

The low-expression programming of 11 β -HSD2 mediates osteoporosis susceptibility induced by prenatal caffeine exposure in male offspring rats

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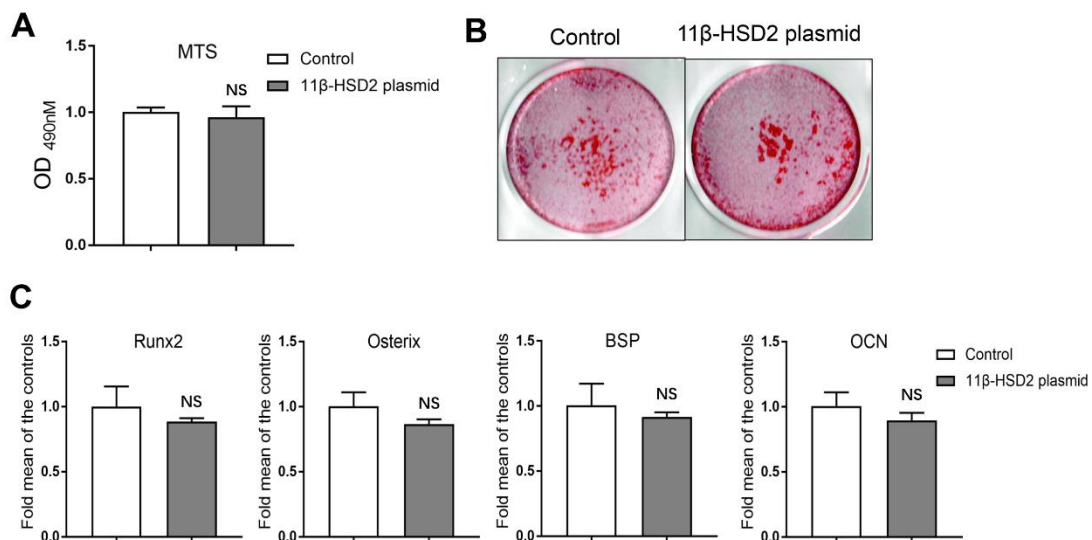


Figure S1. The effects of manipulation of 11 β -HSD2 expression by 11 β -HSD2

plasmid on preosteoblast cells. A. MTS assay for cell activity; B. Alizarin red staining for mineralization detection; C. RT-qPCR analysis of gene expression of Runx2, osterix, bone sialoprotein (BSP), osteocalcin (OCN). $n = 5$ per group, mean \pm S.E.M., NS, no significance.

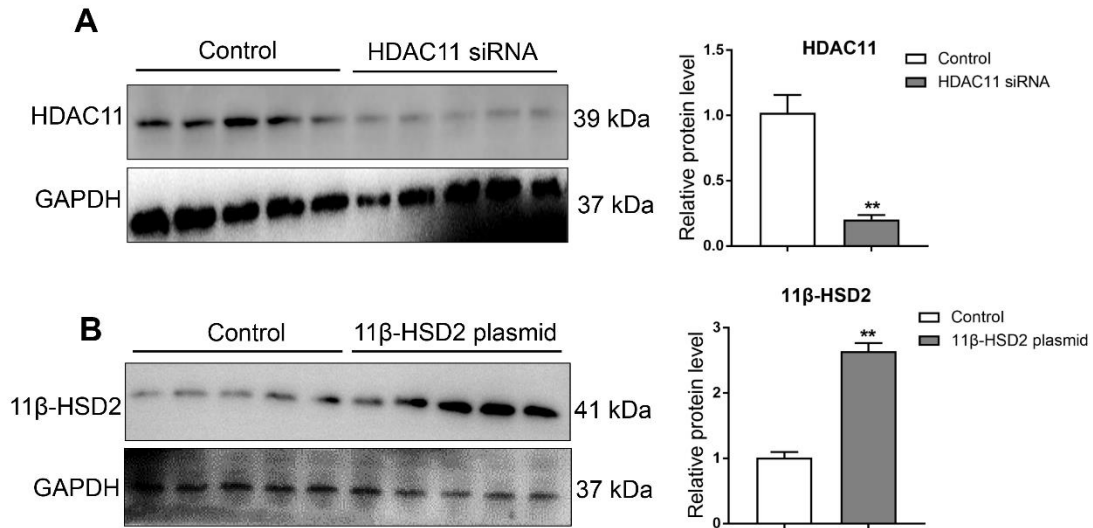


Figure S2. Western blotting assay of HDAC11 or 11β-HSD2 protein level after treating bone marrow mesenchymal stem cells with HDAC11 siRNA or 11β-HSD2 plasmid. $n = 5$ per group, mean \pm S.E.M., ** $P < 0.01$ compared with the control group.

