1 # Activation of autophagy ameliorates cardiomyopathy in *Mybpc3*-
2 targeted knock-in mice

3

4 *Singh et al. Impact of autophagy activation in HCM mice*

5

6 Sonia R. Singh, PhD, Antonia T.L. Zech, MSc, Birgit Geertz, Silke Reischmann-Düsener,
7 Hanna Osinska, PhD, Maksymilian Prondzynski, MSc, Elisabeth Krämer, Qinghang Meng,
8 PhD, Charles Redwood, PhD, Jolanda van der Velden, PhD, Jeffrey Robbins, PhD, Saskia
9 Schlossarek, PhD, Lucie Carrier, PhD

10

11 From the Department of Experimental Pharmacology and Toxicology, Cardiovascular
12 Research Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
13 (S.R.S., A.T.L.Z., B.G., S.R.-D., M.P., E.K.,S.S., L.C.); DZHK (German Centre for
14 Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Germany (S.R.S., A.T.L.Z.,
15 B.G., S.R.-D., M.P., E.K., S.S., L.C.); The Heart Institute, Department of Pediatrics, The
16 Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA (S.R.S., H.O.,
17 Q.M., J.R.); Radcliffe Department of Medicine, University of Oxford, Oxford, UK (C.R.);
18 Department of Physiology, Institute for Cardiovascular Research, VU University Medical
19 Center, Amsterdam, the Netherlands (J.v.d.V.); ICIN-Netherlands Heart Institute, Utrecht, the
20 Netherlands (J.v.d.V.).

21

22 **Correspondence** to Lucie Carrier, Department of Experimental Pharmacology and
23 Toxicology, University Medical Center Hamburg Eppendorf, Martinistraße 52, 20246
24 Hamburg, Germany. Fax: +49-40-7410-54876, Phone: +49-40-7410-57208, Email:
25 l.carrier@uke.de,

26

27 **Total word count**: 5990

28

29 **Journal Subject**: Heart failure and cardiac disease

30 **Abstract**

31 **Background.** Alterations in autophagy have been reported in hypertrophic cardiomyopathy
32 (HCM) caused by Danon disease, Vici syndrome or LEOPARD syndrome, but not in HCM
33 caused by mutations in genes encoding sarcomeric proteins, which account for most of HCM
34 cases. *MYBPC3*, encoding cardiac myosin-binding protein C, is the most frequently mutated
35 HCM gene.

36 **Methods and Results.** We evaluated autophagy in HCM patients carrying *MYBPC3*
37 mutations and in a *Mybpc3*-targeted knock-in (KI) HCM mouse model, as well as the effect of
38 autophagy modulators on the development of cardiomyopathy in KI mice. Microtubule-
39 associated protein 1 light chain 3 (LC3)-II protein levels were higher in HCM septal
40 myectomies than in non-failing control hearts and in 60-week-old KI than wild-type (WT)
41 mouse hearts. In contrast to WT, autophagic flux was blunted and associated with
42 accumulation of residual bodies and glycogen in hearts of 60-week-old KI mice. We found
43 that Akt-mTORC1 signaling was increased, and treatment with 2.24 mg/kgxd rapamycin or
44 40% caloric restriction for 9 weeks partially rescued cardiomyopathy or heart failure and
45 restored autophagic flux in KI mice.

46 **Conclusions.** Altogether, we found that i) autophagy is altered in HCM patients with
47 *MYBPC3* mutations, ii) autophagy is impaired in *Mybpc3*-targeted KI mice and iii) activation
48 of autophagy ameliorated the cardiac disease phenotype in this mouse model. We propose that
49 activation of autophagy might be an attractive option alone or in combination with another
50 therapy to rescue HCM caused by *MYBPC3* mutations.

51

52 **Key Words**: Autophagy; caloric restriction; cardiomyopathy; cMyBP-C; hypertrophic
53 cardiomyopathy; hypertrophy; *MYBPC3*; rapamycin

54

55

56 **Introduction**

57 A well-controlled balance between protein synthesis and degradation is crucial for cellular
58 homeostasis. The major pathways for degradation of cellular proteins are the ubiquitin-
59 proteasome system (UPS) and the autophagy-lysosomal pathway (ALP).[1] Autophagy is
60 defined by the degradation of cellular material within the lysosome. It is a crucial process
61 since it removes damaged proteins and organelles, supplies energy and maintains proper
62 metabolism. Insufficient autophagy may lead to energy deficiency and proteotoxicity, while
63 over-active autophagy can cause cell death. The genes and cellular processes that underlie
64 autophagy are conserved from yeast to mammals and can be selective or nonselective. The
65 most prevalent form of autophagy is called macroautophagy (hereafter autophagy), where a
66 double-membrane vesicle, the phagophore, is formed and subsequently matures into an
67 autophagosome, eventually fusing with a lysosome for degradation of its contents.[2]

68 Postmitotic cells such as neurons or cardiomyocytes are particularly dependent on
69 energy and protein quality control. Whereas altered protein quality control mechanisms have
70 been long correlated to neurological diseases,[3] only a few cardiac diseases are known to be
71 associated with defective autophagy. These include Danon disease,[4, 5] LEOPARD syndrome,[6]
72 Vici syndrome,[7, 8] desmin-related cardiomyopathy,[9-11] diabetic cardiomyopathy,[12] dilated
73 cardiomyopathy (DCM) caused by lamin A/C (*LMNA*) mutations,[13, 14] and left ventricular
74 non-compaction (LVNC) caused by pleckstrin homology domain-containing family M,
75 member 2 (*PLEKHM2*) mutations.[15] In most of these cardiomyopathies there is a defect in a
76 gene encoding a protein, which is involved in the ALP, either by acting directly on it or by
77 inducing protein accumulation.

