

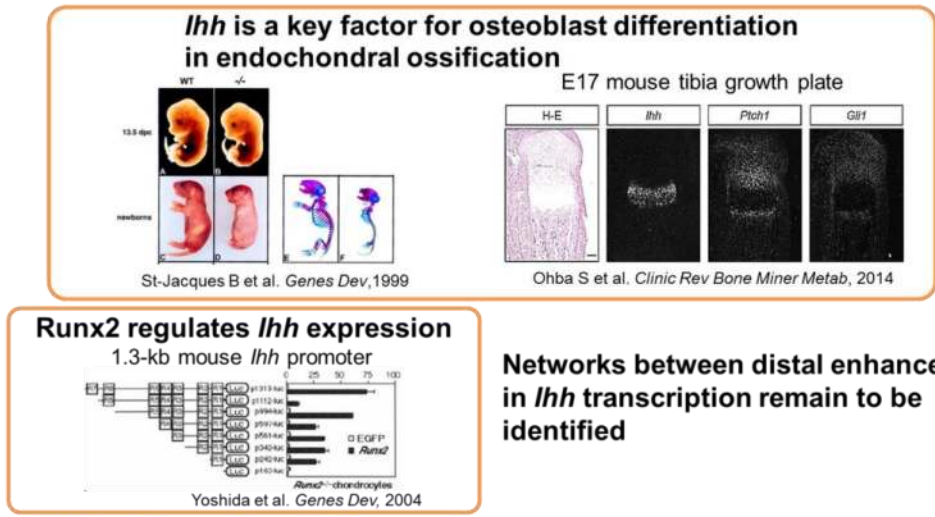
A chondrocyte-specific *Indian hedgehog* enhancer as an OA-driving enhancer

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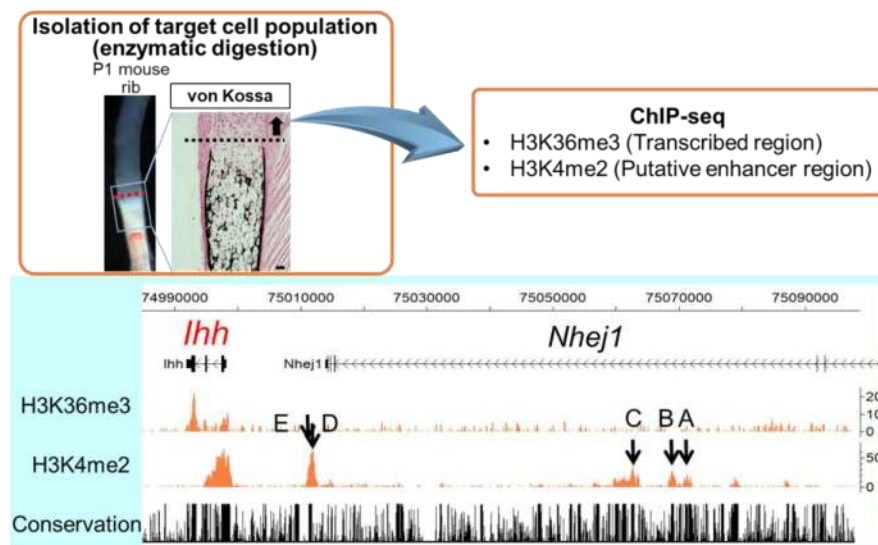
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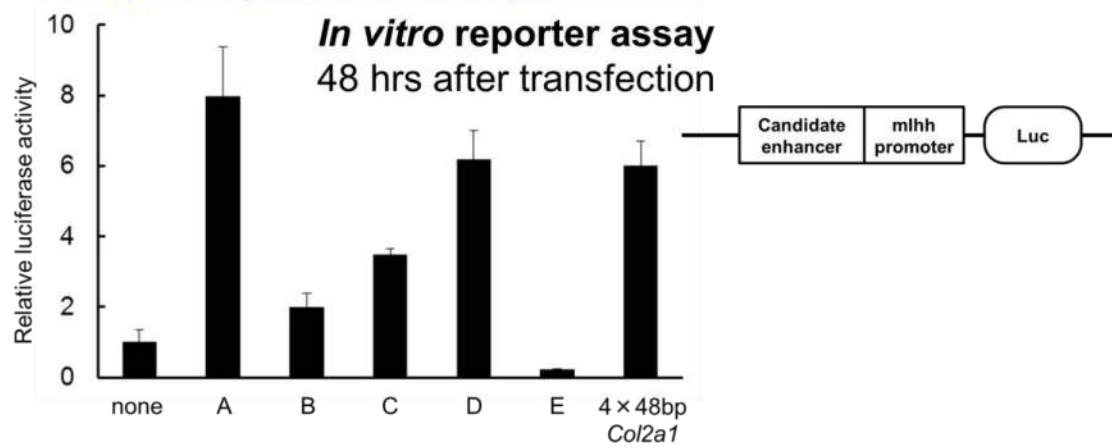
1. Background



