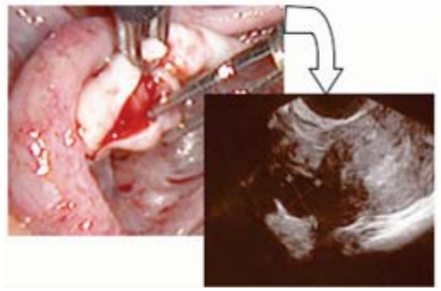
ULB UNIVERSITÉ LIBRE DE BRUXELLES

LABORATOIRE DE RECHERCHE
EN REPRODUCTION HUMAINE

Ultrastructural and functional analysis of human early stage follicles cultured with everolimus

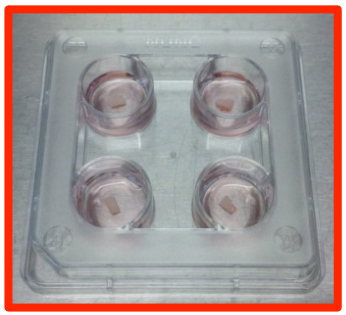
Johanne Grosbois

Research Laboratory on Human Reproduction, ULB

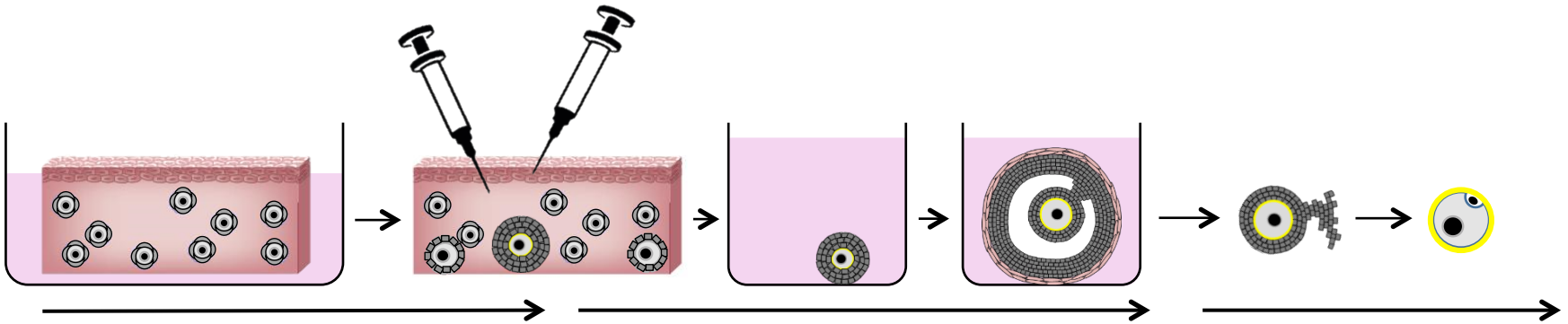


Transplantation

Cryopreserved ovarian tissue



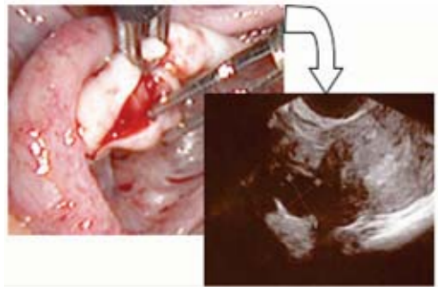
In vitro culture



Step 1:
Primordial follicle activation and growth until the preantral stage

Step 2:
Isolated preantral follicles are further cultured until the antral stage

Step 3:
Oocyte retrieval and in vitro maturation



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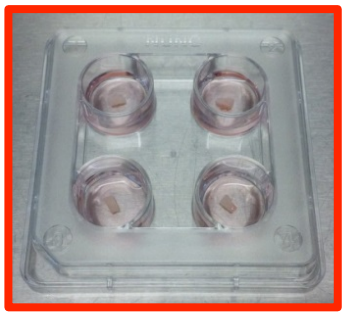
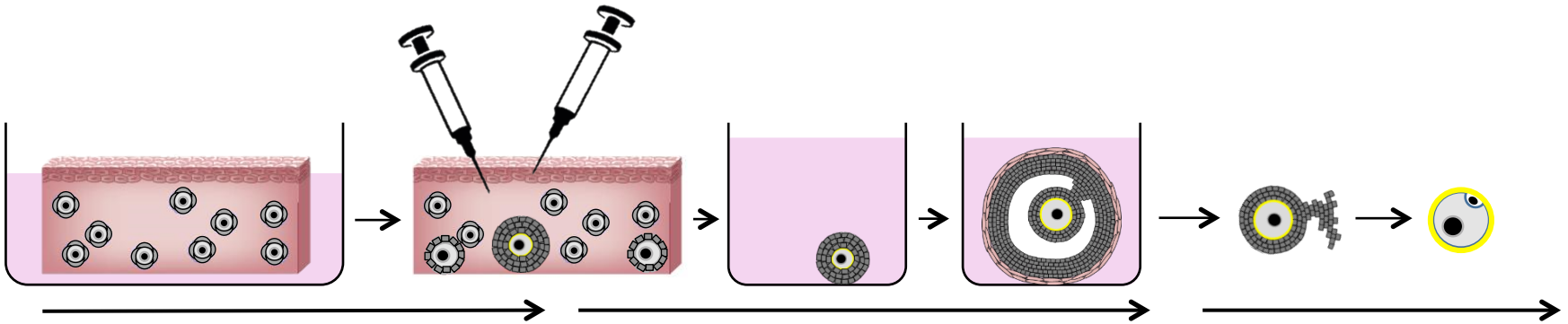


In vitro culture



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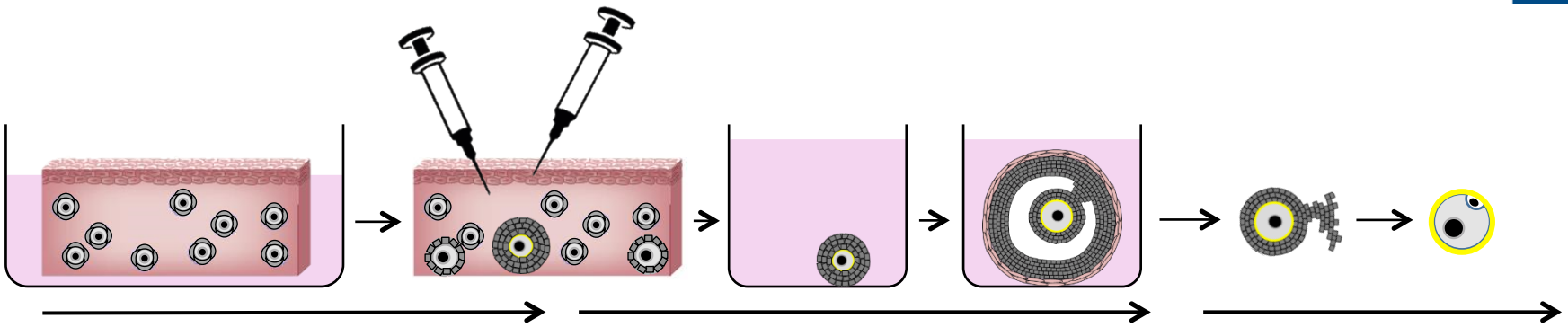


Step 1:
Primordial follicle activation and growth until the preantral stage

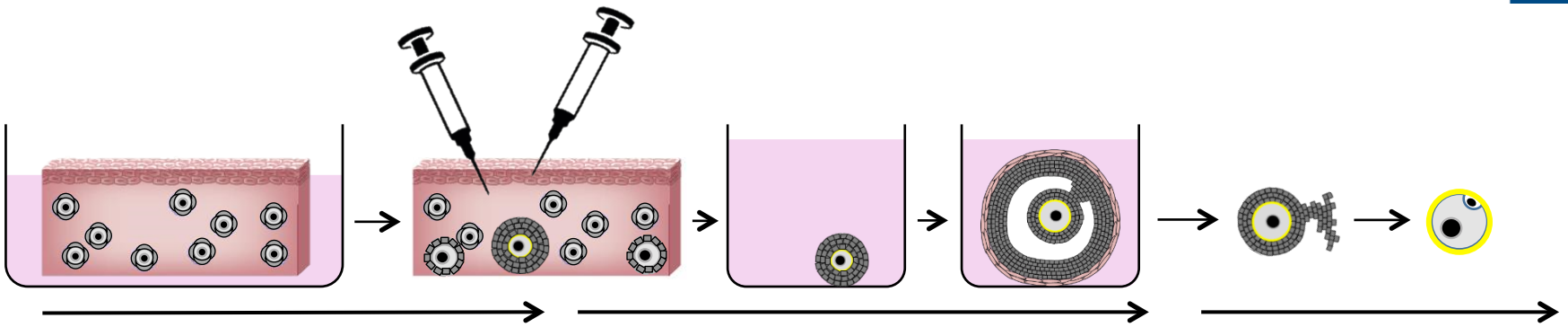
Step 2:
Isolated preantral follicles are further cultured until the antral stage

Step 3:
Oocyte retrieval and in vitro maturation

→ Critical step :

→ exploiting the full reproductive potential of human ovarian tissue

→ improving the outcome of early in vitro growth



Step 1:
Primordial follicle activation and growth until the preantral stage

Step 2:
Isolated preantral follicles are further cultured until the antral stage

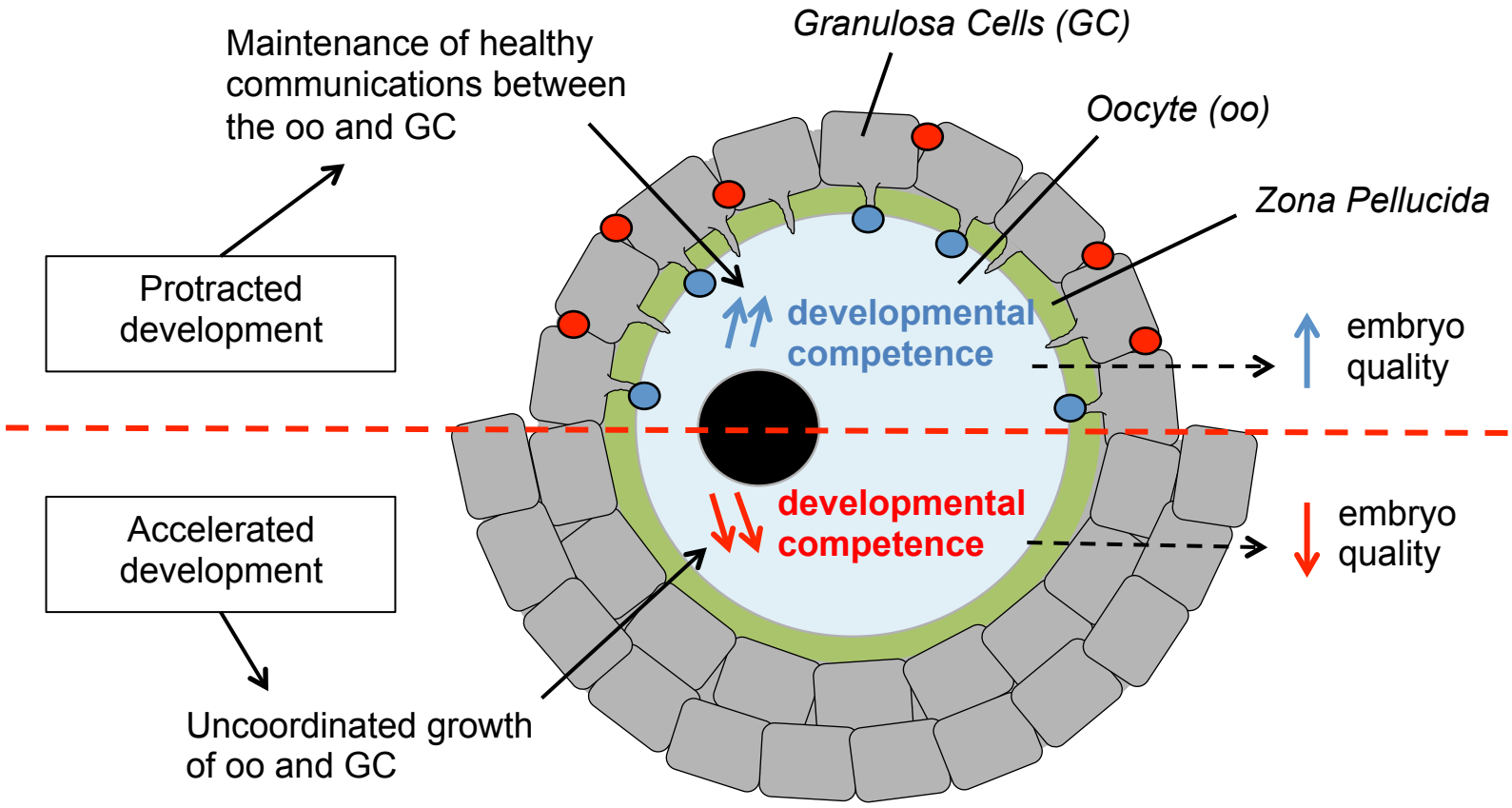
Step 3:
Oocyte retrieval and in vitro maturation

