26º Congresso Português
de Obesidade

SPEO
SOCIEDADE PORTUGUESA PARA O ESTUDO DA OBESIDADE

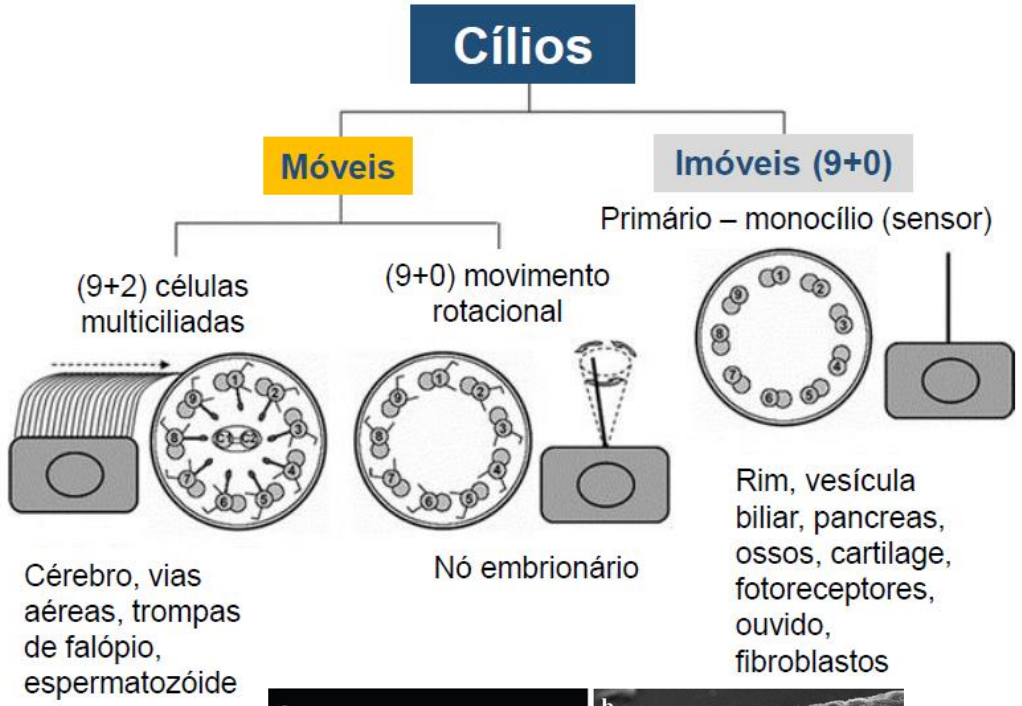
Cílios e sinalização celular na obesidade

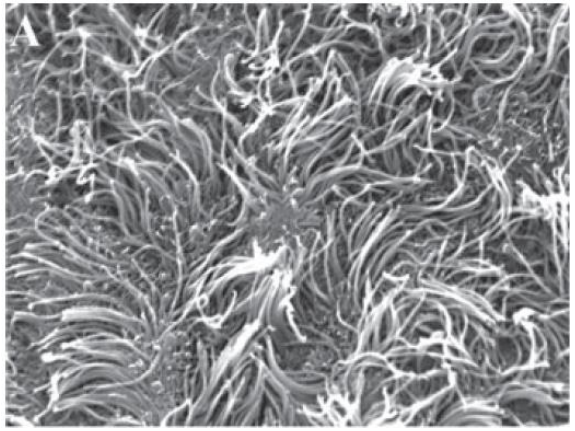
BRUNO CARMONA

@ bfcarmona@fc.ul.pt & bruno.carmona@estesl.ipl.pt

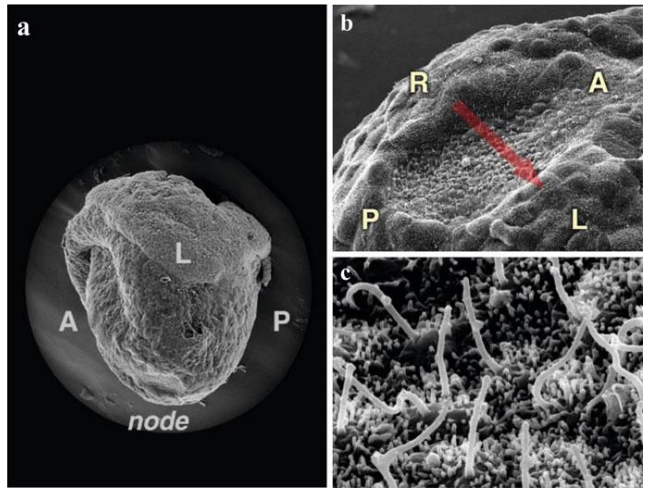
Coimbra, november 25th, 2022

Diversidade da Estrutura ciliar

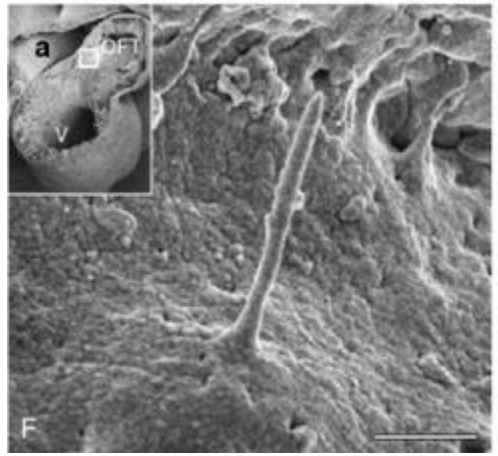




mouse ependymal cells located in the lateral ventricles

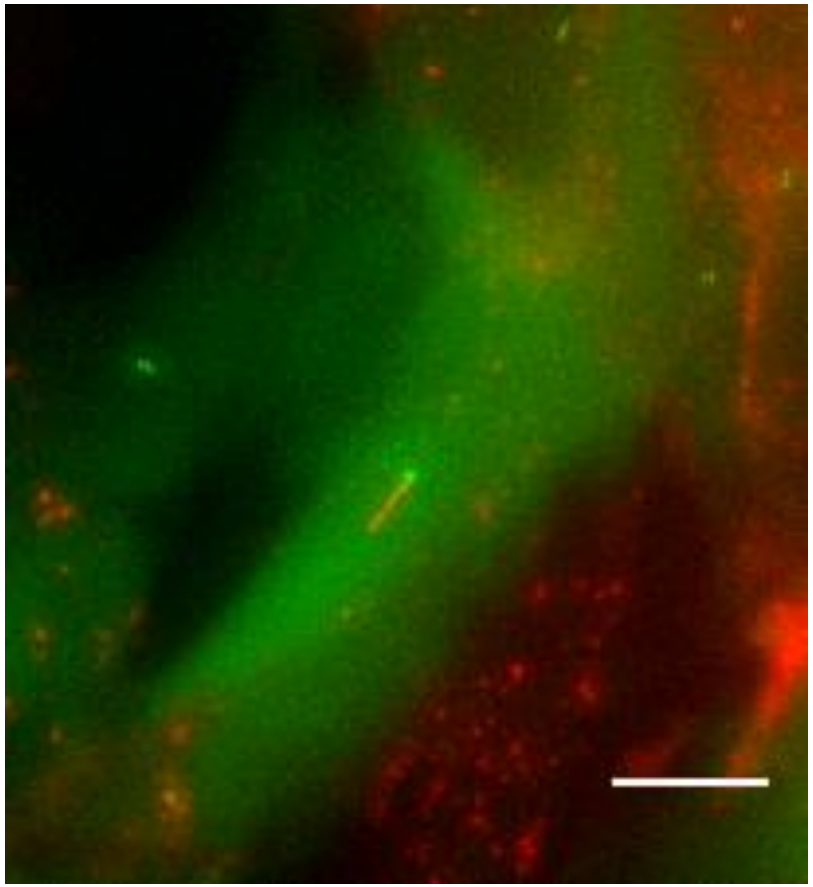


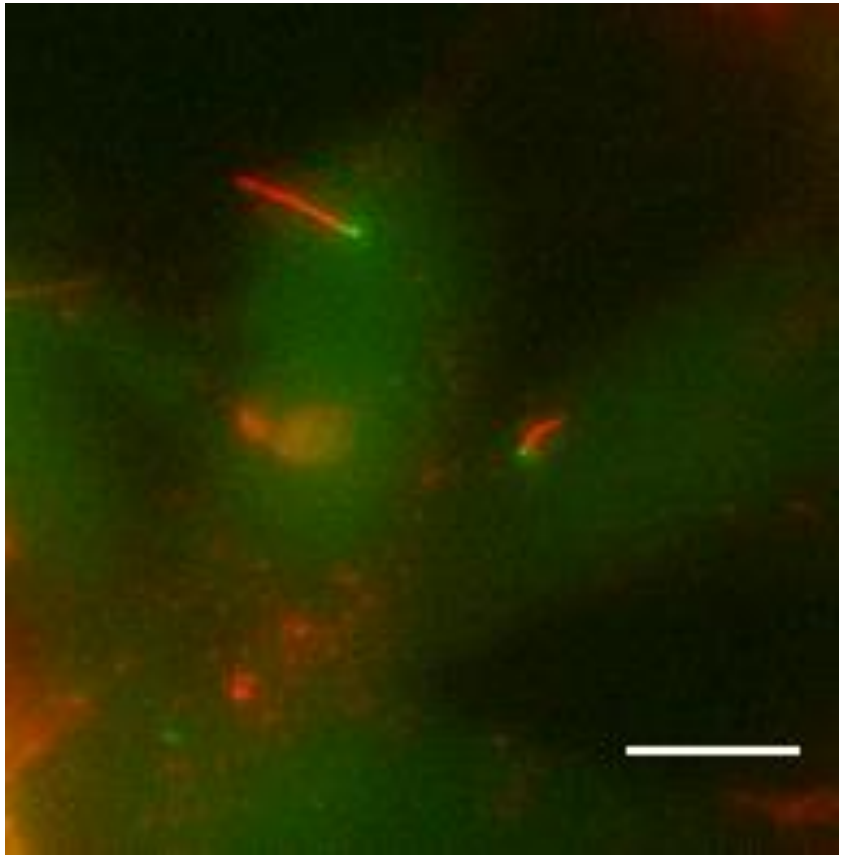
https://www.mskcc.org/videos/distribution-cilia-mouse-embryo



endothelial primary cilium

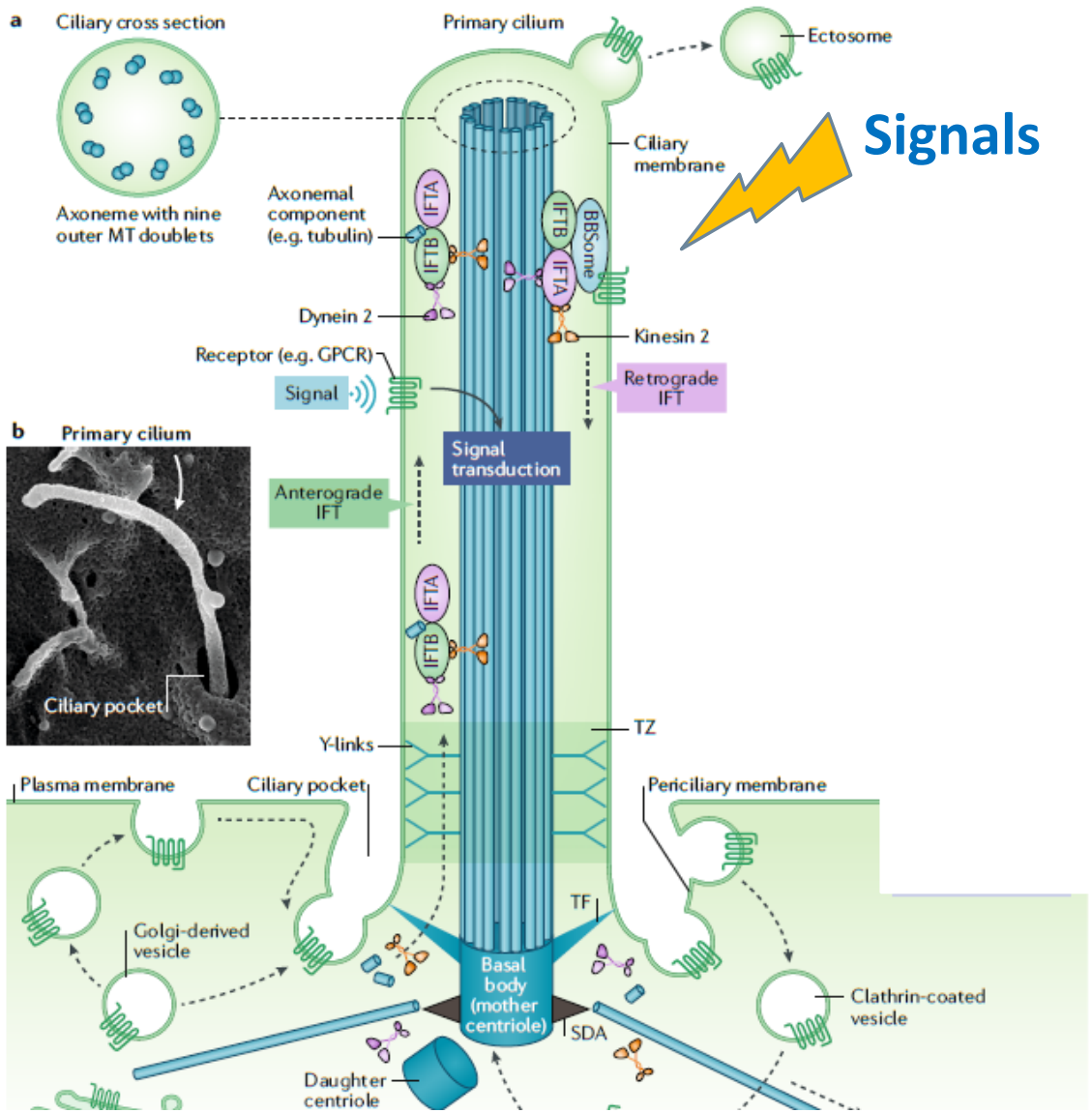
Leigh MW, Pittman JE, Carson JL, et al. Clinical and genetic aspects of primary ciliary dyskinesia/Kartagener syndrome. *Genet Med*. 2009;11(7):473-487. doi:10.1097/GIM.0b013e3181a53562





Gonçalves, J. and Pelletier, L.

