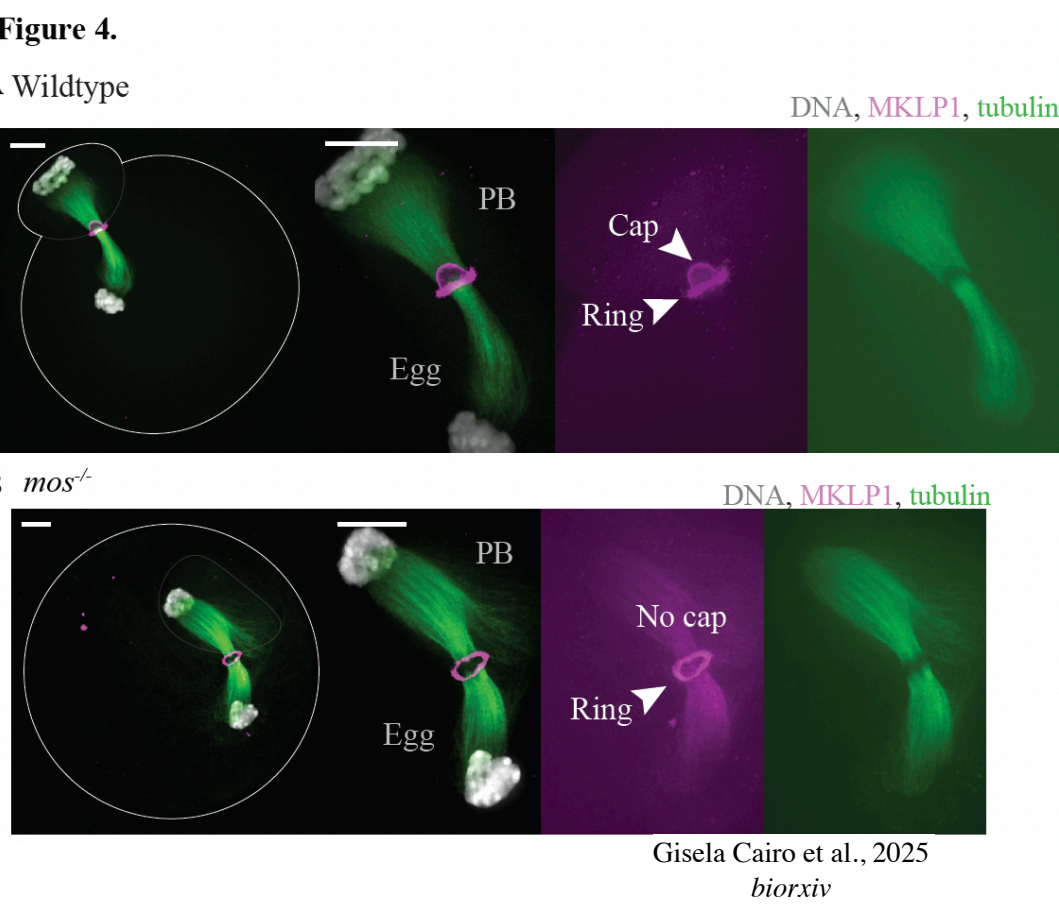


Mitochondrial Leakage and Parthenogenetic Oocyte Activation in Ovarian Teratoma Formation

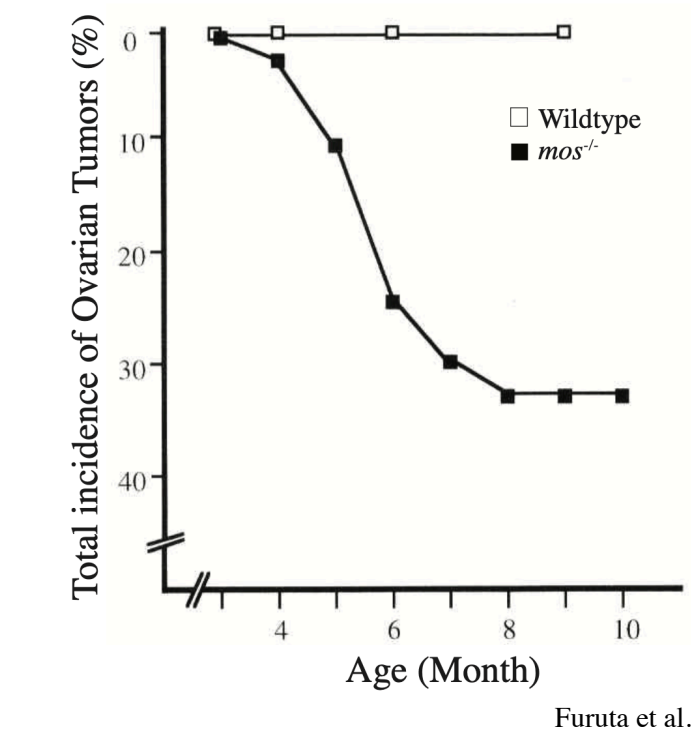
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Background

