# Supporting Information

## Gonzalez-Gutierrez *et al.* 10.1073/pnas.0806558105

### SI Materials and Methods

**Sucrose Density Gradient Centrifugation.** Sucrose gradient analysis on purified proteins was performed as described previously (1). Purified $Ca_V\beta_{2a}$-GK and $Ca_V\beta_{1b}$-GK alone or together with a protein marker were fractionated on a linear sucrose gradient (0–15% in buffer containing 20 mM Tris, 300 mM NaCl, 1 mM EDTA, pH 8.0). Gradients were generated by a gradient mixer to a final volume of 4 ml, and the proteins were layered on top of separate gradients and centrifuged in a Beckman SW-41 Ti rotor at 100,000 $\times$ *g* for 16 h at 4°C. After sedimentation, individual gradients were fractionated bottom-to-top by dropwise collection into 14–16 tubes, and all fractions were analyzed by SDS/PAGE and Western blot analysis. All sucrose gradients were repeated at least twice.

**Western Blot Analysis.** Aliquots from the different sucrose gradient fractions were resolved on a reducing SDS/PAGE, and the gels were electroblotted onto nitrocellulose membrane (Hybond-ECL; GE Healthcare) using sodium carbonate buffer (pH 9.9). After blocking with 3% bovine serum albumin, the membrane was incubated with anti-Penta-His antibody (Qiagen). Secondary horseradish peroxidase-coupled rabbit anti-mouse IgG antibody (Pierce) was used for detection of immune complexes by enhanced chemiluminescence (Super ELISA Femto Maximum Sensitivity Kit; Pierce) using the GeneGnome bioimaging system (Syngene).

**Electrophysiologic Recording.** Electrophysiologic recordings were performed 4–6 days after cRNA injection and 2–7 h after protein injection (50 nl of the protein stock solution) using the cut-open oocyte technique (2) with a Dagan CA-1B amplifier as described previously (3). The external solution contained 10 mM $Ba^{2+}$, 96 mM *N*-methylglucamine, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) (pH 7.0); the internal solution contained 120 mM *N*-methylglucamine, 10 mM EGTA, and 10 mM Hepes (pH 7.0). For a better control of calcium-activated chloride currents, EGTA was replaced by BAPTA in the internal solution when recording oocytes expressing the $Ca_V2.3$ subunit. Data acquisition and analysis were performed using the pCLAMP system and software (Axon Instruments). Currents were filtered at 2 kHz and digitized at 10 kHz. Linear components were eliminated by a P/-4 prepulse protocol (4).


1. Neely A, Garcia-Olivares J, Voswinkel S, Horstkott H, Hidalgo P (2004) Folding of active calcium channel beta(1b) subunit by size-exclusion chromatography and its role in channel function. *J Biol Chem* 279:21689–21694.
2. Taglialatela M, Toro L, Stefani E (1992) Novel voltage clamp to record small, fast currents from ion channels expressed in *Xenopus* oocytes. *Biophys J* 61:78–82.
3. Hidalgo P, Gonzalez-Gutierrez G, Garcia-Olivares J, Neely A (2006) The alpha 1-beta subunit interaction that modulates calcium channel activity is reversible and requires a competent alpha-interaction domain. *J Biol Chem* 281:24104–24110.
4. Neely A, Wei X, Olcese R, Birnbaumer L, Stefani E (1993) Potentiation by the $\beta$ subunit of the ratio of the ionic current to the charge movement in the cardiac calcium channel. *Science* 262:575–578.

