Supplementary Information

Src-transformed cells hijack mitosis to extrude from the epithelium

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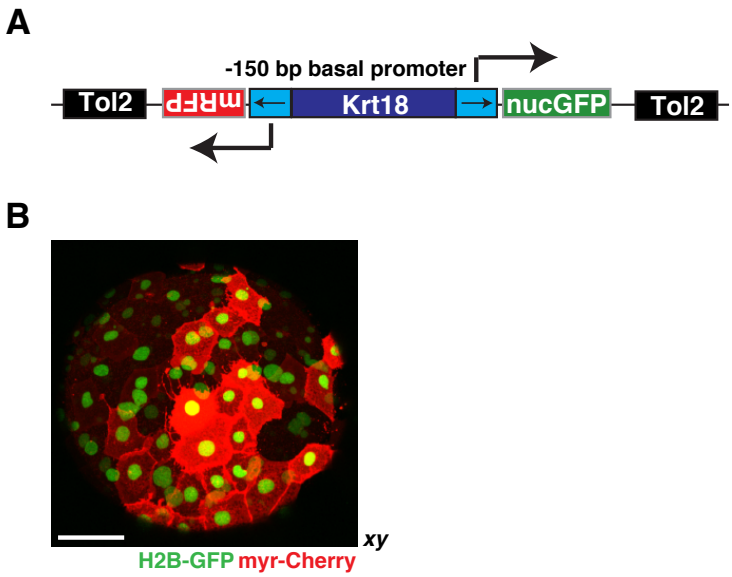
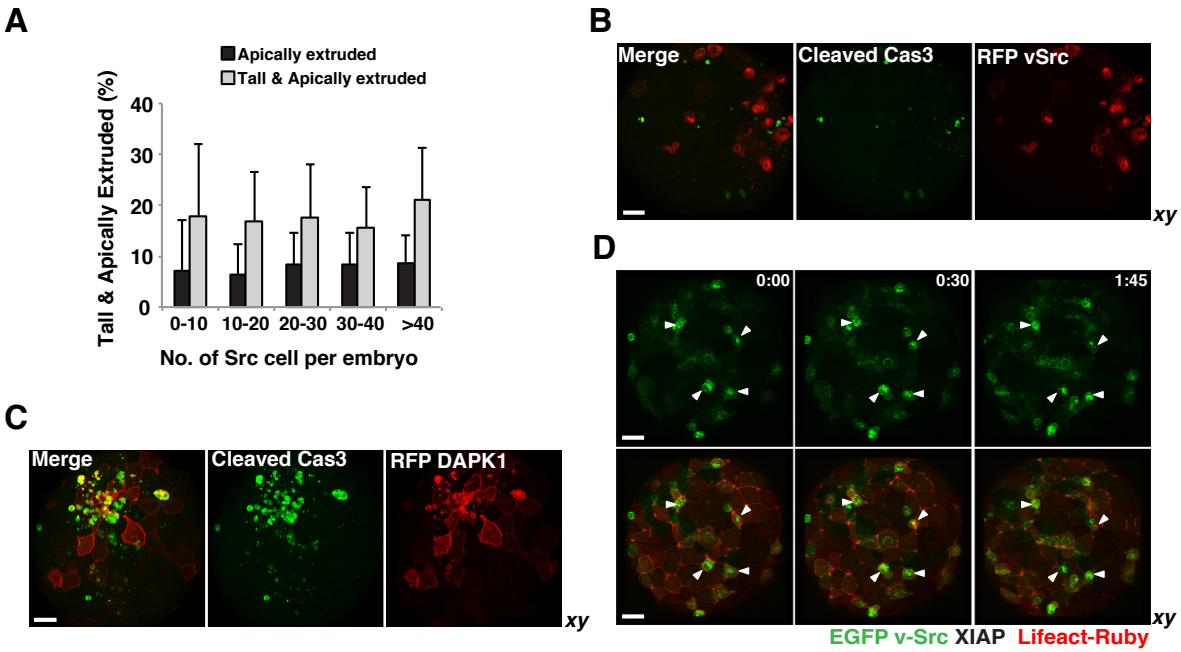
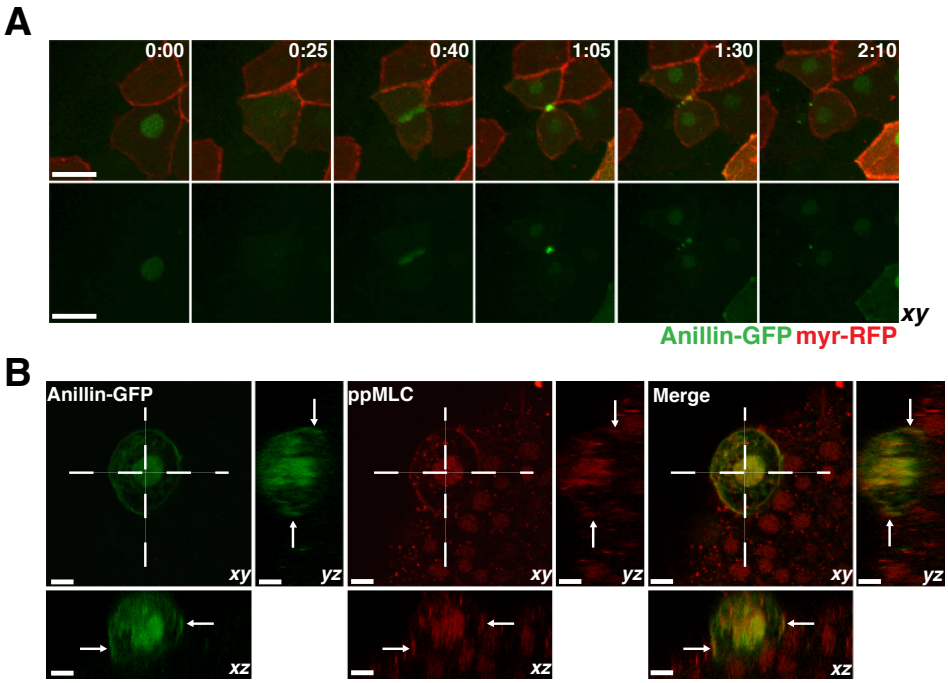
Supplementary Figures