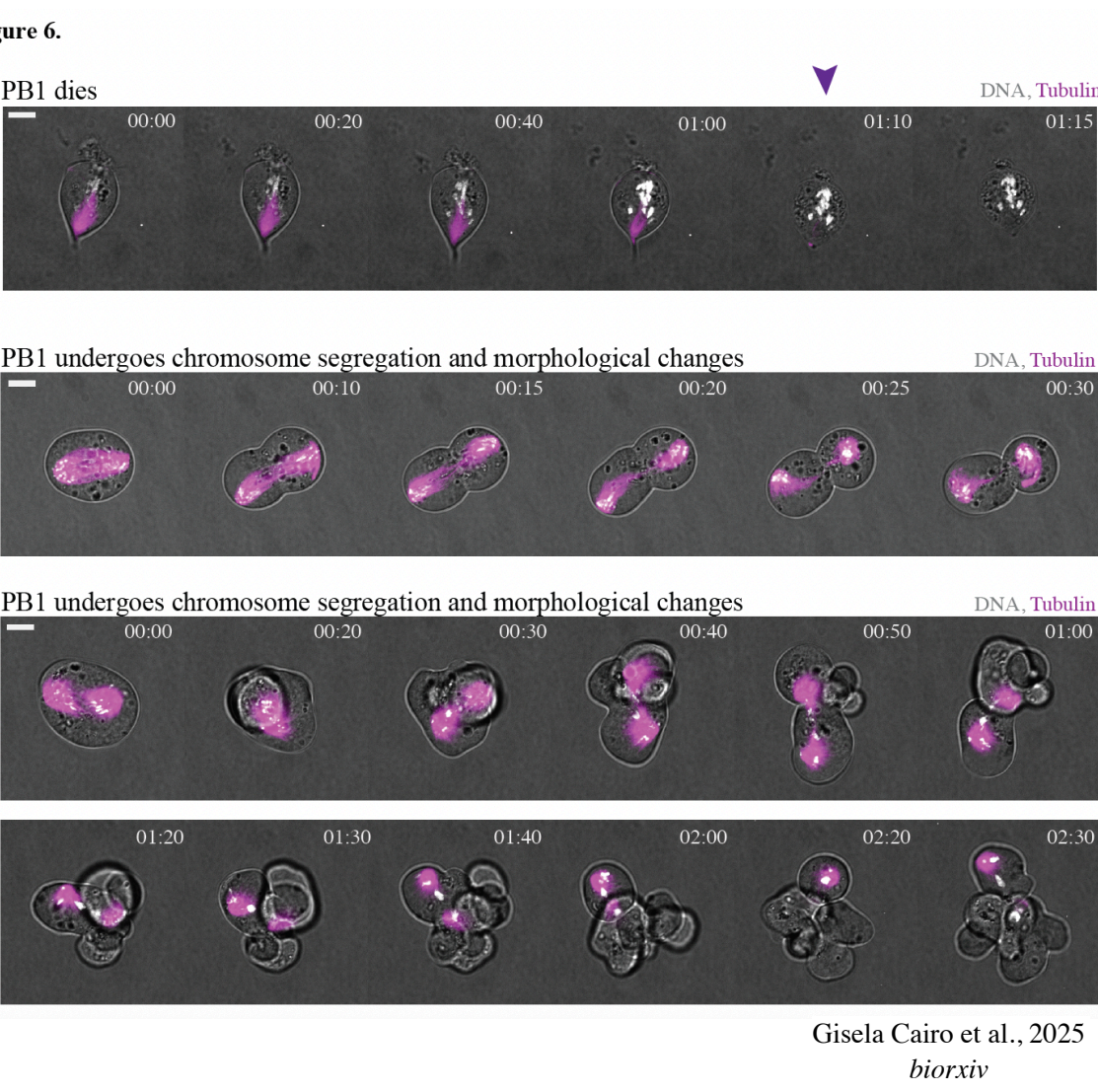
Mos knockout (*mos*^{-/-}) mice serve as a model for studying parthenogenetic activation of oocytes and ovarian teratoma formation. In these oocytes, the meiotic midbody cap structure is absent, permitting cytoplasmic contents to leak into the polar bodies, which can survive and undergo additional divisions, revealing unique defects in meiotic regulation and cytoplasmic partitioning.



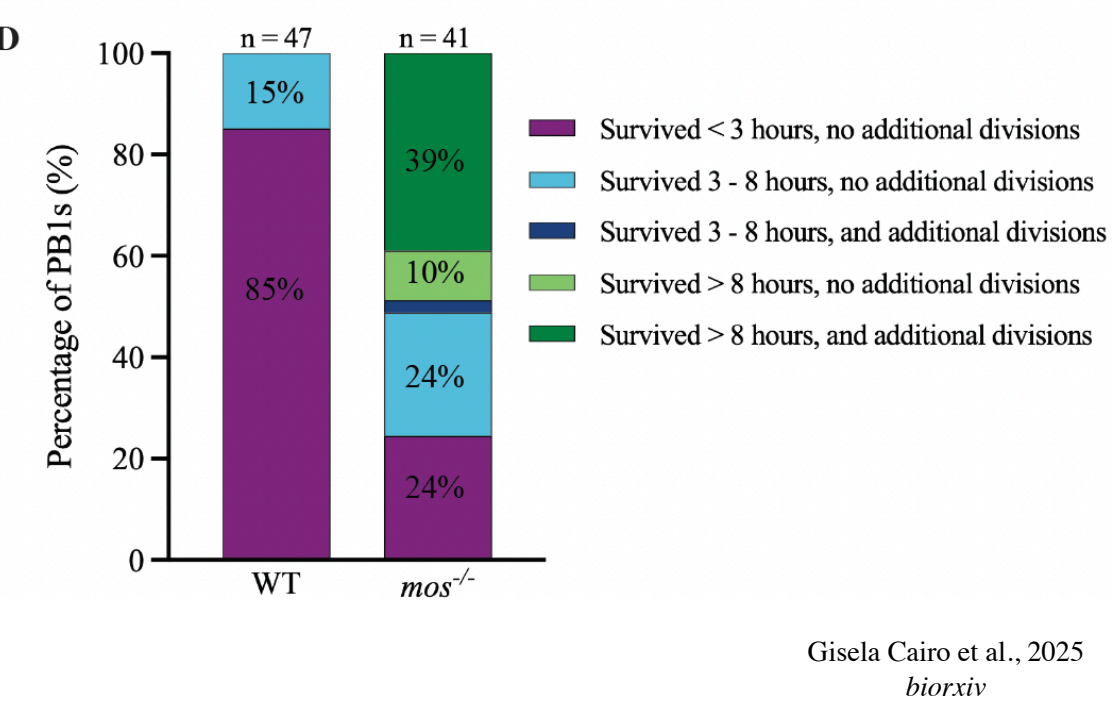
Mos knockout (*mos*^{-/-}) oocytes lack the cap component of the cap-and-ring meiotic midbody structure.



