Supplementary Materials for

Single-cell RNA-sequencing analysis reveals the molecular mechanism of subchondral bone cell heterogeneity in the development of osteoarthritis

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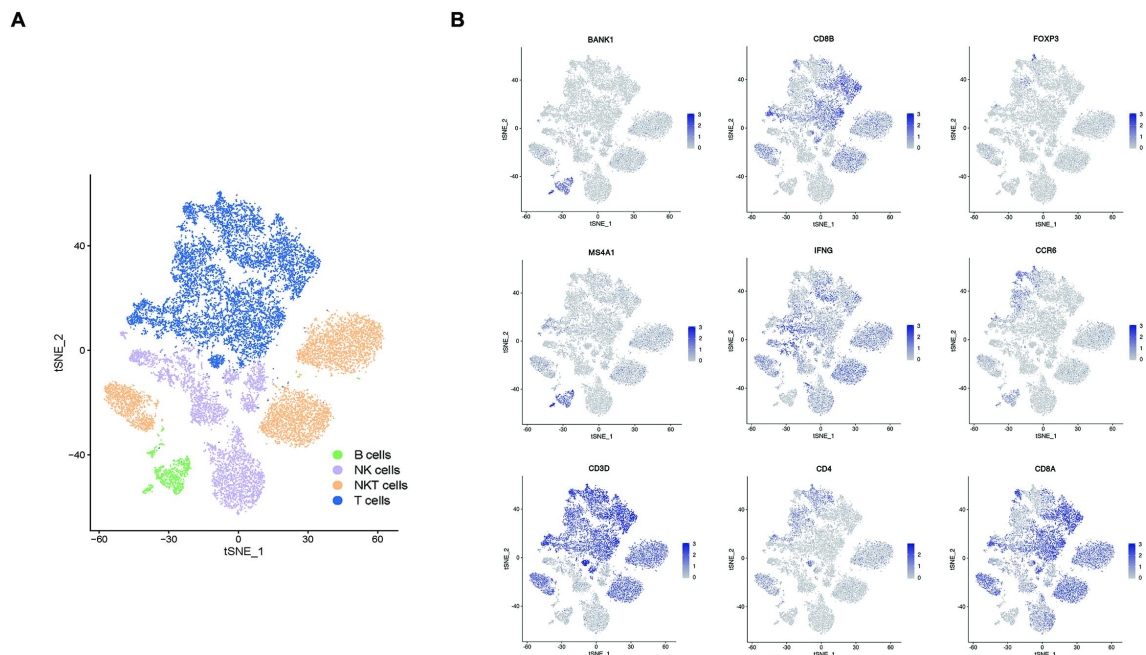
This PDF file includes:

Fig. S1. Single-cell profiling of human OA subchondral bone immune cells. (**A**) t-SNE plots of Immune cells colored by cluster. (**B**) Dot plots showing the expression of indicated markers for each cell types on the t-SNE map.
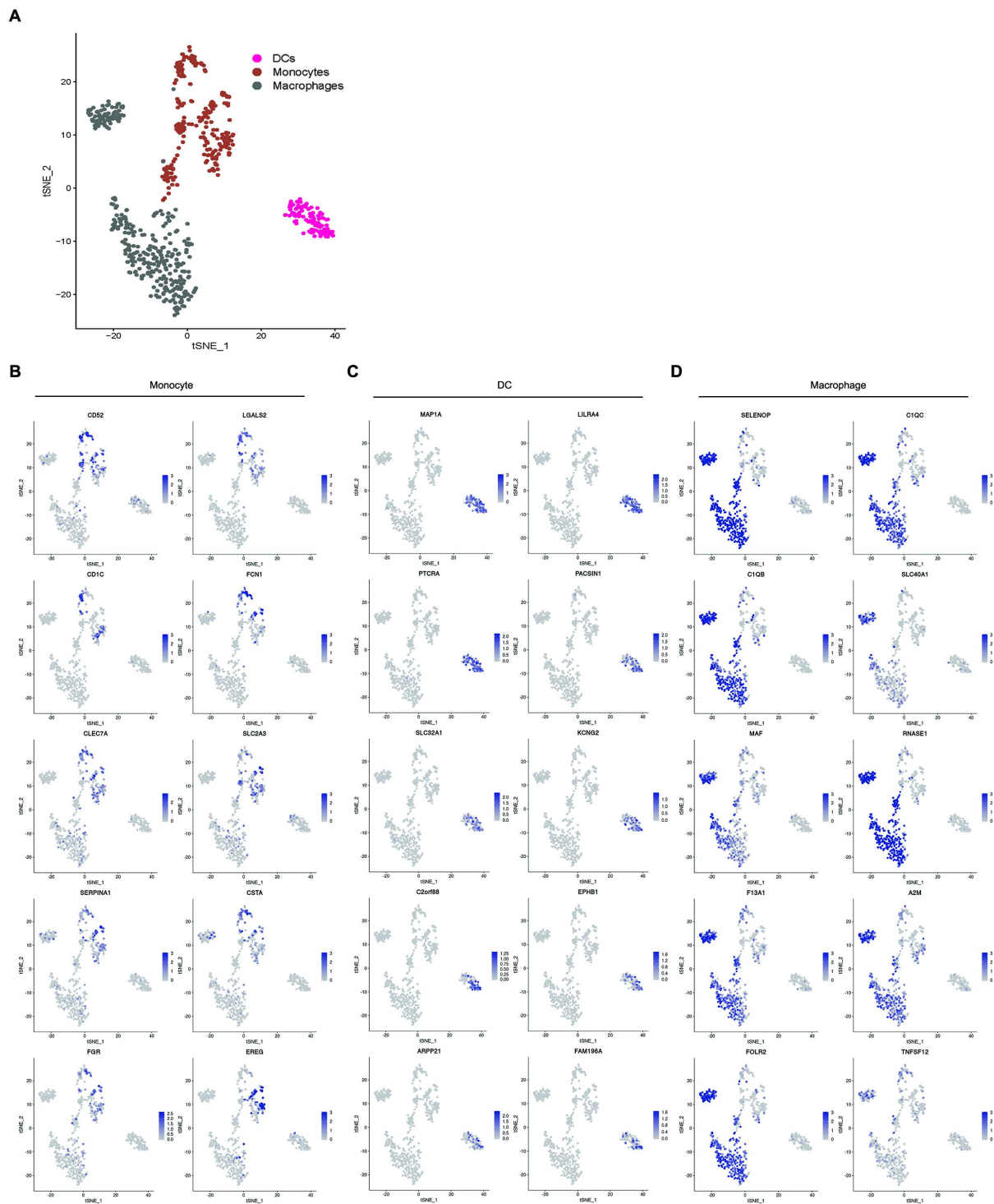
Fig. S2. Single-cell profiling of human OA subchondral bone myeloid