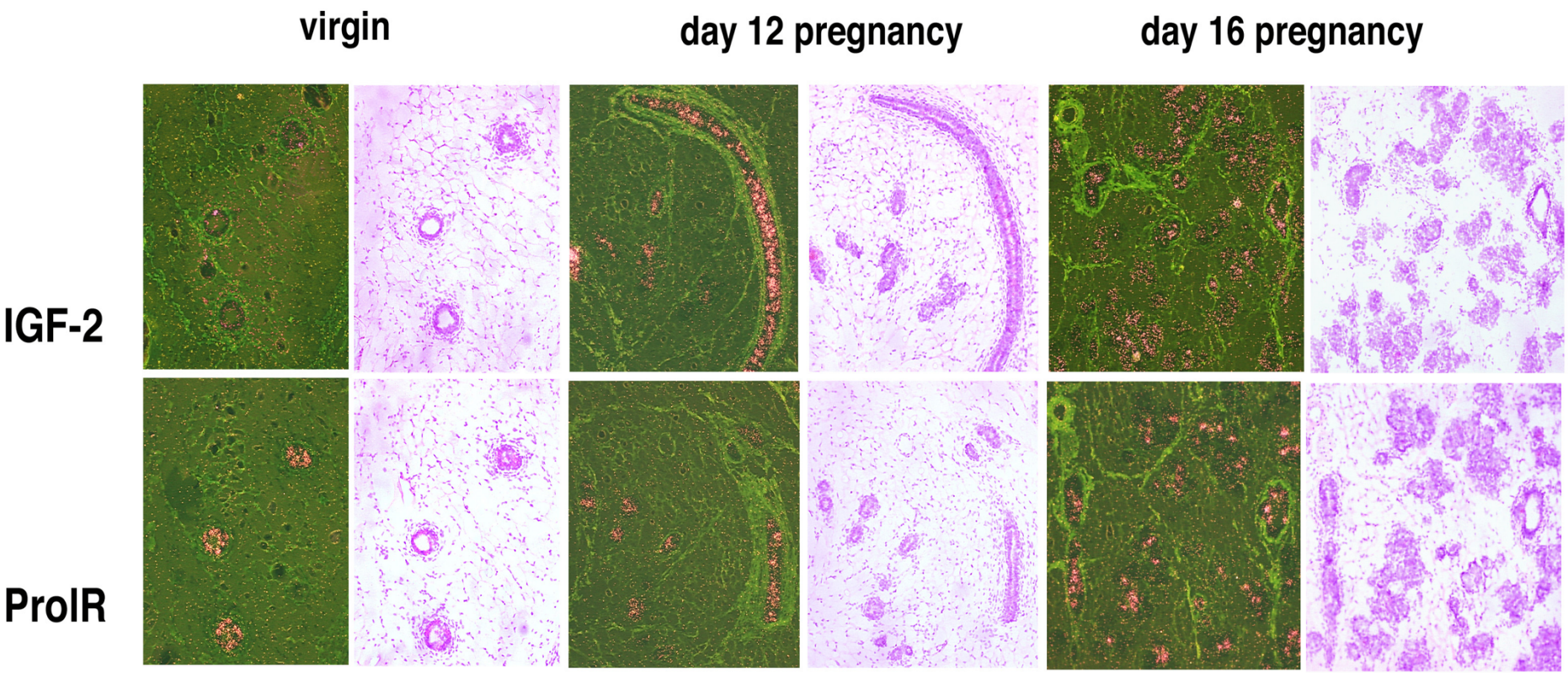
epithelium:	wild type	PrIR -/-.ROSA26+/-	PrIR -/-.ROSA26+/-	PrIR -/-.ROSA26+/-	PrIR -/-.ROSA26+/-
retrovirus:	-	-	PrIR	IGF-2	IGF-2

