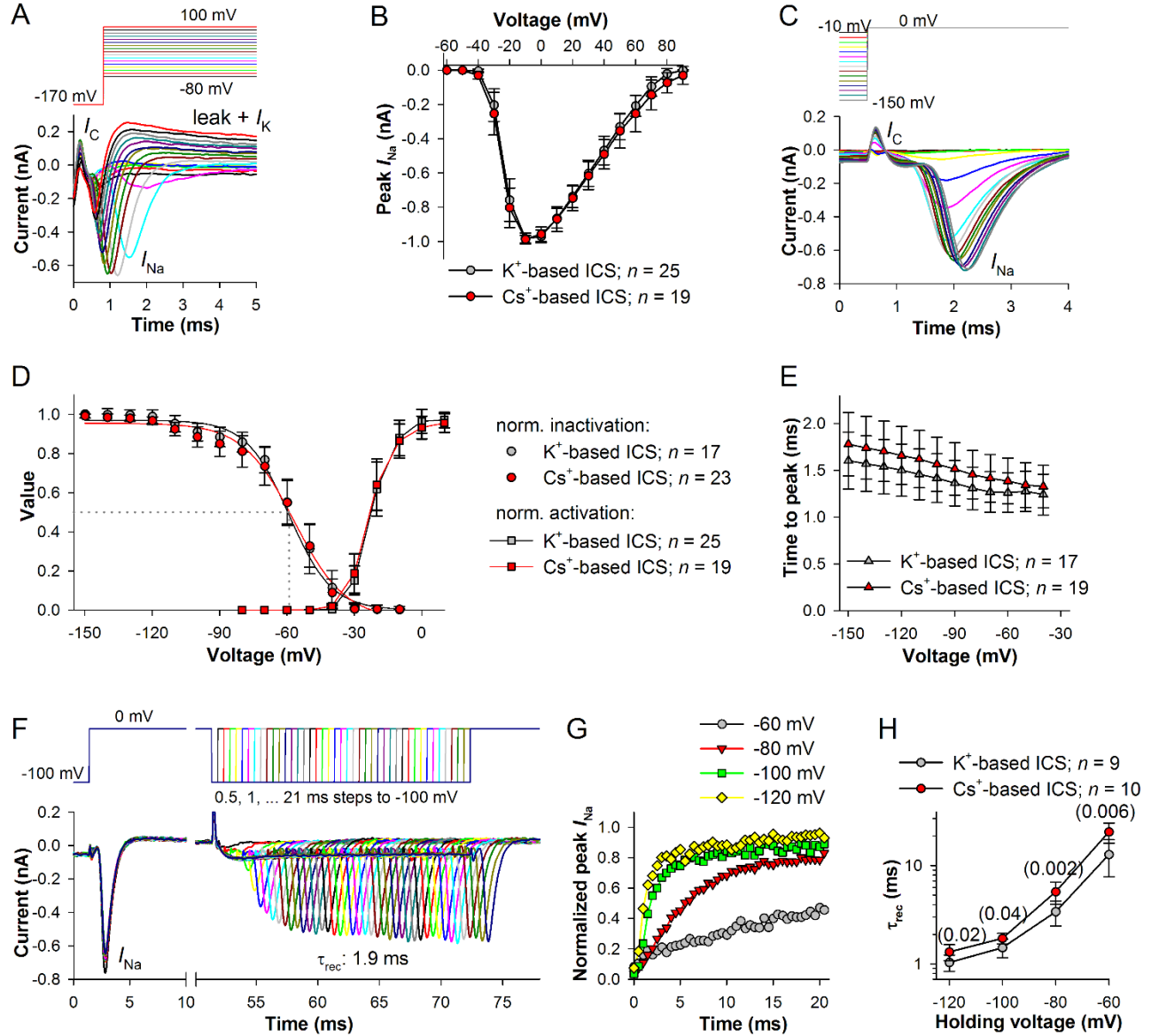


Additional file 1: Figure S1.



Additional file 1: Figure S1. Kinetics of I_{Na} recorded using cesium-based intracellular solution. **A** Typical I_{Na} evoked by depolarizing pulses from -80 to +100 mV in 10 mV increments after holding the cell at -170 mV for 50 ms; I_C , residual capacitive current; I_K , the residual voltage-activated K^+ current. In these experiments K-gluconate (83 mM) and KCl (25 mM) in intracellular solution (ICS) were

replaced with CsCH₃SO₃ (83 mM) and CsCl (25 mM). Cs⁺-based sample also contains several cells recorded in extracellular solution supplemented with 10 μM nifedipine. **B** Comparison of average *I-V* relationships for *I*_{Na} recorded using K⁺-based (25 cells) and Cs⁺-based (19 cells) solutions; here and elsewhere error bars are s.d. (Additional file 2: Table S1). **C** Example of a SSI experiment with Cs⁺-based solution. SSI was studied by evoking *I*_{Na} at 0 mV after a 50 ms conditioning pre-pulses from -150 to -10 mV. **D** Voltage-dependencies of the normalized peak *I*_{Na} from SSI experiments and the normalized peak *I*_{Na} conductance. SSI data were divided by *I*_{Na} after the pre-pulse to -150 mV; average values were fitted with a sigmoidal equation. Conductance at each membrane potential *V* was calculated using peak *I*_{Na} from *I-V* relations as in B and equation $g_{Na}(V) = I_{Na}/(V - E_{rev})$, where *E*_{Na} = 85.8 mV (Additional file 2: Table S1). **E** Voltage-dependencies of the peak *I*_{Na} time to peak from SSI experiments for two intracellular solutions (Additional file 2: Table S1). **F** Recovery of *I*_{Na} from inactivation with Cs⁺-based internal solution. *I*_{Na} was evoked and inactivated by a 50 ms pre-pulse to 0 mV and then hyperpolarizing pulses of increasing duration ranging from 0.5 to 21.0 ms were applied. In this experiment, the test holding potential was -100 mV. **G** By plotting peak *I*_{Na} during recovery from inactivation against the hyperpolarizing pulse duration and then fitting the data with a first-order exponential rise-to-maximum equation, recovery time constants (τ_{rec}) can be obtained; examples are from the same cell as in F. **H** Comparison of average voltage-dependence of the recovery time constants from inactivation for two intracellular solutions; unpaired *t*-test was used for the statistical comparison (Additional file 2: Table S1).

Kinetics of *I*_{Na} were compared between RPE cells recorded using K⁺-based and Cs⁺-based internal solutions. *I-V* relationships, voltage-dependencies of activation and inactivation were identical (Additional file 1: Fig. S1 A-D). *I*_{Na} reached peak more slowly in Cs⁺-based internal solution experiments

compared to K^+ -based solution. However, the differences were not statistically significant (Additional file 1: Fig. S1 E, unpaired t -test). This finding is consistent with results of a previous study [33].

Recovery time constants of I_{Na} from inactivation were statistically significantly slower when Cs^+ -based internal solution was used (Additional file 1: Fig. S1 F-H, unpaired t -test).

Adding 10 μM nifedipine to the extracellular solution did not affect kinetics of I_{Na} ; therefore, Cs^+ -based sample also contains several cells recorded in extracellular solution supplemented with 10 μM nifedipine.