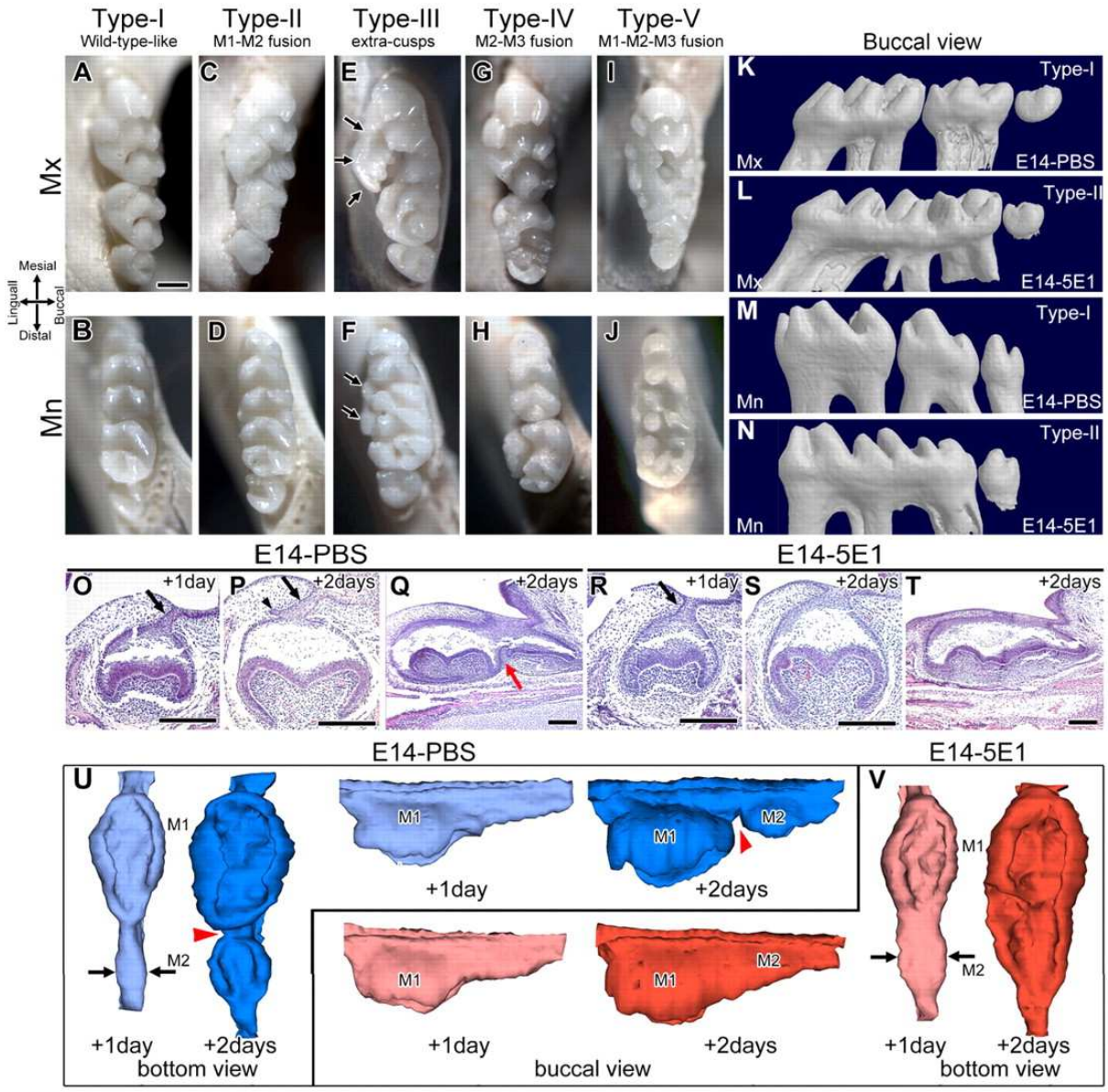

Fig. 1. **Morphological changes in molar spatial patterning in mouse after maternal transfer of 5E1.** (**A-J**) Five types of molar patterns obtained in maxilla (Mx) and mandible (Mn): Type-I, wild-type-like with first (M1), second (M2) and third (M3) molar; Type-II, M1-M2 fusion; Type-III, M1-M2 fusion with extra lingual cusps (arrows); Type-IV, M2-M3 fusion; Type-V, M1-M2-M3 fusion. (**K-N**) Three-dimensional micro-computed-tomography images showing buccal aspect of type-I and type-II patterns at embryonic