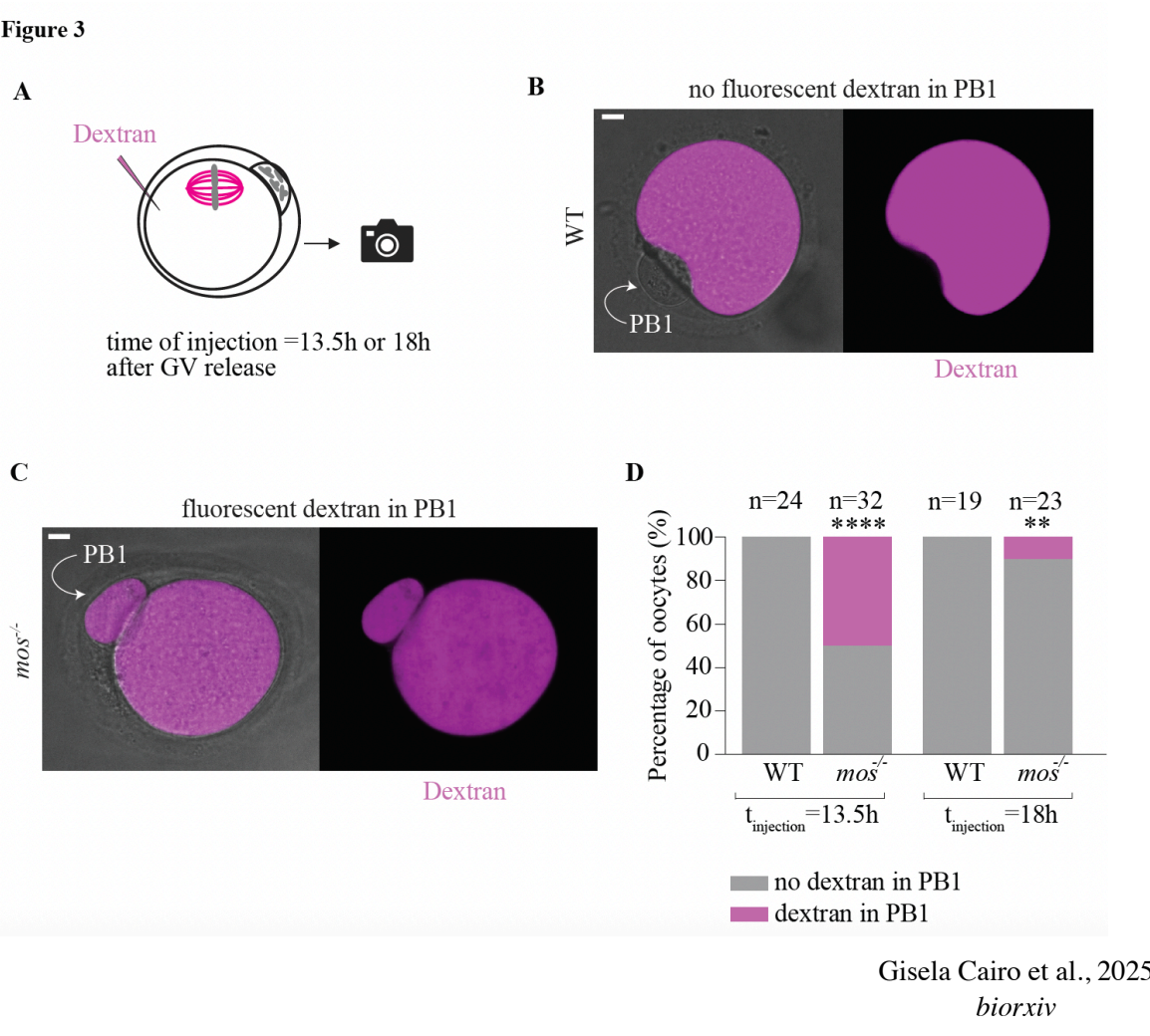
Approximately 30% of *mos*^{-/-} mice develop germ cell tumors between 4 and 8 months of age.



