Supplementary Information

Methods

Assessment of ubiquitinated proteins: Myocardial extracts were subjected to SDS-PAGE separation and immunoblotted for ubiquitinated proteins with anti-ubiquitinated protein antibody, clone FK2 (Millipore).

Cell culture: HL-1 cardiac myocytes were a kind gift from Dr. William Claycomb, Louisiana State University, New Orleans, and were cultured as described previously

Supplementary Figure Legends

Figure S1. Schematic depicting experimental outline. (**A**) In in-vivo studies, young adult mice were subjected to intermittent fasting, for 24 h (black rectangles) either every other day (qOD) or every 4th day (q4D) interspersed with 24 h periods of ad libitum (ad-lib) access to standard chow (white rectangles) for a duration of 2 to 6 weeks (see text for details). After the final 24-h 'fed' period (with ad-lib access to food), mice were subjected to reversible LAD ligation to induce ischemia (for 30 min, red rectangle) followed by reperfusion (23 h and 30 min, blue rectangle). Terminal TTC staining with or without Evans Blue injection to performed to assess infarct size. (**B**) In in vitro studies, neonatal rat cardiac myocytes (NRCMs) were plated for 24 h, washed and subjected to 2 12-h periods of starvation (by culturing in Hanks buffered salt solution), interspersed with 12-h periods of culture in nutrient-replete medium. After the final 12 h period of culture in nutrient-replete medium under normoxic conditions, NRCMs were subjected to 6 h of hypoxia (see methods, red rectangle) followed by 18 h of reoxygenation (blue rectangle); and cell death assessed as an end-point.

Figure S2. Fasting stimulates accumulation of ubiquitinated proteins in the *Lamp2* heterozygous null myocardium. Immunoblot depicting accumulation of ubiquitinated proteins in hearts of *Lamp2* heterozygous null mice, with progressively longer periods of fasting, as compared with non-fasted littermate wild-type female controls (*top*). Ponceau Red-stained membrane is shown (*bottom*) to demonstrate equal protein loading.

Figure S3. *lamp2* null mice demonstrate marked autophagosome accumulation at 8 to 10 weeks of age. (**A**) Representative gray-scale images (*left*, at 630X magnification) of *lamp2* null mice and littermate controls bearing the GFP-LC3 transgene, with (**B**) quantification of punctate GFP-LC3 dots (*right*). N=3 or 4/group. *P* value is by t test.

Figure S4. Echocardiographic signs of cardiomyopathy are not detectable in *lamp2* null mice by 15 weeks of age. (**A**) Representative 2D-directed M mode echocardiographic images in 15-week old *lamp2* null and littermate wild-type males. (**B to D**) Quantitative analysis of left ventricular end-diastolic diameter (LVEDD), % fractional shortening, and LV mass in mice as in (**A**). N=4/group.

Figure S5. Intermittent fasting does not alter abundance of chaperone-mediated autophagy substrates in the myocardium in mice with LAMP2 ablation. (**A**) Immunoblot demonstrating abundance of candidate CMA substrates in *Lamp2* heterozygous null females and littermate control hearts, on a fed day after 6 weeks of intermittent fasting (IF) or ad-lib access to food (non). (**B to F**) Representative immunoblot (**B**) with quantitation (**C to F**) of candidate CMA substrates in cardiac extracts from *lamp2* null and littermate wild-type males on a fed day after 5 weeks of intermittent fasting (IF) or ad-lib access to food (non). N=4/group. No statistically significant differences were detected between groups by one-way ANOVA.

Figure S6. Transcriptional regulation of *Becn1* and *Lamp1* with fasting. (**A and B**) Quantitative PCR analysis of *Becn1* (**A**) and *Lamp1* (**B**) expression (normalized to ribosomal protein gene *Rpl32*) in myocardial extracts from mice subjected to 24 h fasted and nonfasted controls; n=7 or 8/group. *P* values shown are by t test.

Figure S7. Validation of anti-TFEB antibody. (**A**) HL-1 cardiac myocytes were transduced with shRNA targeting *Tfeb*, scrambled shRNA control and a nontargeting shRNA directed against LacZ as previously described [1], and protein extracts subjected to immunoblotting with anti-TFEB antibody in the absence (*left*) or presence (*right*) of saturating concentrations of blocking peptide. (**B**) Immunoblot depicting myocardial extracts from conditional cardiac TFEB overexpressor (oe) mouse and tTA control; and nenonatal rat cardiac myocytes adenovirally transduced with human TFEB or LacZ-expressing control as previously described;[1] and subjected to immunoblotting for TFEB as in (**A**). (**C**) Immunoblot depicting

subcellular fractionation of wild-type heart into nuclear and cytoplasmic extracts; and TFEB-overexpressing heart; and immunoblotted for TFEB as in (**A**). Ponceau Red stained membranes are shown as loading controls.

Table S1. Differentially regulated genes observed only in intermittently fasted wild-type mice; organized by cellular pathways.

