

1 Supplemental Materials and Methods

3 Animals

4 Mice with global deletion of PDE10A (on a C57/Bl6J background) were developed by Pfizer Inc.
5 C57/Bl6J and BALB/cAnNCr-nu/nu mice were ordered from Jackson Labs. All animal procedures
6 were performed in accordance with the National Institutes of Health (NIH) and approved by the
7 animal and care use committee of University of Rochester. For experiments with knockout mice,
8 control mice of the same age/sex from littermates or sibling mating were used.

10 Chronic DOX-induced cardiac dysfunction model

11 Male or female C57BL/6J PDE10A-wildtype (WT) or PDE10A-knockout (KO) mice at the age of
12 12 weeks were treated with DOX (4 mg/kg) on days 0, 7, and 14. Echocardiography was performed
13 prior to the DOX administration as well as 1 week after the last dose of DOX administration to
14 monitor the progression of cardiac structural and functional changes. There were no exclusions
15 among mice. Investigators followed standard laboratory procedures of randomization and the data
16 were analyzed in a blinded manner. Investigators were blinded to the genotypes of the individual
17 animals during the experiments and outcome assessments. Animal protocol was approved by
18 IACUC of University of Rochester.

20 Acute DOX-induced cardiac dysfunction model

21 Male or female C57BL/6J PDE10A-WT or PDE10A-KO mice were received pretreatment of TP-
22 10 (3.2mg/kg/day) or same volume of vehicle (10% DMSO in 40% β -cyclodextrin) via
23 subcutaneous injection before DOX treatment for 2 days via intra-peritoneal injection and then
24 continuously received the same treatment for another 5 days. TP-10 was dissolved in a vehicle
25 consisting of 10% DMSO and 40% β -cyclodextrin. After pretreatment mice received DOX
26 treatment (15mg/kg) in one bolus via intra-peritoneal injection for 7 days. There were no
27 exclusions among mice. Investigators followed standard laboratory procedures of randomization
28 and the data were analyzed in a blinded manner. Investigators were blinded to the genotypes of
29 the individual animals during the experiments and outcome assessments. Animal protocol was
30 approved by IACUC of University of Rochester.

32 Ovarian tumor xenograft model

33 We have tested nude mice with different ages (6-12 weeks) in our preliminary study. We found
34 that the tumor incidence in older mice is much lower compared with that in younger mice.
35 Therefore, we decided to use mice at 6 weeks old to enhance the engraftment rate, and thus, the
36 reproducibility of the studies. A2780 cells (5×10^6 cells) with expression of luciferase were
37 mixed with 50 μ L matrigel matrices. The mixture of cells was then injected into flanks of female
38 BALB/cAnNCr-nu/nu mice at the age of 6 weeks. It takes approximately 2-3 weeks for A2780
39 xenograft tumor to grow to a palpable size and TP-10 exerts a peak anti-tumor effect after two
40 weeks treatment based on tumor growth curve. Therefore, we lowered the dose of DOX to fit a
41 two-week regimen in this model for a better assessment of the effect of TP-10. 10 days post-
42 inoculation of the tumor cells, the animals were randomly divided into four groups and received
43 saline (intra-peritoneal injection), DOX (1.5mg/kg/day, intra-peritoneal injection), vehicle (10%
44 DMSO in 40% β -cyclodextrin v/v, subcutaneous injection), TP-10 succinate (4.5 mg/kg/day,
45 dissolved in a vehicle consisting of 10% DMSO and 40% β -cyclodextrin, subcutaneous
46 injection), or DOX plus TP-10 daily for 2 weeks. We increased the mass of TP-10 succinate

1 because its higher molecular weight (MW: 1301) compared to the free base form (MW: 1003).
2 Tumor development was monitored by Fluorescent Imager (IVIS Spectrum, Caliper Life
3 Sciences, Hopkinton, MA) once a week. Tumor sizes were measured twice weekly and tumor
4 volume was calculated by $ab^2/2$ where “a” and “b” are the long and short axes of tumor. Cardiac
5 function was evaluated via non-invasive echocardiography 1 day before cell injection, 1 day
6 before drug treatment, and at 1 and 2 weeks after drug treatment. Images of tumor were taken
7 and the final tumor weight was measured and recorded. Representative images were chosen
8 based on their quality and to most accurately represent the group average across all the available
9 data. The mice that did not develop tumors after tumor cells inoculation were excluded.
10 Investigators followed standard laboratory procedures of randomization and the data were
11 analyzed in a blinded manner. Investigators were blinded to the genotypes of the individual
12 animals during the experiments and outcome assessments. Animal protocol was approved by
13 IACUC of University of Rochester.

14 15 **Western Blot**

16 Heart tissue and A2780 xenograft lysates were prepared in ice cold RIPA buffer consisting of 50
17 mM Tris-Cl, pH 7.4, 150 mM NaCl, 1% (v/v) NP-40, 0.1% (w/v) SDS, 1 mM EDTA, pH 8.0, 1
18 mM PMSF, 1 mM dithiothreitol, 1 mM sodium orthovanadate and protease inhibitor mixture
19 (Roche). Cell membranes were removed by centrifugation for 25 min at 12000 g, 4 °C. An equal
20 amount of protein was loaded onto a 12% SDS-PAGE gel and transferred to PVDF membrane.
21 Blots were blocked in PBS/0.1% (v/v) Tween-20 (PBS-T) containing and probed with primary
22 antibodies in PBS-T containing 5% (w/v) bovine serum albumin (BSA) (Sigma). Blots were then
23 incubated with horseradish peroxidase-conjugated secondary antibody in PBS-T with 5% (w/v)
24 milk and developed using ECL detection reagent (GE). Normalization to the loading control was
25 performed only when the bands for the target protein and the loading controls are obtained from
26 the same blot. The average value of the control group was set at 1.0 (reference). The relative fold
27 change over the control was calculated relative to the reference. The representative image was
28 chosen based on their quality and to most accurately represent the group average across all the
29 available data.

30 31 **Bioluminescence imaging**

32 Nude mice were anesthetized using isoflurane. Inject mice with 150mg/kg D-Luciferin intra-
33 peritoneally 5 minutes before imaging. Mice were then placed inside the camera box of the IVIS
34 Spectrum imager with abdomen face down. Run sequential of the mice every 2 min until
35 luminescence saturation is reached. Representative images were chosen based on their quality
36 and to most accurately represent the group average across all the available data.

37 38 39 **Echocardiography**

40 Cardiac function was evaluated via non-invasive echocardiography. Mice were anesthetized via
41 continuous administration of inhaled isoflurane (2%). Echocardiography was monitored in
42 anesthetized mice using a Vevo3100 echocardiography machine equipped with an MS-550D 40-
43 MHz frequency probe (VisualSonics) to control for potential differences in heart rate and
44 neurohormonal tone between different mice. M-Mode echocardiography on short axis was used
45 to assess ventricular function. Fractional shortening was calculated by the following
46 formula: $\%FS = (LV \text{ end diastolic diameter} - LV \text{ end systolic diameter}) / (LV \text{ end diastolic diameter})$

1 diameter)*100%. Echocardiography was performed blindly by the core facility. Representative
2 M-mode echocardiographic images were chosen based on their quality and to most accurately
3 represent the group average across all the fractional shortening data.
4

5 **Mouse heart tissue harvesting and processing**

6

7 Mice were anesthetized via intraperitoneal injection of heparin and ketamine/midazolam before
8 euthanasia via cervical dislocation. Hearts were then perfused with saline/heparin and stopped
9 via 10mM KCl injection. Once removed from mice, representative images of the whole hearts
10 were taken. Representative images were chosen based on their quality and to most accurately
11 represent the group average across all the available HW/TL data. Heart weight was measured.
12 Hearts were then divided into three sections. The apex and base were flash frozen in liquid
13 nitrogen. Protein and mRNA expression were assessed in half of the apexes and half of the bases.
14 The center section was fixed with 4% (w/v) paraformaldehyde or methanol/acetic acid [60%
15 methanol and 10% acetic acid in H₂O (v/v)] for histological studies.
16
17

18 **Mouse adult cardiac myocyte isolation and culture**

19 Cardiac myocytes (CMs) were isolated as previously described ^{16, 17}. Briefly, mice were
20 anesthetized via intraperitoneal injection of heparin and ketamine/midazolam, and euthanized via
21 cervical dislocation. The heart was excised and washed briefly to remove blood and excess fat,
22 then was fastened onto a heart perfusion apparatus (Radnoti, Monrovia, California, with VWR
23 heating pump). Perfusion was initiated in the Langendorff mode. Hearts were perfused at 37°C
24 with a Ca²⁺-free Krebs-Henseleit based perfusion buffer (pH 7.4) containing: (120 mmol/l NaCl,
25 15 mmol/l KCl, 0.6 mmol/l Na₂HPO₄, 0.6 mmol/l KH₂PO₄, 1.2 mmol/l MgSO₄, 10 mmol/l HEPES,
26 10 mmol/l creatine monohydrate, 30 mmol/l Taurine, 5.6 mmol/l D-glucose, 4.6 mmol/l NaHCO₃,
27 10 mmol/l BDM, filtered at 0.40 μm). The heart was initially perfused for 3 minutes using
28 perfusion buffer, followed by calcium-free digestion buffer (perfusion buffer with 1.3 mg/ml
29 collagenase II) for 3 minutes, then digestion buffer with 28nmol/l CaCl₂ for 6-10 minutes.
30 Digestion was halted by removing the heart from the cannulating needle and placing it in 2.5mL
31 calcium containing digestion buffer with 5 ml stopping buffer (perfusion buffer with 10% FBS
32 and 12.5 nmol/L CaCl₂). The atria and right ventricle were then removed, and the left ventricle
33 was dissociated using forceps in a petri dish. 10 ml additional stopping buffer was then added to
34 the dissociated heart preparation, and it was filtered through 200 μm mesh into a 50 ml conical
35 tube. This tube was then incubated for 10 minutes at 37 °C followed by 10 minutes at room
36 temperature, allowing a pellet to form. The pellet with CMs was then resuspended in 10ml stopping
37 buffer. Calcium concentration in this buffer was then increased in a stepwise fashion, with 2
38 minutes between each step: first to 112.5 nmol/l, then to 512.5 nmol/l, finally to 1.4 μmol/l. CMs
39 were then visually inspected, and more than 60% of CMs retained rod-shaped morphology, was
40 used for experimentation. CMs were centrifuged at 500 g for 1 minute and resuspended in plating
41 medium (perfusion buffer with 2.5% FBS, 2% P/S, and 1.4 μmol/l CaCl₂) and then plated on dishes
42 coated with 9-10 μg/ml laminin, centrifuged at 500g for 1 minute, and incubated in a cell culture
43 incubator for 2-4 hours. The CMs were then washed twice with phosphate-buffered saline (PBS)
44 to remove dead cells and incubated in CM culture medium (MEM with 0.02% BSA, 10 mmol/l
45 HEPES, 4 mmol/l NaHCO₃, 10 mmol/l creatine, 0.5% insulin-selenium-transferrin, 10 mmol/l
46 BDM, pH 7.4) for 30 minutes to 1 hour before treatments. CMs were cultured in the presence of

1 blebbistatin (a myosin II inhibitor, 10 μ M) to block myocyte contraction and to extend their
2 survival during the culture¹⁸.

3 4 **Cardiac myocyte atrophy**

5 CMs were pre-treated with or without vehicle or the PDE10A inhibitor TP-10 (300 nM) in the
6 presence of vehicle or various reagents and stimulated with DOX (10 μ M) for 24 hours. For
7 morphometric analysis, CMs were fixed in 4% paraformaldehyde and stained with FITC-
8 conjugated WGA at 10 μ g/ml for 1 hour at room temperature to visualize the cell boundary.
9 Fluorescent images were taken at 10x magnification using an Olympus BX-51 fluorescent
10 microscope. Total myocyte surface areas were quantified using NIH Image J software. The average
11 value of the control group was set at 1.0 (reference). The relative fold change over the control was
12 calculated relative to the reference.

13 14 **Cell counting kit-8 assay**

15 Ovarian cancer A2780 cell viability was measured by using the CCK-8 cell counting kit according
16 to the manufacturer's instructions. In brief, cells were seeded into each well of a 96-well plate and
17 cultured in DMEM supplemented with 10% FBS and 1% P/S. After cell attachment, cells were
18 starved in DMEM supplemented with 1% P/S for 24 hours prior to treatment with TP-10 and DOX
19 for 24 hours. CCK-8 reagent (10 μ l) was added to each well (100 μ l medium/well), and the plates
20 were incubated at 37 $^{\circ}$ C. The absorbance of each well was measured at 450 nm using a microplate
21 reader every 1 hour for total 4 hours. The average value of the control group was set at 1.0
22 (reference). The relative fold change over the control was calculated relative to the reference.

23 24 **Gene knock down by shRNA vectors**

25 Lentiviral plasmid vectors encoding mouse PKG1 or scramble shRNA were generated by
26 VectorBuilder. Lentiviral plasmid vectors (6 μ g pLV-shRNA, 3 μ g pspAX2, 3 μ g pMD2G per
27 100mm well) were transfected in HEK293FT cells. Culture medium with viral particles was
28 harvested at 48 h and 72 h after plasmids transfection, filtered at 0.45 μ m and 0.22 μ m, and
29 concentrated by centrifuging at 20,000 rpm for 2 hours. The pallet with viral particles was
30 dissolved in 0.5-1 ml DMEM. CMs were respectively transfected with these lentiviruses for 24
31 hours and further experiments were then carried out. 48 hours after transfection, CMs were
32 harvested to detect the efficiency of gene silencing by real-time PCR.

33 34 **Real-time PCR analysis**

35
36 RNA was extracted from heart tissue, CMs or CFs using either RNeasy kit (Qiagen) or Trizol
37 (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized with
38 iScriptDNA synthesis kit (Bio-Rad). qPCR amplification was performed by using IQ SYBR Green
39 Supermix (Bio-Rad) according to the manufacturer's instructions. Each reaction was performed in
40 triplicate. Relative mRNA expression to that of housekeeping gene GAPDH was calculated. The
41 average value of the control group was set at 1.0 (reference). The relative fold change over the
42 control was calculated relative to the reference. Primers for qPCR were listed in the Major
43 Resource Table.

44 45 **DOX and TP-10 treatment**

46

1 1, To determine PDE10A protein levels upon DOX stimulation in mouse heart, male or female
2 C57BL/6J PDE10A-WT or PDE10A-KO mice at the age of 12 weeks were treated with vehicle or
3 PDE10A inhibitor TP-10 (3.2 mg/kg/day) subcutaneously 2 days prior to a single dose of saline
4 or DOX (15mg/kg), and continued for 1 week. PDE10A protein levels were measured by western
5 blotting in mouse tissues.

6
7 2, To determine the effects of DOX, TP-10 or both on PDE10A mRNA expression in human
8 ovarian cancer cell line A2780 and OCC1, A2780 were treated with vehicle, 300 nM TP-10, 1 μ M
9 DOX or combination of TP-10 and DOX for 6 hours. OCC1 were treated with vehicle, 3 μ M TP-
10 10, 1 μ M DOX or combination of TP-10 and DOX for 6 hours. mRNA was extracted for qPCR
11 analysis.

12
13 3, To determine cancer cell viability or proliferation, human ovarian cancer cell line A2780, human
14 colon cancer cell line HCT116, and human prostate cancer cell line C4-2 and PC-3 were treated
15 with vehicle, 300 nM TP-10, 1 μ M of DOX or combination of 300 nM TP-10 and 1 μ M of DOX
16 for 24 hours. human ovarian cancer cell line OCC1 were treated with vehicle, 3 μ M TP-10, 1 μ M
17 of DOX or combination of 3 μ M TP-10 and 1 μ M of DOX for 24 hours.

18
19 4, We did some preliminary studies, in which we treated CMs for different time points (6, 16 and
20 24h) to determine *Pde10a* expression, *Top2b* and atrophic marker gene expression. We found that
21 *Pde10a*, *Top2b* and atrophic marker gene expression peaks around 6 h. Therefore, we chose 6 h to
22 look at mRNA expression. To determine *Pde10a*, *Top2b*, *Foxo3*, *Mafbx* mRNA expression in CM,
23 CMs isolated from PDE10A-WT or PDE10A-KO mice were stimulated with or without DOX for
24 6 hours. mRNA was extracted for qPCR analysis.

