SUPPLEMENTAL MATERIAL
Data S1.

Supplementary Methods:

Methods

Fasting studies in mice: Mice were housed in groups of up to n = 5 mice/cage and fed standard chow (Lab Diet, 5053; providing 3.4 Kcal/g with 62.1% Kcal derived from carbohydrates, 13.2% from fats, and 24.6% from protein) on a 6:00 PM to 6:00 AM dark-light cycle. Intermittent fasting was performed with total food deprivation and ad libitum access to water while mice were housed on a cedar pine chip bedding from 12:00 PM to 12:00 PM of the following day to implement alternate periods of 24 h fasting and feeding, with change in bedding (AL indicates fed ad libitum and IF indicates that animals were provided access to food every other day). Non-fasted control mice were simultaneously provided fresh food with change in bedding. Terminal studies on mice were initiated between 8:00 to 10:00 AM after an overnight period of feeding (i.e., on a fed day). For fasting studies to determine the effects on HSPB8 transcript levels in the heart, mice were fasted for various durations beginning at 6:00 PM (the onset of the dark cycle during which mice typically feed).

Echocardiography: Echocardiography was performed using a Vevo 2100 Imaging System (VisualSonics, Toronto, Canada) equipped with a 30 MHz linear-array transducer according slight modifications of previously published methods. Briefly, mice were anesthetized using 0.1 g/kg IP Avertin (2,2,2-Tribromoethanol, Sigma-Aldrich, St. Louis, MO). Cardiac images were obtained by a handheld technique. Parasternal long- and short-axis images of the LV were used to guide acquisition of M-mode images at the papillary muscle level. Digitally recorded images were used to measure LV wall thickness (LV posterior wall, LVPW; interventricular septum, IVS) and chamber diameter (LVEDD) in end-diastole (d) and in end-systole (s). These measurements were used to calculate LV fractional shortening (LVFS=(100xLVEDD-LVESD)/LVEDD) and LV mass (LVM=[(LVPWd+IVSd+LVIDd)^3-LVIDd^3]x1.04).
Studies with neonatal rat cardiac myocytes (NRCMs): Primary cultures of neonatal rat cardiac myocytes were prepared as we have previously described.\textsuperscript{4} Hearts were harvested from one-day old neonatal rats, and the ventricles were subjected to trypsin (Invitrogen) digestion in a final concentration of 100 μg/ml in HBSS for 16 – 18 hours at 4°C after removal of the atria. Collagenase digestion (type II collagenase; 150 U/ml; Worthington) was conducted at 37 ° C for 45 min. The mixture of cells was plated for 2 h in flasks to exclude fibroblasts. The remaining non-adherent cardiomyocytes were seeded on collagen-coated four-well chamber slides (Laboratory Tek) at a density of 10^5 cells per square cm. On the 2nd day the culture medium was changed to the Rat Cardiomyocyte Culture Medium (Cell applications INC, cat#R313-500) for at 3-5 days prior to desmin staining. NRCMs were treated with Bafilomycin A1 (BfA, Fisher scientific, cat#NC9686929): 200nM for 2 hours prior to harvest to assess autophagic flux, in vitro.

Isolation of soluble and insoluble fractions: The protocol described by Tannous et al was modified for this assay.\textsuperscript{5} Heart tissue was mechanically homogenized in 500-1000ul (depending on size of tissue) of homogenization buffer (0.3 M KCl, 0.1 M KH2PO4, 50 mM K2HPO4, 10 mM EDTA, 4 mM Na Orthovanadate, 100 mM NaF, Protease inhibitor, pH to 6.5). NRCMs were similarly homogenized with 20 strokes on ice with a Dounce homogenizer. Homogenized samples were passed through mesh basket on ice, followed by collection of the lysate run-through which was incubated on ice for 30 minutes. A known volume of the sample was transferred to another Eppendorf tube and 10% NP-40 was added to for a final concentration of 1% NP-40. Samples were then incubated on ice for 30 minutes, and spun at 13,000 rpm for 15 minutes, 4°C. Supernatant was collected as soluble fraction. The pellet was washed 3 times with cold PBS (following addition of 1ml PBS to each pellet, and spin down at 13,000 rpm for 10 minutes) followed by resuspension in 1% SDS, 10mM Tris buffer to generate the insoluble fraction.

Quantitation of desmin in aggregates by immuno-fluorescence: At least 10 images were obtained per heart from desmin stained whole heart sections of the left ventricle. 20X images were obtained with a 3x
zoom factor using a Zeiss Fluorescent microscope. After acquisition, images were converted into a JPG format using the Zeiss Zen software. Each image was analyzed using ImageJ software with a predetermined size threshold to exclude noise to obtain area of aggregates per section, depicted as a percentage of the total visualized area.

*Puromycin labeling to assess protein synthesis by the SunSET technique:* We utilized the SunSET technique to assess protein synthesis in the mouse heart. Mice were injected with puromycin (Enzo Life Sciences, Inc. cat#BML-GR312-0250) at a dose of 0.04 μmol/g body mass, i.p. 30 minutes prior to sacrifice. Cardiac tissue was homogenized in buffer I (50mM Tris HCl pH7.4, 2.5mM EDTA, 25mM NaCl, 0.2% NP 40, 10mM EGTA, 20mM NaFl, 25mM Na4O7P2, 2mM Na3VO4 with added Halt Protease and Phosphatase Inhibitor™, Thermo Scientific, cat# 78442) and subjected to SDS-PAGE electrophoresis. Anti-puromycin antibody (clone 12D10, cat# MABE343 from Millipore) was employed for immuno-detection of incorporated puromycin.
Table S1. List of primers for quantitative PCR analysis.

