Supplementary Data

The RyR1 P3528S Substitution Alters Mouse Skeletal Muscle Contractile Properties and RyR1 Ion Channel Gating

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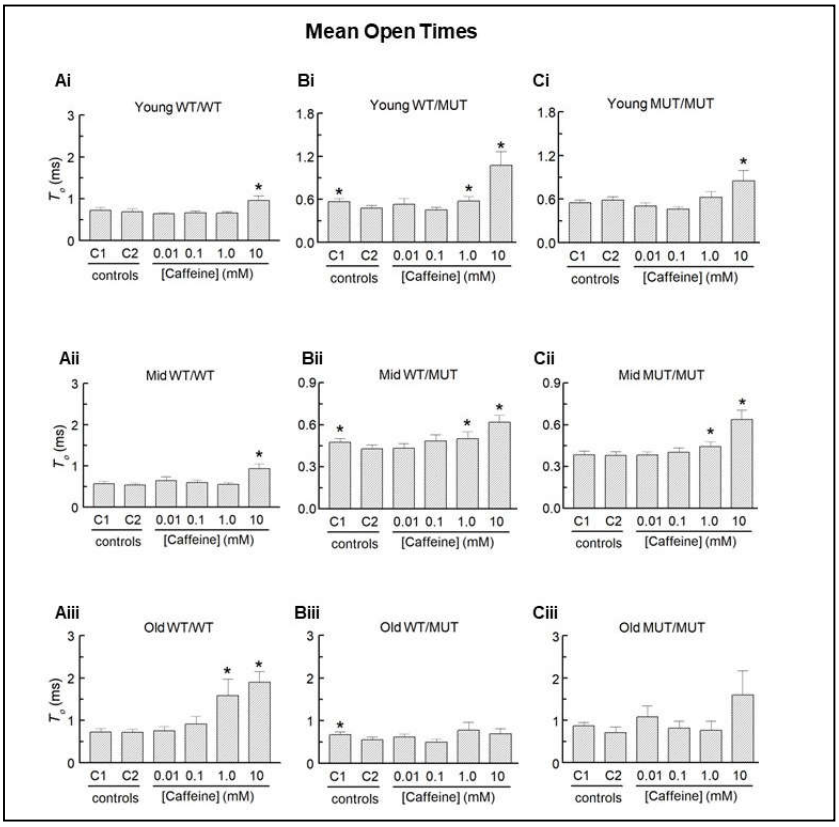

Figure S1. The influence of the RyR1 P3528S mutation on the changes in the mean open time of RyR1 channels in response to a reduction in cytoplasmic [Ca^{2+}] from 1 μM to 300 nM (C1 to C2) and to the addition of caffeine in increasing concentrations from 10 μM to 10 mM. A-C. Graphs of the average channel mean open time (T_o in ms) with 1 μM *cis* Ca^{2+} (C1), 300 nM *cis* Ca^{2+} (C2) and then after progressive increases in cytoplasmic [caffeine] to 10 μM, 100 μM, 1 mM and 10 mM. Average T_o is shown for channels from WT/WT mice (column **A**), WT/MUT mice (column **B**) and MUT/MUT mice (column **C**). Average T_o is shown for channels from young (row **Ai-Ci)**), middle (row **Aii-Cii**) and old (row **Aiii-Ciii**) aged mice. T_o values obtained at +40 mV and -40 mV are included in the average values, shown as mean ± sem. Young WT/WT, n=14; young WT/MUT n=16; young MUT/MUT n=12; middle aged WT/WT n=16; middle aged

WT/MUT n=16; middle aged MUT/MUT n=14; old WT/WT n=8; old WT/MUT n=8; old MUT/MUT n=6. *, significantly different from C2 (300 nM *cis* Ca^{2+}).

