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Supplemental Information

A Selectivity Filter Gate Controls

Voltage-Gated Calcium Channel

Calcium-Dependent Inactivation

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Supplementary material for

A selectivity filter gate controls voltage-gated calcium channel calcium-dependent inactivation

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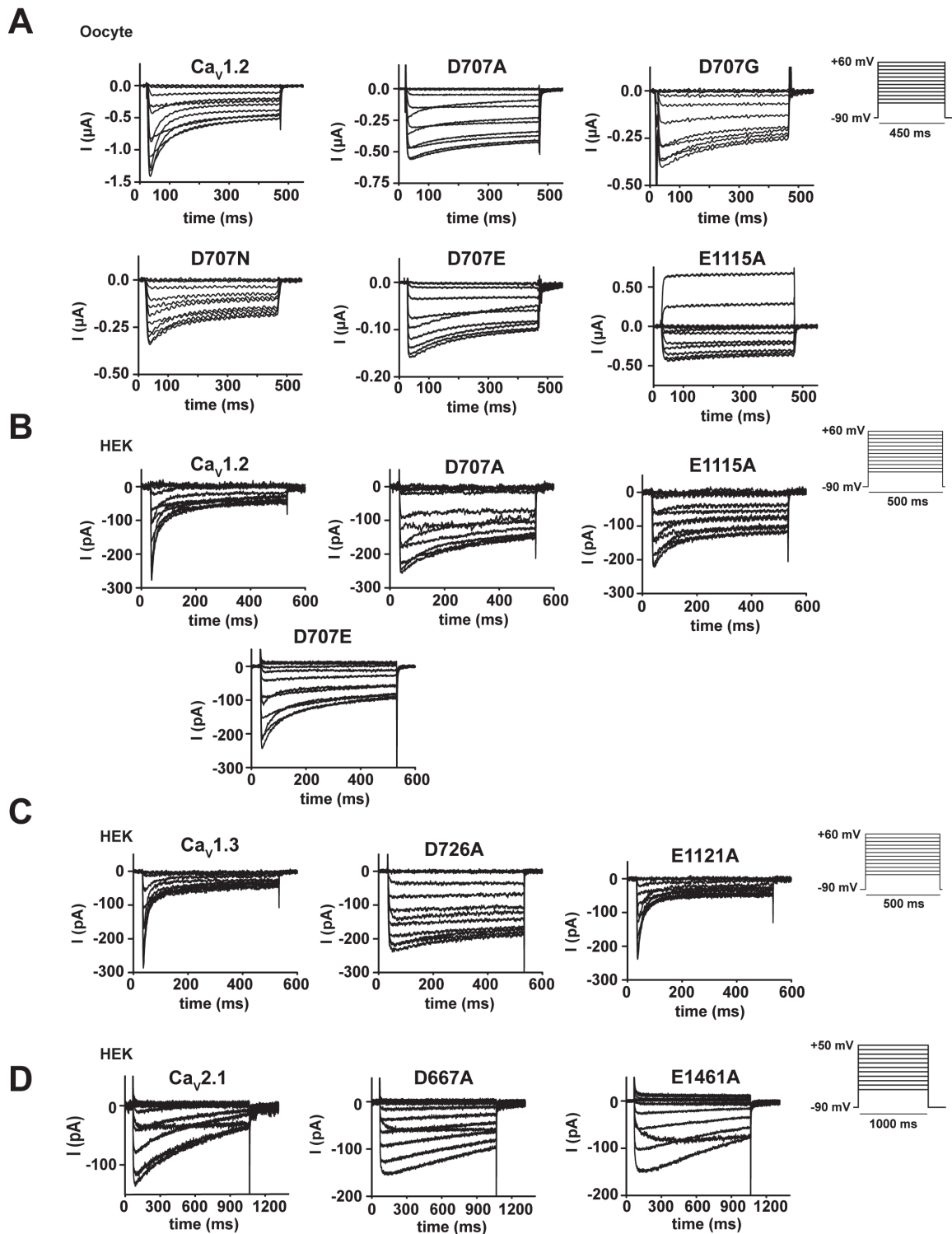