78 To the best of our knowledge, there is no evidence of altered autophagy in
79 sarcomeropathy leading to hypertrophic cardiomyopathy (HCM) or DCM. HCM is an
80 autosomal-dominant disorder, characterized by left ventricular hypertrophy (LVH) and
81 diastolic dysfunction and has an estimated prevalence of 1:500 in the general population.[16]
82 The *MYBPC3* gene, encoding cardiac myosin-binding protein-C (cMyBP-C), is frequently
83 mutated in HCM, representing 40-50% of all HCM mutations.[17, 18] cMyBP-C interacts with
84 myosin, titin and actin and plays an important role in cardiac contraction and relaxation.[17, 19-21]
85 We previously reported impairment of the UPS and elevated protein levels of autophagic
86 markers such as sequestosome-1 protein (p62), a marker for ubiquitinated protein aggregates,
87 and microtubule-associated protein 1 light chain 3 (LC3)-II, an indicator of autophagosome
88 number, in 60-week-old *Mybpc3*-targeted knock-in (KI) mice that develop LVH and cardiac
89 dysfunction.[22-26] These mice carry at the homozygous state the human c.772G>A *MYBPC3*

90 transition that results in a low level of mutant protein. In the present study, we investigated
91 whether autophagy is altered in HCM patients and KI mice and whether activation of
92 autophagy could ameliorate cardiomyopathy in KI mice.
93
94
95 **Methods**

96 *Expanded Methods are available in the Data supplements.*
97
98 ***Human samples***
99 Human samples were obtained from septal myectomies of HCM patients carrying *MYBPC3*
100 mutations, from non-failing human heart tissue not suitable for transplantation or from donors
101 that did not die from cardiac disease but of another cause (=non-failing, NF).[27] All materials
102 from patients and donors were taken with informed consent of the donors and with approval
103 of the local ethical boards and according to the Declaration of Helsinki.
104
105 ***Animals***
106 The investigation conformed to the guide for the care and use of laboratory animals published
107 by the NIH (Publication No. 85-23, revised 2011, published by the National Research
108 Council). The experimental procedures were in accordance with the German Law for the
109 Protection of Animals and approved by the Authority for Health and Consumer Protection of
110 the City State of Hamburg, Germany (no. 118/13 and 100/14).
111
112 ***Echocardiography***
113 Transthoracic echocardiography was performed using the Vevo 2100 System (VisualSonics,
114 Toronto, Canada) as described previously.[25]
115
116 ***Autophagic flux measurement***
117 To measure the autophagic flux *in vivo*, mice were injected i.p. with 40 mg/kg leupeptin
118 (Sigma Aldrich, L-8511) or sodium chloride (500 µL) as described previously.[28]
119
120 ***Experimental diet***
121 Eleven-week-old KI and WT mice were kept on caloric restriction, rapamycin or control diet
122 for 9 weeks. All diets were based on LabDiet 5LG6 (TestDiet) including Eudragit S100
123 (rapamycin coating material). Mice kept on caloric restriction were fed ~20% less in the first

124 week and then ~40% less for the following 8 weeks than control mice. It was assumed that a
125 30 g mouse eats about 5 g per day. Mice were kept under tight observation and were regularly
126 weighed. Mice on rapamycin diet received ~2.24 mg/kg rapamycin (Rapamycin Holdings$^{TM}$)
127 encapsulated in Eudragit S100 daily. Echocardiography was performed at the beginning and
128 at the end of the experiment. The autophagic flux was measured at the end of the experiment.
129

130 ***Electron Microscopy***

131 Mouse hearts were initially fixed by perfusion with 1% paraformaldehyde/2% (vol/vol)
132 glutaraldehyde in cardioplegic solution (50 mmol/L KCl, 5% dextrose in PBS) and next in 1%
133 paraformaldehyde/2% (vol/vol) glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.2. The
134 heart was removed and immersed into the latter fixative (ice cold) and then left and right
135 ventricular free walls and septa were isolated. Each region was divided into small fragments
136 and fixed further in the same fixative at 4 °C, then postfixed/stained in 1% $OsO_4$ (in water)
137 before dehydration in acetone and embedding in epoxy resin. Ultrathin sections were
138 counterstained with uranium and lead salts. Images were acquired on a Hitachi 7600 electron
139 microscope equipped with an AMT digital camera.
140

141 ***Statistical analysis***

142 Data were expressed as mean ± s.e.m. Statistical analyses were performed by one-way
143 ANOVA plus Tukey's or Dunnett's post-test, or by unpaired Student's t-test with GraphPad-
144 Prism7 or by Welch's ANOVA plus Tukey's post-test with R v3.4.0, as indicated in the figure
145 legends. A value of $P < 0.05$ was considered statistically significant.
146

147 **Results**

148

149 ***Autophagy is altered in HCM patients and KI mice***

150 We evaluated p62, beclin-1 and LC3 protein levels in myectomy samples from patients
151 carrying *MYBPC3* mutations (Table S1). Whereas p62, beclin-1 and LC3-I levels did not
152 differ between HCM and NF samples (Figure 1A to 1C), LC3-II protein levels were 2.6-fold
153 higher in HCM (Figure 1A and 1E). We next quantified the expression of a customized panel
154 of human genes regulated in heart failure, arrhythmias and autophagy in cardiac RNA pools
155 of HCM and NF individuals. In addition to the commonly dysregulated genes in HCM, such
156 as markers of hypertrophy or fibrosis and calcium/potassium handling proteins, the expression
157 of several genes regulating autophagy was also altered in HCM, some being up-regulated

(*BCL2, BECN1, CHMP2B, EPG5, FYCO1, HDAC6, LAMP1, MTOR, NBR1, SQSTM1 and TFEB*), others down-regulated (*BAG3, MAP1LC3B,*) when compared to NF (Figure 1F, Table S2).

We then evaluated autophagic markers in 10-week-old and 60-week-old mice to explore changes during disease progression. We confirmed previously described higher p62 (non-significant) and LC3-II protein levels in 60-week-old,[23] but not in 10-week-old KI mice (Figure 2A to 2C), suggesting that accumulation of p62 and LC3-II protein occurs late in the disease progression. Of note, however, LC3-I level was already higher in 10-week-old KI than WT mice (Figure 2A and 2C). We then quantified the expression of a customized panel of mouse genes associated with heart failure, arrhythmias and autophagy in ventricular RNA pools from 60-week-old KI and WT mice. KI exhibited dysregulated expression of proteins regulating hypertrophy, fibrosis, calcium handling, cardiac action potential, and autophagy (*Bag3, Bcl2, Becn1, Epg5, Erbb2, Fyco1, Hdac6, Nrg1, Rab7 and Sqstm1*; Figure 2D, Table S3).

Both human and mouse data suggest that autophagy is altered in HCM caused by *MYBPC3* mutations.

