Signaling pathway inhibition in marine invertebrate embryological development

The goal of this project

To see how inhibitors influence on axis formation (anterior-posterior, dorso-ventral) during embryo development

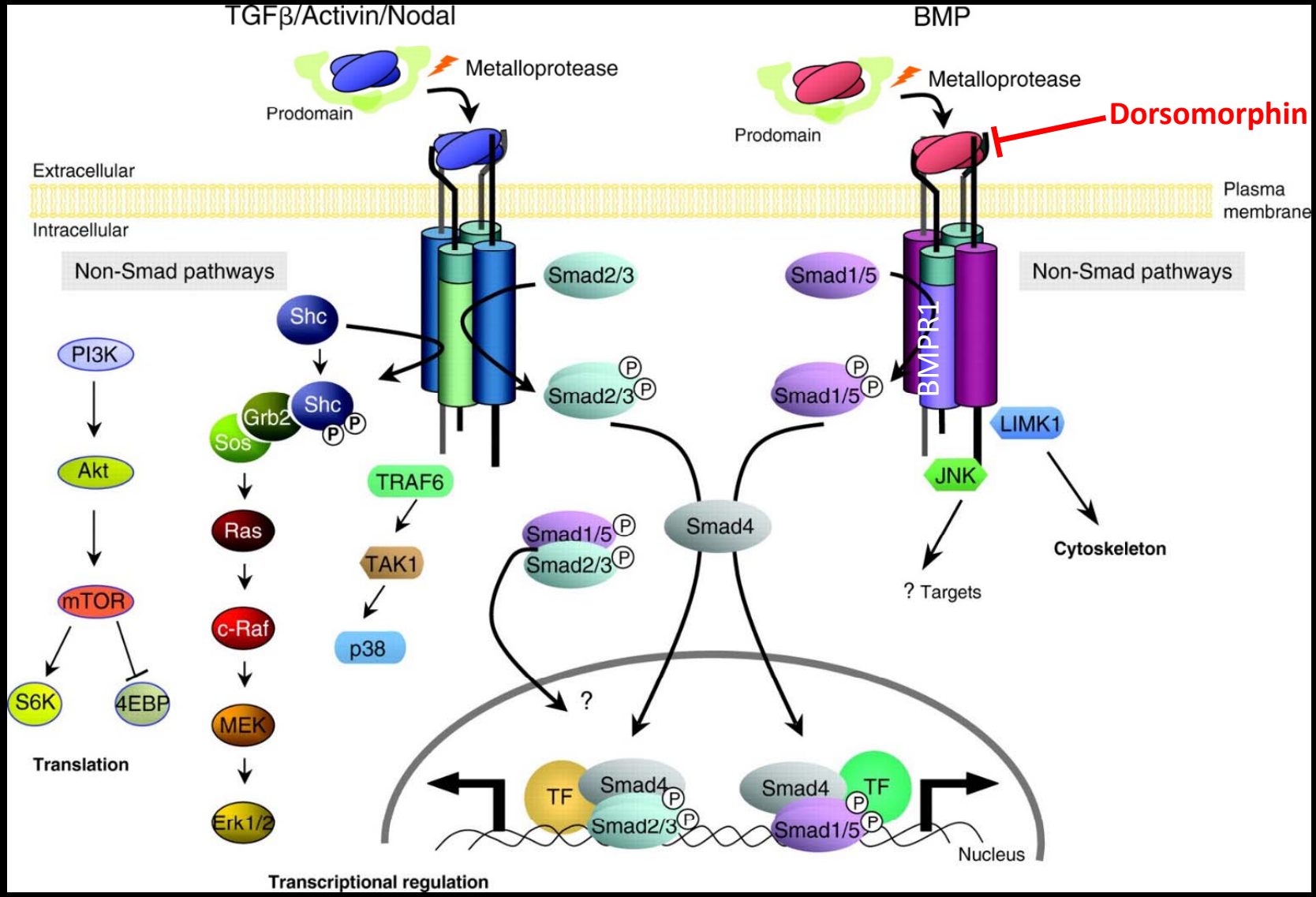
Inhibitors Used	Processes Influenced
• Dorsomorphin	• Blocks BMP Type-1 Receptors
• SB431542 (Nodal)	• TGFβ-R1
• Azakenpaullone	• Blocks GS3KBeta facilitating Beta-catenin
• iCRT14	• WnT/Beta-catenin blocker
• LDN193189	• ALK inhibitor
• SU5402	• fibroblast growth factor receptor (FGFR)
• UO126	• Blocks –P of Mek1/2 (Within FGF)

Signal Pathways

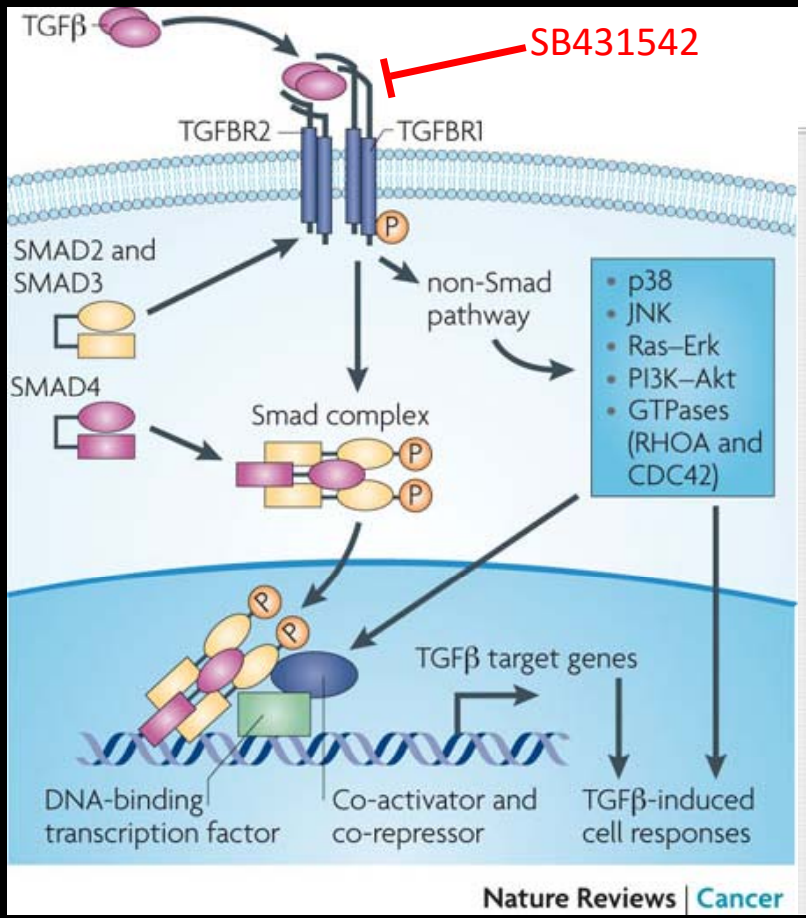
- BMP
- Nodal
- Wnt/β-catenin
- ALK
- FGFR

