

Supplementary Figures

Supplementary Fig 1. Construct *Aebp1* conditional knockout mice. A. Schematic map of the conditional knockout mouse construction strategy. B. Representative gel image of PCR amplification validating the genotyping of conditional knockout mice. C. Relative expression of *Aebp1* in P0 *Aebp1^{fl/fl}* and *Aebp1^{OsxCre}* mice femur (n=3. Student's t-test. The data are shown as means \pm SD). D. Western blotting analyses of P0 pups bone tissue with indicated genotypes.

Supplementary Fig 2. Knockdown *Aebp1* in osteoblasts inhibits osteogenic differentiation. A. Relative expression of *Aebp1* in mouse osteoblasts after siRNA induction were assessed by qRT-PCR. (n=3. one-way ANOVA. The data are shown as means \pm SD) B. Relative expressions of *Sp7*, *Colla1*, and *Bglap* in mouse osteoblasts after siRNA-induced osteogenic differentiation were assessed by qRT-PCR. (n=3. Student's t-test. The data are shown as means \pm SD) C. Western blot analysis of ACLP, COL1A1, RUNX2, and OCN proteins levels in mouse osteoblasts after siRNA-induced osteogenic differentiation. D-E. Representative ALP (D) and ARS (E) staining of mouse osteoblasts after siRNA-induced osteogenic differentiation. Scale bar: 500 μ m. F. Western blot analysis of ACLP, COL1A1 and OCN protein levels of mouse osteoblasts in *Aebp1^{fl/fl}* and *Aebp1^{OsxCre}* mice. G. Western blot analysis of ACLP, COL1A1, OPG and OCN protein levels of *Aebp1^{fl/fl}* mice osteoblasts treated with ad-GFP or ad-Cre. H. Representative ALP staining of *Aebp1^{fl/fl}* mice osteoblasts treated with ad-GFP or ad-Cre.

Supplementary Fig 3. Osteocyte phenotype in *Aebp1^{OsxCre}* mice. A. Dendrite formation of osteocytes stained by phalloidin. Scale bar: 20 μ m. B. Quantification of dendrite numbers and dendrite length of the femur sections from 6-week-old mice with the indicated genotypes. (n=3. Student's t-test. The data are shown as means \pm SD)

Supplementary Fig 4. Single-cell analysis of *Aebp1^{fl/fl}* and *Aebp1^{OsxCre}* mice. A. Quality control plots of scRNA-seq data among each sample. B. Bubble plots of expression level of canonical osteoblast marker genes including *Alpl*, *Colla1*, *Sp7*, *Bglap*, and *Ibsp* among each sample. C. Re-organized clusters of canonical osteoblastic genes expressed cells subset from femoral scRNA-seq data (a total of 5 clusters named S0-S4). D. Expression level of top 100 marker genes among each cluster shown in heatmap. E. Result of GO analysis with top 100 marker genes expressed in Cluster S1. F. Expression score of gene set of GO: Osteoblast differentiation in canonical osteoblastic genes expressed cells subset from femoral scRNA-seq data. G. Bubble plots of expression level of osteocyte marker genes including *Sost*, *Dmp1*, *Phex*, *Fam20c*, *Pdpr*, *Gjal* and *Dkk1* among each cluster. H. Results of GO analysis with top 100 fold change up-regulated DEGs in *Aebp1^{fl/fl}* compare to *Aebp1^{OsxCre}* group in osteocytes. I. Distribution of chondrocyte marker genes-expressed cells shown in dot plots. J. Results of GO analysis with top 100 fold change up-regulated DEGs in *Aebp1^{fl/fl}* compare to *Aebp1^{OsxCre}* group in chondrocytes.

Supplementary Fig 5. Pseudo-time analysis of *Aebp1^{fl/fl}* and *Aebp1^{OsxCre}* mice. A. Differentiated trajectories of osteoblast cells. The direction of the arrow represents the direction of the pseudo-time. B. Distribution of the states of pseudo-time. The dots in each color represents each state, and each state is named numerically. C. Distribution of *Aebp1^{fl/fl}* and *Aebp1^{OsxCre}* cells in pseudo-time. D. Dot

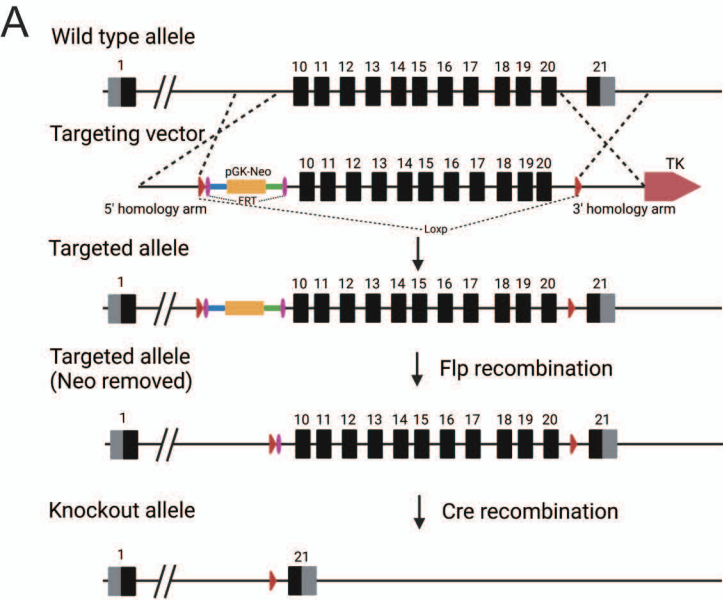
plots of expression level of *Alpl*, *Bglap*, *Colla1*, *Runx2*, *Sox9* and *Sp7* among each state.

