Supplementary Information

Age-dependent Lamin remodeling induces cardiac dysfunction via dysregulation of cardiac transcriptional programs

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RUNNING TITLE

Age-associated Nuclear Remodeling Drives Cardiac Dysfunction
Supplemental Figures
**Supplemental Figure 1: Nuclear Dynamics in Cardiac and Skeletal Muscle Cells. (A)**

Kaplan-Meier survival curve for yw (gray) and w^{1118} (black) flies. n = 103 and flies, respectively, were used in the plot. \( p < 10^{-3} \) based on Log-Rank (Mantel-Cox) test between the two strains.

**(B)** Plots of 2D projected area (left) and circularity data (right) for yw flies. n = 129, 108, and 143 for yw flies at 1-, 3-, and 5-weeks, respectively. **(C)** Cardiomyocyte nuclear area (left) and aspect ratio (right) plotted for w^{1118} and yw flies as a function of adult age. n = 96, 116, and 141 nuclei for for w^{1118} flies and n = 129, 108, and 143 for yw flies at 1-, 3-, and 5-weeks of adulthood, respectively. **(D)** Ventral muscle nuclear area (left), perimeter (center), and aspect ratio (right) from w^{1118} flies at 1-, 3-, and 5-weeks of adulthood. n = 528, 604, 661 ventral muscle nuclei from w^{1118} flies at 1-, 3-, and 5-weeks of adulthood. **(E)** Representative images of the 3D wireframe mesh of cardiomyocyte nuclei from w^{1118} flies at 1- (top), 3- (middle), and 5-weeks (bottom) of adulthood. Scale bar is 5 μm. *\( p < 0.05 \), **\( p < 10^{-2} \), ***\( p < 10^{-3} \), and ****\( p < 10^{-4} \) by one-way ANOVA with Tukey multiple comparisons test.
Supplemental Figure 2: Natural Aging Downregulates LamC and LamB but does not affect their localization. (A) MA plot of all genes of 1- and 5-week adult w^{118} fly hearts for their log₂ normalized expression.
fold change (FC) and mean normalized expression counts. Data is shown in black for genes -
1.25 < FC < 1.25 (dashed lines) or where p-adj > 0.05. Open circles represent genes that do not
map to a nuclear ontological term. Green and purple data represent DEGs that are up- or down-
resulted in 5-week adult flies, respectively. (B) Cellular component and molecular function
ontological terms resulting from genes also associated with the nuclear envelope GO term,
organized by their elimination pruning p-value. (C) Representative images of 1- and 5-week-old
adult w^{1118} fly heart nuclei stained for DNA and Hybridization Chain Reaction (HCR) probes for
LamC (green) and LamB (purple) mRNA transcripts. Scale bar is 5 µm. (D) The left plot shows
the percent area occupied by LamC (green) and LamB (purple) mRNA transcripts per
cardiomyocyte in 1- and 5-week-old adult w^{1118} fly hearts. The right plot normalizes data to mean
area at 1 week for each transcript. n = 49, 36, 49, and 36 nuclei from w^{1118} flies at 1- and 5-
weeks of adulthood for LamC and LamB, respectively. (E) Corrected total nuclear fluorescence
(CTNF) of 1-, 3-, and 5-week-old adult yw flies for LamC (top) and LamB (bottom). n = 94, 106,
and 133 nuclei analyzed for LamC and n = 173, 115, and 90 nuclei analyzed for LamB in of 1-,
3-, and 5-week-old adults, respectively. (F) Image showing a representative nucleus with multiple
lines radiating out from its centroid (left) to create line plots that are averaged into a single radial
profile of the fluorescent intensity (right). At bottom, the ratio of edge to center intensity is plotted
for the indicated fly strains, transcript, and adult ages. n = 72, 63, 73, and 33 nuclei from w^{1118}
flies at 1- and 5-weeks of adulthood for LamC and LamB, respectively. n = 51, 87, 77, and 42
nuclei from yw flies at 1- and 5-weeks of adulthood for LamC and LamB, respectively. *p<0.05,