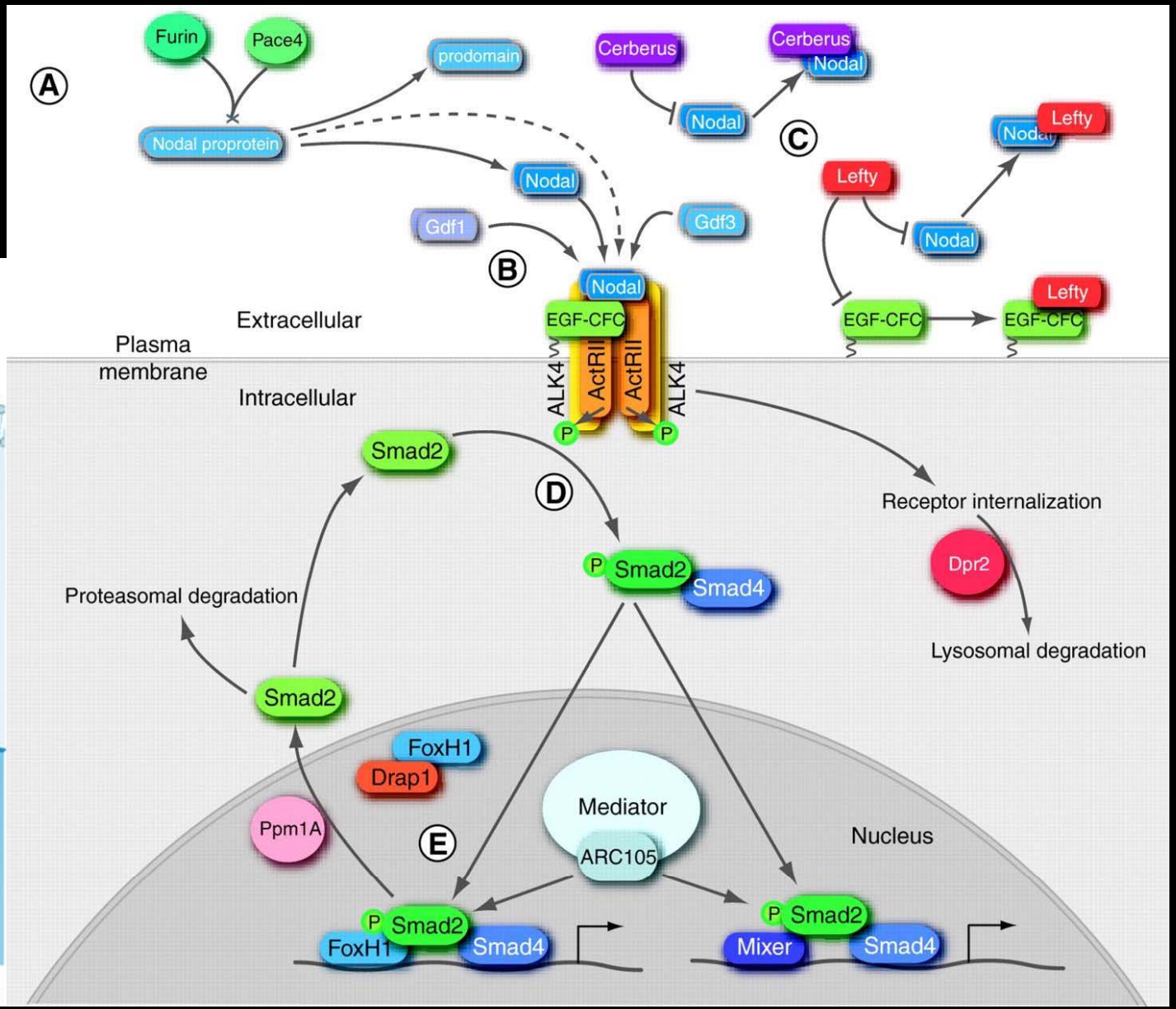
BMP pathway

Dorsomorphin inhibits BMPR1



Nodal Pathway
SB431542 inhibits TGFβR1

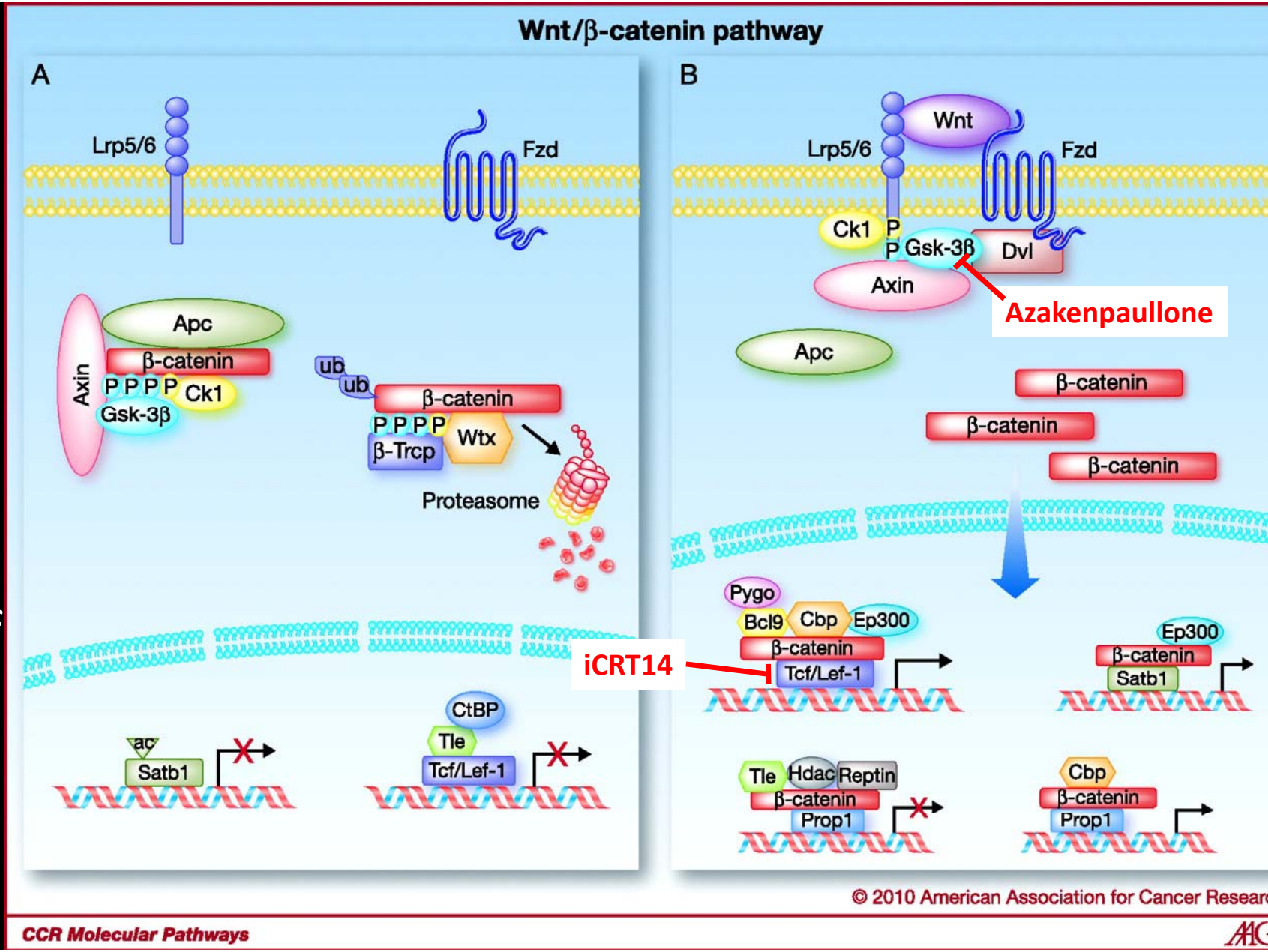




Wnt/ β -catenin Pathway

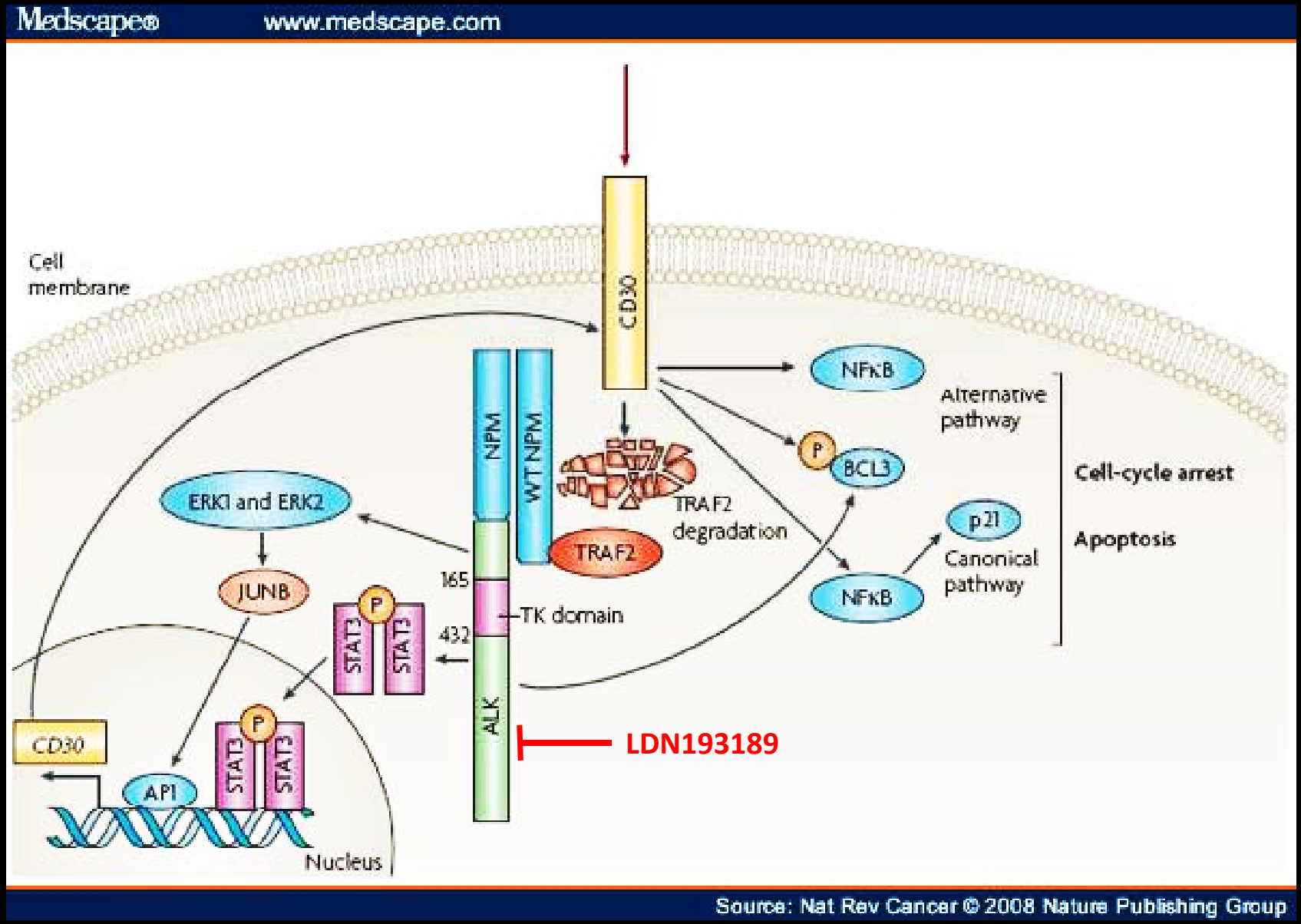
AZK (azakenpaullone) inhibits GSK3β

iCRT14 inhibits Tcf

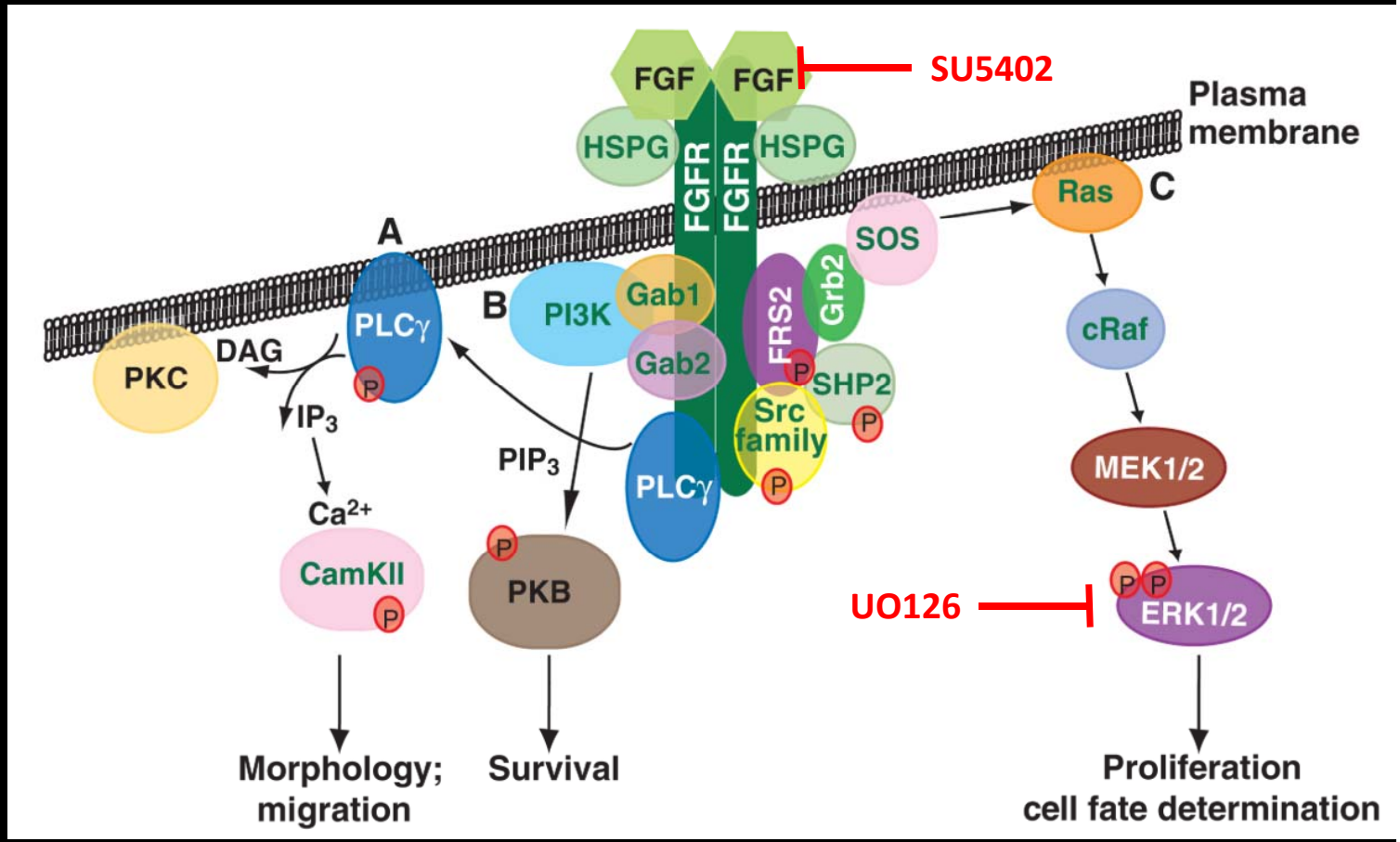


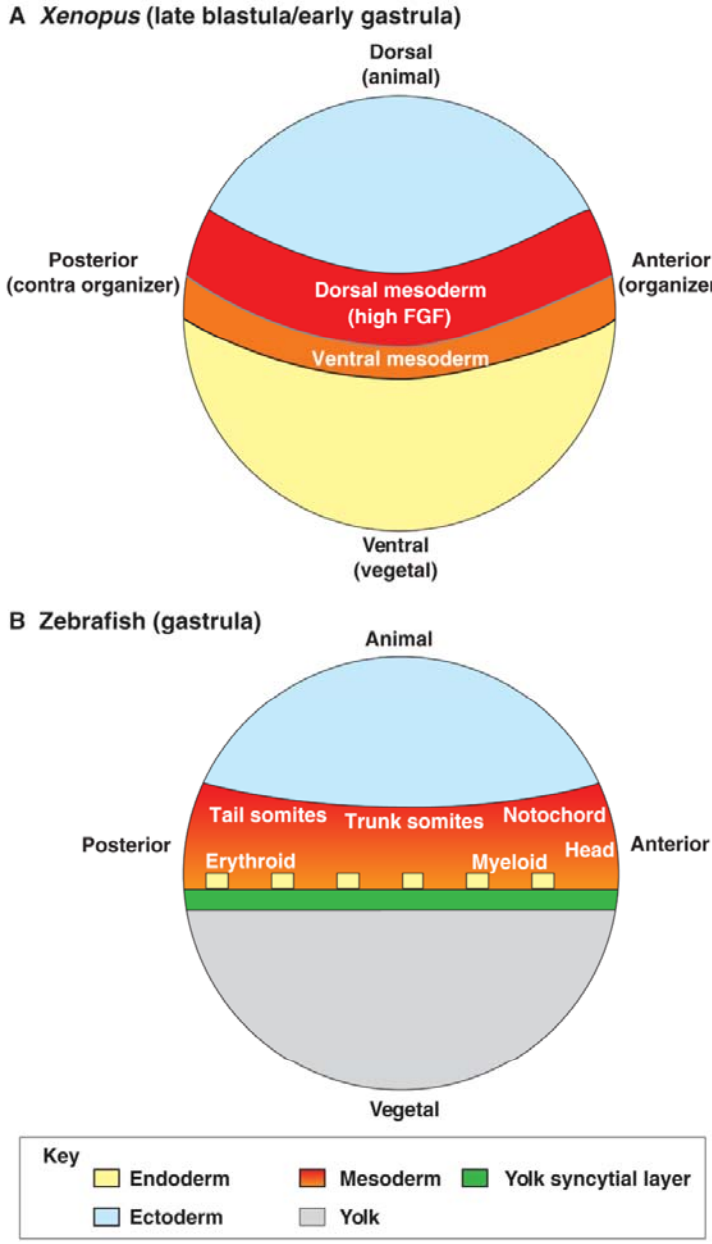
