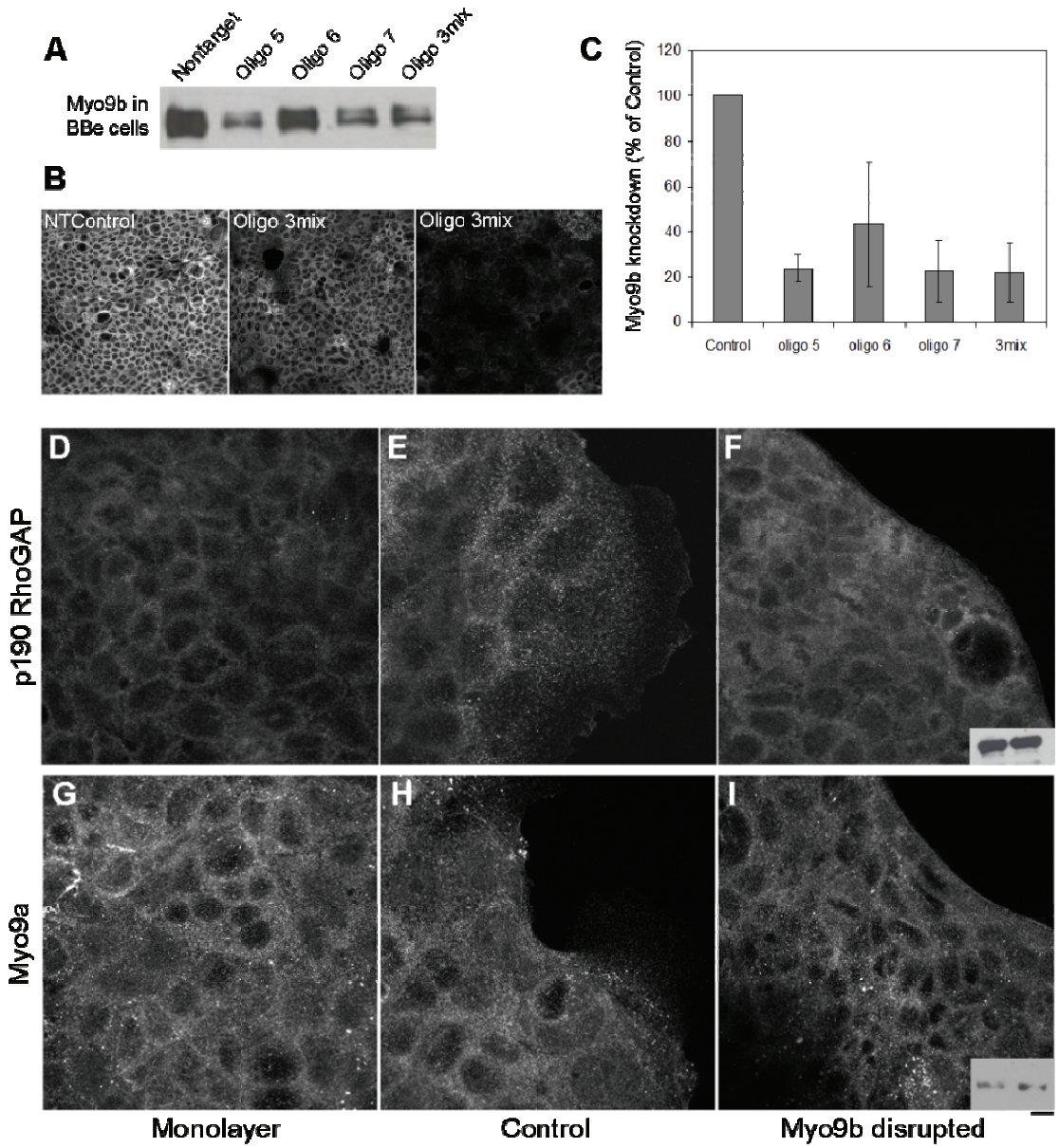

Figure S1. (A-C) RNA interference in BBe cells. (A) Representative western blot of Myo9b expression after siRNA transfection, indicating that a combination of Myo9b targeting oligonucleotides was sufficient to knockdown at least 70% of Myo9b expression. (B) Low magnification images of NTControl cells and two panels of Myo9bKD cells display the heterogeneous nature of Myo9bKD in BBe cells. (C) Quantification of Myo9b protein knockdown from 3 separate experiments. (D-I) Other RhoGAPs do not compensate for loss of Myo9b. (D) p190RhoGAP localization is diffuse within differentiated BBe monolayers and also redistributes to the leading edge of migrating cells (E), but localization is unaffected at the Myo9bKD BBe wound edge (F).

(G) Myo9a localization is diffuse within BBe monolayers and does not redistribute to the leading edge (H). (I) Similarly to p190RhoGAP, Myo9a localization is unaffected at the Myo9bKD wound edge. Western blot insets show protein levels are unchanged with Myo9bKD (NTControl left, Myo9bKD right). Bar, 10 μm.