Primary cilia signaling pathways



Signals

Wnt signaling pathway

cell fate determination, cell migration, cell polarity and organogenesis

- Inhibitor of adipogenesis
- Obesity
- Insulin resistance

Hh signaling pathway

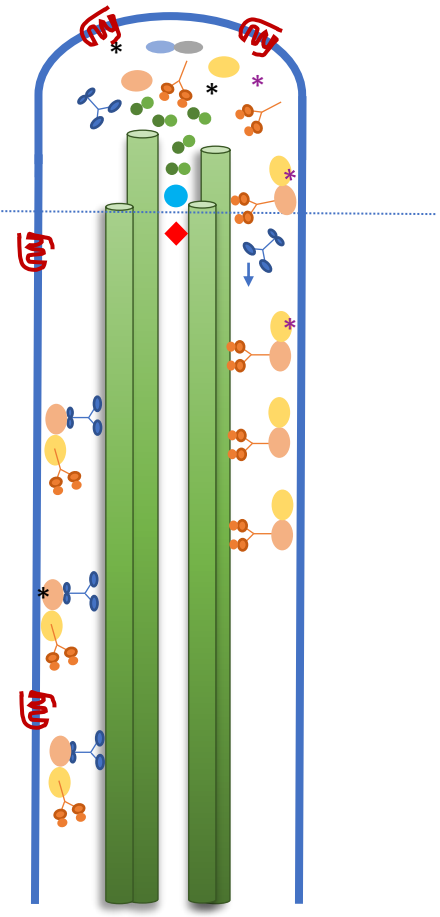
Major regulator of development

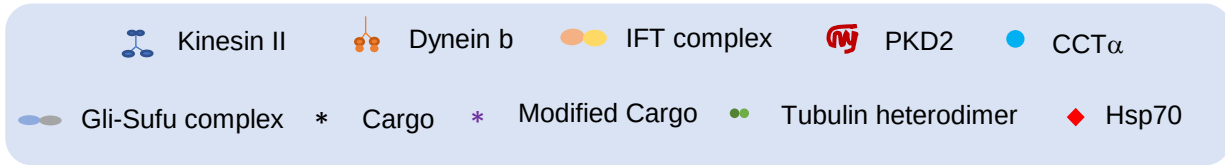
- Inhibitor of adipogenesis
- Energy homeostasis
- Oxidation of glucose

mTOR signaling pathway

Cell proliferation and metabolism regulation

- Glucose metabolism



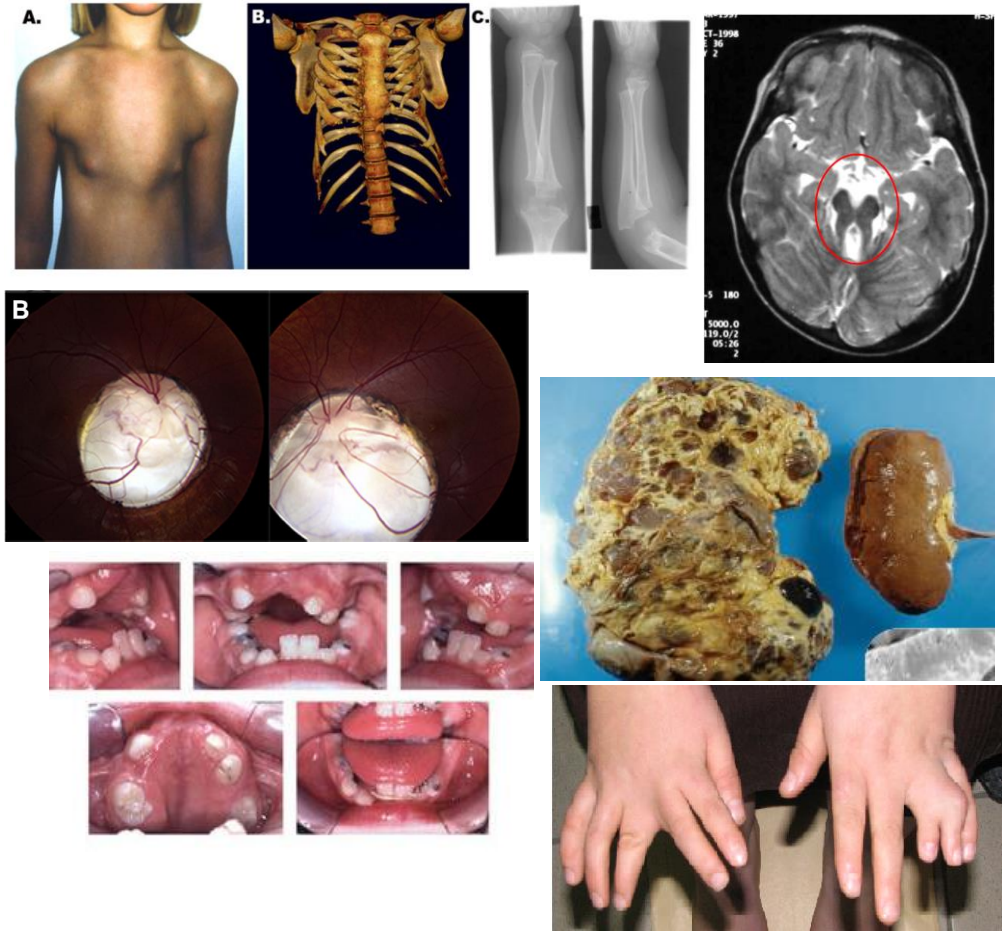


Anvarian, Z., Mykytyn, K., Mukhopadhyay, S., Pedersen, L. B., & Christensen, S. T. (2019). Cellular signalling by primary cilia in development, organ function and disease. *Nature reviews. Nephrology*, *15*(4), 199–219. https://doi.org/10.1038/s41581-019-0116-9

Soares H, Carmona B, Nolasco S, Viseu Melo L, Gonçalves J. Cilia Distal Domain: Diversity in Evolutionarily Conserved Structures. Cells. 2019 Feb 14;8(2). pii: E160. doi: 10.3390/cells8020160. & Rosenbaum, J.L. e Witman G.B. Nature Rev. Mol. Cell Biology ,2002, 3: 813-825

Ciliopathies

- Group of genetic disorders associated with cilia absence/dysfunction
- They are incapacitating chronic diseases and can, in several cases, lead to death
- Several systems can be affected, and ciliopathies can lead to:
  - Deafness
  - Chronic respiratory infections
  - Renal diseases
  - Cardiac diseases
  - Skeleton disorders
  - Infertility
  - Cognitive impairement
  - **Obesity**
  - **Blindness**



Ciliopathies

Although ciliopathies are rare (from a prevalence <1/1000000 - Jeune Asphyxiating Thoracic Dysplasia to a prevalence 1 to 2/1000 - Autosomal dominant polycystic kidney disease), some of the ciliopathies' phenotypes are very common in the general population

Bardet-Biedl Syndrome

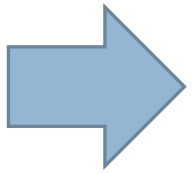
- Obesity and deficiencies in leptin and leptin receptor

Alstrom Syndrome

- Obesity and diabetes
- Retinal dysfunction

Joubert Syndrome

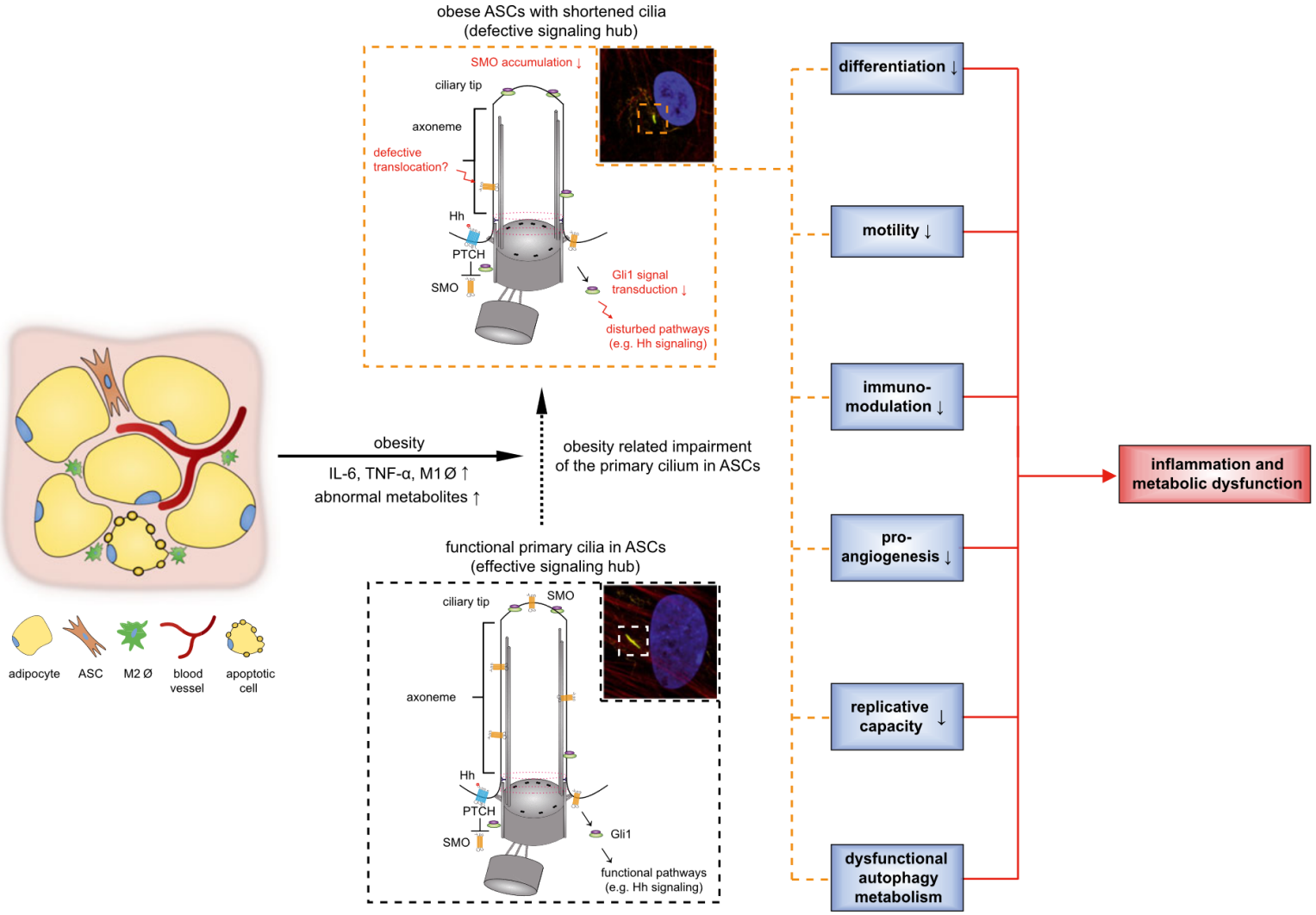
- Retinal dystrophy



Mild defects in cilia may play a role on the onset of more common disorders

Obesity and Cilia

- Leptin signalling in hypotalamus, regulating hunger and saciety
- Adipose-derived mesenchymal stem cells proliferation and differentiation



Ritter, Andreas, Frank Louwen, and Juping Yuan. “Deficient Primary Cilia in Obese Adipose-Derived Mesenchymal Stem Cells: Obesity, a Secondary Ciliopathy?” Obesity Reviews 19, no. 10 (October 1, 2018): 1317–28. https://doi.org/10.1111/obr.12716.

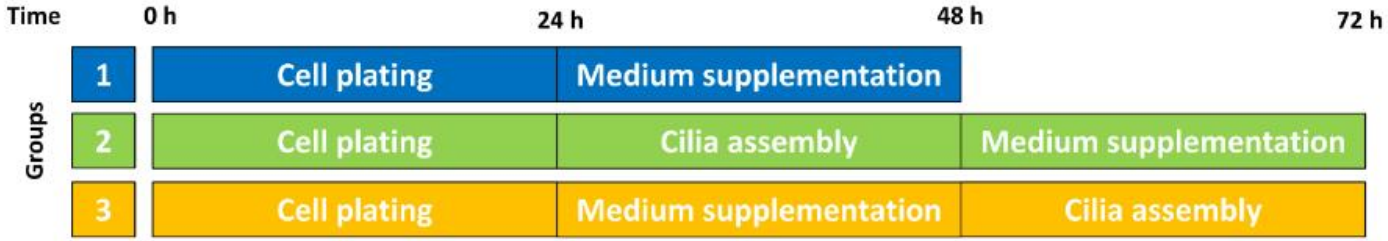
Aims

Determine the functional relevance of cilia in the cellular response to high glucose levels

Using Retinal Pigment Epithelium Cells:

- Assess the impact of high glucose levels on cilia assembly and morphology
- Study the role of cilia in the celullar response to glucose-induced stress
- Understand how the cilia-associated signaling pathways can be involved

Experimental setup

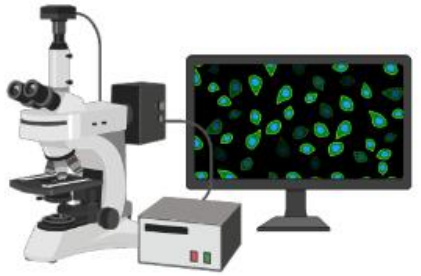


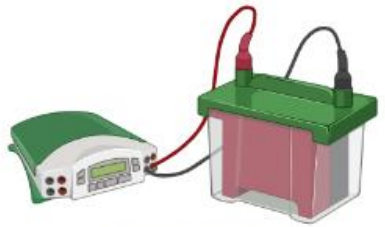
Medium supplementation:

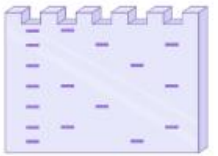
- 0 mM Glucose
- 5 mM Glucose
- 25 mM Glucose
- 5 mM Glucose + 20 mM Mannitol

Cilia assembly:

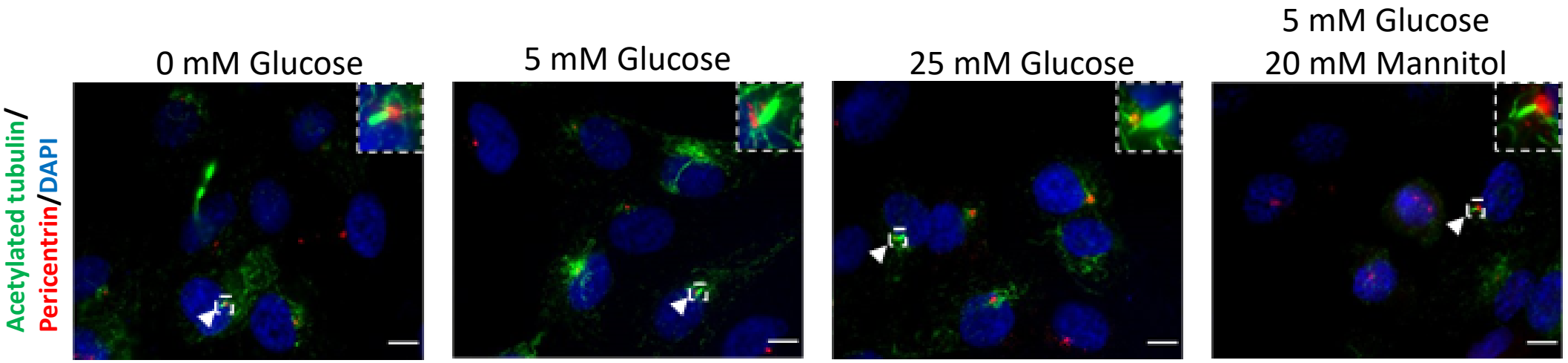
- Serum removal

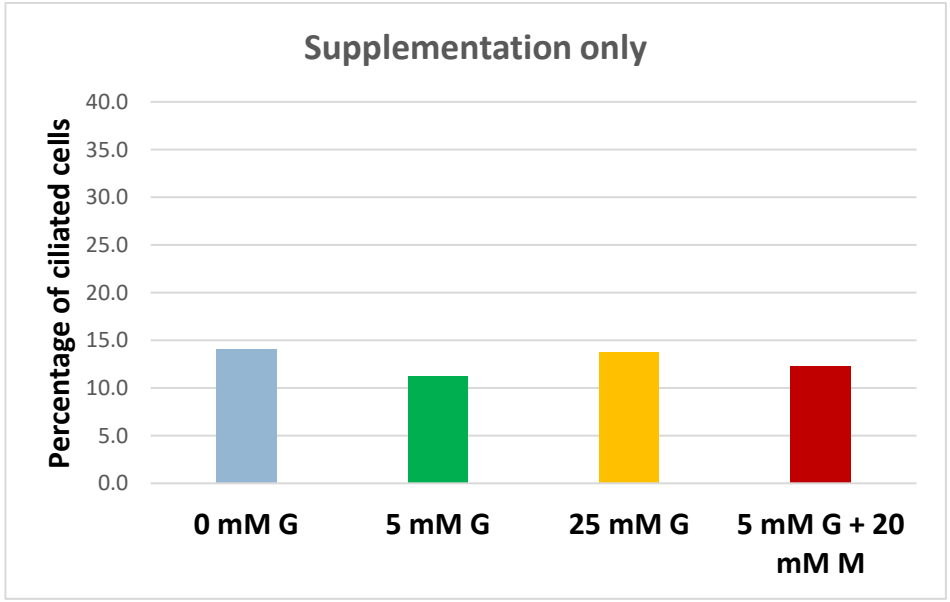


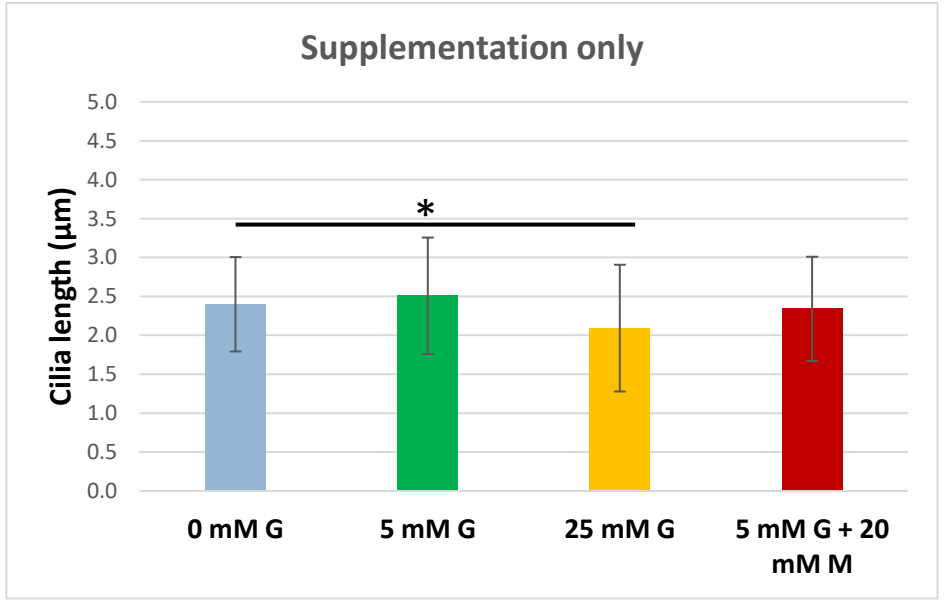




High glucose levels decrease cilia length



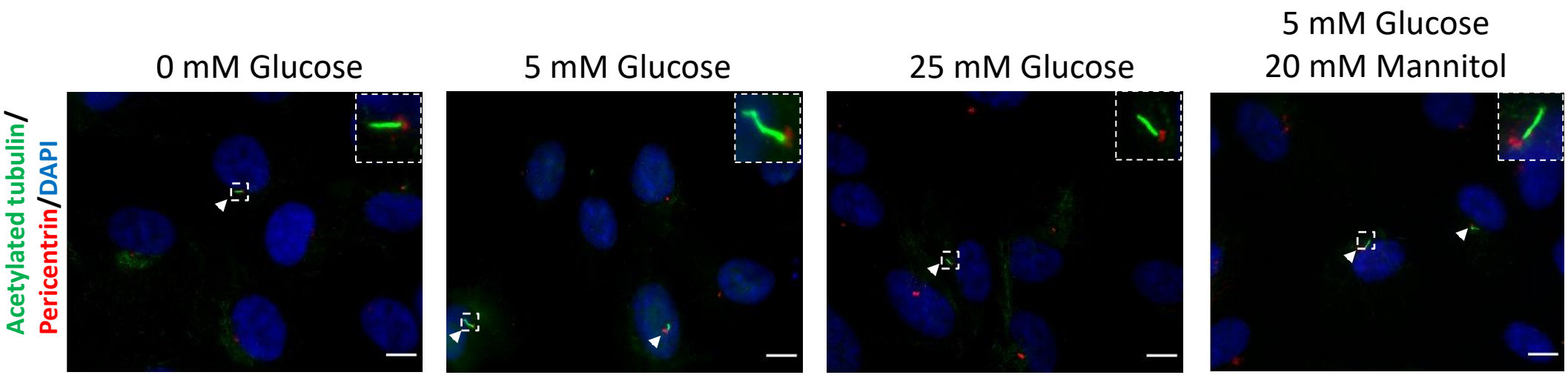


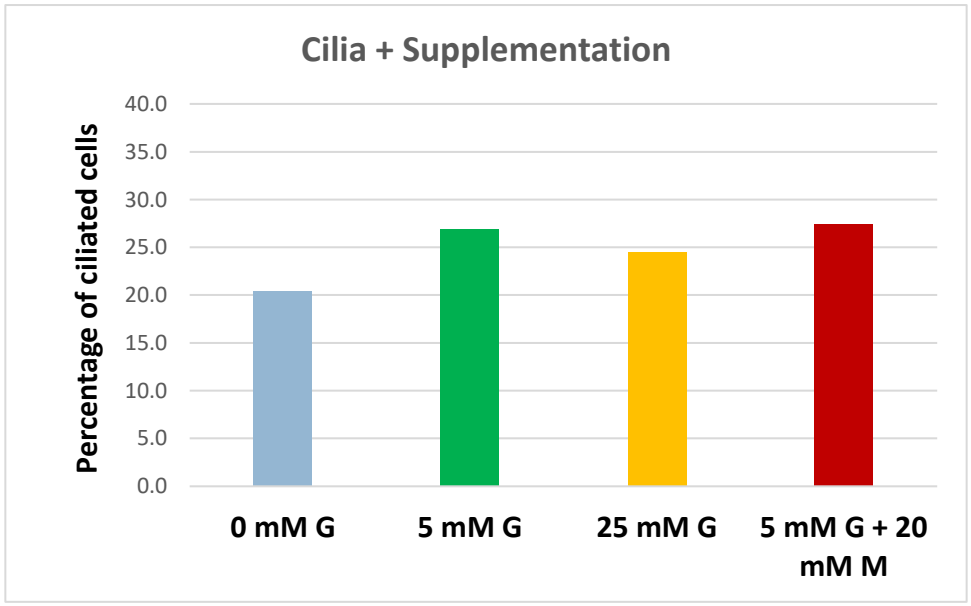


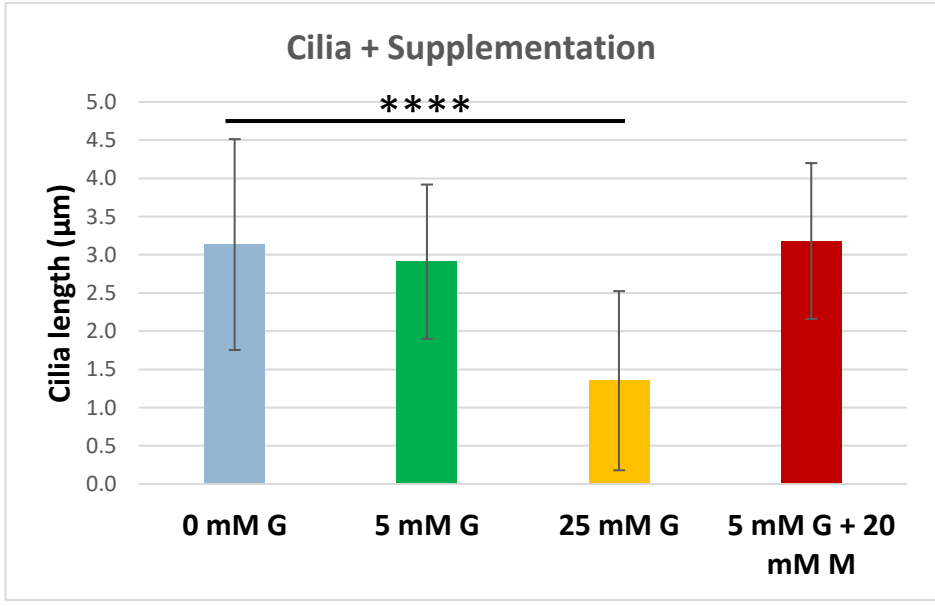
G – Glucose
M - Mannitol

* p < 0,05

High glucose levels decrease cilia length



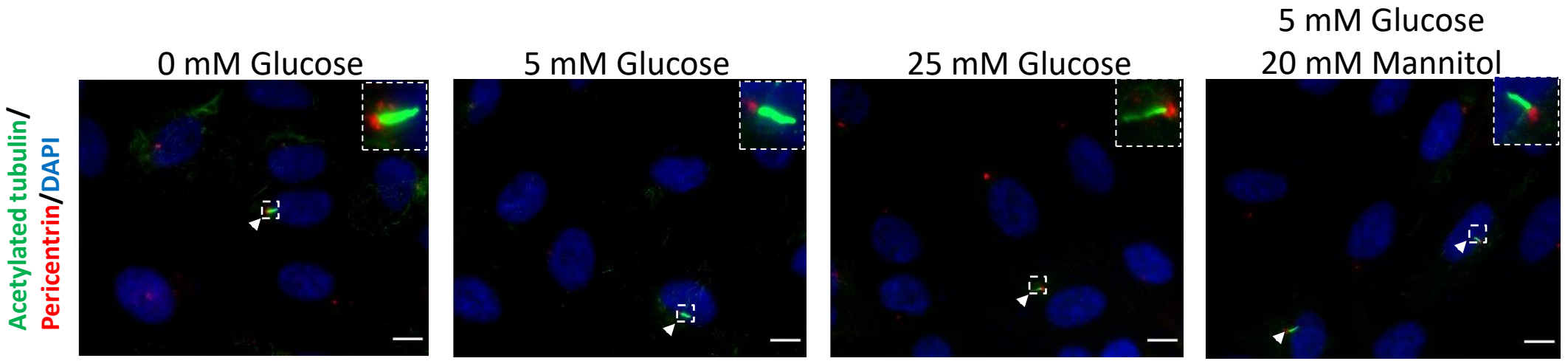


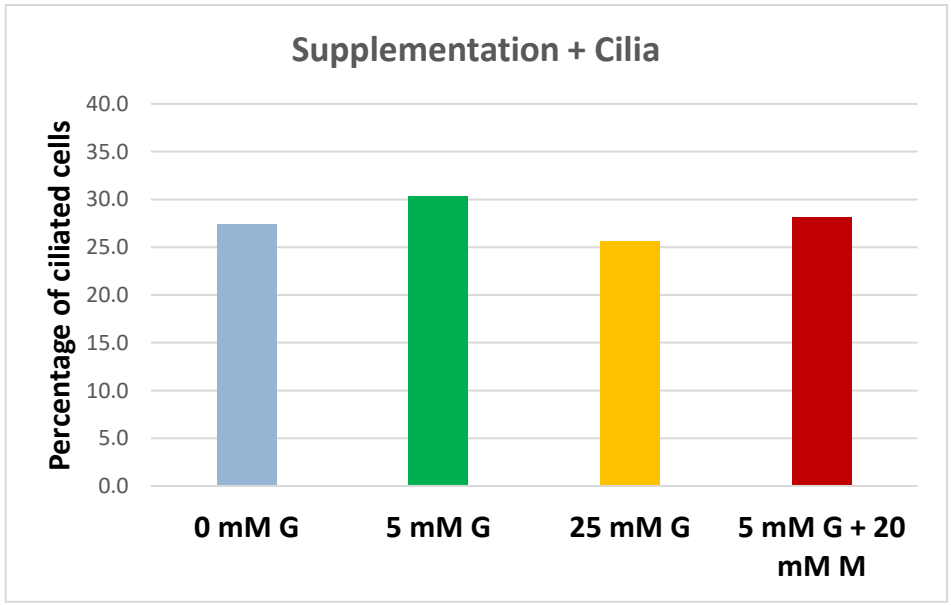


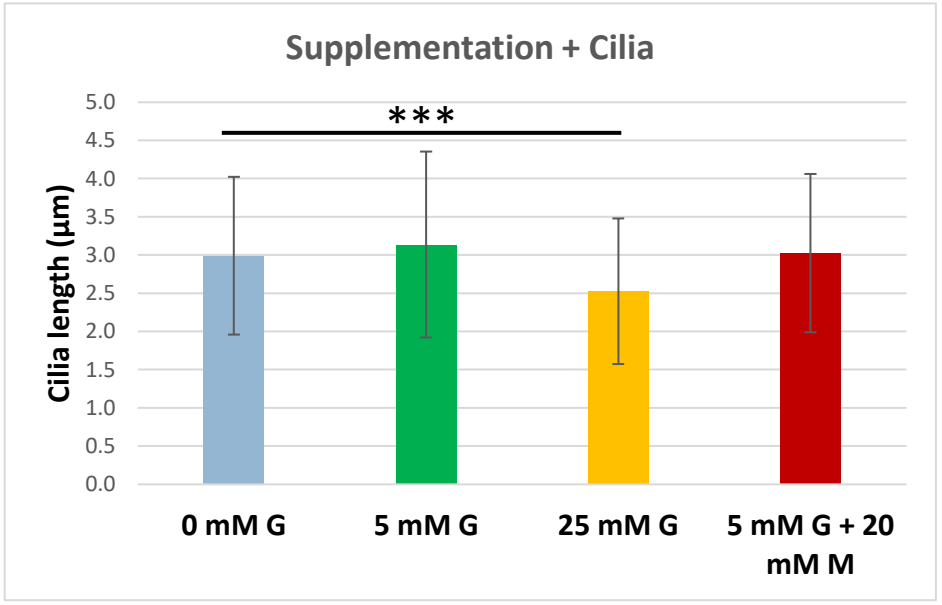
G – Glucose
M - Mannitol

**** p < 0,0001

High glucose levels decrease cilia length





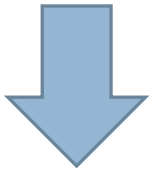


G – Glucose
M - Mannitol

*** p < 0,001

Short Conclusions (1)

