SUPPLEMENTARY MATERIAL

DETAILED METHODS

(All reference numbers refer to references in the main paper)

Animal Model

Animal-care procedures were approved by the Animal Research Ethics Committee of the Montreal Heart Institute (protocol 2018-47-12) and followed Canadian Council on Animal Care Guidelines. A total of 86 adult mongrel dogs were studied, divided into control (n=50, 24.2±3.7 kg, ~2.5 years, female/male (F/M: 26/24)) and AF (n=36, 24.3±2.9 kg, ~2.5 years, F/M: 17/19) groups. All dogs were fed standard dog chow (Hill) diets. AF dogs were anesthetized with ketamine (5.3 mg/kg i.v.) /diazepam (0.25 mg/kg, i.v.) and isoflurane (1.5%), intubated and ventilated. A unipolar pacing lead was inserted into the right-atrial (RA) appendage under fluoroscopic guidance and connected to a pacemaker in the neck. Bipolar electrodes were inserted into the right-ventricular apex and RA-appendage for electrogram-recording. The pacemaker was programmed to maintain AF by pacing the RA at 600 bpm for 1 week. By maintaining AF electrically, this model mimics the atrial remodeling caused by spontaneous arrhythmia.[12, 13] On day 7, dogs were anesthetized with morphine (2 mg/kg, s.c.) and α-chloralose (120 mg/kg, i.v., followed by 29.25 mg/kg/h), and ventilated mechanically. Effective refractory periods (ERPs) were measured at basic cycle lengths of 150, 200, 250, 300, and 350 ms in the RA-appendage, with 10 basic stimuli (S1) followed by a premature extrastimulus (S2) with 5-ms decrements. AF was induced with atrial burst pacing at 50 Hz and 10-V 2-ms square-wave output. Mean AF-duration was based on 10 AF-inductions in each dog. If the mean duration of the first 5 episodes of AF was >2 minutes, AF was induced only 5 times. Hemodynamic data were obtained with fluid-filled catheters and transducers. No major adverse events occurred and all dogs were included in the final analyses. Due to the invasive nature

of the animal model, blinding was not possible for *in vivo* experiments. For *in vitro* experiments, limited blinding would have been possible; however, since this study was a major undertaking performed over a total period of over 7 years, much of it was undertaken before widespread awareness of the need for blinding and its more routine adoption in the laboratory. Formal randomization was not performed; animals were arbitrarily assigned to treatment groups in sequence, attempting to balance control and intervention animals within similar time windows to avoid any time-dependent bias.

Canine Atrial Cardiomyocyte Isolation

Atrial cardiomyocytes (CMs) were isolated with previously described methods.[14,15] Dogs were anesthetized with morphine (2 mg/kg s.c.) and α-chloralose (120 mg/kg i.v.) and mechanically ventilated. The heart was removed after intra-atrial injection of heparin (10,000 U), immersed in 2 mM Ca^{2+}-containing Tyrode's solution, the left coronary artery was cannulated, and left-atrial tissue perfused with Tyrode's solution (37°C, 100% O_2), then with Ca^{2+}-free Tyrode's solution (~10 minutes), followed by ~60-minute perfusion with the same solution containing collagenase (~0.45mg/mL, CLSII, Worthington, Lakewood, NJ) and 0.1% bovine serum albumin (BSA, Sigma–Aldrich, Oakville, ON). Tissues were minced and atrial CMs harvested. Isolated cardiomyocytes were stored in 200-μmol/L Ca^{2+}-containing Tyrode's solution for Ca^{2+}-transient recording and in Kraftbruhe storage solution for immunostaining. For culture, CMs were kept in PC-1 medium (Lonza Walkersville Inc, Walkersville, MD), concentrated by centrifugation at 500 rpm (1 min), and cell-pellets removed.

Canine Atrial CM Culture and in Vitro Pacing

CMs were plated at low density (~104 CMs/cm^2) onto laminin-coated (20-µg/mL) glass coverslips (25*25 mm). Serum-free PC-1 medium supplemented with penicillin/streptomycin (Bioshop Canada Inc, Burlington, ON) was used for cell-culture. After 2 hours, dead and unattached CMs were removed and fresh medium was added. Pacing was accomplished with square-wave, 1-ms pulses (C-pace Cell-Culture Stimulator, IonOptix, Westwood, MA). Isolated atrial cardiomyocytes were subjected to 24-hr in vitro pacing at 1 Hz (P1, sinus rate) or 3 Hz (P3, an in vitro model of AF [15]).

Fluo-4 and Fluo-5N Loading and Cell Permeabilization

Canine atrial CMs were loaded with the Ca^{2+} indicator Fluo-4 AM (10-µM, Thermo Fisher Scientific, Saint-Laurent, QC) for 25-30 minutes and then washed for 30 minutes to allow for de-esterification of the dye. Other CMs were loaded with Fluo-5N AM (10-µmol/L, Thermo Fisher) for 2 hours and deesterified for 1.5 hours. For intact CM physiology studies, the superfusate contained (mM): NaCl 136, $CaCl_2$ 1.8, KCl 5.4, $MgCl_2$ 1, NaH_2PO_4 0.33, dextrose 10, HEPES 5; titrated to pH 7.4 with NaOH, (35°C). For permeabilization, CMs were exposed to a solution containing (mM): KCl 120, EGTA 0.5, MgCl2 0.75, HEPES 10, pH 7.2 with KOH for 5 minutes and then permeabilized by exposure to the same solution containing 50-µg/ml saponin. After 60~90 seconds, the bath solution was changed to a saponin-free internal solution composed of (mM): KCl 115, KH_2PO_4 5; MgATP 5; EGTA 0.5; $MgCl_2$ 0.75; $CaCl_2$ 0.28; phosphocreatine 10; creatine phosphokinase 5 U/ml; dextran (MW: 40,000) 8%, HEPES 10, 2,4-butanedione monoxime (BDM)15, probenecid 1, pH 7.2 (KOH). Free [Ca^{2+}] and [Mg^{2+}] at 23°C calculated

with previously-described approaches[16] were 200 nM and 1.17 mM respectively (https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/).

Line scan Confocal Ca^{2+} Imaging of Nucleoplasmic and Cytosolic Ca^{2+}-transients

Two-dimensional (2D) confocal Ca^{2+}-imaging was performed. Simultaneous imaging of nucleoplasmic and cytosolic Ca^{2+}-transients were recorded in CMs loaded with Fluo-4 AM. An Olympus confocal microscope (Olympus Corp, Tokyo, Japan, with FluoView 1000) equipped with a ×40 oil-immersion objective lens (N.A. 1.3) and an argon-ion laser were used. For Fluo-4, excitation and emission wavelengths were 488 nm and >515 nm, respectively. Images were acquired in line-scan mode (3 or 6 ms $scan^{-1}$; pixel size 0.103μm). CMs were field-stimulated with 2 platinum electrodes.

In studies of miR-26 effects on cell-Ca^{2+}, freshly-isolated cardiomyocytes were cultured and transfected with a noncoding control microRNA (miR-NC), miR-26a and AMO-26a on laminin-coated 42-mm coverslips in Falcon® 60 mm TC-treated Cell Culture Dish. Twenty-four hours later, the medium was removed and coverslips mounted on a ZEISS LSM 5 LIVE confocal fluorescence microscope equipped with a 40x/1.3 N.A. Plan-Apochromat Oil Objective. Cells were incubated with 10-μM Fluo-4 AM (dissolved in Pluronic F-127, Invitrogen, USA) in Tyrode's solution containing 1.8 mM Ca^{2+} for 30 min at room temperature. Cells were then perfused with Tyrode's solution (35 °C) 20 minutes for de-esterification, CM were field-stimulated with 2 platinum electrodes.

In Situ Calibration of Fluo-4 Fluorescence

Calibration solutions were prepared according to the procedure described by Ljubojevic et al.[17] In brief, we calculated the total extracellular [Ca^{2+}] required to obtain the desired free [Ca^{2+}] in the presence of 1-mM EGTA using the MaxChelator program.[16] The EGTA solution contained (mM): KCl 140, EGTA 1, HEPES 10, pH 7.4 (KOH). Calibration solutions were made with calculated $[Ca^{2+}]_{Free}$ of 0 nM, 50 nM, 250 nM, 750 nM, 3000 nM, 0.5 mM and 1 mM. The $[Ca^{2+}]_{Free}$ was confirmed with a Ca^{2+}-sensitive electrode (9720BNWP Calcium Combination Electrode, Thermo Fisher). Calibration solutions also contained metabolic inhibitors (2-deoxy-D-glucose 2 mM; rotenone, 10 mM); a SERCA inhibitor (cyclopiazonic acid 5 mM) to block SR Ca^{2+}-uptake; butanedione monoxime (BDM, 15 mM) to prevent contracture of the myocytes at high [Ca^{2+}] and probenecid (2 mM) to inhibit organic-anion transporters located in the cell membrane to prevent dye leak. Saponin-permeabilized CMs were used for calibration. CMs were equilibrated in each calibration solution for 10 min. During this period, fluorescence was recorded every 30 seconds. Minimal Fluo-4 fluorescence (F_{min}) was measured during exposure to Ca^{2+}-free calibration solution and maximal Fluo-4 fluorescence (F_{max}) during exposure to a calibration solution containing a saturating free [Ca^{2+}] of 1 mM.

Preparation of Nuclear-enriched Fractions from Canine Atrial CMs

Nuclear and non-nuclear fractions were isolated based on a previously-described approach.[18] Isolated CMs were washed with cold Ca^{2+}-free Tyrode's solution, centrifuged at 130×g for 5 minutes to collect the cell pellets. Hypotonic Buffer (20 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$) was prepared with the addition of one tablet of Protease Inhibitor Cocktail (B14012, Bimake, Cedarlane, Burlington, ON) to 50 ml buffer. CMs were gently suspended in 3 volumes of

hypotonic buffer per volume of CMs, and incubated on ice for 20 minutes. 50 µl 10% NP- 40 was added to 1 ml of suspended CMs after incubation and CMs were vortexed for 10 seconds at the highest setting. The homogenate was centrifuged for 5 minutes at 125×g and 4°C. The supernatant containing the non-nuclear and nuclear fractions was centrifuged for 10 minutes at 1910×g and 4 °C to separate the nuclear (pellet) from non-nuclear (supernatant) fractions. The supernatant (non-nuclear fraction) was collected into a new tube, frozen in liquid-N_2 and stored at -80°C for subsequent analysis. The pellet (nuclear fraction) was washed 3 times with Storage Buffer (250-mM sucrose, 20-mM KCl, 1-mM $MgCl_2$, 1-mM dithiothreyitol, 50-mM HEPES, pH 7.0, containing one tablet of Protease Inhibitor Cocktail per 50 ml), frozen in liquid-N_2 and stored at -80°C for subsequent analysis.

Immunostaining and Immunoblot

Atrial CMs were plated on 25*25 mm coverslips and then fixed for 20 minutes with 4% paraformaldehyde in phosphate-buffered saline (PBS) containing (mM): 137 NaCl, 2.68 KCl, 4.23 Na_2HPO_4, 1.76 KH_2PO_4, (pH 7.34) at room temperature to preserve CM morphology. The CMs were then incubated with a blocking/ permeabilizing buffer (PBS, 0.5% Triton X-100, 4% normal donkey serum (NDS, Sigma–Aldrich)) for 1 hour and then incubated overnight at 4°C with the primary antibody against nuclear pore channel (NPC) protein, Lamin A+C, IP_3R1, IP_3R2, sarcoplasmic reticulum Ca^{2+}-ATPase type 2a (SERCA2a), ryanodine receptor channel type-2 (RyR2), Total-Ca^{2+}/calmodulin-dependent protein kinase-II (CaMKII), phospho-Thr305-CaMKII, or histone deacetylase type-4 (HDAC4) diluted in PBS containing 0.05% Triton X-100, 2% NDS (for details, see **Major Resources Table**). After washing (3×5 minutes) with PBS, CMs were incubated in the dark at room temperature for 1 hour, with Alexa-conjugated secondary antibodies

(details in **Major Resources Table**) diluted in PBS containing 0.05% Triton X-100 and 2% NDS. CMs were then carefully washed (3×5 minutes) with PBS, excess buffer was removed with a tissue and CMs were placed face-down onto microscope slides containing a drop of 1,4-diazabicyclo [2.2.2] octane (DABCO, Sigma–Aldrich) antiquenching mounting medium (0.2% DABCO in glycerol), and the edges of the coverslip were sealed with nail polish. Images were acquired with an Olympus FluoView FV1000 confocal laser-scanning microscope equipped with a 40×/1.3 oil immersion objective. To distinguish genuine target staining from background, secondary antibody was used along with an isotype control for primary antibodies (mouse or rabbit IgG; **Online Figure XX**).

Lysis buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 0.1% TritonX-100, 10% glycerol, 0.1% SDS, 1 mM phenylmethane sulfonyl fluoride, PMSF) was prepared by adding one tablet of Protease Inhibitor Cocktail to 10 ml buffer. The nuclear fraction was then lysed by adding to the pellet a volume of lysis buffer three times the volume of the pellet.

The non-nuclear and nuclear fractions were kept at –80°C for subsequent assay. Equivalent amounts of protein (25 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked for 1 hour at room temperature and probed with primary antibodies against IP_3R1, IP_3R2, SERCA2a, Total-RyR2, phospho-Ser2808-RyR2, phospho-Ser2814-RyR2, Total-CaMKII, or phospho-Thr287-CaMKII (for details, see **Major Resources Table**) overnight at 4°C. After extensive washing, membranes were further incubated with secondary antibodies conjugated to horseradish peroxidase and immunoreactive bands detected with enhanced chemiluminescence (ECL). Membranes were probed with appropriate primary and secondary antibodies, and then as needed reprobed up to 3 additional times after stripping with ReBlot Plus

Strong Antibody Stripping Solution. Band intensities were quantified with Bio-Rad Quantity One software. Antibody specificity was determined for HDAC4-antibody studies based on band suppression with knockdown; for IP_3R1, IP_3R2, RyR2 and CaMKII specificity was confirmed based on incubation with antigenic peptide).

