

# Sufficiency of active Rac to drive whole tissue phagocytosis in vivo

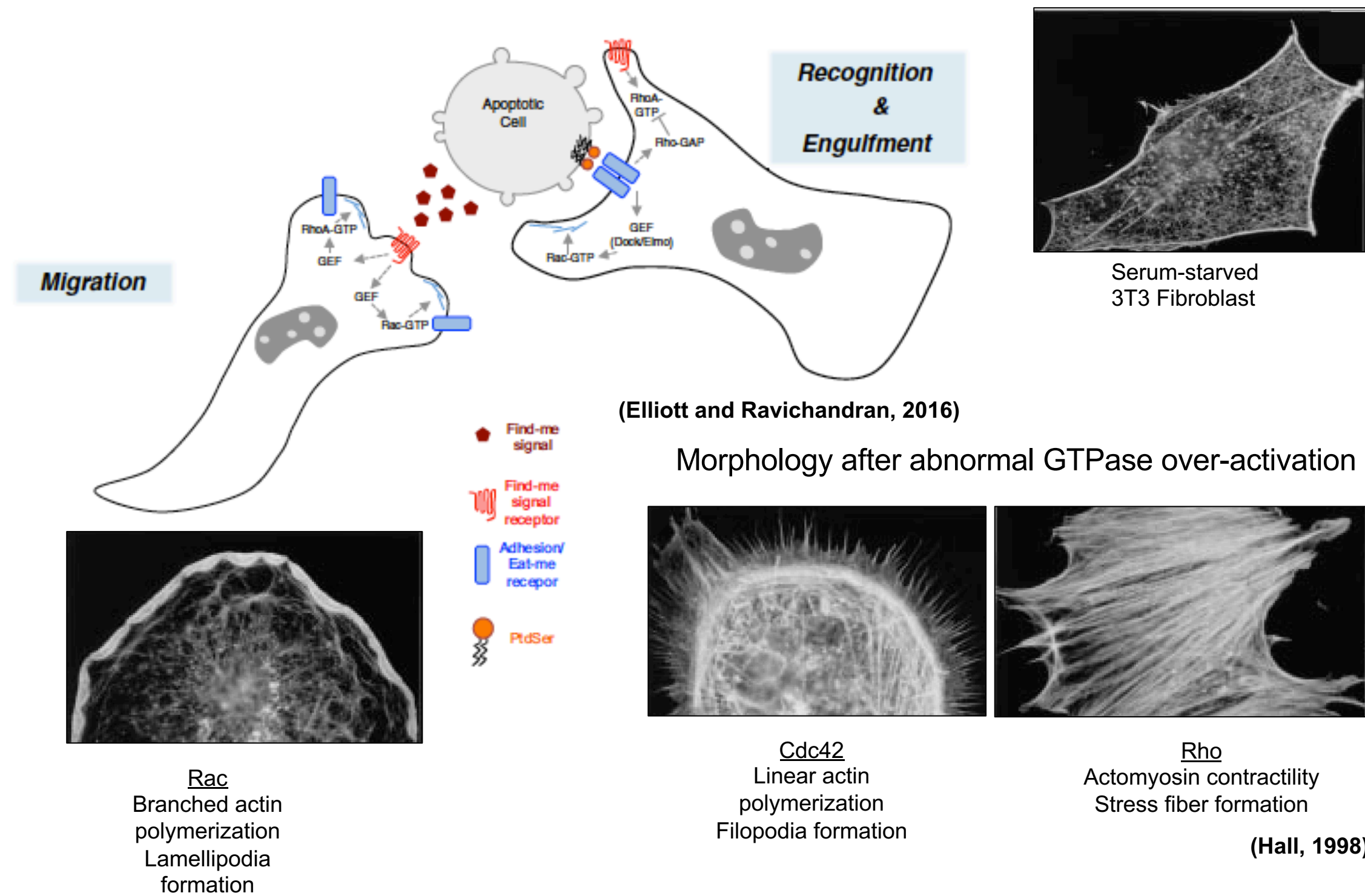
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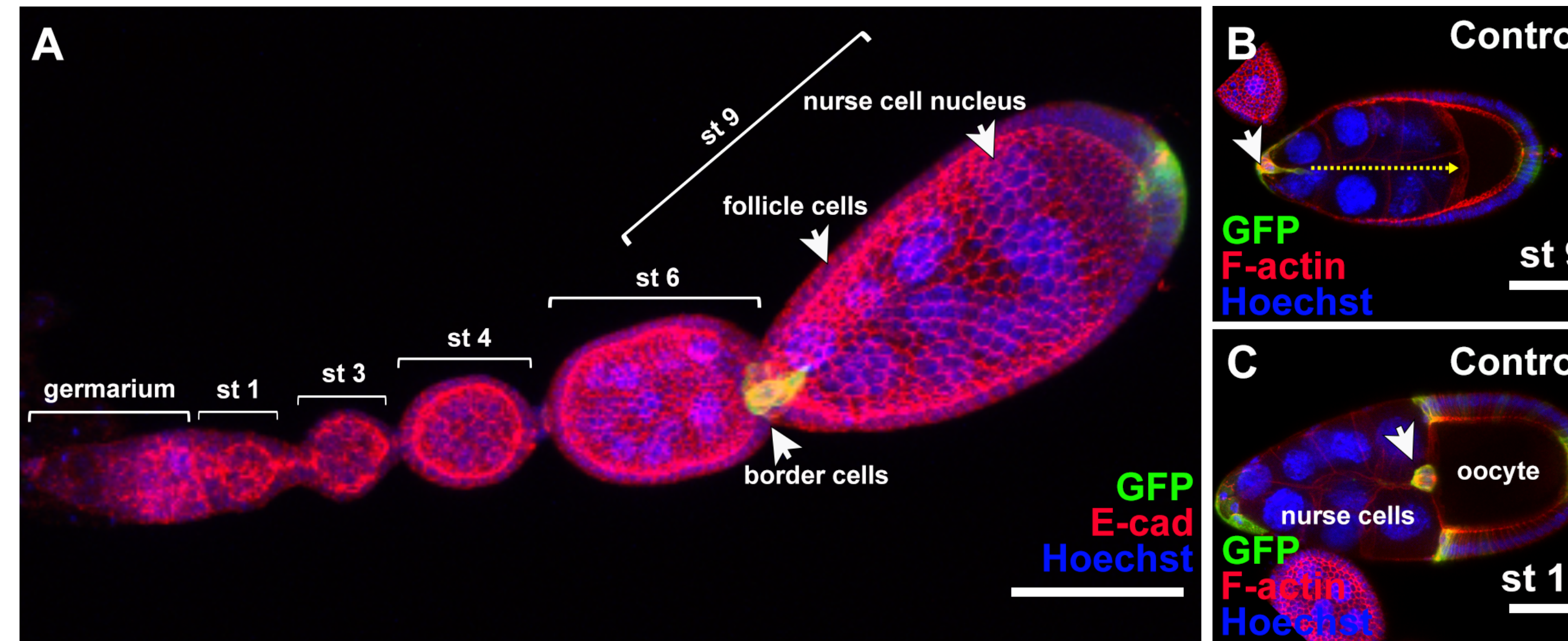


