SUPPLEMENTAL MATERIAL

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

TUNEL staining- TUNEL staining was performed as described previously (4,18-20).

Cell cycle assay- Determination of cell cycle stage of cells from *Tgfbr2*$^{fl/fl}$ and *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* maxilla was performed at different embryonic stages by staining the cells with 5 μg/ml propidium iodide (Sigma). Cells were analyzed by flow cytometry according to standard procedures (Beckman).

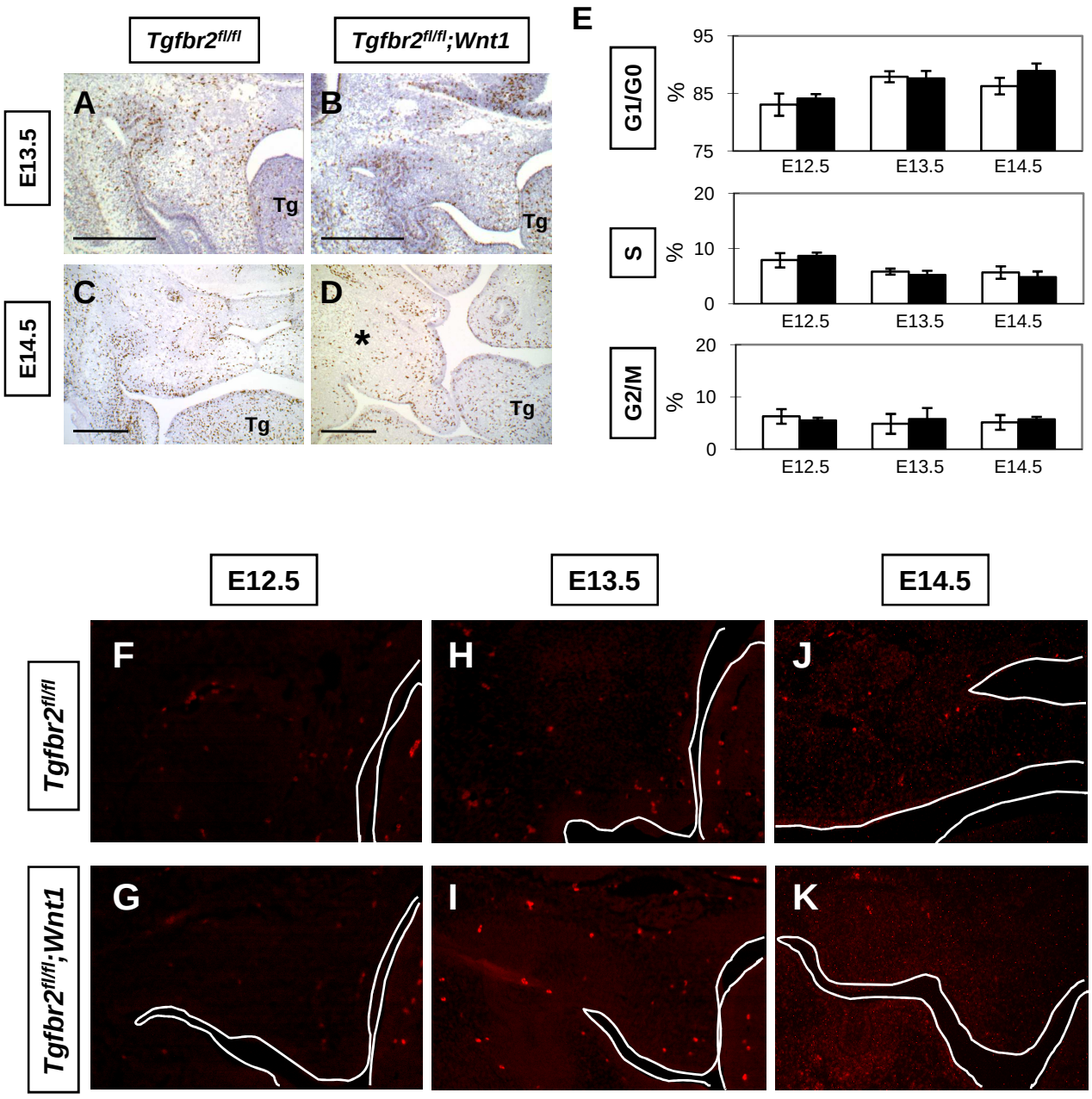
LEGENDS TO SUPPLEMENTARY FIGURES

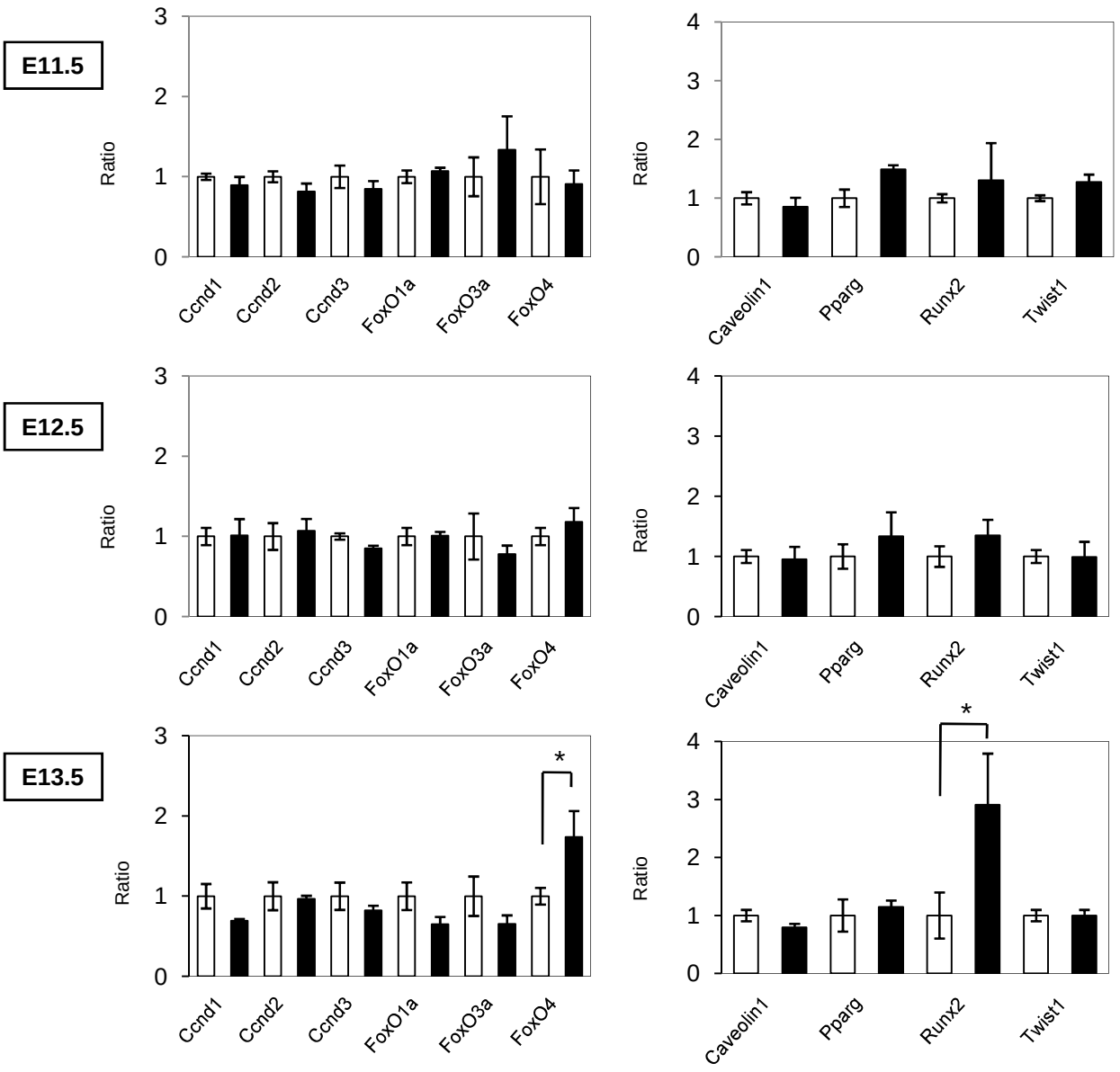
Fig. S1. Cell death in *Tgfbr2*$^{fl/fl}$ and *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* mice during maxillary development. *A-D*. BrdU staining of *Tgfbr2*$^{fl/fl}$ (A, C) and *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* (B, D) maxilla at E13.5 and E14.5. Asterisk indicates the reduced BrdU-positive area in the maxilla of *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* mice. Tg is tongue. Scale bar; 200 μm. *E*. Cell cycle analysis by flow cytometry. The percentage of *Tgfbr2*$^{fl/fl}$ (white bars) and *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* (black bars) maxillary cells in G1/G0, S, G2/M phases of the cell cycle at E12.5, E13.5 and E14.5. *F-K*. TUNEL staining of the maxilla at E12.5, E13.5 and E14.5 in sections from *Tgfbr2*$^{fl/fl}$ (F, H, J) and *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* (G, I, K) mice. Line outlines the edges of palates and tongue.

