

Newly Synthesized Polycystin 1 Takes Different Trafficking Pathways to the Apical and Ciliary Membranes

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Supplementary figure legend

Figure S1: The PC1 N terminal fragment can be recovered from the stripping medium. Western blot analysis demonstrates that the PC1 N-terminal fragment (NTF) can be immunoprecipitated from the medium after stripping, thus confirming that alkaline pH induces the release of the NTF.

Figure S2: Alkaline pH does not affect protein trafficking to the cell surface. Cells were incubated in stripping (pH9.5) or control (pH7.4) medium, followed by labeling with WGA-594 (red) to label a broad cross-section of cell surface glycoproteins. Samples were then incubated with WGA-488 (green) immediately after the first labeling step or following intervals of recovery at 37°C. The images show that immediately after the WGA-594 staining, most of the binding sites for WGA are saturated and cannot be stained by WGA-488. After a 3-hour recovery period, however, WGA-488 staining is visible in both conditions, suggesting that new glyco proteins were delivered to the cell surface. Moreover, similar amounts of internalized WGA-594 (bright red puncta) can be observed under both conditions, further suggesting that recycling of membrane proteins is also not affected by the high pH treatment.

Supplementary methods

Immunoprecipitation

Confluent untransfected (N.T.) and PC1+PC2 LLCPK were incubated in stripping medium (pH 9.5) and control medium (pH 7.4) plus protease inhibitor cocktail (Roche) for 45 minutes at 4°C. After stripping, media were collected, centrifuged at 1000g for 10 minutes to remove cell debris, and the pH of the stripping medium was adjusted to pH 7.4. The supernatant was incubated with anti-FLAG magnetic beads (Sigma) over night with rotation at 4°C. Beads were then washed 5 times in TEN-T buffer (100 mM NaCl, 5 mM EDTA, 50 mM Tris pH 7.4, 1% Tx100). Immunoprecipitates were eluted in SDS-PAGE loading buffer (25 mM Tris-HCl [pH 6.7], 10% glycerol, 1% SDS, 50 mM DTT, bromophenol blue) and loaded on an 8% SDS-PAGE gel, electrophoretically transferred to nitrocellulose, and probed with an antibody directed against the FLAG epitope (Sigma) followed by incubation with an HRP-conjugated secondary antibody (Jackson ImmunoResearch Laboratories). Specific antibody binding was detected by ECL (ThermoFisher).

Protein trafficking assay

In order to test whether exposing cells to the stripping pH has a negative effect on protein trafficking, we took advantage of wheat germ agglutinin (WGA) conjugated to two different fluorescent dyes, WGA-594 and WGA-488 (ThermoFisher) in order to detect the cell surface delivery of a broad collection of glycoproteins. LLC-PK1 cells were plated on Transwell polycarbonate filters (Corning Life Sciences) and allowed to grow to and past confluency for a total of five days. After incubating the cells in stripping (pH 9.5) or control medium (pH 7.4) for 45 minutes at 4°C, surface proteins were labeled with 20 µg/ml WGA-594 in PBS++ for 10 minutes at 37°C, followed by 3 washes in PBS++ to remove excess lectin. Immediate subsequent labeling with WGA-488 showed a low level of staining, indicating that most available sites for binding were successfully labeled. When samples were allowed to recover in regular medium at 37°C for 3 hours, followed by labeling with WGA-488, both stripped and control samples showed similar amounts of WGA-594 internalization and WGA-488 surface staining, suggesting that neither recycling nor delivery of new proteins to the cell surface were hindered by the exposure to high pH.

