Izu et al., http://www.jcb.org/cgi/content/full/jcb.201010010/DC1

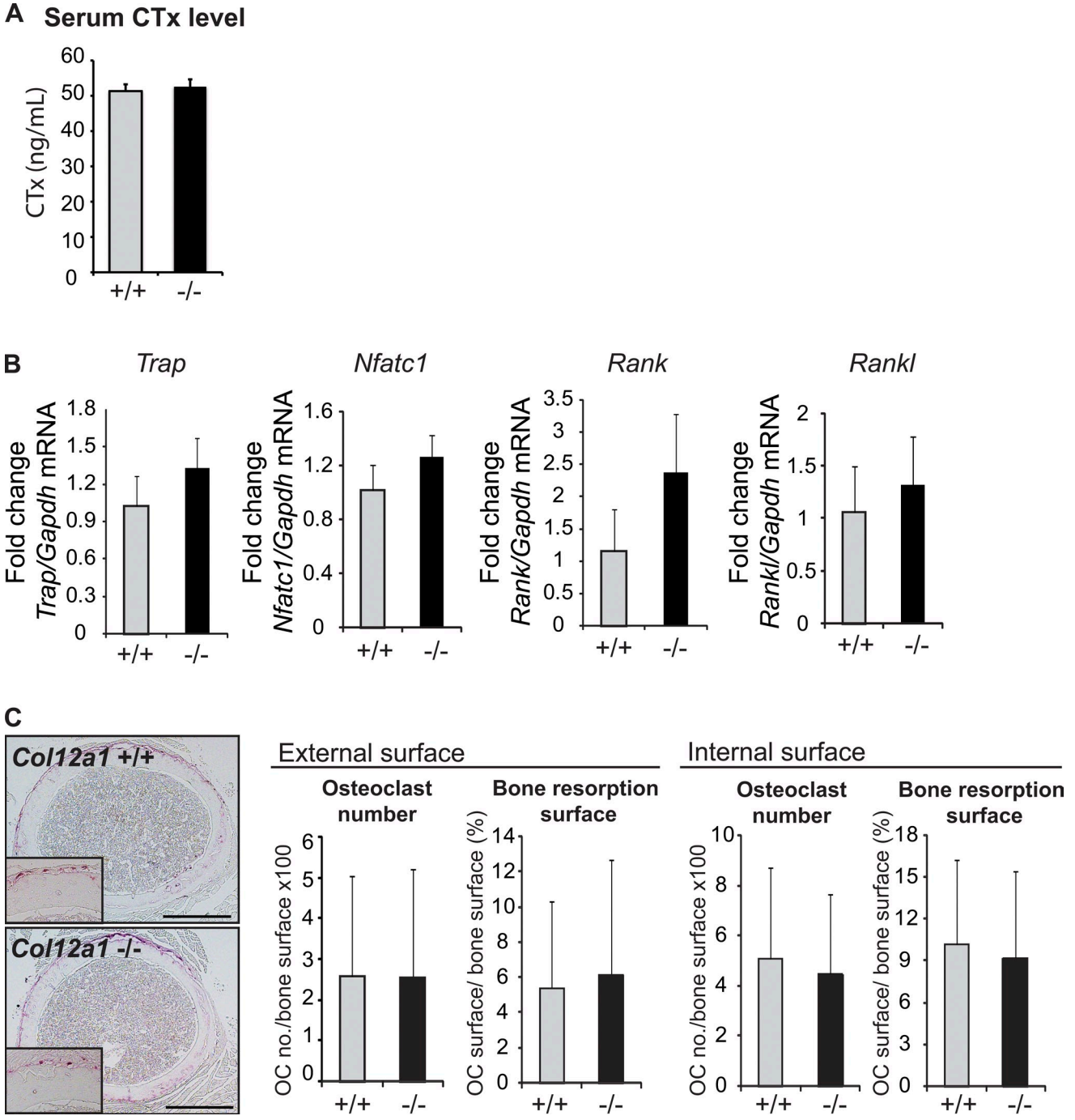


Figure S1. **Type XII collagen deficiency does not affect bone resorption.** (A) Quantitative analysis of the C-terminal telopeptide α1 chain of type I collagen in mouse serum from *Col12a1*$^{+/+}$ ($n = 4$) and *Col12a1*$^{-/-}$ ($n = 5$) mice at P30. (B) mRNA expressions of osteoclast markers in P30 femurs from *Col12a1*$^{+/+}$ and *Col12a1*$^{-/-}$ mice were measured by quantitative real-time PCR normalizing to *Gapdh* expression. *Trap*, *Nfatc1*, *Rank*, and *Rankl* expressions of *Col12a1*$^{-/-}$ femurs tend to increase compared with wild-type controls, but that is not significant. (C) TRAP staining in the cross section of mid-diaphysis of the femur. Insets show high magnification. Quantification of TRAP-positive multinucleated osteoclasts (OC) on the external and internal diaphyseal surfaces of the femur. Type XII collagen deficiency does not affect the number of osteoclasts (osteoclast number per bone surface) and the bone resorption surface (osteoclast surface per bone surface) in both the external and internal surface. Error bars are mean ± SD. Bars, 500 µm.