ALK Pathway

LDN1931189 inhibits ALK-domain in NPM-protein



FGFR – activating pathway
UO126 inhibits ERK - phosphorylation





Methods

1. Exposure (include controls)
2. Allow time to develop
3. Maintain inhibitor concentrations
4. Fix and process similar to ICC protocol

Protocol for ICC

- Relaxation ($MgCl_2$)
- Fixation (4% PFA)
- Washes (3× in 0.1 PBS + 2% TritonX for 10 min at RT)
- Blocking of unspecific binding sites (2% Normal Goat Serum in PBS)
- Primary Antibody binding o/n at RT (1:400)
- Washes **(at least 4×** in 0.1 PBS for **15** min at RT)
- Blocking of unspecific binding sites (2% Normal Goat Serum in PBS)

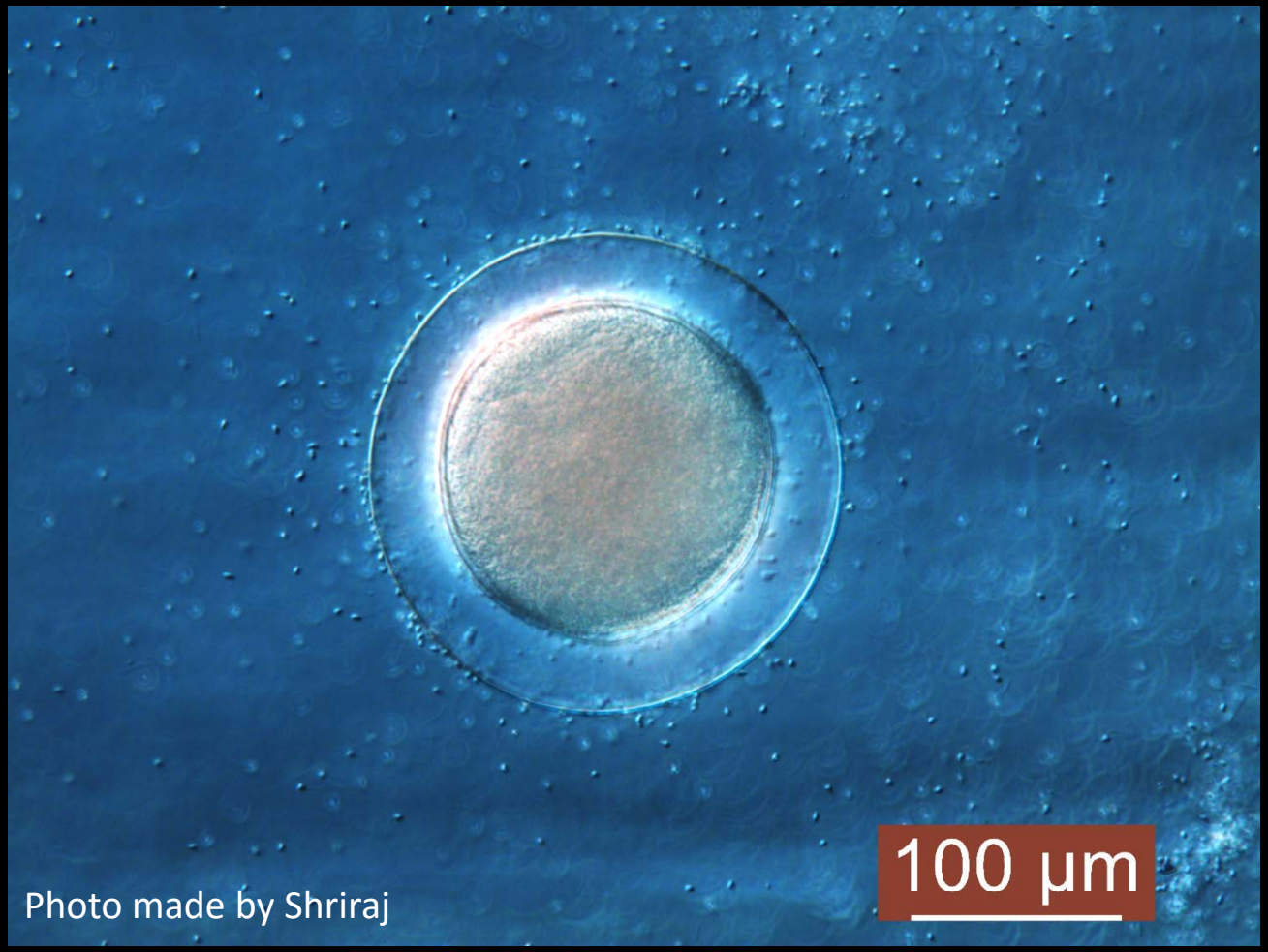
From now on work in the dark conditions:

- Secondary Antibody (Alexa Fluor 488, 1:500)
- Washes **(at least 4×** in 0.1 PBS for **15** min at RT)
- Incubation with 488 phalloidin (F-actin staining)
- Embed specimens in Fluoromount mounting medium (includes DAPI)

1. *Sea urchin*

<u>Inhibitors</u>:

- Dorsomorphin
- SB431542 (Nodal)
- Azakenpaullone
- iCRT14
- LDN193189
- SU5402
- UO126



Why it didn’t work:

- 1. High density of embryos in one dish
- 2. Low salinity of sea water

2. *Littorina saxatilis*

Inhibitor:

- SB431542 (inhibits Nodal pathway)
- @ 50 uM

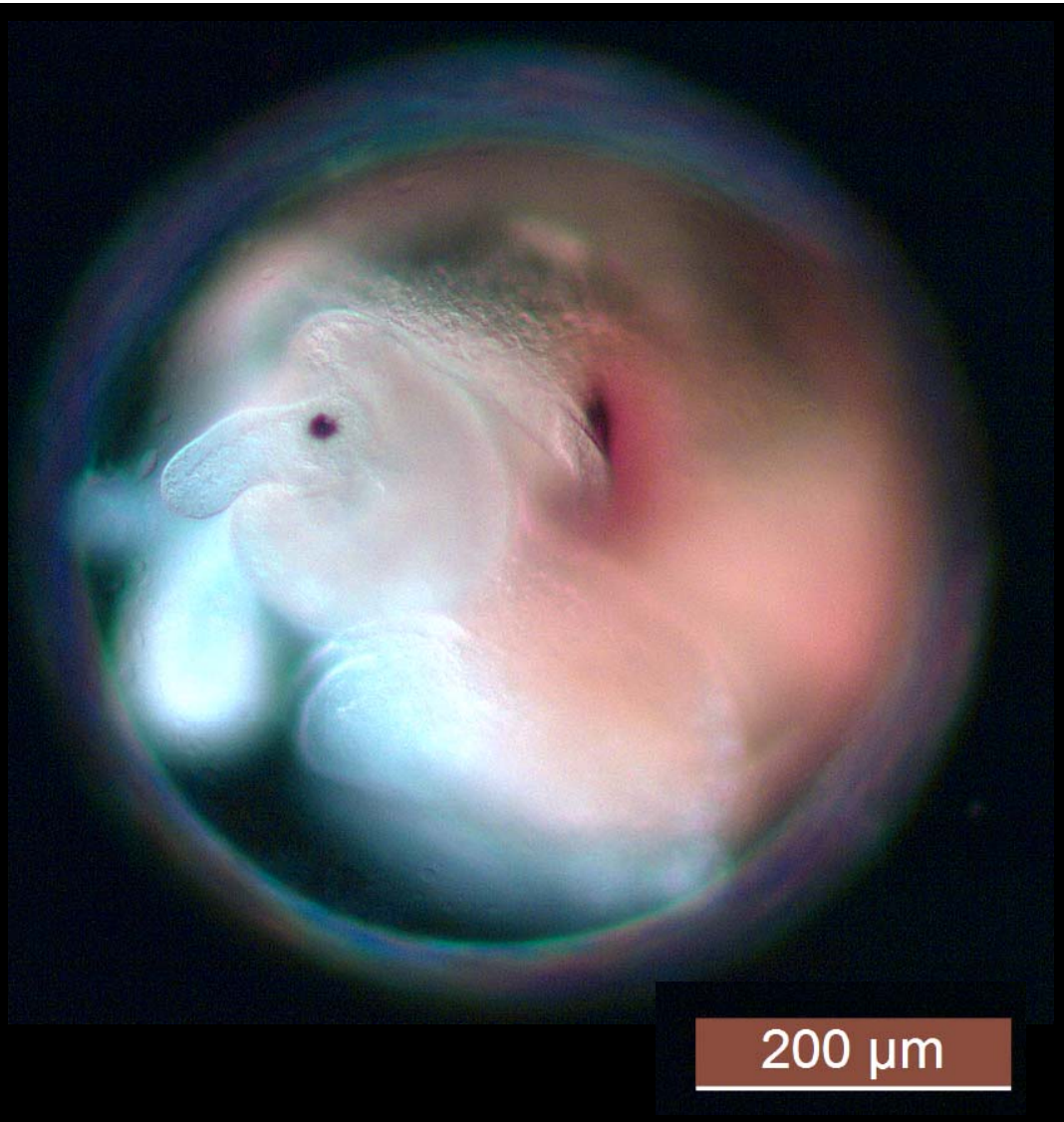
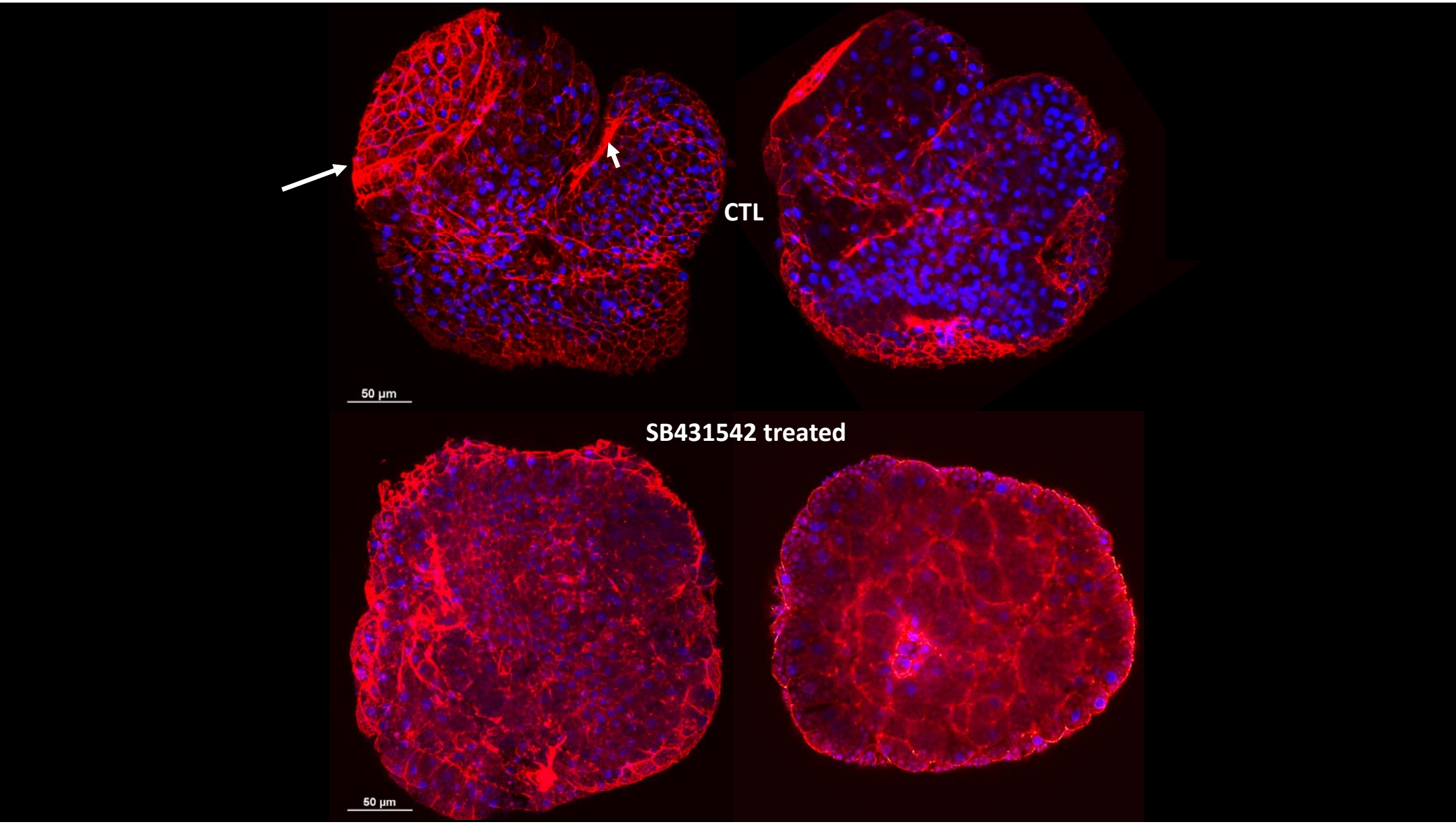
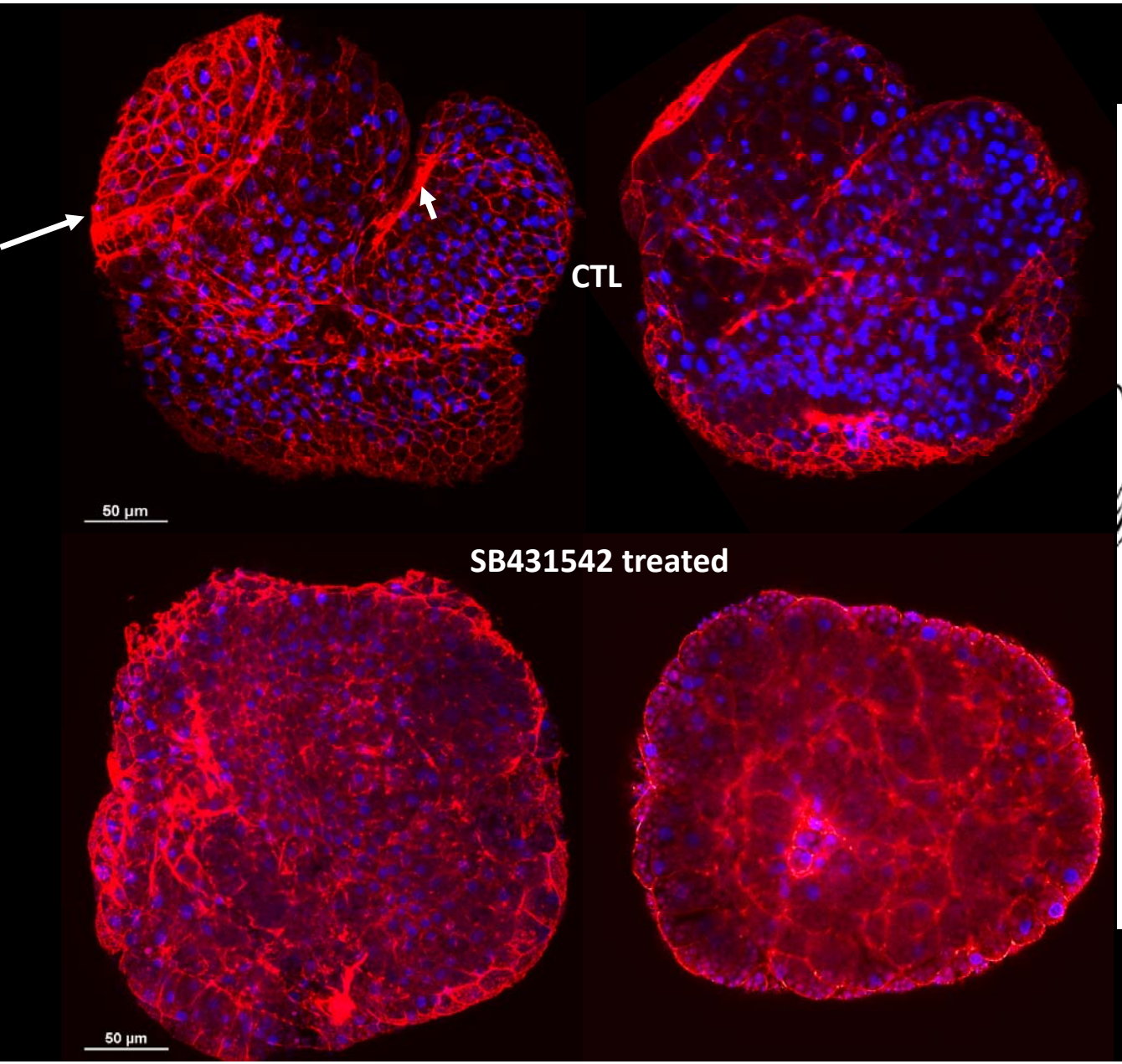
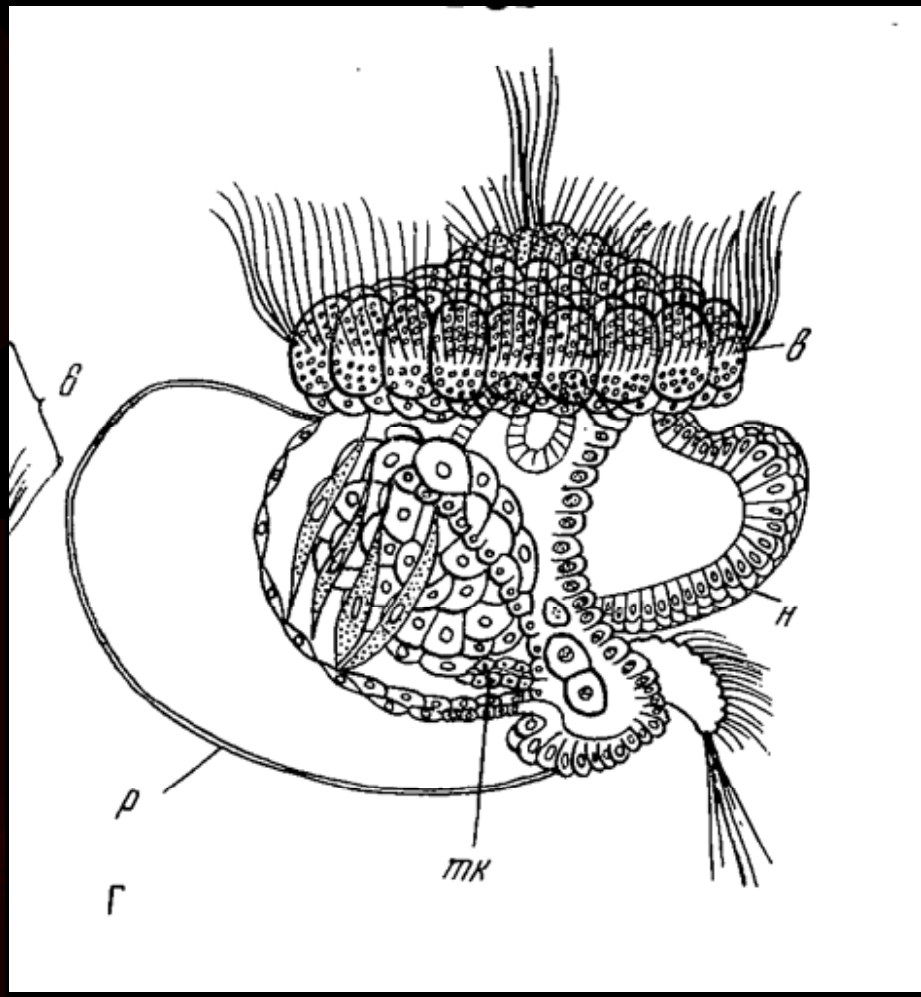