25
26 5, To determine the role of PDE10A, PKA, or PKG on DOX-stimulated CM death, DNA damage,
27 mitochondria dysfunction and atrophy, and to determine the role of PDE10A and DOX on CM
28 PKA and PKG activity, CMs isolated from PDE10A-WT or KO mice were treated with or without
29 vehicle or the PDE10A inhibitor TP-10 (300 nM) in the presence of vehicle or PKA inhibitor PKI
30 (5 μ M), vehicle or Epac inhibitor ESI09 (5 μ M), vehicle or 2 PKG inhibitors Rp-8-Br-PET-cGMPs
31 (10 μ M) and DT-2 (1 μ M), and stimulated with DOX (10 μ M) for 24 hours

32
33 6, To examine the effect of PDE10A deficiency on DOX-induced CM death, we injected PDE10A-
34 WT or PDE10A-KO mice with vehicle or DOX (25 mg/kg, intraperitoneal (i.p.)) once for 16 h.
35 CMs were isolated and cultured for 24 hours to test cell death.

36
37 7, To examine the effect of PDE10A deficiency on DOX-induced mitochondria dysfunction, we
38 injected PDE10A-WT or PDE10A-KO mice with vehicle or DOX (25 mg/kg, intraperitoneal (i.p.))
39 once for 16 h and removed the hearts for determining the expression of genes involved in
40 mitochondria function.

41
42 8, To determine *Pde10a*, *Top2b*, *Foxo3*, *Mafbx* mRNA expression in mouse hearts, PDE10A-WT
43 or PDE10A-KO mice were treated with saline or DOX (15mg/kg, one bolus) for 1 week, mRNA
44 was extracted for qPCR analysis.

45 46 **Immunofluorescence staining**

1 Cells were treated with various reagents. After treatment, cells were then fixed in 4%
2 paraformaldehyde for 20 minutes, followed by permeabilizing and blocking in PBS/0.1% (v/v)
3 Triton-100/10% (v/v) goat serum for 1 hour. Cells were incubated with or without primary
4 antibody (1:200-1:500) diluted in PBS/0.1% (v/v) Triton-100/2% (v/v) goat serum overnight at 4
5 °C. After three PBST washes, cells were then incubated in fluorescent secondary antibody
6 (1:500) diluted in PBS/0.1% (v/v) Triton-100/2% (v/v) goat serum for 1 hour at room
7 temperature following three times PBST washes. Nuclei were counterstained with 4',6-
8 diamidino-2-phenylindole (DAPI). Staining was visualized with an Olympus BX-51 microscope
9 or Olympus IX-81 confocal microscope. Representative images were chosen based on their
10 quality and to most accurately represent the group mean/average across all the available data.
11

12 **Histological analysis**

13 Heart tissues were fixed with methanol/acetic acid [60% methanol and 10% acetic acid in H₂O
14 (v/v)], then were paraffin embedded and transversely sectioned (5 µm).
15

16 For evaluation of cardiac structure, heart tissue slices were deparaffinized and stained by
17 hematoxylin and eosin (H&E). Nucleus was labeled by hematoxylin and cytoplasmic content
18 was stained by eosin. The representative image was chosen based on their quality and to most
19 accurately represent the group average across all the available HW/TL data.
20

21 For analysis of the cell surface area, heart sections were deparaffinized. Heat-induced epitope
22 retrieval (HIER) was performed by boiling deparaffinized sections in 10 mM citrate buffer
23 (pH=6.0). Sections were then incubated in a serum-free protein blocking solution (DAKO) for 1
24 hour and stained with FITC-conjugated WGA at 10 µg/ml for 2 hours at room temperature to
25 visualize cell membrane. Myocyte cell surface area were quantified by using NIH ImageJ
26 software. For quantification, cell surface areas were averaged from n = 300-800 myocytes from
27 2-3 levels (1000 µm between levels) per heart, 2 serial heart sections per level. Each group
28 contains 3-15 hearts. Representative images were chosen based on their quality and to most
29 accurately represent the group mean across all the available data.
30

31 For analysis of cell apoptosis. The terminal deoxynucleotidyl transferase dUTP-mediated nicked
32 end labeling (TUNEL) stain (Roche) was used. Deparaffinized sections were permeabilized and
33 blocked in PBS/0.1% (v/v) Triton-100/10% (v/v) goat serum for 1 hour. 50 µL TUNEL reaction
34 mixture was added to each section and incubated at 37°C for 1 hour. Nuclei were counterstained
35 with DAPI. Staining was visualized with an Olympus BX-51 microscope. The ratio of cell
36 apoptosis was expressed as the percentage of TUNEL staining positive cells versus DAPI
37 staining positive cells. The representative image was chosen based on their quality and to most
38 accurately represent the group average across all the available data.
39

40 For analysis of cardiac fibrosis. Heart sections were deparaffinized. Picro-Sirius Red staining
41 was performed according to the manufacture's instruction. Briefly, deparaffinized sections were
42 hydrated in distilled water. Adequate Picro-Sirius Red Solution was applied to completely cover
43 the heart section for 1 hour. Sections were rinsed quickly in 2 changes of acetic acid solution
44 prior to mounting in absolute alcohol. Slides were then hydrated in 2 changes of alcohol
45 followed by mounting in synthetic resin. Each heart section was scanned by Evos Fl Auto
46 Imaging System (Thermo Fisher Scientific). Total fibrotic area in each section were quantified

1 by using NIH ImageJ software. The representative image was chosen based on their quality and
2 to most accurately represent the group average across all the available data.

3 4 **RNA sequencing**

5
6 Male or female C57BL/6J PDE10A-WT or PDE10A-KO mice at the age of 12 weeks were treated
7 with a single dose of saline or DOX (15mg/kg), and continued for 1 week. We then excised hearts
8 and extracted mRNA for RNA sequencing. Raw reads generated from the Illumina HiSeq2500
9 sequencer were demultiplexed using bcl2fastq version 2.19.0. Quality filtering and adapter
10 removal are performed using Trimmomatic version 0.36 with the following parameters:
11 "TRAILING:13 LEADING:13 ILLUMINACLIP:adapters.fasta:2:30:10
12 SLIDINGWINDOW:4:20 MINLEN:15"⁶⁶. Processed/cleaned reads were then mapped to the *Mus*
13 *musculus* reference genome (GRCm38 + Gencode-M12 Annotation) using STAR_2.5.2b with the
14 following parameters: "--twopass Mode Basic --runMode alignReads --outSAMtype BAM
15 SortedByCoordinate --outSAMstrandField intronMotif --outFilterIntronMotifs
16 RemoveNoncanonical --outReads UnmappedFastx"⁶⁷. Gene-level read quantification was derived
17 using the subread-1.5.0p33 package (featureCounts) with a GTF annotation file (Gencode M12)
18 and the following parameters: "-s 2 -t exon -g gene_name"⁶⁸. Differential expression analysis was
19 performed using DESeq2-1.14.1 with a P-value threshold of 0.05, adjusted by Benjamini &
20 Hochberg multiple test correction within R version 3.3.2 (<https://www.R-project.org/>)⁶⁹. A PCA
21 plot was created within R using the pcaMethods package given the rLog transformed expression
22 values⁷⁰. Heatmaps were generated within R using the pheatmap also given the rLog transformed
23 expression values. Gene set enrichment analysis (GSEA)⁷¹ was performed using the R
24 clusterProfiler package⁷² based on the Kyoto Encyclopedia of Genes and Genomes (KEGG)
25 pathway gene set⁷³. The genes were ranked by $-\log_{10}(\text{p-value})$ with the sign of corresponding
26 $\log_2(\text{fold-change})$ from the differential expression analysis.

27 28 **Measurement of plasma cardiac troponin-I**

29 Plasma cardiac troponin-I was measured with the mouse cardiac troponin-I Elisa kit (Life
30 Diagnostics, PA, USA) to evaluate cardiac injury. The measurement was performed following the
31 manufacturer's instruction. Standard troponin-I solution or plasma samples were incubated with
32 HRP-conjugate in pre-coated plate at room temperature for 1 hour on a plate shaker. Empty and
33 wash plate for five times with washing solution. TMB solution was added into each well and
34 incubated at room temperature for 20 min. Stopping solution was added into each well to stop
35 reaction. Read the absorbance at 450nm in a plate reader. The results were calculated by standard
36 curve.

37 38 **Trypan blue cell viability staining**

39 In order to measure cell death, the trypan blue stain was performed as described previously¹⁷. In
40 brief, 1000-2000 CMs were seeded on 35mm glass coverslip of glass bottom dish with 1.5 mL
41 culture medium. CMs were pre-treated with various test compounds, followed by DOX stimulation
42 for 24 hours. After that, medium was collected for LDH cytotoxicity assay. The cells were then
43 quickly incubated with trypan blue solution (final concentration of 0.2%) for 5 minutes, and then
44 were counted under a phase-contrast inverted microscope, 20 fields in every dish was randomly
45 selected for quantification. The dead cells were expressed as percentage of the total counted cells.

1 Representative images were chosen based on their quality and to most accurately represent the
2 group average across all the available data.

4 **LDH cytotoxicity assay**

5 LDH released in CMs was determined using the Cytotoxicity Detection Kit (Roche) according to
6 the manufacturer's instruction. In brief, 1000-2000 CMs were seeded on 35mm glass coverslip of
7 glass bottom dish with 1.5 mL culture medium. CMs were treated with various test compounds
8 and stimulated with or without DOX as indicated. 24 h after treatment, culture medium was
9 collected. Besides test samples, one positive control culture in medium with 1% Triton X-100 and
10 one negative control culture in medium without any test compounds or Triton X-100 should be
11 included. Medium was transferred into each well of a 96-well plate (100 µl/well). Add 100 µl
12 reaction mixture (freshly prepared) to each well and incubate for up to 30 min at room temperature.
13 Released LDH in the culture media was coupled to an enzymatic assay yielding a red color, the
14 intensity of which was measured at 490 nm by an ELISA microplate reader. The percentage
15 cytotoxicity expressed as percent release of LDH was determined relative to controls as described
16 by the manufacturer. The average value of the control group was set at 1.0 (reference). The relative
17 fold change over the control was calculated relative to the reference.

19 **Apoptosis quantification using annexin V/PI staining**

20 Apoptosis was assessed following the instruction manual of Annexin V-FITC Apoptosis Detection
21 Kit. Annexin V is a protein that binds phosphatidylserine residues exposed on the surface of
22 apoptotic cells. After treatment, A2780 cells were trypsinized, rinsed twice with PBS, re-
23 suspended in 1x binding buffer and labeled with FITC-conjugated Annexin V and propidium
24 iodide. Samples were immediately analyzed with Flow cytometer (BD Accuri C6). The Annexin
25 V-FITC⁻/PI⁻ cell population was considered normal, whereas the Annexin V-FITC⁺/PI⁻ and
26 Annexin V-FITC⁺/PI⁺ were indicative of early and late apoptotic cells.

27 **NucGreen cell viability imaging**

28 A2780 cells were plated on 35mm glass bottom dish and treated with TP-10 or DOX for 24
29 hours. NucGreen (indicator of dead cells) and NucBlue (total cells) were directly added to wells
30 per condition for 15 minutes at 37°C. Images were captured at a magnification of x10, using
31 Olympus BX51 microscope. The percentage of NucGreen positive cells over the total cells was
32 calculated using NIH Image J software. Representative images were chosen based on their
33 quality and to most accurately represent the group average across all the available data.

35 **DNA synthesis by [³H]-thymidine incorporation**

36 To assess DNA synthesis and cell proliferation, [³H]-thymidine incorporation in A2780 cells was
37 performed as described. Briefly, cells were starved in serum-free DMEM medium for 24 hours.
38 Cells were then cultured in DMEM supplemented with 10% FBS. DNA synthesis was determined
39 by incorporating 1 µCi/ml [³H]-thymidine upon TP-10 or DOX treatment for 24 hours. Cells were
40 washed twice with PBS and incubated in 5% trichloroacetic acid for 30 min on ice to precipitate
41 the protein. Precipitates were washed twice with cold 95% ethanol, solubilized in 0.5 N NaOH for
42 30 min, and neutralized with 1N HCl. Radioactivity was quantified using a liquid scintillation
43 counter. For analysis, data were pooled from 3 independent experiments performed in duplicates.

1 The average value of the control group was set at 1.0 (reference). The relative fold change over
2 the control was calculated relative to the reference.

4 **Proximity Ligation Assay**

5 cGMP and PKGI binding, and cAMP and PKA RIIa binding were detected via proximity ligation
6 assay (PLA) using Duolink In Situ Kit (Sigma), following the manufacturer's instructions. Briefly,
7 cells were plated on 35mm glass bottom dishes. Cells were fixed in 4% paraformaldehyde for 20
8 min at room temperature followed by permeabilization in PBS/0.2% Triton-100 for 15 min. Cells
9 were blocked in blocking solution for 1 h at 37 °C, followed by incubation with rabbit anti-cGMP
10 antibody (1:200) and mouse anti-PKGI antibody (1:200), or mouse anti-cAMP antibody and rabbit
11 anti-PKA RIIa antibody (1:200) diluted in Duolink® Antibody Diluent at 4°C overnight. As a
12 negative control, samples were incubated with either antibodies alone. Samples were washed and
13 incubated in "MINUS" mouse and "PLUS" rabbit oligonucleotide probes. Oligonucleotides in
14 close proximity were allowed to hybridize to the two PLA probes using hybridization solution.
15 Hybridized oligonucleotides were joined using a ligase solution to generate a templated and the
16 amplification process was initiated by incubating with a polymerase solution. Rolling circle
17 amplification using the oligonucleotide sequence as a primer generated an extended repeat
18 sequence. Finally, PLA was detected using Alexa 555-conjugated detection oligonucleotide. PLA
19 signals were visualized as distinct red fluorescent dot (representing single protein interactions) and
20 were captured at 40x magnification by using Olympus IX-81 confocal microscope. Representative
21 images were chosen based on their quality and to most accurately represent the group mean across
22 all the available data.

24 **Measurement of mitochondrial membrane potential (TMRE staining)**

25 The mitochondrial membrane potential was measured using TMRE according to the
26 manufacturer's protocol. CMs were treated with various test compounds and stimulated with or
27 without DOX as indicated. 24 h after treatment. CMs were loaded with TMRE at 50 nM
28 concentration for 30 mins at RT. After incubation, cells were washed with 1x PBS for 3 times
29 and submerged in live cell imaging solution. Fluorescence images were captured using a laser
30 scanning confocal microscope (Olympus). Representative images were chosen based on their
31 quality and to most accurately represent the group mean across all the available data.

33 **Dihydroethidium (DHE) staining**

34 DHE staining was performed for detection of intracellular reactive oxygen species (ROS). CMs
35 were treated with various test compounds and stimulated with or without DOX as indicated. 24 h
36 after treatment. The cells were incubated with 10 µM DHE for 20 mins at 37°C in the dark and
37 washed with PBS for 3x 15 mins. Fluorescence images were captured using a laser scanning
38 confocal microscope (Olympus). Representative images were chosen based on their quality and to
39 most accurately represent the group mean across all the available data.

41 **Statistics**

42 The exact sample size in each group is listed in Supplementary Table S5-6 or mentioned in
43 corresponding figure legends. All data are presented as mean ± SEM or median with interquartile
44 range (IQR) based on data distribution. We used the Shapiro-Wilk test and Brown-Forsythe test
45 to help determine the assumptions of normality and variance equality, respectively. Proper
46 corrections were applied to control the inflation of type I error in multiple post-hoc comparisons,

1 and the corresponding adjusted p -values were reported. All parametric and non-parametric tests
2 were two-sided with a significance level set at 5%. Linear mixed effect models were used to test
3 the differences of cell surface area, cell death (as measured by trypan blue staining and LDH
4 cytotoxicity assay), and quantification of fluorescence intensity among groups, with the cell
5 providing animal (mouse) accounted as random effect ¹⁹. The model was fitted using the
6 *lmerTest* packages in R 4.2.2 (<https://www.R-project.org/>). All the other statistical analyses were
7 conducted using GraphPad Prism 8.0. Detailed statistical methods were described in figure
8 legends.

