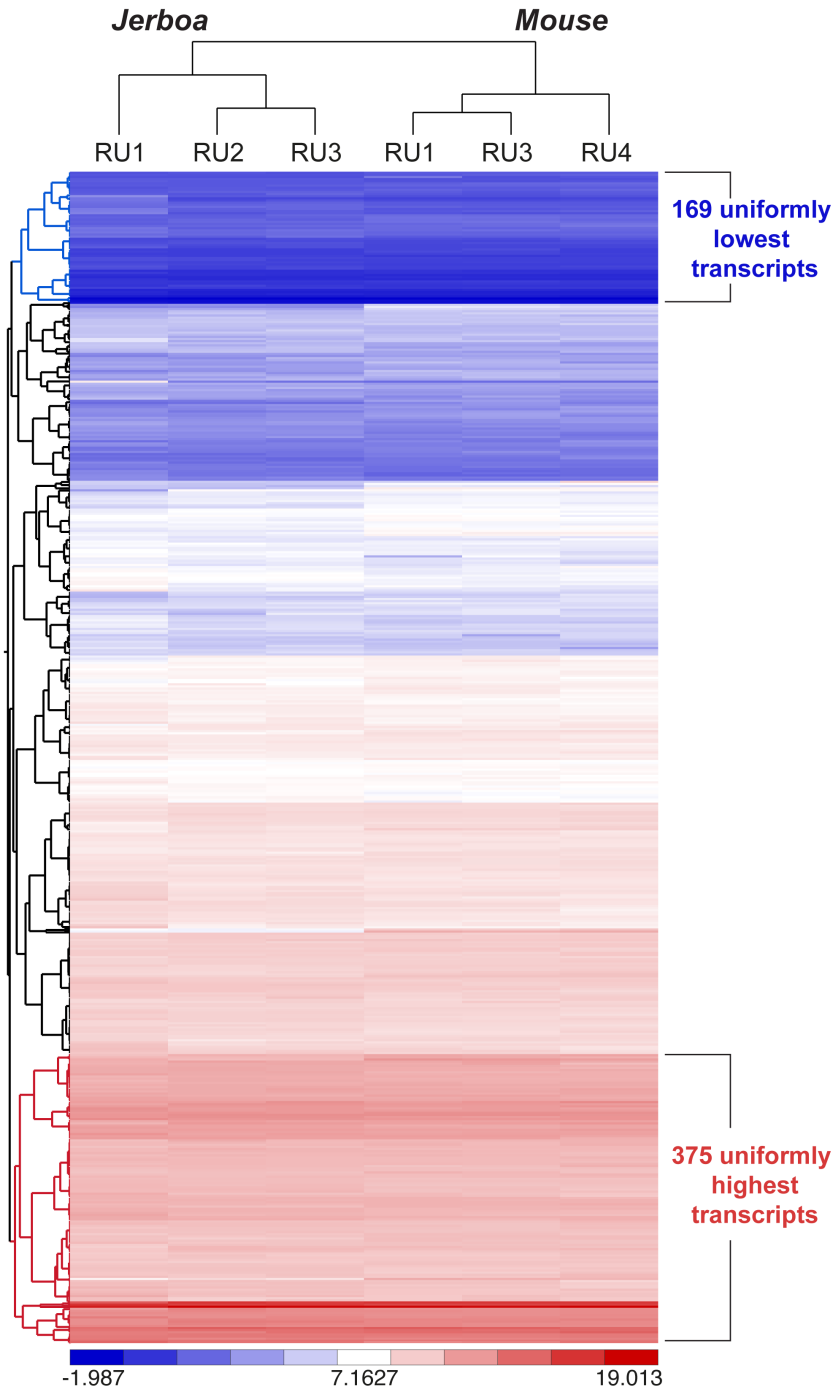

Figure S1 | Genes that are differentially expressed between MT but not RU include a cluster with extremely low to undetectable expression in RU and a cluster with high expression in RU, Related to Figure 2. r-log transformed gene counts for three jerboa (left) and mouse (right) RU samples were hierarchically clustered using Ward's method. Low to high gene expression is marked in blue to red colors, respectively. Clusters of genes with lowest or highest expression in RU of the two species are indicated.