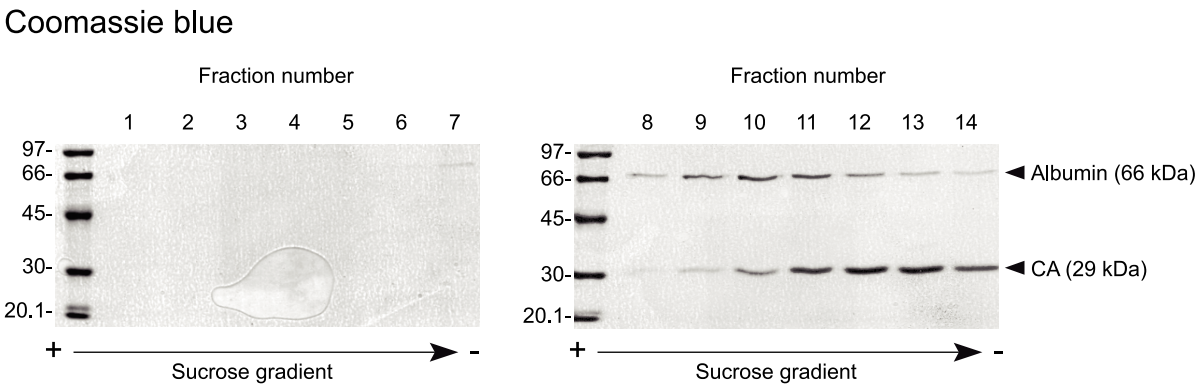


**Fig. S1.** Sucrose gradient analysis of molecular standards. Two molecular mass standards, albumin (66 kDa; Sigma) and carbonic anhydrase (CA) (29 kDa; Sigma), were loaded onto a 0–15% linear sucrose gradient. After sedimentation, the gradient was fractionated bottom-to-top into 14 fractions, and each fraction was resolved onto SDS/PAGE to monitor the protein distribution profile. Albumin and CA distributed throughout the second half of the gradient, with CA appearing at later fractions, as expected due to its reduced molecular mass.

