Supplemental Material

Table S1. RNA expression in wild-type and $L2^{\Delta 6}$ hearts.

Gene category	All genes	$L2^{\Delta 6}$ > WT	P	$L2^{\Delta 6}$ < WT	**P**
All RNAs	31920	1278 (4%)		707 (2.2%)	
Autophagy markers	793	62 (7.8%)	<0.001	15 (1.9%)	0.54
Apoptotic markers	1252	85(6.8%)	<0.001	21(1.7%)	0.2

Percentages represent gene counts in each category out of the total gene counts. A change in gene expression was defined by at least ≥ 1.5 or < 0.67 ratio in the read count (for increase or decrease respectively).

$L2^{\Delta 6}$ > WT, number of genes expressed in $L2^{\Delta 6}$ tissue at a higher level than in wildtype (WT) tissue. $L2^{\Delta 6}$ < WT, number of genes expressed in $L2^{\Delta 6}$ tissue at a lower level than in wild type (WT) tissue. P values - comparison of the percent change between all RNAs and markers of either Autophagy or Apoptosis.

All RNAs, reflects RNAs identified as expressed by RNAseq as described by Christodoulou et al[27].

Autophagy markers is a set of autophagy-associated proteins collected from http://www.tanpaku.org/autophagy/list/GeneList.html. Apoptotic signaling pathway genes collected from http://www.informatics.jax.org/go/term/GO:0097190.

Specific details of the genes and the fold changes in expression are available upon request.

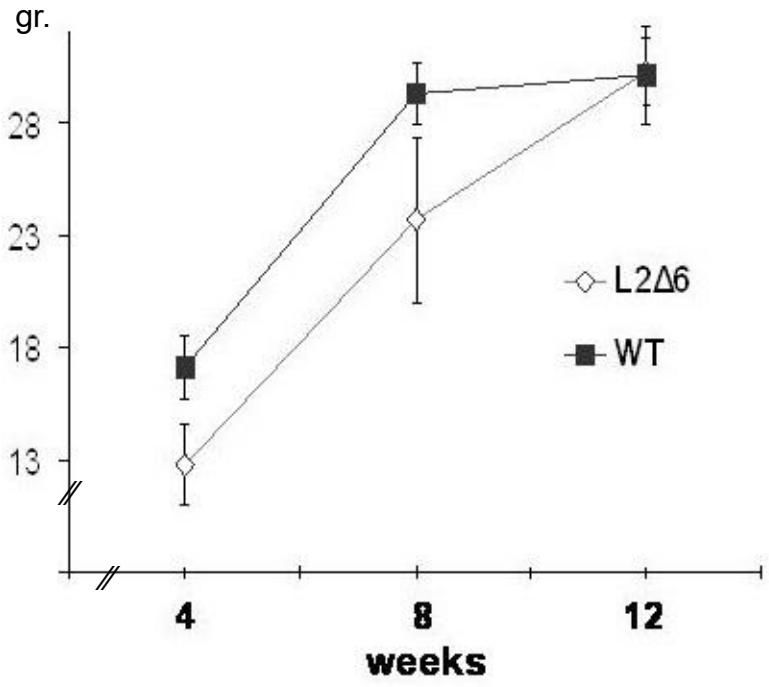
The full results of the transcriptome analysis are provided at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166731 (GEO accession number GSE166731).

Table S2. Autophagy and apoptosis related genes showing the greatest change in expression in hearts of $L2^{\Delta 6}$ mice.

	Gene ID	Gene description	Fold change ($L2^{\Delta 6}$/WT)	WT normalized reads	Regulation	P-value
Autophagy	***Tlr13***	toll-like receptor 13	16.30	0.12	up	<0.0001
	Crocc	ciliary rootlet coiled-coil	3.97	0.83	up	0.0003
	Bcl2l1	BCL2-like 1	3.75	157.54	up	<0.0001
	Sgk1	serum/glucocorticoid regulated kinase 1	3.41	31.15	up	<0.0001
	Ddit4	DNA-damage-inducible transcript 4	3.40	81.68	up	<0.0001
	Rcan1	regulator of calcineurin 1	3.26	103.20	up	<0.0001
	Tecpr1	tectonin beta-propeller repeat containing 1	3.12	25.56	up	<0.0001
	Il18r1	interleukin 18 receptor 1	0.12	16.00	down	0.0005
	Phkg1	phosphorylase kinase gamma 1	0.29	267.00	down	<0.0001
	Alpl	alkaline phosphatase	0.31	146.00	down	<0.0001
Apoptosis	***Bid***	BH3 interacting domain death agonist	49.00	0.00	up	<0.0001
	Lcn2	lipocalin 2	26.66	6.90	up	0.0005
	S100a9	S100 calcium binding protein A9	16.87	0.59	up	0.0003
	Bdkrb2	bradykinin receptor, beta 2	10.22	0.36	up	<0.0001
	Ankrd2	ankyrin repeat domain 2	9.27	0.36	up	<0.0001
	Plaur	plasminogen activator	5.43	0.71	up	<0.0001
	Cdkn1a	cyclin-dependent kinase inhibitor 1A	4.92	85.01	up	<0.0001
	Pawr	PRKC, apoptosis	4.03	1.19	up	<0.0001
	Trp63	transformation related protein 63	0.08	4.52	down	<0.0001
	Cx3cr1	chemokine (C-X3-C motif) receptor 1	0.20	3.45	down	<0.0001