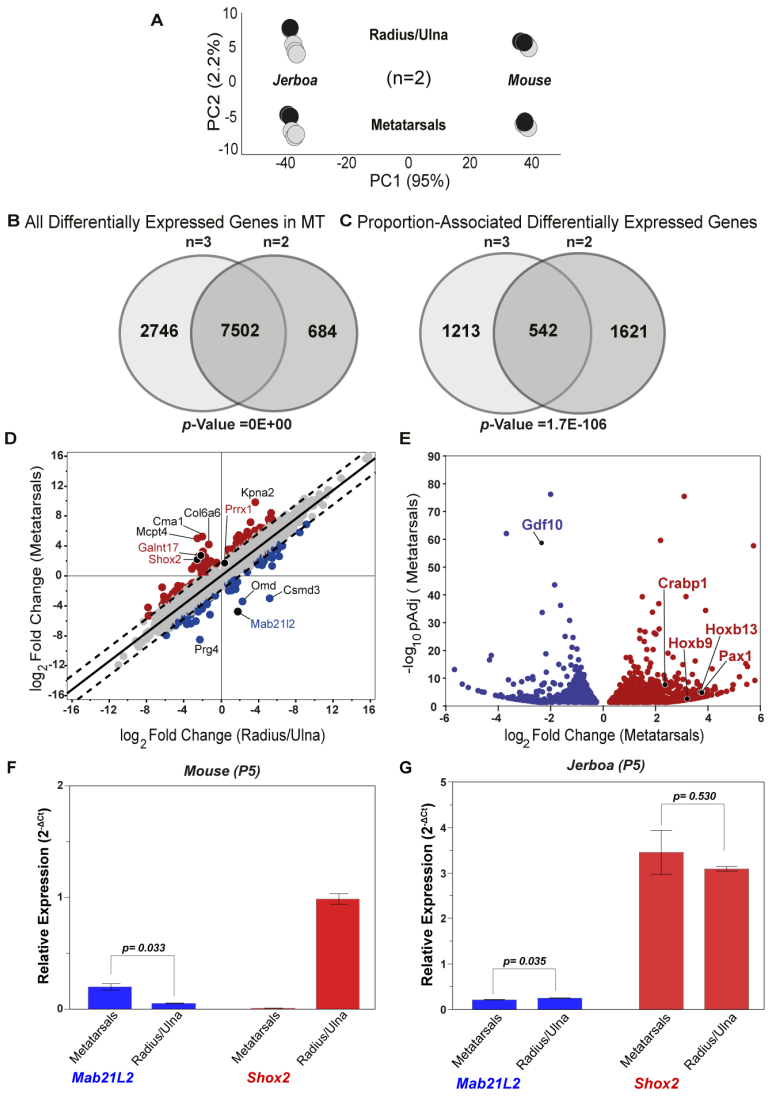

Figure S2 | Verification of interspecies RNASeq using two additional biological replicates and qRT-PCR, Related to Figure 2. (**A**) Principal component (PC) analysis of all five biological replicates with two samples used for verification marked black and the three primary samples in grey**.** Venn diagram overlap of (**B**) all genes differentially expressed between jerboa and mouse MT and (**C**) the proportion-associated differentially expressed genes (i.e., subtracting genes that are equivalently differentially expressed in MT and RU). Significance of overlap (p-value) was calculated using Fisher's exact test. (**D**) Genes that are differentially expressed between species in both growth cartilages (slope=0.952) and (**E**) of genes differentially expressed in MT but not RU. **(F-G)** Quantification of *Mab21L2* and *Shox2* mRNA levels in (**F**) mouse and (**G**) jerboa postnatal day 5 (P5) growth cartilages by qRT-PCR**.** *Mab21L2* and *Shox2* transcript expression was normalized to *Sdha* reference transcript each species. Compared to their respective radius/ulna, *Mab21L2* expression is 3.8-fold higher in mouse MT (**F**) and 1.16-fold lower in jerboa MT (**G**). *Shox2* expression is negligible in mouse MT (**F**) but is similarly expressed in the MT and RU of jerboa (**G**). n=3 each with SEM error bars. Student's t-test (α = 0.05) was used to test significance of differences between means; p value <0.05 indicates a significant difference.

