Supplemental Material

DETAILED METHODS

Animal model

Studies were carried out according to NIH guidelines and approved by the Animal Care and Use Committees of Georgetown University and Vanderbilt University. The generation and in vitro characterization of the transgenic mouse models used here has been reported previously.[1-3] To model the autosomal dominant human disease, we chose transgenic TnT lines (WT line 3, TnT-I79N line 8, TnT-R278C line 5 and TnT-F110I line 1) that have comparable TnT protein expression levels, whereby approximately 50% of the endogenous mouse TnT protein is replaced with either WT or mutant human cardiac TnT.[1-3] The Ca sensitivity of force development (pCa50) in skinned fibers transgenic mice was changed as follows (WT=0): TnT-R278C -0.03±0.01 (n.s.), TnT-F110I +0.12±0.01 and TnT-I79N +0.31±0.01 (both $p<0.01$ vs TnT-WT).[4] All lines have been backcrossed into the BL6SJF1/J mouse strain for more than 7 generations.

Cytosolic Ca buffering and SR Ca content measurements

Mouse ventricular cardiomyocytes were isolated by standard techniques as previously described with minor modifications.[5] Hearts were removed during deep anesthesia with 5% Isoflurane and perfused for 3 min with modified normal tyrode without 2,3 butanedione monoxime (BDM). Myocytes were perfused with normal tyrode (NT) containing in mmol/L: NaCl 134, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1, D-glucose 10, HEPES 10, pH 7.4. Patch pipettes were pulled from borosilicate glass capillaries (resistance 2-3.5 mΩ) and ionic currents were recorded in the whole-cell, voltage-clamp configuration of patch-clamp technique and sampled at 10 kHz using an Axopatch200B amplifier and Clampex 8.2 software (Axon Instruments, Inc, USA).

Rapid caffeine application buffering protocol modified according to Trafford et al.:[6] For this approach 15 mM caffeine was rapidly applied to produce a transient increase of $[Ca]_{free}$ and compared to the change in total cytoplasmic calcium concentration ($\Delta[Ca]_{total}$) derived from the simultaneously integrated NCX current. In detail, the pipette solution contained in mmol/L: KAsp 90, KCl 30, MgATP 5, $MgCl_2$ 1, cAMP 0.050, fluo-4$(K)_5$ 0.025, NaCl 5, HEPES 20, pH 7.2 with KOH. SR Ca content was reduced by approximately 50% by pretreatment with low-dose caffeine (1 mmol/L). This prevented saturation of fluo-4 Ca indicator and reduced cell contraction to prevent movement artifacts. $[Ca]_{free}$ was calculated using the standard equation [eqn. 1] $[Ca]_{free}=K_d*(F-F_0)/(F_{max}-F)$.[7] Fluo-4 is very similar in structure to fluo-3, which is known to have a temperature-sensitive dissociation constant (K_d; approximately 864 nmol/L at 37ºC and 400 nmol/L at 22ºC).[8] Therefore, to calculate $[Ca]_{free}$ we assumed as K_d for fluo-4 for experiments performed at approximately 28ºC 600 nmol/L (TnT mice), but for experiments at approximately 22ºC a K_d of 400 nmol/L (EMD treatment). Each individual myocyte was damaged with the patch pipette at the end of the experiment to assess maximal fluo-4 fluorescence (F_{max}). Myocytes treated with EMD showed systematically lower F_{max} values compared to size-matched controls, possibly because they seized faster than non-sensitized cardiomyocytes when damaged with the pipette. Therefore, we performed linear regression of F_0/pApF (to account for differences in fluo-4 loading) versus F_{max} from control cells and extrapolated F_{max} for EMD-treated cells. Alternatively, we recalculated results using a formula

independent of an F_{max} value[9] and also found an increase in the Ca total/free ratio, even though the effect based on this calculation was not significant.

$\Delta[Ca]_{total}$ was calculated from the integrated NCX current as described by Berlin et al.[10] Cell volume was calculated assuming a capacitance-volume ratio of 6.76 pF/pl as described for 3-month-old rat ventricular myocytes.[11, 12] Only the first rapid caffeine application was used for Ca buffering analysis. Buffering curves were generated by plotting $[Ca]_{total}$ as a function of $[Ca]_{free}$ and K_d and B_{max} estimated for each myocyte using a modified Michaelis-Menten equation [eqn.2]: $[Ca]_{total}= C+(B_{max}*([Ca]_i-x_{off})/(k_d+([Ca]i-x_{off}))$. Only curves that were fit well were used for final analysis. The $\Delta[Ca]_{total}$ was not corrected for non-NCX exchange fluxes (which should be minimal in mouse[13]) and B_{max} values were not corrected for Ca buffering by the Ca indicator as the purpose is only to compare groups. The resulting K_d values of WT (582±57 nM, n=9) were similar to those reported by Trafford et al for ferret myocytes (590±170 nM).[6]

To model the effect of the TnT mutations on systolic Ca transients at different end-diastolic $[Ca]_{free}$, we first generated full buffering curves in MATLAB for WT, R278C, F110I and I79N using the equation $Ca_{bound} = B_{max} / (1 + (K_d)/Ca_{free}))$. Values for B_{max} and K_d were taken from experimental data shown in Figure 2B and C; $[Ca]_{free}$ was iteratively increased from 0 µM to 3 µM in 1 nM steps. Following this, cytosolic buffering capacity was calculated as a function of end-diastolic $[Ca]_{free}$. Diastolic $[Ca]_{free}$ was iteratively increased from 0.13 µM (=the average resting diastolic Ca obtained experimentally in quiescent myocytes) to 0.6 µM in 1 nM steps. At each $[Ca]_{free}$, the result of adding 30 µM of Ca was calculated from the earlier buffering curve to determine the resulting $[Ca]_{free}$. The buffering capacity was calculated as the total added Ca (30 µM) divided by the change in $[Ca]_{free}$ (before and after the addition of 30 µM of Ca). This was repeated for all 4 genotypes.

Ca current pulse protocol according to Berlin et al.:[10] Briefly, using this technique $\Delta[Ca]_{total}$ is determined by integration of Ca influx through the L-type Ca channel. Experiments are carried out in voltage-clamped myocytes in the absence of intra- and extracellular Na^+ (to block NCX-mediated transmembrane Ca flux) and after incubation with 5 µmol/l thapsigargin for 10 min (to block SERCA-mediated Ca uptake into the SR). The internal pipette solution was the same as above, but KAsp and KCl were replaced by $CsCl_2$. The sodium in NT was replaced with 135 mM N-metyl-D-glukomine (NMDG+) and $CaCl_2$ increased to 10 mmol/L to enhance Ca current. In addition, 10 µmol/L Ru360 was used to block mitochondrial Ca uptake.[14] Cells were clamped at a holding potential of -45 mV, and voltage steps to -20-0 mV were applied for 20-50 ms duration at 1 Hz. The impulse duration and voltage step were varied dynamically to produce similar currents for each cell and depolarization step.

SR Ca content measurement modified according to Varro et al.:[15] SR Ca content was determined by releasing all Ca by rapid application of caffeine (10 mmol/l) for 4 s and integrating the resulting NCX in voltage-clamped myocytes. Internal and external solutions were identical to that of the Ca buffering experiments with the Trafford et al method described above, with the only modification that the contractile uncoupler blebbistatin (20 µmol/l) was included in the caffeine solution to prevent the hypercontracture and loss of gigaseal that invariably followed when a full SR was released in I79N myocytes. To avoid any potential carryover effects of blebbistatin, only one caffeine application was done in each myocyte, and the chamber cleaned after each caffeine application. SR Ca content was calculated as $\Delta[Ca]_{total}$ using the same capacitance-volume ratio of 6.76 pF/pl as described above. To measure end-diastolic SR Ca

content during steady-state pacing (2 Hz), caffeine was applied following the last pacing stimulus at the same 0.5 s interval (= pacing cycle length) used in the pacing train. To measure SR content after a pause, caffeine was applied 4 s after the last pacing stimulus.

Protein expression levels

Mouse heart ventricles were mechanically pulverized and then further disrupted using a dounce homogenizer in buffer containing in mmol/l: NP-40 0.5%, NaCl 150, Tris-HCl 50, pH 7.5 in the presence of phosphatase and protease inhibitors. Protein concentration was determined using Lowry assay. Proteins were blotted on nitrocellulose membrane and incubated with primary antibody overnight in LICOR Odyssey blocking buffer. Antibodies used were anti-SERCA, anti-phospholamban (both custom antibodies provided by Dr. M. Periasamy, Columbus, OH), anti-Casq2 (custom antibody provided by Dr. L. Jones, Indianapolis, IN), anti-NCX clone R3F1 (Fitzgerald, MA), anti-CaM clone 05-173 (Fitzgerald, MA) and anti-GAPDH [9484] (abcam, MA). Fluorescent secondary antibodies were obtained from Rockport, MA, and blots were imaged using a LICOR Odyssey scanner. Analysis was performed using Odyssey 3.0 software.

