

Supplementary Materials for  
**Spatial transcriptomics in bone mechanomics: Exploring the  
mechanoregulation of fracture healing in the era of spatial omics**

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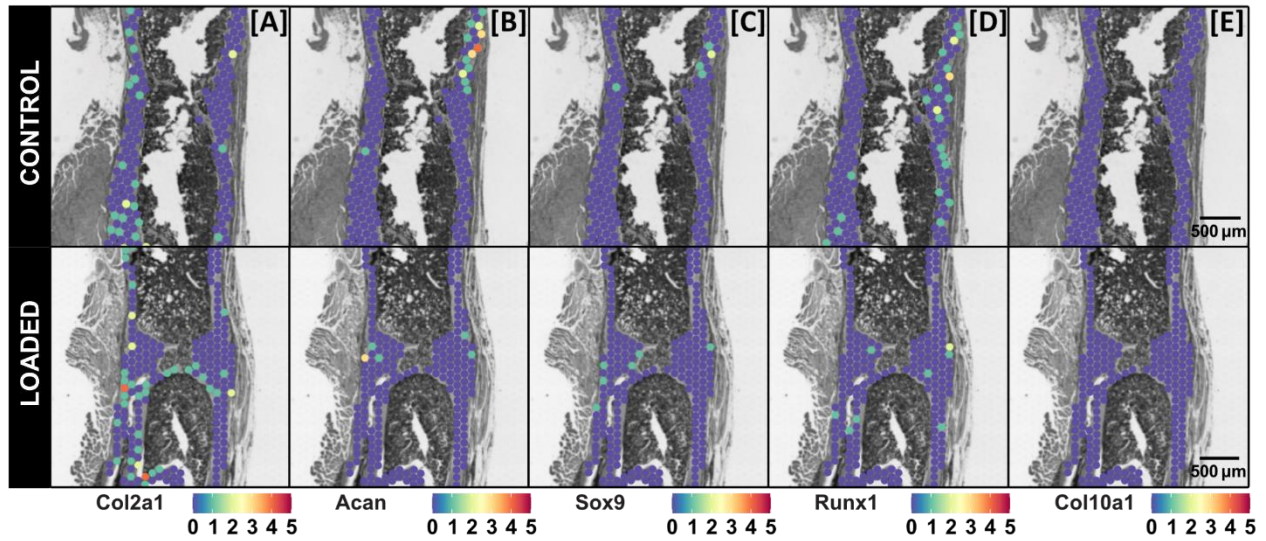
**The PDF file includes:**

