PCR Primers and Information

A-ES Cell clone targeting primers (Fwd primers outside of targeting construct):

PLF: AGTCTCTTCCCTTCAGAGGGTGCCTGACCTACCCG
PLR: TTAAGGGTTATTGAATATGATCGGAATTGGGCTGCAGGAATT
PRF: TGGGGCAGGACAGCAAGGGGGAGGAT
PRR: AACCAAGAACACAGTCTGCAATGGCTGTTGGCTTC

Each Primer set yields 4.5 kB correctly targeted fragment from both left and right arms

B-Primers for positive selection of neo insertion:

FWD CTT GGG TGG AGA GGC TAT TC
REV AGG TGA GAT GAC AGG AGA TC

Product yields 400bp fragment for positive selection (primers within neo cassette)

C- Determination of positive iLS/+ mice:

LSGenoFWD :5'GTATATGTGTGTGCGCGCAC
LSR :5'GTAGGCTCTCCTTTCTCC

Full length WT: 614 bp (cut with HpyCH4v: 387, 226 bp)
Full length iLS/+: 1.3kbp and 614 bp (cut with HpyCH4v: 387, 226 from WT allele, and 226, 226, 60, 378, 441 bp from the LS mutant allele)

(Notes: LSR spans the mutant loxP insertion site and does not amplify (Cre excision regenerates the LSR primer recognition site). The 1. 33 kbp product is from LSGenoFwd to itself. * Y279C mutation introduces two HpyCH4v sites: one in the mutant Exon 7 Y279C site and at the 5' end of the inserted targeted mutant sequence- 35 bps downstream of the 5' mutant loxP site).

Full length LS/+ (after Cre recombination): 614 and 714 (cut with HpyCH4: 387, 226 and 226, 60, 50, 378bps

D-LoxP Genotyping strategy for positivity after Cre recombination:

LoxP Forward: 5'CTTCGTATAATGTATGCTATACGAAG
LSGenoREV: 5'CTACTTAGCTTTCCTGGCCTC

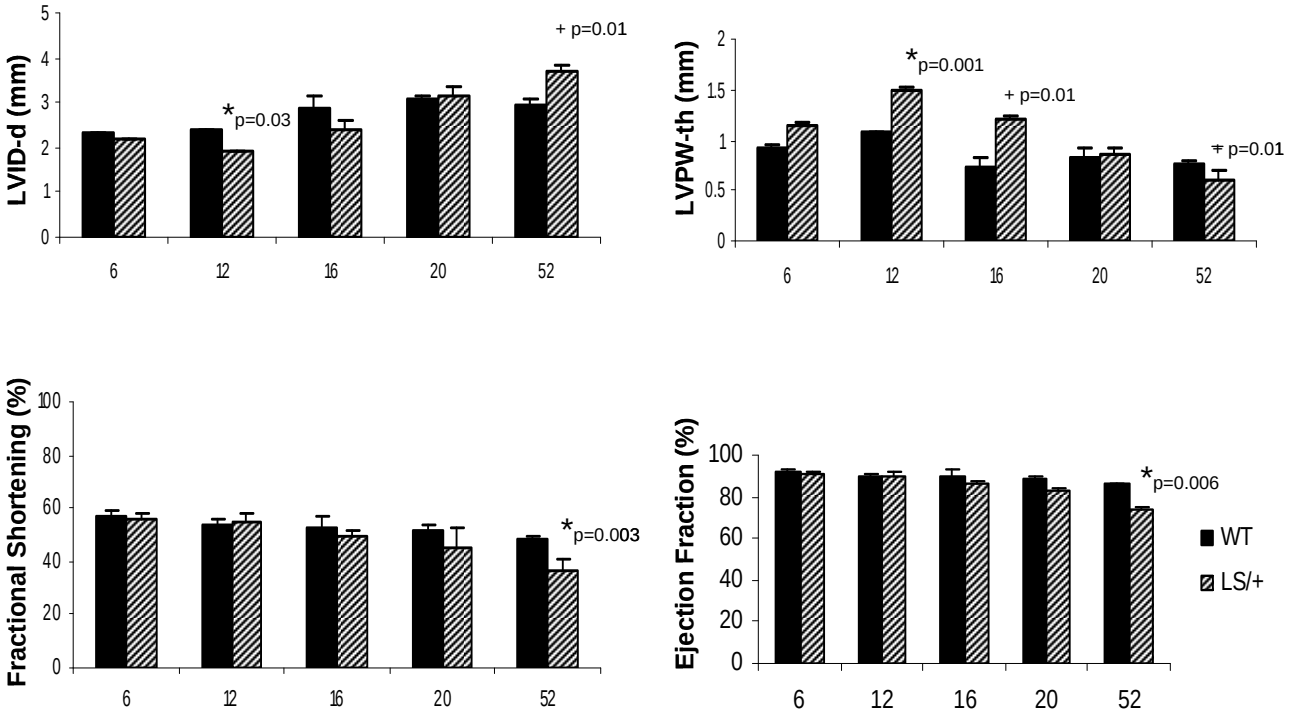
Yields 1 band at 628 bp for LS/+ or LS/LS mice. No band for wt.