cells. (**A**) t-SNE plots of myeloid cells colored by cluster. (**B-D**)Dot plots showing the expression of indicated markers for each cell types on the t-SNE map.
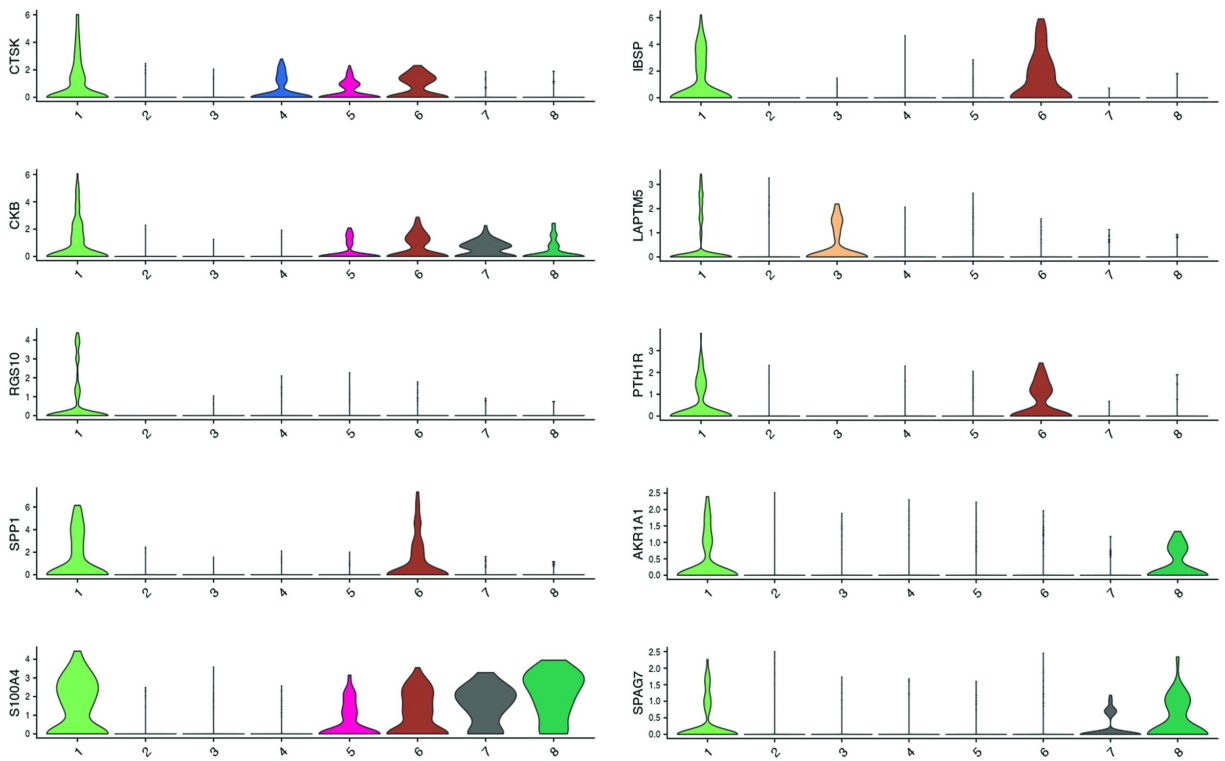
Fig. S3. Violin plots showing expression levels of indicated markers for Cluster 1
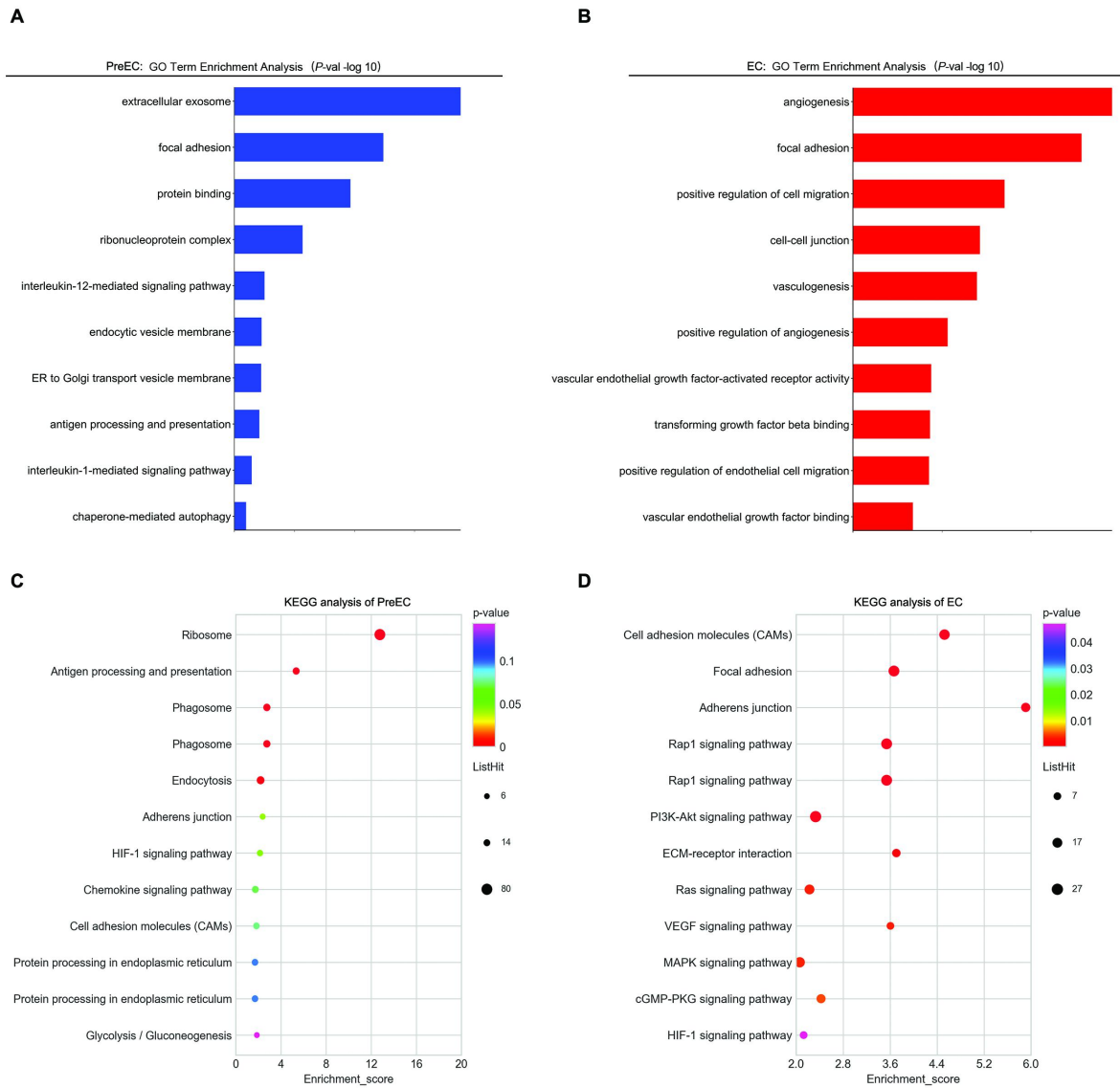
A

