Supplementary materials

Supplementary figure legends

Fig. S1. Knockdown of Par3 prevents *de novo* BC formation and tubular BC extension
(**A**) Representative images of BC formation in the control (si Con) and Par3-knockdown (si Par3) cells. Mdr, green; DNA, green. Scale bar, 10 μm. (**B**) The percentage of cells engaged in BC formation is 61 ± 6% (SD) for siPar3 cells (n = 12 image fields; total 837 cells quantified) compared to control cells (84 ± 3%; n = 8 image fields; total 786 cells quantified) ($p < 0.001$). (**C**) The BC lengths for the siCon (223 BCs for 786 cells quantified) and siPar3 (161 BCs for 837 cells quantified) cells are 15 ± 14 μm, and 8 ± 3 μm, respectively ($p < 0.001$). (**D**) Western blotting indicates that the level of Par3 (relative molecular mass: 150 kD) in the si Par3 cells was reduced to 11% of that in the control cells. Actin was used as a loading control. (**E-F**) Knockdown of Par3 does not appear to affect the maintenance of BC (**E**) or tight junction (**F**). Scale bars, 3 μm. The siRNA against Par3 used in this experiment (AGAGUUGGAUGACAGAGAACGCAGG, sense) was purchased from IDT, and the one used in Figure 4 (GGAGUAGAUUUAGCGGGCA, sense) was purchased from Invitrogen.

Fig. S2. Distinct effects of LatA and NZ treatments on the actin cytoskeleton (F-actin, red), microtubules (tubulin, green), and BC architecture (ZO-1, blue), Related to Fig. 6A
The separate image panels for the same cells presented in Fig. 6A (merged panel only) are shown here for clear visualization of the drug-treatment effects.

Fig. S3. LatA treatment affects BC architecture (Mdr, red), Related to Fig. 6A
Four representatives of the misshaped (cell pairs 1 and 2) and fragmented (cell pairs 3 and 4) BCs caused by LatA treatment. Scale bar, 3 μm.

Fig. S4. Centrosome location (centriolin, green) during different stages of BC formation (ZO-1, red), Related to Fig. 6F
During pre-BC stage, the centrosomes are either away from (cell pair 1) or near (cell pair 2) the BC membrane. However, during the small- and large-BC stages (cell pairs 3 and 4), the centrosomes are invariably close to the BC membrane. Scale bar, 3 μm.

Supplementary movie Legends

Movie 1. BC formation is spatially linked to cytokinesis, Related to Fig. 1A
A time-lapse movie showing a single BC-like structure at the cell division site two hours after cytokinesis. The BC was enlarged over time. Individual frames were acquired every four minutes.

Movie 2. A time-lapse movie showing three cells undergoing cytokinesis and BC formation, Related to Fig. 1B
At the end of this time-lapse analysis, the same three cells were fixed and stained for Mdr (green) to visualize BC formation at the division site. Individual frames were acquired every six minutes.

Movie 3. Endocytosis of a BC structure, Related to Fig. 1
A time-lapse movie showing endocytosis of a BC structure at the division site. Individual frames were acquired every four minutes.

Movie 4. 3D reconstruction of the complex tight junction at the 3-cell stage, Related to Fig. 3D
Tight-junction morphology (ZO-1, red) at the midbody stage (Aurora B, green) of second round of division was reconstructed in 3D using 0.3 μm × 21 optical sections.