Ca transient measurements in intact hearts

Heparinized mice were deeply anesthetized using 5% isofluorane in pure oxygen. A thoracotomy was performed, the hearts rapidly excised and their aortas cannulated using a custom plastic cannula. Hearts were then perfused in the Langendorff mode at constant pressure (70 mmHg) with bicarbonate buffer at a temperature of 36˚C bubbled with 95% O_2 and 5% CO_2. The buffer contained 130 mM NaCl, 4 mM KCl, 23 mM $NaHCO_3$, 1.5 mM NaH_2PO_4, 1 mM $MgCl_2$, 2mM $CaCl_2$, 10 mM Glucose (Sigma Aldrich, Saint Louis, MO and Fisher Scientific, Pittsburgh, PA) and 0.2 µM propranolol, and was prepared just prior to the experiment.

A working dye solution was made by sonicating 50 µg Rhod- 2AM (Molecular Probes, Eugene, OR) dissolved in 22.5µl DMSO (Sigma Aldrich, Saint Louis, MO), 7.5µl 20% pluronic in DMSO (Molecular Probes, Eugene, OR) and 500µl bicarbonate buffer for 10 minutes. This working solution was recirculated through the heart for 15 minutes by using a roller pump connected to a small cup placed under the heart and an injection port directly above the heart. Following dye recirculation, hearts were perfused for 10-15 minutes to allow unloaded dye to wash out of the interstitium and to allow loaded AM dye to de-esterify.

To facilitate external control of heart rate, the atrio-ventricular node was thermally ablated using a fine tip surgical cautery (Cardinal Health, Dublin, OH). Hearts were then immersed into an imaging chamber containing the same warmed, bubbled bicarbonate solution with their left ventricles facing a quartz imaging window. A platinum pacing electrode coated with glass except at the tip was positioned at the apex of the heart. A custom plastic cradle with volume conducting ECG electrodes was used to gently position the heart against the imaging window. The stiff pacing electrode and the ECG cradle mechanically stabilized the hearts during contraction.

Hearts were subjected to a pacing protocol with a 1 minute train of S1 pacing pulses provided at a constant pacing cycle length (PCL) followed by a 1 second pause and a post-post S2 pulse. The S1 PCL was started at 150 ms, then sequentially lowered every minute through 120 ms, 100 ms, and 80 ms. During the last beats of each minute of S1 pacing, the S1-S2 pause and the S2 beat, hearts were uniformly illuminated by a 532 nm diode-pumped laser (Coherent Inc., Santa Clara, CA) through 4 liquid light guides. The resulting Rhod-2 fluorescence was

filtered through a red long-pass optical filter (Tiffen, Long Island, NY) and recorded on a 1000 frame per second 80x80 pixel CCD camera (RedShirtImaging LLC, Decatur, GA).

Data were analyzed offline in MATLAB (Mathworks, Natick, MA) using custom algorithms. Recordings were initially processed with a temporal 3-frame moving average filter and a spatial rotationally-symmetric Gaussian low-pass filter with an SD of 1 applied to a 3x3 matrix. A 10x10 pixel window covering a small region close to the center of the LV was then selected and the fluorescence data spatially averaged; the resulting fluorescence trace was exported to Origin (OriginLab, Northampton, MA). Traces were manually analyzed in Origin to obtain F_0, diastolic and systolic Ca, S1 and S2 amplitudes. The last S1 transient decays were fitted to a single exponent decay function to determine the time constant of cytosolic Ca removal.

Acute MI experiments:

In order to generate an acute MI, a 5-0 silk suture (Ethicon, Somerville, NJ) was threaded under the left anterior descending artery (LAD) and looped without tightening or applying pressure to the heart's surface following cannulation, prior to the start of perfusion. Hearts were then loaded with Rhod-2 and the AV nodes ablated as described above. Following optical confirmation of successful dye loading, the ligature loop was tightened to produce an MI. Depending on the experimental group, either vehicle (DMSO) or EMD 57033 (3mM stock in DMSO) contained in a gastight glass syringe (Hamilton Co., Reno, NV) was continuously perfused into the bubble trap above the heart at a rate $1/1000^{th}$ that of the heart's perfusion rate using a syringe pump (Harvard Apparatus, Holliston, MA) to achieve a final EMD concentration of 3 μM. Hearts were then immersed into the same chamber with the EKG cradle; a platinum pacing electrode coated with plastic except at the tip was positioned at the top of the left ventricle, just below the left atrial appendage.

Hearts were then subjected to a continuous electrical pacing train at a cycle length of 100 ms for 30 minutes. Every 30 seconds, the pacing train was paused for 1 second, following which a post-pause S2 extra stimulus was supplied; the 100 ms cycle length pacing train was then restarted following a post-S2 pause. For 4 seconds around the S1-S2 portion of the pacing protocol alone, hearts were imaged as described above.

Imaging data were analyzed offline as described; the window was selected in a well-perfused region at the base of the LV above the MI and ligature. EKG data were analyzed manually in LabChart (Sydney, Australia); instances of PVC's and post-S2 triggered VT were analyzed in blinded fashion without knowledge of the drug treatment. Sustained VT was defined as VT lasting longer than 30s.

Ca transient measurements in intact myocytes

Single ventricular myocytes were isolated by a modified collagenase/protease method and loaded with Fura-2AM (Molecular Probes, Inc., Eugene, OR) as described.[5, 16] Fura2-loaded cells were placed in heated chamber, superfused for 15-20 minutes with Tyrode buffer (1.8 mmol/L Ca, 33 °C) and then field-stimulated at 1 Hz and 5 Hz (SD4 stimulator, Grass Instruments, Massachusetts). Intracellular Ca transients were measured using a rapidly alternating dual beam excitation fluorescence photometry set-up (Vibraspec Inc., Bear Island, ME) as described.[5] Excitation wavelengths of 340 and 410 nm were used to monitor the fluorescence signals of Ca-bound and Ca-free fura-2.[17] After subtracting the cellular autofluorescence, $[Ca]_i$ is proportional to the fluorescence ratio at 340 nm and 410 excitation (F_{340}/F_{410}). Since fura-2 compartmentalizes into mitochondria,[18] calculating intracellular Ca concentrations from fura-2 fluorescence ratios can be problematic in intact cells. Thus, $[Ca]_i$

measurements were reported as fluorescence ratio (F_{ratio}). After 5 minutes of steady-state pacing, Ca transient amplitude and end-diastolic $[Ca]_i$ were measured using pClamp8 software. The Ca transient decay was fitted with a monoexponential function and the time constant calculated for each pacing rate and genotype.

ECG and monophasic action potential (MAP) recordings

Volume-conducted ECG and monophasic action potential (MAP) recordings have been previously described.[19] In brief, the heart was placed horizontally in a heart cradle and is surrounded by four custom-build silver/silver chloride electrodes in close proximity for recording of volume-conducted ECG signals. Hearts were allowed to equilibrate for at least 10 minutes before any measurements were obtained. A custom-build miniature silver/silver chloride catheter (tip diameter 0.25 mm) was used to record MAP. The catheter was lowered perpendicularly onto the epicardial surface of the heart until gentle, but stable contact pressure was achieved. The ECG and MAP recordings were pre-amplified, digitized and stored using a commercially available data acquisition system (PowerLab, ADInstruments Inc., Menlo Park, CA). MAP data were then analyzed using a custom-built software program written in LabVIEW (National Instruments, Austin, TX).

Our preliminary experiments suggested that afterdepolarizations and triggered arrhythmias occurred more frequently after pauses. Thus, we developed a standardized stimulation protocol to quantify the amount of afterdepolarizations and ventricular arrhythmias. After ablating of the AV node the isolated perfused hearts were paced at twice-diastolic threshold strength with repeated pacing trains of 40 beats at different rates. The last beat of the pacing train (S1) was followed by an extra beat (S2) with S1S2 coupling interval of 500 ms. The next pacing train commenced after another 500 ms pause. Arrhythmic activity following the S2 beat was simultaneously recorded with volume-conducted ECG and monophasic action potential (MAP). Recordings with spontaneous activity within the first pause (S1S2 interval) were discarded. All data were analyzed in blind fashion without knowledge of the genotype. MAP recordings were compared to the simultaneously recorded ECG signal and classified as a) early afterdepolarization (EAD) b) triggered beat c) triggered ventricular arrhythmia d) artefact or not determinable. Disturbances in the ECG recordings were classified as a) triggered beat b) triggered ventricular arrhythmia c) artefact or not determinable.

