Int. J. Dev. Biol. 67: 19-33 (2023)
https://doi.org/10.1387/ijdb.220173db

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Single-cell transcriptome profiling reveals distinct expression patterns among *Sox* genes in the mouse incisor dental pulp

BADAM ENKHMANDAKH[1], DASHZEVEG BAYARSAIHAN*[,1,2]

[1]Center for Regenerative Medicine and Skeletal Development, Department of Reconstructive Sciences, University of Connecticut Health Center, Farmington, CT, USA, [2]Institute for System Genomics, University of Connecticut, Storrs, CT, USA

ABSTRACT SOX transcription factors play key roles in cell differentiation and cell fate determination during development. Using single-cell RNA-sequencing data, we examined the expression profiles of *Sox* genes in the mouse incisor dental pulp. Our analysis showed that *Sox4*, *Sox5*, *Sox9*, *Sox11*, and *Sox12* are mainly expressed in mesenchymal stem/stromal cells (MSCs) representing osteogenic cells at different stages of differentiation. We found that in several MSCs, *Sox* genes co-expressed with regulatory genes such as *Sp7*, *Satb2*, *Msx1*, *Snai2*, *Dlx1*, *Twist2*, and *Tfap2a*. In addition, *Sox* family genes colocalized with *Runx2* and *Lef1*, which are highly enriched in MSCs undergoing osteoblast differentiation. A protein interaction network analysis uncovered that CREBBP, CEBPB, TLE1, TWIST1, and members of the HDAC and SMAD families are interacting partners of RUNX2 and LEF1 during skeletal development. Collectively, the distinct expression patterns of the SOX transcription factors suggest that they play essential regulatory roles in directing lineage-specific gene expression during differentiation of MSCs.

KEYWORDS: dental pulp, single cells, chromatin, incisors, transcriptome

Introduction

SOX transcription factors (TFs) are key regulators of stem cell maintenance, differentiation and cell fate specification (Liu and Guo, 2021; Stepanovic *et al*., 2021; Lefebvre, 2019). Several members of the SOX family play an essential role in the development of the neural crest, which is important for the formation of the craniofacial region (Schock and LaBonne, 2020). Because SOX TFs contribute to the determination of cell fate and lineage specification, mutations within this family often have been associated with birth defects. For instance, mutations in *SOX9* lead to campomelic dysplasia, a severe disorder that affects development of the skeleton, reproductive system, and other parts of the body and heterozygous mutations of *SOX10* cause multiple defects associated with malfunction of neural crest derivatives including melanocytes, the enteric nervous system, Schwann cells, oligodendrocytes and olfactory cells (Angelozzi and Lefebvre, 2019; Ming *et al*., 2022; Pingault *et al*., 2022).

Interestingly, recent research showed that SOX9 behaves like a pioneer TF by establishing accessible chromatin regions (Fuglerud *et al*., 2022). Genome-wide studies have revealed that sequentially expressed SOX TFs bind to the specific regulatory elements to coordinate lineage commitment (Klum *et al*., 2018). Single-cell transcriptomic studies identified *Sox* genes as key regulators of neuroglandular lineages in the cnidarians (Steger *et al*., 2022). In human pluripotent stem cells, SOX TFs establish a permissive chromatin landscape to activate the β-catenin-dependent Wingless-Int (WNT) signaling pathway (Mukherjee *et al*., 2022).

Although the function and regulatory mode of SOX TFs is well established in skeletogenesis and osteoblast and chondrocyte differentiation (Lefebvre, 2019), very little is known about their role in odontogenesis and dentin development. Previous work revealed that *Sox* genes exhibit a dynamic spatial and temporal expression pattern during mouse tooth development (Kawasaki *et al*., 2015). In the current study, we used single-cell RNA-sequencing (RNA-seq) data to demonstrate specific and distinct expression profiles of *Sox* genes in the mouse incisor dental pulp.

***Address correspondence to:** Dashzeveg Bayarsaihan. Center for Regenerative Medicine and Skeletal Development, Department of Reconstructive Sciences, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030, USA. E-mail: dashzeveg@uchc.edu

Submitted: 29 December, 2022; Accepted: 29 March, 2023; Published online: 29 March, 2023.

ISSN: Online 1696-3547, Print 0214-6282

Results

Single-cell RNA expression of Sox genes in different cell subpopulations of the mouse incisor dental pulp

We previously identified 16 distinct cell clusters in the dental pulp of mouse incisors based on the differential expression of marker genes (Bayarsaihan *et al*., 2022). According to the expression of key genes required for osteogenic differentiation, we identified eight clusters of MSCs (Fig. 1A). Differential expression patterns of *Runx2*, *Sp7*, *Satb2*, *Msx1*, *Snai1*, *Dlx1*, *Twist2*, and *Tfap2a* suggest that these MSCs represent osteogenic subpopulations at various stages of differentiation (Fig. 1B).

We found that *Sox1*, *Sox2*, *Sox3*, *Sox6*, *Sox8*, *Sox10*, *Sox15*, *Sox21*, and *Sox30* exhibited weak expression in the mouse incisor dental pulp (Fig. 2). Interestingly, our single-cell RNA-seq failed to detect expression of *Sox14* in any of the dental pulp subpopulations. By contrast, *Sox4* was vigorously expressed in clusters 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13, 14, 15, and 16; *Sox5* in clusters 2, 3, 4, 9, 11, and 14; *Sox9* in clusters 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13, and 14; *Sox11* in clusters 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13, 14, and 15; and *Sox12* in clusters 1, 2, 3, 6, 8, 9, 13, and 14. Interestingly, *Sox7*, *Sox13*, *Sox17*, and *Sox18* were mainly enriched in only a single cluster, cluster 15.

