SUPPLEMENTAL MATERIAL

Supplemental Figure S1

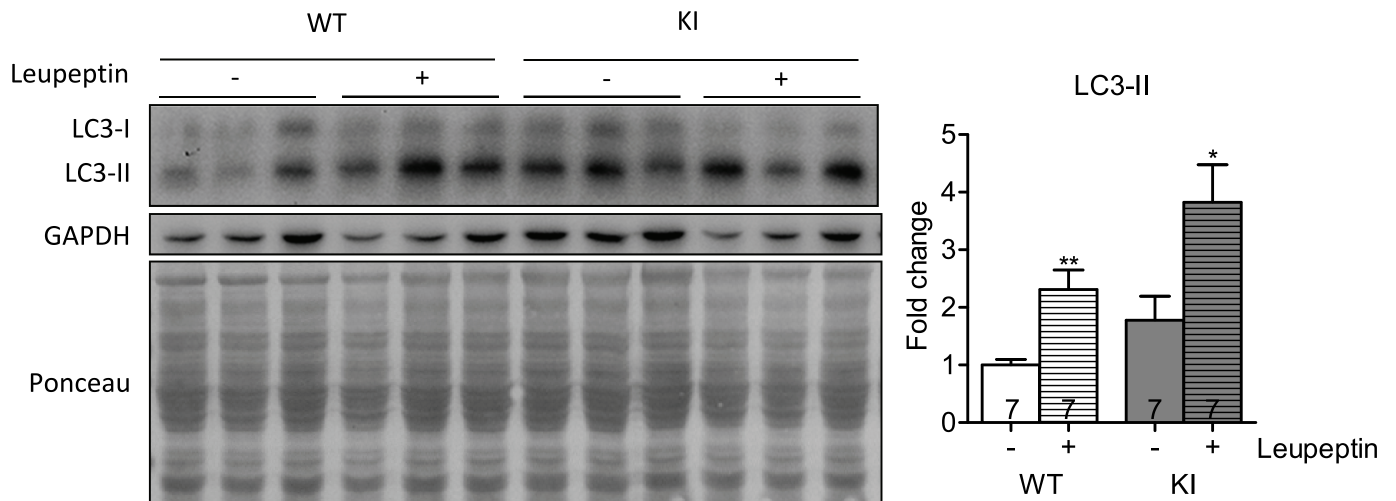

Figure S1. Evaluation of autophagic flux in KI and WT mouse liver. Protein levels of LC3-II in 10-week-old KI and WT mouse livers. Before organ extraction, mice received an i.p. injection of 40 mg/kg leupeptin or sodium chloride for 1 hour. Left, representative Western blots of indicated proteins from liver protein extracts and Ponceau. Right, quantification of LC3-II protein levels normalized to Ponceau and related to WT treated with sodium chloride. Data are expressed as mean + s.e.m. with *P<0.05, and **P<0.01 vs. corresponding control KI and WT, respectively, unpaired Student‘s *t*-test. Number of animals is indicated in the bars.

Figure S2 - Figure 3A extended

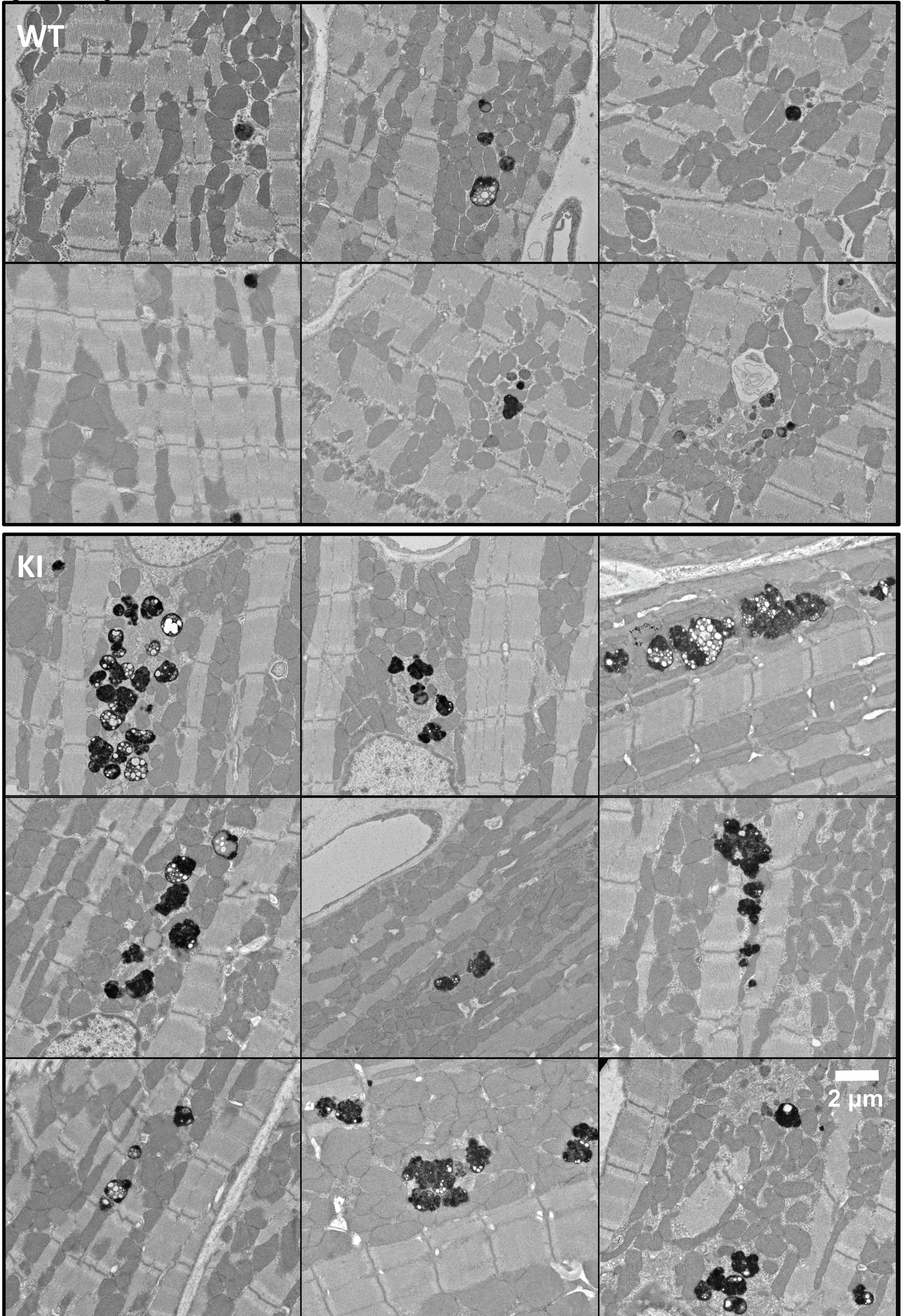

Figure S3-

Figure 3B extended.

