SUPPLEMENTAL FIGURES

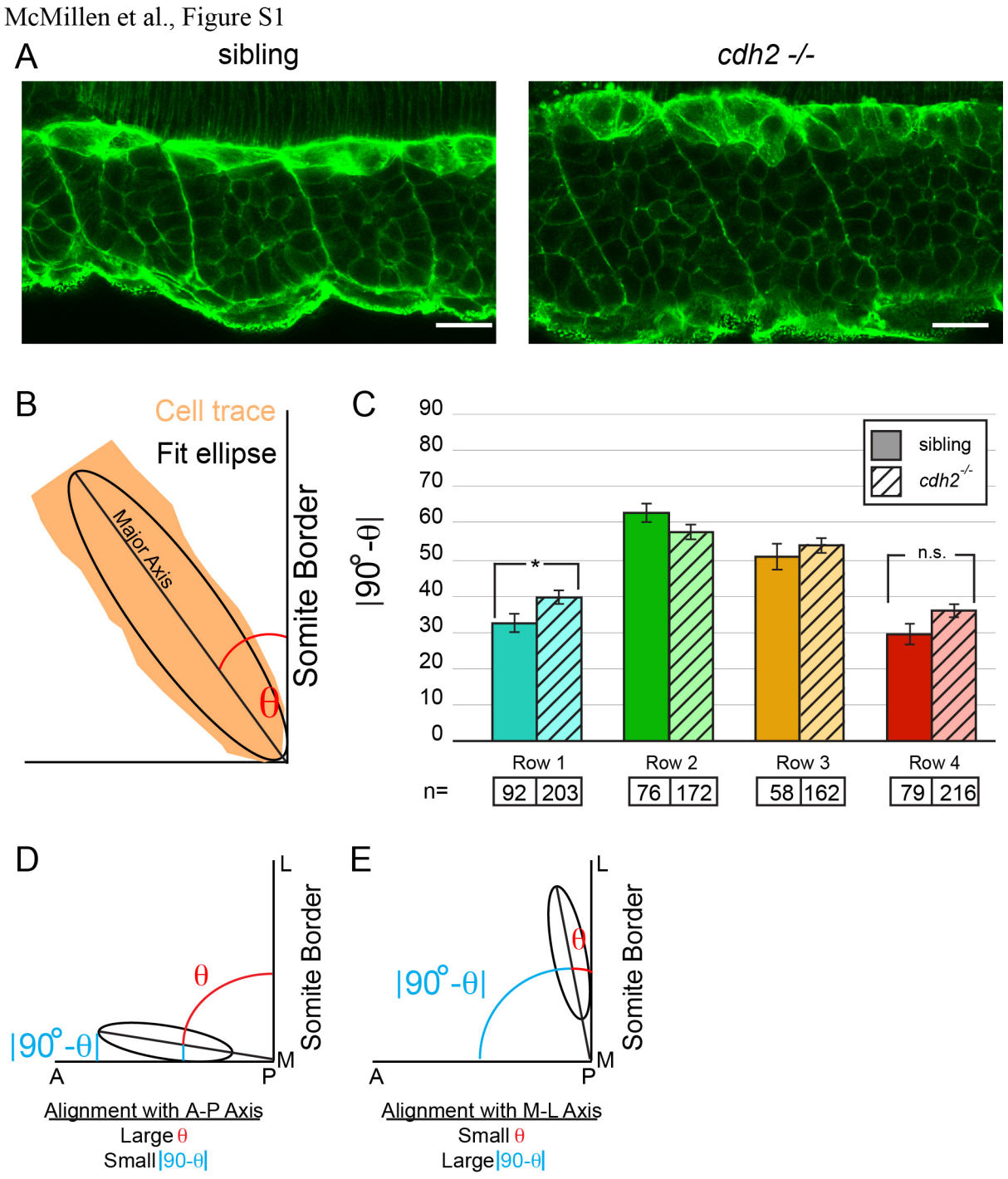

Figure S1. Polarized orientation of posterior somite cells is not affected in *cdh2*$^{-/-}$ embryos; related to Figure 1. **(A)** Phalloidin was used to outline cell cortexes in *cdh2*$^{-/-}$ embryos and wild-type siblings. **(B)** The polarized orientation of cells with respect to the somite boundary was determined by measuring the angle θ between fit ellipses of cell traces and the somite border. The alignment was then calculated using the absolute value of 90-θ, which is small when the cell is aligned along the Anterior-Posterior axis (i.e. perpendicular to the somite border) **(D)** and large when the cell is aligned with the Medial-Lateral axis (i.e. parallel to the somite border) **(E)**. Though the aspect ratio is reduced in row 4 cells in *cdh2*$^{-/-}$ (Figure 1E), the polarized orientation in these cells is not affected by loss of *cdh2* ($p>0.05$). A modest change in polarized orientation is seen in row 1 cells ($p<0.05$). Scale bar = 20 μm.

