Bone & Joint Research

# Supplementary Material

10.1302/2046-3758.111.BJR-2021-0059.R1

**Table i.** Primer sequences for polymerase chain reaction.

| Gene | Forward | Reverse |
|---|---|---|
| *human GAPDH* | GACAGTCAGCCGCATCTTCT | TTAAAAGCAGCCCTGGTGAC |
| *human RUNX2* | TCAACGATCTGAGATTTGTGGG | GGGGAGGATTTGTGAAGACGG |
| *human OCN* | CCACCGAGACACCATGAGAG | TCAGCCAACTCGTCACAGTC |
| *human PPARG2* | GCAAACCCCTATTCCATGCTG | CACGGAGCTGATCCCAAAGT |
| *human LPL* | CAAGAGTGAGTGAACAAC | AATTATGCTGAAGGACAAC |
| *human COL2* | GGCAATAGCAGGTTCACGTACA | CGATAACAGTCTTGCCCCACTT |
| *human SOX9* | ACACACAGCTCACTCGACCTTG | AGGGAATTCTGGTTGGTCCTCT |
| *human ACAN* | TCGAGGACAGCGAGGCC | TCGAGGGTGTAGCGTGTAGAGA |
| *human MMP-13* | GACTGGTAATGGCATCAAGGGA | CACCGGCAAAAGCCACTTTA |
| *human IL-6* | ACTCACCTCTTCAGAACGAATTG | CCATCTTTGGAAGGTTCAGGTTG |
| *rat GAPDH* | AGACAGCCGCATCTTCTTGT | CTCGTGGTTCACACCCATCA |
| *rat TIMP-1* | CTGGCATCCTCTTGTTGCTATCATT | CTTATAACCAGGTCCGAGTTGCAGA |
| *rat TIMP-2* | GGGACACGCTTAGCATCACC | CATCCAGAGGCACTCATCCG |
| *rat ADAMTS-4* | CAGCCATACCCAGAGCGTCAC | GCATCCGAAACCCTGTCAACT |
| *rat ADAMTS-5* | GTTTCGAGGTGCGGGGTTATT | TCTGCCTGCAAGGGAAATGTG |
| *rat IL-1β* | TTGAGTCTGCACAGTTCCCC | GTCCTGGGGAAGGCATTAGG |
| *rat IL-6* | CCGGAGAGGAGACTTCACAG | CAGAATTGCCATTGCAACAAC |
| *rat IL-8* | TGGCCAGAGAAAGAAGTGCC | TGTCTTCAATCCATCCCAGAGC |
| *rat TNF-α* | GCGTGTTCATCCGTTCTCTACC | TACTTCAGCGTCTCGTGTGTTTCT |

ACAN, aggrecan; ADAMTS, A Disintegrin and Metalloproteinase with Thrombospondin motifs; COL2, type II collagen; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; LPL, lipoprotein lipase; MMP, matrix metalloproteinase; OCN, osteocalcin; PPARG, peroxisome proliferator activated receptor gamma; RUNX2, runt-related transcription factor 2; SOX9, SRY-Box Transcription Factor 9; TIMP, tissue inhibitors of matrix metalloproteinases; TNF-α, tumour necrosis factor alpha.

