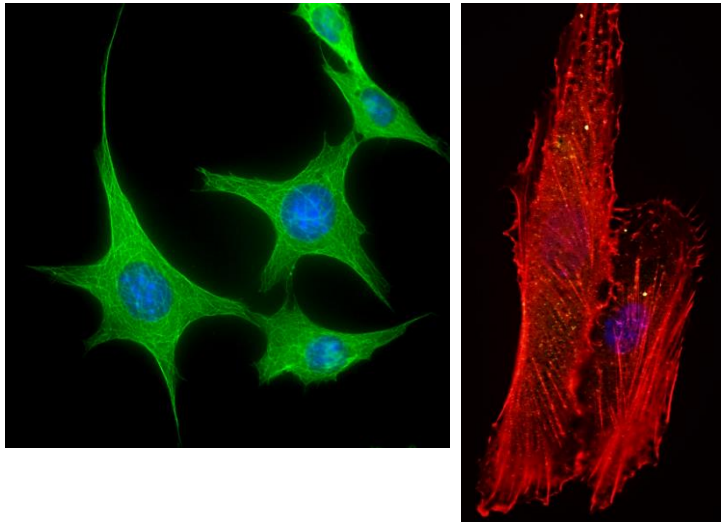


## FROM CILIA TO CANCER: THE TWO SPLICING VARIANTS OF THE HUMAN TBCCD1 GENE

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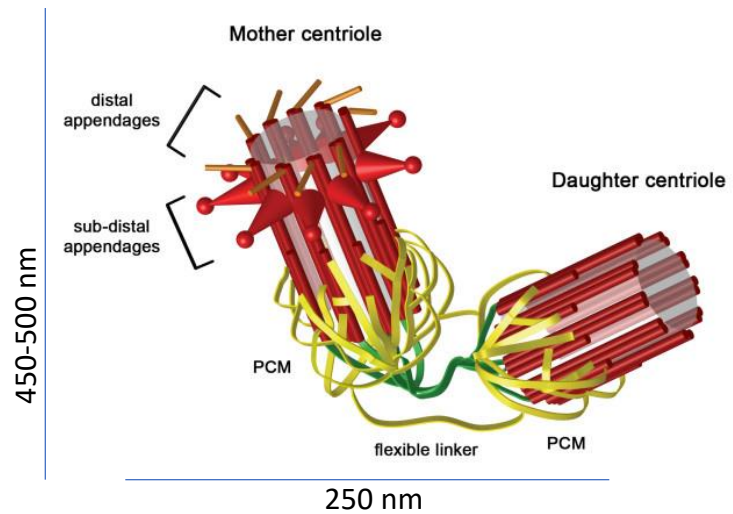
Helena Soares

*H&TRC - Health and Technology Research Center*

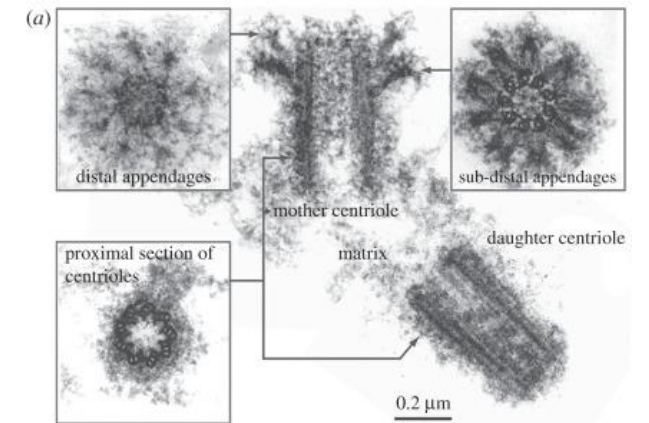
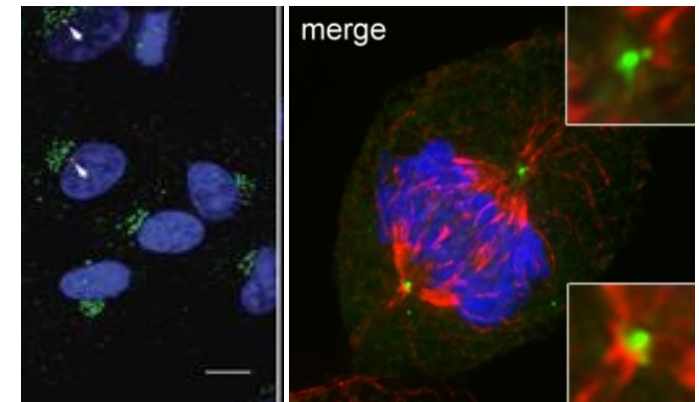


- Organization of the the cytoskeleton,
- Spatial organization of the cytoplasm,
- cell polarity,
- cell motility,
- cell adhesion,
- cell division,
- The centrosome is also critical for cilia assembly

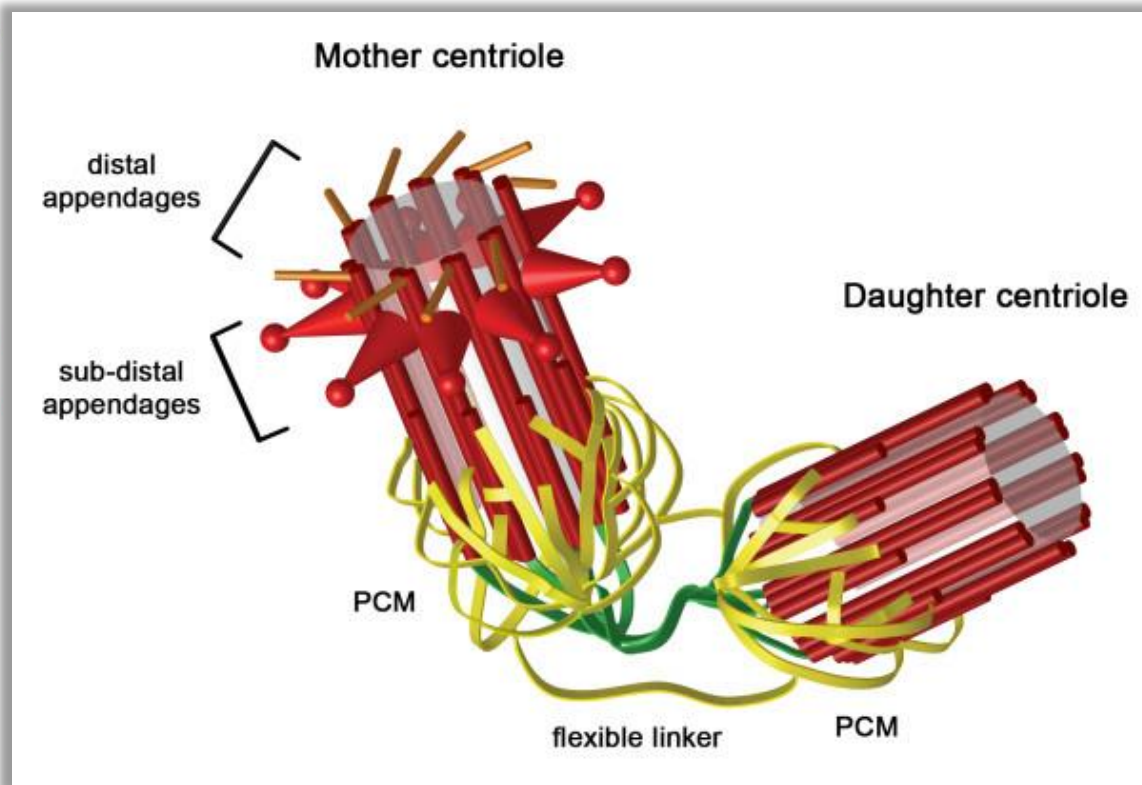
## The centrosome is a microtubule & actin-organizing center in animal cells



Comprises two microtubule-based centrioles around which the pericentriolar material a dynamic matrix of hundreds of proteins



## Multifaceted roles of centrosomes and human diseases



**CENTROSOME ABERRATIONS AND  
SENESCENCE**

**CENTROSOMES AND IMMUNITY**

**CENTROSOME AMPLIFICATION IN CANCER**

**CENTROSOME ABNORMALITIES IN  
INFERTILITY**

**CENTROSOMES AND ASYMMETRIC  
CELL DIVISION IN STEM CELLS**

**CENTROSOME DEFECTS LEADS  
TO PRIMARY MICROCEPHALY**

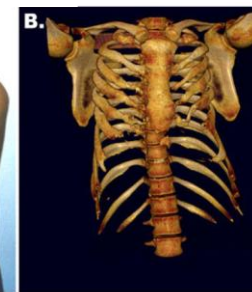
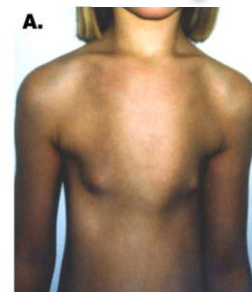
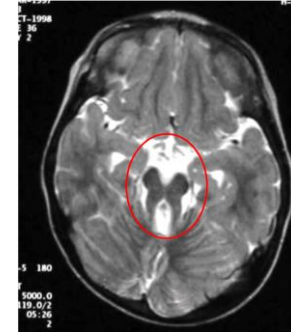
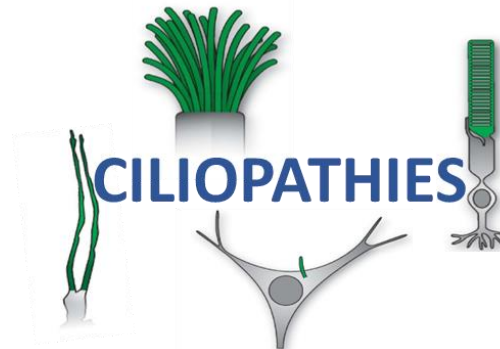
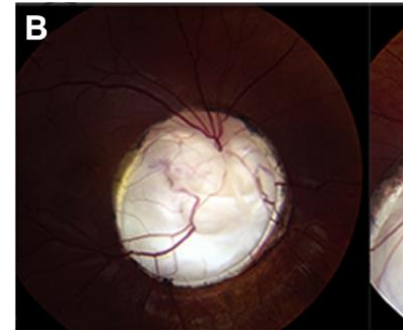
**CENTROSOME DEFECTS  
ASSOCIATED WITH CILIOPATHIES**

