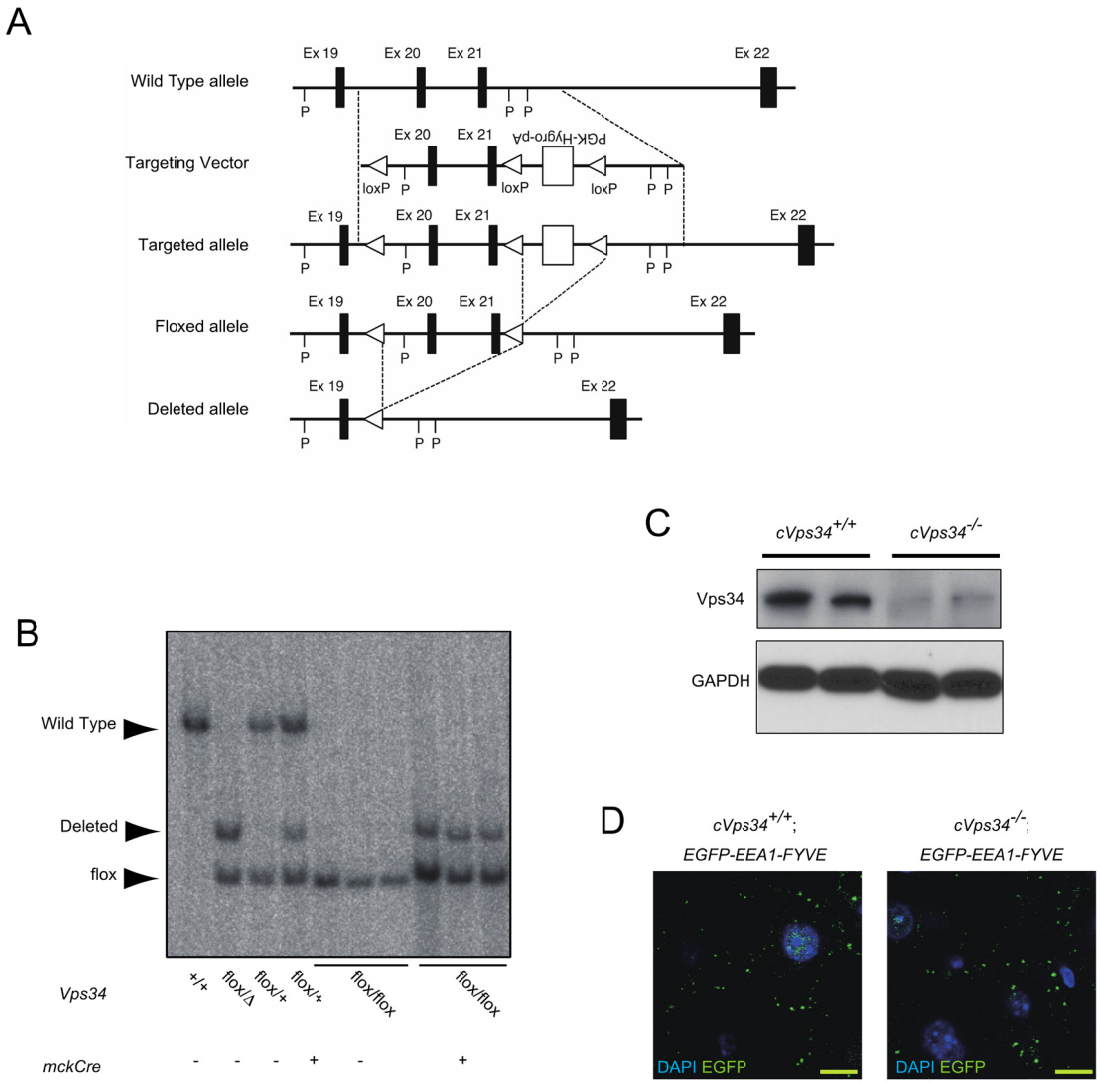
