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Supplemental Material to:

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Modulation of distinct isoforms of L-type calcium channels by Gq-coupled receptors in Xenopus oocytes: Antagonistic effects of Gβγ and protein kinase C

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Channel composition	*n*	G_{max}, *μS*	V_{rev}, *mV*	V_a, *mV*	K_a, *mV*
α_{1C}-LNT, $\alpha_2\delta$	14	6.3±1	66.5±2	9.7±1	8.4±0.3
α_{1C}-LNT, $\alpha_2\delta$, β_{2b}	12	40.2±8.4	63.1±1.8	-4.8±1.1	6.7±0.3
α_{1C}-LNT,+9a, $\alpha_2\delta$	14	7.8±1.5	72.7±6.2	10.6±0.4	8.4±0.2
α_{1C}-LNT,+9a, $\alpha_2\delta$, β_{2b}	20	41.0±5.6	62.8±1.6	-4.8±0.8	6.8±0.2
α_{1C}-SNT, $\alpha_2\delta$	7	20.68±5.5	65.2±1.9	5.1±0.3	8.2±0.5
α_{1C}-SNT, $\alpha_2\delta$, β_{2b}	6	51.4±11.9	61.2±1	-11.6±1.8	6.3±0.5
α_{1C}-SNT,+9a, $\alpha_2\delta$	8	37.2±13.4	74.8±6.8	2.6±0.6	8.7±0.8
α_{1C}-SNT,+9a, $\alpha_2\delta$, β_{2b}	12	43.6±9.9	60.3±0.5	-9.8±1.4	6.5±0.2

Voltage	*isoform*	τ_{slow}, *ms*	A_{slow}	τ_{fast}, *ms*	A_{fast}	*C*
0mV	α_{1C}-LNT (n=5)	2571±52	0.42±0.01	844±46	0.37±0.03	0.21±0.02
	α_{1C}-LNT,+9a (n=6)	4138±707	0.35±0.03	987±66	0.52±0.08	0.12±0.06
20mV	α_{1C}-LNT (n=5)	2056±95	0.46±0.02	634±33	0.44±0.03	0.103±0.01
	α_{1C}-LNT,+9a (n=6)	2672±225	0.46±0.02	619±62	0.44±0.03	0.103±0.02
40mV	α_{1C}-LNT (5)	2148±152	0.51±0.02	490±43	0.45±0.03	0.04±0.02
	α_{1C}-LNT,+9a (n=6)	2577±258	0.55±0.01	377±68	0.39±0.03	0.06±0.03

Weiss 2012. Supplemental Table 1

MLRALVQPATPAYQPLPSHLSAETE***ST***CKGTVVHEAQLNHFYISPGG***S***NY 50
G***S***PRPAHANMNANAAAGLAPEHIP***T***PGAALSWQAAIDAARQAKLMGSAGN 100
ATI***ST***V***SS***TQRKRQQYGKPKKQG***S***TTATRPPRALLCLTLKNPIRRACI***S***I 150
VEWKPFEIII 160

Weiss 2012. Supplemental Figure 1

A

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Rabbit SM, α1Cb         gaggcactccagcgggcttgcatgcccagaagaaagggaagtttgcttggtttagtcact 1450
                        |||||||||| |||||| ||| ||  ||||||||||||||||||||||||||||||||||
Human chromosome 12     gaggcactccggcgggcatgcttgatcagaagaaagggaagtttgcttggtttagtcact 2510936

Rabbit SM, α1Cb         ccacagagacccatg 1472
                        ||||||| |||||||
Human chromosome 12     ccacagaaacccatg 2510951
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B

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α1C, NT-rabbit long     GEFSKEREKAKARGDFQKLREKQQLEEDLKGYLDWITQAEDIDPENEDEGMDEEKPRN-- 493
α1C,77, NT-human short  GEFSKEREKAKARGDFQKLREKQQLEEDLKGYLDWITQAEDIDPENEDEGMDEEKPRN-- 463
α1Cb, rabbit SM         GEFSKEREKAKARGDFQKLREKQQLEEDLKGYLDWITQAEDIDPENEDEGMDEEKPRNRG 465
                        **********************************************************

α1C, NT-rabbit long     -----------------------MSMPTSETESVNTENVAGGDIEGENCGARLAHRISKS 530
α1C,77, NT-human short  -----------------------MSMPTSETESVNTENVAGGDIEGENCGARLAHRISKS 500
α1Cb, rabbit SM         TPAGLHAQKKGKFAWFSHSTETHVSMPTSETESVNTENVAGGDIEGENCGARLAHRISKS 525
                                               :************************************

