Bone & Joint Research

Supplementary Material

10.1302/2046-3758.134.BJR-2023-0292.R1

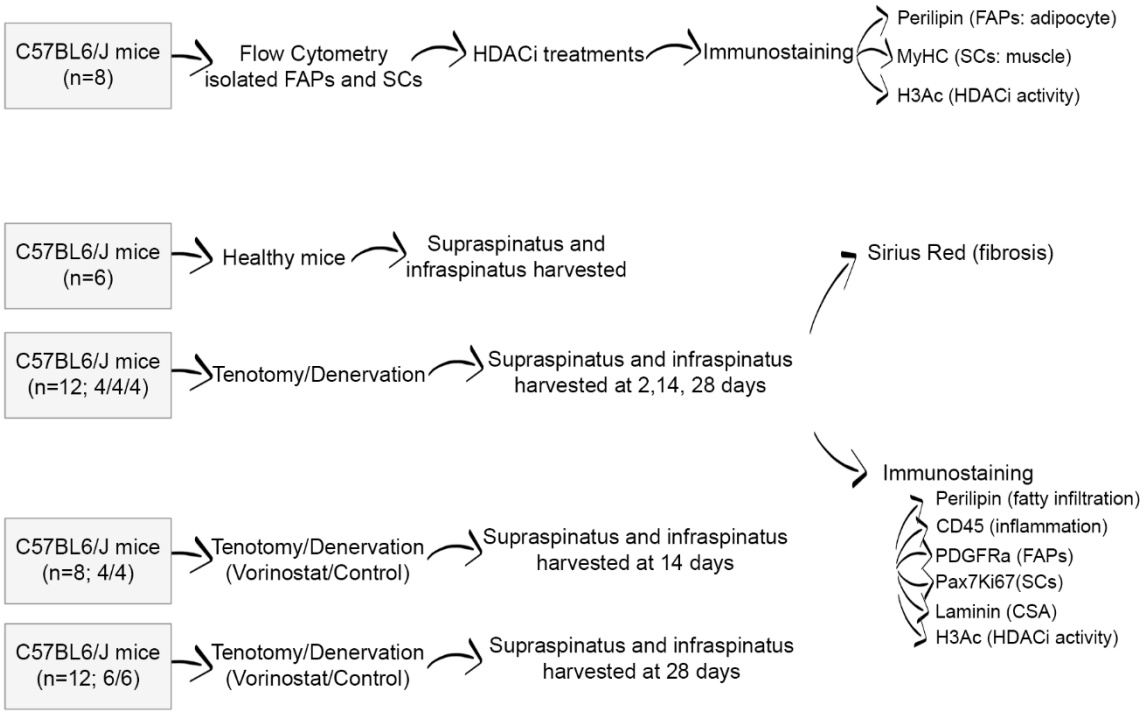

Fig a. Chart depicting the animals used in each set of experiments and the analyses performed. CSA, cross-sectional area; FAPs, fibro-adipogenic progenitors; H3Ac, histone 3 acetylation; HDACi, histone deacetylases inhibitor; MyHC, adult myosin; SCs, satellite cells.

