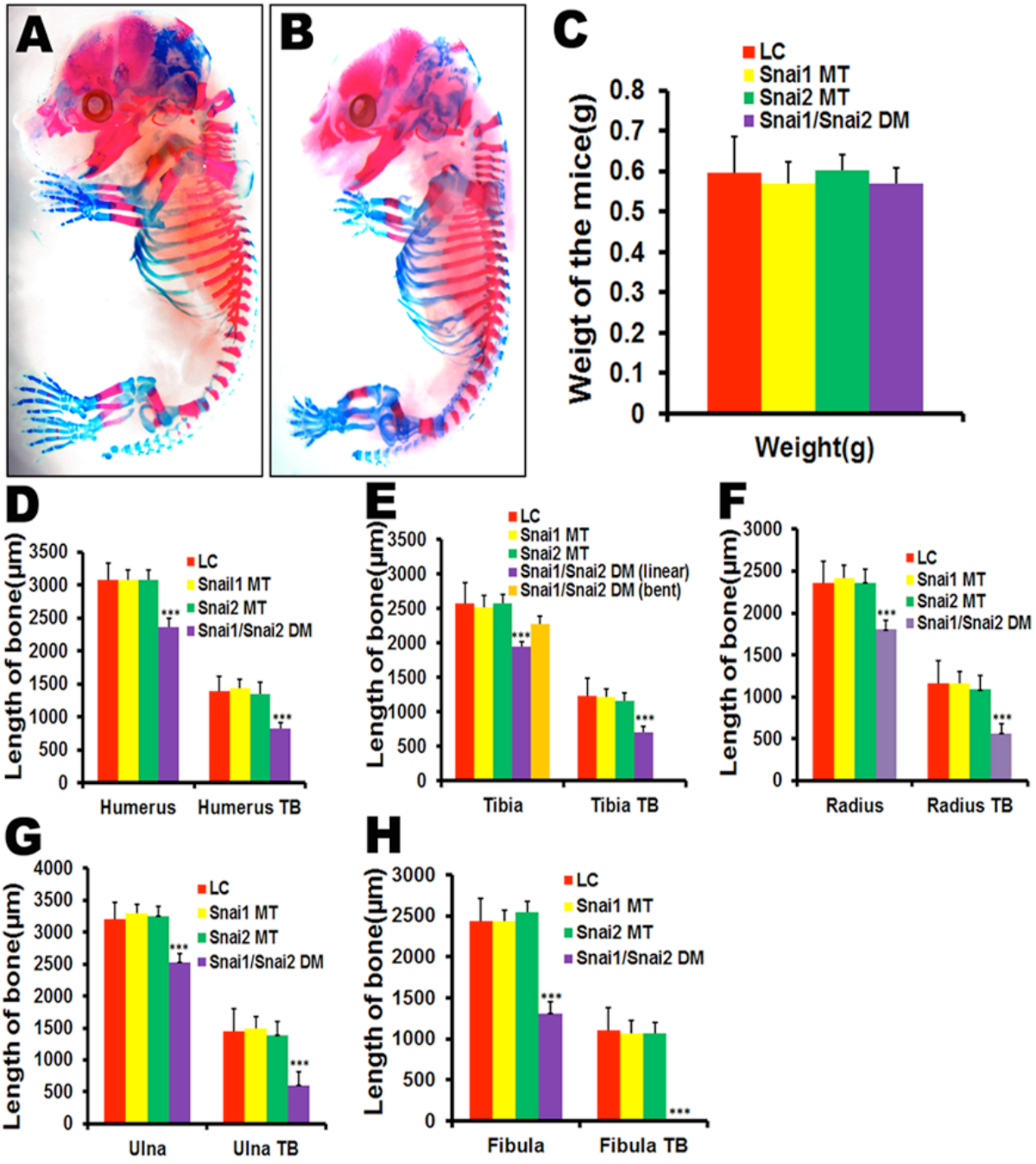
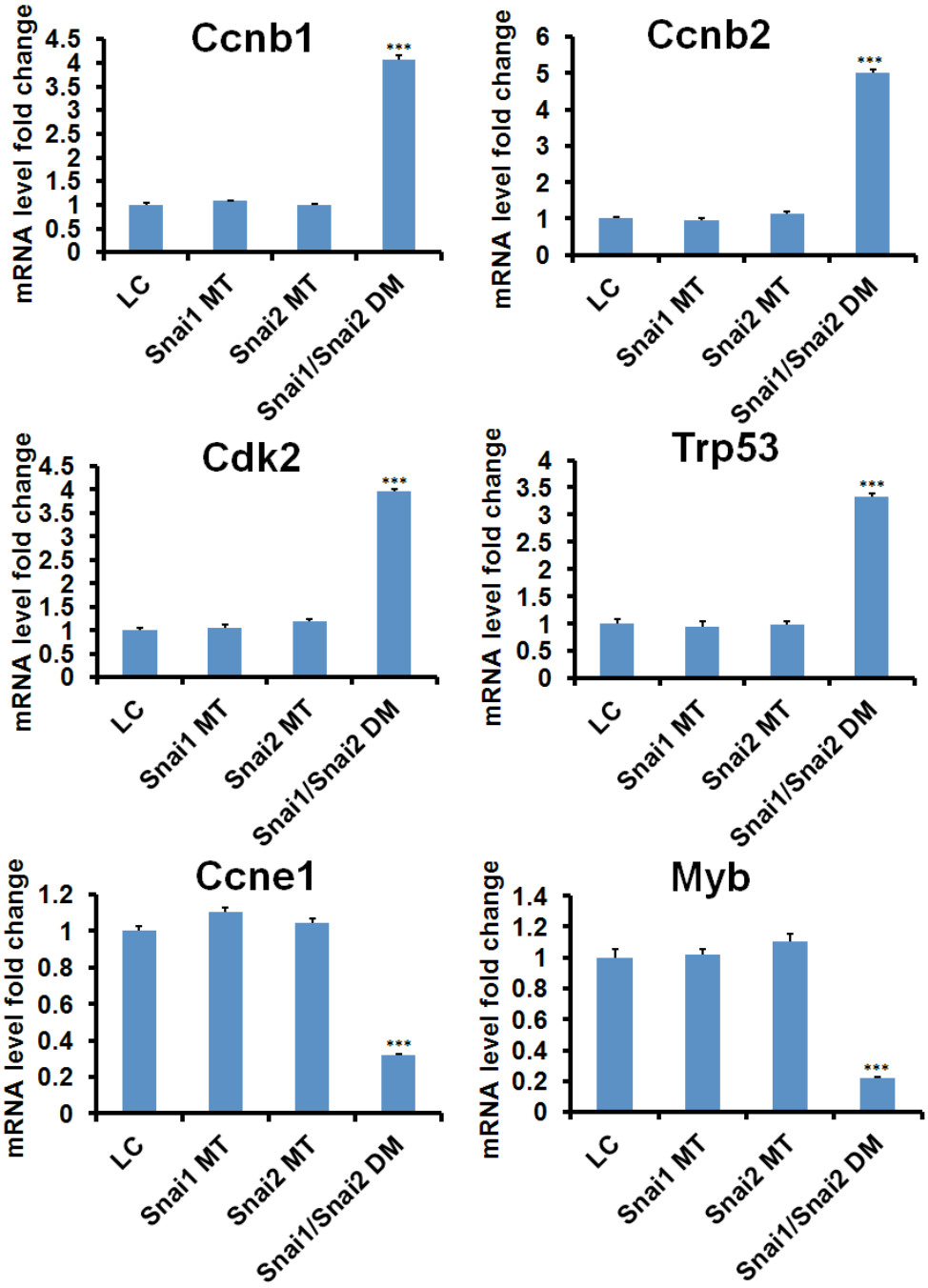
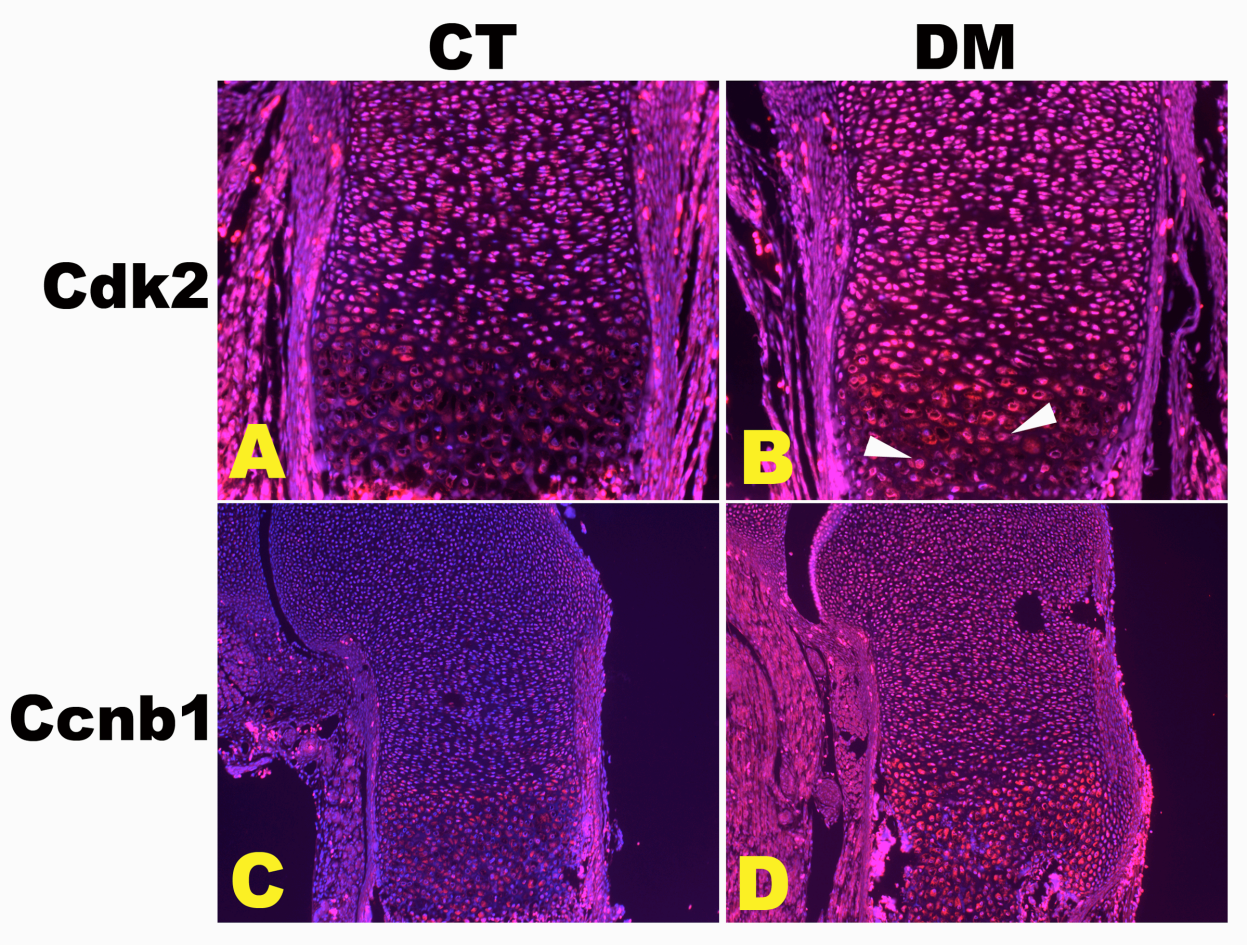


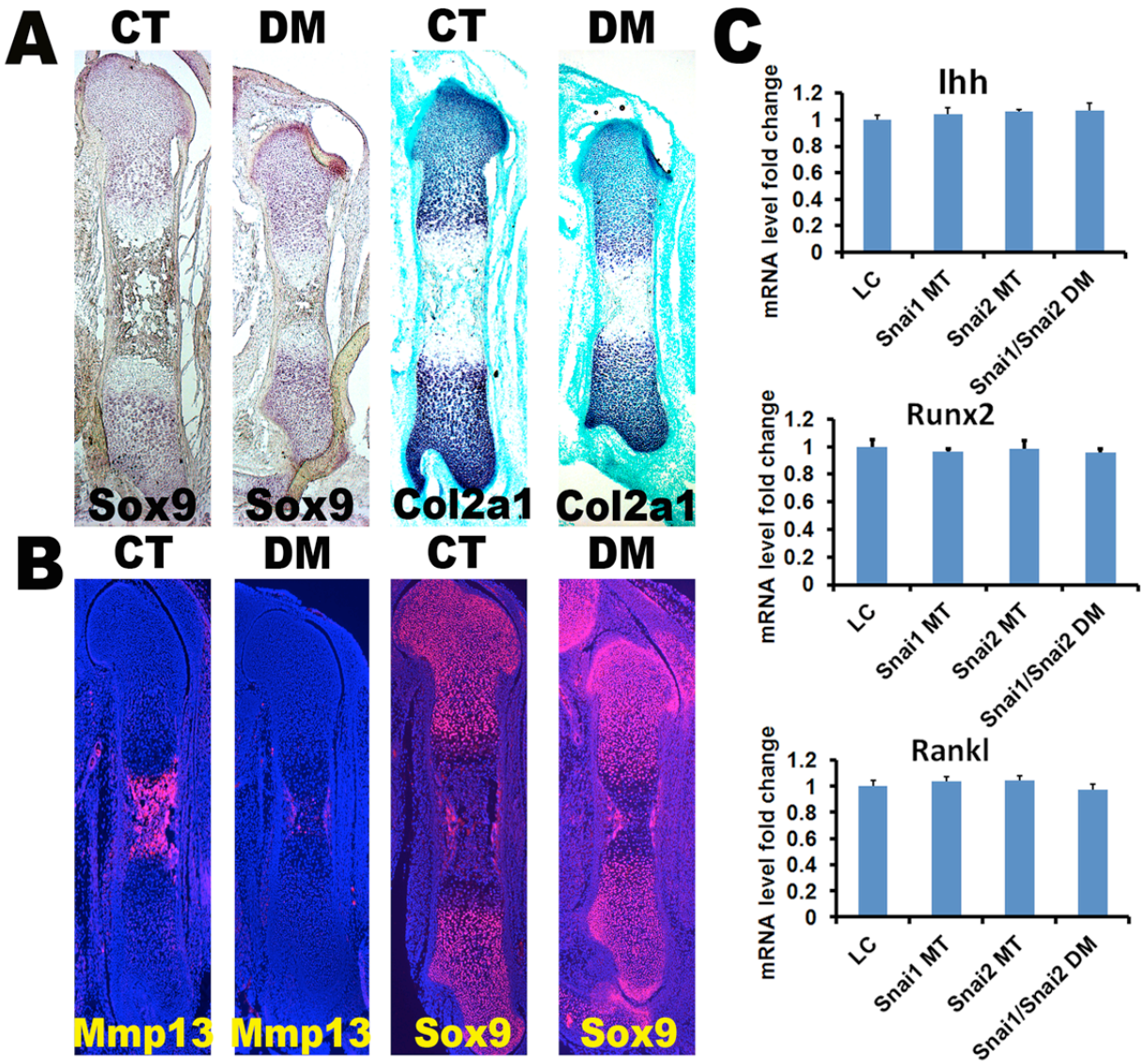
Supplemental Figure S1. (*A*, *B*) Alcian blue-Alizarin red staining of E16.5 control (*A*) and *Snai1/Snai2* (*B*) double mutant (DM) embryos. (*C*) No significant differences