

Proteoglycans in Breast Cancer

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Outline

- Why should we care about context (microenvironment)?
- Why should we care about proteoglycans like versican?
- How can we use 3D tissue culture assays to help understand disease?
- Where is this research currently?

Cancer is a disease of mutation

But ...

REVIEW

FOCUS ON CANCER

nature
medicine

Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression

Mina J Bissell & William C Hines

Cancer is a disease of mutation

But ...

REVIEW

FOCUS ON CANCER

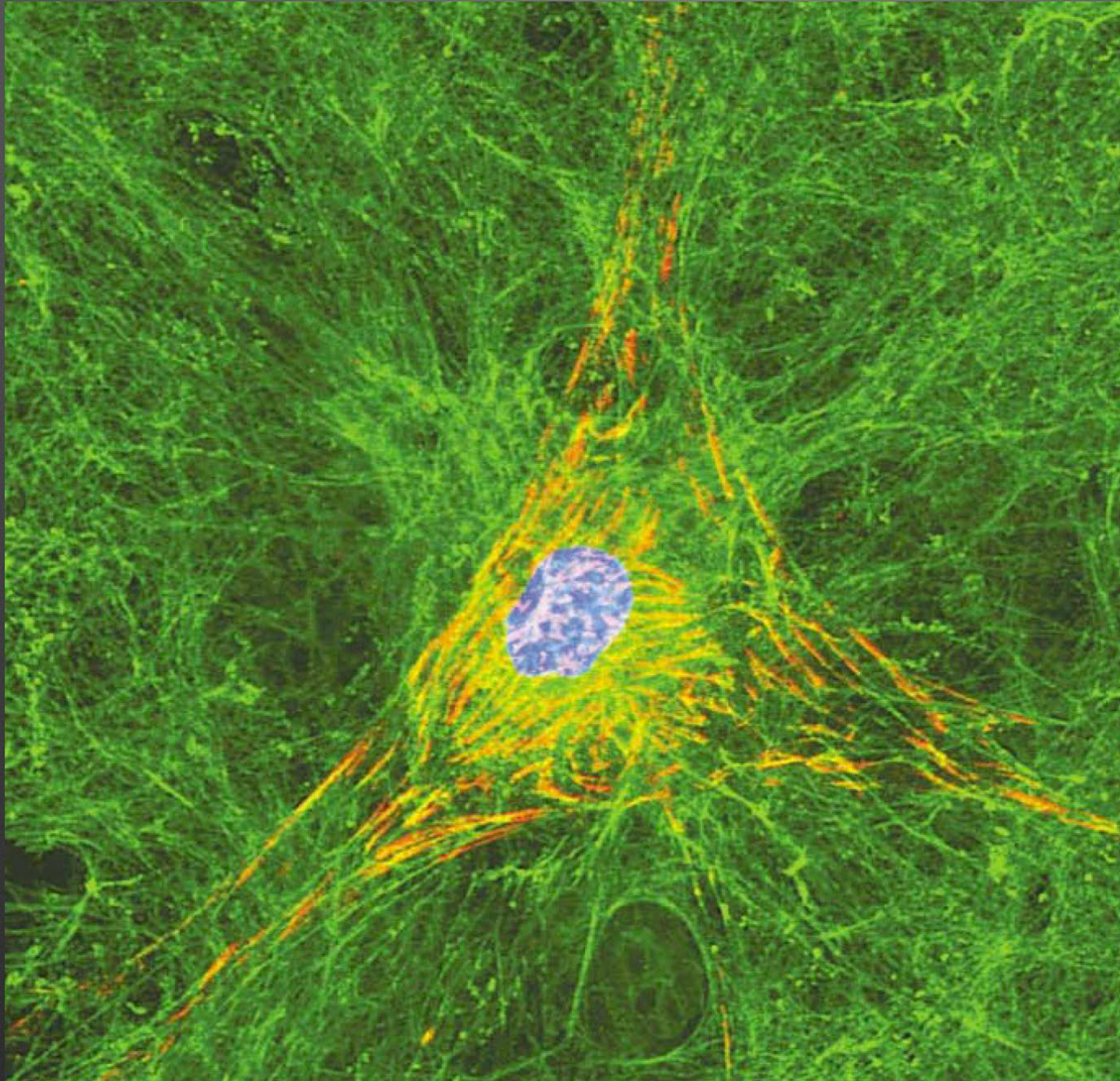
nature
medicine

Phenotype is dominant over genotype

Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression

Mina J Bissell & William C Hines

Context

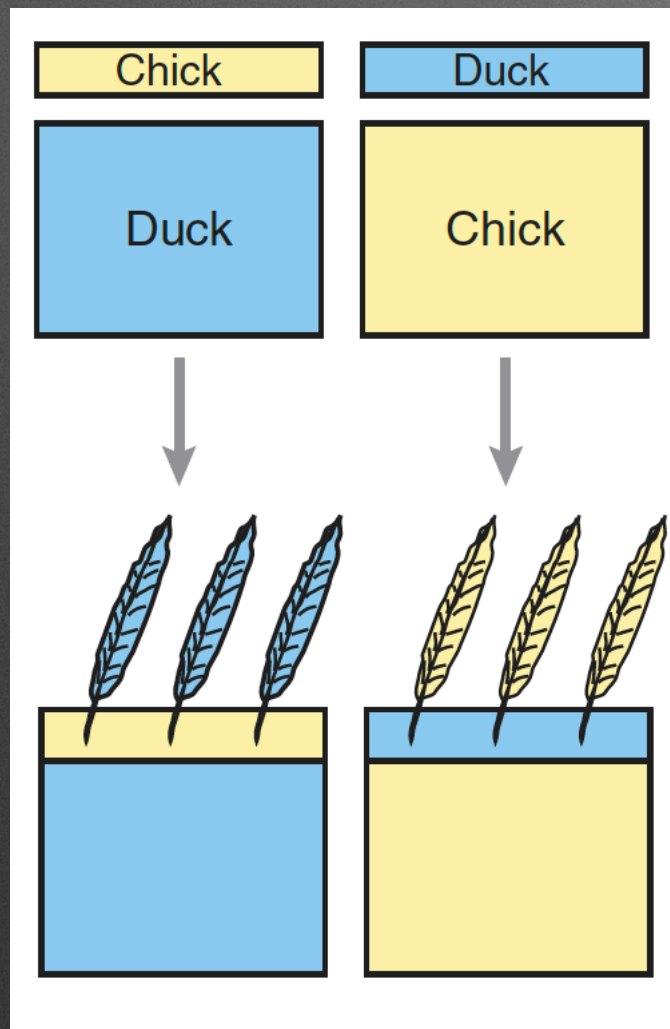


Connective
Tissue = ECM
(extracellular
matrix)