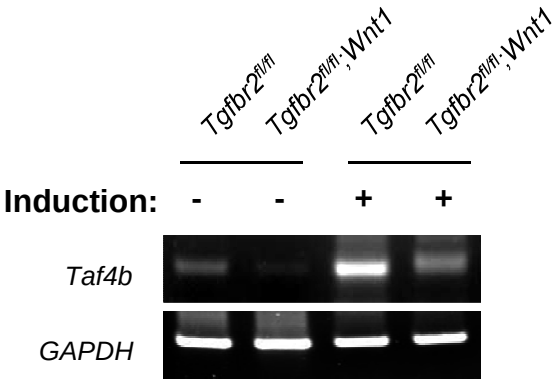
Fig. S2. Time course of expression of genes related to cell proliferation and osteogenic differentiation in *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* maxilla during intramembranous ossification. Quantitative RT-PCR analyses of indicated mRNA from the maxilla of *Tgfbr2*$^{fl/fl}$ (open columns) and *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* (closed columns) mice at E11.5, E12.5 and E13.5. *, $P < 0.05$.

Fig. S3. Gene expression of *Taf4b* during osteogenic differentiation in *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* MEMM cells. mRNA expression of *Taf4b* after no osteogenic induction (-) or induction (+) in *Tgfbr2*$^{fl/fl}$ and *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* MEMM cells.

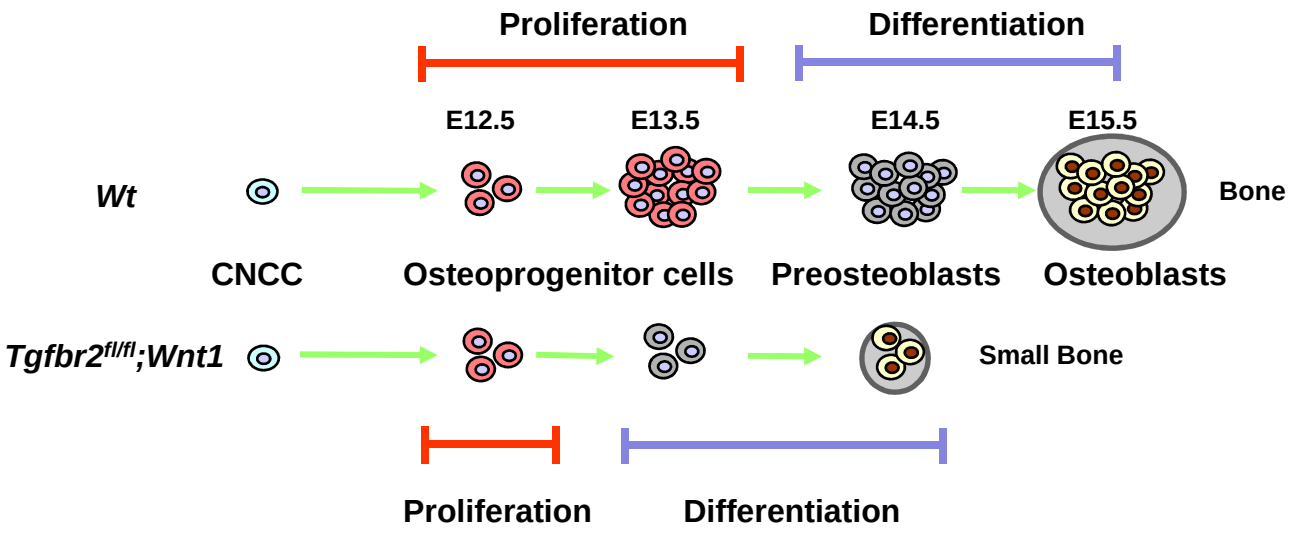
Fig. S4. Model for Tgf-β-mediated effect on proliferation/differentiation and gene regulation during intramembranous ossification. *A*. Schematic diagram depicting our model of the proliferation and differentiation of wild type (*Wt*) and *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* CNCC. The proliferation period of osteoprogenitor cells derived from *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* mice is shorter than that of wild-type mice. The consequence of the decreased proliferation term is the early onset of osteogenic differentiation. The decreased size of the maxilla in *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* mice results from the decreased number of osteoprogenitor cells. *B*. Schematic diagram depicting our model of the network of Tgf-β downstream targets. Tgf-β regulates the gene expressions of basal transcriptional factors *Taf4b* and, to a lesser extent, *Taf1*. Taf4b, which is expressed specifically in osteoprogenitor cells, regulates the downstream targets *FoxO4*, *Runx2*, and *Spp1*. Gene expression of Cyclin D is negatively regulated by FoxO4. *Runx2* is regulated by a combination of *Taf1* and *Taf4*b, and it in turn regulates the expression of osteogenic genes such as *osteonectin* (*On*), *collagen type I* (*ColI*), and *osteocalcin* (*Ocn*).