Group	Upregulated (777 genes)	Downregulated (807)	% of total
Regulation of transcription (excluding known transcription factors)	*Rbbp7, Ikbkap, Mustn1, Zfp691, Isoc2a, tgb3bp, Rdbp, Tspan17, Habp4, Ddx1, Morf4l1, M1ap, Smarca2, Tcf7l2, Aaas, Zfp114, Hira, Dcx, Tbx5, Med25, En2, Ezh1, Zfp786, Eid3, Morf4l1, Lcmt1, Myzap, Zfp189, Suv420h1, Zfp729a, Dhx37, Hnrpdl, Uxt, Hdac10, Ctbp1, Camta1, Zxda, Ebf3, Nr2e3, Nfxl1, Nr2c2ap, Uncx4.1, Crcp, Ccdc85b, Elk1, Taf1a, Arid4a, Mybbp1a, Flii, Ldb1* **(51)**	*Rfx5, Creb5, Ino80e, Zbtb40, Grhl3, Zfp238, Hrsp12, Gspt2, Piwil4, Zbtb43, Zfp82, Fzd4, Atf2, Zscan22, Gm1141, Med11, Tfdp1, Nkrf, Setd8, Prmt2, Carhsp1, Chd1, Hipk2, Alg9, Tmem132a, Scml4, Morf4l2, Rars, Nme1, Cbfb, Tada1l, Phc1, Ing5, Zmiz1, Zfp62, Prkd2, Smarce1, Tceal1, Litaf, Ikbkb* **(40)**	**9.7**
Transcription factors (activators)	*Supt5h, Zfp296, Sox30, Pbx1, Fbxo27, Zfp120, Gata5* **(7)**	*Barx2, Sp1, Gata1, Cux2, Helz2, Tcf12, Sox3, Fosl2, Egr1* **(8)**	**1.7**
Transcription factor (repressors)	*Ccndbp1, Zfp369, Zfp703, Cped1, Zc3h8* **(5)**	*Zfp715, Gatad2b, Bcor, Jdp2, Twist2, Irf2bp2, Ifi205, Mxd4, Zfp174, Cdyl, Id3, Junb, Bcl6, Zfp668* **(14)**	**2.0**
Regulation of translation and ribosomes	*Paip2b, Eif5a, Lars, Eif3s4, Eif3b, Sbds, Gtpbp4, Rpsa, Eif3g, Eif2ak4, Brf1, Eif3s8, Trpt1, Dtd1, Smg6, Eif2b5, Cdc2l6, Mknk1, Ddx17* **(19)**	*Eef1g, Rpl27a, Eef1d, Eif2ak3, Rpl31, Ddx6* **(6)**	**2.6**
RNA processing and binding proteins	*Fus, Syf2, Fip1l1, Sart3, Syf2, Dnd1, Dhx32, Ang, Sart1, Rbpms, Luc7l, Dom3z, Rbm47, Sfrs14, Exosc1, Ddx24, Hnrnph2* **(17)**	*Ints12, Gemin7, Cstf3, Nova1, Ptbp1, Sf3b1, Magoh, Cugbp1, Prpf38a, Caprin1, Tia1, Ints10, Mex3c, Gtf3c6, Pop4, Synpr* **(16)**	**3.3**

Metabolism	*Pfkfb3, Cdo1, Gclm, Pfkm, Pcsk6, Cyp27a1, Slc25a20, Bbox1, Gpt1, Cyp2b19, Haghl, Paics, Pfas, Ormdl2, Adhfe1, Acot11, Alox12, Nudt7, Cryl1, Pmm1, Slc25a21, Fpgs, Gstz1, Dgkb, Cp, Ephx1, Aldob, Apom, Acot12, Pmm1, Galt, Lcmt1, Acsbg1, Asah3, Rpe, Idh1,Gstm2, Gatm, Zdhhc4, Acadl, Gsta4, Gapdhs, Gcnt2, Arel1, Pitpnb, Aloxe3, Gbas, Lrat, Slc9a8, Dhrs2, Adssl1, Edg5, Pdxdc1, Kars, Car11, Dnpep, Abcd3, Aldh5a1, Cps1, Inpp5k, Gstt1, Phkg2, Npepps, Decr1, Gda, Lipe, Dgkq, Ptdss2, Peo1, Pgls, St3gal3, Whsc1l1, Galt, Aldoa-ps1, Suclg1, Dgka* **(77)**	*Parg, Ada, Ncf2, Nadsyn1, Aldh18a1, Pigh, Degs1, Ogdh, Mgat4b, Afmid, Hfm1, Hyal2, Dhdds, St8sia6, Ppap2c, Sgpp1, Osbp2, Car9, Lass6, Inppl1, Prss58, Lepre1, Tpst2, Nudt19, Sulf1, Mdga1, Galnt1, Mettl8, Enpp5, Tyms, Ugt1a6a, Lpin2, Gls, Naprt1, Fahd1, Tpmt, Pigc, B3gnt3, B3galnt2, Galnt1, Zdhhc8, Uevld, Alg14, Zdhhc21, Gch1, Asb7, Pklr, Alas1, Tst* **(50)**	**13.5**
Endocytosis	*Snx1, Dctn1, Myo5a, Vps37b, Slc9a6, Vamp4, Vps8, Ehd4, Vamp2, Ccdc53, Snx3* **(11)**	*Rab8a, Rab22a, Ccdc88a, Snx27, Hip1, Eea1, Lgmn, Sorl1, Mrc1, Sec14l1, Rhod, Rab31* **(11)**	**2.4**
Ubiquitin Proteasome pathway	*Ubqln2, Ubxd4, Fbxw15, Prickle1, Ddrgk1, Loc100047093, Ube3a, Rnf114, Rnf167, Park2, Fbxo43, Usp50, Magea2, Ube2c, Ifrd2, Arih2, Ring1, Ube2h, Ubtd2, Usp33, Asb15, Pomp, Gcnt2, Psmc5, Hectd1, Zer1, Spsb2, Usp13, Rnf135, Cul7, Neurl2, Rnf181, Ubl4b, Ubap2l, Hip2, Trim2, Wdr51b, Rab40c, Usp47* **(39)**	*Ube2f, Fem1c, Rnf182, Serpina3g, Rnf11, Rnf121, Ubash3b, Ttc7b, Rchy1, Ndfip2, Ube2j2, Pdzrn4, Dtx3l, Asb10, Skp2, Ube2f, Cul4b, Rnf2, Mysm1, Sh3md4, Gmcl1, Ercc8, Rnf38, Dcun1d3, Cops7b, Anapc1, Peli2* **(27)**	**7**
Lysosomal pathways (autophagy and exocytosis)	*Vps53, Map1lc3a, Atp6v1g1, Npc1, Stx1b, Vamp1* **(6)**	*Coro1a, Tcirg1, Atp6ap1, Atp6v0a2, Ctsw, Syt4, Ostm1, Syt6, Smpd1, Laptm5, Atg4c* **(10)**	**1.7**
Intracellular trafficking	*Cacng5, Ttll5, Rsn, Rgp1, Rabac1, Ap3s2, Rtn2, Snx21* **(8)**	*Lman1, Vti1, Exoc4, Ift20, Unc119b, Grasp, Lman2* **(7)**	**1.6**