**Other....**

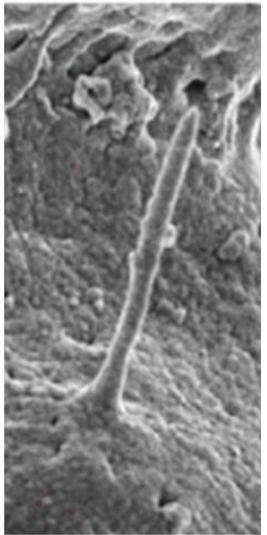
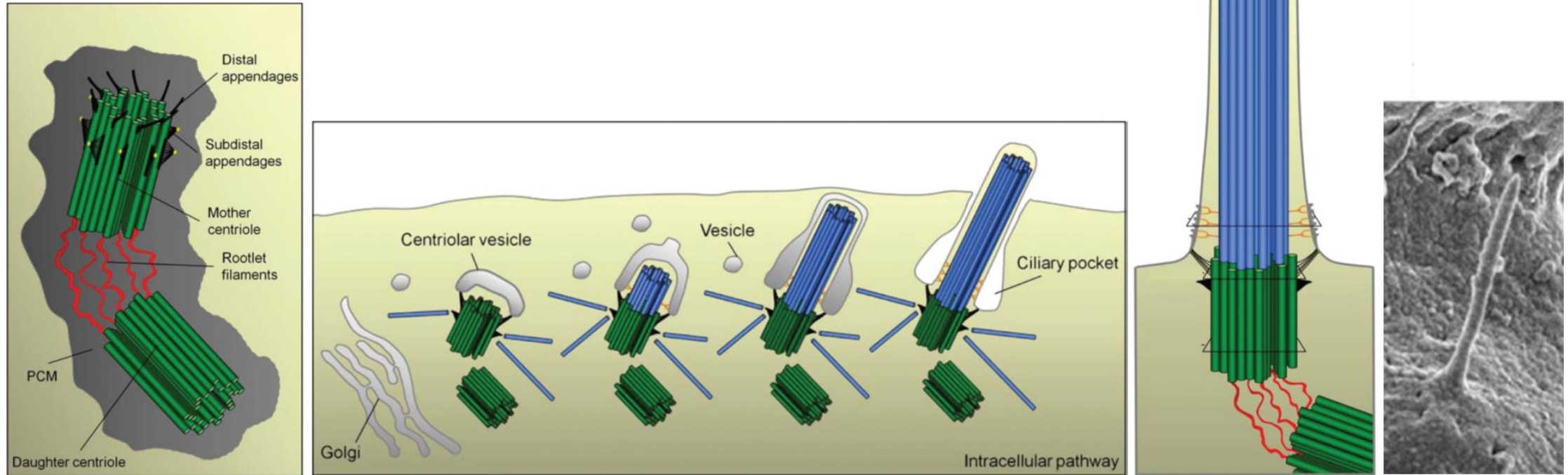
## CILIA DYSFUNCTION UNDERLIES SEVERAL GENETIC DISEASES, OFTEN CHRONICALLY DISABLED WHICH CAN CAUSE DEATH

Multiple systems can be affected, causing:

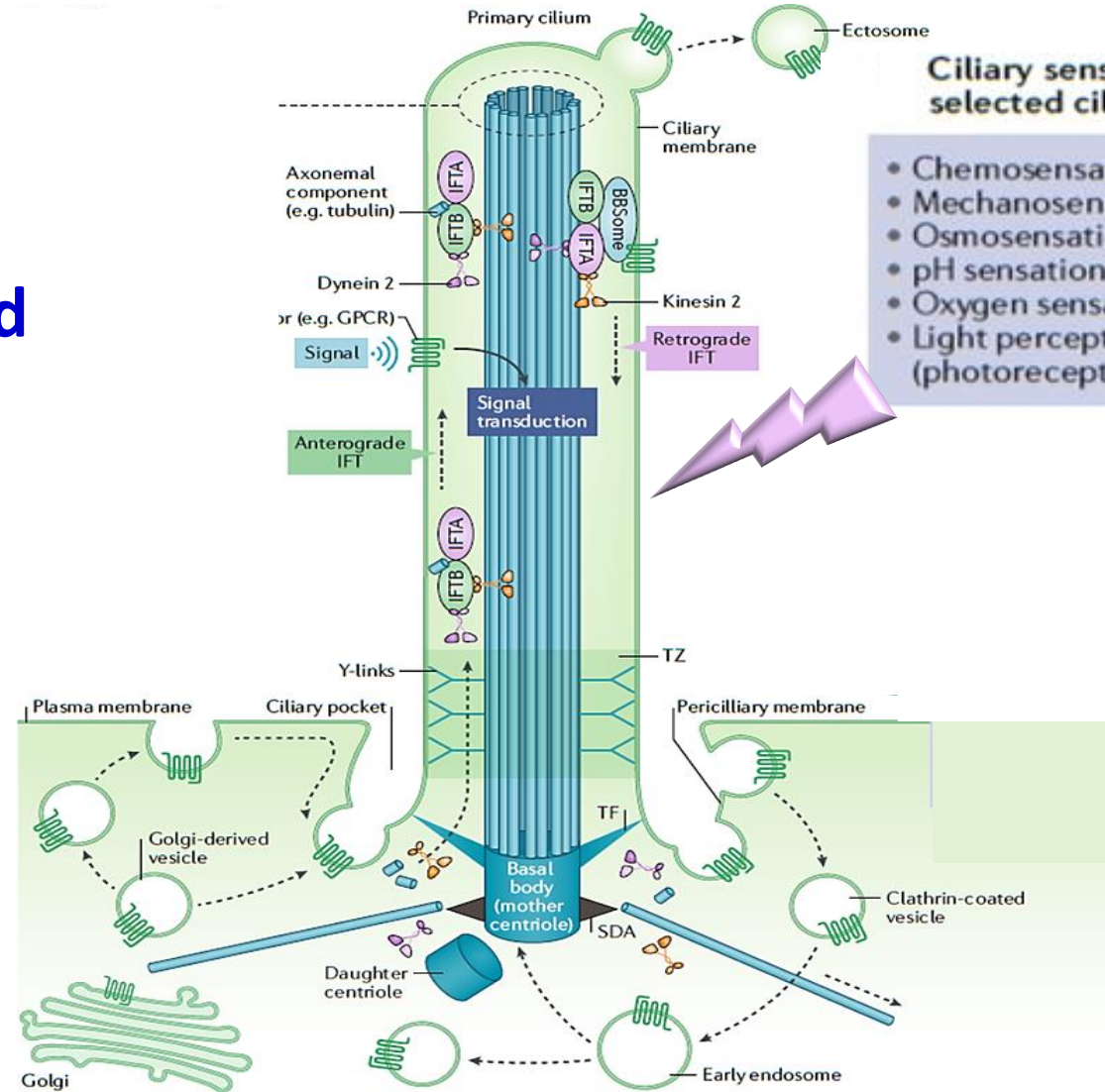
- Blindness
- Deafness
- Chronic respiratory infections (chronic sinusitis; bronchiectasis)
- Kidney disease (polycystic kidney)
- Heart disease
- Skeletal problems
- Infertility
- Cognitive impairment
- Obesity and diabetes.