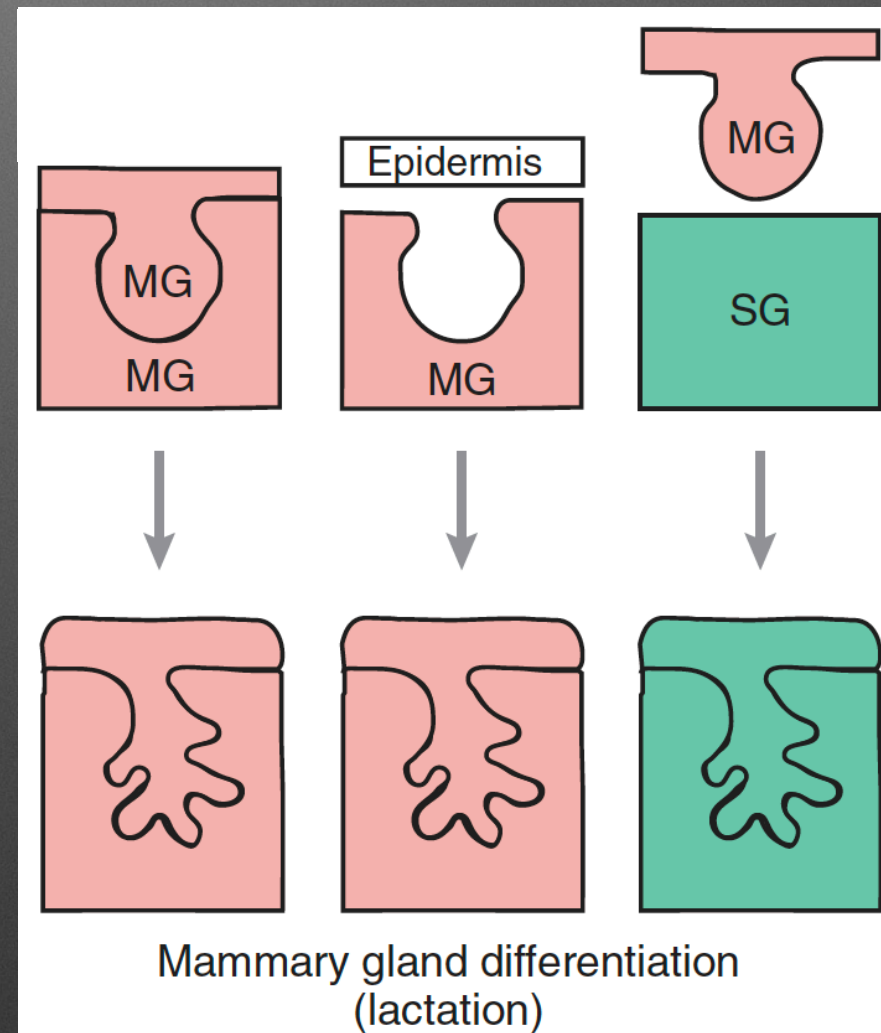
**Dynamic
Reciprocity**

Context

Cells
Connective
Tissue



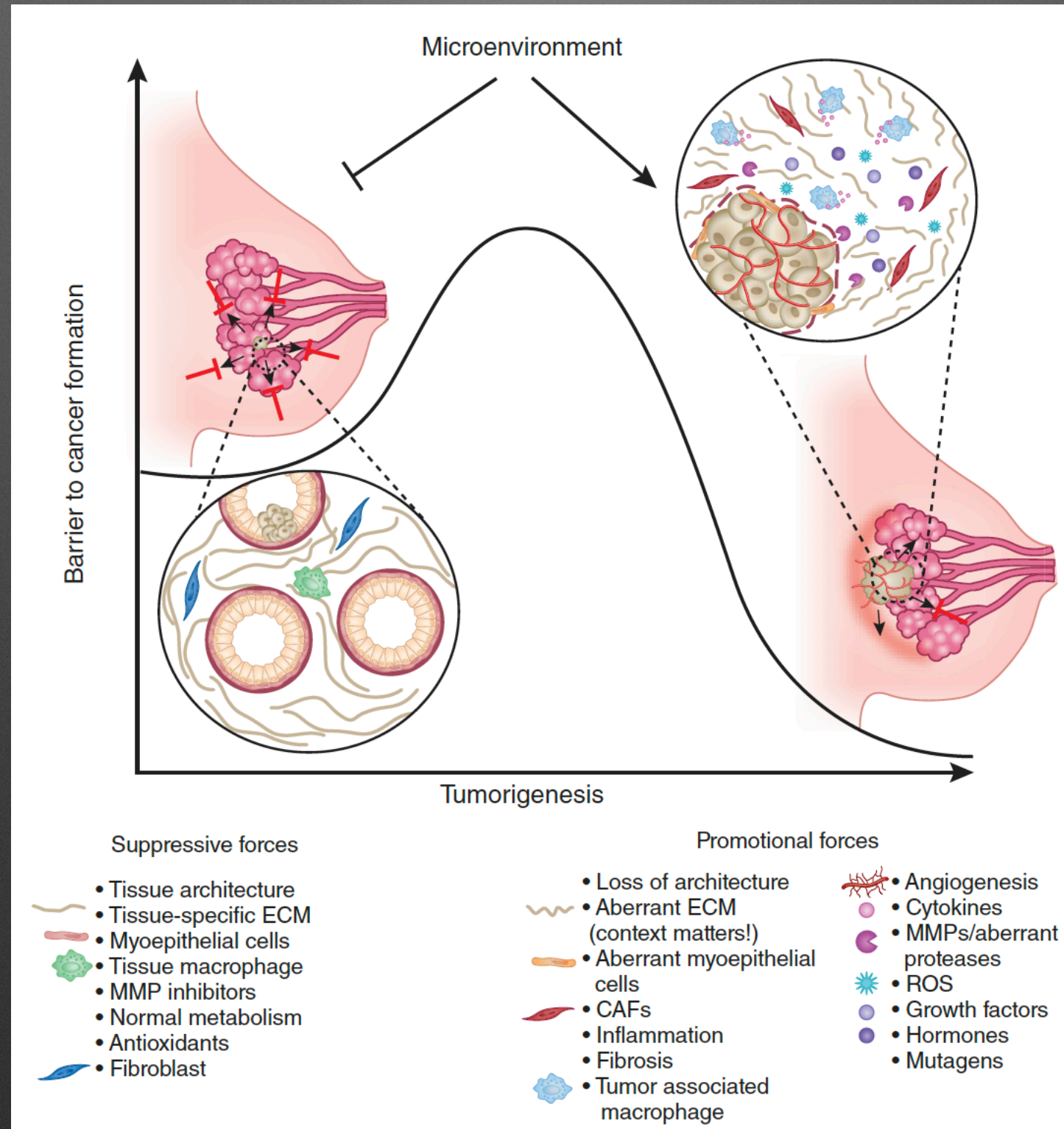
Cells
Connective
Tissue



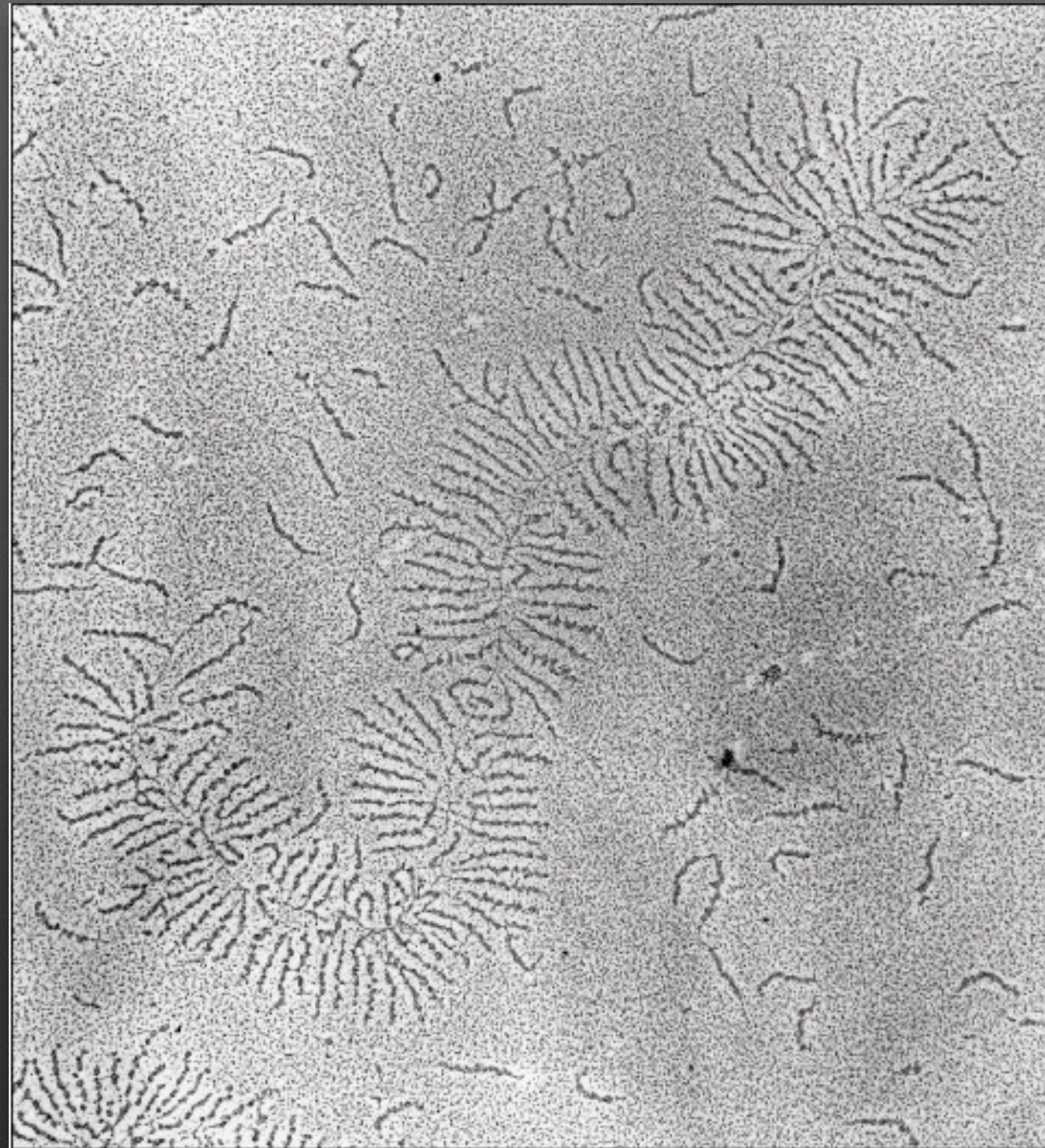
Context

Pre-invasive

Invasive



Versican

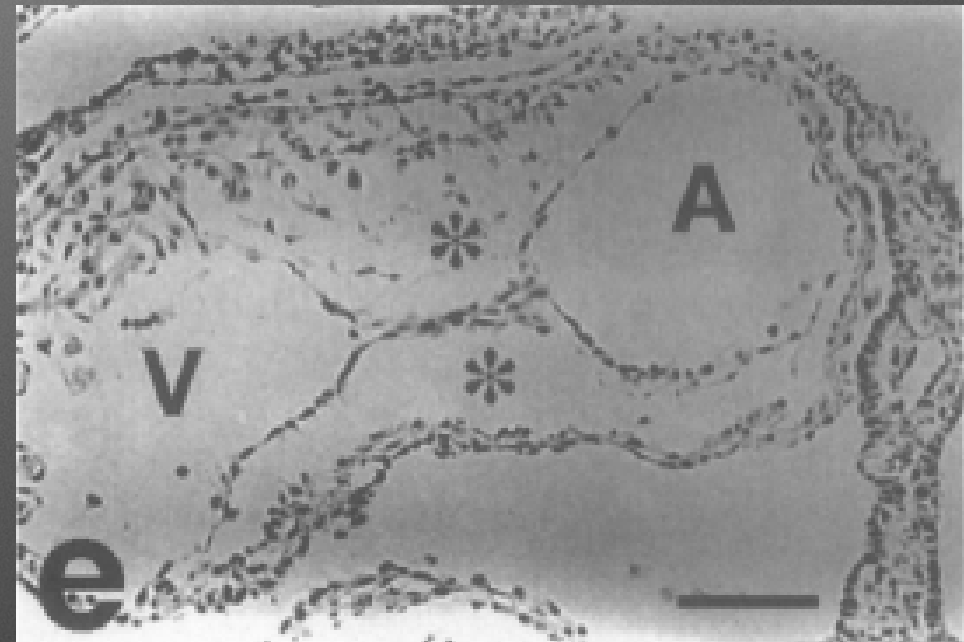


(A)

1 μm

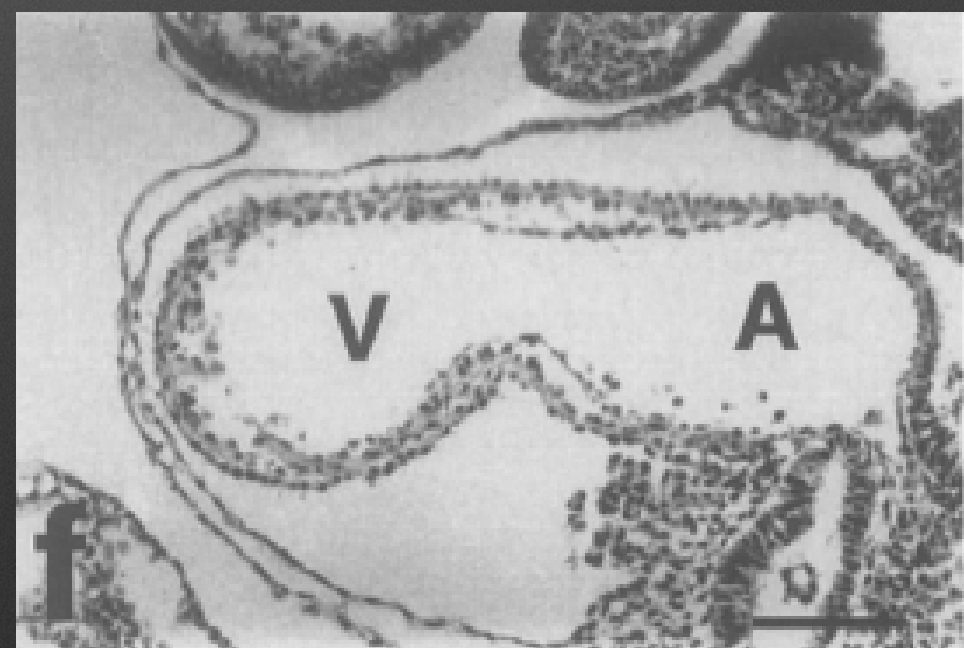
Versican in development

- Knockout is embryonic lethal
- Involved in:
 - Migration and proliferation
 - Tissue visco-elasticity
 - Anti-adhesive
 - Leukocyte homing
 - Binding to cell surface receptors



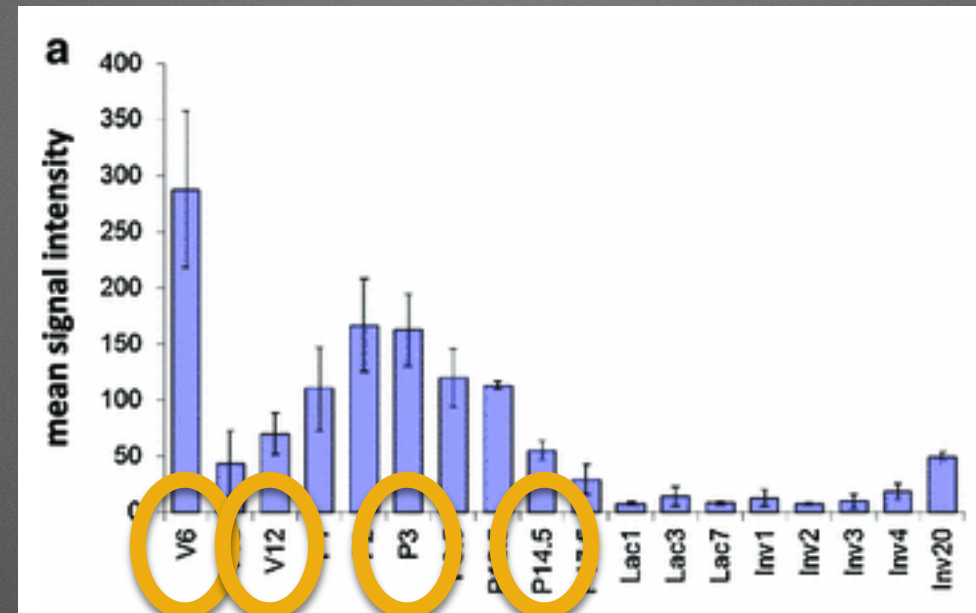
WT

*endocardial cushions

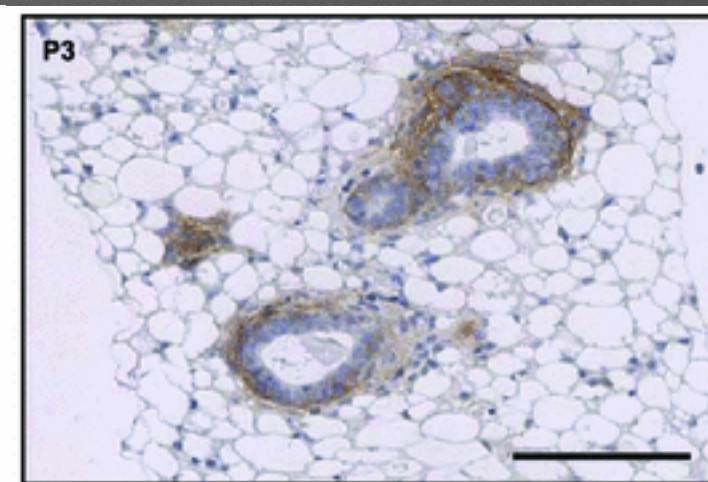
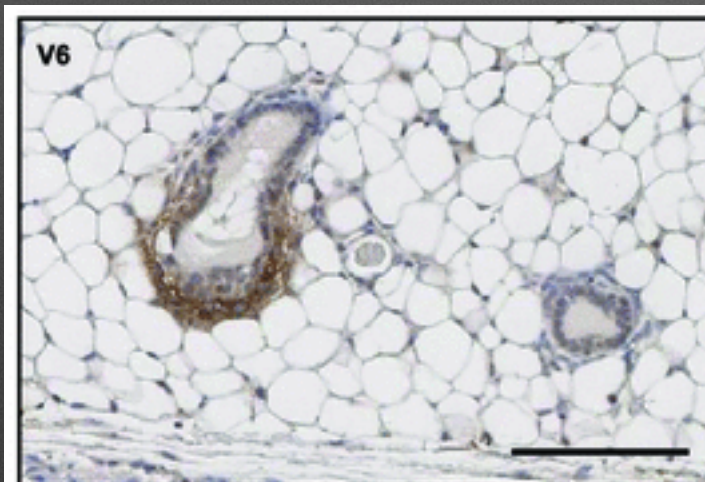


VCAN
-/-

Versican in mammary gland development

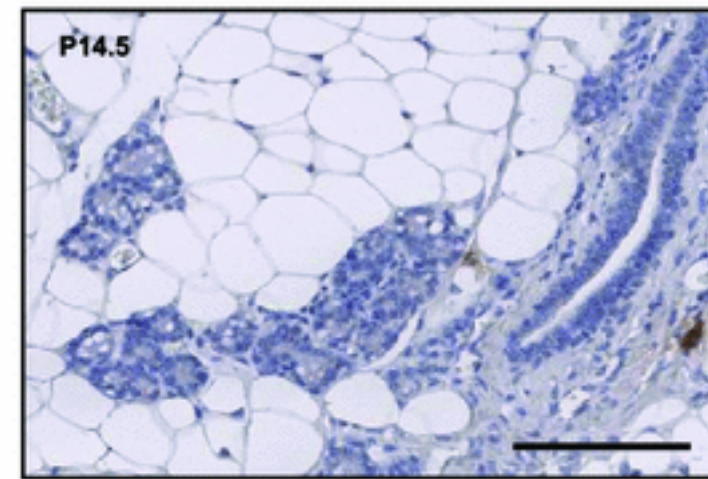
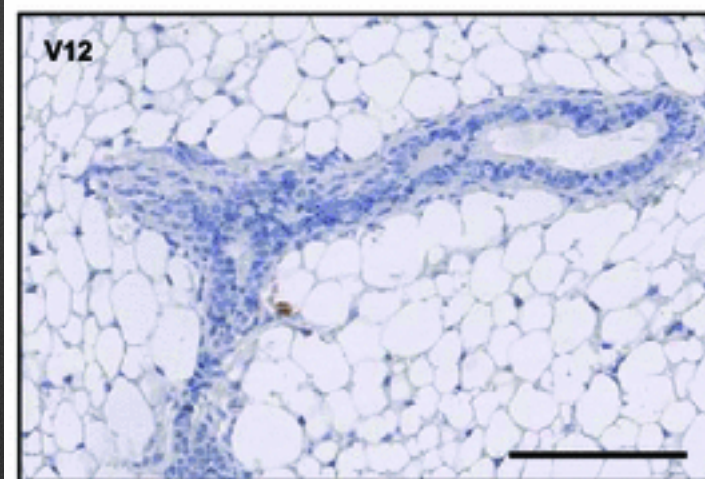