EADs were defined as a positive deflection in the slope of the repolarization curve that exceeds the inflection point. Triggered beats were defined by both QRS complexes in the ECG signal and an action potential in the MAP lead with short coupling intervals (<150 ms) following the last stimulated beat.

Chemicals

EMD 57033 was kept in DMSO as a 3 mmol/l stock solution; when testing the effect of EMD 57033 the final concentration of DMSO in all experimental solutions was adjusted to 0.1%. The concentration of EMD 57033 was 3 μM. Previous studies demonstrate that EMD 57033 possesses minor phosphodiesterase-III (PDE-III) inhibitory properties,[20] although PDE-III inhibition seems to occur principally at concentrations in excess of 20 μM.[21] EMD 57033 was generously provided by Merck (Darmstadt, Germany), all other chemicals were purchased from Sigma Corporation (St. Louis, MO).

Statistical analysis

Mean±SEM are given, unless otherwise indicated. Mean values were compared with single factor analysis of variance (ANOVA). Post hoc Student's t-test was performed whenever significant differences were detected by ANOVA. Incidence rates were compared using χ^2 or Fishers-exact test. Results were considered statistically significant if the p value was less than 0.05

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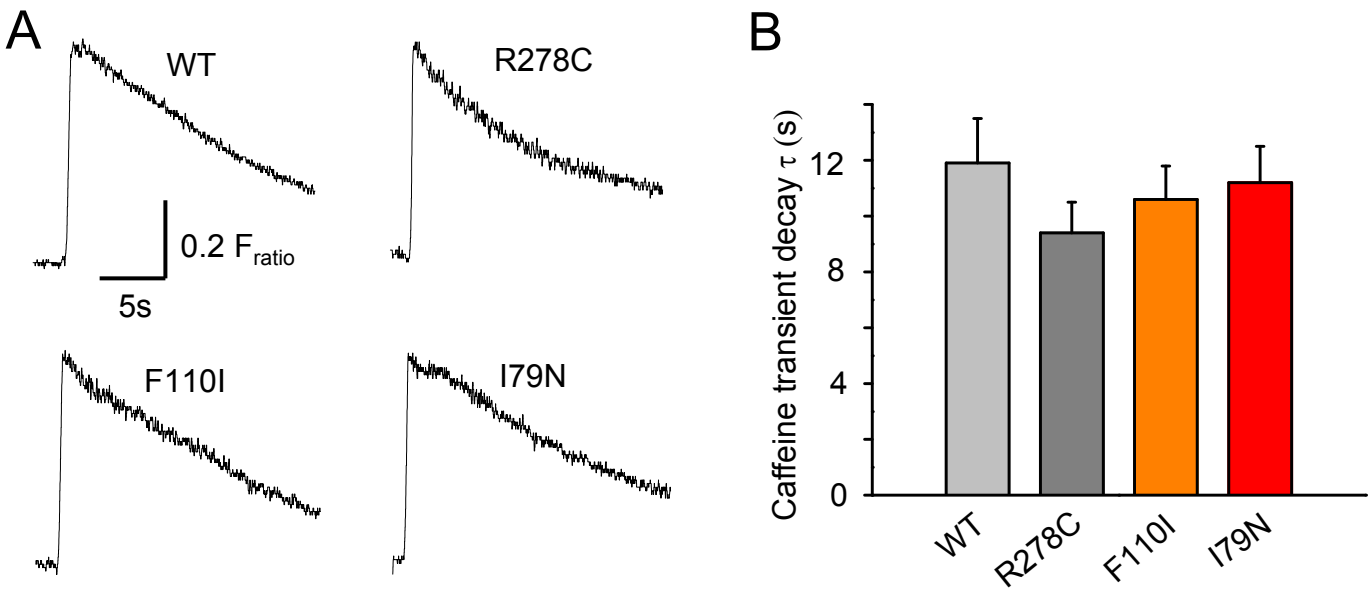
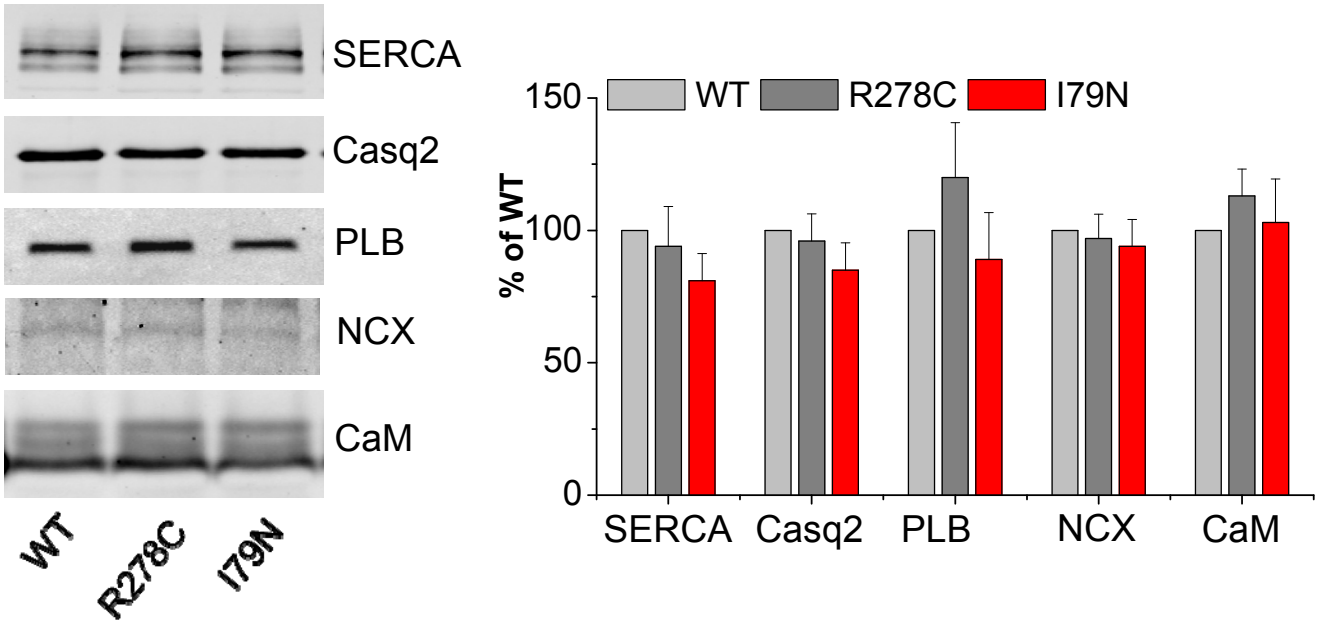
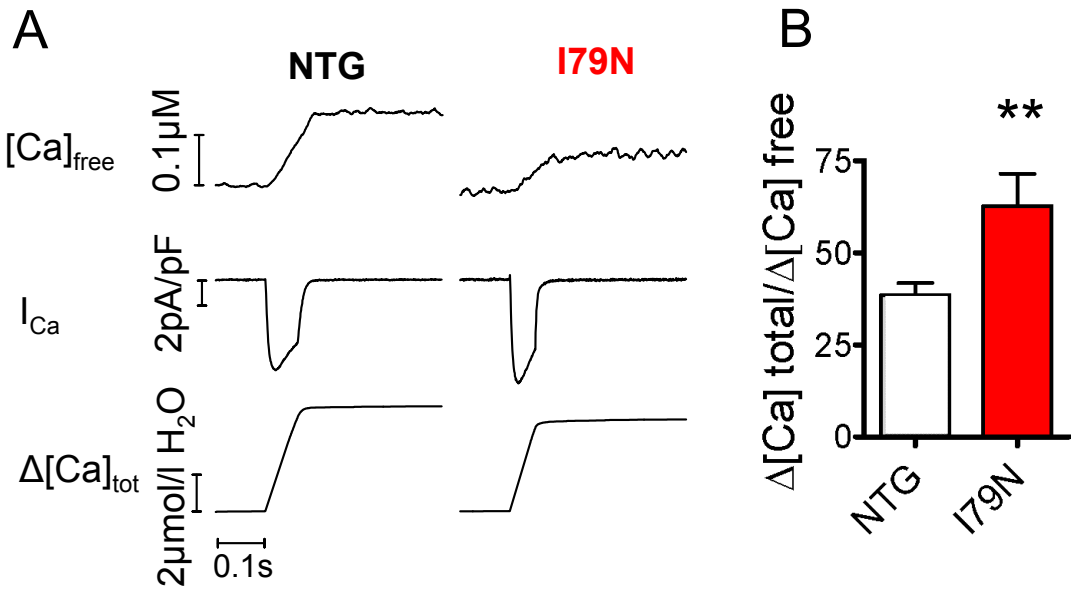