<table>
<thead>
<tr>
<th>Rat Genes</th>
<th>Forward (5’-3’)</th>
<th>Reverse (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>HSP90aa1</em></td>
<td>GCTTTTCAGAGCTGTTGAGATAC</td>
<td>AAAGGCTGAGTTAGCAACCTGG</td>
</tr>
<tr>
<td><em>HSPB1</em></td>
<td>CGGCAACTCAGCGCCTCCTGCT</td>
<td>CATGTTCATCTGCCCTTCTTCGTG</td>
</tr>
<tr>
<td><em>HSPB8</em></td>
<td>CCGGAAGAACTGATGGTAAAGAC</td>
<td>CCTCTGGAGAAAGTGAGGCAAATAC</td>
</tr>
<tr>
<td><em>CryAB</em></td>
<td>CTTCTACCTTCGGCCAACCCTC</td>
<td>GCACCTCAATCACGTCTCCC</td>
</tr>
<tr>
<td><em>HSPBAP1</em></td>
<td>GTACATTGTGGATCGGATCCCT</td>
<td>GGAAGGTGTATCTTCAGGAGG</td>
</tr>
<tr>
<td><em>HSP70</em></td>
<td>ACGAGGGTCTCAAGGGAAGAG</td>
<td>CTCTTTCTCAGCCAGCGTGTAG</td>
</tr>
<tr>
<td><em>RPL32</em></td>
<td>CCTCTGGTGAAGAGCCAAGATC</td>
<td>TCTGGTTCGCAGCCAGT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mouse Genes</th>
<th>Forward (5’-3’)</th>
<th>Reverse (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Desmin</em></td>
<td>GCGGCTAAGAACATCTCTGAGG</td>
<td>ATCTCGCAGGTGTAGGACTGGA</td>
</tr>
<tr>
<td><em>RPL32</em></td>
<td>CCTCTGGTGAAGCCAAGATC</td>
<td>TCTGGTTCGCAGCCAGT</td>
</tr>
</tbody>
</table>

Table S2. Evaluation of cardiac function by m-mode echocardiography in wild type ad lib fed mice and *Myh6CryaABR120G* mice - either treated with ad lib feeding or intermittent fasting. Data shown is Mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Wild type</th>
<th><em>Myh6CryaABR120G</em> ad lib</th>
<th><em>Myh6CryaABR120G</em> IF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>(n=5)</em></td>
<td><em>(n=7)</em></td>
<td><em>(n=7)</em></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>3.3 ± 0.1</td>
<td>4.2 ± 0.1*</td>
<td>3.7 ± 0.1*#</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>1.7 ± 0.1</td>
<td>3.2 ± 0.1*</td>
<td>2.5 ± 0.2**</td>
</tr>
<tr>
<td>FS (%)</td>
<td>49.6 ± 2.2</td>
<td>24.1 ± 1.5*</td>
<td>32.6 ± 3.9**</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>0.70 ± 0.04</td>
<td>1.01 ± 0.06*</td>
<td>0.92 ± 0.03*</td>
</tr>
<tr>
<td>PWTd (mm)</td>
<td>0.71 ± 0.03</td>
<td>0.85 ± 0.03*</td>
<td>0.81 ± 0.03*</td>
</tr>
<tr>
<td>LVM (mg)</td>
<td>70 ± 5</td>
<td>152 ± 8*</td>
<td>114 ± 3**</td>
</tr>
<tr>
<td>r/h</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>603 ± 8</td>
<td>563 ± 18</td>
<td>574 ± 20</td>
</tr>
</tbody>
</table>

* indicates p<0.05 by post-hoc testing after one way ANOVA vs. wild-type control.
# indicates p<0.05 by post-hoc testing after one way ANOVA for *Myh6CryaABR120G* IF vs. ad-lib.

LVEDD = left ventricular end-diastolic dimension. LVESD = left ventricular end-systolic dimension. FS = fractional shortening. IVSd = diastolic thickness of the interventricular septum. PWTd = diastolic thickness of the posterior wall. LVM = left ventricular mass by echocardiography. r/h = radius to thickness ratio. HR = heart rate.
Table S3. Evaluation of cardiac function by M-mode echocardiography in wild type mice and *Myh6CryaABR120G* mice treated with AAV9-GFP or AAV9-TFEB. All data are shown as Mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Wild type AAV9-GFP (n=5)</th>
<th>Wild type AAV9-TFEB (n=5)</th>
<th><em>Myh6CryaABR120G</em> AAV9-GFP (n=8)</th>
<th><em>Myh6CryaABR120G</em> AAV9-TFEB (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LVEDD (mm)</strong></td>
<td>3.3 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>4.2 ± 0.1*</td>
<td>3.9 ± 0.1*</td>
</tr>
<tr>
<td><strong>LVESD (mm)</strong></td>
<td>1.8 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>3.5 ± 0.1*</td>
<td>2.8 ± 0.1*</td>
</tr>
<tr>
<td><strong>FS (%)</strong></td>
<td>46.8 ± 1.3</td>
<td>45.8 ± 2.7</td>
<td>15.3 ± 1.4*</td>
<td>28.1 ± 2.0*</td>
</tr>
<tr>
<td><strong>IVSd (mm)</strong></td>
<td>0.93 ± 0.04</td>
<td>0.97 ± 0.01</td>
<td>1.08 ± 0.02*</td>
<td>1.00 ± 0.05</td>
</tr>
<tr>
<td><strong>PWTd (mm)</strong></td>
<td>0.8 ± 0.02</td>
<td>0.93 ± 0.04</td>
<td>0.96 ± 0.02*</td>
<td>0.89 ± 0.03</td>
</tr>
<tr>
<td><strong>LVM (mg)</strong></td>
<td>97 ± 3</td>
<td>106 ± 2</td>
<td>172 ± 6*</td>
<td>141 ± 6*</td>
</tr>
<tr>
<td><strong>r/h</strong></td>
<td>1.9 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td>621 ± 15</td>
<td>634 ± 13</td>
<td>556 ± 25*</td>
<td>532 ± 17*</td>
</tr>
</tbody>
</table>

* indicates p<0.05 by post-hoc testing after one way ANOVA compared to respective AAV-GFP treated Wild type control group.