Puberty



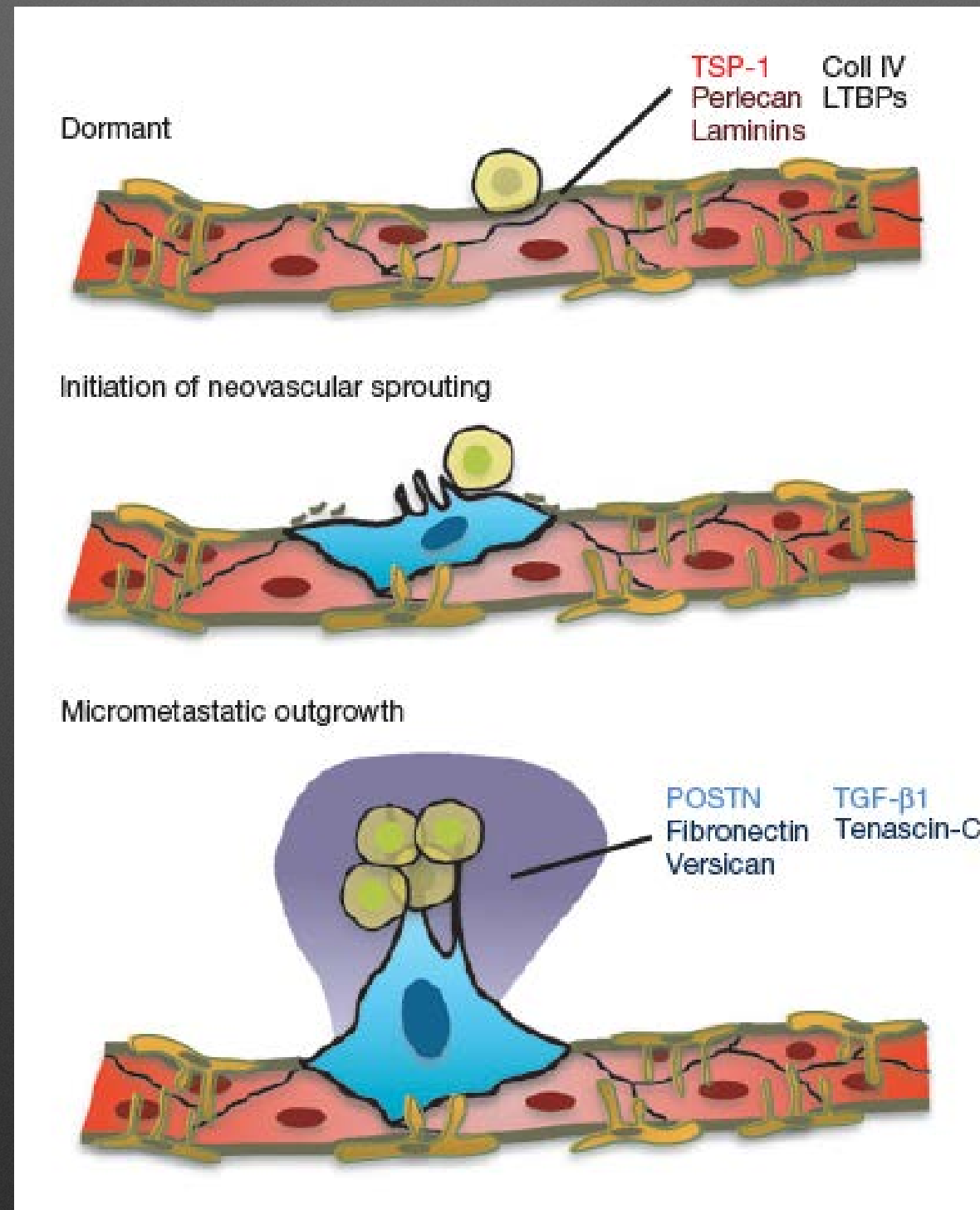
Early pregnancy

Adult virgin

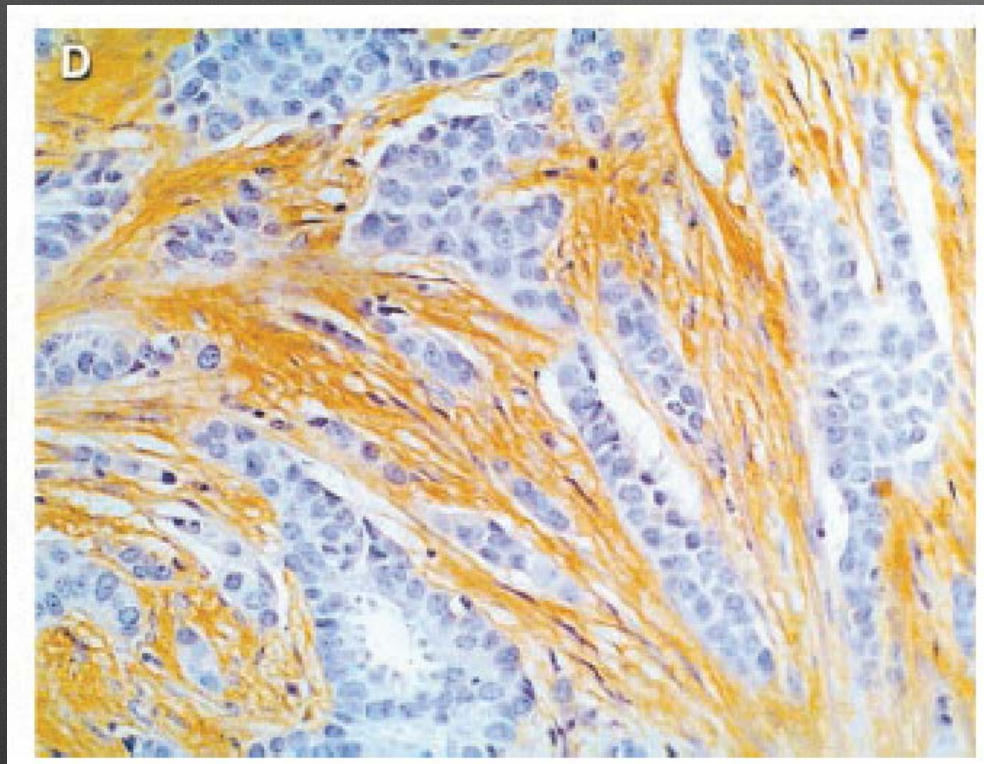


Late pregnancy

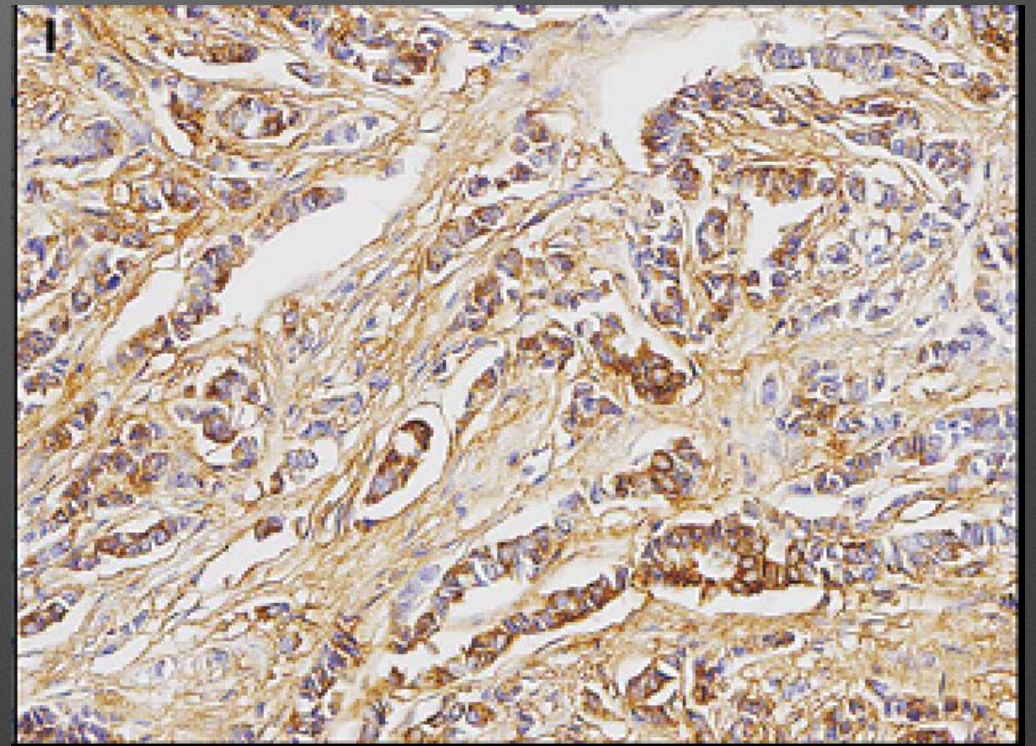
Versican in dormancy



Versican in breast cancer

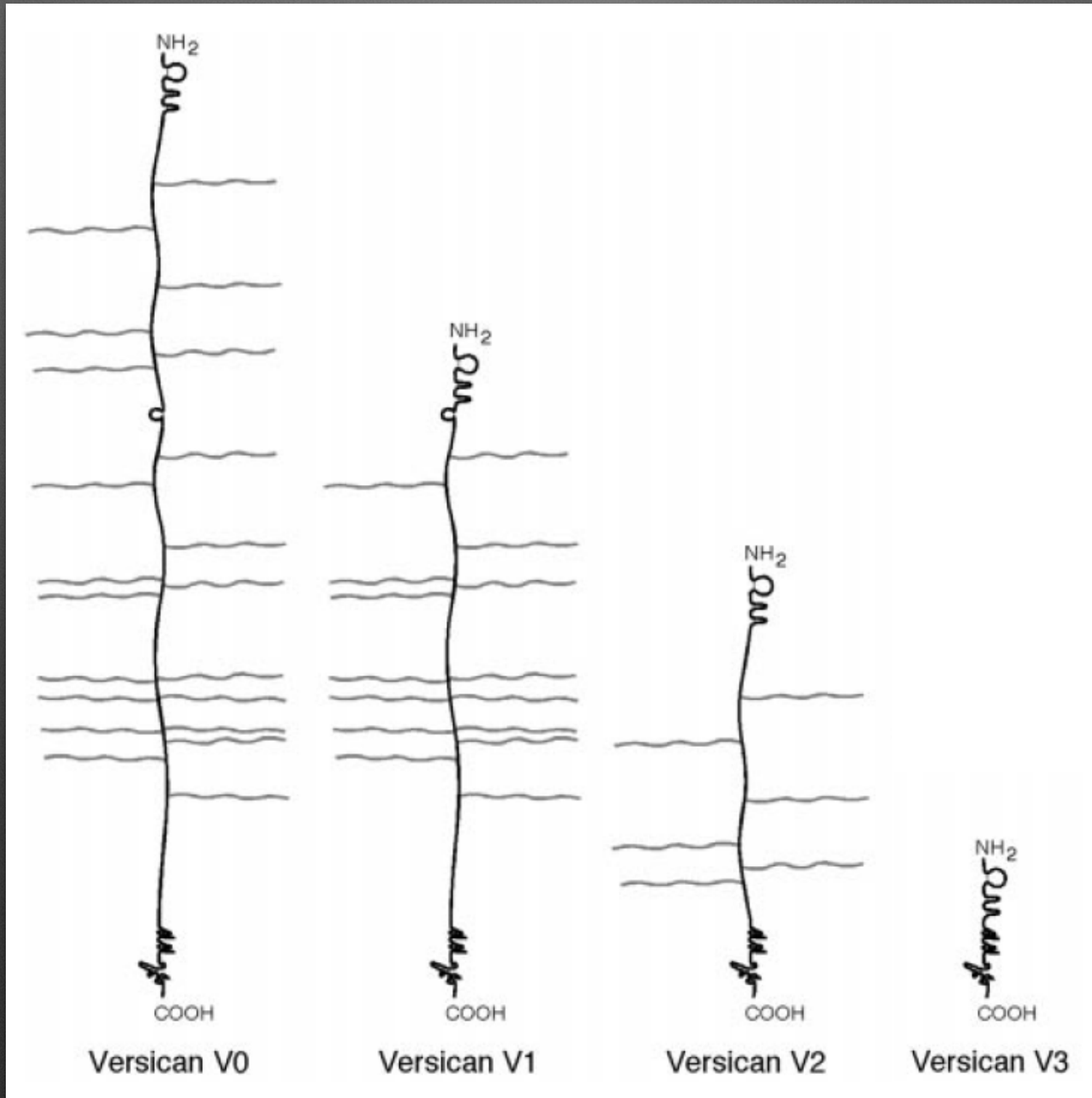


Ricciardelli, C. et al., 2002, Clin. Cancer Res. 8:1054

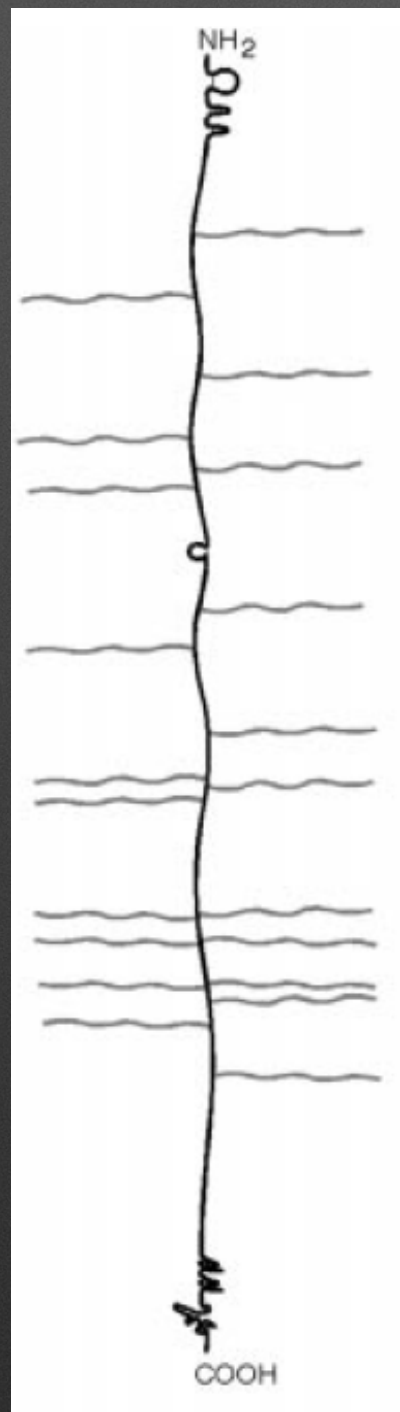


Kharaishvili, G. et al., 2011, J. Clin. Pathol. 64:977

