

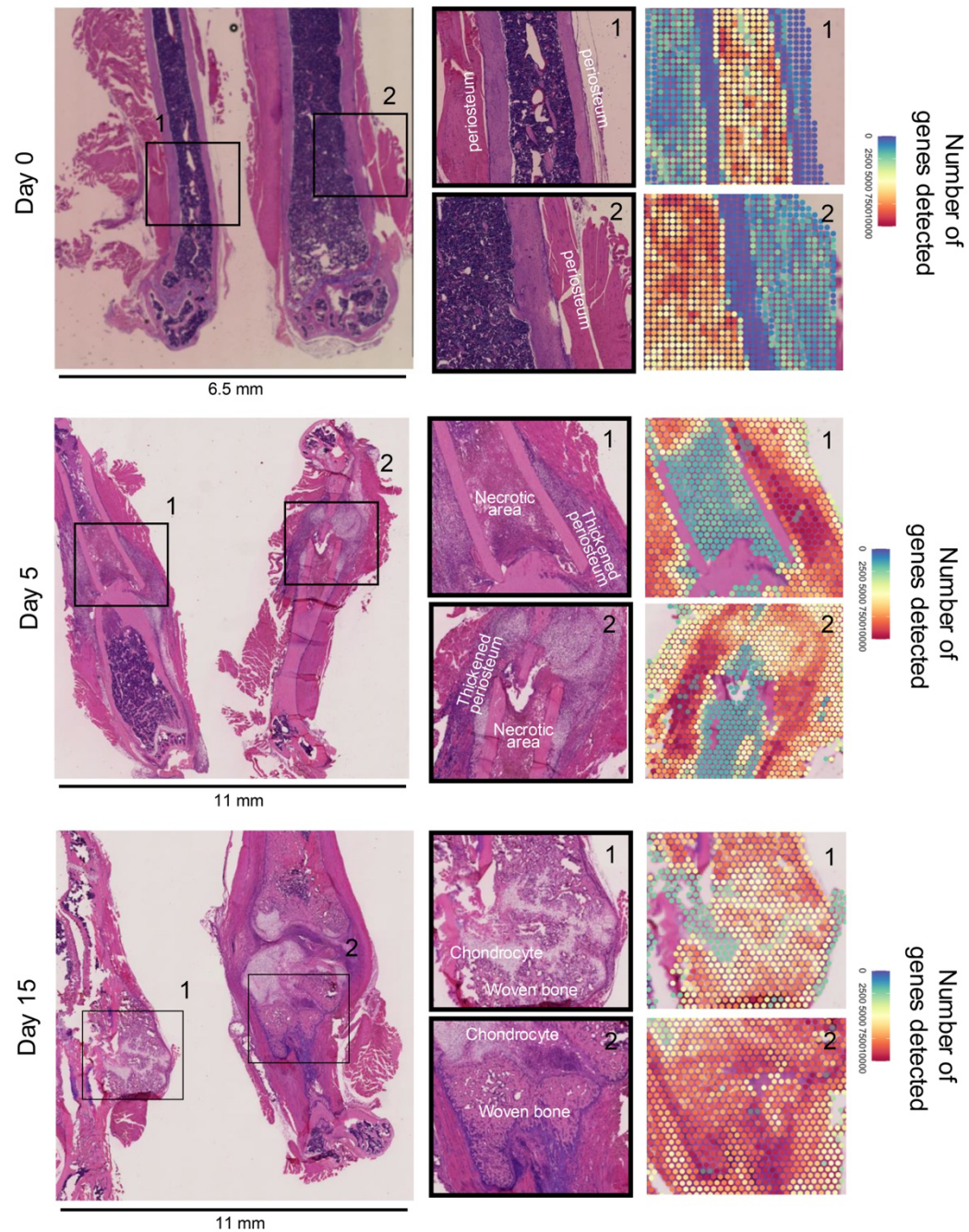
Supplementary information

Supplementary Table 1. Comparative Analysis of Spatial Transcriptomic Datasets Across Different Mouse Tissue Preparation Procedures

Datasets	Method	10X Visium	Spots#	UMIs /spot	Genes/ spot
Mouse IVD (3 week)	Snap frozen, OCT	Four 6.5 X 6.5 mm ²	3860	566	310
Mouse femur (10 week)	10% EDTA decal, Paraffin	Four 6.5 X 6.5 mm ²	4532	415	251
Mouse calvaria (P0)	Snap frozen, OCT	Four 6.5 X 6.5 mm ²	1670	10500	3500
Mouse long bone day 0 before fracture (12 week)	Morse's solution decal, Paraffin	One 6.5 X 6.5 mm ²	10146	7684	2709
Mouse long bone fracture day 5 (12 week)	Morse's solution decal, Paraffin	One 11 X 11 mm ²	6812	17426	4810
Mouse long bone fracture day 15 (12 week)	Morse's solution decal, Paraffin	One 11 X 11 mm ²	7260	21117	5499

