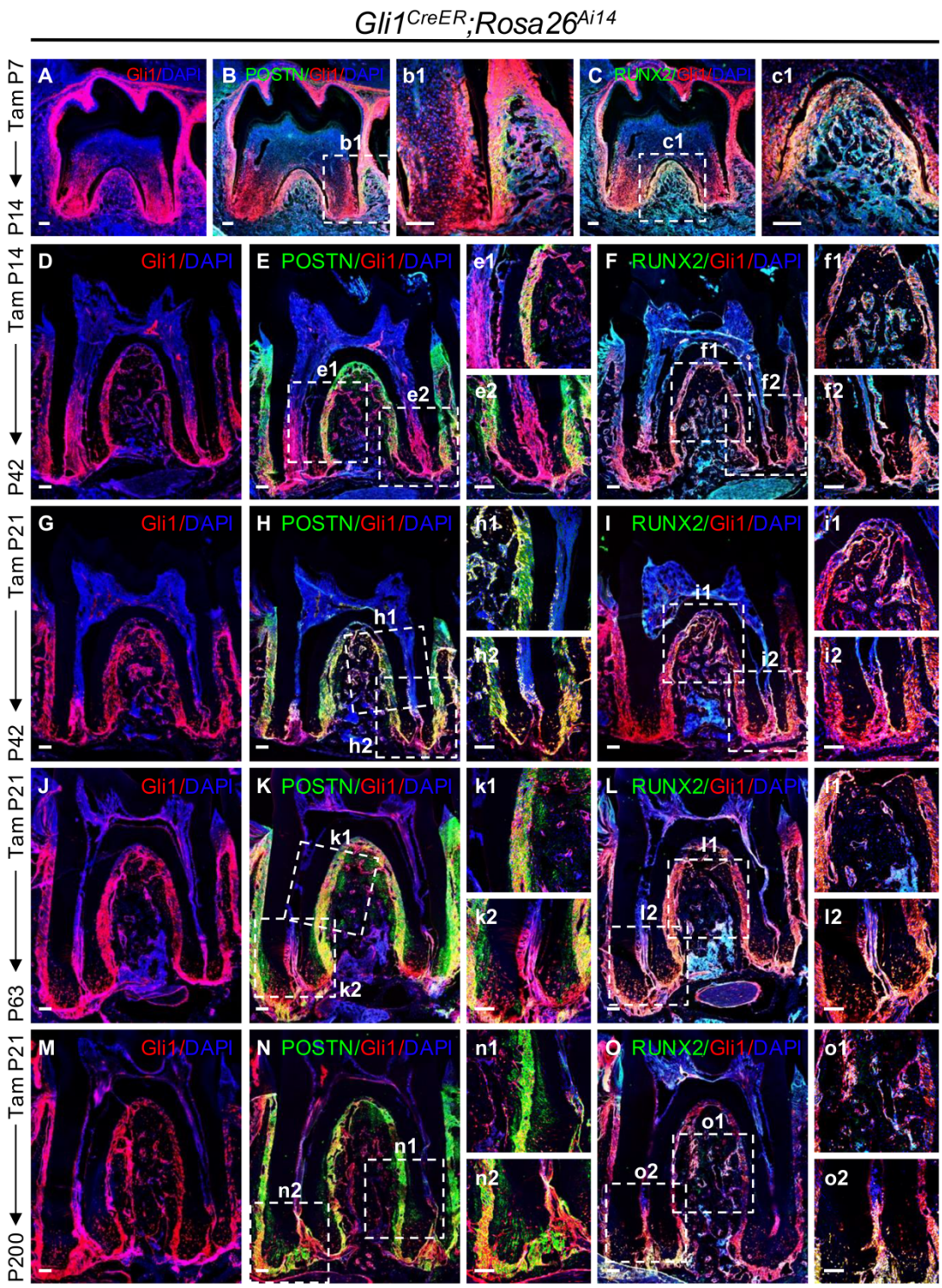
**Supplemental data**

**Supplemental Figures**




**Supplemental Figure 1. Gli1$^{+}$ cells distribution in apical pulp, PDL, and oral bone.**

(**A-C**) Lineage tracing of Gli1$^{+}$ lineage cells on postnatal day 14 (P14) after Tamoxifen induction at P7. Immunofluorescent staining of Periostin (POSTN) (**B**), and RUNX2 (**C**) was performed. Boxed areas are shown at higher magnification in (**b1**), (**c1**). n=3.

