

Supplemental Figure Legends

Supplemental Figure 1 Downregulation of adducin expression does not affect development of the paracellular barrier. SK-CO15 cells were transfected with either control, α -adducin or γ -adducin-specific siRNAs and development of the paracellular barrier was monitored by TEER measurements. Note that depletion of α or γ adducins has no effect on the barrier formation compared to control SK-CO15 cells.

Supplemental Figure 2. Downregulation of α -adducin expression does not affect AJ and TJ in confluent cell monolayers. SK-CO15 cells were transfected with either control or α -adducin-specific siRNAs and the integrity of their AJ and TJ was examined by immunolabeling and confocal microscopy on day 4 post-transfection. Note that depletion of α -adducin has no effect on the integrity of epithelial junctions in confluent epithelial cell monolayers. Bar, 10 μ m.

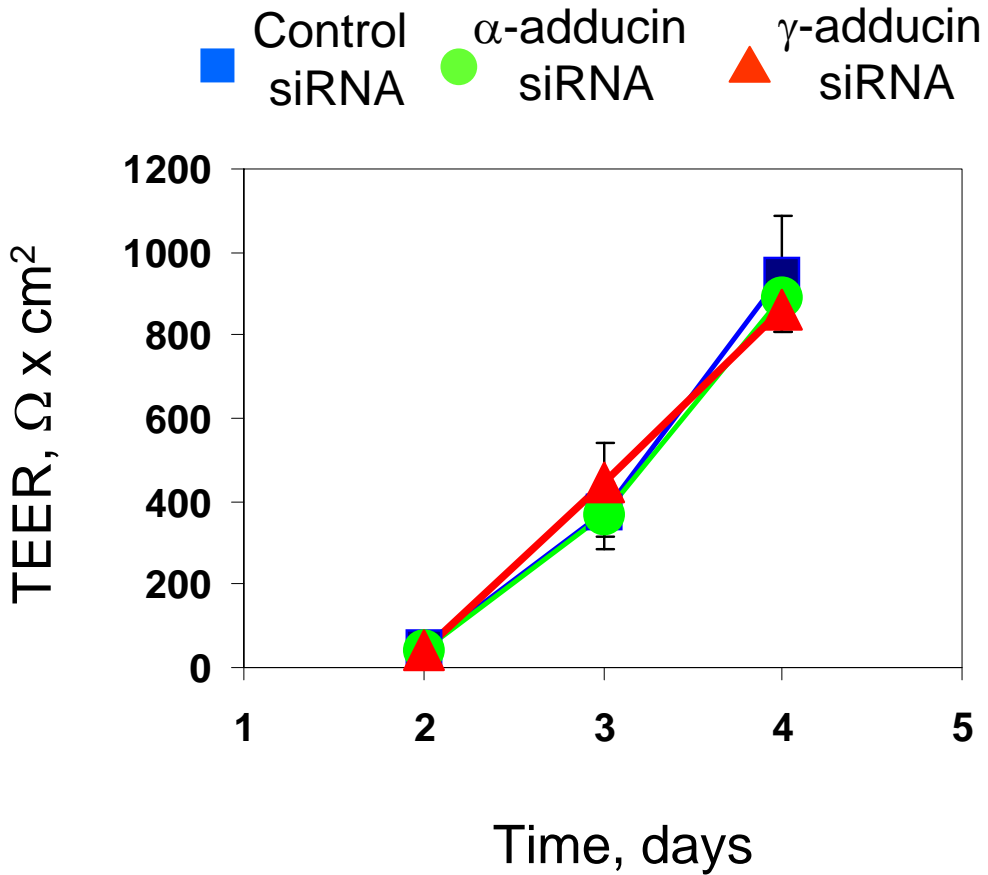
Supplemental Figure 3. Downregulation of γ -adducin expression does not affect AJ and TJ in confluent cell monolayers. SK-CO15 cells were transfected with either control or γ -adducin-specific siRNAs and the integrity of their AJ and TJ was examined by immunolabeling and confocal microscopy on day 4 post-transfection. Note that depletion of γ -adducin has no effect on the integrity of apical junctions in confluent epithelial cell monolayers. Bar, 10 μ m.

