Supplemental Material

Diminished PLK2 induces cardiac fibrosis and promotes atrial fibrillation

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Expanded Materials & Methods

Human sample acquisition. Right atrial appendages were collected in collaboration with the Herzzentrum Dresden GmbH. Patients in SR and permanent AF undergoing open-heart surgery (e.g. bypass surgery or valve replacement) donated their right atrial appendages that incurred as a result of the surgical procedure. The samples were put into 4°C cold isotonic transport solution and immediately processed for fibroblast isolation. Whenever excess material remained, the tissue was frozen and stored in liquid nitrogen. Peripheral blood samples from patients with symptomatic paroxysmal and permanent AF who would undergo ablation of pulmonary veins were collected prior to the intervention in EDTA-tubes. Low voltage zones were assessed by electrophysiological mapping of the left atrium and defined as bipolar voltage < 0.5 mV [38]. Blood samples were kept at 4°C for a maximum of 1 h. The tubes were then centrifuged for 10 min at 1000 g at 4°C. Subsequently, the plasma was transferred carefully into 500 µl Eppendorf tubes and stored at -80° C until analysis. Corresponding patient data can be found in Online Tables I, II and III.

Assessment of low voltage zones by electrophysiological mapping. Left atrial electrophysiological mapping of AF patients undergoing ablation was performed at Herzzentrum Dresden GmbH according to the established clinical routine. In brief, a bipolar voltage map was performed simultaneously with left atrium reconstruction. A detailed description of the electrophysiological studies and ablations was described previously [50].

Blinding. The experimenter was blinded when performing and analysing immunostaining, echocardiography, calcium imaging and Langendorff-perfusion experiments. Unblinding was performed only after analysis.

Technical replicates. In cases we performed technical replicates (qPCR, ELISA) we used the average to represent the value for each biological sample and never considered technical replicates as separate Ns for any experiments.

Representative image selection. Representative images were selected to display the best match of the graphically presented mean values of the data.

PLK2 WT and KO mice. PLK2 WT and KO mice are commercially available via The Jackson Laboratory (Bar Harbor, USA) (129S.B6N-*Plk2*^{tm1Elan}/J, stock number: 017001 Plk2 KO). The mouse model has previously been used to study the alpha-synuclein phosphorylation at serine 129 in the central nervous system [35]. All mice included in the study were genotyped to assure the assumed genetic background

Cell isolation and culture. Primary human right atrial fibroblasts were isolated by outgrowth from right atrial appendage biopsies as published previously [11]. Human ventricular fibroblasts were purchased from abm Inc. (Richmond, Canada) and adapted to the culture conditions of

the primary cells. Primary murine cardiac fibroblasts were isolated enzymatically via Langendorff-perfusion [51]. The supernatant was centrifuged for 1 min at 350 g to remove cardiomyocytes and debris. The resulting supernatant was centrifuged a second time for 1 min at 750 g to sediment fibroblasts. Cells were cultured in Dulbecco modified eagle medium (Life Technologies, Carlsbad CA, USA) supplemented with 10 % fetal calf serum (FCS, Life Technologies, Carlsbad CA, USA) and 1 % penicillin/streptomycin (Life Technologies, Carlsbad CA, USA). The cell culture medium was changed every other day. Equal volumes of the specific PLK2 inhibitor TC-S 7005 (Tocris Bioscience, Bristol, UK) and the solvent control (DMSO) were added with the medium change. Cells were kept in a humidified surrounding at 37°C and 5 % CO_2. In order to generate hypoxic conditions (1 % O_2) cells were cultured in a hypoxia incubator chamber (Coy Laboratory Products Inc, Grass Lake, USA) for 24 up to 96 h. All experiments with primary fibroblasts were performed between passages 2 and 3.

Cell proliferation. To analyze cell proliferation, fibroblasts were seeded at densities of $1x10^4$ cells per well of a 12-well plate. Cells were harvested and counted with a Buerker counting chamber at 5 and 10 days of culture. The stated cell count was calculated for a resuspension volume of 1 ml.

Immunocytofluorescence. In order to perform immunocytoflorescence experiments, fibroblasts were seeded on 1 cm glass cover slips and grown for 7±1 days until they reached approximately 80 % of optical confluence. Cells were then washed with cold PBS, fixed in 4 % PFA for 15 min at RT, washed and subsequently permeabilised using 0.1% Triton X. After blocking with 10% FCS the cover slips were incubated with a mixture of primary antibody against αSMA (Sigma-Aldrich, St. Louis, Missouri, USA) and 4′,6-Diamidin-2-phenylindol (DAPI) (1:200; A5228, Sigma-Aldrich, St. Louis, Missouri, USA) for 1 h in a dark, humidified surrounding at RT. After washing, the secondary antibody Alexa-Fluor 448 (Abcam, Cambridge, UK) was applied for 1 h in a dark, humidified surrounding at RT. The samples were kept in the dark until fluorescence images were obtained with a Zeiss LSM-510 confocal microscope. To rule out potential unspecific binding of the secondary antibody, a secondary antibody only control was performed. Per coverslip, a randomly chosen area containing at least 50 cells (determined by the number of visible nuclei) was captured. Upon expression of orderly arranged αSMA myofilaments, a cell was considered a myofibroblast. Results were calculated as percentage of myofibroblasts relative to all nuclei in the area.

Extracellular collagen deposition. Extracellular collagen deposition was performed as described recently [24,52]. Collagen expression was normalized to control.

Histological analysis. For histological analysis, mid-ventricular short axis sections or long axis sections comprising atria as well as ventricles of the heart were excised and fixed in 4% PFA for 24 h before dehydration using an ethanol gradient. Subsequently the samples were

embedded in paraffin, sectioned at 3 µm thickness and stained with either Masson Trichrome by the histology facility at the Center for Molecular and Cellular Bioengineering (CMCB) Dresden or Sirius red according to the manufacturer's protocol (Sigma-Aldrich, St. Louis, Missouri, USA). Images were acquired using the Keyence BZ-X710 All-in-One Fluorescence Microscope (Keyence Corporation of America, Itasca, USA). Fibrosis was quantified with ImageJ 1.52n software (Wayne Rasband, National Institutes of Health, USA).