***Inositol-trisphosphate Receptor (ITPR)* Gene Knockdown with siRNA**

Freshly-isolated canine atrial CMs were plated on laminin-coated glass cover slips (25*25 mm) in 8-well plates (for functional studies) or 6-well plate (mRNA and protein assay) in serum-free PC-1 medium (Lonza) supplemented with penicillin/ streptomycin for 2 hours before transfection. Atrial CMs were transfected with *inositol-trisphosphate receptor type-1 (ITPR1)* and *type-2 (ITPR2)* gene siRNA (100 nM for each), and negative control with the medium, Lipofectamine® RNAiMAX (Invitrogen, Carlsbad, CA). Sequences of siRNA were: *ITPR1*: GCCAAGCAGTTTGGCTTCATGCAAA; *ITPR2*: CCGAGACTTAGACTTTGCCAATGAT; Stealth RNAi™ siRNA Negative Control, Invitrogen, 12935300**)**. SiRNAs and Lipofectamine® RNAiMAX were separately mixed with Opti-MEM® I Reduced Serum Medium (Gibco, Grand Island, NY) for 5 min. The two mixtures were then combined and incubated for 5 minutes at room temperature. The Lipofectamine® RNAiMAX/siRNA mixture was added to the CMs, which were then incubated at 37°C for 24 hours. CMs were paced at 1 or 3 Hz for 24 hours followed by Ca^{2+}-transient measurement, L-type calcium current recording or were collected for protein/mRNA assay.

Bioinformatic analysis of miRNAs targeting *ITPR1*

We used MirTarget V3, PicTar, TargetScan7.2, and the PubMed database to identify miRNAs predicted to target *ITPR1*. We selected miRNAs with a target score>80 and conserved 7-mer or 8-mer or sites in the 3′UTR of the *ITPR1* mRNA matching the miRNA seed region predicted by at least 2 of the 3 algorithms. We further selected miRNAs based on their strength of expression in human left atrium in public databases. We then looked for miRNAs that were downregulated in AF dog left atrial cardiomyocytes. Only one miRNA, miR-26, fulfilled all of these criteria. The miR26a seed sequence and its complementary sites in the *ITPR1* 3′UTR are:

Position 267-273 of ITPR1 3' UTR (cfa-miR-26a)

```
5'    ...AAGCACCUGAUUUGCACUUGAAC...
              ||||       ||||||
3'     UCGGAUAGGACCUAA--UGAACUU
```

Synthesis of miRNAs and anti-miRNA antisense inhibitors

The synthesis of miR-26a and scrambled miRNA (miR-NC) was performed by Integrated DNA Technologies Inc., Coralville, Iowa. The anti-miRNA oligonucleotide (AMO)-26a, a single-stranded DNA-analog complementary to mature miR-26a, was synthesized by Exiqon-Qiagen, Frederick, MD). Sense and antisense strands of the miR-26 duplex and scrambled miRNA (miR-NC) duplex as negative controls were diluted with RNase-free water to 200 μM, and combined 1:1. The mixture was then heated for 5 minutes to 95°C and kept overnight to cool slowly at room temperature. Freshly-isolated cardiomyocytes cultured in PCELL-100 (Wisent, St-Bruno-de-Montarville, Quebec), containing 1% P/S and 1×insulin-transferrin-selenium-X (Gibco, Grand Island, NY), were transfected with 20 nM (for luciferase activity assay studies) or 100 nM (for others) miR-26a or miR-NC, or 10 nM AMO-26a. Lipofectamine RNAiMAX (Invitrogen,

Carlsbad, CA) was dissolved in reduced serum medium Opti-MEM (Gibco). After 24 hours of transfection, cells were used for Ca^{2+}-transient recording or and kept at -80 °C for immunoblot and RT-qPCR studies. The oligonucleotides used in these studies are listed **Online Table I**.

Online Table I: List of RNA/DNA sequences (5′-sequence-3′) used in luciferase activity assay and transfection studies.

Target	Sequence
miR-NC_S	UCAUAAAGCUGAUAACCUCUAGAU
miR-NC_AS	CUAGAGGUUAUCAGCUUUAUGAAU
miR-26a_S	UUCAAGUAAUCCAGGAUAGGCU
miR-26a_AS	CCUAUUCUUGGUUACUUGCACG
AMO-26a	AGCCTATCCTGGATTACTTGAA
***ITPR1*_T**	CAAAAATATTAAAAATGCCTTTTTTGGAAGGACTAAAGAAAGCACCTGATT TGCACTTGAACCAGATTATAGATTTAAAAGTATATGACATGTATTTTGT
***ITPR1*_F**	ACCGCTCGAGCGGCAAAAATATTAAAAATGC
***ITPR1*_R**	GCTCTAGAGCACAAAATACATGTCATAT

Quantitative Real-Time PCR

Total RNA was purified with the miRNeasy Mini Kit (217004, Qiagen, Germany) according to the manufacturer's instructions. RNA concentration was quantified using Thermo Scientific NanoDrop 2000 spectrophotometer. The primary cDNA was synthesized with TaqMan MicroRNA Reverse Transcription Kit (4366597, Applied Biosystems, Lithuania) using miRNA-specific TaqMan assay primers for miRNA and High-Capacity cDNA Reverse Transcription Kit (4368813, Applied Biosystems, Lithuania) for mRNA. The following settings were used for reverse transcription: miRNA; reverse transcription 16°C 30 min, 42°C 30 min, stop reaction 85°C 5 min, and hold 4°C and for mRNA; reverse transcription 25°C 10 min, 37°C 60 min, stop reaction 85°C 5 min, and hold 4°C. Quantitative PCR was performed with TaqMan probes (**Online Table II**) and TaqMan Universal PCR Master Mix (4444557, Applied Biosystems, Lithuania) on an Applied Biosystems StepOnePlus Real-Time PCR System with the following cycling mode: enzyme activation 95°C 20 s and 40 cycles of denaturation 95°C 1 s and annealing/extension 60°C

20 s. Gene expression was measured in duplicate for each sample and calculated with the $2^{-\Delta Ct}$ method after normalizing to the reference gene *HPRT1* for mRNA and U6 snRNA for miRNA.

Online Table II. List of TaqMan probes for RT-qPCR

Gene	Assay ID	Catalog number
HPRT1	cf02626258_m1	4331182
ITPR1	cf02675749_m1	4351372
ITPR2	cf02675776_m1	4351372
ITPR3	cf02630200_m1	4351372
miR-26a	000405	4427975
U6 snRNA	001973	4427975

Luciferase activity assay

Based on the alignment of canine *ITPR1* 3'UTR and miR-26a with Clustal Omega, 100-bp DNA fragments containing the target site for the miR seed region were synthesized (Invitrogen). These inserts were amplified with forward and reverse primers containing an XhoI and XbaI restriction sites and were digested with XhoI and XbaI (New England Biolabs, Ipswich, CA) and then annealed and ligated into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison WI). The resulting DNA vector was transformed into the high-efficiency chemically competent DH5a (ThermoFisher, Carlsbad, CA), diluted in SOC, and formed colonies were selected from ampicillin-containing plates, and the plasmids were purified with NucleoSpin® Plasmid kit (Macherey-Nagel, Duren, Germany). The final product was sequenced at the McGill University Génome Québec Innovation Centre to confirm the genomic sequences.

Dual-Luciferase Reporter Assay System (Promega) was used to measure luciferase activity in H9C2 cells cultured in DMEM (Gibco) containing 1% P/S and 10% heat-inactivated fetal bovine serum in 24-well plates. Cells were transfected with 20 nM miR-26a or miR-NC, or 10 nM AMO-26a along with 100 ng DNA vector using Lipofectamine 3000 (Invitrogen). Cells were then lysed in 100 ul of 1× passive lysis buffer (Promega), and 10 µl of lysate was used to perform Luciferase reporter assays according to the manufacturer's protocol. Luciferase activity was normalized to Renilla luciferase internal control. Sample luminescence was read with a Synergy 2 Multi-Mode Microplate Reader.

Isolation and Immunoblotting of Nuclear and Non-nuclear Fractions in Human Atrial CMs

Experimental protocols were approved by ethical review board of University Hospital Essen (no. 12-5268-BO) and were conducted in accordance with the Declaration of Helsinki. Each patient provided written informed consent for sample acquisition and subsequent study. Nuclear and non-nuclear fractions were isolated from right atrial (RA) appendages obtained from sinus rhythm (Ctl) and longstanding persistent (>6 months) chronic AF (cAF) patients undergoing cardiac surgery at the University of Duisburg-Essen. Patient characteristics are provided in **Online Table III**.

The CMs were isolated as previously described.[19] In brief, fat residue was removed, and the RAA was chopped into 1-2 mm^3 pieces of. The RAA was washed three times for 3 min in Ca^{2+}-free solution containing (mM): KCl 10, NaCl 100, KH_2PO_4 1.2, $MgSO_4x7\text{-}H_2O$ 1.0, taurine 10, MOPS 1, D-(+)-Glucose 20; pH 7.0). CMs were isolated from the right-atrial (RA) appendage (RAA) using a collagenase-based mixture (21.5 mg collagenase type 1 (330 U/mg) and protease type XXIV (7-14 U/mg) in 20 mL Ca^{2+}-free solution) and incubated at 37°C for 45 minutes. Incubation-

duration was adjusted based on cell quality and yield. Enzymatic action of the collagenase was stopped using 20 mL Feng medium, mM): KCl 20, KH_2PO_4 10, D-(+)-Glucose 10, L-Glutamic acid 70, β-hydroxybutyrate 10, taurine 10, EGTA 10, BSA 1%; pH 7.4). The digested RAAs were centrifuged at 100 g for 10 min. Supernatant was removed and the pellet containing the CMs was resuspended with PBS and centrifuged again at 300 g for 5 min. The CMs were resuspended in buffer A (mM): Tris-HCl 20, NaCl 10, $MgCl_2 \cdot 6H_2O$ 3, PMSF 100, PIC and PhosStop one tablet, and incubated for 60 min on ice. Subsequently, 600 µL 10% IGEPAL was added. The CM-suspension was mixed for 15 seconds and centrifuged at 300 g for 5 min at 4°C. The resulting supernatant contained both nuclear and non-nuclear fractions, and was then centrifuged at 2,000 g for 10 min at 4°C, with the supernatant following this centrifugation contained the non-nuclear fraction. The pellet containing the nuclear fraction was resuspended in buffer B (Tris-HCl 50 mM, NaCl 150 mM, glycerol 10%, triton 0.1%, SDS 0.1%, PMSF 100 mM, PIC and PhosStop one tablet) for 30 min on ice and mixed every 10 min for 15 sec. The nuclear fraction was centrifuged at 10,000 g (10 min) and the supernatant contained the final enriched nuclear fraction. Nuclear and non-nuclear fractions were stored at -80° C for later use. Antibodies for immunoblotting of human samples are provided in the **Major Resources Table**.

Online Table III. Patient characteristics.

	SR	cAF
Patients, n	24	19
Gender, M/F	17/7	15/4
Age, y	66.7±2.2	68.4±2.2
Body mass index, kg/m^2	28.6±0.9	28.9±1.3
CAD, n (%)	11 (46)	3 (16)
AVD/MVD, n (%)	9 (37)	10 (53)
CAD+AVD/MVD, n (%)	4 (17)	7 (37)
Hypertension, n (%)	19 (79)	13 (68)
Diabetes, n (%)	4 (17)	5 (26)
Hyperlipidemia, n (%)	10 (42)	5 (26)
LVEF, %	55.5±1.1	50.1±3.6
LA, mm§	38.0±5.8	43.0±3.3

Ctl, sinus rhythm control group; cAF, long-standing persistent (chronic) atrial fibrillation; CAD, coronary artery disease; AVD, aortic valve disease; MVD, mitral valve disease; LVEF, left ventricular ejection fraction; LA, left atrial diameter. Data are mean ± SEM

Ca^{2+}-current recording

All in-vitro recordings were obtained at 37±2°C. The whole-cell tight-seal patch-clamp to record currents in voltage-clamp mode. Borosilicate glass electrodes (Sutter Instruments) filled with pipette solution were connected to a patch-clamp amplifier (Axopatch 200B, Axon). Electrodes had tip resistances of 2-4 MΩ. Currents are expressed as densities (pA/pF).

The extracellular solution for Ca^{2+} -current (I_{CaL}) measurement contained (mmol/L): tetraethylammonium-chloride 136, CsCl 5.4, $MgCl_2$ 1, $CaCl_2$ 2, NaH_2PO_4 0.33, dextrose 10, and

HEPES 5 (pH 7.4, CsOH). Niflumic acid (50-µmol/L) was added to inhibit Ca^{2+} -dependent Cl^--current, and 4-aminopyridine (2-mmol/L) to suppress I_{to}. The pipette solution for ICa-recording contained (mmol/L): CsCl 120, tetraethylammonium-chloride 20, $MgCl_2$ 1, EGTA 10, MgATP 5, HEPES 10, and Li-GTP 0.1 (pH 7.4, CsOH).

Data Analysis

Custom-made software (available on GitHub, https://github.com/FengXiongCA/Nucleus-Cytosolic-Ca) was used to analyse Ca^{2+}-transients. Image J was used to analyse immunostaining images. GraphPad Prism 8.0, Origin 5.0 and SAS release 9.4 (SAS Institute Inc., Cary, NC, USA) were used for data analysis. Wherever multiple CMs were measured per dog, numbers are given as n/N, where n indicates number of CMs studied from N dogs.

Mixed effect models were used for all analyses unless specified otherwise. For analyses involving the fixed effect condition (AF vs. CTL), the homogeneity of variance between conditions (AF vs. CTL) was verified using the Brown and Forsythe's test. Some of the analyses were multilevel; involving (1) the individual dog and (2) cells within each dog. For these analyses, the random effect within the model was the intercept to take into account correlation of multiple cells within dogs. Other analyses involved fixed effects as repeated factor(s) such as pacing frequency (1 vs 3 Hz), knockdown probe versus control, endothelin-1 (ET-1) exposure (before vs after), exposure to 2APB+IP3 (before versus after), exposure to IP3 (before versus after), 2APB (2APB, 2APB+ET-1, CTL), Tetracaine (CTL+Tetrcaine , CTL+Tetrcaine+IP3), 2APB concentration (5 µM, 25 µM, 50 µM, CTL), AIP (without vs with) and basic cycle length (150, 200, 250, 300, 350).