- Spontaneous activation of primordial follicles *in vitro* *(Wandji et al, 1996; Braw-Tal et Yossefi, 1997; Fortune et al, 1998; Hovatta et al, 1997)*

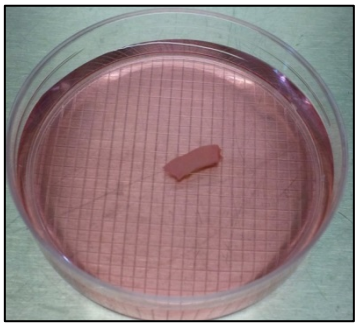
- Clinical application : "reawakened ovaries" (Kawamura et al, 2013; Suzuki et al, 2015; Zhai et al., 2016; Fabregues et al., 2018)

  … **BUT it has been associated with follicular damage** (McLaughlin *et al.*, 2014; Lerer-Serfaty *et al.*, 2013; Grosbois and Demeestere, 2018)

Hypothesis: supporting a protracted culture system may improve oocyte quality and its future acquisition of competence

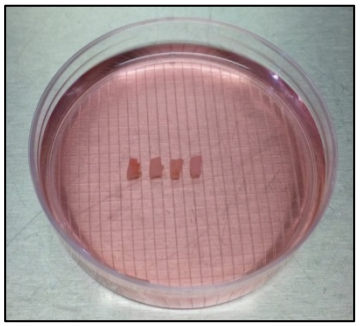


Thawing



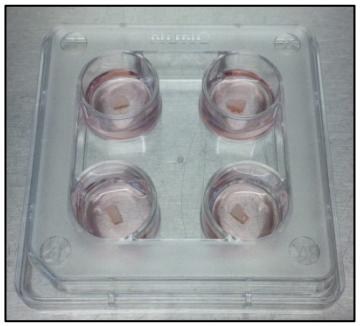
Cryopreserved ovarian cortex

Micro cortex



4 x 2 x 1 mm^3 fragments

Culture



Up to 6 days

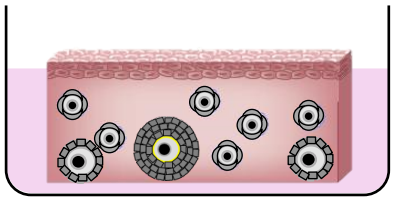
Control medium **DMSO**

mTOR inhibitor **Everolimus (EVE)**

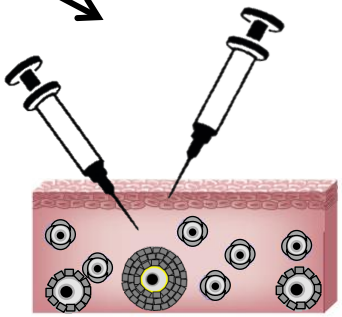
Ultrastructure

Intra-follicular communication

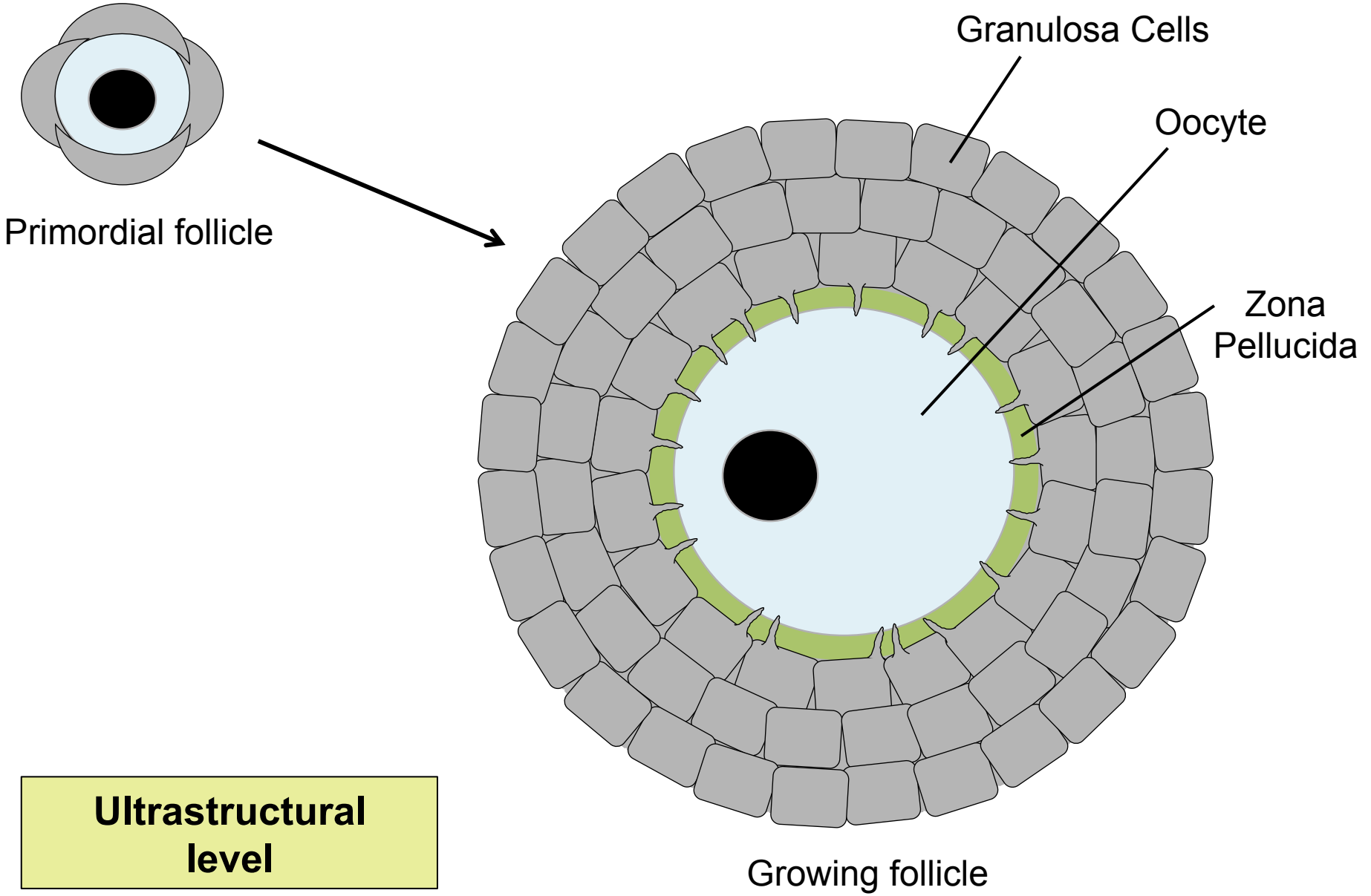
Development



Fixation of the tissue

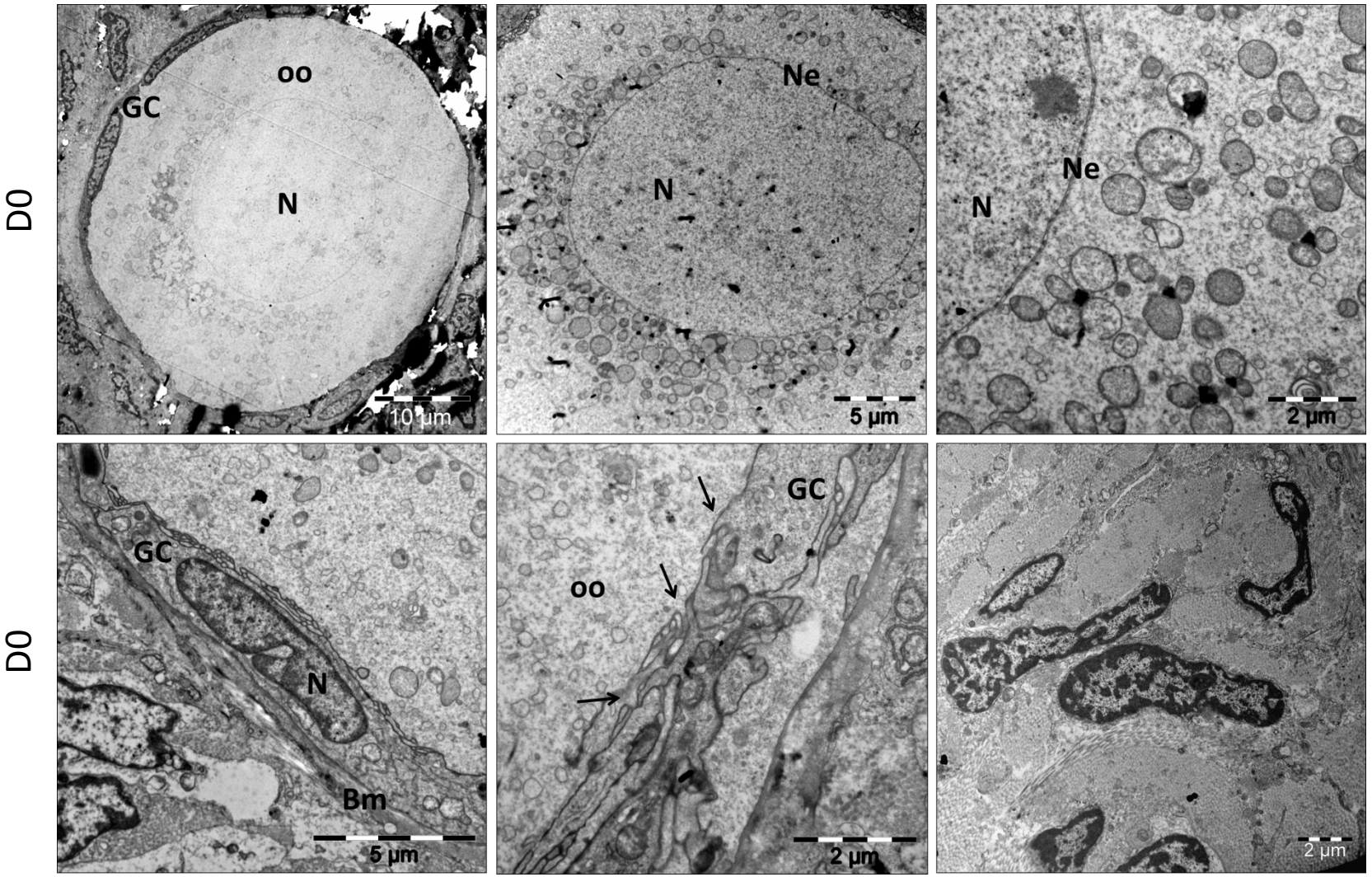


Follicular isolation and fixation or RNA extraction



Ultrastructural level

Ultrastructure at thawing (D0)



