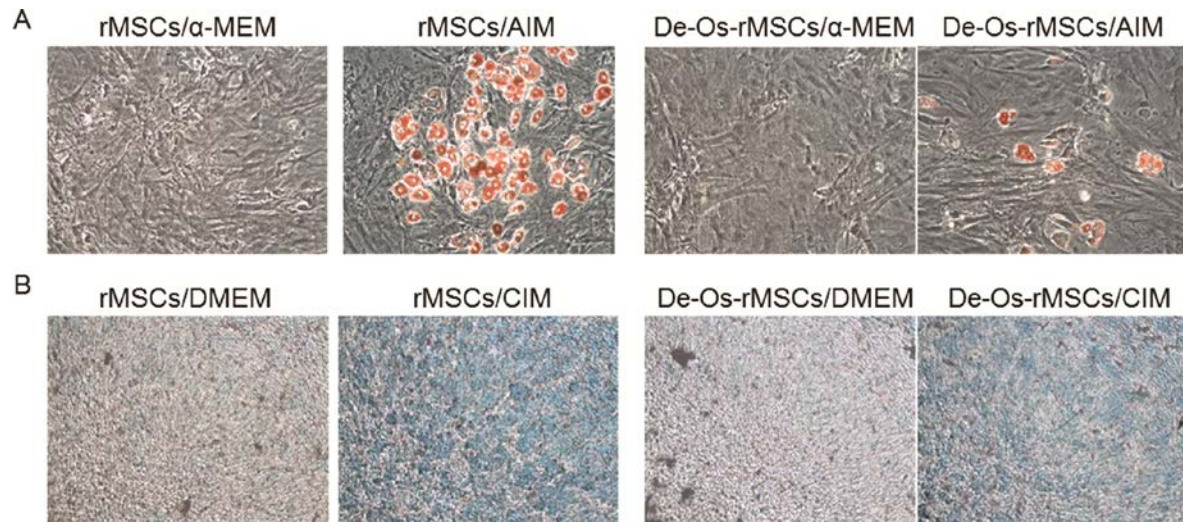


Epigenetic memory gained by priming with osteogenic induction medium improves osteogenesis and other properties of mesenchymal stem cells

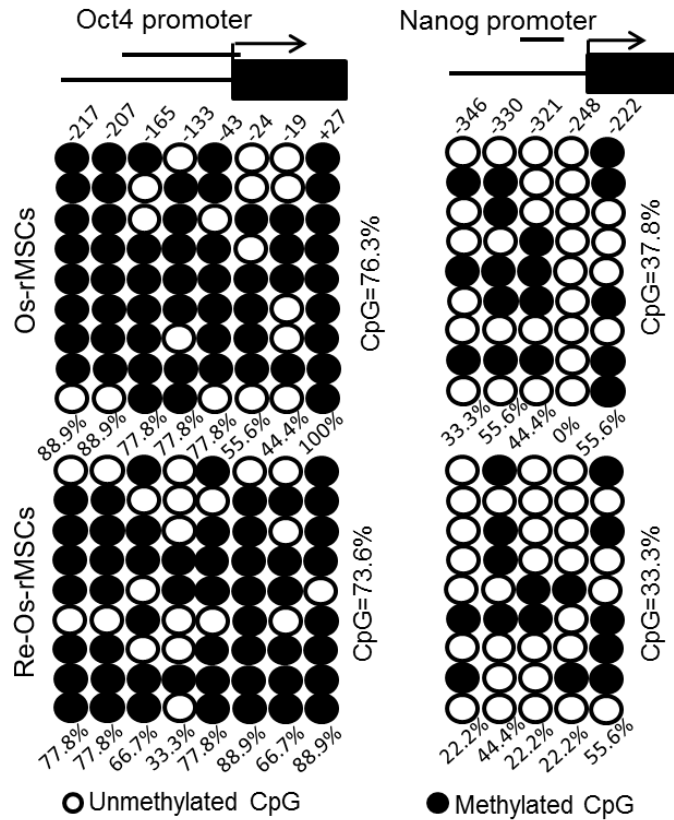
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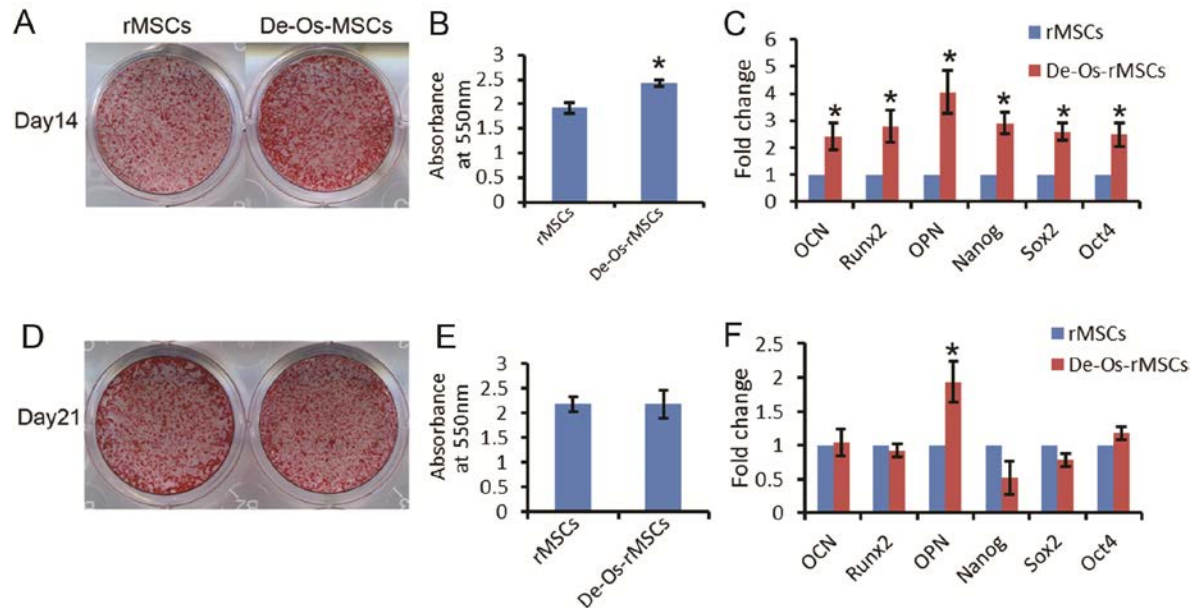
Army, Beijing, PR China.



