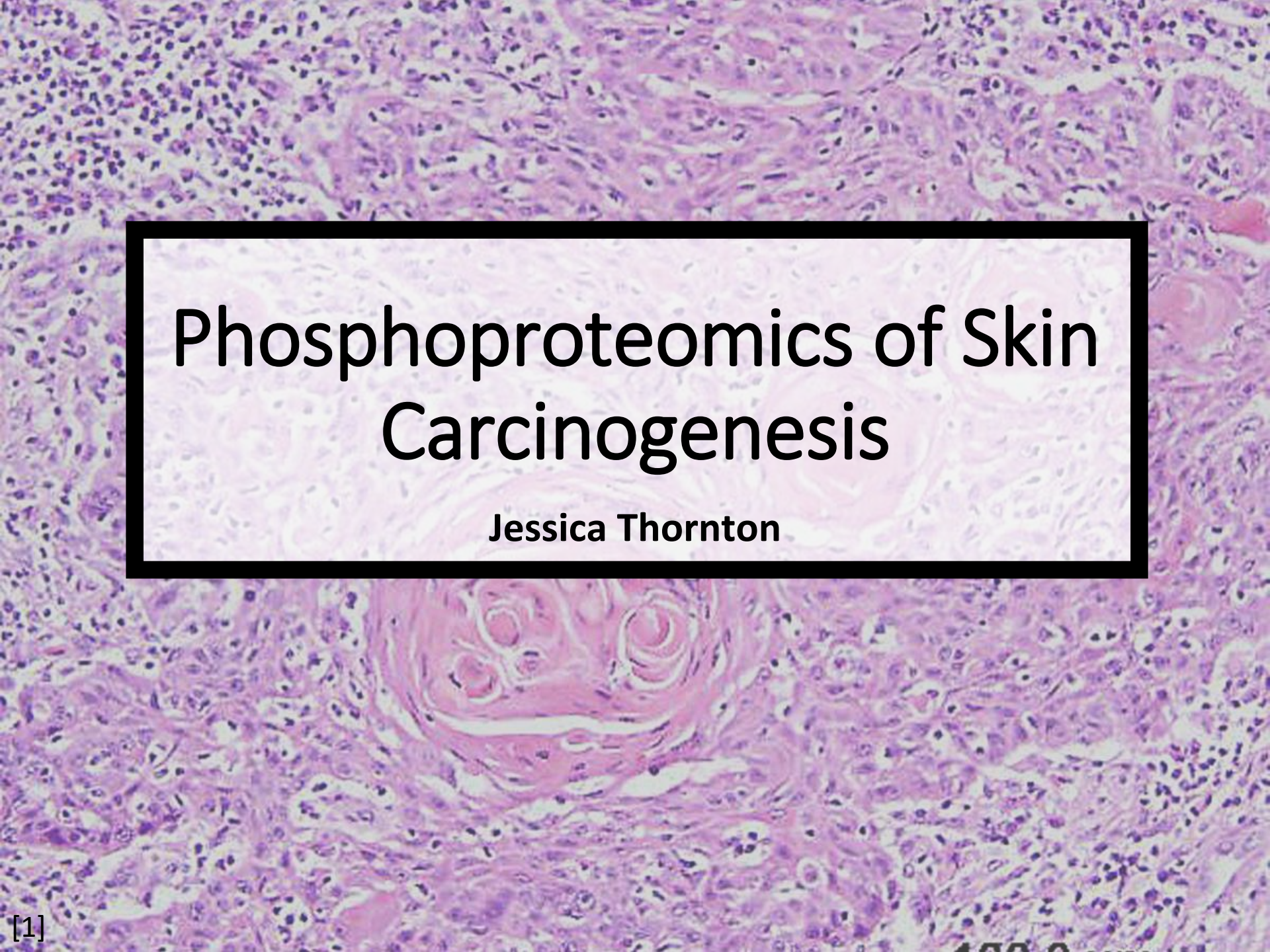
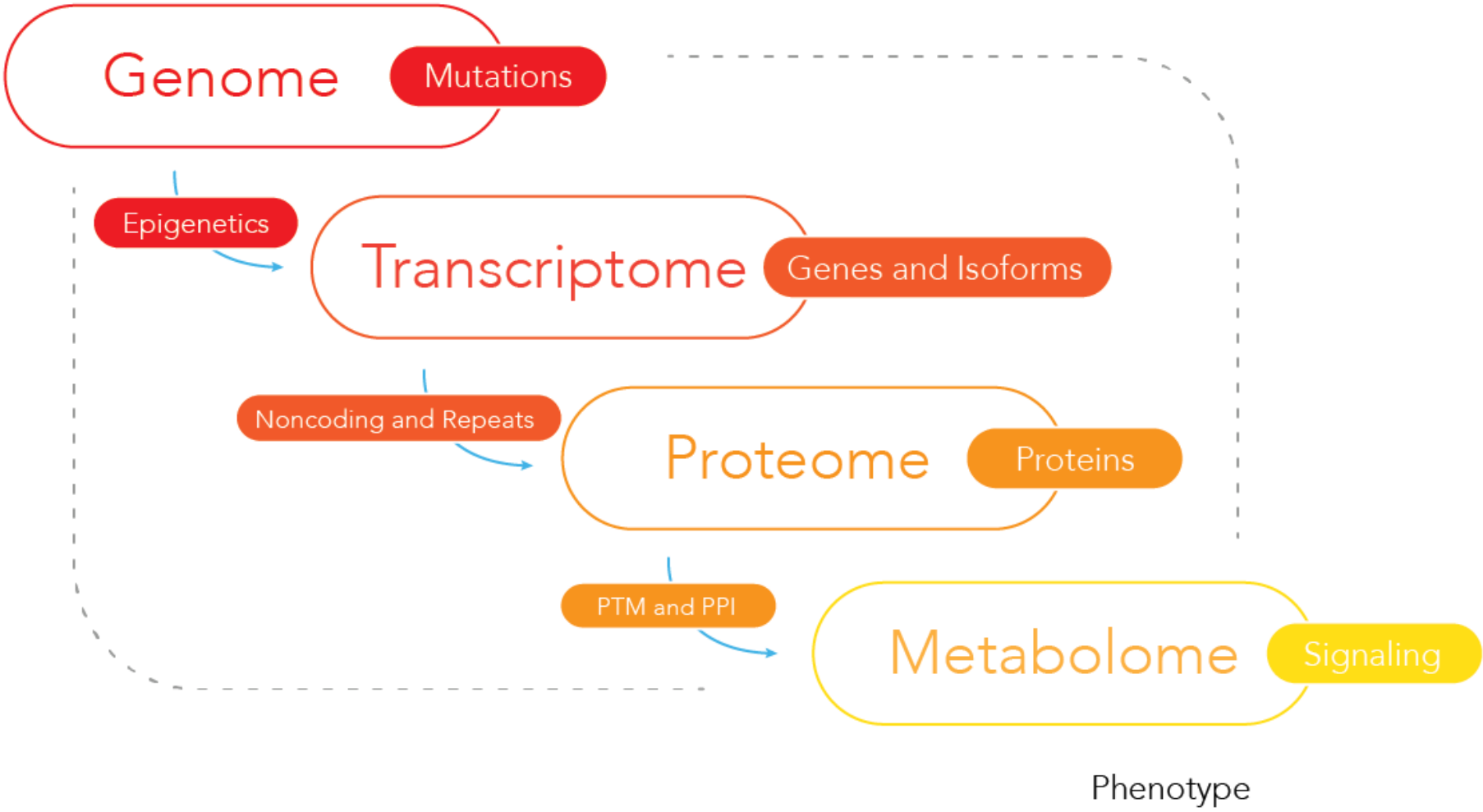


# Phosphoproteomics of Skin Carcinogenesis

**Jessica Thornton**

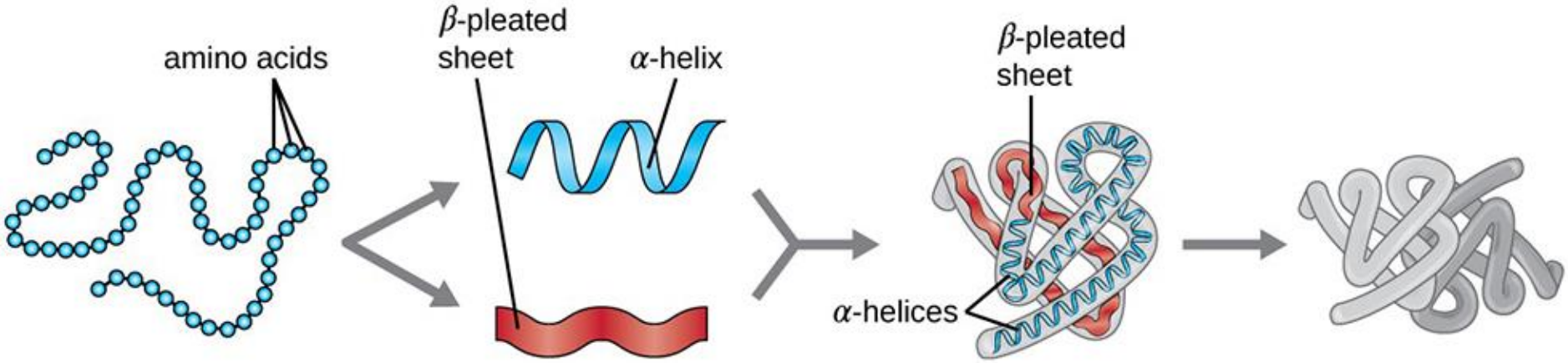
[1]

# What is proteomics?




[2]

# What are proteins?




**Primary Protein Structure**
Sequence of a chain of amino acids

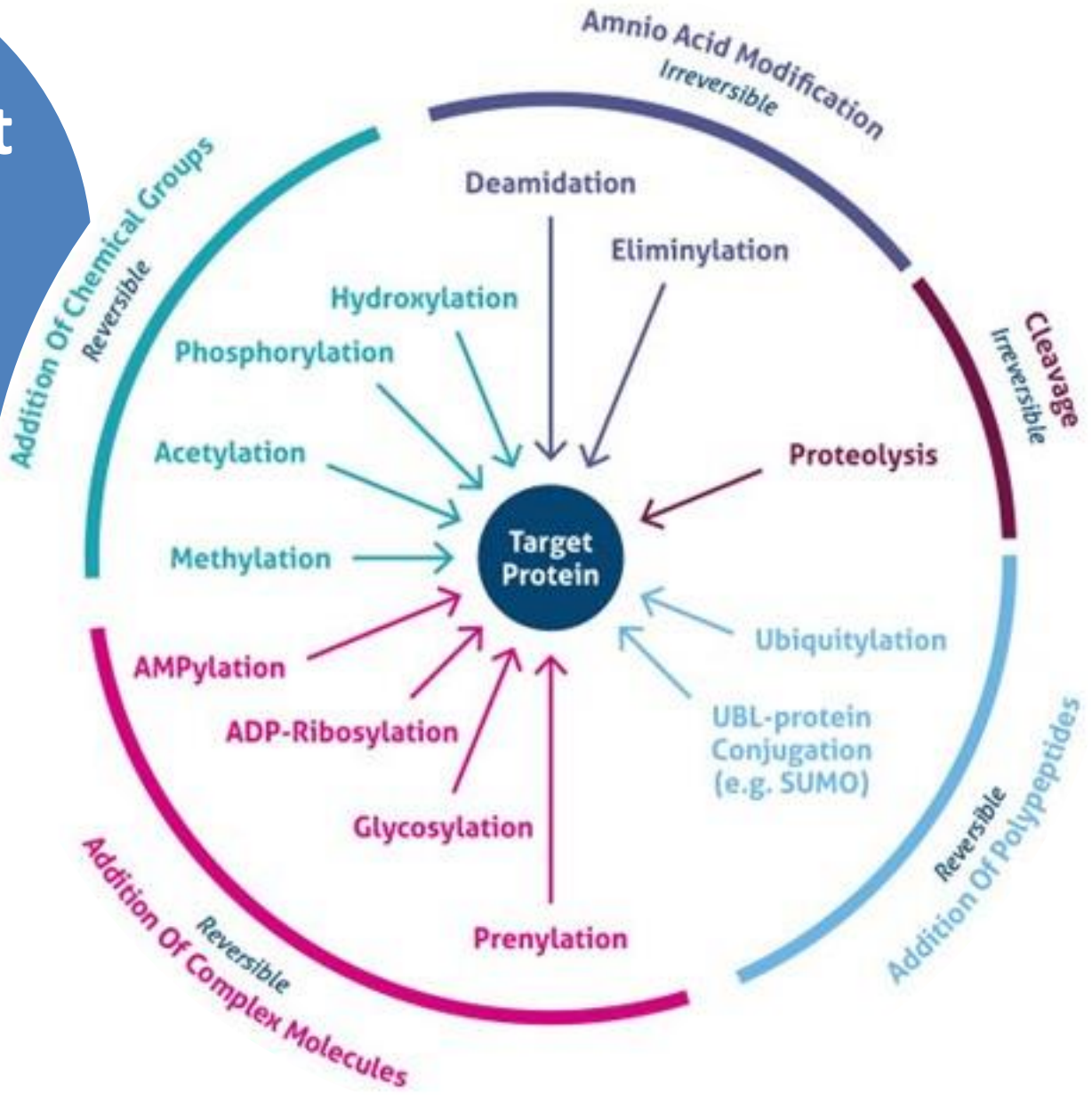
**Secondary Protein Structure**
Local folding of the polypeptide chain into helices or sheets