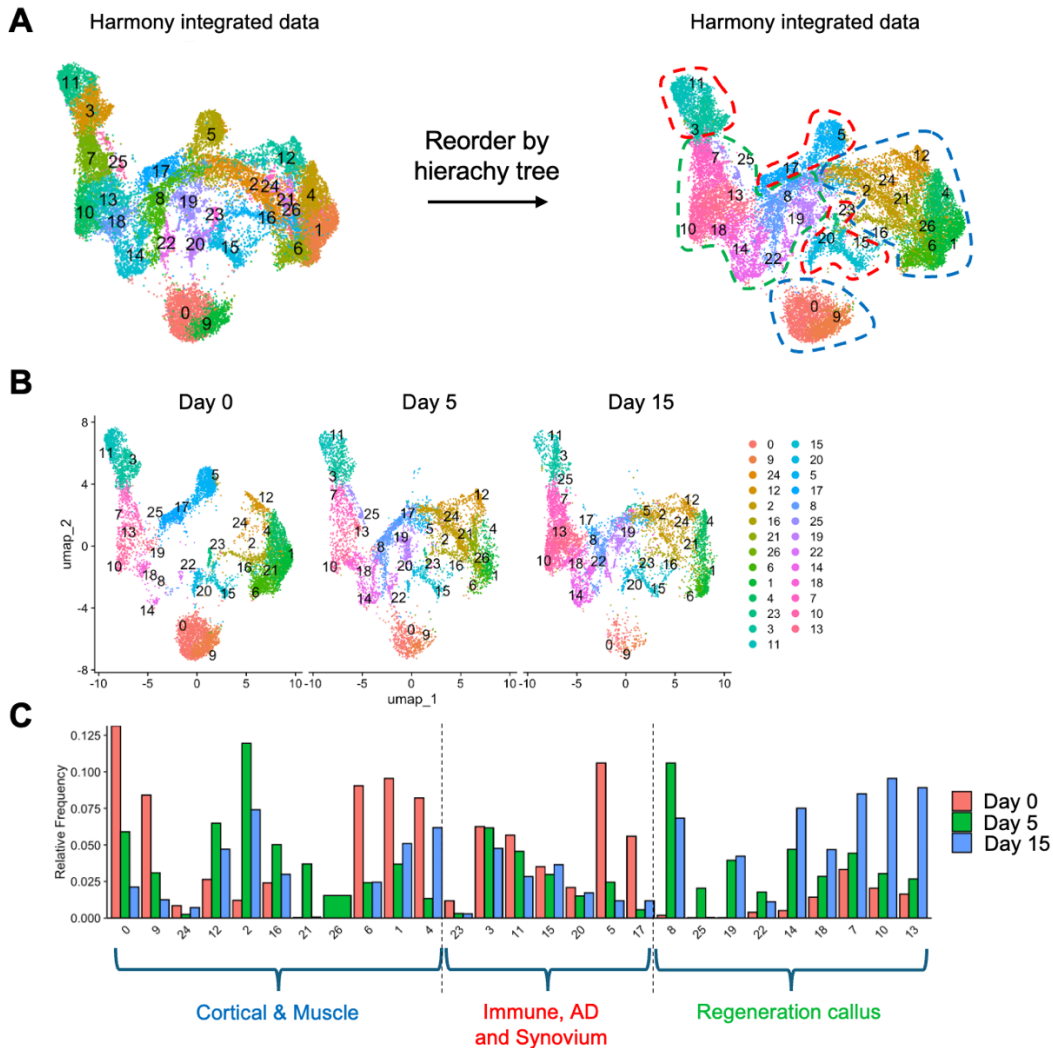
Supplementary Table 2. Spatial clusters nomenclatures by GPTCelltype.

Cluster	Celltype by ChatGPT	Top marker genes
C0	Osteocytes	Sost,Ndrg1,Magi1,Igfbp6,Cadm1,Pcsk6,Pcdh7,Six5,Tle2,Pcdhgc3
C9	low quality cells (Ambient RNA contamination)	D11Wsu47e,Tcerg1,Nipa2,Lmbr11,Slc25a17,Tti2,Hmmr,Bud23,Hpfl,G6pdx
C24	Smooth muscle cells	Sema3g,Myh11,Olf78,Nrip2,C7,Lmod1,Acta2,Pln,Tagln,Mrgprh
C12	Skeletal muscle cells (slow-twitch fibers)	Myl2,Tnnc1,Myh7,Myl3,Tnnt1,Myoz2,Ankrd2,Myh2,Tnni1,Sln
C2	Myoblasts and skeletal muscle progenitors	Col19a1,Myh8,Chma1,Myh3,Gm4841,Aifl1,Myl4,Ankrd1,Myf6,1110002E22Rik
C16	Fibro-adipogenic progenitors	Tigd4,Map3k7cl,Chodl,Thbs4,Mss51,Gdf11,Lama2,Ankrd1,Tceal7,Fmod
C21	Skeletal muscle cells	Trim63,Myf6,Igfn1,Pdlim3,Eda2r,Dupd1,Gadd45a,Cst6,Fbxo32,Musk
C26	Oxidative skeletal muscle cells	Amhr2,3425401B19Rik,Gck,Ckmt2,1700001O22Rik,Tbx1,Cox6a2,Slc25a4,1110002E22Rik,Pdk4
C6	Fast-twitch Skeletal Muscle Fibers	Dhrs7c,Actc1,Xirp2,Ak1,Tmem38a,Rasd2,Tmem233,Prkab2,Cap2,Phka1
C1	Fast-twitch Skeletal Muscle Fibers	Actc1,Plcd4,Mybph,Dhrs7c,Rasd2,Ak1,Mylk4,Pgm1,Ampd1,Tmem233
C4	Skeletal muscle cells (slow-twitch fibers)	Ckmt2,Dusp26,Myh1,Fam57b,Perm1,Dhrs7c,Cox7a1,Atp5j2,Ak1,Tmem38a
C23	Smooth muscle cells	Myh11,Gkn3,Itga8,Lmod1,Tagln,Acta2,Cnn1,Hey2,Kcnmb1,Myocd
C3	Neutrophils	Ighj1,4930438A08Rik,Pglyrp1,Hist1h2ag,Ighg2b,Ngp,Lcn2,Wfdc21,Trpm2,S100a8
C11	Platelets	Ppbp,Gp6,Gp1ba,Gp5,Tubb1,Trem1,Itga2b,Alox12,Clec1b,Mpl
C15	Adipocytes	Cfd,Cyp2e1,Pck1,Plin1,Retn,Cidec,Tmem45b,Adig,Fasn,Adrb3
C20	Synovial cells	Prg4,Htra4,Has1,Hbegf,Fbln7,Pla1a,Ntn1,Ccdc3,Angptl7,Tspan15
C5	Erythrocytes	Hba-a1,Hba-a2,Epb42,Slc4a1,Rrm2,Kif18b,Alad,Alas2,Car2,Gypa
C17	Erythroid progenitors	Rrm2,Carl,Pcna,Cdca7,Stmn1,Ubd,Fcer2a,Cstde4,Slbp,Hba-a1
C8	Mesenchymal progenitors	Myh3,Cxcl5,Tnnt2,Sfrp1,Fbn2,Crabp2,Saa3,Tnfaip6,Ddah1,Mest
C25	Mesenchymal progenitors	Lrrc15,Actg2,Nsg1,Kif26b,Col18a1,Thbs2,Col8a1,Serpine1,Dkk3,Mmm2
C19	Macrophages	Mmp12,Itgax,Gpnmb,Sfrp2,F7,Trem2,Cxcl5,Acod1,Egfl6,Ccl7
C22	M2 macrophages	Arg1,Ill11,Cxcl3,Apln,Igfbp3,Acod1,Inhbb,Serpine1,Npy,Angpt2
C14	Chondrocytes	Col9a1,Col9a2,Matn3,Cnmd,Col9a3,Col2a1,Hapln1,Col10a1,Snorc,Clec3a
C18	Mesenchymal progenitors	Wnt16,Col8a2,Omd,Ndnf,Efna5,Fgfr2,Cpxm1,Ptn,Lum,Pdzrn4
C7	Osteoclasts	Acp5,Ctsk,Oscar,Slc9b2,Dgki,Atp6v0d2,Dcstamp,Slc37a2,Mt3,Arsb
C10	Osteoblasts	Bglap,Phex,Entpd3,Col11a1,Cpz,Slc13a5,Col1a1,Lipc,Col11a2,Kazald1
C13	Bone-resorbing osteoclasts	Ocstamp,Ifitm5,AI593442,Mmp9,Ibsp,Slc13a5,Mt3,Acp5,Cgrefl,Ckb



Supplementary Figure 1: Spatial Transcriptomic Analysis of Bone Fracture Sites at Key Healing Stages

The spatial transcriptomic maps from Day 0, Day 5, and Day 15 postfracture reveal significant changes in the number of detected genes across different regions of the bone healing process.