Quantitative real-time PCR (QPCR). SYBR green (Bio-Rad Laboratories GmbH, Munich, Germany) real-time PCR was used to measure the gene expression *in vitro*. Specific primers for *PLK2, DNMT11, DNMT3a* and *OPN* were purchased from Bio-Rad (Bio-Rad Laboratories GmbH, Munich, Germany). All other primers were designed and validated in house. Sequences are provided in Table S8. GAPDH was used as housekeeping gene. For RNA isolation and subsequent cDNA synthesis the PeqLab total RNA mini and PeqGold cDNA synthesis kits (Peqlab Biotechnologie GmbH, Erlangen, Germany) were used according to the manufacturer's instructions. Optional on-column DNA digestion was performed, in order to remove residual contaminating genomic DNA. PCR runs were performed in a CFX96 Touch Deep Well Real-Time PCR detection system (Bio-Rad Laboratories GmbH, Munich, Germany). Samples were amplified in duplicates or triplicates as indicated in the results part. For data analysis the CFX manager software (Bio-Rad Laboratories GmbH, Munich, Germany) was used. Relative gene expression was calculated to housekeeping gene.

Online Table I: cDNA synthesis protocol

Component	**Volume in well (µl)**
5x Advanced Reaction Mix	4
iScript Advanced RT	1
RNA	15
Total	20
Duration	**Temperature**
5 minutes	25°C
20 minutes	46°C
1 minute	95°C
Hold	4°C

Online Table II: RT-qPCR cycler protocol

Component	**Volume in well (µl)**		
SYBR Green	10		
Water	3,67		
Primer forward	3		
Primer reverse	3		
cDNA	0,33		
Total	20		
Step	**Duration**	**Temperature**	**Repeats**
Denaturation	30s	95°C	
Annealment	15s	95°C	45x
Extension	60s	60°C	
	0,5°C/5s	65°C to 95°C	
	Hold	4°C	

Methylation-specific PCR. The methylation-specific PCR was performed as published by Syed *et al*. and Robinson *et al.* [27,28]. Genomic DNA (gDNA) was isolated using the PureLink Genomic DNA Extraction kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Purified gDNA was subsequently bisulfite-converted using the EZ DNA starter kit according to the manufacturer's instructions. The following PCR protocol was designed according to the suggestions of ZYMO Research:

Online Table III: Methylation specific PCR protocol

Component	**Volume in well (µl)**		
2x ZymoTaq™ PreMix	12.5		
ddH2O	8.5		
Primer I	1		
Primer II	1		
Bisulfite-converted DNA	2		
Total	25		
Step	**Duration**	**Temperature**	**Repeats**
	10min	95°C	
Denaturation	30s	95°C	36x (unmethylated) 38x (methylated)
Annealment	30s	55°C	
Extension	60s	72°C	

	7min	72°C
	Hold	4°C

For unmethylated samples 36 cycles were run and for detection of DNA methylation 38 runs, respectively. For electrophoresis, the PCR products were then applied to a 2 % agarose gel containing HD green (INTAS Science Imaging Instruments GmbH, Göttingen, Germany). Visualization of gel bands was achieved with a Fusion FX (Peqlab Biotechnologie GmbH, Erlangen, Germany) development device. The following primers as published by Syed *et al.* [27] were used for methylation-specific PCR:

PLK2 unmethylated for.: 5′-CACCCCACAACCAACCAAACACACA-3′

PLK2 unmethylated rev.: 5′-GGATGGTTTTGAAGGTTTTTTTGTGGTT-3′ (product = 142 bp)

PLK2 methylated for.: 5′-CCCACGACCGACCGAACGCGCG-3′

PLK2 methylated rev.: 5′-ACGGTTTTGAAGGTTTTTTCGCGGTC-3′ (product = 137 bp)

SDS-PAGE, Western Blotting and Immunodetection. Protein was extracted from whole heart tissue and cells for western blot analysis using RIPA buffer (30 mM Tris, 0.5 mM EDTA, 150 mM NaCl, 1% NP-40, 0.1 % SDS) supplemented with 10 % protease and phosphatase inhibitors. Protein concentration was determined using a BCA kit (Pierce Biotechnology, Waltham, Massachusetts, USA). For SDS-PAGE, 10 % polyacrylamide gels were used. 30 µg of protein were loaded into each lane of the gels. Proteins were subsequently transferred to a 0.45 µm nitrocellulose membrane. Equal loading was ensured by ponceau red staining (Sigma-Aldrich, St. Louis, Missouri, USA) before blocking in 5 % milk. Membranes were incubated with primary antibodies overnight at 4°C under constant gentle shaking. After several washing steps, secondary antibodies (anti mouse or anti rabbit) were applied for 1 h at RT. After final washing membranes were incubated with ECL development solution (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and developed in a Fusion FX device (Vilber Lourmat Deutschland GmbH, Eberhardzell, Germany). Depending on the molecular weight of the proteins of interest either Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), calsequestrin (CSQ) or Eukaryotic elongation factor 2 (EEF2) were used as housekeeping proteins. Online Table VI lists all western blot antibodies used in this study.

Secretome analysis using LC-MS/MS. Secretome analysis was performed as described previously [53,54]**.** Briefly, fibroblasts were isolated from PLK2 wild type and knock out mouse and cultured in serum-free medium for 72 hours. Conditioned media were collected and concentrated using 3kD molecular weight cut off spin columns (Amicon, Millipore, Bedford, USA) and washed 5x with 25mM ammonium bicarbonate. The samples were denatured using 8M urea/ 2M thiourea and reduced by 10mM DTT. After alkylation with 50mM iodoacetamide, the samples were digested using trypsin (enzyme:protein=1:20) overnight. Digested peptides were purified using C18 spin plate (Harvard Apparatus, Holliston, USA). The eluted peptides were resuspended in LC solution (2% acetonitrile, 0.05% TFA) and 1 µg was injected and separated by reverse phase nano-flow HPLC (Dionex UltiMate 3000

RSLCnano, Acclaim PepMap100 C18 column, 75um x 50cm, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The nanoflow mobile phases consisted of HPLC grade water containing 0.1% formic acid (mobile phase A) and acetonitrile/HPLC grade water (80:20, v:v) containing 0.1% formic acid (mobile phase B). The following gradient was run at 250nL/min: 0-10 min, 4-10% B; 10-75 min, 10-30% B; 75-80 min, 30-40% B; 80-85 min, 40-99% B; 85-89.8 min, 99% B; 89.8-90 min, 99-4% B; 90-120 min, 4%B. The nano column was coupled to a nanospray source (Picoview, New Objective, Woburn, USA). Spectra were collected from a Q Exactive HF (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in positive ion mode using Full MS resolution 60,000 (at 200 m/z), scan range 350 to 1600 m/z. Data-dependent MS/MS was performed using higher-energy collisional dissociation (HCD) fragmentation on the 15 most intense ions with resolution 15,000 and dynamic exclusion enabled. Raw files were searched against UniProt/SwissProt Mouse and Bovine database (version 2016_02, 22763 protein entries) using Proteome Discoverer 1.4.1.14. The mass tolerance was set at 10 ppm for the precursor ions and at 20 mmu for fragment ions. Carbamidomethylation of cysteine was set as a fixed modification, oxidation of methionine, proline and lysine was set as variable modifications. Two missed cleavages were allowed. Search result files were loaded into Scaffold software (version 4.3.2) and validated with the following filter: peptide probability > 95% and protein probability > 99% with minimum 2 peptides. Total spectra count was used as quantitative value.