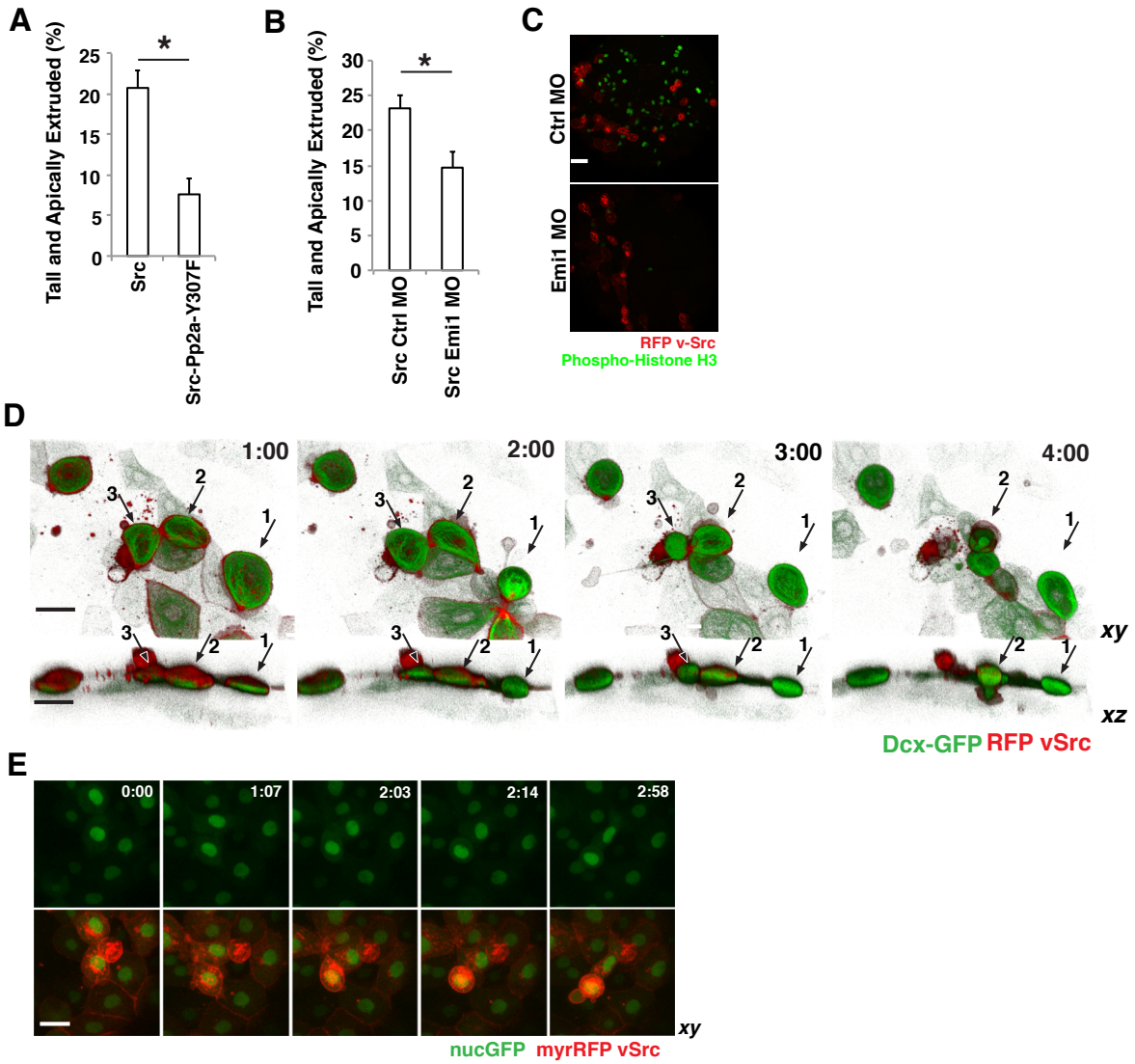
Supplementary Figure 1. Establishment of bidirectional Krt18 promoter. (A) The basal promoter region of the Krt18 promoter, -150 bp, is added to -5kb upstream of the initiation Methionine in the reverse-complement orientation. This bi-directional Krt18 promoter (dKrt18) can drive H2B-GFP and myr-Cherry only in the same EVL cells in a mosaic manner. **(B)** An example of expression of H2B-GFP and myr-Cherry at the tailbud stage. Wild-type embryos were injected with the dKrt18:H2B-GFP;myr-Cherry construct. Scale bar, 100 μm.