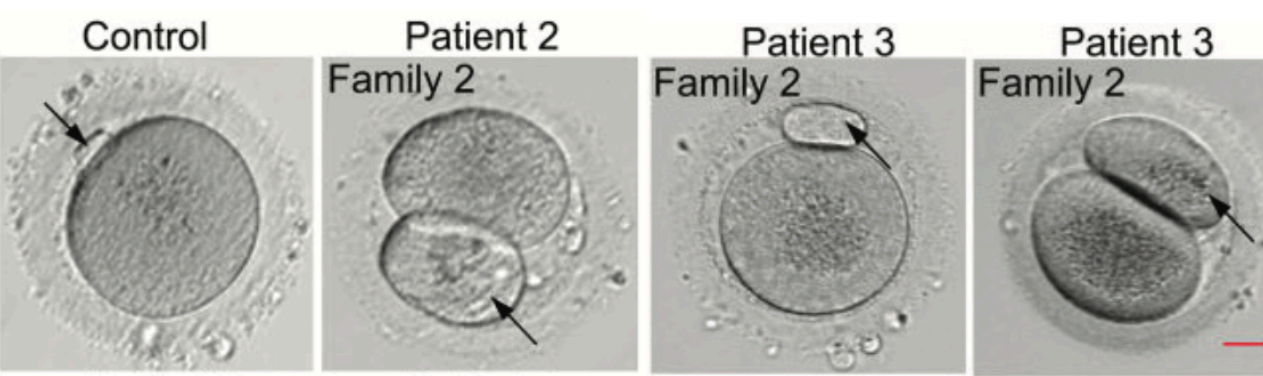
Isolated polar bodies from *mos*^{-/-} oocytes can survive in culture, segregate chromosomes, and develop into a multi-cellular cluster.



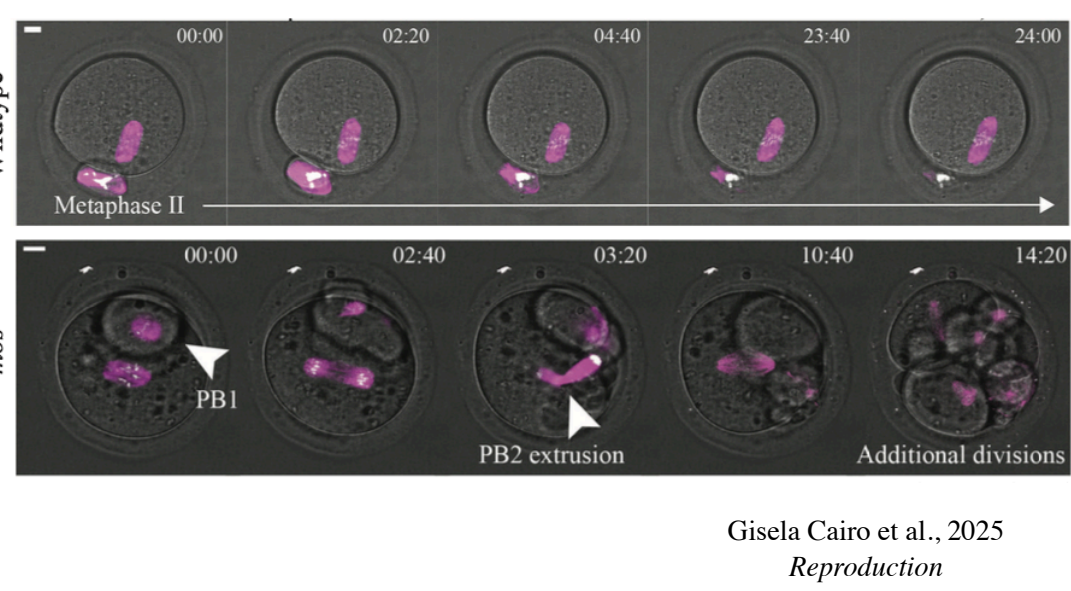
Compared with wild-type (WT) oocytes, *mos*^{-/-} polar bodies show increased survival past 8 hours and a greater tendency to undergo further divisions.