Photo made by Renata


CTL
50 µm
SB431542 treated
50 µm


CTL
50 µm
SB431542 treated
50 µm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Why it didn’t work:

- 1. We stopped our observation **too early**
- 2. **Too high temperature of embryo incubation** (animals develop normally in low temperature)
- 3. Nodal can be responsible for the early formation of embryo (Grande and Patel, 2009)

2. *Ophelia limacina*

- Inhibitor:
- AZK (azakenpaullone)
- @ 10uM

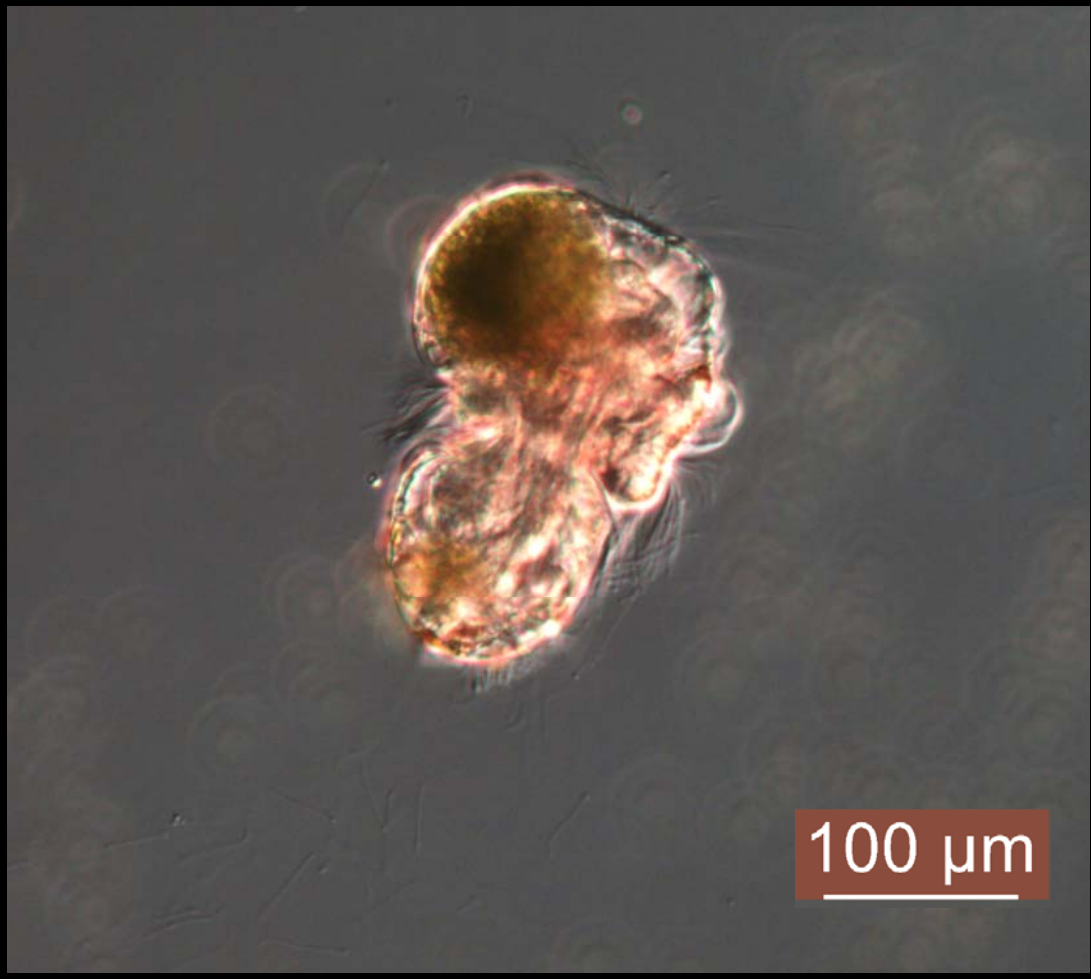
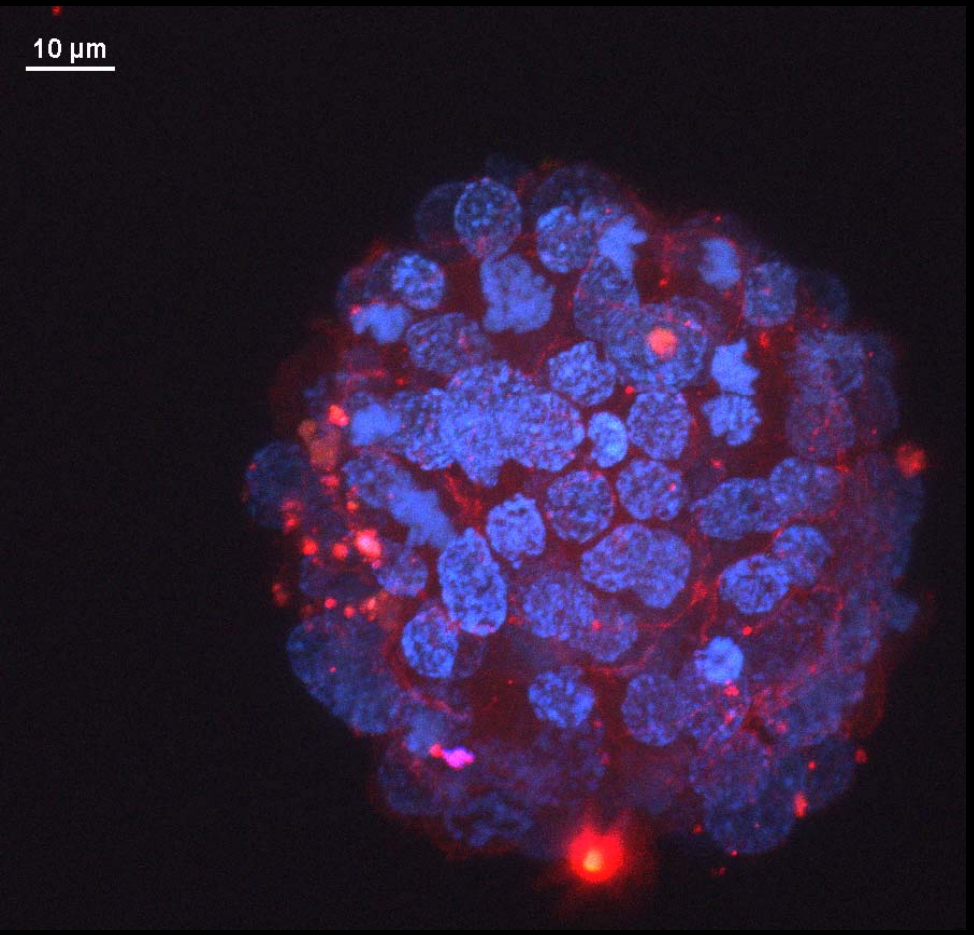
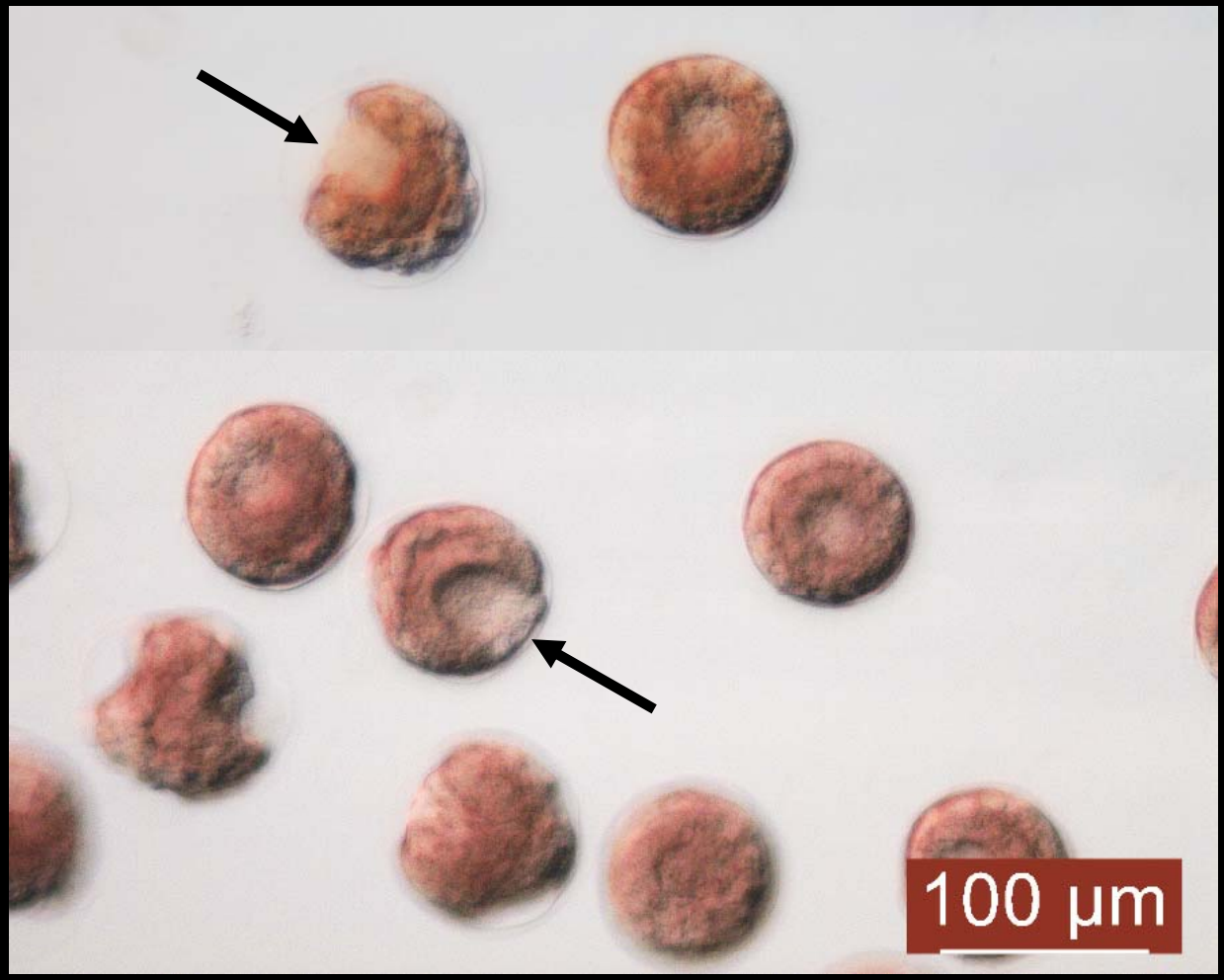


