

1 **Supplementary Methods** – this section provides the sequences of the RNAi
2 constructs, the targets of which affect cell extrusion.

3 4 **Supplementary Video Legends**

5 **Supplementary Video 1 A control embryo extrudes ABplpappap**

6 Time-lapse video of a *ced-3(lf); stls10026[his-72::GFP]; nls632[P_{egl-1}::mCherry::PH]*
7 embryo after RNAi against empty vector control over a 50-minute period ending in
8 ventral enclosure shows ABplpappap (circled at the beginning and end of video) was
9 extruded from this embryo. All nuclei are labeled with GFP (green) and membranes of
10 *egl-1*–expressing cells are labeled with mCherry (magenta). Time-lapse images used to
11 generate this video were obtained using confocal microscopy. Video playback is at 600x
12 real speed.

13 14 **Supplementary Video 2 A *cye-1*(RNAi) embryo does not extrude ABplpappap**

15 Time-lapse video of a *ced-3(lf); stls10026[his-72::GFP]; nls632[P_{egl-1}::mCherry::PH];*
16 *cye-1*(RNAi) embryo over a 50-minute period ending in ventral enclosure shows
17 ABplpappap (circled at the beginning and end of video) was not extruded from this
18 embryo. All nuclei are labeled with GFP (green) and membranes of *egl-1*–expressing
19 cells are labeled with mCherry (magenta). Time-lapse images used to generate this
20 video were obtained using confocal microscopy. Video playback is at 600x real speed.

21 22 **Supplementary Video 3 A *cdk-2*(RNAi) embryos does not extrude ABplpappap**

23 Time-lapse video of a *ced-3(lf); stls10026[his-72::GFP]; nls632[P_{egl-1}::mCherry::PH];*
24 *cdk-2*(RNAi) embryo over a 50-minute period ending in ventral enclosure shows
25 ABplpappap (circled at the beginning and end of video) was not extruded from this
26 embryo. All nuclei are labeled with GFP and membranes of *egl-1*–expressing cells are
27 labeled with mCherry (magenta). Time-lapse images used to generate this video were
28 obtained using confocal microscopy. Video playback is at 600x real speed.

29 30 **Supplementary Video 4 ABplpappap arrests in S phase and is extruded in a** 31 **control embryo**

32 Time-lapse video of a *ced-3(lf); isls17[P_{pie-1}::GFP::pcn-1]; nls861[P_{egl-1}::mCherry::PH]*
33 embryo after RNAi against empty vector control over a 35-minute period ending in
34 ventral enclosure shows ABplpappap (circled at the beginning) arrests in S phase and is
35 extruded (circled at the end of video). All cells express GFP::PCN-1 (green) and
36 membranes of *egl-1*–expressing cells are labeled with mCherry (magenta). Time-lapse
37 images used to generate this video were obtained using confocal microscopy. Video
38 playback is at 600x real speed.

39 40 **Supplementary Video 5 ABplpappap completes the cell cycle and is not extruded** 41 **in a *pig-1*(RNAi) embryo**

42 Time-lapse video of a *ced-3(lf); isls17[P_{pie-1}::GFP::pcn-1]; nls861[P_{egl-1}::mCherry::PH];*
43 *pig-1*(RNAi) embryo over an 80-minute period ending in ventral enclosure shows
44 ABplpappap (circled at the beginning) completed the cell cycle and divided to generate
45 daughters (circled at the end of video) that were not extruded. All cells express
46 GFP::PCN-1 (green) and membranes of *egl-1*–expressing cells are labeled with

47 mCherry (magenta). Time-lapse images used to generate this video were obtained
48 using confocal microscopy. Video playback is at 600x real speed.

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50 **Supplementary Video 6 A vehicle-treated MDCK monolayer extrudes a few cells**

51 A time-lapse video of mammalian MDCK monolayer treated with vehicle control for
52 21.25 h obtained using phase contrast imaging shows that a few cells are extruded
53 during this period. Extruded cells can be identified as bright, white, rounded spots rising
54 from the epithelial plane. Video playback is at 7200x real speed. Scale bar, 100 μm .

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56 **Supplementary Video 7 A HU-treated MDCK monolayer extrudes a large number
57 of cells**

58 A time-lapse video of mammalian MDCK monolayer exposed to HU for 21.25 h
59 obtained using phase contrast imaging shows that many more cells are extruded during
60 this period as a result of HU treatment. Extruded cells can be identified as bright, white,
61 rounded spots rising from the epithelial plane. Video playback is at 7200x real speed.
62 Scale bar, 100 μm .

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