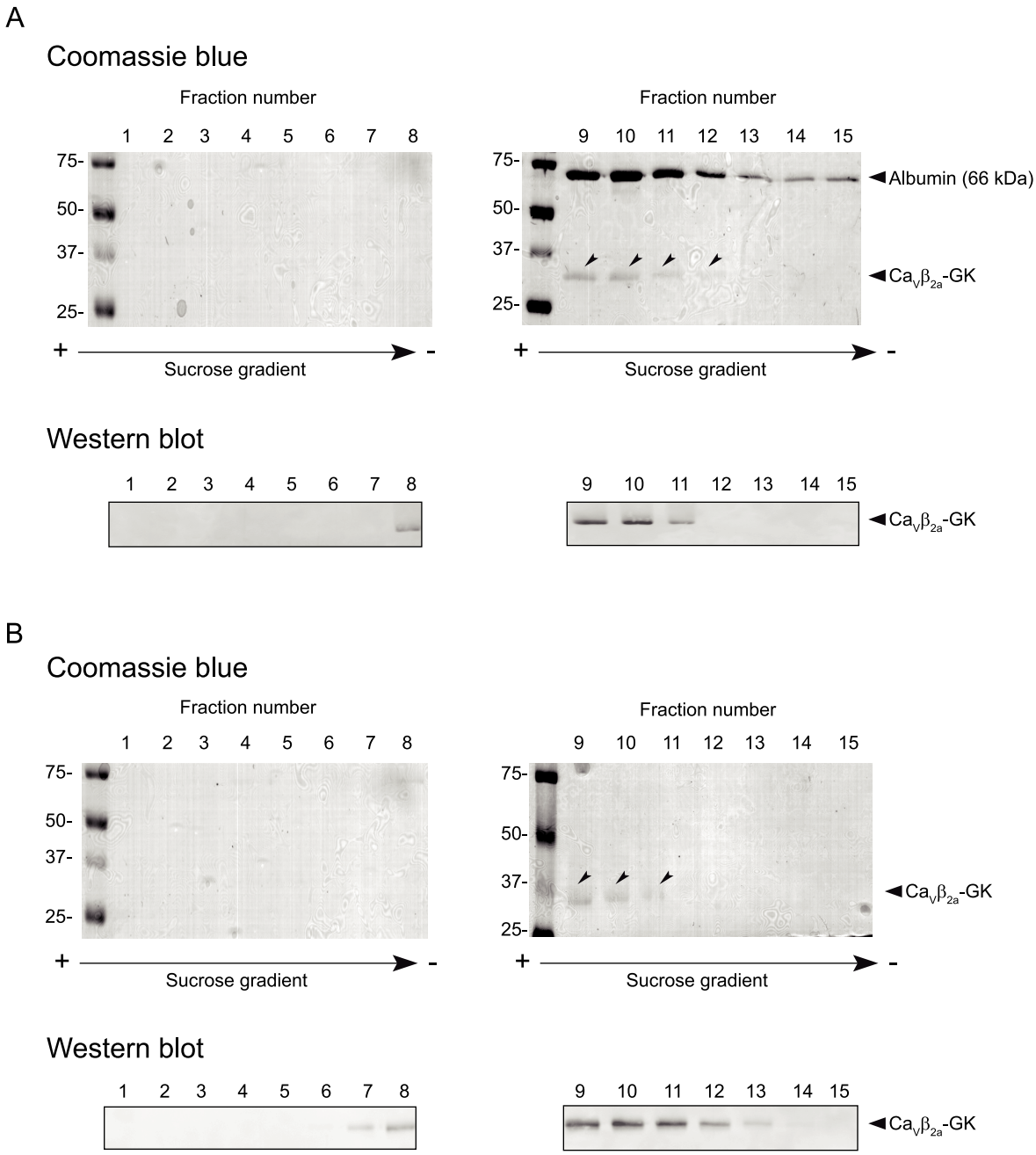


**Fig. S2.** Sucrose gradient analysis of $Ca_V\beta_{2a}$-GK. Two separated 0–15% linear sucrose gradients were loaded with $Ca_V\beta_{2a}$-GK either together with albumin (*A*) or alone (*B*). After sedimentation, the gradient was fractionated bottom-to-top into 15 fractions, and each fraction was resolved onto SDS/PAGE. The presence of albumin did not alter the sedimentation profile of $Ca_V\beta_{2a}$-GK. $Ca_V\beta_{2a}$-GK, with a predicted molecular mass of 28.6 kDa, was co-distributed with albumin, indicating the presence of dimers.