Supplementary Fig 6. Identification of pre-osteoclast cluster in sc-RNA-seq data. A. Bubble plots of expression level of *Tnfrsf11a* and *Csf1r* among each cluster. B. GO enrichment analysis results using top 100 markers of cluster 21 as input. C. GO enrichment analysis results of enriched upregulated markers in *Aebp1^{OssCre}* group in cluster 21. D. Relative expression of Type I interferon signaling pathway related gene *Bst2*, *Irf7*, *Irf9*, *Ifit1*, *Ifit2*, *Isg15* and *Mx2* in 6-week-old mice femur were assessed by qRT-PCR. (n=4. Student's t-test. The data are shown as means \pm SD)

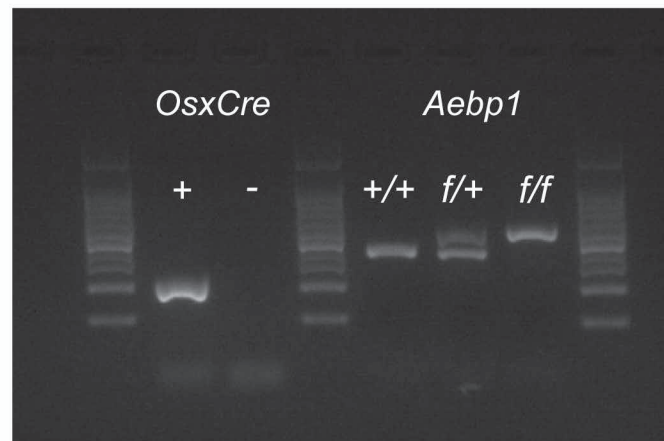
Supplementary Fig 7. *Aebp1* deletion leads to reduction in Wnt/ β -catenin signaling activity in the calvaria. A. Scatter plots shown data quality with different transform algorithms. B. Clustering of bulk-seq data of each calvaria sample. C. Principal component analysis (PCA) of the bulk RNA-seq data of *Aebp1^{fl/fl}* and *Aebp1^{OssCre}* groups. (n=3 for each genotype.) D. Normalized counts of *Aebp1* in *Aebp1^{OssCre}* groups compared to *Aebp1^{fl/fl}* groups. (n=3 for each genotype. Student's t-test. The data are shown as means \pm SD) E. Volcano plot exhibits the DEGs of the *Aebp1^{OssCre}* group compared to the *Aebp1^{fl/fl}* group. (n=3 for each genotype.) Blue dots show genes more highly expressed in the *Aebp1^{fl/fl}* group. Purple dots show genes more highly expressed in the *Aebp1^{OssCre}* group. F. GO enrichment analysis of DEGs downregulated in the *Aebp1^{OssCre}* group. G. Normalized counts of osteoblasts gene in *Aebp1^{OssCre}* groups compared to *Aebp1^{fl/fl}* groups. (n=3 for each genotype. Student's t-test. The data are shown as means \pm SD) H. Normalized counts of Wnt pathway gene in *Aebp1^{OssCre}* groups compared to *Aebp1^{fl/fl}* groups. (n=3 for each genotype. Student's t-test. The data are shown as means \pm SD)

Supplementary Fig 8. BIO treatment alleviate osteoblast and osteoclast abnormalities in vivo. A. Representative Masson's trichrome staining of femurs trabecular bone from 6-week-old mice with indicate genotype. Scale bar: 100 μ m. B. Quantification of N.Ob/B.Pm of A. (n=3 for each genotype. one-way ANOVA. The data are shown as means \pm SD). C. Representative Masson's trichrome staining of femurs cortical bone from 6-week-old mice with indicate genotype. Scale bar: 100 μ m. D. Quantification of cortical bone thickness of C. (n=3 for each genotype. one-way ANOVA. The data are shown as means \pm SD). E. Representative TRAP staining of femurs from 6-week-old mice. Scale bar: 100 μ m. F-G. Quantitative analysis of TRAP staining of E. (n=3 for each genotype. one-way ANOVA. The data are shown as means \pm SD).

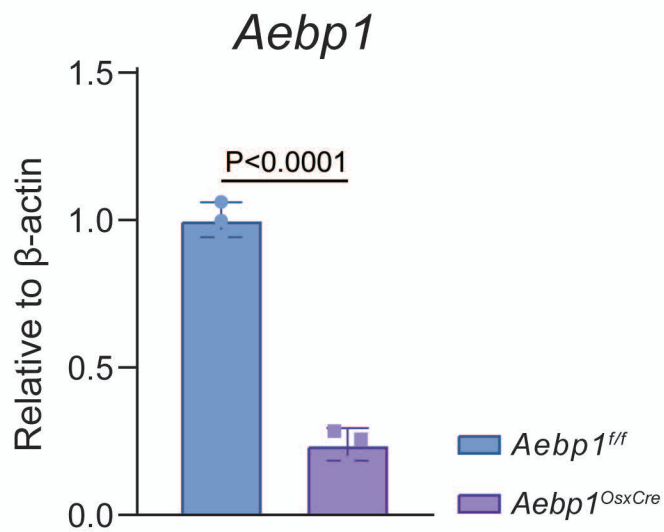
Supplementary Fig 9. Osteoblast differentiation suppression of *Aebp1* deletion was reversed by BIO treatment. A. Representative ARS staining of mouse osteoblasts after osteogenic differentiation with indicate genotype and treatment. B. Relative expressions of *Runx2*, *Sp7*, *Colla1*, and *Bglap* in mouse osteoblasts after osteogenic differentiation were assessed by qRT-PCR. (n=3. one-way ANOVA. The data are shown as means \pm SD)