***Autophagic flux is impaired in KI mice***

Measurement of basal levels of autophagic markers is not sufficient to conclude if autophagy is activated or impaired.[29] Therefore, we next determined autophagic flux (macroautophagic activity) by evaluating LC3 turnover after injecting i.p. 40 mg/kg of the lysosomal protease inhibitor leupeptin in mice for 1 h. In hearts of 10-week-old mice, leupeptin treatment did not have any effect on the LC3-II level (Figure 3A and 3B), although the treatment worked in liver in both KI and WT mice (Figure S1). However, the LC3-II/LC3-I ratio was higher in WT than KI mice (Figure 3B). In hearts of 60-week-old mice, both LC3-II levels and the LC3-II/LC3-I ratio were markedly higher in leupeptin-treated than non-treated WT, whereas they did not differ between leupeptin-treated and non-treated KI mice (Figure 3A and 3B). This finding suggests an increased demand in autophagic activity in WT mice with aging, whereas the LC3 turnover was blunted in KI mice.

To examine whether the impaired autophagic flux in KI mice was due to the presence of mutant cMyBP-C or low level of cMyBP-C, we measured autophagic flux in 60-week-old *Mybpc3*-targeted knock-out (KO) mice that do not express any cMyBP-C but develop a similar cardiac disease phenotype as KI mice.[30] LC3 turnover was blunted in KO mice to the same extent as in KI mice (Figure 3C and 3D). Although we cannot provide direct causality,

192 these data suggest that low level of cMyBP-C rather than mutant cMyBP-C, in combination
193 with pathological remodeling, induce impairment of autophagic flux.
194

195 ***Residual bodies and glycogen accumulate in KI mice***

196 To assess autophagy-related ultrastructural differences between KI and WT mice, we
197 analyzed osmium-stained cryosections from 60-week-old KI and WT mice using electron
198 microscopy (Figure 4A). Terminal autolysosomes (residual bodies) containing cellular waste
199 that was not broken down completely, probably resulting in lipofuscin or similar, markedly
200 accumulated in KI compared to WT mice (Figure 4A, Figure S2). Lipofuscin vesicles usually
201 accumulate with age, indicating an increase in cellular waste and/or deficiency in cellular
202 waste degradation.[31] Furthermore, we observed an accumulation of glycogen granula in KI
203 mice (Figure 4B, Figure S3). Glycogen is degraded by the autophagic pathway, and an
204 accumulation of glycogen granula is thought to be associated with impaired autophagy.[32]
205

206 ***Lysosomes are functional in KI mice***

207 To test if the autophagy impairment is induced by a decrease in number or compromised
208 function of lysosomes, we assessed protein levels and activity of the lysosomal protease
209 cathepsin D and protein levels of the lysosome-associated membrane protein 2 (LAMP-2). No
210 differences in the levels of the different cathepsin D forms were detected between 60-week-
211 old KI and WT mice (Figure S4A and S4B). Consistent with these data, the cathepsin D
212 activity did also not differ between KI and WT (Figure S4C). Protein levels of LAMP-2 were
213 unaltered in the KI mice as well (Figure S4D and S4E). These findings suggest that lysosomal
214 degradation is not affected in KI mice.
215

216 ***Akt-mTORC1 signaling is increased in KI mice***

217 Mammalian target of rapamycin complex 1 (mTORC1) is a key negative regulator of
218 autophagy and a recognized positive regulator of hypertrophy.[33] Hence, we evaluated
219 mTORC1 signaling in 60-week-old KI and WT mice (Figure 5). Levels of phosphorylated
220 proteins (=activation) of mTOR (p-mTOR) and eukaryotic translation initiation factor 4E-
221 binding protein 1 (p-4E-BP1), but not of ribosomal protein S6 (p-S6) were higher in KI than
222 WT (Figure 5A to 5D). Levels of total mTOR and S6 (S6), but not of total 4E-BP1 were
223 higher in KI than WT. Despite no difference in the ratio of phosphorylated-to-total proteins
224 between the groups, increased p-mTOR and p-4E-BP1 levels suggest, at least in part, an
225 increased mTORC1 signaling in KI mice. This increase in mTORC1 signaling was even more

226 pronounced in KO (Figure S5) than in KI mice. The protein levels of Atg5 and Atg7, crucial
227 autophagy enhancers, did not differ between KI, KO and WT mice (Figure S6).
228 We then evaluated which upstream pathways increased mTORC1 activity in KI mice
229 (Figure 5E, Figure S7). Dual phosphorylation (=activation) of serine threonine kinase
230 Akt/protein kinase B, evaluated by $Akt^{Thr308}$/Akt and $Akt^{Ser473}$/Akt ratios, was higher in KI
231 than in WT mice (Figure 5E), whereas p-AMPK/AMPK, p-GSK3β, p-Erk1/2/Erk1/2 and p-
232 p38/p38 ratios did not differ between KI and WT mice (Figure S7). These data suggest that
233 activated mTORC1 results from activation of Akt signaling.
234

235 ***Rapamycin treatment or caloric restriction partially rescues cardiomyopathy in KI mice***

236 To activate autophagy in KI mice, we used rapamycin, an inhibitor of mTORC1, and caloric
237 restriction (CR), which also decreases mTORC1 activity.[34, 35] We evaluated whether these
238 treatments could ameliorate cardiomyopathy in KI mice. Eleven-week-old KI and WT mice
239 were subjected to a 9-week treatment with either 2.24 mg/kgxd rapamycin or 40% CR. At the
240 beginning of the experiment, fractional area shortening (FAS) was lower and left ventricular
241 mass-to-body weight ratio (LVM/BW) was higher in KI than WT mice, whereas BW did not
242 differ between KI and WT, indicating systolic dysfunction and LVH (Figure 6A and 6B,
243 Figure S8). At the end of the treatment, FAS did not significantly differ between rapamycin-
244 treated and untreated WT and KI mice, whereas it was higher in CR-treated than untreated KI
245 and WT mice (Figure 6A; Tables S4 and S5). The FAS difference of 10% between CR-treated
246 and untreated KI mice was significant, suggesting partial amelioration of cardiac function in
247 KI mice. As expected, BW was markedly lower in CR-treated than in untreated KI and WT
248 mice, but was not affected by rapamycin treatment (Figure 6B). Heart weight-to-tibia length
249 ratio (HW/TL) was ~30% higher in untreated KI than in untreated WT mice and ~24% lower
250 in CR-treated KI than in untreated KI mice (Figure 6C), whereas TL did not differ between
251 groups (Figure 6E). Lung weight-to-tibia length ratio (LW/TL) was higher in untreated KI
252 than in untreated WT, indicating pulmonary edema induced by heart failure (Figure 6D). Both
253 rapamycin and CR treatments lowered LW/BW in KI, which did not differ from WT in these
254 conditions (Figure 6D), suggesting regression of heart failure in KI mice.
255 Both treatments normalized the higher *Bcl2* mRNA levels and lower *Kcnj2* mRNA
256 levels in KI mice towards WT levels (Figure 7A, Table S6). In addition, rapamycin partially
257 normalized the levels of markers of hypertrophy/heart failure (*Atp2b4, Myh7, Nppa*), while
258 CR reversed the altered gene expression of hypertrophy and fibrosis markers (*Meox1, Col1a1,*
259 *Postn*), the calcium handling protein *Cacna1g* and autophagy regulating genes (*Map1lc3b,*

