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Transcriptional and electrophysiological consequences of KChIP2-mediated regulation of $Ca_V1.2$

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Keywords

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Potassium channel interacting proteins (KChIP) are Ca^{2+}-binding proteins that originally were identified as auxiliary subunits for K_V4 channels. K_V4 channels encode the voltage gated A-current (I_A) in neuronal tissue and the fast, transient outward current ($I_{to,f}$) in cardiac tissue. Recently, we have reported that KChIP2 functionally modulates the cardiac $Ca_V1.2$-governed L-type Ca^{2+} current ($I_{Ca,L}$) through a direct interaction between KChIP2 and the amino-terminus of $Ca_V1.2$. Here, we show that KChIP2 and $Ca_V1.2$ co-immunoprecipitate enhancing the biochemical support for our previous finding. Using gene-chip and real-time PCR techniques, we find that $KChIP2^{-/-}$ mice have an increased transcriptional activity of the calcium channel β_2 subunit, CACNB2, whereas the expression of $Ca_V1.2$ is preserved. Although $I_{to,f}$ is absent and $I_{Ca,L}$ is decreased in myocytes from $KChIP2^{-/-}$ mice, the action potential morphology is not altered. Furthermore, we show that the ventricular effective refractory period (VERP) is comparable in wild-type (53 ± 5 ms) and $KChIP2^{-/-}$ mice (48 ± 3 ms; $p > 0.05$). In summary, our findings document a novel function of KChIP2 and expand our insights into the in vivo modulation of cardiac ion currents.

The K^+ channel interacting proteins (KChIPs) are small (216–270 amino acids) cytosolic, calcium-binding proteins that were initially identified as subunits for the voltage-gated A-type K^+ current in neuronal tissue and the transient outward K^+ current in cardiac tissue.[1] The pore-forming protein complex governing these K^+ currents is K_V4. KChIPs, dipeptidyl aminopeptidase-like proteins[2] (DPP), $K_V\beta1$,[3] and potentially several other protein structures assemble in macromolecular structures with K_V4 to generate and regulate the native current.[3]

Four *KCHIP* genes have been identified in humans and mice.[1,4,5] The proteins are characterized by a conserved Ca^{2+}-binding region, which places the KChIPs in the family of small Ca^{2+}-binding proteins, including Ca^{2+}-binding protein 1,[6,7] calmodulin[8,9] and neuronal

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Ca^{2+} sensor 1.[10] The conserved Ca^{2+} binding domain of the KChIPs contains 4 EF hands, of which only the last 3 bind Ca^{2+}.[1] A highly variable amino-terminal sequence preceding the Ca^{2+} binding domain distinguishes the KChIPs from other Ca^{2+}-binding proteins and confers variability among the KChIPs themselves.[6] The 4 *KCHIP* genes can, by use of alternative splicing or alternative use of transcription initiation sites, encode several protein isoforms.[5] KChIP2 is the only KChIP expressed in the heart[1] where 4 and 5 transcript isoforms have been identified in mouse and man, respectively.[5]