E-cDNA expression:

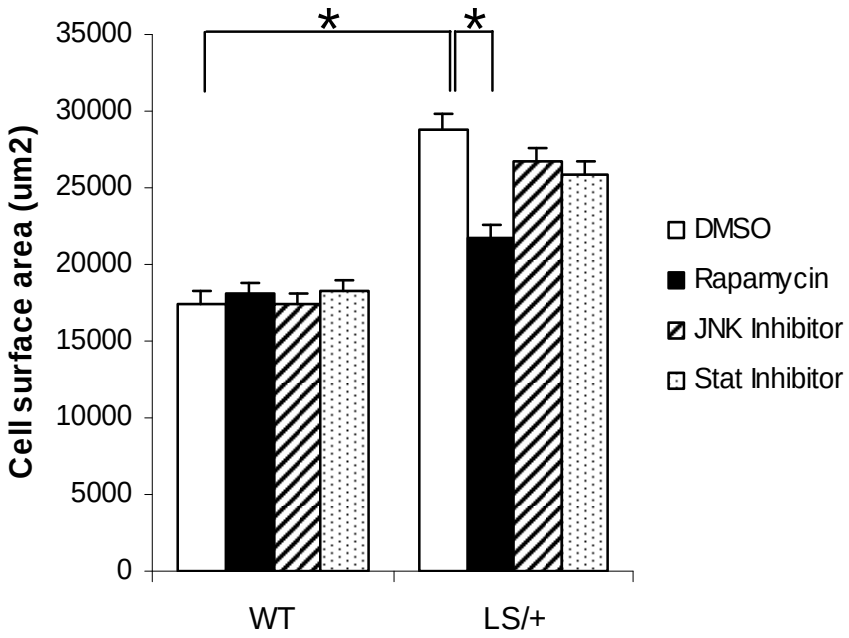
Shp2 900R: CCATCATGCAGAACGACCCTGG
Shp2 721F: GCAAATTAGCTGAGACCACAG

Full length cdna band=180 bp
Cut with HpyCH4v: Wt: 100, 70, 10; LS/+: 100, 70, 10 (WT allele) and 58 and 42 (LS mutant)

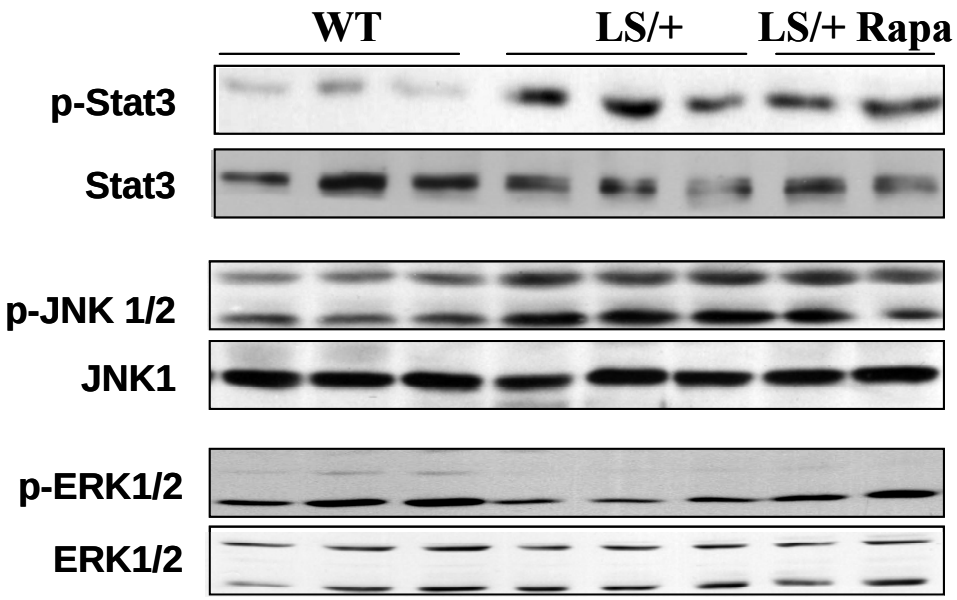
Supplemental Table 1. PCR primers for detecting homologous recombinants and for genotyping. **(A)** ES cell targeting primers. Note that PRF and PLF (the forward primers for each set) lie outside of the targeting construct region. **(B)** Primer set used for detection of the neo resistance gene. **(C)** PCR primers used for genotyping to identify iLS/+ and LS/+ mice, respectively. Expected products after HpyCH4v digestion are also indicated. **(D)** PCR primers used in a second genotyping reaction to identify iLS/+ and LS/+ mice, respectively. A single band is visualized because only the LS allele contains a LoxP site. **(E)** PCR strategy for assessing normal LS expression from cDNA and the expected products after HpyCH4v digestion.



