Supplementary Figures

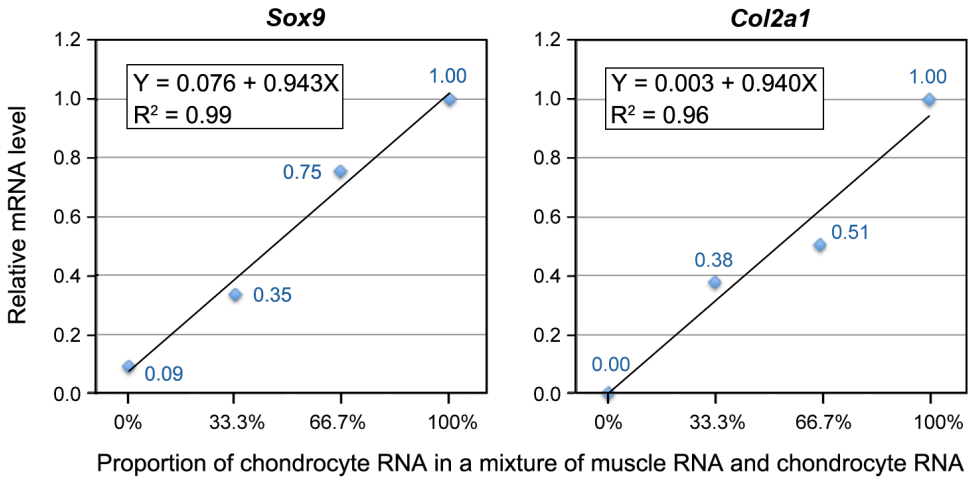

Fig. S1. Validation of qRT-PCR assays. To ensure that our qRT-PCR method correctly measured the relative levels of *Sox9* and *Col2a1* RNA, we prepared RNA from newborn mouse rib cartilage and skeletal muscle, and mixed the samples in distinct ratios: 0% cartilage/100% muscle; 33.3% cartilage/66.7% muscle; 66.7% cartilage/33.3% muscle, and 100% cartilage/0% muscle. All samples had similar levels of β-actin RNA, which were used for normalization. The linear fitness equation and R-squared demonstrate that our assay accurately quantified the relative levels of *Sox9* and *Col2a1* RNAs present in these samples. Importantly, the level of *Col2a1* RNA measured in the sample containing only muscle RNA was zero. This result consolidates evidence that our positive detection of *Col2a1* RNA in *Sox9*-deficient limb buds is real (not due to background noise).

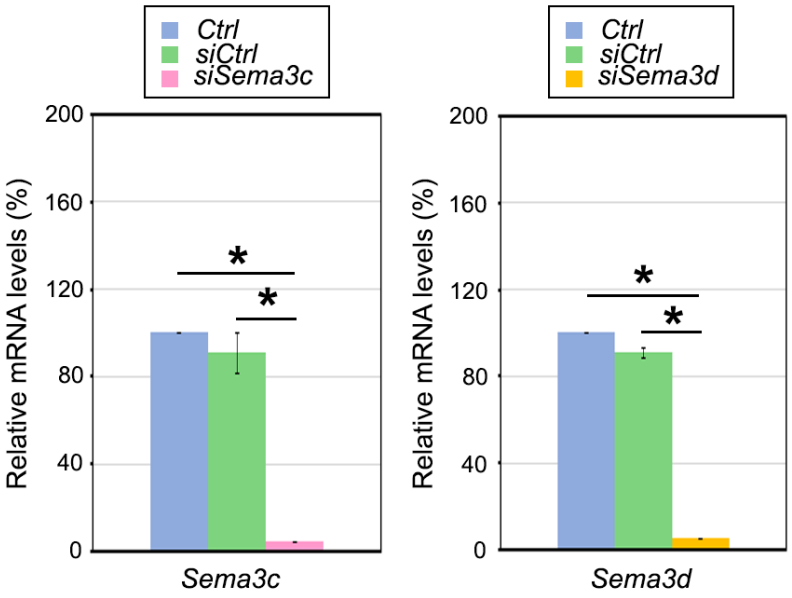

Fig. S2. Test of the efficiency of siRNAs for *Sema3c* (*siSema3c*) and *Sema3d* (*siSema3d*). Chondrogenic ATDC5 cells (Sigma-Aldrich, 99072806-1VL) were cultured in DMEM/F12 with 5% FBS. Cells were plated at 2.5 x 10^4 cells/cm^2. After 2 h, they either were not transfected (*Ctrl*) or were transfected with 10 nM control siRNA (*siCtrl*) or specific siRNA for *Sema3c* or *Sema3d* and the RNAiMAX transfection reagent (Thermo Fisher Scientific). After 48 h, the mRNA levels of *Sema3c* and *Sema3d* were quantified by qRT-PCR relative to the mRNA levels for *Gapdh*. Data are presented as the mean with standard deviation for technical triplicates in percentage of values obtained for untransfected control cells. Asterisks, $p<0.05$ (Student's t-test).

