



THE UNIVERSITY  
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# Taming the Hippo in Mesothelioma using Proteogenomics

Krishna Purohit

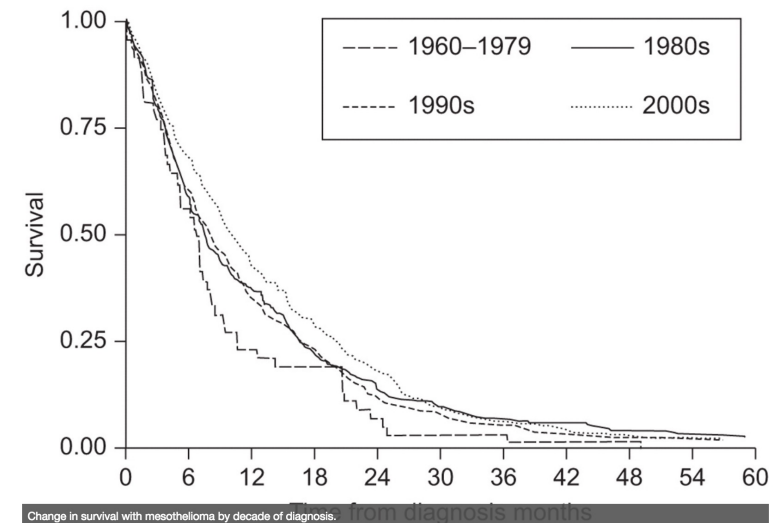
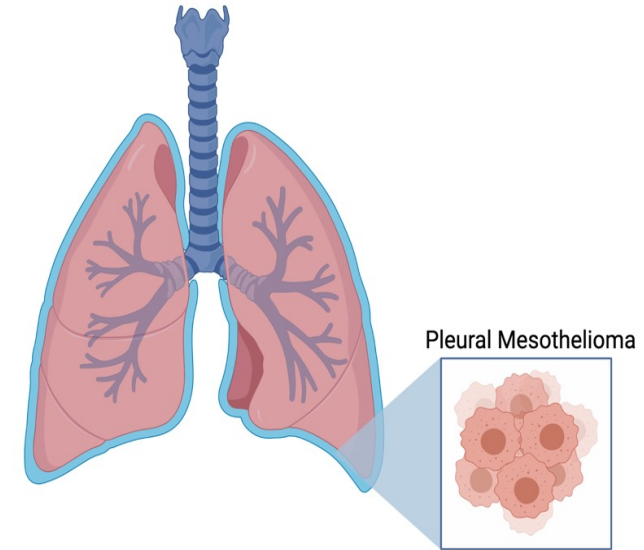
Hansen Lab, CIR, University of Edinburgh  
(In collaboration with Lehtio lab, Karolinska Institute)

Supervisors – Dr Carsten G. Hansen (University of Edinburgh)  
Prof Adriano Rossi (University of Edinburgh)  
Prof Janne Lehtio (Karolinska Institute)

# Pleural Mesothelioma (PM)

## Introduction

- Cancer of the mesothelial cells of the Pleura.
- 80-90% of all mesothelioma cases.
- Rare but aggressive cancer with median survival following diagnosis ~ 8 – 14 months.
- Caused mainly due to asbestos exposure.
- Latency period ~20 – 40 years.



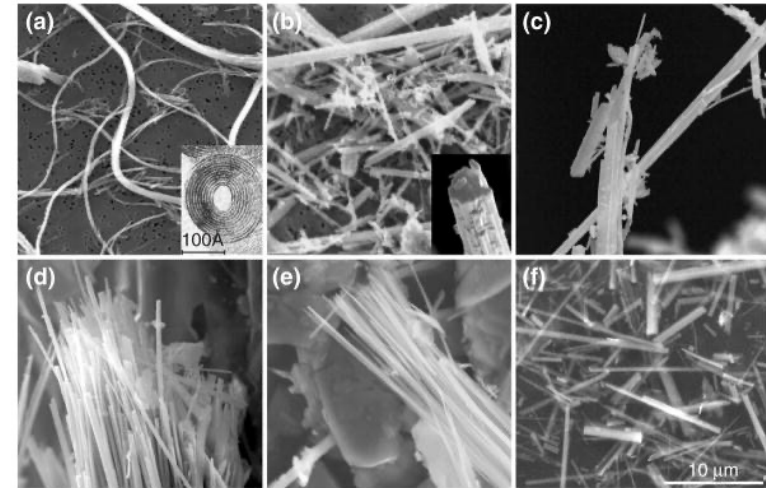
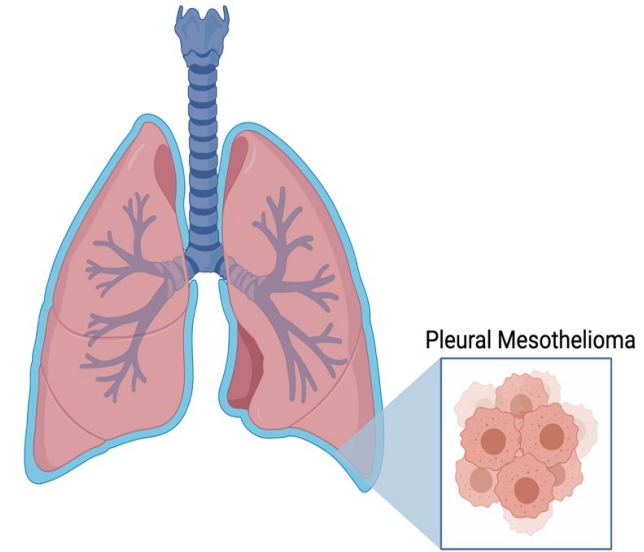
Change in survival with mesothelioma by decade of diagnosis.

Musk et al, Eur Resp, 2011

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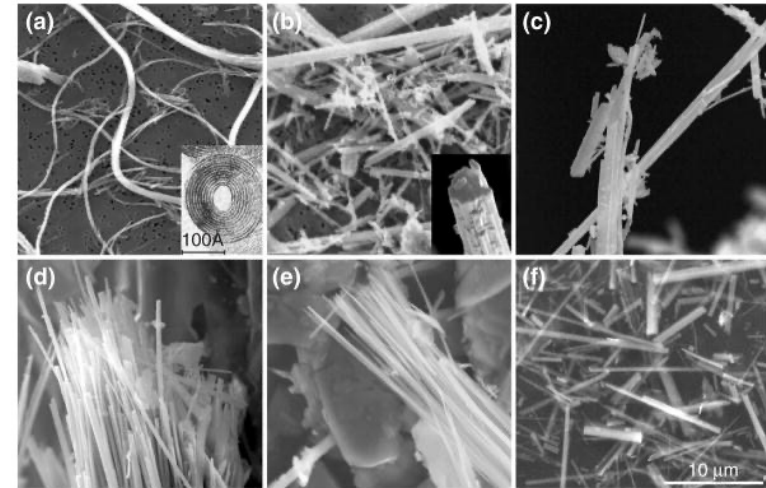
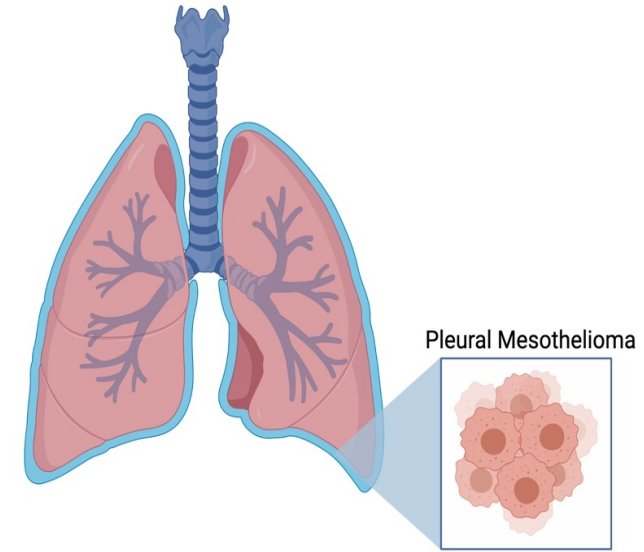


Asbestos fibers

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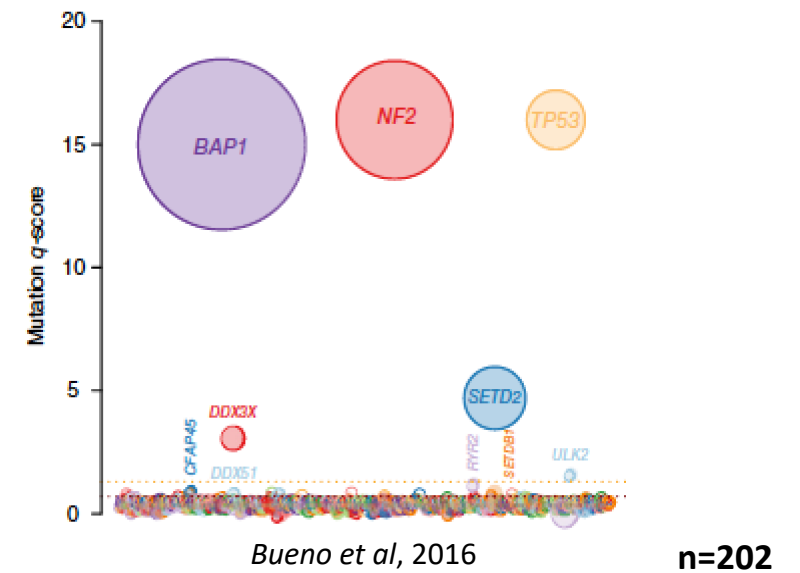
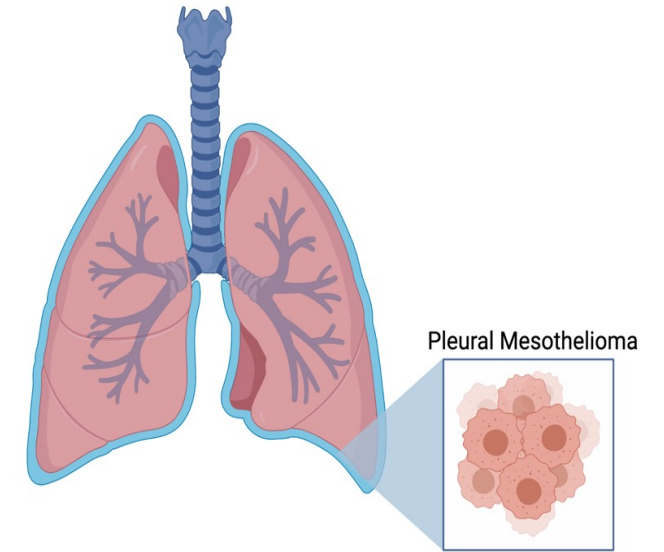
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- Nano-fibres currently used cause mesothelioma like disease in rodents. (Chernova et al., 2017)