# indicates p<0.05 by post-hoc testing after one way ANOVA AAV9-TFEB-treated vs. AAV9-GFP-treated *Myh6CryaABR120G* TG mice.

LVEDD = left ventricular end-diastolic dimension. LVESD = left ventricular end-systolic dimension. FS = fractional shortening. IVSd = diastolic thickness of the interventricular septum. PWTd = diastolic thickness of the posterior wall. LVM = left ventricular mass by echocardiography. r/h = radius to thickness ratio. HR = heart rate.
Table S4. Evaluation of cardiac function by M-mode echocardiography in *Myh6CryABR120G* mice treated with AAV9 control, AAV9 shTFEB, or AAV9shHspB8 and subjected to intermittent fasting (IF). The data are presented at T0 (prior to initiation of IF at 4 weeks post AAV injection), T3 (3 weeks after initiation of IF) and T6 (6 weeks after IF). All data are shown as Mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Myh6CryABR120G AAV9-shControl (n=5)</th>
<th>Myh6CryABR120G AAV9-shTFEB (n=5)</th>
<th>Myh6CryABR120G AAV9-shHspB8 (n=4)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T3</td>
<td>T6</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>4.4 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>3.3 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>FS (%)</td>
<td>25.9 ± 3.3</td>
<td>28.1 ± 3.8</td>
<td>33.7 ± 2.0@</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>0.8 ± 0.04</td>
<td>0.8 ± 0.02</td>
<td>0.8 ± 0.02</td>
</tr>
<tr>
<td>PWTd (mm)</td>
<td>0.7 ± 0.02</td>
<td>0.7 ± 0.01</td>
<td>0.7 ± 0.02</td>
</tr>
<tr>
<td>LVM (mg)</td>
<td>125 ± 15</td>
<td>120 ± 10</td>
<td>114 ± 6</td>
</tr>
<tr>
<td>r/h</td>
<td>3.1 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>556 ± 31</td>
<td>554 ± 13</td>
<td>557 ± 17</td>
</tr>
</tbody>
</table>

@ indicates p<0.05 by post-hoc testing after 2-way ANOVA for comparison of AAV9-shControl at the T6 timepoint as compared to baseline (T0).
* indicates p<0.05 by post-hoc testing after 2-way ANOVA for comparison of AAV9-shTFEB/shHspB8 groups vs. AAV9-shControl at the same time point.
$ indicates p = 0.06 by post-hoc testing after 2-way ANOVA for comparison of AAV9-shTFEB vs. AAV9-shControl at the same time point.

LVEDD = left ventricular end-diastolic dimension. LVESD = left ventricular end-systolic dimension. FS = fractional shortening. IVSd = diastolic thickness of the interventricular septum.
Figure S1. Autophagic flux assessment reveals preserved autophagic flux in skeletal muscle from Myh6-R120G αB-Crystallin TG mice and in the transgenic mouse hearts at a young age. A) Immuno-blot demonstrating LC3 and p62 expression in heart and skeletal muscle (gastrocnemius) tissue from 40 week old Myh6-R120G αB-Crystallin TG mice treated with CQ or diluent for autophagic flux assessment (representative of n=2 experiments). B, C) Representative immuno-blot (B) depicting LC3-II and p62 expression with quantitation (C) in young (8 week) Myh6-R120G αB-Crystallin TG mice treated with CQ or diluent for autophagic flux assessment. N=3/group. * indicates P<0.05 by t-test.
Figure S2. Expression of αB-Crystallin in Myh6-R120G αB-Crystallin TG mice and CryAB null mice demonstrates specificity for immuno-detection of αB-Crystallin. Immunoblot demonstrating expression of αB-Crystallin in hearts from Myh6-R120G αB-Crystallin TG mice, CryAB null mice and wild-type controls. Arrows point to various electrophoretic bands detected with the polyclonal αB-crystallin antibody employed herein.
Supplementary Figure S3: Intermittent fasting reduces aggregate pathology and myocardial cell death in Myh6-R120G αB-Crystallin TG mice. A) Quantitation of aggregates in the myocardium (as % area). B, C) Representative TUNEL stained images (with DAPI-stained nuclei, B) with quantitation of TUNEL+ nuclei (arrows in B, quantified in C) in hearts from Myh6-R120G αB-Crystallin TG mice subjected to intermittent fasting (IF) or ad-lib feeding for 6 weeks beginning at 40 weeks of age (see Fig. 2A for schematic) and WT controls (N=3-5/group). * indicates P<0.05 by t-test for A and by post-hoc test after one-way ANOVA for C.
Figure S4. Intermittent fasting and TFEB activation do not alter desmin transcription. 

A) Transcript abundance for desmin in hearts from Myh6-R120G αB-Crystallin TG mice subjected to intermittent fasting or ad-lib feeding, and littermate wild-type controls. N=4/group. * indicates P<0.05 by post-hoc test after one-way ANOVA.

