

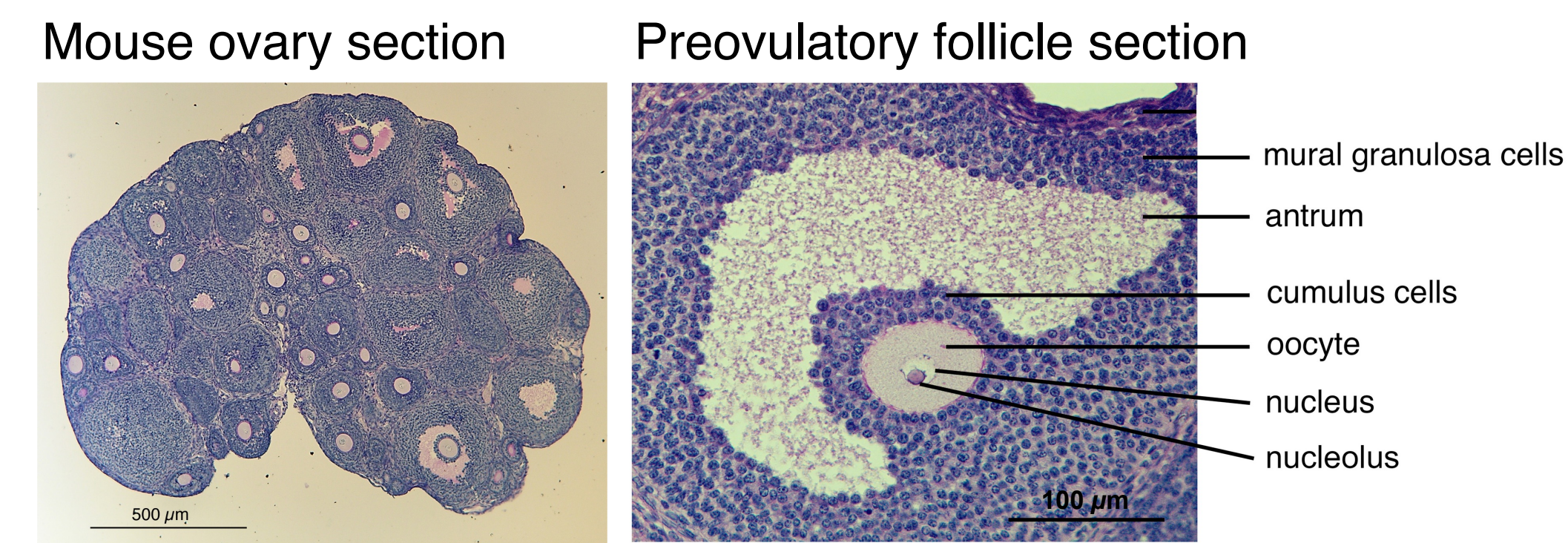
# cAMP Dynamics in Mouse Ovarian Follicles and Oocytes in Response to Luteinizing Hormone (LH): Insights from Mice Expressing the cAMPFIRE-M Sensor