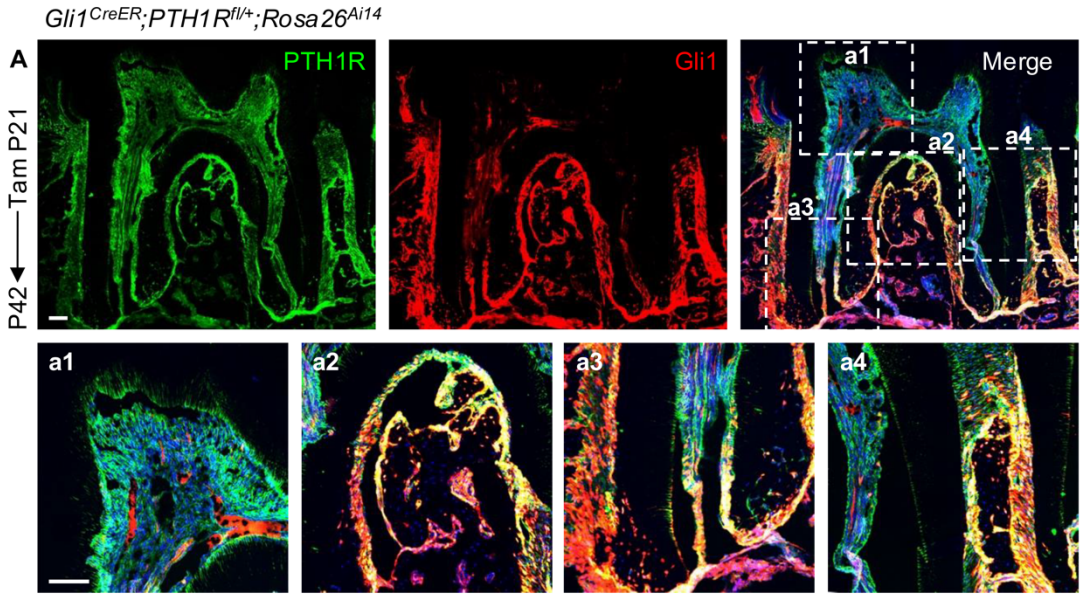
(**D-F**) Lineage tracing of Gli1$^{+}$ lineage cells on P42 after Tamoxifen induction at P14. Immunofluorescent staining of POSTN (**E**), and RUNX2 (**F**) was performed. Boxed areas are shown at higher magnification in (**e1**), (**e2**), (**f1**), (**f2**). n=3. The osteocyte number was calculated in the furcation area of the first mandibular molars in at least six confocal images of each sample.

(**G-I**) Lineage tracing of Gli1$^{+}$ lineage cells on P42 after Tamoxifen induction at P21. Immunofluorescent staining of POSTN (**H**), and RUNX2 (**I**) was performed. Boxed areas are shown at higher magnification in (**h1**), (**h2**), (**i1**), (**i2**). n=3.

(**J-L**) Lineage tracing of Gli1$^{+}$ lineage cells on P63 after Tamoxifen induction at P21. Immunofluorescent staining of POSTN (**K**), and RUNX2 (**L**) was performed. Boxed areas are shown at higher magnification in (**k1**), (**k2**), (**l1**), (**l2**). n=3.

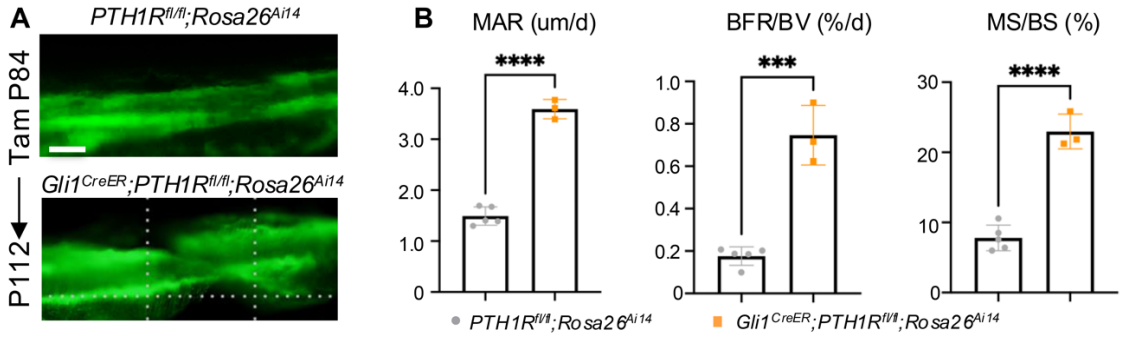
(**M-O**) Long-term lineage tracing of Gli1$^{+}$ lineage cells P200 after Tamoxifen induction at P21. Immunofluorescent staining of POSTN (**N**), and RUNX2 (**O**) was performed. Boxed areas are shown at higher magnification in (**n1**), (**n2**), (**o1**), (**o2**). n=3.