High glucose levels lead to a decrease in cilia length without affecting the number of ciliated cells



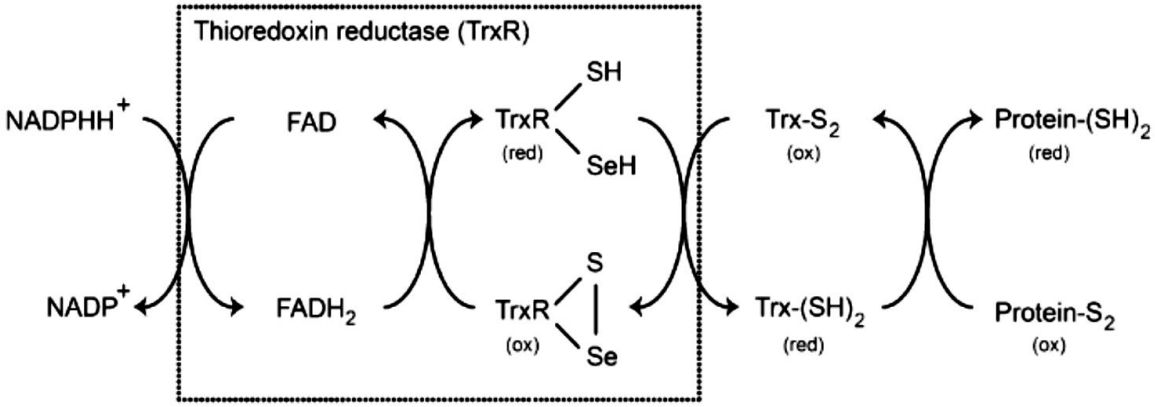
Is the ciliary signaling compromised?

Thioredoxin reductase 1 (TRXR1)

- Increase in glucose levels leads to reactive oxygen species (ROS) production in retina (Chen et. al., 2019)
- In diabetic retinopathy there is an increase in ROS production.

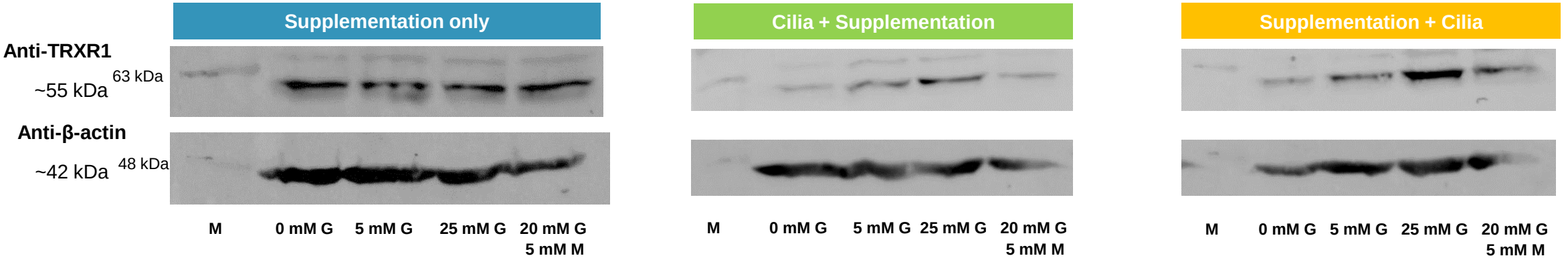
Thioredoxin reductase 1 (TRXR1)

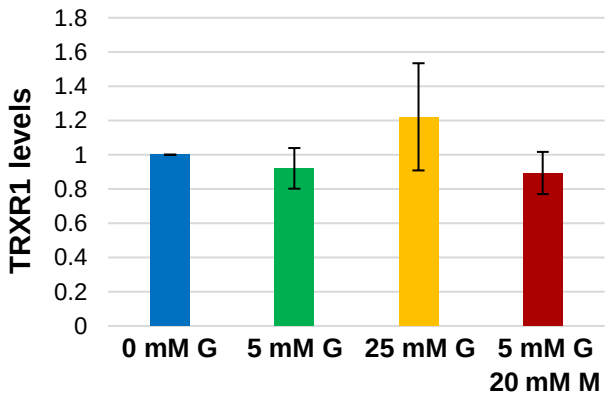
- is a selenoprotein that protects cells against oxidative damage
- localizes in cytoplasm, nucleus and membranes and has as substrate **thioredoxin 1 (TRX1)**
- is essential to elimination of glucose-derived H_2O_2 (Peng et. al., 2014)
- has increased levels in response to high levels of glucose in endotelial cells (Patel et. al., 2013)
- supresses insulin responsiveness, anabolic metabolism and adipocyte differentiation (Peng et. al., 2016)

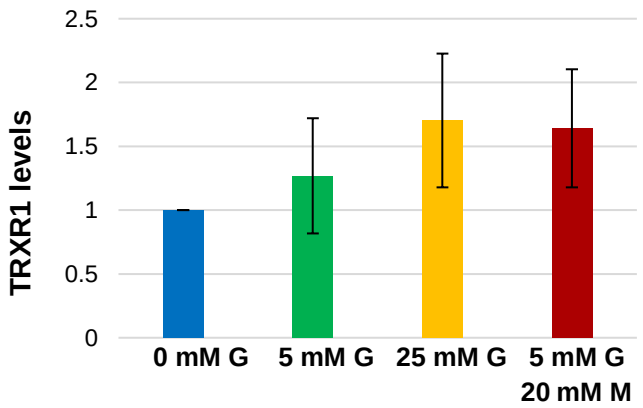


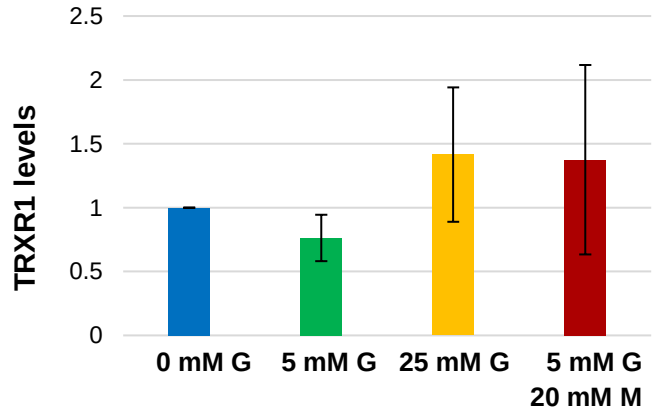
TRXR1/TRX1 impairment → **Cancer, neurodegenerative, cardiovascular and metabolic disorders (Tinkov et.al., 2018)**

TRXR1 levels maintain in response to high glucose in the presence/absence of cilia