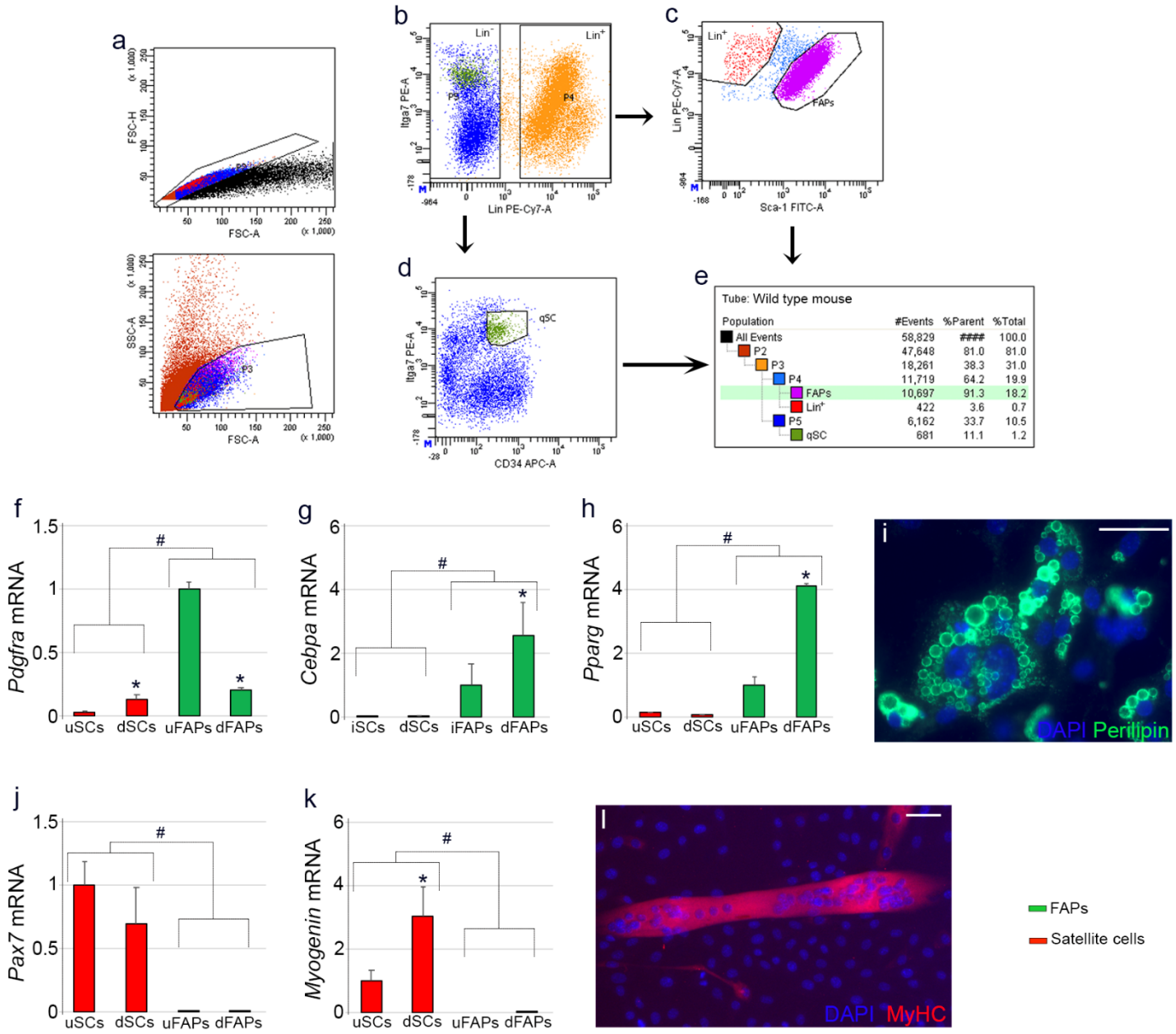

Fig b. a) to e) Flow cytometry gating strategy for sorting fibro-adipogenic progenitors (FAPs) and quiescent satellite cells (qSCs) by FACS is illustrated in panels. a) Muscle-associated cells were initially analyzed for size and complexity to exclude cellular debris and dead cells (gates P2 and P3). Subsequently, these cells were immunostained with specific fluorochrome-labelled antibodies against PE-Cy7-CD31, -CD11b, -CD45, FITC-Sca1, PE-Itga7, and APC-CD34. c) FAPs were sorted from lineage-positive (Lin+) cells (gate P4) as cells expressing Sca1, while d) qSCs were sorted from lineage-negative (Lin-) cells (gate P5) as those co-expressing Itga7 and CD34. e) The gating boxes established for the isolation of FAPs and qSCs. f) to h) Graphs showing the quantification of messenger RNA (mRNA) levels of fibro-adipogenic genes, and j) to k) myogenic genes, related to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), in satellite cells and FAPs at different cellular stages of differentiation. Representative images of FAPs and satellite cells immunostained for i) perilipin and l) adult myosin (MyHC), respectively, are included. 4′,6-diamidino-2-phenylindole (DAPI) was used to identify all nuclei. Scale bar, 10 µm. Measurements are expressed as the mean and standard error of the mean of at least three biological replicates. *Significance between cells of the same origin at different stages of differentiation. '#' defines significance between FAPs and satellite cells ($p < 0.05$). d, differentiated; SCs, satellite cells; u, undifferentiated.

