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Corresponding author(s): Yonglun Lou, Niels Jessen, Jean Farup

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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	NA
Data analysis	Preprocessing and quality control of spatial-sequencing data Space Ranger1.3.0 (10X Genomics) was used to process the raw sequencing data. The detailed report of sequencing and alignment is available through our data-base website. To ensure the quality of data, the generated gene expression matrix was filtered if the UMI count within one spot was less than 700. Identification of spot clusters and cluster markers After filtering, unsupervised clustering was performed using Seurat (v4.0.3, Satija et al., 2015). Briefly, datasets from different sequencing libraries were merged after SCT transformation. After principal components analysis, clusters were identified via the FindClusters function at resolution 1 with PC1-PC30 in Seurat and subsequently visualized using the RunUMAP function (reduction = "pca"). "FindAllMarker" function implemented in Seurat was used to identify DEGs across clusters with the options "min.pct = 0.25, logfc.threshold = 0.25". Adjust P value of 0.05 was set as a threshold to define significance. Furthermore, cluster annotation was assigned by using known region markers. Gene ontology (GO) enrichment analysis The gene lists were subjected to Gene Ontology analysis using the enrichGO function implemented in the clusterProfiler package (v3.18.1). Gene Ontology enrichment level was evaluated by adjusted P values, and multiple test adjustment was conducted using the Benjamini-Hochberg method. Single-cell RNA sequencing data preparation for decomposition

Briefly, single-cell data from a previously published study (30) were cleaned up by removing debris (gene count <200), doublet (gene count > 4000), and dying cells (mitochondrial gene percentage > 15%). Afterward normalization, log1P transformation, and top 2000 variable gene detection, data from different samples were integrated with FindIntegrationAnchors and IntegrateData function in Seurat. After principal component analysis and identification of clusters, scRNAseq data were annotated based on the previous reported markers.

Decomposition and cell type correlations

Each of the spots were decomposed with the SPOTlight package (v0.1.7). Annotated scRNAseq data were SCT transformed, followed by principal component analysis and dimension deduction with UMAP. Cell type markers were identified with FindAllMarkers function in Seurat and used for decomposition of spatial data with spotlight_deconvolution function using the parameter of (cl_n = 100, hvg = 3000, ntop = NULL, transf = "uv", method = "nsNMF", min_cont = 0). Pearson analysis was used to analyze the cell type correlation in the spot, and p-values and confidence were calculated with a significance test using cor.mtest function in corrplot package (v0.89).

Ligand-receptor analysis

Inferring of cell-cell communication by ligand-receptor analysis was performed using CellChat (v1.1.3) with the scRNA-seq data of human skeletal muscle (30). Briefly, we first imported the normalized expression matrix from the scRNA-seq data and created a CellChat object using the 'createCellChat' function. Next, we preprocessed the data by identifying overexpressed genes and interactions with the 'identifyOverExpressedGenes', 'identifyOverExpressedInteraction', and 'projectData' functions. Subsequently, we employed the 'computeCommunProb', 'filterCommunication' (min.cells = 10), and 'computeCommunProbPathway' functions to calculate potential ligand-receptor interactions. Lastly, we utilized the 'aggregateNet' function in CellChat to compute the aggregated cell-cell communication network, providing a comprehensive view of the potential interactions and signaling pathways between distinct cell populations within the studied tissue sections.

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 sequencing data (spot specific gene-count matrix) generated in this study are deposited, available and visualized in DREAMapp (https://dreamapp.biomed.au.dk/HuMdb/).

All other data that support the findings of this study are presented as individual values to allow full transparence and are available in anonymized format upon request to the corresponding author. Source data figures displaying central tendencies are provided in a Source Data file with this paper.

Single nucleus data from a previous trial (43) were downloaded (GSE167186, GSE143704) visualized based on the primary analyses reported in the paper. We utilized this to determine C3 expression in FAPs (PDGFRA enriched fibroblasts, FB) in young (<40 years) versus old (>60 years).