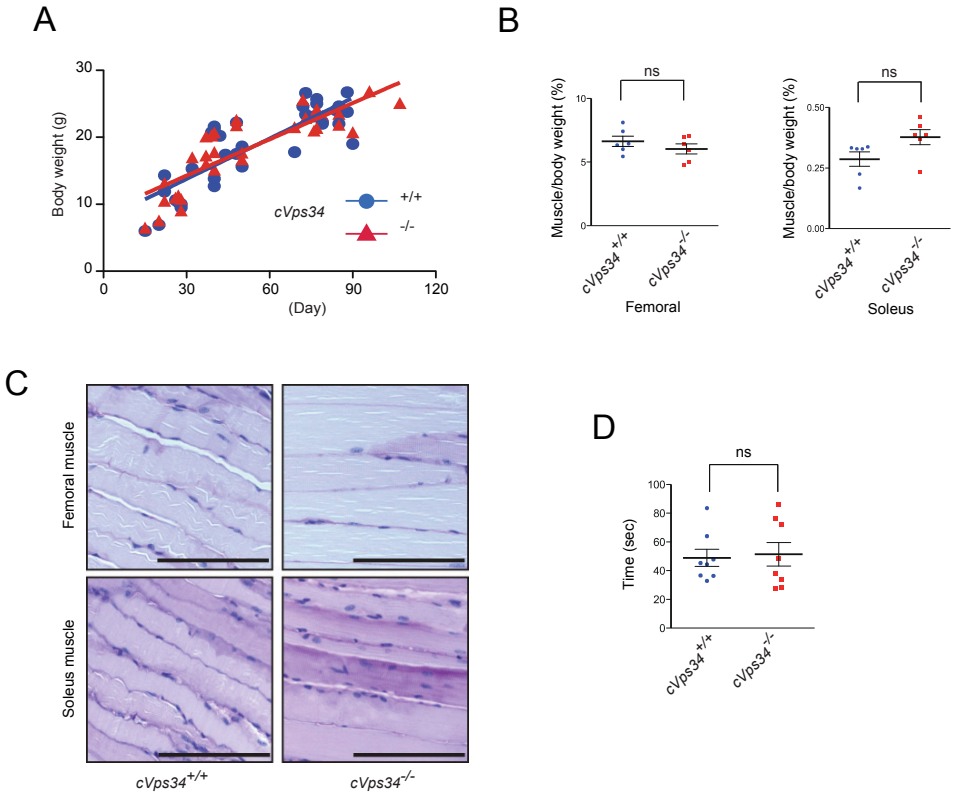
Supplemental Figures

