Supplemental Table 1. Primer sequences used for real-time quantitative PCR.

Gene name	Symbol	GeneID	Forward	Reverse
Tissue nonspecific alkaline phosphatase	*Alpl; Akp2*	11647	GGGGACATGCAGTATGAGTT	GGCCTGGTAGTTGTTGTGAG
Progressive ankylosis protein	*Ank*	11732	GAATCAGTCGGCCCAT	GTTCGCCAGTTTATTGCT
Ectonucleotide pyrophosphatase phosphodiesterase	*Enpp1; PC-1*	18605	CGCCACCGAGACTAAA	TCATAGCGTCCGTCAT
Glyceraldehyde-3 phosphate dehydrogenase	*Gapdh*	14433	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA
Osteopontin	*Opn; Spp1*	20750	TTTACAGCCTGCACCC	CTAGCAGTGACGGTCT

Supplemental Table 2. Measurements and statistics for histomorphometry of *Ank* KO and WT first mandibular molar and incisor at 45 dpc. Independent sample t-tests were performed with α=0.05 used to determine significance. The 95% confidence intervals for observed mean differences are reported in standard deviation (SD) units which were computed using the SD for *Ank* KO in each case. Data are represented graphically in Figure 3.

Tissue	**Mean measurement** (μm) WT KO	**SD** (μm) WT KO	**p-value**	**95% confidence interval of the difference** Lower, Upper (SD units)
Molar – 100 μm				
Cementum (lingual)	2.61 31.58	0.67 2.39	0.001	-14.4, -9.8
Dentin (lingual)	96.00 102.41	16.90 5.48	0.586	-8.0, 5.7
Predentin (lingual)	16.83 16.08	7.50 2.79	0.883	-5.6, 6.1
Pulp	154.17 156.43	14.02 42.45	0.937	-2.3, 2.2
Predentin (buccal)	17.44 16.79	5.04 1.23	0.845	-9.0, 10.0
Dentin (buccal)	101.94 100.29	14.10 8.43	0.872	-3.2, 3.6
Cementum (buccal)	2.94 32.67	0.42 4.64	0.008	-8.9, -4.0
Molar – 300 μm				
Bone (lingual)	151.67 45.38	40.94 41.54	0.093	-1.0, 6.1
PDL (lingual)	97.5 127.2	8.28 0.65	0.024	-76.9, -14.5
Cementum (lingual)	3.00 38.06	0.72 5.83	0.008	-8.4, -3.6
Dentin (lingual)	76.64 83.84	8.87 1.32	0.294	-21.7, 10.8
Predentin (lingual)	14.47 14.40	2.63 1.71	0.973	-3.1, 3.2
Pulp	141.36 149.16	3.16 19.50	0.562	-2.8, 2.0
Predentin (buccal)	12.56 13.18	3.43 1.93	0.801	-4.0, 3.3
Dentin (buccal)	89.33 86.51	12.74 6.14	0.753	-3.9, 4.8
Cementum (buccal)	2.83 26.07	0.17 3.42	0.007	-9.3, -4.3
PDL (buccal)	84.72 98.11	4.86 13.27	0.216	-3.2, 1.2
Bone (buccal)	82.83 68.65	7.52 36.26	0.571	-2.0, 2.7

Molar – 500 μm				
Bone (lingual)	328.04 224.67	22.31 82.02	0.153	-1.0, 3.5
PDL (lingual)	121.17 114.74	16.60 11.65	0.616	-2.4, 3.5
Cementum (lingual)	3.61 29.34	0.35 3.08	0.004	-10.8, -5.9
Dentin (lingual)	67.22 54.26	7.02 12.20	0.204	-1.0, 3.1
Predentin (lingual)	13.56 12.49	1.99 2.80	0.622	-1.7, 2.4
Pulp	151.72 153.22	13.30 10.33	0.885	-2.8, 2.5
Predentin (buccal)	11.06 11.60	0.92 2.09	0.709	-2.4, 1.9
Dentin (buccal)	76.17 71.58	9.18 8.45	0.559	-1.8, 2.9
Cementum (buccal)	3.00 27.13	0.44 0.90	0.000	-28.9, -24.7
PDL (buccal)	66.11 89.91	9.74 3.53	0.039	-12.8, -0.7
Bone (buccal)	88.06 89.63	12.02 15.29	0.895	-2.2, 2.0
Molar – CEJ-ABC				
Lingual aspect	168.44 330.17	5.64 33.51	0.012	-7.2, -2.4
Buccal aspect	111.22 188.74	8.75 17.57	0.007	-3.4, -1.2
Incisor				
PDL	169.49 210.44	25.95 40.29	0.225	-3.1, 1.0
Cementum (lingual)	2.12 21.36	0.11 1.98	0.003	-12.2, -7.2
Dentin (lingual)	110.18 110.89	4.64 9.71	0.916	-2.2, 2.0
Predentin (lingual)	7.51 5.51	0.43 1.77	0.184	-1.2, 3.4
Pulp	387.99 380.28	21.54 14.97	0.641	-2.4, 3.5
Predentin (labial)	8.89 7.91	0.82 1.40	0.368	-1.4, 2.7
Dentin (labial)	142.22 146.86	1.95 9.28	0.480	-2.9, 1.9

Supplemental Table 3. Statistical analysis of OCCM.30 cementoblast gene expression over 9 days, *in vitro*. Intergroup differences were analyzed by one-way ANOVA and post-hoc Tukey test, with α=0.05 used to determine significance. Control (Con) conditions were DMEM with 2% FBS, while "AA" indicates addition of 50 μg/ml ascorbic acid, and "AA + BGP" indicates addition of both 50 μg/ml ascorbic acid and 10 mM β–glycerophosphate. Data are represented graphically in Figure 8.