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1 **Supplemental Table 1**

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5 **PDE10A expression from RNA-seq datasets of previous studies**

6

GEO No.	Reference	Sample	Comparisons	PDE10A fold-change	Raw P-value	Adjusted P-value*
GSE106297	Maillet et al. 2018 [15]	Human ESC-derived CMs	DOX 1uM vs Control	-4.03	9.46×10 ⁻⁸	3.58×10 ⁻⁶
GSE106297	Maillet et al. 2018 [15]	Human ESC-derived CMs	DOX 2.5uM vs Control	-4.60	2.89×10 ⁻⁸	1.57×10 ⁻⁶
GSE157282	Kattih et al. 2020 [16]	Human iPS-derived CMs	DOX 1uM vs Control	1.22	1.40×10 ⁻⁴	3.87×10 ⁻³
GSE206803	Chen et al. 2022	NRVMs	DOX 1uM vs Control	5.09	1.36×10 ⁻⁶	1.47×10 ⁻⁵

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9 **Supplemental Table S1. PDE10A expression from RNA-seq datasets of previous studies.**

10 GEO, gene expression omnibus. DOX, doxorubicin. Human ESC-derived CMs: human
11 embryonic stem cell ESC-derived cardiomyocytes. Human iPS-derived CMs: human induced
12 pluripotent stem cell-derived cardiomyocytes. NRVMs: neonatal rat ventricular myocytes. *The
13 false discovery rate (FDR) was used for adjustment of multiple comparisons within each RNA-
14 seq dataset.

1 **Supplemental Table 2**

Summary of echocardiographic data of PDE10A-WT or PDE10A-KO mice before and after 7-day DOX treatment

Day 0								
Parameters	WT/ Control	WT/ DOX	WT/ TP-10	WT/ DOX+TP-10	KO/ Control	KO/ DOX	KO/ TP-10	KO/ DOX+TP-10
Heart Rate (beats/min)	557.7±8.71	587.88±6.83	556.48±11.94	554.19±6.31	565.32±5.64	562.65±5.75	563.78±3.18	536.30±9.36
LVV, s (μL)	7.58±1.16	5.03±0.82	8.24±1.05	7.76±0.76	7.71±0.79	7.71±1.29	7.82±0.94	8.42±0.96
LVV, d (μL)	42.11±3.62	39.26±2.72	41.63±2.76	41.23±1.90	39.53±3.06	41.86±3.90	46.43±4.3	44.18±2.31
Stroke Volume (μL)	34.52±2.70	34.22±1.95	33.39±2.10	33.47±1.23	31.82±2.42	34.14±2.73	38.61±3.49	35.75±1.63
EF (%)	82.50±1.71	87.16±1.32	80.44±1.78	81.70±1.25	80.63±1.12	83.03±1.70	83.31±1.14	81.39±1.48
FS (%)	50.53±1.85	55.58±1.36	48.20±1.83	49.50±1.37	48.13±1.13	51.29±1.82	51.33±1.31	49.40±1.60
Cardiac Output (mL/min)	19.36±1.76	20.12±1.18	18.48±0.98	18.49±0.82	18.07±1.53	19.18±1.49	21.71±1.86	19.17±0.93
LVM (mg)	95.6.08±10.51	95.578±4.56	99.89±4.79	100.38±5.06	97.89±5.77	107.64±6.73	100.76±8.79	98.90±5.64
LVAW, d (mm)	1.60±0.08	1.61±0.04	1.66±0.02	1.69±0.05	1.53±0.02	1.74±0.04	1.66±0.05	1.33±0.10
LVAW, s (mm)	0.948±0.05	0.98±0.02	0.99±0.03	1.00±0.04	0.94±0.02	1.02±0.03	0.96±0.02	1.21±0.11
LVPW, d (mm)	1.255±0.04	1.36±0.03	1.35±0.03	1.33±0.05	1.16±0.03	1.40±0.05	1.33±0.05	1.12±0.07
LVPW, s (mm)	0.79±0.03	0.87±0.03	0.86±0.02	0.88±0.05	0.72±0.04	0.90±0.04	0.77±0.01	1.03±0.11
BW (g)	27.08±0.89	27.49±0.67	27.77±1.28	27.14±0.74	27.73±1.07	27.41±1.35	27.99±1.24	26.89±1.19

Day 9								
Parameters	WT/ Control	WT/ DOX	WT/ TP-10	WT/ DOX+TP-10	KO/ Control	KO/ DOX	KO/ TP-10	KO/ DOX+TP-10
Heart Rate (beats/min)	577.7±9.77	526.6±10.03**	579.9±3.88	567.7±5.93##	549.8±6.13	526.4±8.10	567.4±8.22	525.7±9.49
LVV, s (μL)	8.07±1.12	11.58±1.15*	8.56±0.79	8.40±0.49#	10.10±1.35	10.37±1.00	8.87±0.98	6.22±0.68
LVV, d (μL)	42.96±1.90	39.85±1.34	41.83±2.29	38.28±0.99	49.09±2.14	44.97±3.17	44.97±3.17	39.94±2.54
Stroke Volume (μL)	34.4±1.96	28.40±0.91**	33.28±1.54	30.86±0.94	39.00±1.16	34.59±2.57†	36.10±2.35	33.73±2.24
EF (%)	80.1±0.83	72.48±1.40**	79.82±0.69	77.66±0.99##	80.05±0.91	78.6±1.54††	81.12±0.91	82.7±1.05
FS (%)	47.62±0.87	40.69±1.10**	47.25±0.69	45.25±0.91##	47.67±0.88	46.31±1.35††	48.77±0.94	50.58±1.22
Cardiac Output (mL/min)	19.86±1.13	15.39±0.58**	18.87±0.75	17.54±0.63#	20.48±0.63	17.72±0.86†	20.37±1.01	18.09±0.86
LVM (mg)	76.08±10.08	55.28±1.45***	74.48±7.11	60.39±1.95	67.18±5.74	60.25±3.06	88.69±10.12	76.81±8.46
LVAW, d (mm)	1.09±0.02	0.71±0.02***	0.83±0.06	0.76±0.02	0.74±0.04	0.72±0.02	0.85±0.05	0.80±0.05
LVAW, s (mm)	1.01±0.04	0.98±0.02	1.29±0.09	1.08±0.026#	1.14±0.05	1.07±0.04	1.34±0.12	1.33±0.11
LVPW, d (mm)	0.87±0.01	0.51±0.01****	0.65±0.03	0.56±0.01	0.57±0.03	0.53±0.02	0.76±0.06	0.70±0.06
LVPW, s (mm)	0.85±0.02	0.77±0.01	1.03±0.07	0.87±0.01##	0.82±0.05	0.79±0.03	1.07±0.08	1.11±0.11
BW (g)	27.27±0.90	23.05±0.54***	27.83±1.05	24.8±0.57	27.27±1.14	24.6±1.06	27.48±1.26	24.3±1.18

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Supplemental Table S2. Summary of echocardiographic data of PDE10A-WT or PDE10A-KO mice before and after drug treatment.

LVV, d/s: left ventricular volume at diastole/systole. EF: ejection fraction. FS: fractional shortening. LVM: left ventricular mass. LVAW, d/s: left ventricular anterior wall diameter at diastole/systole. LVID, d/s: left ventricular internal diameter at diastole/systole. LVPW, d/s: left ventricular posterior wall diameter at diastole/systole. BW: body weight. Values are expressed as mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 WT/DOX vs. WT/Control; # *P* < 0.05, ## *P* < 0.01 WT/DOX+TP-10 vs. WT/DOX. † *P* < 0.05, †† *P* < 0.01 KO/DOX vs. WT/DOX.

1 **Supplemental Table 3**

Summary of echocardiographic data of PDE10A-WT or PDE10A-KO mice before and after 3 week DOX treatment.

Parameters	Week 0				Week 3			
	WT/Control	WT/DOX	KO/Control	KO/DOX	WT/Control	WT/DOX	KO/Control	KO/DOX
Heart Rate (beats/min)	567.02±8.30	594.13±5.96	571.18±8.75	571.62±6.21	568.44±10.59	533.95±8.13*	562.73±7.70	566.57±10.13###
LVV, s (μL)	8.91±1.27	10.12±1.38	9.30±1.45	9.24±1.08	10.25±1.47	12.92±1.75	8.11±0.97	7.72±1.49#
LVV, d (μL)	39.92±3.54	44.76±4.18	47.79±4.59	45.34±3.29	45.23±4.99	35.42±2.60	43.68±2.96	34.93±3.17
Stroke Volume (μL)	31.01±2.36	34.63±2.84	38.49±3.42	36.09±2.37	34.99±3.54	22.5±1.57**	35.57±2.12	27.21±2.22
EF (%)	78.30±1.53	78.24±1.21	80.90±1.56	79.87±1.14	78.09±1.39	64.51±2.94**	81.75±1.15	78.58±2.84##
FS (%)	45.90±1.43	45.92±1.13	48.75±1.60	47.48±1.10	45.80±1.32	34.52±2.16**	49.46±1.14	46.44±2.69##
Cardiac Output (mL/min)	17.57±1.34	20.64±1.81	21.93±1.89	20.61±1.32	19.83±1.95	11.98±0.81***	20.03±1.25	15.46±3.71####
LVM (mg)	95.85±8.68	91.98±7.56	116.47±8.90	109.22±6.09	85.66±9.5	71.02±5.44	88.81±7.87	91.06±4.17##
LVAW, s (mm)	1.60±0.05	1.52±0.04	1.75±0.07	1.69±0.05	1.42±0.07	1.20±0.06*	1.50±0.10	1.50±0.05###
LVAW, d (mm)	0.97±0.07	0.89±0.03	1.05±0.04	1.05±0.03	0.87±0.05	0.88±0.04	0.94±0.06	0.89±0.05
LVPW, s (mm)	1.20±0.05	1.22±0.03	1.31±0.05	1.23±0.02	1.16±0.07	0.93±0.04*	1.06±0.05	1.21±0.05###
LVPW, d (mm)	0.78±0.03	0.77±0.03	0.86±0.04	0.82±0.02	0.71±0.04	0.68±0.06	0.71±0.04	0.75±0.04
BW (g)	24.16±1.62	25.44±1.70	25.25±1.65	25.78±1.71	27.08±1.89	21.16±1.31*	28.00±1.69	20.21±0.88

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Supplemental Table S3. Summary of echocardiographic data of PDE10A-WT or PDE10A-KO mice before and after 3-week DOX treatment.

LVV, d/s: left ventricular volume at diastole/systole. EF: ejection fraction. FS: fractional shortening. LVM: left ventricular mass. LVAW, d/s: left ventricular anterior wall diameter at diastole/systole. LVID, d/s: left ventricular internal diameter at diastole/systole. LVPW, d/s: left ventricular posterior wall diameter at diastole/systole. BW: body weight. Values are expressed as mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 WT/DOX vs. WT/Control at week 3; # *P* < 0.05, ## *P* < 0.01, ### *P* < 0.001, #### *P* < 0.0001 KO/DOX vs. WT/DOX at week 3.

1 **Supplemental Table 4**

Summary of echocardiographic data of nude mice before tumor cell inoculation and 2 weeks after drug treatment.

Parameters	Day -10				Day 14			
	Control	DOX	TP-10	DOX+TP-10	Control	DOX	TP-10	DOX+TP-10
Heart Rate (beats/min)	551.2±15.31	552.93±6.60	560.22±9.18	557.16±14.31	539.71±12.01	508.92±20.65**	574.41±19.8	585.33±11.25##
LVV, s (μL)	10.10±1.87	11.46±1.47	10.79±1.64	11.37±1.34	12.55±1.83	13.01±2.08	10.89±1.25	7.62±1.02[#]
LVV, d (μL)	53.21±4.41	54.31±3.50	50.68±2.54	54.65±3.26	54.39±4.52	43.69±6.39*	51.58±2.62	42.84±3.90
Stroke Volume (μL)	41.42±3.83	42.85±2.48	39.90±1.28	43.28±2.54	41.46±3.06	30.68±4.32	40.69±1.52	34.99±2.90
EF (%)	81.73±2.19	80.24±1.99	81.71±2.46	79.40±1.84	77.49±2.45	70.50±0.65**	80.18±1.53	80.85±1.05#####
FS (%)	50.00±2.41	48.40±2.08	49.61±2.49	47.61±1.97	45.49±2.55	38.81±0.39**	48.04±1.54	48.95±1.14#####
Cardiac Output (mL/min)	23.81±1.81	23.64±1.23	22.35±0.81	23.95±1.18	22.31±1.86	16.14±3.26	23.28±0.73	20.24±1.46
LVM (mg)	97.25±5.11	91.51±3.08	104.78±6.73	98.07±5.26	109.32±5.83	91.27±7.91	99.34±7.90	98.31±9.37
LVAW, d (mm)	0.82±0.05	0.80±0.04	0.93±0.06	0.86±0.03	1.08±0.05	0.84±0.04**	0.83±0.06	0.83±0.03
LVAW, s (mm)	1.49±0.09	1.39±0.05	1.51±0.10	1.53±0.07	1.63±0.08	1.26±0.03**	1.43±0.05	1.48±0.04##
LVPW, d (mm)	0.78±0.04	0.72±0.01	0.80±0.07	0.73±0.03	0.69±0.05	0.85±0.04*	0.81±0.03	0.95±0.07[#]
LVPW, s (mm)	1.30±0.06	1.21±0.03	1.29±0.06	1.15±0.06	1.29±0.03	1.19±0.06	1.32±0.04	1.43±0.07[#]
BW (g)	21.12±1.09	22.75±0.76	22.57±0.59	22.49±0.63	25.80±0.82	21.06±1.40*	23.97±0.82	20.77±0.89*

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4 **Supplemental Table S4. Summary of echocardiographic data of nude mice before tumor cell**
5 **inoculation and 2 weeks after drug treatment.**

6 LVV, d/s: left ventricular volume at diastole/systole. EF: ejection fraction. FS: fractional
7 shortening. LVM: left ventricular mass. LVAW, d/s: left ventricular anterior wall diameter at
8 diastole/systole. LVID, d/s: left ventricular internal diameter at diastole/systole. LVPW, d/s: left
9 ventricular posterior wall diameter at diastole/systole. BW: body weight. Values are expressed as
10 mean ± SEM. **P* < 0.05, ***P* < 0.01 DOX vs. Control and DOX+TP-10 vs. Control; # *P* < 0.05,
11 ## *P* < 0.01, ##### *P* < 0.0001 DOX+TP-10 vs. DOX.