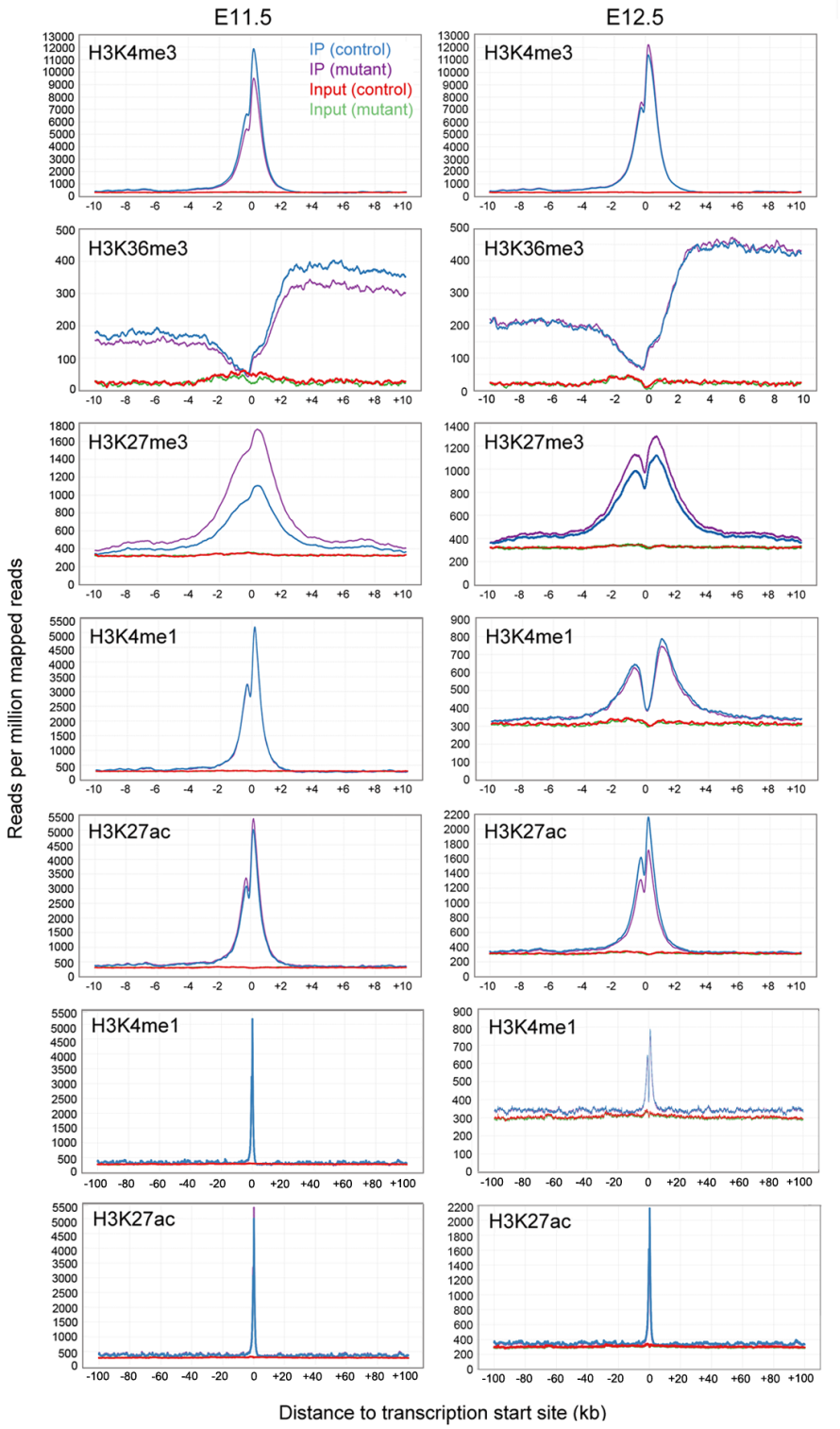

Fig. S3. Average profiles of histone modifications obtained for all genes in ChIP-seq assays for E11.5 *Sox9*$^{+/+}$ and *Sox9*$^{-/-}$ embryo limb buds (left) and for E12.5 *Sox9*$^{fl/fl}$ and

Sox9$^{fl/fl}$Prx1CreER embryo limb buds (right). Data are presented for a window of 10 kb upstream and downstream of transcription start sites. A window of 100 kb is also shown for H3K4me1 and H3K27ac. Note that the control and mutant profiles for H3K4me1 at E11.5 are so similar that they cannot be distinguished. Also note that the characteristic bimodal curving of epigenetic profiles is more readily apparent when the peaks are small than when they are tall.