**Tertiary Protein Structure**
three-dimensional folding pattern of a protein due to side chain interactions

**Quaternary Protein Structure**
protein consisting of more than one amino acid chain

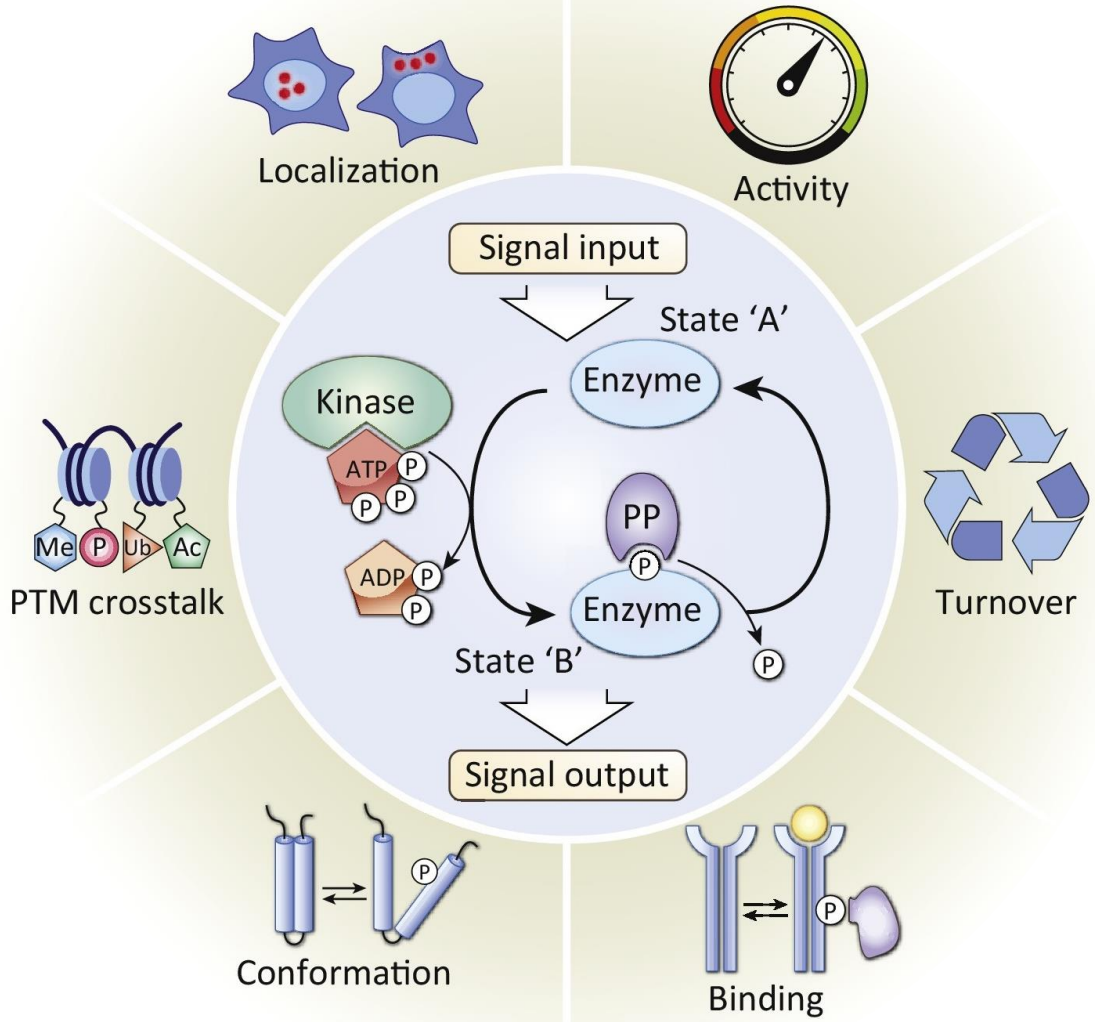
[3]

# What are post translational modifications (PTMs)?



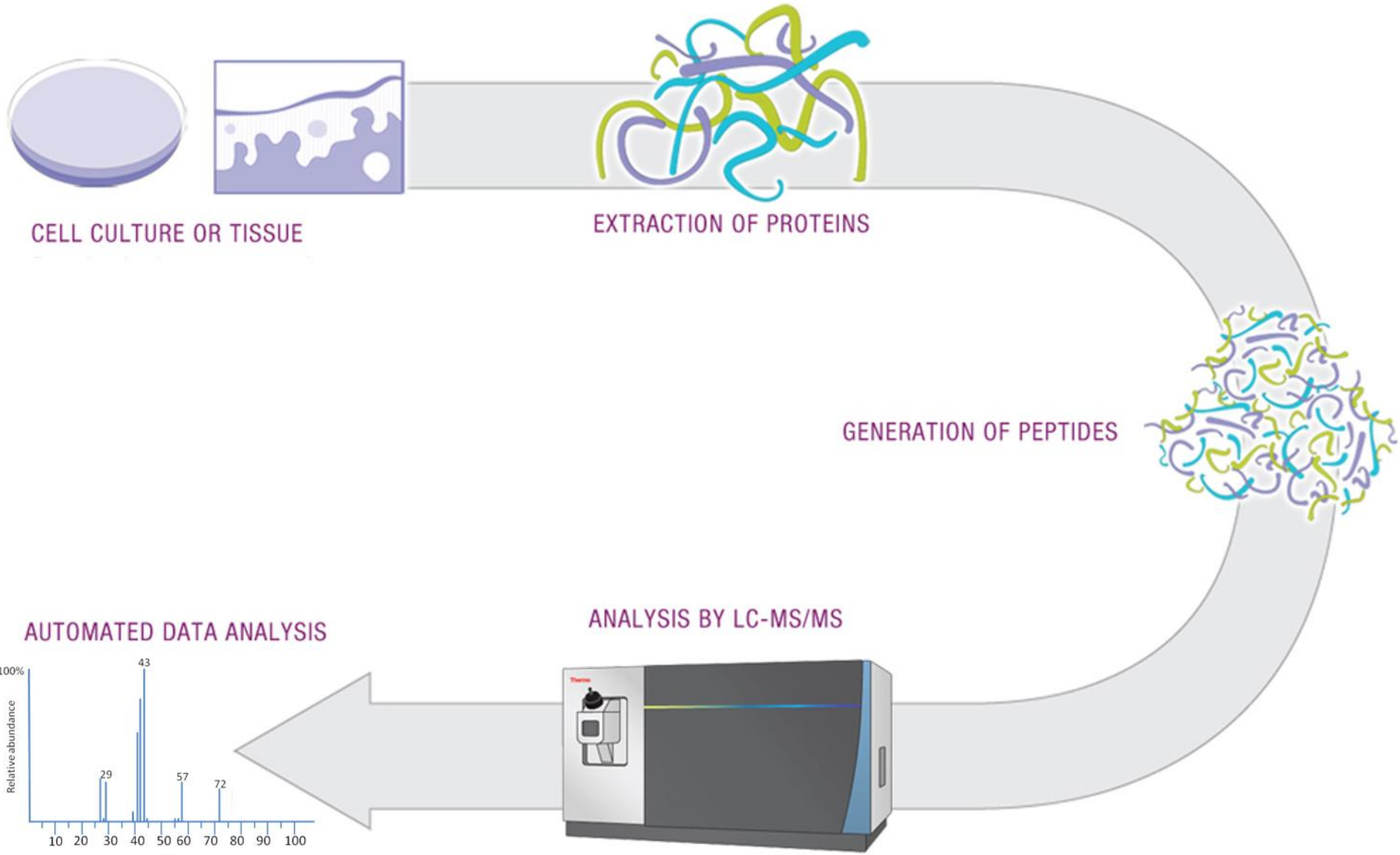

[4]

# What does phosphorylation do?




[5]

# What is the typical proteomics workflow?

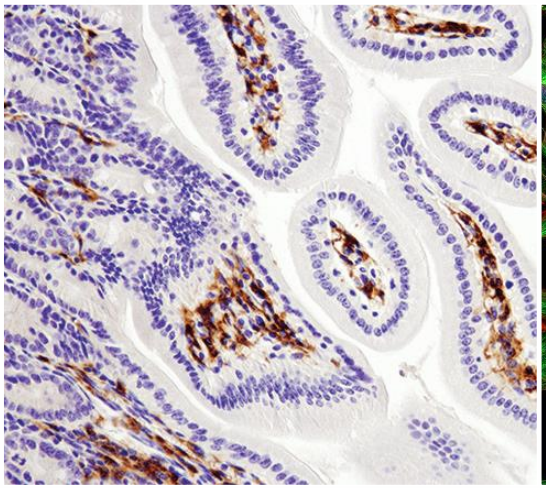


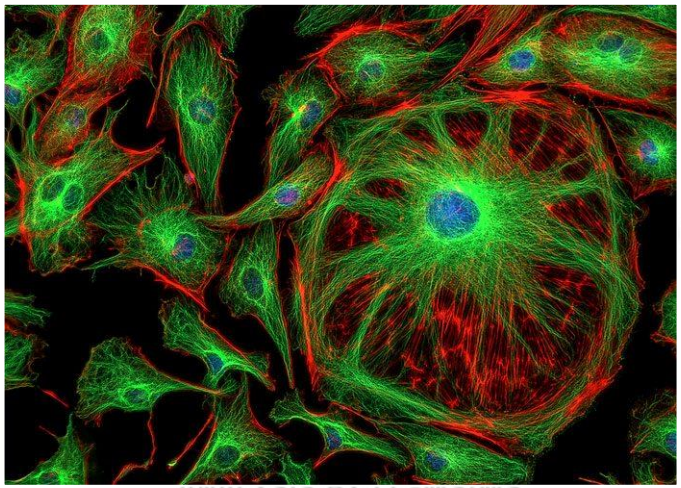

[6]

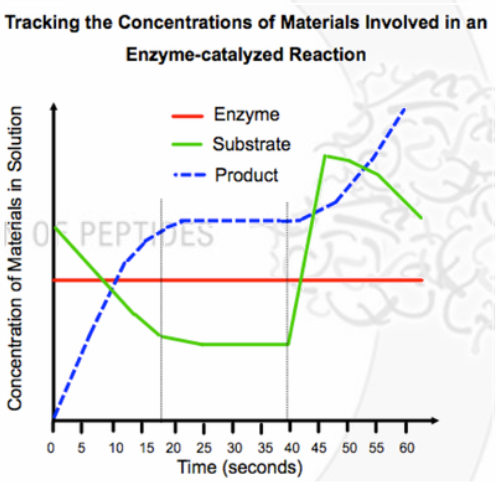
# What is the typical proteomics workflow?

CELL CULTURE OR TISSUE

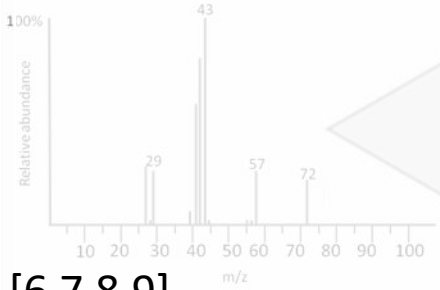
## FUNCTIONAL ASSAYS





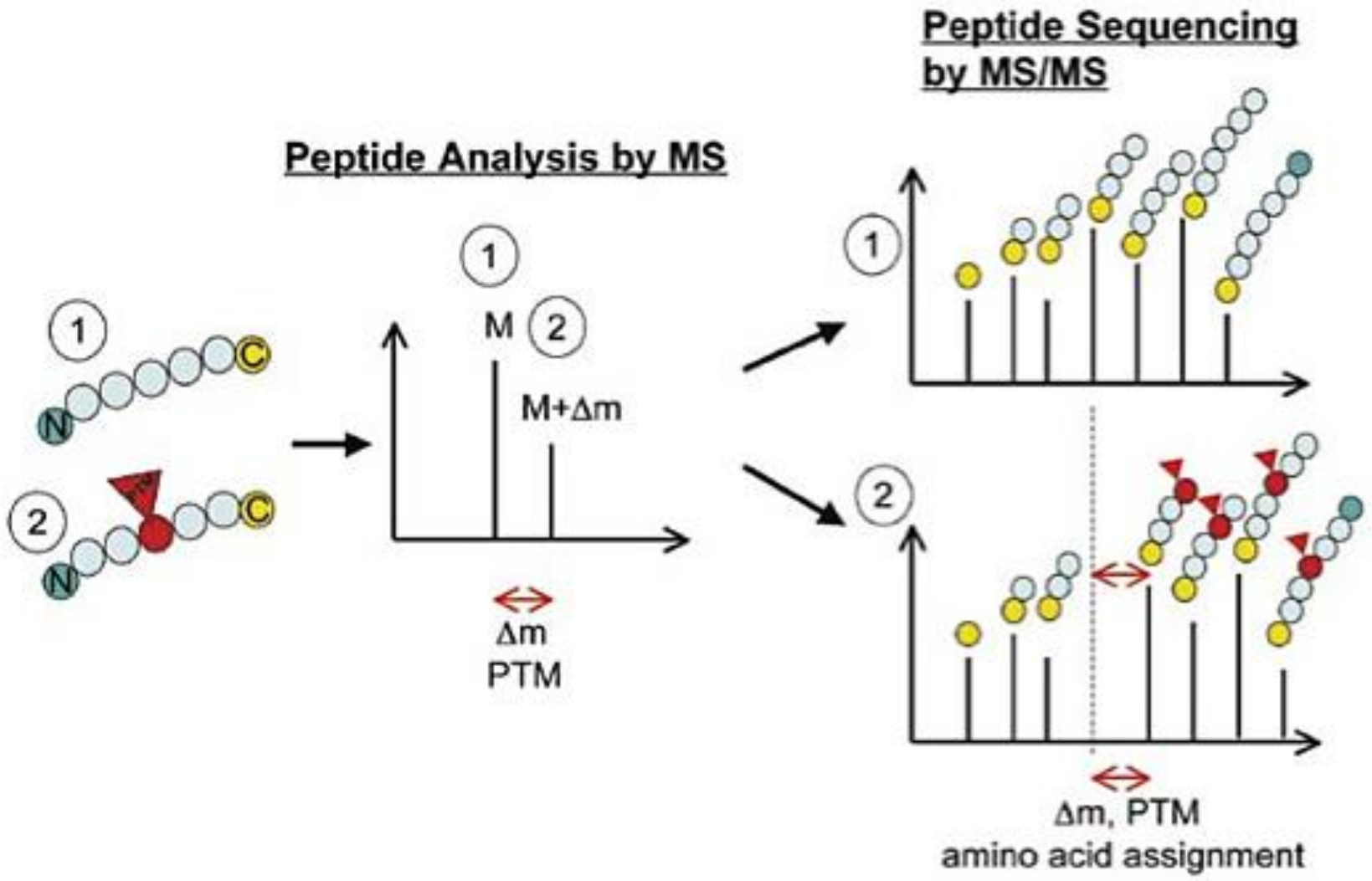



AUTOMATED DATA ANALYSIS




[6,7,8,9]

# How can we detect PTMs?




[10]

# How do we quantitatively analyze proteins?

CELL CULTURE OR TISSUE

**Metabolic Labeling**
(SILAC)

EXTRACTION OF PROTEINS

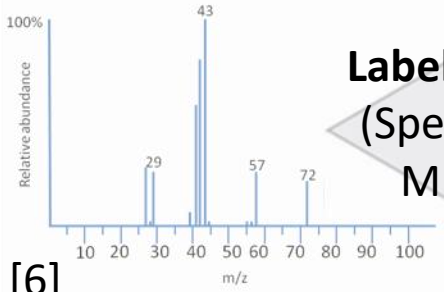
**Spike In Labeling**
(AQUA, QconCAT, Super-SILAC)

GENERATION OF PEPTIDES

**Enzymatic Labeling**
($^{18}O$)

ANALYSIS BY LC-MS/MS

**Isobaric or Chemical Labeling**
(iTRAQ, TMT, TrEnDi)




AUTOMATED DATA ANALYSIS

**Label-Free Methods**
(Spectral Counting, MRM, SWATH)

[6]

