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Supplementary information

Inhibition of PAI-1 shifts cardiomyocyte fate from senescence toward apoptosis and mitigates doxorubicin-induced cardiotoxicity

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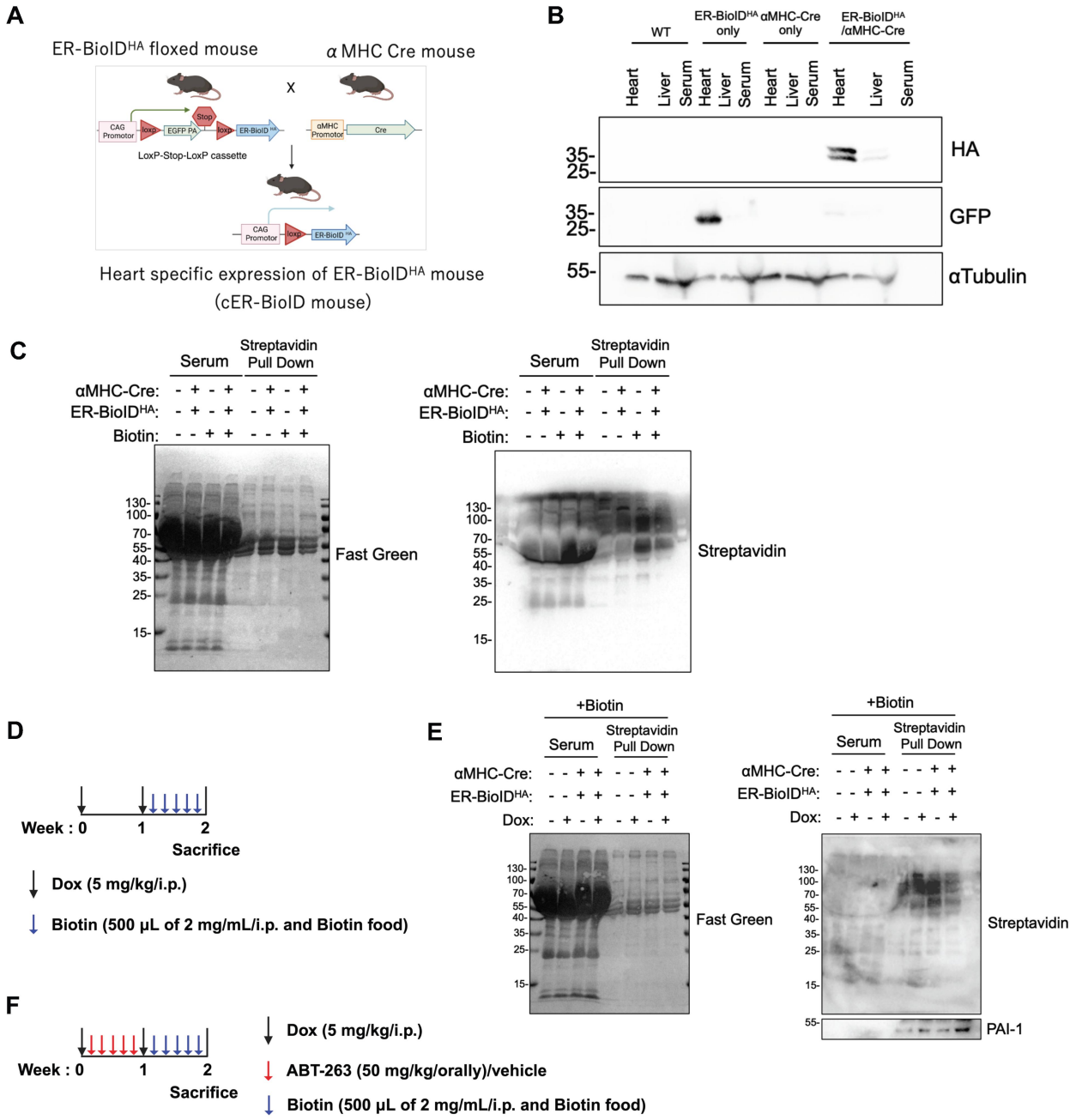


Figure S1. Characterization of the cardiac.specific secretome mouse and experimental protocols. (A) Generation of the cardiac-specific secretome mouse; (B) Cardiac-specific expression of cER-BioID was confirmed in αMHC-Cre-positive mice. Cre-mediated excision within tissues results in an inverse correlation between HA and GFP expression. αTubulin is shown as a loading control; (C) Detection of biotinylation in the srum of mice following biotin administration; (D) Schematic representation of the Dox and biotin administration protocol; (E) Dox-treated Myh6-Cre; ER-BioID mice exhibited increased PAI-1 level; (F) Experimental design for the combined administration of Dox, ABT-263, and biotin. Dox: doxorubicin.