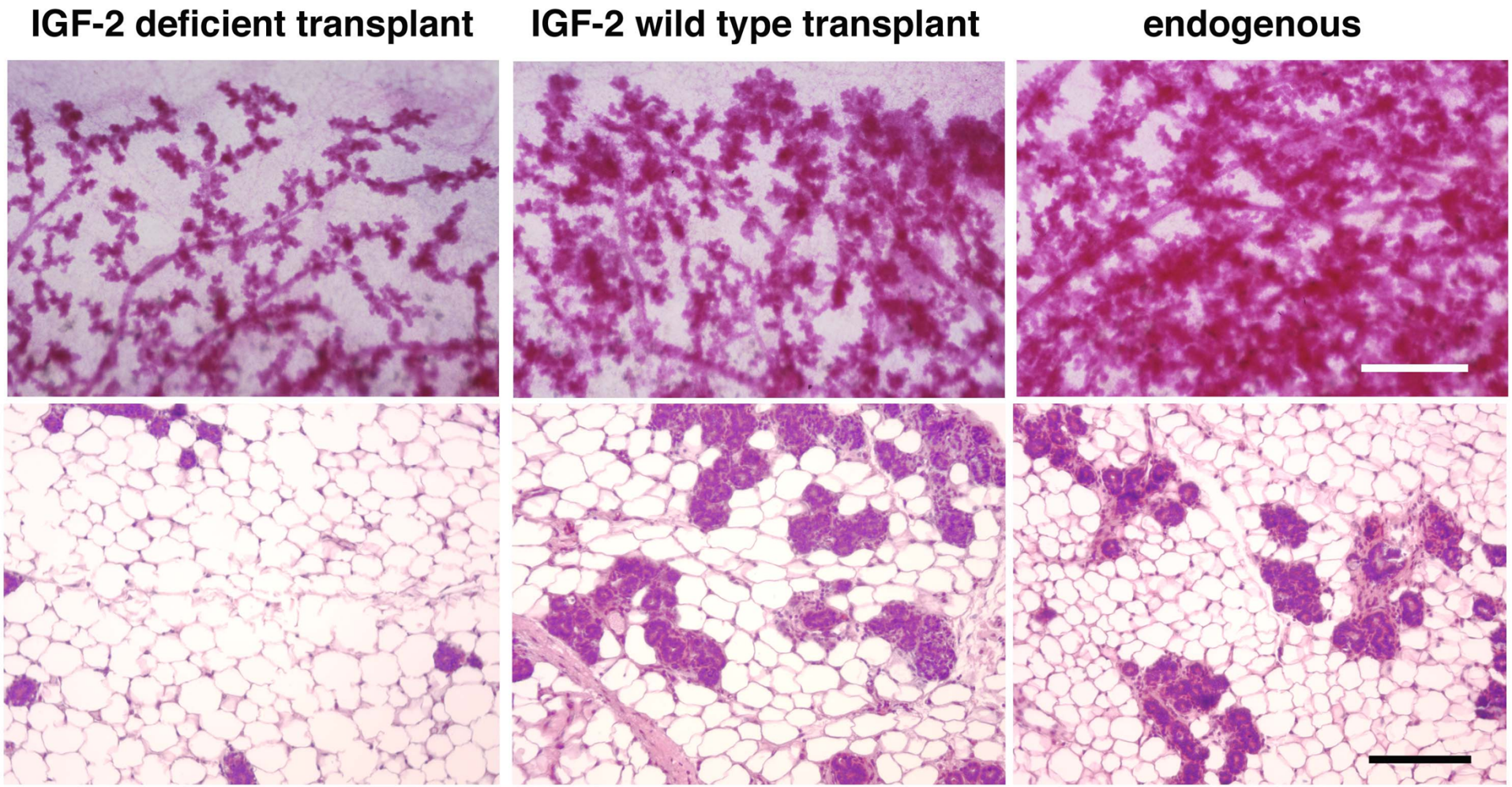


epithelium:	PrIR-/-	PrIR-/-	PrIR-/-	PrIR-/-
retrovirus:	-	IGF-2	-	IGF-2



Prolactin receptor and IGF-2 mRNA colocalize
-in situ hybridisation-



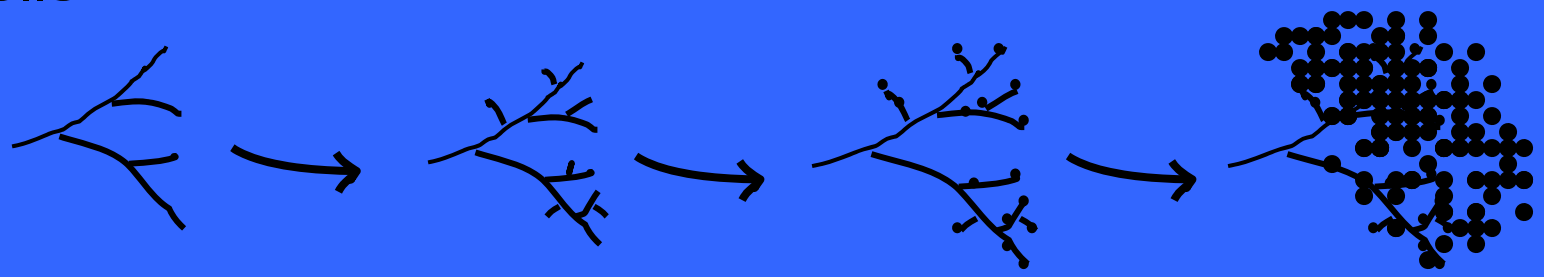

IGF-2 deficient transplant
IGF-2 wild type transplant
endogenous

prolactin

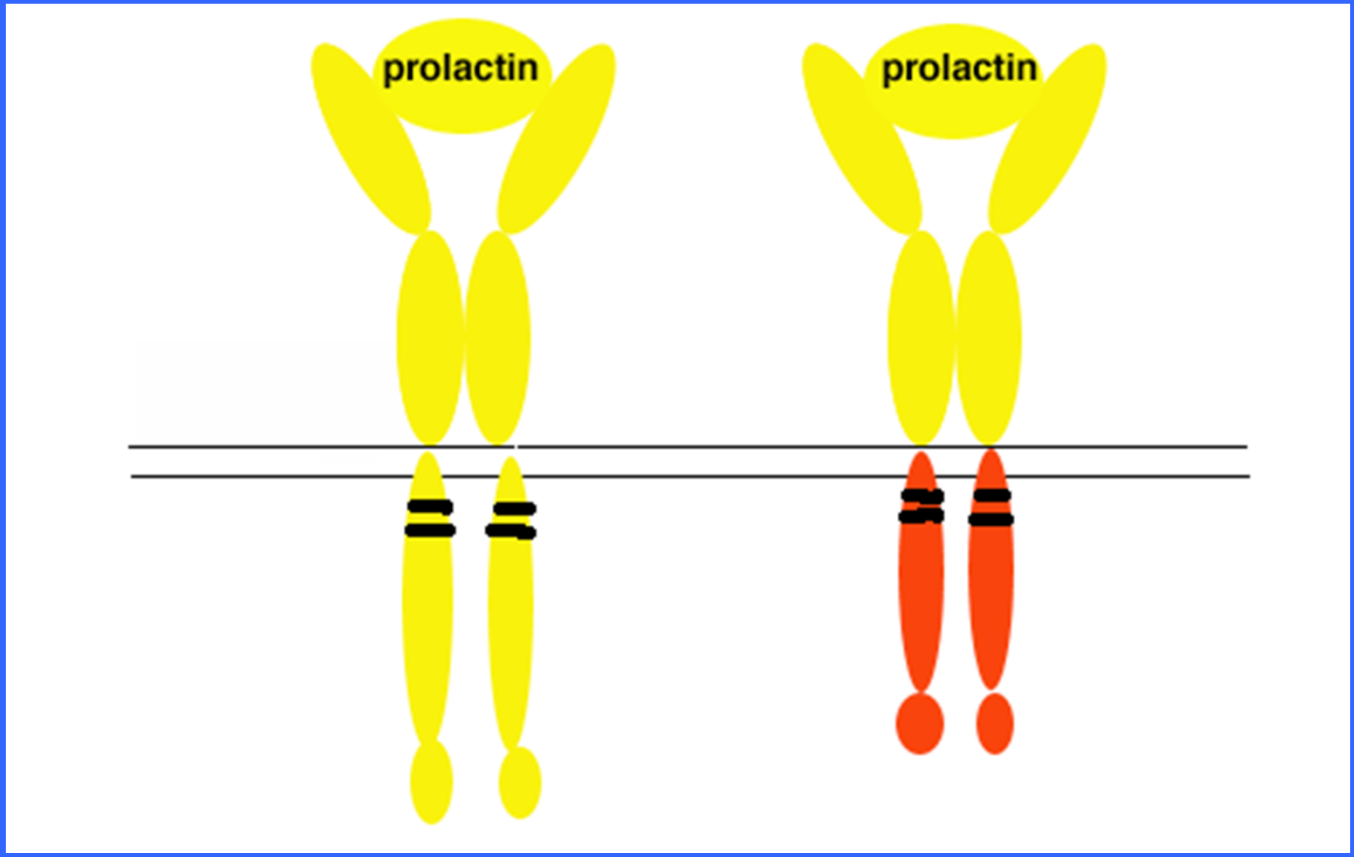
IGF-2

sidebranch

ductal cells → alveolar precursors → alveolar cells → milk-secreting cells

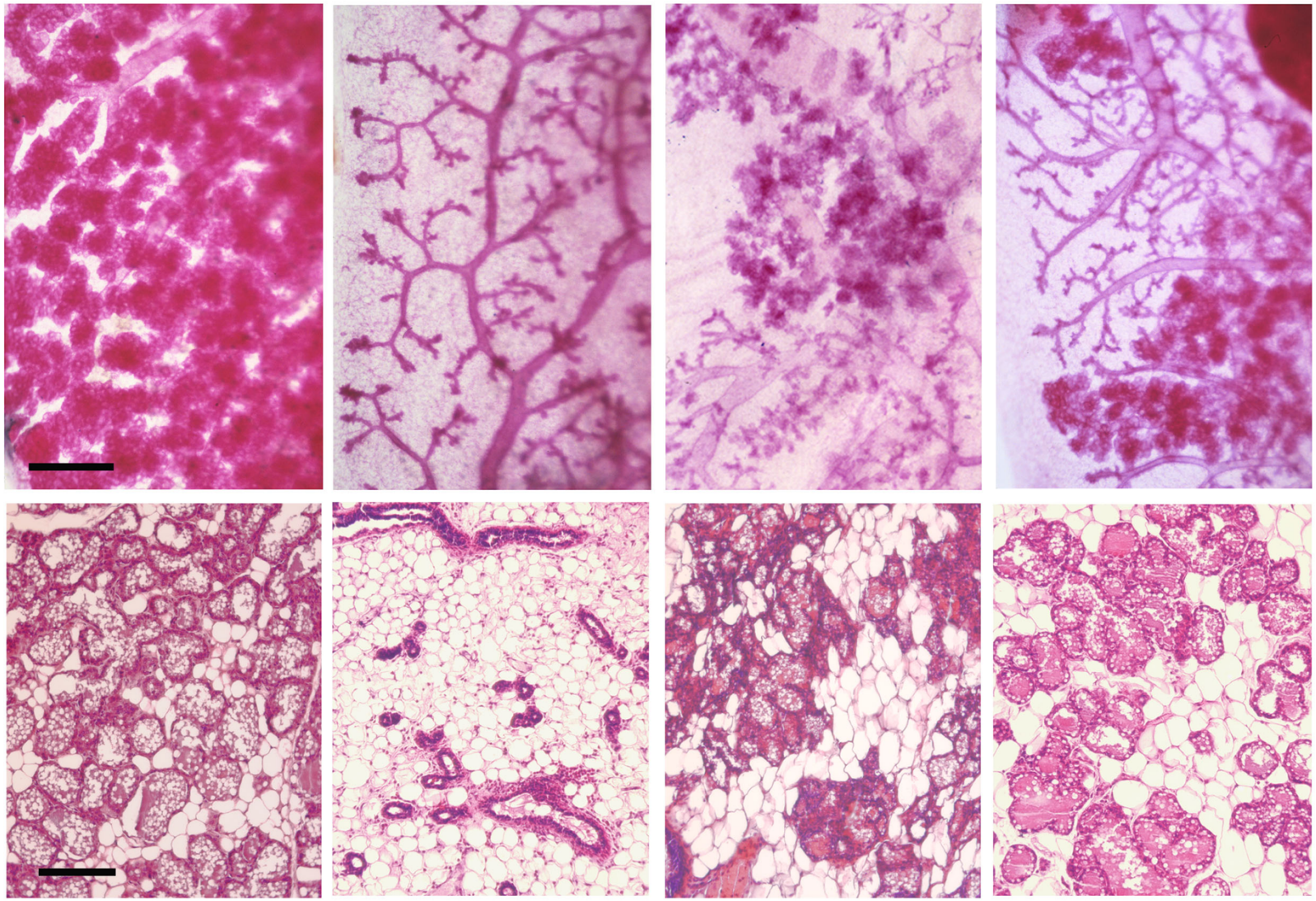


Prolactin and Prolactin-Erythropoietin Receptors

