

Supplementary figures (Tushir and D'Souza-Schorey)

Supplemental figure 1: Expression of ARF6-GTP prevents the development of mature tubules whereas ARF6-GDP mutant expression blocks tubule initiation. MDCK^{ARF6-Q} cells and MDCK^{ARF6-T} cells were grown in 3D to form fully developed cysts and then induced for HA-tagged mutant ARF6 expression by removal of Dox from the growth medium followed by exposure to HGF to promote tubulogenesis. At various time points (days) post HGF treatment cell cultures were fixed and labeled for HA (red) and actin (green). Cell nuclei are labeled blue. ARF6-Q67L expression enhances tubule initiation and is accompanied by the formation of multiple tubules, but prevents tubule maturation. At later time points post HGF treatment, tubules on MDCK^{ARF6-Q} cysts disintegrate. ARF6-T27N expression completely blocks tubule initiation.

Supplemental figure 2: Quantitation of ARF6-Q67L and ARF6-T27N distribution on internal and surface membranes of epithelial cysts. Quantitation of internal and membrane labeling of ARF6-Q67L and ARF6-T27N was performed using the ImageJ program (<http://rsb.info.nih.gov/ij/>). Each image was converted to an 8-bit (grayscale) format and subjected to automatic thresholding, which converts grayscale images into binary (black and white) mode using a cutoff value established by algorithms (Ridler and Calvard, 1978). For comparison purposes, images of the both ARF6-Q67L and ARF6-T27N expressing cysts were analyzed under constant acquisition settings. The best-fit lower threshold to eliminate most of the signal background was determined using the threshold tool and confirmed by visual inspection and count of one-pixel dimension particles. In some cases, the same ARF6-Q67L or ARF6-T27N expressing cysts were analyzed at different lower thresholds to determine the best fit. Greater than 50 cysts were analyzed for each mutant protein and the data is shown \pm SD (error bars).

Supplemental figure 3. Constitutive activation of ARF6 alone promotes ERK activation during tubule initiation. Cysts formed by 3D culture of MDCK^{ARF6-Q} cells were induced for ARF6 mutant expression. At various time points post induction, cysts were collected, lysed, quantitated for protein content, followed by resolving equal amounts of lysates by SDS PAGE. Gels were probed for ERK and phospho-ERK labeling using western blotting procedures.

Supplemental figure 4: Quantitation of Rac1 distribution on internal and surface membranes of MDCK^{ARF6-Q} and MDCK^{ARF6-T} cysts. Quantitation of internal and surface membrane labeling of Rac1 in cysts expressing ARF6-Q67L and ARF6-T27N mutants was performed using the ImageJ program (<http://rsb.info.nih.gov/ij/>) as described above (see legend to supplemental figure 3). Greater than 50 cysts were analyzed for each experimental condition and the data is shown \pm SD (error bars).

Supplemental figure 5: c-Met levels are unchanged in MDCK^{ARF6-Q} and MDCK^{ARF6-T} cysts. Parental MDCK cells, MDCK^{ARF6-Q} cells and MDCK^{ARF6-T} cells were grown in 3D to form fully developed cysts and then induced for HA-tagged mutant ARF6 expression by removal of Dox from the growth medium. At 2 days post induction, cysts were collected, lysed, quantitated for protein content, followed by resolving equal amounts of lysates by SDS PAGE. Gels were probed for c-Met and α -tubulin using western blotting procedures.

Supplemental figure 6: ARF6-GTP-induced E-cadherin internalization during HGF-induced tubulogenesis is dependent on ERK. MDCK^{ARF6-Q} cells and MDCK^{ARF6-T} cells were grown in 3D to form fully developed cysts and then induced for HA-tagged mutant ARF6 expression by removal of Dox from the growth medium followed by exposure to HGF in the presence or absence of the MEK inhibitor, PD98059. 2 days post HGF treatment cell cultures were fixed and labeled for E-cadherin (red) and actin (green). Cell nuclei are labeled blue. Increased internal E-cadherin is observed in MDCK^{ARF6-Q} cysts, a process that is

blocked in the presence of PD98059. Little to no internalized cadherin is detected in MDCK^{ARF6-T} cysts.

Supplemental figure 7: Localization of Rac1 distribution in MDCK^{ARF6-Q} and MDCK^{ARF6-T} cysts. Cysts formed from MDCK^{ARF6-Q} cells and MDCK^{ARF6-T} cells were exposed to HGF to form tubules. Cultures were fixed and labeled for Rac1 (red), and cell nuclei (blue). Only merged images are shown. Bar = 10 μ m.