**p<10^{-2}, ***p<10^{-3}, and ****p<10^{-4} by one-way ANOVA with Tukey multiple comparisons test.
Supplemental Figure 3: Validation and Morphological and Functional Characterization of LamB and LamC RNAi lines. Corrected total nuclear fluorescence (CTNF) for cardiomyocytes from (A) LamC RNAi and (B) LamB RNAi fly lines and their respective controls, i.e., attp2 and attp40. Each RNAi line is stained by its protein of interest to ensure effective knockdown. n = 47, 36, 79, and 69 nuclei from attp2 and LamC RNAi flies at 1- and 4-weeks of adulthood (left to right). n = 53, 29, 65, and 61 nuclei from attp40 and LamB RNAi flies at 1- and 4-weeks of adulthood (left to right). (C) Plots quantifying nuclear perimeter (left) and aspect ratio (right) based on confocal images of LamB and LamC RNAi lines and their genetic control background 4-weeks of adulthood. n = 111, 96, 46, and 89 nuclei/condition for 4-week-old adult control and LamC RNAi flies, respectively. n = 113, 97, 46, and 92 nuclei/condition for 4-week-old adult control and LamB RNAi flies, respectively. (D) Plots quantifying nuclear area (left), perimeter (middle left), aspect ratio (middle right), and circularity (right) based on confocal images of LamB and LamC RNAi lines and their genetic control background at 4-weeks of adulthood. For all plots, n = 136, 101, 86, and 95 nuclei/condition for attp2, LamC RNAi, attp40, and LamB RNAi, respectively. (E) Plots of diastolic and systolic diameters as well as wall ‘shortening’ velocity determined from motion-mode imaging for attp2 control and LamC flies at 1- and 4-weeks of adulthood. For all plots, n = 21, 23, 25, and 27 nuclei/condition for attp2 and LamC RNAi at 1- and 4-weeks of adulthood, respectively. (F) Plots of diastolic and systolic diameters as well as wall ‘shortening’ velocity determined from motion-mode imaging for attp40 control and LamB flies at 1- and 4-weeks of adulthood. For all plots, n = 31, 21, 26, and 25 nuclei/condition for attp40 and LamB RNAi at 1- and 4-weeks of adulthood, respectively. For panels A-D, *p<0.05, **p<10^{-2}, ***p<10^{-3}, and ****p<10^{-4} from a unpaired t-test at each time point and RNAi line. For E-F, *p<0.05, **p<10^{-2}, ***p<10^{-3}, and ****p<10^{-4} by one-way ANOVA with Tukey multiple comparisons test.
Supplemental Figure 4: Validation of transgenic fly background and effects of LamB on myogenic transcription factor expression. (A) Volcano plot and (B) heat map of bulk RNA-seq from surgically dissected attp2 heart tubes. Fold change represents 5-week attp2 hearts normalized to 1-week old hearts and p-adjusted was computed from quintuplicate repeats. 1,998 differentially expressed genes (DEGs) were assessed from cutoffs of -1.25 >
FC > 1.25 and p-adj < 0.05 (dashed lines) from comparisons of 15 fly hearts per replicate; DEGs increasing and decreasing with age are shown in green and purple, respectfully. Heatmap columns were hierarchically clustered using Euclidean distance and linkage shown by the dendrogram. (C) 635 of 688 genes were co-regulated DEGs (92.3%) in the w1118 and attp2 control fly hearts were plotted based on their fold change with age. DEGs were annotated based on their ontological categorization as nuclear- (orange), extracellular matrix (ECM; green)-, or cytoskeletal-related (blue). A subset of DEGs either did not fit those categories (black/white) or lacked a known ontology (gray). Only 7.7% of all DEGs were dysregulated. (D) Representative images of attp40 control and LamB RNAi flies at 1- and 4-weeks of adulthood stained with HCR probes for H15, Hand, and Tin transcription factors and DAPI for DNA. Scale bar is 5 μm. (E) A plot for each transcription factor is shown and quantifies the per cell percent area for each transcript in attp40 control and LamB RNAi flies at 1- and 4-weeks of adulthood. For H15, n = 93, 87, 85, and 71 cells for 1-week control, 1-week LamB RNAi, 4-week control, and 4-week LamB RNAi, respectively. For Hand, n = 111, 96, 105, and 76 cells for 1-week control, 1-week LamB RNAi, 4-week control, and 4-week LamB RNAi, respectively. For Tin, n = 105, 118, 102, and 76 cells for 1-week control, 1-week LamB RNAi, 4-week control, and 4-week LamB RNAi, respectively. *p<0.05, **p<10^{-2}, ***p<10^{-3}, and ****p<10^{-4} by one-way ANOVA with Tukey multiple comparisons test.
Supplemental Figure 5: Effects of Myogenic Transcription Factor Loss on Morphology and Function of Heart Tubes, and its rescue by LamC Overexpression. (A)