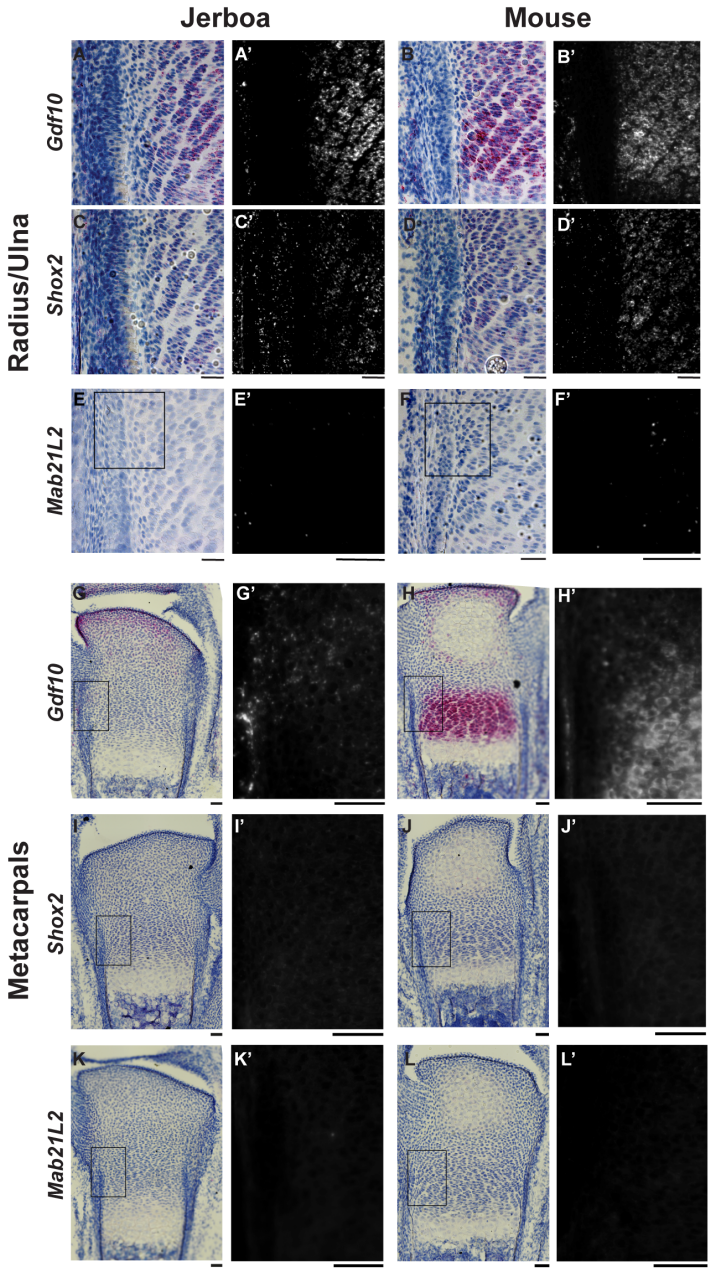

Figure S3 | Spatial pattern of *Gdf10, Shox2,* and *Mab21L2* expression in jerboa and mouse radius/ulna and metacarpal growth cartilage proliferative zones, Related to Figure 4. Expression patterns in distal growth cartilages of P5 jerboa (left) and mouse (right). (**A-L**) RNAScope colorimetric and (**A'-L'**) fluorescence detection. (**A,B,G,H**) *Gdf10*, (**C,D,L,J**) *Shox2*, and (**E,F,K,L**) *Mab21L2*. (**E'-L'**) show regions outlined in (**E-L**) at higher magnification. Scale bars, 50 μm.