Asbestos fibers

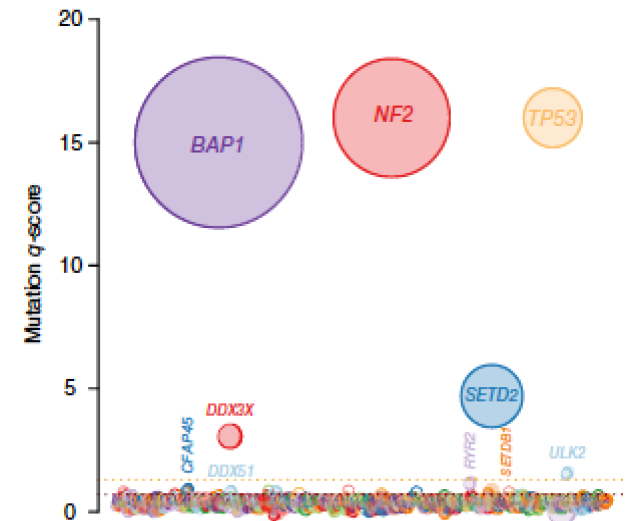
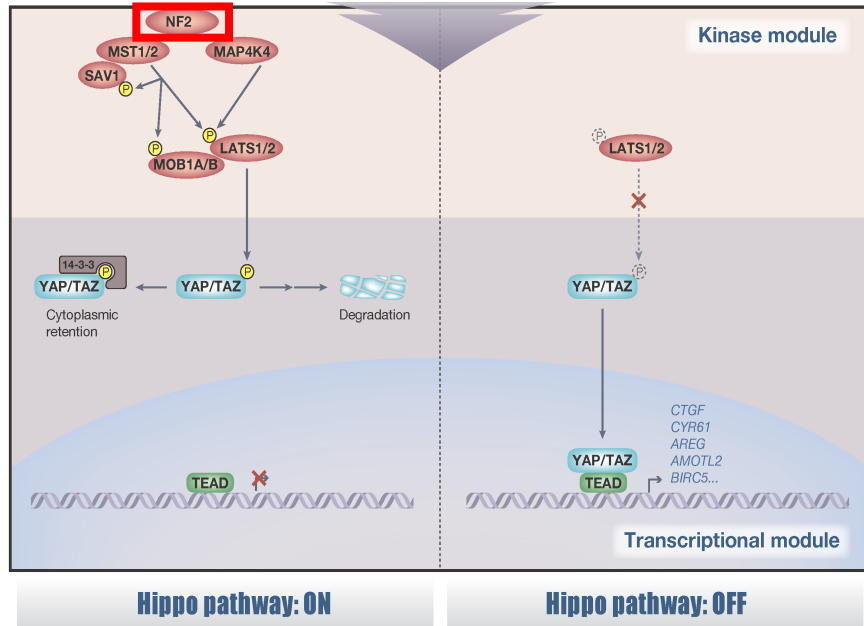
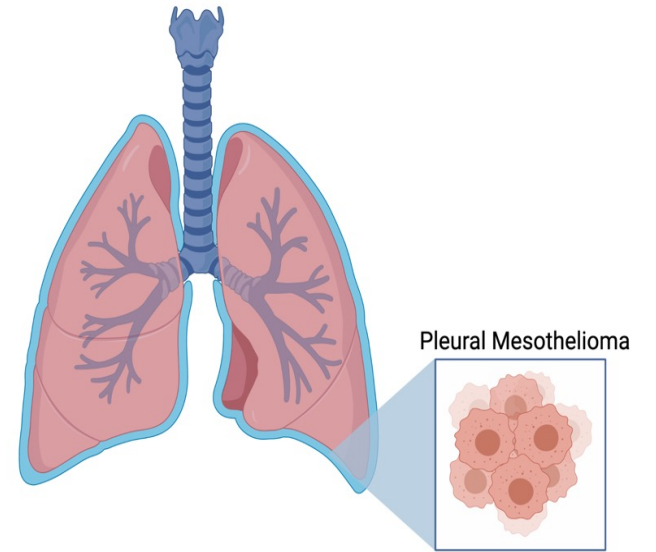
# Pleural Mesothelioma (PM)

- Identification of **NF2** and **BAP1** mutations in a large proportion of PM patients.



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- Hippo pathway of particular interest – **NF2** a regulator.

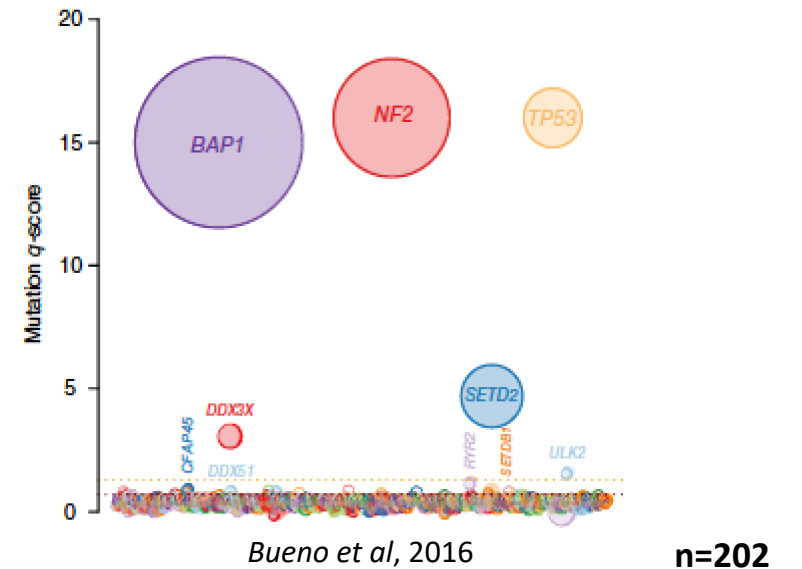
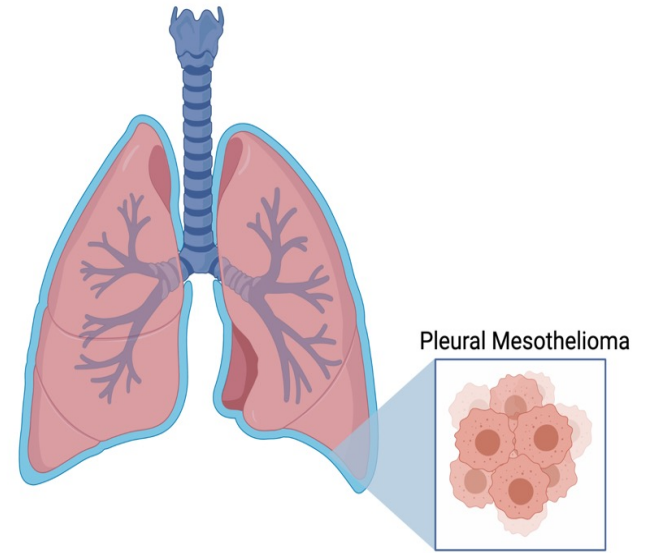
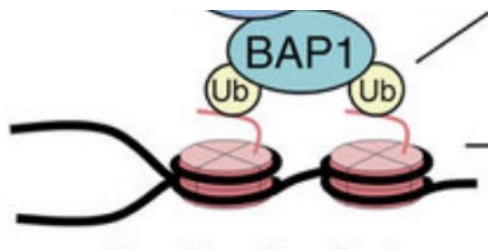


Bueno et al, 2016

n=202

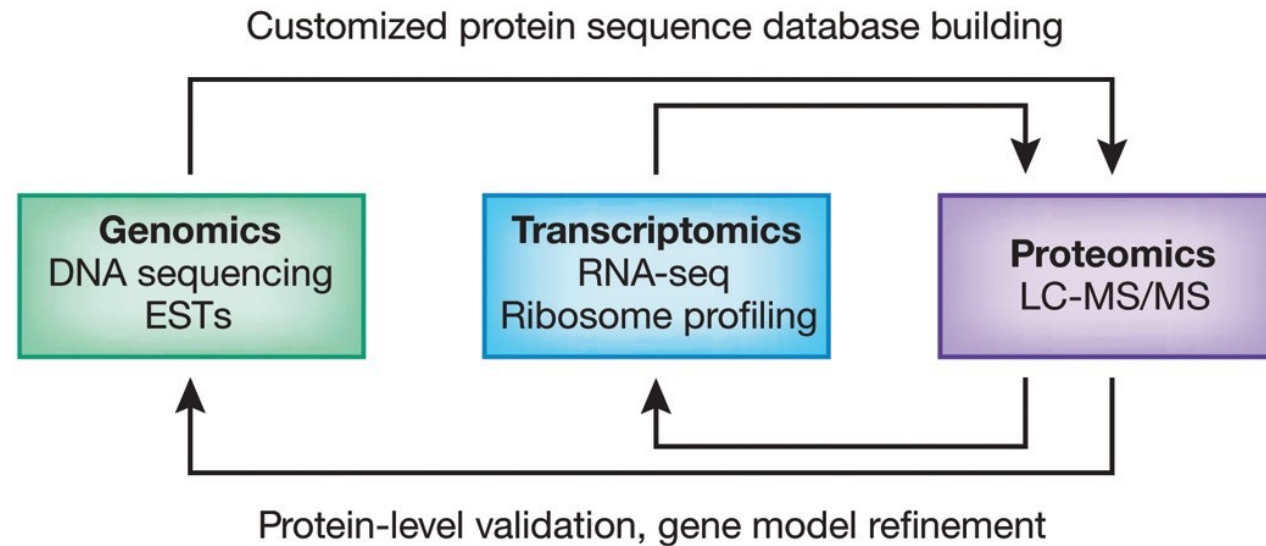
# Pleural Mesothelioma (PM)

- Identification of **NF2** and **BAP1** mutations in a large proportion of PM patients.
- Hippo pathway of particular interest – **NF2** a regulator.
- **BAP1** – a deubiquitylase enzyme.
- De-ubiquitylates Histone H2A and HCF1.



# What is Proteogenomics?

Combination of proteomics with genomics and transcriptomics.