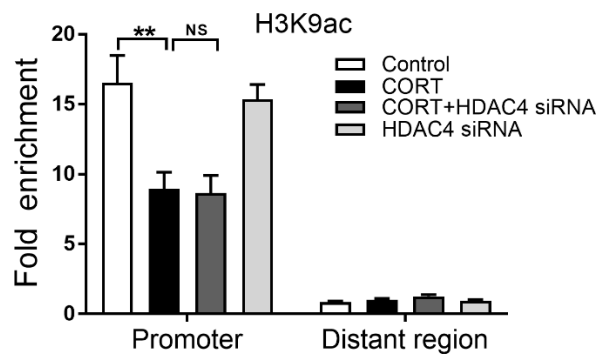


Figure S3. ChIP assay of the H3K9ac level in 11β-HSD2 promoter region after treating bone marrow mesenchymal stem cells with 500 nM CORT or combined with HDAC4 siRNA. $n = 5$ per group, mean \pm S.E.M., ** $P < 0.01$ compared with the control; NS, no significance.

Table S1. Primers used in quantitative real-time PCR

| Genes | Forward primers | Reverse primers | Annealing |
|------------------|--------------------------|--------------------------|-------------|
| Runx2 | TACTTCGTCAGCGTCCTATC | CAGCGTCAACACCATCATT | 60 °C, 30 s |
| BSP | GAGTACAACACTGCGTATGA | GTAATAATCCTGACCCTCGTAG | 58 °C, 20 s |
| OCN | CAGACCTAGCAGACACCATG | GCTTGGACATGAAGGCTTTG | 60 °C, 30 s |
| Osterix | GCACGAGTCACACCGGAAC | CCAATGTCCAAGGGAGCCAC | 58 °C, 20 s |
| 11 β -HSD2 | CAGGCCTATGGTGAAGACTA | CAGGTAGTGGTGGATGAAATAC | 58 °C, 20 s |
| GR | CCAGGCTTCAGAACTTACA | CATGCAGGGTAGAGACATTC | 58 °C, 20 s |
| HDAC1 | GTGGCCCTGGACACAGAGAT | GCTTGAAATCTGGTCCAAAGT | 60 °C, 30 s |
| HDAC2 | CAACCTAACTGTCAAAGGTCACGC | TGAAGTCTGGTCCAAAATACTCGA | 62 °C, 30 s |
| HDAC3 | AGGTGGTGGACTTCTATCAG | CACCAGGAGAGGGATATTGA | 58 °C, 30 s |
| HDAC4 | CTACATCAGAGACCCAATGC | GTGACTGTCTCAGCTTCTTC | 60 °C, 30 s |
| HDAC5 | CCGTGCTCTACATCTCTTTG | GCTGTCAGGTATTCCACATC | 58 °C, 30 s |
| HDAC6 | AGGGA ACTACTGGATCTG | GGCATTGAGGATGGAGAAG | 60 °C, 30 s |
| HDAC7 | TACAGAACTCTTGAGCCCTT | CAGGGATTCTTGGGTTTGT | 60 °C, 30 s |
| HDAC8 | GGCAAGTGTCTGAAGTATGT | TGGATCTCAGAGGATAGTG | 60 °C, 30 s |
| HDAC9 | CCCAGCATCCTGTACATTC | GCTTCAAGGTACTCAACATCTC | 60 °C, 30 s |
| HDAC10 | CTTACAAGTCCCAGTTTCA | ATTCTCCTCTGACCTCTATGG | 60 °C, 30 s |
| HDAC11 | CTTACTTCCCTCCCTCAGTCT | CCTTCTCAGACCTCCAAATC | 60 °C, 30 s |
| GAPDH | GCAAGTTCAACGGCACAG | GCCAGTAGACTCCACGACA | 60 °C, 30 s |

BSP, bone sialoprotein; OCN, osteocalcin; 11 β -HSD2, 11 β -hydroxysteroid

dehydrogenase 2; GR, glucocorticoid receptor; HDAC, histone deacetylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table S2. The offspring's body weight in control and prenatal caffeine exposure (PCE) group

| Age (week) | Male | | Female | |
|------------|---------------|----------------|---------------|---------------|
| | Control | PCE | Control | PCE |
| 0 | 3.81 ± 0.20 | 3.01 ± 0.18* | 3.63 ± 0.12 | 3.11 ± 0.17* |
| 4 | 43.52 ± 3.68 | 33.52 ± 3.05* | 39.32 ± 2.64 | 32.63 ± 1.79* |
| 8 | 146.55 ± 4.43 | 128.27 ± 3.66* | 132.39 ± 4.12 | 131.94 ± 5.30 |
| 12 | 239.26 ± 5.53 | 219.56 ± 6.13* | 223.52 ± 6.29 | 225.47 ± 5.38 |

Data were shown as mean ± S.E.M., n=8 per group, * $P < 0.05$ compared with the control group.