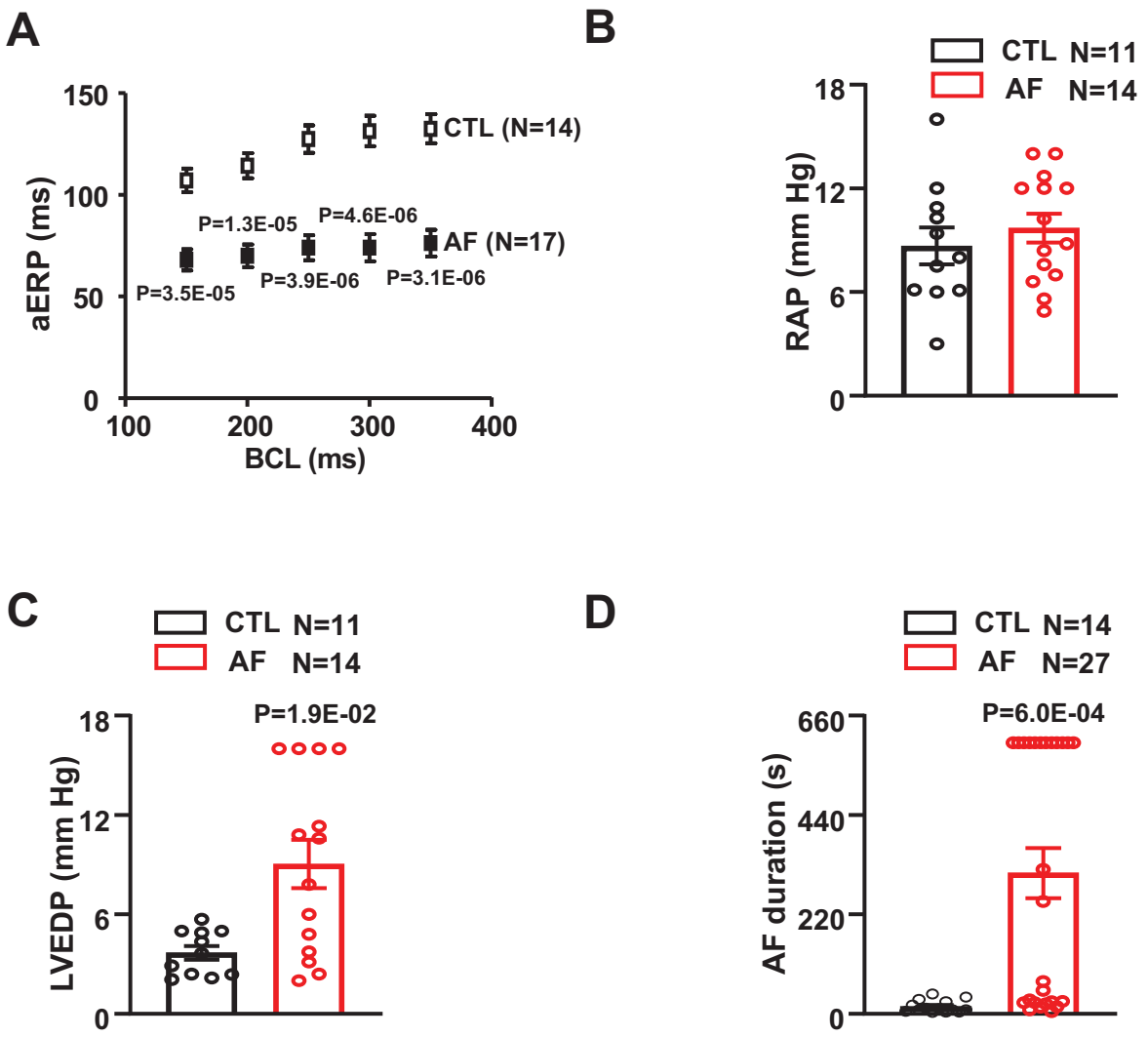
For repeated measure models, the unstructured covariance structure to model the within-dog errors was used. For multilevel repeated models, the random effects included in the model were the intercept, cell and the repeated factor to take into account correlation within dogs, within cells*dogs and within repeated factor*dogs. In addition, the multilevel linear model was used to measure the association between nucleus and cytosol. All variables with a non-normal distribution were transformed using a natural logarithm to produce normally-distributed data for analyses.

Data from human atrial samples, in which each patient contributed a single data point, were compared with unpaired Student's t-test or Mann-Whitney U-test for normally and non-normally distributed data, respectively.

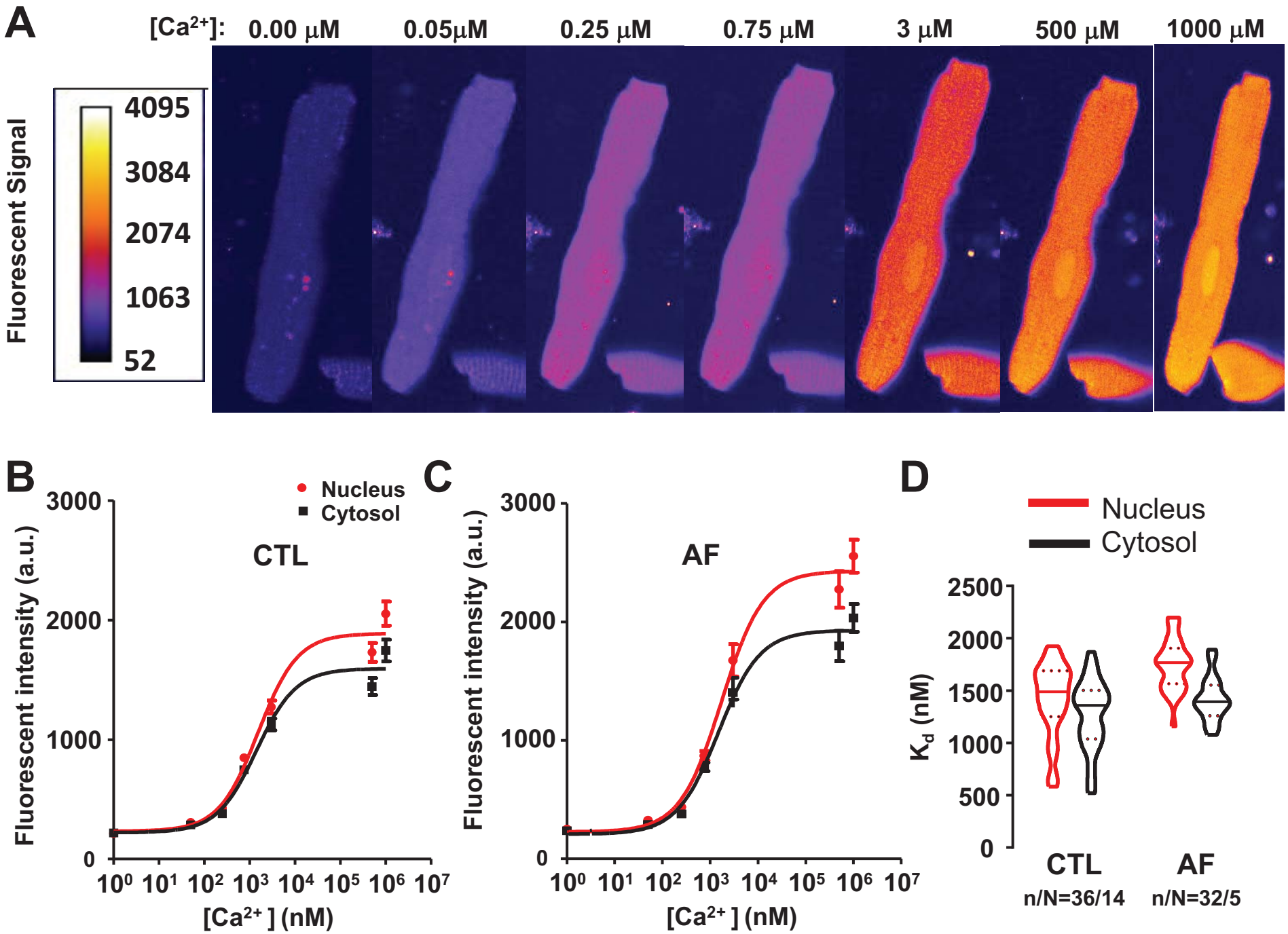
Information about the mechanisms underlying nuclear Ca^{2+}-handling remodeling in AF was not available prior to this study, so sample size calculations could not be performed before commencing the study. Group sizes were planned based on our prior experience and no formal a priori power calculations were done. Results are shown as scatterplots wherever possible and if not, as violin plots in almost all cases. Representative images were selected to represent the mean value of each condition. Group data in scatterplots are presented as geometric mean±standard error and violin plot data show median and interquartile range; any exceptions to these general rules are specified in the figure legends. A 2-tailed $P<0.05$ was considered statistically significant.

ONLINE FIGURES

Online Figure I

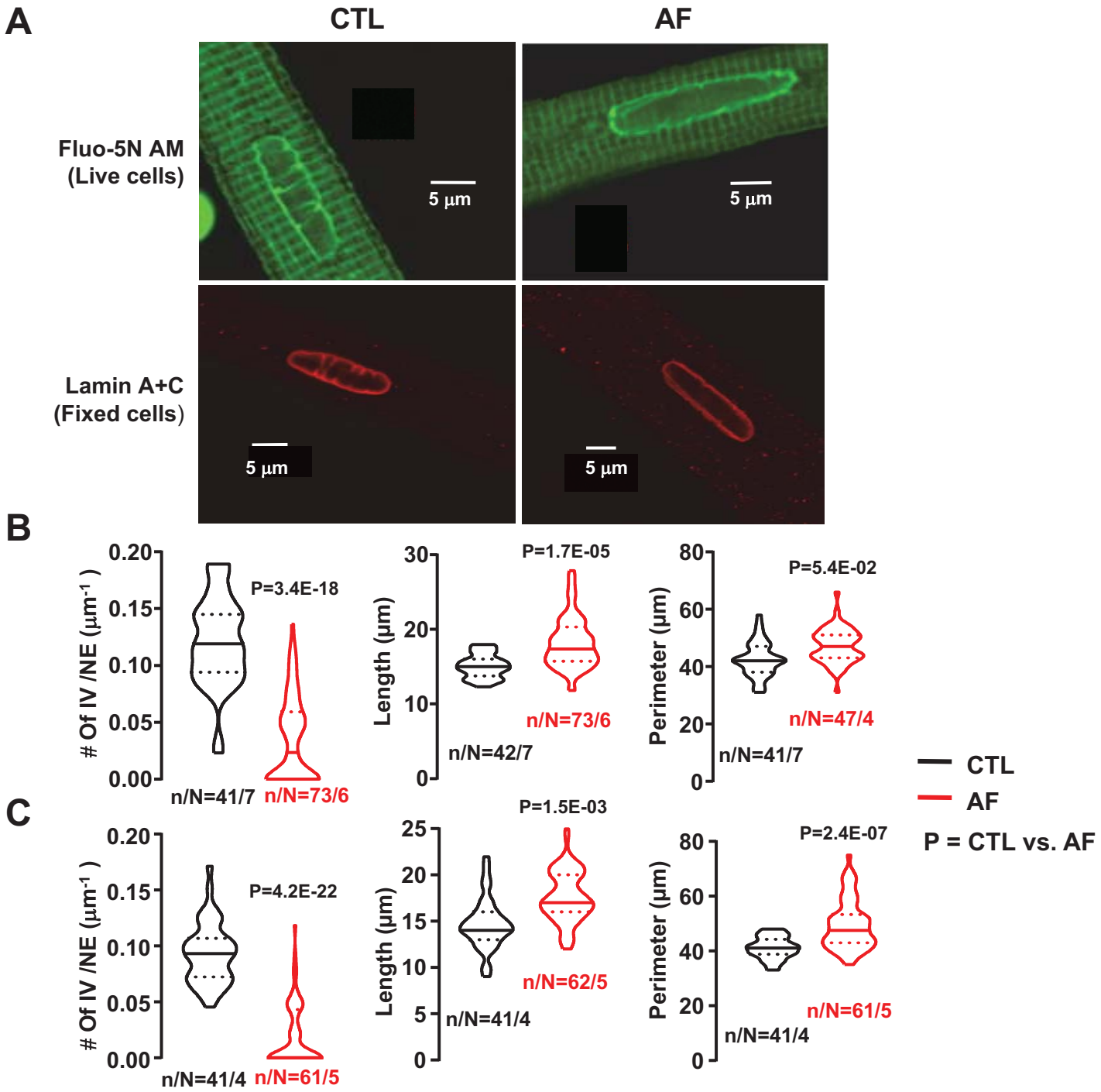


Online Figure I. A. Mean ± SEM atrial effective refractory period (aERP; open squares indicate CTL; filled squares, AF; BCL indicates basic cycle length). **B-D.** Individual data points and mean ± SEM of right atrial pressure (RAP, **B**); left ventricular end-diastolic pressure (LVEDP, **C**) and duration of induced atrial fibrillation (AF, **D**) in control (CTL) and AF dogs. P-values reflect CTL vs. AF based on regular mixed effects models.



