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NOD2 Mediates Odontoblast Differentiation and RANKL Expression

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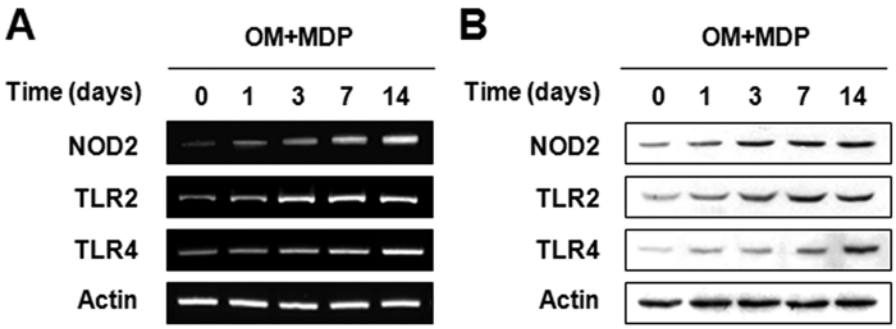
APPENDIX



Appendix Figure. Muramyl dipeptide (MDP) treatment increased NOD2, TLR2, and TLR4 expression during osteoblast/odontoblast differentiation in human dental pulp cells (HDPCs). HDPCs were cultured with MDP (10 μg/mL) for 14 days in osteogenic media (OM) with 10 mM β-glycerophosphate, 10^{-7} M dexamethasone, and 50 μg/mL L-ascorbic acid. The mRNA and protein expression levels were examined by RT-PCR **(A)** and Western blot analysis **(B).** Similar data were obtained from 3 independent experiments.

Appendix Table. RT-PCR Primer Lists

Genes	Primer Sequence (5′-3′)	Annealing Temp. (°C)	Cycle Number	Product Size (bp)
ALP	F : 5′-ACFTFFCTAAFAATFTCATC-3′ R : 5′-CTFFTAFFCFATFTCCTTA-3′	55	30	475
OPN	F : 5′-CCAAGTAAGTCCAACGAAAG-3′ R : 5′-GGTGATGTCCTCGTCTGTA-3′	55	30	347
OCN	F : 5′-CATGAGAGCCCTCACA-3′ R : 5′-AGAGCGACACCCTAGAC-3′	57	30	310
DMP-1	F : 5′-CAGGAGCACAGGAAAAGGAG-3′ R : 5′-CTGGTGGTATCTTCCCCCAGGAG-3′	56	36	213
DSPP	F : 5′-AGAAGGACCTGGCCAAAAAT-3′ R : 5′-TCTCCTCGGCTACTGCTGTT-3′	60	35	280
RANKL	F : 5′-CCAGCATCAAAATCCCAAGT-3′ R : 5′-CCCCTTCAGATGATCCTTC-3′	56	35	605
OPG	F : 5′-TGCAGTACGTCAAGCAGGAG -3′ R : 5′-TGACCTCTGTGAAAACAGC-3′	60	35	481
M-CSF	F: 5′-ATGACAGACAGGTGGAACTGCCAGTGTAGAGG -3′ R : 5′-TCACACAACTTCAGTAGGTTCAGGTGA TGGGC-3′	60	35	437
TNF-α	F: 5′-CTCTFFCCCAFFCAFTCAGA-3′ R: 5′-GGCGTTTGGGAAGGTTGGAT-3′	60	35	519
IL-1β	F: 5′-TGGAGATGACAGTTCAGAAG-3′ R: 5′-GTACTGGTGCCGTTTAtGC-3′	60	35	288
IL-6	F : 5′-TAFCCGCCCCACACAGACAG-3′ R : 5′-GGCTGGCATTTGTGGTTGGG-3′	60	35	408
IL-11	F : 5′-GCAGCGGACAGGGAAGGGTTAAA-3′ R : 5′-TGTGCGACTCAGCGGGGTCTG-3′	59	32	176
IL-17	F : 5′-CGATGACTCCTGGGAAGACCTC-3′ R : 5′-GTGTGGGCTCCCCAGAGCTCTTA-3′	55	25	524
NOD2	F : 5′-CAGAAGGCCGAGCCGCACAA-3′ R : 5′-CCAGACACCAGCGGGCACAG-3′	60	35	145
TLR2	F : 5′-GTGGCCAGCAGGTTCAGGATG-3′ R : 5′-AGGACTTTATCGCAGCTCTCAG-3′	55	641	35
TLR4	F : 5′-AAGTGTCTGAACTCCCTCCAGG-3′ R : 5′-ATGGTCTTATTCATCTGACAGGTGATA-3′	55	278	35
β-actin	F : 5′-CATGGATGATGATATCGCCGCG-3′ R: 5′-ACATGATCTGGGTCATCTTCTCG-3′	55	35	471