2. Identification of putative enhancers around the *Ihh* gene region by ChIP-seq

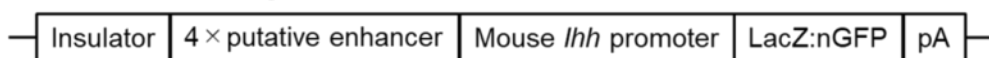


3. Reporter activity of candidate enhancer regions in mouse primary rib chondrocyte

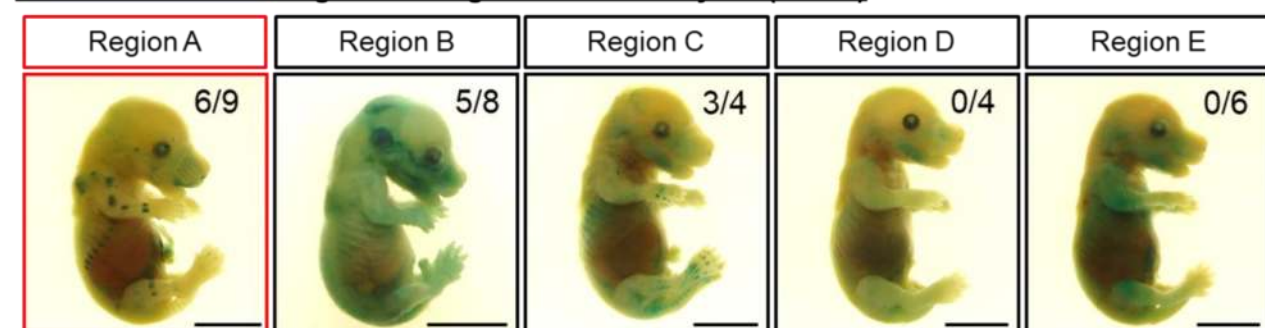


4. In vivo enhancer activity of candidate enhancer regions shown by lacZ reporter transgenic mice

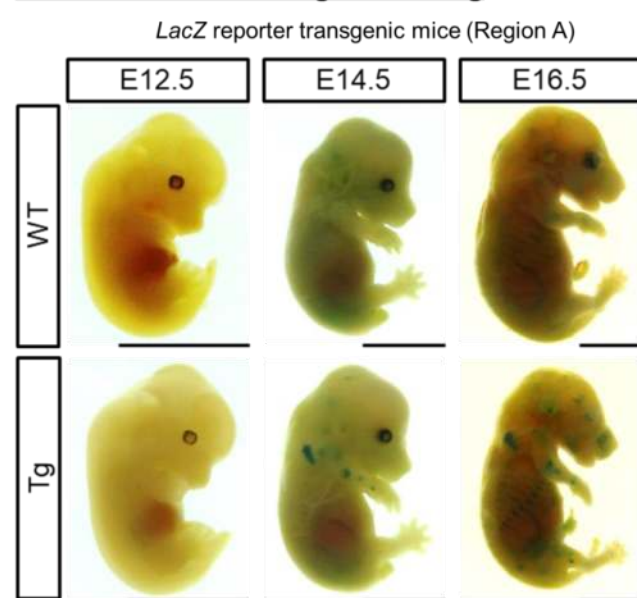
4-1. Schematic of transgene



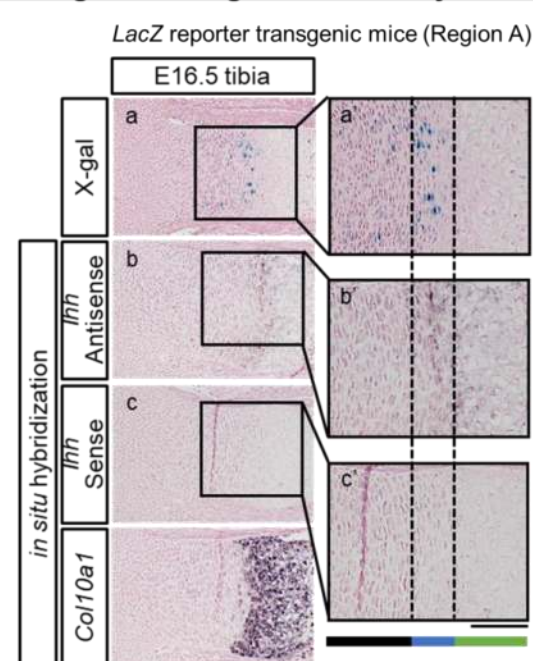
4-2. Whole mount X-gal staining in the G0 analysis (E16.5)



