iScience, Volume *26*

Supplemental information

The Rho1 GTPase controls anillo-septin assembly to facilitate contractile ring closure during cytokinesis

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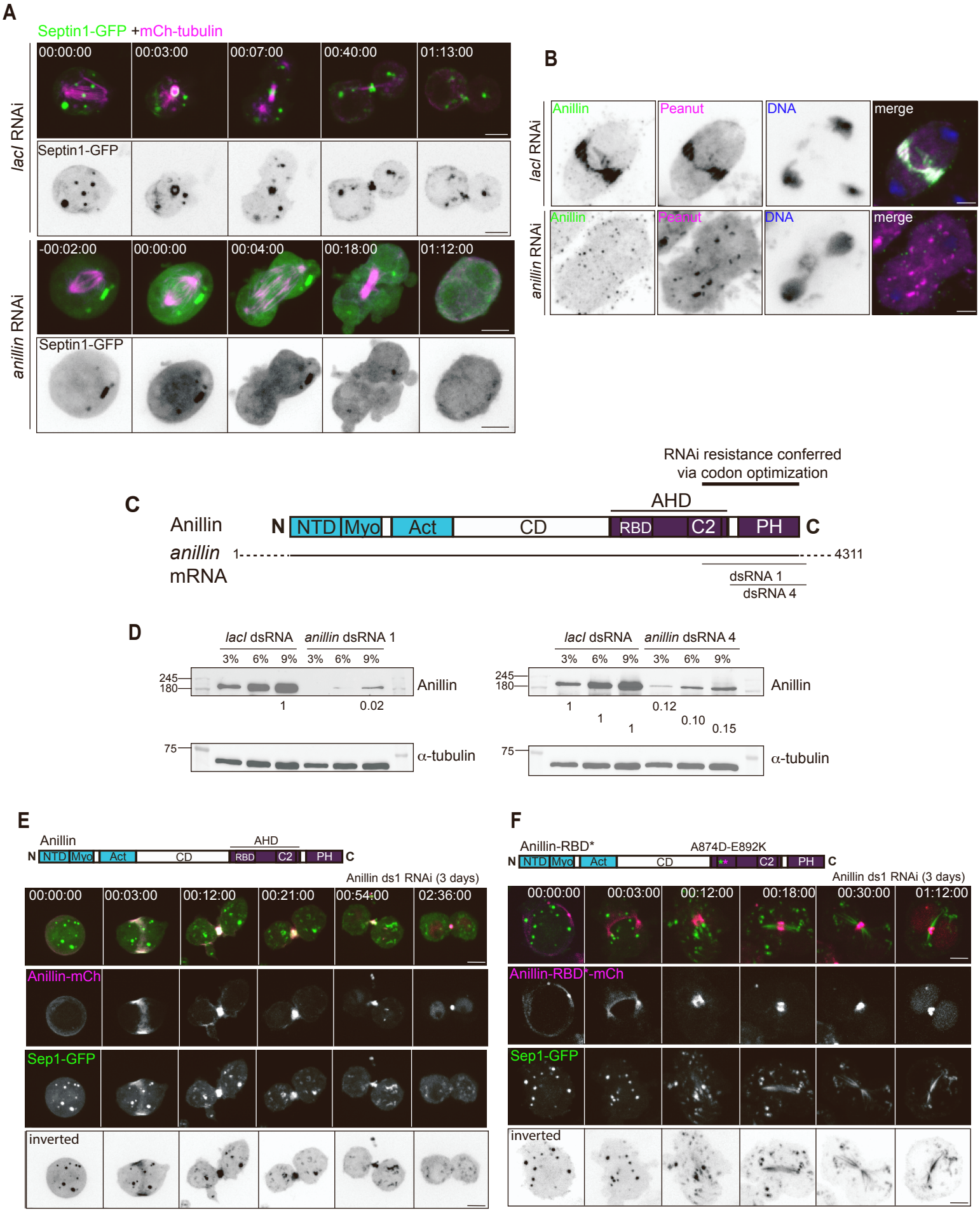