B) Transcript abundance for desmin in hearts from Myh6-R120G αB-Crystallin TG mice and littermate wild-type control mice transduced with AAV-TFEB or AAV-GFP. N=3-6/group. * indicates P<0.05 by post-hoc test after one-way ANOVA.
Figure S5: AAV-mediated TFEB transduction upregulates abundance of candidate autophagy-lysosome target proteins. A) Immunoblot depicting nuclear TFEB abundance in cardiac tissue from CryABR120G mice transduced with AAV-GFP or AAV-TFEB at 36 weeks of age, and sacrificed 10 weeks later. B-F) Representative immuno-blot with quantification of protein abundance for LC3-II (C), LAMP1 (D), p62 (E) and LAMP2 (F) in cardiac tissue from wild-type mice transduced with AAV-GFP or AAV-TFEB at 36 weeks of age, and sacrificed 10 weeks later. * indicates P value by t-test. N=3/group.
Supplementary Figure S6: TFEB transduction does not alter transcript levels of the human CryAB R120G transgene and does not affect cardiac protein synthesis. A) Quantitative PCR analysis for detecting human CRYAB transgene expression with specific primer set (see Supplementary Table 1) in Myh6-R120G dB-Crystallin TG mice transduced with AAV-TFEB or AAV-GFP. N=4/group. B, C) Wild-type male C57bl6 mice (8 weeks old) were transduced with AAV-GFP or AAV-TFEB for 4 weeks and SunSET assay performed for assessing cardiac protein synthesis. Representative immuno-blot (B) with quantitation of puromycin incorporation (C) in cardiac extracts from a non-injected control mouse and AAV transduced mice. D) Representative immuno-blots for detecting mTOR activation state in mice treated as in B. P values were assessed by t-test in A and C.
Figure S7. TFEB transduction reduces aggregate pathology and myocardial cell death in Myh6-R120G αB-Crystallin TG mice. A) Quantitation of aggregates as % area of the myocardium in AAV-GFP and AAV-TFEB transduced mice at 46 weeks of age (see Figure 6A for images). N=3/group. * indicates P<0.05 by t-test. B, C) Representative TUNEL stained images (with DAPI-stained nuclei) with quantitation of TUNEL+ nuclei (pseudo-colored green, arrows in C) in hearts from Myh6-R120G αB-Crystallin TG mice and WT controls transduced with AAV9-TFEB or AAV9-GFP for 10 weeks beginning at 36 weeks of age (see Fig. 5A for schematic) and WT controls (N=3-4/group). * indicates P<0.05 by post-hoc test after one-way ANOVA.
Figure S8. TFEB induces HSPB8 expression. Transcript abundance of various heat shock protein family members in NRCMs adenovirally transduced with HA-tagged TFEB or Ad-LacZ (MOI=10 each) for 24 hours. N=3-4/group. * indicates P<0.05 by t-test.
Figure S9. Fasting increases HSPB8 transcript and protein levels in the myocardium. A) HSPB8 transcript levels in cardiac tissue from C57bl6 wild-type mice fasted for various durations as shown. N=4/group. * indicates P<0.05 by post-hoc analysis after one way ANOVA. B, C) Representative immunoblot (B) and quantitation of HSPB8 protein levels (C) in the myocardium from fed wild-type mice, mice fasted for 24 hours, and mice fasted for 24 hours followed by re-feeding for 24 hours. N=3/group. * indicates P<0.05. D, E) Representative immunoblot (D) and quantitation of HSPB8 protein levels (E) in the myocardium from Myh6-R120G aB-Crystallin TG mice subjected to intermittent fasting (IF) or ad-lib feeding for 6 weeks beginning at 40 weeks of age (see Fig. 2A for schematic) and WT controls (N=4/group). * indicates P<0.05 by post-hoc test after one-way ANOVA.
Figure S10. R120G mutant CRYAB protein interacts more strongly with desmin than the wild-type CRYAB protein, in vivo. A) Male C57bl6 mice at 8 weeks of age were transduced with AAV9 particles with cardiac troponin T promoter driven expression of FLAG-tagged human CRYAB protein or its R120G mutant for 4 weeks. Representative immuno-blot demonstrating expression of FLAG-tagged CRYAB proteins is shown in A. Mice not treated with AAV9 particles are shown as control; ‘ns’ indicates non-specific band. B) Quantitation of FLAG-tagged CRYAB protein and its R120G mutant in cardiac extracts from mice treated as in A. N=3/group. NS indicates not-significant for P value by t-test. C) Immuno-precipitation was performed with anti-FLAG antibody (or IgG control) in cardiac extracts from mice treated as in A and immuno-blot performed for desmin and CRYAB. Representative immuno-blot is shown. D) Quantitation of desmin expression in after immuno-precipitation with anti-FLAG antibody as shown in C. N=3/group. * indicates P<0.05 by t-test.
Figure S11. Intermittent fasting does not alter desmin ubiquitination. Immunoblot demonstrating poly-ubiquitinated desmin in Myh6-CryABR120G TG mice subjected to intermittent fasting (IF) or ad-lib (AL) feeding and littermate control hearts (depicted as "-"). One mg of total protein from cardiac extracts was subjected to immunoprecipitation with anti-desmin antibody or control IgG, followed by immuno-blotting for ubiquitin. The immunoblot for desmin is reproduced from the blot shown in Figure 6F. At the exposure shown to demonstrate desmin in TG samples, a desmin signal was not evident in WT hearts in the input lane (with 20 micrograms/sample; and was seen with longer exposure times (data not shown)).
Figure S12. TFEB promotes clearance of αB-crystallin via induction of autophagic flux, independent of HSPB8 in NRCMs. A) Representative immunoblot demonstrating assessment of autophagic flux and HSPB8 expression in NRCMs adenovirally transduced with R120G mutant of αB-crystallin (MOI=10; for 48 hours) without or with HATagged TFEB (MOI=10; for 24 hours) in the presence of adenoviral particles expressing shRNA targeting rat HSPB8 (shHSPB8; MOI=100 for 72 hours) and treated with bafilomycin A1 (BfA; 200nM or diluent for 2 hours) to inhibit lysosomal acidification. Adenoviral particles coding for LacZ or shLacZ were added as controls simultaneously with Ad-TFEB or Ad-αB-crystallin overexpression and sh-HSPB8, respectively, to equalize the number of viral particles. B-D) Quantitation of HSPB8 (B), LC3-II (C) and p62 (D) expression in NRCMs treated as in A. N=3/group. * indicates \( P<0.05 \) by t-test within BfA and diluent treated groups; and # indicates \( P<0.05 \) by post-hoc test after one-way ANOVA for comparisons within the diluent treated group only. E) Quantitation of αB-crystallin expression in the non-BfA treated samples from A. * indicates \( P<0.05 \) by one-way ANOVA.
Figure S13. TFEB promotes removal of αB-Crystallin aggregates in NRCMs. Immunoblot demonstrating αB-Crystallin expression in soluble and insoluble fractions from NRCMs adenovirally transduced with R120G mutant of αB-Crystallin (MOI=10; for 48 hours) without or with HA-tagged TFEB (MOI=10; for 24 hours). Adenoviral particles coding for LacZ were added as controls simultaneously to equalize the number of viral particles.
Figure S14. Knockdown of TFEB and HSPB8 prevents intermittent fasting-mediated attenuation of proteotoxic cardiomyopathy. A) Schematic depicting experimental intervention with AAV9-mediated shRNA transduction (directed to either TFEB or HSPB8 vs. control shRNA) in 36 week old Myh6-CryAB R120G TG mice. Four weeks after AAV injection, baseline echocardiograms were obtained followed by 6 weeks of intermittent fasting (termed T=0 time point). Serial echocardiograms were then obtained at 3 weeks and 6 weeks (termed T=3 and T=6 time points, respectively), followed by sacrifice and post mortem biochemical, microscopic and immuno-histochemical analysis. B) Immunoblot depicting levels of nuclear TFEB after transduction with shTFEB vs. shCon. C) Left ventricular end-diastolic diameter assessed as fold-change compared to baseline (T=0 weeks) data-point in the control group, in mice treated as in A. N=4-5/group. * indicates P<0.05 by post-hoc test after two-way ANOVA. D) Body weight over the duration of the study in mice treated as in A. N=4-5/group. * indicates P<0.05 by post-hoc test after two-way ANOVA. D) Representative immuno-blot demonstrating expression of LC3 and p62 in mice modeled as in A and injected with chloroquine (40 mg/kg) 4 hours prior to sacrifice to assess autophagic flux. Representative of n=2 experiments.
Figure S15. Knockdown of TFEB and HSPB8 prevents intermittent fasting-mediated removal of aggregates with persistent desmin localization to aggregates. A) Quantitation of aggregates as % area of the myocardium in Myh6-CryABR120G TG mice transduced with AAV9-shControl, AAV9-shTFEB or AAV9-shHSPB8 for 4 weeks at age of 36 weeks and subjected to intermittent fasting, assessed at 46 weeks of age. See Figure 8G for representative images. N=3/group. P value is by post-hoc test after one-way ANOVA. B, C) Representative immuno-blot (B) with quantitation of αB-crystallin in insoluble (C) myocardial fractions from Myh6-CryABR120G TG mice at 46 weeks of age injected with AAV9 particles coding for shRNAs targeting TFEB or HSPB8, or control for 4 weeks and then subjected to intermittent fasting for 6 weeks. N=3/group. P value is by post-hoc test after one-way ANOVA.
Statistical Values calculated using R code for figure panels labeled as below