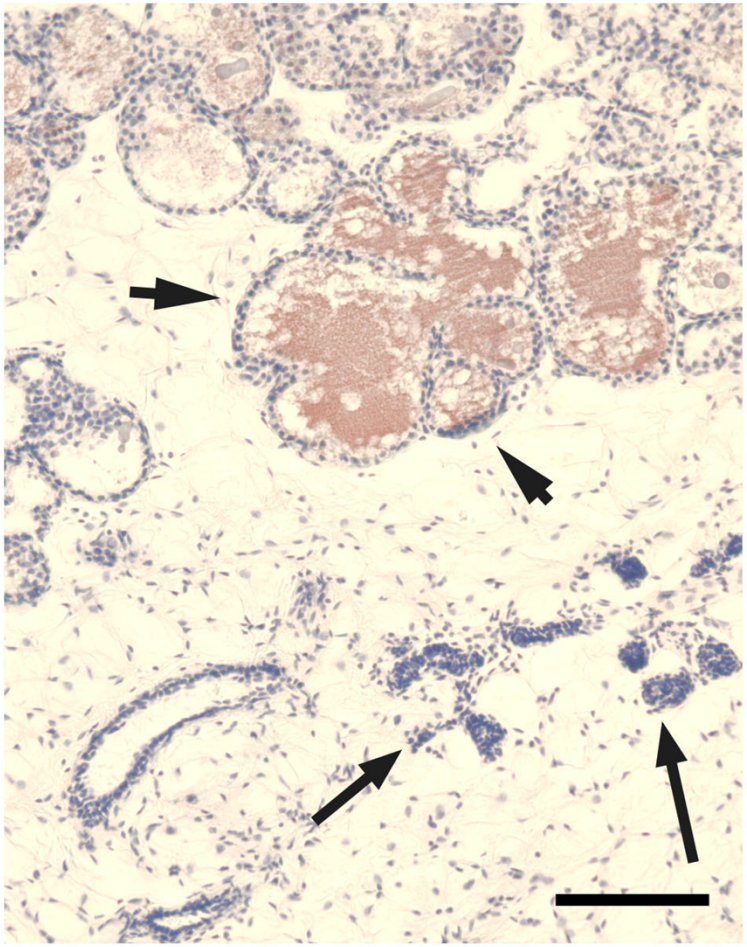


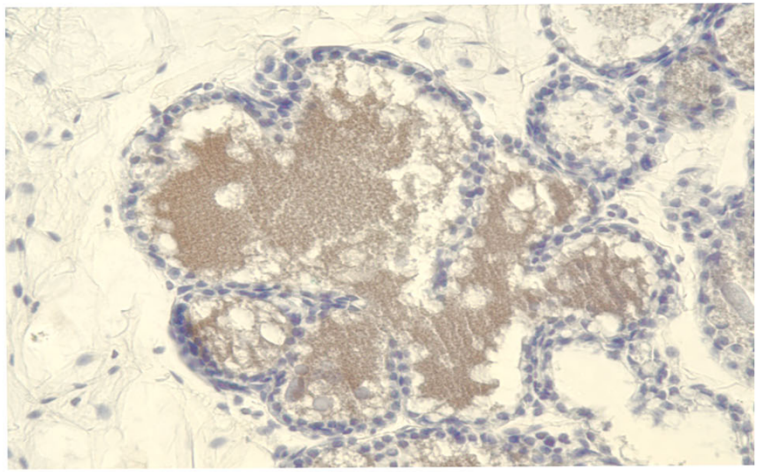
PrlR **Prl- EpoR**

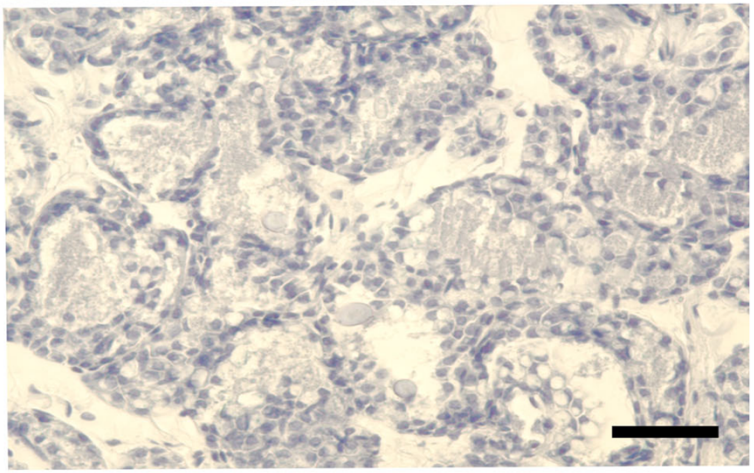
genotype of the epithelium	PrlR+/+	PrlR-/-	PrlR-/-	PrlR-/-
retrovirus	-	-	PrlR	prl-EpoR



β-casein expression







Acknowledgements

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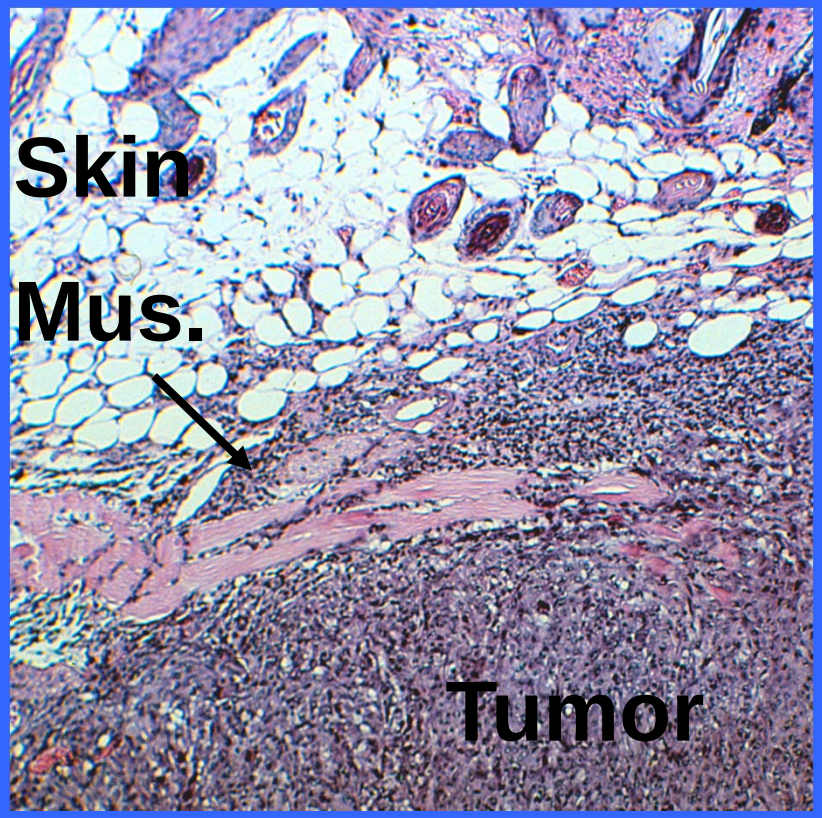
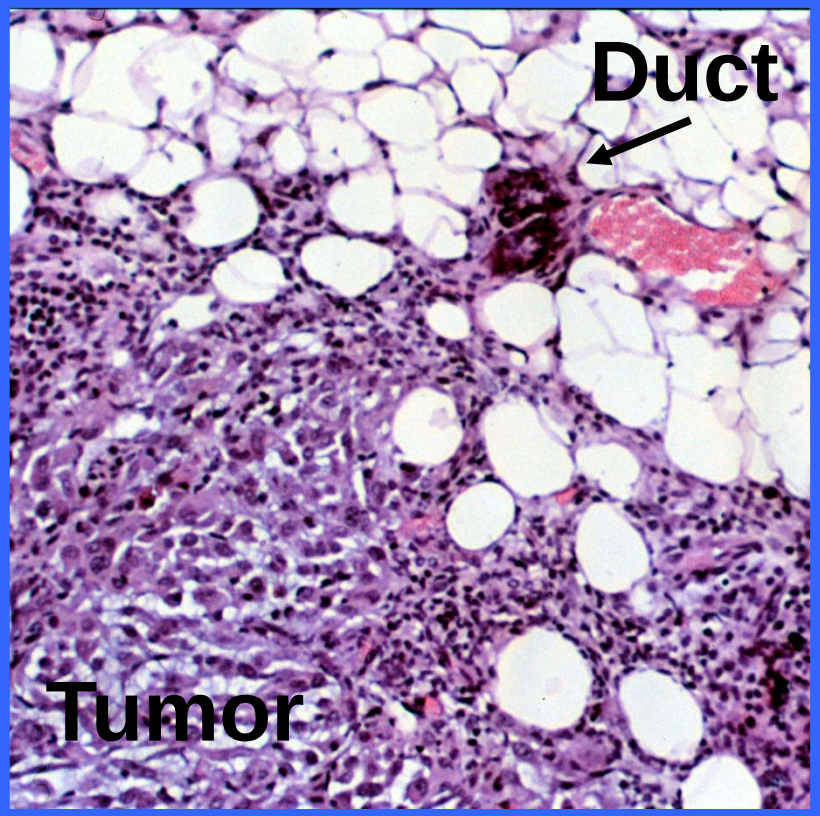
Peter Scinski
Jim Ihle
Chris Ormandy
Jian Tian & SK Dey
Merav Sokolovsky
Harvey Lodish