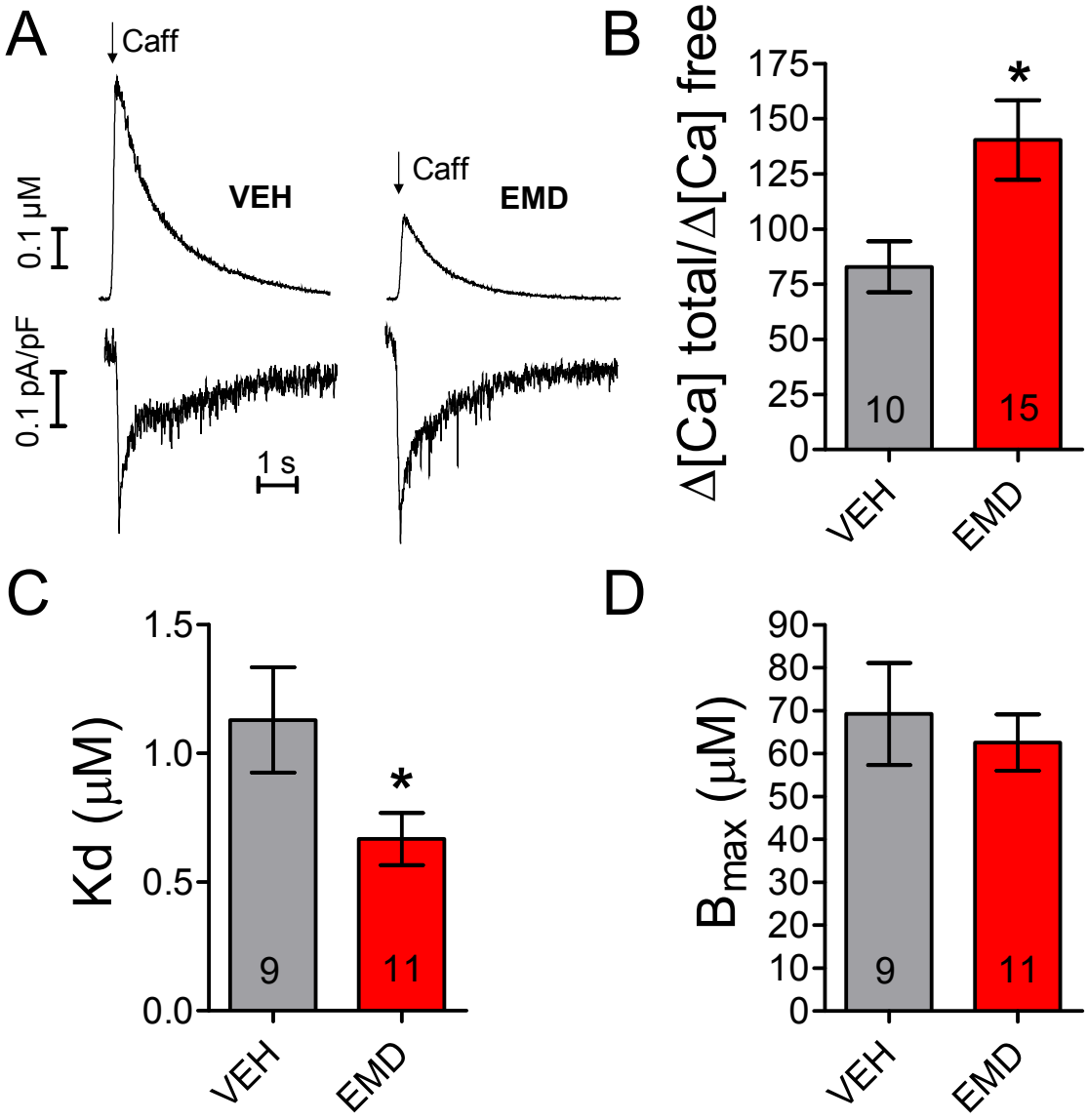
Online Figure I. Non-NCX mediated Ca removal is unchanged in TnT mutants. Non-NCX Ca removal was measured in intact myocytes loaded with Fura-2 AM by rapid application of 10 mM caffeine dissolved in Ca- and Na- free Tyrode's solution. For each myocytes, the decay of the caffeine-induced Ca transient was fitted to a mono-exponential function and the decay time constant τ calculated. Since Ca eflux via NCX is blocked in Na-free solutions, τ provides an estimate of non-NCX Ca removal processes (predominantly via mitochondria and sarcolemmal Ca-ATPase). **A**, Representative examples of caffeine-induced Ca transients. **B**, Average τ for each group. There were no significant differences between wild-type (WT), TnT-R278C, TnT-F110I and TnT-I79N. N = 10-13 myocytes from 3-4 mice per group.



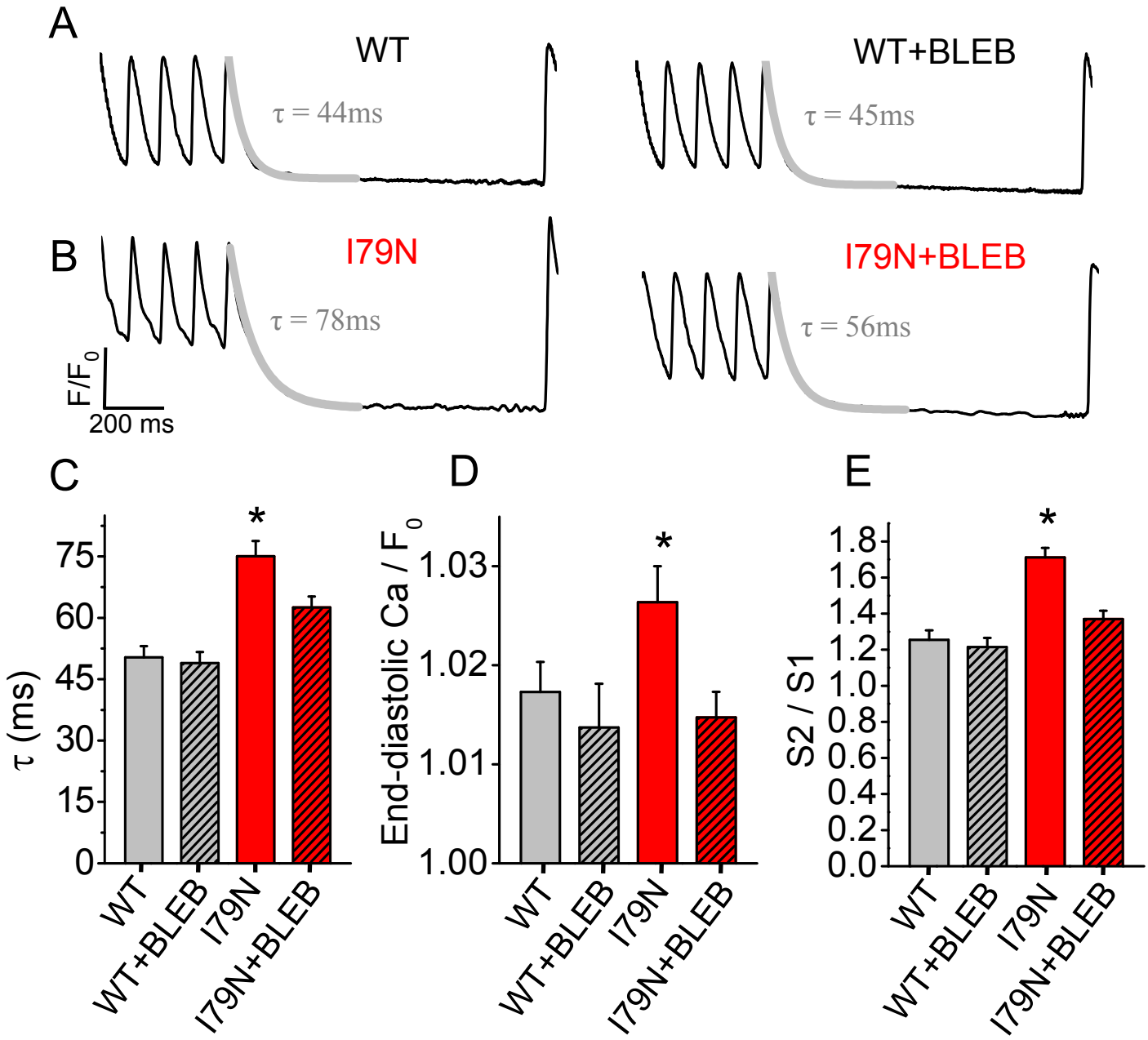
Online Figure II. Key Ca handling proteins are unchanged in TnT mutants. Representative immunoblots of SERCA, Calsequestrin (Casq2), Phospholamban (PLB), Na Ca exchanger (NCX) and Calmodulin (CaM). Band intensity was measured and normalized to GapDH. There were no significant differences between wild-type (WT), TnT-I79N and TnT-R278C. n= 4-6 mice per group.



