Supplementary materials for

Nicotinamide mononucleotide enhances fracture healing by promoting skeletal stem cell proliferation

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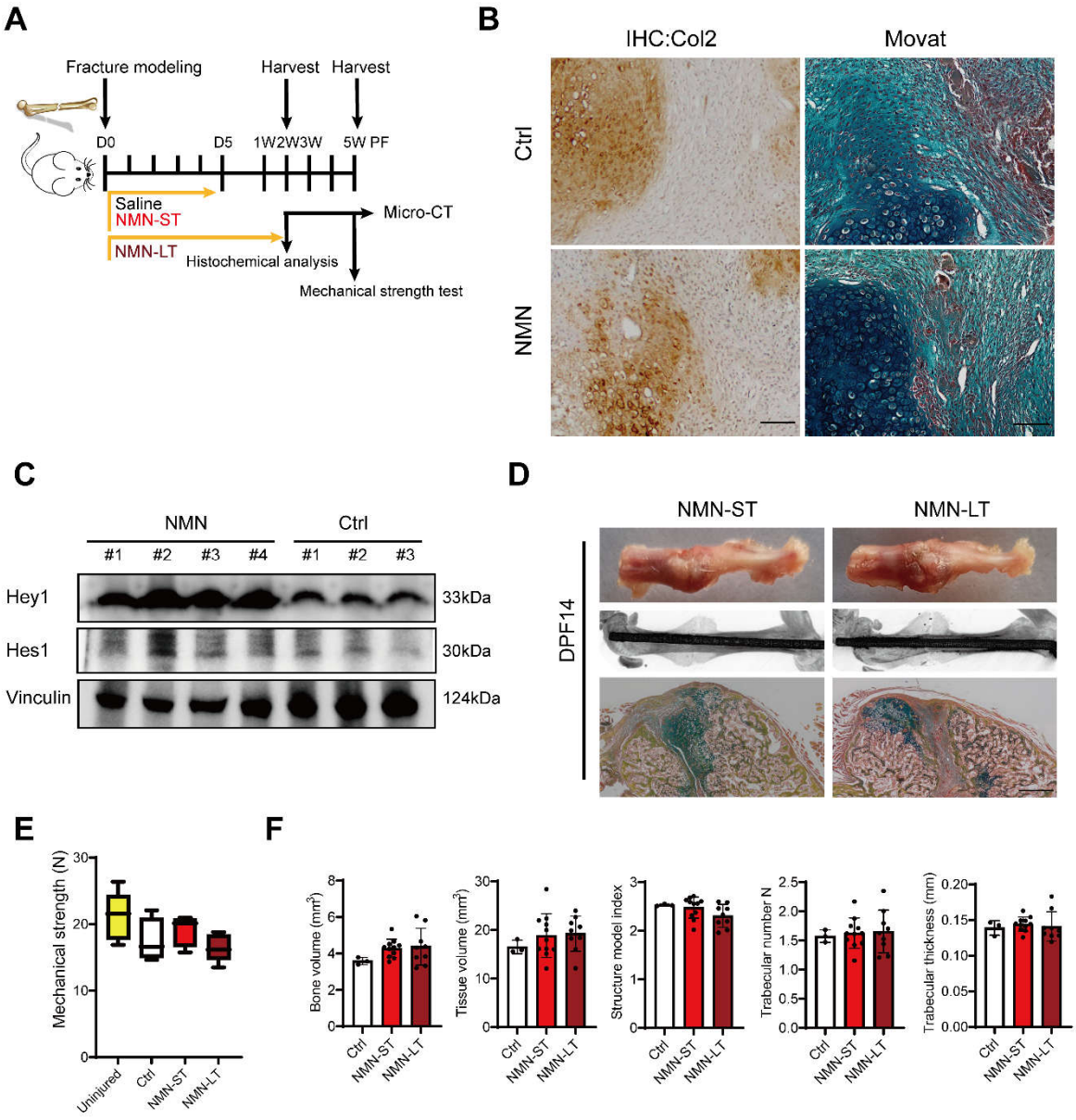

Figure S1. Comparison between long-term and short-term NMN dosing groups for fracture healing.

(A) Schematic illustrating the short-term and long-term administration of NMN, followed by assessment. NMN administration commenced 6 days after fracture in the short-term group and 14 days after fracture in the long-term group.

(B) Characterization of chondrocytes within callus was performed using immunohistochemical staining for type II collagen and Movat staining. Scale bar: 100 μm.

