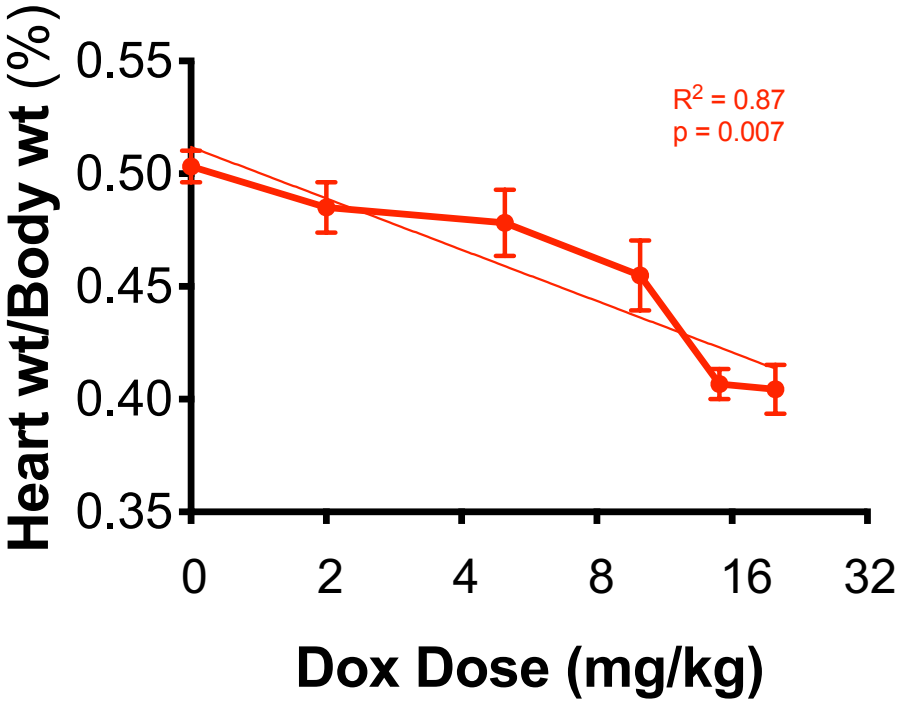
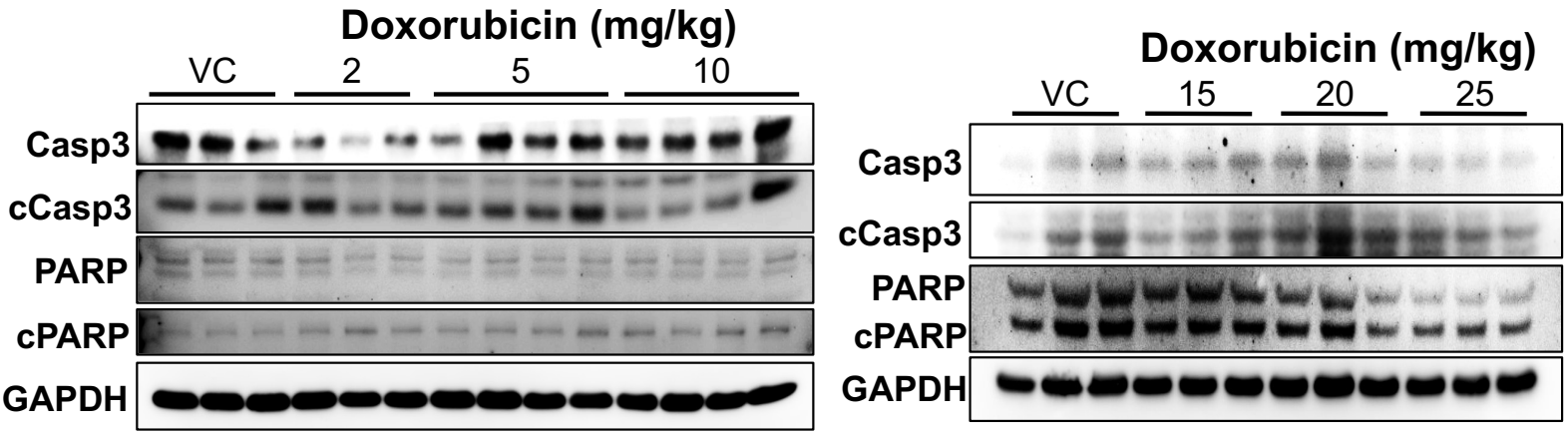
SUPPLEMENTAL MATERIAL

