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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	All data were collected on individual hardware devices(e.g. Visualsonics Vevo 2100 for echocardiographic images, or Licor Odyssey for Western blot, Biorad CFX384 qPCR machine for mRNA expression. No stand-alone open source or commercial software was used for data collection.
Data analysis	Mouse genetic background assessment utilized SNaP-MaP™ and Map-Synth™ performed at the Geisel School of Medicine at Dartmouth University, Hannover, NH; Western blot images were performed using Licor Image Studio Software 3.1, fluorescent images using tandem LC3-GFP-RFP probe was analyzed using Image J (Version 1.52a), echocardiographic images were performed using VisualSonics Vevo 2100, qPCR data was processed using Microsoft Excel, and statistical analysis using Graphpad Prism Version 7.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that [the/all other] data supporting the findings of this study are available within the paper [and its supplementary information files]. Requests for original computer files containing the reported data are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	If variance in the measured variable was known, then sample size for the assay was based either on known variance in the measured variable and thus power calculation of the requisite sample size to determine at least a 20% different in the mean variable (echo studies for example, or immunoblot), at 80% power and 95% confidence. For parameters where a variance was not previously known, studies were replicated at least 6 times, and based on these results, variance assessed and replicates performed to achieve a similar level of power and confidence.
Data exclusions	Data were not exluded
Replication	All of the experiments reported include multiple replications. For immunoblots, only gels in which there was a unambiguous technical error, e.g. incorrect primary or secondary antibody used so no signal detected, inadequate or highly variable loading, were not combined into the final data set. All animals assigned to particular treatment groups for the In vivo studies were used for analysis. If a treatment was employed, the animals were used by intention to treat. Reproducibility was also enhanced by having several investigators perform similar assays. The following list identifies what was done to assure reproducibility for each experiment in this study: Figure 1A, 1B: Based on multiple mice (n=24 total, assigned randomly to 4 groups), all data used and results highly reproducible. Figure 1C: Individual cell images for each condition with overall variance depicted and all data displayed. Results were highly reproducible. Figure 1E (upper) Highly reproducible result, performed by several laboratory members. Figure 1E(lower), 1F, 1G: Based on all mice used in study displayed in Figure 1A. No data excluded. Results highly reproducible. Figure 1H: Sample size of n=12 per group provided sufficient power. Random selected tissues, no bias. Tissue all processed identically. Figure 1I: Reproduced x3 with no disparities, also reproduced with another mutation (x3, n=6 total). Very consistent. Figure 1J: All technical replicates and biological replicates have demonstrated the identical findings. This was repeated x6. Figure 2A: Very strong group differences, and study now shown in entirety using two different serine mutations, fully reproducible. Figure 2B: All cell culture studies used, and data shown in paper demonstrates identical responses with either of the serine mutations. Figure 2C: Same situation as in Figure 1C, all cell data shown, despite variance between cells, group differences are marked, and reproduced. Figure 2D: Data consistent with other results previously shown (without siRNA to Rheb), and technically certified runs used. Very reproduced. Figure 2E: Data from 5 separate experiments, 2 independent investigators with very consistent results. Very reproducible. Figure 3B: All data used from population studied in time course and drug trial. Figure 3C,3D: Example in 3C is confirmed as highly reproducible in 3D. Despite interanimal variance, statistical differences are marked. Figure 3E: Nearly binary results of assay that was very reproducible, all gels for this study were utilized. Figure 3F: Performed in all surviving animals at end-of-study, no bias, and consistent with sample size in other assays from mouse study. Figure 4A: While this entire study was done once, the results were marked, and sample size sufficient to power difference detection. Figure 4B: Very reproducible results as demonstrated by highly disparate effects among groups. Figure 4C,4D: Example echocardiography which is consistent with mean results demonstrated in Figure 4D. Figure 4F: Same animals used to generate imaging and functional data were used for immunoblots. Results marked and reproducible. Figure 4G: Same explanation as provided for Figure 4F. Extended Data 1A-1C: Same experiment shown in Figure 1A, 1B, and same explanation applies. Extended Data 1D: Experiment performed once, though results were striking and differences highly significant. This is further consistent with subsequent data shown in Figure 3F in which protein aggregation was assayed in a different manner. Extended Data 2A: Experiment performed once, all data shown. Little variance in independent samples, supports reproducibility. Extended Data 2B: Experiment performed with multiple biological replicates, PCR results show marked differences. Extended Data 2D: Relates to Figure 1E Upper. Extended Data 2E: Performed multiple times by multiple investigators, panel here shows high concordance of results, reproducible. Extended Data 3A: Given variance, we performed study with larger sample size, providing robust power for interpretation. Extended Data 3B: While there is some inter-group variance, overall analysis shows very consistent result in each group. Extended Data 3C: Relates to Figure 1E lower.