A
Septin1-GFP +mCh-tubulin
lacI RNAi
00:00:00
00:03:00
00:07:00
00:40:00
01:13:00
Septin1-GFP
anillin RNAi
-00:02:00
00:00:00
00:04:00
00:18:00
01:12:00
Septin1-GFP
B
lacI RNAi
Anillin
Peanut
DNA
merge
anillin RNAi
Anillin
Peanut
DNA
merge
C
RNAi resistance conferred via codon optimization
Anillin
N
NTD
Myo
Act
CD
AHD
RBD
C2
PH
C
anillin mRNA
1
4311
dsRNA 1
dsRNA 4
D
lacI dsRNA
anillin dsRNA 1
3%
6%
9%
3%
6%
9%
245
180
Anillin
1
0.02
75
α-tubulin
lacI dsRNA
anillin dsRNA 4
3%
6%
9%
3%
6%
9%
245
180
Anillin
1
1
1
0.12
0.10
0.15
75
α-tubulin
E
Anillin
N
NTD
Myo
Act
CD
AHD
RBD
C2
PH
C
Anillin ds1 RNAi (3 days)
00:00:00
00:03:00
00:12:00
00:21:00
00:54:00
02:36:00
Anillin-mCh
Sep1-GFP
inverted
F
Anillin-RBD*
A874D-E892K
N
NTD
Myo
Act
CD
C2
PH
C
Anillin ds1 RNAi (3 days)
00:00:00
00:03:00
00:12:00
00:18:00
00:30:00
01:12:00
Anillin-RBD*-mCh
Sep1-GFP
inverted

