Supplementary Information

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

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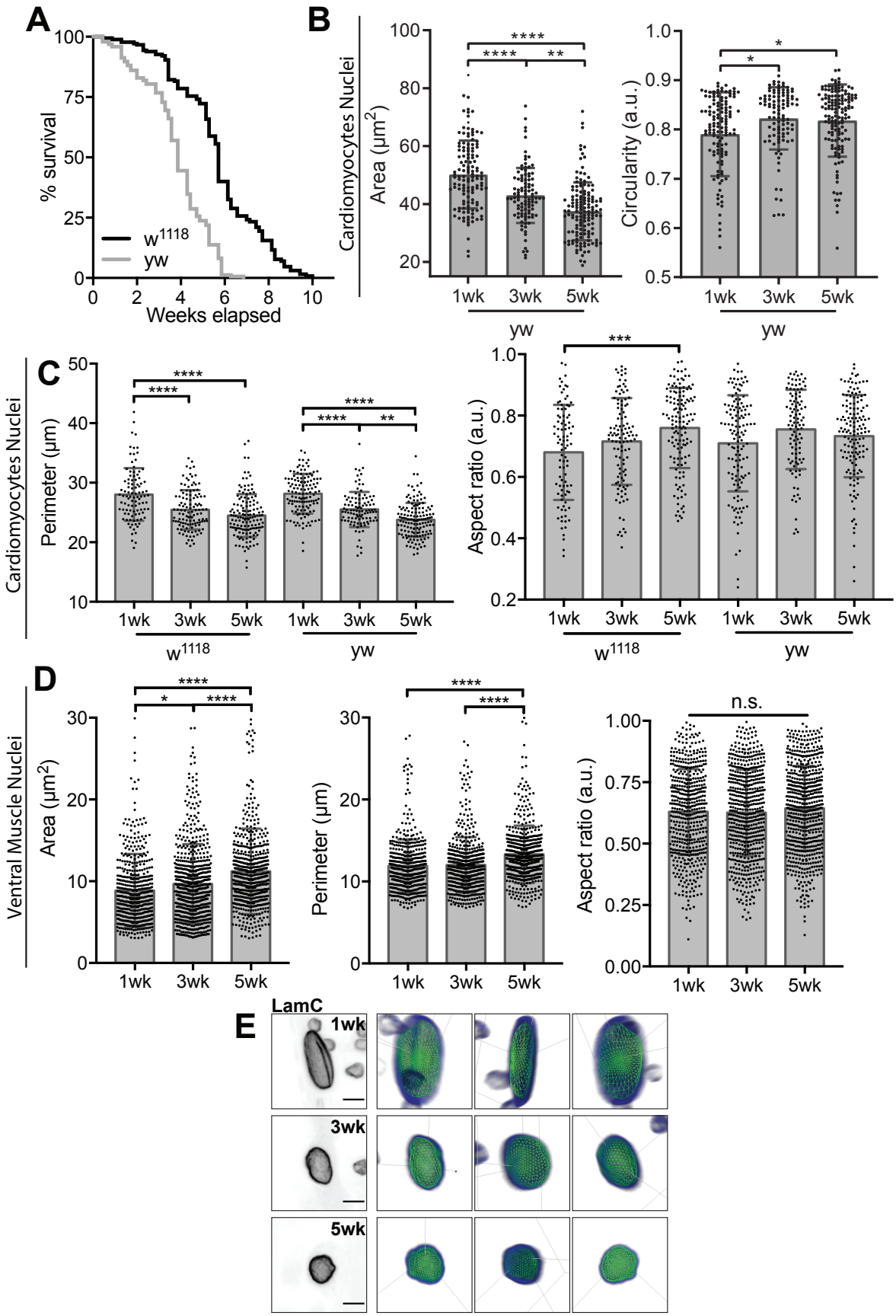
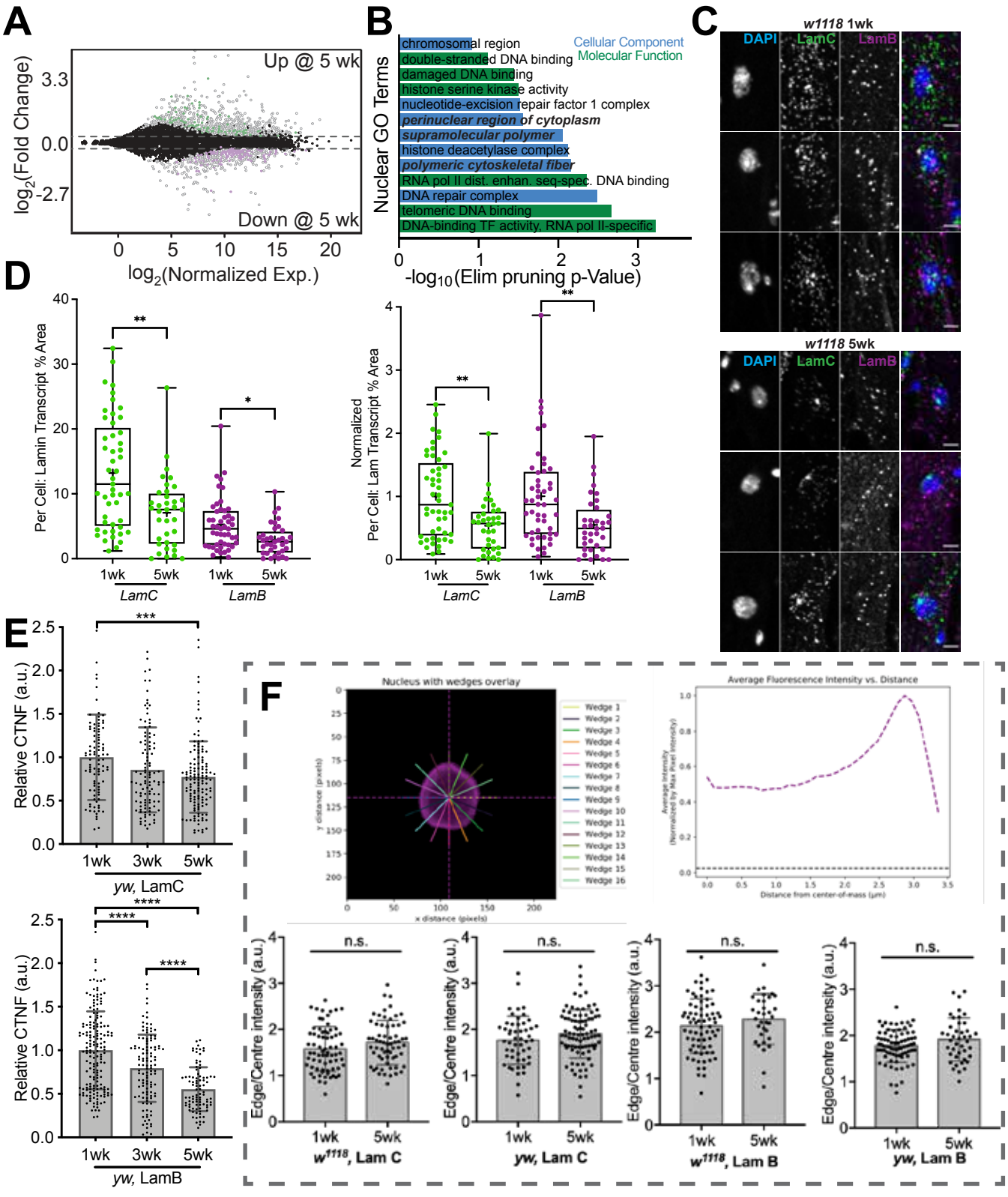
Age-associated Nuclear Remodeling Drives Cardiac Dysfunction