Tumorigenic human mammary epithelial cell

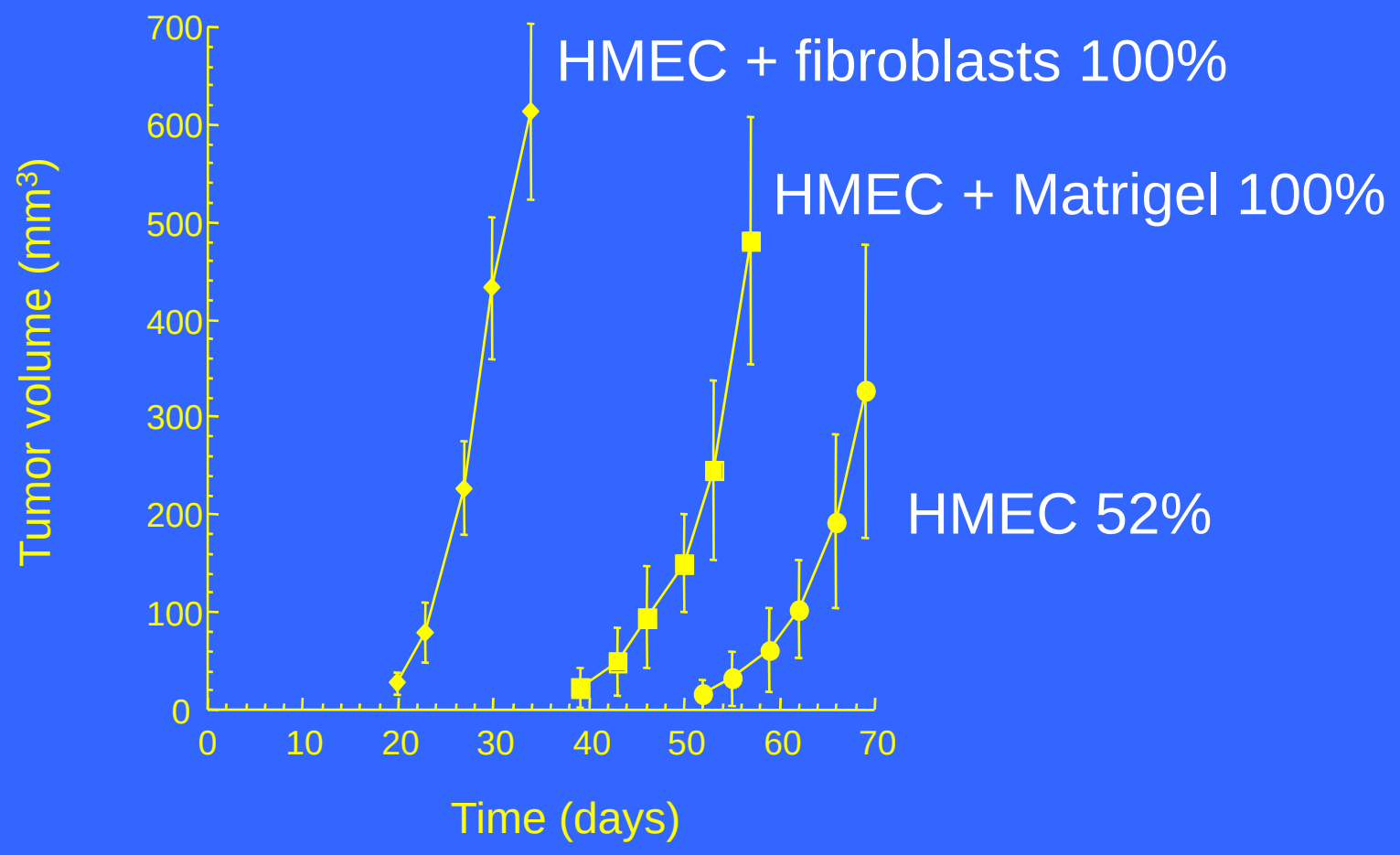


Subcutaneous

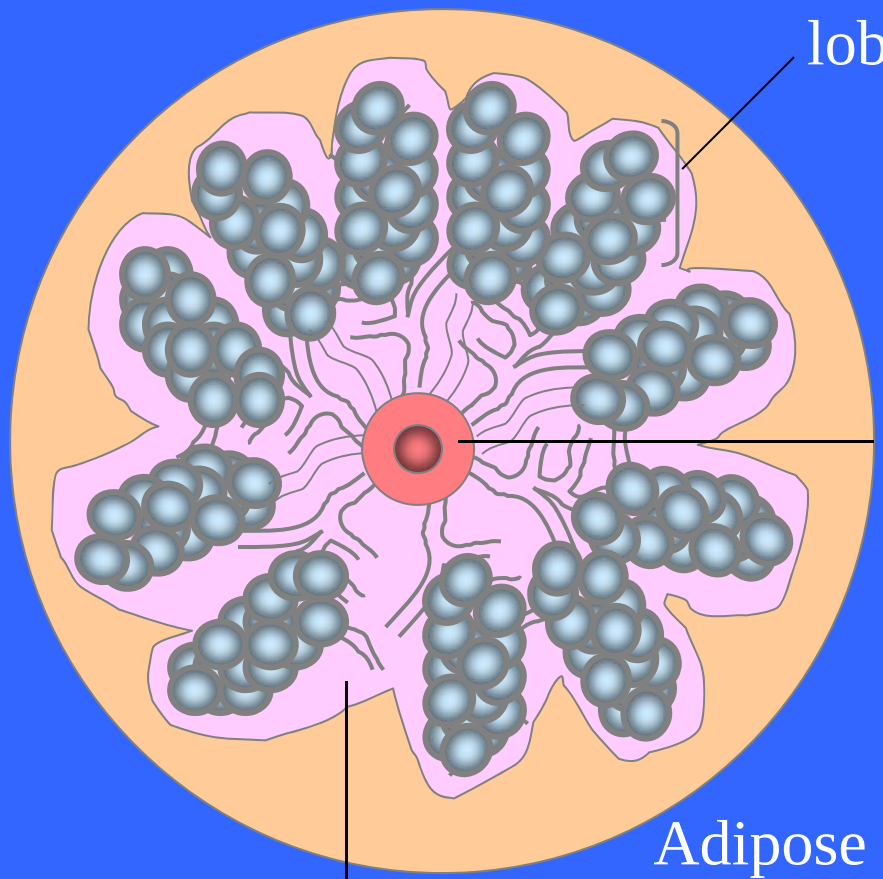


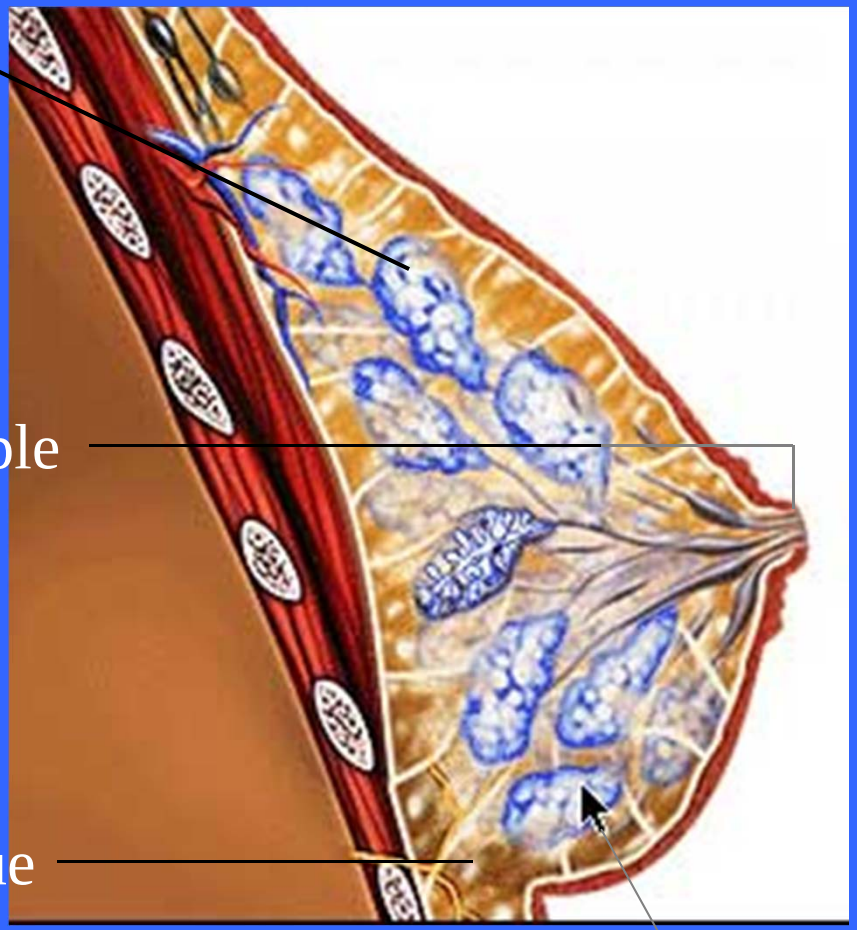
Mammary gland

Enhanced tumorigenicity with Matrigel or primary mammary fibroblasts



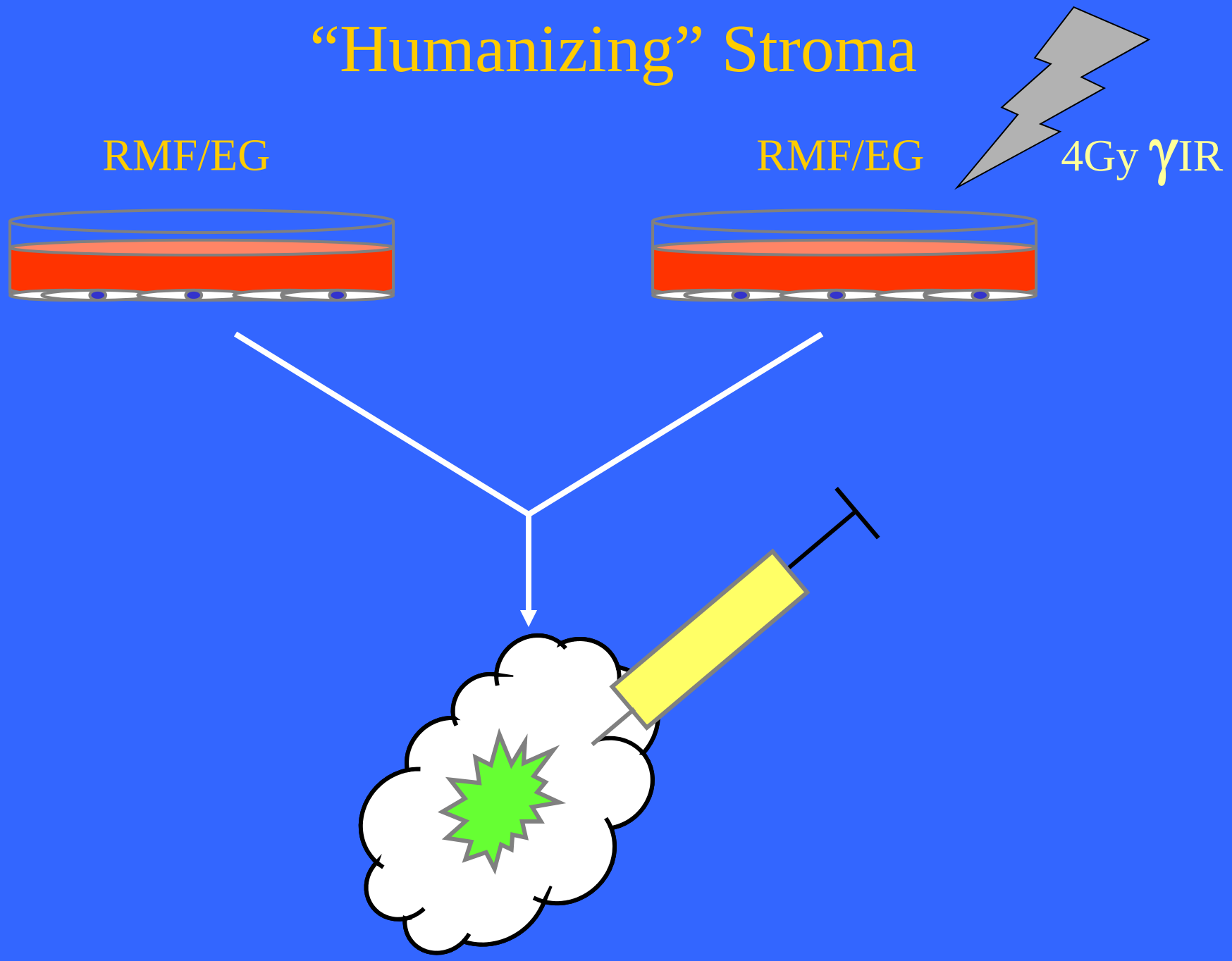
Anatomy of the Human Breast



