

Neutralization of a single voltage sensor (IIS4) affects gating determinants in all four pore forming S6 segments of Ca<sub>v</sub>1.2

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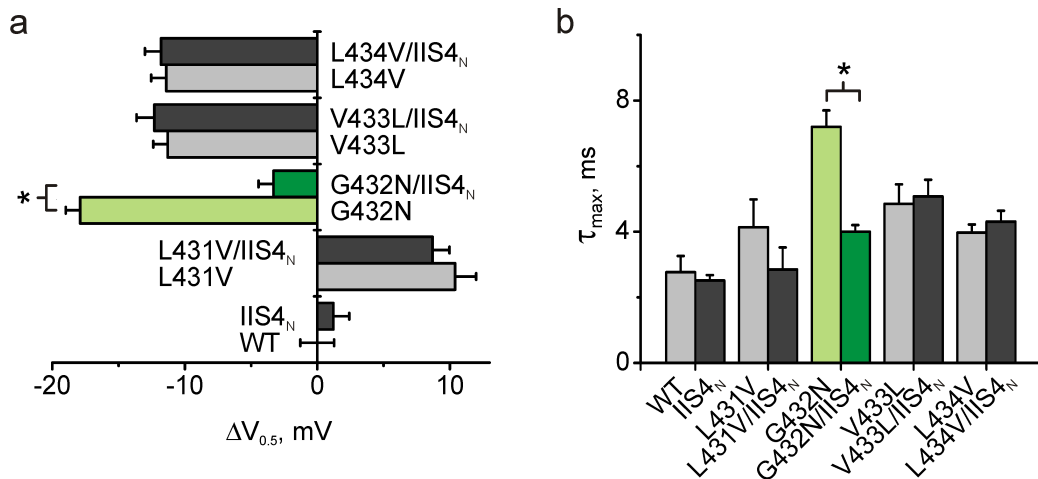
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## SUPPLEMENTAL MATERIALS

### Fingerprints of the IIS4 – IIS6 interaction

A region of gating sensitive residues in IIS6 was previously identified ([3], LAIA motif). Interestingly, these residues were recently suggested to closely interact with the S4-S5 linker region of Ca<sub>v</sub>2.3 suggesting common elements in the transduction mechanism in different Ca<sub>v</sub> [4]. A782P (forming part of LAIA) stabilizes the open state compared to wild type and combination with IIS4<sub>N</sub> resulted in a further shift of the activation curve to the left and a further slowing-down of channel kinetics (Fig. 2c). If we envisage the voltage sensor predominantly “pushing” the gating structures of Ca<sub>v</sub>1.2 into the closed conformation [1], then charge neutralization of IIS4 would be expected to decelerate channel closure at negative voltages (deactivation). These “expected” gating changes were, however, observed only for constructs I781T/IIS4<sub>N</sub> and A782P/IIS4<sub>N</sub>. For the IIS6 mutation A780T, the activation curve was shifted towards wild type but did not completely rescue the gating perturbation (Fig. 2e, colored bars). The half way shift was not accompanied by normalization of channel closure (Fig. 2d and f). This distinguishes A780T from the other mutants of the G/A/G/A ring. We speculate that A780 may participate in two processes: i) stabilization of the open state, like other residues [2] and ii) executing pore closure under downward movement of IIS4. Thus, on the one hand charge neutralization of IIS4 diminishes stabilization of the open state as evident from the shifted the activation curve towards wt. This effect resembles the kinetics of constructs G432W/IIS4<sub>N</sub> G1193T/IIS4<sub>N</sub> and A1503G/IIS4<sub>N</sub>. On the other hand lack of “IIS4 pushing force” on IIS6 destabilizes the closed state, thereby shifting the activation curve leftwards. Consequently, charge neutralization in construct A780T/IIS4<sub>N</sub> induces only “partial rescue” of the gating disturbances.

Figure 1



**Figure 1. Charge neutralization in domain II (IIS4) rescues gating disturbances in position G432 of domains I**

Shift of activation curve (a) and peak activation/deactivation time constants (b) of the studied pore mutations in segments IS6 either alone or in combination with IIS4<sub>N</sub>. Statistically significantly different (t-test) values are marked with asterisks. Error bars indicate SEM.

## REFERENCES

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