

## **Insulin-like growth factor 1 modulates bioengineered tooth morphogenesis**

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## **Supplementary Information**

### **Supplementary Methods**

#### **Measurement of the size of tooth germs and teeth**

Tooth germs were photographed at day 7 and 14 of organ culture using a phase contrast microscope (IX71; Olympus, Tokyo, Japan), and the length and width of tooth germs were measured using image analysis software, Cell Sens (Olympus) (Supplementary Fig. S1a). We first drew a line (line a) connecting cusp tips, and then drew another line (line b) perpendicular to line a. Next, we drew another line (line c) perpendicular to line b through the lowest point of a tooth germ at cervical side. The height of tooth germs was defined as the length between line a and c. We drew a fourth line (line d) perpendicular to line b to measure the maximum width of the tooth germ.

We measured the length and width of the bioengineered teeth developed in subrenal capsules and jawbones on micro-CT images using i-VIEW-3DX (Supplementary Fig. S1b). The height (e) of teeth was defined as the maximum length from the cusp tip to the apex along the long axis of tooth. The width (f) of a tooth was defined as the maximum length of the crown on the horizontal plane being perpendicular to the long axis of tooth.

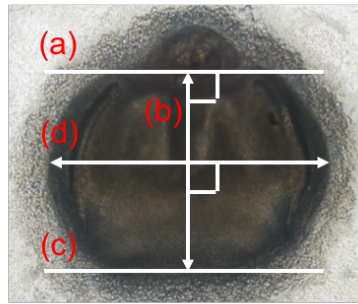
Gene Name	Primer Sequence	Accession No.
<i>Fgf4</i>	5'-TGACATCAGTTTCCAAGGGTGC-3' (S) 5'-CCAGAGTTTTGAGTTGTGGCGAG-3' (AS)	NM_010202

**Supplementary Table S1. Primers used to amplify DNA fragment of *Fgf4*.**

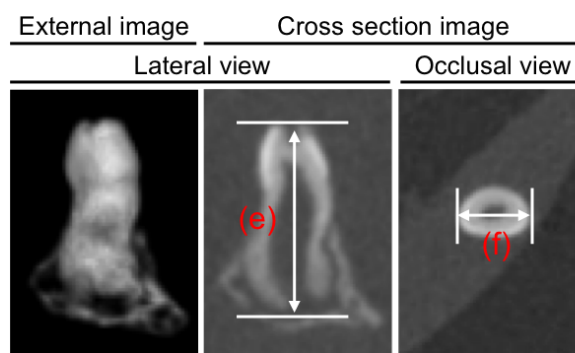
Gene Name	Primer Sequence	Accession No.
<i>Pitx2</i>	5'-ATGTTTTCCCCGCCCAACTC-3' (S) 5'-TTCAGGCTATTGAGGCTGGAGC-3' (AS)	NM_011098
<i>Shh</i>	5'-ACATTGGCACCTGGCTGTTG-3' (S) 5'-AGTTCCTTATTTTCGCAGTCGCTC-3' (AS)	NM_009170
<i>Fgf4</i>	5'-TCCTTTCTGTCTGGTGGCTCAC-3' (S) 5'-ACTGTCTTCTCCCCAACACTCTTG-3' (AS)	NM_010202
<i>Fgf10</i>	5'-CAGCCCCAAACAACAACAACAG-3' (S) 5'-TGAGTAGTTAGGAGAACCCGAAAGC-3' (AS)	NM_008002
<i>Ambn</i>	5'-GGGACTCAACGCACCTTCTCAG-3' (S) 5'-AAGGAGGCTGCTGGGTTTC-3' (AS)	NM_001303431
<i>Dmpl</i>	5'-ACCACACGGACAGCAGTGAATC-3' (S) 5'-CCTCATCGCCAAAGGTATCATCTC-3' (AS)	NM_016779
<i>Dspp</i>	5'-CGACGAATCTAACGGAAGTGACG-3' (S) 5'-TGTAAGAAGCATCTCCACGGCTG-3' (AS)	NM_010080
<i>Map1b</i>	5'-CCTCTCCTACCTGTGTGGCTAATC-3' (S) 5'-CCATTGTGGTGGCTACCTCTAAC-3' (AS)	NM_008634
<i>Igf1</i>	5'-CTGAGCTGGTGGATGCTCTTC-3'(S) 5'-CACTCATCCACAATGCCTGTCT-3'(AS)	NM_001314010
<i>Igf1r</i>	5'-CATTGTGGGTTTGGTTTGGTC-3'(S) 5'-CATAGGAAGCAGACAGGGGTTG-3'(AS)	NM_010513
<i>Bmp2</i>	5'-CAGAGTGGTTGTCCAATCCGTG-3'(S) 5'-TGGTCCTGCTTTGTTCTTGCTG-3'(AS)	NM_007553
<i>Igfbp2</i>	5'-CCCGAGCCATCTCTTCTACAAC-3'(S) 5'-CCCTCCATTTCTGCTGGTGTTC-3'(AS)	NM_008342
<i>Igfbp3</i>	5'-TCTACTTGGGAAGTGTGGGAGAGG-3'(S) 5'-CCTTATCCTTGTCCTTATGGGCTC-3'(AS)	NM_008343
<i>Igfbp4</i>	5'-TGACGAGGTGTTCTCAACTCAAGG-3'(S) 5'-AGGGAGGGCGTTCAATGTTTAC-3'(AS)	NM_010517
<i>Igfbp6</i>	5'-CTATGTGCCAAACTGTGACCTCAG-3'(S) 5'-GTGCTTCCTTGACCATCTGGAG-3'(AS)	NM_008344
<i>Gapdh</i>	5'-TGTGTCCGTCGTGGATCTGA-3' (S) 5'-TTGCTGTTGAAGTCGCAGGAG-3' (AS)	NM_001289726