PreEC: GO Term Enrichment Analysis (*P*-val -log 10)

- extracellular exosome
- focal adhesion
- protein binding
- ribonucleoprotein complex
- interleukin-12-mediated signaling pathway
- endocytic vesicle membrane
- ER to Golgi transport vesicle membrane
- antigen processing and presentation
- interleukin-1-mediated signaling pathway
- chaperone-mediated autophagy

B

EC: GO Term Enrichment Analysis (*P*-val -log 10)

- angiogenesis
- focal adhesion
- positive regulation of cell migration
- cell-cell junction
- vasculogenesis
- positive regulation of angiogenesis
- vascular endothelial growth factor-activated receptor activity
- transforming growth factor beta binding
- positive regulation of endothelial cell migration
- vascular endothelial growth factor binding

C

KEGG analysis of PreEC

- Ribosome
- Antigen processing and presentation
- Phagosome
- Phagosome
- Endocytosis
- Adherens junction
- HIF-1 signaling pathway
- Chemokine signaling pathway
- Cell adhesion molecules (CAMs)
- Protein processing in endoplasmic reticulum
- Protein processing in endoplasmic reticulum
- Glycolysis / Gluconeogenesis

p-value
0.1
0.05
0

ListHit
6
14
80

Enrichment_score
0 4 8 12 16 20

D

KEGG analysis of EC

- Cell adhesion molecules (CAMs)
- Focal adhesion
- Adherens junction
- Rap1 signaling pathway
- Rap1 signaling pathway
- PI3K-Akt signaling pathway
- ECM-receptor interaction
- Ras signaling pathway
- VEGF signaling pathway
- MAPK signaling pathway
- cGMP-PKG signaling pathway
- HIF-1 signaling pathway