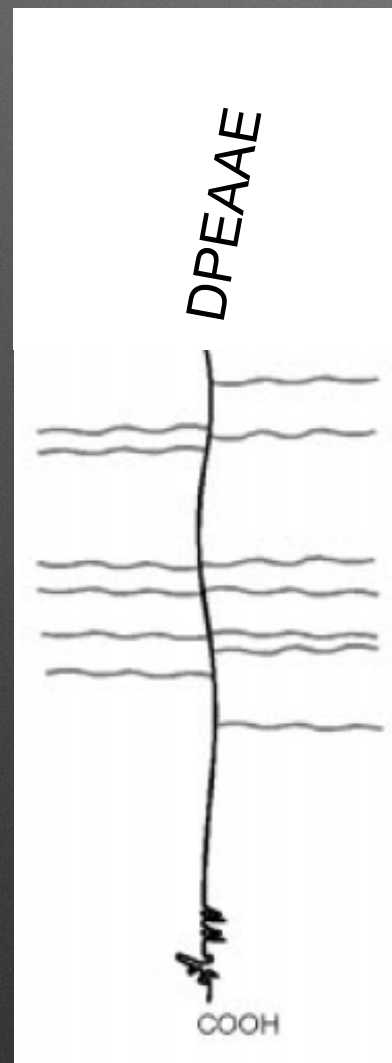
Versican isoforms



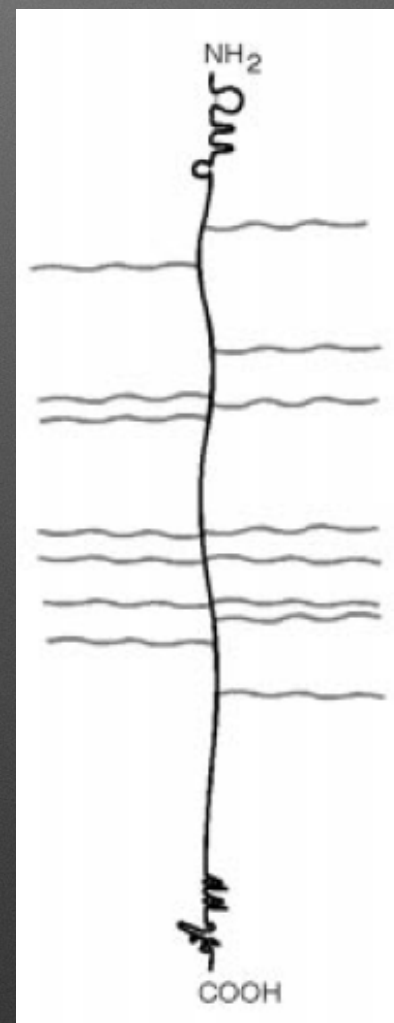
Versican cleavage



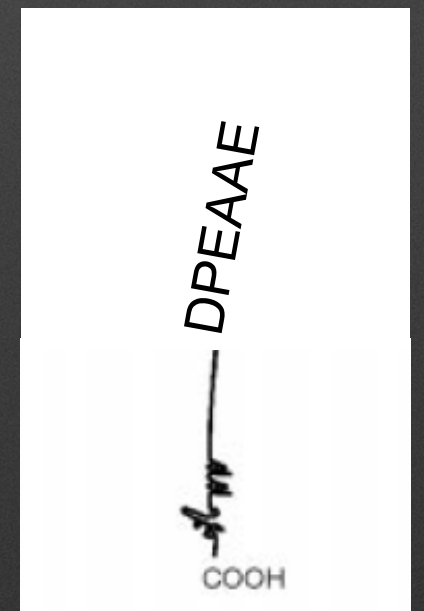
V0



V0-DPEAAE

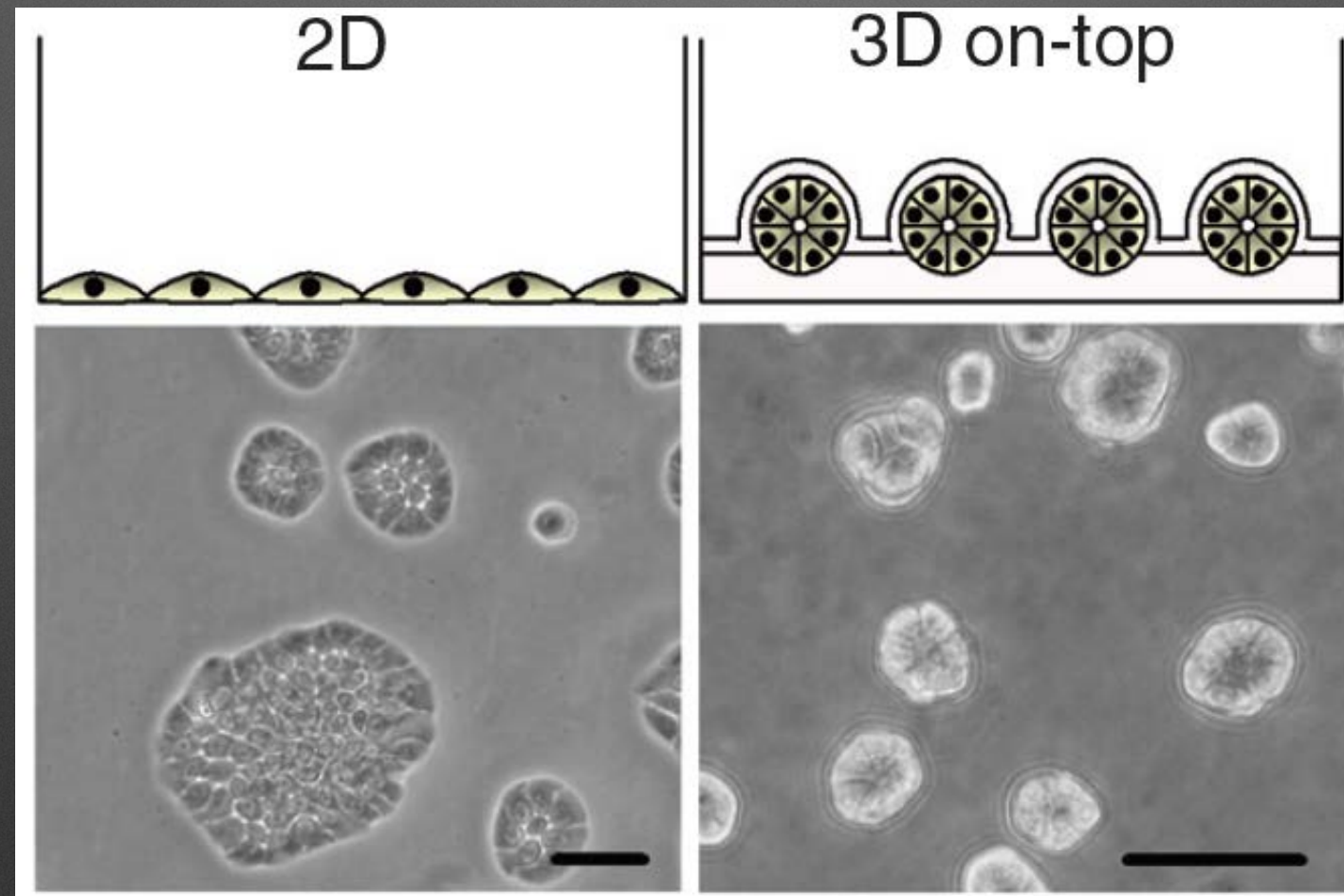


V1



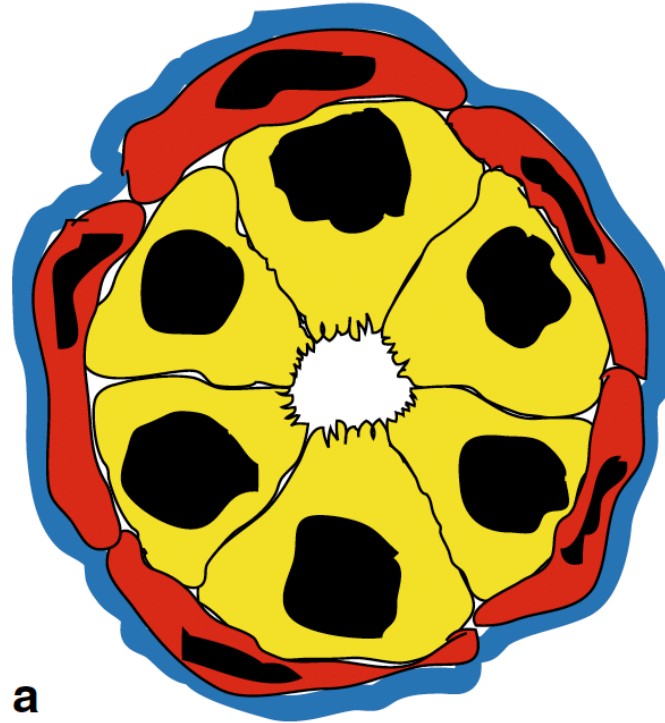
V1-DPEAAE

3D cell culture



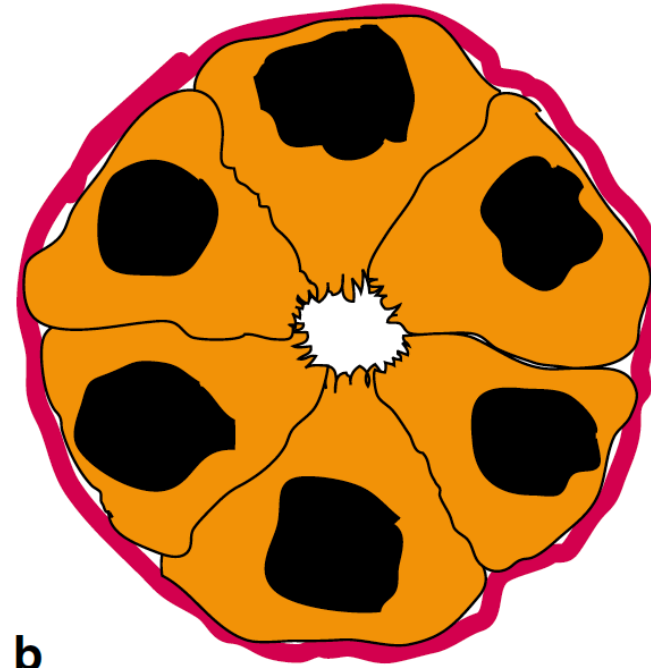
3D cell culture

Cross section
of a TDLU (an acinus)