Scale bar=100 μm.




**Supplemental Figure 2. Distribution of PTH1R and Gli1$^{+}$ lineage cells.**

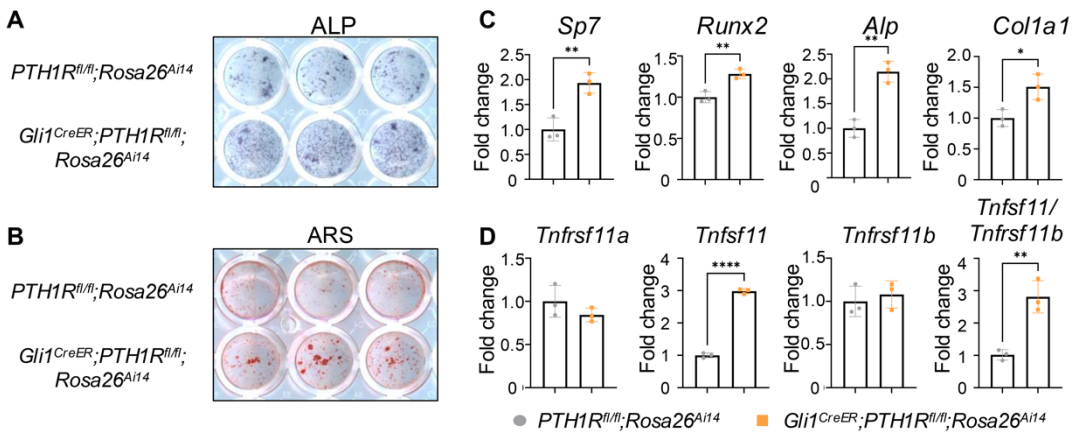
(**A**) Immunofluorescent staining of PTH1R in *Gli1$^{CreER}$;PTH1R$^{fl/+}$;Rosa26$^{Ai14}$* mice showed co-localization of PTH1R and Gli1$^{+}$ lineage cells. Boxed areas are shown at higher magnification. n=3. Scale bar=100 μm.




**Supplemental Figure 3. Bone histomorphometry analysis at P112.**

(**A**) Double calcein labeling in the alveolar bone region of control and PTH1R-cKO mice at P112.

(**B**) Histomorphometry analysis of dynamic bone formation parameters at P112. n=5 in control and n=3 in PTH1R-cKO mice. Male mice were used. Data are mean ± SEM. ***$P$<0.001, ****$P$< 0.0001.

