Supporting Information

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SI Text

Calculation of FRET and Correction of Fc. The spectral separation method for measuring FRET has several advantages. This method eliminates errors from variability in the quantum yield or the expression level of donor and acceptor molecules. Ratiometric analysis of spectra also has the advantage of an internally consistent control because, over a range of emission wavelengths, a properly subtracted FRET ratio remains constant (1). The measurement of FRET using spectra is complicated by emission from the donor (in this case, CFP) and direct excitation of the acceptor (in this case, YFP) by the 458-nm laser line. To measure the component of the spectra due to FRET, we first measured the ratio (Ratio A) of F^{458} to the emission of YFP by the 488 laser line (Fig. 2*A*, F_{488}, black trace) as in Eq. **1**. F^{458} is determined by subtracting the donor-only component.

$$\text{Ratio A} = F_{458}/F_{488} = (F_{458\ \text{FRET}}/F_{488}) + (F_{458\ \text{direct}}/F_{488}) \quad [1]$$

The value $F_{458\ \text{FRET}}/F_{488}$ can be solved by first solving for the ratio of $F_{458\ \text{direct}}$ to F_{488} (Ratio A_0) as in Eq. **2**. Ratio A_0 was experimentally determined in a separate control experiment in oocytes expressing an YFP-hERG ΔN S620T channel (Fig. 2*B*) as the ratio of peak intensity from YFP at 488 excitation (Fig. 2*B*, F_{488}, black trace) versus 458 excitation (Fig. 2*B*, $F_{458\ \text{direct}}$, red trace) as in Eq. **2**.

$$\text{Ratio } A_0 = F_{458\ \text{direct}}/F_{488} \quad [2]$$

Solving Ratio A − Ratio A_0 yields $F_{458\ \text{FRET}}/F_{488}$, as in Eq. **3**, which is a value related to FRET efficiency, and decreases as FRET decreases and increases as FRET increases (Fig. 2*E*).

$$\text{Ratio A} - \text{Ratio } A_0 = F_{458\ \text{FRET}}/F_{488} \quad [3]$$

In our experiments, some of the observed CFP intensities are reduced because of transfer of energy to YFP due to FRET. We corrected the FRET-reduced CFP intensity using a method described previously (2) in which the FRET ratio is calculated as

$$\text{FR} = \text{Ratio A}/\text{Ratio } A_0 \quad [4]$$

and the effective FRET efficiency is calculated as

$$E_{\text{eff}} = (\varepsilon \text{YFP}_{458}/\varepsilon \text{CFP}_{458})(\text{FR} - 1) \quad [5]$$

where ε is the molar extinction coefficients for monomeric citrine and mCFP (3, 4). The true CFP emission (Fc) was then calculated as

$$F_{\text{CFP\_true}} = F_{\text{CFP measured}}/(1 - E_{\text{Eff}}) \quad [6]$$

and was reported in Figs. 2–4.

1. Selvin PR (1995) Fluorescence resonance energy transfer. *Methods Enzymol* 246:300–334.
2. Erickson MG, Alseikhan BA, Peterson BZ, Yue DT (2001) Preassociation of calmodulin with voltage-gated Ca(2+) channels revealed by FRET in single living cells. *Neuron* 31:973–985.
3. Rizzo MA, Springer G, Segawa K, Zipfel WR, Piston DW (2006) Optimization of pairings and detection conditions for measurement of FRET between cyan and yellow fluorescent proteins. *Microsc Micro* 12:238–254.
4. Shaner NC, Steinbach PA, Tsien RY (2005) A guide to choosing fluorescent proteins. *Nat Methods* 2:905–909.