260 *Nrg1 and Rab7;* Figure 7A, Table S6). Furthermore, both treatments increased the LC3-II
261 levels in both KI and WT mice, indicating activation of autophagy (Figure 7B and 7C).
262 Finally, LC3-II levels increased after leupeptin in untreated WT, but not in KI mice,
263 suggesting blunted LC3 turnover in KI mice (Figure 7B and 7C). The autophagic flux was
264 restored in rapamycin-and CR-treated KI mice.
265
266
267 **Discussion**
268
269 In this study, we investigated autophagy in cardiomyopathy associated with *MYBPC3*
270 mutations in human HCM septal myectomies and in a *Mybpc3*-targeted KI mouse model. Our
271 major findings were: (1) autophagy is altered in *MYBPC3* mutation-carrying HCM patients
272 (2) autophagy is impaired in KI mice and (3) activation of autophagy by rapamycin or CR
273 ameliorates cardiomyopathy and autophagic flux in KI mice.
274 LC3-II protein levels were higher in septal myectomies from HCM patients with
275 *MYBPC3* mutations, indicating an alteration of autophagy. Although we cannot conclude
276 whether there is activation or inhibition of autophagy in patients, data obtained in KI mice
277 argue for autophagy impairment. This was associated with dysregulated gene expression of
278 several proteins regulating autophagy in both HCM patients and KI mice. LC3-II
279 accumulation in the KI mouse hearts was progressive and accompanied by autophagic flux
280 impairment with age. Furthermore, residual bodies and glycogen, which are both degraded by
281 autophagy,[31, 32] accumulated in KI mice. Glycogen accumulation was associated with LVH
282 and was found in a number of diseases involving defective autophagy, e.g. Pompe, Danon and
283 Fabry disease.[36] In contrast to the UPS impairment, which was found only in aged KI mice
284 with markedly low amounts of mutant cMyBP-C,[23] autophagy impairment was common in
285 both KI and KO mice, suggesting that cMyBP-C haploinsufficiency alone or in combination
286 with cardiomyopathy is a trigger.
287 The involvement of autophagy in HCM patients and animal models with mutations in
288 sarcomeric proteins has not been studied in depth. Only a few inherited cardiomyopathies are
289 known to be associated with a defect in autophagy. Deficiency of the principal lysosomal
290 membrane protein LAMP-2 causes Danon disease involving severe HCM.[4, 5] LEOPARD
291 syndrome, caused by mutations in *PTPN11* (protein tyrosine phosphatase, non-receptor type
292 11) leads to increased phosphatidylinositol 3-kinase (PI3K) signaling associated with reduced
293 autophagy and HCM.[6] *Lamp2*-deficient mice (Danon disease) showed accumulation of

294 autophagic vesicles,[37] while *PTPN11*-targeted knock-in mice (LEOPARD syndrome)[6] and
295 *PTEN*-targeted knock-out[38] showed increased mTORC1 signaling and decreased autophagic
296 flux, all suggesting autophagy impairment. Vici syndrome, a rare autosomal-recessive
297 inherited multisystem disorder involving cardiomyopathy[7] is caused by mutations in *EPG5*,
298 which encodes the ectopic P-granules autophagy protein 5, an essential protein for autophagic
299 degradation.[39] Defective autophagy has been also reported in desmin-related cardiomyopathy
300 caused by αB-crystallin or desmin mutations and associated with the accumulation of
301 cytotoxic misfolded proteins,[9] in DCM caused by *LMNA* mutations[13, 14] or mutations in
302 *BAG3*,[40, 41] encoding human BCL2 associated athanogene 3 gene involved in selective
303 macroautophagy.[42] LVNC caused by a *PLEKHM2* mutation was associated with a defective
304 ALP and impairment of autophagic flux in patients' fibroblasts.[15]
305 In the present study, mTORC1 signaling was elevated in KI and KO mice. mTORC1
306 negatively regulates autophagy initiation, but can also inhibit autophagosome-lysosome
307 fusion.[43-45] In addition, mTORC1 negatively regulates transcription factor EB and thus
308 inhibits transcription of autophagy and lysosomal genes.[46] Out of the several signaling
309 pathways that are known to activate mTOR, we found that Akt/PKB signaling was increased
310 and likely contributed to mTORC1 activation in KI mice. This was associated with the up-
311 regulation of *Ctgf*, encoding the connective tissue growth factor, which induced
312 cardiomyocyte hypertrophy via Akt signaling,[47] and *Bcl2*, encoding B-cell CLL/lymphoma 2
313 (BCL2), which can be up-regulated via Akt signaling (Figure 2D).[48] Accumulation of BCL2
314 can sequester Beclin-1 or inhibit Bax/Bak-mediated apoptosis and thus inhibits autophagy.[49,
315 50] Similarly, *BCL2*, *CTGF* and *MTOR* genes were up-regulated in HCM (Figure 1F),
316 suggesting activated mTORC1 in HCM.
317 Treatment with rapamycin or caloric restriction to inhibit mTORC1 activity, and
318 thereby activate autophagy partially rescued the cardiomyopathy phenotype or heart failure
319 and restored the autophagic flux in KI mice. The mechanism of action is not fully certain and
320 rapamycin and CR may affect other cellular functions besides autophagy. However, our
321 customized transcriptome analysis indicates normalization of expression of markers of
322 hypertrophy, fibrosis and also autophagy regulating genes. Specifically, the expression levels
323 of both *Bcl2* and *Kcnj2*, encoding the potassium inwardly-rectifying channel, subfamily J,
324 member 2 (Kir2.1), were both markedly dysregulated in KI mice, and were partially
325 normalized toward levels found in WT mice with either treatment. Our data are in agreement
326 with previous findings showing that rapamycin administration to mice with *PTPN11*
327 mutation, which resulted in activation of PI3K pathway and increased mTORC1 activity,

