H&TRC Health and Technology Research Center

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ORAL COMMUNICATIONS I

THE NUCLEAR LEVELS OF THIOREDOXIN REDUCTASE 1, GAMMA-H2AX, AND YAP ARE MODULATED BY PRIMARY CILIA IN RESPONSE TO HIGH GLUCOSE LEVELS

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CQE Química Estrutural

Ciências ULisboa

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ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA
INSTITUTO POLITÉCNICO DE LISBOA

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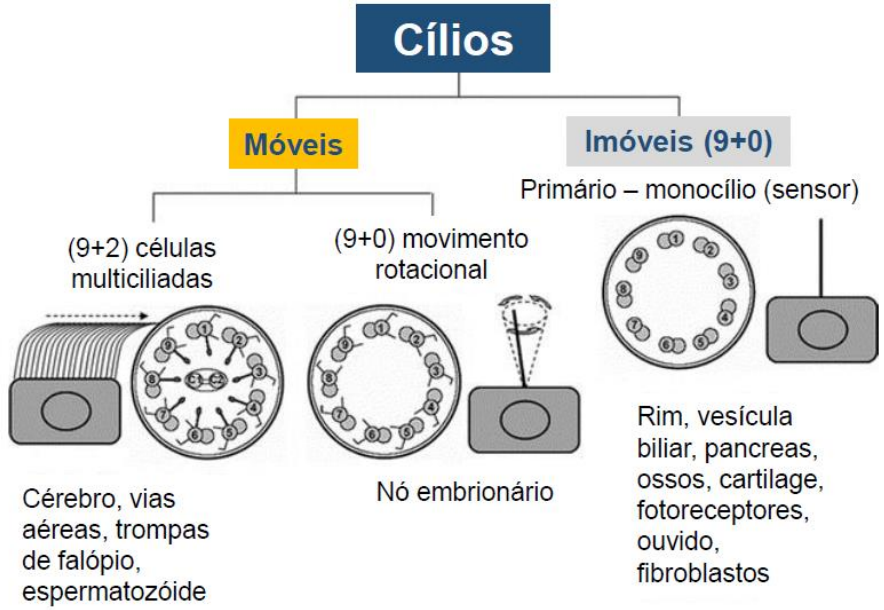
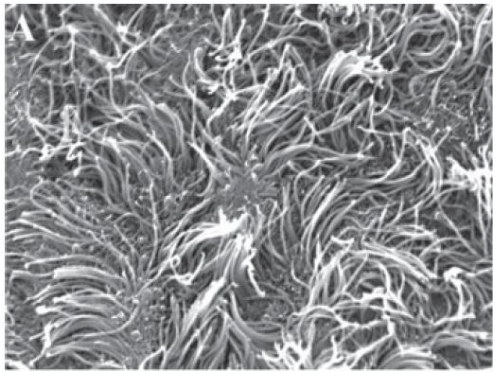
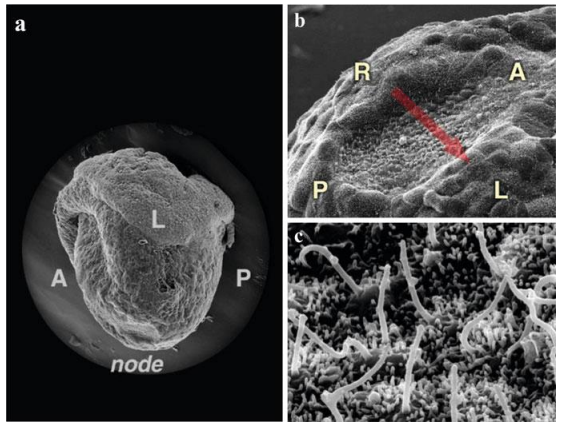
Diversidade Ciliar

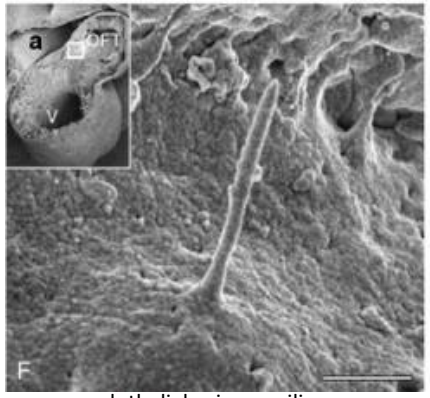




mouse ependymal cells located in the lateral ventricles



Cilia node mouse embryo



endothelial primary cilium

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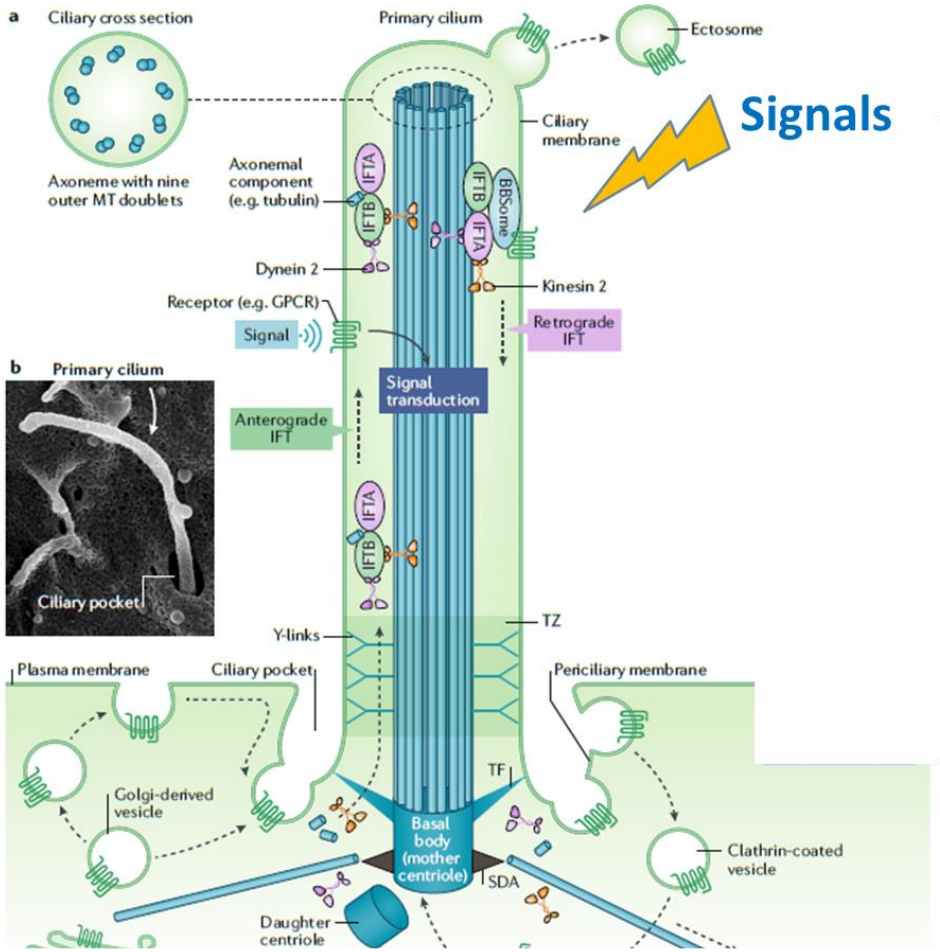
VIAS DE SINALIZAÇÃO DOS CÍLIOS PRIMÁRIOS



