

# **Insights into the molecular characteristics of embryonic cranial neural crest cells and their derived mesenchymal cell pools**

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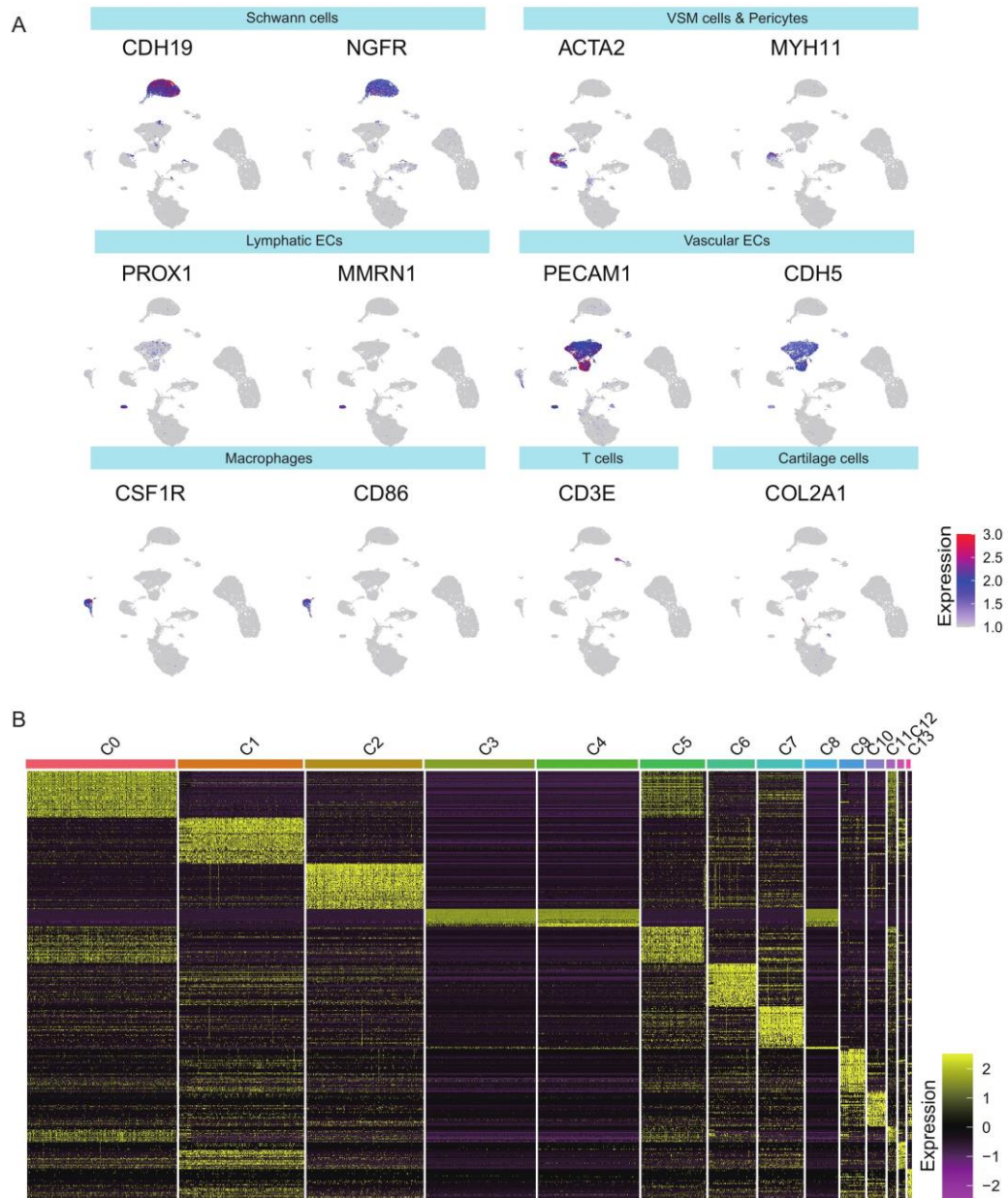
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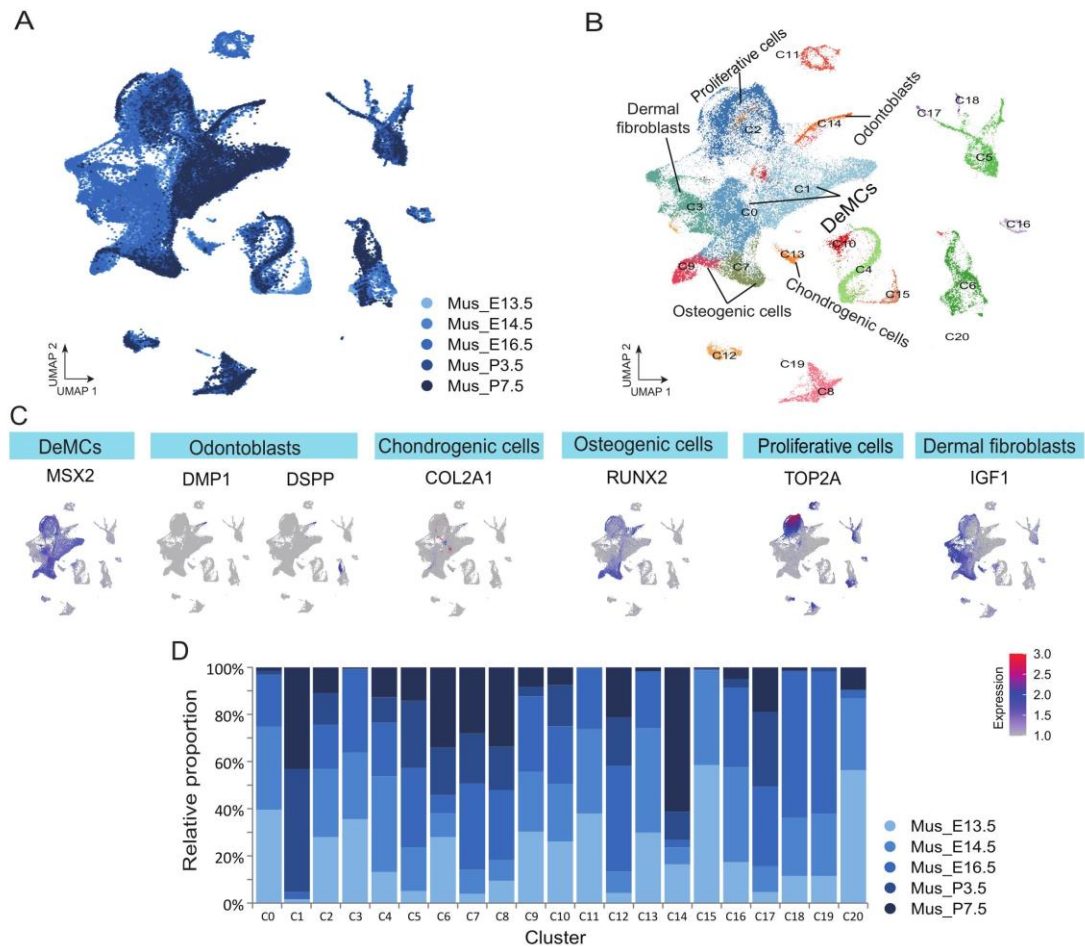