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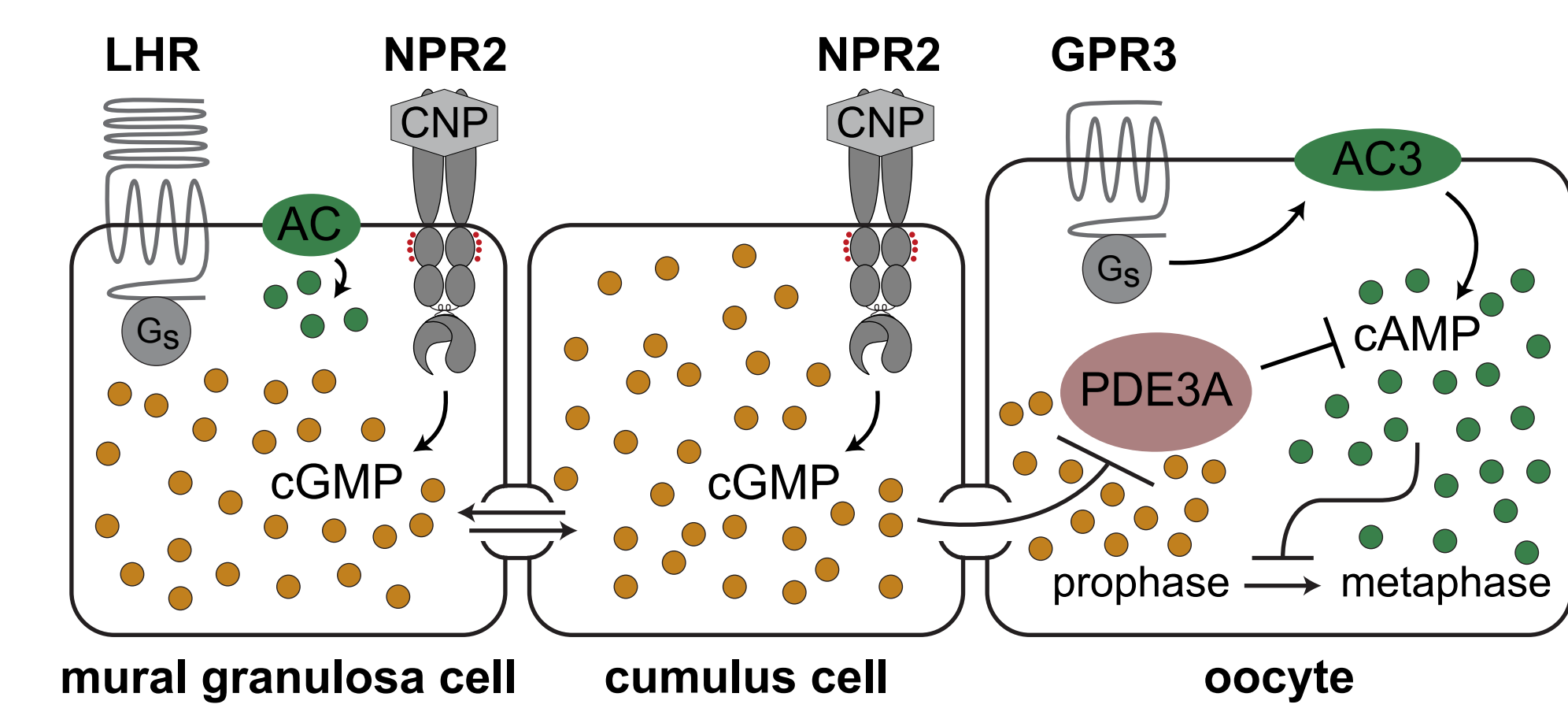
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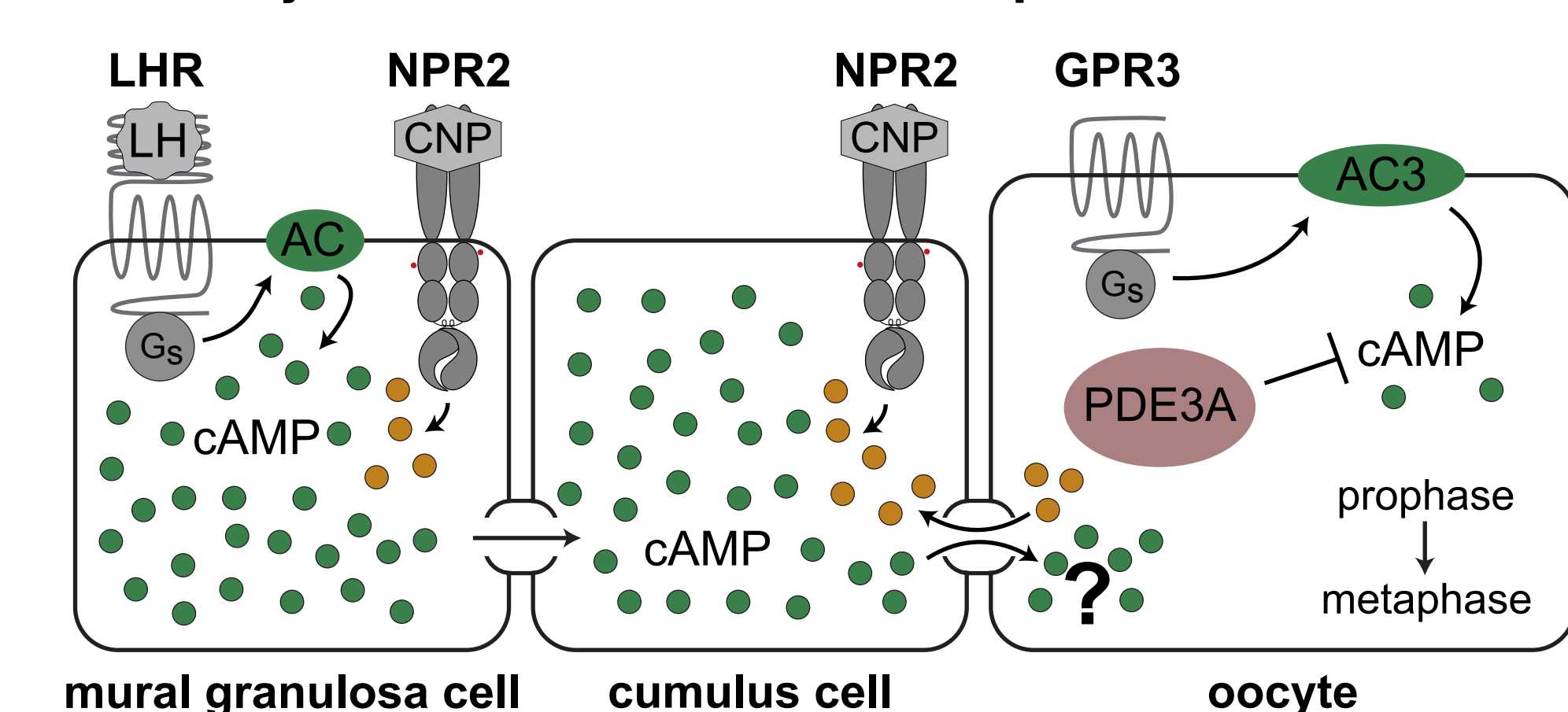
## Background