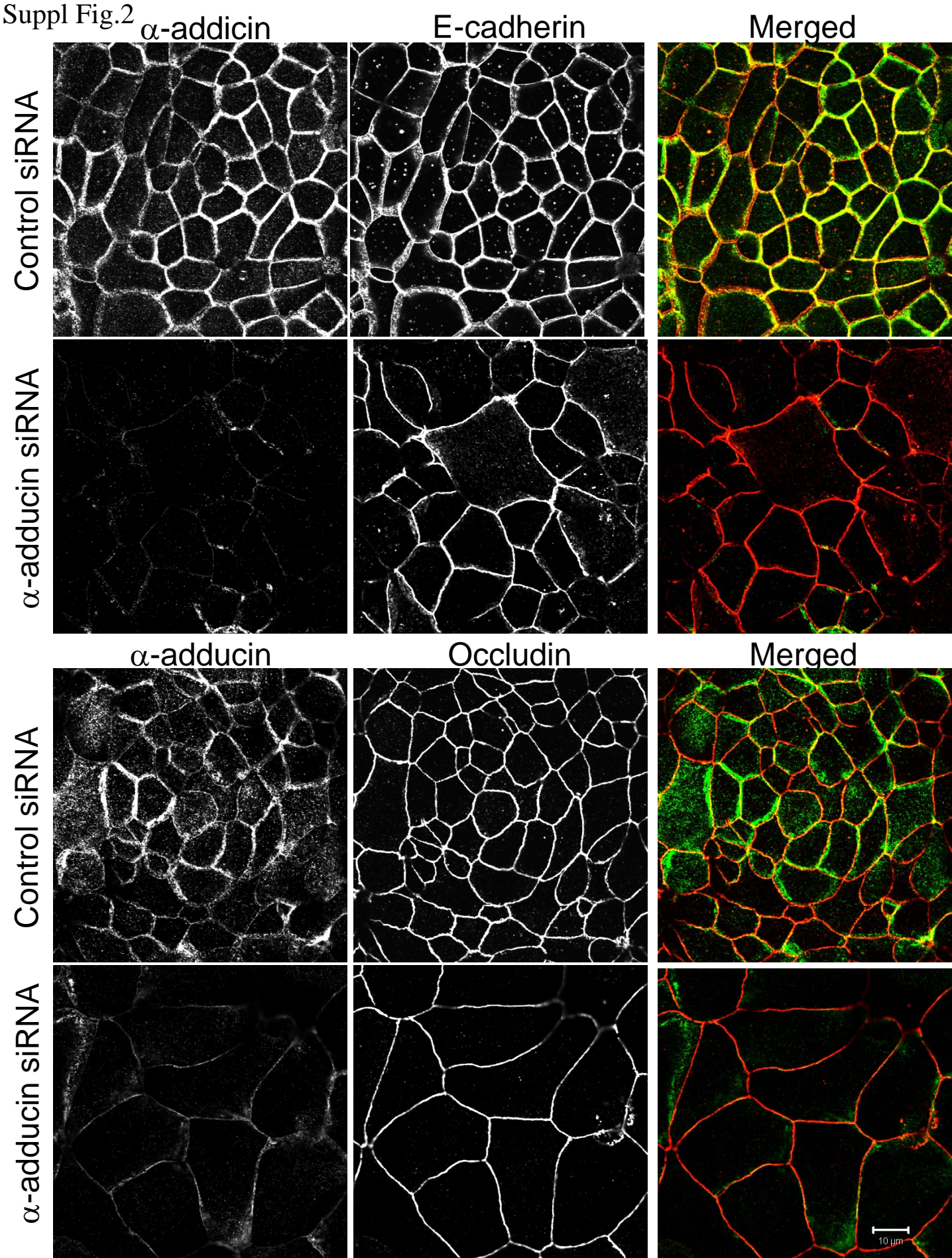
Supplemental Figure 4. Downregulation of α and γ adducin expression does not induce collapse of the lateral plasma membrane. (A) Representative xz images and (B) image quantification show that siRNA-mediated depletion of α -adducin does not effect the average height in confluent SK-CO15 cell monolayers, whereas γ -adducin depletion induces just a small (< 10%) decrease in the cell height. *p < 0.05 compared to control siRNA-treated cells (n = 20).