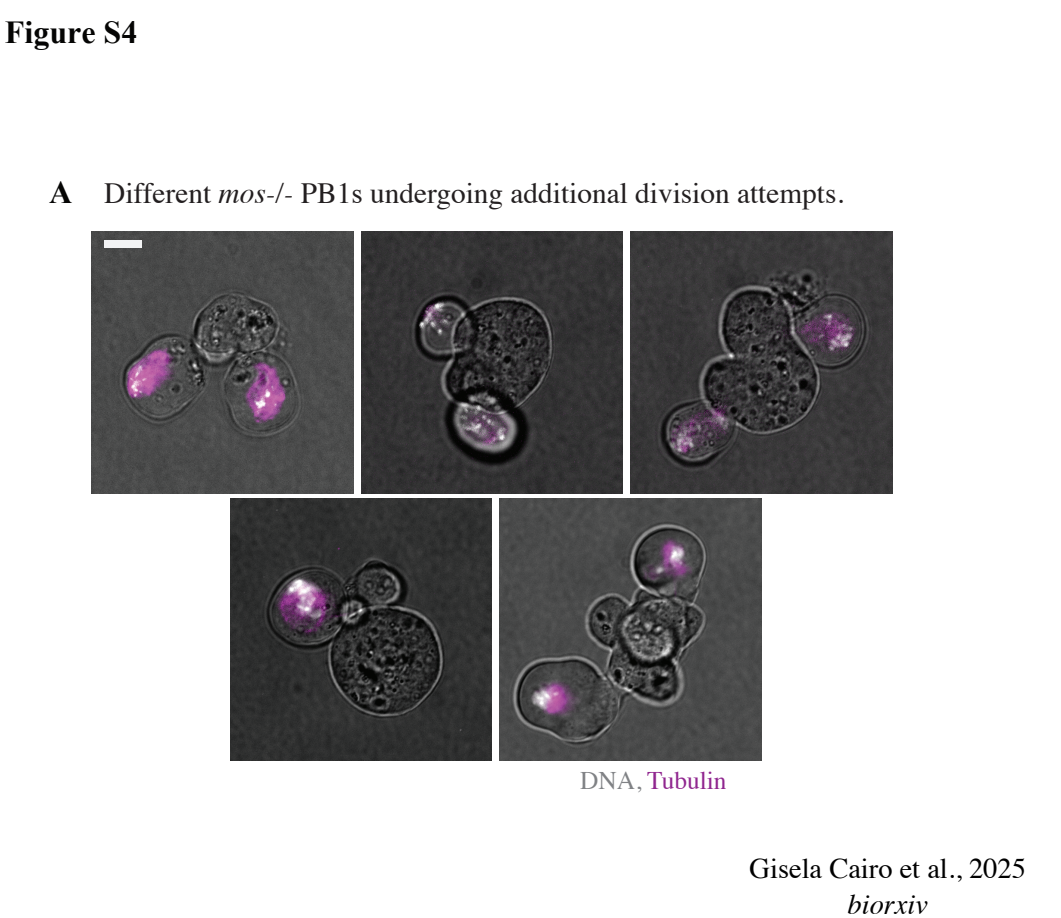
Dextran detected in the first polar body of *mos*^{-/-} oocytes indicates cytoplasmic leakage.



In vitro-cultured oocytes from *mos*-deficient females.



In contrast to WT eggs, which remain arrested at metaphase II, *mos*-deficient eggs continue meiosis, releasing a second polar body and undergoing further abnormal divisions.



Polar bodies from *mos* knockout (*mos*^{-/-}) oocytes develop a variety of abnormal multicellular morphologies.

Does the midbody cap prevent mitochondria from entering the polar body?

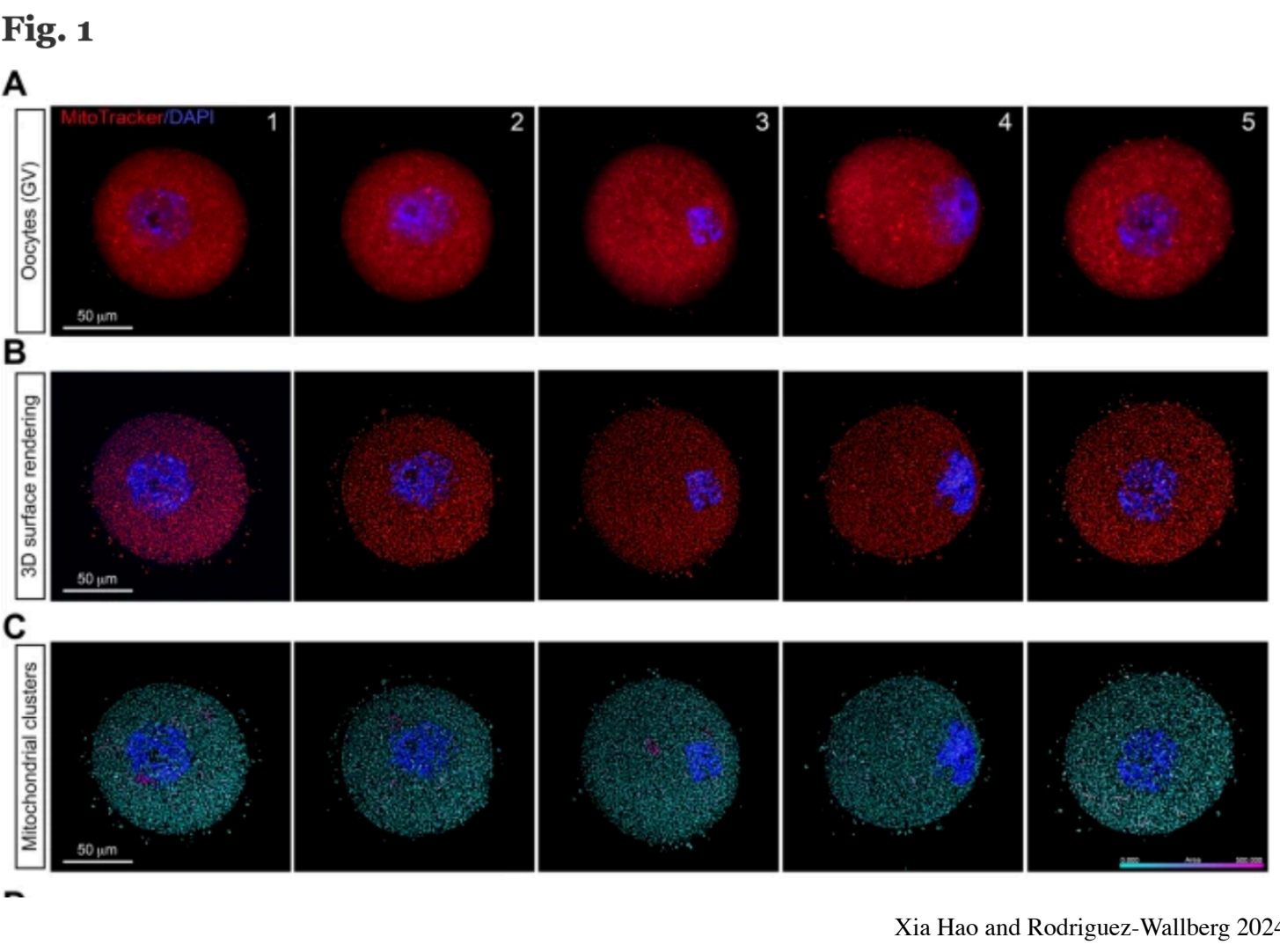
Mitochondria are typically excluded from the polar body and provide the energy necessary for cellular division.