328 ameliorated cardiomyopathy.[6] Similarly, rapamycin treatment reversed hypertrophy in a
329 PTEN-deficient mouse model with increased mTORC1 activity.[38] Moreover, rapamycin
330 treatment or CR has been shown to have positive effects in different models of pressure
331 overload-induced hypertrophy and age-related hypertrophy.[6, 38]
332 Up to now, there are only general treatments like calcium channel- and β-blockers,
333 septal myectomy, ethanol ablation and heart transplantation available for human HCM. Here,
334 we provide evidence that autophagy is defective in HCM patients and mice carrying *MYBPC3*
335 mutations and that activation of autophagy ameliorates cardiomyopathy in mice. We therefore
336 propose that activation of autophagy might be an attractive option alone or in combination
337 with another approach to rescue HCM induced by *MYBPC3* mutations.
338
339
340 **Acknowledgments**
341
342 We thank Julia Münch and Monica Patten (University Heart Center Hamburg, Hamburg,
343 Germany) for patients' recruitment, Giulia Mearini, Frederik Flenner and Felix Friedrich
344 (UKE-Pharmacology, Hamburg, Germany) for help in preservation of human septal
345 myectomies and database maintenance, Jutta Starbatty (UKE-Pharmacology, Hamburg,
346 Germany) for protein preparations, and Konstantina Stathopoulou and Frederik Flenner
347 (UKE-Pharmacology, Hamburg, Germany) for selection/validation of NanoString probe
348 sequences.
349
350
351 **Funding**
352
353 This work was supported by the Fondation Leducq (Research grant Nr. 11, CVD 04), the
354 DZHK (German Centre for Cardiovascular Research; Grants B12-011 and B14-016), the
355 German Ministry of Research Education (BMBF), the seventh Framework Program of the
356 European Union (Health-F2-2009-241577-Big-Heart project), and the Netherlands
357 Cardiovascular Research Initiative CVON2014-40 DOSIS, an initiative with support of the
358 Dutch Heart Foundation.
359
360
361

362 **Disclosure of Potential Conflicts of Interest**

363

364 No potential conflicts of interest were disclosed.

365

366

367 **References**

368

369 1. Rubinsztein DC. The roles of intracellular protein-degradation pathways in
370 neurodegeneration. *Nature*. 2006;443:780-6.
371 2. Ravikumar B, Sarkar S, Davies JE, Futter M, Garcia-Arencibia M, Green-Thompson
372 ZW, Jimenez-Sanchez M, Korolchuk VI, Lichtenberg M, Luo S, Massey DC, Menzies FM,
373 Moreau K, Narayanan U, Renna M, Siddiqi FH, Underwood BR, Winslow AR and
374 Rubinsztein DC. Regulation of mammalian autophagy in physiology and pathophysiology.
375 *Physiol Rev*. 2010;90:1383-435.
376 3. Menzies FM, Fleming A and Rubinsztein DC. Compromised autophagy and
377 neurodegenerative diseases. *Nat Rev Neurosci*. 2015;16:345-57.
378 4. Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, Koori T, Mora M, Riggs JE, Oh SJ,
379 Koga Y, Sue CM, Yamamoto A, Murakami N, Shanske S, Byrne E, Bonilla E, Nonaka I,
380 DiMauro S and Hirano M. Primary LAMP-2 deficiency causes X-linked vacuolar
381 cardiomyopathy and myopathy (Danon disease). *Nature*. 2000;406:906-10.
382 5. Danon MJ, Oh SJ, DiMauro S, Manaligod JR, Eastwood A, Naidu S and Schliselfeld
383 LH. Lysosomal glycogen storage disease with normal acid maltase. *Neurology*. 1981;31:51-7.
384 6. Marin TM, Keith K, Davies B, Conner DA, Guha P, Kalaitzidis D, Wu X, Lauriol J,
385 Wang B, Bauer M, Bronson R, Franchini KG, Neel BG and Kontaridis MI. Rapamycin
386 reverses hypertrophic cardiomyopathy in a mouse model of LEOPARD syndrome-associated
387 PTPN11 mutation. *J Clin Invest*. 2011;121:1026-43.
388 7. del Campo M, Hall BD, Aeby A, Nassogne MC, Verloes A, Roche C, Gonzalez C,
389 Sanchez H, Garcia-Alix A, Cabanas F, Escudero RM, Hernandez R and Quero J. Albinism
390 and agenesis of the corpus callosum with profound developmental delay: Vici syndrome,
391 evidence for autosomal recessive inheritance. *Am J Med Genet*. 1999;85:479-85.
392 8. Cullup T, Dionisi-Vici C, Kho AL, Yau S, Mohammed S, Gautel M and Jungbluth H.
393 Clinical utility gene card for: Vici Syndrome. *Eur J Hum Genet*. 2014;22.
394 9. McLendon PM and Robbins J. Desmin-related cardiomyopathy: an unfolding story.
395 *Am J Physiol Heart Circ Physiol*. 2011;301:H1220-8.

396 10. Tannous P, Zhu H, Johnstone JL, Shelton JM, Rajasekaran NS, Benjamin IJ, Nguyen
397 L, Gerard RD, Levine B, Rothermel BA and Hill JA. Autophagy is an adaptive response in
398 desmin-related cardiomyopathy. *Proc Natl Acad Sci U S A*. 2008;105:9745-50.
399 11. Sanbe A, Osinska H, Saffitz JE, Glabe CG, Kayed R, Maloyan A and Robbins J.
400 Desmin-related cardiomyopathy in transgenic mice: a cardiac amyloidosis. *Proc Natl Acad*
401 *Sci U S A*. 2004;101:10132-6.
402 12. Delbridge LM, Mellor KM, Taylor DJ and Gottlieb RA. Myocardial autophagic
403 energy stress responses--macroautophagy, mitophagy, and glycophagy. *Am J Physiol Heart*
404 *Circ Physiol*. 2015;308:H1194-204.
405 13. Choi JC, Muchir A, Wu W, Iwata S, Homma S, Morrow JP and Worman HJ.
406 Temsirolimus activates autophagy and ameliorates cardiomyopathy caused by lamin A/C
407 gene mutation. *Sci Transl Med*. 2012;4:144ra102.
408 14. Ramos FJ, Chen SC, Garelick MG, Dai DF, Liao CY, Schreiber KH, MacKay VL, An
409 EH, Strong R, Ladiges WC, Rabinovitch PS, Kaeberlein M and Kennedy BK. Rapamycin
410 reverses elevated mTORC1 signaling in lamin A/C-deficient mice, rescues cardiac and
411 skeletal muscle function, and extends survival. *Sci Transl Med*. 2012;4:144ra103.
412 15. Muhammad E, Levitas A, Singh SR, Braiman A, Ofir R, Etzion S, Sheffield VC,
413 Etzion Y, Carrier L and Parvari R. PLEKHM2 mutation leads to abnormal localization of
414 lysosomes, impaired autophagy flux and associates with recessive dilated cardiomyopathy and
415 left ventricular noncompaction. *Hum Mol Genet*. 2015;24:7227-40.
416 16. Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT and Bild DE. Prevalence
417 of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic
418 analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in
419 (Young) Adults. *Circulation*. 1995;92:785-9.
420 17. Carrier L, Mearini G, Stathopoulou K and Cuello F. Cardiac myosin-binding protein C
421 (MYBPC3) in cardiac pathophysiology. *Gene*. 2015;573:188-97.
422 18. Behrens-Gawlik V, Mearini G, Gedicke-Hornung C, Richard P and Carrier L.
423 MYBPC3 in hypertrophic cardiomyopathy: from mutation identification to RNA-based
424 correction. *Pflugers Arch*. 2014;466:215-23.
425 19. Cazorla O, Szilagyi S, Vignier N, Salazar G, Kramer E, Vassort G, Carrier L and
426 Lacampagne A. Length and protein kinase A modulations of myocytes in cardiac myosin
427 binding protein C-deficient mice. *Cardiovasc Res*. 2006;69:370-80.