## How oocyte meiotic arrest is maintained<sup>1,2</sup>

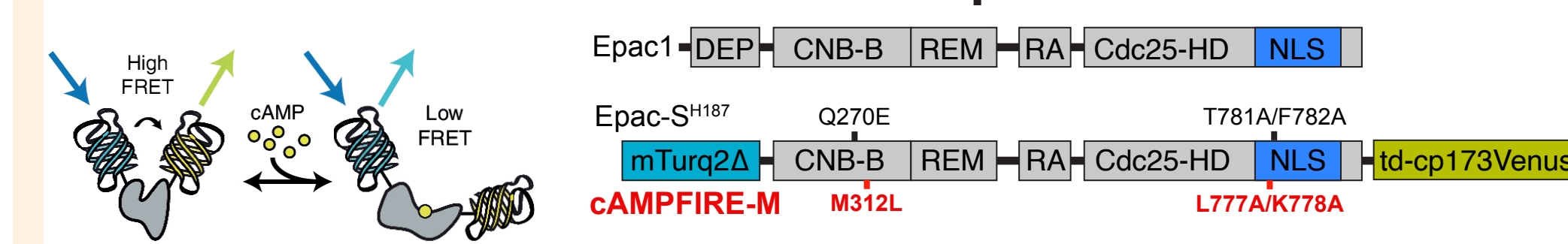


## How oocyte meiosis resumes in response to LH<sup>3,4,5</sup>



## Methods

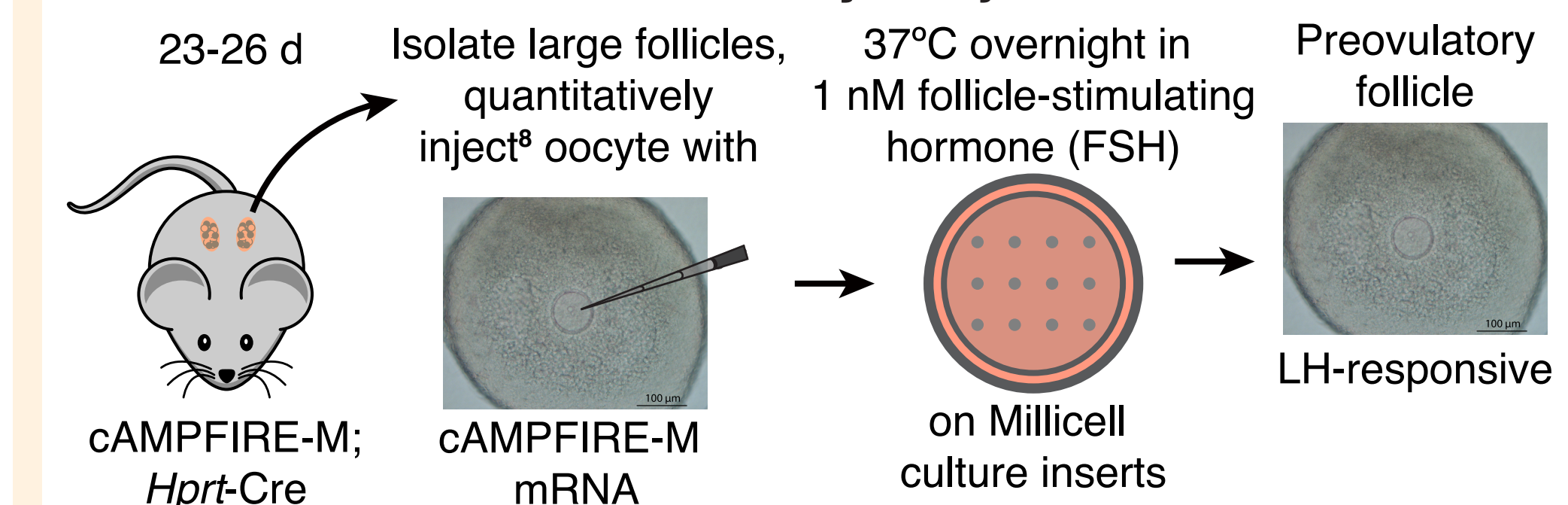
### Generation of cAMPFIRE-M<sup>6</sup> from Epac-S<sup>H187</sup> FRET sensor<sup>7</sup>