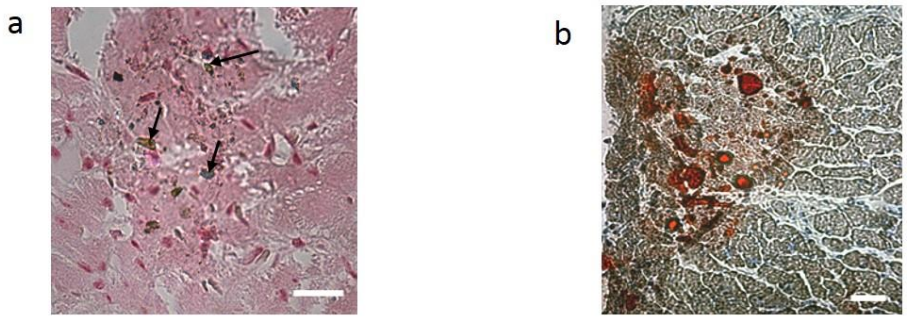
Ten genes which changed most (in either direction) are shown in each category. RNAseq libraries were constructed from hearts of adult $L2^{\Delta 6}$ and WT mice and data were analyzed as described[27].

Figure S1. Average weight of 10 WT and 10 L2$^{\Delta 6}$ mice at 4, 8, and 12 weeks.



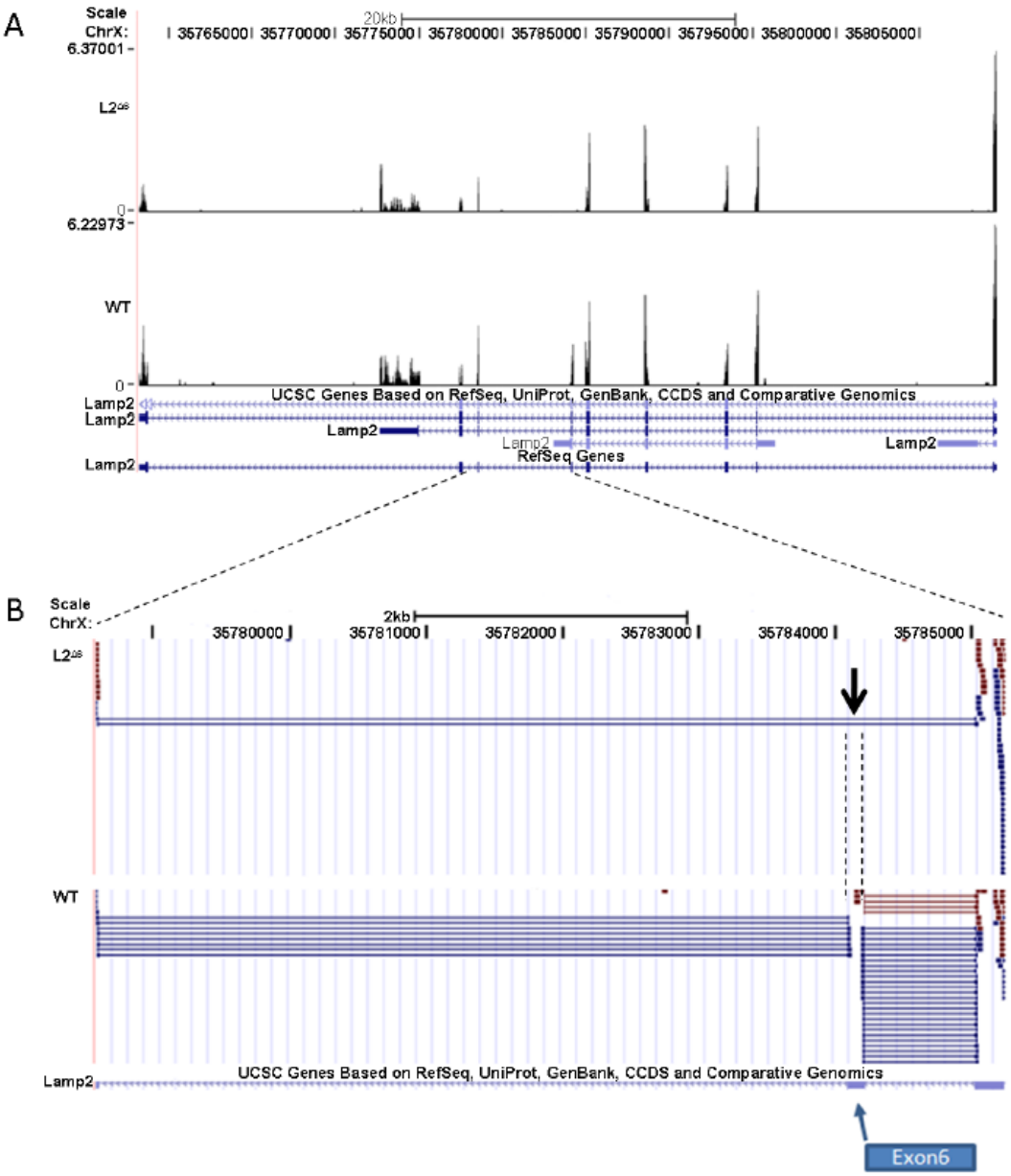
The L2$^{\Delta 6}$ had a lower body weight at 4 weeks but the differences were not anymore apparent at 12 weeks age.