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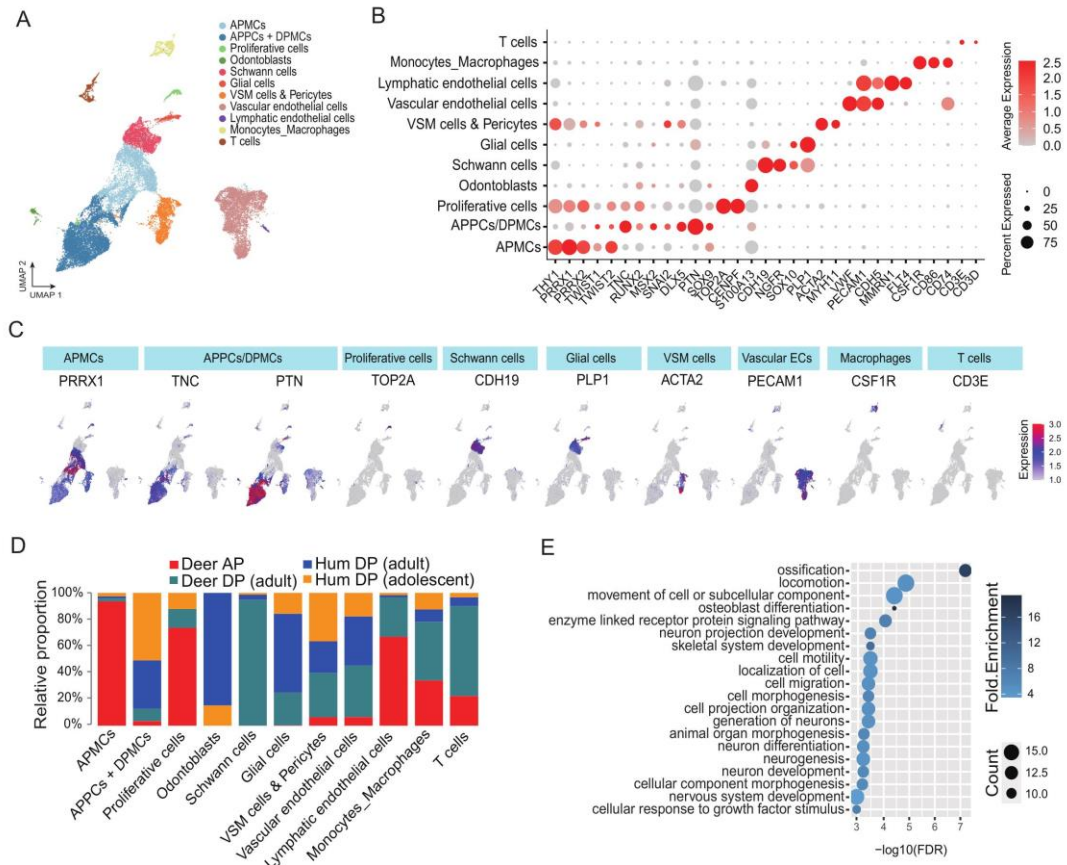
**Figure S1 Integrated analysis of scRNA-seq from mouse E8.5 Wnt1-traced NT/NC, deer AP and DP**