428 20. Pohlmann L, Kroger I, Vignier N, Schlossarek S, Kramer E, Coirault C, Sultan KR,
429 El-Armouche A, Winegrad S, Eschenhagen T and Carrier L. Cardiac myosin-binding protein
430 C is required for complete relaxation in intact myocytes. *Circ Res*. 2007;101:928-38.
431 21. Moss RL, Fitzsimons DP and Ralphe JC. Cardiac MyBP-C regulates the rate and force
432 of contraction in mammalian myocardium. *Circ Res*. 2015;116:183-92.
433 22. Vignier N, Schlossarek S, Fraysse B, Mearini G, Kramer E, Pointu H, Mougenot N,
434 Guiard J, Reimer R, Hohenberg H, Schwartz K, Vernet M, Eschenhagen T and Carrier L.
435 Nonsense-mediated mRNA decay and ubiquitin-proteasome system regulate cardiac myosin-
436 binding protein C mutant levels in cardiomyopathic mice. *Circ Res*. 2009;105:239-48.
437 23. Schlossarek S, Englmann DR, Sultan KR, Sauer M, Eschenhagen T and Carrier L.
438 Defective proteolytic systems in Mybpc3-targeted mice with cardiac hypertrophy. *Basic Res*
439 *Cardiol*. 2012;107:235.
440 24. Gedicke-Hornung C, Behrens-Gawlik V, Reischmann S, Geertz B, Stimpel D,
441 Weinberger F, Schlossarek S, Precigout G, Braren I, Eschenhagen T, Mearini G, Lorain S,
442 Voit T, Dreyfus PA, Garcia L and Carrier L. Rescue of cardiomyopathy through U7snRNA-
443 mediated exon skipping in Mybpc3-targeted knock-in mice. *EMBO Mol Med*. 2013;5:1060-
444 77.
445 25. Mearini G, Stimpel D, Geertz B, Weinberger F, Kramer E, Schlossarek S, Mourot-
446 Filiatre J, Stoehr A, Dutsch A, Wijnker PJ, Braren I, Katus HA, Muller OJ, Voit T,
447 Eschenhagen T and Carrier L. Mybpc3 gene therapy for neonatal cardiomyopathy enables
448 long-term disease prevention in mice. *Nat Commun*. 2014;5:5515.
449 26. Mearini G, Stimpel D, Kramer E, Geertz B, Braren I, Gedicke-Hornung C, Precigout
450 G, Muller OJ, Katus HA, Eschenhagen T, Voit T, Garcia L, Lorain S and Carrier L. Repair of
451 Mybpc3 mRNA by 5'-trans-splicing in a Mouse Model of Hypertrophic Cardiomyopathy. *Mol*
452 *Ther Nucleic Acids*. 2013;2:e102.
453 27. Thottakara T, Friedrich FW, Reischmann S, Braumann S, Schlossarek S, Kramer E,
454 Juhr D, Schluter H, van der Velden J, Munch J, Patten M, Eschenhagen T, Moog-Lutz C and
455 Carrier L. The E3 ubiquitin ligase Asb2beta is downregulated in a mouse model of
456 hypertrophic cardiomyopathy and targets desmin for proteasomal degradation. *J Mol Cell*
457 *Cardiol*. 2015;87:214-224.
458 28. Haspel J, Shaik RS, Ifedigbo E, Nakahira K, Dolinay T, Englert JA and Choi AM.
459 Characterization of macroautophagic flux in vivo using a leupeptin-based assay. *Autophagy*.
460 2011;7:629-42.

461 29. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena
462 A, Adachi H, Adams CM, Adams PD, Adeli K, Adhihetty PJ, Adler SG, Agam G, Agarwal
463 R, Aghi MK, Agnello M, Agostinis P, Aguilar PV, Aguirre-Ghiso J, Airoldi EM, et al.
464 Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition).
465 *Autophagy*. 2016;12:1-222.
466 30. Carrier L, Knoll R, Vignier N, Keller DI, Bausero P, Prudhon B, Isnard R, Ambroisine
467 ML, Fiszman M, Ross J, Jr., Schwartz K and Chien KR. Asymmetric septal hypertrophy in
468 heterozygous cMyBP-C null mice. *Cardiovasc Res*. 2004;63:293-304.
469 31. Brunk UT and Terman A. Lipofuscin: mechanisms of age-related accumulation and
470 influence on cell function. *Free Radic Biol Med*. 2002;33:611-9.
471 32. Kotoulas OB, Kalamidas SA and Kondomerkos DJ. Glycogen autophagy. *Microsc Res*
472 *Tech*. 2004;64:10-20.
473 33. Sciarretta S, Volpe M and Sadoshima J. Mammalian target of rapamycin signaling in
474 cardiac physiology and disease. *Circ Res*. 2014;114:549-64.
475 34. Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS and Schreiber SL.
476 A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature*.
477 1994;369:756-8.
478 35. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, Iemura S, Natsume
479 T, Takehana K, Yamada N, Guan JL, Oshiro N and Mizushima N. Nutrient-dependent
480 mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol*
481 *Biol Cell*. 2009;20:1981-91.
482 36. Arad M, Maron BJ, Gorham JM, Johnson WH, Jr., Saul JP, Perez-Atayde AR, Spirito
483 P, Wright GB, Kanter RJ, Seidman CE and Seidman JG. Glycogen storage diseases
484 presenting as hypertrophic cardiomyopathy. *N Engl J Med*. 2005;352:362-72.
485 37. Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lullmann-Rauch R, Janssen
486 PM, Blanz J, von Figura K and Saftig P. Accumulation of autophagic vacuoles and
487 cardiomyopathy in LAMP-2-deficient mice. *Nature*. 2000;406:902-6.
488 38. Xu X, Roe ND, Weiser-Evans MC and Ren J. Inhibition of mammalian target of
489 rapamycin with rapamycin reverses hypertrophic cardiomyopathy in mice with
490 cardiomyocyte-specific knockout of PTEN. *Hypertension*. 2014;63:729-39.
491 39. Cullup T, Kho AL, Dionisi-Vici C, Brandmeier B, Smith F, Urry Z, Simpson MA,
492 Yau S, Bertini E, McClelland V, Al-Owain M, Koelker S, Koerner C, Hoffmann GF, Wijburg
493 FA, ten Hoedt AE, Rogers RC, Manchester D, Miyata R, Hayashi M, et al. Recessive