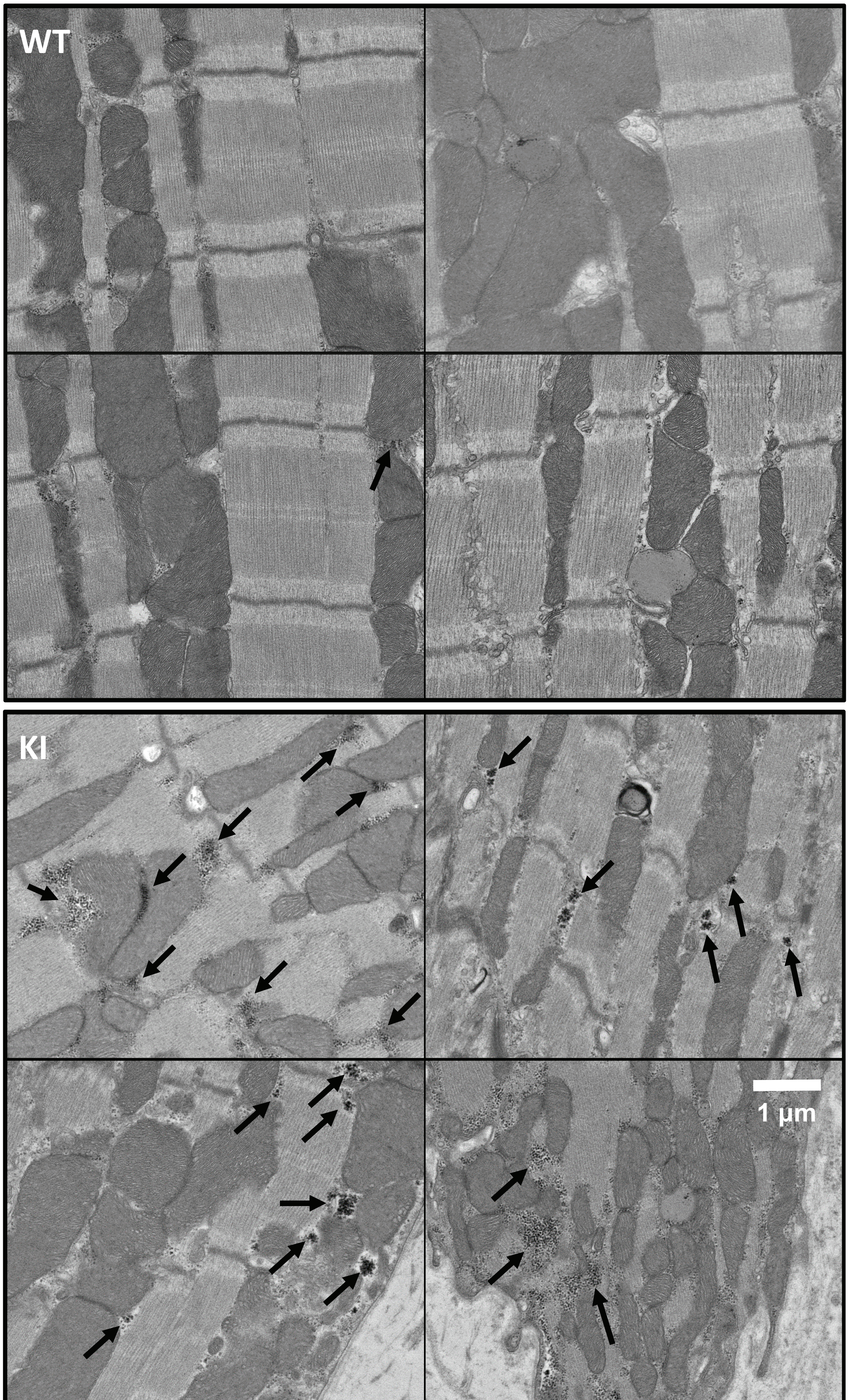

Figure S4

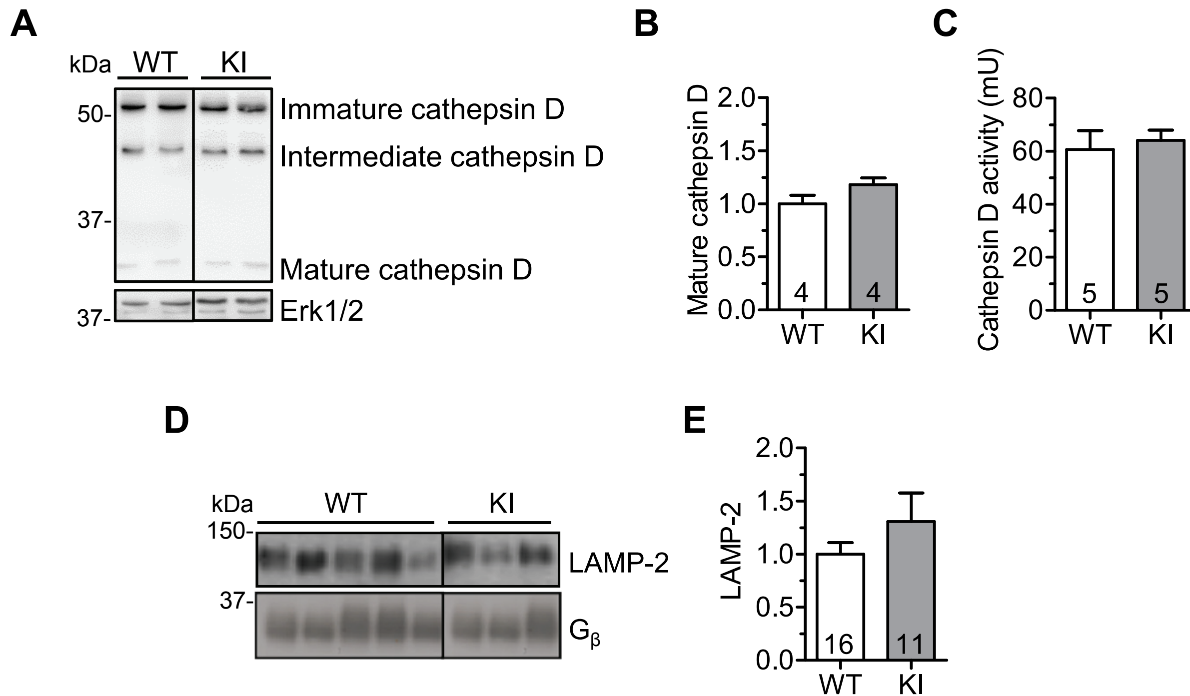

Figure S4. Functional lysosomes in KI mice. Determination of cathepsin D and LAMP-2 protein levels, and cathepsin D activity in 60-week-old KI and WT mice. **A**, Representative Western blots of indicated proteins of ventricular protein extracts (cytosolic fraction). Erk1/2 was used as loading control. **B**, Quantification of mature cathepsin D protein level normalized to Erk1/2 and related to WT. **C**, Cathepsin D activity in milliunits (mU) measured in ventricular protein extracts. **D**, Representative Western blots of indicated proteins from ventricular protein extracts (membrane-enriched fraction). Gβ was used as a loading control. **E**, Quantification of LAMP-2 protein level normalized to Gβ and related to WT. Data are expressed as mean + s.e.m. Number of animals is indicated in the bars.

Figure S5

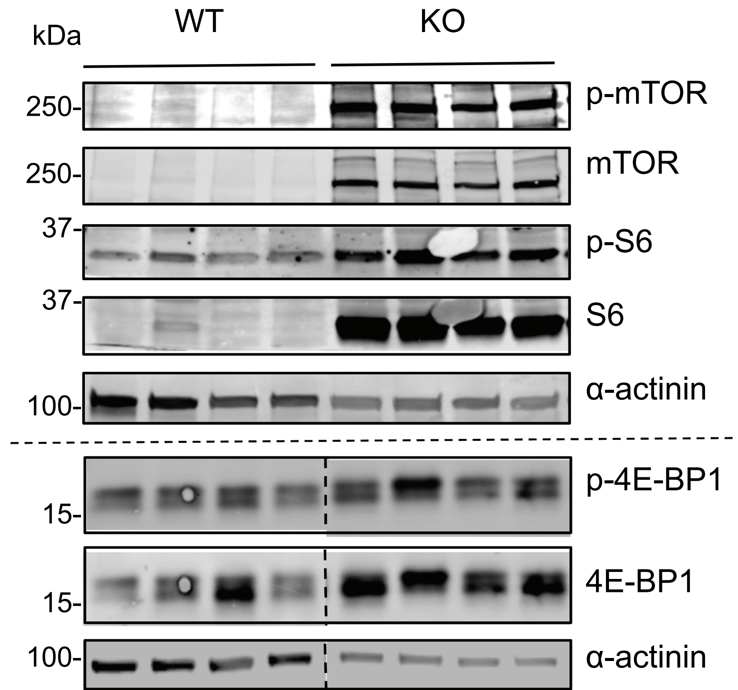