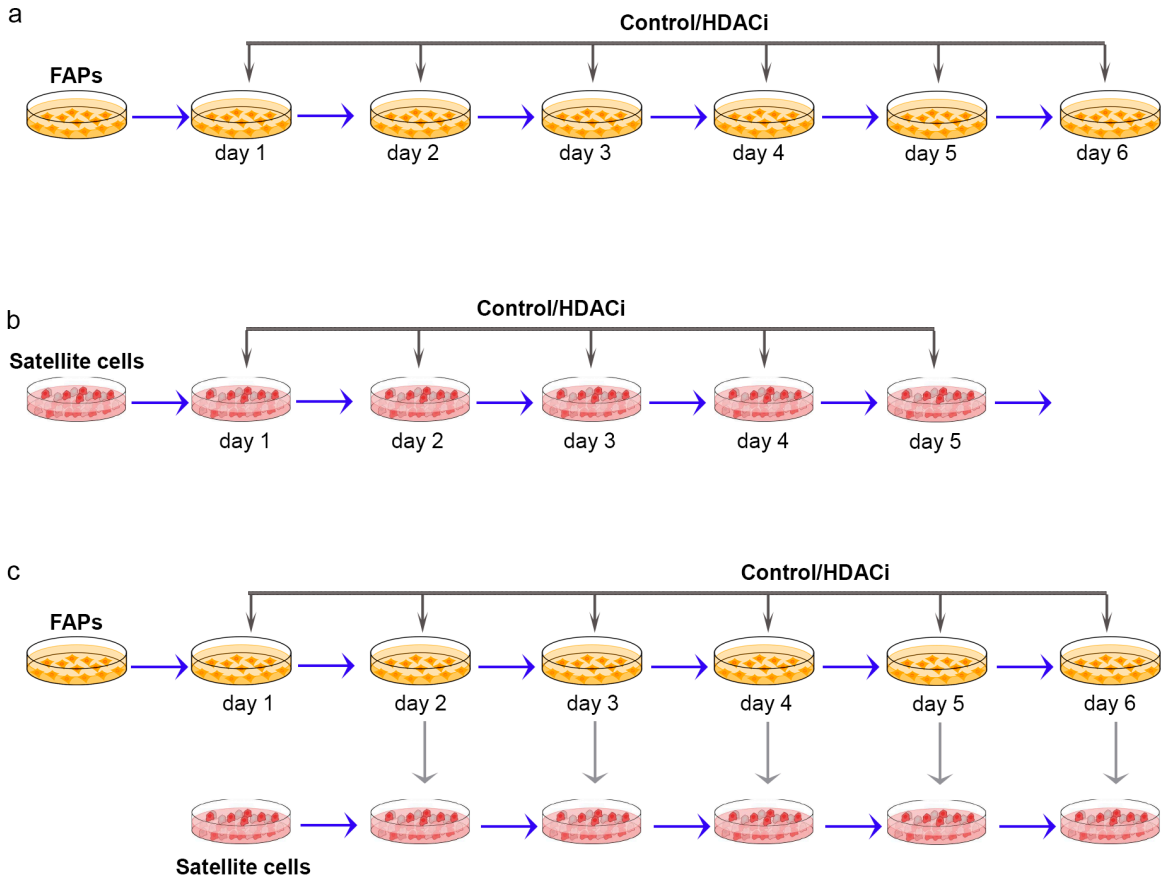

Fig c. a) Fibro-adipogenic progenitors (FAPs) and b) satellite cells cultured with histone deacetylase inhibitors (HDACis) in growth media for six or five days, respectively. c) Supernatant of daily HDACi-treated FAPs was added to satellite cells for five consecutive days. Control cells were treated with dimethylsulfoxide diluted into phosphate-buffered saline and daily added to growth media.