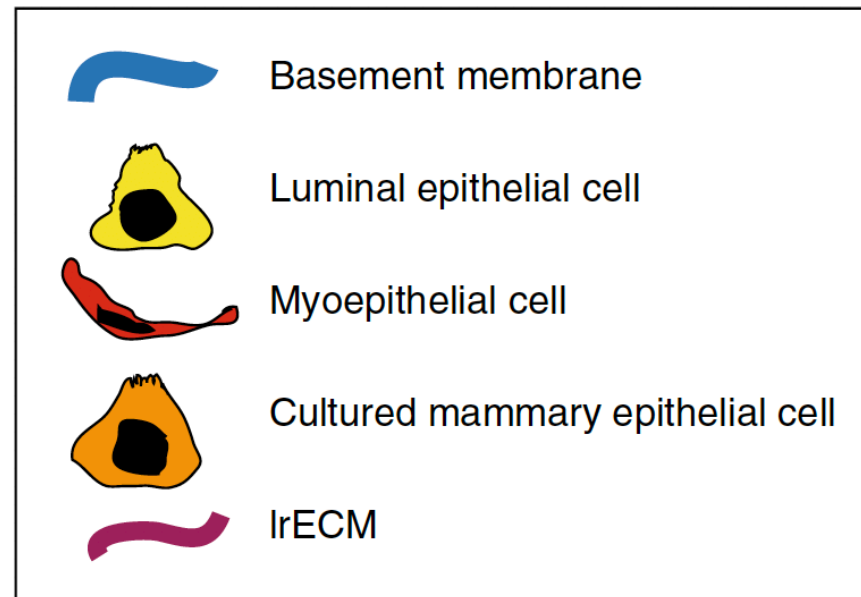


a

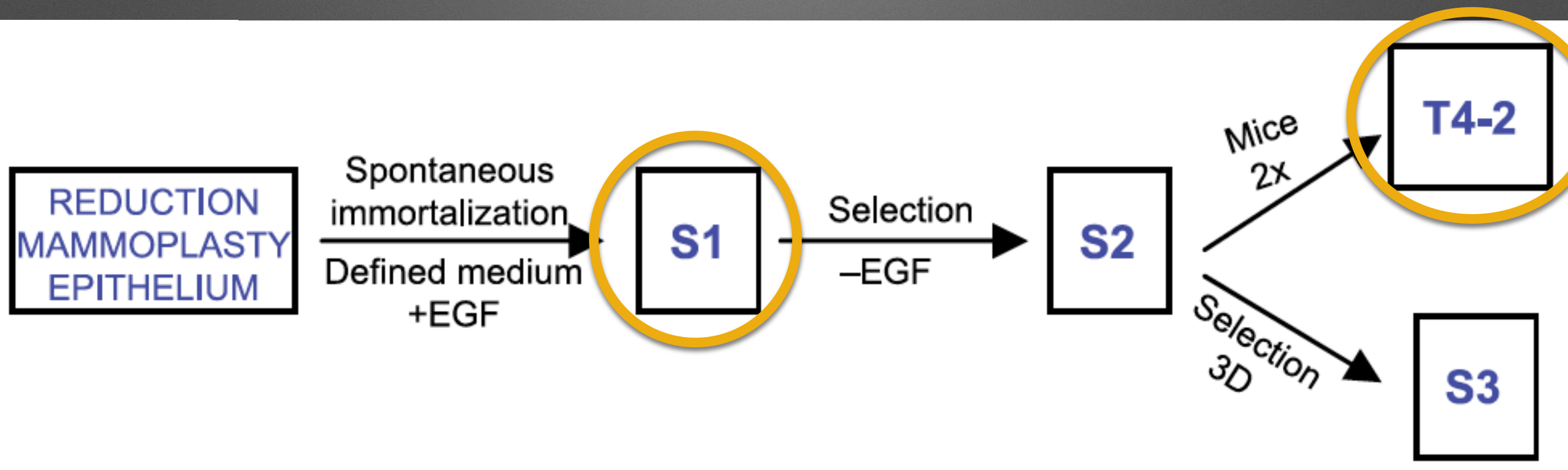
An acinus-like
structure in
3D culture



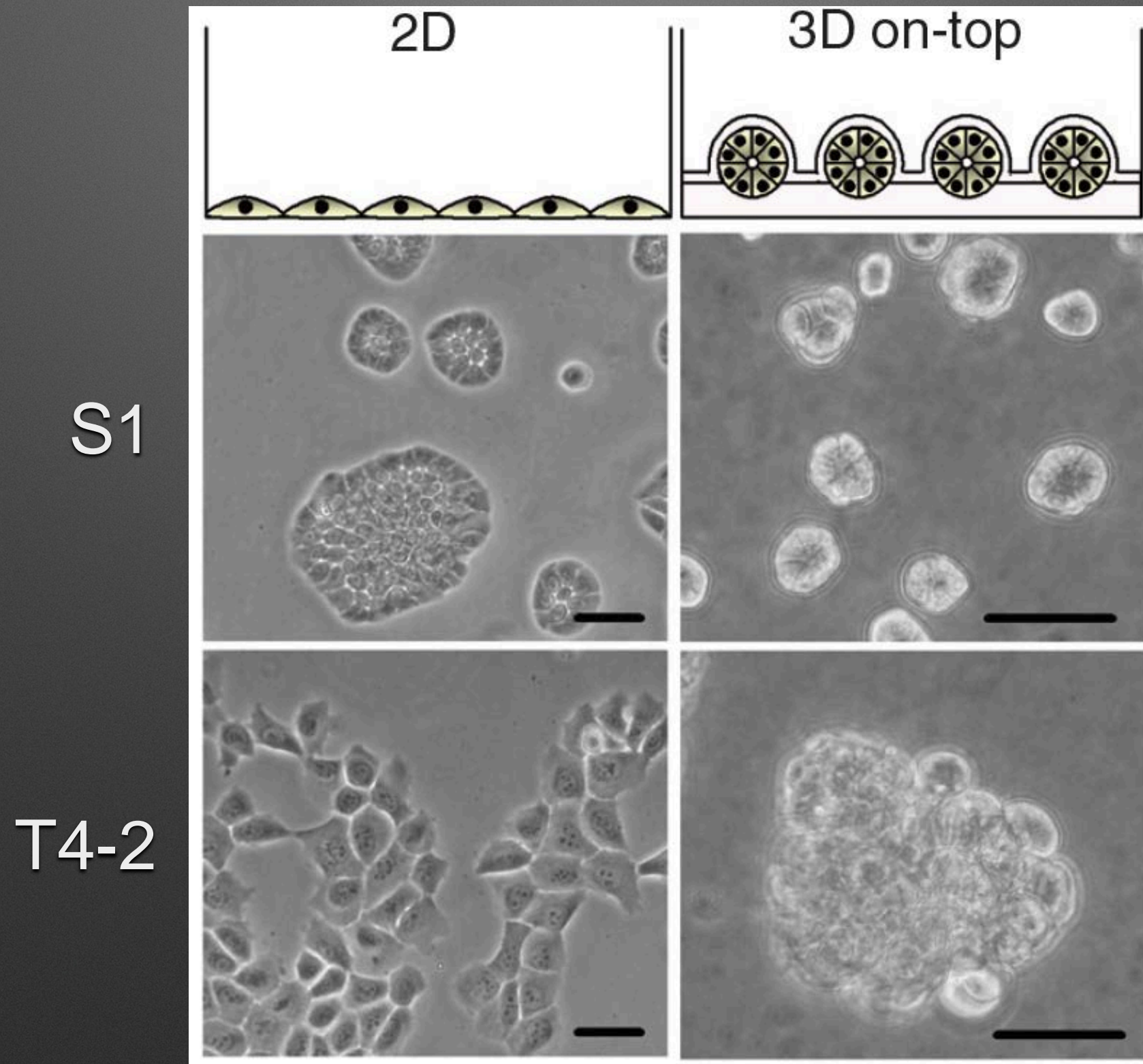
b



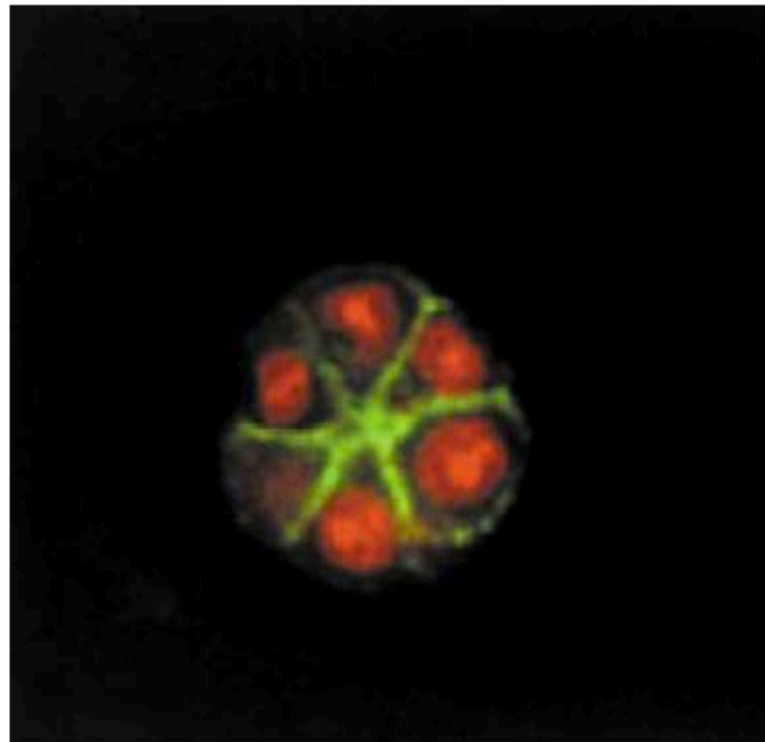
A preinvasive to invasive model



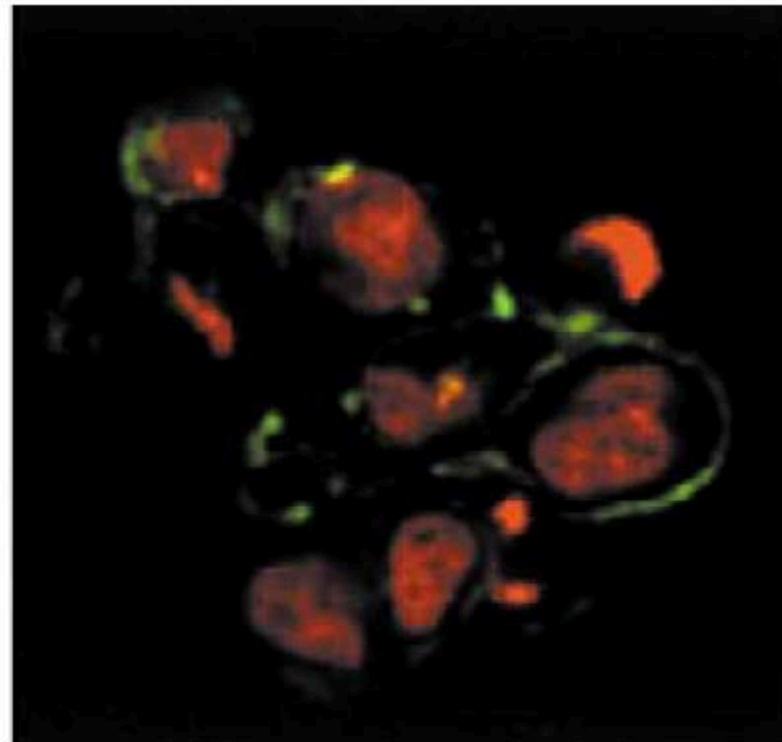
A preinvasive to invasive model



'Normal'

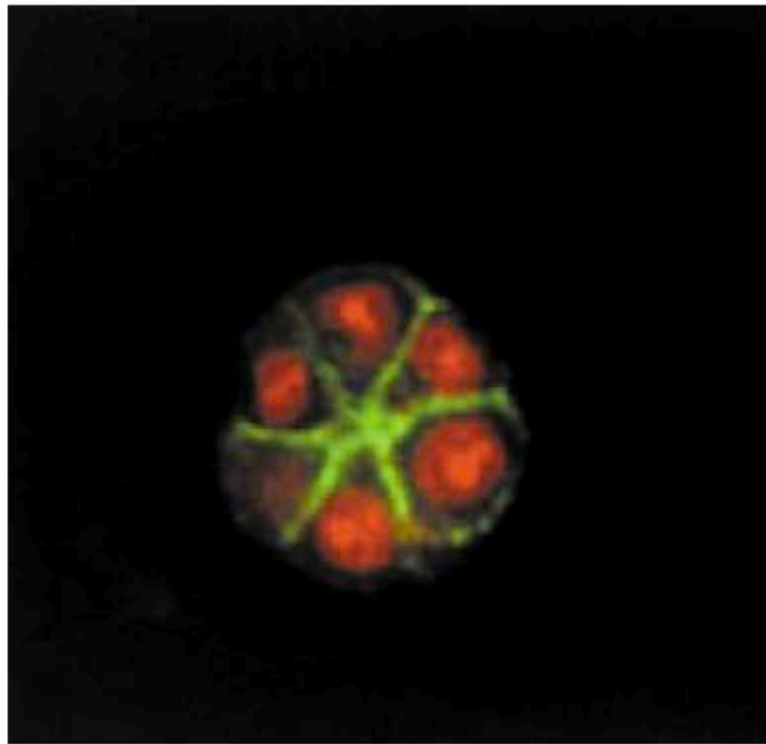


Tumor

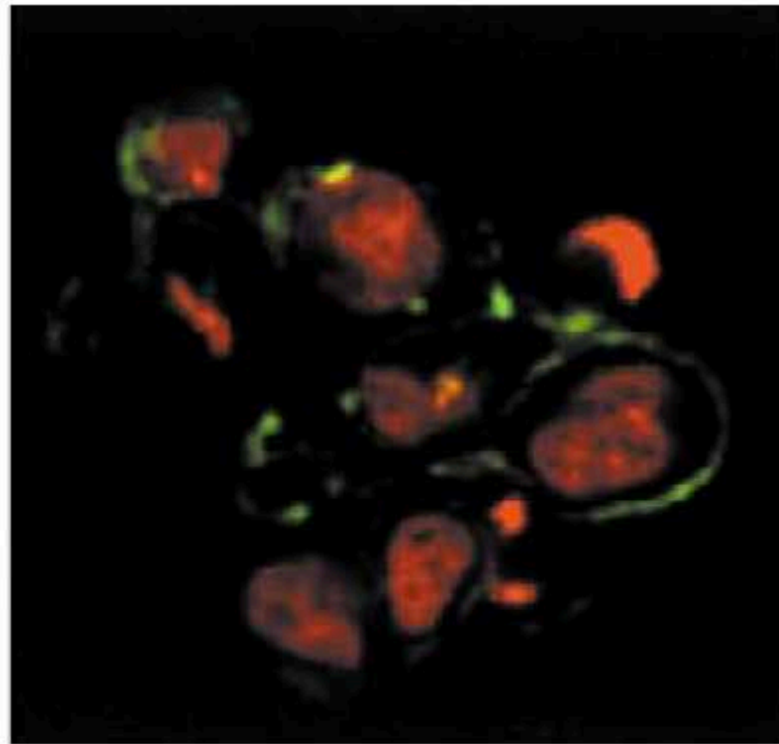


“Reversion”

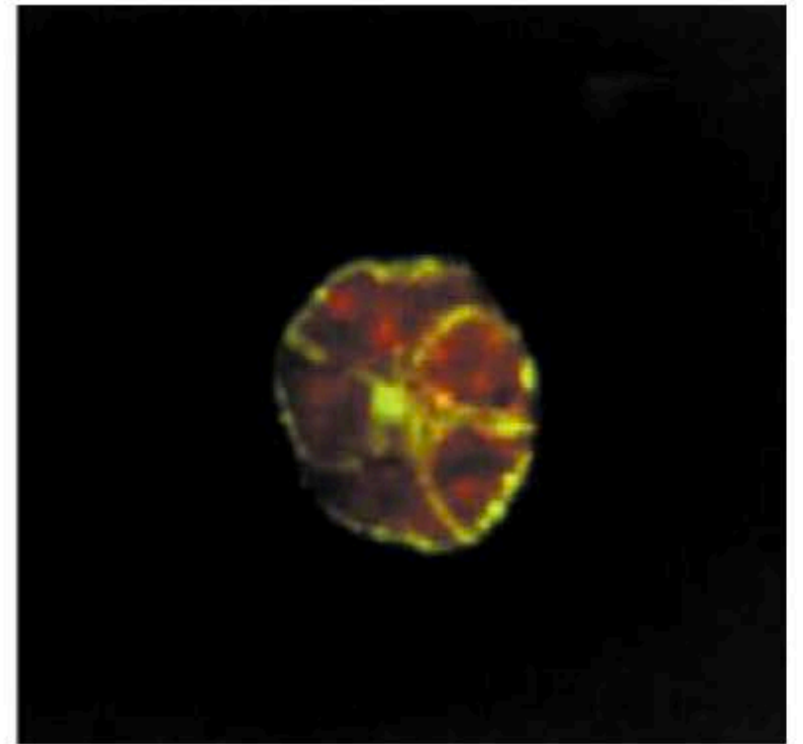
'Normal'



Tumor



Reverted



Phenotype is dominant over genotype

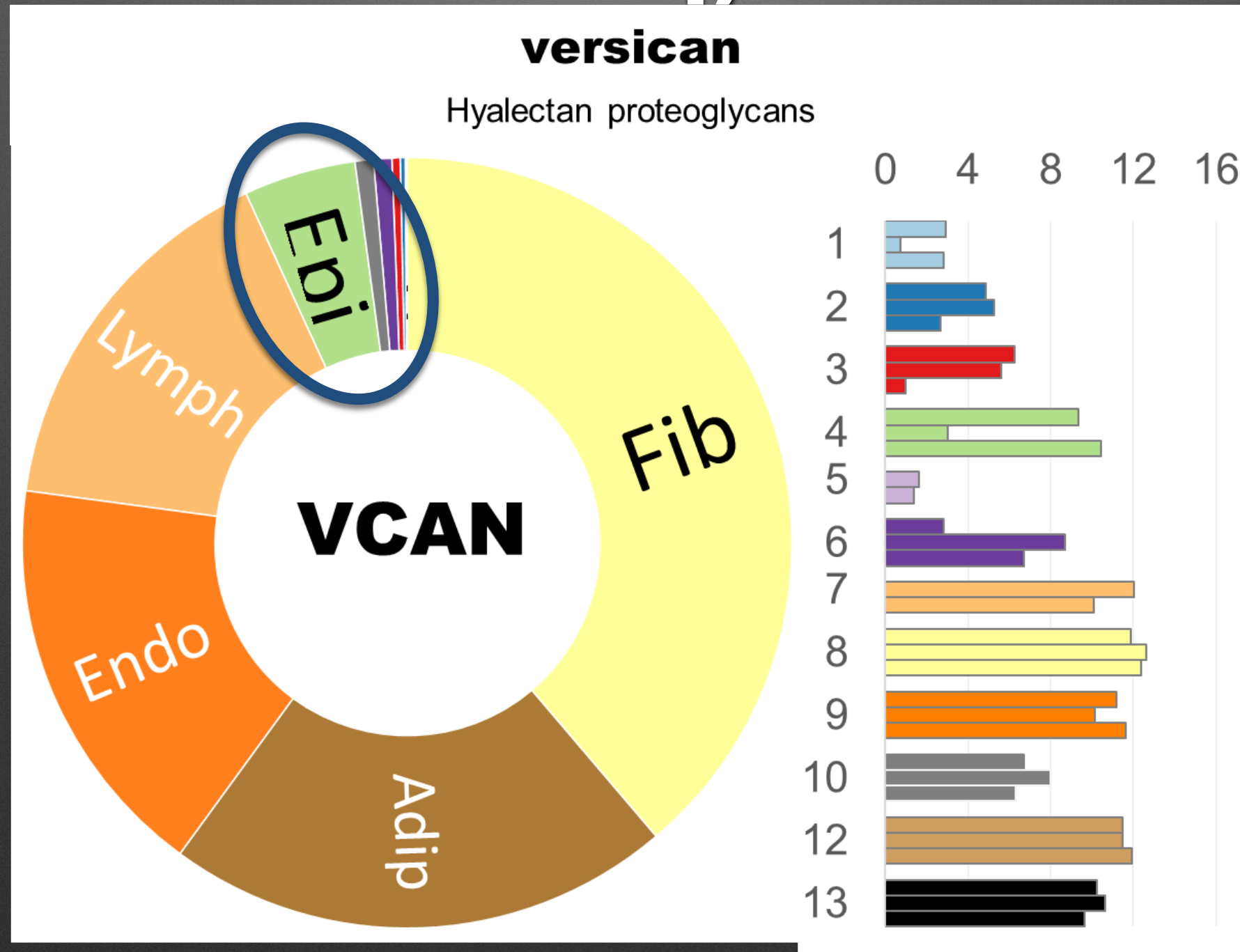
Hypothesis

Versican expression and turnover are central events in the transition from pre-invasive to invasive phenotype in breast cancer cells