# What is SILAC?

**S**table

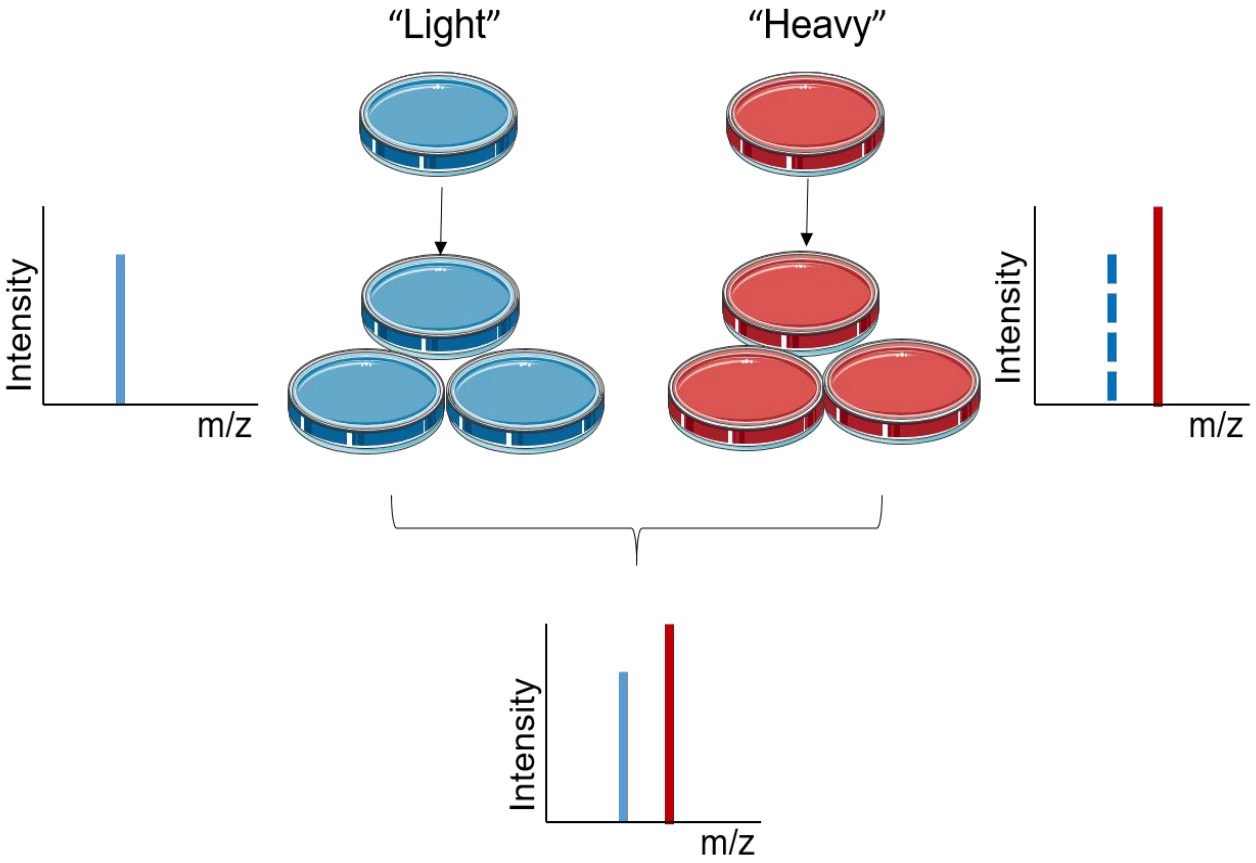
**I**sotope

**L**abeling by

**A**mino acids

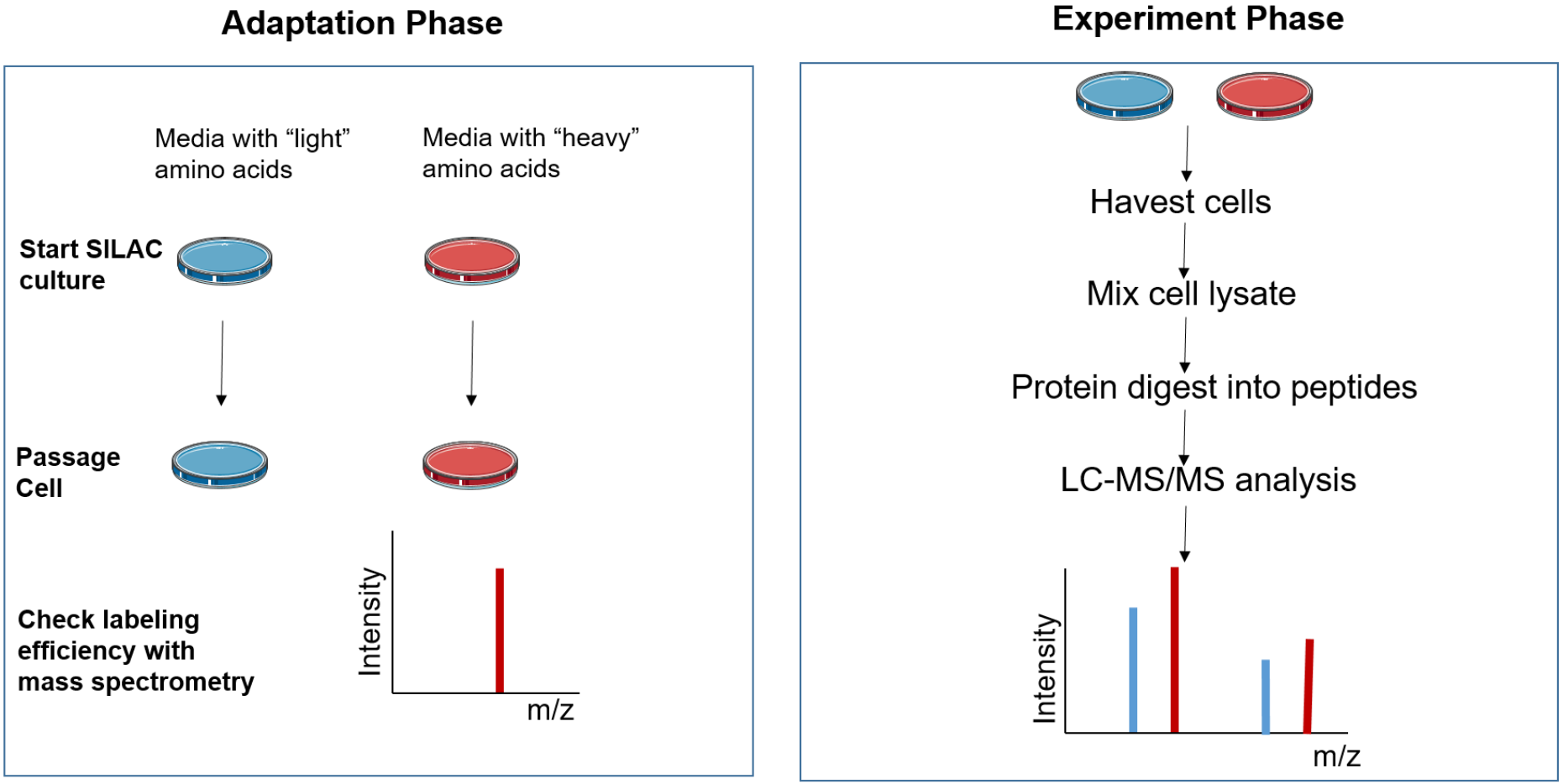
**I**n

**C**ell culture



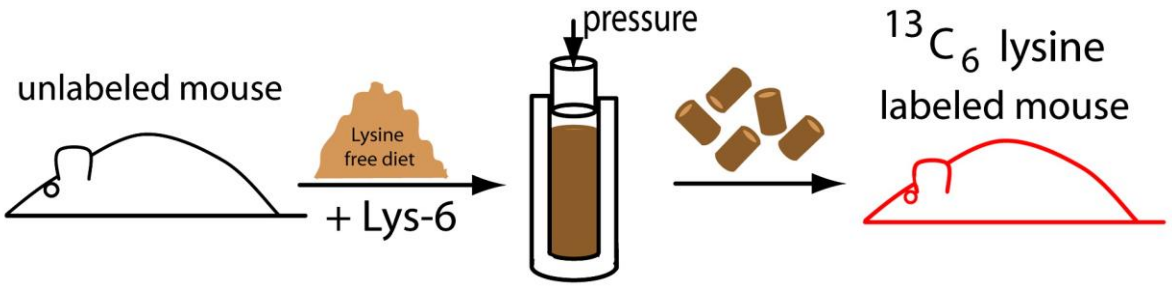

[12]

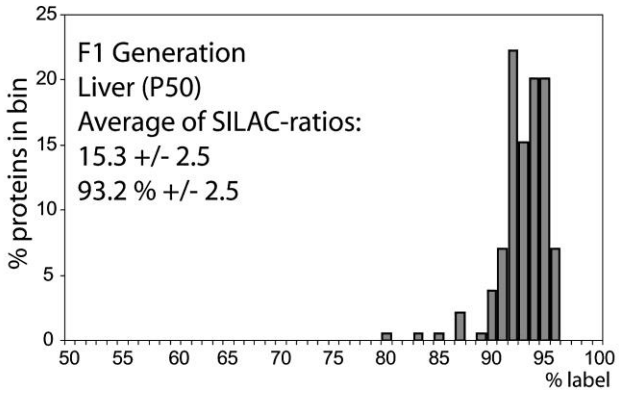
# How do you perform SILAC?

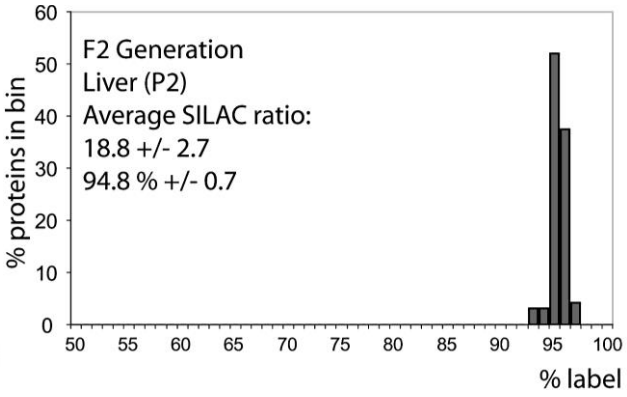


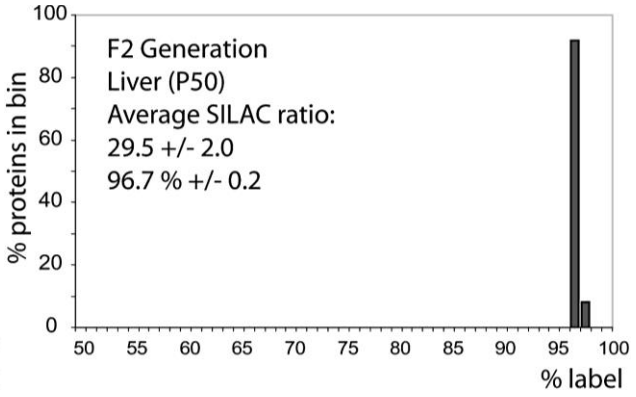

[12]

# How can you use SILAC in mice?



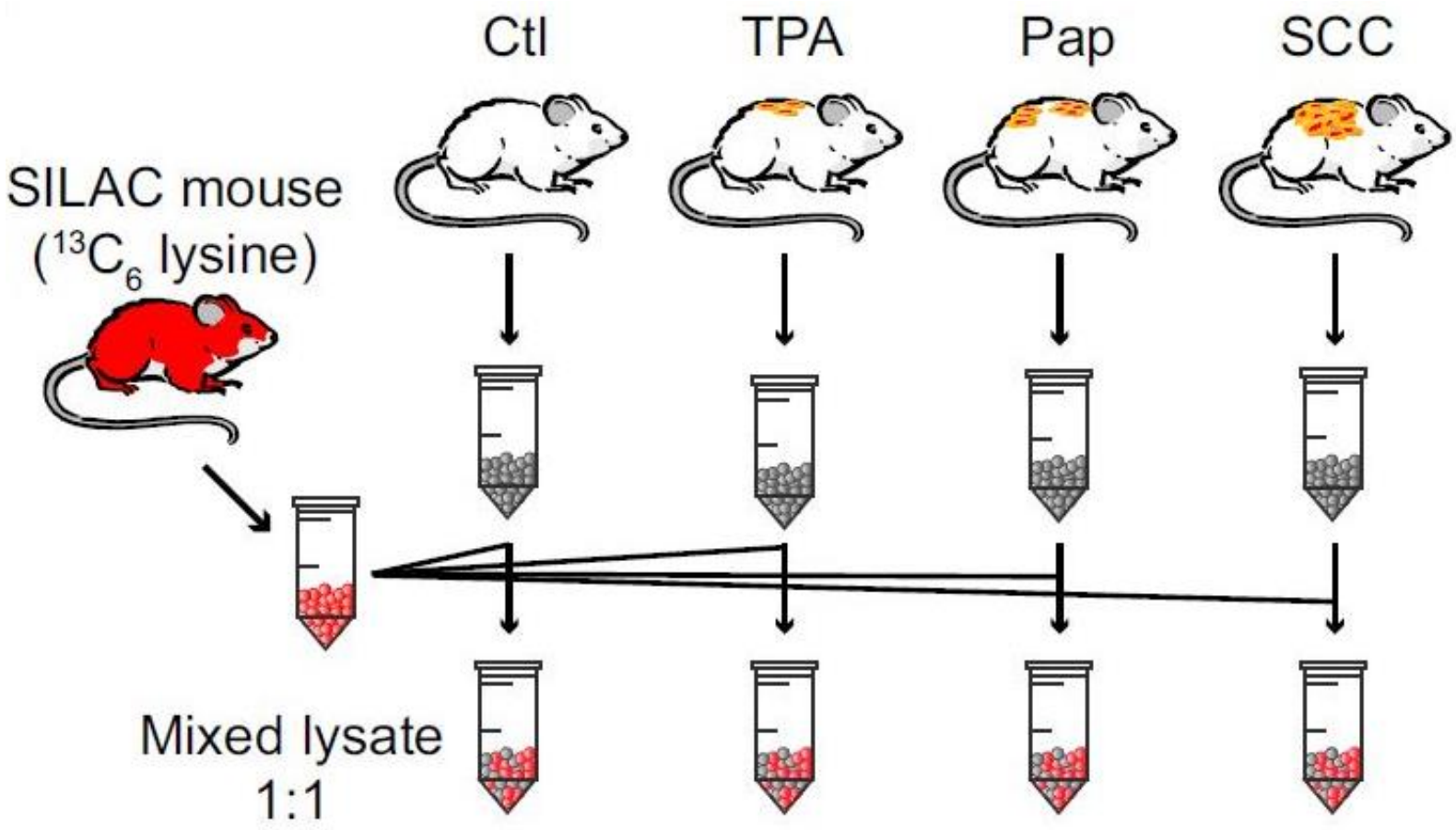



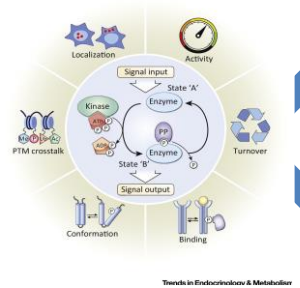




[13]

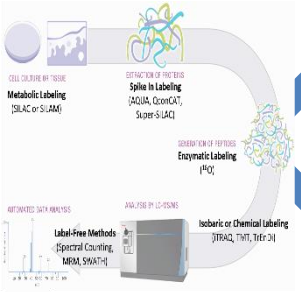
# How did the authors use SILAC?