**Supplementary Table S2. Primers used for RT-PCR and real-time PCR.**

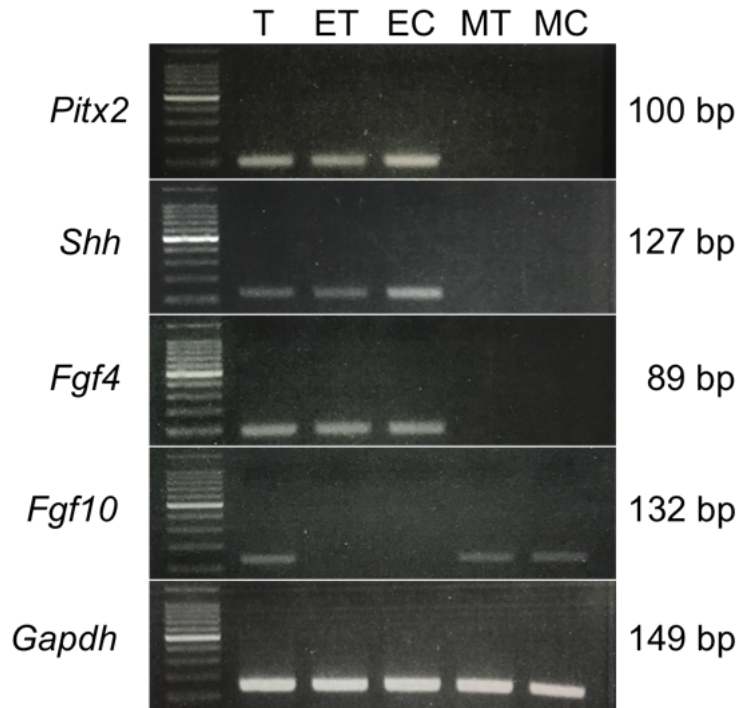
**a**



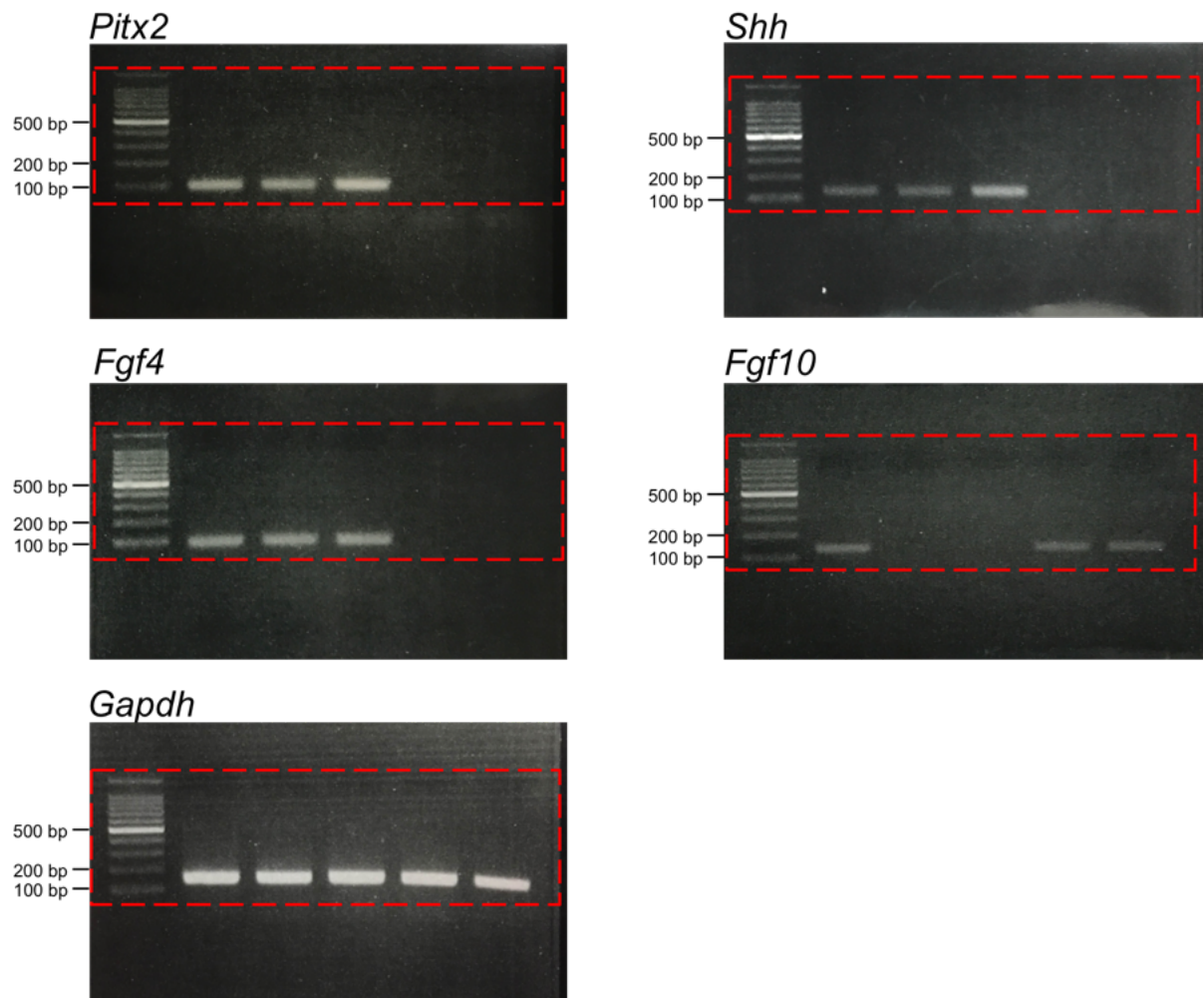
**b**



**Supplementary Figure S1. Measurement of the size of tooth germs and teeth.** (a) Phase contrast images of bioengineered tooth germs. (b) Micro-CT images of bioengineered teeth. Details about the measurement of the size of tooth germs and teeth were described in the Supplementary Methods.