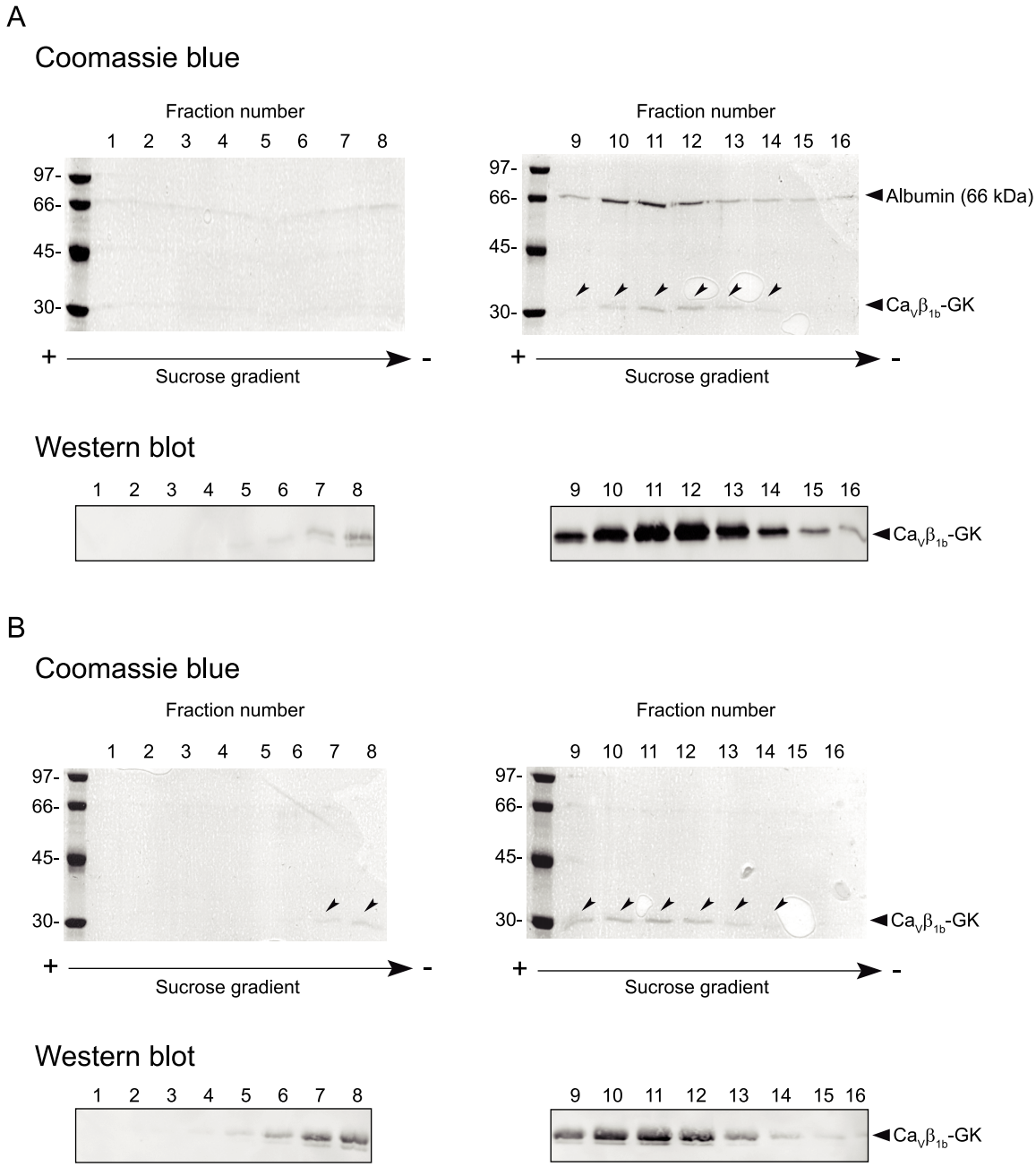


**Fig. S3.** Sucrose gradient analysis of Ca$_V$β$_{1b}$-GK. Two separated 0–15% linear sucrose gradients were loaded with Ca$_V$β$_{1b}$-GK either together with albumin (*A*) or alone (*B*). After sedimentation, the gradient was fractionated bottom-to-top into 16 fractions, and each fraction was resolved onto SDS/PAGE. The presence of albumin did not alter the sedimentation profile of Ca$_V$β$_{1b}$-GK. Ca$_V$β$_{1b}$-GK was distributed along with albumin and CA (see Fig. S1), suggesting the coexistence of dimers and monomers.

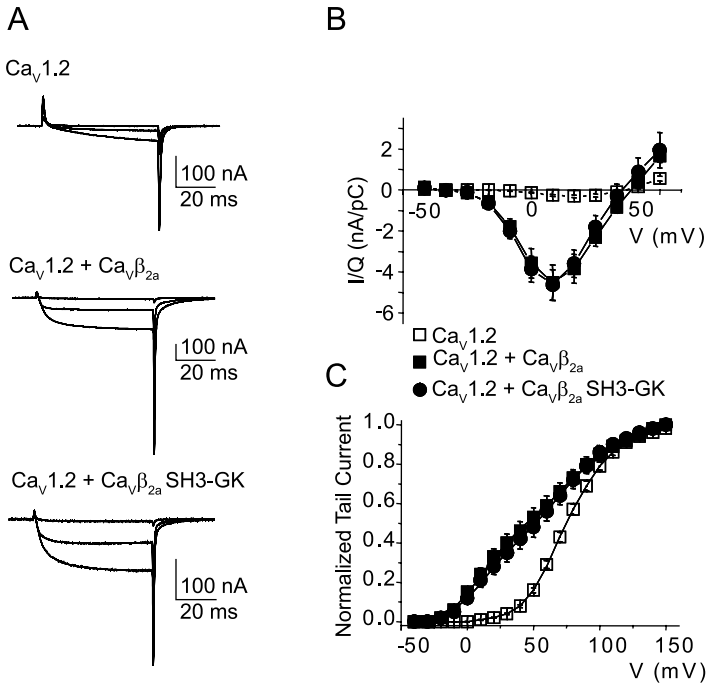


**Fig. S4.** $Ca_V\beta_{2a}$-SH3-GK is as effective as full-length $Ca_V\beta_{2a}$ in modulating activation of $Ca_V1.2$-mediated currents. (*A*) Representative gating and ionic currents traces from oocytes expressing $Ca_V1.2$ cRNA alone and after injection of $Ca_V\beta_{2a}$ or $Ca_V\beta_{2a}$-SH3-GK during a 60-ms pulse to −30, 0, and +30 mV from a holding potential of −80 mV. (*B*) Current–voltage plot normalized by charge movement (I/Q) from oocytes expressing the different subunit combinations shown in (*A*). The peak I/Q for oocytes expressing $Ca_V1.2$ alone was 0.30 ± 0.06 nA/pC ($n$ = 15), 4.6 ± 0.8 nA/pC ($n$ = 17) for $Ca_V1.2 + Ca_V\beta_{2a}$-SH3-GK, and 4.5 ± 0.9 nA/pC ($n$ = 18) for $Ca_V1.2 + Ca_V\beta_{2a}$. (*C*) Normalized tail currents from oocytes expressing the different subunit combinations shown in (*B*). The continuous lines correspond to the fit of the sum of two Boltzmann distributions that best describe each set of data (for details, see Table S1 and *SI text*).

