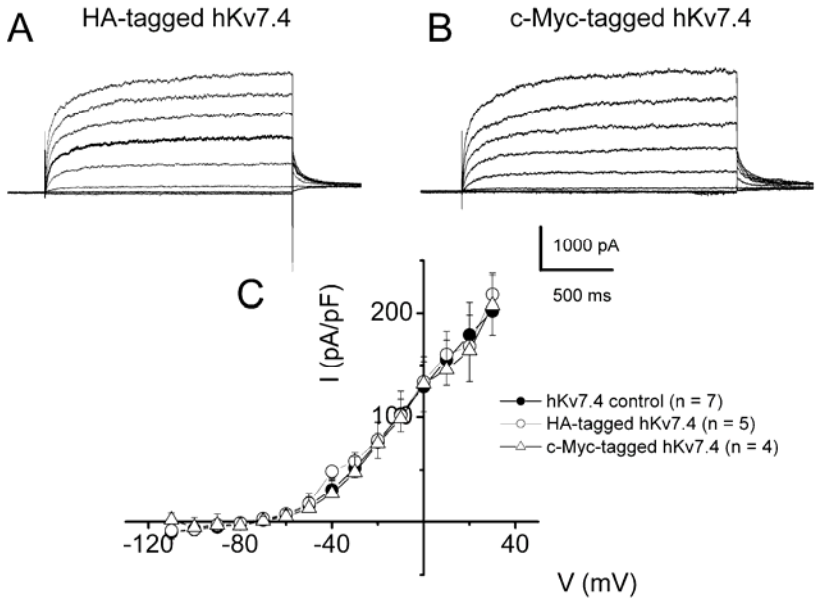


**CELLULAR AND MOLECULAR MECHANISMS OF AUTOSOMAL DOMINANT FORM  
OF PROGRESSIVE HEARING LOSS, DFNA2**

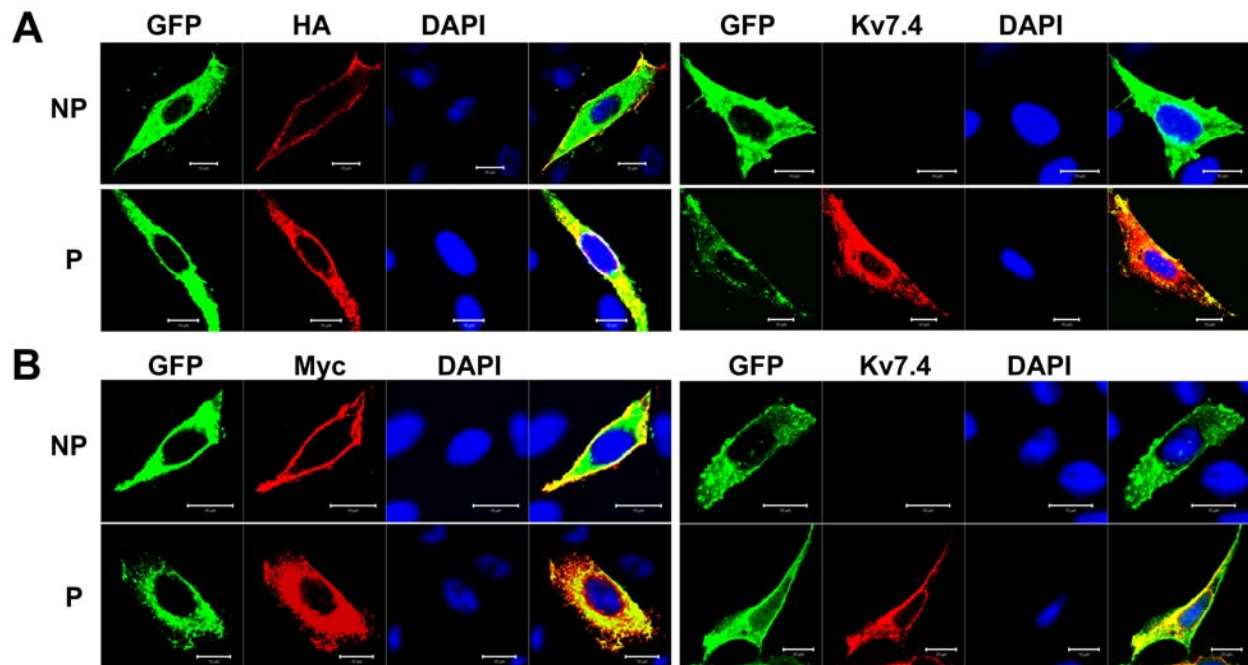
**Hyo Jeong Kim, Ping Lv, Choong-Ryoul Sihm, and Ebenezer N. Yamoah**