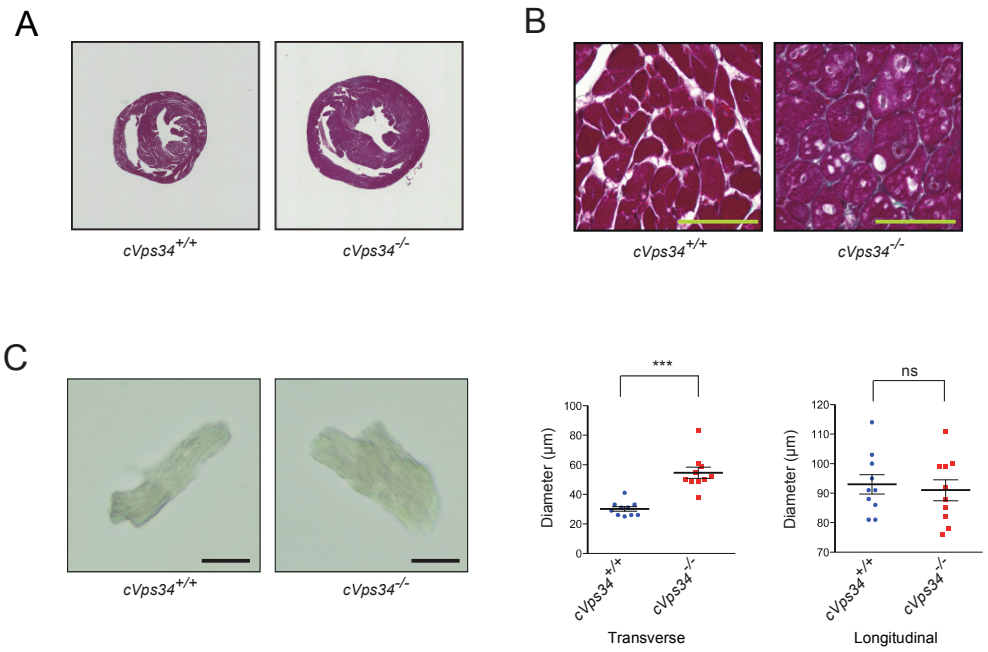


Supplemental Figure 1. **Targeted disruption of the *Vps34/Pik3c3* gene in murine hearts. (A)** Schematic representation of the WT mouse *Vps34* gene showing exons (Ex) 19-22; the targeting vector with loxP sites and a hygromycin resistance cassette; the targeted *Vps34* allele, the floxed *Vps34* allele; and the deleted *Vps34* allele after *Cre*-mediated removal of a fragment containing exons (Ex) 20 and 21. P, *Pst*I site. **(B)** Southern blot analysis of genomic DNA from hearts of mice bearing the indicated *Vps34* alleles. Genomic DNA was digested with PstI

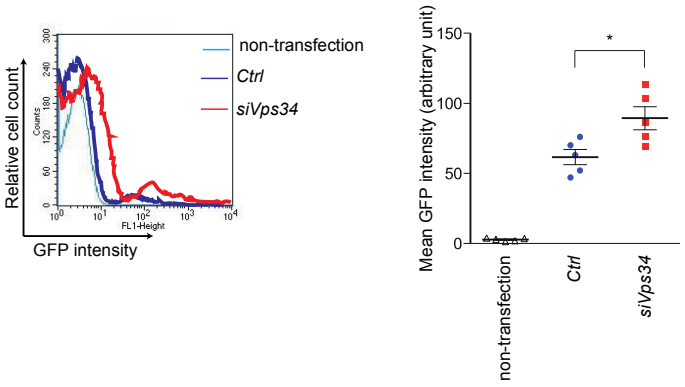
for hybridization. The probe detected a 3.0 kbp *PstI* fragment from the wild type (WT) allele, a 1.9 kbp fragment from the floxed *Vps34* allele, and a 1.5 kbp fragment from the disrupted *Vps34* allele. **(C)** Levels of Vps34 protein in *mckCre-Vps34*$^{flox/flox}$ (*cVps34*$^{-/-}$) mouse hearts. **(D)** Indirect immunofluorescence analysis to detect PI(3)P in liver sections from the indicated mouse strains. GFP-EEA1-FYVE-positive puncta (green) were normal in muscle-specific-Vps34-deficient liver. Blue, DAPI staining to detect nuclei. Scale bars, 10 μm.



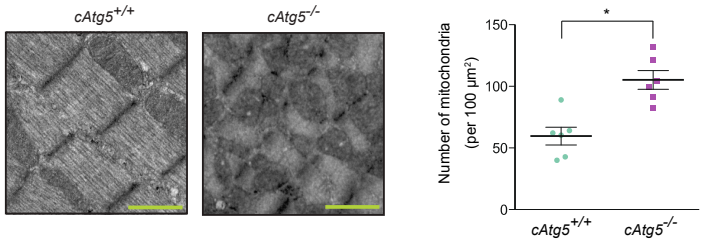
Supplemental Figure 2. Analyses of body weight and skeletal muscles in the absence of Vps34. Body weights of male *mckCre-Vps34$^{flox/flox}$* (*cVps34$^{-/-}$*) (n=37) mice and littermate control *Vps34$^{flox/flox}$ (cVps34$^{+/+}$*) (n=35) mice over the indicated period. **(B) (**Left panels) quantitation of muscle weight/body weight ratios for femoral muscle (white muscle) and (right panels) soleus muscle (red muscle) in male *cVps34$^{+/+}$* (n=6) and *cVps34$^{-/-}$* mice (n=6) at postnatal day 80 (P80). **(C)** Representative images of longitudinal sections of *cVps34$^{+/+}$* and *cVps34$^{-/-}$* skeletal muscles stained with PAS. Scale bars, 100 μm. **(D)** Quantitation of hanging wire test results for *cVps34$^{+/+}$* (n=8) and *cVps34$^{-/-}$* (n=8) male mice at P80. Individual data points and the group mean ± SEM are shown. ns, not significant.