1) Figure 1B:

Exact Permutation Test (complete enumeration)

data:  WT dil and WT CQ
p-value = 0.002165
alternative hypothesis: true mean s10 - mean s20 is 0
sample estimates:
mean WT dil - mean WT cq
-0.5683489

data:  CryAB dil and CryAB CQ
p-value = 0.9524
alternative hypothesis: true mean s11 - mean s21 is 0
sample estimates:
mean CryAB dil - mean CryAB CQ
0.1116057
2) Figure 1C:

Exact Permutation Test (complete enumeration)

data: WT dil and WT CQ
p-value = 0.002165
alternative hypothesis: true mean s10 - mean s20 is 0
sample estimates:
mean WT dil - mean WT CQ
-0.5683489

data: CryAB dil and CryAB CQ
p-value = 0.9524
alternative hypothesis: true mean s11 - mean s21 is 0
sample estimates:
mean CryAB dil - mean CryAB CQ
0.1116057
3) Figure 1D

Exact Permutation Test (complete enumeration)

data:  WT dil and WT CQ
p-value = 0.002165
alternative hypothesis: true mean s10 - mean s20 is 0
sample estimates:
mean WT dil - mean WT CQ
-0.5683489

data:  CryAB dil and CryAB CQ
p-value = 0.9524
alternative hypothesis: true mean s11 - mean s21 is 0
sample estimates:
mean CryAB dil - mean CryAB CQ
0.1116057
4) **Figure 2D**

- **Exact Permutation Test (complete enumeration)**

**data:** WT and CryAb AL  
*p-value* = 0.02857  
*alternative hypothesis:* true mean s10 - mean s11 is 0  
*sample estimates:*  
mean s10 - mean s11  
0.5142135

**data:** Cyto: CryAB AL and CryAB IF  
*p-value* = 0.08571  
*alternative hypothesis:* true mean s11 - mean s12 is 0  
*sample estimates:*  
mean s11 - mean s12  
-0.2665246

**data:** Nuc: CryAB AL and CryAB IF  
*p-value* = 0.02857  
*alternative hypothesis:* true mean s21 - mean s22 is 0  
*sample estimates:*  
mean s21 - mean s22  
-0.5184553
Figure 2F

Exact Permutation Test (complete enumeration)

data: LC3-II CryAB R120G IF dil vs. CRYAB R120G IF CQ
p-value = 0.0079
alternative hypothesis: true mean s12 - mean s13 is
sample estimate
mean s12 - mean s1
-0.391546

data: p62 CryAB R120G IF dil vs. CRYAB R120G IF CQ
p-value = 0.0079
alternative hypothesis: true mean s22 - mean s23 is
sample estimate
mean s22 - mean s2
-0.3453506
6) Figure 2H

Exact Permutation Test (complete enumeration)

Data: LAMP1: WT and CRYAB AL
p-value = 0.02857
alternative hypothesis: true mean s10 - mean s11 is 0
sample estimates:
mean s10 - mean s11
0.3220731