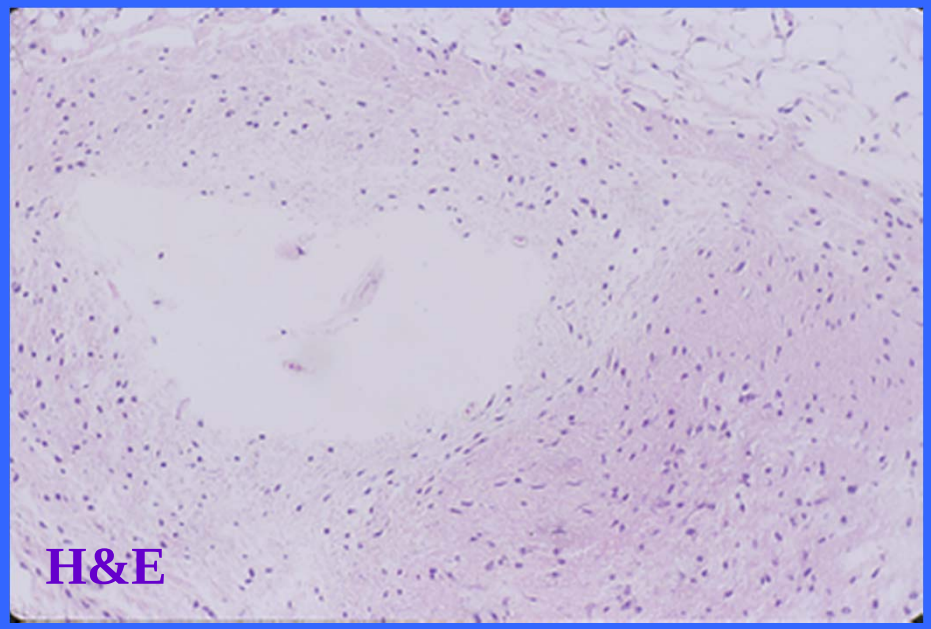


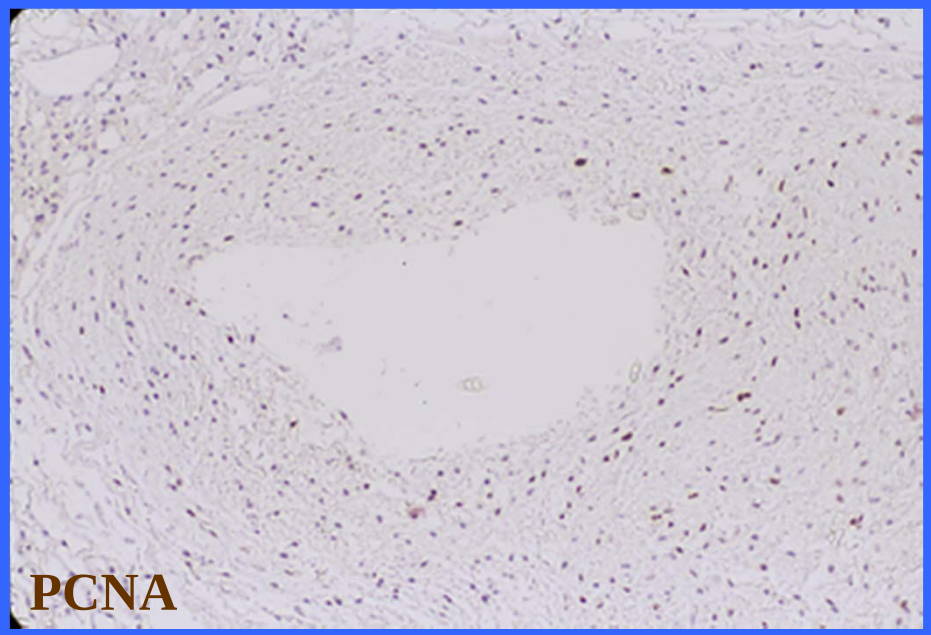
TDLU = ductles + lobule + terminal ducts

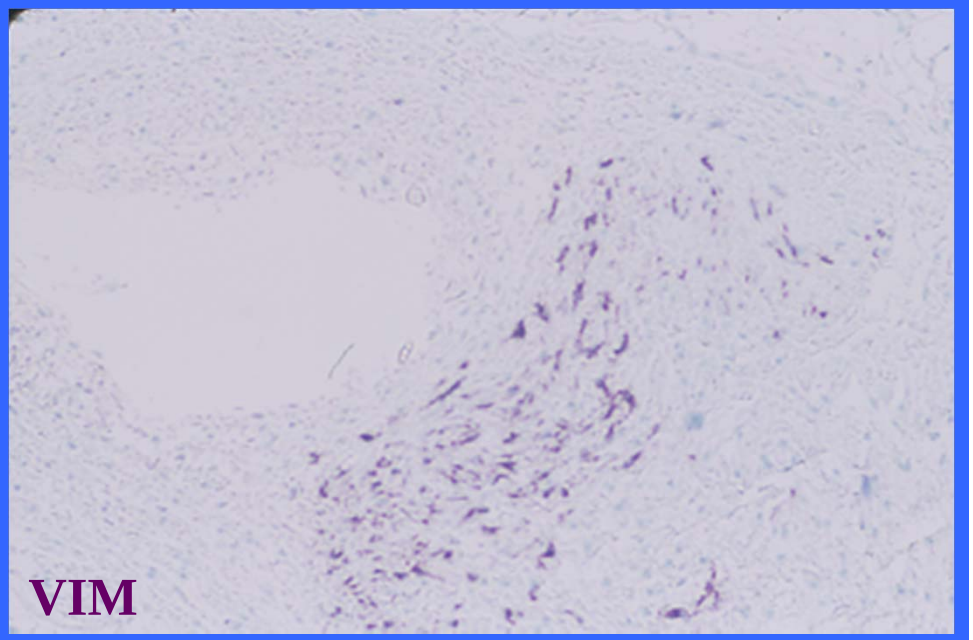
“Humanizing” Stroma



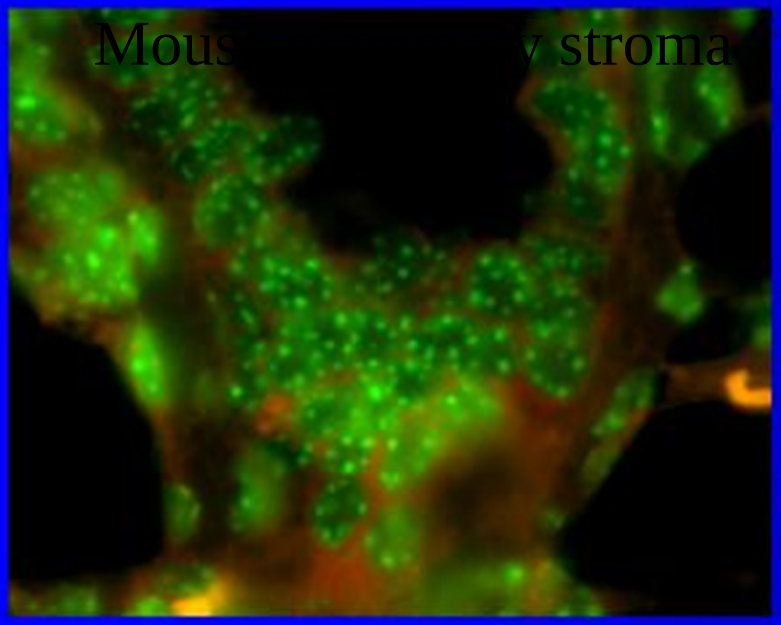
Human Breast Fibroblasts Proliferate and Intermingle with Mouse Stroma in Cleared Fat Pad