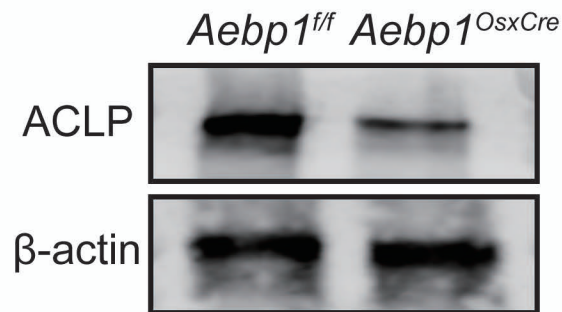
B

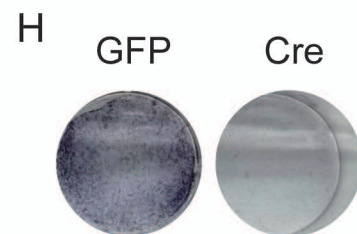
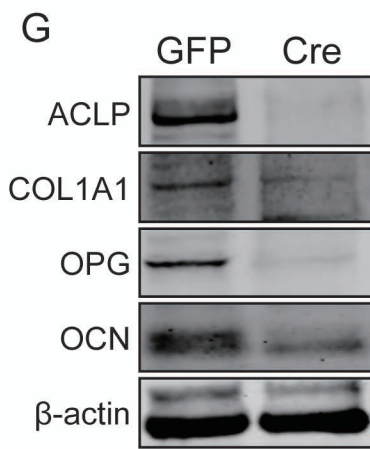
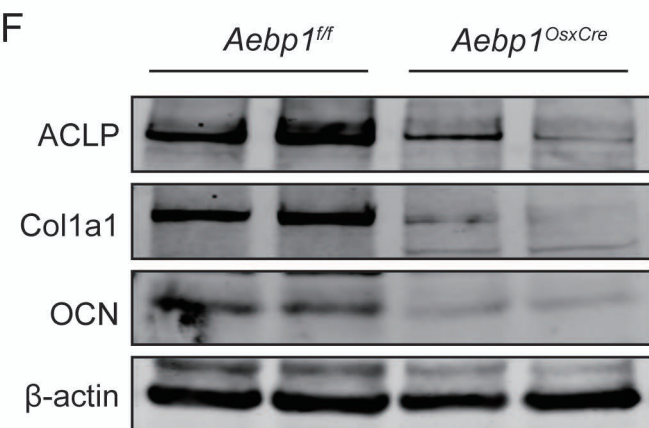
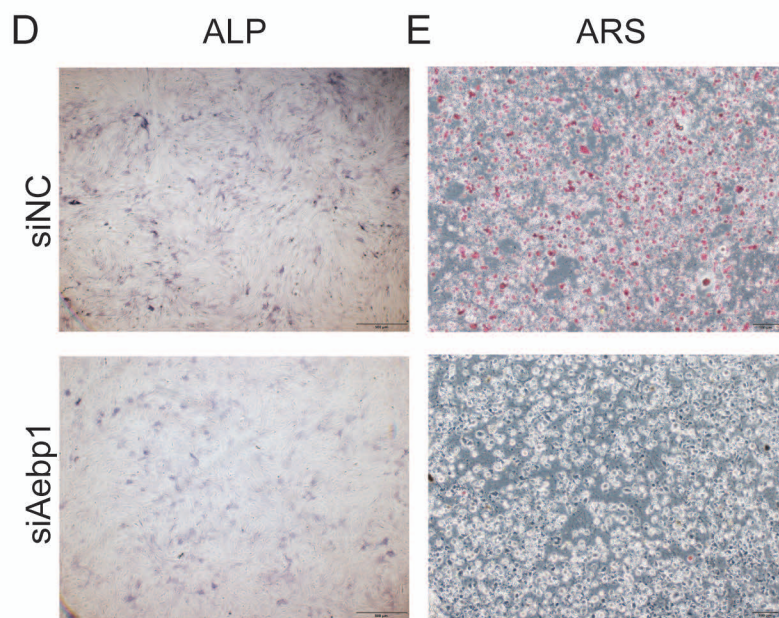
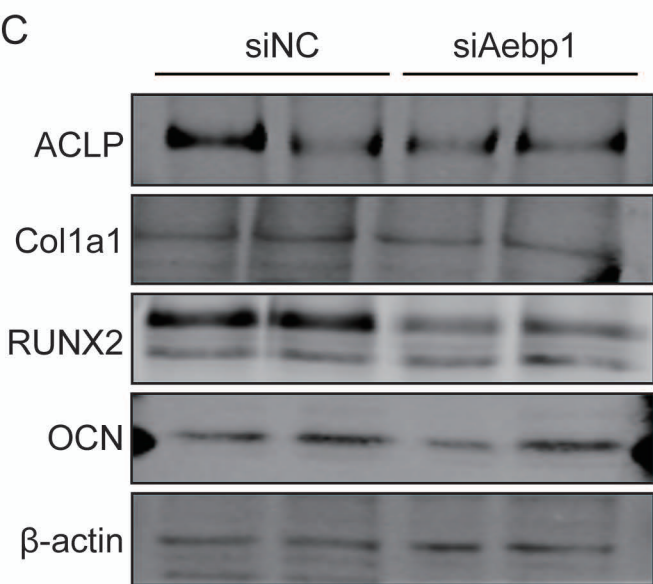
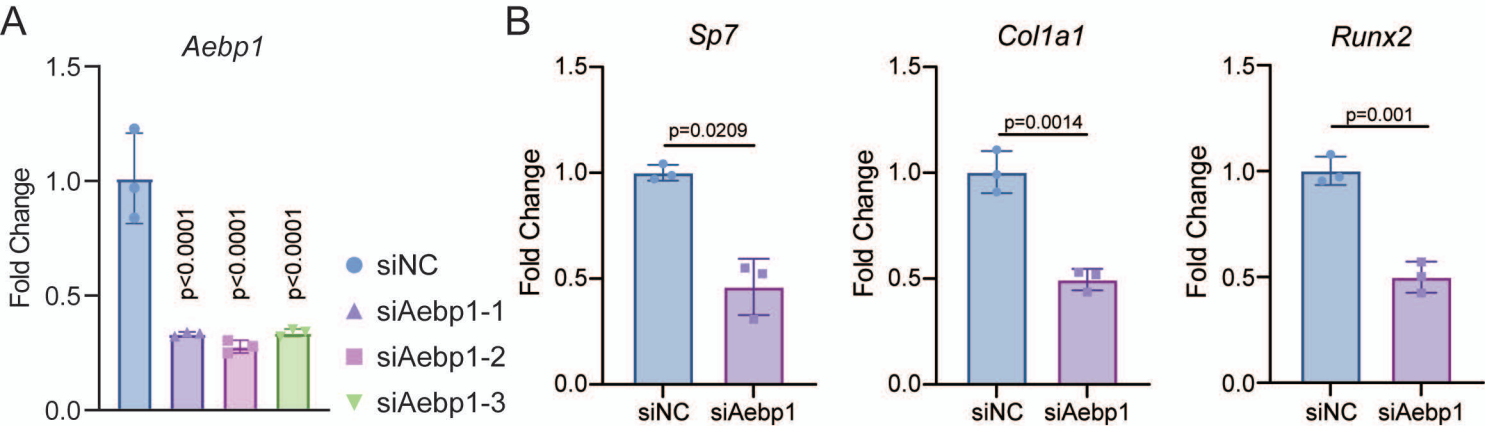