Figure S1 Exemplar Ca²⁺ currents for Ca_v1.2, Ca_v1.3, Ca_v2.1, and mutants co-expressed with Ca_vβ_{2a}. Related to Figures 1, 2, and 4. Exemplar traces recorded in **A, *Xenopus* oocytes expressing Ca_v1.2 or the indicated mutants, **B**, HEK 293 cells expressing Cav1.2 or the indicated mutants, **C**, HEK 293 cells expressing Ca_v1.3 or the indicated mutants, and **D**, HEK 293 cells expressing Ca_v2.1 or the indicated mutants. Currents were evoked using a multi-step activation protocol (insets).**

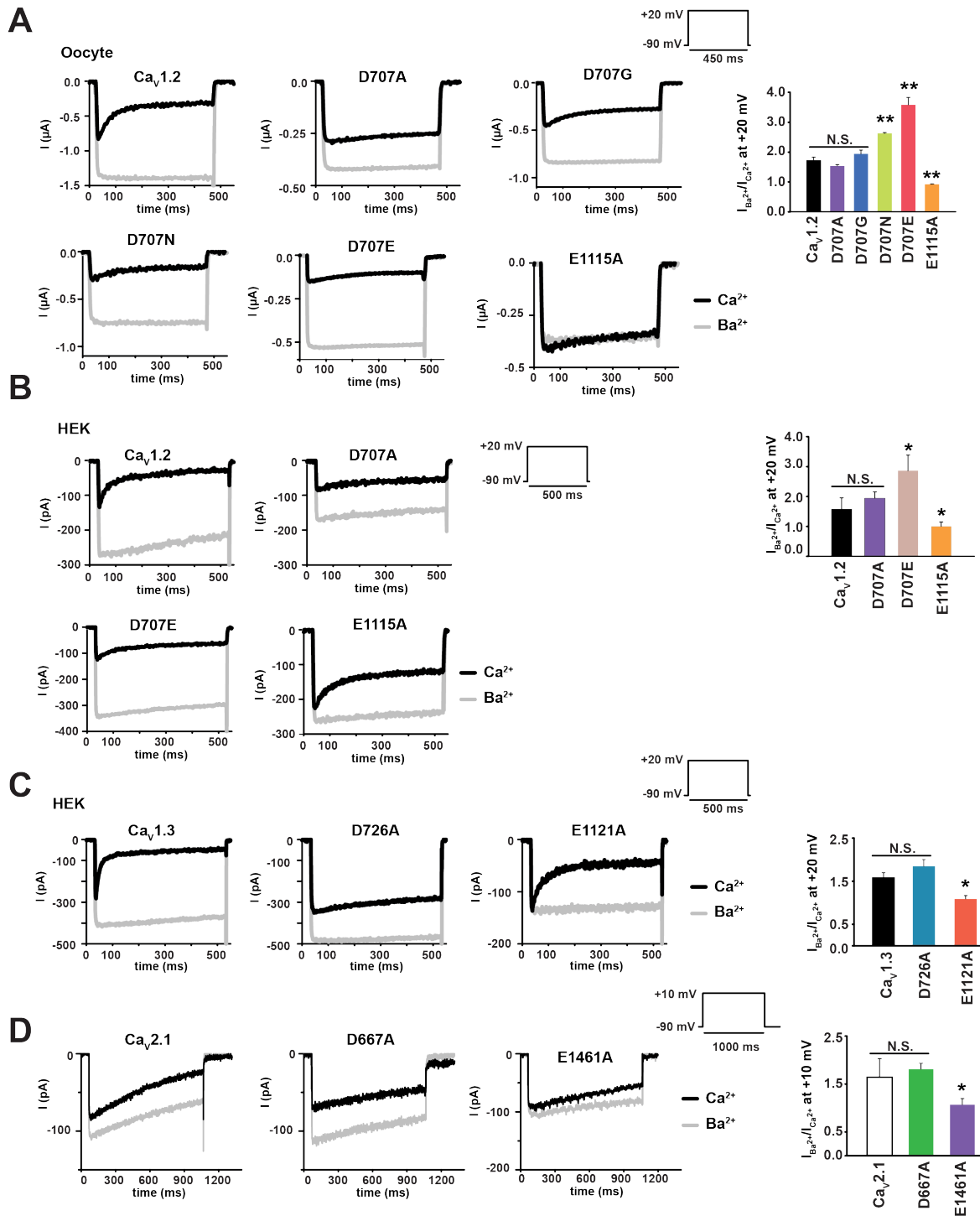


Figure S2 SFII (+1) mutants do not dramatically reduce Ca_v selectivity properties. Related to Figures 1, 2, and 4. A, Exemplar recordings at +20 mV in Ca^{2+} (black) or Ba^{2+} (grey) from *Xenopus* oocytes expressing $\text{Ca}_v1.2$ or indicated mutants, **B**, from HEK293 cells expressing $\text{Ca}_v1.2$ or indicated mutants, **C**, from HEK293 cells expressing $\text{Ca}_v1.3$ or indicated mutants, and **D**, HEK293 cells expressing $\text{Ca}_v2.1$ or indicated mutants. Right panels represent averaged values of ratios between Ba^{2+} and Ca^{2+} peak current amplitude at +20 mV or $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ or at +10 mV for $\text{Ca}_v2.1$. ‘*’ indicates $p < 0.01$, ‘***’ indicates $p < 0.001$, and ‘N.S.’ indicates ‘not statistically significant’ compared to wild-type channels. n values for all bar graphs are in Table 1. All recordings were made with $\text{Ca}_v\beta_{2a}$.