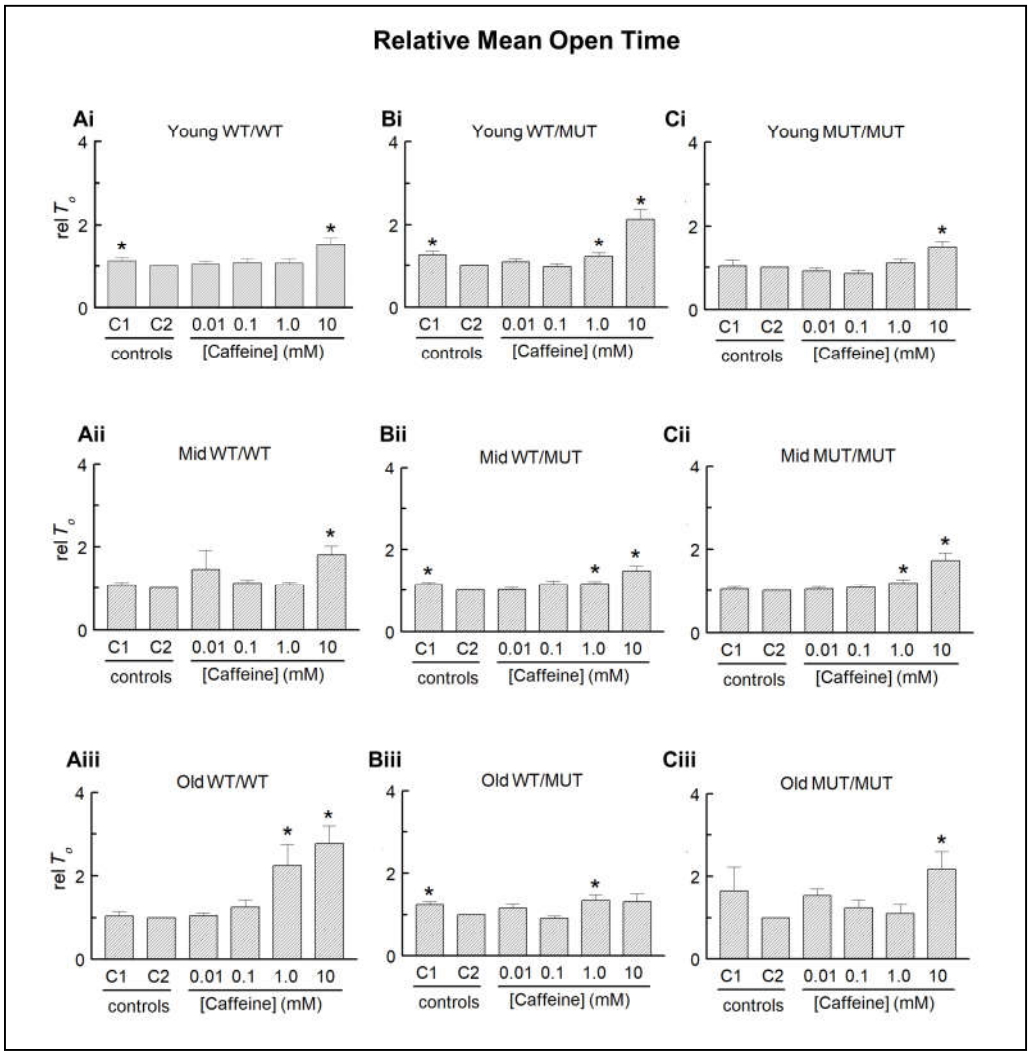

Figure S2. **The influence of the RyR1 P3528S mutation on the changes in the relative mean open time of RyR1 channels in response to a reduction in cytoplasmic [Ca^{2+}] from 1 μM to 300 nM (C1 to C2) and to the addition of caffeine in increasing concentrations from 10 μM to 10 mM.** **A-C**. Graphs of average relative mean open time (rel T_o) with 1 μM *cis* Ca^{2+} (C1), 300 nM *cis* Ca^{2+} (C2) and then after progressive increases in cytoplasmic [caffeine] to 10 μM, 100 μM, 1 mM and 10 mM. Average rel P_o is shown for channels from WT/WT mice (column **A**), WT/MUT mice (column **B**) and MUT/MUT mice (column **C**). Data is shown for channels from young (**Ai - Aiii**), middle (**Bi - Biii**) and old (row **Ci - Ciii**) aged mice. Rel T_o values obtained at +40 mV and -40 mV are included in the average values which are shown as mean ± sem. The numbers of observations in each group were as follows. Young WT/WT, n=14; young WT/MUT n=16; young MUT/MUT n=12; middle aged WT/WT n=16; middle aged WT/MUT n=16; middle aged MUT/MUT n=14; old WT/WT n=8; old WT/MUT n=8; old MUT/MUT n=6. *, significantly different from C2 (300 nM cis Ca^{2+}).