Chromatin accessibility and 5hmC of Sox genes in the mouse incisor dental pulp

Genomic regions of actively transcribed genes retain open chromatin and are characterized by the enrichment of 5hmC, a hallmark feature of DNA demethylation. Hence, we investigated chromatin accessibility and DNA demethylation by performing transposase-accessible chromatin sequencing (ATAC-seq) and hydroxymethylation DNA immunoprecipitation sequencing (hMeDIP-seq). Using these assays, we demonstrated that actively transcribed *Sox* genes retain characteristics of open chromatin. Transcriptome profiling using bulk RNA-seq showed that *Sox4, Sox9*, *Sox11*, and *Sox12* are strongly expressed in the molar dental pulp (Fig. 3). We observed elevated ATAC-seq peaks within the transcription start sites and promoter regions of these genes as well as within the gene body regions. Using hMeDIP-seq, we detected 5hmC enrichment across the gene bodies and the proximal and distal regions of *Sox4, Sox9*, *Sox11*, and *Sox12*, whereas input hMeDIP-seq showed low levels of 5hmC. We also analyzed the genomic regions of *Sox1* and *Sox21*, which displayed very weak or no expression in dental pulp. Both genes retained closed chromatin and we failed to detect 5hmC marks along the genomic regions of these genes. Collectively, our results support the previous observation that genes associated with cell differentiation and lineage commitment retain an open chromatin configuration and are enriched in 5hmC in the mouse dental pulp (Pujan *et al*., 2022a; Pujan *et al*., 2002b).

Single-cell RNA expression profile of interacting partners of Sox genes in MSCs of the mouse incisor dental pulp

Our research demonstrated that only a few members of the *Sox* gene family, specifically *Sox4*, *Sox9*, *Sox11*, and *Sox12*, retain a highly specific expression pattern within different clusters of the mouse incisor dental pulp (Fig. 2). SOX4, SOX11, and SOX12 comprise the SOXC group of TFs, which play key functions in cell fate determination (Lefebvre and Bhattaram, 2016). Conversely, SOX9 is essential for chondrocyte fate maintenance and differentiation (Lefebvre, 2019).

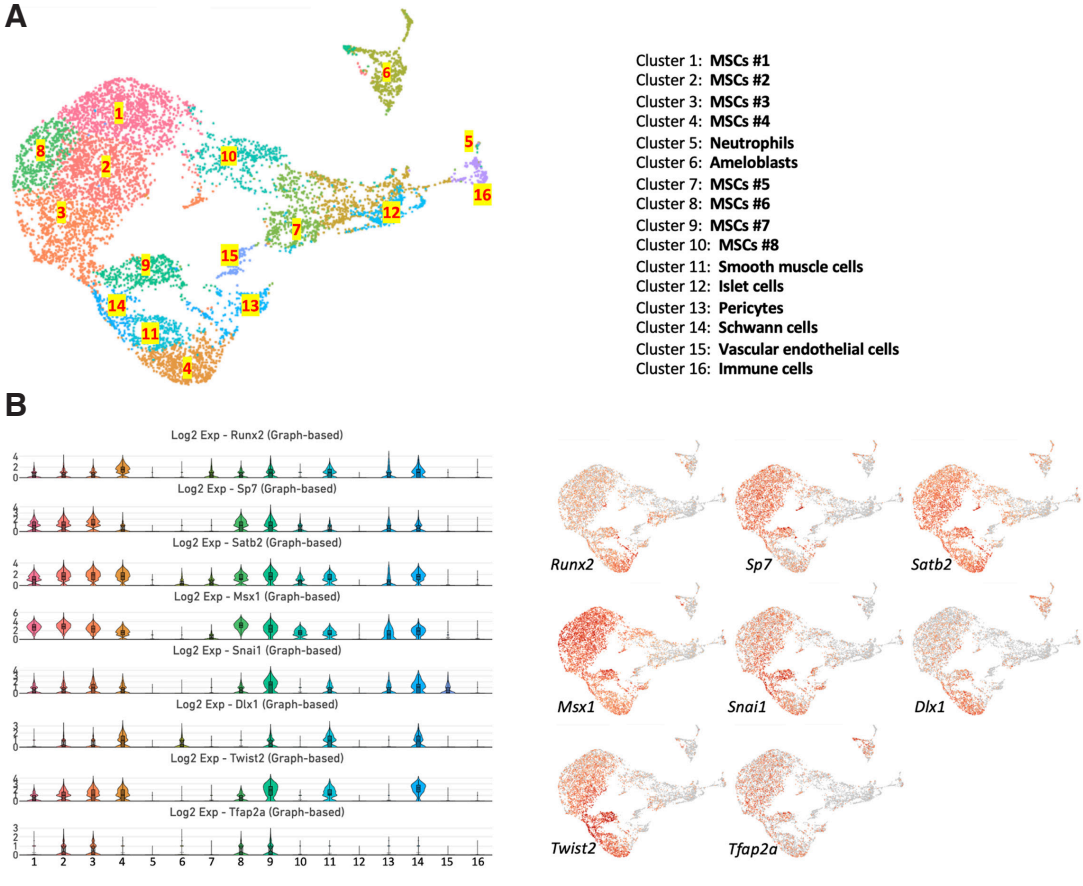

Fig. 1. Expression of genes encoding osteogenic transcription factors. (A) Uniform manifold approximation and projection (UMAP) visualization depicts the single-cell RNA expression profile of 16 cell types in the incisor dental pulp. **(B)** Violin plots (left) and UMAP visualization (right) of the *Runx2, Sp7, Satb2, Msx1, Snai1, Dlx1, Twist2*, and *Tfap2a* genes.

TABLE 1

THE EXPRESSION PATTERN OF SOX GENES IN THE MOUSE INCISOR DENTAL PULP

	MSCs #1	MSCs #2	MSCs #3	MSCs #4	Ameloblasts	MSCs #5	MSCs #6	MSCs #7	MSCs #8	Smooth muscle cells
Sox4	+	+	+	+	+	+	+	+	+	+
Sox5		+	+	+				+		+
Sox9	+	+	+	+	+	+	+	+	+	+
Sox11	+	+	+	+	+	+	+	+	+	+
Sox12	+	+	+		+	+	+			

By analyzing the STRING interaction network (https://string-db.org), we discovered that the SOXC group and SOX9 have a specific set of interacting partners (Fig. 4). Among these interacting partners, we identified TEAD2, ATF3, QSOX1, RUNX2, MAF, CREBBP, LEF1, and CTNNB1. We found that within MSC subpopulations, *Tead2* was enriched in clusters 1, 2, 3, 4, 8, and 9, while *Atf3* was weakly expressed in clusters 3, 4, and 9. *Qsox1* encodes an enzyme that catalyzes disulfide bond formation and is involved in redox regulation (Reznik and Fass, 2022). In our analysis, *Qsox1* was expressed in MSC clusters 1, 2, 3, 4, 8, 9, and 10; *Runx2* in cluster 1, 2, 3, 4, 7, 8, and 9; and *Maf* in clusters 1, 2, 3, 4, 8, and 10. *Lef1* exhibited a very restricted expression pattern in clusters 4 and 9. Both *Crebbp* and *Ctnnb1* were vigorously expressed in clusters 1, 2, 3, 4, 7, 8, 9, and 10.

