**Supplementary information**

**Loss of Stemness, EMT and Supernumerary Tooth Formation in *Cebpb*-/-*Runx2*+/- Murine Incisors**

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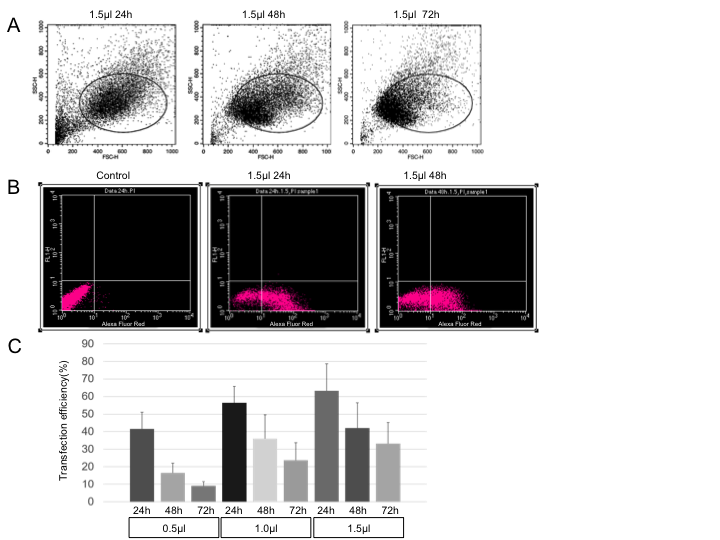
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**Supplementary information**

The PDF file includes:

Supplementary Figure 1, 2, 3, 4, 5, 6 and table 1, 2



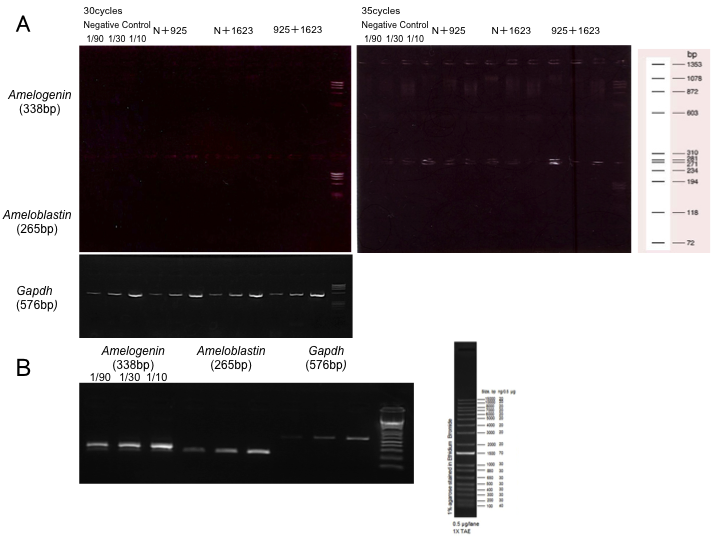
**Supplementary Figure 1 | Transfection efficiency of mHAT9d cells with stealth siRNA.**

Efficiency was measured by fluorescence-activated cell sorting (FACS caliber; BD, NJ, USA) 24, 48, and 72 h after transfection of BLOCK-iT (TM) Alexa Fluor (R) Red Fluorescent Control (Thermo Fisher Scientific, Waltham, MA, USA).

A: Gated dot plots generated by Cell QuestTM Pro Software (BD) indicate forward scatter (X-axis) and side scatter (Y-axis) following transfection with 1.5 μL Lipofectamine® RNAiMAX (Thermo Fisher Scientific).

B: Comparison between control (without transfection), 24h and 48h after transfection with 1.5 μL Lipofectamine® RNAiMAX. Gated dot plots generated by Cell QuestTM Pro Software indicate Alexa Fluor Red (X-axis) and FL-1H (Y-axis)).