Supplemental Figure 1



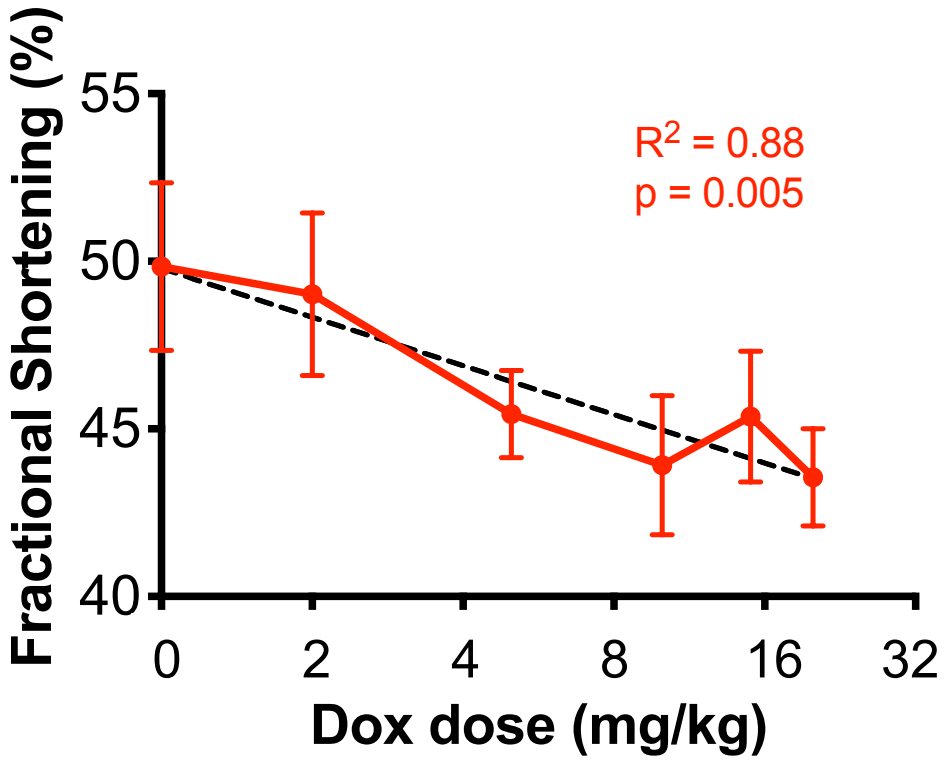
Supplemental Figure 1. Heart weight/body weight by log-2 transformation of doxorubicin dose. C57Bl6J mice were give a single dose of intraperitoneal doxorubicin (dox) 7 days prior to sacrifice (n=9 for 20 mg/kg, n=6 for all other doses)**.** Heart weight was indexed to body weight. Dox dose was log-2 transformed for logistic regression.

