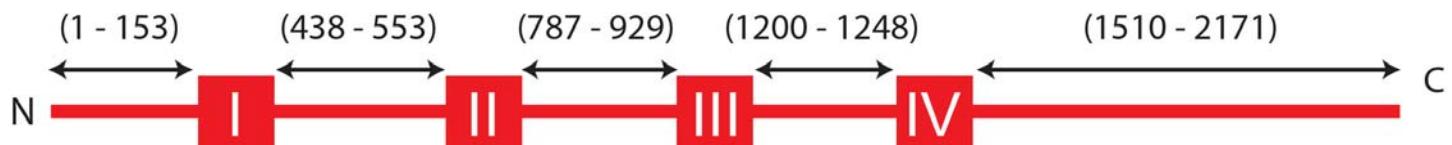


Supplemental Information: Figure S1

Ca_V1.2 (α_{1C}) : Accession # CAA33546



Ca_V3.1 (α_{1G}) : Accession # AAC67372

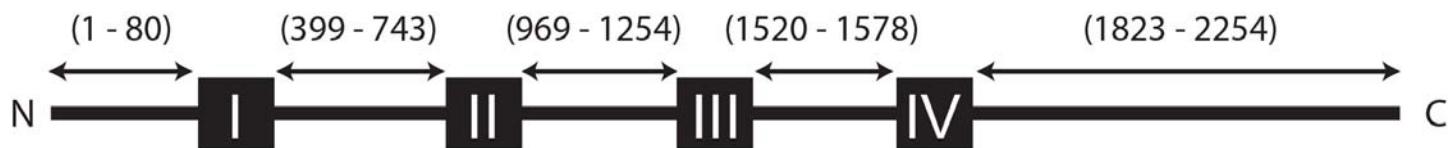


Figure S1. Schematic showing precise boundaries of the N- and C-termini, and intracellular loops of CaV1.2 (top) and CaV3.1 channels (bottom) used to generate chimeric channels.

Supplemental Information: Figure S2

	AID	PEER
Human Ca _v 2.1 (NP_000059)	GEFAKERERVENRRAFLKLRRQQQIERELNGYMEWI SKAEEVILA EDE TDGEQRH PFDGA	420
Human Ca _v 2.2 (NP_000709)	GEFAKERERVENRRAFLKLRRQQQIERELNGYLEWIFKAEEVMLAEEDRNAEEKSPLD-V	415
Rabbit Ca _v 1.2 (CAA33546)	GEFSKEREKAKARGDFQKLREKQQLEEDLKGYLDWI TQAEDIDPENEDEGMDEEKPRN--	493
Human Ca _v 1.2 (NP_001123302)	GEFSKEREKAKARGDFQKLREKQQLEEDLKGYLDWI TQAEDIDPENEDEGMDEEKPRN--	463
	*** : * *** : . : * * * . : * : * : * : * : * : * : * : * : * : * : * :	
Human Ca _v 2.1 (NP_000059)	LRR TT IKKS KTDLLN PEEAEDQLADIASVGS PFARASI KSALENST FFHKKERRMRFYI	480
Human Ca _v 2.2 (NP_000709)	LKRAATKKSRNDL IHAEEGEDRFADLCAVGS PFARASL KSGKT ESSSY FRRKEKMFRFFI	475
Rabbit Ca _v 1.2 (CAA33546)	--MSMPTSETES VNTENVAGGDI EGENCG --ARLAHRISKSKFSRYWRRWNRF CRRKC	547
Human Ca _v 1.2 (NP_001123302)	--MSMPTSETES VNTENVAGGDI EGENCG --ARLAHRISKSKFSRYWRRWNRF CRRKC	517
	: . * . : : . * : . : * * : : . * : * : * : * : * : * : * : * :	
Human Ca _v 2.1 (NP_000059)	RRMVKTQ	487
Human Ca _v 2.2 (NP_000709)	RRMVKAQ	482
Rabbit Ca _v 1.2 (CAA33546)	RAAVKSN	554
Human Ca _v 1.2 (NP_001123302)	RAAVKSN	524
	* ** ::	

Figure S2. ClustalW alignment of the intracellular I-II loop of distinct Ca_v1 and 2 channels showing regions of sequence conservation and homology. The previously identified α interaction domain (AID) is shown. Immediately downstream of the AID is the acidic-residue-rich putative ER export region (PEER) identified in this study.