Wnt signaling pathway

cell fate determination, cell migration, cell polarity and organogenesis

- Inhibitor of adipogenesis
- Obesity
- Insulin resistance

Hh signaling pathway

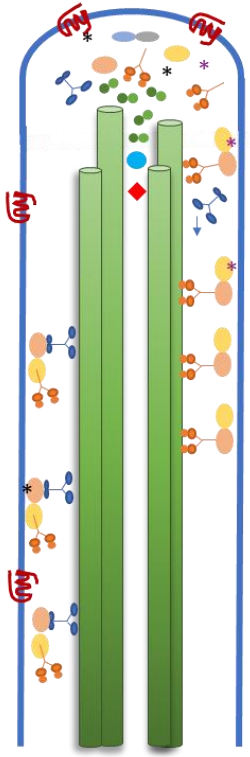
Major regulator of development

- 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

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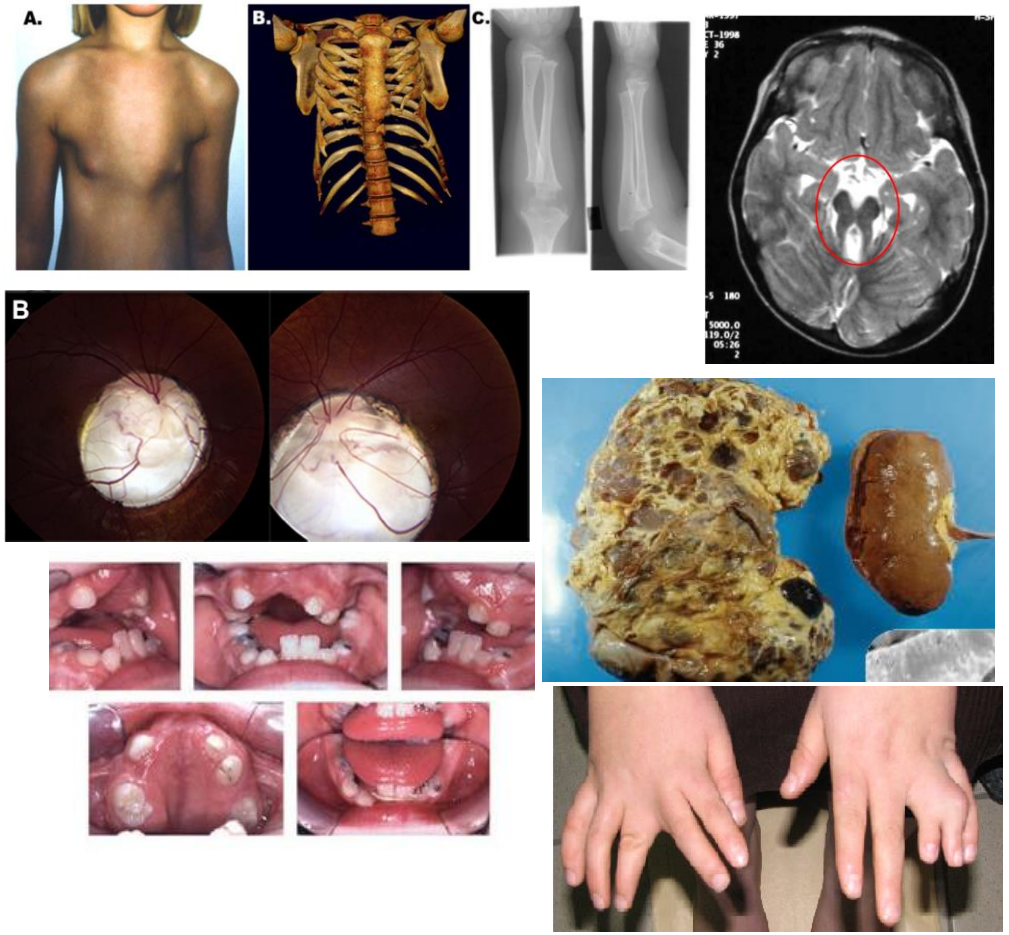
CILIOPATIAS

- 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**
  - **Diabetes**
  - **Blindness**



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CILIOPATIAS

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

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

Using Retinal Pigment Epithelium (RPE-1) 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

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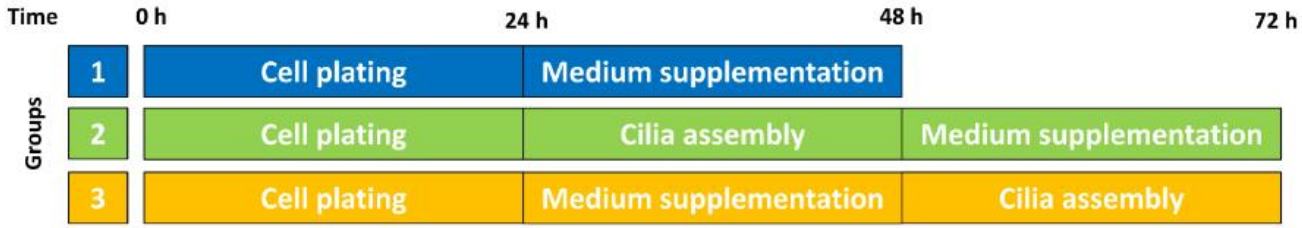
SETUP EXPERIMENTAL

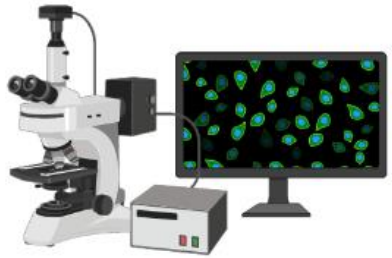


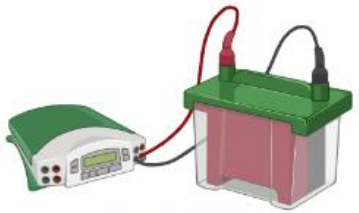
Medium supplementation:

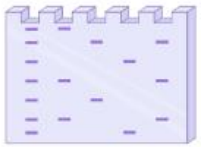
- 0 mM Glucose
- 5 mM Glucose
- 25 mM Glucose
- 5 mM Glucose + 20 mM Mannitol

Cilia assembly:

- Serum removal







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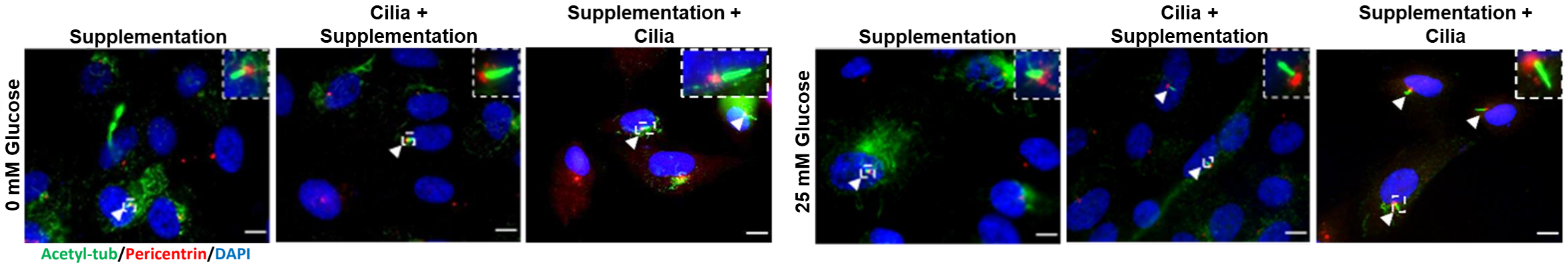
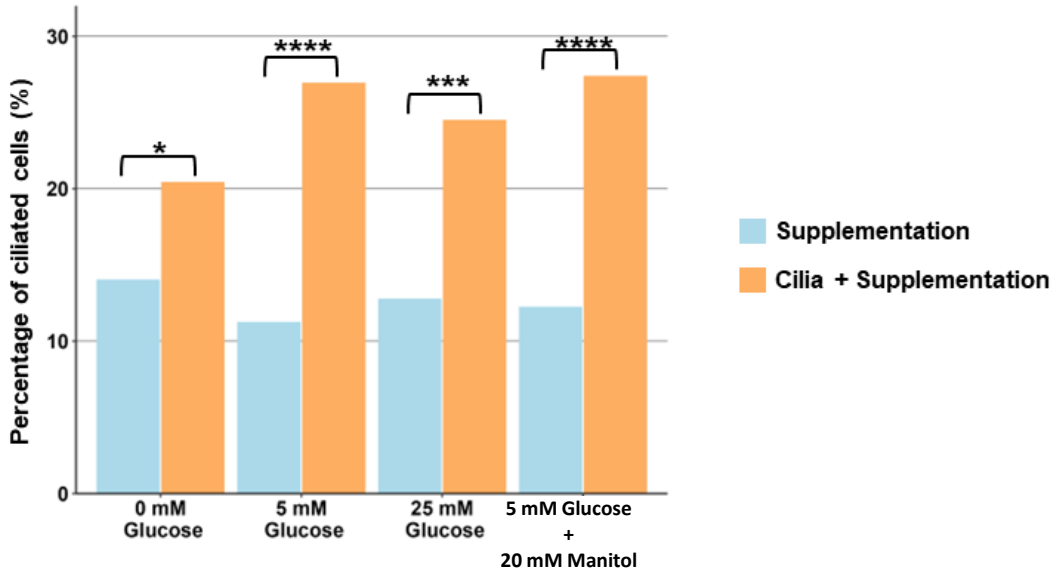
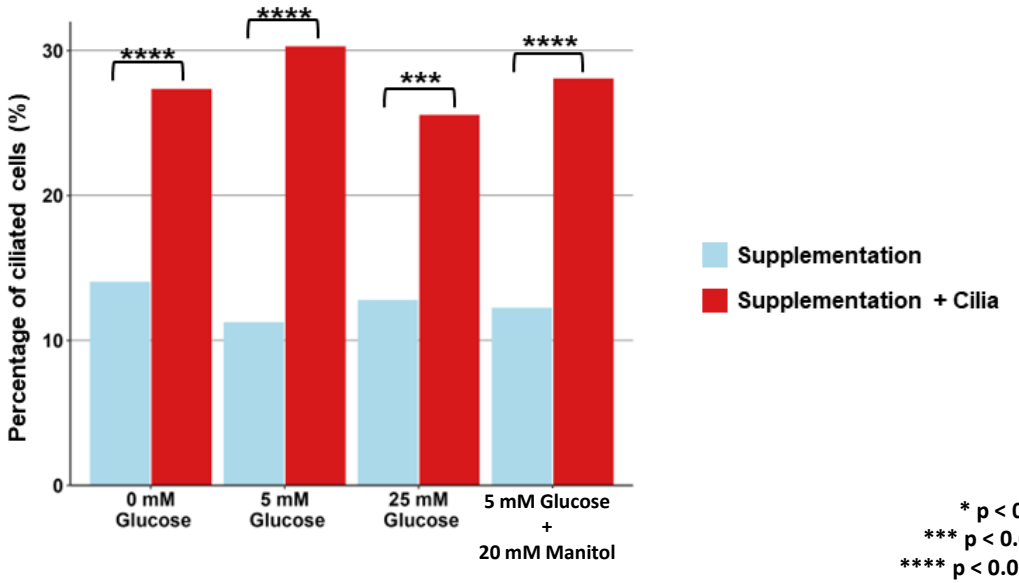
CONCENTRAÇÕES ELEVADAS DE GLUCOSE NÃO AFETAM A PERCENTAGEM DE CÉLULAS CILIADAS







* p < 0.05
*** p < 0.001
**** p < 0.0001

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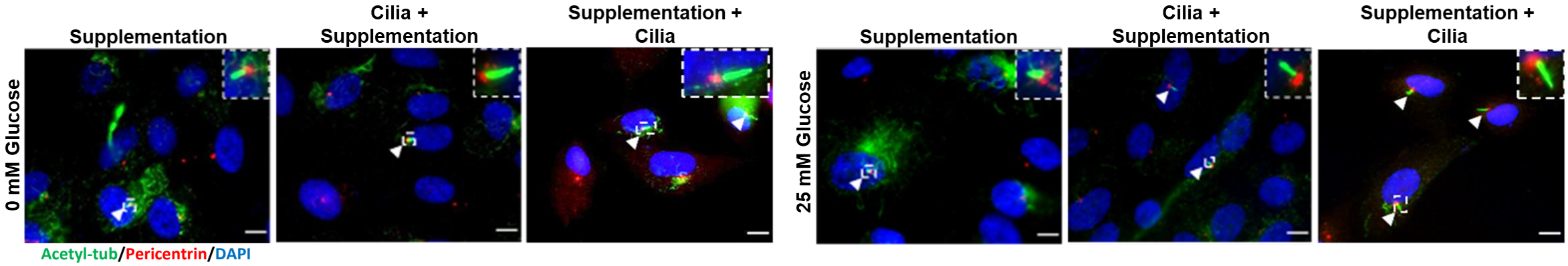
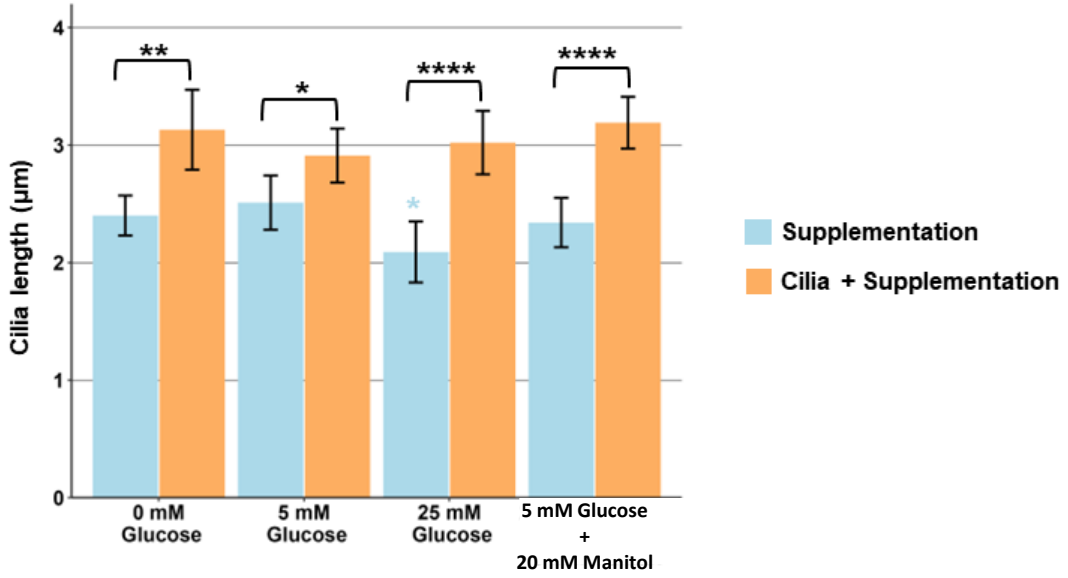
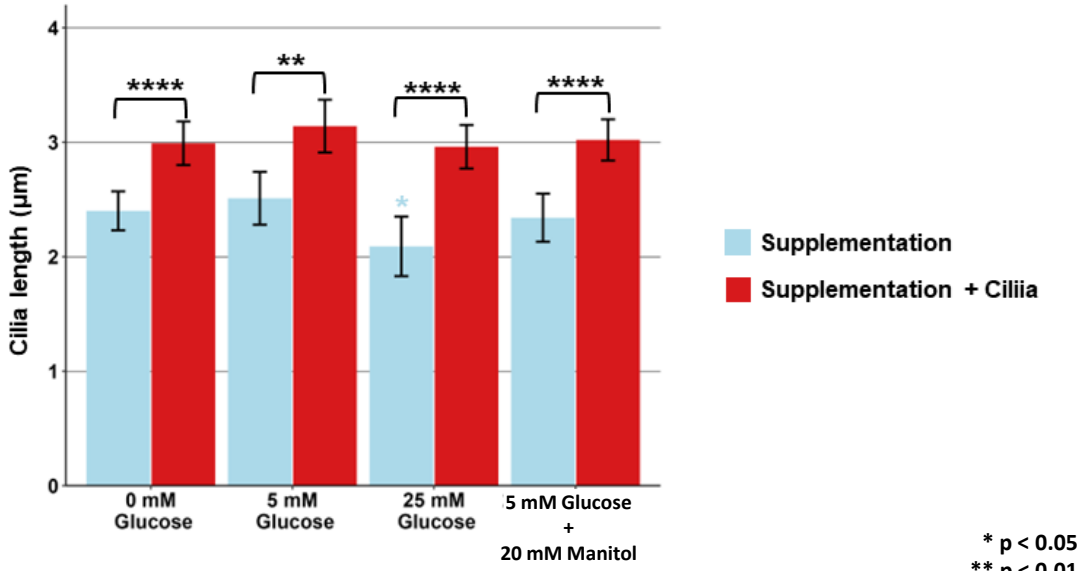
CONCENTRAÇÕES ELEVADAS DE GLUCOSE DIMINUEM O TAMANHO DO CÍLIO







* p < 0.05
** p < 0.01
**** p < 0.0001

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

Is the ciliary signaling compromised?

