

denotes a statistically significant difference, $p < 0.05$. (C) Quantitative RT-PCR analysis of *cyclin E* (top) and *cyclin A* (bottom) mRNA levels from $Rb^{c-/c-}$ and control littermates. mRNA was isolated from the calvaria of e16.5 embryos. Analysis performed as described in Figure 3. $Rb^{c-/c-}$ calvaria (blue bars) express increased levels of *cyclin A* and *cyclin E* relative to wild-type littermates (red bars). Error bars signify one standard deviation. Abbreviations: br, brain; de, dermis; fr, frontal bone. WT = $Rb^{+/c};Mox2^{+/+}$, Rb = $Rb^{c-/c-};Mox2^{+/Cre}$.

Figure 6. Deletion of *E2f1* suppresses the bone defects due to the loss of pRb. (A)

Immunohistochemical analysis of BrdU incorporation (top) or PCNA protein expression (bottom) in coronal sections of frontal bones from e17.5 embryos, performed with 4 to 6 samples of each genotype. Deletion of *E2f1* suppresses the increased BrdU incorporation and PCNA expression observed in $Rb^{c-/c-}$ frontal bone osteoblasts. (B) Skeletal staining of e17.5 embryos as described in Figure 1. Deletion of *E2f1* suppresses the decreased ossification found in the $Rb^{c-/c-}$ calvaria (first column) and hyoid bone (second column). Deletion of *E2f1* also suppresses the aberrant formation of the palatine process and pterygoid bone (third column) and xiphoid process (fourth column) observed in $Rb^{c-/c-}$ skeletons. An aberrant palatine process in the $Rb^{c-/c-}$ and a suppressed palatine process in the double mutant are circled in the respective third column figures. Error bars signify one standard deviation. * denotes a statistically significant difference between $Rb^{c-/c-}$ and wild-type, $E2f1^{-/-}$, or $Rb^{c-/c-};E2f1^{-/-}$, $p < 0.05$. WT = $Rb^{+/c};Mox2^{+/+};E2f1^{+/+}$, $E2f1 = Rb^{+/c};Mox2^{+/+};E2f1^{-/-}$, Rb = $Rb^{c-/c-};Mox2^{+/Cre};E2f1^{+/+}$, RbE2f1 = $Rb^{c-/c-};Mox2^{+/Cre};E2f1^{-/-}$.

Supplemental Figure 1. Deletion of *Rb* *in vivo* does not affect osteoclast activity. TRAP

analysis of coronal sections from wild-type and $Rb^{c-/c-}$ frontal bones of e17.5 embryos. Neither wild-type nor $Rb^{c-/c-}$ frontal bones exhibit osteoclasts as assessed by TRAP staining. A positively

stained osteoclast (arrow) within a facial bone from the same section as the wild-type frontal bone is shown (“+ Ctrl” and “+ Ctrl 40X”). 20X magnification shown except for “+ Ctrl 40X,” which is 40X magnification. Frontal bones are marked with the bar. Abbreviations: S, suture. WT = $Rb^{+/c};Mox2^{+/+}$, Rb = $Rb^{c-/c-};Mox2^{+/Cre}$.

Supplemental Figure 2. $Rb^{c-/c-}$ calvaria display increased mRNA levels of $E2f1$.

Quantitative RT-PCR analysis of Rb (left) and $E2f1$ (right) mRNA levels from $Rb^{c-/c-}$ and control littermates. mRNA was isolated from the calvaria of e16.5 embryos. Analysis performed as described in Figure 3. (Left) Rb mRNA is expressed in the calvaria of $E2f1^{-/-}$, Rb heterozygous, and wild-type embryos. (Right) $Rb^{c-/c-}$ calvaria express increased levels of $E2f1$ relative to control littermates. Error bars signify one standard deviation. WT = $Rb^{+/c};Mox2^{+/+};E2f1^{+/+}$, $E2f1 = Rb^{+/c};Mox2^{+/+};E2f1^{-/-}$, Rb = $Rb^{c-/c-};Mox2^{+/Cre};E2f1^{+/+}$.