Supplemental Information: Figure S3

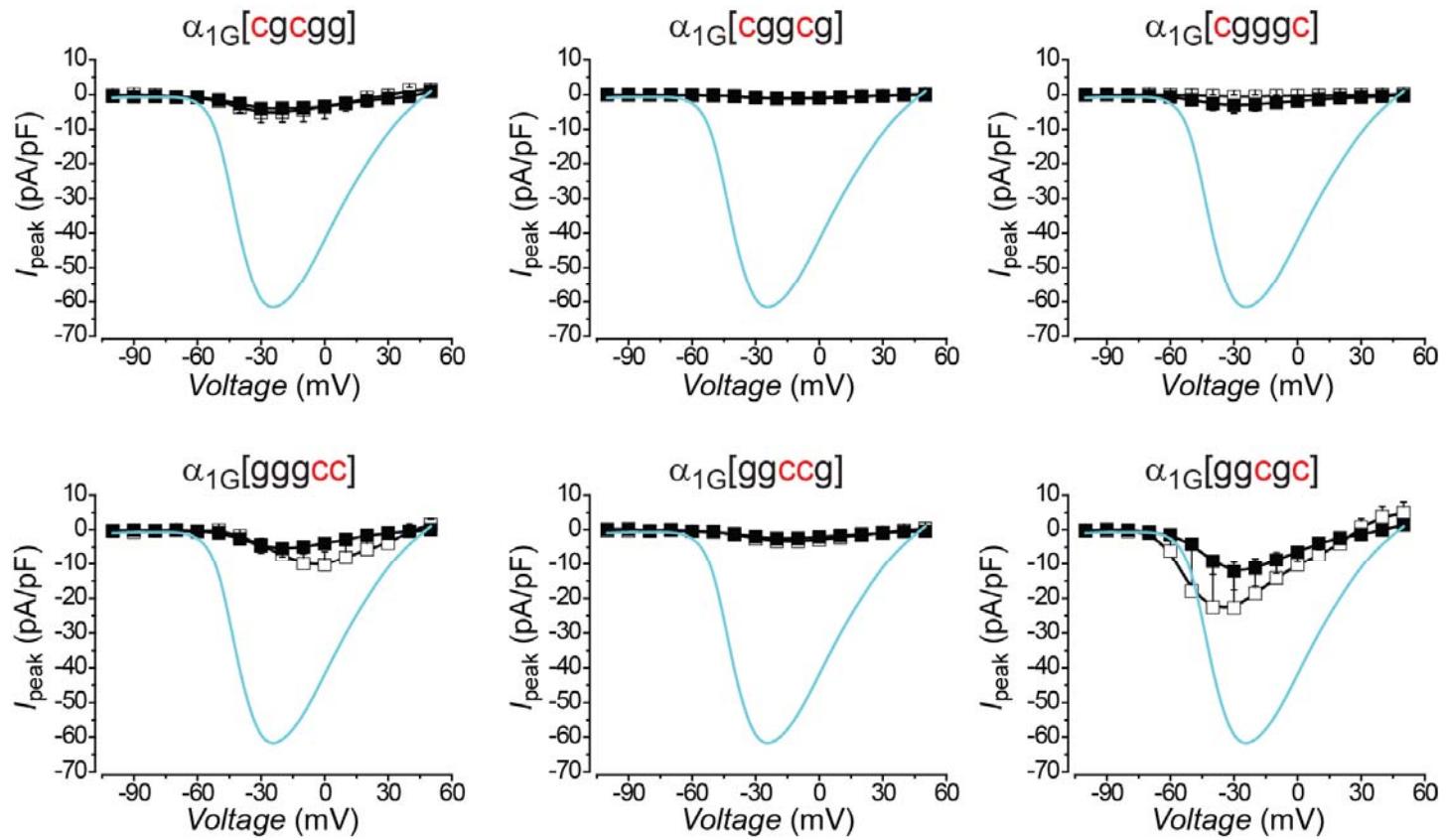


Figure S3. I_{peak} - V curves of double-intracellular-domain substituted chimeras either expressed alone (open symbols) or with β_{2a} subunits (solid symbols).

