

Supplementary Material

Supplementary Table 1: IC₅₀ values for tetracaine at different holding potentials (HP) for an average of 4 chips in standard internal solution and 3 chips in fluoride-free internal solution. Single addition of compound to each well was performed and the concentration response curve calculated across multiple wells. Shown are mean ± S.E.M.

Parameter	Standard internal	Fluoride-free internal
IC ₅₀ Tetracaine HP -120 mV (μM)	35.60 ± 9.41	41.76 ± 9.29
IC ₅₀ Tetracaine HP -100 mV (μM)	14.76 ± 4.43	21.28 ± 5.29
IC ₅₀ Tetracaine HP -80 mV (μM)	5.20 ± 1.64	10.28 ± 2.49

Supplementary Table 2: Success rate, R_{Seal} and I_{Peak} parameters for CHO cells expressing Nav1.5 at physiological temperature. R_{Seal} values > 4 GΩ could not be accurately calculated, therefore only R_{Seal} ≤ 4 GΩ were used for the calculation.

Parameter	Standard internal	Fluoride-free internal
Success rate (% available wells) R _{Seal} ≥ 1 GΩ at start of experiment	79.6 ± 4.7 (10 chips)	30.0 ± 7.1 (6 chips)
Success rate (% available wells) R _{Seal} ≥ 250 MΩ at start of experiment	88.8 ± 4.5 (10 chips)	35.5 ± 9.2 (6 chips)
R _{Seal} (GΩ)	2.11 ± 0.06 (286)	1.41 ± 0.07 (156)
I _{Peak} (nA)	-2.40 ± 0.20 (357)	-1.61 ± 0.09 (194)

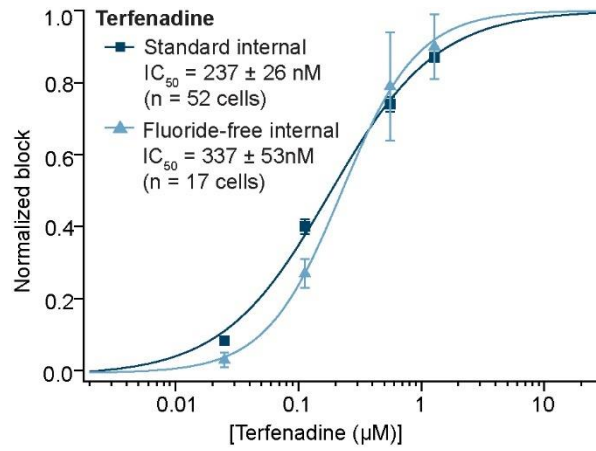
Supplementary Table 3: Success rate in % for the different channels/cell lines tested with different fluoride free approaches, R_{Seal} and $I_{Peak/Tail}$ cut off at $R_{Seal} > 1 \text{ G}\Omega$ or $R_{Seal} > 0.25 \text{ G}\Omega$ as indicated. n.d = not done

Channel/ Cell line	Parameter	Fluoride-free internal (K-Gluconate based)	Fluoride-free internal (K-Chloride based)
Nav1.5- CHO	Success rate (% available wells) $R_{Seal} \geq 1 \text{ G}\Omega$ & $I < -100 \text{ pA}$	Start: 41.9 ± 9.3 (17 chips) End: 33.0 ± 5.7 (17 chips)	Start: 37.8 ± 6.6 (6 chips) End: 30.2 ± 4.4 (6 chips)
Nav1.5- CHO	Success rate (% available wells) $R_{Seal} \geq 250 \text{ M}\Omega$ & $I < -100 \text{ pA}$	Start: 55.0 ± 11.7 (17 chips) End: 46.3 ± 8.6 (17 chips)	Start: 57.4 ± 5.8 (6 chips) End: 48.9 ± 6.1 (6 chips)
hERG- CHO	Success rate (% available wells) $R_{Seal} \geq 1 \text{ G}\Omega$ & $I > 150 \text{ pA}$	n.d	Start: 38.0 ± 7.1 (2 chips) End: 32.0 ± 8.5 (2 chips)
hERG- CHO	Success rate (% available wells) $R_{Seal} \geq 250 \text{ M}\Omega$ & $I > 150 \text{ pA}$	n.d	Start: 63.5 ± 9.2 (2 chips) End: 57.5 ± 6.4 (2 chips)
hERG- HEK	Success rate (% available wells) $R_{Seal} \geq 1 \text{ G}\Omega$ & $I > 150 \text{ pA}$	Start: 35.9 ± 7.9 (32 chips) End: 33.6 ± 7.8 (32 chips)	Start: 36.9 ± 8.3 (2 chips) End: 32.2 ± 6.4 (2 chips)
hERG- HEK	Success rate (% available wells) $R_{Seal} \geq 250 \text{ M}\Omega$ & $I > 150 \text{ pA}$	Start: 59.0 ± 8.5 (32 chips) End: 57.0 ± 8.8 (32 chips)	Start: 47.8 ± 3.8 (2 chips) End: 45.3 ± 7.0 (2 chips)

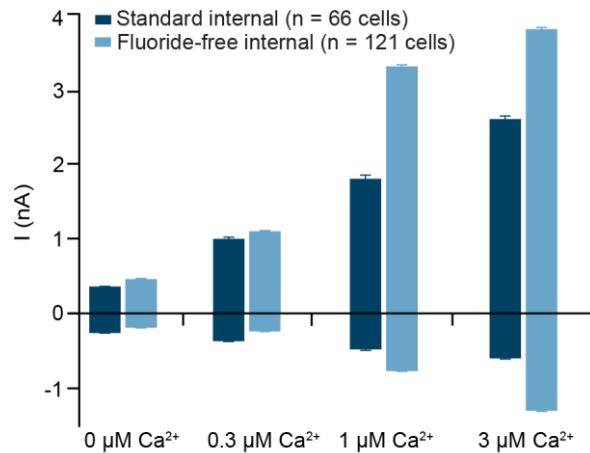
1 Supplementary Figures and Tables

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1.1 Supplementary Figures



Supplemental Figure S1: Concentration response curve for Terfenadine in standard internal solution or fluoride-free solution are shown overlaid.



Supplementary Figure S2. Mean I_{Peak} values observed after applying 0, 0.3, 1 and 3 μM free internal Ca²⁺ in standard internal solution or fluoride-free solution at -120 mV (bars below 0 nA) or 60 mV (bars above 0 nA).