

MOL231: Role of Unc13 in cell division of *Salpingoeca rosetta*



Eline Sunnanå Kleppan*, Aishwarya Ravi, and Pawel Burkhardt

Sars International Center for Marine Molecular Biology, University of Bergen, Bergen, Norway

Background and Aim

Choanoflagellates are the closest unicellular relatives of animals and they are therefore an ideal model to use for studying the origin of multicellularity in animals and cell types. Unc13 is a multi-domain protein which has multiple functions in animals, fungi and plants. In animals, the protein is an essential neurosecretory protein in synapses. It is however a regulator in cytokinesis in fission-yeast and is important for stomatal responses in plants. The aim of the project is to study the function of Unc13 in choanoflagellates. Unc13 knockout reveals multinucleated cells and a profound effect on cell division.

Keywords: Neurosecretion, synapses, choanoflagellates, Unc13

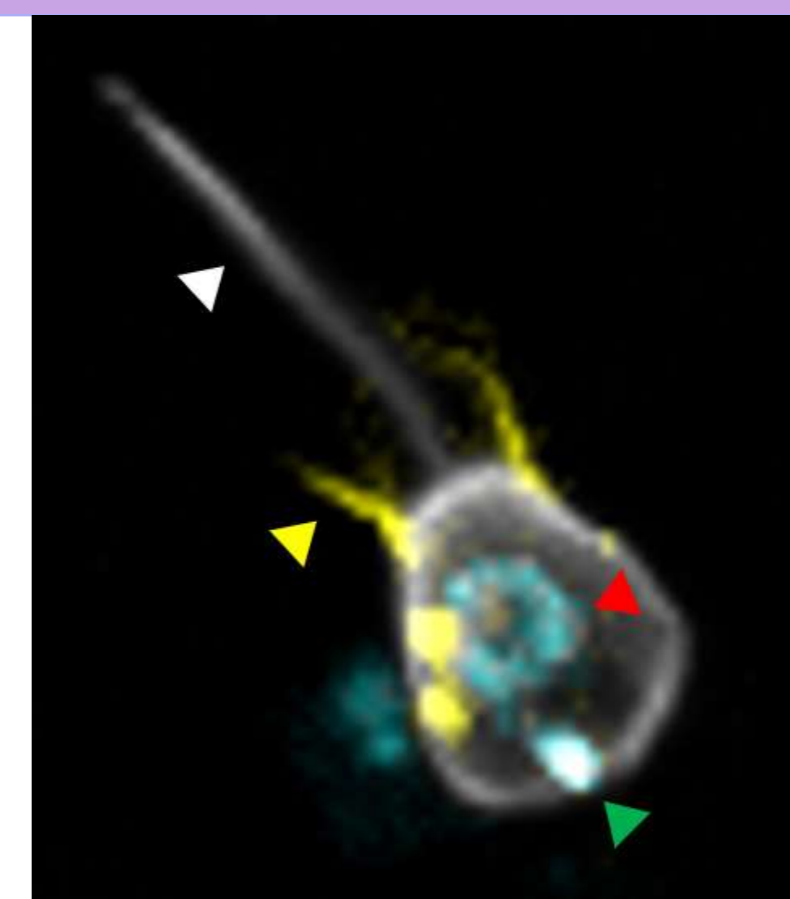


Figure 1. Choanoflagellate morphology.

White: Flagellum.
Yellow: Collar.
Red: Nucleus.
Green: Bacteria.