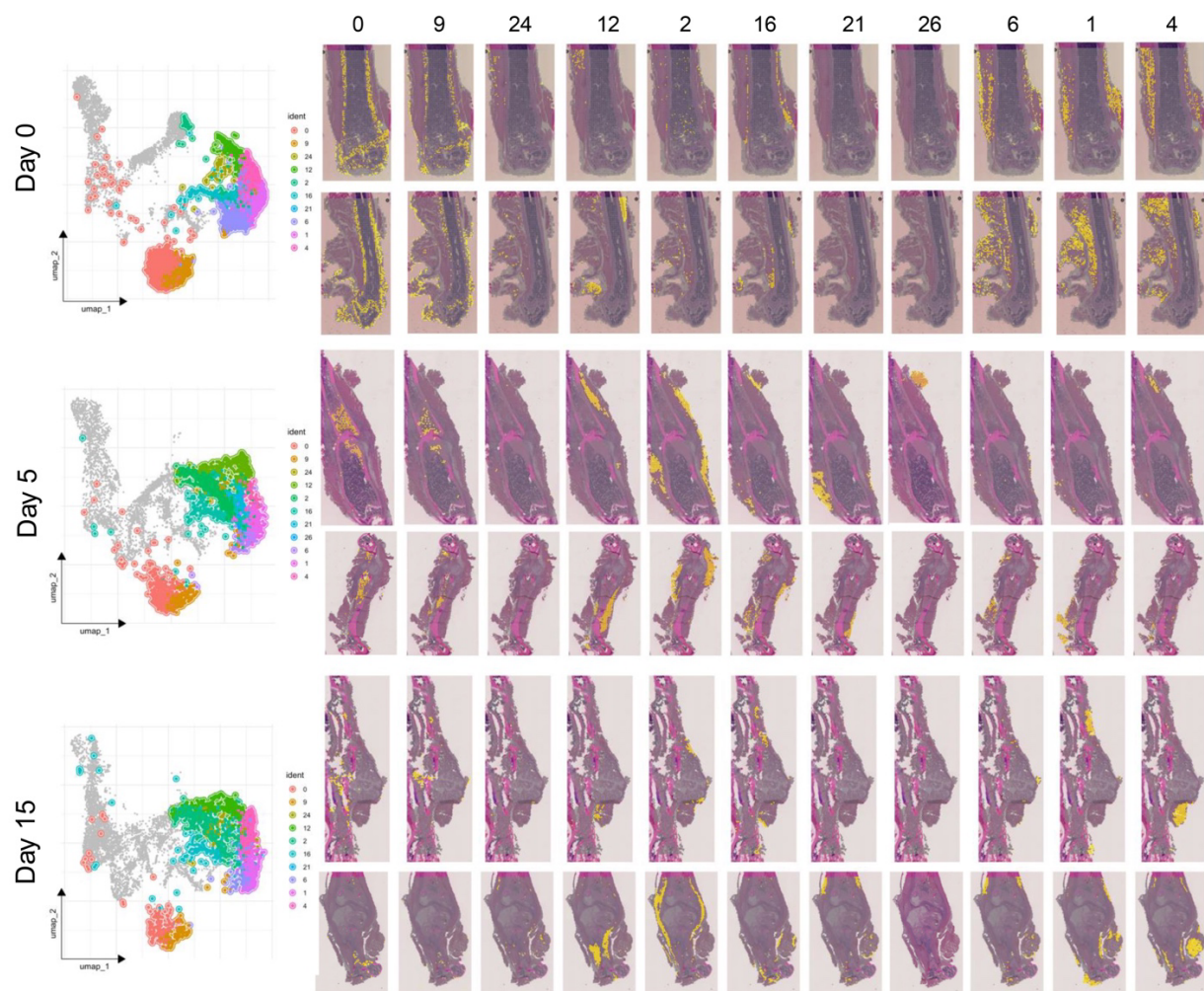


Supplementary Figure 2: Harmony Integration of Spatial Transcriptomic Data Across Bone Healing Stages

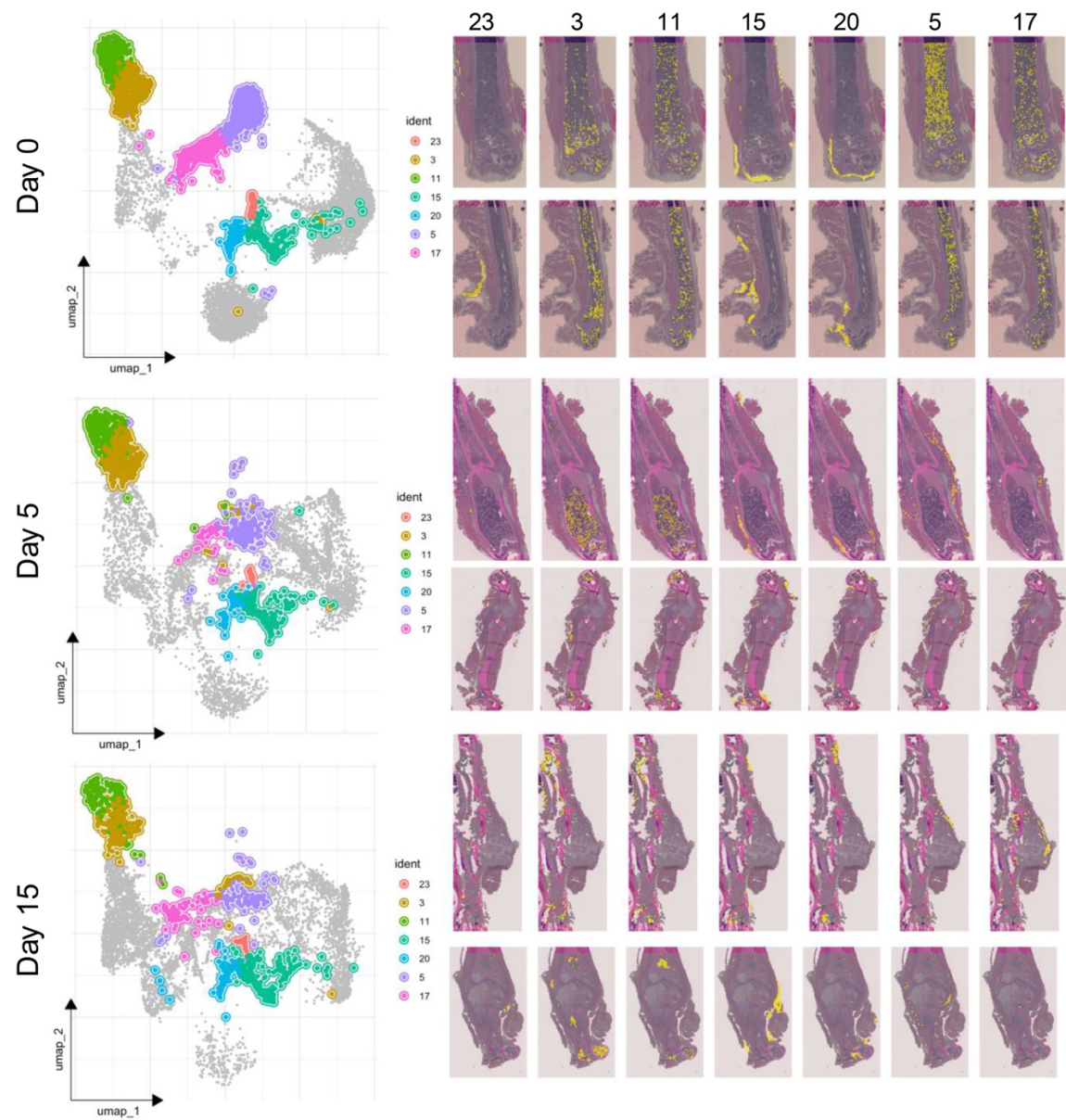
A: Left: UMAP visualization showing the integrated transcriptomic data across different healing stages (Day 0, Day 5 and Day 15), with each cluster color-coded to represent unique cell populations involved in the healing process. Right: The same data reordered according to a hierarchy tree to emphasize the relationships and transitions between different cell types, highlighted by dashed outlines around grouped clusters.

