# nature portfolio

Corresponding author(s): Osama F. Harraz

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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

#### **Statistics**

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	firmed
	×	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. <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> .
×		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 <u>statistics for biologists</u> contains articles on many of the points above.

#### Software and code

 Policy information about availability of computer code

 Data collection
 Laser Doppler flowmetry: LabChart 8 (AD Instruments)

 Patch clamp electrophysiology: Clampex v10
 LSCI: software based on the Basler Pylon SDK (https://github.com/BUNPC/laserSpeckleImaging; https://www.dropbox.com/s/6hklpf33nu1jycf/

 Basler%20pylon%20x64%204.2.2.5468.exe?dl=0; https://www.dropbox.com/s/9kwiopruryq7je7/Speckle%20Recorder%20v2.1%20by%

 20Dmitry%20D%20Postnov.zip?dl=0).

 Data analysis
 Laser Doppler flowmetry was analyzed with LabChart 8 (AD Instruments).

 Electrophysiology was analyzed with commercially available Clampfit 10.3 (Molecular Devices).

 LSCI was analyzed using publicly available MATLAB functions (https://github.com/BUNPC/laserSpeckleImaging).

 Brain sample imaging was analyzed using ImageJ/Fiji (NIH).

 All graphs and statistics were performed using GraphPad Prism 9 (GraphPad software).

 Behavioral tests were analyzed using EthoVision XT 16 software (Noldus Information Technology Inc., The 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 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.

Policy information about <u>availability of data</u>

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 and supporting information are included in the article and the supplementary materials. A Data Source file is provided.

#### Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race</u>, <u>ethnicity and racism</u>.

Reporting on sex and gender	NA
Reporting on race, ethnicity, or other socially relevant groupings	ΝΑ
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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 <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes are similar to those reported in previous publications (e.g., Harraz et al., Circulation Research 2022; Dabertrand, Harraz et al., PNAS 2021). No statistical methods were used to predetermine sample sizes.
Data exclusions	Some data were excluded following pre-established exclusion criteria.
	- I SCI: depending on baseline stability before and after whisker or CO2 stimulation
	- Two-photon imaging: depending on whether arteriolar diameter returned to baseline after whisker stimulation.
Replication	All data were replicated.
Randomization	All experiments were performed in a randomized manner (animals, pharmacological treatments).
Blinding	Whenever possible experimenters were blinded for genotype and treatment. Imaging and analyses of immuno-histology were performed in a blinded fashion. LSCI and behavioral data analysis was performed in a blinded fashion.

## 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

Method	S

n/a	Involved in the study	n/a	Involved in the study
	X Antibodies	×	ChIP-seq
×	Eukaryotic cell lines	×	Flow cytometry
×	Palaeontology and archaeology	×	MRI-based neuroimaging
	× Animals and other organisms		
×	Clinical data		
×	Dual use research of concern		
×	Plants		

#### Antibodies

Antibodies used	<ul> <li>- Anti-caveolin 1 antibody (Cell Signaling Technology, #3267, 1:400, RRID: AB_2275453)</li> <li>- Anti-CD31 antibody (Bio-Rad, #MCA2388, 1:200, RRID: AB_2161026)</li> <li>- Anti-collagen IV antibody (Bio-Rad, #134001, 1:200, RRID: 797 AB_2082646)</li> <li>- Anti-GFAP antibody (Millipore, #AB5541, 1:200, RRID: AB_177521)</li> <li>- Anti-Iba1 antibody (Abcam, #ab5076, 1:400, RRID: 2224402)</li> <li>- Anti-NeuN antibody (Millipore, #MAB377, 1:500, RRID: AB_2298772)</li> <li>- Anti-NF200 antibody (Abcam, #ab8135, 1:1,000, RRID: AB_306298)</li> <li>- Anti-PDGFRβ antibody (Cell Signaling Technology, #3169, 1:100, RRID: AB_2162497)</li> <li>- Anti-α-SMA antibody (Millipore, #C6198, 1:200, RRID: AB_476856)</li> <li>- Anti-goat IgG coupled to Alexa Fluor 647 (Thermo Fisher Scientific, #A-21447, RRID: AB_2535864)</li> </ul>
	<ul> <li>- Anti-rabbit IgG coupled to Alexa 488 (Thermo Fisher Scientific, #A-21206, RRID: AB_2535792)</li> <li>- Anti-chicken IgG coupled to Cy3 (Jackson ImmunoResearch, #703-165-155, RRID: AB_2340363)</li> <li>- Anti-mouse coupled to Alexa Fluor 488 (Life Technologies, #A-21202, RRID: AB_141607)</li> <li>- Anti-rat coupled to Alexa Fluor 647 (Abcam, #ab150155, RRID: AB_2813835)</li> </ul>
Validation	<ul> <li>All antibodies are commercially available:</li> <li>Anti-caveolin 1 : Monoclonal rabbit antibody, specific in immunoblotting according to manufacturer</li> <li>Anti-CD31 : Monoclonal antibody used by numerous studies according to manufacturer, validated by the staining pattern</li> <li>Anti-collagen IV : Used by 4 publications according to manufacturer, validated by the staining pattern</li> <li>Anti-GFAP : Anti-Glial Fibrillary Acidic Protein Antibody is validated for use in Immunohistological staining according to manufacturer for the detection of GFAP.</li> <li>Anti-Iba1 : Validated by cell morphology</li> <li>Anti-NeuN : detects level of NeuN and has been published and validated for use in immunohistological staining according to manufacturer</li> <li>Anti-AcSMA : Skalli et al. A monoclonal antibody against alpha-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 103, 2787-2796 (1986).</li> </ul>

### 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
 Adult (3–5-month-old) male and female C57BL/6J, Piezo1flox/flox;Cdh5-Cre, Piezo1cx/cx;Cdh5-Cre, Piezo1cx/cx;Slco1c1-Cre, Piezo1flox/flox;Slco1c1-Cre mice were used in this study.

 Wild animals
 No wild animals were used.

 Reporting on sex
 Males and females

 Field-collected samples
 NA

 Ethics oversight
 All experimental protocols used in this study are in accord with institutional guidelines approved by the Institutional Animal Care and Use Committee of the University of Vermont and the University of Maryland.

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

## Plants

Seed stocks	(NA			
Novel plant genotypes	NA			
Authentication	NA			