Supplementary Figure 1: De-Os-rMSCs showed lower adipogenic and chondrogenic differentiation potential. (A) Adipogenic differentiation assay. The untreated rMSCs and De-Os-rMSCs were plated in complete culture medium in a 6-well plate and incubated at 37°C with 5% CO₂, respectively. When the cells became confluent, the medium was changed with adipogenic induction medium. 21 days later, the cells were fixed and stained with Oil Red O. (B) Chondrogenic differentiation assay. The untreated rMSCs and De-Os-rMSCs were plated in a 24-well plate to form micromasses. Then the medium was changed with chondrogenic induction medium. 14 days later, the cells were fixed and stained with Alcian Blue.



Supplementary Figure 2: DNA methylation status of Oct4 and Nanog promoters in Os-rMSCs and Re-Os-rMSCs using sodium bisulfite sequencing. The top panel indicates the CpG dinucleotide position of the Oct4 and Nanog promoter regions and the numbers show positions of CpGs relative to the translation start site. Each PCR product was subcloned and subjected to nucleotide sequencing analysis. Nine representative sequenced clones were depicted by filled (methylated) and open (unmethylated) circles for each CpG site.



Supplementary Figure 3: De-Os-rMSCs maintained osteogenic differentiation advantage for at least 14 days. (A-C) De-Os-rMSCs were cultured in α -MEM for 14 days, then the cells were induced to differentiate in OIM for 10 days and stained with Alizarin Red S. The mineralization was quantified by extraction of Alizarin Red S dye with 10% CPC (B). * $p < 0.05$. (C) QRT-PCR showing the expression of osteogenic differentiation genes and pluripotency-related genes.* $p < 0.05$. (D-F) De-Os-rMSCs were cultured in α -MEM for 21 days, then the cells were induced to differentiate in OIM for 10 days and stained with Alizarin Red S. The mineralization was quantified by extraction of Alizarin Red S dye with 10% CPC (E). (F) QRT-PCR showing the expression of osteogenic differentiation genes and pluripotency-related genes.* $p < 0.05$.

Supplemental Table 1. Sequences of primers for real time PCR analysis

Gene Name	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
<i>Nanog</i>	GCCCTGATTCTTCTAGCAAT	AGAACACAGTCCGCATCTT
<i>OPN</i>	TCCAAGGAGTATAAGCAGCG GGCCA	CTCTTAGGGTCTAGGACTAGCTT T
<i>OCN</i>	GAGCTGCCCTGCACTGGGTG	TGGCCCCAGACCTCTTCCCG
<i>Runx2</i>	CCGATGGGACCGTGGTT	CAGCAGAGGCATTTTCGTAGCT
<i>β-Actin</i>	CGTAAAGACCTCTATGCCAACAT	CGGACTCATCGTACTCCTGCT
<i>Oct4</i>	AGAACCGTGTGAGGTGGAAC	CTCAATGCTAGTCCGCTTTC
<i>Sox2</i>	CCCACCTACAGCATGTCCTA	TGGAGTGGGAGGAAGAGGTA
<i>KDM5C</i>	GAATGTGATGCCTGTGTTGG	CAGGCTGGCTATCAAAAAGC
<i>ESCO1</i>	CCTGTGCCTGTAAGTCTGA	TTTGCTCTTTCCAGGTTGCT
<i>ESCO2</i>	ATCACCACCGATTTCTGGAG	GGCAGGACCAACACAATCTT
<i>HDAC2</i>	TGGCCTTTCTGAGCTGATTT	AGAGGGTCTCTGCCACTGAA
<i>p53</i>	ATGGAGGAGTCACAGTCGGATA	GACTTCTTGTAGATGGCCATGG
<i>CXCR4</i>	TCCGTGGCTGACCTCCTCTT	CAGCTTCCTCGGCCTCTGGC
<i>Dnmt1</i>	GGAAGGTGAGCATCGACGAA	GATCATCCGGAATGACCGAG
<i>ALP</i>	TCCGTGGGTCCGATTCCT	GCCGGCCCAAGAGAGAA

Supplemental Table 2. Sequences of primers for bisulfite sequencing analysis

Promoters	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
<i>Nanog</i>	TATTATAATGTTTTTGGTGGGTTT G	TCAACCTATCTAAAAACCAACAA CTC
<i>Oct4</i>	AGTTTTGAGGTGTTTAGGGATTTA T	CCCCACCAAATAAAAAATAAAAAA A

Supplemental Table 3. Sequences of primers for CHIP-PCR analysis

Promoters	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
<i>Nanog-1</i>	GCTTGAGGGGGAGGAGTAAG	GCTTGAGGGGGAGGAGTAAG
<i>Nanog-2</i>	GCTTGAGGGGGAGGAGTAAG	GCTTGAGGGGGAGGAGTAAG
<i>Oct4</i>	GTGAGTCGTCCTTCCACCAG	GAGAAGGCGAAGTCTGAAGC