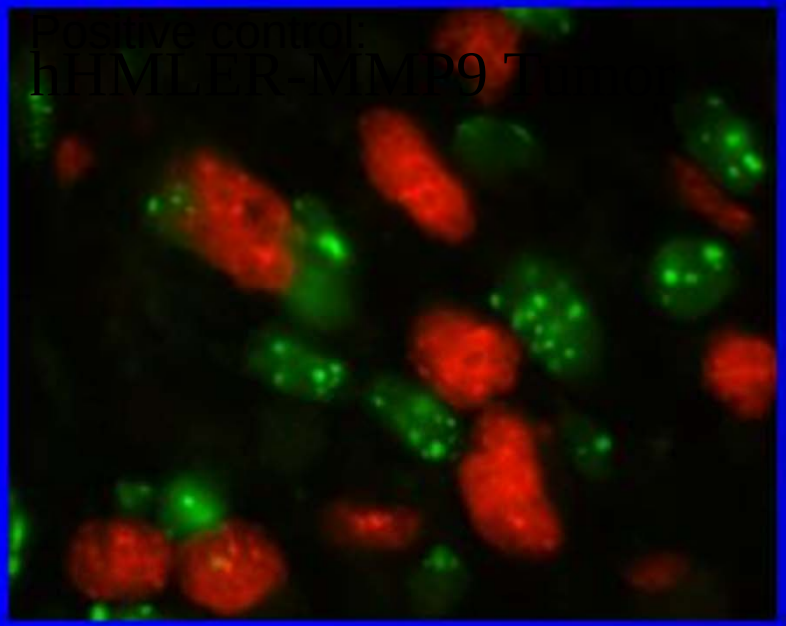


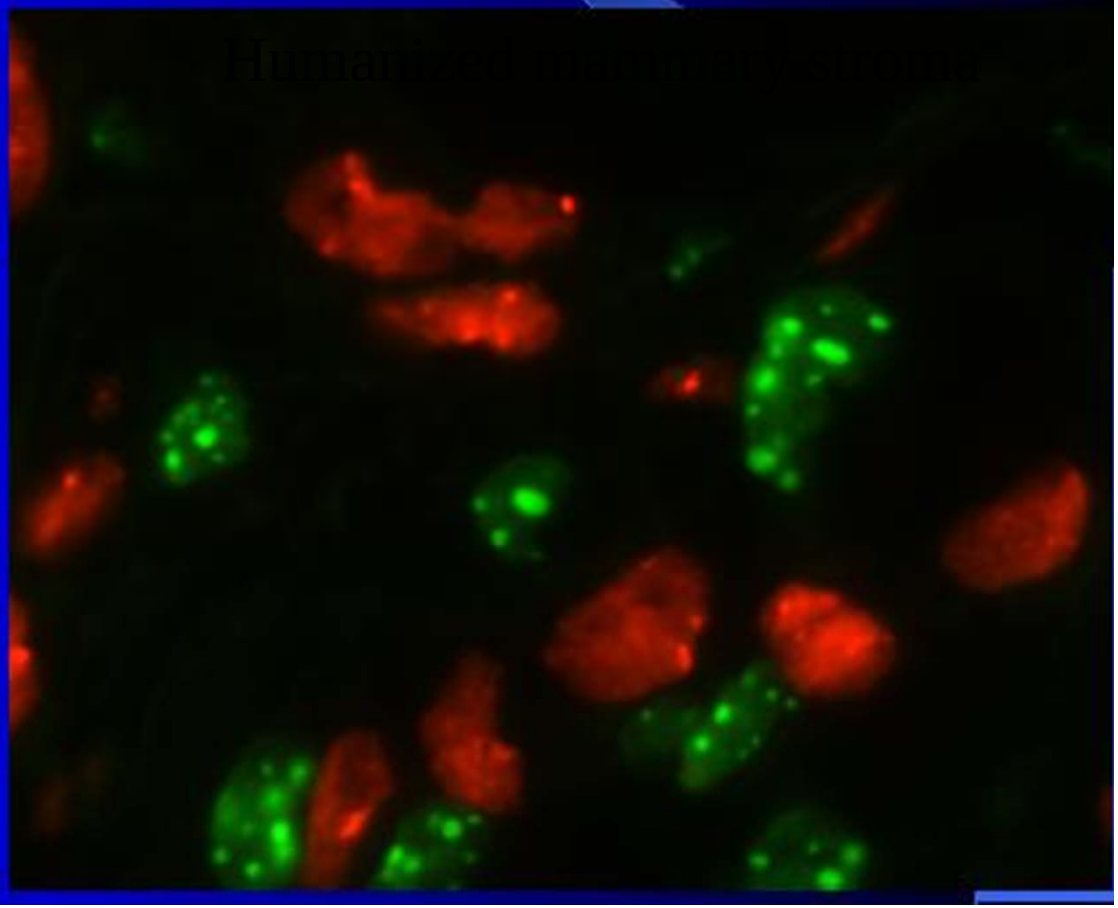


Genomic FISH on “Humanized” Stroma

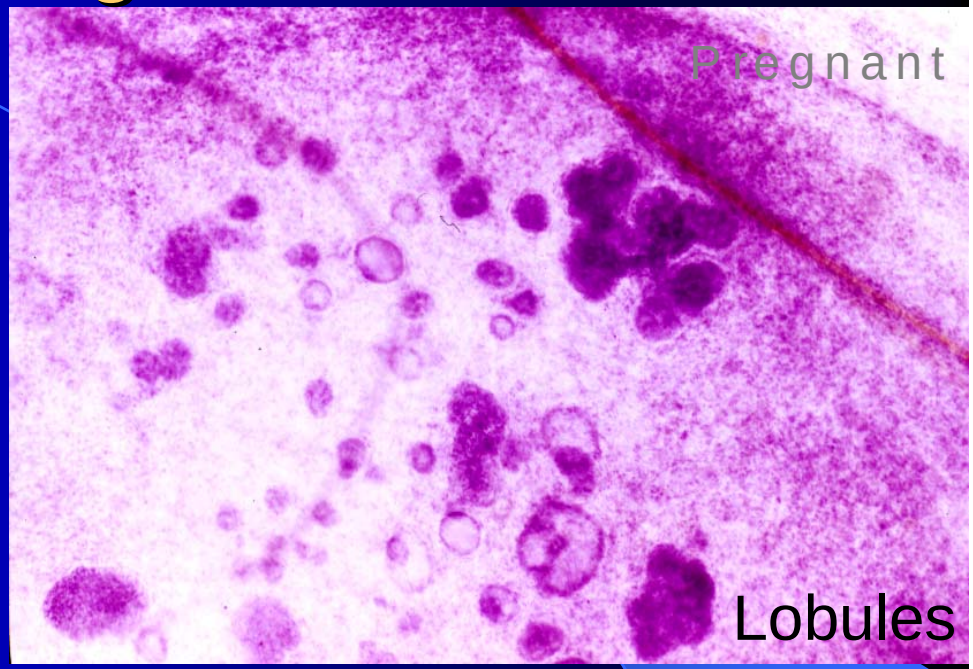


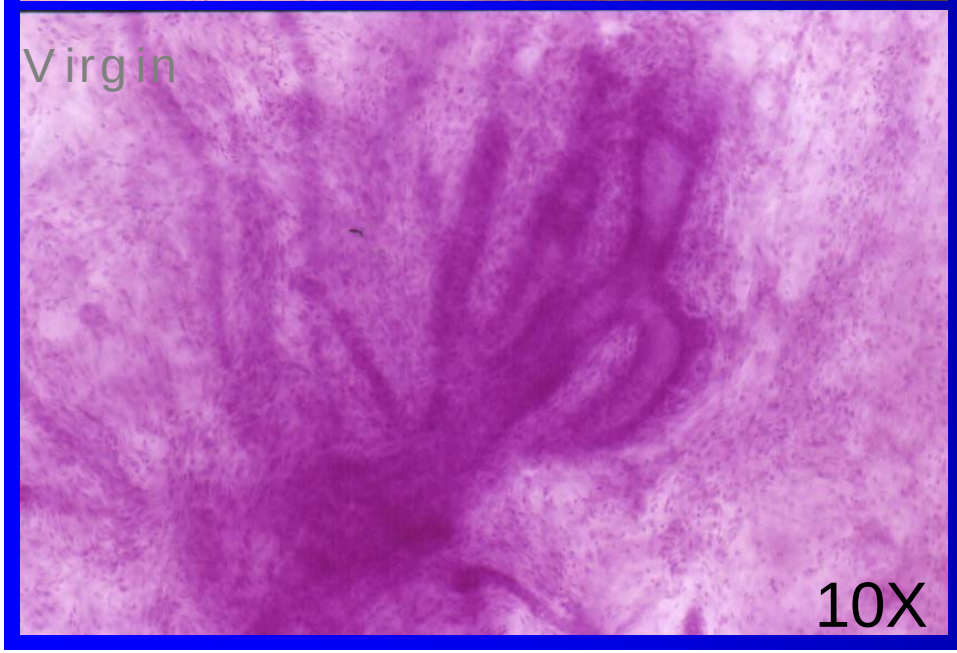
Mouse Cot I DNA
Human Genomic DNA

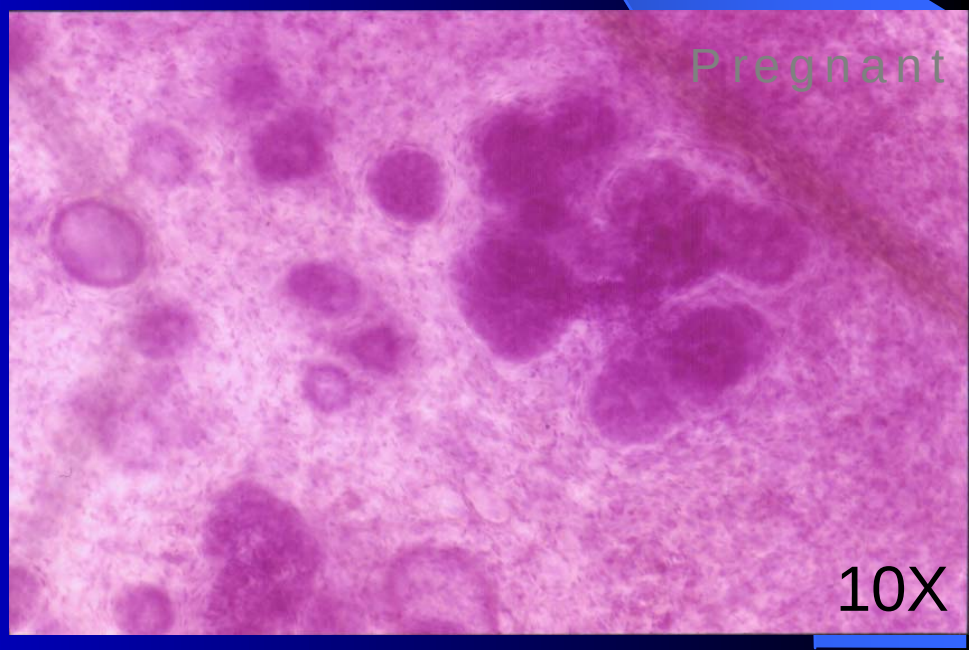




Wholemount of Xenograft Breast Tissue





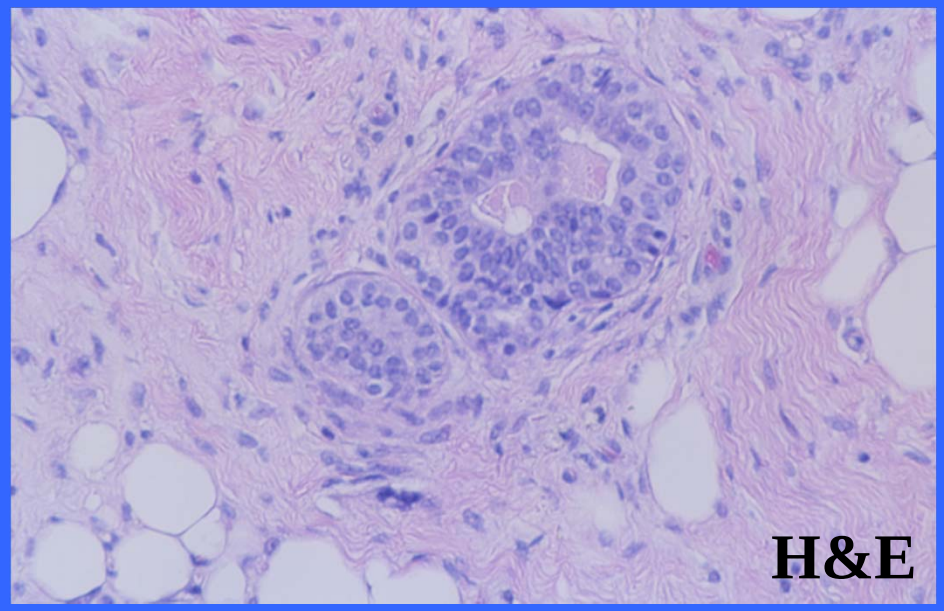


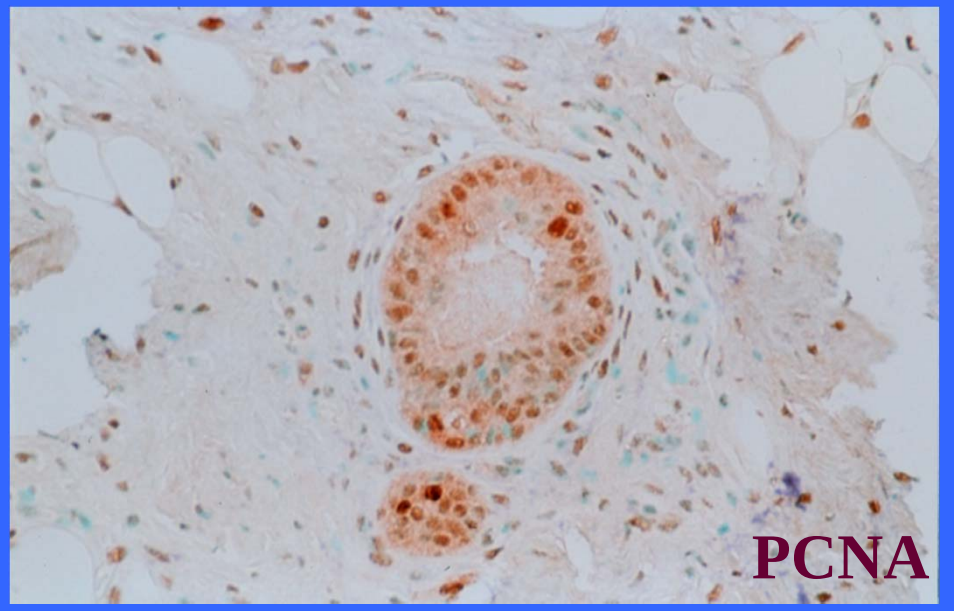
Genomic FISH of Normal Human Organoids engrafted in a “humanized” mammary fat pad