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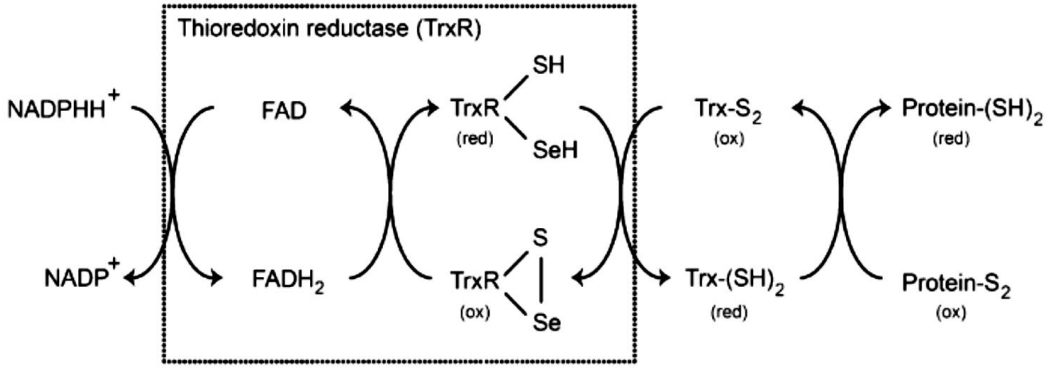
TIORREDOXINA REDUCTASE 1 (TRXR1)

- 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)**

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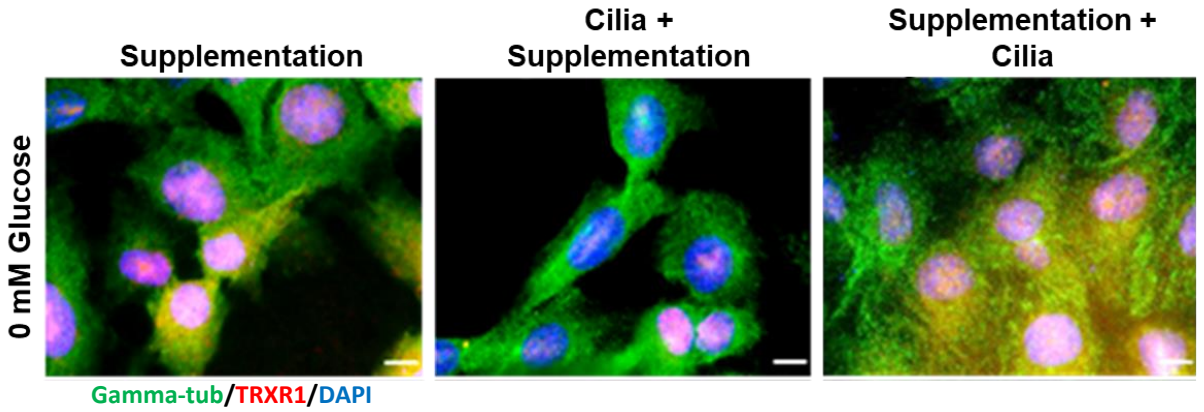
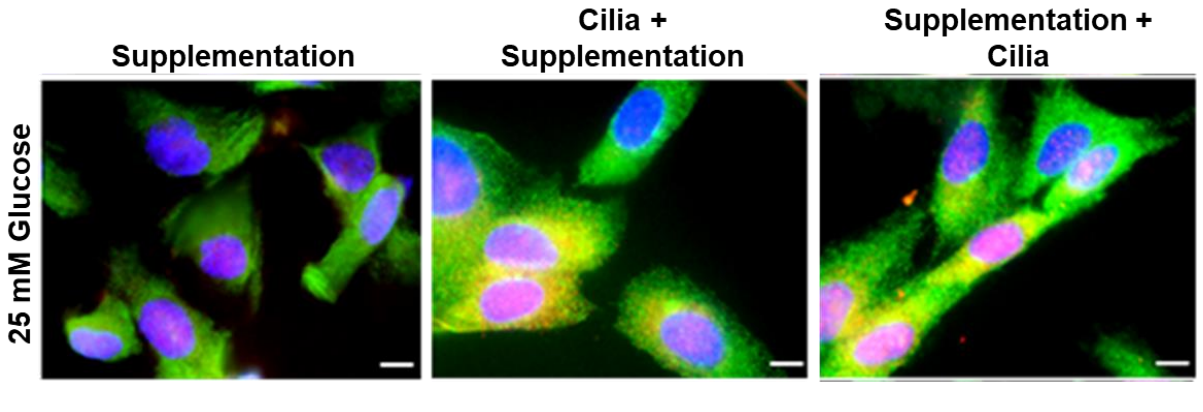
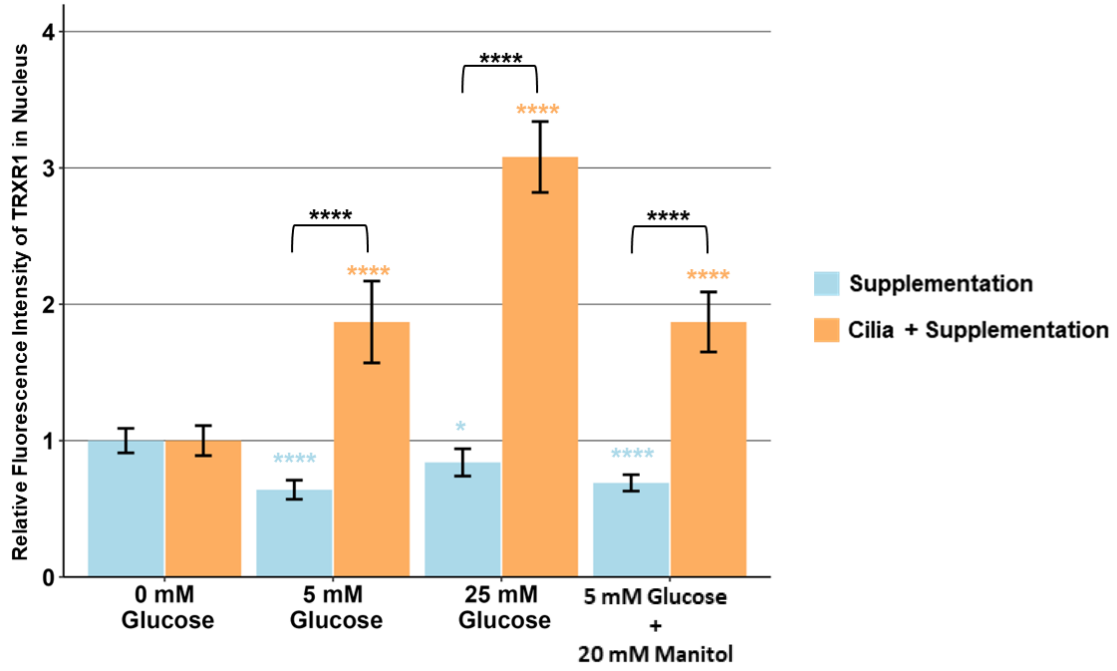
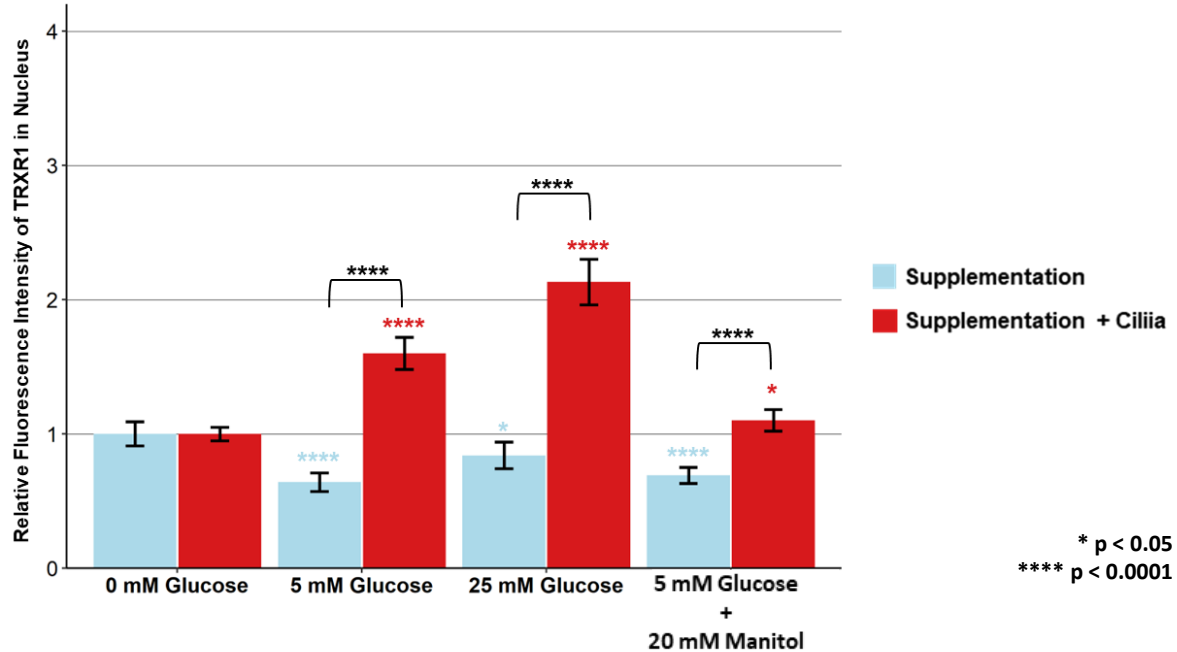
A TRXR1 AUMENTA OS SEUS NÍVEIS NUCLEARES EM RESPOSTA A CONCENTRAÇÕES ELEVADAS DE GLUCOSE NA PRESENÇA DE CÍLIOS