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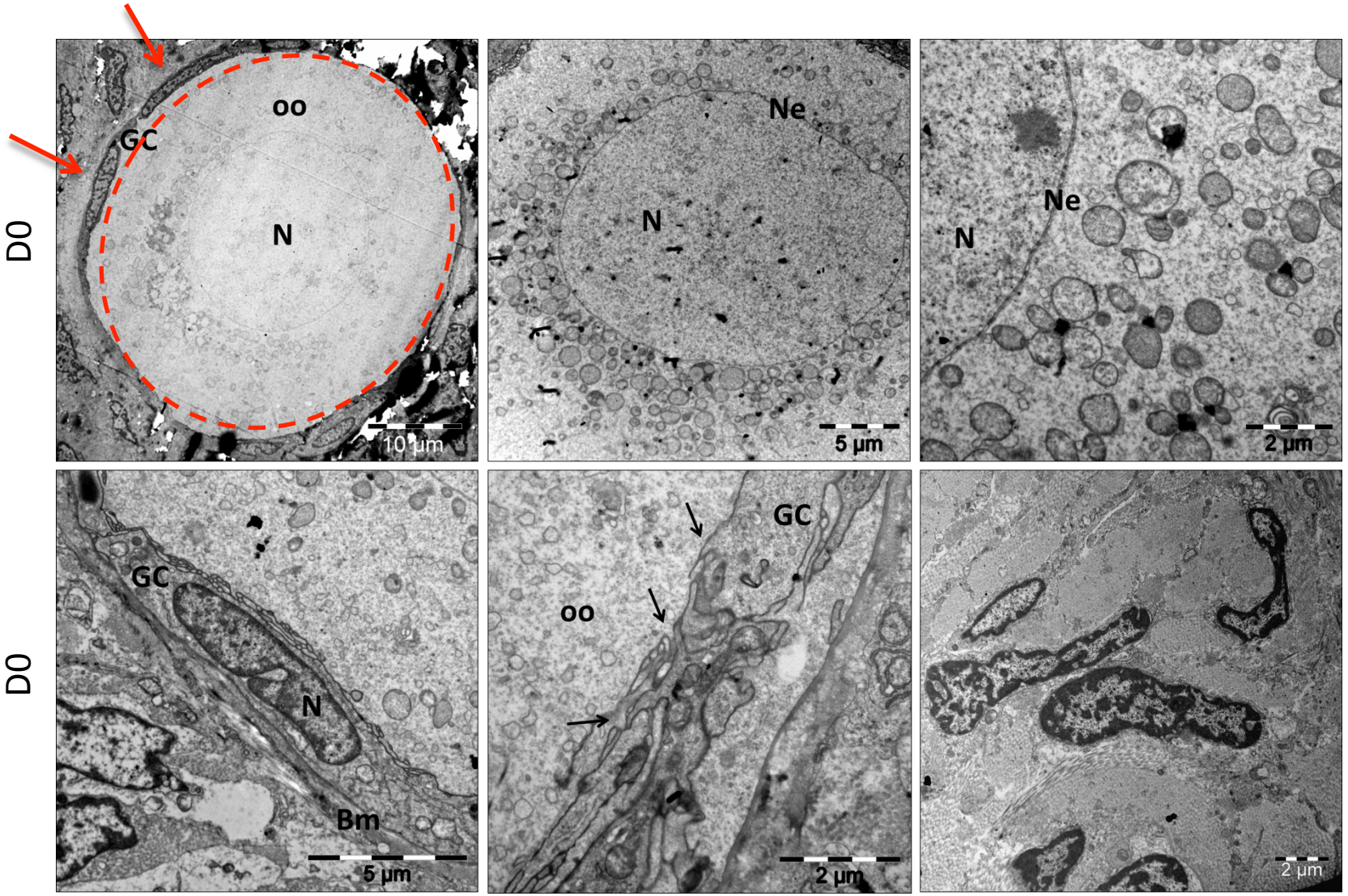

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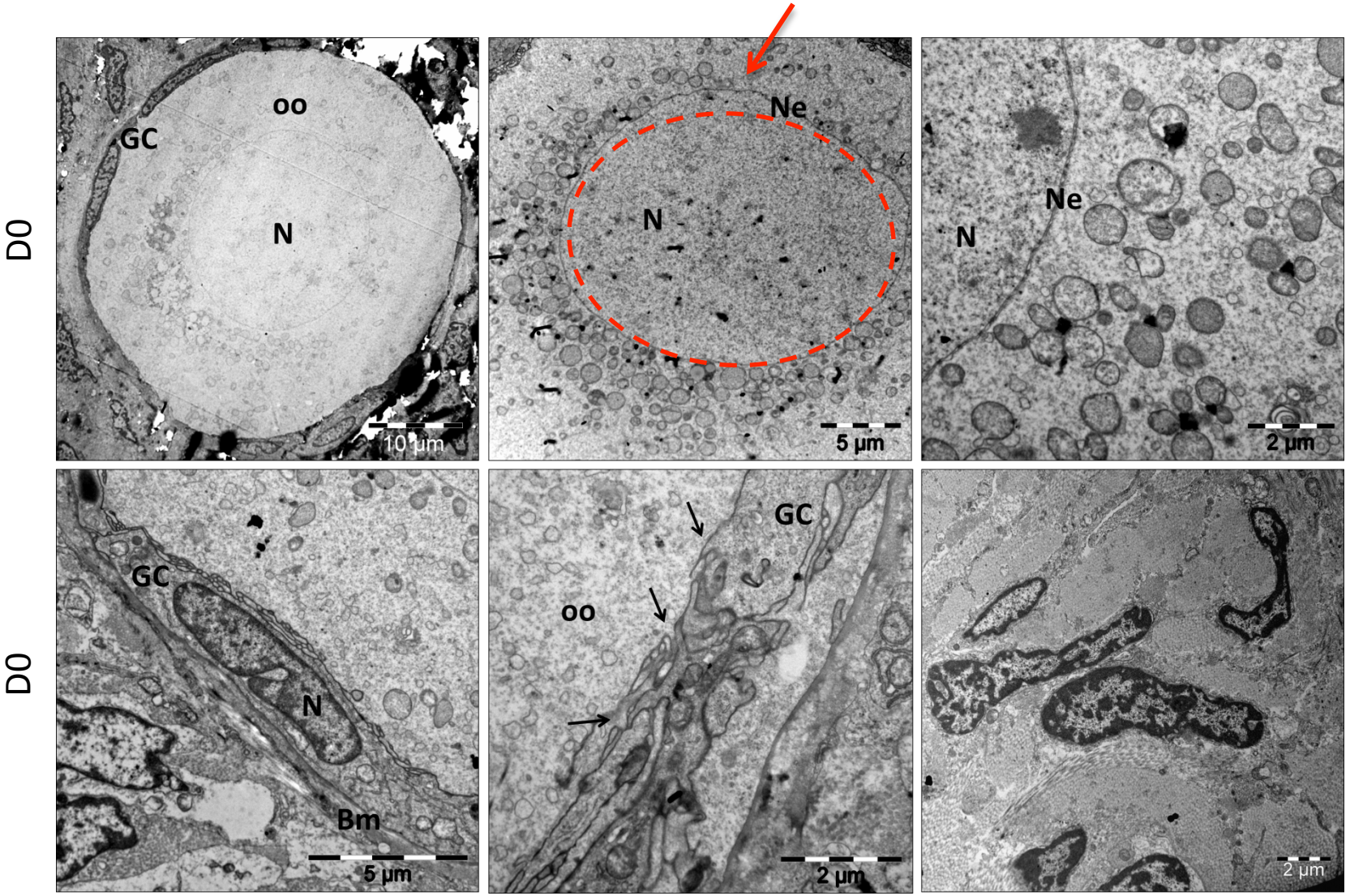

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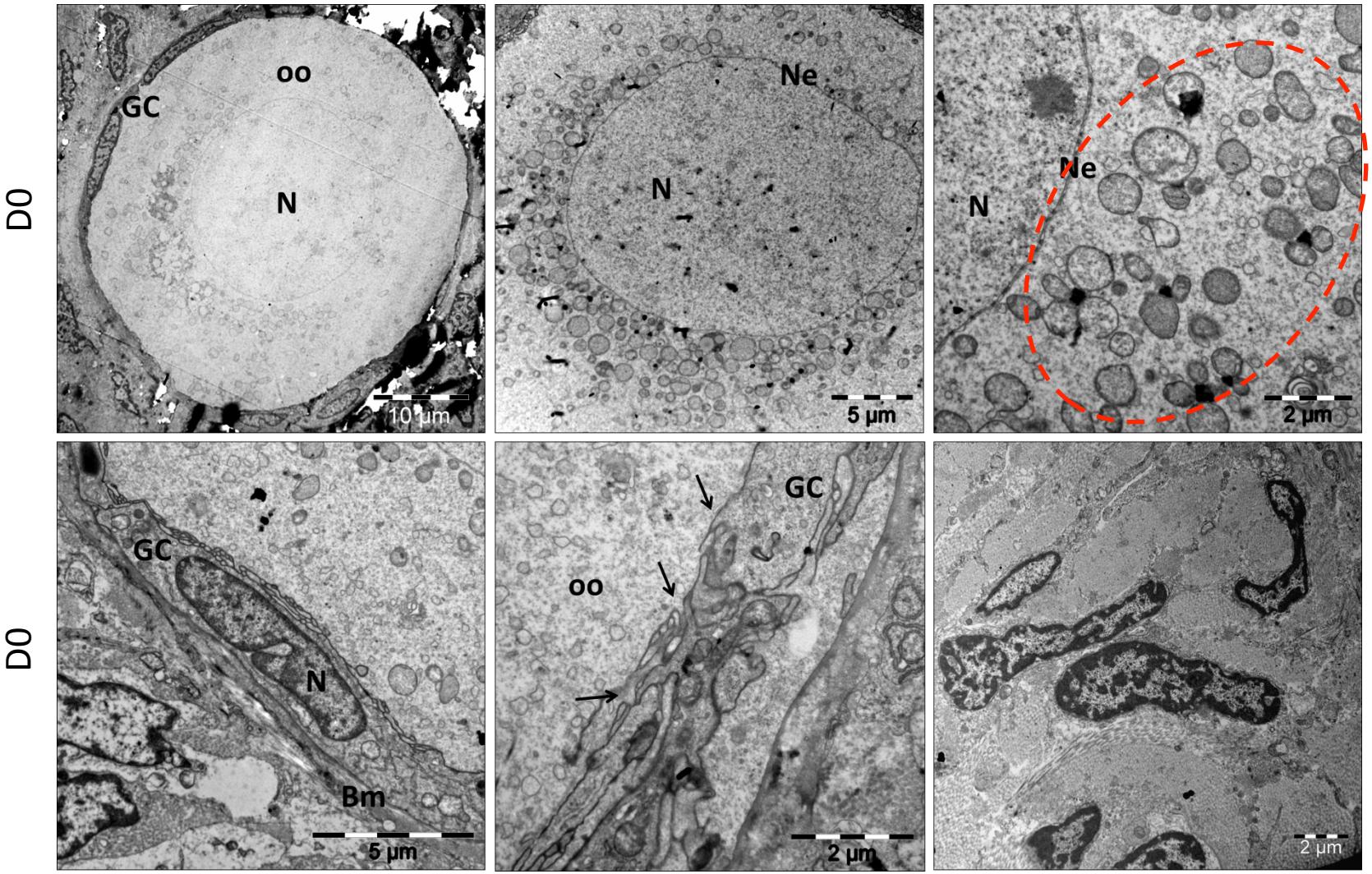

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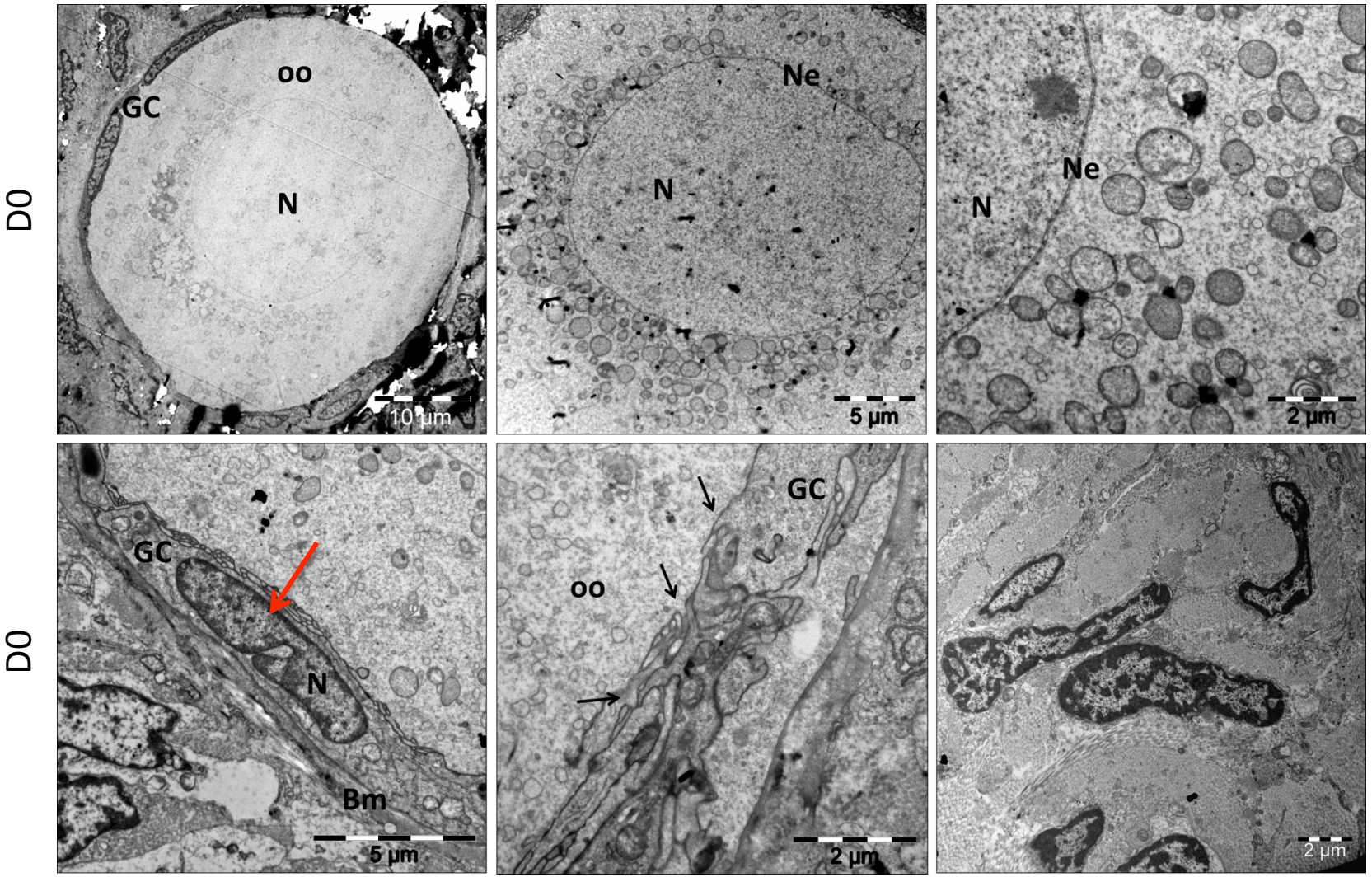