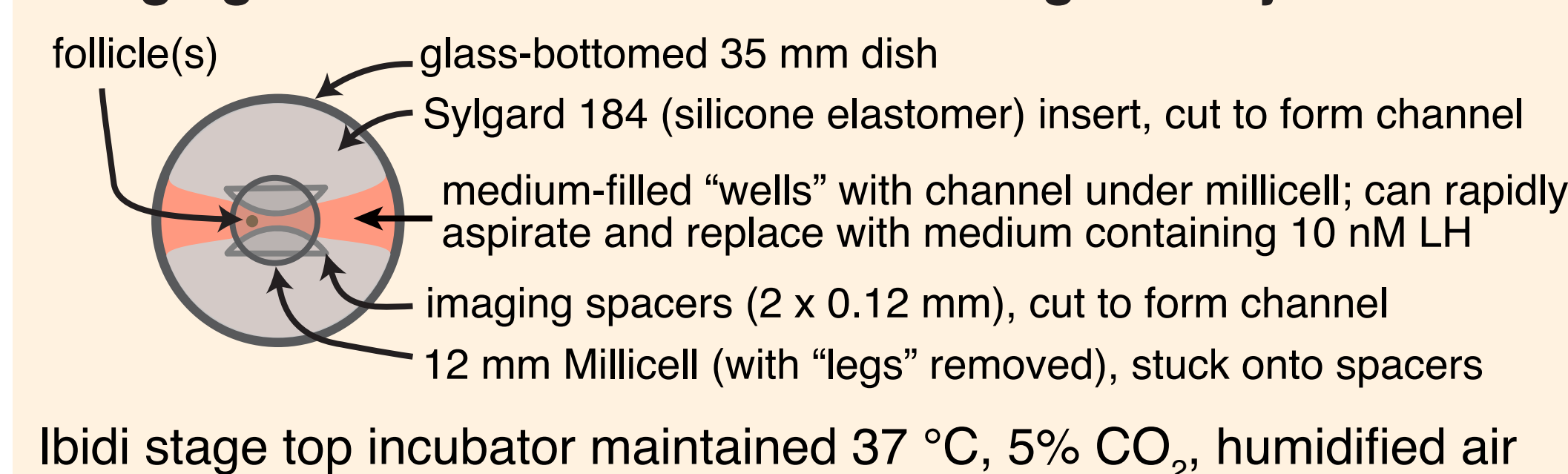
### Generation of cAMPFIRE-M-lox-STOP-lox mouse line

- Created a CAG-driven conditional (LSL) overexpression allele
- Inserted into Rosa26 locus using CRISPR/Cas9 tools
- Bred with *Hprt-Cre* mice for global cAMPFIRE-M overexpression