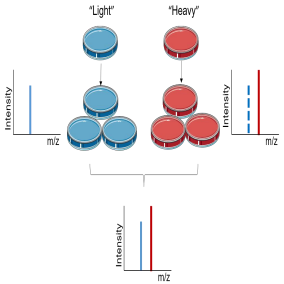
# Summary




**Knowledge of proteins and their PTMs is important to know their function**

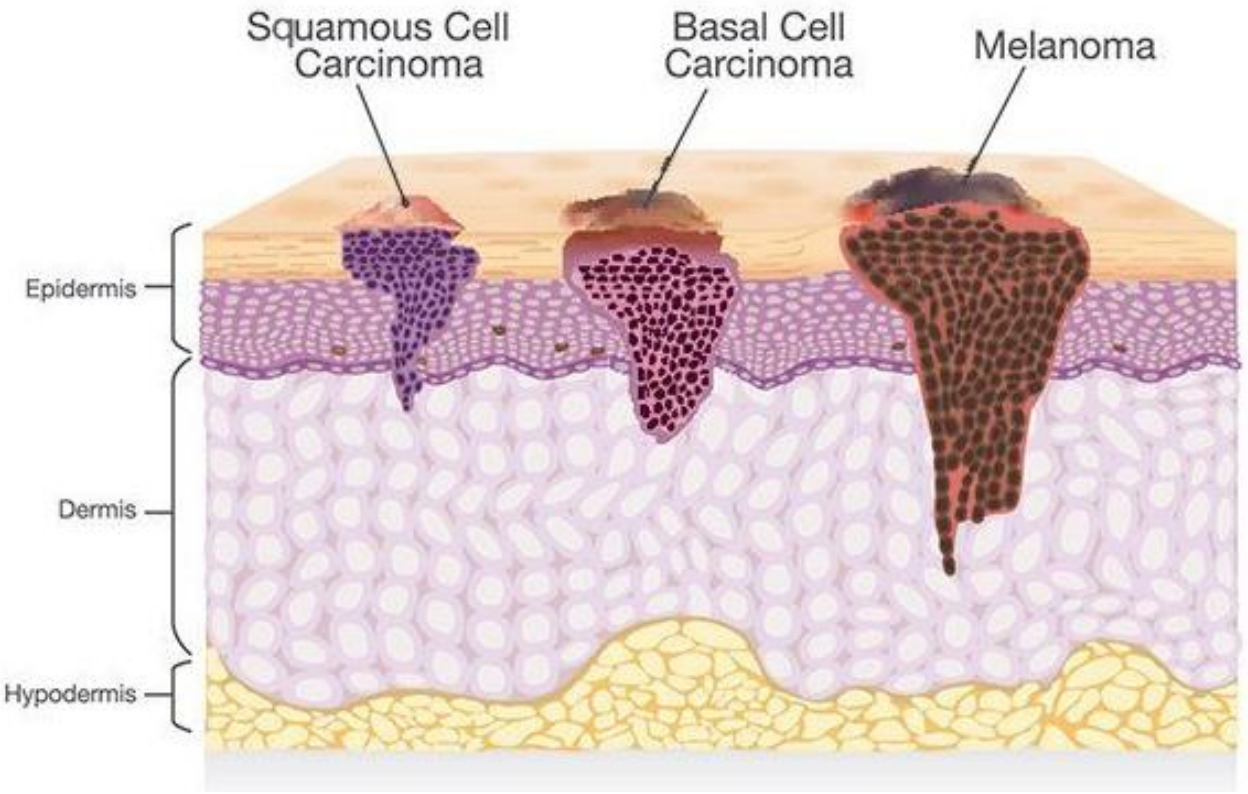



**Quantitative proteomics requires labeling of peptides**




**SILAC is a good way to label proteins in vivo**

# What is skin cancer?




[14]

# Why study skin cancer?


Squamous cell carcinoma (SCC) is diagnosed in more than **1 million** Americans each year

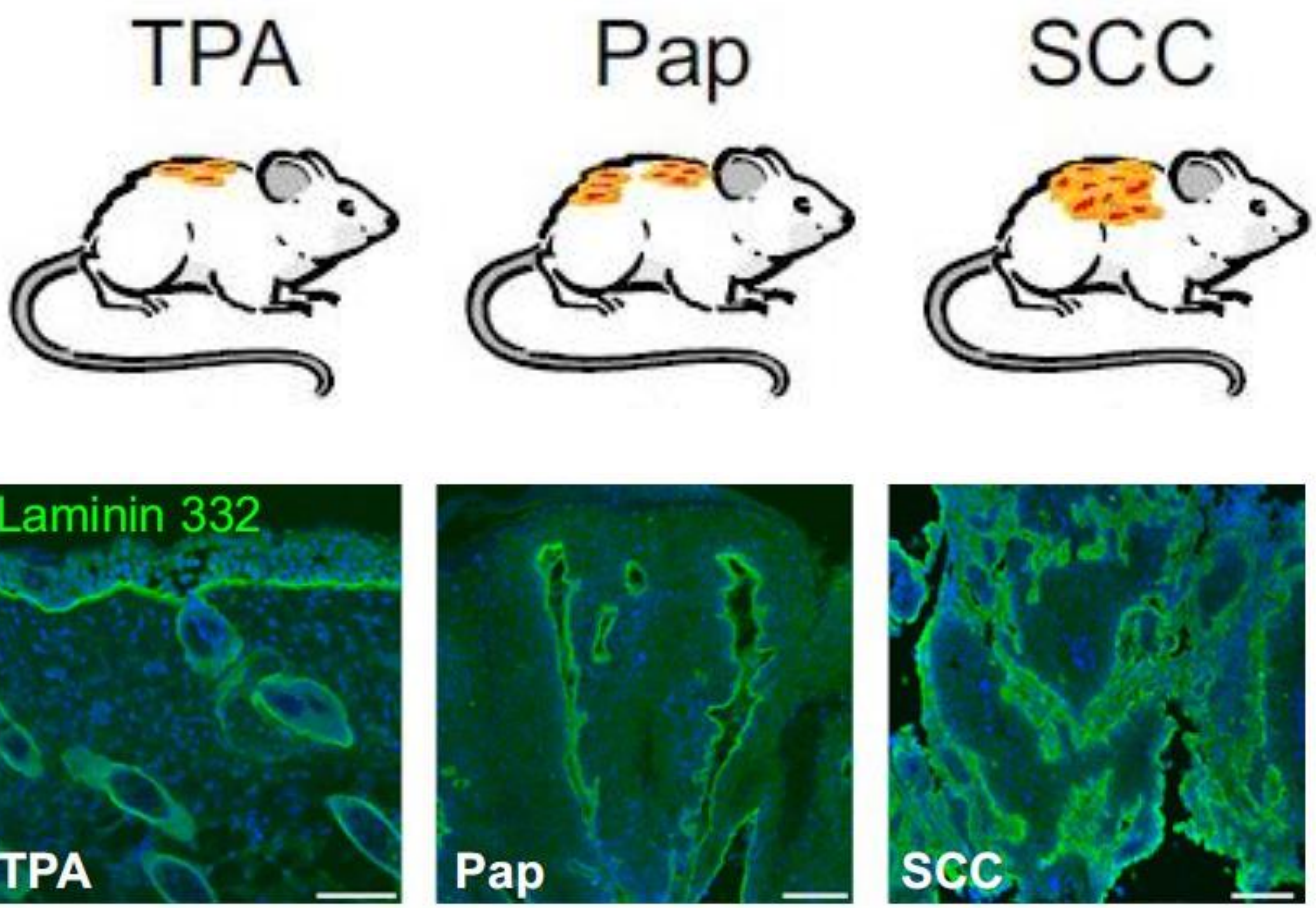
1m+

92k

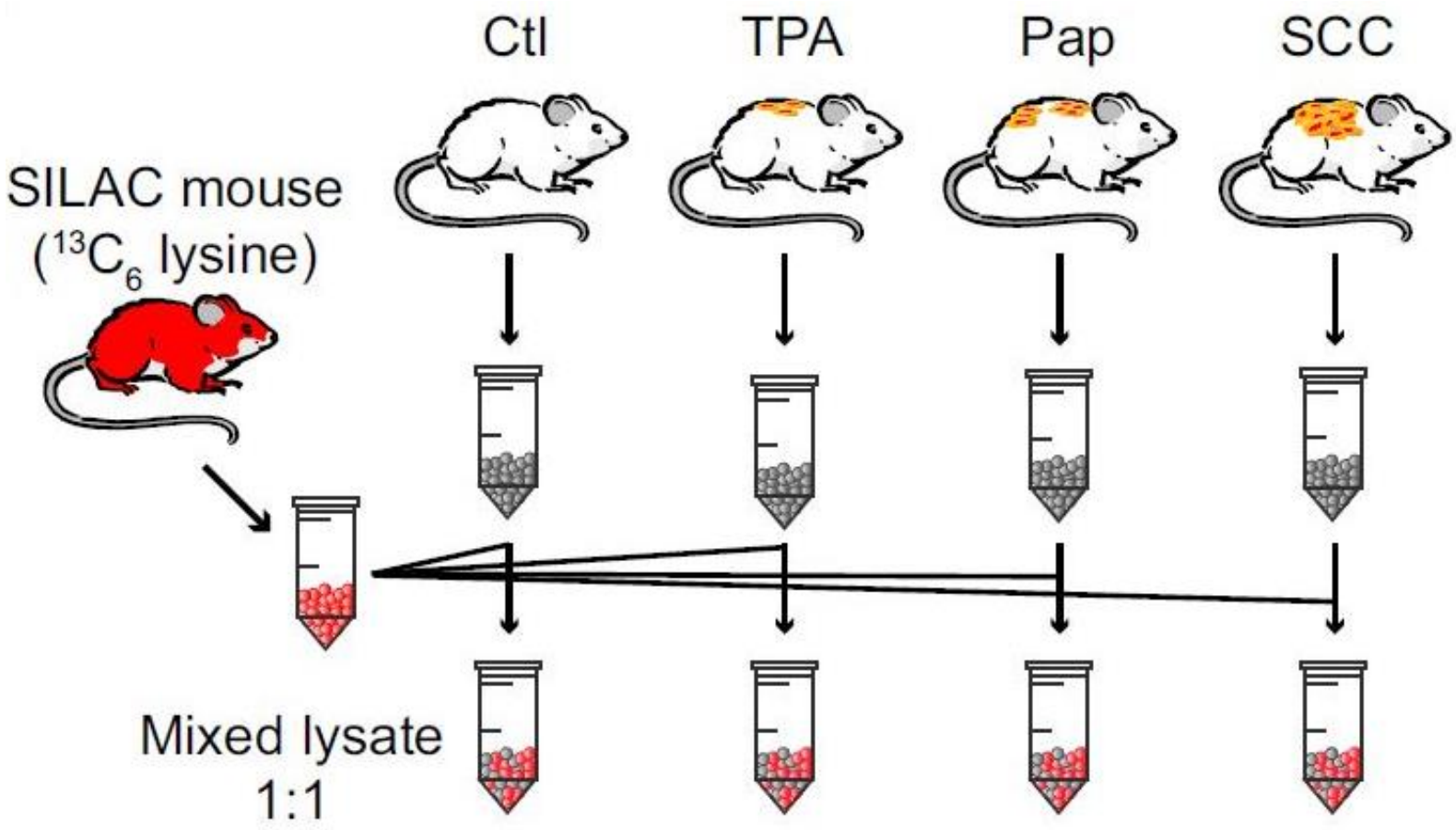
In 2018, melanoma will account for nearly **92,000** new cases of skin cancer

[15]

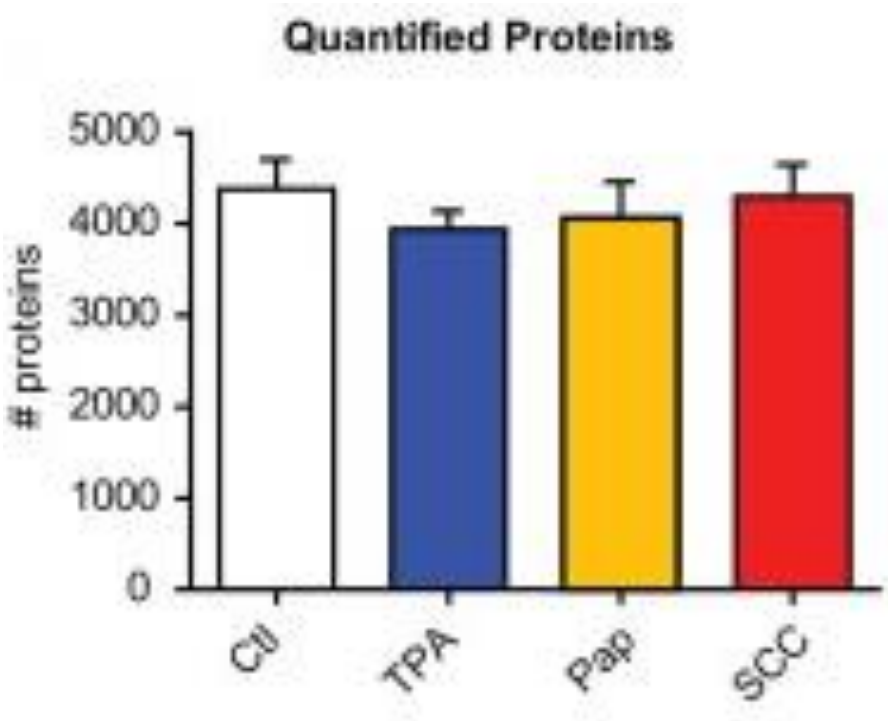
# How can we model skin cancer?