(C) Western blot was used to detect the expression of Hey1 and Hes1 (n = 3-4).

(D) Representative photographs and radiographs of the fractured femur in NMN-ST and NMN-LT group at DPF14 (top and middle). Bottom, images of Movat's pentachrome staining.

(E) Biomechanical assessment of the fractured femur was conducted at DPF35 (n = 4-

6).
(F) Quantification of fracture callus parameters by Micro-CT measurements at DPF14 (n = 3-11).
Data are presented as mean ± SEM; Statistical significance was determined by one-way ANOVA (E, F).

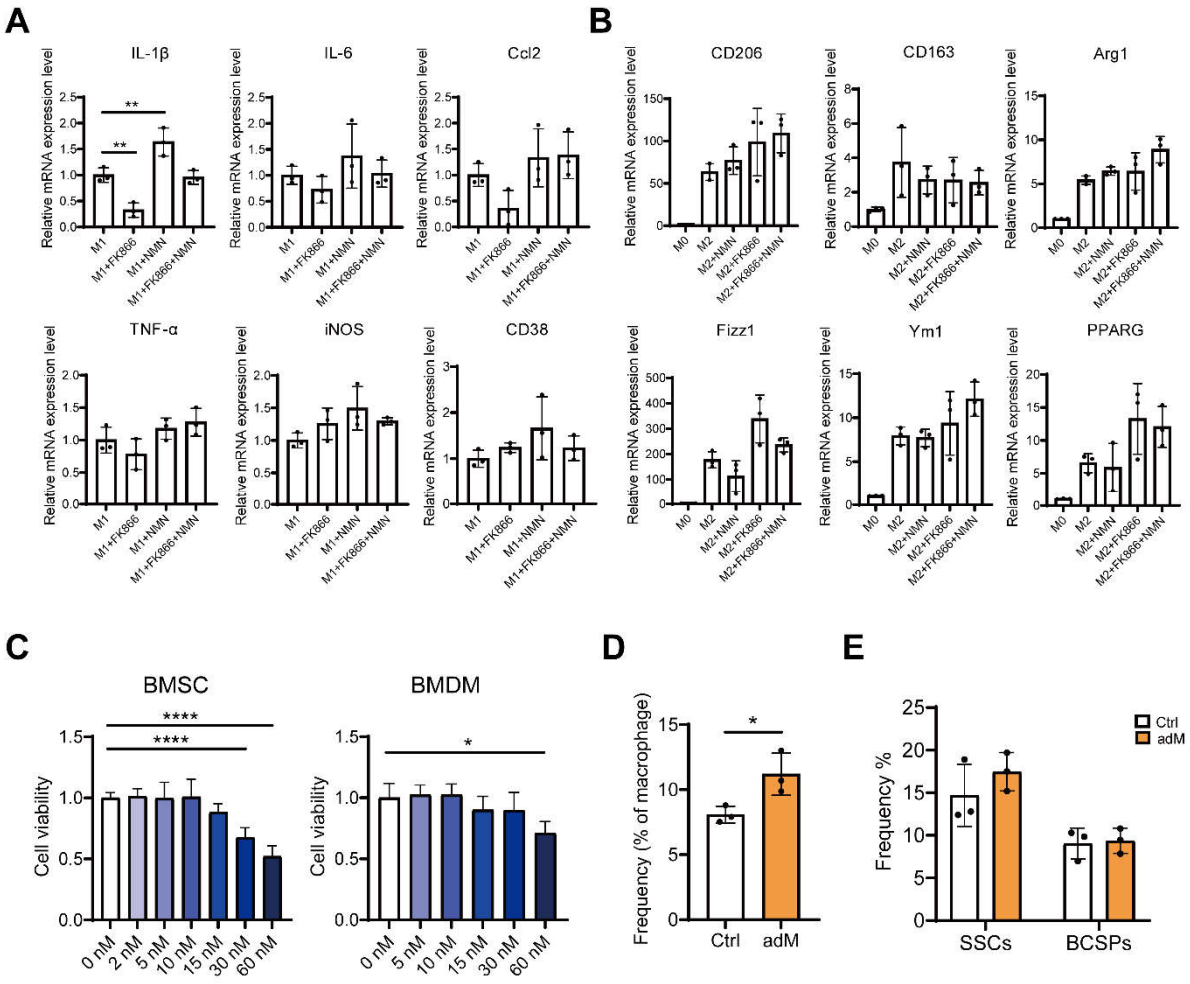

Figure S2. The effect of modulation of macrophage NAD levels on their polarization and activity *in vitro*; changes in the frequency of macrophages and skeletal stem cells in callus generated by the transplantation of macrophages at the time of fracture.