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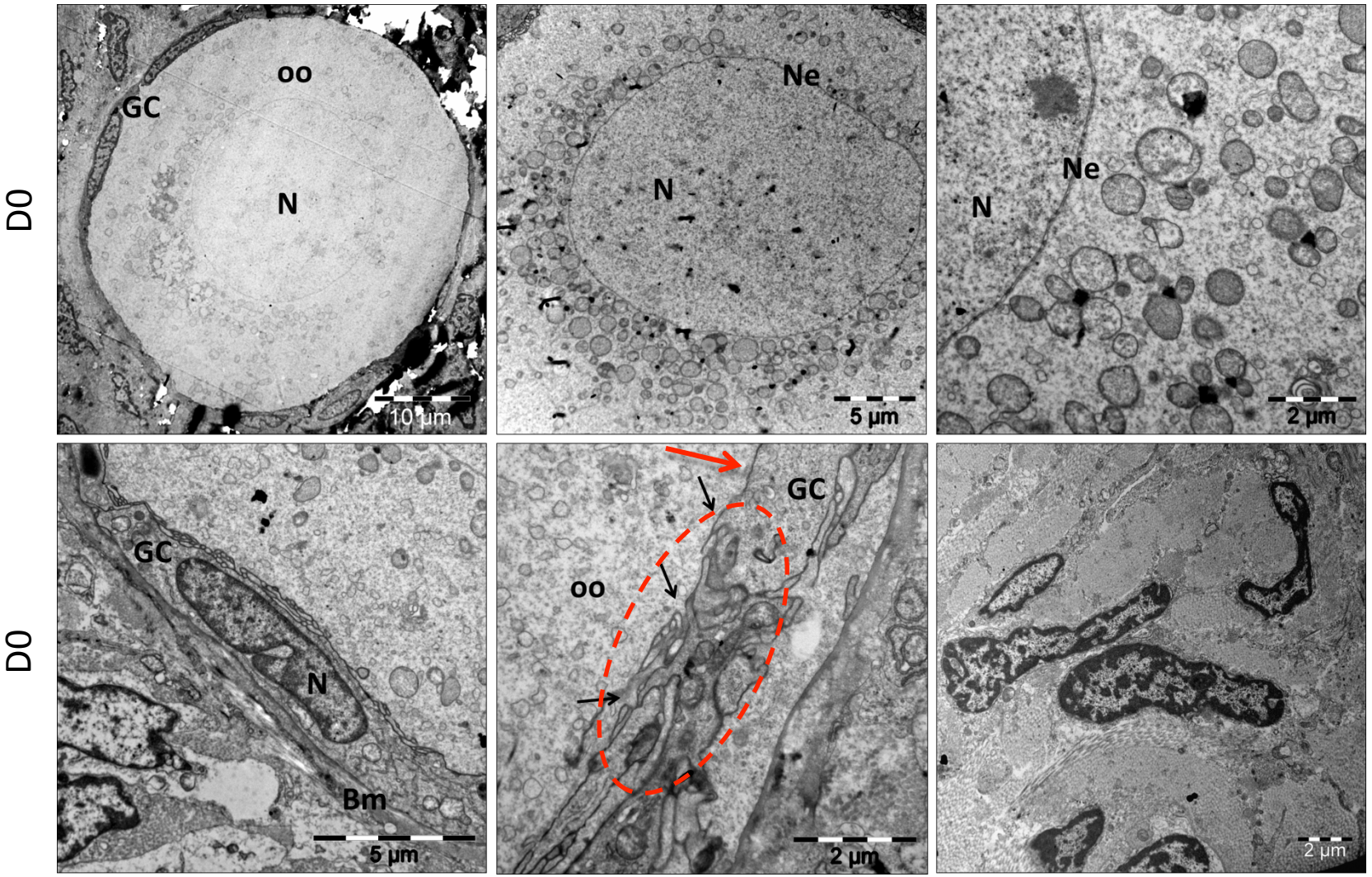

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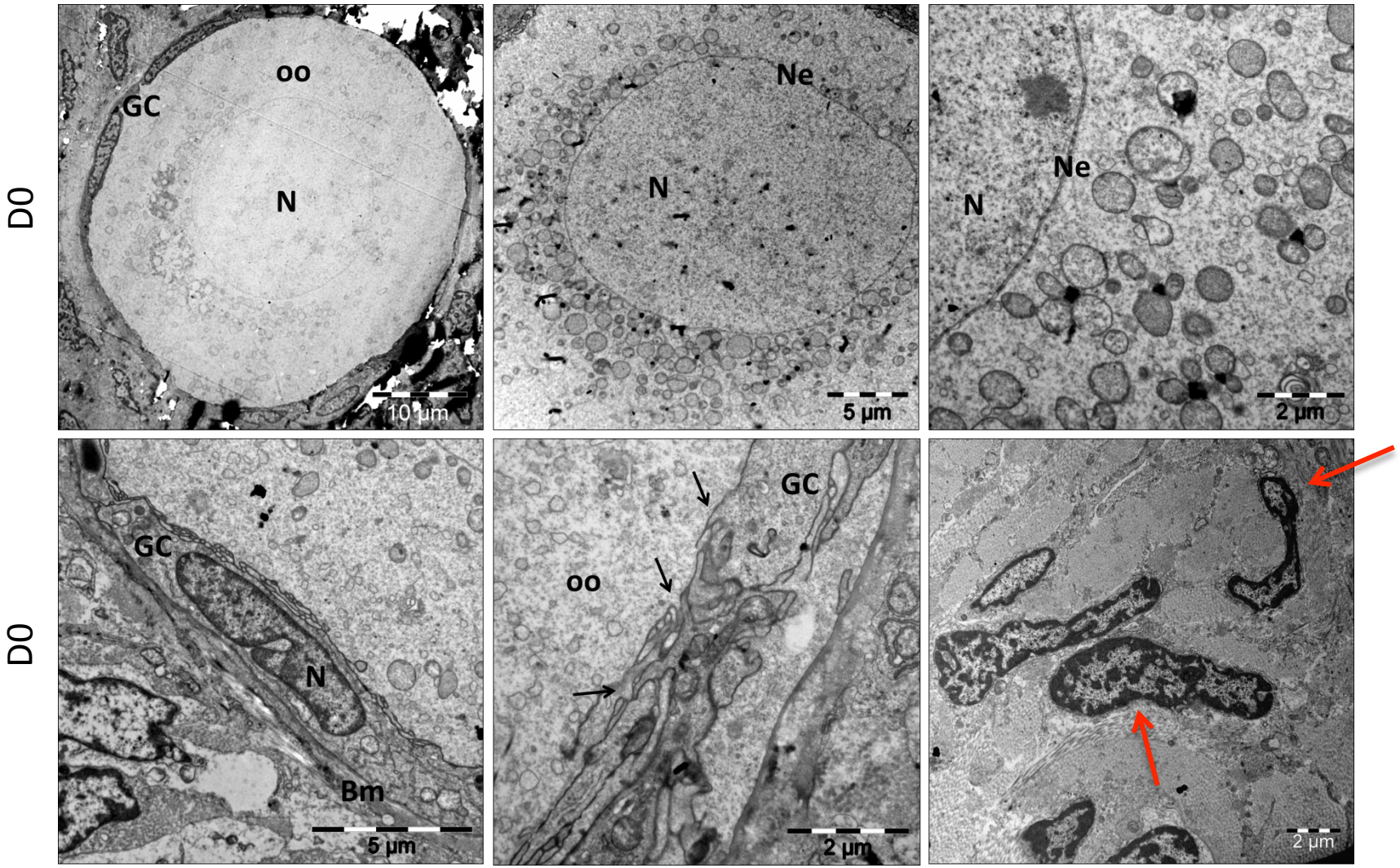
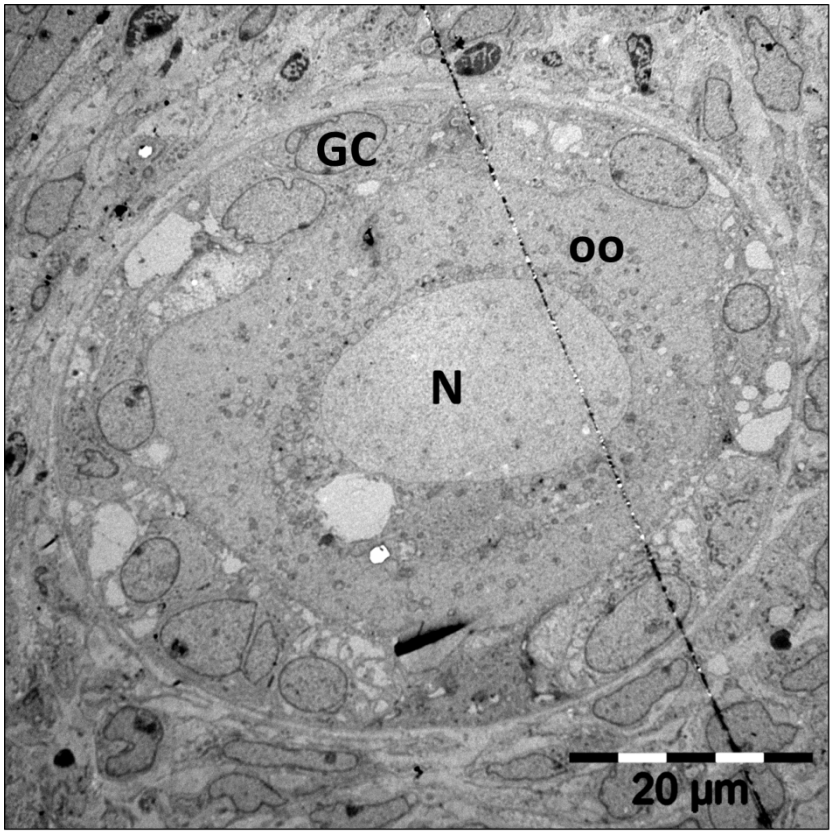
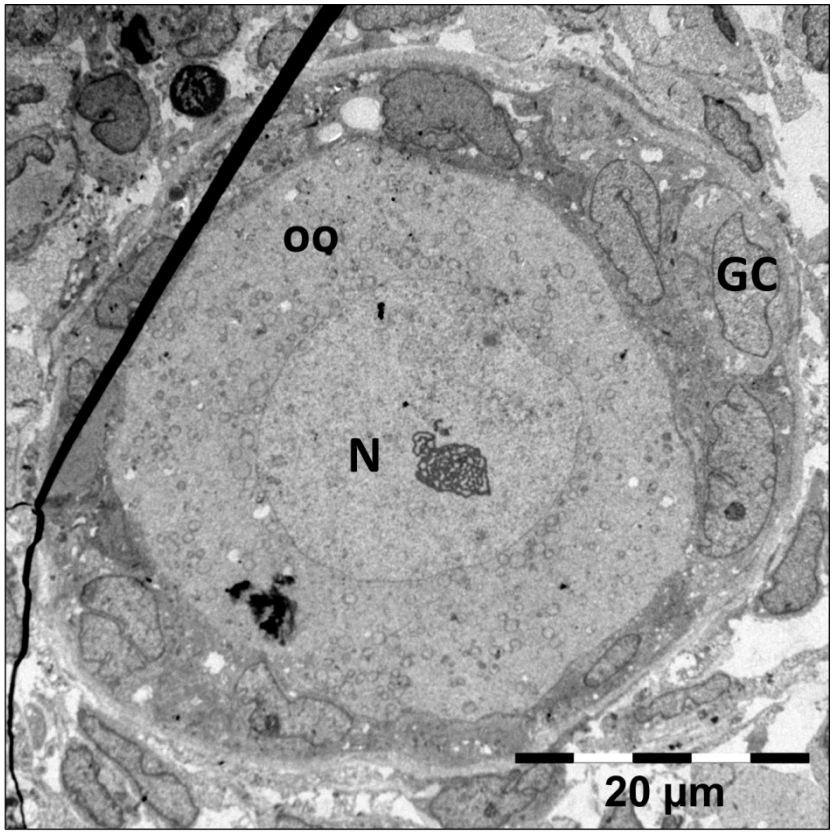