Supplemental Figure 5. siRNA-mediated depletion of α -adducin attenuates post-EGF reassembly of AJ. Serum-starved control and α -adducin-depleted A549 cells were exposed to EGF for 24 h followed by additional 24 h post-EGF recovery in a complete medium. Immunofluorescence labeling and confocal microscopy show reversible disassembly of β -catenin based AJ by the EGF treatment and complete restoration of AJ in control cells after EGF removal (arrows). This AJ recovery is attenuated in α -adducin-depleted A549 cells (arrowheads). Bar, 10 μ m.

Supplemental Figure 6. Downregulation of α and γ adducins does not affect mRNA level of β II-spectrin. A Real time RT-PCR analysis presented as individual amplification graphs (A) and the calculated normalized delta threshold cycle number (B), demonstrates that siRNA-mediated downregulation of α and γ adducins in SK-CO15 cells does not alter mRNA expression of β II-spectrin.

Supplemental Figure 7. Downregulation of γ -adducin does not affect organization of the perijunctional F-actin belt in stationary epithelial cells. SK-CO15 cells were transfected with either control or α -adducin-specific siRNAs and the integrity of their junction-associated actin cytoskeleton was examined by fluorescence labeling and confocal microscopy on day 4 post-transfection. Note normal organization of the perijunctional F-actin belt in confluent control and α -adducin depleted cell monolayers (arrows). Bar, 20 μ m.



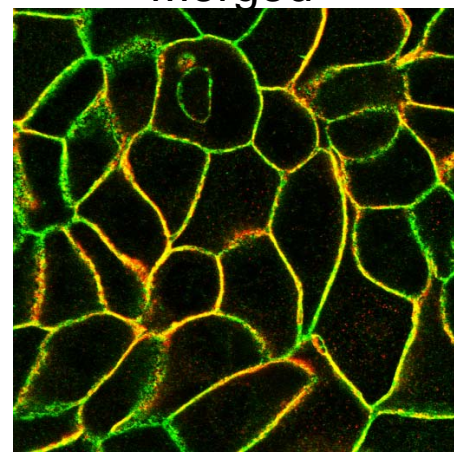
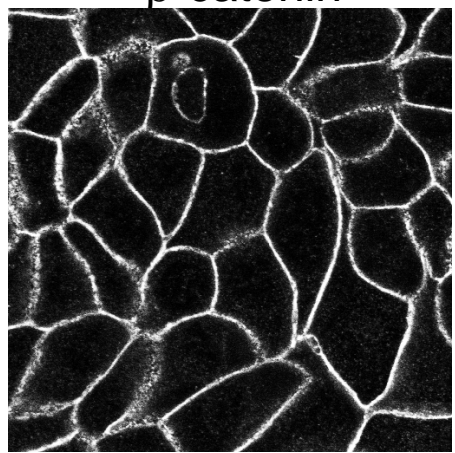
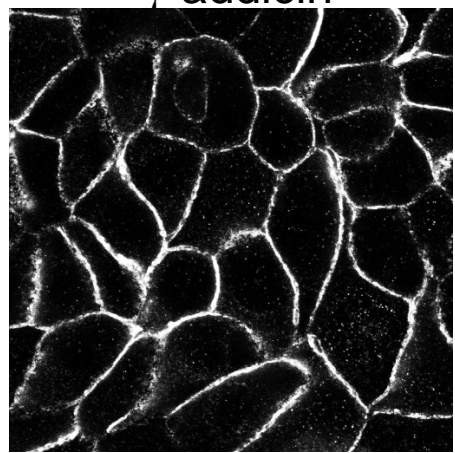