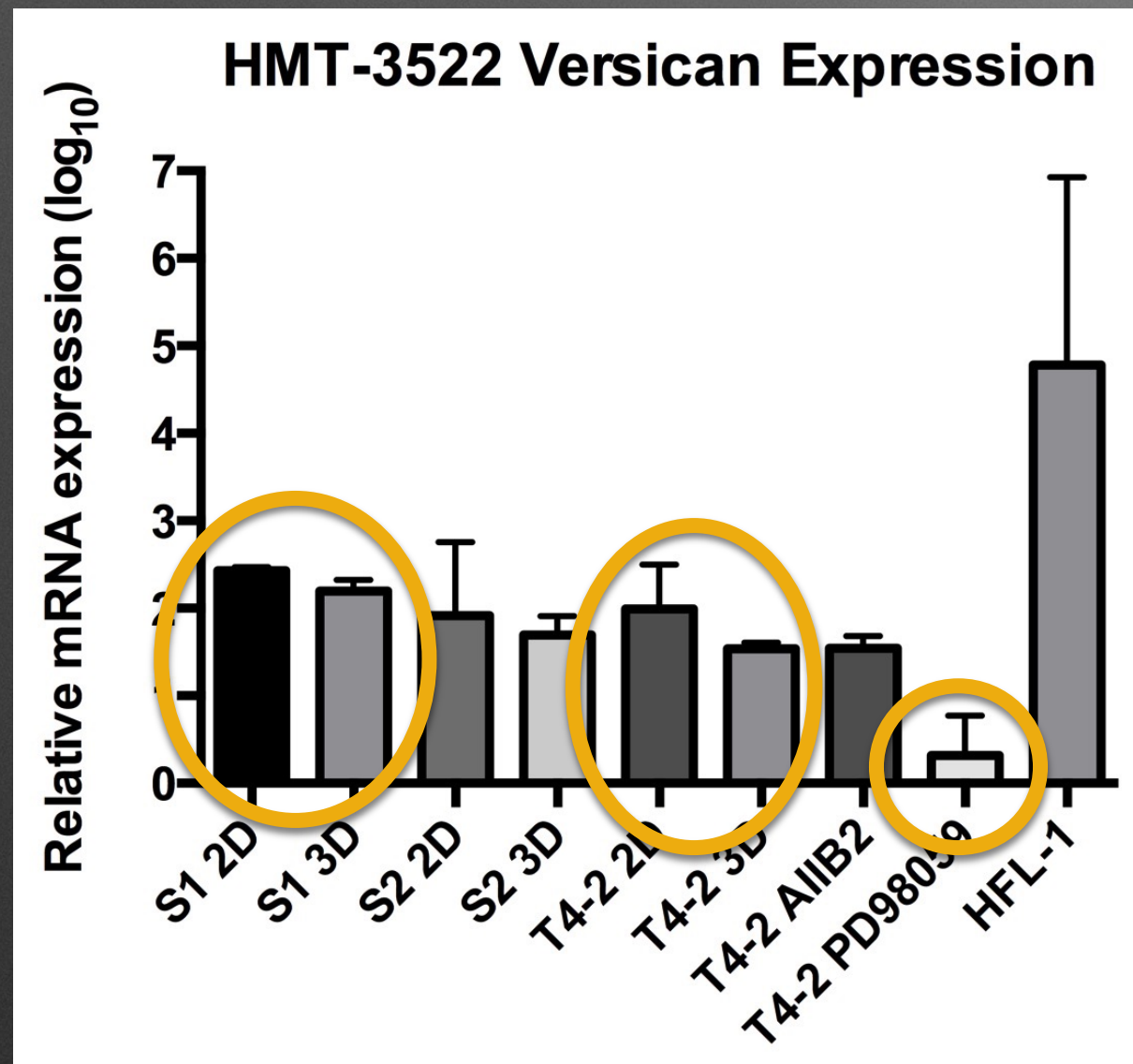
What have we done?

- Is versican expressed by breast epithelial cells?
- Is versican found in human breast tissue?
- Isoform expression in breast epithelial cells?
- Versican knockdown in T4-2 cells
- Does versican contribute to epithelial cell invasion?

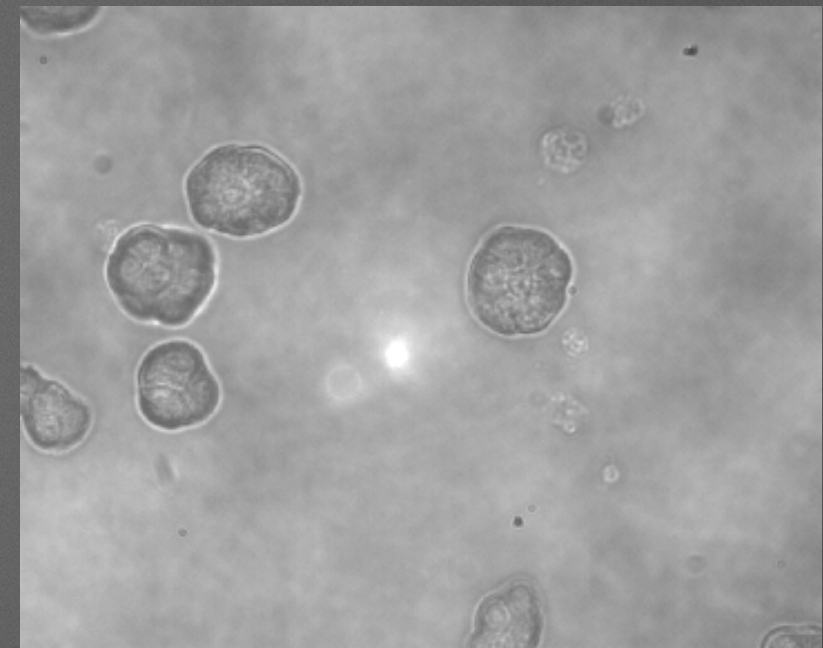
Normal human breast versican expression (RNA seq)



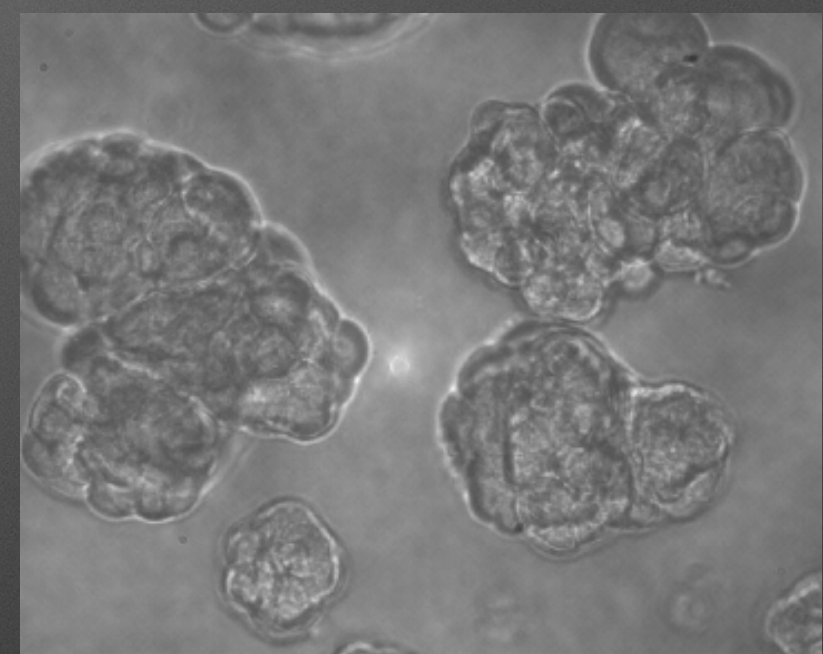
Do S1 or T4-2 cells make versican?



S1



T4-2

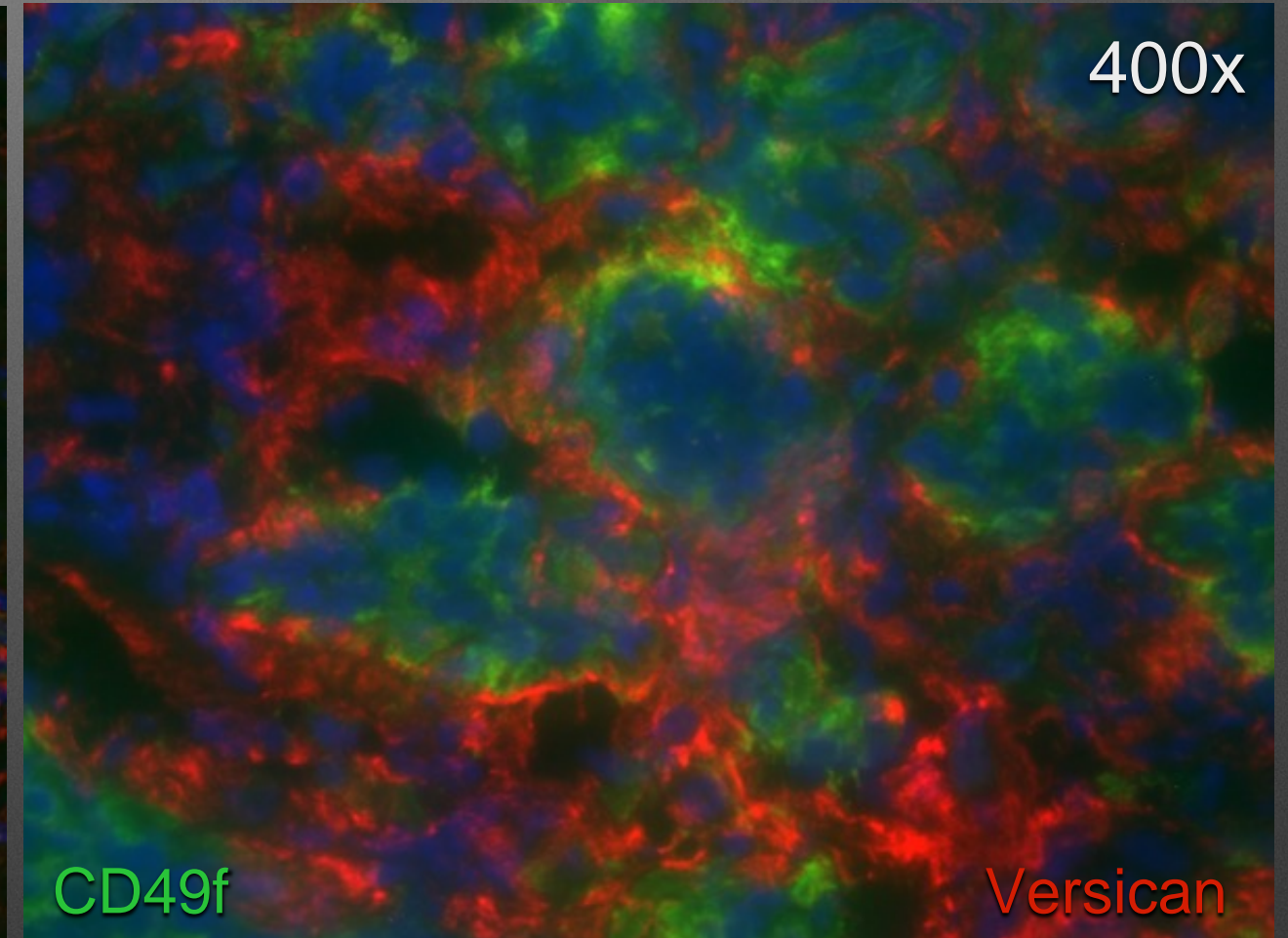
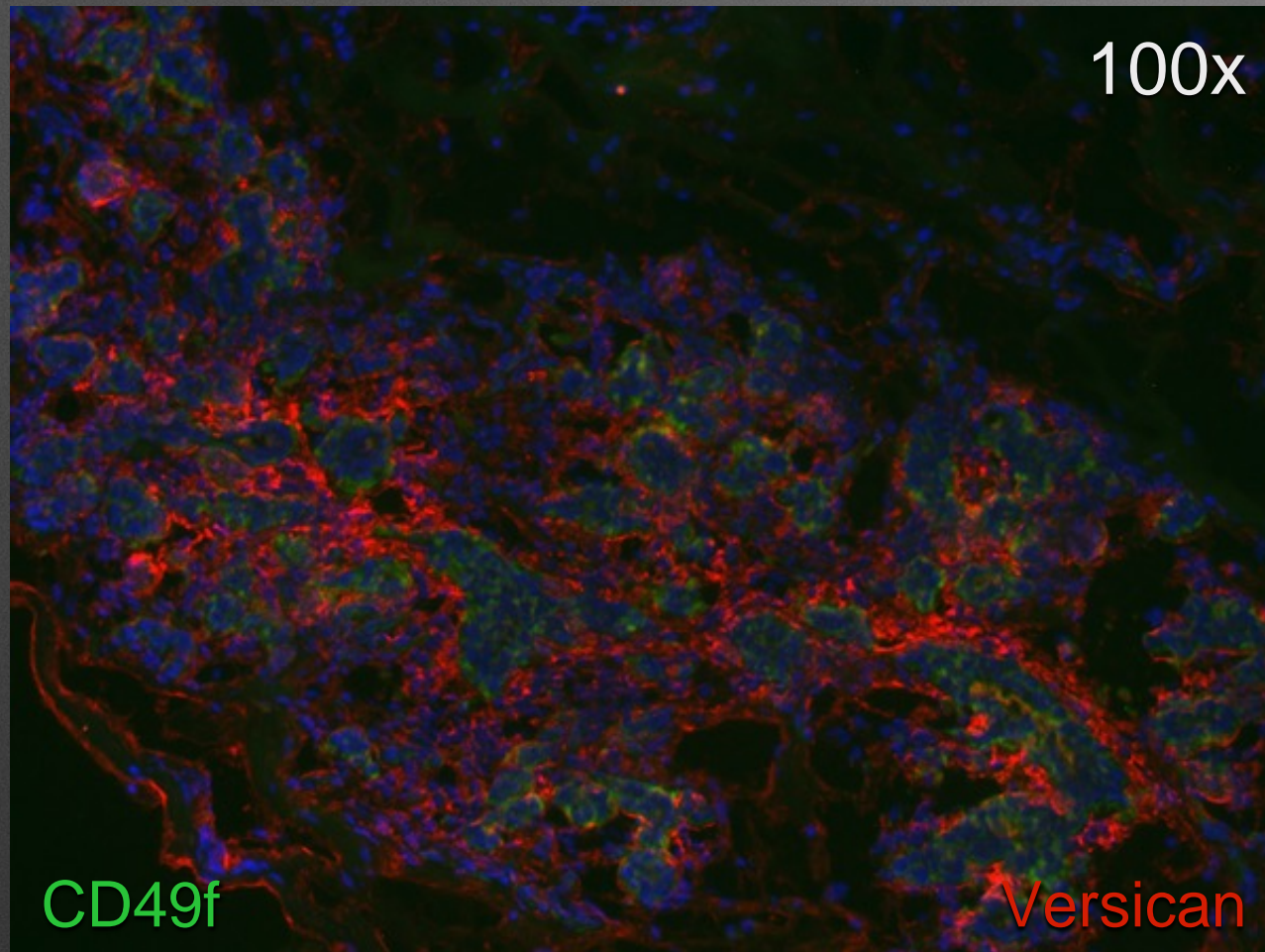


T4-2 reverted
(PD98059)