Recently, we have shown that KChIP2, in addition to facilitating K_V4-mediated current in vivo,[11,12] also modulates the cardiac L-type Ca^{2+} current ($I_{Ca,L}$).[13] The hypothesis was triggered by the finding that KChIP2 immunoprecipitates with $Ca_V1.2$ α_{1C} from brain tissue (Fig. 1). We found that $I_{Ca,L}$ density is significantly reduced in myocytes disaggregated from KChIP2$^{-/-}$ mice compared to myocytes from wild-type (WT) mice. The decreased current density in the absence of KChIP2 did not result from reduced protein expression or trafficking of the pore-forming $Ca_V1.2$ α_{1C} subunit. Rather, we showed that the channel modulation results from a direct interaction between KChIP2 and the intracellular amino-terminus of the α_{1C} subunit. We were unable to show a direct association between $Ca_V1.2$ and KChIP2 in cardiac tissue, despite the demonstrated functional effects.[13] Several reasons could underlie this, including antibodies with higher affinity for neuronal proteins and higher concentration of ion-channel proteins in brain. However, KChIP2 does copurify with the amino-terminus of $Ca_V1.2$ when co-expressed in a bacterial system.[13] Moreover, co-expression of KChIP2 increased the current density governed by recombinant $Ca_V1.2$ in a transfected, mammalian cell line, further substantiating our finding that KChIP2 directly augments $I_{Ca,L}$.[13] We proposed a model, in which KChIP2 indirectly increases the open probability of $Ca_V1.2$ by alleviating the inhibitory effects of the $Ca_V1.2$ amino-terminus, as previously characterized.[14] Deleting the amino-terminus of $Ca_V1.2$ causes a large increase in the whole-cell current amplitude,[13,15] and studies have shown that the β_2 subunit of $I_{Ca,L}$ in part increases $Ca_V1.2$ open probability by interacting with the amino-terminus.[14] Additional studies showed that KChIP2 could not fully replace the β_2 subunit, likely due to the pivotal role of β_2 in $Ca_V1.2$ trafficking.[16,17] Nevertheless, β_2 and possibly KChIP2 both increase the open probability of $Ca_V1.2$ via modulation of the aminoterminal inhibitory segment of $Ca_V1.2$. Single-channel recordings of $Ca_V1.2$ are required to determine the exact mechanism of KChIP2-induced augmentation of whole-cell Ca^{2+} current.

Microarray analysis with Mouse Genome 430A 2.0 array chips (Affymetrix) of cDNA from total RNA isolated from hearts of 4 KChIP2$^{-/-}$ and 4 C57BL6/J mice revealed that transcription of several genes was comparatively altered in the KChIP2$^{-/-}$ mice. We used quantitative, real-time PCR to verify the microarray results for the tran-scriptional activity of $Ca_V1.2$ and a number of associated subunits in KChIP2$^{-/-}$ mice (Fig. 2). As anticipated, KChIP2 mRNA contents in KChIP2$^{-/-}$ were below the detection levels of both assays, confirming the successful deletion of all KChIP2 isoforms (Fig. 2 and Table 1). Despite the increased protein levels of $Ca_V1.2$ that we observed in KChIP2$^{-/-}$ ventricles,[13] we found no increases in mRNA levels of $Ca_V1.2$ in either assay. This would be compatible with an increased protein stability or decreased protein degradation in the absence of KChIP2; however further experimental work is required to test this hypothesis and elucidate the mechanism. Transcriptional activity of the β_2 subunit was increased in KChIP2$^{-/-}$ ventricles, compared to WT ventricles ($p < 0.05$ in both assays; Fig. 2).

In addition to a reduced $I_{Ca,L}$, the KChIP2$^{-/-}$ mice had no residual $K_V4.2$-mediated $I_{to,f}$;[11,12] however we were unable to identify any differences in action potential morphology in multicelluar preparations.[11] This is in accordance with ECG analysis performed by Kuo et al.[12] and by us,[13] showing a J-wave elevation in KChIP2$^{-/-}$ mice, but no difference in QT intervals between WT and KChIP2$^{-/-}$ mice. Furthermore, the ventricular effective refractory period

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(VERP), established at a basic pacing rate of 100 ms is 53 ± 5 ms in anesthetized WT mice (n = 5) compared to 48 ± 3 ms in KChIP2$^{-/-}$ mice (n = 7; p > 0.05). Hence, although the amplitudes of several depolarizing and repolarizing currents are altered, the overall repolarization reserve seems preserved.[11,18]

It has been reported that KChIP2$^{-/-}$ mice have an increased incidence of ventricular polymorphic tachyarrhythmias in response to programmed electrical stimulation.[12] Interestingly, $K_V4.2^{-/-}$ mice have no arrhythmogenic phenotype,[19,20] suggesting that the augmented susceptibility to arrhythmia in KChIP2$^{-/-}$ mice does not result exclusively from the absence of a functional $I_{to,f}$. Given the prominent J-wave elevation, the KChIP2$^{-/-}$ mice may serve as a model of the early repolarization syndrome, a familial disease characterized by ventricular ectopic activity and sudden death related to an accentuation of the J wave.[21]