Figure S2

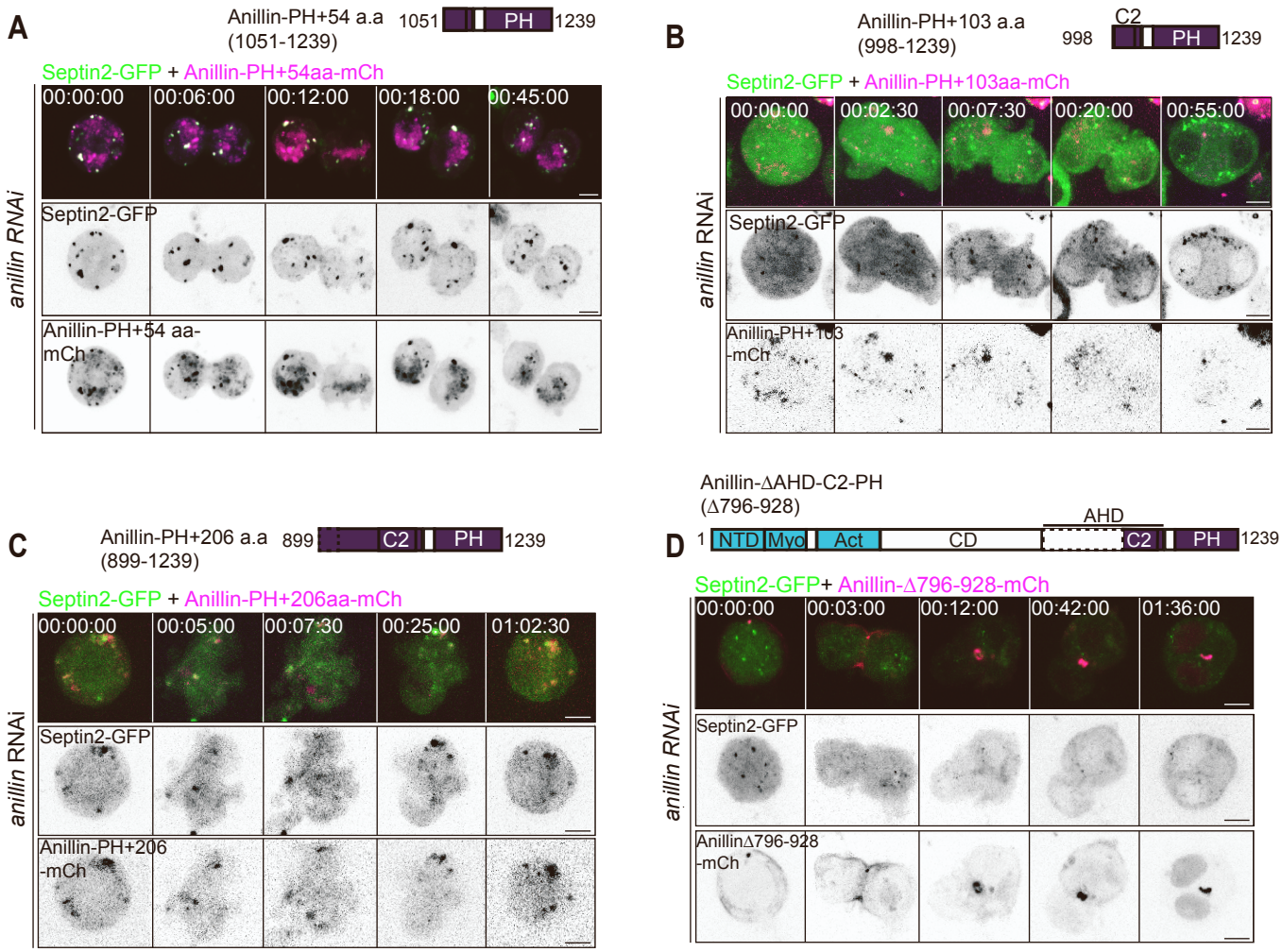
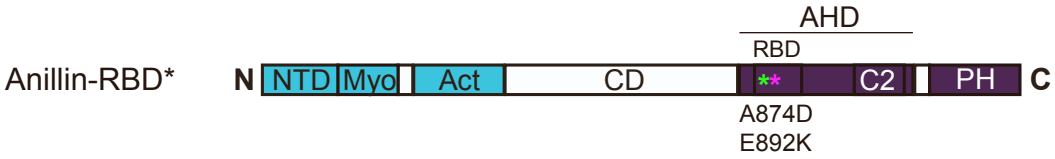
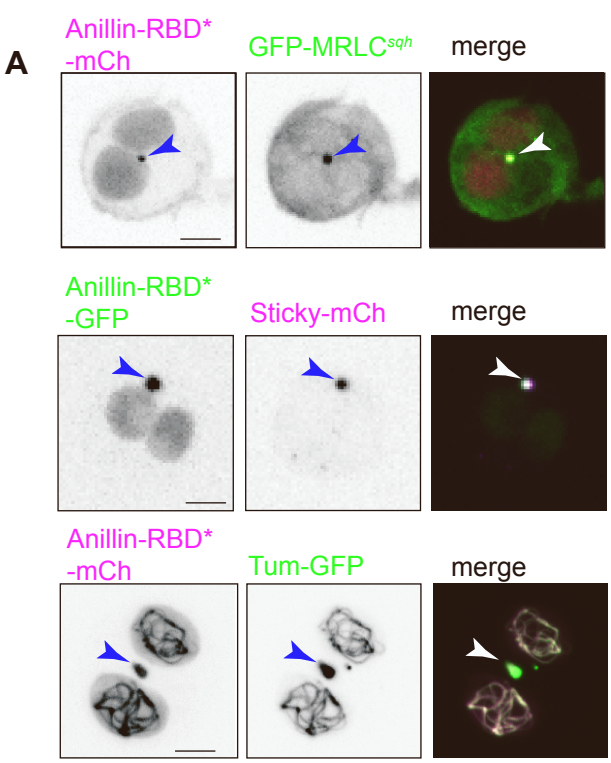

A
Anillin-PH+54 a.a
(1051-1239)
1051
PH
1239
Septin2-GFP + Anillin-PH+54aa-mCh
00:00:00
00:06:00
00:12:00
00:18:00
00:45:00
anillin RNAi
Septin2-GFP
Anillin-PH+54 aa-mCh
B
Anillin-PH+103 a.a
(998-1239)
998
C2
PH
1239
Septin2-GFP + Anillin-PH+103aa-mCh
00:00:00
00:02:30
00:07:30
00:20:00
00:55:00
anillin RNAi
Septin2-GFP
Anillin-PH+103 -mCh
C
Anillin-PH+206 a.a
(899-1239)
899
C2
PH
1239
Septin2-GFP + Anillin-PH+206aa-mCh
00:00:00
00:05:00
00:07:30
00:25:00
01:02:30
anillin RNAi
Septin2-GFP
Anillin-PH+206 -mCh
D
Anillin-ΔAHD-C2-PH
(Δ796-928)
1
NTD
Myo
Act
CD
AHD
C2
PH
1239
Septin2-GFP+ Anillin-Δ796-928-mCh
00:00:00
00:03:00
00:12:00
00:42:00
01:36:00
anillin RNAi
Septin2-GFP
AnillinΔ796-928 -mCh