## Rho family GTPases: Regulators of Actin cytoskeleton network



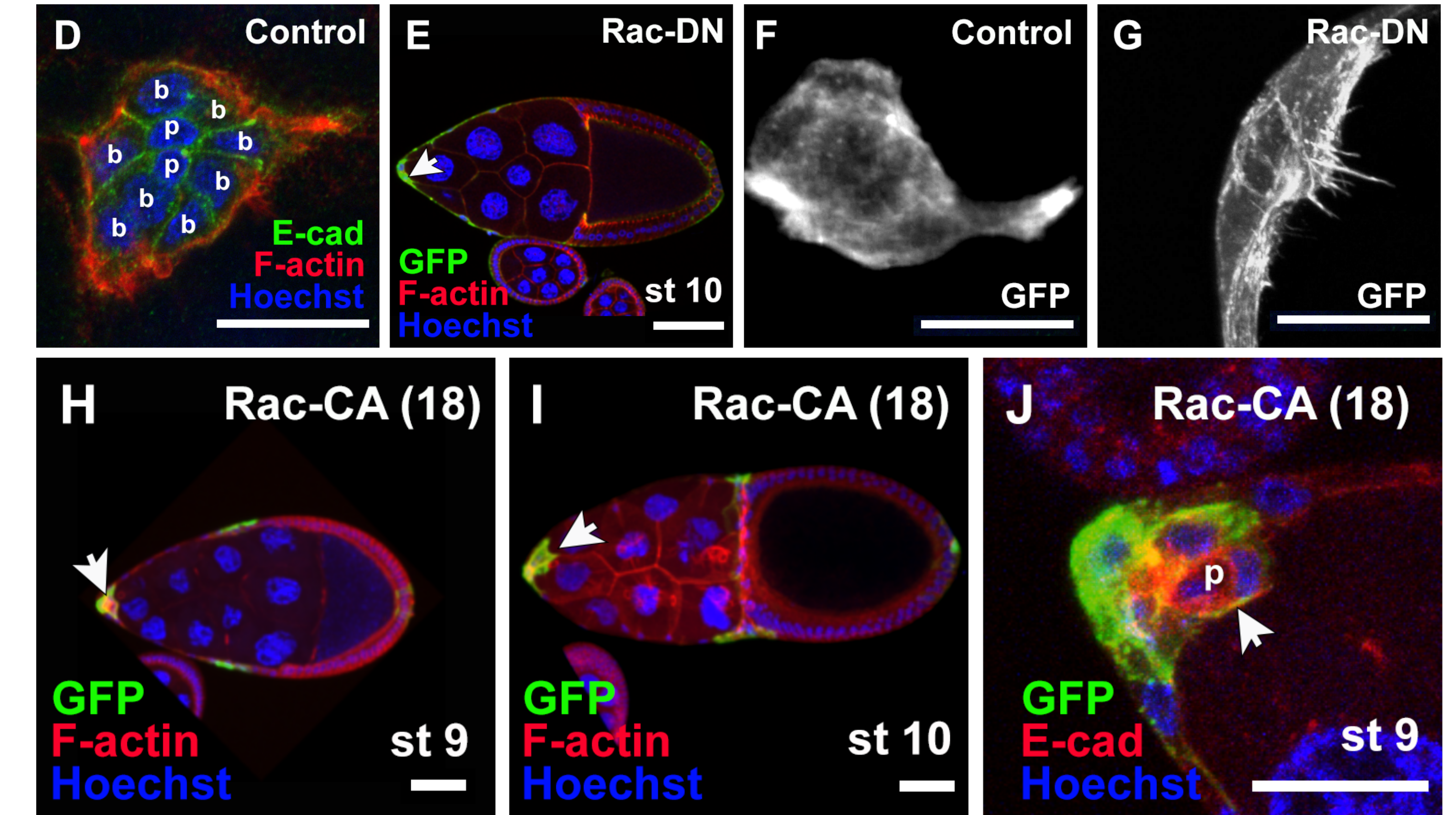
The Rho family of small GTPases, Rac, Rho, and Cdc42 represent central nodes in the cytoskeletal and signaling networks that drive cell migration and engulfment. How the cells utilize the same RhoGTPase networks to promote different processes in different contexts is not thoroughly understood. We address this question in the *Drosophila* ovary, which contains ~850 somatic follicle cells and 16 much larger germline cells.