γ -adducin

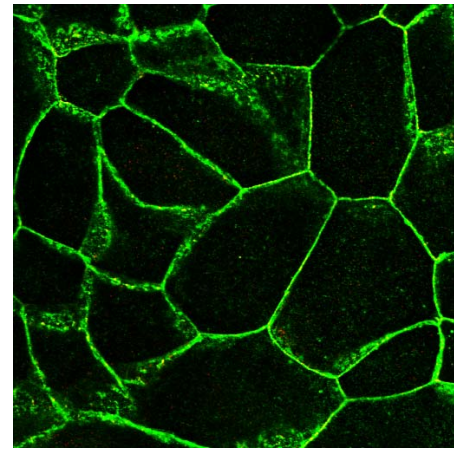
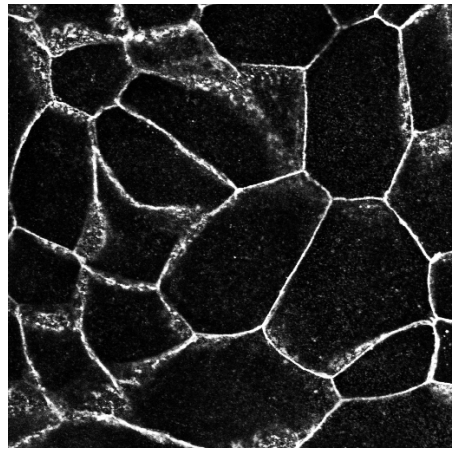
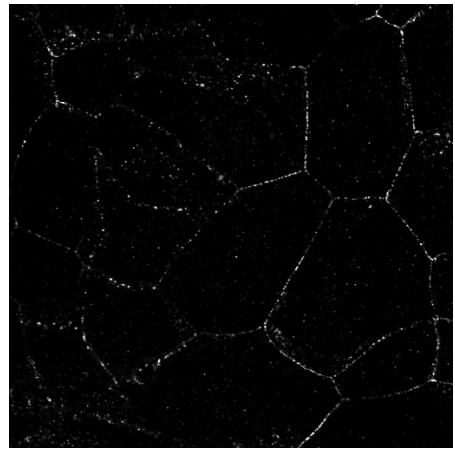
β -catenin

Merged

Control siRNA



γ -adducin siRNA

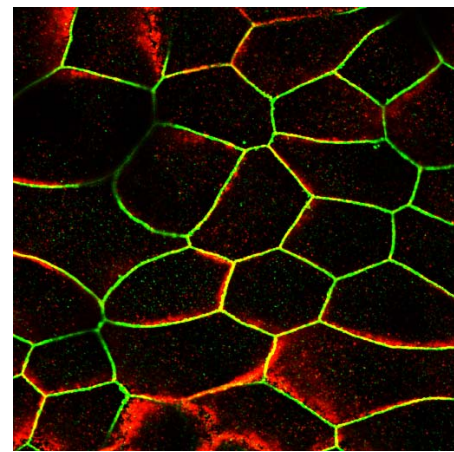
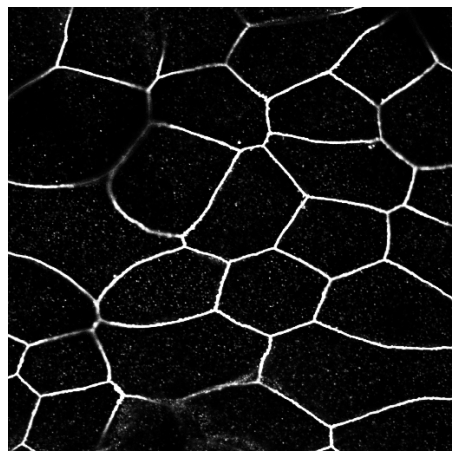
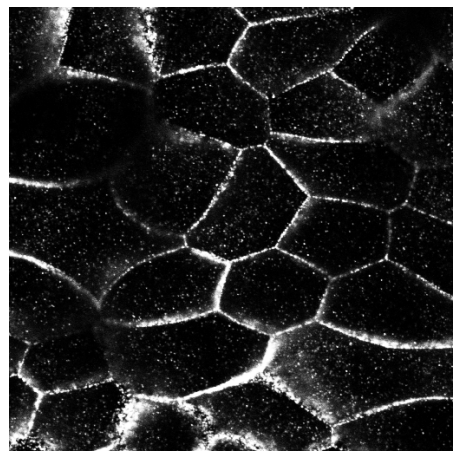


