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**Supplementary Material**

# A $Ca_V1.1$ $Ca^{2+}$ Channel Splice Variant with High Conductance and Voltage-Sensitivity Alters EC-Coupling in Developing Skeletal Muscle

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**Supplementary results:**

**Table 1:** Putatively non-funtional splicing variants in the *CACNA1S* gene

| *variant* | *protein length* | *nonsense residues* | *location in channel* | *% transcripts in adult muscle* |
|---|---|---|---|---|
| *deletion of exons 3-5* | 187 | 101 | IS1-S2 | 3,0 |
| *deletion of exons 3-7* | 111 | 25 | IS1-S2 | 1,7 |
| *deletion of exons 4-5* | 140 | 8 | IS3 | 1,0 |
| *insertion of intron 4* | 208 | 28 | IS4-S5 | 2,4 |
| *insertion of intron 8* | 461 | 78 | I-II | 3,0 |
| *insertion of intron 25* | 1115 | 30 | III-IV | 3,6 |
| *insertion of intron 26* | 1147 | 9 | IVS1 | 2,1 |
| *insertion of intron 28* | 1244 | 41 | IVS3-S4 | 1,3 |
| *deletion of exon 19* | 1853 | missing 20 in frame | IIIS1-S2, IIIS2 | 1,4 |

**Table 2:** Putatively funtional splicing variants in the *CACNA1S* gene

| *variant* | *protein length* | *nonsense residues* | *location in channel* | *% transcripts in adult muscle* |
|---|---|---|---|---|
| *deletion of exons 39-40* | 1559 | 3 | C-terminus | 1,6 |
| *alternate 5' site of exon 40* | 1847 | missing 26 in frame | C-terminus | 1,5 |
| *insertion of intron 43* | 1851 | 61 | C-terminus | 3,0 |

