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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	Histological images were acquired using Leica Application Suite V3.5.0 acquisition software (Leica) or with a scanner NanoZoomer-2.ORS C110730 and NDP.view 2 V2.7.43 sofware. Fluorescence or immunofluorescence images were acquired using LAS-AF V2.7.3. acquisition software (Leica). Ultrasound data were collected using a Vevo 2100 1.5.0 software (Visual Sonics) qPCR data were acquired using the Bio-Rad CFX Manager 3.1 software.
Data analysis	Images were analyzed and quantified using ImageJ V1.52a software. NDP.view 2 V2.7.43 software was used to visualize images from the scanner. qPCR data were analyzed using the Bio-Rad CFX Manager 3.1 software and further analysis was performed in Excel software. Statistics analysis was performed using Excel and GraphPad Prism 7.05 software. Additional layout was made using Adobe Photoshop software.

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 data generated or analysed during this study are included in this published article (and its supplementary information files). Other relevant datasets generated and/or analyzed during the current study are available from the corresponding authors on reasonable request.

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	The initial estimation of sample size was estimated using software Gpower3.1.94. In brief, with a 0.8 experiment power, a significance level of 0.05, the possible mortality associated to a genotype or treatment and considering that differences around 1 SD would be informative when comparing strains and/or treatments, the estimated number of animales per condition would be 10-20 mice, to divide in the different individual experiments. After this inital estimation, the number of animals were reduced based on the results obtained from previous experiments. The specific numbers of animals used in each type of experiment are described in the corresponding figure legends or throughout the text. Phenotypic sex differences were not observed in this study and, therefore, they are not considered in the study, although we have reported the sexes of the animals in the Source Data file.
Data exclusions	no data was excluded
Replication	Most of the experiments, RNA-seq, etc were performed at least in triplicates
Randomization	No randomization was performed to allocate animals into experimental groups. The random allocation of the mice, either Wild Type versus mutant or females versus males was the result of the Mendelian inheritance. Usually males Floxed/WT CRE positive and females Floxed/ Floxed Cre negative mice were used in the breedings, so 50% of pups were WT, 25% heterozygous and 25% KO.
Blinding	Echocardiographies were recorded and blindingly analysed by CNIC image facility personnel and other measures were checked by at least 2 authors. Moreover, each independent experiment was performed by a different person to avoid bias. ECG parameters measurements were blindingly analyzed by eletrophysiologisc not knowing what samples were WT or KO

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	1) rabbit polyclonal anti-Dhx36 (Proteintech # 13159-1-AP). 1/500-1/1000. 2) mouse monoclonal anti-α-Tubulin (T 6074, Sigma-Aldrich; St. Louis, MO, USA). 1/40000 dilution

3) Goat anti-Human/Mouse/Rat Contactin-2 Antibody (Cat# AF117, R&D Systems): 1/500-1/1000
4) AlexaFluor-568 conjugated Donkey anti-goat IgG (A-11057, Molecular Probes)
5) Anti-PSF. Sigma P2860 . 1/1000
6) anti-Nkx2-5.(Proteintech 13921-1-AP). 1/500 dilution

Validation

All antibodies used were validated in the manufacturer's website for our species and application. Specificity was determined by substituting the primary antibody with unrelated IgG at the same dilutions as the antigen-specific antibodies.
anti-tubulin was used 1:40,000 for immunoblot and anti Dhx36 1:500 for immunoblot and 1:100 of IHC. Anti.Contactin 2 was used 1:250 for IF. Secondary Antibodies were diluted 1:4,000 for western blot and 1:100 for IF or IHC.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HL-1, Hela, HEK293 and C2C12 cell lines used in this study were used only after reception from ATCC or after resucitation from early stocks at low passage number (p10 top).

Authentication

HL-1 cells expressed cardiac tropomyosin and alfa actinin in more than 85% of the cells, so they beat. C2C12 formed myotubes after differentiation at higher than 90% sucess. HeLa and HEK cells were originally certified by ATCC and used only up to 5-8 pases.

Mycoplasma contamination

They were free of mycoplasma tested with the MycoAlert Mycoplasma detection Kit Lonza (#LT07-318)

Commonly misidentified lines
(See ICLAC register)

No misidentified cell line was used

Animals and other organisms

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

Laboratory animals

Laboratory conditional KO mice were used in this study; ages ranging from embryos to 10 month old mice. Dr. Lucile Miquerol provided the Cx40/eGFP tansgenic line, Dr. Yoshikuni Nagamine provided the Dhx36 floxed mice and the acquisition of the cre lines were previously described in Gómez del Arco et al 2016 (doi:10.1016/j.cmet.2016.04.008). The mice were maintained in a mixed C57bl/6-CD1 background.

Wild animals

The study did not involve wild animals

Field-collected samples

The study did not involve samples collected from the field

Ethics oversight

The use of animals was ethically oversighted by the CNIC Animal Experimentation Ethics Committee and licensed by the authorities of the Madrid Region (PROEX 346/15) and conformed to EU Directive 2010/63EU and Recommendation 2007/526/EC regarding the protection of animals used for experimental and other scientific purposes, enforced in Spanish law under Real Decreto 53/2013.

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