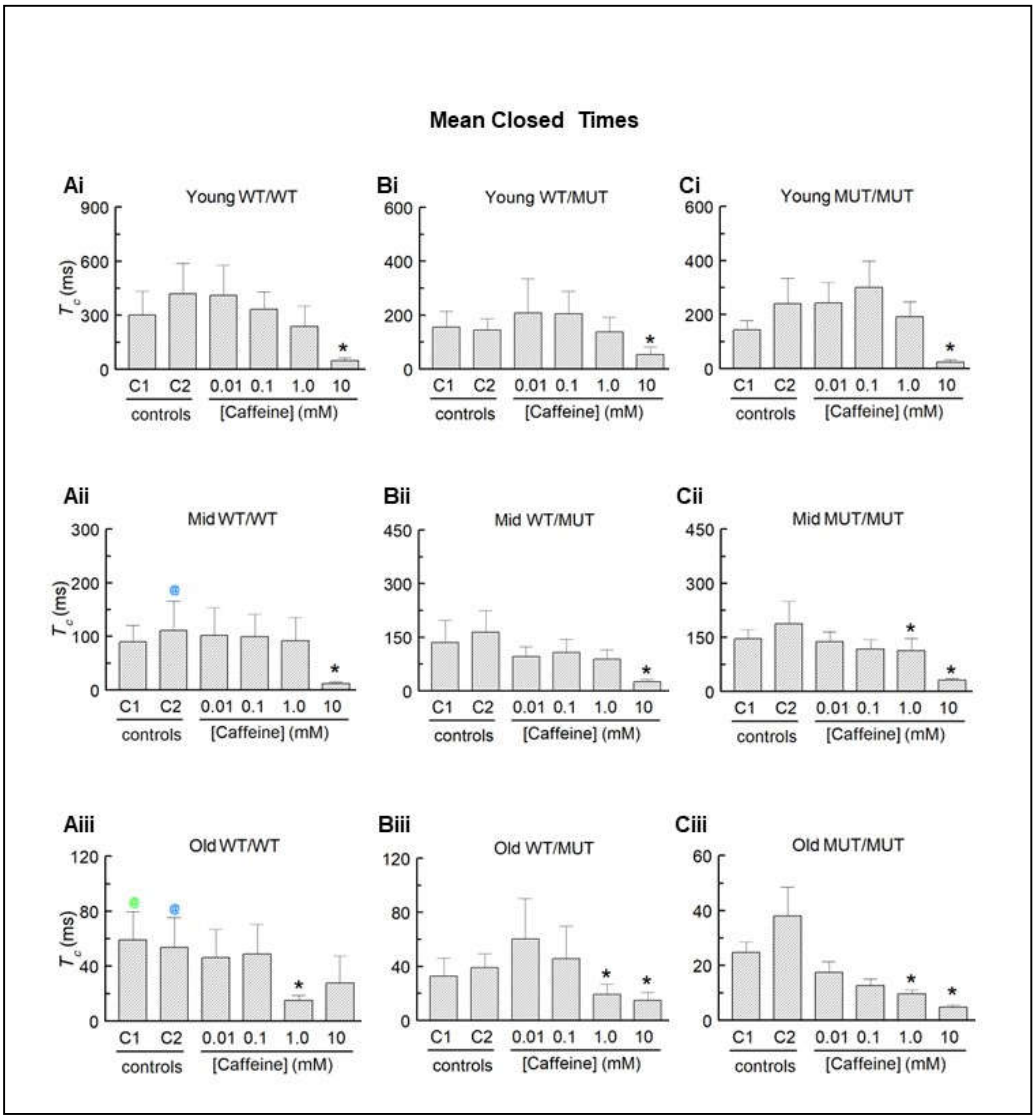

Figure S3. **The influence of the RyR1 P3528S mutation on the changes in the mean closed duration of RyR1 channels in response to a reduction in cytoplasmic [Ca^{2+}] from 1 μM to 300 nM (C1 to C2) and to the addition of caffeine in increasing concentrations from 10 μM to 10 mM.** **A-C**. Graphs of average mean closed time (T_c) with 1 μM *cis* Ca^{2+} (C1), 300 nM *cis* Ca^{2+} (C2) and then after progressive increases in cytoplasmic [caffeine] to 10 μM, 100 μM, 1 mM and 10 mM. Average T_c is shown for channels from WT/WT (column **A**), WT/MUT (column **B**) and MUT/MUT (column **C**) mice. Data is shown for channels from young (**Ai - Aiii**), middle (**Bi - Biii**) and old (row **Ci - Ciii**) aged mice. T_c values obtained at +40 mV and -40 mV are included in the average values which are shown as mean ± sem. The numbers of observations in each group were as follows. Young WT/WT, n=14; young WT/MUT n=16; young MUT/MUT n=12; middle aged WT/WT n=16; middle aged WT/MUT n=16; middle aged MUT/MUT n=14; old WT/WT n=8; old WT/MUT n=8; old MUT/MUT n=6. *, significantly different from C2 (300 nM *cis* Ca^{2+}); @, significantly different from C1 (1 μM cis Ca^{2+}) in channels from young WT/WT mice; @, significantly different from C2 (300 nM cis Ca^{2+}) in channels from young WT/WT mice.