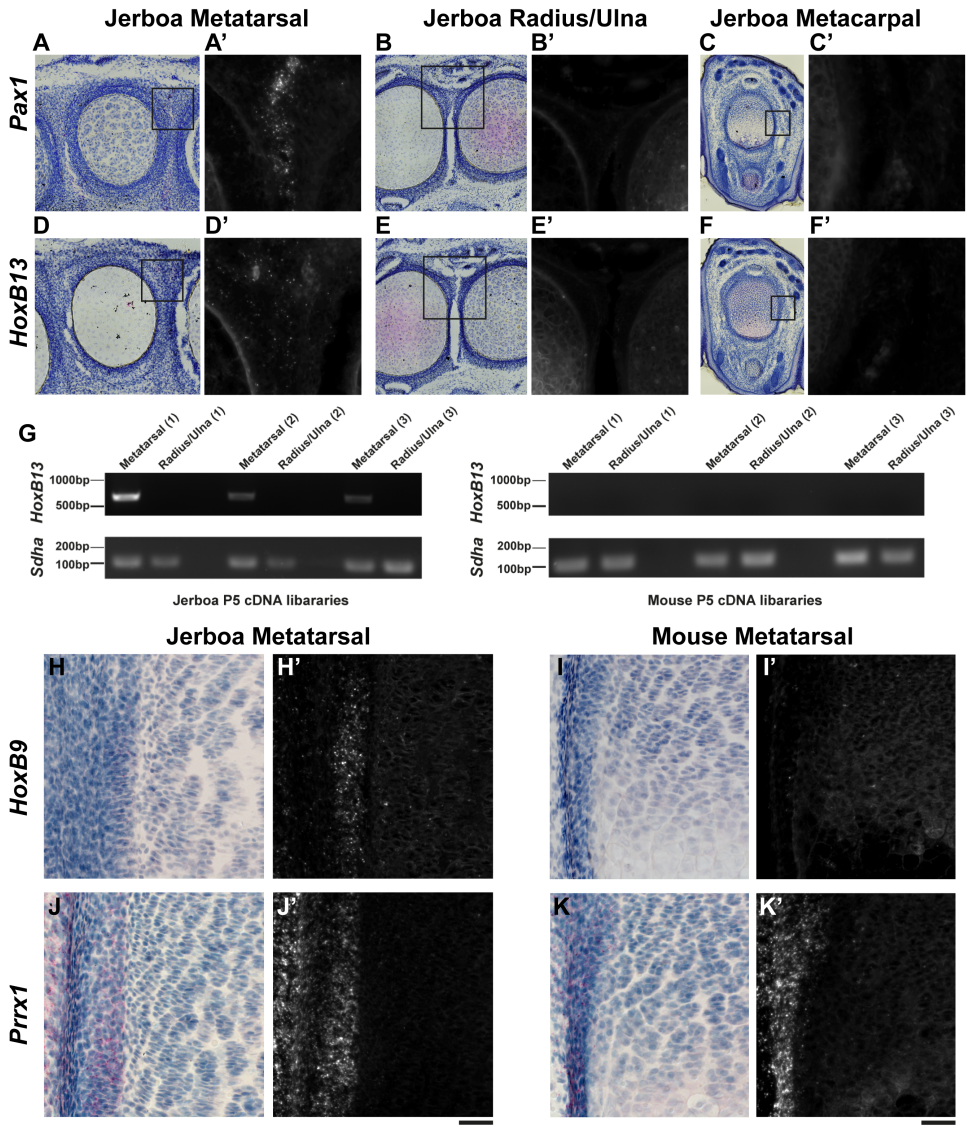

Figure S4 | Expression of transcriptional regulators *Pax1*, *HoxB13, HoxB9, and Prrx1* mRNA in growth cartilages, Related to Figure 4. Transverse sections through the distal growth cartilage of P5 (**A,D**) metatarsals, (**B,E**) radius/ulna and (**C,F**) metacarpals. (**A-F**) RNAScope colorimetric and (**A'-F'**) fluorescence detection shows expression in connective tissue between jerboa metatarsals. *HoxB13* is present as sparse bright dots throughout the perichondrial connective tissue. Cartilage and/or the edge of cartilage is apparent by autofluorescence (**A'-F'**). (**G**) Endpoint PCR amplifications of *HoxB13* transcripts with primers spanning exon-exon junctions show presence of a 552 bp amplicon only in jerboa metatarsal cDNA libraries (**left**). No product was detected in mouse metatarsal or in the radius/ulna of the two species. Amplification of *Sdha* transcripts with exon-exon junction spanning primers was used