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Corresponding author(s): Christine L. Mummery
Valeria V. Orlova
Milena Bellin

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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 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	N/A
Data analysis	MUSCLEMOTION software for contraction analysis is freely accessible on the web at https://gitlab.com/bjvanmeer/MUSCLEMOTION or https://github.com/l-sala/MUSCLEMOTION/. The license is a GNU GENERAL PUBLIC LICENSE version 3 (GPL v3), which can be found here: https://github.com/l-sala/MUSCLEMOTION/blob/master/LICENSE . After downloading the macro, users can access the code through a text editor or directly through FIJI. When using MUSCLEMOTION, users are encouraged to read Sala, L. et al., 2018 PMID: 29282212 (https://www.ahajournals.org/doi/suppl/10.1161/CIRCRESAHA.117.312067).

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 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

The datasets generated and/or analysed during the current study are available from Giacomelli et al. 2020 and from the corresponding author upon request MUSCLEMOTION software for contraction analysis is freely accessible on the web at https://gitlab.com/bjvanmeer/MUSCLEMOTION or https://github.com/l-sala/MUSCLEMOTION/.

The license is a GNU GENERAL PUBLIC LICENSE version 3 (GPL v3), which can be found here: https://github.com/l-sala/MUSCLEMOTION/blob/master/LICENSE . After downloading the macro, users can access the code through a text editor or directly through FIJI. When using MUSCLEMOTION, users are encouraged to read Sala, L. et al., 2018 PMID: 29282212 (https://www.ahajournals.org/doi/suppl/10.1161/CIRCRESAHA.117.312067).

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 sizes are specified for each data figure where applicable.
Data exclusions	N/A
Replication	N/A
Randomization	N/A
Blinding	N/A

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	Complete data for all antibodies used are included in the Materials section of the manuscript.
Validation	Unstained samples and samples stained with isotype controls were used to validate the reliability of the results.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	hiPSC line LUMC0020iCTRL-06 and hiPSC line LUMC0099iCTRL04 were generated from primary skin fibroblasts using Sendai virus by the LUMC hiPSC core facility
Authentication	hiPSC line LUMC0020iCTRL-06 and hiPSC line LUMC0099iCTRL04 were authenticated at human pluripotent stem cell registry (https://hpscreg.eu/cell-line/LUMCi028-A, https://hpscreg.eu/cell-line/LUMCi004-A)
Mycoplasma contamination	All cell lines were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	The procedures are described in Box1 and Box3 of the manuscript.
Instrument	MACSQuant VYB (Miltenyi Biotech) equipped with violet (405 nm), blue (488 nm) and yellow (561 nm) lasers
Software	Miltenyi Biotech MACSQuantify Software, FlowJo Software
Cell population abundance	Cell populations are defined in data graphs.
Gating strategy	Side and forward scatter gates of the starting cell population were used to exclude dead cells and debris, as well as gating to discriminate doublets and aggregates. Detailed description of gating strategy is described in the Supplementary Information.

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