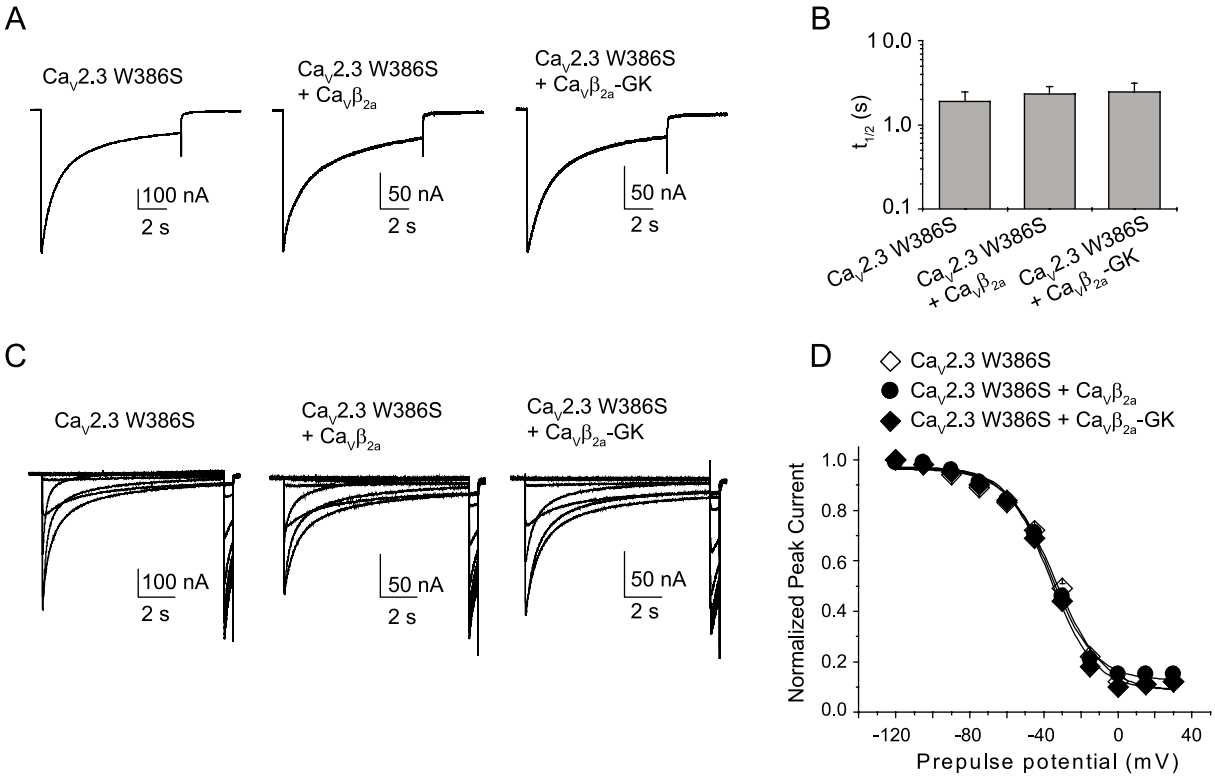


**Fig. S5.** Mutation of a fully conserved tryptophan within the AID sequence yields Ca$_V$2.3 channels insensible to full-length Ca$_V\beta_{2a}$ and refolded Ca$_V\beta_{2a}$-GK. (*A*) Representative current traces from oocytes expressing Ca$_V$2.3 W386S cRNA alone or after injection of either full-length Ca$_V\beta_{2a}$ or Ca$_V\beta_{2a}$-GK during a 10-s pulse to 0 mV from a holding potential of −90 mV. (*B*) Average decay times to t½ for the different subunit combinations shown in (*A*). (*C*) Representative current traces evoked with the steady-state inactivation pulse protocol consisting of a 10-s conditioning period to voltages of increasing amplitude, from −120 mV to + 30 mV in 15-mV increments, followed by a 0.4-s test pulse to 0 mV. (*D*) Average steady-state inactivation curves from oocytes expressing Ca$_V$2.3 W386S cRNA alone or after injection of either full-length Ca$_V\beta_{2a}$ or Ca$_V\beta_{2a}$-GK. The continuous lines correspond to the fit to Boltzmann distributions plus a noninactivating current component that best describes each set of data (for details, see Table S2).