## Overview of ovary development in *Drosophila*

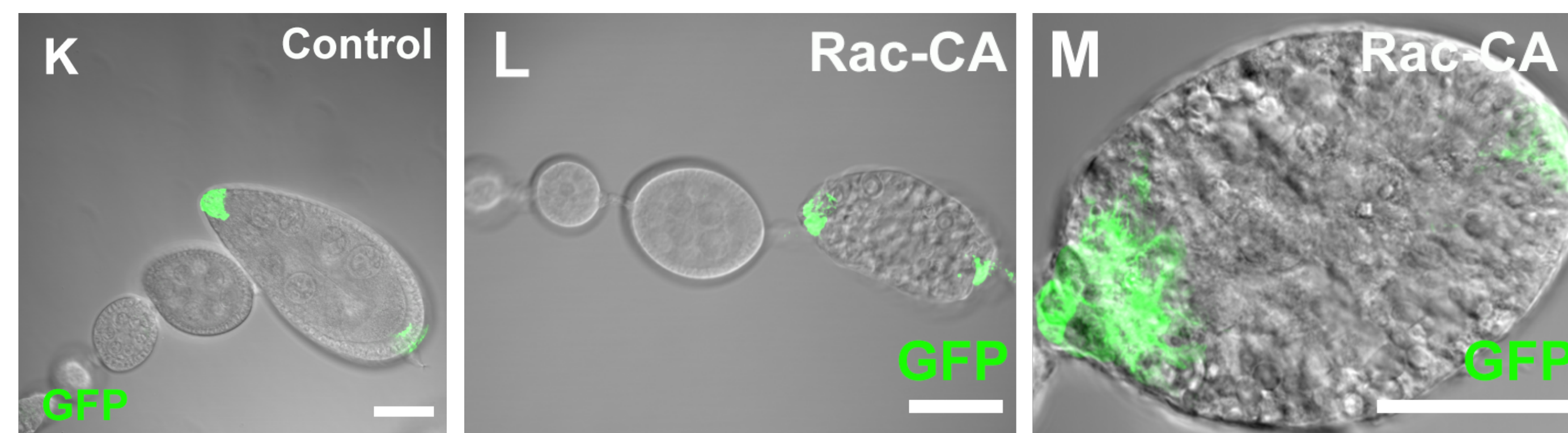


A *Drosophila* ovariole containing developing egg chambers is shown (A). A group of six to ten epithelial follicle cells in *Drosophila* ovary called border cells (b), along with a pair of non-motile polar cells (p), migrate collectively through the egg chamber to the anterior border of oocyte (B,C,D). Together they form the micropyle, a structure required for sperm entry. Lack of proper migration causes sterility. High magnification image of a border cell cluster with 6-10 border cells (b) surrounding a pair of non-motile polar cells (p) (D). Expression of dominant-negative Rac (Rac1N17) blocks protrusion and chemotaxis (E,F,G). Expression of even low levels of constitutively active (CA) Rac (Rac1V12) in border cells blocks their chemotaxis (H,I,J). Remarkably, expression of high levels of constitutively active Rac, only in a subset of follicle cells, leads to destruction of the entire egg chamber (K,L,M). Previously we reported that local and transient activation of Rac using a photoactivatable Rac (PA-Rac) induced protrusions and motility in border cells. These results indicate that focal and transient activation of Rac promotes protrusion and motility, whereas high and sustained Rac activation promotes phagocytosis, initially of living cells, ultimately resulting in destruction of the whole tissue.