Data: LAMP1: CRYAB AL and CRYAB IF
p-value = 0.02857
alternative hypothesis: true mean s11 - mean s12 is 0
sample estimates:
mean s11 - mean s12
-0.2118281

Data: LAMP2: WT and CRYAB AL
p-value = 0.02857
alternative hypothesis: true mean s20 - mean s21 is 0
sample estimates:
mean s20 - mean s21
0.3928372

Data: LAMP2: CRYAB AL and CRYAB IF
p-value = 0.02857
alternative hypothesis: true mean s21 - mean s22 is 0
sample estimates:
mean s21 - mean s22
-0.2607011
7) Figure 2J

Exact Permutation Test (complete enumeration)

data: pMTOR/mTOR WT vs. CRYAB AL
p-value = 0.02857
alternative hypothesis: true mean s10 - mean s11 is 0
sample estimates:
mean s10 - mean s11
-1.810188

data: pMTOR/mTOR CRYAB AL vs. CRYAB IF
p-value = 0.02857
alternative hypothesis: true mean s11 - mean s12 is 0
sample estimates:
mean s11 - mean s12
0.9711987

data p-S6K/S6K WT vs. CRYAB AL
p-value = 0.02857
alternative hypothesis: true mean s20 - mean s21 is 0
sample estimates:
mean s20 - mean s21
-1.134393

data: S6K/S6K CRYAB AL vs. CRYAB IF
p-value = 0.02857
alternative hypothesis: true mean s21 - mean s22 is 0
sample estimates:
mean s21 - mean s22
0.7850461

data: p-4EBP1/4EBP1 WT vs. CRYAB AL
p-value = 0.02857
alternative hypothesis: true mean s30 - mean s31 is 0
sample estimates:
mean s30 - mean s31
   -1.134386

data:  p-4EBP1/4EBP1 CRYAB AL vs. CRYAB IF
p-value = 0.02857
alternative hypothesis: true mean s31 - mean s32 is  0
sample estimates:
mean s31 - mean s32
   0.7850461
Exact Permutation Test (complete enumeration)

data:  CRYAB AL vs. IF
p-value = 0.02857
alternative hypothesis: true mean s1 - mean s2 is  0
sample estimates:
mean s1 - mean s2
  -0.2432008
Exact Permutation Test (complete enumeration)

data: CRYAB AL vs. IF
p-value = 0.02857
alternative hypothesis: true mean s1 - mean s2 is 0
sample estimates:
mean s1 - mean s2
-0.3708712
data: WTAL vs. CRYAB AL
p-value = 0.001263
alternative hypothesis: true mean s2 - mean s3 is 0
sample estimates:
mean s2 - mean s3
-0.8959714

data: CRYAB IF and CRYAB AL
p-value = 0.006993
alternative hypothesis: true mean s1 - mean s3 is 0
sample estimates:
mean s1 - mean s3
-0.4657143

data: CRYAB IF and WT AL
p-value = 0.005051
alternative hypothesis: true mean s1 - mean s2 is 0
sample estimates:
mean s1 - mean s2
0.4302571
Exact Permutation Test (complete enumeration)

data: CRYAB AL vs. IF

Exact Permutation Test (complete enumeration)

data: WTAL vs. CRYAB AL
p-value = 0.001263
alternative hypothesis: true mean s2 - mean s3 is 0
sample estimates:
mean s2 - mean s3
25.54954

Exact Permutation Test (complete enumeration)

data: CRYAB IF and CRYAB AL
p-value = 0.08159
alternative hypothesis: true mean s1 - mean s3 is 0
sample estimates:
mean s1 - mean s3
8.524286

Exact Permutation Test (complete enumeration)

data: CRYAB IF and WT AL
p-value = 0.01136
alternative hypothesis: true mean s1 - mean s2 is 0
sample estimates:
mean s1 - mean s2
-17.02526
Exact Permutation Test (complete enumeration)

data:  CRYAB AL vs. IF
p-value = 0.001263
alternative hypothesis: true mean s2 - mean s3 is  0
sample estimates:
mean s2 - mean s3
        -82.17463
Exact Permutation Test (complete enumeration)

data:  CRYAB AL vs. IF
p-value = 0.0001998
alternative hypothesis: true mean s2 - mean s3 is 0
sample estimates:
mean s2 - mean s3
-4.391861

data:  CRYAB IF and CRYAB AL
p-value = 0.01132
alternative hypothesis: true mean s1 - mean s3 is 0
sample estimates:
mean s1 - mean s3
-1.983246

data:  CRYAB IF and WT AL
p-value = 4.114e-05
alternative hypothesis: true mean s1 - mean s2 is 0
sample estimates:
mean s1 - mean s2
2.408615
14) Figure 5C

Exact Permutation Test (complete enumeration)

data:  TFEB WT AAV GFP and WT AAV TFEB
p-value = 0.004329
alternative hypothesis: true mean s2 - mean s3 is 0
sample estimates:
mean s2 - mean s3
0.9304463

data:  TFEB CRYAB AAV GFP and CRYAB AAV TFEB
p-value = 0.0005828
alternative hypothesis: true mean s1 - mean s4 is 0
sample estimates:
mean s1 - mean s4
0.995846
data: Crystallin WT AAV GFP and WT AAV TFEB
p-value = 0.002165
alternative hypothesis: true mean s2 - mean s3 is 0
sample estimates:
mean s2 - mean s3
0.9835765

data: Crystallin WT AAV GFP and CRYAB AAV TFEB
p-value = 0.002165
alternative hypothesis: true mean s2 - mean s4 is 0
sample estimates:
mean s2 - mean s4
-17.61125

data: Crystallin CRYAB AAV GFP and CRYAB AAV TFEB
p-value = 0.0005828
alternative hypothesis: true mean s1 - mean s4 is sample estimates:
mean s1 - mean s4
-6.151504
Exact Permutation Test (complete enumeration)

data:  WT + AAV GFP VS. CRYAB R120 + AAV GFP
p-value = 0.000777
alternative hypothesis: true mean s3 - mean s4 is 0
sample estimates:
mean s3 - mean s4
-0.8395

data:  CRYABR120G + AAV GFP VS. CRYAB R120 + AAV TFEB
p-value = 0.1593
alternative hypothesis: true mean s1 - mean s4 is 0
sample estimates:
mean s1 - mean s4
-0.2455

