Supplemental Material for:

Identification and functional characterization of malignant hyperthermia mutation T1354S in the outer pore of the $Ca_V\alpha_{1S}$ subunit

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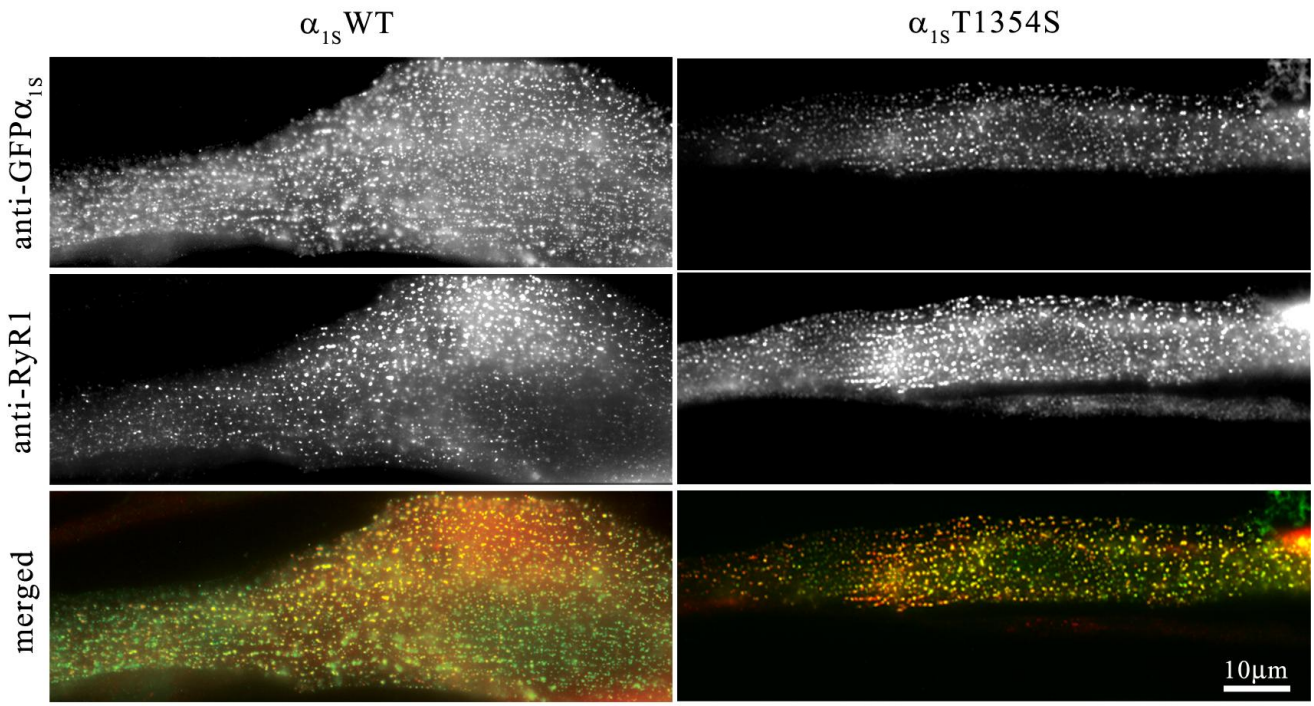
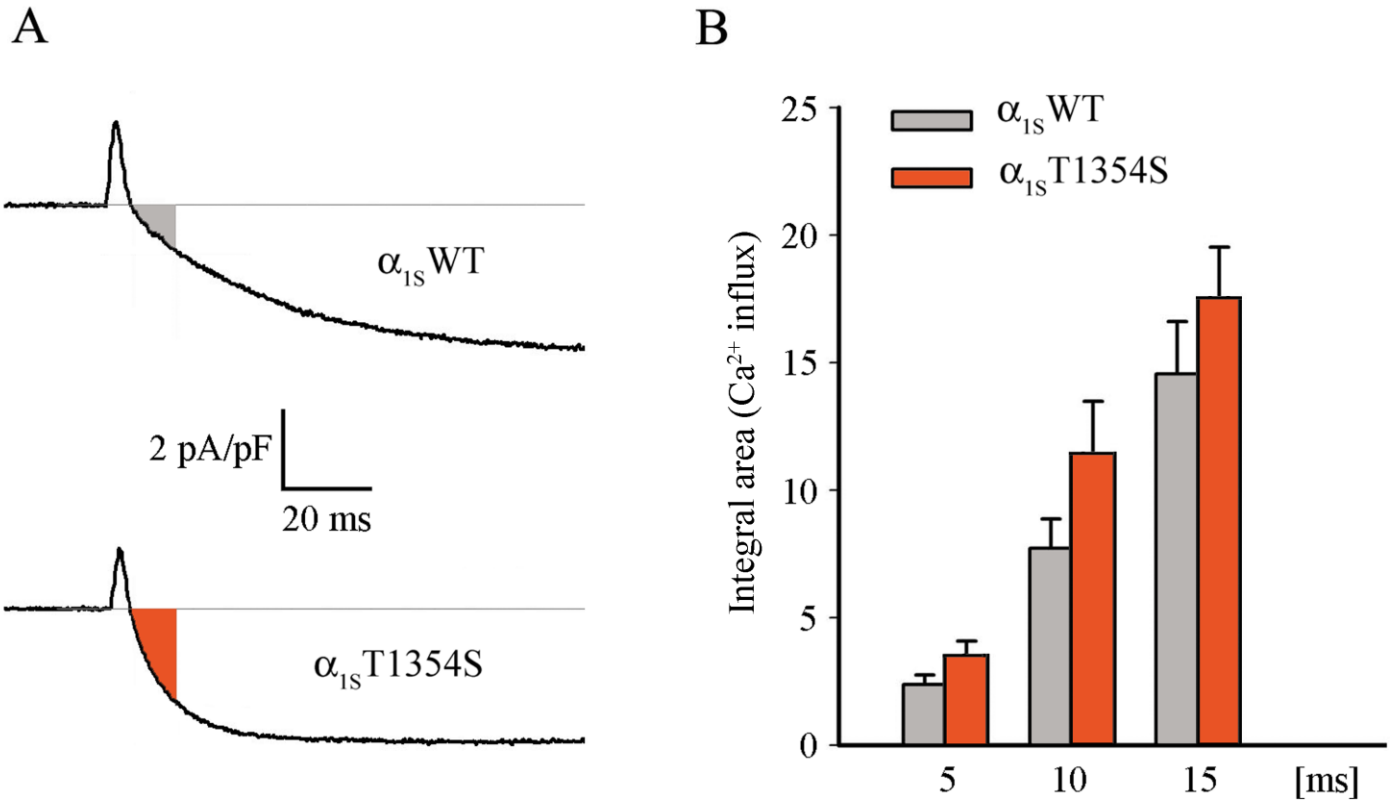
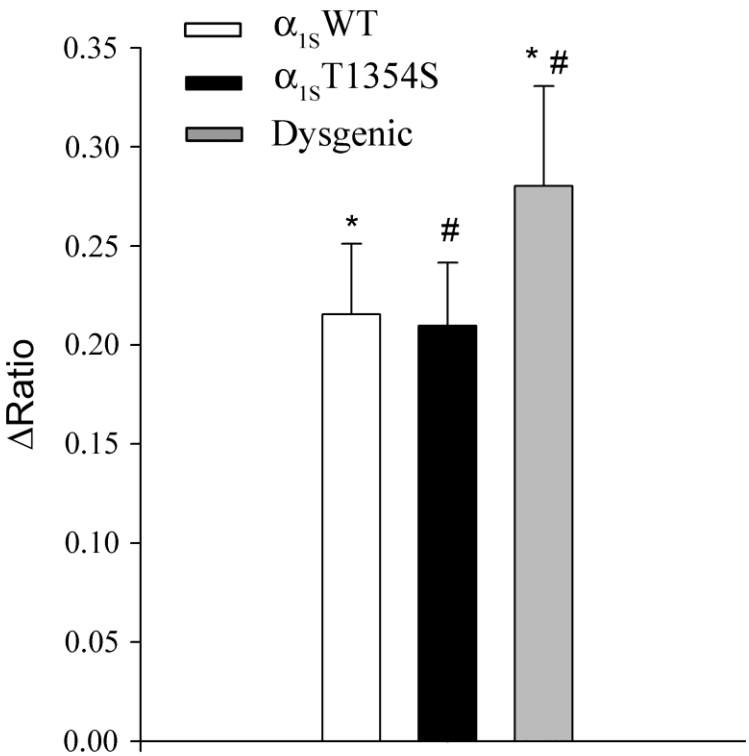
SUPPLEMENTAL FIGURES AND TABLES