Mitochondrial structure and function	*Cox6b2, Mrps10, c11orf31, Chchd6, Mrps26, Atp5h, Slc25a23, Cmc1, Oxa1l, Cyp2a12, Cox17* **(13)**	*Mfn2, Coa5, Tomm40l, Slc5a2, Cyb561d1, Oma1, Mrpl18, Tmem126a, Mrps25* **(9)**	**2.2**
Endoplasmic reticulum stress	*Os9, Naca, Dnajb12, Tram1l1* **(4)**	*Cyb5r4, Derl2, Ergic1* **(3)**	**7.4**
Ion channels and Transporters	*Slc40a1, Cutc, Slc39a10, Accn1, Slc14a1, Slc4a3, Prdx5, Kcnip2, Slc10a7, Slc1a5, Trpv6, Abcb4, Slc16a1, Kcnk10, Slc29a4, Bsnd, , Cacnb3, Xk* **(18)**	*Slc2a12, Slc39a5, Slc31a1, Slc11a1, Slc39a11, Trpm7, Trpv5, Kcnk4, Slc4a4, Kcnk6, Atp1b3, Trpc1,Slc7a9, Tmco3, Slc7a4, Slc26a9, Scn3a, Osta, Slc1a1, Slc6a14, Tfrc* **(19)**	**3.9**
Signal transduction	*Sbk, Camk2n1, Klrk1, Tmeff1, Spnb3, Adcy6, C1s, Pik3ap1, Nosip, Spinlw1, Cat, Ripk2, Mapk1ip1, Mapk7, Dctn3, Nrxn2, Cd200r2, Braf, Cpn1, Senp5, Chat, Klk5, Clps, Chd5, Masp2, Arpp21, Bmp1, Pik3cd, Gpsm1, Slc6a9, Traf3ip1, Inadl, Tulp1, P2ry13, Grid2, Tnf, Pla2g12a, Arl2, Ankrd6, Ctf1, Bambi-ps1, Serinc1, Ktn1, Arfgap2, Fn1, Txndc1, Mertk, Acpp, Akap7, Dctn4, Nsmce2, Dcc, Gps2, Gna13, Mif* **(54)**	*Nck1, Mpl, Capn10, Dab2, Jak1, Dusp19, Nov, Mobkl2c, Epha3, Trpc3, Snx22, Tnfaip2, Olfm1, Gabrb2, Crk, Irf1, Rasa3, Ywhah, Htr1d, Prkx, Pank4, Gabrg2, Robo4, Cd97, Shank3, Plekha2, Nat5, Mcam, Npy2r, Chek2, Pdgfrb, Akap2, Cnga3, Wdr25, Btn1a1, Adora3, Ifngr2, Fcna, Ddx10, Centd1, Pald1, Calu, Sh2b3, Il6ra, Esr1, Rtp1, Lynx1, Lrrc67, Sppl2b, Gria2, Nos3, Il13ra1, Arfrp1, Ppp1r12a, Nell2, Ctnnbip1, Ankrd28, Apmap, Dusp3, Tmprss13, Itgb1bp3, Ptpn14, Acap2, Camk2d, P2ry4, Nuak1, Ppp1r3c, Gnb5, Prkx, Il22ra1, Il21r, Tas2r140, Crb2, Itpripl2, Sh3pxd2b, Gpr4, Serpina3b, Vill, Amigo1, Flt4, Akap12, Grb14, Anxa3, C1qb,Tuba1b, Adamts9* **(86)**	**14.9**