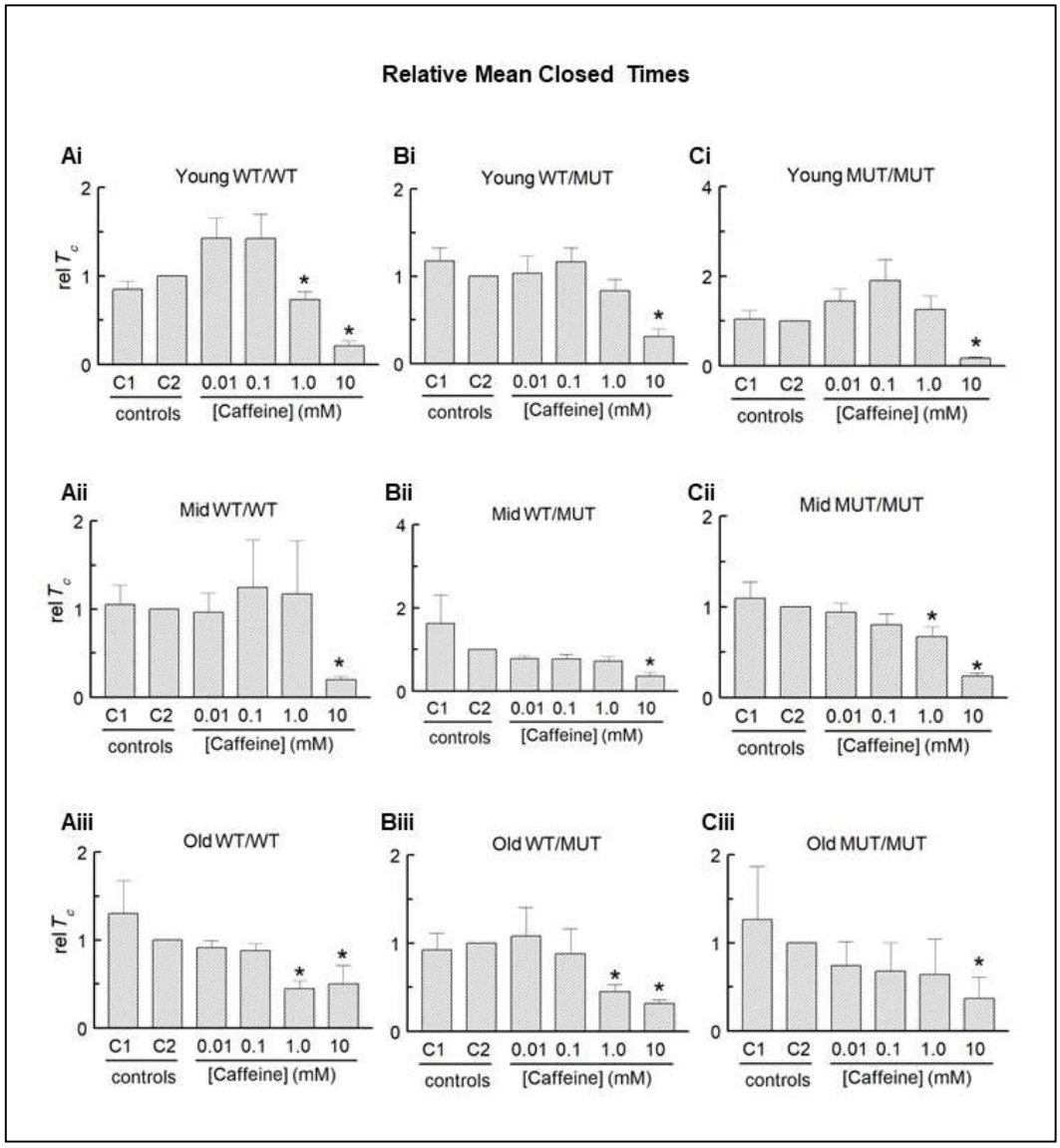

Figure S4. The influence of the RyR1 P3528S mutation on the changes in the relative mean closed duration of RyR1 channels in response to a reduction in cytoplasmic [Ca^{2+}] from 1 μM to 300 nM (C1 to C2) and to the addition of caffeine in increasing concentrations from 10 μM to 10 mM. **A-C**. Graphs of average relative mean closed time (rel T_c) with 1 μM *cis* Ca^{2+} (C1), 300 nM *cis* Ca^{2+} (C2) and then after progressive increases in cytoplasmic [caffeine] to 10 μM, 100 μM, 1 mM and 10 mM. Average rel T_c is shown for channels from WT/WT mice (column **A**), WT/MUT mice (column **B**) and MUT/MUT mice (column **C**). Data is shown for channels from young (**Ai - Aiii**), middle (**Bi - Biii**) and old (row **Ci - Ciii**) aged mice. Rel T_c values obtained at +40 mV and -40 mV are included in the average values which are shown as mean ± sem. The numbers of observations in each group were as follows. Young WT/WT, n=14; young WT/MUT n=16; young MUT/MUT n=12; middle aged WT/WT n=16; middle aged WT/MUT n=16; middle aged MUT/MUT n=14; old WT/WT n=8; old WT/MUT n=8; old MUT/MUT n=6. The asterisks indicate significant differences from the C2 control values obtained with 300 nM *cis* Ca^{2+}. *, significantly different from C2 (300 nM cis Ca^{2+}).