were observed in the weights of E16.5 embryos of the four different genotypes. (*D-E*) Length measurements of other fore- and hindlimb long bones, including humerus (*D*), tibia (*E*), radius (*F*), ulna (*G*), and fibula (*H*). All long bones, as well as their trabecular regions, were significantly shorter in *Snai1/Snai2* DM embryos than in the other genotypes. TB: trabecular bone.



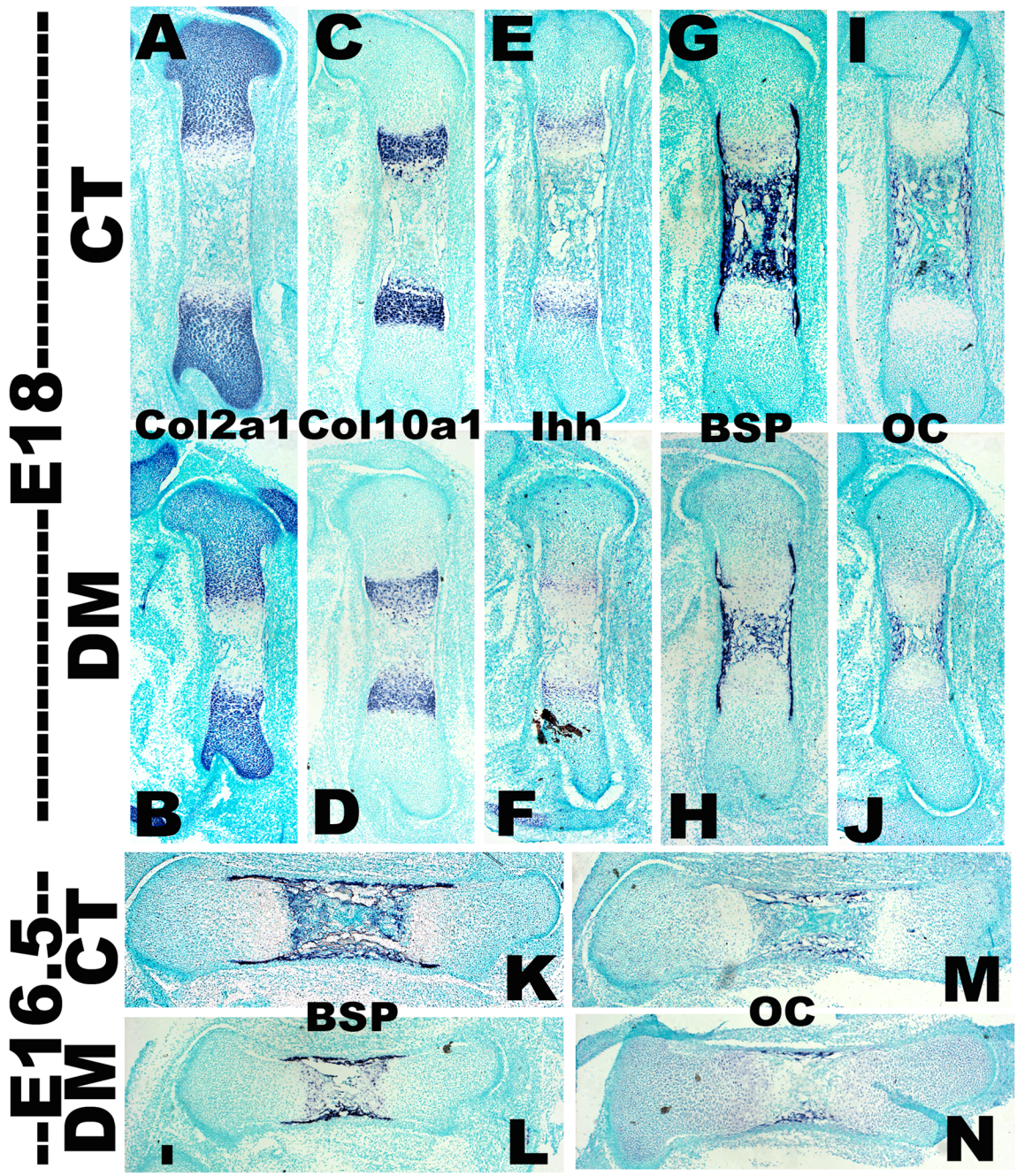
Supplemental Figure S2. Quantitative RT-PCR of cell cycle regulators in femurs at E16.5. mRNAs encoding *Ccnb1*, *Ccnb2*, *Cdk2*, and *Trp53* were significantly increased in *Snai1/Snai2* DM femurs compared to the other genotypes, whereas *Ccne1* and *Myb* mRNA levels were significantly decreased in DM femurs. *** $p < 0.001$.



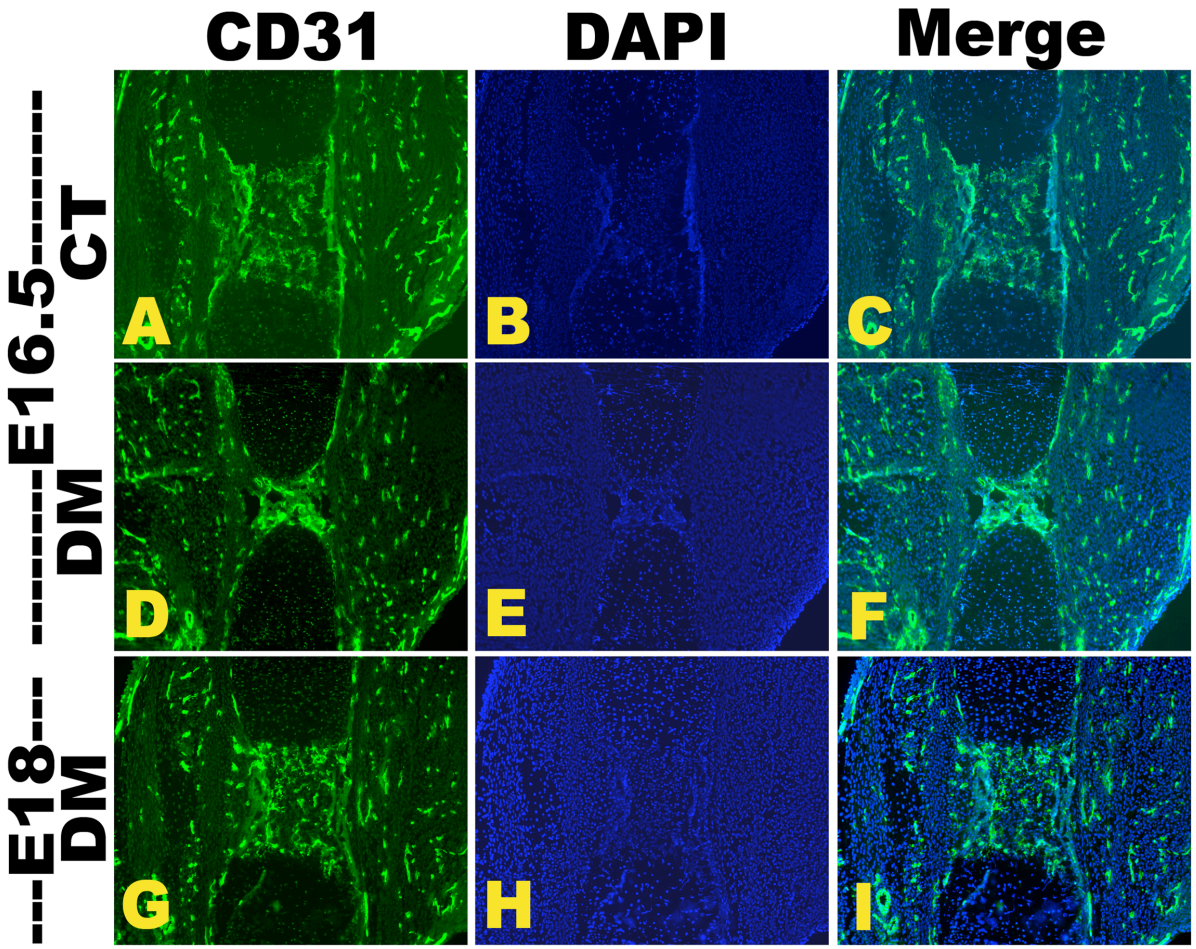
Supplemental Figure S3. Immunofluorescent staining with antibodies to the (*A*, *B*) cyclin dependent kinase 2 (Cdk2) and (*C-D*) cyclin B1 (Ccnb1) proteins revealed increased protein expression in DM femurs.



