

<https://doi.org/10.1038/s42003-026-11007-z>

Multi-omics insight into cardiac myofibril remodeling in post-prandial Burmese pythons



Thomas G. Martin^{1,2,15,16}, Lorena Suarez-Artiles^{3,16}, Kathleen C. Woulfe⁴, Elise G. Melhedegaard⁵, Yuxiao Tan^{1,2}, Dakota R. Hunt^{2,6}, Bruce E. Kirkpatrick^{2,7,8}, Lia Nguyen^{2,9}, Joseph Lee^{1,2}, Isabella Laskey^{2,9}, Kristi S. Anseth^{2,7,10}, Julien Ochala⁵, Michael Gotthardt^{3,11,12}✉, Philipp Mertins^{3,11,13,14}✉ & Leslie A. Leinwand^{1,2}✉

Burmese pythons exhibit cardiac remodeling in response to increased metabolic rate during digestion. Here, we performed single-myofibril mechanics measurements and myosin biochemical assays to evaluate the impact of feeding on the cardiomyocyte sarcomere in two experimental paradigms: normal feeding (one meal per month) and frequent feeding (eight meals per month). Myofibril tension and rate of relaxation increased during digestion in both paradigms, while frequent feeding was further associated with slower myofibril activation. To gain potential mechanistic insight, we performed multi-omics analyses. RNA sequencing identified increased relative concentration of some sarcomeric mRNAs during digestion; however, proteomics suggested a delay in sarcomere protein synthesis at the peak of remodeling, as the relative abundance of most sarcomere proteins declined. Analysis of post-translational modifications identified hundreds of sites on sarcomere proteins, including on the tension-regulating proteins titin and myosin. Our results detail the molecular underpinnings of cardiac remodeling in digesting Burmese pythons and suggest the mechanism for rapidly increasing myofibril function is a post-translational program.

Burmese pythons (*Python bivittatus*) utilize an ambush-hunting predation strategy that is associated with consuming large prey across months-long intervals of fasting¹. Consequently, they exhibit a tenfold or greater increase in metabolic rate during digestion, which coincides with increased size and activity of most organs, including the heart^{2,3}. Cardiac adaptations in digesting pythons include modest, but rapid development of cardiac hypertrophy, increased heart rate, and increased ventricular stroke volume² stemming from enhanced venous and mean circulatory filling pressures, which increase cardiac filling⁴. These factors contribute to approximately a

fivefold increase in cardiac output at the peak of digestion², which is comparable to the change in the human heart going from rest to exercise⁵. The factorial increase in these parameters and rapid timescale of cardiac remodeling in Burmese pythons positions them as a useful model for deciphering the molecular regulation of cardiac plasticity^{2,3}. Previous studies of Burmese pythons identified important roles for hypertrophy-promoting circulating factors^{6,7}, lipid regulation⁸, cardiomyocyte cell cycle re-entry⁹, and systemic oxygen supply-demand mismatch¹⁰ during adaptive cardiac remodeling. Nevertheless, the molecular mechanisms underlying cardiac

¹Department of Molecular, Cellular, and Developmental Biology, University of Colorado Boulder, Boulder, CO, USA. ²BioFrontiers Institute, University of Colorado Boulder, Boulder, CO, USA. ³Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany. ⁴Department of Medicine, Division of Cardiology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA. ⁵Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark. ⁶Department of Biochemistry, University of Colorado Boulder, Boulder, CO, USA. ⁷Department of Chemical and Biological Engineering, University of Colorado Boulder, Boulder, CO, USA. ⁸Medical Scientist Training Program, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA. ⁹Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO, USA. ¹⁰Materials Science and Engineering Program, University of Colorado Boulder, Boulder, CO, USA. ¹¹German Center for Cardiovascular Research (DZHK), partner site, Berlin, Germany. ¹²Department of Cardiology, Charité Universitätsmedizin Berlin, Berlin, Germany. ¹³German Cancer Consortium (DKTK), partner site, Berlin, Germany. ¹⁴Berlin Institute of Health (BIH), Berlin, Germany. ¹⁵Present address: Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE, USA. ¹⁶These authors contributed equally: Thomas G. Martin, Lorena Suarez-Artiles. ✉e-mail: gotthardt@mdc-berlin.de; philipp.mertins@mdc-berlin.de; leslie.leinwand@colorado.edu

functional changes in digesting Burmese pythons remain incompletely understood.

The smallest molecular unit of striated muscle contraction is the sarcomere¹¹. A paracrystalline structure composed of hundreds of proteins, the sarcomere mediates calcium (Ca^{2+})-dependent shortening of cardiac muscle via tightly-regulated interactions between actin thin filaments and myosin thick filaments¹¹. Sarcomere function can be tuned through changes in sarcomere protein isoform expression and post-translational modifications (PTMs), which contribute both to increased myofilament function with exercise^{12,13} and impaired function in heart failure^{14,15}. In Ball pythons (*Python regius*), a smaller snake species separated from Burmese pythons by ~20 million years of evolution¹⁶, increased active (Ca^{2+} -dependent) and decreased passive (Ca^{2+} -independent) tension in cardiac myofibrils were observed one day after feeding¹⁷. These results suggest that myofibril properties are modified to support enhanced cardiac function during digestion in pythons, but the enabling molecular changes at the sarcomere were not investigated. Previous work in Burmese pythons identified increased relative abundance of myosin heavy chain 6 (*MYH6*) mRNA post-prandially¹⁸, suggesting that myosin isoform switching may contribute to myofibril functional changes, as the predominant myosin in the python heart is myosin heavy chain 15 (*MYH15*)¹⁹. Further, studies in pythons and broad-snouted caimans—another reptile that consumes infrequent large meals—identified increased relative abundance of Ca^{2+} -handling proteins during digestion and enhanced ex vivo cardiac contractility^{20,21}. Whether sarcomere modifications contribute to changes in contractility during digestion in these extreme-feeding reptiles has not been explored.

We hypothesized that feeding engages coordinated, PTM-driven sarcomere adaptations that rapidly adjust myofibril mechanics to meet the transient metabolic demands of digestion. To test this, we investigated the effects of feeding on single-myofibril mechanics, relative sarcomere gene and protein abundance, and sarcomere PTMs in the Burmese python heart. We studied the responses to normal (one meal per month) and frequent (eight meals per month) feeding intervals^{8,9}, the latter of which is expected to induce sustained elevation of metabolism from constant digestion. Myofibril tension increased during digestion in both groups, and this effect was more pronounced after frequent feeding. Digestion was also generally associated with an increased rate of myofibril relaxation, while frequent feeding also reduced myofibril activation rate. Analysis of sarcomeric mRNAs by bulk RNA sequencing revealed increased relative abundance of several after feeding, yet proteomics identified a net decrease in relative sarcomere protein abundance, which coincided with decreased ventricular tissue density—consistent with an apparent dilution of sarcomere content during rapid tissue remodeling rather than a simple transcription-to-protein gain. Enrichment and quantification of PTMs (ubiquitination, phosphorylation, and acetylation) via mass spectrometry identified hundreds of sarcomere modifications that were significantly affected during digestion. Dozens of modifications were identified on the tension-regulating titin and myosin heavy chain proteins, which may contribute to the observed changes in myofibril function with feeding. Altogether, our results highlight the robust sarcomere remodeling induced by feeding in Burmese pythons and indicate that enhanced cardiac contractility during digestion is accompanied by increased myofibril performance and extensive PTM remodeling of key mechano-active proteins.

Results

Digestion is associated with increased myofibril active tension and altered activation and relaxation kinetics

Adult Burmese pythons were randomly assigned to receive 25% bodyweight rat meals in two different feeding intervals: normal feeding (one meal per month) and frequent feeding (FF, eight meals per month) (Fig. 1A)⁹. After 2 months of feeding in these intervals, the pythons were humanely euthanized, and cardiac ventricular tissue was collected. The timepoint of 3 days post-feeding (3DPF and FF-3DPF) was chosen for the study as this had previously been identified as the peak of metabolic activity and organ

remodeling post-prandially^{2,3,6}. Fasted pythons (28DPF and FF-28DPF) were included as controls for both feeding groups.

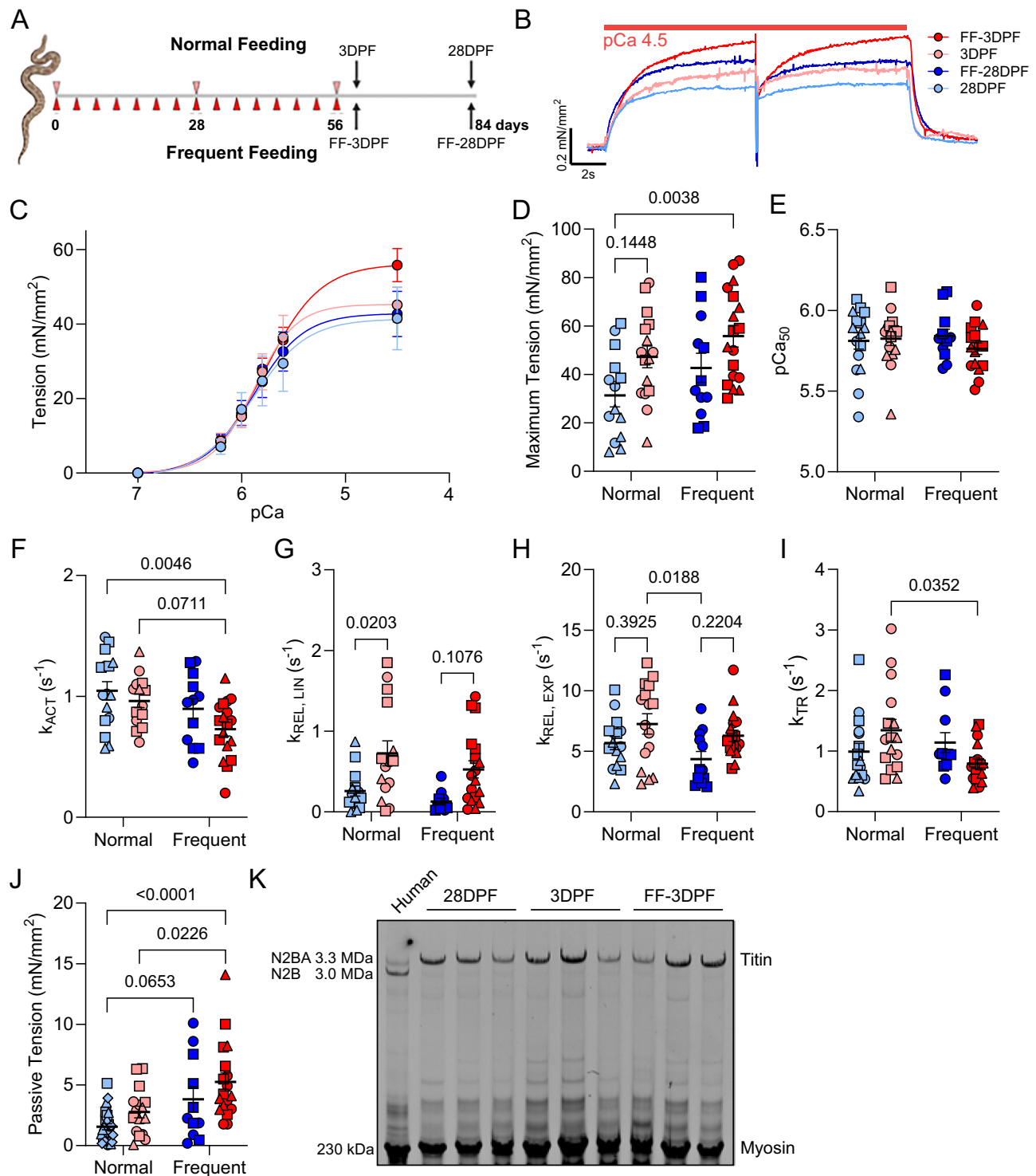
To determine the effects of digestion and feeding frequency on myofibril properties, single myofibrils were isolated, attached to a force transducer and high-speed length controller, and the kinetics of activation and relaxation (Fig. 1B and Fig. S1) were measured over a range of Ca^{2+} concentrations. Myofibril tension and Ca^{2+} sensitivity were determined by force-pCa measurements (Fig. 1C). There was no interaction between feeding frequency and time after feeding for maximal tension by two-way ANOVA; however, tension was significantly affected by both feeding frequency (frequent vs. normal) and time after feeding (3DPF vs. 28DPF), and the increase in tension was more pronounced during digestion after frequent feeding (Fig. 1D). There was no difference in Ca^{2+} sensitivity between groups (Fig. 1E). Myofibril rate of activation (k_{ACT}) was significantly affected by feeding frequency but not time after feeding, where frequent feeding led to decreased k_{ACT} (Fig. 1F). Meanwhile, the rates of the linear ($k_{\text{REL,LIN}}$) and exponential ($k_{\text{REL,EXP}}$) phases of relaxation both increased as functions of time after feeding but not feeding frequency (Fig. 1G, H). For the rate of tension redevelopment (k_{TR}), representing the sum of myosin cross-bridge attachment and detachment rates²², there was a significant interaction between feeding frequency and time after feeding, with decreased k_{TR} in FF-3DPF versus 3DPF (Fig. 1I).