## THE ROLE OF CENTROSOME IN CILIA ASSEMBLY



## Cilia, cellular signalling and ciliopathies



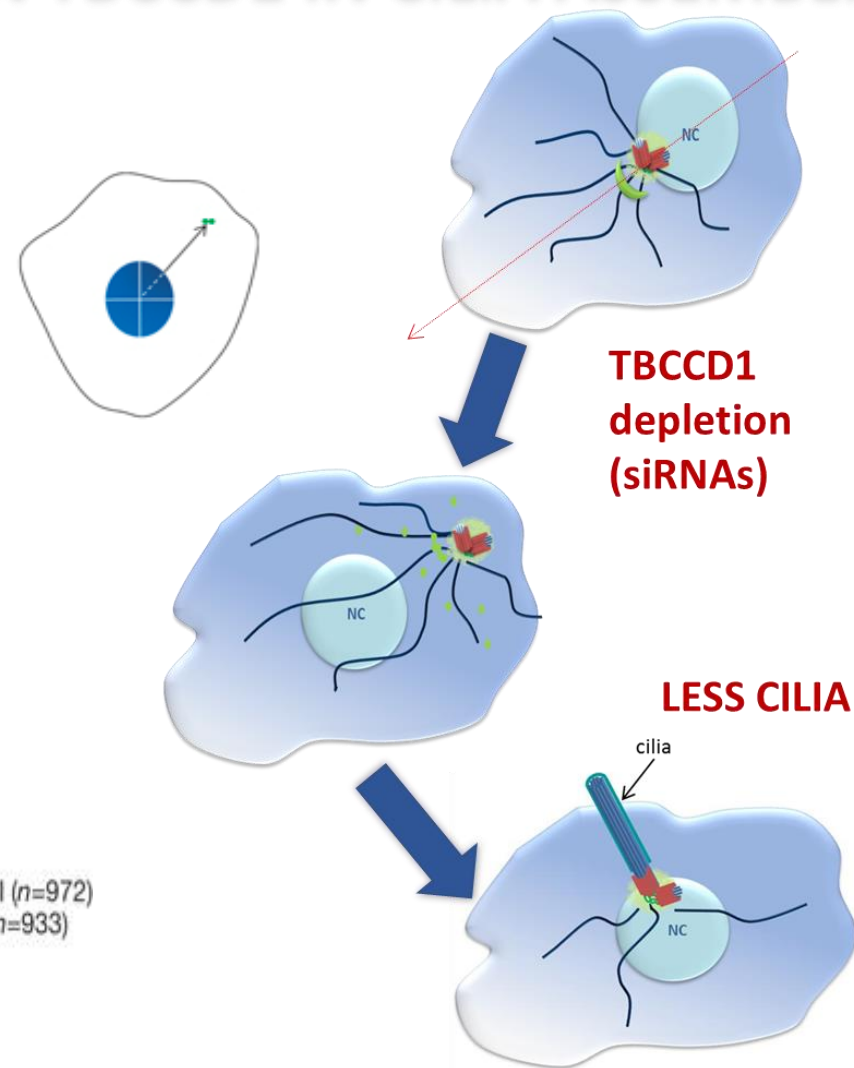
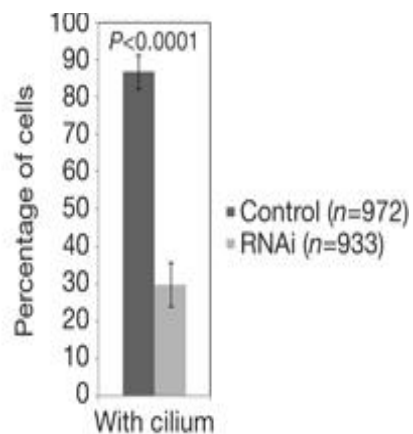
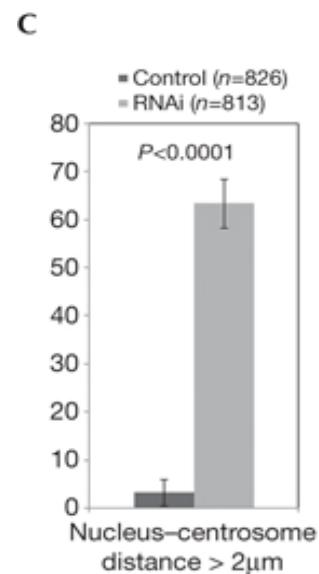
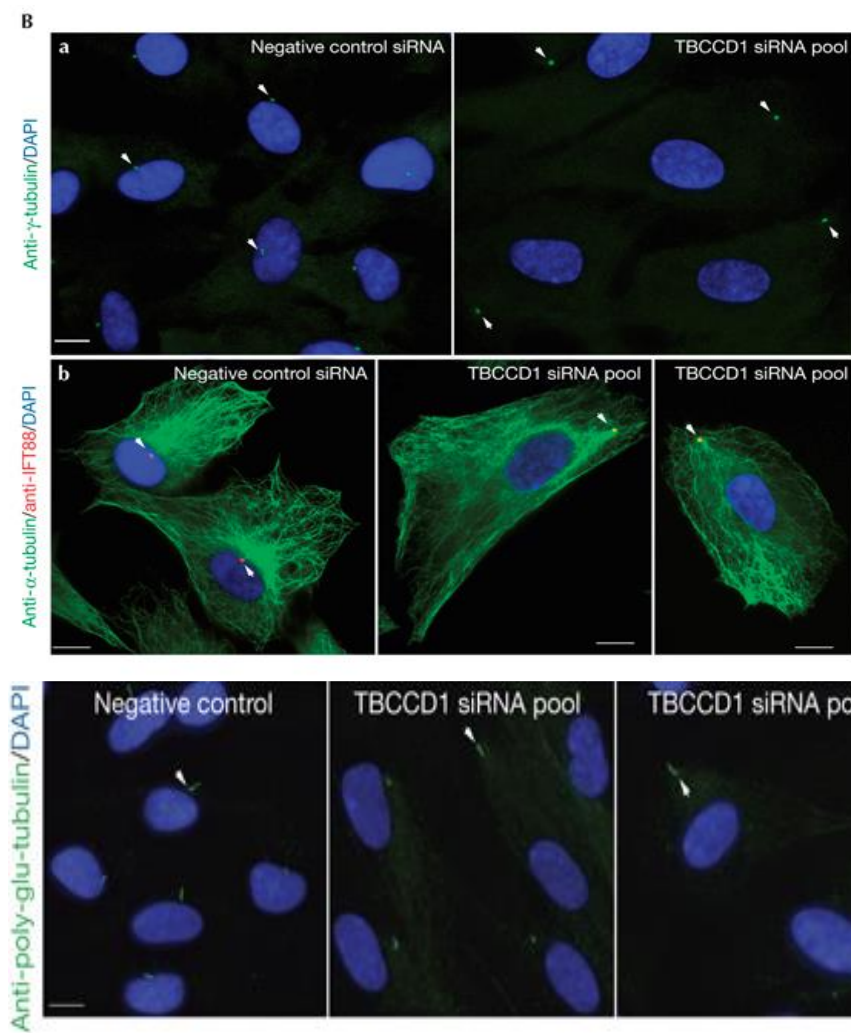
### Ciliary sensing capabilities and selected ciliary signalling pathways

- Chemosensation
  - Mechanosensation
  - Osmosensation
  - pH sensation
  - Oxygen sensation
  - Light perception (photoreceptors)
- Hedgehog signalling
  - Class A/B GPCR signalling
  - WNT signalling
  - RTK signalling
  - TGF $\beta$ /BMP signalling
  - Notch signalling
  - ECM receptor signalling
  - Hippo signalling
  - mTOR signalling
  - NF- $\kappa$ B signalling

### Selected ciliopathies and syndromes (primary cilia)

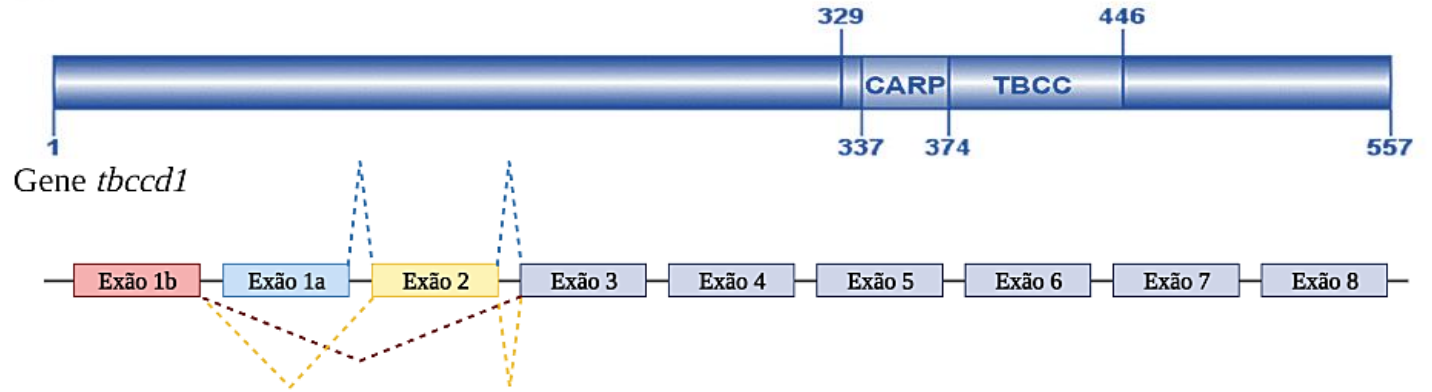
- Alström syndrome (ALMS)
- Bardet–Biedl syndrome (BBS)
- Jeune syndrome (JATD)
- Joubert syndrome (JBTS)
- Leber's congenital amaurosis (LCA)
- Meckel syndrome (MKS)
- Nephronophthisis (NPHP)
- Orofaciodigital syndrome (OFDS)
- Polycystic kidney disease (PKD)
- Senior Løken syndrome (SLS)
- Usher syndrome (USH)

## THE ROLE OF HUMAN CENTROSOMAL PROTEIN TBCCD1 IN CILIA ASSEMBLY



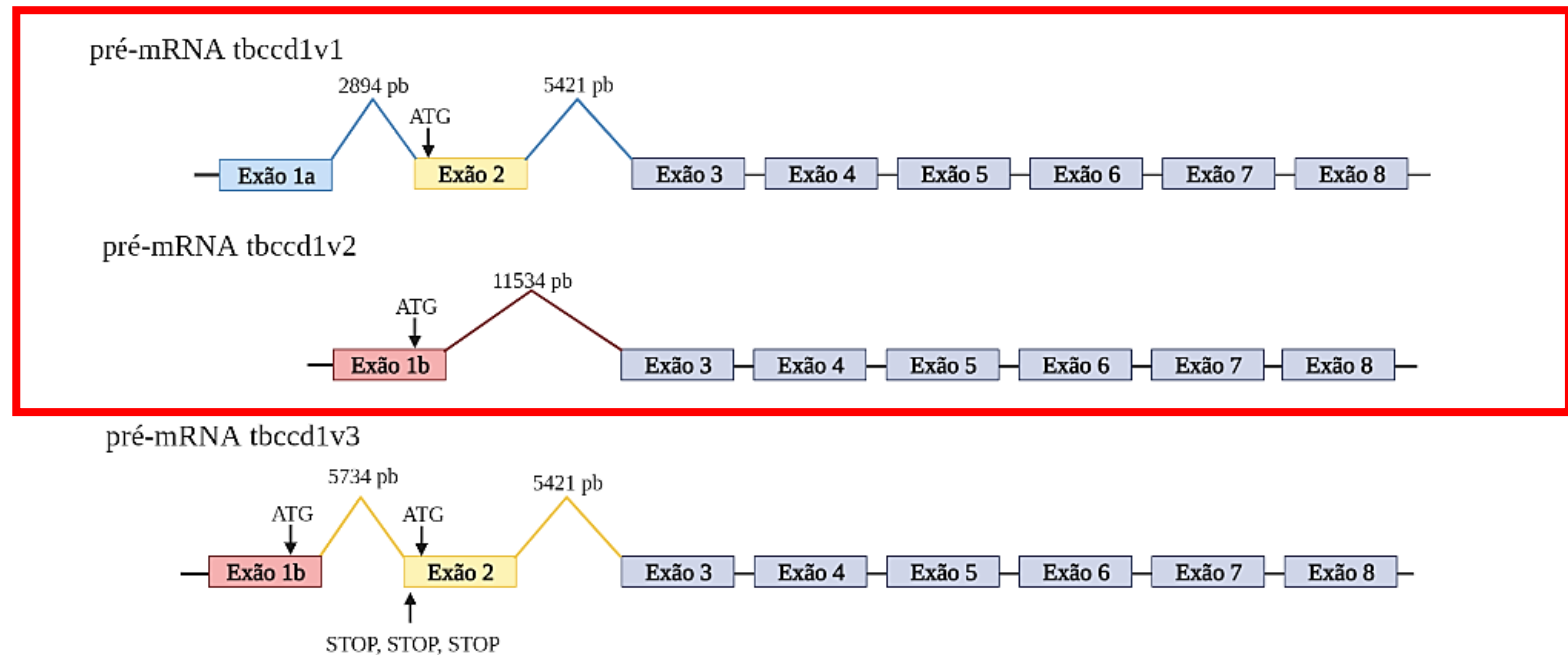
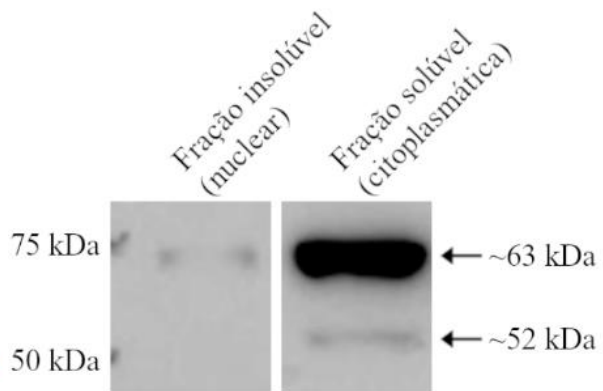
## The alternative splicing of *tbccd1* gene

