Supporting Information

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SI Methods

Measurement of Colocalization in Immunolabeled Sections of Uroepithelium. Stacks of dual-labeled confocal sections were imported into Volocity (PerkinElmer), background noise was removed by using the fine (3 × 3) median noise reduction filter, and a scatter plot of green and red pixel intensities (associated with each of the two markers in question) was generated by using the colocalization function. Regions of the image that contained no visual signal were selected and used to threshold the image. Colocalization coefficients for each of two markers (M_x and M_y, respectively) were calculated for the entire image by using the following equations (1):

$$M_x = \frac{\Sigma_i X_{i,colocalized}}{\Sigma_i X_i}$$

$$M_Y = \frac{\Sigma_i Y_{i,colocalized}}{\Sigma_i Y_i},$$

where X_1 is equal to the intensity of marker X at a given pixel and $X_{\text{i,colocalized}} = X_\text{i}$ if the associated intensity of the other marker (Y_i) is above the threshold value (i.e., it colocalizes). However, $X_{\text{i,colocalized}} = 0$ if Y_i is below the threshold value (i.e., does not colocalize). Y_i is equal to the intensity of marker Y at a given pixel and $Y_{\text{i,colocalized}} = Y_\text{i}$ if X_i is above the threshold value, and $Y_{\text{i,colocalized}} = 0$ if X_i is below the threshold value. An M_x or M_y value of 1.0 indicates 100% colocalization, while a value of 0.0 indicates no colocalization. The values for M_x and M_y can be similar or not, as one marker may have a broader distribution than the other in the sampled region of the tissue.

Preparation of Adenoviruses and Infection of Umbrella cells *in Situ*. Rats were anesthetized with 5% (vol/vol) halothane, and then kept under halothane (2% vol/vol) anesthesia for the subsequent steps. A 22-gauge catheter was passed through the urethra into the bladder, and residual urine was emptied. The bladder was subsequently washed with 450 μl PBS three times, and then treated with 450 μl of 0.1% (wt/vol) dodecyl-β-D-maltoside (DDM) dissolved in PBS for 5 min as described (2). For capacitance studies, adenoviruses encoding DA-Rab11a (2.5×10^8 infectious virus particles; ivp), DN-Rab11a (2.25×10^8 ivp), or GFP (2.1×10^8 ivp) were diluted in 450 μl of PBS containing 5×10^7 ivp of adenovirus encoding the tetracycline-repressible transactivator (TA) (3), and then instilled in the bladder. After a 30-min incubation, the bladder was washed three times with PBS, and the rats were allowed to revive. After 24 h the animals were euthanized, and the rat bladder was removed for further study. For studies examining secretion of hGH the rat bladder was first infected with adenovirus expressing hGH, and then after 18–20 h infected with adenovirus expressing GFP, DA- or DN-Rab11a with TA. After an additional 24 h, the bladders were filled and hGH release was measured as described below.

Mounting of Rat Bladder Uroepithelium in Ussing Stretch Chambers and Measurements C_T. Excised rat bladders were cut open and pinned mucosal side up onto a rubber sheet, and a plastic tissue ring with an exposed tissue area of 0.65 cm^2 and sharp pins at the edges were placed onto the tissue. The tissue was transferred to the ring, the ring was mounted in an Ussing stretch chamber (4, 5), and the tissue was equilibrated for 1 h in Kreb's buffer (100 mM NaCl, 5.8 mM KCl, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 2.0 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 11.1 mM glucose) gassed with 95% (vol/vol) air/ 5% (vol/vol) CO_2. To simulate bladder filling, Kreb's buffer was slowly added to the mucosal hemichamber at 25 μl/min using a NE-1600 pump (New Era Pump Systems), to fill the chamber to its capacity. The chamber was then sealed, and an additional 0.25 ml of Krebs solution was added at the same rate of infusion to further stimulate mechanical stretch across the apical plasma membrane of the umbrella cell layer. C_T was measured as described (4, 6); however, the voltage response was fit to a single exponential by using PRISM software (GraphPad). The fits had R^2 values of ≥0.98.

Uptake of FITC-WGA, FITC-Con A, or FITC-Dextran. Rats were anesthetized and catheterized as described above. The bladder was washed with 150 μl of PBS^+ (PBS containing 1 mM $MgCl_2$ and 1 mM $CaCl_2$) three times, and then filled with 550 μl of PBS^+ supplemented with either 25 μg/ml FITC-WGA (Vector Laboratories), 20 μg/ml FITC-Con A (Vector Laboratories), or 2.5 mg/ml FITC-dextran. The bladder was clamped for 30 min and then released to allow for a voiding response. The bladder was washed twice with 150 μl of buffer, excised, cut open, and incubated in Kreb's buffer for up to 45 min at 37°C. The tissue was then washed 3 × 30 min on a shaker at 4°C with either 50 mM *N*-acetyl glucosamine (FITC-WGA), or both 200 mM α-methyl mannoside and 200 mM α-methyl glucoside (FITC-Con A) to remove cell surface-bound fluorescent marker and then fixed with 4% paraformaldehyde as described above. The tissue incubated with FITC-dextran was washed 3 × 5 min with Kreb's buffer and fixed in periodate-lysine-paraformaldehyde, pH 7.4, for 2 h at 37°C.