B: Individual UMAP plots for Day 0, Day 5, and Day 15, illustrating the distribution and evolution of cellular clusters over time.

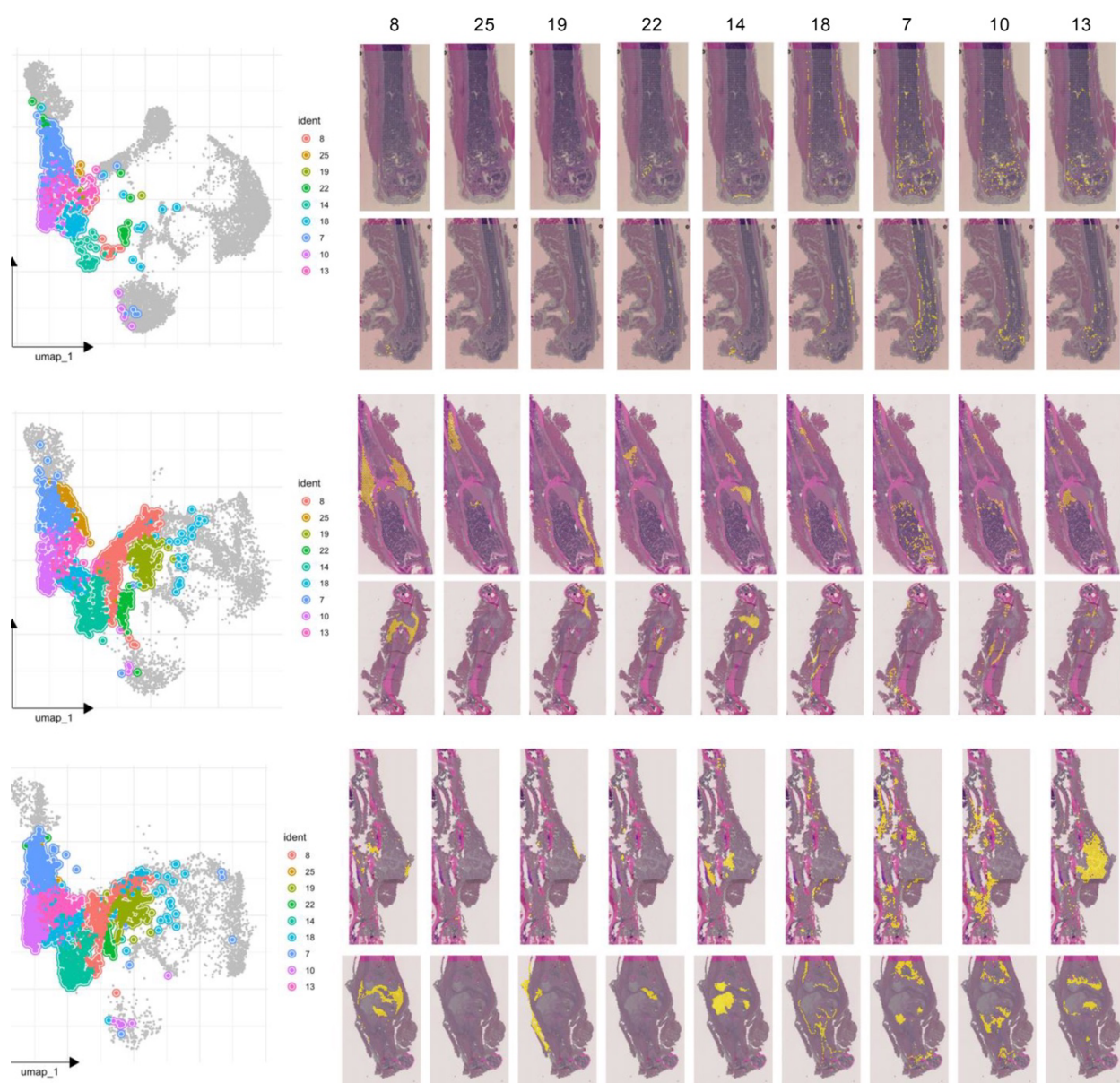
C. Bar charts showing the relative frequency of clusters across the three major groups: Cortical & Muscle, Immune, Adipose Tissue and Synovium, and Regeneration Callus.