494 mutations in EPG5 cause Vici syndrome, a multisystem disorder with defective autophagy.
495 *Nat Genet*. 2013;45:83-7.
496 40. Villard E, Perret C, Gary F, Proust C, Dilanian G, Hengstenberg C, Ruppert V,
497 Arbustini E, Wichter T, Germain M, Dubourg O, Tavazzi L, Aumont MC, DeGroote P,
498 Fauchier L, Trochu JN, Gibelin P, Aupetit JF, Stark K, Erdmann J, et al. A genome-wide
499 association study identifies two loci associated with heart failure due to dilated
500 cardiomyopathy. *Eur Heart J*. 2011;32:1065-76.
501 41. Norton N, Li D, Rieder MJ, Siegfried JD, Rampersaud E, Zuchner S, Mangos S,
502 Gonzalez-Quintana J, Wang L, McGee S, Reiser J, Martin E, Nickerson DA and Hershberger
503 RE. Genome-wide studies of copy number variation and exome sequencing identify rare
504 variants in BAG3 as a cause of dilated cardiomyopathy. *Am J Hum Genet*. 2011;88:273-82.
505 42. Gamerdinger M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU and Behl C. Protein
506 quality control during aging involves recruitment of the macroautophagy pathway by BAG3.
507 *EMBO J*. 2009;28:889-901.
508 43. Kim YC and Guan KL. mTOR: a pharmacologic target for autophagy regulation. *J*
509 *Clin Invest*. 2015;125:25-32.
510 44. Yu L, McPhee CK, Zheng L, Mardones GA, Rong Y, Peng J, Mi N, Zhao Y, Liu Z,
511 Wan F, Hailey DW, Oorschot V, Klumperman J, Baehrecke EH and Lenardo MJ.
512 Termination of autophagy and reformation of lysosomes regulated by mTOR. *Nature*.
513 2010;465:942-6.
514 45. Puertollano R. mTOR and lysosome regulation. *F1000Prime Rep*. 2014;6:52.
515 46. Settembre C, Zoncu R, Medina DL, Vetrini F, Erdin S, Erdin S, Huynh T, Ferron M,
516 Karsenty G, Vellard MC, Facchinetti V, Sabatini DM and Ballabio A. A lysosome-to-nucleus
517 signalling mechanism senses and regulates the lysosome via mTOR and TFEB. *EMBO J*.
518 2012;31:1095-108.
519 47. Hayata N, Fujio Y, Yamamoto Y, Iwakura T, Obana M, Takai M, Mohri T, Nonen S,
520 Maeda M and Azuma J. Connective tissue growth factor induces cardiac hypertrophy through
521 Akt signaling. *Biochem Biophys Res Commun*. 2008;370:274-8.
522 48. Pugazhenthi S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, Heasley LE and
523 Reusch JE. Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response
524 element-binding protein. *J Biol Chem*. 2000;275:10761-6.
525 49. Lindqvist LM, Heinlein M, Huang DC and Vaux DL. Prosurvival Bcl-2 family
526 members affect autophagy only indirectly, by inhibiting Bax and Bak. *Proc Natl Acad Sci U S*
527 *A*. 2014;111:8512-7.

528 50. Zou H, Lai Y, Zhao X, Yan G, Ma D, Cardenes N, Shiva S, Liu Y, Bai X, Jiang Y and
529 Jiang Y. Regulation of mammalian target of rapamycin complex 1 by Bcl-2 and Bcl-XL
530 proteins. *J Biol Chem*. 2013;288:28824-30.
531

## Figure legends

**Figure 1. Dysregulation of autophagy in HCM patients with *MYBPC3* mutations.** Myectomy samples of HCM patients or heart samples from non-failing (NF) individuals were analyzed. **A**, Representative Western blots of p62, beclin-1 and LC3. Ponceau was used as loading control. Quantification of **B**, p62, **C**, beclin-1, **D**, LC3-I and **E**, LC3-II. Data are expressed as mean + s.e.m with *P<0.05 vs. NF, unpaired Student's t-test. Number of individuals is indicated in the bars. **F**, Heatmap of selected genes comparing gene expression of proteins modulating hypertrophy, fibrosis, calcium handling, autophagy and potassium handling in NF and HCM (threshold <0.8- or >1.2-fold change to NF).

**Figure 2. Dysregulation of autophagy in KI mice.** Protein levels of p62 and LC3 in 10- and 60-week-old KI and WT mouse hearts. **A**, Representative Western blots of indicated proteins from mouse ventricular protein extracts (membrane-enriched fraction) of indicated ages. Calsequestrin and Ponceau were used as loading controls. Quantification of **B**, p62 and **C**, LC3-I and LC3-II protein levels normalized to Ponceau and related to WT. Data are expressed as mean + s.e.m. with *P<0.05 and **P<0.01 vs. WT, unpaired Student's *t*-test (Welch's test). Number of animals is indicated in the bars. **D**, Heatmap of selected genes (threshold <0.8 or >1.2 fold change to WT) comparing gene expression of hypertrophy, fibrosis, calcium handling, autophagy and potassium and sodium regulation between WT and KI mice.