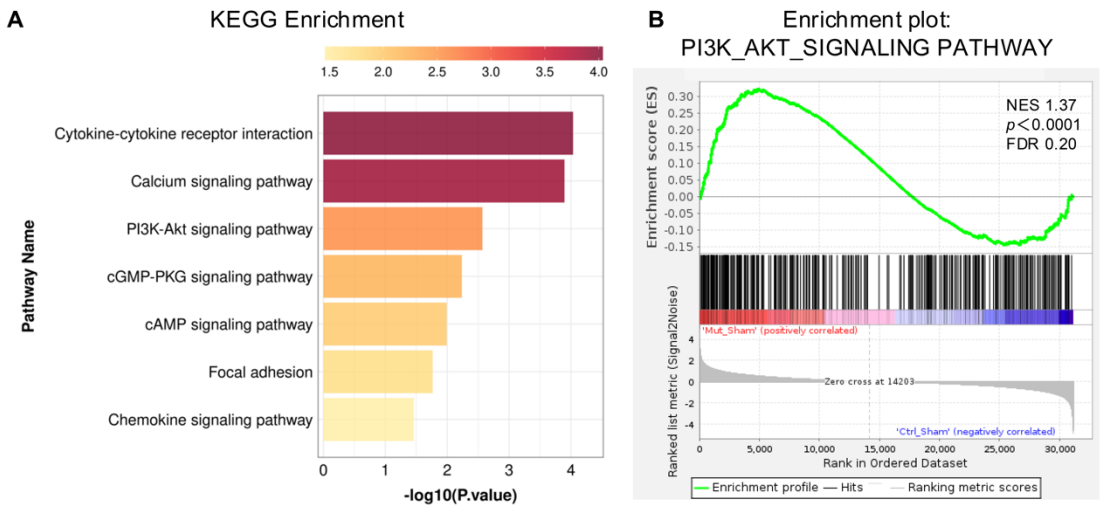



**Supplemental Figure 4. PTH1R-deficient OMSCs presented increased osteogenic differentiation potential.**

(**A-B**) Alkaline phosphatase (ALP) staining at 7-day of osteogenic induction and alizarin red staining (ARS) at 14-day of osteogenic induction reveal a significantly increased osteogenesis of OMSCs extracted from *Gli1*$^{CreER}$;*PTH1R*$^{fl/fl}$;*Rosa26*$^{Ai14}$ mice. n=3.

(**C, D**) RT-qPCR analysis showed upregulated osteogenic-related gene expression (*Sp7, Runx2, Alp, Col1a1, Tnfsf11*) of PTH1R-cKO OMSCs after 14 d of osteogenic induction. n=3.

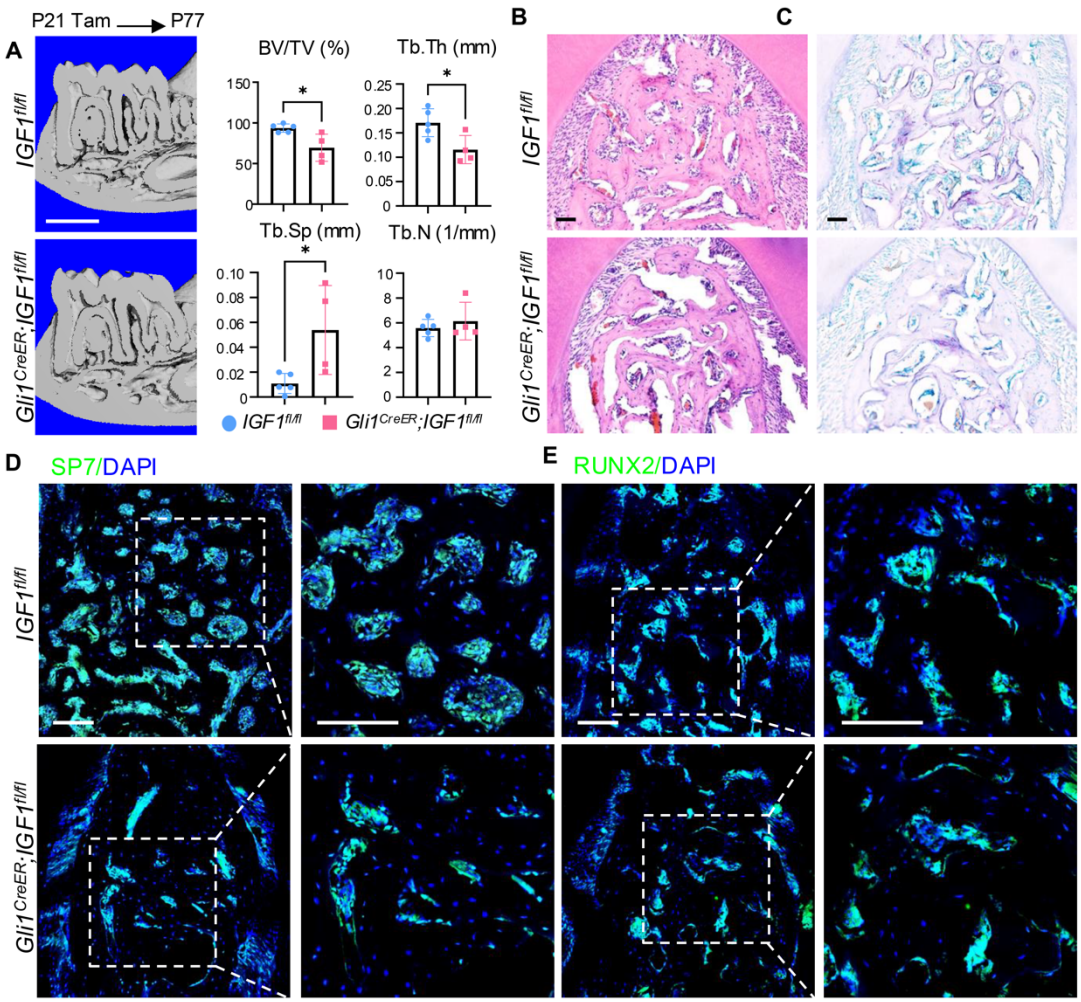
Significance is determined using unpaired two-sided student's t-tests. Data are mean ± SEM. **P*<0.05, ***P*<0.01, *****P*<0.0001.




