

Supplemental Materials

Molecular Biology of the Cell

van Loon *et al.*

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Additional quantification of morphological changes in maturing microridges

A) Violin and box-and-whisker plot of protrusion length for periderm cells at the specified stages. 48hpf, n=34 cells from 12 fish; 72hpf, n=24 cells from 10 fish; 96hpf, n=34 cells from 15 fish. $P < 2.2 \times 10^{-16}$, Kruskal-Wallis test followed by Dunn test with Benjamini-Hochberg p-value adjustment: 48-72hpf, $P = 1.36 \times 10^{-14}$; 48-96hpf, $P = 2.67 \times 10^{-59}$; 72-96hpf, $P = 6.08 \times 10^{-14}$.

B) Dot and box-and-whisker plot of average protrusion length on periderm cells at the specified stages. 48hpf, n=34 cells from 12 fish; 72hpf, n=24 cells from 10 fish; 96hpf, n=34 cells from 15 fish. $P = 1.81 \times 10^{-10}$, Kruskal-Wallis test followed by Dunn test with Benjamini-Hochberg p-value adjustment: 48-72hpf, $P = 0.019$; 48-96hpf, $P = 8.60 \times 10^{-11}$; 72-96hpf, $P = 3.05 \times 10^{-4}$.

C) Box-and-whisker plot of protrusion number distributed among pegs ($< 0.75 \mu\text{m}$), short microridges ($0.75 - 5 \mu\text{m}$), and long microridges ($> 5 \mu\text{m}$) on periderm cells at the specified stages. 48hpf, n=34 cells from 12 fish; 72hpf, n=24 cells from 10 fish; 96hpf, n=34 cells from 15 fish. Two-way ANOVA with interaction: hpf, $P = 3.12 \times 10^{-12}$; protrusion type, $P = 4.84 \times 10^{-3}$; hpf-protrusion type interaction, $P = 3.03 \times 10^{-5}$.

D) Dot and box-and-whisker plot of periderm cell apical area at the specified stages. 48hpf, n=34 cells from 12 fish; 72hpf, n=24 cells from 10 fish; 96hpf, n=34 cells from 15 fish. $P = 0.011$, Kruskal-Wallis test followed by Dunn test with Benjamini-Hochberg p-value adjustment: 48-72hpf, $P = 0.722$; 48-96hpf, $P = 0.014$; 72-96hpf, $P = 0.041$.

E) Dot and box-and-whisker plot of microridge spacing mean for periderm cells at the specified stages. 48hpf, n=34 cells from 12 fish; 72hpf, n=24 cells from 10 fish; 96hpf, n=34 cells from 15 fish. $P = 0.308$, one-way ANOVA.

F) Dot and box-and-whisker plot of microridge spacing median for periderm cells at the specified stages. 48hpf, n=34 cells from 12 fish; 72hpf, n=24 cells from 10 fish; 96hpf, n=34 cells from 15 fish. P=0.569, one-way ANOVA.

'*' $p \geq 0.05$ and '***' $p \geq 0.001$.

For box-and-whisker plots, the middle line is the median, and lower and upper ends of boxes are 25th and 75th percentiles, respectively.

Figure S2. Additional images of NMII minifilament association with protrusions and rearrangement events

A) NMII minifilaments associate with protrusions. Airyscan images of a 16hpf zebrafish periderm cell expressing fluorescent reporters for actin (Lifeact-Ruby) and NMII light chain (Myl12.1-GFP). NMII minifilaments (green doublets) appear in the cortex near adjacent pegs (magenta puncta) in the aligned channels. NMII minifilaments were associated with pegs less frequently when the NMII fluorescence channel was rotated 90° relative to the actin channel.

B) Dot plot of the sum of NMII intensity within 0.25µm of protrusions on 16hpf periderm cells expressing fluorescent reporters for actin (Lifeact-Ruby) and NMII light chain (Myl12.1-GFP). Images with unrotated channels were compared to the same images with the NMII fluorescence channel rotated 90° relative to the actin channel as a control. n=7 cells from 3 fish. $P=3.86 \times 10^{-5}$, paired t-test.

C) Stills from Airyscan time-lapse movies of microridge fusion (white arrowheads) events in periderm cells expressing fluorescent reporters for actin (Lifeact-mRuby) and NMII (Myl12.1-GFP). Four different fusion events in different microridges are shown.

