nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the 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
☐	☒	A statement on 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 statistical parameters 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	BioRad real-time PCR detection system (TOYOBO). Microscope system (BZ-X, KEYENCE). Echocardiography (Vevo 1100, Fujifilm VisualSonics).
Data analysis	GraphPad Prism 9 (GraphPad Software Inc.). Image J software ver1.53k (National Institute of Health). R (4.1.0). Seurat (4.3.0). scDblFinder (1.6.0).

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All the data are included in the main text, Supplementary information and Source data. Any additional information of data can be available from the corresponding authors on request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A.
Reporting on race, ethnicity, or other socially relevant groupings	N/A.
Population characteristics	N/A.
Recruitment	N/A.
Ethics oversight	N/A.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	Sample size was determined in accordance to standard practices in this field of research.
Data exclusions	No data were excluded.
Replication	All studies were not replicated but they included sufficient numbers to account for biological variability. In addition, multiple techniques and models were used to validate the same findings, each with 3 or more biological replicated.
Randomization	Randomization was not a relevant feature as a uniform set of animal or cultured cells were applied to all experiments.
Blinding	Blinding was not a relevant feature as a uniform set of animal or cultured cells were applied to all experiments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Antibodies to vimentin (1:1000, ab45939), Ki67 (1:1000, ab15580) and phospho-Smad3 (1:100, ab52903) were obtained from Abcam (Cambridge, UK). PKN1(1:1000, sc-1842) and MRTFA (1:1000, sc-21558) were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Antibodies to CD45 (1:100, #70257), PKN2 (1:1000, #2612S), phospho-PKN1/2 (1:1000, #2611S), phospho-p38 MAPK (1:1000,

#4511), p38 MAPK (1:1000, #8690), phospho-MKK3/6 (1:1000, #12280), phospho-SMAD2 (1:1000, #18338), and smad2/3 (1:1000, #8685) were obtained from Cell Signaling Technology (Danvers, MA, USA). We used antibodies to CD31 (1:200, #553370, BD Biosciences, San Jose, CA, USA), PDGFRα (1:1000, AF1062, R &D Systems, Minneapolis, MN, USA), αSMA (1:1000, C6198, Sigma-Aldrich, St. Louis, MO, USA), and DAPI (1:1000, #340-07971, Dojindo, Kumamoto, Japan).

Validation

All antibodies in this study were used and validated according to the provided data sheets and reference for the specific technique (Western blotting, immunostaining) fond directly on the manufacture's website.
1. Vimentin: https://www.abcam.com/products/primary-antibodies/vimentin-antibody-cytoskeleton-marker-ab45939.html
2. Ki67: https://www.abcam.com/products/primary-antibodies/ki67-antibody-ab15580.html
3. phospho-Smad3: https://www.abcam.com/products/primary-antibodies/smad3-phospho-s423--s425-antibody-ep823y-ab52903.html
4. PKN1: https://datasheets.scbt.com/sc-1842.pdf
5. MRTFA: https://datasheets.scbt.com/sc-21558.pdf
6. CD45: https://www.cellsignal.com/products/primary-antibodies/cd45-d3f8q-rabbit-mab/70257
7. PKN2: https://www.cellsignal.jp/products/primary-antibodies/prk2-antibody/2612
8. phospho-PKN1/2: https://www.cellsignal.jp/products/primary-antibodies/phospho-prk1-thr774-prk2-thr816-antibody/2611
9. phospho-p38: https://www.cellsignal.jp/products/primary-antibodies/phospho-p38-mapk-thr180-tyr182-d3f9-xp-rabbit-mab/4511
10. p38: https://www.cellsignal.jp/products/primary-antibodies/p38-mapk-d13e1-xp-rabbit-mab/8690
11. phospho MKK3: https://www.cellsignal.jp/products/primary-antibodies/phospho-mkk3-ser189-mkk6-ser207-d8e9-rabbit-mab/12280
12. phospho-SMAd2: https://www.cellsignal.jp/products/primary-antibodies/phospho-smad2-ser465-ser467-e8f3r-rabbit-mab/18338
13. SMAD2/3: https://www.cellsignal.jp/products/primary-antibodies/smad2-3-d7g7-xp-rabbit-mab/8685
14: CD31: https://www.bdbiosciences.com/ja-jp/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-rat-anti-mouse-cd31.553370
15. PDGFRα: https://www.rndsystems.com/products/mouse-pdgf-ralpha-antibody_af1062
16. αSMA: https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/247/283/c6198dat.pdf
17. DAPI: https://www.dojindo.com/JP-EN/products/D523/

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

C57BL/6 (The Jackson Laboratory, JAX#000664) mice were utilized.
PKN1/2 flox mice and aMHC-PKN floxed mice (https://doi.org:10.1161/circulationaha.119.041019) in a background of C57BL/6 were utilized.
PDGFRα-CreERT2-mice (https://doi.org:10.1002/dvg.22853) in a a background of C57BL/6 were utilized.
All mice were housed at 20-22° and 50% relative humidity in a 12 h light/dark cycle. Mice had free access to water and standard chow (CE-2, CLEA Japan Inc.).

Wild animals

No wild animals were used in this study.

Reporting on sex

Male and Female mice were used for HFpEF heart failure model.
Male mice were used for MI- and AngII-induced heart failure models, because the previous our and other group's studies (https://doi.org/10.1038/s41467-023-42760-y, https://doi.org/10.1038/s41467-023-37832-y, https://doi.org:10.1161/circulationaha.119.041019) were performed using male mice and be easy to confirm the relevance of study results.
In discussion, we mention that further research is warranted to confirm the role of PKN1/2 in sex differences.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All animal procedures were approved by the Institutional Animal Care and Use Committee of Nagoya University School of Medicine.

Note that full information on the approval of the study protocol must also be provided in the manuscript.