TBCCD1 protein

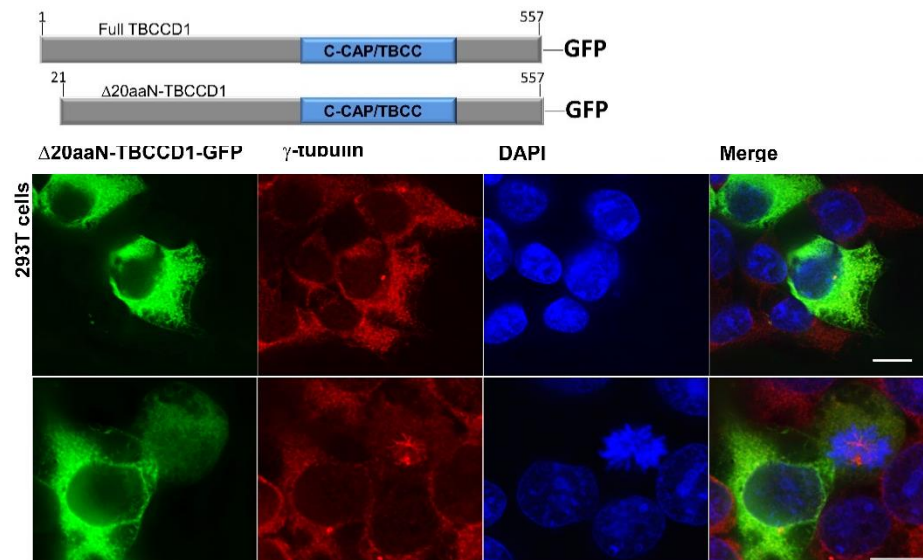
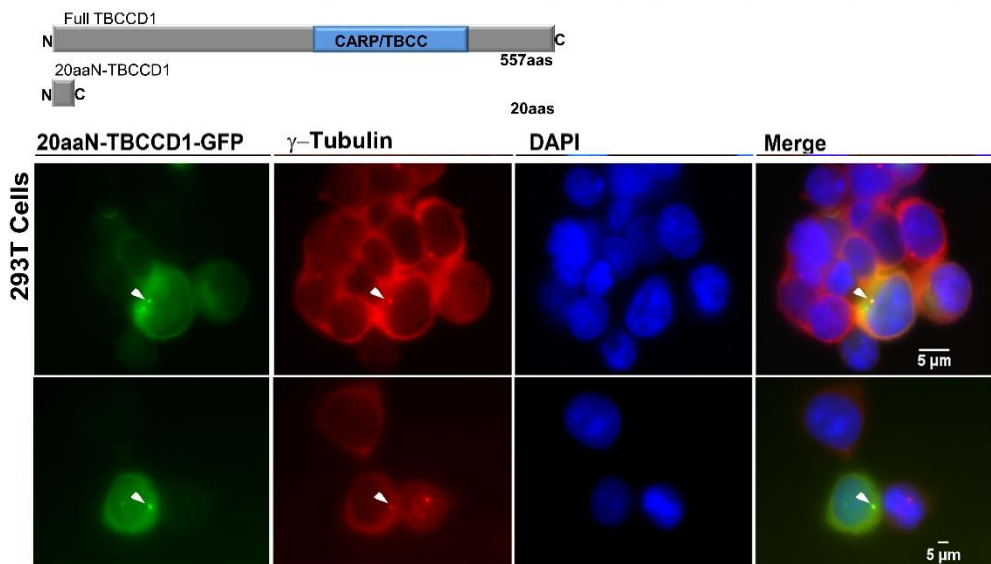


Human *tbccd1* gene localizes in chromosome 3 (3q27.3)

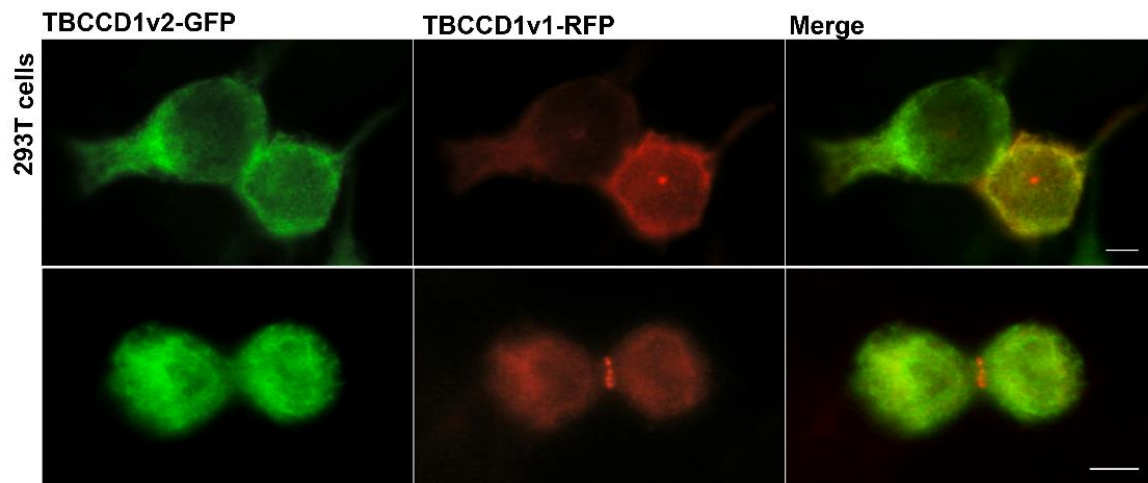
Anti-TBCCD1



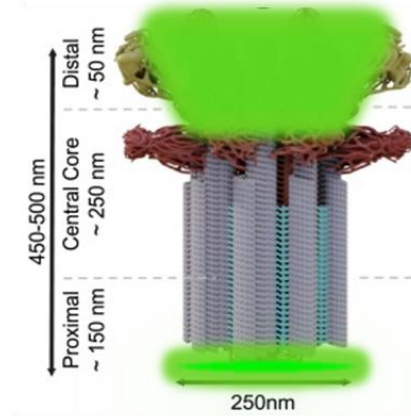
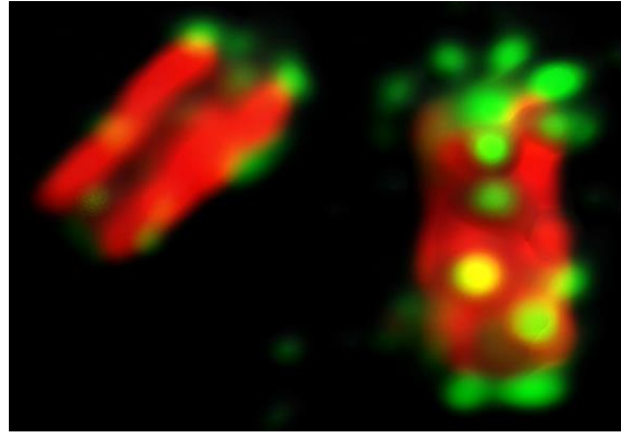
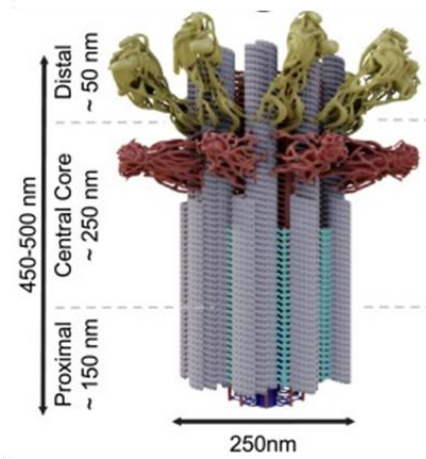
## The minimal domain of TBCCD1v1 centrosomal localization



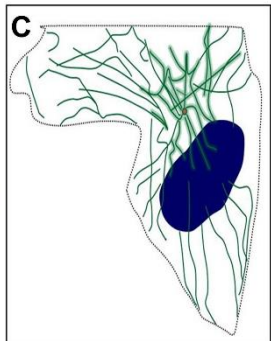
The cytoplasmic localization of TBCCD1v2



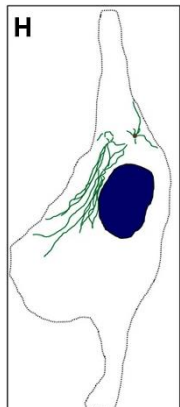
## The centrosomal localization of TBCCD1v1



