



Supplemental Material to:

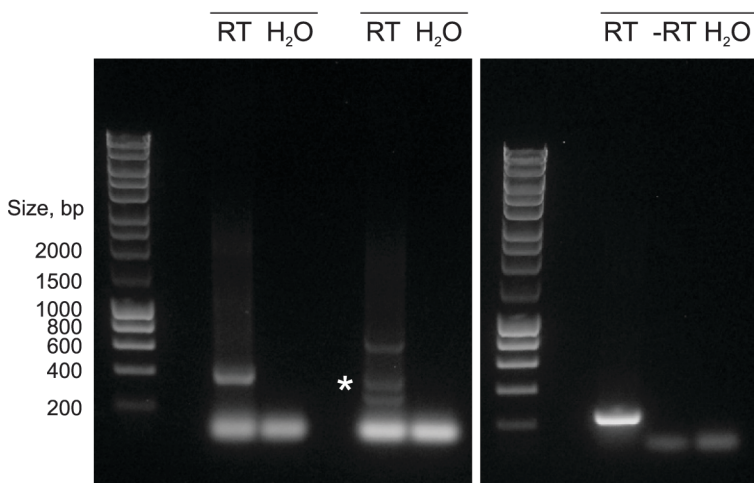
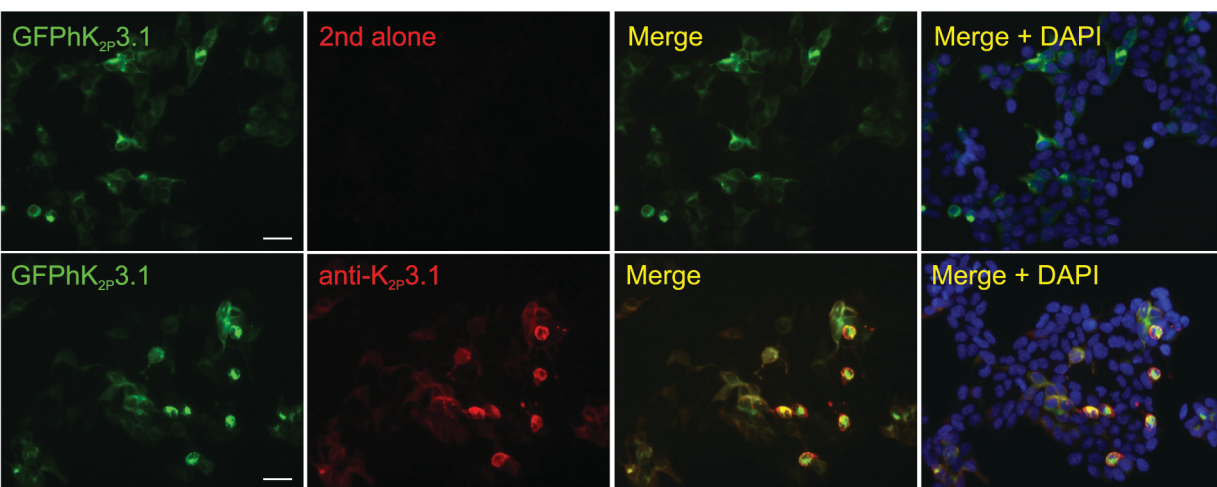
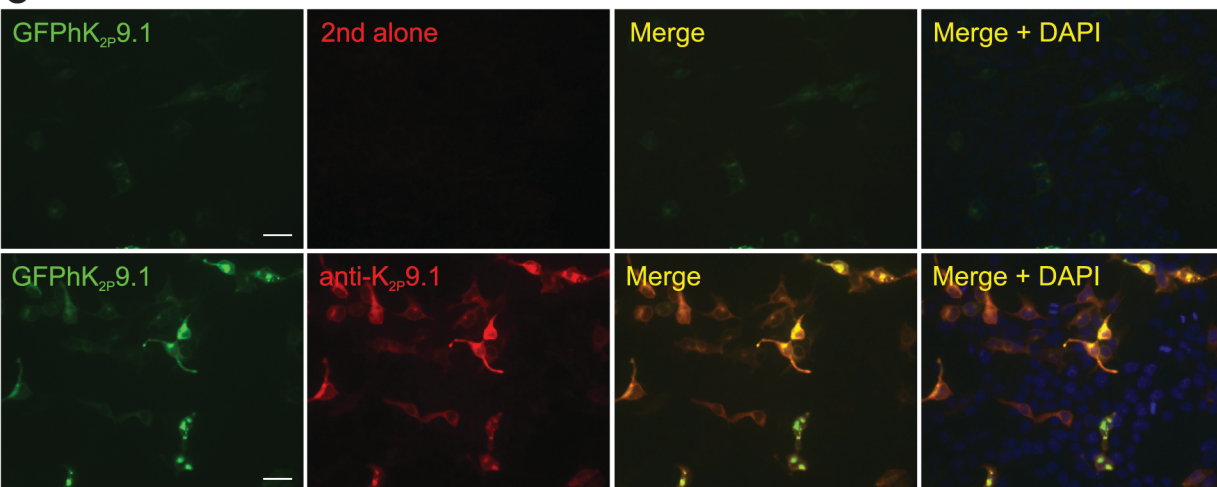
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Acid sensitive background potassium channels K2P3.1 and K2P9.1 undergo rapid dynamin-dependent endocytosis

