Supplementary Figures

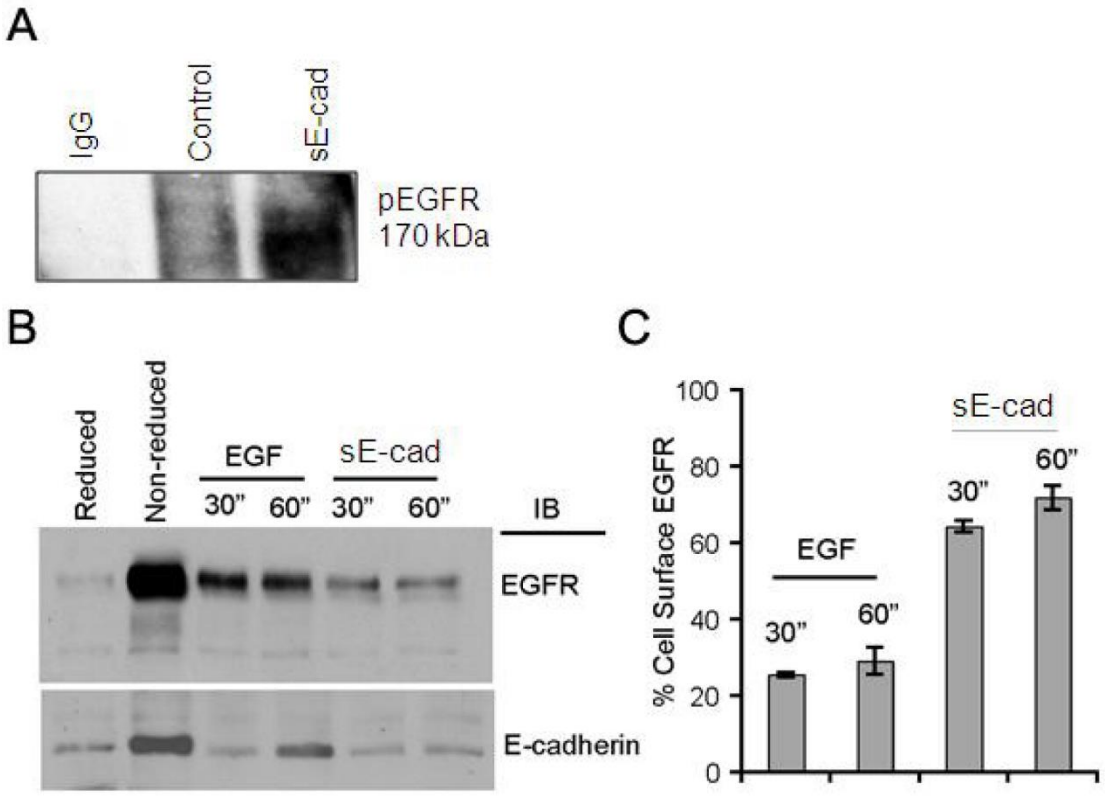


Fig. S1. sE-cad associates with EGFR and attenuates its internalization. A, Immunoblot showing pEGFR levels co-immunoprecipitated with sE-cad using an anti-myc antibody. Control lane: MDCK cells alone, sE-cad lane: Treatment with 10µg/ml sE-cad for 2 h. B, Serum starved MDCK cells were treated with sE-cad (10µg/ml) or EGF (10ng/ml) for indicated times after biotinlaytion as described in Materials and Methods. For controls, two plates were kept on ice and either reduced or non-reduced to determine the basal levels of internalization and total amount of cell surface protein. Samples were immunoprecipitated as described in Materials and Methods and immunoblotted for EGFR and E-cadherin. sE-cad reduces internalization of EGFR, compared to EGF. Blot represents data from two independent experiments. C, Quantification of % of cell surface EGFR. Amount of cell surface EGFR was calculated from blots. Bars represent standard error. Note sE-cad maintains EGFR on the cell surface.