Human OPN assay. The human OPN ELISA kit was purchased from Abcam (Abcam, Cambridge, UK) and used according to the manufacturer's instructions to detect the concentration of OPN in the peripheral blood of control SR controls and AF patients.

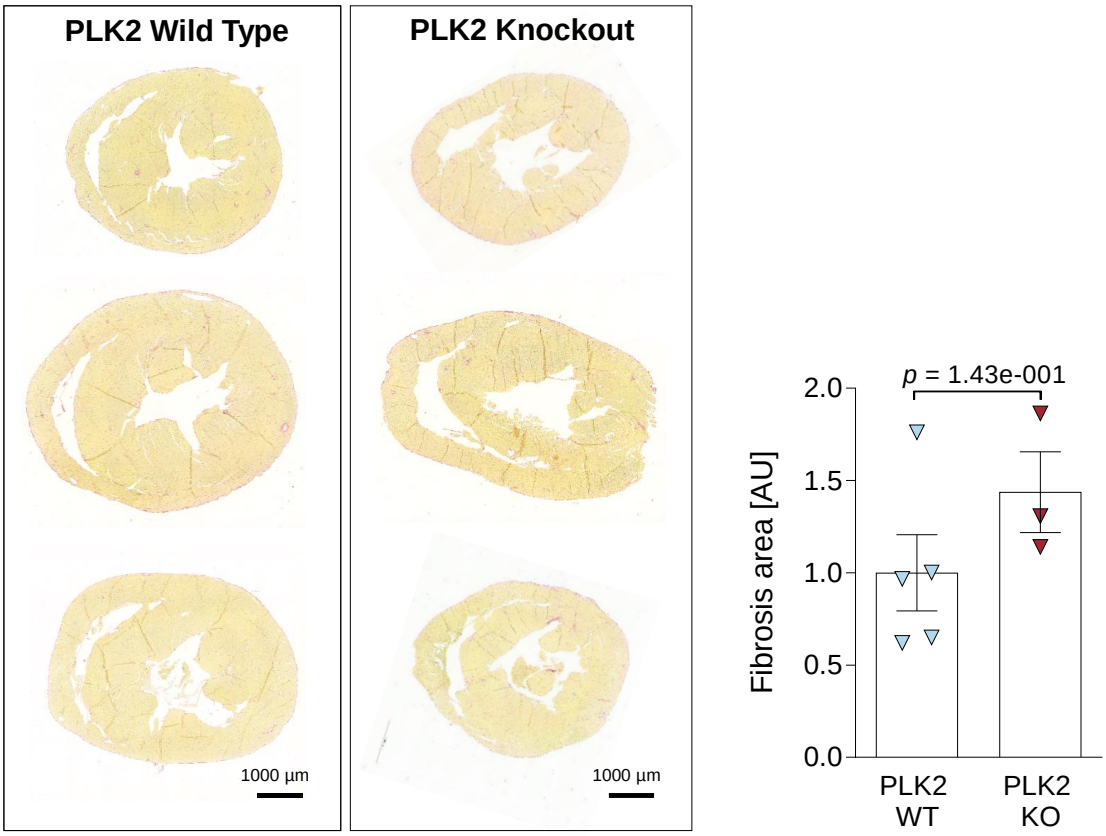
Echocardiography. Echocardiography was performed as reported previously [55]. Briefly, animals were kept under light anesthesia (isoflurane, 1.5% v/v) while echocardiographic images were obtained on a Vevo 3100 System (FUJIFILM Visual Sonics Inc., Toronto, Canada) in the parasternal long axis view and a short axis view at midpapillary and apical muscle level. Three PLK2 WT mice have been excluded from echocardiographic analysis due to poor technical quality of the images.

Electrophysiological study in the isolated heart. Electrophysiological studies on isolated PLK2 WT and KO hearts were performed as described previously [56,57]. Murine hearts were rapidly extracted under deep terminal anaesthesia by inhalation of 5% isoflurane with an O2 flow rate of 3 L/min under heparinisation; the aorta was cannulated, retrogradely perfused and mounted on a vertical Langendorff-apparatus (Hugo Sachs/Harvard Apparatus, Germany) for coronary perfusion with Krebs-Henseleit buffer aiming for constant aortic pressure (100 ± 5 mmHg), coronary flow rate (4 ± 1 mL/min) and temperature (36 ± 1 °C). After 10 min of perfusion, stimulation was conducted via an octapolar EP-catheter, inserted through a small