Supplemental Figure 2



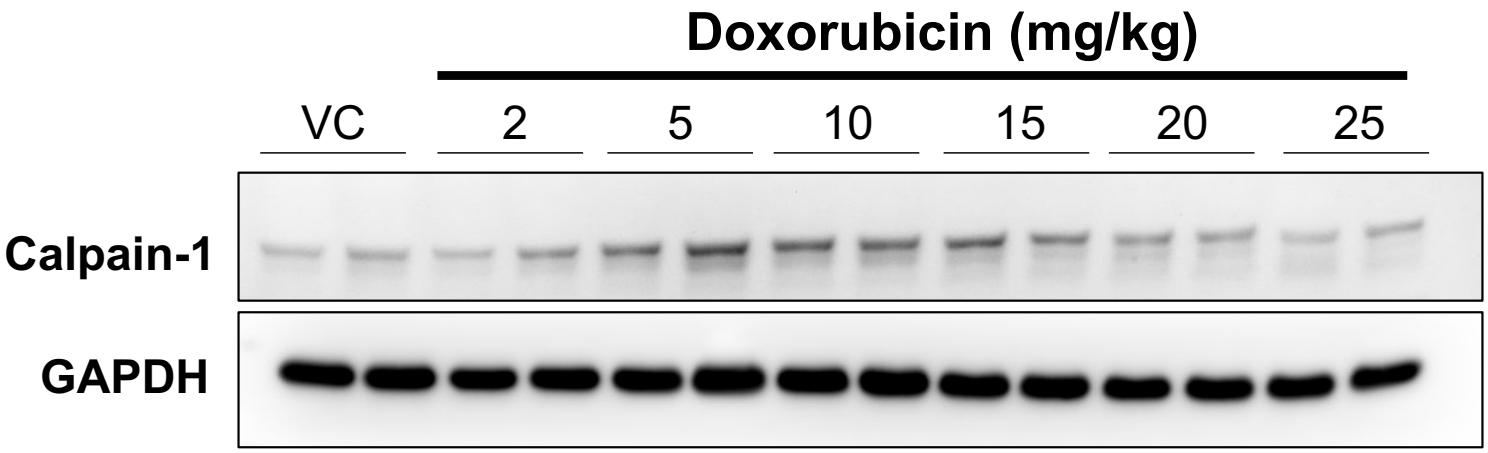
Supplemental Figure 2. **Doxorubicin does not predictably affect abundance of apoptotic markers in the heart.** Mice were sacrificed 7 days after treatment with vehicle control (VC) or doxorubicin I.P. one time at the indicated doses. Heart lysates were prepared for immunoblotting. Summary densitometry is displayed in **Figure 2C**. Casp3= caspase 3; cCasp3 = cleaved caspase 3; GAPDH = glyceraldehyde phosphate dehydrogenase; PARP = poly ADP ribose polymerase; cPARP = cleaved poly ADP ribose polymerase