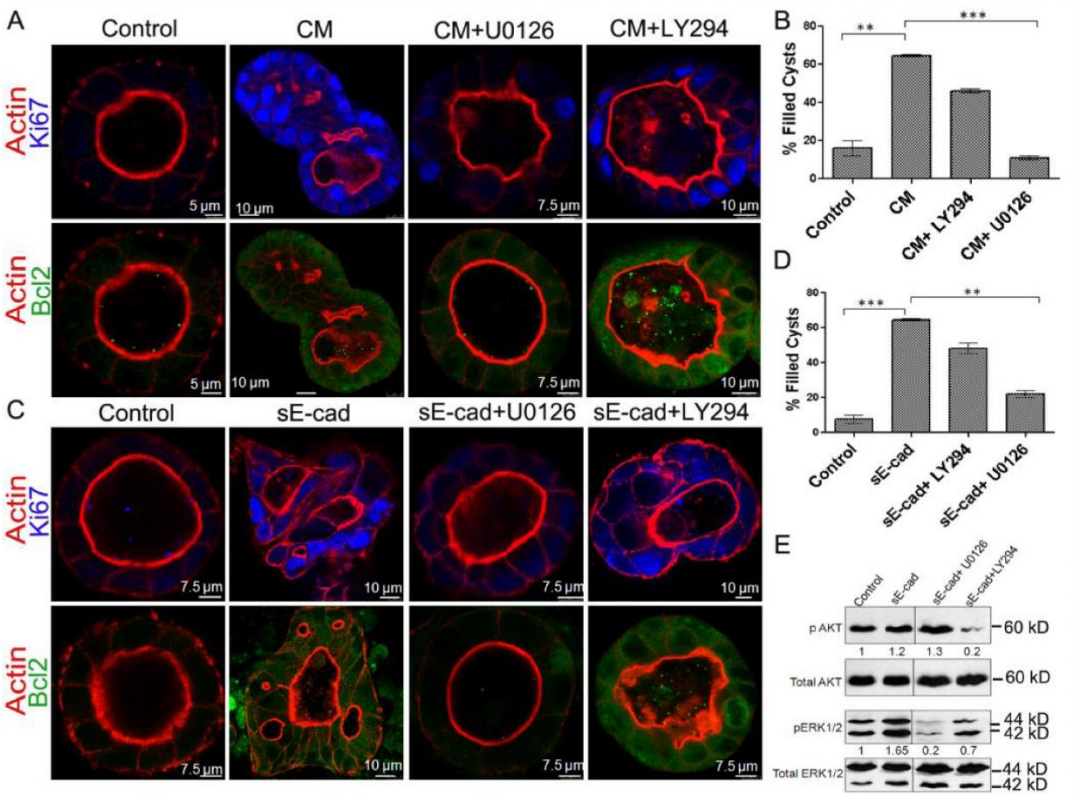


Fig. S2. ERK1/2 is involved in lumen filling in MDCK cysts. Immunofluorescence showing lumen filling in MDCK cysts treated with (A) CM or with sE-cad (C) in presence of 1 µM U0126 to inhibit MEK/ERK pathway or 1 µM LY294002 to block PI3K-AKT pathway at 48hr. Images were obtained from staining cysts with anti Bcl2 antibody(green), phalloidin-Alexa Fluor 546 (for actin, red) and Ki67 (blue). B & D, Quantification showing percent lumen filled cysts with CM and sE-cad treatment in presence of inhibitors from three independent experiments. One hundred cysts under each condition were analyzed for the presence of hollow lumen and filled lumens. Error bars represent standard error mean. **P<.01, *** P<.005. E, Representative immunoblot from two independent experiments showing phosho ERK 1/2, phospho AKT and total ERK1/2 and total AKT levels in cysts treated with sE-cad in presence of inhibitors. (U0126 and LY294002 were used at 1 µM concentration)