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References

1. An WF, Bowlby MR, Betty M, Cao J, Ling HP, Mendoza G, et al. Modulation of A-type potassium channels by a family of calcium sensors. Nature 2000;403:553–6. [PubMed: 10676964]
2. Nadal MS, Ozaita A, Amarillo Y, Vega-Saenz de Miera E, Ma Y, Mo W, et al. The CD26-related dipeptidyl aminopeptidase-like protein DPPX is a critical component of neuronal A-type K^+ channels. Neuron 2003;37:449–61. [PubMed: 12575952]
3. Nerbonne JM, Kass RS. Molecular physiology of cardiac repolarization. Physiol Rev 2005;85:1205–53. [PubMed: 16183911]
4. Morohashi Y, Hatano N, Ohya S, Takikawa R, Watabiki T, Takasugi N, et al. Molecular cloning and characterization of CALP/KChIP4, a novel EF-hand protein interacting with presenilin 2 and voltage-gated potassium channel subunit K_V4. J Biol Chem 2002;277:14965–75. [PubMed: 11847232]
5. Pruunsild P, Timmusk T. Structure, alternative splicing and expression of the human and mouse KCNIP gene family. Genomics 2005;86:581–93. [PubMed: 16112838]
6. Burgoyne RD, Weiss JL. The neuronal calcium sensor family of Ca^{2+}-binding proteins. Biochem J 2001;353:1–12. [PubMed: 11115393]
7. Haeseleer F, Imanishi Y, Sokal I, Filipek S, Palczewski K. Calcium-binding proteins: intracellular sensors from the calmodulin superfamily. Biochem Biophys Res Commun 2002;290:615–23. [PubMed: 11785943]
8. Zuhlke RD, Pitt GS, Deisseroth K, Tsien RW, Reuter H. Calmodulin supports both inactivation and facilitation of L-type calcium channels. Nature 1999;399:159–62. [PubMed: 10335846]
9. Peterson BZ, DeMaria CD, Adelman JP, Yue DT. Calmodulin is the Ca^{2+} sensor for Ca^{2+}-Dependent inactivation of L-type calcium channels. Neuron 1999;22:549–58. [PubMed: 10197534]
10. Zhou H, Yu K, McCoy KL, Lee A. Molecular mechanism for divergent regulation of $Ca_V1.2$ Ca^{2+} channels by calmodulin and Ca^{2+}-binding protein-1. J Biol Chem 2005;280:29612–9. [PubMed: 15980432]
11. Thomsen MB, Sosunov EA, Anyukhovsky EP, Özgen N, Boyden PA, Rosen MR. Deleting the accessory subunit KChIP2 results in loss of $I_{to,f}$ and increased $I^{K,slow}$ that maintains normal action potential configuration. Heart Rhythm 2009;6:370–7. [PubMed: 19251214]
12. Kuo HC, Cheng CF, Clark RB, Lin JJ, Lin JL, Hoshijima M, et al. A defect in the K_V channel-interacting protein 2 (KChIP2) gene leads to a complete loss of I_{to} and confers susceptibility to ventricular tachycardia. Cell 2001;107:801–13. [PubMed: 11747815]

