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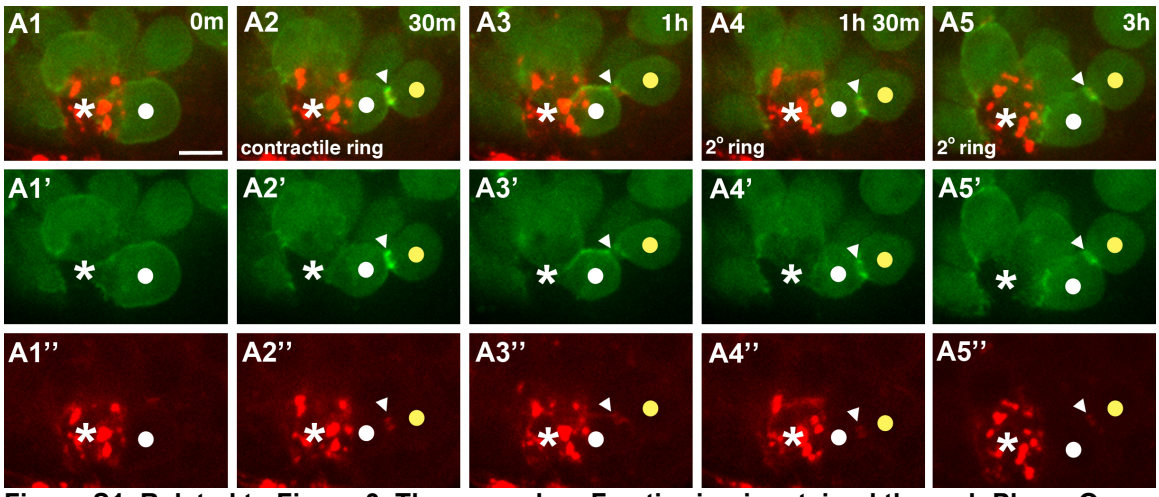

Figure S1, Related to Figure 3. The secondary F-actin ring is retained through Phase One.
Time lapse imaging of ABD-moeGFP and Myo-mCherry. Each panel is a projection of 2-5 Z planes. White dot=GSC. Yellow dot=Gb. Arrowhead=IC bridge. *=Hub. m=min. h=hour.

(A1-A2'')A GSC divided and was connected by an IC bridge containing the contractile ring (arrowhead) to its nascent daughter Gb.

(A3-A3'')The F-actin of the contractile ring was depolymerized and the GSC-Gb pair remained visibly attached through an IC bridge containing a Myo-mCherry-labeled midbody ring (arrowhead).

(A4-A4'')Within 30min, a secondary F-actin ring was formed at the IC bridge (arrowheads)

(A5-A5'')and was retained for the remainder of this imaging session (another 1hr 30min); this is well into Phase One of delay.

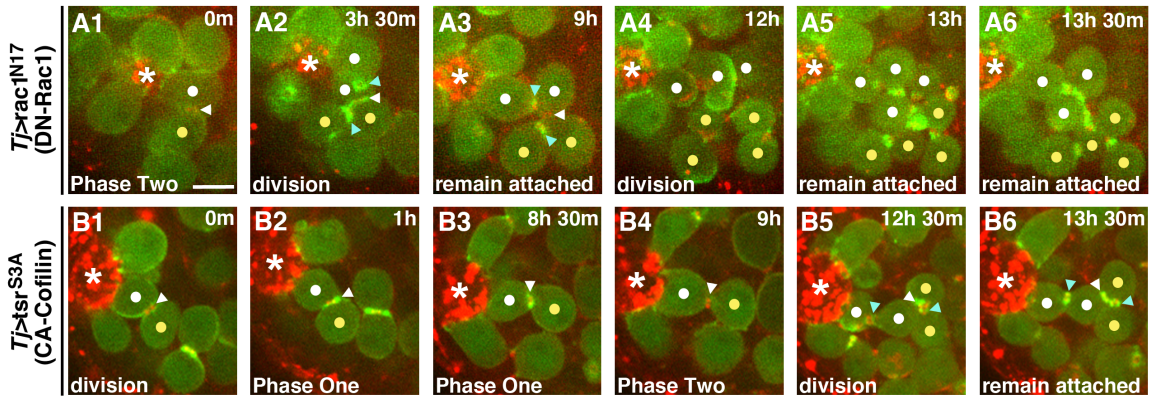

Figure S2, Related to Figure 7. Somatic cell encystment controls GSC abscission
Time lapse imaging of ABD-moeGFP and Myo-mCherry in the indicated genotypes. Each panel is a 2-6 Z plane projection. m=min. h=hour. White dot=GSC and "new" GSC daughter generated after second division. Yellow dot=Gb and "new" Gb daughter generated after second division. White arrowhead="original" IC bridge. Blue arrowheads="new" IC bridges. Arrow=midbody remnant. *=Hub. Scale bar=5 microns.

(A1-A6) A GSC-Gb pair in testes with somatic expression of DN-Rac1 fails to abscise prior to a second round of mitosis (A2), remains attached as a four-cell grouping at the hub for the entirety of the following cell cycle (A3). The four cells then synchronously enter another round of mitosis (A4), resulting in the formation of an interconnected cyst of 8 cells connected to the hub (A5-A6). One cell part of the "GSC" four-cell grouping is out of the planes visible in the Z projection shown in A6.

(B1-B6)A GSC-Gb pair in testes with somatic expression of CA-Cofilin showing that disruption of actin dynamics within somatic cells also blocks the ability of GSC-Gb pairs to abscise.