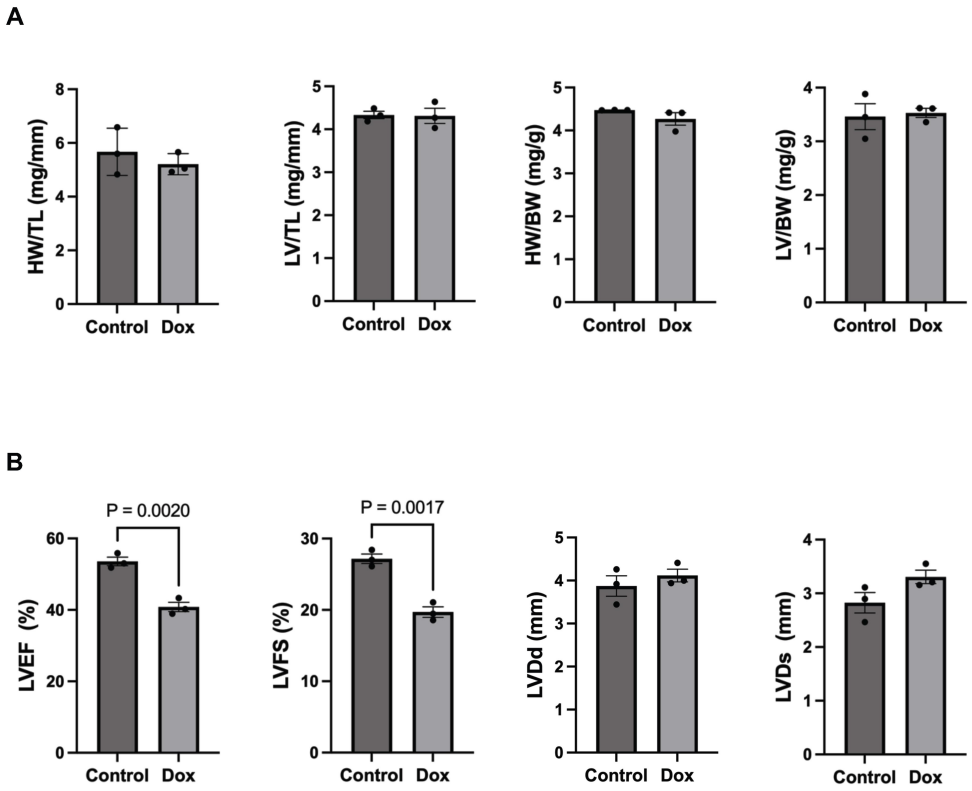
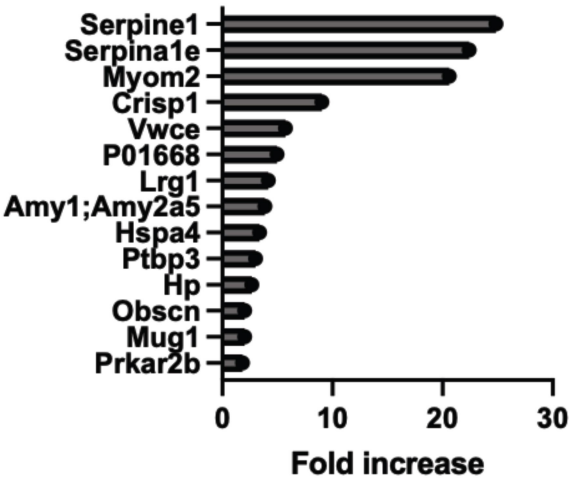