Nesvizhskii *et al*, *nature*, 2014



# KI – Lehtio Lab



SciLifeLab, Stockholm

# KI – Lehtio Lab

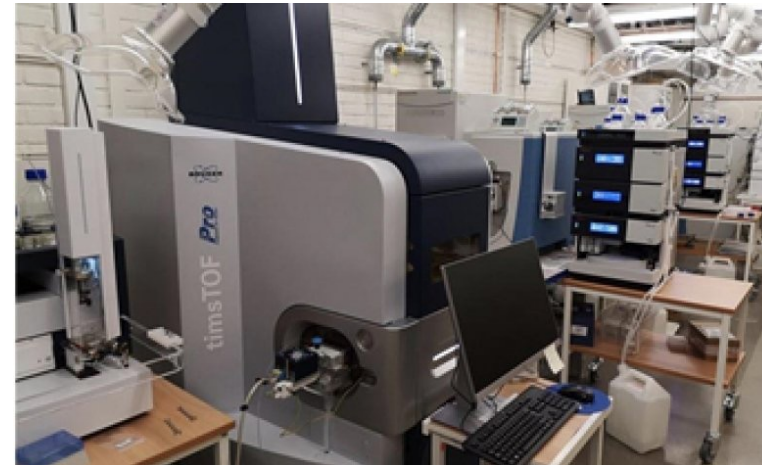


**Proteogenomics** to study cancer and for precision medicine

- MS based techniques for studying the proteome.
- Combining it with genomic and transcriptomic data to gather further molecular insights.



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# KI – Lehtio Lab



**Proteogenomics** to study cancer and for precision medicine

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**Proteogenomics of non-small cell lung cancer reveals molecular subtypes associated with specific therapeutic targets and immune-evasion mechanisms**

[Janne Lehtiö](#) , [Taner Arslan](#), [Ioannis Siavelis](#), [Yanbo Pan](#), [Fabio Socciarelli](#), [Olena Berkovska](#), [Husen M. Umer](#), [Georgios Mermelekas](#), [Mohammad Pirmoradian](#), [Mats Jönsson](#), [Hans Brunnström](#), [Odd Terje Brustugun](#), [Krishna Pinganksha Purohit](#), [Richard Cunningham](#), [Hassan Foroughi Asl](#), [Sofi Isaksson](#), [Elsa Arbajian](#), [Mattias Aine](#), [Anna Karlsson](#), [Marija Kotevska](#), [Carsten Gram Hansen](#), [Vilde Drageset Haakensen](#), [Åslaug Helland](#), [David Tamborero](#), [Henrik J. Johansson](#), [Rui M. Branca](#), [Maria Planck](#), [Johan Staaf](#) & [Lukas M. Orre](#)



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# Tandem Mass Tag (TMT) proteomics

Whole proteome quantification from samples

MeT5A genotypes:

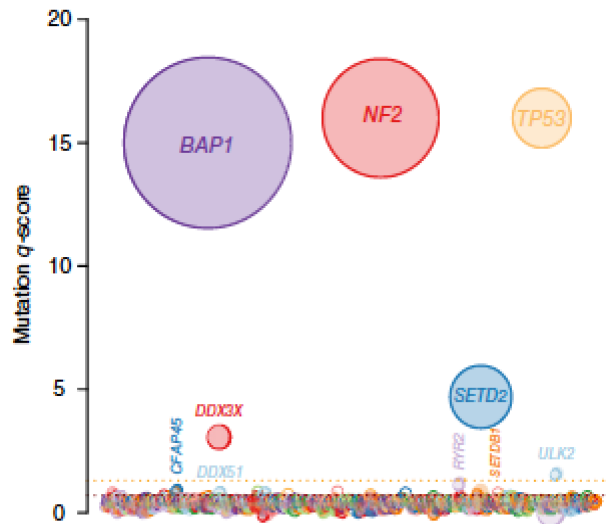
WT

WT high density

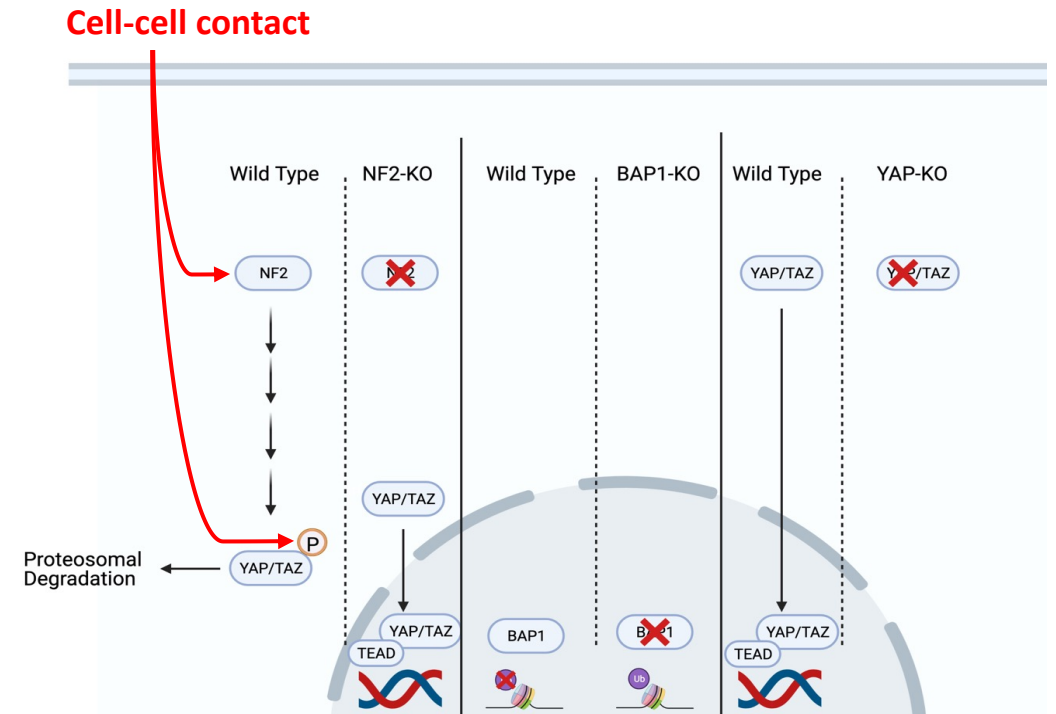
NF2 KO

BAP1 KO

YAP KO



**MeT5A** – non-malignant human isogenic mesothelial cell line.





# Tandem Mass Tag (TMT) proteomics

Whole proteome quantification from samples

MeT5A genotypes:

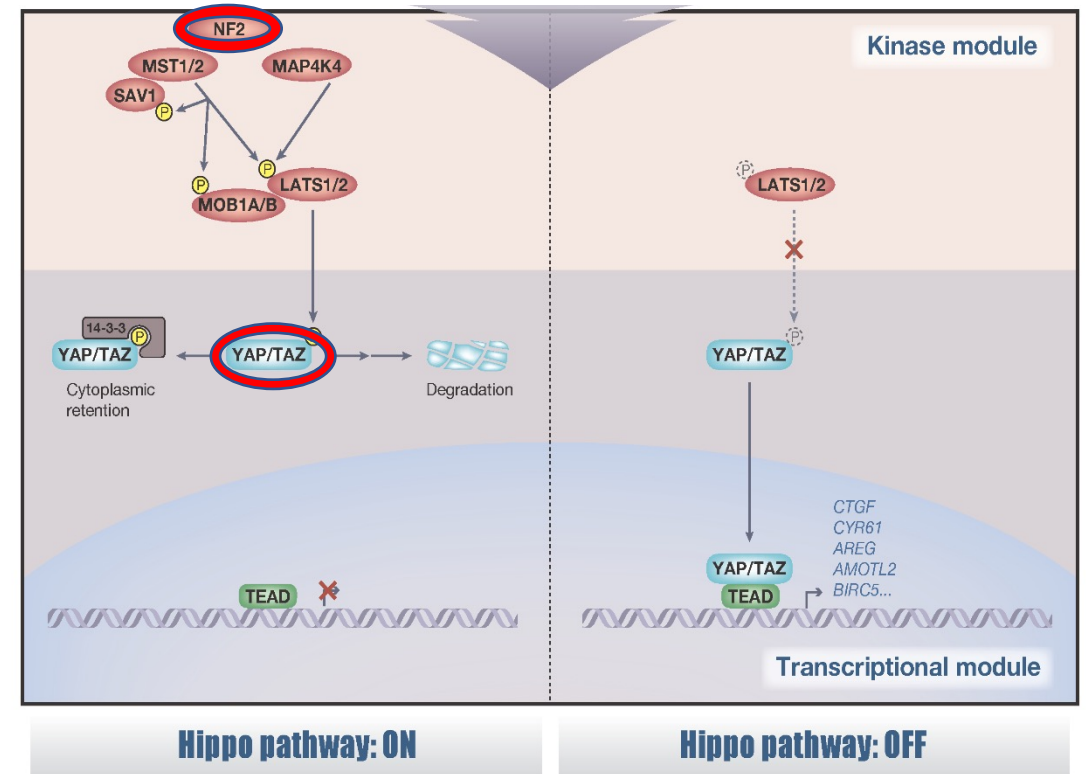
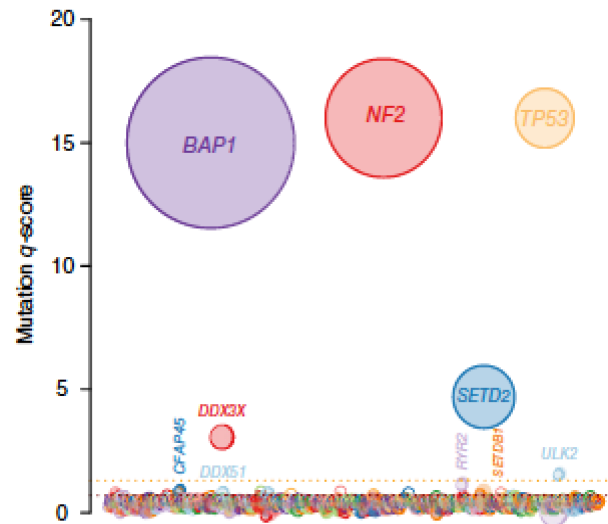
WT