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	Both male and female participants were included in the injury trial (see references in methods to view distribution). We do not have permission to identify individuals of a specific sex on an individual level.
Reporting on race, ethnicity, or other socially relevant groupings	Not relevant.
Population characteristics	Analysis of skeletal muscle injury and subsequently regeneration was carried out on skeletal muscle samples from the placebo group of a previously published study (25). In brief, skeletal muscle injury was induced in a dynamometer by 200 involuntary muscle contractions initiated by electrical stimulation of the vastus lateralis part of the quadriceps femoris muscle simultaneously with a greater opposite force applied by the dynamometer arm (eccentric muscle work). Analyses of FAP function and validation of C3 expression in young subjects was carried out in a second cohort of healthy subjects with no muscle injury, no smoking, chemotherapy, no use of glucocorticoids or performance enhancing drugs or chronic diseases.
Recruitment	Participants were recruited through online advertisement, through the university and university hospital websites/social media channels and newspapers.
Ethics oversight	The study was conducted in accordance with the Declaration of Helsinki after approval by the local Research Ethics Committee in Region Midtjylland, Denmark. All participants received oral and written information before written consent was obtained.

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	Sample size was calculated based on primary endpoint estimates as detailed in the online protocol (NCT03754842) at clinicaltrials.gov. All sample sizes for each experiment are detailed in the figure lengend and the majority of data are displayed as individual values to increase transparency whenever possible.
Data exclusions	Only when no data point were obtained to to equipment failure or similar were data points excluded.
Replication	All key experiments were performed with several biological or technical replica.
Randomization	In the initial study-design subjects were randomized to either a placebo or intervention group (NCT03754842). However, in this manuscript we only analyzed the placebo group.
Blinding	Blinding was not possible due to the nature of the muscle injury protocol.

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

CD11c (rabbit anti human, 1:100, cat. no.: ab52632)
CD68 (mouse anti human, 1:100, cat. no.: M0718, Dako Norden, Glostrup Denmark)
PDGFR-α (goat anti human, 1:300, cat. no.: AF-307-NA; RRID: AB_354459, R&D Systems, Bio-Techne)
C3 (rabbit anti human, 1:100, cat. no.: PA5-21349, Thermo Fisher Scientific).
CD56-BV421 (5µl/sample, cat. no.: 562751, BD Biosciences, CA, USA)
CD82-PE-Vio770 (10µl/sample, cat. no.: 130-101-302, Miltenyi Biotec)
CD34-APC (20µl/sample, cat 555824, BD Biosciences)
CD31-PerCPvio 700 (2µl/sample, cat. no.: 700 130-110-673, Miltenyi Biotec)
CD90-PE (3,6µl/sample, cat. no.: 12-0909-42, Invitrogen, ThermoFisher Scientific)
CD45-VioBright FITC (12µl/sample, cat. no.: 130-114-567, Miltenyi Biotec)
CD14-BV605 (5µl/sample, cat. no.: 564054, BD Biosciences)
CD11c-APCvio770 (2µl/sample, cat. no.: 130-113-585, Miltenyi Biotec)
CD206-BV711 (5µl/sample, cat. no.: 740797, BD Biosciences)
CD68 BV421 (5µl/sample, cat. no.: cat. no.: 564943, BD Bioscience).
CD14-magnetic beads (5ul/test, cat. no.: 130-050-201, Miltenyi Biotec)
Complement Factor Bb (mouse anti-human, 1:100, cat. no.: MA528085, Thermo Fisher Scientific)
Donkey anti-rabbit Alexa Fluor 488 (1:500), cat. no.: A21206, Thermo Fisher Scientific
Goat anti-rabbit Alexa Fluor 568 (1:500), cat. no.: A11011, Thermo Fisher Scientific
Goat anti-rabbit Alexa Fluor 647 (1:500), cat. no.: A21245, Thermo Fisher Scientific
Goat anti-mouse Alexa Fluor 488 (1:500), cat. no.: A11001, Thermo Fisher Scientific
Goat anti-mouse Alexa Fluor 568 (1:500), cat. no.: A11004, Thermo Fisher Scientific

Goat anti-mouse Alexa Fluor 647 (1:500), cat. no.: A21236, Thermo Fisher Scientific
Donkey anti-goat Alexa Fluor 568(1:500), cat. no.: A11057 Thermo Fisher Scientific

