Appendix III

1. Special considerations for the ablation step:
   1. It is important to know junctions are effectively ablated. This, could be done by acquiring the full field of view and compare the changes in junctional length between the junction that has been ablated and the surrounding junctions and determine how much difference there is between these two groups (See also Supplementary Video 4). This would help to determine whether or not ablations have been successful or just so minor that they are undistinguishable from the underlying junctional or cell movements in the monolayer.
2. It is important to check the infrared laser is properly collimated. As infrared lasers have a very different wavelength compared to more commonly used visible lasers, special attention is needed to check perfect alignment of the collimator between the infrared laser and the blue (488) and green (546) lasers. This could be done by doing imaging with the same settings during pre and post ablation and acquire images of GFP fluorescence using either the 488 nm laser or the two-photon laser (*i.e.*, doing multi-photon microscopy). By comparing these two images and using pinhole 1 Airy unit, one should note that the two images are equal and the fields of view match perfectly. In the case that the image on the two photon channel exhibits a different focus from the 488 nm channel, the position of the collimator then has to be varied slightly to minimize the differences between the images. If the fields of view are different, then the collimators XY needs to be adjusted in order to center both the multiphoton image and the visible image at the same position.

Tuning the wavelength of the infrared laser. Ablations can be done using short pulse length (femtosecond) infrared lasers (as described here) or UV lasers (see Smutny *et al.*, 2015). In our experience infrared lasers produces ablations that have a good resolution in the Z axis, which we found very useful to measure differences in tension between the apical and lateral cell-cell junctions (Wu et al., 2014). In addition, as these lasers are have tunable wavelengths, it is required to set the wavelength with greater power output for efficient ablation. In the case of the Chameleon laser, this corresponds to 790 nm.