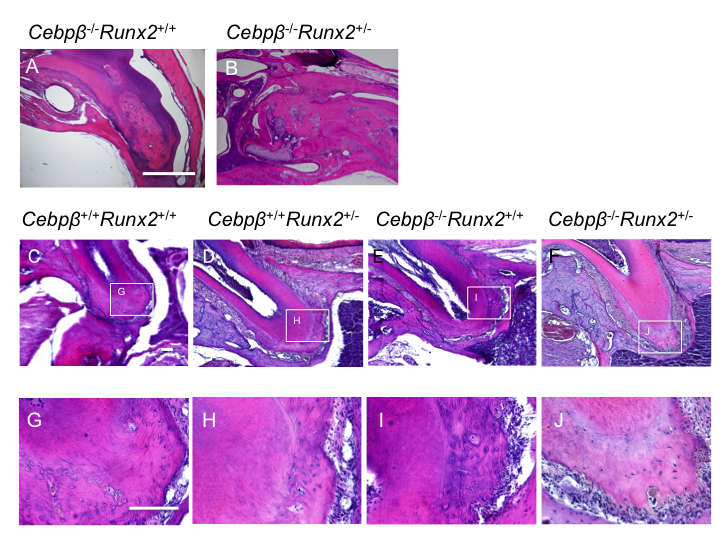
C: Efficiency is represented as mean ± standard deviation following transfection with 0.5, 1.0, and 1.5 μL Lipofectamine® RNAiMAX. Number of samples is 4.



**Supplementary Figure 2 | Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) of mHAT9d cells transfected with stealth siRNA for amelogenin (*Amelx*)and ameloblastin (*Ambn*).**

A: Total RNA (3μg) was extracted from 70% confluent mHAT9d cells and reverse transcribed using a SuperScript ®IV First-Strand Synthesis System (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The cDNAs were serially diluted and PCR amplification was performed using KOD FX (KFX-101, TOYOBO, Osaka, Japan) and specific oligonucleotide primers for *Amelx,* *Ambn* and glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*)(Supplemental Table 1). No PCR products were detected in 30-35 cycles using a 2% agarose gel electrophoresis with φ×174-HaeⅢdigest (3405A, TaKaRa Bio, Shiga, Japan). N indicates negative control stealth siRNA; 925 and 1623 indicate *Cebpb* and *Runx2* type1siRNA, respectively (final concentration 20 nM). Each siRNA was the same in quantity (10 nM each) when mixed.

B: Total RNA (0.5µg) was extracted from labial cervical loop epitheliums in apical end of four bimaxillary incisors of a 129sv wild-type mouse and reverse transcribed as stated above. The cDNAs were serially diluted and PCR amplification was performed using KOD FX and specific oligonucleotide primers for *Amelx*, *Ambn*, and *Gapdh*. The PCR products were detected in 25 cycles using a 2% agarose gel electrophoresis with 1kb plus DNA Ladder (10787-018, ThermoFisher scientific).

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**Supplementary Figure 3 | Hematoxylin-eosin (H&E) staining of cementum-like hard tissue in the dental pulp and cementum in adult maxillary molars in *Cebpβ**and/or Runx2* double genetically modified mice (F2: 129Sv/C57BL/6) at 3 months after birth.**

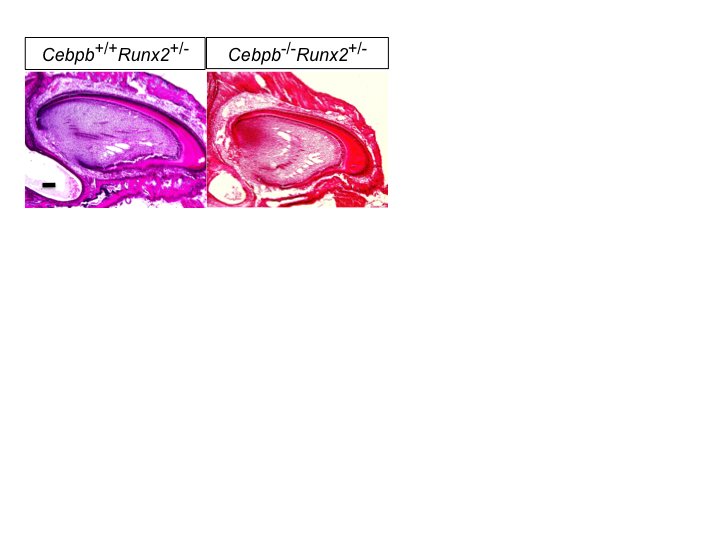
A, B: Cementum-like hard tissue of dental pulp in maxillary incisors in *Cebpb*-/-*Runx2*+/+ or *Cebpb*-/-*Runx2*+/- mice. Scale bar: 1mm, 40×.