Figure S5. Increased mTORC1 signaling in KO mice. Protein levels of phosphorylated mTOR (p-mTOR), mTOR, phosphorylated S6 (p-S6), S6, phosphorylated 4E-BP1 (p-4E-BP1) and 4E-BP1 in 60-week-old KO and WT mouse hearts. Representative Western blots of indicated proteins from mouse ventricular protein extracts (cytosolic fraction). α-actinin was used as loading control.

Figure S6

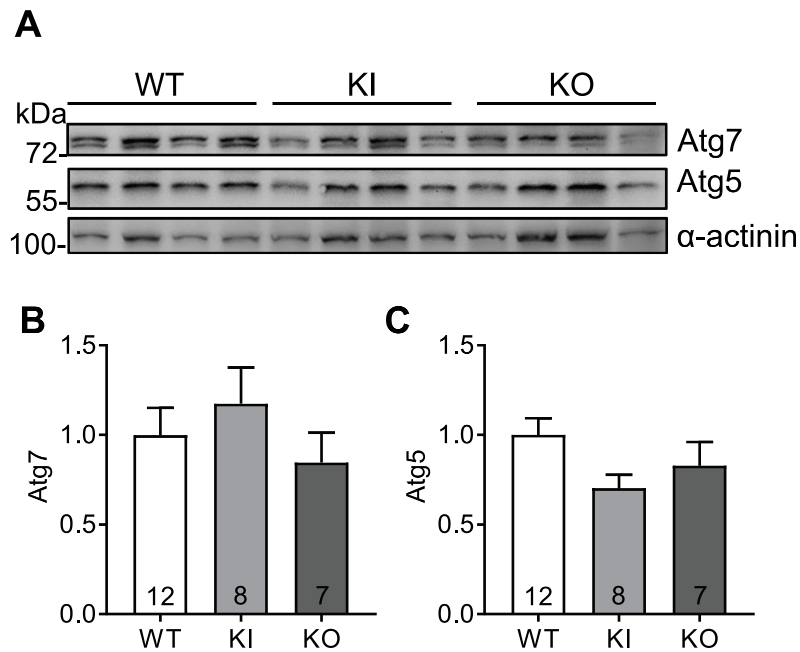

Figure S6. Atg7 and Atg5 protein levels are not affected in KI and KO mice. Protein levels of Atg7 and Atg5 in 60-week-old KI, KO and WT mouse hearts. **A**, Representative Western blots of indicated proteins from mouse ventricular protein extracts (membrane-enriched fraction). α-actinin was used as loading control. Quantification of **B**, Atg7 and **C**, Atg5. Protein levels were normalized to α-actinin and related to WT. Data are expressed as mean + s.e.m.. Number of animals is indicated in the bars.