Supplemental Information: Figure S4

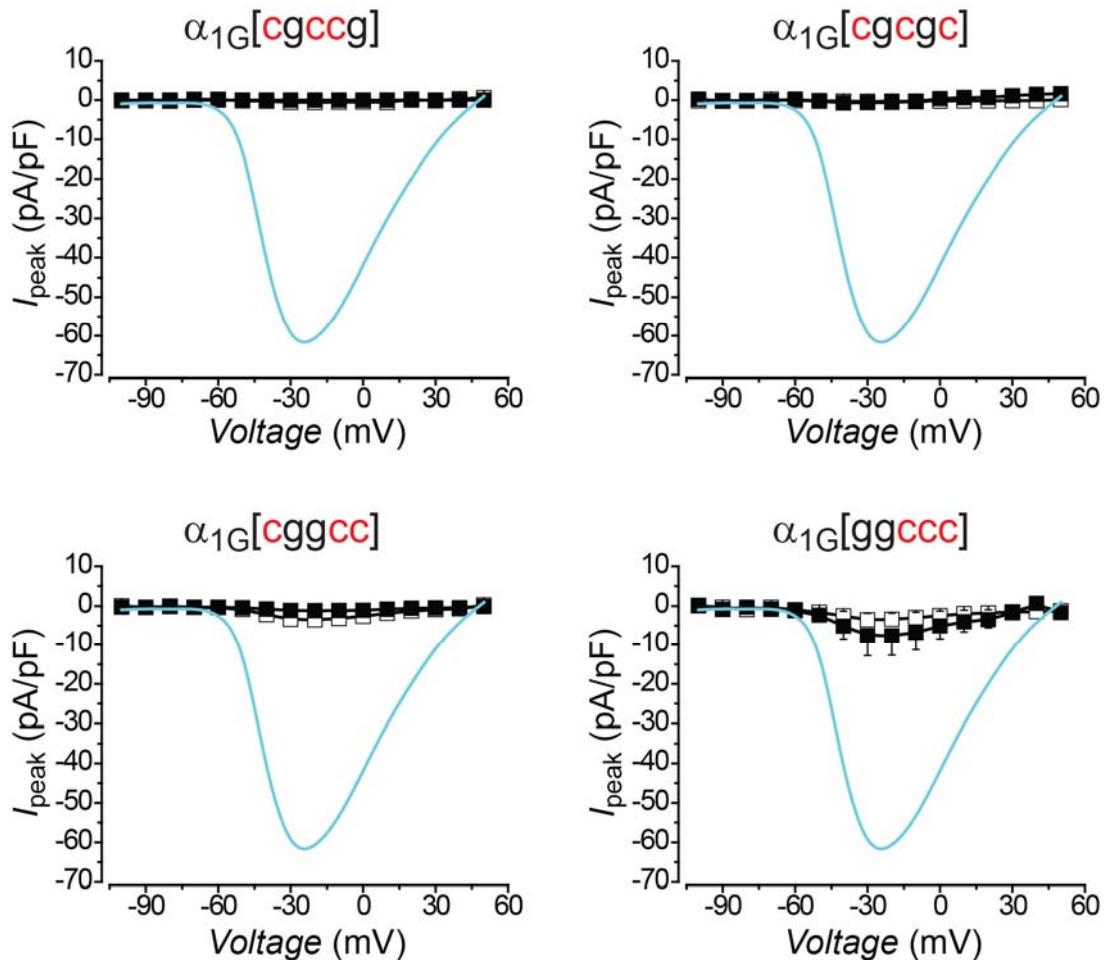


Figure S4. $I_{\text{peak}}\text{-}V$ curves of triple-intracellular-domain substituted chimeras either expressed alone (open symbols) or with $\beta_{2\text{a}}$ subunits (solid symbols).