McMillen et al., Figure S2.

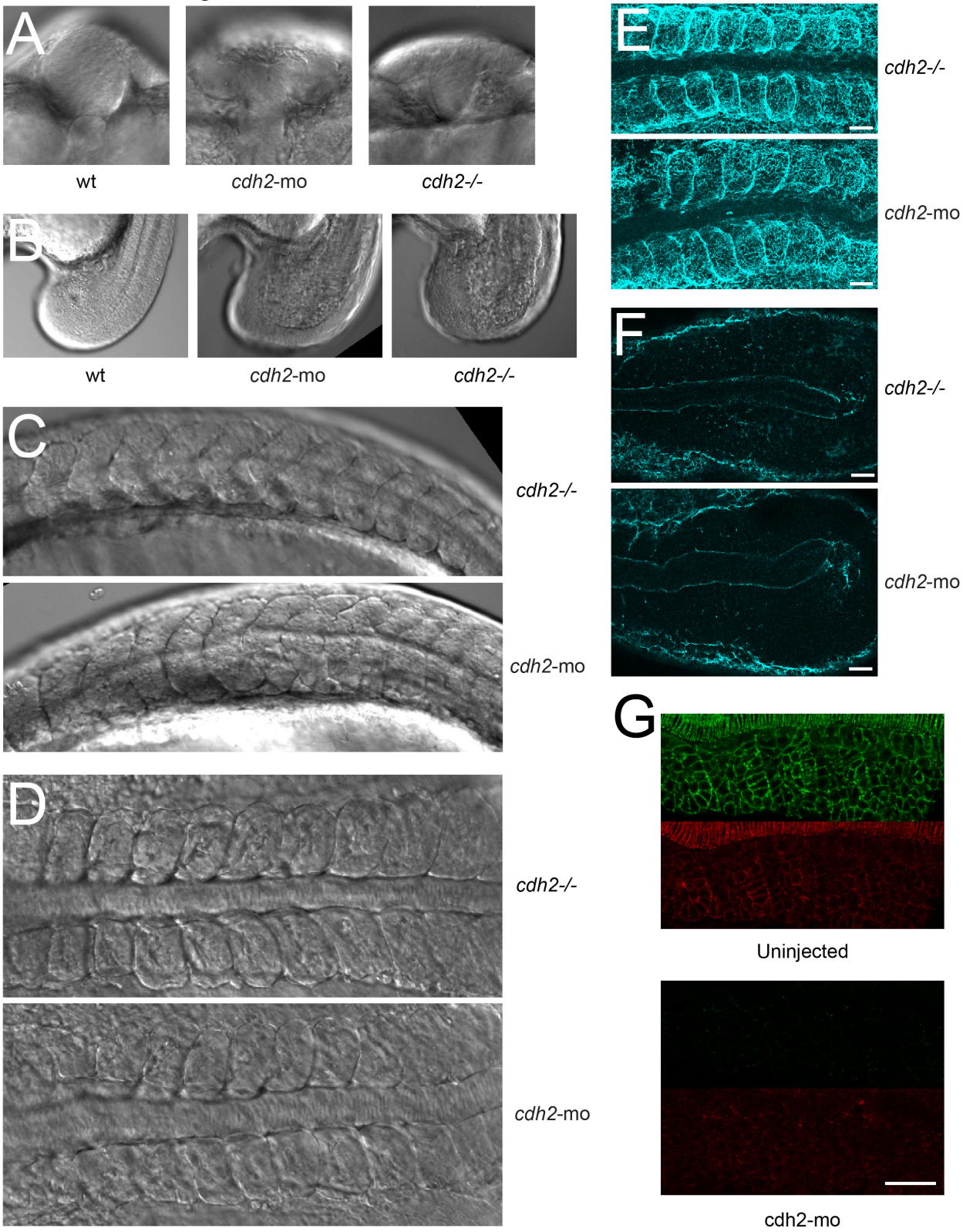

Figure S2. The *cdh2*^{mo} phenotype recapitulates the *cdh2*$^{-/-}$ phenotype; related to Figure 2.
(A) Both mutant and morphant phenotypes include abnormal neural tube morphogenesis and **(B)** cell de-adhesion within the tailbud. Neither manipulation affects somite boundary formation as visualized by either DIC (**C,D**) or FN immunolocalization **(E)**. Furthermore, both phenotypes exhibit FN formation within the mesenchymal presomitic mesoderm **(F)**. The *cdh2*^{mo} also strongly inhibits both total and stable Cdh2 as reported by the *cdh2-sfGFP-TagRFP* transgene (n=9). **(G)**. Scale bar = 40 μm.