Figure S7

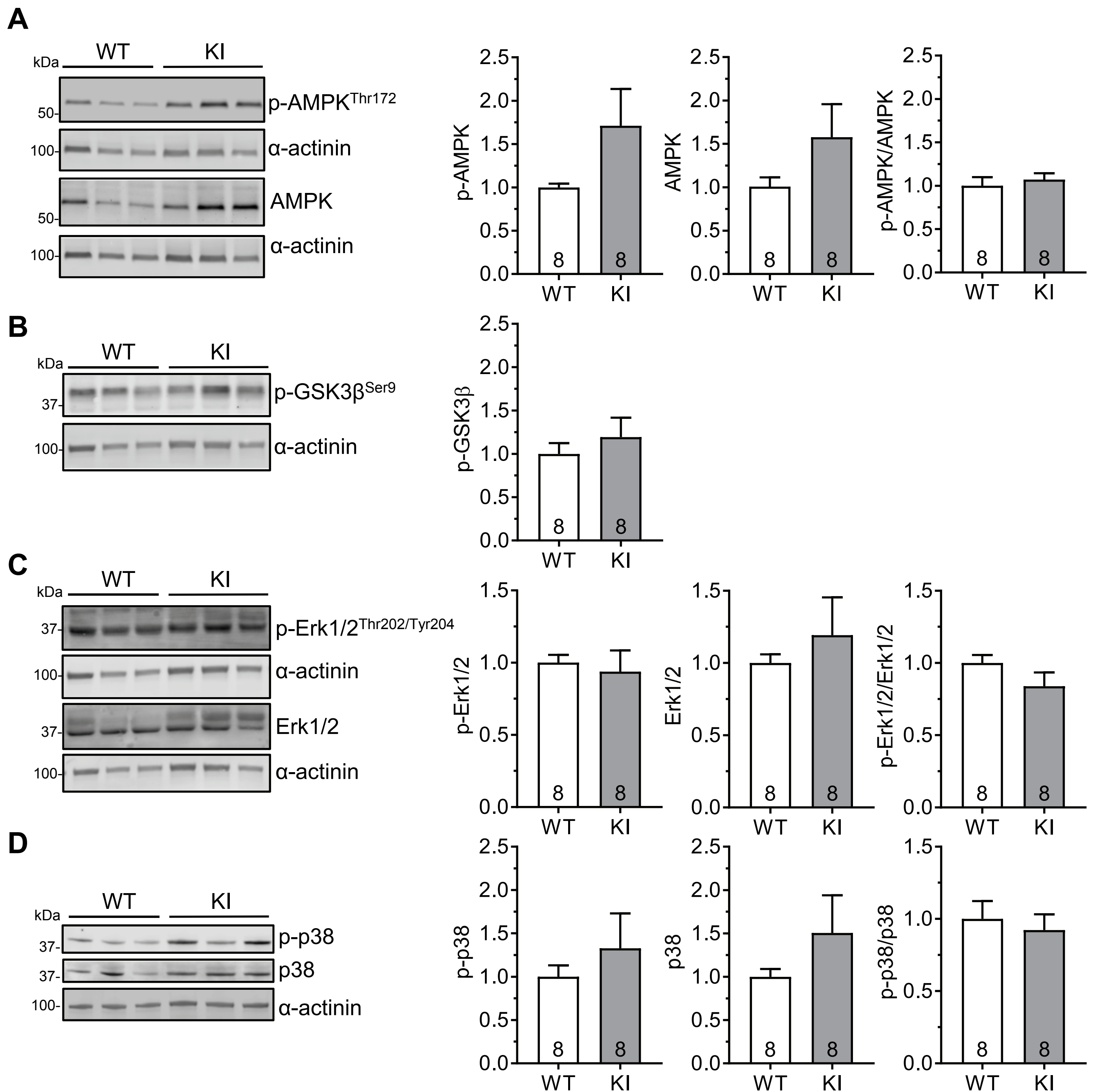

Figure S7. Evaluation of signaling pathways upstream of mTORC1. Protein levels of phosphorylated AMPK (p-AMPK), AMPK, phosphorylated GSK3β (p-GSK3β), phosphorylated Erk1/2 (p-Erk1/2), Erk1/2, phosphorylated p38 (p-p38) and p38 in 60-week-old KI and WT mouse hearts. **A**, Representative Western blots of indicated proteins from mouse ventricular protein extracts (cytosolic fraction). α-actinin was used as loading control. Quantification of p-AMPK, AMPK and p-AMPK/AMPK. **B**, Representative Western blots of indicated proteins from mouse ventricular protein extracts (cytosolic fraction). α-actinin was used as loading control. Quantification of p-GSK3β. **C**, Representative Western blots of indicated proteins from mouse ventricular protein extracts (cytosolic fraction). α-actinin was used as loading control. Quantification of p-Erk1/2, Erk1/2 and p-Erk1/2/Erk1/2. **D**, Representative Western blots of indicated proteins from mouse ventricular protein extracts (cytosolic fraction). α-actinin was used as loading control. Quantification of p-p38, p38 and p-p38/p38. Protein levels were normalized to α-actinin and related to WT. Data are expressed as mean + s.e.m. Number of animals is indicated in the bars.

