

Figure 1. RGA-3/4 localizes to the cortex, cortical waves, the cytokinetic apparatus, and suppresses Rho activity in starfish eggs and embryos.

(A) Time course of mitosis in starfish blastomeres (8 of 16 cells) expressing mNeon-RGA-3/4^{R96E}. mNeon-RGA-3/4^{R96E} first localizes to the cortex, then the equatorial cortex, then the cytokinetic apparatus. Time in min:s; scale bar = 50 μm. (B) Time course of an Ect2-loaded starfish oocyte undergoing first and second meiosis; animal pole is to the right; time in min:s; active Rho labeled with mCherry-rGBD (cyan) and mNeon-RGA-3/4^{WT} (orange). Waves develop coincident with polar body emission and subside between meiosis I and II. Single-channel insets of the animal pole use the deepest slice to show cortical recruitment: RGA-3/4 is cortical as the cell approaches meiosis I (M1) metaphase, while Rho is not (00:00); RGA-3/4 appears brightly in the polar body furrow (20:30); RGA-3/4 departs the cortex between M1 and MII (28:00) but returns in metaphase (41:15) before waves develop. Scale bar = 50 μm. (C and C') Waves

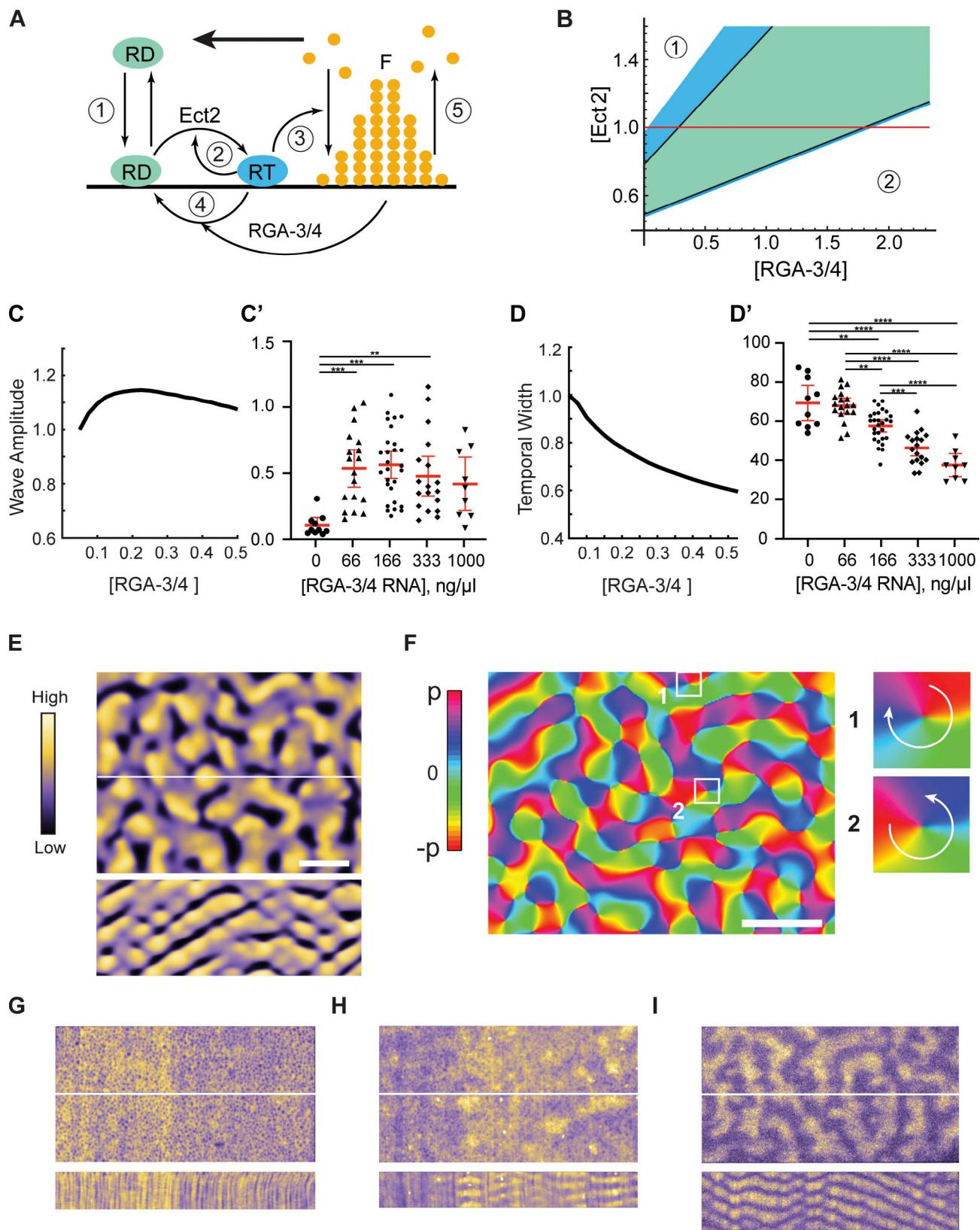


Figure 7. The model robustly predicts wave dynamics preceded by a turbulence regime. **(A)** The major reactions described by the model (see text and Materials and methods for details). F, F-actin; RD, inactive Rho; RT, active Rho. Bold arrow indicates the direction of wave propagation. **(B)** Diagram of the model behavior. Waves are predicted in the domains of wave instability (blue) and oscillations (green); zone 1 is the higher and zone 2 is the lower uniform state. **(C and C')** Modeling (C) vs. in vivo (C') data of normalized active Rho wave amplitude over changing $[RGA-3/4^{WT}]$. **(D and D')** Modeling (D) vs. in vivo (D') data of normalized Rho wave temporal width over changing $[RGA-3/4^{WT}]$. In C' and D', each dot represents a single oocyte; group mean \pm 95% confidence