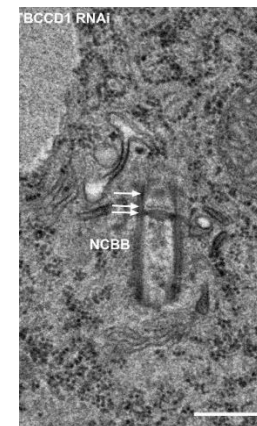
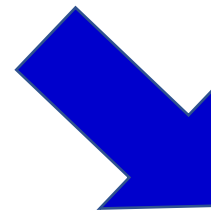
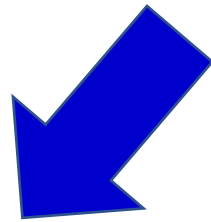
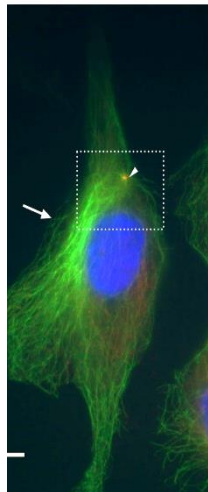
### COMPROMISED CYTOSKELETON ORGANIZATION



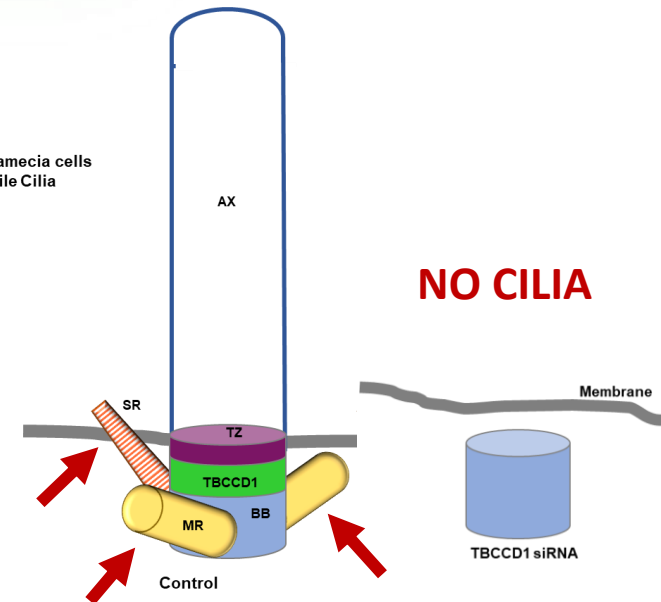
Control



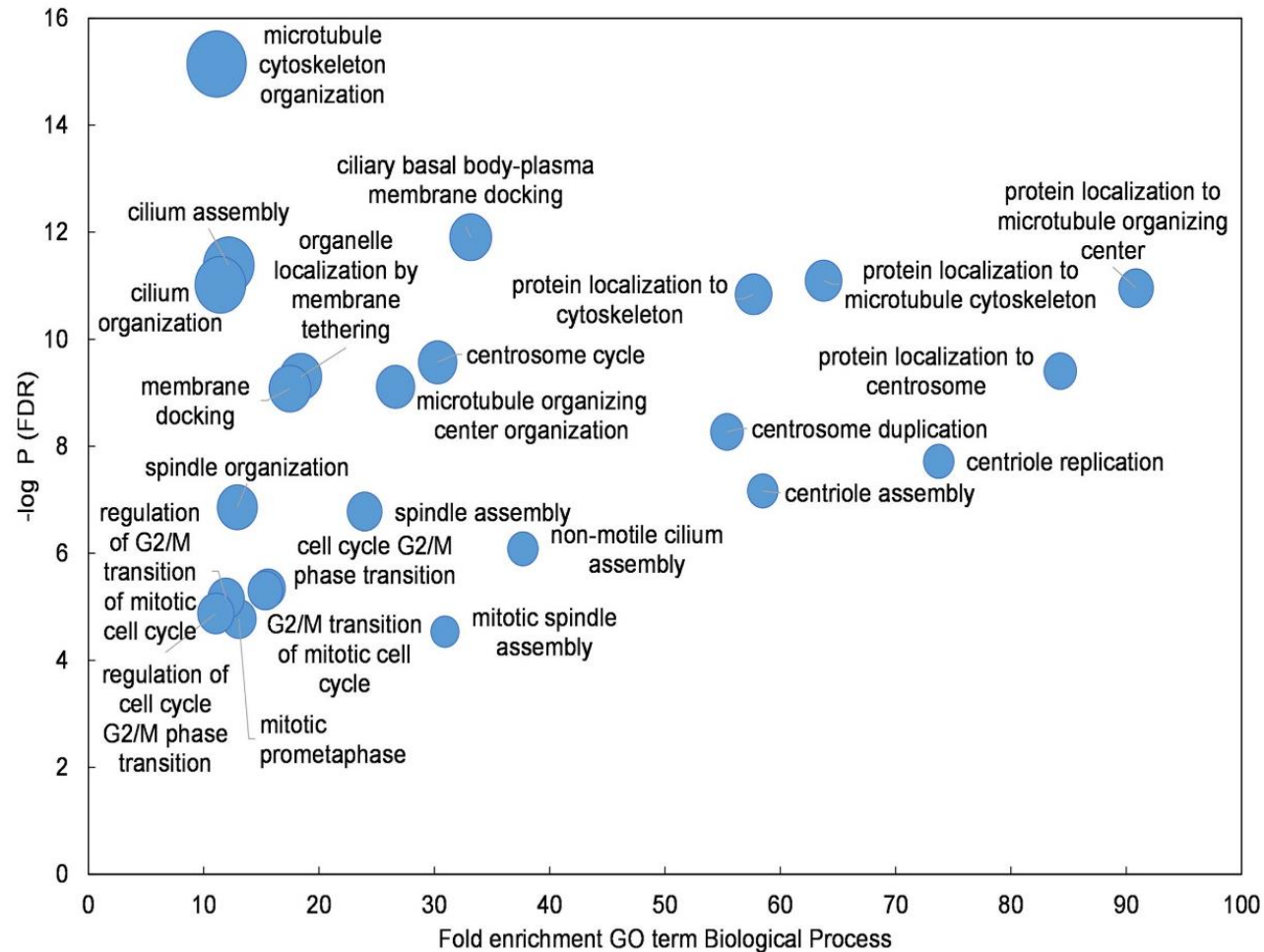
TBCCD1 siRNAs



Paramecia cells  
Motile Cilia

