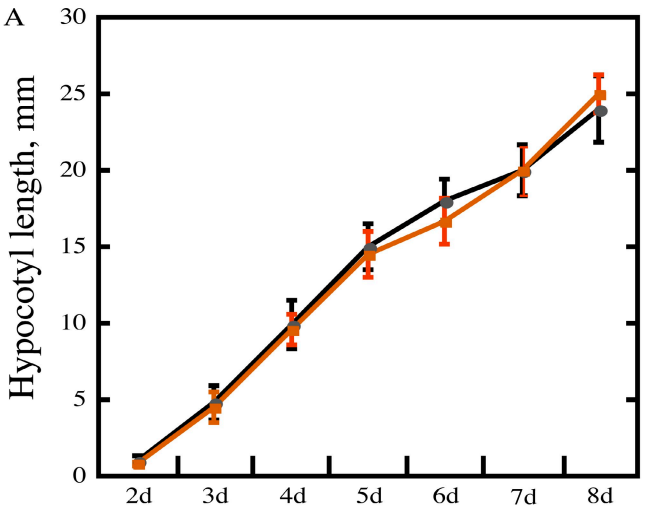
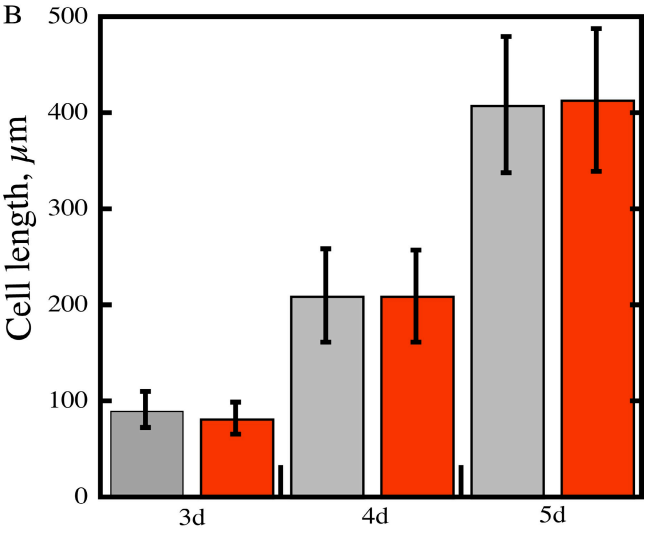
Staiger et al., http://www.jcb.org/cgi/content/full/jcb.200896185/DC1





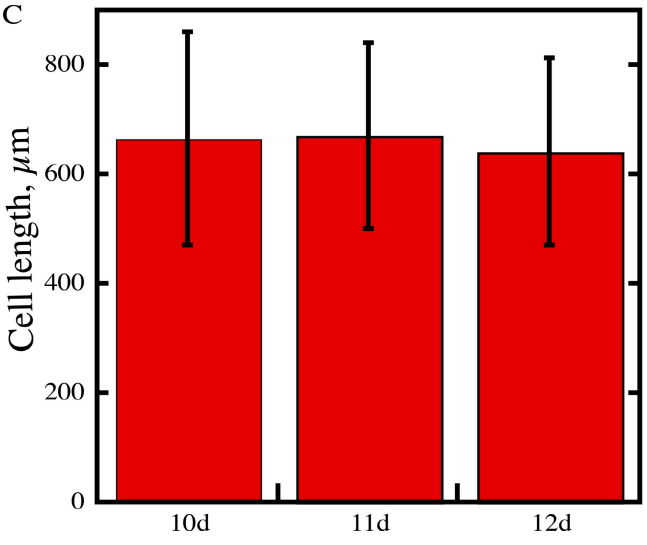


Figure S1. **Hypocotyls and epidermal cells expressing GFP-fABD2 grow similarly to those from wild-type seedlings.** (A) Hypocotyl length plotted as a function of time shows little difference when wild-type seedlings are compared with GFP-fABD2 expressing seedlings. Red squares, GFP-fABD2; black circles, wild-type etiolated seedlings. Error bars represent SD. Measurements were taken from 35–66 hypocotyls of each genotype grown on the same plate, and individual plates were killed on each day of data acquisition. (B) Epidermal cell lengths in wild-type (gray bars) and GFP-fABD2 expressing (red bars) seedlings were not markedly different during active cell expansion on days 3–5. Etiolated hypocotyls stained with FM4-64 to outline cell boundaries were imaged with fluorescence microscopy. Length measurements were recorded from epidermal cells in the top third of the hypocotyl, but below the hook region. Cell length values are the average (±SD) from at least 51 cells in ≥20 hypocotyls. (C) Epidermal cells cease expanding after day 10 in etiolated seedlings. Length measurements were recorded from cells in the bottom half of the hypocotyl. Values are the average (±SD) from at least 51 cells in ≥12 hypocotyls.