Feeding increases myofibril passive tension independent of titin isoform changes

At fixed sarcomere lengths, myofibril force-pCa and activation/relaxation kinetics describe Ca^{2+} -dependent properties and are predominantly driven by thin filament Ca^{2+} -responsiveness and myosin cross-bridge cycling. However, Ca^{2+} -independent properties also play a role in regulating sarcomere function. Passive tension describes the elastic force generated by a myofibril at a given sarcomere length in the absence of Ca^{2+} and is primarily due to the spring-like sarcomere protein titin (interchangeable with TTN hereafter)^{23,24}. Passive tension, measured in a pCa 9 solution at a sarcomere length of 2.2 μm , increased as a function of time after feeding and feeding frequency, but the effect was more pronounced during digestion after a frequent feeding interval (Fig. 1J). Rheological assessment of stiffness and elastic properties at the ventricular tissue level revealed that stiffness (increased) and elasticity (decreased) were also significantly affected by increased feeding frequency, irrespective of time after feeding (Fig. S2A, B). In humans and other mammals, increased myofibril passive tension can result from expression of shorter titin isoforms²⁵ or changes in stiffness-modifying titin post-translational modifications²⁶. To determine whether feeding altered titin isoform expression, we performed agarose gel electrophoresis to separate titin isoforms (Fig. 1K). Compared to the human heart, which displayed the expected N2BA (3.3 MDa) and N2B (3.0 MDa) titin isoforms, Burmese python ventricular samples had only one dominant titin isoform that migrated more slowly than human N2BA (~3.4 MDa) (Fig. 1K), suggesting passive tension changes are not mediated by titin isoform switching.

Cardiac myosin heavy chain SRX:DRX ratio is unaffected by feeding

Myofilament force generation is directly proportional to the number of actin-myosin cross-bridges formed²⁷. Myosin can exist in at least three distinct states: actin-bound, disordered relaxed (DRX), and super-relaxed (SRX)²⁸. The relative proportion of myosins in the DRX (fast ATP turnover) versus SRX (slow ATP turnover) states contributes significantly to cardiomyocyte contractility and energetics²⁹. To determine whether digestion impacted myosin DRX:SRX ratio, we pulsed skinned ventricular strips from 28DPF, 3DPF, and FF-3DPF pythons with fluorescently labeled ATP (Mant-ATP) and then chased with dark ATP to assess turnover kinetics (Fig. 2A). The FF-28DPF group was not analyzed as no myofibril functional differences were identified between this group and 28DPF. Fluorescence in this assay decays according to a two-phase exponential model from which DRX and SRX states can be deconvoluted (Fig. 2A)³⁰. In fasted pythons,



~40% of myosins were in the DRX state, similar to the proportion in hibernating mammals³¹, and this was not altered during digestion after normal or frequent feeding intervals (Fig. 2B, C). While the proportion of myosin in these states was unchanged, the time constant of ATP turnover in the DRX state (T1) reached the statistical threshold for a significant decrease in FF-3DPF versus 3DPF (Fig. 2D). The SRX myosin time constant (T2) was not significantly different between groups (Fig. 2E). There was also no difference in relative ATP consumption by myosin in FF-3DPF versus 3DPF (Fig. 2F). These data indicate that the myosin SRX/DRX states are not affected during digestion, although frequent feeding may modestly decrease DRX myosin ATP turnover time.

Increased relative sarcomere mRNA abundance with feeding is insufficient to maintain sarcomere protein density at the peak of remodeling

To gain insight into molecular regulation of myofibril function during digestion, we next measured relative sarcomere mRNA and protein abundance. First, we performed bulk mRNA sequencing to assess the transcriptional response to feeding (Supplementary Data 1). Principal component dimensionality reduction analysis (PCA) revealed that the transcriptional profiles of the four groups clustered based on time after feeding and were less affected by feeding frequency (Fig. S3A). Digestion after each type of feeding was associated with ~3000 differentially expressed

Fig. 1 | Digestion in pythons is associated with altered cardiac myofibril tension and kinetics. **A** Experimental paradigm; python art was generated using a licensed version of BioRender (Created in BioRender. Martin, T. (2026) <https://BioRender.com/11kitnf>). **B** Representative myofibril mechanics traces from the four groups at pCa 4.5. **C** Myofibril tension-pCa curve; $n = 18$ 28DPF, 17 3DPF, 12 FF-28DPF, and 18 FF-3DPF. **D** Maximum tension normalized to cross-sectional area; $n = 14$ 28DPF, 16 3DPF, 12 FF-28DPF, and 18 FF-3DPF; interaction: $p = 0.782$, feeding frequency: $p = 0.049$, time after feeding: $p = 0.005$. **E** Myofibril pCa at 50% maximum tension (pCa_{50}); $n = 16$ 28DPF, 16 3DPF, 12 FF-28DPF, 16 FF-3DPF. **F** Rate of myofibril activation (k_{ACT}); $n = 17$ 28DPF, 14 3DPF, 12 FF-28DPF, 18 FF-3DPF; interaction: $p = 0.491$, feeding frequency: $p = 0.008$, time after feeding: $p = 0.077$. **G** Rate of the linear phase of relaxation ($k_{REL,LIN}$); $n = 15$ 28DPF, 15 3DPF, 10 FF-28DPF, 18 FF-3DPF; interaction: $p = 0.763$, feeding frequency: $p = 0.148$, time after feeding: $p = 0.0003$. **H** Rate of the exponential phase of relaxation ($k_{REL,EXP}$); $n = 15$

28DPF, 16 3DPF, 12 FF-28DPF, 18 FF-3DPF; interaction: $p = 0.789$, feeding frequency: $p = 0.077$, time after feeding: $p = 0.008$. **I** Rate of tension redevelopment (k_{TR}); $n = 16$ 28DPF, 16 3DPF, 11 FF-28DPF, 17 FF-3DPF; interaction: $p = 0.019$, feeding frequency: $p = 0.172$, time after feeding: $p = 0.983$. **J** Passive tension at sarcomere length $2.2 \mu m$ normalized to cross-sectional area; $n = 26$ 28DPF, 16 3DPF, 11 FF-28DPF, 18 FF-3DPF; interaction: $p = 0.854$, feeding frequency: $p = 0.0002$, time after feeding: $p = 0.031$; myofibrils from 2–3 biological replicates per group (**D–I**) or 2–4 biological replicates per group (**J**) were analyzed; biological replicates are denoted by different data point shapes. **K** Titin protein isoform gel for a human LV sample and python ventricular samples. For (**D–J**), outliers determined by the ROUT outlier test were removed. Statistical analysis was performed by two-way ANOVA; if significance was detected, Sidak's post hoc test for multiple independent pairwise comparisons was employed. All data were presented as the mean $\pm$ SEM.

genes (Fig. S3B, C). Gene Ontology (GO) Biological Process analysis identified protein translation, RNA processing, and autophagy among the downregulated processes in both feeding paradigms, while protein folding and cell metabolism increased after a normal feeding interval (Fig. S3D–F). Focusing on genes encoding sarcomere proteins, 17 were significantly altered at 3DPF (13 up and 4 down) (Fig. 2H) and *MYH6* was among the genes with increased relative abundance, matching previous observations¹⁸. Relative abundance of 12 sarcomere genes changed after frequent feeding (7 up and 5 down), although here *MYH6* decreased (Fig. S3I). *MYH6*, *MYH7*, *MYL9*, *FHL3*, and *PDLIM7* were significantly increased in 3DPF versus FF-3DPF hearts (Fig. S3J). Frequent feeding was uniquely linked to increased relative abundance of mitosis and cell cycle genes (Fig. S4), matching previous observations⁹.

To determine if transcriptome-level differences translated to the proteome, we performed label-free data-independent acquisition (DIA) quantitative proteomics (Supplementary Data 2). PCA of the proteomics data showed no overlap between the four groups (Fig. 3A). Over 800 and 2000 proteins were differentially expressed during digestion after normal and frequent feeding intervals, respectively (Fig. 3B, C). GO Biological Process analysis of the down- and up-regulated proteins identified decreased cellular respiration and increased protein synthesis with both feeding intervals (Figs. 3D–G and Fig. S5). At the sarcomere level, we found 12 differentially abundant proteins, 11 of which had reduced relative abundance during digestion (Fig. 3H). Digestion after frequent feeding was associated with more robust sarcomere protein changes, although here too the majority (31/35) were found to decrease (Fig. 3I). Recent work in the post-prandial Ball python heart identified increased cytoplasmic area by electron microscopy, suggesting reduced myofibril density during digestion¹⁷. To determine whether ventricular tissue density decreased post-prandially, we loaded tissue samples with a fluorescent molecule (calcein) and then bleached the signal with a high-powered laser and measured fluorescence recovery over time to monitor diffusion rate through the tissue. We found a faster diffusion rate in the 3DPF and FF-3DPF groups, suggesting decreased ventricular tissue density during digestion (Fig. 3J).

Feeding-associated ubiquitinome changes largely track with protein abundance

We reasoned that the disconnect between sarcomere transcript and protein abundance could be due to two possibilities: (1) New sarcomere protein synthesis was unable to match the rate of tissue growth, despite increased mRNA abundance (i.e., delayed protein translation); (2) Sarcomere proteins were being actively removed through degradation pathways. Given the rapid timescale of cardiac remodeling in pythons, we hypothesized that the former was likely true. However, to assess the second possibility, we performed ubiquitin-enrichment proteomics (ubiquitinomics) (Supplementary Data 3). Ubiquitin is a post-translational modification that marks proteins for degradation by the ubiquitin-proteasome and autophagy-lysosome systems¹¹. Our analysis revealed that ubiquitination generally increased during digestion at 3DPF, and protein synthesis pathways were

among the top-enriched processes (Fig. 4A, B). Comparison of ubiquitination site fold changes with total protein abundance showed a strong positive correlation (Fig. 4C). Digestion after a frequent feeding interval coincided with more significant remodeling of the ubiquitinome, including decreased ubiquitination of sarcomere proteins and increased ubiquitination of protein synthesis factors (Fig. 5D–F). In most cases, the decreased ubiquitination of sarcomere proteins tracked with their total protein abundance; however, sites with increased ubiquitination were found on some proteins with reduced total protein abundance (TTN, MYH15, and FLNC) (Fig. 5G). GO Biological Process analysis of proteins with decreased relative abundance and at least one increased ubiquitination site identified enrichment of plasma membrane and sarcomere components (Fig. 4H). No significant ubiquitination sites were identified between FF-3DPF and 3DPF (Fig. 4I).

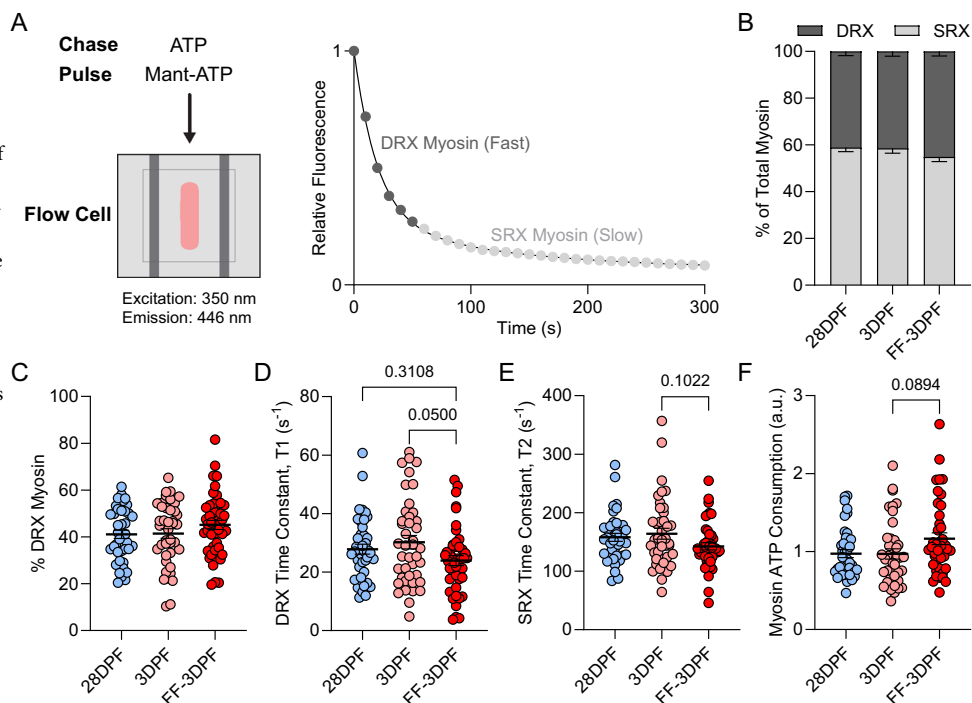
The sarcomere phosphoproteome is dramatically remodeled post-prandially

Decreased sarcomere protein density with digestion could contribute to altered tissue-level contractility; however, it cannot explain functional changes at the single-myofibril level. Therefore, we analyzed post-translational modifications that have previously been described to modulate sarcomere function: phosphorylation^{26,32–34} and acetylation^{26,35–37}. Enrichment of phospho-peptides and analysis by mass spectrometry (Supplementary Data 4) revealed that, based on PCA, the digestive responses were largely similar irrespective of feeding interval (Fig. 5A). Digestion in both paradigms was associated with potent remodeling of the phosphoproteome, with 3638 and 6911 significantly regulated sites identified in 3DPF and FF-3DPF, respectively (Fig. 5B, C). Over 2500 phospho-sites were uniquely regulated during digestion after frequent feeding (Fig. 5D), and 161 remained significant between FF-28DPF and 28DPF (Fig. S6). To deconvolute changes in phosphorylation from relative protein abundance changes, we analyzed phospho-site fold-change versus total protein fold-change and selected only those sites with >1 or <-1 log₂ fold-change at the phospho-site level versus total protein for enrichment analyses (Fig. 5E, F). In both feeding intervals, GO Biological Process analysis identified decreased phosphorylation of nuclear pore proteins post-prandially, while myofibril proteins were enriched among both the down- and up-regulated phospho-sites (Fig. 5G–J). PTM signature enrichment analysis identified shared kinase activation signatures associated with digestion in both paradigms (CK2A2, MAPK12, and ERK2) and others that were specific to the frequent feeding interval (DYRK4, JNK1, and MARK2) (Fig. 5K). That distinct kinase activation patterns might contribute to distinct sarcomere remodeling was supported by the identification of over 350 significantly differentially regulated sarcomere phospho-sites between FF-3DPF and 3DPF (Fig. S7).