Figs. S1 and S2  
Legends for tables S1 to S6

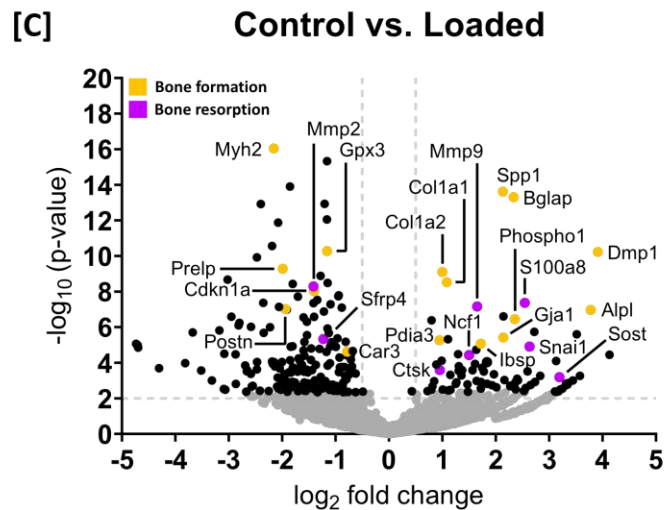
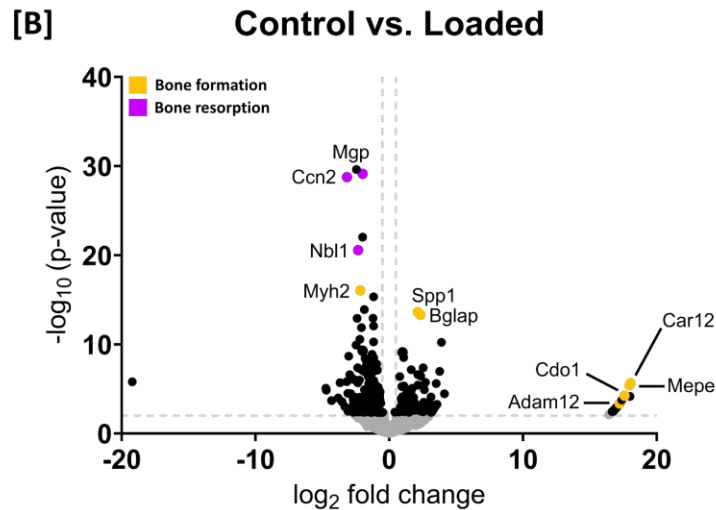
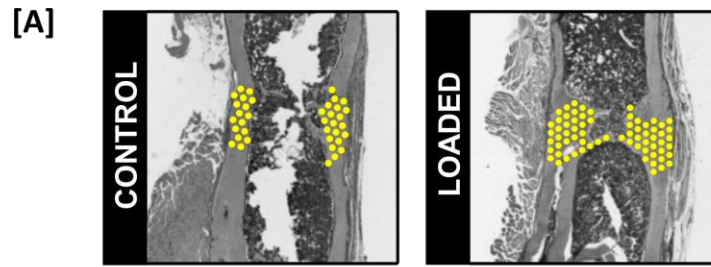
**Other Supplementary Material for this manuscript includes the following:**

Tables S1 to S6

## Supplementary Materials



**Supplementary Fig. S1. Spatial gene expression maps of selected chondrocyte markers at the fracture site of Control and Loaded mice.** Visualization of the spatial expression patterns of chondrocyte markers: [A] *Col2a1*, [B] *Acan*, [C] *Sox9*, [D] *Runx1* and [E] *Col10a1* within the fracture sites of Control and Loaded mice are presented. Each legend denotes the normalized expression of each gene. Data presented (n = 1 per group) corresponds to samples at 5 weeks post-surgery. 3D visualizations of the morphology of these Control and Loaded fracture sites are presented in Figure 2. Spatial transcriptomics spot size = 55  $\mu\text{m}$ .



**Supplementary Fig. S2. Gene expression profiling in DC + DP regions of Control vs. Loaded fracture sites.** Data presented ( $n = 1$  per group) corresponds to samples at 5 weeks post-surgery. [A]: Areas of interest defined in each fracture site for the analysis. [B]: Volcano plot to visualize differentially expressed genes (DEGs) (significance criteria: FDR-adjusted p value cutoff  $< 0.05$  and an absolute  $\log_2$  fold change  $> 0.5$ ). Significant DEGs associated with bone formation are identified in orange and significant DEGs associated

with bone resorption (or are inhibitors / antagonists of bone formation) are identified in purple. Non-DEGs are represented in gray. [C]: Magnified view of volcano plot to highlight DEGs of interest.

**Supplementary Table S1.** Barcodes corresponding to all spots of the Control and Loaded bone fracture sites.

**Supplementary Table S2.** Differential gene expression analyses (DEG) between Control and Loaded fracture sites.

**Supplementary Table S3.** Differential gene expression (DEG) analysis of high strain region with respect to reference region.

**Supplementary Table S4.** Differential gene expression (DEG) analysis of low strain region with respect to reference region.

**Supplementary Table S5.** Gene-set enrichment analysis between mean expression of high strain versus reference strain regions.

**Supplementary Table S6.** Gene-set enrichment analysis between mean expression of low strain versus reference strain regions.