**Biophysical Journal, Volume *118***

## Supplemental Information

# The Selectivity Filter Is Involved in the U-Type Inactivation Process of Kv2.1 and Kv3.1 Channels

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**Supplemental Information**

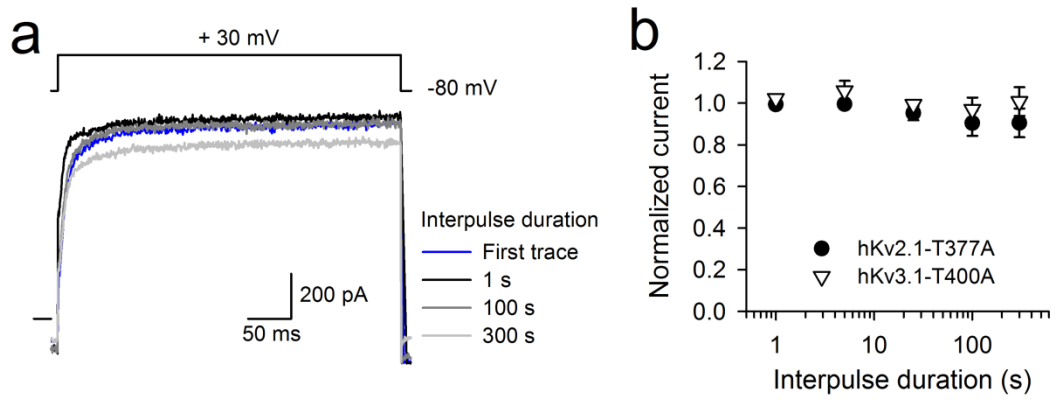


**Figure S1**: Prolonged membrane hyperpolarization does not reveal closed-state inactivation in hKv2.1-T377A and hKv3.1-T400A. **a.** Representative current recording of hKv2.1-T377A obtained by applying the pulse protocol shown on top. Inter-pulse duration was increased from 1 s (black trace) to 100 s (dark gray trace) and 300 s (light gray trace) at -80 mV. The current elicited by the first pulse is depicted in blue. Aside of a very small decrease in current amplitude after 300 s at -80 mV, prolonged hyperpolarizations did not decrease hKv2.1-T377A currents. **b.** Normalized **c**urrent amplitude at +30 mV for Kv2.1-T377A and Kv3.1-T400A as a function of inter-pulse duration. There is no significant decrease in current amplitude with prolonged hyperpolarization at -80 mV. Data is shown as mean values ± SEM from 4 and 6 individual cells for hKv2.-T377A and hKv3.1-T400A, respectively.

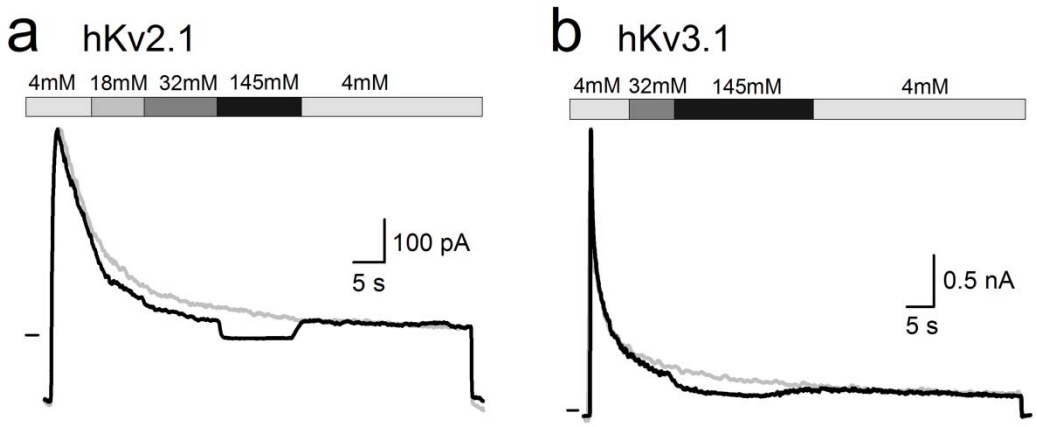


**Figure S2**: Increased external $K^+$ reduces current amplitude in hKv2.1 and hKv3.1.

**a.** Representative hKv2.1 current recording showing the effect of increasing the external $K^+$ concentration on current amplitude. During a prolonged depolarization to +20 mV, starting from -80 mV holding potential, the external $K^+$ concentration was changed, using a pressurized perfusion system, from 4 mM to 145 mM as indicated on top. The current amplitude decreased with increasing $K^+$ concentrations, as is predicted from a reduced effective driving force ($V_{effective} = V_{applied} - V_{reversal}$). The gray trace is the current elicited by the same pulse protocol in 4 mM [$K^+$]. **b.** A representative hKv3.1 recording during prolonged depolarization to +40 mV and increasing the extracellular $K^+$ concentration. Gray recording is again the reference current obtained in 4 mM [$K^+$].