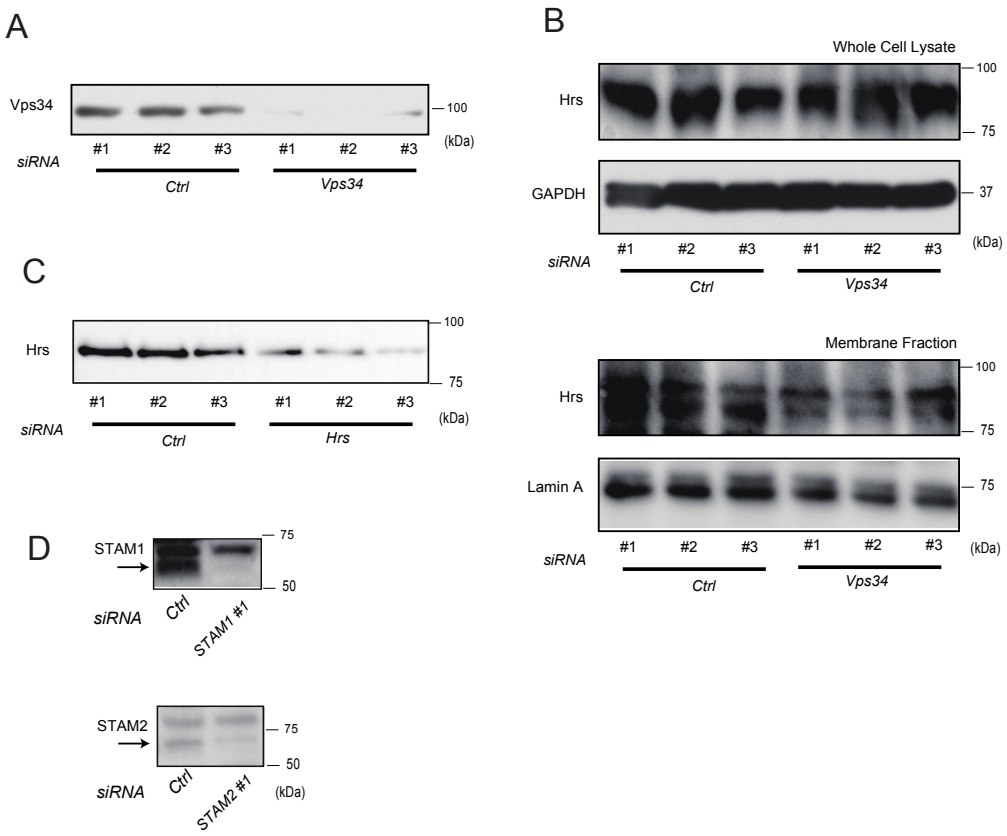
Supplemental Figure 3. Cardiac hypertrophy in the absence of Vps34. (A) Elastica Masson-stained cross-sections of ventricles from *mckCre-Vps34*$^{flox/flox}$ (*cVps34*$^{-/-}$) mice and *Vps34*$^{flox/flox}$ (*cVps34*$^{+/+}$) mice at P80. Results are representative of at least 10 ventricles examined/group. (**B)** Representative Elastica-Masson-stained sections of myocardium from *cVps34*$^{+/+}$ and *cVps34*$^{-/-}$ mice at P80 (n=6). Scale bars, 100 µm. **(C)** Left panels: Phase contrast microscopy of cardiomyocytes isolated from *cVps34*$^{+/+}$ and *cVps34*$^{-/-}$ mice at P80. Scale bars, 25 µm. Right panels: Quantitation of transverse and longitudinal diameters of the *cVps34*$^{+/+}$ and *cVps34*$^{-/-}$ cardiomyocytes in the left panels. Individual data points and the group mean ± SEM are shown (n=10 cells examined/group). ***p<0.001, two-tailed Student's t test. ns, not significant.