MovieS1, Related to Figure 1: Mitosis through abscission in a wild type GSC reveals two phases of abscission delay

GSC, white dot. Gb, yellow dot. Each frame is a single Z plane. Images taken every 25min. Movie is 3 frames per second (fps). The GSC divided, progressed through Phase One and Phase Two of delay and ultimately abscises, with the Gb inheriting the midbody remnant. In the final frames, the Gb has moved out of the plane of view and at 1,050min the GSC has entered a second round of mitosis.

MovieS2, Related to Figure 2: Shrub-GFP accumulates at the IC bridge during Phase Two

GSC followed from mitosis through abscission marked by an arrow in first frames prior to division. Each frame is a projection of 2-6 Z planes. Images taken every 25min. Movie is 3fps. Shrub-GFP does not localize to the IC bridge following mitosis and instead does not begin to accumulate until 7hrs after GSC division. Shrub-GFP becomes tightly localized to the midbody ring/midbody during Phase Two and is retained at the midbody remnant inherited by the Gb following abscission (arrowhead at 900m).

MovieS3, Related to Figure 3: Control photo-activation of PAtub-GFP in an unattached GSC and one cell of a two-cell cyst

GSC and cell of two-cell cyst indicated by arrows in first frames, prior to UV exposure. Each frame is a single Z plane. Images taken every 2s. Movie is 3fps. GFP was visible immediately following UV exposure (at 8sec). GFP remained restricted to the individual GSC at the hub (arrow at 64s) while GFP diffused rapidly into the connected, untargeted germ cell of the two-cell cyst (arrowhead at 64s).

MovieS4, Related to Figure 3: GSC-Gb pairs in Phase One share cytoplasm

GSCs of two different pairs, both in Phase One and connected via a midbody ring indicated by arrows in first frames, prior to UV exposure. Each frame is a single Z plane. Images taken every 2s. Movie is 3fps. GFP was visible immediately following UV exposure (at 16s) and rapidly diffused into the connected Gbs (arrowheads at 80s) of the two targeted GSCs (arrows at 80s).

MovieS5, Related to Figure 3: GSC-Gb pairs in Phase Two no longer share cytoplasm

GSC of a pair in Phase Two and connected via a midbody dot indicated by arrow in first frames, prior to UV exposure. Each frame is a single Z plane. Images taken every 2s. Movie is 3fps. GFP was visible immediately following UV exposure (at 6s) but no diffusion of GFP into the attached Gb daughter was observed (arrowhead at 68s).

MovieS6, Related to Figure 2: The central spindle is disassembled during Phase One

GSC followed from mitosis through central spindle disassembly marked by an arrow in first frames, prior to division. Each frame is a projection of 2-6 Z planes. Images taken every 10min. Movie is 3fps. Tubulin-GFP accumulated at the centrosomes in early mitosis immediately prior to formation of the metaphase spindle. The central spindle was first visible in the next frame (at 140min) as densely compacted microtubules spanning the newly formed IC bridge. The density of central spindle microtubules was progressively diminished throughout the course of the time lapse until the structure was fully disassembled.

MovieS7, Related to Figure 7: Somatic cell encystment promotes GSC-Gb abscission

GSC, white dot. Gb, yellow dot. Images taken every 25min. Each frame is a projection of 2-6 Z planes. Images taken every 25min. Movie is 3fps. After division, the GSC properly transitioned through both Phases One and Two of delay. However, rather than abscising, the GSC-Gb pair remained connected and synchronously entered a second round of mitosis to form a group of four connected cells attached to the hub. These cells remain physically associated through the remainder of our imaging.

Table S1, Related to Figure 5. Average timing of cytokinesis progress with manipulation of Cofilin activity

	Division to 2° Ring Disassembly PHASE ONE	2° Ring Disassembly to Abscission PHASE TWO	Division to Abscission	Division to Division
Wild Type	504.8min (*n*=63)	266.2min (*n*=48)	776.1min (*n*=23)	841.7min (*n*=12)
***nos*>tsr^{S3A}**	370.8min (*n*=37)	287.5min (*n*=14)	706.3min (*n*=11)	709.4min (*n*=8)
***nos*>Ssh**	337.1min (*n*=29)	296.9min (*n*=16)	605min (*n*=10)	592.9min (*n*=7)
LimK^{2}	321.8min (*n*=47)	355.8min (*n*=26)	630.6min (*n*=18)	850min (*n*=2)
ROK Inhibitor	272.9min (*n*=12)	337.5min (*n*=12)	614.6min (*n*=12)	-

Table S2, Related to Figures 6 and 7. Average timing of cytokinesis progress with manipulation of AurB/Svn activity or encystment

	Division to 2° Ring Disassembly PHASE ONE	2° Ring Disassembly to Abscission PHASE TWO	2° Ring Disassembly to Division	Division to Abscission	Division to Division
Wild Type (*n*=12)	500min	258.3min	341.7min	758.3min	841.7min
***nos*>SvnS125E (*n*=6)**	308.3min	387.5min	475min	695.8min	800min
***aurB^{1689}* (*n*=12)**	570.8min	-	181.3min	-	752.1min
***tj*>tsr^{S3A} (*n*=10)**	557.5min	-	240min	-	797.5min
***c587*>egfr^{DN} (*n*=14)**	430.4min	-	321.4min	-	751.8min
***c587* control (*n*=13)**	373.1min	186.5min	225min	559.6min	603.8min