Method

Genotyping

Sequencing

Immunofluorescence

Imaging

Live imaging

Results

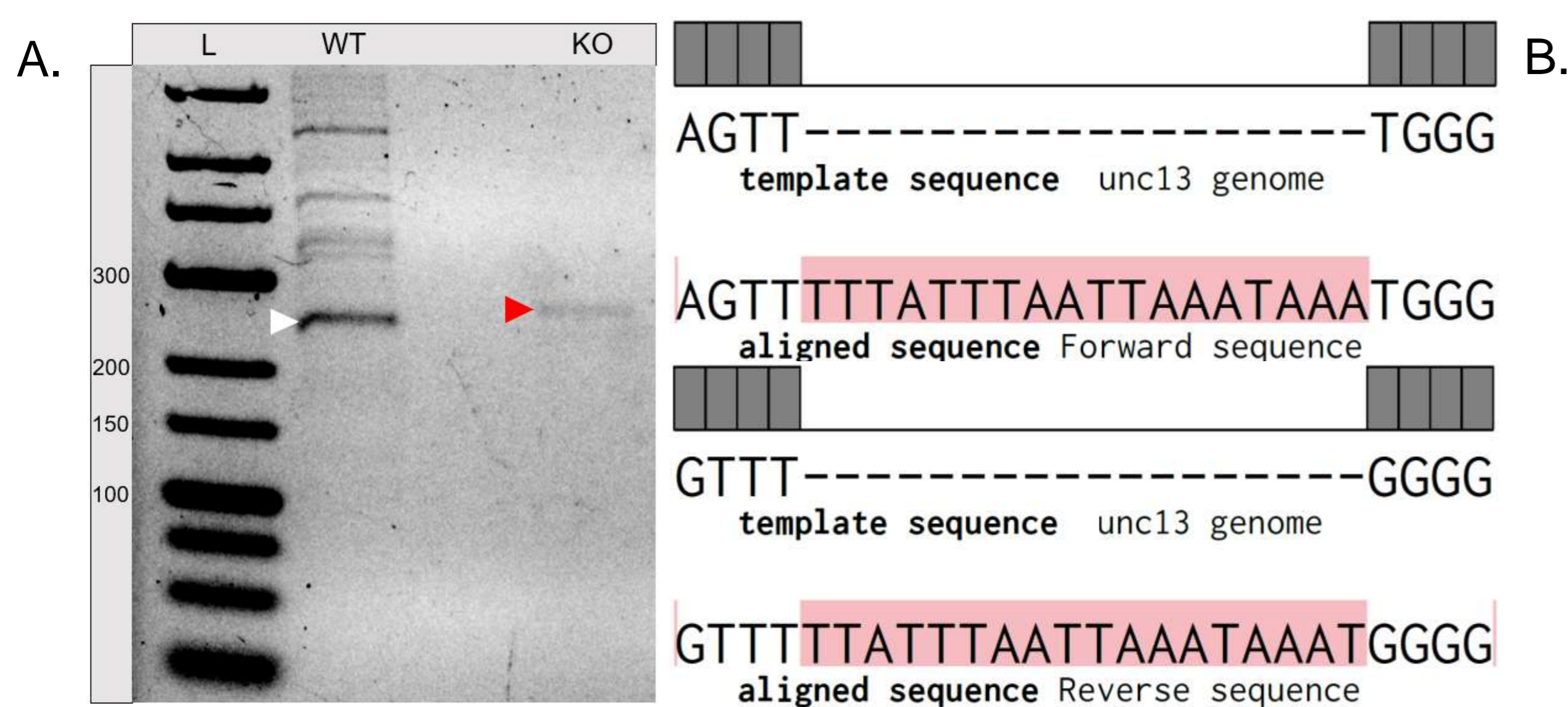


Figure 2. Genotyping of Unc13 knockout cells with wildtype cells as control. Unc13 knockout cells expected band length 264 bp. Slightly higher than wildtype cells band with 246 bp. White arrow shows WT band. Red arrow shows KO band. Run on 3% agarose. (A) (L) Low range DNA ladder. (WT) Wildtype cells. (KO) Unc13 knockout cells. (B) Sequencing confirms presence of premature termination sequence.

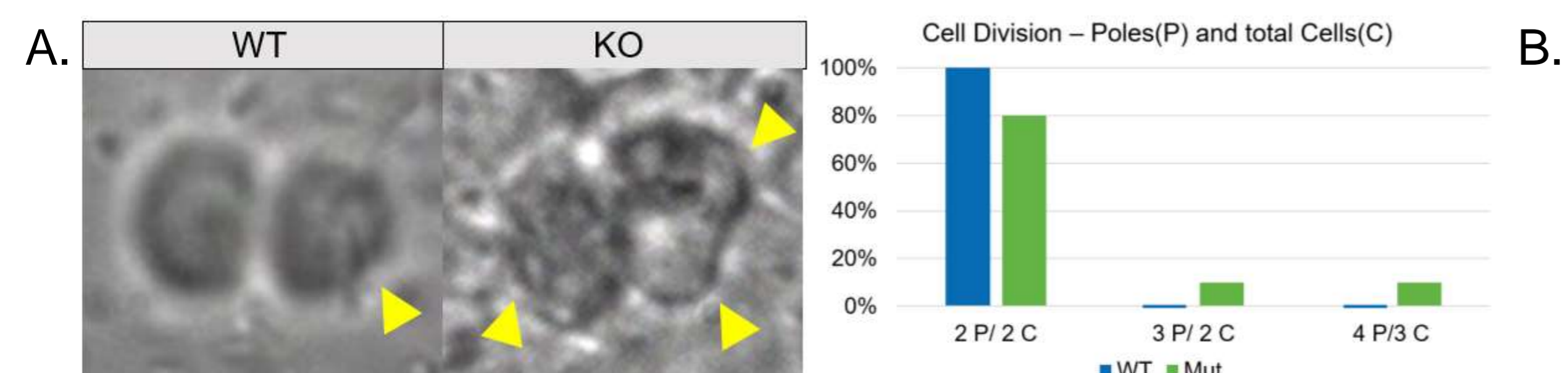


Figure 4. Comparison of cell division in WT and Unc13 KO mutant. Poles are defined as a set of flagellum and collar. Arrows show poles. (A) Left: Normal cell division in WT. Right: Cell dividing with three poles in KO-Unc13. (B) Total cells observed, 11 in WT and 10 in KO. Only mutant showed abnormal cell division.