19 **Supplemental Figures**

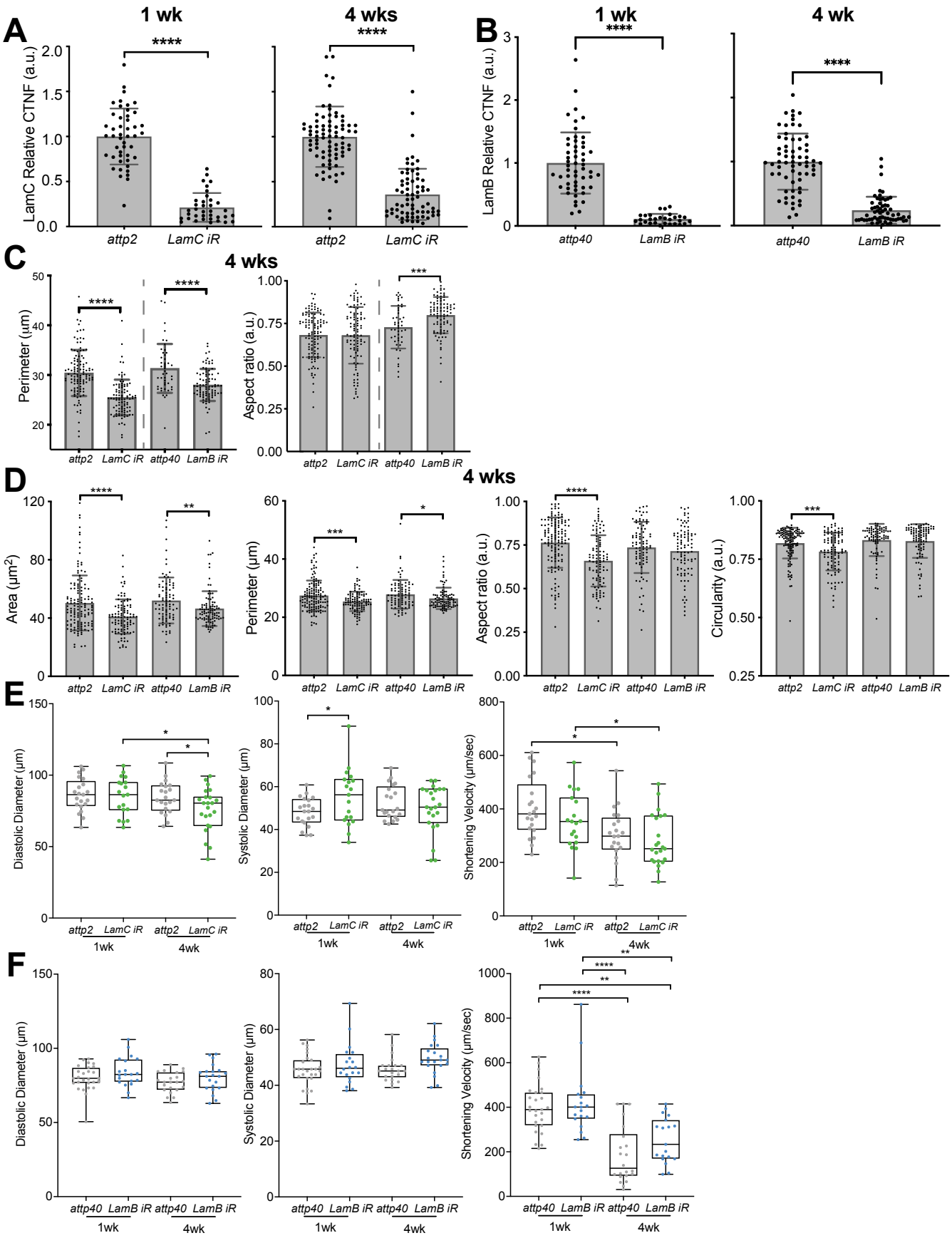

A
% survival
Weeks elapsed
w1118
yw
B
Cardiomyocytes Nuclei
Area (μm2)
Circularity (a.u.)
1wk
3wk
5wk
yw
C
Cardiomyocytes Nuclei
Perimeter (μm)
Aspect ratio (a.u.)
w1118
yw
D
Ventral Muscle Nuclei
Area (μm2)
Perimeter (μm)
Aspect ratio (a.u.)
n.s.
E
LamC
1wk
3wk
5wk

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.