Figure S8

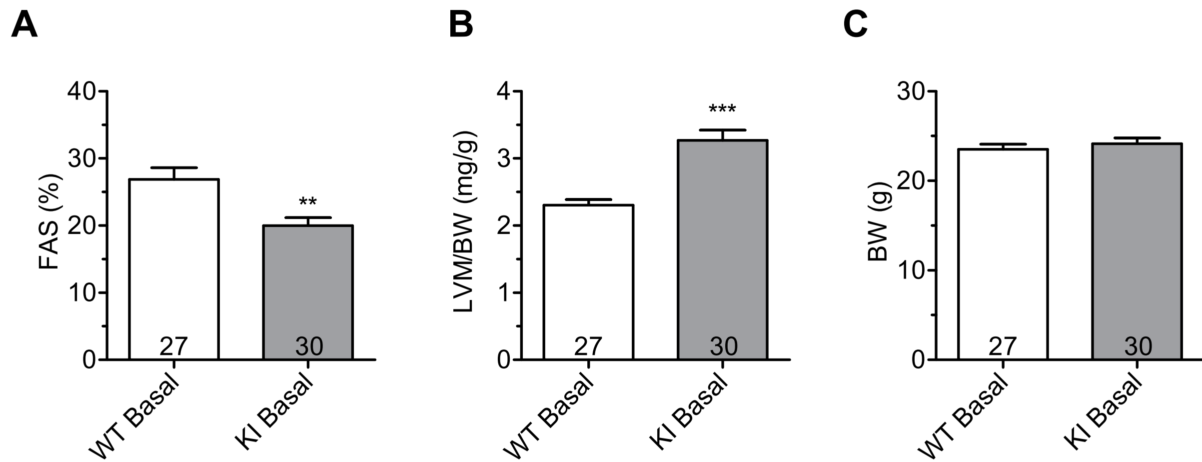

Figure S8. Basal echocardiographic parameters of cardiac function and hypertrophy in KI and WT mice. Transthoracic echocardiography was performed in 11-week-old KI and WT mice with the Vevo 2100 System (VisualSonics, Toronto, Canada). Data are expressed as mean ± s.e.m with **P<0.01 and ***P<0.001 vs. WT, unpaired Student's *t*-test. Abbreviations: BW, body weight; FAS, fractional area shortening; LVM/BW, left ventricular mass-to-body weight ratio. Number of animals is indicated in the bars.

Singh et al., Supplemental Tables

Table S1. Description of *MYBPC3* mutations in patients with hypertrophic cardiomyopathy

Patient #	Exon	Mutation (cDNA)	Mutant protein	Prediction
1	16	c.1358dupC	p.Val454CysfsX21	Disease causing (1.0; MutationTaster)
2	21	c.1960C>T	p.Arg654Cys	Disease causing (0.99; MutationTaster)
	26	c.2686G>A	p.Val896Met	Polymorphism (rs35078470; MutationTaster) Benign (0.2 PolyPhen-2)
3	24	c.2373dupG	p.Trp792ValfsX41	Disease causing (1.0; MutationTaster)
4	24	c.2373dupG	p.Trp792ValfsX41	Disease causing (1.0; MutationTaster)
5	33	c.3776delA	p.Gln1259ArgfsX72	Disease causing (1.0; MutationTaster)
6	27	c.2827C>T	p.Arg943X	Disease causing (1.0; MutationTaster)
7	24	c.2373dupG	p.Trp792ValfsX41	Disease causing (1.0; MutationTaster)
8	23	c.2308G>A	p.Lys717fsX51	Disease causing (1.0; MutationTaster)
9	23	c.2234A>G potential cryptic donor splice site	p.Asp745Gly p.Asp745del25	Disease causing (1.0; MutationTaster)

Note: Patient #2 is compound heterozygote. However, the second mutation is considered as a polymorphism. Prediction was obtained from the following websites: MutationTaster (http://www.mutationtaster.org/), Exome Variant Server (http://evs.gs.washington.edu/EVS/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/). The numbering of cDNA and amino acid is given with number 1 being the A of ATG (cDNA) or ATG (protein). Abbreviations: del, deletion; dup, duplication; Xn, X represents the stop codon that followed n number of new amino acids introduced by the frameshift.

Table S2. Gene acronym, name and expression in human non-faling (NF) and hypertrophic cardiomyopathy (HCM). Data in HCM are expressed in fold-change over NF. Values represent the mean of 2 independent experiments.

Gene acronym	Gene full name	Expression	
		NF	HCM
		n=9	n=12
ACTN2	actinin, alpha 2	1,00	1,39
ATP1A1	ATPase, Na+/K+ transporting, alpha 1 polypeptide	1,00	0,59
ATP1A2	ATPase, Na+/K+ transporting, alpha 2 polypeptide	1,00	0,65
ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	1,00	0,50
BAG3	BCL2-associated athanogene 3	1,00	0,66
BCL2	B-cell CLL/lymphoma 2	1,00	1,68
BECN1	beclin 1	1,00	1,36
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	1,00	1,65
CASQ2	calsequestrin 2	1,00	1,33
CHMP2B	charged multivesicular body protein 2B	1,00	1,37
COL1A1	collagen, type I, alpha 1	1,00	1,44
COL3A1	collagen, type III, alpha 1	1,00	0,82
CTGF	connective tissue growth factor	1,00	1,41
EPG5	ectopic P-granules autophagy protein 5 homolog	1,00	1,43
FHL2	four and a half LIM domains 1	1,00	0,80
FN1	fibronectin 1	1,00	0,64
FOXO1	forkhead box O1	1,00	1,35
FYCO1	FYVE and coiled-coil domain containing 1	1,00	1,89
HDAC6	histone deacetylase 6	1,00	1,73
KCNA4	potassium voltage-gated channel, shaker-related subfamily, member 4	1,00	2,12
KCNA5	potassium voltage-gated channel, shaker-related subfamily, member 5	1,00	1,86
KCNE1	potassium voltage-gated channel, Isk-related family, member 1	1,00	1,63
KCNIP2	Kv channel interacting protein 2	1,00	0,31
KCNJ12	potassium inwardly-rectifying channel, subfamily J, member 12	1,00	1,53
KCNJ2	potassium inwardly-rectifying channel, subfamily J, member 2	1,00	1,74
KCNJ5	potassium inwardly-rectifying channel, subfamily J, member 5	1,00	0,69
KCNMA1	potassium large conductance calcium-activated channel, subfamily M, alpha member 1	1,00	2,61
KCNN3	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3	1,00	2,43
LAMP1	lysosomal-associated membrane protein 1	1,00	1,30
MAP1LC3B	microtubule-associated protein 1 light chain 3 beta	1,00	0,65
MTOR	mechanistic target of rapamycin (serine/threonine kinase)	1,00	1,72
MYH6	myosin, heavy chain 6, cardiac muscle, alpha	1,00	0,05
MYH7	myosin, heavy chain 7, cardiac muscle, beta	1,00	1,63
NBR1	neighbor of BRCA1 gene 1	1,00	1,44
NPPA	natriuretic peptide A	1,00	2,36
PLN	phospholamban	1,00	1,33
POSTN	periostin	1,00	3,02
PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A	1,00	0,58
RYR2	ryanodine receptor 2 (cardiac)	1,00	1,31
SLC8A1	solute carrier family 8 (sodium/calcium exchanger), member 1	1,00	1,68
SQSTM1	sequestosome 1	1,00	1,59
SRF	serum response factor (c-fos serum response element-binding transcription factor)	1,00	1,27
STAT3	signal transducer and activator of transcription 3 (acute-phase response factor);	1,00	0,79
TFEB	transcription factor EB	1,00	1,42

Table S3. Gene acronym, name and expression in wild-type and *Mybpc3*-targeted KI mice. Data in KI are expressed in fold-change over WT. Values represent the mean of 2 independent experiments.