### Mouse follicle culture and oocyte injection

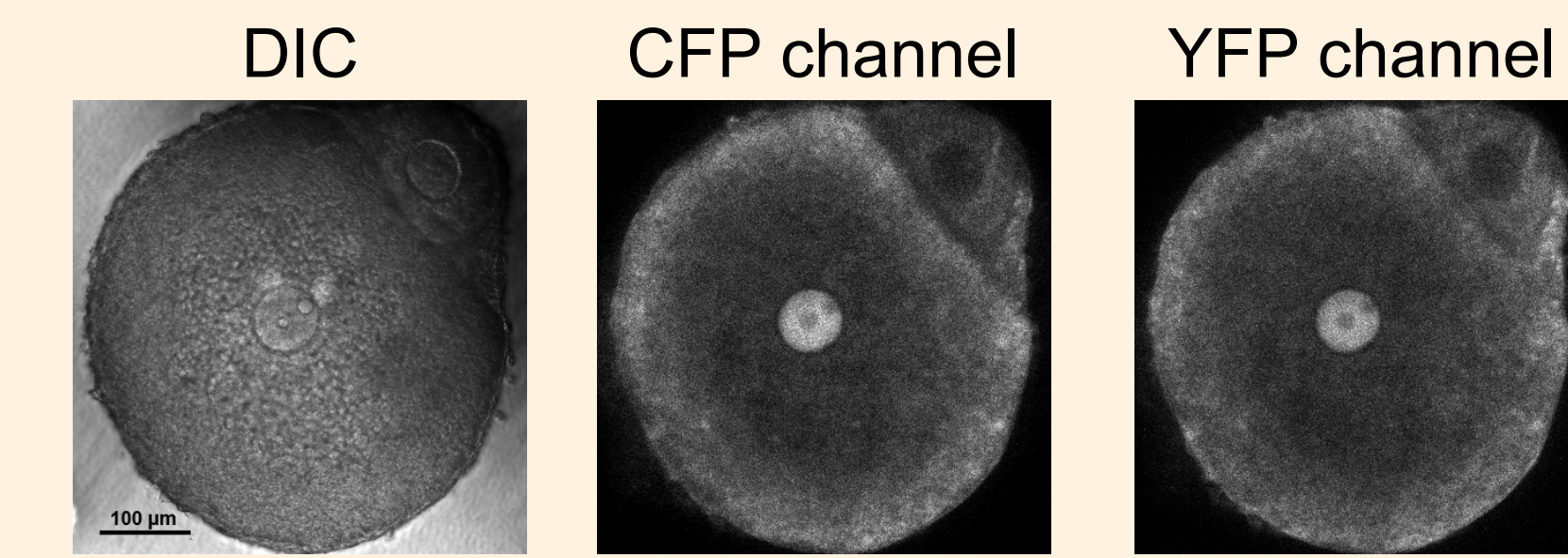


### Imaging: Zeiss 980 with 20X/0.5 NA long WD objective



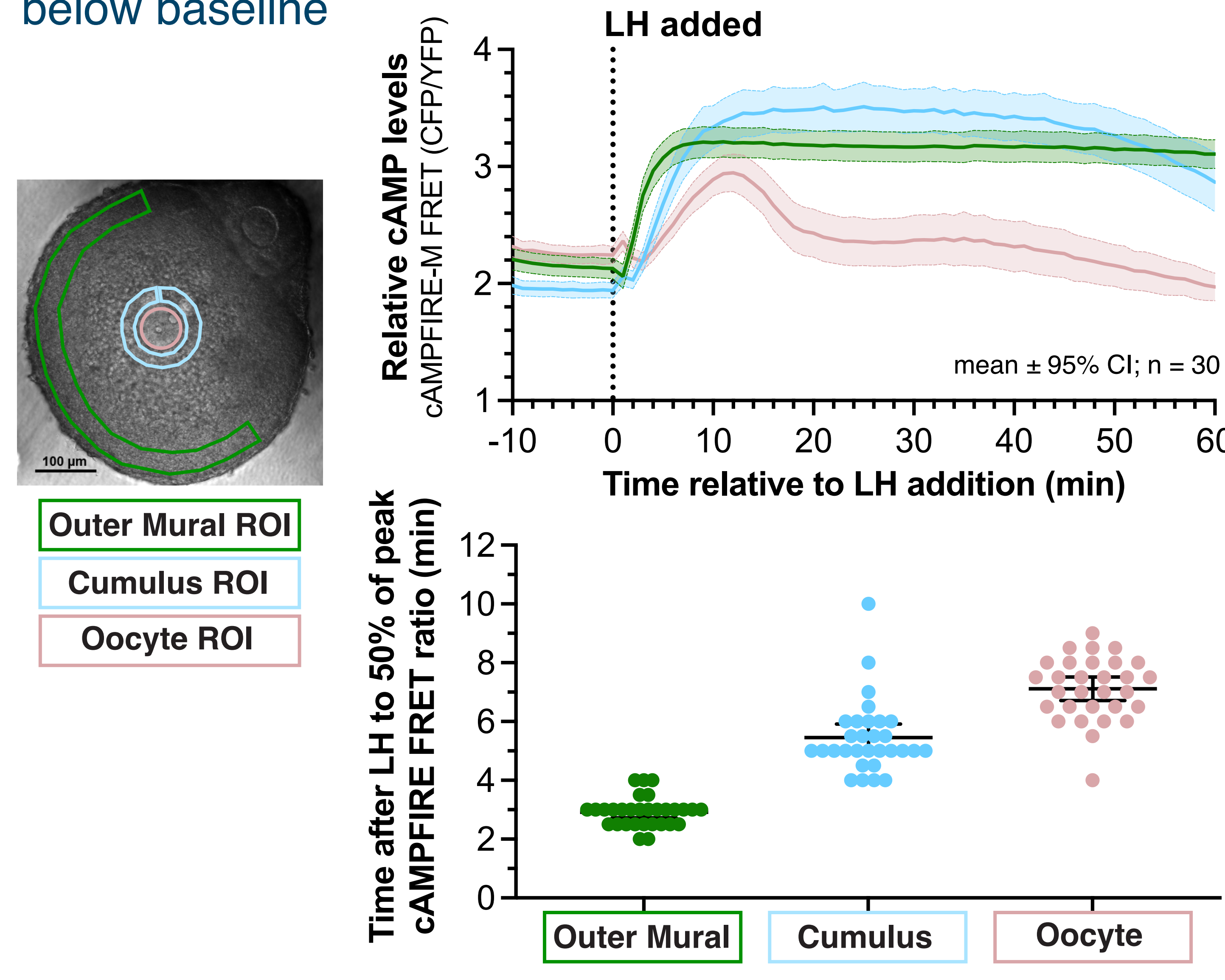
## Questions and Approach

- Does the LH-induced cAMP increase in somatic cells spread to the oocyte?
- If so, what are its temporal dynamics and is it dependent on gap junctions?