C-F: Root with cementum in mouse molar (M1)×100 Scale bar: 100 μm, 100×.

G-J: Higher magnification image of cellular intrinsic fiber cementum (CIFC). Scale bar: 100 μm, 400×.

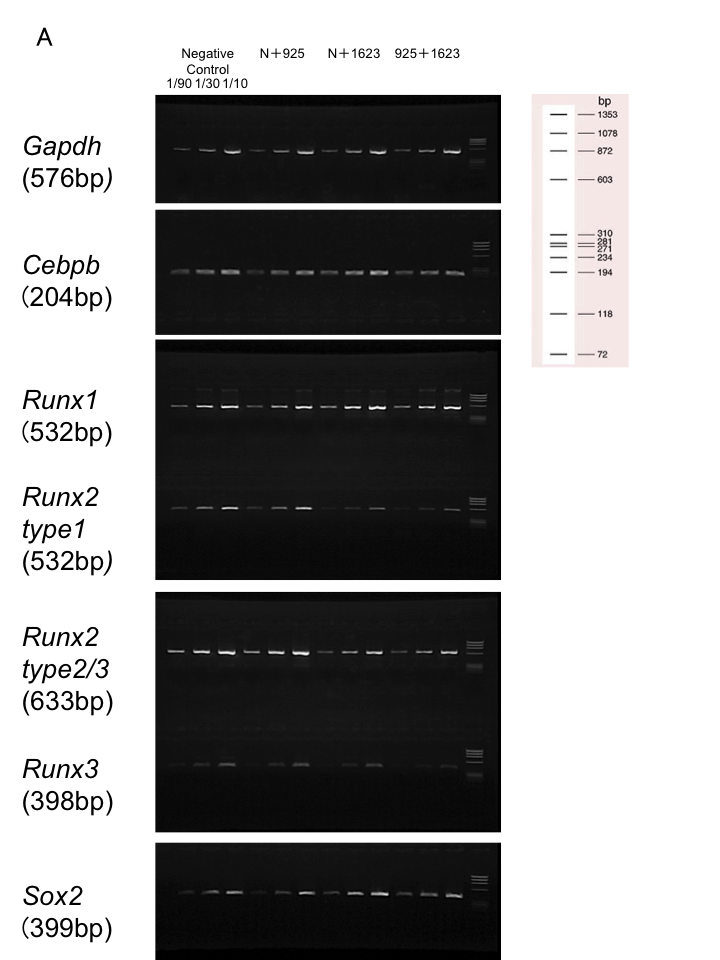
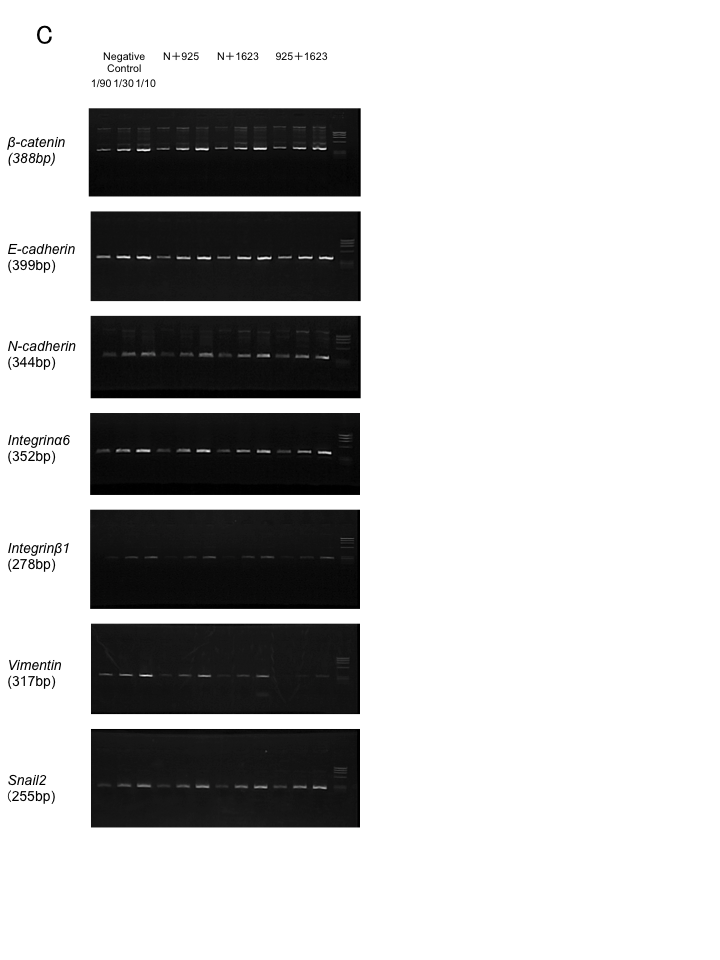
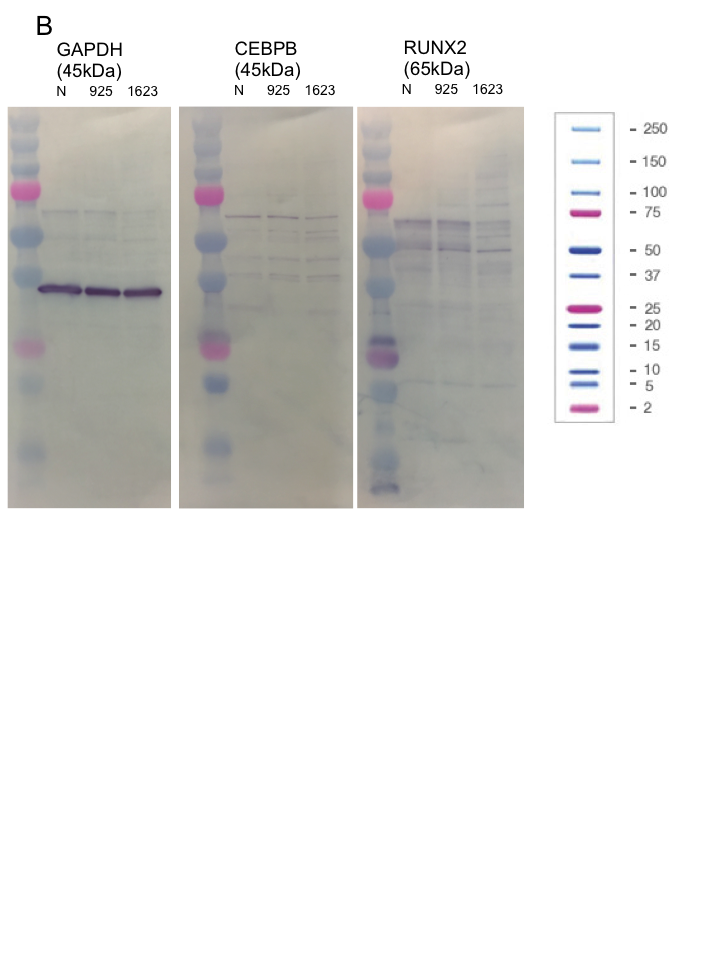
C, D, G, H: Margin of CIFC is smooth shaped in wild-type or *Runx2* heterozygnous mice.