**Supplemental Figure 5. KEGG enrichment analysis of control and PTH1R-cKO mice.**

(**A**) KEGG enrichment result bar graph of PTH1R-cKO vs. control mandibular bones.

(**B**) GSEA plots showed the upregulation of PI3K-Akt signaling pathway in PTH1R-cKO mice.




**Supplemental Figure 6. *Gli1*$^{CreER}$*;IGF1*$^{fl/fl}$ mice exhibit low bone turnover.**

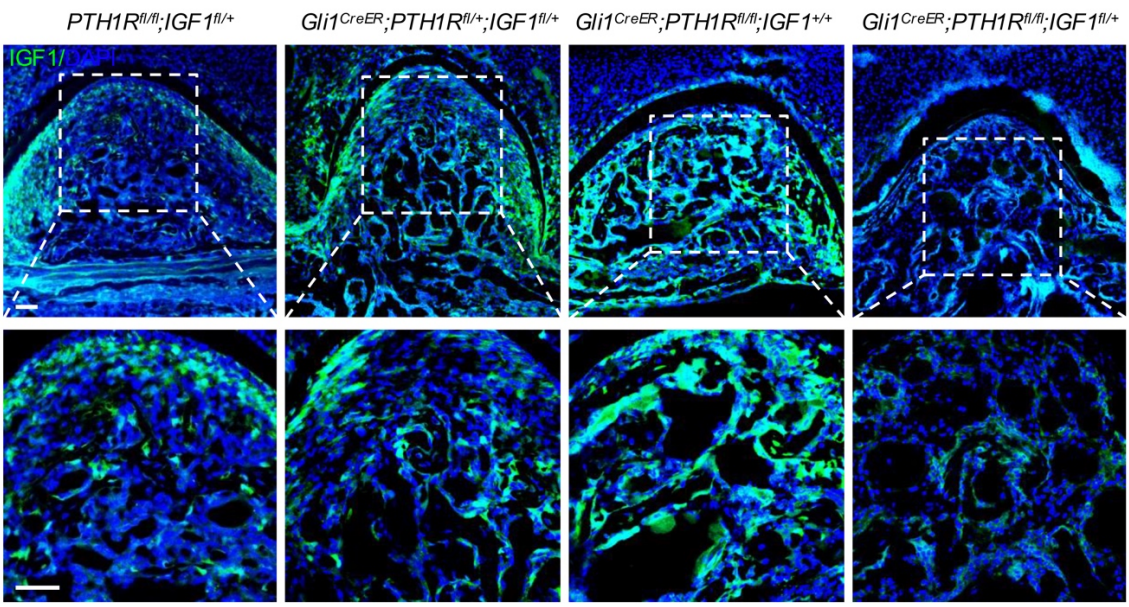
(**A**) Three-dimensional micro-CT reconstruction and quantitative analysis of alveolar bone parameters including BV/TV (%), Tb. Th (mm), Tb. Sp (mm) and Tb. N (1/mm) of male control and IGF1-cKO mice. n=5 for *IGF1*$^{fl/fl}$ and n=4 for *Gli1*$^{CreER}$*;IGF1*$^{fl/fl}$ mice.