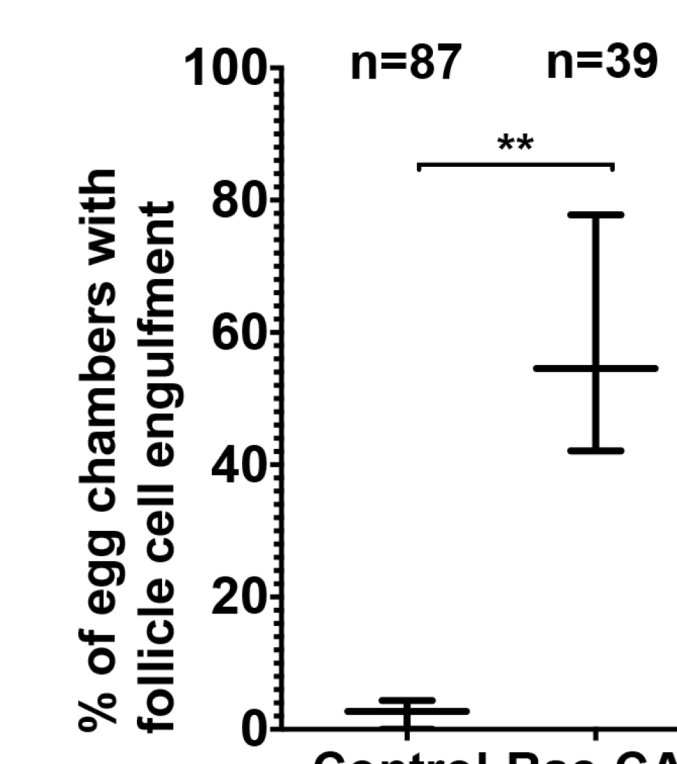
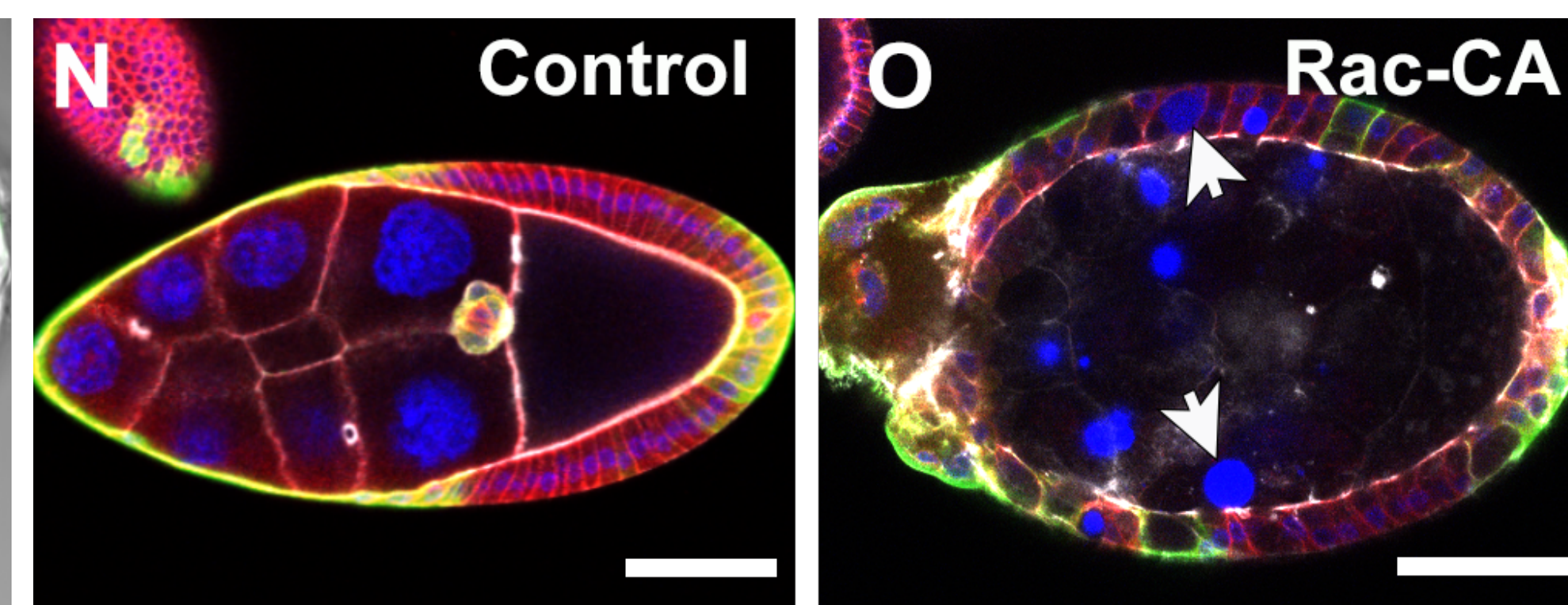
## Expression of dominant negative Rac in border cells causes protrusion defects and blocks migration and low expression of active Rac also blocks border cells migration



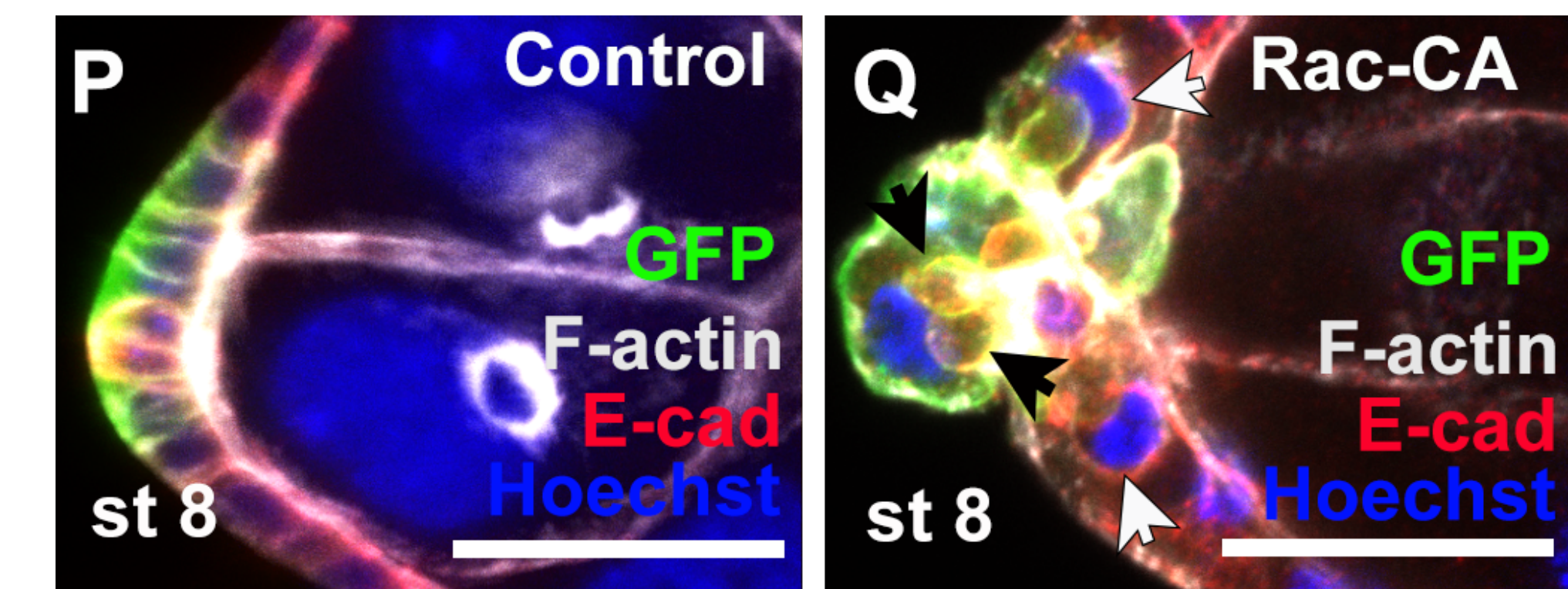
## Sustained and high expression of active Rac in a subset of follicle cells causes wholesale tissue destruction