Short term exposure to EVE does not impair follicular ultrastructure

D6 Control



D6 EVE



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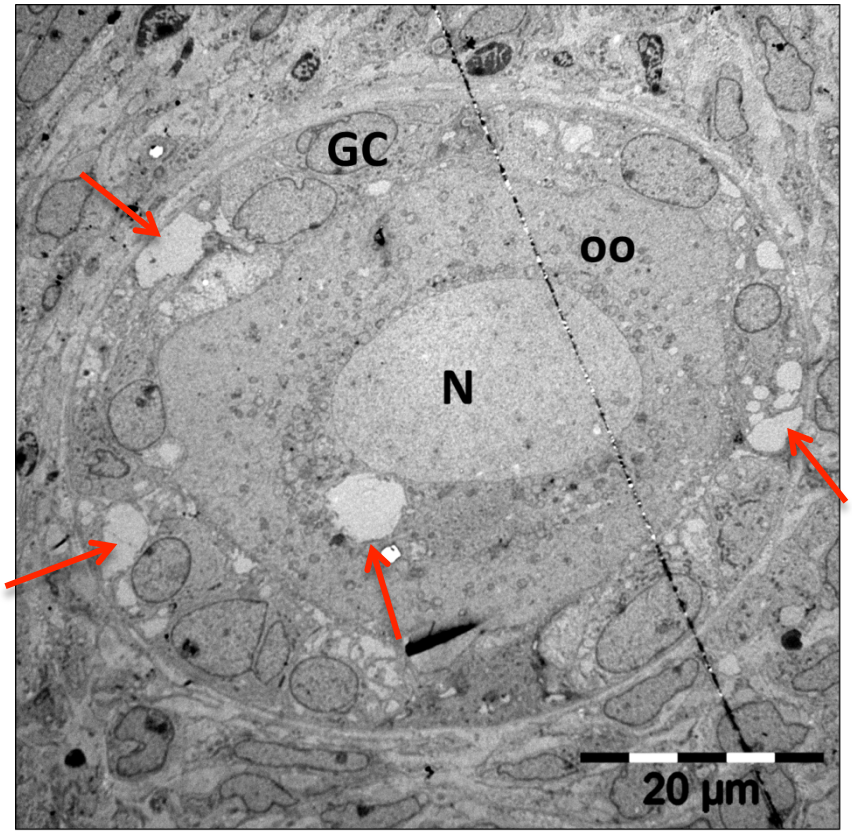

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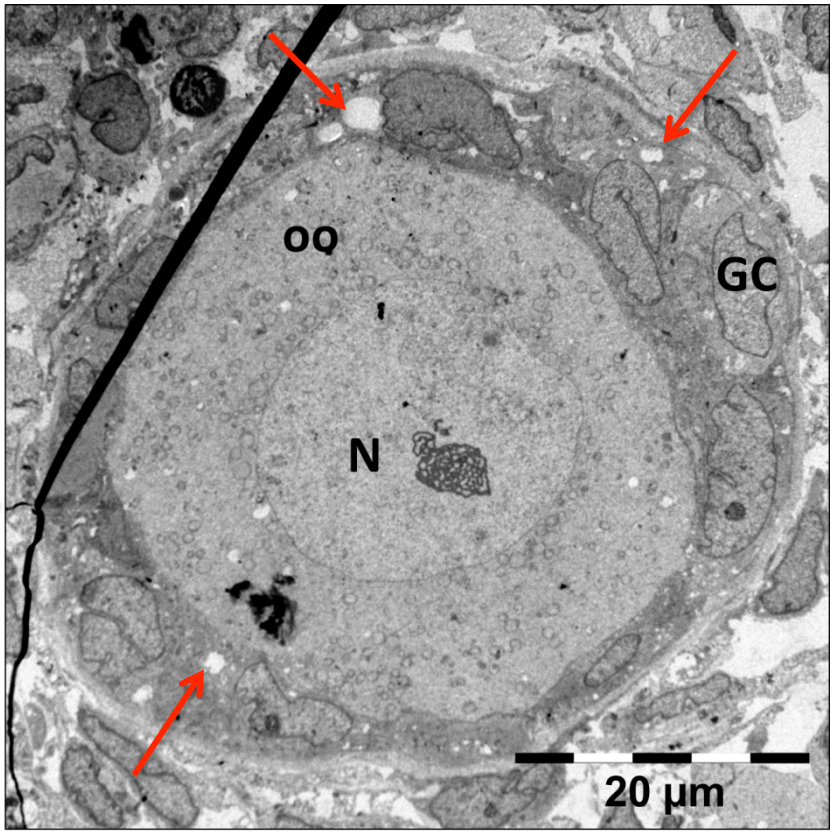

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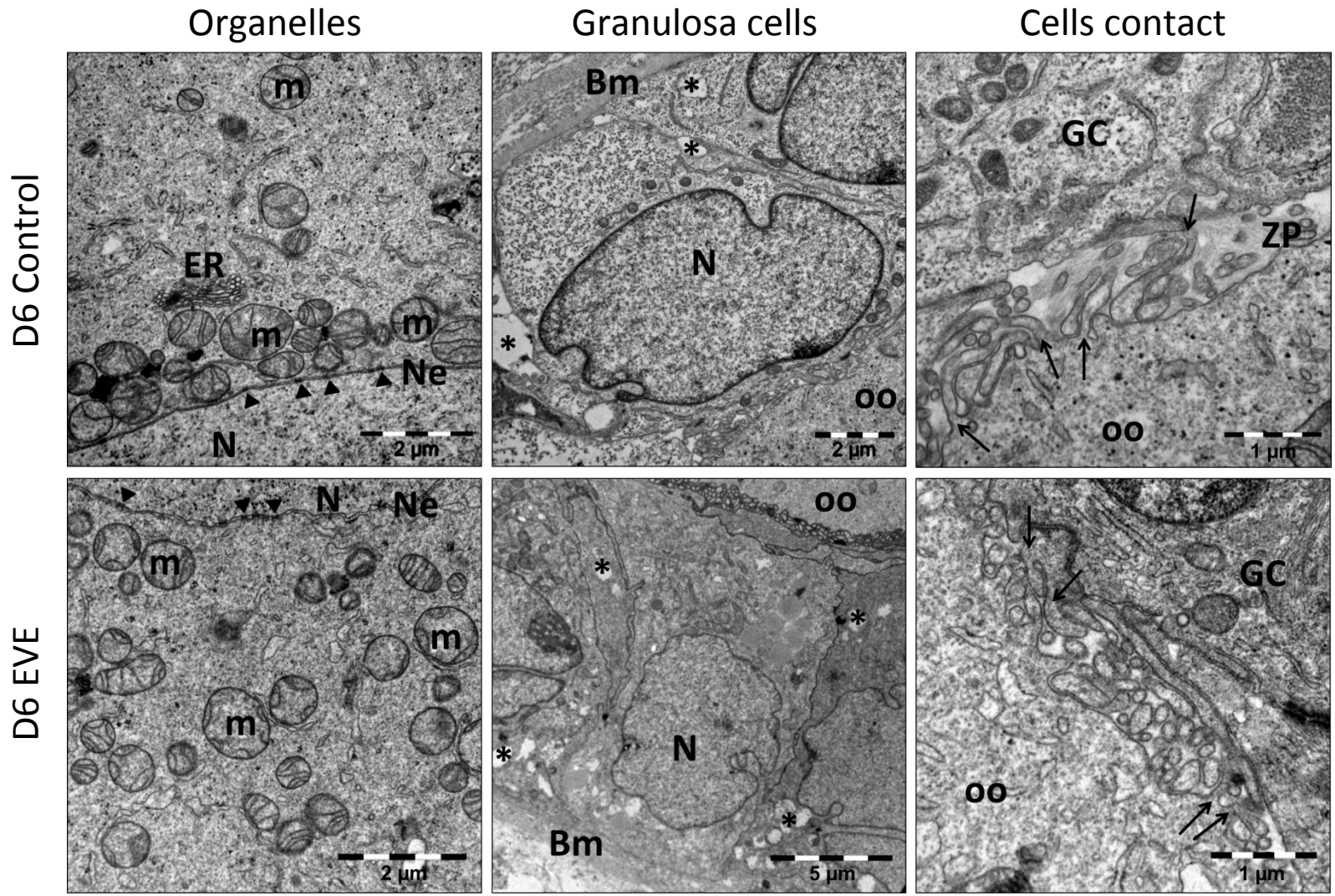

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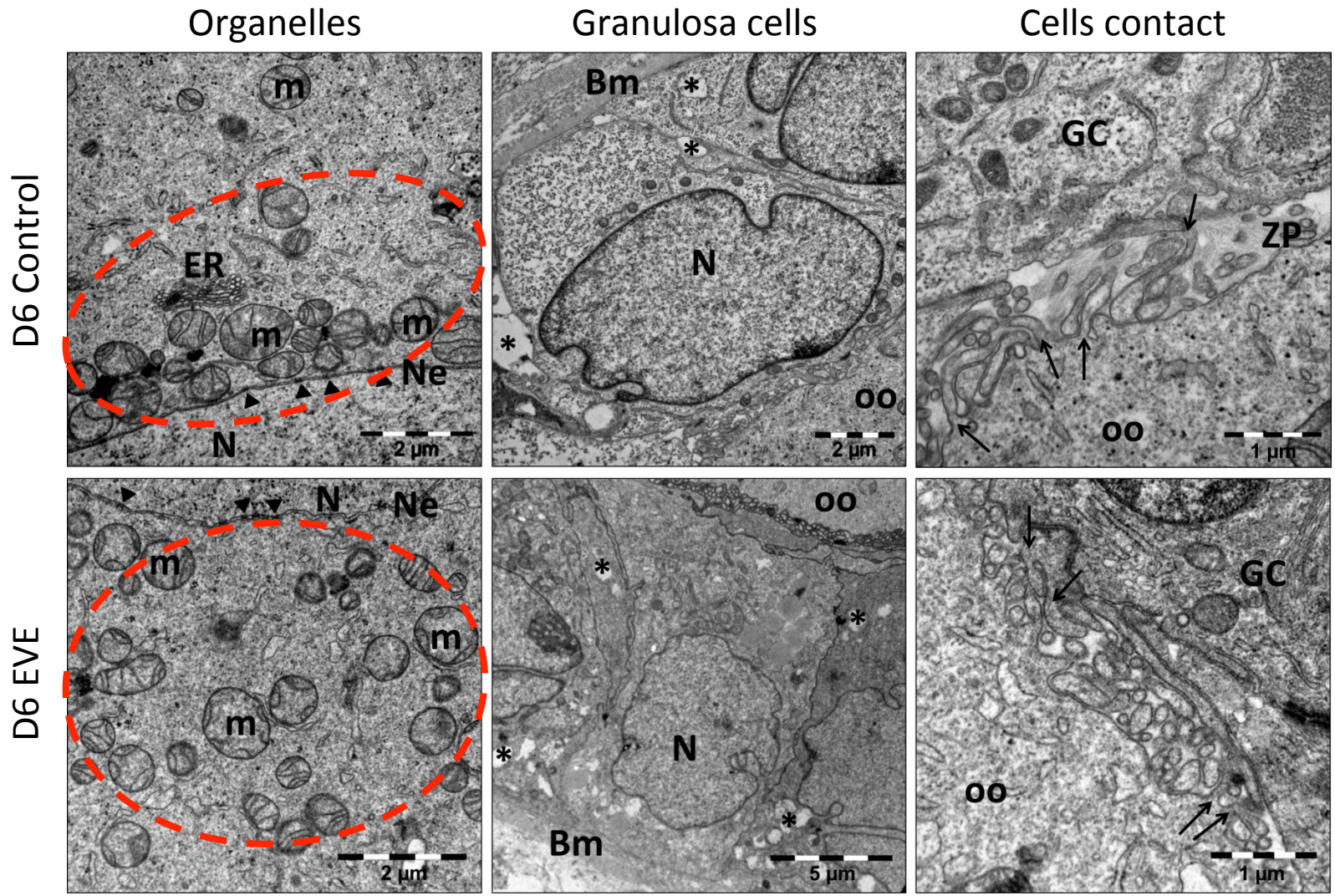