Feeding induces increased protein acetylation of multiple sarcomere proteins

To determine the effects of digestion and feeding frequency on the acetylome, we enriched acetyl-lysine peptides and analyzed them by mass

Fig. 2 | Cardiac myosin heavy chain SRX/DRX states are not affected by feeding. **A** Schematic of the experimental setup for the Mant-ATP pulse-chase assay and a representative graph displaying the two-phase exponential decay of fluorescence attributed to DRX and SRX myosin. **B** Proportion of myosin in the DRX and SRX states in skinned cardiac ventricular tissue strips. **C** Proportion of myosin in the DRX state. **D** Time constant of ATP turnover for myosin in the DRX state (T1). **E** Time constant of ATP turnover for myosin in the SRX state (T2). **F** Relative myosin ATP consumption represented as a fold-change vs. the 28DPF group. For all, $n = 41$ 28DPF, 42 3DPF, 45 FF-3DPF from $n = 3$ –5 biological replicates. Statistical analysis was performed by one-way ANOVA with Tukey's post hoc test for multiple independent pairwise comparisons. Data were presented as the mean $\pm$ SEM.



spectrometry (Supplementary Data 5). This analysis identified 85 (75 up and 10 down) significant sites at 3DPF versus 28DPF (Fig. 6A). Proteins with increased acetylation included factors involved in protein acetylation and chromatin organization (Fig. 6B), suggesting increased chromatin accessibility during digestion, matching previous observations in Ball pythons¹⁷. Significantly increased acetylation sites were identified on the sarcomere proteins TTN, MYH15, and PDLIM5-like (Fig. 6C). Digestion after a frequent feeding interval was associated with even further remodeling of the acetylome (104 up 25 and down) (Fig. 6D). Here too, most proteins with increased acetylation were involved in chromatin remodeling and protein acetylation (Fig. 6E); however, three acetylated lysine residues on TTN and one on MYH15 were also identified that were similarly regulated in 3DPF. Most of the downregulated acetylation sites in FF-3DPF were on sarcomere proteins, reflecting their reduced total protein abundance in this group (Fig. 6F). When we compared the impact of the different feeding intervals on acetylome remodeling, we identified three sarcomeric protein sites (MYH15 Ac-K1395, ACTN2 Ac-K792, and MYOZ2 Ac-K172) that were significantly affected by frequency but not time after feeding (Fig. 6H–J). Acetylation at these sites remained elevated following a subsequent 28-day fast (Fig. 6H–J).

Protein acetylation after both feeding paradigms generally increased, suggesting global activation of acetyltransferases and/or inactivation of deacetylases during digestion. This observation agrees with prior work in Ball pythons, where cardiac histone deacetylase activity was found to decrease post-prandially¹⁷. Our phosphoproteomics data revealed significant changes in phosphorylation shared between feeding paradigms for HDAC4 and HDAC5, but not HDAC7 (Fig. 6K–M). According to the PhosphoSite Plus database, S34 in HDAC4 and S633 in HDAC5 are conserved in humans (S265 and S661, respectively), and changes in their phosphorylation regulate HDAC subcellular localization^{38,39}. S633 phosphorylation of HDAC5 is reduced after feeding, and PKD-dependent phosphorylation of this site regulates 14-3-3 binding, and subsequent cytoplasmic retention in rat ventricular myocytes³⁸. It has been shown that cAMP induces hypo-phosphorylation of 14-3-3 binding sites in HDAC5, leading to nuclear accumulation and subsequent inhibition of MEF2 in cardiomyocytes⁴⁰. Thus, the HDAC-MEF2 axis may regulate feeding-induced hypertrophy.

Summary of modifications on the tension-regulating titin and myosin heavy chain proteins

Considering key mechano-active sarcomere proteins, our analyses identified dozens of modifications on TTN that were shared between feeding groups (Fig. 7A) and over 100 more that were specific to frequent feeding (Fig. 7B). The Z-disk and M-band regions of TTN were PTM hotspots, although modifications were found throughout the molecule, including many in the I-band region, which generates most of TTN-based passive tension^{41,42}. We identified six feeding-regulated modifications on MYH15 that were shared between feeding groups, two specific to 3DPF, and dozens exclusively in FF-3DPF (Fig. 7C, D). Most of these PTMs were in the rod domain, which regulates sarcomere incorporation⁴⁰; however, several were also in the N-terminal motor domain that is responsible for ATP-dependent cross-bridge cycling¹¹. Some PTMs on TTN and MYH15 were in domains that have previously been implicated in tension generation, including the PEVK domain, immunoglobulin-like, and fibronectin-3-like domains in I-band TTN (Fig. 7E)^{26,43} and the converter domain of MYH15 (Fig. 7F)⁴⁴.

Discussion

Digesting Burmese pythons are a model of extreme cardiometabolic plasticity³. Compared to mammalian models of adaptive cardiac remodeling, which develop hypertrophy and enhanced cardiac function over weeks⁴⁵, the python heart reaches its peak structural and functional remodeling within 72 h of feeding^{2,3}. The rapid timescale and magnitude of remodeling position pythons as a valuable model for investigating the molecular regulation of cardiac plasticity⁷. Previous studies of the post-prandial Burmese python heart identified changes in cardiac physiology^{10,46–48}, mechanisms of cardiac hypertrophy and regression^{6,7,10}, transcriptional remodeling^{9,49–53}, and increased ex vivo cardiac contractility⁴. However, cardiac remodeling at the sarcomere scale had not been assessed. In this study, we investigated the impact of feeding on sarcomere function and found that digestion was associated with increased myofibril tension and rate of relaxation, while frequent feeding additionally altered myofibril activation kinetics. Furthermore, we characterized the changes in relative sarcomere mRNA/protein abundance and sarcomere PTMs that coincided with these functional differences via multi-omics analyses, lending potential molecular insight into mechanisms for tuning myofibril function.

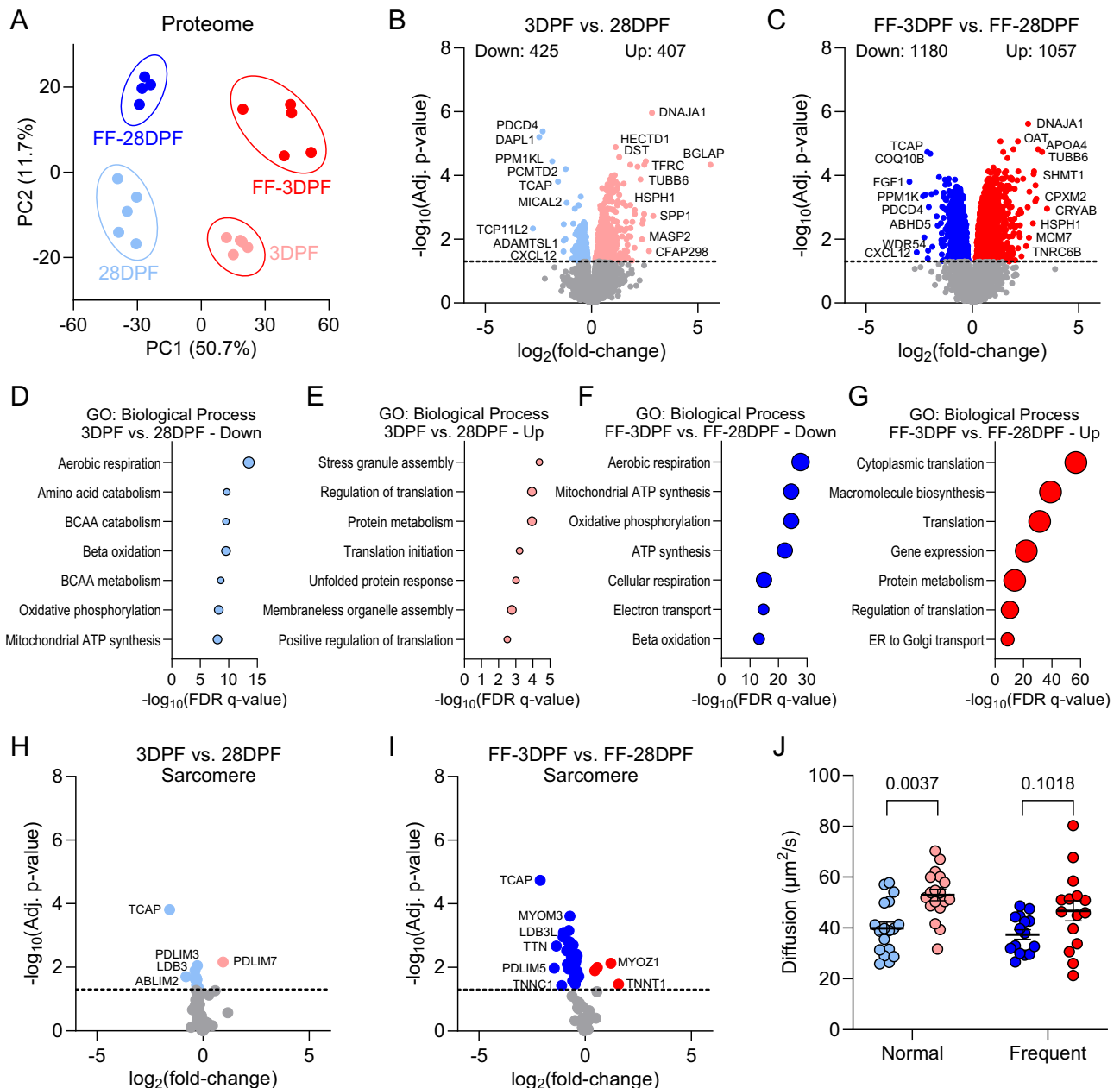


Fig. 3 | Relative sarcomere protein abundance and cardiac tissue density decrease post-feeding. **A** PCA plot for quantitative proteomics data from post-fed Burmese python cardiac ventricular samples. **B,C** Volcano plots depicting fold-change, statistical significance, and number of significantly differentially expressed proteins in the 3DPF vs. 28DPF (**B**) and FF-3DPF vs. FF-28DPF (**C**) comparisons. **D, E** GO: Biological Process enrichment for the downregulated (**D**) and upregulated (**E**) proteins in the 3DPF vs. 28DPF comparison. **F, G** GO: Biological Process enrichment for the downregulated (**F**) and upregulated (**G**) proteins in the FF-3DPF vs. FF-28DPF comparison. **H, I** Volcano plots depicting fold-change and statistical significance showing only the sarcomere proteins from the 3DPF vs. 28DPF (**H**) and

FF-3DPF vs. FF-28DPF (**I**) comparisons; for (**A–I**), $n = 5$ 28DPF, 5 3DPF, 4 FF-28DPF FF-3DPF; data were analyzed using two-sample moderated t -test (two-sided) pairwise comparison of the normalized $\log_2$ intensities to determine the significantly regulated proteins between groups with an FDR cutoff of 5%. **J** Calcein fluorescence recovery after photobleaching (FRAP), denoting the diffusion rate of the molecule through python ventricular tissue (faster recovery/increased diffusion rate = decreased tissue density); $n = 15$ – 18 samples from three biological replicates per group; two-way ANOVA with Tukey's post hoc test: interaction: $p = 0.492$, feeding frequency: $p = 0.114$, time after feeding: $p = 0.0001$; data were plotted as the mean $\pm$ SEM.