γ -adducin

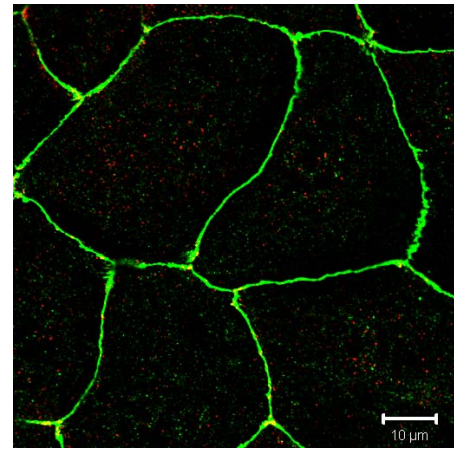
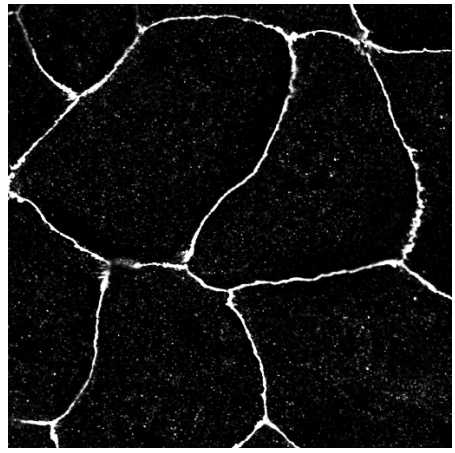
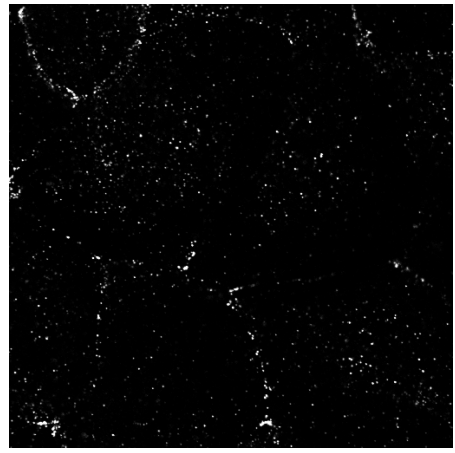
Occludin

Merged

Control siRNA



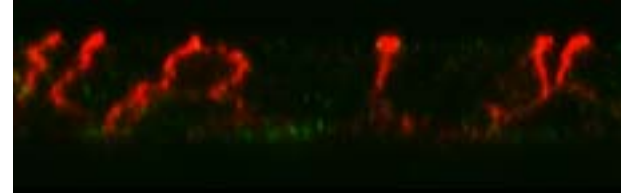
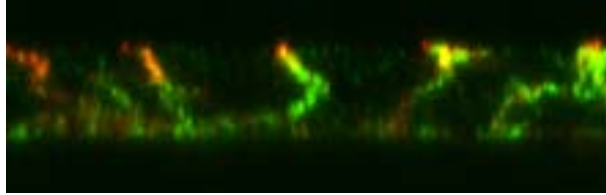
γ -adducin siRNA



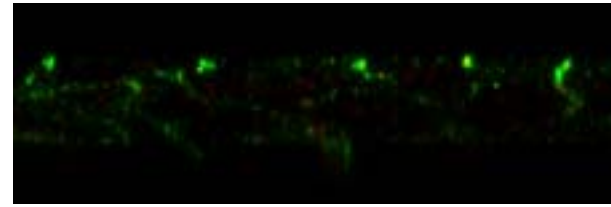
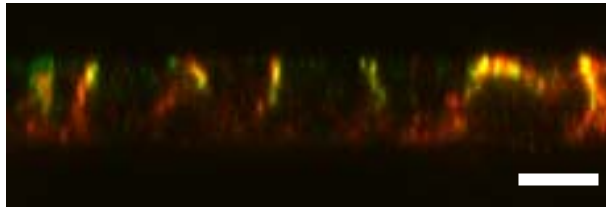
10 μ m