Extended Data 3D: Identical experiment to that shown in Figure 1E, and Extended Data 3C, but with hsS1365 mutated.
Extended Data 3E: Identical experiment to that shown in Figure 1J. Reproduced 6 independent times, with identical results.
Extended Data 3F: Both experiments were fully reproduced (total 6 replicates each) and are internally consistent.
Extended Data 4A: Same experiment as in Figure 2A, but using hsS1365 as the target for SA/SE mutations. Near identical to Fig 2A.
Extended Data 4B: Relates to Figure 2B.
Extended Data 4C/D: Shows identical experiment to that of Figure 2B, ED Fig 4B, but with hsS1365 mutated. Near identical findings.
Extended Data 5A: One of two gels for replicated study, that is consistent with many other studies in paper using ET1 as stimulus.
Extended Data 5B: Experiment x1, with 3 replicates. Results were highly consistent between each lane within group, thus reproducible.
Extended Data 5C: Relates to data in Figure 2D.
Extended Data 6B: Consistent with many gels since performed using out KI mouse models. Highly reproducible.
Extended Data 6C: Data from intact animal experiment shown in Figure 3B, 3F. Same extent of reproducibility.
Extended Data 6D-F: All analysis from same intact animal experiments displayed in Figure 3B, 3F, ED Fig 6C. Reproducibility supported by very marked statistical differences throughout.
Extended Data 7A: As shown by example on left, the results were very consistent. Entire analysis was performed using ventricular tissue from the same set of animals used for the other molecular assays.
Extended Data 7B: Molecular analysis from same animals displayed in Figure 4A-4C, no selection bias, results markedly different supporting reproducibility.
Extended Data 8B: Relates to Figure 4F - summary analysis.
Extended Data 8C: Study performed twice in vivo, all animals included, and variance between groups is small enough to provide highly significant differences.

Randomization

Mouse models involving drug treatment were randomized up front prior to the procedure being performed - pressure overload with or without concomitant drug treatment. Individuals providing drug (oral or IP), or performing echocardiographic analysis were blinded to group allocation. All controls were performed in littermates using heterozygote crossing strategies to yield 25% WT, 50% heterozygote, and 25% homozygote. Male and female mice were equally included in this study and were age-matched and weight-matched.

Blinding

We blinded all in vivo functional analysis involving genotype and/or drug treatment - with the echo-sonographer assessing images blinded to both. Molecular analysis (western blots, immunoprecitipation, etc) was performed by several individuals, where the experiment and sampling was obtained by one, isolates analyzed by another. In these instances, the individuals were aware of the experimental groups involved.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

The study involves two novel custom developed KI mouse models. We will provide these mice to other investigators under an MTA, once an initial study is published. These models will be subject to an anticipated patent that was already filed and is related to this discovery.

Antibodies

Antibodies used

The following primary antibodies were used in this study: phospho-Akt (S473) #9271 lot 14 used at 1:1,000, Akt #9272 lot 28 used at 1:1,000, phospho-70 S6K (T389) #9205 lot 21 used at 1:1,000, p70 S6K #9202 lot 20 used at 1:1,000, phospho-4EBP1 (S65) #9451 lot 14 used at 1:1,000, 4EBP1 #9452 lot 12 used at 1:1,000, phospho-Ulk-1 (S757) #1420 clone D706U lot 4 used at 1:1,000, Ulk-1 #8054 clone D8H5 lot 5 used at 1:1,000, phospho-FoxO1/3 #9464 lot 7 used at 1:1,000, FoxO1 #2880 clone C29H4 lot 11 used at 1:500, phospho-NRDG-1 #3217 clone D98G11 lot 3, NRDG-1 #9395 clone D6C2 lot 1, GAPDH #2118 clone 14C10lot 10 used at 1:1,000, Rheb #13879 clone E1G1Rlot 1 used at 1:1,000, phospho-TSC2 (S1387) #5584 lot 5 used at 1:1,000, TSC2 #3612 clone D93F12lot 5 used at 1:1,000, and α-tubulin #3873 clone DM1Alot 12 used at 1:1,000 (Cell Signaling Technology), phospho-TSC2 (S1365) #120718 lot NFSA12072OAH used at 1:500 (NovoPro Labs), LC3 #ab192890 lot GR321-3 used at 1:1,000, thiophosphate ester #ab92570 lot GR237393-18 used at 1:5,000, and p62 #ab109012 lot GR12843-70 used at 1:1,000 (Abcam), FLAG #F3165 lot SLBK1346V used at 1:1,000, ubiquitin #SAB4503053 lot 310385 used at 1:1,000 (Sigma), and a total protein stain #926-11016 lot C80522-02 used at 5 ml/membrane (Li-Cor).

All antibodies used were either well established in the literature, for which validation in the model system (mouse or human)

was previously determined. We present a novel antibody in this study as well, and we validated this using KO models, mutated protein studies where the phosphorylation site is deleted.

Validation

All of the commercial antibodies were previously well described and validated. The antibody for S1365 TSC2 phosphosphorylation obtained from NovoPro had not been previously reported. Validation was performed in MEFs lacking the protein, with re-expression of the protein with and without site mutations at the S1365 that impact phosphorylation of the residue (e.g. S1365A which prevents it), and then with and without kinase activation (PK). These validation studies are presented in the manuscript. Each commercial antibody was validated by the manufacturer as noted at their website. The information for this validation is listed for each antibody used in the study in a separate Antibody Validation Table Document.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

We used mouse embryonic fibroblasts (standard cell line, or MEFs with TSC2 knocked-out) provided from the laboratory of Brendan Manning. These have been used in many prior studies and are well established models. We also generated a new HEK TSC2 KO cell line and present these methods and line details in the revision. Other cells are primary isolated neonatal ventricular myocytes from rat - so not a cell line per se. Methods for this preparation are well established and referenced.

Authentication

We did not perform specific authentication procedures for the cells used in this study, as the cell lines are well established and widely used, and the primary isolations are not cell lines.

Mycoplasma contamination

Our cultured cells are assessed for evidence of bacterial contamination in a more general manner, as part of maintenance of our incubator systems and being sure we have not infectious contamination. However, we did not specifically test for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

None were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

For all in vivo studies the mice were aged around 2-3 months at the time of pressure overload or sham surgeries. Wild type C57 black mice were purchased from Charles River. TSC2 SA and SE knock in mice were designed by our lab and generated at the Johns Hopkins Mouse Transgenics Core using CRISPR/Cas9 technology. They are also raised in the C57 black background. All protocols and procedures were approved by the Johns Hopkins IUCAC. The studies were in compliance with all ethical regulations. Both male and female mice were utilized in this study.

Wild animals

The study did not involve the use of wild animals.

Field-collected samples

The study did not involve the use of samples collected from the field.

Human research participants

Policy information about studies involving human research participants

Population characteristics

De-identified human tissue was made available to study through an agreement between Johns Hopkins (David Kass, PI) and University of Pennsylvania (Ken Margulies, PI). Patient information including demographics, clinical diagnosis and hemodynamic, treatment, and other relevant clinical parameters are provided. Left ventricular tissue from non-failing control hearts are obtained from organ donors whose heart was not used for transplantation (Gift of Life). The research is approved by both Johns Hopkins and Univ. Pennsylvania respective IRBs. There were two cohorts of samples. For the non-failing controls, the age was 52.8 ± 15.4, 6 males, 6 females, LV function in normal range, and no clinical history of heart failure; for the heart failure group, the mean age was 51.3 ±12.1, 8 males, 4 females, all with severe dilated non-ischemic cardiomyopathy who then all underwent subsequent cardiac transplantation.

Recruitment

The samples are obtained from either deceased patients or patients receiving a heart transplant - the latter are alive and recruited from those subjects receiving transplantation at the University of Pennsylvania. The particular samples reported in this study were randomly taken from a database of many such patients in each group, selected purely based on their inclusion in either the control or heart failure cohort. Informed consent was obtained from failing heart human tissue donors. The family or legal representative provided consent for organ harvesting from deceased donor controls.