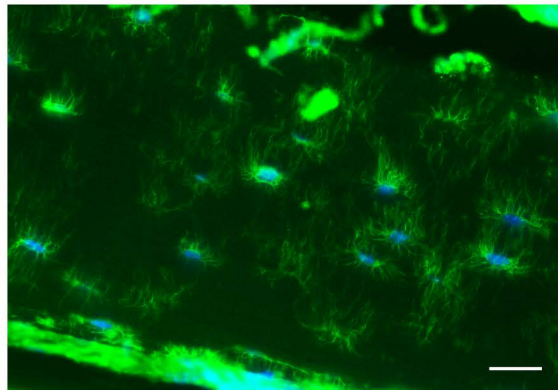
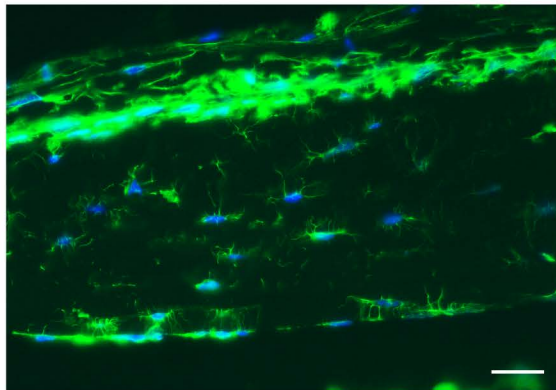
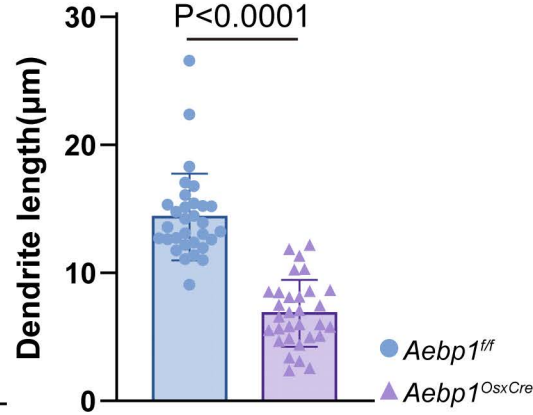
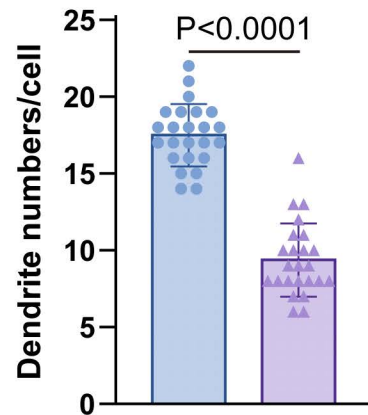
C