- A. UMAP plots to visualize the expression levels of marker genes in the mouse E8.5 Wnt1-traced NT/NC, AP and DP.
- B. Heat map plot to illustrate the expression profiles of the top 50 highly expressed genes in all clusters of the mouse E8.5 Wnt1-traced NT/NC, AP and DP. These clusters are color-coded in the top bar.



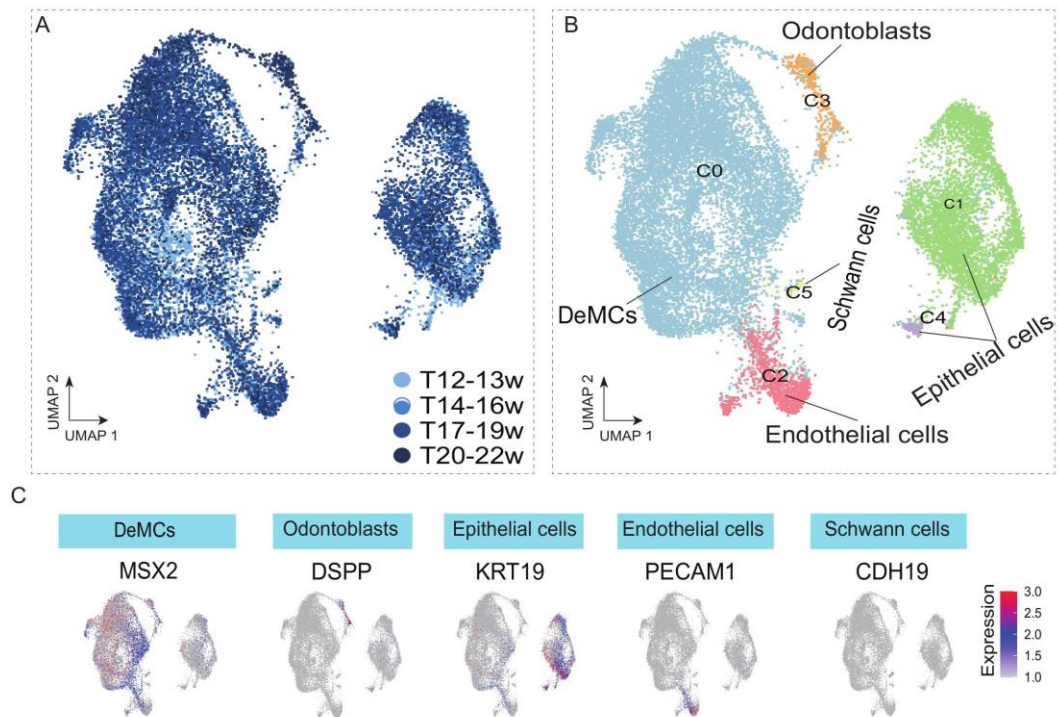
**Figure S2 Analysis of scRNA-seq from mouse molar tissues at various developmental stages, including tooth germ stages E13.5, E14.5, E16.5, postnatal stages P3.5 and P7.5, as well as deer AP**

- A. UMAP plot to visualize all cells from mouse teeth tissues at various developmental stages; developmental stages were color-coded.
- B. UMAP plot to visualize unsupervised clusters along with the identification of key cell types in mouse teeth tissues at various developmental stages.
- C. UMAP plot to visualize the expression levels of marker genes for key cell types.
- D. Bar plot giving the relative proportions of unsupervised clusters in mouse teeth tissues at various developmental stages.