**Figure 3. Impaired autophagic flux in KI and KO mice.** Evaluation of the autophagic flux in hearts of KI and WT mice. Either 40 mg/kg leupeptin (inh., inhibitor) or sodium chloride was injected i.p. into mice. After 1 h, hearts were extracted. **A**, Representative Western blots of indicated proteins from ventricular protein extracts of KI and WT mice of indicated ages. Calsequestrin and Ponceau were used as loading controls. **B**, Quantification of LC3-II (normalized to calsequestrin) and LC3-II/LC3-I ratio of KI and WT mice of indicated ages. **C**, Evaluation of the autophagic flux in hearts of KO and WT mice. Representative Western blots of indicated proteins from ventricular protein extracts of 60-week-old KO and WT mice. Ponceau and Erk1/2 were used as loading controls. **D**, Quantification of LC3-II (normalized to Erk1/2) of 60-week-old KO and WT mice. Quantifications are related to WT control. Data are expressed as mean + s.e.m. with *P<0.05, **P<0.01 and ***P<0.001 vs. corresponding control, one-way ANOVA (Welch's test) plus Tukey's post-test. Number of animals is indicated in the bars.

566

567 **Figure 4. Accumulation of residual bodies and glycogen granula in KI mice.** Electron
568 microscope images of (osmium-stained) left ventricular tissues of 60-week-old WT and KI
569 mice. Electron-dense structures like lipids stain dark. **A**, Residual bodies (black vesicular
570 structures). **B**, Glycogen granula (indicated by arrows).

571

572 **Figure 5. Increased Akt-mTORC1 signaling in KI mice.** Protein levels of phosphorylated
573 mTOR (p-mTOR), mTOR, phosphorylated S6 (p-S6), S6, phosphorylated 4E-BP1 (p-4E-
574 BP1), 4E-BP1, phosphorylated Akt (p-Akt$^{Thr308}$ and p-Akt$^{Ser473}$) and Akt in 60-week-old KI
575 and WT mouse hearts. **A**, Representative Western blots of indicated proteins from mouse
576 ventricular protein extracts (cytosolic fraction). α-actinin was used as loading control.
577 Quantification of **B**, p-mTOR, mTOR and p-mTOR/mTOR, **C**, p-S6, S6 and p-S6/S6, **D**, p-
578 4E-BP1, 4E-BP1 and p-4E-BP1/4E-BP1 and **E**, p-Akt$^{Thr308}$, Akt, p-Akt$^{Thr308}$/Akt, p-Akt$^{Ser473}$
579 and p-Akt$^{Ser473}$/Akt. Protein levels were normalized to α-actinin and related to WT. Data are
580 expressed as mean + s.e.m. with *P<0.05, **P<0.01 vs. WT, unpaired Student‘s *t*-test.
581 Number of animals is indicated in the bars.

582

583 **Figure 6. Partial rescue of cardiomyopathy by 9-week rapamycin treatment or caloric**
584 **restriction in KI mice.** Determination of cardiac function by echocardiography and
585 parameters of hypertrophy and heart failure in KI and WT mice after 9-week rapamycin
586 treatment (rapa), 40% caloric restriction (CR) or control treatment (ctrl). Mice were fed with
587 chow containing either 2.24 mg/kg rapamycin or coating material (control). Mice on caloric
588 restriction were fed with 60% of control diet. **A**, Fractional area shortening (FAS). **B**, Body
589 weight (BW). **C**, Heart weight-to-tibia length ratio (HW/TL). **D**, Lung weight-to-tibia length
590 ratio (LW/TL) **E**, Tibia length (TL). Data are expressed as mean + s.e.m. with $^{*}$P<0.05,
591 $^{**}$P<0.01, and $^{****}$P<0.0001 vs. WT ctrl, and $^{+}$P<0.05, $^{++}$P<0.01 and $^{+++}$P<0.001 vs. KI ctrl,
592 one-way ANOVA plus Tukey's post-test. Number of animals is indicated in the bars.

593

594 **Figure 7. Gene expression analysis and autophagic flux in rapamycin-treated or calorie-**
595 **restricted KI and WT mice.** KI and WT mice were treated for 9 weeks with either 2.24
596 mg/kg rapamycin (rapa), 40% caloric restriction (CR) or control treatment (ctrl). **A**, Heatmap
597 of selected genes (threshold <0.8 or >1.2 fold change to KI ctrl) comparing gene expression
598 of hypertrophy, fibrosis, calcium handling, autophagy and potassium and sodium regulation
599 between KI ctrl, KI rapa or KI CR and WT ctrl mice. **B**, Representative Western blots of

600 indicated proteins from mouse ventricular protein extracts (membrane-enriched fraction). α-
601 actinin was used as loading control. **C**, LC3-II quantification (normalized to α-actinin) related
602 to WT ctrl. Data are expressed as mean + s.e.m. with $^{*}P<0.05$, $^{**}P<0.01$ and $^{****}P<0.0001$ vs.
603 WT ctrl, one-way ANOVA plus Dunnett's post-test, and non-significant (NS) and $^{+}P<0.05$ vs.
604 indicated group (comparing with and without inhibitor), unpaired Student's *t*-test. Number of
605 animals is indicated in the bars.

606 **Clinical Perspective**

607

608 Hypertrophic cardiomyopathy (HCM) is an autosomal-dominant disorder, characterized by
609 left ventricular hypertrophy and diastolic dysfunction, and has an estimated prevalence of
610 1:500 in the general population. HCM can result in serious conditions, such as heart failure
611 cardiac arrhythmias, and sudden cardiac death. The *MYBPC3* gene, encoding cardiac myosin-
612 binding protein-C (cMyBP-C), is frequently mutated in HCM, representing 40-50% of all
613 HCM mutations. Up to now, there are only common treatments like calcium channel- and β-
614 blockers, septal myectomy, ethanol ablation and heart transplantation available for HCM. It is
615 known that postmitotic cells such as neurons or cardiomyocytes are particularly dependent on
616 energy and protein quality control. A major pathway for energy supply and degradation of
617 cellular proteins is the autophagy-lysosomal pathway. Whereas altered autophagy has been
618 long correlated to neurological diseases, only a few cardiac diseases are known to be
619 associated with it. To the best of our knowledge, there is no evidence of altered autophagy in
620 sarcomeropathies. Here, we show that protein levels of the autophagy marker LC3-II were
621 higher and gene expression of several autophagy markers was altered in HCM patients and
622 mice carrying *MYBPC3* mutations, autophagy was blunted and Akt-mTORC1 signaling
623 increased in *Mybpc3*-knock in mice and that rapamycin treatment or caloric restriction
624 ameliorated cardiomyopathy or heart failure in these mice. We therefore propose that
625 activation of autophagy might be an attractive option alone or in combination with another
626 approach to rescue HCM induced by *MYBPC3* mutations.