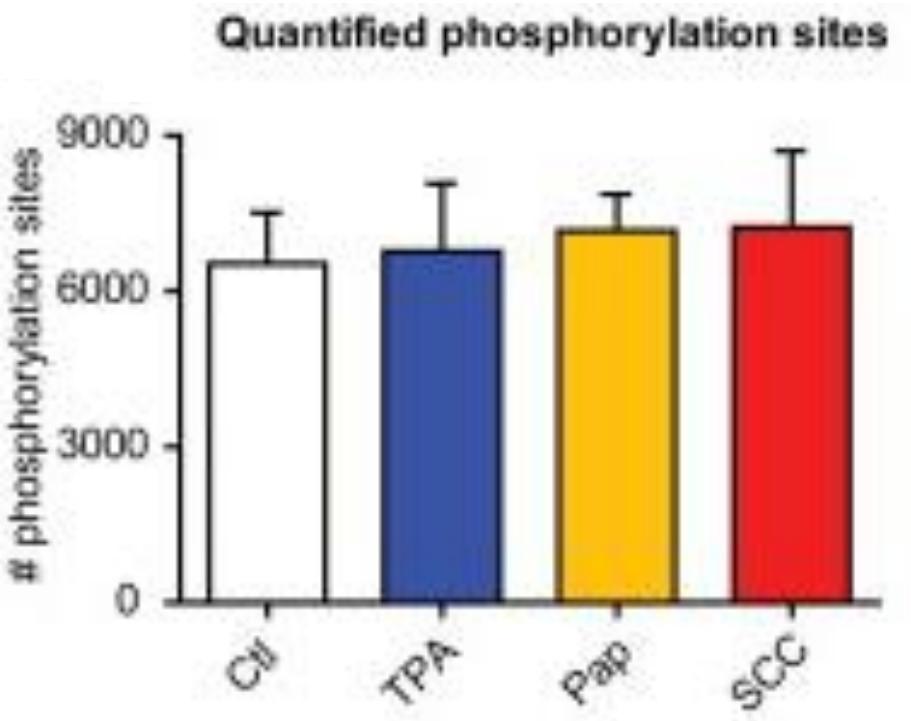


# How can we study the skin carcinogenesis proteome?



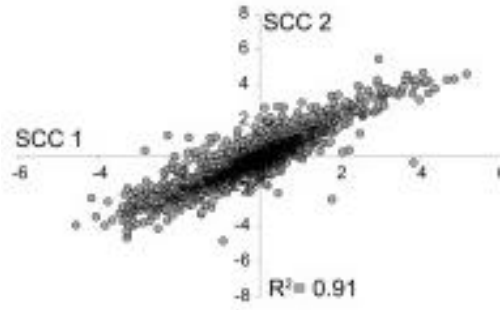
# How well does the SILAC model label the proteome?

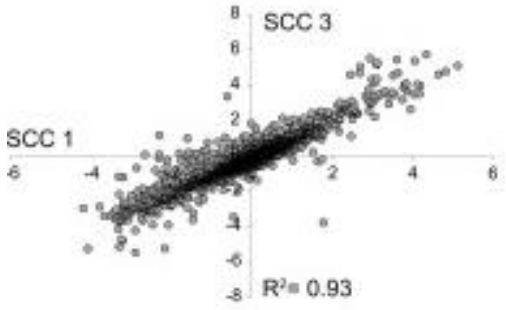


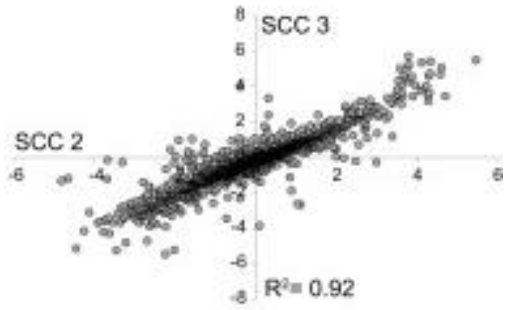



# How accurate is this method across samples?

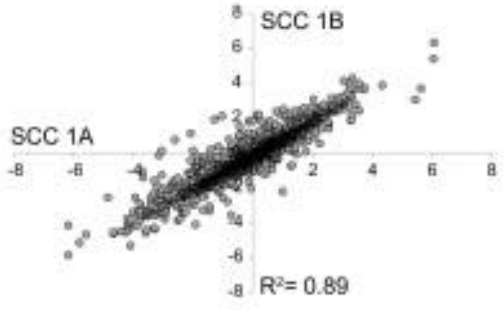
Proteome experiment

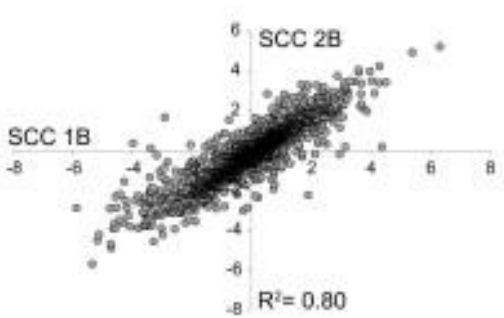


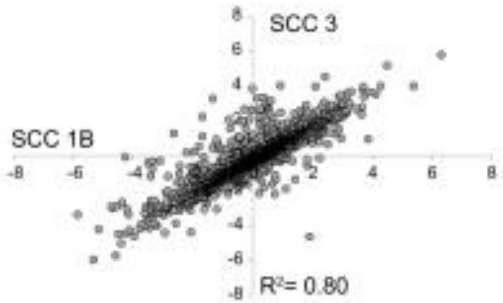



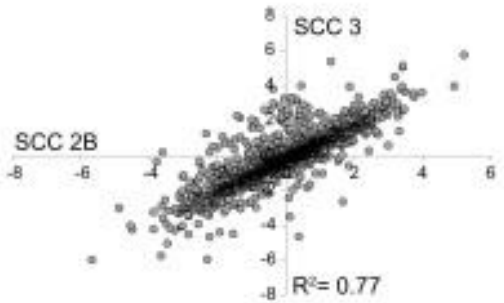




Phosphoproteome experiment

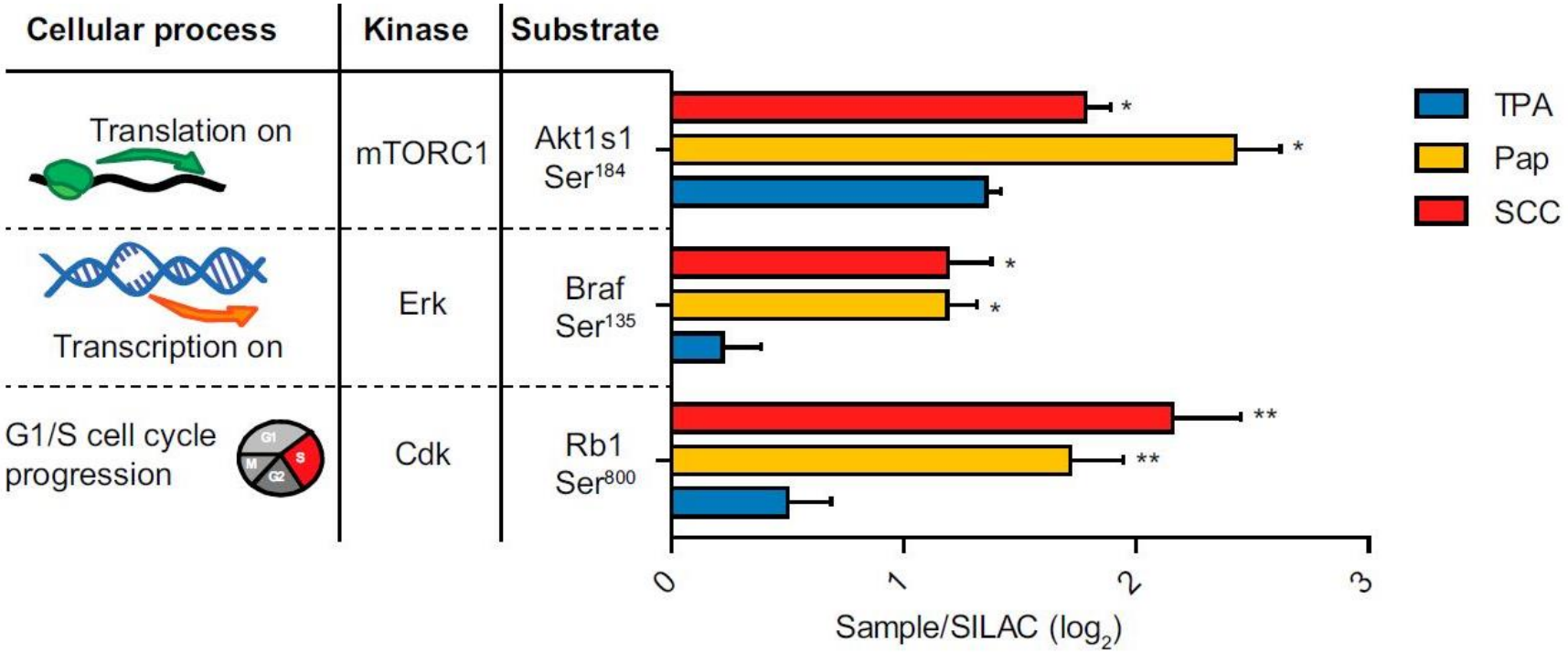


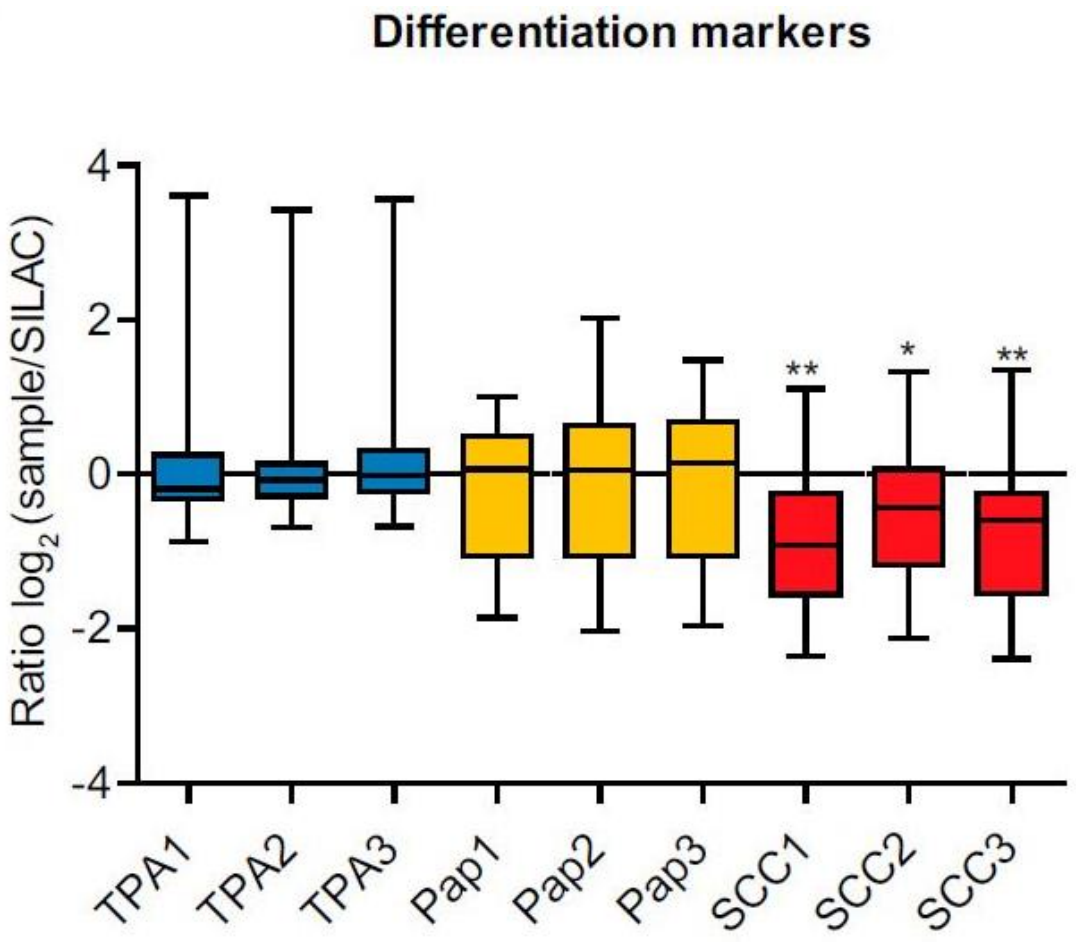



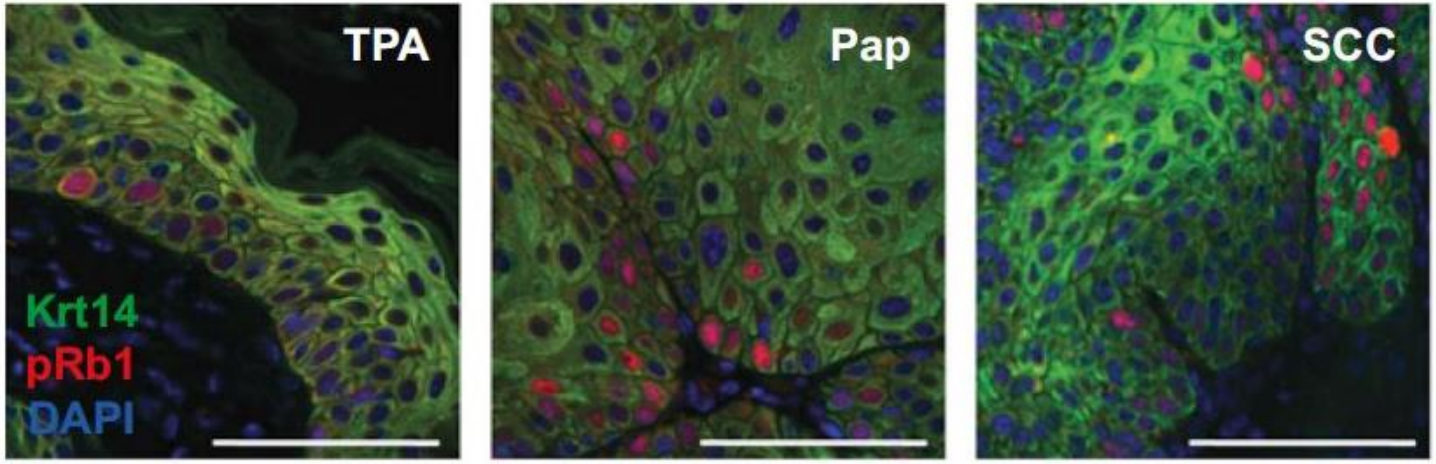
# Can this method rediscover known cancer traits?

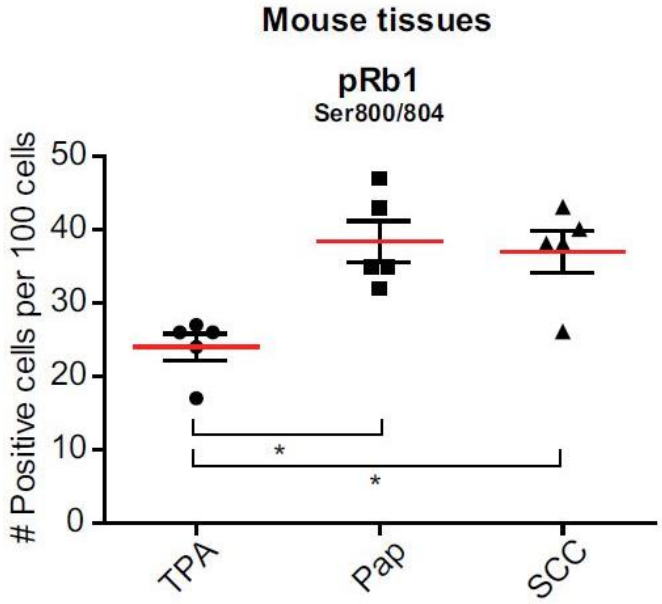


# Can this method rediscover known cancer traits?

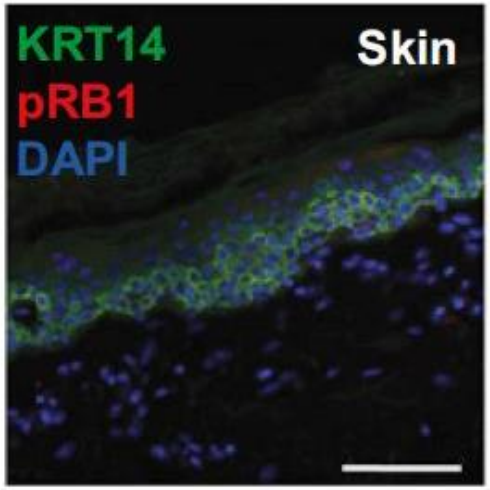


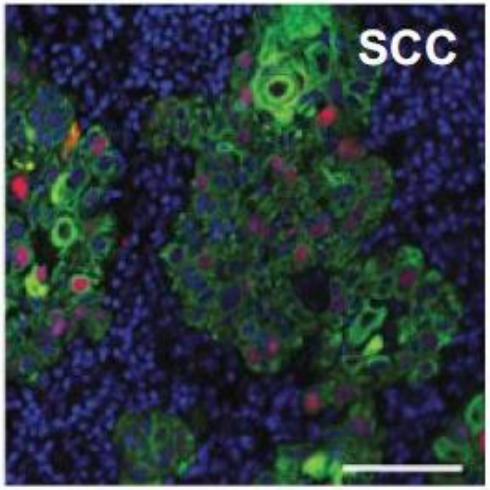
# Is this a valid model for human skin carcinogenesis?