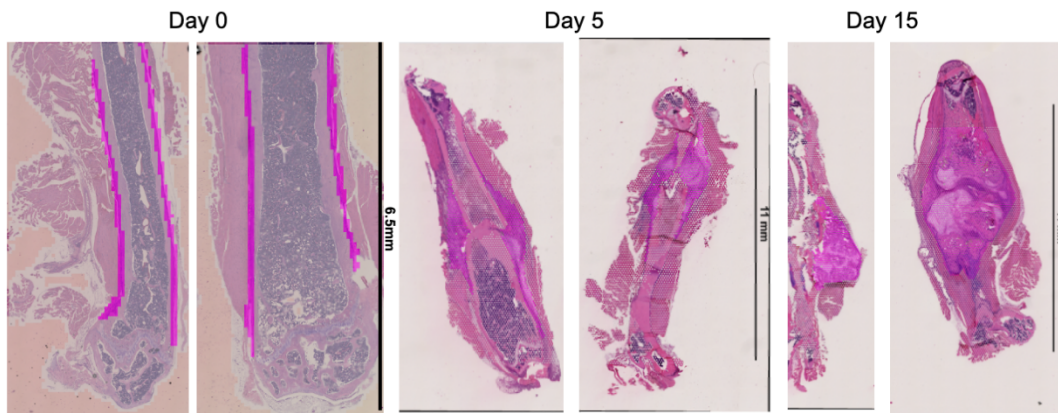
Supplementary Figure 3: Spatial Distribution of Group 1 clusters (Cluster: 0, 9, 24, 12, 2, 16, 21, 26, 6, 1, 4)



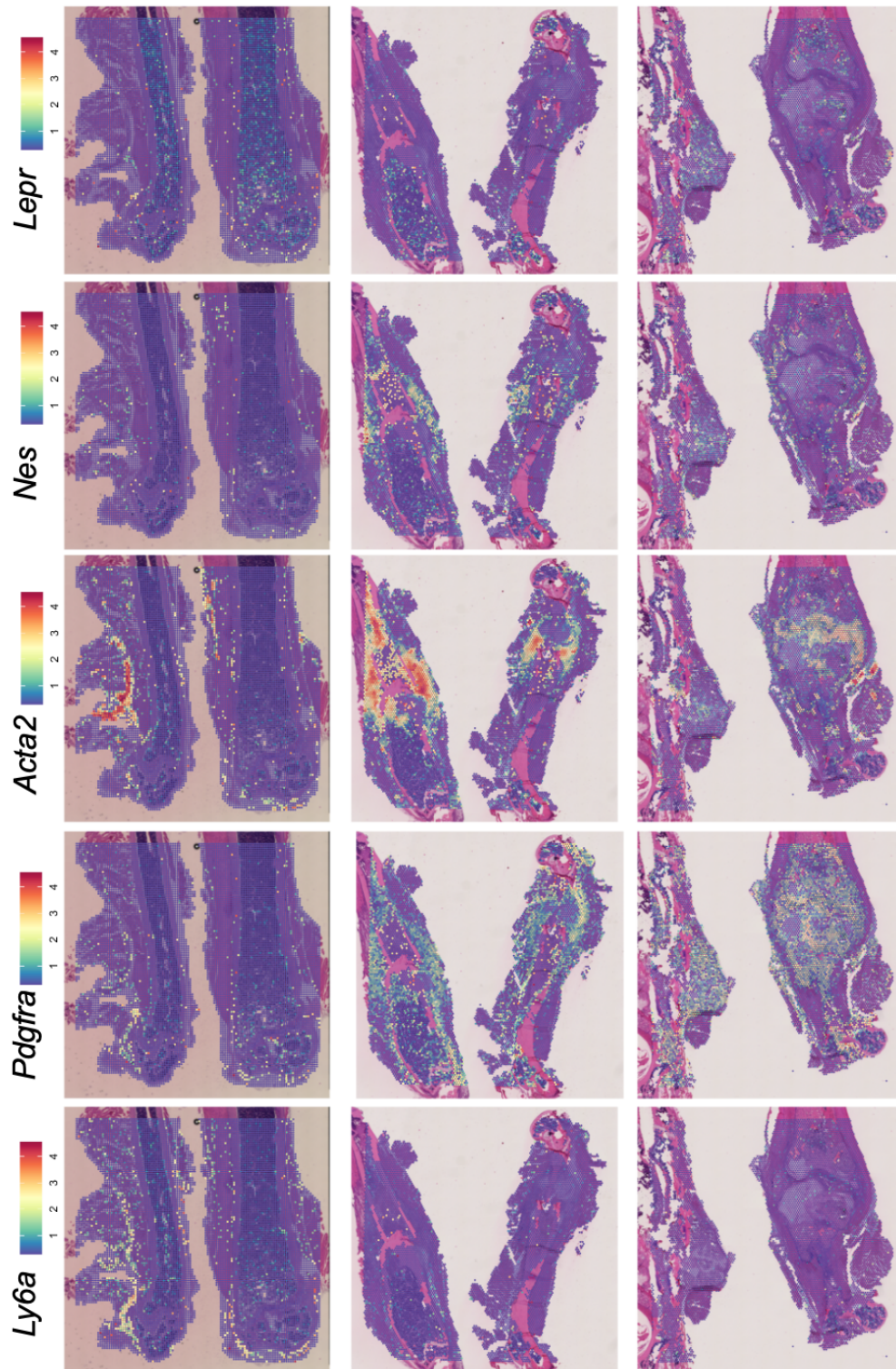
Supplementary Figure 4: Spatial Distribution of Group 2 clusters (Cluster: 23, 3, 11, 15, 20, 5, 17)



