Supplementary Figure Legends

Supplementary Figure 1. Anti-N-terminal Sec3 antibody and an anti-V5 antibody recognize the immunogen. Increasing quantities (0.01, 0.025, 0.05, 0.075, 0.1, 0.5 μg) of the immunogen (Sec3NT-His/V5) were resolved by SDS-PAGE and transferred to Immobilon P membranes for Western blotting with anti-Sec3NT and anti-V5 antibodies. The increase in protein led to a linear increase in anti-Sec3NT and anti-V5 signal. Anti-Sec3 antibodies are more sensitive then the commercially available anti-V5 antibodies.

Supplementary Figure 2. Sec8 colocalizes with Sec3 at desmosomes. Polarized MDCK cells were pre-extracted in CSK, fixed in methanol and co-labeled with anti-Sec3CT and a cocktail of anti-Sec8 mAbs (2E9, 5C3, 7E8 and 17A10). Although a high level of nuclear background was observed following this approach, clear co-labeling of Sec3 and Sec8 within discontinuous puncta at the lateral plasma membrane was also detected (arrows).

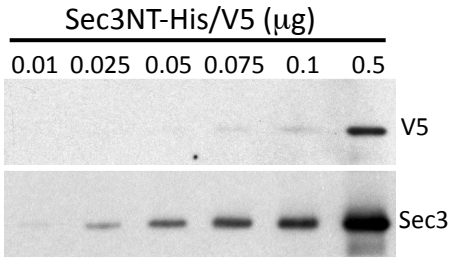
Supplementary Figure 3. Endogenous Exocyst subunits localize to distinct punctate structures at sites of cell-cell contact in A431 cells. A431 cells were fixed and immunolabeled with antibodies against Sec15, Exo70, and Exo84. This pattern is identical to anti-Sec3 immunolabeling in MDCK cells.

Supplementary Figure 4. Triton solubility of Exocyst subunits. (A) Triton-X-100 soluble and insoluble fractions were resolved by SDS-PAGE, and proteins were transferred to PVDF membranes for Western blotting with antibodies specific for Sec3, Sec5, Sec6, Sec8, Sec10,

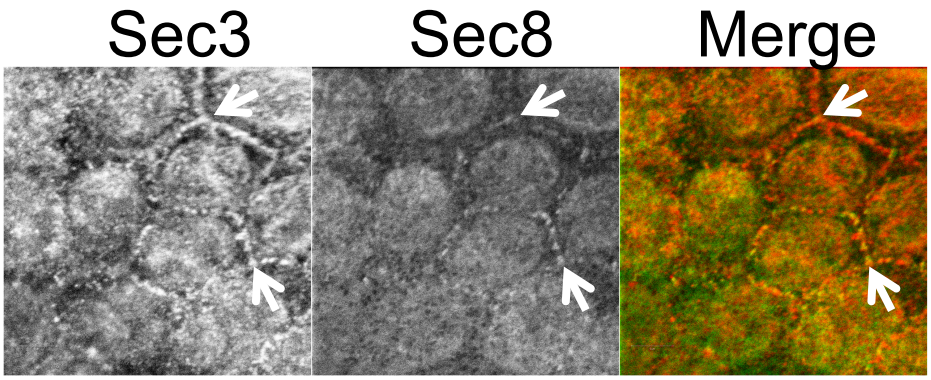
Sec15, Exo70, and Exo84. (B) Levels of protein recovered in Triton-X-100 soluble and insoluble fractions were quantified by phosphorimaging, and the results are presented as % of total.

Supplementary Figure 5. Sec3 knockdown selectively elevates expression of desmosomal proteins. Parental MCF-10A cells, or stably transduced MCF-10A cell lines expressing either shSec3 hairpin 1 or hairpin 2, or a control non-targeting hairpin (shControl) were assessed for expression of desmosomal and adherens junction proteins. RIPA lysates, in triplicate, were immunoblotted for indicated proteins. Note that steady state levels of each of the desmosomal cadherins (Dsg1, Dsg2 and Dsc2/3) were substantially elevated in shSec3 cells relative to controls. PG and DP1/2 levels were also increased in the Sec3 knockdown cells, but to a lesser extent. In contrast, levels of adherens junction components (E-cadherin and α-catenin) were not significantly altered.