GTPase regulation	*Stard13, Rasl11b, Rasa2, Cdc42se1, Tbc1d17, Elmod3, Rasl11b, Arap1, Gpsm1, Ric8, Rasgrp1, Eral1, Arfgap2, Arhgap10, Was* **(51)**	*Ralb, Daam1, Pscd3, Fgd3, Chn1, Mcf2l, Rhoj, Rab27b, Arhgdib, Kras, Plekhg2, Mcf2l, Rgs7bp, Mcf2l, Iigp2* **(15)**	**7.0**
Cell death (pro-death)		*Isg20l1, Gzmb, Bax, Casp9, Tnfrsf22, Ppm1f, Bok, Pdcd10, Cln8, Ltbr, Trp53bp2, Nek5, Irs2, Snf1lk* **(13)**	**1.3**
Cell death (prosurvival)	*Pea15b, Ssty2, Pim2, Prkci, Lif, Parl, Mil1* **(7)**	*Diap2, Ndnf,* **(2)**	**0.9**
Cytoskeleton	*Gsn, Cetn3, Stmn3, Rcsd1, Tubgcp2, Vasp, Eml2, Tbcel, Cep164, Xirp2* **(10)**	*Macf1, Actn1, Ank2, Elmo1, Cotl1, Dnahc6, Incenp, Arpc1b, Clasp1, Sept6, Sept 11, Tubb5, Katna1, Mapt, Ppp1r18, Mapre2, Mtap1b, Knsl5, Tmsb10, Fkbp4* **(20)**	**0.3**
Structural/ contractile proteins	*Pdlim3, Itgae, Crb3, Ergic3, Sgca, Dsg2, Mylc2b, Myh7, Mpp7, Gjd4* **(10)**	*Ttn, Flnc, Crtap, Myo5a,* **(4)**	**0.1**
Others	*Sult1a1, Hbb-b1, Prom2, Corin, Hba-a1, Nphp1, Ncapd2, Serpind1, Ache, Per3, Gstm2, Agtpbp1, Pold2, Rfc1, Olfml1, Rad51, Caps2, Trhr, Tas2r126, Clec4a3, Stard9, Zwint, Obfc2b, Mms22l, Bpifb4, Olfr1265, Olfr478, Cdc6, Col11a2, Top2a, Rptn, Spaca4, Olfr411,Yjefn3 , Acp1, Olfr883, Peg3, Pcnt, Tg, Flrt2, Pcdh9, Hspa1a, Cct6b, Igl-5, Xpo7, p3r, Gh, Cenpo, Dbf4, Cpne3, Olfr251, Agpt, Eya1, Hist1h4j, Pex11c, Hsp90aa1, Pcdha10, Olfr159, Olfr1324, Chrna6, Tinf2, Olfr406, Mcm3, Glg1* **(63)**	*Cd93, Gprc2a-rs5, Tsn, Pold3, Mkks, Hspd1, Pex1, Lhx4, Cep152, Cdh8, Adamts2, Tob2, Gm88, Cdc20, Pcolce, Olfr1325, Plat, Pigo, Blcap, Rbp2, Uhrf1bp1, Cenpo, Fxyd2, Fcrl5, Pvalb, lfr556, Olfr1396, Pex2, Upk1a, Olfr320, Olfr105, Olfr877, Ly6a, Olfr845, Nup205, Egln1, Ddx60, Olfr234, Lip1, Dchs1, Abhd2, Olfr137, Hspg2, Lce1a2, Alox5, Sit1, Krit1, Sln, Lmnb2, Olfr860, H2-aa, Eln, Oas1g, Egln1, App, Nes, Hspg2, Als2cr4, Phf13, Emcn, Cd99l2, lfr344, Gpx6, Taar8b, Prelp, Bc004004, Mad2l1bp, Smc1a, Olfr1042, C1qc, Esam, Ifitm2, Cdh5, Fgf9, Nav1, Sost,*	**19.1**

		Atp2c1, Polq, Cdkl4, Pdlim7, Dag1, Emilin1, Hsd3b7, Folr2, Olfr976, Chrnb1, Prlr, Ifitm3, Reep3, Dchs1, Parp3, Srl, Col5a1, P4ha2, Fstl1, Chsy1, Sobp, Cyp2a5, Fxyd5, Slamf9, Cxcl9, Mmp8, Tagln2, Col1a1, Cd74, Coq10b, Loc100044842, Ccnd1, Rad54l, Ednrb, H2-dmb2, c15orf23, Sema3f, B2m, H2-dma, H2-eb1 **(116)**	
Duplicates	*Supt5h, Rpap3, Itgae, Rnf167, Eif5a, c11orf31, Sgca, Hbb-b1, Ddx1, Hbb-b1, Kcnv1, Chchd5, Tmem66, Kcnip2, Rbbp7, Gstm2* **(16)**	*Tnfsf10, Afmid, Tmem35, Rnf11, Galnt1, Parg, Prmt2, Akap2, Dusp6, Dusp15, Smpd1, C1qa, Bcl6b, H2-dma, Anxa3, Nuak1,Sema3f, B2m, Coro1a, Cd74, H2-ab1, Loc641240, H2-ab1, Tfrc* **(24)**	
Not found	**208**	**194**	
Unknown	**93**	**115**	
Total excluded	**317**	**333**	
Total number of Identified genes in denominator	**460**	**474**	

Supplementary References

1. Ma X, Godar RJ, Liu H, Diwan A. Enhancing lysosome biogenesis attenuates BNIP3-induced cardiomyocyte death. Autophagy 2012; 8:297-309.