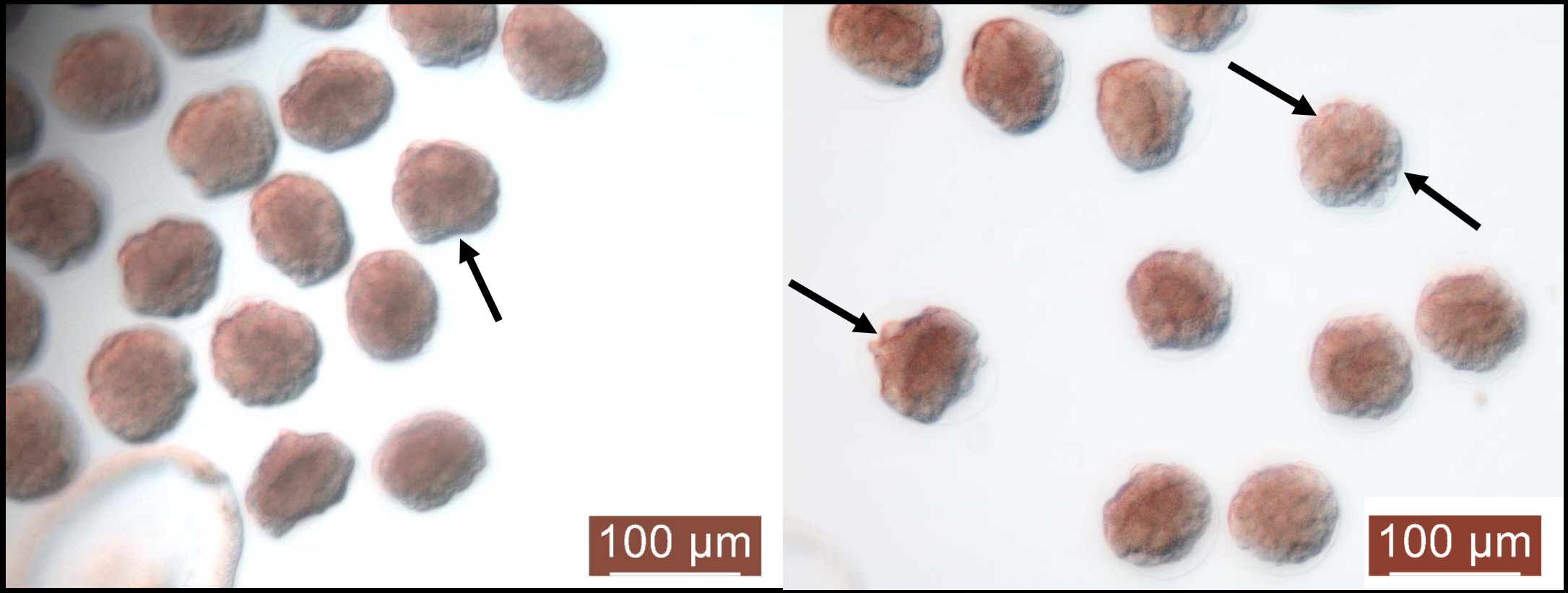
Photo made by Olga



~ 500 cell-stage



Control



@ 10 uM (AZK), 25h post exposure

Some advices for further experiments

- 1. Keep animals in low temperature sea water (if it’s needed)
- 2. During the whole protocol: always check embryos (whether they are still there)
- 3. Change tips for each solution and procedure steps
- 4. Do not put a huge amount of embryos to one dish
- 5. Write down the whole information about experiment stages (time, stage in protocol)

Thank you! ☺