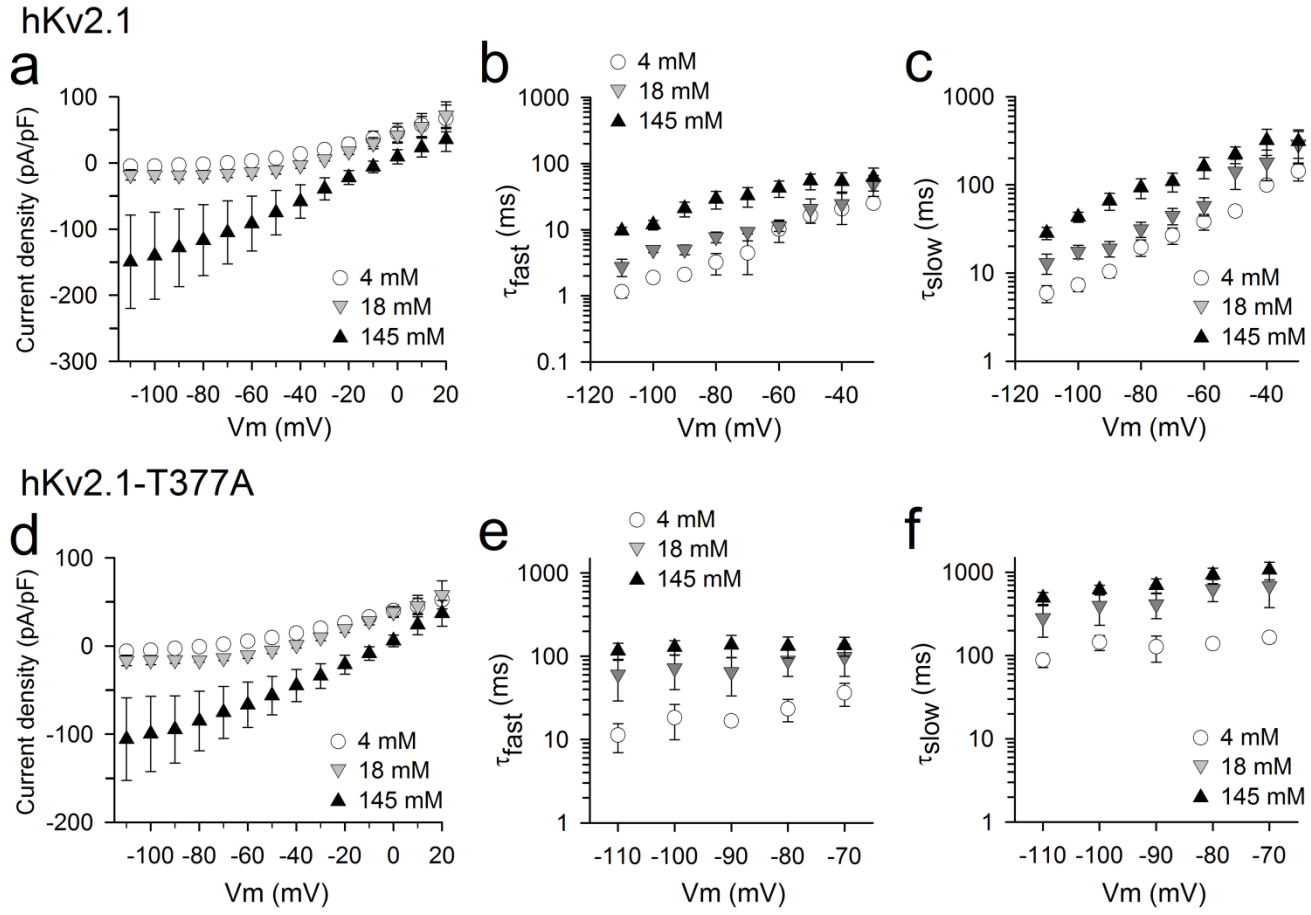


**Figure S3**: Increased external $K^+$ concentration slowed down deactivation in both hKv2.1 and hKv2.1-T377A. **a.** Current-voltage (IV) relationship of hKv2.1 obtained in different external $K^+$ concentrations (4 mM, 18 mM and 145 mM). As expected for $K^+$ selective channels, the reversal potential shifts from -80 mV to -45 and 7 mV at 4 mM, 18 mM and 145 mM external $K^+$ respectively. **b and c.** Time constants of deactivation at 4 mM (white circles), 18 mM (gray inverted triangles) and 145 mM (black triangles) external $K^+$ for hKv2.1. Values were obtained by fitting the current decay with a double exponential function yielding a fast (panel b) and slow (panel c) component. Time constants are represented as mean ± SEM from 3 to 6 independent recordings. Both the fast and the slow component are slowed down by increased extracellular $K^+$. **d.** IV relationship of hKv2.1-T377A obtained in 4 mM, 18 mM and 145 mM external $K^+$ concentration. **e and f.