as a positive control for each of the cDNA libraries. n=3 for all samples. Longitudinal sections through distal metatarsal growth cartilages of (**H,J**) jerboa and (**I,K**) mouse showing (**H-K**) RNAScope colorimetric (red) and (**H'-K'**) fluorescence detection of mRNA expression. (**H**) *HoxB9* expression is detected in the jerboa perichondrium. (**J-K**) *Prrx1* expression in the perichondrium of jerboa and mouse metatarsals.

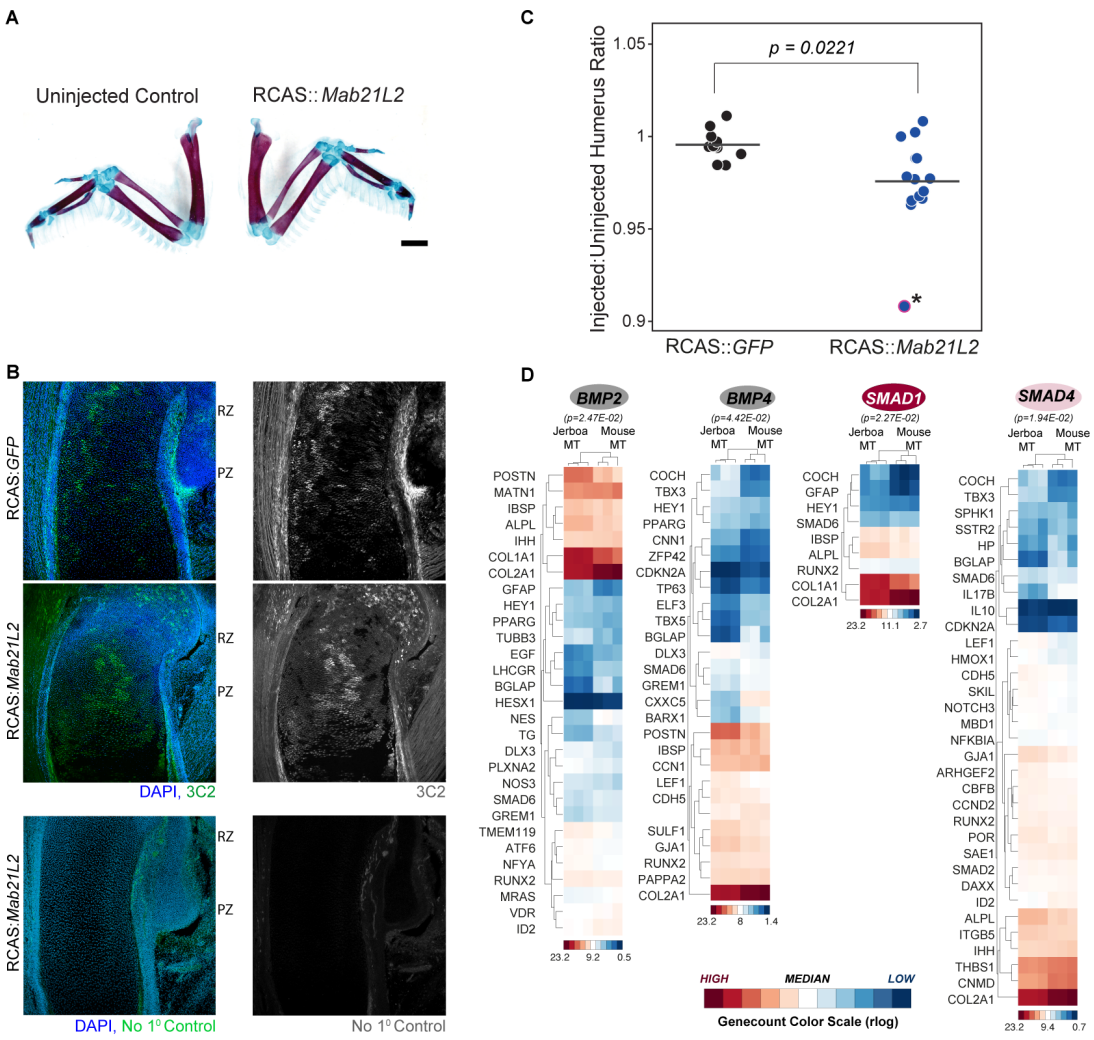

Figure S5 | *Mab21L2* over-expression reduces humerus length, Related to Figure 4. (**A**) Skeletal preparations show the left unmanipulated and right RCAS::*Mab21L2* infected wings of a representative chicken embryo. (**B**) 3C2 antibody staining shows wide-spread RCAS infection in longitudinal sections of growth cartilage from RCAS::*GFP* (n=4) and RCAS::*Mab21L2* (n=5) infected humeri (top and middle panels). A no primary (1^0) antibody staining control is shown in the bottom panels. Resting (RZ) and proliferative (PZ) zones are indicated next to each growth cartilage. (**C**) Ratio of the lengths of unmanipulated to infected humerus in RCAS::*GFP* control and RCAS::*Mab21L2* chicken embryos shows a 1.9% ratio reduction in *Mab21L2* overexpressing humerus (p-value derived from a Wilcoxon/Mann-Whitney nonparametric test). The RCAS::*Mab21L2* outlier marked with an asterisk (*) was excluded from statistical analysis. Scale bar in **A** = 200 µm. Note: humerus, radius, ulna, and third metacarpals were measured in each wing. Only RCAS::*Mab21L2* infected humerus showed a significant change in length. (**D**) All differentially expressed genes in the significantly enriched *BMP2*, *BMP4, SMAD1*, and *SMAD4* target networks are shown. These targets predict activation (red) of *SMAD1* and *SMAD4* in jerboa metatarsals (Table S7). Intensity of red indicates confidence of activation status prediction (z-score). p-values for enrichments are indicated in (**C**) and (**D**).