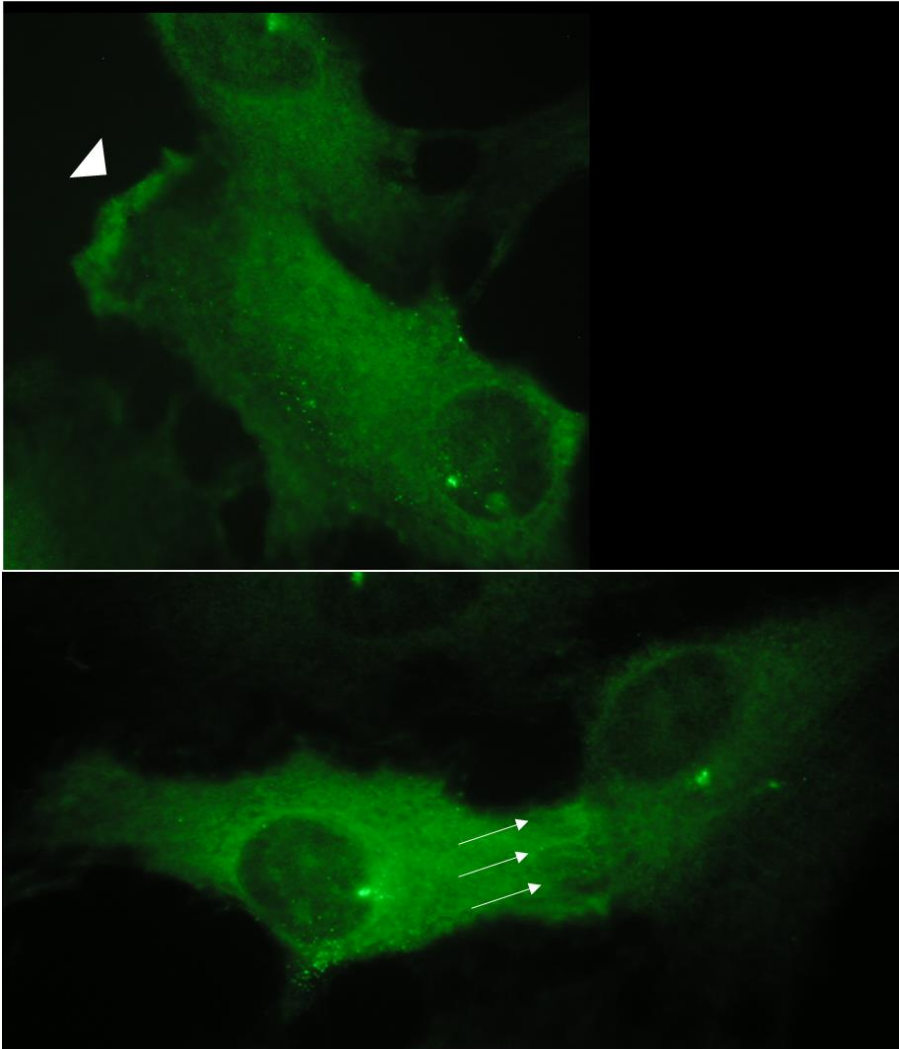


## The proximity interactome of TBCCD1v1



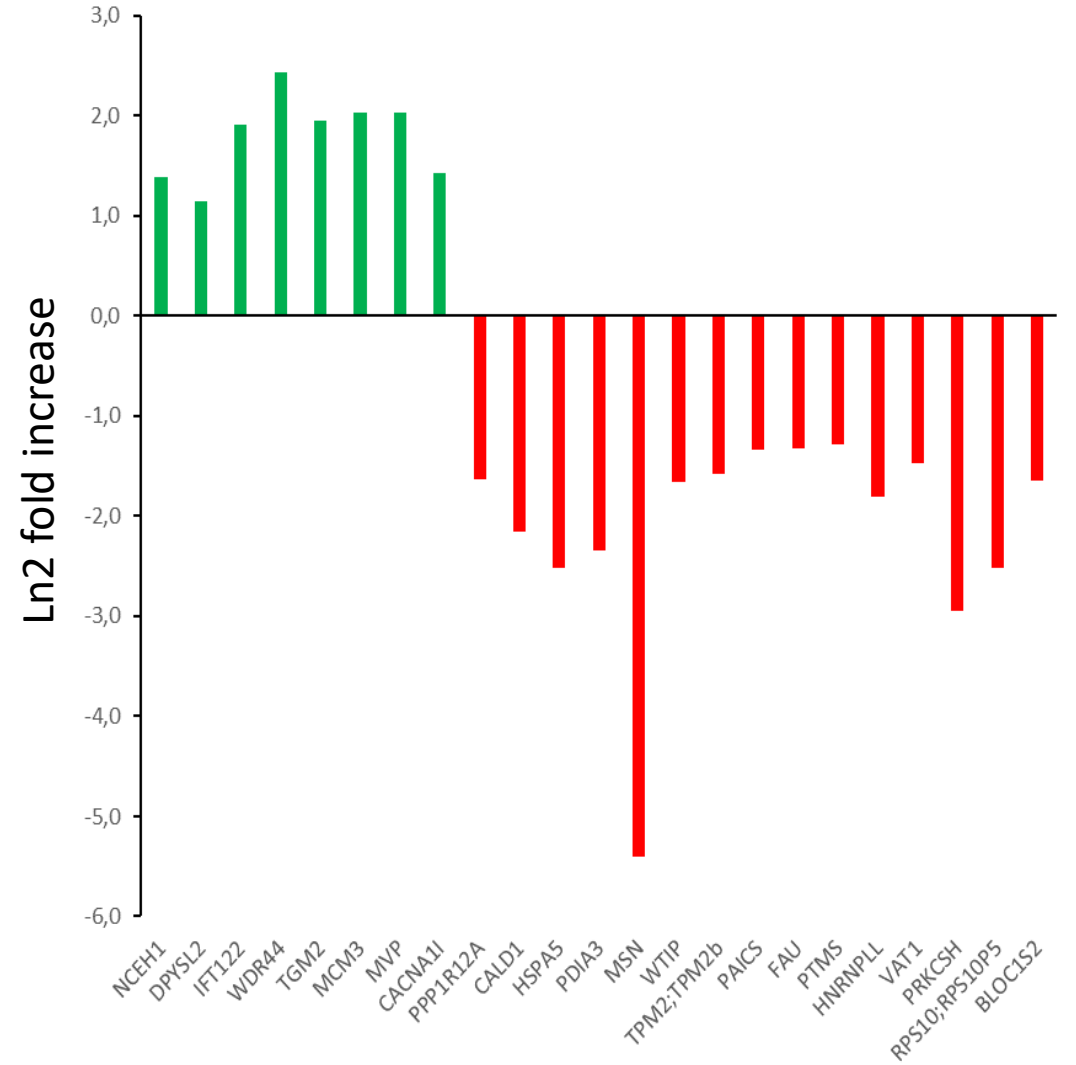
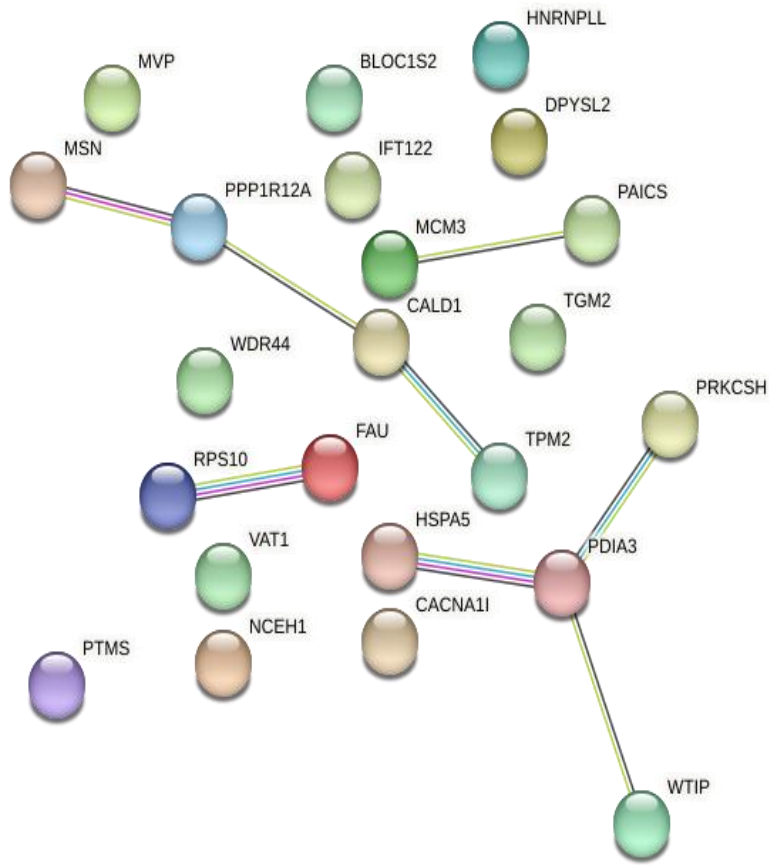
By a BioID screening

## What about the role of the TBCCD1 cytoplasmic pool?

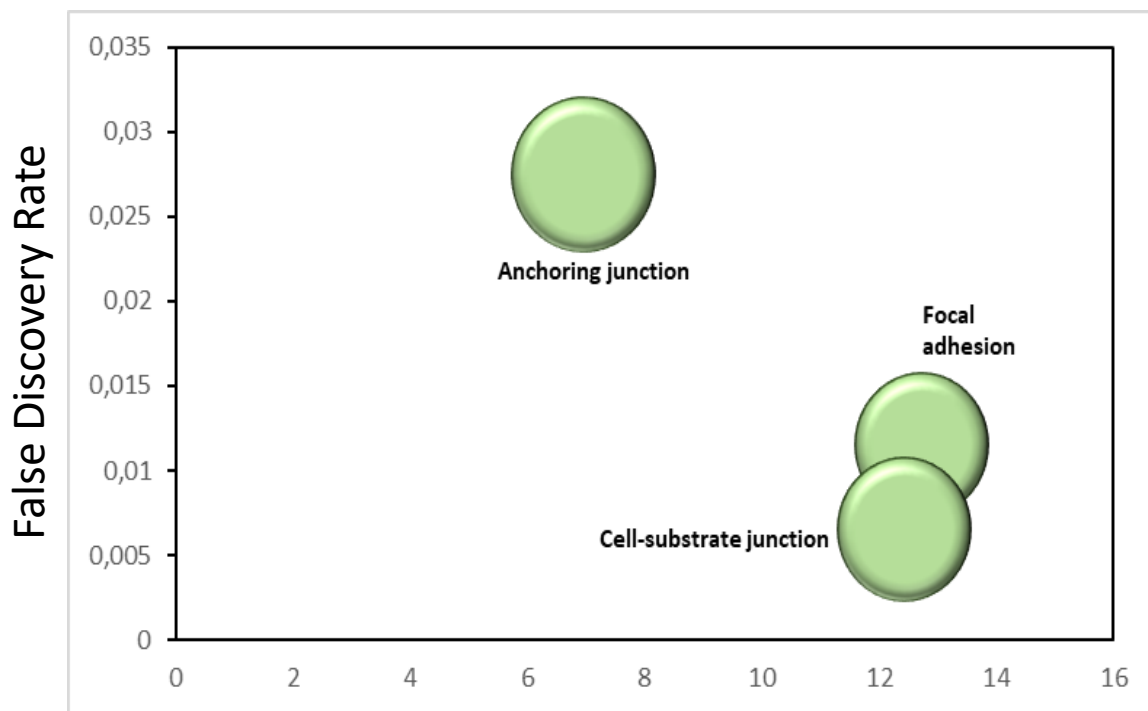


we decided to investigate the impact of altered levels of TBCCD1 in cellular physiological proteome