Figure 5E
Exact Permutation Test (complete enumeration)

data: CRYAB R120G AAV GFP VS. CRYAB R120G AAV TFEB
p-value = 0.001554
alternative hypothesis: true mean s1 - mean s4 is 0
sample estimates:
mean s1 - mean s4
12.76925

data: CRYAB R120G AAV TFEB and WT AAV9 GFP
p-value = 0.007937
alternative hypothesis: true mean s1 - mean s3 is 0
sample estimates:
mean s1 - mean s3
-18.662

data: CRYAB R120G AAV GFP and WT AAV9 GFP
p-value = 0.000777
alternative hypothesis: true mean s3 - mean s4 is 0
sample estimates:
mean s3 - mean s4
31.43125
Figure 5G

Exact Permutation Test (complete enumeration)

data:  CRYAB R120G AAV GFP vs. CRYAB R120G AAV TFEB
p-value = 0.003108
alternative hypothesis: true mean s1 - mean s4 is 0
sample estimates:
mean s1 - mean s4
-31.3035

data:  CRYAB R120G AAV TFEB and WT AAV9 GFP
p-value = 0.007937
alternative hypothesis: true mean s1 - mean s3 is 0
sample estimates:
mean s1 - mean s3
44.326

data:  CRYAB R120G AAV GFP and WT AAV9 GFP
p-value = 0.000777
alternative hypothesis: true mean s3 - mean s4 is 0
sample estimates:
mean s3 - mean s4
-75.6295
data: CRYAB R120G AAV GFP vs. CRYAB R120G AAV TFEB
p-value = 0.003108
alternative hypothesis: true mean s1 - mean s4 is 0
sample estimates:
mean s1 - mean s4
-31.3035

data: CRYAB R120G AAV TFEB and WT AAV9 GFP
p-value = 0.007937
alternative hypothesis: true mean s1 - mean s3 is 0
sample estimates:
mean s1 - mean s3
44.326

data: CRYAB R120G AAV GFP and WT AAV9 GFP
p-value = 0.000777
alternative hypothesis: true mean s3 - mean s4 is 0
sample estimates:
mean s3 - mean s4
-75.6295
Exact Permutation Test (complete enumeration)

data:  HSPB8/ACTIN WT AAV GFP vs. WT AAV TFEB
p-value = 0.0303
alternative hypothesis: true mean s2 - mean s3 is 0
sample estimates:
mean s2 - mean s3
 1.144588

data:  HSPB8/ACTIN WT AAV GFP vs. CRYAB R120G AAV GFP
p-value = 0.002165
alternative hypothesis: true mean s3 - mean s4 is 0
sample estimates:
mean s3 - mean s4
-3.923404

data:  HSPB8/ACTIN CRYAB R120G AAV GFP vs. CRYAB R120G AAV TFEB
p-value = 0.01166
alternative hypothesis: true mean s1 - mean s4 is 0
sample estimates:
mean s1 - mean s4
 2.077214

data:  HSPB8/ACTIN WT AAV GFP vs. CRYAB R120G AAV TFEB
p-value = 0.001263
alternative hypothesis: true mean s1 - mean s3 is 0
sample estimates:
mean s1 - mean s3
6.000618
Exact Permutation Test (complete enumeration)

data:  HSPB1/ACTIN WT AAV GFP vs. CRYABR120G AAV GFP
p-value = 0.01786
alternative hypothesis: true mean s1 - mean s3 is 0
sample estimates:
mean s1 - mean s3
3.960078

data:  HSPB1/ACTIN WT AAV TFEB vs. CRYABR120G AAV TFEB
p-value = 0.007937
alternative hypothesis: true mean s1 - mean s2 is 0
sample estimates:
mean s1 - mean s2
3.93306
data: DESMIN/ACTIN WT AAV GFP vs. CRYAB R120G AAVGFP
p-value = 0.002165
alternative hypothesis: true mean s3 - mean s4 is 0
sample estimates:
mean s3 - mean s4
-1.840548

data: DESMIN/ACTIN WT AAV TFEB vs. CRYAB R120G AAVTFEB
p-value = 0.001166
alternative hypothesis: true mean s1 - mean s2 is 0
sample estimates:
mean s1 - mean s2
1.977417
Figure 7D

Exact Permutation Test (complete enumeration)

data: JC-1 DEPOLARIZATION: Ad LacZ + Ad CRYAB R120G vs. Ad TFEB + Ad CRYAB R120G
p-value = 0.0005828
alternative hypothesis: true mean s13 - mean s14 is 0
sample estimates:
mean s13 - mean s14
1.747366

data: JC-1 depol.: Ad TFEB + Ad CRYAB R120G vs. Ad TFEB + Ad CRYAB R120G + Ad sh-HSPB8
p-value = 0.0005828
alternative hypothesis: true mean s14 - mean s15 is 0
sample estimates:
mean s14 - mean s15
-1.709391

data: JC-1 DEPOLARIZATION: Ad LacZ vs. Ad LacZ + Ad CRYAB R120G
p-value = 0.0005828
alternative hypothesis: true mean s10 - mean s13 is 0
sample estimates:
mean s10 - mean s13
-2.200875

data: JC-1 DEPOLARIZATION: Ad LacZ vs. Ad TFEB + Ad CRYAB R120G
p-value = 0.0005828
alternative hypothesis: true mean s10 - mean s14 is 0
sample estimates:
mean s10 - mean s14
-0.4535086

data: JC-1 DEPOLARIZATION: Ad LacZ vs. Ad LacZ + Ad shHSPB8
p-value = 0.0005828
alternative hypothesis: true mean s10 - mean s12 is 0
sample estimates:
mean s10 - mean s12
-0.4109876

data: JC-1 DEPOLARIZATION: Ad LacZ vs. Ad TFEB+ AD CRYABR120G + Ad shHSPB8
p-value = 0.0005828
alternative hypothesis: true mean s10 - mean s15 is 0
sample estimates:
mean s10 - mean s15
-2.1629
23) Figure 7E