Supplemental Figure 3



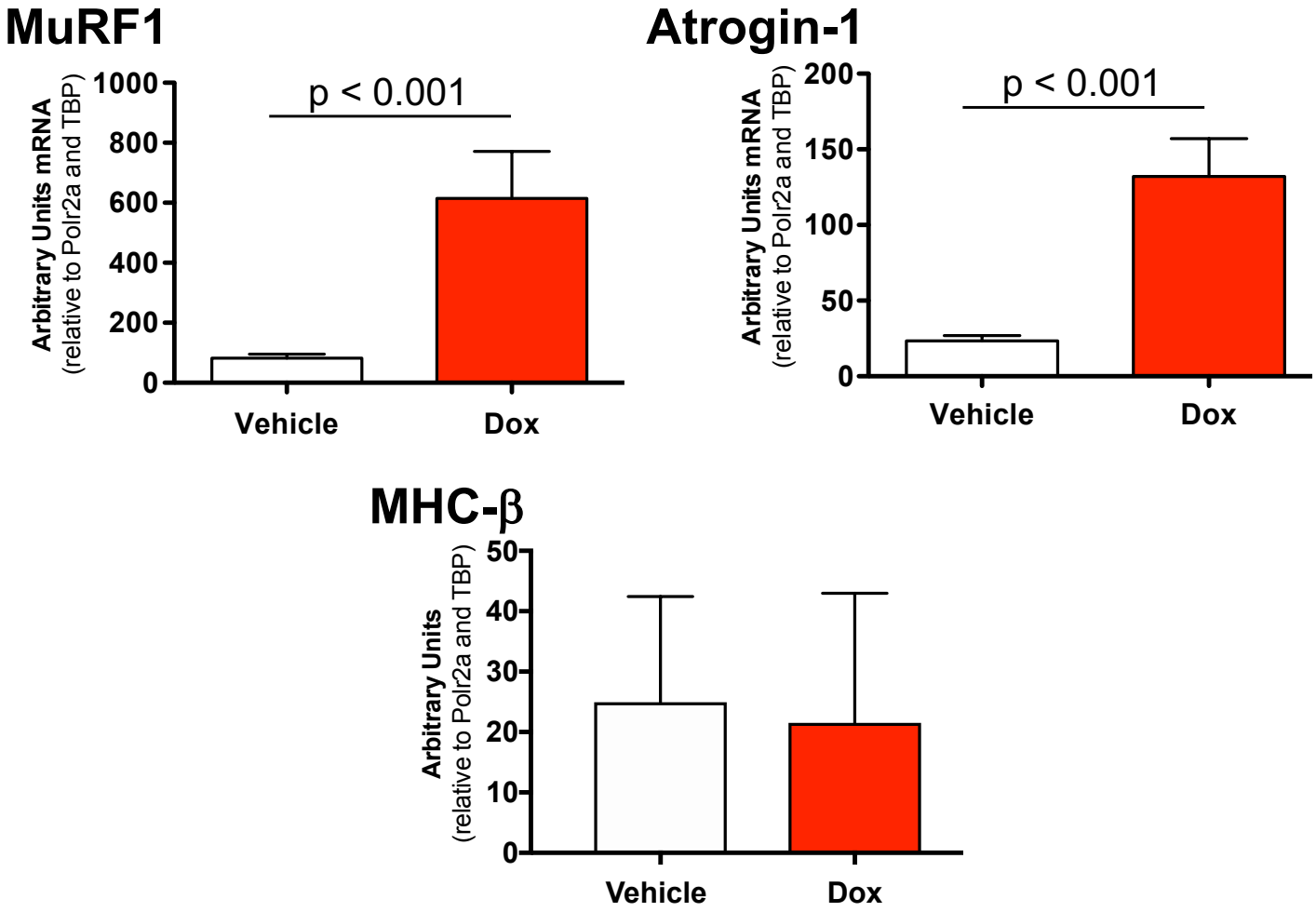
Supplemental Figure 3. Fractional shortening by log-2 transformation of doxorubicin dose. C57Bl6J mice were give a single dose of intraperitoneal doxorubicin (dox) 7 days prior to sacrifice (n=15 for vehicle, n=9 for 20 mg/kg, n=6 for all other doses)**.** Contractile function (fractional shortening) was measured by conscious echocardiography. Dox dose was log-2 transformed for logistic regression.

Supplemental Figure 4



Supplemental Figure 4. Doxorubicin does not affect the abundance of calpain-1 in the heart. Mice were sacrificed 7 days after intraperitoneal injection of doxorubicin or vehicle control (VC). Immunoblotting of heart lysates compared the abundance of calpain-1 protein with the loading control, GAPDH.

Supplemental Figure 5



Supplemental Figure 5. Doxorubicin increases skeletal muscle expression of MuRF-1 and Atrogin-1 in vivo. Mice were sacrificed 7 days after intraperitoneal injection of doxorubicin 20 mg/kg or vehicle control. Quantitative RT-PCR assayed abundance of MuRF1, Atrogin-1, and myosin heavy chain β relative to two reference genes.

Materials and Methods

Animals: C57Bl6J mice were from Jackson Laboratory (dose-response experiments) or from our MuRF1$^{-/-}$ breeding colony (MuRF1$^{-/-}$ and MuRF1$^{+/+}$ littermates).[1] 8-12 week old males and females were used in all experiments. Animal care and experimental protocols were approved by the UNC IACUC and complied with *Guide for the Care and the use of Laboratory Animals* (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Mice were euthanized and hearts weighed and flash frozen in liquid nitrogen and stored at -80°C. Legs were digested overnight in 1 M NaOH, tibias isolated, and measured to 0.01mm using digital calipers for normalization of heart weights.

