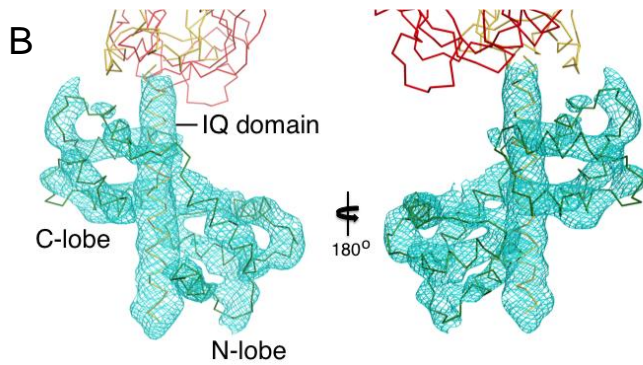
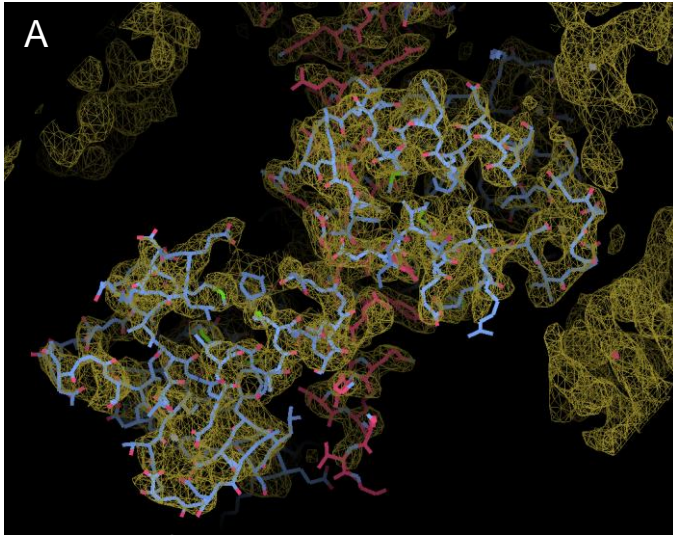
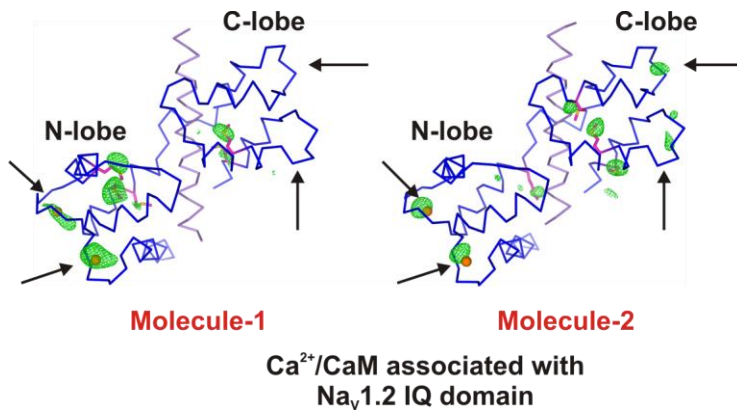