Validation

https://www.abcam.com/en-us/products/primary-antibodies/cd11c-antibody-ep1347y-c-terminal-ab52632?srsltid=AfmBOorY9-AVmuCLY7nIg70sbHHd3vc4QkdQdl5oZNMDPIBdPeq5db5e
https://www.citeab.com/antibodies/2414830-m0718-cd68-concentrate
https://www.bio-techne.com/p/antibodies/human-pdgf-ralpha-antibody_af-307-na
https://www.citeab.com/antibodies/109320-pa5-21349-complement-c3-polyclonal-antibodyhttps://www.bdbiosciences.com/en-dk/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-mouse-anti-human-cd34.555824?tab=product_details
https://www.bdbiosciences.com/en-dk/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv421-mouse-anti-human-cd56.562751
https://www.miltenyibiotec.com/DK-en/products/cd82-antibody-anti-human-reafinity-rea221.html#conjugate=pe-vio-770:size=100-tests-in-1-ml
https://www.bdbiosciences.com/en-dk/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-mouse-anti-human-cd34.555824?tab=product_details
https://www.miltenyibiotec.com/DE-en/products/cd31-antibody-anti-human-reafinity-rea730.html#conjugate=pe-vio-670:size=100-tests-in-200-ul
https://www.thermofisher.com/antibody/product/CD90-Thy-1-Antibody-clone-eBio5E10-5E10-Monoclonal/15-0909-42
https://www.miltenyibiotec.com/UN-en/products/cd45-antibody-anti-human-reafinity-rea747.html#conjugate=fitc:size=100-tests-in-200-ul
https://www.bdbiosciences.com/en-dk/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv605-mouse-anti-human-cd14.564054
https://www.miltenyibiotec.com/UN-en/products/cd11c-antibody-anti-human-reafinity-rea618.html#conjugate=pe-vio-670:size=100-tests-in-200-ul
https://www.bdbiosciences.com/en-dk/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv711-mouse-anti-human-cd206.740797?tab=product_details
https://www.bdbiosciences.com/en-dk/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv421-mouse-anti-human-cd68.564943?tab=product_details
https://www.miltenyibiotec.com/DK-en/products/cd14-microbeads-human.html#130-050-201
https://www.thermofisher.com/antibody/product/Complement-Factor-Bb-Antibody-clone-10-09-Monoclonal/MA5-28085
https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206
https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11011
https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21245
https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001
https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11004
https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21236
https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11057

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Mouse C2C12 cell line, CRL-1772, ATCC, https://www.atcc.org/products/crl-1772
Authentication	Differentiation into myotubes upon serum starvation
Mycoplasma contamination	Rutinely tested
Commonly misidentified lines (See ICLAC register)	NA

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completedCONSORT checklist must be included with all submissions.

Clinical trial registration	NCT03754842; NCT04998500
Study protocol	clinicaltrials.gov
Data collection	Data were collected from 2019-2024 at Aarhus University Hospital, Denmark.
Outcomes	Endpoints for NCT03754842: • Satellite cell count assessed via histology on muscle biopsies. Secondary Endpoints • Activation and presence of muscle stem cells, fibro/adipogenic progenitor cells, mitochondrial markers and function, inflammatory response, and fibrosis markers. • Muscle strength measured using a dynamometer. • Muscle soreness following electrical stimulation and eccentric exercise. • Fat content in muscle and liver assessed using MR spectroscopy. • Blood glucose levels post-NR/PT supplementation and muscle injury. • Bone density measured using DXA scanning. PPrimary Endpoint • Satellite cell count assessed via histology on muscle biopsies. Secondary Endpoints • Activation and presence of muscle stem cells, fibro/adipogenic progenitor cells, mitochondrial markers and function, inflammatory response, and fibrosis markers. • Muscle strength measured using a dynamometer. • Muscle soreness following electrical stimulation and eccentric exercise. • Fat content in muscle and liver assessed using MR spectroscopy. • Blood glucose levels post-NR/PT supplementation and muscle injury. • Bone density measured using DXA scanning. Primary endpoint is reported elsewhere: Jensen JB, Dollerup OL, Møller AB, Billeskov T, Dalbram E, Chubanava S, et al. A randomized placebo-controlled trial of nicotinamide riboside+pterostilbene supplementation in experimental muscle injury in elderly subjects. Journal of Clininal Investigation - Insight. 2022 Endpoint for NCT04998500 : Primary Endpoints • FAPα concentration in blood and adipose tissue • Quantification of FAP cells in adipose tissue and in vitro determination of proliferation and fibro-/adipogenic differentiation potential • Fibrosis in adipose tissue assessed microscopically and immunohistochemically, and via deuterium oxide (heavy water)-determined connective tissue protein synthesis Secondary Endpoints • Temperature measurement • Indirect calorimetry and substrate oxidation • Isotope-determined lipolysis • Heavy water-determined protein synthesis in muscle tissue for comparison with adipose tissue Primary outcome is to be reported in 2025-2026, as per guideline in the ethical approval.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