Gene acronym	Gene full name	WT	KI
		n=12	n=7
Adrb2	adrenoceptor beta 2	1,00	0,68
Atp2a2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	1,00	0,71
Atp2b4	ATPase, Ca++ transporting, plasma membrane 4	1,00	6,78
Bag3	BCL2-associated athanogene 3	1,00	1,21
Bcl2	B-cell CLL/lymphoma 2	1,00	1,46
Becn1	beclin 1	1,00	1,20
Cacna1c	calcium channel, voltage-dependent, L type, alpha 1C subunit	1,00	0,68
Cacna1g	calcium channel, voltage-dependent, T type, alpha 1G subunit	1,00	1,21
Col1a1	collagen, type I, alpha 1	1,00	2,02
Col3a1	collagen, type III, alpha 1	1,00	1,35
Ctgf	connective tissue growth factor	1,00	5,27
Epg5	ectopic P-granules autophagy protein 5 homolog	1,00	0,71
Erbb2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2	1,00	1,38
Fhl1	four and a half LIM domains 1	1,00	1,83
Fhl2	four and a half LIM domains 1	1,00	0,61
Fn1	fibronectin 1	1,00	0,64
Fyco1	FYVE and coiled-coil domain containing 1	1,00	0,71
Gja1	gap junction protein, alpha 1, 43kDa	1,00	0,59
Hdac6	histone deacetylase 6	1,00	1,29
Kcna5	potassium voltage-gated channel, shaker-related subfamily, member 5	1,00	1,23
Kcnb1	potassium voltage-gated channel, Shab-related subfamily, member 1	1,00	0,75
Kcnd2	potassium voltage-gated channel, Shal-related subfamily, member 2;	1,00	0,20
Kcnj2	potassium inwardly-rectifying channel, subfamily J, member 2	1,00	0,47
Map1lc3b	microtubule-associated protein 1 light chain 3 beta	1,00	0,88
Mcu	mitochondrial calcium uniporter;	1,00	0,68
Meox1	mesenchyme homeobox 1	1,00	3,06
Myh6	myosin, heavy chain 6, cardiac muscle, alpha	1,00	0,49
Myh7	myosin, heavy chain 7, cardiac muscle, beta	1,00	6,77
Nppa	natriuretic peptide A	1,00	2,58
Nppb	natriuretic peptide B	1,00	2,93
Nrg1	neuregulin 1	1,00	1,34
Pln	phospholamban	1,00	0,73
Postn	periostin	1,00	2,30
Ppp1r1a	protein phosphatase 1, regulatory (inhibitor) subunit 1A	1,00	1,21
Rab7	RAB7, member RAS oncogene family	1,00	0,84
Rcan1	regulator of calcineurin 1	1,00	4,60
Ryr2	ryanodine receptor 2 (cardiac)	1,00	0,68
Scn5a	sodium channel, voltage-gated, type V, alpha subunit	1,00	0,56
Slc8a1	solute carrier family 8 (sodium/calcium exchanger), member 1	1,00	0,74
Sqstm1	sequestosome 1	1,00	1,17

Table S4. Cardiac parameters of wild-type mice under control treatment, rapamycin treatment or caloric restriction.

	WT before	WT after	WT rapa before	WT rapa after	WT CR before	WT CR after
IVSd	0.35 ± 0.02	0.38 ± 0.02	0.33 ± 0.02	0.44 ± 0.02	0.34 ± 0.04	0.37 ± 0.02
IVSs	0.70 ± 0.03	0.74 ± 0.06	0.69 ± 0.03	0.64 ± 0.03	0.55 ± 0.06	**0.83 ± 0.06^{++}**
LVIDd	4.37 ± 0.11	4.38 ± 0.18	4.43 ± 0.11	4.48 ± 0.08	4.44 ± 0.23	**3.94 ± 0.17^{+}**
LVIDs	3.65 ± 0.10	3.53 ± 0.10	3.54 ± 0.11	3.64 ± 0.10	3.75 ± 0.21	**2.95 ± 0.30^{++}**
LVPWd	0.41 ± 0.02	0.44 ± 0.03	0.41 ± 0.02	0.46 ± 0.02	0.40 ± 0.05	0.44 ± 0.03
LVPWs	0.61 ± 0.03	0.57 ± 0.05	0.67 ± 0.06	0.62 ± 0.04	**0.68 ± 0.04***	**0.71 ± 0.05^{++}**
LVM	56 ± 3	61 ± 4	55 ± 3	61 ± 4	55 ± 6	49 ± 6
FAS	28 ± 3	34 ± 3	32 ± 3	31 ± 2	24 ± 2	**43 ± 4**$^{++}$**
HR	423 ± 12	472 ± 15	428 ± 8	**471 ± 10^{++}**	419 ± 20	**407 ± 23***
BW	23 ± 1	**27 ± 2^{+}**	24 ± 1	25 ± 1	24 ± 1	**19 ± 1*$^{++}$**
Age (weeks)	12	21	12	21	12	21
n-number	8	8	10	10	5	5

Data are expressed as mean ± s.e.m. with *P<0.05 and **P<0.01 vs. WT ctrl (before vs. before and after vs. after), one-way ANOVA plus Tukey's post-test, or $^{+}$P<0.05 and $^{++}$P<0.01 vs. same group before treatment, paired Student's *t*-test. IVSd – intraventricular septum end diastole (mm); IVSs – intraventricular septum end systole (mm); LVIDd – left ventricular internal diameter end diastole (mm); LVIDs – left ventricular internal diameter end systole (mm); LVPWd – left ventricular posterior wall end diastole (mm); LVPWs – left ventricular posterior wall end systole (mm); LVM – left ventricular mass (mg); FAS – fractional area ahortening (%); HR – heart rate (bpm); and BW – body weight (g).

Table S5. Cardiac parameters of KI mice under control treatment, rapamycin treatment or caloric restriction.