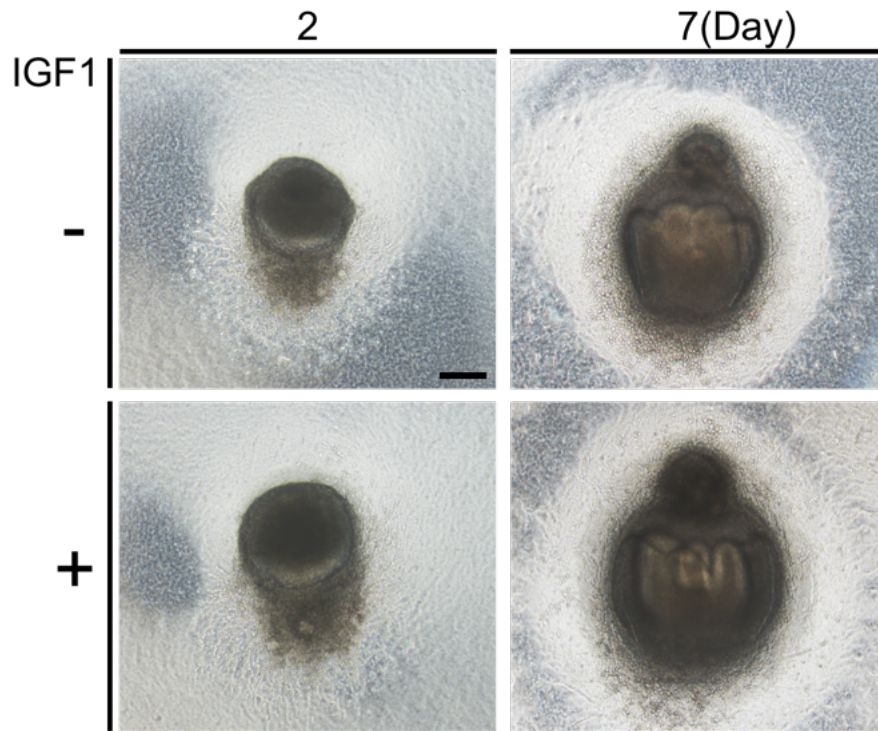


**Supplementary Figure S2. Analysis of gene expressions in epithelial and mesenchymal cells isolated from the ED 14.5 mouse mandibular molar tooth germs.** The mRNA expressions of *Pitx2*, *Shh*, *Fgf4*, *Fgf10* and *Gapdh* were analysed by RT-PCR. The product size of the amplified cDNA was *Pitx2*: 100 bp, *Shh*: 127 bp, *Fgf4*: 89 bp, *Fgf10*: 132 bp, *Gapdh*: 149 bp. T, tooth germ; ET, dental epithelial tissue; EC, dental epithelial cell; MT, dental mesenchymal tissue; MC, dental mesenchymal cell