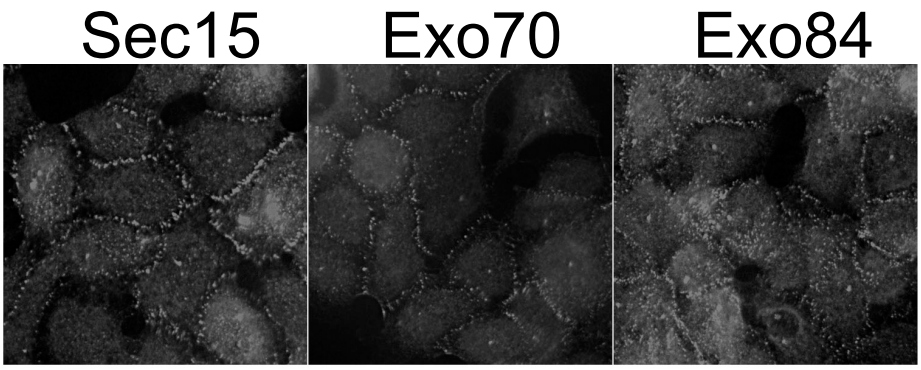
S1



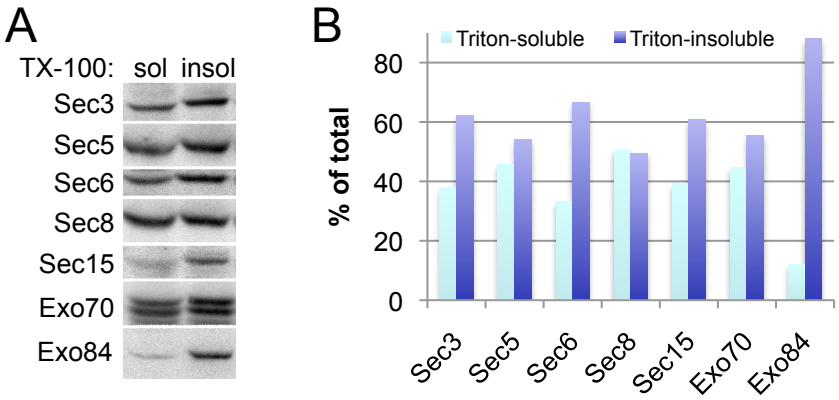
S2



S3

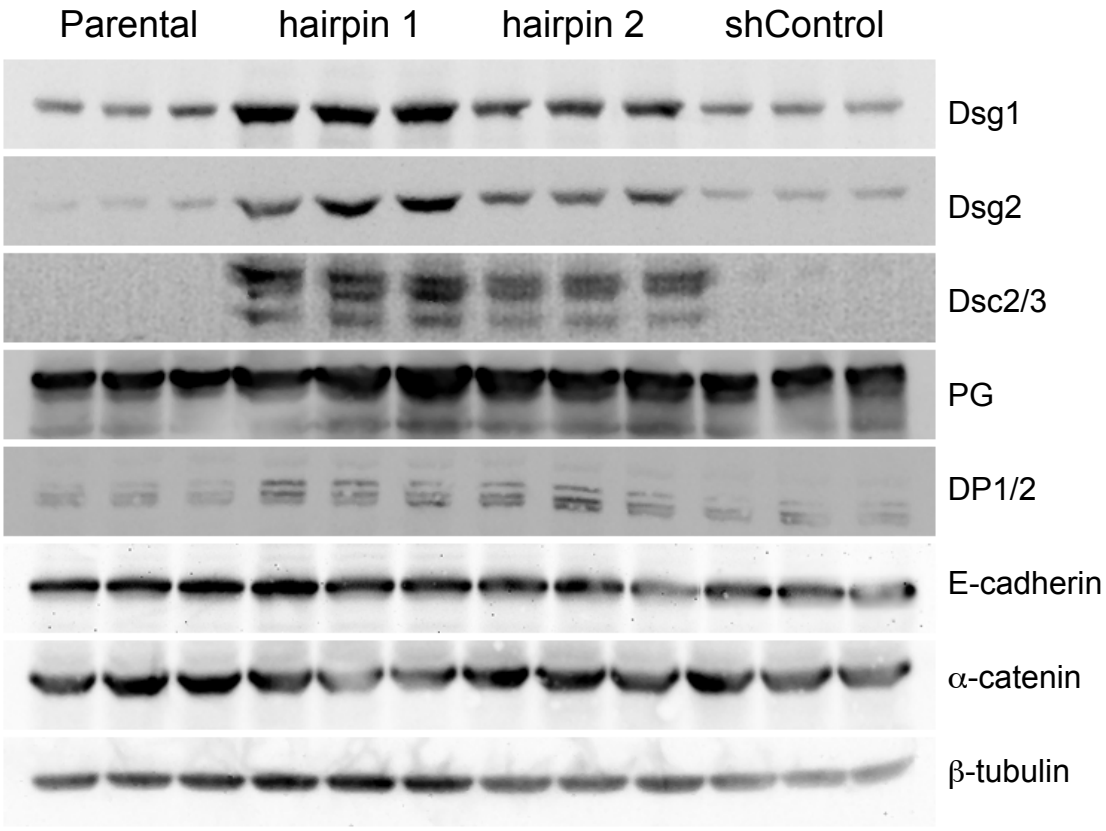


S4



Andersen & Yeaman, Supplemental Figures

S5


Parental
hairpin 1
hairpin 2
shControl
Dsg1
Dsg2
Dsc2/3
PG
DP1/2
E-cadherin
α-catenin
β-tubulin