Tissue nonspecific alkaline phosphatase (*Akp2*)			
Time point (days)	**Intergroup comparison**	**Mean difference (1-2), relative expression units**	**p-value**
1	Con vs. AA	-1,373	0.488
	Con vs. AA+BGP	-3,370	0.027
	AA vs. AA+BGP	-1,997	0.215
3	Con vs. AA	-7,595	0.213
	Con vs. AA+BGP	-20,720	0.002
	AA vs. AA+BGP	-13, 130	0.024
5	Con vs. AA	-9,233	0.003
	Con vs. AA+BGP	-1,957	0.674
	AA vs. AA+BGP	7,276	0.012
6	Con vs. AA	-4,946	0.042
	Con vs. AA+BGP	3,977	0.105
	AA vs. AA+BGP	8,923	0.001
7	Con vs. AA	-1,193	0.343
	Con vs. AA+BGP	9,417	0.000
	AA vs. AA+BGP	10,610	0.000
8	Con vs. AA	-1,066	0.893
	Con vs. AA+BGP	11,060	0.000
	AA vs. AA+BGP	12,130	0.000
9	Con vs. AA	1,006	0.914
	Con vs. AA+BGP	9,047	0.002
	AA vs. AA+BGP	8,041	0.004
Osteopontin (*Opn*)			
Time point (days)	**Intergroup comparison**	**Mean difference (1-2), relative expression units**	**p-value**
1	Con vs. AA	-0.0119	0.948
	Con vs. AA+BGP	-0.0725	0.046
	AA vs. AA+BGP	-0.0606	0.098
3	Con vs. AA	0.0606	0.278
	Con vs. AA+BGP	0.0510	0.407
	AA vs. AA+BGP	-0.00967	0.989
5	Con vs. AA	0.106	0.937
	Con vs. AA+BGP	-1.01	0.003
	AA vs. AA+BGP	-1.11	0.001
6	Con vs. AA	0.137	0.999
	Con vs. AA+BGP	-8.82	0.000
	AA vs. AA+BGP	-8.95	0.000
7	Con vs. AA	0.0749	0.995

	Con vs. AA+BGP	-3.36	0.000
	AA vs. AA+BGP	-3.44	0.000
8	Con vs. AA	-0.0529	0.992
	Con vs. AA+BGP	-2.12	0.00
	AA vs. AA+BGP	-2.07	0.00
9	Con vs. AA	0.0388	1.000
	Con vs. AA+BGP	-2.50	0.007
	AA vs. AA+BGP	-2.54	0.006
Progressive ankylosis (*Ank*)			
Time point (days)	**Intergroup comparison**	**Mean difference (1-2), relative expression units**	**p-value**
1	Con vs. AA	-0.0813	0.698
	Con vs. AA+BGP	-0.334	0.008
	AA vs. AA+BGP	-0.252	0.037
3	Con vs. AA	-0.114	0.118
	Con vs. AA+BGP	-0.353	0.000
	AA vs. AA+BGP	-0.239	0.003
5	Con vs. AA	-0.0935	0.278
	Con vs. AA+BGP	-2.65	0.002
	AA vs. AA+BGP	-0.171	0.029
6	Con vs. AA	-0.0124	0.999
	Con vs. AA+BGP	-0.471	0.005
	AA vs. AA+BGP	-0.458	0.006
7	Con vs. AA	-0.0410	0.961
	Con vs. AA+BGP	-0.449	0.003
	AA vs. AA+BGP	-0.408	0.006
8	Con vs. AA	0.00297	1.000
	Con vs. AA+BGP	-0.343	0.001
	AA vs. AA+BGP	-0.346	0.001
9	Con vs. AA	0.0448	0.389
	Con vs. AA+BGP	-0.219	0.000
	AA vs. AA+BGP	-0.264	0.000
Ectonucleotide pyrophosphatase/phosphodiesterase (*Enpp1*)			
Time point (days)	**Intergroup comparison**	**Mean difference (1-2), relative expression units**	**p-value**
1	Con vs. AA	-424	0.413
	Con vs. AA+BGP	-1,300	0.005
	AA vs. AA+BGP	-874	0.039
3	Con vs. AA	-1,930	0.000
	Con vs. AA+BGP	-2,990	0.000
	AA vs. AA+BGP	-1,060	0.007
5	Con vs. AA	-570	0.724
	Con vs. AA+BGP	-2,810	0.004
	AA vs. AA+BGP	-2,240	0.014
6	Con vs. AA	-584	0.997
	Con vs. AA+BGP	-14,700	0.005
	AA vs. AA+BGP	-12,700	0.006

7	Con vs. AA	-1,340	0.980
	Con vs. AA+BGP	-8,010	0.179
	AA vs. AA+BGP	-6,670	0.297
8	Con vs. AA	-1,560	0.694
	Con vs. AA+BGP	-3,690	0.113
	AA vs. AA+BGP	-2,130	0.472
9	Con vs. AA	-345	0.975
	Con vs. AA+BGP	-1,520	0.330
	AA vs. AA+BGP	-1,180	0.528