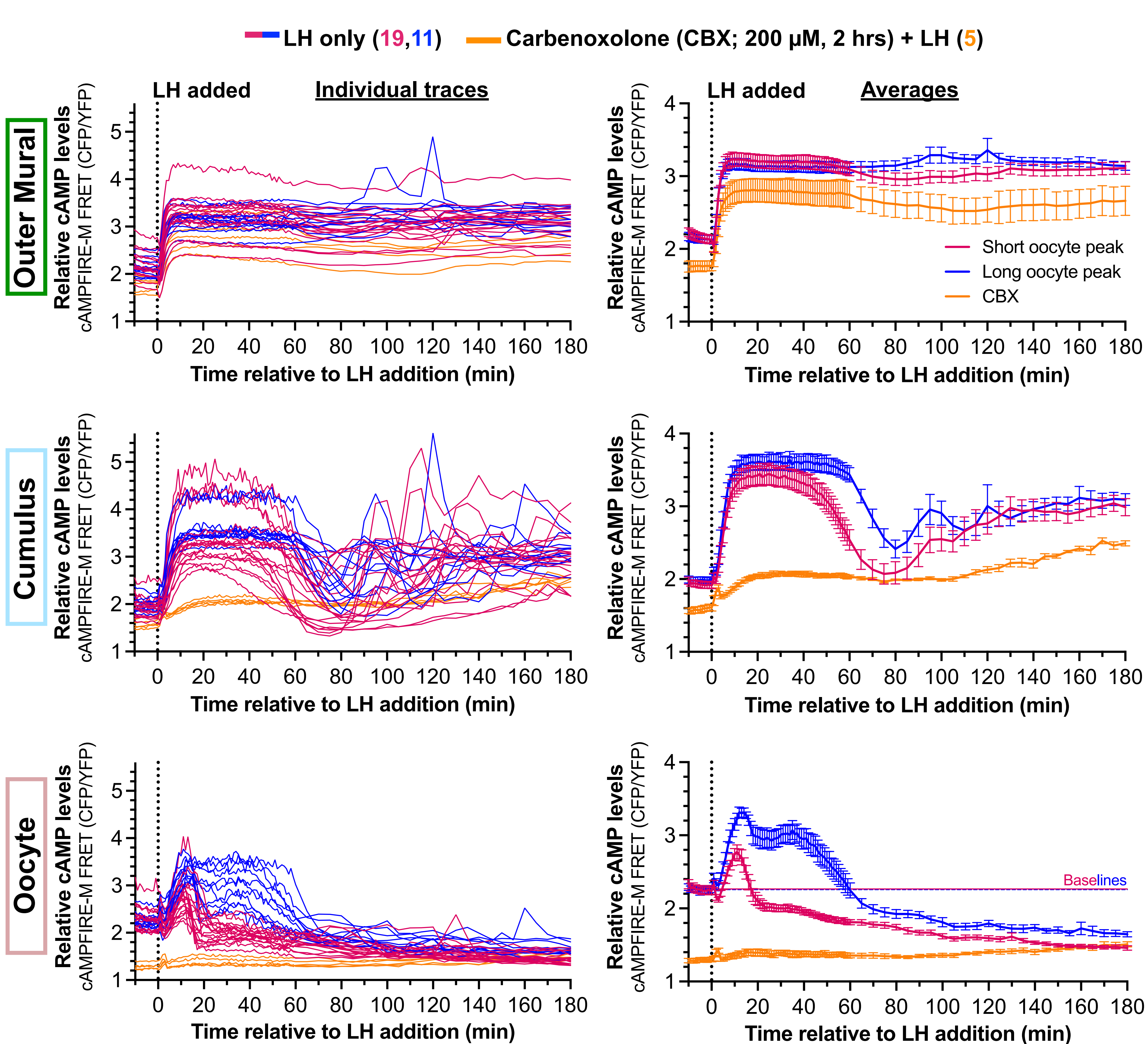


Isolated follicle from a mouse endogenously expressing cAMPFIRE-M in somatic cells, ~19 hours after microinjection of cAMPFIRE-M mRNA into the oocyte.

