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	No commercial, open source, or custom code was used to collect the data in this study.
Data analysis	GERP (Genomic Evolutionary Rate Profiling), phastCons, phyloP (http://genome.ucsc.edu), SIFT (Sorting Intolerant From Tolerant) and PolyPhen-2 (Polymorphism Phenotyping v2) were used to predict conservation and deleteriousness scores for receptor variants. Harmony High Content Imaging and Analysis Software (PerkinElmer) was used to analyse high content imaging experiments. EBDA and LIGAND components of the KELL (Kinetic, EBDA, Ligand, Lowry) software package (Biosoft) were used to analyse radioligand binding data. GraphPad Prism version 6.07 for Windows (GraphPad Software) was used to analyse and present numerical data. AlphaFold2 (https://www.readcube.com/library/5d1671d8-5c39-40fd-8d64-7a7d3a0c899e:ccb52d59-1461-4b7a-a85e-1e21631710a1) was used to model apelin receptor variants, using Schrodinger's Maestro suite (Schrödinger Release 2022-2: Maestro, Schrödinger, LLC, New York, NY, 2021.). The binding surface was calculated using GRID from Molecular Discovery (https://pubmed.ncbi.nlm.nih.gov/3892003/). The structure of the apelin receptor StaR bRIL-CMF-019 complex was determined by molecular replacement (MR) with Phaser (McCoy et al., 2007). Model refinement was performed first using phenix.refine65 and further using BUSTER (Global Phasing Limited, Cambridge, UK).

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

The data that support the findings of this study are available from the corresponding author (A.P.D.) upon reasonable 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	Patient identities, and all associated information (sex, gender, age, race, ethnicity etc.), were anonymised and will not be disclosed. This study used anonymised genetic patient data from the 100,000 Genomes Project (Genomics England, Smedley et al., 2021) solely to identify rare variants in the apelin receptor for artificial experimentation in an in vitro setting and the identities of the patients likely has no bearing on the methodology, results, or interpretation of the data.
Reporting on race, ethnicity, or other socially relevant groupings	Patient identities, and all associated information (sex, gender, age, race, ethnicity etc.), were anonymised and will not be disclosed. This study used anonymised genetic patient data from the 100,000 Genomes Project (Genomics England. Smedley et al., 2021) solely to identify rare variants in the apelin receptor for artificial experimentation in an in vitro setting and the identities of the patients likely has no bearing on the methodology, results, or interpretation of the data.
Population characteristics	See above
Recruitment	Participants were not recruited by the authors of this study
Ethics oversight	Ethical approval and written informed consent was obtained from the participants by the National Institute for Health Research (NIHR) BioResource for Rare Diseases as part of the 100,000 Genomes Project

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	For the in vitro biological experiments outlined in this study, n = 3 independent experiments were performed with at least duplicate technical replicates per experiment. These sample sizes are typical for such experimentation and allow for appropriate statistical analysis
Data exclusions	No data were excluded from the analyses
Replication	For the in vitro biological experiments outlined in this study, all attempts at replication were successful
Randomization	This is not applicable to this study as it does not contain any clinical research or data
Blinding	This is not applicable to this study as it does not contain any clinical research or data

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: Primary apelin receptor antibody (Sigma-Aldrich, SAB2700205, 1:50); primary cardiac Troponin T-APC antibody (Miltenyi Biotec, 130-120-54300, 1:50); primary CD90.1 (Thy1.1) antibody conjugated to PE (CD90 (Thy-1) Monoclonal Antibody (eBio5E10 (5E10)), PE, eBioscience (Invitrogen, 12-0909-42), 1:50); secondary antibody Donkey Anti-Rabbit IgG H&L (abcam, ab150073, 1:200)

Validation: The primary apelin receptor antibody (Sigma-Aldrich, SAB2700205) has been used and reported on previously (Georgiadou et al., 2019). The primary cardiac Troponin T-APC antibody (Miltenyi Biotec, 130-120-54300, 1:50) has been validated by the supplier and is deemed suitable for flow cytometry (https://www.miltenyibiotec.com/GB-en/products/cardiac-troponin-t-antibody-anti-human-mouse-rat-reafinity-rea400.html#conjugate=apc:size=100-tests-in-200-ul). The primary CD90.1 (Thy1.1) antibody conjugated to PE (CD90 (Thy-1) Monoclonal Antibody (eBio5E10 (5E10)), PE, eBioscience (ThermoFisher, 12-0909-42)) has been validated by the supplier and is deemed suitable for flow cytometry (https://www.thermofisher.com/antibody/product/CD90-Thy-1-Antibody-clone-eBio5E10-5E10-Monoclonal/12-0909-42),The secondary antibody Donkey Anti-Rabbit IgG H&L (abcam, ab150073) has been validated by the supplier and is deemed suitable for immunocytochemistry applications (https://www.abcam.com/products/secondary-antibodies/donkey-rabbit-igg-hl-alexa-fluor-488-ab150073.html).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): CHO-K1 were sourced from the European Collection of Cell Cultures (ECACC, 85051005). H9 human embryonic stem cells were obtained from WiCell (WA09)

Authentication: None of the cell lines used in this study were authenticated

Mycoplasma contamination: All cell lines tested negative for mycoplasma contamination

Commonly misidentified lines (See ICLAC register): n/a

Plants

Seed stocks: n/a

Novel plant genotypes: n/a

Authentication: n/a

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	Populations of stem cell-derived cardiomyocytes were harvested and pelleted by centrifugation at 300 xg for 3 mins. Pellets were resuspended in PBS supplemented with 0.1 % BSA and 2 mM EDTA (PBE), with CD90 (Thy-1) Monoclonal Antibody directly conjugated to PE diluted at 1:50, for 1 hour at 4 °C. Cells were then washed with PBE and resuspended in Fixation/ Solubilization solution (BD Cytofix/Cytoperm Fixation/Permeabilization Kit, Biosciences) for 20 mins at 4 °C. Following incubation, cells were washed using 1X BD Perm/Wash Buffer (Biosciences) and then resuspended in 1X BD Perm/Wash Buffer containing directly conjugated Anti-Cardiac Troponin T-APC antibody diluted at 1:50 and incubated for 2 hours at 4 °C. Cells were then washed in 1X BD Perm/Wash Buffer, resuspended in PBE and transferred to flow tubes.
Instrument	LSRFortessa Cell Analyzer (BD Biosciences)
Software	FlowJo v10.8.1 software
Cell population abundance	A mean purity of 80 ± 9 % was observed for wild-type stem cell-derived cardiomyocytes determined using positive staining for the cardiac specific marker troponin T
Gating strategy	The gating strategy was the same as that used and reported in Williams et al. 2021 (https://doi.org/10.1038/s42003-021-02453-y); Macrae et al., 2023 (https://doi.org/10.1093/cvr/cvac065)

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