Figure S2. Cell death in L2 $^{\Delta 6}$ hearts.



(a) Von Kossa stains of 40-week old L2 $^{\Delta 6}$ hearts show dystrophic calcification (arrows) consistent with necrosis (scale bar = 50 μm). **(b)** Oil-red-O staining of frozen section of 40 week old L2$^{\Delta 6}$ heart showing fat depositions (in red) within the myocardium. Scale bar = 50μm.

Figure S3. RNAseq analysis.



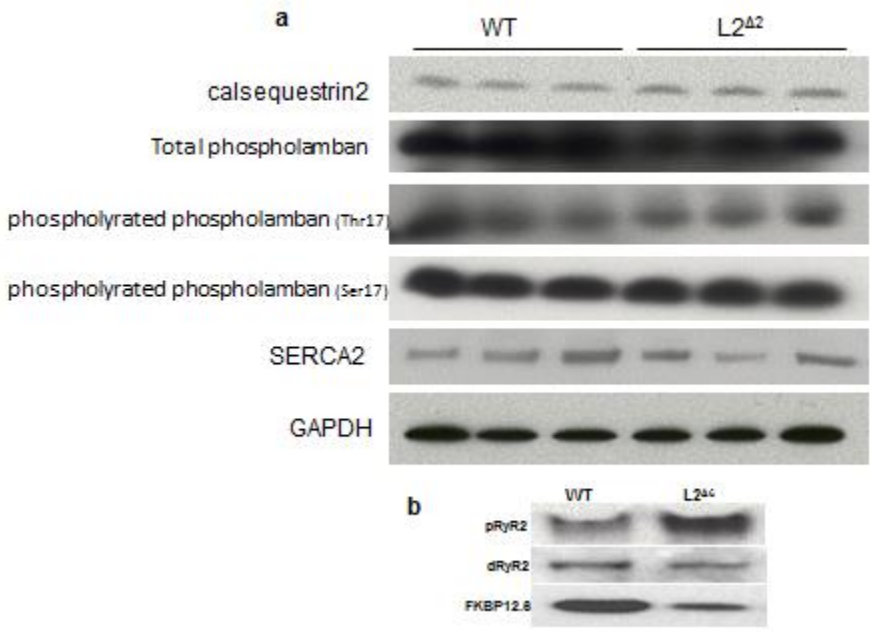
RNAseq analysis is demonstrated by the assessment of Lamp2 RNA expression in WT and $L2^{\Delta 6}$ hearts.