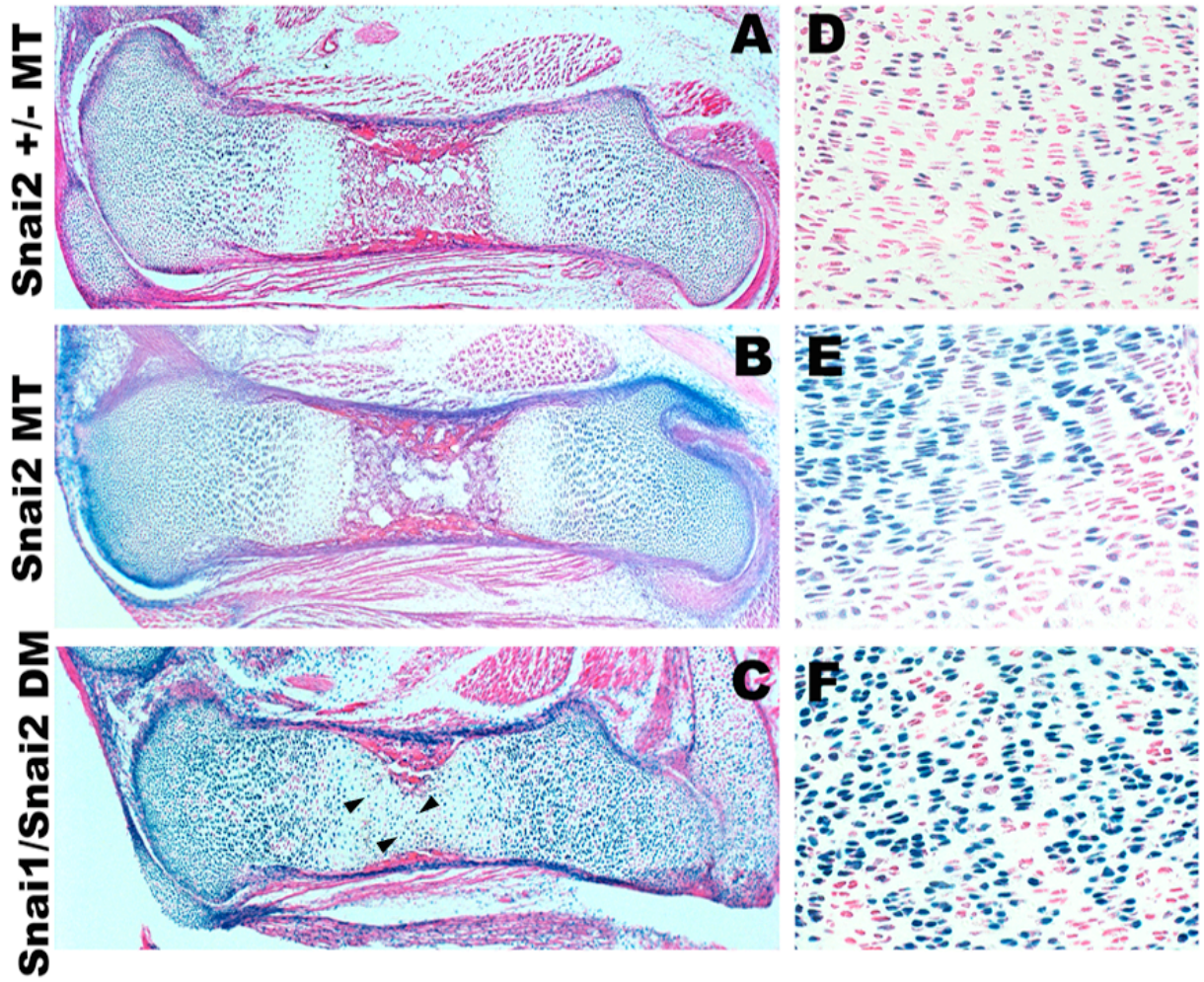
Supplemental Figure S4. (*A*) In situ hybridization of E16.5 femurs with probes to *Sox9* and *Col2a1* demonstrate no significant differences between their expression pattern in control (CT) and *Snai1/Snai2* double mutant (DM) femurs. (*B*) Analysis of protein expression by immunofluorescence