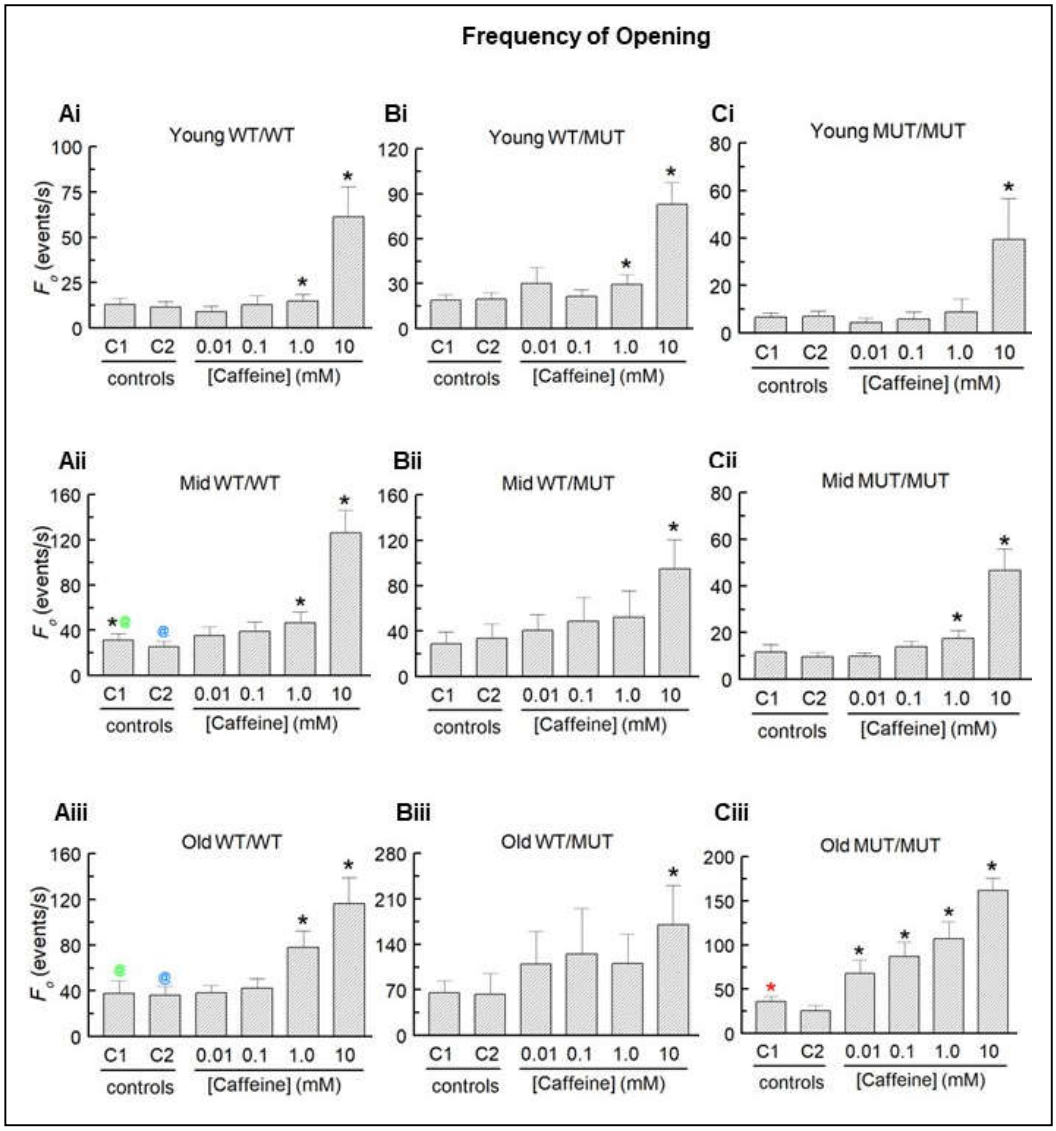

Figure S5. **The influence of the RyR1 P3528S mutation on the changes in the frequency of open events in RyR1 channels in response to a reduction in cytoplasmic [Ca^{2+}] from 1 μM to 300 nM (C1 to C2) and response to the addition of caffeine in increasing concentrations from 10 μM to 10 mM.** **A-C**. Graphs of average frequency (F_o) of channel open events with 1 μM *cis* Ca^{2+} (C1), 300 nM *cis* Ca^{2+} (C2) and then after progressive increases in cytoplasmic caffeine concentrations to 10 μM, 100 μM, 1 mM and 10 mM. Average F_o is shown for channels from WT/WT mice (column **A**), WT/MUT mice (column **B**) and MUT/MUT mice (column **C**). Data is shown for channels from young (**Ai - Aiii**), middle (**Bi - Biii**) and old (row **Ci - Ciii**) aged mice. F_o values obtained at +40 mV and -40 mV were included in the average values which are shown as mean ± sem. The numbers of observations in each group were as follows. Young WT/WT, n=14; young WT/MUT n=16; young MUT/MUT n=12; middle aged WT/WT n=16; middle aged WT/MUT n=16; middle aged MUT/MUT n=14; old WT/WT n=8; old WT/MUT n=8; old MUT/MUT n=6. * or *, significantly different from corresponding C2 value (300 nM cis Ca^{2+}); @, significantly different from C1 (1 μM cis Ca^{2+}) in channels from young WT/WT mice; @, significantly different from C2 (300 nM cis Ca^{2+}) in channels from young WT/WT mice.