Supplementary Figure S1

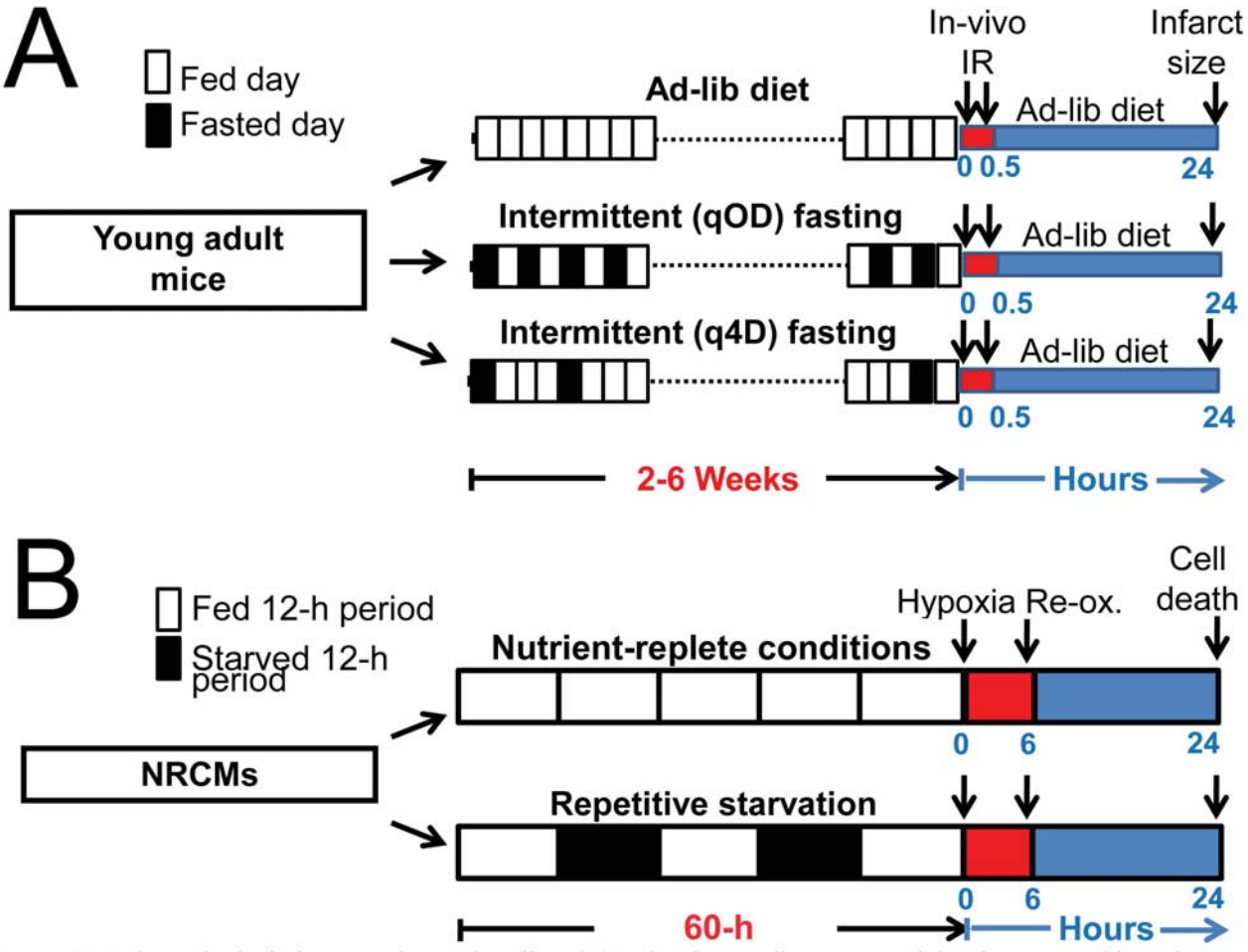

Figure S1. Schematic depicting experimental outline. (A) In in-vivo studies, young adult mice were subjected to intermittent fasting, for 24 h (black rectangles) either every other day (qOD) or every 4th day (q4D) interspersed with 24 h periods of ad libitum (ad-lib) access to standard chow (white rectangles) for a duration of 2 to 6 weeks (see text for details). After the final 24-h 'fed' period (with ad-lib access to food), mice were subjected to reversible LAD ligation to induce ischemia (for 30 min, red rectangle) followed by reperfusion (23 h and 30 min, blue rectangle). Terminal TTC staining with or without Evans Blue injection to performed to assess infarct size. (B) In in vitro studies, neonatal rat cardiac myocytes (NRCMs) were plated for 24 h, washed and subjected to 2 12-h periods of starvation (by culturing in Hanks buffered salt solution), interspersed with 12-h periods of culture in nutrient-replete medium. After the final 12 h period of culture in nutrient-replete medium under normoxic conditions, NRCMs were subjected to 6 h of hypoxia (see methods, red rectangle) followed by 18 h of reoxygenation (blue rectangle); and cell death assessed as an end-point.

Supplementary Figure S2

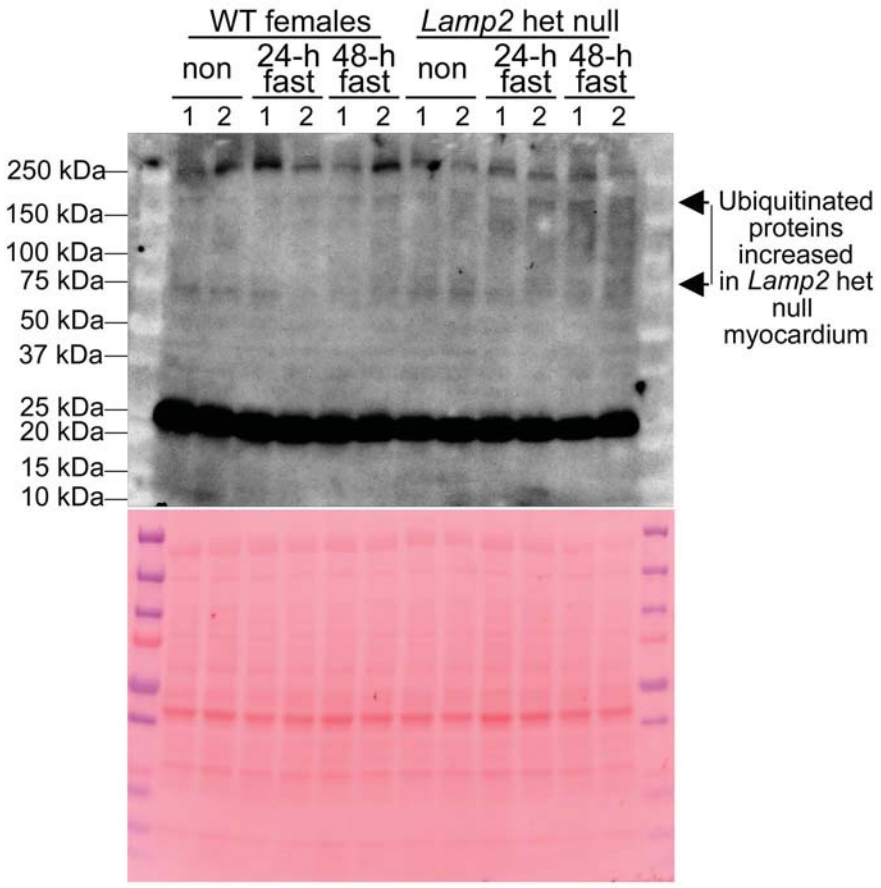

Figure S2. Fasting stimulates accumulation of ubiquitinated proteins in the *Lamp2* heterozygous null myocardium. Immunoblot depicting accumulation of ubiquitinated proteins in hearts of *Lamp2* heterozygous null mice, with progressively longer periods of fasting, as compared with non-fasted littermate wild type female controls (*top*). Ponceau Red stained membrane is shown (*bottom*) to demonstrate equal protein loading.