Online Figure III. Caffeine-independent measurement of cytosolic Ca binding. A, Myocytes were voltage clamped, dialyzed with Fluo-4 and cytosolic Ca fluorescence recorded ($[Ca]_{free}$). Ca current was triggered with a voltage step and $\Delta[Ca]_{total}$ was calculated from the Ca current integral. **B**, Cytosolic Ca binding as calculated by the ratio of total and free cytosolic [Ca] is significantly higher in TnT-I79N compared to non-transgenic (NTG) myocytes. n=8-11 myocytes per group from 3-4 mice. **p<0.01 vs NTG.



Online Figure IV. The Ca sensitizer EMD 57033 increases cytosolic Ca buffering. A, Cytosolic Ca fluorescence was recorded from voltage-clamped myocytes loaded with the fluorescent indicator Fluo-4. **Upper trace**: Rapidly applied caffeine was used to release Ca from the SR. **Lower trace**: Integration of the Na Ca exchanger current yielded the total amount of Ca released from the SR. **B,** Myocytes treated with the Ca sensitizer EMD 57033 show a higher net cytosolic Ca binding calculated by a smaller rise in $[Ca]_{free}$ and an higher $\Delta[Ca]_{total}$ compared to myocytes treated with vehicle (VEH). **C and D,** The increased buffering capacity with EMD is caused by an increased cytosolic Ca binding affinity (K_d), whereas maximal Ca binding capacity is unchanged (B_{max}). N = 9-15 myocytes per group. *p<0.05 vs VEH.



Online Figure V. Blebbistatin normalizes Ca transients in response to an extrastimulus after a pause in TnT-I79N hearts but does not affect WT hearts. Ca transients were measured from intact hearts loaded with Rhod-2AM. Hearts were paced at 100 ms pacing cycle length (S1), followed by a 1 second pause and an extrastimulus (S2 pulse). Next, (-)-Blebbistatin (BLEB, 3μM) was added to the perfusate, and the pacing protocol was repeated in presence of BLEB. **A-B**, Representative traces demonstrating the pacing protocol in wild-type (WT) and I79N hearts at pacing cycle length 100 ms under control and BLEB conditions. **C-D**, BLEB treatment accelerated Ca removal from the cytosol and decreased end-diastolic [Ca] during the pacing train only in I79N hearts. **E**, BLEB normalized Ca release in response to a post-pause S2 extrastimulus. n=7-10 mice per group. * p<0.01 TnT-I79N vs WT, and also TnT-I79N vs TnT-I79N+BLEB.

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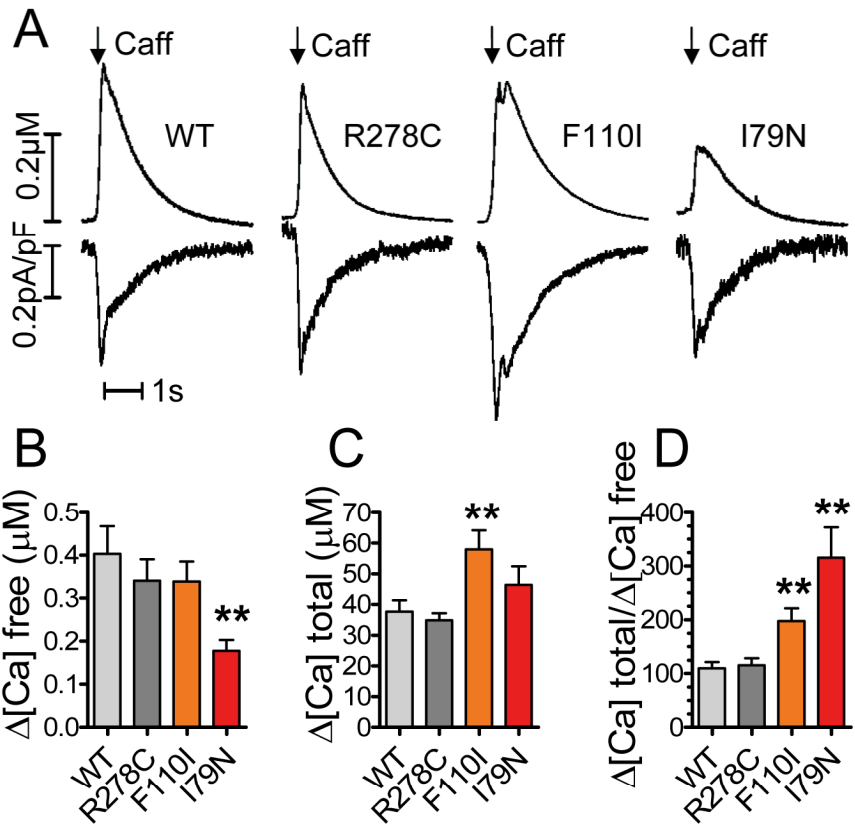
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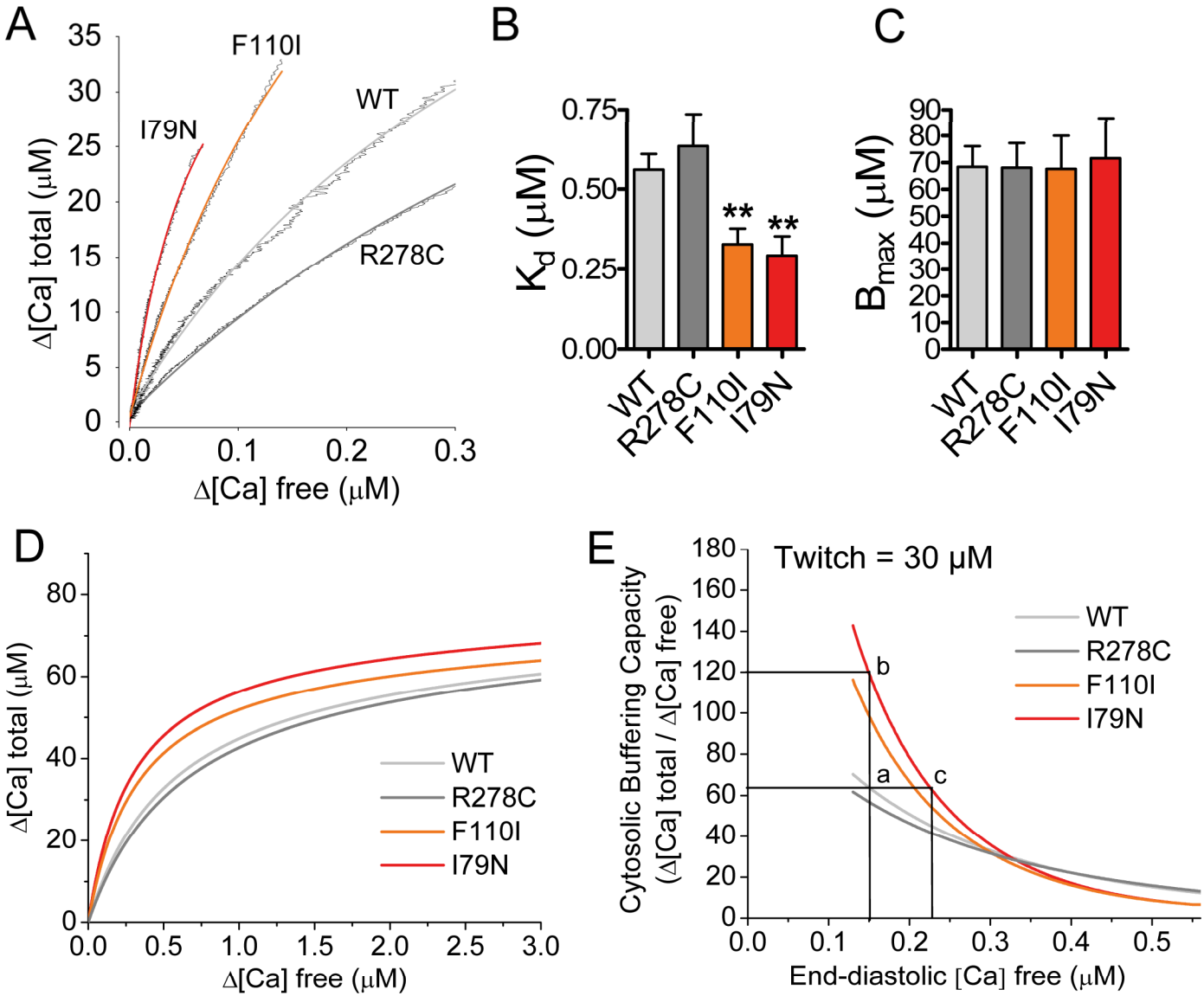
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B_{max} (μM)
**
**
WT
R278C
F110I
I79N
Twitch = 30 μM
Cytosolic Buffering Capacity
(Δ[Ca] total / Δ[Ca] free)
End-diastolic [Ca] free (μM)
a
b
c

Figure 2.