p-value
0.04
0.03
0.02
0.01

ListHit
7
17
27

Enrichment_score
2.0 2.8 3.6 4.4 5.2 6.0

Fig. S4. Identification of Pre-ECs and ECs. (**A**)Enriched GO functions of upregulated genes in Pre-ECs. (**B**)Enriched GO functions of upregulated genes in ECs. (**C**)Enrichment plot from KEGG pathway analysis for Pre-ECs. (**D**)Enrichment plot from KEGG pathway analysis for ECs. Type or paste caption here. Create a page break and paste in the Figure above the caption.
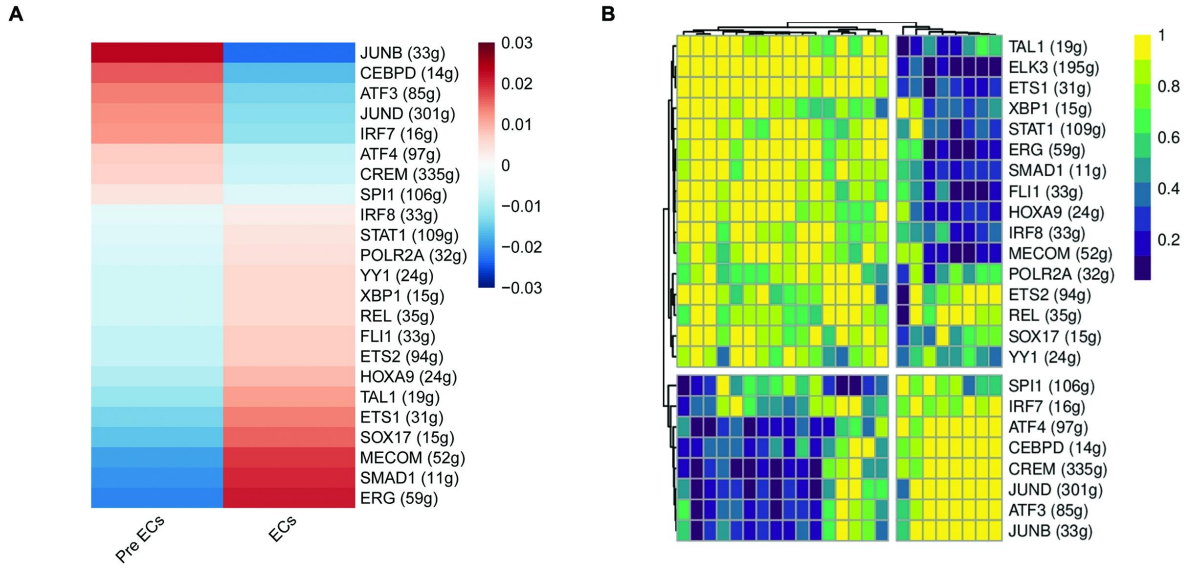
Fig. S5.The difference between Pre-ECs and ECs in transcriptional regulation. Single-cell regulatory network inference and clustering (SCENIC) analysis showing distinct regulons between ECs and Pre-ECs.The heatmap listing only the regulons with significant differences.