Methodology

Oocyte Retrieval from Mice:

Ovaries were mechanically harvested from young (8-12 week old) female mice. Oocytes were initially incubated in minimal essential medium (MEM) with milrinone to maintain meiotic arrest at prophase I. Milrinone was then washed out of the medium and oocytes were release from arrest in CZB medium for meiotic resumption. Oocytes were incubated in CZB at 37C and 5% CO2 until completion of meiosis I, resulting in polar body extrusion and metaphase II (MII) eggs.

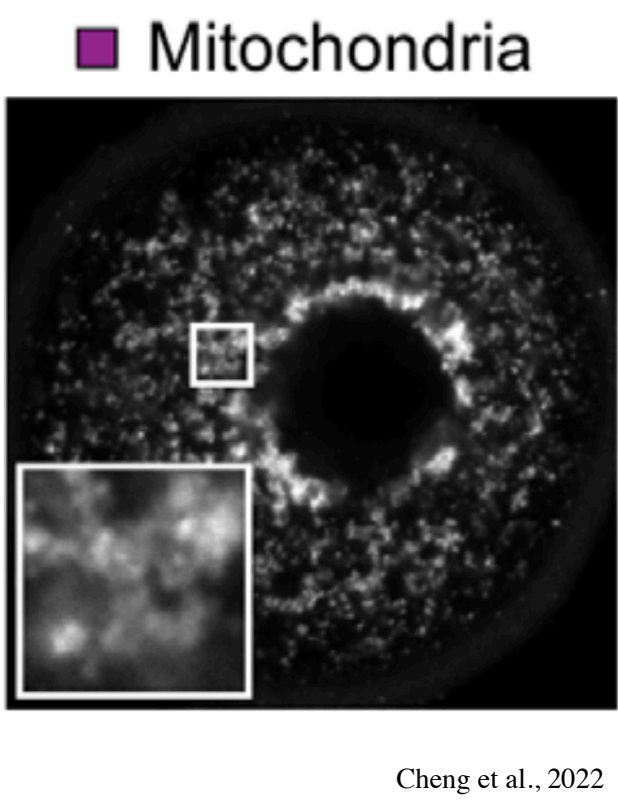
MitoTracker (a membrane permeable dye) was used to stain mitochondria in oocytes and to visualize their intracellular distribution.



The mitochondria have an intracellular pattern of diffuse puncta.

The use of MitoTracker allows for real-time visualization of oocytes as they undergo polar body extrusion. Live imaging with Mitotracker was performed; however, several challenges were encountered, including cytotoxic effects that resulted in premature tubulin disassembly.

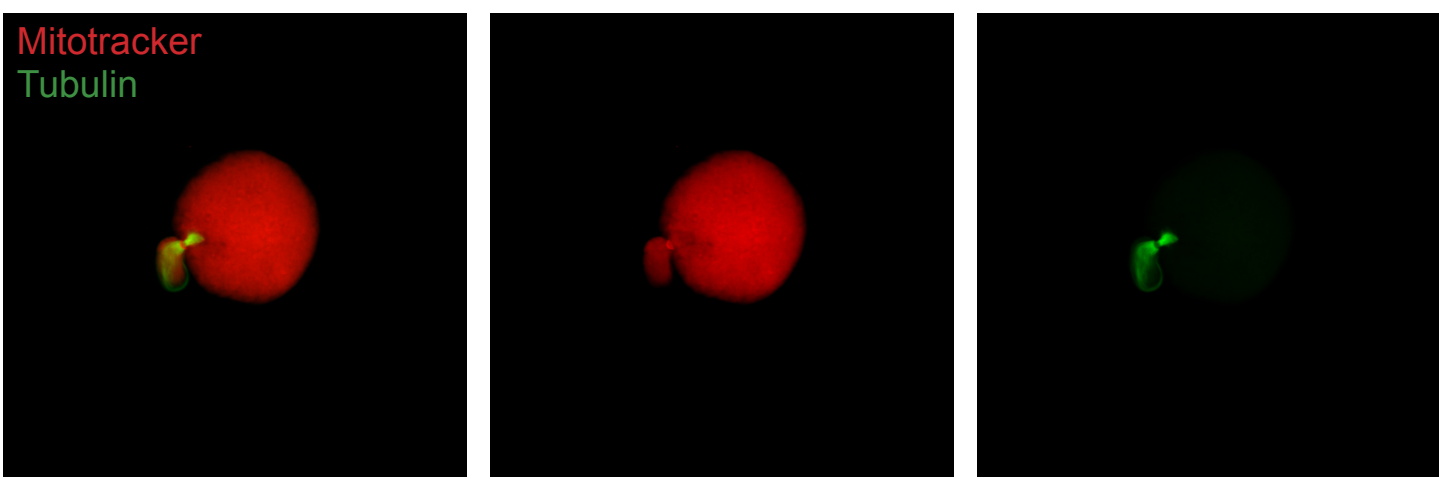
Due to the challenges with MitoTracker, we employed a cytochrome c antibody for mitochondrial staining. Although this approach did not permit live imaging, it yielded improved staining quality and reproducibility.