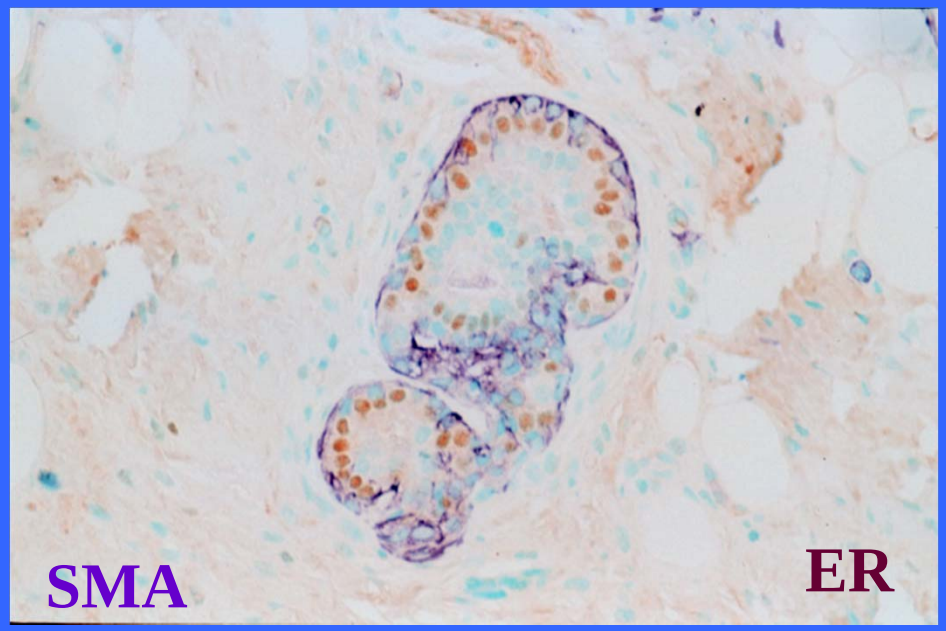
Human Genomic DNA

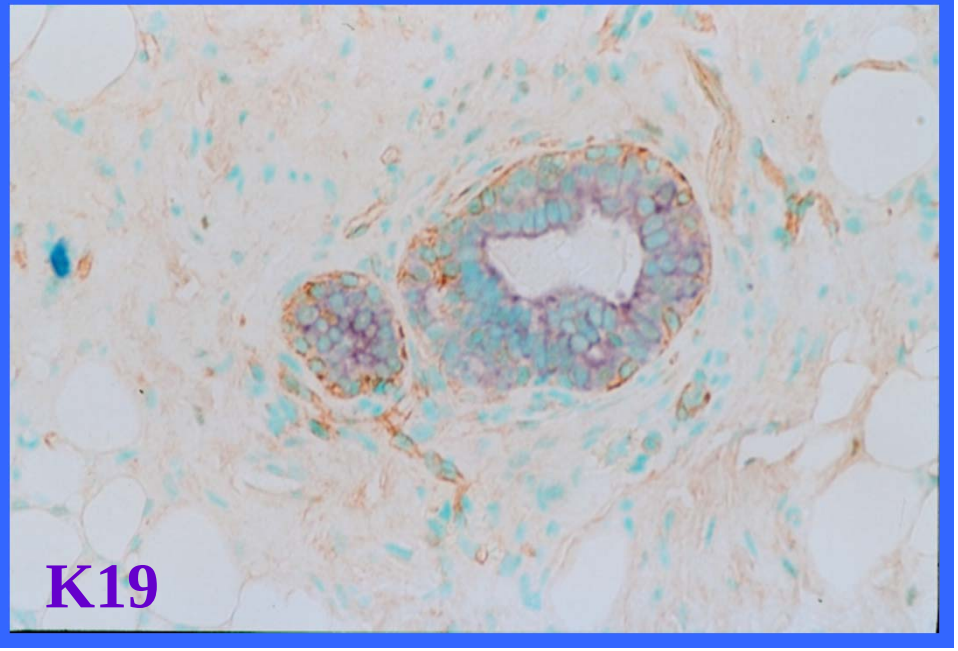
Mouse Cot I DNA

Breast Marker Expression in Xenograft Model

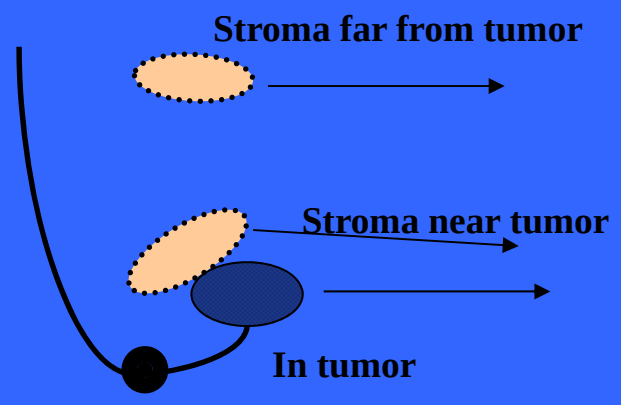








Isolation of human fibroblasts and CAF from human mammary tissue

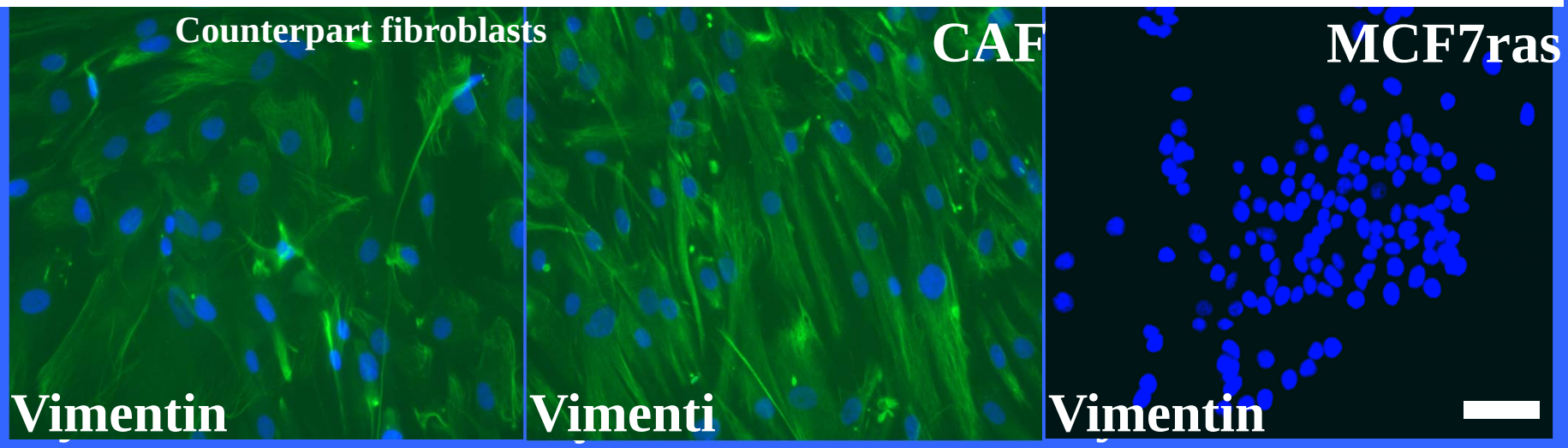


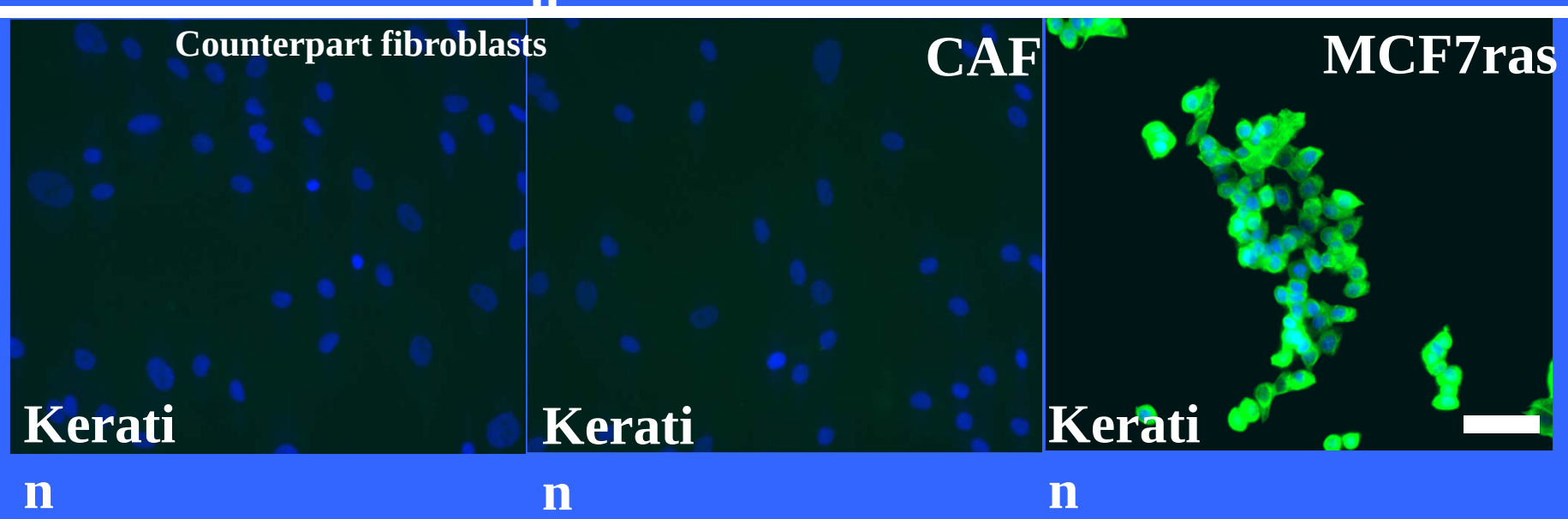
Human breast tissue

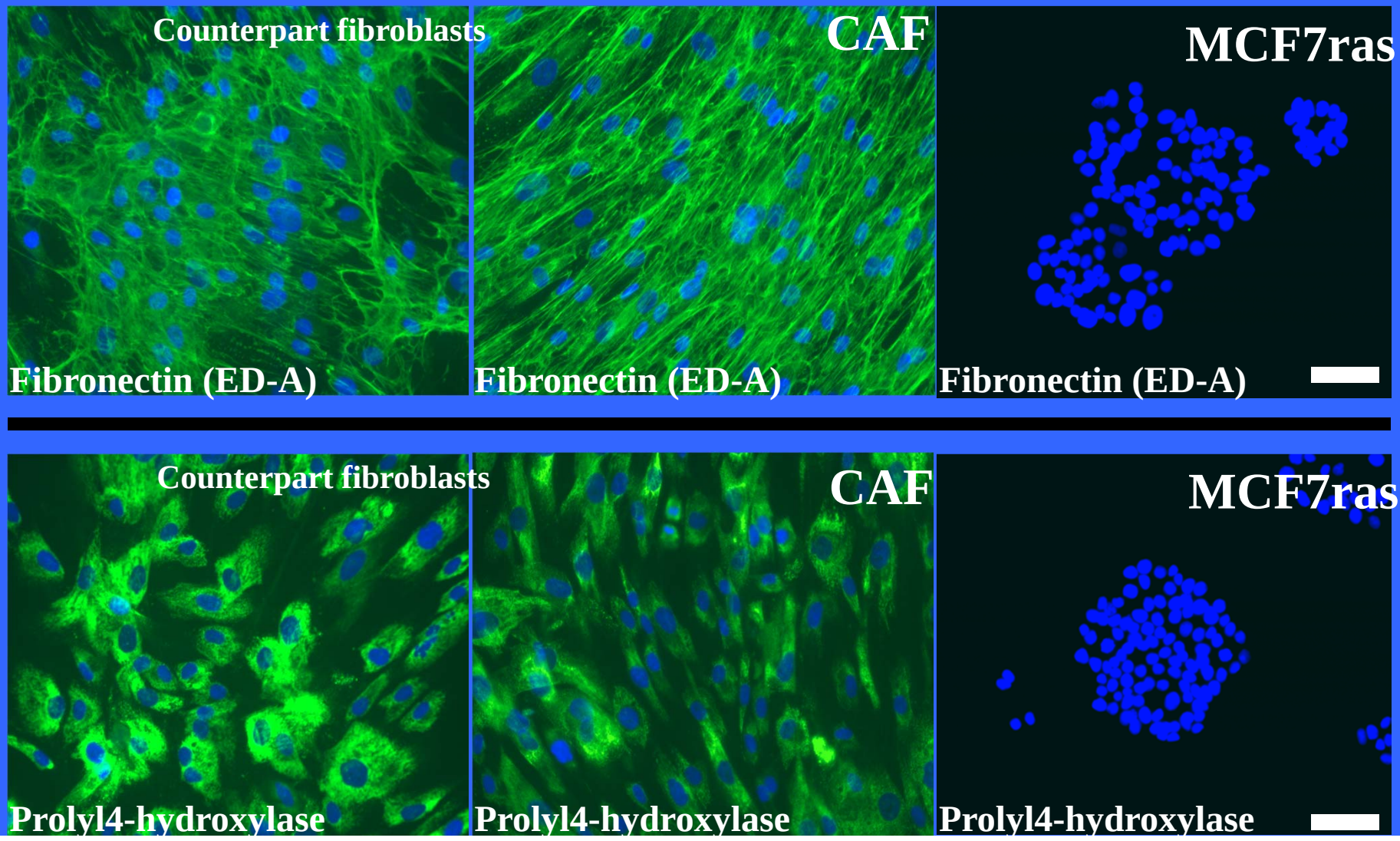
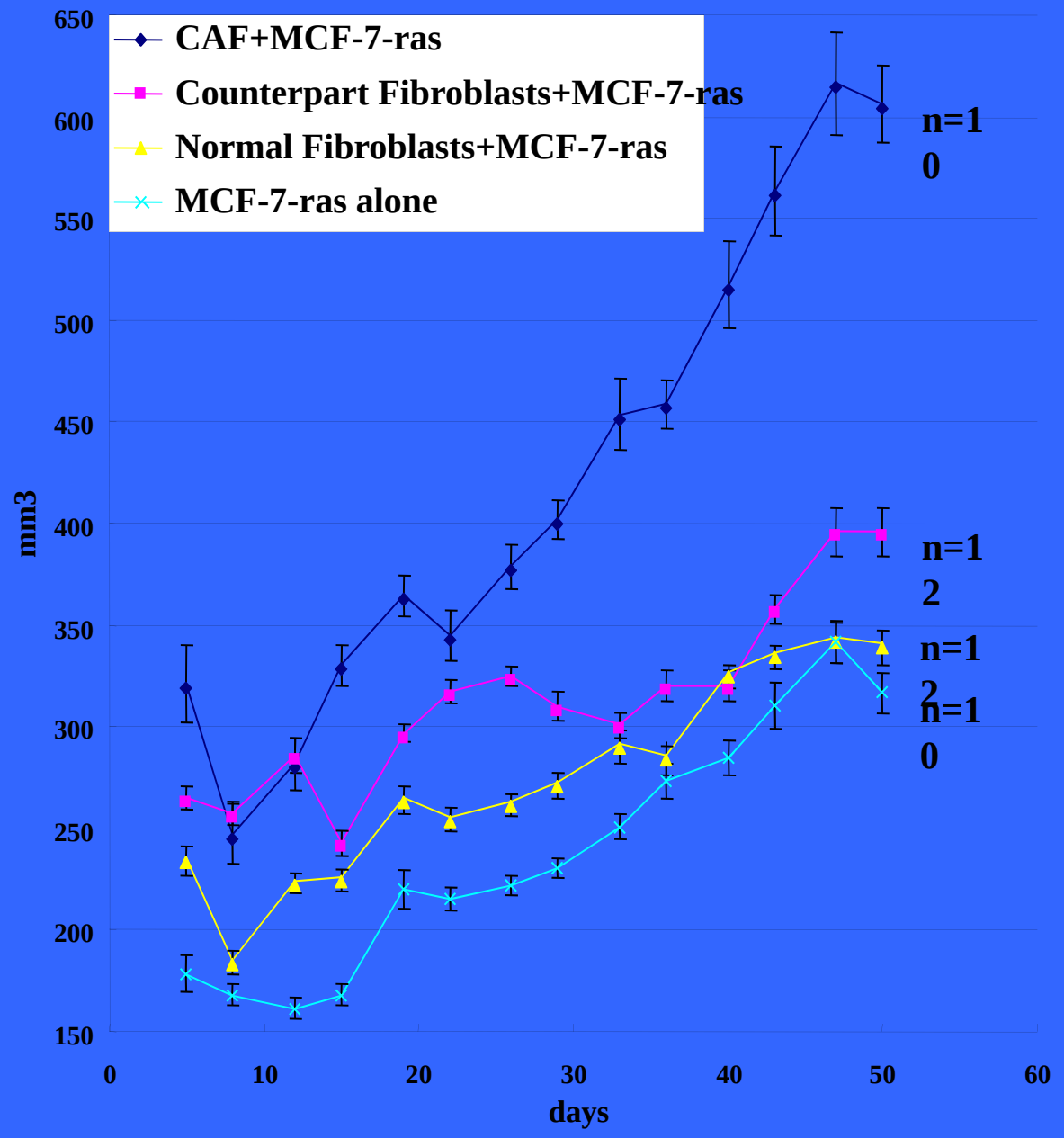
1. Enzyme treatment
2. Differential centrifugation
3. Differential trypsin treatment

↓

Isolation of each fibroblast


Counterpart fibroblasts
CAF
MCF7ras
Vimentin
Vimenti
Vimentin


Counterpart fibroblasts
CAF
MCF7ras
Kerati
Kerati
Kerati
n
n
n


Counterpart fibroblasts
CAF
MCF7ras
Fibronectin (ED-A)
Fibronectin (ED-A)
Fibronectin (ED-A)
Counterpart fibroblasts
CAF
MCF7ras
Prolyl4-hydroxylase
Prolyl4-hydroxylase
Prolyl4-hydroxylase

CAF stimulate MCF-ras tumor growth in nude mice