Supplemental Information: Figure S5

	EF-hand
	*Δ1540
Human Ca _v 1.2 (NP_001123302)	DNFDYLTRDWSILGPHHLDEFKRIWAELYDPEAKGRIKHLDVVTLLRRIQPPPLGFGKLCPH 1536
Rabbit Ca _v 1.2 (CAA33546)	DNFDYLTRDWSILGPHHLDEFKRIWAELYDPEAKGRIKHLDVVTLLRRIQPPPLGFGKLCPH 1566
Human Ca _v 2.2 (NP_000709)	DNFELYLTDSSILGPHHLDEFIRVWAELYDPAACGRISYNDMFEMLKHMSPPGLGKKCPA 1768
Human Ca _v 2.1 (NP_000059)	DNFELYLTDSSILGPHHLDEFIRVWAELYDPAACGRISYNDMFEMLKHMSPPGLGKKCPA 1876
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
	Pre-IQ
Human Ca _v 1.2 (NP_001123302)	RVACKRLVSMNMLPLNS-DGTVMFNATLFALVRTALRIKTEGN---LEQANEELRAIJKKI 1592
Rabbit Ca _v 1.2 (CAA33546)	RVACKRLVSMNMLPLNS-DGTVMFNATLFALVRTALRIKTEGN---LEQANEELRAIJKKI 1622
Human Ca _v 2.2 (NP_000709)	RVAYKRLVRMNMPISNEDMTVHFSTLMAIRALEIKLAPAGTKHQQCDAELRKESVV 1828
Human Ca _v 2.1 (NP_000059)	RVAYKRLRMDLPVAD-DNTVHFNSTLMAIRALDIKIAKGGADKQQMDAELRKEMMAI 1935
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
	*Δ1632
Human Ca _v 1.2 (NP_001123302)	WKRTSMKLLDVVFPAGDDEVTVGFYATFLIQEYFRKFKKRKEQGLVGKPQRNALSLQ 1652
Rabbit Ca _v 1.2 (CAA33546)	WKRTSMKLLDVVFPAGDDEVTVGFYATFLIQEYFRKFKKRKEQGLVGKPQRNALSLQ 1682
Human Ca _v 2.2 (NP_000709)	WANLPQKTLDLLVPPHKPDEMTPGVKVAALMIFDFYKQNKTTRDQMQQAPG---GLSQM 1884
Human Ca _v 2.1 (NP_000059)	WPNLSSQKTLDLLVTPHKSTDLTGVKHYAAMMIMEYYRQSNAKKLQAMREE-----QD 1987
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
	*Δ1732
Human Ca _v 1.2 (NP_001123302)	AGLRTLHDIGPEIRRAISGDLTAEEELDKAMKEAVSAASEDDIFRAGGLFGNHVSYYQS 1712
Rabbit Ca _v 1.2 (CAA33546)	AGLRTLHDIGPEIRRAISGDLTAEEELDKAMKEAVSAASEDDIFRAGGLFGNHVSYYQS 1742
Human Ca _v 2.2 (NP_000709)	GPVSLFHPKATLEQTQPAVLRGARVFLRKQSSTSLSN-----GGAIQNQESGIKE 1935
Human Ca _v 2.1 (NP_000059)	RTPLMFQRMEP----PSPTQEPPGQNALPSTQLDP-----GGALMAHESGLKE 2032
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Human Ca _v 1.2 (NP_001123302)	DGRSAFPQTFTTQRPLHINKAGSSQGDTESPSSHEKLVDSFTTPSSYSSSTGSANINNANN 1772
Rabbit Ca _v 1.2 (CAA33546)	DSRSAFPQTFTTQRPLHISKAGNNQGDTESPSSHEKLVDSFTTPSSYSSSTGSANINNANN 1802
Human Ca _v 2.2 (NP_000709)	S-----VSGWTQRTQDAPHEARP-PLERGHSTEIPVGRSGALAVDQMQSITRRG-PDG 1987
Human Ca _v 2.1 (NP_000059)	S-----PSWVQTQRAQEMFQKTGTWSPEQGPPTDMPN--SQPNQSVEVEMREMRGDRGYSDS 2084
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Human Ca _v 1.2 (NP_001123302)	TALGRLPRPAGYPSTVSTVEGHGPPPLSPAIRVQEVAWLSSNRERHVPMCEDLELRRDSG 1832
Rabbit Ca _v 1.2 (CAA33546)	TALGRLPRPAGYPSTVSTVEGHGPPLSPLSPAVRAQEAAWLSSKR----- 1845
Human Ca _v 2.2 (NP_000709)	EPQPGLIE-SQGRAASMPRLAAETQP-----VTDASP----- 2017