confirmed the in situ hybridization results for Mmp13 and Sox9. (*C*) Quantitative RT-PCR demonstrated that transcript levels of *Ihh*, *Runx2*, and *Rankl* did not differ significantly in any of the genotype groups.



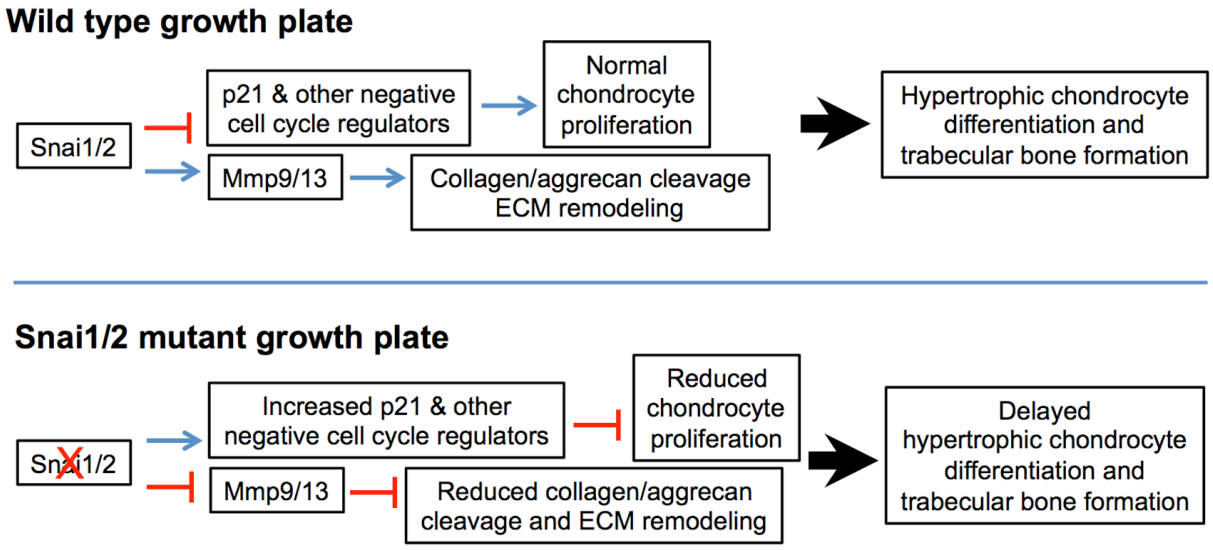
Supplemental Figure S5. Chondrocyte and osteoblast marker gene expression in *Snai1/Snai2* DM femurs. (A-J) E18 femurs. (K-N) E16.5 femurs. The DM femurs exhibited decreased expression of the osteoblast differentiation markers bone sialoprotein (BSP) and osteocalcin (OC), particularly at E16.5. However, the pattern of expression of both chondrocyte and osteoblast markers was unaltered in the DM femurs.