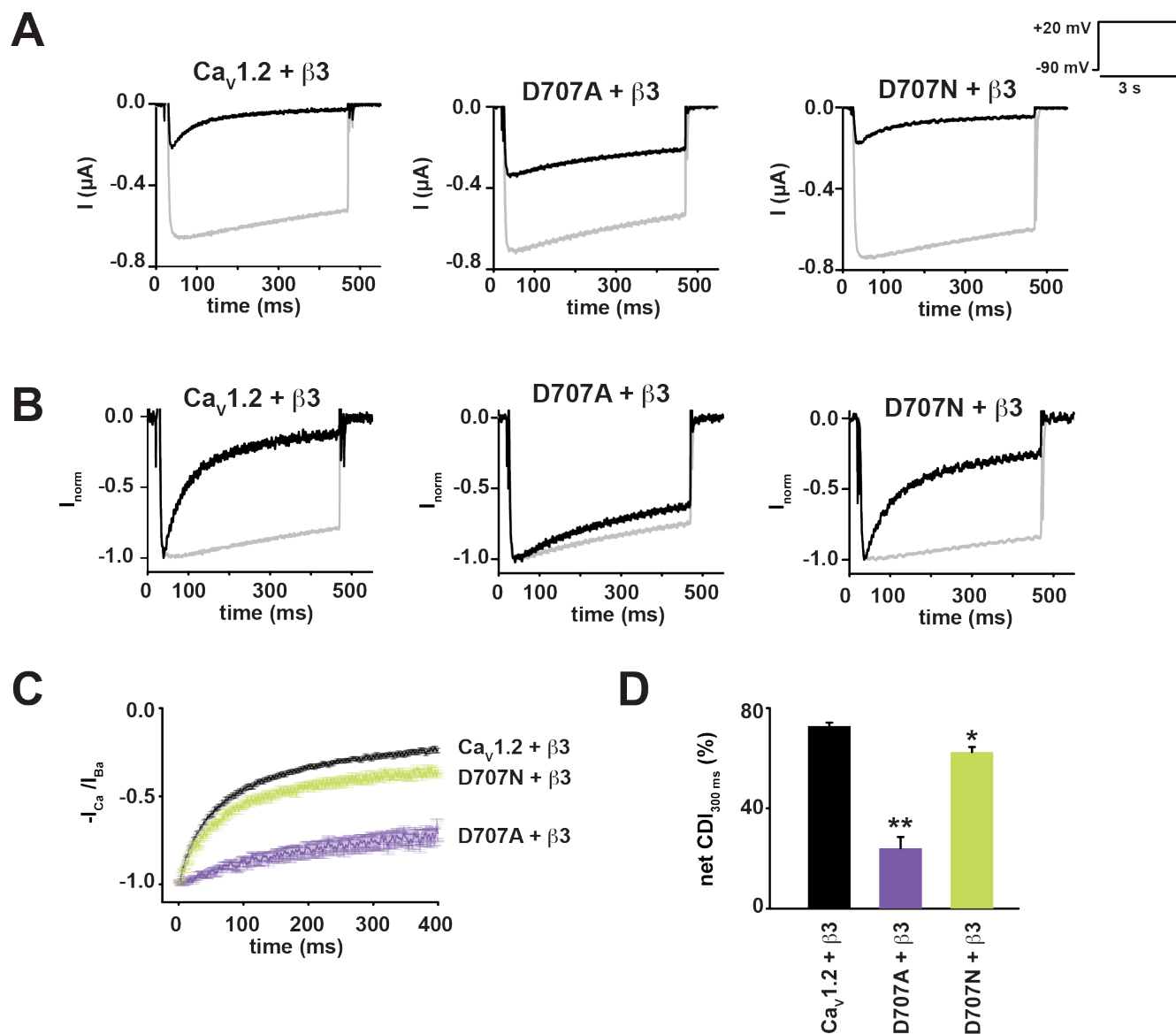


Figure S3 Ca_v1.2 selectivity filter mutations affect CDI independently of Ca_vβ. Related to Figure 1. **A**, Exemplar raw traces recorded at +20 mV in Ca²⁺ (black) or Ba²⁺ (grey) from *Xenopus* oocytes expressing Ca_v1.2 or the indicated mutants with Ca_vβ₃. **B**, Normalized traces from 'A'. **C**, Ratio of