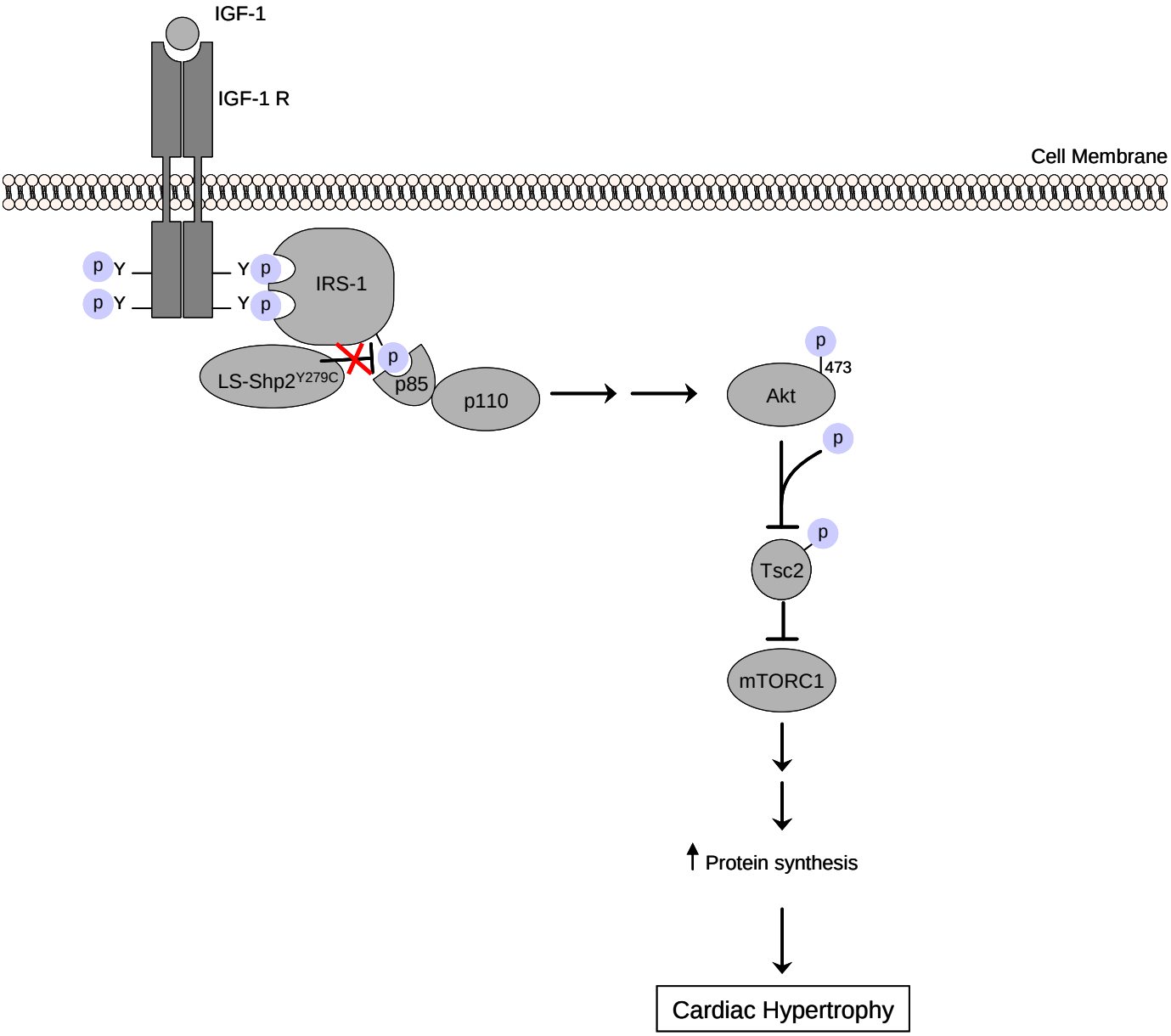
Supplemental Figure 1. Cardiac anatomic and functional parameters. Cardiac anatomic and functional parameters, as assessed by echocardiography, in WT and LS/+ mice at indicated ages. (LVPW-th=left ventricular posterior wall thickness, LVID-d=left ventricular chamber dimension in diastole). n=5-12 mice per group at each age; *p values are as indicated. Statistics are based on ANOVA and Bonferroni post-test when ANOVA was significant.



