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## Reporting Summary

X Life sciences

Behavioural & social sciences

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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

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 sam	The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement				
A statement of	n whether measurements were taken from distinct samples or whether the same sample was measured repeatedly				
The statistical Only common to	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	A description of all covariates tested				
A description	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 Give <i>P</i> values as exact values whenever suitable.					
For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings					
For hierarchic	al and complex designs, identification of the appropriate level for tests and full reporting of outcomes				
Estimates of e	ffect 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 c	ode				
Policy information abou	ut <u>availability of computer code</u>				
Data collection	MATLAB R2016b, Femtonics MES v.6.2.4858, Spike2 v.7.12, Olympus Fluoview FVMPE-R, Zen 2.6				
Data analysis	Python 2, Prism 5, RStudio v.1.1.463, ImageJ v.1.51j8, Amira Avizo FEI v.6.5, velocity_from_tif v.02-07-09 (Schaffer Lab)				
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. 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 <u>availability of data</u> All manuscripts must include a <u>data availability statement</u> . 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 data that supports the findings of this study are available from the corresponding author upon request.					
Field-specific reporting					
Please select the one h	elow that is the hest fit for your research. If you are not sure, read the appropriate sections before making your selection				

Ecological, evolutionary & environmental sciences

### Life sciences study design

All studies must dis	sclose on these points even when the disclosure is negative.
Sample size	We did not predetermine sample size but aimed for a sample of 8 independent mice in each group? Using LME as a statistical tool, we could image across multiple sites in each animal increasing the statistical power of our analysis
Data exclusions	In Figure 3, we excluded RBC velocity measurements from precapillary sphincters where there were no diameter changes in response to whisker stimulation, or where there was no precapillary sphincter. Otherwise, we did not exclude data from the analysis.
Replication	We used a sample size (independent animals) of at least 8 per sub-study. In different sub-studies, the same sample size applied, effectively adding the total sample size if the same characteristic was under observation
Randomization	In this observational study we did not assess the effect of treatments but rather the different behavior of vessel segments to various treatments. Therefore randomization was not applicable to our study.

Blinding

We did not have blinding in our experiments. First, this is an observational study. Second, we used a particular procedure to induce stimulation, CSD, or cardiac arrest that did not lend to proper blinding. As we did not look at the effect of different treatments (but rather vessel segment differences) blinding was also not applicable to data analyses.

### 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		Methods	
n/a	Involved in the study	n/a	Involved in the study
	Antibodies	$\boxtimes$	ChIP-seq
$\boxtimes$	Eukaryotic cell lines	$\boxtimes$	Flow cytometry
$\boxtimes$	Palaeontology	$\boxtimes$	MRI-based neuroimaging
	Animals and other organisms		
$\boxtimes$	Human research participants		
$\boxtimes$	Clinical data		

#### **Antibodies**

Antibodies used

Primary Antibodies: mouse ACTA2-FITC (1:200; Sigma; F3777), Rabbit anti-collagen-I (1:50; ab34710), Mouse Vitronectin antibody (1:100, R & D systems, #347317), Goat Aminopeptidase N/CD13 Antibody (1:100, R & D Systems, AF2335), Rat MCAM/CD146 Antibody (1:100, R & D Systems, MAB7718), Rabbit Anti-Collagen Antibody, Type IV (1:100, Merck Millipore, AB756P). Secondary antibody: Rabbit Alexa488 (1:500, ThermoFisher SCIENTIFIC), Mouse Alexa488 (1:500, ThermoFisher SCIENTIFIC), Rat Alexa488 (1:500, ThermoFisher SCIENTIFIC), Goat Alexa488 (1:500, ThermoFisher SCIENTIFIC).

Validation

Primary Antibodies:

mouse ACTA2-FITC (1:200; Sigma; F3777).

Details: Monoclonal Anti-Actin, Smooth Muscle-FITC, clone 1A4 Produced in mouse, purified immunoglobulin, FITC conjugate

Source: mouse Isotype: IgG2a

Tested application: immunohistochemistry

Validation Species: rabbit, guinea pig, mouse, chicken, snake, sheep, goat, human, frog, rat, canine, bovine

UniProt accession no: P62736

References:

https://www.sigmaaldrich.com/catalog/product/sigma/f3777?lang=en&region=DK

https://www.sigmaaldrich.com/catalog/papers/8698823

Rabbit anti-collagen-I (1:50; ab34710).