Supplementary figures



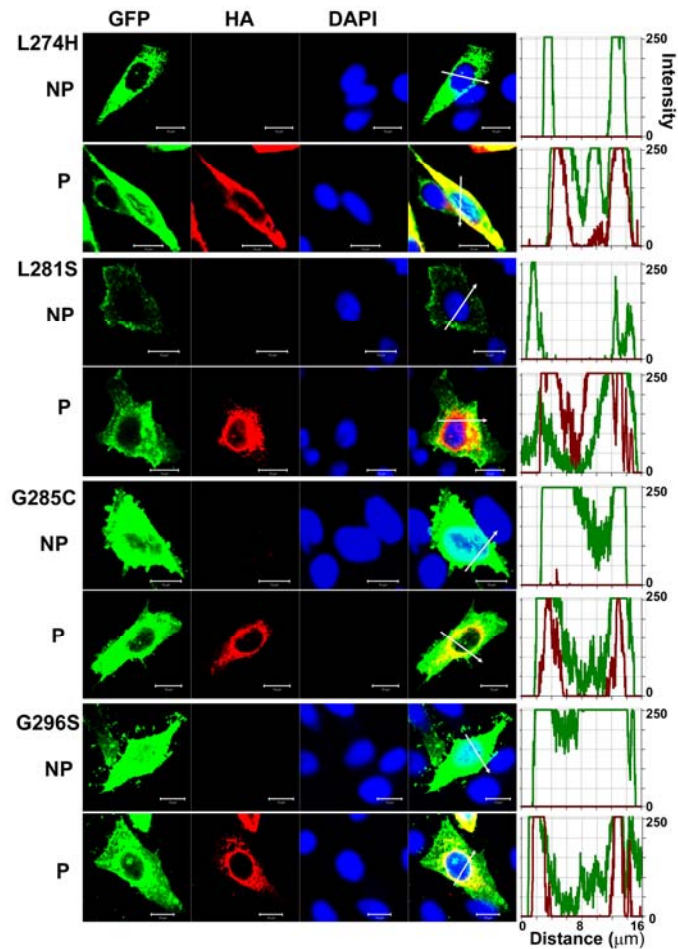
**Suppl. Fig 1 (SI)**

**Whole-cell  $K^+$  currents derived from HA- and c-Myc-tagged-hKv7.4 channels in CHO cells. A-B.** HA- (A) and c-Myc (B) -tagged hKv7.4 channels transfected CHO cells yielded measurable current. Shown are examples of current traces recorded from CHO cells that were transfected with HA- and c-Myc-tagged hKv7.4. CHO cells were held at -80 mV and the step voltages ranged from -110 to 40 mV at 10 mV increments. C. The corresponding current density-voltage relation and untagged-hKv7.4 channel currents are shown for comparisons.