(A) qRT-PCR results show the effect of NMN or FK866 on M1 marker expression during macrophage M1 polarization (n = 3).

(B) qRT-PCR results show the effect of NMN or FK866 on M2 marker expression during macrophage M2 polarization (n = 3).

(C) The results of the CCK-8 toxicity assay showed that FK866 was more toxic to BMSCs than to macrophages (n = 5-6).

(D) Macrophage frequency within the callus was measured at DPF7 when macrophages were transplanted at the time of fracture (n = 3).

(E) Frequency of SSCs and BCSPs within the callus was measured at DPF7 when macrophages were transplanted at the time of fracture (n = 3).

Data are presented as mean ± SEM; Statistical significance was determined by two-tailed unpaired Student's t test (D, E) or one-way ANOVA (A, B, C).

(*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$).

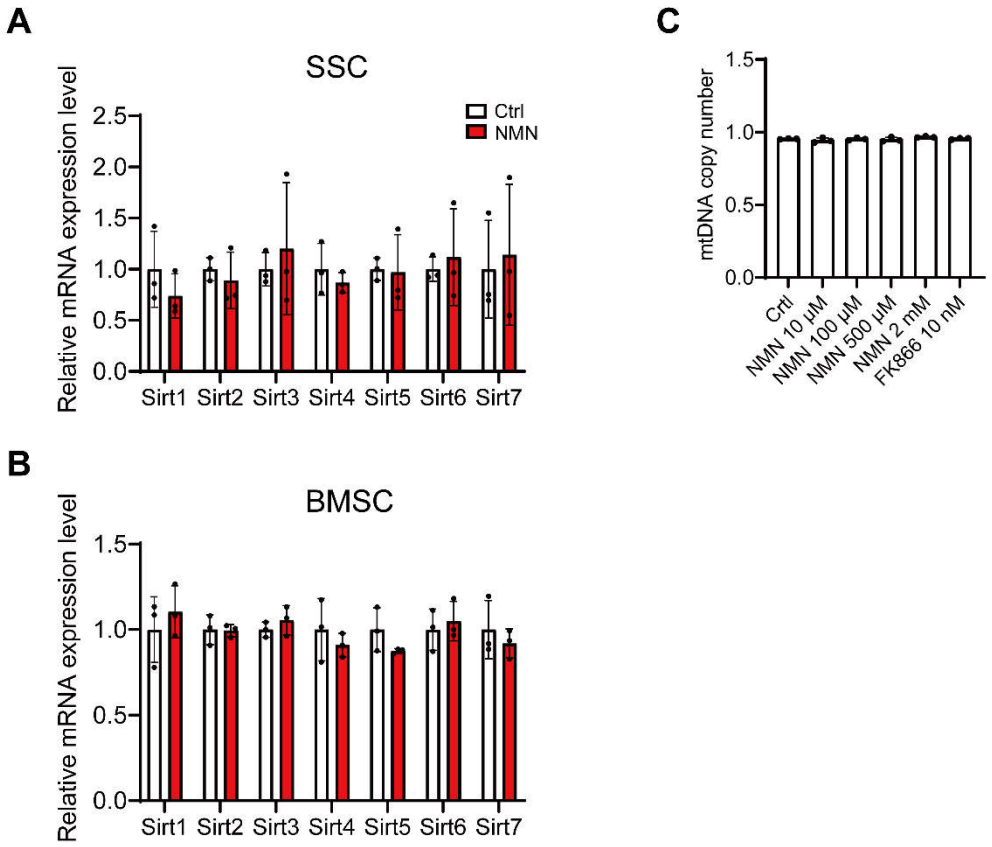

Figure S3. The effect of NMN on the transcript levels of the Sirtuin family and mitochondrial DNA copy number in stem cells.

(A) qRT-PCR results show that NMN does not alter the expression levels of the SIRT family in SSCs (n = 3).

(B) qRT-PCR results show that NMN does not alter the expression levels of the SIRT family in BMSCs (n = 3).

(C) qRT-PCR results show that NMN does not affect the copy number of mitochondrial DNA in BMSCs (n = 3).

Data are presented as mean ± SEM; Statistical significance was determined by two-tailed unpaired Student's t test (A, B) or one-way ANOVA (C).

Table S1: List of primer sequences used for qRT-PCR.