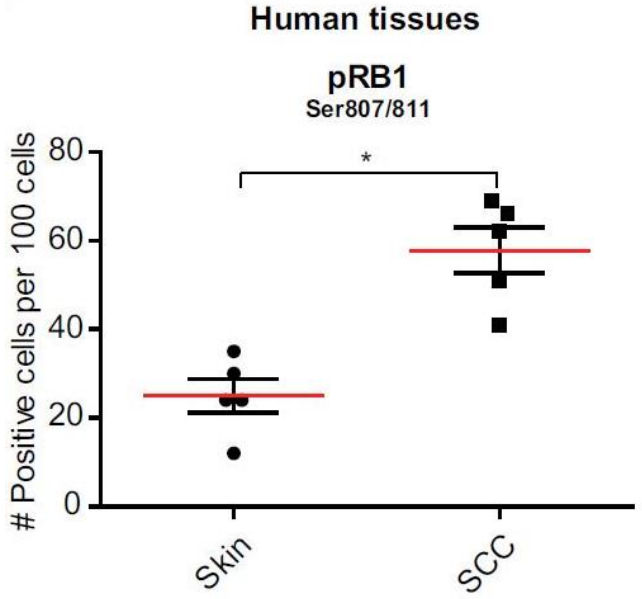


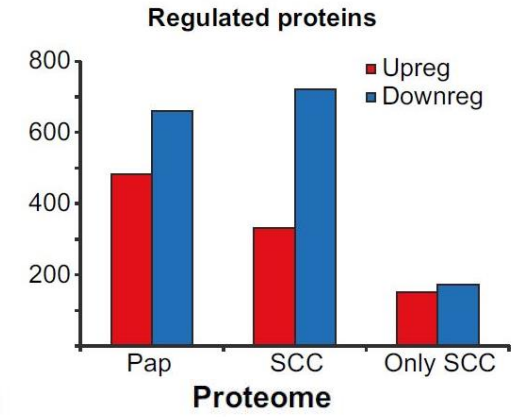
# Is this a valid model for human skin carcinogenesis?