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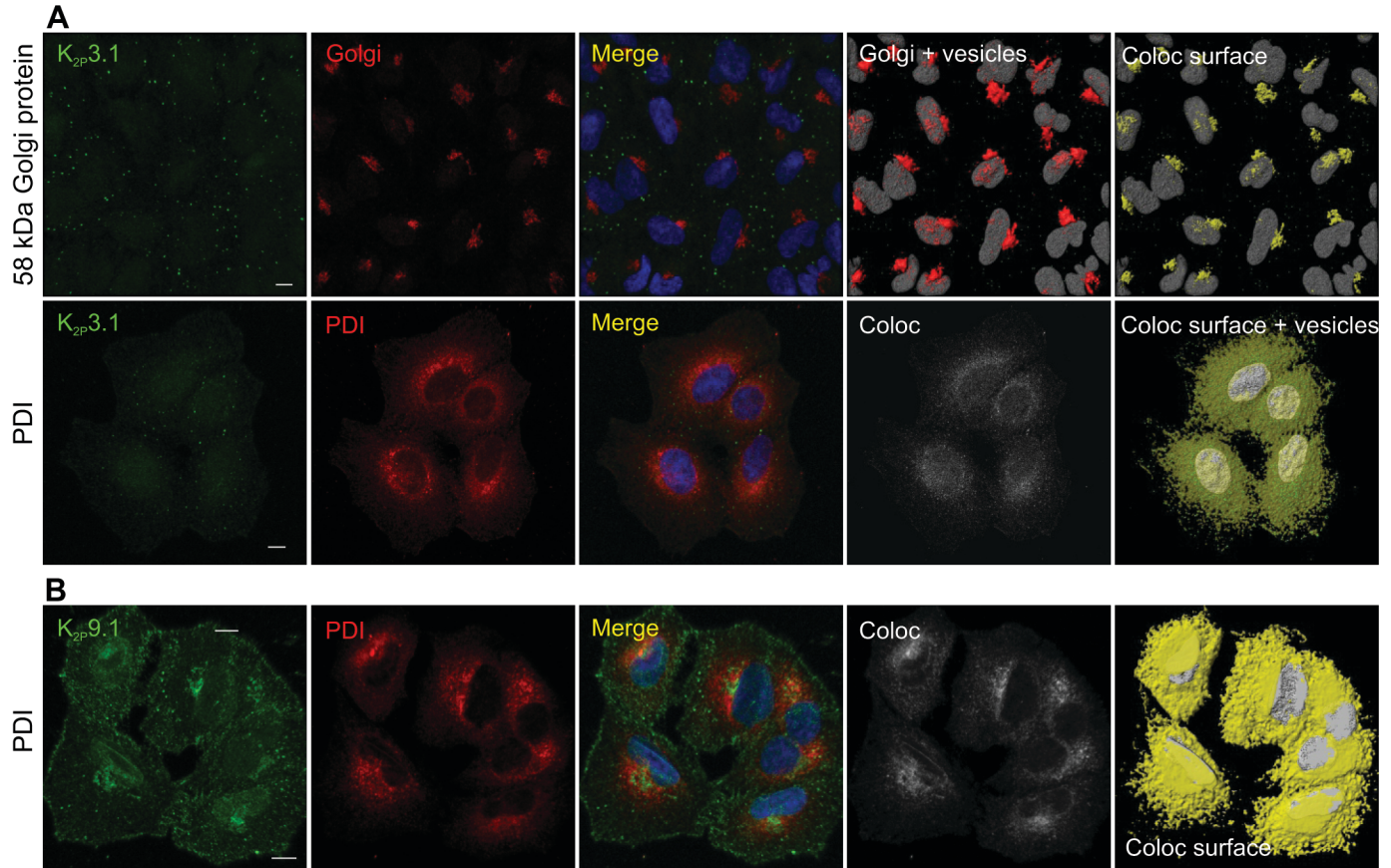
<http://dx.doi.org/10.4161/chan.25120>