normalized I_{Ca}/I_{Ba} currents (net CDI, (Barrett and Tsien, 2008; Findeisen and Minor, 2009)) showing average plots ± s.e.m. **D**, net CDI 300-ms post-depolarization. '**' indicates p<0.01 and '***' indicates p<0.001 compared to Cav1.2. n = 5-10.

Figure S4

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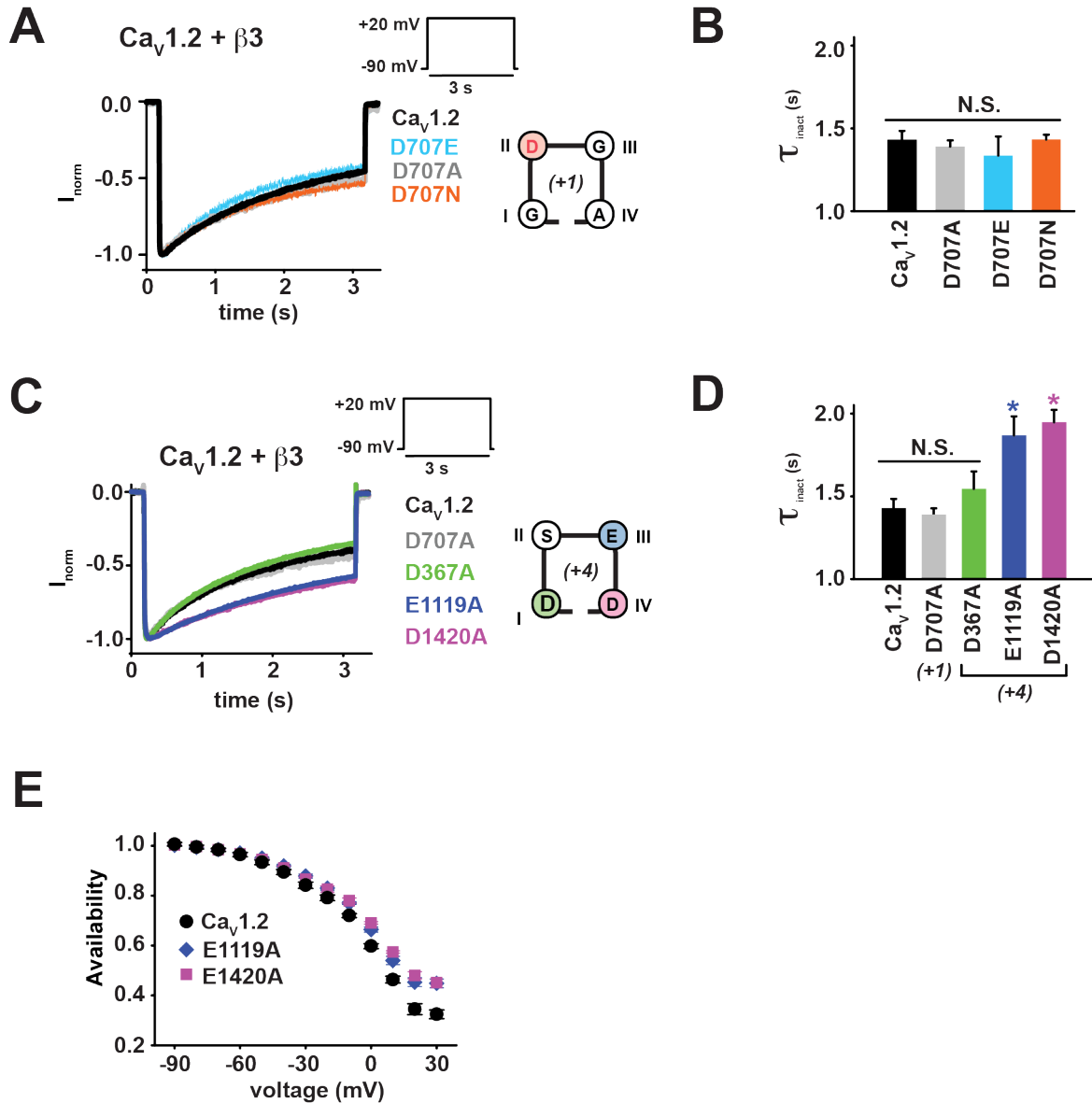