## Expression of active Rac in a subset of follicle cells promotes nurse cell death and engulfment by follicle cells

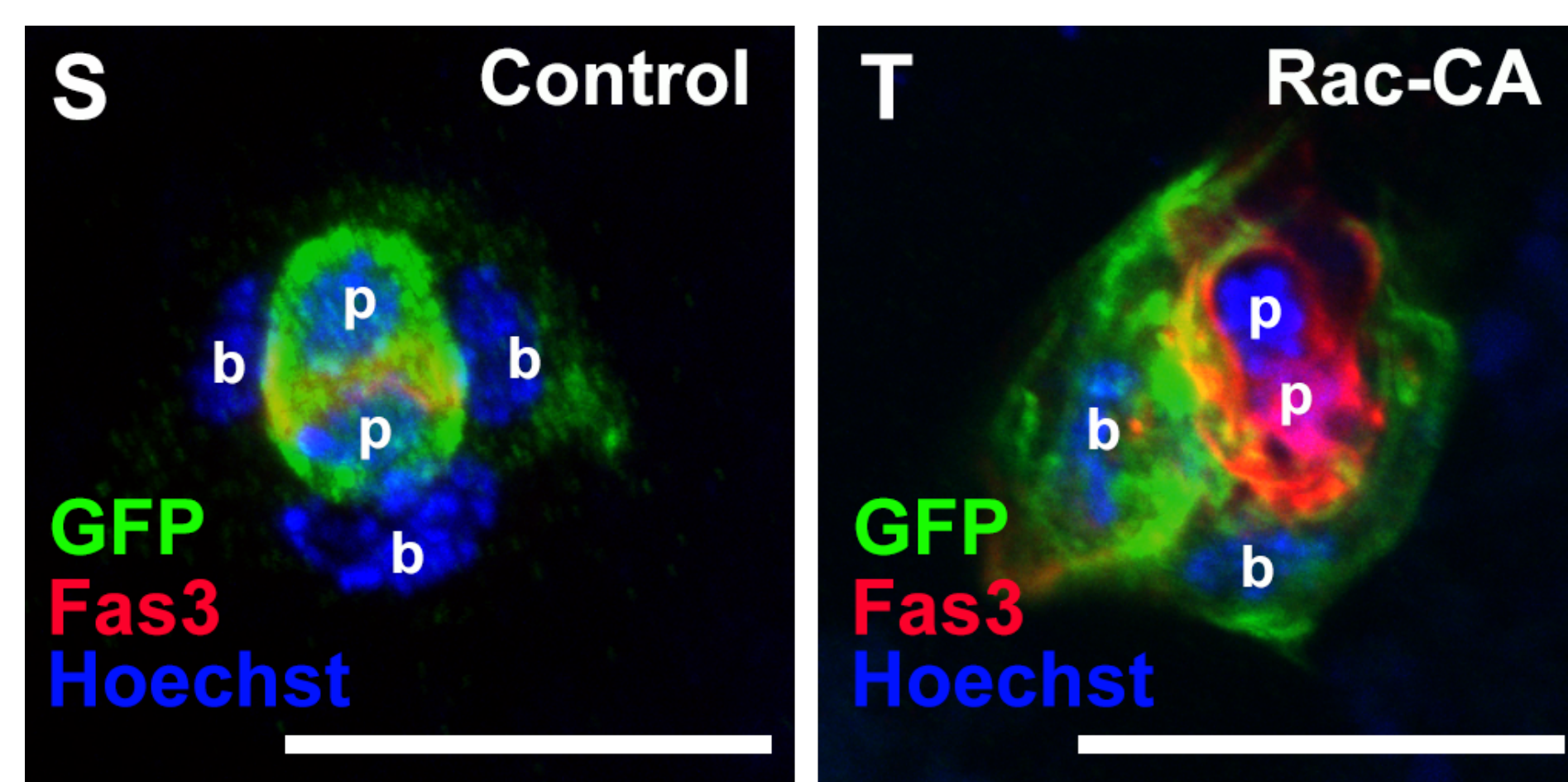


## Expression of active Rac in border cells causes engulfment of polar cells and neighboring follicle cells



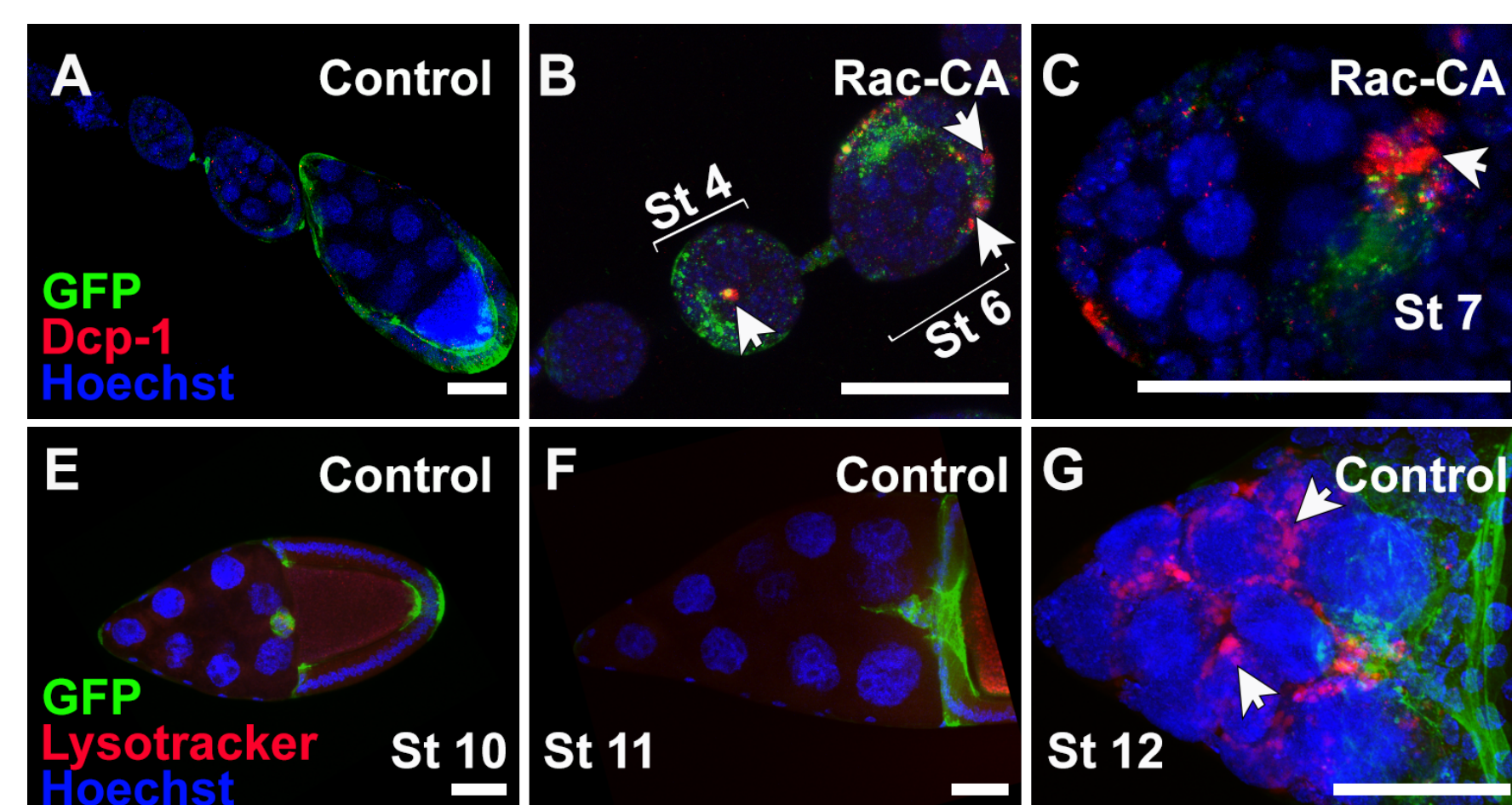
Interestingly, we observe that the Rac-CA-expressing follicle cells occasionally engulf nurse cells (N,O -arrows) as well as non Rac-CA-expressing polar cells (black arrows) and neighboring follicle cells (white arrows) in earlier stages (stage 8) (P,Q) suggesting an engulfment dependent destruction of egg chambers.

