

Figure S1. **PR\_GEF recruitment as a function of photoactivation pulse duration.** (left) Images of HeLa cell expressing PR\_GEF or RhoA Biosensor just before (5:00), during (15:00), and after (20:00) simultaneous local illumination at the designated regions (boxes) for varying pulse lengths. (right) Quantification of the normalized intensity of the mCherry-tagged probes (mCh) in each region as a function of time. Cells were locally illuminated with 405-nm light every 20 s for designated pulse lengths for the designated photoactivation period (blue-shaded region), and 561-nm images were taken every 20 s. Bar, 10  $\mu$ m.

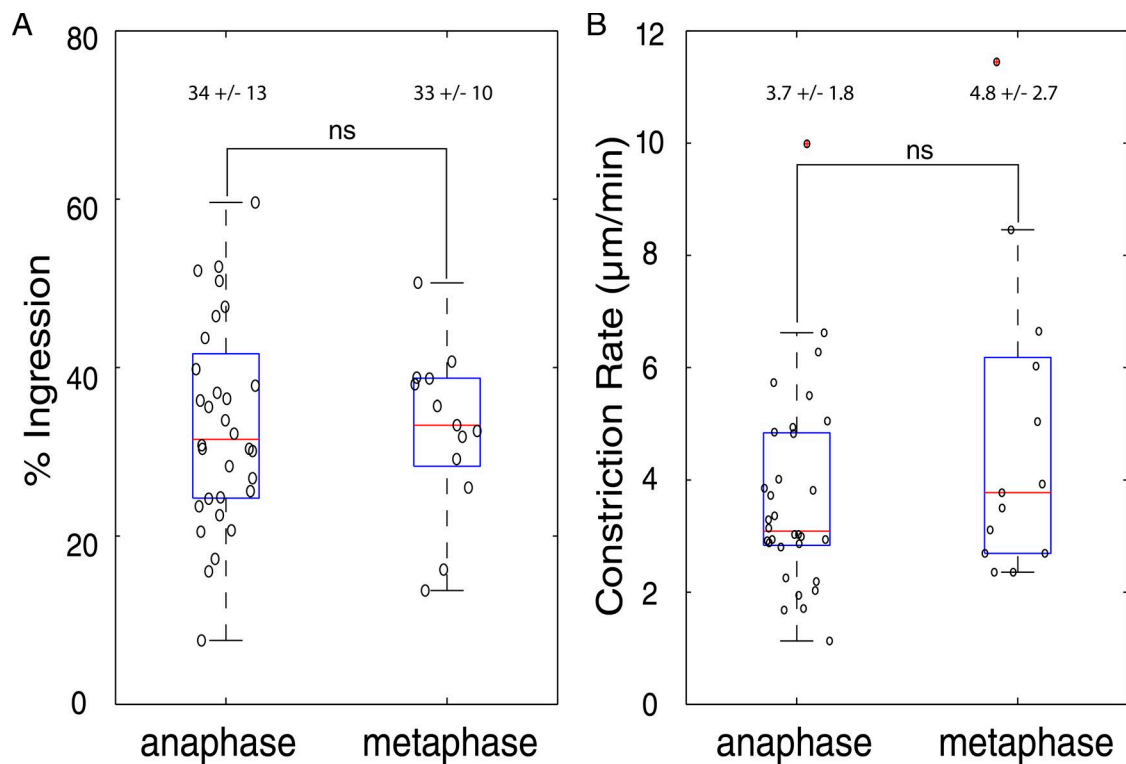


Figure S2. **Response to RhoA activation is similar in anaphase and metaphase.** Quantification of extent of furrow ingression (A) and constriction rate ( $\mu\text{m}/\text{min}$ ; B) of HeLa cells locally illuminated at the midzone during metaphase (results with Plk1-inhibited anaphase cells from Fig. 3 [B and C] is shown for comparison). ns, not significant.

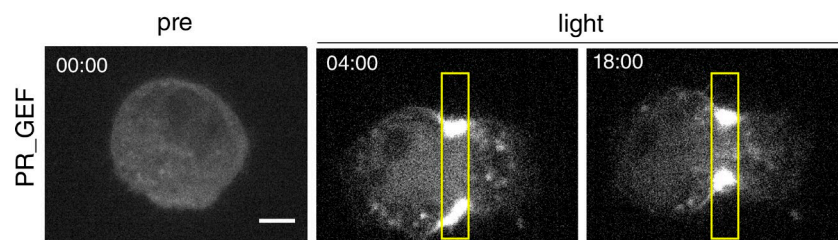
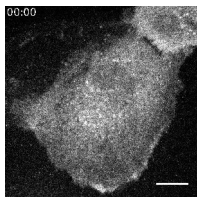
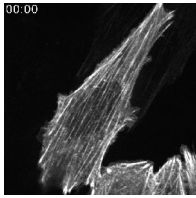


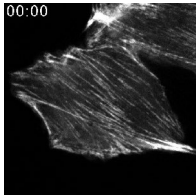
Figure S3. **Local activation of RhoA is sufficient to generate furrow formation in nonadherent interphase HeLa cells.** Images of locally illuminated, nonadherent interphase HeLa cell. For  $n = 4$  cells, the mean ingression was  $69 \pm 6.3\%$  and the mean constriction rate was  $7.34 \pm 4.59 \mu\text{m}/\text{min}$ . The increased adhesive nature of NIH3T3 cells when replated onto glass coverslips stabilized cells for imaging, whereas HeLa cells frequently moved out of the imaging field. Bar,  $5 \mu\text{m}$ .