Figure S3


AHD
RBD
Anillin-RBD*
N
NTD
Myo
Act
CD
**
C2
PH
C
A874D
E892K


A
Anillin-RBD*
-mCh
GFP-MRLC^sqh
merge
Anillin-RBD*
-GFP
Sticky-mCh
merge
Anillin-RBD*
-mCh
Tum-GFP
merge

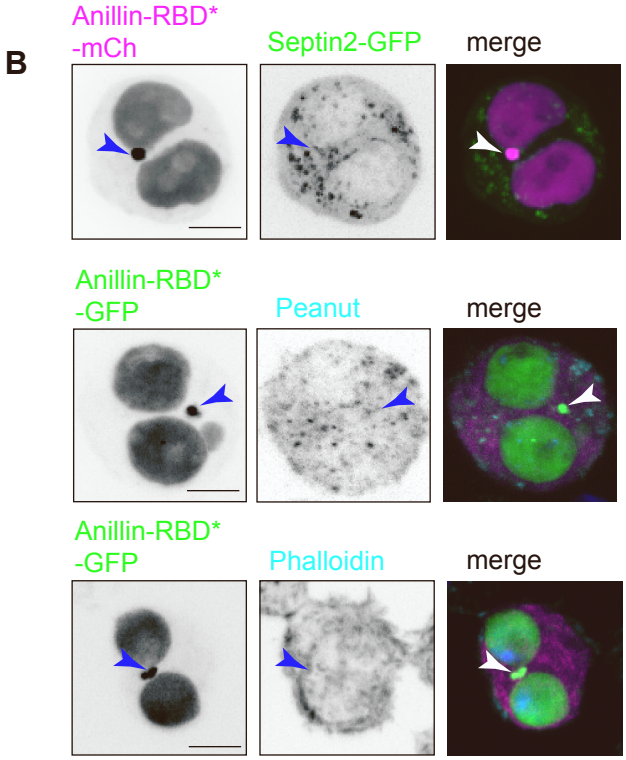
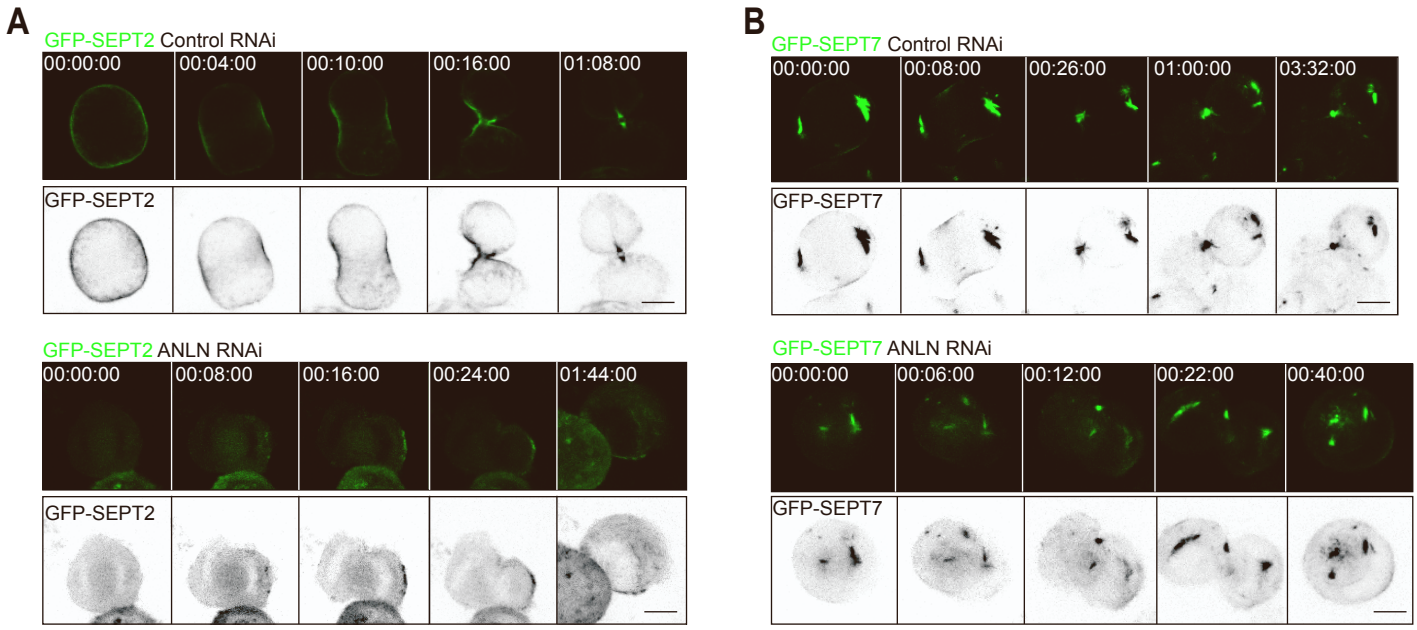