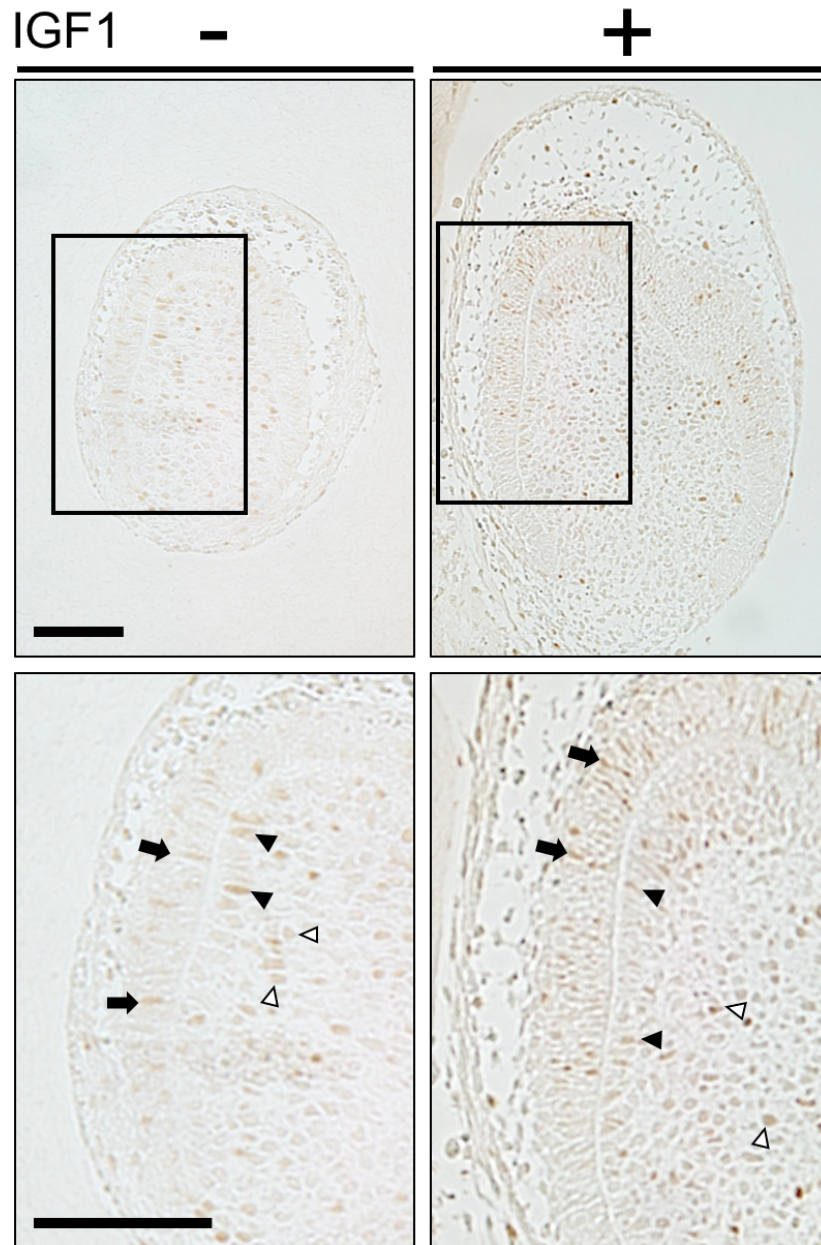
**Supplementary Figure S3. Original photos corresponding to Supplementary Figure S2.**

Dotted red lines show the cropped region.

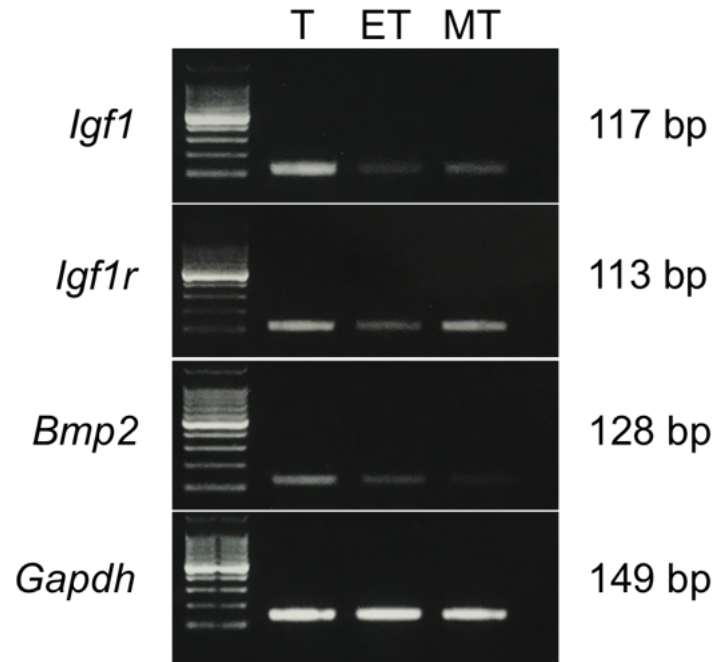


**Supplementary Figure S4. Phase contrast images of bioengineered tooth germs at days 2 and 7 of organ culture in the control and IGF1-treated groups. Scale bar, 200  $\mu$ m.**