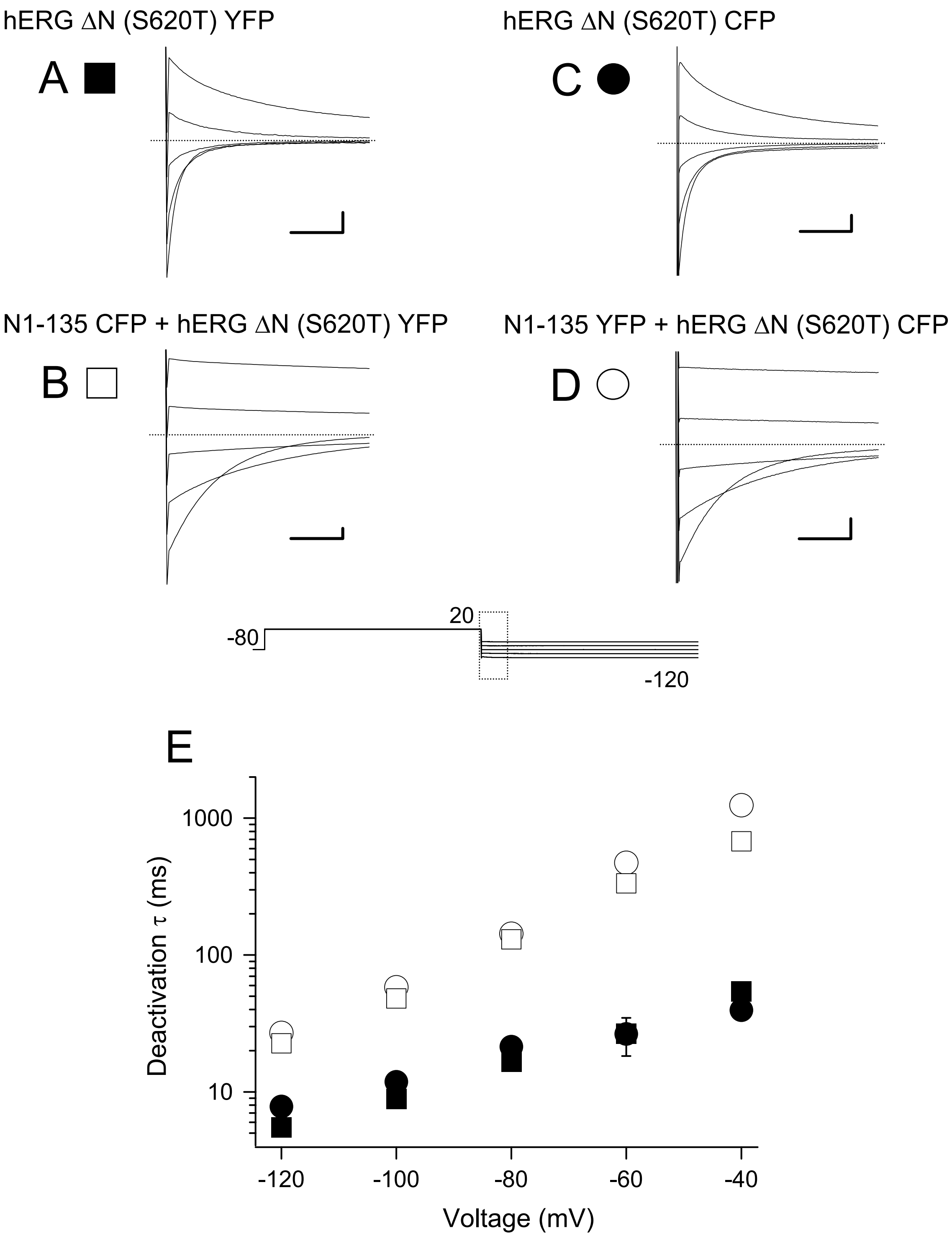

Fig. S1. Regulation of deactivation gating in N-truncated hERG S620T channels by soluble eag domains. Two-electrode voltage-clamp recordings of current families from hERG ΔN S620T channels with eag domain fragments. To better visualize tail currents, traces are shown just from the region of the voltage protocol indicated (box). (*A*) hERG ΔN S620T YFP (closed square); (*B*) N1–135 CFP + hERG ΔN S620T YFP (open square); (*C*) hERG ΔN S620T CFP (closed circle); (*D*) N1–135 YFP + hERG ΔN S620T CFP (open circle); and (*E*) plot of time constant (τ) of deactivation from single exponential fit versus voltage. Error bars denote the SEM and are inside the points when not visible. Calibration bars, 1 μA and 25 ms. $n \geq 4$ for each.