WT high density

NF2 KO

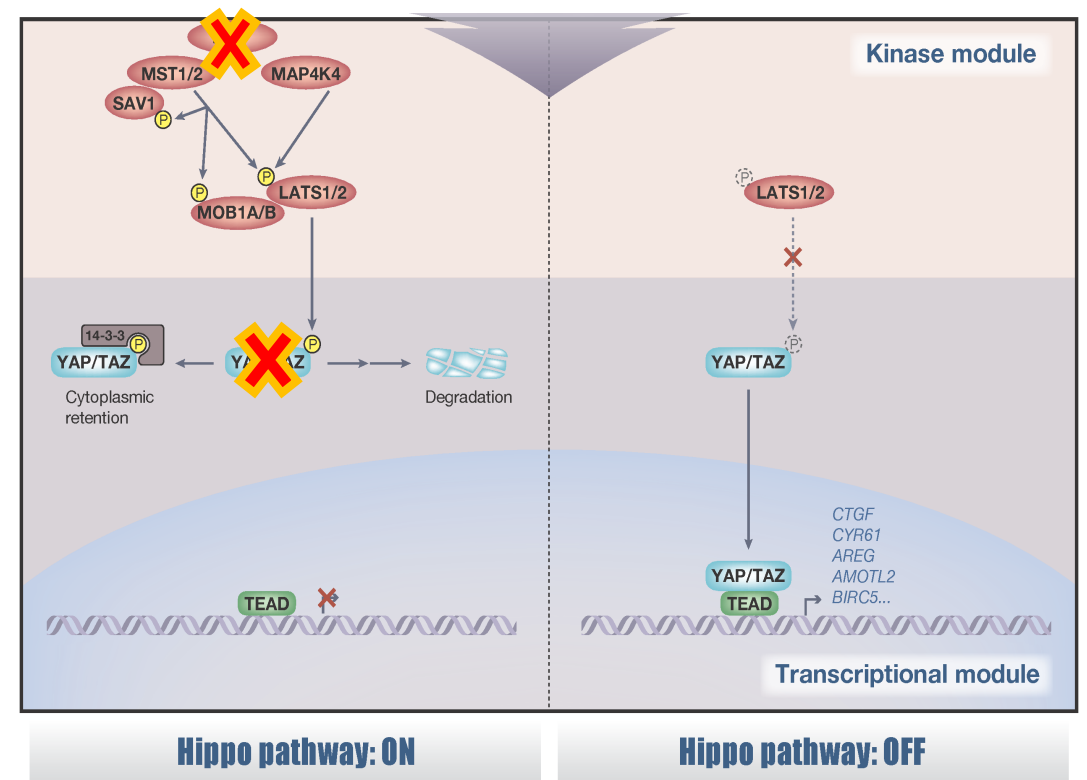
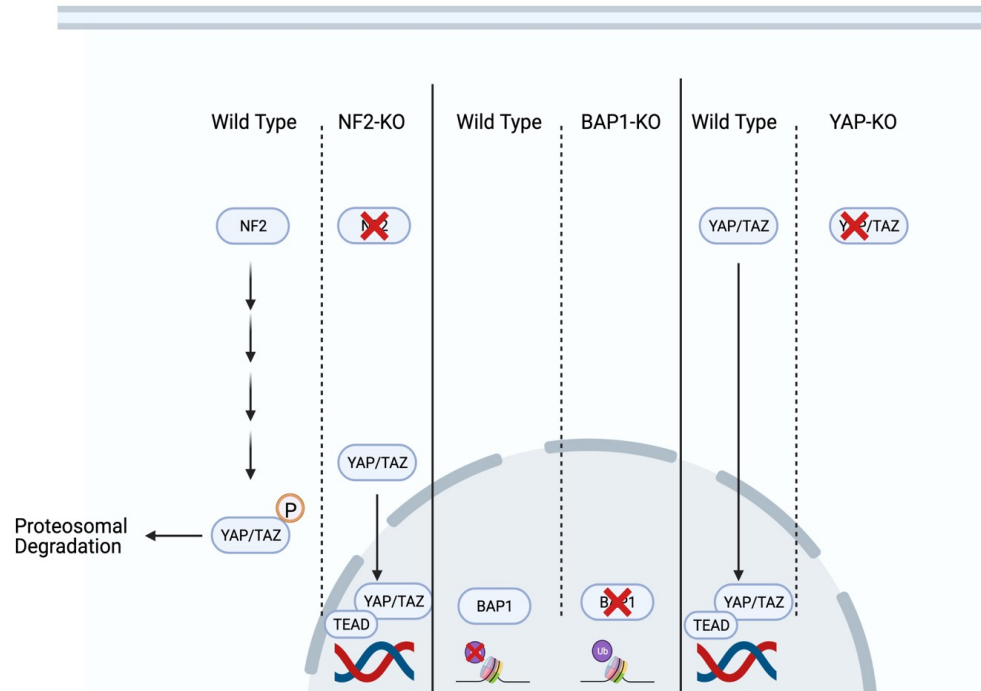
BAP1 KO

YAP KO



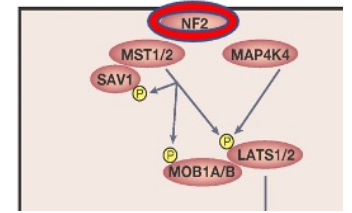
# Research question

Effect of KO of driver genes (**NF2** and **BAP1**) and **Hippo targets** on the proteome in the cell lines?

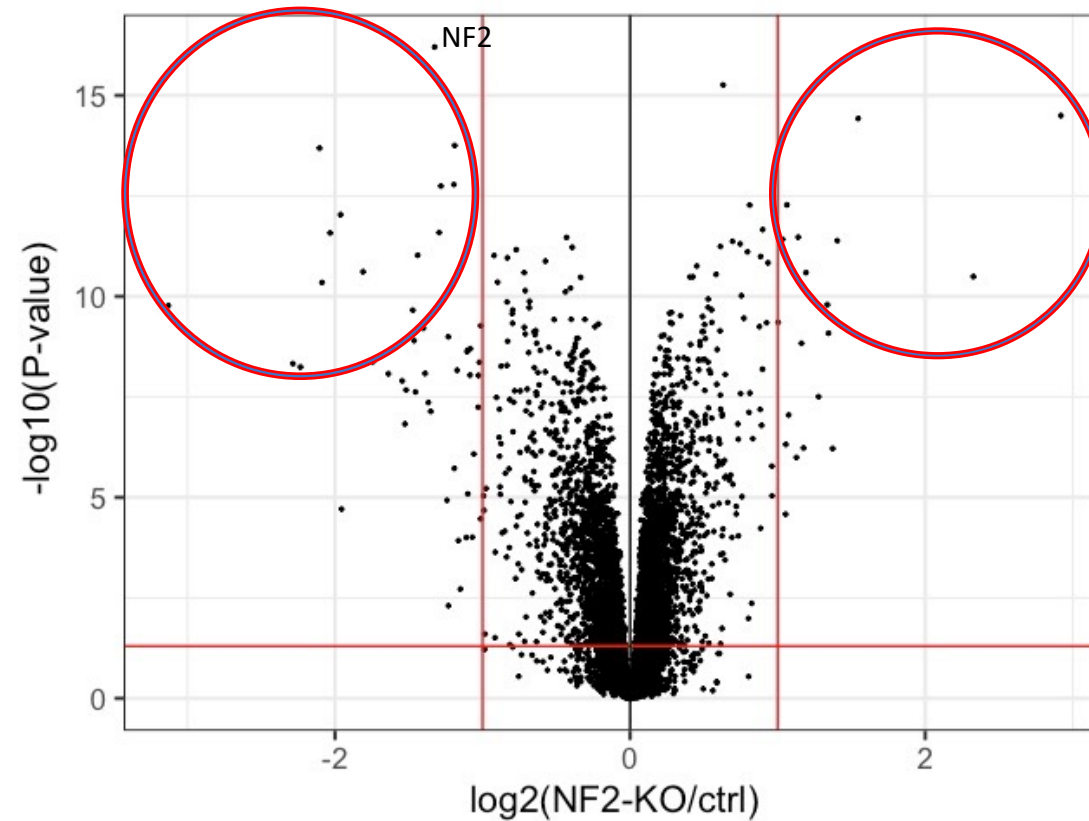


# Differential expression (DE) analysis

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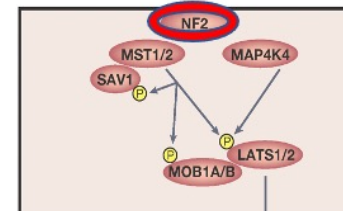