Figure S4 DII (+1) aspartate mutations affecting CDI spare VDI. Related to Figure 1. **A**, Exemplar two electrode voltage clamp recordings at +20 mV from *Xenopus* oocytes expressing $Ca_v1.2$ (black), $Ca_v1.2$ D707A (grey), $Ca_v1.2$ D707E (light blue), or $Ca_v1.2$ D707N (orange) with $Ca_v\beta_3$. **B**, τ_{inact} for 'A'. $n = 6-15$. **C**, Exemplar two electrode voltage clamp recordings at +20 mV from *Xenopus* oocytes expressing $Ca_v1.2$ (black), $Ca_v1.2$ D707A (grey), $Ca_v1.2$ D367A (green), $Ca_v1.2$ E1119A (blue), or $Ca_v1.2$ D1420A (pink) with $Ca_v\beta_3$. **D**, τ_{inact} for 'C'. $n = 9-19$. '*' indicates $p < 0.001$, and 'N.S.' indicates 'not statistically significant' compared to $Ca_v1.2$. **E**, Voltage-dependent inactivation curves representing channel availability after a steady-state inactivation at the indicated potentials.

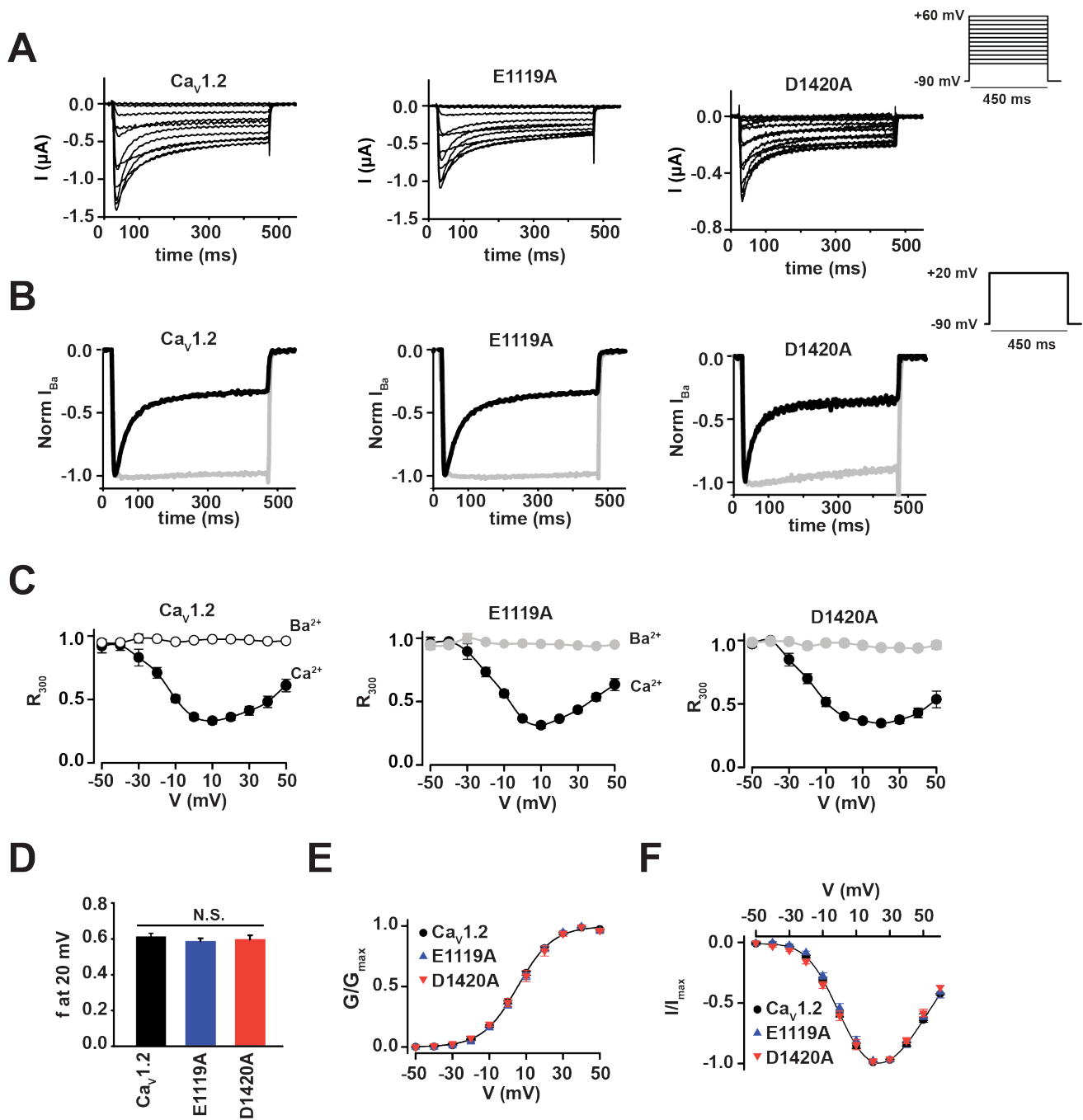


Figure S5 SF (+) mutations affecting VDI spare CDI. Related to Figure 1. **A**, Exemplar traces recorded in *Xenopus* oocytes co-expressing Ca_v1.2, Ca_v1.2 E1119A, or Cav1.2 D1420A with Cavβ_{2a} in response to the indicated protocol. **B**, Exemplar normalized recordings at +20 mV in Ca²⁺ (black) or Ba²⁺ (grey) from *Xenopus* oocytes expressing Ca_v1.2, Ca_v1.2 E1119A, or Cav1.2 D1420A. **C**, Fractional current remaining 300 ms post-depolarization (R₃₀₀) as a function of the membrane potential for channels in 'B'. **D**, Average fraction CDI (f) at +20 mV. 'N.S.' indicates 'not statistically significant'. n values are found in Table 1. **E**, Voltage-dependent activation curves for Ca_v1.2 (black circles), Ca_v1.2 E1119A (blue triangles), and Cav1.2 D1420A (red inverted triangles). **F**, I-V relationships for the indicated channels. Symbols are the same as 'E'.