day (E) 14 after injection of phosphate-buffered saline (E14-PBS) and after 5E1 injection (E14-5E1) in maxilla and mandible. (**O-T**) Dental lamina (arrow) is evident in the frontal section of M1 in both E14-PBS and E14-5E1 at one day after injection (O,R). After two days, dental lamina and lingual epithelial bud (arrowhead) are observed only in an E14-PBS (compare P with S), and M1-M2 separation (red arrow) is evident in E14-PBS (Q). M1-M2 fusion is evident in E14-5E1 (T). (**U**,**V**) Three-dimensional images of dental epithelium from bottom view show the larger buccolingual diameter of M2 (between arrows) in E14-5E1 (V) than in E14-PBS (U) after one day. The boundary (red arrowheads) between M1 and M2 is clearly shown in E14-PBS after two days both from bottom and buccal view (U), whereas M1-M2 fusion is evident in E14-5E1 (V). Scale bars: 500 μm.

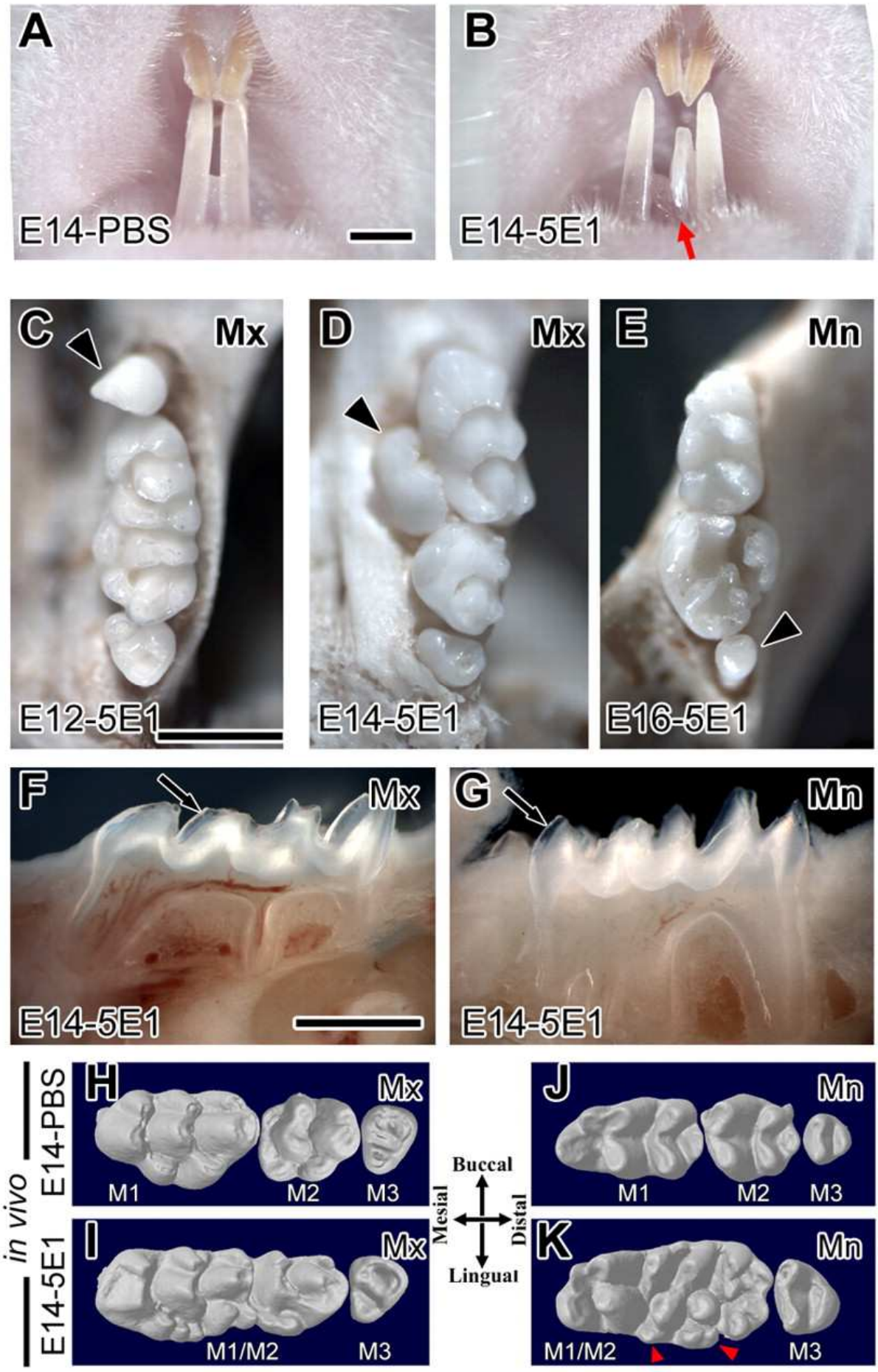
A
E14-PBS
B
E14-5E1
C
Mx
E12-5E1
D
Mx
E14-5E1
E
Mn
E16-5E1
F
Mx
E14-5E1
G
Mn
E14-5E1
in vivo
E14-PBS
H
Mx
M1
M2
M3
E14-5E1
I
Mx
M1/M2
M3
Buccal
Mesial
Distal
Lingual
J
Mn
M1
M2
M3
K
Mn
M1/M2
M3

