

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input type="checkbox"/>	<input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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	For data collection, we used the Visual ASonics Vevo 2100 with MS400 linear array transducer machine for echocardiographic image acquisition, the Illumina NovaSeq 6000 sequencer for RNA sequencing, and the Moxi GO II (Orflo) with the "Apoptosis (Annexin V – FITC&PI)" app for apoptosis measurement. For immunofluorescence staining, we utilized the Revolve Microscope with ECHO Revolution™ Software. Endothelial barrier function analysis data was collected using the ECIS Zθ device by Applied Biophysics. For qRT-PCR, data collection was performed using the CFX Connect Real-time System (Bio-Rad)
Data analysis	Western Blot Quantification was performed using Image J software, while Luciferase Reporter Assays were conducted using the SpectraMax ID3 plate reader. For RNA-seq, the RNA integrity was confirmed using a Cytation3 microplate reader (BioTek, VT), and the mRNA profiling was conducted using the Illumina NovaSeq 6000 sequencer at the University of Chicago Genomics Facility (Chicago, IL). Libraries were prepared using the Illumina TruSeq Small RNA Sample Preparation Kit (RS-930-1012, Illumina, CA). Gene feature counting was done using "featureCounts," and differential gene expression detection was performed using edgeR, DESeq2, and Limma-voom. Pathway and network analysis for gene sets enrichment were conducted using clusterProfiler and GsorViz. Echocardiography analysis was conducted using Vevo LAB software, and ex vivo tubule formation was analyzed using Image J software. Statistical analysis was performed using Prism version 8.0.

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](#)

Provide your data availability statement here.

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](https://www.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

In our study design, most experiments were performed at least in triplicate, which generally ensures biological reproducibility in cell culture and most in vitro studies. For in vivo studies, we used an initial sample size of 25-30 mice per group: Control HIF2 α flox/flox and ecHIF2 α -/-, both with sham operation or under MI, measured at three different time points. This approach was taken to ensure the reliability and robustness of our findings. The sample size was chosen based on standard practices in the field to provide sufficient statistical power and reproducibility.

Data exclusions

1. Death during or immediately after surgery
2. Failure of the ligation to induce a sufficient MI.
3. Signs of severe illness or distress during the experiment

Replication

All experiments for Western blot, fluorescence, RT-PCR, tubule formation, permeability assays, luciferase activity, and in vitro studies, including supplemental experiments, were performed in duplicates. We validated our findings by repeating the experiments on different dates to ensure reproducibility.

Randomization

The animals have been randomized to treatment groups to minimize bias and ensure validity of the results.

Blinding

In our study, blinding was implemented to minimize bias. For in vivo experiments, echocardiography measurements were conducted without knowledge of the animals' genotyping, using coded identifiers. For in vitro experiments, a third party labeled samples and reagents with unique codes, ensuring researchers were unaware of the treatment assignments during data collection and analysis. Blinding was only broken after all data were collected and analyzed.

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		Methods	
n/a	Involved in the study	n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies	<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines	<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology	<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms		
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data		
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern		
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants		

Antibodies

Antibodies used	The primary antibodies used in Western blotting were IL6 (#P620, Invitrogen, 1:2000), VE-Cadherin (#sc-9989, Santa Cruz, 1:500), ARNT (#5537, Cell Signaling, 1:500), GAPDH (#5174, Cell Signaling, 1:5000), HIF1A (#NB100-479, Novus, 1:500), HIF2A (#NB100-122, Novus, 1:500), and ZO-1 (#sc-33725, Santa Cruz, 1:1000). HRP-conjugated anti-rabbit IgG (#31460, Invitrogen, 1:10000) or anti-mouse IgG (#62-6520, Invitrogen, 1:10000) were used as the secondary antibodies. Alexa Fluor 594 Phalloidin (A12381, 1:1000), Alexa Fluor 488
Validation	These antibodies are commercially validated and available. (anti ve-Cadherin, https://www.scbt.com/p/ve-cadherin-antibody-f-8?gclid=Cj0KCQjwspKUBhCvARIsAB2IYut91HGfacI5qpcavgI049nnMfnjlt_c6l4L6eyDiOM4jEnOAw8VF0aAqRsEALw_wcB); anti HIF2A, https://www.novusbio.com/products/hif-2-alpha-epas1-antibody_nb100-122?gclid=CjwKCAjwsMGYBhAEEiwAGUXJaTIMQ7v8ct_bp0fG4T1X_V-2KffSI4z5fjcgwAJ06BIsYiUV_F5U0hoCUpMQAvD_BwE&gclidsrc=aw.ds ; Anti GAPDH, https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118 ; Anti IL6, https://www.thermofisher.com/antibody/product/IL-6-Antibody-Polyclonal/P620 ; Anti zo-1, https://www.scbt.com/p/zo-1-antibody-r40-76?requestFrom=search ; Anti ARNT, https://www.cellsignal.com/products/primary-antibodies/hif-1b-arnt-d28f3-xp-rabbit-mab/5537 ; Anti Rabbit IgG, https://www.bio-rad.com/en-us/sku/1721019-goat-anti-rabbit-igg-hl-hrp-conjugate?ID=1721019 ; Anti Mouse IgG, https://www.bio-rad.com/en-us/sku/1706516-goat-anti-mouse-igg-h-l-hrp-conjugate?ID=1706516 ; Anti Alexa Fluor 594 Phalloidin, https://www.thermofisher.com/order/catalog/product/A12381 ; Anti Fluor488, https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008 ; Alexa Fluor 594, https://www.thermofisher.com/order/catalog/product/A12381