A

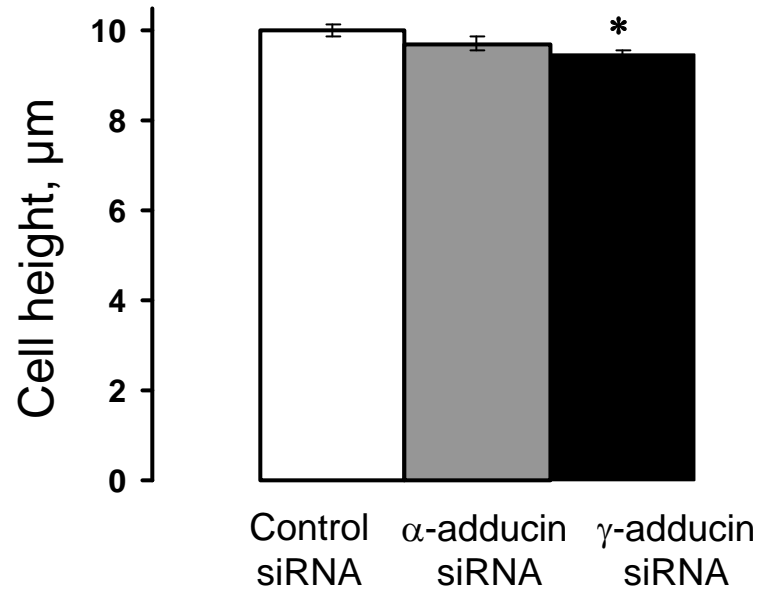
Control siRNA

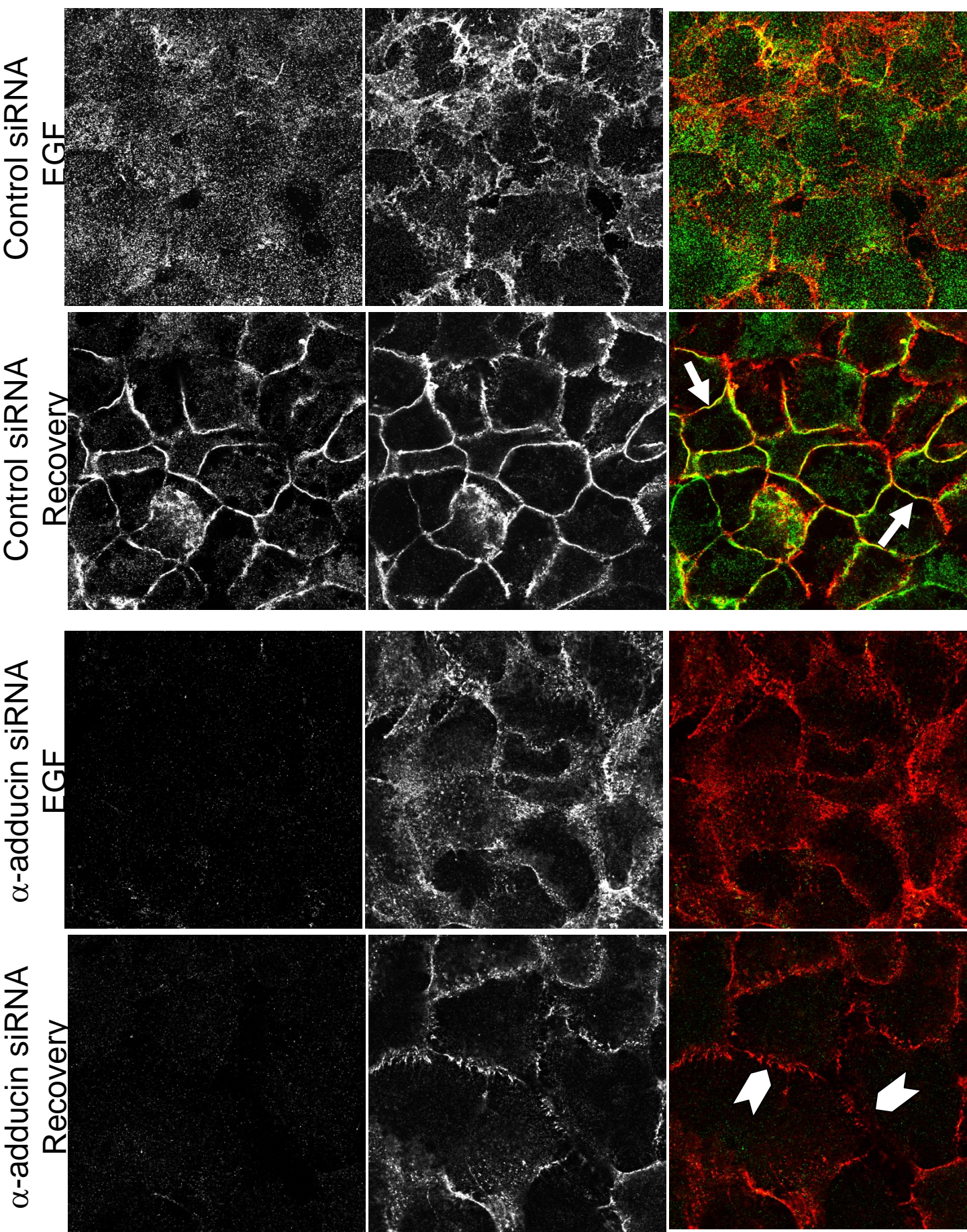
 α -adducin siRNA α -Adducin/
E-cadherin