Fig. 2.

Morphological changes in mouse molars after 5E1 treatment in vivo. (**A**,**B**) Supernumerary incisors (red arrow) develop in embryonic day (E) 14 mice treated with 5E1 (B, E14-5E1; 2/82 in mandibular quadrants) but not in control mice treated with PBS (A, E14-PBS). (**C-E**) Supernumerary molars (arrowheads) develop in E12-5E1 [1/40 in the maxillary quadrants (C), 1/32 in the mandibular quadrants], E14-5E1 (4/78 in the maxillary quadrants, D) and E16-5E1 (1/54 in the mandibular quadrants, E). (**F**,**G**) Transparent enamel (arrows) is seen covering the dentine in sagittal sections of fused molars in Mx and Mn of E14-5E1. (**H-K**) Three-dimensional images from occlusal view show pattern of molars in 4-week-old E14-PBS and E14-5E1. E14-PBS mice have three molars in Mx (H) and Mn (J). M1-M2 fusion is observed in Mx (I) and Mn (K) of E14-5E1. Extra cusps are evident on lingual side of a fused molar (red arrowheads in K). Scale bars: 1 mm. Mx, maxilla; Mn, mandible.

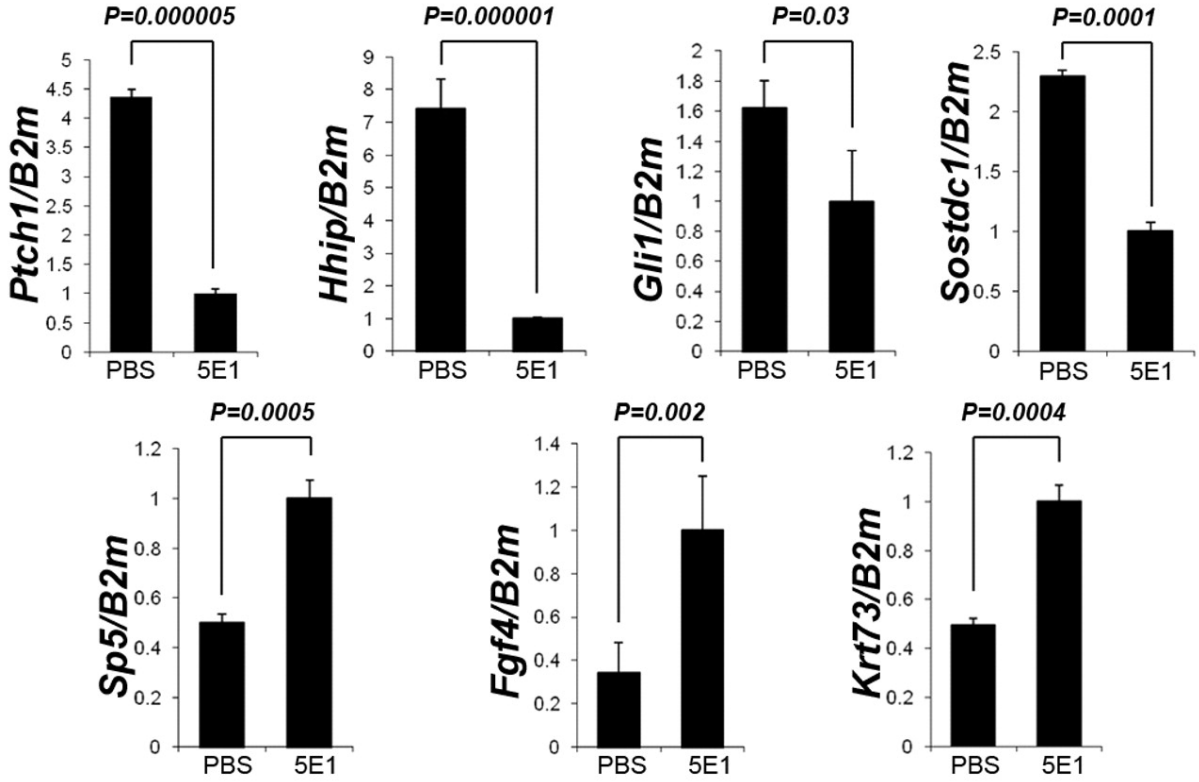

Fig. 3.

Transcriptional changes in molars after maternal transfer of 5E1 into mouse embryos at E14. RT-qPCR analysis shows up- or downregulation of *Ptch1, Hhip, Gli1, Sostdc1, Sp5, Fgf4* and *Krt73*. The amount of each of the RT-qPCR products was normalized using β-2-microglobulin (*B2m*) as an internal control. Student's *t*-test was performed for statistical analysis with level of statistical significance set at $P<0.05$. Error bars indicate s.d. on the normalized ratio.