	KI before	KI after	KI rapa before	KI rapa after	KI CR before	KI CR after
IVSd	0.39 ± 0.01	0.31 ± 0.04	0.46 ± 0.05	0.37 ± 0.03	0.46 ± 0.04	**0.37 ± 0.02**$^{+}$
IVSs	0.60 ± 0.05	0.47 ± 0.05	0.64 ± 0.06	0.57 ± 0.03	0.71 ± 0.04	0.64 ± 0.05
LVIDd	4.80 ± 0.18	4.82 ± 0.09	4.70 ± 0.18	4.83 ± 0.08	4.75 ± 0.10	4.61 ± 0.09
LVIDs	4.25 ± 0.14	4.49 ± 0.11	4.22 ± 0.16	4.41 ± 0.09	4.25 ± 0.09	**3.97 ± 0.11***
LVPWd	0.44 ± 0.02	0.42 ± 0.04	0.51 ± 0.04	0.48 ± 0.03	0.51 ± 0.04	0.45 ± 0.03
LVPWs	0.61 ± 0.03	0.66 ± 0.03	0.61 ± 0.03	0.66 ± 0.03	0.51 ± 0.04	0.66 ± 0.03
LVM	74 ± 6	64 ± 9	83 ± 6	76 ± 6	87 ± 8	67 ± 5^{+}
FAS	20 ± 3	16 ± 2	19 ± 2	21 ± 1	21 ± 2	**26 ± 2***
HR	385 ± 14	**456 ± 10**$^{+++}$	**434 ± 15***	**497 ± 13**$^{+++}$	415 ± 8	417 ± 16
BW	23 ± 1	**26 ± 2**$^{++}$	24 ± 1	**29 ± 2**$^{+++}$	25 ± 1	**20 ± 1*****$^{+++}$
Age (weeks)	11	20	11	20	11	20
n-number	9	9	9	9	9	9

Data are expressed as mean ± s.e.m. with *P<0.05 and ***P<0.001 vs. KI ctrl (before vs. before and after vs. after), one-way ANOVA plus Tukey‘s post-test, or $^{+}$P<0.05, $^{++}$P<0.01 and $^{+++}$P<0.001 vs. same group before treatment, paired Student‘s *t*-test. IVSd – intraventricular septum end diastole (mm); IVSs – intraventricular septum end systole (mm); LVIDd – left ventricular internal diameter end diastole (mm); LVIDs – left ventricular internal diameter end systole (mm); LVPWd – left ventricular posterior wall end diastole (mm); LVPWs – left ventricular posterior wall end systole (mm); LVM – left ventricular mass (mg); FAS – fractional area ahortening (%); HR – heart rate (bpm); and BW – body weight (g).

Table S6. Gene acronym, full name and expression in wild-type (WT) and *Mybpc3*-targeted KI mice, treated with rapamycin (rapa) or caloric restriction (CR). Data in rapa-treated-KI, in CR-treated-KI and WT-Ctrl are expressed in fold-change over untreated-KI (n=4).

Rapamycin				
Gene acronym	**Gene full name**	**KI Ctrl**	**KI rapa**	**WT Ctrl**
Atp2b4	ATPase, Ca++ transporting, plasma membrane 4	1,00	0,70	0,56
Bcl2	B-cell CLL/lymphoma 2	1,00	0,73	0,64
Kcnj2	potassium inwardly-rectifying channel, subfamily J, member 2	1,00	1,39	2,14
Myh7	myosin, heavy chain 7, cardiac muscle, beta	1,00	0,68	0,04
Nppa	natriuretic peptide A	1,00	0,74	0,17

Caloric restriction				
Gene acronym	**Gene full name**	**KI Ctrl**	**KI CR**	**WT Ctrl**
Bcl2	B-cell CLL/lymphoma 2	1,00	0,78	0,64
Col1a1	collagen, type I, alpha 1	1,00	0,34	0,68
Cacna1g	calcium channel, voltage-dependent, T type, alpha 1G subunit	1,00	0,59	0,89
Kcnj2	potassium inwardly-rectifying channel, subfamily J, member 2	1,00	1,80	2,14
Map1lc3b	microtubule-associated protein 1 light chain 3 beta	1,00	1,60	1,06
Meox1	mesenchyme homeobox 1	1,00	0,51	0,40
Nrg1	neuregulin 1	1,00	1,62	2,30
Postn	periostin	1,00	0,51	0,36
Rab7	RAB7A, member RAS oncogene family	1,00	1,28	1,15

Singh SR et al. **Expanded Methods**

Animals

The investigation conformed to the guide for the care and use of laboratory animals published by the NIH (Publication No. 85-23, revised 2011, published by the National Research Council). The experimental procedures were in accordance with the German Law for the Protection of Animals and approved by the Authority for Health and Consumer Protection of the City State of Hamburg, Germany (no. 118/13 and 100/14). Generation and characterization of *Mybpc3*-targeted KI and knock-out (KO) mice were described previously.[1, 2] The homozygous KI mice carry a founder mutation that was found in ~14% of HCM patients in Tuscany,[3] and results in low levels (10% of wild-type (WT)) of mutant cMyBP-C in KI mice.[2] Homozygous KO mice that do not express cMyBP-C served as a model of pure cMyBP-C deficiency.[1] Both models develop LVH, and diastolic and systolic dysfunction. Mice were maintained on Black Swiss background.

Echocardiography

Transthoracic echocardiography was performed using the Vevo 2100 System (VisualSonics, Toronto, Canada). Mice were anesthetized with isoflurane (1–2%, Abbott Inc.) and placed on a warming platform in a supine position. B-mode images were obtained using a MS400 transducer with a frame rate of 230-400 frames/s. Two-dimensional short axis views were recorded at the mid-papillary muscle level. The dimensions of the left ventricle were measured in a short axis view in diastole and systole. All images were recorded digitally and off-line analysis was performed using the Vevo 2100 software.

Autophagic flux measurement

To measure the autophagic flux *in vivo*, mice were injected i.p. with 40 mg/kg leupeptin (Sigma Aldrich, L-8511) or sodium chloride (500 µL) as described previously.[4] After 1h, mice were sacrificed by cervical dislocation under CO_2-anesthesia and hearts were extracted for further analyses. The autophagic flux was measured by LC3 turnover via Western blot.