Proteins differentially expressed in **NF2 KO** MeT5A



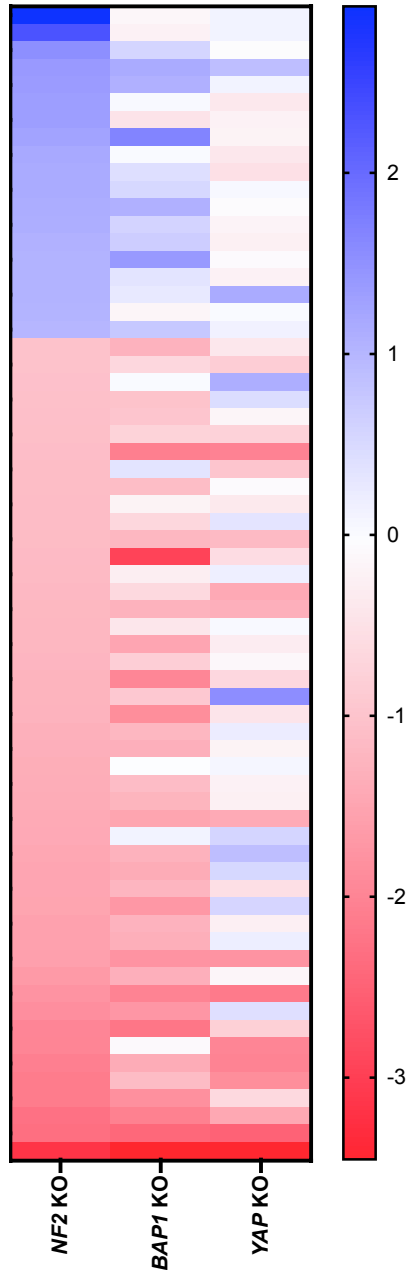


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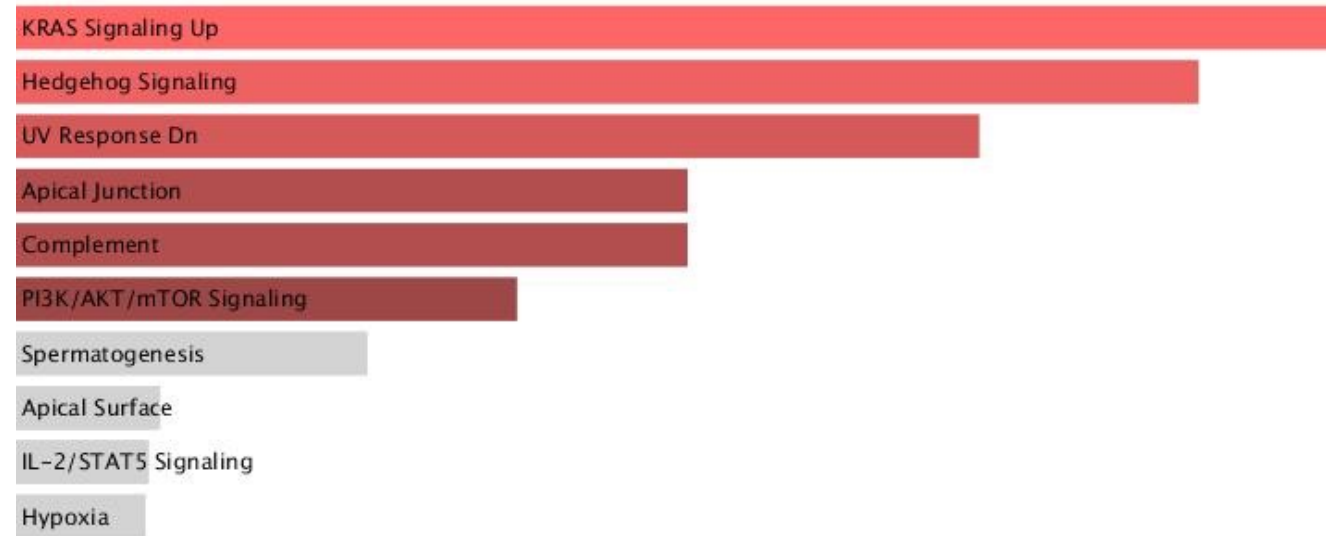


Proteins differentially expressed in **NF2 KO** MeT5A

**N = 3**

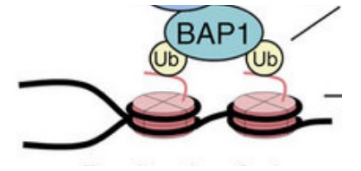


## Pathway enrichment

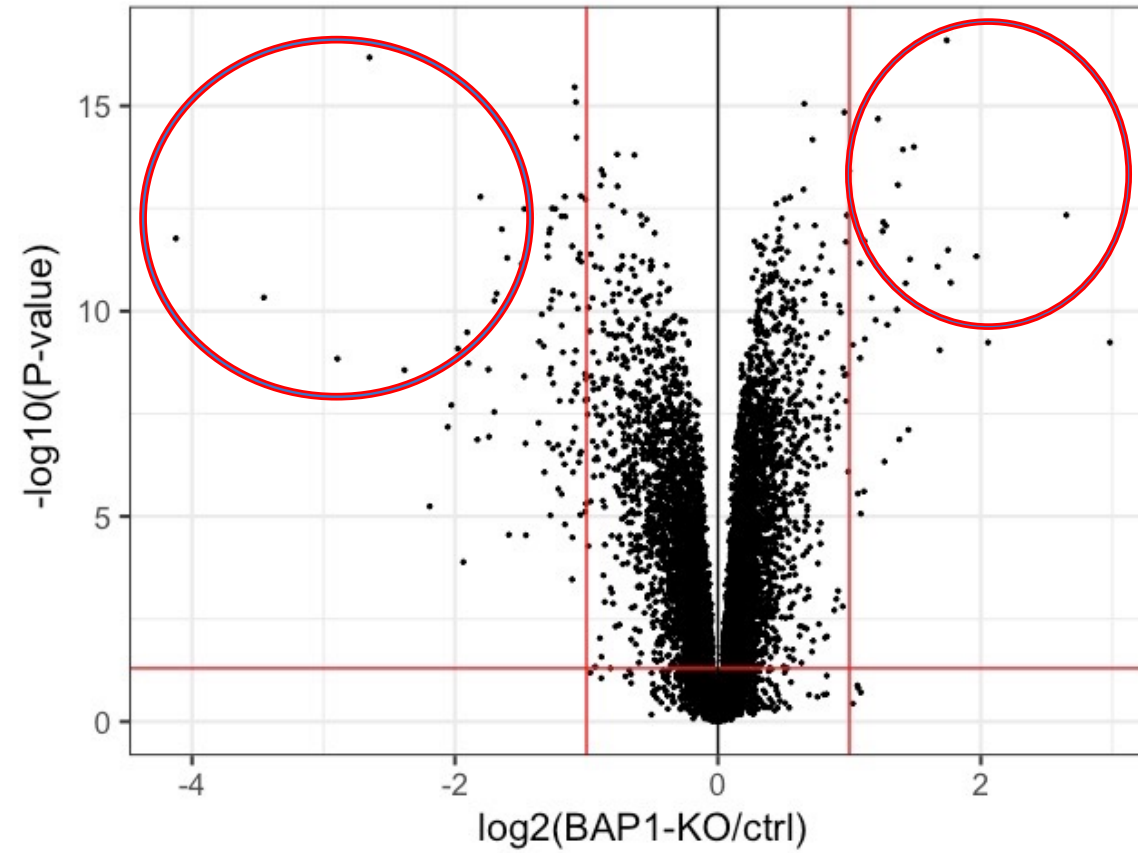


MSigDB

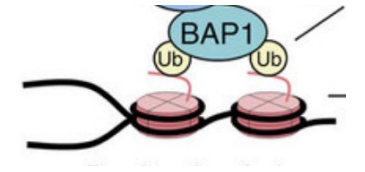
# Differential expression (DE) analysis



Proteins differentially expressed in **BAP1 KO** MeT5A

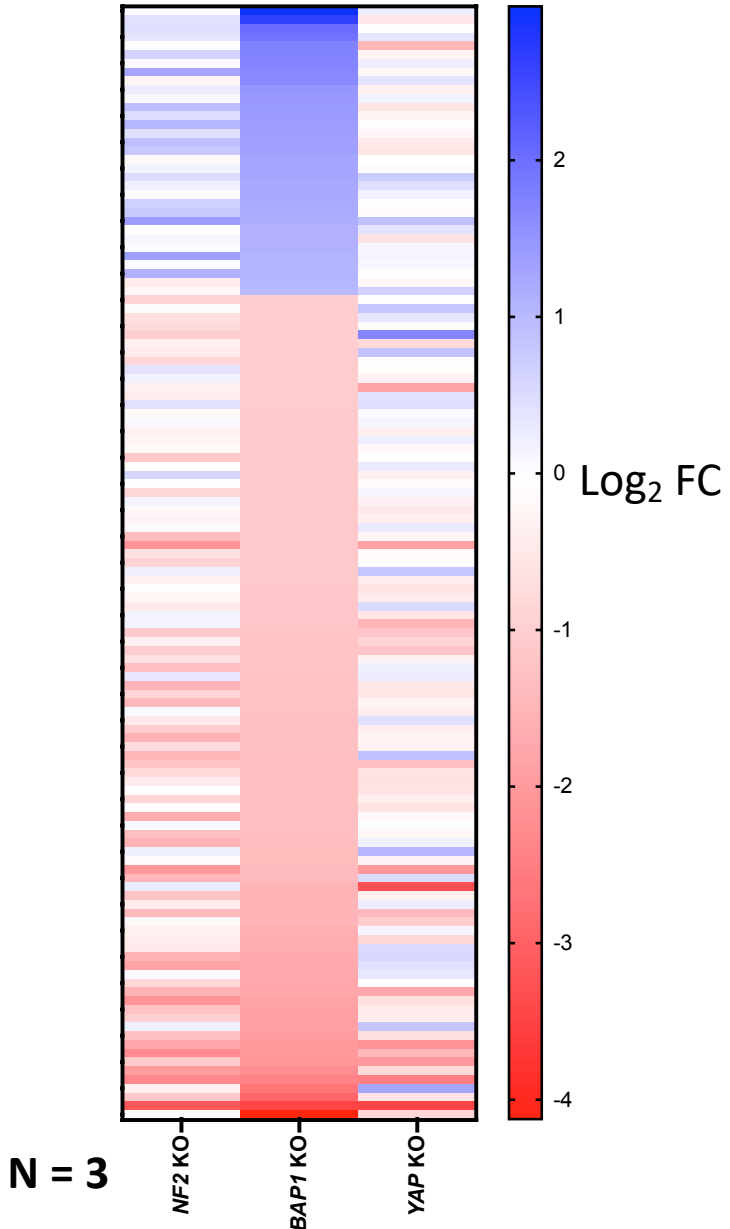
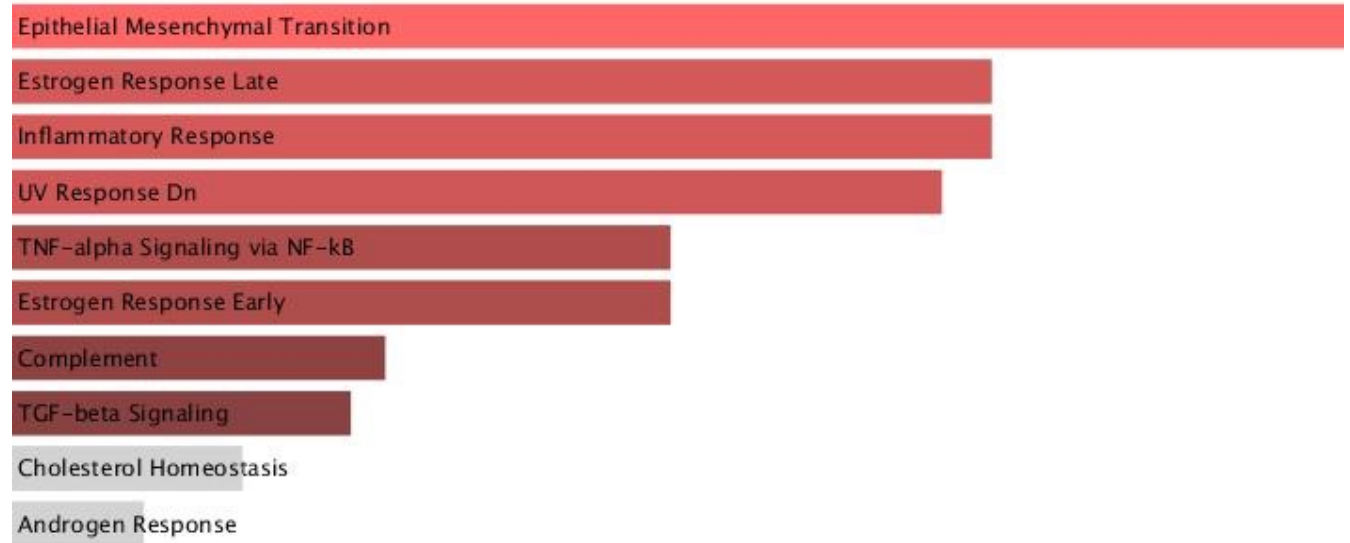