## Clonal expression of active Rac in border cells promotes engulfment of polar cells by border cells



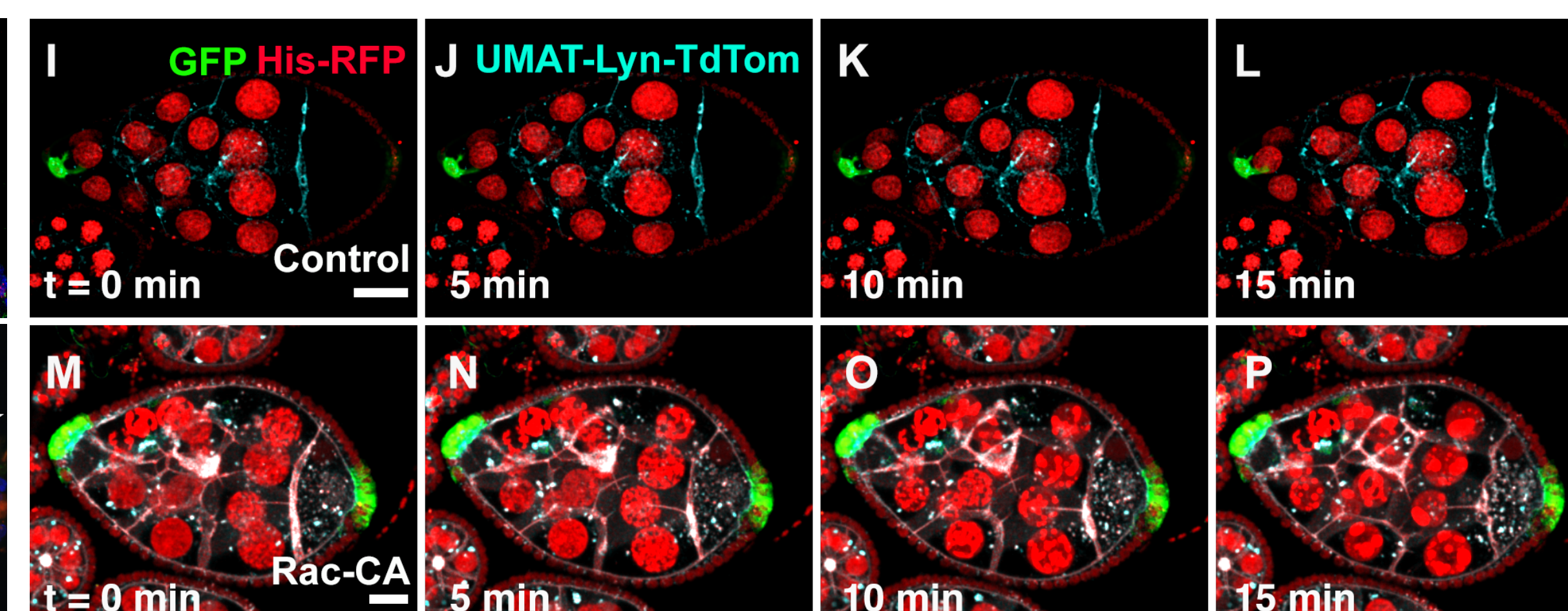
FipoutGal4 driven clones expressing control/Rac-CA in a subset of border cells display clonal cells (green) engulfing the non-clonal polar cell (marked by Fas3) altering their Fas3 Localization in Rac-CA expressing border cell clusters (S,T).

## Expression of active Rac causes non-autonomous caspase activation in follicle cells and lysosome mediated death of germline

