Figure S6

a

Stage	Sample	Dpf[a]	Tissue	Strategy	Gender	Cell Number	Doublets	Cell Number (QC[b])	Gene number	UMI number	Perc.mito[c]
8 WPC	Embryo 24	56	Calvarial bone	scRNA-seq on 7AAD⁻CD235A⁻ cells	Male	3,786	338	2,932	3,126	14,860	2.7%
	Embryo 25					6,476	116	4,355	4,330	17,899	6.5%
	Embryo 26-27	56	Calvarial bone	IF[e] staining							

[a]WPC: weeks post conception; [b]Dpf: days post fertilization; [c]QC: quality control; [d]Pec.mito: percentage of mitochondrial genes; [e]IF: immunofluorescence
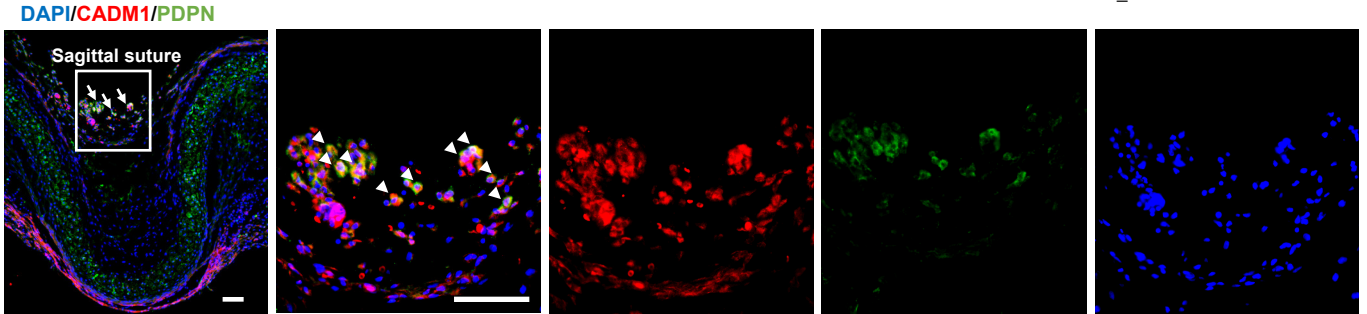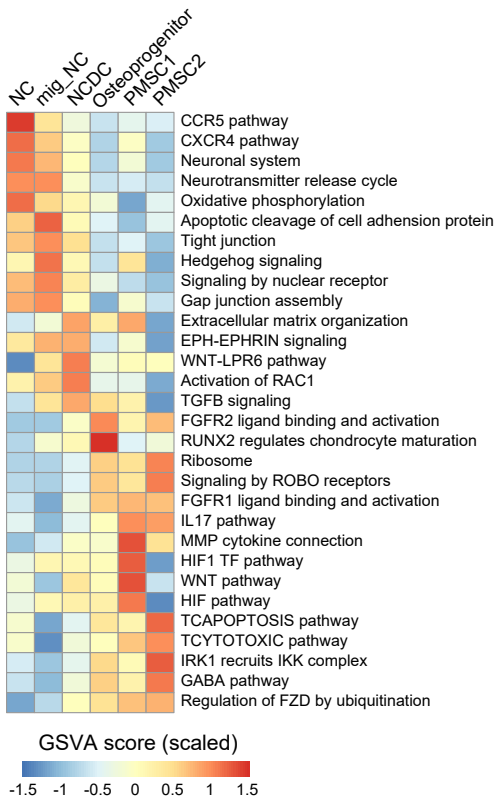
b

c

d

DAPI/CADM1/PDPN

Sagittal suture

e

GSVA score (scaled)