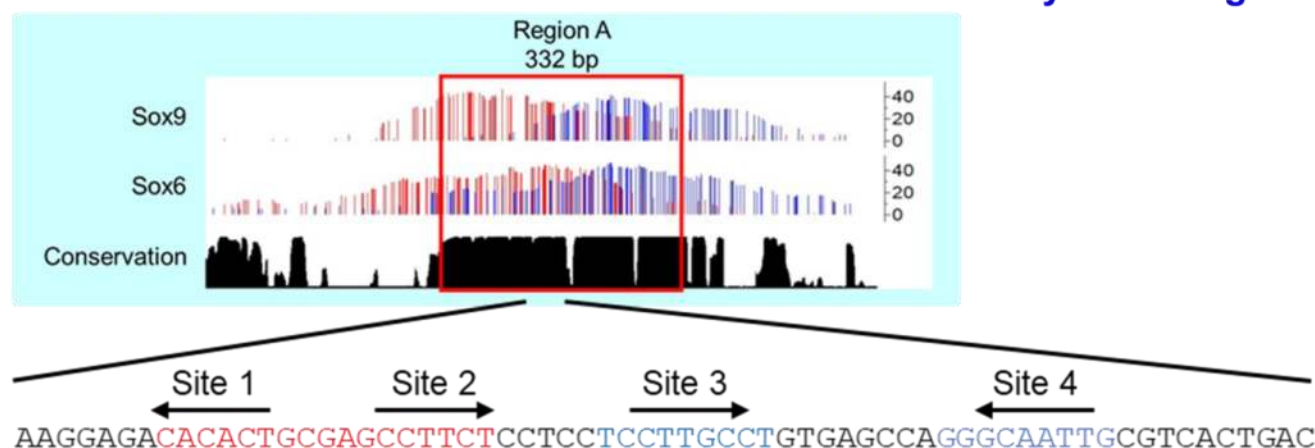
4-3. Whole mount X-gal staining



4-4. X-gal staining and in situ hybridization

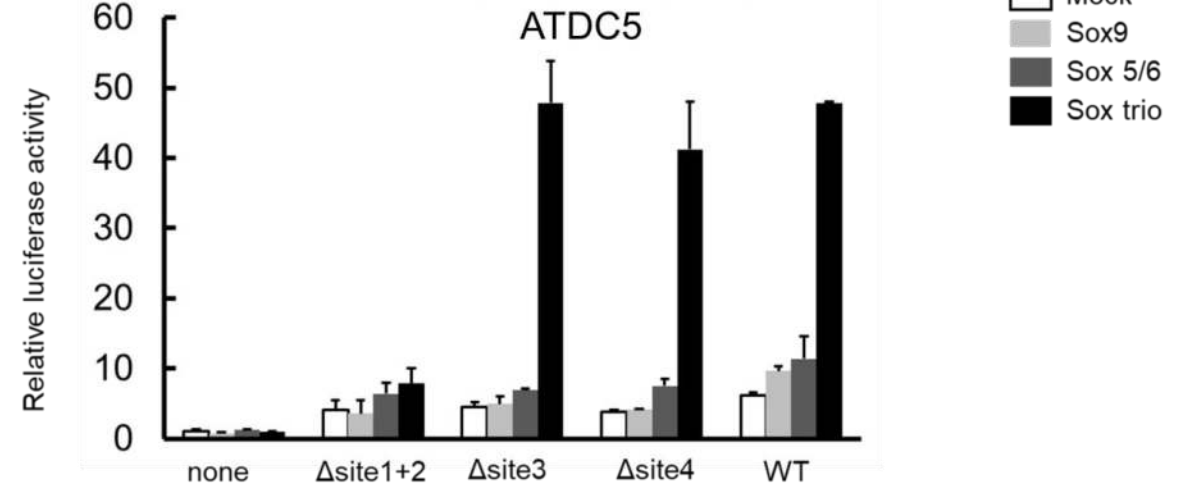


5. Involvement of Sox9 and Sox5/6 in the enhancer activity of the region A