Type of file: figure
Label: 3
Filename: RES201280Fig3.tif

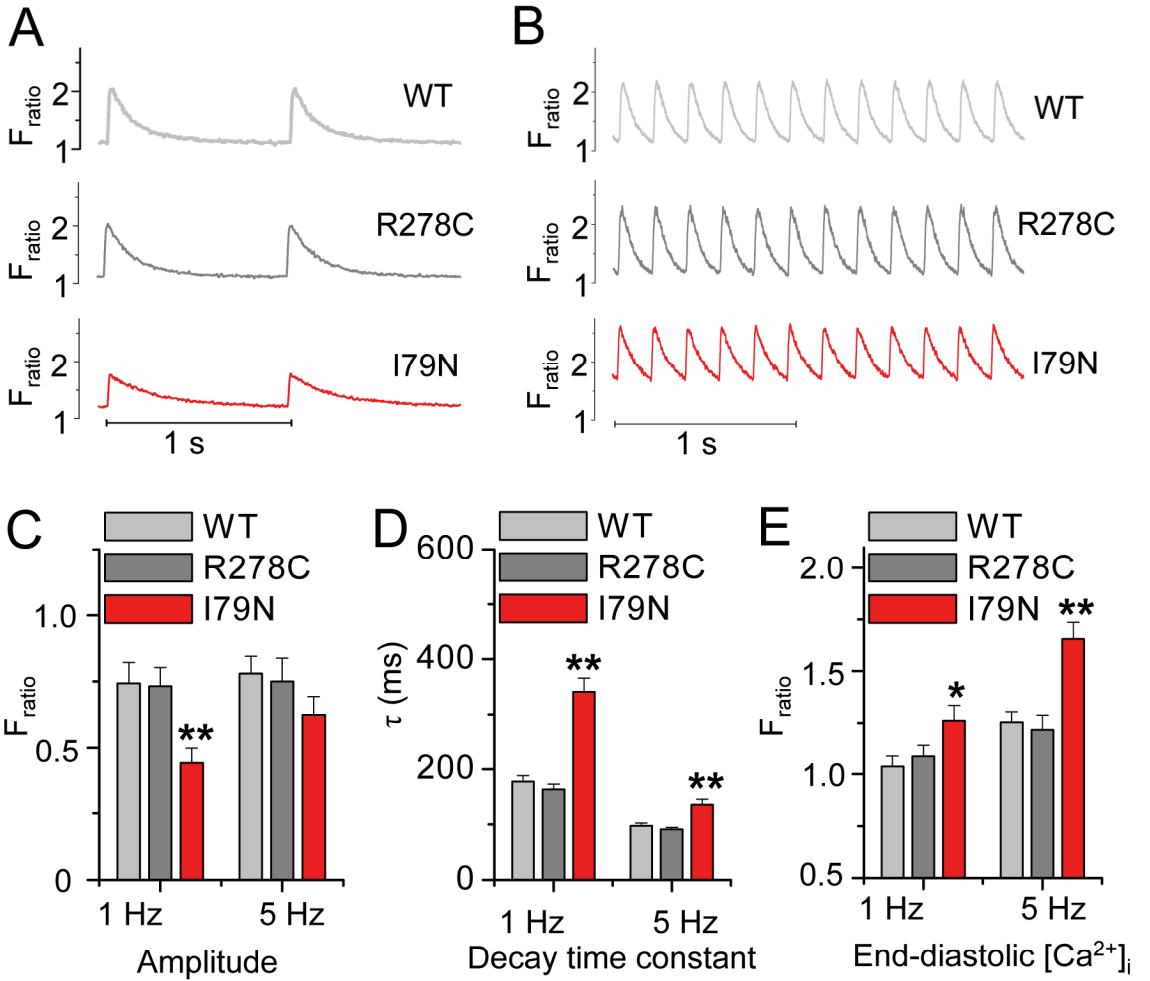

A
B
WT
R278C
I79N
F ratio
1 s
C
D
E
Amplitude
Decay time constant
End-diastolic [Ca2+]i
1 Hz
5 Hz
τ (ms)
**
*

Figure 3.

Type of file: figure
Label: 4
Filename: RES201280Fig4.tif

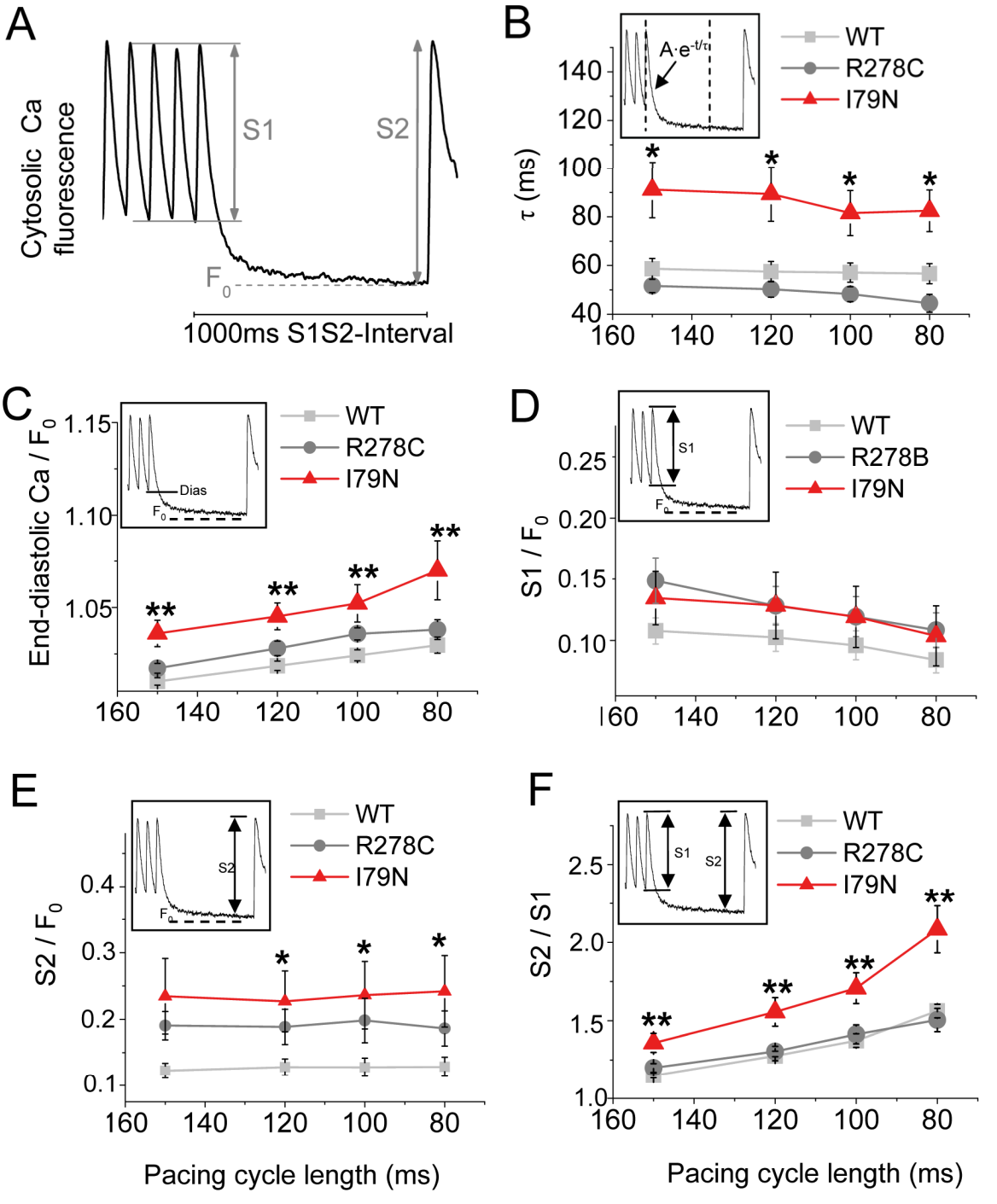

A
Cytosolic Ca fluorescence
S1
S2
F0
1000ms S1S2-Interval
B
A·e-t/τ
WT
R278C
I79N
τ (ms)
C
End-diastolic Ca / F0
Dias
D
S1 / F0
R278B
E
S2 / F0
Pacing cycle length (ms)
F
S2 / S1
Pacing cycle length (ms)

Figure 4.