1 **Supplemental Table 5**

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Figure Number	Description	N per group							
		1	2	3	4	5	6	7	8
1E	Male	9	13	8	14	7	6	7	8
	Female	3	6	3	5	3	4	3	5
1G	Male	4	10	8	10	3	6	7	8
	Female	3	6	3	4	3	5	3	5
1H	Male	8	11	6	10	6	8	6	10
	Female	3	4	3	4	3	3	3	3
1L	Male	4	6	4	3	4	4	3	3
	Female	4	4	3	4	4	4	3	3
2C	Male	4	6	4	6				
	Female	3	4	3	4				
2E	Male day 0	4	6	4	6				
	Female day 0	3	5	3	4				
	Male day 21	4	6	4	6				
	Female day 21	3	5	3	4				
2F	Male	4	6	4	6				
	Female	3	5	3	4				
2I	Male	4	6	4	6				
	Female	3	4	3	4				
2K	Male	4	6	4	6				
	Female	3	5	3	4				
3L	Replicates	9	11	9	11				
4F	Tumors	11	9	7	12				
5C	Mouse	6	8	6	8				
5E	Mouse	9	8	7	7				
5H	Mouse	3	6	3	4				
6D	Replicates	10	10	6	10				
6E	Replicates	6	6	6	6				
6F	Replicates	12	5	14	8				
7H	Mouse	3	5	3	5				
8J	Mouse	4	5	3	5				
8K	Mouse	4	10	3	5				

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6 **Supplemental Table S5. Summary of N in each group in main figures.**

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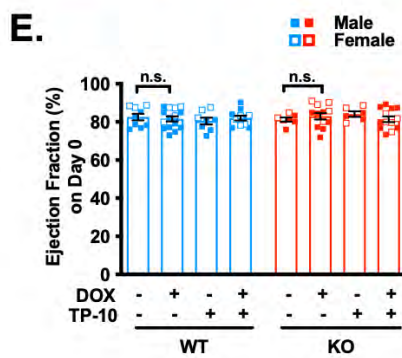
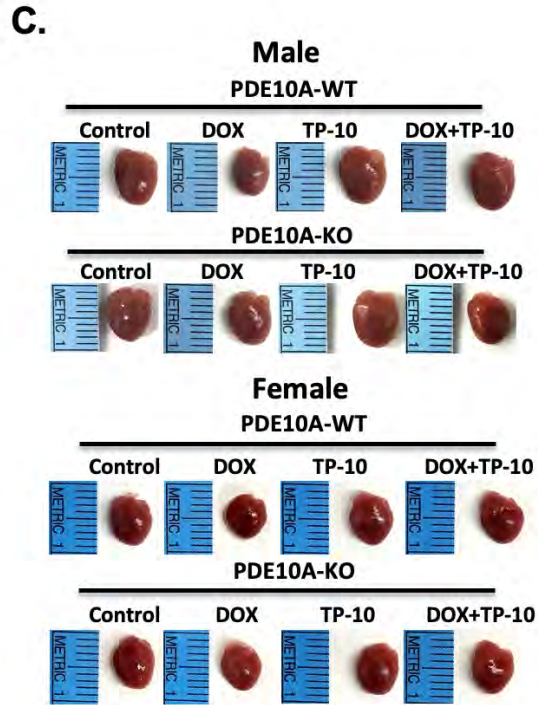
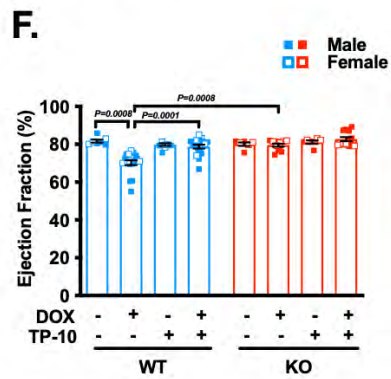
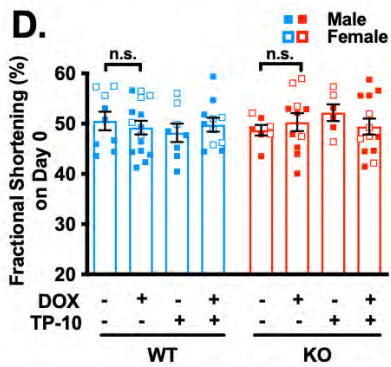
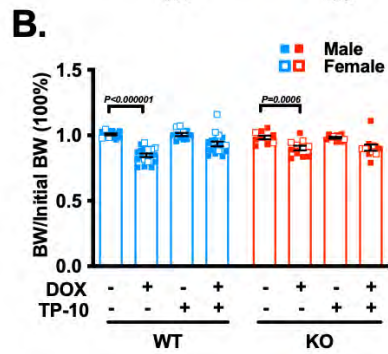
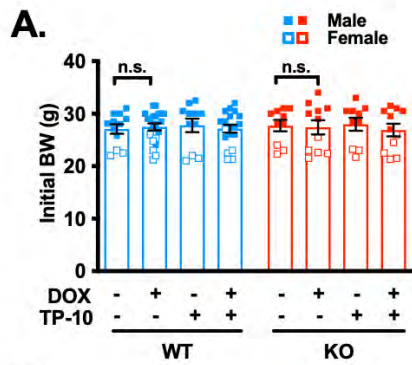
1 **Supplemental Table 6**

Figure Number	Description	N per group							
		1	2	3	4	5	6	7	8
S1A	Male	9	15	8	14	7	6	7	6
	Female	3	6	3	5	3	5	3	5
S1B	Male	8	11	7	12	6	5	6	5
	Female	3	6	3	5	3	5	3	5
S1D	Male	6	10	5	6	4	7	3	8
	Female	3	5	3	5	3	4	3	5
S1E	Male	6	10	5	6	4	7	3	8
	Female	3	5	3	5	3	5	3	5
S1F	Male	4	13	8	14	3	6	7	8
	Female	3	6	3	5	3	5	3	5
S2A	Male	9	15	8	14	7	6	7	6
S2B	Female	3	6	3	5	3	5	3	5
S2C	Male	8	11	7	12	6	5	6	5
S2D	Female	3	6	3	5	3	5	3	5
S2E	Male	9	13	8	14	7	6	7	8
S2F	Female	3	6	3	5	3	4	3	5
S2G	Male	6	10	5	6	4	7	3	8
S2H	Female	3	5	3	5	3	4	3	5
S2I	Male	4	13	8	14	3	6	7	8
S2J	Female	3	6	3	5	3	5	3	5
S2K	Male	6	10	5	6	4	7	3	8
S2L	Female	3	5	3	5	3	4	3	5
S2M	Male	4	13	8	14	3	6	7	8
S2N	Female	3	6	3	5	3	5	3	5
S2O	Male	8	11	6	10	6	9	6	10
S2P	Female	3	4	3	4	3	3	3	3
S2S	Male	4	6	4	3	4	4	3	3
S2T	Female	4	4	3	4	4	4	3	3
S3A	Male	4	6	4	6				
	Female	3	4	3	4				
S3B	Male	4	6	4	6				
	Female	3	4	3	4				
S3D	Male day 0	4	6	4	6				
	Female day 0	3	5	3	4				
	Male day 21	4	6	4	6				
	Female day 21	3	5	3	4				
S4A	Male	4	6	4	6				
S4B	Female	3	4	3	4				
S4C	Male	4	6	4	6				
S4D	Female	3	4	3	4				
S4E	Male	4	6	4	6				
S4F	Female	3	4	3	4				
S4G	Male day 0	4	6	4	6				
	Male day 21	4	6	4	6				
S4H	Female day 0	3	5	3	4				
	Female day 21	3	5	3	4				
S4I	Male day 0	4	6	4	6				
	Male day 21	4	6	4	6				
S4J	Female day 0	3	5	3	4				
	Female day 21	3	5	3	4				
S4K	Female	3	5	3	4				
S4L	Male	4	6	4	6				
S4O	Male	4	6	4	6				
S4P	Female	3	4	3	4				
S4Q	Male	4	6	4	6				
S4R	Female	3	5	3	4				
S13A	Mouse	6	6	6	8				
S13B	Mouse	6	6	6	8				
S15H	Mouse	6	6	6	6				
S15I	Mouse	6	6	6	6				

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3 **Supplemental Table S6. Summary of N in each group in supplementary figures.**

1 Supplemental Figure S1

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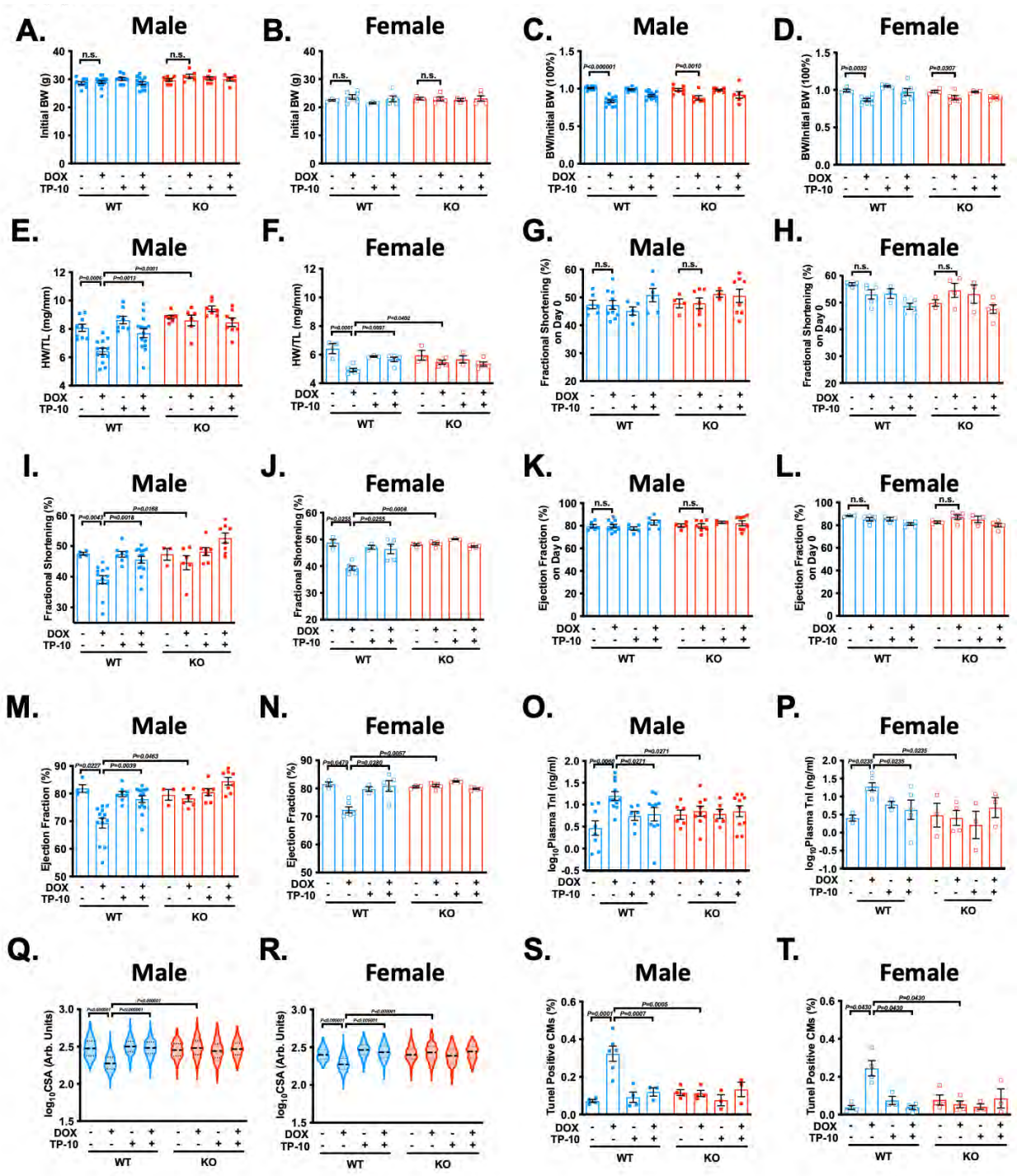
1 **Supplemental Figure S1: Supplemental Figure S1: PDE10A inhibition or deficiency**
2 **alleviates doxorubicin-induced cardiac dysfunction *in vivo*.**

3 Male or female C57BL/6J PDE10A-WT or PDE10A-KO mice at the age of 12 weeks were
4 treated with vehicle or PDE10A inhibitor TP-10 (3.2 mg/kg/day) subcutaneously 2 days prior to
5 a single dose of saline or DOX (15mg/kg), and continued for 1 week. (A) Quantification of
6 initial body weight. (B) Quantification of body weight/initial body weight. (C) Representative
7 images of whole hearts from male and female mice. (D-F) Echocardiography was performed
8 before and 1 week after DOX administration to monitor the progression of cardiac structural and
9 functional changes. (D) Percent fractional shortening before drug treatment. (E) Analysis of
10 ejection fraction before drug treatment. (F) Analysis of ejection fraction at 1 week point. Data
11 were represented as mean \pm SEM (A, B, E, F) or median with IQR (D). Statistics: two-way
12 ANOVA with Holm-Sidak post-hoc test for 2 comparisons in A, B, E and 3 comparisons in F;
13 Kruskal-Wallis's test with Dunn's corrections for 2 comparisons in D. n.s.: no significance
14 difference. Animal numbers: A-B and D-F, n = 6-19.

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1 Supplemental Figure S2

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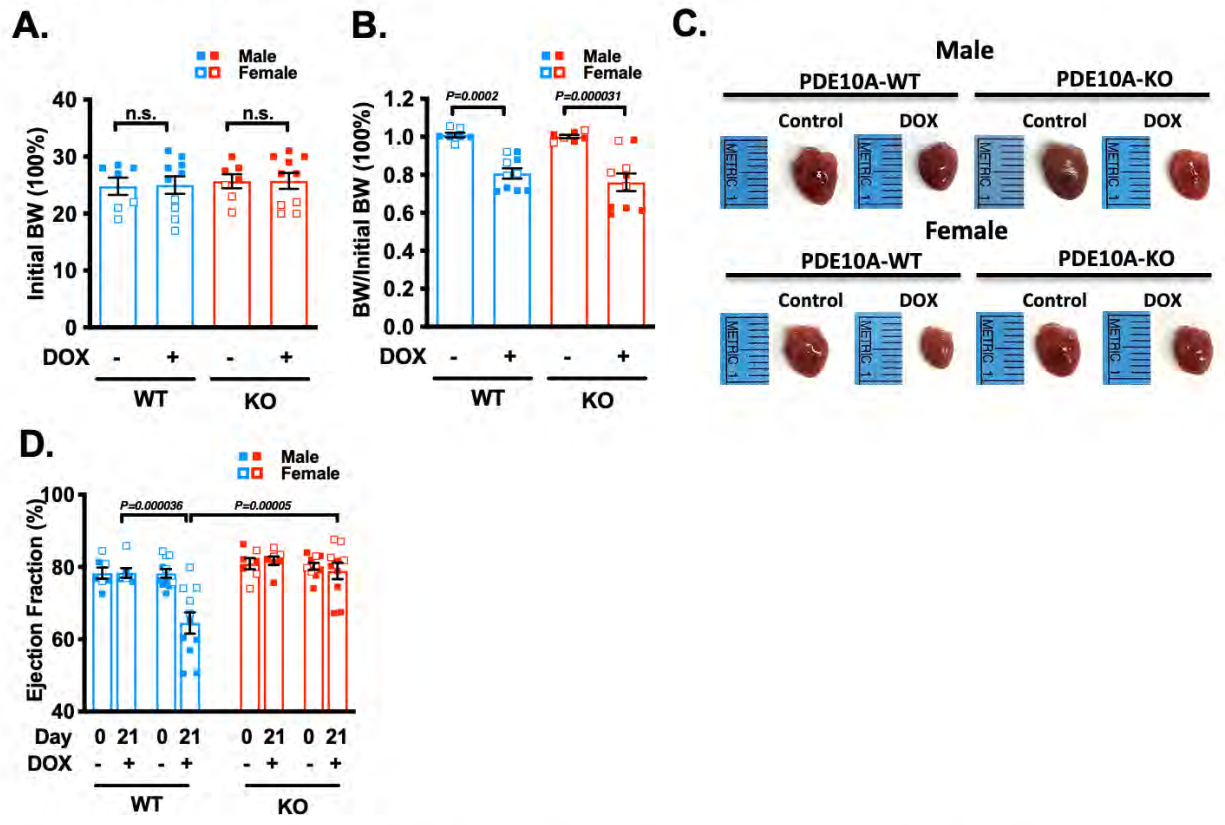
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1 **Supplemental Figure S2: PDE10A inhibition or deficiency alleviates doxorubicin-induced**
2 **cardiac dysfunction in male and female mice.**