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

Part of the biopsy for fluorescence activated cell sorting (FACS), were immediately put into a C-tube (cat. no.: 130-093-237, Miltenyi Biotec, Lund, Sweden) containing ice cold wash buffer (HAMS F10 incl. glutamine and bicarbonate (cat. no.: N6908, Sigma-Aldrich, Denmark) + 1% Penicillin-Streptomycin (cat. no.: 15140122, Gibco, ThermoFisher Scientific, MA, USA) + 10 % horse serum (cat. no.: 26050088, Gibco, ThermoFisher Scientific, MA, USA). The C-tube was weighted before and after the biopsy was added to obtain total milligram of skeletal muscle. Before digestion, Collagenase II (cat. no.: 46D16552, Worthington, Lakewood, NJ, USA) and Dispase II (cat. no.: 04 942 078 001, Roche Diagnostics, Basel, Switzerland) were added to the C-tube making a final concentration of 700 U/ml and 3.27 U/ml, respectively. The biopsy was then digested using a gentleMACS (cat. no.: 130-096-427, Miltenyi Biotec) for 60 min at a skeletal muscle program (37C_mr_SMDK1). Afterwards, 10 µl of wash buffer was added before filtering through a 70 µm cell strainer. The cell strainer was washed twice and the cell suspension was centrifuged at 500g for 5 min before supernantant was removed and the pellet was frozen in 1 ml Cryobrew (cat. no.: 130-109-558, StemMACS, Miltenyi Biotec) before further analysis. Biopsies were obtained pre-injury as well as 2h, 2, 8, and 30 days after injury. FAP quantification from one individual from the injury study was excluded from further analysis due to exceptional high FAP count in the pre sample.

Skeletal muscle tissue for ex vivo studies (C3 ELISA and C3 staining of isolated cells, Macrophage subset isolation to generate conditioned media) was obtained from donors undergoing leg amputation at Aarhus University Hospital, Aarhus, Denmark. The biopsies were obtained from the amputated part as close to the vital amputation line as possible.

Monocytes from peripheral blood for ex vivo studies of monocytes and macrophages were obtained from three healthy male donors.

Samples were thawed in a 37 deg C water bath and added 10 ml of wash buffer before centrifuged at 500g for 5 min. The supernatant was removed, and the pellet was resuspended in 400 µl wash buffer and added:

- FcR-block (20µl/sample, cat. no.: 130-059-901, Miltenyi Biotec)
- Anti-CD56-BV421 (5µl/sample, cat. no.: 562751, BD Biosciences, CA, USA)
- Anti-CD82-PE-Vio770 (10µl/sample, cat. no.: 130-101-302, Miltenyi Biotec)
- Anti-CD34-APC (20µl/sample, cat 555824, BD Biosciences)
- Anti-CD31-PerCPvio 700 (2µl/sample, cat. no.: 700 130-110-673, Miltenyi Biotec)
- Anti-CD90-PE (3,6µl/sample, cat. no.: 12-0909-42, Invitrogen, ThermoFisher Scientific)
- Anti-CD45-VioBright FITC (12µl/sample, cat. no.: 130-114-567, Miltenyi Biotec)
- Anti-CD14-BV605 (5µl/sample, cat. no.: 564054, BD Biosciences)
- Anti-CD11c-APCvio770 (2µl/sample, cat. no.: 130-113-585, Miltenyi Biotec)

The suspension was incubated at 5 deg C for 30 min protected from light before washed in 10 ml wash buffer and centrifuged at 500g for 5 min. The supernatant was removed, and the pellet was resuspended in wash buffer and filtered through a 30 µm cell strainer. For viability staining, propidium iodide (PI, 10µl/sample, cat. no.: 556463, BD Bioscience) was added before cell sorting. For absolute quantification, CountBright™ counting beads (10µl/sample, cat. no.: C36950, ThermoFisher Scientific) were used together with total milligram of tissue.

For flow-cytometry analysis of primary human macrophages, the cells were fixed in 4% PFA, permeabilized (cat no.: 554723, BD Biosciences), blocked in FcR-block (Miltenyi Biotec) and stained with antibodies against; CD11c-APCvio770 (Miltenyi Biotec), CD206-BV711 (5µl/sample, cat. no.: 740797, BD Biosciences) and anti-CD68 BV421 (5µl/sample, cat. no.: cat. no.: 564943, BD Bioscience).