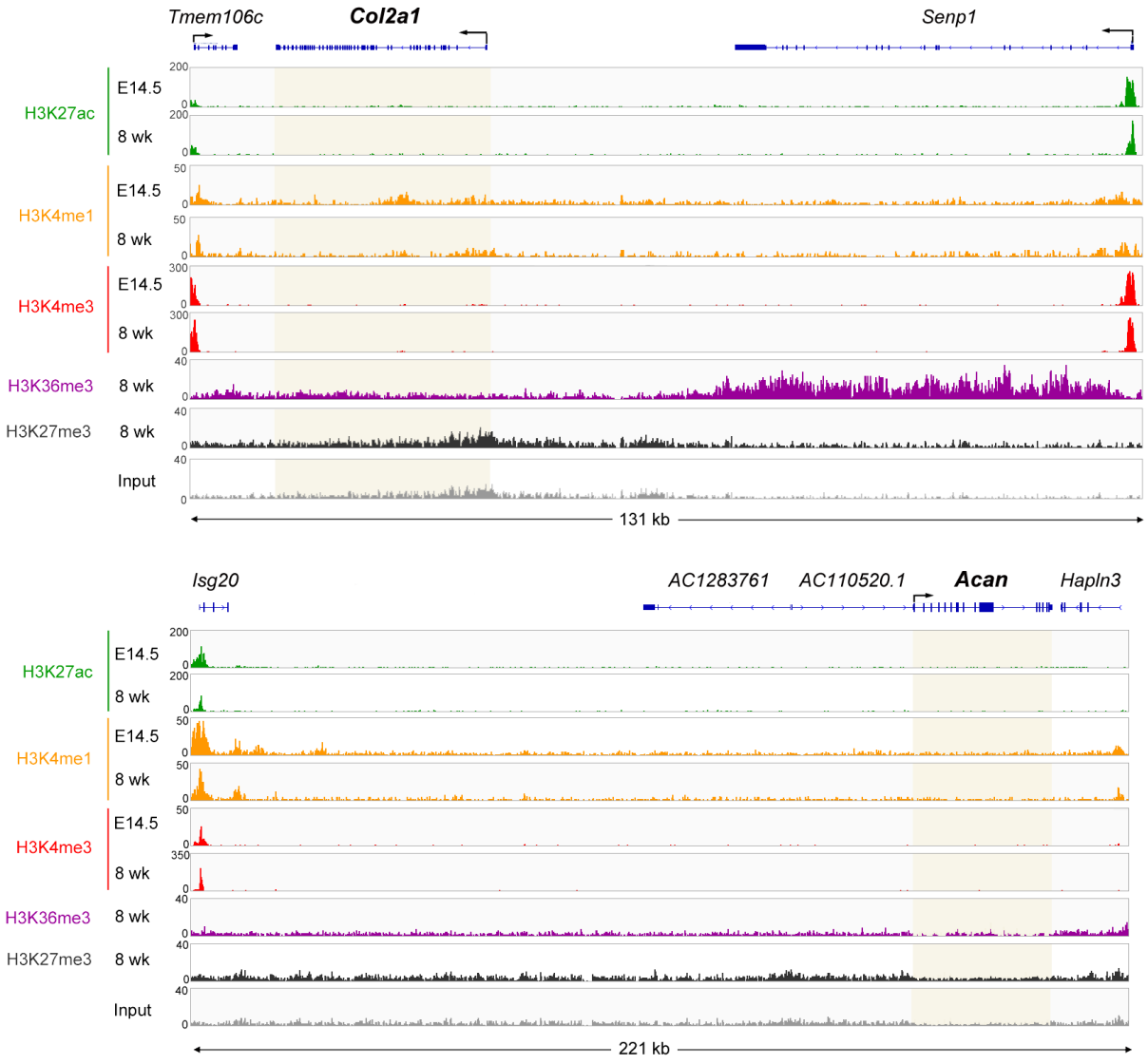

Fig. S4. Epigenetic profiles of the *Col2a1* and *Acan* loci in liver of E14.5 and 8-week-old mice. Data were downloaded from the GEO database (GSE31039). H3K36me3 and H3K27me3 profiles were not available for fetal liver.

Supplementary Tables

Table S1. List of all genes expressed in E11.5 *Sox9*$^{fl/-}$ and *Sox9*$^{-/-}$ mouse limb buds and in E12.5 *Sox9*$^{fl/fl}$ and *Sox9*$^{fl/fl}$*Prx1CreER* mouse limb buds, as detected by RNA-seq assay

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Table S2. Lists of genes upregulated and downregulated in *Sox9*-deficient limb buds, as detected by RNA-seq assay

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Table S3. Ingenuity Pathway Analysis of biological process categories and disease/function subcategories containing genes downregulated ≥1.5 fold in *Sox9*-deficient limb buds at E12.5

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Table S4. Lists of genes upregulated and downregulated in limb buds from E11.5 to E12.5, as detected in RNA-seq assays

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Table S5. Ingenuity Pathway Analysis of biological processes involving genes upregulated or downregulated in limb buds between E11.5 and E12.5

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Table S6. Lists of primers and enhancer features

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Table S7. List of 623 cartilage-related genes used for generating average histone modification profiles in Fig. 7

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