Histology and immunofluorescence microscopy: Mice were heparinized and the heart was perfused with 10 mL PBS followed by 20 mL of 4% PFA/PBS through a 23G butterfly needle, then excised and placed in 4% PFA/PBS for 24h prior to transfer to 70% EtOH. Hearts were sectioned and stained using standard methods in the UNC Histology Research Core. Slides were scanned using an Aperio ScanScope (Aperio Technologies, Vista, CA) and analyzed in Aperio ImageScope software. For immunohistochemistry, hearts were fixed overnight in 4% PFA/PBS, incubated in 30% sucrose/PBS, and then frozen in O.T.C. medium (Tissue-Tek, Hatfield, PA, https://www.emsdiasum.com/). Frozen sections (10 microns), obtained with a Leica cryostat (Leica, Buffalo Grove, IL, http://www2.leicabiosystems.com), were placed on glass slides, dried at room temperature, and then incubated with primary antibodies against Additional slides were stained with wheat-germ-agglutinin-Alexa Fluor 488 conjugate (W11261; Invitrogen) and counterstained with DAPI (17985-50, EMS) to identify sarcolemmal membranes to visualize the myofiber diameter for quanitification. Slides were scanned on a Leica Aperio VERSA epifluorescent microscope digital scanner (DM6000 B, Leica) at 20X with an Andor Zyla sCMOS camera. Ventricular sections were visualized using Aperio ImageScope (Version 12.1.0.5029; Aperio Technologies) and exported as tiff files with scale bars (at 5X) and myocyte diameter was

quantified in a blinded manner using ImageJ software (by R.M.) on fluorescent micrographs from 4 hearts per genotype, analyzing 200 cells per heart across 3-6 sections.

Troponin I ELISA: Just prior to euthanasia ~200 microliters of whole blood was collected via retro-orbital bleed directly into serum separator tubes with clot activator (Cat.#367983, Becton Dickinson and Co., Franklin Lakes, NJ). Blood was incubated for 1 hour at room temperature, centrifuged for 10 minutes at 1300g, then serum was collected and immediately stored at -80°C. Subsequently, serum was used for Troponin I ELISA (CTNI-1-HS, Life Diagnostics, West Chester, PA) per manufacturer's protocol.

Mouse echocardiography: Conscious transthoracic echocardiography was performed on loosely restrained mice in the McAllister Heart Institute Animal Models Core using a VisualSonics Vevo 2100 ultrasound system (VisualSonics, Inc., Toronto, Ontario, Canada). Two-dimensional and M-mode echocardiography were performed in the parasternal long-axis view at the level of the papillary muscle. Left ventricular systolic function was assessed by fractional shortening (%FS = [(LVEDD − LVESD)/LVEDD] × 100). Reported values represent the average of at least five cardiac cycles per mouse. Sonographers and investigators were blinded to mouse treatment condition during image acquisition and analysis.

Quantitative reverse transcriptase PCR (qRT-PCR): Total RNA was isolated from cells and tissue (QiagenRNeasy Plus mini kit #74134) and analyzed using a NanoDrop (ThermoScientific). For qRT-PCR, one μg of RNA was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Life Technologies #4368814). Two step qRT-PCR reactions contained 2% of the cDNA product. All reactions were performed in triplicate in a Roche 480 Light Cycler. Relative quantitation of PCR products used the ΔΔCt method relative to two

validated reference genes (Tbp and Polr2a). Similar efficiencies were confirmed for all primers. All probes and primers were from Roche.

qRT-PCR primers:

Reference genes:

Tbp mouse F:ggcggtttggctaggttt; R:gggttatcttcacacaccatga; UPL Probe # 107

Polr2a mouse F:aatccgcatcatgaacagtg, R:tcatcatccattttatccacca; UPL Probe # 69

Target genes:

ANF mouse F: cacagatctgatggatttcaaga; R: cctcatcttctaccggcatc; UPL Probe # 25

Atrogin 1 mouse F: gccagagctgggtgaagac R: cttactgtatacctccttgttgcagt UPL Probe # 11