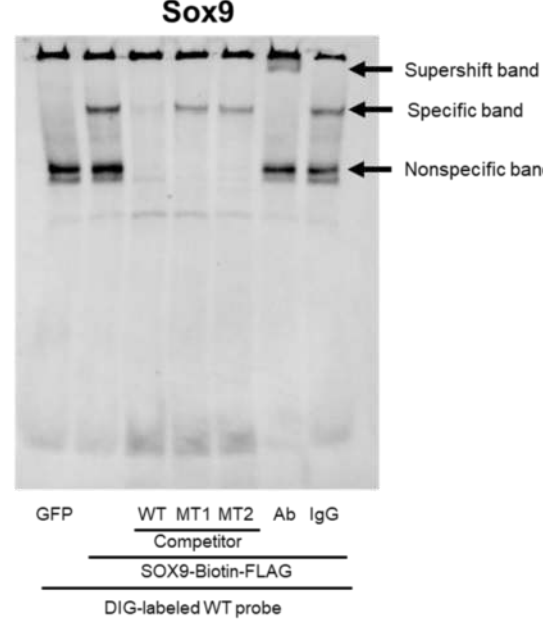


In vitro reporter assay

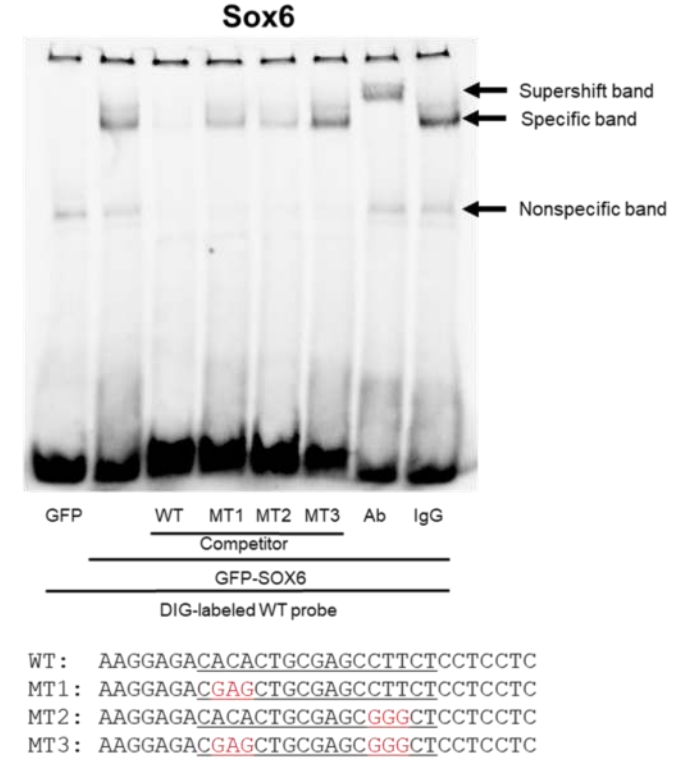
48 hrs after transfection



Gel shift assay

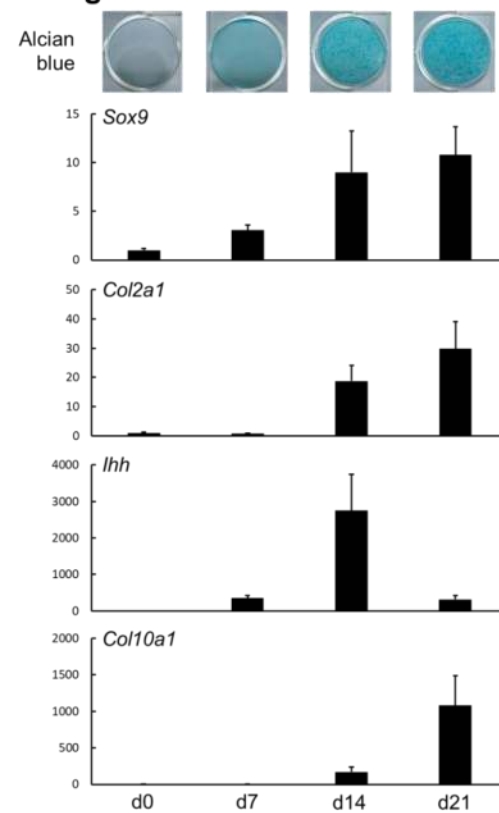