MuSCs were quantified and sorted as CD31-CD45-CD34-CD56+CD82+, FAP as CD31-CD45-CD34+, monocytes as CD45+CD14 +CD11c+/- and endothelial cells as CD45-CD31+. Lymphocytes were defined as CD45+ with small size and granularity (Forward-scatter low, Side-scatter low). A 4-laser FACSAria III (405 nm, 488 nm, 561 nm, 633 nm) with FACSDiva software v. 8.0.2 (BD Biosciences, San Jose, CA), was used for sorting. Sorting was performed using a 100 µm nozzle at 4 deg C. Cells were sorted into 5 ml FACS tubes containing 500 µl wash buffer. Compensation was performed on single stained beads (eComp Ultrabeads, ThermoFisher Scientific).

Primary human monocytes were isolated from healthy human blood samples using CD14-magnetic beads (cat. no.: 130-050-201, Miltenyi Biotec). In brief, following sample collection, the erythrocytes were lysed in a lysis buffer (cat. no.: 130-094-183, Miltenyi Biotec) for 1 min. Leukocytes were then centrifuged and washed once in PBS. Following this the leukocytes were incubated with anti-CD14-Microbeads or anti-CD14-BV421 (5ul/test, Cat. No.: 564943, BD) in stain buffer (PBS + 2mM EDTA + 1% BSA) for 15 min at room temperature. The cells were then washed twice in stain buffer and applied to a magnetic column (cat. no.: 130-042-201, Miltenyi Biotec). The column was washed x 3 with stain buffer, removed from the magnet, and column retained cells were flushed out. Flow cytometry analysis of flow-through (CD14-) and the retained cells (CD14+) revealed a marked enrichment for CD14+ monocytes (Supplementary Figure 6A). For phagocytosis experiments enriched monocytes were plated in ECM-coated wells (54000/cm2) in Advanced RMPI 1640 medium (cat. no.: 12633012, Gibco, Thermo Fisher Scientific) + 10% FBS (cat. no.: 16000044, Thermo Fisher Scientific) + 50 ng/ml M-CSF (cat. no.: 130-096-491, Miltenyi Biotec) + 1% Penicillin-Streptomycin (cat. no.: 15140122, Gibco, ThermoFisher Scientific, MA, USA) for six days to induce differentiation into macrophages.

Instrument	FACSAria III (405 nm, 488 nm, 561 nm, 633 nm), Bigfoot cell sorter equipped with six lasers (349 nm, 405 nm, 488 nm, 561 nm and 640 nm) and 52 fluorescence detectors (Thermo Fisher, Fort Collins, CO), NovoCyte Penteon flow cytometer equipped with five lasers (349 nm, 405 nm, 488 nm, 561 nm and 637 nm) and 30 fluorescence detectors (Agilent, Santa Clara, CA).
Software	FACSDiva software v. 8.0.2 (BD Biosciences, San Jose, CA), SQS software (v. 1.9.4, Thermo Fisher, Fort Collins, CO) ,FlowJo (v 10.10.0, BD).and NovoExpress (v. 1.6.2, Agilent, Santa Clara, CA)
Cell population abundance	Sorted cell populations were genereally >95% pure and this was rutinely checked during sorting as described in detail elsewhere: Jensen JB, Dollerup OL, Møller AB, Billeskov T, Dalbram E, Chubanava S, et al. A randomized placebo-controlled trial of nicotinamide riboside+pterostilbene supplementation in experimental muscle injury in elderly subjects. Journal of Clininal Investigation - Insight. 2022; Farup J, Just J, de Paoli F, Lin L, Jensen JB, Billeskov T, et al. Human skeletal muscle CD90(+) fibro-adipogenic progenitors are associated with muscle degeneration in type 2 diabetic patients. Cell Metab. 2021;33(11):2201-14 e11.
Gating strategy	MuSCs were quantified and sorted as CD31-CD45-CD34-CD56+CD82+, FAP as CD31-CD45-CD34+, monocytes as CD45+CD14+CD11c+/- and endothelial cells as CD45-CD31+. Lymphocytes were defined as CD45+ with small size and granularity (Forward-scatter low, Side-scatter low).

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