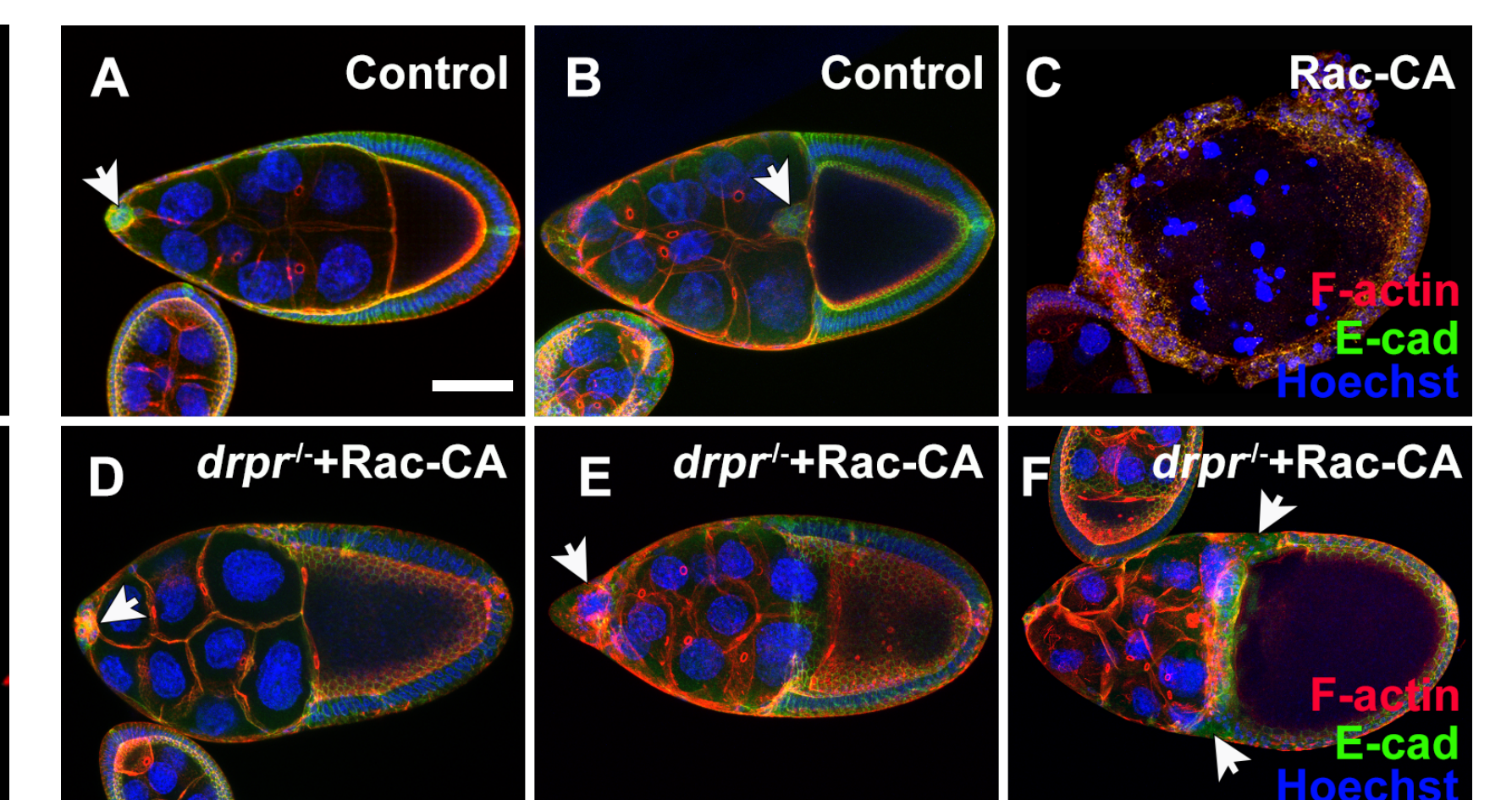


FipoutGal4 driven clones of follicle cells expressing control/active Rac (green) stained for the active executioner caspase (c-Dcp1) show a non-autonomous, occasionally long-range c-Dcp1 expression in follicle cells but not in germline nurse cells (A, B,C,D). Nurse cells are surrounded and engulfed by stretch follicle cells at the end of oogenesis undergoing a lysosome dependent death (E,F,G). Nurse cells in stage 9 egg chambers expressing active Rac show intense lysotracker staining (H) suggesting that they were killed precociously. Further, live imaging suggests the process of nurse cell nuclear condensation is synchronous and quick (I-P).

## Expression of active Rac causes quick and synchronous death of germline nurse cells



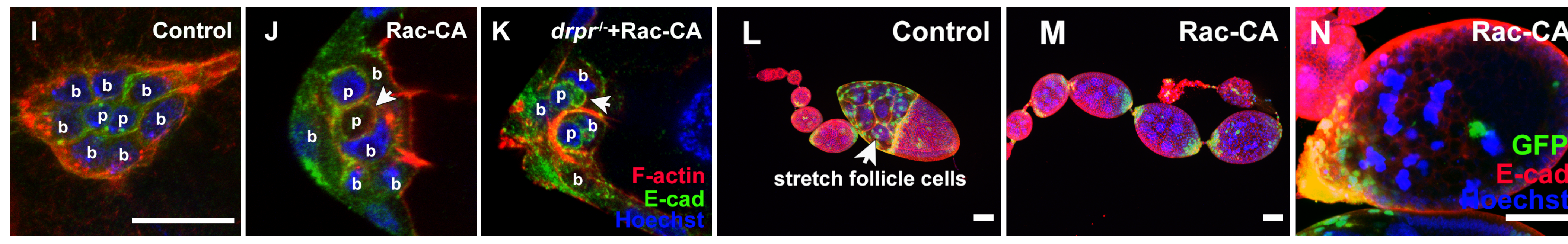
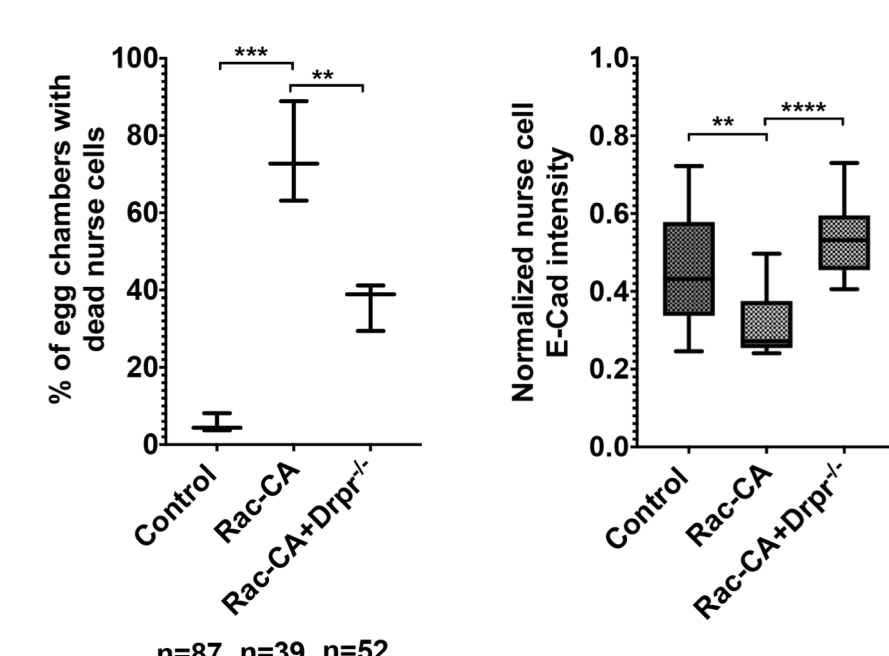
## Loss-of-function of *Drosophila* engulfment receptor Draper rescues the germline nurse cell death



The loss-of-function of *Drosophila* engulfment receptor draper rescues the germline nurse cell death but doesn't rescue the Rac dependent border cell migration (arrows-D,E). Centripetal cell migration appears complete (arrows-F). The follicle cell engulfment however is not rescued suggesting involvement of other receptors in that process (I,J,K).

## Draper does not rescue active Rac mediated follicle cell engulfment

## Expression of active Rac in stretch follicle cells causes early and synchronous onset of developmental programmed cell death



Expression of active Rac using a different Gal4 line, one that expresses in small subset of stretch follicle cells early, similarly cause germ cell death and tissue destruction suggesting that the active Rac causes early and synchronous onset of a late stage developmental programmed cell death (L,M,N).

## References

- Hall, a. Rho GTPases and the actin cytoskeleton. *Science* 279, 509–14 (1998).
- Elliott, M. R. & Ravichandran, K. S. The Dynamics of Apoptotic Cell Clearance. *Dev. Cell* 38, 147–160 (2016).

## Acknowledgements

- Bloomington *Drosophila* Stock Center
- Developmental Studies Hybridoma Bank
- Celeste Berg
- Ilich Rodriguez
- Amanda Cecil