Online Figure II

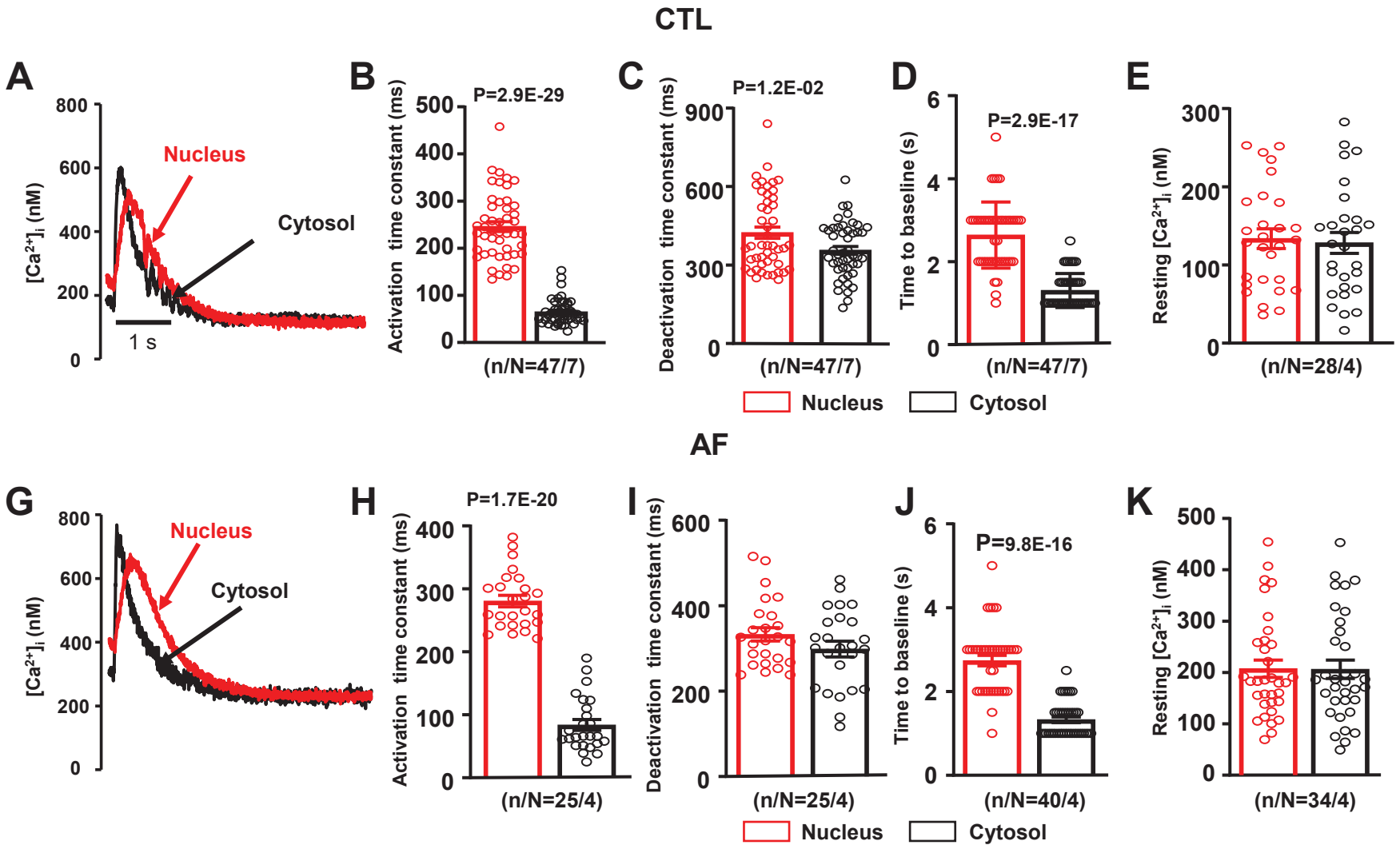
Online Figure II. In situ calibration of Ca^{2+} indicator Fluo-4 in the nucleus versus the cytosol of control (CTL) and AF canine atrial cardiomyocytes. **A.** Original 2-dimensional confocal image of Fluo-4 fluorescence of canine CTL atrial cardiomyocytes at different [Ca^{2+}] during calibration protocol. **B.** Concentration-response curves with seven different [Ca^{2+}] values from CTL atrial cardiomyocytes. **C.** Concentration-response curves with seven different [Ca^{2+}] values from AF atrial cardiomyocytes. **D.** Violin plots with median (solid lines) and interquartile ranges (dotted lines) of Fluo-4 dissociation constants for Ca^{2+} binding (*Kd*). (n/N=31-47/5-14 CMs/dogs).



Online Figure III

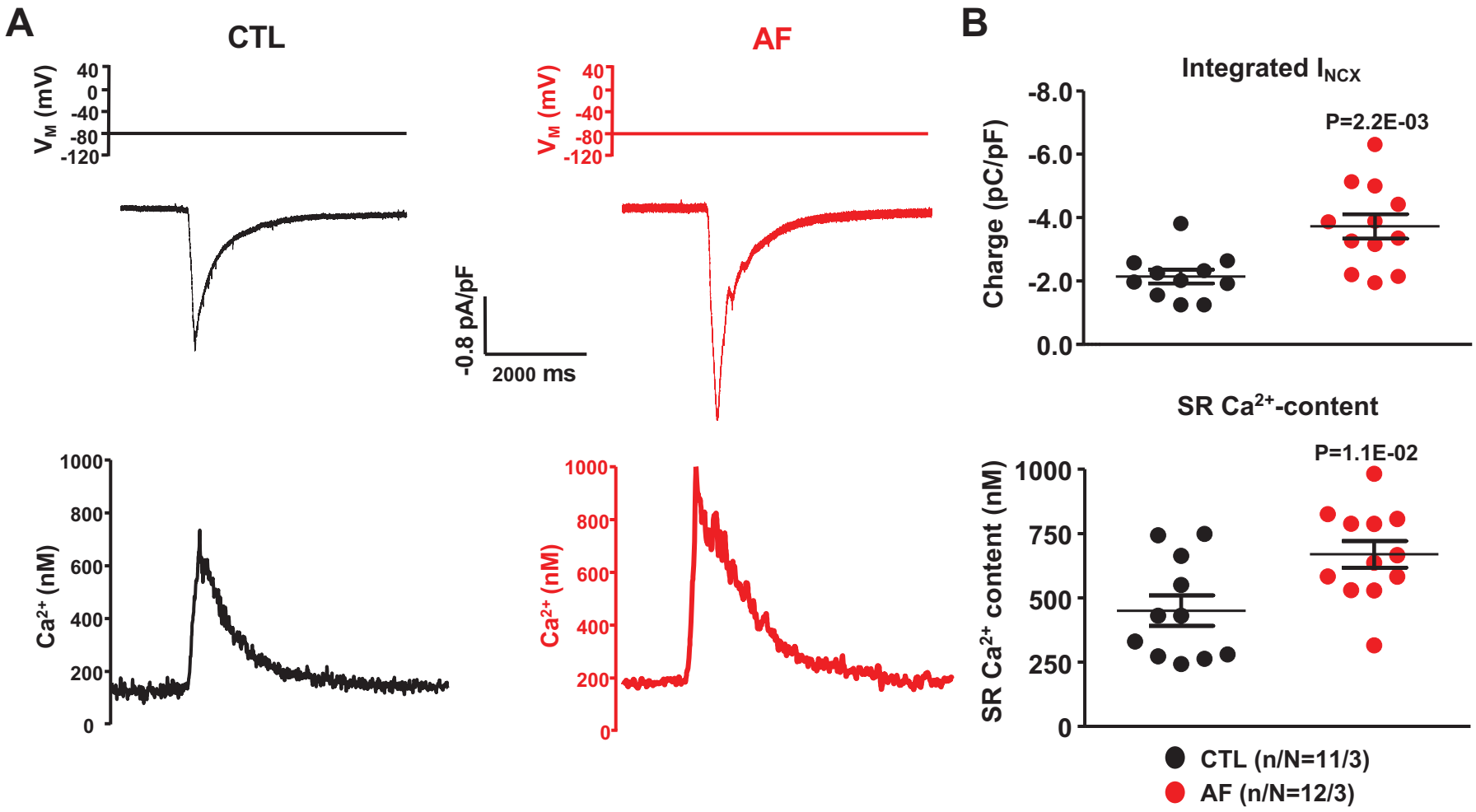
Online Figure III. A. Top: Original 2-dimensional confocal images of Fluo-5N fluorescence of nuclei from freshly isolated control (CTL) and AF atrial cardiomyocytes. Bottom: Original 2-dimensional confocal images from CTL and AF atrial cardiomyocytes after immunostaining for LaminA+C. **B.** Violin plots with median (solid lines) and interquartile ranges (dotted lines) of nuclear envelope (NE) invagination density (calculated as number of invaginations per NE circumference), nuclear length, and perimeter; as obtained in living cells with multiphoton confocal microscopy. **C.** Similar to panel **B**, as obtained in fixed cells. P-values reflect CTL vs. AF based on multilevel mixed effects models (n/N=cells/dogs).

Online Figure IV



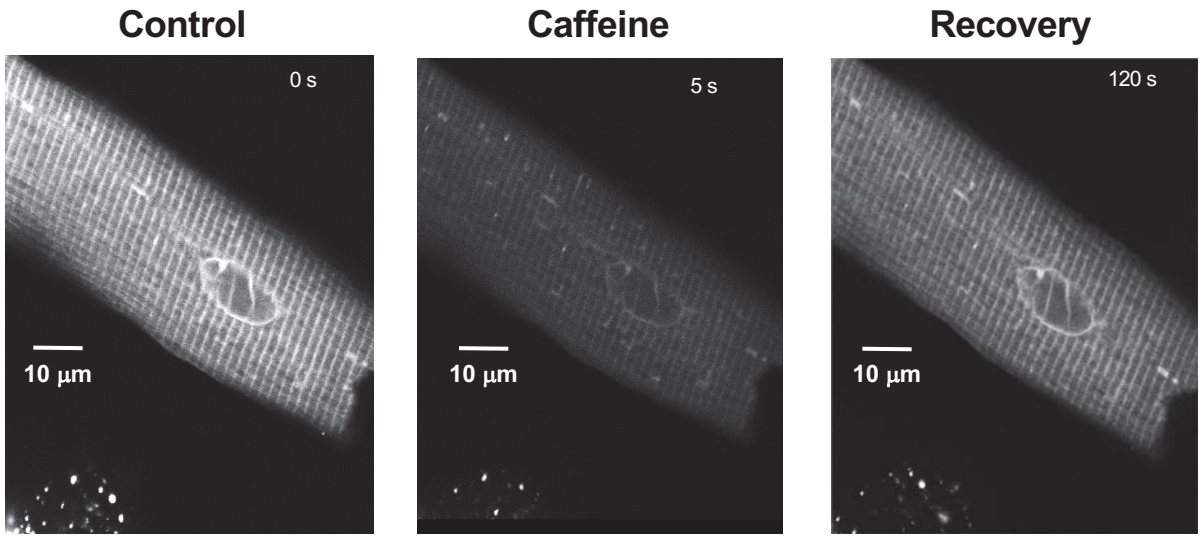
Online Figure IV. A. Original recordings of $[Ca^{2+}]_{nuc}$ and $[Ca^{2+}]_{cyto}$ transients at 1 Hz followed by a 10-second pause to assess resting $[Ca^{2+}]$ in CTL atrial cardiomyocytes. **B-E.** Individual data points and mean ± SEM of activation time constant (**B**), decay time constant (**C**), time to baseline (**D**) and resting $[Ca^{2+}]_{nuc}$ and $[Ca^{2+}]_{cyto}$ (**E**). **G-K**. Similar to A-E for AF cardiomyocytes. P-values reflect nucleus vs. cytosol based on multilevel mixed effects models.

Online Figure V

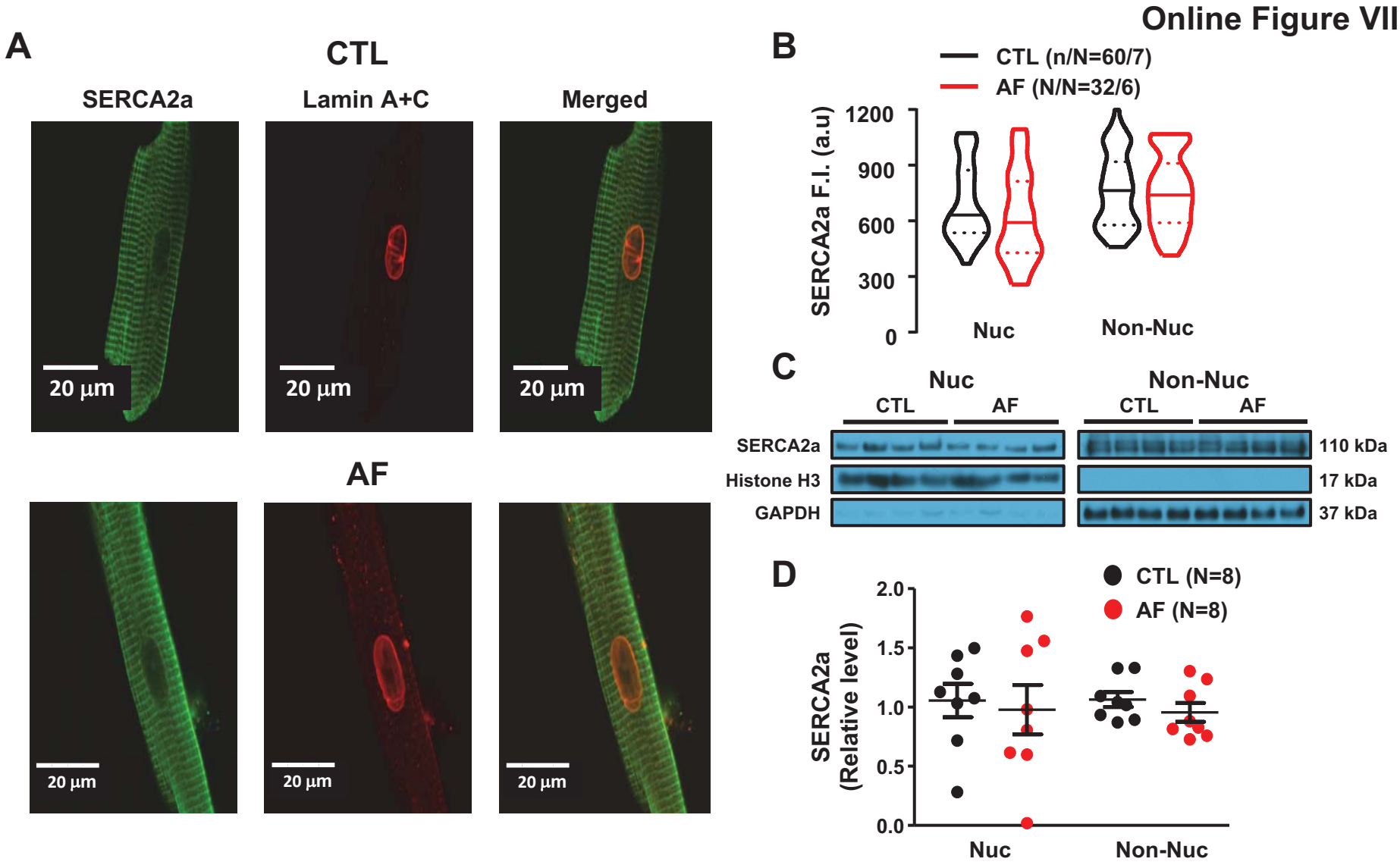


Online Figure V. A. Voltage-clamp protocol (top), corresponding recording of I_{NCX} (middle), and calibrated intracellular [Ca^{2+}] (bottom) during rapid caffeine (10 mM) application following steady-state stimulation for 1 minute at 0.5 Hz. **B.** Individual data points and mean ± SEM of integrated NCX current (top) and total SR Ca^{2+} content (bottom). P-values reflect CTL vs. AF based on multilevel mixed effects models.