Supplemental Figure S6. Pecam1 (CD31) protein expression in control littermate (*A-C*) and *Snai1/Snai2* DM (*D-I*) femurs at E16.5 (*A-F*) and E18 (*G-I*). Blood vessels were observed growing into the region of trabecular bone formation in DM femurs at both time points.



Supplemental Figure S7. (*A*-*F*) Femurs at E16.5 were analyzed for β-galactosidase expression from the *Snai2*^{lacZ} null allele. In *Snai2*$^{+/-}$ heterozygotes (*A*, *D*), β-galactosidase expression was observed in proliferating chondrocytes in the growth plate. In *Snai2*$^{-/-}$ homozygotes (*B*, *E*), the expression pattern was not changed; however, compared to the *Snai2*$^{+/-}$ heterozygote, β-galactosidase expression was increased (compare *A*, *D* to *B*, *E*). In the *Snai1/Snai2* DM femur (*C*, *F*), β-galactosidase expression was again increased compared to both *Snai2*^{lacZ} heterozygotes and homozygotes. In addition, β-galactosidase expression in *Snai1/Snai2* DM femurs was expanded to hypertrophic chondrocytes (arrowheads in *C*).



Supplemental Figure S8. Model for *Snai1/Snai2* function in the growth plate. We propose that a major function of the SNAI1 and SNAI2 proteins in the growth plate is to cooperatively repress transcription of the *Cdkn1a* gene (encoding the $p21^{Waf1/Cip1}$ protein) and other negative cell cycle regulators. Either directly or indirectly, the SNAI1 and SNAI2 proteins also promote transcription of the *Mmp9* and *Mmp13* genes. The consequence of loss of both SNAI1 and SNAI2 function in the growth plate is reduced chondrocyte cell proliferation and delayed hypertrophic chondrocyte differentiation and trabecular bone formation.

Supplementary Table 1. qRT-PCR primers

Gene	Forward	Reverse	Product size
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT	154 bp
Snai1	CTTGTGTCTGCACGACCTGT	CTTCACATCCGAGTGGGTTT	167 bp
Snai2	GGCTGCTTCAAGGACACATT	TTGGAGCAGTTTTTGCACTG	151 bp
Col1a1	CTGGCGGTTCAGGTCCAAT	TTCCAGGCAATCCACGAGC	141 bp
Col2a1	CAGGATGCCCGAAAATTAGGG	ACCACGATCACCTCTGGGT	132 bp
Col10a1	TTCTGCTGCTAATGTTCTTGACC	GGGATGAAGTATTGTGTCTTGGG	115 bp
Sox9	AGTACCCGCATCTGCACAAC	TACTTGTAATCGGGGTGGTCT	145 bp
Acan	TTGGAGATCCAGAACCTTCG	TGTGCTCGATCAAAGTCCAG	164 bp
Mmp9	CTGGACAGCCAGACACTAAAG	CTCGCGGCAAGTCTTCAGAG	145 bp
Mmp13	ACCTCCACAGTTGACAGGCT	AGGCACTCCACATCTTGGTTT	114 bp
Ihh	CTCTTGCCTACAAGCAGTTCA	CCGTGTTCTCCTCGTCCTT	156 bp
Cdkn1a	CGAGAACGGTGGAACTTTGAC	CAGGGCTCAGGTAGACCTTG	106 bp
Ccnb1	GCGTGTGCCTGTGACAGTTA	CCTAGCGTTTTTGCTTCCCTT	135 bp
Ccnb2	GCCAAGAGCCATGTGACTATC	CAGAGCTGGTACTTTGGTGTTC	114 bp
Cdk2	CTCTCACGGGCATTCCTCTTC	CCCTCTGCATTGATAAGCAGG	133 bp
Ccne1	GTGGCTCCGACCTTTCAGTC	CACAGTCTTGTCAATCTTGGCA	101 bp
Myb	AGACCCCGACACAGCATCTA	CAGCAGCCCATCGTAGTCAT	81 bp
Trp53	GCGTAAACGCTTCGAGATGTT	TTTTTATGGCGGGAAGTAGACTG	144 bp