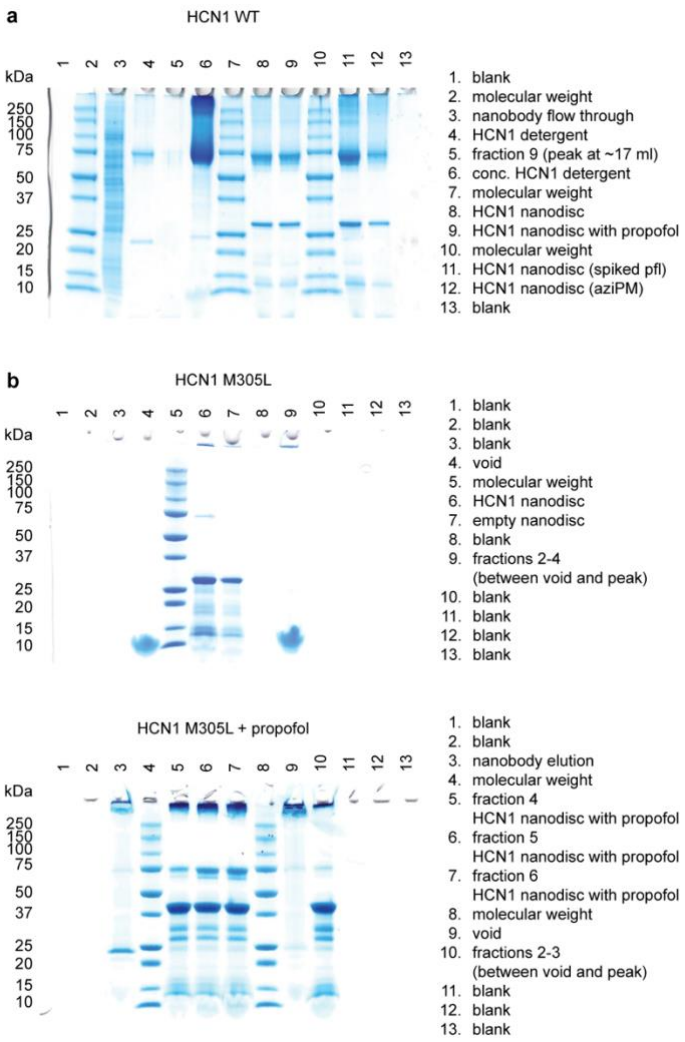
nature portfolio

https://doi.org/10.1038/s41586-024-07743-z

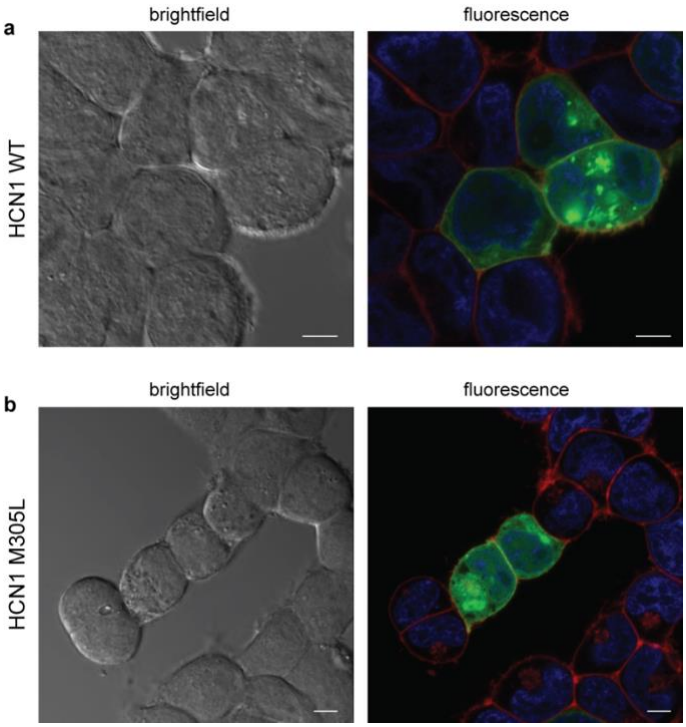
Supplementary information

Propofol rescues voltage-dependent gating of HCN1 channel epilepsy mutants

In the format provided by the authors and unedited



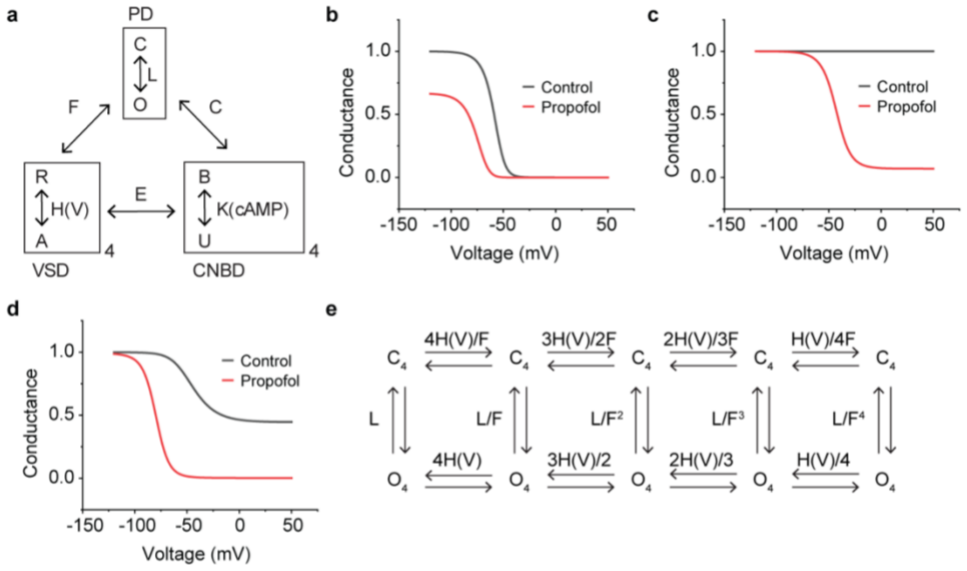
Supplementary Fig. 1 | Uncropped HCN1 WT and M305L purifications. SDS-PAGE of HCN1 **a,** WT and WT + propofol and **b,** M305L and M305L + propofol purifications. Gels were stained with InstantBlue.



Supplementary Fig. 2 | Uncropped HCN1 WT and M305L confocal images. HEK293S GnTI$^-$ cells were transfected with HCN1 **a,** WT and **b,** M305L and stained with NucBlue Live ReadyProbes and CellMask Deep Red. HCN1 is in green, nuclei in blue, and the plasma membrane in red. Shown are brightfield and fluorescent overlay images. Scale bar represents 5 μM.

Supplementary Appendix

To investigate whether our results can be rationalized within the framework of a previously developed state model for HCN channel gating[1] (Appendix Fig. 1a), we simulated the effects of propofol on WT and mutant (M305L and D401H) HCN channel. The model includes three linked equilibria: the PD closed-open (top, described by the equilibrium constant L), The VSD resting-activated (bottom left, described by the equilibrium constant H), and the CNBD resting-activated (bottom right, described by the equilibrium constant K). In the model, the PD is coupled allosterically to the VSD (described by factor F), as well as to the CNBD (described by factor C), indicating that VSD activation and cAMP binding in each of the four subunits respectively leads to an F-fold and C-fold change in the closed-open equilibrium constant, L. Hence, a value of 1 for each of these factors (F, C, E) indicates no coupling. To simulate WT, we used the same parameters as previously[1] (Appendix Fig. 1b), where it was assumed that the outward movement of the voltage sensor to the resting state closes the channel gate and that in an uncoupled HCN channel the gate is mainly open. The M305L mutation was simulated by simply reducing the VSD-PD coupling factor F to 1 (from about 10 in WT) to make the M305L channels voltage independent (Appendix Fig. 1c). The D401H mutation was simulated by mainly increasing the equilibrium constant L about 50-fold compared to WT, as suggested earlier for a homologous mutation in spHCN channels[1] (Appendix Fig. 1d). Both propofol inhibition of WT and restoration of voltage-dependent closing in the two mutants were well-captured by simply reducing the equilibrium constant between the closed and open state fourfold and by increasing the voltage sensor-to-gate coupling threefold for all channels (decreasing L and increasing F both stabilize the closed resting state) (Appendix Fig. 1b-d), in good agreement with our findings.