**Suppl. Fig 2 (S2)**

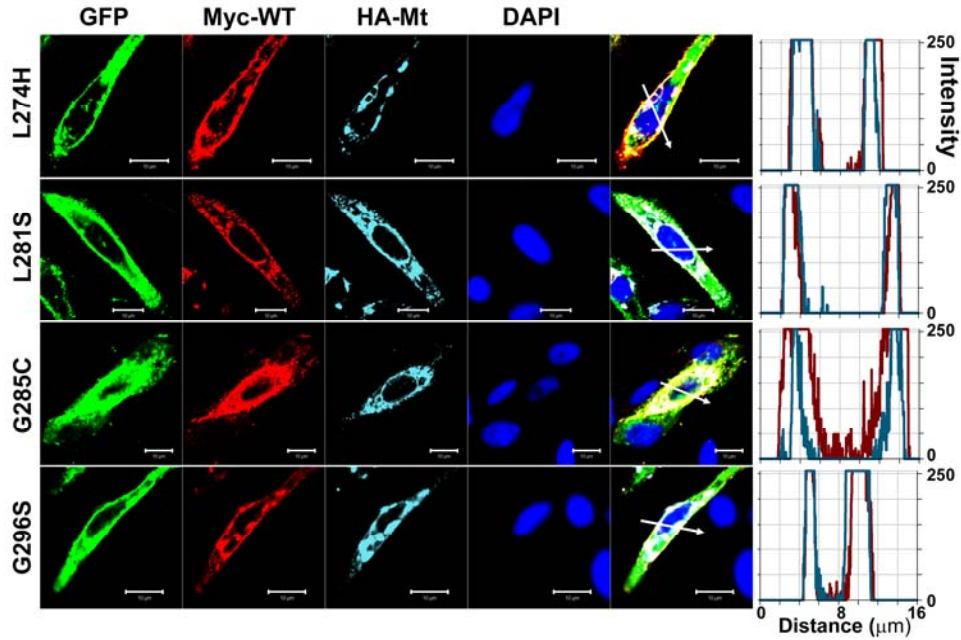
**Detection of epitope-tagged wildtype Kv7.4 channels.** HA-(A) and c-Myc-epitope (B) were inserted into wildtype Kv7.4. Farnesylated green fluorescent protein (GFP) was used as a reporter that binds to the plasma membrane. Expression of Kv7.4 protein was detected using anti-HA or anti-c-Myc antibody and anti-Kv7.4 antibody in non-permeabilized (NP) and permeabilized (P) conditions. Only anti-epitope antibodies stained membrane-localized proteins but Kv7.4 antibody, which binds to cytoplasmic N-terminus (amino acids 2-77), could not detect proteins in non-permeabilized condition. In permeabilized condition, all antibodies stained both the cell surface and cytoplasmic expressed proteins. Scale bar is 10  $\mu\text{m}$ .



**Suppl. Fig 3 (S3)**

**Detection of cell-surface expression of HA-tagged Kv7.4 channels with mutation in P-loop.**

Farnesylated green fluorescent protein (GFP) was used as a reporter that binds to the plasma membrane. Anti-HA antibody did not stain Kv7.4 protein expressed on the cell surface in a detectable level in non-permeabilized cells (NP), but stained cytoplasmic expressed proteins in permeabilized cells (P). Fluorescent intensity of GFP (green) and Kv7.4 channel (red) were plotted against distance, which was marked in a merged image with a white arrow. The overlap of green and red signals in NP cells indicates the surface expression of Kv7.4 channels. Scale bar is 10  $\mu\text{m}$ .



Suppl. Fig 4 (S4)

**Detection of cell-surface expression of c-Myc-tagged wildtype and HA-tagged mutant  $K_V7.4$  channels in co-expression.** Farnesylated green fluorescent protein (GFP) was used as reporter which bound to plasma membrane. Anti-c-Myc and anti-HA antibodies double staining detected wildtype and mutant  $K_V7.4$  protein respectively, which expressed on the cell surface in non-permeabilized conditions. Fluorescent intensity of wildtype (red) and mutant (cyan)  $K_V7.4$  channel were plotted against the distance, which was marked in a merged image with a white arrow. The overlap of red and cyan signals in NP cells indicates their co-localization on the cell surface. Scale bar is 10  $\mu\text{m}$ .