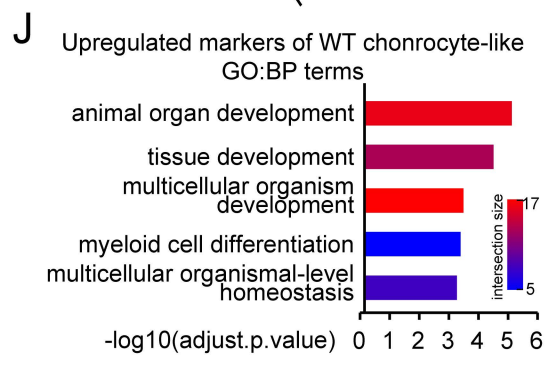
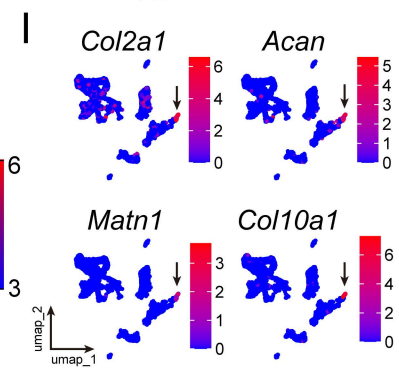
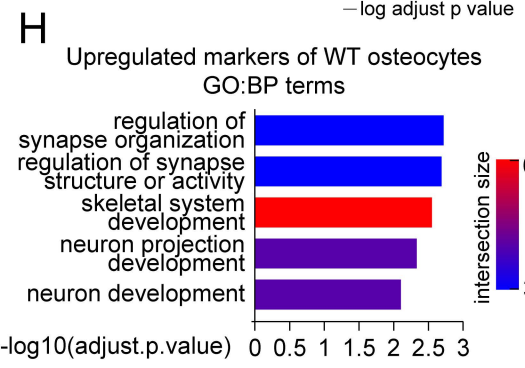
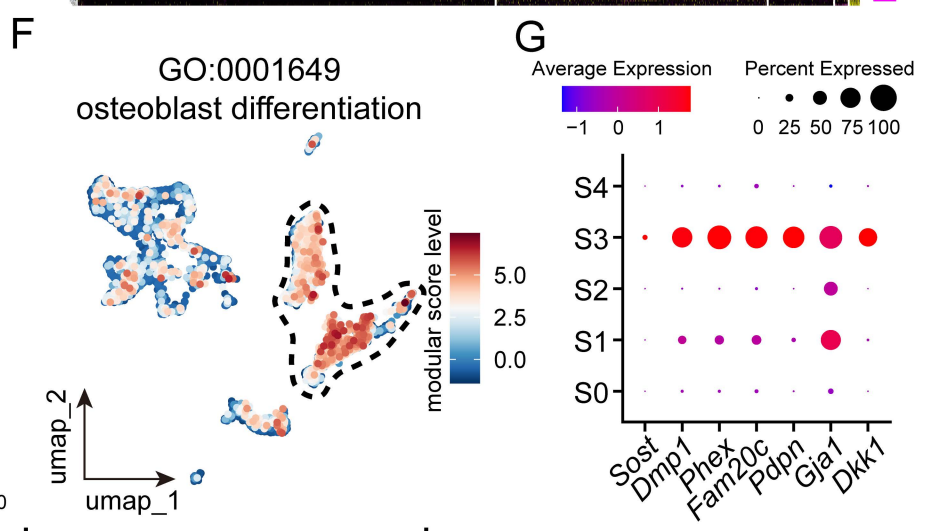
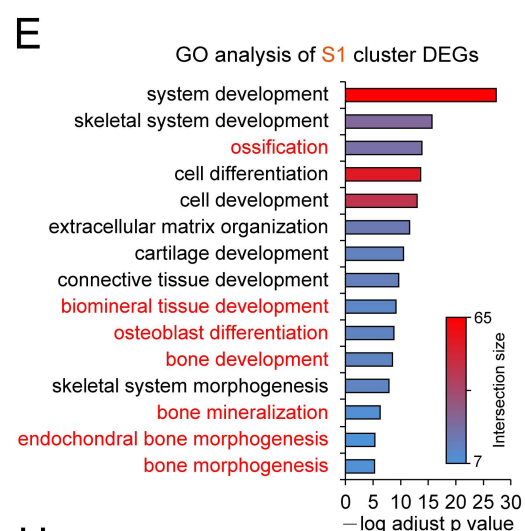