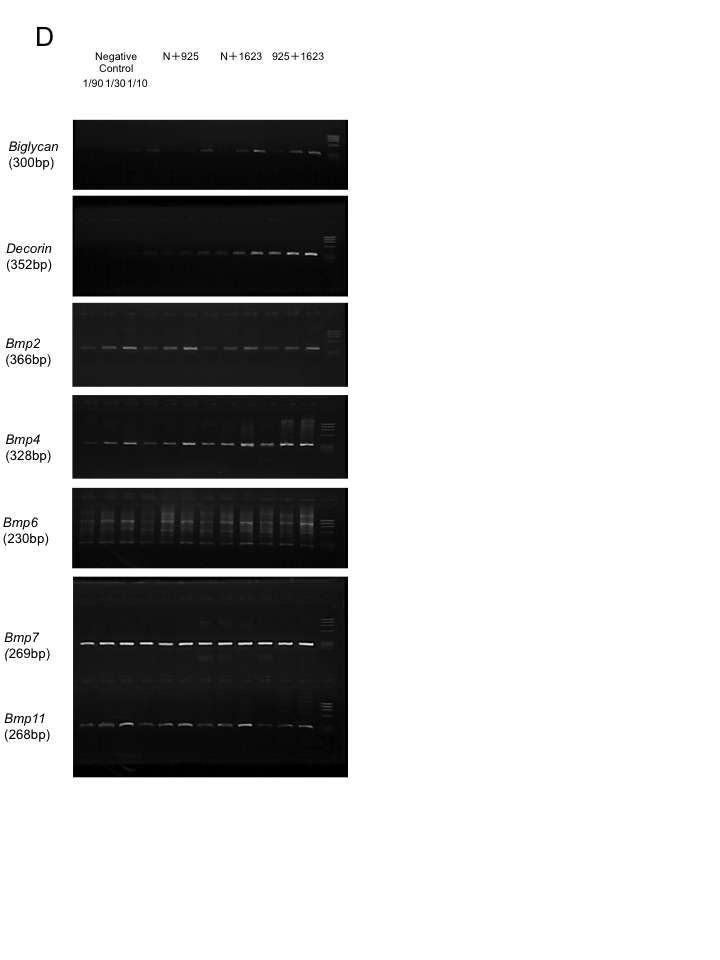
E, F, I, J: Margin of CIFC is invasive type and rough-edged in *Cebpb* null mice.

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**Supplementary Figure 4 |** **the upper incisors of *Cebpb*+/+*Runx2*+/- and *Cebpb*-/-*Runx2*+/- F2 mice (129Sv/C57BL/6) on day 7 after birth.**