McMillen et al., Figure S3.

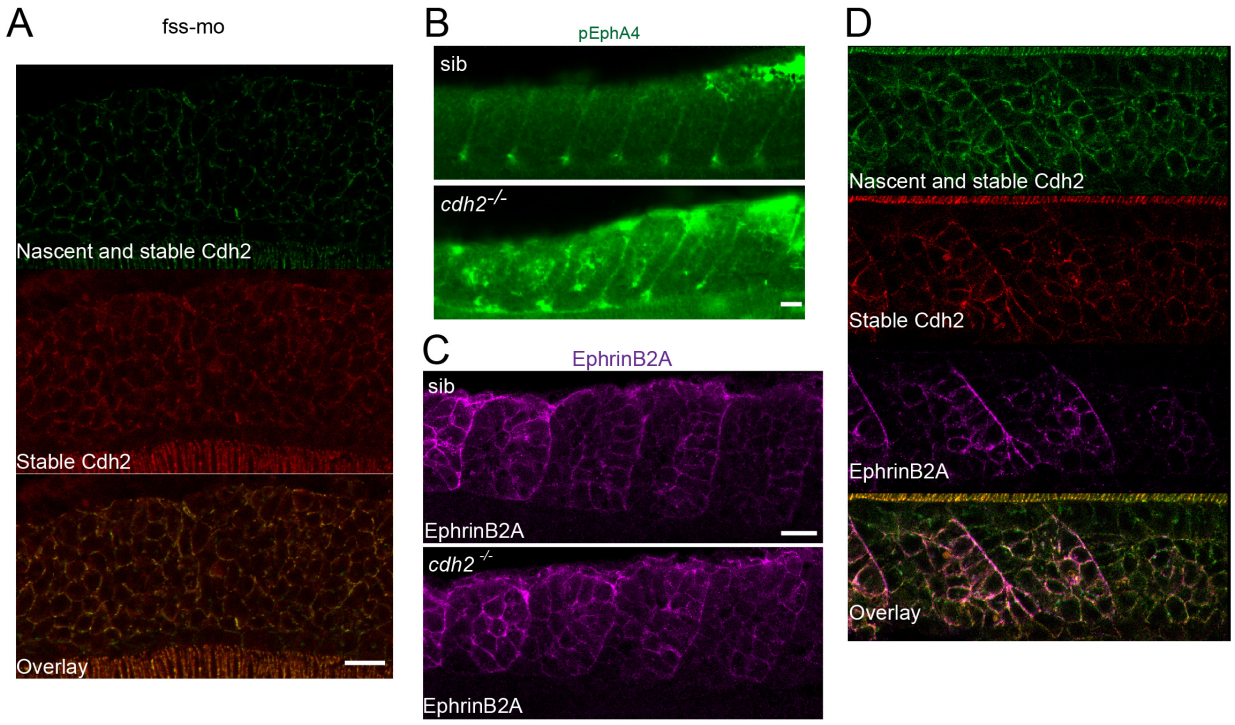

Figure S3. Sawtooth Cdh2 stabilization is downstream of patterning by the segmentation clock and regulates EphA4 phosphorylation; related to Figure 4.

(A) Injection of 200 μM *fss*^{mo} [S1] inhibits the formation of sawtooth gradients of Cdh2 stability in *cdh2-sfGFP-TagRFP* transgenic embryos (n=11). **(B)** Loss of *cdh2* does not affect activation of EphA4 at somite boundaries, but does increase of EphA4 activation within the mesenchymal core of somites. An average of 55.4% of somites in *cdh2*$^{-/-}$ embryos exhibited mesenchymal EphA4 phosphorylation (n=25 embryos) vs. 26.9% in (n=28) sibling control embryos ($p<0.001$, Mann-Whitney-Wilcoxon test). **(C)** EphrinB2A localization is unaffected in *cdh2*$^{-/-}$ embryos (n=9). **(D)** EphrinB2A colocalizes with stable Cdh2 in the posterior somite (n=29). Scale bar = 20 μm.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Phalloidin staining protocol

Phalloidin stains were performed as described [S2], except that Alexa Fluor 488 Phalloidin was used at a 1:125 dilution.