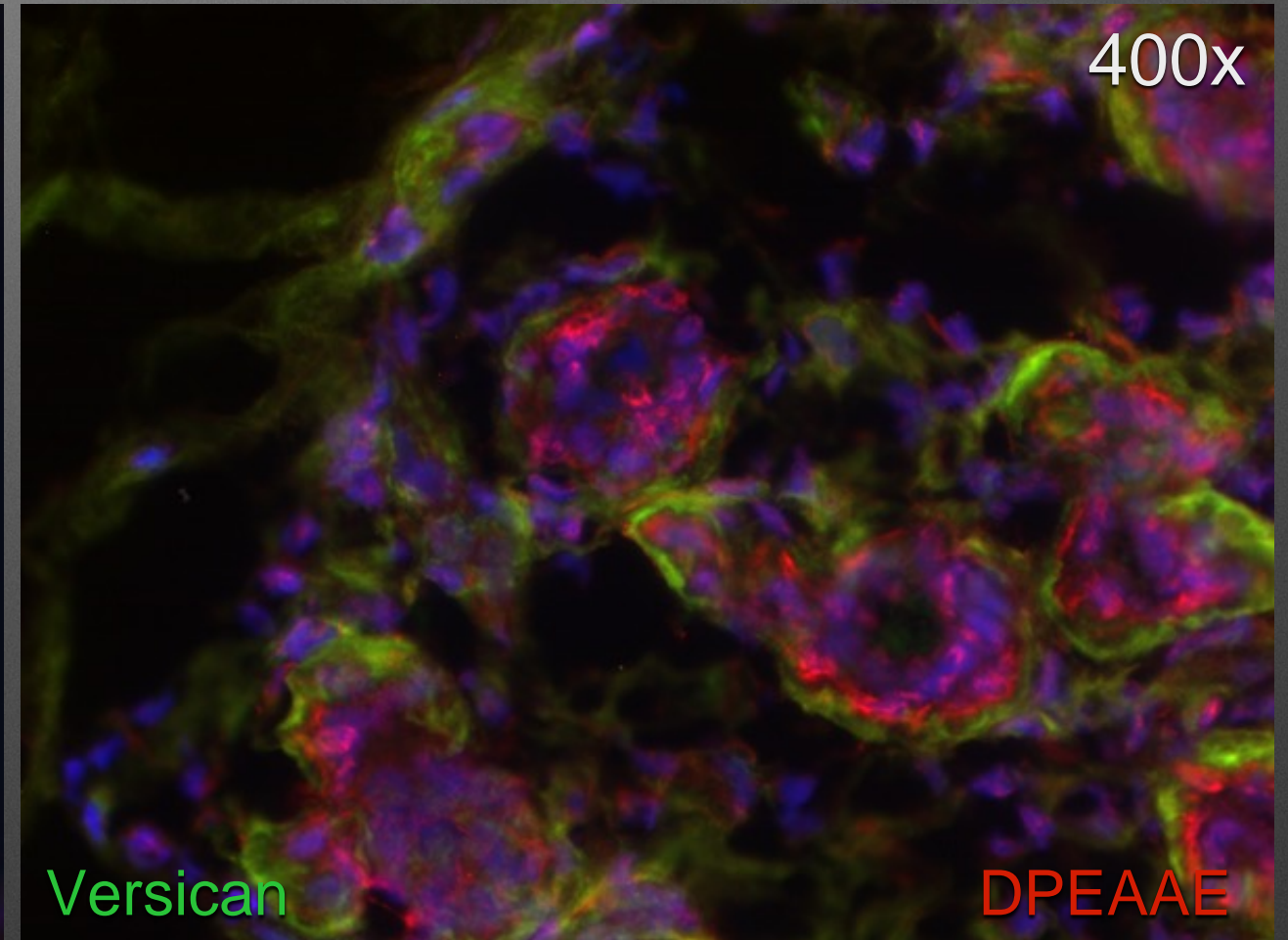
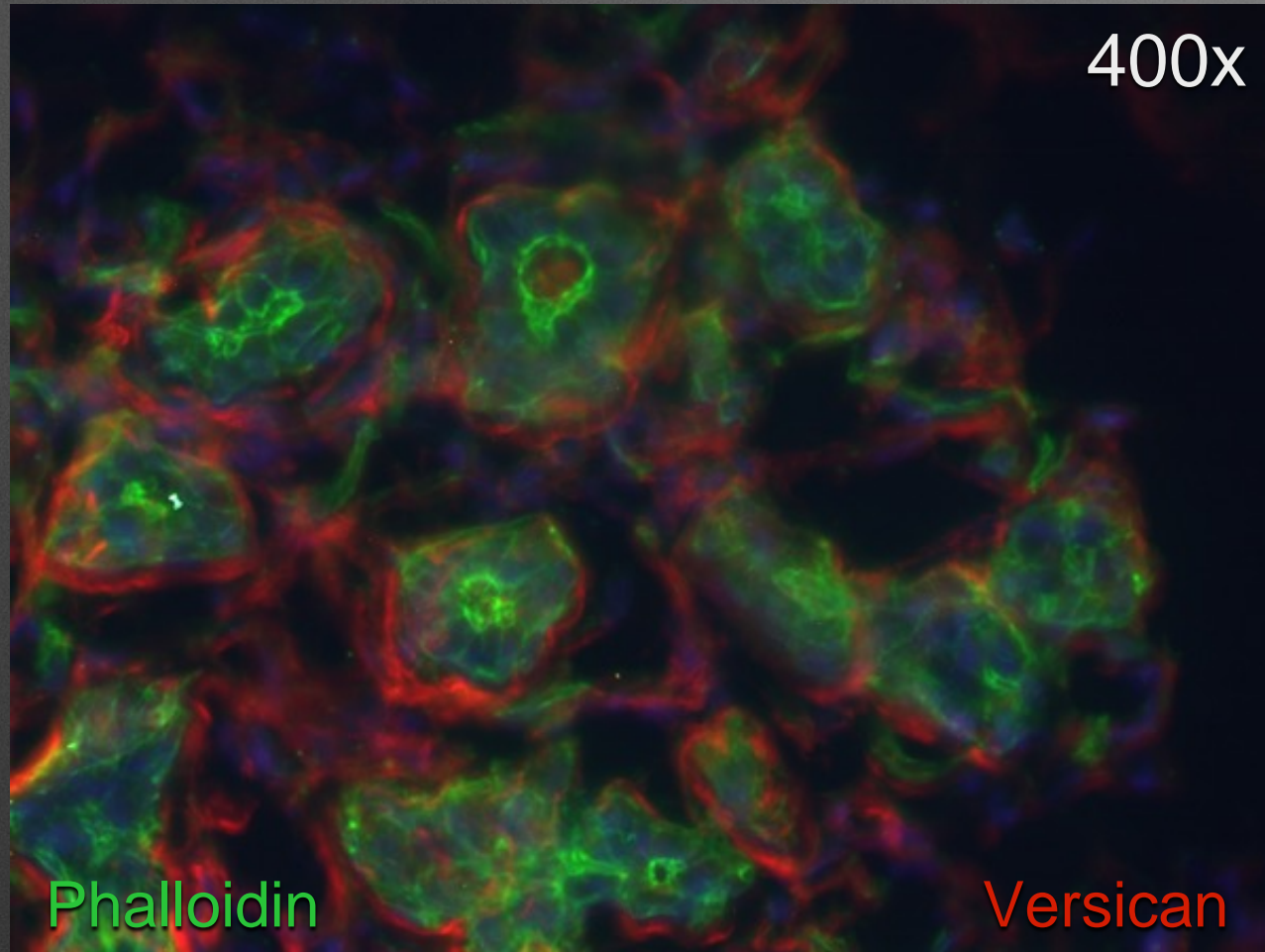


Lower levels of versican expression
but still detectable

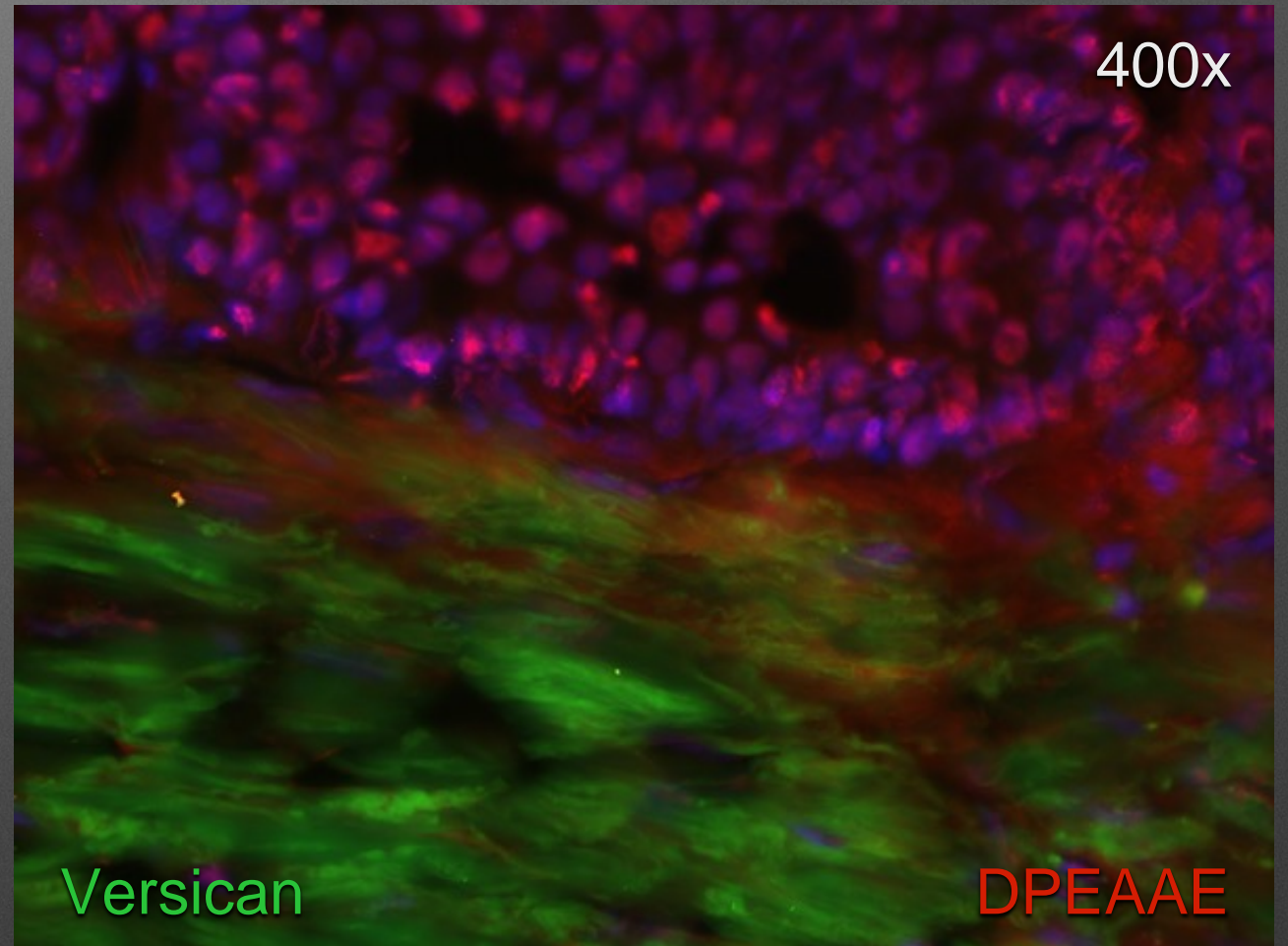
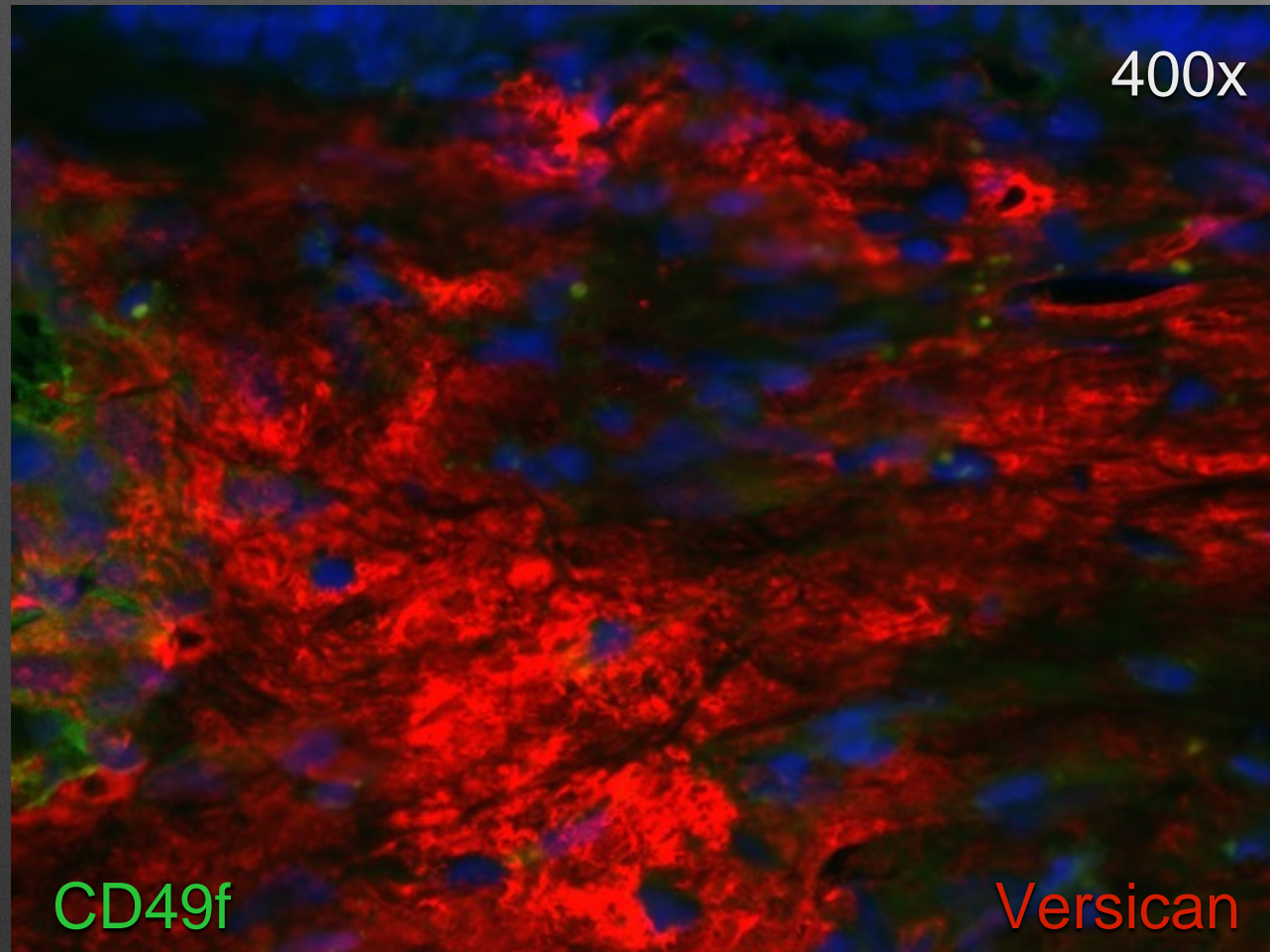
Normal breast - N239



