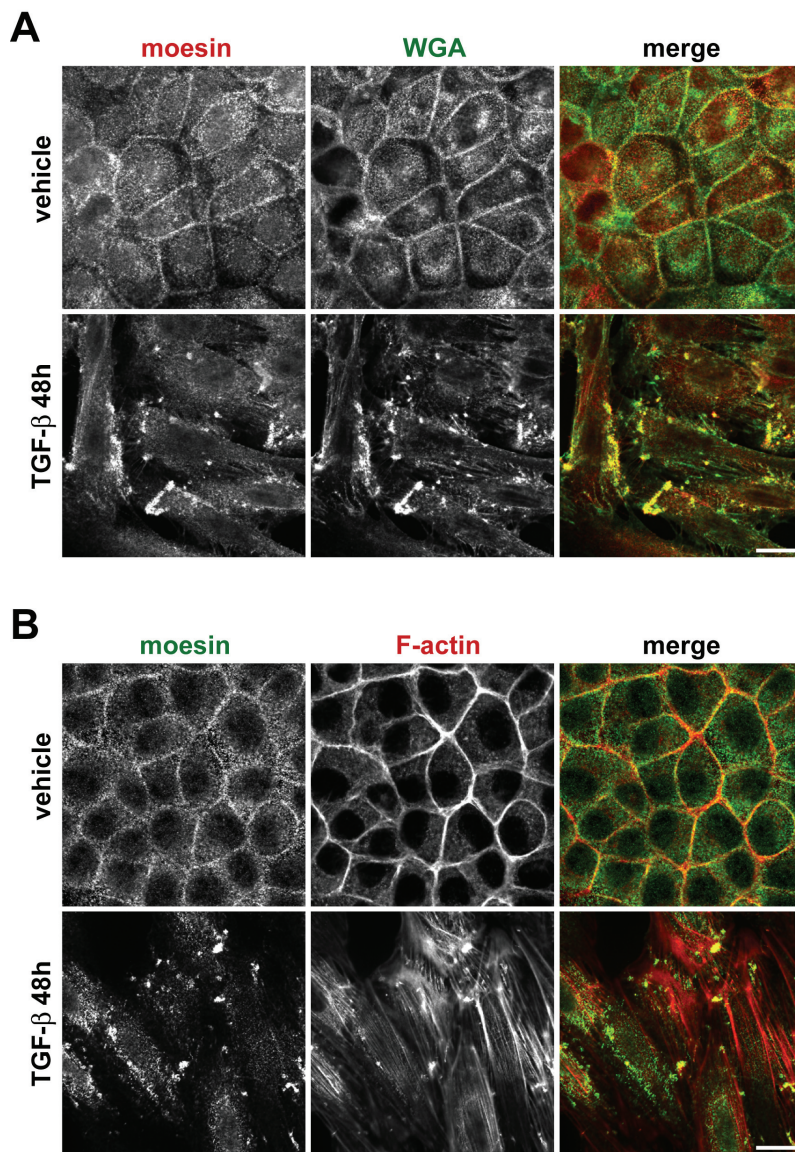
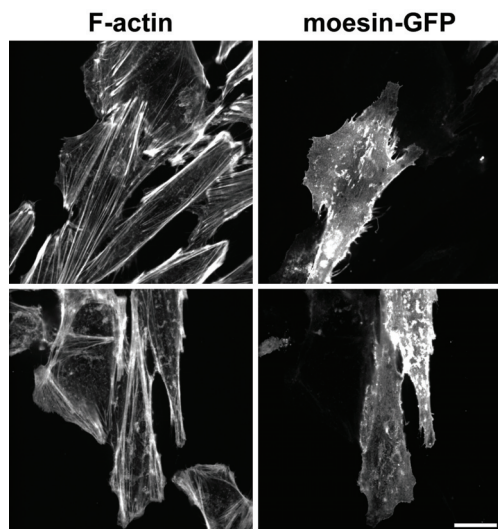