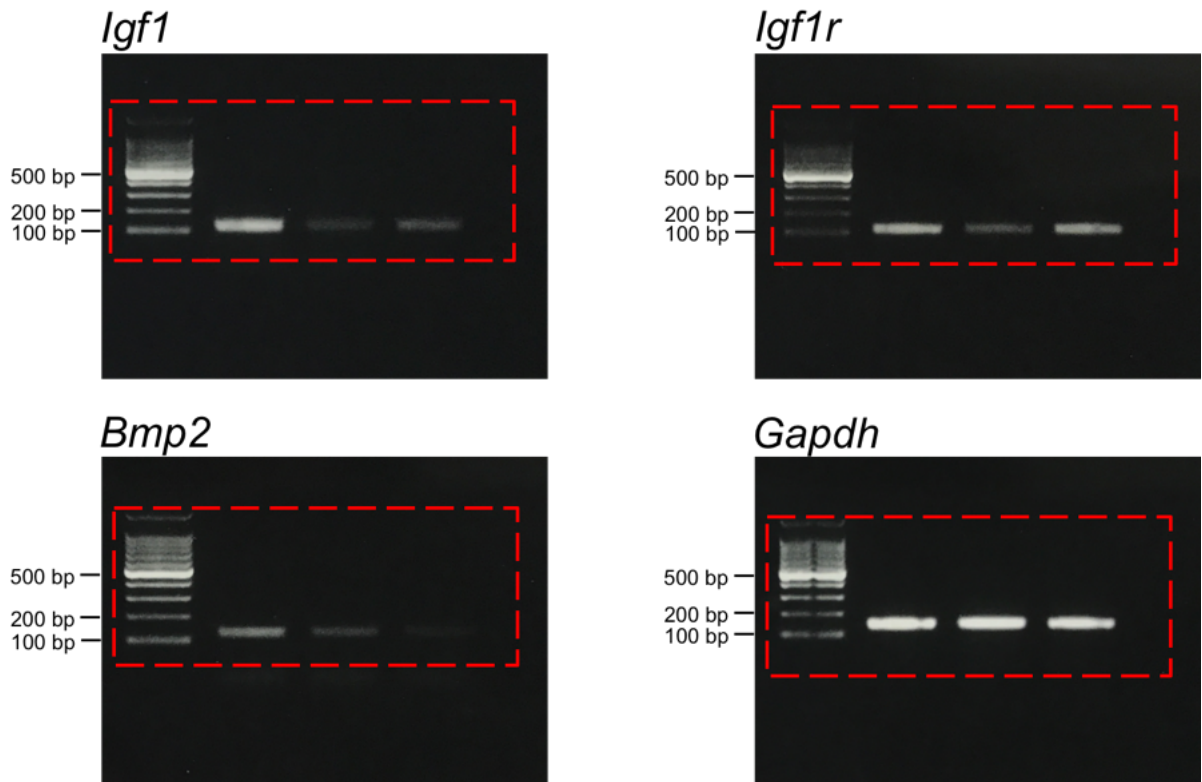




**Supplementary Figure S5. Immunohistochemical staining of IGF1R in bioengineered tooth germs at day 7 of culture in the control and IGF1-treated groups. IGF1R was expressed in ameloblasts (black arrows), odontoblasts (black arrowheads) and dental papilla cells (white arrowheads). The boxed areas in the upper panels are shown as enlarged images in the lower panels. Scale bar, 100  $\mu$ m.**



**Supplementary Figure S6. Analysis of gene expressions in epithelial and mesenchymal tissues isolated from the ED 14.5 mouse mandibular molar tooth germs.** The mRNA expressions of *Igf1*, *Igf1r*, *Bmp2* and *Gapdh* were analysed by RT-PCR. The product size of the amplified cDNA was *Igf1*: 117 bp, *Igf1r*: 113 bp, *Bmp2*: 128 bp, *Gapdh*: 149 bp. T, tooth germ; ET, dental epithelial tissue; MT, dental mesenchymal tissue



**Supplementary Figure S7. Original photos corresponding to Supplementary Figure S4.**

Dotted red lines show the cropped region.