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	The cell lines used in this study include isolated human primary cardiac microvascular endothelial cells (hCMVEC) from Cell Biologics, https://cellbiologics.com/index.php?route=product/product&product_id=2341 ; HUVEC from ATCC, https://www.atcc.org/products/pcs-100-013?_gl=1*19p37od*_up*MQ..&gclid=CjwKCAjwsMGYBhAEEiwAGUXJaUr4f543zOShYXTa4jlUxd2qAzZFsSrMlrT2EhGYrxeaMDZh0HGk_xoCWHUQA_VD_BwE ; HEK293T from ATCC, https://www.atcc.org/products/crl-1573 ; HAEC from ATCC, https://www.atcc.org/products/crl-4052
Authentication	The cell lines used in this study underwent authentication procedures as follows: Isolated Human Primary Cardiac Microvascular Endothelial Cells, hCMVEC, (Cell Biologics): Authentication was performed by Cell Biologics, involving morphological analysis, specific endothelial marker expression (CD31, vWF), and functional assays (tube formation on Matrigel). HUVEC (ATCC): The HUVEC cells from ATCC were authenticated by the supplier using short tandem repeat (STR) profiling, karyotyping, and verification of endothelial marker expression (e.g., CD31, vWF). HEK293T (ATCC): The HEK293T cells from ATCC were authenticated by the supplier using STR profiling and karyotyping. HAEC (ATCC): The HAEC cells from ATCC were authenticated by the supplier using STR profiling, karyotyping, and endothelial marker expression (e.g., CD31, vWF). STR profiling was used to ensure the identity of each cell line by amplifying and analyzing specific STR loci, followed by comparison with known reference profiles. This method helps in detecting any cross-contamination and confirms the authenticity of the cell lines used.
Mycoplasma contamination	All cells used in this study were tested and confirmed to be free of mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used in this study.H

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	In this study, we used mice (<i>Mus musculus</i>) of the C57BL/6 background strain, aged 10 to 12 weeks old. Both male and female mice were utilized
Wild animals	No
Reporting on sex	In this study, we used both male and female mice. The sex of the animals was considered in the study design to ensure a balanced representation. Methods Used for Assigning Sex: The sex of the mice was determined by visual inspection of the genitalia at the time of weaning. Random assignment to experimental groups was employed to minimize any potential bias. Data Disaggregation: While both male and female mice were included in the study, the data was not disaggregated by sex in the analysis. As such, sex-specific differences were not evaluated or reported separately in this study. Overall Numbers: Each experimental group consisted of a sample size of n=25-30 mice, encompassing both male and female subjects. Detailed numbers for each experimental condition are provided in the main figures and supplementary materials of the manuscript. Justification for Lack of Sex-Based Analysis: The primary objective of this study was to investigate the overall impact of Hif2 α deletion on myocardial infarction (MI) outcomes. Due to the constraints of sample size and the scope of the study, a detailed sex-based analysis was not performed. Future studies will be designed to specifically address and analyze potential sex-based differences in greater detail. Conclusion: The inclusion of both male and female mice ensures that the findings are broadly applicable, although specific sex-based effects were not examined in this study. Future research will aim to incorporate sex-based analyses to further elucidate any differential impacts.
Field-collected samples	All laboratory works conducted with animals were maintained under specific conditions to ensure their well-being and the integrity of the experimental results. The housing and maintenance of the animals were carried out in accordance with the relevant institutional guidelines and permits. The animals were kept in a controlled environment with a temperature maintained at 22 \pm 2°C and a 12-hour light/12-hour dark photoperiod. All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Chicago. The end-of-experiment protocol included humane euthanasia of the animals using CO ₂ asphyxiation, followed by cervical dislocation to ensure death
Ethics oversight	All animal research was conducted in accordance with the National Health and Medical Research Council of America guidelines. The study was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Chicago.

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

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>