Human Ca _v 2.1 (NP_000059)	EHYLPME-GQGRAASMPRLPAENQRRGRPRGNLNLSTISDTP----- 2126
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Human Ca _v 1.2 (NP_001123302)	SAGTQAHCLLRKANPSRCHSRESQAAMAGQEETSQDETYEVKMNHDEACSEPSILLSTE 1892
Rabbit Ca _v 1.2 (CAA33546)	-----CHSQESQIAMAQCQEGASQDDNYDVRIGEDAECCESEPSILLSTE 1887
Human Ca _v 2.2 (NP_000709)	-----MKRSISTLAQRPRGTHLCSTTPDRPPPSQASSHHHHH--R 2055
Human Ca _v 2.1 (NP_000059)	-----MKRSASVLG--PKARRDDYSLERVPPEENQRHHQ----- 2159
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
	*Δ1906
Human Ca _v 1.2 (NP_001123302)	MLSYQDDENRQLTLPEEDKRDIRQSPKRGFLRSASLGRRASFHLECLKRQKDRGGDISQK 1952
Rabbit Ca _v 1.2 (CAA33546)	MLSYQDDENRQLLAPPEEKDRDPLSPKGFLRSASLGRRASFHLECLKRQKDRGGDISQK 1947
Human Ca _v 2.2 (NP_000709)	CHRRRDRKQRSLEKGPSLSDAMDGAPEAVGPGLPPGEPTGCRERERRQERGRGSQERR 2115
Human Ca _v 2.1 (NP_000059)	--RRDRSHRASERSLGRTVDTGLGTDLSMTTQSGDLP-----KERDQERGRPKDR- 2211
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Human Ca _v 1.2 (NP_001123302)	TVLP---LHLVHHQALAVAGLSPLLQRSHSPASFPFRPFATPPATPGSRGWPPQPVPTLRL 2009
Rabbit Ca _v 1.2 (CAA33546)	TVLP---LHLVHHQALAVAGLSPLLQRSHSPSLPSCPATPPATPGSRGWPPQPIPTLRL 2004
Human Ca _v 2.2 (NP_000709)	QPSSSSSEKQRFYSCDRFGREPCKPKPSLSSHPTSPTAGQEPGPHFQGSGSVNGSPLLS 2175
Human Ca _v 2.1 (NP_000059)	-----KHRQHHHHHHHHHHHHHHPPPDP----- 2230
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Human Ca _v 1.2 (NP_001123302)	EGVESSEKLNSSFFPSIHCGSWA-EETTPGGGSSAARRVRPVSIMVPSQAGAPGRQFHGSA 2068
Rabbit Ca _v 1.2 (CAA33546)	EGADSSEKLNSSFFPSIHCGSWGENSPCRGDSSAARRARPVSLTVPSQAGAQGRQFHGSA 2064
Human Ca _v 2.2 (NP_000709)	TSGASTPGRGGRRQLPQTPLTPRPSITYKTANSSPIHFAGAQTSPLAFSPGRLSRLSEH 2235
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Human Ca _v 1.2 (NP_001123302)	SSLVEAVLISEGLGQFAQDPKFIETTTQELADACDMTIEEMESAADNILSGGAPQSPNGA 2128
Rabbit Ca _v 1.2 (CAA33546)	SSLVEAVLISEGLGQFAQDPKFIETTTQELADACDMTIEEMENAADDILSGGARQSPNGT 2124
Human Ca _v 2.2 (NP_000709)	NALLQRDPLSQLAPGSRIGSDPYLQLDSEASVHALPEDLTLEEAVATNSGRSSRTS 2295
Human Ca _v 2.1 (NP_000059)	-----KDRYAQERPD-----HGRAR--- 2245
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Human Ca _v 1.2 (NP_001123302)	LLPFVNCRDAGQDRAG-GEEDAGCVRARGR-PSSEELQDSRVYVSSL 2173
Rabbit Ca _v 1.2 (CAA33546)	LLPFVNRRDPGRDRAGQNEQDASGACAPGCGQSEEALADRRAVGVSSL 2171
Human Ca _v 2.2 (NP_000709)	YVSSLTSQSHPLRVPNGYHCTLGLSSGGRARHSYHHPDQDHWC--- 2310
Human Ca _v 2.1 (NP_000059)	-----ARDQRWSRSP-----SEGREHMAHRQ----- 2266
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:

Figure S5. ClustalW alignment of the C-termini of distinct Cav1 and 2 α₁ subunits.

Supplemental Information: Figure S6

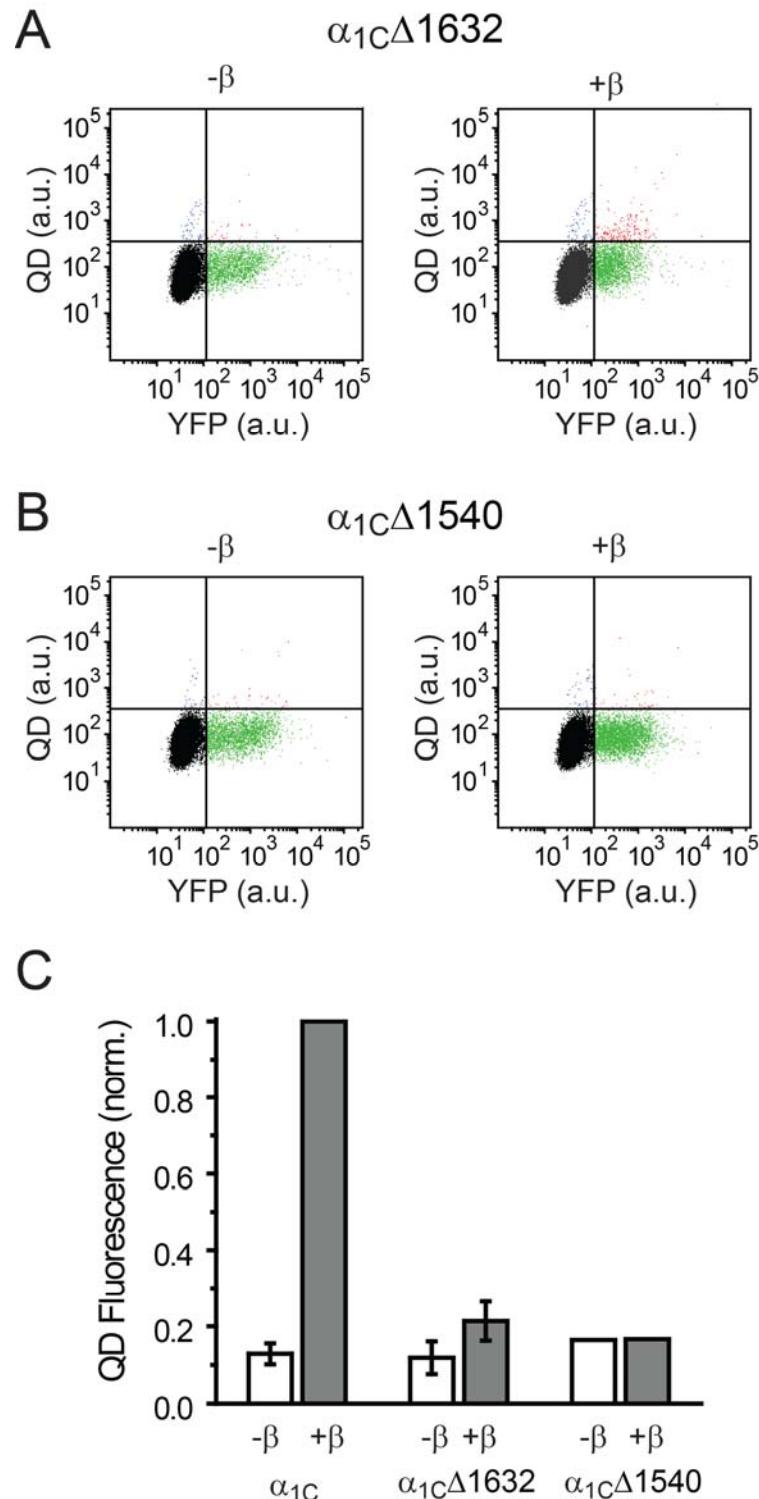


Figure S6. Impact of C-terminus truncations on β -dependent membrane-targeting of α_{1C} subunits. (A, B) Exemplar flow cytometry results for $\alpha_{1C}\Delta 1632 \pm \beta_{2a}$ and $\alpha_{1C}\Delta 1540 \pm \beta_{2a}$, respectively. (C) Normalized QD fluorescence intensity for truncated mutants compared to full-length α_{1C} .

Table S1. PCR primers used to generate single-intracellular-domain substituted chimeras by using in-fusion technique. For each chimera, four primers were required. The underlined 15-nucleotide segments are within the boundary of α_{1G} at the α_{1G}/α_{1C} junctions. The red 15-nucleotide segments in forward primer for insert and in reverse primer for vector, and the blue 15-nucleotide segments in forward primer for vector and in reverse primer for insert, are complementary reversal, respectively, thus the purified PCR insert and vector could be directly linked together with the consequent in-fusion reaction.

Constructs	PCR Targets	Templates	Primer 1 (Forward)	Primer 2 (Reverse)