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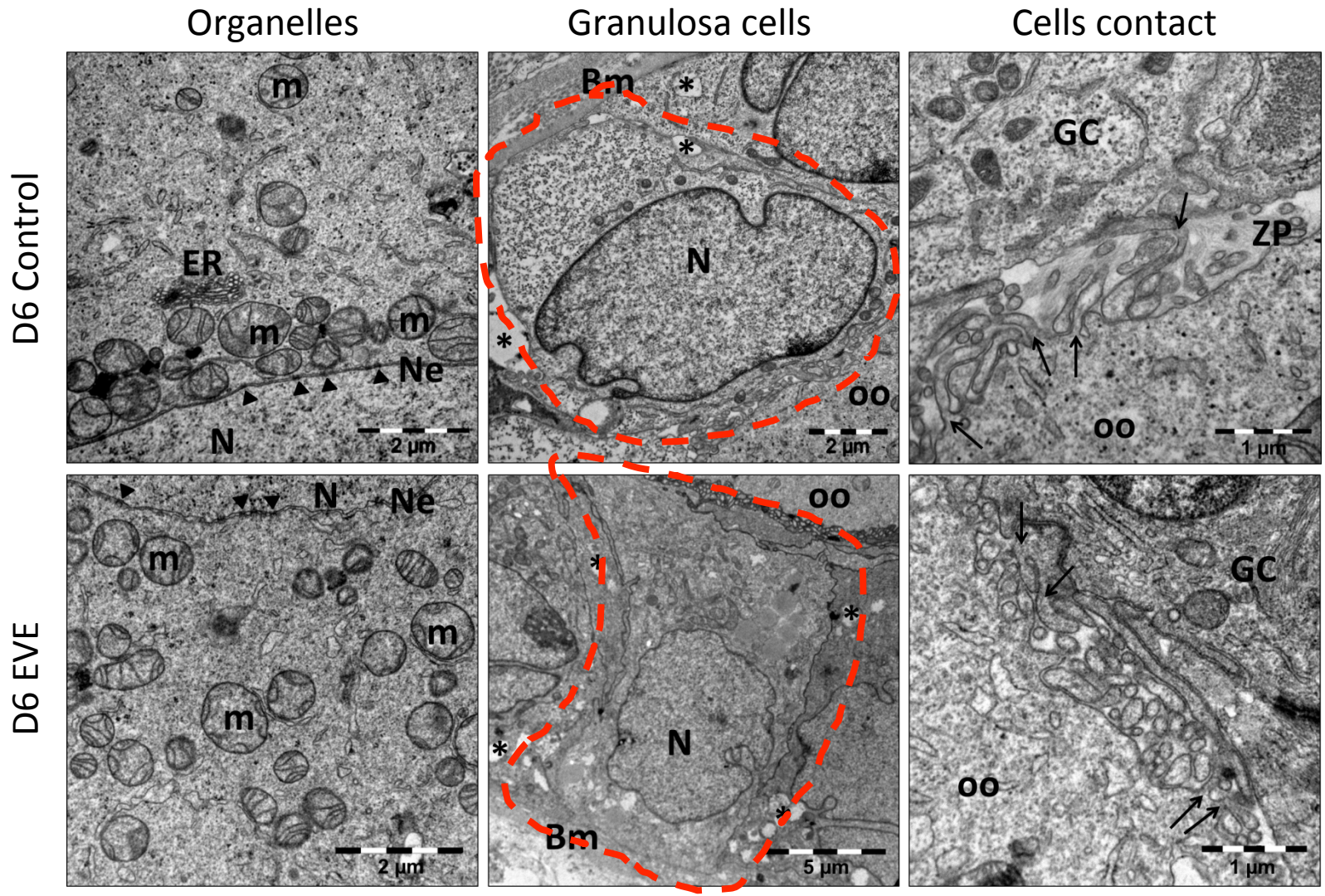

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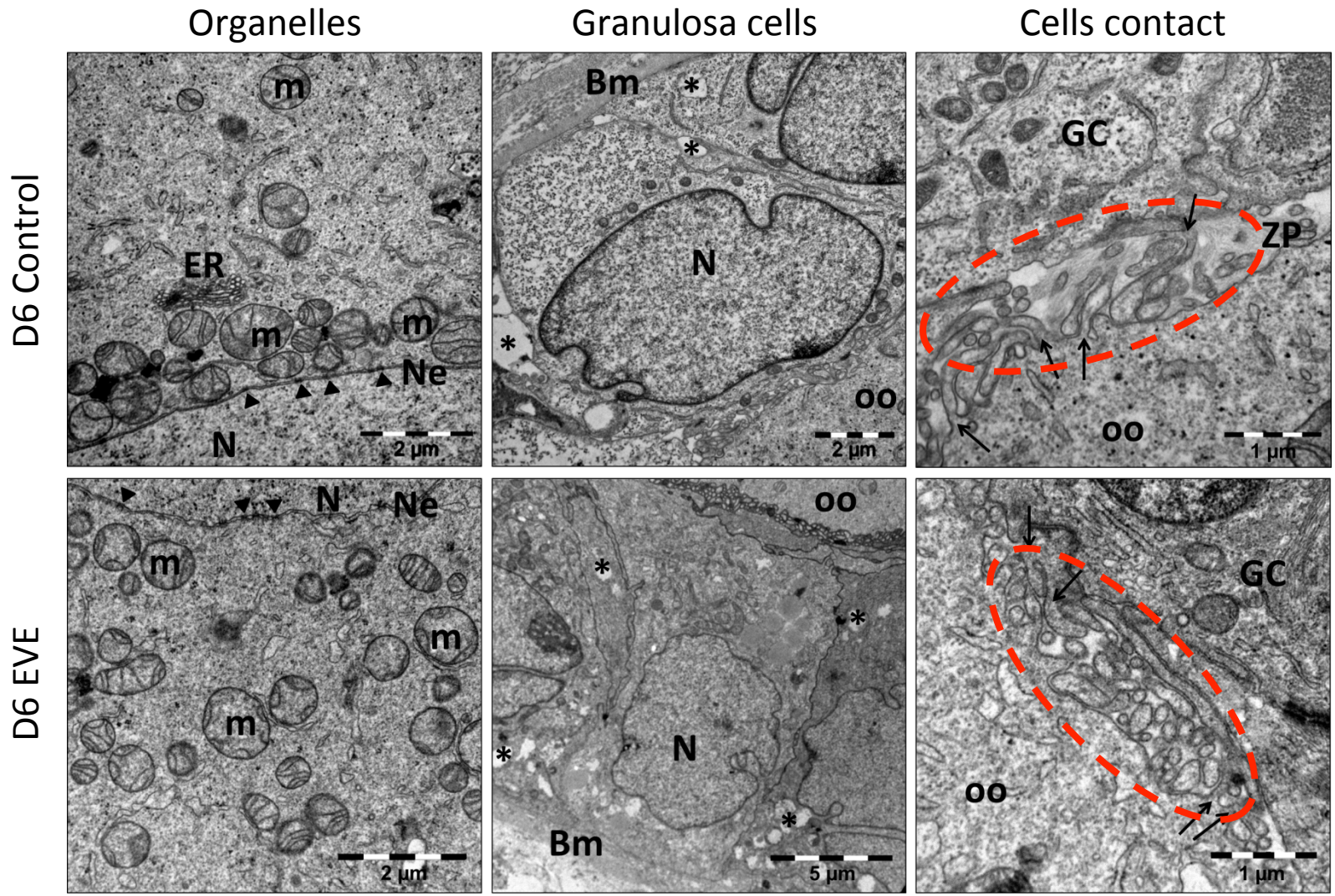
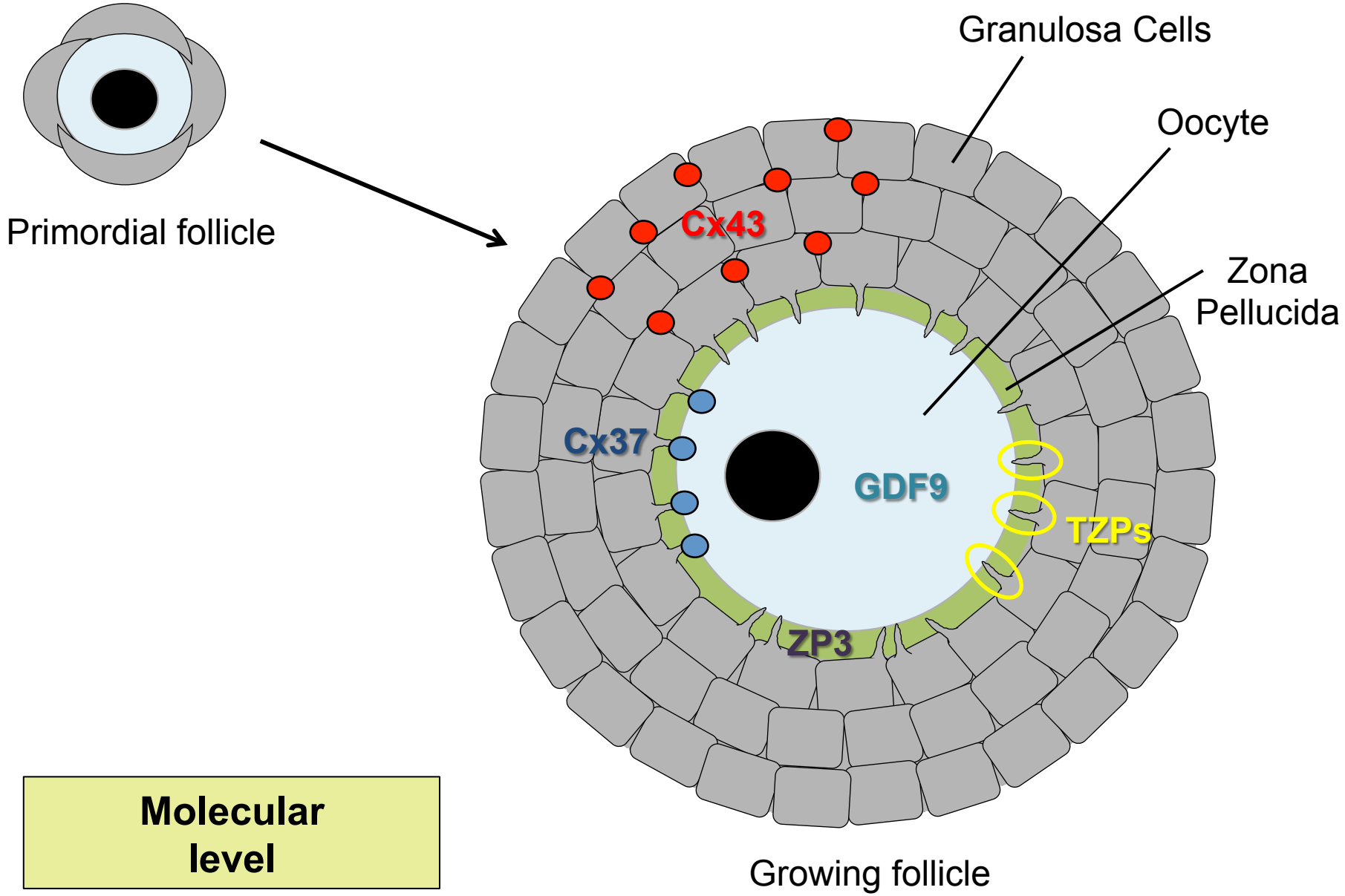
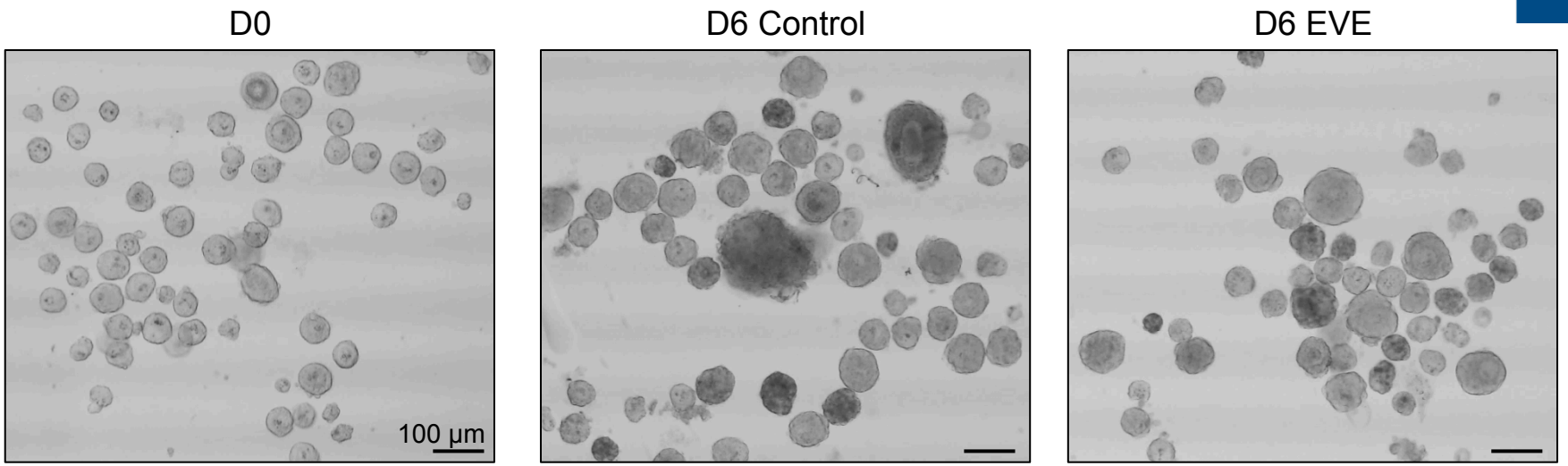