Figure S2. Validation of Dox.induced cardiac dysfunction in *cER.BioID* mice. (A) Body and organ weight indices in control and Dox-treated *cER-BioID* mice. HW heart weight; TL tibial length; LV left ventricular weight; BW body weight; (B) Echocardiographic assessment of cardiac function in control and Dox-treated *cER-BioID* mice. LVEF, LVFS, LVDd, and LVDs in control and Dox-treated *cER-BioID* mice. n= 3 per group. Data are presented as mean ± SEM. Statistical significance was assessed using unpaired Student's t-test. $P < 0.05$ was considered statistically significant. LVEF: left ventricular ejection fraction; LVFS: left ventricular fractional shortening; LVDd: left ventricular end-diastolic diameter; LVDs: left ventricular end-systole.

A

PG.Protein Descriptions	PG.Genes	No. of Peptides	Ratio (PBS+Biotin/ PBS)	Ratio (Dox+Biotin/ PBS)	Ratio (Dox+Biotin/ PBS+Biotin)
Plasminogen activator inhibitor 1	Serpine1	17	0.3	7.8	24.8
Alpha-1-antitrypsin 1-5	Serpina1e	6	2.1	46.5	22.4
Myomesin 2	Myom2	58	0.1	2.6	20.6
Cysteine-rich secretory protein 1	Crisp1	2	1.6	14.9	9.0
von Willebrand factor C and EGF domain-containing protein	Vwce	1	0.3	1.9	5.7
Ig kappa chain V-III region CBPC 101		1	1.2	5.8	5.0
Leucine-rich HEV glycoprotein	Lrg1	3	0.6	2.3	4.2
Alpha-amylase 1	Amy1;Amy2a5	2	1.1	4.2	3.9
Heat shock 70 kDa protein 4	Hspa4	3	0.6	2.0	3.4
Polypyrimidine tract binding protein 3	Ptbp3	1	1.2	3.7	3.0
Haptoglobin	Hp	2	1.6	4.3	2.7
non-specific serine/threonine protein kinase	Obscn	2	0.8	1.6	2.0
Murinoglobulin-1	Mug1	82	1.9	3.7	2.0
cAMP-dependent protein kinase type II-beta regulatory subunit	Prkar2b	7	0.9	1.6	1.8

B



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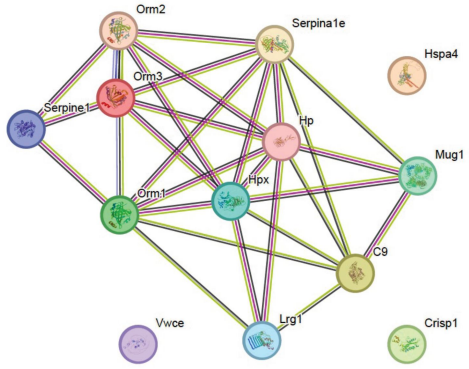

Figure S3. Identification of PAI-1 as a secreted protein induced by Dox using *cER.BioID* mice. (A) Mass spectrometry analysis of serum collected from mice treated with PBS, PBS + biotin, or Dox + biotin; (B) Secreted proteins ranked by fold change between Dox + biotin and PBS + biotin treatment groups; (C)Protein-protein interaction network of the identified secreted proteins generated by STRING analysis. Dox: doxorubicin; STRING: Search Tool for the Retrieval of Interacting Gene/Protein.