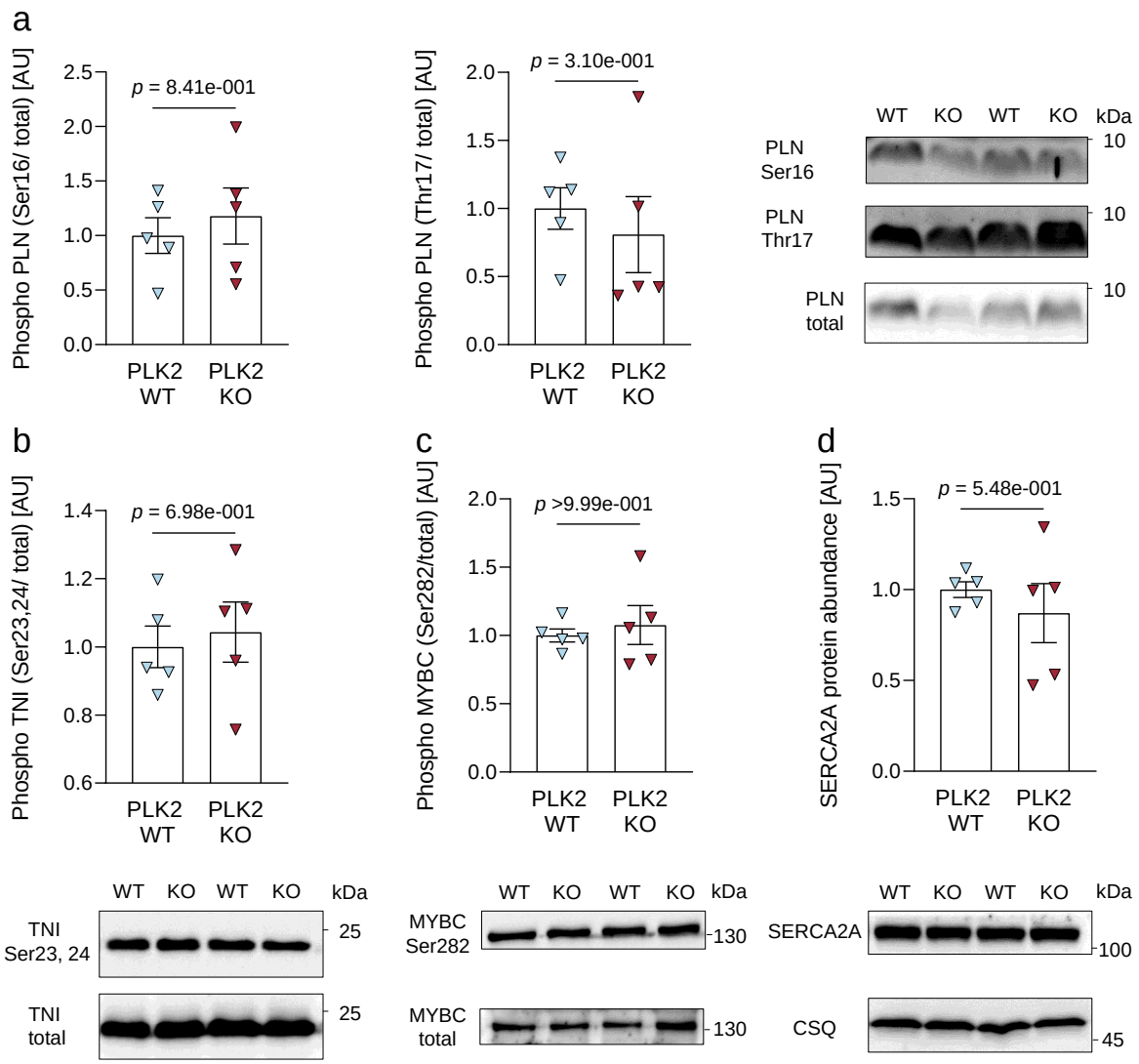
hole in the right atrium and monophasic action potentials (MAPs) were recorded using epicardial spring-loaded MAP-catheters. Pre-amplified (Model 2000, EP Technologies Inc, California) MAP signals were analyzed semi-automatically using a commercially available software (ecgAUTO, EMKA Technologies, France). MAP signals were subjected to manual quality control using established criteria [58]. The right atrial septum was paced at constant cycle length (80, 100, or 120 ms) for 60 sec with S1-stimuli for action potential duration (APD) assessment. Effective refractory period (ERP) was determined using trains of 8 S1-stimuli at 80, 100 or 120 ms cycle length followed by a single early S2-stimulus with an initial coupling interval of 50 ms, incrementally decreased by 1 ms. ERP was defined as the shortest S2-coupling-interval still provoking left atrial depolarization.

Calcium dynamics. Primary cardiomyocytes were isolated from of PLK2 WT and KO as well as C57B6J mice. To assess the effects of pharmacological PLK2 inhibition, the specific PLK2 inhibitor TC-S 7005 was applied at 100 nM throughout the isolation process and during the subsequent experiments. Freshly isolated atrial and ventricular myocytes were plated on laminin-coated cover slips (2.5 cm) and loaded with 5 µM Fura-2 AM (25 min, 37°C). After washing, cardiomyocytes were field stimulated (20 V, 10 ms at 35°C, MyoPacer MYP100). Fura-2 ratio was measured at 340 and 380 nm using an IonOptix system. An adapted protocol to that described in Vettel et al., 2017, was implemented to measure Ca^{2+} transients as well as SR Ca^{2+} leak. After reaching steady state, Ca^{2+} transients were measured at 1Hz. Pacing at 2 Hz (30 sec) was applied to detect SR Ca2+ leak and spontaneous Ca^{2+} waves (sCaWs). Therefore, normal Tyrode bath solution was substituted by $0Na^{+}/0Ca^{2+}$ Tyrode containing Li^{+} and the RyR2 inhibitor Tetracaine (1 mM, 30 sec) to exclude trans-sarcolemmal and intracellular SR Ca^{2+} fluxes. To detect SR Ca^{2+} leak, tetracaine was washed out for 30 sec with $0Na^{+}/0Ca^{2+}$ Tyrode allowing SR Ca^{2+} fluxes. SR Ca^{2+} leak and sCaWs were quantified during this period. Finally, 10 mM caffeine was applied to release SR Ca^{2+} stores in order to evaluate the total SR Ca^{2+} content.The analysis was performed using the IonWizard 6.5 Monotonic Transient Analysis Tool and Fura 2 ratios were filtered (Butterworth Lowpass, Cutoff frequency: 100.000, Number of Poles: 2) after background signal subtraction. Ca^{2+} transient amplitudes were measured as the difference between peak systolic and end diastolic ratios ($\Delta F_{trans} = F_{0,\,systolic} - F_{0,\,diastolic}$). Transient properties were determined upon assessing transient peak velocity ($\Delta F_{trans}/\Delta t$) and transient decay ($\tau$ of sin exponential fit). SR Ca^{2+} leak was measured as the area under the curve of the fluorescence ratio during the 30 sec tetracaine wash out period (AUC $F_{0,\,tetra}$:$F_{0,\,wash}$) and number of sCaWs was quantified. Finally, SR Ca^{2+} load was determined as the amplitude of the caffeine-induced twitch and the fractional release was calculated by dividing the ΔF_{trans} (2 Hz) by the caffeine-induced transient amplitude reflecting the total SR Ca^{2+} content and fraction of Ca^{2+} released from the SR during a paced contraction.