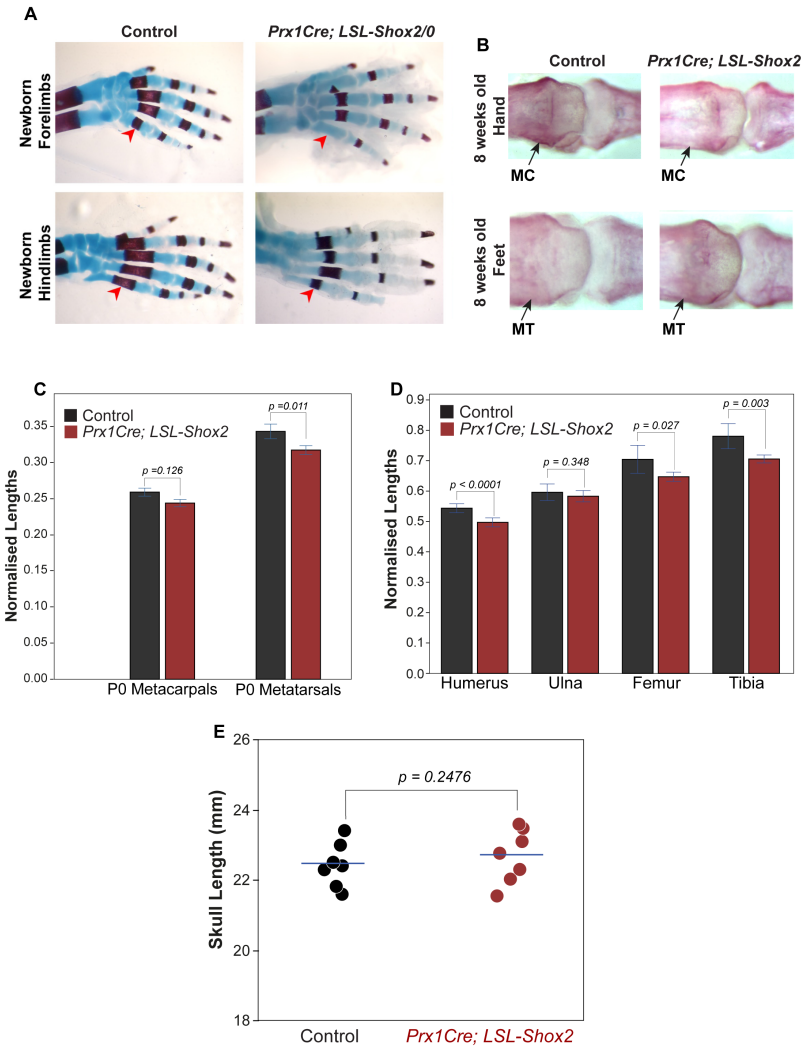

Figure S6 | Affects on limb skeletal element lengths upon *Shox2* overexpression, Related to Figure 5. (**A**) In newborn hemizygotes (*Prx1-Cre*; LSL-*Shox2*/0, n=5), metacarpal and metatarsal ossifications are reduced (red arrowheads) compared to litter mate controls. All limbs are oriented with digit 1 (anterior) toward the top. (**B**) Alizarin-stained metacarpals (MC) and metatarsals (MT) show no remnant cartilage at their distal ends (arrows) in 8-week old control and *Prx1-Cre*; LSL-*Shox2*/0 hemizygotes (n = 7). (**C**) Newborn metacarpal lengths are similar between control littermates (black, n=6) and *Prx1-Cre; LSL-Shox2* hemizygous (red, n=10) mice. Newborn metatarsals in these same animals are significantly shorter than littermate controls. p-values derived from a t-test (α = 0.05). (**D**) Although the distal elements are significantly longer at 8 weeks (Fig. 5d-h), proximal forelimb and hindlimb elements are significantly shorter (except for ulna) at this later age in *Prx1-Cre; LSL-Shox2* hemizygous animals (red) compared to littermate controls (black). (**E**) Skull lengths are similar between control littermates (black) and *Prx1-Cre; LSL-Shox2* hemizygous (red) mice at 8 weeks. p-values in (**D**) and (**E**) are derived from a paired t-test (α = 0.05) between matched-sex littermates (n=5 females and 2 males of each genotype).