* p < 0.05
**** p < 0.0001

Thioredoxin Reductase 1 levels are not affected by high glucose levels

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

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

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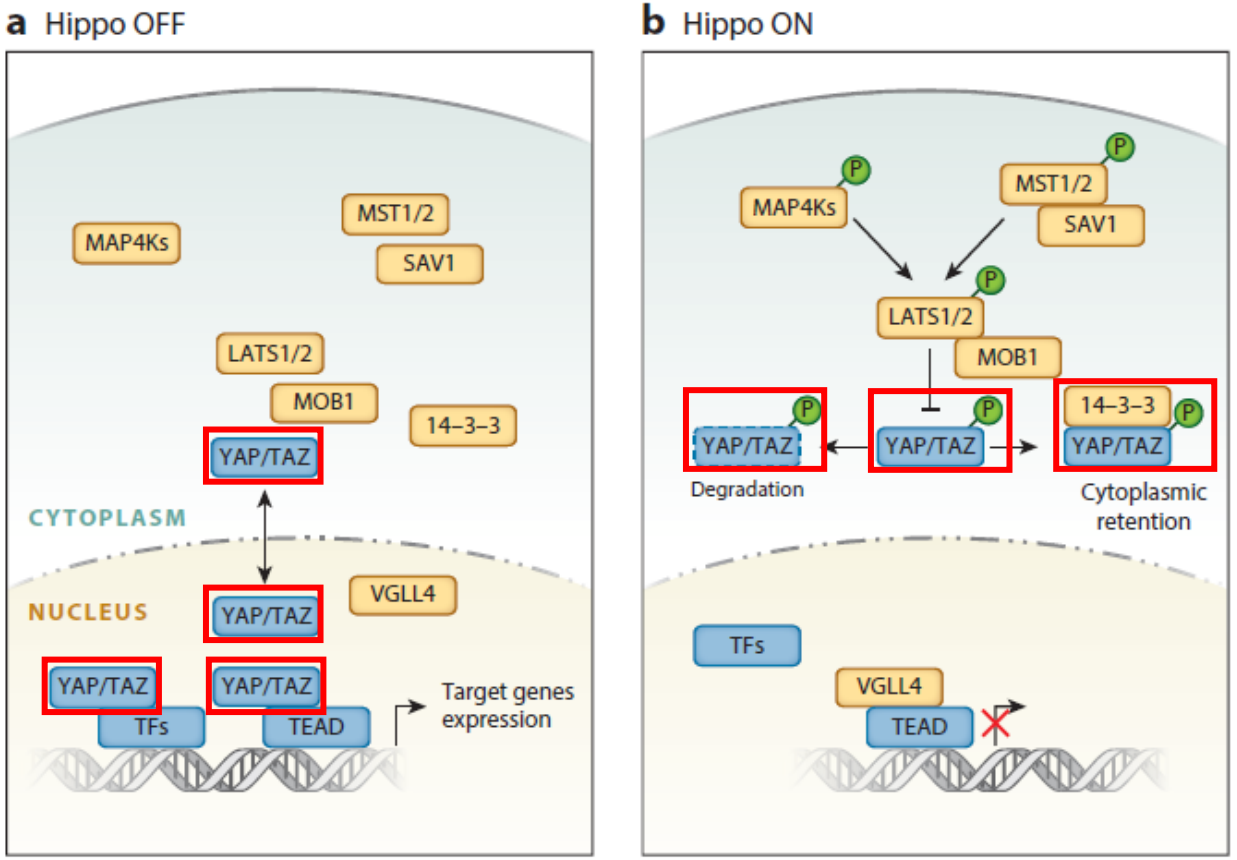
HIPPO PATHWAY

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.