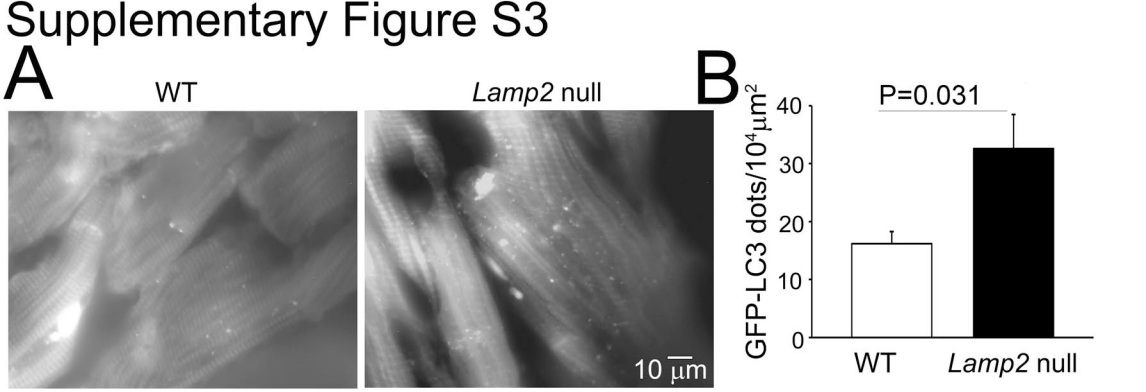

Figure S3. *Lamp2* null mice demonstrate marked autophagosome accumulation at 8-10 weeks of age. (**A**) Representative gray-scale images (*left*, at 630X magnification) of *Lamp2* null mice and littermate controls bearing the GFPL-LC3 transgene, with (**B**) quantification of punctate GFP-LC3 dots (*right*). N=3-4/group. P value is by t-test.