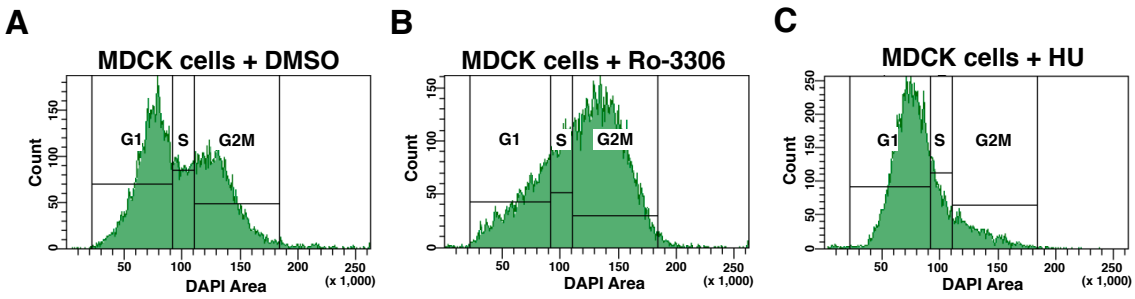
Supplementary Figure 2. vSrc-driven extrusion is cell-death independent. (A) Quantification of vSrc-driven cell extrusion and rounding rates based on cell density in fixed embryos. Embryos were injected with the dUAS:EGFP-vSrc. Data represent 34 independent experiments (number of embryos: n_{Src} = 344). Error bars represent s.d. **(B)** Immunofluorescence images of Cleaved-Cas3 (green) in EVL cells expressing myr-Cherry-vSrc (red). Scale bar, 50 μm. **(C)** Immunofluorescence images of Cleaved-Cas3 (green) in EVL cells expressing myr-Cherry and DAPK1 (death-associated kinase 1; red). Scale bar, 50 μm. **(D)** The effect of inhibiting apoptosis in vSrc-mediated extrusion visualised in time-lapse imaging. Embryos obtained from the Krt18:Lifeact-Ruby) line expressing crossed with the Krt18:KalTA4-ERT2 line were injected with the dUAS:myr-Cherry-vSrc;XIAP construct (red). Movies were taken over 4 hours. Frames were extracted from a representative movie at indicated times from the tailbud stage. White arrowheads indicate cells that become extruded. Scale bars, 50 μm.



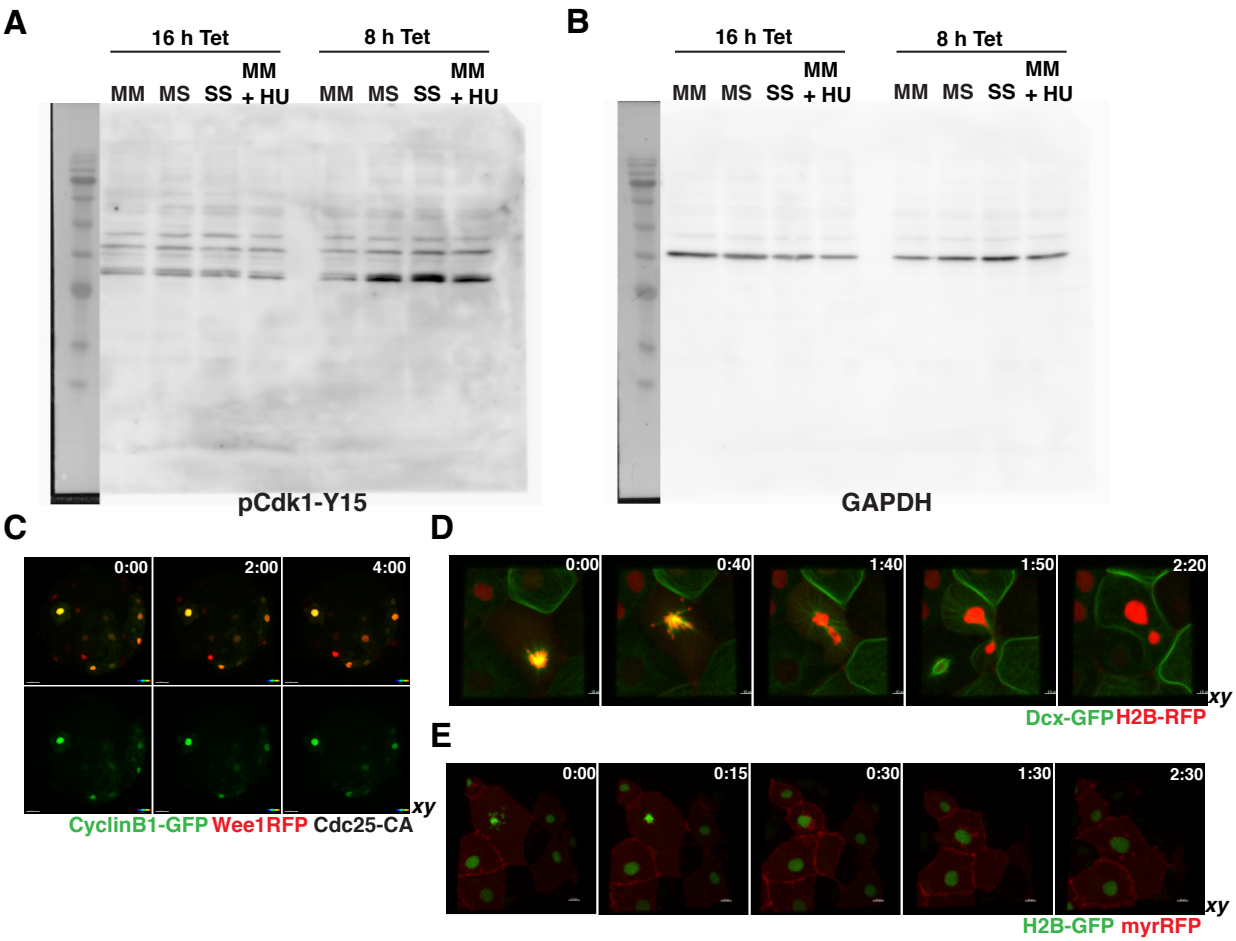
Supplementary Figure 3. Analyses of the cytokinetic ring components in vSrc cell extrusion. (A) Time-lapse imaging of Anillin-GFP in mitosis. Embryos were injected with the dUAS:myr-Cherry;Anillin-GFP construct. Movies were taken over 4 hours. Frames were extracted from a representative movie at indicated times from the tailbud stage. Scale bars, 25 μm. **(B)** Immunofluorescence images of phosphorylated myosin light chain (pMLC Thr18, Ser19; red) in the EVL cells expressing the Anillin-GFP (green) alongside vSrc. Scale bar, 10 μm. Arrows indicate lateral enrichment of Anillin-GFP.



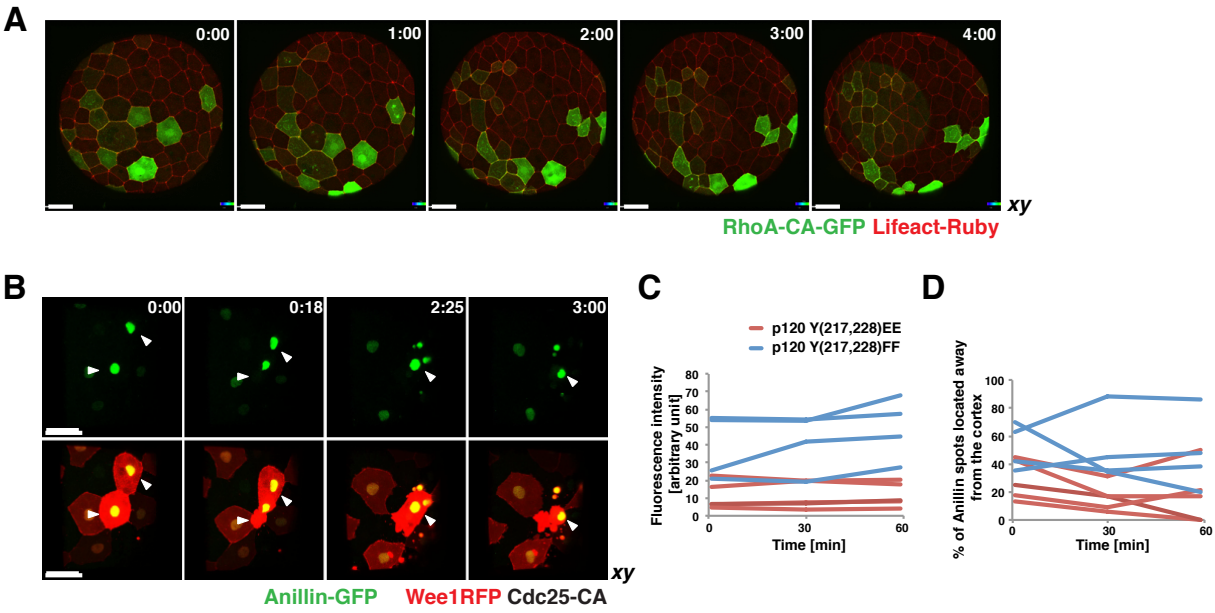
Supplementary Figure 4. Analyses of mitotic markers in vSrc-driven extrusion. **(A)** The effect of CA-Pp2a on vSrc-driven extrusion. Embryos were injected with the following constructs: dUAS:EGFP-vSrc and dUAS:EGFP-vSrc;Pp2a-Y307F (CA-Pp2a). Data are mean ± s.d. of 3 independent experiments (total number of embryos: $n_{Src} = 35$; $n_{Src,CA\text{-}pp2a} = 40$). $^*P < 0.05$ (Student's t-test). **(B)** The effect of *Emi1* morpholino on vSrc-driven extrusion. Embryos were injected with the UAS:EGFP-vSrc construct and the control or *Emi1* morpholinos (G2/M arrest[1]). Data are mean ± s.d. of 3 independent experiments (total number of embryos: $n_{Ctrl} = 15$; $n_{Emi1MO} = 15$). $^*P < 0.05$ (Student's t-test). **(C)** The effect of the *Emi1* MO on cell proliferation in the embryo. Embryos from (B) were fixed and stained for pH3 (green). A representative image was selected. Scale bar, 50 μm. **(D)** Time-lapse imaging of a microtubule marker Dcx-GFP in extrusion. Embryos were injected with the dUAS:myr-Cherry-vSrc;Dcx-GFP construct. Movies were taken over 4 hours. Frames were extracted from a representative movie at indicated from the tailbud stage ($t = 0$). Image is presented in the blend projection view of Imaris. Black arrows indicate cells that become extruded. Scale bars, 25 μm (*xy*) 25 μm (*xz*). **(E)** Time-lapse imaging of nucGFP in extrusion (3xNLS-Azami-GFP fusion). Embryos were injected with the dUAS:myr-Cherry-vSrc;nucGFP construct. Movies were taken over 4 hours. Frames were extracted from a representative movie at indicated from the tailbud stage ($t = 0$). Scale bars, 25 μm.



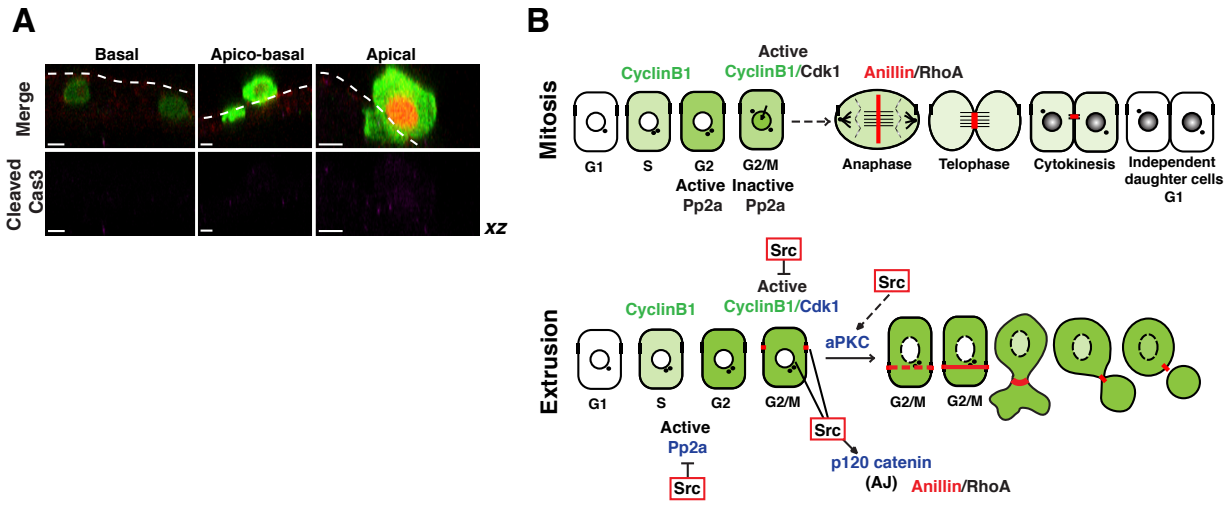
Supplementary Figure 5. FACS analyses of the effect of proliferation inhibitors on DNA content in MDCK cells. Prior to FACS analyses, MDCK populations were treated with DMSO (A), 10μM Ro-3306 (B), 2 mM hydroxyurea (C) and stained with DAPI.



Supplementary Figure 6. Analyses of cell cycle progression in MDCK cells and the EVL. (A, B) The effect on phosphorylation of CDK1 after 16 and 8 hours from Src activation in MDCK cells. MM – MDCK cells alone, SS – Src cells alone, MS – cultures mixed 1:1, MM+HU – MDCK cells treated with 2mM hydroxyurea (HU). Original blots corresponding to Fig. 6C with pCDK staining (A) and GAPDH loading control (B). **(C)** Time-lapse imaging of CyclinB1-GFP localisation in cells arrested at the G2/M transition. Embryos were injected with a combination of the following constructs: dUAS:Cherry-Wee1;CA-Cdc25 and Krt18:CcnB1-GFP. Movies were taken over 4 hours. Frames were extracted from a representative movie at indicated times from the tailbud stage. Scale bars, 50 μm. **(D,E)** The effect of inducing monopolar spindles in the EVL in time-lapse imaging. Embryos were injected with the following constructs: dUAS:Dcx-GFP;H2B-RFP (D) and dUAS:myr-Cherry;H2B-GFP (E). At 50-70% epiboly embryos were treated for 3 hours with the Kif11 inhibitor, 0.875 mM STLC. Movies were taken over 4 hours in the presence of the inhibitor. Frames were extracted from a representative movie at indicated times from the tailbud stage. Scale bars, 10 μm.



Supplementary Figure 7. Determining conditions for misoriented cytokinetic ring assembly. (A) The effect of RhoA activation in the EVL. Transgenic embryos obtained from a line expressing an RFP-actin marker (red) specifically in the EVL (Krt18:Lifeact-Ruby) line crossed with the Krt18:KalTA4-ERT2 line were injected with the dUAS:GFP-CAAX;CA-RhoA construct. Movies were taken over 4 hours. Frames were extracted from a representative movie at indicated times from the tailbud stage. Scale bars, 50 μm. **(B)** Time-lapse imaging of Anillin-GFP localization in cells arrested at the G2/M transition. Embryos were injected with a combination of the following constructs: dUAS:Cherry-Wee1;CA-Cdc25 and dUAS:myr-Cherry;Anillin-GFP. Movies were taken over 4 hours. Frames were extracted from a representative movie at indicated times from the tailbud stage. White arrowheads indicate cells that become extruded due to protein overexpression. Scale bars, 50 μm. **(C,D)** Quantification of the average fluorescence intensity (C) and position in regards to the cortex (D) of Anillin GFP spots over time in cells expressing either p120-mutEE or p120-mutFF. Embryos were injected with the following constructs: dUAS:Cherry-Wee1;CA-Cdc25 and either dUAS:p120-mutEE;AnillinGFP or dUAS:p120-mutFF;AnillinGFP. Data from 4 cells p120-mutFF (in blue) and 5 cells for p120-mutFF (in red) were acquired in 6 independent experiments and plotted.



Supplementary Figure 8. Key conditions for vSrc-mediated extrusion. (A) Immunofluorescence images of Cleaved-Cas3 (magenta) in EVL cells expressing p120EE-GFP (green), aPKC-delN, wee1-RFP (nuclear red), cdc25, XIAP and stained with phalloidin (cortical red). The dashed white line indicates the surface of the embryo. Scale bars, 10 μm. Note that there is no cleaved Cas3 staining in the extruding vSrc cells. **(B)** vSrc interferes with the cell cycle, adherens junctions and nuclear composition as well as polarity, leading to apico-basal extrusion. The top panel illustrates a cell undergoing mitosis; the bottom panel, a cell where vSrc hijacked the cell cycle regulators. In the vSrc cell, Pp2A is inactivated earlier in the cell cycle, but counteracting Cdk1 inhibition results in G2/M arrest instead of mitosis. In the vSrc cell, Cyclin B1 (green) is accumulated to a greater extent, the nuclear envelope becomes partially permeable and Anillin (in red) is recruited to adherens junctions by modified p120-catenin. A contractile junctional ring assembles parallel to the plane of epithelium, constricts and releases the cell from the epithelium. vSrc-mediated modulation of the apico-basal polarity complex (e.g. aPKC) promotes the predominantly apical direction of extrusion. Immediate cell death is avoided due to vSrc promoting cell survival.