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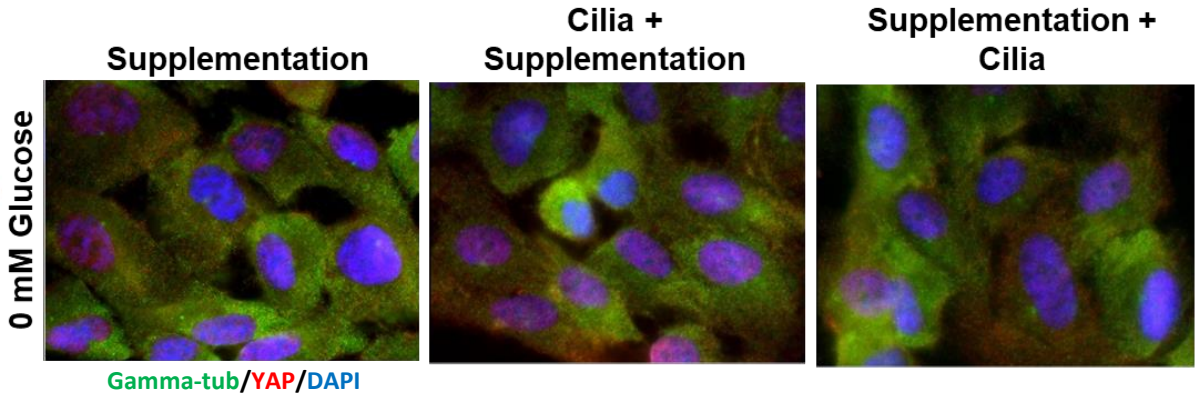
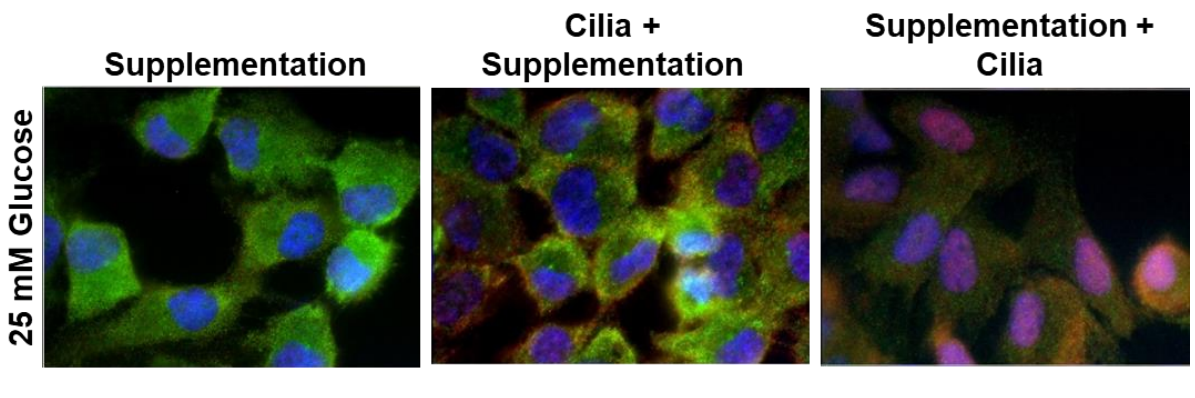
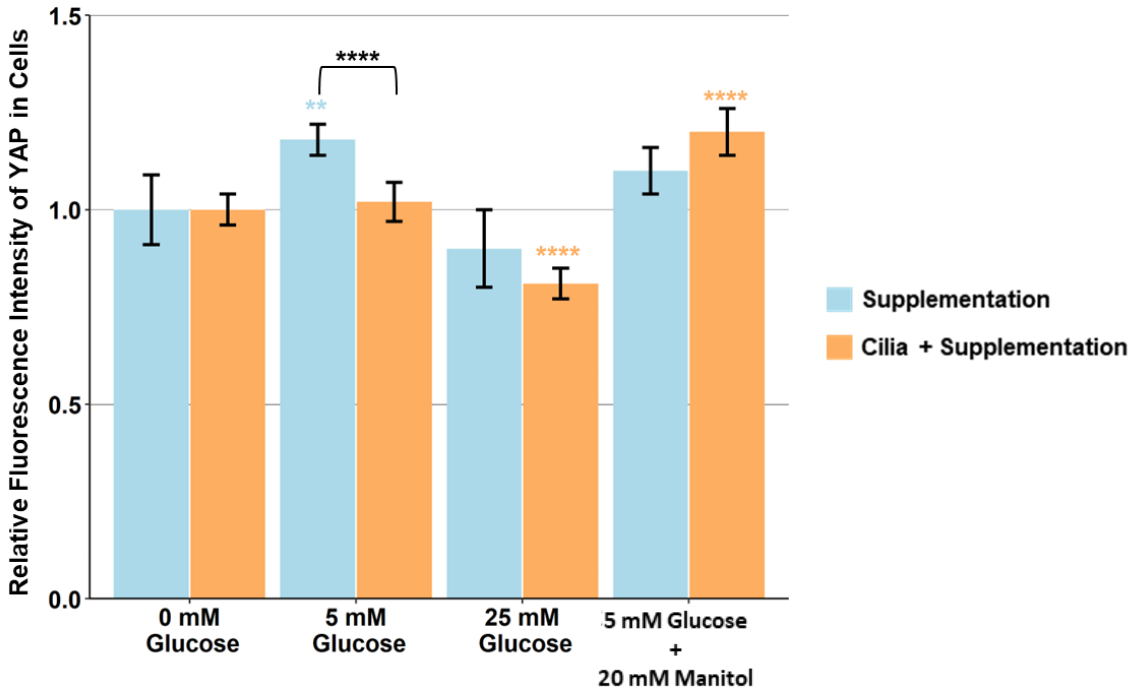
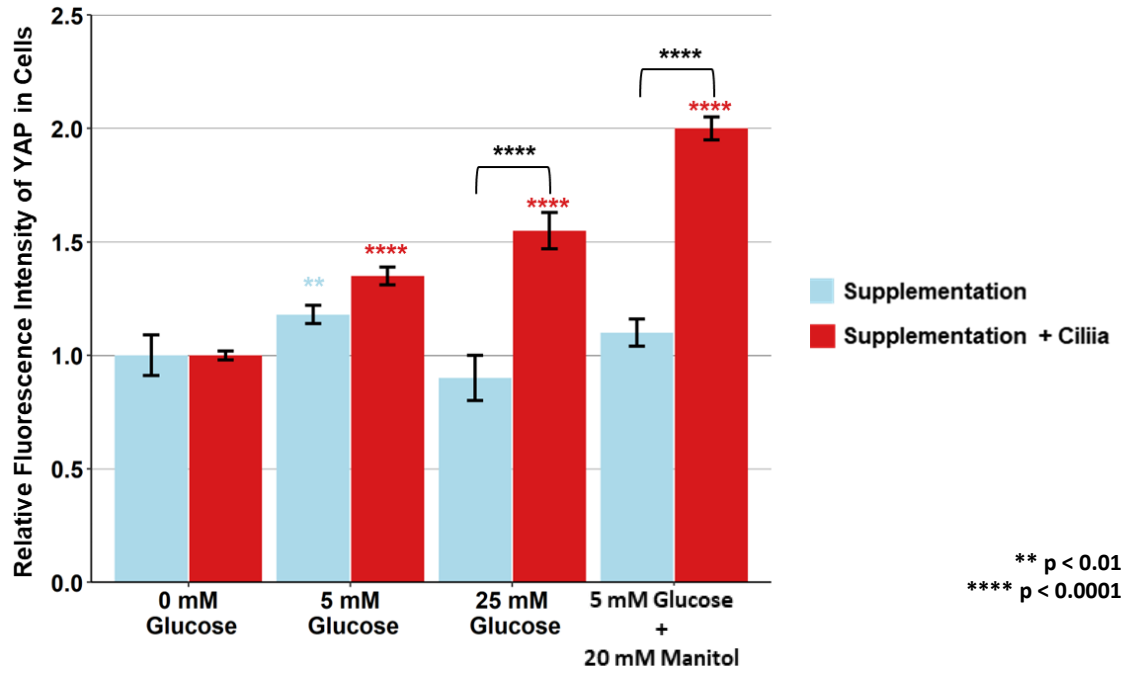
A PRESENÇA DE CÍLIOS PRIMÁRIOS AFETA OS NÍVEIS DE YAP NO NÚCLEO EM RESPOSTA A CONCENTRAÇÕES ELEVADOS DE GLUCOSE