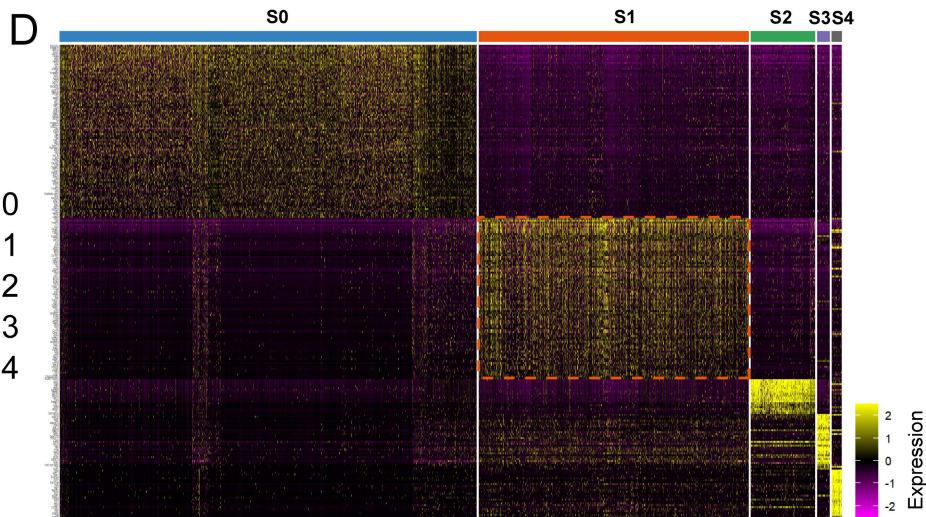
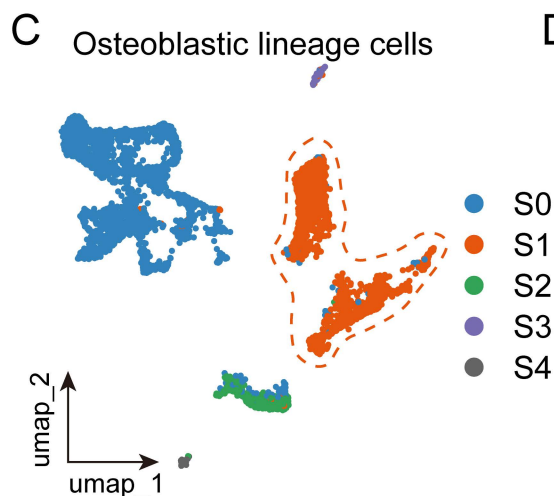
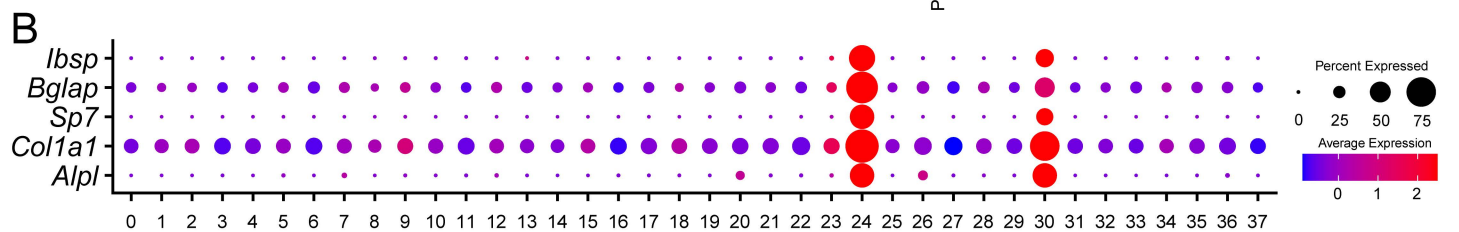
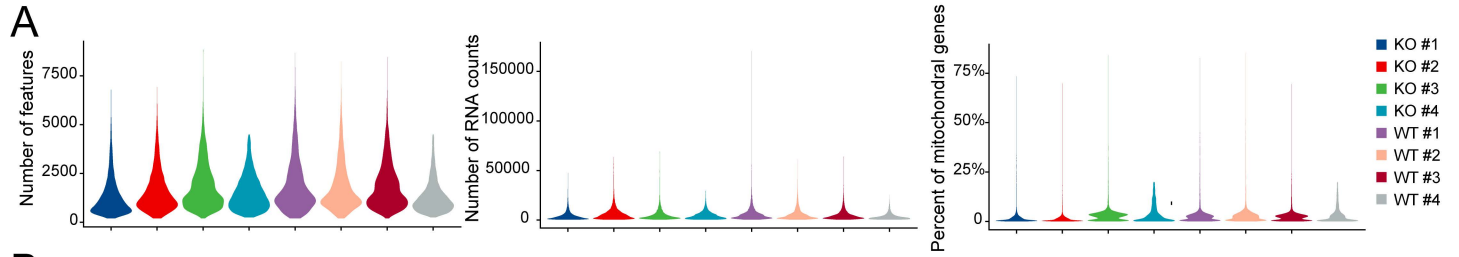