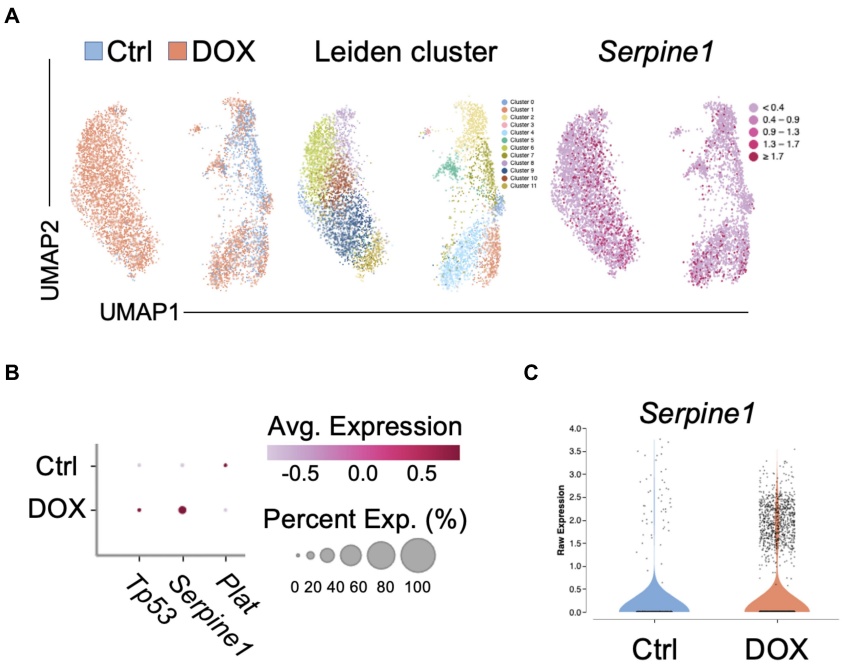

Figure S4. Dox treatment upregulated *Serpine1* expression in cardiomyocytes. (A) UMAP plots of single-cell transcriptomes from Ctrl (blue) and Dox-treated (orange) mouse hearts. Cells are annotated by treatment condition, Leiden clusters and *Serpine1* expression levels; (B) Dot plot showing expression of *Tp53* and *Serpine1* and *Plat* across conditions, where dot size represent the percentage of cells expressing each gene and color scale reflects average expression; (C) Violin plots comparing *Serpine1* expression in control versus Dox-treated cardiomyocytes. Ctrl: control; Dox: doxorubicin.