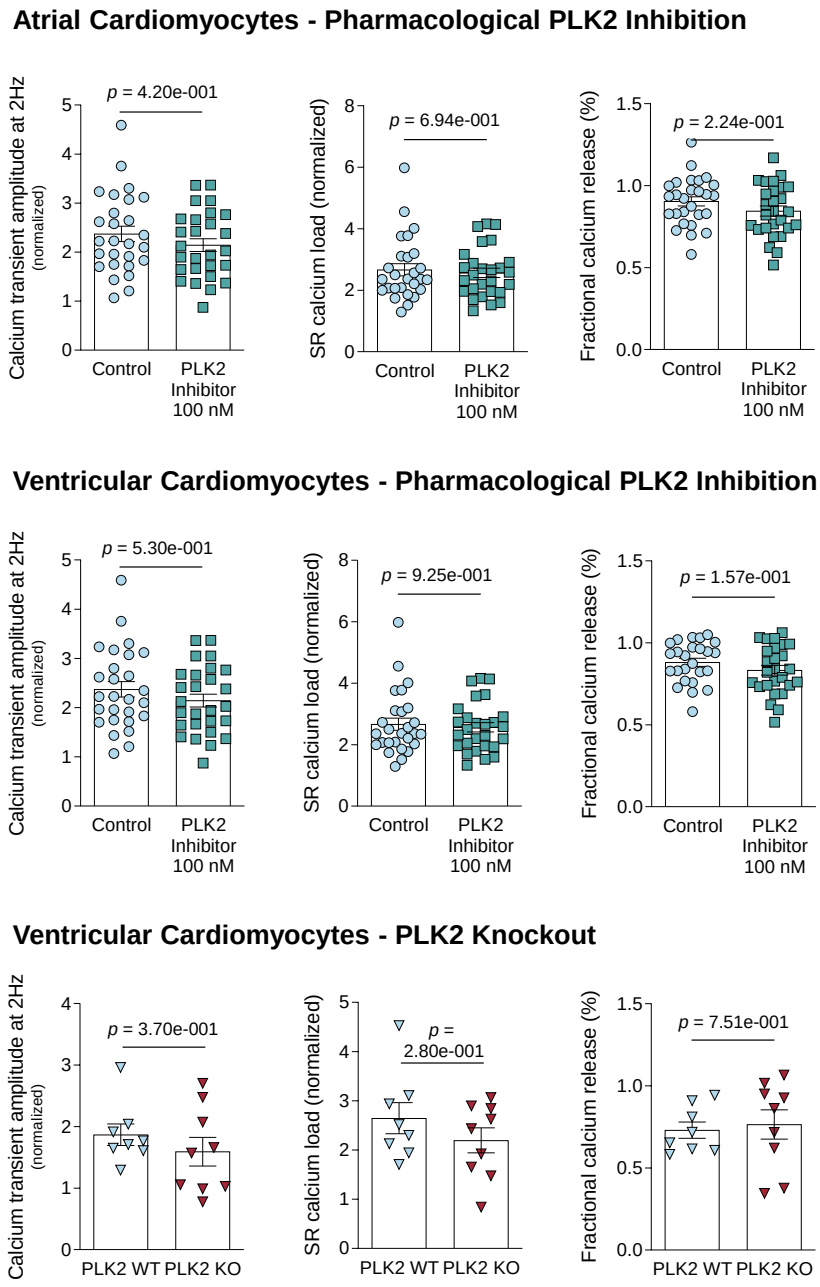
Mesalazine treatment. Male and female PLK2 KO mice were randomly assigned to control and treatment group. Treatment was performed with 100 µg/g body weight mesalazine (5-amino salicylic acid) dissolved in drinking water for 6 months starting at 7±2 weeks of age. The water was protected from light by tinfoil wrapping and changed every Monday, Wednesday and Friday. Mice were weighed every other week to adjust the mesalazine dose.



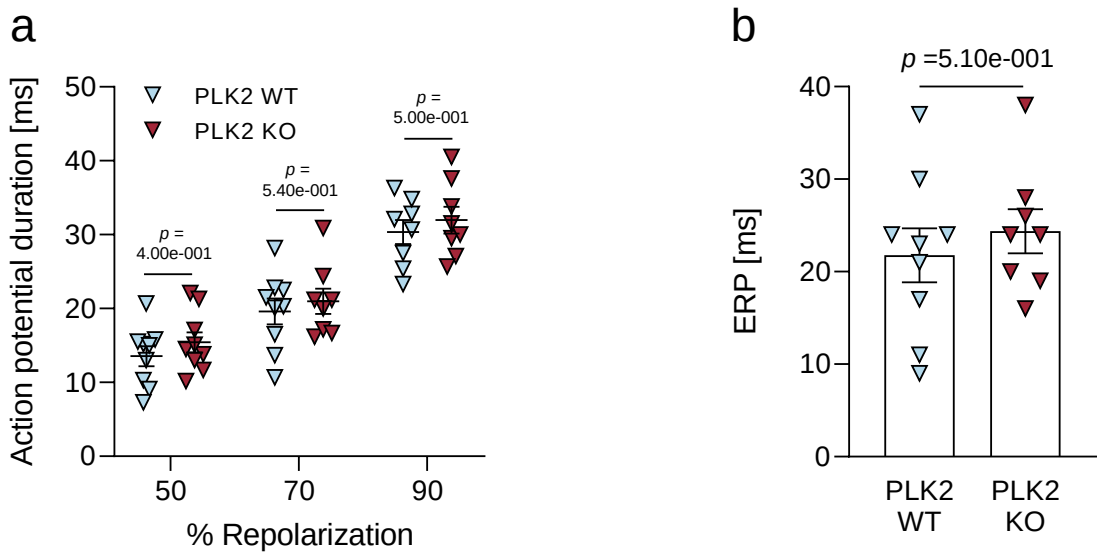
Online Figure I. Sirius red staining of ventricular sections of 4 months-old PLK2 WT and KO mice. a) Histological sections of murine hearts stained with sirius red. Collagen is stained in red. Microscopic pictures were taken along the short axis at mid-ventricular level. **b)** Quantification of fibrosis with ImageJ software (WT (n = 5) KO (n=3); Stated is the mean ± SEM; *p* was determined by Mann Whitney test.). AU: arbitrary units – red (fibrotic) area normalized to the total area of the respective section and to PLK2 WT control.