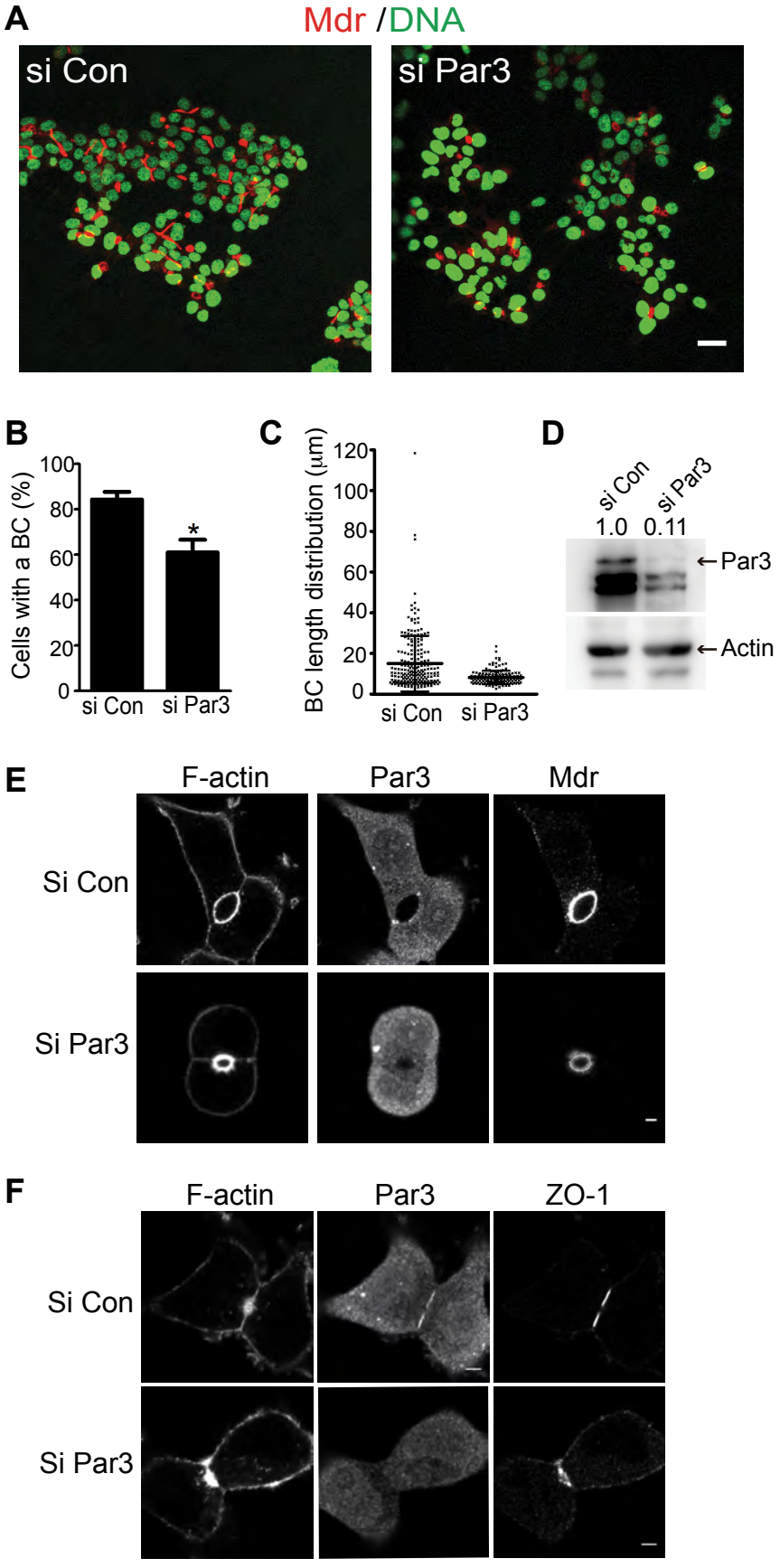


Figure S1. Wang et al.

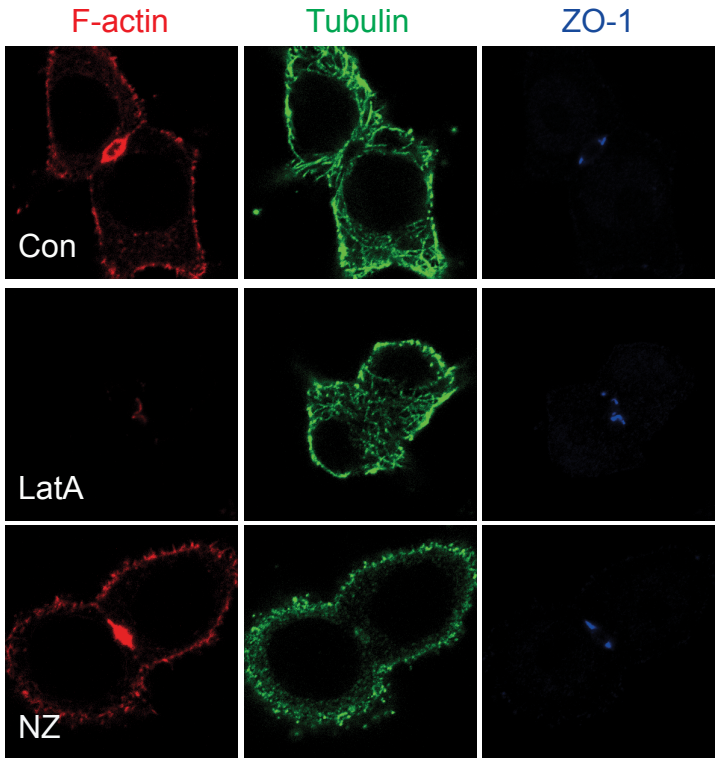


Figure S2. Wang et al.