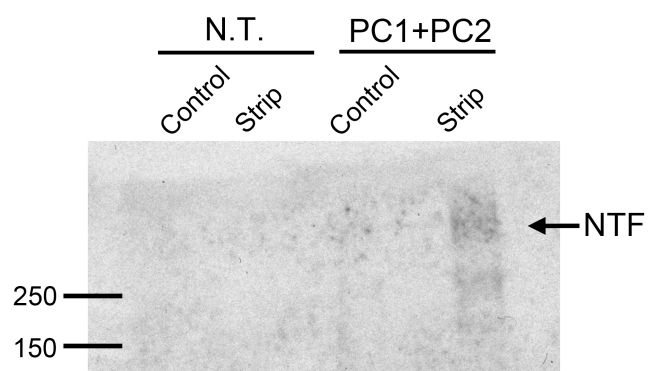


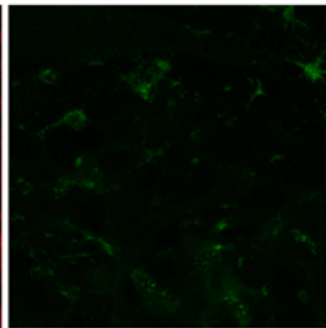
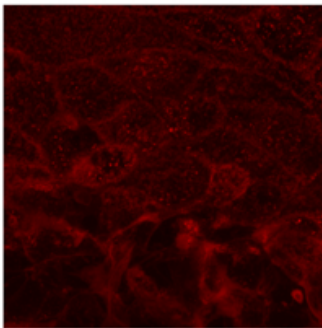
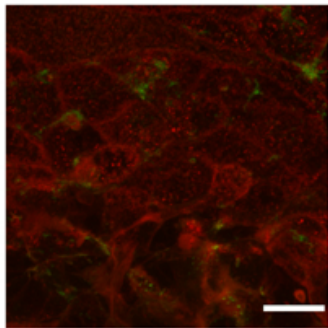
Figure S1

WGA-594/WGA-488

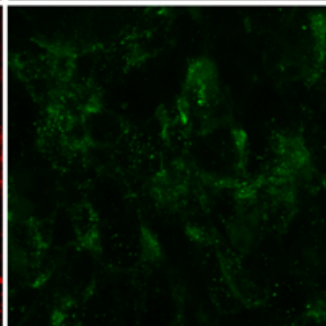
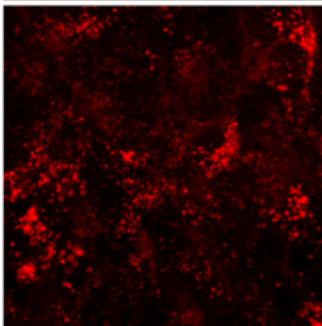
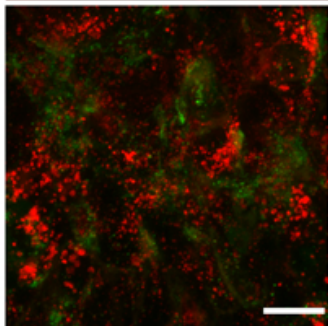
WGA-594

WGA-488

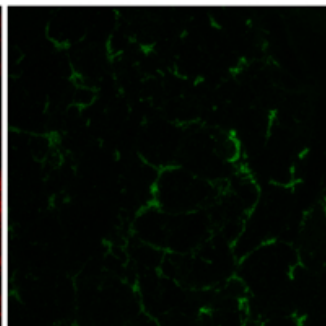
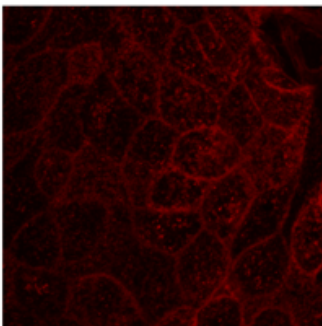
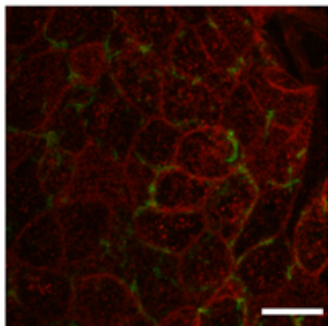
Control



Control + Recovery



Strip



Strip + Recovery

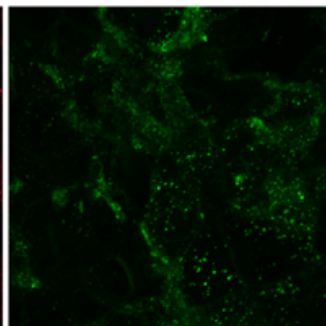
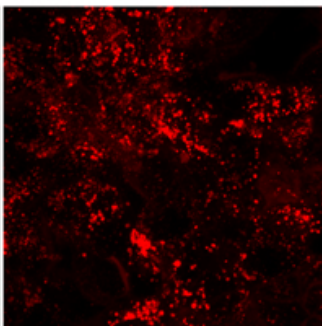
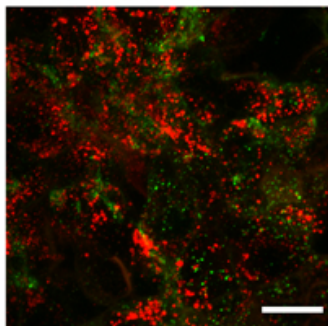


Figure S2