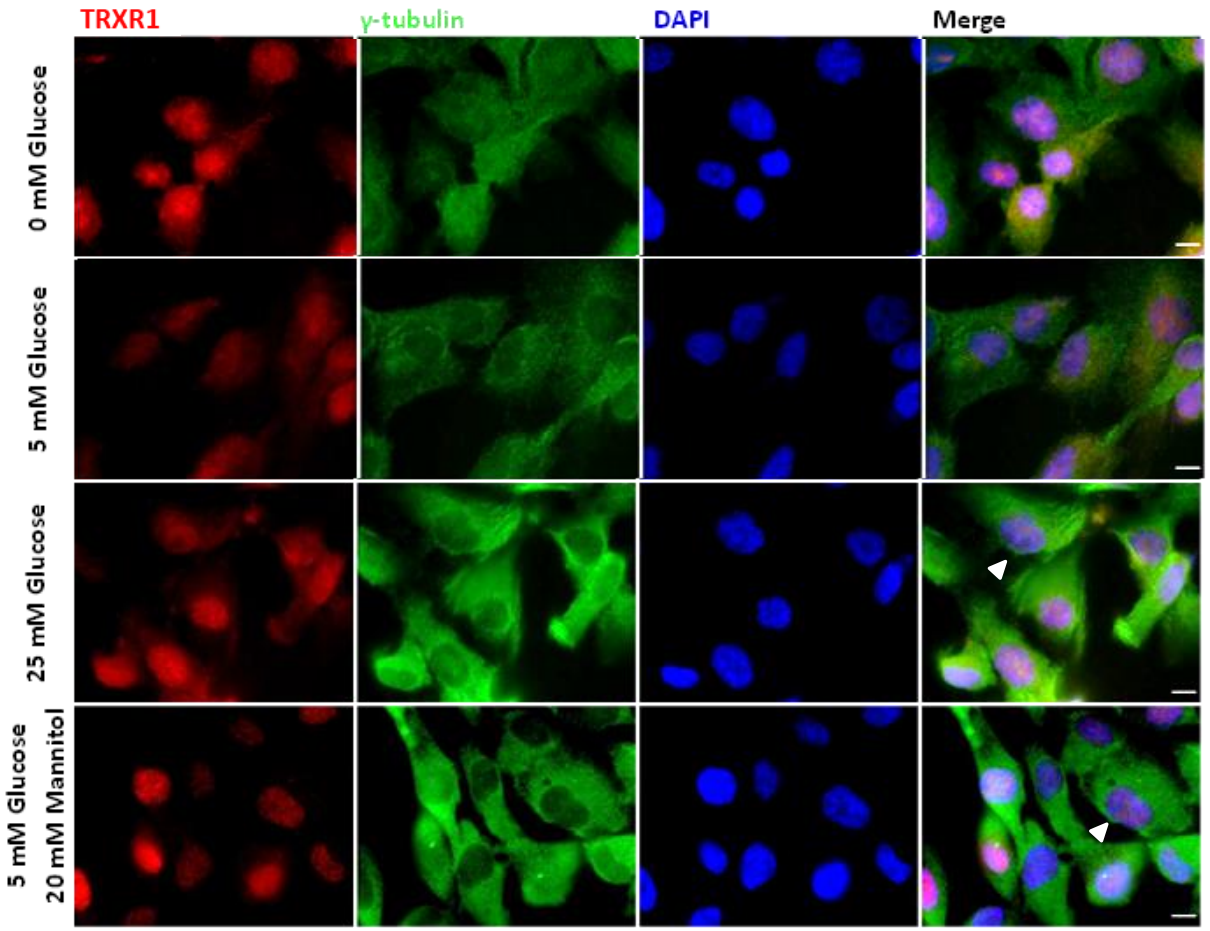




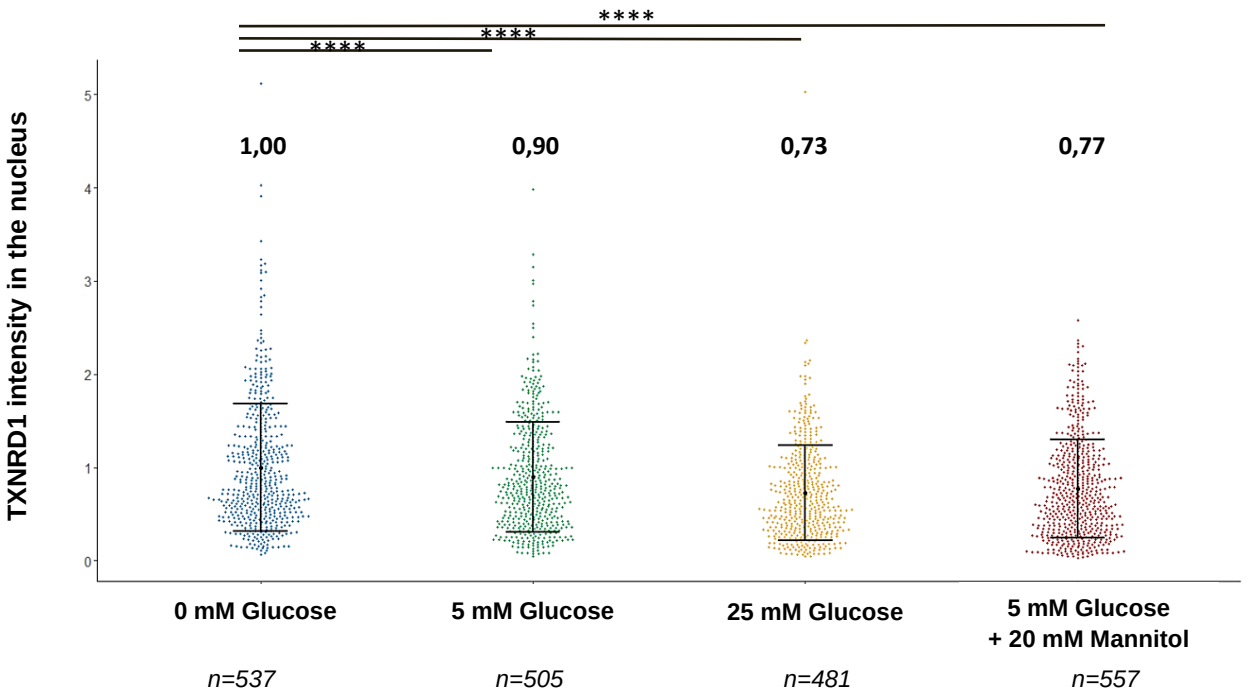


G – Glucose
M - Mannitol

High glucose decreases TRXR1 nuclear levels

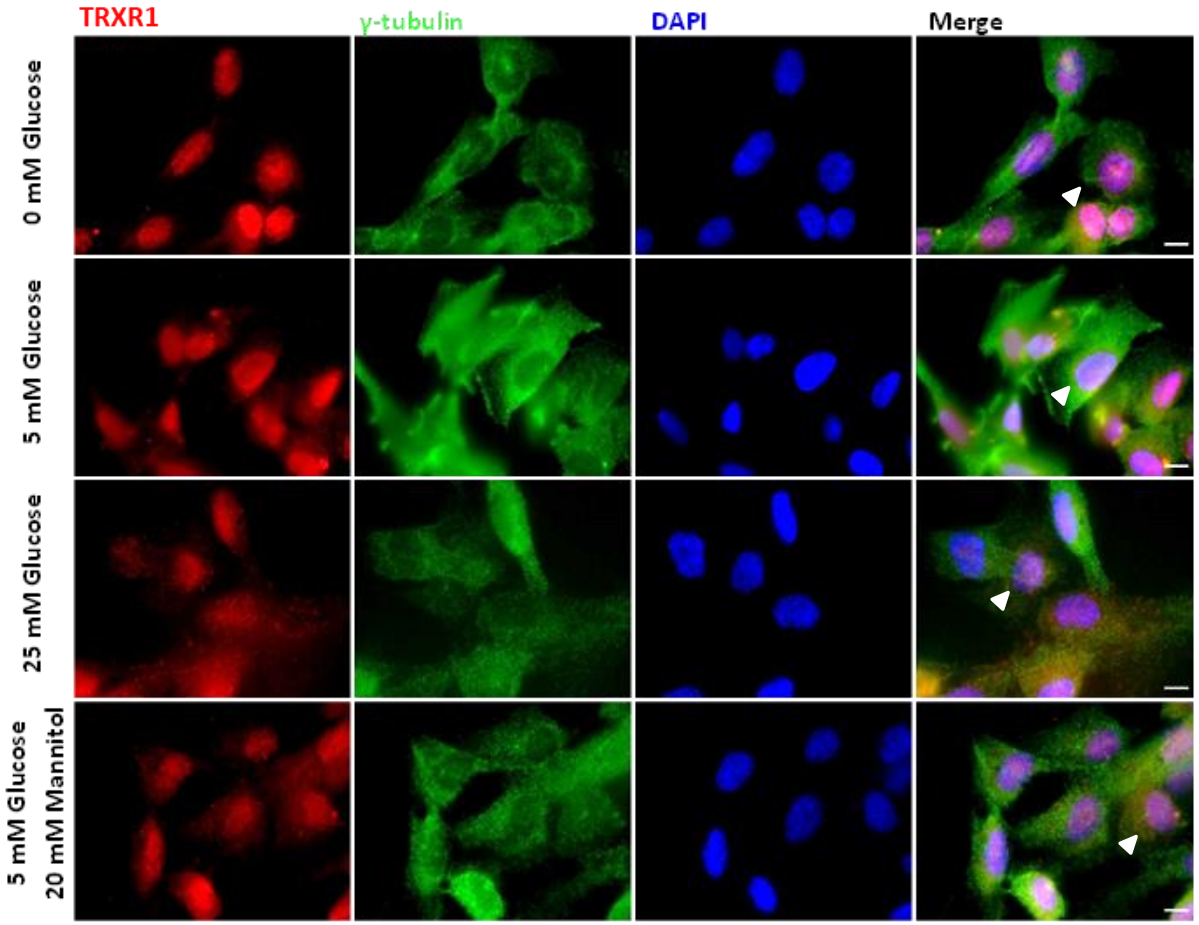


Supplementation only

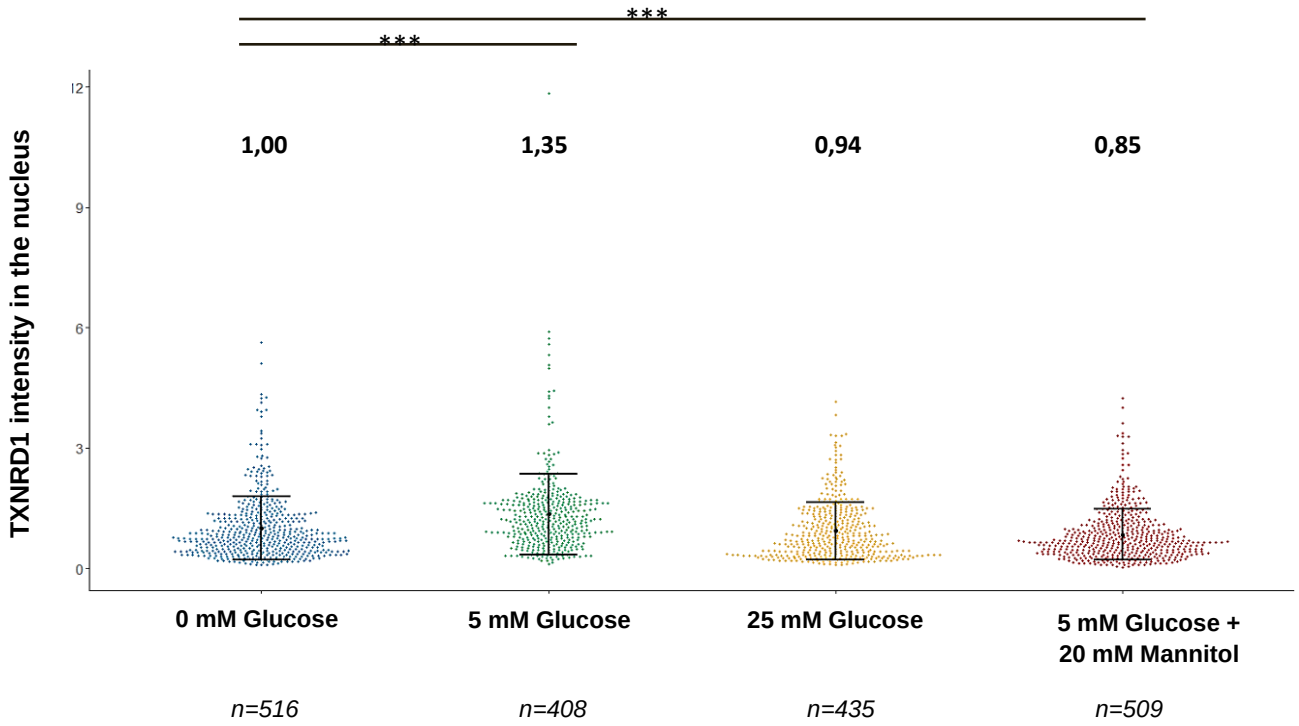


**** p < 0,0001

Cilia modulate TRXR1 nuclear transport in response to glucose levels

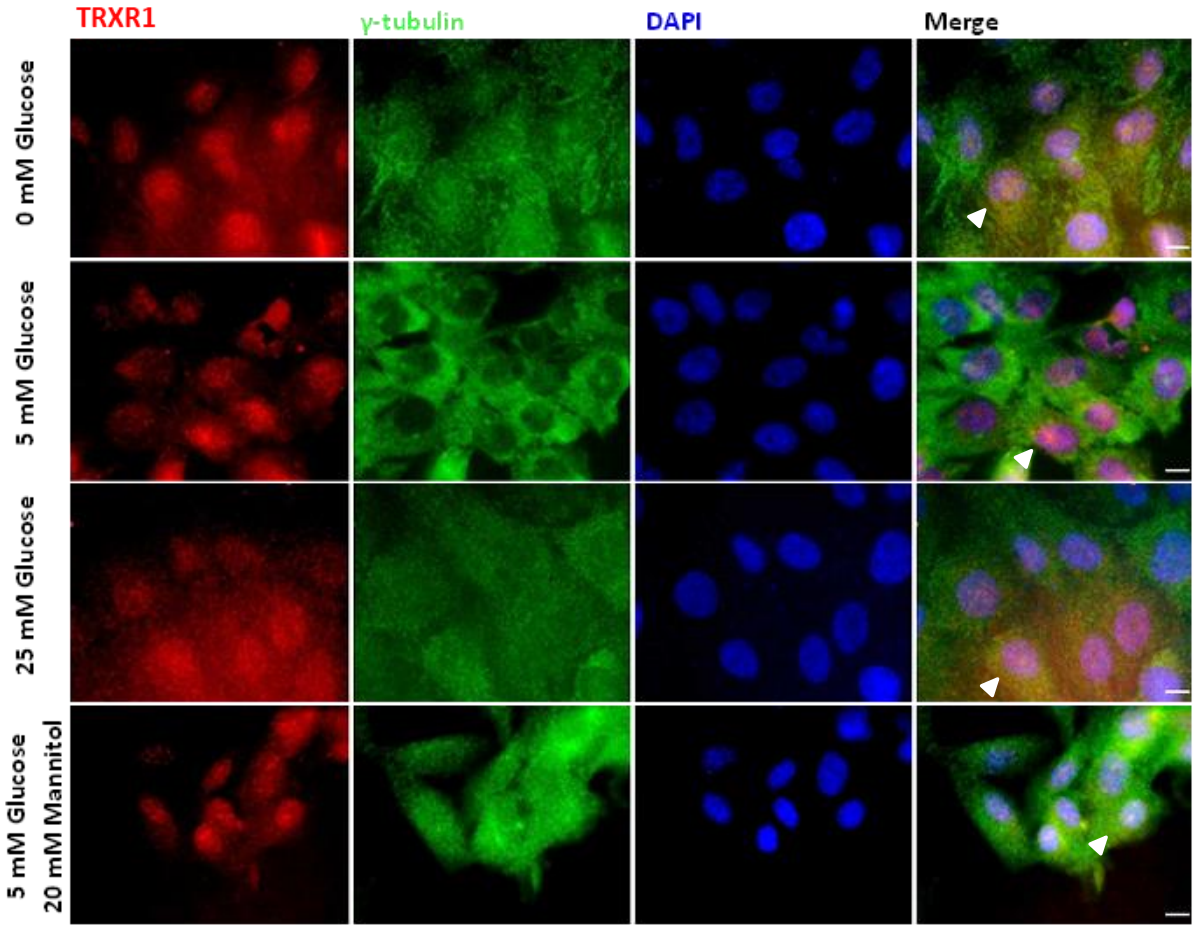


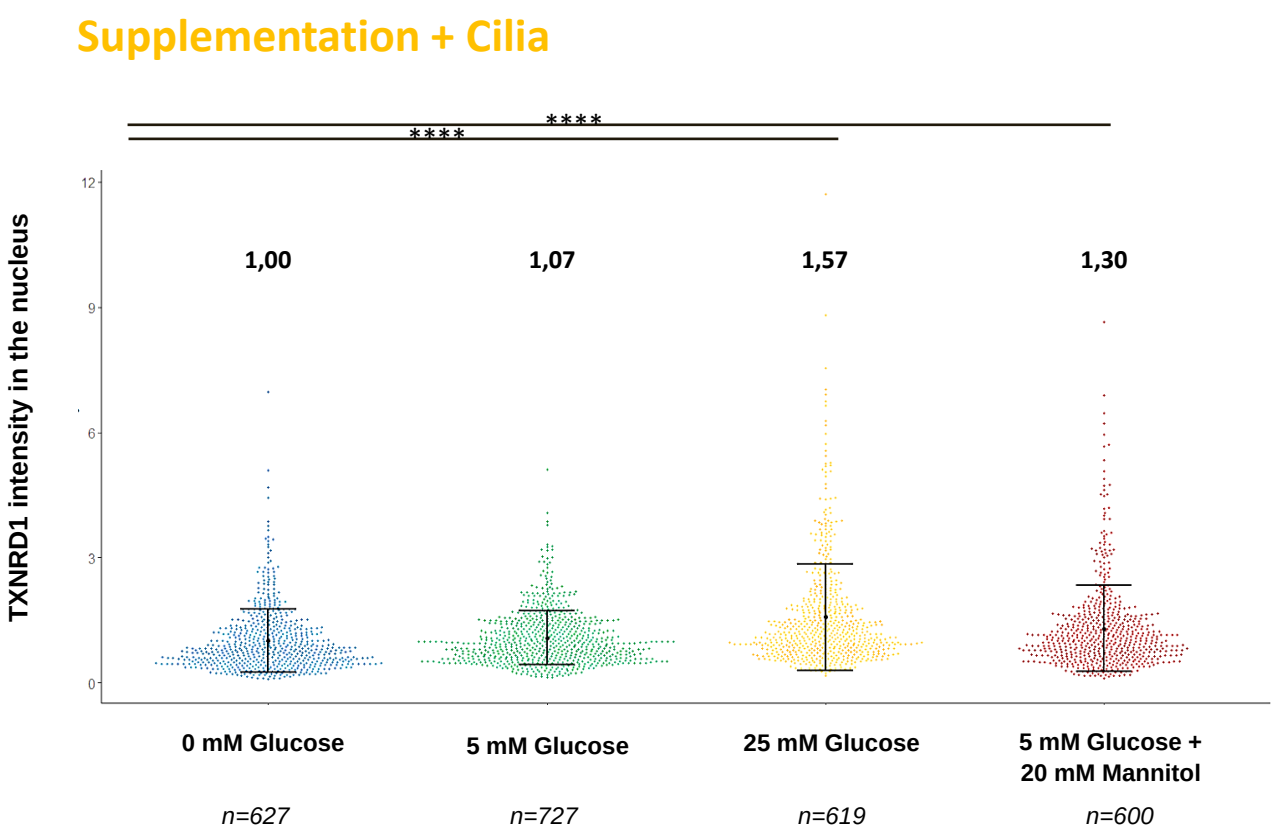
Cilia + Supplementation



*** p < 0,001

Cilia modulate TRXR1 nuclear transport in response to glucose levels





**** p < 0,0001

Short Conclusions (2)

Thioredoxin Reductase 1 levels are not affected by high glucose levels

Thioredoxin Reductase 1 has lower levels in the cell's nucleus in response to high glucose levels

When cells present cilia, either before or after glucose supplementation, Thioredoxin Reductase 1 translocates to the nucleus in high glucose levels

In fact… (2)