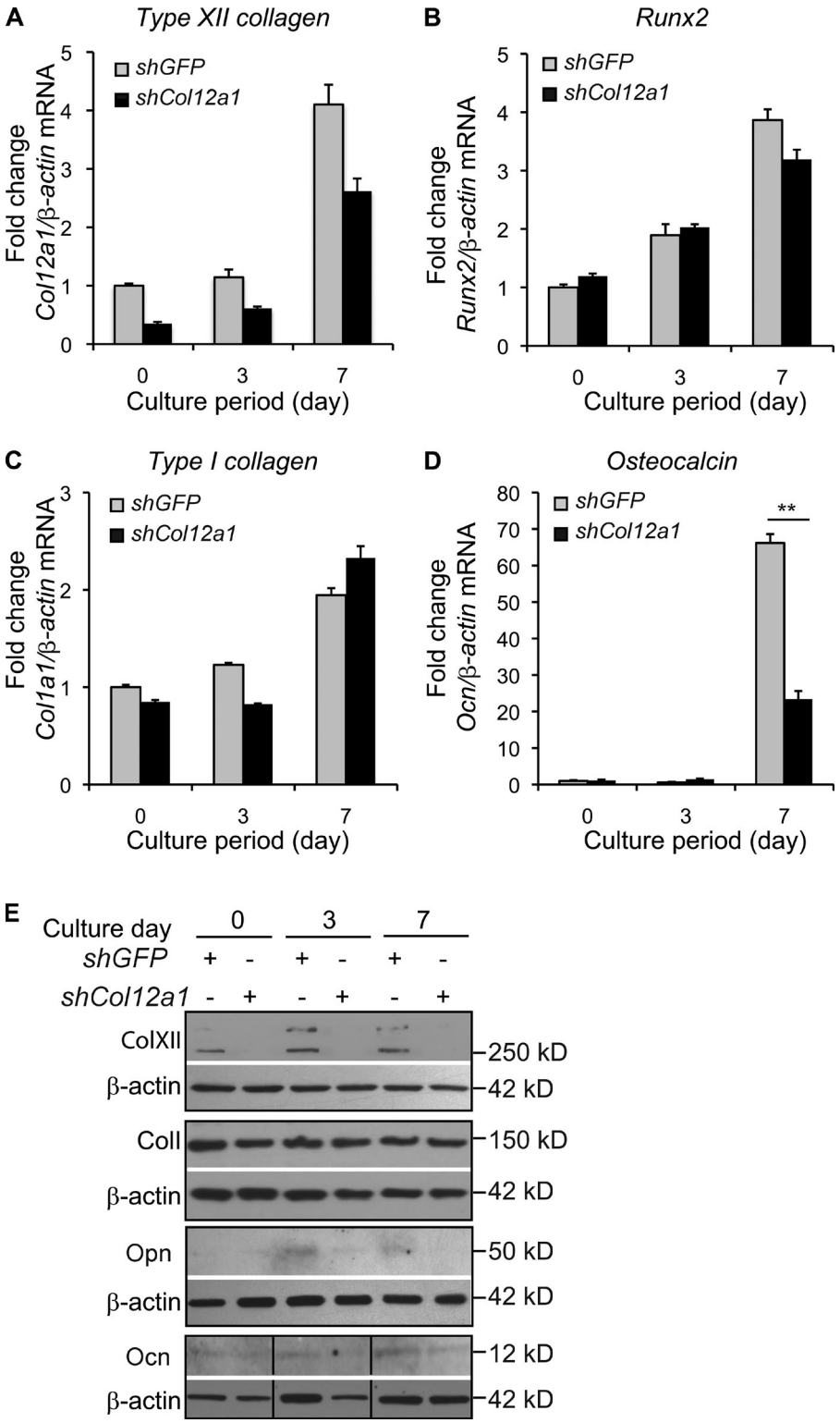


Figure S2. **Delayed terminal differentiation in type XII collagen knockdown MC3T3-E1 cells.** (A–E) A mouse preosteoblast cell line, MC3T3-E1, was lentivirus transduced with *shGFP* (control vector) and *shCol12a1* (knockdown vector). The cells are cultured in osteogenic medium, harvested after 0, 3, and 7 d in culture, and used for quantitative real-time PCR normalized to β-actin expression (A–D) and immunoblot (E) analysis. (A) Type XII collagen mRNA expression is decreased ~40% when transduced with *shCol12a1* lentivirus. (B and C) Similar expression patterns are detected in mRNA expression of *Runx2* (B) and *type I collagen* (C) in control versus knockdown cultures. (D) The expression of *Ocn* is significantly decreased at day 7 in *shCol12a1*-treated cells. **, $P < 0.01$. Error bars are mean ± SD. (E) Type I collagen (Col I) expression is stable, whereas Opn and Ocn