**Table S1. Parameters defining the sum of two Boltzmann distributions that best fit the normalized tail currents for the specified $Ca_V1.2$ subunit combinations**

| | Max I/Q, nA/pC | | $G_1$ fraction, | $V_1$, mV, | $Z_1$, | $V_2$, mV, | $Z_2$ | |
|---|---|---|---|---|---|---|---|---|
| Combination | Mean ± SEM | *n* | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM | *n* |
| $Ca_V1.2$ | 0.30 ± 0.06 | 15 | 0.64 ± 0.02 | 68.5 ± 2.9 | 2.0 ± 0.1 | 97.3 ± 2.6 | 1.5 ± 0.1 | 16 |
| $Ca_V1.2$ + $Ca_V\beta_{2a}$ | 4.51 ± 0.85* | 18 | 0.47 ± 0.04* | 15.9 ± 3.5* | 2.1 ± 0.2 | 77.5 ± 4.8* | 1.4 ± 0.1 | 11 |
| $Ca_V1.2$ + $Ca_V\beta_{2a}$-SH3-GK | 4.63 ± 0.75* | 17 | 0.47 ± 0.08* | 19.0 ± 5.8* | 2.1 ± 0.4 | 80.4 ± 6.3* | 1.5 ± 0.1 | 6 |
| $Ca_V1.2$–$Ca_V\beta_{2a}$ concatamer | 2.44 ± 0.44* | 17 | 0.58 ± 0.02 | 38.4 ± 4.8* | 1.3 ± 0.1* | 97.1 ± 2.9 | 1.5 ± 0.1 | 17 |
| $Ca_V1.2$–$Ca_V\beta_{2a}$-GK concatamer | 2.71 ± 0.52* | 17 | 0.58 ± 0.01 | 48.6 ± 5.8* | 1.3 ± 0.1* | 90.1 ± 4.5 | 1.5 ± 0.0 | 15 |
| $Ca_V1.2$ W470S–$Ca_V\beta_{2a}$-GK concatamer | 0.35 ± 0.03† | 17 | 0.60 ± 0.01 | 65.9 ± 1.1† | 2.1 ± 0.1† | 95.8 ± 1.6 | 1.5 ± 0.0 | 17 |

Mean ± SEM of maximal current normalized by total charge movement (I/Q) and parameters defining the best fit to the sum of two Boltzmann distributions that describe the activation curves. Peak tail currents ($I_{tail}$) were measured during deactivation at −40 mV after a 65-ms pulse of increasing voltage, normalized and plotted versus to pre-pulse potential to yield the GV curve that was then fitted to

$$I_{tail}(V) = \left(\frac{I_{MAX1}}{1 + \exp[(V_1 - V)\cdot z_1 F/RT]} + \frac{I_{MAX2}}{1 + \exp[(V_2 - V)\cdot z_2 F/RT]}\right)$$

where $R$ is the universal gas constant, $F$ is the Faraday constant, $T$ is temperature, $V$ is the voltage preceding repolarization to −40 mV, $I_{MAX1}$ and $I_{MAX2}$ are the contribution of each Boltzmann distribution, characterized by slope factors $z_1$ and $z_2$ and half activation potentials $V_1$ and $V_2$. Normalized conductances were obtained by $I_{tail}/(I_{MAX1} + I_{MAX2})$, yielding $G_1$ and $G_2$ fractions.

*$t$ test; $P < 0.01$ compared with values measured in oocytes expressing $Ca_V1.2$ alone.

†$t$ test; $P < 0.01$ compared with values measured in oocytes expressing $Ca_V1.2$–$Ca_V\beta_{2a}$ concatamer.

**Table S2. Average $t_{1/2}$, and parameters defining the Boltzmann distribution and the percentage of noninactivating current component that best fit the steady-state inactivation for the specified $Ca_V2.3$ subunit combinations**