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13. Thomsen MB, Wang C, Ozgen N, Wang HG, Rosen MR, Pitt GS. Accessory subunit KChIP2 modulates the cardiac L-type calcium current. Circ Res 2009;104:1382–9. [PubMed: 19461043]
14. Kanevsky N, Dascal N. Regulation of maximal open probability is a separable function of $Ca_V\beta$ subunit in L-type Ca^{2+} channel, dependent on NH2 terminus of alpha1C ($Ca_V1.2\alpha$). J Gen Physiol 2006;128:15–36. [PubMed: 16801381]
15. Wei X, Neely A, Olcese R, Lang W, Stefani E, Birnbaumer L. Increase in Ca^{2+} channel expression by deletions at the amino terminus of the cardiac α_{1C} subunit. Receptors Channels 1996;4:205–15. [PubMed: 9065969]
16. Chien AJ, Zhao X, Shirokov RE, Puri TS, Chang CF, Sun D, et al. Roles of a membrane-localized β subunit in the formation and targeting of functional L-type Ca^{2+} channels. J Biol Chem 1995;270:30036–44. [PubMed: 8530407]
17. Colecraft HM, Alseikhan B, Takahashi SX, Chaudhuri D, Mittman S, Yegnasubramanian V, et al. Novel functional properties of Ca^{2+} channel β subunits revealed by their expression in adult rat heart cells. J Physiol 2002;541:435–52. [PubMed: 12042350]
18. Roden DM. Taking the “idio” out of “idiosyncratic”: predicting torsades de pointes. Pacing Clin Electrophysiol 1998;21:1029–34. [PubMed: 9604234]
19. Nerbonne JM. Studying cardiac arrhythmias in the mouse—a reasonable model for probing mechanisms? Trends Cardiovasc Med 2004;14:83–93. [PubMed: 15121155]
20. Guo W, Jung WE, Marionneau C, Aimond F, Xu H, Yamada KA, et al. Targeted deletion of $K_V4.2$ eliminates $I_{to,f}$ and results in electrical and molecular remodeling, with no evidence of ventricular hypertrophy or myocardial dysfunction. Circ Res 2005;97:1342–50. [PubMed: 16293790]
21. Haissaguerre M, Derval N, Sacher F, Jesel L, Deisenhofer I, de Roy L, et al. Sudden cardiac arrest associated with early repolarization. N Engl J Med 2008;358:2016–23. [PubMed: 18463377]
22. Wang HG, George MS, Kim J, Wang C, Pitt GS. Ca^{2+}/calmodulin regulates trafficking of $Ca_V1.2$ Ca^{2+} channels in cultured hippocampal neurons. J Neurosci 2007;27:9086–93. [PubMed: 17715345]

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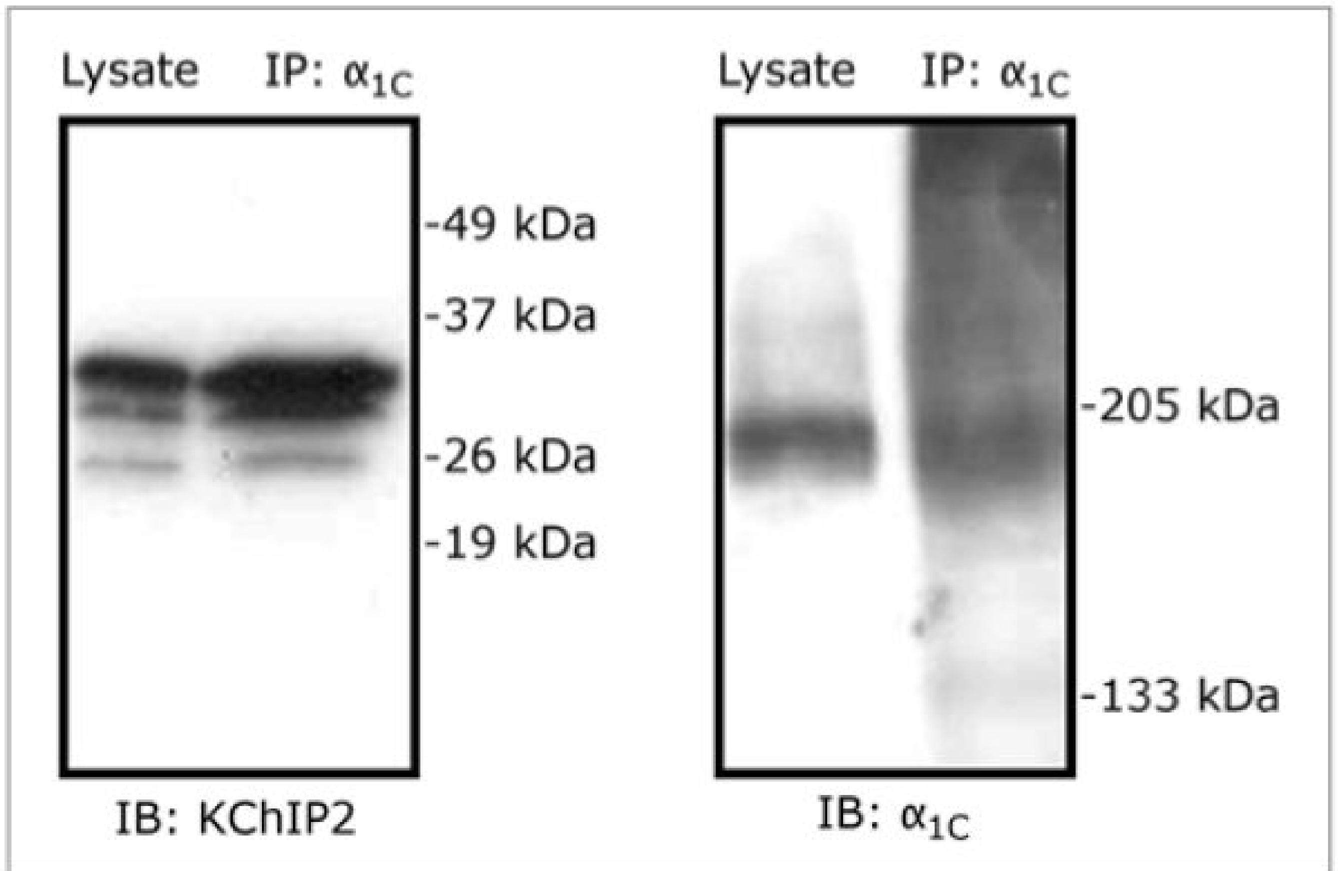