expression is decreased in knockdown compared with control cells. β-Actin is used for internal control. The Opn loading control is duplicated in Fig. S4 B for Cad2 and 11, which is from the same gel/filter. Black lines indicate that intervening lanes have been spliced out.

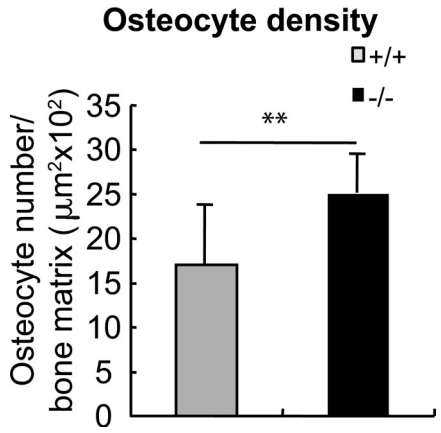
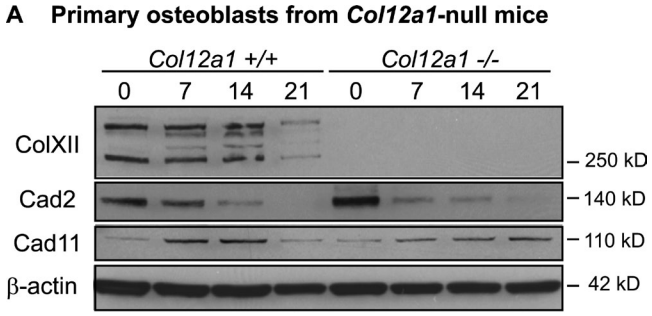


Figure S3. **Osteocyte density is increased in type XII collagen–null cortical bone matrix.** Osteocyte density was determined as osteocyte number/100 μm^2 from cortical bone matrix in H&E-stained cross sections of the femur diaphysis from *Col12a1*$^{+/+}$ (n = 3) and *Col12a1*$^{-/-}$ (n = 3) mice at P14. Five different areas per individual mouse were randomly selected, and the numbers of osteocytes in cortical bone areas were calculated. The osteocyte density in *Col12a1*$^{-/-}$ bone is ∼60% greater compared with wild-type bone. **, P < 0.01. Error bars are mean ± SD.