** p < 0.01
**** p < 0.0001

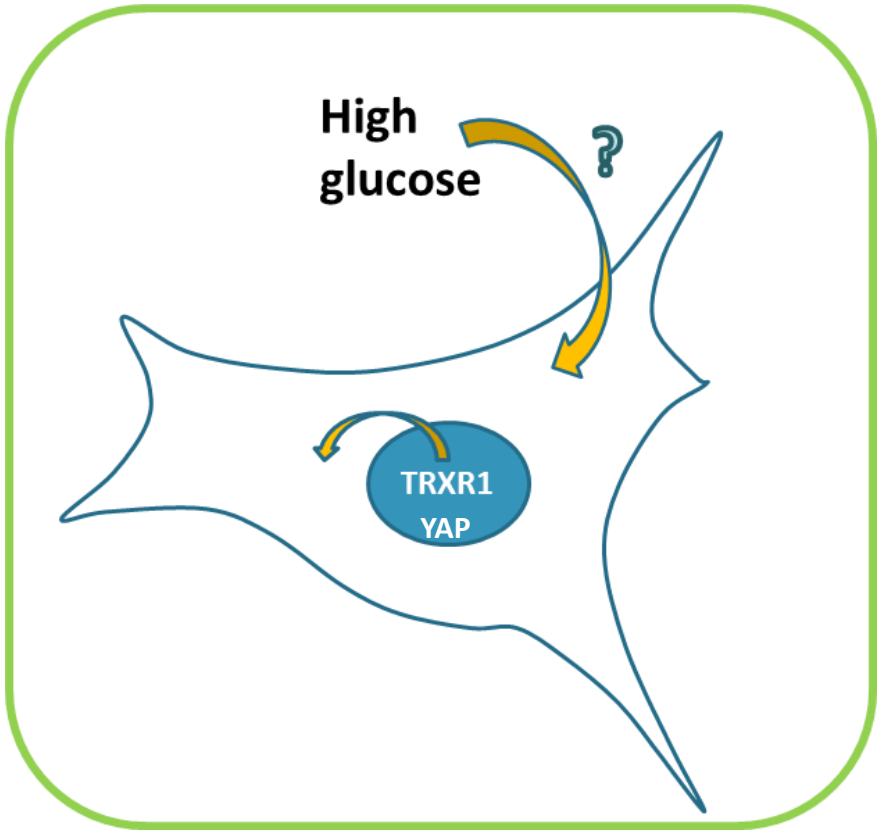
When cells present cilia after glucose supplementation YAP translocates to the nucleus

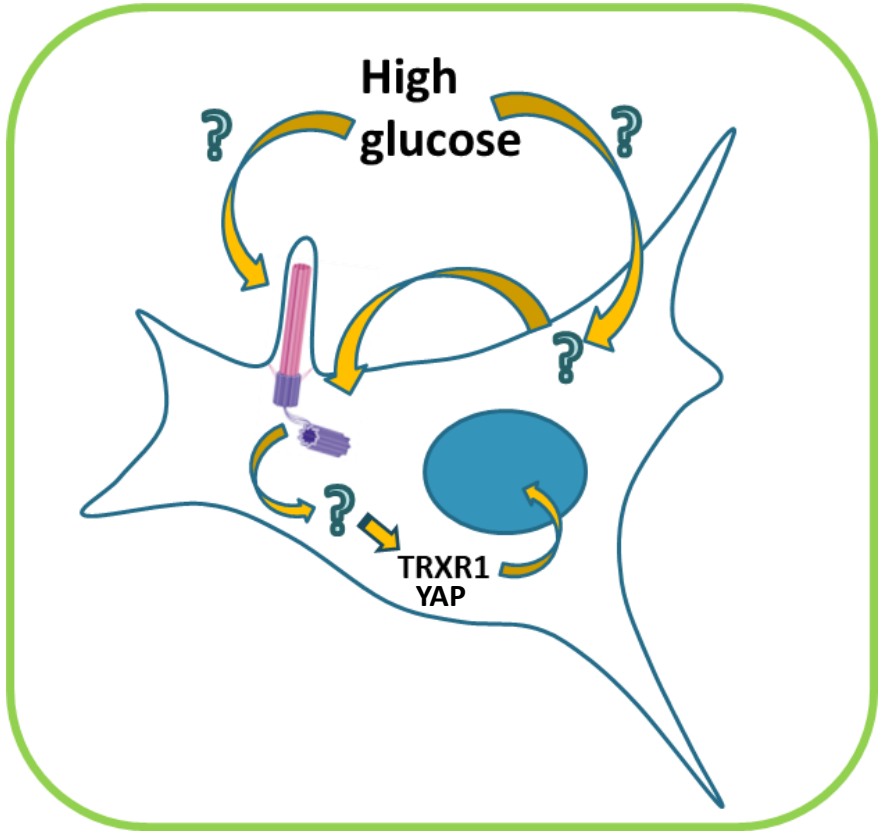
"Adipogenesis was attenuated in 3T3-L1 cells stably expressing Bcl-2 or YAP" Chang et al., 2017

"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

"PPP1CA/YAP/GS/Gln/mTORC1 pathway activates retinal Müller cells during diabetic retinopathy" Guo et al., 2021

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?

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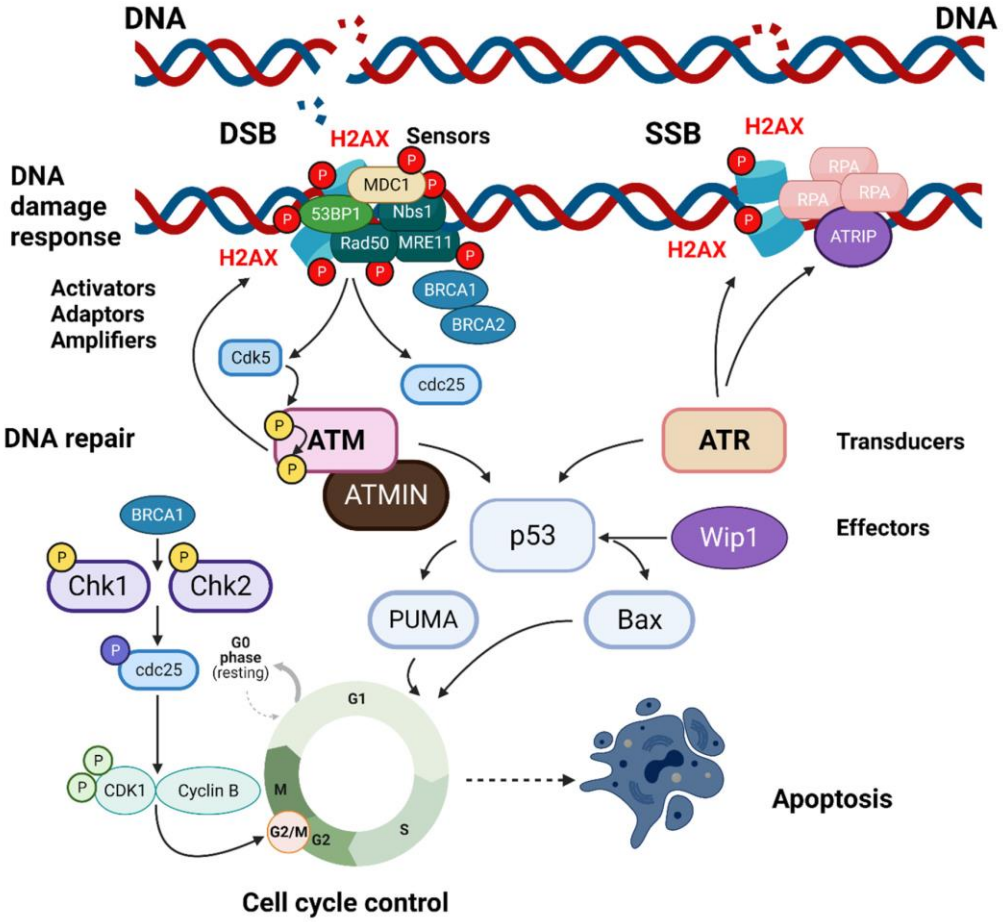
GAMMA-H2AX

- 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
- **<u>Histone γ-H2AX is the second most common marker of cellular senescence after SA-βgal</u>** Bernadotte et al., 2016