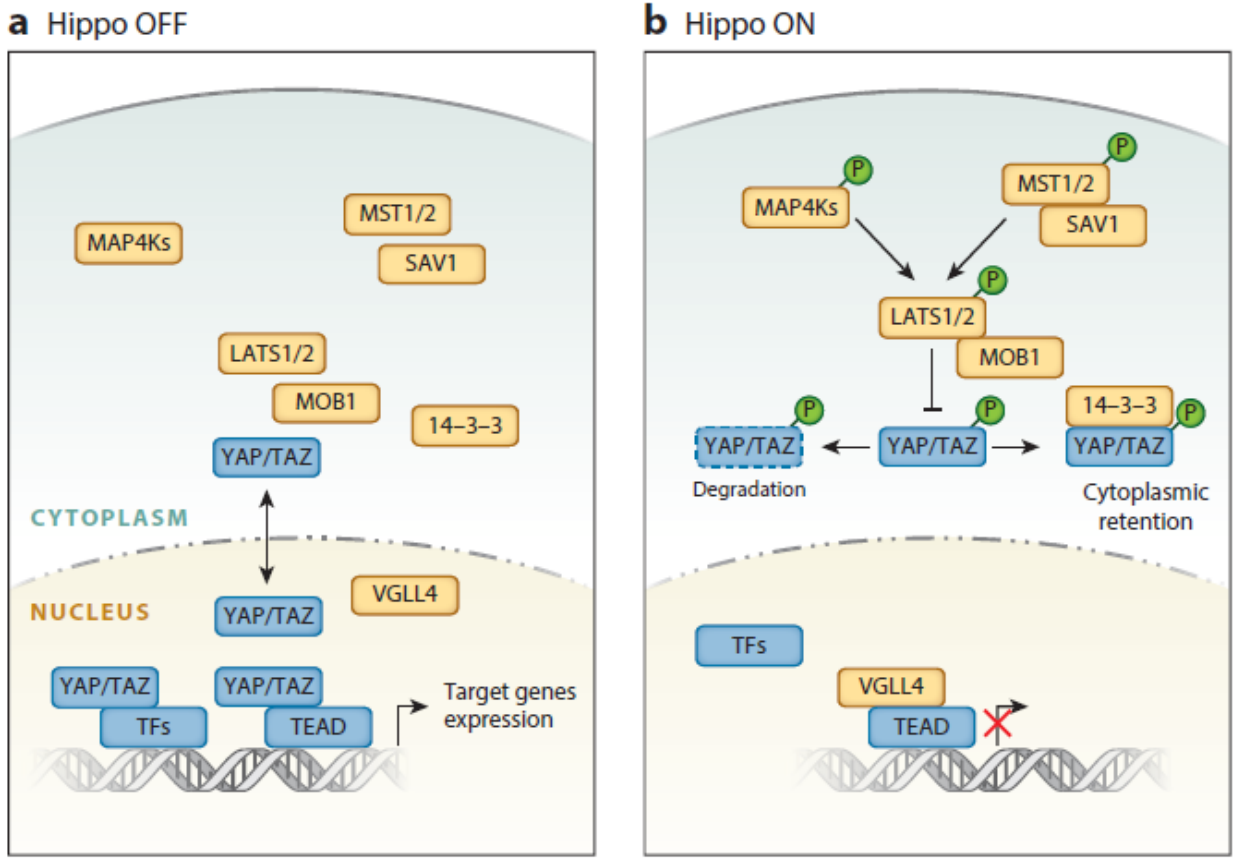
"Thioredoxin reductase 1 suppresses adipocyte differentiation and insulin responsiveness" Peng et al., 2016

Hippo Pathway - YAP

Hippo pathway has a major role in regulating many fundamental biological processes, namely cell proliferation and apoptosis

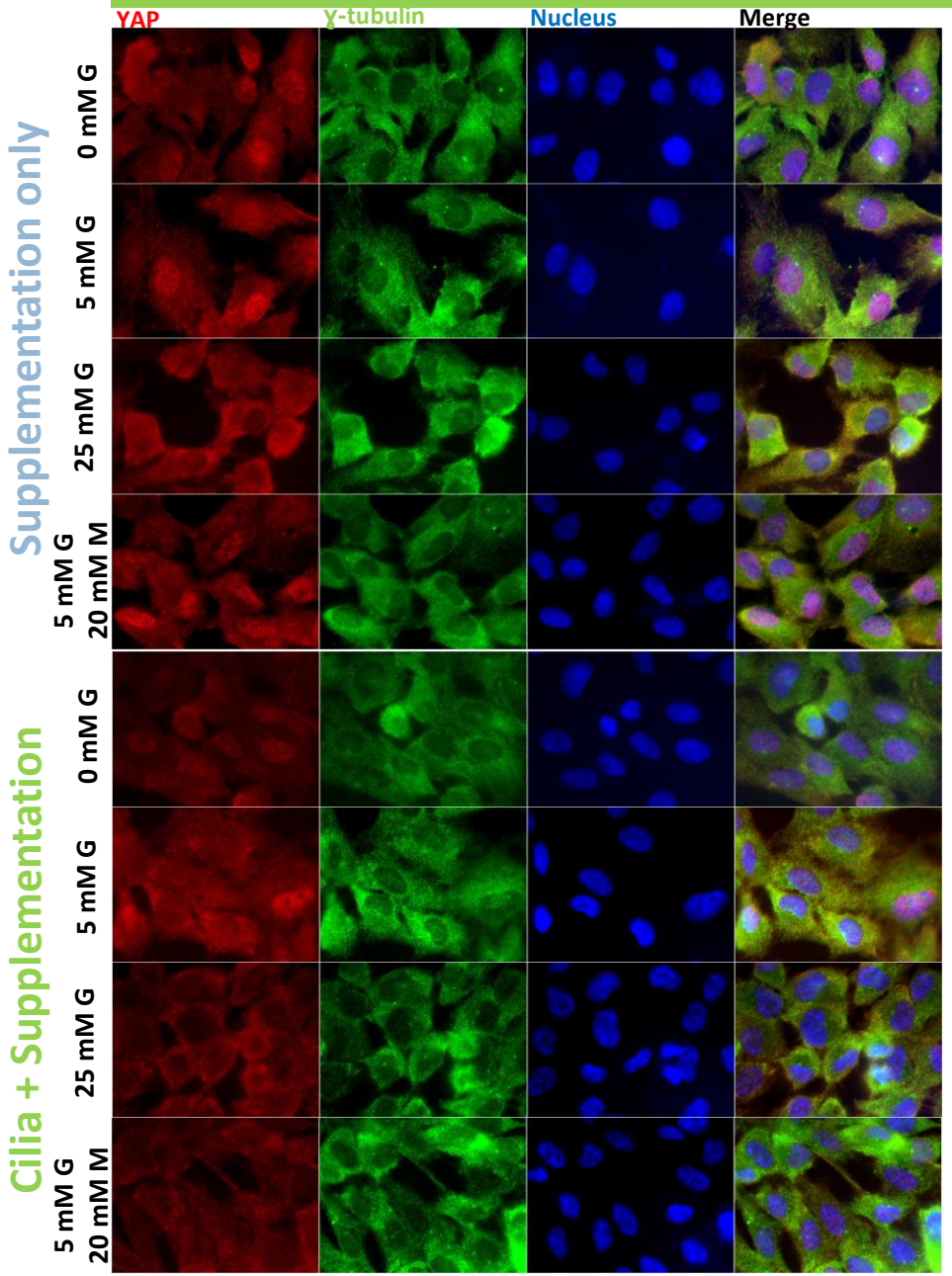
YAP

- Transcriptional regulator that does not contain DNA-binding domains.
- Once activated, the Hippo pathway limits tissue growth and cell proliferation by phosphorylating and inhibiting YAP/TAZ.
- In contrast, when the Hippo pathway is off, YAP/TAZ are dephosphorylated and translocated into the nucleus
- Induce transcriptional programs important for cell proliferation, survival, and migration.

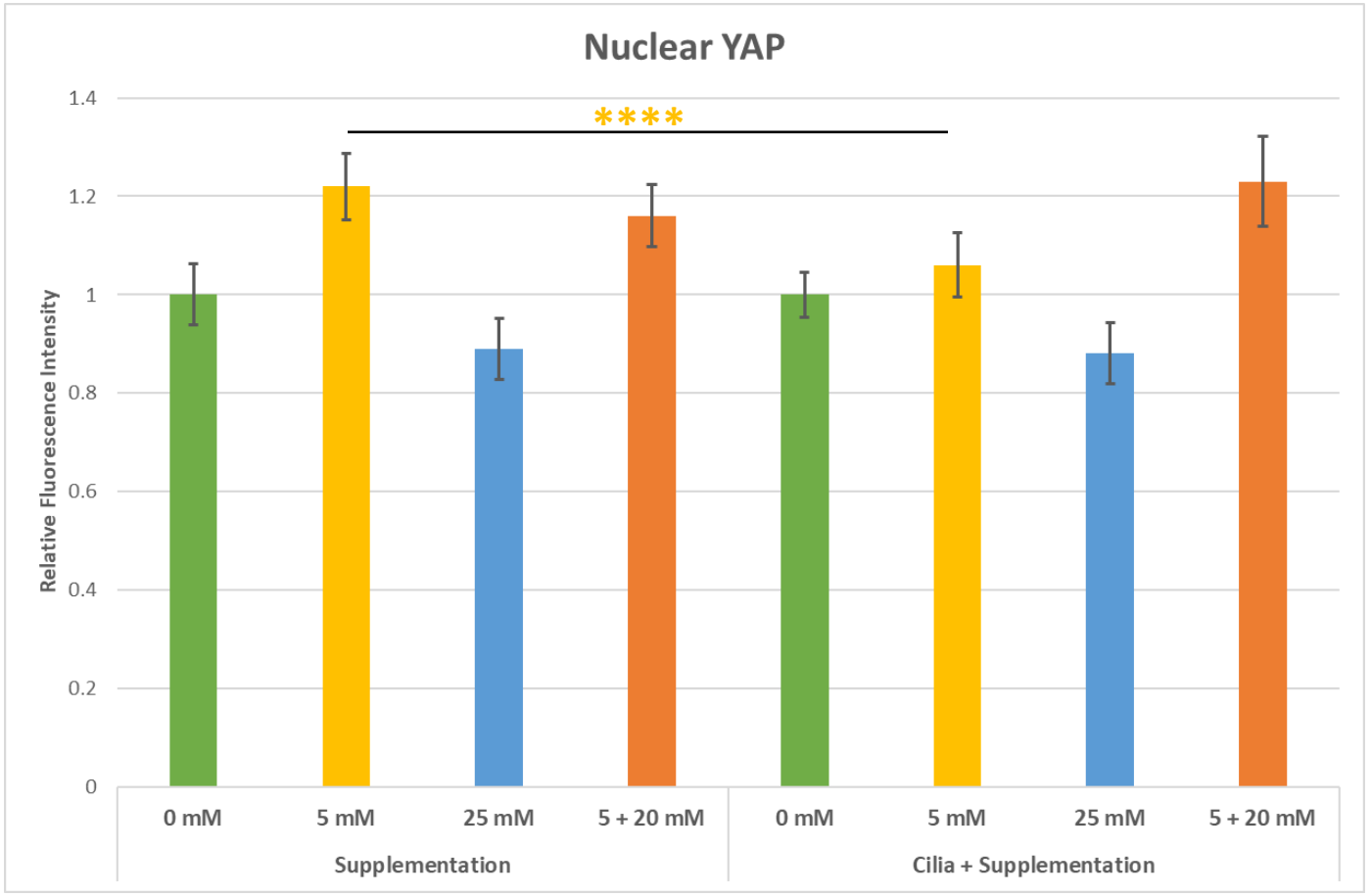


Ma, Shenghong, Zhipeng Meng, Rui Chen, and Kun-Liang Guan. “The Hippo Pathway: Biology and Pathophysiology.” Annual Review of Biochemistry 88, no. 1 (2019): 577–604. https://doi.org/10.1146/annurev-biochem-013118-111829.