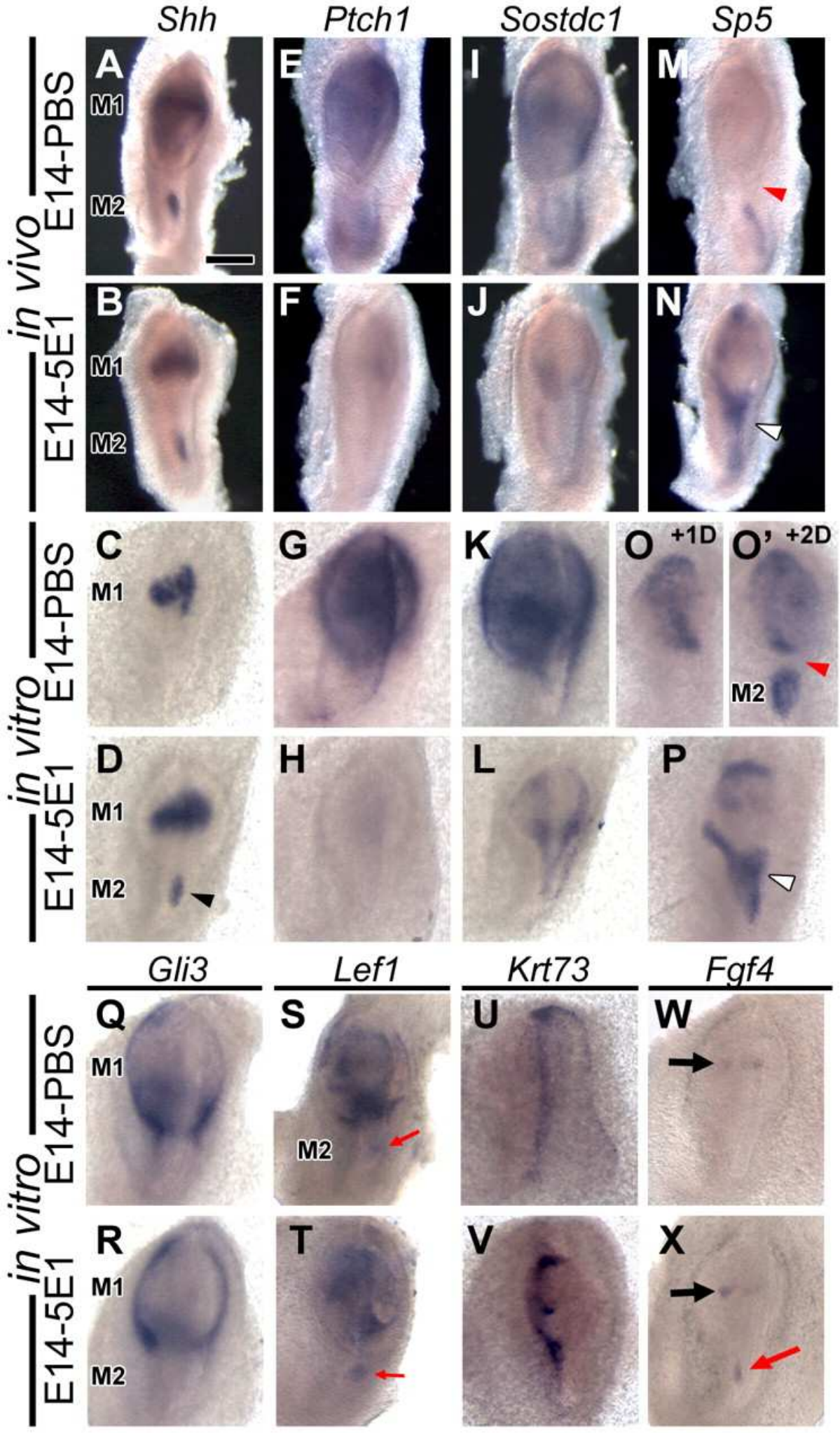
Shh
Ptch1
Sostdc1
Sp5
in vivo
E14-PBS
E14-5E1
A
B
E
F
I
J
M
N
M1
M2
in vitro
C
D
G
H
K
L
O
+1D
O'
+2D
P
Gli3
Lef1
Krt73
Fgf4
Q
R
S
T
U
V
W
X

Fig. 4.