f

	WT	*WT + pfl*	*M305L*	*M305L + pfl*	*D401H*	*D401H + pfl*
L	5	1.25	5	1.25	500	125
F	10.1	30.3	1	3	5	15
C	1.5	1.5	1.5	1.5	1.5	1.5
E	1	1	1	1	1	1
H_0	0.024	0.024	0.024	0.024	0.024	0.024
S (mV)	9	9	9	9	15	15

Appendix Fig. 1 | HCN model recapitulates effects of propofol on wildtype and M305L channels. a, Model of HCN channel gating developed by Flynn and Zagotta[1] with three channel modules: a pore domain (PD) in either a closed (C) or open (O) state, four voltage sensing domains (VSD) in either a resting (R) or activated (A) state, and four cyclic nucleotide-binding domains (CNBD) in either a bound (B) or unbound (U) state. The three types of domains are coupled by the three coupling factors F, C, and E. Simulations of

G(V)s for HCN **b,** wildtype (WT), **c,** M305L, and **d,** D401H channels in the presence or absence of propofol using the model in *A*. WT Control: L = 5, F = 10.1, C = 1.5, E = 1, H(V) = 0.024exp(V/s) with s = 9.3 mV, and fully bound cAMP (parameters identical to[1]). The M305L mutation is reproduced by decreasing F = 1 to remove voltage-dependent closing in control conditions. The D401H mutation is reproduced by increasing L to 500, as suggested by[1], to prevent complete closure at positive voltages. We also slightly decreased F = 5 and increased s = 15 mV to simulate the shallower slope of the G(V) for D401H in control conditions. Propofol is simulated to stabilize the closed state by reducing L by a factor of 4 and increasing F by a factor of 3 in all three panels.

Kinetic Modeling Methods

We used a previously developed 20-state model of HCN channels[1] (Appendix Fig. 1a) to explain the effects of propofol and the M305L mutation on HCN channel gating. The model has 4 subunits, with one pore domain (PD) with S5-S6 from all four subunits that can be either closed (C) or open (O) governed by the equilibrium constant L when all VSDs are activated, 4 voltage sensing domains (VSD) that can be in the resting (R) or activated (A) state governed by the equilibrium constant H(V), and 4 cyclic nucleotide-binding domains (CNBD) that can be either with cAMP bound (B) or unbound (U) (here all assumed to be in the bound state in our simulations). Deactivation of each VSD decreases the closed-open transition by a factor F, and binding of cAMP to each CNBD increases the closed-open transition by a factor C. The only voltage dependent transitions are the independent movements of the voltage sensors (S4) modeled by H(V) = H0 exp(-V/s) with H0 = 0.024, s = 9.3 mV. All parameters for control conditions on WT channels were as in Flynn and Zagotta[1]. The conductance versus voltage curves were simulated with the equation $G(V) = L(1/F + H/F)^4/\{ (1 + H/F)^4 + L(1 + H/F)^4 \}$ which is a simplified version of the equation 5[1]under the assumption that cAMP is bound to the channel in whole oocyte recordings ($\alpha = 1$) and without the normalization factor, (1 + L)/L . It has previously been shown that cAMP concentration in oocytes is 3-5 μM[2] and the Km for spHCN for cAMP is < 1 μM[3], so we think it is a good approximation that cAMP is bound in our experiments. This reduces the model in Appendix Fig. 1a to the model shown in Appendix Fig. 1e. The parameters used for the simulations are shown in Appendix Fig. 1f.

Supplementary References

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