Supplementary methods

List of primers used

To clone the dKrt18 promoter:
Krt18-0-S1: 5'-CTCGAGGCGCGCCAGTAACACAGCAGAGCAGAGC-3'
Krt18-150-A1: 5'-GAGCTCCAGTAACACAGCAGAGCAGAGC-3'

To clone from zebrafish gastrula cDNA:
- Pp2a (ZDB-GENE-050417-441)
PP2A-F: 5'-GTTCTTTTTGCAGGAATGGACGAAAAGGTTTTTACGAAAGAACTG-3
PP2A-R: 5'-TCTAGAGGCTCGAGATTACAGGAAATAATCTGGAGTGCGAC-3'

- RhoA (ZDB-GENE-040426-2150)
RhoA-F: 5'-GTTCTTTTTGCAGGAATGGCTGCGATTCGTAAGAAG-3'
RhoA-R: 5'-TCTAGAGGCTCGAGACTATAGCAAAGCGCAGGCATT-3'

- CcnB1 (ZDB-GENE-000406-10)
Ccnb-EcoRI-F: 5'-CCATCGATTCGAATTATGATGGCTCTCCGTGTCACA-3'
Ccnb-EcoRI-R: 5'-AGTAGGCCTTGAATTTGAGATCTGCTTAGCCAGGTC-3'

- Cdk1 (ZDB-GENE-010320-1)
CDK1-InF-F: 5'-GTTCTTTTTGCAGGAATGGATGACTATCTGAAGATAG-3'
CDK1-InF-R: 5'-TCTAGAGGCTCGAGATTATATCTTGAGGTTGCTGGC-3'

- XIAP (ZDB-GENE-030825-7)
Xiap-S1: 5'-ATGGCACACTCAACTCATAAT-3'
Xiap-A1: 5'-TTAGGCATTGTAAGTCTTTAT-3'

To create indicated point mutations and deletions:
- Y307F in CA-Pp2a
PP2A-Y307F-F: 5'-CGCACTCCAGATTTTTTCCTGTAATCT-3'
PP2A-Y307F-R: 5'-AGATTACAGGAAAAAATCTGGAGTGCG-3'

- Q63L in CA-RhoA
RhoA-CA-F: 5'-ATACAGCTGGCCTAGAGGACTACGAC-3'
RhoA-CA-R: 5'-GTCGTAGTCCTCTAGGCCAGCTGTAT-3'

- T19N in DN-RhoA
RhoA-DN-2-F: 5'-AACTGTTTACTCATCGTTTTCAGTAAAGACC-3'
RhoA-DN-2-R: 5'-GATGAGTAAACAGTTCTTTCCACAGGCTCCATCT-3'

- T14A and Y15F in CA-Cdk1
T14AY15F-InF-F: 5'-GGTGCGTTTGGTGTAGTATATAAGGGCAGGAATAAA-3'
T14AY15F-InF-R: 5'-TACACCAAACGCACCTTCACCAATTTTCTCTATCTT-3'

- Δ(1-740) in DN-Anillin
Anillin-DN-F: 5'-GTTCTTTTTGCAGGAAGCTCCAGTCACATCAACATC-3'

p120-del346-R: 5’-TCCTGCAAAAAGAACAAGTAGCTTGTATTC-3’

- Δ(201-591) in DN-aPKC
aPKC-RD-F: 5’-GAACGATGGTCTCGAGAAGCTGAACCCTCCT-3’
aPKC-RD-R: 5’-TCGAGACCATCGTTCTTGTCATCTACTGGAG-3’
TCTAGAGGCTCGAGA

- Y417F in aPKC-mutF
aPKC-Y417F-F: 5’-CCGAACTTTATCGCCCCCGAAATCC-3’
aPKC-Y417F-R: 5’-GGCGATAAAGTTCGGGGTTCCACA-3’

- Δ(1-201) in aPKC-delN
aPKC-delN-F: 5’-
GTTCTTTTTGCAGGAATGGTAGACCTTCCTTCAGAAGAAACTGATG-3’
p120-del346-R: 5’-TCCTGCAAAAAGAACAAGTAGCTTGTATTC-3’

- Y217,228F in p120-mutFF
p120_Y217_Y228F_F: 5’-GGTTATCCAGGTGGCAGTGACAACTtCGGCAGT-3’
p120_Y217_Y228F_R: 5’-GCCACCTGGATAACCATCTTCAaAGTGTCGGC-3’

- Y217,228E in p120-mutEE
p120_Y217,228E_F: 5’-GGTTATCCAGGTGGCAGTGACAACGAAGGCAGTCTGTCC-3’
p120_Y217,228E_R: 5’-GCCACCTGGATAACCATCTTCTTCGTGTCGGCCATACCC-3’

- Δ(375-1127) in DN-ParD3
Par3DN-R: 5’-TCCTGAGCTCCCTCCATTATGGGATATCTGCTCATACTGGGAC-3’
EGFP-Par3DN-F: 5’-GGAGGGAGCTCAGGAATGGTGAGCAAGGGCGAGG-3’