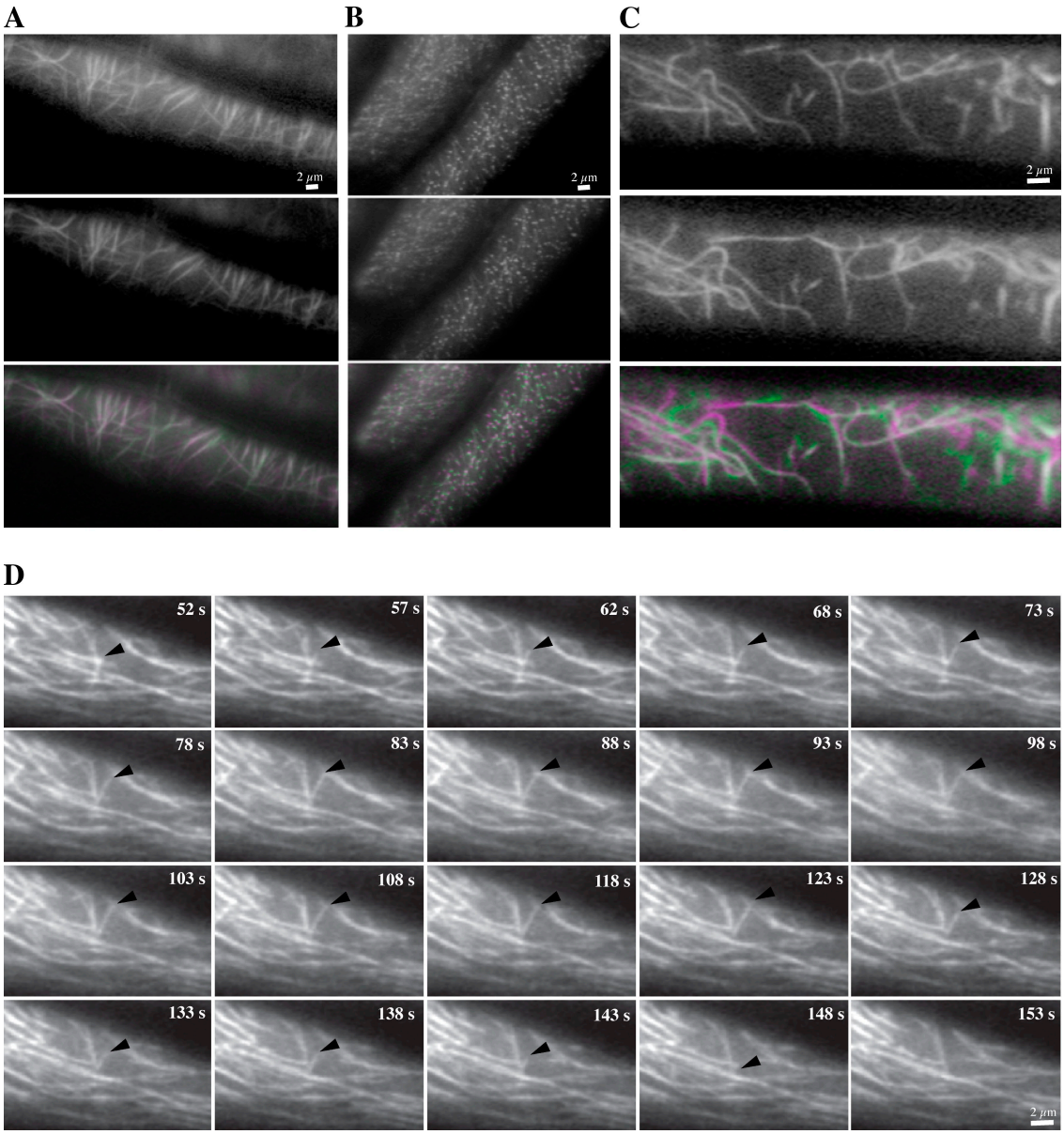


Figure S2. **VAEM is useful for documenting the organization and dynamics of the cortical cytoskeleton.** (A) Time-lapse composite of microtubules in an elongating hypocotyl epidermal cell expressing EYFP-TUB5. Two images taken 20 s apart (top and middle), were pseudo-colored green, or magenta and blue. The three pseudo-color images were then overlaid to produce a color composite (bottom). Microtubules that remain stationary or unchanged are white, whereas microtubule growth and shrinkage are magenta and green, respectively. The predominant orientation of the array was transverse and only modest microtubule movement or growth and shrinkage was observed during this time interval. (B) Time-lapse composite of microtubule plus-ends marked with GFP-EB1. Two images taken 10 s apart (top and middle) were pseudo-colored and a composite overlay created (bottom). Many unique magenta and green spots are obvious, but a region of white overlap can often be distinguished. See Video 1 for full time-lapse sequence. (C) Time-lapse composite of cortical actin filaments in an elongating hypocotyl epidermal cell expressing GFP-fABD2. Two images taken 3 s apart (top and middle) were pseudo-colored and a composite overlay image generated (bottom). The actin filaments do not show an overall orientation with respect to the cell main axis, most are quite convoluted in appearance, and very few remain constant in location or shape during the short interval between exposures. (D) Microtubules display dynamic instability. A montage of successive frames at ~5-s intervals shows a microtubule plus end (black arrowhead) that transitions between phases of growth (52–103 s) and catastrophic shrinkage (123–153 s). Time points indicate elapsed time from start of video sequence. Bars, 2 μm.