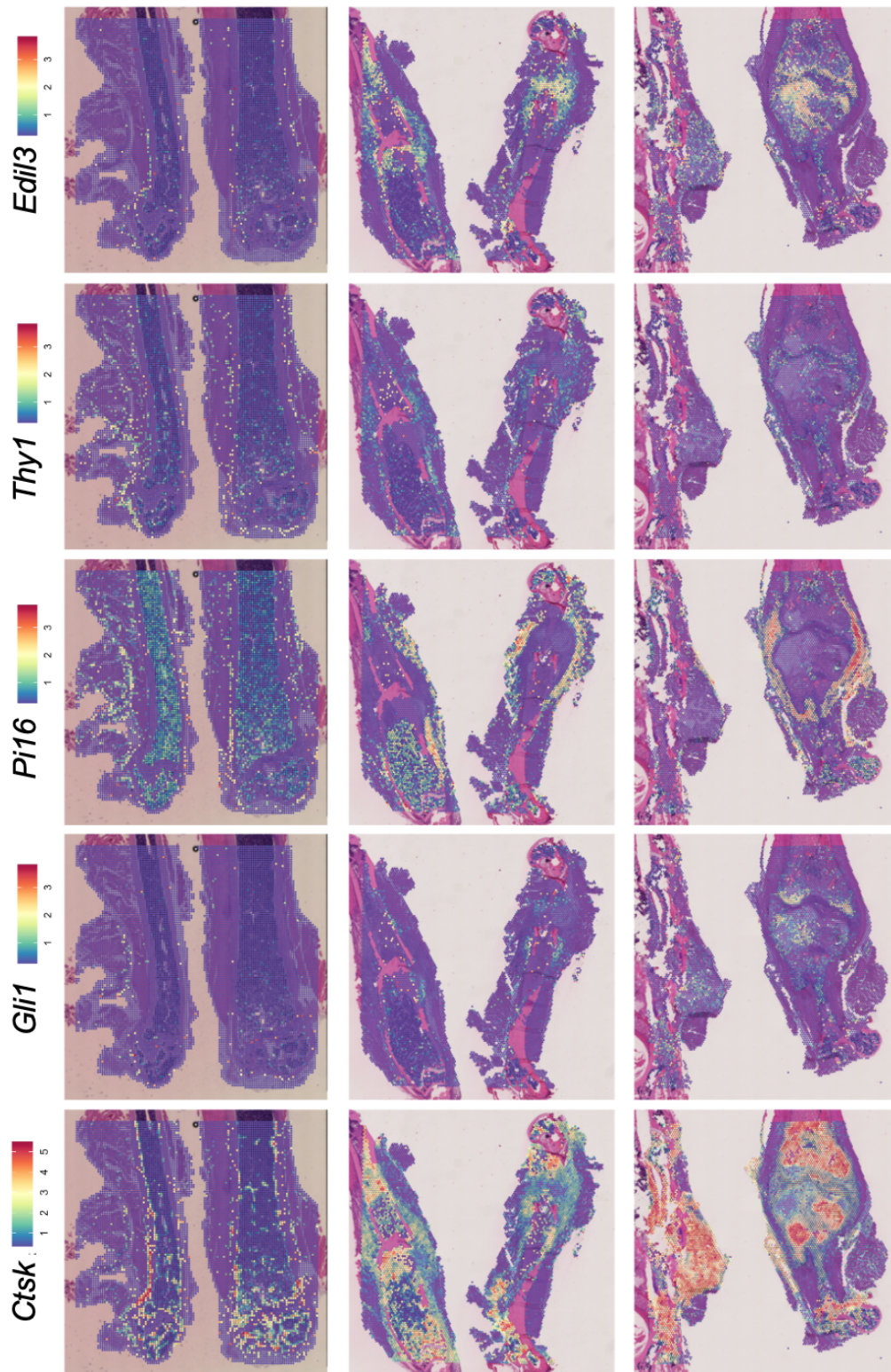
Supplementary Figure 5: Spatial Distribution of Group 2 clusters (Cluster: 8, 25, 19, 22, 14, 18, 7, 10, 13)



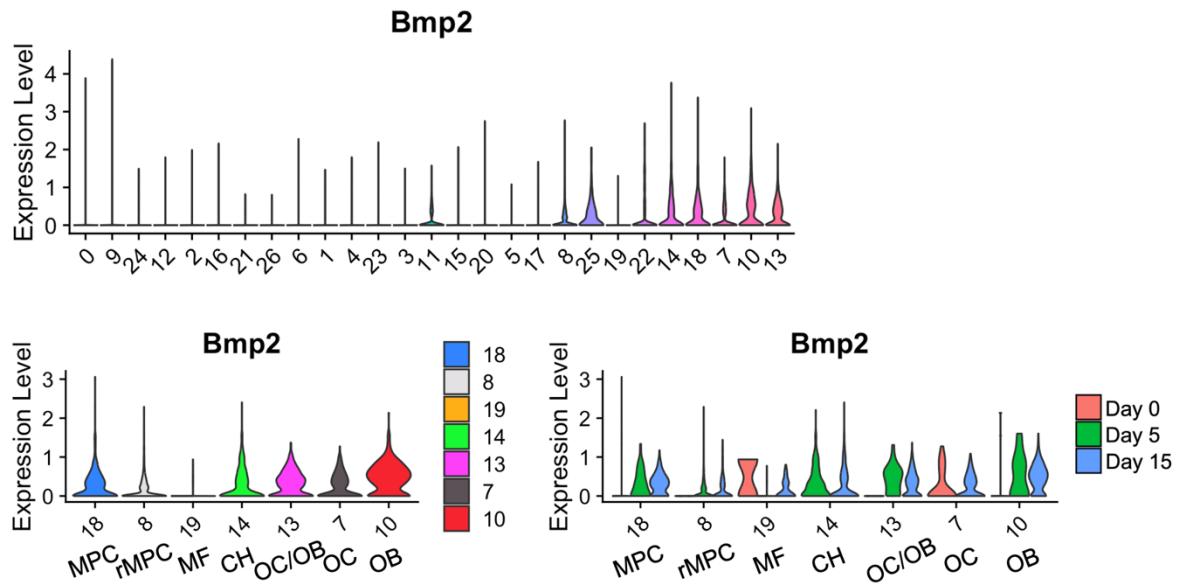
Supplementary Figure 6: Loupe Cell software selection of periosteal or callus region spots.



Supplementary Figure 7: Spatial expression pattern of literature reported periosteal progenitor markers-1



Supplementary Figure 8: Spatial expression pattern of literature reported periosteal progenitor markers-2

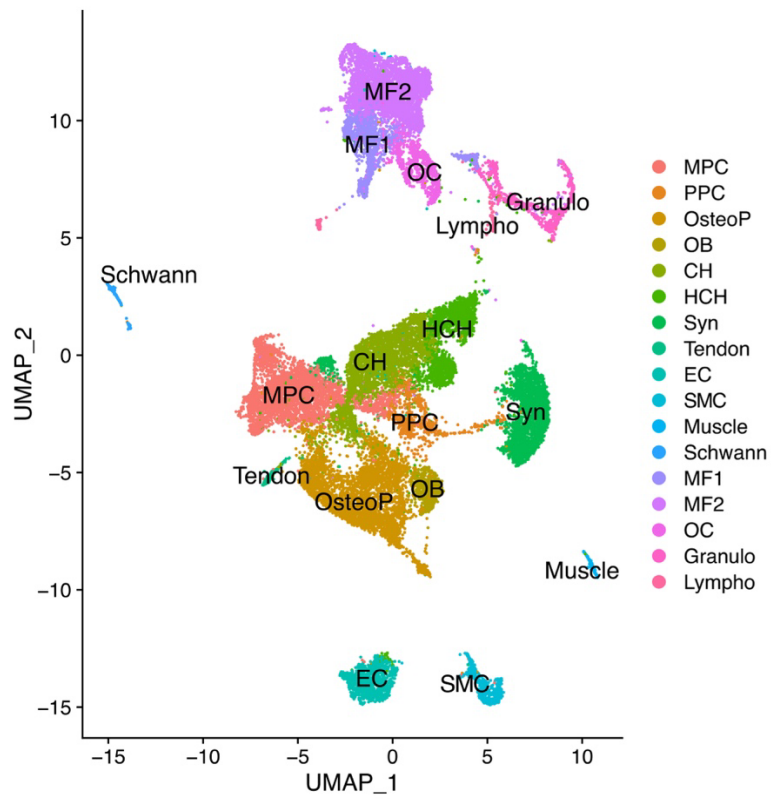


Supplementary Figure 9: Expression of Bmp2 across identified cellular clusters during bone fracture healing.