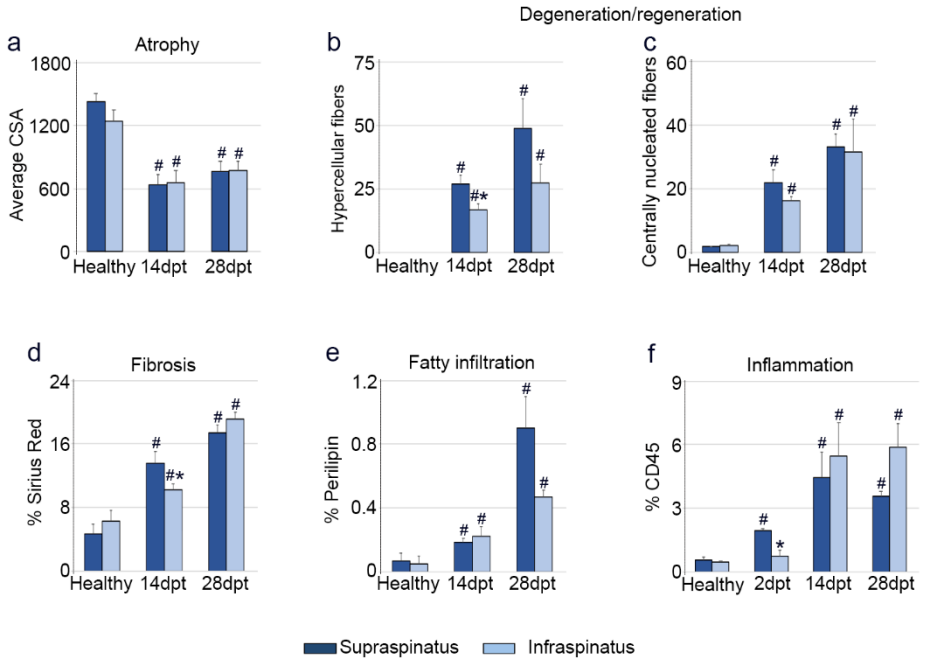

Fig d. Graphs show a) the muscle fibre cross-sectional area (CSA), b) the presence of hypercellular and c) centrally nucleated fibres per mm^2, the accumulation of d) fibrotic and e) fat tissues, and f) the presence of cellular infiltration in the supra- and infraspinatus muscles from healthy and rotator cuff (RC)-injured mice. Fibre CSA was measured in μm^2. Data were expressed as the mean and standard error of the mean of at least four biological replicates per condition. *Statistically significant differences between supraspinatus and infraspinatus muscles, where $p < 0.05$. '#' defines significance between healthy and RC injured mice. dpt, days post-tenotomy.