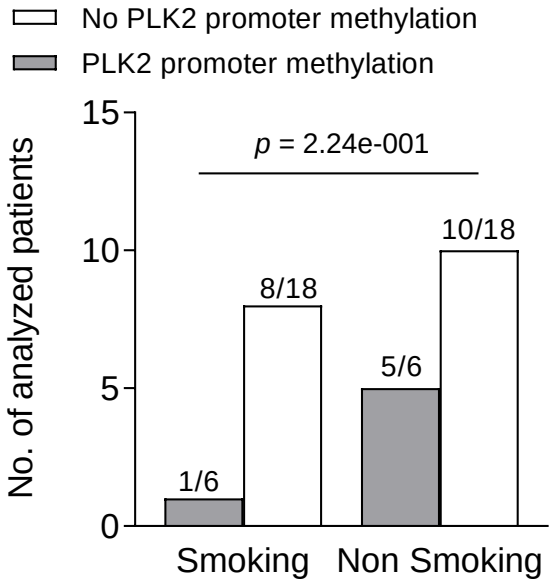
Online Figure II. Quantification and representative western blot images of **a)** phospholamban (PLN) phosphorylation (WT (n = 5) KO (n=5); Stated is the mean ± SEM; *p* was determined by Mann Whitney test), **b)** Troponin I (TNI) phosphorylation (WT (n = 5) KO (n=5); Stated is the mean ± SEM; *p* was determined by Mann Whitney test), **c)** myosin binding protein c (MYBC) phosphorylation (WT (n = 5) KO (n=5); Stated is the mean ± SEM; *p* was determined by Mann Whitney test), and **d)** SERCA protein expression normalized to calsequestrin (CSQ) (WT (n = 5) KO (n=5); Stated is the mean ± SEM; *p* was determined by Mann Whitney test). AU: arbitrary units – relative protein expression normalized to the indicated housekeeping protein and the respective control group.



Online Figure III. Calcium handling in primary atrial and ventricular murine cardiomyocytes. Calcium transient amplitude (at 2 Hz), sarcoplasmic reticulum calcium load and fractional calcium release in **a)** atrial cardiomyocytes of male C57B6N mice (Control (A1, A2, A3: n=25, N=3 animals), PLK2-Inhibitor (A1, A2, A3: n=25, N=3 animals); Results are given as mean ± SEM. *p*-values were determined by a hierarchical model and **b)** ventricular cardiomyocytes of male C57B6N mice in the absence and presence of 100 nM of the selective PLK2 inhibitor TC-s 7005 (Control (B1, B2, B3: n=27, N=3 animals), PLK2-Inhibitor (B1, B2, B3: n=27; N=3 animals); Results are given as mean ± SEM. *p* was determined by a hierarchical model (for SR calcium load (mid panel) log-transformed data was used for normality reasons). **c)** Similar experiments in ventricular cardiomyocytes of male PLK2 WT and KO mice (Control (C1-C3: n=8, N=1 animal), KO (C1-C3: n=9, N=1 animal); Results are given as mean ± SEM. *p* was determined by a hierarchical model. Normalized expression: unit-less result resulting from normalization of treatment or PLK2 KO group to the respective control.



Online Figure IV. Analysis of left atrial monophasic action potentials in Langendorff hearts during right atrial pacing at 100 ms cycle length. a) Action potential duration for implicated % of repolarization (WT n=9), KO (n=9); Results are given as mean ± SEM determined by multiple t-tests with Two-stage step-up method of Benjamini, Krieger and Yekutieli). **b)** Effective refractory period (ERP) determined during S1-S2 pacing protocol. (WT n=9), KO (n=8); Results are given as mean ± SEM, *p* was determined by an unpaired t test).



Online Figure V. Analysis of PLK2 promoter methylation and smoking status. The patients' smoking status and the presence of PLK2 promoter methylation (see Figure 2a) was analyzed by chi-square test. N=24; n (smoking) = 9; n (non smoking) = 15; n (PLK2 promoter methylation) = 6; n (No PLK2 promoter methylation) = 18).

Online Table IV: Patient data for cell culture, western blot and methylation-specific PCR

	SR (n = 40)	**AF (n = 34)**	***p-value***
Average age [mean ± SD, years]	68.8 (± 7.7)	72.7 (± 6.5)	0.02*
Gender			
(Male)	31	24	0.63#
(Female)	9	10	0.49#
Average BMI [mean ± SD, kg/m²]	29.9 (± 4.3)	28.7 (± 6.3)	0.47*
Disease			
Hypertension	38	25	0.009#
Diabetes mellitus	14	11	0.81#
Hyperlipidemia	20	10	0.07#
Chronic kidney disease (GFR 40 - 90)	11	7	0.49#
Chronic lung disease	7	5	0.75#
Current smoking	19	4	0.0009#
Alcohol addiction	1	0	0.35#
OSAS[1]	3	2	0.78#
Diagnosis			
CABG[2]	28	7	0.0001#
Valvular replacement	19	16	0.96#
Ablation	0	13	0.0001#
Echocardiography			
Ejection fraction [mean ± SD, %]	55.8 (± 11.4)	50.6 (± 10.7)	0.15*
Left ventricular hypertrophy	12	7	0.45#
Drugs			
ACE inhibitors	22	14	0.23#
AT_1 receptor blockers	6	4	0.68#
β-AR blockers	23	29	0.009#

[1] Obstructive sleep apnea syndrome
[2] Coronary artery bypass graft

Calcium channel blockers	6	2	0.21[#]
Antiarrhythmic drugs	0	2	0.11[#]
Glycosides	0	13	0.0001[#]
Statin	27	11	0.005[#]
Allopurinol	2	5	0.15[#]
Diuretics	14	11	0.96[#]
Aldosterone inhibitor	1	3	0.23[#]
Oral anticoagulants	3	14	0.0006[#]
Antidepressant	2	1	0.65[#]
Oral antidiabetic drugs	7	6	0.99[#]
α-AR blocker	2	2	0.87[#]
PPI[3]	2	3	0.51[#]
ASS	23	6	0.0005[#]
Insulin	2	2	0.87[#]

* Welsh's t-test; [#] Chi squared test

[3] Proton-pump inhibitors

Online Table V: Patient data for real-time PCR

	SR (n = 7)	**AF (n = 5)**	***p-value***
Average age [mean ± SD, years]	68.0 (± 8.5)	69.2 (± 9.8)	0.83*
Gender			
(Male)	7	4	0.21#
(Female)	0	1	0.22#
Average BMI [mean ± SD), kg/m²]	28.0 (± 2.8)	30.2 (±3.1)	0.23*
Disease			
Hypertension	6	5	0.38#
Diabetes mellitus	1	1	0.79#
Hyperlipidemia	6	4	0.38#
Chronic kidney disease (GFR 40 - 90)	2	2	0.68#
Chronic lung disease	0	1	0.21#
Current smoking	3	3	0.56#
Epilepsy	1	0	0.38#
OSAS[4]	1	0	0.38#
Diagnosis			
CABG[5]	7	3	0.06#
Valvular replacement	2	5	0.01#
Ablation	0	3	0.01#
Echocardiography			
Ejection fraction [mean ± SD), %]	45.9 (± 16.6)	38 (± 9.1)	0.32+
Left ventricular hypertrophy	2	2	0.68#
Drugs			
ACE inhibitors	5	4	0.58#
AT_1 receptor blockers	2	1	0.74#
β-AR blockers	6	5	0.38#