Normal breast - N239



D23698T (DCIS w/ minimal invasion)



Versican isoform expression in S1 & T4-2 cells

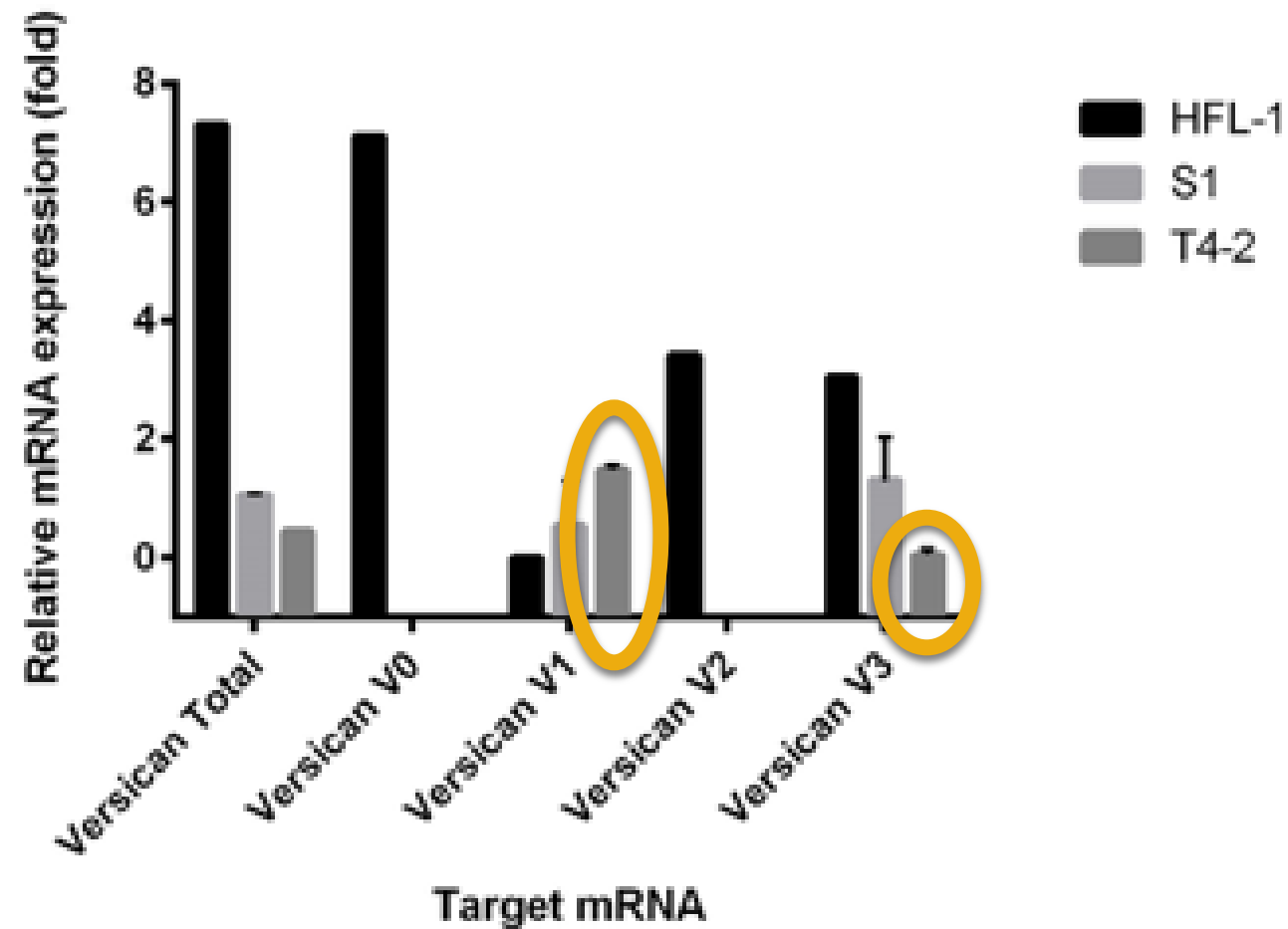


Figure 10. Relative mRNA expression of versican in S1, T4-2 and HFL-1 cells. HFL-1 (n=1 for total and all isoforms); S1 (n=2 for total and all isoforms); T4-2 (n=2 for all isoforms and n=1 for total versican). Columns show mean value and error bars represent SD.

Versican V1-DPEAAE secretion increases in T4-2 cells

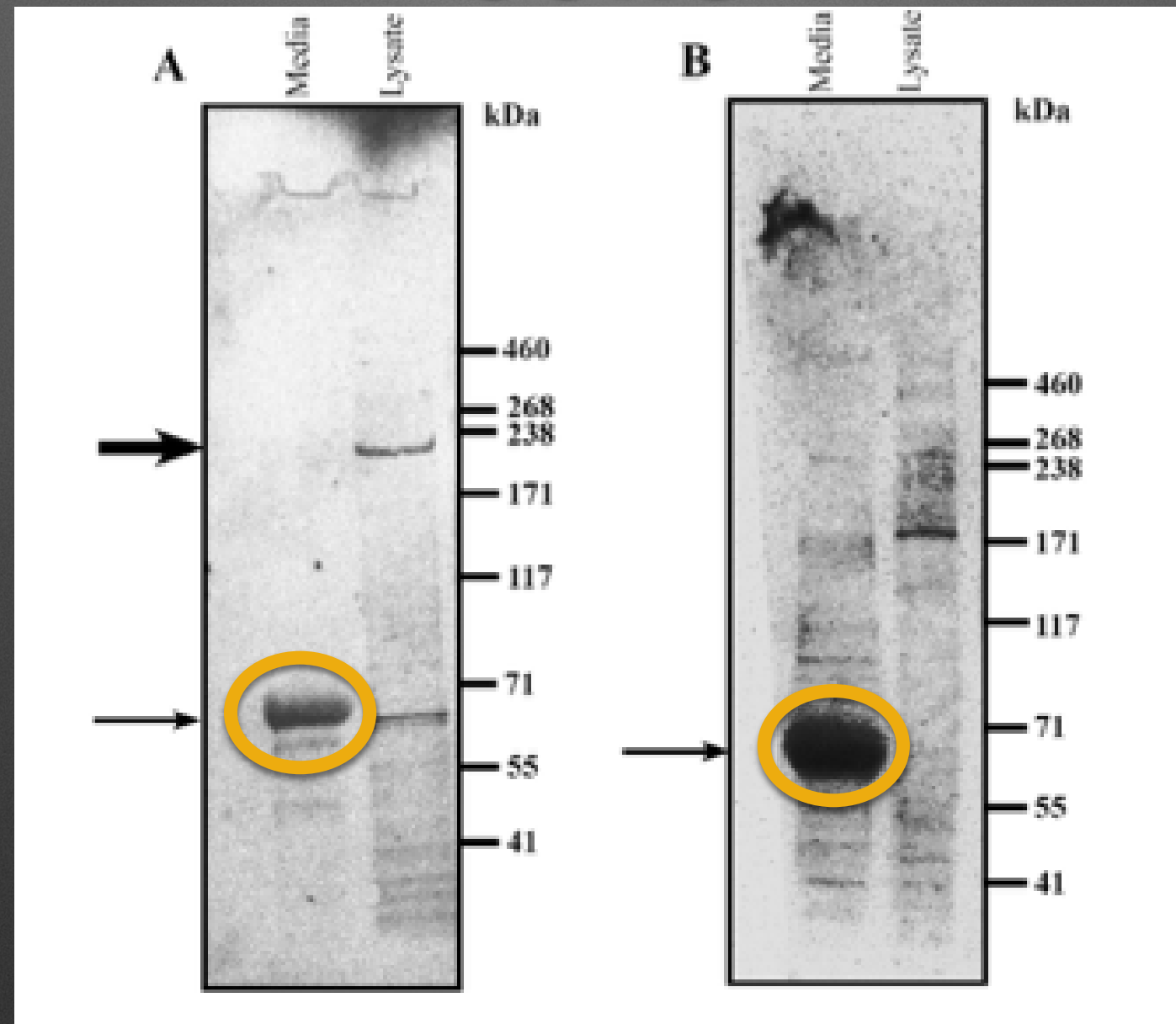
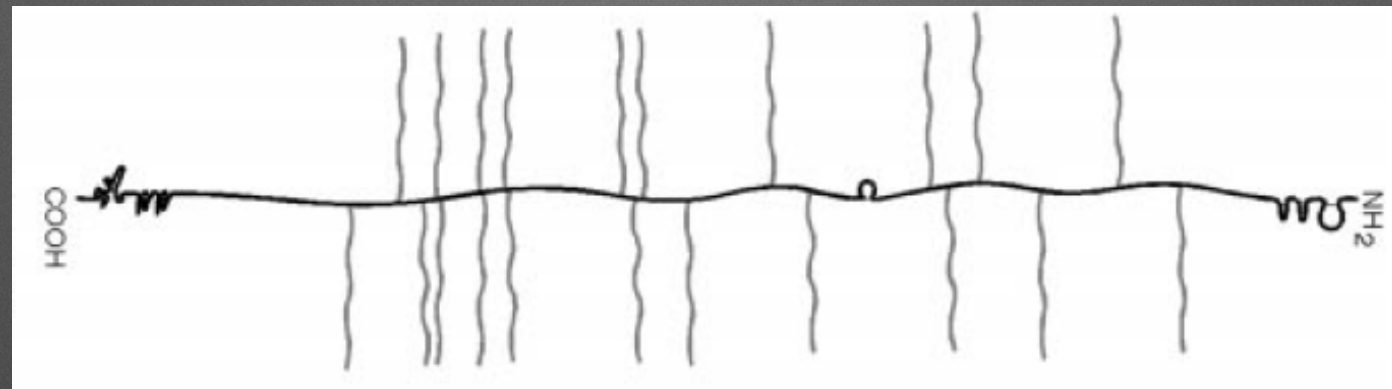


Figure 7. Detection of proteolytic fragments in HMT-3522 cells. S1 (A) and T4-2 (B) RIPA lyse and secreted media extracts immuno-blotted with the versican DPEAAE neo-epitope antibody. Large arrows indicate the V0 DPEAAE¹⁴⁹¹ fragment and small arrows indicate the V1 DPEAAE⁴⁴¹ fragment.

Lentiviral knockdown

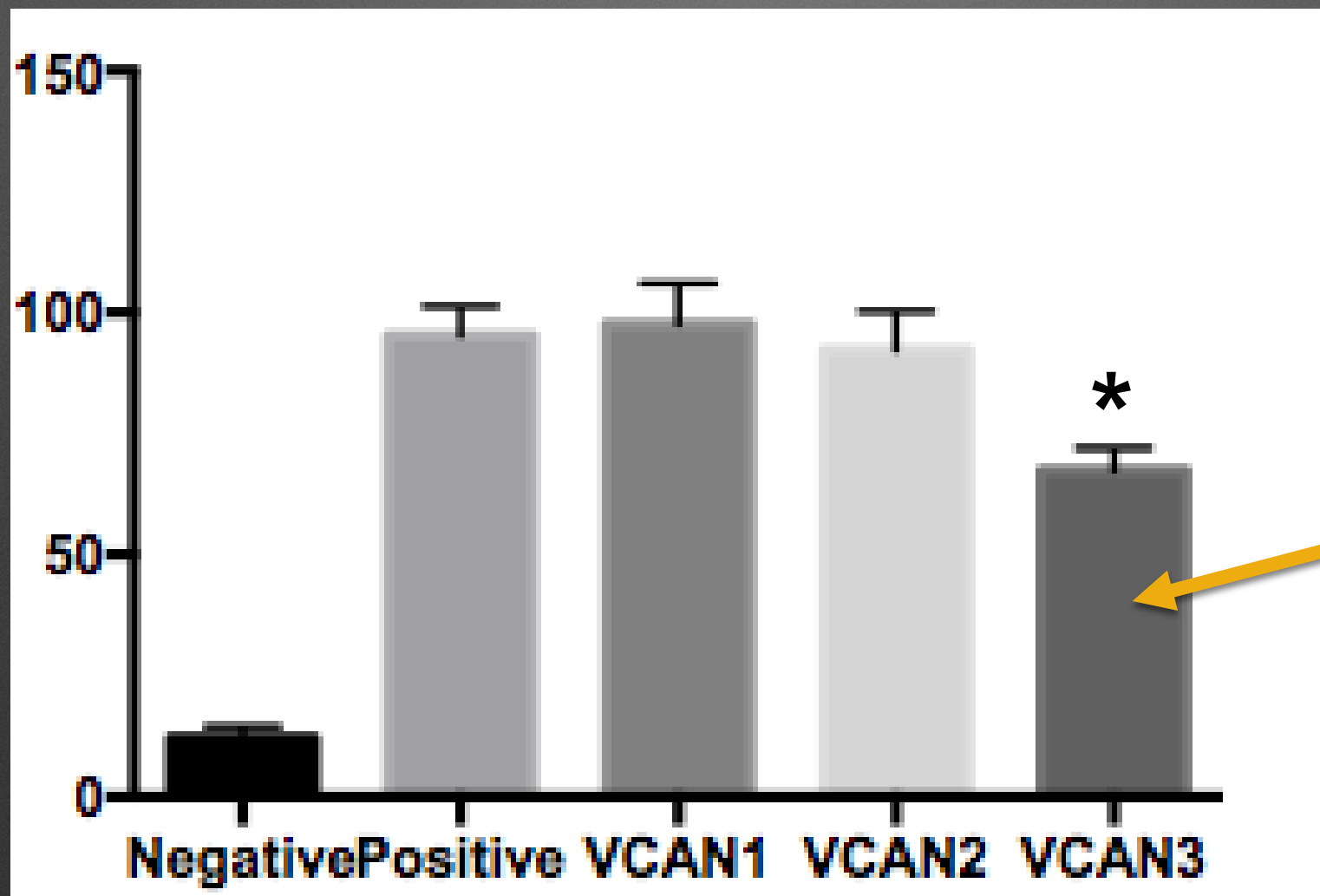


VCAN1 (C-terminal) = All isoforms

VCAN2 (signal) = All isoforms

VCAN3 (GAG-beta) = V0 & V1

Versican knockdown inhibits T4-2 invasion



No V0 or V1
No DPEAAE
signalling

What's next

- Do T4-2 knockdowns behave differently in 3D cell culture (morphology)
- Influence of T4-2 versican knockdown on communication with other cells

Thanks!

- Katie Timms
- Mina Bissell
- Curt Hines
- Sun-Young Lee
- Kate Thi
- Northern Medical Program
- You!

D23698T (DCIS w/ minimal invasion)