Table S1. Mean time constants of deactivation

Construct	N	−120 mV	−100 mV	−80 mV	−60 mV	−40 mV
hERG YFP	6	59.49 ± 5.05	117.42 ± 8.94	336.55 ± 33.65	1027.96 ± 75.49	1463.61 ± 82.32
hERG ΔN YFP	8	10.00 ± 0.53	16.65 ± 0.85	27.12 ± 1.67	47.54 ± 3.14	149.19 ± 20.49
N1–135 CFP + hERG ΔN YFP	7	55.25 ± 4.79	131.25 ± 9.37	407.67 ± 29.18	1103.91 ± 35.21	2087.84 ± 121.67
CFP + hERG ΔN YFP	4	11.03 ± 0.43	18.14 ± 1.39	30.68 ± 0.85	49.78 ± 2.29	144.77 ± 19.15
hERG ΔN (S620T) YFP	7	5.53 ± 0.49	8.90 ± 0.81	16.70 ± 1.52	26.74 ± 3.09	54.19 ± 4.03
N1–135 CFP + hERG ΔN (S620T) YFP	11	22.61 ± 1.00	48.13 ± 2.88	130.33 ± 9.97	333.89 ± 24.33	678.39 ± 33.92
hERG ΔN (S620T) CFP	4	7.83 ± 1.00	11.85 ± 1.32	21.44 ± 1.41	26.48 ± 8.21	39.51 ± 2.77
N1–135 YFP + hERG ΔN (S620T) CFP	5	26.99 ± 0.94	58.30 ± 2.06	143.25 ± 17.12	473.33 ± 32.19	1246.16 ± 53.12
N1–135 (Y43A) CFP + hERG ΔN YFP	3	24 ± 2.15	35.96 ± 2.69	55.67 ± 2.78	77.84 ± 4.79	207.24 ± 26.29
N1–135 (R56Q) CFP + hERG ΔN YFP	5	10.35 ± 0.39	17.02 ± 0.36	34.09 ± 3.36	45.10 ± 0.83	114.24 ± 5.63
YFP hERG ΔN (S620T)	5	7.87 ± 0.93	14.35 ± 1.31	24.50 ± 3.77	28.53 ± 3.97	66.68 ± 2.64
N1–135 CFP + YFP hERG ΔN (S620T)	5	24.28 ± 1.57	49.22 ± 4.27	128.93 ± 10.60	311.84 ± 51.16	878.91 ± 74.04
N1–135 (Y43A) CFP + YFP hERG ΔN (S620T)	3	8.35 ± 0.97	14.79 ± 1.96	26.90 ± 4.27	44.51 ± 10.04	89.77 ± 8.92
N1–135 (R56Q) CFP + YFP hERG ΔN (S620T)	3	7.29 ± 0.51	11.00 ± 0.63	17.39 ± 0.76	31.47 ± 0.59	66.06 ± 5.55
hERG Y43A	4	25.61 ± 2.12	47.21 ± 4.97	86.87 ± 22.32	272.55 ± 27.37	671.73 ± 38.69
N1–135 CFP + hERG Y43A	4	45.41 ± 4.68	85.48 ± 6.41	180.35 ± 8.07	490.11 ± 31.04	958.51 ± 61.57
hERG R56Q	5	27.80 ± 2.09	46.65 ± 2.68	87.77 ± 14.32	190.78 ± 4.15	649.02 ± 77.49
N1–135 CFP + hERG R56Q	5	50.39 ± 3.18	88.28 ± 5.60	194.03 ± 10.66	509.84 ± 59.53	1298.28 ± 112.48
N1–135 CFP + hERG YFP	4	60.54 ± 3.48	138.72 ± 9.38	514.93 ± 106.75	ND	ND
hERG (S620T) YFP	6	42.08 ± 6.59	132.20 ± 21.72	443.13 ± 65.81	ND	ND
N1–135 CFP + hERG (S620T) YFP	4	38.28 ± 7.06	107.60 ± 22.77	352.61 ± 52.05	ND	ND
hERG Y43A (S620T) YFP	4	21.99 ± 0.76	40.05 ± 2.10	87.21 ± 2.47	271.31 ± 83.06	402.52 ± 46.34
N1–135 CFP + hERG Y43A (S620T) YFP	5	29.05 ± 3.06	57.97 ± 5.72	151.45 ± 12.44	495.39 ± 36.14	883.44 ± 91.40
hERG R56Q (S620T) YFP	4	14.21 ± 1.88	25.08 ± 2.79	50.49 ± 4.37	123.05 ± 20.85	357.29 ± 21.54
N1–135 CFP + hERG R56Q (S620T) YFP	5	24.81 ± 1.89	52.34 ± 4.88	134.02 ± 15.08	544.08 ± 78.97	833.47 ± 28.11

Values shown are the mean ± SEM. Tail currents are fit with a single exponential function $y = Ae^{-1/\tau}$. ND indicates that fits were not able to be determined for those points.