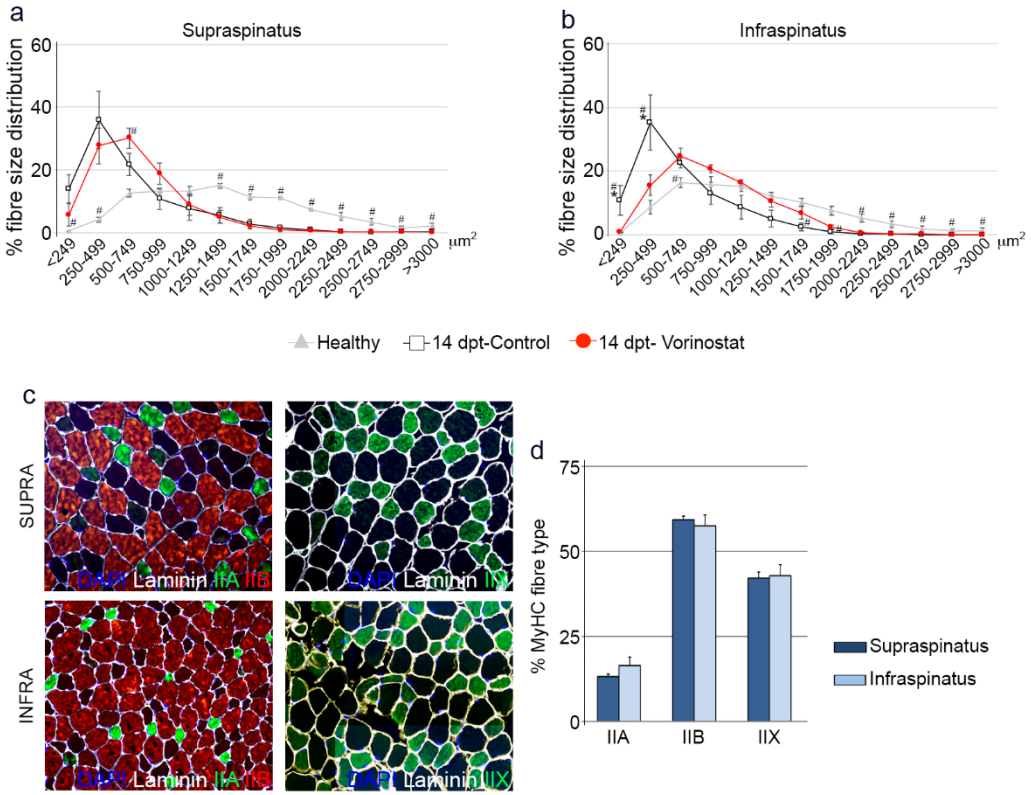

Fig e. Fibre size distribution of the a) supra- and b) infraspinatus muscles from healthy and vorinostat-treated/untreated rotator cuff-injured mice. Data were expressed as the mean and standard error of the mean of at least four biological replicates per condition. Representative images of c) supra- and infraspinatus muscles isolated from three healthy adult mice immunostained for laminin and MyHC isoform IIA and IIB or IIX, and d) quantification of fibre type II composition. *Significance between vorinostat-treated and control-treated injured mice. '#' defines significance between healthy and injured mice, where $p < 0.05$. DAPI, 4',6-diamidino-2-phenylindole; dpt, days post-injury; IIA, fibre type IIA; IIB, fibre type IIB; IIX, fibre type IIX; INFRA, infraspinatus; SUPRA, supraspinatus.