Previous investigations into myofibril function in digesting Ball pythons identified increased myofibril maximum Ca^{2+} -dependent tension¹⁷. However, whereas we observed increased myofibril relaxation rate in Burmese pythons at 72 h in the present study, this property was significantly reduced at 24 h in Ball pythons¹⁷. Also, opposite to the findings presented herein, Ball python myofibrils displayed decreased passive tension with feeding¹⁷. These differences in functional adaptations to digestion may reflect the divergence between these species with respect to meal size, metabolic rate regulation, and growth potential. Ball pythons are much

more limited in size, reaching lengths of up to 1.8 m compared to 6 m for Burmese pythons. In our experience, Ball pythons are also unlikely to eat prey larger than 25% of their bodyweight, while Burmese pythons can consume prey up to their own size^{1,54}. The consequent differences in peak metabolic requirements may contribute to species differences in feeding-associated cardiac adaptations. Future investigations into digestion-associated sarcomere PTMs in Ball pythons may help to explain the opposite myofibril relaxation kinetics and passive tension responses to those observed in Burmese pythons. Additionally, an assessment of myofibril

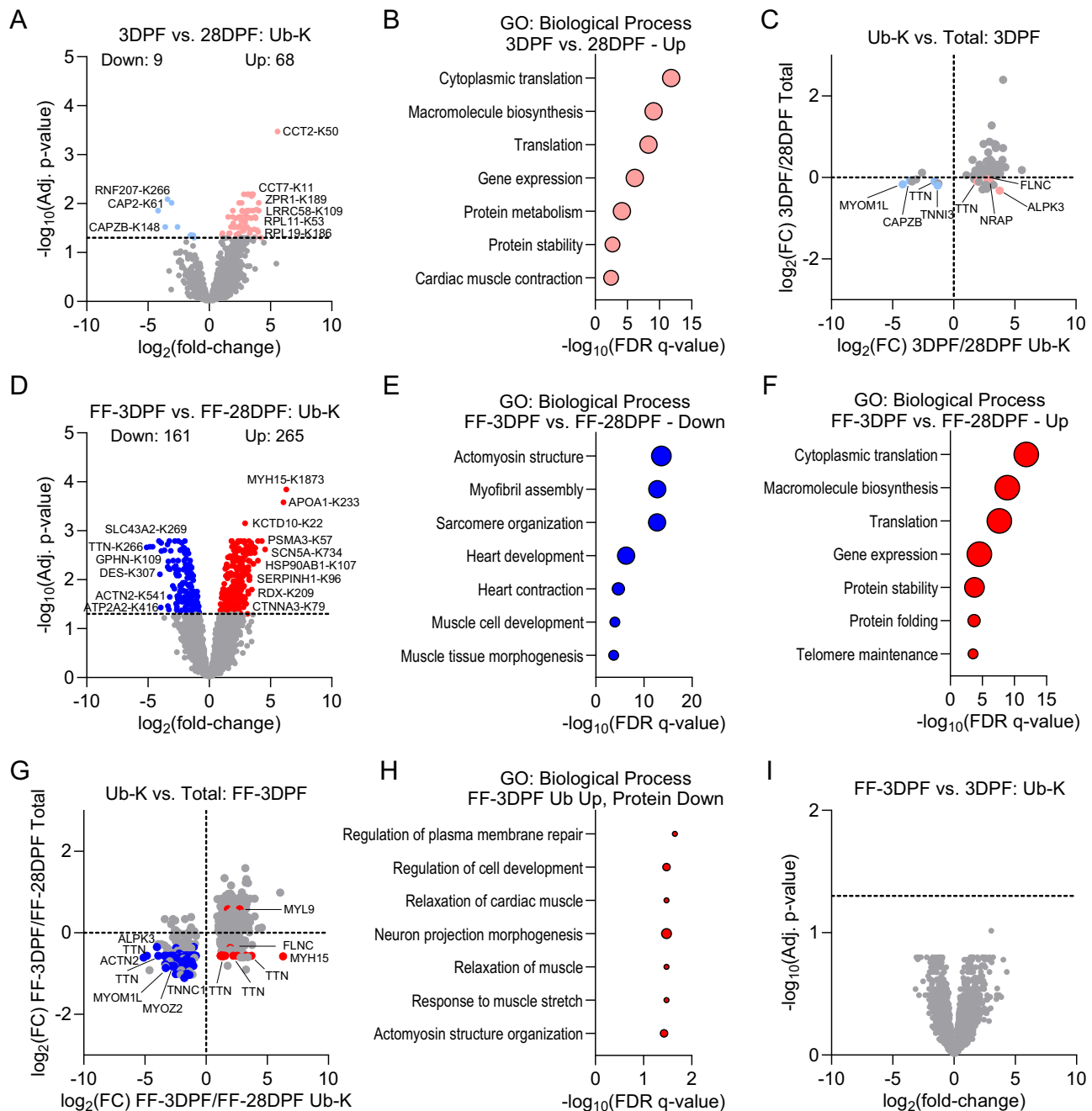


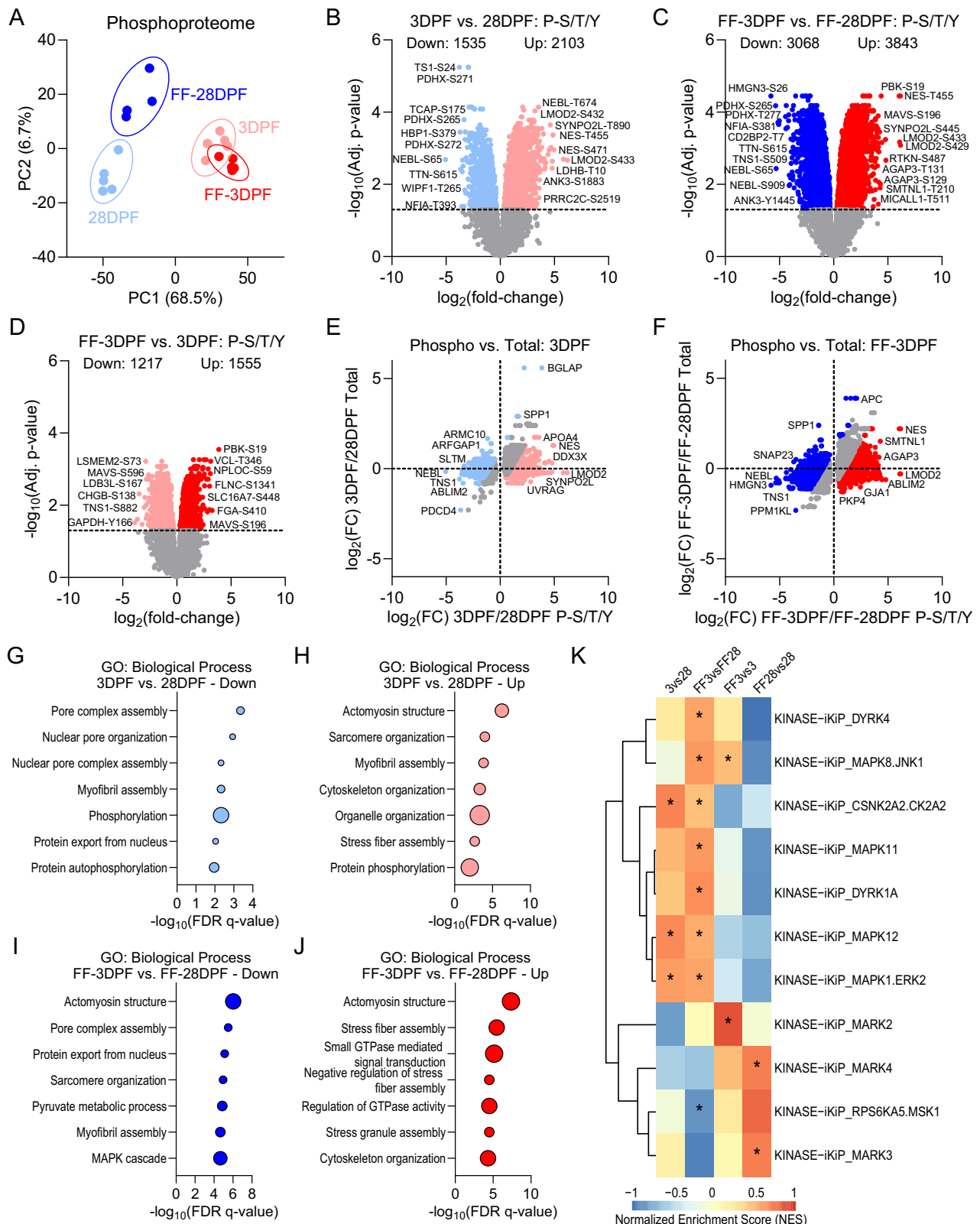
Fig. 4 | Cardiac ubiquitinome changes with feeding track with relative protein abundance. **A** Volcano plot depicting fold-change, statistical significance, and number of significantly differentially ubiquitinated sites at 3DPF vs. 28DPF. **B** GO: Biological Process enrichment for the upregulated ubiquitination sites. **C** Total protein fold-change vs. ubiquitination site fold-change for the significantly differentially abundant ubiquitination sites at 3DPF vs. 28DPF; sarcomere proteins are annotated. **D** Volcano plot depicting fold-change, statistical significance, and number of significantly differentially ubiquitinated sites at FF-3DPF vs. FF-28DPF. **E, F** GO: Biological Process enrichment for the downregulated (**E**) and upregulated

(**F**) ubiquitination sites. **G** Total protein fold-change vs. ubiquitination site fold-change for the significantly differentially abundant ubiquitination sites at FF-3DPF vs. FF-28DPF; sarcomere proteins are annotated. **H** GO: Biological Process enrichment of the proteins with increased ubiquitination and decreased total abundance. **I** Volcano plot depicting fold-change and significance for ubiquitination sites at FF-3DPF vs. 3DPF. For all analyses, $n = 5$ 28DPF, 5 3DPF, 4 FF-28DPF, FF-3DPF; data were analyzed using a two-sample moderated *t*-test (two-sided) pairwise comparison of the normalized $\log_2$ intensities to determine the significantly regulated PTMs between groups with an FDR cutoff of 5%.

properties across a range of post-feeding timepoints in both species will be informative.

In this study, we identified increased maximum myofibril tension independent of changes in Ca^{2+} sensitivity. Since Ca^{2+} sensitivity is primarily regulated by thin filament proteins⁵⁵, this result implicates altered thick filament properties as the underlying mechanism. Myofibril active tension represents the ensemble of forces produced by hundreds of individual myosin molecules. Therefore, mechanisms that increase cross-bridge

number (e.g., increased myosin DRX state) and efficiency (e.g., optimal filament overlap and decreased lattice spacing), promote greater myosin step sizes (the distance a myosin head moves actin per ATP), or increase duty ratio (the time myosin heads spend bound to actin) represent possible explanations^{56–62}. While we cannot rule out the contribution of changing sarcomere lengths to altered in vivo cardiac contractility during digestion, our single-myofibril studies were performed at fixed sarcomere lengths. Thus, differences in optimal filament overlap or lattice spacing (titin-



dependent properties⁶³ do not underlie the observed differences in active tension. Our analysis of DRX/SRX myosin states also suggests that myosin head availability is not a major contributor. Thus, changes in myosin intrinsic properties represent the most logical mechanism. The myosin converter domain regulates force transmission and step size^{64–66}. Converter domain mutations in MYH7 (the predominant cardiac myosin in humans)

cause hypertrophic cardiomyopathy (HCM)⁶⁵, a disease associated with hypercontractility, and biopsies from patients with converter domain mutations display increased maximum tension^{67,68}. Our analysis of digestion-associated PTMs identified modifications in (Ac-K824, homologous to human MYH7 Q734) and structurally adjacent (p-S235, homologous to human MYH7 S148) to the Burmese python MYH15 converter

Fig. 5 | Feeding dramatically remodels the cardiac ventricular phosphoproteome. **A** PCA plot for phospho-proteomics data from post-fed Burmese python cardiac ventricular samples. **B–D** Volcano plots depicting fold-change, statistical significance, and number of significantly differentially expressed phospho-sites in the 3DPF vs. 28DPF (**B**), FF-3DPF vs. FF-28DPF (**C**), and FF-3DPF vs. 3DPF comparisons. **E, F** Total protein fold-change vs. phospho-site fold-change for the significantly differentially abundant phospho-sites at 3DPF vs. 28DPF (**E**) and FF-3DPF vs. FF-28DPF (**F**). **G, H** GO: Biological Process enrichment for the phospho-sites that decreased by $\log_2$ fold-change ≥ 1.0 (**G**) or increased by $\log_2$ fold-change ≥ 1.0 (**H**) vs. total protein abundance in the 3DPF vs. 28DPF comparison. **I, J** GO:

Biological Process enrichment for the phospho-sites that decreased by $\log_2$ fold-change ≥ 1.0 (**G**) or increased by $\log_2$ fold-change ≥ 1.0 (**H**) vs. total protein abundance in the FF-3DPF vs. FF-28DPF comparison. **K** Heat map depicting normalized enrichment scores (NES, columns) of signatures (rows) for significant in vitro kinase-to-phosphosite (iKIP) database hits in the different post-feeding group comparisons by ptmSEA; asterisks in heat map denote statistical significance. For all analyses, $n = 5$ 28DPF, 5 3DPF, 4 FF-28DPF FF-3DPF; data were analyzed using two-sample moderated *t*-test (two-sided) pairwise comparison of the normalized $\log_2$ intensities to determine the significantly regulated PTMs between groups with an FDR cutoff of 5%.

domain. MYH15 K824 acetylation is a particularly interesting modification that increased during digestion, irrespective of the feeding interval. Glutamine (Q) structurally mimics acetylation, suggesting that its substitution for lysine in human MYH7 may have stemmed from positive selection for permanent acetylation at this site in mammals. Notably, two mutations at this position, MYH7 Q734P and Q734E, are listed as likely pathogenic for HCM in ClinVar. Future studies investigating the functional effects of the myosin motor domain PTMs identified herein will be valuable.

The increased myofibril passive tension identified during digestion was particularly pronounced after a frequent feeding interval and coincided with changes in ventricular tissue stiffness and elasticity. In myofibrils from mammals at the same sarcomere length (2.2 μm), passive tension arises from sarcomere components (i.e., titin), while non-myofibril structures like microtubules only make significant contributions at longer sarcomere lengths^{23,24}. Therefore, we expect the increased passive tension during digestion in Burmese python myofibrils is due to changes in titin stiffness. It should be noted that the passive tension measurements were conducted without the addition of 2,3-butanedione (BDM), so the potential contribution of residual cross-bridge attachment to this parameter cannot be ruled out. We also found that Burmese python myofibrils contain only a single major titin isoform, unlike mammals, which have two. Thus, changes in titin isoforms cannot explain the phenotype. Titin PTMs are also known to contribute to passive tension²⁶, and we identified hundreds of digestion-responsive titin PTMs. The functional effects of most titin PTMs are not known. However, in mammals, phosphorylation at two regions has been linked to stiffness regulation: the N2B-unstructured region (phosphorylation = decreased tension) and the region immediately following the PEVK domain (phosphorylation = increased tension)²⁶. Further, a recent study identified that acetylation of titin in the lysine-rich PEVK domain increases passive tension⁶⁹. The Burmese python N2B domain is poorly conserved, with human making inferences about PTMs in this region difficult; however, we did identify increased phosphorylation at multiple PEVK-adjacent sites (including T10373, S10375, T10870, and T10876) and increased acetylation at three sites within the PEVK domain (K9007, K9039, and K10301). In total, over 30 PTMs in the I-band region of titin increased during digestion, with roughly half of these being specific to frequent feeding. A single PTM is not likely to confer the passive tension changes observed during digestion, but the ensemble effect of many modifications together in and near the PEVK offers a potential explanation.

This study highlights the myofibril functional remodeling that occurs during digestion in the Burmese python heart and provides an atlas of feeding-regulated sarcomere PTMs in these snakes. We also identified potential mechanisms responsible for these changes, including postprandial activation of kinase pathways (CK2A2, MAPK12, and ERK2) and suggested altered subcellular localization of deacetylases (HDAC4 and HDAC5). The combination of these datasets provides a launchpad for future mechanistic investigations that interrogate the individual and collective contributions of sarcomere protein PTMs to cardiac functional regulation and prioritizes specific, testable candidate sites on titin and myosin. A key limitation is that our multi-omics analyses are associative; establishing causality will require targeted perturbation of prioritized PTM sites coupled with single-molecule and myofibril functional assays. Toward this end, we believe that examinations of myosin and titin properties will be most tractable, due to the already-developed assays for studying these

proteins at a single molecule level^{19,43}. Finally, we chose to focus our analysis of the multi-omics data on the sarcomere. However, there is a wealth of information regarding transcriptome and proteome remodeling present in these datasets that researchers interested in other aspects of cardiac plasticity regulation may find valuable.

Methods

Ethical oversight

All animal experiments were performed with strict adherence to institutional and national guidelines for the care and use of laboratory animals. Procedures involving animals were conducted at the University of Colorado Boulder after approval by the Institutional Animal Care and Use Committee (IACUC). The University of Colorado Boulder Office of Animal Resources is an AAALAC International-accredited facility.

Burmese python husbandry and experimentation

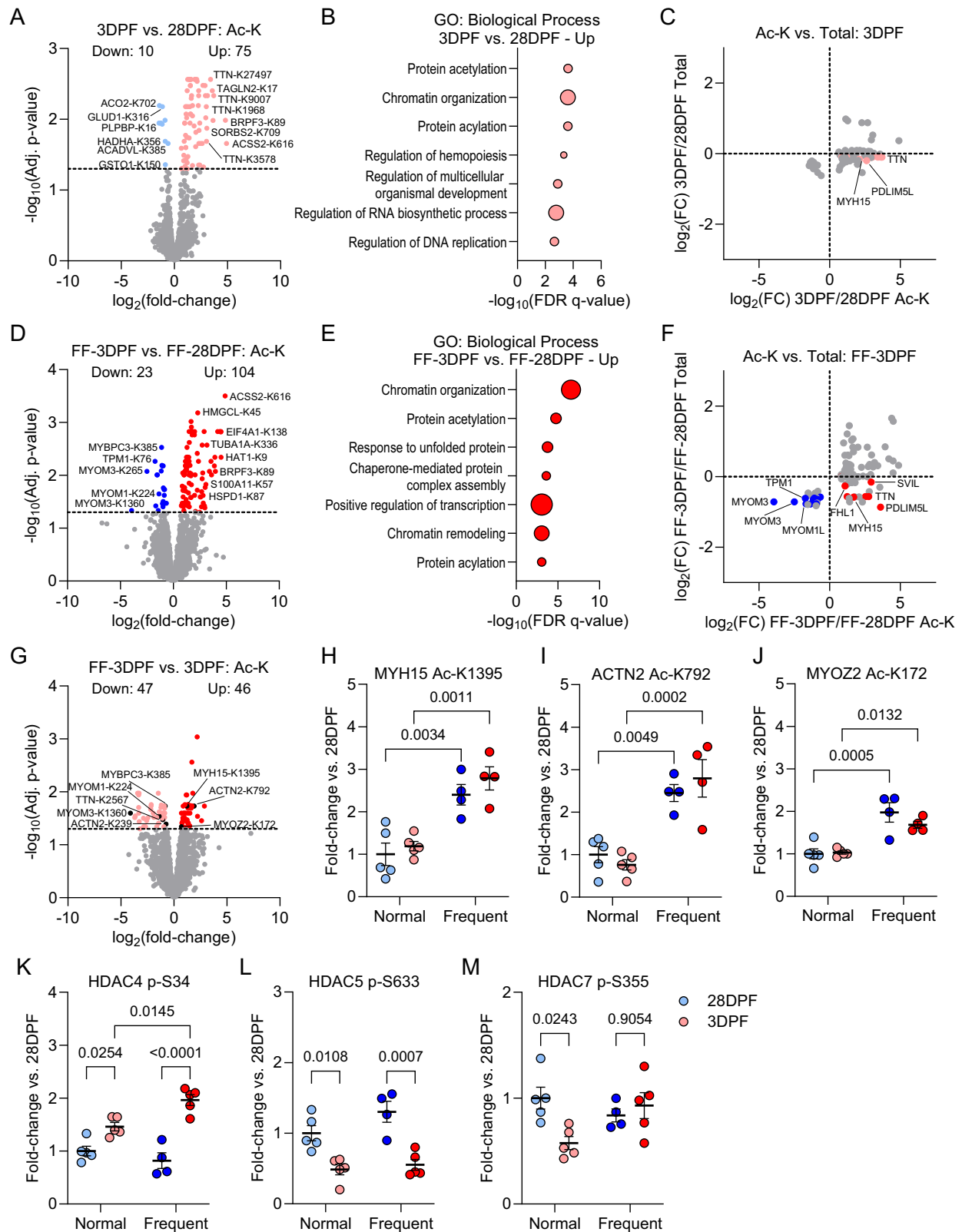
Captive-bred Burmese pythons (*Python bivittatus*) were acquired as hatchlings from Bob Clark Reptiles, Oklahoma City, Oklahoma. The pythons were singly housed at the University of Colorado Boulder in a room maintained at 30 °C with 12-h light-dark cycles. Pythons weighed 1.5–2 kg and were randomly assigned to treatment groups using block randomization at the start of the study, when they were ~2 years old. There were 4–5 pythons per group for this study (28DPF: $n = 3$ male, 2 female; 3DPF: $n = 4$ male, 1 female; FF-28DPF: $n = 2$ male, 2 female; FF-3DPF: $n = 3$ male, 2 female). Sample sizes were chosen based on expected variability from previous experiments studying cardiac molecular biology in Burmese pythons. For the normal feeding frequency groups, pythons were fed two 25% bodyweight rat meals at 28-day intervals. After the second feeding, pythons were euthanized by isoflurane overdose and rapid decapitation at 3- or 28-days post-feeding (DPF), and their cardiac ventricle tissue was collected, snap frozen in liquid nitrogen, and stored at –80 °C until downstream processing and analysis. For the frequent feeding groups, pythons were fed a 25% bodyweight meal once every four days for eight weeks. After the final feeding, pythons were euthanized at 3- and 28-days post-frequent feeding (FF-3DPF and FF-28DPF). Changes in the mass of the heart and other organs in these cohorts in response to feeding were reported previously⁹.

Myofibril isolation

Myofibrils were isolated as previously described¹⁷. Frozen python ventricle tissues were skinned in Linke's solution (132 mM NaCl, 5 mM KCl, 1 mM MgCl_2 , 10 mM Tris-base, 5 mM EGTA, 1 mM sodium azide, and pH 7.0) with 1% Triton X-100 and protease inhibitors (10 μM leupeptin, 5 μM pepstatin, 200 μM phenyl-methylsulfonyl fluoride (PMSF), 10 μM E64, 500 μM NaN_3 , and 2 mM dithioerythritol) for 1 h at 4 °C. Skinned myofibrils were washed in rigor solution (50 mM Tris, 100 mM KCl, 2 mM MgCl_2 , 1 mM EGTA, and pH 7.0), then placed in bath solution (100 mM Na_2EGTA ; 1 M potassium propionate; 100 mM Na_2SO_4 ; 1 M MOPS; 1 M MgCl_2 ; 6.7 mM ATP; and 1 mM creatine phosphate; pH 7.0) with protease inhibitors, then homogenized (Tissue-Tearor).

Myofibril functional measurements

Myofibril mechanical function was assessed on the day of isolation using the Poggessi fast solution switching method as previously described^{17,70}. Briefly, a droplet of myofibril suspension was added to a temperature-controlled



chamber (15 °C), and a relaxing solution was added. Single myofibrils were then picked up using two custom microtools and positioned within 1 mm of a double-barreled pipette at a right angle to the myofibril. We used a customized program to initiate the start of flow for the relaxing solution (pCa 9.0; delivered from the right side of the pipette). Myofibrils were maintained within the relaxing solution stream for 3 s, at which time passive tension was measured. After three seconds, a stepper-motor-controlled system rapidly

switched the pipette so that the myofibril was engulfed in the pCa 4.5 activating solution, which remained in place for 17 s. After 17 s, the pipette was switched back to relaxation solution, and the motor relaxation kinetics were measured. Following the return to baseline, the traces were captured. The myofibril remained upright in relaxation solution for one minute prior to switching the calcium solution to a different pCa, and then the measurements were performed again for each of the decreasing pCa solutions

Fig. 6 | Digestion is associated with sarcomere acetylome changes. **A** Volcano plot depicting fold-change, statistical significance, and number of significantly differentially acetylated sites at 3DPF vs. 28DPF. **B** GO: Biological Process enrichment for the upregulated acetylation sites at 3DPF. **C** Total protein fold-change vs. acetylated lysine site fold-change for the significantly differentially abundant acetylation sites at 3DPF vs. 28DPF; sarcomere proteins are annotated. **D** Volcano plot depicting fold-change, statistical significance, and number of significantly differentially acetylated sites at FF-3DPF vs. FF-28DPF. **E** GO: Biological Process enrichment for the upregulated acetylation sites at FF-3DPF. **F** Total protein fold-change vs. acetylated lysine site fold-change for the significantly differentially abundant acetylation sites at FF-3DPF vs. FF-28DPF; sarcomere proteins are annotated.

(5.6, 5.8, 6.0, and 6.2). Following the final pCa measurement, the myofibril tension was again measured at pCa 4.5 to account for rundown. If the force collected at the final pCa 4.5 was less than 80% of the force measured at the first pCa 4.5, the entire set of data was excluded. Myofibril sarcomere length was set at ~2.2 μm . Mounted myofibrils were activated and relaxed by rapidly translating the interface between two flowing streams of solutions (pCa 4.5–maximum calcium concentration, and pCa 9.0–relaxation solution). Data were collected and analyzed using customized LabVIEW software.

Large-format agarose gel electrophoresis

The protocol for separation and visualization of titin protein isoforms was performed as previously described⁷¹. Briefly, 20 mg of frozen myocardium was pulverized using a 2 mL Dounce homogenizer submerged in liquid nitrogen. After tempering at -20°C for 10 min, the samples were solubilized in 1:40 (w/v) 8 M urea, 2 M thiourea buffer, 30% glycerol, 50 mM Tris, 75 mM dithiothreitol, 0.03% (w/v) bromophenol blue buffer and incubated at 60°C for 10 min. Samples were clarified by centrifugation, snap frozen, and stored at -80°C . A 1.5 mm thick 1% Seakem Gold agarose (Lonza) gel was assembled using the Hoefer SE6015 system. The gel was run on the day it was made. Protein samples were thawed at room temperature, and 7.5 μL loaded per well. The gel apparatus was then moved to a container with cold running buffer (SDS-Tris-Glycine) in a 4°C cold room and run at 15 mA constant current for 3 h and 15 min. The gel was removed from the glass plates and fixed in 50% methanol, 12.5% glacial acetic acid, 5% glycerol buffer overnight with gentle agitation and then stained with 0.05% (w/v) Coomassie brilliant blue for 3 h. Destaining was performed with 25% methanol, 10% acetic acid for 5 h. Imaging was performed using an Amersham Typhoon 5 imager (Cytiva) using the Coomassie in IR setting.

Mant-ATP chase experiments

As previously published³⁰, the relaxing solution contained 4 mM Mg-ATP, 1 mM free Mg^{2+} , 10–6.0 μM free Ca^{2+} (pCa 9.0), 10 mM imidazole, 7 mM EGTA, 14.5 mM creatine phosphate. Ionic strength was adjusted to 180 mM, and pH to 7.0. Additionally, the rigor buffer for Mant-ATP chase experiments contained 120 mM K acetate, 5 mM Mg acetate, 2.5 mM K_2HPO_4 , 50 mM MOPS, 2 mM DTT, with a pH of 6.8. On the day of the experiments, thin cardiac strips were isolated. Their ends were individually clamped to half-split copper meshes designed for electron microscopy (SPI G100 2010C-XA, width 3 mm), which had been glued to glass slides (Academy, 26×76 mm, thickness 1.00–1.20 mm). Cover slips were attached to the top using double-sided tape to create flow chambers (Menzel-Gläser, 22×22 mm, thickness 0.13–0.16 mm)³⁰. Subsequently, at 22°C (or 4°C), for each strip, the sarcomere length was measured using the brightfield mode of a Zeiss Axio Scope A1 microscope, a Zeiss AxioCam ICm 1 camera and a LD Plan-Neofluar 40x/0.60 objective. Preparations with a sarcomere length of 1.9–2.0 μm were further subjected to the protocol. Indeed, similar to previously published protocols³⁰, each thin strip was first incubated for 5 min with a rigor buffer. A solution containing the rigor buffer with 250 μM Mant-ATP ((2'-(or-3')-O-(N-methylantraniloyl) adenosine 5'-triphosphate) was then flushed and kept in the chamber for five minutes. At the end of this step, another solution made of the rigor buffer with 4 mM ATP was added with simultaneous acquisition of the

G Volcano plot depicting fold-change, statistical significance, and number of significantly differentially acetylated sites at FF-3DPF vs. 3DPF. **H–J** Examples of differentially acetylated sites uniquely modified according to feeding frequency. **K–M** Phosphorylation sites are regulated after feeding on Histone deacetylase-4, -5, and -7. For (A–G), $n = 5$ 28DPF, 5 3DPF, 4 FF-28DPF FF-3DPF; data were analyzed using a two-sample moderated t -test (two-sided) pair-wise comparison of the normalized $\log_2$ intensities to determine the significantly regulated PTMs between groups with an FDR cutoff of 5%. For H–M, the data represent $n = 5$ 28DPF, 5 3DPF, 4 FF-28DPF, FF-3DPF, were analyzed by two-way ANOVA with Tukey's post hoc test for multiple independent pairwise comparisons, and are presented as the mean $\pm$ SEM.

Mant-ATP chase. For fluorescence acquisition, a Zeiss Axio Scope A1 microscope was used with a Plan-Apochromat 20x/0.8 objective and a Zeiss AxioCam ICm 1 camera. Frames were acquired every five seconds with a 20 ms exposure time using a DAPI filter set for 5 min. Tests were run prior to starting the current study, where images were collected for 15 min instead of 5 min—these tests did not reveal any significant difference in the parameters calculated. Three regions of each individual strip were sampled for fluorescence decay using the ROI manager in ImageJ as previously published³⁰. The mean background fluorescence intensity was subtracted from the average of the fiber fluorescence intensity (for each image taken). Each timepoint was then normalized by the fluorescence intensity of the final Mant-ATP image before washout ($T = 0$). These data were then fit to an unconstrained double exponential decay using GraphPad Prism (version 10.4):

$$\text{Normalised Fluorescence} = 1 - P1 \left(1 - \exp(-t/T_1) \right) - P2 \left(1 - \exp(-t/T_2) \right)$$

Where P1 is the amplitude of the initial rapid decay approximating the DRX state with T_1 as the time constant for this decay. P2 is the slower second decay approximating the proportion of myosin heads in the SRX state with its associated time constant T_2 . In the present article, P1, P2, T_1 , and T_2 are presented without any correction for the rapid washout of nonspecific binding of Mant-ATP³⁰. Solely based on P1, P2, T_1 , and T_2 , we estimated the theoretical ATP consumed by myosin³⁰.

Fluorescence recovery after photobleaching (FRAP)

FRAP was performed as previously described⁷². Briefly, tissue was equilibrated in a solution of calcein (2 μM in water) for at least 30 min before the sample was placed on a laser scanning confocal microscope (Nikon A1R). At least three regions of interest from separate imaging planes were selected in each tissue, with baseline fluorescence intensity ($\lambda_{\text{ex}} = 488$ nm) approximately 60% of the detection limit of the microscope. Following bleaching with 405 nm laser light at 100% power, fluorescence recovery was monitored for 90 s and fit to a closed formula in MATLAB to calculate the characteristic timescale of diffusion.

Tissue rheology

The modulus of ventricles was measured with oscillatory shear rheology (1 rad/s, 1% strain). A shear rheometer (DHR-3, TA Instruments) equipped with a sandblasted parallel plate geometry was used to measure the storage (G') and loss (G'') moduli and $\tan \delta$ (G''/G') of the ventricle tissue at room temperature; tissue samples were compressed to an axial force of ~0.1 N before measurement. Young's modulus was calculated by multiplying G' by a value of 3 (i.e., assuming a Poisson ratio of 0.5, representing tissue incompressibility)⁷³. Prior to measurement, frozen python ventricle tissues ($n = 3$) were thawed on ice and cut to size using an 8 mm cylindrical punch. Ventricle tissue was measured within 1 h of defrosting. Tissue was trimmed to ensure a level height of ~1 mm, and sandpaper was affixed to the Peltier plate to prevent tissue slippage.

RNA isolation

Cardiac ventricle tissue (~50 mg) was added to 5 mL polypropylene tubes containing 1 mL of Trizol and homogenized using a mechanical

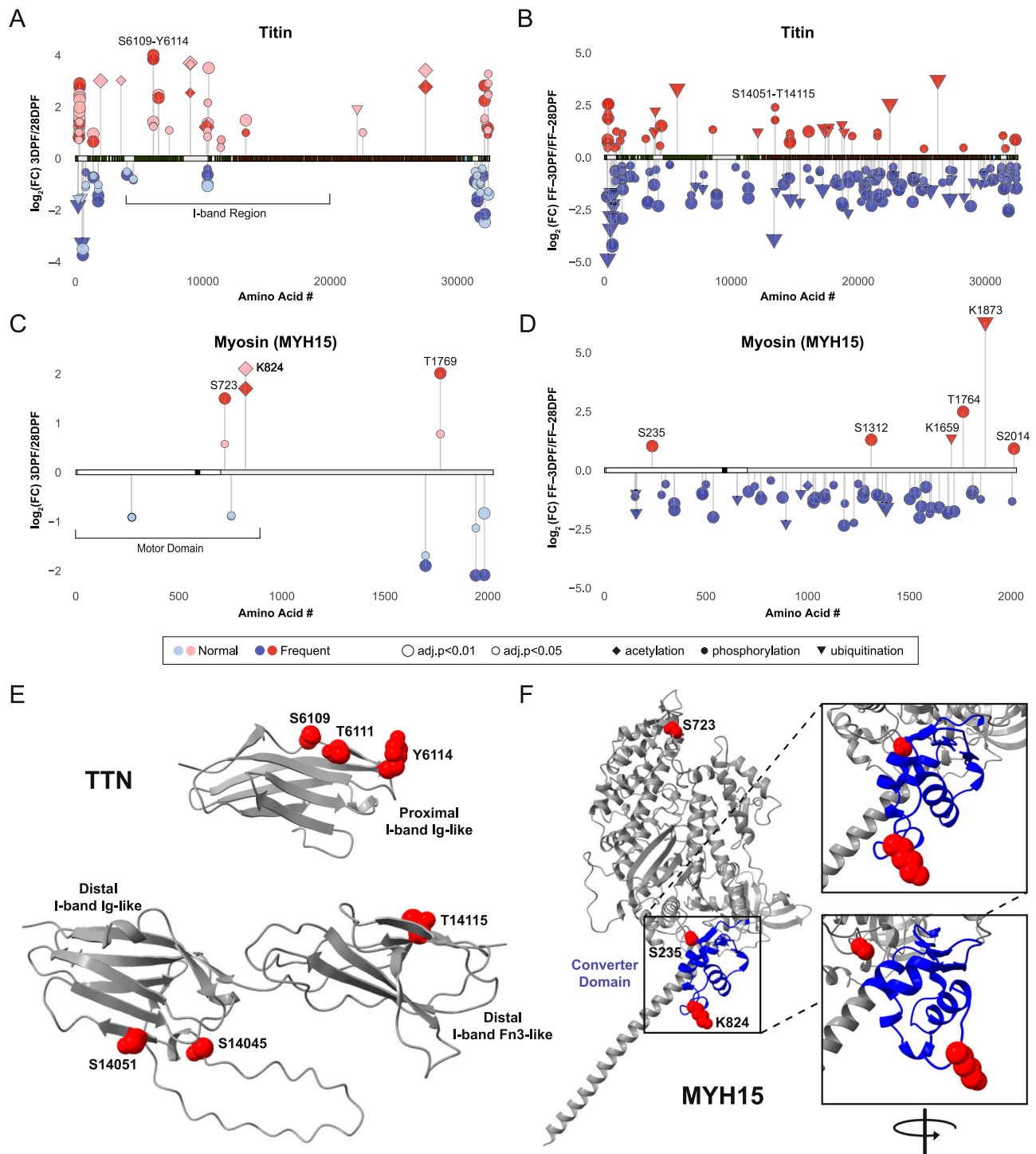


Fig. 7 | Feeding-induced post-translational modifications on titin and myosin heavy chain. **A** Summary of increased and decreased PTMs on titin that were shared in both feeding groups. **B** Summary of titin PTMs that were uniquely regulated in the frequent feeding group. **C** Summary of increased and decreased PTMs on myosin heavy chain (MYH15) that were shared in both feeding groups. **D** Summary of MYH15 PTMs that were uniquely regulated in the frequent feeding group. **E** AlphaFold3 structural predictions and amino acid residue annotations for phosphorylation sites on titin I-band immunoglobulin (Ig)-like and fibronectin-3

(Fn3)-like domains that were particularly enriched after frequent feeding. “Proximal” and “Distal” denotations are in reference to the Z-disk.

F AlphaFold3 structural predictions and amino acid residue annotations for PTM sites in the MYH15 motor domain that were altered by feeding: S723 and K824 in 3DPF and FF-3DPF; S235 only in FF-3DPF. All PTMs portrayed in this figure were determined to be statistically significant using a two-sample moderated *t*-test (two-sided) pair-wise comparison of the normalized $\log_2$ intensities to determine the significantly regulated PTMs between groups with an FDR cutoff of 5%.

homogenizer (Omni International). Samples were then incubated for 5 min at room temperature and transferred to 1.5 mL Eppendorf tubes. Chloroform was added at 1:5 (vol/vol), the tubes were shaken vigorously, and incubated at room temperature for 15 min prior to centrifugation at 12,000

RCF for 15 min at 4 °C. The upper aqueous layer (~500 μ L) was collected into a clean tube, isopropanol was added at 1:1 (vol/vol), and the samples were briefly vortexed. To facilitate increased RNA precipitation, the tubes were incubated at -20 °C for 30 min. The samples were then centrifuged at

12,000 RCF for 10 min to pellet the RNA. The supernatant was decanted, and the RNA pellet was washed twice with ice-cold 75% ethanol by resuspension and centrifugation. Following the last ethanol wash, the sample was air-dried for ~10 min at room temperature, and then the RNA was dissolved in 200 µL milli-Q water.

RNA sequencing

Poly-A RNA enrichment, cDNA library preparation, and short-read Illumina mRNA sequencing were performed by Novogene Corporation (Sacramento, CA) with the Illumina NovaSeq X system. Sample quality was assessed using FastQC version 0.11.51, and reads in FASTQ format were cleaned up using Trimmomatic version 0.36⁷⁴ with the following parameters: ILLUMINACLIP:/opt/trimmomatic/0.36/adapters/TruSeq3-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36. Trimmed paired-end reads were mapped to the *Python molurus bivittatus* reference genome (NCBI RefSeq #GCF_000186305.1) using HISAT2 version 2.1.0⁷⁵ with the --sensitive and --dta flags. Samtools version 1.8⁷⁶ was used to convert SAM files to sorted and indexed BAM files. The Rsubread version 2.0.1⁷⁷ featureCounts function was used to generate raw counts for each sample using the indexed BAM files and the reference annotation GTF file. Gene count normalization and differential gene expression analysis was conducted with DESeq2 version 1.36.0⁷⁸. Pairwise comparisons between each group were conducted, and the normal shrinkage estimator was used to produce shrunken log fold changes for visualization. Differentially expressed genes were defined as those with an adjusted *p* value <0.01. Over-representation analysis was performed on differentially expressed genes identified from RNA-Seq data. A gene annotation database for *Python bivittatus* was obtained through the AnnotationHub⁷⁹ (v3.14.0) and AnnotationDbi⁸⁰ (v1.68.0) packages in R. The complete set of genes tested for differential expression served as the background gene set. Functional enrichment of Gene Ontology (GO) biological processes was conducted separately for upregulated and down-regulated gene sets using the clusterProfiler package (v4.14.4)⁸¹. Over-representation analysis was performed with the enrichGO function using the Benjamini–Hochberg method for multiple testing correction, and cut-offs of *p* < 0.05 and *q* < 0.1. Minimum and maximum gene set sizes were restricted to 10 and 500, respectively.