Mitochondrial DNA quantitation

Exact Permutation Test (complete enumeration)

data: MitoDNA: Ad LacZ + Ad CRYAB R120G vs. Ad TFEB + Ad CRYAB R120G
p-value = 0.02857
alternative hypothesis: true mean s13 - mean s14 is 0
sample estimates:
mean s13 - mean s14
0.4075

data: MitoDNA: Ad TFEB + Ad CRYAB R120G vs. Ad TFEB + Ad CRYAB R120G + Ad sh-HSPB8
p-value = 0.02857
alternative hypothesis: true mean s14 - mean s15 is 0
sample estimates:
mean s14 - mean s15
-0.44

data: MitoDNA: Ad LacZ vs. Ad LacZ + Ad CRYAB R120G
p-value = 0.02857
alternative hypothesis: true mean s10 - mean s13 is 0
sample estimates:
mean s10 - mean s13
data: MitoDNA: Ad LacZ vs. Ad TFEB + Ad CRYAB R120G
p-value = 0.02857
alternative hypothesis: true mean s10 - mean s14 is 0
sample estimates:
mean s10 - mean s14
-0.29

data: MitoDNA: Ad LacZ vs. Ad LacZ + Ad shHSPB8
p-value = 0.02857
alternative hypothesis: true mean s10 - mean s12 is 0
sample estimates:
mean s10 - mean s12
-0.3775

data: MitoDNA:Ad LacZ vs. Ad TFEB+ AD CRYABR120G + Ad shHSPB8
p-value = 0.02857
alternative hypothesis: true mean s10 - mean s15 is 0
sample estimates:
mean s10 - mean s15
-0.73
Figure 7F

![Graph showing cell death percentages for different combinations of Ad-LacZ, Ad-CRYAB R120G, Ad-TFEB, and Ad-shHSPB8.

Exact Permutation Test (complete enumeration)

data: Ad LacZ + Ad CRYAB R120G vs. Ad TFEB + Ad CRYAB R120G
p-value = 0.0001554
alternative hypothesis: true mean s4 - mean s5 is 0
sample estimates:
mean s4 - mean s5
6.829617

data: Ad TFEB + Ad CRYAB R120G vs. Ad TFEB + Ad CRYAB R120G + Ad sh-HSPB8
p-value = 0.0001554
alternative hypothesis: true mean s5 - mean s6 is 0
sample estimates:
mean s5 - mean s6
-7.874323

data: Ad LacZ vs. Ad LacZ + Ad CRYAB R120G
p-value = 0.0001554
alternative hypothesis: true mean s1 - mean s4 is 0
sample estimates:
mean s1 - mean s4
-9.49469

data: Ad LacZ vs. Ad TFEB + Ad CRYAB R120G
p-value = 0.006838
alternative hypothesis: true mean s1 - mean s5 is 0
sample estimates:
mean s1 - mean s5
  -2.665073

data: Ad LacZ vs. Ad LacZ + Ad shHSPB8
p-value = 0.02455
alternative hypothesis: true mean s1 - mean s3 is 0
sample estimates:
mean s1 - mean s3
  -1.78758

data: Ad LacZ vs. Ad TFEB+ AD CRYABR120G + Ad shHSPB8
p-value = 0.0001554
alternative hypothesis: true mean s1 - mean s6 is 0
sample estimates:
mean s1 - mean s6
  -10.5394
data: AAV sh control and sh TFEB
p-value = 0.02857
alternative hypothesis: true mean s10 - mean s11 is 0
sample estimates:
mean s10 - mean s11
0.7235474

data: sh TFEB and sh HSPB8
p-value = 0.02857
alternative hypothesis: true mean s11 - mean s12 is 0
sample estimates:
mean s11 - mean s12
-0.4478951
26) Figure 8C

data:  sh Control and sh TFEB
p-value = 0.02857
alternative hypothesis: true mean s10 - mean s11 is  0
sample estimates:
mean s10 - mean s11
  0.2165965


data:  sh TFEB and sh HSPB8
p-value = 0.02857
alternative hypothesis: true mean s11 - mean s12 is  0
sample estimates:
mean s11 - mean s12
  0.5273424


data:  sh Control and sh HSPB8
p-value = 0.02857
alternative hypothesis: true mean s10 - mean s12 is  0
sample estimates:
mean s10 - mean s12
  0.743939
Exact Permutation Test (complete enumeration)

data:  sh Control baseline and sh Control 6wk
p-value = 0.03968
alternative hypothesis: true mean s10 - mean s30 is  0
sample estimates:
mean s10 - mean s30
   -7.7552

data:  sh control baseline and shHSPB8 baseline
p-value = 0.07937
alternative hypothesis: true mean s20 - mean s21 is  0
sample estimates:
mean s20 - mean s21
     9.0979

data:  sh Control 6wk and sh TFEB 6wk
p-value = 0.007937
alternative hypothesis: true mean s30 - mean s31 is  0
sample estimates:
mean s30 - mean s31
    14.0881

data:  sh Control 6wk and sh HSPB8 6wk
p-value = 0.007937
alternative hypothesis: true mean s30 - mean s32 is  0
sample estimates:
mean s30 - mean s32
    9.2062
Supplemental References:


