Figure S1. LiCl activates β-catenin and Tcf-1 in cementoblast cell line. (a) Western blot analyses displayed a time-dependent activation of β-catenin by 10 mM LiCl in cementoblasts; (b) Cells were cultured with or without 10mM LiCl for 12 hrs. Immunofluorescence assay was performed to detect the nuclear translocation of β-catenin (β-catenin signal, red; DAPI stained nucleus, blue; Scale bar, 10μm); and (c) Real-time RT-PCR analyses revealed a similar TCF-1 reaction to LiCl with a peak time at 12 and 24 hours after addition of 10 mM LiCl. Results were shown as mean ±SD of three separate experiments, each performed with triplicate samples.
Figure S2. DKK1 attenuates Wnt3a-induced Wnt/β-catenin signaling and promotes cementoblast differentiation. (a) Western blot images revealed a partial attenuation of activation of β-catenin by Wnt3; (b) The RT-PCR data showed a blockage of activation of Tef-1 by Wnt3; (c) The immuno staining assay presented an increase in translocation of β-catenin into the nucleus by Wnt3, which was partially blocked by addition of DKK1; (d) The cell proliferation assay using CCK-8 kit revealed a time-dependent increase in cell proliferation by Wnt3a; (e) RT-PCR data showed that Wnt3a inhibits expression of OPN, OCN and BSP by Wnt3, and that DKK1 increases their expression; (f) Western blot data displayed a similar inhibition of expression of OPN, OCN and BSP by Wnt3, which is partially blocked by DKK1; and (g) ALP assays showed an inhibition in ALP activity by Wnt3 (dark blue bar), which is partially reversed by an addition of DKK1. Data represented as mean ±SD of three separate experiments; Statistical differences: *P<0.05.
Figure S3. A decrease in DKK1 expression is correlated with the increase in β-catenin expression in the 6-week-old Osx cKO lower jaw (crossing between Osx/fx and 2.3 Col 1-Cre mice, right panels), which is likely responsible for the poorly formed cementum and bone. (a) The H&E images revealed the reduced cementum and porotic bone in the Osx cKO jaw; and (b-c) Immunostained images displayed a reduced DKK1 expression (b) and an increase in β-catenin (c) in the Osx cKO lower jaw.