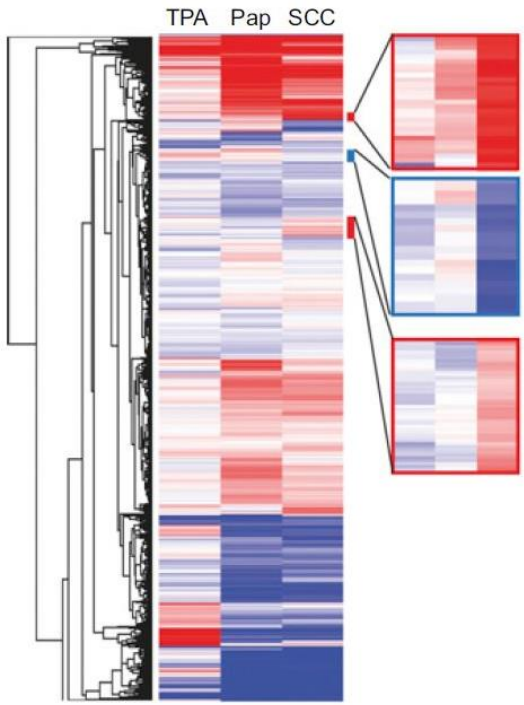


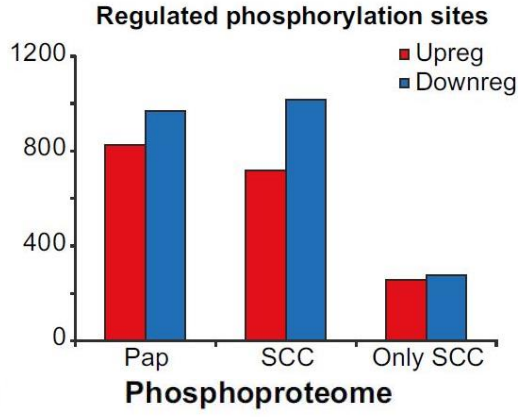



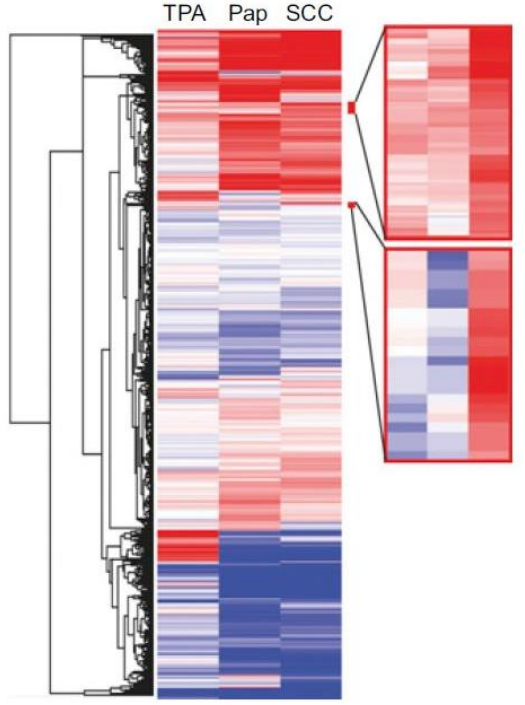



# What changes throughout carcinogenesis?

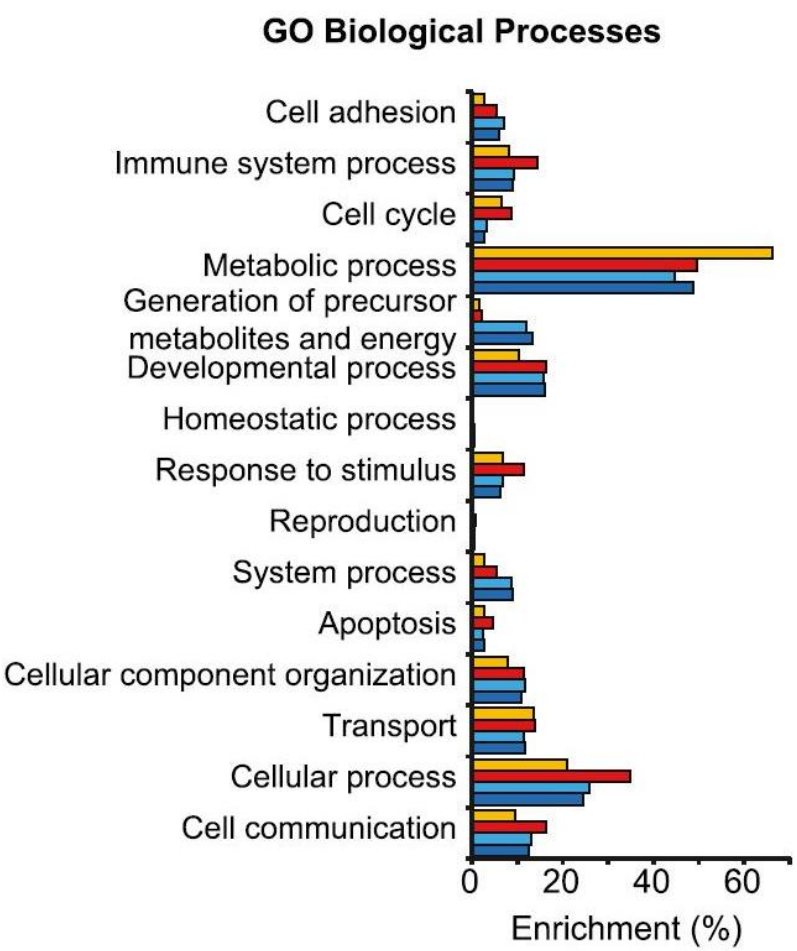


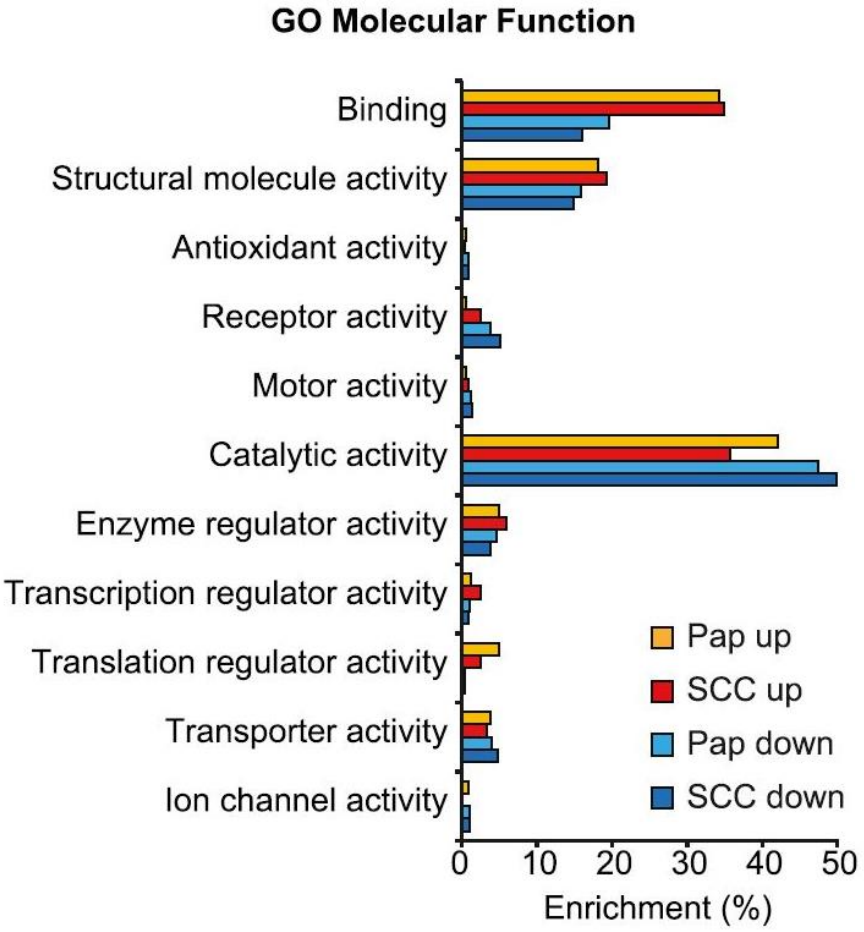



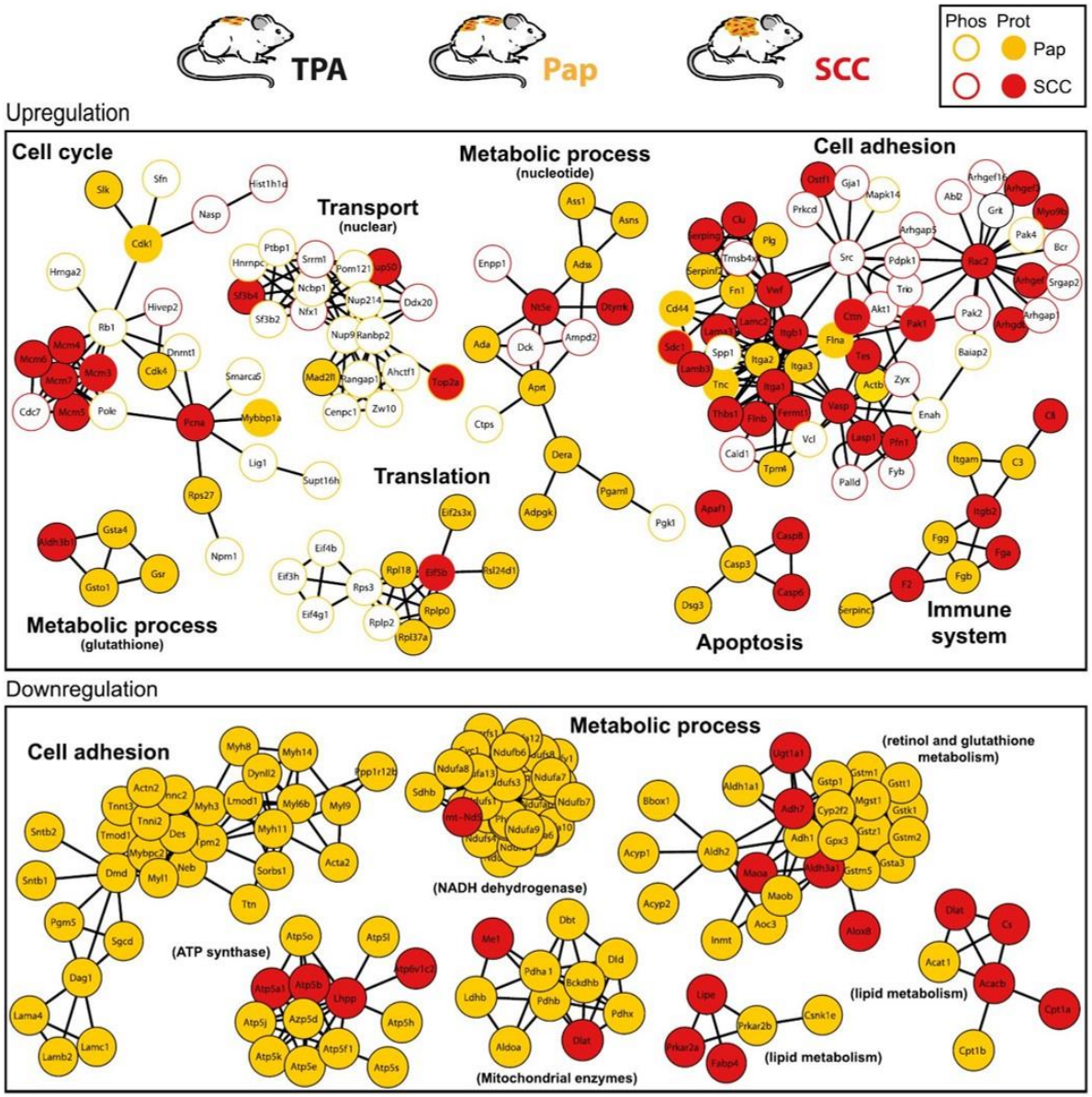



# What changes throughout carcinogenesis?

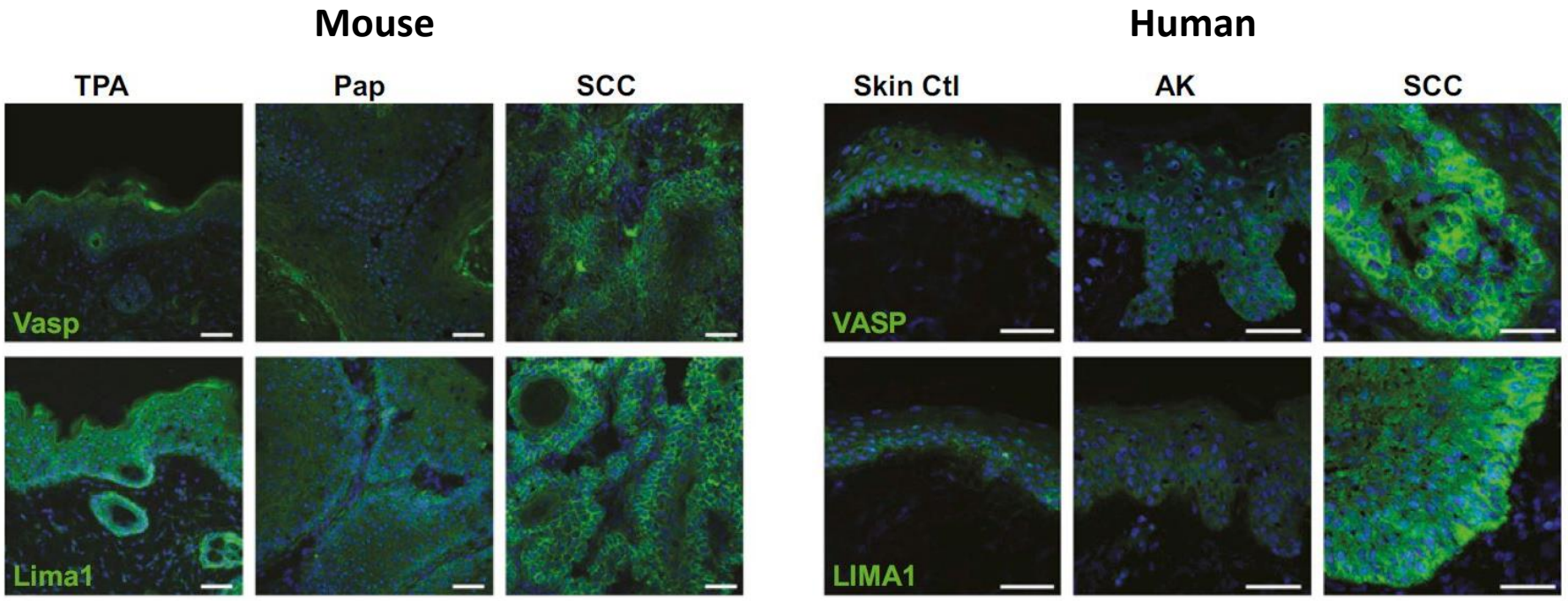


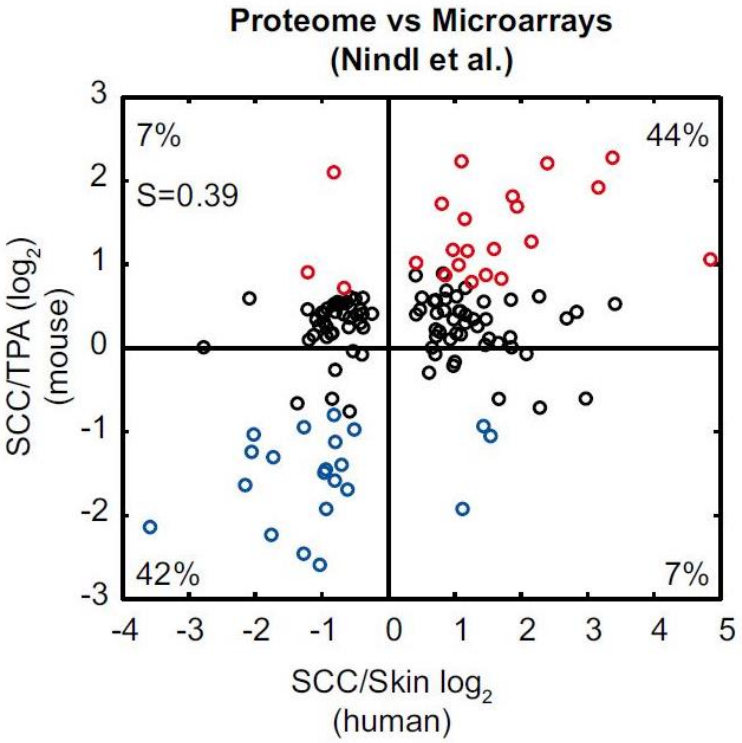
# How are involved proteins related?

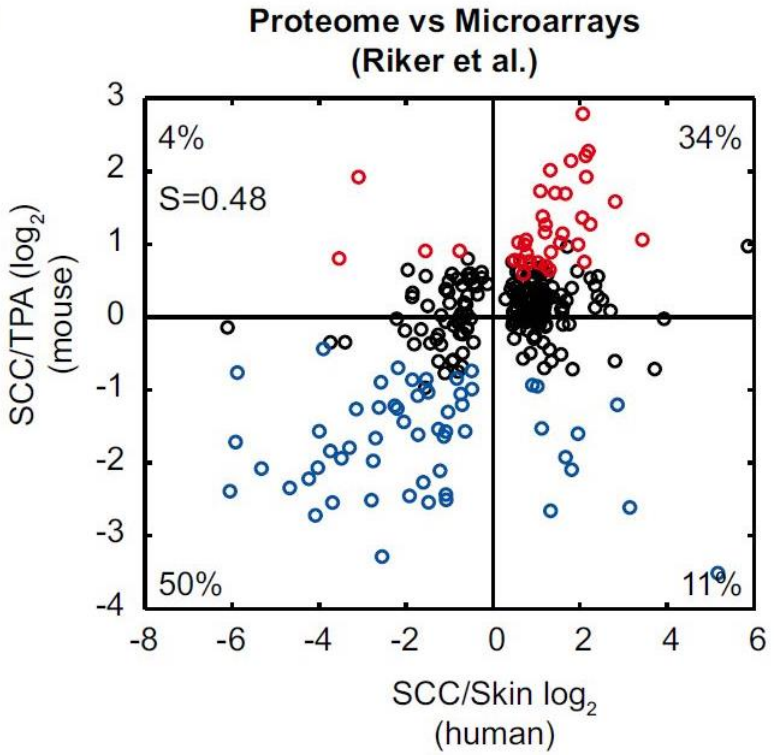


# How are cell adhesion proteins affected in humans?

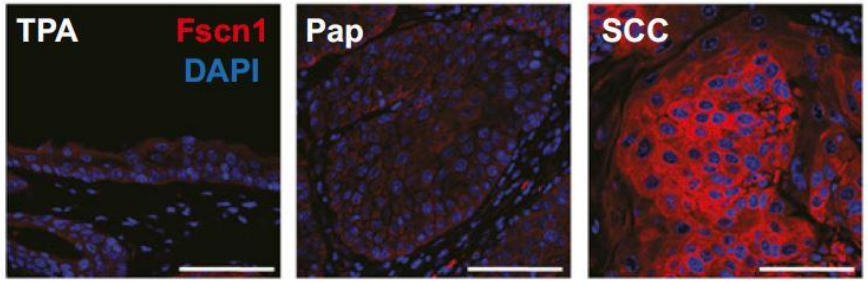


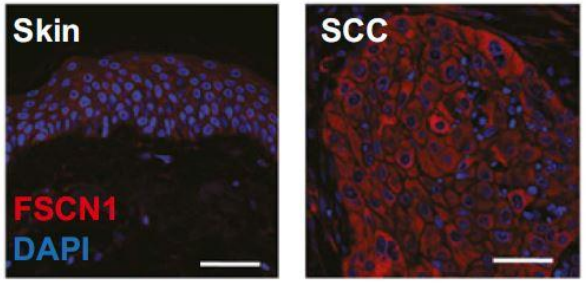
# Are these networks similar in human skin carcinogenesis?

