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Supplemental information

ac4C acetylation of RUNX2 catalyzed by NAT10 spurs osteogenesis of BMSCs and prevents ovariectomy-induced bone loss

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Supplementary data

Table S1: Characteristics of the study subjects.

	Normal control	Postmenopausal osteoporosis Patients
Number	6	6
Age (years)	56.38±6.98	65.45±6.82
Sex	Female	Female
Hight (cm)	156.35±7.49	154.94±5.64
Weight (kg)	56.85±7.68	58.16±5.37
BMI (kg/m^2)	23.79±2.74	22.62±3.53
Age of menarche (years)	14.25±2.04	13.16±1.18
Age of menopause (years)	50.35±3.57	52.43±2.82
Lumbar spine BMD (g/cm^2)	1.53±0.24	0.56±0.31*
Lumbar spine *T* score	0.35±1.07	-2.63±1.16*
Total hip BMD (g/cm^2)	1.15±0.24	0.61±0.17*
Total hip *T* score	0.37±1.12	-1.83±0.58*

Data are shown as the mean ± SD, n=6 in each group. P values for all variables are the result of independent t tests between the control and osteoporosis groups. *$p < 0.05$, **$p < 0.01$. BMI: body mass index; BMD: bone mineral density.

Supplementary Table 2: Primers of the analyzed genes

Species	NCBI Gene ID	Gene Name	Forward Primer	Reverse Primer
Human	2597	GAPDH	AAGGTGAAGGTCGGAGTCAA	AATGAAGGGGTCATTGATGG
Human	55226	NAT10	ATTCACACCGTAAGCAGCGA	CAGGTCATTCGGGGTCTGTC
Mouse	98956	NAT10	CACAAACATTCGCTACTGCTACT	AACGCTTCAAAATCCTGGAGG
Human	860	RUNX2	AAAGACAAGCACAAGTAAATC	CATAATTGAACCCTCTATCCA
Human	4088	SMAD3	ATAGGTGCTTTGGGCGTATG	CTCTTGCCCTTTTCAACTGTCC
Human	3371	TNC	CAAAGATGTCCCAGTGACTGTC	CGCATTGTCCTAAGTTGTTGC
Human	1277	COL1A1	GCCTCAAGGTATTGCTGGAC	ACCTTGTTTGCCAGGTTCAC
Human	121340	Osterix	CCTCTGCGGGACTCAACAAC	AGCCCATTAGTGCTTGTAAAGG

Supplementary Table 3: The siRNA sequences of the analyzed genes.

Gene Name	Sense (5’-3’)	Antisense (5’-3’)
NAT10 siRNA1	GGGCCAGGCUGAACUAGUUTT	AACUAGUUCAGCCUGGCCCTT
NAT10 siRNA2	GCAUUUGGGUACUCCAAUATT	UAUUGGAGUACCCAAAUGCTT
Negative control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