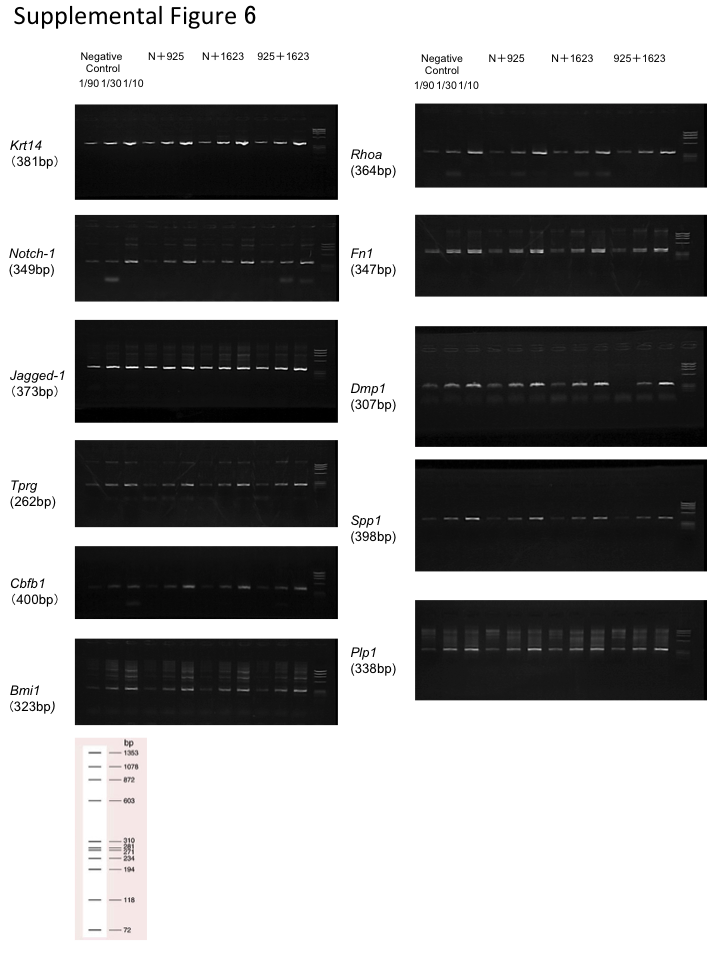
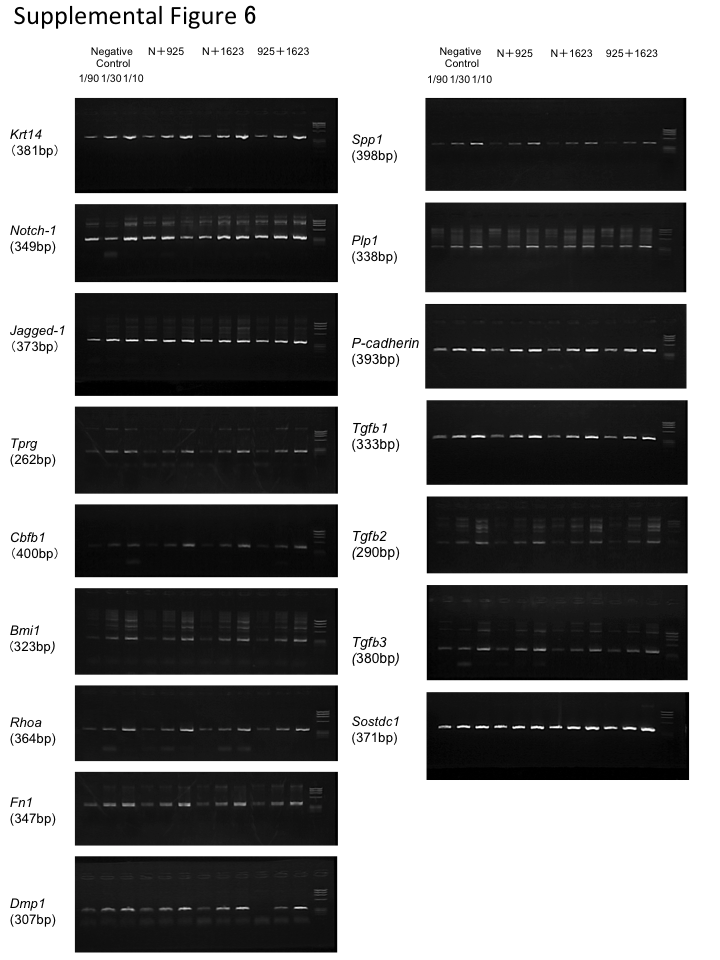
No supernumerary tooth formation of the incisors of bothmice. Scale bar: 100μm.

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**Supplementary Figure 5 |Full-length gels and blots in Figure 5.**

A, C, D: Total RNA (3μg) was extracted from 70% confluent mHAT9d cells and reverse transcribed using a SuperScript ®IV First-Strand Synthesis System (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The cDNAs were serially diluted and PCR amplification was performed using KOD FX (KFX-101, TOYOBO, Osaka, Japan) and specific oligonucleotide primersand glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*)(Supplemental Table 1). PCR products were detected using a 2% agarose gel electrophoresis with φ×174-HaeⅢdigest (3405A, TaKaRa Bio, Shiga, Japan). N indicates negative control stealth siRNA; 925 and 1623 indicate *Cebpb* and *Runx2* type1siRNA, respectively (final concentration 20 nM). Each siRNA was the same in quantity (10 nM each) when mixed. B: Western blotting results.Western blotting of mHAT9d cells transfected with *Cebpb* and *Runx2* type1stealth siRNA was performed using the following antibodies:anti-*Gapdh* primary rabbit polyclonal (1:1000, sc-150; Santa Cruz Biotechnology), secondary anti-rabbit IgG (1:5000, #7074; Cell Signaling Technology), anti-C/EBP beta primary rabbit polyclonal (1:200, #3087; Cell Signaling Technology), anti-*Runx2* primary rabbit polyclonal (1:200, M-70 and sc-10758; Santa Cruz Biotechnology), and secondary anti-rabbit IgG (1:1000, #7074; Cell Signaling Technology). Ez West Blue (AE-1490; ATTO, Tokyo, Japan) was used to stain the membranes and detect the proteins of interest.