# Differential expression (DE) analysis



Proteins differentially expressed in **BAP1 KO** MeT5A

## Pathway enrichment

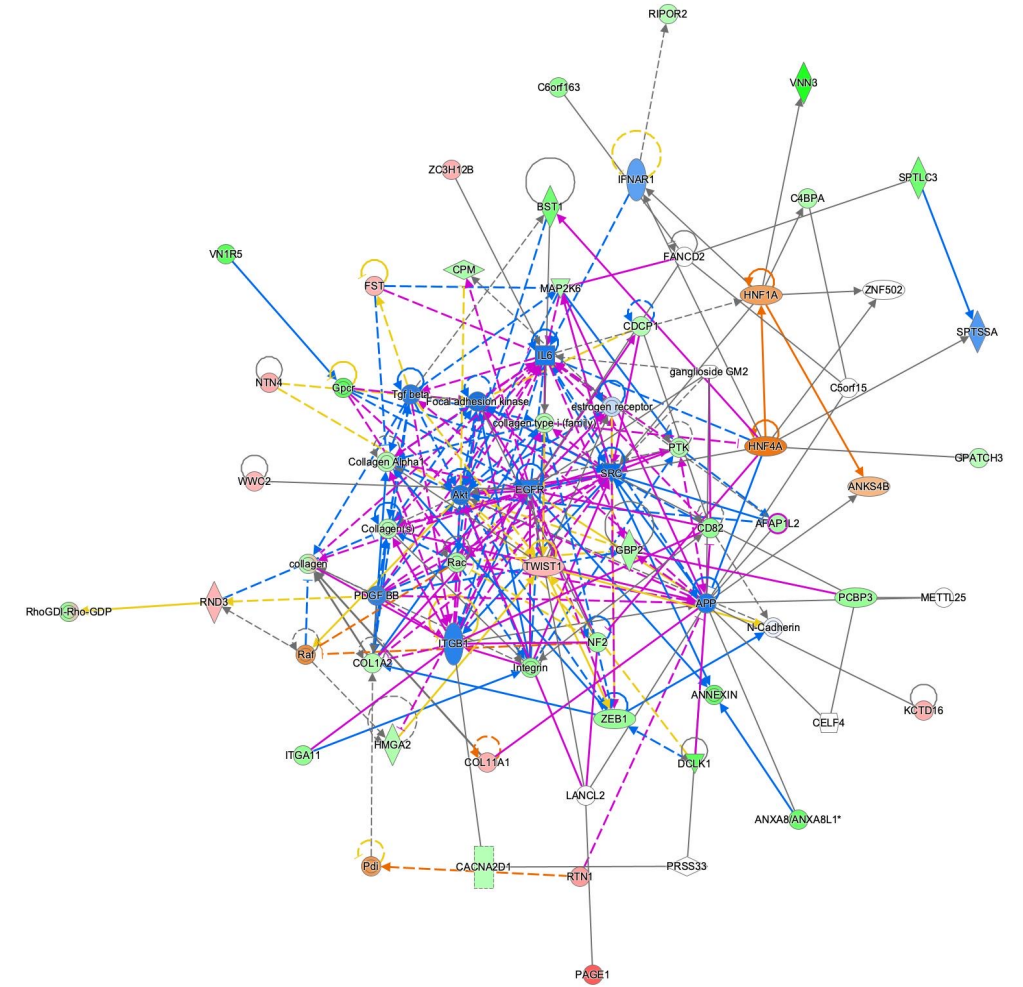
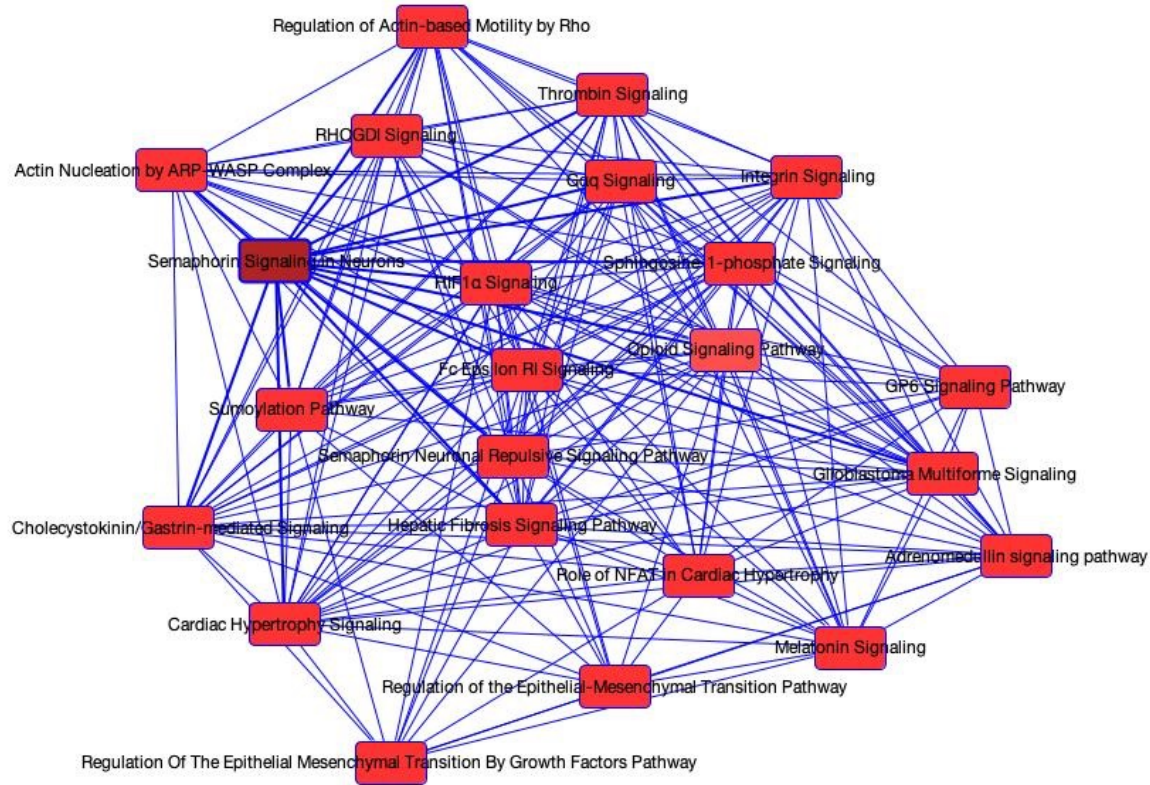