Estimation of hGH Secretion. Rats infected with adenoviruses were anesthetized, a lower ventral incision was made, and the ureters were isolated and dissected free from the surrounding connective tissue. A loop of silk suture thread was placed around the ureters and tightly knotted, the abdomen was closed, and the bladder was catheterized and emptied by carefully pressing the lower abdomen. The bladder was washed three times with 150 μl of Kreb's buffer, and slowly filled with 550 μl of Kreb's buffer over 2–3 min, and then sealed. In a separate experiment, we determined that no hGH was released during the initial PBS washes. At the indicated time, the bladder contents were removed by manual compression of the abdomen, the bladder was washed in 2 × 150 μl of Kreb's buffer, and the original solution and washes were pooled. In some experiments the bladder was refilled and incubated for the indicated time, and the released solution and washes were also pooled. The empty bladder was then excised and cut open, and the uroepithelium was then collected by scraping and lysed in SDS-lysis buffer [0.5% (wt/vol) SDS, 5 mM EDTA, 100 mM NaCl, and 50 mM triethanolamine-HCl, pH 8.6]. Cellular material and membranes were removed from the hGH-containing Kreb's buffer by ultracentrifugation at 100,000 × *g* for 1 h, and the secretory proteins in the resulting supernatant were precipitated with ice-cold 10% (vol/vol) trichloroacetic acid for 30 min at 4°C. After centrifugation for 10 min at 20,000 × *g* at 4°C, the pellet was washed with ice-cold acetone at 4°C, and then incubated at room temperature until dry. Aliquots of the hGH-rich pellet or uroepithelial lysate were resuspended in Laemmli loading buffer, the proteins were resolved by 15% SDS/PAGE, hGH was detected by immunoblot, and the relative amount of hGH released versus cell associated was quantified by densitometry using Quantity One software (Bio-Rad).

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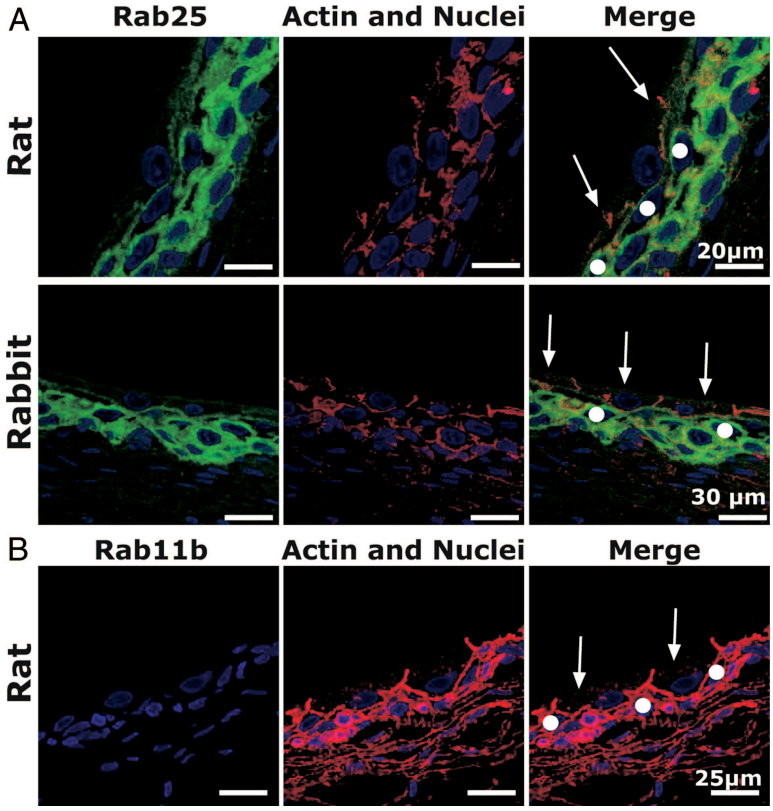

Fig. S1. Expression of Rab25, but not Rab11b, in uroepithelium. (*A*) Distribution of Rab25 in rat and rabbit uroepithelium. (*B*) Distribution of Rab11b in rat uroepithelium. All images are projections of confocal optical sections. Rab25 and Rab11b are shown in green, nuclei in blue, and actin in red. Arrows indicate umbrella cells, and solid circles indicate the first layer of intermediate cells.

