

SUPPLEMENTAL DATA

Supplemental data on the investigation of potential interacting partners with the N-terminus of Ca_v2.2 using the yeast two hybrid assay

Methods

Assays were carried out using the MATCHMAKER GAL4 yeast two hybrid kit (Clontech). Fragments of Ca_v2.2 within the first domain (amino acids 96-355, 180-225, 246-330), within the Ca_v2.2 N-terminus (1-95, 45-95, 56-95, 45-55), the Ca_v2.2 I-II loop (360-483), II-III loop (711-1154), III-IV loop (1422-1476), C-terminus (1713-2339) and Ca_vβ1b were generated by PCR and subcloned in-frame into the vectors pACT2 and pAS2-1. Plasmids were cotransformed into the yeast strain Y190 and transformants were selected by plating onto minimal selective dropout (SD) *-Leu*, *-Trp* agar. Protein interactions were identified by re-streaking colonies onto SD *-Leu*, *-Trp* plates and carrying out colony-lift β-galactosidase assays according to the supplied protocol.

Results

We were unable to demonstrate any positive interactions between the N-terminus of Ca_v2.2 and the Ca_v2.2 I-II loop or Ca_v2.2 Dom I using the yeast two hybrid assay, in contrast to a previous study (1). The Ca_v2.2 N-terminus was also truncated into smaller fragments (amino acids 45-95, 56-95 and 45-55), none of which were shown to interact with Ca_v2.2 I-II linker. We also made fusion proteins containing regions of domain I of the Ca_v2.2; the transmembrane domains (amino acids 96-355), the Domain IS4 trans-membrane region (180-225) and the p-loop region (246-330). None of these were found to interact with the full-length (1-95) or the truncated (45-95) N-terminus of Ca_v2.2 in this assay. In addition, dimerization was investigated for each insert and found to be negative for all except Ca_vβ1b. All fragments were tested and found negative for autoactivation. As a positive control in all assays, the Ca_v2.2 I-II loop was shown to interact with Ca_vβ1b, as described previously (2). Therefore we cannot identify a high affinity interaction of the Ca_v2.2 N-terminus with a particular peptide domain in the yeast two hybrid system, providing some support for the view that the N-terminus is more likely to interact in a complex binding pocket, involving multiple elements (Table 1).

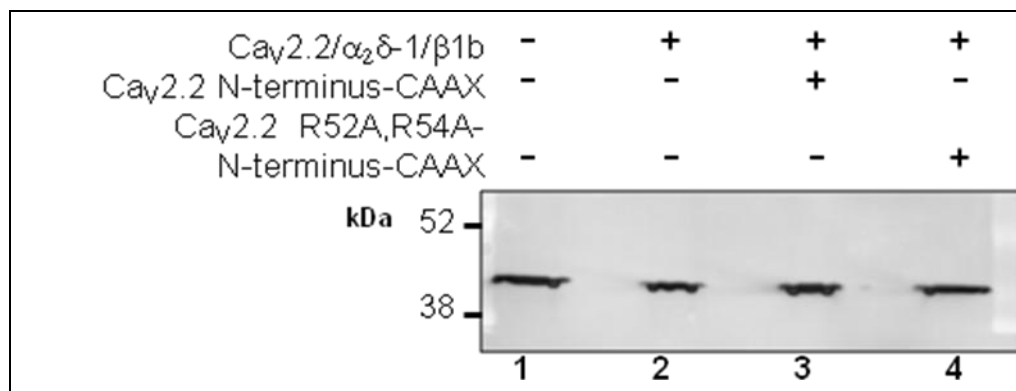
Table 1**Summary of potential interactions examined by yeast two hybrid**

	Ca_v2.2 N-term (1-95)	Ca_v2.2 N-term (45-95)	Ca_v2.2 N-term (56-95)	Ca_v2.2 N-term (45-55)	Ca_vβ1b
Ca_v2.2 N-term (1-95)	-	ND	ND	ND	-
Ca_v2.2 I-II (360-483)	-	-	-	-	+++
Ca_v2.2 II-III (711-1154)	-	-	ND	ND	-
Ca_v2.2 III-IV (1422-1476)	-	ND	ND	ND	-
Ca_v2.2 C-term (1713-2339)	-	-	-	-	-
Ca_v2.2 Dom I (96-355)	-	-	ND	ND	-
Ca_v2.2 Is4 (180-225)	-	-	ND	ND	ND
Ca_v2.2 I-p-loop (246-330)	-	-	ND	ND	ND
Ca_vβ1b	-	-	ND	ND	+

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Supplemental Figure 1

Expression of GAPDH as a loading control



Expression of GAPDH protein in untransfected tsA-201 cells (lane 1), when Ca_v2.2/α₂δ-1/β1b were expressed, alone (lane 2) and together with Ca_v2.2 N-terminus-CAAX (lane 3), or Ca_v2.2 R52A, R54A-N-terminus-CAAX (lane 4). The same amount of total protein was loaded for all samples on a gel, for accurate comparison between lanes. These samples were the same as those illustrated in Fig. 6A. The primary Ab was a mouse anti-GAPDH monoclonal at 1:25,000. This experiment was replicated 3 times for other samples used for the bar chart in Fig. 6B, with identical results that GAPDH was unchanged.

References

1. Agler, H. L., Evans, J., Tay, L. H., Anderson, M. J., Colecraft, H. M., and Yue, D. T. (2005) *Neuron* **46**, 891-904
2. Dresviannikov, A. V., Page, K. M., Leroy, J., Pratt, W. S., and Dolphin, A. C. (2009) *Pflugers Arch.* **457**, 743-756