MSigDB

# Network analysis

## Ingenuity Pathway Analysis (IPA)

NF2 KO MeT5A

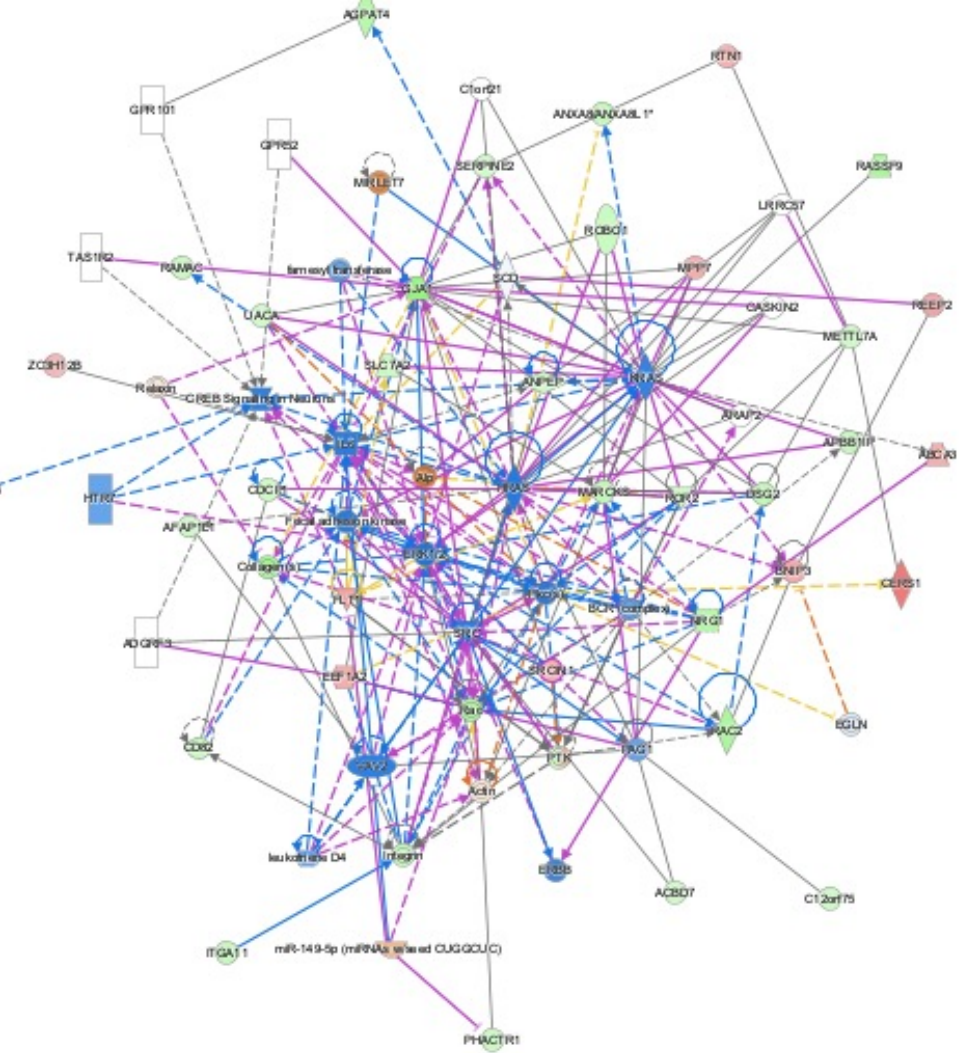
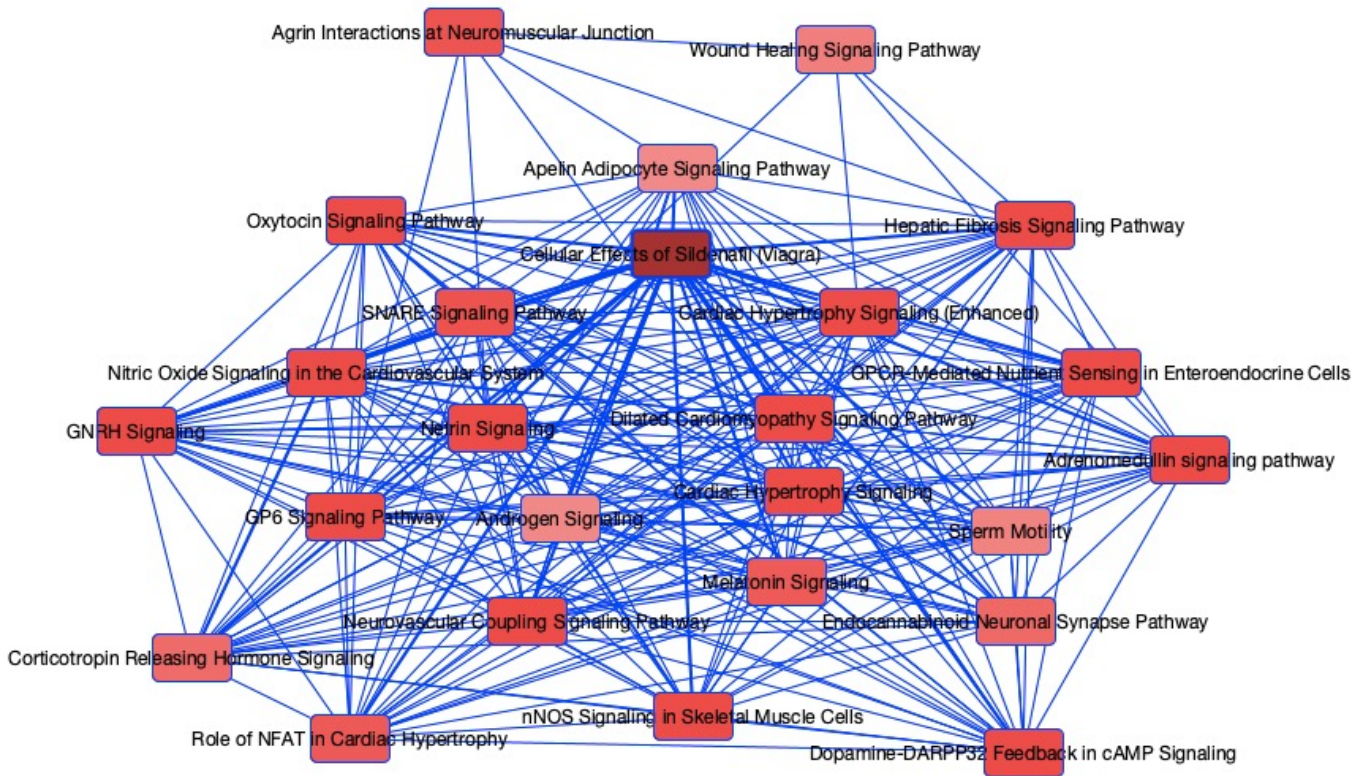




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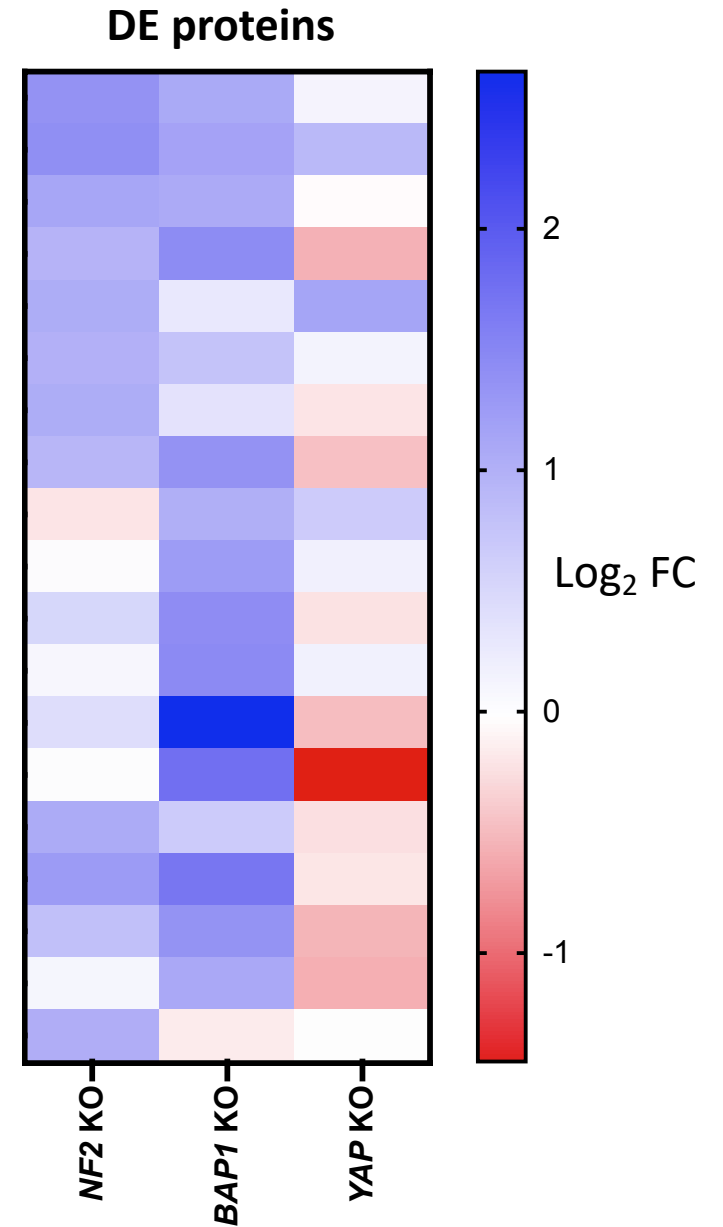
## Ingenuity Pathway Analysis (IPA)

BAP1 KO Met5A

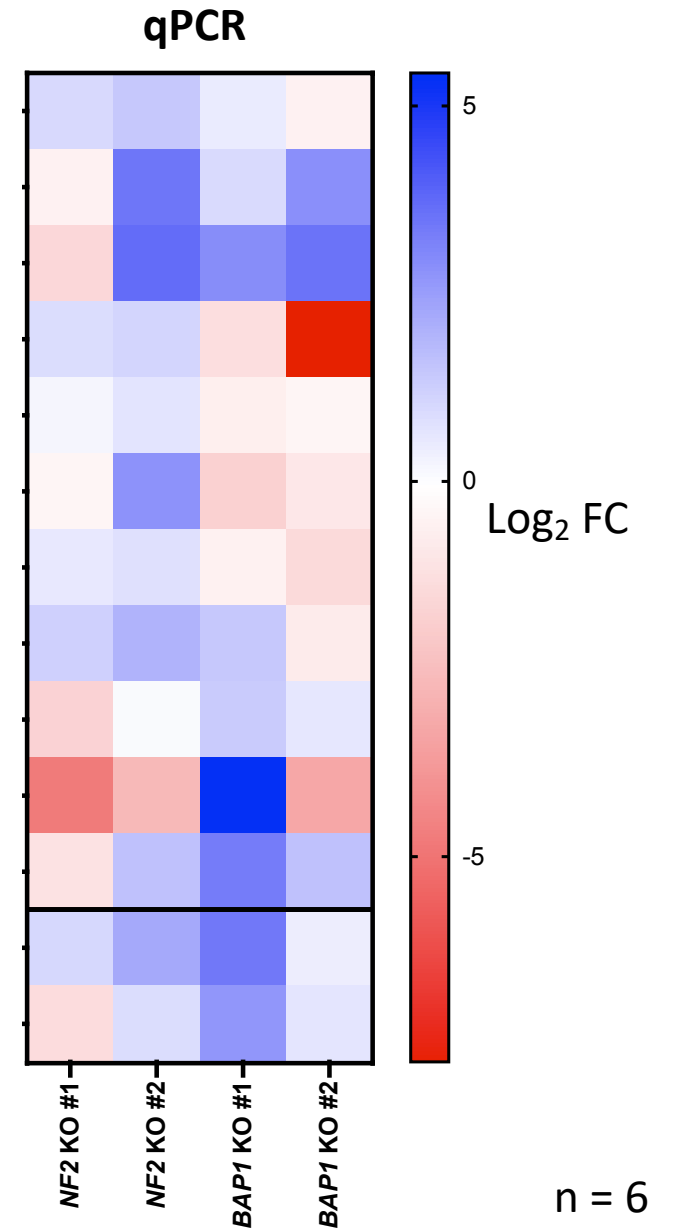
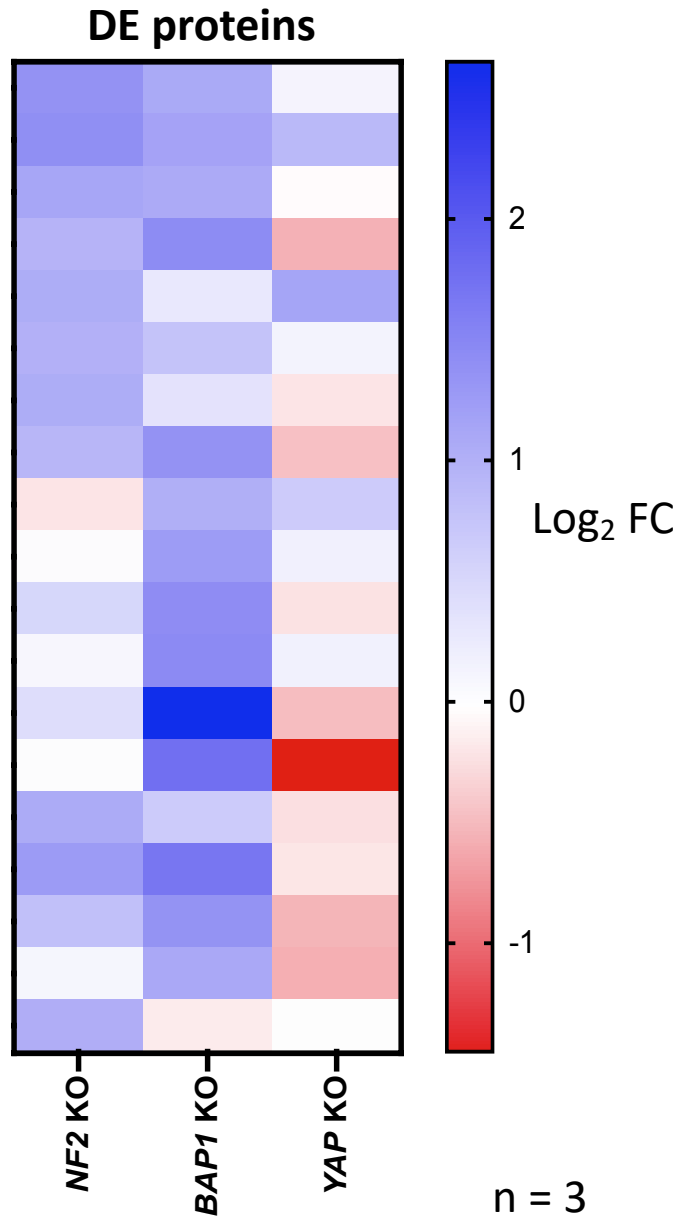


