Supplementary data

Effect of Nano-Montmorillonite on Osteoblast Differentiation, Mineral Density, and Osteoclast Differentiation in Bone Formation

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Table S1. Bodyweight and food intake.

	Sham	Low Ca	Normal Diet	NM[a]
Feed intake (g/day)	**16.78 ± 2.09[a]**	**17.62 ± 1.82[a]**	**16.75 ± 1.82[a]**	**16.59 ± 0.36[a]**
Body weight (g)				
Initial	**117.33 ± 8.41[a]**	**118.75 ± 7.68[a]**	**118.50 ± 6.95[a]**	**119 ± 6.89[a]**
Final	**339.75 ± 16.34[a]**	**324.25 ± 30.89[a]**	**338.25 ± 32.34[a]**	**344.25 ± 16.19[a]**
Body weight gain (g/week)	**27.8 ± 4.2[a]**	**25.68 ± 3.6[a]**	**27.46 ± 3.62[a]**	**27.46 ± 3.62[a]**
FER[b]	**0.23 ± 0.02[a]**	**0.2 ± 0.01[a]**	**0.23 ± 0.01[a]**	**0.23 ± 0.01[a]**

[a] **NM:** Nano-Montmorillonite

[b] **FER:** Feed efficiency ratio = body weight gain (g/week)/feed intake (g/week)

Table S2. Primers used for RT-PCR analysis.

Target Gene	Species	Primer Sequence (5' → 3')	Annealing Temperature
GAPDH	Human	F: GATGGTACATGACAAGGTGC R: CAAGAAGGTGGTGAAGCAGG	60°C
BMP-2	Human	F: TCATAAAACCTGCAACAGCCAACTCG R: GCTGTACTAGCGACACCCAC	60°C
RUNX2	Human	F: CCGCACGACAACCGCACCAT R: CGCTCCGGCCCACAAATCTC	60°C
Osteocalcin	Human	F: GGCCAGGCAGGTGCGAAGC R: GCCAGGCCAGCAGAGCGACAC	65°C
COL-1	Human	F: AGCGCTGGTTTCGACTTCAGCTTCC R: CATCGGCAGGGTCGGAGCCCT	60°C
GAPDH	Mouse	F: ACTTTGTCAAGCTCATTTCC R: TGCAGCGAACTTTATTGATG	58°C
RANK	Mouse	F: AAGATGGTTCCAGAAGACGGT R: CATAGAGTCAGTGCTCGGA	63°C
TRAP	Mouse	F: ACTTCCCCAGCCCTTACTACCG R: TCAGCACATAGCCCACACCG	63°C
Cathepsin K	Mouse	F: CTGAAGATGCTTTCCCATATGTGGG R: GCAGGCGTTGTTCTTATTCCGAGC	63°C
GAPDH	Rat	F: AACTCCATTCCACCTT R: GAGGGCCTCTCTCTTGCTCT	48°C
BMP-2	Rat	F: AAGGCACCCTTTGTATGTGGACT R: CATGCCTTAGGGATTTTGGA	55°C
RUNX2	Rat	F: TCCAGCCACCTTCACTTACAC R: GCGTCAACACCATCATTCTG	53°C
Osteocalcin	Rat	F: AGCTCAACCCCAATTGTGAC R: AGCTGTGCCGTCCATACTTT	55°C
COL-1	Rat	F: TTGACCCTAACCAAGGATGC R: CACCCCTTCTGCGTTGTATT	57°C
Wnt3a	Rat	F: TCCGACTCTTGGCAGAACTT R: AATGGAATAGGTCCCGAACA	51°C
RANK	Rat	F: GTGACTCTCCAGGTCACTCC R: GGCAGACACACACTGTCG	60°C
TRAP	Rat	F: CGCCAGAACCGTG CAGA R: TCAGGCTGCTGGCTGAC	60°C
Cathepsin K	Rat	F: CCCGAGCTCCATCGACTATCG R: CTGTACCCTCTGCACTTAGCTGCC	60°C

Table S3. Composition of the experimental diets.

Composition	Sham	Low Ca	Normal Diet	NM[a]
Casein (g/kg)	**200.0**	**200.0**	**200.0**	**200.0**
L-Cystine (g/kg)	**3.0**	**3.0**	**3.0**	**3.0**
Sucrose (g/kg)	**334.288**	**342.188**	**334.288**	**342.188**
Corn Starch (g/kg)	**313.0**	**320.0**	**313.0**	**320.0**
Soybean Oil (g/kg)	**60.0**	**60.0**	**60.0**	**60.0**
Cellulose (g/kg)	**40.0**	**40.0**	**40.0**	**40.0**
Mineral Mix, (g/kg)[b]	**13.37**	**13.37**	**13.37**	**13.37**
Potassium Phosphate, Monobasic (g/kg)	**11.43**	**11.43**	**11.43**	**11.43**
Vitamin Mix, (g/kg)[c]	**10.0**	**10.0**	**10.0**	**10.0**
Calcium (%)	**0.6**	**0.01**	**0.6**	**0.01**
P (%)[d]	**0.4**	**0.4**	**0.4**	**0.4**
NM (mg/kg)	**-**	**-**	**-**	**50**

[a] **NM :** Nano-Montmorillonite

[b] **Mineral Mix (g/kg) :** (NaCl: 193.7325, $C_6H_7K_3O_8$: 575.9615, K_2SO_4: 136.1363, MgO: 62.8322, $MnCO_3$: 9.163, $C_6H_5FeO_7$: 15.708, $ZnCO_3$: 4.1888, $CuCO_3$: 0.7854, KIO_3: 0.0262, $Na_2SeO_3 \cdot 5H_2O$: 0.0262, $CrK(SO_4)_2 \cdot 12H_2O$: 1.4399).