| Combination | Inactivation rate, $t_{1/2}$, s | | Steady-state inactivation parameters | | | |
|---|---|---|---|---|---|---|
| | Mean ± SEM | *n* | $V_{1/2}$, mV, mean ± SEM | *Z*, mean ± SEM | % $I_{res}$, mean ± SEM | *n* |
| $Ca_V2.3$ | 0.33 ± 0.03 | 26 | −48.7 ± 1.0 | −2.2 ± 0.1 | 0.6 ± 0.5 | 22 |
| $Ca_V2.3$ + $Ca_V\beta_{2a}$ | 4.76 ± 0.70* | 16 | −41.3 ± 1.4* | −2.6 ± 0.2 | 35.2 ± 4.2* | 13 |
| $Ca_V2.3$ + $Ca_V\beta_{2a}$ cRNA | 4.11 ± 0.65* | 12 | −42.4 ± 2.2 | −2.2 ± 0.1 | 19.5 ± 2.2*† | 12 |
| $Ca_V2.3$ + $Ca_V\beta_{2a}$-SH3-GK | 3.57 ± 0.42* | 21 | −44.3 ± 1.1* | −2.1 ± 0.0 | 8.4 ± 1.2*† | 23 |
| $Ca_V2.3$ + $Ca_V\beta_{2a}$-SH3 | 0.35 ± 0.04 | 13 | NA | NA | NA | NA |
| $Ca_V2.3$ + $Ca_V\beta_{2a}$ GK cRNA | 0.41 ± 0.05 | 13 | −45.9 ± 1.6 | −2.6 ± 0.1 | 1.4 ± 1.8† | 12 |
| $Ca_V2.3$ + $Ca_V\beta_{2a}$-GK | 1.85 ± 0.44*† | 13 | −41.5 ± 1.8* | −2.3 ± 0.1 | 6.3 ± 1.6*† | 15 |
| $Ca_V2.3$ + $Ca_V\beta_{1b}$-GK | 4.20 ± 0.87* | 16 | −40.9 ± 1.4* | −1.8 ± 0.1*† | 8.1 ± 1.2*† | 14 |
| $Ca_V2.3$ + $Ca_V\beta_{1b}$ cRNA | 0.29 ± 0.02 | 11 | −59.1 ± 1.2* | −2.7 ± 0.1* | 0.7 ± 0.4 | 11 |
| $Ca_V2.3$ + $Ca_V\beta_{1b}$ late-injection | 2.19 ± 0.25*‡ | 18 | −46.8 ± 0.9‡ | −2.1 ± 0.1‡ | 7.0 ± 0.8*‡ | 18 |
| $Ca_V2.3$ + $Ca_V\beta_{1b}$ co-injection | 0.27 ± 0.02* | 14 | −59.3 ± 0.8* | −2.2 ± 0.1‡ | 5.1 ± 0.7*‡ | 14 |
| $Ca_V2.3$ + $Ca_V\beta_{2a}$ C3,4S late-injection | 2.86 ± 0.48*‡ | 16 | −42.9 ± 1.6*‡ | −2.1 ± 0.1‡ | 10.5 ± 2.4*‡ | 12 |
| $Ca_V2.3$ + $Ca_V\beta_{2a}$ C3,4S co-injection | 0.80 ± 0.08*‡ | 13 | −55.7 ± 0.8* | −2.4 ± 0.1‡ | 2.1 ± 0.7 | 14 |
| $Ca_V2.3$ W386S | 1.92 ± 0.54* | 19 | −34.3 ± 1.1* | −2.0 ± 0.1 | 8.5 ± 1.5 | 17 |
| $Ca_V2.3$ W386S + $Ca_V\beta_{2a}$ | 2.35 ± 0.50* | 17 | −36.8 ± 1.0* | −2.1 ± 0.1 | 7.1 ± 2.1 | 15 |
| $Ca_V2.3$ W386S + $Ca_V\beta_{2a}$-GK | 2.48 ± 0.66* | 15 | −36.8 ± 1.5* | −2.2 ± 0.1 | 13.2 ± 3.4 | 14 |

Steady-state parameters were obtained by plotting the peak currents in a test pulse to 0 mV after a prepulse to different voltages and fitting to the following equation:

$$I = I_{res} + \frac{(I_{max} - I_{res})}{1 + e^{-\frac{zF}{RT}(V - V_{1/2})}}$$

$I_{res}$ is the noninactivating current, *F* is the Faraday constant, *R* is the universal gas constant, *T* is temperature (298 K), *V* is the membrane voltage, and $V_{1/2}$ is the voltage at which the fraction of inactivated and non-inactivated channels is equal. NA, not applicable.

*$t$ test; $P < 0.01$ compared with values measured in oocytes expressing $Ca_V2.3$ alone.

†$t$ test; $P < 0.01$ compared with values measured in oocytes expressing $Ca_V2.3$ + $Ca_V\beta_{2a}$.

‡$t$ test; $P < 0.01$ compared with values measured in oocytes expressing $Ca_V2.3$ + $Ca_V\beta_{1b}$ cRNA.