Supplemental Figure 1. α_{1S}T1354S correctly incorporates into the plasma membrane and co-localizes with RyR1. Double immunocytochemical staining of dysgenic myotubes expressing either α_{1S}WT (*left*) or α_{1S}T1354S (*right*) show indistinguishable localization patterns for both constructs. Correct triad targeting is indicated by a punctuate pattern for the α_{1S} subunit (*upper panel*) and for RyR1 (*middle panel*). The merged image (*lower panel*) shows the high degree of co-localization of α_{1S} and RyR1 clusters (*yellow foci*).



Supplemental Figure 2. Ca^{2+} influx in the initial phase of depolarization-induced α_{1S}WT and α_{1S}T1354S currents. *A*: The initial depolarization phase (indicated are 10 ms of each 30-mV trace) of normalized (pA/pF) depolarization-induced Ca^{2+} currents (same data pool as Fig. 3) was integrated to estimate the net Ca^{2+} influx through α_{1S}WT (upper trace, *gray*) and α_{1S}T1354S (lower trace, *red*). *B*: Integration of the first 5, 10 and 15 ms of the currents revealed a tendency to higher Ca^{2+} influx (at 5 ms, $P = 0.08$) through α_{1S}T1354S- (n = 26, *red bars)* compared to α_{1S}WT expressing myotubes (n = 31, *gray bars*).



Supplemental Figure 3. Relative cytosolic Ca^{2+} resting levels in myotubes measured as F_{405}/F_{485} Indo-1 ratios at the beginning of each caffeine dose response experiment before depolarizations were applied (see Fig. 5). Ca^{2+} resting levels were significantly ($p < 0.01$) higher in untransfected *dysgenic* myotubes (n = 21) compared to α_{1S}WT (*; n = 19) and α_{1S}T1354S (#; n = 22) expressing myotubes.

SUPPLEMENTARY TABLES

Supplemental Table 1. IVCT results of family NA-6

Individuals	Halothane^						Caffeine^						Diagnosis
	Threshold* [%]		Contracture [g]				Threshold* [mM]		Contracture [g]				
			at threshold		**at 2%**				at threshold		**at 2 mM**		
	I	II	I	II	**I**	**II**	I	II	I	II	**I**	**II**	
II:1	1.5	0.5	0.3	0.2	**0.6**	**1.2**	2	0.5	0.2	0.2	**0.2**	**0.8**	MHS
II:2	n.d.	n.d.	n.d.	n.d.	**-0.2**	**0**	4	4	0.2	n.d.	**-0.2**	**0**	MHN
II:3	1	1	0.4	0.2	**2**	**0.6**	1	1	0.4	0.2	**1.2**	**1**	MHS
II:6	0.5	1	0.6	0.4	**3.2**	**1**	1.5	2	0.2	0.6	**1**	**0.6**	MHS
III:1	1	1	0.8	0.8	**2.4**	**2.6**	0.5	1	0.6	0.4	**1.4**	**2.4**	MHS
III:2	2	2	0.6	0.8	**0.6**	**0.8**	2	2	0.6	0.8	**0.6**	**0.8**	MHS
III:4	n.d.	n.d.	n.d.	n.d.	**-0.4**	**0.1**	>4	4	n.d.	0.4	**-1**	**-0.3**	MHN
III:5	1.5	-	0.2	-	**0.4**	**-**	1.5	1.5	0.2	0.2	**0.4**	**0.4**	MHS
III:6	n.d.	2	n.d.	0.25	**-0.3**	**0.25**	4	4	1.1	0.9	**0**	**0.1**	MHE
III:7	n.d.	1.5	n.d.	0.3	**-0.3**	**0.8**	4	4	0.6	0.45	**0**	**0**	MHE
III:8	0.5	0.5	0.2	0.25	**1.4**	**1.6**	1.5	-	0.5	-	**0.9**	**-**	MHS

^ results obtained from two different fibers (I and II) are reported
* at which a contraction ≥ 0.2 g of a muscle fiber was obtained
n.d. = not determined

Members of family NA-6 were subjected to the standardized European *in-vitro* contracture test (IVCT). Standardized muscle biopsy material was used to determine the threshold concentration for halothane (in %) and caffeine (in mM) at which a force of ≥ 0.2 g was generated as well as the force production upon application of 2% halothane or 2 mM caffeine. Samples which generated a force of ≥ 0.2 g at 2% halothane and 2 mM caffeine were judged MH susceptible (MHS). According contractions induced by only one of the triggering agents were classified as MH equivocal (MHE).

Supplemental Table 2. CACNA1S sequence variants in family NA-6

Exon	Polymorphism	Substitution	Amino Acid	Reference
5	c.579C>T	AT**C**/AT**T**	Ile199	this work
11	c.1491C>T	AA**C**/AA**T**	Asn497	this work
11	c.1515C>T	TG**C**/TG**T**	Cys505	this work
11	c.1551C>T	GG**C**/GG**T**	Gly517	this work
11	c.1564C>T	**C**TG/**T**TG	Leu522	this work
18	c.2403T>C	TT**T**/TT**C**	Phe 801	(ref. 6)
33	c.4060A>T	**A**CC/**T**CC	T1354S	this work
37	c.4475C>A	G**C**C/G**A**C	A1492D	(ref. 6)
44	c5398T>C	**T**TG/**C**TG	Leu1800	(ref. 6)