<http://www.landesbioscience.com/journals/channels/article/25120/>

A**B****C**

Supplementary Figure 1 A549 cells express KCNK3 (K_{2P}3.1) and KCNK9 (K_{2P}9.1)

A Reverse-transcription PCR was carried out using cDNA prepared from A549 cells, as described in Materials and Methods. KCNK3-specific primers amplified a 430 bp product that was sequenced and identified as the desired KCNK3 amplicon (>95% homology). Three products, including a 413 bp fragment were amplified using KCNK9-specific primers and sequenced. The middle fragment (*) was >90% homologous to KCNK9. A 252 bp fragment from a housekeeping gene, transferrin receptor (TFRC), was amplified from the same cDNA sample. Lanes RT: reverse transcriptase included in the cDNA synthesis reaction; H₂O: water instead of cDNA template; -RT: reverse transcriptase omitted during cDNA synthesis. **B, C** Commercially available antibodies detect GFP-tagged human K_{2P}3.1 and K_{2P}9.1 HEK293 cells were transiently transfected with plasmids encoding GFP-tagged hK_{2P}3.1 and hK_{2P}9.1 fusion proteins, as described in Materials and Methods. Cells were fixed and stained with antibodies directed against K_{2P}3.1 (panels **B**) or K_{2P}9.1 (panels **C**). No signal was detected from secondary antibody alone samples (2nd alone images). No signal could be detected in untransfected cells (Merge + DAPI images). Scale bars represent 32 μ m.

Subcellular distribution of hK_{2p}3.1 and hK_{2p}9.1Supplementary Figure 2 Subcellular distribution of hK_{2p}3.1 and hK_{2p}9.1

A K_{2p}3.1 Golgi panels: fixed A549 cells were stained with anti-K_{2p}3.1 (green) and anti-58 kDa Golgi protein (red). Merge: superimposed K_{2p}3.1 and 58 kDa Golgi protein; nuclei in blue. Golgi + vesicles: 3D reconstruction of the volume occupied by the 58 kDa Golgi signal (red), together with the most prominently stained K_{2p}3.1 spots (green); nuclei in grey. Coloc surface: 3D reconstruction of the colocalized volume of K_{2p}3.1 and 58 kDa Golgi protein (yellow), together with the most prominently stained K_{2p}3.1 spots (green). PDI panels: fixed A549 cells were stained with anti-K_{2p}3.1 (green) and anti-protein disulfide isomerase (PDI, red). Merge: superimposed K_{2p}3.1 and PDI; nuclei in blue. Coloc: colocalized K_{2p}3.1 and PDI. The mean Mander's coefficient for hK_{2p}3.1 with PDI is 0.51 ± 0.05 SEM; n=3 fields of view. Coloc surface and vesicles: 3D reconstruction of colocalized volume of K_{2p}3.1 and PDI (yellow), together with the most prominently stained K_{2p}3.1 vesicles (green); nuclei in grey. **B** K_{2p}9.1 PDI panels: fixed A549 cells were stained with anti-K_{2p}9.1 (green) and anti-PDI (red). Merge: superimposed K_{2p}9.1 and PDI. Coloc: colocalized K_{2p}9.1 and PDI. The mean Mander's coefficient for hK_{2p}9.1 with PDI is 0.56 ± 0.07 SEM; n=3. Coloc surface and vesicles: 3D reconstruction of colocalized volume of K_{2p}3.1 and PDI (yellow). Scale bars: 10 μ m. All images are whole cell projections of confocal z-stacks. Image analysis was performed using Imaris 7.6.1.