Single-cell RNA expression profile of interacting partners of Runx2 and Lef1 in MSCs of the mouse incisor dental pulp

The expression of *Runx2* and *Lef1* was highly enriched in cluster 4, which represents the osteoblastic lineage. RUNX2 interacts with LEF1 to induce canonical WNT signaling during skeletal development (Reinhold and Naski, 2007). Therefore, we analyzed the expression pattern of interacting partners of RUNX2 and LEF1 in cluster 4. Using the STRING interaction network, we uncovered the interacting partners of RUNX2 and LEF1 in the dental pulp (Fig. 5). We found that genes encoding several canonical interacting partners of RUNX2 and LEF1, such as *Crebbp*, *Hdac1*, *Hdac4*, *Tle1*, *Smad1*, *Smad4*, *Cebpb*, and *Twist1*, were enriched in cluster 4.

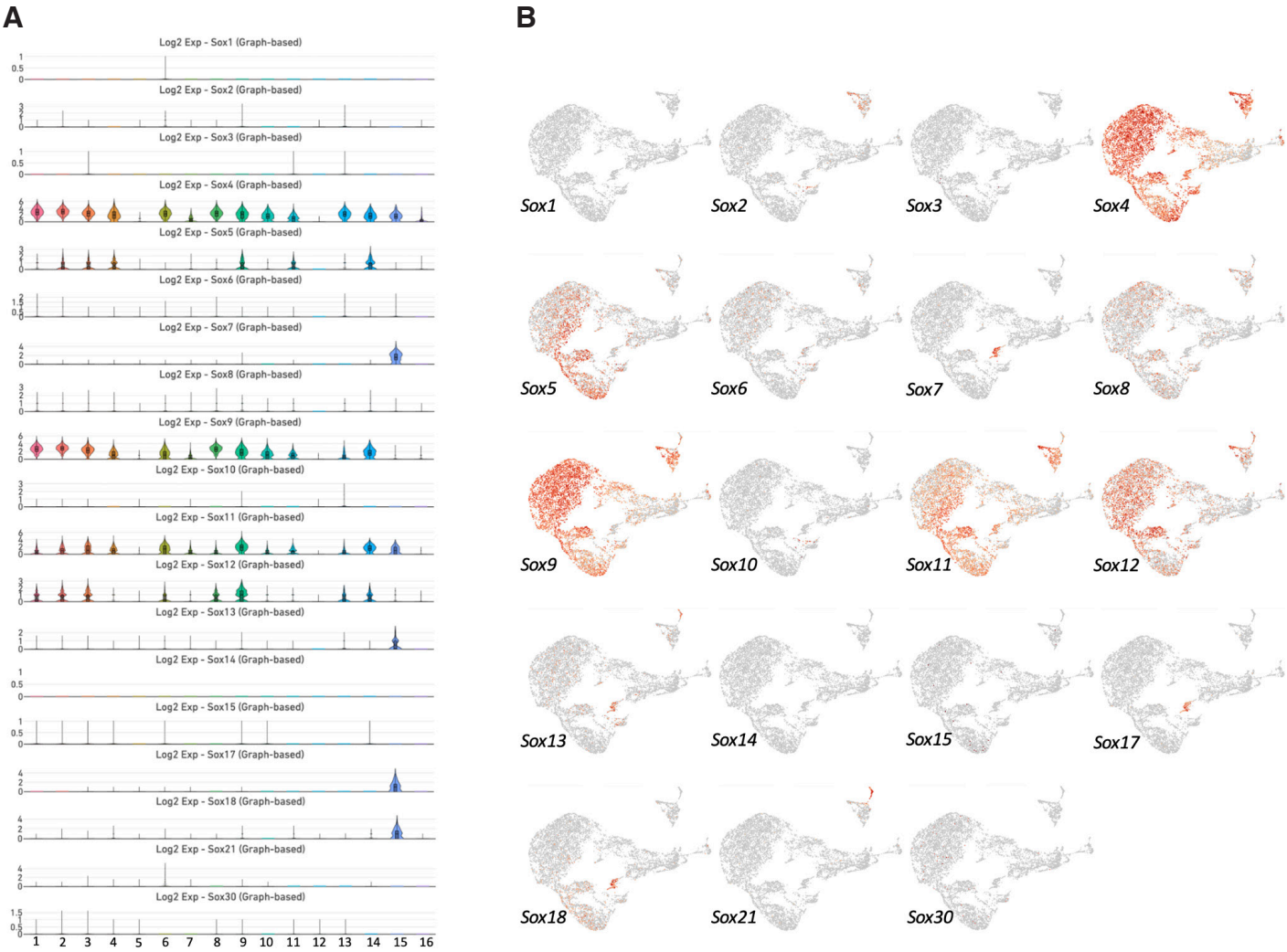

Fig. 2. Expression of genes encoding members of the SOX family of transcription factors. (A) Violin plots and **(B)** UMAP visualization revealed that *Sox4, Sox5, Sox9, Sox11,* and *Sox12* are mainly expressed in mesenchymal stem/stromal cells (MSCs), whereas *Sox7, Sox13, Sox17,* and *Sox18* are enriched in vascular endothelial cells.