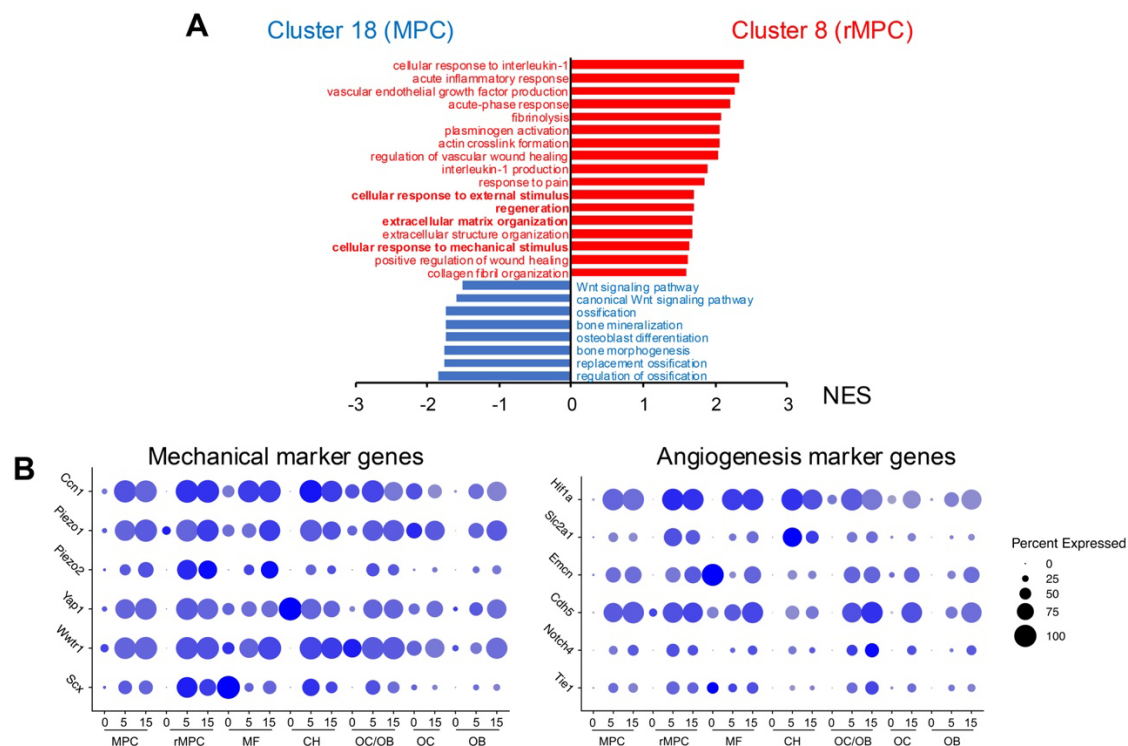
Top panel: Violin plot showing the expression level of Bmp2 across all spatial clusters identified in the fracture healing tissue.

Bottom left panel: Violin plot depicting Bmp2 expression across major cell types identified in the periosteal region, color-coded by cell cluster identity: mesenchymal progenitor cells (MPC), regenerative MPC (rMPC), macrophages (MF), chondrocytes (CH), osteoclasts/osteoblasts (OC/OB), osteoclasts (OC), and osteoblasts (OB).

Bottom right panel: Violin plot illustrating temporal changes in Bmp2 expression in periosteal cells at Day 0, Day 5, and Day 15 postfracture, color-coded by time point.



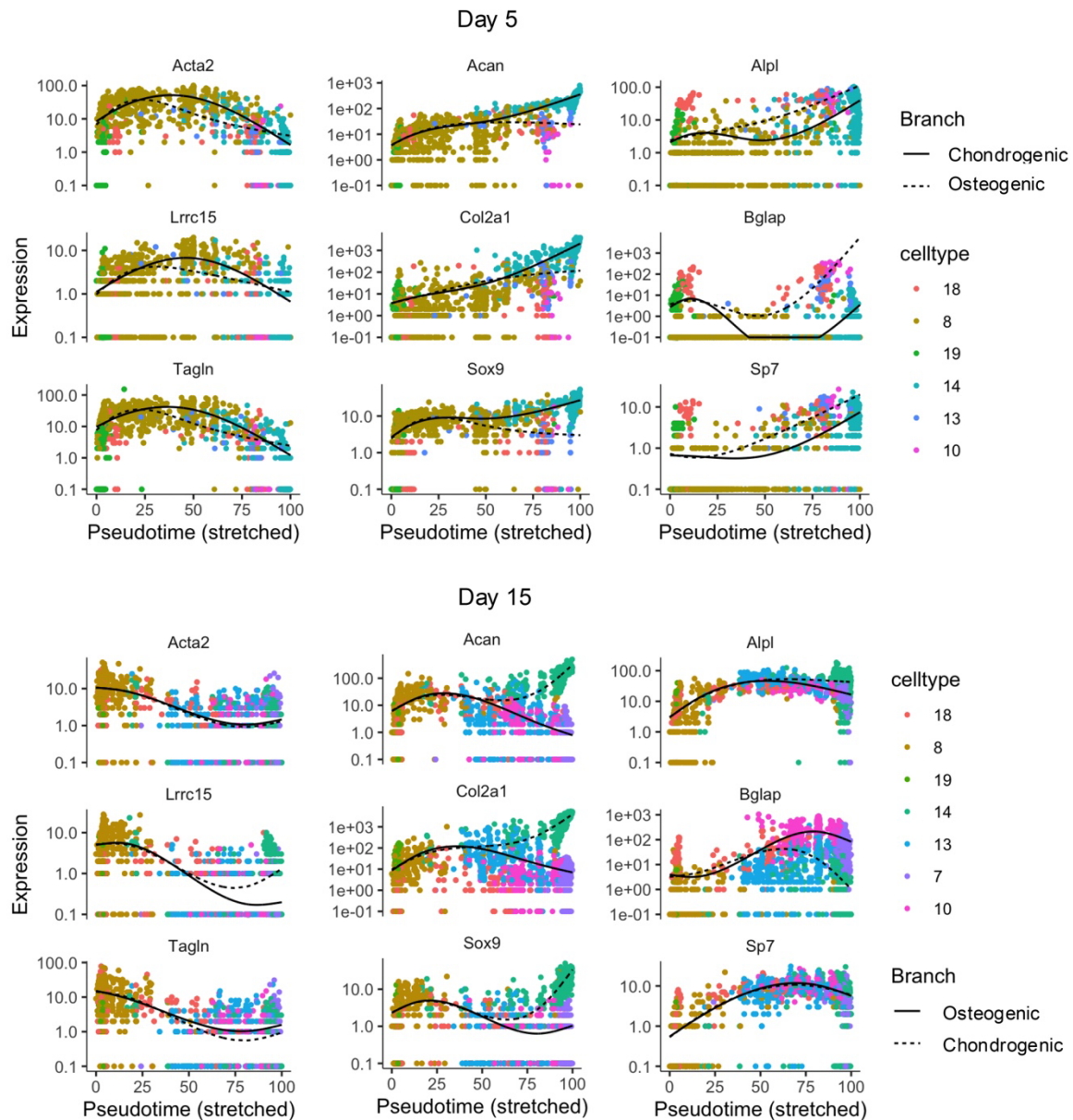
Supplementary Figure 10. UMAP plot of ScRNA seq of periosteal population during fracture healing.