Proteomics sample preparation

Frozen left ventricle tissue samples were cryo-pulverized using the Covaris CP02 CryoPrep Pulverizer with an impact level of 1. Pulverized samples were transferred to 2 mL Eppendorf tubes and resuspended in 500 µL of chilled 8 M urea lysis buffer (8 M urea in water, 75 mM NaCl, 50 mM Tris pH 8.0, and 1 mM EDTA, supplemented with 2 µg/mL Aprotinin, 10 µg/mL Leupeptin, 1 mM PMSF, 10 mM NaF, 1:100 phosphatase-inhibitor cocktails 2 and 3, and 5 mM CAA). Samples were mixed by vortex for 10 s and incubated on ice for 15 min; this step was repeated twice. Tubes were centrifuged at maximum speed for 10 min to remove cell debris, and the supernatants were transferred to new 2 mL Eppendorf tubes. Protein concentration was estimated using the BCA assay, and 1.5 mg protein aliquots were taken for in-solution digestion. Denatured proteins were reduced by adding a final concentration of 5 mM DTT and incubating for 1 h at 37 °C, and alkylated by incubation in the dark with 10 mM iodoacetamide (IAA) for 45 min at 25 °C. Samples were diluted 1:4 with 50 mM Tris-HCl, pH 8.0, to decrease the Urea concentration below 2 M. Proteins were predigested with LysC (1:50 enzyme to substrate ratio) for 2 h at 25 °C and digested overnight with Trypsin (1:50, 25 °C). Peptides were acidified by adding formic acid (FA) to a final concentration of 1% and desalted using SepPak C18 columns (Waters) following the manufacturer's instructions. For PTM analysis, 100 µg of peptides were used for IMAC phosphopeptide enrichment, automated on an AssayMap Bravo System (Agilent) equipped with AssayMAP Fe(III)-NTA cartridges. About 1 mg of peptides were used for serial ubiquitination and acetylation enrichment following the UbiFast ubiquitin profiling protocol⁸² using the PTMScan® HS Ubiquitin/SUMO

Remnant Motif (K-ε-GG) Kit and the PTMScan® HS Acetyl-Lysine Motif (Ac-K) Kit (Cell Signaling Technology, 59322S and 46784S).

Label-free proteomics analysis

Dried, desalted peptides were reconstituted in MS sample buffer containing 3% acetonitrile (ACN)/0.1% formic acid (FA) in water. For global proteome analysis, 1 µg of peptides was injected into the system. One sixth or half of the enrichment output was used for phosphoproteome or ubiquitinome and acetylome analysis respectively. Samples were measured using a Vanquish Neo UHPLC system in nanoflow mode coupled with an Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific). Peptides were separated on a 20-cm reverse-phase column (inner diameter 75 µm, packed in-house with 1.9 µm C18 Reprosil beads) at a flow rate of 250 nL/min at 55 °C. Mobile phase A was 0.1% (vol/vol) FA and 3% (vol/vol) ACN in water, while mobile phase B was 90% (vol/vol) ACN and 0.1% (vol/vol) FA in water. The gradient was increased from 2 to 90% B in 99 min, followed by a plateau at 90% B for 5 min. MS data were acquired in data-independent acquisition (DIA) mode. The spray voltage was set to 2.2 kV in positive ion mode. The expected LC peak was 10 s. Full scan MS spectra were acquired at a resolution of 120 K in a scan range of 350–1650 *m/z*, 55% RF lens, 300% normalized automated gain control (AGC) target, 20 milliseconds maximum injection time (IT). DIA scans were done at a maximum injection time of 54 ms at 30 K resolution, 12 *m/z* isolation window, 26, 29, and 32% stepped normalized collision energy (NCE), and 1000% normalized AGC target.

Raw proteomics data processing

Raw data were processed using Spectronaut 18 software (Biognosys) with the spectral library-free (directDIA+) workflow and the Uniprot Burmese python (*Python bivittatus*) proteome as database (UP000695026, May 2024), using the default parameters. For the global proteome, carbamidomethyl (C) was used as a fixed modification, and acetyl (protein N-term), oxidation (M), and deamidation (NQ) as variable modifications. For PTM analysis, the PTM workflow was used, including Phospho (STY), GlyGly (K), and Acetyl (K), respectively, as variable modifications and setting the PTM probability cutoff to zero. Spectronaut intensity data matrices were imported to Perseus (1.6.10.45) and transformed into PTM site tables using the peptide collapse plugin.

Proteomics data analysis

The statistical analysis was done using the R software (R version 3.5.0, RStudio version 1.0.143) and the proteomics toolset for integrative data analysis Shiny app (ProTIGY, Broad Institute). Output data from Spectronaut 18 was filtered to remove protein groups with more than 20% missing values and protein groups with less than two peptides for global proteome analysis. For PTM analysis, sites with three valid values in at least one group and a localization probability above 0.5 were kept. Intensities were normalized by median subtraction, and missing values were imputed by random sampling of the lower end of the normal distribution (width 0.3 and downshift 1.8). Two-sample moderated *t*-test pair-wise comparison of the normalized log₂ intensities was used to determine the significantly regulated proteins and PTM sites among groups with an FDR cutoff of 5%. GO Biological Process over-enrichment analysis was performed using Enrichr⁸³. PTM set enrichment analysis of the phospho-proteomics data was performed using ptmSEA, as previously described⁸⁴.

Protein structural modeling and annotation

AlphaFold3⁸⁵ was used for protein structural predictions with *Python bivittatus* MYH15 and TTN sequences. The Burmese python MYH15 converter domain sequence was ascertained by sequence homology with *Homo sapiens* MYH7. The highest confidence level AlphaFold3 structures were used for downstream annotation of post-translationally modified residues. ChimeraX⁸⁶ (version 1.10) was used to annotate feeding-regulated residues in MYH15 and TTN.

Statistical analysis and presentation

Statistical significance cut-offs for the RNA sequencing and proteomics datasets were established using adjusted *p* values after Benjamini–Hochberg false discovery rate correction. Comparisons of two groups were made using an unpaired, two-sided *t*-test. Comparisons of more than two groups with a single variable were performed by one-way ANOVA. Comparisons of more than two groups and two independent variables were performed by two-way ANOVA. If statistical significance was reached, Sidak's or Tukey's post-hoc test for multiple independent pairwise comparisons was used, as detailed in the figure legends. Unless otherwise noted in the figure legends, a *p* value or adjusted *p* value of <0.05 was considered statistically significant. Data presentation was performed using GraphPad Prism (version 10) and Adobe Illustrator.

Data availability

Data types associated with this study include RNA sequencing, proteomics, agarose gel protein electrophoresis, myofibril mechanics measurements, and myosin ATP turnover kinetics. The raw RNA sequencing data were deposited at the National Center for Biotechnology Information (NCBI) (BioProject ID: PRJNA1335253) and are publicly available⁸⁷. The raw proteomics data were deposited at ProteomeXchange using the PRIDE partner repository (Dataset ID: PXD073863)⁸⁸. An uncropped image of the titin agarose gel is available in the Source Data File (Supplementary Data 6). All other data associated with this study can be found in the text, figures, figure legends, or supplementary material. The source data for all figures and Supplementary Figs. are available in Supplementary Data 1–6 and were deposited at FigShare (10.6084/m9.figshare.33340383). Requests for information or materials associated with this study should be directed to the corresponding authors.

Received: 11 March 2026; Accepted: 9 September 2026;

Published online: 30 September 2026

References

- Secor, S. M. & Diamond, J. A vertebrate model of extreme physiological regulation. *Nature* **395**, 659–662 (1998).
- Martin, T. G. & Leinwand, L. A. Molecular regulation of reversible cardiac remodeling: lessons from species with extreme physiological adaptations. *J. Exp. Biol.* **227**, jeb247445 (2024).
- Tan, Y., Martin, T. G., Harrison, B. C. & Leinwand, L. A. Utility of the Burmese python as a model for studying plasticity of extreme physiological systems. *J. Muscle Res. Cell Motil.* **44**, 95–106 (2023).
- Sanne, E. et al. Improved cardiac filling facilitates the postprandial elevation of stroke volume in *Python regius*. *J. Exp. Biol.* **219**, 3009–3018 (2016).
- Lavie, C. J. et al. Exercise and the cardiovascular system: clinical science and cardiovascular outcomes. *Circ. Res.* **117**, 207–219 (2015).
- Riquelme, C. A. et al. Fatty acids identified in the Burmese python promote beneficial cardiac growth. *Science* **334**, 528–531 (2011).
- Martin, T. G. et al. Regression of postprandial cardiac hypertrophy in Burmese pythons is mediated by FoxO1. *Proc. Natl. Acad. Sci. USA* **121**, e2408719121 (2024).
- Tan, Y., Peter, A. K., Ozeroff, C. D. & Leinwand, L. A. Python cardiomyocytes store lipids to buffer against hyperlipidemia. *Ann. N. Y. Acad. Sci.* **1551**, 159–166 (2025).
- Tan, Y. et al. Dynamic hyperplastic cardiac growth in Burmese pythons. *Am. J. Physiol. Cell Physiol.* **331**, C641–C652 (2026).
- Slay, C. E., Enok, S., Hicks, J. W. & Wang, T. Reduction of blood oxygen levels enhances postprandial cardiac hypertrophy in Burmese python (*Python bivittatus*). *J. Exp. Biol.* **217**, 1784–1789 (2014).
- Martin, T. G. & Kirk, J. A. Under construction: the dynamic assembly, maintenance, and degradation of the cardiac sarcomere. *J. Mol. Cell Cardiol.* **148**, 89–102 (2020).
- Sarin, V., Muthuchamy, M. & Heaps, C. L. Ca²⁺ sensitization of cardiac myofilament proteins contributes to exercise training-enhanced myocardial function in a porcine model of chronic occlusion. *Am. J. Physiol. Heart Circ. Physiol.* **301**, H1579–H1587 (2011).
- Müller, A. E. et al. Acute exercise modifies titin phosphorylation and increases cardiac myofilament stiffness. *Front. Physiol.* **5**, 449 (2014).
- Bilchick, K. C. et al. Heart failure-associated alterations in troponin I phosphorylation impair ventricular relaxation-afterload and force-frequency responses and systolic function. *Am. J. Physiol. Heart Circ. Physiol.* **292**, H318–H325 (2007).
- Hamdani, N., Bishu, K. G., von Frieling-Salewsky, M., Redfield, M. M. & Linke, W. A. Deranged myofilament phosphorylation and function in experimental heart failure with preserved ejection fraction. *Cardiovasc. Res.* **97**, 464–471 (2013).
- Xiao, S. et al. Python metabolomics uncovers a conserved postprandial metabolite and gut-brain feeding pathway. *Nat. Metabol.* **8**, 869–884 (2026).
- Crocini, C. et al. Postprandial cardiac hypertrophy is sustained by mechanics, epigenetic, and metabolic reprogramming in pythons. *Proc. Natl. Acad. Sci. USA* **121**, e2322726121 (2024).
- Andersen, J. B., Rourke, B. C., Caiozzo, V. J., Bennett, A. F. & Hicks, J. W. Postprandial cardiac hypertrophy in pythons. *Nature* **434**, 37–38 (2005).
- Lee, L. A. et al. Functional divergence of the sarcomeric myosin, MYH7b, supports species-specific biological roles. *J. Biol. Chem.* **299**, 102657 (2022).
- da Silva Vasconcelos, E. et al. Effects of feeding and digestion on myocardial contractility and expression of calcium-handling proteins in Burmese pythons (*Python molurus*). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **240**, 110371 (2020).
- Guelli et al. Contractile function of fasting and digesting broad-snouted caiman (*Caiman latirostris*). *Can. J. Zool.* **101**, 614–622 (2023).
- Wang, L. & Kawai, M. A re-interpretation of the rate of tension redevelopment (kTR) in active muscle. *J. Muscle Res. Cell Motil.* **34**, 407–415 (2013).
- Linke, W. A., Popov, V. I. & Pollack, G. H. Passive and active tension in single cardiac myofibrils. *Biophys. J.* **67**, 782–792 (1994).
- Loescher, C. M. et al. Titin governs myocardial passive stiffness with major support from microtubules and actin and the extracellular matrix. *Nat. Cardiovasc. Res.* **2**, 991–1002 (2023).
- Trombitás, K. et al. Extensibility of isoforms of cardiac titin: variation in contour length of molecular subsegments provides a basis for cellular passive stiffness diversity. *Biophys. J.* **79**, 3226–3234 (2000).
- Koser, F., Loescher, C. & Linke, W. A. Posttranslational modifications of titin from cardiac muscle: how, where, and what for? *FEBS J.* **286**, 2240–2260 (2019).
- Rassier, D. E. Sarcomere mechanics in striated muscles: from molecules to sarcomeres to cells. *Am. J. Physiol. Cell Physiol.* **313**, C134–C145 (2017).
- Schmid, M. & Toepfer, C. N. Cardiac myosin super relaxation (SRX): a perspective on fundamental biology, human disease and therapeutics. *Biol. Open* **10**, bio057646 (2021).
- Toepfer, C. N. et al. Myosin sequestration regulates sarcomere function, cardiomyocyte energetics, and metabolism, informing the pathogenesis of hypertrophic cardiomyopathy. *Circulation* **141**, 828–842 (2020).
- Jones, S. T. M. et al. Assaying the myosin super-relaxed state across muscle types, cells and proteins for understanding muscle biology and use in drug discovery. *Nat. Protoc.* <https://doi.org/10.1038/s41596-025-01291-0> (2025).
- Van der Pijl, R. J. et al. Increased cardiac myosin super-relaxation as an energy saving mechanism in hibernating grizzly bears. *Mol. Metab.* **92**, 102084 (2024).