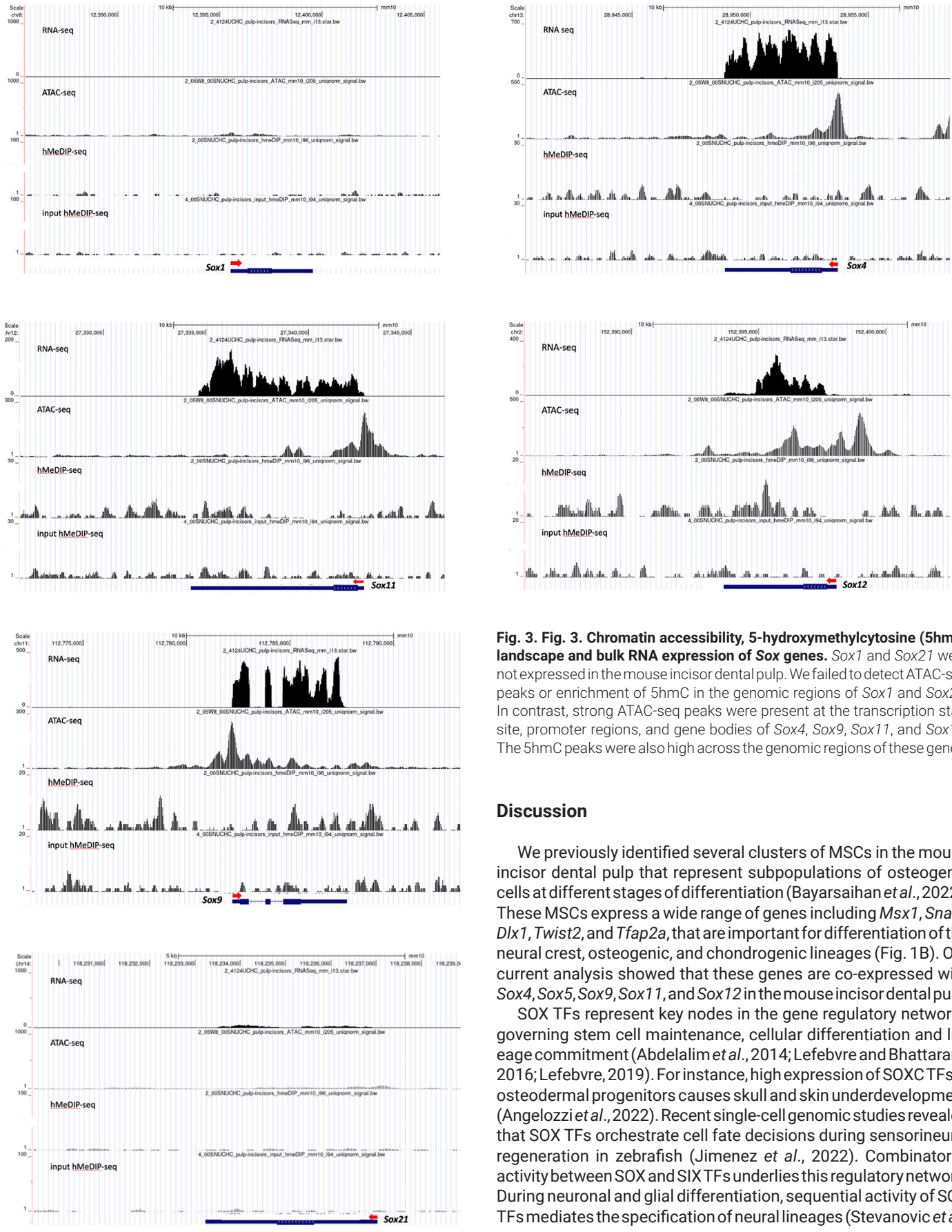

Fig. 3. Fig. 3. Chromatin accessibility, 5-hydroxymethylcytosine (5hmC) landscape and bulk RNA expression of *Sox* genes. *Sox1* and *Sox21* were not expressed in the mouse incisor dental pulp. We failed to detect ATAC-seq peaks or enrichment of 5hmC in the genomic regions of *Sox1* and *Sox21*. In contrast, strong ATAC-seq peaks were present at the transcription start site, promoter regions, and gene bodies of *Sox4*, *Sox9*, *Sox11*, and *Sox12*. The 5hmC peaks were also high across the genomic regions of these genes.

Discussion

We previously identified several clusters of MSCs in the mouse incisor dental pulp that represent subpopulations of osteogenic cells at different stages of differentiation (Bayarsaihan *et al*., 2022). These MSCs express a wide range of genes including *Msx1*, *Snai1*, *Dlx1*, *Twist2*, and *Tfap2a*, that are important for differentiation of the neural crest, osteogenic, and chondrogenic lineages (Fig. 1B). Our current analysis showed that these genes are co-expressed with *Sox4*, *Sox5*, *Sox9*, *Sox11*, and *Sox12* in the mouse incisor dental pulp.

SOX TFs represent key nodes in the gene regulatory networks governing stem cell maintenance, cellular differentiation and lineage commitment (Abdelalim *et al*., 2014; Lefebvre and Bhattaram, 2016; Lefebvre, 2019). For instance, high expression of SOXC TFs in osteodermal progenitors causes skull and skin underdevelopment (Angelozzi *et al*., 2022). Recent single-cell genomic studies revealed that SOX TFs orchestrate cell fate decisions during sensorineural regeneration in zebrafish (Jimenez *et al*., 2022). Combinatorial activity between SOX and SIX TFs underlies this regulatory network. During neuronal and glial differentiation, sequential activity of SOX TFs mediates the specification of neural lineages (Stevanovic *et al*.,

2021). A recent study suggested that SOX11 is a novel regulator of odontoblast differentiation (Inoue *et al*., 2022). The regulatory mode of SOX TFs is achieved by forming functional interactions with other TFs and chromatin remodeling and epigenetic complexes (Weider and Wegner, 2017). In this regard, RUNX2 and LEF1 are essential components of gene regulatory cross-wirings during bone formation and homeostasis (Rodríguez-Carballo *et al*., 2011). It is well established that the interaction of RUNX2 with LEF1 triggers the canonical WNT signaling pathway during skeletal development (Reinhold and Naski, 2007). In our analysis, *Runx2* and *Lef1* were highly enriched in cluster 4, which represents MSCs undergoing osteoblastic differentiation. Our STRING interaction network analysis uncovered that both TFs interact with CREBBP, CEBPB, TLE1, TWIST1, and members of the histone deacetylases (HDAC) and SMAD families. Genes encoding these regulatory proteins were co-expressed with *Runx2* and *Lef1* in cluster 4. *Crebbp* encodes the CBP co-activator of the CBP/p300 histone acetyltransferase family, which plays an important role in MSC osteogenic and chondrogenic differentiation (Ma *et al*., 2022).

