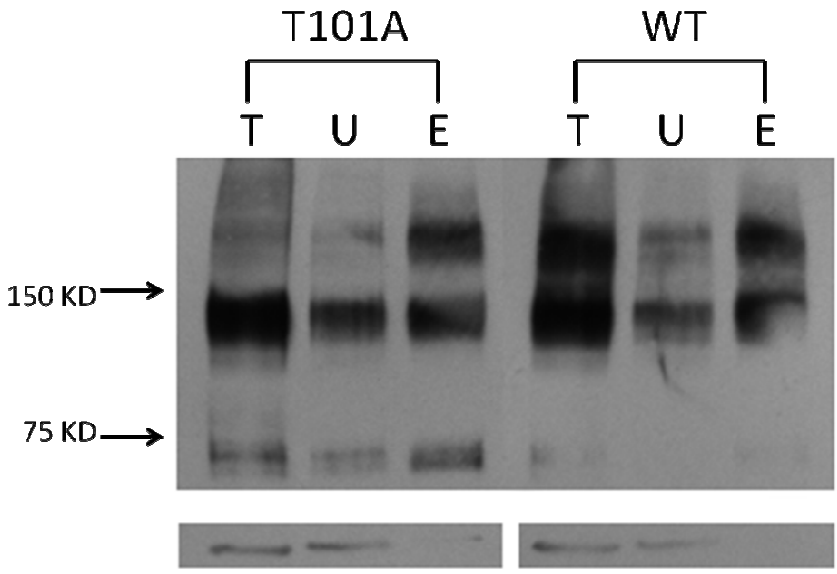
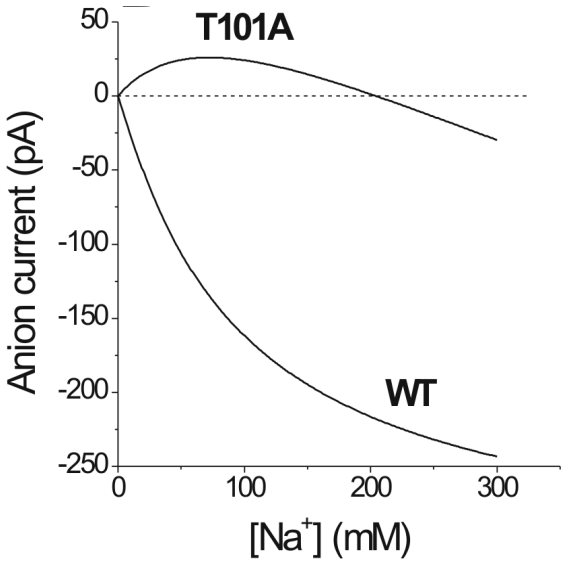
**Online Supplementary Material**




**Supplementary Figure 1:** Biotinylation was performed as described in (Davis et al., 1998) with some modifications. Briefly, confluent HEK293T cells in 10 cm plates were transfected with transporter cDNA by using FuGENE 6 Transfection Reagent (Roche) according to the supplied protocol. Cells were washed 2~3 times with ice cold PBS [1] plus 0.1 mM $Ca^{2+}$, 1 mM $Mg^{2+}$ 48 hours after transfection; 2 ml biotinylation reagent (2mg/ml, EZ-Link$^{TM}$Sulfo-NHS-Biotin, PIERCE) was added to the plate and kept at 4˚C, 30 min while gently shaking; cells were washed with ice cold PBS plus 0.1 mM $Ca^{2+}$, 1 mM $Mg^{2+,}$ 100 mM glycine three times; then the same solution was added to the plate, 4˚C, 45 min to quench any un-reacted biotin reagent; cells were lysed by adding 1 ml RIPA/lysis buffer (100 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA[2], 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS[3], plus protease inhibitors (PI)) to the cell and vortex for 1 hour at 4˚C; then 20,000 X g to sediment nucleic acid and debris, 10 min, 4˚C; 100 µl 50% slurry protein A beads (Upstate) in PBS were added to pre-clear the sample at RT for 1 hour. The beads were spun down and the supernatant transferred to a new tube (this

[1] PBS: Phosphate-buffered saline
[2] EDTA: Ethylenediaminetetraacetic acid
[3] SDS: Sodium dodecyl sulfate

is the total fraction, T). After adding 300 µl 50% slurry Avidin beads (PIERCE) in PBS to 300 µl pre-cleared sample and keeping at RT for 1 hour, the beads were sedimented by centrifugation and the supernatant was transferred to a new tube (this is the unbound fraction, U). The beads were washed with RIPA/lysis buffer 6 times, 1 ml each time (this is the wash fraction, W, not shown). The protein was eluted from beads by adding 150 µl 2 X Laemmli buffer (1 X Laemmli buffer, 62.5 mM Tris, pH 6.8, 2% SDS, 20% glycerol and 5% 2-mercaptoethanol, this is the eluted fraction, E). Each fraction was adjusted to comparable volumes with Laemmli buffer and analyzed by Western-blotting. The blots were visualized with enhanced chemiluminescence (Amersham Biosciences). After 15 s of exposure, the blots were in the linear range, as tested with time-dependent exposures up to 2 min. From densitometry, integrating over all bands (ImageJ software), the intensity of the $EAAC1_{T101A}$-associated bands was found to be 104% of that of $EAAC1_{WT}$ for the biotinylated membrane fraction (fraction E). Anti-actin antibodies reacted with the total protein fraction, but not with the biotinylated fraction, indicating that the biotinylated fraction did not contain detectable amounts of intracellular proteins (data not shown).




**Supplementary Figure 2:** Simulations of the biphasic $Na^+$ dose response curve of the leak anion current. In this model, two $Na^+$ ions associate with the empty transporter before inhibitor (or substrate) binding takes place. The transporter with only one $Na^+$ ion bound (state TN in Fig. 8) has the lowest anion conductance. Thus, $Na^+$ application results in an apparent outward current, which in fact is an inhibition of a tonic inward anion current carried by the $Na^+$-free transporter (state T in Fig. 8). State T has a higher anion conductance than state TN. As $[Na^+]$ increases, state $TN_2$ is populated, which has the highest relative anion conductance, resulting in formation of an inward anion current. The simulated anion current fits well with the experimental data (Fig. 1), and demonstrates that at least one $Na^+$ ion binds with very low affinity ($K_{Na2}$ = 1400 mM, $K_{Na1}$ = 110 mM). These dissociation constants are well defined by the fit, with an accuracy of ± 20%.