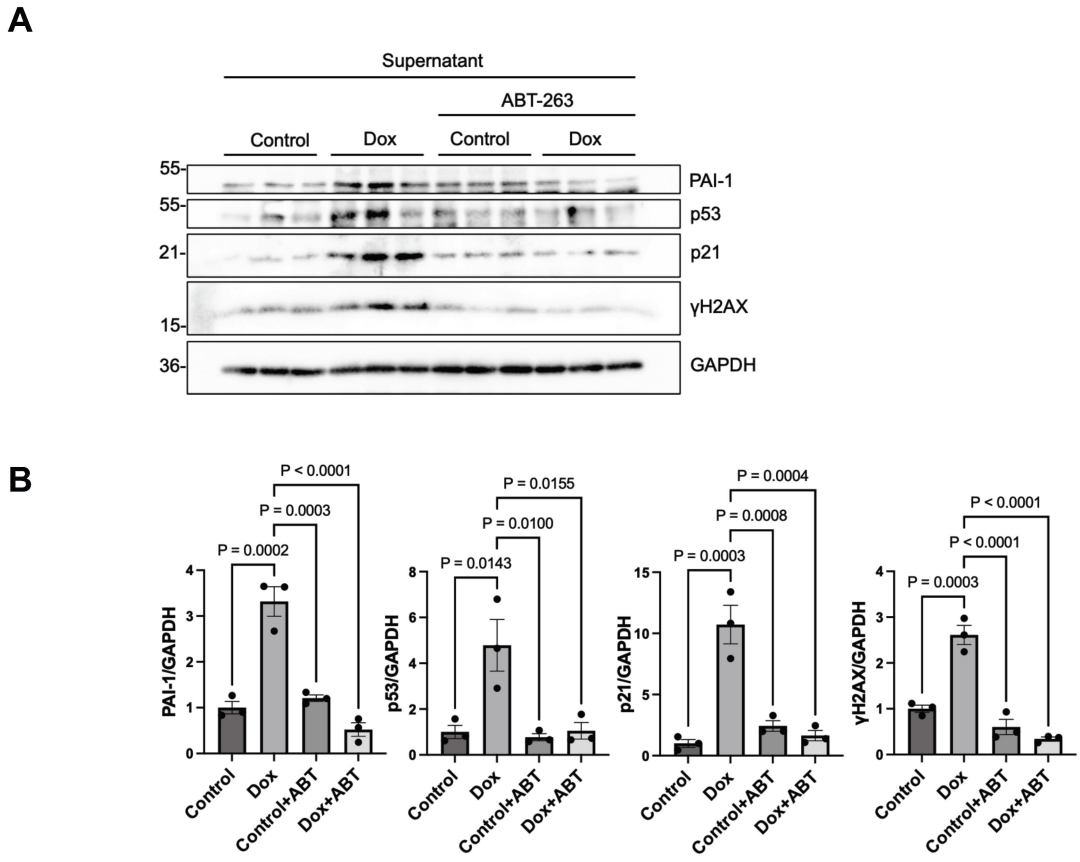

Figure S5. Supernatant from NRVMs treated with Dox in the presence of ABT.263 failed to upregulate senescence markers. (A) Western blot analysis of PAI-1, p53, p21, and γ H2AX in NRVMs treated with conditioned media collected from NRVMs exposed to PBS (Control), Dox, PBS + ABT-263, or Dox + ABT-263 for 72 hours; (B) Quantification of band intensity for each protein. n = 3 per group. Data are presented as mean ± SEM. Statistical significance was assessed using one-way ANOVA followed by Bonferroni–Dunn *post hoc* test. $P < 0.05$ was considered statistically significant.