(**B**) HE staining of mandibular first molar furcation area showed reduced bone volume of IGF1-cKO mice. n=3.

(**C**) TRAP staining displayed reduced TRAP$^{+}$ osteoclasts in IGF1-cKO mice. n=3.

(**D, E**) Immunofluorescent staining of SP7 (**D**) and RUNX2 (**E**) revealed decreased Sp7$^{+}$ and Runx2$^{+}$ osteoblast numbers in IGF1-cKO mice alveolar bone. Boxed areas are shown at higher magnification. n=3.

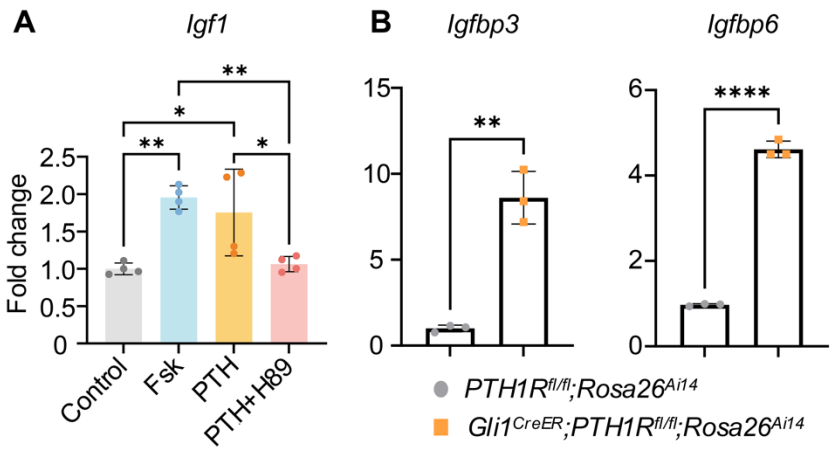
Significance is determined using unpaired two-sided student's t-tests. Data are mean ± SEM. *$P$<0.05. Scale bar=1 mm in A, 100 μm (B-E).




**Supplemental Figure 7. IGF1 expression in alveolar bone of PTH1R and IGF1 deficient mouse models.**

Immunofluorescent staining revealed IGF1 expression was increased in alveolar bone of PTH1R-cKO mice, which was reversed in *Gli1*$^{CreER}$;*PTH1R*$^{fl/fl}$;*IGF1*$^{fl/+}$ mice. Boxed areas are shown at higher magnification. n=3.

Scale bar=100 μm in lower magnification and 200 μm in higher magnification.