[4] Obstructive sleep apnea syndrome
[5] Coronary artery bypass graft

Nitrates	1	1	0.79[#]
Calcium channel blockers	2	3	0.27[#]
Antiarrhythmic drugs	0	1	0.22[#]
Glycosides	1	2	0.31[#]
Statin	6	4	0.79[#]
Diuretics	2	4	0.04[#]
Aldosterone inhibitor	0	1	0.21[#]
Oral anticoagulants	1	3	0.10[#]
Antidepressant	1	1	0.79[#]
Oral antidiabetic drugs	1	1	0.79[#]
PPI[6]	2	1	0.74[#]
ASS	4	3	0.55[#]

* Mann Whitney Test; [#] Chi squared test

[6] Proton-pump inhibitors

Online Table VI: Patient data for the human osteopontin ELISA

	Healthy (n = 4)	**AF without LVZs [7] (n = 8)**	**AF with LVZs (n = 9)**	***p-value***
Average age [mean ± SD, years]	54 (± 5.2)	71.3 (± 11.9)	71.4 (± 8.7)	0.03*
Gender				
Male	2	5	5	0.91#
Female	2	3	4	0.91#
Disease				
Hypertension	1	8	9	0.0006#
Diabetes mellitus	0	4	3	0.223#
Hyperlipidemia	0	5	5	0.10#
Chronic kidney disease (GFR 40 - 90)	0	2	3	0.43#
Chronic lung disease	0	1	2	0.56#
Thyroid disease	0	0	2	0.23#
Adipositas	0	1	0	0.43#
Current smoking	0	1	1	0.77#
Atrial fibrillation characteristics				
Persistent AF	0	4	7	0.03#
Paroxysmal AF	0	3	1	0.21#
Atrial flutter	0	1	1	0.77#
Drugs				
ACE inhibitors	1	0	6	0.01#
AT_1 receptor blockers	0	5	1	0.02#
β-AR blockers	0	7	9	0.0003#
Calcium channel blockers (nifedipine)	0	2	4	0.25#
Calcium channel blockers (verapamil)	0	1	0	0.28#

[7] Low voltage zones

Antiarrhythmic drugs	0	0	3	0.43[#]
Glycosides	0	2	1	0.47[#]
Statin	1	5	5	0.46[#]
Allopurinol	0	1	3	0.31[#]
Diuretics	0	5	6	0.07[#]
Aldosterone inhibitor	0	1	1	0.77[#]
Oral anticoagulants	0	7	9	0.35[#]
Antidepressant	0	1	1	0.77[#]
Oral antidiabetic drugs	0	3	2	0.35[#]
α-AR blocker	0	1	0	0.43[#]
PPI[8]	1	7	9	0.01[#]
NSAID	0	2	1	0.54[#]
Insulin	0	3	1	0.21[#]
Osteopontin [mean ± SD, ng/ml]	9.65 (± 1.87)	16.78 (± 1.81)	25.99 (± 11.24)	0.001

* Kruskal Wallis Test with Dunn's posttest; [#] Chi squared test

[8] Proton-pump inhibitors

Online Table VII: Differentially regulated proteins from the fibroblast secretome analysis

Proteomics data was filtered for missing values, logarithmised and statistical significance was calculated with Bayesian statistics using the ebayes function of the limma package in R. Final *p*-values were corrected for multiple testing using the Benjamini Hockberg method.

#	Protein name	UniProt Accession No.	Molecular Weight	*p-Value*	Adjusted *p-value*	Number of identified spectra					
						KO 1	KO 2	KO 3	WT 1	WT 2	WT 3
1	Macrophage metalloelastase	MMP12_MOUSE	55 kDa	3.30E-06	**0.0015**	9	8	7	0	0	0
2	**Osteopontin**	OSTP_MOUSE	66 kDa	9.20E-05	**0.0213**	4	4	3	0	0	0
3	Glycine-tRNA ligase	SYG_MOUSE	82 kDa	0.0022	0.2013	3	2	2	0	0	0
4	Transcription elongation factor B polypeptide 1	ELOC_MOUSE	12 kDa	0.0022	0.2013	0	0	0	3	2	2
5	Properdin	PROP_MOUSE	50 kDa	0.0022	0.2013	3	2	2	0	0	0
7	40S ribosomal protein S3	RS3_MOUSE	27 kDa	0.0064	0.4963	5	4	5	3	2	2
13	Cathepsin S	CATS_MOUSE	38 kDa	0.0095	0.6286	10	5	8	3	2	4
6	A disintegrin and metalloproteinase with thrombospondin motifs 5	ATS5_MOUSE	102 kDa	0.0200	0.9643	7	9	8	12	12	14
10	Ribonuclease T2	RNT2_MOUSE	30 kDa	0.0249	0.9643	4	2	3	5	5	6
9	Glutaminyl-peptide cyclotransferase	QPCT_MOUSE	41 kDa	0.0309	0.9643	4	3	4	6	6	5
11	Lysosomal alpha-glucosidase	LYAG_MOUSE	106 kDa	0.0373	0.9643	9	5	6	10	11	11
12	Serotransferrin	TRFE_MOUSE	77 kDa	0.0429	0.9643	58	59	45	43	29	33
8	Protein disulfide-isomerase A6	PDIA6_MOUSE	48 kDa	0.0464	0.9643	17	17	15	10	11	13

Online Table VIII: Echocardiographic parameters of PLK2 deficient and WT mice at 8 months. (Figure 6)