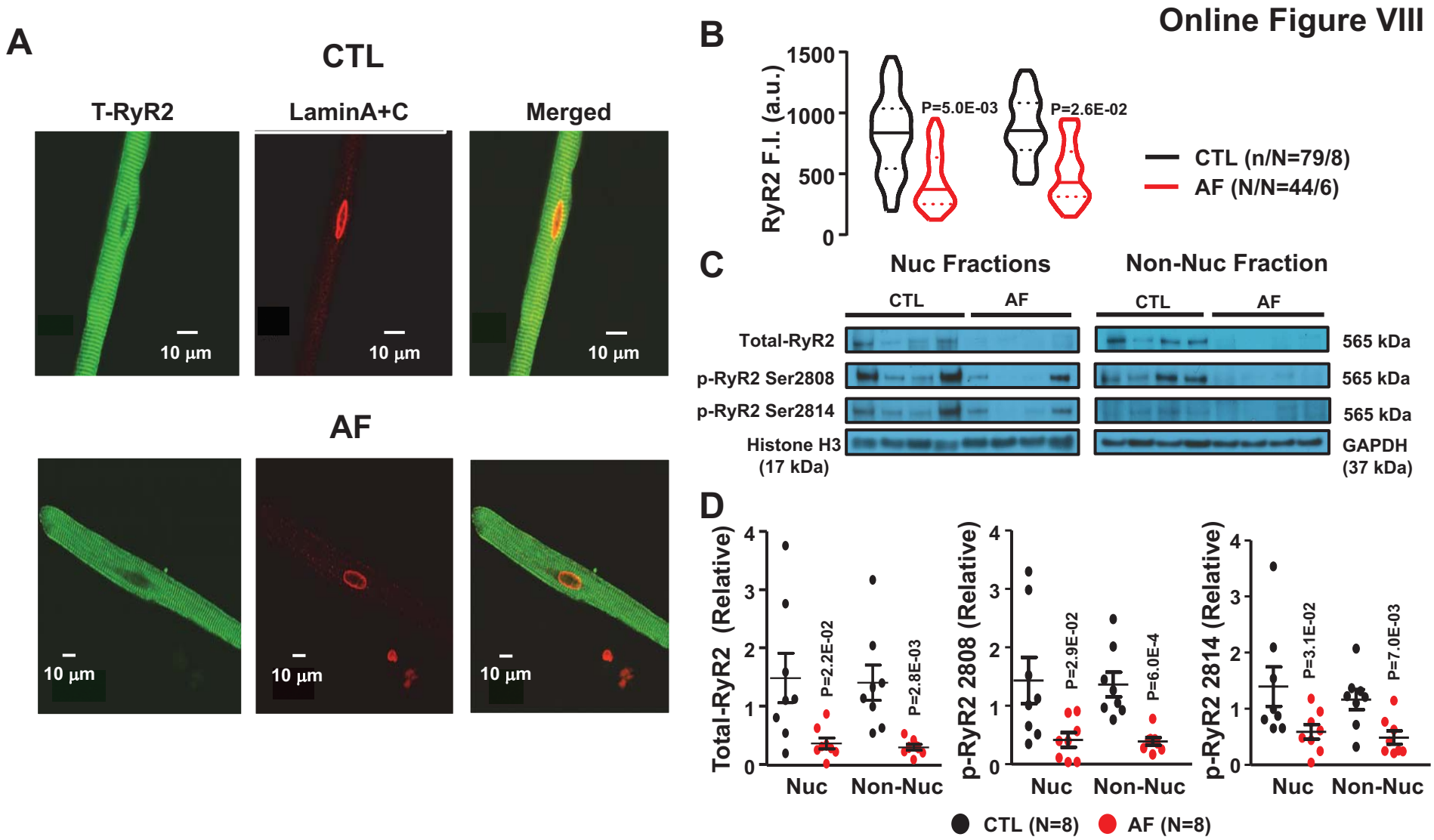
Online Figure VI



Online Figure VI. Original 2-dimensional confocal images of Fluo-5N fluorescence of a CTL atrial cardiomyocyte before, immediately after, and following washout of caffeine application. Similar results were obtained in 20 cells from 4 dogs.

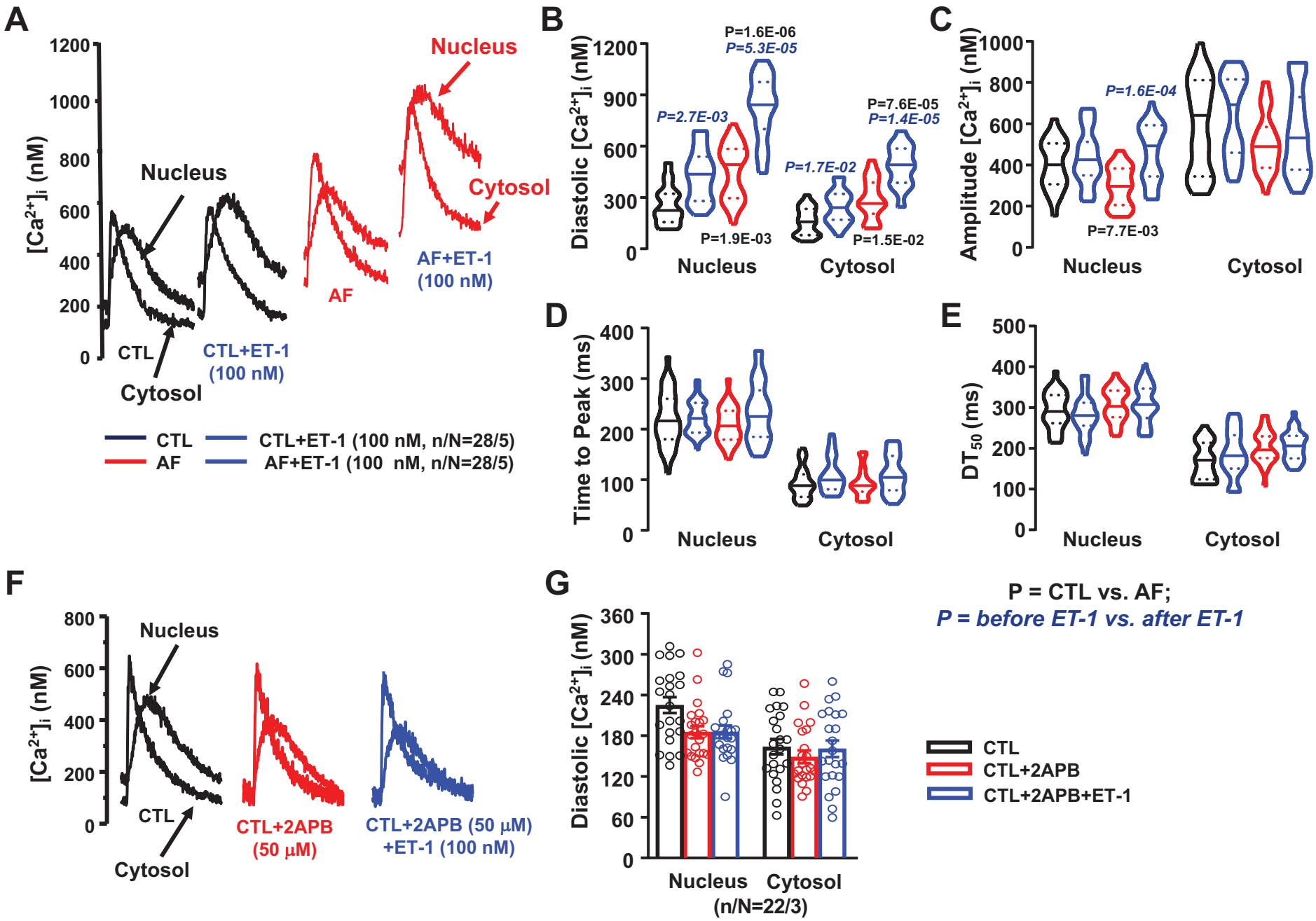


Online Figure VII. A. Original 2-dimensional confocal images of cardiomyocytes isolated from control (CTL) and AF dogs stained with anti-SERCA2a antibody and anti-LaminA+C to visualize the nuclei. **B.** Violin plots with median (solid lines) and interquartile ranges (dotted lines) of SERCA2a fluorescence intensity from isolated CTL and AF atrial cardiomyocytes. **C.** Immunoblots for SERCA2a from CTL and AF atrial myocyte nuclei fraction and non-nuc fraction. **D.** Individual data points and mean ± SEM protein expression levels.



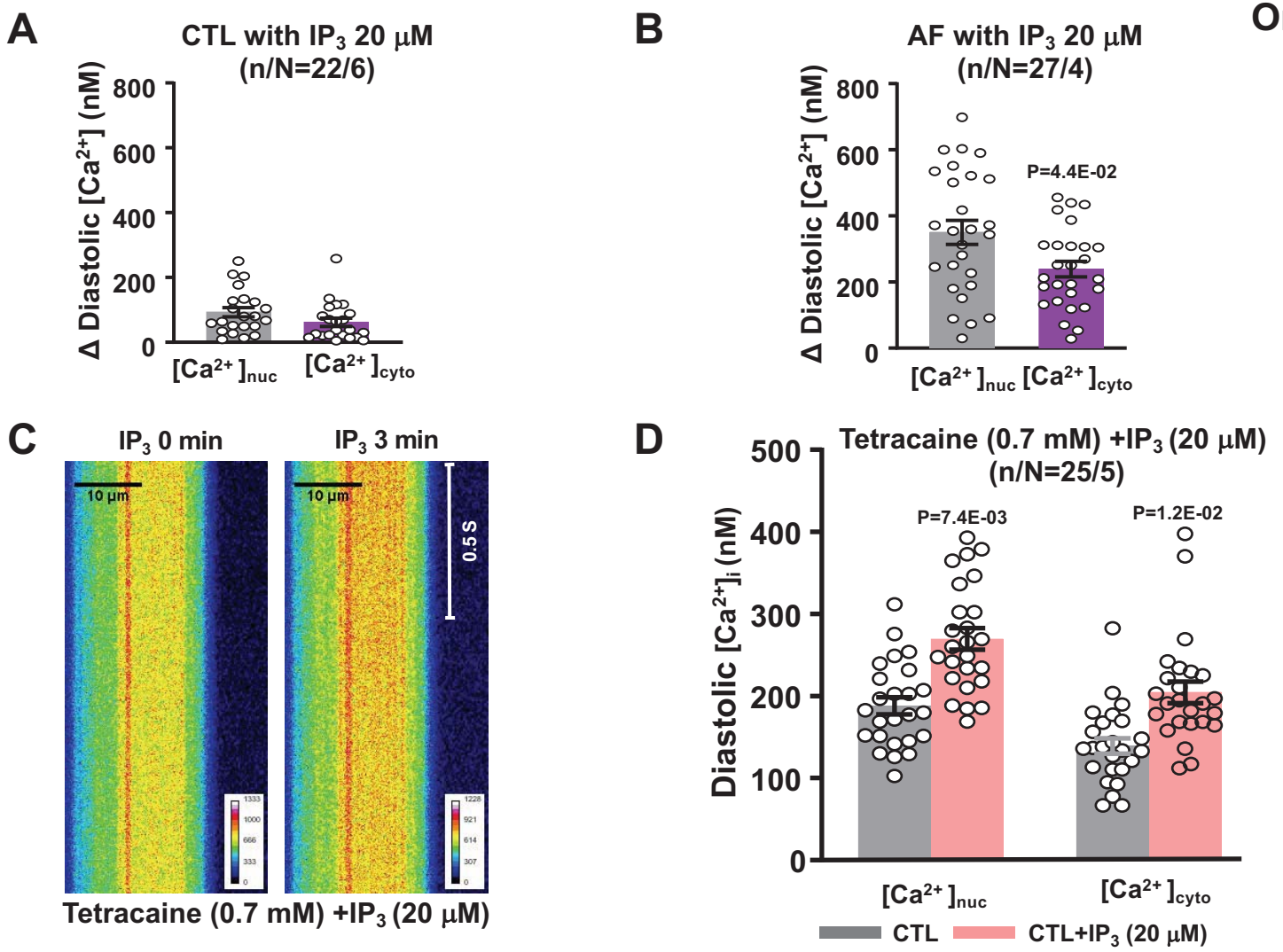
Online Figure VIII. A. Original 2-dimensional confocal images of cardiomyocytes isolated from control (CTL) and AF dogs and double staining with an anti-RyR2 antibody and LaminA+C to visualize the nuclei. **B.** Violin plots with median (solid lines) and interquartile ranges (dotted lines) of RyR2 fluorescence intensity. **C.** Immunoblots for Total-RyR2, p-RyR2 Ser2808 and p-RyR2 Ser2814 from CTL and AF atrial myocyte nuclear fraction and non-nuclear fractions. **D.** Individual data points and mean ± SEM protein expression. P-values reflect CTL vs. AF based on multilevel mixed effects models (**B**) or regular mixed effects models (**D**).