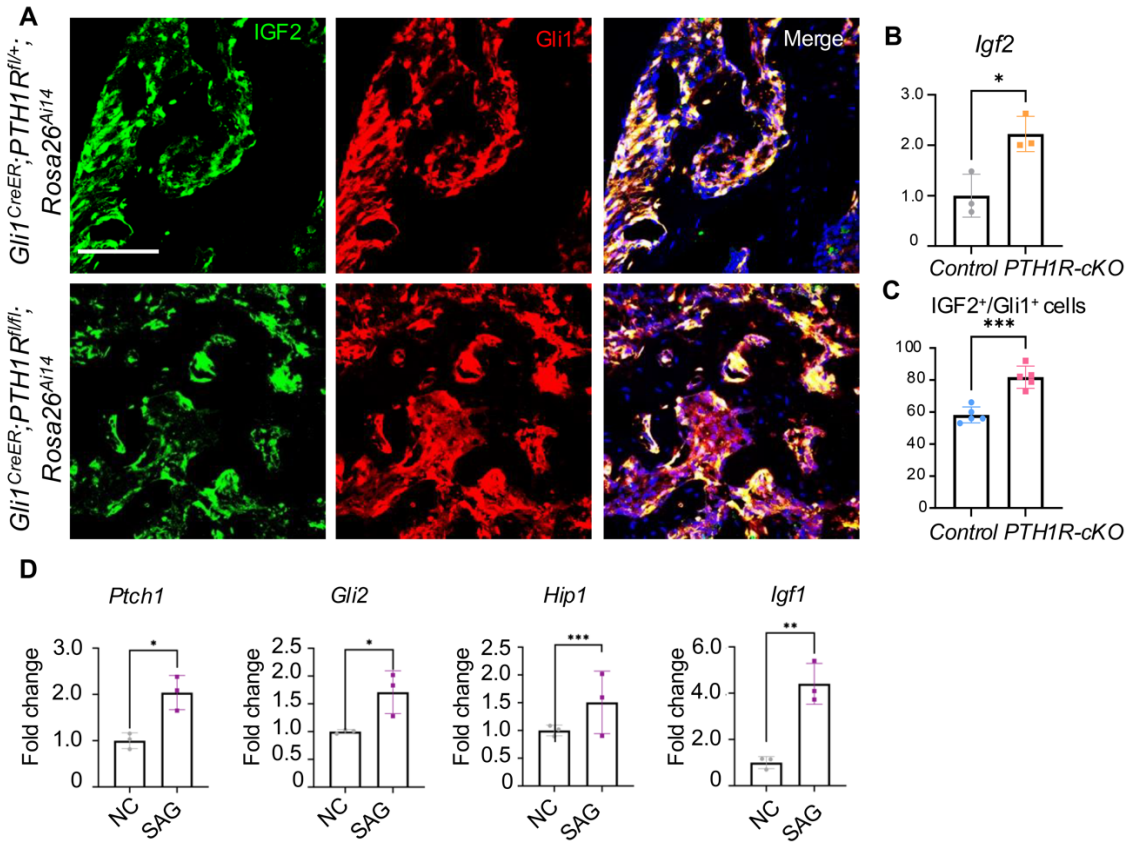



**Supplemental Figure 8. Gene expression levels of *Igf1* and *Igfbps*.**

(**A**) Pre-osteoblast cell line MC3T3-E1 were treated with vehicle, 100 nM PTH (1-34) (Bachem), 20 μM Forskolin (Fsk) (Selleck), 100 nM PTH + 10 μM H89 (Selleck) respectively, for 6 hours prior to RNA extraction. RT-qPCR showed *Igf1* expression upon Fsk, PTH (1-34), or PTH (1-34)+H89 treatment. n=4.

(**B**) RT-qPCR analysis of *Igfbp3* and *Igfbp6* gene expression in OMSCs extracted from control or PTH1R-cKO mice. n=3.

Significance is determined using one-way ANOVA with Tukey's correction in **A** and unpaired two-sided student's t-tests in **B**. Data are mean ± SEM. *$P$<0.05, **$P$<0.01, ****$P$<0.0001.




**Supplemental Figure 9. IGF2 expression in control and PTH1R-cKO mice and Hedgehog signaling related gene expressions following treatment with Smoothened Agonist (SAG).**

(**A, C**) Immunofluorescent staining and quantification showed increased IGF2$^{+}$/Gli1$^{+}$ cell number in root furcation area of *Gli1*$^{CreER}$*;PTH1R*$^{fl/fl}$*;Rosa26*$^{Ai14}$ female mice at P42. n=5.

(**B**) RT-qPCR results showed upregulated *Igf2* in PTH1R-cKO OMSCs. n=3.

(**D**) Gene expression levels of Hedgehog transcriptional targets (*Ptch1*, *Gli2*, and *Hip1*) and *Igf1* in OMSCs at 48 hours post SAG treatment. Significance is determined using unpaired two-sided student's t-tests. Data are mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001.

**Supplemental Tables.**

**Supplemental Table 1. Mouse genotyping primers.**

| Gene | Forward primer | Reverse primer | Expected |
|---|---|---|---|
| *Rosa26*$^{Ai14}$ | GGCATTAAAGCAGCGTATCC | CTGTTCCTGTACGGCATGG | 196 bp |
| WT | AAGGGAGCTGCAGTGGAGTA | CCGAAAATCTGTGGGAAGTC | 297 bp |
| *Gli1*$^{CreER}$ | GCGGTCTGGCAGTAAAAACTATC | GTGAAACAGCATTGCTGTCACTT | 100 bp |
| WT | CTAGGCCACAGAATTGAAAGATCT | GTAGGTGGAAATTCTAGCATCATCC | 136 bp |
| IGF1 mutant | AAACCACACTGCTCGACATTG | CACTAAGGAGTCTGTATTTGGACC | 275 bp |
| WT | GGCAAATGGAAATCCTATGTCT | CACTAAGGAGTCTGTATTTGGACC | 398 bp |
| PTH1R | ATGAGGTCTGAGGTACATGGCTCTGA | CCTGCTGACCTCTCTGAAAGAATGT | 280 bp (mutant) |
| | | | 210 bp (WT) |