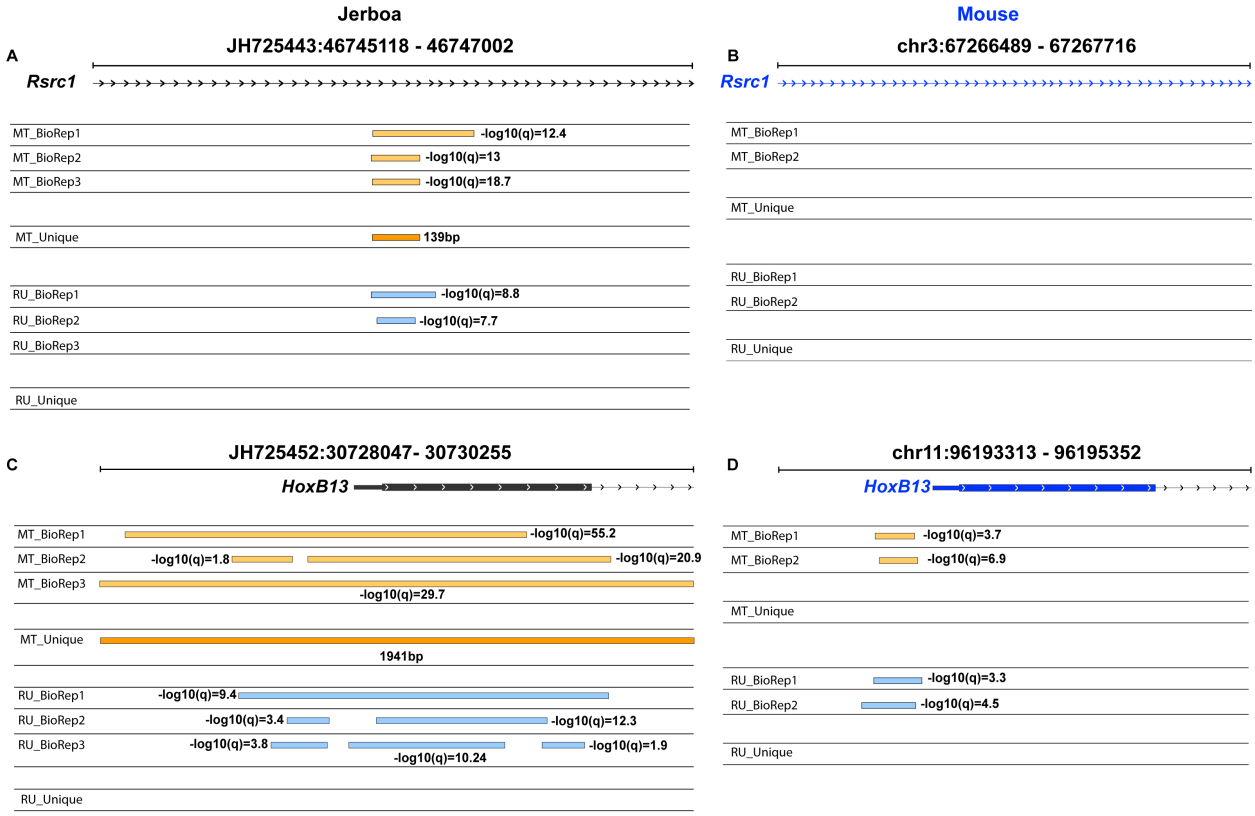

Figure S7 | Open chromatin regions in each individual replicate of jerboa and mouse growth cartilage, Related to Figure 6. ATAC-Seq tracks show all MACS2 called peaks in individual biological replicates (BioRep) of (**A,C**) jerboa and (**B,D**) mouse in two genomic regions. False discovery rate for each peak is indicated as the negative log10 of the q-value [-log10(q)]. (**A**) An intronic interval in *Rsrc1* shows overlapping open chromatin regions in all jerboa metatarsal biological replicates (MT_BioRep) resulting in a robustly accessible 139 bp region. The radius/ulna peak in this region is detected in only two of three biological replicates (RU_BioRep) with lower statistical confidence (higher q-values). This peak therefore surpasses the 'unique to metatarsals' threshold. (**B**) The mouse *Rsrc1* intronic region orthologous to the 139 bp jerboa metatarsal peak was identified with LiftOver. This region lacks any detectable accessibility in mouse metatarsals or in radius/ulna. (**C**) Peaks with high statistical confidence in all jerboa metatarsal biological replicates delineate a 1941 bp region associated with *HoxB13* that is also 'unique' to metatarsals. Peaks in this region in jerboa radius/ulna and (**D**) in LiftOver orthologous regions in mouse did not pass the statistical threshold. Genomic coordinates in jerboa and mouse are indicated on top in **A-D**. Metatarsal and radius/ulna tracks are shown in orange and blue, respectively.