Supplementary Figure 11. Functional Profiling of Mesenchymal Progenitor Cells (MPCs) and Regenerative MPCs (rMPCs) Through GSEA and Marker Gene Analysis

A. Left (Cluster 18 - MPC): Bar graph displaying negative enrichment scores for pathways involved in inflammation, wound healing, and extracellular matrix organization, indicating lower activity of these processes in standard MPCs. Right (Cluster 8 - rMPC): Bar graph showing positive enrichment scores for pathways related to Wnt signaling, ossification, and bone differentiation, reflecting the enhanced regenerative capabilities and activation of osteogenic pathways in rMPCs.

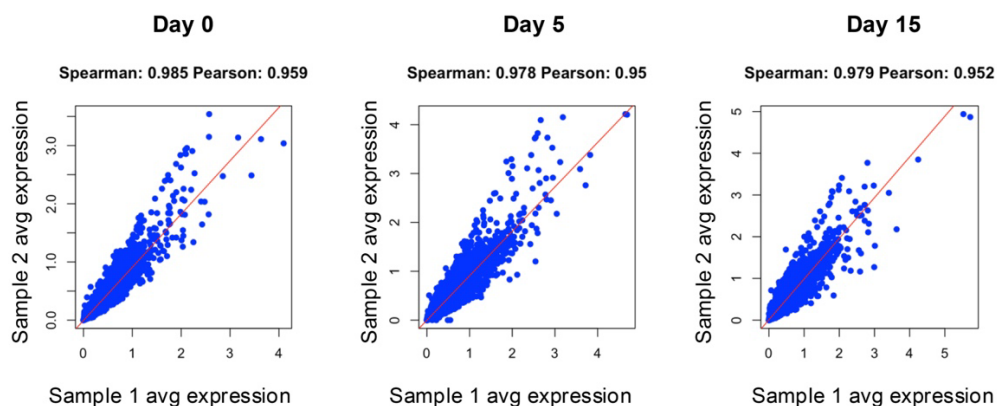
B. Left: Dot plot visualization of the expression of mechanical transduction-related genes across different cell types. Right: Dot plot showing the expression of angiogenesis-related genes in the same cell types.



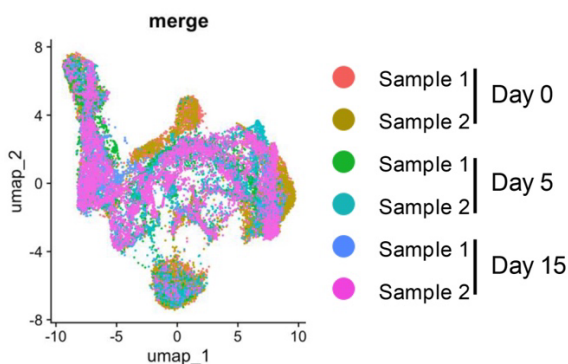
Supplementary Figure 12. Expression Dynamics of Key Differentiation Marker Genes During Bone Healing

Several key differentiation marker genes expression evolves in various cell types as they progress through the stages of differentiation during bone healing.

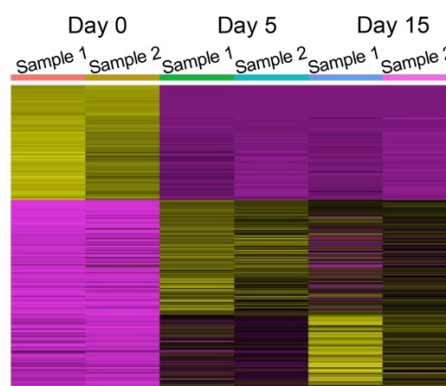
A



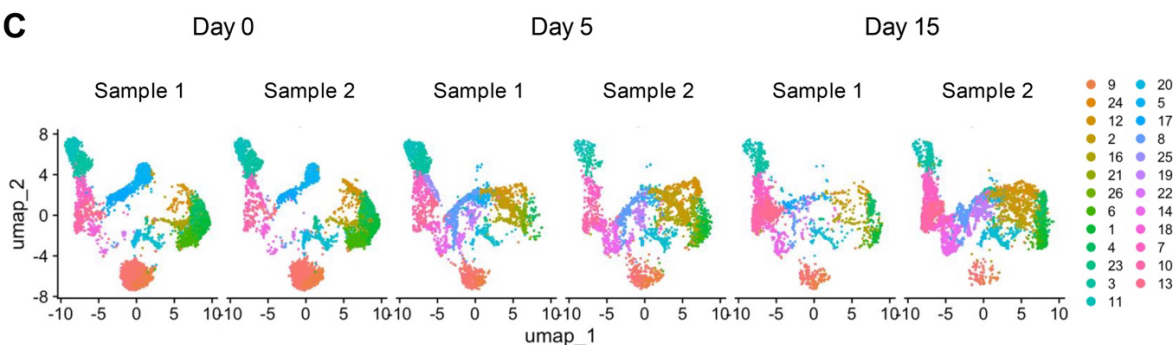
B



D



C



Supplementary Figure 13. Correlation analysis and reproducibility across biological replicates.

A. Scatter plots showing high correlation of gene expression between biological replicates at Days 0, 5, and 15 postfracture.

B. UMAP plot merging samples from all time points, with different colors indicating sample replicates and time points.

C. Individual UMAP plots showing cluster distribution separately for each replicate at each time point.

D. Heatmap of differentially expressed genes across samples and replicates at Days 0, 5, and 15, highlighting reproducibility between samples.