α1C, NT-rabbit long     KFSRYWRRWNRFCRRKCRAAVKSN  554
α1C,77, NT-human short  KFSRYWRRWNRFCRRKCRAAVKSN  524
α1Cb, rabbit SM         KFSRYWRRWNRFCRRKCRAAVKSN  549
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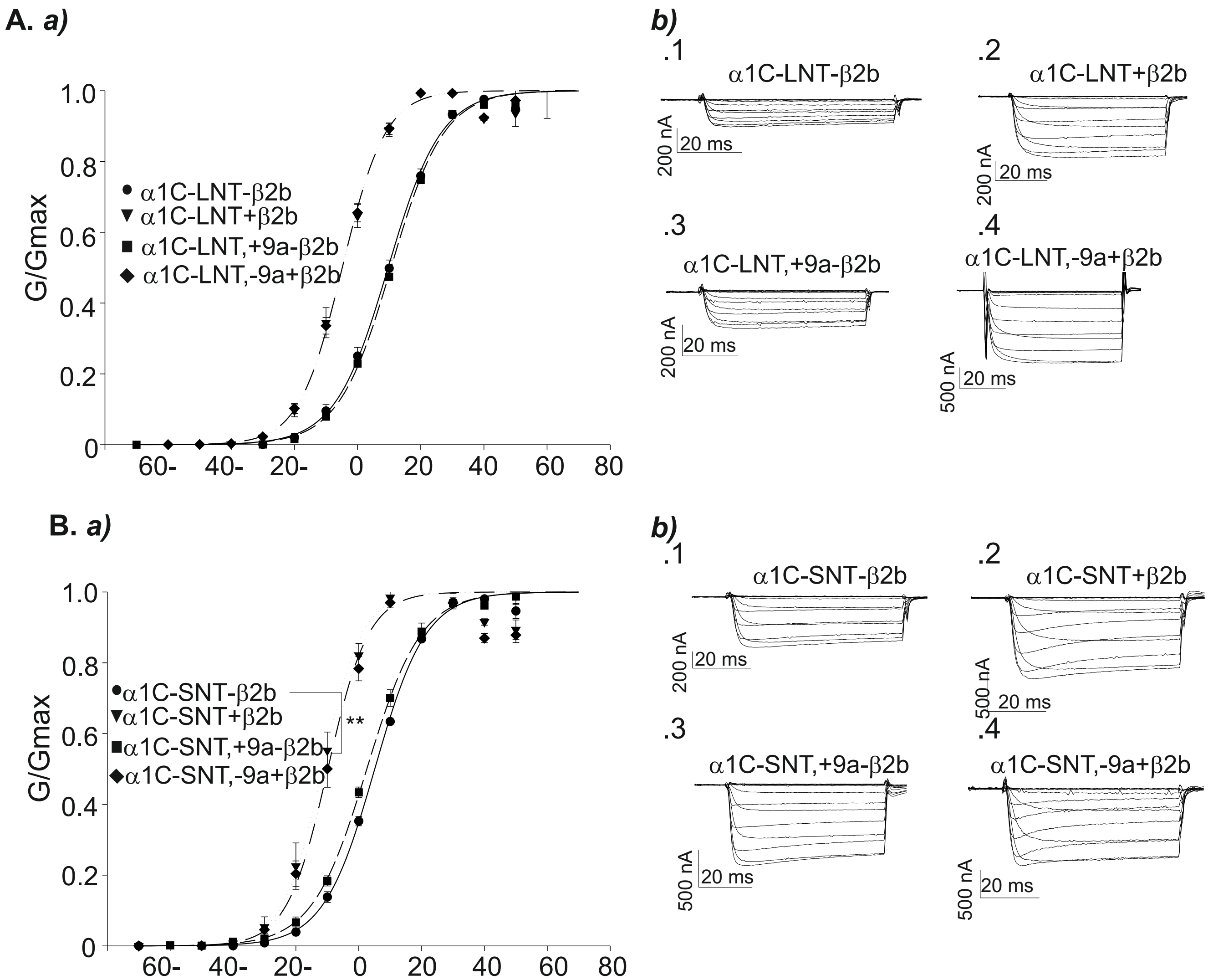
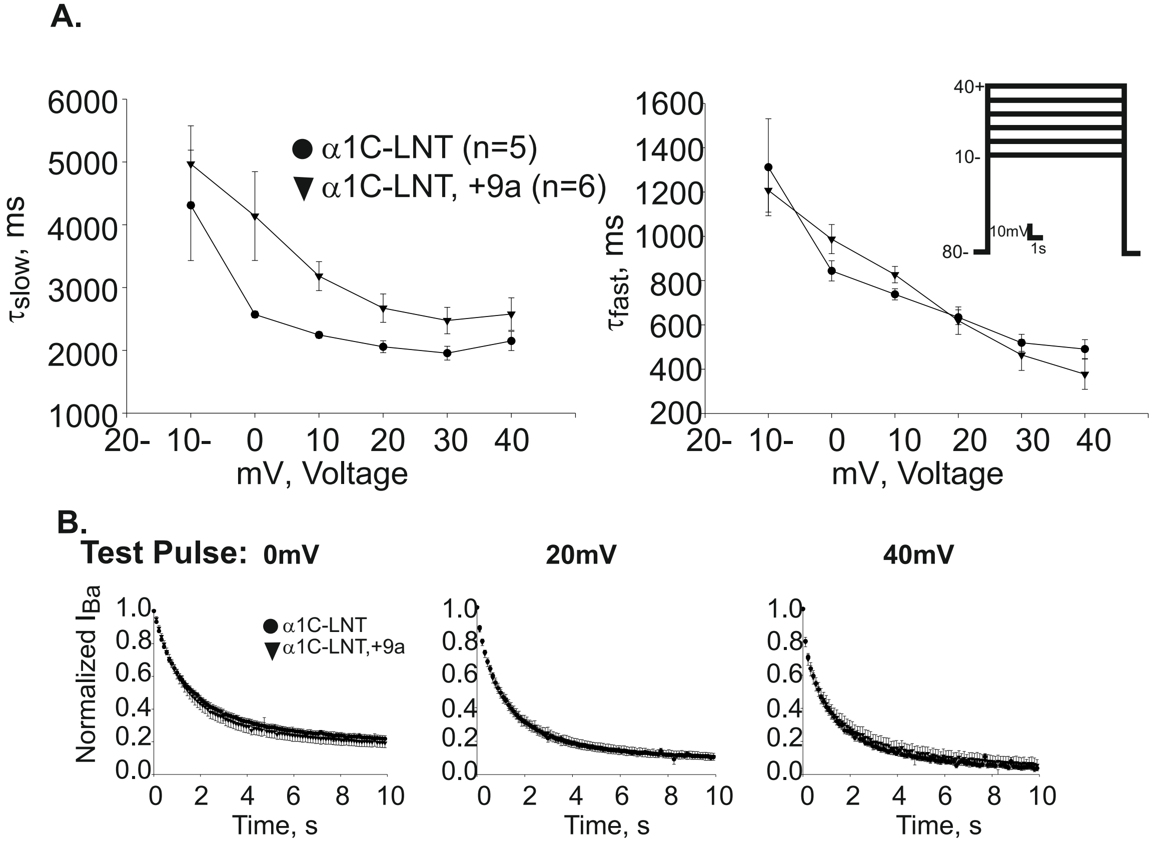
Weiss 2012. Supplemental Figure 2