High glucose decreases YAP nuclear levels



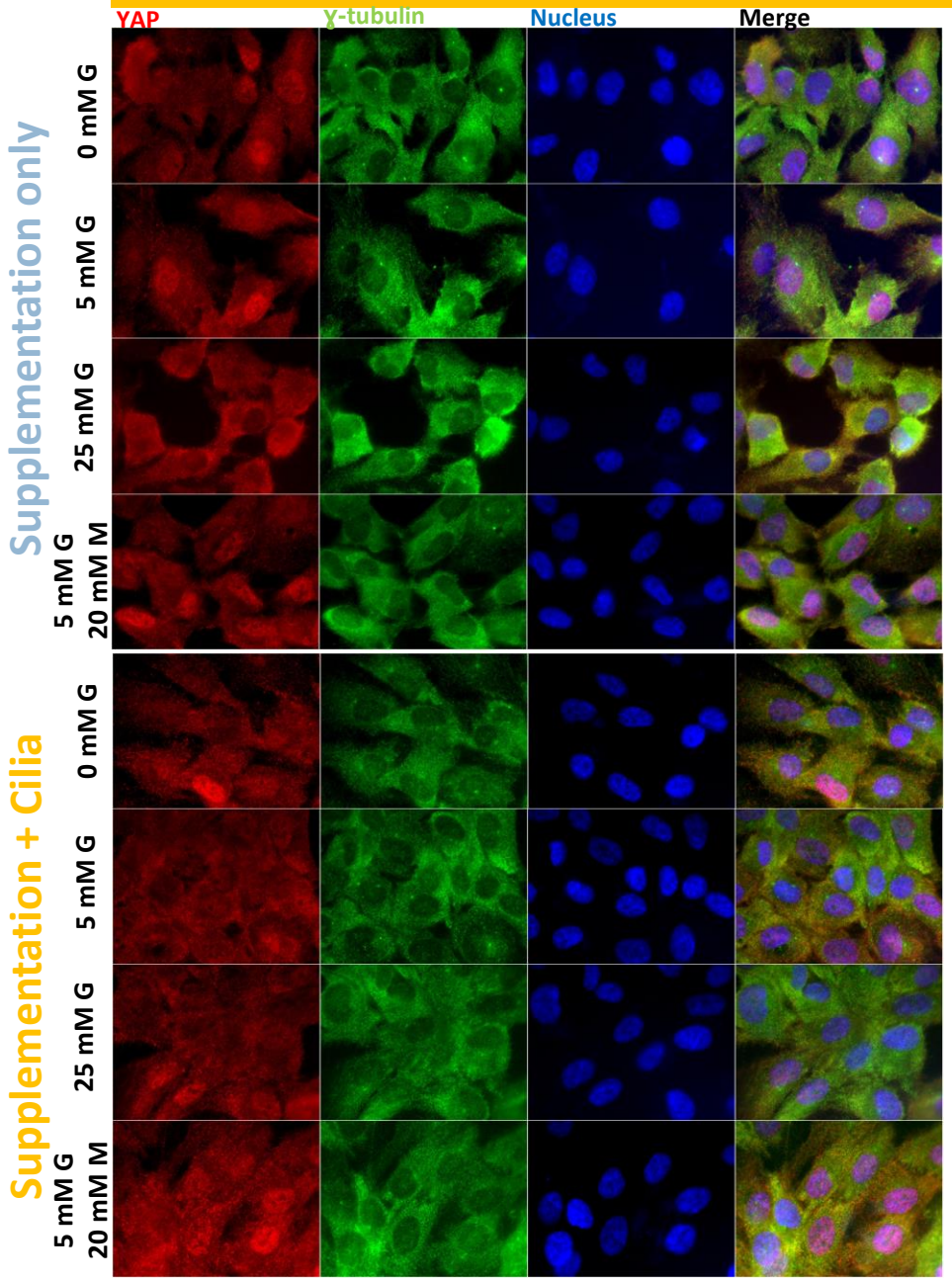
Cilia + Supplementation



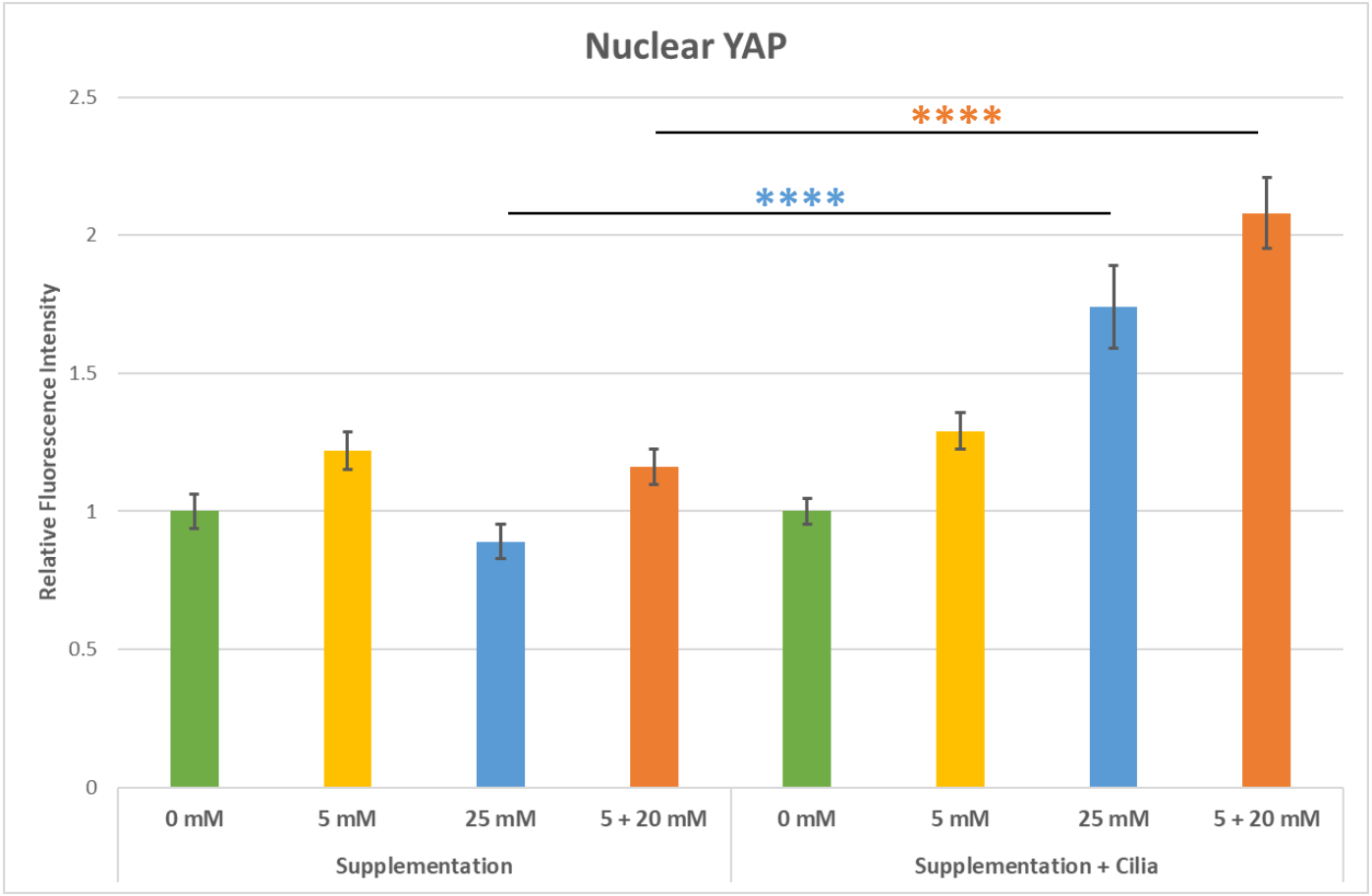
G – Glucose
M - Mannitol

**** p < 0,0001

Cilia assembly affects YAP nuclear levels



Supplementation + Cilia



G – Glucose
M - Mannitol

**** p < 0,0001

Short Conclusions 3

YAP has lower levels in the cell's nucleus in response to high glucose levels

When cells present cilia after glucose supplementation YAP translocates to the nucleus

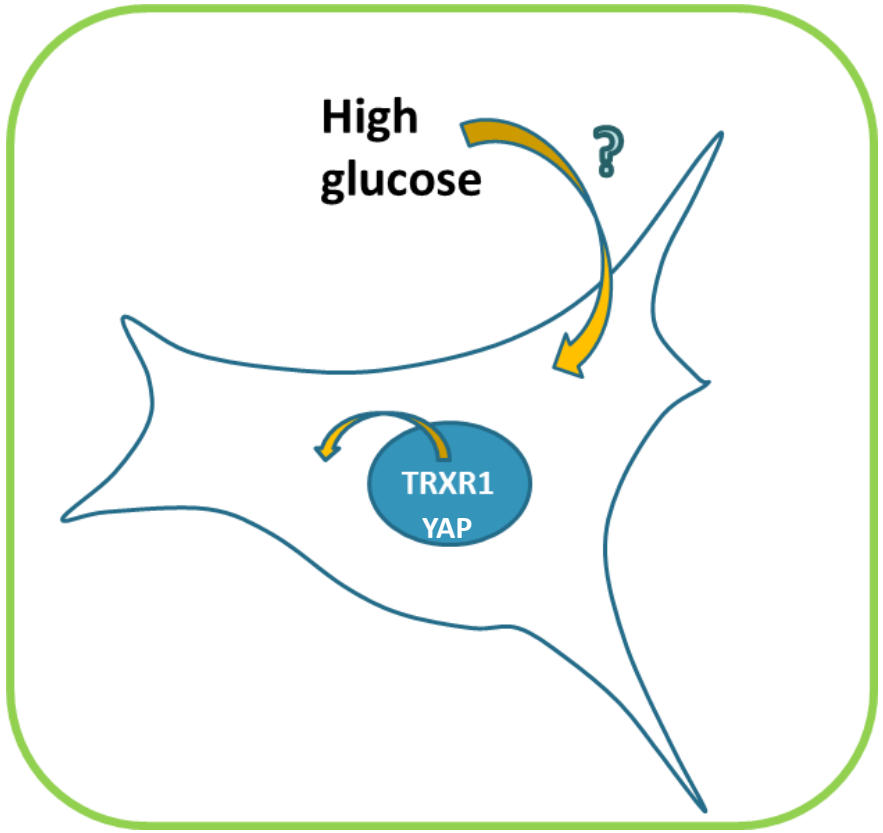
In fact… (3)

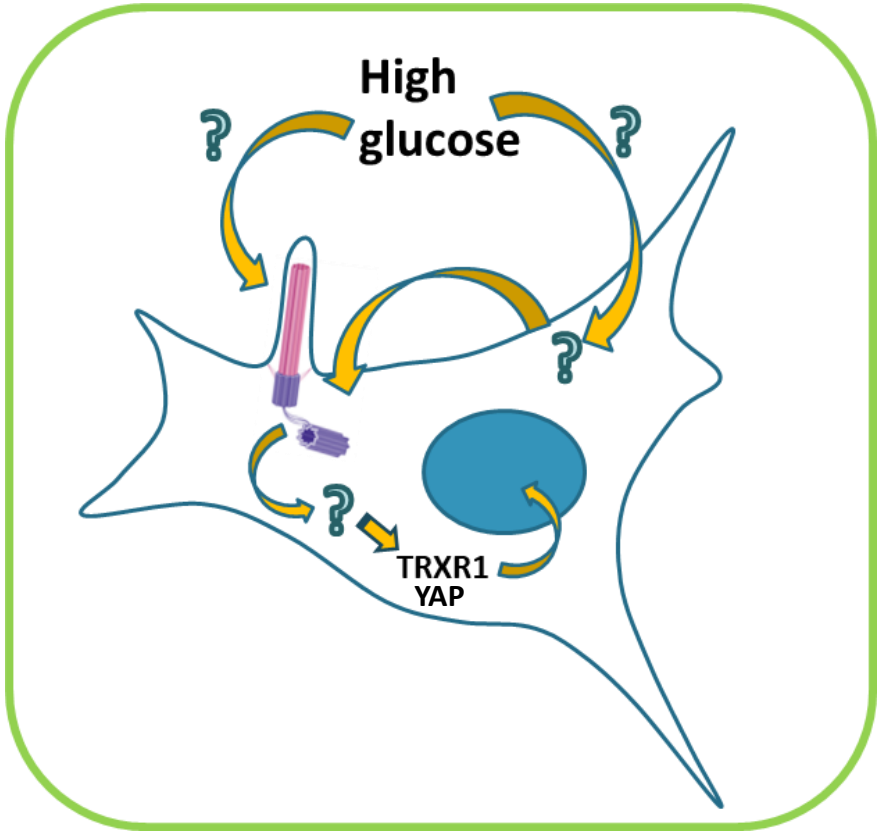
"Adipogenesis was attenuated in 3T3-L1 cells stably expressing Bcl-2 or YAP" Chang et al., 2017

"Overexpression of YAP remarkably induced β cell proliferation…" & "The small redox proteins thioredoxin-1 and thioredoxin-2 (Trx1/2) were upregulated by YAP…"
Yuan et al., 2016

In Summary

The presence of cilia modulates cells' response to high glucose levels by shifting TRXR1 and YAP localization to the nucleus

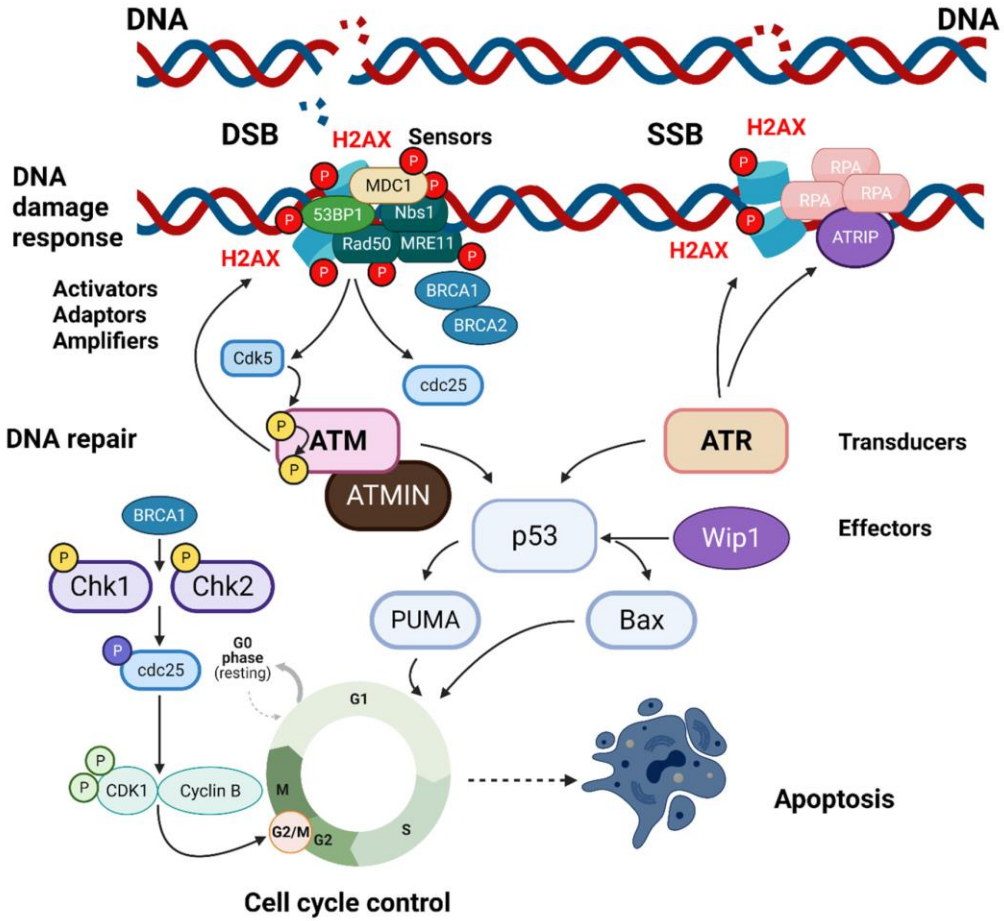




What are TRXR1 and YAP doing in the nucleus?