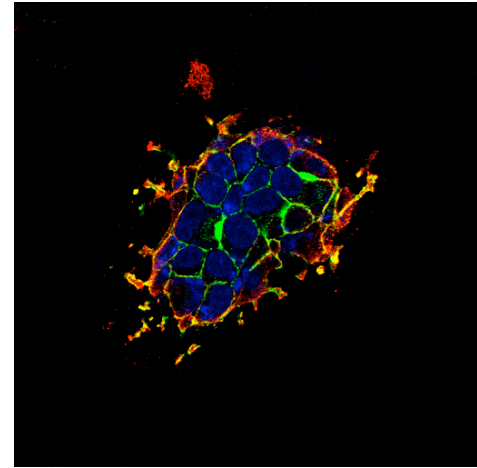
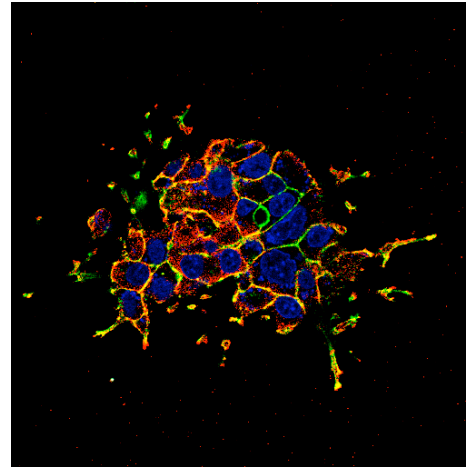
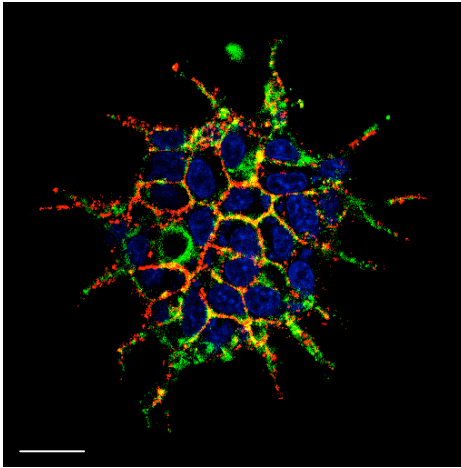
Days post HGF
treatment