$\alpha_{1G}[cgggg]$	Insert (α_{1C} N-Terminus)	α_{1C} -YFP	<u>GCTTCTGGCCAGAGG</u> ATGCTTCGAGCCCTTG TTCAGGCCAGCTAC	<u>TCGCTCGAACACGG</u> CCACTCGACGATGCTTA TGCACGCC
	Vector	α_{1G} -YFP	<u>CCGTGGTTCGAGCGA</u> GTCAGTATG	<u>CCTCTGGCCAGAAC</u> TTAAGTTAAAC
$\alpha_{1G}[gcggg]$	Insert (α_{1C} I-II loop)	α_{1C} -YFP	<u>ATTGCCACGCAGTC</u> TCCAAGAGAGGGAGA AGGCCAAAGC	<u>CCGGCCAAAGTATT</u> CGACTTGACCGCTGCGC GGCACTTTCTCCTG
	Vector	α_{1G} -YFP	<u>AAATACTTTGGCCGG</u> GGAATCATG	<u>GAACTGCGTGGCAAT</u> CACCACCAGGCAC
$\alpha_{1G}[ggcgg]$	Insert (α_{1C} II-III loop)	α_{1C} -YFP	<u>CTTGTGGAAGGATTC</u> GCTGATGCTGAGAGCC TTACTTCTGC	<u>ATGGTCAAACATCTT</u> GTCGTTGACGATAACGGT GACACTG
	Vector	α_{1G} -YFP	<u>AAGATTTGACCAT</u> GTGGTCCTCG	<u>GAATCCTTCCACAAG</u> AATGGCCACCAGCAGGT TAAAG
$\alpha_{1G}[ggcgc]$	Insert (α_{1C} III-IV loop)	α_{1C} -YFP	<u>GTGGTGGAGAACTTC</u> CAGGAGCAGGGGGAGC AGGAGTAC	<u>GAGGTCCAGGTAGTG</u> GGAGTTGACCACGTACC ACACTTTG
	Vector	α_{1G} -YFP	<u>CACTACCTGGACCTC</u> TTCATCACTG	<u>GAAGTTCTCCACCAAC</u> CACGCCAACAAACATG
$\alpha_{1G}[ggggc]$	Insert (α_{1C} C-Terminus)	α_{1C} -YFP	<u>CTGATGAAGCACCTG</u> GACTACCTGACAAGGG ACTGGTCAATC	<u>CTTGCTCACATCGAT</u> CAGGCTGCTGACGCCGG CCCTGCGGTCCCGCG
	Vector	α_{1G} -YFP	<u>ATCGATGTGAGCAAC</u> GGCGAGGAGCTGTTTC	<u>CAGGTGCTTCATCAG</u> CACAGCTATG