[c] **Vitamin Mix (g/kg) :** (*p*-Aminobenzoic Acid: 11.0132, Vitamin C, Ascorbic Acid, Coated (97.5%): 101.6604, Biotin: 0.0441, Vitamin B_{12} (0.1% in mannitol): 2.9736, Calcium Pantothenate: 6.6079, Choline Dihydrogen Citrate: 349.6916, Folic Acid: 0.1982, Inositol: 11.0132, Vitamin K_3, Menadione: 4.9559, Niacin: 9.9119, Pyridoxine HCl: 2.2026, Riboflavin: 2.2026, Thiamin (81%): 2.2026, Vitamin A Palmitate (500,000 IU/g): 3.9648, Vitamin D_3, Cholecalciferol (500,000 IU/g): 0.4405, Vitamin E, DL-Alpha Tocopheryl Acetate (500 IU/g): 24.2291, Corn Starch: 466.6878).

[d] **P :** Phosphorus

Supplementary Figure Legends

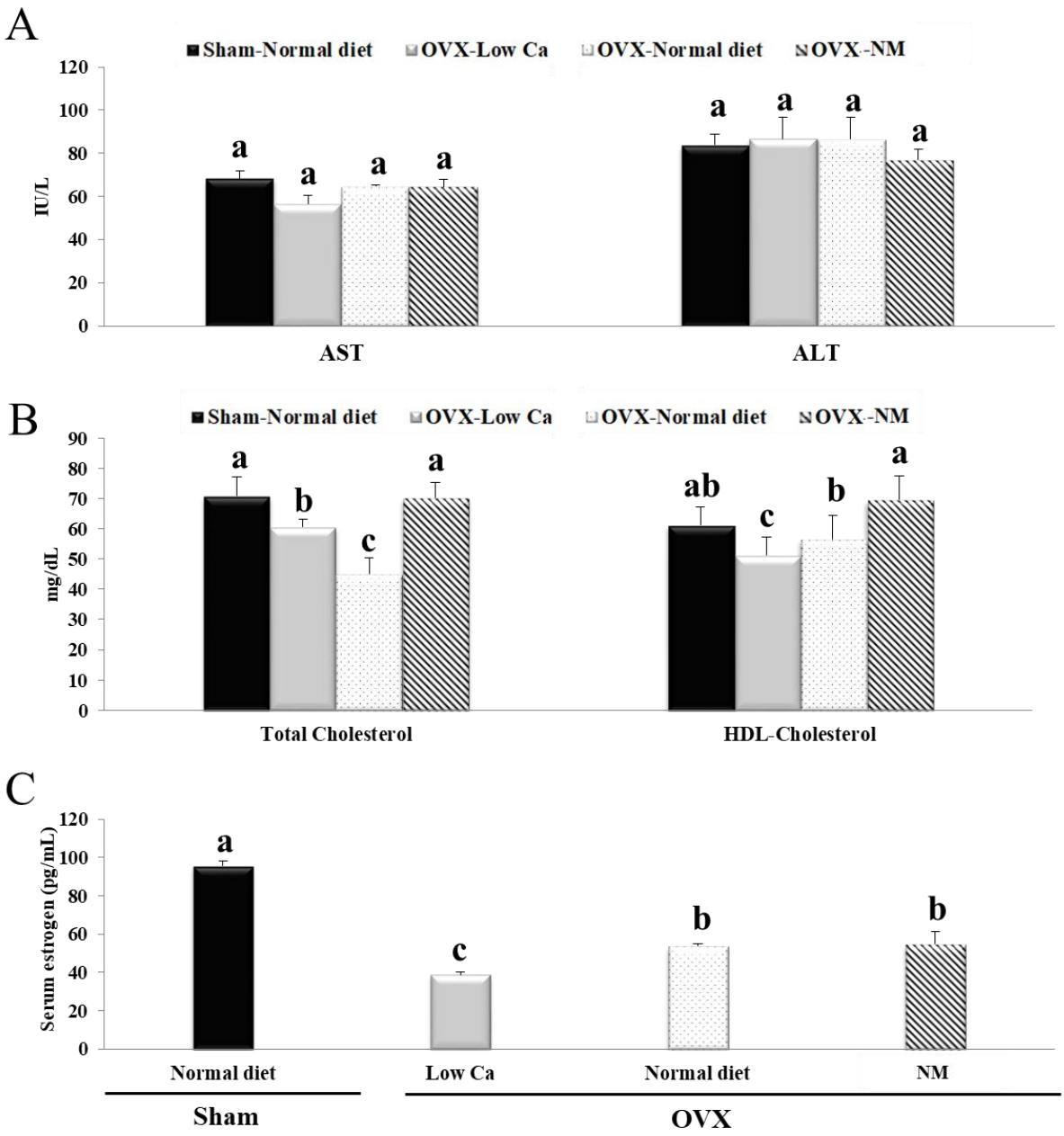

Figure S1. *In vivo* biochemical analyses of sham and ovariectomized (OVX) rats. Serum levels were determined for (A) alanine aminotransferase (ALT) and aspartate aminotransferase (AST); (B) total cholesterol and high-density lipoprotein (HDL-Cholesterol); and (C) estrogen. Values represent the mean ± SD (n = 3). Values not sharing a common superscript (a-c) differed significantly (Duncan's multiple range test) ($p < 0.05$).