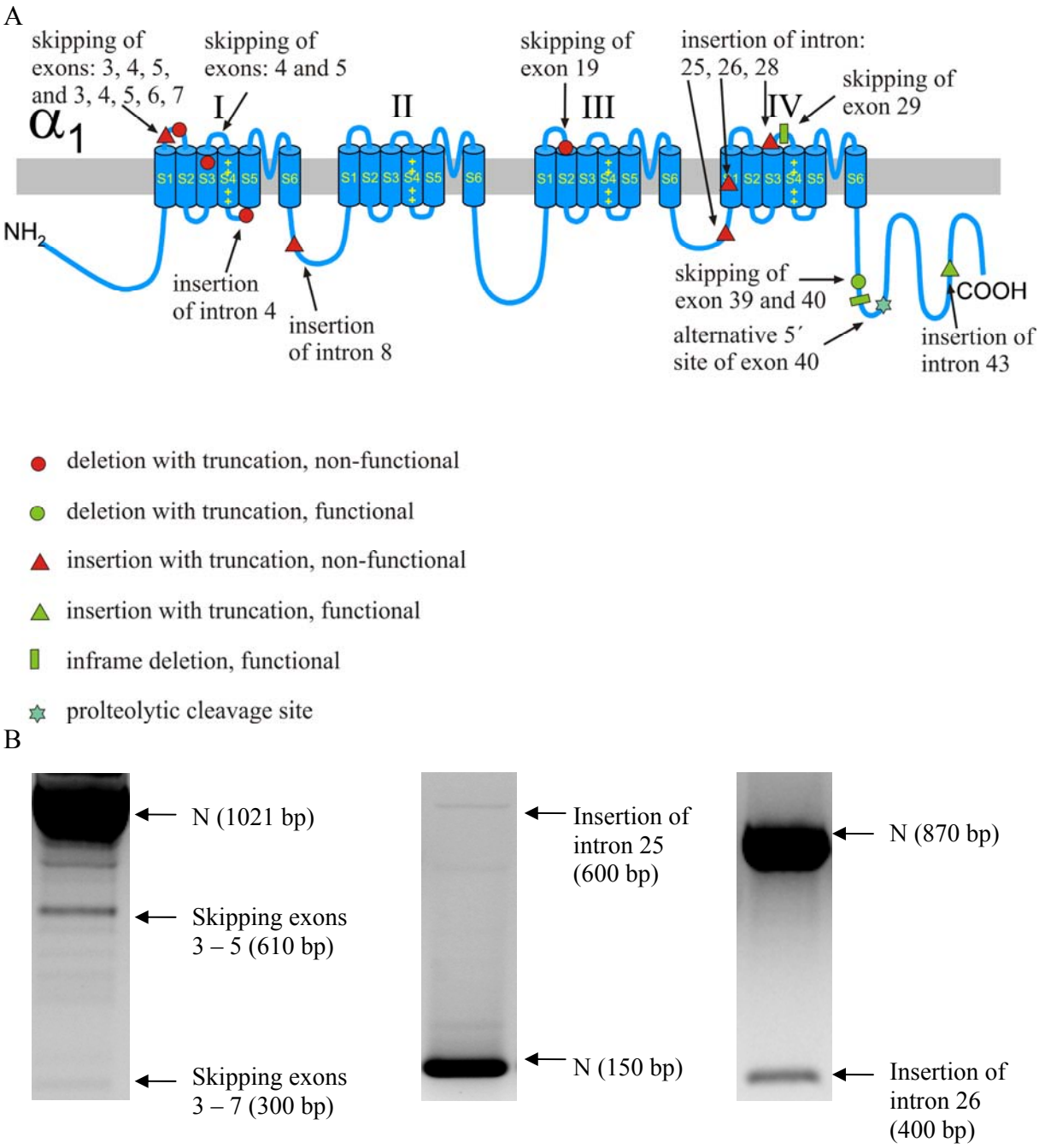


**Figure 1. 13 splicing variants found in the *CACNA1S* gene. A.** Schematic representation of pore forming $\alpha_1$ subunit of $Ca_V1.1$. Arrows point to segments encoded by alternative splice variants. **B.** Examples of scanning of *CACNA1S* gene uncovered splice variations revealed by different reverse transcriptase PCR product size. Standard band marked with N.

**Table 3: RT-PCR and cloning primers**

| *Exons/name* | *Forward Primer* | *Reverse Primer* | *Tm °C* | *Product length bp* |
|---|---|---|---|---|
| *1-9* | ttcctgagattctgccaagg | ctgtgtcagagccaccttca | 51 | 1129 |
| *2-8* | atcatcttgctcaccatctttgc | ccccgaaggtcctcatcta | 51 | 929 |
| *7-10* | tattttgtcaccctcattttgctg | tgcggctggttgtggtgct | 54 | 437 |
| *8-10* | accaaggagcgggagaag | aggcgatagacagggtgttg | 52 | 340 |
| *9-15* | atgaaggtggctctgacaca | ggtttctgctccagcttcttg | 51 | 961 |
| *10-14* | ccgacattggaggcagtgga | atcttcctgcgtttttctcctc | 51 | 825 |
| *13-18* | acttcatcatccttttcgtctgt | catggaatcagcccggatg | 52 | 563 |
| *14-18* | cccagaaggccaaggctgag | cgatgcggtgacacaggacacg | 55 | 369 |
| *17-26* | acgaggaagatgagcctgag | tgggtttttgggaatgtagc | 51 | 1084 |
| *18-25* | atgccacctggtttaccaac | tcatgaagaaggcaatgagg | 52 | 780 |
| *24-27* | tctcagccatgatgtccctctt | gtggttcatctgctccgactg | 54 | 443 |
| *25-26* | catctttgtgggcttcgtcatt | tggtatgggtttttgggaatgt | 54 | 150 |
| *26-35* | cacctcctcctactttgaatacct | agaacttcccaaagcccaga | 52 | 971 |
| *27-34* | agtcggagcagatgaaccac | atggccttgaactcatccag | 51 | 790 |
| *34-40* | actacctcacccgggactg | cgttggcattgttggtattg | 51 | 843 |
| *37-42* | aaccagcatgaagctcttgg | agcagtcccttcagcatctc | 52 | 678 |
| *39-44* | cggaccattgaggaagagg | cattggtcatgccagctcta | 51 | 990 |
| *34-44* | actacctcacccgggactg | cattggtcatgccagctcta | 51 | 1514 |
| *P1/P4* | gcacaagggctccttctgccgcaac | atgcgggcgctctcgtctgggtcgtcgatctcg ctgaggatgacgtcaatgat | 60 | 1059 |
| *P3/P2* | atcattgacgtcatcctcagcgagatcg acgacccagacgagagcgcccgcat | ggaagtgctcctgaatgaggaatgtggc | 60 | 1021 |
| *Taqman RT-PCR probes* | | | | |
| *28/29* | ctaatcgtcatcggcagcat | cagtctcatgacccggaaca (for template only) | | 168 |
| | ctaatcgtcatcggcagcat | ctccacccaggcaatacagt | | 91 |
| | **Taqman MGB Probe:** | attgacgtcatcctgagc | | |
| *28/30* | ctaatcgtcatcggcagcat | tctcatctgggtcatcgatct | | 61 |
| | **Taqman MGB Probe:** | attgacgtcatcctgagc | | |