# Identified targets

Selected based on **functional enrichment, interaction network, and DE in previous datasets.**



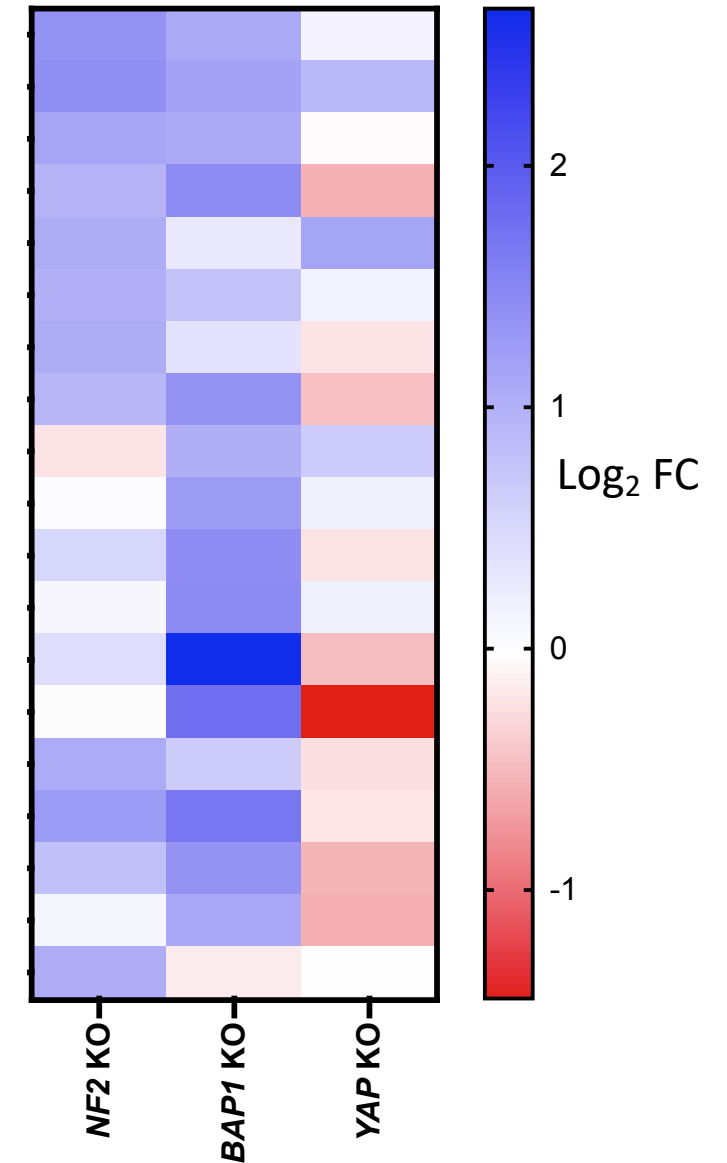
# mRNA level validation



# In the process...

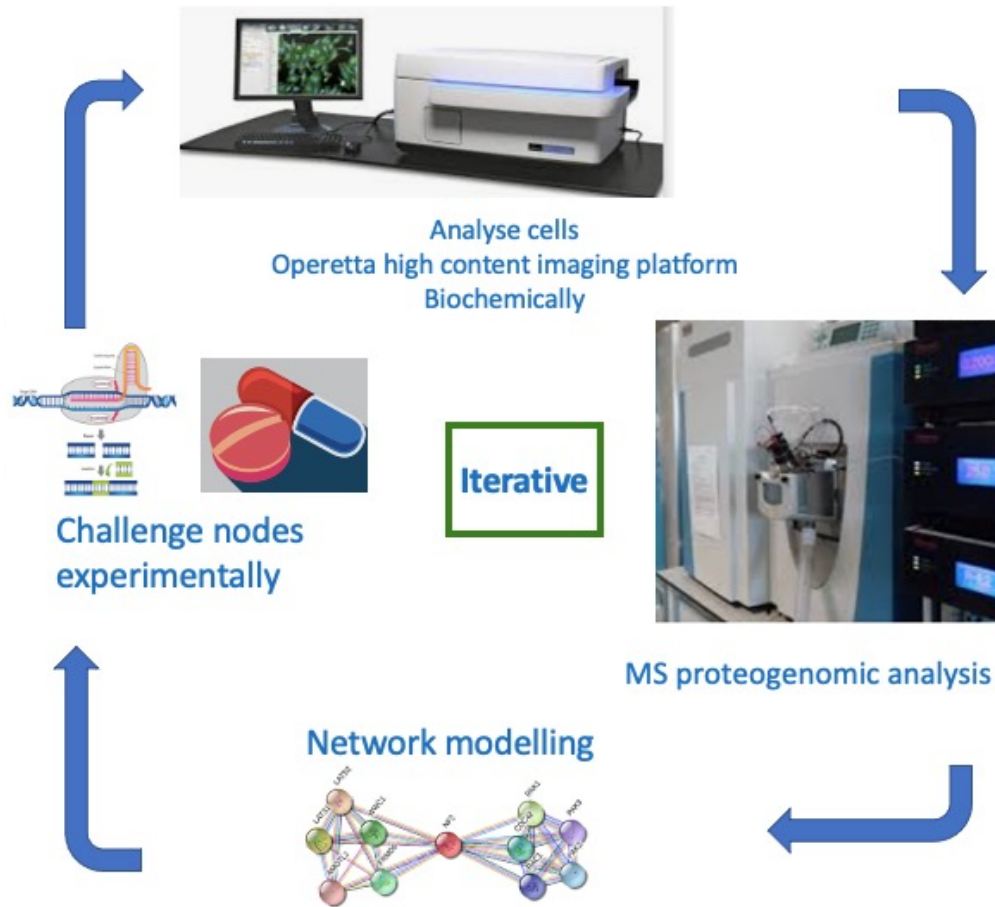
- Targets for KO in current MeT5A genotypes (**NF2** KO and **BAP1** KO).
- Iterative proteomics to investigate change in molecular phenotype.
- Functional in vitro assays for characterization.

Proteomics DE targets

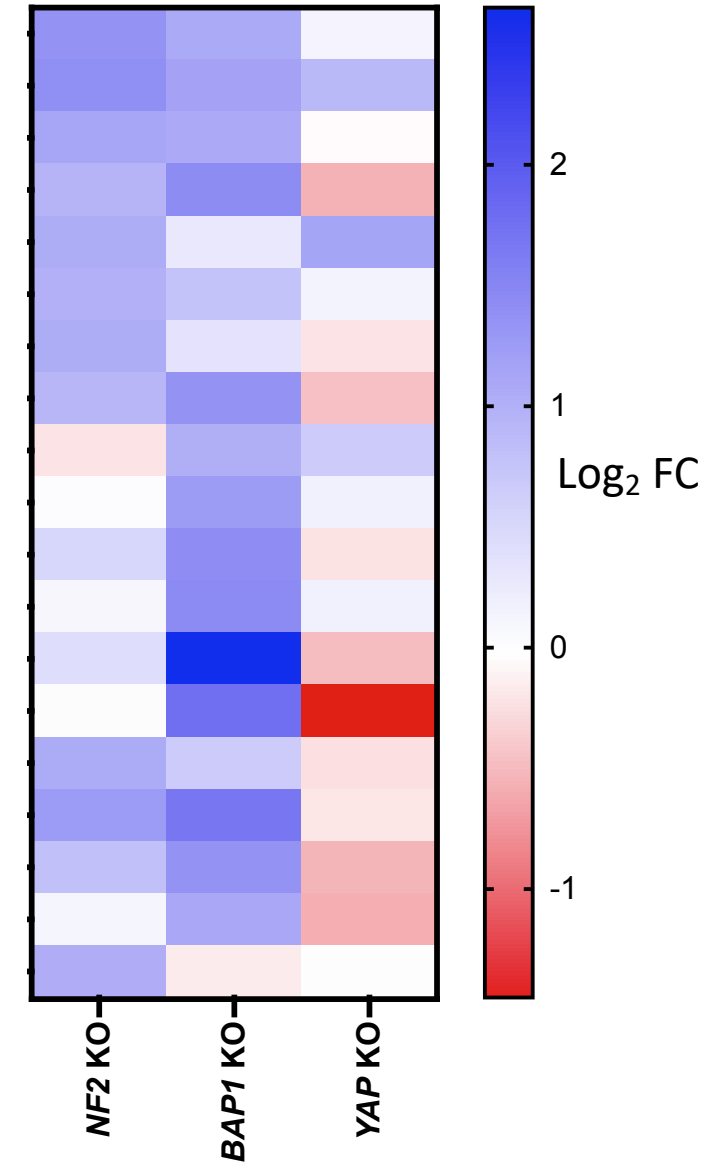




# In the process...



## Proteomics DE targets





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Medical  
Research  
Council



## Hansen Lab

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Dr Richard Cunningham

Dr Omar Salem

Krishna Purohit

Susanna Riley

Siyang Jia

Nancy Hui

### Supervisory Team:

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Prof Adriano Rossi (UoE)

Prof Janne Lehtio (Karolinska Institute)



### Thesis committee:

Prof Martin Taylor (UoE)

Dr Alexander von Kriegsheim (UoE)



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## **MRC Precision medicine DTP**

Prof Susan Farrington

Prof Rob Semple

& Administrators

**MRC**

Medical  
Research  
Council