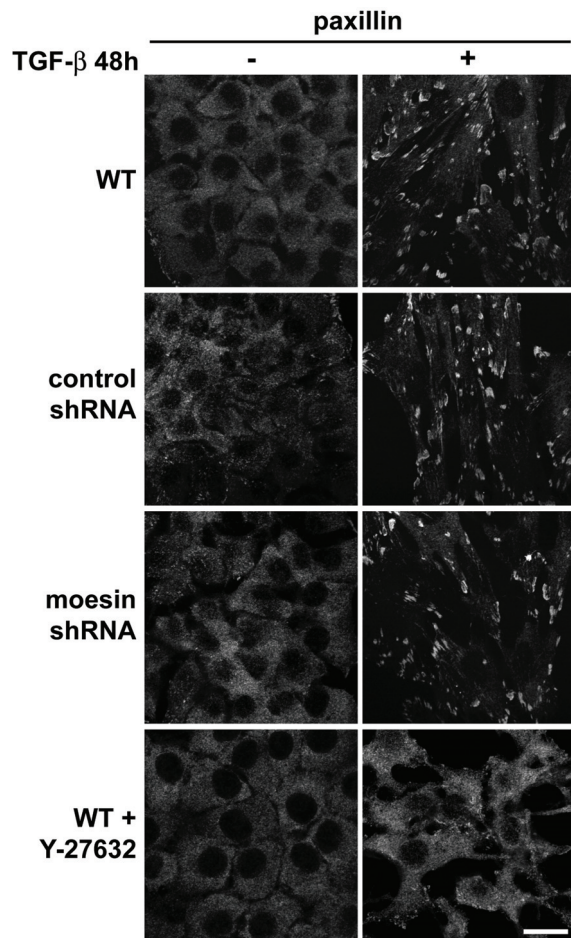
## Supplemental Figure Legends



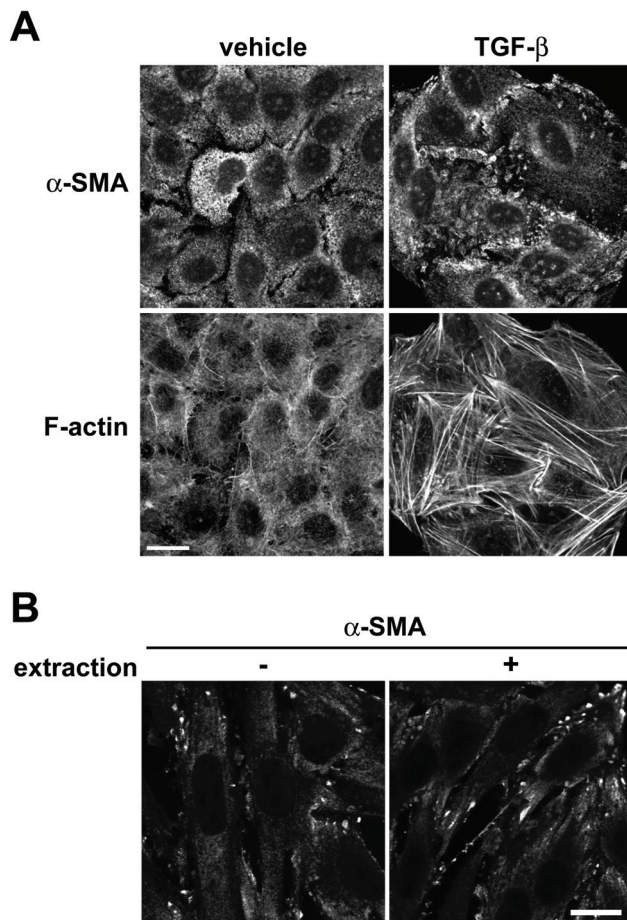
**Figure S1.** Moesin localizes to the plasma membrane and co-labels with membrane-associated F-actin during TGF- $\beta$ -induced EMT. NMuMG cells were maintained in the absence or presence TGF- $\beta$  for 48 h, fixed, and immunolabeled with antibodies for moesin. (A) Cells were incubated with fluorophore-conjugated wheat germ agglutinin (WGA) to label the plasma membrane prior to fixation. (B) Fixed cells were labeled with rhodamine-phalloidin to stain F-actin. Bars, 20  $\mu$ m.



**Figure S2.** Expression of moesin-GFP in moesin shRNA cells increases the number and size of parallel actin stress fibers traversing the ventral cell surface. Moesin shRNA NMuMG cells transiently expressing moesin-GFP not targeted by moesin shRNA sequences were maintained in the presence of TGF- $\beta$  for 48h, fixed, and labeled with rhodamine-phalloidin to stain F-actin. Bar, 20  $\mu$ m.