Online Figure IX



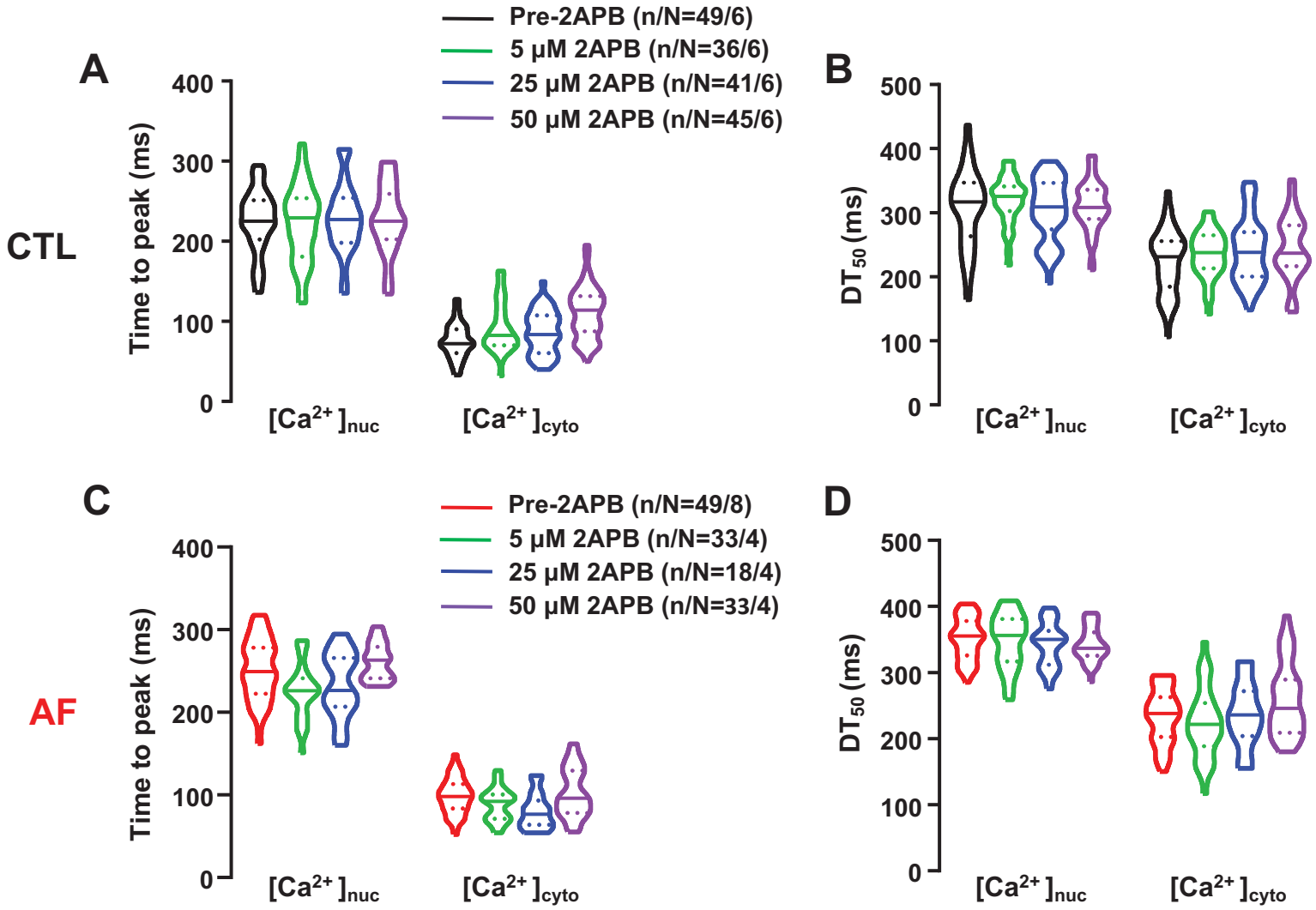
Online Figure IX. Effects of endothelin-1 (ET-1) on nucleoplasmic and cytosolic CaTs. **A.** Original recordings of $[Ca^{2+}]_{nuc}$ vs $[Ca^{2+}]_{cyto}$ of one CTL and AF atrial cardiomyocyte before (black and red, respectively) and after (blue) application of 100 nM ET-1. **B-E.** Violin plots with median (solid lines) and interquartile ranges (dotted lines) of diastolic $[Ca^{2+}]$ (**B**), $[Ca^{2+}]$ amplitude (**C**), time to peak (**D**) and DT_{50} (**E**) in CTL and AF atrial cardiomyocytes before and after application of ET-1 (100 nM). P-values in black reflect CTL vs. AF, whereas P-values in blue italics reflect before ET-1 vs. after ET-1 based on multilevel mixed effects models (n/N=21-36/4-6). **F.** Original recordings of $[Ca^{2+}]_{nuc}$ vs $[Ca^{2+}]_{cyto}$ in one CTL atrial cardiomyocyte at baseline (black), with 50 μM 2APB (red) and with 50 μM 2APB+ ET-1 (blue). **G.** Individual data points and mean ± SEM diastolic $[Ca^{2+}]_{nuc}$ and $[Ca^{2+}]_{cyto}$.

Online Figure X



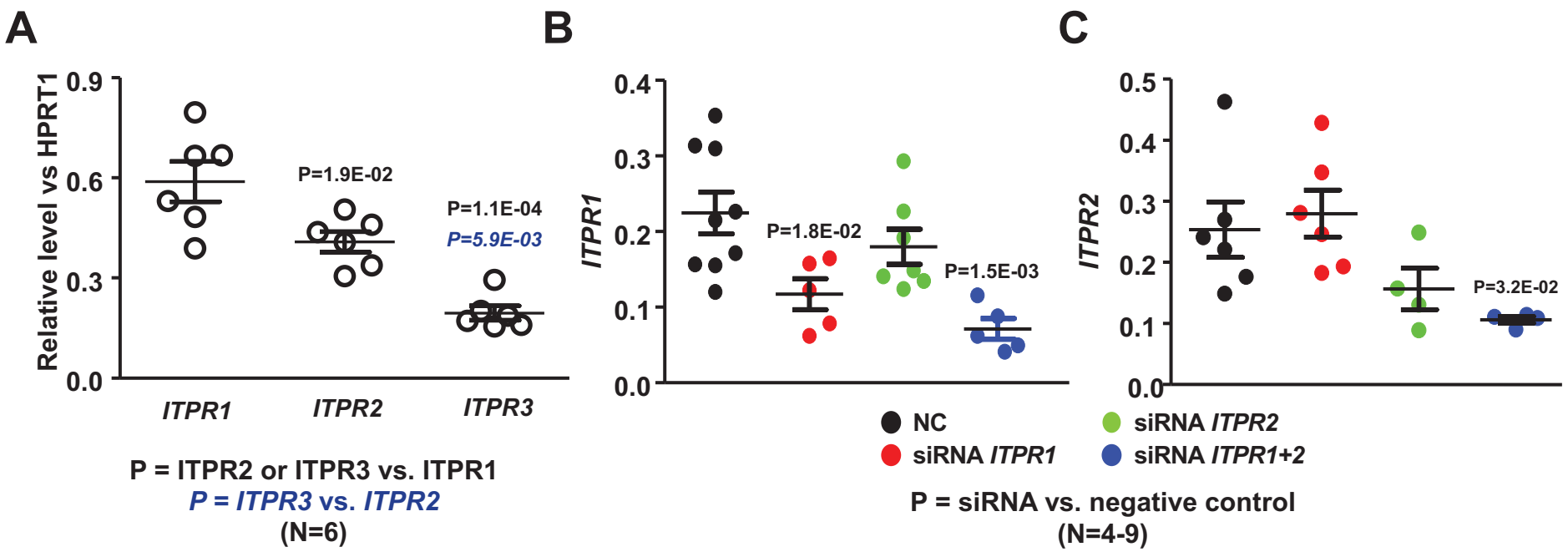
Online Figure X. A and **B.** Response of diastolic $[Ca^{2+}]$ to 20-µM IP3 (difference between value before vs after application of 20-µM IP3) in permeabilized control (CTL, **A**) and AF (**B**) atrial cardiomyocytes. P-values reflect nucleus vs. cytosol based on repeated measures multilevel mixed effects models. **C.** 2-dimensional line-scan image of cytosolic and nucleoplasmic CaTs in a control permeabilized atrial cardiomyocyte pretreated with tetracaine and exposed to 20-µM IP3. **D.** Individual data points and mean ± SEM diastolic $[Ca^{2+}]_{nuc}$ and $[Ca^{2+}]_{cyto}$. P-values reflect CTL vs. CTL+IP_3 based on repeated measures multilevel mixed effects models.

Online Figure XI

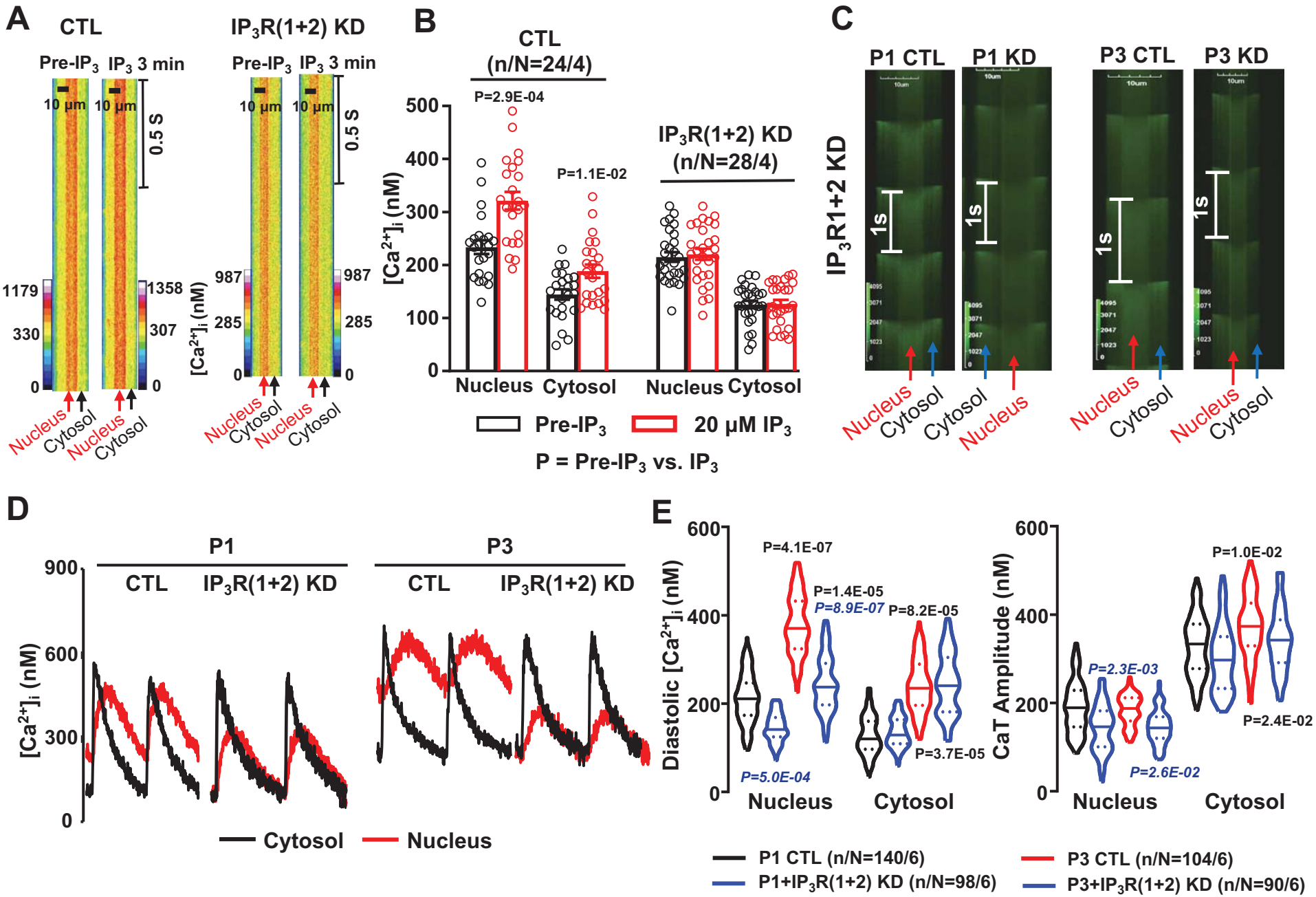


Online Figure XI. A and **B.** Violin plots with median (solid lines) and interquartile ranges (dotted lines) of time to peak and time from peak $[Ca^{2+}]$ to 50% decline (DT_{50}) of nucleoplasmic and cytoplasmic CaTs from CTL atrial cardiomyocytes with and without 2APB. **C** and **D.** Similar to panels A and B for AF atrial cardiomyocytes with and without 2APB.

Online Figure XII



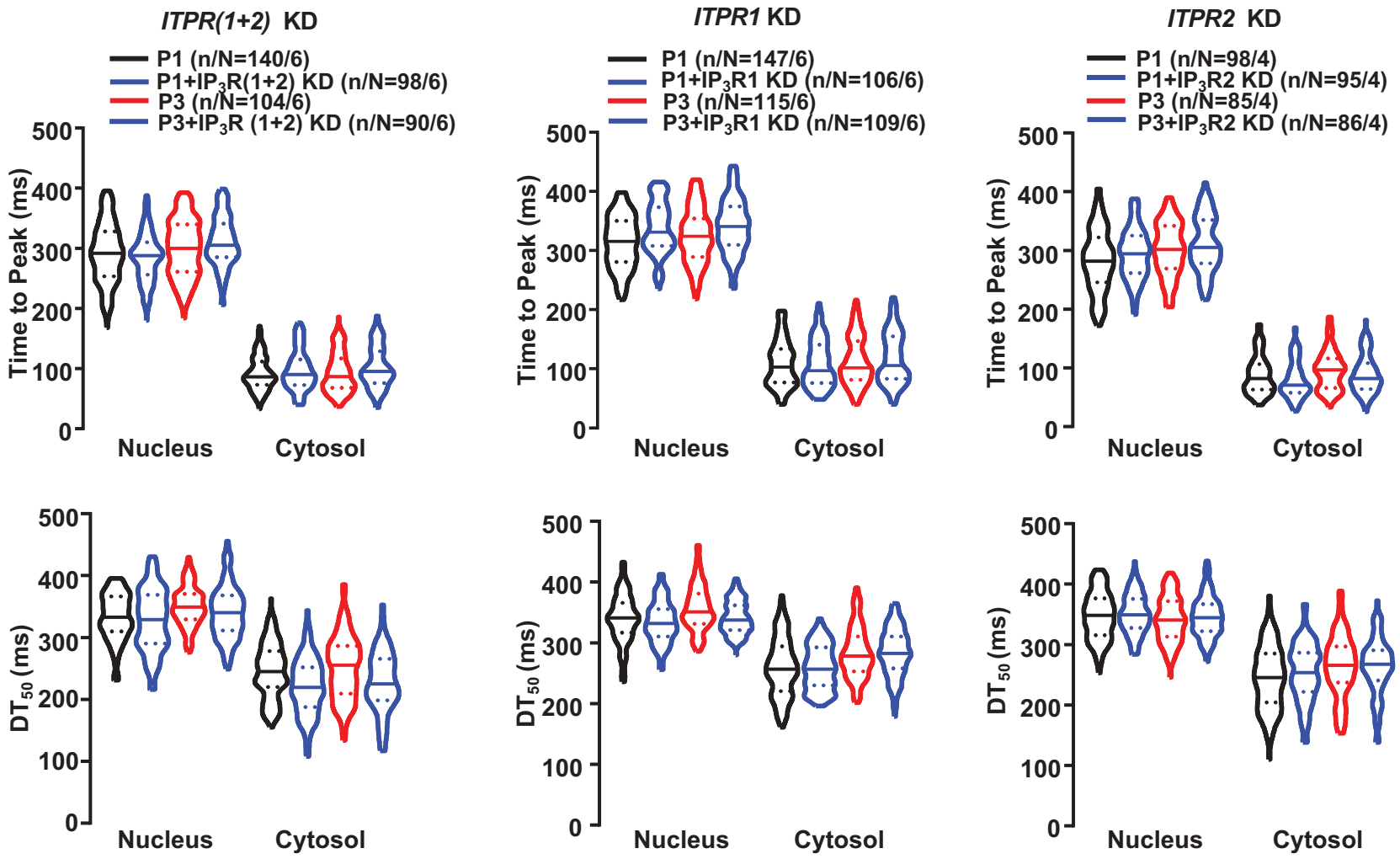
Online Figure XII. A. Expression of *ITPR1*, *ITPR2* and *ITPR3* as determined by qPCR. P-values in black reflect ITPR2 or ITPR3 vs. ITPR1, whereas P-values in blue italics reflect ITPR3 vs. ITPR2 based on 1-way ANOVA (N=dogs). **B.** Efficiency of *ITPR1* knockdown by siRNA. **C.** Efficiency of *ITPR2* knockdown by siRNA. P-values reflect siRNA vs. negative control (NC) based on 1-way ANOVA.



Online Figure XIII

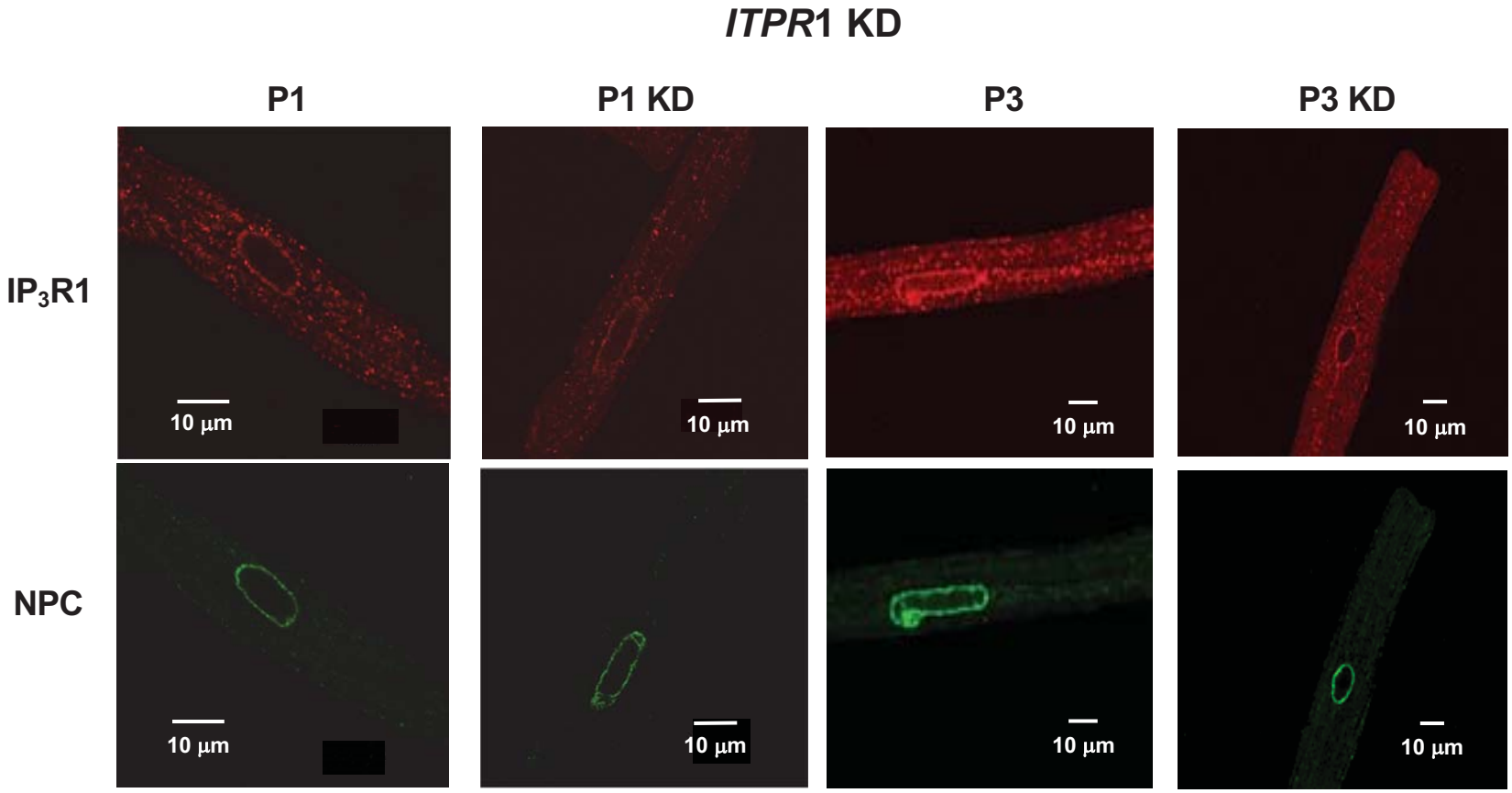
Online Figure XIII. Effects of *ITPR (1+2)* knockdown on nucleoplasmic and cytosolic cytosolic Ca^{2+}-transients (CaTs). **A.** Confocal line-scan images from permeabilized CTL (left) and *ITPR(1+2)* knockdown (KD, right) atrial cardiomyocytes. **B.** Individual data point and mean ± SEM showing the effect of IP_3 on $[Ca^{2+}]$. P-values reflect pre-IP3 vs. IP3 based on multilevel mixed effects models (n/N=24-28/6 CMs/dogs). **C.** Line-scan images of CaTs in P1 and P3 atrial CMs recorded at 1 Hz with or without *ITPR(1+2)* knockdown (KD). **D.** Original recordings of $[Ca^{2+}]_{nuc}$ and $[Ca^{2+}]_{cyto}$ transients from atrial CMs recorded at 1 Hz, for P1 and P3 CMs with or without *ITPR(1+2)* KD). **E.** Violin plots with median (solid lines) and interquartile ranges (dotted lines) of diastolic $[Ca^{2+}]$ and CaT-amplitude in cytosol and nucleus of P1 and P3 CMs. P-values in black reflect P1 vs. P3, whereas P-values in blue italics reflect CTL vs. KD based on multilevel mixed effects models (n/N=CMs/dogs).

Online Figure XIV



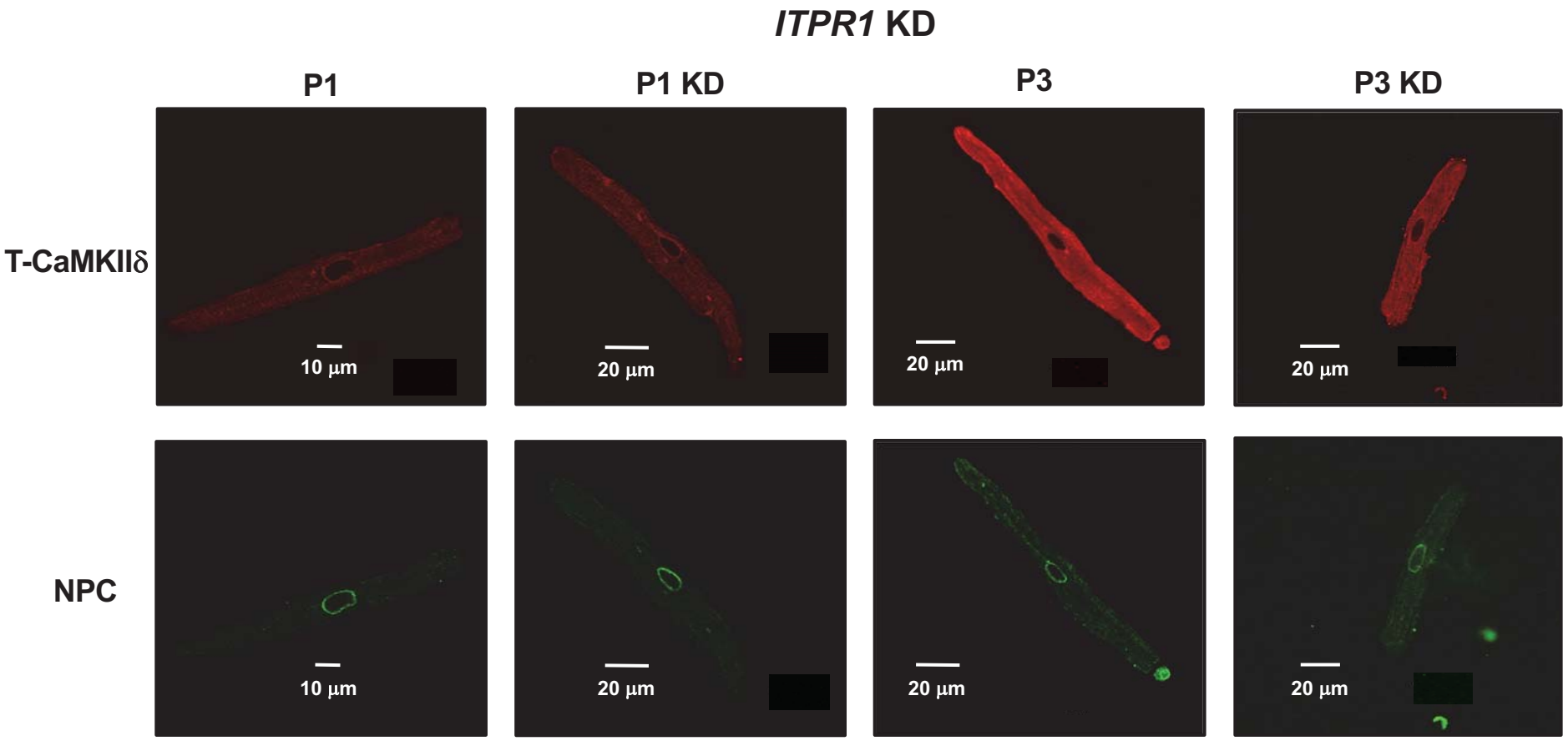
Online Figure XIV. Violin plots with median (solid lines) and interquartile ranges (dotted lines) of time to peak and DT_{50} of nucleoplasmic and cytosolic CaTs from 1-Hz paced (P1) and 3-Hz paced (P3) atrial cardiomyocytes before and after *ITPR* knockdown.

Online Figure XV



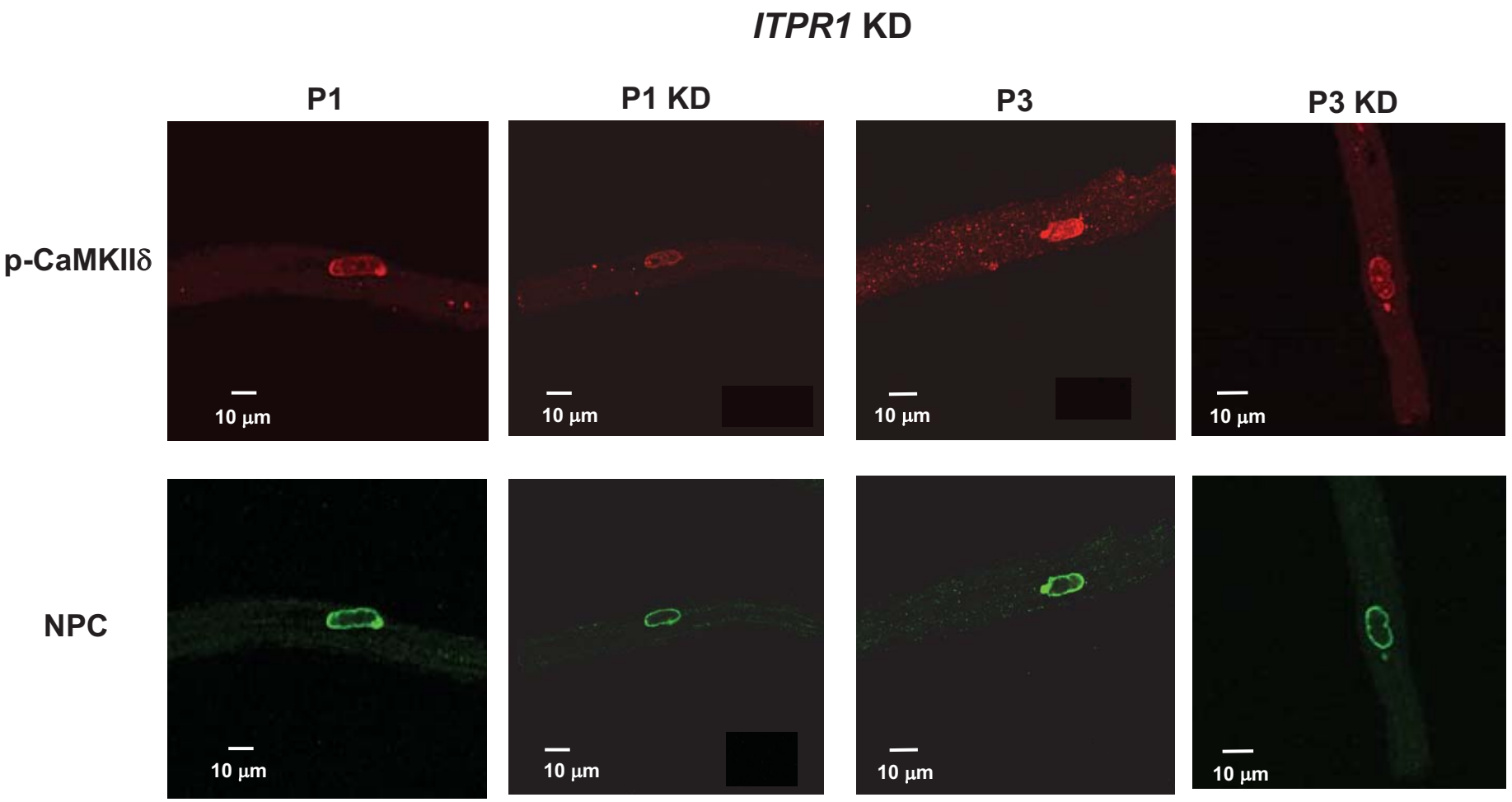
Online Figure XV. Original 2-dimensional confocal images from *ITPR1* knockdown P1 and P3 atrial cardiomyocytes after immunostaining for IP_3R1 (red, upper panel) and NPC (green, lower panel).

Online Figure XVI



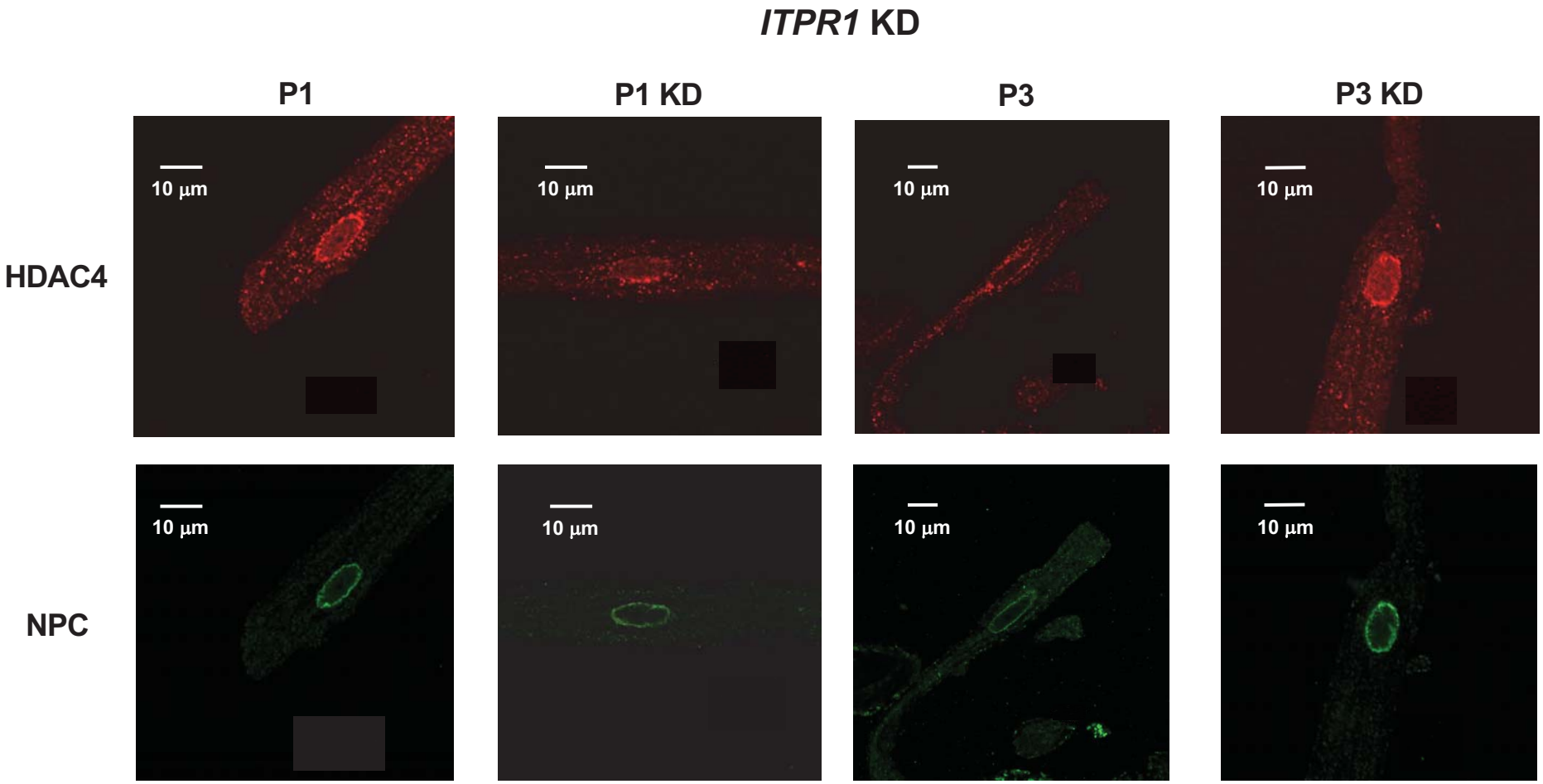
Online Figure XVI. Original 2-dimensional confocal images from *ITPR1* knockdown P1 and P3 atrial cardiomyocytes after immunostaining for T-CaMKIIδ (red, upper panel) and NPC (green, lower panel).

Online Figure XVII



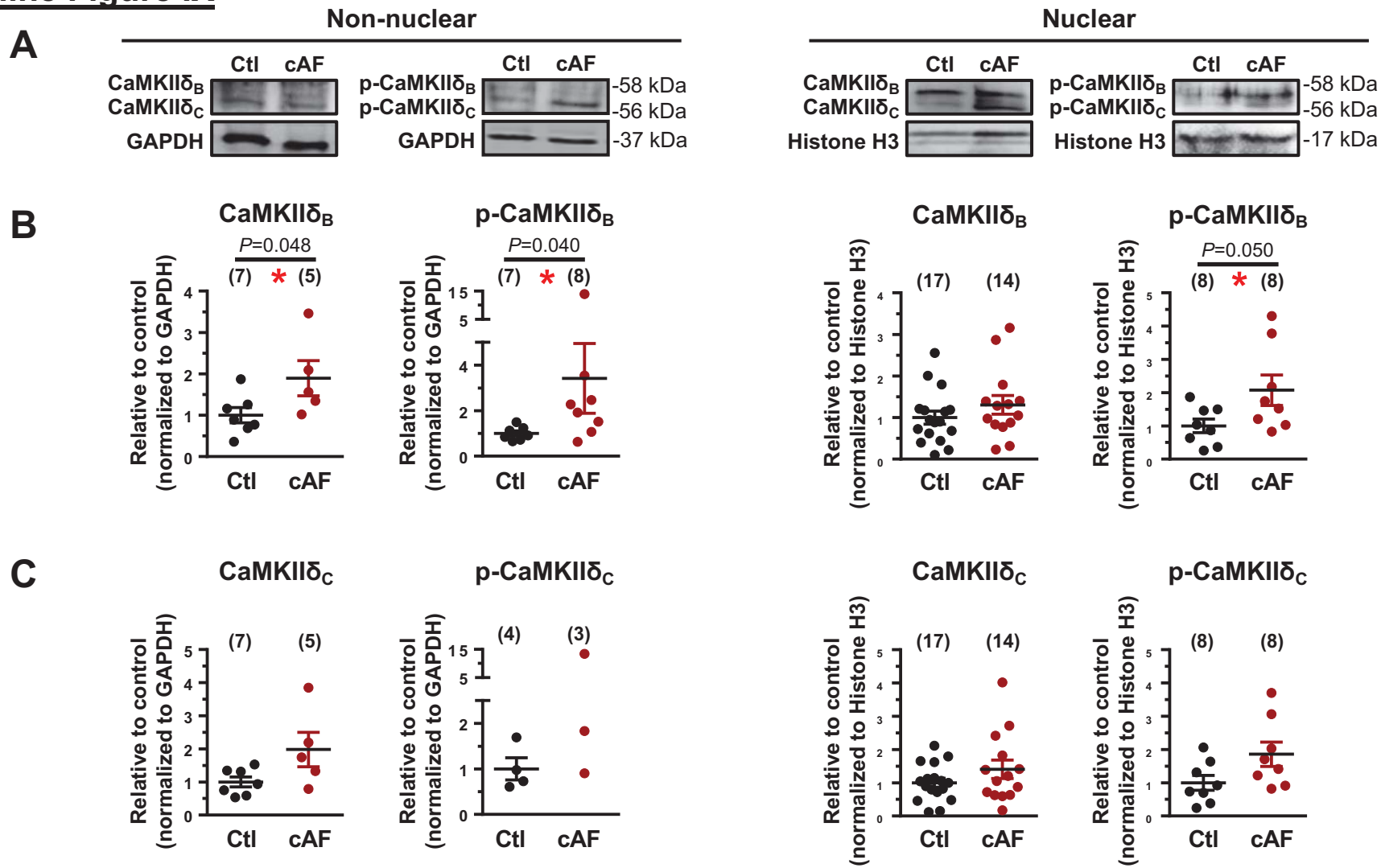
Online Figure XVII. Original 2-dimensional confocal images from *ITPR1* knockdown P1 and P3 atrial cardiomyocytes after immunostaining for P-CaMKII (red, upper panel) and NPC (green, lower panel).

Online Figure XVIII



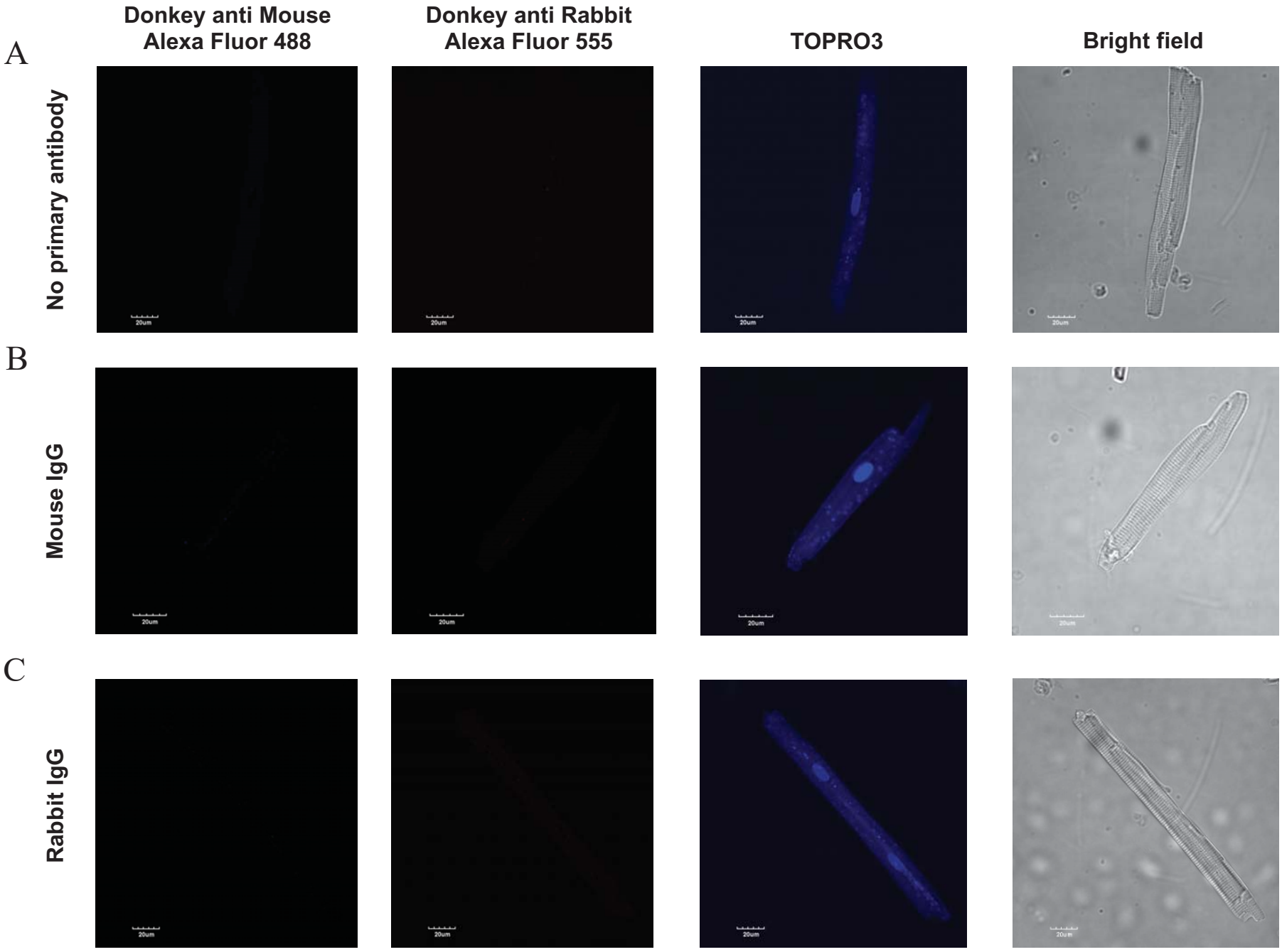
Online Figure XVIII. Original 2-dimensional confocal images from *ITPR1* knockdown P1 and P3 atrial cardiomyocytes after immunostaining for HDAC4 (red, upper panel) and NPC (green, lower panel).

Online Figure IX



Online Figure IX. A. Western blot of total and Thr287-phosphorylated CaMKIIδ_B (upper band) and CaMKIIδ_C (lower band) in nuclear and non-nuclear fractions from human atrial cardiomyocytes isolated from Ctl or long-term (>6 months) persistent 'chronic' atrial fibrillation (cAF) patients. **B.** Quantification of total CaMKIIδ_B and p-CaMKIIδ_B in non-nuclear fractions (left) and nuclear fractions (right) of Ctl (black symbols) and cAF (red symbols) patients. **C.** Similar to panel B, but for CaMKIIδ_C. P-values reflect Ctl vs. cAF based on unpaired Student's t-test (nuclear CaMKIIδ_B, nuclear p-CaMKIIδ_B, nuclear p-CaMKIIδ_C) or Mann-Whitney test (all other comparisons).

Online Figure XX



Online Figure XX. Negative controls for immunostaining. **A.** Dog atrial cardiomyocytes without primary antibody showed no immunofluorescence for both donkey anti-mouse 488 and donkey anti-rabbit 555 secondary antibodies. Third and fourth columns show TOPRO-3 and bright field images as controls. **B-C.** Similar to panel A for Mouse (**B**) or rabbit (**C**) IgG isotype controls.