To create nucGFP:
nucGFP was created by fusing 2xNLS Sv40 with NLS from Wee1 (RNNRKRSHWN), hmAzami-Green and EGFP.
NLS-AG-F: 5’-
CCAAAGAAGAAGCGTAAGGTAAATCGATCGATGGTGAGCGTGATCAAGCCC-3’
NLS-AG-R: 5’-TCCTGATCCACCACTTCCCTTGGCCTGGCTGGGCAG-3’
NLS-fix-F: 5’-AGAAGCGTAAGGTAAATCGATCGGGGGTGAGCGTGA-3’
NLS-fix-R: 5’-TTACCTTACGCttCTTCTTTGGCATGAATCGATGGG-3’
Alt-NLS-F: 5’-
CCGAAGAAAAAAAGGAAAGTGGACCCAAAGAAGAAGCGTAAGGTAAATC-3’
Alt-NLS-R: 5’-CCTTTTTTTCTTCGGACTTCCcatGAATCGATGGGATCCTGCAAA-3’
NLS-wee1-F: 5’-CAGGAAACGATCACACTGGAATAGTGGTGGAGGGAGCTCAGGA-3’
NLS-wee1-R: 5’- TGTGATCGTTTCCTGTTGTTTCTTCCGCCACCTCCTGATCCA-3’

To create CDK1pep:
mKO2-FLAG-F: 5’-TACAAGGATGACGACGATAAGGTGAGTGTGATTAAACCAGAG-3’
CS2-FLAG-R: 5’-GTCGTCATCCTTGTAATCCATTCCTGCAAAAAGAACAAGTAG-3’
CB1-CDK1pep-F: 5’-
GGAGAAGGTACCTATGGAGTTGTGTATAAGAGTGGTGGAGGGAGCTCA-3’

mKO2-CKD1pep-R: 5'-
ATAGGTACCTTCTCCAATTTTCTCTATTTTTCCGCCACCTCCTGATCC-3'
CDK1pep-HA-F: 5'-TGACTATGCAGGCCTGTAATCTCGAGCCTCTAGAAC-3'
CDK1pep-HA-R: 5'-AACATCGTATGGATAGATCCCTCCACCACTCTT-3'
2xHA-PCR-F: 5'-GATCTATCCATACGATGTTCCAGAT-3'
2xHA-PCR-R: 5'-ATTACAGGCCTGCATAGTCAGGG-3'

To create a variety of bidirectional pBR-Tol2-dUAS constructs we used two unique restriction sites (PmeI and EcoRV) on either side of the dUAS element. We cloned multiple CDS sequences into either of them from the pCS2 vector using primers:
dUAS-PmeI-IF-S1: 5'-TAATGAGCTCGGTTTGGAGCAAGCTTGATTTAGGTG-3'
dUAS-PmeI-IF-A1: 5'-GGATCGAATTCGTTTCCGCGAATTAAAAAACCTCCC-3'
dUAS-EcoRV-IF-S2: 5'-GATCCTGGTACCGATTTGATTTAGGTGACACTATAG-3'
dUAS-EcoRV-IF-A2: 5'-CCGCAAGTCGACGATCCGCGAATTAAAAAACCTCCC-3'

To create pBR-Tol2-UAS constructs we used a unique restriction site (EcoRV). We cloned CDS sequences from the pCS2 vector using primers dUAS-EcoRV-IF-S2 and dUAS-EcoRV-IF-A2.

To create bidirectional pBR-Tol2-dKrt18 constructs we used two unique restriction sites (PmeI and AscI) on either side of the dUAS element. We cloned multiple CDS sequences into either of them from the pCS2 vector using primers:
Krt18-PmeI-IF-S1: 5'-AGCTCGGATCCGTTTGGAGCAAGCTTGATTTAGGTG-3'
Krt18-PmeI-IF-A1: 5'-GCCGCGTCGACGTTTCCGCGAATTAAAAAACCTCCC-3'
Krt18-AscI-IF-S2: 5'-TGTGTTACTGGCGCGGGAGCAAGCTTGATTTAGGTG-3'
Krt18-AscI-IF-A2: 5'-CGCGCCTCGAGGGCCCCGCGAATTAAAAAACCTCCC-3'

To create pBR-Tol2-Krt18 constructs we used a unique restriction site (PmeI). We cloned CDS sequences along with Poly A signal from the pCS2 vector with primers Krt18-PmeI-IF-S1 and Krt18-AscI-IF-S2 using the In-Fusion cloning system (Takara Bio).

Supplementary references

1 Buckley, C. E. *et al.* Mirror-symmetric microtubule assembly and cell interactions drive lumen formation in the zebrafish neural rod. *EMBO J* **32**, 30-44, doi:10.1038/emboj.2012.305 (2013).