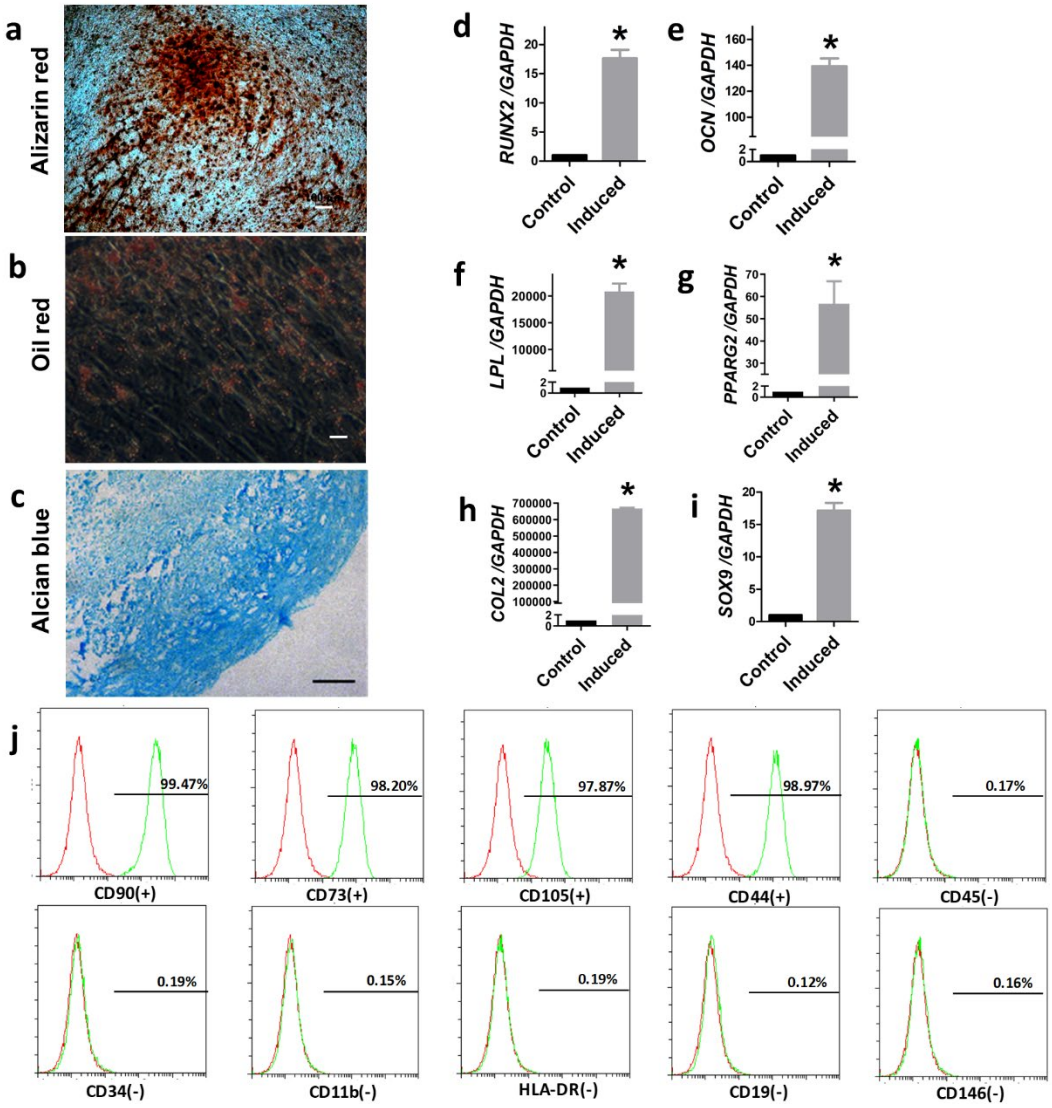


**Fig. a.** Characterization of synovium-derived mesenchymal stem cells (SMSCs) from the temporomandibular joint. a) Alizarin red staining of calcium deposits in SMSCs after osteogenic induction. b) Oil red staining of lipid droplets in SMSCs after adipogenic induction. c) Alcian blue staining of the cartilage matrix in histological sections of cartilage pellets after chondrogenic induction. d) and e) Expression of the osteogenic markers, RUNX family transcription factor 2 (*RUNX2*) and osteocalcin (*OCN*) using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). f) and g) Expression of the adipogenic markers lipoprotein lipase (*LPL*) and peroxisome proliferator activated receptor gamma (*PPARG2*) using RT-qPCR. h) and i) Expression

of the chondrogenic markers type II collagen (*COL2*) and SRY-box transcription factor 9 (*SOX9*) using RT-qPCR. j) Flow cytometric analysis of surface-marker expression on SMSCs. Green: target marker; Red: isotype control group. SMSCs are positive for CD90, CD73, CD105, and CD44, but negative for CD45, CD34, CD11b, human leucocyte antigen (HLA)-DR, CD19, and CD146. Scale bar in a) to c) = 100 μm. Data represent the mean and standard deviation of three experiments. *p < 0.05. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