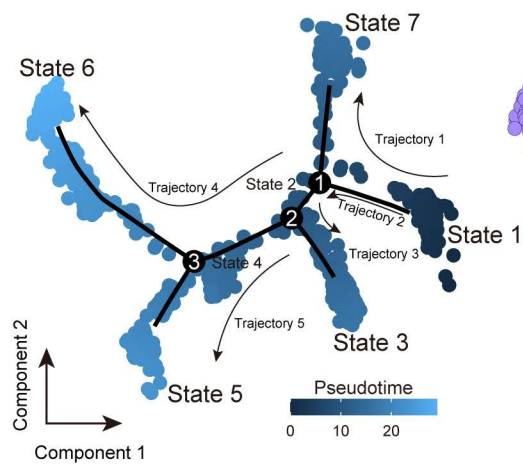
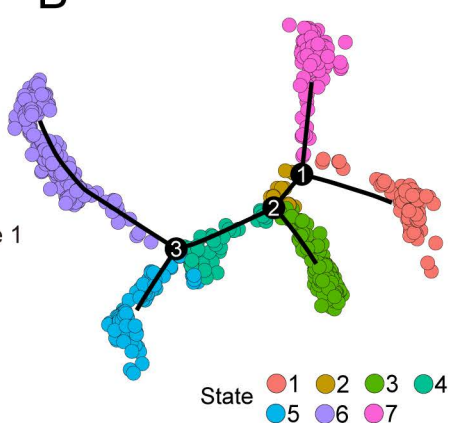
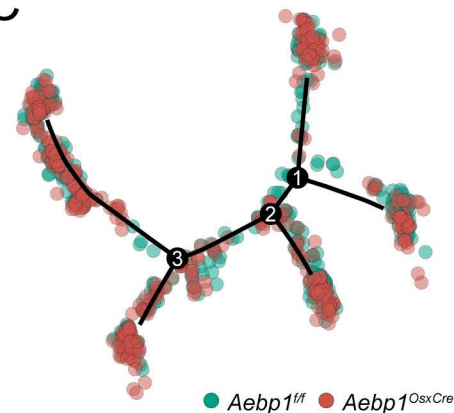
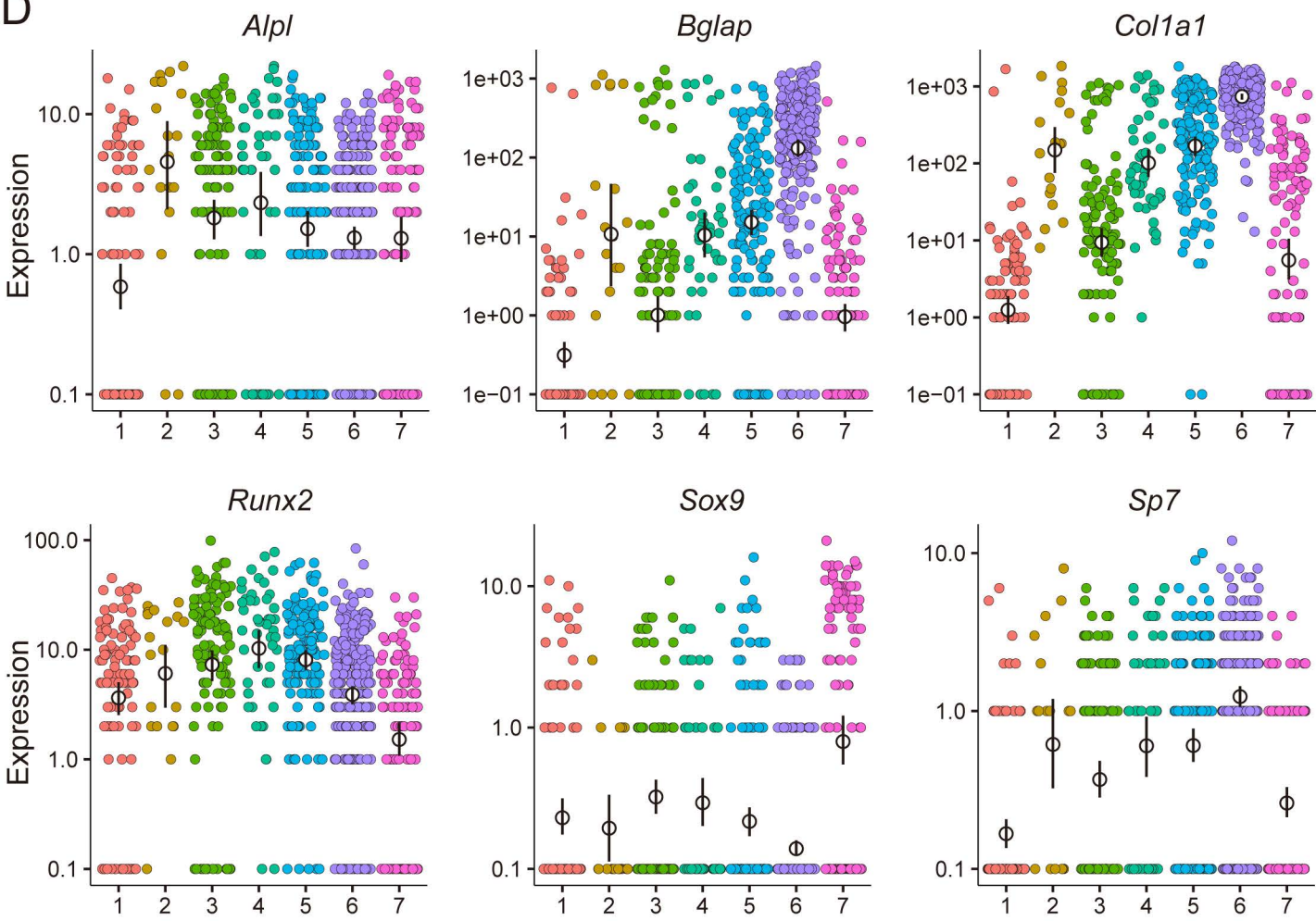
D