Alterations in gene expression pattern in 5E1-treated mouse molars. (**A-D**) Whereas the pattern of *Shh* expression is the same in E14-PBS and E14-5E1 mice after one day in vivo (A,B), *Shh* expression in M2 (black arrowhead) is evident only in E14-5E1 in vitro, showing accelerated M2 development (compare D with C). (**E-H**) *Ptch1* is strongly expressed in E14-PBS in vivo (E) and in vitro (G), but disappeared in E14-5E1 (F,H). (**I-L**) *Sostdc1*-expressing areas are markedly reduced in E14-5E1 (J,L) compared with E14-PBS (I,K). (**M-P**) *Sp5* expression in M1 and M2 is separated in E14-PBS in vivo (red arrowhead in M). *Sp5* is observed in M1 of E14-PBS after one day (+1D) culture in vitro (O) and expressed in M1 and M2 separately after two days (+2D) (red arrowhead in O′), but connected in E14-5E1 in vivo and from one day in culture (white arrowheads in N and P). (**Q**,**R**) *Gli3* expression pattern of E14-PBS is the same as that of E14-5E1 after one day in vitro. (**S**,**T**) *Lef1* expression is observed in M1 after one day in vitro in both E14-PBS and E14-5E1, but its domain of expression in M2 (red arrows) is larger in E14-5E1 (T) than in E14-PBS (S). (**U**,**V**) *krt73* is expressed in a line in E14-PBS (U), but is widely expressed throughout M1 and M2 in E14-5E1 (V). (**W**,**X**) *Fgf4* expression in M1 (black arrow) is evident in both E14-PBS (W) and E14-5E1 (X), but its expression in M2 (red arrow) is only present in E14-5E1 (X). Scale bar: 200 μm