SNAKA51 Immunohistochemistry protocol

FN/SNAKA51 immunohistochemistry was performed as described [S3].

Phospho-EphA4 staining protocol

Phosoph-EphA4 was conducted as described [S2] except that the Alexa Fluor 488 secondary antibody and 0.5% blocking reagent were used.

EphrinB2A staining protocol

Phosoph-EphA4 was conducted as described in [S2] except that the primary antibody was used at a concentration of 1:100 and an Alexa Fluor 488 secondary antibody was used.

Cell Shape Analysis

Ellipses were fit to manual cell traces using the following workflow:

1.) Run "Rainbowmask macro.ijm" on image of interest

```
>run("Duplicate...", "title=rainbowmask");
>rename("rainbowmask (green)");
>selectWindow("rainbowmask (green)");
>run("Set...", "value=0");
>run("Merge Channels...", "c1=[rainbowmask (green)] c2=[rainbowmask (green)] c3=[rainbowmask (green)] c4=[rainbowmask (green)] c5=[rainbowmask (green)] c6=[rainbowmask (green)] c7=[rainbowmask (green)] create ignore");
>run("Split Channels");
```

2.) Arrange "Composite windows" so that all are visible

3.) Trace a cell (include cell edges in cell) and run one of the "color" macros. A cell trace will show up on the relevant window. Do this for each cell in the somite. Make sure that no adjacent cells share the same color.

```
>selectWindow("C1-Composite");
>run("Restore Selection");
>setForegroundColor(255, 255, 255);
>run("Fill", "slice");

>selectWindow("C2-Composite");
>run("Restore Selection");
>setForegroundColor(255, 255, 255);
>run("Fill", "slice");

>selectWindow("C3-Composite");
>run("Restore Selection");
>setForegroundColor(255, 255, 255);
>run("Fill", "slice");

>selectWindow("C4-Composite");
>run("Restore Selection");
>setForegroundColor(255, 255, 255);
>run("Fill", "slice");

>selectWindow("C5-Composite");
```

```
>run("Restore Selection");
>setForegroundColor(255, 255, 255);
>run("Fill", "slice");

>selectWindow("C6-Composite");
>run("Restore Selection");
>setForegroundColor(255, 255, 255);
>run("Fill", "slice");

>selectWindow("C7-Composite");
>run("Restore Selection");
>setForegroundColor(255, 255, 255);
>run("Fill", "slice");
```

4.) Run "Combine and Analize" to calucate the magnitude of the major axis, the magnitude of the minor axis, and the angle of the major axis with respect to the X-Axis. Correct for the fact that the somite border and the X-Axis are not parallel by rotating the somite so that they are parallel, and correcting the calculated angle by the angle of rotation.

```
>run("Merge Channels...", "c1=C1-Composite c2=C2-Composite c3=C3-Composite c4=C4-Composite c5=C5-Composite c6=C6-Composite c7=C7-Composite create keep");
>run("Set Measurements...", "  fit redirect=None decimal=3");
>run("Analyze Particles...", "size=0-Infinity circularity=0.00-1.00 show=Masks display clear add in_situ");
```

Cadherin2-sfGFP-TagRFP Analysis

1.) Rename the Green and Red Channels "Green" and "Red", respectively.

2.)Make a green mask using the following macro:

```
>selectWindow("Green");
>run("Duplicate...", "title=[Green Mask]");
>run("Auto Local Threshold", "method=Bernsen radius=25 parameter_1=0 parameter_2=0 white");
```

3.) Remove saturated pixels:

```
>selectWindow("Green");
>run("Duplicate...", "title=Green-1");
>setAutoThreshold("Default");
>//run("Threshold...");
>setThreshold(255, 255);
>run("Convert to Mask");
>selectWindow("Red");
>run("Duplicate...", "title=Red-1");
>setAutoThreshold("Default");
>//run("Threshold...");
>setThreshold(255, 255);
>run("Convert to Mask");
>imageCalculator("Subtract", "Red","Green-1");
>imageCalculator("Subtract", "Red","Red-1");
>imageCalculator("Subtract", "Green","Red-1");
>imageCalculator("Subtract", "Green","Green-1");
>selectWindow("Green-1");
>close();
>selectWindow("Red-1");
>close()
```

