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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	• Echocardiography was done using a commercially available ultrasound system with a 5 MHz probe (Vivid-q, GE Healthcare, Horten, Norway). • EKG recordings were acquired using a Vmed Vetcheck telemetry system. • Hamamatsu Nanozoomer whole slide scanner was used to get the whole slide images. The images from the scanner is viewed with NDP software (NDP.view 2.6.13). • Microscopic histological images were taken using NIS Elements Version 5.00. • Activation mapping was performed using the CARTO III system with either a 7-French NaviStar B- or D-curve catheter or a 7-French D-curve PENTARAY catheter (Biosense Webster Inc, South Diamond Bar, CA, USA)
Data analysis	• Statistical analysis was performed using Graphpad Prism software version 7. • The images from Hamamatsu Nanozoomer whole slide scanner is viewed with NDP software (NDP.view 2.6.13). • Image J (version 1.51s) was used to view and adjust brightness/contrast of the histological images. • Histological images were taken using NIS Elements Version 5.00. • Cardiac MRI analysis was done using Intellispace Portal version 9.0, Philips, Best, Netherlands.

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

This study did not generate genomic data sets. Most of the data associated with the study have been included in the main figures and table, or the supplementary figures and tables. Any other datasets generated during and/or analysed during the current study are available from the corresponding author on 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	Our principal endpoint was ejection fraction, determined by MRI. We performed a power analysis using pilot data from infarcted animals. This showed we could detect a 25% increase in ejection fraction with an alpha of 0.05 and a beta of 0.8 using group sizes of 4.
Data exclusions	Several animals were excluded due to complications leading to early euthanasia. The flow chart including every enrolled animal and its fate is presented in Suppl. Fig. 2.
Replication	The nature of primate work does not readily lend itself to doing multiple batches of experiments. We continuously enrolled animals over a 4-year period to complete this project. The results were quite consistent from animal to animal.
Randomization	The animals were not randomized due to the complexities of having a 4-person cell preparation team come in early on the mornings of cell transplantation experiments. We enrolled approximately twice as many cell-treated animals as controls, due to the need to study the arrhythmias induced by the cell transplantation.
Blinding	The MRI scans were performed and evaluated by 3 investigators who were blinded to treatment. There was excellent inter-observer variability. The electrophysiology analyses were performed and analyzed principally by one investigator who was blinded to treatment. A subset of his analyses were repeated by a second blinded reviewer, and there was excellent inter-observer variability.

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

Antibodies

Antibodies used

The following data are presented in a more legible format in Supplementary Table 2.

Antigen Antibody type Company Catalog # Lot # Dilution
cTnI Rabbit monoclonal Abcam Ab52862 GR116627-7 and GR116627-10 1:200
ssTnI Mouse monoclonal Novus NBP2-46170 F001 1:200

cTnT Mouse monoclonal Invitrogen MA5-12960 SH2439467A 1:100
Collagen 1 Goat polyclonal Abcam Ab24821 GR3175980-1 1:200
MLC2a Mouse monoclonal Becton-Dickinson 565496 5082617 1:500
MLC2v Rabbit polyclonal Proteintech 10906-1-AP 39557 1:100
Cav 3 Rabbit polyclonal Abcam Ab2912 GR2939265 1:200
Pan-cadherin Rabbit polyclonal Sigma C3678 030M4772 1:100
Connexin 43 Rabbit polyclonal Sigma C6219 045M4882V 1:200
Desmin Goat polyclonal Santa Cruz Sc-7559 G1014 1:50
CD31 Mouse monoclonal Abcam Ab9498 GR3192748-2 1:20
PCM-1 Rabbit polyclonal Sigma HPA023370 GR304183-6 1:100
Ki67 Mouse monoclonal BD Bioscience BDB558615 7242989 1:20

Validation

Each antibody was validated using established positive and negative controls and titered for an optimal signal:noise ratio. Both human and macaque monkey samples were used to establish which antibodies to use and the specifics of their immunostaining assays.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

We used RUES2 (Rockefeller University) and H7 (University of Wisconsin/WiCell) human embryonic stem cell lines.

Authentication

We used a commercial short tandem repeat assay and matched our results against published standards for both cell lines.

Mycoplasma contamination

All cells were routinely tested for mycoplasma and were negative.

Commonly misidentified lines
(See ICLAC register)

Neither of our cell lines are on the ICLAC registry.

Animals and other organisms

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

Laboratory animals

The subjects in our study were pigtailed macaques, Macaca nemestrina. They were provided by the Washington National Primate Research Center. Both male and female animals were studied. Specific details on gender, age, weight are pasted below. They also can be found in Table 1 of the manuscript in a more legible format.