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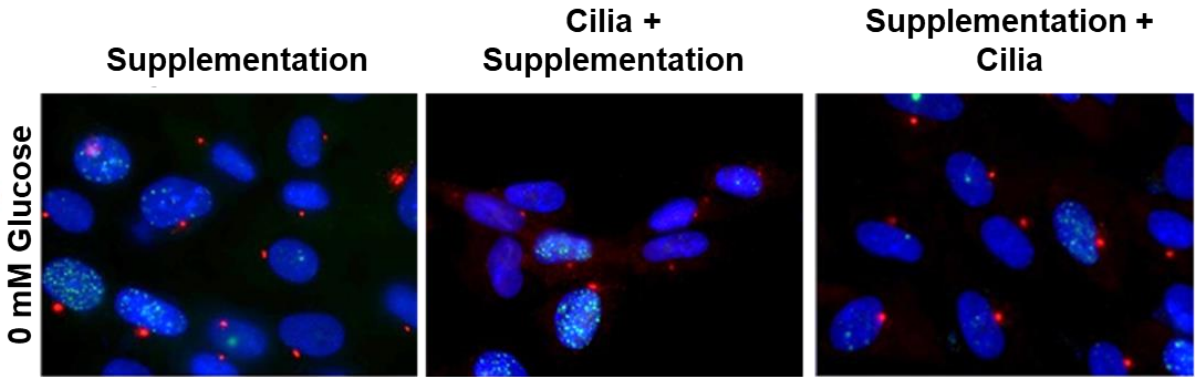
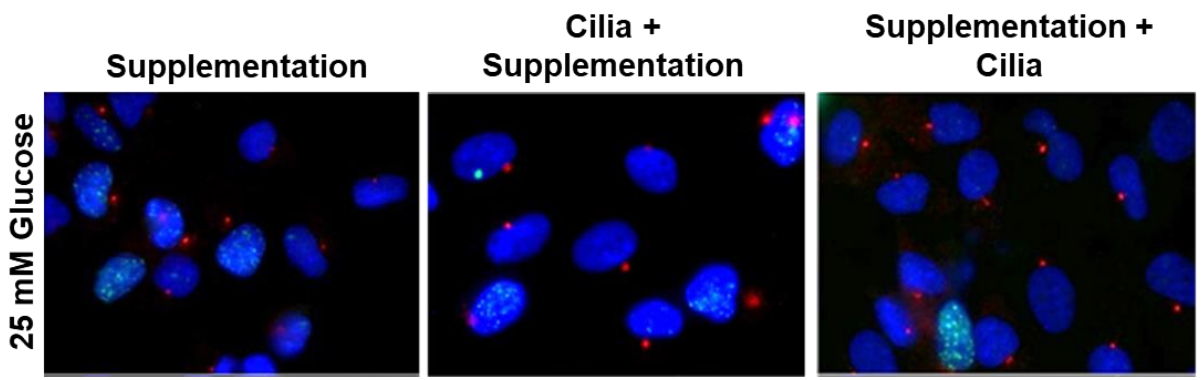
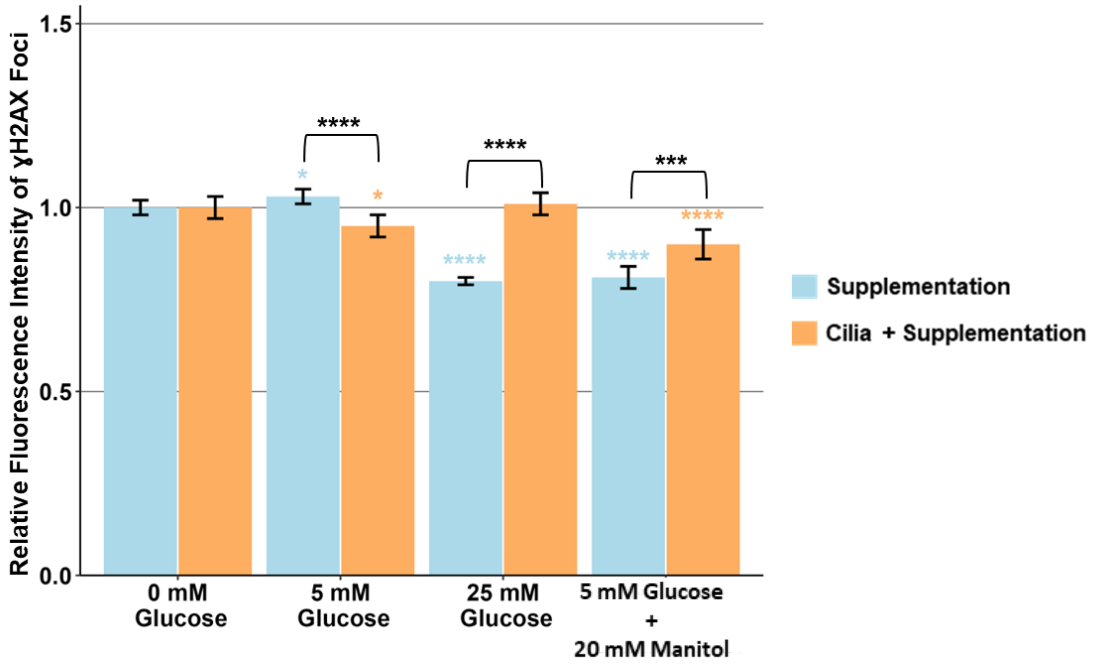
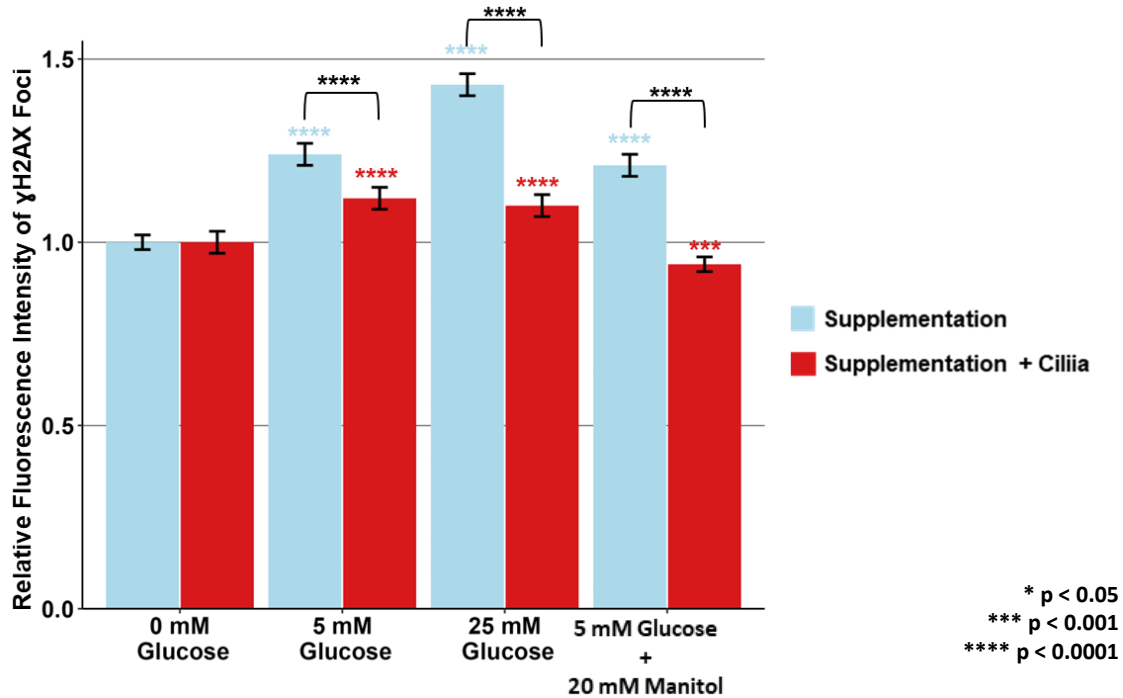
A PRESENÇA DE CÍLIOS PRIMÁRIOS ATENUA A VARIAÇÃO DOS NÍVEIS DE GAMMA-H2AX NO NÚCLEO EM RESPOSTA A NÍVEIS ELEVADOS DE GLUCOSE









* p < 0.05
*** p < 0.001
**** p < 0.0001

"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

Hipótese atual

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

Potential impact on obesity and diabetes development, progression and comorbidities by controlling cellular differentiation and regeneration, e.g., adipocytes and retinal pigment epithelium cells

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