Supplementary Figure S4

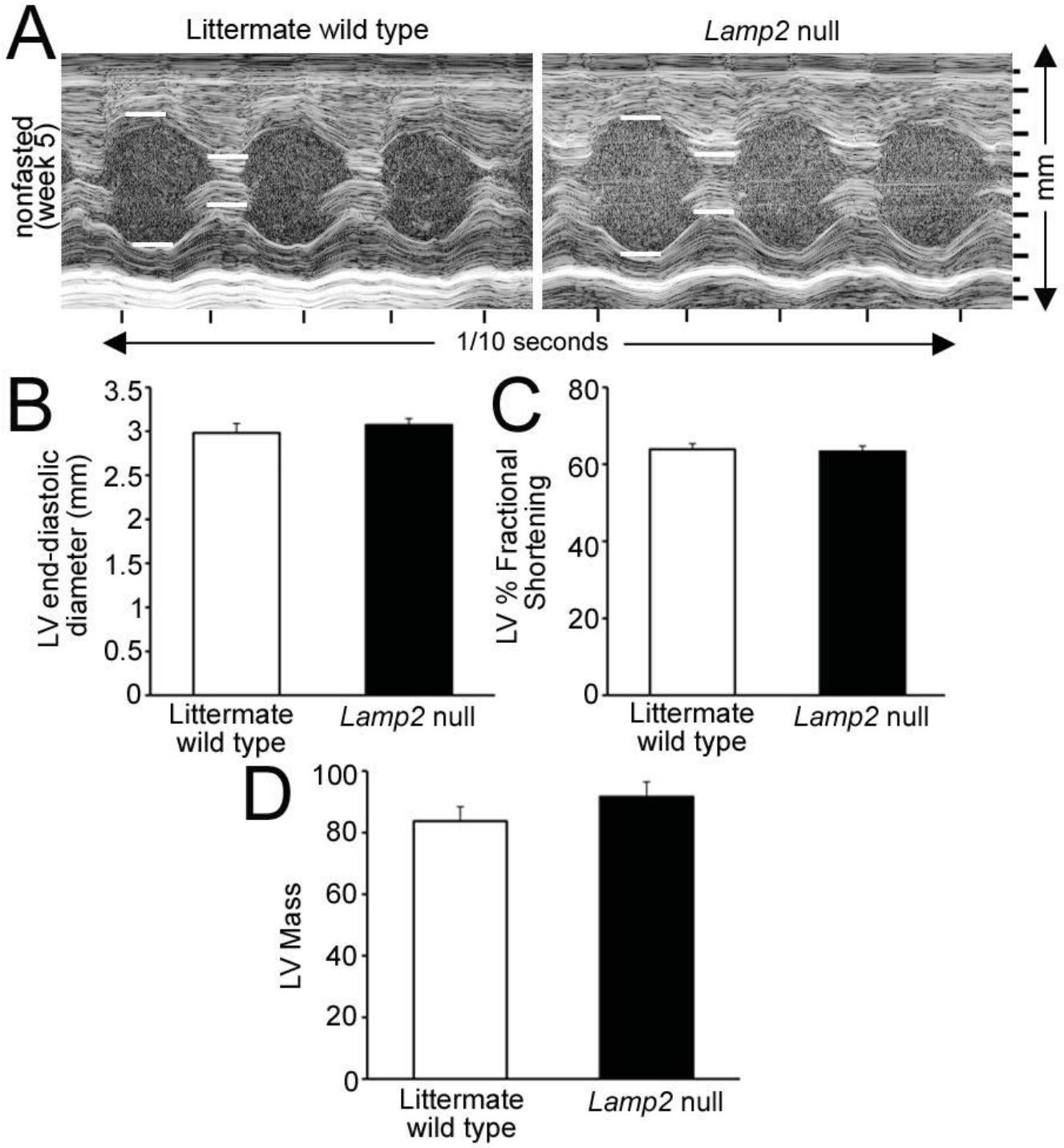

Figure S4. Echocardiographic signs of cardiomyopathy are not detectable in *lamp2* null mice by 15 weeks of age. (**A**) Representative 2D-directed M mode echocardiographic images in 15-week old *lamp2* null and littermate wild-type males. (**B to D**) Quantitative analysis of left ventricular end-diastolic diameter (LVEDD), % fractional shortening, and LV mass in mice as in (**A**). N=4/group.

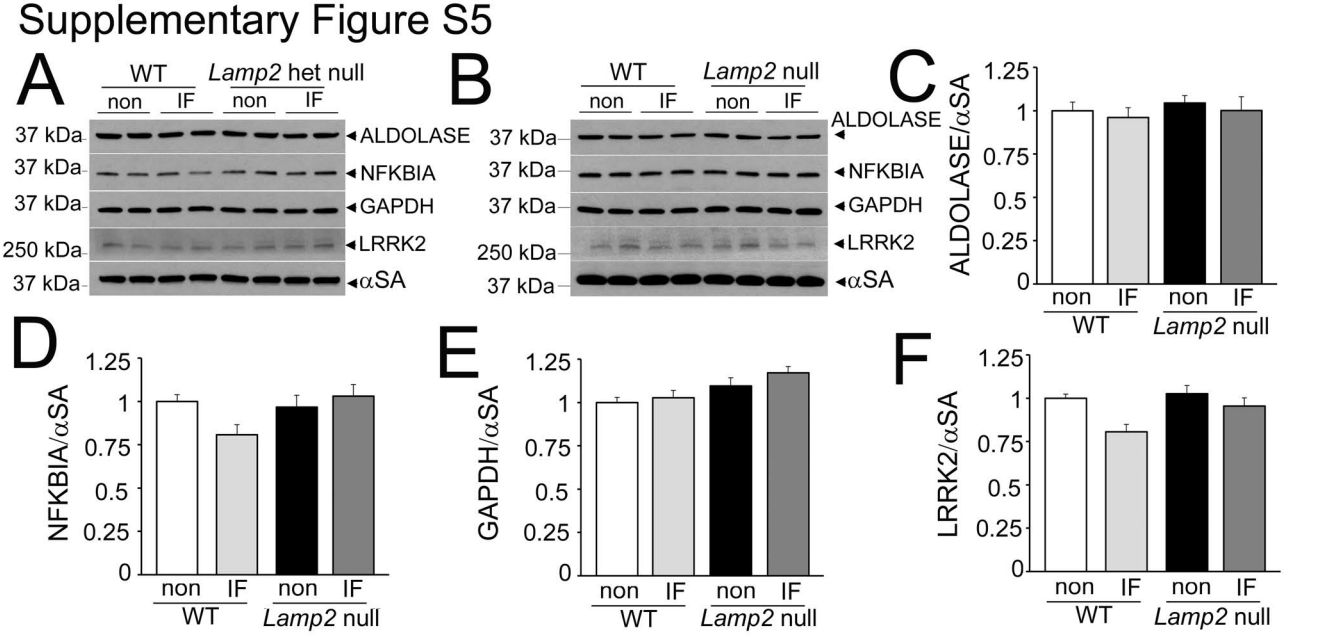

Figure S5. Intermittent fasting does not alter abundance of chaperone mediated autophagy substrates in the myocardium in mice with LAMP2 ablation. (**A**) Immunoblot demonstrating abundance of candidate CMA substrates in *Lamp2* heterozygous null females and littermate control hearts, on a fed day after 6 weeks of intermittent fasting (IF) or ad-lib access to food (non). (**B-F**) Representative immunoblot (B) with quantitation (C-F) of candidate CMA substrates in cardiac extracts from *Lamp2* null and littermate wild-type males on a fed day after 5 weeks of intermittent fasting (IF) or ad-lib access to food (non). N=4/group. No statistically significant differences were detected between groups by one-way ANOVA.