	WT (n = 7)		KO (n = 8)		
Atrial parameters	Mean	SD	Mean	SD	p-value (unpaired Welsh's t-test)
Diastolic LA Area [mm^2]	9.30	1.2	11.84	2.2	0.010
Systolic LA Area [mm^2]	7.26	1.6	9.34	2.0	0.024
Ventricular parameters					
HR [bpm]	401.50	21.74	389.13	46.71	0.478
Diastolic Anterior Wall Thickness [mm]	0.43	0.11	0.37	0.07	0.157
Systolic Anterior Wall Thickness [mm]	0.72	0.08	0.64	0.05	0.066
Diastolic Posterior Wall Thickness [mm]	0.38	0.09	0.34	0.10	0.423
Systolic Posterior Wall Thickness [mm]	0.67	0.14	0.54	0.12	0.095
Fractional Shortening [%]	22.14	4.51	20.49	5.68	0.552
Fractional Area Shortening [%]	39.06	4.57	41.21	5.60	0.486
Systolic Volume [µl]	53.75	7.26	48.90	10.17	0.196
Diastolic Volume [µl]	97.54	9.94	89.43	11.98	0.056
Ejection Fraction [%]	44.86	5.22	45.60	6.11	0.880
Stroke Volume [µl]	43.78	6.88	40.53	6.55	0.181
Cardiac Output [ml/min]	17.61	3.12	15.82	3.46	0.164
LV Weight [mg]	71.25	12.69	60.69	11.75	0.064
LV Weight / Body Weight [mg/g]	2.53	0.14	2.53	0.32	0.991
Body Weight [g]	28.35	6.11	23.94	3.66	0.077

Online Table IX: Echocardiographic parameters of PLK2 KO. PLK2 WT and PLK2 KO + mesalazine mice at 8 months. (Figure 8)

	PLK2 WT (n = 5)		PLK2 KO (n = 7)		PLK2 KO + Mesa (n = 7)		Kruskal Wallis Test	Multiple Comparisons with Dunn's posttest		
	Mean	SD	Mean	SD	Mean	SD	p-value	WT *vs* KO	WT *vs* KO + Mesa	KO *vs* KO + Mesa
Atrial parameters										
Diastolic LA Area [mm^2]	8.07	0.65	10.35	1.08	8.67	1.28	0.004	0.01	>0.99	0.08
Systolic LA Area [mm^2]	6.10	0.30	7.69	1.13	7.13	1.58	0.06	0.07	0.28	>0.99
Ventricular parameters										
HR [bpm]	371.2	47.28	401.4	41.91	371.0	19.82	0.41	>0.99	>0.99	0.55
Diastolic Anterior Wall Thickness [mm]	0.45	0.12	0.46	0.15	0.32	0.08	0.13	>0.99	0.34	0.19
Systolic Anterior Wall Thickness [mm]	0.78	0.18	0.46	0.15	0.61	0.15	0.16	>0.99	0.33	0.26
Diastolic Posterior Wall Thickness [mm]	0.47	0.07	0.49	0.07	0.38	0.11	0.09	>0.99	0.52	0.09
Systolic Posterior Wall Thickness [mm]	0.74	0.18	0.72	0.10	0.65	0.23	0.90	>0.99	>0.99	>0.99
Fractional Shortening [%]	25.44	14.32	25.11	4.92	19.14	4.54	0.17	>0.99	0.56	0.21
Fractional Area Shortening [%]	42.33	15.75	47.20	4.67	39.98	4.75	0.13	0.85	>0.99	0.14
Systolic Volume [µl]	49.91	17.38	36.08	10.42	49.71	10.70	0.11	0.21	>0.99	0.21

Diastolic Volume [µl]	97.44	17.19	73.47	18.71	88.21	12.38	0.09	0.10	>0.99	0.46
Ejection Fraction [%]	50	12	51	5	44	5	0.08	>0.99	0.81	0.08
Stroke Volume [µl]	47.53	7.02	37.39	9.78	38.49	3.34	0.10	0.22	0.14	>0.99
Cardiac Output [ml/min]	17.50	2.47	15.13	4.47	14.95	2.61	0.36	0.89	0.46	>0.99
LV Weight [mg]	74.84	12.90	67.33	11.53	60.70	12.69	0.19	>0.99	0.23	0.71
LV Weight / Body Weight [mg/g]	2.88	0.465	2.94	0.47	2.52	0.35	0.25	>0.99	0.59	0.35
Body Weight [g]	25.98	0.96	22.96	1.99	23.03	2.30	0.03	0.047	0.13	>0.99

Online Table X: Western blot antibodies

Antibody	**Usage**[9]	**Dilution**	**Origin**	**Supplier**
Primary antibodies				
GAPDH	WB	1 : 1.000	Mouse	Santa-Cruz. sc-365062
EEF2	WB	1 : 50.000	Rabbit	Abcam. ab40812
CSQ	WB	1 : 1.000	Rabbit	Abcam. ab3516
PLK2	WB	1 : 1.000	Rabbit	Cell Signaling. #14812
αSMA	WB. ICC	1 : 1.000 (WB) 1: 200 (ICC)	Mouse	Sigma-Aldrich. A5228
ERK1/2	WB	1 : 1.000	Rabbit	Cell Signaling. 9102
Phospho-ERK1/2 (Thr202/Tyr204)	WB	1 : 1.000	Rabbit	Cell Signaling. 9101
OPN	WB	1 : 1.000	Rabbit	Abcam. ab8448
FAPα	WB	1 : 1.000	Rabbit	Abcam. ab53066
Vimentin	WB	1 : 1.000	Rabbit	Abcam. ab137321
Collagen 1	ICC	1 : 200	Rabbit	Abcam. ab34710

[9] ICC: Immunocytochemistry; WB: Western Blot

Secondary antibodies				
Goat anti-mouse	WB	1 : 10:000	Peroxidase	Sigma-Aldrich. A3682
Goat anti-rabbit	WB	1 : 10.000	Peroxidase	Jackson ImmunoResearch. 111-035-045
Alexa fluor 546 (goat-anti-mouse)	ICC	1 : 400	Streptavidin	ThermoFisher. Z25004
Alexa fluor 546 (goat-anti-rabbit)	ICC	1 : 400	Streptavidin	ThermoFisher. Z25304

Online Table XI: RT-qPCR Primer

Species	**Target**	**Fwd**	**Rev**	**Supplier**
Homo sapiens	Plk2	N/A	N/A	qHsaCED0045278; BioRad
	Dnmt1	N/A	N/A	qHsaCED0044343; BioRad
	Dnmt3a	N/A	N/A	qHsaCID0010863; BioRad
Mus musculus	Opn	N/A	N/A	qMmuCED0040763; BioRad

Online Table XII: Contingency table for Figure 2a

		Heart Rhythm	
		SR	**AF**
PLK2 promoter methylation	**Yes**	0	6
	No	11	7

Online Table XIII: Contingency table for Online Figure 5

		Smoking	
		Yes	**No**
PLK2 promoter methylation	**Yes**	1	5
	No	8	10

Online Table XIV: Contingency table for Figure 7b

		Genotype	
		PLK2 WT	**PLK2 KO**
AF induction	**Yes**	0	4
	No	9	5