Control
P14 M 6y 10mo 12.6
P15 F 9y 7mo 11.7
P18 F 10y 9mo 11.4
P20 F 6y 0mo 10.6
Mean 8 11.6
SEM 1 0.4

hESC-CM
P22 F 15y 7mo 7.0
P23 F 11y 6mo 10.7
P24 F 12y 2mo 9.5
P25 F 6y 7mo 5.2
P39 F 14y 6 mo 9.2
Mean 11 8.6
SEM 2 0.9

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cardiomyocytes derived from Rues or H7 embryonic stem cells were dissociated into single cells by consecutive treatment of Liberase TH solution (ROCHE, cat. 5401151001) and TryPLE (ThermoFisher, cat. A1285901) and fixed by 4% paraformaldehyde and incubated with a mouse monoclonal cTnT antibody (clone 1C11, Abcam, cat. ab8295) and isotype control (mouse IgG1, kappa antibody clone B11/6, Abcam, cat. ab91353), followed by incubation with an PE-conjugated goat anti-mouse secondary antibody.
Instrument	BD Accuri C6
Software	BD Accuri C6 software, version 1.0. 264.21
Cell population abundance	Cell populations that had 86 to 98.7% cTnT-positive cardiomyocytes were used for transplantation.
Gating strategy	a) The major cell population which was considered as single cells was gated from forward scatter/side scatter plot of isotype staining. b) The PE channel of the isotype control was gated at approximately 1.0% to be used for cTnT-positive cell gating. c) Use the gating of isotype control to determine population size of cTnT-positive cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

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☐ Initial submission ☐ Revised version ☒ Final submission

MRI Studies Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Experimental design

1. Describe the experimental design.	Cardiac MRI was performed prior to cell injection (2 weeks after myocardial infarction) and at 4 week and 12 week post cell-injection. In vivo imaging studies were conducted on a 3-Tesla Achieva clinical scanner (Philips, Best, Netherlands). Depending on the animal size, two overlapped Flex-M and Flex-L coils (dual-element, approximately 15 and 20 cm diameter, respectively) or an 8-element knee coil was used. Pediatric ECG leads were used for gating of MRI acquisitions. ECG, respiration rate, arterial oxygen saturation and body temperature were monitored continuously.
2. Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.	One animal was scanned in each imaging session. Length of each imaging session was about 2 hours.
3. Describe how behavioral performance was measured.	N/A.

Acquisition

4. Imaging	
a. Specify the type(s) of imaging.	Structural and functional imaging of the heart.
b. Specify the field strength (in Tesla).	3 Tesla
c. Provide the essential sequence imaging parameters.	CINE images were acquired with a RF-spoiled turbo field echo (T1-TFE) sequence that generated 25-30 cardiac phases for 10-12 short axis slices spanning the left ventricle. Acquisition parameters included a repetition time (TR) 3.9 msec, echo time (TE) 1.9 msec, a 15° flip angle (FA), 4 mm slice thickness (no gap), field of view (FOV) 120x120 mm2, 1 mm2 voxel size, 6-8 signal averages, retrospective ECG gating without breath hold. Animals subsequently received an intravenous bolus injection of gadolinium-based contrast agent ProHance (Bracco Diagnostics Inc., Princeton, NJ) 0.2 mmol/kg, followed by a saline flush. At 8 minutes after injection, TI-scout images were acquired for determination of inversion time (TI) to null signal from non-infarcted myocardium. At nine minutes post-injection of contrast agent, phase-sensitive inversion recovery (PSIR) multislice images were acquired at the LV short axis to visualize infarct: TR/TE 7.3/3.5 msec, FA 25°, voxel size 1.3 mm2, FOV 150x150 mm2, slice thickness 4 mm without gap, four averages. This was repeated in the long axis plane. Inversion time range 280-350 msec, adjusted by the operator based on TI-scout values.
d. For diffusion MRI, provide full details of imaging parameters.	N/A.
5. State area of acquisition.	Heart.

Preprocessing

6. Describe the software used for preprocessing.

A standard Philips Intellispace Portal software version is v7.0.1.20482 was used for cardiac analysis (Best, Netherlands).

7. Normalization

   a. If data were normalized/standardized, describe the approach(es).

N/A.

   b. Describe the template used for normalization/ transformation.

N/A.

8. Describe your procedure for artifact and structured noise removal.

ECG gating was complicated by a weak signal and presence of arrhythmias in some infarcted animals. Prospective ECG triggering was used when retrospective gating was not successful or if the scan was abrupted due to arrhythmia. Respiratory gating was not used because of the absence of the plateau segments in respiration waves. No mechanical respiration devices were used. Respiratory motion was noticeable in imaging, but not critical to image quality. Blood flow artifacts are common in the infarcted animals. Blood flow correction techniques are not always effective in fast heart rates.

9. Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

N/A.

Statistical modeling & inference

10. Define your model type and settings.

Univariate and multivariate statistics were used.

11. Specify the precise effect tested.

Paired t-test statistical method was used to compare means for the same studied animals imaged in different time points. For comparison of two means of the different studied groups, a t-test assuming unequal variances was used. One-way ANOVA was used to test the difference in left ventricle dimensions and contractile function between control and cell-treated group. The differences were considered statistically significant with p value less than 0.05.

12. Analysis

   a. Specify whether analysis is whole brain or ROI-based.

Whole heart analysis.

   b. If ROI-based, describe how anatomical locations were determined.

Regional left ventricle wall thickness in end-systole and end-diastole was measured from CINE images in three infarcted slices in the middle of the infarcted area and a border zone and then averaged. Extension of the infarcted zone was determined from the late gadolinium enhanced images.

13. State the statistic type for inference. (See Eklund et al. 2016.)

N/A.

14. Describe the type of correction and how it is obtained for multiple comparisons.

N/A.

15. Connectivity

   a. For functional and/or effective connectivity, report the measures of dependence used and the model details.

N/A.

   b. For graph analysis, report the dependent variable and functional connectivity measure.

N/A.

16. For multivariate modeling and predictive analysis, specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.