CCAAT/enhancer binding protein beta (C/EBPβ), encoded by *Cebpb*, is a critical regulator of both adipocyte and osteoblast differentiation (Motyl *et al*., 2011). The transcriptional corepressor transducin-like enhancer-1 (TLE1), a product of the *Tle1* gene, functions with RUNX2 in the epigenetic suppression of ribosomal RNA genes (Ali *et al*., 2010). An interaction between TWIST1 and RUNX2 coordinates craniofacial muscle development (Han *et al*., 2021). Studies have shown that both the canonical and non-canonical SMAD-dependent signaling pathways control the proliferation and differentiation of dental MSCs as well as osteogenesis and skeletal development (Liu *et al*., 2022; Wu *et al*., 2016). Histone deacetylation via HDACs is an important function of the multiprotein epigenetic complexes that interact with many TFs, including RUNX2 and LEF1, during skeletal development (Jensen *et al*., 2010; Huynh *et al*., 2017).

Coordinated crosstalk among the bone morphogenic protein (BMP), fibroblast growth factor (FGF) and WNT signaling pathways from the dental epithelium to the mesenchyme underlies the regulation of the odontogenic regulatory network during early tooth development (Chen *et al*., 2009). BMP signaling utilizes the canonical and non-canonical SMAD signaling pathways, which engage different TFs including RUNX2 and SP7 to initiate the differentiation of MSCs along the odontoblastic lineage (Liu *et al*., 2022).

RUNX2 is an essential TF in osteogenic differentiation and early tooth development (Wen *et al*., 2020). In committed odontoblasts, RUNX2 is required for bone matrix protein gene expression (Qin *et al*., 2021). Depletion of the *Runx2* gene in odontoblasts leads to shorter and irregularly aligned incisors. Wen *et al.,* showed that *Runx2* plays a crucial role in tooth root development and the differentiation of root progenitor cells. Ablation of *Runx2* results in upregulation of canonical WNT signaling and interference of odontoblast differentiation (Wen *et al*., 2020). At the late stages of odontoblast differentiation and dentin formation, RUNX2 attenuates terminal differentiation of odontoblasts while enhancing differentiation of odontoblasts to osteoblasts (Li *et al*., 2011). Interestingly, SOX9 directly interacts with RUNX2 and inhibits its activity during skeletogenesis (Zhou *et al*., 2006).

It is well accepted that TFs exert their function by interacting with epigenetic enzymes, coactivators and chromatin remodeling complexes (Dubois-Chevalier *et al*., 2015; Hernandez-Hernandez *et al*., 2020). In eukaryotes, TFs form large regulatory domains known as super-enhancers that define cell identity by expressing cell-type-specific genes (Whyte *et al*., 2013). Although we know very little about the contribution of the SOX family to dentin formation, the modular nature of TFs in forming gene regulatory networks suggests that crosstalk between SOX factors and bone-associated TFs could underlie the odontoblast-specific regulatory program. For example, SOX5, SOX6, and SOX9 cooperate genome-wide through super-enhancers to regulate chondrogenesis (Liu and Lefebvre, 2015). During skeletal development, SOX9 cooperates with RUNX2 and SP7 to coordinate cell-fate specification of osteoblasts and chondrocytes (Ohba, 2021).

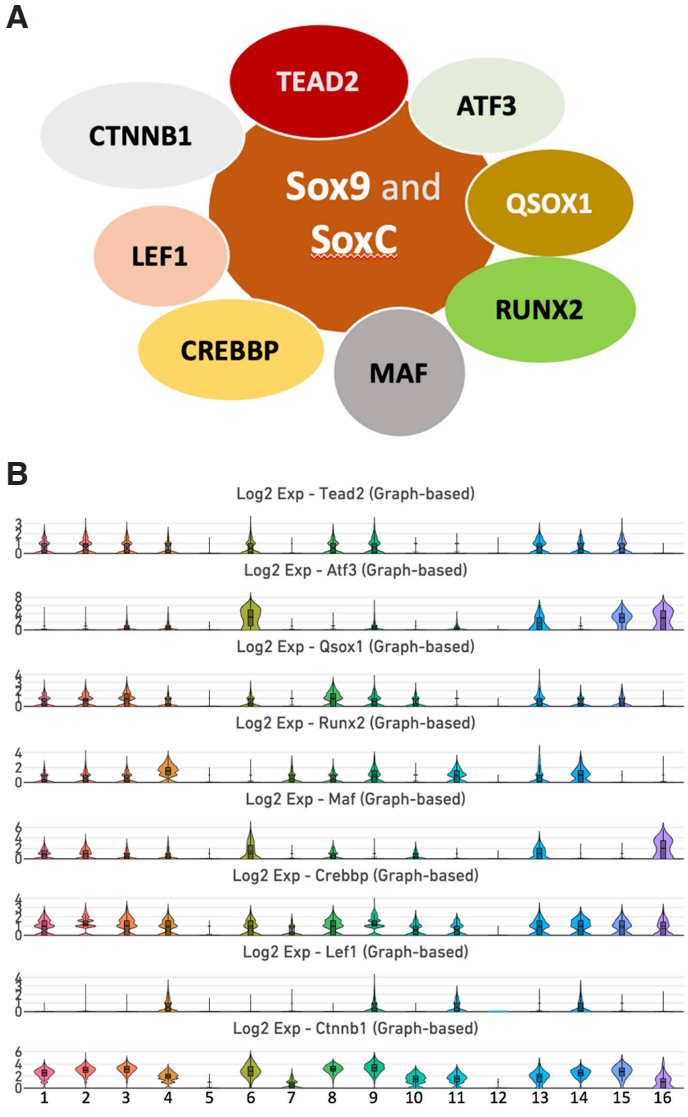

Fig. 4. Interaction network of SOX9 and SOXC and single-cell transcriptome clustering analysis. (A) STRING interaction network analysis revealed that SOX factors associate with TEAD2, ATF3, QSOX1, RUNX2, MAF, CREBBP, LEF1, and CTNNB1. **(B)** Violin plots revealed that *Tead2*, *Qsox1*, *Runx2*, *Crebbp*, and *Ctnnb1* co-express with *Sox9* and *SoxC* group in the MSC clusters 1, 2, 3, 4, 8, and 9. The expression of *Atf3* was limited to clusters 3, 4, and 9, whereas *Maf* exhibited wide expression in clusters 1, 2, 3, 4, 7, 8, and 9. *Lef1* was enriched in clusters 4 and 9.