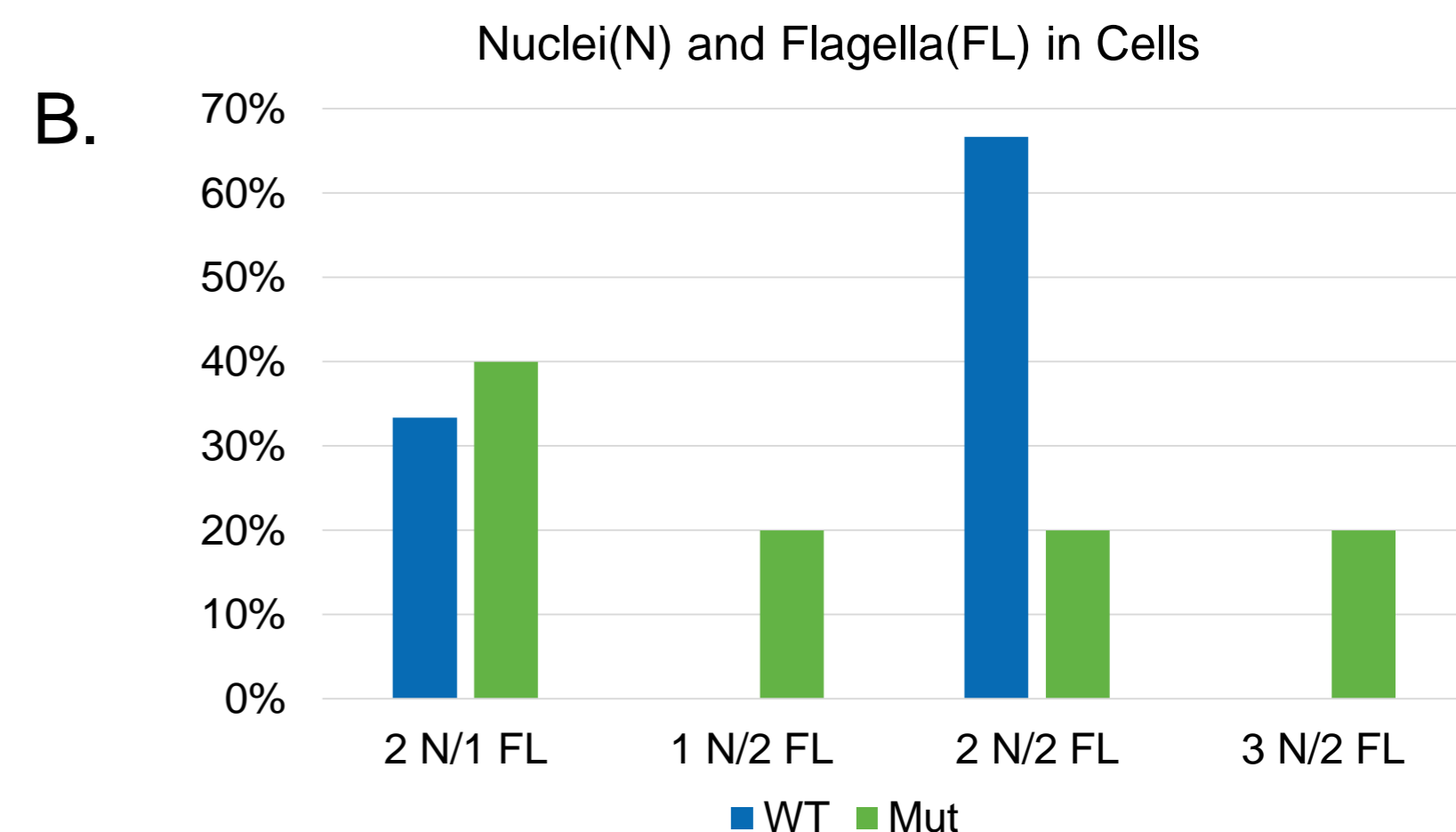
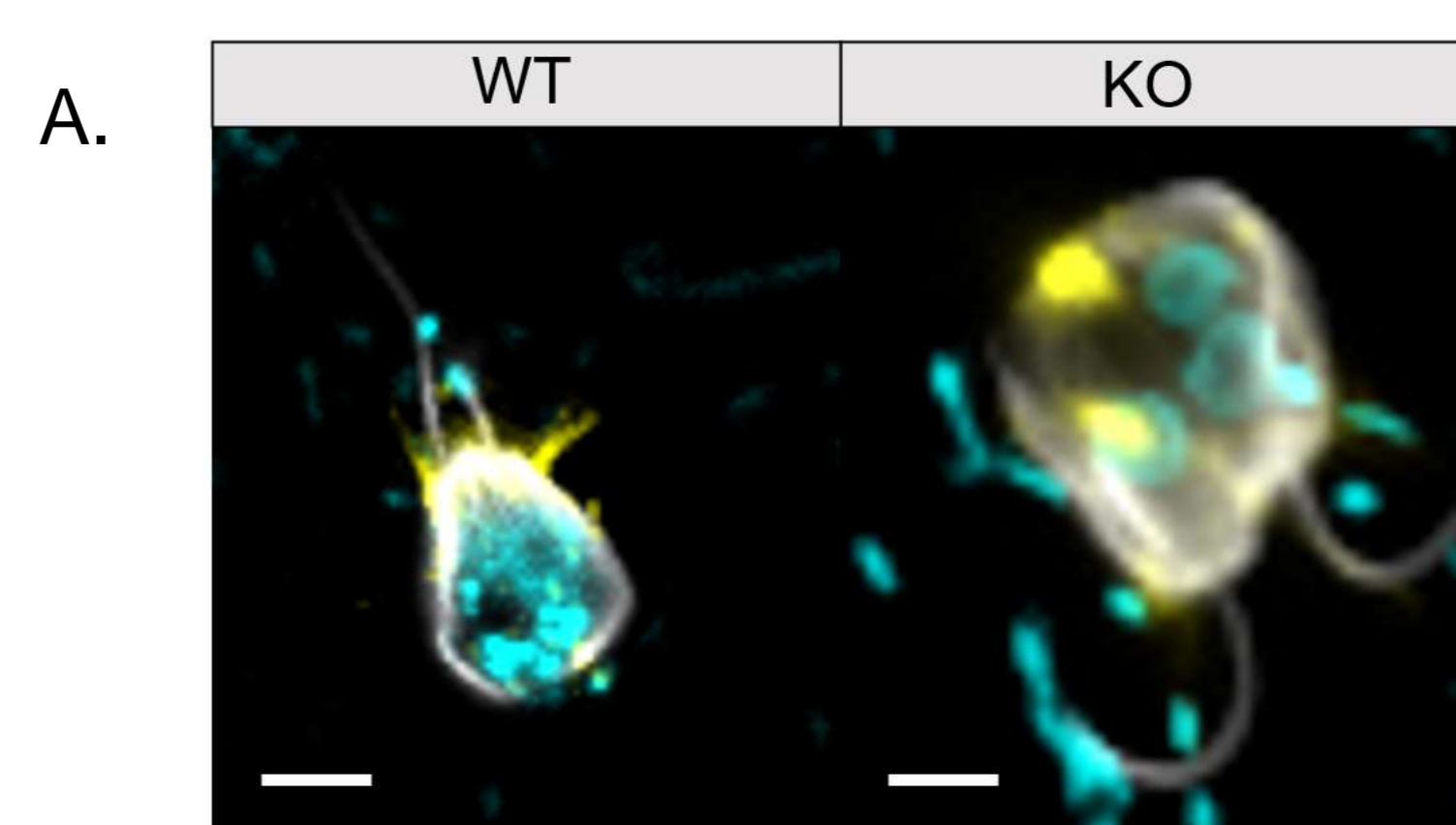