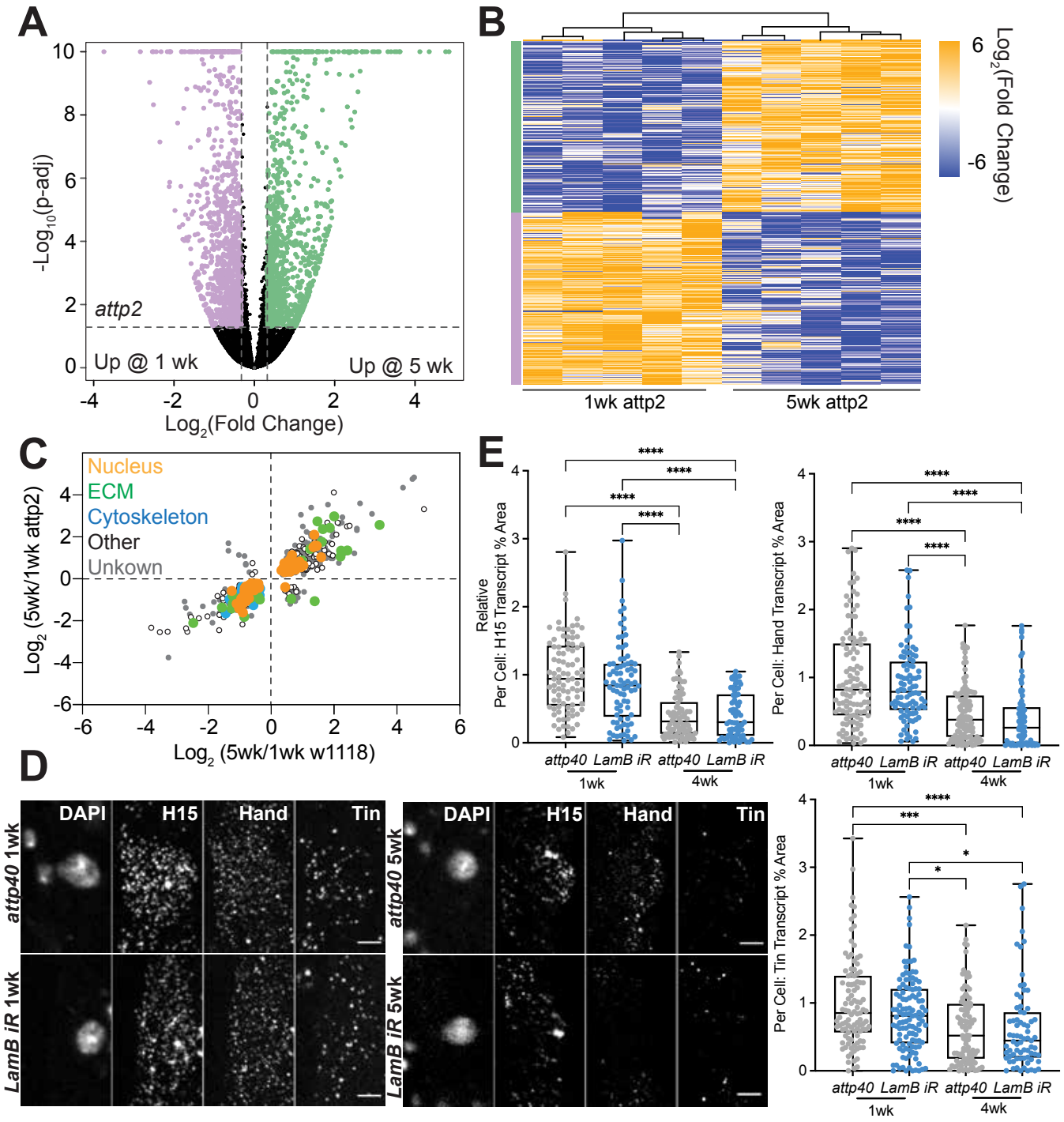


Supplemental Figure 2: Natural Aging Downregulates LamC and LamB but does not affect their localization. (A) MA plot of all genes of 1- and 5-week adult *w*^{1118} fly hearts for their $\log_2$

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.