2

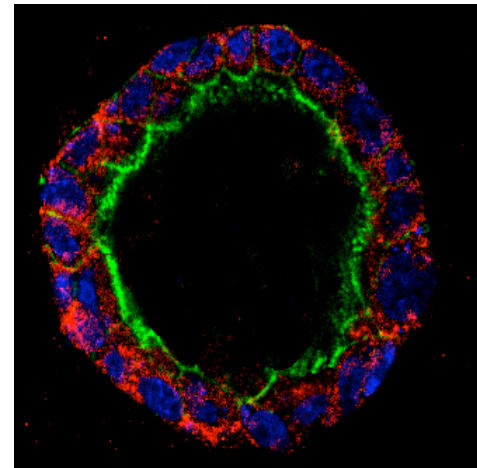
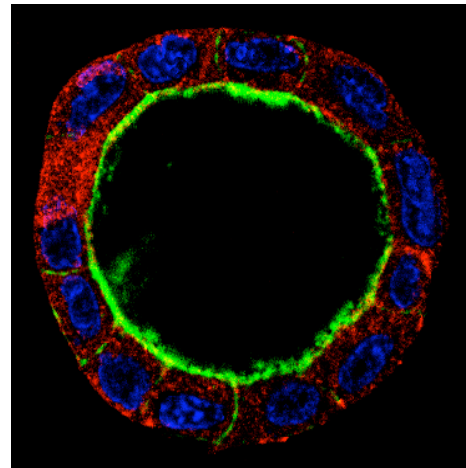
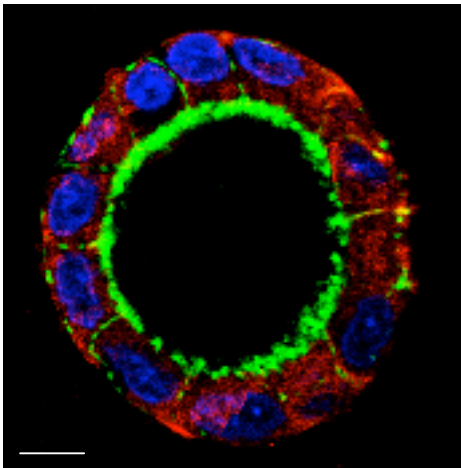
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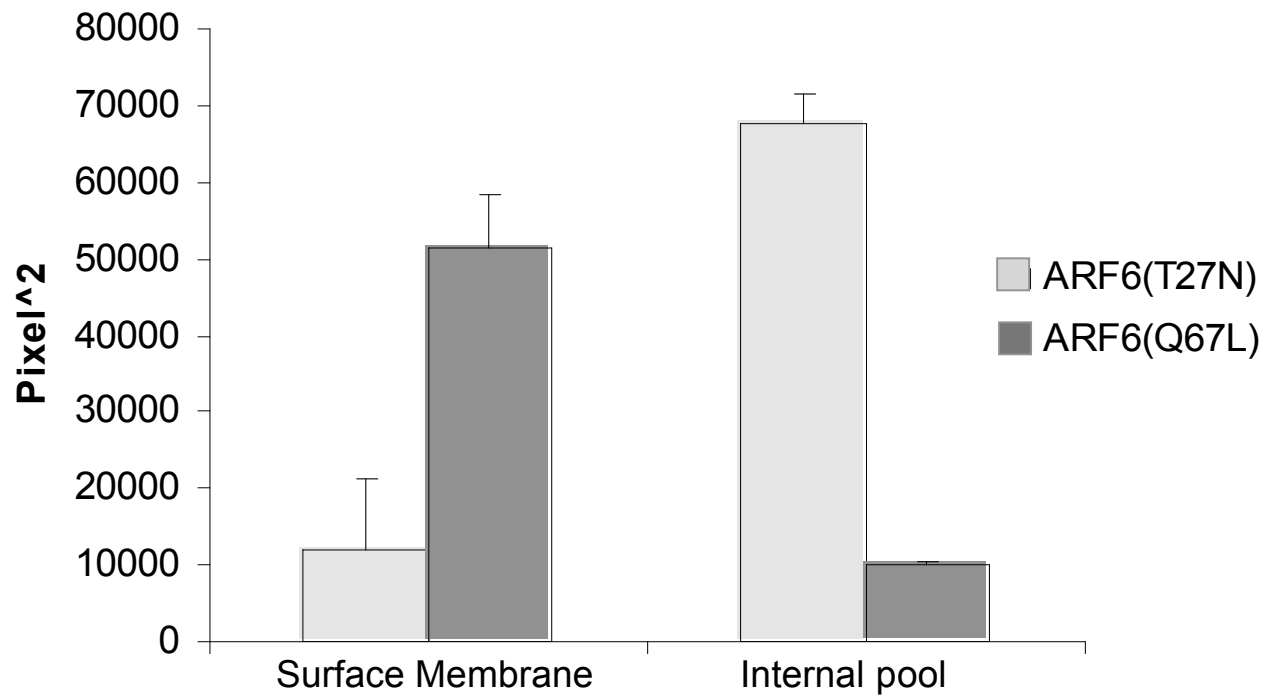
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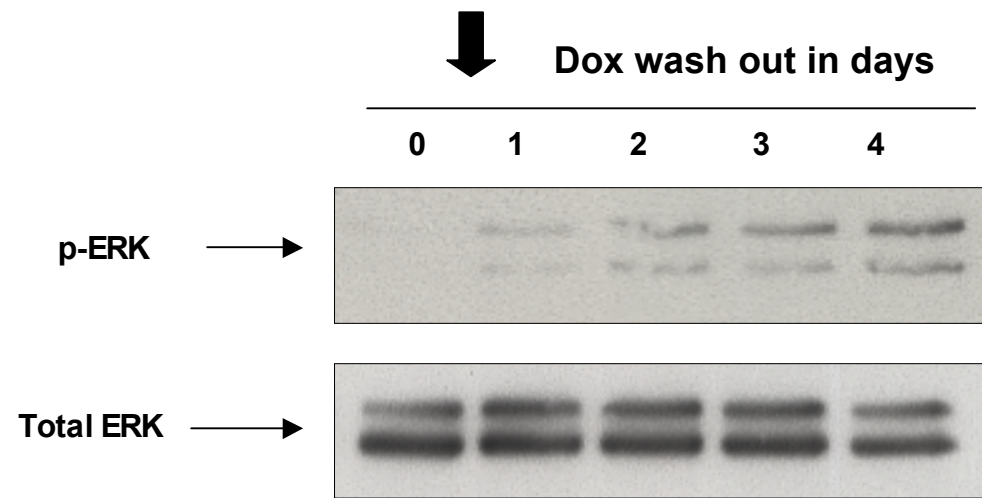
ARF6(Q67L)