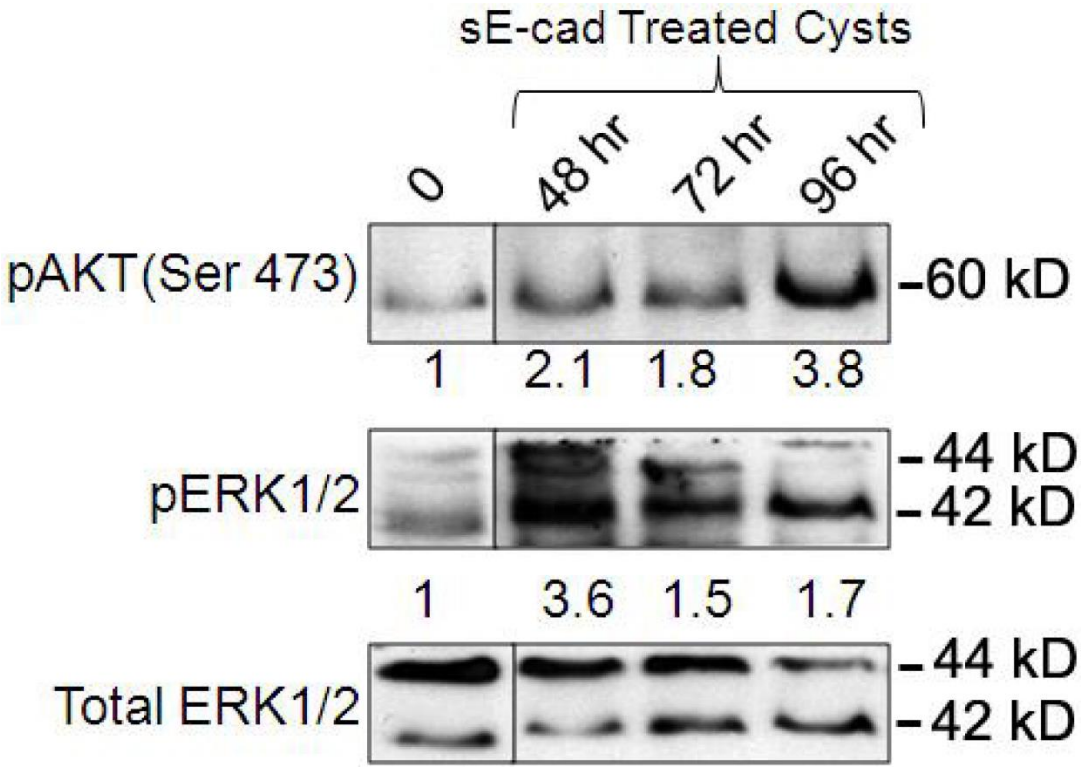
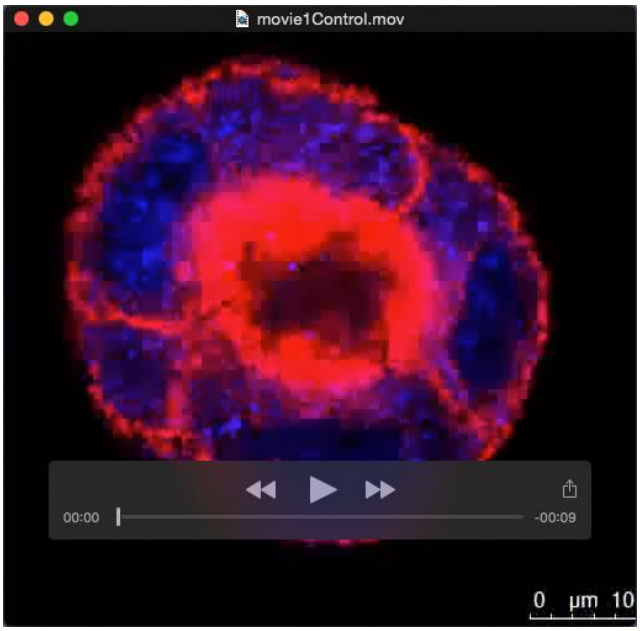
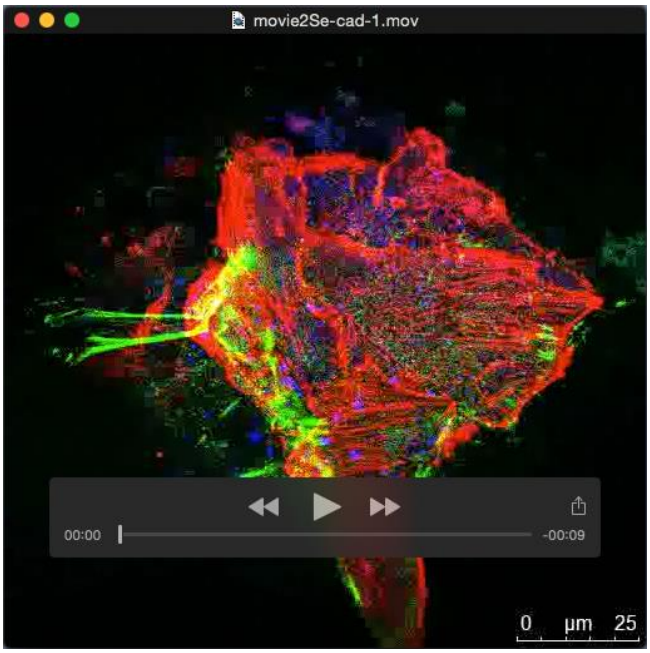


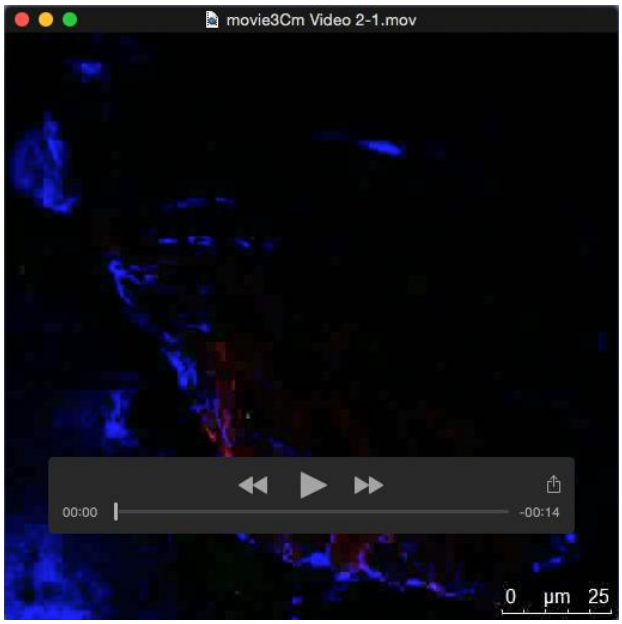
Fig. S3. Immunoblot showing pAKT, pERK1/2 and total ERK1/2 in sE-cad treated cysts at 48, 72 and 96 h.



Movie 1. Avi file showing z-stacks of control. Cysts were stained for N-cadherin (blue), fibronectin (green) and Actin (red).



Movie 2. Avi file showing z-stacks of cysts treated with sE-cad for 96 h. Cysts were stained for N-cadherin (blue), fibronectin (green) and Actin (red).



Movie 3. Avi file showing z-stacks of cysts treated with CM for 96 h. Cysts were stained for N-cadherin (green), fibronectin (blue) and Actin (red).