3 Male or female C57BL/6J PDE10A-WT or PDE10A-KO mice at the age of 12 weeks were treated
4 with vehicle or PDE10A inhibitor TP-10 (3.2 mg/kg/day) subcutaneously 2 days prior to a single
5 dose of saline or DOX (15mg/kg), and continued for 1 week. (A-B) Quantification of initial body
6 weight in male (A) or female (B) mice. (C-D) Quantification of body weight/initial body weight
7 in male (C) or female (D) mice. (E-F) Quantification of heart weight/tibia length in male (E) or
8 female (F) mice. (G-H) Percent fractional shortening before drug treatment in male (G) or female
9 (H) mice. (I-J) Percent fractional shortening at 1 week point in male (I) or female (J) mice. (K-L)
10 Analysis of ejection fraction before drug treatment in male (K) or female (L) mice. (M-N) Analysis
11 of ejection fraction at 1 week point in male (M) or female (N) mice. (O-P) Plasma troponin I levels
12 in male (O) or female (P) mice. (Q-R) Quantitative data of cardiomyocyte (CM) atrophy assessed
13 by CSA in male (Q) or female (R) mice, n = 4-10 hearts per group with 150-500 CMs analyzed
14 per heart. (S-T) Quantification of TUNEL staining in male (S) or female (T) mice, n = 3-6 hearts
15 per group with 10 random fields analyzed per heart. Data were represented as mean \pm SEM (A-F,
16 K-P, and S-T) or median with IQR (G-J, and Q-R). Statistics: one-way ANOVA with Holm-Sidak
17 post-hoc test for 2 comparisons in A-D, K-L and 3 comparisons in E-F, M-N, O-P; Kruskal-
18 Wallis's test with Dunn's corrections for 2-3 comparisons as indicated in G-J; mixed effect model
19 with Sidak corrections for 3 comparisons in Q-R; Welch ANOVA with Dunnett's T3 corrections
20 for 3 comparisons in S-T. n.s., no significance difference. Animal numbers: A-T, n = 3-14
21

1 Supplemental Figure S3

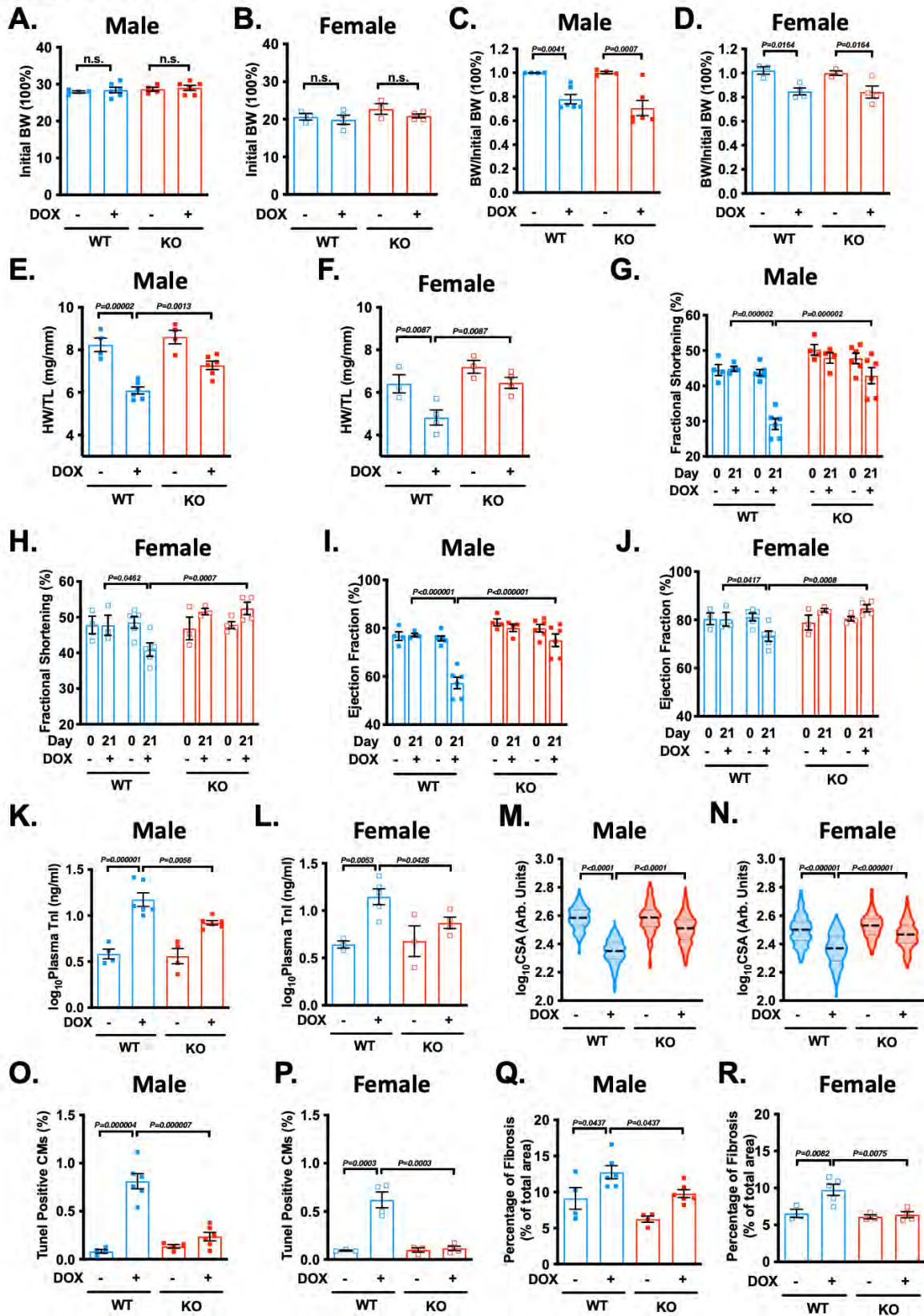
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1 **Supplemental Figure S3: PDE10A deficiency alleviates DOX-induced cardiotoxicity and**
2 **dysfunction in mice.**
3 Male or female C57BL/6J PDE10A-WT or PDE10A-KO mice at the age of 12 weeks were treated
4 with DOX (4 mg/kg) on days 0, 7, and 14. (A) Quantification of initial body weight. (B)
5 Quantification of body weight/initial body weight. (C) Representative images of whole hearts from
6 male and female mice. (D) Echocardiography was performed prior to the DOX administration as
7 well as 1 week after the last dose of DOX administration to monitor the progression of cardiac
8 structural and functional changes. Ejection fraction of male and female mice. Data were
9 represented as mean \pm SEM. Statistics: two-way ANOVA with Holm-Sidak post-hoc test for 2
10 comparisons in A, B, and D. n.s., no significance difference. Animal numbers: n = 7-10.
11

1 Supplemental Figure S4



2

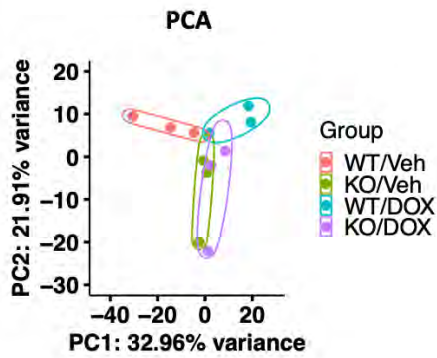
1 **Supplemental Figure S4: PDE10A deficiency alleviates DOX-induced cardiotoxicity and**
2 **dysfunction in male and female mice.**

3 Male or female C57BL/6J PDE10A-WT or PDE10A-KO mice at the age of 12 weeks were treated
4 with DOX (4 mg/kg) on days 0, 7, and 14. (A-B) Quantification of initial body weight in male (A)
5 or female (B) mice. (C-D) Quantification of body weight/initial body weight in male (C) or female
6 (D) mice. (E-F) Quantification of heart weight/tibia length in male (E) or female (F) mice. (G-H)
7 Percent fractional shortening at 0- or 21-day point in male (G) or female (H) mice. (I-J) Analysis
8 of ejection fraction in male (I) or female (J) mice. (K-L) Plasma troponin I levels in male (K) or
9 female (L) mice. (M-N) Quantitative data of cardiomyocyte (CM) atrophy assessed by CSA in
10 male (M) or female (N) mice, n = 3-6 hearts per group with 150-200 CMs analyzed per heart. (O-
11 P) Quantification of TUNEL staining in male (O) or female (P) mice, n = 3-5 hearts per group with
12 10 random fields analyzed per heart. (Q-R) Quantification of total fibrosis in male (Q) or female
13 (R) mice. Data were represented as mean \pm SEM (A-F, I-L, and O-R) or median with IQR (G-H,
14 and M-N). Statistics: one-way ANOVA with Holm-Sidak post-hoc test for 2 comparisons in A-F,
15 I-L, and Q-R; Kruskal-Wallis's test with Dunn's corrections for 2 comparisons in G-H; mixed
16 effect model with Sidak corrections for 2 comparisons in M-N; Welch ANOVA with Dunnett's
17 T3 corrections for 2 comparisons in O-P. Animal numbers: A-L, and Q-R, n = 3-6.

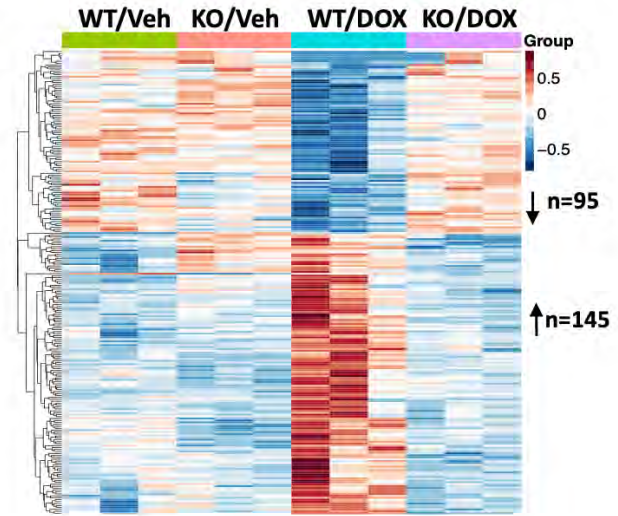
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1 Supplemental Figure S5

A.

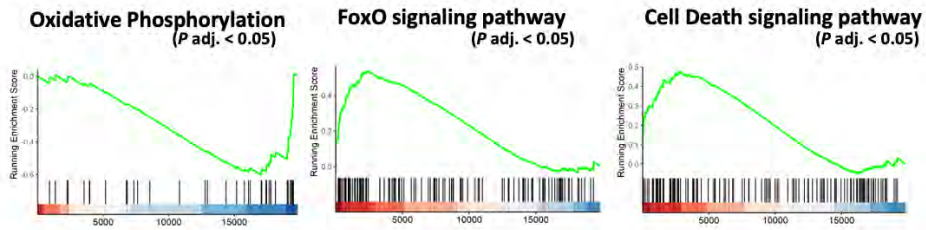


B.



C.

Gene Set Enrichment Analysis

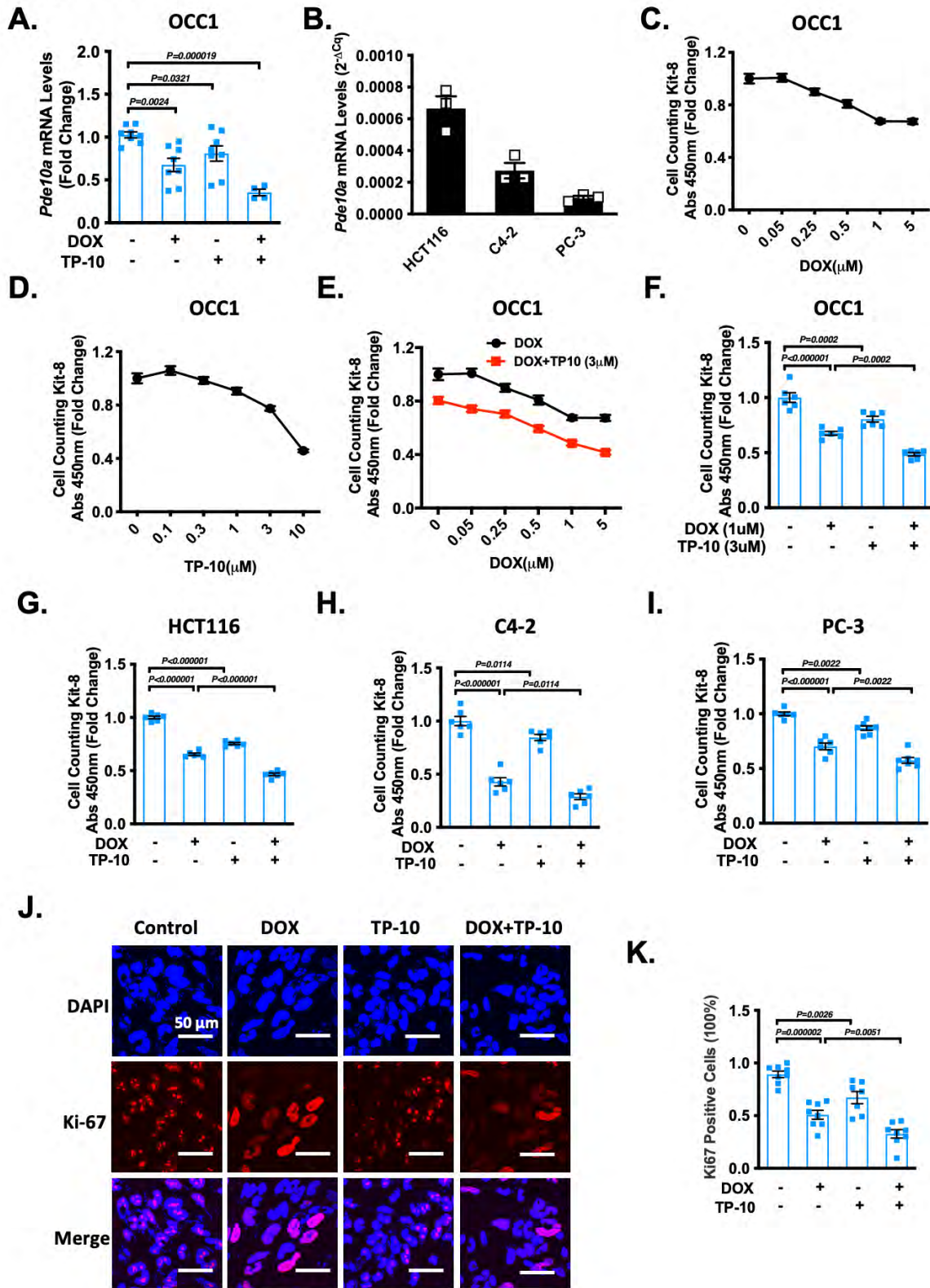


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1 **Supplemental Figure S5: RNA-sequencing identifies transcriptome that is regulated by**
2 **PDE10A.**

3 Male C57BL/6J PDE10A-WT or PDE10A-KO mice at the age of 12 weeks were treated with a
4 single dose of saline or DOX (15mg/kg), and continued for 1 week. mRNA was extracted to
5 perform RNA-sequencing. (A) Principle component analysis (PCA) demonstrates distinct patterns
6 of transcriptomes in four groups, including PDE10A-WT/Vehicle (WT/Veh), PDE10A-WT/DOX
7 (WT/DOX), PDE10A-KO/Vehicle (KO/Veh), and PDE10A-KO/DOX (KO/DOX). Each point
8 represents the projections of individual hearts onto principle component (PC). The majority of
9 genetic variation is addressed by the first (PC1, 32.96%), followed by the second (PC2, 21.91%).
10 (B) Heatmap of genes differentially expressed in the hearts of PDE10A-WT or PDE10A-KO mice
11 after vehicle or DOX treatment. Each column represents an individual replicate and there are 3
12 replicates per group. Each row represents an individual gene. The top of heatmap is the cluster of
13 genes that are downregulated in WT/DOX, while the bottom is the cluster of genes upregulated in
14 WT/DOX compared to WT/Veh or KO/Veh and reversed in KO/DOX. The color bar represents
15 relative expression of log-transformed, normalized counts with upregulated genes shown in red
16 and downregulated genes in blue. (C) Gene set enrichment analysis (GSEA) revealed that
17 oxidative phosphorylation was among the most significantly ($P_{adj} < 0.05$) upregulated gene sets,
18 while Forkhead box containing O family (FoxO) signaling pathway and cell death signaling
19 pathway were among the most significantly ($P_{adj} < 0.05$) downregulated gene sets in KO/DOX
20 group versus WT/DOX group.
21