BNIP3 ThermoFisher Mm01185722_m1

Foxo3a ThermoFisher Mm01275601_g1

MuRF1 mouse F: tcctgcagagtgaccaagg. R: atggcgtagagggtgtcaaa UPL Probe # 17

MHC-alpha mouse F: ccaagactgtccggaatga R: tccaaagtggatcctgatga UPL Probe # 52

MHC-beta mouse F: ctgcaggacctggtggac, R: ggaacttggacaggttggtg; UPL Probe # 64

skAct mouse F: cctgccatgtatgtggctatc, R: ccagaatccaacacgatgc; UPL Probe # 56

Immunoblotting: Whole tissue or cell lysates were produced in RIPA buffer supplemented with PhosSTOP (Roche Diagnostics Corporation, Indianapolis, IN, USA) and protease inhibitor cocktail (Roche Diagnostics Corporation). Subsequently samples were incubated in 4× LDS sample buffer, including 2% β-mercaptoethanol, for 10 min at 70 °C. SDS–PAGE and immunoblotting were performed using the 4-12% Nupage gel system (Life technology, Foster City, CA, USA). Membranes were blocked in 5% milk/TBS-Tween, incubated in primary antibody overnight at 4°C, then secondary HRP-conjugated antibodies for 1h at room temperature. Images were generated using Amersham ECL Select Western Blotting Detection Reagent (GE

Healthcare life sciences, Marlborough, MA, USA) and the MultiDoc-It™ Imaging System (UVP gel image system, Upland, CA, USA).

Antibodies:

Atrogin-1 (sc-33782 H-300) and MuRF1 (sc-27642 C-20) were from Santa Cruz Biotechnology, Inc. (Dallas, TX). Myosin heavy chain, beta (M8421) was from Sigma-Aldrich (St. Louis, MO).

Human cardiac MRI: Cardiac MRI data were extracted from the DETECT-I study that was approved by the institutional review board of the Wake Forest School of Medicine. [2] All participants provided witnessed, written informed consent. The study population consisted of 70 participants receiving an anthracycline for the first time. Participants were ineligible for enrollment if they had a contraindication to CMR (implanted metal or electronic devices). No participants received radiation therapy during the study. They were recruited over 1.5 years from the hematology/oncology outpatient clinics and inpatient hospital facilities in the Wake Forest School of Medicine Comprehensive Cancer Center. Cardiac magnetic resonance (CMR) was performed at baseline and 1, 3, and 6 months after initiation of low to moderately dosed anthracycline administration according to specifications used in other large National Institutes of Health sponsored population-based studies of CV disease such as MESA (Multi-Ethnic Study of Athero-sclerosis).[3] CMR imaging was obtained early in the morning prior to the scheduled administration of chemotherapy. Analyses of all images were performed by individuals blinded to all study participant identifiers, including previous imaging and biomarker results (i.e., a blinded, unpaired read).

Statistics. For CMR analysis, the individual growth curves were visually assessed, with an average trend line fit by ordinary least squares regression. Rate of change in LVM was analyzed

by constructing a linear mixed model[4] with random intercepts and time effects. Time was modeled as a predictor of LV mass to assess within-subject change as well as the overall mean change for the population. All statistical analyses were carried out using SAS 9.4 (SAS Institute; Cary, NC). All other results are presented as mean ± SEM. Comparisons were made using t-test (groups of 2) or one-way ANOVA (groups of 3) with Tukey's post-hoc analysis (GraphPad Prism).

REFERENCES

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3. Rerkpattanapipat P, D'Agostino RB, Jr., Link KM, Shahar E, Lima JA, Bluemke DA, Sinha S, Herrington DM and Hundley WG. Location of arterial stiffening differs in those with impaired fasting glucose versus diabetes: implications for left ventricular hypertrophy from the Multi-Ethnic Study of Atherosclerosis. *Diabetes*. 2009;58:946-53.
4. Cheng J, Edwards LJ, Maldonado-Molina MM, Komro KA and Muller KE. Real longitudinal data analysis for real people: building a good enough mixed model. *Stat Med*. 2010;29:504-20.