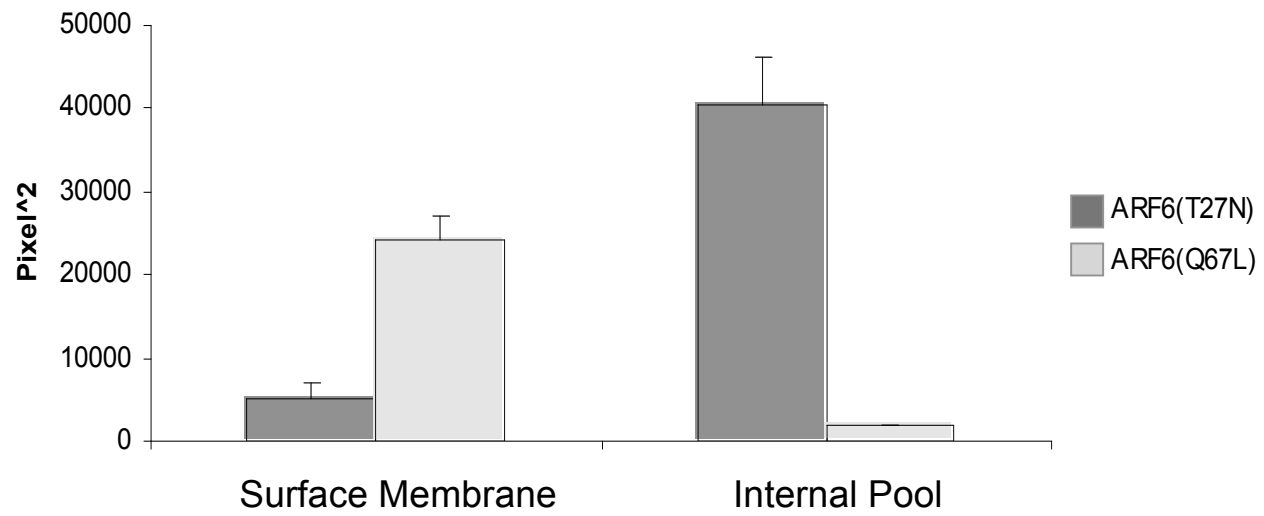


ARF6(T27N)