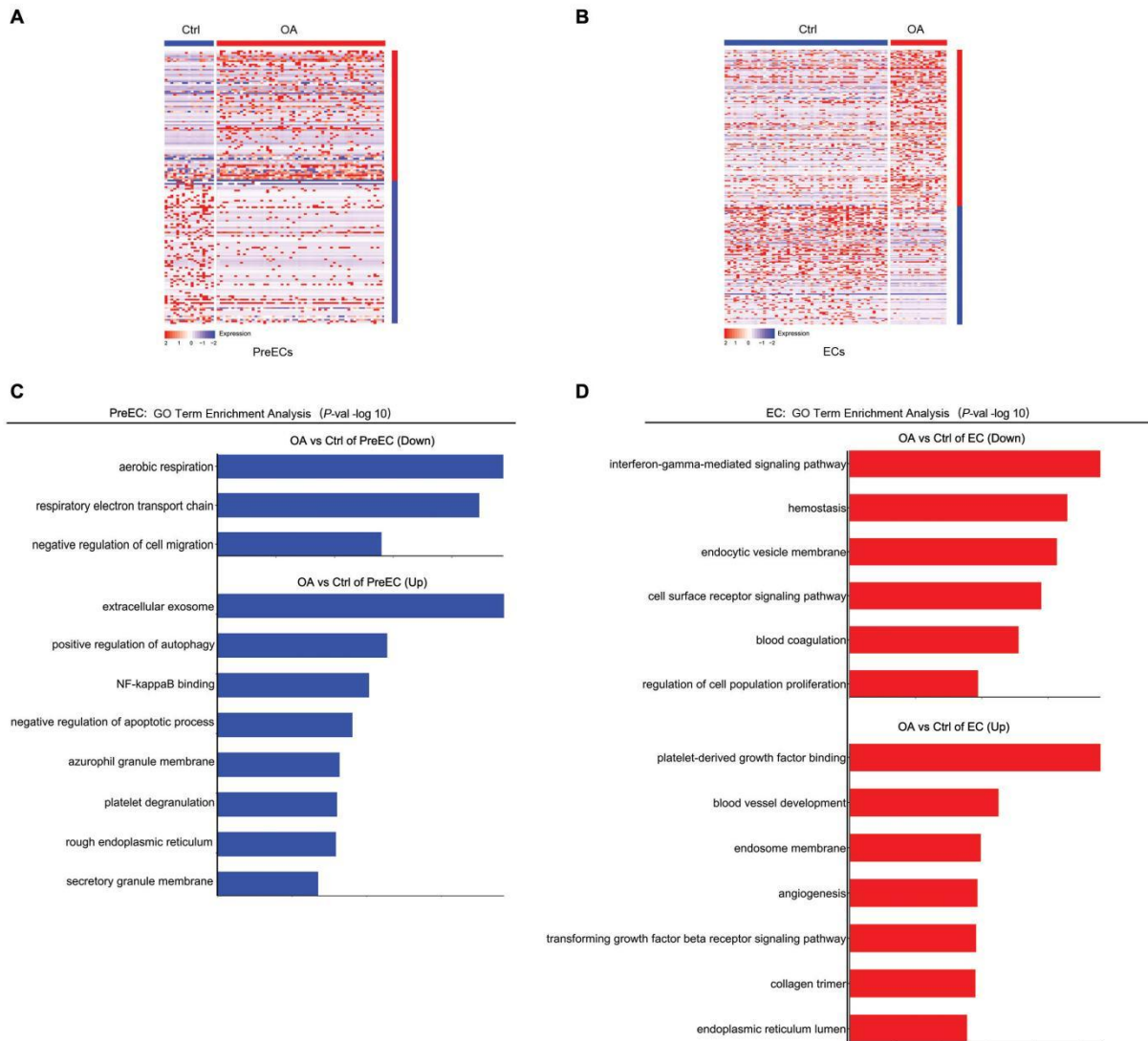
Fig. S6. Identification of OA group and Ctrl group in endothelial cells. (**A**)Heatmap of DEGs between OA group and Ctrl group in Pre-ECs and ECs. (**B**)GO functions enrichment analysis of OA versus ctrl upregulated genes in Pre-ECs and ECs
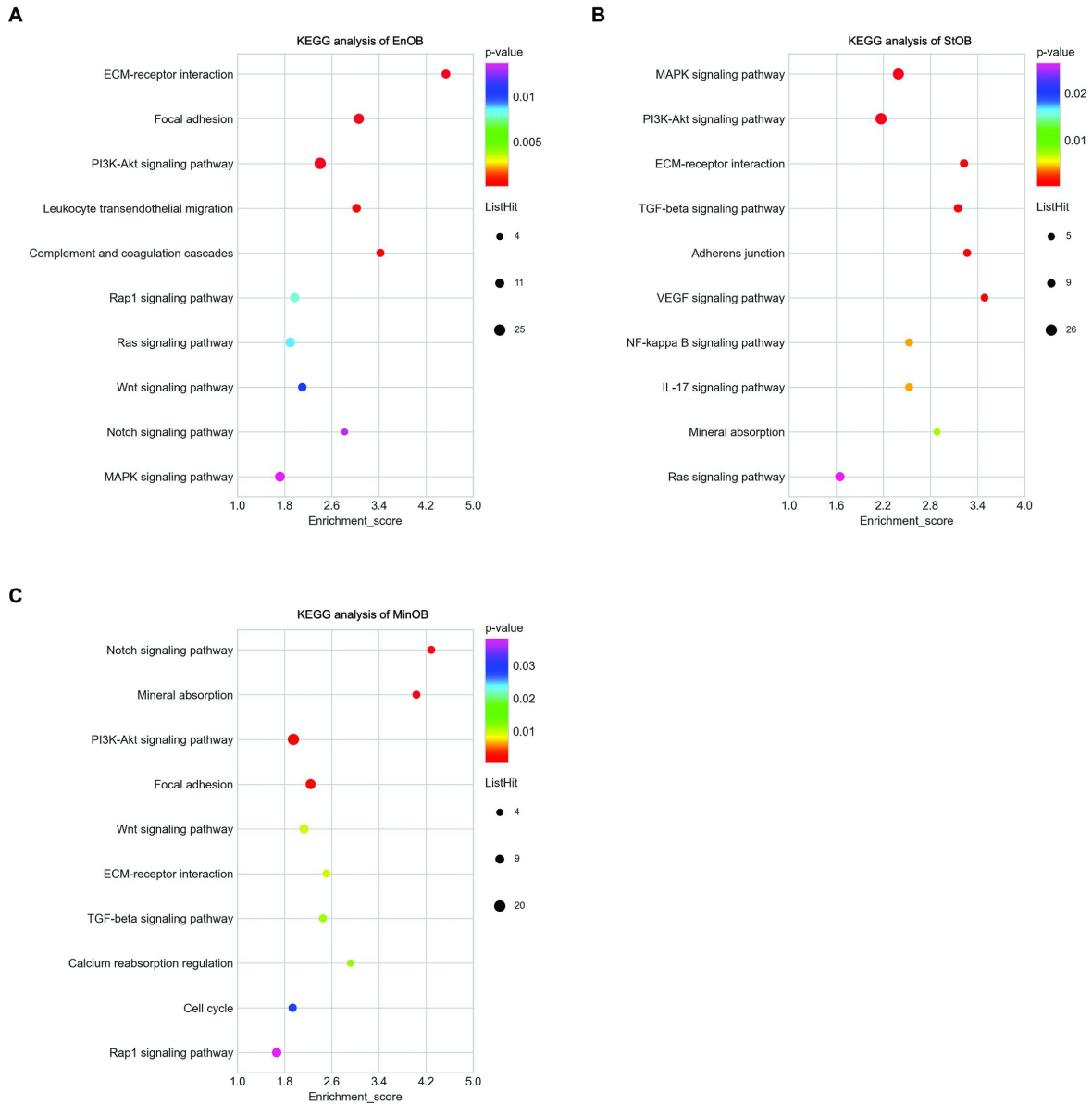
Fig. S7. KEGG showing enrichment of pathways among EnOBs, StOBs and MinOBs.
(**A**)Enrichment plot from KEGG pathway analysis for EnOBs. (**B**)Enrichment plot from KEGG pathway analysis for StOBs. (**C**)Enrichment plot from KEGG pathway analysis for MinOBs
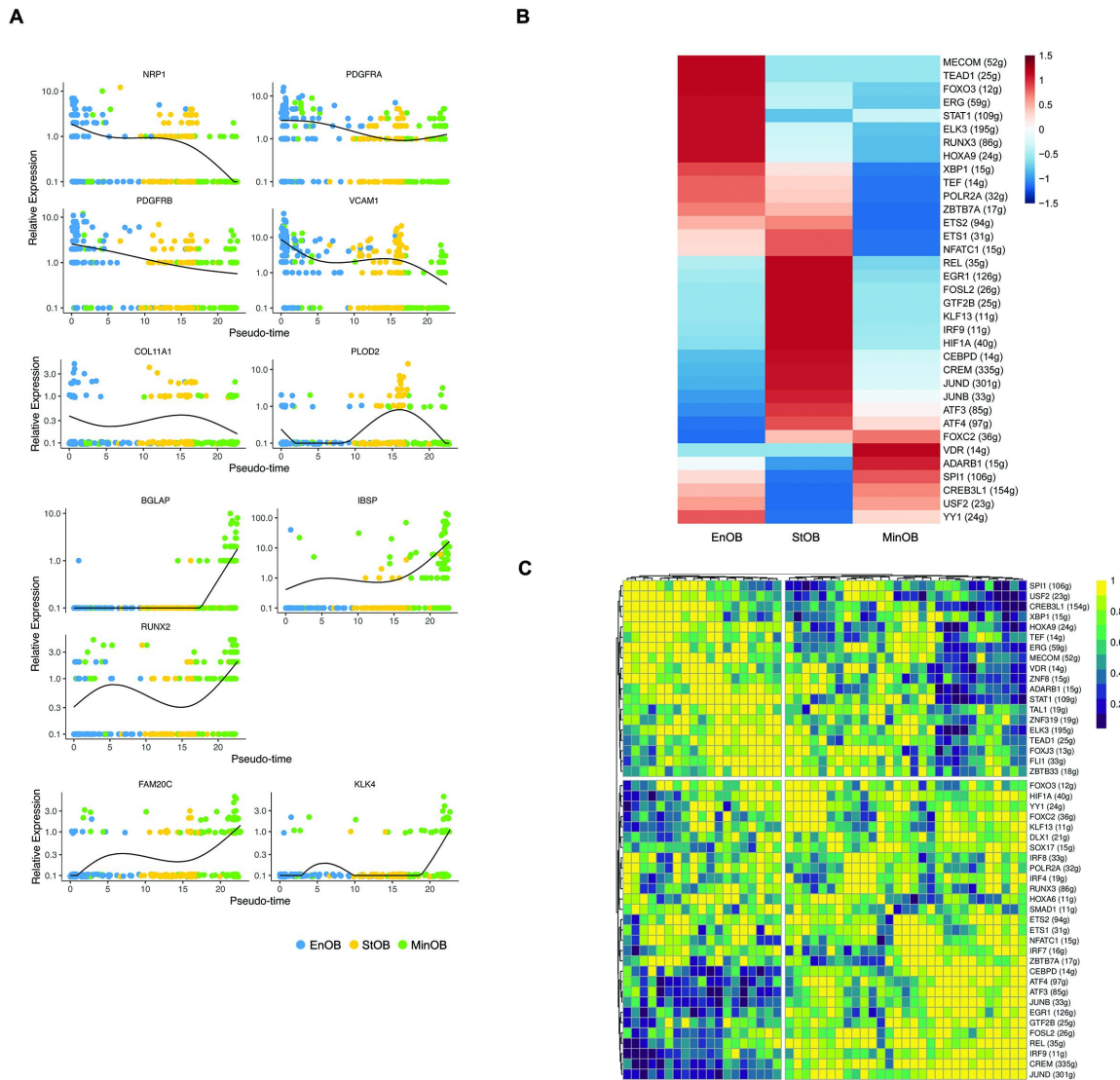
Fig. S8. Identification of EnOB, StOB and MinOB. (**A**)Pseudotemporal expression dynamics of marker gene in EnOBs,StOBs and