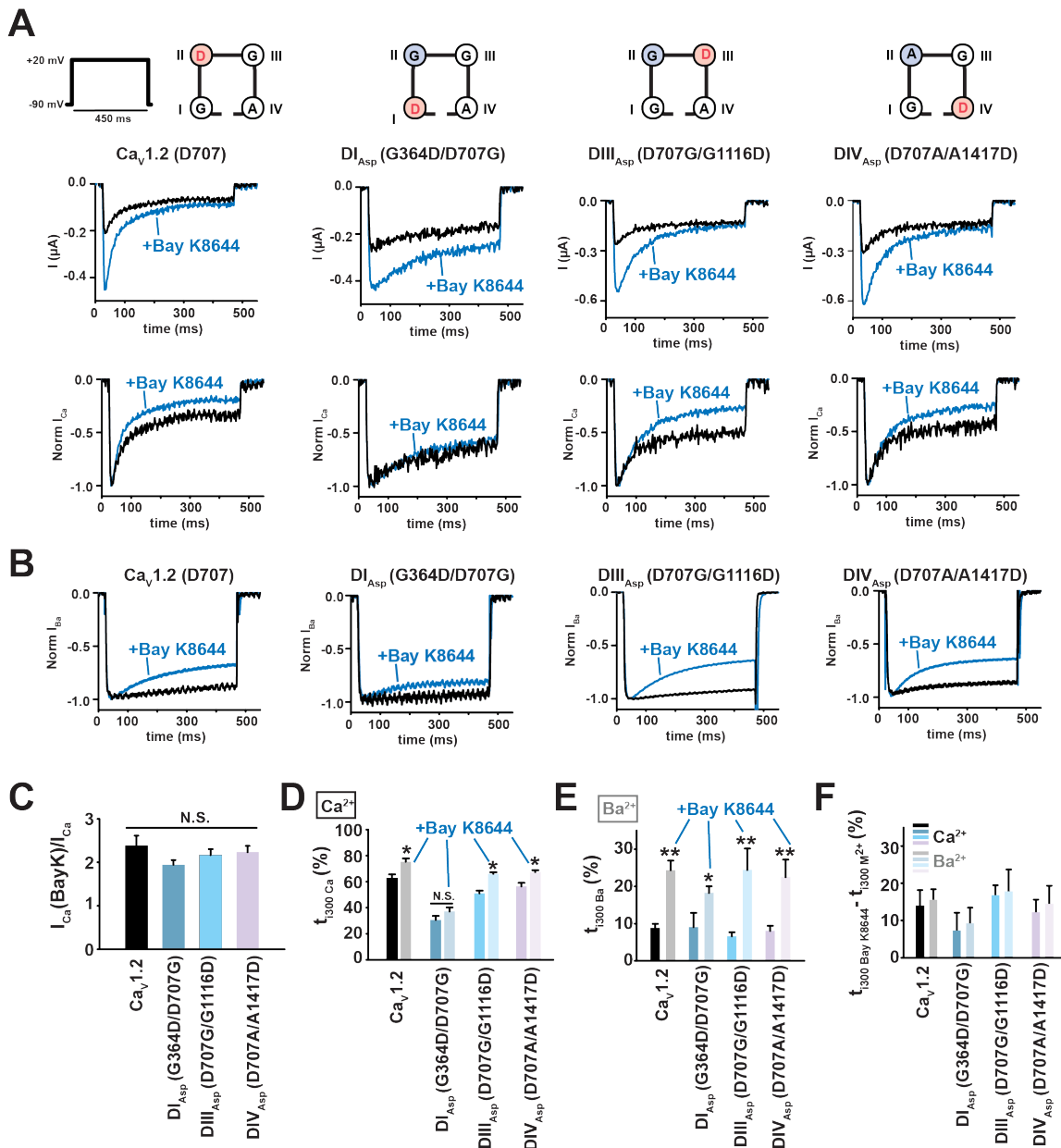


Figure S6 Ca_v1.2 CDI requires a negative charge at +1 position on DII, DIII, or DIV, not DI, regardless of the amount of Ca²⁺ influx. Related to Figures 3 and 5. A, Exemplar Ca²⁺ currents in response to a +20 mV depolarization from *Xenopus* oocytes, co-expressing Ca_v1.2 or the indicated mutants with Cavβ_{2a} in the absence (black) or presence of 5 μM Bay K8644 (blue). Raw traces (upper panels) and normalized traces (lower panels) are shown to illustrate Bay K8644 effects on both the peak current and inactivation, respectively. **B**, Exemplar normalized Ba²⁺ currents from *Xenopus* oocytes expressing Ca_v1.2 or the indicated mutants, in response to a +20 mV depolarization in absence (black) or in presence of 5 μM Bay K8644 (blue). **C**, Ratio of Ca²⁺ current amplitudes in presence, I_{Ca}(BayK), and in absence, I_{Ca}, of Bay K8644. ‘N.S.’ indicates not statistically different. n = 4-7. **D** and **E**, Percentage of inactivation 300 ms post-depolarization (t₃₀₀) in the absence (dark bars) and presence of Bay K8644 (light bars) on recordings performed using **D**, Ca²⁺, or **E**, Ba²⁺ as the charge carrier. ‘*’ indicates p<0.01 and ‘***’ indicates p<0.001. n= 6-11. **F**, Difference in t₃₀₀ induced by Bay K8644 in Ca²⁺ (dark bars) and Ba²⁺ (light bars).