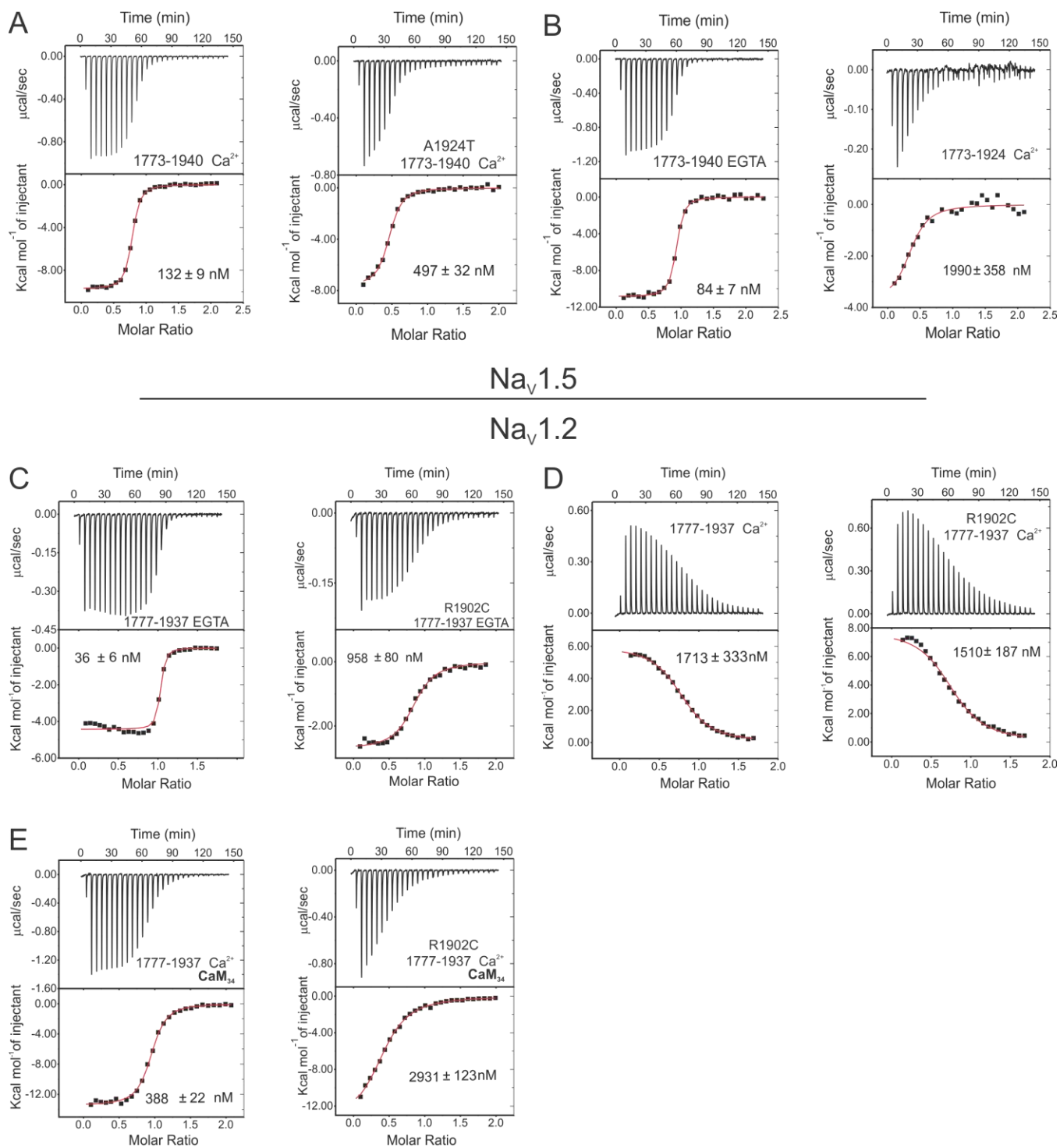
Supplementary Figure 1: Gel filtration chromatography profiles of the ternary complex of Na_v1.2 CTD, FGF13U, and Ca²⁺/CaM (left); Na_v1.5 CTD, FGF12B, and Ca²⁺/CaM (Right). Migration of standards (Ferritin, MW 440 kDa; Human IgG, MW 150 kDa; Human transferrin, MW 81 kDa; Ovalbumin, MW 43 kDa; and Myoglobin, MW 17.6 kDa) is indicated by arrows. The inset shows Coomassie blue-stained samples from the peak fractions used for crystallization. Molecular weight markers are indicated.



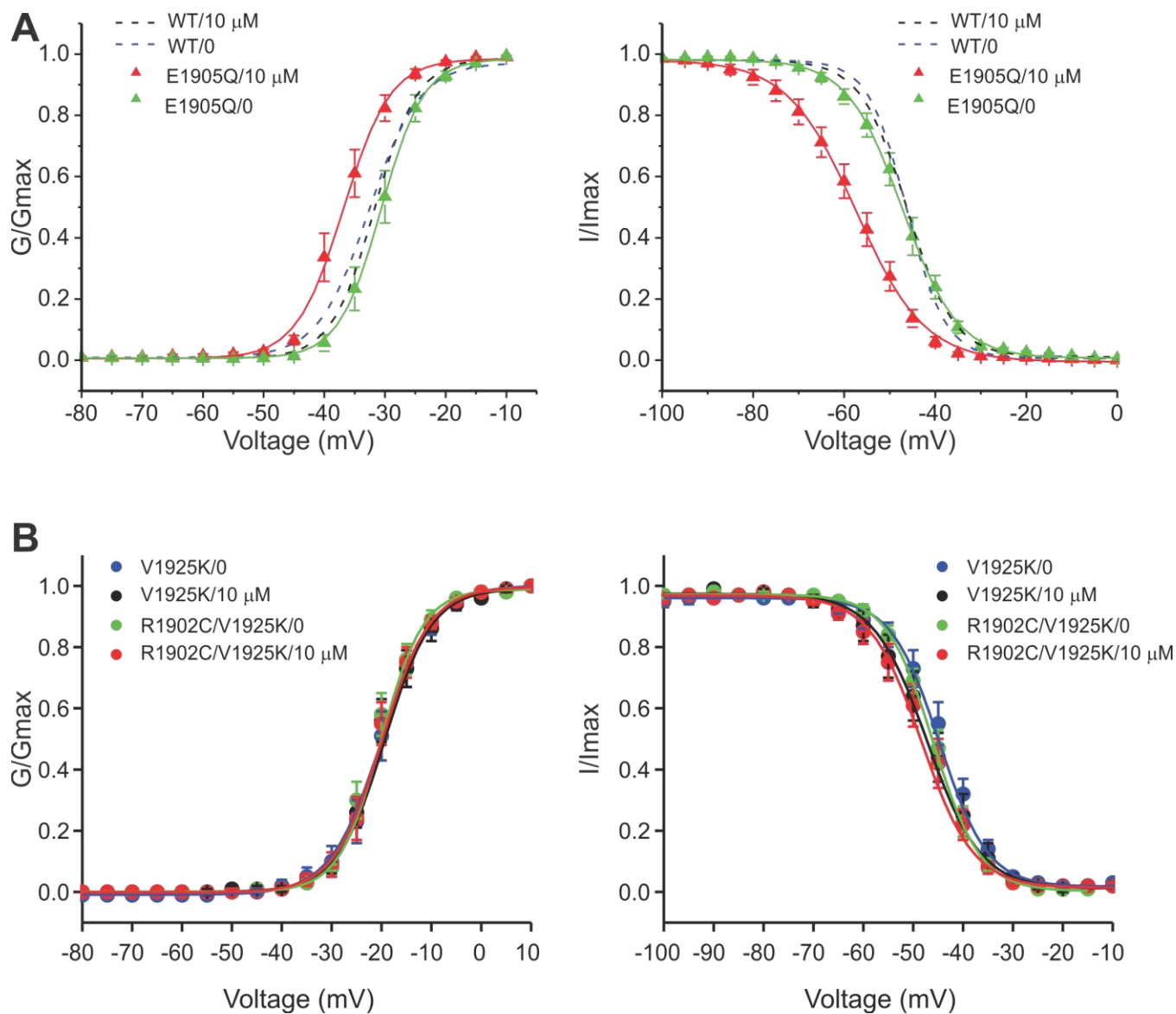
Supplementary Figure 2: A. Experimental phasing of the Na_v1.2 CTD/FGF13/Ca²⁺-CaM crystal. The electron density peaks, contoured at 1 σ , are shown in yellow mesh. Phases were obtained by single anomalous dispersion (SAD) from a SeMet-substituted crystal followed by density modification. The final model containing CaM (cyan) and Na_v1.2 CTD (red) is shown. **B.** 2Fo-Fc OMIT map of Na_v1.5/Ca²⁺. The map was calculated using phases derived from the final model without CaM. The final models for CaM (green), Nav1.5 CTD (yellow), and FHF (red) are shown in C α .