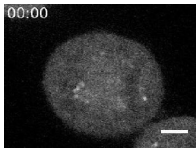
Video 1. **Light-mediated membrane recruitment of PR\_GEF.** A NIH3T3 cell expressing Stargazin-GFP-LOVpep and PR\_GEF was locally photoactivated with 405-nm light every 20 s with a 960-ms pulse (yellow box). To visualize PR\_GEF recruitment, 561-nm images were taken every 20 s before (5 min), during (5 min), and after (5 min) photoactivation.



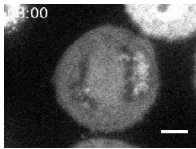
Video 2. **Light-mediated activation of RhoA induces actin polymerization.** A NIH3T3 cell expressing Stargazin-GFP-LOVpep, PR\_GEF<sup>YFP</sup>, and mApple-actin was locally photoactivated with 405-nm light every 20 s with a 960-ms pulse (yellow box). To visualize the effects of RhoA activation on the actin network, 561-nm images were taken every 20 s before (15 min), during (15 min), and after (15 min) photoactivation.



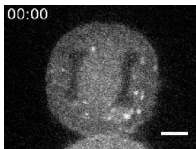
Video 3. **Light-mediated activation of RhoA induces myosin II accumulation.** A NIH3T3 cell expressing Stargazin-GFP-LOVpep, PR\_GEF<sup>YFP</sup>, and mApple-MLC was locally photoactivated with 405-nm light every 20 s with a 960-ms pulse (yellow box). To visualize the effects of RhoA activation on myosin localization, 561-nm images were taken every 20 s before (15 min), during (15 min), and after (15 min) photoactivation.



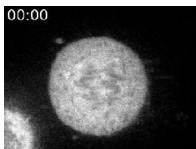
Video 4. **Light-mediated activation of RhoA in noncontractile anaphase HeLa cell induces furrow formation at the midzone.** A noncontractile anaphase HeLa cell (200 nM BI 2536) expressing Stargazin-GFP-LOVpep and PR\_GEF was locally photoactivated with 405-nm light every 20 s with a 960-ms pulse (yellow box) at the midzone. To visualize PR\_GEF recruitment and furrow induction, 561-nm images were taken every 1 min during (20 min) and after (6 min) photoactivation.



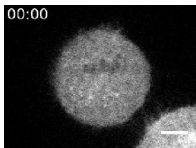
Video 5. **Light-mediated activation of RhoA in noncontractile anaphase HeLa cell induces furrow formation at the poles.** A noncontractile anaphase HeLa cell (200 nM BI 2536) expressing Stargazin-GFP-LOVpep and PR\_GEF was locally photoactivated with 405-nm light every 20 s with a 960-ms pulse (yellow box) at the poles. To visualize PR\_GEF recruitment and furrow induction, 561-nm images were taken every 20 s during (23 min) and after (14 min) photoactivation.



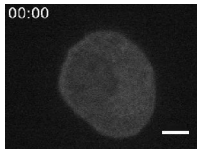
Video 6. **Simultaneous photoactivation of midzone and polar regions induces a similar response in noncontractile anaphase HeLa cells.** A noncontractile anaphase HeLa cell (200 nM BI 2536) expressing Stargazin-GFP-LOVpep and PR\_GEF was simultaneously photoactivated with 405 nm light every 20 s with a 960-ms pulse (yellow box) at both the midzone and polar regions. To visualize PR\_GEF recruitment and furrow induction, 561-nm images were taken every 20 s during (20 min) and after (5 min) photoactivation.



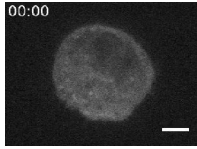
Video 7. **Exogenous activation of RhoA in the poles competes with endogenous furrow formation.** A dividing HeLa cell expressing Stargazin-GFP-LOVpep and PR\_GEF was locally photoactivated with 405-nm light every 20 s with a 960-ms pulse (yellow box) at the poles. To visualize PR\_GEF recruitment and the effect on endogenous furrow formation, 561-nm images were taken every 20 s during (30 min) photoactivation.



Video 8. **Light-mediated activation of RhoA induces furrow formation during metaphase.** A metaphase HeLa cell expressing Stargazin-GFP-LOVpep and PR\_GEF was locally photoactivated with 405-nm light every 20 s with a 960-ms pulse (yellow box). To visualize PR\_GEF recruitment and furrow induction, 561-nm images were taken every 20 s during (23 min) and after (14 min) photoactivation.



**Video 9. Light-mediated activation of RhoA induces rapid and nearly complete furrow formation in nonadherent interphase NIH3T3 cells.** A nonadherent interphase NIH3T3 cell expressing Stargazin-GFP-LOVpep and PR\_GEF was locally photoactivated with 405-nm light every 20 s with a 960-ms pulse (yellow box). To visualize PR\_GEF recruitment and furrow induction, 561-nm images were taken every 1 min during (20 min) and after (10 min) photoactivation.



**Video 10. Low levels of global RhoA activation dampen the extent and rate of furrow induction upon local RhoA activation in nonadherent interphase NIH3T3 cells.** A nonadherent interphase NIH3T3 cell expressing Stargazin-GFP-LOVpep and PR\_GEF was photoactivated both globally (10-ms pulse) and locally (960-ms pulse, yellow box) with 405-nm light every 20 s. To visualize PR\_GEF recruitment and furrow induction, 561-nm images were taken every 20 s during (10 min) and after (6 min) photoactivation.