Control siRNA

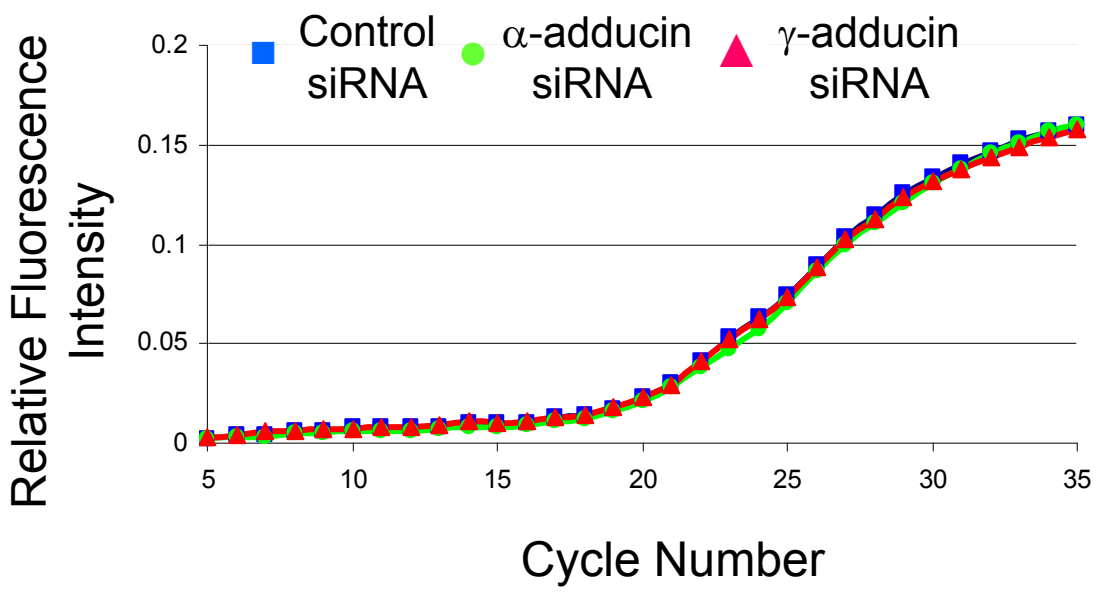
 γ -adducin siRNA γ -Adducin/
 β -cadherin

B





A β II-spectrin mRNA expression



B