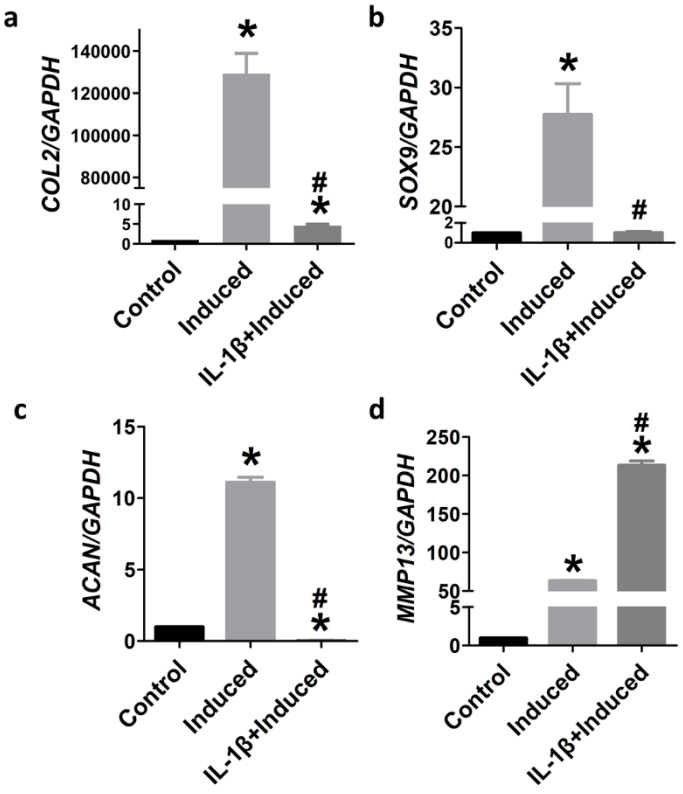


**Fig. b.** Interleukin (IL)-1β inhibits chondrogenic differentiation of synovium-derived mesenchymal stem cells (SMSCs). a) to d) Expression of type II collagen (COL2), SRY-box transcription factor 9 (SOX9), aggrecan (ACAN), and matrix metalloproteinase 13 (MMP13) using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) after SMSCs underwent chondrogenic differentiation for 14 days with or without 10 ng/ml IL-1β treatment. RNA extracted from SMSC-cultured six-well plates without chondrogenic induction is considered the control group. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is used as an endogenous control. Data represent the mean and standard deviation of three experiments, *p < 0.05 compared to the control group. #p < 0.05 compared to the chondrogenic-induced group.

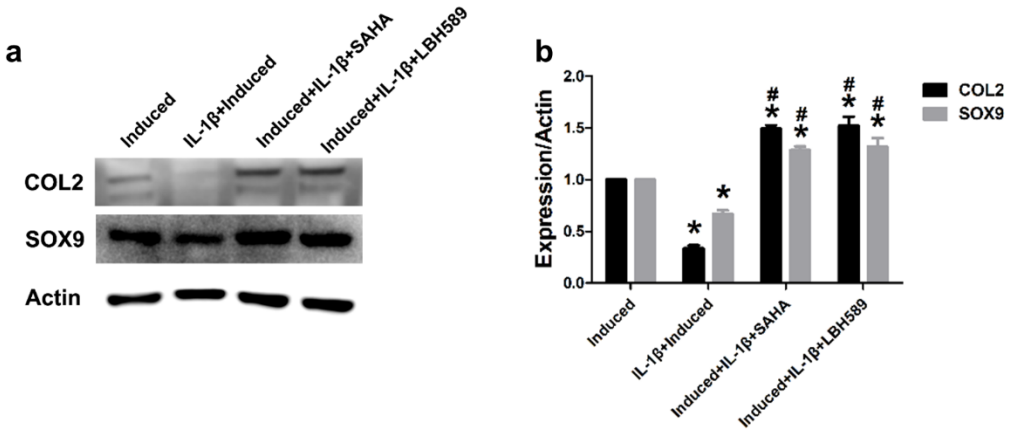


**Fig. c.** Histone deacetylase (HDAC) inhibitor reverses interleukin (IL)-1β-induced inhibition of synovium-derived mesenchymal stem cell (SMSC) chondrogenesis. a) Expression of type II collagen (COL2) and SRY-box transcription factor 9 (SOX9), as estimated by western blotting. Actin is used as an endogenous control. b) Quantification of the blot. *p < 0.05 compared to the induced group. #p < 0.05 compared to the IL-1β-induced group.