Supplemental material

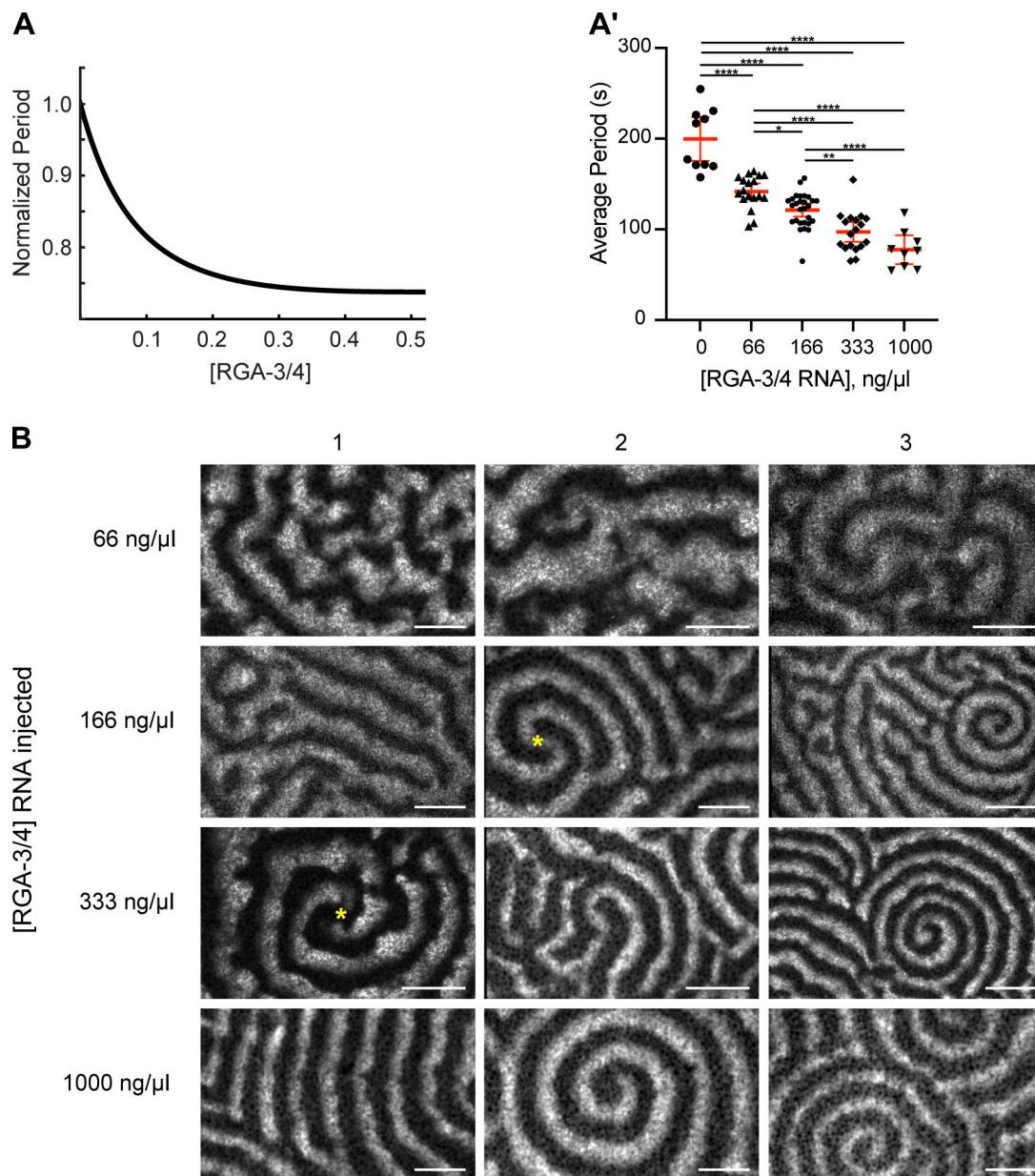


Figure S4. Changes in the activator:inhibitor ratio produce a wide range of cortical behaviors. (A and A') Modeling (A) and in vivo (A') data of normalized Rho wave period over changing RGA-3/4^{WT}. Each dot represents a single oocyte; group mean \pm 95% confidence interval; 0 ng/μl, n = 10; 66 ng/μl, n = 18; 166 ng/μl, n = 28; 333 ng/μl, n = 18; 1,000 ng/μl, n = 9; seven experiments. One-way ANOVA with Tukey post hoc test for multiple comparisons; data distribution was assumed to be normal but was not formally tested. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. **(B)** Representative oocytes from the quantifications shown in Fig. 7, C' and D', and Fig. S4 A'. Each row represents three individual cells at the noted RGA-3/4^{WT}. Waves progress from choppy/turbulent spirals to long unbroken spiral wave chains that dominate the cortex. Scale bar = 25 μm. Yellow asterisks represent double spiral cores.

Video 1. Postmeiotic starfish oocytes expressing mCh-rGBD (cyan; right), excess WT Ect2, and varying doses of mNeon-RGA-3/4^{WT} (orange; left): 25 ng/μl (needle concentration), 75 ng/μl, and 200 ng/μl. Corresponds to Fig. 1 C. Time-lapse confocal microscopy; time in min:s from start of recording; all are single superficial optical planes at 4-s intervals at 15 fps. Sequences highlight two key points: (1) RGA-3/4 recruits in the wake of Rho activity waves, and (2) increasing dose of RGA-3/4 modulates waves, first regularizing them before suppressing their amplitude and continuity. Scale bar = 50 μm.