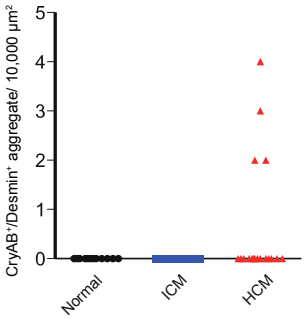
Supplemental Figure 4. Suppression of autophagic flux by siRNA silencing of Vps34. P19.CL6 cells were co-transfected with GFP-LC3 and control siRNA (*Ctrl*) or siRNA-Vps34 (*siVps34*). Total cellular GFP–LC3 signals were analyzed by flow cytometry. Left panel, a representative flowcytometry profile. Right panel, quantification of mean fluorescent intensity (n=5). Individual data points and the group mean ± SEM are shown. *p<0.05, two-tailed Student's t test.



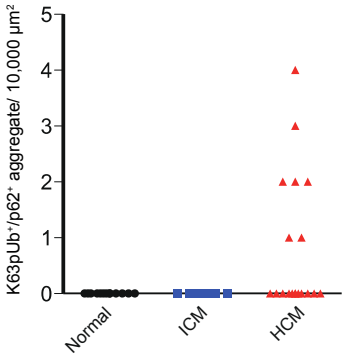
Supplemental Figure 5. *mckCre-Atg5*$^{flox/flox}$ mice exhibit accumulation of mitochondria in cardiomyocytes. Electron micrographs of myocardia from *Atg5*$^{flox/flox}$ (*cAtg5*$^{+/+}$) and *mckCre-Atg5*$^{flox/flox}$ (*cAtg5*$^{-/-}$) mice at postnatal day 80 and quantitation of numbers of mitochondria (n=6 examined/group). Individual data points and the group mean ± SEM are shown. *p<0.05, two-tailed Student's t test. Scale bars, 1 μm.



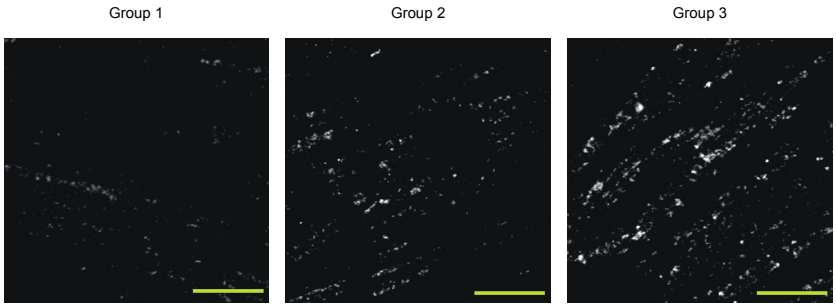
Supplemental Figure 6. Knockdown of Vps34 dissociates Hrs from intracellular membranes. (A) Suppression of Vps34 protein expression by siRNAs. P19.CL6 cells were transfected with control *siRNAs* (*Ctrl*) or *siRNAs against Vps34* (*#1-3*). **(B)** Levels of Hrs protein in whole cell lysate or total membrane fraction of P19.CL6 cells transfected with *Ctrl* or *siRNA-Vps34*, as indicated. GAPDH and Lamin A, loading controls. **(C, D)** Confirmation of knockdown of Hrs, STAM1 or STAM2 by siRNAs in P19.CL6 cells. *siRNA-Ctrl#2*, *siRNA-Vps34#2*, *siRNA-Hrs#3*, *siRNA-STAM1#1* and *siRNA-STAM2#1* were used for the experiments shown in Figures 5 and 7.