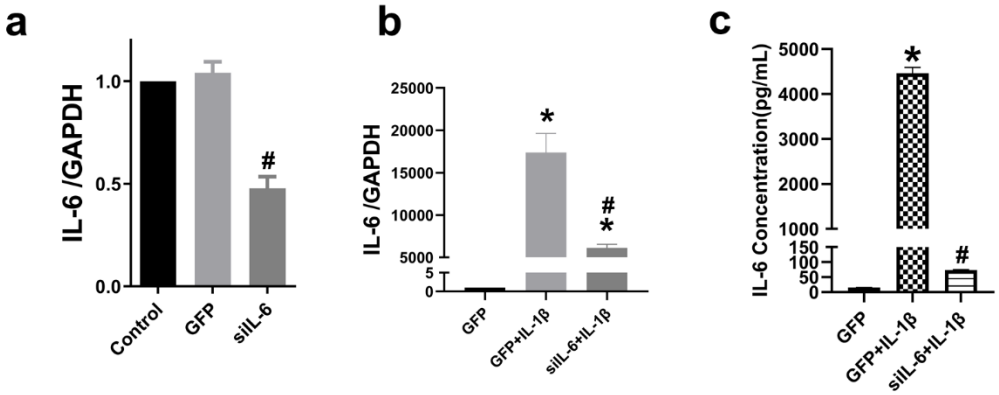


**Fig. d.** Silencing interleukin (IL)-6 could reduce its expression level. a) After synovium-derived mesenchymal stem cells (SMSCs) were transfected with the empty green fluorescent protein (GFP) vector or the knock-down IL-6 GFP vector, the efficiency of silencing IL-6 was determined by reverse transcription quantitative polymerase chain reaction (RT-qPCR). #p < 0.05 compared to the GFP group. b) and c) Expression levels of IL-6 were detected after SMSCs were pre-transfected with the empty GFP vector or the silencing IL-6 GFP vector for 48 hours and then co-treated with 10 ng/ml IL-1β for 24

hours, as detected by RT-qPCR and cytometric bead array (CBA). *p < 0.05 compared to the GFP group. #p < 0.05 compared to the GFP+IL-1β group. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