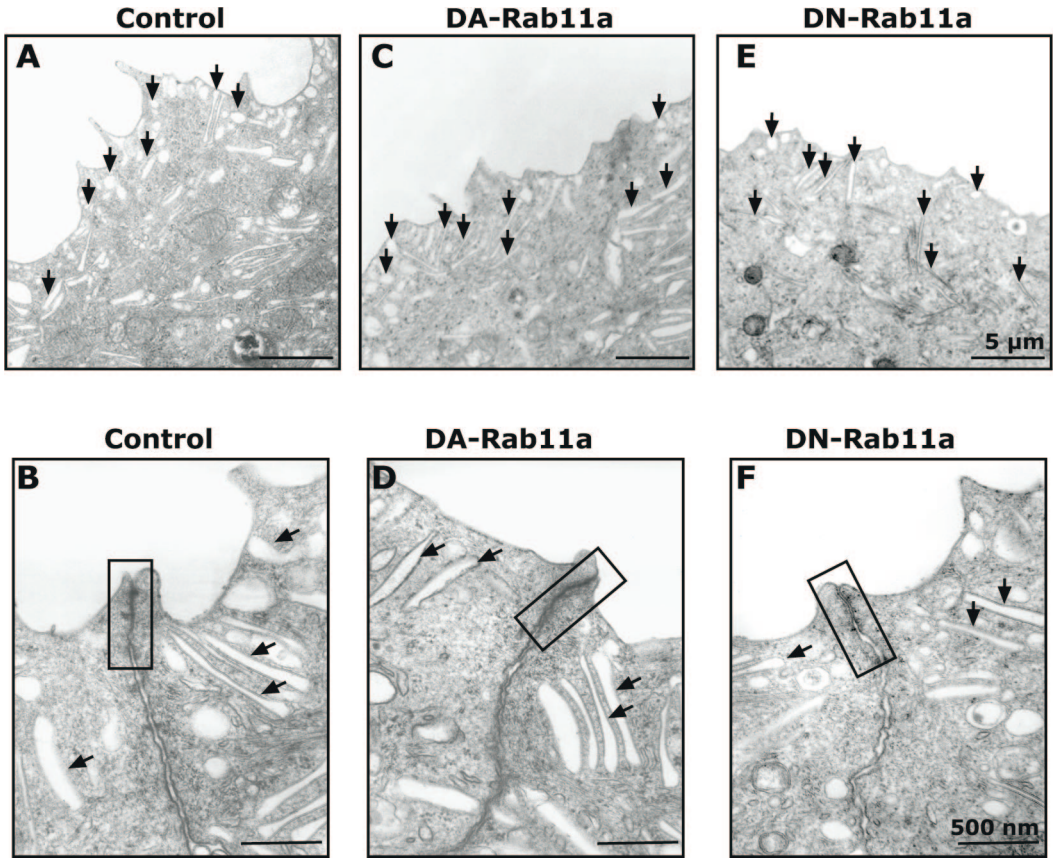

Fig. S2. Ultrastructure of control umbrella cells or those infected with adenovirus expressing DA- or DN-Rab11a. Examples of untreated control tissue (*A* and *B*), or tissue that was infected with adenoviruses expressing DA-Rab11a (*C* and *D*) or DN-Rab11a (*E* and *F*). Several blocks of tissue from different animals were examined, and representative images are shown. Both control tissues or those infected with adenovirus showed a single layer of umbrella cells that contained DFV (examples of which are indicated by arrows) and had intact junctional complexes (enclosed by boxes).

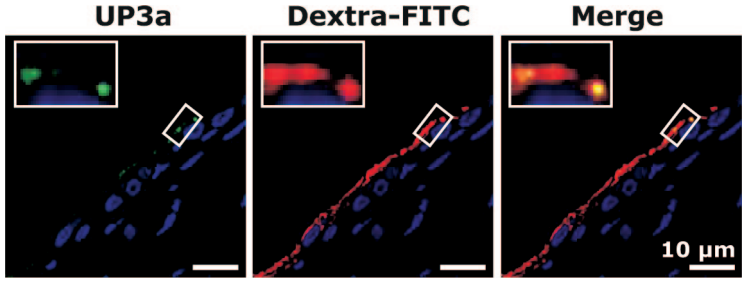

Fig. S3. Localization of internalized FITC-dextran and UP3a in bladder umbrella cells. Bladders were filled with FITC-dextran, the marker was released, and the tissue was excised, fixed, cryosectioned, and then immunolabeled. The distributions of UP3a (red), FITC-Dextran (green), and nuclei (blue) are shown in cross-sections of rat uroepithelium. Images are projections of confocal optical sections; boxed regions are magnified in the *Insets*.

Table S1. Colocalization coefficients for various markers

Marker	Endogenous Rab11a
Rab11a and UP3a*	
M_X (Rab11a colocalizing with UP3a)	0.89 ± 0.03
M_Y (UP3a colocalizing with Rab11a)	0.83 ± 0.07
M_X (DA-Rab11a colocalizing with UP3a)	0.89 ± 0.02
M_Y (UP3a colocalizing with DA-Rab11a)	0.85 ± 0.03
M_X (DN-Rab11a colocalizing with UP3a)	0.87 ± 0.05
M_Y (UP3a colocalizing with DN-Rab11a)	0.83 ± 0.04
hGH and endogenous Rab11a	
M_X (hGH colocalizing with Rab11a)	1.0 ± 0.0
M_Y (Rab11a colocalizing with hGH)	0.69 ± 0.01
hGH and UP3a	
M_X (hGH colocalizing with UP3a)	0.94 ± 0.02
M_Y (UP3a colocalizing with hGH)	0.56 ± 0.03

Data are mean ± SEM ($n \geq 4$).
*There is no significant difference between any of these values ($P > 0.05$).