** Time constants of deactivation at 4 mM (white circles), 18 mM (gray inverted triangles) and 145 mM (black triangles) external $K^+$ for hKv2.1-T377A. Note that the T-to-A mutant behaved as WT and the deactivation kinetics gradually slowed down with increasing extracellular $K^+$ concentrations.

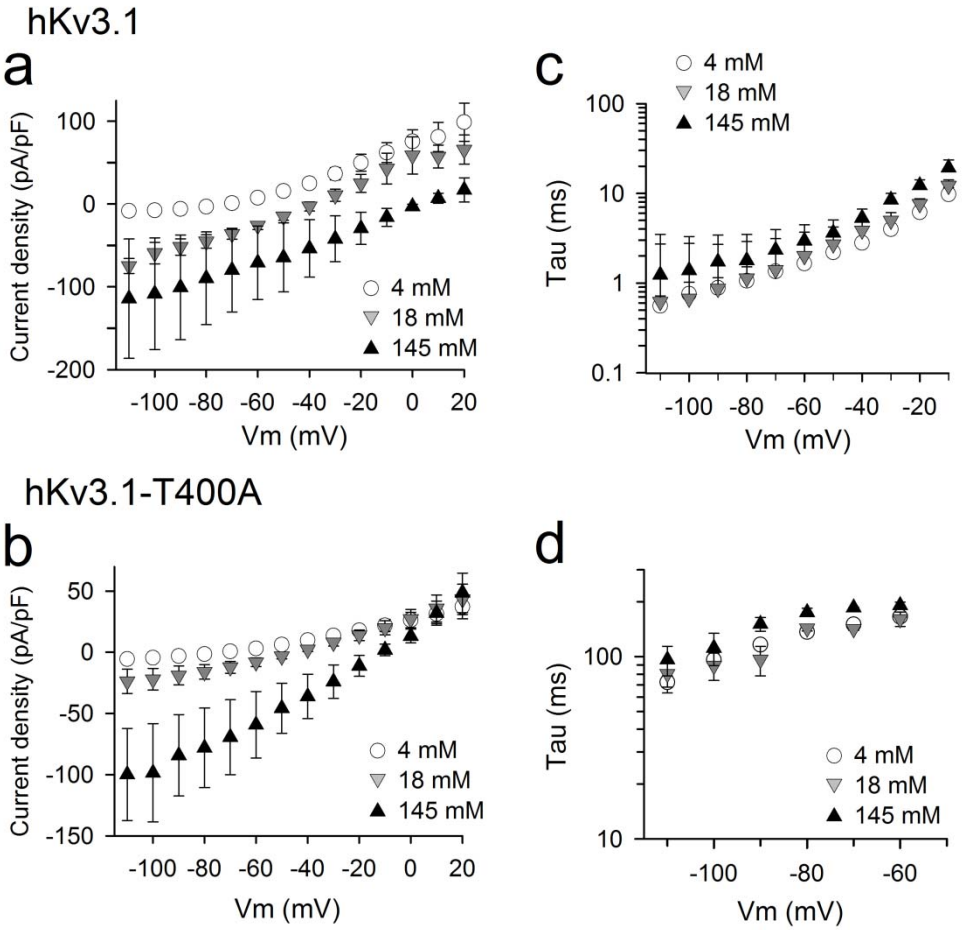


**Figure S4**: Increased external $K^+$ concentration did not affect deactivation in both hKv3.1 and hKv3.1-T400A. **a and b.** IV relationships of hKv3.1 and hKv3.1-T400A at different external $K^+$ concentrations: 4 mM (white circles), 18 mM (gray inverted triangles) and 145 mM (black triangles). As expected for $K^+$ selective channels, the reversal potential shifts from -80 mV to -45 and 7 mV at 4 mM, 18 mM and 145 mM external $K^+$, respectively. **c and d.** Time constants of deactivation in 4 mM (white circles), 18 mM (gray inverted triangles) and 145 mM (black triangles) external $K^+$ for WT hKv3.1 (panel c) and hKv3.1-T400A (panel d). Current decay was fitted with a single exponential function and time constants are depicted as mean ± SEM from 4 to 5 independent cells. Elevated external [$K^+$] appeared to have no significant effect on deactivation kinetics in both the WT and the T-to-A mutant channels.