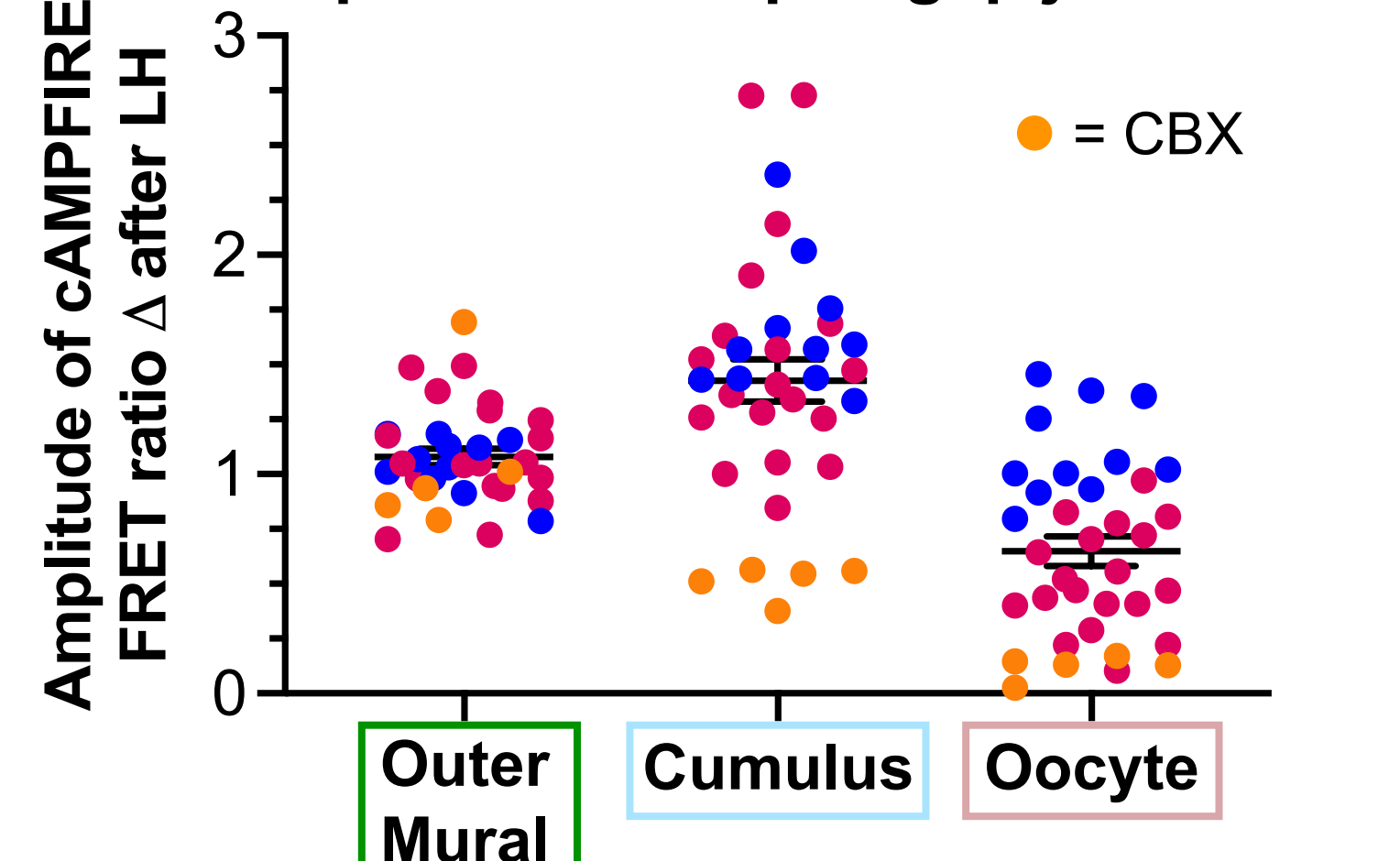
**Results:** LH-induced cAMP elevation progresses inward, causing a transient oocyte cAMP increase which later falls below baseline