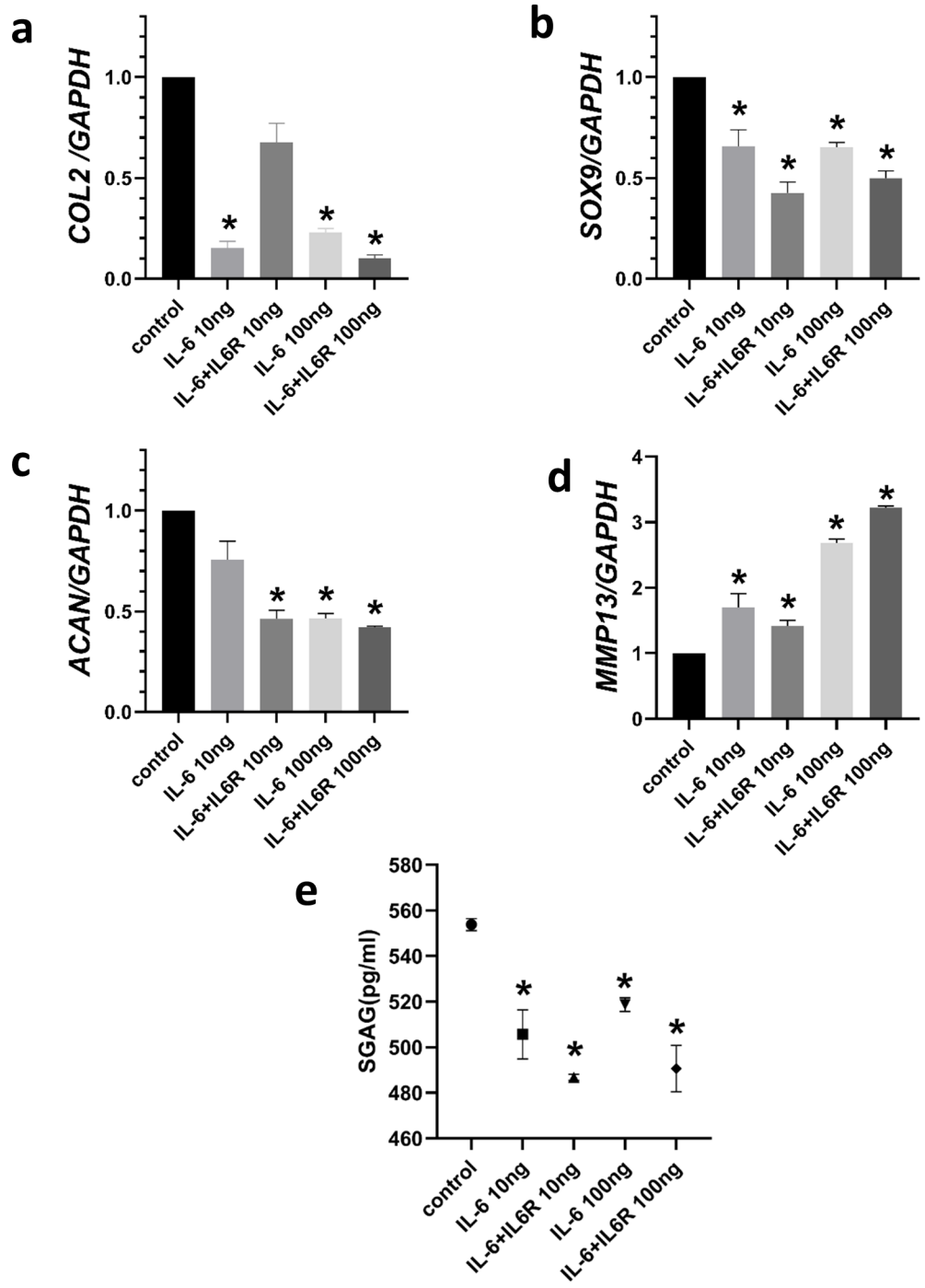


**Fig. e.** Interleukin (IL)-6 inhibits chondrogenic differentiation of synovium-derived mesenchymal stem cells (SMSCs). a) to d) Expression of type II collagen (COL2), SRY-box transcription factor 9 (SOX9), aggrecan (ACAN), and matrix metalloproteinase (MMP)-13 using reverse transcription quantitative polymerase chain reaction (RT-

qPCR) after SMSCs underwent chondrogenic differentiation for 14 days with 10 ng/ml or 100 ng/ml IL-6 with or without the same concentration of IL-6 soluble receptor (IL-6R) treatment. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is used as an endogenous control. Data represent the mean and standard deviation of three experiments, *$p < 0.05$ compared to the control group. e) Soluble sulphated glycosaminoglycan (sGAG) in culture supernatant, as determined by enzyme-linked immunosorbent assay. *$p < 0.05$ compared to the control group.

# 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: | Animal experiments |
| | | a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. | Animal experiments |
| | | b. The experimental unit (e.g. a single animal, litter, or cage of animals). | |
| **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. | Animal experiments |
| | | b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done. | Animal experiments |
| **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. | Animal experiments |
| | | 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. | All animals were included in the analysi |
| | | c. For each analysis, report the exact value of *n* in each experimental group. | Animal experiments |
| **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. | Animal experiments.We used a computer to generate a random number to group the m |
| | | 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. | Animal experiments |
| **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). | Animal experiments |
| **Outcome measures** | 6 | a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). | Animal experiments |
| | | b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. | Animal experiments |
| **Statistical methods** | 7 | a. Provide details of the statistical methods used for each analysis, including software used. | Statistical analysis |
| | | 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. | Statistical analysis |
| **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. | Animal experiments |
| | | b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. | Animal experiments |
| **Experimental procedures** | 9 | For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: | Animal experiments |
| | | a. What was done, how it was done and what was used. | Animal experiments |
| | | b. When and how often. | Animal experiments |
| | | c. Where (including detail of any acclimatisation periods). | Animal experiments |
| | | d. Why (provide rationale for procedures). | |
| **Results** | 10 | For each experiment conducted, including independent replications, report: | result |
| | | a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). | result |
| | | 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.<br>b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology. | Introduction<br>The use of rat OA mo del in this experime nt is an auxiliary e |
| **Objectives** | 13 | Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested. | Article Summary |
| **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. | This study was appro ved by the Instituti onal Ethics Board of the Hospital of Sto matology, Sun Yat-se n University, Guangz |
| **Housing and husbandry** | 15 | Provide details of housing and husbandry conditions, including any environmental enrichment. | sed in a temperature - |
| **Animal care and monitoring** | 16 | a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.<br>b. Report any expected or unexpected adverse events.<br>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. | Animal experiments s etion<br>Animal experiments s etion<br>The intervetion in o ur study do not caus e mass loss of weig |
| **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.<br>b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results. | disccusion setion<br>disccusion setion |
| **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). | disccusion setion |
| **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. | method |
| **Data access** | 20 | Provide a statement describing if and where study data are available. | result |
| **Declaration of interests** | 21 | a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.<br>b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study. | The authors declare that there is no con flict of interests.<br>This study was supported by grants from National Natural |