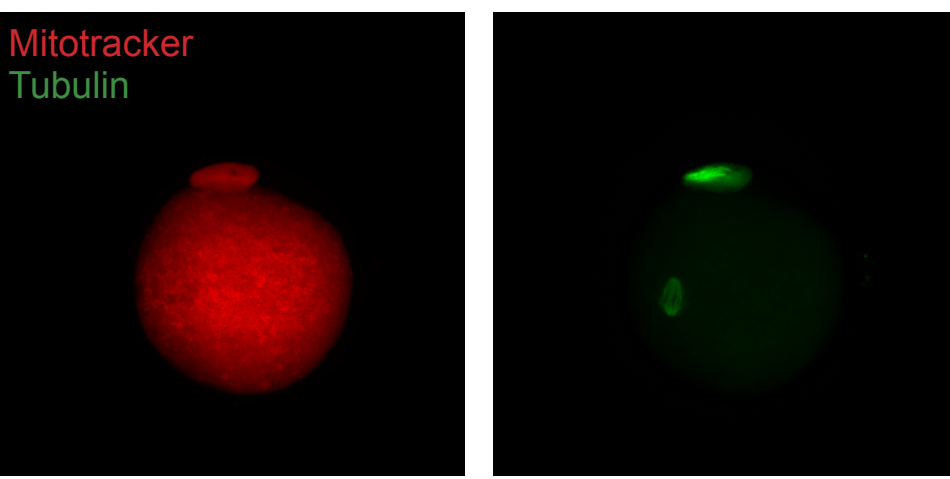
Mitochondria in an oocyte arrested at prophase I is visualized through anti cytochrome c staining.

With using the anti cytochrome c antibodies, we were able to visualize a similar peri-nuclear distribution of puncta and did not observe any cytotoxic effects.