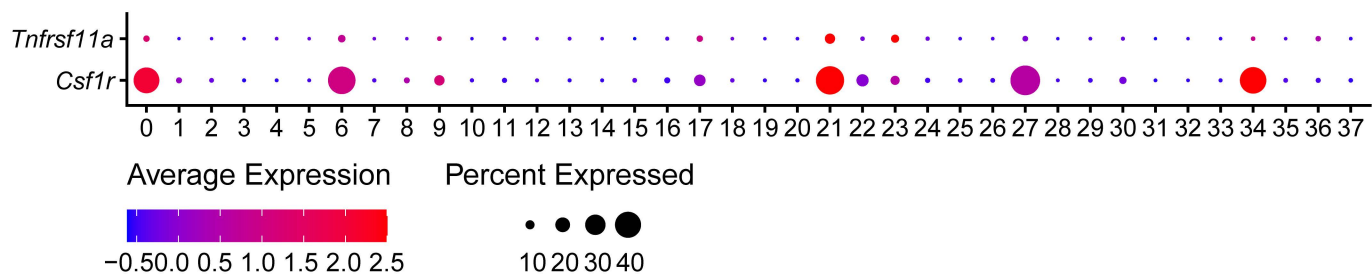


A*Aebp1*^{f/f}*Aebp1*^{OsxCre}**B**● *Aebp1*^{f/f}▲ *Aebp1*^{OsxCre}

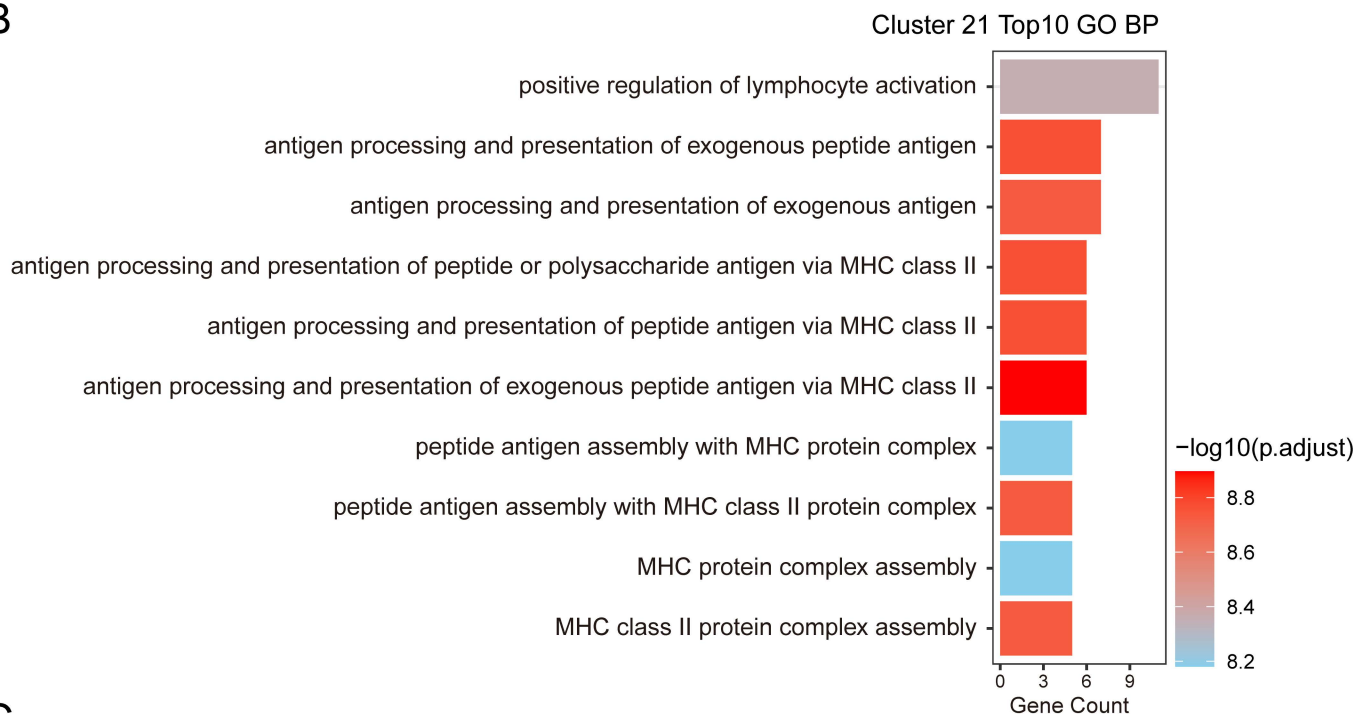


A**B****C****D**

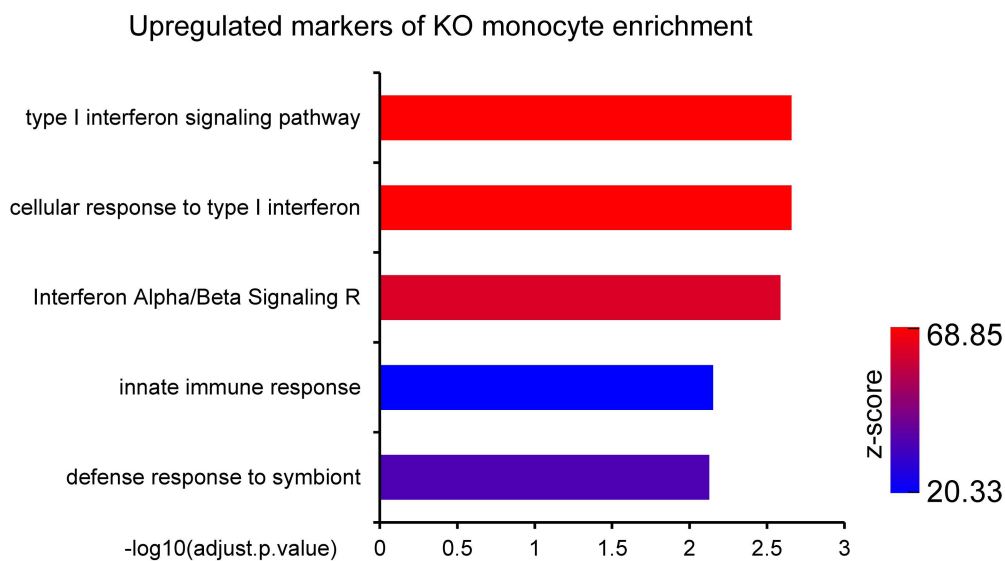
A



B



C



D