Supplementary Figure S6

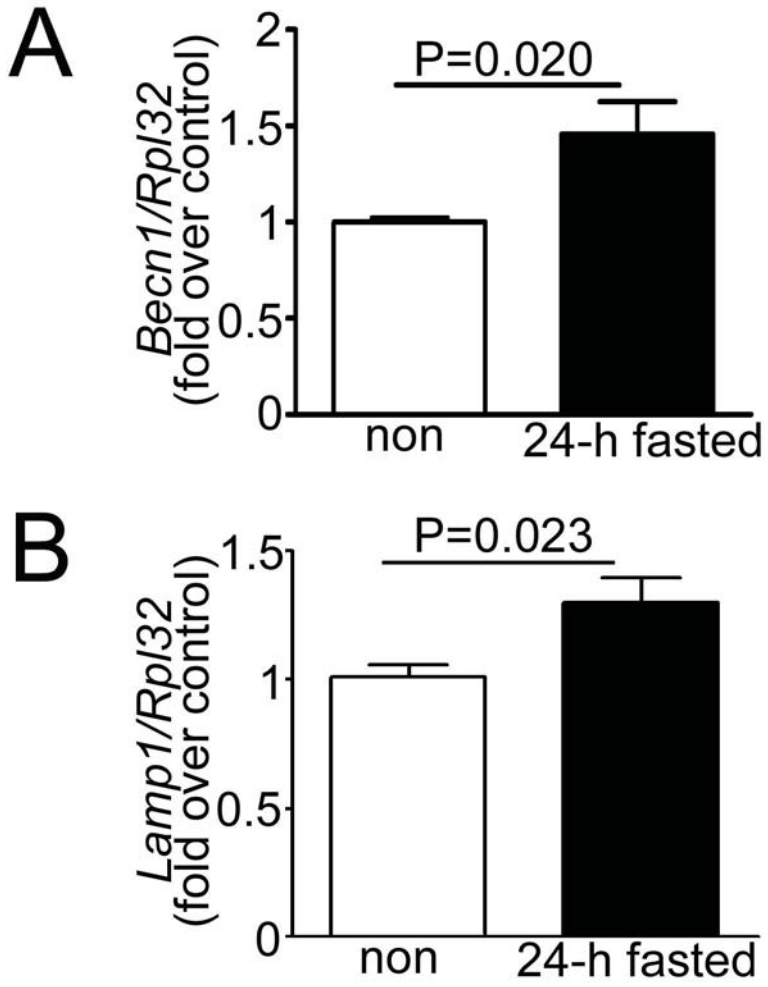

Figure S6. Transcriptional regulation of *Becn1* and *Lamp1* with fasting. (**A and B**) Quantitative PCR analysis of *Becn1* (**A**) and *Lamp1* (**B**) expression (normalized to ribosomal protein gene *Rpl32*) in myocardial extracts from mice subjected to 24 h fasted and nonfasted controls; n=7 or 8/group. *P* values shown are by t test.

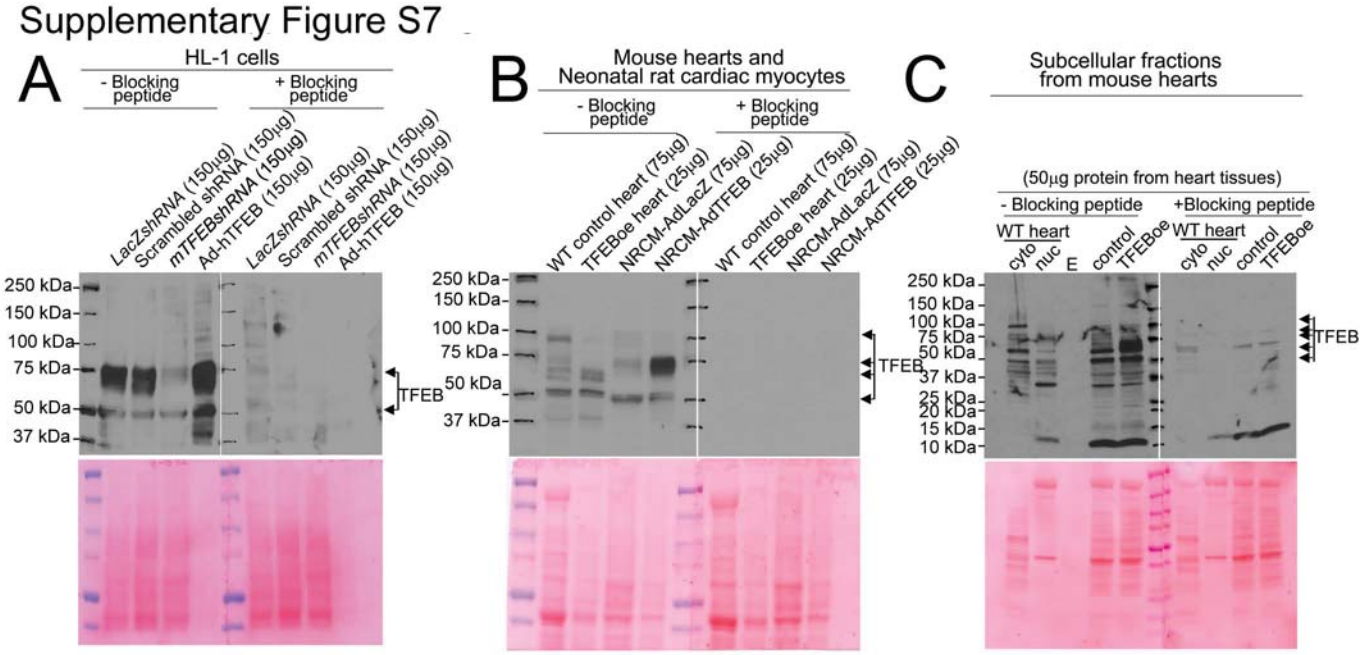

Figure S7. Validation of anti-TFEB antibody. (**A**) HL-1 cardiac myocytes were transduced with shRNA targeting *Tfeb*, scrambled shRNA control and a nontargeting shRNA directed against LacZ as previously described [1], and protein extracts subjected to immunoblotting with anti-TFEB antibody in the absence (*left*) or presence (*right*) of saturating concentrations of blocking peptide. (**B**) Immunoblot depicting myocardial extracts from conditional cardiac TFEB overexpressor (oe) mouse and tTA control; and nenonatal rat cardiac myocytes adenovirally transduced with human TFEB or LacZ-expressing control as previously described;[1] and subjected to immunoblotting for TFEB as in (**A**). (**C**) Immunoblot depicting subcellular fractionation of wild-type heart into nuclear and cytoplasmic extracts; and TFEB-overexpressing heart; and immunoblotted for TFEB as in (**A**). Ponceau Red stained membranes are shown as loading controls.