Representative images of sarcomeres visualized by phalloidin (F-Actin) staining in heart tubes with the indicated transgenes expressed under the control of the Hand promoter. Scale bar is 10 μm. (B) Diastolic (top) and systolic (bottom) diameters of heart tubes from control fly lines (attp40 and attp2) and their corresponding transgenic flies expressing the indicated RNAi. Diameters are shown for non-permissive (29°C) and permissive (18°C) temperatures for transgene expression. n = 13, 15, 18, and 17 hearts/condition for attp40 at 18°C and 29°C and H15 RNAi at 18°C and 29°C, respectively. Also n = 33, 31, 20, 23, 33, 31, 33, and 24 hearts/condition for attp2, Tin RNAi, and Hand RNAi at 18°C and 29°C, respectively. (C) Corrected total nuclear fluorescence (CTNF) of LamC protein expressed in nuclei from flies at 18°C and 29°C expressing a generic GFP<sub>NLS</sub> transgene or an overexpression of LamC (LamC OE). n = 41, 41, 45, and 42 nuclei (left to right). (D) Representative images (top) of HCR probes for LamC stained in LamC OE and GFP<sub>NLS</sub> flies in permissive (29°C) and non-permissive (18°C) conditions. A plot quantifying the area containing transcripts, normalized to mean 18°C GFP<sub>NLS</sub>, is shown at bottom. n = 30, 42, 27, and 46 nuclei analyzed/condition (left to right). (E) Plots of perimeter (left) and circularity data (right) for cardiomyocyte nuclei from GFP<sub>NLS</sub> and LamC OE fly lines grown at 18°C and 29°C. n = 39, 40, 46, and 41 nuclei (left to right). (F) Diastolic (left) and systolic (right) diameters of heart tubes from GFP<sub>NLS</sub> and LamC OE fly lines grown at permissive (29°C) and non-permissive (18°C) temperatures for transgene expression. n = 36, 23, 33, and 39 hearts/condition (left to right). *p<0.05, **p<10<sup>-2</sup>, ***p<10<sup>-3</sup>, and ****p<10<sup>-4</sup> by independent t-test.
Supplemental Figure 6: Myogenic transcription factor expression in the left ventricles of aged non-human primates and mice. (A) Expression of three housekeeping genes for mice are plotted as a function of age with a linear and associated p-value shown. Data is plotted for
raw Cq values. (B) Expression of four transcription factors in mice is shown, normalized to each housekeeper gene. Data normalized to Eef1e1 (black), Rpl4 (medium gray), and ACTB (light gray) are plotted using the left and right y-axes, depending on the axis label colors. P-values for each fit are shown in the upper right corner. (C) Expression of three housekeeping genes for rhesus macaques are plotted as a function of age with a linear and associated p-value shown. Data is plotted for raw Cq values. (D) Expression of four transcription factors in rhesus macaques is shown, normalized to each housekeeper gene. Data normalized to Rpl13a (black) and TUBB2 (light gray) use the left y-axis whereas Rpl32 uses the right y-axis (medium gray). P-values for each fit are shown in the upper right corner.
Supplemental Tables

**Supplemental Table 1: Age-Associated Transcriptome Changes of \( w^{1118} \) flies.** Table showing changes in the transcriptome of \( w^{1118} \) flies. Data listed is annotated for common gene name followed by (left to right): gene description, fold change for the comparison of 5-week/1-week adults, log₂ Fold Change value, p-Value, p-Adj, Average Log2 Expression, raw Fragments Per Kilobase of transcript per Million mapped reads (FPKM), gene ID, and any gene aliases, if available.

**Supplemental Table 2: Gene Ontologies of Age-Associated Transcriptome Changes of \( w^{1118} \) flies.** Table showing gene ontologies associated with transcriptome changes in \( w^{1118} \) flies. Data listed is annotated for (left to right): gene ontology identifier, common name, p-value, false discovery rate (FDR)-adjusted p-Value, Elim pruning p-Value, Number of Genes in Term, Number of Genes that are also in this Filter or Cluster, Number of Up-regulated genes, Number of Down-regulated genes, and the negative Log10 Elim pruning p-Value.