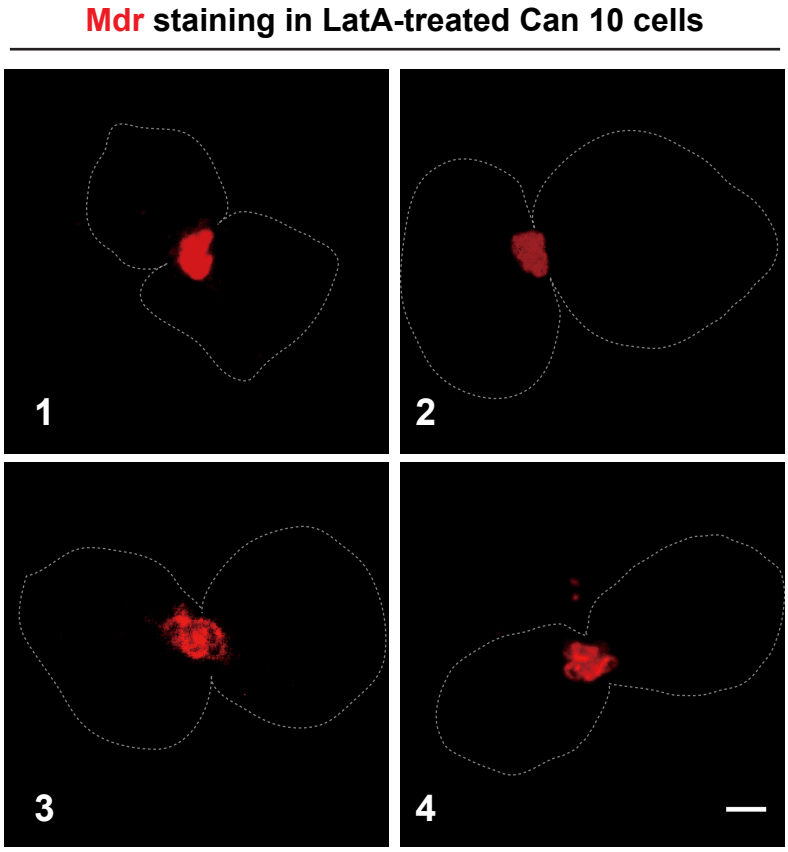


Figure S3. Wang et al.

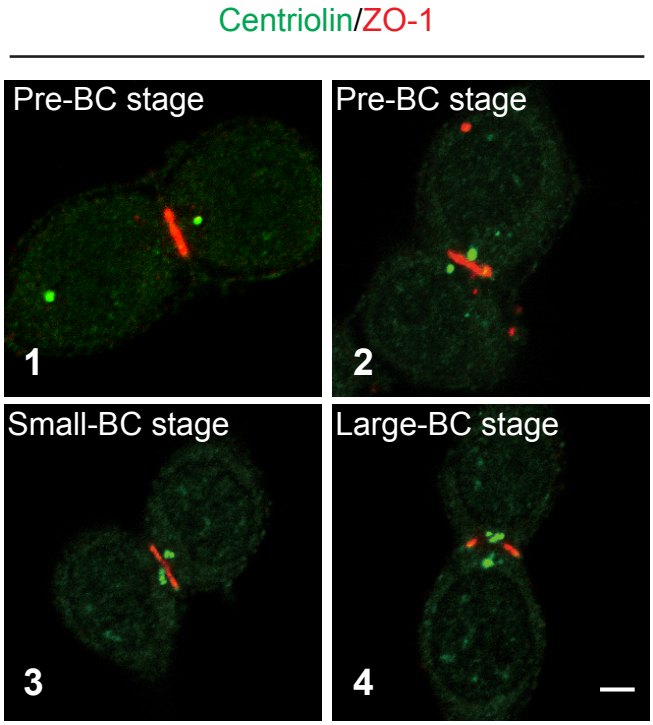
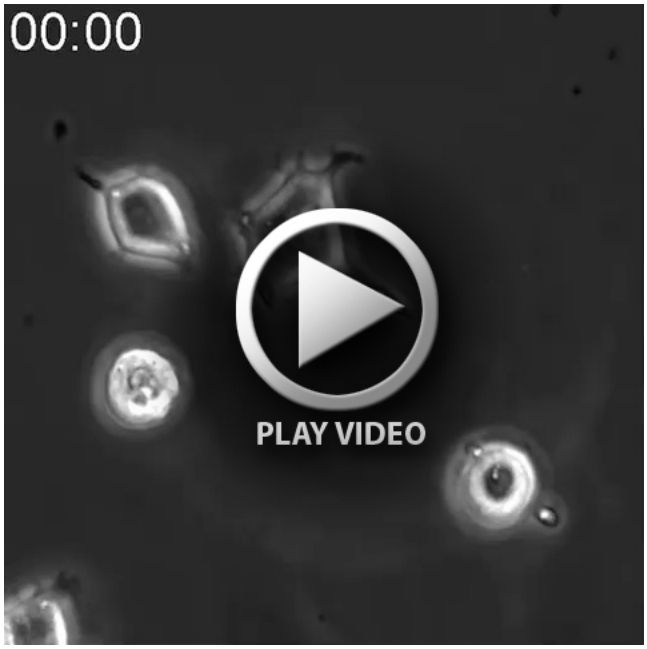


Figure S4. Wang et al.

Movie 1.



Movie 2.

Movie 3.

Movie 4