Type of file: figure
Label: 5
Filename: RES201280Fig5.tif

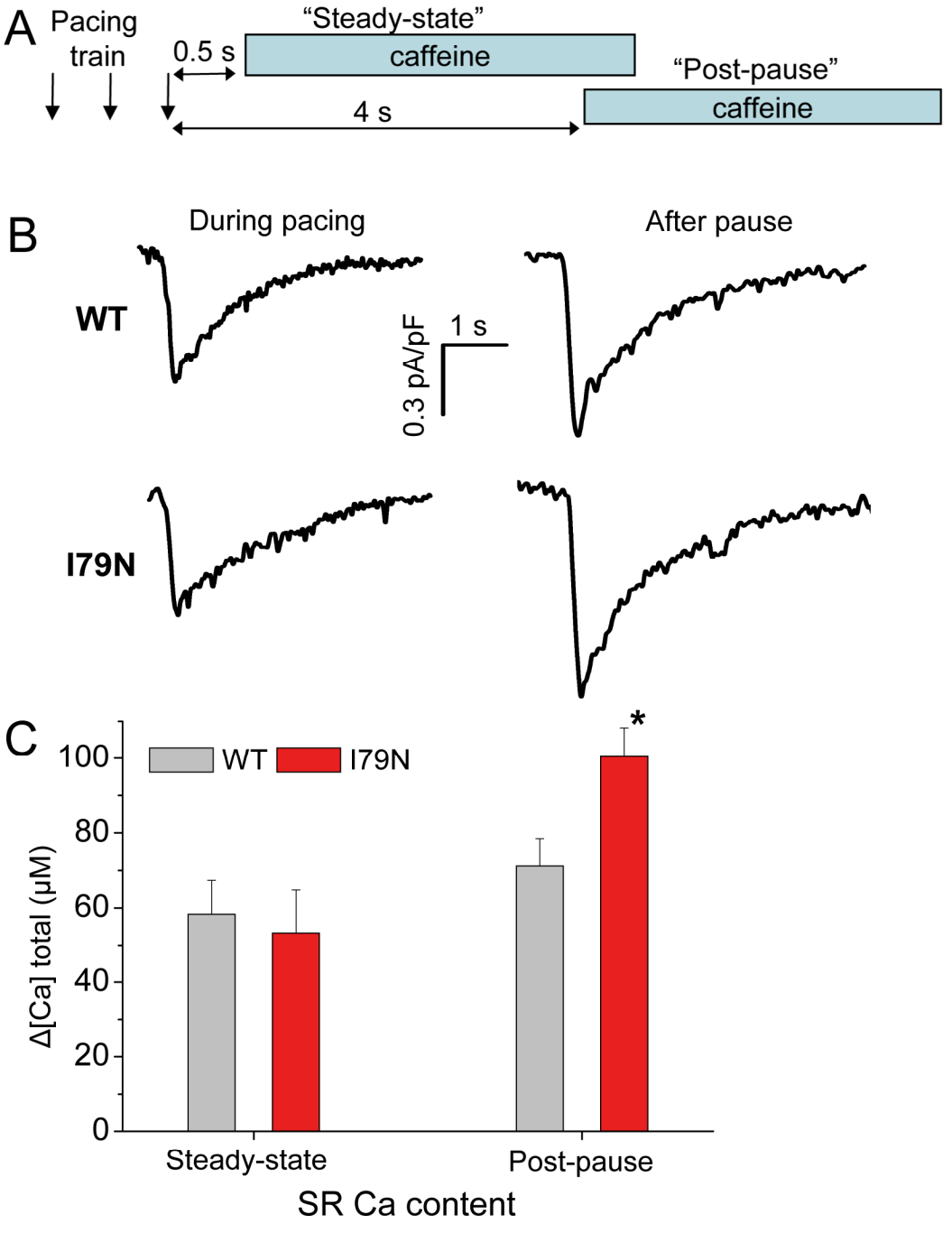

A
Pacing train
0.5 s
"Steady-state"
caffeine
4 s
"Post-pause"
caffeine
B
During pacing
After pause
WT
1 s
0.3 pA/pF
I79N
C
WT
I79N
100
80
60
40
20
0
Δ[Ca] total (μM)
*
Steady-state
Post-pause
SR Ca content

Figure 5.

Type of file: figure
Label: 6
Filename: RES201280Fig6.tif

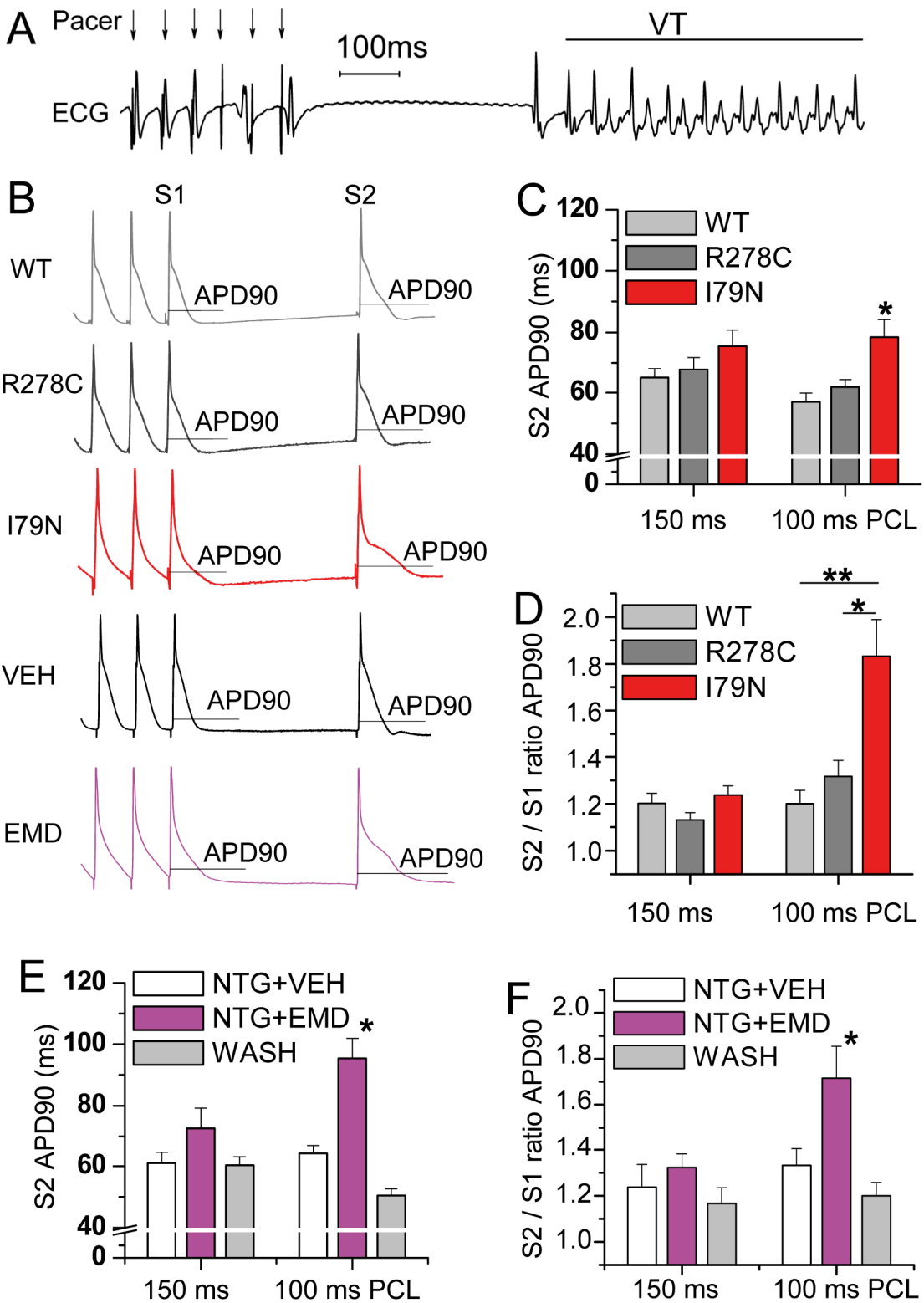

A
Pacer
100ms
VT
ECG
B
S1
S2
WT
APD90
APD90
R278C
APD90
APD90
I79N
APD90
APD90
VEH
APD90
APD90
EMD
APD90
APD90
C
120
100
80
60
40
0
WT
R278C
I79N
S2 APD90 (ms)
*
150 ms
100 ms PCL
D
**
*
2.0
1.8
1.6
1.4
1.2
1.0
WT
R278C
I79N
S2 / S1 ratio APD90
150 ms
100 ms PCL
E
120
100
80
60
40
0
NTG+VEH
NTG+EMD
WASH
*
S2 APD90 (ms)
150 ms
100 ms PCL
F
2.0
1.8
1.6
1.4
1.2
1.0
NTG+VEH
NTG+EMD
WASH
*
S2 / S1 ratio APD90
150 ms
100 ms PCL

Figure 6.