B
Anillin-RBD*
-mCh
Septin2-GFP
merge
Anillin-RBD*
-GFP
Peanut
merge
Anillin-RBD*
-GFP
Phalloidin
merge

Figure S4


A
GFP-SEPT2 Control RNAi
00:00:00
00:04:00
00:10:00
00:16:00
01:08:00
GFP-SEPT2
GFP-SEPT2 ANLN RNAi
00:00:00
00:08:00
00:16:00
00:24:00
01:44:00
GFP-SEPT2
B
GFP-SEPT7 Control RNAi
00:00:00
00:08:00
00:26:00
01:00:00
03:32:00
GFP-SEPT7
GFP-SEPT7 ANLN RNAi
00:00:00
00:06:00
00:12:00
00:22:00
00:40:00
GFP-SEPT7

SUPPLEMENTAL FIGURE LEGENDS

Figure S1 (Related to Figure 1). Recruitment of Septin1 and Peanut to the CR is dependent on Anillin.

(A) Representative time-lapse sequences of dividing cells transiently expressing mCherry-tubulin (magenta) and inducibly expressing (in green in the merged top panels, or inverted grayscale in the bottom panels) wild-type Septin1 after a 3-day incubation with either LacI (negative control) (top) or Anillin dsRNA (bottom). (B) Cells after 3 days of LacI (left) or Anillin RNAi (right) fixed and stained with antibodies against Anillin (green in merged panel and inverted grayscale, left) and Peanut (magenta in merged panel and inverted grayscale, second to left) and the DNA dye Hoechst 33242 (blue in merge panel). (C) Cartoon of the *Drosophila* orthologue of full-length Anillin depicting its domain organization and underneath, anillin mRNA showing the regions targeted by the different dsRNAs used to deplete anillin. Anillin constructs were engineered by codon optimization to be resistant to dsRNA1, which was used in rescue experiments. (D) Western blot showing efficacy on untransfected S2 cells of *anillin* RNAi for 3 days, with 2 different dsRNAs, and with *lacI* as a negative control. Three different dilutions of each whole cell extract were run on SDS-PAGE gels, proteins were transferred to nitrocellulose and the upper portions blotted for Anillin, while the lower portions were blotted for α–tubulin. (E-F) Representative time-lapse sequences of dividing cells transiently expressing Septin1-GFP (in green in the merged top panels, or inverted grayscale in the bottom panels) and Anillin-mCherry (E, magenta) or Anillin-RBD*-mCherry (F, magenta) after a 3-day incubation with Anillin dsRNA1. Time in h:min:s, scale bars 5 μm.

Figure S2 (related to Figure 2) The PH domain cannot recruit septins to the plasma membrane without the RBD.