Tgfbr2fl/fl
Tgfbr2fl/fl;Wnt1
E13.5
E14.5
A
B
C
D
Tg
*
E
G1/G0
S
G2/M
%
95
85
75
20
10
0
E12.5
E13.5
E14.5
E12.5
E13.5
E14.5
Tgfbr2fl/fl
Tgfbr2fl/fl;Wnt1
F
H
J
G
I
K

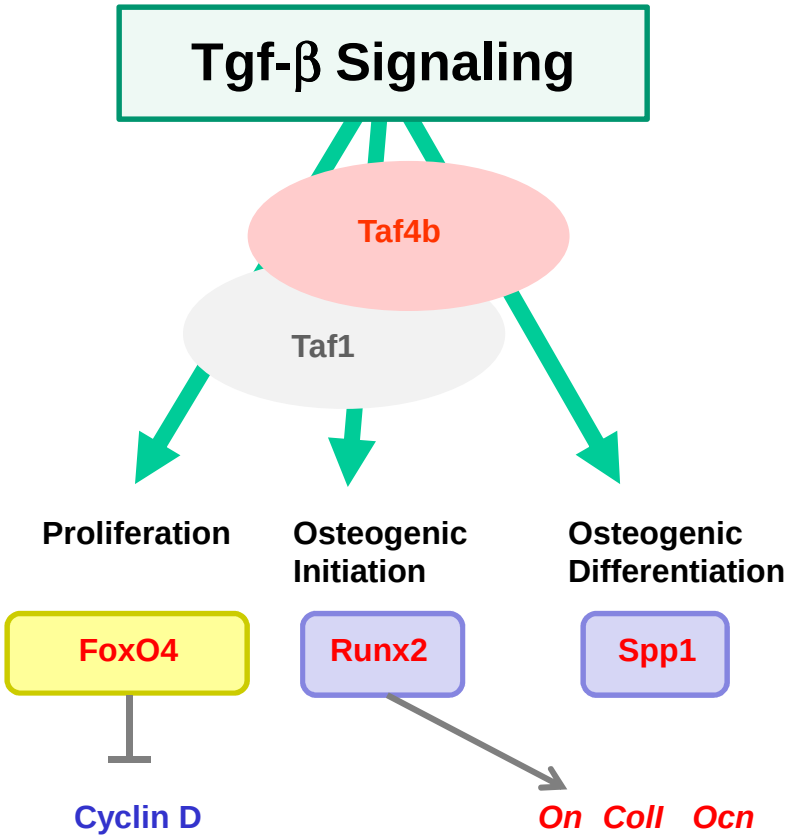

E11.5
E12.5
E13.5
Ratio
Ccnd1
Ccnd2
Ccnd3
FoxO1a
FoxO3a
FoxO4
Caveolin1
Pparg
Runx2
Twist1
*


Tgfbr2fl/fl
Tgfbr2fl/fl;Wnt1
Tgfbr2fl/fl
Tgfbr2fl/fl;Wnt1
Induction: - - + +
Taf4b
GAPDH

A


Proliferation
Differentiation
E12.5
E13.5
E14.5
E15.5
Wt
Bone
CNCC
Osteoprogenitor cells
Preosteoblasts
Osteoblasts
Tgfbr2fl/fl;Wnt1
Small Bone
Proliferation
Differentiation

B


Tgf-β Signaling
Taf4b
Taf1
Proliferation
Osteogenic Initiation
Osteogenic Differentiation
FoxO4
Runx2
Spp1
Cyclin D
On Coll Ocn