**Figure S3 Analysis of scRNA-seq from deer AP, deer and adult and growing human molar dental pulps**

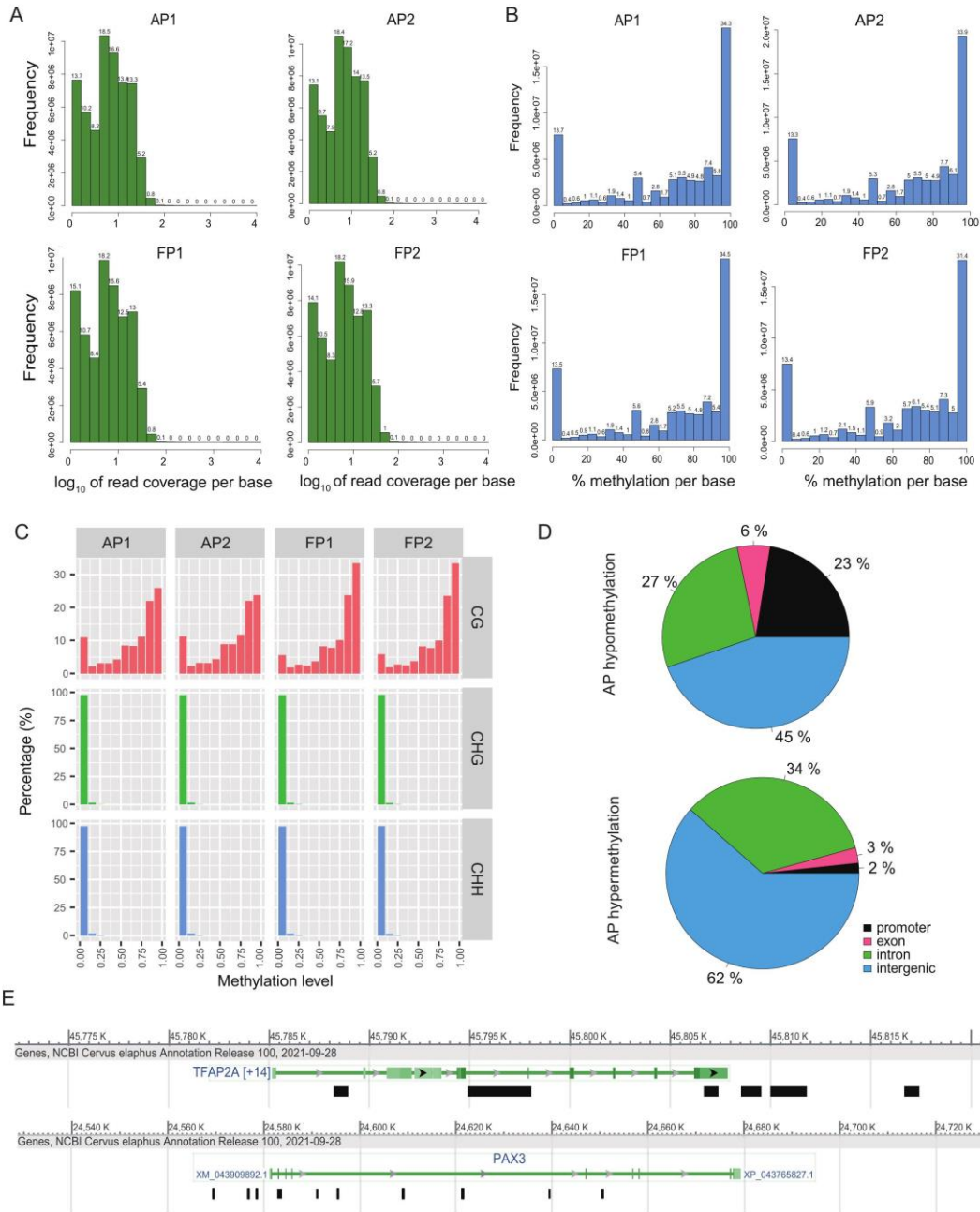
- A. UMAP plot to visualize all cells from deer AP, deer and human (adult and growing) molar dental pulp, cell types were color-coded.
- B. Dot plot of the expression levels of marker genes for the different cell types.
- C. UMAP plots to visualize the expression levels of a subset of marker genes for the different cell types.
- D. Bar plot to show the relative proportions of cell types in these samples.
- E. Dot plot giving DAVID enriched Gene Ontology terms (top 10) using 35 co-expressed genes among the APPCs and deer and human DPMCs.



**Figure S4 Analysis of scRNA-seq from human fetal tooth germ.**

- A. UMAP plot to visualize all cells from human fetal tooth germ at 12-22 weeks, developmental stages were color-coded.
- B. UMAP plot to visualize unsupervised clusters along with the identification of DeMCs and odontoblasts.
- C. UMAP plots to visualize the expression levels of marker genes for DeMCs and odontoblasts.





**Figure S5 Analysis of whole genome bisulfite sequencing of AP and FP tissues**

A. Bar plot to show read coverage per base.

B. Bar plot to show the percentage of methylation per base.

C. Bar plot to show methylation levels for three types of cytosine methylation.

D. Pie chart to show the percentage of hypermethylation and hypomethylation based on  $|\text{diff. Methy}| > 0.1$  in different genetic regions (promoter, exon, intron, intergenic) in AP vs. FP. Promoters for each gene are limited to  $\pm 1000$  bp around the TSS. Note the significant proportion of hypomethylation in the promoter region in the AP.

E. Hypomethylated regions (indicated by black bars) in AP vs. FP are located within or around CNCC derivate signature genes *TFAP2A* and *PAX3*.