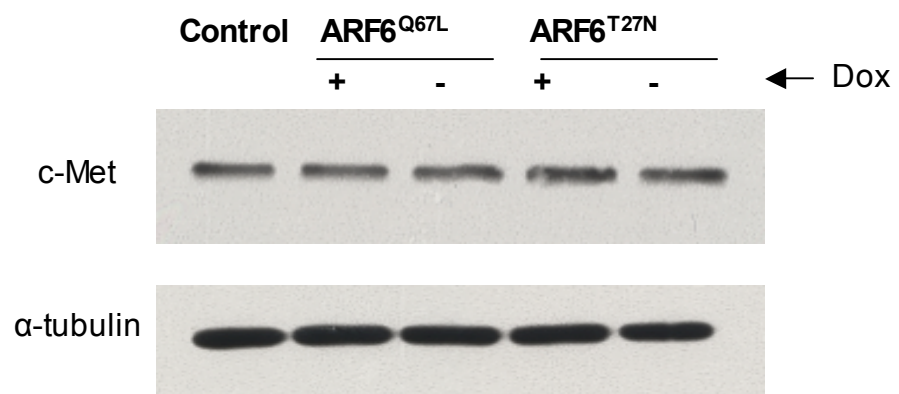


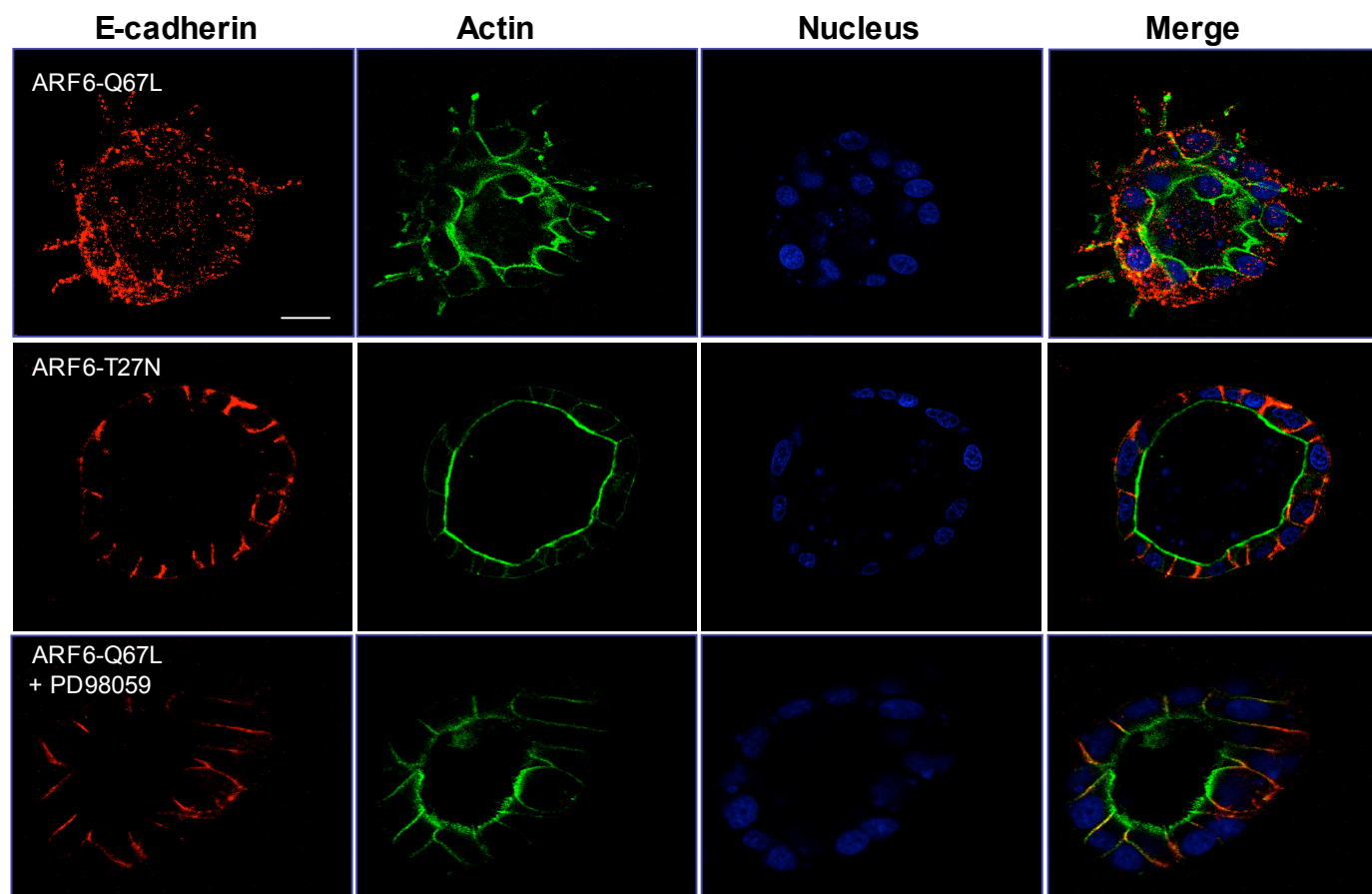






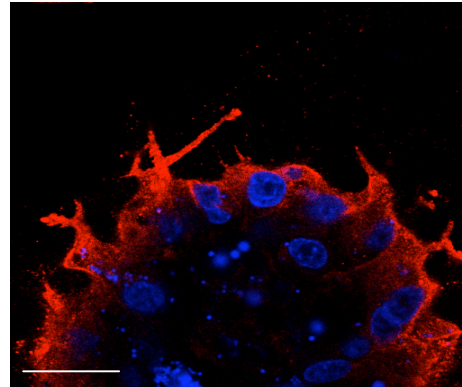
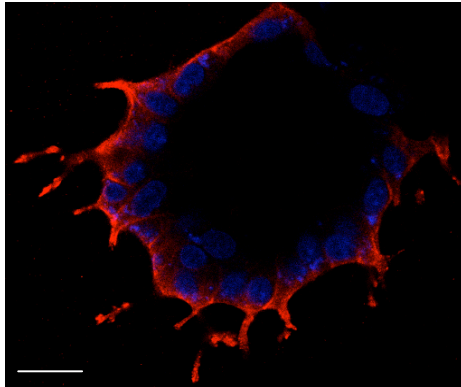
Tushir and D'Souza-Schorey, Supplemental figure 4





Tushir and D'Souza-Schorey, Supplemental figure 6

ARF6-Q67L



ARF6-T27N