**Supplemental Table 3: Differentially Accessible Chromatin Regions for Age, Lam C RNAi and LamB RNAi.** This table annotates ATAC-sequencing data for age comparisons in the w1118 background, in the LamB RNAi vs. attp40 flies, and in the LamC RNAi vs. attp2 flies. In each tab, data annotates (left to right): peak number, chromosome, peak start location, peak end location, peak annotation, detailed peak annotation, distance to transcription start site (TSS), nearest promoter ID, Entrez ID of nearest gene, Unigene ID for nearest gene, nearest Refseq, gene name, gene alias, gene description, gene type, gene concentration, conc_group1, conc_group2, fold change, p-value, and false discovery rate (FDR).

**Supplemental Table 4: Analysis of DARs common to Aging vs LamC RNAi and Aging vs LamB RNAi.** This table annotates co-regulated DAR from ATAC-sequencing data for LamB RNAi vs. attp40 flies and for LamC RNAi vs. attp2 flies. In tabs one (Age_and_LamC_EdgeR) and three (Age_and_LamB_EdgeR), each comparison is denoted with “x” and “y” corresponding to RNAi vs. control and 5wk vs. 1wk aging flies, respectively, and annotated for (left to right): peak number, chromosome, peak start location, peak end location, peak annotation, detailed peak annotation, distance to transcription start site (TSS), nearest promoter ID, Entrez ID of...
nearest gene, Unigene ID for nearest gene, nearest Refseq, gene name, gene alias, gene description, gene type, gene concentration, conc_group1, conc_group2, fold change, p-value, and false discovery rate (FDR). In tabs two (Age and LamC Genes Only) and four (Age and LamB Genes Only), data is annotated just for the fold change for each gene associated with the DAR.

Supplemental Table 5: Gene Ontologies for co-downregulated DAR in Aging and LamC iR. This table annotates the gene ontologies for biological process (BP) and cellular component (CC) for co-downregulated genes based on the closest assigned gene for the peak and associated DAR. Data is annotated (left to right) for: GO complete name, Drosophila melanogaster peak, number of genes in dataset, expected gene number, gene over/under, fold Enrichment, raw P-value, FDR, and -Log10 (FDR p-val).

Supplemental Table 6: LamC RNAi-mediated Transcriptome Changes in attp2 fly background. Table showing changes in the transcriptome of LamC RNAi flies at 1-week of adulthood versus their control attp2 background flies. Data plotted is annotated for common gene name followed by (left to right): gene description, fold change for the comparison of LamC/attp2, log2 Fold Change value, p-Value, p-Adj, Average Log2 Expression, raw Fragments Per Kilobase of transcript per Million mapped reads (FPKM), gene ID, and any gene aliases, if available.

Supplemental Table 7: Age-Associated Transcriptome Changes of attp2 flies. Table showing changes in the transcriptome of attp2 flies. Data plotted is annotated for common gene name followed by (left to right): gene description, fold change for the comparison of 5-week/1-week adults, log2 Fold Change value, p-Value, p-Adj, Average Log2 Expression, raw Fragments Per Kilobase of transcript per Million mapped reads (FPKM), gene ID, and any gene aliases, if available.

Supplemental Table 8: Co-regulated Transcriptome Changes in attp2 vs w1118 fly background. Table showing differentially expressed genes as a function of age between the attp2 and w1118 fly genotypes. Only genes differentially expressed with age in both systems are
shown. Data is annotated for common gene symbol followed by (left to right): gene ID, fly base alias, gene description, and then in the *attp2* fly first and *w1118* second, we show the log₂ fold change value with age, p-value for the age fold change, and p-adj value for the age fold change.

**Supplemental Table 9: Co-regulated Transcriptome Changes in LamC RNAi vs attp2 and Biological Process ontology terms.** Mutually significant DEGs from *LamC RNAi* and aged fly hearts are listed in this table. In the first tab listing co-regulated genes, data is annotated (left to right) by: symbol, gene ID, gene alias, gene description, base mean gene expression with age, log₂ fold change with age, p-adjusted for age fold change, base mean gene expression for *LamC RNAi vs. attp2*, log₂ fold change for *LamC RNAi vs. attp2*, p-adjusted for *LamC RNAi vs. attp2* fold change, protein type, additional protein description, protein classification, and the type of cellular component ontological terms associated with the gene, e.g., cytoskeleton, nucleus, extracellular matrix, other, or unknown. In the second tab, PANTHER biological process ontological terms are annotated for co-regulated terms.

**Supplemental Table 10: Primer Sequences.** All forward and reverse primer sequences for mouse and rhesus macaque are shown here.