Supplemental Figure 2. Response of LS/+ cardiomyoctyes to rapamycin treatment. Quantification of the overall change in area of WT or LS/+ cardiomyocytes treated with either DMSO, rapamycin (0.02 μM), JNK Inhibitor (10 μM), or Stat3 Inhibitor (1 μM) for 12 hours. Cell surface area was quantified from 100-200 cells from each group. Results are the mean ± SEM; *$p<0.001$, 2-tailed Student's t test. Note significant difference in total area between WT and LS/+ cardiomyocytes only when treated with rapamycin.



Supplemental Figure 3. Rapamycin treatment does not affect other signaling pathways in LS/+ hearts. Whole heart lysates from 4-week daily vehicle-treated WT, vehicle-treated LS/+, and rapamycin-treated LS/+ mice were harvested, lysed and immunoblotted with anti-phospho–Stat3, -Jnk1/2, -and –Erk1/2 antibodies followed by anti-Stat3, Jnk1, and Erk1/2 antibodies to control for loading, respectively. Note that rapamycin treatment had no effects these downstream signaling pathways.



Supplemental Figure 4. Model for LS hypertrophic pathology. Our results suggest that LS mutations render Shp2 catalytically inactive, yet preferentially in the open conformation, thereby reducing the ability of Shp2 to dephosphorylate the binding sites for the p85 subunit of PI3K on IRS1. This results in increased PI3K activity and enhanced activation of the Akt/mTorc1 pathway. Increased mTorc1 activity results in increased p70S6K activity, enhanced protein synthesis and cardiac hypertrophy in LS/+ patients.

A.

ANF-FWD	CCT GGA GGA GAA GAT GCC GGT AGA A
ANF-REV	CCC CAG TCC AGG GAG GCA CCT CGG
BNP-FWD	CAC TTC AAA GGT GGT CCC AGA GCT GC
BNP-REV	GAC CGG ATC GGA TCC GTC AGT CG
αMHC-FWD	GTC CCG GAC ACT GGA CCA GGC C
αMHC-REV	CTC CTT TTC TTC CAG TTG CCT AGC CAA
βMHC-FWD	GAG CAA GGC CGA GGA GAC GCA GCG T
βMHC-REV	GAG CCT CCT TCT CGT CCA GCT GCC GG
SERCA2a-FWD	GGC TTT TAC AGG GCG AGA GT
SERCA2a-REV	ACC AGA TTG ACC CAG AGT AAC TG
Phospholamban-FWD	GAT TCT GAC GTG CTT GCT GAG G
Phospholamban-REV	CAC ATA GAA GCC TAG CCC ACA C

B.

	qPCR Conditions
Step 1	50ºC 2 minutes
Step 2	95ºC 10 minutes
Step 3	95ºC 15 seconds
Step 4	60ºC 1min
Step 5	Go to step 3 for 39 times

Supplemental Table 2. PCR primers for quantitative real-time (qRT) PCR. (A) PCR primer sequences for fetal genes. **(B)** qPCR conditions for analysis of fetal gene expression profiles.