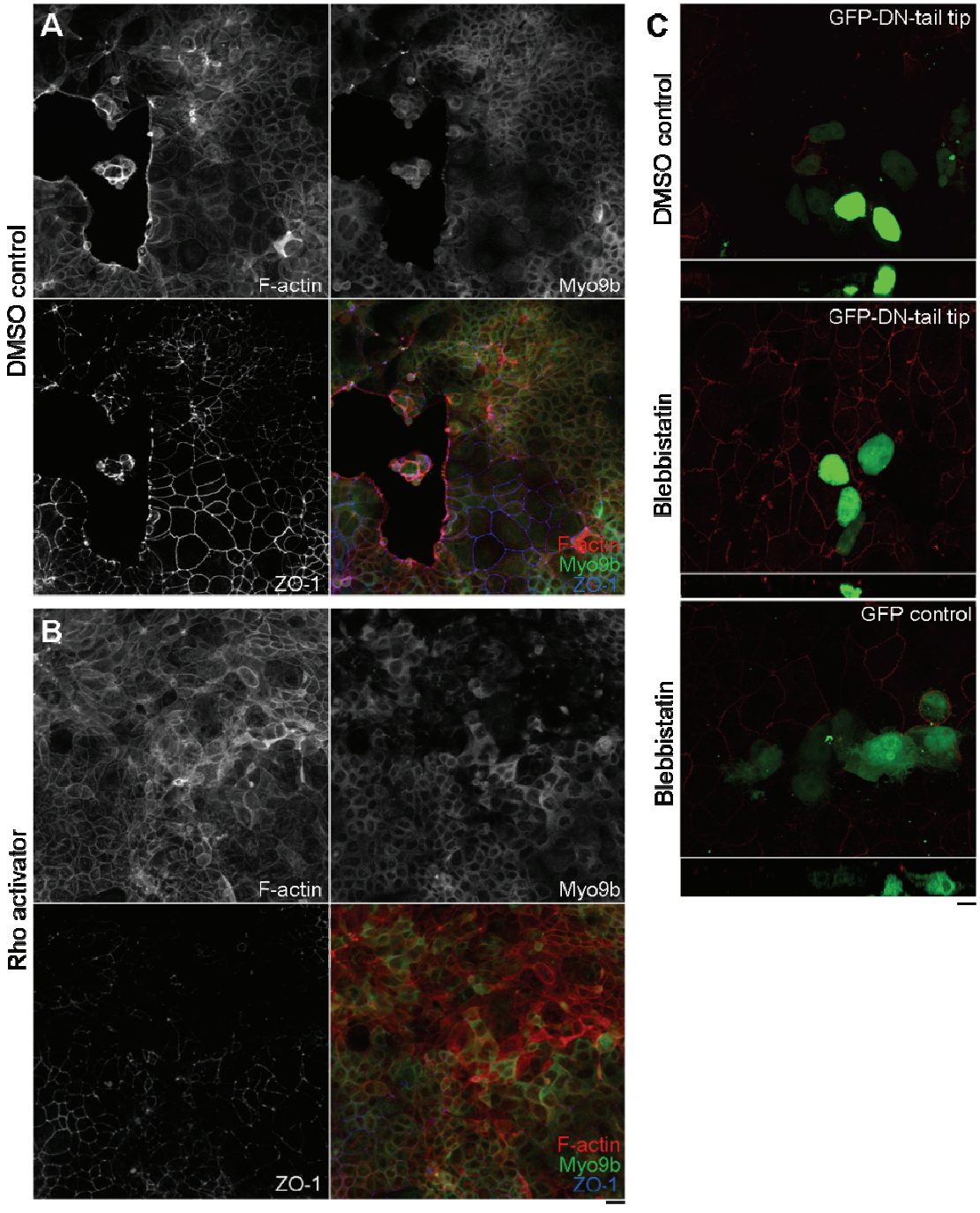

Figure S2. (A-B) Hyper-activation of Rho signaling disrupts TJs. Differentiated BBe monolayers were incubated with PBS containing 3mM EDTA (30 min) to disrupt junctions. This was followed by incubation with DMEM media containing DMSO alone (control) or Rho activator. (A) Control cells were able to reform junctions following

EDTA incubation, with TJ localization of ZO-1 (blue). (B) Similar to Myo9bKD cells, cells incubated with Rho activator exhibited loss of TJ ZO-1 localization. F-actin (red) and Myo9b localization (green) were unaffected. (C) Blebbistatin rescues ZO-1 localization in GFP-DN-tail-tip expressing cells. DN-tail-tip cells incubated with DMSO exhibit loss of TJ protein localization (top), which is rescued with 20μM Blebbistatin (middle). ZO-1 localization is slightly affected with Blebbistatin treatment in GFP control cells (bottom). Insets are Z-plane views to display apical ZO-1 localization. Bar, 10 μm.