**Figure S3.** Moesin shRNA knockdown has no effect on the abundance or size of focal adhesions during TGF- $\beta$ -induced EMT. Paxillin immunolabeling of wild-type (WT), control shRNA, and moesin shRNA NMuMG cells in the absence (-) or presence (+) of TGF- $\beta$  after 48 h. Also shown are wild-type cells co-treated with the ROCK inhibitor Y-27632. Bar, 20  $\mu$ m.



**Figure S4.** Cytoskeleton-associated  $\alpha$ -SMA cortical patches assemble during EMT. (A)  $\alpha$ -SMA relocalizes to cortical patches during TGF- $\beta$ -induced EMT of A549 cells. A549 cells were maintained in the absence or presence of TGF- $\beta$  for 48 h, fixed, and immunolabeled with antibodies for  $\alpha$ -SMA and with rhodamine-phalloidin. (B)  $\alpha$ -SMA in cortical patches is associated with the cytoskeleton during TGF- $\beta$ -induced EMT.  $\alpha$ -SMA immunolabeling of wild-type NMuMG cells treated with TGF- $\beta$  for 48 h in the absence (-) or presence (+) of triton extraction. For extraction, soluble proteins were removed with detergent prior to fixation. Bars, 20  $\mu$ m.

## **Supplemental Video Legends**

**Video 1.** Morphological changes of NMuMG cells during TGF- $\beta$ -induced EMT. Wild-type cells were treated with TGF- $\beta$  for 48 h. Images were acquired every 10 min for 48 h. The video plays at 15 frames/s. Corresponds to Figure 1A.

**Video 2.** Actin cytoskeleton remodeling of NMuMG cells during TGF- $\beta$ -induced EMT. Wild-type cells expressing Lifeact-GFP were treated with TGF- $\beta$  for 33 h. Images were acquired every 10 min for 27 h, beginning at 6 h after TGF- $\beta$  treatment. The video plays at 15 frames/s. Corresponds to Figure 1D.

**Video 3.** Actin filament dynamics of NMuMG cells treated with TGF- $\beta$  for 24 h. Wild-type cells expressing Lifeact-GFP were treated with TGF- $\beta$  for 24 h. Images were acquired every 1 min for 1 h. The video plays at 15 frames/s. Corresponds to Figure 1E.

**Video 4.** Actin filament dynamics of NMuMG cells treated with TGF- $\beta$  for 48 h. Wild-type cells expressing Lifeact-GFP were treated with TGF- $\beta$  for 48 h. Images were acquired every 1 min for 1 h. The video plays at 15 frames/s. Corresponds to Figure 1E.

**Video 5.** Actin filament dynamics of NMuMG cells not treated with TGF- $\beta$ . Wild-type cells expressing Lifeact-GFP were treated with vehicle control for 48 h. Images were acquired every 1 min for 1 h. The video plays at 15 frames/s. Corresponds to Figure 1E.

**Video 6.** Actin filament dynamics of wild-type and control shRNA NMuMG cells treated with TGF- $\beta$  for 48 h. Wild-type (left) and control shRNA (right) cells expressing Lifeact-GFP were treated with TGF- $\beta$  for 48 h. Images were acquired every 1 min for 1 h. The video plays at 15 frames/s. Corresponds to Figure 5A.

**Video 7.** Actin filament dynamics of moesin shRNA NMuMG cells treated with TGF- $\beta$  for 48 h. Moesin shRNA cells expressing Lifeact-GFP were treated with TGF- $\beta$  for 48 h. Images were acquired every 1 min for 1 h. The video plays at 15 frames/s. Corresponds to Figure 5A.

**Video 8.** Dynamics of moesin-GFP in NMuMG cells maintained in the absence or presence of TGF- $\beta$  for 48 h. Wild-type cells expressing moesin-GFP were treated with vehicle control (left) or TGF- $\beta$  (right) for 48 h. Images were acquired every 1 min for 90 min. The video plays at 15 frames/s. Arrows indicate contractile moesin-GFP clusters.

**Video 9.** Migration after monolayer wounding of control shRNA NMuMG cells treated with TGF- $\beta$  for 48 h. Control shRNA cells were treated with TGF- $\beta$  for 48 h before wounding. Images were acquired every 15 min for 20 h. The video plays at 10 frames/s. Corresponds to Figure 9A.

**Video 10.** Migration after monolayer wounding of moesin shRNA NMuMG cells treated with TGF- $\beta$  for 48 h. Moesin shRNA cells were treated with TGF- $\beta$  for 48 h before wounding. Images were acquired every 15 min for 20 h. The video plays at 10 frames/s. Corresponds to Figure 9A.