Gel shift assay

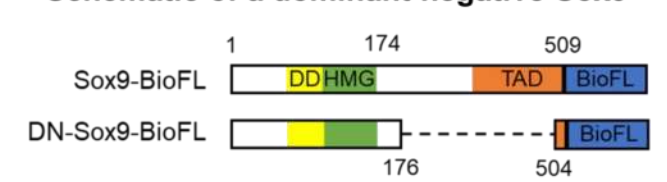


6. Loss of function of Sox9 protein on Ihh expression in vitro

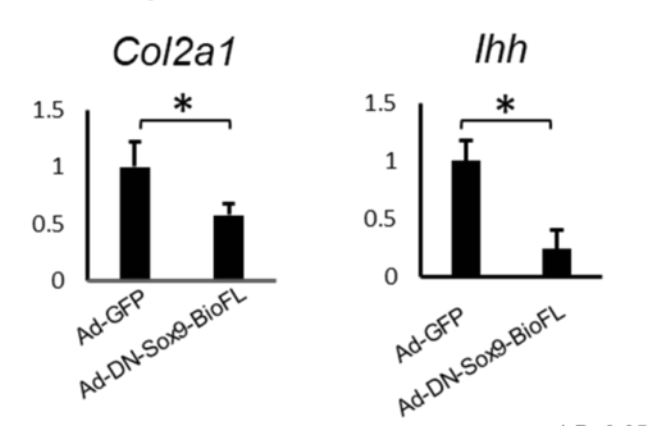
Chondrogenic differentiation of ATDC5 cells