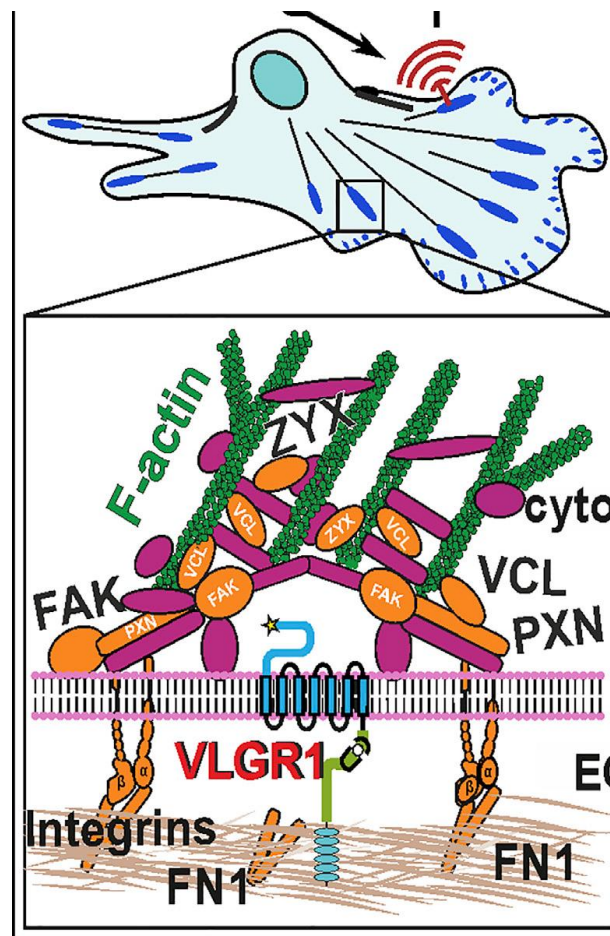
## TBCCD1 overexpression proteomics



## TBCCD1 overexpression proteomics: GO enrichment analysis cellular component



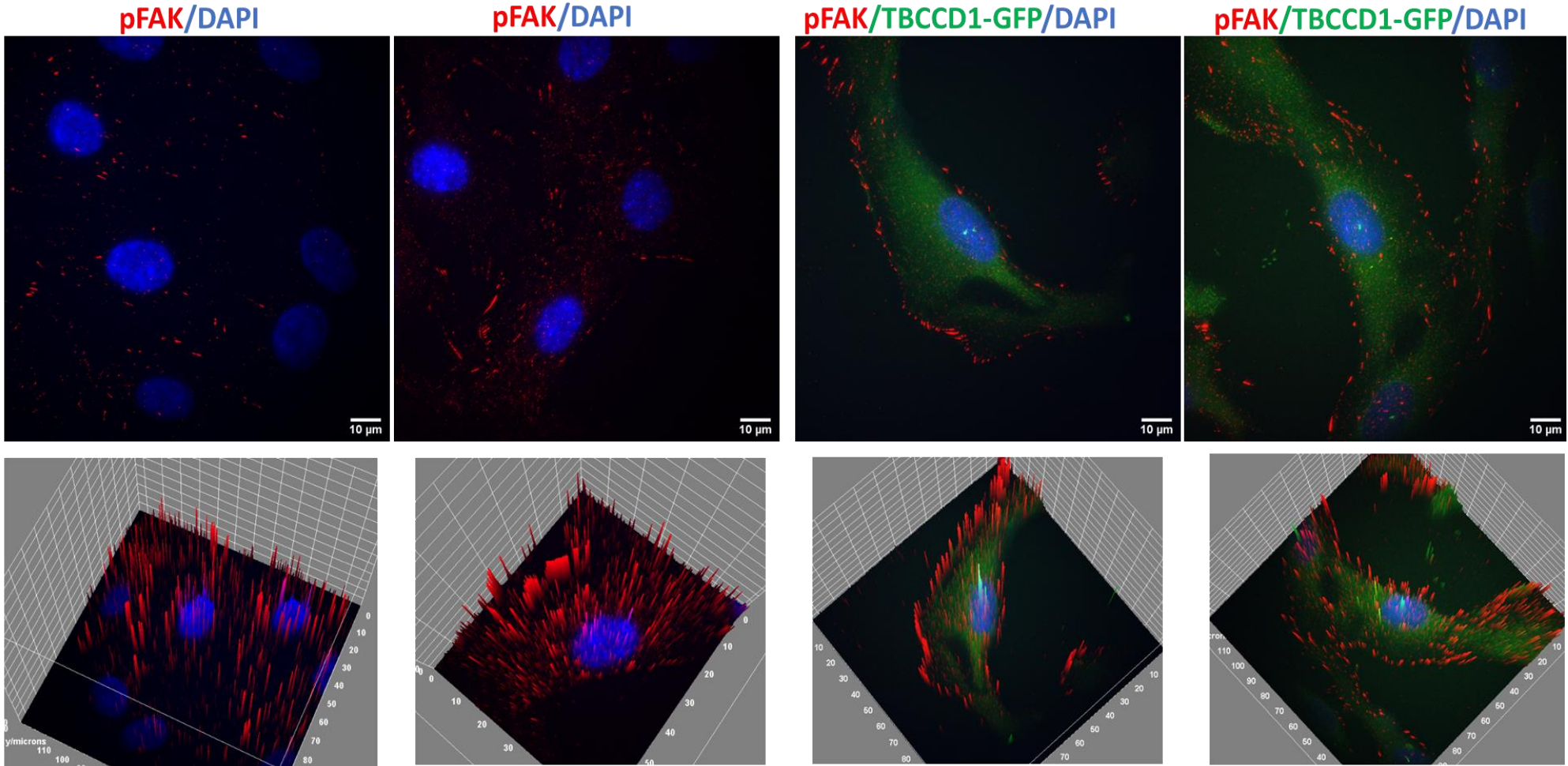
Fold Enrichment for the cellular component Go term



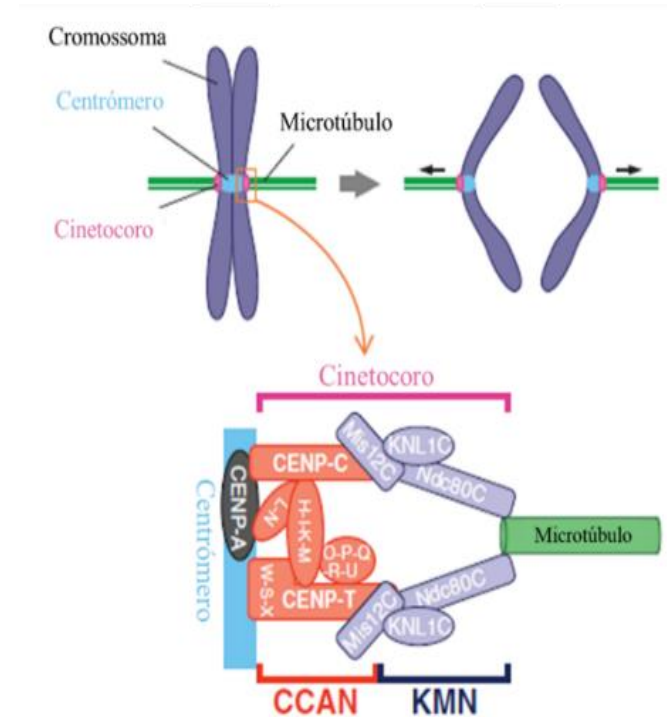
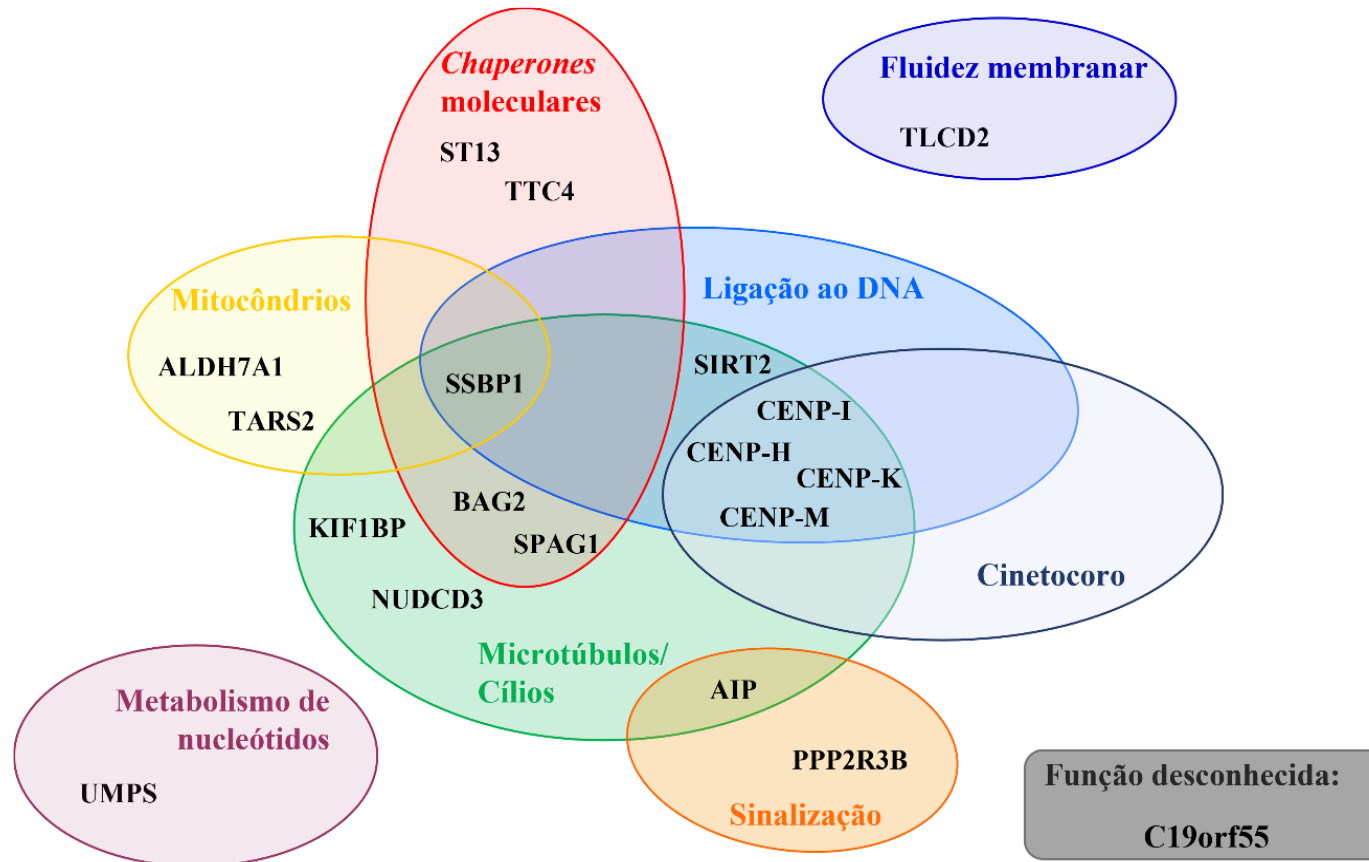
## Pattern of localization of pFAK in TBCCD1-overexpressing RPE1 cells