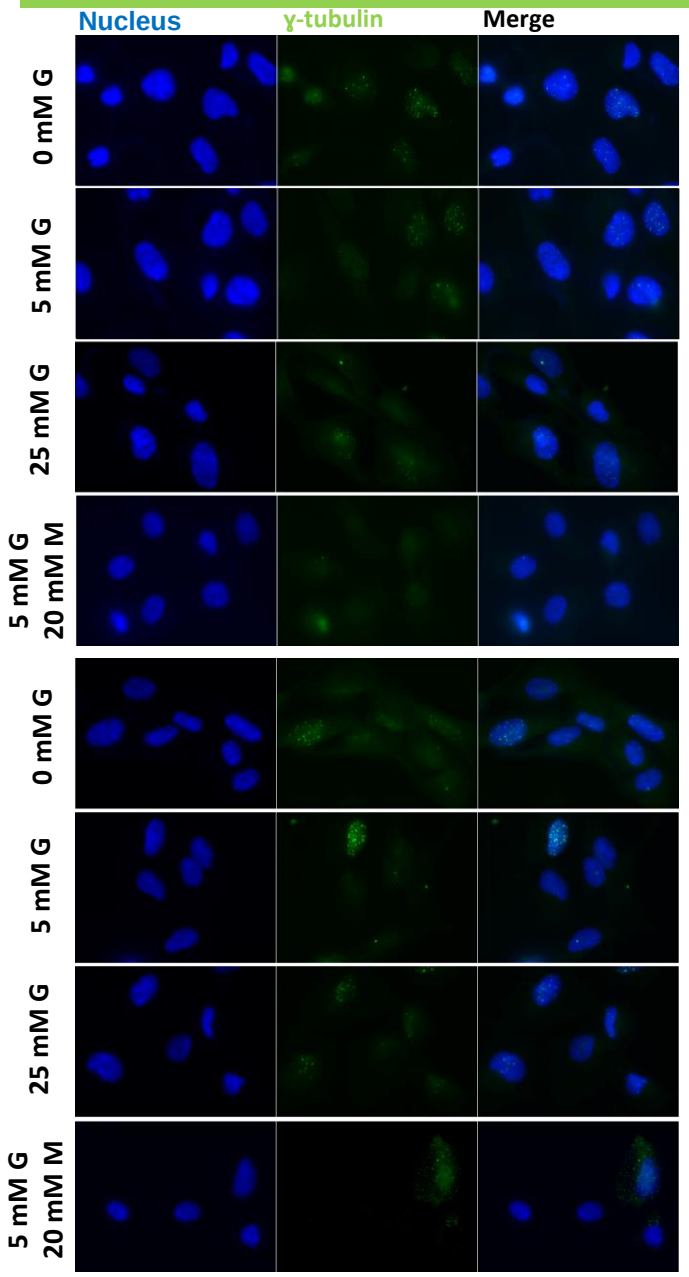
γ-H2AX

- Variant of the histone H2A
- represents 2.5–25% of H2A in the total mammalian genome and is specifically phosphorylated in response to DNA damage
- H2AX has a primary role in the repair of DNA DSBs, but it also intervenes in the mending of SSBs
- **Histone γ-H2AX is the second most common marker of cellular senescence after SA-βgal** Bernadotte et al., 2016

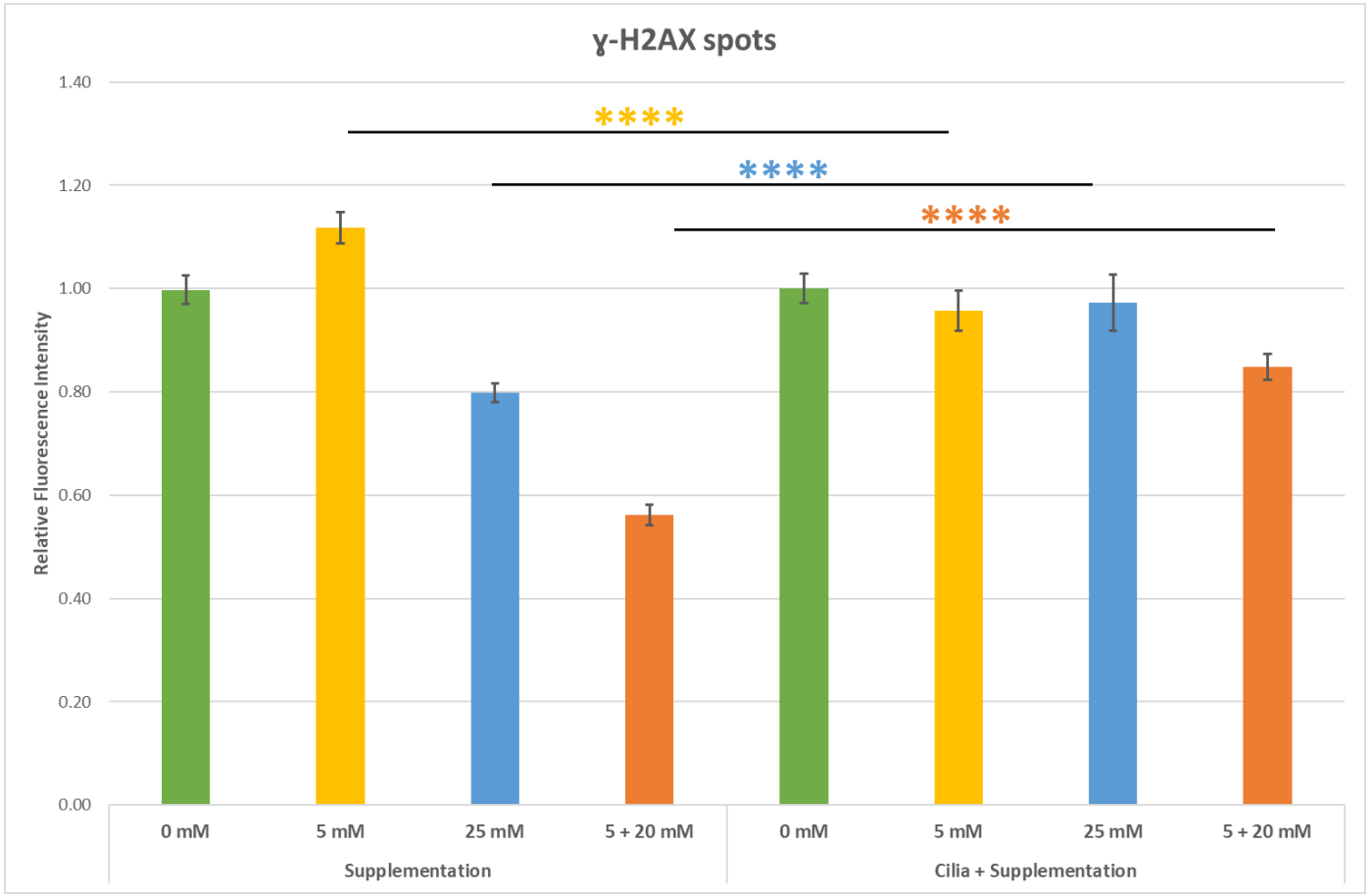


Merighi, Adalberto, Nadia Gionchiglia, Alberto Granato, and Laura Lossi. "The Phosphorylated Form of the Histone H2AX (ΓH2AX) in the Brain from Embryonic Life to Old Age." *Molecules (Basel, Switzerland)* 26, no. 23 (November 27, 2021): 7198.

Cilia assembly affects ɣ-H2AX



Cilia + Supplementation



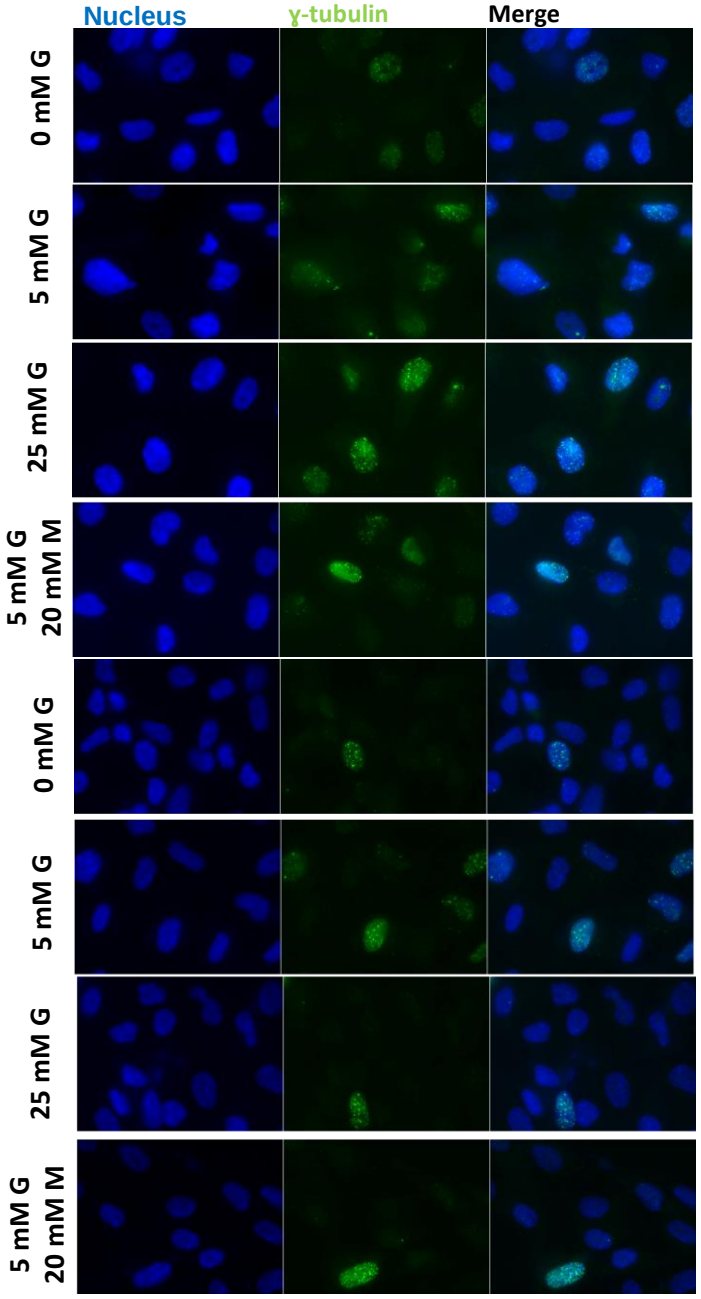
G – Glucose
M - Mannitol

**** p < 0,0001

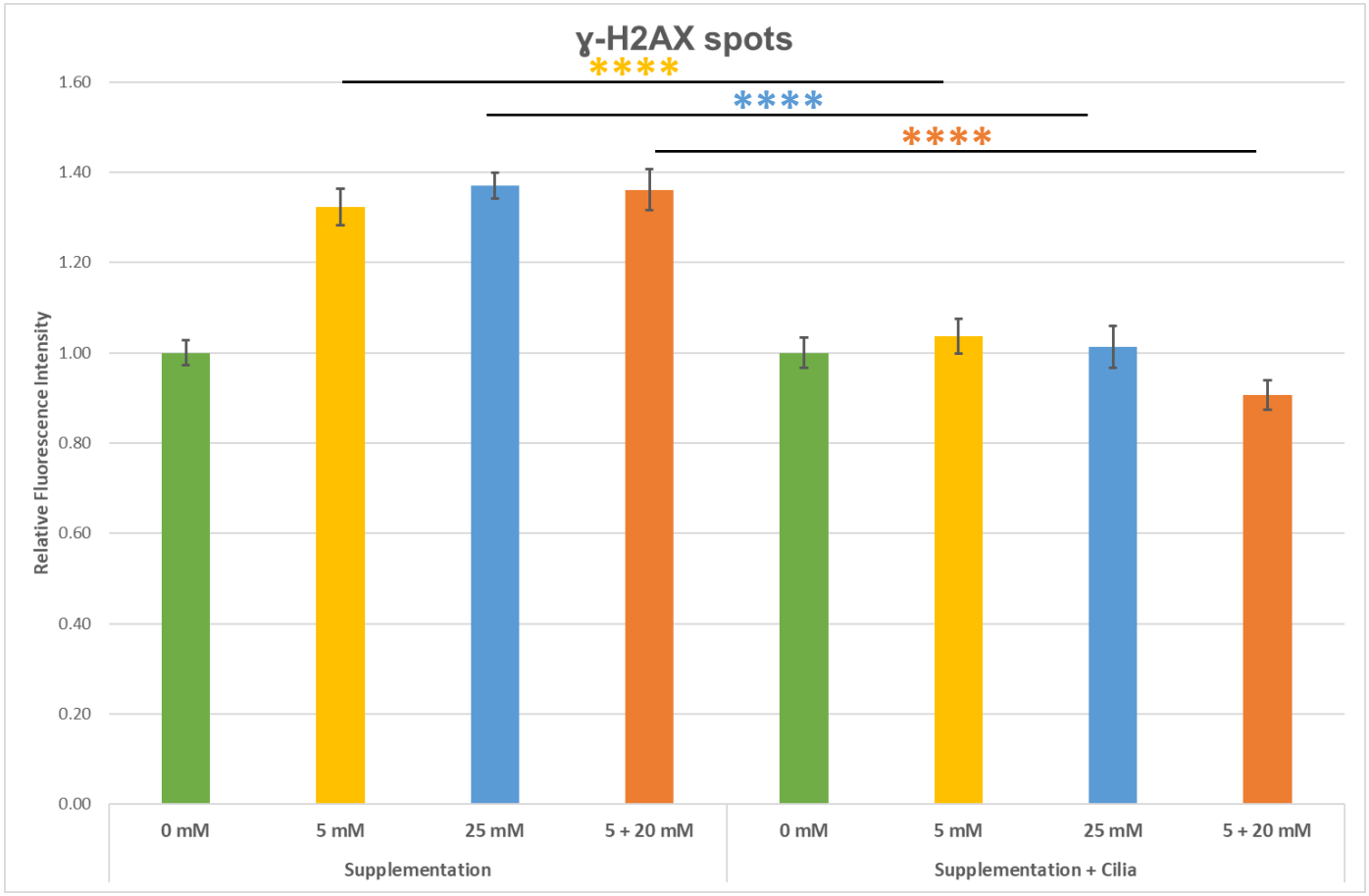
Cilia assembly affects γ-H2AX

Supplementation only

Supplementation + Cilia



Supplementation + Cilia



G – Glucose
M - Mannitol

**** p < 0,0001

In fact… (4)

"Inhibition of thioredoxin reductase 1 by caveolin 1 promotes stress-induced premature senescence" Volonte et al., 2009

"Overexpression of YAP partially ameliorated PCAF-induced endothelial senescence…" Kong et al., 2022

"Obesity and hyperinsulinemia drive adipocytes to activate a cell cycle program and senesce" Li et al., 2021

Current hypothesis

Cilia assembly delays cellular senescence by translocating TRXR1 and YAP to the cell nucleus

Potential impact on obesity development and progression by controlling cellular differentiation and regeneration, e.g., adipocytes

Acknowledgements

Helena Soares

Mariana Paiva
Rita Marques

Susana Marinho
Fernando Antunes
Catarina Matos
João Santos

Colaborations

- Escola Superior da Tecnologia da Saúde de Lisboa, IPL
  Sofia Nolasco (FMV, UL); Miguel Brito; Luísa Veiga; Catarina Ginete

- Centro de Química Estrutural is a Research Unit funded by Fundação para a Ciência e Tecnologia through projects UIDB/00100/2020 and UIDP/00100/2020.
- Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020.
- This work was funded by Instituto Politécnico de Lisboa IPL/2021/ObeCil_ESTeSL & IPL/2022/WintCilGlu_ESTeSL.