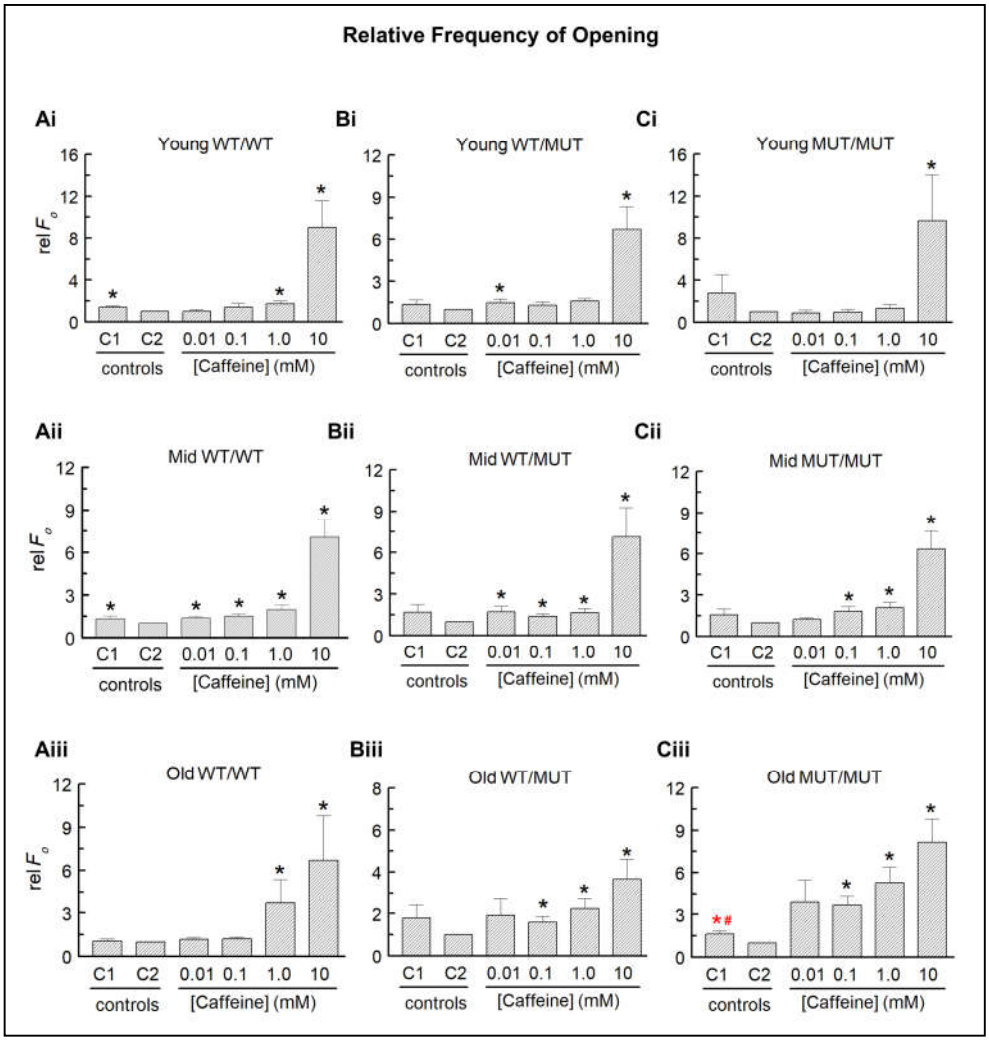

Figure S6. **The influence of the RyR1 P3528S mutation on changes in the relative frequency of open events in RyR1 channels in response to a reduction in cytoplasmic [Ca^{2+}] from 1 μM to 300 nM (C1 to C2) and response to the addition of caffeine in increasing concentrations from 10 μM to 10 mM.** **A-C**. Graphs of average relative event frequency (rel F_o) with 1 μM *cis* Ca^{2+} (C1), 300 nM *cis* Ca^{2+} (C2) and then after progressive increases in cytoplasmic caffeine concentrations to 10 μM, 100 μM, 1 mM and 10 mM. Average rel F_o is shown for channels from WT/WT mice (column **A**), WT/MUT mice (column **B**) and MUT/MUT mice (column **C**). Data is shown for channels from young (**Ai - Aiii**), middle (**Bi - Biii**) and old (row **Ci - Ciii**) aged mice. Rel F_o values obtained at +40 mV and -40 mV were included in the average values which are shown as mean ± sem. The numbers of observations in each group were as follows. Young WT/WT, n=14; young WT/MUT n=16; young MUT/MUT n=12; middle aged WT/WT n=16; middle aged WT/MUT n=16; middle aged MUT/MUT n=14; old WT/WT n=8; old WT/MUT n=8; old MUT/MUT n=6. * or *, significantly different from C2 (300 nM cis Ca^{2+}); # significantly different from old WT/WT.