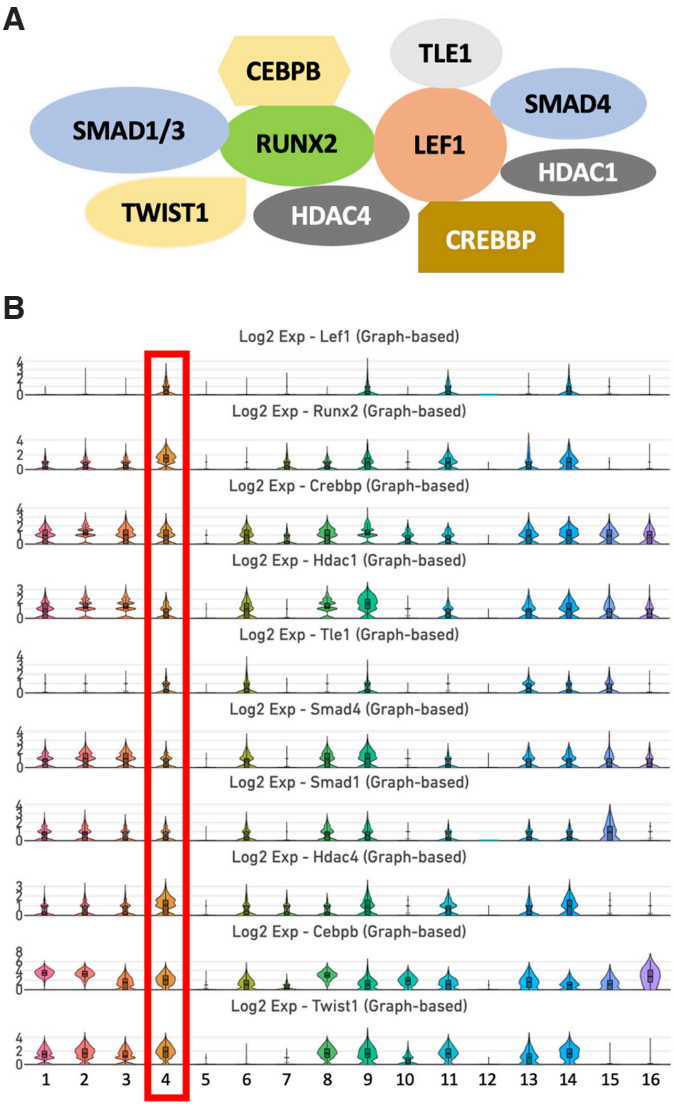

Fig. 5. Interaction network of RUNX2 and LEF1 and single-cell transcriptome clustering analysis. (A) STRING interaction network analysis revealed that RUNX2 and LEF1 associate with TLE1, SMAD4, CREBBP, TWIST1, CEBPB, and members of the HDAC and SMAD families. **(B)** Violin plots showed that *Crebbp*, *Hdac1*, *Hdac4*, *Tle1*, *Smad1*, *Smad4*, *Cebpb*, and *Twist1* co-express with *Runx2* and *Lef1* in cluster 4, which represents MSCs of the osteoblastic lineage.

In summary, we determined distinct cell-specific expression patterns of *Sox* genes in the mouse incisor dental pulp. A few members of the *Sox* family colocalize with key regulatory genes encoding TFs, epigenetic enzymes, and signaling molecules that play significant roles during skeletal and dental development.

Materials and Methods

Single-cell RNA-seq, genome-wide ATAC-seq, hMeDIP-seq, and bulk RNA-seq

Primary pulp isolation and viability of each single-cell suspension was performed as previously described (Bayarsaihan *et al*., 2022; Enkhmandakh *et al*., 2022). Suspensions of dissociated cells were loaded for capture using a Chromium Single Cell 3' Reagent kit, v2 Chemistry (10× Genomics). Following capture and lysis, complementary DNA was synthesized and amplified (14 cycles) as per the 10×Genomics protocol. The amplified cDNA was used to construct an Illumina sequencing library and was sequenced on a single lane of a HiSeq 4000 (Illumina, San Diego, CA). ATAC-seq, hMeDIP-seq, and bulk RNA-seq services were performed as previously described (Joshi *et al*., 2022a; Joshi *et al*., 2022b).

Computational analysis

For single-cell RNA-seq, we excluded reads with more than one mismatch in the 8-bp i7 index. Using the STAR aligner, we retained only reads with MAPQ scores greater than 255. We excluded reads containing bases with Q30 scores below 3. After alignment, cell barcodes were filtered (up to one mismatch) against a whitelist of 737,500 barcodes provided by 10×Genomics. Cell-associated barcodes were distinguished from those associated with ambient mRNA using an adaptively computed unique molecular identifier threshold. After this filtering step, a digital counts matrix for the pulp sample was generated. The uniform manifold approximation and projection (UMAP), batch correction software BBKNN and FASTQ generation, MACS2 peak-calling algorithm, STAR aligner, and feature counts (FPKM assignment to genes) were performed as previously described (Bayarsaihan *et al*., 2022; Enkhmandakh *et al*., 2022).

Acknowledgments
This project was funded by the grant REP-401754-20144-20 from the University of Connecticut to DB. The data that support the findings of this study are available on request.

Author contributions
DB: conceptualization, funding acquisition and supervision. BE: methodology. DB and BE: data analysis and writing. All authors gave final approval and agreed to be accountable for all aspects of the work.

References

ABDELALIM E. M., EMARA M. M., KOLATKAR P. R. (2014). The SOX Transcription Factors as Key Players in Pluripotent Stem Cells. *Stem Cells and Development* 23: 2687-2699. https://doi.org/10.1089/scd.2014.0297

ALI S. A., ZAIDI S. K., DOBSON J. R., SHAKOORI A. R., LIAN J. B., STEIN J. L., VAN WIJNEN A. J., STEIN G. S. (2010). Transcriptional corepressor TLE1 functions with Runx2 in epigenetic repression of ribosomal RNA genes. *Proceedings of the National Academy of Sciences* 107: 4165-4169. https://doi.org/10.1073/pnas.1000620107

ANGELOZZI M., LEFEBVRE V. (2019). SOXopathies: Growing Family of Developmental Disorders Due to SOX Mutations. *Trends in Genetics* 35: 658-671. https://doi.org/10.1016/j.tig.2019.06.003

