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.

**Mdr** staining in LatA-treated Can 10 cells

1 2
3 4
Figure S4. Wang et al.
Movie 1.

Movie 2.

Movie 3.
Movie 4