1 Supplemental Figure S6



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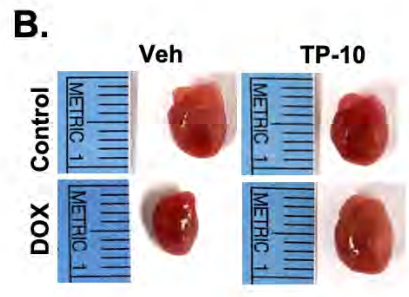
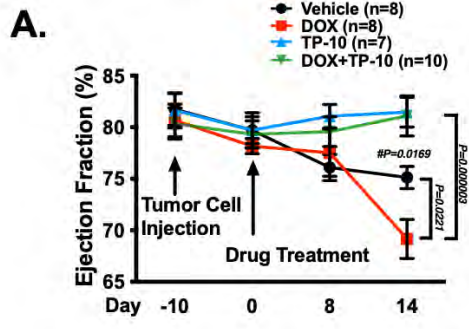
1 **Supplemental Figure S6: PDE10A inhibition enhanced DOX-induced ovarian cancer cell**
2 **growth.**

3 (A) Human ovarian cancer cell line OCC1 were treated with vehicle, 3 μ M TP-10, 1 μ M DOX or
4 combination of TP-10 and DOX as indicated for 6 hours. qPCR analysis of *Pde10a*, normalized to
5 *Gapdh*, n = 4-8 replicates for each group. (B) qPCR analysis of *Pde10a* in human colon cancer
6 cell line HCT116, human prostate cancer cell line C4-2 and PC-3, normalized to *Gapdh*, n = 3
7 replicates for each group. (C-F) OCC1 were treated with vehicle or increasing dose of DOX (C),
8 vehicle or increasing dose of TP-10 (D), 3 μ M TP-10 plus vehicle or increasing dose of DOX (E),
9 vehicle, 3 μ M TP-10, 1 μ M of DOX or combination of 3 μ M TP-10 and 1 μ M of DOX (F) as
10 indicated for 24 hours. Cell viability was measured by cell counting kit-8 (CCK8) assay, n = 6-7
11 replicates from 3 independent experiments for each group. (G-I) Human colon cancer cell line
12 HCT116, human prostate cancer cell line C4-2 and PC-3 were treated with vehicle, TP-10 (300nM),
13 DOX (1 μ M) or combination of TP-10 and DOX as indicated for 24 hours. CCK8 assay was done
14 to measure cell viability. n = 6-7 replicates for each group. (J-K) Human ovarian cancer cell line
15 A2780 were treated with vehicle, 3 μ M TP-10, 1 μ M of DOX or combination of 3 μ M TP-10 and
16 1 μ M of DOX as indicated for 24 hours. (J) Representative images of immunostaining of Ki-67,
17 A2780 cells were fixed, and immunostained for Ki-67, and counterstained for nuclei with DAPI.
18 Scale bars: 50 μ m. (K) Quantification of percentage of Ki-67 positive cells, n = 8 random fields
19 from 3 independent experiments for each group. Data were represented as mean \pm SEM. Statistics:
20 Welch ANOVA with Dunnett's T3 corrections for 3 comparisons in A; one-way ANOVA with
21 Holm-Sidak post-hoc test for 3 comparisons in F-I and K.

22

Supplemental Figure S7

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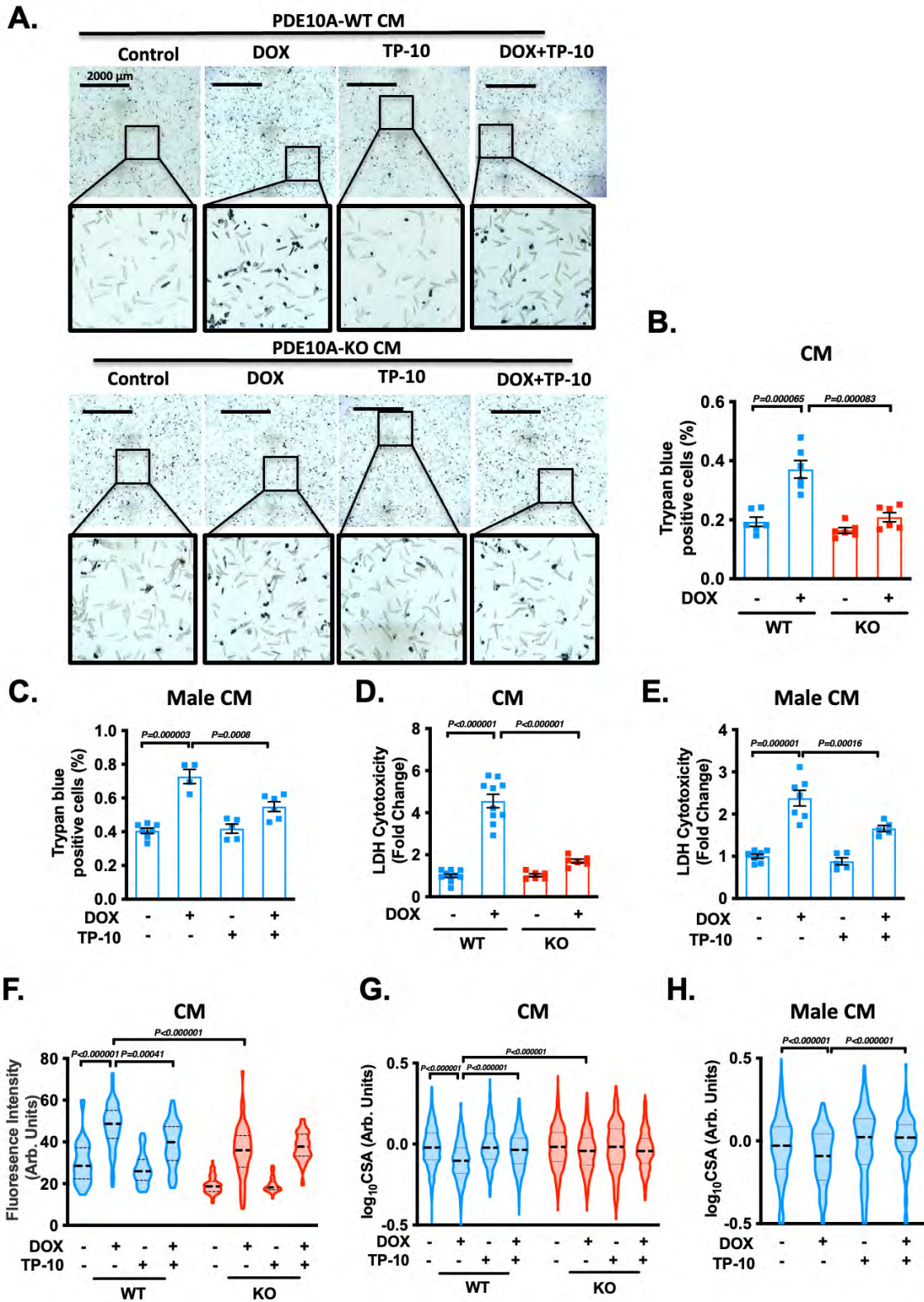
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1 **Supplemental Figure S7: PDE10A inhibition protects doxorubicin-induced cardiotoxicity in**
2 **nude mice.**

3 A2780 cells with expression of luciferase were injected into flanks of female nude mice at the age
4 of 6 weeks. 10 days post-inoculation of the tumor cells, the animals received the injection of saline,
5 DOX (1.5mg/kg), TP-10 (succinate form, 4.5 mg/kg/day), or DOX plus TP-10 daily for 2 weeks.
6 Cardiac function was monitored by echocardiography throughout the study. (A) Longitudinal
7 echocardiographic analysis of ejection fraction. (B) Representative images of whole hearts. Data
8 were represented as mean \pm SEM. Statistics: repeated measures ANOVA with Holm-Sidak post-
9 hoc test for 2 comparisons at day 14 and 1 comparison between Vehicle/day 14 vs. Vehicle/day -
10 10 (#) in A. Animal numbers: n = 7-10.

11

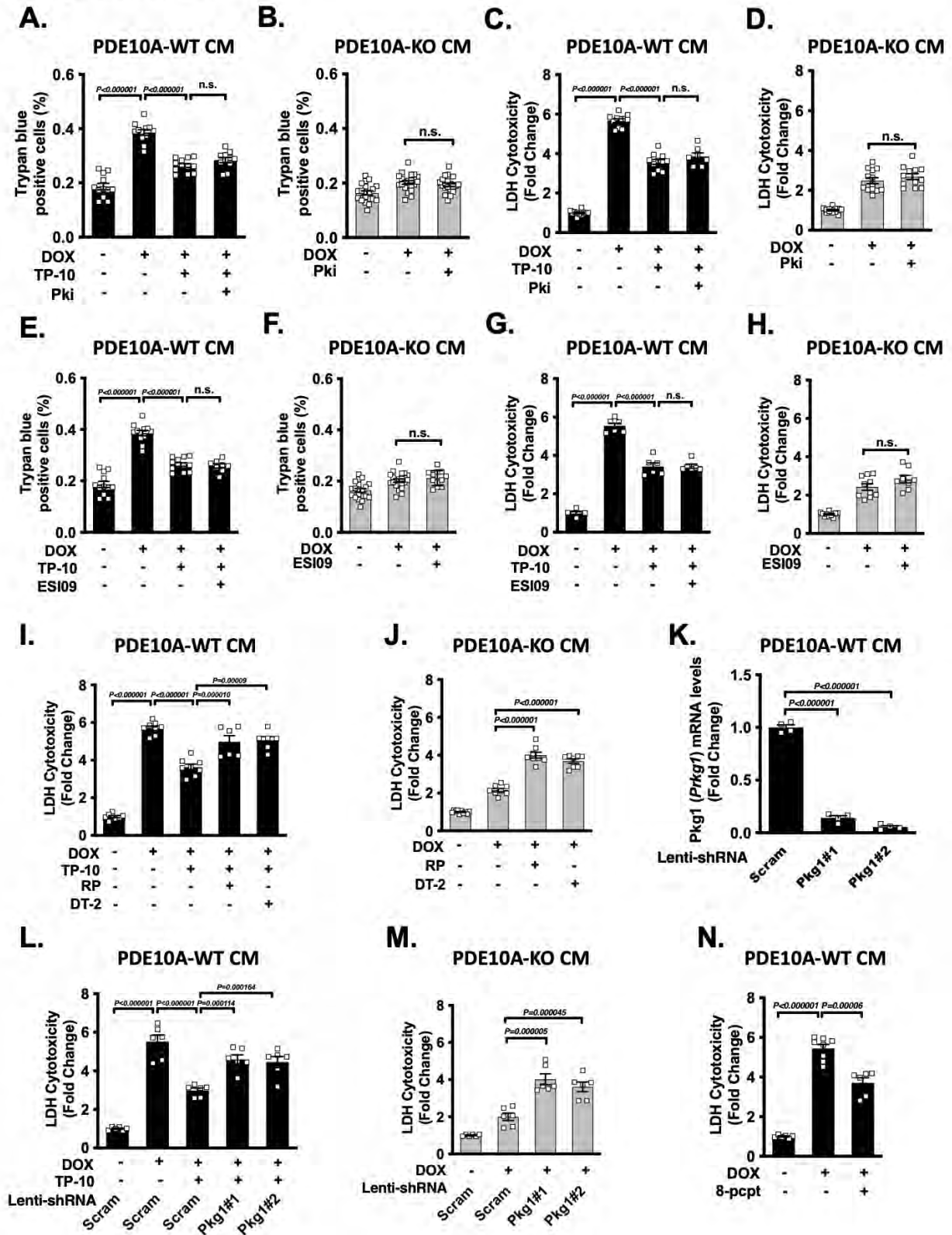
1 Supplemental Figure S8



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1 **Supplemental Figure S8: PDE10A inhibition or deficiency alleviates doxorubicin-induced**
2 **cardiomyocyte death and atrophy *in vitro*.**
3 Cardiomyocytes (CMs) isolated from PDE10A-WT or KO mice were stimulated with 10 μ M DOX
4 in the presence of 300 nM TP-10 or vehicle; (A) Trypan blue staining after 24 hours. The scale bar
5 is 2000 μ m. (B) Quantification of trypan blue staining. 20 random fields were assessed per
6 treatment group, n = 6 replicates from 3 mice for each group. (C) Quantification of trypan blue
7 staining of CMs isolated from male PDE10A-WT mice. 20 random fields were assessed per
8 treatment group, n = 4-7 replicates from 3 mice for each group. (D) Quantification of lactate
9 dehydrogenase (LDH) cytotoxicity, n = 6 replicates from 3 mice for each group. (E) Quantification
10 of lactate dehydrogenase (LDH) cytotoxicity of CMs isolated from male PDE10A-WT mice, n =
11 5-7 replicates from 3 mice for each group. (F) Quantification of p-H2AX fluorescence intensity in
12 PDE10A-WT and PDE10A-KO CM, n = 50-80 CMs from 3 isolations. (G) Basal cell surface areas
13 (CSAs) were quantified from CMs isolated from PDE10A-WT and PDE10A-KO mice. CSAs were
14 averaged from n = 500-1500 myocytes from 3 isolations per group. (H) CSAs were quantified
15 from CMs isolated from male PDE10A-WT mice. CSAs were averaged from n = 150-300
16 myocytes from 3 isolations per group. Data were represented as mean \pm SEM (B-E) or median
17 with IQR (E-H). Statistics: mixed effect model with Sidak corrections for 2-3 comparisons as
18 indicated in B-H.
19

1 Supplemental Figure S9

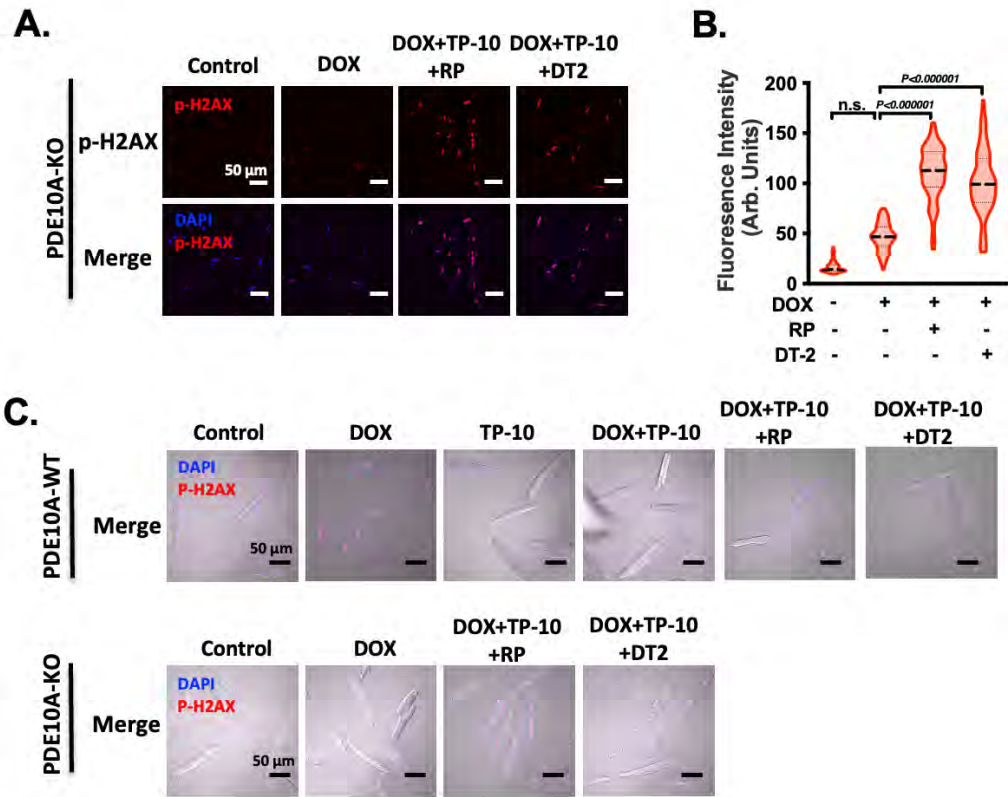


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1 **Supplemental Figure S9: Role of cAMP and cGMP signaling in PDE10A-mediated**
2 **regulation of CM death.**