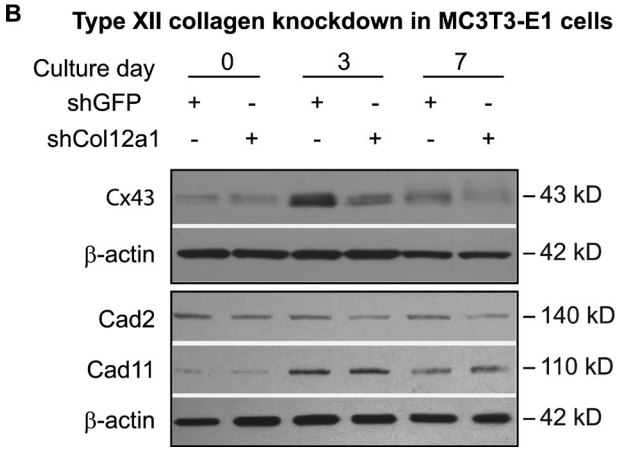


Figure S4. **Osteoblast expression of Cad2 and Cad11 is not altered in the absence of type XII collagen.** (A) Cad2 and Cad11 expression was analyzed using immunoblots in cultures of primary osteoblasts. Cad2 expression is gradually decreased during osteoblast differentiation in both genotypes. Cad11 expression increases in day 7 and 14 wild-type osteoblasts, whereas this is not observed in *Col12a1*$^{-/-}$ osteoblasts. (B) Immunoblot analysis of Cx43, Cad2, and Cad11 in *Col12a1* knockdown MC3T3-E1 cells. Cx43 expression is decreased at all time points in *shCol12a1*-treated cells. Cad2 expression is moderately decreased at day 3 and 7 in *shCol12a1*-treated cells. No reduction was detected in Cad11 expression between control and *shCol12a1*-treated cells. The Cad2 and 11 loading control is duplicated in Fig. S2 E for Opn, which is from the same gel/filter.