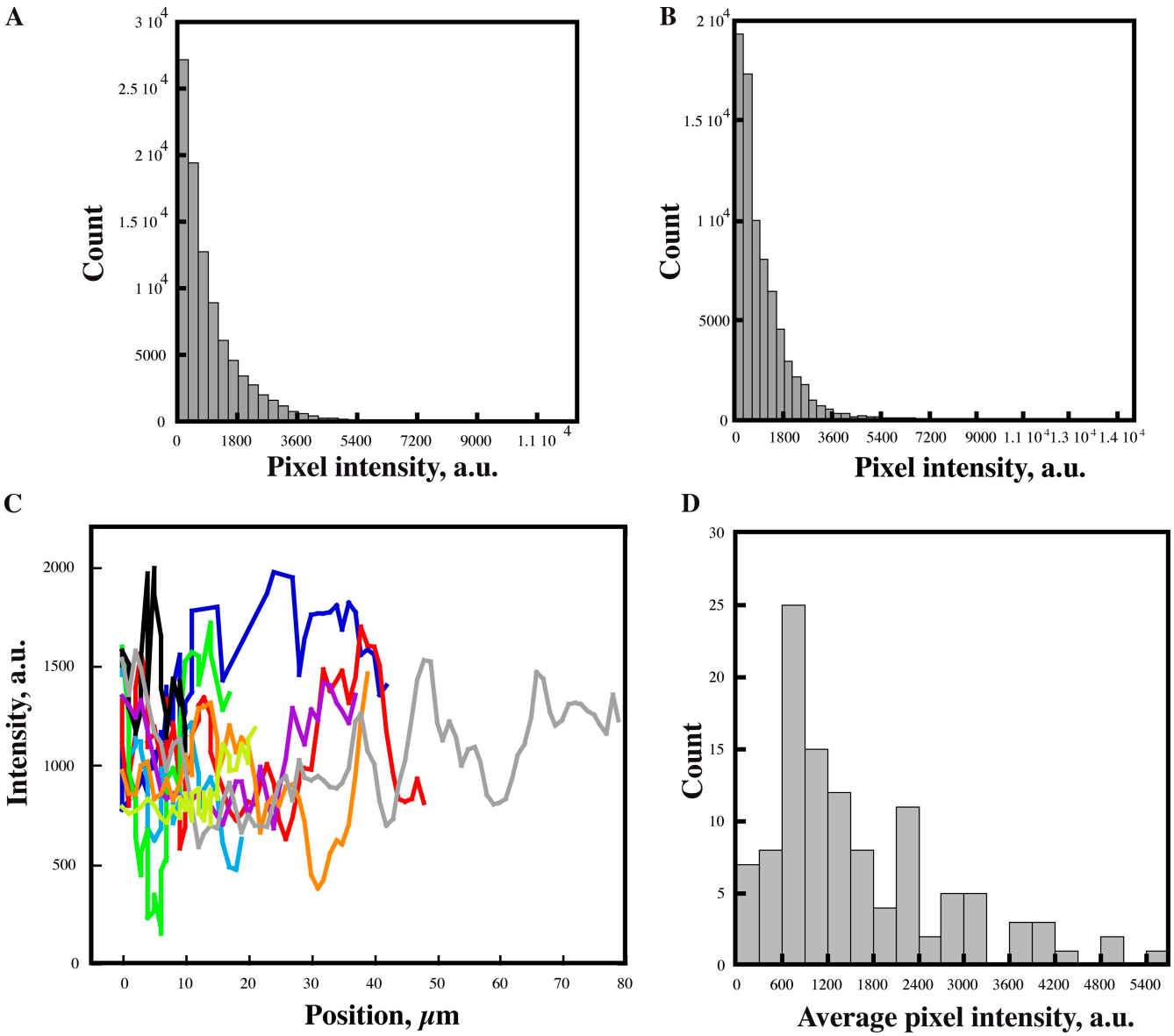


Figure S3. **Analysis of pixel intensities for actin filaments and actin-based structures in the cortical array.** (A) Range of pixel intensities for the epidermal cell shown in Fig. 1. Individual pixel intensity over time was computer-generated using multiple line-scans along the cell, represented by the red lines in the first panel of Fig. 1, allowing a full analysis of the pixel intensity for the different cellular actin structures. (B) Range of pixel intensities for an 11-d-old, non-elongating epidermal cell (see Video 8; frames 55–84). For both growing (A) and nongrowing (B) cells, the majority of actin-based structures have pixel intensities <1800. (C) Several actin filaments were hand-selected from the time-lapse series shown in Videos 2 and 3 and scanned using MetaMorph. Plots of pixel intensity as a function of position along each filament highlights intensity fluctuations between 150 and 1990 a.u. (D) Histogram of average pixel intensity for >100 hand-selected actin-based structures from numerous cells in different hypocotyls. The faintest filamentous structures, with average intensities between 0 and 2100 a.u., have a Gaussian distribution that peaks around 1100 a.u. Average intensity values (±SEM) for the two populations, thick and thin actin-based structures, are given in Table I.