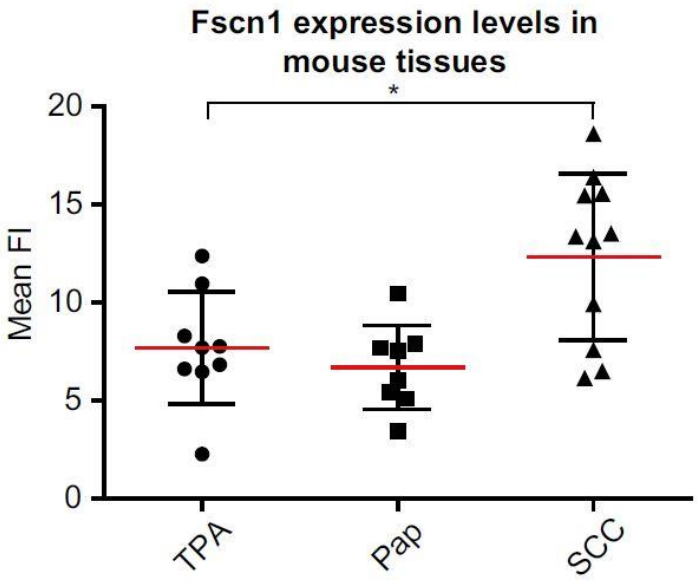


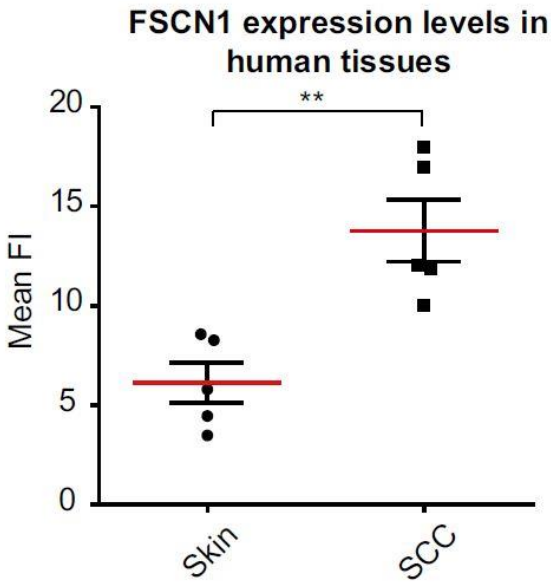



# Is FSCN1 involved in skin SCC?

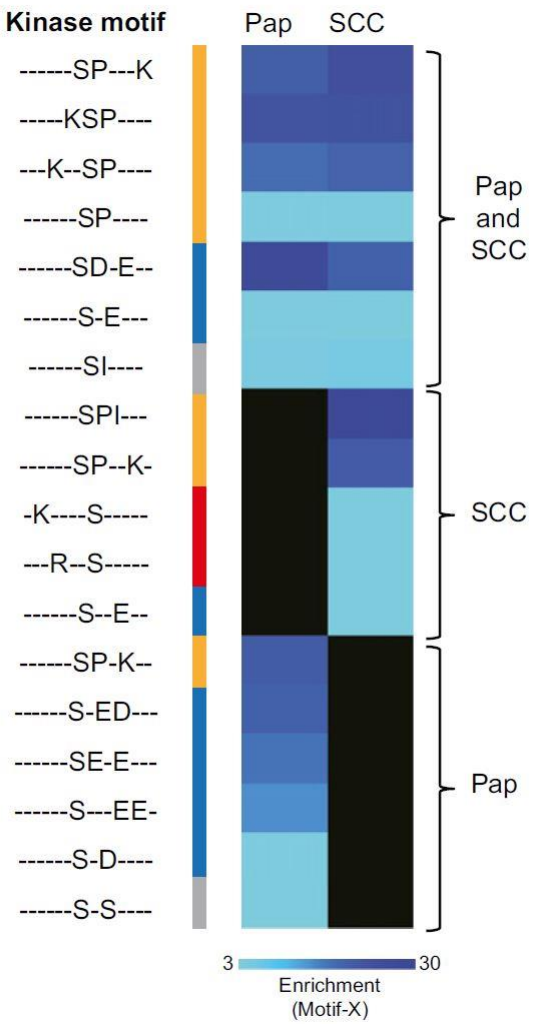


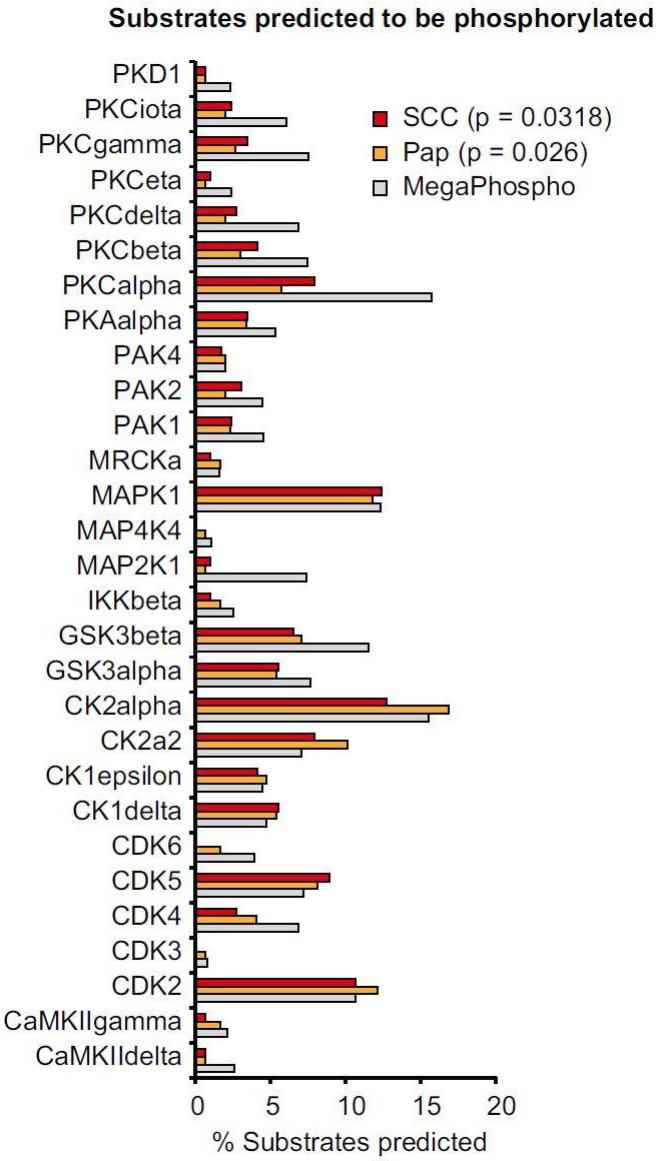



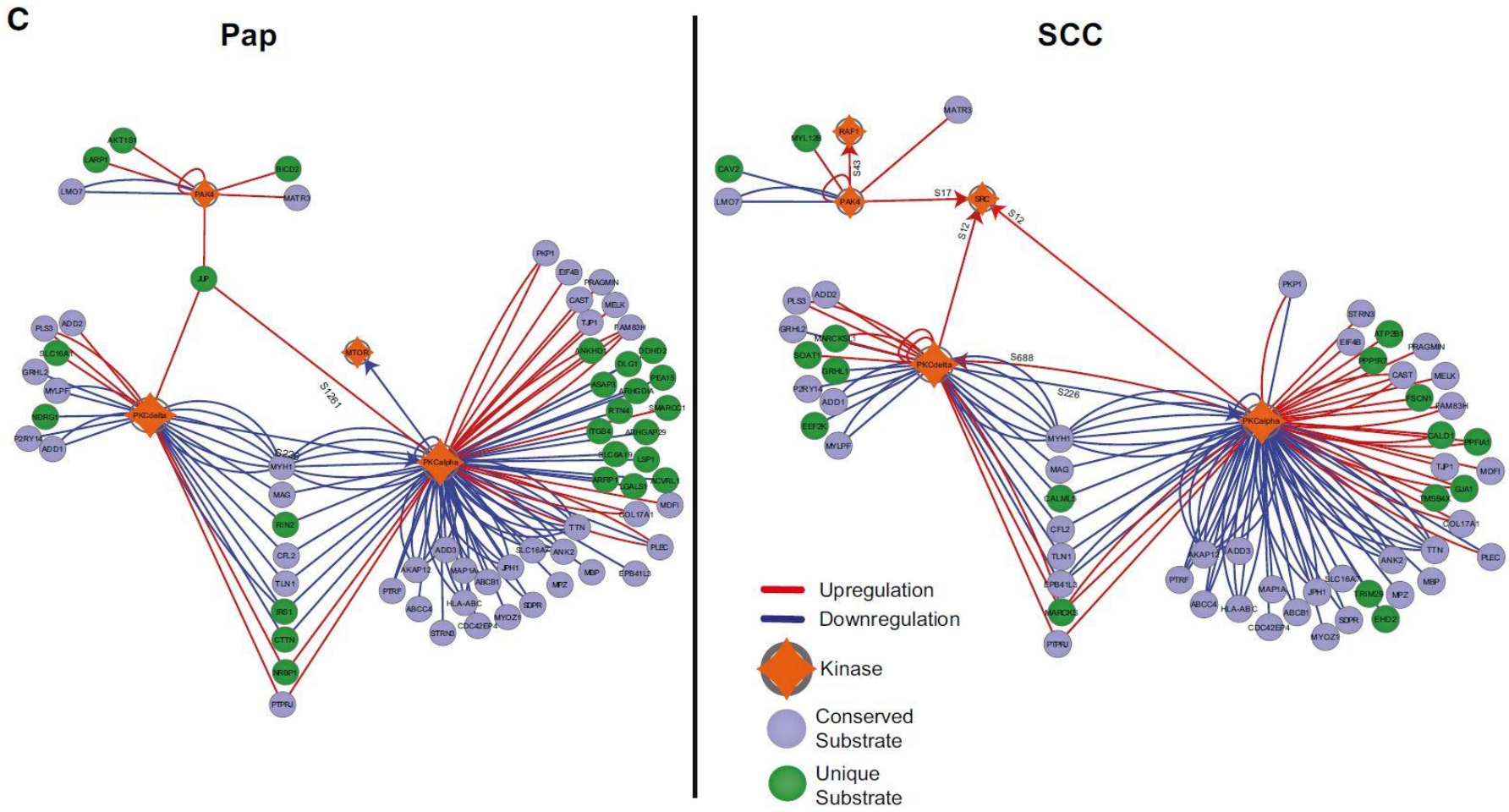



# How did kinase regulation change throughout carcinogenesis?

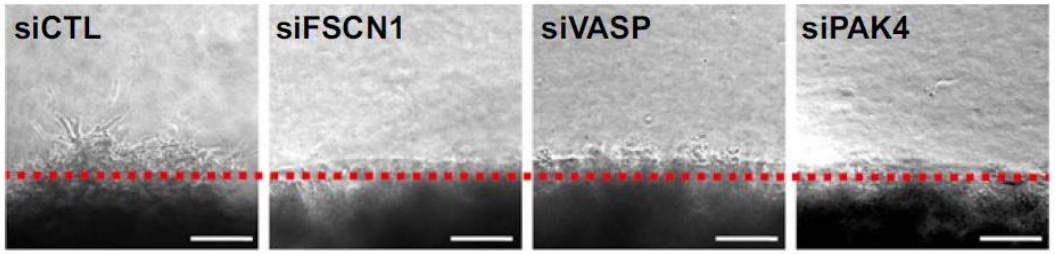


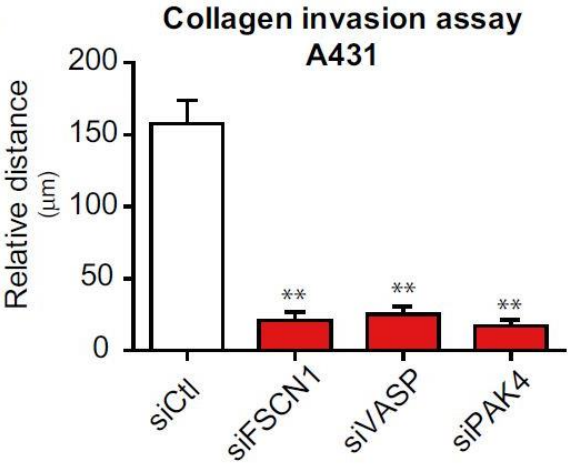



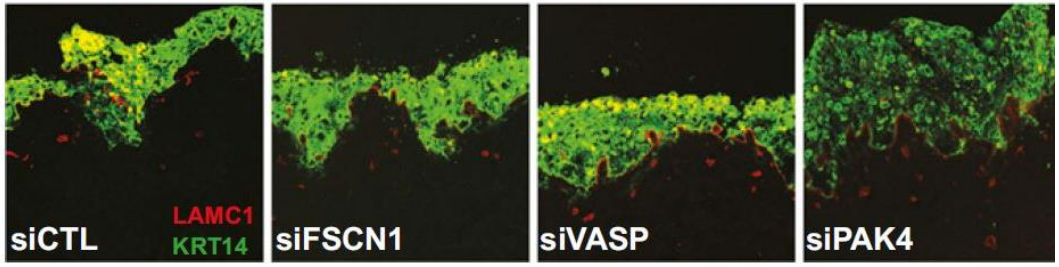
# How is PAK4 involved?

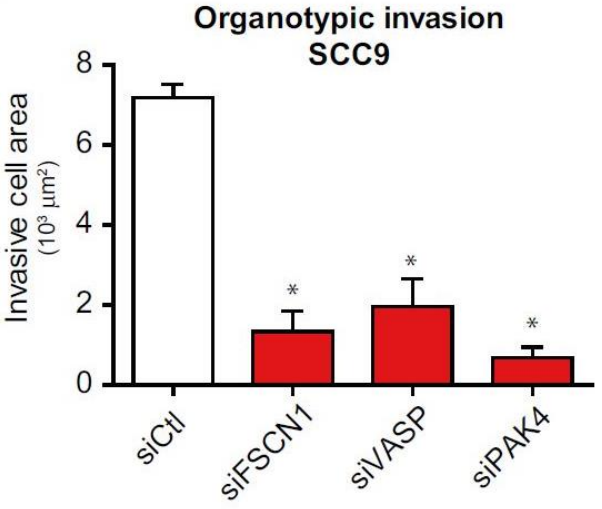


# Do VASP, FSCN1, or PAK4 affect invasion?

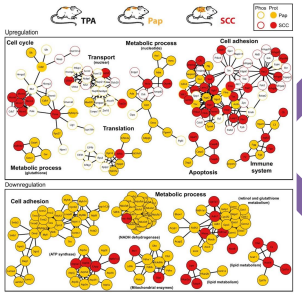


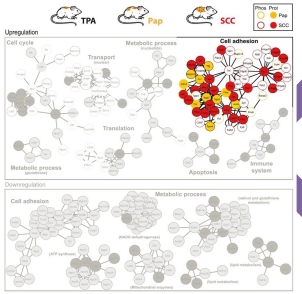



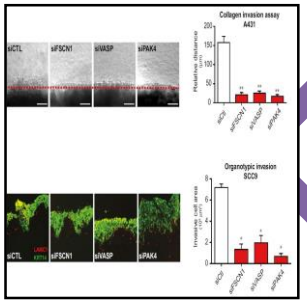
# So what's the point?



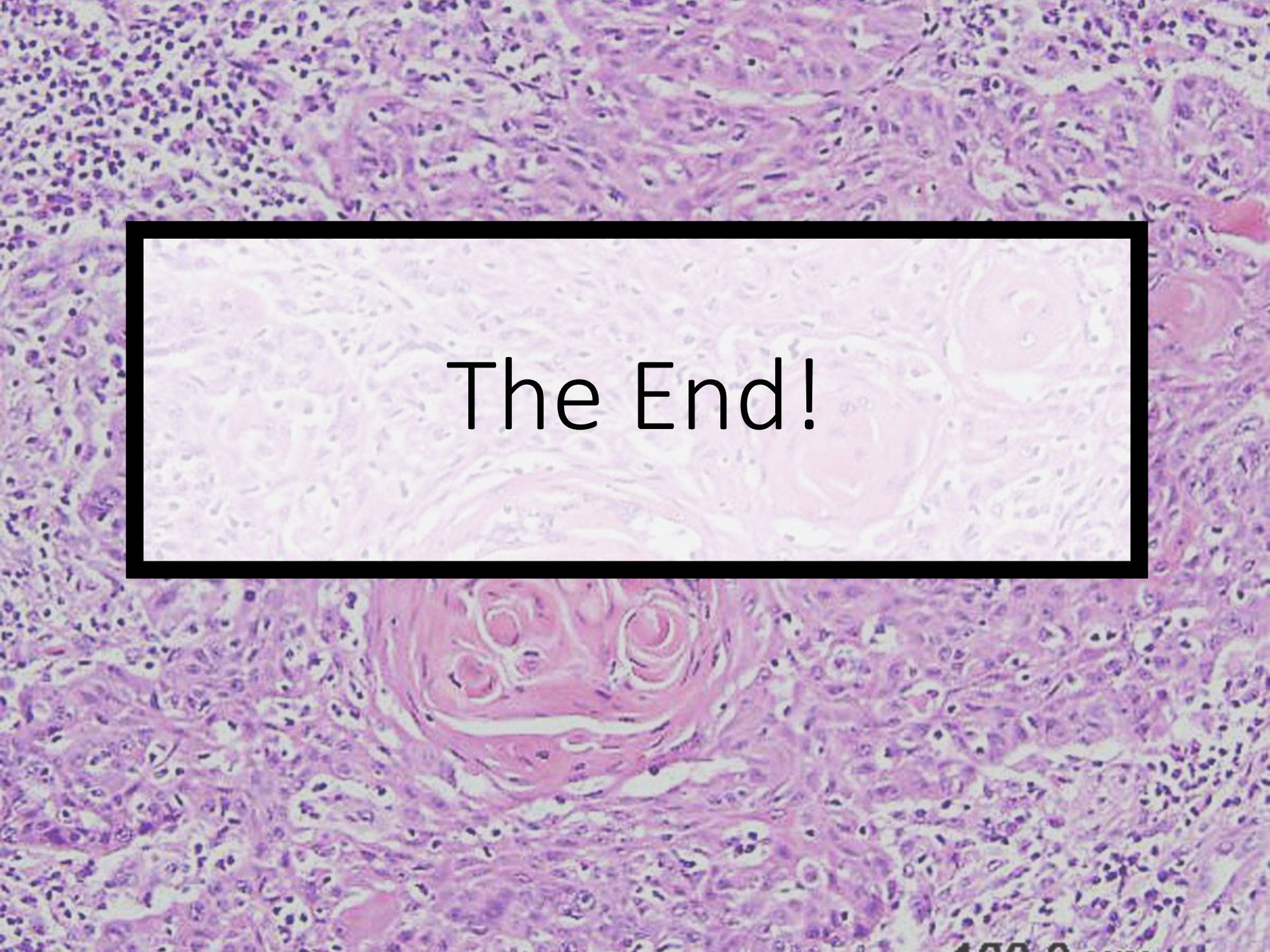

**There are clear changes in the proteome and phosphoproteome during carcinogenesis**




**Cell-adhesion proteins are altered during carcinogenesis**




**The cell adhesion proteins, VASP, FSCN1, and PAK4, are important for SCC invasion**


The End!

# References

## FIGURES:


[1] https://www.glowm.com/section_view/heading/Pathology%20of%20Cervical%20Carcinoma/item/230

[2] https://edu.t-bio.info/wp-content/uploads/2016/08/Omics-cascade.png

[3] https://growersnetwork.org/cultivation/what-is-dna-what-is-a-gene-what-is-protein/

[4] https://www.ptglab.com/news/blog/post-translational-modifications-an-overview/

[5] https://www.cell.com/trends/endocrinology-metabolism/fulltext/S1043-2760(15)00196-4

[6] https://bitesizebio.com/6016/how-does-mass-spec-work/

[7] https://www.cellsignal.com/contents/resources-applications/understand-protein-expression-in-tissue-with-immunohistochemistry-(ihc)/apps-immunohistochemistry

[8] https://www.europeanpharmaceuticalreview.com/news/70957/precision-nanosystems-named-one-british-columbias-fastest-growing-companies/

[9] https://www.albert.io/learn/ap-biology/intermolecular-interactions-1/enzyme-activity-graph

[10] https://www.researchgate.net/figure/Tandem-mass-spectrometry-MS-MS-of-posttranslational-modifications-PTMs-A-MS-MS-of_fig1_7009815

[11] https://slideplayer.com/slide/10725521/

[12] https://www.creative-proteomics.com/blog/index.php/stable-isotope-labeling-using-amino-acids-in-cell-culture-silac-principles-workflow-and-applications/

[13] https://www.sciencedirect.com/science/article/pii/S0092867408006958#app2

[14] https://www.everydayhealth.com/skin-cancer/types/

[15] https://www.healthline.com/health/skin-cancer/facts-and-stats#16


## ALL UN-REFERENCED FIGURES ARE FROM:


Zanivan, Sara, et al. “In Vivo SILAC-Based Proteomics Reveals Phosphoproteome Changes during Mouse Skin Carcinogenesis.” *Cell Reports*, vol. 3, no. 2, 2013, pp. 552–566., doi:10.1016/j.celrep.2013.01.003.