Appendix II

1. Special considerations for imaging pre and post ablation:
2. It is important to consider how long it takes to cause a proper ablation and how long the junctions take to recoil after ablation. Ideally, the time scale of the ablation process should be shorter (30 to 100 times shorter) than the time scale of the recoil process; as for tension measurements, the velocity of recoil immediately after ablation is an index of the underlying tension applied on the junction before ablation. For example, in Figure 2 and 3, the recoil process takes ~40 sec. For this particular experiment, ablation was conducted in aprox. 1 sec.
3. For acquisition post ablation, the recoil needs to be captured until it reaches its maximal value, i.e., it plateaus. In our experimental conditions plateau is reached within 1 min after ablation. This facilitates the fitting procedure, and therefore the model and data conclusions, that are based in non-linear regression of curves.
4. Bleaching and noise in images. Unlike FRAP, the images for ablation experiments only require to be of enough quality to allow tracking of the positions of junctional vertices over time. Therefore, some degree of bleaching and image noise is acceptable during the post-acquisition phase of ablation. Note that sometimes ablation could cause movements in Z direction that drive the imaging of the vertices out of focus. It is possible to address this issue when the displacement in XY is significantly larger than it in Z, by acquiring 3 Z-slices: one above, one below and one at the plane of the zonula adherens; then using maximal projections of the resulting movie for vertex tracking.