Toret et al., http://www.jcb.org/cgi/content/full/jcb.201406135/DC1

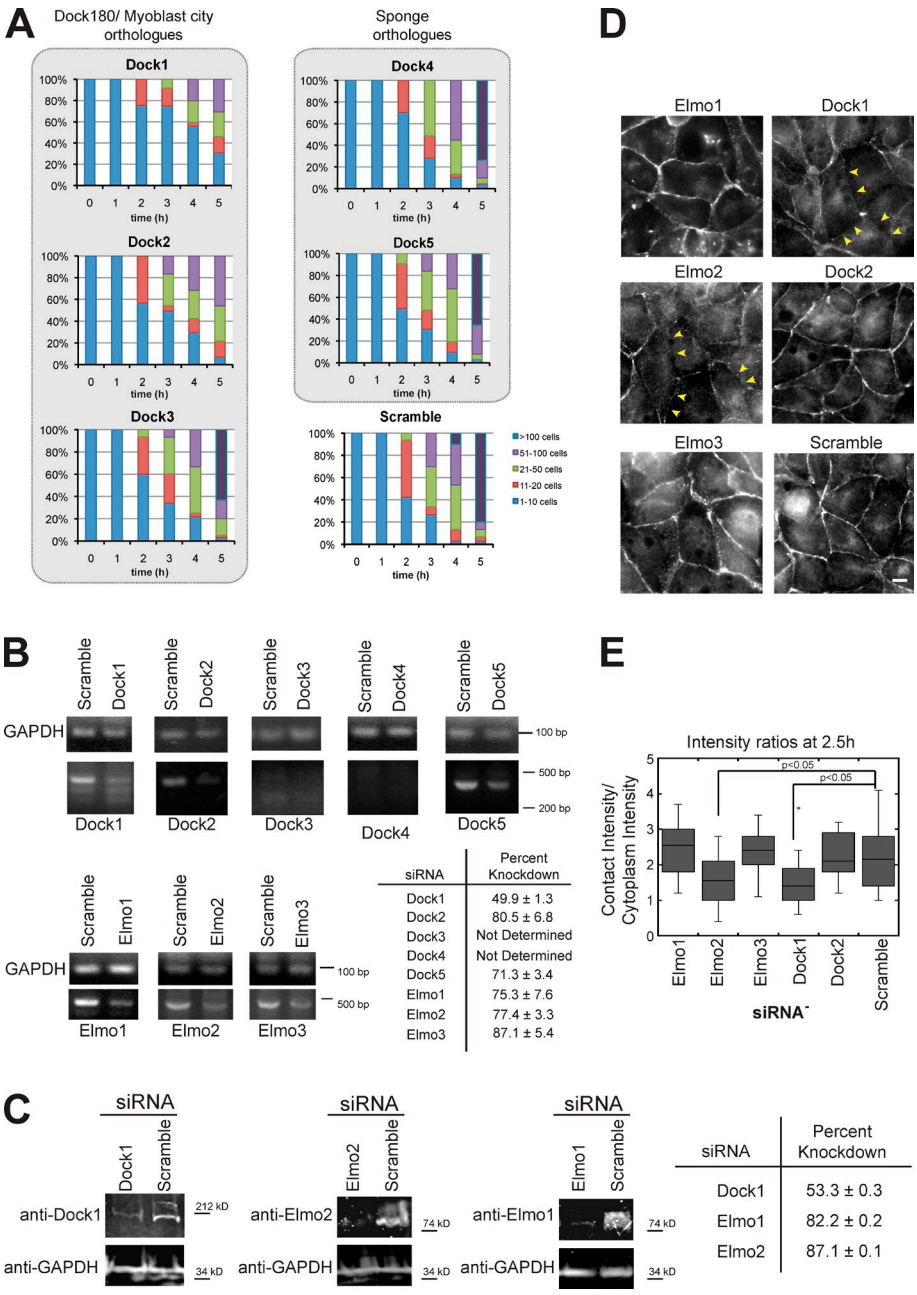


Figure S1. **Verification of Dock/Elmo knockdown phenotypes using additional siRNAs.** (A) Quantification of hanging drop assays for the indicated siRNAs in which the cells were binned into cluster classes: 1–10, 11–20, 21–50, 51–100, or >100 cells (Toret et al., 2014). The percentage of cells in each category is shown for each time point. The data shown are from a representative experiment of four repeats in which ~5 × 10^4 cells were analyzed for each time point. (B) RT-PCR analysis of transcript levels using a second, independent siRNA sequence for each gene. Dashes indicate molecular masses, and the percentage of knockdown was calculated by taking the mean from three independent experiments. (C) Western blot analysis of protein expression in the indicated siRNA-treated cells. Hash lines indicate molecular mass standards. Quantification of protein intensities from three independent Western blots for the indicated siRNAs. (D) E-cadherin immunofluorescence for the indicated siRNA-treated cells at 2.5 h after cell plating. Yellow arrowheads indicate reduced E-cadherin staining at cell–cell contacts. Bar, 5 µm. (E) Box plot quantification of the ratio of E-cadherin fluorescence intensity at a region of cell–cell contact normalized to the intensity of an equal region of the cytoplasm underlying the contact (n = 25 for each condition). Results are presented in a box and whisker format, in which the ends of the box mark the upper and lower quartiles, the horizontal line in the box indicates the median, and the whiskers outside the box extend to the highest and lowest value within 1.5× the interquartile range. P-values were determined by unpaired t test for the indicated samples.