ANGELOZZI M., PELLEGRINO DA SILVA R., GONZALEZ M. V., LEFEBVRE V. (2022). Single-cell atlas of craniogenesis uncovers SOXC-dependent, highly proliferative, and myofibroblast-like osteodermal progenitors. *Cell Reports* 40: 111045. https://doi.org/10.1016/j.celrep.2022.111045

BAYARSAIHAN D., ENKHMANDAKH B., VIJAYKUMAR A., ROBSON P., MINA M. (2022). Single-cell transcriptome analysis defines mesenchymal stromal cells in the mouse incisor dental pulp. *Gene Expression Patterns* 43: 119228. https://doi.org/10.1016/j.gep.2021.119228

DUBOIS-CHEVALIER J., STAELS B., LEFEBVRE P., EECKHOUTE J. (2015). The ubiquitous transcription factor CTCF promotes lineage-specific epigenomic remodeling and establishment of transcriptional networks driving cell differentiation. *Nucleus* 6: 15-18. https://doi.org/10.1080/19491034.2015.1004258

ENKHMANDAKH B., ROBSON P., JOSHI P., VIJAYKUMAR A., SHIN D.G., MINA M., BAYARSAIHAN D. (2022). Single-Cell Transcriptome Analysis Defines Expression of Kabuki Syndrome-Associated KMT2D Targets and Interacting Partners. *Stem Cells International* 2022: 1-9. https://doi.org/10.1155/2022/4969441

FUGLERUD B. M., DRISSLER S., LOTTO J., STEPHAN T. L., THAKUR A., CULLUM R., HOODLESS P. A. (2022). SOX9 reprograms endothelial cells by altering the chromatin landscape. *Nucleic Acids Research* 50: 8547-8565. https://doi.org/10.1093/nar/gkac652

CHEN J., LAN Y., BAEK J.A., GAO Y., JIANG R. (2009). Wnt/beta-catenin signaling plays an essential role in activation of odontogenic mesenchyme during early tooth development. *Developmental Biology* 334: 174-185. https://doi.org/10.1016/j.ydbio.2009.07.015

HAN X., FENG J., GUO T., LOH Y.H. E., YUAN Y., HO T.V., CHO C. K., LI J., JING J., JANECKOVA E., HE J., PEI F., BI J., SONG B., CHAI Y. (2022). Runx2-Twist1 interaction coordinates cranial neural crest guidance of soft palate myogenesis. *eLife* 10: e62387. https://doi.org/10.7554/eLife.62387

HERNÁNDEZ-HERNÁNDEZ O., ÁVILA-AVILÉS R. D., HERNÁNDEZ-HERNÁNDEZ J. M. (2020). Chromatin Landscape During Skeletal Muscle Differentiation. *Frontiers in Genetics* 11: 578712. https://doi.org/10.3389/fgene.2020.578712

HUYNH N. C.N., EVERTS V., AMPORNARAMVETH R. S. (2017). Histone deacetylases and their roles in mineralized tissue regeneration. *Bone Reports* 7: 33-40. https://doi.org/10.1016/j.bonr.2017.08.001

INOUE K., MATSUZAKA K., INOUE T. (2022). Identification of Novel Regulator Involved in Differentiation of Mouse iPS Cells into Odontoblast-like Cells. *The Bulletin of Tokyo Dental College* 63: 119-128. https://doi.org/10.2209/tdcpublication.2021-0045

JENSEN E. D., GOPALAKRISHNAN R., WESTENDORF J. J. (2010). Regulation of gene expression in osteoblasts. *BioFactors* 36: 25-32. https://doi.org/10.1002/biof.72

JIMENEZ E., SLEVIN C. C., SONG W., CHEN Z., FREDERICKSON S. C., GILDEA D., WU W., ELKAHLOUN A. G., OVCHARENKO I., BURGESS S. M. (2022). A regulatory network of Sox and Six transcription factors initiate a cell fate transformation during hearing regeneration in adult zebrafish. *Cell Genomics* 2: 100170. https://doi.org/10.1016/j.xgen.2022.100170

JOSHI P., VIJAYKUMAR A., ENKHMANDAKH B., MINA M., SHIN D.G., BAYARSAIHAN D. (2022a). Genome-wide distribution of 5hmC in the dental pulp of mouse molars and incisors. *The Journal of Biochemistry* 171: 123-129. https://doi.org/10.1093/jb/mvab114

JOSHI P., VIJAYKUMAR A., ENKHMANDAKH B., SHIN D.G., MINA M., BAYARSAIHAN D. (2022b). The chromatin accessibility landscape in the dental pulp of mouse molars and incisors. *Acta Biochimica Polonica* 69: 131-138. https://doi.org/10.18388/abp.2020_5771

LEFEBVRE V., BHATTARAM P. (2016). SOXC Genes and the Control of Skeletogenesis. *Current Osteoporosis Reports* 14: 32-38. https://doi.org/10.1007/s11914-016-0296-1

LEFEBVRE V. (2019). Roles and regulation of SOX transcription factors in skeletogenesis. In *Vertebrate Skeletal Development*. Current Topics in Developmental Biology, Vol. 133. Elsevier, , pp. 171-193. https://doi.org/10.1016/bs.ctdb.2019.01.007

LI S., KONG H., YAO N., YU Q., WANG P., LIN Y., WANG J., KUANG R., ZHAO X., XU J., ZHU Q., NI L. (2011). The role of runt-related transcription factor 2 (Runx2) in the late stage of odontoblast differentiation and dentin formation. *Biochemical and Biophysical Research Communications* 410: 698-704. https://doi.org/10.1016/j.bbrc.2011.06.065

LIU C.F., LEFEBVRE V. (2015). The transcription factors SOX9 and SOX5/SOX6 cooperate genome-wide through super-enhancers to drive chondrogenesis. *Nucleic Acids Research* 43: 8183-8203. https://doi.org/10.1093/nar/gkv688

LIU M., GOLDMAN G., MACDOUGALL M., CHEN S. (2022). BMP Signaling Pathway in Dentin Development and Diseases. *Cells* 11: 2216. https://doi.org/10.3390/cells11142216

LIU Y., GUO W. (2021). SOX factors as cell-state regulators in the mammary gland and breast cancer. *Seminars in Cell & Developmental Biology* 114: 126-133. https://doi.org/10.1016/j.semcdb.2021.01.002

