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

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