Type of file: figure
Label: 7
Filename: RES201280Fig7.tif

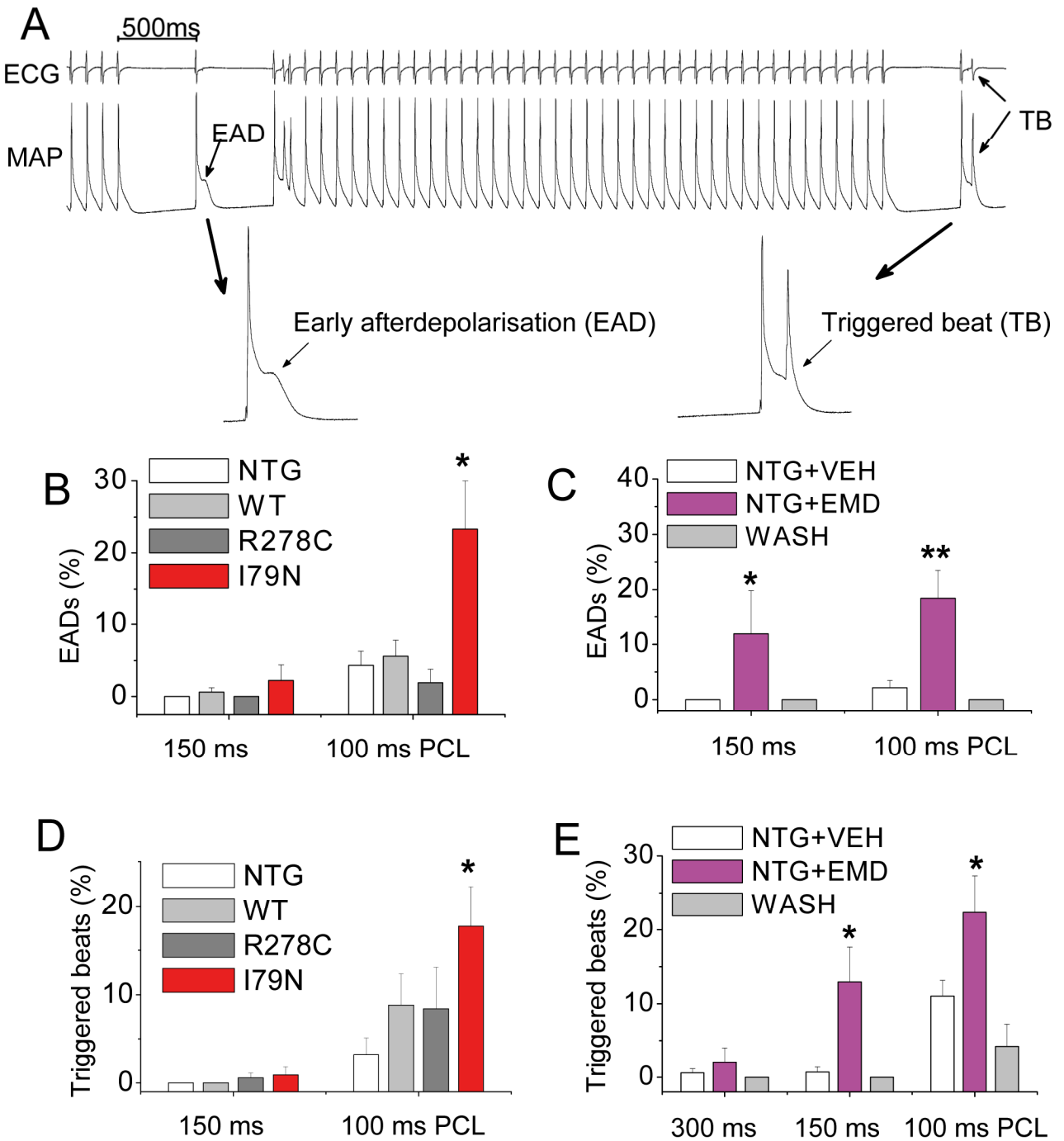
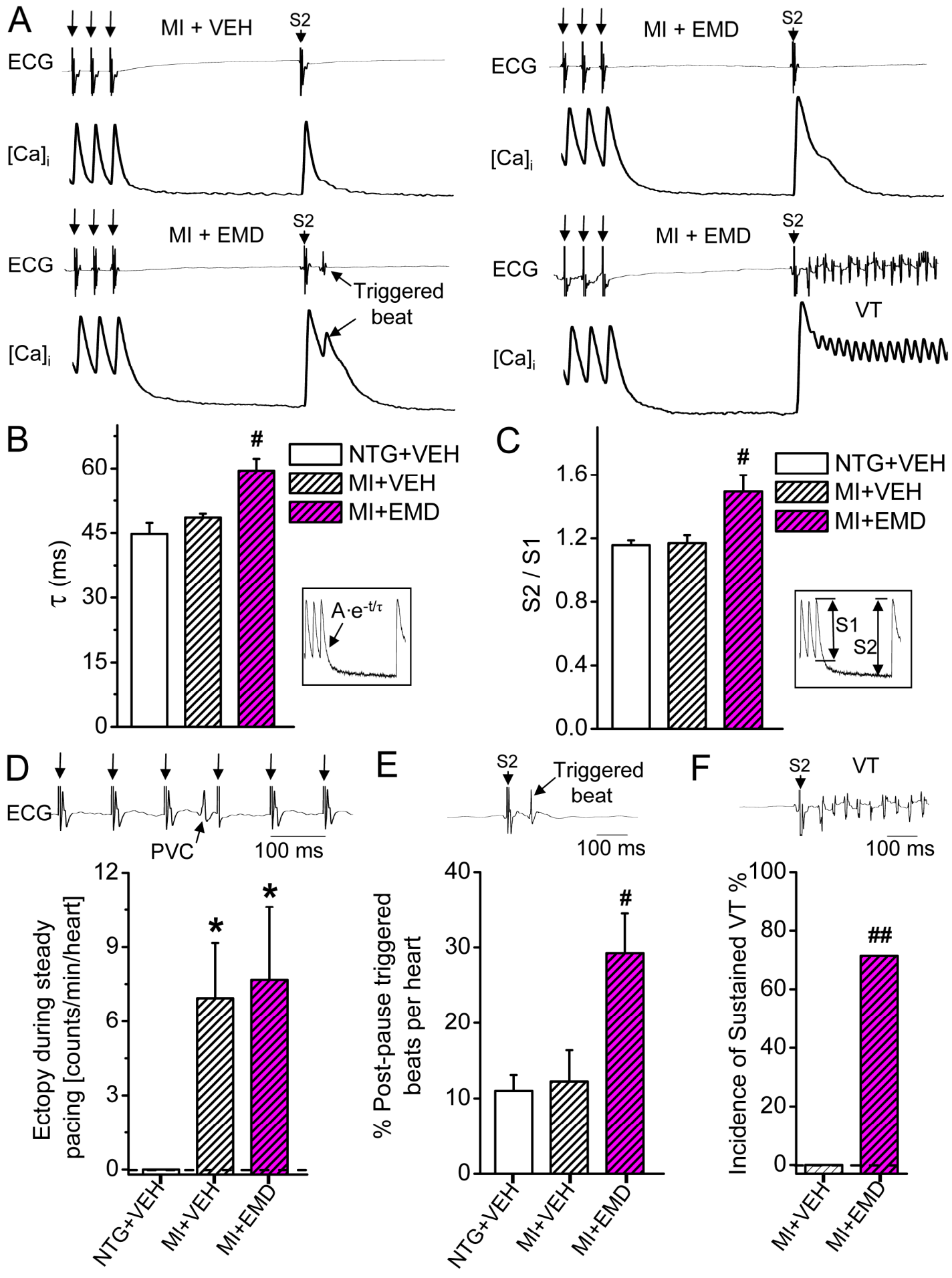

A
500ms
ECG
MAP
EAD
TB
Early afterdepolarisation (EAD)
Triggered beat (TB)
B
NTG
WT
R278C
I79N
EADs (%)
150 ms
100 ms PCL
C
NTG+VEH
NTG+EMD
WASH
EADs (%)
150 ms
100 ms PCL
D
NTG
WT
R278C
I79N
Triggered beats (%)
150 ms
100 ms PCL
E
NTG+VEH
NTG+EMD
WASH
Triggered beats (%)
300 ms
150 ms
100 ms PCL

Figure 7.

Type of file: figure
Label: 8
Filename: RES201280Fig8.tif


A
MI + VEH
S2
ECG
[Ca]i
MI + EMD
Triggered beat
VT
B
τ (ms)
NTG+VEH
MI+VEH
MI+EMD
A·e-t/τ
C
S2 / S1
S1
S2
D
PVC
100 ms
Ectopy during steady pacing [counts/min/heart]
E
Triggered beat
% Post-pause triggered beats per heart
F
Incidence of Sustained VT %