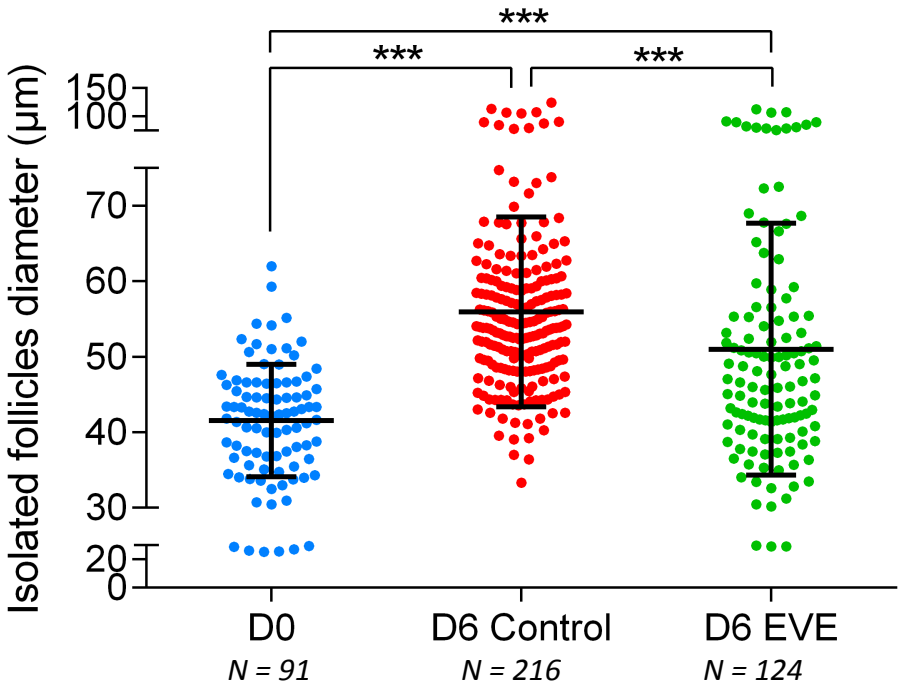


Molecular level

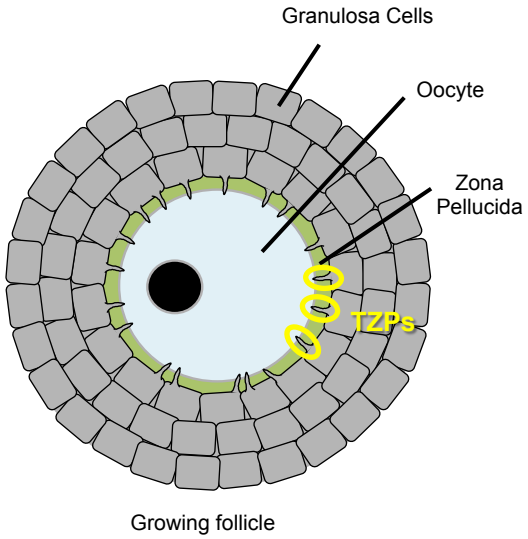


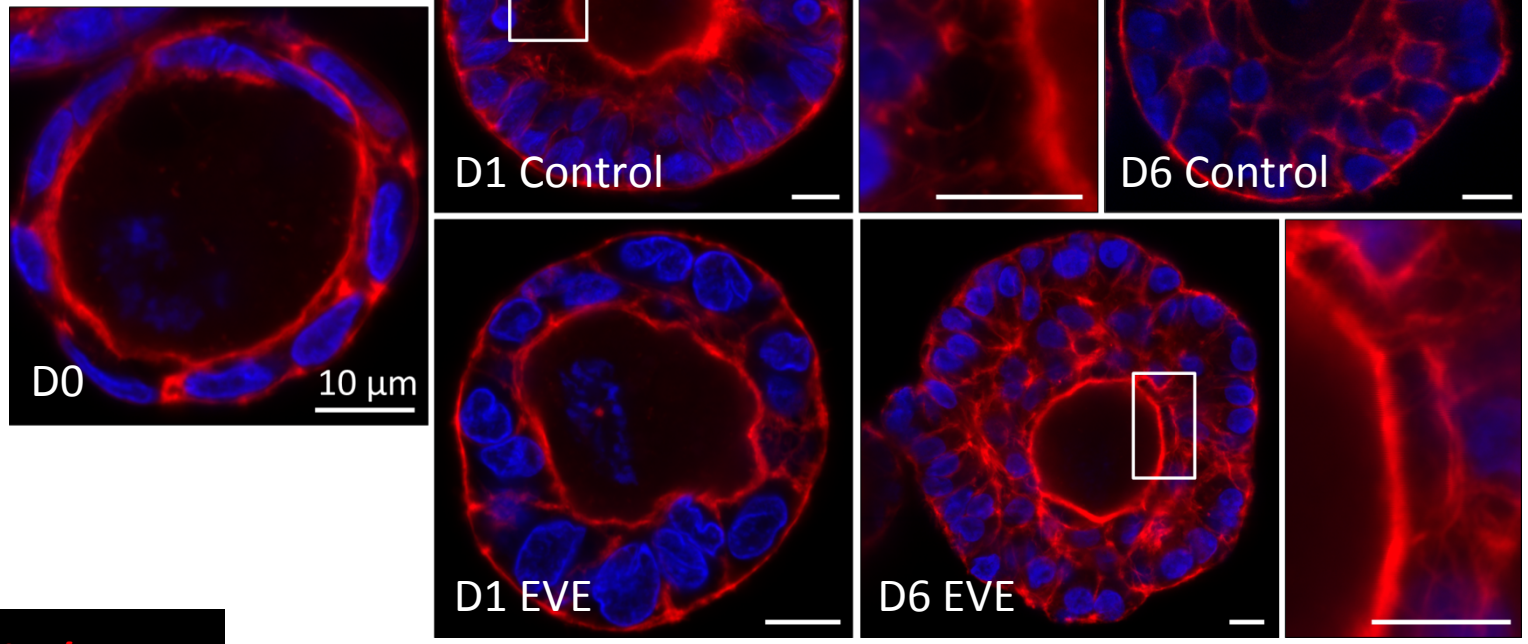
Follicles increase in size during culture

EVE-treated follicles are smaller than those spontaneously activated after 6 days of culture



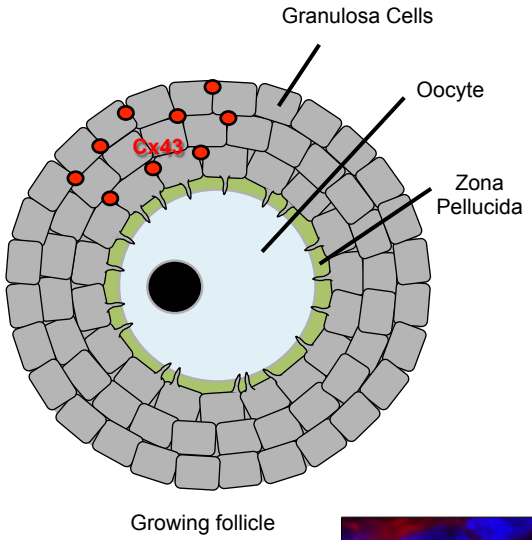
Intra-follicular communication forms and expands during in vitro culture

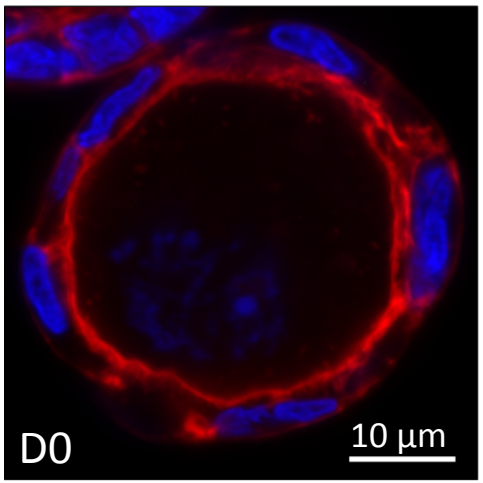


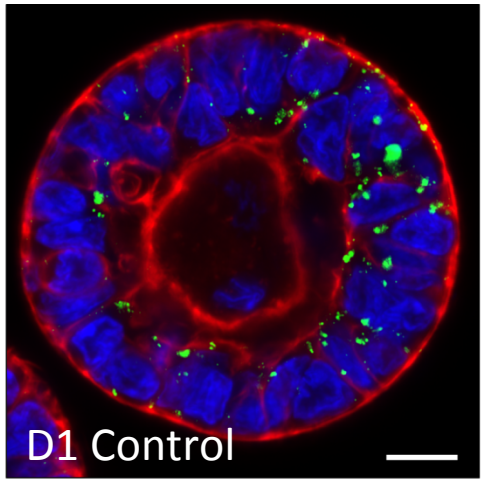


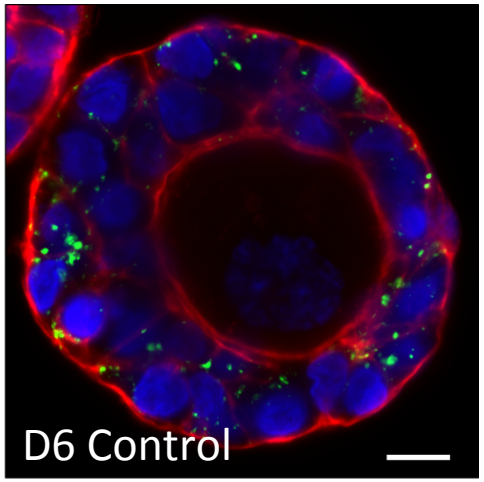
Phalloidin/DAPI

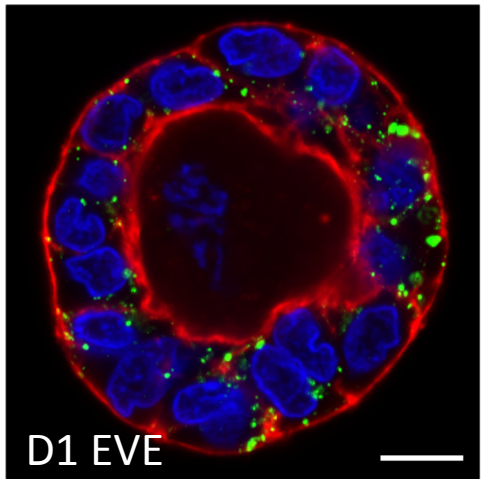
Intra-follicular communication forms and expands during in vitro culture