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**Supplementary Figure 6 | Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) of mHAT9d cells transfected with stealth siRNA for genes characteristic of them.**

Total RNA was extracted from 70% confluent mHAT9d cells and reverse transcribed using a SuperScript ®IV First-Strand Synthesis System (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The cDNAs were serially diluted and PCR amplification was performed using KOD FX (KFX-101, TOYOBO, Osaka, Japan) and specific oligonucleotide primers (Supplemental Table 2), and glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) (Supplemental Table 1). PCR products were detected using a 2% agarose gel electrophoresis with φ×174-HaeⅢdigest (3405A, TaKaRa Bio, Shiga, Japan). N indicates negative control stealth siRNA; 925 and 1623 indicate *Cebpb* and *Runx2* type1siRNA, respectively (final concentration 20 nM). Each siRNA was the same in quantity (10nM each) when mixed.

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Forward sequence | Reverse sequence | NCBI accession number |
| *Gapdh* | CCATCACCATCTTCCAGGAG | CCTGCTTCACCACCTTCTTG | NM\_008084.3 |
| *Cebpb* | ACACGTGTAACTGTCAGCCG | GCTCGAAACGGAAAAGGTTC | NM\_001287738.1 |
| *Runx1* | AGAAGTGTAAGCCCAGCACA | TTCTCAGTTCTGCCGAGTAG | NM\_001111021.2 |
| *Runx2type2/3* | GAGGGCACAAGTTCTATCTG | CGCTCCGGCCCACAAATCTC | NM\_001146038.2 XM\_006523544.2 |
| *Runx2type1* | CACTTCGCTAACTTGTGGCTGT | TTCATAACAGCGGAGGCATTT | NM\_001145920.2 |
| *Runx3* | GGCTTCCAACAGCATCTTTG | CGGAGTAGTTCTCATCATTG | NM\_019732.2 |
| *Sox2* | AACCACCAATCCCATCCAA | CCAGCAAGAACCCTTTCCTC | NM\_011443.4 |
| *β-catenin* | TGCTGGTGACAGGGAAGACA | CCGAGCAAGGATGTGGAGAG | NM\_007614.3 |
| *E-cadherin* | CCTGTCTTCAACCCAAGCAC | GATTTCCTGACCCACACCAA | NM\_009864.2 |
| *N-cadherin* | CGCCTATGAGTGGGACAGGA | ACGCAGGATGGAAATGTTGG | NM\_007664.5 |
| *Integrin α6* | CATCCTCCTGGCTGTTCTTG | GGGGCTTTGGGTAGTGTGAG | NM\_008397.4 |
| *Integrin β1* | TTAGCACAACCCCAGCAAAG | CCCATCTCCAGCAAAGTGAA | NM\_010578.2 |
| *Vimentin* | CAAGAACACCCGCACCAAC | TGCTTTCGGCTTCCTCTCTC | NM\_011701.4 |
| *Snai2* | CCTCCAAGAAGCCCAACTACA | TGGATGAAGTGTCAGAGGAAGG | NM\_011415.2 |
| *Biglycan* | CCCTGAGACCCTGAACGAAC | ACTCCGAAGCCCATAGGACA | NM\_007542.5 |
| *Decorin* | TGCCTGGGCTGCATAGTTAG | GGTTGTGTCGGGTGGAAAA | NM\_001190451.2 |
| *Bmp2* | CTGTCTTCTAGTGTTGCTGCTTCC | GCCGTTTTCCCACTCATCTC | NM\_007553.3 |
| *Bmp4* | AGGAGGAGGAGGAAGAGCAGA | TCCAGTAGTCGTGTGATGAGGTG | NM\_007554.3 |
| *Bmp6* | TCCCACTCAACGCACACA | CACCCACACACACACACCA | NM\_007556.3 |
| *Bmp7* | GAGGGCTGGTTGGTGTTTG | TGGTTCTTTGGCGTCTTGG | NM\_007557.3 |
| *Bmp11* | AAGTCGCAGATCCTGAGCAAA | TGGGGCTGAAGTGGAAATG | NM\_010272.2 |
| *Ameloblastin* | GCCTGATCCTGTTCCTGTCC | GTTTCATGTTCCCTTGGTCCTATC | NM\_009666 |
| *Amelogenin* | TTTGTTTGCCTGCCTCCTG | GCTGATGGTGTTGGGTTGG | NM\_009664.2 |