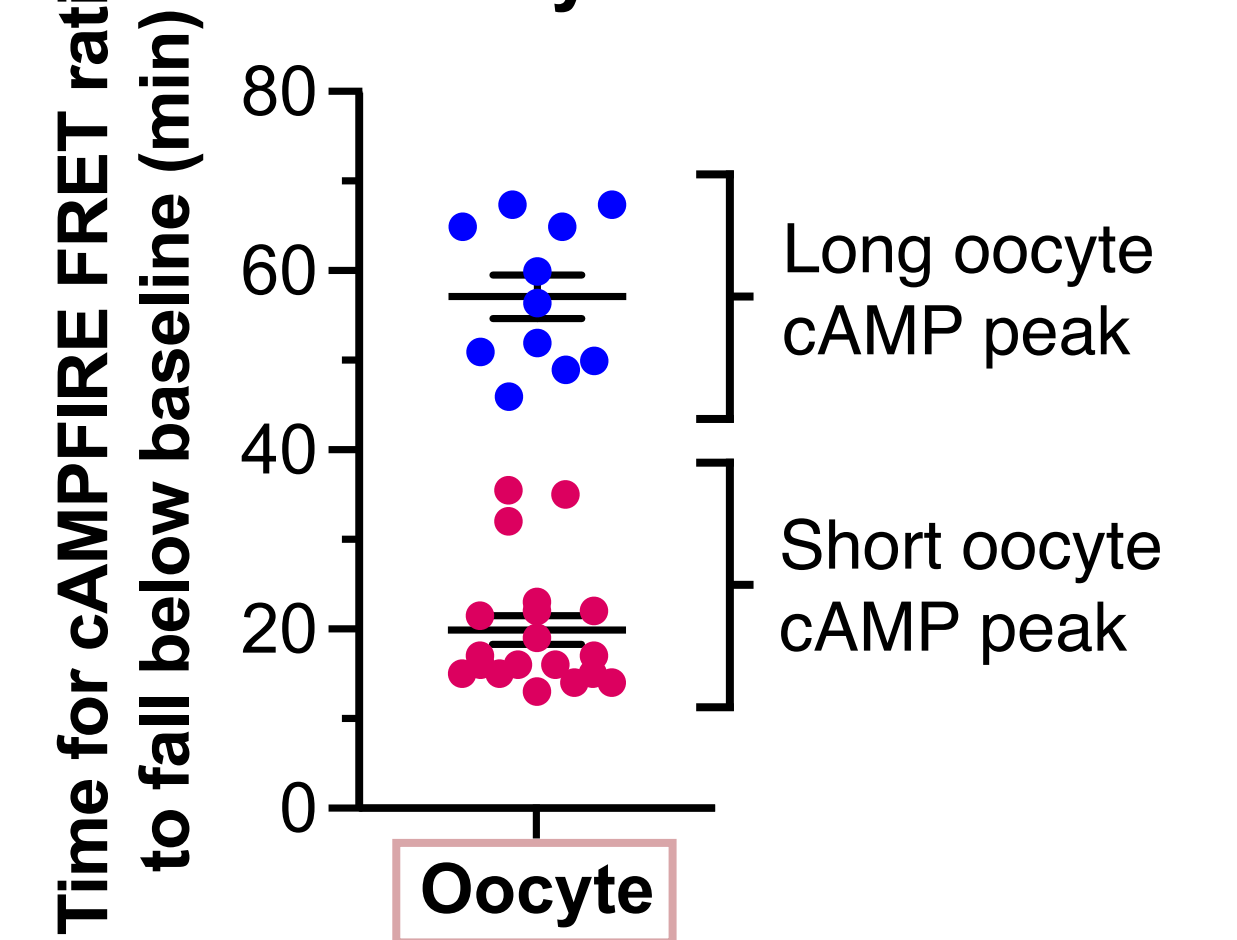
**Results:** cAMP traces reveal unexpected patterns and the effect of gap junction inhibition on follicle cAMP dynamics



cAMP elevation in oocyte and cumulus is dependent on open gap junctions



Two patterns in timing of the oocyte cAMP elevation



## Conclusions

- We report the generation of a mouse line globally expressing an improved cAMP FRET sensor, cAMPFIRE-M<sup>6</sup>.
- For the first time, we have shown that LH causes a transient increase in oocyte cAMP mediated by elevated cAMP levels in the somatic cells diffusing through gap junctions into the oocyte.
- The oocyte cAMP increase represents a barrier to meiotic resumption that must be overcome.
- As previously reported<sup>3</sup>, LH signaling causes a rapid increase in cAMP in somatic cells that is not dependent on gap junction communication in the outer mural, but is gap junction-dependent for cumulus cells.
- cAMP levels in the cumulus region also fell transiently, possibly reflecting the dynamics of LH-induced inhibition of gap junction communication<sup>9</sup>.

## Future Directions

- Determine the mechanism by which oocyte cAMP falls after the transient increase:
  - Is it entirely due to an increase in cAMP hydrolysis following the decrease in oocyte cGMP?
  - Or is it also mediated by a decrease in gap junction permeability?

## References and Funding

- Jaffe and Egbert. 2017. *Annu Rev Physiol* 79: 237-260
  - Norris et al. 2009. *Development* 136: 1869-1878
  - Lyga et al. 2016. *Endocrinology* 157: 1613-1621
  - Egbert et al. 2021. *Biol Reprod* 104: 939-941
  - Shuhaibar et al. 2015. *PNAS* 112: 5527-5532
  - Massengill et al. 2022. *Nat Methods* 19: 1461-1471
  - Klarenbeek et al. 2015. *PLoS One* 10: e0122513
  - Jaffe et al. 2009. *Methods Mol Biol* 518: 157-173
  - Norris et al. 2008. *Development* 135: 3229-3238
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