Figure 1.

Co-immunoprecipitation showing a biochemical association between $Ca_V1.2$ α_{1C} and KChIP2. Tissue samples from adult rat brain were lysed and immunoprecipitated (IP) with anti-α_{1C}, according to methods described earlier.[22] The lysate lanes represent total tissue lysates. The membranes are immunoblotted (IB) using anti-KChIP2 (left) and anti-α_{1C} (right).

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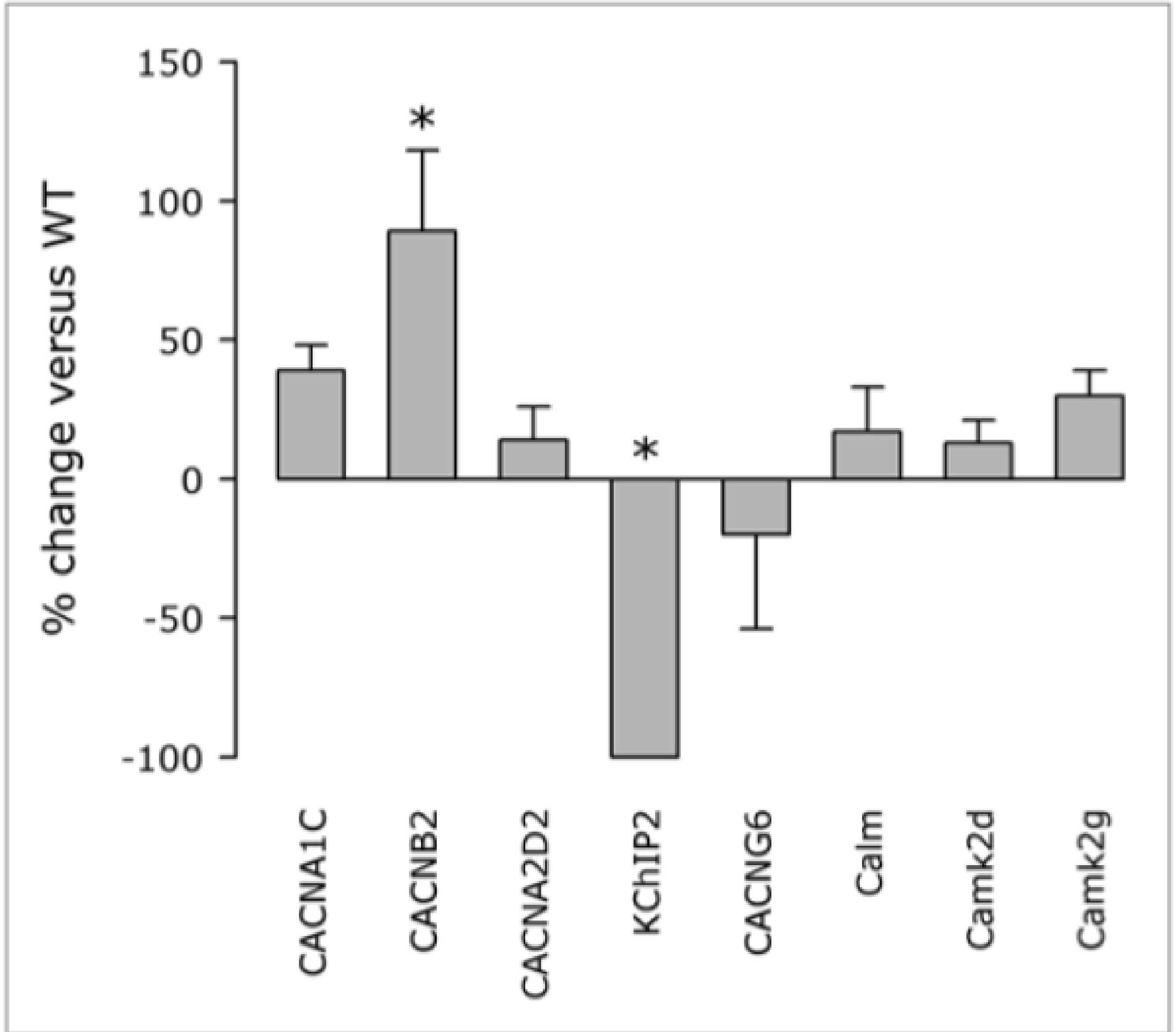