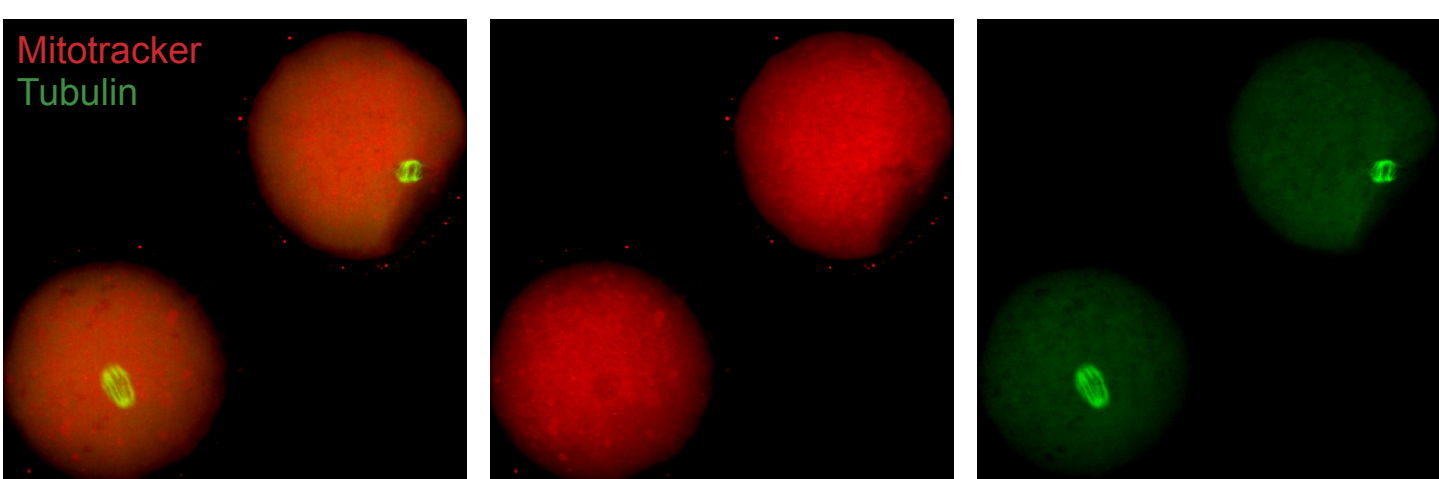
Results



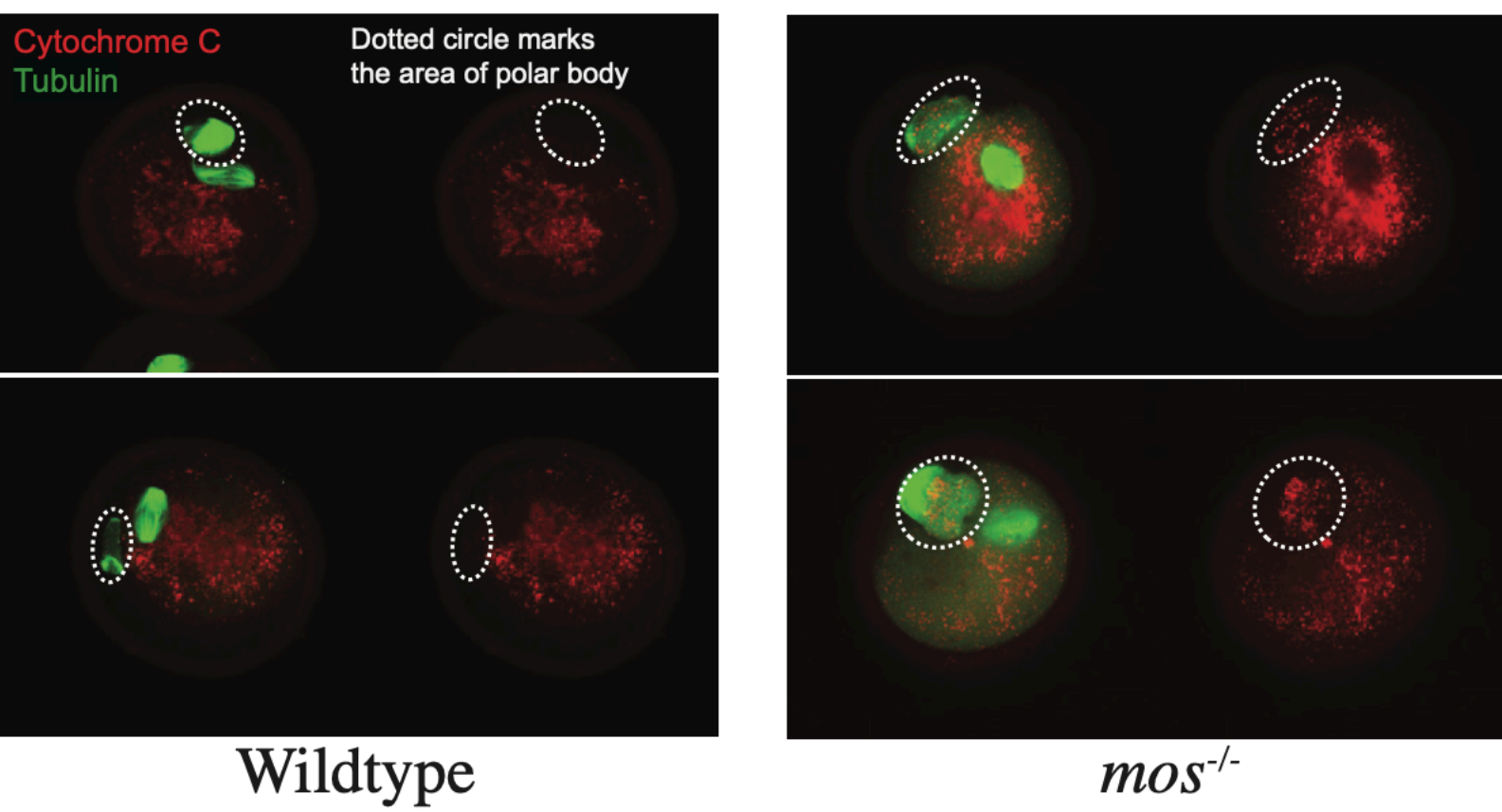
In WT MII eggs, MitoTracker staining reveals the characteristic midbody cap structure.



Polar bodies of WT MII eggs were also stained with MitoTracker.



In WT oocytes, MitoTracker exposure resulted in premature tubulin disassembly.



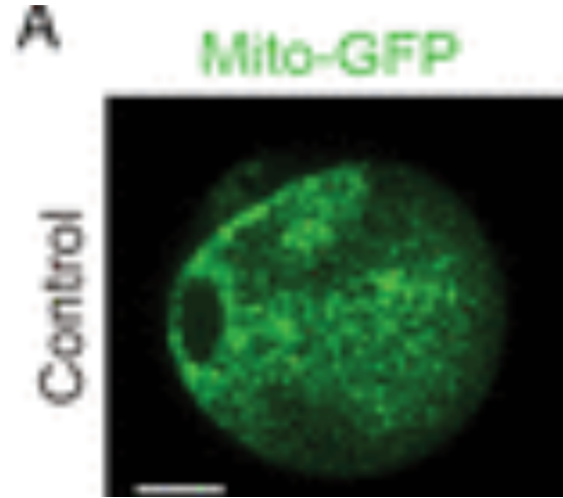
In contrast to WT MII eggs, mitochondria are able to leak into the polar bodies of *mos*^{-/-} MII eggs.

Conclusion

Therefore, the leakage of mitochondria into the polar bodies of *mos*^{-/-} eggs—resulting from the absence of the cap component of the meiotic midbody structure—may enhance polar body survival and subsequently contribute to ovarian teratoma formation through continued divisions.

Beyond mitochondria, might other organelles like lysosomes and peroxisomes also contribute to polar body survival?

In order to perform live tracking of mitochondria movement in future experiments, mRNA of Mito-GFP can be injected into the oocytes, which is a GFP tagged with a mitochondria localization signal.



Microinjecting with fluorescent proteins is not expected to be cytotoxic and has been previously used to track organelles in oocytes.