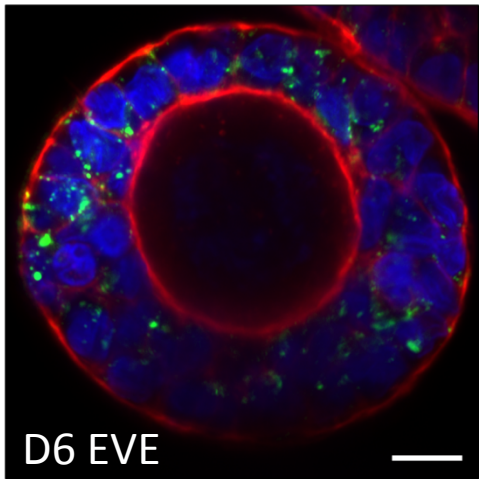




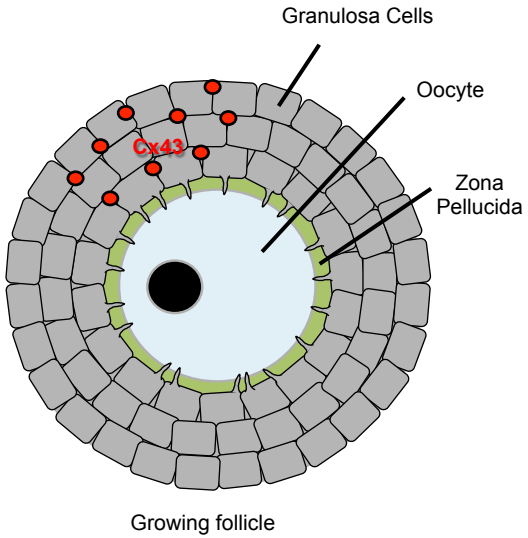








Cx43/Phalloidin/DAPI



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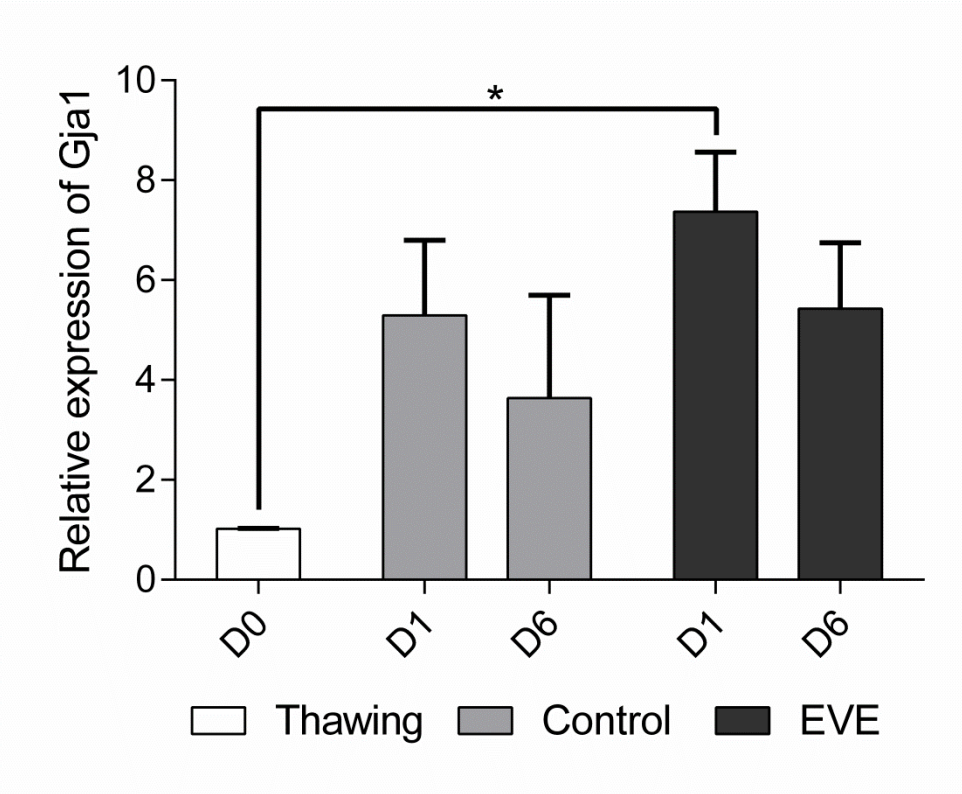
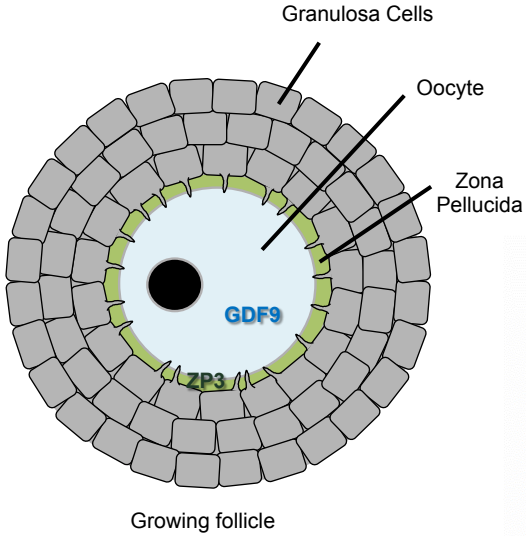
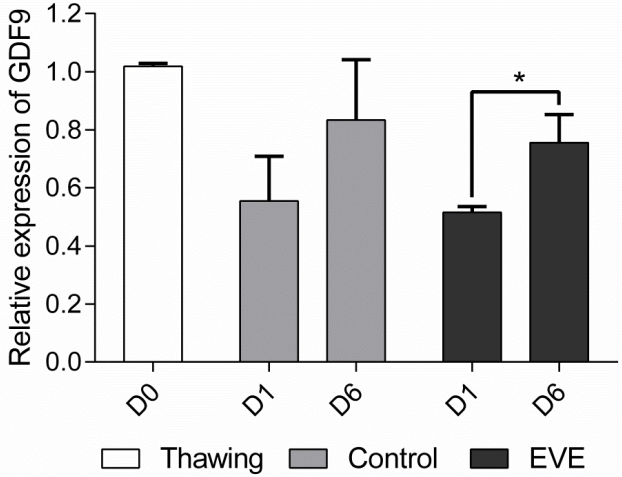
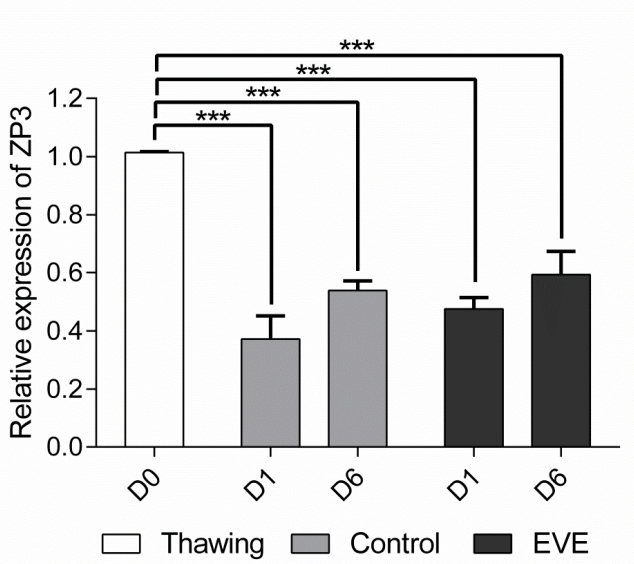


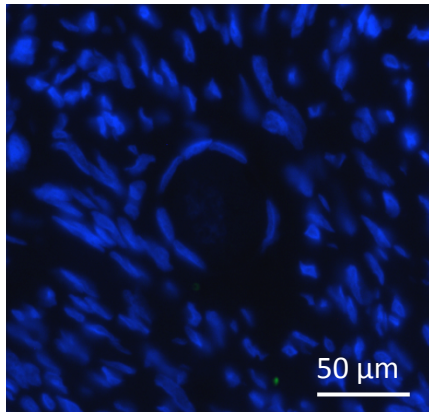
Follicles developmental markers tend to increase throughout in vitro culture



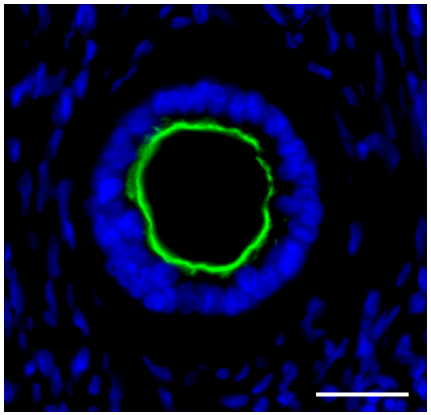




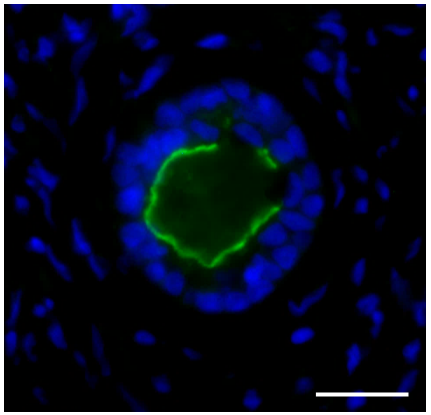
D0 - primordial follicle



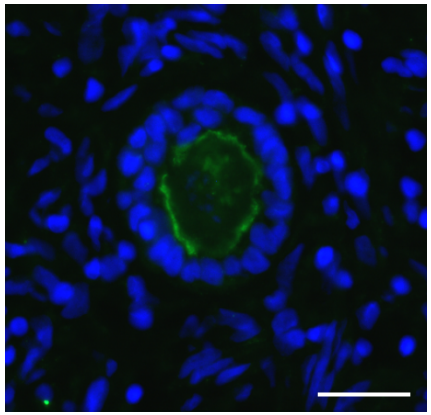
D0 – growing follicle

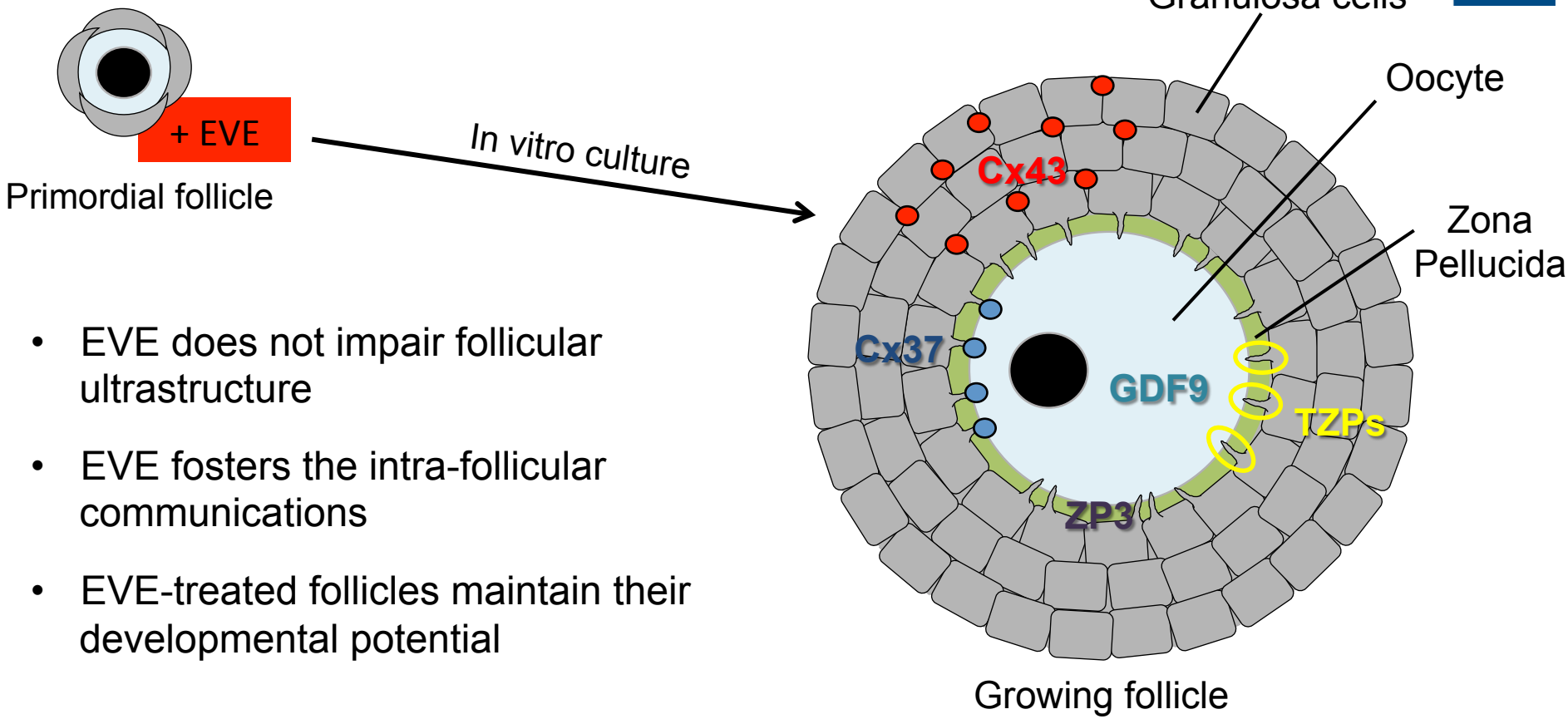


D6 Control



D6 EVE





- EVE does not impair follicular ultrastructure
- EVE fosters the intra-follicular communications
- EVE-treated follicles maintain their developmental potential

→ **Slowing down the spontaneous activation may garantee the generation of growing follicles of good quality**

→ **Using mTORC1 inhibitors may represent a potentially powerful pharmacological tool to support a protracted growth system**

Isabelle Demeestere
Chrysanthi Alexandri
Margherita Condorelli
Julie Dechène
Melody Devos
Eric Gonzalez
Matteo Lambertini
Géraldine Van den Steen

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