Supplemental Figure 7. Quantification of CryAB$^+$/Desmin$^+$ aggregates in cardiomyocytes. CryAB$^+$/Des$^+$ aggregate (> 5 μm in diameter) were counted in 5 randomly chosen fields (10,000 μm^2) for each sample. Human myocardial specimens from normal heart (n=12), ICM (n=15) and HCM (n=18) were examined.



Supplemental Figure 8. Quantification of K63pUb$^+$/p62$^+$ aggregates in cardiomyocytes. K63pUb$^+$/p62$^+$ aggregate (> 5 μm in diameter) were counted in 5 randomly chosen fields (10,000 μm^2) for each sample. Human myocardial specimens from normal heart (n=12), ICM (n=7) and HCM (n=18) were examined.



Supplemental Figure 9. Vps34 protein expression score.
Representative fluorescent immunohistochemical analyses of sections of human hearts classified in the Groups 1, 2 and 3 of Vps34 protein expression (see Methods). Scale bars, 10 μm.

Supplemental Tables

Parameter	*cAtg5*$^{+/+}$	*cAtg5*$^{-/-}$
LVPWd (mm)	0.75±0.05	0.79±0.02
LVDd (mm)	3.26±0.08	3.90±0.10*
LVDs (mm)	1.65±0.03	2.07±0.05*
FS (%)	49.6±1.8	47.5±0.9
EF (%)	83.8±3.7	78.6±2.3
LV mass (mg)	91.0±6.6	104±4.1
Heart rate (bpm)	477±25	489±17

Supplemental Table 1. Echocardiographic measurements and cardiological parameters in muscle-specific *Atg5* deficient mice. *mckCre-Atg5*$^{flox/flox}$ (*cAtg5*$^{-/-}$) mice and littermate *Atg5*$^{flox/flox}$ (*cAtg5*$^{+/+}$) at postnatal day 80 were examined. LVPWd, left ventricle posterior wall thickness in diastolic phase; LVDd, left ventricle internal dimension in end diastolic phase; LVDs, left ventricle internal dimension in end systolic phase; FS, fraction shortening; and EF, ejection fraction. Data are the mean ± SEM (n=6 examined/group). *p<0.05, two-tailed Student's t test.

No.	Gender	Vps34 score	CryAB	Desmin	K63 pUb	p62
1	M	2	N	N	N	N
2	M	2	N	N	N	N
3	M	2	N	N	N	N
4	F	2	N	N	N	N
5	F	2	N	N	N	N
6	F	2	N	N	N	N
7	F	2	N	N	N	N
8	F	2	N	N	N	N
9	F	2	N	N	N	P
10	M	3	N	N	N	N
11	M	3	N	N	N	N
12	M	3	N	N	N	N

Supplemental Table 2. A list of characteristics of myocardial tissue specimens from 12 normal individuals analyzed by immunohistochemistry. N, normal localization of CryAB or desmin, or no detectable signal for K63pUb or p62. Vps34 protein expression score was determined as described in Method and illustrated in Supplemental Figure 9.

No.	Age	Gender	LV wall (mm)	Fibrosis	Vps34	K63	p62	Des	CryAB
1#	52	f	14	PF	2	N	N	N	N
2	80	m	20	PF	2	N	N	N	N
3	62	m	20	NF	2	P	N	N	N
4	70	m	16	NF	3	N	N	N	N
5	93	f	16	NF	3	N	N	N	N
6	84	m	20	NF	3	N	N	N	N
7	72	m	18	NF	3	P	N	N	N

Supplemental Table 3. A list of characteristics of myocardial tissue specimens from 7 ICM patients analyzed by immunohistochemistry. PF, presence of myocardial fibrosis within left ventricle. NF, absence of myocardial fibrosis. N, normal localization of CryAB or desmin, or no detectable signal for K63pUb or p62. P, presence of abnormal aggregates containing the indicated proteins. Vps34 expression levels were scored as described in Method and illustrated in Supplemental Figure 9.