Weiss 2012. Supplemental Figure 3



Weiss 2012. Supplemental Figure 4

Supplemental Material

Supplemental Table 1. Top. Activation parameters of four α_{1C} isoforms. Bottom. Inactivation parameters of two α_{1C}-LNT isoforms.

Supplemental Figure 1. The amino acid composition of rabbit long-NT. Ser/Thr mutations are underlined: mutant 1 (mut I), single underline; mutant 2 (mut II), double underline.

Supplemental Figure 2. A 75 nucleotide long insertion in L1 of α_{1C}. *A.* Nucleotide alignment of the L1 insertion in rabbit and the part of human chromosome 12 encoding α_{1C}. *B.* a.a. alignment of the entire L1 reveals a highly homologous sequence among the different species and isoforms. Identical a.a. are marked by asterisk, gaps are marked by dashes. Only short-NT human L1 is shown, as this region is identical in human long-NT isoform.

Supplemental Figure 3. α_{1C},+9a constructs have similar activation parameters as α_{1C},-9a constructs. Oocytes were injected with one of four different α_{1C} isoforms, $\alpha_2\delta$ with or without β_{2b}. G-V curves of the +9a isoforms vs. their controls reveal no significant changes in activation kinetics, when expressed with or without the auxiliary β_{2b} subunit. *Aa)* G-V curves of long-NT isoforms. *b)* representative traces of two LNT isoforms. I_{Ba} measured at 10 mV steps from -70 mV to +50 mV. *Ba)* G-V curves of short-NT isoforms. *b)* representative traces of two SNT isoforms. I_{Ba} measured at 10 mV steps from -70 mV to +50 mV. **, p<0.01 by t-test; brackets indicate the two groups with a statistical significance effect.

Supplemental Figure 4. α_{1C},+9a constructs have similar inactivation kinetics as α_{1C},-9a constructs. *A.* Oocytes were injected with α1C-LNT ±9a, $\alpha_2\delta$, β_{2b}. The inactivation curve was fitted with two time constants, τ_{slow} and τ_{fast} (see Experimental Procedures for details). There was no significant difference between the two isoforms. *Indent:* The inactivation protocol consists of 6, 10s, incrementing voltage steps (from -10mV to +40mV). B. Representative normalized Ba^{2+} currents at 3 selected test pulses. Each point is mean±S.E.M. α_{1C},-9a n=5; α_{1C},+9a n=6.