3 (A-D) Role of PKA in cardiomyocyte (CM) death induced by DOX. PDE10A-WT CMs (A) and
4 PDE10A-KO CMs (B) were treated with or without vehicle or the PDE10A inhibitor TP-10 (300
5 nM) in the presence of vehicle or PKA inhibitor PKI (5 μ M) and stimulated with DOX (10 μ M)
6 for 24 hours. Cell death was measured by trypan blue staining. Cell death of PDE10A-WT CMs
7 (C) and PDE10A-KO CMs (D) was measured by LDH cytotoxicity assay. (E-H) Role of Epac in
8 CM death induced by DOX. PDE10A-WT CMs (E) and PDE10A-KO CMs (F) were treated with
9 or without vehicle or the PDE10A inhibitor TP-10 (300 nM) in the presence of vehicle or Epac
10 inhibitor ESI09 (5 μ M) and stimulated with DOX (10 μ M) for 24 hours. Cell death was measured
11 by trypan blue staining. Cell death of PDE10A-WT CMs (G) and PDE10A-KO CMs (H) was
12 measured by LDH cytotoxicity assay. (I-N) Role of PKG on CM death induced by DOX. PDE10A-
13 WT CMs (I) and PDE10A-KO CMs (J) were treated with or without vehicle or the PDE10A
14 inhibitor TP-10 (300 nM) in the presence of vehicle or 2 PKG inhibitors Rp-8-Br-PET-cGMPs (10
15 μ M) and DT-2 (1 μ M), and stimulated with DOX (10 μ M) for 24 hours. Cell death was measured
16 by LDH cytotoxicity assay. (K) WT CMs were transduced with lentivirus expressing scramble
17 shRNA, and 2 different PKG1 shRNAs for 48 hours. PKG1 (*Prkg1*) mRNA levels were measured
18 by qPCR. n = 6 replicates from 3 isolations for each group. (L-M) PDE10A-WT CMs (L) and
19 PDE10A-KO CMs (M) were transduced with lentivirus expressing scramble shRNA, and 2
20 different PKG1 shRNAs, and treated with or without vehicle or the PDE10A inhibitor TP-10 (300
21 nM) and stimulated with DOX (10 μ M) for 24 hours. Cell death was measured by LDH
22 cytotoxicity assay. (N) PDE10A-WT CMs were treated with PKG activator 8-pCPT-cGMP (10
23 μ M) and stimulated with DOX (10 μ M) for 24 hours. Cell death was measured by LDH
24 cytotoxicity assay. Data were represented as mean \pm SEM. Statistics: mixed effect model with
25 Sidak corrections for 1-4 comparisons as indicated in A-J and L-N. Welch ANOVA with Dunnett's
26 T3 corrections for 2 comparisons in K. n.s.: no significance difference. For trypan blue staining,
27 20 random fields were assessed per treatment group, n = 6-22 replicates from 3-5 mice for each
28 group. For LDH cytotoxicity assay, n = 6 -11 replicates from 4 isolations.
29

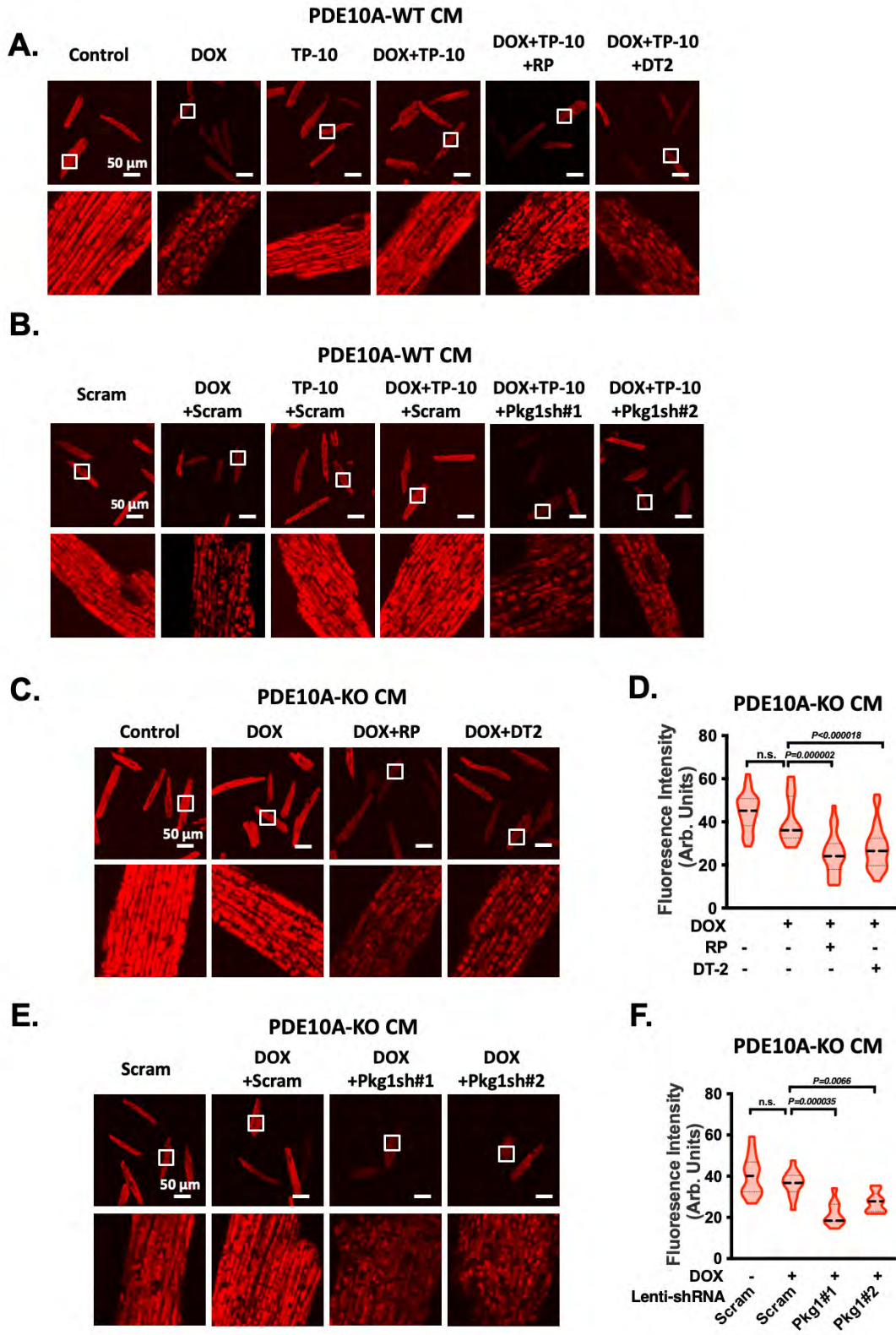
1 Supplemental Figure S10



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1 **Supplemental Figure S10: Effect of DOX and TP-10 on DNA damage in cardiomyocyte.**
2 (A) Representative images of immunostaining of p-H2AX in PDE10A-KO CMs were fixed, and
3 immunostained for p-H2AX, and counterstained for nuclei with DAPI. Scale bars: 50 μ m. (B)
4 Quantification of p-H2AX fluorescence intensity in PDE10A-KO CM, n = 50-100 CMs from 3
5 isolations. (C) Representative bright field images merged with fluorescence images of p-H2AX in
6 PDE10A-WT or PDE10A-KO CMs. CMs were fixed, and immunostained for p-H2AX, and
7 counterstained for nuclei with DAPI. Scale bars: 50 μ m. Data were represented as median with
8 IQR. Statistics: mixed effect model with Sidak corrections for 3 comparisons in B. n.s.: no
9 significance difference.
10

1 Supplemental Figure S11

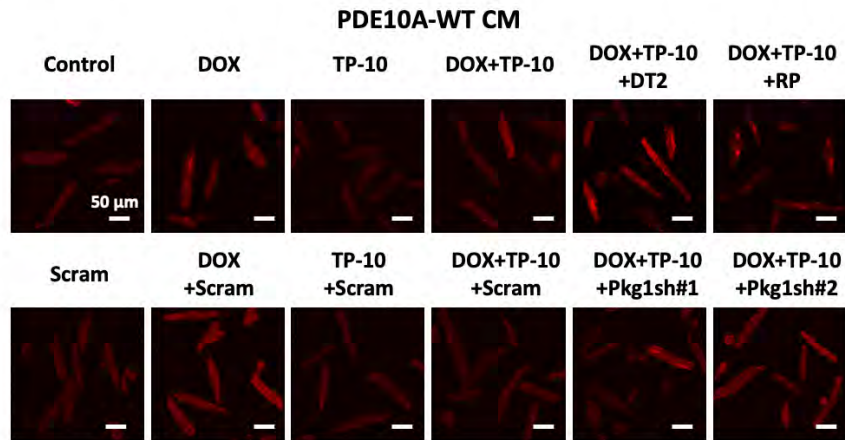


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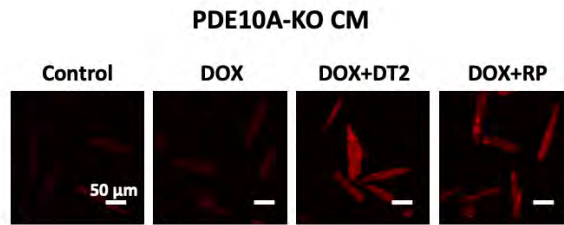
1 **Supplemental Figure S11: Role of cGMP signaling in PDE10A-mediated regulation of DNA**
2 **damage and mitochondria membrane potential in cardiomyocyte.**
3 (A-B) Representative images of PDE10A-WT CMs loaded with TMRE as a probe for
4 mitochondria membrane potential. Scale bars: 50 μm . (A) PDE10A-WT CMs were treated with or
5 without vehicle or the PDE10A inhibitor TP-10 (300 nM) in the presence of vehicle or 2 PKG
6 inhibitors Rp-8-Br-PET-cGMPs (10 μM) and DT-2 (1 μM), and stimulated with DOX (10 μM) for
7 24 hours. (B) PDE10A-WT CMs were transduced with lentivirus expressing scramble shRNA,
8 and 2 different PKG1 shRNAs, and treated with or without vehicle or the PDE10A inhibitor TP-
9 10 (300 nM) and stimulated with DOX (10 μM) for 24 hours. (C) Representative images of
10 PDE10A-KO CMs loaded with TMRE. PDE10A-KO CMs were treated with 2 PKG inhibitors
11 Rp-8-Br-PET-cGMPs (10 μM) and DT-2 (1 μM), and stimulated with DOX (10 μM) for 24 hours.
12 (D) Quantification of TMRE fluorescence intensity. (E) Representative images of PDE10A-KO
13 CMs loaded with TMRE. PDE10A-KO CMs were transduced with lentivirus expressing scramble
14 shRNA, and 2 different PKG1 shRNAs, and stimulated with DOX (10 μM) for 24 hours. (F)
15 Quantification of TMRE fluorescence intensity. Data were represented as median with IQR.
16 Statistics: mixed effect model with Sidak corrections for 3 comparisons in D and F. n.s.: no
17 significance difference. For TMRE staining, 8-10 random fields were assessed per treatment group,
18 n = 20-60 cells from 3 mice for each group.
19

1 Supplemental Figure S12

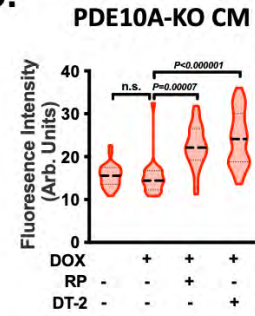
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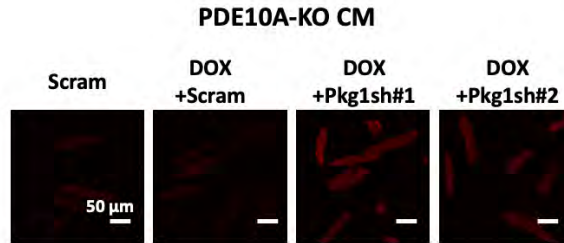
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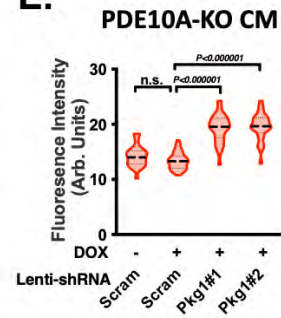
C.



D.



E.



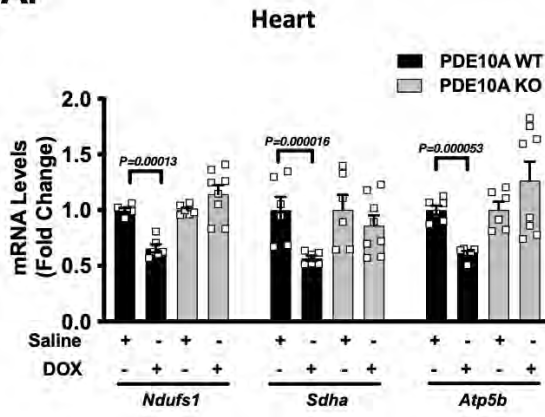
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1 **Supplemental Figure S12: Role of cGMP signaling in PDE10A-mediated regulation of ROS**
2 **generation.**

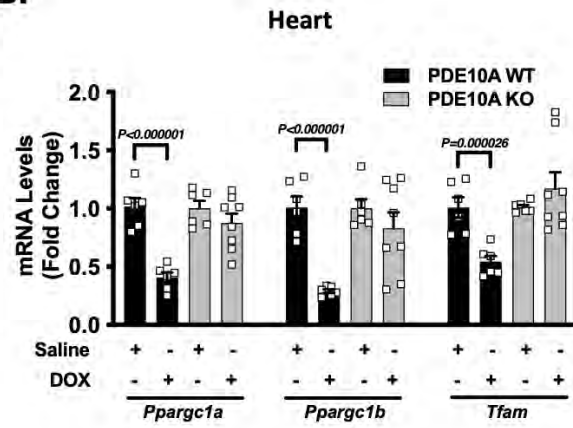
3 (A) Representative images of PDE10A-WT cardiomyocyte (CM) loaded with DHE as probes for
4 ROS generation. (B) Representative images of PDE10A-KO CM loaded with DHE as probes for
5 ROS generation. (C) Quantification of DHE fluorescence intensity. PDE10A-KO CMs were
6 treated with 2 PKG inhibitors Rp-8-Br-PET-cGMPs (10 μ M) and DT-2 (1 μ M), and stimulated
7 with DOX (10 μ M) for 24 hours. (D) Representative images of PDE10A-KO CM loaded with
8 DHE as probes for ROS generation. (E) Quantification of DHE fluorescence intensity. PDE10A-
9 KO CMs were transduced with lentivirus expressing scramble shRNA, and 2 different PKG1
10 shRNAs, and stimulated with DOX (10 μ M) for 24 hours. Data were represented as median with
11 IQR. Statistics: mixed effect model with Sidak corrections for 3 comparisons in C and E. n.s.: no
12 significance difference. For DHE staining, 8-10 random fields were assessed per treatment group,
13 n = 20-60 cells from 3 mice for each group.
14

1 Supplemental Figure S13

A.



B.