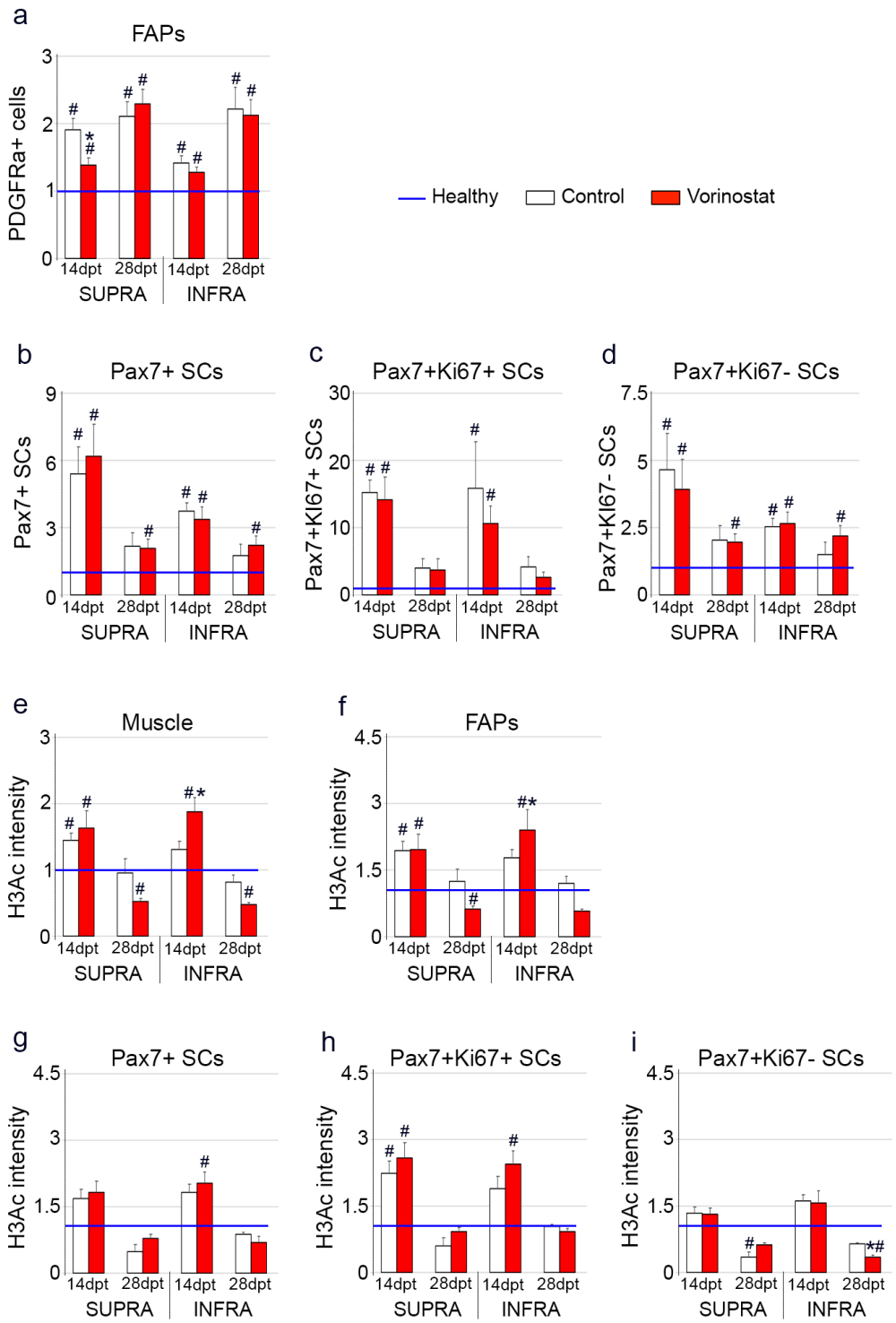

Fig f. Quantification of the number of a) fibro-adipogenic progenitors (FAPs) and b) total Pax7+, c) proliferating, and d) quiescent satellite cells in the supra- and infraspinatus muscles from injured mice treated with or without vorinostat for 12 or 26 days, two days after tenotomy. Mean intensity of H3Ac was measured in e) the muscles, f) FAPs, and g) Pax7+ satellite cells, and specifically in the Pax7+ h) proliferating and i) quiescent muscle stem cells. Values were expressed as the mean and standard error of the mean of at least four biological replicates per condition, where expression levels were related to those found in healthy muscles, which were considered as 1 (blue line). *Significance between control and vorinostat-treated injured mice. '#' defines significance between healthy and damaged mice, where $p < 0.05$. dpt, days post-injury; H3Ac, histone 3 acetylation; INFRA, infraspinatus; PDGFR, platelet-derived growth factor receptor α; SCs, satellite cells; SUPRA, supraspinatus.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including:	
		a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.	Methods
		b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.	Methods; Supplementary Figure a
		b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Methods
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly.	No animal was excluded
		b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.	No experimental group, animal, or experimental unit was excluded
		c. For each analysis, report the exact value of *n* in each experimental group.	Methods; Supplementary Figure a
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.	Randomization was not applied
		b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Methods
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Methods
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).	Methods
		b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Fatty infiltration
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used.	Methods
		b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.	Methods
		b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Methods
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:	Methods
		a. What was done, how it was done and what was used.	Methods
		b. When and how often.	Methods
		c. Where (including detail of any acclimatisation periods).	Methods
		d. Why (provide rationale for procedures).	Methods
Results	10	For each experiment conducted, including independent replications, report:	Results; Figure and Supplementary Figure legends
		a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).	not applicable
		b. If applicable, the effect size with a confidence interval.	

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction Discussion
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Abstract
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Methods
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Methods
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Methods Methods Methods section. The human endpoints were included in the ethical animal protocol 039-18. They were not applied.
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Results; Discussion Discussion
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Discussion
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	Yes. Ethical animal protocol 039-18, which was approved and authorized.
Data access	20	Provide a statement describing if and where study data are available.	Data sharing
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	ICMJE COI statement Funding statement