D) Stills from Airyscan time-lapse movies of microridge fission (white arrowheads) events in periderm cells expressing fluorescent reporters for actin (Lifeact-mRuby) and NMII (Myl12.1-GFP). Four different fission events in different microridges are shown.

E) Stills from an Airyscan time-lapse movie of the spacing between adjacent microridges narrowing and then subsequently widening in a periderm cell expressing fluorescent reporters for actin (Lifeact-mRuby) and NMII (Myl12.1-GFP). Dotted lines highlight narrowing and widening regions.

Scale bars: 1 μ m (A, C, D and E).

Figure S1

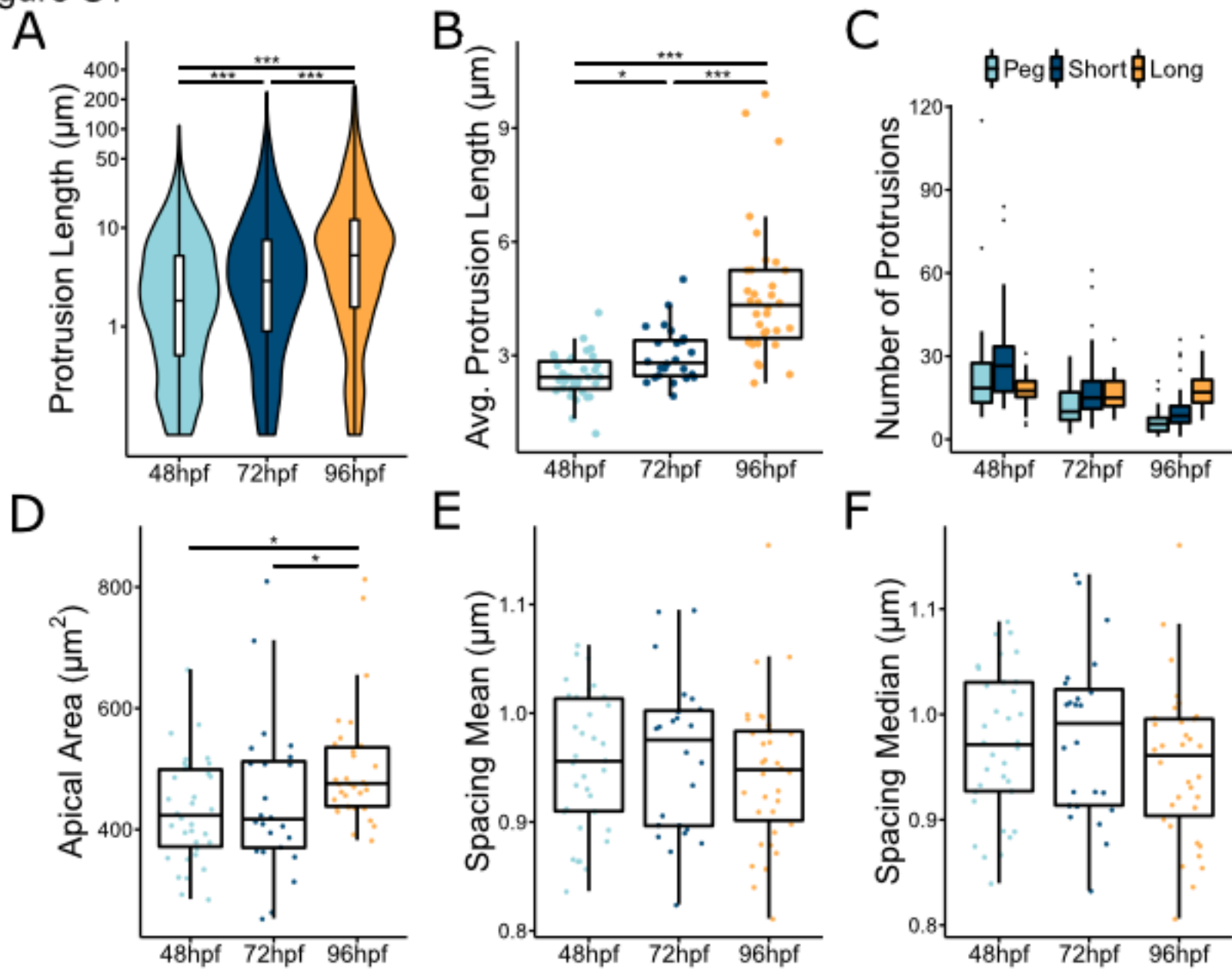


Figure S2