-1.5 -1 -0.5 0 0.5 1 1.5

f

FOXP1 targets
FOXP2 targets
FOXP4 targets
FOXP1/2 shared targets
FOXP1/4 shared targets
FOXP2/4 shared targets
FOXP1/2/4 shared targets
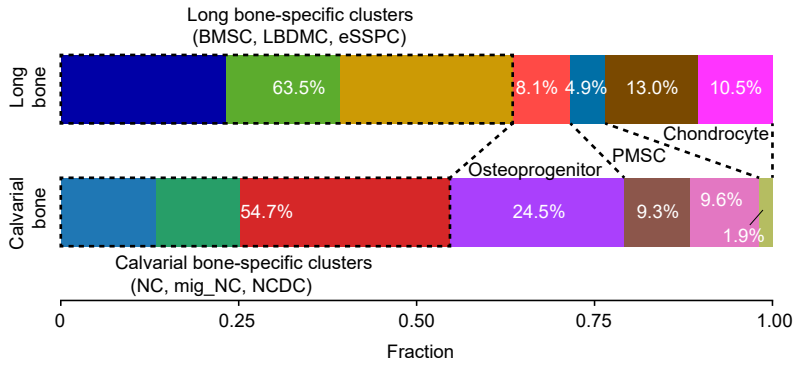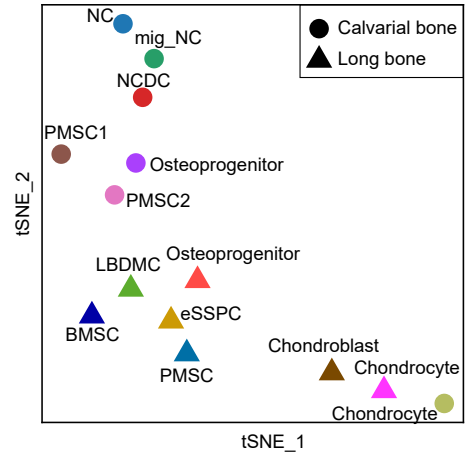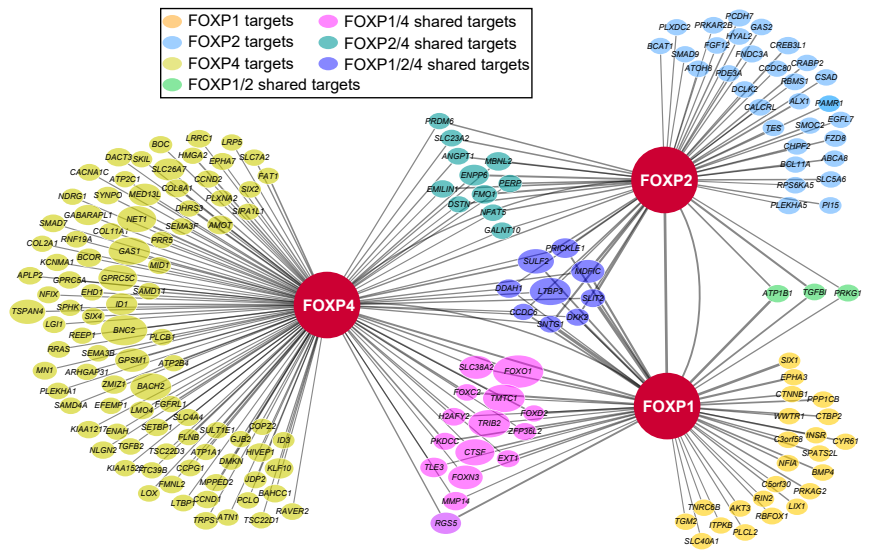
Supplementary Fig. 6. Further characterizations of human embryonic calvaria
a, Table summary of the 8 WPC human embryonic calvarial bone samples for scRNA-seq and immunostaining.
b, Stacked bar charts comparing the distribution of 8 WPC long bone and calvarial subsets. Dashed boxes indicated skeletal site-specific clusters. The three shared clusters (osteoprogenitor, PMSC and chondrocyte) were highlighted by dash lines.
c, t-distributed stochastic neighbor embedding(t-SNE) projection of indicated subsets from long bones and calvarial bones to compare the transcriptomic similarities at the pseudo-bulk level.
d, Immunofluorescent images of PDPN⁺CADM1⁺ cells in 8 WPC human calvarial bones. Snapshot image of the calvarial region surrounding sagittal suture was shown on the left. PDPN⁺CADM1⁺ cells (arrows) were found in the outer layer of sagittal mesenchyme. Arrow heads indicated enlarged PDPN⁺CADM1⁺ cells. Merged and single-channel images of DAPI (blue), CADM1 (red) and PDPN (green) were shown (n=2 embryos). Scale bars: 100 μm.
e, Heatmap showing pathways differentially enriched in calvarial bone subsets by GSVA, colored by scaled mean of GSVA scores.
f, The FOXP1/2/4 regulon network in 8 WPC human calvarial bone subsets. Line thickness indicated the level of GENIE3 weights. Dot size indicated the number of enriched TF-motif.