(A-D) Representative time-lapse sequences of dividing cells after 3 days of Anillin RNAi, inducibly expressing Septin2-GFP (green in merged top panels, or inverted grayscale in middle panels) and either Anillin-PH+52aa-mCherry (A), Anillin-PH+103aa-mCherry (B), Anillin-PH+206aa (C) or Anillin-Δ796-928-mCherry (D) (magenta in merged top panels, or inverted grayscale in bottom panels). None of these constructs were able to recruit Sep2 to the cleavage furrow. Time is h:min:s, scale bars 5 μm.

Figure S3 (Related to Figure 4) Characterization of internal midbody ring-like structures formed by Anillin-RBD*

(A) Identification of components present in the internal midbody ring-like structures formed by Anillin-RBD*. Stills of live cells after 3 days of Anillin RNAi and inducibly expressing Anillin-A874D-E892K-mCherry (or -GFP) and constitutively expressing GFP-MRLC^{sqh} (upper) or inducibly expressing Citron Kinase^{Sticky}-mCherry (middle) or RacGAP50C/Tumbleweed^{Tum}-GFP (bottom). (B) Identification of components absent in the internal midbody ring-like structures formed by Anillin-RBD*. Upper panel: Live still of cells after 3 days of Anillin RNAi and inducibly expressing Anillin-A874D-E892K-mCherry and Septin2-GFP (upper). Middle and lower panels: cells inducibly expressing Anillin-A874D-E892K-GFP after 3 days of Anillin RNAi fixed and stained with either an antibody against Peanut (middle, cyan in merged) or Phalloidin conjugated to Alexa Fluor 647 (bottom, cyan in merged). Arrowheads point to Anillin-RBD*-positive, internal midbody ring like structures that remain following furrow regression and cytokinesis failure. Scale bars, 5 μm.

Figure S4 (Related to Figure 5) The furrow localization of SEPT2 and SEPT7 in HeLa cells depends on ANLN.

Representative time-lapse sequences of HeLa cells transiently expressing GFP-SEPT2 (A) or GFP-SEPT7 (B) following either control RNAi (luciferase, upper panels) or ANLN RNAi (lower panels). Time is h:min:s, scale bars 5 μm.

SUPPLEMENTAL TABLES

Table S1: Primers used in this study, related to STAR Methods.

lacI dsRNA F:	5′-GGGCGGGTTGGTGGTGTCGATGGTAGAA-3′
lacI dsRNA R:	5′-GGGCGGGTCGGTATCGTCGTATCCCACT-3′
anillin dsRNA1 F:	5′-GGGCGGGTTAGAAATCTATGGCATGTTGGC-3′
anillin dsRNA1 R:	5′-GGGCGGGTGAGAAAACTGTTAACAACCCGC-3′
anillin dsRNA4 F:	5'-GCGAACTATCCGTAAGCGTG-3'
anillin dsRNA4 R:	5′-GGGCGGGTGAGAAAACTGTTAACAACCCGC-3′
anillin dsRNA 3′UTR F:	5′-GGGCGGGTGGAACCACCCACTGACCCGCT-3′
anillin dsRNA 3′UTR R:	5′-GGGCGGGTGCGAGTCATCCTAAATTAAATG-3′
septin2 dsRNA (G-domain) F:	5'-GGGCGGGTACAAGGACGACTCGTTCAAGG-3'
septin2 dsRNA (G-domain) R:	5'-GGGCGGGTTTGCTCCAGCCTCTTCTGGCG-3'
Anillin 796 F:	5'-CACCATGCGATCAGCCCTAGCAGGC-3'
Anillin_ds res 3' ORF R:	5'- ATGCGTCGTACCCCACGC-3'
Anillin 1105 F:	5'-GTTAGTGTCGAATATAAAGGT-3'
Anillin 1051 F:	5'-CCCGCAGGCCCGCACGTCGTT-3'
Anillin 1002 F:	5'-ACCCTGGAGATATACGGGATG-3'
Anillin 899 F:	5'-CTTGCAACCCATCGACGCCAG-3'
Anillin ORF F:	5'-CACCATGGACCCGTTTACTCAGCACA-3'
Anillin 1104 ds resistant R:	5'-CGAAAGCTCACAATTCACCTT-3'