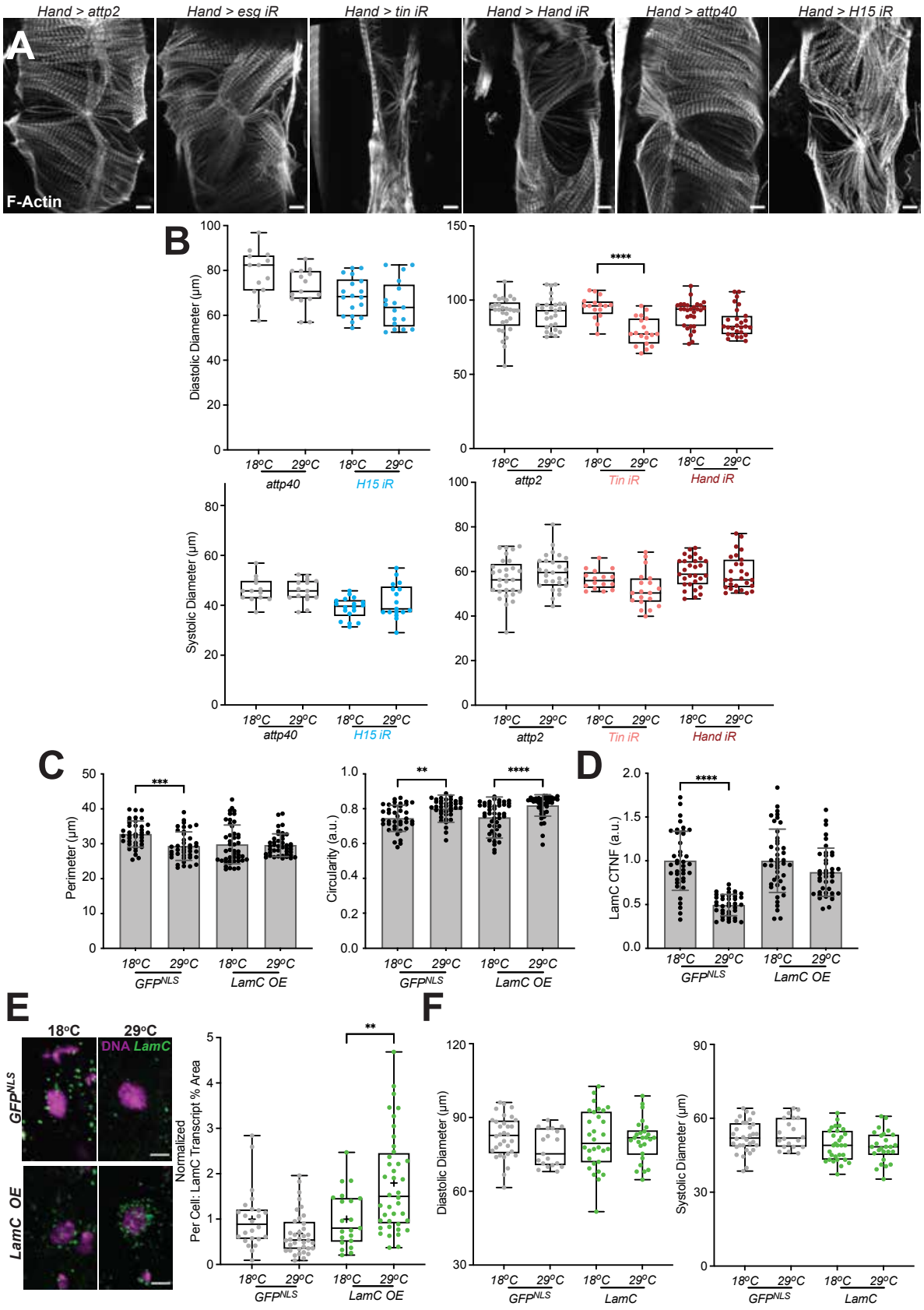

A
1 wk
4 wks
LamC Relative CTNF (a.u.)
attp2
LamC iR
B
1 wk
4 wk
LamB Relative CTNF (a.u.)
attp40
LamB iR
C
4 wks
Perimeter (µm)
Aspect ratio (a.u.)
D
4 wks
Area (µm²)
Perimeter (µm)
Aspect ratio (a.u.)
Circularity (a.u.)
E
Diastolic Diameter (µm)
Systolic Diameter (µm)
Shortening Velocity (µm/sec)
1wk
4wk
F
Diastolic Diameter (µm)
Systolic Diameter (µm)
Shortening Velocity (µm/sec)
1wk
4wk

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.