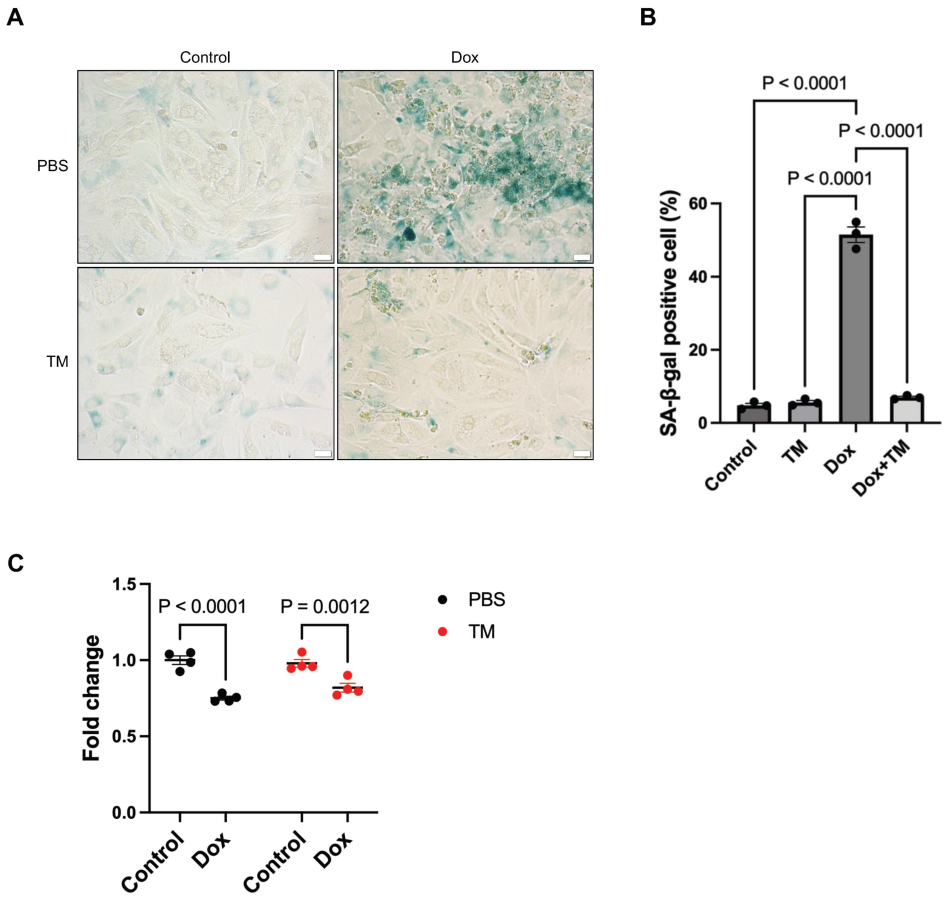

Figure S6. PAI-1 inhibition reduces cardiomyocyte senescence without affecting doxorubicin cytotoxicity in cancer cells. Neonatal rat ventricular myocytes (NRVMs) were treated with Dox (100 nM) and/or TM5275 (10uM) for 72 hours. (A) Representative images of SA-β-gal staining. Scale bar = 20 μm; (B) Quantification of SA-β-gal-positive cells; (C) EO771 murine breast cancer cells were treated with Dox (100 nM) in the presence or absence of the PAI-1 inhibitor TM5275 (25 μM) for 48 hours. Cell viability was assessed using CellTiter-blue assay. TM5275 did not attenuate Dox-induced cytotoxicity in EO771 cells. n = 3 per group in (B); n=4 per group in (C). Data are presented as mean ± SEM. Statistical significance was assessed using one-way ANOVA followed by Bonferroni–Dunn *post hoc* test in (B); Statistical significance was assessed using two-way ANOVA followed by Sidak's multiple comparison test in (C). $P < 0.05$ was considered statistically significant. Dox: doxorubicin; SA-β-gal: SA-β-galactosidase.

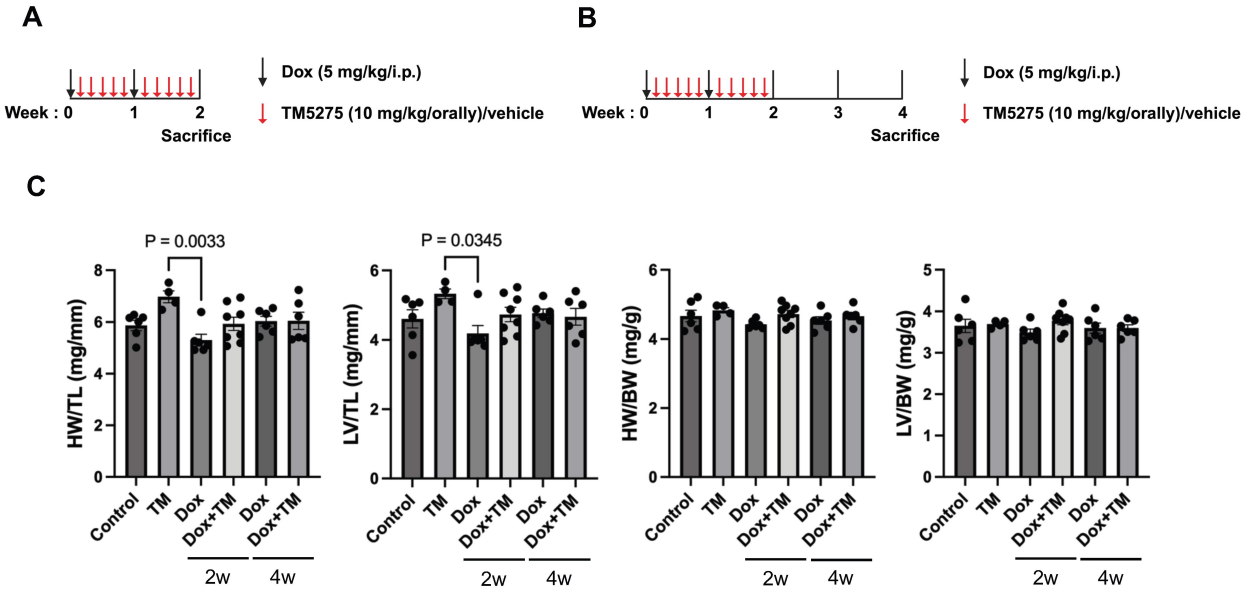

Figure S7. Experimental protocols and evaluation of cardioprotective effects of PAI-1 inhibition. (A) Schematic representation of the acute-phase protocol used to evaluate the cardioprotective effects of PAI-1 inhibition. Wild-type mice were treated with Dox with or without the PAI-1 inhibitor TM5275 during the acute phase, without a subsequent rest period; (B) Schematic representation of the delayed protocol. Mice received the same Dox and TM5275 treatments as in (A), followed by a 2-week rest period before evaluation; (C) Physiological measurements of control, TM5275-treated, Dox-treated, and Dox + TM5275-treated mice during the acute phase and after the 2-week rest period. HW heart weight; TL tibial length; LV left ventricular weight; BW body weight. $n = 6$ per group. Data are presented as mean ± SEM. Statistical significance was assessed using one-way ANOVA followed by Bonferroni–Dunn *post hoc* test. $P < 0.05$ was considered statistically significant. Dox: doxorubicin.