Figure 2.
Analysis of the molecular remodelling in KChIP2$^{-/-}$ ventricles reveals a significantly increased transcriptional activity of the β_2 subunit. The transcription levels of $I_{Ca,L}$ subunits and associated proteins in WT (n = 6) and KChIP2$^{-/-}$ (n = 6) ventricles were examined using quantitative real-time PCR, as previously described.[11] Values are normalized to cyclophilin A level; error bars represents SEM; *p < 0.05. All samples are tested in triplicate. Primers and abbreviations are provided in Table 1. These analyses revealed that β_2 expression is increased significantly, whereas KChIP2 mRNA could not be detected in KChIP2$^{-/-}$ ventricular tissue.

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Table 1

Primers used for quantitative real-time PCR

Gene	Forward primer	Reverse primer
CACNA1C ($Ca_V1.2$)	TGACTACCTGACTAGGGATTGGTCTA	TGCTCTAGGTTCCCTTCTGTTTTG
CACNB2 (β_2)	TTGCAAGAACACTGCAATTGG	GGGCCAGTTTATCAGCTGCTA
CACNA2D2 ($\alpha_2\delta_2$)	TCCAAGACCAGCGCCTTAAG	ACGTTCCACTAACTGCTGTGTAATG
KChIP2	AGCGTGGAGGATGAGTTTGAAC	TTCCCCGAAGAATCACTGACA
CACNG6 (γ_6)	AGCGAGAGGGCAAGATCAAG	TTGGTGGTTCGCTGGAAGAT
Calm	AGCCTTCTCCCTCTTCGACAA	TAATCATGTCCTGCAGCTCCG
Camk2d	CAGACTTCGGCTTAGCCATAGAA	GGTGTGCCAGCAAAACCAA
Camk2g	TTGAAGACATTGTGGCCAGAGA	GCCCAGATGTCCACAGGTTT
Cyclophilin A	TGGCGGCAGGTCCATCTA	TCCACAATGTTCATGCCTTCTT

Abbreviations: Calm, calmodulin; Camk2d and -g, Ca^{2+}/calmodulin-dependent protein kinase II, subtype d and g, respectively.