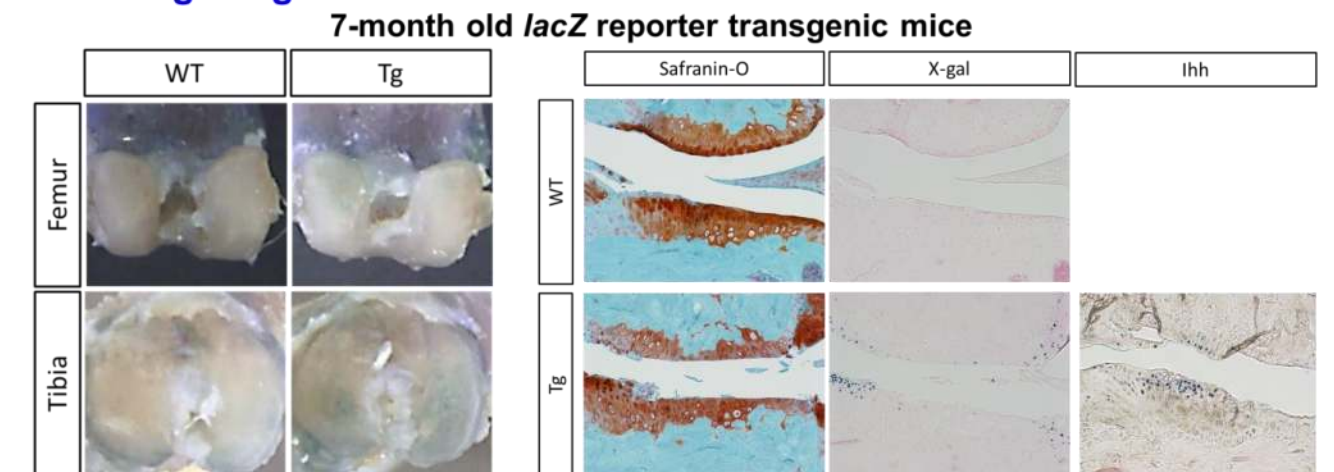
Schematic of a dominant negative Sox9



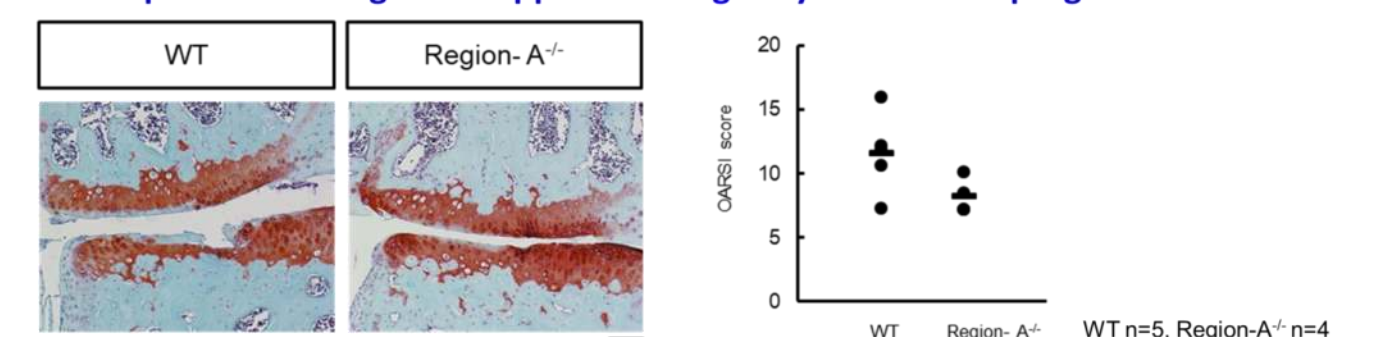
mRNA expression on day16



7. Region A activation and Ihh expression during naturally occurring cartilage degeneration in mice



8. Disruption of the region A suppressed surgically induced OA progression



Summary

- Region A, identified by a genome-wide approach, works as a chondrocyte-specific *Ihh* enhancer.
- Sox trio positively regulates *Ihh* transcription at least partially through distal enhancers including the region A.
- Region A activates *Ihh* transcription in articular chondrocytes during OA progression.