

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

- | | | |
|-------------------------------------|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | 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 Zeiss Zen Blue version 3.2, VisualSonics 3100 Echocardiography System

Data analysis Vevo LAB 5.7.1, QuPath 0.5.1, GraphPad Prism 10.0, FlowJo 10.9.0.
All code used to analyse the data in this manuscript is available publicly at https://github.com/SchapiroLabor/mi_spatialomics.

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](#)

A data availability statement is included in the manuscript. All relevant images and data for Molecular Cartography and SeqIF described in this study are publicly available via Synapse (project SynID : syn51449054): <https://www.synapse.org/#!Synapse:syn54235747>. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD066993.

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

Use the terms *sex* (biological attribute) and *gender* (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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](https://www.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

Molecular Cartography was performed using two biological replicates per time point (i.e., two mouse hearts per time point). In addition, a second slide with consecutive sections from the same mouse hearts was analyzed to replace samples from the first slide that did not pass quality control (see Supplementary Figure 1a and Supplementary Figure 2 for a comparison of the two slides). For the final analysis presented in Figures 1 and 2, only the two biological replicates per time point from these two slides were used. For SeqIF (COMET) experiments, 3 biological replicates were used for controls and 2 biological replicates for 4 hours, 24 hours and 2 days. All of the SeqIF samples as well as the conventional immunofluorescence samples are from different mice than the Molecular Cartography experiments and are therefore completely independent. For in-vivo outcome studies, sample size calculations were performed in G*Power 3.1 in accordance with the approved animal protocol and were based on our experience with comparable experimental studies to achieve 80% power at a significance level of 0.05.

Data exclusions

No exclusion of specific animals from the experiments was performed.

Replication

Since findings were consistent across all technologies, experiments were not repeated due to ethical reasons.

Randomization

Mice were randomly assigned to different experimental groups.

Blinding

Echocardiographic and histological analyses were performed in a blinded fashion if applicable.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

All antibodies used in this studies are listed in supplementary tables 2-4: Trem2 (Abcam ab245227), CD68 (Abcam ab53444), aSMA (Cell Signaling 19245), CD11b (Thermo Fisher 14-0112-82), CD31 (Abcam ab182981), CD45 (Novus Biosciences NB100-77417), Ccr2 (Abcam ab273050), Postn (Abcam ab215199), Pdgfra (Abcam ab203491), Icam1 (Thermo Fisher MA5-43363), LaminB1 (Abcam ab133741), Mpo (Abcam ab208670), Gpnmb (Abcam ab188222), Ankrd1 (Thermo Fisher BS-8074R), Tnnt2 (Thermo Fisher ab209813), WGA-AF555 (Thermo Fisher W32464), anti-CD45-PerCP-Cy5.5 (BD Biosciences clone 30-F11), anti-Ter119-PE (BD Biosciences clone TER-119), anti-CD4-PE (Biolegend clone RM4-5), anti-CD19-PE (BD Biosciences clone 1D3), anti-NK1.1-PE (BD Biosciences clone PK136), anti-Ly6G-PE (BD Biosciences clone 1A8), anti-CD11b-APC-Cy7 (BD Biosciences clone M1/70), anti-F4/80-PE-Cy7 (Biolegend clone BM8), anti-Ly6C-APC (BD Biosciences clone AL-2), vWF (Dako A0082), CD41 (Abcam ab33661), PSGL-1 (BioXCell/Biozol BE0186), CCR2 (Abcam ab273050), CD68 (Abcam ab245227), CD31 (R&D Systems AF3628), anti-Rabbit IgG, Alexa Fluor 555 (Thermo Fisher Scientific A-31572), anti-Goat IgG, Alexa Fluor 555 (Thermo Fisher Scientific A-21432), anti-Rat IgG, Alexa Fluor 647 (Thermo Fisher Scientific A-48272), anti-Rabbit, Alexa Fluor 750 (Abcam ab175728), anti-Rabbit IgG, Alexa Fluor 647 (Thermo Fisher Scientific A-32733), anti-Rabbit IgG, Alexa Fluor 555 (Thermo Fisher Scientific A-32732), anti-Rat IgG, Alexa Fluor 647 (Thermo Fisher Scientific A-48265), anti-Rat IgG, Alexa Fluor 555 (Thermo Fisher Scientific A-48263).

Validation

Antibodies used in this study were from well-established commercial vendors that perform rigorous species-specific QC testing for each lot. Please see details on manufacturers websites (Abcam, Agilent, BD Biosciences, BioXCell, Novus Biosciences, Thermo Fisher Scientific). anti-human vWF antibody A0082 is a validated antibody with reported mouse cross-reactivity (cited in over 1060 publications). For validation of antibodies for SeqIF, performed test stainings at the vendor-recommended concentrations. If staining was too strong or weak, dilution curves were performed, and staining specificity was manually evaluated. The final evaluation was subjective, relying on whether the staining pattern aligned with expectations based on literature and established markers. For challenging markers like Trem2, which tended to form aggregates, larger aggregates were excluded, and coexpression with CD68 was used to confirm specificity. SeqIF requires additional considerations compared to standard immunofluorescence, such as determining the optimal fluorescence channel (e.g., Cy5 or TRITC) and cycle placement. Fluorescence signal acquisition in the Cy5 channel demonstrated higher signal-to-noise ratios and overall cleaner results, whereas the TRITC channel exhibited increased autofluorescence. Of the 16 antibodies tested, 12 were imaged in the Cy5 channel due to the predominance of functional antibodies raised in rabbit (Supplementary Table 2). The panel was finalized after 58 optimization runs, supported by the Lunaphore COMET PA platform for evaluating intensity, sensitivity, elution efficacy, incubation time, antibody dilution, exposure time, and cycle position.

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

C57BL/6Nrf female mice were obtained from Janvier labs (Saint-Berthevin, France) and were studied at 10 - 12 weeks of age. Mice were housed under standard laboratory conditions with a 12h light-dark cycle and access to water and food ad libitum.

Wild animals

No wild animals were used in this study.

Reporting on sex

All experiments in this study were performed in female mice, which is common practice for murine MI models due to higher resilience to cardiac stress. Validation experiments were performed in male mice which confirmed a similar pattern of vWF expression and endocardial monocyte accumulation.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All animal procedures were approved by the institutional review board of the University of Heidelberg, Germany, and the responsible government authority of Baden-Württemberg, Germany (project number G-106/19 and G-94/21).

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

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

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	Single-cell suspensions of infarcted hearts were obtained by mincing the tissue with fine scissors and digesting it with a solution containing 450 U/mL collagenase I, 125 U/mL collagenase XI, 60 U/mL DNase I, and 60 U/mL hyaluronidase (MilliporeSigma) for 1 hour at 37°C while shaking. For flow cytometry of blood samples, erythrocytes were lysed in RBC lysis buffer (Milenyi Biotec). The fluorescent antibodies are described in Supplementary Table 3.
Instrument	Flow cytometry was performed on a FACSVerse (BD Biosciences).
Software	Data was analyzed using FlowJo v10.9.0 software
Cell population abundance	No sorting of certain cell populations was performed.
Gating strategy	Mo/Mφ were identified as CD45+, Lin-(CD19;CD4;NK1.1;Ly6G;Ter119), CD11b+. Neutrophils were identified as CD45+, Lin+(CD19;CD4;NK1.1;Ly6G;Ter119), CD11b+. Lymphoid cells were gated CD45+, Lin-(CD19;CD4;NK1.1;Ly6G;Ter119), CD11b-.

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