Primer Name	Sequence (5'-3')
β-actin	FOR - AGCCTCGCCTTTGCCGA REV - CTGGTGCCTGGGGCG
Gapdh	FOR - CTACACTGAGGACCAGGTTGTCT REV - TTGTCATACCAGGAAATGAGCTT
Nampt	FOR - GCAGAAGCCGAGTTCAACATC REV - TTTTCACGGCATTCAAAGTAGGA
Nmnat1	FOR - GAAATTGCTGTGTGGGGCAG REV - CCACGATTTGCGTGATGTCC
Nmnat2	FOR - GATGTTCGAGAGAGCCAGGG REV - AAGGCCCTGTTTTCCGTAGG
Nmnat3	FOR - AAGACACCATCAGCCTCTGC REV - CCAAGCCGAACTTCTCCACT
Sirt1	FOR - AGTTCCAGCCGTCTCTGTGT REV - GATCCTTTGGATTCCTGCAA
Sirt2	FOR - CAAGGAAAAGACAGGCCAGACGG REV - CCTGACTGGGCATCTATGTTGGC
Sirt3	FOR - CCCTGTCTGTACTGGCGTTGTGA REV - TCCATCCAGCTTGCCACGTTCC
Sirt4	FOR - TTCTCCTCTCACCAACCCAACCC REV - TTCAGGCAAGCCAAATCGTCAG
Sirt5	FOR - ATGCGACCTCTCCTGATTGCTCC REV - CCTCCCTCCGGTAGTGGTAAAAC
Sirt6	FOR - TGCAACCCACAAAACATGACCG REV - GTATAGGGCTGTTGGGCTTGGAC
Sirt7	FOR - GAGCGAGGATCTGGTGACCGAG REV - CAGGAGGTGCAGACTTCAATATACAT
Parp1	FOR - GGCAAGCACAGTGTCAAAGG REV - TGTCGTTGACACCAGATGGG
PCNA	FOR - TGGAATCCCAGAACAGGAG REV - TCAGAGCAAACGTTAGGTG
Sox9	FOR - AGCTCAACCAGACCCTGAGAA REV - TCCCAGCAATCGTTACCTTC
Hes1	FOR - TGCCAGCTGATATAATGGAG REV - CTTTGATGACTTTCTGTGCTC
Hey1	FOR - ACTACAGCTCCTCAGATAGTG REV - AACTCAAGTTTCCATTCTCGTC
CD115	FOR - TGTGCAAGACCATGGTGAAT REV - TTTTATCTGTGGGGGCTCTG
CD206	FOR - CAAGGAAGGTTGGCATTTGT

	REV - CCTTTCAGTCCTTTGCAAGC
CD163	FOR - TCAGCGACTTACAGTTTCCTC REV - GCCTTTGAATCCATCTCTTG
Arg1	FOR - CTCCAAGCCAAAGTCCTTAGAG REV - GGAGCTGTCATTAGGGACATCA
Fizz1	FOR - CCAATCCAGCTAACTATCCCTCC REV - ACCCAGTAGCAGTCATCCCA
Ym1	FOR - CAGGTCTGGCAATTCTTCTGAA REV - GTCTTGCTCATGTGTGTAAGTGA
IL10	FOR - CAGGGATCTTAGCTAACGGAAA REV - GCTCAGTGAATAAATAGAATGGGAAC
iNOS	FOR - GGAGTGACGGCAAACATGACT REV - TCGATGCACAACTGGGTGAAC
TNF-α	FOR - AGTGACAAGCCTGTAGCCC REV - GAGGTTGACTTTCTCCTGGTAT
Ccl2	FOR - CCAGCAAGATGATCCCAATG REV - TGGTTCCGATCCAGGTTTT
IL6	FOR - TGTATGAACAACGATGATGCACTT REV - ACTCTGGCTTTGTCTTTCTTGTTATCT
IL1	FOR - CTGGTACATCAGCACCTCAC REV - AGAAACAGTCCAGCCCATAC
PPARG	FOR - ACGATCTGCCTGAGGTCTGT REV - CATCGAGGACATCCAAGACA
CD38	FOR - CGAAGGAGCTTCCAGTAACG REV - TGGCAGGCCTGTAGTTATCC
36B4	FOR - ACTGGTCTAGGACCCGAGAAG REV - TCAATGGTGCCTCTGGAGATT
Cytb	FOR - CCCACCCCATATTAAACCCG REV - GAGGTATGAAGGAAAGGTATTAGGG