32. Kampourakis, T., Ponnamp, S., Campbell, K. S., Wellette-Hunsucker, A. & Koch, D. Cardiac myosin binding protein-C phosphorylation as a function of multiple protein kinase and phosphatase activities. *Nat. Commun.* **15**, 5111 (2024).
33. Davis, J. S., Satorius, C. L. & Epstein, N. D. Kinetic effects of myosin regulatory light chain phosphorylation on skeletal muscle contraction. *Biophys. J.* **83**, 359–370 (2002).
34. Franks, K., Cooke, R. & Stull, J. T. Myosin phosphorylation decreases the ATPase activity of cardiac myofibrils. *J. Mol. Cell Cardiol.* **16**, 597–604 (1984).
35. Romanick, S. S. et al. Skeletal muscle alpha actin acetylation enhances myosin binding and increases calcium sensitivity. *Biophys. Rep.* **5**, 100226 (2025).
36. Eaton, D. M. et al. HDAC inhibition regulates cardiac function by increasing myofilament calcium sensitivity and decreasing diastolic tension. *Pharmacother.* **14**, 1509 (2022).
37. Lin, Y. H. et al. Site-specific acetyl-mimetic modification of cardiac troponin I modulates myofilament relaxation and calcium sensitivity. *J. Mol. Cell Cardiol.* **139**, 135–147 (2020).
38. Carnegie, G. K. et al. AKAP-Lbc mobilizes a cardiac hypertrophy signaling pathway. *Mol. Cell* **32**, 169–179 (2008).
39. Liu, Y. & Schneider, M. F. Opposing HDAC4 nuclear fluxes due to phosphorylation by β -adrenergic activated protein kinase A or by activity or Epac activated CaMKII in skeletal muscle fibres. *J. Physiol.* **591**, 3605–3623 (2013).
40. He, T. et al. Cyclic AMP represses pathological MEF2 activation by myocyte-specific hypo-phosphorylation of HDAC5. *J. Mol. Cell Cardiol.* **145**, 88–98 (2020).
41. Anderson, B. R. & Granzier, H. L. Titin-based tension in the cardiac sarcomere: molecular origin and physiological adaptations. *Prog. Biophys. Mol. Biol.* **110**, 204–217 (2012).
42. Freundt, J. K. & Linke, W. A. Titin as a force-generating muscle protein under regulatory control. *J. Appl. Physiol.* **126**, 1474–1482 (2018).
43. Linke, W. A., Stockmeier, M. R., Ivemeyer, M., Hosser, H. & Mundel, P. Characterizing titin's I-band Ig domain region as an entropic spring. *J. Cell Sci.* **111**, 1567–1574 (1998).
44. Gunther, L. K. et al. Converter domain mutations in myosin alter structural kinetics and motor function. *J. Biol. Chem.* **294**, 1554–1567 (2019).
45. Allen, D. L. et al. Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. *J. Appl. Physiol.* **90**, 1900–1908 (2001).
46. Jensen, B., Larsen, C. K., Nielsen, J. M., Simonsen, L. S. & Wang, T. Change of cardiac function, but not form, in postprandial pythons. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **160**, 35–42 (2011).
47. Last, K. B., Malte, H., Rindom, E., Guagnoni, I. N. & Wang, T. Proportional increment of oxygen consumption, heart rate and core body temperature in the digesting *Python bivittatus*. *J. Exp. Biol.* **227**, jeb248021 (2024).
48. Secor, S. M. Digestive physiology of the Burmese python: broad regulation of integrated performance. *J. Exp. Biol.* **211**, 3767–3774 (2008).
49. Andrew, A. L. et al. Growth and stress response mechanisms underlying post-feeding regenerative organ growth in the Burmese python. *BMC Genomics* **18**, 338 (2017).
50. Castoe, T. A. et al. A multi-organ transcriptome resource for the Burmese python (*Python molurus bivittatus*). *BMC Res. Notes* **4**, 310 (2011).
51. Castoe, T. A. et al. The Burmese python genome reveals the molecular basis for extreme adaptation in snakes. *Proc. Natl. Acad. Sci. USA* **110**, 20645–20650 (2013).
52. Duan, J. et al. Transcriptome analysis of the response of Burmese python to digestion. *Gigascience* **6**, 1–18 (2017).
53. Wall, C. E. et al. Whole transcriptome analysis of the fasting and fed Burmese python heart: insights into extreme physiological cardiac adaptation. *Physiol. Genomics* **43**, 69–76 (2011).
54. Jayne, B. C., Bamberger, A. L., Mader, D. R. & Bartoszek, I. A. Scaling relationships of maximal gape in two species of large invasive snakes, brown treesnakes and Burmese pythons, and implications for maximal prey size. *Integr. Org. Biol.* **4**, obac033 (2022).
55. Kobayashi, T. & Solaro, R. J. Calcium, thin filaments, and the integrative biology of cardiac contractility. *Annu. Rev. Physiol.* **67**, 39–67 (2005).
56. Zhang, X. et al. Distinct contributions of the thin and thick filaments to length-dependent activation in heart muscle. *eLife* **6**, e24081 (2017).
57. Cazorla, O., Wu, Y., Irving, T. C. & Granzier, H. Titin-based modulation of calcium sensitivity of active tension in mouse skinned cardiac myocytes. *Circ. Res.* **88**, 1028–1035 (2001).
58. Kampourakis, T., Sun, Y.-B. & Irving, M. Myosin light chain phosphorylation enhances contraction of heart muscle via structural changes in both thick and thin filaments. *Proc. Natl. Acad. Sci. USA* **113**, E3039–E3047 (2016).
59. Nag, S. et al. Contractility parameters of human β -cardiac myosin with the hypertrophic cardiomyopathy mutation R403Q show loss of motor function. *Sci. Adv.* **1**, e1500511 (2015).
60. Reconditi, M. et al. Motion of myosin head domains during activation and force development in skeletal muscle. *Proc. Natl. Acad. Sci. USA* **108**, 7236–7240 (2011).
61. Rassier, D. E. & Månsson, A. Mechanisms of myosin II force generation: insights from novel experimental techniques and approaches. *Physiol. Rev.* **105**, 1–93 (2025).
62. Bell, K. M., Huang, A., Kronert, W. A., Bernstein, S. I. & Swank, D. M. Prolonged myosin binding increases muscle stiffness in *Drosophila* models of Freeman-Sheldon syndrome. *Biophys. J.* **120**, 844–854 (2021).
63. Li, Y., Lang, P. & Linke, W. A. Titin stiffness modifies the force-generating region of muscle sarcomeres. *Sci. Rep.* **6**, 24492 (2016).
64. Kawana, M., Sarkar, S. S., Sutton, S., Ruppel, K. M. & Spudich, J. A. Biophysical properties of human β -cardiac myosin with converter mutations that cause hypertrophic cardiomyopathy. *Sci. Adv.* **3**, e1601959 (2017).
65. Vera, C. D. et al. Myosin motor domains carrying mutations implicated in early or late onset hypertrophic cardiomyopathy have similar properties. *J. Biol. Chem.* **294**, 17451–17462 (2019).
66. Liu, C. et al. Homologous mutations in human β , embryonic, and perinatal muscle myosins have divergent effects on molecular power generation. *Proc. Natl. Acad. Sci. USA* **121**, e2315472121 (2024).
67. Köhler, J. et al. Mutation of the myosin converter domain alters cross-bridge elasticity. *Proc. Natl. Acad. Sci. USA* **99**, 3557–3562 (2002).
68. Seeböhm, B. et al. Cardiomyopathy mutations reveal variable region of myosin converter as major element of cross-bridge compliance. *Biophys. J.* **97**, 806–824 (2009).
69. Lin, Y.-H. et al. HDAC6 modulates myofibril stiffness and diastolic function of the heart. *J. Clin. Invest.* **132**, e148333 (2022).
70. Piroddi, N., Tesi, C., Tobacman, P., elligrinoM. A., Homsher, L. S. & Poggese, E. C. Contractile effects of the exchange of cardiac troponin for fast skeletal troponin in rabbit psoas single myofibrils. *J. Physiol.* **552**, 917–931 (2003).
71. Warren, C. M., Krzesinski, P. R. & Greaser, M. L. Vertical agarose gel electrophoresis and electroblotting of high-molecular-weight proteins. *Electrophoresis* **24**, 1695–1702 (2003).
72. Schnell, E. A. et al. Diffusion measured by fluorescence recovery after photobleaching based on multiphoton excitation laser scanning microscopy. *J. Biomed. Optics* **13**, e064037 (2008).
73. Choi, A. P. C. & Zheng, Y. P. Estimation of Young's modulus and Poisson's ratio of soft tissue from indentation using two different-sized indentors: finite element analysis of the finite deformation effect. *Med. Biol. Eng. Comput.* **43**, 258–264 (2005).
74. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
75. Kim, D., Paggi, J. M., Park, C., Bennett, C. & Salzberg, S. L. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat. Biotechnol.* **37**, 907–915 (2019).

76. Danecek, P. et al. Twelve years of SAMtools and BCFtools. *Gigascience* **10**, giab008 (2021).
77. Liao, Y., Smyth, G. K. & Shi, W. The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. *Nucleic Acids Res.* **47**, e47 (2019).
78. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
79. Morgan, M. & Shepherd, L. AnnotationHub: client to access AnnotationHub resources. <https://doi.org/10.18129/B9.bioc.AnnotationHub> (2026).
80. Pages, H., Carlson, M., Falcon, S. & Li, N. AnnotationDbi: manipulation of SQLite-based annotations in Bioconductor. <https://doi.org/10.18129/B9.bioc.AnnotationDbi> (2026).
81. Xu, S. et al. Using clusterProfiler to characterize multiomics data. *Nat. Protoc.* **19**, 3292–3320 (2024).
82. Udeshi, N. D. et al. Rapid and deep-scale ubiquitylation profiling for biology and translational research. *Nat. Commun.* **11**, 359 (2020).
83. Chen, E. Y. et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* **14**, 128 (2013).
84. Krug, K. et al. A curated resource for phosphosite-specific signature analysis. *Mol. Cell. Proteomics* **18**, 576–593 (2019).
85. Abramson, J. et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493–500 (2024).
86. Meng, E. C. et al. UCSF ChimeraX: tools for structure building and analysis. *Protein Sci.* **32**, e4792 (2023).
87. Martin T. G. et al. Data from “multi-omics insight into cardiac myofibril remodeling in post-prandial Burmese pythons.” National Center for Biotechnology Information (NCBI), BioProject ID: PRJNA1335253. <https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1335253> (2025).
88. Martin T. G. et al. Data from “study of post-prandial response in the Burmese python heart.” Proteomics Identifications Database (PRIDE), Dataset ID: PXD073863. <https://www.ebi.ac.uk/pride/archive/projects/PXD073863> (2026).

Acknowledgements

We thank Dr. Jack Gugel, Dr. Skip Maas, Marcus Mullen, and Brooke Shepard for valuable scientific discussions and feedback on this study. We also acknowledge Aaron Rothchild and the staff in the Office of Animal Resources at CU Boulder for assistance with python colony husbandry. We thank the Shared Instruments Pool (RRID: SCR_018986), Department of Biochemistry, University of Colorado Boulder, for the use of the Amersham Typhoon 5 imager (funded by NIH S10OD034218). We thank the BioFrontiers Computing Facility at the University of Colorado Boulder for high-performance computing and data storage resources supported by BioFrontiers IT.

Author contributions

Conceptualization: T.G.M., L.-S.A., Y.T., M.G., P.M., and L.A.L. Methodology: T.G.M., L.-S.A., K.C.W., E.G.M., Y.T., D.R.H., and B.E.K. Investigation: T.G.M., L.-S.A., K.C.W., E.G.M., Y.T., D.R.H., B.E.K., L.N., J.L., and I.L. Formal analysis: T.G.M., L.-S.A., K.C.W., E.G.M., D.R.H., and J.L. Visualization: T.G.M., L.-S.A., D.R.H., and J.L. Funding acquisition: T.G.M., D.R.H., J.O., M.G., and L.A.L. Resources: K.S.A., M.G., P.M., and L.A.L.

Project administration: M.G., P.M., and L.A.L. Supervision: K.S.A., J.O., M.G., P.M., and L.A.L. Writing—original draft: T.G.M. Writing—review and editing: L.-S.A., K.C.W., E.G.M., Y.T., D.R.H., B.E.K., J.L., I.L., L.N., K.S.A., J.O., M.G., P.M., and L.A.L.

Funding

This study was supported by the Fondation Leducq Transatlantic Network of Excellence (21CVD02 to M.G. and L.A.L.), the United States National Institutes of Health (F32HL170637 to TGM), and the American Heart Association (24PRE1195130 to D.R.H.).

Competing interests

L.A.L. was a Co-Founder of MyoKardia, acquired by Bristol Myers Squibb, and Kardigan. T.G.M. and L.A.L. are Co-Founders of Arkana Therapeutics. These companies were not involved in the present study. The remaining authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s42003-026-11007-z>.

Correspondence and requests for materials should be addressed to Michael Gotthardt, Philipp Mertins or Leslie A. Leinwand.

Peer review information *Communications Biology* thanks William Joyce and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editors: Dr Aylin Bircan and Professor Simona Chera. A peer review file is available.

Reprints and permissions information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2026