**Supplemental Table 1 Mutagenic primers used for introduction of variants**

|  |  |  |
| --- | --- | --- |
| Variant | Forward primer sequence | Reverse primer sequence |
| c.14570T>C \*p.Phe4857Ser† | CACCGTGGTGGCCT**C**CAACTTCTTCCGC | GCGGAAGAAGTTG**G**AGGCCACCACGGTG |
| c.14582G>A \*p.Arg4861His† | CCTTCAACTTCTTCC**A**CAAGTTCTACAACAA | TTGTTGTAGAACTTG**T**GGAAGAAGTTGAAGG |
| c.14752G>A \*p.Asp4918Asn† | ACAGGGTGGTCTTC**A**ACATCACCTTCTTCTT | AAGAAGAAGGTGATGT**T**GAAGACCACCCTGT |
| c.14497C>T\*p.His4833Tyr† | TGTCCTCTGTCACC**T**ACAATGGGAA | TTCCCATTGT**A**GGTGACAGAGGACA |
| c.13920G>C\*p.Met4640Ile† | GCACAGGCTACAT**C**GAACCCGCCCTGCG | CGCAGGGCGGGTTC**G**ATGTAGCCTGTGC |
| c.14545G>A\*p.Val4849Ile† | CCTTCTGGCGGTGGTC**A**TCTACCTGTACACCG | CGGTGTACAGGTAGA**T**GACCACCGCCAGAAGG |

Mutated codon is shown underlined with the altered nucleotide in bold.

\* GenBank accession NM\_000540.2.

† GenBank accession NP\_000531.2.

**Supplemental Table 2 EC50 values and statistical significance of RyR1 variants**

|  |  |  |
| --- | --- | --- |
| Variant | EC50 ± SEM (μM 4-c*m*c) | *p*-value† |
| Wild-type | 468.8 ± 17.2 |  |
| p.Met4640Ile | 487.2 ± 28 | 0.582 |
| p.His4833Tyr | 290.2 ± 11.5 | 2.85 x 10-8 \* |
| p.Val4849Ile | 411.6 ± 18.5 | 0.039  |
| p.Phe4857Ser | 0 | 7.65 x 10-15 \* |
| p.Arg4861His | 322.4 ± 19.7 | 4.81 x 10-5 \* |
| p.Asp4918Asn | 0 | 7.65 x 10-15 \* |

All variants show the amino acid change, GenBank accession NP\_000531.2.

† For comparisons with wild-type

\* Statistical significance, *p* < 0.01

**Supplemental Figure**

**Sequence alignment surrounding the de novo c.14570T>C, p.Phe4857Ser variant**

Sequence alignment was carried out using ClustalW (http://www.ch.embnet.org/software/ClustalW.html ) and shows this residue is conserved in RyR1, RyR2 and RyR3 in all species.