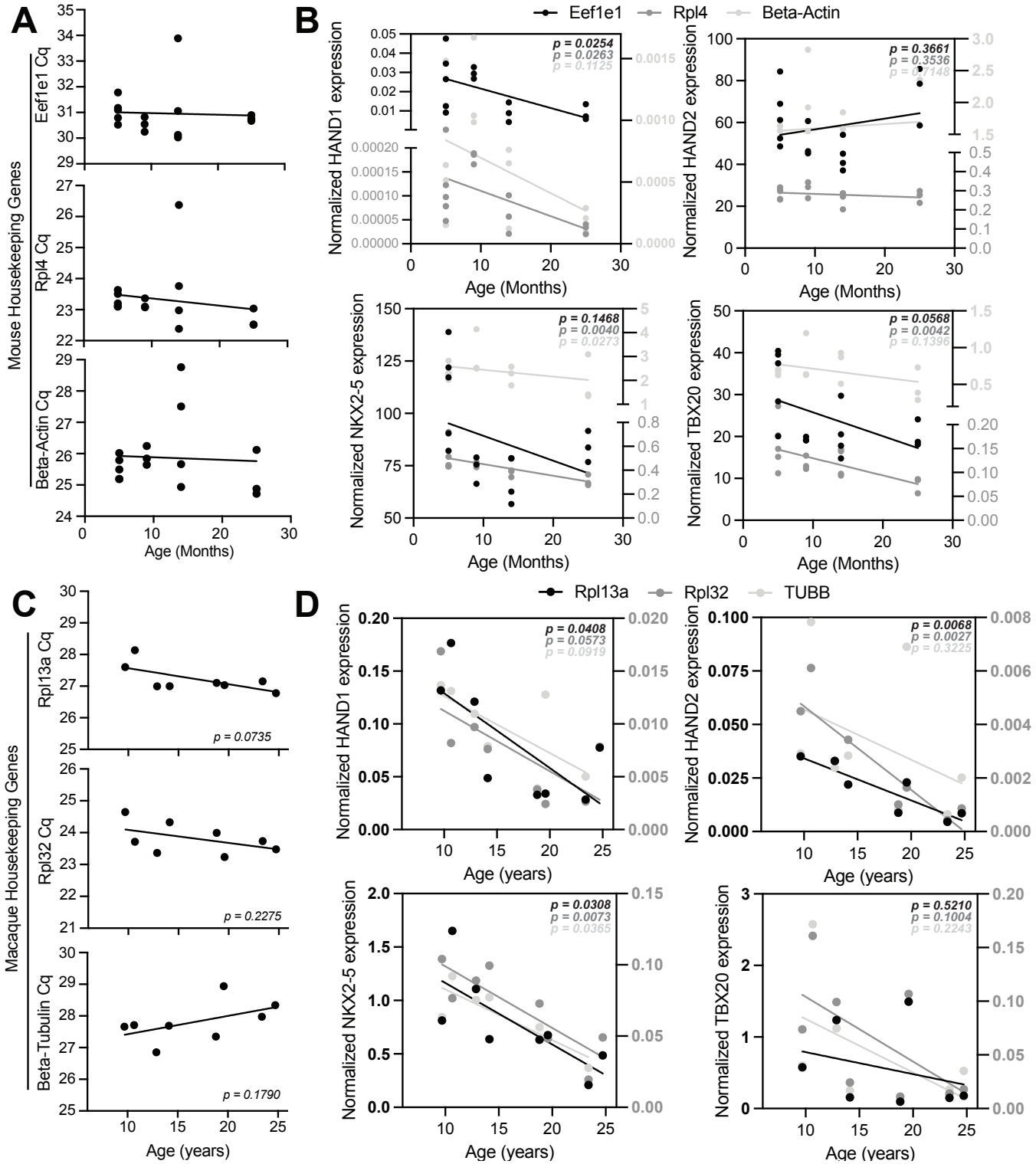


Supplemental Figure 4: Validation of transgenic fly background and effects of LamB on myogenic transcription factor expression. (A) Volcano plot and **(B)** heat map of bulk RNA-seq from surgically dissected *attp2* heart tubes. Fold change represents 5-week *attp2* fly hearts normalized to 1-week old 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 w^{1118} 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.


A
Hand > attp2
Hand > esg iR
Hand > tin iR
Hand > Hand iR
Hand > attp40
Hand > H15 iR
F-Actin
B
Diastolic Diameter (µm)
Systolic Diameter (µm)
18°C 29°C 18°C 29°C
attp40
H15 iR
attp2
Tin iR
Hand iR
****
C
Perimeter (µm)
Circularity (a.u.)
***
**
****
GFPNLS
LamC OE
D
LamC CTNF (a.u.)
****
E
18°C
29°C
DNA LamC
GFPNLS
LamC OE
Normalized Per Cell: LamC Transcript % Area
**
F
Diastolic Diameter (µm)
Systolic Diameter (µm)
GFPNLS
LamC

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 (29ºC) and permissive (18ºC) temperatures for transgene expression. n = 13, 15, 18, and 17 hearts/condition for *attp40* at 18ºC and 29ºC and *H15* RNAi at 18ºC and 29ºC, respectively. Also n = 33, 31, 20, 23, 33, 31, 33, and 24 hearts/condition for *attp2*, *Tin* RNAi, and *Hand* RNAi at 18ºC and 29ºC, respectively. **(C)** Corrected total nuclear fluorescence (CTNF) of LamC protein expressed in nuclei from flies at 18ºC and 29ºC expressing a generic GFP^{NLS} 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^{NLS} flies in permissive (29ºC) and non-permissive (18ºC) conditions. A plot quantifying the area containing transcripts, normalized to mean 18ºC GFP^{NLS}, 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^{NLS} and *LamC* OE fly lines grown at 18ºC and 29ºC. n = 39, 40, 46, and 41 nuclei (left to right). **(F)** Diastolic (left) and systolic (right) diameters of heart tubes from GFP^{NLS} and *LamC* OE fly lines grown at permissive (29ºC) and non-permissive (18ºC) temperatures for transgene expression. n = 36, 23, 33, and 39 hearts/condition (left to right). *$p<0.05$, **$p<10^{-2}$, ***$p<10^{-3}$, and ****$p<10^{-4}$ 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, $\log_2$ 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*, $\log_2$ 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, $\log_2$ 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 log_2 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, log_2 fold change with age, p-adjusted for age fold change, base mean gene expression for *LamC RNAi* vs. *attp2*, log_2 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, extracelluar 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.