Figure 3. Immunostaining of *S. rosetta* to quantify the nuclei. Scale: 2 μ m. Staining used: tubulin (white), actin (yellow), and DAPI (blue). (A) Left: Representative WT cell with two nuclei and two flagella. Right: Example of multinucleated Unc13 knockout cell. (B) Quantification of nuclei and flagella. Total cells observed 127 WT and 132 mutant. Representative graph only show cells with aberrant nuclei count. Total cells: 3 WT and 5 mutant.

Take-home message

- Unc13 has probably an effect on cell division of choanoflagellates.
- We can assume that the multinucleated cells after division are a cause or consequence of KO-Unc13.
- To get higher statistical significance, more quantification must be made.

References

1. Dittman, J. (2019) *Unc13: a multifunctional synaptic marvel*, Molecular neuroscience, 57, 17-25
2. Hashimoto-Sugimoto, M. et al. (2013) *A Munc13-like protein in Arabidopsis mediates H⁺-ATPase translocation that is essential for stomatal responses* Nature Communications, 4, 2215
3. Hoffmeyer, T et al. (2016) *Choanoflagellatamodels- Monosiga brevicollis and Salpingoeca rosetta* Current opinion in genetics development, 39, 42-47.
4. Y.-H. Zhu et al. (2018) *Roles of the fission yeast UNC-13/Munc13 protein Ync13 in late stages of cytokinesis* Molecular Biology of the Cell, 29(19), 2259-2279.