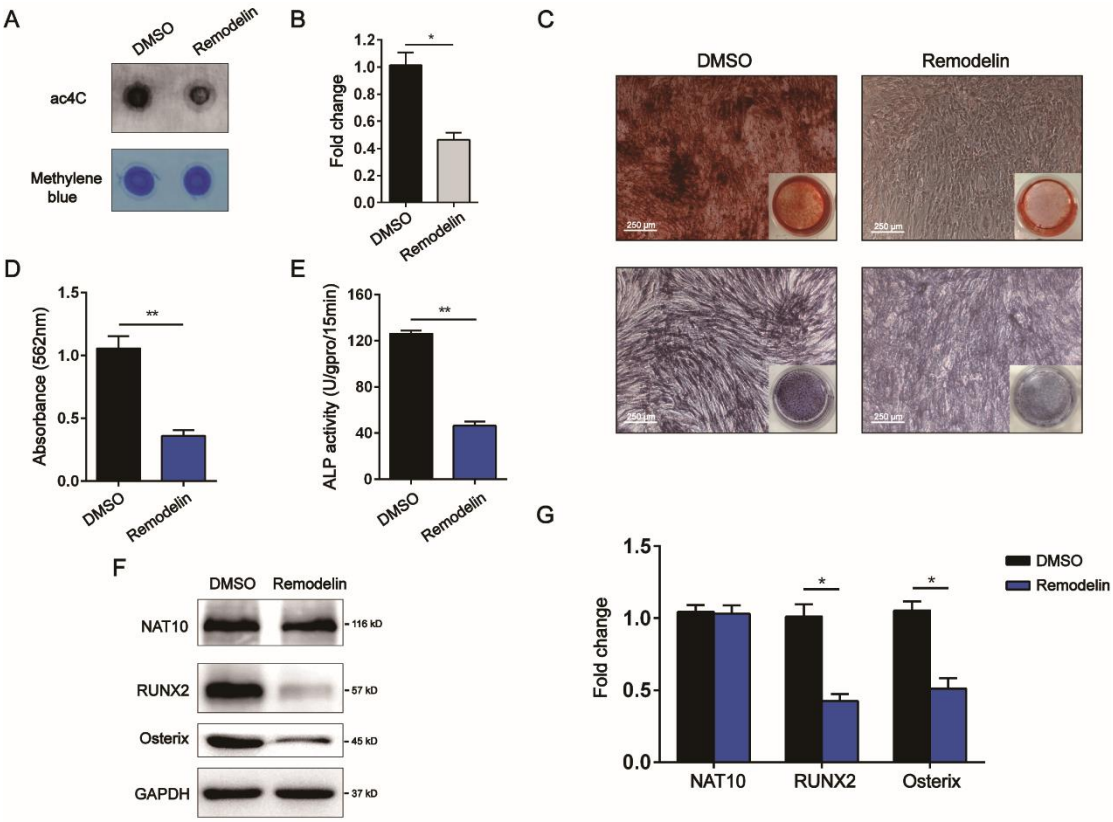

Figure S1. Remodelin inhibits the osteogenic differentiation of BMSCs in vitro. (A) Dot blot analysis demonstrated that the ac4C level of total RNA decreased in BMSCs treated with 20 μM Remodelin, n=6. (B) Densitometry quantitation of (A), n=6. (C) Remodelin decreased calcium nodule formation (upper panels) and ALP staining (lower panels) (scale bar=250 μm), n=6. (D) ARS staining was quantified as the absorbance at 562 nm, n=6. (E) ALP activity was determined as units per gram of protein per 15 min, n=6. (F) Remodelin decreased protein levels of the markers of osteoblast differentiation, RUNX2 and Osterix during osteogenic induction, n=6. (G) Quantification of band intensities, n=6. All data are presented as the means ± SDs. *$p < 0.05$, **$p < 0.01$ (n = 3 independent experiments).

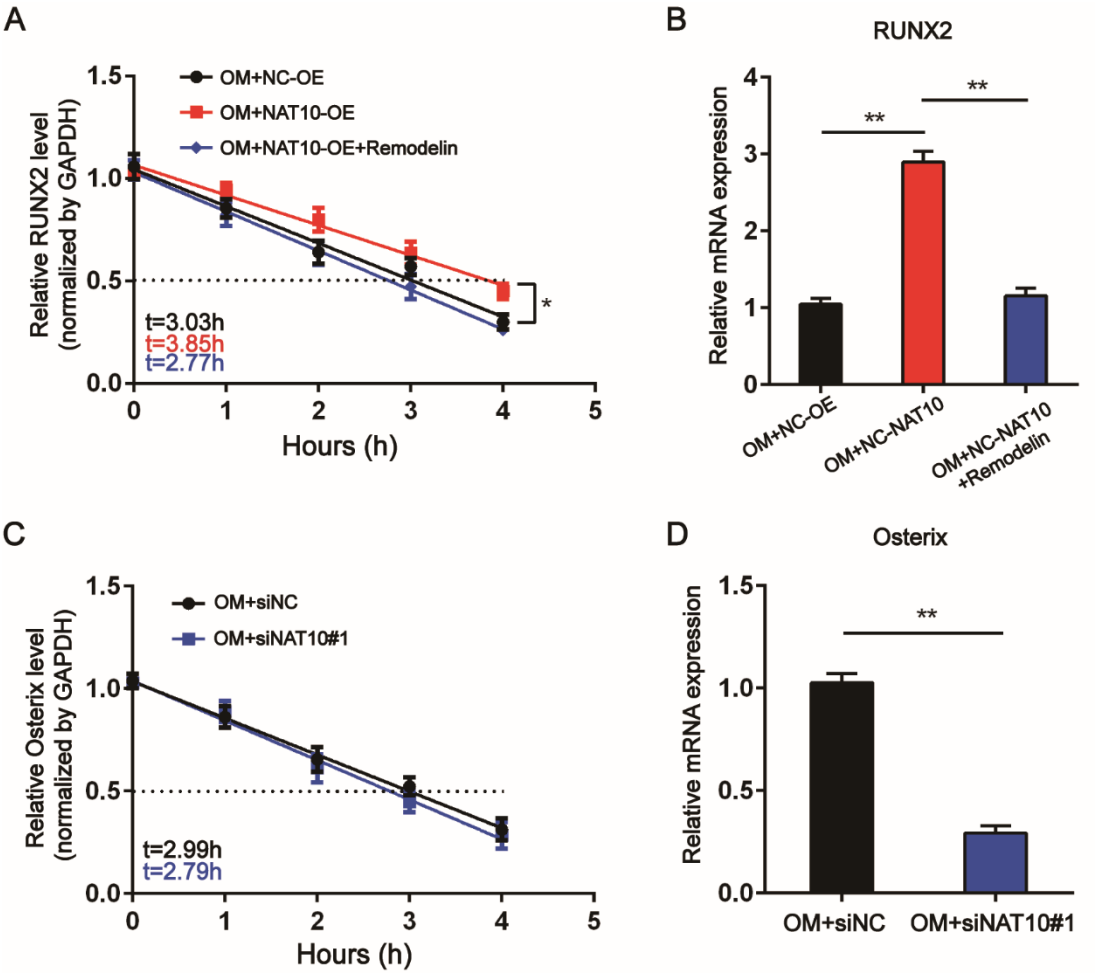

Figure S2. NAT10 does not regulate the decay rate of Osterix during osteogenic differentiation. (A) The decay rate of mRNA and qPCR analysis of RUNX2 at the indicated times after overexpressing NAT10 and treating with Remodelin, n=6. (B) RUNX2 expression was quantified by qPCR after overexpressing NAT10 and treating with Remodelin, n=6. (C) qPCR analysis of Osterix at the indicated times after silencing NAT10, n=6. (D) Osterix expression was quantified by qPCR after silencing NAT10, n=6. All data are presented as the means ± SDs. *p < 0.05, **p < 0.01 (n = 3 independent experiments).

Data S1. The ac4C Peak locations of differentially acetylated genes.

Data S2. Gene ontology (GO) enrichment analysis of the differentially acetylated genes.