Supplementary Figure 3: Identification of Ca²⁺ within CaM. Anomalous difference Fourier map for two CaMs and in the asymmetric unit from the Na_v1.2/+Ca²⁺ crystal. The map was calculated using data from 25.0 Å- 5.5 Å of the native crystal using the final model phases. The anomalous difference peaks, colored in green mesh, are contoured at 2.8 σ . The arrows indicate the expected positions of Ca²⁺ in CaM. Side-chains of methionines are shown. Ca²⁺ is shown as a red ball.



Supplementary Figure 4: Exemplar ITC data for CaM – Na_v CTD interactions. See Tables 1 and 2 in the main text for specific fitting parameters.



Supplementary Figure 5: Electrophysiological characterization of Na_v1.2 mutants. A., Activation and steady-state inactivation relationships in 0 mM Ca²⁺ or 10 μ M free Ca²⁺ in the recording pipette for E1905Q mutant. The data for wild type are repeated from Fig. 3. See Supp. Table 2 for parameters. **B.**, Activation and steady-state inactivation relationships in 0 mM Ca²⁺ or 10 μ M free Ca²⁺ in the recording pipette for V1925K and the double mutant V1925K/R1902C. See Supp. Table 2 for parameters.

Supplementary Table 1. Summary of Electrophysiological Data

	Free [Ca ²⁺] _i (μ M)	V _{1/2} of activation (mV)	k of activation (pA/mV)	V _{1/2} of inactivation (mV)	k of inactivation (pA/mV)	N
WT	0	-32.6 ± 1.6	1.5 ± 0.2	-46.1 ± 0.7	4.0 ± 0.2	14-16
	10	-31.8 ± 0.9	1.5 ± 0.2	-46.1 ± 1.2	3.7 ± 0.1	21-22
R1902C	0	-30.3 ± 1.6	2.0 ± 0.2	-47.3 ± 1.5	4.9 ± 0.2	12-13
	10	-39.2 ± 1.2 *	1.5 ± 0.2	-55.0 ± 0.1 *	3.9 ± 0.7	25-27
E1905Q	0	-30.8 ± 1.1	2.3 ± 0.4	-47.4 ± 1.3	4.8 ± 0.3	15-16
	10	-36.8 ± 1.0 *	1.8 ± 0.1	-58.1 ± 1.9 *	6.3 ± 0.4	17-18
V1925K	0	-20.2 ± 1.7	3.8 ± 0.4	-44.1 ± 1.5	4.5 ± 0.3	10
	10	-20.3 ± 1.3	4.0 ± 0.4	-47.1 ± 2.0	4.5 ± 0.3	8-9
R1902C/ V1925K	0	-20.8 ± 1.3	3.6 ± 0.3	-45.7 ± 1.1	4.3 ± 0.2	10-11
	10	-20.5 ± 1.4	3.4 ± 0.4	-47.5 ± 1.8 *	4.4 ± 0.3	11

* P < 0.05 vs. Ca²⁺ free conditions