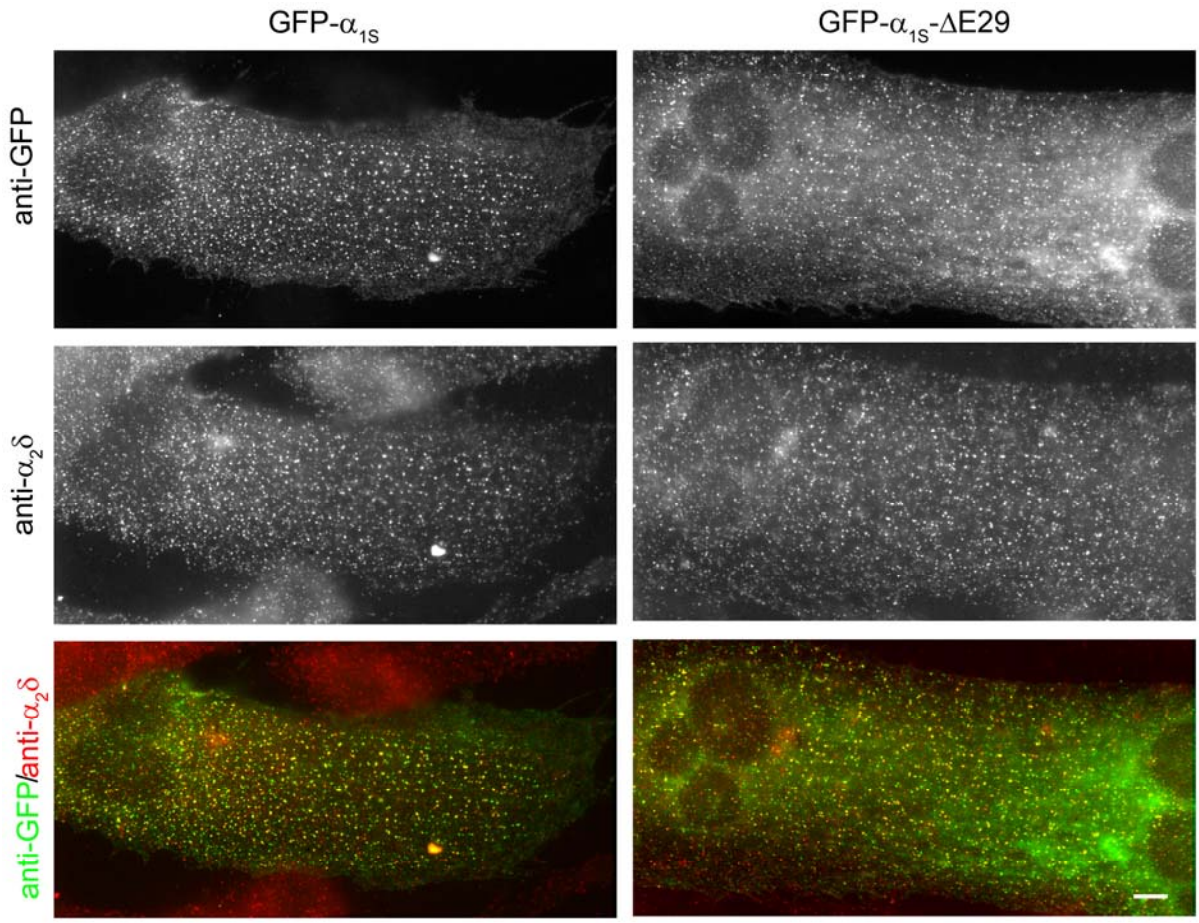


**Figure 2. Deletion of exon 29 does not disturb the colocalization of $\alpha_{1S}$ and $\alpha_2\delta$-1 subunit.** Dysgenic myotubes transfected with GFP-$\alpha_{1S}$ (**left**) or GFP-$\alpha_{1S}\Delta29$ splice variant (**right**) were double immunolabeled with anti-GFP (**top**) and anti-$\alpha_2\delta$-1 (**middle**). Clusters of GFP-$\alpha_{1S}$ or GFP-$\alpha_{1S}\Delta29$ colocalize with clusters of $\alpha_2\delta$-1 subunit (**color overlay**). Bar, 10 μm.