**Supplementary Table 1| Specific oligonucleotide primers for reverse transcription polymerase chain reaction (RT-PCR) regarding genes presenting changes of mRNA expression in mHAT9d cells 48 h after transfection with *Cebpb* and *Runx2* type1 stealth siRNA.**

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Forward sequence | Reverse sequence | NCBI accession number |
| *Krt 14* | GCGAGATGGAGCAGCAGAA | GGACAAGGGTCAAGTAAAGAGTGAA | NM\_001313956 |
| *Notch1* | CGCAAGCACCCAATCAAG | CACAGCCCACAAAGAACAGG | NM\_008714 |
| *Jag1* | GCTGGATGGGTCCTGATTG | TTATGGCAGGGGTCAGAGAGA | NM\_013822 |
| *p63* | TTCCCTATGCCACCTTCACA | CAAAAGCCAATACTCCCACGA | NM\_175165 |
| *Cbfb1* | CAGGAACCAATCTGTCTCTCCA | AAGCTGTGCTCCACTTAACGAA | NM\_022309 |
| *Bmi1* | TACGATGCCCAGCAGCAA | ACAGGAAGAGGTGGAGGGAAC | NM\_007552 |
| *Rhoa* | AATGACGAGCACACGAGACG | CGTGGTTGGCTTCTAAATACTGG | NM\_001313961 |
| *Fn1* | CGGGAATGGAAAGGGAGAA | TAGGGTGGGGCTGGAAAGA | NM\_010233 |
| *Dmp1* | TGTCATTCTCCTTGTGTTCCTTTG | ACTGTCGTCTTCATCATCCTCCTT | NM\_016779 |
| *Spp1* | GATTTGCTTTTGCCTGTTTGG | CTGTAGGGACGATTGGAGTGAA | NM\_001204233 |
| *Plp1* | AGCAAAGTCAGCCGCAAAA | TAGAAGCCCTCAGCCAGCA | NM\_011123 |
| *P-cadherin* | TTTCCAGGCCCAGCTAACAC | TCACCACCACCCTCTTCTCC | NM\_001037809.5 |
| *Tgfb1* | TGACAGCAAAGATAACAAACTCCAC | TACTGTGTGTCCAGGCTCCAA | NM\_011577.2 |
| *Tgfb2* | AACCAGAGCGGAGGGTGAA | AGGGCAACAACATTAGCAGGAG | NM\_009367.4 |
| *Tgfb3* | CGCAGACACAACCCATAGCA | ACCAACCCACACTTTCTTTACCAC | NM\_009368.3 |
| *Sostdc1* | TGGAGGCAGGCATTTCAGTAG | AGTTGTGGCTGGACTCGTTG | NM\_025312.3 |

**Supplementary Table 2 | Specific oligonucleotide primers for reverse transcription polymerase chain reaction (RT-PCR) regarding characteristic genes of mHAT9d.**