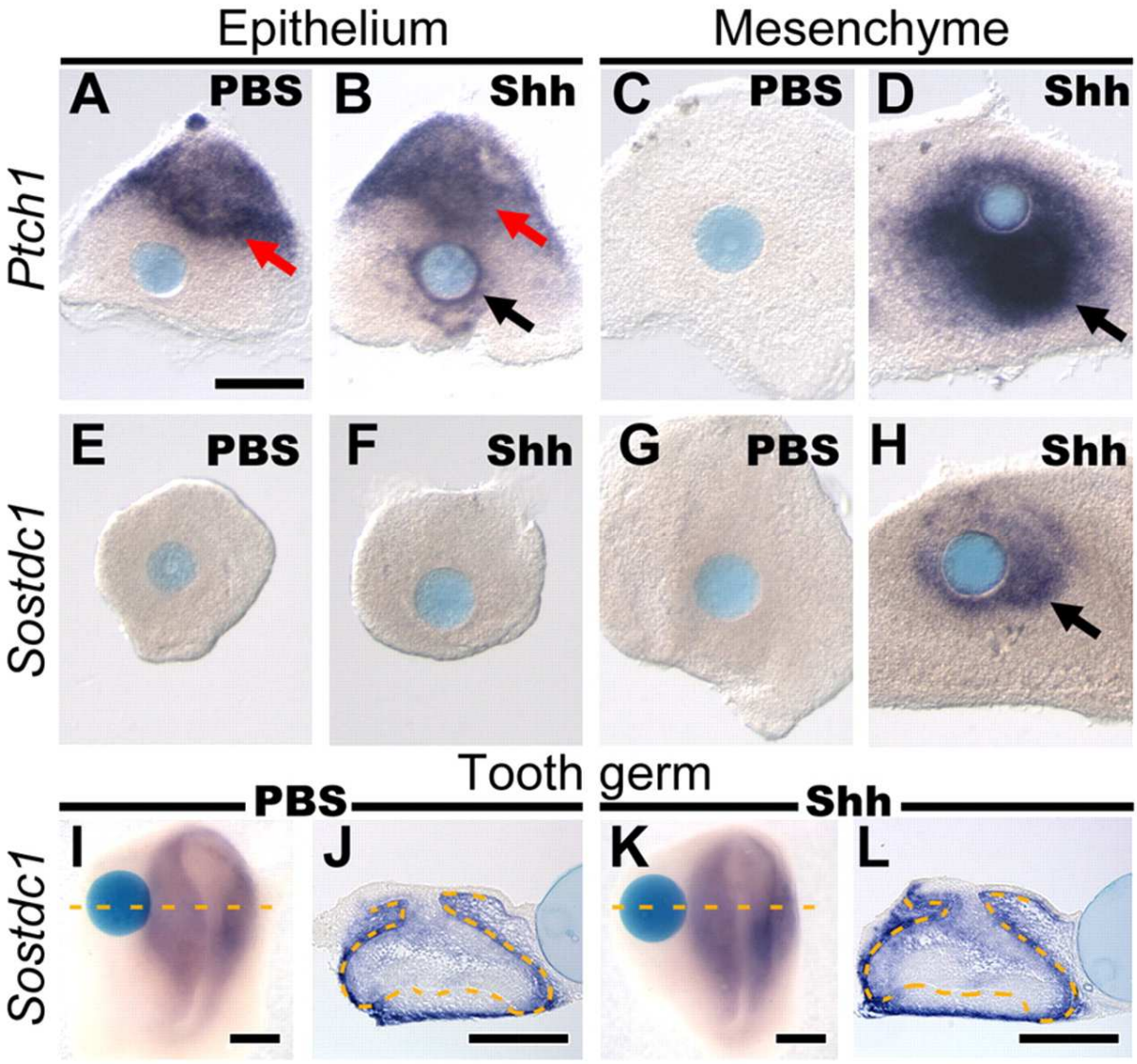

Fig. 5.

Effects of Shh on *Sostdc1* expression in mouse. (**A-D**) Apart from endogenous *Ptch1* (red arrows in A and B), exogenous *Ptch1* is expressed around the Shh protein bead in both dental epithelium and mesenchyme (black arrows in B,D), but not around PBS beads (A,C). (**E-H**) *Sostdc1* is not expressed around the control PBS beads (E,G). *Sostdc1* is induced by exogenous Shh protein in mesenchyme (arrow in H), but not in epithelium (F). (**I-L**) *Sostdc1* is expressed in dental epithelium and mesenchyme around wild-type tooth germs at E14 but is not induced in either epithelium or mesenchyme around beads soaked in Shh protein. Occlusal views are shown in I and K. Frontal section is shown in J and L at the level of the dashed lines in I and K, respectively. Yellow dashed lines indicate the boundary of the dental epithelium and mesenchyme. Scale bars: 200 μm.