MinOBs. All single cells in the EnOBs,StOBs and MinOBs cell lineage are ordered based on pseudotime. (**B-C**) Single-cell regulatory network inference and clustering (SCENIC) analysis showing distinct regulons among EnOBs,StOBs and MinOBs.The heatmap listing only the regulons with significant differences.
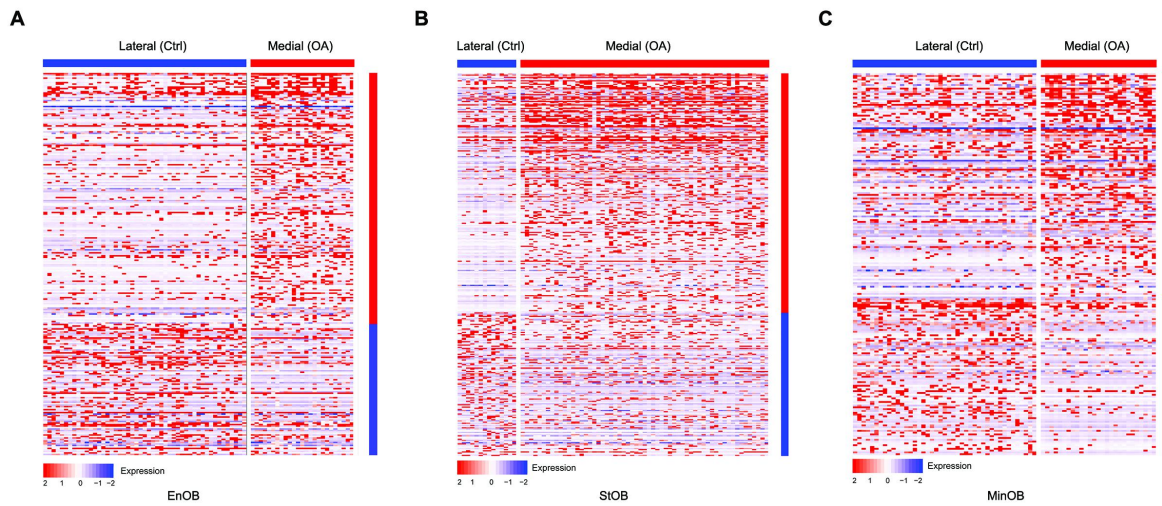
Fig. S9. Identification of OA group and Ctrl group in osteoblasts. (**A**)Heatmap of DEGs between OA group and Ctrl group in EnOBs. (**B**)Heatmap of DEGs between OA group and Ctrl group in StOBs. (**C**)Heatmap of DEGs between OA group and Ctrl group in MinOBs
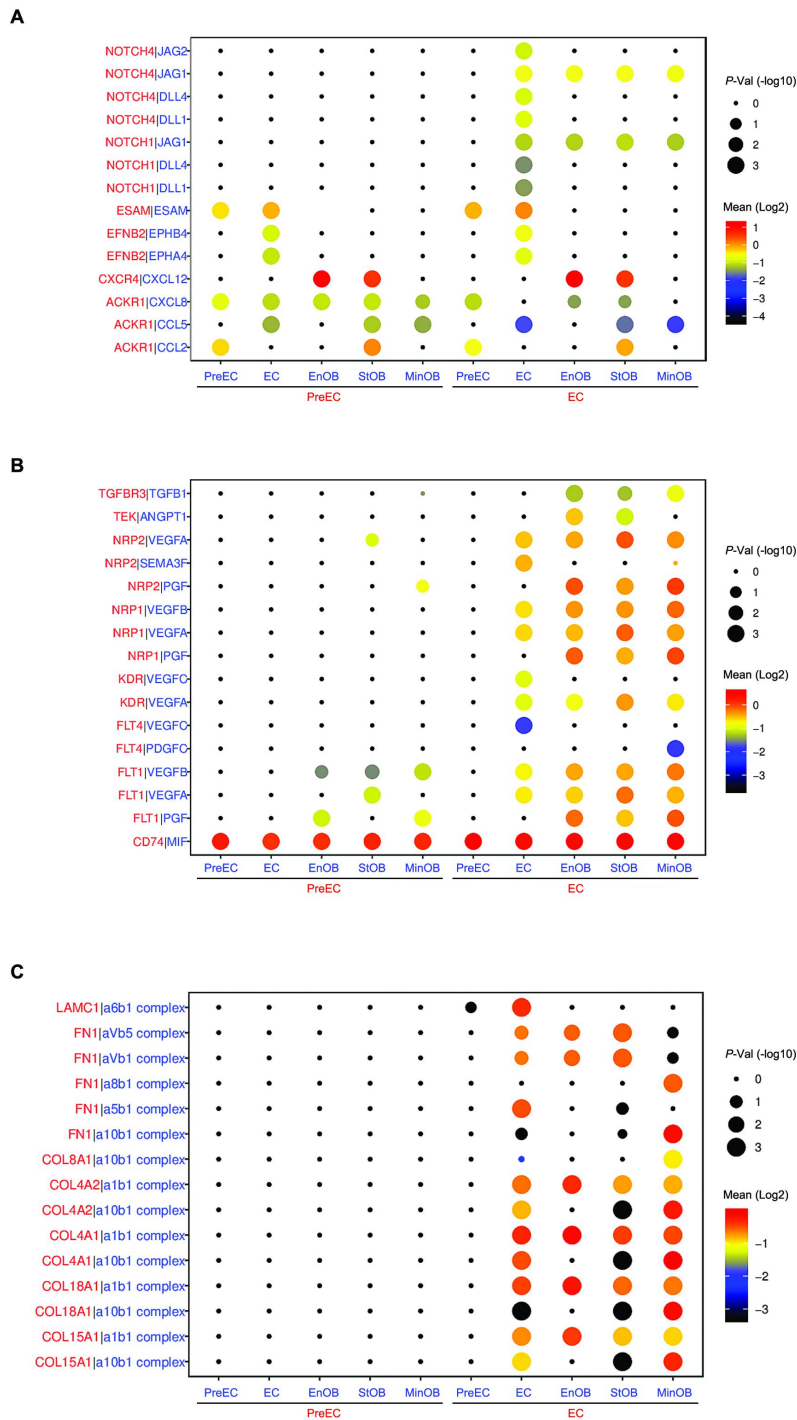
Fig. S10. Vascular endothelial cell and osteoblast subtype interaction. CellPhoneDB analysis showing the number of ligand-receptor interactions between endothelial cell subpopulation and osteoblast subpopulation. Bubble plots show ligand-receptor pairs of cytokines (**A**), growth factors (**B**) and integrin (**C**) between endothelial cells subpopulation.

Table S1. Clinical and demographic characteristics of the patients

	Patient 1	Patient 2
Gender	Female	Female
Age (years)	74	72
BMI	26.89	29.14
TKA	Right	Left
KSS score	54	58
Chronic conditions	Hypertension (grade Ⅱ)	Type 2 diabetes, hypertension (grade Ⅰ)

Table S2. Markers of each cell type

Cell type	Markers
B cell	CD79A, BANK1, MS4A1
NK cell	GZMB, NKG7
NKT cell	NKG7, CD3D
T cell	CD3D, CD3G
DC	LILRA4, PTCRA
Monocyte	CSTA, FCN1
Macrophage	CD14, CD68, CSF1R, C1QC, F13A1
EC	PECAM1, CLDN5
MSC	MCAM
OB	RUNX2, CDH11