Experimental diet

Eleven-week-old KI and WT mice were kept on caloric restriction, rapamycin or control diet for 9 weeks. All diets were based on LabDiet 5LG6 (TestDiet) including Eudragit S100 (rapamycin coating material). Mice kept on caloric restriction were fed ~20% less in the first

week and then ~40% less for the following 8 weeks than control mice. It was assumed that a 30 g mouse eats about 5 g per day. Mice were kept under tight observation and were regularly weighed. Mice on rapamycin diet received ~2.24 mg/kg rapamycin (Rapamycin Holdings^{TM}) encapsulated in Eudragit S100 daily. This protocol was adapted from Dai *et al.*[5] Echocardiography was performed at the beginning and at the end of the experiment. The autophagic flux was measured at the end of the experiment.

Organ extraction

Mice were sacrificed by cervical dislocation under CO_2-anesthesia. After median thoracotomy, organs were extracted, rinsed in sodium chloride and shortly dried on a paper tissue. Organs were shock frozen in liquid nitrogen and tissue samples were powdered and stored at -80 °C until utilization.

Protein analysis

Human and mouse tissue samples were powdered and protein extraction was performed in two steps. First, the organ powder (about 30 mg) was dissolved in 180 µL water or CelLytic M (Sigma-Aldrich) with protease inhibitor cocktail (complete mini^{TM,}, Roche Diagnostics) and homogenized by using Tissue Lyser (2 x 30 s at 20 Hz) and centrifuged at 4 °C, full speed for 30 min in a table-top centrifuge. The supernatant was kept as the cytosolic fraction. Second, the pellet of the first step was homogenized in 180 µL SDS-buffer (3% SDS, 30 mM Tris-base, pH 8.8, 5 mM EDTA, 30 mM NaF, 10 % glycerol and 1 mM DTT) and centrifuged at room temperature, full speed for 15 min in a table-top centrifuge and the supernatant was kept as the membrane-enriched fraction. Proteins were loaded on acrylamide/bisacrylamide (29:1) gels and electrotransferred to nitrocellulose or polyvinylidene fluoride (PVDF) membranes. For LC3 analysis, proteins were electrotransferred to PVDF membranes. Antibodies against following proteins were used for Western blots: p-4E-BP1 (Cell Signaling Technology, 2855 or 9459), 4E-BP1 (Cell Signaling Technology, 9452 or 9644), α-actinin (Sigma Aldrich, a-7811), p-Akt^{Ser273} (Cell Signaling Technology, 4060), p-Akt^{Thr308} (Cell Signaling Technology, 13038), Akt (Cell Signaling Technology, 4691), p-AMPK (Cell Signaling Technology, 2535), AMPK (Cell Signaling Technology, 2532), calsequestrin (Dianova, ABR-01164), cathepsin D (Santa Cruz, SC-6486), p-Erk1/2 (Cell Signaling Technology, 4376), Erk1/2 (Cell Signaling Technology, 9102), $G_β$ (Santa Cruz, sc-378), p-GSK-3β (Cell Signaling Technology, 5558), LAMP-2 (Abcam, ab13524), LC3 (Novus Biologicals, NB100-2331), p-mTOR (Cell Signaling Technology, 2971), mTOR (Cell

Signaling Technology, 2972), p-p38 (Cell Signaling Technology, 4511), p38 (Cell Signaling Technology, 9212), p62 (Sigma Aldrich, P0067 for mouse and BD Biosciences 610833 for human samples), p-S6 (Cell Signaling Technology, 2215) and S6 (Cell Signaling Technology, 2217). All proteins were analyzed in ventricular cytosolic protein extracts, except for LAMP-2, LC3 and p62, which were analyzed in ventricular membrane-enriched protein extracts.

Electron Microscopy

Hearts of isoflurane-anesthetized mice were initially fixed by perfusion with 1% paraformaldehyde/2% (vol/vol) glutaraldehyde in cardioplegic solution (50 mmol/L KCl, 5% dextrose in PBS) and next in 1% paraformaldehyde/2% (vol/vol) glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.2. The heart was removed and immersed into the latter fixative (ice cold) and then left and right ventricular free walls and septa were isolated. Each region was divided into small fragments and fixed further in the same fixative, at 4 °C, then postfixed/stained in 1% OSO_4 (in water) before dehydration in acetone and embedding in epoxy resin. Ultrathin sections were counterstained with uranium and lead salts. Images were acquired on a Hitachi 7600 electron microscope equipped with an AMT digital camera.

Cathepsin D activity

Cathepsin D activity was determined in cytosolic ventricular protein fractions using the Sigma-Aldrich Cathepsin D Assay Kit according to manufacturer's instructions.

RNA isolation and expression analysis with the NanoString nCounter® Elements

Total RNA was extracted from septal myectomies of HCM patients (n=12), non-failing human heart tissue not suitable for transplantation or from donors that did not die from cardiac disease but of another cause (=non-failing, NF, n=9), from 60-week-old WT and KI mice (n=12 for WT and n=7 for KI) and from 20-week-old WT and KI treated or not with rapamycin or CR (n=4 in each group). For gene expression analysis, we designed for both human and mice a NanoString's nCounter® Elements TagSet panel of 78 genes coding for proteins regulated in hypertrophy/heart failure, including Ca^{2+} and K^+ handling proteins and proteins involved in the autophagy process. RNAs of each group were pooled, and 50-ng RNA pool was hybridized to target-specific capture and reporter probes at 67 °C over night (16 h) according to manufacturer's instructions. Samples were cooled down at 4 °C, loaded into the NanoString cartridge and nCounter Gene Expression Assay started immediately. Raw data were analyzed with nCounter® Sprint Profiler including background subtraction using

negative controls and normalization to 5 housekeeping genes (*ABCF1, CLTC, GAPDH, PGK1, TUBB* for human and *Abcf1, Cltc, Gapdh, Pgk1 and Tubb5* for mice). Data represented the mean of normalized counts of two independent experiments and were expressed as fold-change in HCM vs NF and in KI vs WT. We selected genes that were lower than 0.8-fold and higher than 1.2-fold dysregulated in cardiomyopathy. Heatmaps were generated with Morpheus online tool (https://software.broadinstitute.org/morpheus/).

Statistical analysis

Data were expressed as mean ± s.e.m. Statistical analyses were performed by one-way ANOVA followed by Newmann-Keuls post-test, or by unpaired or paired Student's *t*-test as indicated in the figure legends. All analyses were performed using GraphPad Prism5 or newer (Software Inc.). A value of $P < 0.05$ was considered statistically significant.

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