Rabbit polyclonal to Collagen I

Source: Rabbit Isotype: IgG

Tested application: Immunohistochemistry, Immunocytochemistry, ELISA, Western Blot Validation species: Mouse, rat, sheep, goat, horse, cow, human, pig, common marmoset

Database link: P02452

References:

• Yan Y et al. Vascularized 3D printed scaffolds for promoting bone regeneration. Biomaterials190-191:97-110 (2019).

• Wang D et al. Insulin-like growth factor-1 engaged in the mandibular condylar cartilage degeneration induced by experimental unilateral anterior crossbite. Arch Oral Biol 98:17-25 (2019).

Rat Vitronectin antibody (1:100, R & D systems, #347317)

Details: Monoclonal Rat IgG2A, Clone #347317

Source: Rat Isotype: IgG2a

Tested application: immunohistochemistry Validation species: mouse

References:

https://www.rndsystems.com/products/mouse-vitronectin-antibody-347317 mab38751

He L, Vanlandewijck M, Raschperger E, Andaloussi Mäe M, Jung B, Lebouvier T, Ando K, Hofmann J, Keller A, Betsholtz C. 2016. Analysis of the brain mural cell transcriptome. Sci Rep. 6:35108. d Franco M, Roswall P, Cortez E, Hanahan D, Pietras K. 2011. Pericytes promote endothelial cell survival through induction of autocrine VEGF-A signaling and Bcl-w expression. Blood 118:2906-2917.

Goat Aminopeptidase N/CD13 Antibody (1:100, R & D Systems, AF2335)
Details: Antigen Affinity-purified Polyclonal Goat IgG, Catalog Number: AF2335

Source: goat Isotype: IgG

Tested application: Immunohistochemistry Validation species: mouse

References:

https://www.rndsystems.com/products/mouse-aminopeptidase-n-cd13-antibody\_af2335

He L, Vanlandewijck M, Raschperger E, Andaloussi Mäe M, Jung B, Lebouvier T, Ando K, Hofmann J, Keller A, Betsholtz C. 2016. Analysis of the brain mural cell transcriptome. Sci Rep. 6:35108.

Rat MCAM/CD146 Antibody (1:100, R & D Systems, MAB7718)

Details: Monoclonal Rat IgG2A Clone #733216

Source: Rat Isotype: IgG

Tested application: immunohistochemistry in mouse cell line

References: https://www.rndsystems.com/products/mouse-mcam-cd146-antibody-733216\_mab7718

Rabbit Anti-Collagen Antibody, Type IV (1:100, Merck Millipore, AB756P)

Details: Polyclonal to Collagen alpha 1 Type IV (LOT: 2972819)

Source: Rabbit Isotype: IgG

Tested application: immunohistochemistry

References:

http://www.merckmillipore.com/DK/en/product/Anti-Collagen-Antibody-Type-IV,MM NF-AB756P

Secondary antibody:

Rabbit Alexa488 (1:500, ThermoFisher SCIENTIFIC)

Source: goat Isotype: Goat IgG

Tested application: Flow cytometry, Immunohistochemistry, Immunocytochemistry, CHIP assay, Western Blot

Validation species: rabbit

References:

https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/secondary-Antibody-Polyclonal-secondar

A-11008

Recombinant Adeno-Associated Virus Serotype 6 (rAAV6) Potently and Preferentially Transduces Rat Astrocytes In vitro and In vivo. Schober AL,Gagarkin DA,Chen Y,Gao G,Jacobson L,Mongin AA

Mouse Alexa488 (1:500, ThermoFisher SCIENTIFIC)

Source: Goat Isotype: Goat IgG Validation species: Mouse

Rat Alexa488 (1:500, ThermoFisher SCIENTIFIC)

Source: Rabbit Isotype: Rabbit IgG Validation species: Rat

Goat Alexa488 (1:500, ThermoFisher SCIENTIFIC)

Source: Chicken Isotype: Chicken IgG Validation species: Goat

#### Animals and other organisms

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

Laboratory animals

A total of 38 male or female NG2-dsRed mice (Tg(Cspg4-DsRed.T1)1Akik/J; Jackson Laboratory; 19 to 60 weeks old) and 27 male or female wild-type mice (C57bl/6j; Janvier-labs, France; 16 to 32 week) were used.

Wild animals

The study did not involve wild animals

The study did not involve wild animals

The study did not involve samples collected from the field

Ethics oversight

Animal procedures were approved by The Danish National Ethics committee according to the guidelines set forth in the European Council's Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

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