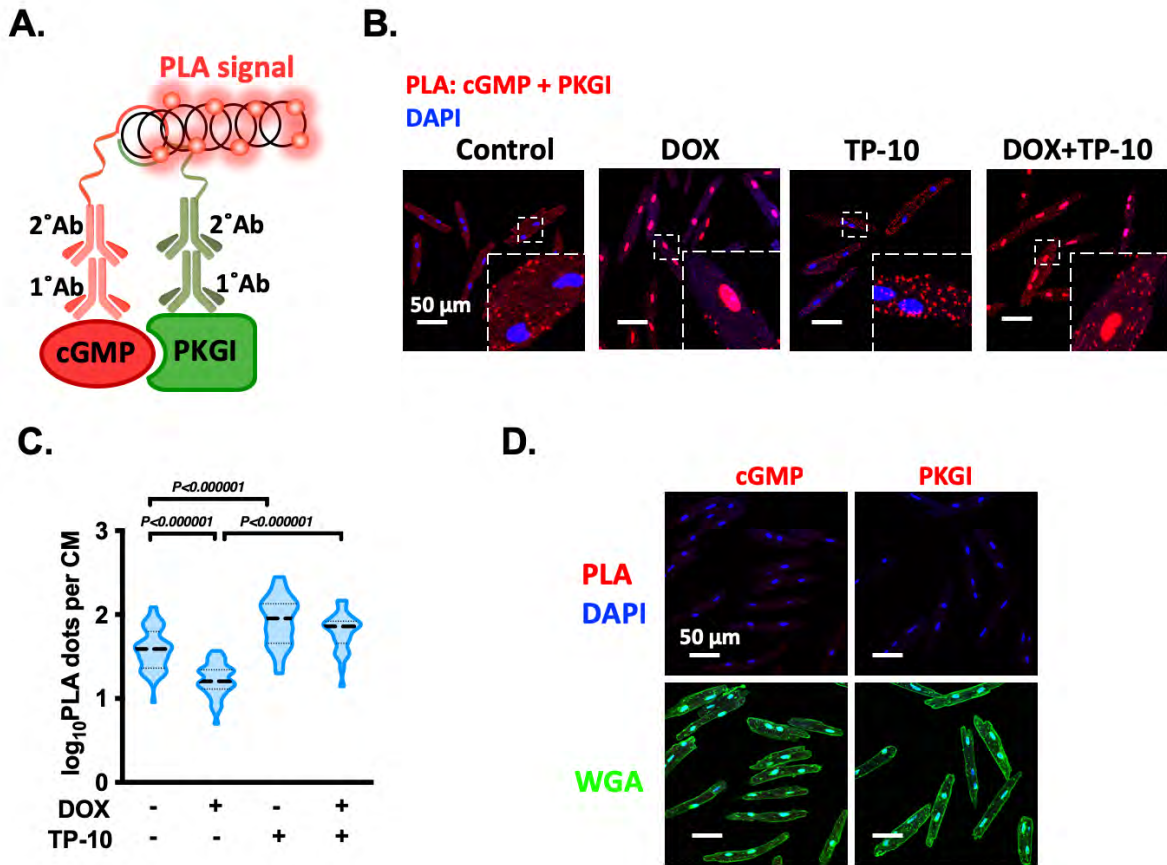
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1 **Supplemental Figure S13: Role of PDE10A in regulation of mitochondria function in the**
2 **heart.**

3 (A-B) The mRNA expression of genes involved in the electron transport chain (*Ndufs1*, *Sdha*, and
4 *Atp5b*) (A), and mitochondria biogenesis (*Ppargc1a*, *Ppargc1b*, and *Tfam*) (B) was determined in
5 hearts from PDE10A-WT or PDE10A-KO mice treated with DOX (25 mg/kg by intraperitoneal
6 injection (i.p.)) for 16 hours, normalized to *Gapdh*. n = 6-8 replicates. Data were represented as
7 mean \pm SEM. Statistics: Welch ANOVA with Dunnett's T3 corrections for 3 comparisons as
8 indicated in A-B.

9

1 Supplemental Figure S14

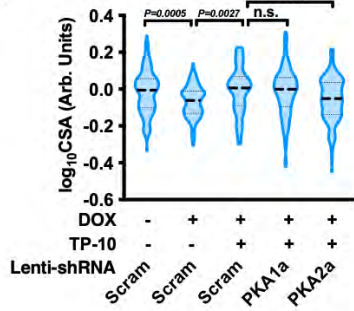


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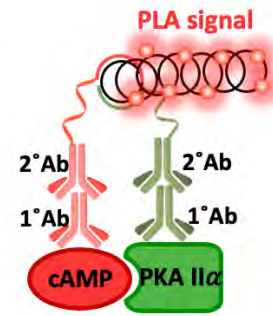
1 **Supplemental Figure S14: Effect of DOX and TP-10 on PKG activation in cardiomyocyte.**
2 (A) Proximity ligation assay (PLA) principal paradigm. (B-C) PLA images (B) and quantitative
3 results (C) showing PKG activity in cardiomyocytes (CMs) treated with DOX (10 μ M for 6 hours)
4 with or without TP-10 (300 nM for 20 minutes). PKG activity was detected by PLA (red) using a
5 cGMP antibody and PKGI antibody. The insets are the zoomed areas corresponding to the areas
6 with white dash borders in the whole CMs. CMs were counterstained for nuclei with DAPI. The
7 scale bar is 50 μ m. (D) PLA results of negative control performed by cGMP or PKGI antibody
8 alone in CMs. WGA image showed CM in the corresponding field. CMs were counterstained for
9 nuclei with DAPI. The scale bar is 50 μ m. Data were represented as median with IQR. Statistics:
10 one-way ANOVA with Holm-Sidak post-hoc test for 3 comparisons in C.
11

1 Supplemental Figure S15

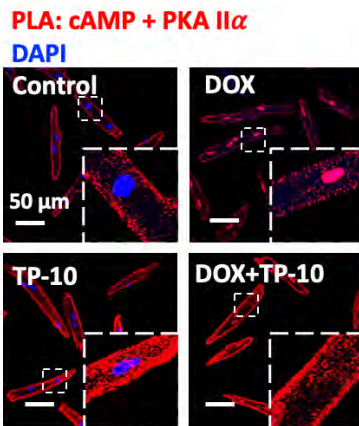
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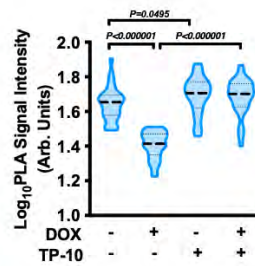
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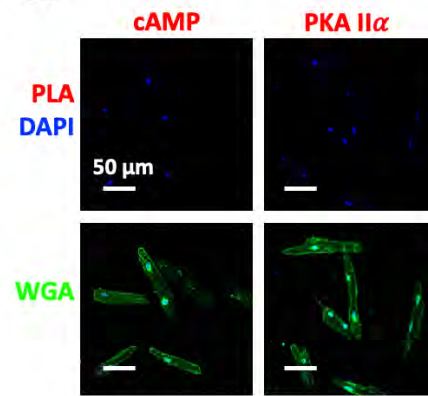
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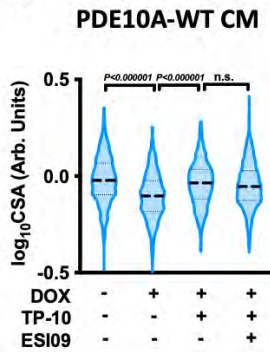
D.



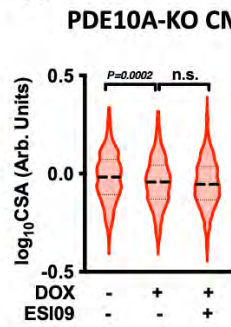
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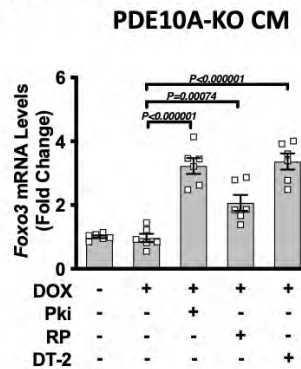
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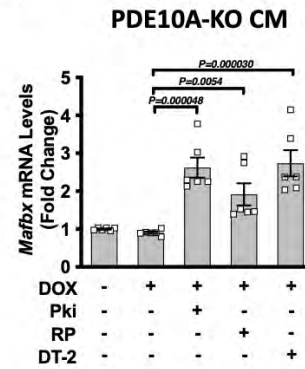
G.



H.



I.



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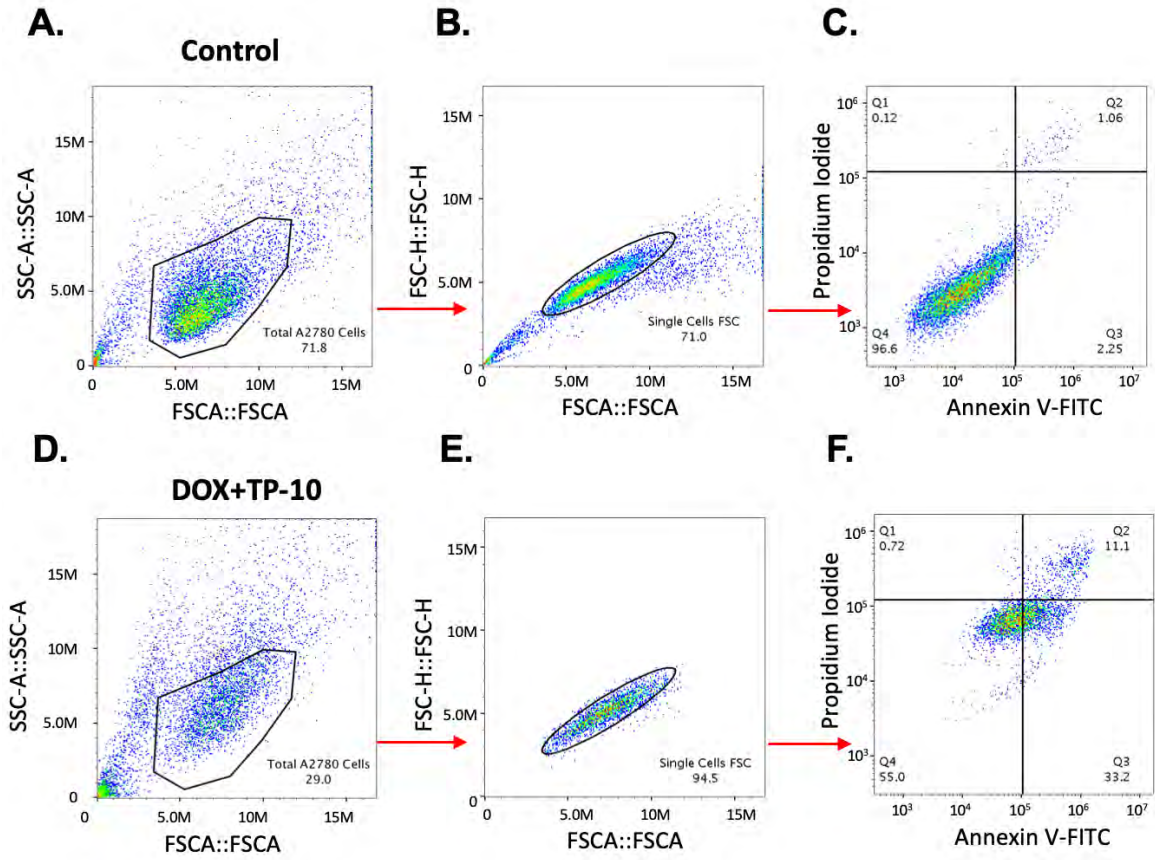
1 **Supplemental Figure S15: Role of cAMP and cGMP signaling in PDE10A-mediated**
2 **regulation of cardiomyocyte atrophy.**

3 (A) Quantified data of cell surface area of PDE10A-WT CMs transduced with lentivirus
4 expressing scramble shRNA, pka1a, and pka2a shRNA, and treated with or without vehicle or the
5 PDE10A inhibitor TP-10 (300 nM) and stimulated with DOX (10 μ M) for 24 hours. n > 100 CMs
6 from 3 isolations for each group. (B) Proximity ligation assay (PLA) principal paradigm. (C-D)
7 PLA images (C) and quantitative results (D) showing PKA activity in CMs treated with DOX (10
8 μ M for 6 hours) with or without TP-10 (300 nM for 20 minutes). PKA activity was detected by
9 PLA (red) using a cAMP antibody and PKA regulatory subunit PKAII α antibody. The insets are
10 the zoomed areas corresponding to the areas with white dash borders in the whole CMs. CMs were
11 counterstained for nuclei with DAPI. The scale bar is 50 μ m. (E) PLA results of negative control
12 performed by cAMP or PKAII α antibody alone in CMs. WGA image showed CM in the
13 corresponding field. CMs were counterstained for nuclei with DAPI. The scale bar is 50 μ m. (F-
14 G) Role of Epac in cardiomyocyte (CM) atrophy induced by DOX. PDE10A-WT CMs (E) and
15 PDE10A-KO CMs (F) were treated with or without vehicle or the PDE10A inhibitor TP-10 (300
16 nM) in the presence of vehicle or Epac inhibitor ESI09 (5 μ M) and stimulated with DOX (10 μ M)
17 for 24 hours. For quantification of cell surface area, n > 1000 CMs from 3-4 isolations for each
18 group. (H-I) CMs isolated from PDE10A-KO mice were treated with vehicle or PKA inhibitor
19 PKI (5 μ M), or 2 PKG inhibitors Rp-8-Br-PET-cGMPs (10 μ M) and DT-2 (1 μ M), and stimulated
20 with DOX (10 μ M) for 6 hours. qPCR analysis of cardiac atrophic genes *Foxo3* (H) and *Mafbx* (I),
21 normalized to *Gapdh*, n = 6 replicates from 3 isolations for each group. Data were represented as
22 mean \pm SEM (H-I) or median with IQR (A, D, and F-G). Statistics: mixed effect model with Sidak
23 corrections for 2-4 comparisons as indicated in A, F-G; one-way ANOVA with Holm-Sidak post-
24 hoc test for 3 comparisons in D; Welch ANOVA with Dunnett's T3 corrections for 3 comparisons
25 in H-I. n.s.: no significance difference.

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1 Supplemental Figure S16

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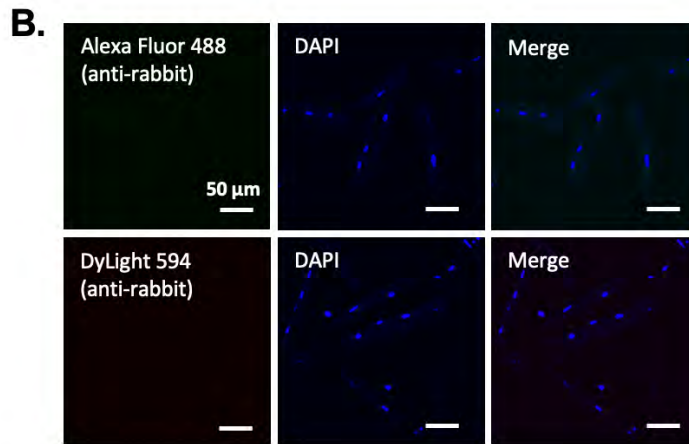
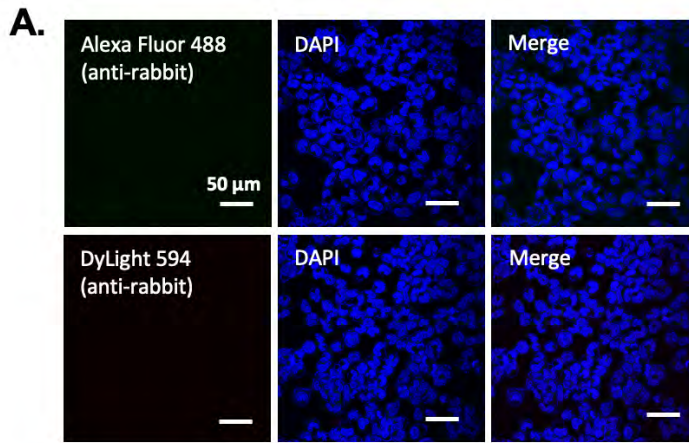


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1 **Supplemental Figure S16: The gating strategy for the Annexin-V/PI analysis.**
2 (A-F) Representative flow cytometry images of A2780 cells from control (upper panel) and
3 DOX+TP-10 (lower panel) treated groups. (A and D) A2780 were gated on forward (FSC) versus
4 side scatter (SSC) to select the cell population. (B and E) Next, the cells were gated on FSC-Height
5 (FSC-H) vs FSC-Area (FSC-A) to exclude all the doublets and to generate the singlets gate. (C
6 and F) Finally, all the subpopulations were analyzed on the Annexin V-FITC versus PI scatter for
7 live, early and late apoptosis.
8

1 Supplemental Figure S17

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1 **Supplemental Figure S17: Negative controls for immunostaining.**
2 (A) A2780 cells were incubated with anti-rabbit Alexa Fluor 488 (top panel) and DyLight 594
3 (bottom panel), and counterstained for nuclei with DAPI. Scale bars: 50 μm . (B) Cardiomyocytes
4 were incubated with anti-rabbit Alexa Fluor 488 (top panel) and DyLight 594 (bottom panel), and
5 counterstained for nuclei with DAPI. Scale bars: 50 μm .
6