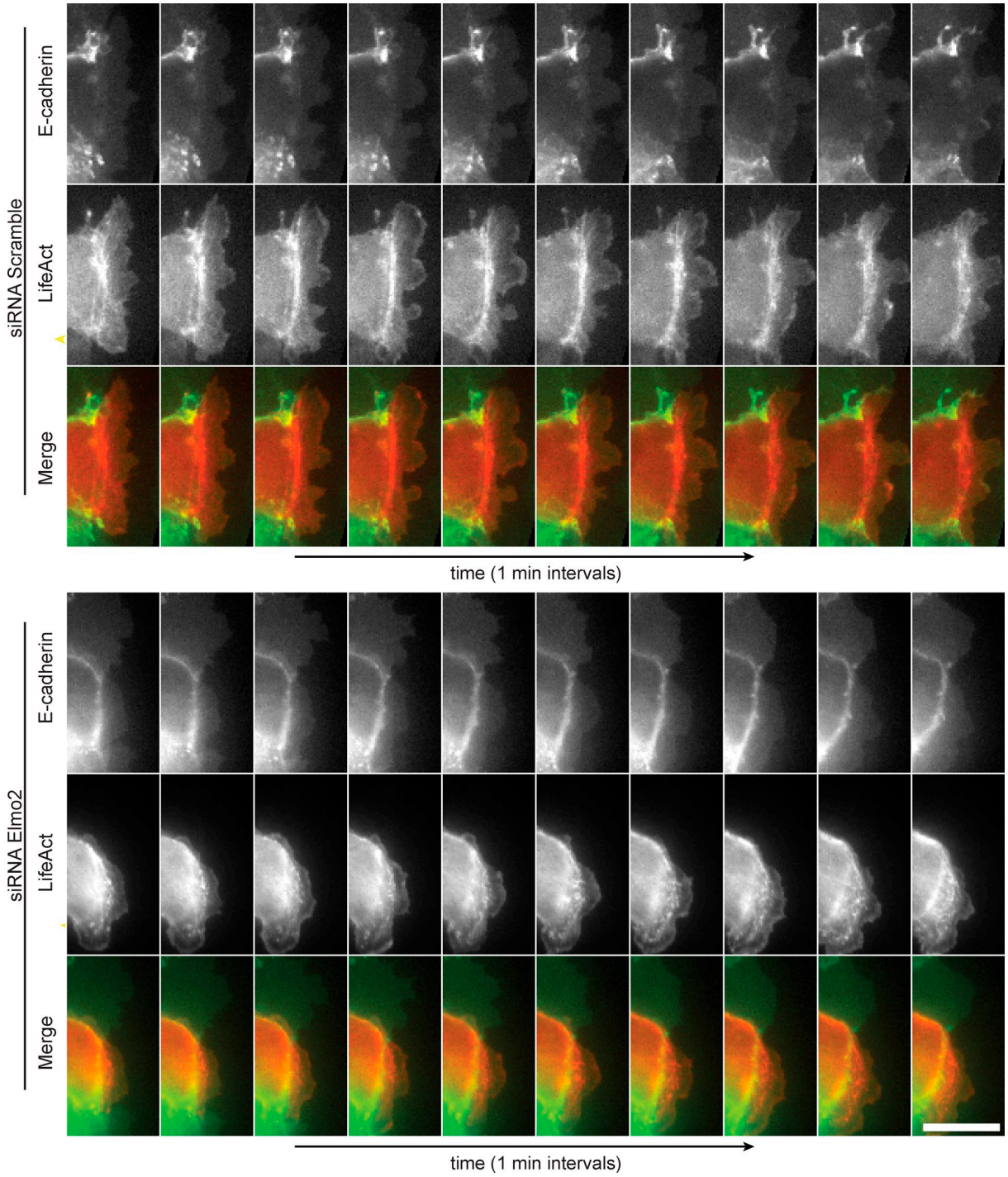
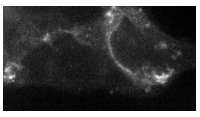
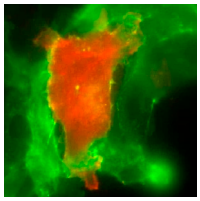


Figure S2. **Actin dynamics at lamellipodia.** Montage of images from videos of lamella from live cells expressing E-cadherin–GFP and LifeAct-RFP and treated with scramble or Elmo2 siRNAs. Bar, 5 µm.



Video 1. **E-cadherin–GFP dynamics at cell–cell contacts.** Cells stably expressing E-cadherin–GFP were transfected with scramble or Elmo2 siRNA. Cells were visualized by epifluorescence time-lapse microscopy. 1-s exposures captured over 1 h with a 1-min interval. Bar, 5 µm.



Video 2. **E-cadherin–GFP and LifeAct-RFP dynamics at cell–cell contacts.** Cells stably expressing E-cadherin–GFP were transfected with LifeAct-RFP and scramble or Elmo2 siRNA. Cells were visualized by epifluorescence time-lapse microscopy. 1-s exposures captured over 30 min with a 1-min interval. Bar, 5 µm.

Reference

Toret, C.P., M.V. D'Ambrosio, R.D. Vale, M.A. Simon, and W.J. Nelson. 2014. A genome-wide screen identifies conserved protein hubs required for cadherin-mediated cell–cell adhesion. *J. Cell Biol.* 204:265–279. http://dx.doi.org/10.1083/jcb.201306082