**Supplemental Table 2. RT-qPCR primer sequences.**

| Gene | Forward primer | Reverse primer |
|---|---|---|
| Mouse *Actin* | AAGGCCAACCGTGAAAAGAT | GTGGTACGACCAGAGGCATAC |
| Mouse *Pth1r* | CCAGCATCTACGTCAGGGAAG | CAAACGATGTTGTCCCACTCT |
| Mouse *Sp7* | CTCCTTGGTGGGACATGC | GTAGGCAGCTGGGGGTTC |
| Mouse *Runx2* | TCCACAAGGACAGAGTCAGATTACAG | CAGAAGTCAGAGGTGGCAGTGTCATC |
| Mouse *Alp* | CACGGCCATCCTATATGGTAA | GGGCCTGGTAGTTGTTGTGA |
| Mouse *Opn* | ATCTCACCATTCGGATGAGTCT | TGTAGGGACGATTGGAGTGAAA |
| Mouse *Ctsk* | CAGCTTCCCCAAGATGTGAT | AAAAATGCCCTGTTGTGTCC |
| Mouse *Mmp9* | CTGGACAGCCAGACACTAAAG | CTCGCGGCAAGTCTTCAGAG |
| Mouse *Nfatc1* | TGCCTTTTGCGAGCAGTATCT | CAGGCAAGGATGGGCTCATAT |
| Mouse *Atp6v0d2* | CAGAGCTGTACTTCAATGTGGAC | AGGTCTCACACTGCACTAGGT |
| Mouse *Rank* | CCAGGAGAGGCATTATGAGCA | ACTGTCGGAGGTAGGAGTGC |
| Mouse *Rankl* | GCAGAAGGAACTGCAACACA | GATGGTGAGGTGTGCAAATG |
| Mouse *Opg* | CAGCATCGCTCTGTTCCTGTA | CTGCGTTTTCATGGAGTCTCA |
| Mouse *Col1a1* | GCGCTAAAGGTGCCAATG | AGCACCAGGTTCACCACTG |
| Mouse *Igf1* | CACATCATGTCGTCTTCACACC | GGAAGCAACACTCATCCACAATG |
| Mouse *Igf2* | GTGCTGCATCGCTGCTTAC | CGGTCCGAACAGACAAACTG |
| Mouse *Ptch1* | AAAGAACTGCGGCAAGTTTTTG | CTTCTCCTATCTTCTGACGGGT |
| Mouse *Smo* | GTGCTGTCTACATGCCCAAGT | GCAACGCAGAAAGTCAGGC |
| Mouse *Gli2* | CAACGCCTACTCTCCCAGAC | GAGCCTTGATGTACTGTACCAC |
| Mouse *Hip1* | GGTCAGCGTCAATAAGGCCAT | GGTCTGCGCTCCTTTCTCA |

| | | |
|---|---|---|
| Mouse *Igfbp3* | CACACCGAGTGACCGATTCC | GTGTCTGTGCTTTGAGACTCAT |
| Mouse *Igfbp6* | TGCTAATGCTGTTGTTCGCTG | CACGGTTGTCCCTCTCTCCT |
| Human *GAPDH* | ACTGAGGACCAGGTTGTC | TGCTGTAGCCGTATTCATTG |
| Human *PTH1R* | CAGGCGCAATGTGACAAGC | TTTCCCGGTGCCTTCTCTTTC |
| Human *IGF1* | CACATCATGTCGTCTTCACACC | GGAAGCAACACTCATCCACAATG |

**Supplemental Table 3. Sequences for RNAi experiments.**

| | |
|---|---|
| *Igf1* shRNA target sequence | TATTATTGTTATTTGGTAGGTG |
| NC shRNA hairpin loop sequence | TAGTGAAGCCACAGATGTA |
| Gli1 siRNA sequence | CCCAACAUGGAGUGGGUAACAUGA<br>UCAUGUUACCCACUGCCAUGUUGGG |
| Gli2 siRNA sequence | CCACAACCACAACGUUGCUCAGACA<br>UGUCUGAGCAAGCUUGUGGUUGUGG |