MA Q., SONG C., YIN B., SHI Y., YE L. (2022). The role of Trithorax family regulating osteogenic and Chondrogenic differentiation in mesenchymal stem cells. *Cell Proliferation* 55: e13233. https://doi.org/10.1111/cpr.13233

MING Z., VINING B., BAGHERI-FAM S., HARLEY V. (2022). SOX9 in organogenesis: shared and unique transcriptional functions. *Cellular and Molecular Life Sciences* 79: 522. https://doi.org/10.1007/s00018-022-04543-4

MOTYL K. J., RAETZ M., TEKALUR S. A., SCHWARTZ R. C., MCCABE L. R. (2011). CCAAT/enhancer binding protein β-deficiency enhances type 1 diabetic bone phenotype by increasing marrow adiposity and bone resorption. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 300: R1250-R1260. https://doi.org/10.1152/ajpregu.00764.2010

MUKHERJEE S., LUEDEKE D. M., MCCOY L., IWAFUCHI M., ZORN A. M. (2022). SOX transcription factors direct TCF-independent WNT/β-catenin responsive transcription to govern cell fate in human pluripotent stem cells. *Cell Reports* 40: 111247. https://doi.org/10.1016/j.celrep.2022.111247

OHBA S. (2021). Genome-scale actions of master regulators directing skeletal development. *Japanese Dental Science Review* 57: 217-223. https://doi.org/10.1016/j.jdsr.2021.10.001

PINGAULT V., ZERAD L., BERTANI-TORRES W., BONDURAND N. (2022). SOX10: 20 years of phenotypic plurality and current understanding of its developmental function. *Journal of Medical Genetics* 59: 105-114. https://doi.org/10.1136/jmedgenet-2021-108105

KAWASAKI K., KAWASAKI M., WATANABE M., IDRUS E., NAGAI T., OOMMEN S., MAEDA T., HAGIWARA N., QUE J., SHARPE P. T., OHAZAMA A. (2015). Expression of Sox genes in tooth development. *The International Journal of Developmental Biology* 59: 471-478. https://doi.org/10.1387/ijdb.150192ao

KLUM S., ZAOUTER C., ALEKSEENKO Z., BJÖRKLUND K., HAGEY D. W., ERICSON J., MUHR J., BERGSLAND M. (2018). Sequentially acting SOX proteins orchestrate astrocyte- and oligodendrocyte-specific gene expression. *EMBO reports* 19: e46635. https://doi.org/10.15252/embr.201846635

QIN X., JIANG Q., KOMORI H., SAKANE C., FUKUYAMA R., MATSUO Y., ITO K., MIYAZAKI T., KOMORI T. (2021). Runt-related transcription factor-2 (Runx2) is required for bone matrix protein gene expression in committed osteoblasts in mice . *Journal of Bone and Mineral Research* 36: 2081-2095. https://doi.org/10.1002/jbmr.4386

REINHOLD M. I., NASKI M. C. (2007). Direct Interactions of Runx2 and Canonical Wnt Signaling Induce FGF18. *Journal of Biological Chemistry* 282: 3653-3663. https://doi.org/10.1074/jbc.M608995200

REZNIK N., FASS D. (2022). Disulfide bond formation and redox regulation in the Golgi apparatus. *FEBS Letters* 596: 2859-2872. https://doi.org/10.1002/1873-3468.14510

RODRÍGUEZ-CARBALLO E., ULSAMER A., SUSPERREGUI A. R.G., MANZANARES-CÉSPEDES C., SÁNCHEZ-GARCÍA E., BARTRONS R., ROSA J. L., VENTURA F. (2011). Conserved regulatory motifs in osteogenic gene promoters integrate cooperative effects of canonical Wnt and BMP pathways. *Journal of Bone and Mineral Research* 26: 718-729. https://doi.org/10.1002/jbmr.260

SCHOCK E. N., LABONNE C. (2020). Sorting Sox: Diverse Roles for Sox Transcription Factors During Neural Crest and Craniofacial Development. *Frontiers in Physiology* 11: 606889. https://doi.org/10.3389/fphys.2020.606889

STEGER J., COLE A. G., DENNER A., LEBEDEVA T., GENIKHOVICH G., RIES A., REISCHL R., TAUDES E., LASSNIG M., TECHNAU U. (2022). Single-cell transcriptomics identifies conserved regulators of neuroglandular lineages. *Cell Reports* 40: 111370. https://doi.org/10.1016/j.celrep.2022.111370

STEVANOVIC M., DRAKULIC D., LAZIC A., NINKOVIC D. S., SCHWIRTLICH M., MOJSIN M. (2021). SOX Transcription Factors as Important Regulators of Neuronal and Glial Differentiation During Nervous System Development and Adult Neurogenesis. *Frontiers in Molecular Neuroscience* 14: 654031. https://doi.org/10.3389/fnmol.2021.654031

WEIDER M., WEGNER M. (2017). SoxE factors: Transcriptional regulators of neural differentiation and nervous system development. *Seminars in Cell & Developmental Biology* 63: 35-42. https://doi.org/10.1016/j.semcdb.2016.08.013

WEN Q., JING J., HAN X., FENG J., YUAN Y., MA Y., CHEN S., HO T.V., CHAI Y. (2020). Runx2 Regulates Mouse Tooth Root Development Via Activation of WNT Inhibitor NOTUM . *Journal of Bone and Mineral Research* 35: 2252-2264. https://doi.org/10.1002/jbmr.4120

WHYTE W. A., ORLANDO D. A., HNISZ D., ABRAHAM B. J., LIN C. Y., KAGEY M. H., RAHL P. B., LEE T. I., YOUNG R. A. (2013). Master Transcription Factors and Mediator Establish Super-Enhancers at Key Cell Identity Genes. *Cell* 153: 307-319. https://doi.org/10.1016/j.cell.2013.03.035

WU M., CHEN G., LI Y.P. (2016). TGF-β and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. *Bone Research* 4: 16009. https://doi.org/10.1038/boneres.2016.9

ZHOU G., ZHENG Q., ENGIN F., MUNIVEZ E., CHEN Y., SEBALD E., KRAKOW D., LEE B. (2006). Dominance of SOX9 function over RUNX2 during skeletogenesis. *Proceedings of the National Academy of Sciences* 103: 19004-19009. https://doi.org/10.1073/pnas.0605170103