A) Screen shot of UCSC Genome Browser custom tracks (WIG files;http://genome.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=JonSeidman&hgS_other

UserSessionName=Lamp2_mm9), displaying the LAMP2 gene and normalized number of DNA sequence reads per million in $L2^{\Delta 6}$ and wildtype LV RNAseq libraries (see Methods). RNAseq libraries were constructed from 2 micrograms of total left ventricular RNA pooled from two mice of each genotype (see Methods). Four different LAMP2 isoforms are presented in the UCSC gene list. LAMP2 extends from exon 1 (right) to exon 10 (left). Note that 5' exons are more highly represented in this RNAseq data than 3' exons because of the method used for RNAseq library construction (our unpublished results).

B) Screen shot from custom tracks (BAM files) of UCSC Genome Browser displaying DNA sequence reads obtained from $L2^{\Delta 6}$ and WT LV RNAseq libraries between exon 5 and 7. Exon 6 is skipped in $L2^{\Delta 6}$ RNA as predicted by PCR analysis (black arrow).

Figure S4. Expression of key calcium handling proteins in L2$^{\Delta 6}$ hearts.

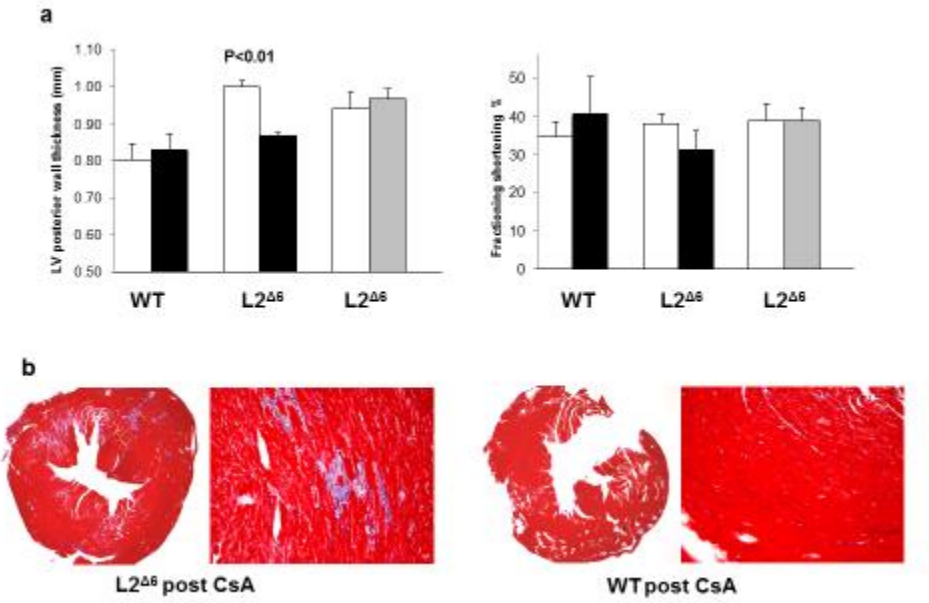


a) Western blot analysis of Calsequestrin2, Phospholamban (both total and the phosphorylated forms) and the cardiac sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) revealed no differences in the expression between wild type and L2$^{\Delta 6}$.

b) Increased expression of phosphorylated RyR2 and decreased expression of FKBP12.6 (calstabin) in L2$^{\Delta 6}$ hearts. See also figure 5.

All analyses were done on sarcoplasmic reticulum fraction of ventricular muscle extracts.

Figure S5. Cyclosporin A inhibits hypertrophy in L2$^{\Delta 6}$ hearts.



a) Left ventricular posterior wall thickness (left panel) and left ventricular fractional shortening (right panel) before (white bars) and after (black bars) 2 weeks of treatment with Cyclosporin A (CsA) in L2$^{\Delta 6}$ and wild type (WT) mice (two subcutaneous injections of

15 mg/kg in PBS daily). The gray bars represent control age-matched L2$^{\Delta 6}$ mice that were followed for 2 weeks without treatment (mean ± SEM , n=3/group, age at examination 32 weeks). Left ventricular posterior wall thickness was significantly reduced in treated L2$^{\Delta 6}$ hearts compared to untreated ($p<0.01$). No significant differences were found in the interventricular septum thickness, left ventricular dimension or fractional shortening b) Histology section stained with Masson's trichrome for fibrosis (in blue) of L2$^{\Delta 6}$ and wild type hearts assessed two weeks after treatment with CsA. The amount of fibrosis in L2$^{\Delta 6}$ hearts was comparable to untreated hearts (see Figure 1 for reference). There was no significant fibrosis in age-matched wild type hearts before or after treatment (data not shown).