4.) Remove noise objects less than 25 pixels (removes intracellular artifacts)

```
>run("Analyze Particles...", "size=25.00-100000000000.00 pixel >circularity=0.00-1.00 show=Masks in_situ");
```

5.) Apply mask and ratio:

```
>selectWindow("Green Mask");
```

```
>run("32-bit");
>//run("Threshold...");
>setAutoThreshold("Default dark");
>run("NaN Background");
>run("Subtract...", "value=254");
>imageCalculator("Divide create 32-bit", "Green","Green Mask");
>imageCalculator("Divide create 32-bit", "Red","Green Mask");
>imageCalculator("Divide create 32-bit", "Result of Red","Result of Green");
```

6.) Select a somite, and rotate it so that it is in plane with the vertical axis

```
>selectWindow("Result of Red");
>imageCalculator("Divide create 32-bit", "Result of Red","Green Mask");
>run("Enhance Contrast", "saturated=0.35");
>run("Restore Selection");
>run("Make Inverse");
>run("Set...", "value=NaN");
```

7.) Apply ratio mapper, which calculates the mean pixel intensities in columns moving from left to right. Note: Copy and paste the below code multiple times, increasing X at intervals of 20 until 2048 is reached

```
>makeRectangle(X, 0, 20, 2048);
>run("Measure");
...
```

8.) Average red values for each column across somites. For our analysis we discard the posteriormost columns that occur only in a minority of especially wide somites.

Quantification of percent FN coverage in genetic mosaics

This workflow is optimized for Fiji ImageJA 1.45k (Mac) and may need to be modified slightly to work correctly for other versions or operating systems.

1) We select the region of interest, ensuring that the image is in composite mode, and run Macro 1.ijm. This macro first performs a Gaussian blur (sigma=2) on both the rhodamine channel (red) and the FN immunohistochemistry channel (blue). It then runs a Bernsen local thresholding mask included with Fiji [S4] to both channels to convert them to binary images. A local threshold is used in lieu of a global threshold to prevent artifacts arising from varying signal intensity outside of the ROI. The mask of the red channel is then traced using the analyze particles tool, with objects smaller than 100 square pixels being discarded.

2) Because it uses a local threshold, Macro 1.ijm will occasionally make obvious errors where very little signal is present. These errors can be verified using Macro5.ijm as regions of red (i.e. mask where there is no FN immunohistochemistry signal), and we remove them using Macro 2.ijm. We also use Macro 2.ijm to remove any FN contained in the medial or lateral surface of the paraxial mesoderm, in this case removing the pixels from both C1-1.tif and C2-1.tif. Finally, we use Macro2.ijm to remove any artifacts where Macro1.ijm has clearly mis-traced the rhodamine signal.

3) Macro 3 produces a composite image consisting of pixels in which the rhodamine trace overlaps with the FN immunohistochemistry signal (white) and pixels in which the two channels do not overlap (red). The macro also produces a read-out of the total raw integrated density of the trace as well as the integrated density of the white signal pixels.

-Under Analyze --> Set Measurements, ensure that Area and Integrated Density boxes are checked

4) We then add the number of pixels of the relevant FN type in each Z-slice and divide by the total areas of all ROIs to get a single value for each Z-stack.

5) Macro4.ijm can be used to close any windows remaining open from the analysis workflow. This step is highly recommended as any open windows may interfere with analysis of subsequent clones.

SUPPLEMENTAL REFERENCES

S1. Nikaido, M., Kawakami, A., Sawada, A., Furutani-Seiki, M., Takeda, H., and Araki, K. (2002). Tbx24, encoding a T-box protein, is mutated in the zebrafish somite-segmentation mutant fused somites. Nat Genet *31*, 195-199.

S2. Julich, D., Mould, A.P., Koper, E., and Holley, S.A. (2009). Control of extracellular matrix assembly along tissue boundaries via Integrin and Eph/Ephrin signaling. Development *136*, 2913-2921.

S3. Jülich, D., Cobb, G., Melo, A.M., McMillen, P., Lawton, A.K., Mochrie, S.G.J., Rhoades, E., and Holley, S.A. (2015). Cross-scale Integrin regulation organizes ECM and tissue topology. Developmental cell *34,* 33-44.

S4. Bernsen, J. (1986). Dynamic thresholding of gray-level images. Proc 8th Conf on Pattern Recognition *Paris*, 1251-1255.