Focal adhesion kinase (FAK) is a key signaling molecule regulating cell adhesion, migration, and survival

hTERT-RPE1

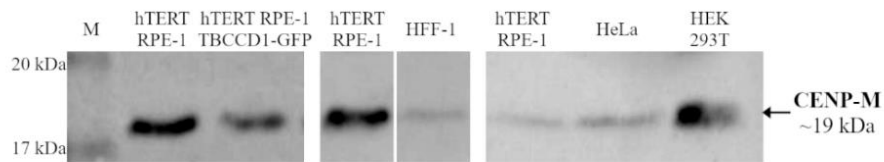


## The proximity interactome of TBCCD1v2

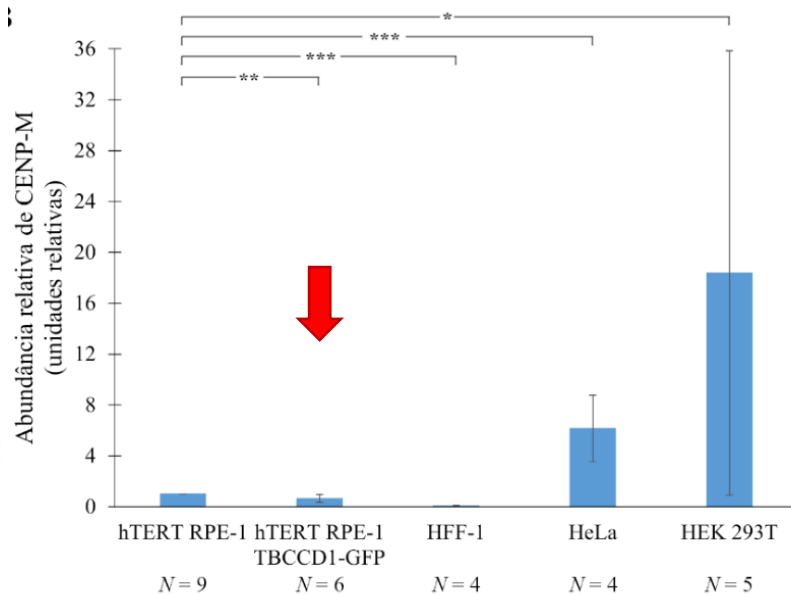
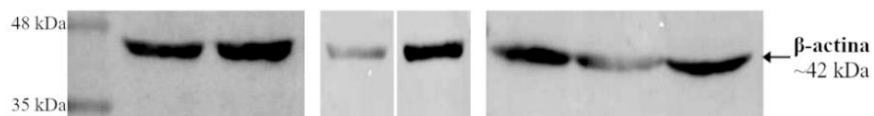


## Analysis of relative levels of CENP-M in different cell lines

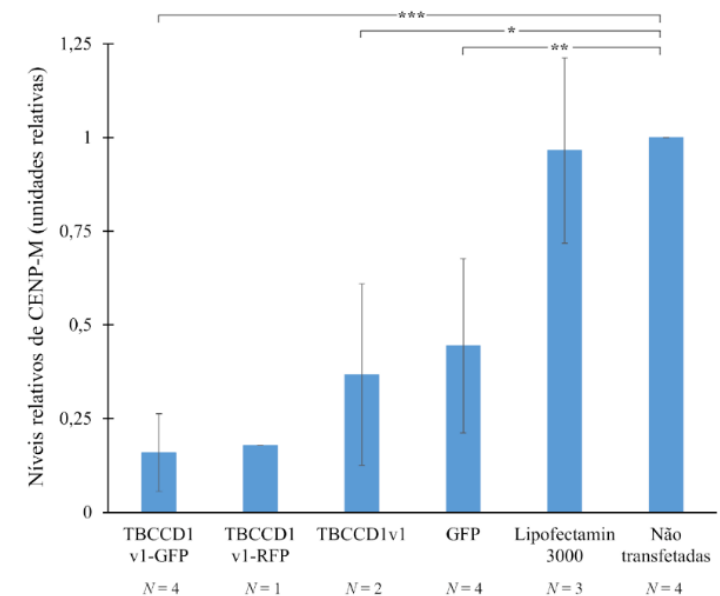
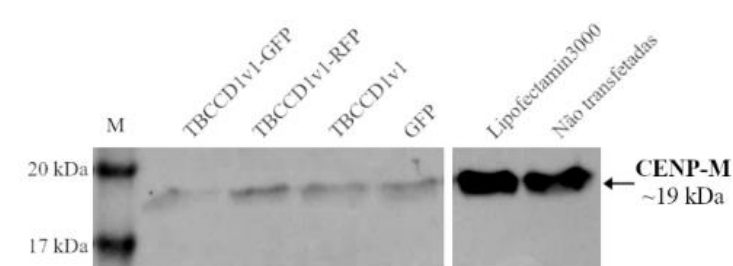
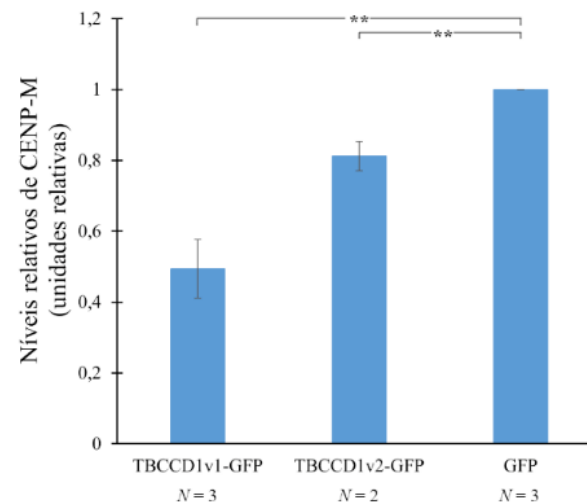
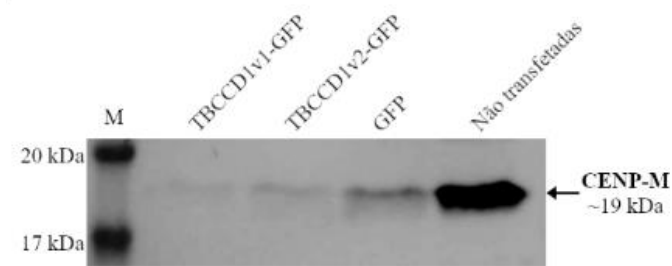
### Anti-CENP-M

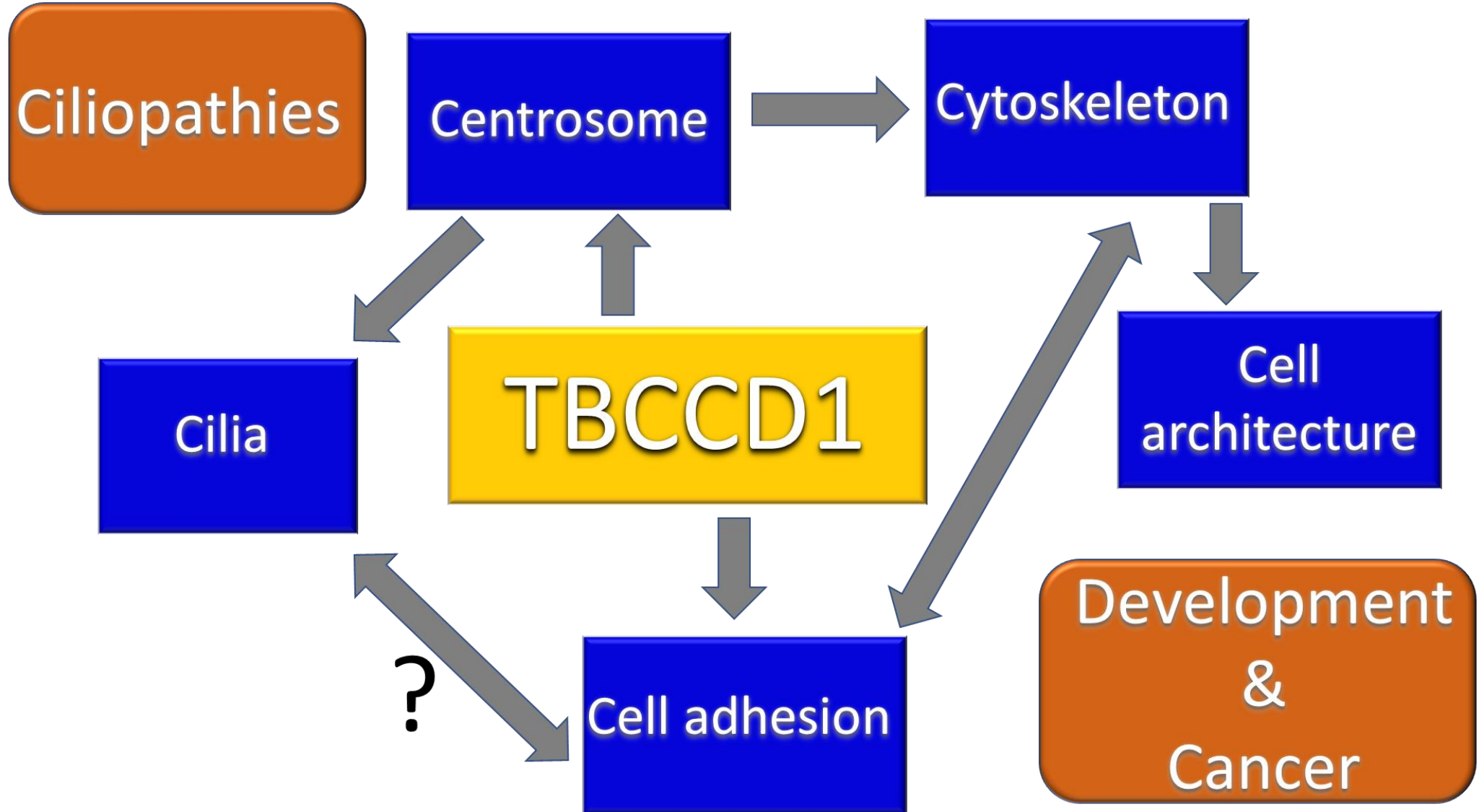


### Anti-β-actina



## Overexpression of TBCCD1v1-GFP causes a decrease in relative CENP-M levels in HEK 293T cells





# V H&TRC BOOTCAMP 2023

## Acknowledgements

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**Inês Pereira**  
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