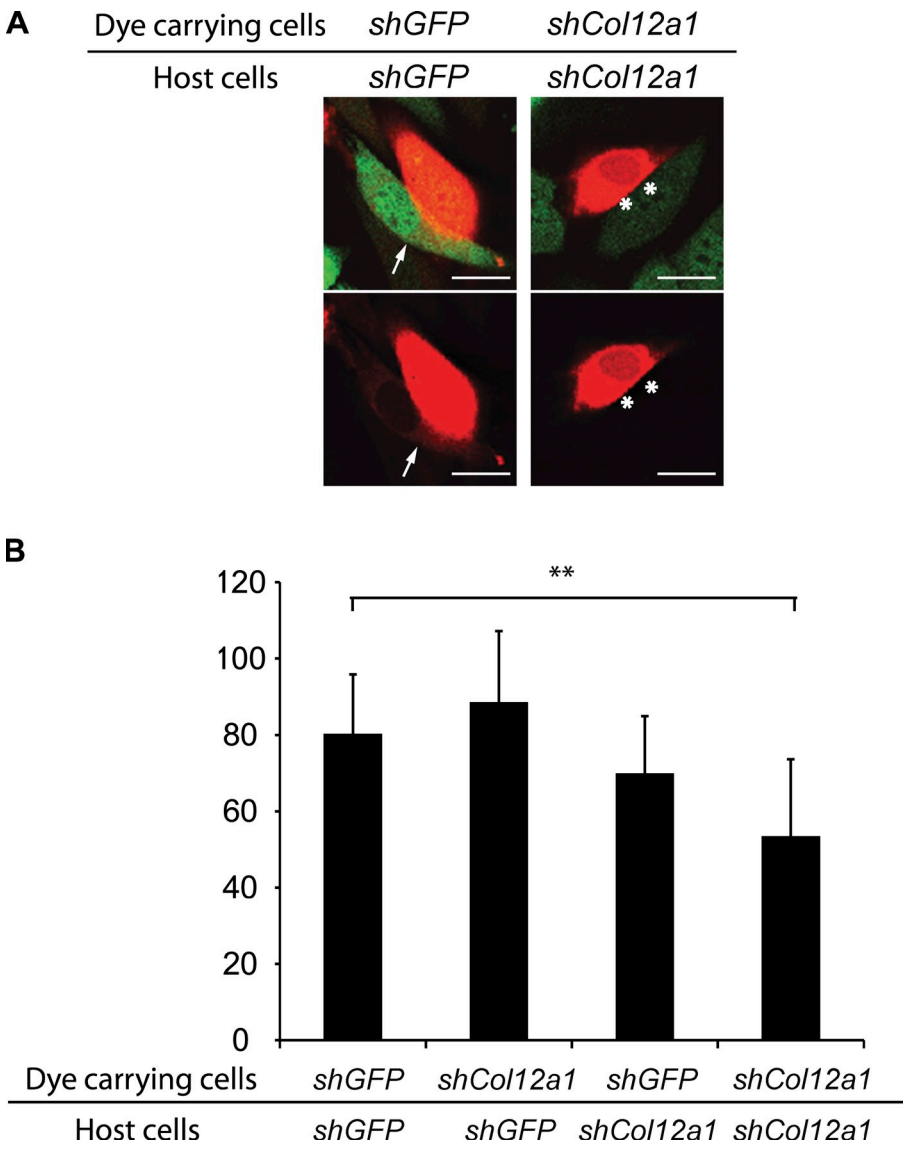
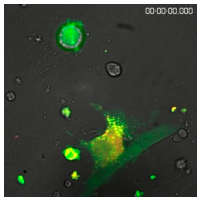
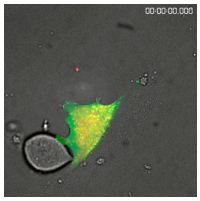


Figure S5. **Altered cell–cell communication is associated with the absence of type XII collagen in vitro.** (A) A dye coupling study was performed in *shCol12a1*-treated MC3T3-E1 cells. Calcein red-orange AM–labeled cells (red, dye-carrying cells) were seeded onto confluent osteoblasts (green, host cells) and observed by confocal microscopy. Calcein dye (red) is transferred to host cells (arrows) in control cells (*shGFP*), whereas no red signal is seen (asterisks) in *Col12a1* knockdown cells (*shCol12a1*). Bars, 20 µm. (B) The percentage of calcein dot–carrying cells per total dye-labeled cells is significantly decreased in *shCol12a1* versus *shCol12a1* when compared with control *shGFP* versus control *shGFP* cells ($P < 0.01$). Error bars are mean ± SD.



Video 1. **Gap junction communication in *Col12a1*$^{+/+}$ osteoblasts.** Dye coupling study in primary osteoblasts from *Col12a1*$^{+/+}$. Calcein AM– (green) and Cell Tracker (orange)–labeled osteoblasts (dye-carrying cells) were seeded onto confluent osteoblasts (host cells) with no color and observed by spinning disc confocal microscopy. Dye-carrying wild-type osteoblasts (+/+) attaching to the wild-type host osteoblasts transfer green dots to the host cell.



Video 2. **Impaired gap junction communication in *Col12a1*$^{-/-}$ osteoblasts.** Dye coupling study in primary osteoblasts from *Col12a1*$^{-/-}$. Calcein AM– (green) and Cell Tracker (orange)–labeled osteoblasts (dye-carrying cells) were seeded onto confluent osteoblasts (host cells) with no color and observed by spinning disc confocal microscopy. Dye-carrying *Col12a1*$^{-/-}$ osteoblasts attach to the *Col12a1*$^{-/-}$ host cell, but the calcein dye remains within the donor cell.