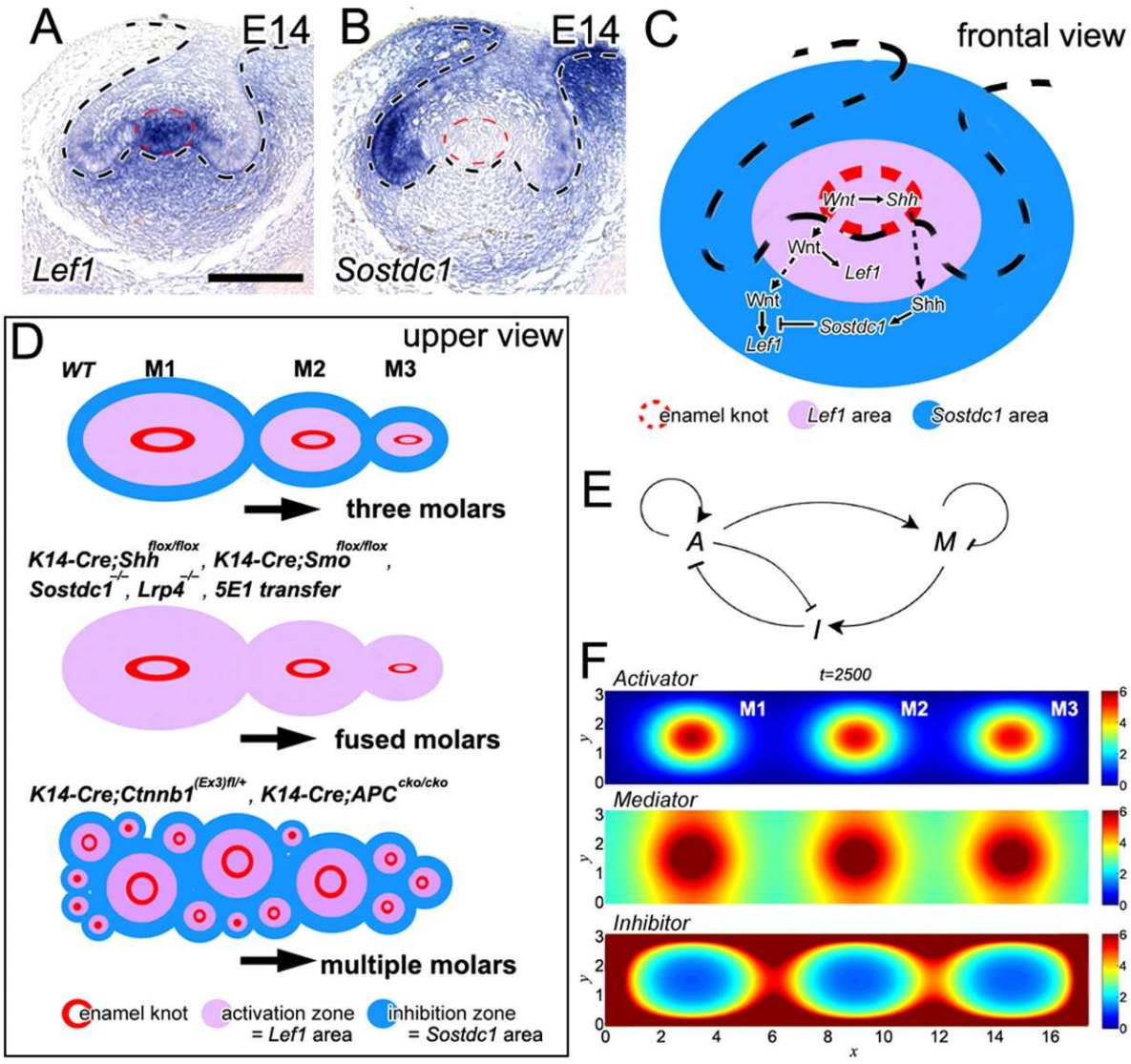

Fig. 6.

Wnt-Shh-Sostdc1 negative feedback loop for the regulation of the tooth spatial patterning. (**A**,**B**) Frontal section of M1 at E14 shows that *Lef1* is expressed in the enamel knot (inside the red dashed circles) and dental mesenchyme surrounding the enamel knot, whereas *Sostdc1* is expressed mainly outside the *Lef1*-expressing area. (**C**) Schematic of Wnt-Shh-Sostdc1 negative feedback loop in M1 at E14 from frontal view. Wnt signals in the enamel knot induce *Shh* in the enamel knot and Wnt moves laterally to induce *Lef1* and *Sp5*. Secreted Shh in the enamel knot also moves laterally to induce *Sostdc1*. The *Sostdc1*-expressing area (*Sostdc1* area) is non-overlapping with the

Lef1-expressing area (*Lef1* area). (**D**) Wild-type mouse has three molars, each of which has an activation zone (*Lef1* area) and an inhibition zone (*Sostdc1* area). Loss of the inhibition zone in *K14-Cre;Shh*$^{flox/flox}$, *K14-Cre;Smo*$^{flox/flox}$, *Sostdc1*$^{-/-}$, *Lrp4*$^{-/-}$ and 5E1-transferred mice enhances molar fusion. Sustained Wnt/β-catenin signals in *K14-Cre;Ctnnb1*$^{(Ex3)fl/+}$ and *K14-Cre;APC*$^{cko/cko}$ induce multiple activation and inhibition zones to form multiple molars. (**E**) Schematic of the proposed reaction-diffusion model showing interactions between activator (*A*), mediator (*M*) and inhibitor (*I*). (**F**) Simulated patterns of activator, mediator and inhibitor show three molars in wild-type mice. The black dotted lines in A-C indicate the boundary of the dental epithelium and mesenchyme. Scale bar: 100 μm.